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(54) Titre : NOUVELLE APPROCHE POUR CONCEVOIR DES GLYCOPEPTIDES A BASE DE O-SPECIFIQUE POLYSACCHARIDE DE SHIGELLA FLEXNERI SEROTYPE 2A

(54) Title: NOVEL APPROACH TO DESIGN GLYCOPEPTIDES BASED ON O-SPECIFIC POLYSACCHARIDE OF SHIGELLA FLEXNERI SEROTYPE 2A

(57) Abrégé/Abstract:
As part of a program for the development of synthetic vaccines against the pathogen Shigella flexneri, the conformational behavior of the O-specific polysaccharide (O-SP) of S. flexneri 5a and of four related synthetic pentasaccharide fragments was studied with a combination of NMR and molecular modeling methods. The NMR conformational analysis based on ^{1}H and ^{13}C chemical shifts analysis, inter-residue distances evaluation as well as one- and three-bond heteronuclear coupling constants measurements shows that the conformation of one of the four related pentasaccharides closely mimics the conformational features of the native O-SP in solution. Inhibition ELISA demonstrated that a protective monoclonal antibody specific for S. flexneri 5a shows a higher affinity for this pentasaccharide when compared with the others. A complete conformational search was carried out on the pentasaccharides using the CICADA algorithm interfaced with MM3 force field. Boltzman-averaged inter-residue distances and $^{3}J_{C,H}$ coupling constants were calculated for the different conformational families and compared to data obtained by NMR on all pentasaccharides. Experimental data are consistent with only one conformational family. From these informations, several models of the O-SP have been built with the molecular builder POLYS. The models in agreement with NMR data consist of right-handed helices presenting the branched glucosyl residue toward the external surface of their cylinder.

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ABSTRACT

As part of a program for the development of synthetic vaccines against the pathogen *Shigella flexneri*, the conformational behavior of the *O*-specific polysaccharide (O-SP) of *S. flexneri* 5a and of four related synthetic pentasaccharide fragments was studied with a combination of NMR and molecular modeling methods. The NMR conformational analysis based on ^{1}H and ^{13}C chemical shifts analysis, inter-residue distances evaluation as well as one- and three-bond heteronuclear coupling constants measurements shows that the conformation of one of the four related pentasaccharides closely mimics the conformational features of the native O-SP in solution. Inhibition ELISA demonstrated that a protective monoclonal antibody specific for *S. flexneri* 5a shows a higher affinity for this pentasaccharide when compared with the others. A complete conformational search was carried out on the pentasaccharides using the CICADA algorithm interfaced with MM3 force field. Boltzman-averaged inter-residue distances and $^{3}J_{C,H}$ coupling constants were calculated for the different conformational families and compared to data obtained by NMR on all pentasaccharides. Experimental data are consistent with only one conformational family. From these informations, several models of the O-SP have been built with the molecular builder POLYS. The models in agreement with NMR data consist of right-handed helices presenting the branched glucosyl residue toward the external surface of their cylinder.

INTRODUCTION

Capsular polysaccharides (CPS) and lipopolysaccharides (LPS) of Gram-negative bacteria are known as important virulence factors as well as major targets of the host's immune response upon infection (1). The potential of CPSs as vaccine candidates against bacterial infections has been demonstrated in the early 30s, and several polysaccharide vaccines such as those targetting

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Blockwise approach to fragments of the O-specific polysaccharide of *Shigella flexneri* serotype 2a: Convergent synthesis of a decasaccharide representative of a dimer of the branched repeating unit[1]

ABSTRACT

Introduction

Shigellosis or bacillary dysentery is a worldwide disease, occurring in humans only, caused by organisms of the genus *Shigella*. Responsible for an estimated 200 million cases annually, *Shigella* is increasingly resistant to antimicrobial drugs. [2]Shigellosis is a priority target as defined by the World Health Organization since this disease is a major cause of mortality in developing countries, especially among children under 5 years of age and in the immunocompromised population. [3]Although no vaccine is yet available against shigellosis, several programs targeting the eradication of this bacterial infection are under development, with emphasis on vaccination strategies involving either live attenuated strains of *Shigella*[4] or acellular vaccines based on lipopolysaccharide (LPS) antigens and derivatives thereof. [5]Of particular interest in the later approach is the design of glycoconjugate vaccines based on the use of detoxified LPS. Indeed, there is evidence that natural and experimental infections with *Shigella* confer type-specific immunity[6] which points to the O-specific polysaccharide (O-SP) moiety of the LPS as the target antigen of the host's protective immune response to infection. Besides, data show that significant levels of pre-existing antibodies specific for the O-SP correlate with a diminished attack rate of

shigellosis.[7] Furthermore, it was recently demonstrated in field trials that protein conjugates of detoxified LPS provided protection to human volunteers against infections caused by *S. sonnei*.[8] As was particularly emphasized in the case of *S. dysenteriae* type 1, conjugates incorporating oligosaccharide fragments of the native bacterial polysaccharides may be even more immunogenic than their counterparts made of the detoxified LPS.[9]

Of most concern amongst the different species of *Shigella*, is *S. flexneri* serotype 2a, the prevalent infective agent responsible for the endemic form of the disease.[10] Indeed, major efforts from different laboratories including the development of conventional polysaccharide-protein conjugates,[11] aim at the development of a vaccine against the disease associated with this particular serotype. In parallel, a program aimed at the design of chemically defined glycoconjugate vaccines based on the use of synthetic fragments of the O-SP of *S. flexneri* 2a, is under development in this laboratory. We adopted a rational approach, involving a preliminary study of the interaction between the bacterial O-SP and homologous protective monoclonal antibodies.

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit **I**.[12,13] It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens and comprises a *N*-acetyl glucosamine and three rhamnose residues, together with an α-D-glucopyranose residue branched at position 4 of one of the rhamnoses. Besides the known methyl glycoside of the **EC** disaccharide,[14,15] a set of di- to pentasaccharides[16-18] and more recently an octasaccharide[19] representative of fragments of *S. flexneri* 2a O-SP have been synthesized recently. The use of these compounds as molecular probes for mapping at the molecular level the binding characteristics of a set of protective antibodies against *S. flexneri* 2a infection indicated that access to larger oligosaccharides would help the characterization of the carbohydrate antigenic determinants. For this purpose, methodologies allowing a straightforward access to *S. flexneri* 2a oligosaccharides of larger size are under study in this laboratory. We now report the synthesis of the first decasaccharide in the series, namely the **D'A'B'(E')C'DAB(E)C** fragment, which was prepared as its methyl glycoside (**1**).

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Results and discussion

Considering its dimeric nature, a convergent synthetic strategy to the target **1** was considered. Indeed, retrosynthetic analysis, supported by previous work in the field,[19-22] indicated that disconnections at the **C-D** linkage, thus based on two **DAB(E)C** branched pentasaccharides corresponding to a frame-shifted repeating unit **I**, would be the most advantageous (Scheme 1). Such a strategy would involve a pentasaccharide acceptor easily derived from the known methyl glycoside **2**[17] or from the corresponding *N*-acetylated analogue **3** and a pentasaccharide donor bearing a 2-*O*-acyl protecting group at the reducing residue (**C**) in order to direct glycosylation towards the desired stereochemistry. Depending on the nature of the 2-*N*-acyl group in residue **D**, the latter could derive from the allyl glycosides **4** or **5**. Besides, bearing in mind that the major drawbacks of the linear synthesis of pentasaccharide **2** reported so far[17] dealt with the selective deblocking of key hydroxyl groups to allow further chain elongation, we describe herein various attempts at a convergent synthesis of the fully protected **DAB(E)C** pentasaccharide as its methyl (**2**, **3**) or allyl (**4**, **5**) glycosides. Precedents concerning a related serotype of *S. flexneri* have indicated that disconnection at the **D-A** linkage should be avoided. [21,22] However to our knowledge, disconnection at the **B-C** or **A-B** linkages was never attempted in the series. Both routes were considered in the following study. The nature of the repeating unit **I** indicated that any blockwise synthesis involving such linkages would rely on donors lacking any participating group at position 2 of the reducing residue, thus the relevance of this strategy may be questioned. Nevertheless, although β-glycoside formation was observed occasionally,[23] the good α-stereoselectivity reported on several occasions in the literature for glycosylation reactions based on mannopyranosyl[24,25] and derivatives such as perosaminyl[26,27] **(cf KOVAC)** or rhamnopyranosyl donors that were either glycosylated at C-2,[28] or blocked at this position with a non participating group,[29] encouraged the evaluation of the above mentioned block strategies. To follow up the work developed thus far in the *S. flexneri* 2a series, emphasis was placed on the use of the use of trichloroacetimidate (TCA) chemistry.[30]

*Strategy based on the disconnection at the **A-B** linkage (Scheme 1, route a):* Such a strategy involves the coupling of suitable **DA** donors to an appropriate **B(E)C** acceptor. Taking into

account the glycosylation chemistry, two sets of disaccharide building blocks (**6**, **7**, **8**), easily obtained from known monosaccharide precursors which were readily available by standard protecting group/activation strategies, were selected (Scheme 1). Thus, condensation of the allyl rhamnopyranoside **14**, [31] as precursor to residue **A**, with the glucosaminyl trichloroacetimidate **16**, [32] as precursor to residue **D**, was performed in the presence of a catalytic amount of TMSOTf to give the fully protected disaccharide **17** (99%). Selective deallylation of **17** proceeded in two steps involving (i) iridium(I)-catalysed isomerisation of the allyl glycoside into the corresponding 1-*O*-propenyl glycoside[33] and (ii) hydrolysis of the latter. [34,35] The resulting hemiacetal **18** (81%) was converted into the trichloroacetimidate **6** (78%) by treatment with trichloroacetonitrile in the presence of a catalytic amount of DBU (Scheme 2). Knowing from previous experience that conversion of the 2_D-trichloroacetyl moiety into the required 2_D-*N*-acetyl group could be somewhat low-yielding, we took advantage of the blockwise approach to perform the above mentioned transformation at an early stage in the synthesis. Thus, the disaccharide intermediate **17** was converted to the corresponding **19** (90%) upon overnight treatment with a saturated ammonia methanolic solution and subsequent peracetylation. Conversion of **19** into the hemiacetal **20** (69%), and next into the required trichloroacetimidate donor **7** (86%), followed the procedure described above for the preparation of **6** from **17**. Where glycosylation is concerned, the bifunctional role of thioglycosides as protected acceptors and masked donors is highly appreciated.(***Ref***) Thus, the thiophenyl disaccharide **8** was considered as a possible alternative to the use of the more reactive trichloroacetimidates **6** and **7**. It was synthesized in 97% yield by condensing the known thiophenyl rhamnopyranoside **15**,[36] and **16** in the presence of a catalytic amount of TMSOTf. To fulfil the requirements of the synthesis of **1**, two different trisaccharide building blocks were used, namely either the known methyl glycoside **9**[17] or the corresponding allyl glycoside **10**, obtained from the known 2_B-*O*-acetylated trisaccharide **42** (see below and Scheme 5).[18] Condensation of the trisaccharide acceptor **9** and the trichloroacetimidate donor **6** was attempted under various conditions of solvent, temperature and promoter. The α-linked condensation product, ie the known pentasaccharide **2**,[17] was at best isolated in 41% yield providing that the glycosylation reaction was run in acetonitrile in the presence of a catalytic amount of TMSOTf, following the inverted procedure protocol[37,38] in order to minimize the degradation of the donor.

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Although the α-selectivity of the glycosylation reaction was good, yields of pentasaccharide remained low, and, as anticipated, use of the alternate trichloroacetimidate donor **7** to give **3** did not result in any improvement (not described). Rearrangement of the activated donor into the corresponding inert trichloroacetamide was observed previously in glycosylation reactions based on trichloroacetimidate donors lacking a participating group at position 2 of the reducing residue.(***Ref***) Although the expected side-product was not isolated in any of the attempted glycosylation with **6** or **7**, it was anticipated that the use of an alternate glycosylation chemistry would prevent such side-reaction, and possibly favour the condensation. However, reaction of thiophenyl donor **8** and acceptor **10** in the presence of *N*-iodosuccinimide and catalytic triflic acid did not prove any better as it resulted in mixtures of products from which the target **4** was isolated in very low yield, 10% at best. This strategy was thus not considered any further.

*Strategy based on the disconnection at the **B-C** linkage (Scheme 1, route b):* It was hypothesized that the good α-selectivity, but poor yields, of the condensation of the various **DA** donors with the **B(E)C** acceptors **9** and **10** might result from the poor nucleophilicity of the axial hydroxyl at position 2_B. Thus, we next turned to the 3_C-OH as a possible elongation site in the design of a block synthesis of pentasaccharide **5**. Considering such a disconnection approach suggests the use of a **DAB** trisaccharide donor for coupling to an **EC** disaccharide acceptor. As the target pentasaccharide should serve as an appropriate donor in the construction of **1**, we reasoned that an acyl participating group had to be present at its position 2_C. Thus, two 2_C-*O*-acylated **EC** building blocks, **11** or **12**, were considered. In order to avoid any unnecessary deprotection step at the pentasaccharide level, the trisaccharide **13**, bearing an acetamido functionality at position 2_D, was selected as the donor. Indeed, as it involves the less readily available **EC** structure in fewer synthetic steps and does not rely on selective deprotection at the 2_A position, this path was found particularly attractive. Again, it relies on the use of appropriately functionalized known monosaccharide intermediates.

The known key di-rhamnoside core structure **23**[39] was formed by glycosylation of the allyl rhamnoside **21(in fact 14)** with the trichloroacetimidate donor **22**[40] in the presence of a catalytic amount of TMSOTf. It should be pointed out that using diethyl ether as the

solvent, the isolated yield of **23** was 92%, which compares favourably with those obtained previously, 60% and 76.2%,[39] when running the reaction in dichloromethane under promotion by TMSOTf or $BF_3.OEt_2$, respectively. De-*O*-acetylation under Zemplén conditions afforded the 2_A-*O*-unprotected acceptor **24**[21] in quantitative yield.

As shown previously in the construction of the **DA** intermediate **17**, the *N*-trichloroacetyl trichloroacetimidate **16** appears to be a highly suitable precursor to residue **D** when involved in the formation of the β-GlcNAc linkage at the poorly reactive 2_A position. Indeed, reaction of **16** with the acceptor **24** in 1,2-dichloroethane in the presence of TMSOTf went smoothly and gave the trisaccharide **26** in 96% yield. However, conversion of the *N*-trichloroacetyl group to the *N*-acetyl derivative **27** was rather less successful as the desired trisaccharide was obtained in only 42 % yield when treated under conditions that had previously been used in the case of a related oligosaccharide (sodium methoxide, Et_3N, followed by re-*N,O*-acetylation).[17] This result led us to reconsider the protection pattern of the glucosamine donor. The *N*-tetrachlorophthalimide group has been proposed as an alternative to overcome problems associated with the widely spread phthalimido procedure when introducing a 2-acetamido-2-deoxy-β-D-glucopyranosidic linkage.[41] Thus, the *N*-tetrachlorophthalimide trichloroacetimidate donor **25** was selected as an alternative. It was prepared as described from commercially available D-glucosamine,[42] apart from in the final imidate formation step, where we found the use of potassium carbonate as base to be more satisfactory than DBU. Glycosylation of **24** with **25** in the presence of TMSOTf resulted in the trisaccharide **28** in 65% yield. The tetrachlorophthaloyl group was then removed by the action of ethylenediamine, and subsequent re-*N,O*-acetylation gave the trisaccharide **27** in 65% yield. The latter was next converted into the donor **13** in two steps, analogous to those described for the preparation of **6** from **17**. Indeed, de-*O*-allylation of **27** cleanly gave the hemiacetal **29** (83%), which was then activated into the required trichloroacetimidate (94%). It is worth mentioning that although they involve a different **D** precursor, both strategies give access to the intermediate **27** in closely related yields, 40 and 42%, respectively.

Initial attempts to form the pentasaccharide **5** from **13** and the previously described acceptor **11**[18] in the presence of TMSOTf as promoter were rather unsuccessful, resulting in at best 17% of the desired product, accompanied by decomposition of the donor into the

hemiacetal **29** (75%). Using $BF_3.OEt_2$ as the promoter in place of TMSOTf, reaction of **11** with **13** at room temperature provided **5** in 44% yield, with the acceptor **11** and hemiacetal **29** also recovered in 54 and 29% yield, respectively. We considered that the poor reactivity of the acceptor was responsible for these results, as the ^{13}C NMR of **13**, showing several distorted signals **(notably ???)**, suggests that there is considerable steric hindrance around the position 3_C. For that matter, the 2_C-*O*-acetylated disaccharide **12** was considered as an alternate acceptor. Analogously to the preparation of **11**, it was obtained from the known diol **30** through regioselective opening of the intermediate orthoester. However, coupling of the potentially less hindered acceptor **12** and the trisaccharide donor **13** resulted, at best, in the isolation of the condensation product **31** in 42% yield (not described).

The modest yield of **1** obtained by this route made the alternative reaction path (Scheme 4) worth investigating, despite the more numerous synthetic steps required. Indeed, it was found rather appealing when evaluated independently in a closely related series (unpublished results). By this route, a tetrasaccharide acceptor can be formed from two disaccharide building blocks (**EC** and **AB**), and coupled with an appropriate monosaccharide donor as precursor to **D**. Considering that selective deprotection of the 2_A hydroxyl group would occur in the course of the synthesis, glycosylation attempts were limited to the 2-*O*-benzoylated acceptor **11**. The disaccharide donor necessary for this path could be derived from the building block **24**, already in hand. The choice of temporary protecting group at position 2_A was determined by our experience of the stepwise synthesis of the corresponding methyl pentasaccharide,[17] where we noted that an acetate group at this position may not be fully orthogonal to the benzoate located at position 2_C. The chosen group had also to support removal of the anomeric allyl group and the subsequent conversion to the trichloroacetimidate. At first, a chloroacetate group was anticipated to fulfil these requirements. Thus, the disaccharide **24** was treated with chloroacetic anhydride and pyridine to give the derivative **32** (57%). Anomeric deprotection to give the hemiacetal **33** (84%) and subsequent trichloroacetimidate activation of the latter into the donor **34** (83%) were performed in the same way as before. Coupling of **11** with **34**, carried out in the presence of TMSOTf at –40°C, yielded a complex mixture of products. When the temperature was lowered to –60°C, the condensation product **38** could be isolated in 22% yield. The α-stereoselectivity of the glycosylation was ascertained from the value of the $^1J_{C,H}$

coupling constant at C-1_B which was **XX** Hz.[43,44]**(A faire ?)** Alternative donor protection was attempted. Treatment of **24** with *p*-methoxybenzyl chloride and sodium hydride gave the fully protected derivative **35** (97%), which was cleanly converted into the trichloroacetimidate donor **37** (82%) in two steps involving the hemiacetal intermediate **36** (73%). Glycosylation of **11** with **37** in the presence of TMSOTf at –40°C gave the desired tetrasaccharide **39** in 44% yield. Again, the stereochemistry of the newly created linkage was ascertained based on the $^1J_{C,H}$ heteronuclear coupling constants. When the temperature was lowered to –60°C, the yield of **39** fell to 34% and a second major product **40** (21%) was observed in the mixture. Indeed, examination of the NMR spectra of this product revealed that the *p*MeOBn group had been lost. That **40** was the acceptor required for the next step brought the estimated yield of condensation to 55%. Nevertheless, the overall outcome of this blockwise strategy did not match our expectations, and this route was abandoned.

*Linear strategy to the fully protected pentasaccharide **4**:* As preliminary studies have demonstrated, rapid access to suitable building blocks allowing the synthesis of higher-order oligosaccharides representative of fragments of the O-SP of *S. flexneri* 2a remains a challenge. Major conclusions drawn from our studies favour the design of a linear synthesis of the target **4**. Indeed, when put together with our previous work, such as the synthesis of tetrasaccharide **41** (95%)[17] or that of trisaccharide **42** (97%),[18] all the above described attempted couplings outlined the loss of efficiency of glycosylation reactions involving rhamnopyranosyl donors glycosylated at position 2 in comparison to those involving the corresponding acetylated donor. Thus, matching the linear strategy of the methyl pentasaccharide **2** described previously,[17] a synthesis of **4**, based on donors bearing a participating group at O-2, was designed. Three key building blocks were selected. These were the readily accessible **EC** disaccharide acceptor **11** benzoylated at C-2 as required for the final condensation step leading to the fully protected decasaccharide intermediate; the rhamnopyranosyl trichloroacetimidate **22**, which serves as a precursor to residues **A** and **B**, and bears a both temporary and participating group at position 2; and the trichloroacetamide glucosaminyl donor **16** as a precursor to residue **D**. As stated above, coupling of **11** and **22** gave **42** in high yield. As observed in the methyl glycoside series,[17] de-*O*-acetylation using MeONa or methanolic HCl was poorly selective. Although,

guanidine/guanidinium nitrate was proposed as a mild and selective *O*-deacetylation reagent compatible with the presence of benzoyl protecting groups,[45] none of the conditions tested prevented partial debenzoylation leading to diol **43**, as confirmed from mass spectroscopy and NMR analysis **(cf Anne-Laure)**. The required alcohol **10** was readily obtained in an acceptable yield of 84% yield by a five-day acid catalysed methanolysis, using HBF_4 in diethyl ether/methanol,[17,46] of the fully protected intermediate **42** (Scheme 5). Repeating this two-step process using **10** as the acceptor and **22** as the donor resulted first in the intermediate **44** (90%), and next in the tetrasaccharide acceptor **40** (84%). Glycosylation of the latter with **16** gave the fully protected pentasaccharide **4** in high yield (98%), thus confirming that the combination of the trichloroacetamide participating group and the trichloroacetimidate activation mode in **16** results in a potent donor to be used as a precursor to residue **D** in the *S. flexneri* series, where low-reactive glycosyl acceptors are concerned. Following the above described procedure, selective anomeric deprotection of **4** furnished the hemiacetal **45** which was smoothly converted to the trichloroacetimidate donor **46** (66% from **4**). From these data, the linear synthesis of **4,** truly benefiting from the use of **22** as a common precursor to residue **A** and **B**, appears as a reasonable alternative to the block syntheses which were evaluated in parallel.

Synthesis of the target decasaccharide 1: Having a pentasaccharide donor in hand, focus was next placed on the synthesis of an appropriate pentasaccharide acceptor. In our recent description of the convergent synthesis of the **B'(E')C'DAB(E)C** octasaccharide,[19] the pentasaccharide **48**, bearing a $4_D,6_D$-*O*-isopropylidene protecting group, was found a most convenient acceptor which encouraged its selection in the present work. Briefly, **48** was prepared in two steps from the known **2**. Thus, mild transesterification of **2** under Zemplen conditions allowed the selective removal of the acetyl groups to give triol **47**, which was converted to the required acceptor **48** (72% from **2**) upon subsequent treatment with 2,2-dimethoxypropane. Relying on previous optimisation of the glycosylation step,[19] the condensation of **48** and **46** was performed in the presence of a catalytic amount of triflic acid. However, probably due to the closely related nature of the donor and acceptor, the reaction resulted in an inseparable mixture of the fully protected **49** and the hemiacetal **45** resulting from partial hydrolysis of the donor. Most conveniently, acidic hydrolysis of the

mixture, allowing the selective removal of the isopropylidene group in **49**, gave the intermediate diol **50** in a satisfactory yield of 72% for the two steps. According to the deprotection strategy used for the preparation of the closely related octasaccharide,[19] diol **50** was engaged in a controlled de-*O*-acylation process upon treatment with hot methanolic sodium methoxide. However, partial cleavage of the trichloroacetyl moiety, leading to an inseparable mixture, was observed which prevented further use of this strategy. Indeed, it was assumed that besides being isolated and therefore resistant to Zemplén transacetylation conditions,[47-49] the 2_C-*O*-benzoyl groups were most probably highly hindered which contributed to their slow deprotection. Alternatively, **50** was submitted to an efficient two-step in-house process involving first, hydrogenolysis under acidic conditions which allowed the removal of the benzyl groups and second, basic hydrochlorination which resulted in the conversion of the *N*-trichloroacetyl groups into the required *N*-acetyl ones, thus affording **51**. Subsequent transesterification gave the final target **1** in 37% yield from **50**.

CONCLUSION

The decasaccharide **1**, corresponding to two consecutive repeating units of the O-Ag of *S. flexneri* 2a was synthesized successfully based on the condensation of two key pentasaccharide intermediates, the donor **46** and acceptor **48**. Several routes to these two building blocks were investigated, involving either blockwise strategies or a linear one. The latter was the preferred one based on yields of condensation and the number of steps.

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(1) Alme *Part 10 of the series Synthesis of ligands related to the O-specific polysaccharides of Shigella flexneri serotype 2a and Shigella flexneri serotype 5a. For part 9, see ref. XX.*

(2) World; Health; Organisation *WHO Weekly Epidemiol. Rec.* **1997**, *72*, 73-80.

(3) Kotloff, K. L.; Winickoff, J. P.; Ivanoff, B.; Clemens, J. D.; Swerdlow, D. L.; Sansonetti, P. J.; Adak, G. K.; Levine, M. M. *Bull. WHO* **1999**, *77*, 651-666.

(4) Coster, T. S.; Hoge, C. W.; Verg, L. L. v. d.; Hartman, A. B.; Oaks, E. V.; Venkatesan, M. M.; Cohen, D.; Robin, G.; Fontaine-Thompson, A.; Sansonetti, P. J.; Hale, T. L. *Infect. Immun.* **1999**, *67*, 3437-3443.

(5) Fries, L. F.; Montemarano, A. D.; Mallett, C. P.; Taylor, D. N.; Hale, T. L.; Lowell, G. H. *Infect. Immun.* **2001**, *69*, 4545-4553.

(6) DuPont, H. L.; Hornick, R. B.; Dawkins, A. T.; Synder, M. J.; Formal, S. B. *J. Infect. Dis.* **1969**, *119*, 296-299.

(7) Cohen, D.; Green, M. S.; Block, C.; Slepon, R.; Ofek, I. *J. Clin. Microbiol.* **1991**, *29*, 386-389.

(8) Cohen, D.; Ashkenazi, S.; Green, M. S.; Gdalevich, M.; Robin, G.; Slepon, R.; Yavzori, M.; Orr, N.; Block, C.; Ashkenazi, I.; Shemer, J.; Taylor, D. N.; Hale, T. L.; Sadoff, J. C.; Pavliovka, D.; Schneerson, R.; Robbins, J. B. *The Lancet* **1997**, *349*, 155-159.

(9) Pozsgay, V.; Chu, C.; Panell, L.; Wolfe, J.; Robbins, J. B.; Schneerson, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194-5197.

(10) Sansonetti, P. *Rev. Infect. Dis.* **1991**, *13*, S285-???

(11) Passwell, J. H.; Harlev, E.; Ashkenazi, S.; Chu, C.; Miron, D.; Ramon, R.; Farzan, N.; Shiloach, J.; Bryla, D. A.; Majadly, F.; Roberson, R.; Robbins, J. B.; Schneerson, R. *Infect. Immun.* **2001**, *69*, 1351-1357.

(12) Simmons, D. A. R. *Bacteriol. Reviews* **1971**, *35*, 117-148.

(13) Lindberg, A. A.; Karnell, A.; Weintraub, A. *Rev. Infect. Dis.* **1991**, *13*, S279-S284.

(14) Berry, J. M.; Dutton, G. G. S. *Can. J. Chem.* **1974**, *54*, 681-683.

(15) Lipkind, G. M.; Shashkov, A. S.; Nikolaev, A. V.; Mamyan, S. S.; Kochetkov, N. K. *Bioorg. Khim.* **1987**, *13*, 1081-1092.

(16) Mulard, L. A.; Costachel, C.; Sansonetti, P. J. *J. Carbohydr. Chem.* **2000**, *19*, 849-877.

(17) Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150.

(18) Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 000-000.

(19) Bélot, F.; Costachel, C.; Wright, K.; Phalipon, A.; Mulard, L. A. *Tetrahedron. Lett.* **2002**, 000-000.

(20) Kochetkov, N. K.; Byramova, N. E.; Tsvetkov, Y. E.; Backinovsky, L. V. *Tetrahedron* **1985**, *41*, 3363-3375.

(21) Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650-2656.

(22) Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293-299.

(23) Srivastava, O. P.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 2324-2330.

(24) Ogawa, T.; Kitajma, T.; Nukada, T. *Carbohydr. Res.* **1983**, *123*, c5-c7.

(25) Ogawa, T.; Sugimoto, M.; Kitajma, T.; Sadozai, K. K.; Nukuda, T. *Tetrahadron Lett.* **1986**, *27*, 5639-5742.

(26) Kihlberg, J.; Eichler, E.; Bundle, D. R. *Carbohydr. Res.* **1991**, *211*, 59-75.

(27) Peters, T.; Bundle, D. R. *Can. J. Chem.* **1989**, *67*, 491-496.

(28) Reimer, K. B.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1992**, *228*, 399-414.

(29) Varga, Z.; Bajza, I.; Batta, G.; Liptak, A. *Tetrahedron Lett.* **2001**, *42*, 5283-5286.

(30) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123.

(31) Westerduin, P.; Haan, P. E. d.; Dees, M. J.; Boom, J. H. v. *Carbohydr. Res.* **1988**, *180*, 195-205.

(32) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189-202.

(33) Oltvoort, J. J.; Boeckel, C. A. A. v.; Koning, J. H. d.; Boom, J. v. *Synthesis* **1981**, 305-308.

(34) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903-1911.

(35) Gigg, R.; Payne, S.; Conant, R. *J. Carbohydr. Chem.* **1983**, *2*, 207-223.

(36) Lau, R.; Schuele, G.; Schwaneberg, U.; Ziegler, T. *Liebigs Ann. Org. Bioorg. Chem.* **1995**, *10*, 1745-1754.

(37) Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353-3356.

(38) Bommer, R.; Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1991**, 425-433.

(39) Zhang, J.; Mao, J. M.; Chen, H. M.; Cai, M. S. *Tetrahedron: Asymmetry* **1994**, *5*, 2283-2290.

(40) Castro-Palomino, J. C.; Rensoli, M. H.; Bencomo, V. V. *J. Carbohydr. Chem.* **1996**, *15*, 137-146.

(41) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302-3303.

(42) Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343-5346.

(43) Bock, K.; Pedersen, C. *Acta Chem. Scand. Ser. B* **1975**, *29*, 258-264.

(44) Bock, K.; Pedersen, C. *J. Chem. Soc. Perkin Trans 2* **1974**, 293-297.

(45) Ellervik, U.; Magnusson, G. *Tetrahedron Lett.* **1997**, *38*, 1627-1628.

(46) Pozsgay, V.; Coxon, B. *Carbohydr. Res.* **1994**, *257*, 189-215.

(47) Liptak, A.; Szurmai, Z.; Nanasi, P.; Neszmelyi, A. *Carbohydr. Res.* **1982**, *99*.

(48) Szurmai, Z.; Liptak, A.; Snatzke, G. *Carbohydr. Res.* **1990**, *200*, 201-208.

(49) Szurmai, Z.; Kerékgyarto, J.; Harangi, J.; Liptak, A. *Carbohydr. Res.* **1987**, *174*, 313-325.

LMPP10exp-brevet-decaOMe

General methods

Optical rotations were measured for $CHCl_3$ solutions at 25°C, expect where indicated otherwise, with a Perkin-Elmer automatic polarimeter, Model 241 MC. TLC were performed on precoated slides of Silica Gel 60 F_{254} (Merck). Detection was effected when applicable, with UV light, and/or by charring in 5% sulfuric acid in ethanol.

Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 0.040-0.063 mm). For all compounds the NMR spectra were recorded at 25°C for solutions in $CDCl_3$, on a Bruker AM 400 spectometer (400 MHz for ^1H, 100 MHz for ^{13}C). External references : for solutions in $CDCl_3$, TMS (0.00 ppm for both ^1H and ^{13}C). Proton-signal assignements were made by first-order analysis of the spectra, as well as analysis of 2D ^1H-^1H correlation maps (COSY) and selective TOCSY experiments. Of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. The ^{13}C NMR assignments were supported by 2D ^{13}C-^1H correlations maps (HETCOR). Interchangeable assignments are marked with an asterisk in the listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the O-SP and identified by a subscript in the listing of signal assignments. Fast atom bombardment mass spectra (FAB-MS) were recorded in the positive-ion mode using dithioerythridol/dithio-L-threitol (4 :1, MB) as the matrix, in the presence of NaI, and Xenon as the gas. Anhydrous DCM, 1,2-DCE and Et_2O, sold on molecular sieves were used as such. 4 Å powder molecular sieves was kept at 100°C and activated before use by pumping under heating at 250°C.

Phenyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside) (8). A mixture of alcohol **15** (0.12 g, 0.27 mmol) and imidate **16** (0.245 g, 0.41 mmol) in anhydrous DCM (10 mL) was stirred for 15 min under dry ar. After cooling at 0°C, Me_3SiOTf (28 μL) was added dropwise and the mixture was stirred for 0.5 h. Triethylamine (60 μL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane-EtOAc to give **8** (227 mg, 97 %) as a colorless foam; $[\alpha]_D$ -63° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.10-7.40 (m, 15H, Ph), 6.73 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH_D), 5.47 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1_A), 5.07 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3_D), 4.99 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4_D), 4.80-4.55 (m,

4H, CH_2Ph), 4.52 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1$_D$), 4.13-3.95 (m, 2H, $J_{5,6}$ = 5.3 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a$_D$, 6b$_D$), 4.10 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-5$_A$), 4.00 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2$_A$), 3.99 (m, 1H, H-2$_D$), 3.77 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3$_A$), 3.50 (m, 1H, H-5$_D$), 3.39 (dd, 1H, H-4$_A$), 1.90, 1.93, 1.95 (3s, 9H, OAc), 1.23 (d, 3H, H-6$_A$). ^{13}C NMR ($CDCl_3$):δ 171.1, 170.9, 169.6, 162.1 (C=O), 127-138 (Ph), 102.1 (C-1$_D$), 92.7 (CCl_3), 87.4 (C-1$_A$), 81.3 (C-4$_A$), 80.5 (C-3$_A$), 79.1 (C-2$_A$), 76.4, 74.1 (*C*H$_2$Ph), 72.4 (C-5$_D$), 72.4 (C-3$_D$), 69.8 (C-5$_A$), 68.7 (C-4$_D$), 62.3 (C-6$_D$), 56.2 (C-2$_D$), 21.0, 20.9, 20.8 (3C, OAc), 18.1 (C-6$_A$). FABMS of $C_{40}H_{44}Cl_3NO_{12}S$ (M, 867), *m/z* 890 ([M+Na]$^+$). Anal. Calcd for $C_{40}H_{44}Cl_3NO_{12}S$: C, 55.27 ; H, 5.10 ; N, 1.61. Found C, 55.16 ; H, 5.18 ; N, 1.68.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (17). A mixture of alcohol **14** (1.86 g, 4.86 mmol) and imidate **16** (3.85 g, 6.47 mmol) in anhydrous CH_3CN (80 mL) was stirred for 15 min under dry Ar. After cooling at 0°C, Me_3SiOTf (46 μL) was added dropwise and the mixture was stirred for 0.5 h. Triethylamine (150 μL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 7:3 cyclohexane-EtOAc to give **17** (4.0 g, 99 %) as a white solid; $[\alpha]_D$ -3° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 7.18-7.32 (m, 10H, Ph), 6.70 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH$_D$), 5.78-5.82 (m, 1H, All), 5.05-5.20 (m, 2H, All), 5.00 (m, 2H, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-3$_D$, 4$_D$), 4.45-4.75 (m, 4H, CH_2Ph), 4.76 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1$_A$), 4.60 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1$_D$), 4.05-4.15 (m, 2H, $J_{5,6}$ = 4.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a$_D$, 6b$_D$), 3.98 (m, 1H, H-2$_D$), 3.90 (m, 2H, All), 3.86 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2$_A$), 3.81 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3$_A$), 3.62 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-5$_A$), 3.50 (m, 1H, H-5$_D$), 3.32 (dd, 1H, H-4$_A$), 1.93, 1.97, 2.02 (3 s, 9H, OAc), 1.24 (d, 3H, H-6$_A$). ^{13}C NMR ($CDCl_3$):δ 171.0, 170.9, 169.6, 162.1 (C=O), 117.1-138.5 (Ph, All), 101.8 (C-1$_D$), 98.5 (C-1$_A$), 92.6 (CCl_3), 81.4 (C-4$_A$), 80.4 (C-3$_A$), 77.1 (C-2$_A$), 75.9, 74.1 (*C*H$_2$Ph), 72.7 (C-3$_D$), 72.5 (C-5$_D$), 68.6 (C-4$_D$), 68.3 (C-5$_A$), 68.1 (All), 62.3 (C-6$_D$), 56.1 (C-2$_D$), 21.1, 20.9, 20.9 (OAc), 18.2 (C-6$_A$). FABMS of $C_{37}H_{44}Cl_3NO_{13}$ (M, 815), *m/z* 838 ([M+Na]$^+$). Anal. Calcd for $C_{37}H_{44}Cl_3NO_{13}$: C, 54.39 ; H, 5.43 ; N, 1.71. Found C, 54.29 ; H, 5.45 ; N: 1.72.

(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranose) (18). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (120 mg, 140 μmol) was

dissolved tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **17** (1.46 g, 1.75 mmol) in tetrahydrofuran (20 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (27 mL), and water (3 mL) was added. Mercuric bromide (949 mg, 2.63 mmol) and mercuric oxide (761 mg, 3.5 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (Cyclohexane-AcOEt 4:1) to give **18** (1.13 g, 81 %) as a white foam. $[\alpha]_D$ +4° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 7.05-7.35 (m, 10H, Ph), 6.74 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH_D), 5.10 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_A), 5.02 (m, 2H, $J_{2,3} = J_{3,4} = J_{4,5}$ = 9.5 Hz, H-3_D, 4_D), 4.50-4.80 (m, 4H, CH_2Ph), 4.61 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.08-4.15 (m, 2H, $J_{5,6}$ = 4.5 Hz, $J_{6a,6b}$ = 12.3 Hz, H-$6a_D$, $6b_D$), 4.00 (m, 1H, H-2_D), 3.90 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2_A), 3.86 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.85 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.50 (m, 1H, H-5_D), 3.30 (dd, 1H, H-4_A), 2.85 (d, 1H, $J_{1,OH}$ = 3.5 Hz, OH), 1.94, 1.97, 2.02 (3s, 9H, OAc), 1.23 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 171.1, 170.0, 169.6, 162.1 (C=O), 127.1-138.5 (Ph), 101.7 (C-1_D), 94.1 (C-1_A), 92.6 (CCl_3), 81.4 (C-4_A), 79.9 (C-2_A), 77.3 (C-3_A), 75.9, 74.1 (CH_2Ph), 72.7 (C-3_D), 72.5 (C-5_D), 68.6 (C-4_D), 68.4 (C-5_A), 62.2 (C-6_D), 56.1 (C-2_D), 21.1, 21.0, 20.9 (OAc), 18.3 (C-6_A). FABMS of $C_{34}H_{40}Cl_3NO_{13}$ (M, 775), *m/z* 789 ($[M+Na]^+$) ; Anal. Calcd for $C_{34}H_{40}Cl_3NO_{13}$: C, 52.55 ; H, 5.19 ; N, 1.80. Found C, 52.48 ; H, 5.37 ; N, 1.67.

(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranose trichloroacetimidate (6). The hemiacetal **18** (539 mg, 0.68 mmol) was dissolved in CH_2Cl_2 (50 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (0.6 mL, 6.8 mmol), then DBU (10 µL, 70 µmol) were added. The mixture was stirred at 0°C for 30 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 7:3 cyclohexane-EtOAC and 0.2 % of Et_3N to give **6** (498 mg, 78 %) as a colourless foam; $[\alpha]_D$ -18° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 8.48 (s, 1H, N=H), 7.15-7.40 (m, 10H, Ph), 6.75 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH_D), 6.18 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_A), 5.15 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-

3_D), 5.07 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4_D), 4.50-4.82 (m, 4H, CH_2Ph), 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.03-4.20 (m, 2H, $J_{5,6}$ = 4.5 Hz, $J_{6a,6b}$ = 12.3 Hz, H-$6a_D$, $6b_D$), 3.98 (m, 1H, H-2_D), 3.85 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.84 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2_A), 3.83 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.55 (m, 1H, H-5_D), 3.45 (dd, 1H, H-4_A), 1.93, 1.96, 1.98 (3s, 9H, OAc), 1.23 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 171.1, 170.0, 169.6, 162.1 (C=O), 127.2-138.4 (Ph), 101.7 (C-1_D), 97.2 (C-1_A), 92.6 (CCl_3), 80.5 (C-4_A), 79.1 (C-3_A), 76.2 (C-2_A), 76.2, 74.1 (CH_2Ph), 74.4 (C-3_D), 74.1 (C-5_D), 71.3 (C-5_A), 68.6 (C-4_D), 62.3 (C-6_D), 56.3 (C-2_D), 21.1, 21.0, 20.9 (3C, OAc), 18.2 (C-6_A). Anal. Calcd for $C_{36}H_{40}Cl_6N_2O_{13}$: C, 46.93 ; H, 4.38 ; N, 3.04. Found C, 46.93 ; H, 4.52 ; N, 2.85.

Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (19). A mixture of the protected disaccharide **17** (3.0 g, 3.61 mmol) in MeOH (50 mL) was cold to 0°C and treated by NH_3 gaz overnight. The solution was concentrated and the residue (2.02 g) was dissolved again in MeOH (50 mL) and treated by Ac_2O (3.98 mL, 36.1 mol). The solution was stirred for 2 h and then concentrated. The residue was eluted from a column of silica gel with 95:5 DCM-EtOAC to give the intermediate triol which was dissolved in Pyridine (5 mL), cold to 0°C and treated by Ac_2O (2.4 mL). The mixture was stirred overnight and concentrated. The residue was eluted from a column of silica gel with 3:2 cyclohexane-EtOAC to give **19** (2.3 g, 90 %) was obtained as a colourless foam. $[\alpha]_D$ -12° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 7.18-7.32 (m, 10H, Ph), 5.70-5.80 (m, 1H, All), 5.40 (d, 1H, $J_{2,NH}$ = 8.1 Hz, NH), 5.10-5.20 (m, 2H, All), 4.96 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_D), 4.90 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-3_D), 4.52-4.76 (m, 4H, CH_2Ph), 4.80 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1_A), 4.46 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.02-4.10 (m, 2H, $J_{5,6}$ = 4.7 Hz, $J_{6a,6b}$ = 11.2 Hz, H-$6a_D$, $6b_D$), 3.92 (m, 1H, H-2_D), 3.87 (m, 2H, All), 3.86 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-2_A), 3.82 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.62 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.52 (m, 1H, H-5_D), 3.30 (dd, 1H, H-4_A), 1.92, 1.94, 1.98 (3 s, 9H, OAc), 1.26 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 171.1, 171.0, 170.3, 169.6 (C=O), 117-138 (Ph, All), 103.4 (C-1_D), 98.5 (C-1_A), 81.3 (C-4_A), 80.4 (C-3_A), 78.5 (C-2_A), 75.9, 73.9 (CH_2Ph), 73.6 (C-3_D), 72.4 (C-5_D), 68.7 (C-4_D), 68.2 (C-5_A), 68.1 (All), 62.5 (C-6_D), 54.5 (C-2_D), 23.4 (AcNH), 21.2, 21.1, 21.0 (OAc), 18.1 (C-6_A). FABMS of $C_{37}H_{47}NO_{13}$ (M, 713.3), *m/z* 736.2 ([M+Na]$^+$) Anal. Calcd for $C_{37}H_{47}NO_{13}$: C, 62.26 ; H, 6.64 ; N, 1.96. Found C, 62.12 ; H, 6.79 ; N, 1.87.

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranose) (20). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (10 mg, 12 μmol) was dissolved tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **19** (830 mg, 1.16 mmol) in tetrahydrofuran (40 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (90 mL), and water (10 mL) was added. Mercuric chloride (475 mg, 1.75 mmol) and mercuric oxide (504 mg, 2.32 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (Cyclohexane-AcOEt 3:7) to give **20** (541 mg, 69 %) as a white foam; $[\alpha]_D$ +16° (*c* 1, $CHCl_3$).^{1}H NMR ($CDCl_3$):δ 7.05-7.35 (m, 10H, Ph), 5.50 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH_D), 5.22 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_A), 5.06 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4_D), 5.00 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-3_D), 4.60-4.85 (m, 4H, CH_2Ph), 4.56 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1_D), 4.13-4.22 (m, 2H, $J_{5,6}$ = 4.5 Hz, $J_{6a,6b}$ = 12.3 Hz, H-$6a_D$, $6b_D$), 4.03 (m, 1H, H-2_D), 4.00 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.96 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2_A), 3.90 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.60 (m, 1H, H-5_D), 3.48 (d, 1H, $J_{1,OH}$ = 3.5 Hz, OH), 3.40 (dd, 1H, H-4_A), 2.01, 2.03, 2.08 (3s, 9H, OAc), 1.65 (s, 3H, AcNH), 1.30 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 171.2, 171.0, 170.4, 169.6 (C=O), 128.0-138.2 (Ph), 103.3 (C-1_D), 94.1 (C-1_A), 81.4 (C-4_A), 79.9 (C-2_A), 78.7 (C-3_A), 75.8, 73.9 (*C*H_2Ph), 73.6 (C-3_D), 72.4 (C-5_D), 68.7 (C-4_D), 68.2 (C-5_A), 62.4 (C-6_D), 54.5 (C-2_D), 23.3 (AcNH), 21.1, 21.0, 21.0 (3C, OAc), 18.3 (C-6_A). FABMS of $C_{34}H_{43}NO_{13}$ (M, 673.2), *m/z* 696.3 ($[M+Na]^+$) Anal. Calcd for $C_{34}H_{43}NO_{13}$: C, 60.61 ; H, 6.43 ; N, 2.08. Found C, 60.46 ; H, 6.61 ; N, 1.95.

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranose trichloroacetimidate (7). The hemiacetal **20** (541 mg, 0.80 mmol) was dissolved in CH_2Cl_2 (20 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (0.810 mL, 8 mmol), then DBU (10 μL, 80 μmol) were added. The mixture was stirred at 0°C for 1 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 1:1 cyclohexane-EtOAC and 0.2 % of

Et_3N to give **7** (560 mg, 86 %) as a colourless foam; $[\alpha]_D$ +2° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 8.56 (s, 1H, N-H), 7.20-7.50 (m, 10H, Ph), 6.29 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1_A), 5.50 (d, 1H, $J_{2,NH}$ = 8.3 Hz, NH_D), 5.17 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3_D), 5.09 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4_D), 4.60-4.85 (m, 4H, CH_2Ph), 4.68 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1_D), 4.10-4.22 (m, 2H, $J_{5,6}$ = 5.0 Hz, $J_{6a,6b}$ = 12.2 Hz, H-$6a_D$, $6b_D$), 4.00 (m, 1H, H-2_D), 3.99 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-2_A), 3.90 (m, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.89 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.62 (m, 1H, H-5_D), 3.50 (dd, 1H, H-4_A), 1.98, 2.00, 2.02 (3s, 9H, OAc), 1.65 (s, 3H, AcNH), 1.32 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 171.2, 171.0, 170.4, 169.6 (C=O), 160.5 (C=NH), 128-138 (Ph), 103.3 (C-1_D), 97.3 (C-1_A), 91.4 (CCl_3), 80.3 (C-4_A), 79.9 (C-3_A), 77.5 (C-2_A), 76.0, 73.8 (2C, CH_2Ph), 73.1 (C-3_D), 72.2 (C-5_D), 71.1 (C-5_A), 68.8 (C-4_D), 62.5 (C-6_D), 54.8 (C-2_D), 23.3 (AcNH), 21.4, 21.1, 21.0 (3C, OAc), 18.4 (C-6_A). Anal. Calcd for $C_{36}H_{43}Cl_3N_2O_{13}$: C, 52.85 ; H, 5.30 ; N, 3.42. Found C, 52.85 ; H, 5.22 ; N, 3.47.

Allyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (23). The acceptor **21** (1.78 g, 4.65 mmol) and the trichloroacetimidate donor **22** (2.96 g, 5.58 mmol) were dissolved in anhydrous ether (100 mL). The mixture was placed under argon and cooled to –55°C. TMSOTf (335 µL, 1.86 mmol) was added dropwise. The mixture was stirred at –55°C to –20°C over 3 h. Triethylamine (0.75 mL) was added, and the mixture was allowed to warm to rt. The mixture was concentrated. The residue was purified by column chromatography (solvent x, 80 :20) to give **23** as a colourless syrup (3.21 g, 92 %) ; $[\alpha]_D$ –16° (*c* 0.55, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.30-7.42 (m, 20H, Ph), 5.82-5.92 (m, 1H, All), 5.62 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.2 Hz, H-2_A), 5.20-5.32 (m, 2H, All), 5.07 (d, 1H, H-1_A), 4.82 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_B), 4.60-4.95 (m, 8H, CH_2Ph), 4.15-4.20 (m, 1H, All), 4.09 (d, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 4.05 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.95-4.05 (m, 1H, All), 3.96 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_B), 3.89 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5_A), 3.76 (dd, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_B), 3.52 (m, 1H, H-4_B), 3.50 (m, 1H, H-4_A), 2.18 (s, 3H, OAc), 1.39 (d, 3H, H-6_A), 1.36 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 170.8 (C=O), 117.1-138.4 (Ph, All), 99.5 (C-1_A), 98.4 (C-1_B), 80.5 (2C, C-4_A, 4_B), 80.0 (C-3_B), 78.1 (C-3_A), 75.8, 75.7 (CH_2Ph), 74.9 (C-2_B), 72.5, 72.2 (CH_2Ph), 69.3 (C-2_A), 68.6 (C-5_A), 68.4 (C-5_B), 68.0 (All), 21.5 (OAc), 18.4, 18.2 (2C, C-6_A, 6_B). CI-MS for $C_{45}H_{52}O_{10}$ (M = 752) *m/z* 770 $[M + NH_4]^+$. Anal. Calcd. for $C_{45}H_{52}O_{10}$: C, 71.79 ; H, 6.96. Found C, 70.95 ; H, 7.01.

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (24). A 1M solution of sodium methoxide in methanol (1.1 mL) was added to a solution of **23** (3.10 g, 4.13 mmol) in methanol. The mixture was stirred at rt for 3 h. The mixture was neutralised with Amberlite IR-120 (H^+) resin, filtered and concentrated to give **24** (2.72 g, 93%) as a colourless syrup which crystallised on standing ; mp 98-99°C ; $[\alpha]_D$ –30° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.30-7.42 (m, 20H, Ph), 5.80-5.90 (m, 1H, All), 5.20-5.32 (m, 2H, All), 5.13 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_A), 4.82 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.60-4.95 (m, 8H, CH_2Ph), 4.12-4.20 (m, 1H, All), 4.19 (m, 1H, $J_{2,3}$ = 3.2 Hz, $J_{2,OH}$ = 1.8 Hz, H-2_A), 4.09 (d, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 3.95-4.00 (m, 1H, All), 3.95 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.93 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3_B), 3.87 (m, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.74 (dd, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5_B), 3.53 (m, 1H, H-4_A), 3.46 (m, 1H, H-4_B), 2.52 (d, 1H, OH), 1.35 (m, 6H, H-6_A, 6_B). ^{13}C NMR ($CDCl_3$):δ 117.1-138.4 (Ph, All), 101.2 (C-1_A), 98.4 (C-1_B), 80.8, 80.4 (2C, C-4_A, 4_B), 80.3 (C-3_B), 80.0 (C-3_A), 75.8, 75.7 (*C*H_2Ph), 75.0 (C-2_B), 72.7, 72.6 (*C*H_2Ph), 69.1 (C-2_A), 68.4 (C-5_B), 68.3 (C-5_A), 68.1 (All), 18.4, 18.3 (2C, C-6_A, 6_B). CI-MS for $C_{43}H_{50}O_9$ (M = 710) *m/z* 728 $[M + NH_4]^+$.

Allyl (3,4-di-*O*-benzyl-2-*O*-paramethoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (35). The alcool **24** (3.8 g, 5.35 mmol) was dissolved in DMF (25 mL). The mixture was cold to 0°C and NaH (320 mg, 8.02 mmol) was added in 3 parts each 10 min. Then pMeOBnCl (1.8 mL, 13.34 mmol) was added and the mixture was stirred overnight at rt. MeOH (5 mL) was added and the solution stirred for 10 min. The solution was concentrated and the residue was eluted from a column of silica gel with 95:5 cyclohexane-acetone to give **35** (4.34 g, 97 %) as a colorless syrup; $[\alpha]_D$ -8° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 6.80-7.20 (m, 24H, Ph), 5.80-5.90 (m, 1H, All), 5.15-5.30 (m, 2H, All), 5.12 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_A), 4.73 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 4.40-4.70 (m, 10H, $PhCH_2$), 4.08-4.20 (m, 1H, All), 4.10 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 3.88-3.95 (m, 3H, H-3_A, 3_B, All), 3.78-3.80 (m, 2H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-2_A, 5_A), 3.72 (s, 3H, OCH_3), 3.70 (m, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.61 (dd, 1H, H-4_A), 3.32 (dd, 1H, H-4_B), 1.18 (d, 3H, H-6_A), 1.10 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 113.8-133.9 (Ph, All), 99.0 (C-1_A), 97.8 (C-1_B), 80.4 (C-4_A), 80.2 (C-4_B), 80.0 (C-3_B), 79.0 (C-3_A), 75.2, 72.3, 71.8, 71.5, 71.3, 67.5 (Ph*C*H_2, All), 74.1 (C-2_A), 73.8 (C-2_B), 68.3 (C-5_A), 67.8 (C-5_B), 55.0 (OCH_3), 17.8, 17.9 (2C, C-6_A, 6_B).

FAB-MS for $C_{51}H_{58}O_{10}$ (M = 830.4) *m/z* 853.5 $[M + Na]^+$. Anal. Calcd. for $C_{51}H_{58}O_{10}$: C, 73.71 ; H, 7.03. Found C, 73.57 ; H, 7.21.

(3,4-di-*O*-benzyl-2-*O*-paramethoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranose) (36). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 60 μmol) was dissolved tetrahydrofuran (6 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **35** (4.23 g, 5.09 mmol) in tetrahydrofuran (24 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (45 mL), and water (5 mL) was added. Mercuric chloride (2.07 g, 7.63 mmol) and mercuric oxide (2.2 g, 10.2 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (Cyclohexane-AcOEt 4:1) to give **36** (2.97 g, 73 %) as a white foam; $[\alpha]_D$ +8° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.25-7.40 (m, 20H, Ph), 6.73-7.18 (m, 4H, Ph), 5.12 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_A), 5.05 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 4.40-4.80 (m, 10H, $PhCH_2$), 4.08 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 3.80-3.90 (m, 2H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-3_B, 5_B), 3.78-3.80 (m, 2H, $J_{2,3}$ = 3.1 Hz, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-2_A, 5_A), 3.73 (m, 1H, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.72 (s, 3H, OCH_3), 3.60 (dd, 1H, H-4_A), 3.33 (dd, 1H, H-4_B), 1.34 (d, 3H, H-6_A), 1.24 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 113.2-129.8 (Ph), 99.1 (C-1_A), 93.8 (C-1_B), 80.7 (C-4_A), 80.3 (C-4_B), 79.7 (C-3_B), 79.2 (C-3_A), 75.5, 75.4, 72.6, 72.5, 72.4, ($PhCH_2$), 74.2 (C-2_A), 74.1 (C-2_B), 68.5 (C-5_A), 68.1 (C-5_B), 55.3 (OCH_3), 18.1 (2C, C-6_A, 6_B). FAB-MS for $C_{48}H_{54}O_{10}$ (M = 790.4) *m/z* 813.4 $[M + Na]^+$. Anal. Calcd. for $C_{48}H_{54}O_{10}$: C, 72.89 ; H, 6.88. Found C, 72.86 ; H, 6.98.

(3,4-di-*O*-benzyl-2-*O*-paramethoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L- rhamnopyranose trichloroacetimidate (37). The hemiacetal **36** (2.1 g, 2.66 mmol) was dissolved in CH_2Cl_2 (20 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (2.7 mL, 26 mmol), then DBU (40 μL, 0.26 mmol) were added. The mixture was stirred at 0°C for 30 min. The mixture was concentrated and toluene was co-

evaporated from the residue. The residue was eluted from a column of silica gel with 8:2 cyclohexane-EtOAC and 0.2 % Et_3N to give **37** (2.03 g, 82 %) as a colourless foam; $[\alpha]_D$ -10° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 8.50 (s, 1H, C=NH), 7.05-7.25 (m, 20H, Ph), 6.62-7.05 (m, 4H, Ph), 6.08 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 5.10 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_A), 4.40-4.80 (m, 10H, $PhCH_2$), 4.10 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 3.80-3.90 (m, 4H, H-3_B, 2_A, 3_A, 5_A), 3.72-3.80 (m, 1H, H-5_B), 3.72 (s, 3H, OCH_3), 3.63 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_A), 3.42 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_B), 1.30 (d, 3H, H-6_B), 1.25 (d, 3H, H-6_A). ^{13}C NMR ($CDCl_3$):δ 161.1 (C=NH), 113.4-129.5 (Ph), 99.6 (C-1_A), 97.0 (C-1_B), 80.6 (C-4_A), 79.6 (C-4_B), 79.3 (2C, C-3_A, 3_B), 75.7, 75.5, 72.8, 72.3, 72.0, ($PhCH_2$), 74.4 (C-2_A), 72.6 (C-2_B), 71.1 (C-5_A), 68.9 (C-5_B), 55.3 (OCH_3), 18.1 (2C, C-6_A, 6_B). Anal. Calcd. for $C_{50}H_{54}Cl_3NO_{10}$: C, 64.21 ; H, 5.82; N, 1.50. Found C, 64.67 ; H, 6.01; N, 1.28.

Allyl (3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside) (32). To a mixture of **24** (3.8 g, 5.35 mmol) in pyridine (40 mL) was added chloroactic anhydride (1.83 g, 10.7 mmol) at 0°C. The solution was stirred overnight at 0°C. MeOH (10 mL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 95:5 cyclohexane-acetone to give **32** (2.4 g, 57 %) as a colorless syrup; $[\alpha]_D$ -15° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.15-7.30 (m, 20H, Ph), 5.71-5.81 (m, 1H, All), 5.49 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2_A), 5.08-5.20 (m, 2H, All), 4.90 (d, 1H, H-1_A), 4.50-4.84 (m, 8H, $PhCH_2$), 4.65 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 3.85-4.04 (m, 2H, All), 4.02 (m, 2H, CH_2Cl), 3.93 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 3.88 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_A), 3.81 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_B), 3.62 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.73 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.34 (dd, 1H, H-4_B), 3.30 (dd, 1H, H-4_A), 1.22 (d, 3H, H-6_A), 1.21 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 166.9 (C=O), 117.2-138.5 (Ph, All), 99.2 (C-1_A), 98.2 (C-1_B), 80.4 (C-4_A), 80.3 (C-3_B), 80.2 (C-4_B), 77.9 (C-3_A), 75.8, 75.7, 72.6, 72.4 ($PhCH_2$), 74.9 (C-2_B), 71.2 (C-2_A), 68.6 (C-5_A), 68.4 (C-5_B), 68.0 (All), 41.3 (CH_2Cl), 18.3 (2C, C-6_A, 6_B). FABMS of $C_{45}H_{51}ClO_{10}$ (M, 786.3), *m/z* 809.3 ($[M+Na]^+$). Anal. Calcd for $C_{45}H_{51}ClO_{10}$: C, 68.65 ; H, 6.53. Found C, 68.51 ; H, 6.67.

(3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α/β-L-rhamnopyranose) (33). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (40 mg, 46 μmol) was dissolved tetrahydrofuran (7 mL), and the

resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **32** (2.39 g, 3.04 mmol) in tetrahydrofuran (18 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (30 mL), and water (5 mL) was added. Mercuric chloride (1.24 g, 4.56 mmol) and mercuric oxide (1.3 g, 6.08 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (Cyclohexane-AcOEt 4:1) to give **33** (1.91 g, 84 %) as a white foam. $[\alpha]_D$ -2° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 7.10-7.40 (m, 20H, Ph), 5.49 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2_A), 4.99 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 4.90 (d, 1H, H-1_A), 4.45-4.85 (m, 8H, $PhCH_2$), 4.01 (m, 2H, CH_2Cl), 3.93 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 3.90 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3_A), 3.84 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3_B), 3.81 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5_B), 3.72 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.33 (dd, 1H, H-4_B), 3.30 (dd, 1H, H-4_A), 2.81 (d, 1H, $J_{2,OH}$ = 3.4 Hz, OH), 1.22 (d, 3H, H-6_A), 1.20 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 167.0 (C=O), 127.2-138.5 (Ph), 99.1 (C-1_A), 93.9 (C-1_B), 80.3 (C-4_B), 80.2 (C-4_A), 79.7 (C-3_B), 77.8 (C-3_A), 75.8, 75.7, 72.6, 72.4 ($PhCH_2$), 75.0 (C-2_B), 71.1 (C-2_A), 68.6 (C-5_A), 68.4 (C-5_B), 41.3 (CH_2Cl), 18.1 (2C, C-6_A, 6_B). FABMS of $C_{42}H_{47}ClO_{10}$ (M, 746.3), *m/z* 769.3 ($[M+Na]^+$). Anal. Calcd for $C_{42}H_{47}ClO_{10}$: C, 67.51 ; H, 6.34. Found C, 67.46 ; H, 6.39.

(3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranose trichloroacetimidate (34). The hemiacetal **33** (1.80 g, 2.41 mmol) was dissolved in CH_2Cl_2 (25 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (2.4 mL, 24 mmol), then DBU (35 µL, 0.24 mmol) were added. The mixture was stirred at 0°C for 40 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 4:1 cyclohexane-EtOAC and 0.2 % Et_3N to give **34** (1.78 g, 83 %) as a colourless foam; $[\alpha]_D$ -12° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 8.60 (s, 1H, C=NH), 7.30-7.50 (m, 20H, Ph), 6.21 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_B), 5.63 (dd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.2 Hz, H-2_A), 5.07 (d, 1H, H-1_A), 4.65-5.00 (m, 8H, $PhCH_2$), 4.19 (m, 2H, CH_2Cl), 4.09 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 4.04 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3_B), 3.95 (m, 3H, H-3_A, 5_A, 5_B), 3.58 (dd, 1H, H-4_A), 3.48 (dd, 1H, H-4_B), 1.39 (m, 6H, H-6_A, 6_B). ^{13}C NMR

($CDCl_3$):δ 167.1 (C=O), 160.7 (C=N), 127.0-138.3 (Ph), 99.4 (C-1_A), 97.5 (C-1_B), 91.4 (CCl_3), 80.1 (C-4_B), 80.0 (C-4_A), 79.2 (C-3_A), 77.9 (C-3_B), 75.9, 75.8, 73.0, 72.6 (Ph*C*H_2), 73.7 (C-2_B), 71.4 (C-2_A), 71.2, 68.9 (2C, C-5_A, 5_B), 41.3 (CH_2Cl), 18.4, 18.2 (2C, C-6_A, 6_B). Anal. Calcd for $C_{44}H_{47}Cl_4NO_{10}$: C, 59.27 ; H, 5.31 ; N, 1.57. Found C, 59.09 ; H, 5.49 ; N, 1.53.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (26). 1,2-Dichloroethane (35 mL) was added to the trichloroacetimidate donor **16** (2.49 g, 4.20 mmol), the acceptor **24** (2.48 g, 3.50 mmol) and 4Å-MS powder (4 g). The mixture was stirred for 1.5 h at rt under argon. The mixture was cooled to -20°C and TMSOTf (230 μL, 1.26 mmol) was added. The temperature was allowed to rise to 0°C over 1 h, and the mixture was stirred for an additional 2 h at this temperature. Triethylamine (0.5 mL) was added and the mixture was allowed to warm to rt. The mixture was diluted with CH_2Cl_2 and filtered. The filtrate was concentrated. The residue was purified by column chromatography with 3 :1 cyclohexane-AcOEt to give **26** (3.83 g, 96 %) as a colourless amorphous solid ; $[\alpha]_D$ –6° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$) : δ 7.28-7.52 (m, 20H, Ph), 6.83 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH), 5.85 (m, 1H, All), 5.09-5.26 (m, 4H, H-3_D, 4_D, All), 4.98 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_A), 4.58-4.98 (m, 10H, H-1_B, 1_D, CH_2Ph), 4.08 (m, 4H, H-2_A, 2_D, $6a_D$, All), 3.91 (m, 5H, H-2_B, 3_A, 3_B, $6b_D$, All), 3.79 (m, 2H, H-5_A, 5_B), 3.45 (m, 3H, H-4_A, 4_B, 5_D), 1.97, 2.02, 2.04 (3s, 9H, OAc), 1.30 (m, 6H, H-6_A, 6_B). ^{13}C NMR ($CDCl_3$) : δ 170.6, 170.3, 169.1, 163.2, 161.6 (C=O), 138.4-117.1 (Ph, All), 101.3 (C-1_D), 100.9 (C-1_A), 97.6 (C-1_B), 92.0 (CCl_3), 80.9, 80.4 (2C, C-4_A, 4_B), 79.1, 79.0 (2C, C-3_A, 3_B), 77.3 (C-2_A), 76.5 (C-2_B), 75.4, 75.2, 73.6 (CH_2Ph), 72.2 (C-3_D), 71.9 (C-5_D), 71.6 (CH_2Ph), 68.2 (C-5_B*), 67.8 (C-4_D), 67.5 (C-5_A*), 67.5 (CH_2O), 61.3 (C-6_D), 55.7 (C-2_D), 20.5 (OAc), 17.9, 17.7 (2C, C-6_A, 6_B). FAB-MS for $C_{57}H_{66}Cl_3NO_{17}$ (M = 1141.3) *m/z* 1166.3, 1164.3 $[M + Na]^+$. Anal. Calcd. for $C_{57}H_{66}Cl_3NO_{17}$: C, 59.87 ; H, 5.82 ; N, 1.22. Found C, 59.87 ; H, 5.92 ; N, 1.16.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (28). Anhydrous ether (30 mL) and CH_2Cl_2 (15 mL) were added to the trichloroacetimidate donor **25** (3.34 g, 4.66 mmol), the acceptor **24** (2.20 g, 3.10 mmol). The mixture was cooled

to 0°C and TMSOTf (85 µL, 0.466 mmol) was added dropwise. The mixture was stirred at 0°C for 1 h, then at rt for 3 h. Triethylamine (1 mL) was added and the mixture was stirred for 10 min., then concentrated. The mixture was taken up in ether and the resulting precipitate was filtered off. The filtrate was concentrated. The residue was purified by column chromatography with 7:3 cyclohexane-AcOEt to give **28** (2.57 g, 65 %) as a colourless amorphous solid ; $[\alpha]_D$ +22° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) : δ 7.16-7.42 (m, 20H, Ph), 5.91 (dd, 1H, H-3_D), 5.81 (m, 1H, All), 5.10-5.24 (m, 4H, H-1_D, 4_D, All), 4.93 (s, 1H, H-1_A), 4.53-4.81 (m, 5H, H-1_B, CH_2Ph), 4.23-4.45 (m, 5H, H-2_D, CH_2Ph), 4.05 (m, 2H, H-$6a_D$, All), 3.58-3.91 (m, 8H, H-2_A, 2_B, 3_A, 3_B, 5_A, 5_B, $6b_D$, All), 3.38 (m, 1H, H-5_D), 3.13-3.21 (m, 2H, H-4_A, 4_B), 2.00, 2.02, 2.05 (3s, 9H, OAc), 1.24 (m, 6H, H-6_A, 6_B). ^{13}C NMR δ 170.4, 169.3 (C=O), 117.1-138.4 (Ph, All), 101.1 (C-1_A), 99.9 (C-1_D), 97.7 (C-1_B), 80.6 (2C, C-4_A, 4_B), 78.9, 79.7 (2C, C-3_A, 3_B), 78.2 (C-2_A), 76.3 (C-2_B), 75.2, 75.1, 72.6, 71.3 (CH_2Ph), 71.2 (C-5_D), 70.1 (C-3_D), 68.4 (C-5_B*), 68.4 (C-4_D), 67.6 (C-5_A*), 67.6 (All), 61.3 (C-6_D), 55.4 (C-2_D), 20.6 (OAc), 18.0, 17.6 (2C, C-6_A, 6_B). FAB-MS for $C_{63}H_{65}Cl_4NO_{18}$ (M = 1263.3) *m/z* 1288.4, 1286.4 [M + Na]$^+$. Anal. Calcd. for $C_{63}H_{65}Cl_4NO_{18}$: C, 59.77 ; H, 5.17 ; N, 1.11. Found C, 60.19 ; H, 5.53 ; N, 1.18.

Allyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (27). The trisaccharide **26** (1.71 g, 1.50 mmol) was dissolved in MeOH (20 mL). A 1M solution of sodium methoxide in methanol (9 mL) and triethylamine (5 mL) were added, and the mixture was stirred at rt for 18 h. The mixture was cooled to 0°C and acetic anhydride was added dropwise until the pH reached 6. A further portion of acetic anhydride (0.4 mL) was added, and the mixture was stirred at rt for 30 min. The mixture was concentrated, and toluene was co-evaporated from the residue. The residue was purified by column chromatography with 95 :5 DCM-MeOH to give **27** (623 mg, 45 %) as a colourless amorphous solid ; $[\alpha]_D$ −16° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) : δ 7.24-7.48 (m, 20H, Ph), 6.79 (d, 1H, NH), 5.73 (m, 1H, All), 5.12 (m, 3H, H-1_A, All), 4.52-4.86 (m, 9H, H-1_B, CH_2Ph), 4.34 (d, 1H, H-1_D), 3.79-4.08 (m, 6H, H-2_A, 2_B, 3_A, 3_B, All), 3.53-3.74 (m, 3H, H-5_A, 5_B, $6a_D$), 3.24-3.45 (m, 6H, H-2_D, 3_D, 4_A, 4_B, 4_D, $6b_D$), 3.20 (m, 1H, H-5_D), 1.46 (s, 3H, OAc), 1.24 (m, 6H, H-6_A, 6_B). ^{13}C NMR δ 173.6 (C=O), 117.3-137.4 (Ph, All), 103.2 (C-1_D), 100.3 (C-1_A), 97.9 (C-1_B), 81.3, 80.4 (2C, C-4_A, 4_B), 79.9 (2C, C-3_A, 3_B), 79.9 (C-2_B*), 78.9 (C-3_D), 75.7 (C-5_D), 75.6 75.3, 74.5 (CH_2Ph), 73.6 (C-2_A*), 72.5

(CH_2Ph), 71.9 (C-4_D), 68.2, 68.0 (2C, C-5_A, 5_B), 67.7 (CH_2O), 62.5 (C-6_D), 58.8 (C-2_D), 22.3 (OAc), 18.0,17.8 (2C, C-6_A, 6_B). FAB-MS for $C_{51}H_{63}NO_{14}$ (M = 913.4) *m/z* 936.6 $[M + Na]^+$. Anal. Calcd. for $C_{51}H_{63}NO_{14}.H_2O$: C, 65.72 ; H, 7.03 ; N, 1.50. Found C, 65.34 ; H, 7.03 ; N, 1.55.

Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (27). (a) Pyridine (5 mL) was added to **27a** (502 mg, 0.55 mmol) and the mixture was cooled to 0°C. Acetic anhydride (3 mL) was added. The mixture was stirred at rt for 18 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was taken up in CH_2Cl_2 and washed successively with 5% aq HCl and saturated aq $NaHCO_3$. The organic phase was dried and concentrated to give **27** (538 mg, 94 %) as a colourless foam.

(b) Tetrahydrofuran (3 mL) and ethanol (3.3 mL) were added to **28** (384 mg, 0.303 mmol). Ethylenediamine (90 µL, 1.36 mmol) was added and the mixture was heated at 55°C for 4 h. The mixture was allowed to cool to rt. Acetic anhydride (1.0 mL) was added, and the mixture was stirred at rt for 1.5 h. The mixture was concentrated. The residue was taken up in pyridine (5 mL) and the mixture was cooled to 0°C. Acetic anhydride (2.5 mL) was added. The mixture was stirred at rt for 18 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was taken up in CH_2Cl_2, which caused the formation of a white precipitate. The mixture was filtered through a plug of silica gel, eluting with 7:3 Cyclohexane-acetone. The filtrate was concentrated to give **27** (215 mg, 68 %) as a colourless foam ; $[\alpha]_D$ –7° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$) : δ 7.24-7.48 (m, 20H, Ph), 5.84 (m, 1H, All), 5.53 (d, 1H, NH), 5.19 (m, 2H, All), 5.03 (dd, 1H, H-4_D), 4.98 (m, 2H, H-1_A, 3_D), 4.54-4.95 (m, 10H, H-1_B, 1_D, CH_2Ph), 4.07 (m, 4H, H-2_A, 2_D, $6a_D$, All), 3.88 (m, 5H, H-2_B, 3_A, 3_B, $6b_D$, All), 3.79, 3.68 (2m, 2H, H-5_A, 5_B), 3.42 (m, 3H, H-4_A, 4_B, 5_D), 2.02, 2.01, 1.97, 1.64 (4s, 12H, OAc), 1.30 (m, 6H, H-6_A, 6_B). ^{13}C NMR ($CDCl_3$) δ 170.7, 170.4, 169.9, 169.1 (C=O), 117.1-138.5 (Ph, All), 102.9 (C-1_D), 101.2 (C-1_A), 97.7 (C-1_B), 81.0, 80.5 (2C, C-4_A, 4_B), 79.5, 79.1 (2C, C-3_A, 3_B), 78.2 (C-2_A), 76.1 (C-2_B), 75.5, 75.2, 73.6 (CH_2Ph), 73.3 (C-3_D), 71.9 (C-5_D), 71.7 (CH_2Ph), 68.3 (C-5_A*), 68.0 (C-4_D), 67.6 (C-5_B*), 67.6 (CH_2O), 61.6 (C-6_D), 54.1 (C-2_D), 22.9 (AcNH), 20.6 (OAc), 18.0, 17.7 (2C, C-6_A, 6_B). FAB-MS for $C_{57}H_{69}NO_{17}$ (M = 1039.5) *m/z* 1062.4 $[M + Na]^+$. Anal. Calcd. for $C_{57}H_{69}NO_{17}$: C, 65.82 ; H, 6.69 ; N, 1.35. Found C, 65.29 ; H, 6.82 ; N, 1.29.

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α/β-L-rhamnopyranose (29). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (30 mg, 35 μmol) was dissolved tetrahydrofuran (5 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **27** (805 mg, 0.775 mmol) in tetrahydrofuran (10 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (15 mL), and water (1.5 mL) was added. Mercuric chloride (315 mg, 1.16 mmol) and mercuric oxide (335 mg, 1.55 mmol) were added to the mixture, protected from light. The mixture was stirred for 1 h at rt, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography with 4 :6 AcOEt-cyclohexane to give **29** (645 mg, 83 %) as a white foam. The ^{1}H NMR spectra showed the α :β ratio to be 3.3 :1 ; $[\alpha]_D$ +3° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) α-anomer : δ 7.30-7.47 (m, 20H, Ph), 5.53 (d, 1H, NH), 5.17 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1_B), 5.08 (m, 1H, H-4_D), 5.03 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_A), 4.99 (m, 1H, H-3_D), 4.62-4.92 (m, 8H, CH_2Ph), 4.60 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1_D), 4.01-4.18 (m, 3H, H-2_A, 2_D, $6a_D$), 3.90-3.97 (m, 5H, H-2_B, 3_A, 3_B, 5_A*, $6b_D$), 3.83 (m, 1H, H-5_B*), 3.37-3.45 (m, 3H, H-4_A, 4_B, 5_D), 2.04, 2.03, 1.99, 1.68 (4s, 12H, OAc, AcNH), 1.32 (m, 6H, H-6_A, 6_B). ^{13}C NMR ($CDCl_3$) δ 170.7, 170.4, 169.9, 169.1 (C=O), 129.3-138.5 (Ph), 103.3 (C-1_D), 101.6 (C-1_A), 93.9 (C-1_B), 81.5, 80.8 (2C, C-4_A, 4_B), 79.9, 78.9 (2C, C-3_A, 3_B), 78.6 (C-2_A), 76.8 (C-2_B), 76.0, 75.5, 74.0 (CH_2Ph), 73.7 (C-3_D), 72.4 (C-5_D), 72.2 (CH_2Ph), 68.7 (C-5_A*), 68.5 (C-4_D), 68.2 (C-5_B*), 62.0 (C-6_D), 54.6 (C-2_D), 23.4 (AcNH), 21.1 (OAc), 18.5, 18.1 (2C, C-6_A, 6_B). FAB-MS for $C_{54}H_{65}NO_{17}$ (M = 999.4) *m/z* 1022.5 $[M + Na]^+$. Anal. Calcd. for $C_{54}H_{65}NO_{17}$: C, 64.85 ; H, 6.55 ; N, 1.40. Found C, 64.55 ; H, 7.16 ; N, 1.15.

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α/β-L-rhamnopyranosyl trichloroacetimidate (13). The hemiacetal **29** (595 mg, 0.59 mmol) was dissolved in CH_2Cl_2 (10 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (0.6 mL, 6 mmol), then DBU (10 μL, 59 μmol) were added. The mixture was stirred at 0°C for 20 min., then at rt for 20 min. The mixture was concentrated and toluene was co-evaporated from the residue. The

residue was purified by flash chromatography with 1:1 cyclohexane-AcOEt and 0.2 % of Et_3N to give **13** (634 mg, 94 %) as a colourless foam. The ^1H NMR spectra showed the α :β ratio to be 10 :1. $[\alpha]_D$ -20° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$) α-anomer : δ 8.47 (s, 1H, C=NH), 7.20-7.38 (m, 20H, Ph), 6.10 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1_B), 5.40 (d, 1H, NH), 5.01 (m, 1H, H-4_D), 4.95 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1_A), 4.89 (m, 1H, H-3_D), 4.55-4.85 (m, 9H, H-1_D, CH_2Ph), 4.07 (dd, 1H, H-$6a_D$), 4.03 (m, 1H, H-2_A), 3.97 (m, 1H, H-2_D), 3.91 (dd, 1H, H-$6b_D$), 3.71-3.85 (m, 5H, H-2_B, 3_A, 3_B, 5_A, 5_B), 3.31-3.45 (m, 3H, H-4_A, 4_B, 5_D), 1.58, 1.91, 1.96, 1.99 (4s, 12H, OAc, AcNH), 1.26 (m, 6H, H-6_A, 6_B). ^{13}C NMR ($CDCl_3$) δ 171.1, 170.9, 170.3, 169.6 (C=O), 160.6 (C=NH), 128.1-138.6 (Ph), 103.3 (C-1_D), 101.6 (C-1_A), 96.9 (C-1_B), 91.3 (CCl_3), 81.4, 80.2 (2C, C-4_A, 4_B), 79.9, 78.5 (2C, C-3_A, 3_B), 78.3 (C-2_A), 75.9 (CH_2Ph), 75.0 (C-2_B), 73.7 (CH_2Ph), 73.7 (C-3_D), 72.4 (CH_2Ph), 72.4 (C-5_D), 71.0, 69.0 (2C, C-5_A, 5_B), 68.5 (C-4_D), 62.1 (C-6_D), 54.6 (C-2_D), 23.4 (AcNH), 21.1 (OAc), 18.5,18.0 (2C, C-6_A, 6_B). Anal. Calcd. for $C_{56}H_{65}Cl_3N_2O_{17}$: C, 58.77 ; H, 5.72 ; N, 2.45. Found C, 58.78 ; H, 5.83; N, 2.45.

Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (5). Anhydrous ether (5 mL) was added to the donor **13** (500 mg, 0.437 mmol) and the acceptor **11** (242 mg, 0.29 mmol) and powdered 4Å-MS. The mixture was placed under argon and cooled to 0°C. Boron trifluoride etherate (415 µL, 3.27 mmol) was added. The mixture was stirred at 0°C for 1 h, then at rt for 18 h. The mixture was diluted with CH_2Cl_2 and triethylamine (1 mL) was added. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was purified by column chromatography with 3:2 cyclohexane-AcOEt to give, in order, the acceptor **11** (132 mg, 54 %), **5** (231 mg, 44 %) and the hemiacetal **29** (129 mg, 29 %). The desired pentasaccharide **5** was obtained as a colourless foam ; $[\alpha]_D$ +10° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.09-8.02 (m, 45H, Ph), 5.92 (m, 1H, All), 5.65 (d, 1H, NH), 5.37 (m,1H, H-2_C), 5.19 (m, 2H, All), 5.13 (bs, 1H, H-1_A), 4.35-4.96 (m, 15H, H-1_B, 1_C, 1_D, 1_E, 2_B, 3_D, 4_D, CH_2Ph), 4.17 (m, 2H, H-2_A, All), 3.87-4.04 (m, 8H, H-2_D, 3_A, 3_C, 3_E, 5_A, 5_E, $6a_D$, All), 3.63-3.81 (m, 7H, H-3_B, 4_C, 4_E, 5_C, $6a_E$, $6b_E$, $6b_D$), 3.59 (m, 1H, H-5_B), 3.43 (m, 3H, H-2_E, 4_A, 5_D), 3.28 (t, 1H, H-4_B), 1.66, 1.71, 1.99, 2.01 (4s, 12H, OAc, AcNH), 1.34 (m, 6H, H-6_A, 6_C), 1.00 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.0, 169.3, 165.8, 163.5 (C=O), 117.6-138.7 (Ph, All), 102.7 (C-1_D), 100.8 (2C, C-1_A, 1_B), 98.1

(C-1_E), 95.9 (C-1_C), 81.8 (C-3_E), 81.2 (2C, C-2_E, 4_A), 80.0 (C-4_B), 79.7 (2C, C-3_A, 3_C), 78.2 (C-3_B), 77.7 (C-2_A), 77.3 (2C, C-4_C, 4_E), 75.6, 75.4, 74.9 (CH_2Ph), 74.3 (C-2_B), 73.8 (CH_2Ph), 73.7 (C-3_D), 72.8 (CH_2Ph), 72.3 (C-2_C), 72.1 (C-5_D), 71.5 (C-5_E), 70.2 (CH_2Ph), 68.5 (C-5_B), 68.4 (C-5_A, CH_2O), 68.2 (C-4_D), 67.9 (C-6_E), 67.4 (C-5_C), 61.8 (C-6_D), 54.3 (C-2_D), 23.1 (AcNH), 20.7, 20.6, 20.4 (OAc), 18.6 (C-6_A), 18.0 (C-6_C), 17.8 (C-6_B). FAB-MS for $C_{104}H_{117}NO_{27}$ (M = 1812.1) *m/z* 1836.2, 1835.2 $[M + Na]^+$. Anal. Calcd. for $C_{104}H_{117}NO_{27}$: C, 68.90 ; H, 6.50 ; N, 0.77. Found C, 68.64 ; H, 6.66 ; N, 1.05.

Allyl (3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (38). A mixture of alcohol **11** (212 mg, 0.255 mmol) and imidate **34** (270 mg, 0.33 mmol) in anhydrous Et_2O (4 mL) was stirred for 15 min under dry Ar. After cooling at -60°C, Me_3SiOTf (30 μL, 0.166 mmol) was added dropwise and the mixture was stirred overnight and allowed to reach rt. Triethylamine (120 μL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 7:1 cyclohexane-EtOAc to give **38** (86 mg, 22 %) as a foam; $[\alpha]_D$ +5° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 6.95-8.00 (m, 45H, Ph), 5.80-6.00 (m, 1H, All), 5.56 (dd, 1H, H-2_A), 5.40 (dd, 1H, $J_{1,2}$ < 1.0 Hz, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.20-5.37 (m, 2H, All), 5.08 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.04 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_A), 5.00 (d, 1H, $J_{1,2}$ < 1.0 Hz, H-1_B), 4.99 (d, 1H, H-1_C), 4.30-4.90 (m, 16H, CH_2Ph), 4.35 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_B), 4.14 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_C), 4.03 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3_E), 3.90-4.20 (m, 2H, All), 3.75-4.00 (m, 4H, ClAc, H-$6a_E$, $6b_E$), 3.96 (dd, 1H, H-3_A), 3.95 (m, 1H, H-5_A), 3.95 (dd, 1H, H-5_E), 3.83 (dd, 1H, H-4_C), 3.80 (m, 1H, H-5_C), 3.72 (dd, 1H, H-4_E), 3.64 (dd, 1H, H-3_B), 3.60 (m, 1H, H-5_B), 3.52 (dd, 1H, H-2_E), 3.39 (dd, 1H, H-4_A), 3.30 (dd, 1H, H-4_B), 1.35 (d, 1H, H-6_A), 1.30 (d, 1H, H-6_C), 1.00 (d, 1H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 166.1, 165.7 (C=O), 117.0-133.4 (Ph), 100.9 (C-1_B), 98.9 (C-1_A), 97.8 (C-1_E), 96.0 (C-1_C), 81.8 (C-3_E), 80.9 (C-2_E), 79.9 (C-4_A), 79.6 (C-4_B), 79.6 (C-3_C), 78.9 (C-3_B), 78.0 (C-4_C), 77.5 (C-4_E), 77.3 (C-3_A), 75.6, 75.3, 75.0, 74.7, 73.9, 73.5, 72.8, 70.9, (CH_2Ph, All), 74.9 (C-2_B), 72.5 (C-2_C), 71.2 (C-5_E), 70.9 (C-2_A), 68.8 (C-5_B), 68.5 (C-6_E), 68.3 (C-5_A), 67.5 (C-5_C), 40.9 (ClAc), 18.8 (C-6_A), 18.2 (C-6_C), 17.8 (C-6_B). FAB-MS for $C_{92}H_{99}ClO_{20}$ (M = 1558.6) *m/z* 1581.7 $[M + Na]^+$. Anal. Calcd. for $C_{92}H_{99}ClO_{20}$: C, 70.82 ; H, 6.40. Found C, 70.67 ; H, 6.58.

LMPP10exp-brevet-decaOMe

Allyl (3,4-di-*O*-benzyl-2-*O*-*p*methoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (39). A mixture of alcohol **11** (125 mg, 0.15 mmol) and 4Å molecular sieves in anhydrous Et_2O (3 mL) was stirred for 45 min under dry Ar. After cooling at -40°C, Me_3SiOTf (20 μL, 0.112 mmol) was added dropwise. A solution of the donor **37** (210 mg, 0.225 mmol) in anhydrous Et_2O (2 mL) was added dropwise to the solution of the acceptor during 1 h. The mixture was stirred for 3 h at -40°C. Triethylamine (100 μL) was added and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 85:15 cyclohexane-EtOAc to give **39** (107 mg, 44 %) as a foam; $[\alpha]_D$ +12° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.1-8.1 (m, 45H, Ph), 6.50-7.00 (m, 4H, CH_2PhOMe), 5.70-5.90 (m, 1H, All), 5.32 (dd, 1H, $J_{1,2}$ = 1.6, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.10-5.25 (m, 2H, All), 5.05 (d, 1H, H-1_B), 4.98 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.85 (m, 2H, H-1_A, 1_C), 4.20-4.80 (m, 18H, CH_2Ph), 3.90-4.20 (m, 2H, All), 3.00-4.20 (m, 20H, H-2_A, 2_B, 2_E, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_E, 5_A, 5_B, 5_C, 5_E, $6a_E$, $6b_E$, OCH_3), 0.82-1.30 (3 d, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR ($CDCl_3$):δ 166.3 (C=O), 118.2-138.5 (Ph, All), 99.5, 99.3 (2C, C-1_A, 1_B), 98.4 (C-1_E), 96.4 (C-1_C), 82.3, 81.4, 81.1, 80.5, 80.3, 79.5, 78.2, 77.6 (8C, C-2_E, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C), 76.0, 75.5, 75.3, 74.9, 74.3, 73.3, 72.3, 71.8, 71.6, (*C*H_2Ph), 72.5 (C-2_C), 72.0 (C-4_E), 69.2, 69.0, 68.9 (3C, C-5_A, 5_B, 5_C), 68.8, 68.6 (All, C-6_E), 67.8 (C-5_E), 55.5 (OCH_3), 19.0, 18.8, 18.4 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{98}H_{106}O_{20}$ (M = 1603.8) *m/z* 1626.6 $[M + Na]^+$. *Correct elem. analysis could not be obtain for this compound.*

Allyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (42).

A mixture of alcohol **11** (6.5 g, 7.8 mmol) and imidate **22** (6.5 g, 12.2 mmol) in anhydrous Et_2O (86 mL) was stirred for 15 min under dry Ar. After cooling at –50°C, Me_3SiOTf (560 μ L, 3.1 mmol) was added dropwise and the mixture was stirred and allowed to rt overnight. Triethylamine (1.1 mL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc to give **42** (8.0 g, 84 %) as a colorless foam; $[\alpha]_D$ +21° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.1-8.2 (m, 35H, Ph), 5.95 (m, 1H, All), 5.72 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.1 Hz, H-2_B), 5.44 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.30 (m, 2H, All), 5.07 (d, 1H, $J_{1,2}$ = 3.05 Hz, H-1_E), 5.05 (d, 1H, H-1_B), 4.95 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_C), 4.35-4.90 (m, 12H, CH_2Ph), 4.00-4.20 (m, 2H, All), 4.20 (dd, 1H,

$J_{3,4}$ = 8.5 Hz, H-3_C), 4.05 (dd, 1H, $J_{2,3}$ = 9.7, $J_{3,4}$ = 10.0 Hz, H-3_E), 3.80-3.90 (m, 2H, H-$6a_E$, $6b_E$), 3.82 (m, 1H, $J_{5,6}$ = 6.0 Hz, H-5_C), 3.80 (m, 2H, H-4_E, 5_E), 3.76 (m, 1H, H-4_C), 3.75 (dd, 1H, $J_{3,4}$ = 8.5Hz, H-3_B), 3.69 (m, 1H, $J_{4,5}$ = 8.5, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.53 (dd, 1H, H-2_E), 3.35 (dd, 1H, H-4_B), 2.15 (s, 3H, OAc), 1.40 (d, 3H, H-6_C), 1.01 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$): δ 170.3, 166.1 (C=O), 118.2-138.6 (Ph, All), 99.7 (C-1_B), 98.6 (C-1_E), 96.4 (C-1_C), 82.2 (C-3_E), 81.7 (C-2_E), 80.2 (C-4_B), 80.1 (C-3_C), 78.0 (C-4_C), 77.8 (C-3_B), 75.9, 75.4, 75.2, 74.3, 73.3, 70.9 (6C, CH_2Ph), 72.5 (C-2_C), 72.0 (C-4_E), 69.0 (C-5_C), 69.0 (C-5_B), 68.9 (2C, All, C-2_B), 68.0 (C-6_E), 67.8 (C-5_E), 21.1 (OAc), 19.0 (C-6_C), 18.1 (C-6_B). FABMS of $C_{72}H_{78}O_{16}$ (M, 1198.5), *m/z* 1221.4 ($[M+Na]^+$).

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (10). A mixture of the trisaccharide **42** (8.0 g, 6.5 mmol) in MeOH (128 mL) was treated with 5.7 mL of HBF_4/Et_2O at rt. The solution was stirred during 4 days. Et_3N was added until neutralization and concentrated. The residue was diluted with DCM, washed with satd aq $NaHCO_3$ and water. The organic layer was dried on $MgSO_4$, filtered and concentrated. The residue was eluted from a column of silica gel with 15:1 toluene-AcOEt to give **10** (6.31 g, 84 %) as a foam; $[\alpha]_D$ +14° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$):δ 7.05-8.10 (m, 35H, Ph), 5.82 (m, 1H, All), 5.25 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.19 (m, 2H, All), 5.00 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.87 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_B), 4.81 (d, 1H, H-1_C), 4.35-4.90 (m, 12H, CH_2Ph), 4.00-4.20 (m, 2H, All), 4.10 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3_C), 4.09 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 3.95 (m, 1H, $J_{4,5}$ = 9.5 Hz, H-5_E), 3.92 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3_E), 3.78 (m, 1H, $J_{5,6}$ = 6.0 Hz, H-5_C), 3.70 (m, 1H, H-4_C), 3.58-3.62 (m, 2H, H-$6a_E$, $6b_E$), 3.59 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5_B), 3.54 (dd, 1H, H-4_E), 3.48 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3_B), 3.45 (dd, 1H, H-2_E), 3.31 (dd, 1H, H-4_B), 2.68 (d, 1H, $J_{2,OH}$ = 2.3 Hz, O-H), 1.29 (d, 3H, H-6_C), 1.09 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 166.2 (C=O), 118.2-137.5 (Ph, All), 103.1 (C-1_B), 98.5 (C-1_E), 96.6 (C-1_C), 82.1 (C-3_E), 81.4 (C-2_E), 80.4 (C-4_B), 79.7 (C-3_B), 79.4 (C-4_C), 78.9 (C-3_C), 78.1 (C-4_E), 76.0, 75.5, 74.5, 74.2, 73.6, 72.1 (CH_2Ph), 73.7 (C-2_C), 68.9 (C-6_E), 68.8 (C-5_B), 68.7 (All, C-5_E), 68.1 (C-5_C), 19.1 (C-6_C), 18.2 (C-6_B). FABMS of $C_{70}H_{76}O_{15}$ (M, 1156.5), *m/z* 1179.5 ($[M+Na]^+$). Anal. Calcd for $C_{70}H_{76}O_{15}$: C, 72.64; H, 6.62. Found C, 72.49; H, 6.80.

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Allyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (44).

A mixture of alcohol **10** (5.2 g, 4.49 mmol), imidate **2** (3.58 g, 6.74 mmol) and 4Å molecular sieves in anhydrous Et_2O (117 mL) was stirred for 1 h under dry ar. After cooling at –30°C, Me_3SiOTf (580 μL, 3.2 mmol) was added dropwise and the mixture was stirred and allowed to rt overnight. Triethylamine (1.2 mL) was added and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 9:1 cyclohexane-EtOAc to give **44** (6.16 g, 90 %); $[\alpha]_D$ +13° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.00-8.10 (m, 45H, Ph), 5.82 (m, 1H, All), 5.45 (dd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 2.5 Hz, H-2_A), 5.29 (dd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 2.5 Hz, H-2_C), 5.19 (m, 2H, All), 4.97 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.95 (d, 1H, H-1_A), 4.91 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.84 (d, 1H, H-1_C), 4.35-4.90 (m, 16H, CH_2Ph), 4.29 (dd, 1H, $J_{2,3}$ = 2.6 Hz, H-2_B), 4.00-4.10 (m, 2H, All), 4.02 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3_C), 3.90 (m, 2H, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-3_E, 5_E), 3.85 (m, 2H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.5 Hz, H-3_A, 5_A), 3.72 (m, 2H, $J_{5,6}$ = 6.0 Hz, H-4_C, 5_C), 3.62-3.66 (m, 2H, H-$6a_E$, $6b_E$), 3.61 (dd, 1H, H-4_E), 3.54 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3_B), 3.45 (dd, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.39 (dd, 1H, H-2_E), 3.34 (dd, 1H, H-4_A), 3.21 (dd, 1H, H-4_B), 1.89 (s, 3H, OAc), 1.26 (2d, 6H, H-6_A, 6_C), 0.89 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 170.2, 166.1 (2C, C=O), 118.1-138.4 (Ph, All), 101.3 (C-1_B), 99.8 (C-1_A), 98.2 (C-1_E), 96.4 (C-1_C), 82.2 (C-3_E), 81.4 (C-2_E), 80.6 (C-4_A), 80.5 (C-3_C), 80.1 (C-4_B), 79.3 (C-3_B), 78.5 (C-4_C), 78.1 (C-3_A), 78.0 (C-4_E), 76.0, 75.9, 75.7, 75.2, 74.3, 73.3, 72.1, 71.1 (*C*H_2Ph), 75.2 (C-2_B), 72.9 (C-2_C), 71.7 (C-5_E), 69.5 (C-2_A), 69.2 (2C, C-5_A, 5_B), 68.9 (All, C-2_B), 68.9 (C-6_E), 67.9 (C-5_C), 21.4 (OAc), 19.1 (C-6_A), 18.7 (C-6_C), 18.1 (C-6_B). FABMS of $C_{90}H_{100}O_{20}$ (M, 1524.7), *m/z* 1547.8 ($[M+Na]^+$). Anal. Calcd for $C_{92}H_{100}O_{20}$: C, 72.42; H, 6.61. Found C, 72.31; H, 6.75.

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (40).

A mixture of **44** (6.0 g, 3.93 mmol) in MeOH (200 mL) was treated with 10 mL of HBF_4/Et_2O at rt. The solution was stirred during 5 days. Et_3N was added until neutralization and concentrated. The residue was diluted with DCM, washed with satd aq $NaHCO_3$ and water. The organic layer was dried on $MgSO_4$, filtered and concentrated. The residue was eluted

from a column of silica gel with 6:1 cyclohexane-AcOEt to give **40** (5.0 g, 84 %) as a colorless foam; $[\alpha]_D$ +12° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.00-8.00 (m, 45H, Ph), 5.83 (m, 1H, All), 5.29 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 2.9 Hz, H-2_C), 5.19 (m, 2H, All), 4.99 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_A), 4.97 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 4.94 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_B), 4.83 (d, 1H, H-1_C), 4.35-4.90 (m, 16H, CH_2Ph), 4.30 (dd, 1H, $J_{2,3}$ = 2.7 Hz, H-2_B), 4.00-4.10 (m, 2H, All), 4.02 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 8.5 Hz, H-3_C), 3.98 (dd, 1H, H-2_A), 3.91-3.95 (m, 3H, H-5_E, $6a_E$, $6a_E$), 3.90 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.4 Hz, H-3_E), 3.73-3.82 (m, 4H, H-3_A, 5_A, 4_C, 5_C), 3.66 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4_E), 3.53 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_B), 3.48 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 5.1 Hz, H-5_B), 3.40-3.44 (m, 2H, H-4_A, 2_E), 3.17 (dd, 1H, H-4_B), 2.18 (d, 1H, $J_{2,OH}$ = 2.0 Hz, O-H), 1.26 (d, 3H, H-6_C), 1.25 (d, 3H, H-6_A), 0.90 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 166.2 (C=O), 118.0-138.3 (Ph, All), 101.5 (C-1_B), 101.4 (C-1_A), 98.2 (C-1_E), 96.4 (C-1_C), 82.2 (C-3_E), 81.4 (C-2_E), 80.6 (C-4_A), 80.3 (C-4_B), 79.9 (2C, C-3_C, 3_A), 79.2 (C-3_B), 78.3 (C-4_C), 78.0 (C-4_E), 75.9, 75.6, 75.5, 74.8, 74.2, 73.5, 72.4, 71.0 (*C*H_2Ph), 75.3 (C-2_B), 72.9 (C-2_C), 71.6 (C-2_A), 69.2, 69.1, 68.6, 68.3, 67.9 (5C, C-5_A, 5_B, 5_C, 5_E, 6_E), 68.9 (All), 19.1 (C-6_C), 18.6 (C-6_A), 18.1 (C-6_B). FABMS of $C_{90}H_{98}O_{19}$ (M, 1482.7), *m/z* 1505.8 ($[M+Na]^+$). Anal. Calcd for $C_{90}H_{98}O_{19}.2H_2O$: C, 71.12; H, 6.77. Found C, 71.21; H, 6.78.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (4). A mixture of alcohol **10** (5.0 g, 3.37 mmol), imidate **16** (3.0 g, 5.04 mmol) and 4Å molecular sieves in anhydrous DCM (120 mL) was stirred for 1 h under dry Ar. After cooling at 0°C, Me_3SiOTf (240 μL, 1.32 mmol) was added dropwise and the mixture was stirred for 2.5 h while coming back to rt. Et_3N (800 μL) was added, and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 4:1 to 2:1 cyclohexane-EtOAc to give **X4** (6.27 g, 98 %); $[\alpha]_D$ +1.5° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.00-8.00 (m, 45H, Ph), 6.68 (d, 1H, $J_{2,NH}$ = 8.5 Hz, N-H_D), 5.82 (m, 1H, All), 5.29 (dd, 1H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.3 Hz, H-2_C), 5.19 (m, 2H, All), 5.00 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 4.96 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 10.5 Hz, H-3_D), 4.88 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 4.85 (d, 1H, H-1_C), 4.82 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_B), 4.81 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4_D), 4.72 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_D), 4.35-4.90 (m, 16H, CH_2Ph), 4.38 (m, 1H, H-2_B), 4.00-4.10 (m, 2H, All), 4.05 (dd, 1H, $J_{2,3}$ = 2.7 Hz, H-2_A), 3.95 (dd, 1H, $J_{2,3}$ = 3.5 Hz $J_{3,4}$ = 8.5 Hz, H-

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3_C), 3.90 (m, 2H, H-5_E, 4_E), 3.82-3.86 (m, 2H, H-$6a_D$, $6b_D$), 3.70-3.84 (m, 6H, H-3_E, $6a_E$, $6b_E$, 3_A, 5_A, 2_D), 3.68 (m, 1H, H-5_C), 3.61 (dd, 1H, $J_{4,5}$ = 9.0 Hz, H-4_C), 3.56 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_B), 3.47 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.33-3.35 (m, 3H, H-4_A, 5_D, 2_E), 3.17 (dd, 1H, H-4_B), 1.98, 2.00, 2.02 (3s, 9H, OAc), 1.24 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_A), 1.23 (d, 3H, $J_{5,6}$ = 5.9 Hz, H-6_C), 0.90 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 170.9, 170.7, 169.6, 166.1, 162.1 (C=O), 118.1-138.3 (Ph, All), 101.5 (C-1_D), 101.4 (C-1_B), 101.1 (C-1_A), 98.5 (C-1_E), 96.4 (C-1_C), 92.6 (CCl_3), 82.1 (C-3_E), 81.7 (C-3_C), 81.6 (C-2_E), 80.4 (C-4_B), 80.1 (C-3_A), 79.1 (C-4_C), 78.5 (C-3_B), 77.9 (C-4_A), 77.6 (C-4_E), 76.4 (C-2_A), 76.1, 75.8, 75.4, 74.7, 74.3, 74.2, 73.2, 70.4 (CH_2Ph), 74.9 (C-2_B), 72.9 (C-3_D), 72.7 (C-2_C), 72.5 (C-5_D), 71.9 (C-5_E), 68.4 (C-6_E), 68.8 (All), 68.9, 68.7, 68.5, 67.7 (4C, C-4_D, 5_A, 5_B, 5_C), 62.1 (C-6_D), 56.2 (C-2_D), 20.9, 20.9, 20.7 (3C, OAc), 19.0 (C-6_A), 18.5 (C-6_C), 18.2 (C-6_B). FABMS of $C_{104}H_{114}Cl_3NO_{27}$ (M, 1916.4), *m/z* 1938.9 $[M+Na]^+$. Anal. Calcd for $C_{104}H_{114}Cl_3NO_{27}$: C, 65.18 ; H, 6.00 ; N, 0.73. Found C, 64.95 ; H, 6.17 ; N, 0.76.

(2,3,4-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (46). Compound **4** (3.5 g, 1.8 mmol) was dissolved in anhydrous THF (35 mL). The solution was degassed and placed under Ar. 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (81 mg) was added, and the solution was degassed again. The catalyst was activated by passing over a stream of hydrogen until the solution has turned yellow. The reaction mixture was degassed again and stirred under an Ar atmosphere, then concentrated to dryness. The residue was dissolved in acetone (15 mL), then water (3 mL), mercuric chloride (490 mg) and mercuric oxide (420 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspensionwas taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 2:1 petroleum ether-EtOAc to give the corresponding hemiacetal **45**. Trichloroacetonitrile (6.5 mL) and DBU (97 μL) were added to a solution of the residue in anhydrous dichloromethane (33 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 5:2 cyclohexane-EtOAC and 0.2 % Et_3N to give **46** (2.48 g, 66 %); $[\alpha]_D$ +4° (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):δ 8.71 (s, 1H, N=H), 7.00-

8.00 (m, 45H, Ph), 6.80 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH_D), 6.37 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1_C), 5.59 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.10 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 5.05 (dd, 1H, H-3_D), 4.98-5.00 (m, 2H, H-1_E, 1_B), 4.97 (dd, 1H, H-4_D), 4.00-5.00 (m, 19H, 8 CH_2Ph, H-3_C, 2_A, 2_B), 3.20-4.00 (m, 17H, H-2_E, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 4_C, 5_C, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A, 5_D, $6a_D$, $6b_D$), 1.80, 2.02, 2.03 (3s, 9H, OAc), 1.39, 1.32 and 1.00 (3d, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR ($CDCl_3$):δ 169.7, 169.5, 168.3, 164.5, 160.9 (C=O, C=N), 126.2-137.5 (Ph), 101.6 (C-1_D), 101.3 (2C, C-1_A, 1_B), 98.7 (C-1_E), 94.8 (C-1_C), 91.3 (CCl_3), 82.1, 81.5, 80.4, 80.1, 78.4, 77.9, 77.6, 76.5 (10C, C-2_A, 2_E, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_E), 76.0, 75.9, 75.5, 74.9, 74.3, 73.3 (CH_2Ph), 72.9, 72.6, 71.9, 70.9, 70.6, 69.1, 68.8, 68.5 (9C, C-2_B, 2_C, 3_D, 4_D, 5_A, 5_B, 5_C, 5_D, 5_E), 68.3, 62.1 (2C, C-6_D, 6_E), 56.2 (C-2_D), 21.0, 20.9, 20.8 (3 OAc), 19.1, 18.3, 18.1 (3C, C-6_A, 6_B, 6_C). Anal. Calcd for $C_{103}H_{110}Cl_6N_2O_{27}$ C: 61.22, H: 5.49, N: 1.39. Found C: 61.24, H: 5.50, N: 1.21.

Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (49). A mixture of **46** (154 mg, 76 μmol) and **48** (92 mg, 51 μmol), 4Å molecular sieves and dry 1,2-DCE (3 mL), was stirred for 1 h, then cooled to –35°C. Triflic acid (6 μL) was added. The stirred mixture was allowed to reach 10°C in 2.5 h. Et_3N (25 μL) was added and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAC and 0.5 % of Et_3N to give **49** which could not be obtained as pure material at this stage, and was directly engaged in the next reaction.

Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-

rhamnopyranoside (50). To a solution of the residue **49** (186 mg, 51 µmol) in DCM (3 mL) was added dropwise, at 0°C, a solution of TFA (0.5 mL) and water (0.5 mL). The mixture was stirred for 3 h, then concentrated by co-evaporation with water then toluene. The residue was eluted from a column of silica gel with 2:1 to 1:1 petroleum ether-EtOAC to give **50** (134 mg, 72 %, 2 steps); $[\alpha]_D$ +6° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.10-8.05 (m, 90H, Ph), 6.82-6.86 (2d, 2H, $J_{2,NH}$ = 8.0 Hz, $J_{2,NH}$ = 8.5 Hz, NH_D, $NH_{D'}$), 5.19-5.35 (m, 2H, H-2_C, $2_{C'}$), 5.20, 5.08 (2s, 2H, H-1_A, $1_{A'}$), 5.05 (dd, 1H, H-$3_{D'}$), 4.99-4.80 (m, 9H, H-1_B, $1_{B'}$, 1_C, $1_{C'}$, 1_D, $1_{D'}$, 1_E, $1_{E'}$, $4_{D'}$), 4.30-4.80 (m, 32H, OCH_2Ph), 3.15-4.10 (m, 44H, H-2_A, $2_{A'}$, 2_B, $2_{B'}$, 2_D, $2_{D'}$, 2_E, $2_{E'}$, 3_A, $3_{A'}$, 3_B, $3_{B'}$, 3_C, $3_{C'}$, 3_D, 3_E, $3_{E'}$, 4_A, $4_{A'}$, 4_B, $4_{B'}$, 4_C, $4_{C'}$, 4_D, 4_E, $4_{E'}$, 5_A, $5_{A'}$, 5_B, $5_{B'}$, 5_C, $5_{C'}$, 5_D, $5_{D'}$, 5_E, $5_{E'}$, $6a_D$, $6b_D$, $6a_{D'}$, $6b_{D'}$, $6a_E$, $6b_E$, $6a_{E'}$, $6b_{E'}$), 3.42 (3H, s, OMe), 2.02, 2.04, 2.08 (9H, 3s, OAc), 1.40-0.96 (18H, m, H-6_A, $6_{A'}$, 6_B, $6_{B'}$, 6_C, $6_{C'}$). ^{13}C NMR ($CDCl_3$) : δ 171.5, 170.9, 170.8, 169.6, 166.2, 162.4, 162.1 (C=O), 127.2-139.5 (Ph), 101.9, 101.6, 101.5, 101.3, 99.2, 98.8, 98.2 (10C, C-1_A, $1_{A'}$, 1_B, $1_{B'}$, 1_C, $1_{C'}$, 1_D, $1_{D'}$, 1_E, $1_{E'}$), 92.7, 92.6 (2C, CCl_3), 82.1, 81.8, 81.7, 80.5, 80.3, 80.1, 79.3, 77.9, 77.8, 73.0, 72.6, 72.5, 72.0, 69.4, 69.0, 68.9, 67.4, (39C, C-2_A, $2_{A'}$, 2_B, $2_{B'}$, 2_C, $2_{C'}$, 2_E, $2_{E'}$, 3_A, $3_{A'}$, 3_B, $3_{B'}$, 3_C, $3_{C'}$, 3_D, $3_{D'}$, 3_E, $3_{E'}$, 4_A, $4_{A'}$, 4_B, $4_{B'}$, 4_C, $4_{C'}$, 4_D, $4_{D'}$, 4_E, $4_{E'}$, 5_A, $5_{A'}$, 5_B, $5_{B'}$, 5_C, $5_{C'}$, 5_D, $5_{D'}$, 5_E, $5_{E'}$, $6_{D'}$), 76.0, 75.9, 74.8, 74.3, 73.6, 73.2, 68.6 (CH_2Ph), 62.3, 62.2, 60.7 (3C, C-6_D, 6_E, $6_{E'}$), 55.5, 56.2 (3C, C-2_D, $2_{D'}$, OCH_3), 20.97, 20.94, 20.77 (OAc), 19.01, 18.72, 18.62, 18.15, 17.90 (6C, C-6_A, $6_{A'}$, 6_B, $6_{B'}$, 6_C, $6_{C'}$). FABMS for $C_{197}H_{214}Cl_6N_2O_{50}$ (M, 3622.5), *m/z* 3645.3 $[M+Na]^+$. Anal. Calcd for $C_{197}H_{214}Cl_6N_2O_{50}$ C: 65.32, H: 5.95, N: 0.77. Found C: 65.20, H: 6.03, N: 0.78.

Methyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (1). A solution of **50** (183 mg, 50 µmol), in EtOH (3 mL), EtOAc (0.3 mL), 1M HCl (100 µL) was hydrogenated in the presence of Pd/C (250 mg) for 72 h at rt. The mixture was filtered and concentrated. A solution of the residue in MeOH (4 mL) and Et_3N (200 µL) was hydrogenated in the presence of Pd/C (200 mg) for 24 h at rt. The mixture was filtered and concentrated. A solution of the residue (50 mg, 25 µmol) in MeOH (3 mL) and DCM (0.5 mL) was treated by MeONa until pH=10. The mixture was stirred overnight at 55°C. After cooling at rt, IR 120 (H^+) was added until neutral pH, and the solution was filtered and

concentrated, then was eluted from a column of C-18 with water/CH_3CN and freeze-dried to afford amorphous **1** (30 mg, 37 %); $[\alpha]_D$ -1° (*c* 1, H_2O). ^1H NMR (D_2O): δ 5.13 (2d, 2H, $J_{1,2}$ = 3.5Hz, H-1_E, $1_{E'}$), 4.75, 4.95, 5.05 (m, 5H, H-1_A, 1_B, $1_{A'}$, $1_{B'}$, $1_{C'}$), 4.62-4.64 (2d, 2H, $J_{1,2}$ = 7.0 Hz, $J_{1,2}$ = 8.0 Hz, H-1_D, $1_{D'}$), 4.58 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1_C), 3.20-4.10 (m, 51H, H-2_A, $2_{A'}$, 2_B, $2_{B'}$, 2_C, $2_{C'}$, 2_D, $2_{D'}$, 2_E, $2_{E'}$, 3_A, $3_{A'}$, 3_B, $3_{B'}$, 3_C, $3_{C'}$, 3_D, $3_{D'}$, 3_E, $3_{E'}$, 4_A, $4_{A'}$, 4_B, $4_{B'}$, 4_C, $4_{C'}$, 4_D, $4_{D'}$, 4_E, $4_{E'}$, 5_A, $5_{A'}$, 5_B, $5_{B'}$, 5_C, $5_{C'}$, 5_D, $5_{D'}$, 5_E, $5_{E'}$, $6a_D$, $6b_D$, $6a_{D'}$, $6b_{D'}$, $6a_E$, $6b_E$, $6a_{E'}$, $6b_{E'}$, OCH_3), 1.97, 1.99 (2s, 6H, 2 AcNH), 1.15-1.33 (6d, 18H, $J_{5,6}$ = 6.3Hz, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$). ^{13}C NMR (D_2O): δ 175.2, 174.7 (C=O), 103.1 (2C, C-$1_{D'}$, 1_D), 102.6, 101.7, 101.3, 100.8 (6C, C-1_A, 1_B, 1_C, $1_{A'}$, $1_{B'}$, $1_{C'}$), 98.0 (2C, C-1_E, $1_{E'}$), 81.6, 79.7, 79.6, 79.1, 76.2, 76.1, 73.9, 73.0, 72.7, 72.6, 72.5, 72.2, 72.1, 71.6, 70.1, 70.0, 69.7, 69.0, 68.5 (38C, C-2_A, $2_{A'}$, 2_B, $2_{B'}$, 2_C, $2_{C'}$, 2_E, $2_{E'}$, 3_A, $3_{A'}$, 3_B, $3_{B'}$, 3_C, $3_{C'}$, 3_D, $3_{D'}$, 3_E, $3_{E'}$, 4_A, $4_{A'}$, 4_B, $4_{B'}$, 4_C, $4_{C'}$, 4_D, $4_{D'}$, 4_E, $4_{E'}$, 5_A, $5_{A'}$, 5_B, $5_{B'}$, 5_C, $5_{C'}$, 5_D, $5_{D'}$, 5_E, $5_{E'}$), 60.9 (4C, C-6_E, $6_{E'}$, 6_D, $6_{D'}$), 56.20, 56.00, 55.31 (3C, C-2_D, $2_{D'}$, OCH_3), 22.7, 22.6 (2C, AcNH), 18.3, 18.1, 17.2, 17.1, 16.95, 16.90 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$). HRMS: calculated for $C_{65}H_{110}N_2O_{45}$+Na: 1661.6278. Found 1661.6277.

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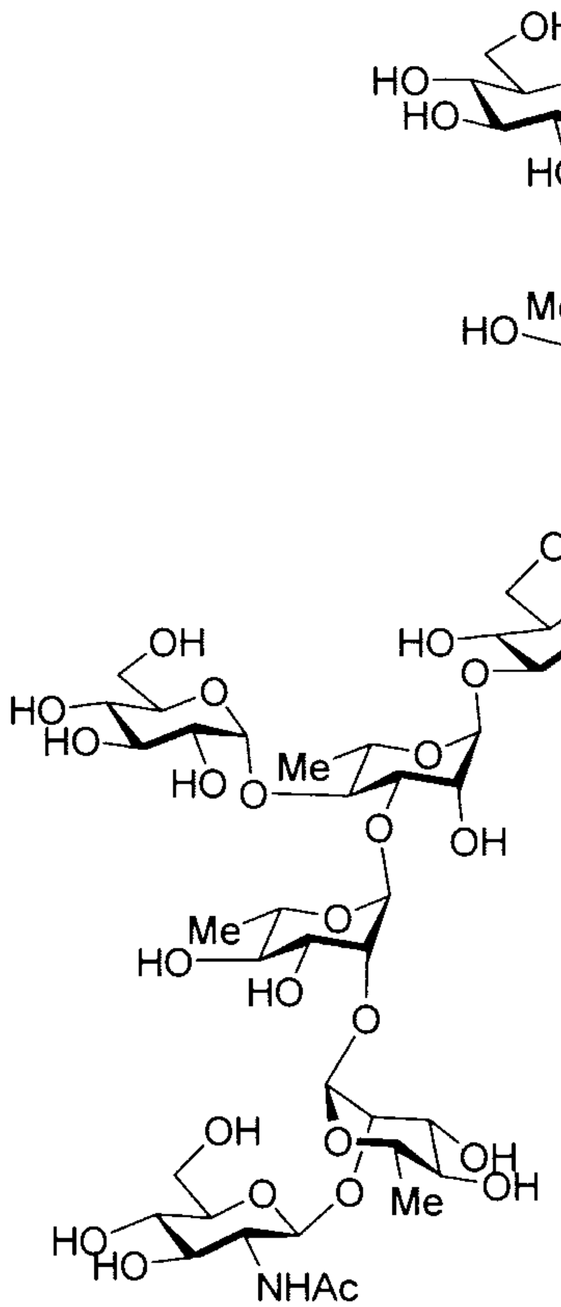
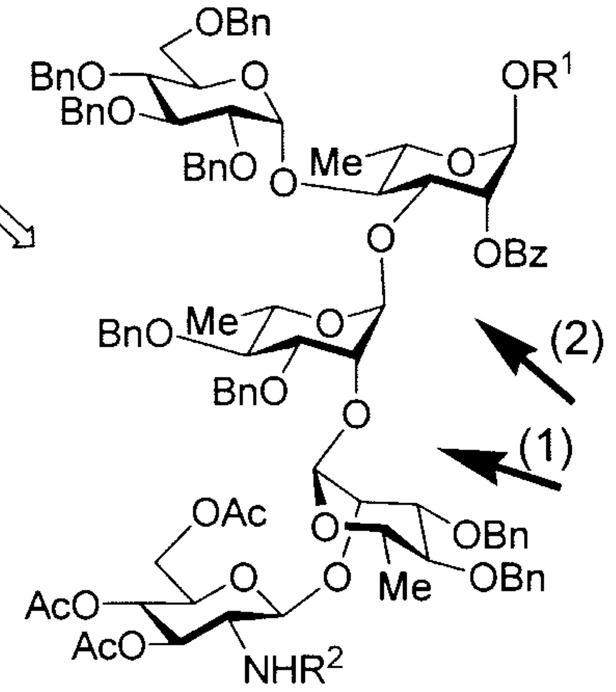
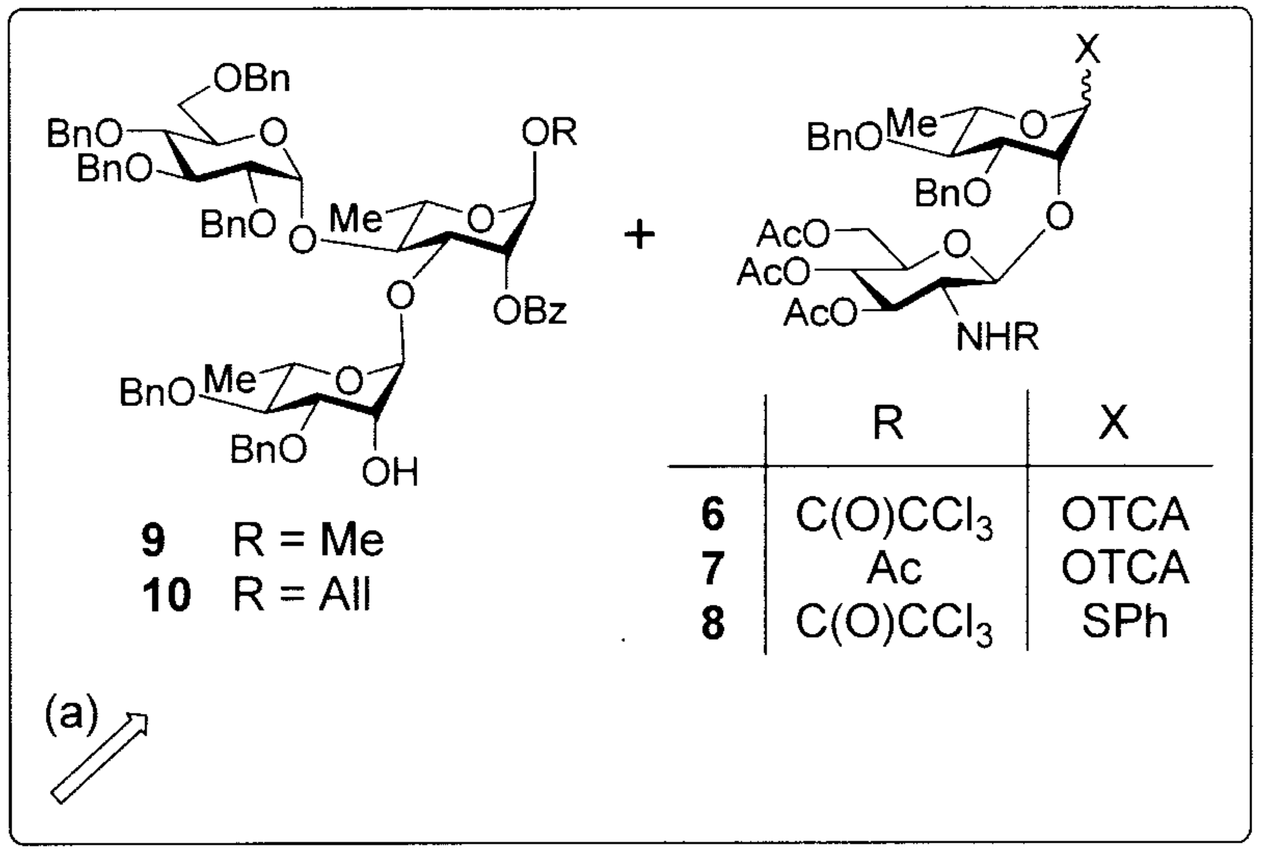


1

	R^1	R^2
2	Me	$C(O)CCl_3$
3	Me	Ac
4	All	$C(O)CCl_3$
5	All	Ac

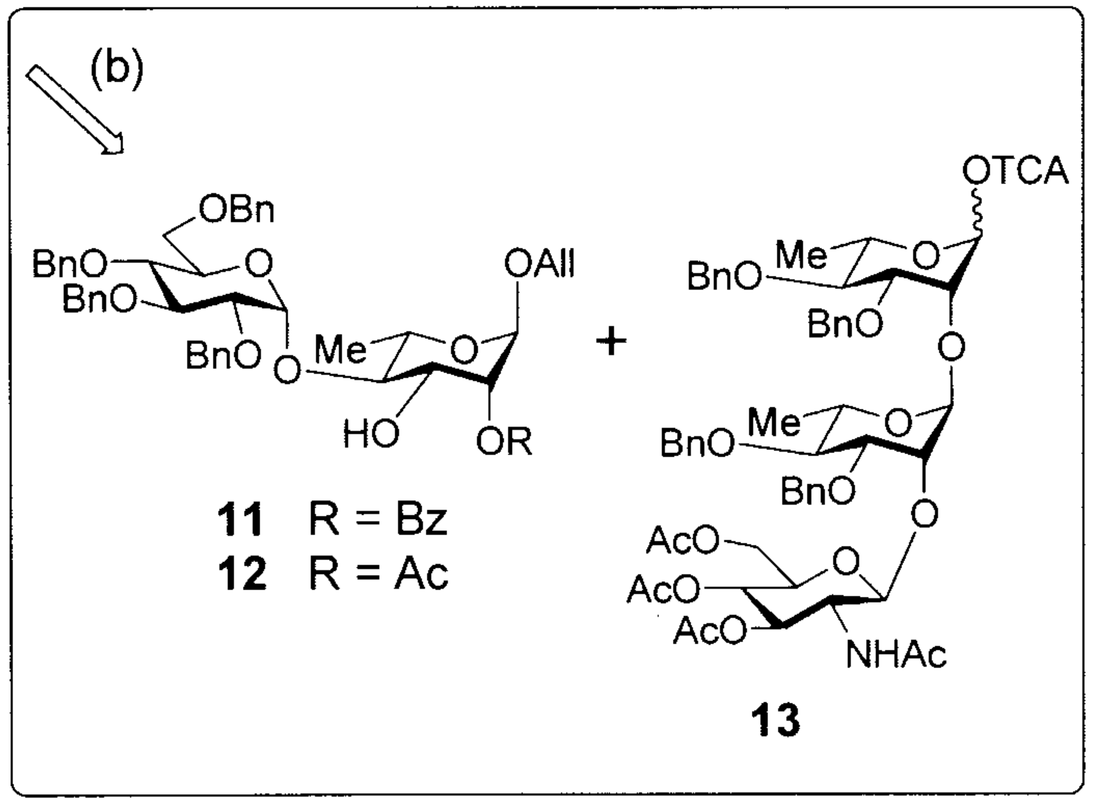


(c) linear approach



9 R = Me
10 R = All

	R	X
6	$C(O)CCl_3$	OTCA
7	Ac	OTCA
8	$C(O)CCl_3$	SPh



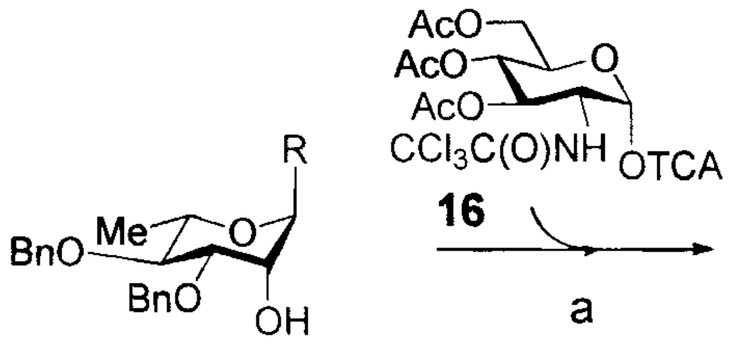
11 R = Bz
12 R = Ac

13

l

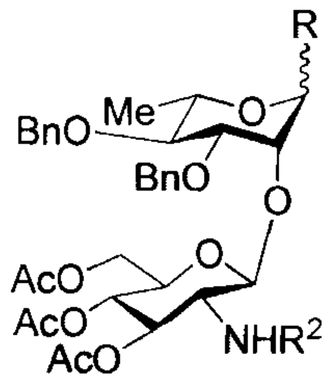
CA 02434668 2003-07-07

Scheme 1 :



	R
14	OAll
15	SPh

a



d (b, c)

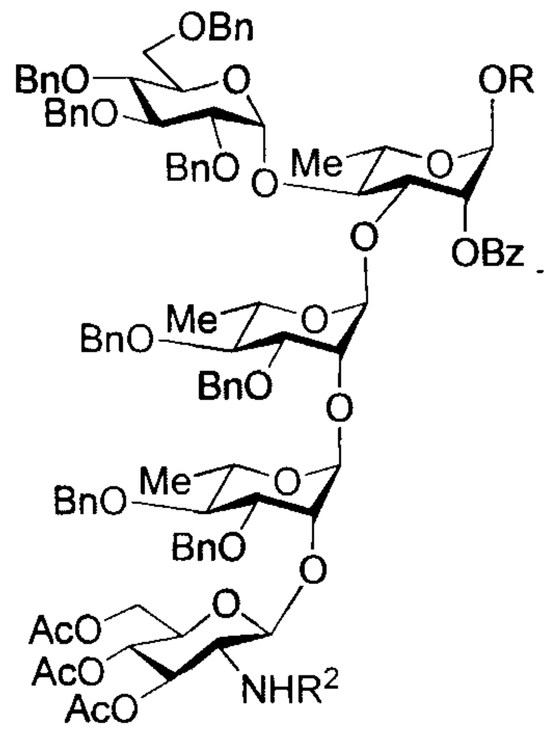
b (c)

	R	R^2	
17	OAll	$C(O)CCl_3$	α
18	OH	$C(O)CCl_3$	α/β
6	OTCA	$C(O)CCl_3$	α/β
19	OAll	Ac	α
20	OH	Ac	α/β
7	OTCA	Ac	α/β
8	SPh	$C(O)CCl_3$	α

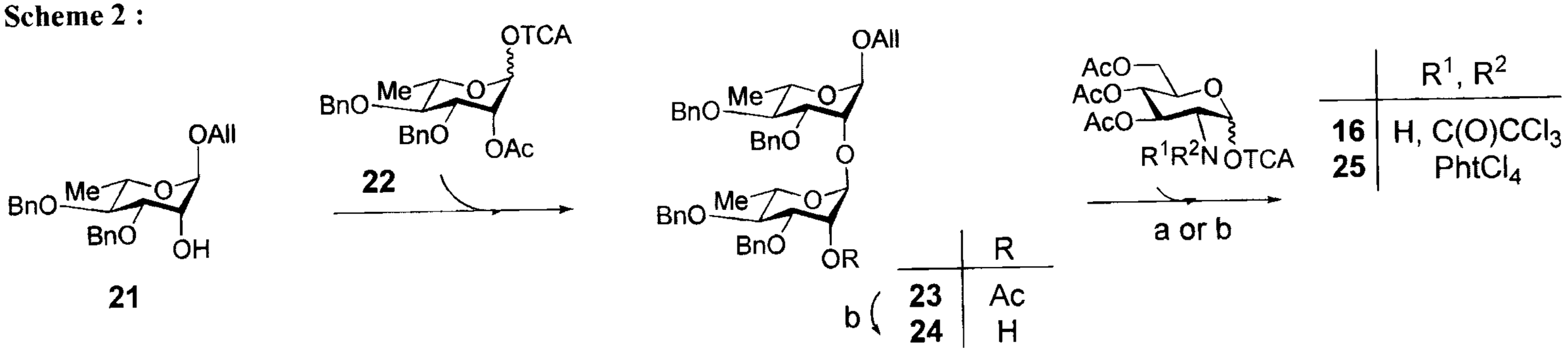
9, 10

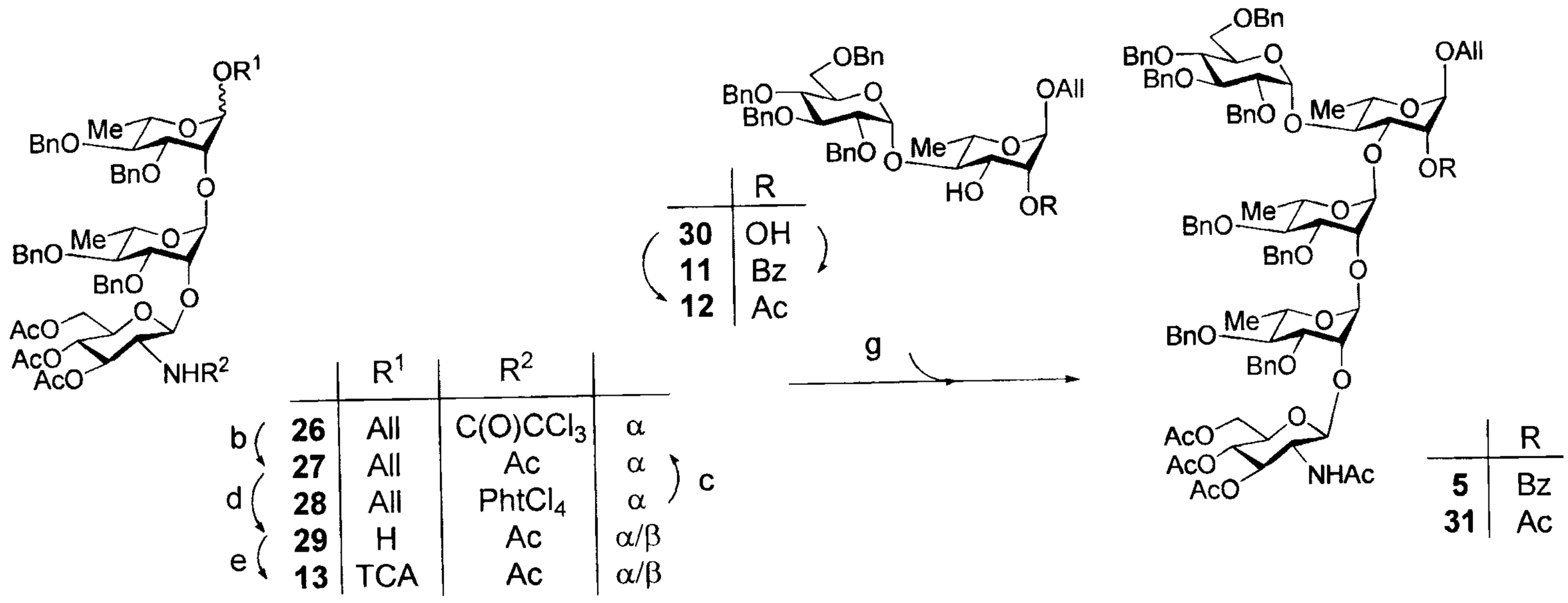
e or f

	R	R^2
2	OMe	$C(O)CCl_3$
3	OMe	Ac
4	OAll	$C(O)CCl_3$

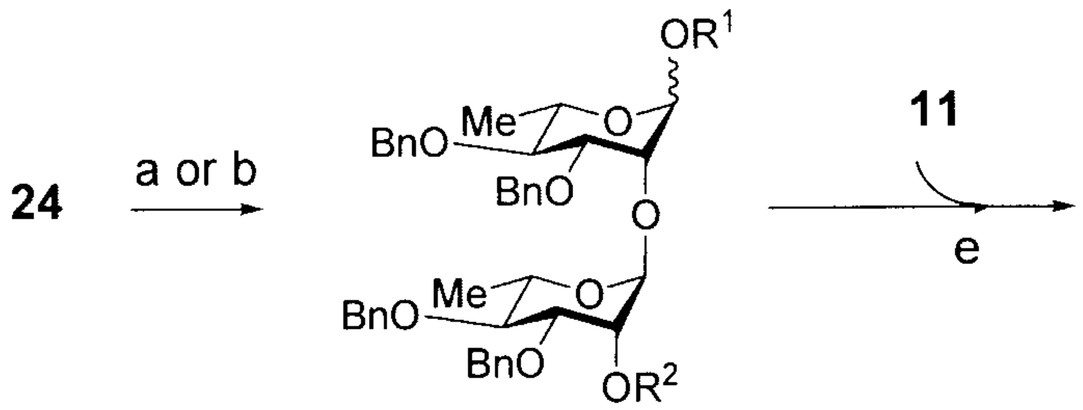


Scheme 2 :

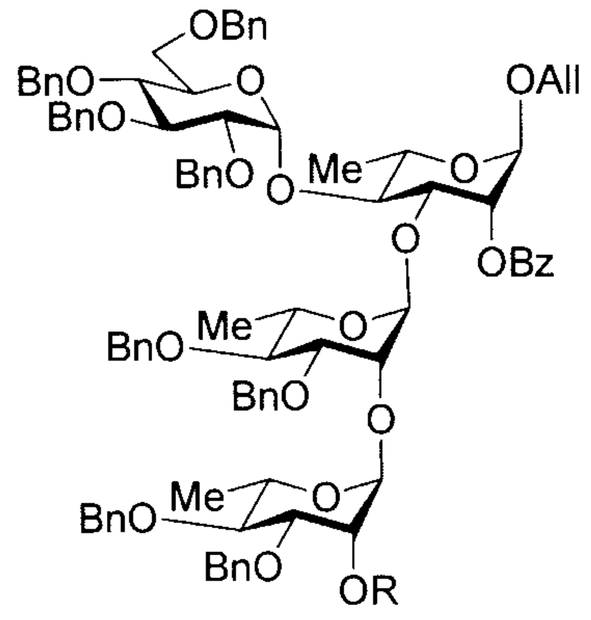




Scheme 3 :

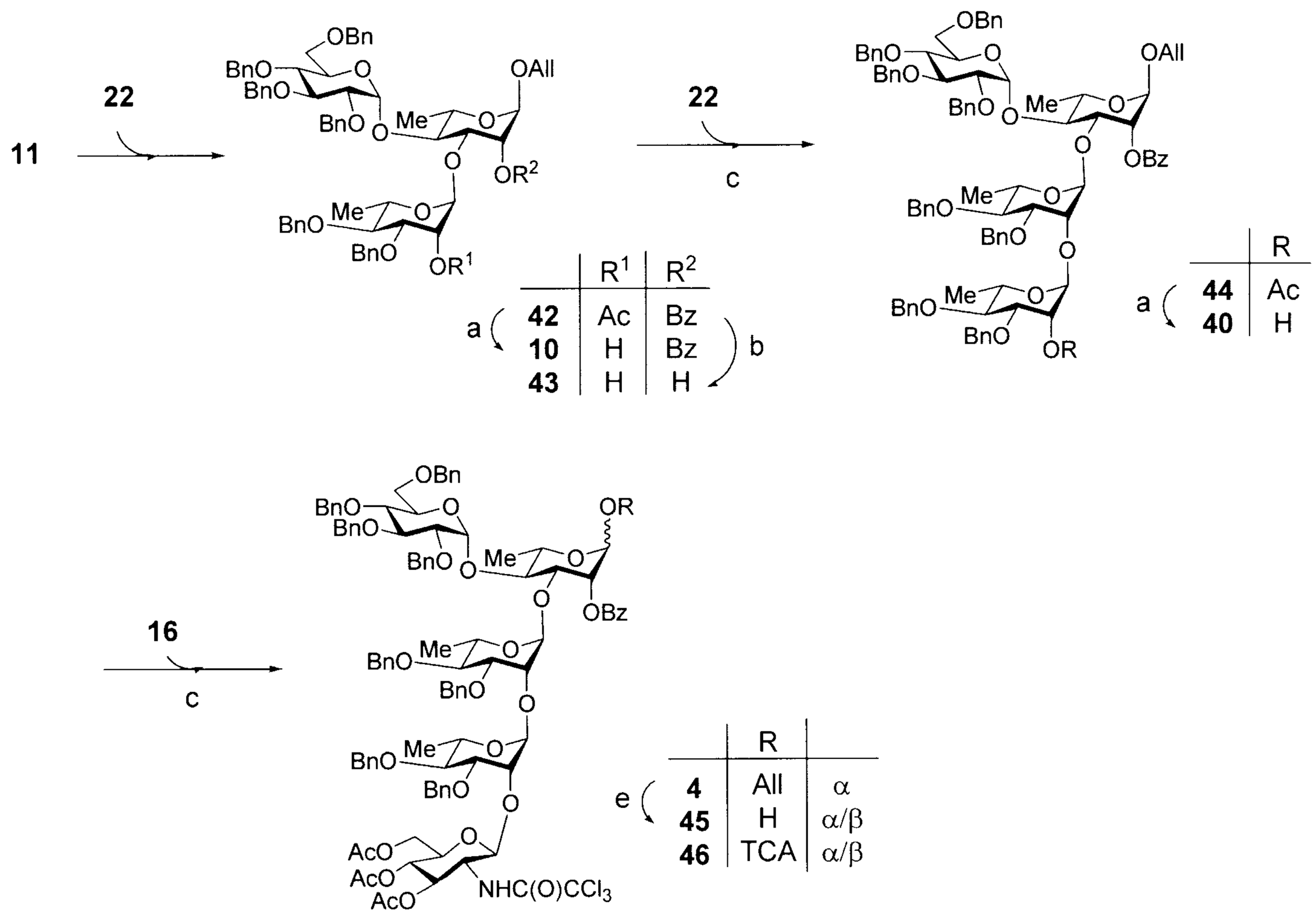


		R^1	R^2	
c	**32**	All	ClAc	α
d	**33**	H	ClAc	α/β
	34	TCA	ClAc	α/β
c	**35**	All	*p*MeOBn	α
d	**36**	H	*p*MeOBn	α/β
	37	TCA	*p*MeOBn	α/β

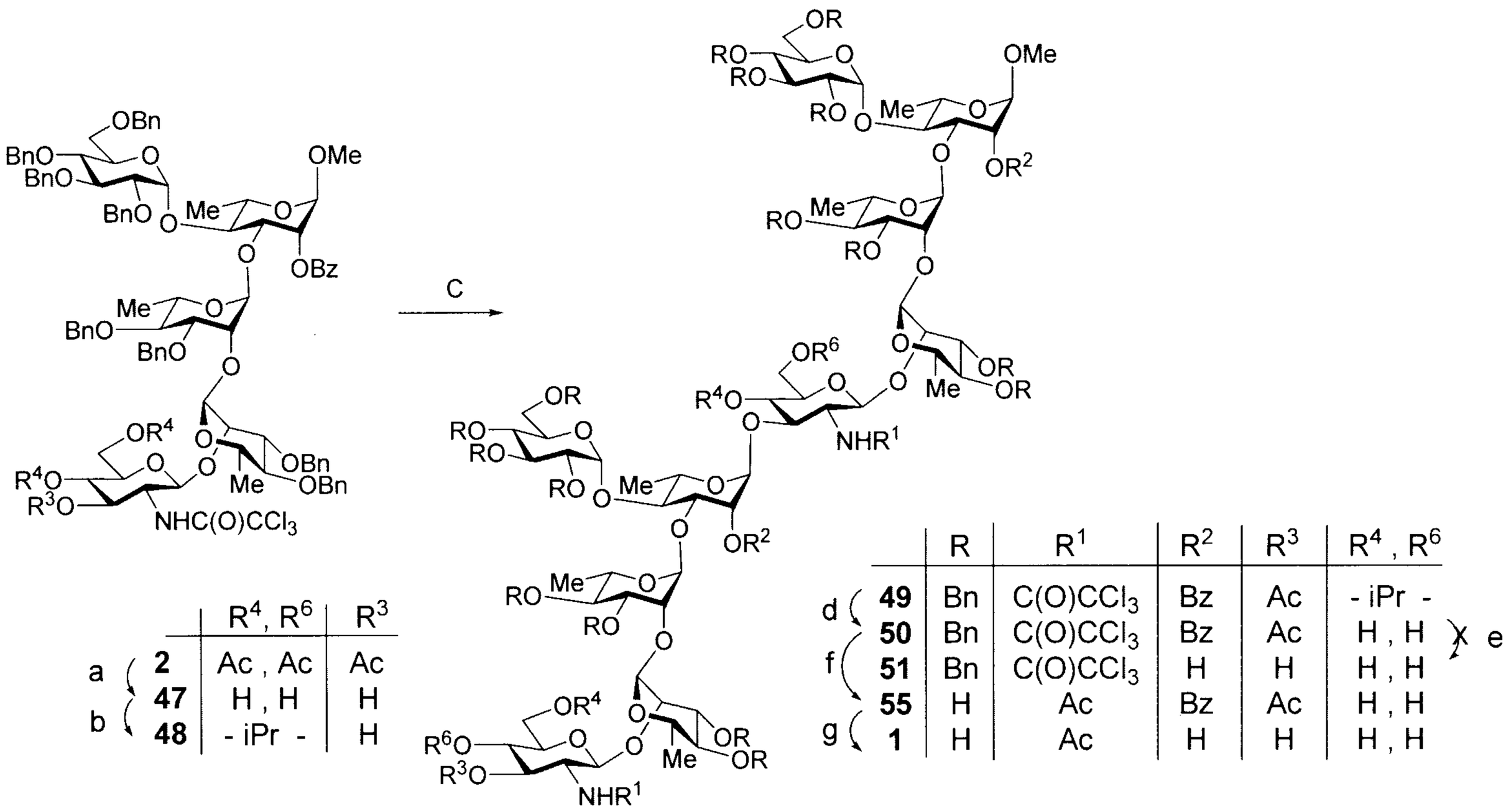


	R
38	ClAc
39	*p*MeOBn
40	H

Scheme 4 :



Scheme 5:



		R^4, R^6	R^3
a	**2**	Ac , Ac	Ac
b	**47**	H , H	H
	48	- iPr -	H

		R	R^1	R^2	R^3	R^4 , R^6
d	**49**	Bn	$C(O)CCl_3$	Bz	Ac	- iPr -
	50	Bn	$C(O)CCl_3$	Bz	Ac	H , H
f	**51**	Bn	$C(O)CCl_3$	H	H	H , H
	55	H	Ac	Bz	Ac	H , H
g	**1**	H	Ac	H	H	H , H

e

Scheme 6:

LMPP11-theo-brevet-pentaOMe

Synthesis of a tetra- and two pentasaccharide fragments of the O-specific polysaccharide of *Shigella flexneri* serotype 2a

INTRODUCTION

Shigellosis, also known as bacillary dysentery, is a major enteric disease which accounts for some 165 million annual episodes, among which 1.1 million deaths, occurring mostly in developing countries.{Kotloff, 1999 #147} Young children and immunocompromised individuals are the main victims. Occurrence of the disease is seen as a correlate of sanitary conditions, and those are not likely to improve rapidly in areas at risk. The financial status of the populations in which shigellosis stands in its endemic or epidemic forms, as well as the emerging resistance to antimicrobial drugs,{Khan, 1985 #367}*{Salam, 1991 #368}*{Ashkenazi, 1995 #328}*{Iversen, 1998 #357}*{Iwalokun, 2001 #358}* limit the impact of the latter. Some 15 years ago, vaccination was defined as a priority by the WHO in its program on enteric diseases.**REF** However, there is still no license vaccine against this bacterial infection although intensive research is ongoing in the field.{Hale, 1995 #102}**(voir si ref récente PS)** *Shigellae* are Gram negative bacteria. As for other bacterial pathogens, their lipopolysaccharide (LPS) is an important virulence factor. It is also a major target of the host's protective immunity against infection. Indeed, data from infected patients indicated that circulating anti-LPS antibodies were strong markers of acquired immunity.{Cohen, 1988 #329}{Cohen, 1991 #52} It was also demonstrated in a murine model that the presence locally, preliminary to infection, of a secretory antibody of isotype A specific for an epitope located on the O-specific polysaccharide (O-SP) moiety of the LPS of *Shigella flexneri* 5a, prevented any host's homologous infection.{Phalipon, 1995 #228} Importantly, field trial of an investigational *Shigella sonnei* O-SP conjugate, which was shown to induce anti-LPS secretory IgAs, thus suggesting mucosal stimulation,{Cohen, 1996 #360} demonstrated 75% efficacy.{Cohen, 1997 #54}

Shigella flexneri 2a is the prevalent serotype in developing countries, where it is responsible for the endemic form of the disease. Based on the early hypothesis that a critical

level of serum IgG antibodies specific for the O-specific polysaccharide (O-SP) moiety of the LPS was sufficient to confer protection against homologous infections,{Robbins, 1992 #256}{Robbins, 1994 #257} several *S. flexneri* 2a O-SP-protein conjugates were designed. They were found safe and immunogenic in both adults and children.{Ashkenazi, 1965 #362}{Passwell, 2001 #220}

Allowing a better control of the various structural parameters possibly involved in the immunogenicity of glycoconjugate vaccines, oligosaccharide-protein conjugates were proposed as alternatives to polysaccharide-protein conjugate vaccines against bacteria.{Pozsgay, 2000 #247} Indeed, such constructs were found immunogenic on several occasions, including examples whereby the oligosaccharide portion was made of one repeating unit only.{Benaissa-Trouw, 2001 #363}{Mawas, 2002 #364} We reasoned that glycoconjugates incorporating chemically synthesized oligosaccharides, appropriately selected for their ability to mimic the native O-SP in terms of both antigenicity and solution conformation, may offer an alternative to the *S. flexneri* 2a O-SP-protein conjugates currently under study. Our approach relies on a rational basis. Indeed, in order to select the best oligosaccharide mimic, we have undertaken the characterization of the antigenic determinants of *S. flexneri* 2a O-SP recognized by serotype-specific protective monoclonal antibodies. The synthesis of a panel of methyl glycoside oligosaccharides representative of fragments of *S. flexneri* 2a O-SP was thus undertaken to be used as probes in the study of antibody recognition.

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit **I**.{Simmons, 1971 #88; Lindberg, 1991 #46} It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens and comprises a *N*-acetyl glucosamine and three rhamnose residues, together with an α-D-glucopyranose residue branched at position 4 of one of the rhamnoses. We have already reported on the synthesis of the methyl glycosides of various fragments of the O-SP, including the known **EC** disaccharide,{Berry, 1974 #224; Lipkind, 1987 #223}{Mulard, 2000 #52} the **ECD**{Mulard, 2000 #52} and **B(E)C**{Mulard, 2000 #52} trisaccharides, the **ECDA**{Segat, 2002 #225} and **AB(E)C**{Costachel, 2000 #101} tetrasaccharides, the **B(E)CDA**{Segat, 2002 #225} and **DAB(E)C**{Costachel, 2000 #101} pentasaccharides and more recently the **B(E)CDAB(E)C** octasaccharide.{Bélot, 2002 #314} In the following, we report on the synthesis of the

LMPP11-theo-brevet-pentaOMe

ECDAB, **AB(E)CD** pentasaccharides as well on that of the **B(E)CD** tetrasaccharide as their methyl glycosides, **1**, **2** and **3**, respectively.

RESULTS AND DISCUSSION

Analysis of the targets shows that all the glycosylation reactions to set up involve 1,2-*trans* glycosidic linkages except for that of the **E-C** junction which is 1,2-*cis*. Consequently, the syntheses described herein rely on key **EC** disaccharide building blocks as well as on appropriate **A**, **B** and **D** monosaccharide precursors.

*Synthesis of the linear **ECDAB**-OMe pentasaccharide (1):* Based on earlier findings in the series which have demonstrated that the **C-D** linkage was an appropriate disconnection site,{Segat, 2002 #283} a blockwise synthesis of **1** was designed (Scheme 1). It is based on the glycosylation of the known **EC** trichloroacetimidate donor **XX**,{Mulard, 2000 #196} obtained in three steps (69%) from the key intermediate **XX**,{Segat, 2002 #283} and the **DAB** trisaccharide acceptor **XX**. The latter was obtained by the stepwise condensation of known monosaccharide precursors, readily available by selective protection, deprotection and activation sequences. Thus, TMSOTf-catalysed condensation of the rhamnopyranoside acceptor **XX**{Pozsgay, 1987 #241} with the trichloroacetimidate donor **XX**{Castro-Palomino, 1996 #47} in diethyl ether to give the fully protected rhamnobioside **XX**, and subsequent de-*O*-acetylation under Zemplén conditions gave the **AB** disaccharide acceptor **XX** in XX% overall yield, which compares favourably with the previously described preparation.{Pozsgay, 1987 #241} Conventional glycosylation of **XX** with the known glucosaminyl bromide,{Debenham, 1995 #63}chosen as the precursor to residue **D**, under base-deficient conditions in order to avoid orthoester formation, smoothly afforded the fully protected **DAB** trisaccharide (XX%). Removal of the tetrachlorophtalimide and concomitant deacetylation by action of XXX in XXX, followed by *N*-acetylation furnished the triol **XX** (XX%), which was next protected at positions 4_D and 6_D by regioselective introduction of an isopropylidene acetal upon reaction with 2,2-dimethoxypropane under acid-catalysis (XX%). *(mentionner produit vert fluo)* Indeed, data previously obtained when synthesizing shorter fragments in the series outlined the interest of using 4,6-*O*-isopropylidene-glucosaminyl intermediates instead of the more common benzylidene analogs.{Mulard, 2000 #196} Once the two key building blocks made available, their condensation was performed in dichloromethane in the presence of a catalytic amount of TMSOTf to give the fully protected pentasaccharide **XX** (XX%). Conventional stepwise deprotection involving (i) acidic

hydrolysis of the isopropylidene acetal using 90% aq TFA to give diol **XX** (XX%), (ii) conversion of the latter into the corresponding tetraol **XX** under Zemplén conditions (XX%), and (iii) final hydrogenolysis of the benzyl protecting groups, gave the linear pentasaccharide **1** in XX% yield.

*Synthesis of the **AB(E)CD** pentasaccharide **2** and of the **B(E)CD** tetrasaccharide **3** (Scheme 2).* For reasons mentioned above, compound **XX**,{Mulard, 2000 #196} protected at its 4 and 6 hydroxyl groups by an isopropylidene acetal was the precursor of choice for residue **D**. In the past, introduction of residue **B** at position 3_C was performed on a 2_C-*O*-benzoylated **EC** acceptor resulting from the regioselective acidic hydrolysis of the corresponding 2,3-orthoester intermediate.{Costachel, 2000 #55}{Segat, 2002 #283} It rapidly occurred to us that opening of the required phenyl orthoester was not compatible with the presence of an isopropylidene acetal. For that reason, the trichloroacetimidate donor **XX**, suitably benzoylated at 2_C and orthogonally protected by a chloroacetyl group at position 3_C was used as the **EC** building block instead of the previously mentioned **XX**. The choice of protecting group at position 2 of the rhamnosyl precursor to residue B was again crucial in the synthesis of 2. Indeed, most of our previous work in the series relied on the use of the known 2-*O*-acetyl rhamnopyranosyl donor **XX**, {Castro-Palomino, 1996 #47} as an appropriate precursor to residue **B**. In the reported syntheses,{Costachel, 2000 #55} selective 2_B-*O*-deacetylation the presence of a 2_C-*O*-benzoate was best performed by treatment with methanolic $HBF_4.OEt_2$ for five days. Clearly, such deacetylation conditions are not compatible with the presence of isopropylidene group on the molecule either. To overcome this limitation, the corresponding 2-*O*-chloroacetyl rhamnopyranosyl trichloroacetimidate **XX** was selected as an alternate donor. The latter could indeed also serve as an appropriate precursor to residue **A**.

Regioselective conversion of diol **XX** into its 2-*O*-benzoylated counterpart **XX** was performed as described.{Segat, 2002 #283} Treatment of the latter with chloroacetic anhydride and pyridine gave the orthogonally protected **XX** (XX%), which was smoothly de-*O*-allylated to yield the corresponding hemiacetal **XX** (XX%) by a two-step process, involving (i) iridium (I)-promoted isomerisation{Oltvoort, 1981 #216} of the allyl glycoside and (ii) subsequent hydrolysis in the presence of iodine.(Ref, cf Nacira) The selected trichloroacetimidate leaving group was successfully introduced by treatment of **XX** with trichloroacetonitrile in the presence of DBU, which resulted in the formation of **XX** (XX%). TMSOTf-mediated glycosylation of donor **XX** and acceptor **XX** furnished the fully protected **ECD** trisaccharide (XX%), which was as expected readily converted to the required acceptor **XX** upon selective

deblocking of the chloroacetyl protecting group with thiourea (XX%). Following the two-step protocol described above for the preparation of **XX**, the known allyl rhamnopyranoside **XX**,{Westerduin, 1988 #348} bearing a 2-*O*-choloacetyl protecting group, was converted to the hemiacetal **XX** (XX%). Next, treatment of the latter with trichloroacetonitrile and a slight amount of DBU gave donor **XX** in an acceptable yield of XX%. Glycosylation of the **ECD** acceptor **XX** and the **B** donor **XX** was attempted under various conditions of solvent and catalyst. Whatever the conditions, hardly separable mixtures of compounds were obtained. When using TMSOTf as the promoter and XXX as the solvent, the expected tetrasaccharide **XX** was indeed formed, although it was often markedly contaminated with glycosylation intermediates such as the silylated **XX** as well as the orthoester side-product **XX**, as suggested from mass spectroscopy analysis and NMR data. In fact, the nature of the latter was fully ascertained at the next step in the synthesis. Indeed, treatment of a mixture of the condensation products **XX** and supposedly **XX** resulted in the expected tetrasaccharide acceptor **XX** contaminated by the trisaccharide acceptor **XX,** whereas the corresponding β**B**-tetrasaccharide isomer could not be detected at this stage, which indicated that the corresponding chloroacetylated **XX** was not part of the initial mixture. Formation of the starting **XX** during the dechloroacetylation step is not unexpected as it may be explained by intramolecular rearrangement leading to expulsion of the **B** residue, following dechlorination in the presence of XXX. Starting from **XX**, the isolated yield of the tetrasaccharide acceptor **XX** was XX%, which encouraged us to reconsider the use of the 2-*O*-acetyl analogue **XX** as a precursor to residues **B** and **A** in the synthesis of **2**.

A suivre …

CONCLUSION

The synthesis of the methyl glycoside (**2**) of the repeating unit **I** of the *S. flexneri* 2a O-SP, together with that of the corresponding pentasaccharide **1** and tetrasaccharide **3** were described. All the methyl glycosides of the di- to pentasaccharides obtained by circular permutation of the monosaccharide residues partaking in the linear backbone of **I**, and comprising the **EC** portion, are now available in the laboratory. Their binding to a set of available protective IgG antibodies will be reported elsewhere.

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EXPERIMENTAL

General Methods. General experimental methods not referred to in this section were as described previously.(REF) TLC on precoated slides of Silica Gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, dichloromethane-methanol; *B*, cyclohexane-ethyl acetate, *C*, cyclohexane-diethyl ether, *D*, water-acetonitrile, *E*, *iso*-propanol-ammonia-water, *F*, cyclohexane-diethyl ether-ethyl acetate. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in 4N aq H_2SO_4. In the NMR spectra, of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. Interchangeable assignments in the ^{13}C NMR spectra are marked with an asterisk in listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the *O*-SP and identified by a subscript in listing of signal assignments. Low-resolution mass spectra were obtained by either chemical ionisation (CIMS) using NH_3 as the ionising gas, by electrospray mass spectrometry (ESMS), or by fast atom bombardment mass spectrometry (FABMS).

Methyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside (XX). 2,2-dimethoxypropane (4.9 mL, 39.8 mmol) and *para*-toluenesulfonic acid (18 mg, 95 µmol) were added to a solution of the triol **XX** (964 mg, 1.09 mmol) in acetone (3 mL) and the mixture was stirred at rt for 1h. Et_3N was added, and volatiles were evaporated. Column chromatography of the residue (solvent *D*, 99:1) gave the acceptor **XX** as a white solid (969 mg, 96%) which could be crystallized from AcOEt:iPr_2O; mp XX°C; $[\alpha]_D$ XX (*c* 1.0); NMR: ^1H, δ 7.45-7.31 (m, 20H, Ph), 6.98 (d, 1H, $J_{NH,2}$ = 2.4 Hz, NH), 6.37 (bs, 1H, OH), 5.07 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1_A), 4.90 (d, 1H, J = 10.8 Hz, OCH_2), 4.85 (d, 1H, J = 10.1 Hz, OCH_2), 4.84 (d, 1H, J = 10.8 Hz, OCH_2), 4.76 (d, 1H, OCH_2), 4.69 (d, 1H, OCH_2), 4.68 (s, 2H, OCH_2), 4.65 (d, 1H, OCH_2), 4.61 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.48 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.09 (dd, 1H, H-2_A), 4.01 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.91 (dd, 1H, H-2_B), 3.89-3.84 (m, 2H, $J_{5,6}$ = 6.3, $J_{4,5}$ = 9.4, $J_{2',3'}$ = 3.3, $J_{3',4'}$ = 9.4 Hz, H-5_A, 3_B), 3.68 (dq, partially overlapped, $J_{5,6}$ = 6.2, $J_{4,5}$ = 9.5 Hz, H-5_B), 3.66-3.58 (m, 4H, H-$6a_D$, $6b_D$, 2_D, 4_D), 3.44 (pt, 1H, H-4_A), 3.41 (pt, 1H, H-4_B), 3.32 (s, 3H, OCH_3), 3.16 (m, 1H, H-5_D), 1.60 (s, 3H, $C(O)CH_3$), 1.54, 1.48 (2s, 6H, $C(CH_3)_2$), 1.35 (d, 6H, H-6_A, 6_B); ^{13}C, δ 173.9 (CO), 138.8-128.0 (Ph), 103.7 (C-1_D), 101.3 (C-1_A), 100.3 ($C(CH_3)_2$), 100.2 (C-1_B), 81.9 (C-4_A), 80.8 (C-4_B), 80.5 (C-3_A), 79.7 (C-3_B), 79.4 (C-2_A), 76.2 (OCH_2), 76.0 (C-2_B), 75.6, 75.1 (2C, OCH_2), 74.4 (C-4_D), 74.4 (C-3_D), 72.6 (OCH_2), 68.6 (C-5_A), 68.0, 67.9 (2C, C-5_B, 5_D), 62.2 (C-6_D), 60.6 (C-2_D), 55.1 (OCH_3), 29.5 ($C(CH_3)_2$), 22.7 ($C(O)CH_3$), 19.4 ($C(CH_3)_2$), 18.5, 18.2 (2C, C-6_A, 6_B). FABMS for $C_{52}H_{65}NO_{14}$ (M, 927.44) *m/z* 950.5 $[M+Na]^+$.

Anal. Calcd for $C_{52}H_{65}NO_{14}$: C, 67.30; H, 7.06; N, 1.51%. Found: C, 67.15; H, 7.24; N,

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1.44%.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranoside (XX). Activated powdered 4Å molecular sieves were added to a solution of the trisaccharide acceptor **XX** (202 mg, 0.22 mmol) and the disaccharide donor **XX** (263 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (5 mL) and the suspension was stirred for 30 min at –15°C. Trifluoromethanesulfonic acid (7 μL, 34 μmol) was added and the mixture was stirred for 2 h while the bath temperature was slowly coming back to 10°C. TLC (solvent *D*, 49:1) showed that no **XX** remained. Et_3N was added and after 30 min, the suspension was filtered through a pad of Celite. Concentration of the filtrate and chromatography of the residue (solvent *B*, 9:1 → 17:5) gave the fully protected pentasaccharide **XX** (330 mg, 84%) as a white foam; $[\alpha]_D$ XX (*c* 1.0); NMR: ^1H, δ 8.07-6.96 (m, 50H, Ph), 5.82 (d, 1H, $J_{NH,2}$ = 7.4 Hz, NH), 5.63 (dd, 1H, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.5 Hz, H-3_C), 5.43 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2_C), 5.09 (bs, 1H, H-1_B), 5.02 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 4.99 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.95 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_C), 4.94-4.63 (m, 13H, OCH_2), 4.63 (s, 1H, H-1_A), 4.37 (d, 1H, J = 11.0 Hz, OCH_2), 4.29 (dq, 1H, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, H-5_C), 4.25 (d, 1H, J = 9.5 Hz, OCH_2), 4.23 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-3_D), 4.01 (m, 1H, H-2_B), 3.97-3.86 (m, 5H, H-3_E, 2_A, 3_E, 4_C, OCH_2), 3.82 (m, 1H, H-3_A, 5_B), 3.71-3.57 (m, 7H, H-5_D, 4_E, 5_A, 4_D, 4_E, $6a_E$, $6b_E$), 3.54-3.41 (m, 3H, H-2_E, 4_B, 2_D) 3.38-3.31 (m, 2H, H-4_A, $6a_D$), 3.31 (s, 3H, OCH_3), 3.17 (m, 1H, H-5_E), 3.08 (d, 1H, $J_{6a,6b}$ = 10.1 Hz, H-$6b_D$), 1.84 (s, 3H, NHAc), 1.46 (s, 3H, $C(CH_3)_2$), 1.45 (d, 3H, $J_{5,6}$ = 5.9 Hz, H-6_C), 1.35 (m, 6H, $J_{5,6}$ = 5.9 Hz, H-6_B, $C(CH_3)_2$), 1.31 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A); ^{13}C, δ 171.7, 165.9, 165.8 (3C, CO), 138.9-127.9 (Ph), 102.3 (C-1_X, J = 167 Hz), 101.5 (C-1_X, J = 170 Hz), 100.3 (C-1_X, J = 170 Hz), 99.8 ($C(CH_3)_2$), 99.6 (C-1_X, J = 172 Hz), 98.2 (C-1_X, J = 172 Hz), 82.0 (C-X_A), 81.2 (C-X_B), 80.9 (C-X_A), 80.7 (C-X_B), 79.7, 79.3 (3C, OCH_2), 78.1 (C-X_A), 77.8, 77.4, 75.5, 71.8 (5C, OCH_2), 71.7 (C-X_B), 71.6 (C-X), 68.8 (C-X), 68.0 (C-6_E), 67.6 (C-X_D), 62.5 (C-6_D), 58.9 (C-2_D), 55.0 (OCH_3), 29.5 ($C(CH_3)_2$), 23.8 ($C(O)CH_3$), 19.8 ($C(CH_3)_2$), 18.6 (C-6_C), 18.5 (C-6_A), 18.3 (C-6_B). XXMS for $C_{106}H_{117}NO_{25}$ (M, 1803.79) *m/z* XXX $[M+H]^+$.

Anal. Calcd for $C_{106}H_{117}NO_{25}$: C, 70.53; H, 6.53; N, 0.78%. Found: C, XXXX; H, XXX; N, XXX%.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (XX). 90% aq TFA (750 μL) was added at 0°C to a solution of the fully protected **XX** (588 mg, 326 μmol) in CH_2Cl_2 (6.7 mL) and the mixture was stirred at this temperature for 1 h. TLC (solvent *B*, 1.5:1) showed that no **XX** remained. Volatiles were evaporated by repeated addition of toluene. Chromatography of the residue (solvent *B*, 4:1 → 1:1) gave **XX** (544 mg, 95%) as a white foam;

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$[\alpha]_D$ XX° (*c* 1.0); NMR: ^1H, δ 8.06-7.06 (m, 50H, Ph), 5.82 (d, 1H, $J_{NH,2}$ = 7.1 Hz, NH), 5.65 (dd, 1H, $J_{2,3}$ = 3.8, $J_{3,4}$ = 9.0 Hz, H-3_C), 5.53 (m, 1H, H-2_C), 5.34 (s, 1H, H-1_B), 5.04 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.00 (m, 2H, H-1_C, 1_E), 4.97-4.63 (m, 13H, OCH_2), 4.48 (bs, 1H, H-1_A), 4.40 (d, 1H, J = 8.4 Hz, OCH_2), 4.29 (d, 1H, J = 8.0 Hz, OCH_2), 4.28-4.21 (m, 2H, H-3_D, 5_C), 4.10 (m, 1H, H-2_A), 4.04 (m, 1H, H-2_B), 3.99 (d, 1H, OCH_2), 3.95-3.89 (m, 3H, H-3_B, 3_E, 4_C), 3.87 (dd, 1H, $J_{2,3}$ = 2.7, $J_{3,4}$ = 9.7 Hz, H-3_A), 3.81-3.64 (m, 5H, H-5_E, 5_B, $6a_D$, 4_E, 5_A), 3.54 (dd, 1H, $J_{1,2}$ = 3.2, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.51 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_B), 3.45-3.37 (m, 4H, H-4_A, 4_D, $6a_E$, 2_D), 3.33 (m, 5H, H-5_D, $6b_D$, OCH_3), 3.12 (d, 1H, $J_{6a,6b}$ = 10.6 Hz, H-$6b_E$), 2.28 (bs, 1H, OH),1.97 (bs, 1H, OH), 1.84 (s, 3H, NHAc), 1.54 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_C), 1.37 (m, 6H, H-6_B, 6_A); ^{13}C, δ 171.5, 165.8, 165.6 (3C, CO), 138.8-127.9 (Ph), 101.6 (C-1_D), 100.8 (C-1_B), 100.5 (C-1_A), 100.1 (C-1_E*), 99.9 (C-1_C*), 84.9 (C-3_D), 82.1 (C-3_E), 80.9, 80.7, 80.6, 80.5 (4C, C-4_B, 3_A, 4_A, 2_E), 79.7 (C-4_C), 79.3 (C-3_B), 77.8 (2C, C-2_B, 4_E), 76.0, 75.9 (2C, OCH_2), 75.8 (C-5_D), 75.6, 75.1, 74.6, 73.7, 73.1 (5C, OCH_2), 72.8 (C-2_A), 72.6 (OCH_2), 71.8 (C-5_E), 71.6 (C-4_D), 71.3 (C-3_C), 71.1 (C-2_C), 69.4 (C-5_C), 68.8 (C-5_B), 68.3 (C-5_A), 68.1 (C-6_E), 63.0 (C-6_D), 57.6 (C-2_D), 55.0 (OCH_3), 23.8 (NHAc), 18.8 (C-6_C), 18.6, 18.5 (2C, C-6_A, 6_B). XXMS for $C_{103}H_{113}N0_{25}$ (M, 1763.76) *m/z* XXX $[M+H]^+$.

Anal. Calcd for $C_{103}H_{113}N0_{25}$: C, XX; H, XX; N, XX%. Found: C, XXXX; H, XXX; N, XXX%.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (XX). 1M Methanolic sodium methoxide was added to a solution of **XX** (277 mg, 157 μmol) in a 1:1 mixture of CH_2Cl_2 and MeOH (6 mL) until the pH was 10. The mixture was stirred overnight at rt and neutralized with Amberlite IR-120 (H^+). The crude material was chromatographed (solvent *D*, 49:1) to give **XX** (211 mg, 86%) as a white foam; $[\alpha]_D$ XX° (*c* 1.0); NMR: *^1H, δ 8.07-7.06 (m, 50H, Ph), 5.82 (d, 1H, $J_{NH,2}$ = 7.1 Hz, NH), 5.65 (dd, 1H, $J_{2,3}$ = 3.8, $J_{3,4}$ = 9.0 Hz, H-3_C), 5.53 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2_C), 5.34 (s, 1H, H-1_B), 5.04 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.00 (m, 2H, H-1_C, 1_E), 4.97-4.63 (m, 13H, OCH_2), 4.48 (bs, 1H, H-1_A), 4.40 (d, 1H, J = 8.4 Hz, OCH_2), 4.29 (d, 1H, J = 8.0 Hz, OCH_2), 4.28-4.21 (m, 2H, H-3_D, 5_C), 4.10 (m, 1H, H-2_A), 4.04 (m, 1H, H-2_B), 3.99 (d, 1H, OCH_2), 3.95-3.89 (m, 3H, H-3_E, 3_B, 4_C), 3.87 (dd, 1H, $J_{2,3}$ = 2.7, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.81-3.64 (m, 5H, H-5_B, 5_E, $6a_D$, 5_A, 4_E), 3.54 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.7 Hz, H-2_E), 3.51 (pt, 1H, $J_{4,5}$ = $J_{3,4}$ = 9.5 Hz, H-4_B), 3.45-3.37 (m, 4H, H-4_A, 4_D, $6a_E$, 2_D), 3.33 (m, 5H, H-5_D, $6b_D$, OCH_3), 3.12 (d, 1H, $J_{6a,6b}$ = 10.6 Hz, H-$6b_E$) 2.28 (bs, 1H, OH), 1.97 (bs, 1H, OH), 1.84 (s, 3H, NHAc), 1.53 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_C), 1.37 (m, 6H, H-6_B, 6_A); ^{13}C, δ 171.5, 165.8, 165.6 (3C, CO), 138.8-127.9 (Ph), 101.6 (C-1_D), 100.8 (C-1_B), 100.5 (C-1_A), 100.1, 99.9 (2C, C-1_E, 1_C), 84.9 (C-3_D), 82.1 (C-3_E), 80.9, 80.7, 80.6, 80.5, 79.7 (5_C, C-4_B, 3A, 4A, 2E, 4C) (3C, OCH_2), 78.1 (C-X_A), 77.8, 77.4, 75.5, 71.8 (5C, OCH_2), 71.7 (C-X_B), 71.6 (C-X), 68.8 (C-X), 68.0 (C-6_E), 67.6 (C-X_D), 62.5 (C-6_D), 58.9 (C-2_D), 55.0 (OCH_3), 29.5 ($C(CH_3)_2$), 23.8 ($C(O)CH_3$), 19.8 ($C(CH_3)_2$), 18.6 (C-6), 18.5 (C-6_A,), 18.3*

(C-6$_B$). XXMS for $C_{103}H_{113}N0_{25}$ (M, 1763.76) *m/z* XXX $[M+H]^+$.

Anal. Calcd for $C_{103}H_{113}N0_{25}$: C, XX; H, XX; N, XX%. Found: C, XXXX; H, XXX; N, XXX%.

Methyl α-D-Glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (1). *The benzylated tetrasaccharide **23** (484 mg, 394 μmol) was dissolved in a mixture of methanol (10 mL) and AcOH (1 mL), treated with 10% Pd-C catalyst (200 mg), and the suspension was stirred overnight at rt. TLC (solvent D, 3:2) showed that the starting material had been transformed into a more polar product. The suspension was filtered on a pad of Celite. The filtrate was concentrated and coevaporated repeatedly with cyclohexane. Reverse phase chromatography of the residue (solvent F, 100:0 → 49:1), followed by freeze-drying, gave the target tetrasaccharide **1** as an amorphous powder (230 mg, 85%); $[\alpha]_D$ +3° (c 1.0, water); NMR: ^1H, δ 5.04 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1$_E$), 4.87 (bs, 1H, H-1$_C$), 4.84 (bs, 1H, H-1$_A$), 4.76 (d, overlapped, 1H, H-1$_D$), 4.10 (dq, 1H, $J_{4,5}$ = 9.5 Hz, H-5$_C$), 4.01 (m, 1H, H-2$_A$), 4.00 (m, 1H, H-5$_E$), 3.92 (dd, 1H, $J_{6a,6b}$ = 12.0, $J_{5,6a}$ = 1.8 Hz, H-6a$_D$), 3.87-3.73 (m, 7H, H-3$_C$, 3$_A$, 6a$_E$, 6b$_E$, 2$_D$, 2$_C$, 6b$_D$), 3.73-3.61 (m, 3H, H-3$_E$, 3$_D$, 5$_A$), 3.59-3.43 (m, 5H, H-2$_E$, 4$_D$, 4$_C$, 5$_D$, 4$_E$), 3.39 (s, 3H, OCH$_3$), 3.32 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4$_A$), 2.07 (s, 3H, C(O)CH$_3$), 1.32 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_C$), and 1.28 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$); ^{13}C, δ 175.3 (C(O)), 102.7 (C-1$_D$, J = 163 Hz), 102.0 (C-1$_C$, J = 170 Hz), 100.5 (2C, C-1$_A$, 1$_E$, J = 170 Hz), 82.3 (C-3$_D$), 81.8 (C-4$_C$), 79.3 (C-2$_A$), 76.7 (C-4$_E$), 73.6 (C-3$_E$), 73.1 (C-4$_A$), 72.6 (C-5$_E$), 72.4 (C-2$_E$), 71.8 (C-2$_C$), 70.7 (C-3$_A$), 70.1 (C-5$_D$), 69.7 (C-3$_C$), 69.3 (C-5$_A$), 69.2 (C-4$_D$), 68.9 (C-5$_C$), 61.4 (C-6$_D$), 60.9 (C-6$_E$), 56.4 (C-2$_D$), 55.6 (OCH$_3$), 23.0 (C(O)CH$_3$), 17.5 (C-6$_A$), and 17.3 (C-6$_C$). FABMS for $C_{27}H_{47}NO_{19}$ (M, 689.3) m/z 712.2 $[M+Na]^+$.*

3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranose (XX). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (Ir(I), 25 mg) was dissolved in dry THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **XX** (3.28 g, 7.12 mmol) in THF (30 mL) was degassed and added. The mixture was stirred overnight at rt, and a solution of iodine (3.6 g, 14.2 mmol) in a mixture of THF (70 mL) and water (20 mL) was added. The mixture was stirred at rt for 1 h, **then concentrated**. The residue was taken up in CH_2Cl_2 and washed twice with 5% aq $NaHSO_4$. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, X:X) to give **XX** (2.53 g, 85%) *as a slightly yellow foam*. , $[\alpha]_D$ +25° (c 1.0); ^1H NMR: δ 7.40-7.28 (m, 10H, Ph), 5.57 (bd, 0.2H, H-2β), 5.45 (dd, 0.8H, $J_{1,2}$ = 2.0 Hz, H-2α), 5.13 (bd, 0.8H, H-1α), 4.92 (d, 1H, J = 10.9 Hz, $OCH_2\alpha$, $OCH_2\beta$), 4.79 (d, 0.2H, J = 11.2 Hz, $OCH_2\beta$), 4.74 (d, 1H, J = 11.2 Hz, $OCH_2\alpha$, H-1β), 4.65 (d, 0.8H, $OCH_2\alpha$), 4.64 (d, 0.2H, $OCH_2\beta$), 4.58 (d, 0.8H, $OCH_2\alpha$), 4.54 (d, 0.2H, $OCH_2\beta$),

4.30 (d, 0.2H, J = 15.1 Hz, $CH_2Cl\beta$), 4.26 (d, 0.2H, $CH_2Cl\beta$), 4.20 (s, 1.6H, $CH_2Cl\alpha$), 4.08 (dd, 0.8H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.6 Hz, H-3α), 4.04 (dq, 0.8H, $J_{4,5}$ = 9.5 Hz, H-5α), 3.66 (dd, 0.2H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 8.7 Hz, H-3β), 3.44 (pt, 2H, H-4α, 5β, OH-1α, 1β), 3.38 (pt, 0.2H, $J_{4,5}$ = 9.5 Hz, H-4β), 1.37 (d, 0.6H, $J_{5,6}$ = 5.7 Hz, H-6β), and 1.34 (d, 2.4H, $J_{5,6}$ = 6.2 Hz, H-6α); ^{13}C NMR: δ167.8 (C=Oβ), 167.4 (C=Oα), 138.6-128.2 (Ph), 93.0 (C-1β), 92.4 (C-1α), 80.3 (C-4α), 80.2 (C-3β), 79.6 (C-4β), 77.8(C-3α), 75.9 ($OCH_2\beta$), 75.8 ($OCH_2\alpha$), 72.5 ($OCH_2\alpha$), 72.3 (0.4C, C-5β, $OCH_2\beta$), 71.9 (C-2-β), 71.7 (C-2α), 68.2(C-5α), 41.3 ($CH_2Cl\alpha$, $CH_2Cl\beta$), and 18.3 (C-6α, 6β); *CIMS for $C_{70}H_{76}O_{16}$ (M, 1172.51) m/z 1190.8 $[M+NH_4]^+$.*

Anal. Calcd for $C_{70}H_{76}O_{16}$: C, 71.65; H, 6.53%. Found: C, 71.52; H, 6.56%.

3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl Trichloroacetimidate (XX). The hemiacetal **XX** (700 mg, 1.66 mmol) was dissolved in CH_2Cl_2 (6 mL) and the solution was cooled to 0°C. Trichloroacetonitrile (1.7 mL) and DBU (26 μL) were added. The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent B 4:1 + 0.1% Et_3N) to give **XX** as a white foam (687 mg, 73%, α/β: 4/1). *$[\alpha]_D$ +25° (c 1.0);* ^{1}H NMR (α-anomer): δ 8.71 (s, 1H, NH), 7.40-7.30 (m, 10H, Ph), 6.24 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.57 (dd, 1H, H-2), 4.94 (d, 1H, J = 10.8 Hz, OCH_2), 4.76 (d, 1H, J = 11.2 Hz, OCH_2), 4.67 (d, 1H, OCH_2), 4.62 (d, 1H, OCH_2), 4.22 (s, 2H, CH_2Cl), 4.04 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-3), 3.99 (dq, 1H, $J_{4,5}$ = 9.6 Hz, H-5), 3.53 (pt, 1H, H-4), and 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (α-anomer): δ 166.9 (C=O), 160.4 (C=NH), 138.4-128.3 (Ph), 95.2 (C-1), 91.1 (CCl_3), 79.5 (C-4), 77.6 (C-3), 76.1, 72.9 (2C, OCH_2), 71.2 (C-5), 69.8 (C-2), 41.1 (CH_2Cl), and 18.3 (C-6).

Anal. Calcd for $C_{70}H_{76}O_{16}$: C, 71.65; H, 6.53%. Found: C, 71.52; H, 6.56%.

Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranoside (XX). To a solution of the known **XX** (7.10 g, 8.55 mmol) in a mixture of DCM (40 mL) and pyridine (5 mL) at 0°C was added chloroacetic anhydride (3.65 g, 21.3 mmol), and the mixture was stirred at this temperature for 2 h. TLC (solvent C, 9:1) showed the complete disappearance of the starting material. MeOH (10 mL) was added, and after 30 min, volatiles were evaporated. Column chromatography (solvent B, 1:0 → 4:1) of the crude yellow oil afforded **XX** as a *XXXXXX* (7.34 g, 95%). *$[\alpha]_D$ +25° (c 1.0);* ^{1}H NMR: δ 8.12-7.13 (m, 25H, Ph), 5.95 (m, 1H, CH_{All}), 5.50-5.42 (m, 2H, $J_{2,3}$ = 3.6 Hz, H-2_C, 3_C), 5.37 (m, 1H, CH_{2All}), 5.28 (m, 1H, CH_{2All}), 4.96 (d, 1H, J = 11.0 Hz, OCH_2), 4.93 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_C), 4.90 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 4.87-4.81 (m, 3H, OCH_2), 4.67 (d, 1H, J = 12.1 Hz, OCH_2), 4.64 (d, 1H, J = 12.8 Hz, OCH_2), 4.47 (d, 1H, J = 10.8 Hz, OCH_2), 4.43 (d, 1H, J = 12.0 Hz, OCH_2), 4.25 (m, 1H, OCH_{2All}), 4.09 (d, 1H, J = 15.5 Hz, CH_2Cl), 3.99-3.93 (m,

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3H, CH_2Cl, H-5_C, 3_C), 3.84 (m, 1H, H-5_E), 3.78-3.74 (m, 2H, H-$6a_E$, 4_E), 3.70 (pt, 1H, $J_{4,5}$ = $J_{3,4}$ = 9.3 Hz, H-4_C), 3.58-3.54 (m, 2H, H-$6b_E$, 2_E), and 1.50 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: δ167.0 (C=O, ClAc), 166.0 (C=O, Bz), 139.1-128.0 (Ph, All), 118.5 (All), 99.5 (C-1_E), 96.8 (C-1_C), 81.9 (C-3_E), 81.0 (C-2_E), 79.7 (C-4_C), 77.7 (C-4_E), 76.0, 75.4, 74.1, 73.8 (4C, OCH_2), 73.5 (C-3_C), 71.8 (C-5_E), 70.9 (C-2_C), 68.8 (OCH_{2All}), 68.1 (C-6_E), 67.7 (C-5_C), 41.5 (CH_2Cl), and 18.6 (C-6_C); FAB-MS for $C_{52}H_{55}O_{12}$ (M, 906.5) *m/z* 929.3 $[M+Na]^+$.

Anal. Calcd for $C_{52}H_{55}ClO_{12}$: C, 68.83; H, 6.11%. Found: C, 68.74; H, 6.19%.

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α/β-L-rhamnopyranose (XX). A solution of **XX** (7.21 g, 7.95 mmol) in THF (80 mL) containing activated iridium complex (60 mg) was treated as describd for the preparation of **XX**. The mixture was stirred at rt for 3 h, at which point a solution of iodine (4.0 g, 15.7 mmol) in a mixture of THF (90 mL) and water (24 mL) was added. The mixture was stirred at rt for 30 min, then concentrated. The residue was taken up in CH_2Cl_2 and washed twice with 5% aq $NaHSO_4$, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 4:1) to give **XX** (6.7 g, 97%) as a slightly yellow foam. *$[\alpha]_D$ +25° (c 1.0);* ^1H NMR: δ 8.10-7.09 (m, 25H, Ph), 5.47 (dd, 1H, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.3 Hz, H-3_C), 5.41 (bs, 1H, H-2_C), 5.03 (bs, 1H, H-1_C), 4.94 (d, 1H, J = 10.9 Hz, OCH_2), 4.87 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 4.85 (d, 1H, OCH_2), 4.80 (m, 2H, OCH_2), 4.64 (m, 2H, OCH_2), 4.45 (d, 1H, J = 10.7 Hz, OCH_2), 4.41 (d, 1H, J = 12.1 Hz, OCH_2), 4.16 (dq, 1H, $J_{4,5}$ = 9.3 Hz, H-5_C), 4.09 (d, 1H, J = 15.6 Hz, CH_2Cl), 3.96 (d, 1H, CH_2Cl), 3.93 (pt, 1H, H-3_E), 3.83 (m, 1H, H-5_E), 3.77-3.68 (m, 2H, H-4_E, $6a_E$), 3.65 (pt, 1H, H-4_C), 3.54 (m, 2H, H-$6b_E$, 2_E), and 1.48 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: δ167.0 (C=O, ClAc), 166.0 (C=O, Bz), 139.1-127.9 (Ph), 99.5 (C-1_E), 92.3 (C-1_C), 81.9 (C-3_E), 81.0 (C-2_E), 79.9 (C-4_C), 77.6 (C-4_E), 76.0, 75.6, 74.2, 74.1 (4C, OCH_2), 72.1 (C-3_C), 71.7 (C-4_E), 71.1 (C-2_C), 68.0 (C-6_E), 67.5 (C-5_C), 41.5 (CH_2Cl), and 18.9 (C-6_C); FAB-MS for $C_{49}H_{51}ClO_{12}$ (M, 866.3) *m/z* 889.3 $[M+Na]^+$.

Anal. Calcd for $C_{49}H_{51}ClO_{12}$: C, 67.85; H, 5.93%. Found: C, 67.72; H, 6.00%.

(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl trichloroacetimidate (XX). Trichloroacetonitrile (1.1 mL, 10.9 mmol) and DBU (17 μL) were added to a solution of the hemiacetal **XX** (950 mg, 1.09 mmol) in dry DCM (8 mL), and the mixture was stirred at 0°C for 1.5 h. Toluene was added, and volatiles were evaporated. The residue was purified by flash chromatography (solvent B, 3:2 containing 0.1% Et_3N) to give **XX** (930 mg, 84%) as a *XXXXXXX*. Further elution gave some remaining starting material **XX** (136 mg, 14%). *$[\alpha]_D$ +25° (c 1.0);* ^1H NMR: δ 8.76 (s, 1H, NH), 8.12-7.17 (m, 25H, Ph), 6.34 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_C), 5.67 (dd, 1H, H-2_C), 5.54 (dd, 1H, $J_{2,3}$ = 3.4, $J_{3,4}$ = 8.8 Hz, H-3_C), 4.98 (d, 1H, OCH_2), 4.88 (d, 1H, $J_{1,2}$ = 3.4 H-1_E), 4.84 (d, 1H, J = 11.1 Hz, OCH_2),

4.82 (d, 1H, J = 11.2 Hz, OCH_2), 4.65 (d, 1H, OCH_2), 4.62 (d, 1H, OCH_2), 4.44 (d, 1H, J = 11.4 Hz, OCH_2), 4.41 (d, 1H, J = 11.8 Hz, OCH_2), 4.14 (dq, 1H, $J_{4,5}$ = 9.5 Hz, H-5_C), 4.11 (d, 1H, J = 15.5 Hz, CH_2Cl), 3.98 (d, 1H, CH_2Cl), 3.94 (pt, 1H, H-3_E), 3.83-3.71 (m, 4H, H-5_E, $6a_E$, 4_E, 4_C), 3.56-3.51 (m, 2H, H-$6b_E$, 2_E), and 1.51 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: □167.1 (C=O, ClAc), 165.7 (C=O, Bz), 169.6 (C=NH), 139.0-127.9 (Ph), 99.9 (C-1_E), 95.2 (C-1_C), 82.1 (C-3_E), 80.9 (C-2_E), 79.0 (C-4_C), 77.6 (C-4_E), 76.0, 75.6, 74.2, 73.8 (4C, OCH_2), 73.0 (C-3_C), 71.9 (C-5_E), 70.7 (C-5_C), 69.2 (C-2_C), 68.0 (C-6_E), 67.7 (C-5_C), 41.4 (CH_2Cl), and 18.6 (C-6_C).

Anal. Calcd for $C_{51}H_{51}Cl_4NO_{12}$: C, 60.54; H, 5.08; N, 1.38%. Found: C, 60.49; H, 5.01; N, 1.34%.

Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). The acceptor **XX** (500 mg, 1.82 mmol) was dissolved in DCM (5.5 mL) and 4Å-MS (300 mg) were added. The mixture was cooled to -60°C and stirred for 15 min. TMSOTf (35 µL, mmol) and a solution of the disaccharide donor **XX** (2.39 g, 2.36 mmol) in DCM (7.5 mL) were added. The mixture was stirred for 45 min while the cooling bath was coming back to rt, and for more 3 h at rt. The mixture was then heated at 65°C for 1 h 30 min. Et_3N was added and the mixture was stirred at rt for 20 min, then diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 85:15 → 1:1) to give **XX** (1.64 g, 80%) as a *XXXXXX*. *[α]$_D$ +25° (c 1.0);* ^{1}H NMR: δ 8.06-6.93 (m, 25H, Ph), 6.18 (d, 1H, $J_{NH,2}$ = 7.3 Hz, NH_D), 5.40 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-3_C), 5.38 (bs, 1H, H-2_C), 4.98 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.94 (bs, 1H, H-1_C), 4.94 (d, 1H, OCH_2), 4.93 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 4.83 (d, 2H, J = 10.7 Hz, OCH_2), 4.81 (d, 1H, J = 10.6 Hz, OCH_2), 4.67 (d, 1H, J = 11.7 Hz, OCH_2), 4.62 (d, 1H, J = 11.4 Hz, OCH_2), 4.47 (m, 3H, H-3_D, OCH_2), 4.22 (dq, 1H, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.2 Hz, H-5_C), 4.10 (d, 1H, J = 15.5 Hz, CH_2Cl), 3.96 (m, 2H, H-$6a_D$, CH_2Cl), 3.91 (pt, 1H, H-3_E), 3.82 (m, 2H, H-5_E, $6b_D$), 3.72 (m, 3H, H-$6a_E$, 4_E, 4_C), 3.62 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_D), 3.55 (m, 2H, H-$6b_E$, 2_E), 3.51 (s, 3H, OMe), 3.41 (m, 1H, H-5_D), 3.15 (m, 1H, H-2_D), 2.04 (s, 3H, NHAc), 1.51 (s, 3H, CMe_2), 1.42 (m, 6H, H-6_C, CMe_2), and 1.51 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: δ 171.8 (C=O, NHAc), 167.3 (C=O, ClAc), 166.1 (C=O, Bz), 139.0-128.0 (Ph), 101.1 (C-1_D, J_{CH} < 164 Hz), 99.9 (CMe_2), 99.4 (C-1_E, J_{CH} > 165 Hz), 98.2 (C-1_C, J_{CH} = 172 Hz), 81.8 (C-3_E), 80.9 (C-2_E), 79.0 (C-4_C*), 77.7 (C-4_E*), 76.7 (C-3_D), 75.9, 75.3, 74.2, 73.9 (4C, OCH_2), 73.7 (C-4_D), 73.4 (C-3_C), 71.9 (C-5_E), 71.2 (C-2_C), 68.2 (C-6_E), 67.8 (C-5_C), 67.4 (C-5_D), 62.7 (C-6_D), 59.6 (C-2_D), 57.6 (OMe), 41.5 (CH_2Cl), 29.5 (CMe_2), 27.3 (NHAc), 19.7 (CMe_2), and 18.6 (C-6_C); FAB-MS for $C_{61}H_{70}ClNO_{17}$ (M, 1123.4) *m/z* 1146.5 $[M+Na]^+$.

Anal. Calcd for $C_{61}H_{70}ClNO_{17}$: C, 65.15; H, 6.27; N, 1.25%. Found: C, 65.13; H, 6.23; N, 1.22%.

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Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). To a solution of the fully protected **XX** (1.40 g, 1.25 mmol) in a mixture of methanol (18 mL) and pyridine (18 mL) was added thiourea (951 mg, 12.5 mmol). The mixture was stirred at 65°C for 5 h at which time no TLC (solvent D, 4:1) that no starting material remained. Evaporation of the volatiles and co-evaporation of petroleum ether form the residue resulted in a crude solid which was taken up in a minimum of methanol. A large excess of DCM was added and the mixture was left to stand at 0°C for 1 h. The precipitate was filtrated on a pad of Celite and the filtrated was concentrated. Column chromatography of the residue (solvent C, 4:1) gave the trisaccharide acceptor **XX** (1.28 g, 97%) as a **XXXX**. *[α]$_D$ +25° (c 1.0);* ^{1}H NMR: δ 8.10-6.96 (m, 25H, Ph), 6.09 (d, 1H, $J_{NH,2}$ = 7.9 Hz, NH_D), 5.26 (dd, 1H, $J_{1,2}$ = 1.6, $J_{2,3}$ = 3.4 Hz, H-2_C), 4.97 (m, 3H, H-1_C, 1_E, OCH_2), 4.86 (m, 3H, H-1_D, OCH_2), 4.81 (d, 1H, OCH_2), 4.72 (d, 1H, OCH_2), 4.58 (d, 1H, J = 12.2 Hz, OCH_2), 4.51 (d, 1H, J = 10.9 Hz, OCH_2), 4.48 (d, 1H, J = 12.2 Hz, OCH_2), 4.23 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3_D), 4.18-4.10 (m, 2H, H-5_C, 5_E), 4.06-3.95 (m, 3H, H-3_C, 3_E, $6a_D$), 3.80 (pt, 1H, $J_{5,6b}$ = $J_{6a,6b}$ = 10.4 Hz, H-$6b_D$), 3.66 (m, 2H, H-$6a_E$, $6b_E$), 3.62 (dd, 1H, $J_{2,3}$ = 9.8, $J_{1,2}$ = 4.1 Hz, H-2_E), 3.59 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, H-4_E), 3.55 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4_D), 3.51 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4_C), 3.49 (s, 3H, OCH_3), 2.22 (s, 3H, NHAc), 1.90 (bs, 1H, OH), 1.49 (s, 3H, CMe_2), 1.43 (s, 3H, CMe_2), and 1.40 (s, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: δ171.8, 166.6 (2C, C=O), 138.9-128.1 (Ph), 101.6 (C-1_D), 99.8 (CMe_2), 98.6 (C-1_E*), 98.3 (C-1_C*), 85.4 (C-4_C), 82.0 (C-3_E), 80.4 (C-2_E), 78.2 (C-4_E), 77.1 (C-3_D), 75.9, 75.5, 74.2, 73.9 (4C, OCH_2), 73.6 (C-4_D*), 73.5 (C-2_C*), 71.7 (C-5_E), 69.0 (C-6_E), 68.3 (C-3_C), 67.5 (C-5_D), 66.9 (C-5_C), 62.7 (C-6_D), 58.9 (C-2_D), 57.5 (OMe), 29.5 (CMe_2), 24.0 (NHAc), 19.7 (CMe_2), and 18.2 (C-6_C); FAB-MS for $C_{59}H_{69}NO_{16}$ (M, 1047,5) *m/z* 1070.4 $[M+Na]^+$.

Anal. Calcd for $C_{70}H_{76}O_{16}$: C, 67.61; H, 6.64; N, 1.34%. Found: C, 67.46; H, 6.78; N, 1.24%.

Methyl (3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). The trisaccharide acceptor **XX** (615 mg, 0.58 mmol) was dissolved in Et_2O (10 mL) and the solution was cooled to -60°C. TMSOTf (32 μL) and donor **XX** (497 mg, 0.88 mmol) in Et_2O (12 mL) were added, and the mixture was stired for 1 h while the bath was slowly coming back to -20°C. The mixture was stirred for 4 h at this temperature, then at 0°C overnight. More **XX** (50 mg, 88 μmol) was added, and the mixture was stirred at rt for 3 h more at 0°C. Et_3N was added, and the mixture was concentrated. Column chromatography of the residue (solvent B, 9:1 → 1:1) gave the orthoester **XX** (44 mg, 5%) then the fully protected **XX** (445 mg, 52%) contaminated with the trimethylsilyl side product **XX** (**XX**/**XX**: XX:XX) together with a mixture of **XX** and **XX** (65 mg, 8%), and the starting **XX** (27 mg, 4%). Compound **XX (alpha)** had *[α]$_D$ +25° (c 1.0);* ^{1}H

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NMR: δ 8.07-7.12 (m, 35H, Ph), 5.96 (d, 1H, $J_{NH,2}$ = 7.9 Hz, NH_D), 5.82 (m, 1H, H-2_C), 5.33 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 5.07 (m, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.05 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_C), 4.98 (d, 1H, OCH_2), 4.97 (bs, 1H, H-1_B), 4.91-4.78 (m, 5H, H-1_D, OCH_2), 4.64 (d, 1H, J = 11.6 Hz, OCH_2), 4.60-4.45 (m, 5H, OCH_2), 4.36 (d, 1H, J = 11.9 Hz, OCH_2), 4.26 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3_D), 4.17 (dd, 1H, $J_{2,3}$ = 3.4 Hz, H-3_B), 4.16 (d, 1H, J = 15.1 Hz, CH_2Cl), 4.11 (d, 1H, CH_2Cl), 4.10 (dq, 1H, $J_{4,5}$ = 9.1, $J_{5,6}$ = 6.3 Hz, H-5_B), 4.06 (m, 1H, H-5_E), 4.00 (pt, 1H, $J_{3,4}$ = $J_{2,3}$ = 9.4 Hz, H-3_E), 3.97 (dd, 1H, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 10.8 Hz, $6a_D$), 3.89 (m, 1H, H-$6a_E$), 3.88-3.68 (m, 4H, H-$6b_E$, $6b_D$, 4_B, 3_C), 3.67 (m, 1H, H-5_C), 3.58 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_D), 3.52 (dd, 1H, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.8 Hz, H-2_E), 3.49 (s, 3H, OCH_3), 3.39 (m, 1H, H-5_D), 3.30 (m, 2H, H-2_D, 4_C), 2.12 (s, 3H, NHAc), 1.52 (s, 3H, CMe_2), 1.42 (s, 3H, CMe_2), 1.33 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_X), and 0.96 (s, 3H, $J_{5,6}$ = 6.2 Hz, H-6_X); ^{13}C NMR: □171.9 (C=O, NHAc), 167.0 (C=O, CH_2Cl), 166.3 (C=O, Bz), 138.8-128.0 (Ph), 101.4 (C-1_D, J_{CH} = 164 Hz), 99.9 (CMe_2), 99.3 (C-1_C, J_{CH} = 168 Hz), 98.3 (C-1_E, J_{CH} = 168 Hz), 97.9 (C-1_B, J_{CH} = 171 Hz), 82.1 (C-3_E), 81.8 (C-2_E), 80.4 (bs, C-3_B), 80.0 (C-4_C), 78.8 (bs, C-4_E*), 78.3 (C-4_B*), 77.7 (C-3_C*), 76.9 (C-3_D), 75.9, 75.5, 75.3, 74.3 (4C, OCH_2), 73.4 (C-4_D), 73.2 (OCH_2), 72.7 (C-2_B), 72.1 (C-5_E), 69.1 (C-5_C), 67.7(C-5_D*), 67.6 (C-5_B*), 62.7 (C-6_D), 59.1 (C-2_D), 57.5 (OMe), 41.4 (CH_2Cl), 29.5 (CMe_2), 24.0 (NHAc), 19.7 (CMe_2), 18.8 (C-$6_{\underline{XX}}$), and 18.2 (C-$6_{\underline{XX}}$); FAB-MS for $C_{81}H_{92}NClO_{21}$ (M, 1449.5) *m/z* 1472.7 $[M+Na]^+$.

Anal. Calcd for $C_{81}H_{92}NClO_{21}$: C, 67.05; H, 6.39; N, 0.97%. Found: C, 66.21; H, 6.46; 1.01%.

Compound **XX (orthoester)** had *[α]$_D$ +25° (c 1.0);* ^{1}H NMR: δ 8.07-7.15 (m, 35H, Ph), 5.47 (d, 1H, $J_{NH,2}$ = 7.4 Hz, NH_D), 5.45 (bs, 1H, H-2_C), 5.42 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1_B), 5.24 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 4.94 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1_D), 4.91-4.82 (m, 7H, H-1_C, OCH_2), 4.80 (d, 1H, J = 11 Hz, OCH_2), 4.75 (d, 1H, J = 11.6 Hz, OCH_2), 4.68 (dd, 1H, $J_{1,2}$ = 2.4, $J_{2,3}$ = 4.0 Hz, H-2_B), 4.65-4.47 (m, 4H, OCH_2), 4.44-4.32 (m, 4H, H-5_E, 3_D, 3_C, OCH_2), 4.15 (m, 1H, H-5_C), 4.05 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3_E), 4.03 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_C), 3.94 (dd, 1H, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 10.7 Hz, H-$6a_D$), 3.83-3.75 (m, 4H, H-$6a_E$, $6b_D$, CH_2Cl), 3.74-3.70 (m, 3H, H-4_E, 6_E, 3_B), 3.65 (dd, 1H, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.4 Hz, H-2_E), 3.48 (pt, 2H, H-4_B, 4_D), 3.46 (s, 3H, OCH_3), 3.38 (m, 1H, H-5_D), 3.22 (dq, 1H, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, H-5_B), 2.88 (m, 1H, H-2_D), 1.90 (s, 3H, NHAc), 1.42 (s, 3H, CMe_2), 1.36 (s, 6H, CMe_2, H-6_C), and 1.30 (s, 3H, $J_{5,6}$ = 6.3 Hz, H-6_B); ^{13}C NMR: δ171.8 (C=O, NHAc), 166.4 (C=O, Bz), 139.1-122.5 (Ph), 101.0 (C-1_D, J_{CH} = 165 Hz), 99.7 (CMe_2), 98.3 (C-1_C, J_{CH} = 172 Hz), 97.8 (bs, C-1_E, J_{CH} = 170 Hz), 97.5 (C-1_B, J_{CH} = 176 Hz), 82.2 (C-3_E), 80.7 (C-2_E), 79.3 (bs, C-4_B), 78.8 (C-3_B), 78.1 (bs, C-4_E), 77.3 (C-2_B), 76.2 (bs, C-3_C), 75.8, 75.6, 74.9, 74.6, 73.9 (6C, C-4_C, OCH_2), 73.5 (2C, C-4_D,2_C), 71.4 (OCH_2), 71.0 (C-3_D), 70.7 (2C, C-5_E, 5_B), 69.0 (C-5_C), 68.8 (C-6_E), 67.2 (C-5_D), 62.5 (C-6_D), 60.0 (C-2_D), 57.6 (OMe), 46.9 (CH_2Cl), 29.5 (CMe_2), 23.9 (NHAc), 19.7 (CMe_2), 19.0 (C-6_B), and 18.4 (C-6_C); FAB-MS for $C_{81}H_{92}NClO_{21}$ (M, 1449.5) *m/z* 1472.7 $[M+Na]^+$.

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Anal. Calcd for $C_{81}H_{92}NClO_{21}\cdot H_2O$: C, 66.23; H, 6.34; N, 0.96%. Found: C, 66.11; H, 6.62; N, 0.85%.

Voir tentative déblocage orthoester seul ou bien mélange α/orthoester issu du couplage dans des proportions connues au départ.(644 11-12 par ex)

Methyl (2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). The trisaccharide acceptor **XX** (500 mg, 0.47 mmol) was dissolved in DCM (5 mL) and the solution was cooled to -40°C. TMSOTf (21 μL) and donor **XX** (328 mg, 0.62 mmol) were added and the mixture was left under stirring while the bath was slowly coming back to rt. After 5 h, more **XX** (50 mg, 94 μmol) was added and the mixture was stirred at rt for 1 h more at rt. Et_3N was added and the mixture was concentrated. Column chromatography of the residue (solvent B, 4:1 → 1:1) gave the fully protected **XX** (484 mg, 72%) slightly contaminated with the corresponding trimethylsilyl side-product **XX**. The **XX**:**XX** ratio was estimated to be XX:XX from the ^1H NMR spectrum.

RMN à faire

FAB-MS for $C_{81}H_{93}NO_{21}$ (M, 1415) *m/z* XXXX $[M+Na]^+$. *Voir si presence silyl*

Anal. Calcd for $C_{81}H_{93}NO_{21}\cdot H_2O$: C, 68.69; H, 6.57; N, 0.98%. Found: C, 67.64; H, 6.67; N, 0.88%.

Methyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). (a) Thiourea (362 mg, 4.76 mmol) was added to an unseparable mixture of **XX** and **XX** (689 mg, 0.48 mmol) in MeOH/pyridine (1/1, 16 mL), and the mixture was heated overnight at 65°C. Volatiles were evaporated, and the solid residue thus obtained was taken up in the minimum of MeOH. DCM was added, and the suspension was left standing at 0°C for 1 h. The precipitate was filtrated on a pad of Celite, and the filtrate was concentrated. Column chromatography of the residue (solvent B, 9:1 → 1:1) gave the trisaccharide acceptor **XX** (107 mg, 22%) as the first eluting product. Further elution gave the tetrasaccharide acceptor (419 mg, 63%) together with a mixture of **XX** and **XX** (66 mg).

(b) The monoacetylated **XX** (52.3 mg, 37 μmol) was dissolved in a mixture of EtOH (10 mL) and DCM (100 μL). A freshly prepared 0.4M ethanolic solution of guanidine (92 μL, 37 μmol) was added and the mixture was stirred at rt overnight. Volatiles were evaporated, and the residue taken up in DCM was washed with water. The organic phase was dried and concentrated. Column chromatography of the crude product gave **XX** (42 mg, 83%). Compound **XX** had

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RMN à faire

FAB-MS for $C_{79}H_{91}NO_{20}$ (M, 1373) *m/z* 1396.5 $[M+Na]^+$.

Anal. Calcd for $C_{79}H_{91}NO_{20}\cdot 0.5\ H_2O$: C, 68.56; H, 6.65; N, 1.01%. Found: C, 68.53; H, 6.71; N, 1.01%.

Methyl (2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (XX). 4Å Molecular sieves and TMSOTf (16 µL) were added to a solution of the tetrasaccharide acceptor **XX** (406 mg, 0.29 mmol) in Et_2O (10 mL), and the mixture was stirred at -60°C for 30 min. The donor **XX** (234 mg, 0.44 mmol) in DCM (7 mL) was added, and the mixture was stirred for 1 h while the bath temperature was reaching rt. After a further 1 h at this temperature, more **XX** (50 mg, 94 µmol) was added, and the mixture was stirred for 1 h before Et_3N was added. Filtration through a pad of Celite and evaporation of the volatiles gave a residue which was column chromatographed twice (solvent B, 4:1; then solvent D, 17:3) to give **XX** (262 mg, 52%); *$[\alpha]_D$ +25° (c 1.0);* ^{1}H NMR: δ 8.07-7.13 (m, 45H, Ph), 6.03 (bs, 1H, NH_D), 5.59 (bs, 1H, H-2_A), 5.35 (bs, 1H, H-2_C), 5.16 (bs, 1H, H-1_E), 5.13 (bs, 1H, H-1_A), 5.06 (bs, 1H, H-1_B), 5.02-4.97 (m, 4H, H-1_D, 1_C, OCH_2), 4.91-4.50 (m, 12H, OCH_2), 4.44-4.32 (m, 4H, H-2_B, 3_D, OCH_2), 4.20-3.96 (m, 7H, H-5_E, 5_A, 3_C, 3_E, $6a_D$, 5_C, 3_A), 3.87-3.68 (m, 6H, H-4_E, $6a_E$, $6b_E$, $6b_D$, 4_C, 3_B), 3.64-3.47 (m, 7H, H-5_B, 4_D, 2_E, 4_A, OCH_3), 3.42 (m, 1H, H-5_D), 3.34 (pt, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4_B), 3.17 (m, 1H, H-2_D), 2.13 (s, 3H, NHAc), 1.49 (s, 3H, CMe_2), 1.43 (s, 6H, CMe_2, H-6_C), 1.33 (d, 3H, $J_{5,6} = 6.1$ Hz, H-6_A), and 1.01 (s, 3H, $J_{5,6} = 5.8$ Hz, H-6_B); ^{13}C NMR: δ 171.9 (C=O, NHAc), 170.3 (C=O, Ac), 166.3 (C=O, Bz), 139.2-127.6 (Ph), 101.5 (bs, C-1_B, J_{CH} = 171 Hz), 101.2 (C-1_D, J_{CH} = 163 Hz), 99.8 (CMe_2), 99.7 (C-1_A, J_{CH} = 171 Hz), 97.9 (2C, C-1_E, 1_C, J_{CH} = 172, 169 Hz), 82.4 (C-3_E), 82.1 (C-2_E), 80.5 (C-4_A), 80.2 (C-3_C), 80.1 (C-4_B), 79.4 (C-3_B*), 78.1 (2C, C-4_E*, 3_A), 78.0 (C-4_C), 76.6 (C-3_D), 75.9, 75.8, 75.4 (3C, OCH_2), 74.8 (2C, C-2_B, OCH_2), 73.5 (C-4_D), 73.4 (OCH_2), 73.2 (C-2_C), 72.1 (OCH_2), 71.8 (C-5_A), 71.2 (OCH_2), 69.4 (C-2_A), 69.2 (C-5_B), 68.9 (C-6_E), 68.7 (C-5_C), 67.8 (C-5_E), 67.5 (C-5_D), 62.7 (C-6_D), 59.6 (C-2_D), 57.6 (OMe), 29.5 (CMe_2), 24.0 (NHAc), 21.4 (OAc), 19.7 (CMe_2), 19.1 (C-6_A), 18.8 (C-6_C), and 18.2 (C-6_B); FAB-MS for $C_{101}H_{115}NO_{25}$ (M, 1741.7) *m/z* 1765.9 $[M+Na]^+$.

Anal. Calcd for $C_{101}H_{115}NO_{25}$: C, 69.60; H, 6.65; N, 0.80%. Found: C, 69.56; H, 6.75; N, 0.73%.

Methyl α-L-rhamnopyranosyl-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (XX). 50% aq TFA (1 mL) was added at 0°C to a solution of the fully protected pentasaccharide **XX** (155 mg, 89 µmol) dissolved in DCM (4 mL). After 1 h at this temperature, volatiles were evaporated.

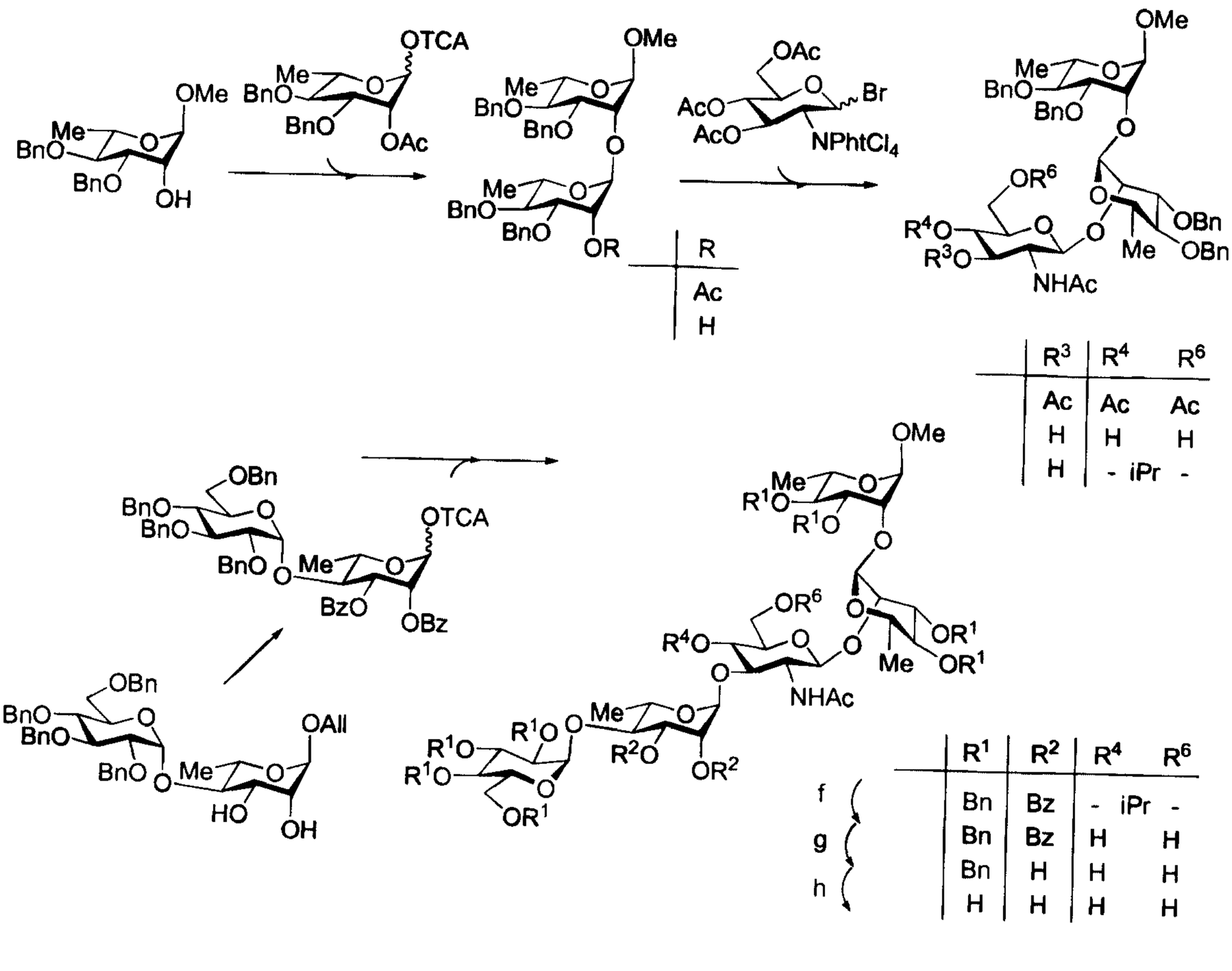
LMPP11-exp-brevet-pentaOMe

The residue was taken up in 0.5M methanolic sodium methoxide (8 mL) and the mixture was heated overnight at 55°C. Neutralisation with Dowex X8 (H^+), evaporation of the volatiles, and column chromatography of the residue gave **XX** (121 mg, 98%). Compound **XX** (111 mg, 81 µmol) was dissolved in a mixture of ethanol (13 mL) and ethyl acetate (2.6 mL) containing 1N aq HCl (130 µL). Palladium on charcoal (130 mg) was added and the suspension was stirred under a hydrogen atmosphere for 2 h. Filtration of the catalyst and reverse phase chromatography gave the target pentasaccharide (60 mg, 88%) as a slightly yellow foam. RP-HPLC purification (solvent XX, XXX) followed by freeze-drying gave pure **XX** (36 mg).

RMN

HRMS (MALDI) Calcd for $C_{33}H_{57}NO_{23}$ + Na: 858.3219. Found: 858.3089.

LMPP11-Sheme-brevet-pentaOMe



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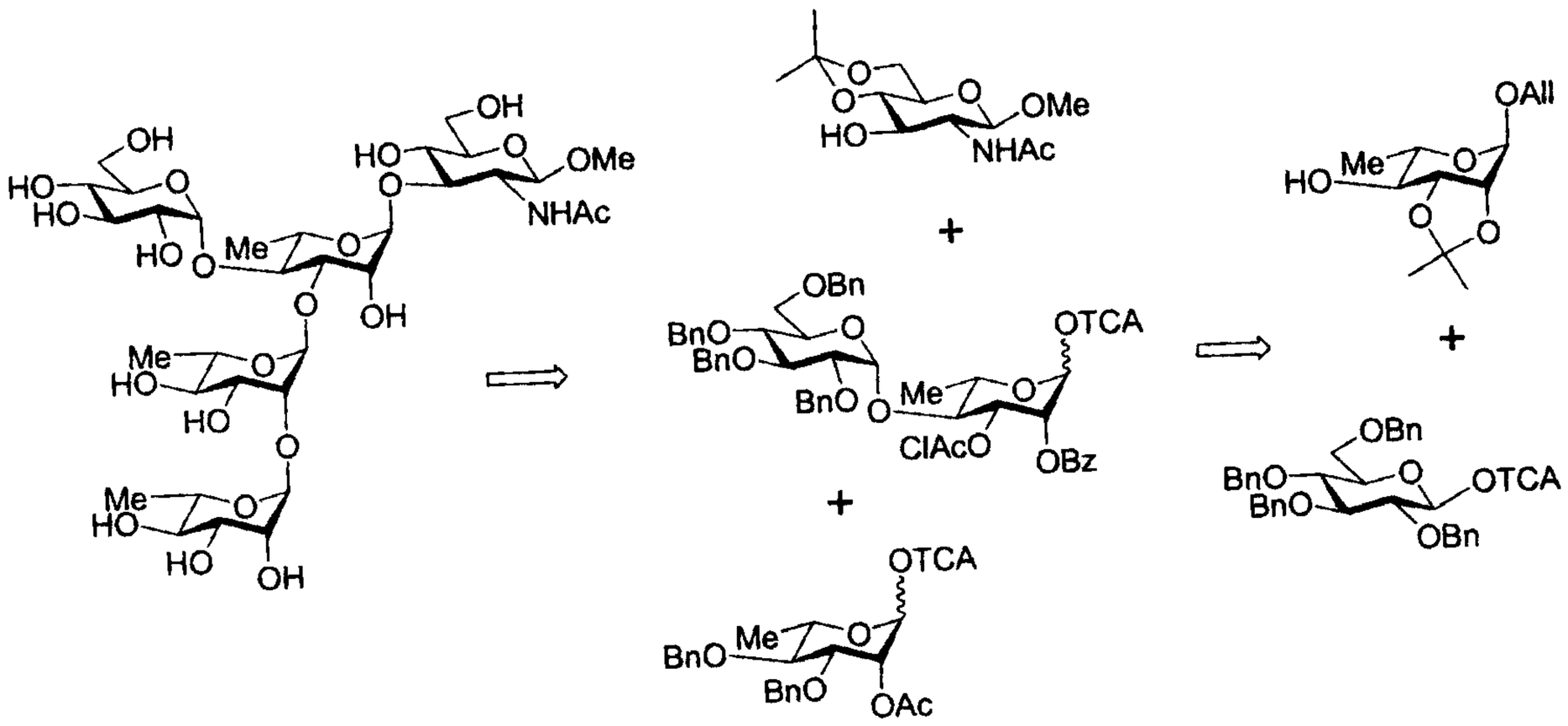

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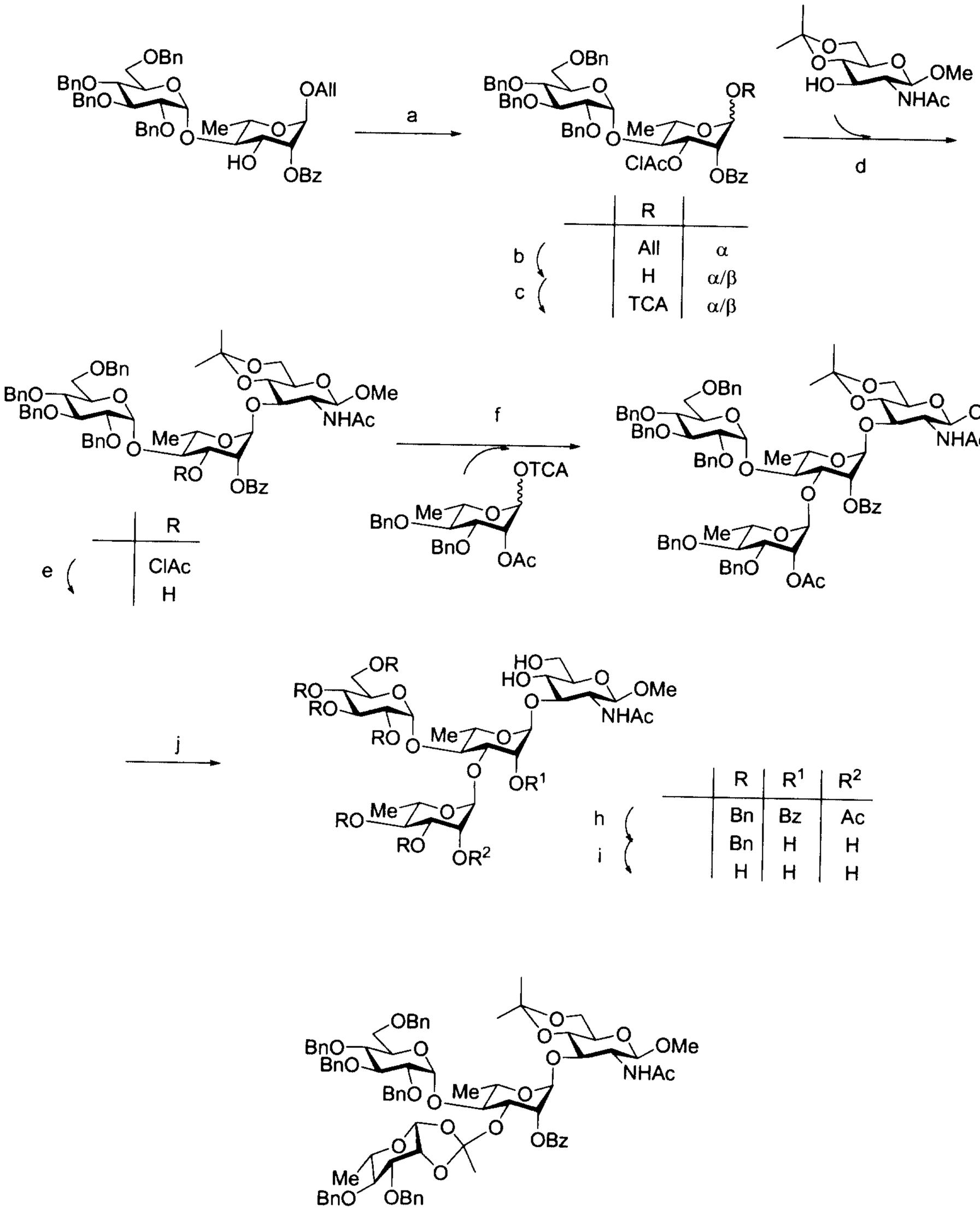

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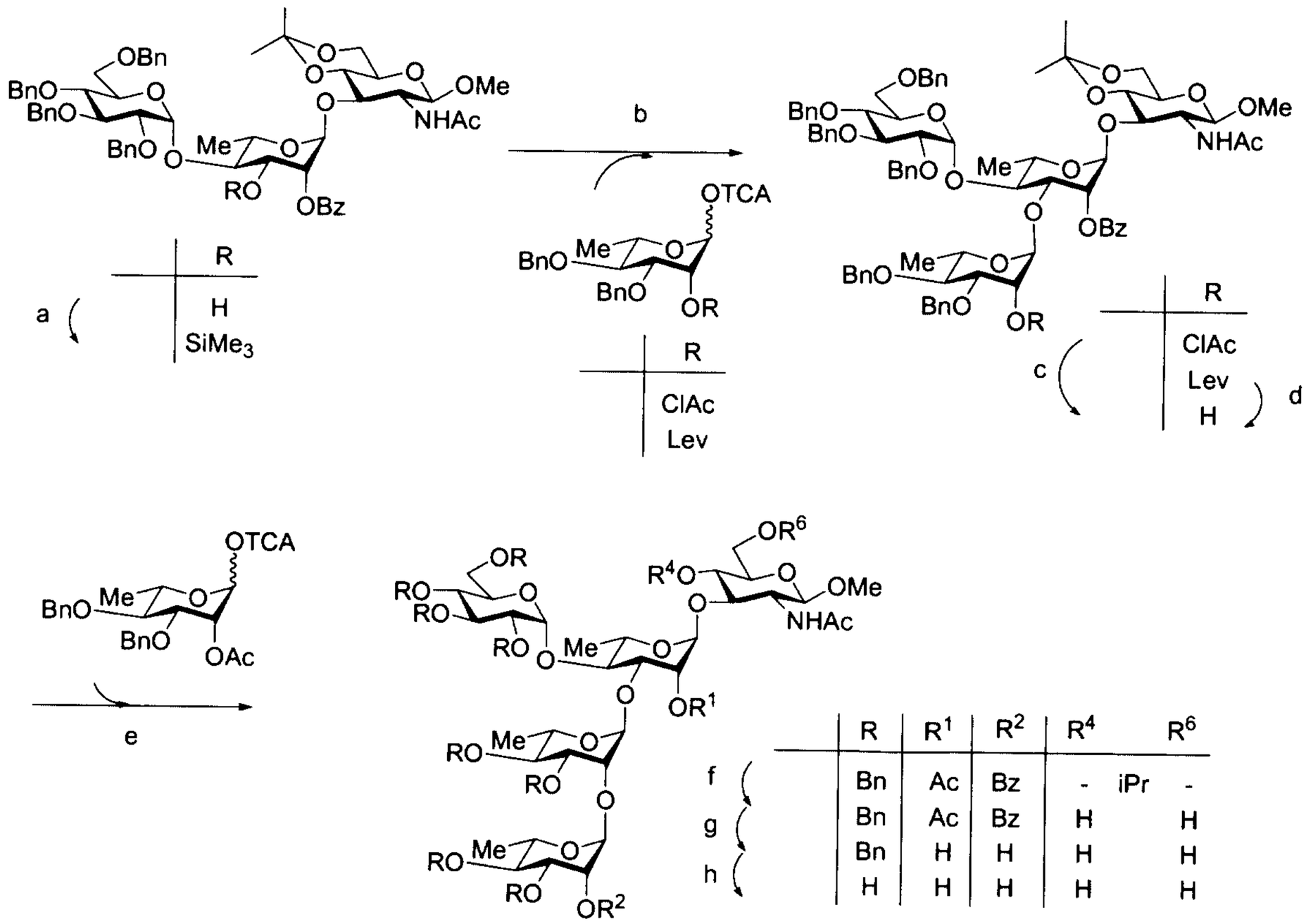

	R	R1	R2	R4		R6
	Bn	Ac	Bz	-	iPr	-
	Bn	Ac	Bz	H		H
	Bn	H	H	H		H
	H	H	H	H		H

LMPP12-theo-brevetgp

Preparation of chemically defined glycopeptides as potential synthetic conjugate vaccines against *Shigella flexneri* serotype 2a disease[1]

Abstract

INTRODUCTION

Since the discovery of *Shigella dysenteriae* type 1 (Shiga's bacillus) more than a century ago, [2]shigellosis or bacillary dysentery has long been known as a serious infectious disease, occurring in humans only. [3]In a recent survey of the literature published between 1966 and 1977, [4]the number of episodes of shigellosis occurring annually throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries. Up to 1.1 million annual deaths were associated to shigellosis during the same period. Of the four species of *Shigellae*, *Shigella flexneri* is the major responsible of the endemic form of the disease, with serotype 2a being the most prevalent. The critical importance of the development of a vaccine against *Shigellae* infections was first outlined in 1987. [5]Due to increasing resistance of all groups of *Shigellae* to antibiotics, [6]it remained a high priority as stated by the World Health Organization ten years later. [7]In the meantime, several experimental vaccines have gone through field evaluation, [8-10]but there are yet no licensed vaccines for shigellosis.

Shigella's lipopolysaccharide (LPS) is a major surface antigen of the bacterium. The corresponding O-specific polysaccharide domain (O-SP) is both an essential virulence factor

and the target of the infected host's protective immune response. [11,12]Indeed, using the pulmonary murine model for shigellosis, it was recently demonstrated that secretory IgA specific for the O-SP of *S. flexneri* serotype 5a were protective against an homogolous infection when present locally prior to the challenge. [13]Based on the former hypothesis that serum IgG anti-LPS antibodies may confer specific protection against shigellosis, [14]several polysaccharide-proteine conjugates, targeting either *Shigella sonnei*, *S. dysenteriae* 1 or *S. flexneri* serotype 2a, were evaluated in humans. [10,15]In the case of *S. sonnei*, recent field trials allowed Robbins and co-workers to demonstrate the efficacy of a vaccine made of the corresponding detoxified LPS covalently linked to recombinant exoprotein A. [16]Conversion of polysaccharide T-independent antigens to T-depend ones through their covalent attachment to a carrier protein had a tremendous impact in the field of bacterial vaccines. Several such neoglycoconjugate vaccines are currently in use against *Haemophilus influenzae*, [17]*Neisseria meningitidis*, [18]and *Streptococcus pneumoniae*. [19]These polysaccharide-protein conjugate vaccines are highly complex structures, whose immunogenicity depends of several parameters amongst which the length and nature of the saccharide component as well as its loading on the protein. It is reasonably admitted that control of these parameters is somewhat difficult when dealing with polysaccharides purified from bacterial cell cultures. As recent progress in carbohydrate synthesis allows access to complex saccharides, it was suggested that the use of well-defined synthetic oligosaccharides may allow a better control, and consequently the optimisation, of these parameters. Indeed, available data on *S. dysenteriae* type 1 indicate that neoglycoconjugates incorporating di-, tri- or tetramers of the O-SP repeating unit were more immunogenic than a detoxified LPS-human serum albumin conjugate of reference. [20]Besides, recent reports demonstrate that short oligosaccharides comprising one repeating unit or less may be immunogenic in animal models. [21,22]Another critical parameter in the design of neoglycoconjugate vaccines is the carrier protein. As potential applications for these vaccines are expanding, the need for new carrier proteins licensed for human use is growing. [23]That synthetic peptides representing immunodominant T-cell epitopes could act as carriers in polysaccharide and oligosaccharide conjugates has been suggested, [24]and latter on demonstrated. [25,26]Besides, the use of T-cell epitopes offer several advantages, including potential access to well-defined conjugates with no risk of epitopic suppression, as the latter phenomenon appeared as a major drawback of protein carriers. [27-30]Polypeptides containing multiple T-cell epitopes have been generated in order to address the extensive polymorphism of HLA molecules. [31,32]In other strategies, universal T-helper epitopes compatible with human use have been characterized, for example from tetanus toxoid, [33]or engineered such as the pan

HLA DR-binding epitope (PADRE). [34]Recently, covalent attachment of the human milk oligosaccharide, lacto-*N*-fucopentose II, to PADRE resulted in a linear glycopeptide of comparable immunogenicity to that of a glycoconjugate employing HSA as the carrier. [35]
Based on these converging data, we focused on the development of well-defined neoglycopeptides as an alternative to polysaccharide-proteine conjugate vaccines targeting infections caused by *S. flexneri* 2a. The target neoglycopeptides were constructed by covalently linking a short peptide, serving as a T-helper epitope, to appropriate carbohydrate haptens, serving as B epitopes mimicking the *S. flexneri* 2a O-Ag. Our approach is based on rational bases involving a preliminary study of the interaction between the bacterial O-SP and homologous protective monoclonal antibodies, which helped to define the carbohydrate haptens.

RESULTS AND DISCUSSION

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

I

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit **I**. [36,37]It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens and comprises a *N*-acetyl glucosamine (**D**) and three rhamnose residues (**A**, **B**, **C**) The specificity of the serotype is associated to the α-D-glucopyranose residue linked to position 4 of rhamnose **C**. Besides the known methyl glycoside of the **EC** disaccharide, [38,39]a set of di- to pentasaccharides corresponding to frame-shifted fragments of the repeating unit **I**, [40-43]an octasaccharide[44] and more recently a decasaccharide[45] representative of fragments of *S. flexneri* 2a O-SP have been synthesized in this laboratory. Based on the use of these compounds as molecular probes for mapping at the molecular level the binding characteristics of a set of protective monoclonal antibodies against *S. flexneri* 2a infection, [46]fragments **ECD**, **B(E)CD** and **AB(E)CD** were selected as haptens that will act as B-epitopes in the conjugates. Three fully synthetic linear neoglycopeptides **1**, **2** and **3**, corresponding to haptens **ECD**, **B(E)CD**, and **AB(E)CD**, respectively, were synthesized according to a strategy built up on the concept of chemoselective ligation which allows the selective one-point attachment of the free B and T epitopes in aqueous media. All conjugates involve the peptide PADRE as the universal T-cell epitope.

LMPP12-theo-brevetgp

Scheme 1:

Retrosynthetic analysis of the saccharidic haptens (Scheme 1): Analysis of *S. flexneri* 2a O-SP suggests that, due to the 1,2-cis glycosidic linkage involved, construction of the **EC** disaccharide is probably the most demanding. Besides, prior work in this laboratory has shown that the **C-D** glycosidic linkage was an appropriate disconnection site when dealing with the blockwise synthesis of oligosaccharide fragments of *S. flexneri* O-2a SP. [40,42,44]These observations supported the design of a synthetic strategy common to all three targets. Basically, it relies on (i) the condensation of an **EC** (**4**), [41]**B(E)C** (**5**) [42]or **AB(E)C** (**6**) donor to a **D** acceptor (**7**), functionalized at the anomeric position with an azidoethyl spacer; (ii) elongation of the spacer with introduction of a masked thiol group to allow its coupling onto a PADRE peptide derivatized by a maleimido group on a C-terminal Lysine (**8**). The carbohydrate synthesis relies on the trichloroacetimidate methodology[47] and the use of known building blocks whenever possible.

Scheme 2:

*Synthesis of the aminoethyl **ECD** building block **18** (Scheme 2):* The now easily accessible disaccharide donor **4**, [42]with a benzoyl participating group at position 2_C, was used as the precursor to the **EC** moiety in the construction of **1**. It was prepared, as described, [41]in 5 steps and 45% overall yield from 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate (**9**) [48,49]and allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside (**10**) [50]through the key intermediate diol **11** (69% from **10**). Introduction of the azidoethyl spacer on a glucosaminyl intermediate was performed according to a known procedure[51] by coupling of azidoethanol[52] onto the oxazoline[53] **12** to give the triacetate **13**. [51,54]We have shown on several occasions in the *S. flexneri* series, that regioselective protection of the 4- and 6-OH groups of precursors to residue **D** with an isopropylidene acetal was appropriate, especially when such precursors are involved in a blockwise synthesis based on the disconnection at the **C-D** linkage. [42,44]Thus, Zemplén deacetylation of **13** gave the triol **14** which was converted to the key acceptor **7** (81% from **13**) upon reaction with 2,2-dimethoxypropane under acid catalysis. When the latter was glycosylated with the donor **4** in the presence of $BF_3.OEt_2$ in dichloromethane, the fully protected trisaccharide **15** was isolated in 58% yield together with the diol **16** (30%), resulting from partial loss of the isopropylidene acetal. When **4** and **7** were glycosylated in the presence of a catalytic amount of TMSOTf, no side-reaction was observed, and the condensation product **15** was obtained in 86% yield. Quantitative conversion of **15** into **16**

was more conveniently achieved by acidic hydrolysis of the former with 95% aq TFA. Zemplén debenzoylation of **16** gave the tetraol **17** (94%) which was subsequently transformed into the aminoethyl-armed trisaccharide **18** (69%) by hydrogenation in the presence of palladium-on-charcoal (Pd/C) and 1N aq HCl to convert the formed amine to its hydrochloride salt. Indeed, others have pointed out that hydrogenolysis using Pd/C in the presence of a free amine was sluggish and low-yielding. [55-57]In order to prevent any side-reaction at a latter stage of the synthesis, isolation of pure **18** was performed by reversed-phase HPLC (RP-HPLC).

Scheme 3:

Synthesis of the aminoethyl ***B(E)CD*** *building block* ***25*** *(Scheme 3):* The known rhamnopyranosyl tricholoracetimidate **20**, [58]acetylated at its 2-, 3-, and 4-OH groups thus acting as a chain terminator, was chosen as the precursor to residue **C**. Benzoylation of diol **11** to give **19** was performed by regioselective opening of the cyclic orthoester intermediate as described. [42]Glycosylation of the latter by donor **20**, with activation by a catalytic amount of TMSOTf proceeded smoothly in Et_2O to yield the fully protected trisaccharide **21** (89%), which was de-*O*-allylated into the hemiacetal **22** (80%) following a two step process involving (i) iridium(I)-catalysed isomerisation of the allyl glycoside to the prop-1-enyl glycoside[59] and (ii) subsequent hydrolysis. [50,60]The selected trichloroacetimidate leaving group was introduced by treatment of **22** with trichloroacetonitrile in the presence of a catalytic amount of DBU, which resulted in the formation of **5** (99%). Condensation of the latter with acceptor **7** was performed in CH_2Cl_2 in the presence of a catalytic amount of trifluoromethanesulfonic acid (TfOH) to give the required tetrasaccharide **23** (76%). Acidic hydrolysis of the latter using 95% aq TFA gave the intermediate diol **24** in 95% yield. Deacylation of the resulting diol under Zemplén conditions followed by debenzylation and concomitant conversion of the azide into the corresponding amine to give the key aminoethyl-armed tetrasaccharide **25** (77%) was performed by treatment of **24** with hydrogen in the presence of Pd/C under acidic conditions. Again, compound **25** was purified by RP-HLPC before elongation of the spacer or conjugation.

Scheme 4:

Synthesis of the aminoethyl ***AB(E)CD*** *building block* ***37*** *(Scheme 4):* The synthesis of **37** is based on the condensation of acceptor **7** and donor **6**, which resulted from the selective deallylation and anomeric activation of the key intermediate tetrasaccharide **33**. The latter was

obtained according to two routes following either a block strategy (route 1) based on the condensation of an **AB** disaccharide donor (**30**) and the **EC** disaccharide acceptor **16**, or a linear strategy (route 2) involving the stepwise elongation of **16**. The construction of the donor **30** was based on the use of the known allyl rhamnopyranoside **26**, [61]having permanent protecting groups at position 3 and 4, as the precursor to residue **B**, and the trichloroacetimidate chain terminator **27**, [62]acting as a precursor to residue **A**. Condensation of the two entities in the presence of a catalytic amount of TMSOTf resulted in the fully protected **28** (96%), which was selectively de-*O*-allylated into **29** (84%) according to the protocol described above for the preparation of **22**. Subsequent treatment of **29** with trichloroacetonitrile and a catalytic amount of DBU gave the required **30** (96%). Glycosylation of **16** with the latter under TMSOTf promotion afforded the fully protected tetrasaccharide **34** in 55% yield. No β-anomer was detected. The stereochemical outcome of this glycosylation step involving a rhamnosyl donor glycosylated at C-2, thus lacking any participating group at this position is not without precedent. Related examples involving rhamnopyranosyl donors may be found in the synthesis of oligosaccharides representative of the capsular polysaccharide of the β-hemolytic *Streptococcus* Group A, [63]or of the O-Ag of *Serratia marcescens* O18[64] as well as in our own work on *S. flexneri* serotype 2a. [45]Route 1 was considered initially in order to prevent extensive consumption of the **EC** disaccharide **11**. Given the relatively low yield of coupling of **16** and **30**, route 2 was considered as well. Of all precursors to **34**, only that to residue **B**, namely the donor and potential acceptor **31**, differed from those used in route 1. Conventional glycosylation of disaccharide **16** and **31** and subsequent selective deacetylation using methanolic HBF_4, gave the acceptor **32** in 70% yield from **16**. [45]The trisaccharide **32** was glycosylated with trichloroacetimidate **27** in an analogous fashion to its glycosylation with **30**, yielding **34** (92%). Deallylation of this key intermediate, as described above for the preparation of **22**, gave the corresponding hemiacetal **35** (90%) which was converted into the required trichloroacetimidate **6** (88%) upon treatment with trichloroacetonitrile and DBU. Condensation of donor **6** with the glucosaminyl acceptor **7** was performed under promotion by TfOH or TMSOTf, which resulted in the fully protected pentasaccharide **35** in 62% and 80% yield, respectively. Following the process described for the preparation of **25**, compound **35** was submitted to acetolysis (97%) and subsequent Zemplén deacylation to give the partially deblocked **36** (87%), which was next converted to the aminoethyl-spacer pentasaccharide **37** upon treatment with hydrogen in the presence of Pd/C. Final RP-HPLC purification resulted in the isolation of **37** in 53% yield.

Scheme 5:

*Synthesis of the target neoglycopeptides **1-3** (Scheme 5):* In all cases, chemoselective ligation of the B and T epitopes was achieved through coupling of the carbohydrate haptens pre-functionalized with a thiol function and a maleimido group properly introduced at the C terminus of the T helper peptide. Such a strategy was chosen in order to exploit the high reactivity and specificity of thiol groups towards the maleimide functionality, [65]which allows specific and high-yielding modification of the former in the presence of other nucleophiles. [66]It was used previously under various forms in the coupling of carbohydrate haptens to either proteins[67,68] or peptides. [26,68]To our knowledge, in all the reported cases the maleimide functionality was introduced onto the carbohydrate hapten. On the contrary, our strategy relies on the introduction of this activating group on the T helper peptide. The immunogenicity of various maleimide-derived coupling reagents was evaluated in a model system. [69]Based on the reported data, [69]4-(*N*-maleimido)-*n*-butanoyl was selected as the linker, and incorporated by covalent linkage to the side chain amino group of a Lysine residue added at the C-terminus of the PADRE sequence (PADRE-Lys). It is worth mentioning that the strategy described herein somewhat differs from that described by others when demonstrating the usefulness of PADRE in the construction of immunogenic neoglycopeptides. [35]

The Lysine-modified PADRE (**8**) was assembled using standard Fmoc chemistry for solid-phase peptide synthesis. [70]Standard side chain protecting groups were used, except for that of the C-terminal Lysine side chain which was protected by the 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) group. [71]Indeed, this orthogonal protecting group strategy allows specific introduction of the maleïmide group on the C-terminal Lysine, upon selective cleavage of the ivDde by hydrazine. The thiol functionality was introduced onto the carbohydrate haptens as a masked thiol function (acetylthioester), which is easily generated *in situ* during the conjugation process Thus, reaction of **15**, **25**, and **27** with S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-oPfp) resulted in the site-selective elongation of their aminoethyl spacer via a thioacetyl acetamido linker. Derivatization could be monitored by RP-HPLC with detection at 215 nm. Under these conditions, the required thioacetyl-armed intermediates, **38**, **39** and **40** were isolated in 53%, 74%, and 75% yield, respectively. Their structure was confirmed based on MS and NMR analysis. Conjugation of the carbohydrate haptens to the maleimido activated PADRE-Lys (**8**) was run in phosphate buffer at pH 6.0 in presence of hydroxylamine[72]and monitored by RP-HPLC. Lastly, RP-

HPLC purification gave the target neoglycopeptides **1**, **2**, and **3** as single products, which identity was assessed based on MS analysis, in yields of 58%, 48% and 46%, respectively.

CONCLUSION

The synthesis of three fully synthetic glycopeptides incorporating a tri-, tetra-, and pentasaccharide haptens representative of fragments of the O-Ag of *S. flexneri* serotype 2a covalently linked to the PADRE-sequence, which acts as a universal T cell epitope is reported. The carbohydrate haptens were selected based on a preliminary study of the recognition of synthetic oligosaccharides with homologous protective antibodies. They were synthesized following a common block strategy, in a form allowing their coupling by chemical ligation onto a maleimido-activated PADRE. Evaluation of the immunogenicity of the conjugates in mice is ongoing.

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REFERENCES

(1) Almg *Part 12 of the series Synthesis of ligands related to the O-specific polysaccharides of Shigella flexneri serotype 2a and Shigella flexneri serotype 5a. For part 11, see ref. XX.* **2003**.

(2) Shields, R.; Burnett, W. *Zentl. Bakteriol.* **1898**, *24*, 817-828.

(3) Keusch, T. G.; Bennish, M. L. *Shigellosis*; Plenum Medical Book Company: New York, 1991.

(4) Kotloff, K. L.; Winickoff, J. P.; Ivanoff, B.; Clemens, J. D.; Swerdlow, D. L.; Sansonetti, P. J.; Adak, G. K.; Levine, M. M. *Bull. WHO* **1999**, *77*, 651-666.

(5) World; Health; Organization *Bull. W.H.O.* **1987**, *65*, 17-25.

(6) Ashkenazi, S.; May-Zahav, M.; Sulkes, J.; Samra, Z. *Antimicrob. Agents Chemother.* **1995**, *39*, 819-823.

(7) World; Health; Organisation *WHO Weekly Epidemiol. Rec.* **1997**, *72*, 73-80.

(8) Coster, T. S.; Hoge, C. W.; Verg, L. L. v. d.; Hartman, A. B.; Oaks, E. V.; Venkatesan, M. M.; Cohen, D.; Robin, G.; Fontaine-Thompson, A.; Sansonetti, P. J.; Hale, T. L. *Infect. Immun.* **1999**, *67*, 3437-3443.

(9) Fries, L. F.; Montemarano, A. D.; Mallett, C. P.; Taylor, D. N.; Hale, T. L.; Lowell, G. H. *Infect. Immun.* **2001**, *69*, 4545-4553.

(10) Passwell, J. H.; Harlev, E.; Ashkenazi, S.; Chu, C.; Miron, D.; Ramon, R.; Farzan, N.; Shiloach, J.; Bryla, D. A.; Majadly, F.; Roberson, R.; Robbins, J. B.; Schneerson, R. *Infect. Immun.* **2001**, *69*, 1351-1357.

(11) Cohen, D.; Green, M. S.; Block, C.; Rouach, T.; Ofek, I. *J. Infect. Dis.* **1988**, *157*, 1068-1071.

(12) Cohen, D.; Green, M. S.; Block, C.; Slepon, R.; Ofek, I. *J. Clin. Microbiol.* **1991**, *29*, 386-389.

(13) Phalipon, A.; Kauffmann, M.; Michetti, P.; Cavaillon, J.-M.; Huerre, M.; Sansonetti, P.; Krahenbuhl, J.-P. *J. Exp. Med.* **1995**, *182*, 769-778.

(14) Robbins, J. B.; Chu, C.; Schneerson, R. *Clin. Infect. Dis.* **1992**, *15*, 346-361.

(15) Taylor, D. N.; Trofa, A. C.; Sadoff, J.; Chu, C.; Bryla, D.; Shiloach, J.; Cohen, D.; Ashkenazi, S.; Lerman, Y.; Egan, W.; Schneerson, R.; Robbins, J. B. *Infect. Immun.* **1993**, *61*, 3678-3687.

(16) Cohen, D.; Ashkenazi, S.; Green, M. S.; Gdalevich, M.; Robin, G.; Slepon, R.; Yavzori, M.; Orr, N.; Block, C.; Ashkenazi, I.; Shemer, J.; Taylor, D. N.; Hale, T. L.; Sadoff, J. C.; Pavliovka, D.; Schneerson, R.; Robbins, J. B. *The Lancet* **1997**, *349*, 155-159.

(17) Ellis, R. W.; Granoff, D. M. *Development and clinical use of Haemophilus b conjugate vaccines*; Dekker: New York, 1994.

(18) Richmond, P.; Borrow, R.; Miller, E.; Clark, S.; Sadler, F.; Fox, A.; Begg, N.; Morris, R.; Cartwright, K. *J. Infect. Dis.* **1999**, *179*, 1569-1572.

(19) Rennels, M. B.; Edwards, K. M.; Keyserling, H. L.; Reisinger, K. S.; Hogerman, D. A.; Madore, D. V.; Chang, I.; Paradiso, P. R.; Malinoski, F. J.; Kimura, A. *Pediatrics* **1998**, *101*, 604-611.

(20) Pozsgay, V.; Chu, C.; Panell, L.; Wolfe, J.; Robbins, J. B.; Schneerson, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194-5197.

(21) Benaissa-Trouw, B.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Kraaijeveld, K.; Snippe, H. *Infect. Immun.* **2001**, *69*, 4698-4701.

(22) Mawas, F.; Niggemann, J.; Jones, C.; Corbet, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Infect. Immun.* **2002**, *70*, 5107-5114.

(23) Robbins, J. B.; Schneerson, R.; Szu, S. C.; Pozsgay, V. In *Vaccinia, Vaccination, Vaccinology: Jenner, Pasteur and their successors*; Plotkin, S., Fantini, B., Eds.; Elsevier: Paris, 1996, pp 135-143.

(24) Boons, G. J. P. H.; Hoogerhout, P.; Poolman, J. T.; Marel, G. A. v. d.; Boom, J. H. v. *Bioorg. Med. Chem.* **1991**, *1*, 303-308.

(25) Lett, E.; Gangloff, S.; Zimmermann, M.; Wachsmann, D.; Klein, J.-P. *Infect. Immun.* **1994**, *62*, 785-792.

(26) Kandil, A.; Chan, N.; Klein, M.; Chong, P. *Glycoconjugate J.* **1997**, *14*, 13-17.

(27) Barington, T.; Skettrup, M.; Juul, L.; Heilmann, C. *Infect. Immunol.* **1993**, *61*, 432-438.

(28) Barington, T. A.; Gyhrs, K.; Kristensen, K.; Heilman, C. *Infect. Immun.* **1994**, *62*, 9-14.

(29) Peeters, C. C. A. M.; Tenbergen-Meekes, A.-M.; Poolman, J. T.; Beurret, M.; Zegers, B. J. M.; Rijkers, G. T. *Infect. immun.* **1991**, *59*, 3504-3510.

(30) Schutze, M.-P.; Leclerc, C.; Jolivet, M.; XXXX, X.; Chedid, L. *J. Immunol.* **1985**, *135*, 2319-XXX.

(31) Paradiso, P. R.; Dermody, K.; Pillai, S. *Vaccine Res.* **1993**, *2*, 239-248.

(32) Falugi, F.; Petracca, R.; Mariani, M.; Luzzi, E.; Mancianti, S.; Carinci, V.; Melli, M. L.; Finco, O.; Wack, A.; Tommasco, A. D.; Magistris, M. T. d.; Costantino, P.; Giudice, G. d.; Abrignani, S.; Rappuoli, R.; Grandi, G. *Eur. J. Immunol.* **2001**, *31*, 3816-3824.

(33) Valmori, D.; Pessi, A.; Bianchi, E.; Corradin, G. P. *J. Immunol.* **1992**, *149*, 717-721.

(34) Alexander, J.; Sidney, J.; Southwood, S.; Ruppert, J.; Oseroff, C.; Maewal, A.; Snoke, K.; Serra, H. M.; Kubo, R. T.; Sette, A.; Grey, H. M. *Immunity* **1994**, *1*, 751-761.

(35) Alexander, J.; Guercio, A.-F. d.; Maewal, A.; Qiao, L.; Fikes, J.; Chesnut, R. W.; Paulson, J.; Bundle, D. R.; DeFrees, S.; Sette, A. *J. Immunol.* **2000**, *164*, 1625-1633.

(36) Simmons, D. A. R. *Bacteriol. Reviews* **1971**, *35*, 117-148.

(37) Lindberg, A. A.; Karnell, A.; Weintraub, A. *Rev. Infect. Dis.* **1991**, *13*, S279-S284.

(38) Berry, J. M.; Dutton, G. G. S. *Can. J. Chem.* **1974**, *54*, 681-683.

(39) Lipkind, G. M.; Shashkov, A. S.; Nikolaev, A. V.; Mamyan, S. S.; Kochetkov, N. K. *Bioorg. Khim.* **1987**, *13*, 1081-1092.

(40) Mulard, L. A.; Costachel, C.; Sansonetti, P. J. *J. Carbohydr. Chem.* **2000**, *19*, 849-877.

(41) Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150.

(42) Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222.

(43) Mulard, L.; Guerreiro, C.; Costachel, C.; Phalipon, A. *in preparation* **2003**.

(44) Bélot, F.; Costachel, C.; Wright, K.; Phalipon, A.; Mulard, L. A. *Tetrahedron. Lett.* **2002**, *43*, 8215-8218.

(45) Bélot, F.; Wright, K.; Costachel, C.; Phalipon, A.; Mulard, L. A. *J. Org. Chem.* **2003**, *sumitted*.

(46) Mulard, L. A.; Nato, F.; Marcel, V.; Thuizat, A.; Sansonetti, P.; Phalipon, A. *in preparation* **2003**.

(47) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123.

(48) Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343-1357.

(49) Schmidt, R. R.; Michel, J. *Tetrahedron Lett.* **1984**, *25*, 821-824.

(50) Gigg, R.; Payne, S.; Conant, R. *J. Carbohydr. Chem.* **1983**, *2*, 207-223.

(51) Eklind, T.; Gustafsson, R.; Tidén, A.-K.; Norberg, T.; Åberg, P.-M. *J. Carbohydr. Chem.* **1996**, *15*, 1161-???

(52) Chernyak, A.; Sharma, G.; Konokov, L.; Krishna, P.; Levinsky, A.; Kochetkov, N.; Rao, A. R. *Carbohydr. Res.* **1992**, *223*, 303-309.

(53) Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4063.

(54) Hasegawa, A.; Terada, T.; Ogawa, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 319-???

(55) Stahl, W.; Sprengard, U.; Kretschmar, G.; Kunz, H. *Angew. Chem. Int. Ed.* **1994**, *33*, 2096-2098.

(56) Spikjer, N. M.; Keuning, C. A.; Hooglugt, M. *Tetrahedron* **1996**, *52*, 5945-5960.

(57) Li, Q.; Li, H.; Lou, Q.-H.; Su, B.; Cai, M.-S.; Li, Z.-J. *Carbohydr. Res.* **2002**, *337*, 1929-1934.

(58) Kitagawa, I.; Baek, N. I.; Ohashi, K.; Sakagami, M.; Yoshikawa, M.; Shibuya, H. *Chem. Pharm. Bull.* **1989**, *37*, 1131-1133.

(59) Oltvoort, J. J.; Boeckel, C. A. A. v.; Koning, J. H. d.; Boom, J. v. *Synthesis* **1981**, 305-308.

(60) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903-1911.

(61) Westerduin, P.; Haan, P. E. d.; Dees, M. J.; Boom, J. H. v. *Carbohydr. Res.* **1988**, *180*, 195-205.

(62) Ziegler, T.; Bien, F.; Jurish, C. *Tetrahedron: Asymmetry* **1998**, *9*, 765-780.

(63) Reimer, K. B.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1992**, *228*, 399-414.

(64) Zhang, J.; Mao, J. M.; Chen, H. M.; Cai, M. S. *Tetrahedron: Asymmetry* **1994**, *5*, 2283-2290.

(65) Marrian, D. H. *J. Chem. Soc. C* **1949**, 1515.

(66) Hermanson, G. T. *Bioconjugate techniques*; Academic Press: New York, 1996.

(67) Ragupathi, G.; Koganty, R. R.; Qiu, D.; Llyod, K. O.; Livingston, P. O. *Glycoconjugate J.* **1998**, *15*, 217-221.

(68) Shin, I.; Jung, H.; Lee, M. *Tetrahedron Lett.* **2001**, *42*, 1325-1328.

(69) Peeters, J. M.; Hazendonk, T. G.; Beuvery, E. C.; Tesser, G. I. *J. Immunol. Methods* **1989**, *120*, 133-143.

(70) Chan, W. C.; White, P. D. *Fmoc solid phase peptide synthesis*; Oxford University Press: New York, 2000.

(71) Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1998**, *39*, 1603-1606.

(72) Brugghe, H. F. *Int. J. Peptide Protein Res.* **1994**, *43*, 166-172.

LMPP12exp-brevet-gp

General Methods. General experimental methods not referred to in this section were as described previously.(REF) TLC on precoated slides of Silica Gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, dichloromethane-methanol; *B*, cyclohexane-ethyl acetate, *C*, cyclohexane-diethyl ether, *D*, toluene-acetone. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in 4N aq H_2SO_4. NMR Spectra were measured in $CDCl_3$ unless stated otherwise. In the NMR spectra, of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. Interchangeable assignments in the ^{13}C NMR spectra are marked with an asterisk in listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the O-SP and identified by a subscript in listing of signal assignments. Low-resolution mass spectra were obtained by either chemical ionisation (CIMS) using NH_3 as the ionising gas, by electrospray mass spectrometry (ESMS), or by fast atom bombardment mass spectrometry (FABMS). High-resolution mass spectra were obtained by MALDI-MS.

Solid phase peptide synthesis was performed using standard Fmoc chemistry protocols on a Pioneer peptide synthesiser (AppliedBiosystem). Fmoc-Lys(iv-Dde)-OH, Fmoc-Cha-OH, Fmoc-D-Ala-OH, Fmoc-eAhx-OH and Boc-D-Ala-OH were purchased from NovaBiochem (VWR). All others reagents and amino acids were purchased from Applied Biosystem.

2-Azidoethyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (7). Camphorsulfonic acid (200 mg, 0.9 mmol) was added to a solution of triol **14** (1.31 g, 4.52 mmol) in a mixture of DMF (4 mL) and 2,2-dimethoxypropane (4 mL). After 3 h at rt, low boiling point solvents were evaporated under reduced pressure and more 2,2-dimethoxypropane (2 mL, 15.8 mmol) was added. The mixture was stirred for 2h at rt, Et_3N was added, and the mixture was concentrated. The crude product was purified by column chromatography (solvent A, 19:1) to give **7** as a white solid (1.21 g, 81%), $[\alpha]_D$ −89.8; ^1H NMR: δ 6.15 (d, 1H, J = 5.9 Hz, NH), 4.70 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.05 (m, 1H, OCH_2), 3.97-3.89 (m, 2H, H-6a, 3), 3.79 (pt, 1H, $J_{5,6b}$ = $J_{6a,6b}$ = 10.5 Hz, H-6b), 3.70 (m, 1H, OCH_2), 3.62-3.46 (m, 3H, H-2, 4, OCH_2), 3.35-3.26 (m, 2H, H-5, CH_2N_3), 2.05 (s, 3H, Ac), 1.52 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$); ^{13}C NMR: δ 100.9 (C-1), 74.3 (C-4), 81.8 (C-3), 68.6 (OCH_2), 67.3 (C-5), 62.0 (C-6), 58.7 (C-2), 50.7 (CH_2N_3), 29.0 ($C(CH_3)_2$), 23.6 (CH_3CO), 19.1 ($C(CH_3)_2$). CIMS for $C_{13}H_{22}N_4O_6$ (330) *m/z* 331 $[M+H]^+$. Anal. Calcd. for $C_{67}H_{74}N_4O_{17}\cdot 0.5H_2O$: C, 46.01; H, 6.83; N, 16.51. Found C, 46.37; H, 6.69; N, 16.46.

2-Azidoethyl

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyra

nosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (15). (a) The disaccharide donor **4** (1.425 g, 1.37 mmol) and the acceptor **7** (377 mg, 1.14 mmol) with 4Å-MS (2 g) were placed under argon and CH_2Cl_2 (15 mL) was added. The mixture was stirred for 1 h at rt, then cooled to –40°C. A solution of $BF_3.OEt_2$ (0.5 mL, 4.11 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The mixture was stirred at -40°C to –15°C over 3 h. Triethylamine (2.5 mL) was added and the mixture stirred for 20 min. The mixture was filtered through a pad of Celite, and the filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 2:3) to give **15** (803 mg, 58%) as a colourless foam. Further elution (solvent B, 9:1) gave **16** (395 mg, 30%) as a colourless foam.

(b) 4 Å Molecular sieves (560 mg) were added to a solution of donor **4** (565 mg, 0.54 mmol) and acceptor **7** (150 mg, 0.45 mmol) in DCM (3 mL) and the suspension was stirred for 15 min –40°C. Triflic acid (16 μL) was added and the mixture was stirred for 3h at rt once the cooling bath had reached rt. Et_3N was added and after 15 min, the mixture was filtered through a pad of Celite. Volatiles were evaporated and the residue was column chromatographed (solvent B, 9:1) to give **15** (475 mg, 87%). $[\alpha]_D$ +87.7 (*c* 0.32); ^1H NMR: δ 6.99-8.07 (m, 30H, Ph), 6.21 (d, 1H, NH), 5.58 (dd, 1H, H-3_C), 5.44 (m, 1H, H-2_C), 5.13 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.02 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 4.97 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_C), 4.64-4.90 (m, 5H, CH_2Ph), 4.45 (t, 1H, H-3_D), 4.27 (m, 3H, H-5_C, CH_2Ph), 3.79-4.05 (m, 7H, H-3_E, 4_C, 5_D, $6a_D$, $6b_D$, CH_2O, CH_2Ph), 3.60-3.76 (m, 4H, H-4_D, 4_E, 5_E, CH_2O), 3.37-3.51 (m, 3H, H-2_E, 5_D, CH_2N_3), 3.16-3.34 (m, 3H, H-2_D, $6a_E$, CH_2N_3), 3.04 (d, 1H, H-$6b_E$), 2.01 (s, 3H, CH_3C=O), 1.43 (s, 6H, $(CH_3)_2C$), 1.36 (d, 3H, H-6_C); ^{13}C NMR: δ 171.7, 165.6, 163.4 (C=O), 127.3-138.6 (Ph), 99.6 (C-1_D), 99.1 (C-1_E), 97.7 (C-1_C), 91.9 ($(CH_3)_2C$), 81.4 (C-3_E), 80.3 (C-2_E), 79.4 (C-4_C), 77.1 (C-4_D), 76.0 (C-3_D), 75.3, 74.6, 73.9, 73.2 (4C, CH_2Ph), 73.1 (C-4_E), 71.2 (2C, C-2_C, 3_C), 71.1 (C-5_E), 68.6 (CH_2O), 67.5 (C-5_C), 67.4 (C-6_E), 67.1 (C-5_D), 62.1 (C-6_D), 59.0 (C-2_D), 50.5 (CH_2N_3), 28.9 ($(CH_3)_2C$), 23.4 (CH_3CO), 19.2 ($(CH_3)_2C$), 18.1 (C-6_C). FAB-MS for $C_{67}H_{74}N_4O_{17}$ (1206) *m/z* 1229 $[M+Na]^+$.

Anal. Calcd. for $C_{67}H_{74}N_4O_{17}$: C, 60.41; H, 5.66; N, 4.82. Found: C, 60.36; H, 5.69; N, 4.78.

2-Azidoethyl

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (16). Compound **15** (95 mg, 79 μmol) was dissolved in 80% aq AcOH (2.5 mL), and the mixture was heated at 60°C for 1 h. After cooling to rt and repeated co-evaporation with toluene, the crude residue was column chromatographed (solvent B, 1:4 → 0:1) to give **16** (80 mg, 87%) as a white foam. $[\alpha]_D$ +91.5

(*c* 0.18); ^{1}H NMR: δ 6.99-8.02 (m, 30H, Ph), 6.10 (d, 1H, NH), 5.60 (dd, 1H, H-3$_C$), 5.52 (m, 1H, H-2$_C$), 5.20 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1$_D$), 5.00 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1$_C$), 4.95 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1$_E$), 4.63-4.89 (m, 5H, 5 CH_2Ph), 4.47 (pt, 1H, H-3$_D$), 4.25 (d, 1H, CH_2Ph), 4.19 (m, 2H, H-5$_C$, CH_2Ph), 4.06 (m, 1H, CH_2O), 3.87 (m, 5H, H-3$_E$, 4$_C$, 6a$_D$, 6b$_D$, CH_2Ph), 3.58-3.74 (m, 4H, H-4$_E$, 5$_D$, 5$_E$, CH_2O), 3.50 (m, 3H, H-2$_E$, 4$_D$, CH_2N_3), 3.29 (m, 2H, H-6a$_E$, CH_2N_3), 3.04 (d, 2H, H-2$_D$, 6b$_E$), 2.02 (s, 3H, CH_3CO), 1.51 (d, 3H, H-6$_C$); ^{13}C NMR: δ 171.5, 165.6, 165.2 (3C, C=O), 127.3-138.6 (Ph), 99.6 (C-1$_C$), 99.5 (C-1$_E$), 99.0 (C-1$_D$), 83.4 (C-3$_D$), 81.6 (C-3$_E$), 80.1 (C-2$_E$), 79.2 (C-4$_C$), 77.2 (C-4$_E$), 75.5 (CH_2Ph), 75.1 (C-4$_D$), 74.7, 74.0, 73.2 (3C, CH_2Ph), 71.3 (C-5$_D$*), 70.9 (C-5$_E$*), 70.8 (C-3$_C$), 70.4 (C-2$_C$), 69.0 (C-5$_C$), 68.8 (CH_2O), 67.5 (C-6$_E$), 62.6 (C-6$_D$), 57.9 (C-2$_D$), 50.5 (CH_2N_3), 23.4 (*C*H_3CO), 18.2 (C-6$_C$). FAB-MS for $C_{64}H_{70}N_4O_{17}$ (1166) *m/z* 1185 $[M+Na]^+$.

Anal. Calcd. for $C_{64}H_{70}N_4O_{17}\cdot H_2O$: C, 64.85; H, 6.12; N, 4.73. Found: C, 64.71; H, 6.01; N, 4.83.

2-Azidoethyl

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (17). An ice cold solution of 95% aq TFA (1.5 mL) in CH_2Cl_2 (13.5 mL) was added to the trisaccharide **15** (730 mg, 0.60 mmol). The mixture was kept at 0°C for 15 min, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (20 mL), and a 1M solution of sodium methoxide in MeOH (1.5 mL) was added. The mixture was left to stand at rt for 3 h. The mixture was neutralised with Amberlite IR-120 (H^+) resin and filtered. The filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 9:1) to give **17** (548 mg, 94%) as a colourless foam. $[\alpha]_D$ +9.7 (*c* 0.48, MeOH); ^{1}H NMR: δ 7.13-7.31 (m, 8H, Ph), 5.99 (d, 1H, NH), 4.79-4.97 (m, 7H, H-1$_C$, 1$_D$, 1$_E$, CH_2Ph), 4.35-4.74 (m, 4H, CH_2Ph), 3.91-4.10 (m, 7H, H-2$_C$, 3$_D$, 3$_E$, 5$_C$, 5$_E$, 6a$_D$, CH_2O), 3.80 (m, 2H, H-3$_E$, 6b$_D$), 3.73 (m, 1H, CH_2O), 3.40-3.63 (m, 8H, H-2$_E$, 4$_C$, 4$_D$, 4$_E$, 5$_D$, 6a$_E$, 6b$_E$, CH_2N_3), 3.27 (m, 2H, H-2$_D$, CH_2N_3), 1.99 (s, 3H, CH_3CO), 1.41 (d, 3H, H-6$_C$); ^{13}C NMR: δ 170.7 (C=O), 127.6-138.4 (Ph), 101.2 (C-1$_C$), 99.7 (C-1$_E$), 99.0 (C-1$_D$), 84.7 (C-4$_C$), 84.3 (C-3$_D$), 81.5 (C-3$_E$), 79.6 (C-2$_E$), 77.6 (C-4$_D$*), 75.6 (CH_2Ph), 75.3 (C-4$_E$*), 74.9, 73.5, 73.4 (3C, CH_2Ph), 71.2 (C-5$_E$), 70.8 (C-5$_C$), 70.8 (C-5$_D$), 69.4 (C-3$_C$), 68.6 (C-6$_E$), 68.4 (CH_2O), 67.6 (C-2$_C$), 62.6 (C-6$_D$), 56.4 (C-2$_D$), 50.5 (CH_2N_3), 23.5 (*C*H_3CO), 17.6 (C-6$_C$). FAB-MS for $C_{50}H_{62}N_4O_{15}$ (958) *m/z* 981 $[M+Na]^+$.

Anal. Calcd. for $C_{50}H_{62}N_4O_{15}.H_2O$: C, 61.46; H, 6.60; N, 5.73. Found: C, 61.41; H, 6.61; N, 5.97.

LMPP12exp-brevet-gp

2-Aminoethyl α-D-Glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (18). The trisaccharide **17** (368 mg, 0.38 mmol) was dissolved in a mixture of EtOH (10 mL) and EtOAc (1 mL). A 1N solution of aqueous HCl (0.77 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (400 mg) for 24 h. The mixture was diluted with water and filtered. The filtrate was concentrated, then lyophilised. The residue was dissolved in a solution of $NaHCO_3$ (75 mg) in water (1 mL) and purified by passing first through a column of C_{18} silica (eluting with water), then through a column of Sephadex G_{10} (eluting with water) to give, after lyophilisation, **18** (151 mg, 69%). HPLC (230 nm): Rt 4.09 min (Kromasil 5 µm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^1H NMR (D_2O): δ 5.03 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1_E), 4.84 (bs, 1H, H-1_C), 4.58 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.10 (m, 1H, H-5_C), 3.98 (m, 3H, H-5_E, $6b_D$, CH_2O), 3.79 (m, 6H, H-2_C, 2_D, 3_D, $6a_E$, $6b_E$, CH_2O), 3.68 (pt, 1H, H-3_E), 3.42-3.60 (m, 6H, H-2_E, 3_D, 4_C, 4_D, 4_E, 5_D), 3.03 (m, 2H, CH_2NH_2), 2.06 (s, 3H, CH_3CO), 1.31 (d, 3H, H-6_C); ^{13}C δ 175.2 (C=O), 101.9 (C-1_C), 101.0 (C-1_D), 100.3 (C-1_E), 82.4 (C-3_D), 81.6 (C-4_C), 76.5 (C-2_E), 73.3 (C-3_E), 72.4 (C-5_E), 72.1 (C-4_D), 71.6 (C-2_C), 69.9 (C-4_E), 69.5 (C-3_C), 69.0 (C-5_D), 68.7 (C-5_C), 68.7 (CH_2O), 61.2 (C-6_D), 60.7 (C-6_E), 55.8 (C-2_D), 40.3 (CH_2NH_2), 22.7 (CH_3CO), 17.3 (C-6_C). Electrospray-MS for $C_{22}H_{40}N_2O_{15}$ (572) *m/z* 573 $[M+H]^+$. ***Manque $6a_D$ et 3_C ; $2x3_D$***

HRMS (MALDI) Calcd for $C_{22}H_{40}N_2O_{15}$+Na: 595.2326. Found: XXXXX.

Allyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→ 3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (21). TMSOTf (100 µL) was added to a solution of donor **20** (2.5 g, 5.78 mmol) and acceptor **19** (4.0 g, 4.80 mmol) in Et_2O (40 mL) at –50°C. The mixture was stirred for 2.5 h, at which time the cooling bath had reached rt. Et_3N was added and after 15 min, volatiles were evaporated. Column chromatography (solvent C, 4:1) of the crude product gave the fully protected **21** (4.74 g, 89%) as a white solid. ^1H NMR: δ 8.00-6.90 (m, 25H, Ph), 5.92 (m, 1H, CH=), 5.53 (dd, 1H, H-2_B), 5.40-5.20 (m, 4H, H-1_E, 2_C, CH_2=), 5.18 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = ***10.X*** Hz, H-3_B), 5.10 (d, 1H, H-1_B), 5.00-4.40 (m, 10H, H-4_B, 1_C, OCH_2), 4.30-4.00 (m, 5H, H-3_E, 3_C, 5_E, OCH_2), 4.00-3.50 (m, 7H, H-2_E, 4_E, $6a_E$, $6b_E$, 5_B, 5_C, 4_C), 1.90 (s, 3H, Ac), 1.60 (s, 3H, Ac), 1.22 (s, 3H, Ac), 1.20 (d, 3H, $J_{5,6}$ = **X.X** Hz, H-6_C), 0.80 (d, 3H, $J_{5,6}$ = **X.X** Hz, H-6_B); ^{13}C NMR: δ ***16.89-16.55***, 166.1 (4C, C=O), 133.4-127.3 (Ph), 117.5 (=CH_2), 9.8 (C-1_B), 96.9 (C-1_E), 95.7 (C-1_C), 81.4 (C-3_E), 80.7 (C-2_E), 7.3 (C-3_C), 77.7 (C-4_E), 77.5 (C-4_C), ***75.6-72.6*** (4C, OCH_2Ph), 72.7 (C-2_C), **7.9** (2C, C-5_E, 4_B), **6.0** (C-2_B), 68.7

($C\text{-}6_E$), 68.6 ($C\text{-}3_B$), 68.2 (OCH_2), 67.2 ($C\text{-}5_C$), 66.8 ($C\text{-}5_B$), ***20.7-20.2*** (3C, $C(O)CH_3$), 18.5 ($C\text{-}6_C$), 16.8 ($C\text{-}6_B$). CI-MS for $C_{62}H_{70}O_{18}$ (1102) *m/z* 1125 $[M+Na]^+$.
Anal. Calcd. for $C_{62}H_{70}O_{18}$: C, 67.50; H, 6.40. Found: C, 67.51; H, 6.52.

(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→ 3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)]-2-*O*-benzoyl-α-L-rhamnopyranose (22).
1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (33 mg) was dissolved in THF (10 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, until the colour had changed to yellow. The solution was then degassed again in an argon stream. A solution of **21** (4.59 g, 4.16 mmol) in THF (30 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in a mixture of acetone (10:1, 44 mL). Mercuric bromide (1.78 g, 8.32 mmol) and mercuric oxide (1.69 g, 6.24 mmol) were added to the mixture, which was protected from light. The suspension was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat aq KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 3:1) to give **22** (3.52 g, 80%) as a colourless foam; $[\alpha]_D$ +17.7; ^1H NMR: δ 7.15 (m, 25H, Ph), 5.50 (dd, 1H, $H\text{-}2_B$), 5.30-5.27 (m, 2H, $H\text{-}1_C$, $H\text{-}2_C$), 5,23 (d, 1H, $J_{1,2}$ = 3.3 Hz, $H\text{-}1_E$), 5.18 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.0 Hz, $H\text{-}3_B$), 5.10 (d, 1H, $J_{1,2}$ = 1.2 Hz, $H\text{-}1_B$), 5.00-4.35 (m, 9H, $H\text{-}4_B$, OCH_2), 4.28 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 8.6 Hz, $H\text{-}3_C$), 4.20-4.00 (m, 3H, $H\text{-}3_E$, 5_E, 5_C), 3.80-3.50 (m, 6H, $H\text{-}2_E$, $6a_E$, $6b_E$, 5_B, 4_E, 4_C), 3.05 (d, 1H, $J_{OH,1}$ = 4.0 Hz, OH), 2.09, 1.81, 1.44 (3s, 9H, $CH_3C{=}O$), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, $H\text{-}6_C$), 0.95 (d, 3H, $J_{5,6}$ = 6.2 Hz, $H\text{-}6_B$); ^{13}C NMR: δ **169.9-169.6,** 166.2 (4C, C=O), 138.9-127.5 (Ph), 99.8 ($C\text{-}1_B$), 97.3 ($C\text{-}1_E$), 91.3 ($C\text{-}1_C$), 81.7 ($C\text{-}3_E$), 80.7 ($C\text{-}2_E$), 78.8 ($C\text{-}3_C$), 78.1-78.0 (2C, $C\text{-}4_E$, 4_C), 76.6, 75.5 (2C, CH_2Ph), 74.9 (2C, $C\text{-}2_E$, CH_2Ph), 73.8 (CH_2Ph), 73.3 (2C, $C\text{-}4_B$, 5_E), 72.9 ($C\text{-}2_B$), 71.2 (2C, $C\text{-}3_B$, 6_E), 67.5 ($C\text{-}5_C$), 67.1 ($C\text{-}5_B$), **21.0-20.6** (3C, $CH_3C{=}O$), 18.9 ($C\text{-}6_C$), 17.1 ($C\text{-}6_B$).
FAB-MS for $C_{59}H_{66}O_{18}$ (1062) *m/z* 1085 $[M+Na]^+$.
Anal. Calcd. for $C_{59}H_{66}O_{18}\cdot H_2O$: C, 65.54; H, 6.34. Found: C, 65.68; H, 6.41.

(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→ 3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)]-2-*O*-benzoyl-α-L-rhamnopyranose trichloroacetimidate (5). DBU (100 µL) was added at 0°C to a solution of the hemiacetal **22** (3.8 g, 3.58 mmol) in DCM (40 mL) containing trichloroacetonitrile (4 mL). The mixture was stirred for 30 min at 0°C, and volatiles were evaporated. Flash chromatography (solvent B, 7:3 + 0.2% Et_3N) of the crude material gave the donor **5** (3.9 g, 90%) as a white solid; $[\alpha]_D$ +2.8 (c ?);

NMR ???

Anal. Calcd. for $C_{61}H_{66}Cl_3NO_{18}$: C, 60.67; H, 5.51; N, 1.16. Found: C, 60.53; H, 5.48; N, 1.38.

LMPP12exp-brevet-gp

2-Azidoethyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (23). The trisaccharide donor **5** (1.86 g, 1.54 mmol) and the acceptor **7** (712 mg, 2.16 mmol) were dissolved in 1,2-dichloroethane (15 mL) and 4Å-MS (2 g) were added. The mixture was stirred at rt for 1 h. The mixture was cooled to 0°C and triflic acid (34 μL, 0.385 mmol) was added. The mixture was stirred at 0°C for 30 min, then at rt for 30 min. The mixture was then heated at 65°C for 1 h. The mixture was allowed to cool, Et_3N (0.5 mL) was added, and the mixture was stirred at rt for 20 min. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 1:1) to give **23** (1.61 g, 76%). ^{1}H NMR: δ 7.90-6.90 (m, 25H, Ph), 5.92 (d, 1H, J = 7.5 Hz, NH), 5.53 (dd, 1H, $J_{1,2}$ = 1.8 Hz, H-2_B), 5.29 (d, 1H, H-1_E), 5.19 (m, 2H, H-2_C, 3_B), 5.09 (m, 2H, H-1_C, 1_D), 4.97 (bs, 1H, H-1_B), 4.96-4.70 (m, 9H, CH_2Ph, H-4_B), 4.54-4.41 (m, H, CH_2Ph), 4.34 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-3_D), 4.19-3.89 (m, 6H, H-3_C, 5_C, 5_E, 3_E, $6a_D$, OCH_2), 3.79-3.60 (m, 5H, H-$6b_D$, 4_C, 5_B, 2_E, OCH_2), 3.56-3.33 (m, 4H, H-5_D, 4_E, 4_D, CH_2N_3), 3.27-3.12 (m, 2H, CH_2N_3, H-2_D), 2.10, 2.09 (2s, 6H, $C(CH_3)_2$), 1.78 (s, 3H, OAc), 1.73 (s, 3H, NHAc), 1.42, 1.35 (2s, 6H, OAc), 1.30 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 0.90 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B); ^{13}C NMR: δ 171.4, 169.7, 169.6, 169.5, 166.0 (5C, C=O), 138.7-127.2 (Ph), 99.8, 99.7 (C-1_D, 1_C), 97.1 (C-1_B), 96.4 (C-1_E), 81.5 (C-3_E), 81.1 (C-2_E), 79.5 (bs, C-3_C), 77.9 (C-4_D), 77.0 (bs, C-4_C), 75.4 (C-3_D), 75.3, 74.7, 73.6 (3C, CH_2Ph), 73.0, 72.9 (2C, C-2_C, 4_E), 72.9 (CH_2Ph), 71.2 (C-5_E), 71.1 (C-4_B), 69.9 (C-2_B), 69.2 (C-6_E), 68.8 (C-3_B), 68.7 (OCH_2), 67.2, 67.1 (3C, C-5_C, 5_B, 5_D), 62.2 (C-6_D), 59.0 (C-2_D), 50.6 (CH_2N_3), 29.0, 23.4 (2C, $C(CH_3)_2$), 20.9, 20.4 (3C, OAc), 19.0 (NHAc), 18.4 (C-6_C), 17.0 (C-6_B). FAB-MS for $C_{72}H_{86}N_4O_{23}$ (1374) *m/z* 1397 $[M+Na]^+$.

Anal. Calcd. for $C_{72}H_{86}N_4O_{23}$: C, 62.87; H, 6.30; N, 4.07. ***Found: C, ??; H, ??; N, ??.***

2-Azidoethyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (24). 50% aq TFA (1.3 mL) was added to a solution of the fully protected tetrasaccharide **23** (210 mg, 111 μmol) in DCM (6 mL). The mixture was stirred at 0°C for 1 h. Volatiles were evaporated and toluene was co-evaporated from the residue. Column chromatography (solvent B, 7:3 → 1:1) of the crude product gave **24** (195 mg, 95%). $[\alpha]_D$ –6.9 (*c* 0.5, MeOH);

NMR ?

FAB-MS for $C_{69}H_{82}N_4O_{23}$ (1334) *m/z* 1357.5.

Anal. Calcd. for $C_{69}H_{82}N_4O_{23}\cdot H_2O$: C, 60.43; H, 6.32; N, 4.09. Found: C, 60.56; 6.22, 3.92.

LMPP12exp-brevet-gp

2-Aminoethyl α-L-Rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (25). An ice cold solution of 95% aqueous trifluoroacetic acid (2.4 mL) in CH_2Cl_2 (21.6 mL) was added to the tetrasaccharide **23** (1.93 g, 1.40 mmol). The mixture was kept at 0°C for 5 min., then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (65 mL), and a 1M solution of sodium methoxide in MeOH (3 mL) was added. The mixture was left to stand at rt for 18 h, then neutralised with Amberlite IR-120 (H^+) resin, and filtered. The filtrate was concentrated, and the residue was purified by column chromatography (solvent B, 9:1) to give **24** (1.38 g, 89%) as a colourless foam. The tetrasaccharide **24** (1.38 g, 1.25 mmol) was dissolved in a mixture of EtOH (35 mL) and EtOAc (3.5 mL). A 1N solution of aq HCl (2.5 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (1.5 g) for 72 h, then diluted with water and filtered. The filtrate was concentrated, then lyophilised. The residue was dissolved in a solution of 5% aqueous $NaHCO_3$ and purified by passing first through a column of C_{18} silica (eluting with water), then through a column of Sephadex G_{10} (eluting with water) to give, after lyophilisation, **25** (693 mg, 77%). HPLC (230 nm): Rt 4.78 min (Kromasil 5 µm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^{1}H NMR (D_2O): δ 5.10 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 4.89 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_B), 4.73 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_C), 4.50 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_D), 4.08 (m, 1H, H-5_C), 3.96 (m, 1H, H-2_B), 3.91 (m, 2H, H-$6a_D$, CH_2O), 3.68-3.88 (m, 12H, H-2_C, 2_D, 3_B, 3_C, 4_B, 4_C, 5_B, 5_E, $6b_D$, $6a_E$, $6b_E$, CH_2O), 3.59 (pt, 1H, H-3_E), 3.52 (pt, 1H, H-3_D), 3.33-3.48 (m, 4H, H-2_E, 4_D, 4_E, 5_D), 3.01 (m, 2H, CH_2NH_2), 1.99 (s, 3H, CH_3C=O), 1.28 (d, 3H, H-6_C), 1.18 (d, 3H, H-6_B); ^{13}C δ 174.8 (C=O), 103.2 (C-1_B), 101.4 (C-1_C), 100.9 (C-1_D), 98.6 (C-1_E), 81.9 (C-3_D), 79.0 (C-4_B), 76.6 (C-4_C), 76.3 (C-2_E), 72.9 (C-3_E), 72.3 (C-5_E), 72.3 (C-4_D), 71.8 (C-3_C), 71.1 (C-2_C), 70.5 (C-2_B, 3_B), 69.7 (C-4_B), 69.5 (C-4_E), 69.2 (C-5_D), 68.8 (2C, C-5_B, 5_C), 67.9 (CH_2O), 61.0 (C-6_D), 60.8 (C-6_E), 55.5 (C-2_D), 40.0 (CH_2NH_2), 22.6 (CH_3C=O), 18.0 (C-6_C). 17.0 (C-6_B). **XX**MS for $C_{28}H_{50}N_2O_{19}$ (718) *m/z* 741 $[M + Na]^+$. HRMS (MALDI) Calcd for $C_{28}H_{50}N_2O_{19}$: 741.2905. **Found: XXXX.**

Allyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (28). TMSOTf (11 µL, 59 µmol) was added to a solution of the rhamnoside **26** (2.26 g, 5.88

mmol) and the trichloroacetimidate **27** (4.23 g, 6.82 mmol) in anhydrous Et_2O (60 mL) at –70°C. The reaction mixture was stirred for 8 h while the cooling bath was slowly coming back to rt. Et_3N (100 µL) was added, and the mixture was stirred at rt for 15 min. Solvents were evaporated, and the crude material was purified by column chromatography (solvent B, 49:1 → 9:1), to give **28** as a white foam (4.78 g, 96%). ^{1}H NMR: δ 8.17-7.12 (m, 25H, Ph), 5.97-5.85 (m, 3H, H-2_A, 3_A, CH=), 5.67 (pt, 1H, $J_{3,4}$ = 9.6 Hz, H-4_A), 5.34-5.19 (m, 3H, H-1_A, CH_2=), 5.01 (d, 1H, J = 9.0 Hz, CH_2Ph), 4.92 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1_B), 4.82-4.74 (m, 2H, CH_2Ph), 4.71 (d, 1H, J = 11.8 Hz, OCH_2), 4.31 (dq, 1H, $J_{4,5}$ = 9.7 Hz, H-5_A), 4.21 (m, 1H, OCH_2), 4.10 (dd, 1H, H-2_B), 4.02 (m, 1H, OCH_2), 3.97 (dd, 1H, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.2 Hz, H-3_B), 3.82 (dq, 1H, $J_{4,5}$ = 9.4 Hz, H-5_B), 3.71 (pt, 1H, H-4_B), 1.43 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_B), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A); ^{13}C NMR: δ 166.3, 165.9, 165.7 (3C, C=O), 139.0-127.9 (CH=, Ph), 117.8 (CH_2=), 99.9 (C-1_A), 98.3 (C-1_B), 80.6 (C-4_B), 80.2 (C-3_B), 76.5 (C-2_B), 76.0, 72.9 (2C, CH_2Ph), 72.3 (C-4_A), 71.0 (C-2_A*), 70.4 (C-3_A*), 68.7 (C-5_B), 68.1 (OCH_2), 67.5 (C-5_A), 18.4 (C-6_B), 18.1 (C-6_A). FAB-MS for $C_{50}H_{50}O_{12}$ (M = 842.3) *m/z* 865.1 $[M+Na]^+$.

Anal. Calcd. for $C_{50}H_{50}O_{12}$: C, 71.24; H, 5.98. Found C, XX; H, XXX.

(2,3,4-tri-*O*-Benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranose (29). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg) was dissolved in THF (10 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, until the colour had changed to yellow. The solution was then degassed again in an argon stream. A solution of **28** (4.71 g, 5.59 mmol) in THF (40 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in acetone (350 mL) and water (82 mL). Mercuric bromide (3.23 g, 8.96 mmol) and mercuric oxide (2.64 g, 12.3 mmol) were added to the mixture, which was protected from light. The suspension was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat aq KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 3:1) to give **29** (3.87 g, 84%) as a colourless foam. ^{1}H NMR: δ 8.15-7.12 (m, 25H, Ph), 5.94-5.88 (m, 3H, H-2_A, 3_A, CH=), 5.70 (pt, 1H, $J_{3,4}$ = 9.7 Hz, H-4_A), 5.31 (dd, 1H, $J_{1,OH}$ = 3.0 Hz, H-1_B), 5.28 (bs, 1H, H-1_A), 4.98 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.82-4.68 (m, 3H, CH_2Ph), 4.31 (dq, 1H, $J_{4,5}$ = 9.8 Hz, H-5_A), 4.13 (dd, 1H, $J_{1,2}$ = 2.1 Hz, H-2_B), 4.06-3.99 (m, 2H, H-3_B, 5_B), 3.72 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_B), 2.79 (bs, 1H, OH-1_B), 1.41

(d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B), 1.37 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6_A); ^{13}C NMR: δ 166.2, 165.9, 165.7 (3C, C=O), 138.9-127.9 (Ph), 99.7 (C-1_A), 94.2 (C-1_B), 80.5 (C-4_B), 79.6 (C-3_B), 77.6 (C-2_B), 76.5, 72.5 (2C, CH_2Ph), 72.3 (C-4_A), 71.0 (C-2_A*), 70.4 (C-3_A*), 68.8 (C-5_B), 67.6 (C-5_A), 18.5 (C-6_B*), 18.1 (C-6_A*). FAB-MS for $C_{47}H_{46}O_{12}$ (M = 802.3) *m/z* 825.1 $[M+Na]^+$.

Anal. Calcd. for $C_{47}H_{46}O_{12}$: C, 70.31; H, 5.78. Found C, XX; H, XXX.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (30). The hemiacetal **29** (3.77 g, 4.71 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0°C. Trichloroacetonitrile (2.5 mL) was added, then DBU (200 μL). The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent B, 4:1 + 0.1% Et_3N) to give **30** as a white foam (4.29 g, 96%). Some hydrolyzed material **29** (121 mg, 3%) was eluted next. The trichloroacetimidate **30**, isolated as an α/β mixture had ^{1}H NMR (α anomer): δ 8.62 (s, 1H, NH), 8.20-7.18 (m, 25H, Ph), 6.31 (s, 1H, H-1_B), 5.94 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2_A), 5.89 (dd, 1H, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.9 Hz, H-3_A), 5.71 (pt, 1H, H-4_A), 5.27 (bs, 1H, H-1_A), 5.02 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.84 (d, 1H, J = 11.9 Hz, CH_2Ph), 4.79 (d, 1H, CH_2Ph), 4.72 (d, 1H, CH_2Ph), 4.36 (dq, 1H, $J_{4,5}$ = 9.8 Hz, H-5_A), 4.13 (dd, 1H, H-2_B), 4.03-3.97 (m, 2H, H-3_B, 5_B), 3.80 (pt, 1H, $J_{3,4}$ = 9.5 Hz, H-4_B), 1.45 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_B), 1.40 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A); ^{13}C NMR (α anomer): δ 166.2, 165.9, 165.7 (3C, C=O), 160.8 (C=NH), 138.6-128.2 (Ph), 99.9 (C-1_A), 97.2 (C-1_B), 91.4 (CCl_3), 79.9 (C-4_B), 79.1 (C-3_B), 76.2 (CH_2Ph), 74.9 (C-2_B), 73.3 (CH_2Ph), 72.1 (C-4_B), 71.7 (C-5_B), 71.0 (C-2_A), 70.2 (C-3_A), 67.8 (C-5_A), 18.4 (C-6_B), 18.0 (C-6_A).

Anal. Calcd. for $C_{49}H_{46}Cl_3NO_{12}$: C, 62.13; H, 4.89; N, 1.48. Found C, XX; H, XXX, N, X.XX.

Allyl

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (33). (a) The acceptor **16** (465 mg, 0.56 mmol) was dissolved in ether (3 mL). The solution was cooled to –60°C and TMSOTf (65 μL, 0.36 mmol) was added. The donor **30** (690 mg, 0.73 mmol) was dissolved in ether (6 mL) and added to the acceptor solution in two portions with an interval of 30 min. The mixture was stirred at –60°C to –30°C over 2 h. Et_3N

(100 µL) was added. The mixture was concentrated and the residue was purified by column chromatography (solvent B, 7:1) to give **33** (501 mg, 55%).

(b) A solution of the donor **27** (1.41 g, 2.25 mmol) and the acceptor **32** (1.07 g, 1.79 mmol) in anhydrous Et_2O (88 mL) was cooled to –60°C. TMSOTf (63 µL) was added, and the mixture was stirred at –60°C to –20°C over 2.5 h. Et_3N was added (100 µL). The mixture was concentrated and the residue was purified by column chromatography (solvent D, 49:1) to give 33 (2.66 g, 92%); $[\alpha]_D$ +74.1 (*c* 0.5); ^{1}H NMR: δ 7.06-8.11 (m, 50H, Ph), 5.88-6.05 (m, 3H, H-2_A, 3_A, CH =), 5.71 (t, 1H, H-4_A), 5.51 (dd, 1H, H-2_C), 5.22-5.41 (m, 3H, H-1_A, CH_2 =), 5.14 (d, 1H, $J_{1,2}$ = 0.9 Hz, H-1_B), 5.10 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.97 (bs, 1H, H-1_C), 4.35-5.00 (m, 14H, H-2_B, 5_A, 12 x CH_2Ph), 3.98-4.19 (m, 5H, H-3_C, 3_E, 5_E, OCH_2), 3.43-3.87 (m, 9H, H-2_E, 3_B, 4_B, 4_C, 4_E, 5_B, 5_C, 6_E, 6'$_E$), 1.44 (d, 3H, H-6_A), 1.40 (d, 3H, H-6_C), 1.13 (d, 3H, H-6_B); ^{13}C NMR: δ 165.9, 165.4, 165.1 (C=O), 127.1-138.7 (CH=, Ph), 117.8 (CH_2=), 101.3 (C-1_B), 99.6 (C-1_A), 97.9 (C-1_E), 96.1 (C-1_C), 81.9 (C-3_E), 81.0 (C-2_E), 80.1 (C-3_C), 79.8 (C-4_B), 78.9 (C-3_B), 77.9 (C-4_C), 77.4 (C-4_E), 75.9 (C-2_B), 75.6, 75.0, 74.9, 73.9, 72.9 (CH_2Ph), 72.4 (C-2_C), 71.9 (C-4_A), 71.2 (C-5_E), 70.9 (CH_2Ph), 70.7 (C-2_A*), 70.0 (C-3_A*), 69.2 (C-5_B), 68.5 (OCH_2), 68.1 (C-6_E), 67.6 (C-5_C), 67.2 (C-5_A), 18.8 (C-6_A), 18.1 (C-6_C), 17.8 (C-6_B). FAB-MS for $C_{97}H_{98}O_{22}$ (1614) *m/z* 1637 $[M+Na]^+$.

Anal. Calcd. for $C_{97}H_{98}O_{22}$: C, 72.10; H, 6.11. Found: C, 71.75; H, 6.27.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α/β-L-rhamnopyranose (34). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (12.5 mg) was dissolved in THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **33** (1.138 g, 0.70 mmol) in THF (15 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (7 mL) and water (0.7 mL). Mercuric chloride (285 mg, 1.05 mmol) and mercuric oxide (303 mg, 1.4 mmol) were added to the mixture, which was protected from light. The mixture was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 7:3) to give **34** (992 mg, 90%) as a colourless foam. ^{1}H NMR: δ 7.05-8.16 (m, 50H, Ph), 5.88-5.93 (m, 2H, H-2_A, 3_A), 5.73 (pt, 1H, H-4_A),

5.55 (m, 1H, H-2_C), 5.37 (bs, 1H, H-1_A), 5.28 (bs, 1H, H-1_C), 5.14 (bs, 1H, H-1_B), 5.07 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.78-4.99 (m, 6H, CH_2Ph), 4.31-4.68 (m, 8H, H-2_B, 5_A, CH_2Ph), 4.24 (dd, 1H, H-3_C), 3.99-4.09 (m, 3H, H-3_E, 5_C, 5_E), 3.82 (pt, 1H, H-4_C), 3.57-3.76 (m, 5H, H-3_B, 4_E, 5_B, $6a_E$, $6b_E$), 3.48 (dd, 1H, H-2_E), 3.17 (d, 1H, OH), 1.43 (d, 6H, H-6_A, 6_C), 1.14 (d, 3H, H-6_B); ^{13}C NMR: δ 166.0, 165.6, 165.2 (4C, C=O), 127.2-138.9 (Ph), 101.1 (C-1_B), 99.7 (C-1_A), 98.1 (C-1_E), 91.6 (C-1_C), 81.9 (C-3_E), 81.1 (C-2_E), 79.9 (C-4_B), 79.4 (C-3_C), 78.9 (C-3_B), 78.3 (C-4_C), 77.6 (C-4_E), 76.1 (C-2_B), 75.8, 75.3, 75.1, 74.0, 73.1 (**XX**C, CH_2Ph), 72.7 (C-2_C), 72.1 (C-4_A), 71.4 (C-5_E), 71.1 (CH_2Ph), 70.8 (C-2_A*), 70.2 (C-3_A*), 69.4 (C-5_B), 68.3 (C-6_E), 67.7 (C-5_C), 67.3 (C-5_A), 19.0 (C-6_A), 18.2 (C-6_C), 17.9 (C-6_B). FAB-MS for $C_{94}H_{94}O_{22}$ (1574) *m/z* 1597 $[M+Na]^+$.

Anal. Calcd. for $C_{94}H_{94}O_{22}$: C, 71.65; H, 6.01. Found: C, 71.48; H, 6.17.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α/β-L-rhamnopyranosyl trichloroacetimidate (35). The hemiacetal **34** (412 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to 0°C. Trichloroacetonitrile (0.26 mL) was added, then DBU (4 μL). The mixture was stirred at 0°C for 1 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was purified by flash chromatography (solvent B, 4:1 + 0.1% Et_3N) to give **34** (393 mg, 88%). ^1H NMR: δ 8.74 (s, 1H, NH), 7.03-8.10 (m, 50H, Ph), 6.42 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1_C), 5.87 (m, 2H, H-2_A, 3_A), 5.67 (m, 2H, H-2_C, 4_A), 5.30 (bs, 1H, H-1_A), 5.14 (bs, 1H, H-1_B), 5.08 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.74-4.98 (m, 6H, CH_2Ph), 4.23-4.69 (m, 9H, H-2_B, 3_C, 5_A, CH_2Ph), 3.88-4.07 (m, 3H, H-3_E, 5_B, 5_E), 3.57-3.74 (m, 7H, H-2_E, 4_B, 4_C, 4_E, 5_C, $6a_E$, $6b_E$), 3.50 (dd, 1H, H-3_B), 1.38 (d, 6H, H-6_A, 6_B), 1.07 (d, 3H, H-6_C); ^{13}C NMR: δ 165.9, 165.5, 165.4, 165.1 (4C, C=O), 160.1 (C=NH), 127.2-138.7 (Ph), 101.2 (C-1_B), 99.7 (C-1_A), 98.3 (C-1_E), 94.3 (C-1_C), 90.9 (CCl_3), 81.7 (C-3_E), 80.9 (C-2_E), 79.6 (C-3_C, 4_B), 78.5 (C-3_B), 77.2 (C-4_C), 77.5 (C-4_E), 75.9 (C-2_B), 75.6, 75.1, 75.0, 74.0, 72.9 (CH_2Ph), 71.8 (C-2_C), 71.3 (C-4_A), 71.0 (CH_2Ph), 70.7 (C-5_E), 70.5 (C-2_A*), 70.3 (C-3_A*), 70.0 (C-5_B), 69.5 (C-5_C), 67.9 (C-6_E), 67.2 (C-5_A), 18.7 (C-6_A), 17.8 (C-6_C), 17.7 (C-6_B).

Anal. Calcd. for $C_{96}H_{94}Cl_3NO_{22}$: C, 67.03; H, 5.51; N, 0.81. Found: C, 63.14, H, 5.14; N, 1.00.

2-Azidoethyl

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyrano

syl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α-L-rham nopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (35). (a) The tetrasaccharide donor **6** (500 mg, 0.29 mmol) and the acceptor **7** (140 mg, 0.42 mmol) were dissolved in 1,2-dichloroethane (5 mL) and 4Å-MS (400 mg) were added. The mixture was stirred at rt for 2 h. The mixture was cooled to 0°C and triflic acid (7 μL, 0.072 mmol) was added. The mixture was stirred at 0°C to rt over 1 h 30 min. The mixture was then heated at 65°C for 1 h 30 min. The mixture was allowed to cool, Et_3N (0.5 mL) was added, and the mixture was stirred at rt for 20 min. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 4:3) to give **35** (340 mg, 62%).

(b) The tetrasaccharide donor **6** (250 mg, 145 μmol) and the acceptor **7** (67 mg, 204 μmol) were dissolved in DCM (1.5 mL) and 4Å-MS (200 mg) were added. The mixture was stirred at –40°C for 30 min and triflic acid (5 μL) was added. The mixture was stirred at rt over 3 h, triethylamine was added, and the mixture was stirred at rt for 15 min. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 9:1 → 1:1) to give **35** (219 mg, 80%). **$[\alpha]_D$ +29.0 (*c* 0.25, MeOH);** ^{1}H NMR: δ 7.04-8.06 (m, 50H, Ph), 6.24 (d, 1H, NH), 5.90 (m, 2H, H-2_A, 3_A), 5.70 (t, 1H, H-4_A), 5.42 (m, 1H, H-2_C), 5.35 (bs, 1H, H-1_A), 5.13 (m, 3H, H-1_B, 1_D, 1_E), 4.77-5.00 (m, 5H, H-1_C, CH_2Ph), 4.29-4.66 (m, 11H, H-2_B, 3_D, 5_A, CH_2Ph), 3.80-4.11 (m, 6H, H-3_C, 3_E, 5_C, 5_E, $6a_D$, CH_2O), 3.45-3.78 (m, 12H, H-2_E, 3_B, 4_B, 4_C, 4_D, 4_E, 5_B, 5_D, $6b_D$, $6a_E$, $6b_E$, CH_2O), 3.39 (m, 1H, CH_2N_3), 3.23 (m, 2H, H-2_D, CH_2N_3), 2.13 (s, 3H, CH_3CO), 1.43 (d, 9H, H-6_A, $(CH_3)2C$), 1.29 (d, 3H, H-6_C), 1.11 (d, 3H, H-6_B); ^{13}C NMR: δ 171.8, 165.9, 165.5, 165.0, 163.5 (5C, C=O), 127.1-138.7 (Ph), 101.3 (C-1_B), 99.8 (C-1_D), 99.3 (C-1_A), 97.7 (C-1_C), 97.6 (C-1_E), 91.8 ($C(CH_3)_2$), 81.6 (C-3_E), 81.0 (C-2_E), 80.0 (C-3_C), 79.7 (C-4_D), 78.9 (C-4_B), 77.5 (C-3_B, 4_C), 76.4 (C-3_D), 75.6 (C-2_B), 75.5, 74.9, 74.8, 73.8, 73.0 (5C, CH_2Ph), 72.9 (C-4_E), 72.7 (C-2_C), 71.8 (C-4_A), 71.3 (C-5_E), 71.0 (CH_2Ph), 70.6 (C-2_A*), 70.0 (C-3_A*), 69.3 (C-5_B), 68.6 (OCH_2), 68.3 (C-6_E), 67.5 (C-5_C), 67.3 (C-5_A), 67.1 (C-5_D), 62.2 (C-6_D), 58.9 (C-2_D), 50.6 (CH_2N_3), 29.1 (CH_3C), 23.6 (CH_3C=O), 19.2 (CH_3C), 18.6 (C-6_A), 18.0 (C-6_C), 17.6 (C-6_B). FAB-MS for $C_{107}H_{114}N_4O_{27}$ (1886) *m/z* 1909 $[M + Na]^+$.

Anal. Calcd. for $C_{107}H_{114}N_4O_{27}$: C, 68.07, H, 6.09; N, 2.97. Found: contient du CCl3CN

2-Aminoethyl

α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl)-(1→4

)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (37). An ice cold solution of 95% aq TFA (1 mL) in CH_2Cl_2 (9 mL) was added to the pentasaccharide **35** (645 mg, 0.34 mmol). The mixture was kept at 0°C for 10 min, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (20 mL), and a 1M solution of methanolic sodium methoxide (3.5 mL) was added. The mixture was stirred at 50°C for 18 h. The mixture was neutralised with Amberlite IR-120 (H^+) resin and filtered. The filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 9:1) to give **36** (374 mg, 77%) as a colourless foam. The crude pentasaccharide **36** (360 mg, 0.25 mmol) was dissolved in a mixture of EtOH (10 mL) and EtOAc (1 mL). A 1N solution of aq HCl (0.5 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (400 mg) for 18 h. The mixture was diluted with water and filtered. The filtrate was concentrated, then lyophilised. The residue was dissolved in a solution of $NaHCO_3$ (75 mg) in water (1 mL) and purified by passing first through a column of C_{18} silica (eluting with water), then through a column of Sephadex G_{10} (eluting with water) to give, after lyophilisation, **37** (138 mg, 64%). HPLC (230 nm): Rt 5.87 min (Kromasil 5 µm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^1H NMR (D_2O): δ 5.15 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 5.00 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_B), 4.76 (bs, 1H, H-1_C), 4.53 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_D), 4.10 (m, 1H, H-5_C), 4.03 (m, 2H, H-2_A, 2_B), 4.01 (m, 3H, H-4_A, 4_B, CH_2O), 3.83-3.88 (m, 7H, H-2_C, 2_D, 3_A, $6a_D$, $6b_D$, $6a_E$, CH_2O), 3.69-3.76 (m, 7H, H-3_B, 3_C, 3_E, 4_C, 5_A, 5_B, $6b_E$), 3.52 (pt, 1H, H-3_D), 3.33-3.54 (m, 5H, H-2_E, 4_D, 4_E, 5_D, 5_E), 3.09 (m, 2H, CH_2NH_2), 1.98 (s, 3H, CH_3C=O), 1.28 (d, 3H, H-6_C), 1.22 (m, 6H, H-6_A, 6_B); ^{13}C NMR (D_2O): δ 175.3 (C=O), 103.4 (C-1_B), 101.9 (C-1_A), 101.4 (C-1_C, 1_D), 98.4 (C-1_E), 82.3 (C-3_D), 80.2 (C-2_B), 79.9, 76.7 (C-2_E), 72.9, 72.4, 72.4, 72.2, 71.8, 71.6, 70.5, 70.4, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 66.7 **(?????CH_2O)**, 61.0 (2C, C-6_D, 6_E), 55.5 (C-2_D), 39.9 (CH_2NH_2), 22.6 (CH_3C=O), 18.2 (C-6_C), 17.2 (C-6_A), 17.0 (C-6_B). MS for HRMS (MALDI) Calcd for $C_{34}H_{60}N_2O_{23}$+H: 865.3665. Found: 865.3499.

Maleimido activated PADRE Lys (8).

Starting from 0.1 mmol of Fmoc Pal Peg Ps resin, amino acids (0.4 mmol) were incorporated using HATU/DIEA (0.4 mmol) activation. The N-terminal D-Ala was incorporated as Boc-D-Ala-OH. After completion of the chain elongation, the resin was treated three times with hydrazine monohydrate (2% solution in DMF, 25 mL/g of peptide resin) for 3min, which allowed the selective deblocking of the Dde protecting group. To a solution of maleimide

butyric acid (183 mg, 1.0 mmol) in DCM (2 mL) was added DCC (103 mg, 0.5 mmol). After stirring for 10 min, the suspension was filtered, and the filtrate was added to the drained peptide resin. DIEA (17 µL, 0.5 mmol) was added. After 30 min, the peptide resin was washed with DMF (100 mL), MeOH (100 mL), and dried under vacuum. After TFA/TIS/H_2O (95/2.5/2.5) cleavage (10 mL/g of resin, 1.5 h), the crude peptide (157 mg) was dissolved in 16 mL of 15% CH_3CN in 0,08% aq TFA, and purified by reverse phase Medium Pressure Liquid Chromatography (MPLC) on a Nucleoprep 20 µm C18 100 Å column, using a 15-75% linear gradient of CH_3CN in 0,08% aq TFA over 60 min at 25 mL/min flow rate (214 nm detection) to give **8** (107 mg, 61%). HPLC (214 nm): Rt 13.4 min (94% pure, Nucleosil 5 µm C18 300 Å analytical column, using a 15-45% linear gradient over 20 min of CH_3CN in 0,08% aq TFA at 25 mL/min flow rate). Positive ion ESMS Calcd for $C_{85}H_{139}N_{21}O_{19}$: 1759.18. Found: 1758.83 (SD: 0.40).

(*S*-Acetylthiomethyl)carbonylaminoethyl α-D-Glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (38). The trisaccharide **18** (58 mg, 0.1 mmol) was dissolved in DMF (1 mL). SAMA-Pfp (33 mg, 0.11 mmol) was added, and the mixture was left to stand at rt for 40 min. Toluene was added and the mixture was concentrated. Ether was added to the residue. The resulting precipitate was collected and purified by passing through a column of C_{18} silica (solvent D, gradient) to give **38** (36 mg, 53%). HPLC (230 nm): Rt 13.74 min (99% pure, Kromasil 5 µm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^{13}C NMR (D_2O): δ 200.3 (SC=O), 175.2, 171.9 (NC=O), 102.1 (C-1_C), 101.2 (C-1_D), 100.5 (C-1_E), 82.7 (C-3_D), 81.8 (C-4_C), 76.8 (C-2_E), 73.6 (C-3_E), 72.6 (C-5_E), 72.4 (C-4_D), 71.8 (C-2_C), 70.2 (C-4_E), 69.7 (C-3_C), 69.4 (C-5_D), 68.9 (C-5_C), 68.9 (CH_2O), 61.6 (C-6_D), 60.9 (C-6_E), 56.1 (C-2_D), 40.6 (CH_2NH), 33.7 (CH_2S), 30.4 ($CH_3C(O)S$), 23.0 ($CH_3C(O)N$), 17.5 (C-6_C). ES-MS for $C_{26}H_{44}N_2O_{17}S$ (688) *m/z* 689 $[M+H]^+$.

HRMS (MALDI) Calcd for $C_{26}H_{44}N_2O_{17}S$ +Na: 711.2258. Found: XXXXX.

(*S*-Acetylthiomethyl)carbonylaminoethyl α-L-Rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (39). A solution of SAMA-Pfp (16.7 mg, 40 µmol) in acetonitrile (150 µL) was added to the tetrasaccharide **25** (20 mg, 28.8 µmol) in 0.1M phosphate buffer (pH 7.4, 600 µL). The mixture was stirred at rt for 45 min and purified by RP-HPLC to give **39** (17 mg, 74%). HPLC (230 nm): Rt 13.63 min (98% pure, Kromasil 5 µm

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C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^1H NMR (D_2O): δ 5.10 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 4.91 (d, 1H, $J_{1,2}$ = 0.8 Hz, H-1_B), 4.73 (bs, 1H, H-1_C), 4.45 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.09 (m, 1H, H-5_C), 3.97 (m, 1H, H-2_B), 3.87 (m, 4H, H-2_C, 3_C, $6a_D$, CH_2O), 3.62-3.78 (m, 8H, H-2_D, 3_B, 4_C, 5_B, $6b_D$, $6a_E$, $6b_E$, 1 x CH_2O), 3.60 (m, 3H, H-3_E, CH_2S), 3.48 (pt, 1H, H-3_D), 3.39-3.46 (m, 6H, H-2_E, 4_B, 4_D, 4_E, 5_D, 5_E), 3.33 (m, 2H, CH_2NH_2), 2.35 (s, 3H, $CH_3C(O)S$), 1.98 (s, 3H, $CH_3C(O)N$), 1.27 (d, 3H, H-6_C), 1.23 (d, 3H, H-6_B): ^{13}C NMR (D_2O): δ 199.8 (SC=O), 174.5, 171.3 (NC(O)), 103.2 (C-1_B), 101.4 (C-1_C), 100.9 (C-1_D), 98.6 (C-1_E), 82.0 (C-3_D), 79.0 (C-4_B), 76.6 (C-4_C), 76.3 (C-2_E), 72.9 (C-3_E), 72.3 (C-5_E), 72.2 (C-4_D), 71.8 (C-3_C), 71.0 (C-2_C), 70.5 (C-2_B, 3_B), 69.7 (C-4_B), 69.5 (C-4_E), 69.1 (C-5_C, 5_D), 68.8 (C-5_B), 68.7 (CH_2O), 61.1 (C-6_D), 60.7 (C-6_E), 55.5 (C-2_D), 40.1 (CH_2NH), 33.2 (CH_2S), 29.9 ($CH_3C(O)S$), 22.6 ($CH_3C(O)N$), 17.9 (C-6_C), 16.9 (C-6_B). MS for $C_{32}H_{54}N_2O_{21}S$ (834) *m/z* 857 $[M + Na]^+$. HRMS-MALDI Calcd for $C_{32}H_{54}N_2O_{21}S$+Na: 857.2838. Found: 857.2576.

(*S*-Acetylthiomethyl)carbonylaminoethyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl)-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (40). The pentasaccharide **37** (6.4 mg, 7.4 μmol) was dissolved in 0.1M phosphate buffer (pH 7.4, 1.0 mL). SAMA-Pfp (6.6 mg, 22 μmol) was added, and the mixture was stirred at rt for 5 h. More SAMA-Pfp (6.6 mg, 22 μmol) was added and the mixture was stirred for 1 h more at rt. RP-HPLC of the mixture gave **40** (5.4 mg, 75%). HPLC (230 nm): Rt 13.86 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ^1H NMR (D_2O): δ 5.13 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 4.98 (bs, 1H, H-1_A), 4.90 (bs, 1H, H-1_B), 4.74 (bs, 1H, H-1_C), 4.47 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.09 (m, 1H, H-5_C), 4.00 (m, 2H, H-2_A, 2_B), 3.79-3.85 (m, 8H, H-2_C, 2_D, 3_A, 4_A, 4_B, $6a_D$, $6b_D$, CH_2O), 3.65-3.74 (m, 9H, H-3_B, 3_C, 3_E, 4_C, 5_A, 5_B, $6a_E$, $6b_E$, CH_2O), 3.60 (m, 2H, CH_2S), 3.53 (pt, 1H, H-3_D), 3.13-3.49 (m, 7H, H-2_E, 4_D, 4_E, 5_D, 5_E, CH_2NH), 2.35 (s, 3H, CH_3C=OS), 1.99 (s, 3H, CH_3C=ON), 1.28 (d, 3H, H-6_C), 1.20 (m, 6H, H-6_A, 6_B); ^{13}C NMR (D_2O): δ 199.9 (SC=O), 174.5, 171.4 (NC=O), 102.8 (C-1_B), 101.7 (C-1_A), 101.4 (C-1_C), 100.9 (C-1_D), 97.9 (C-1_E), 82.0 (C-3_D), 79.7 (C-2_B), 79.0, 76.3, 72.9, 72.4, 72.2, 71.8, 71.0, 70.5, 69.7, 69.5, 69.1, 68.8, 68.5 (**XXXX, CH_2O**), 61.1 (2C, C-6_D, **6_E**), 60.7 (C-**6_E**), 55.6 (C-2_D), 40.1 (CH_2NH), 33.2 (CH_2S), 29.9 (CH_3C=OS), 22.7 (CH_3C=ON), 18.2 (C-6_C), 17.2 (C-6_A), 17.0 (C-6_B). HRMS (MALDI) Calcd for $C_{38}H_{64}N_2O_{25}S$+Na: 1003.3417. Found: 1003.3426.

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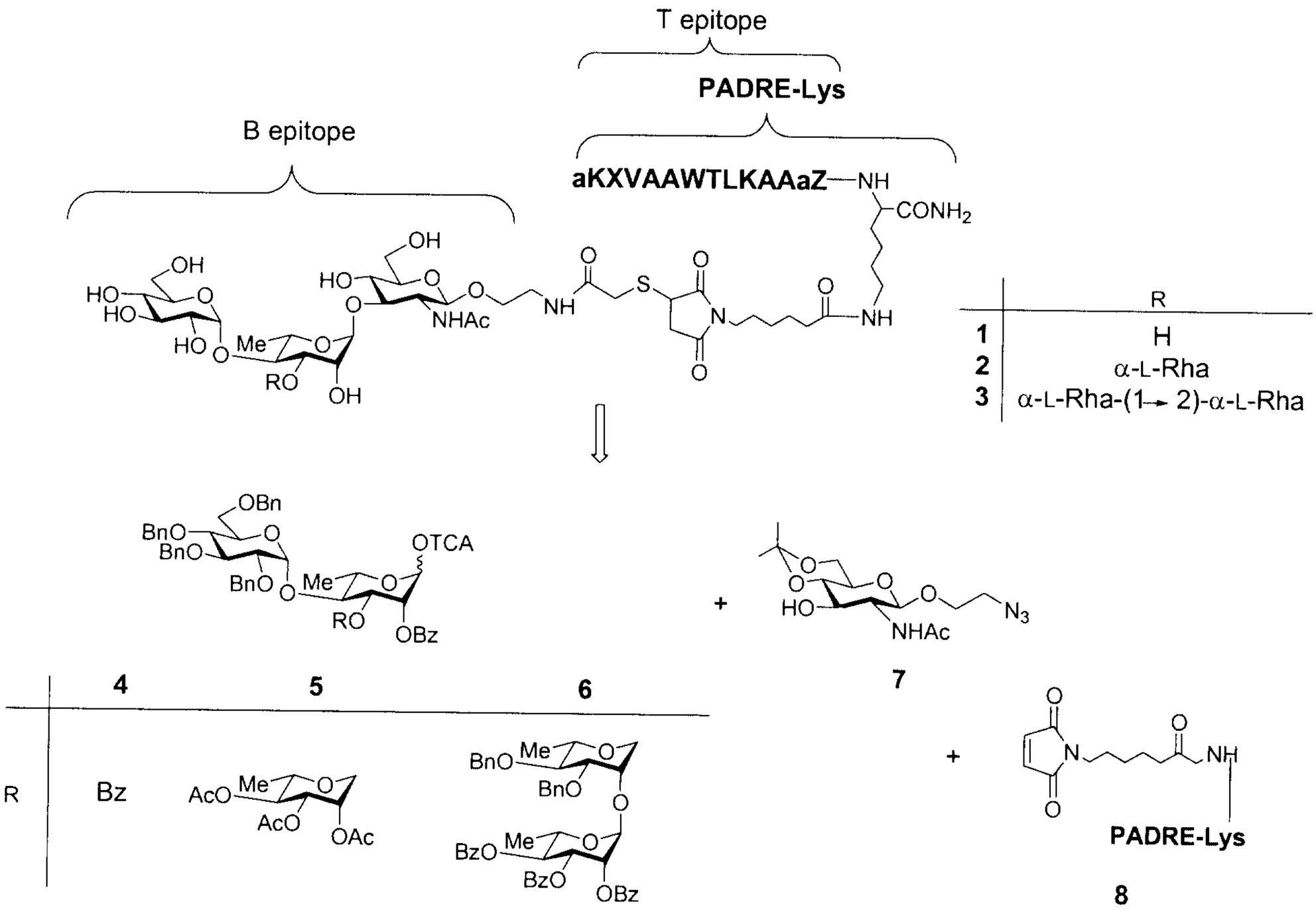
PADRE (thiomethyl)carbonylaminoethyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1). Compound **38** (5.0 mg, 7.3 μmol) was dissolved in water (500 μL) and added to a solution of PADRE-Mal (10 mg, 5.68 μmol) in a mixture of water (900 μL), acetonitrile (100 μL) and 0.1M phosphate buffer (pH 6.0, 1 mL). 117 μL of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.1M phosphate buffer (pH 6.0) was added and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide **1** (8.5 mg, 62%). HPLC (230 nm): Rt 10.40 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ESMS Calcd for $C_{109}H_{181}N_{23}O_{35}S$: 2405.85. Found: 2405.52.

PADRE (thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl)-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2). Compound **39** (4.9 mg, 5.8 μmol) was dissolved in water (500 μL) and added to a solution of PADRE-Mal (13 mg, 7.4 μmol) in a mixture of water (1 mL), acetonitrile (200 μL) and 0.5M phosphate buffer (pH 5.7, 1.2 mL). 117 μL of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5M phosphate buffer (pH 5.7) was added, and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide **2** (6.7 mg, 48%). HPLC (230 nm): Rt 11.60 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 20-50% linear gradient over 20 min of CH_3CN in 0,01M aq TFA at 1 mL/min flow rate). ESMS Calcd for $C_{125}H_{191}N_{23}O_{39}S$: 2552. Found: 2551.90.

PADRE (thiomethyl)carbonylaminoethyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl)-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (3). Compound **40** (5.59 mg, 5.7 μmol) was dissolved in water (500 μL) and added to a solution of PADRE-Mal (12.6 mg, 7.2 μmol) in a mixture of water (1 mL), acetonitrile (200 μL), which had been previously diluted with 0.5M phosphate buffer (pH 5.7, 1.2 mL). A solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5M phosphate buffer (pH 5.7, 117 μL) was added and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide **3** (7.1 mg, 46%). HPLC (230 nm): Rt 10.33 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250

mm analytical column, using a 20-50% linear gradient over 20 min of CH_3CN in 0.01M aq TFA at 1 mL/min flow rate). ESMS Calcd for $C_{121}H_{201}N_{23}O_{43}S$: 2698. Found: 2698.09.

LMPP12-schema-brevet-gp



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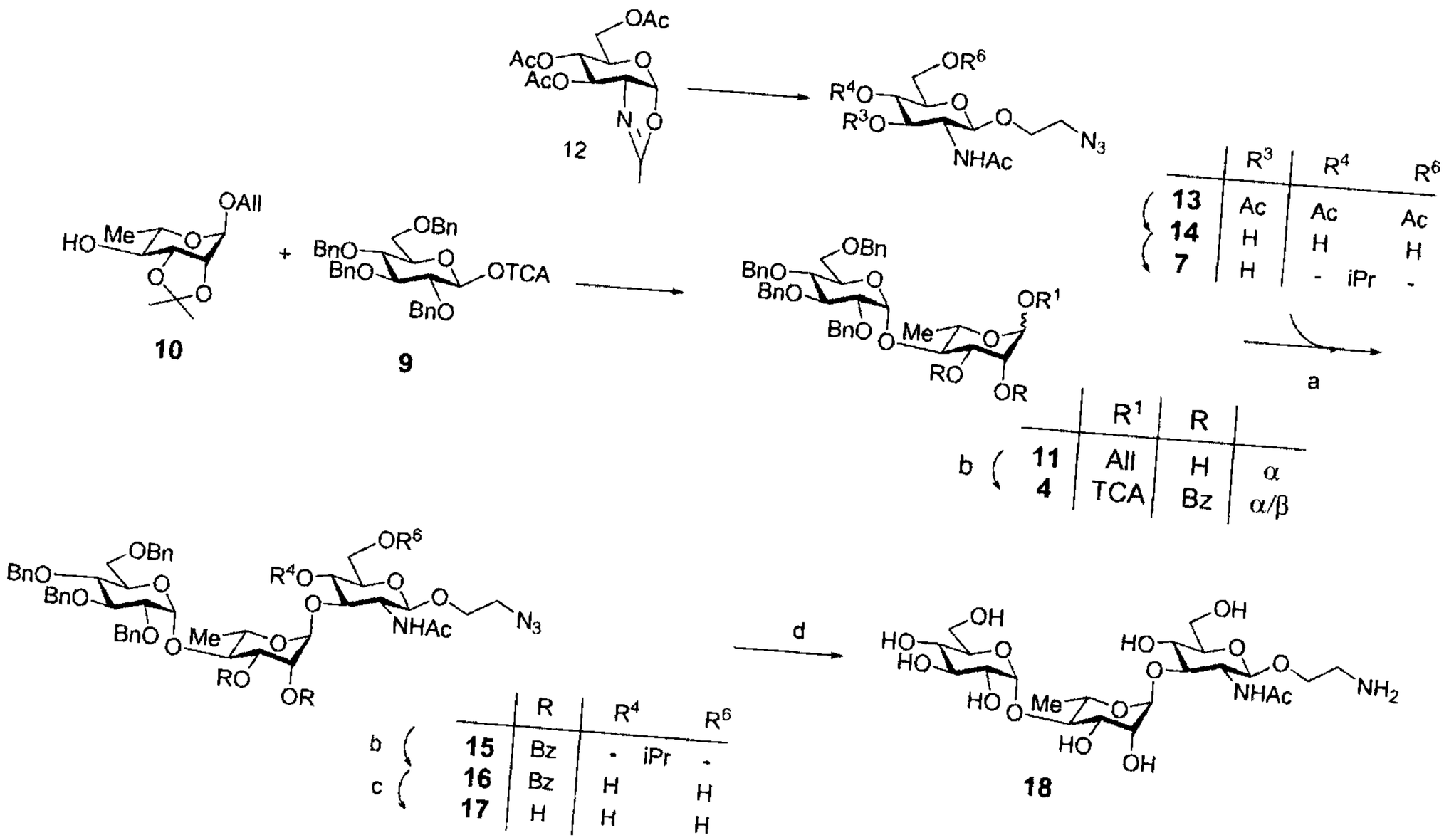

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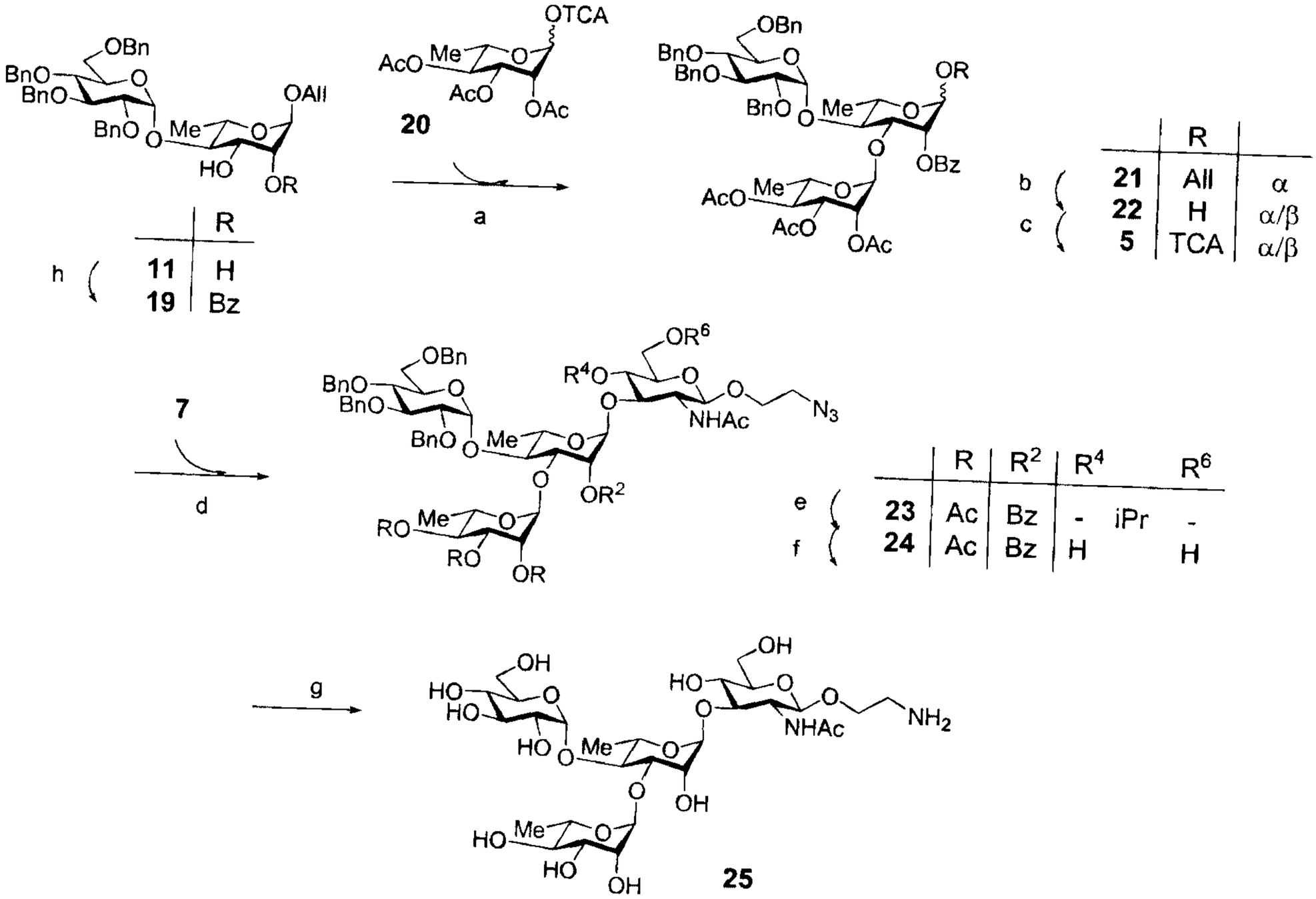

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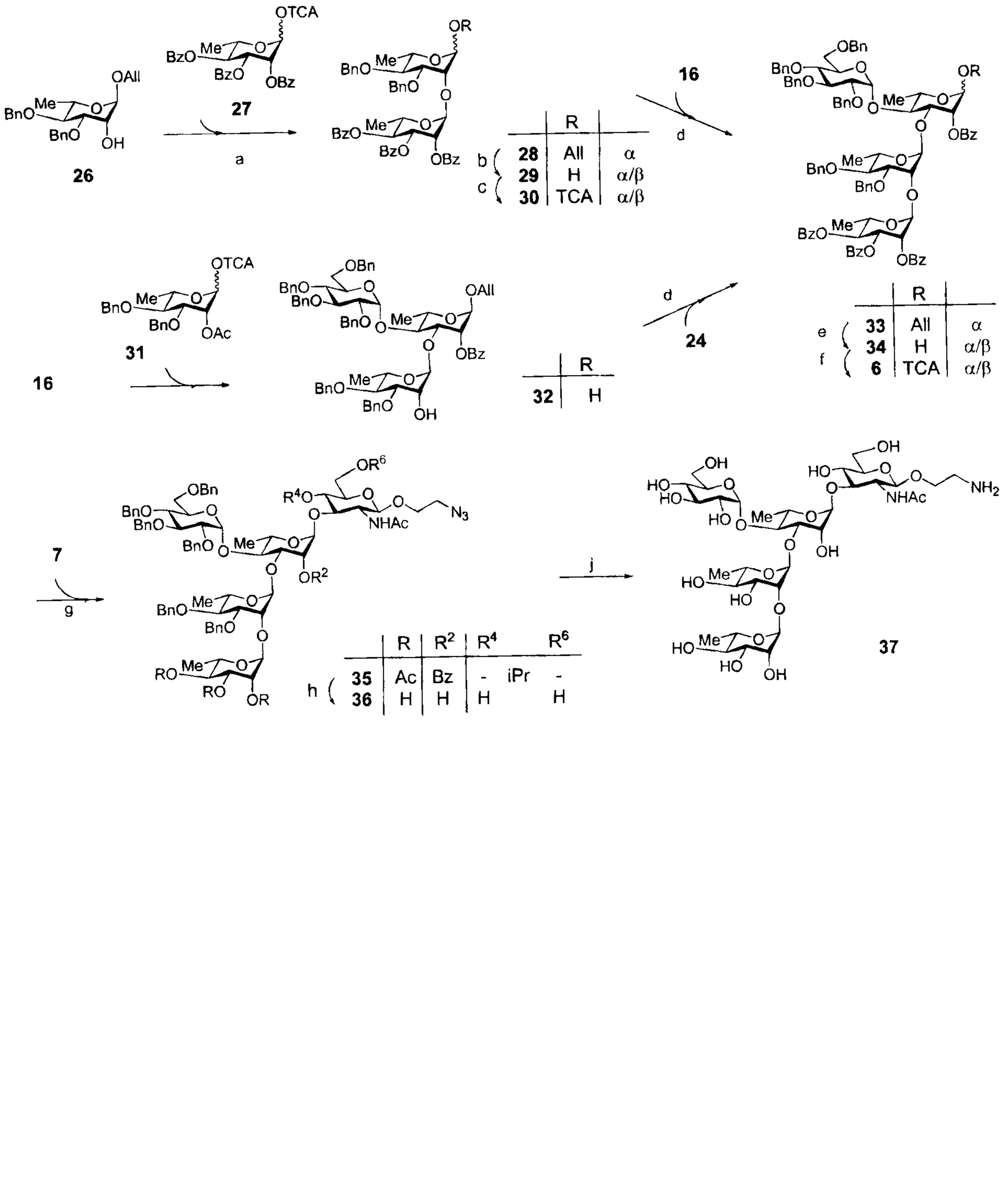

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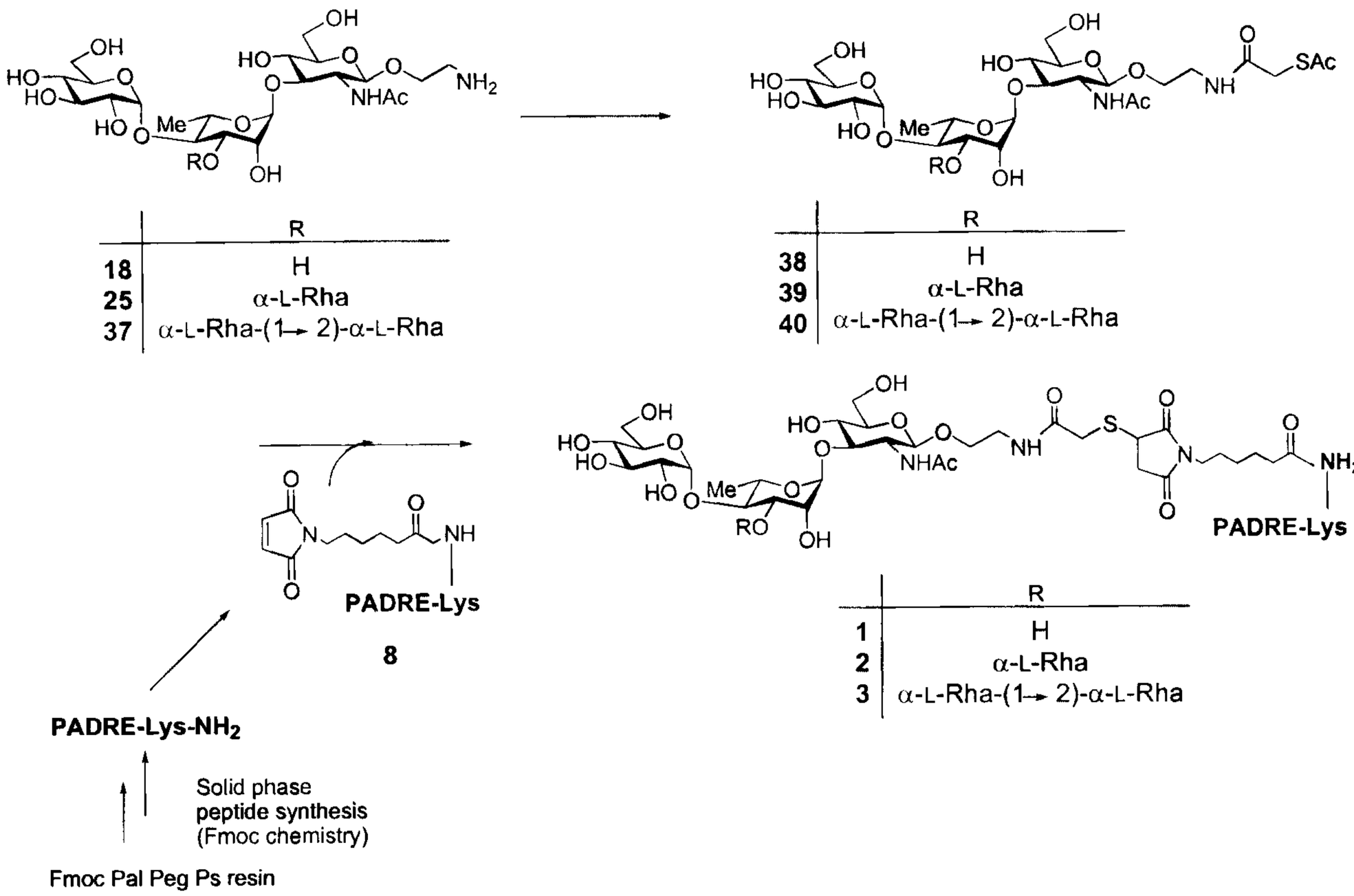

LMPP13-theo-brevet-pentablock

Synthesis of a pentasaccharide building block of the O-specific polysaccharide of *Shigella flexneri* serotype 2a[1]

Abstract

INTRODUCTION

Shigellosis or bacillary dysentery is a serious infectious disease, responsible for some 200 million episodes annually, mostly in children and immunocompromised individuals living in areas were sanitary conditions are insufficient. [2] Of the four species of *Shigellae*, *Shigella flexneri* is the major responsible of the endemic form of the disease, with serotype 2a being the most prevalent. Due to increasing resistance of all groups of Shigellae to antibiotics, [3] the development of a vaccine against shigellosis is of high priority as stated by the World Health Organization in its program against enteric diseases. [4] However, there are yet no licensed vaccines for shigellosis.

Shigella's lipopolysaccharide (LPS) is a major surface antigen of the bacterium. The corresponding O-antigen (O-Ag) is both an essential virulence factor and the target of the infected host's protective immune response. [5, 6] Based on the former hypothesis that serum IgG anti-LPS antibodies may confer specific protection against shigellosis, [7] several polysaccharide-proteine conjugates, targeting either *Shigella sonnei*, *Shigella dysenteriae* 1 or *S. flexneri* serotype 2a, were evaluated in humans. [8, 9] In the case of *S. sonnei*, recent field trials allowed Robbins and co-workers to demonstrate the efficacy of a vaccine made of the corresponding detoxified LPS covalently linked to recombinant exoprotein A. [10] Even though efficient, polysaccharide-protein conjugate vaccines remain highly complex structures, whose immunogenicity depends on several parameters amongst which the length and nature

of the saccharide component as well as its loading on the protein. It is reasonably admitted that the standardization of these parameters is somewhat difficult when dealing with polysaccharides purified from bacterial cell cultures. That short oligosaccharides were immunogenic when conjugated onto a protein carrier was demonstrated on several occasions. [11] It may be assumed that the use of well-defined synthetic oligosaccharides would allow a better control, and consequently the optimisation, of the above mentioned parameters. Indeed, available data on *S. dysenteriae* type 1 indicate that neoglycoconjugates incorporating di-, tri- or tetramers of the O-Ag repeating unit were more immunogenic than a detoxified LPS-human serum albumin conjugate of reference. [12] Others have shown that conjugates incorporating oligosaccharides comprising one repeating unit or smaller fragments were immunogenic in mice. [13, 14]

Along this line, we recently prepared three neoglycoproteins as potential semi-synthetic vaccines against *Shigella flexneri* 2a infection. These incorporated short oligosaccharide haptens, representative either of part or of the whole repeating unit of the O-Ag of *S. flexneri* serotype 2a. Preliminary data indicate that two out of the three conjugates are immunogenic in mice.(Phalipon et al, unpublished results) However, parallel studies on the recognition of synthetic fragments of the O-Ag by protective homologous monoclonal antibodies suggested that sequences comprising more than one repeating unit of the O-Ag were more antigenic, thus probably better mimicking the natural polysaccharide. [15] It is anticipated that better mimics of the O-SP would lead to conjugates of higher immunogenicity. Thus, the preparation of oligo- or polysaccharides[16] made of two repeating units or more was considered. We reasoned that it would best rely on the use of a pre-functionalized building block, representative of the repeating unit of the O-Ag, or of a frame-shifted sequence thereof, and susceptible to act either as a donor and potential acceptor, or as an acceptor and potential donor. The synthesis of such a key synthetic intermediate is described in the following, together with its conversion in the form of either a donor or an acceptor.

RESULTS AND DISCUSSION

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

I

LMPP13-theo-brevet-pentablock

The O-SP of *S. flexneri* 2a is a branched heteropolysaccharide defined by the pentasaccharide repeating unit **I**. [17, 18] It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens and comprises a *N*-acetyl glucosamine (**D**) and three rhamnose residues (**A**, **B**, **C**). The specificity of the serotype is associated to the α-D-glucopyranose residue linked to position 4 of rhamnose **C**.

As part of a study of the mapping at the molecular level of the binding of protective monoclonal antibodies to *S. flexneri* 2a O-antigen, a set of of di- to pentasaccharides corresponding to frame-shifted fragments of the repeating unit **I**, [19-22] an octasaccharide[23] and more recently a decasaccharide[24] have been synthesized in this laboratory. The latter, namely **D'A'B'(E')C'DAB(E)C**, was synthesized as its methyl glycoside by condensing a chain terminator pentasaccharide donor and a methyl glycoside pentasaccharide acceptor. In the following, the key intermediate is the **DAB(E)C** pentasaccharide **1**, which is protected in an orthogonal fashion at position O-3_D with an acetyl group and at the reducing end by an allyl group. At this stage, the acetamido function is already present at position 2_D. Compound **1** may be converted to the corresponding alcohol **2**, which acts as an acceptor and a masked donor, or to the trichloroacetimidate **3** which acts as an acceptor allowing subsequent chain elongation at the non-reducing end (Scheme 1). Previous work in the laboratory has shown that in order to construct the **DAB(E)C** sequence, the linear approach involving stepwise elongation at the non-reducing end, was more suitable than the blockwise one.

*D-glucosamine unit(**D**).* In order to limit the number of steps at the pentasaccharide level, we reasoned that an appropriate precursor to residue **D** should have (i) permanent protecting groups at positions 4 and 6, (ii) a participating group at position 2 and (iii) an orthogonal protecting group at position 3, allowing easy cleavage. As they allow a wide range of protecting group manipulations previously to ultimate activation, thioglycosides are highly convenient masked donors. Recently, two sets of non-malodorous thioglycosyl donors have been proposed[25]**Ref**??, among which the thiododecanyl moiety was selected. Thus, the known peracetylated trichloroacetamide **XX**[26] was reacted with dodecanthiol in the presence of $BF_3.OEt_2$ to give thioglycoside **XX** in high yield (97%). Zemplén deacetylation cleanly afforded the corresponding triol **XX**, which was selectively protected at position 4 and 6 upon reaction with 2,2-dimethoxypropane (80% from **XX**). Indeed, previous observations in the series have demonstrated that 4,6-*O*-isopropylidene-D-glucosaminyl derivatives were highly

suitable precursors to residue **D**. [19, 23] Next, conventional acetylation of **XX** gave the required donor thioglycoside **XX**.

*L-Rhamnose units (**A**, **B**):* Previous work in the series was mostly based on the use of the 2-*O*-acetyl trichloroacetimidate rhamnopyranosyl donor **XX**. [20, 24] Condensation yields were excellent. However, the acetyl protecting group not being fully orthogonal to the benzoyl one, the weak point of the strategy resides in the de-*O*-acetylation step which, in fact, is required twice. The levulinate on the contrary is fully orthogonal to either benzyl or allyl ethers, and to benzoates. The 2-*O*-levulinoyl trichloroacetimidate donor **XX** was thus evaluated as an alternative to **XX**. It was prepared from the known allyl rhamnopyranoside **XX**[27] in three steps. Indeed, treatment of **XX** with levulinic acid gave the fully protected **XX** (XX%, *ALG/GL*), deallylation of which proceeded in two steps based on (i) isomerisation of the allyl group into the propen-1-yl ether using an iridium complex, [28] and (2) subsequent oxidative cleavage of the latter to give the hemiacetal **XX** (XX%, *ALG/GL*). [29] Reaction of the latter with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in the required donor **XX** (XX%, *ALG/GL*). One should note that several routes to the known **XX** have been described including opening of the intermediate 2,3-*O*-benzylidene derivative[27]or regioselective benzylation of the corresponding 2,3-diol via the stannylidene intermediate.**(ref?)** Alternatively, **XX** could be prepared from the orthoester **XX**, readily available from acetobromorhamnose **XX** upon reaction with allylic alcohol in the presence of lutidine (XX% from L-rhamnose, *MP et ???*). Deacetylation of **XX** in methanolic ammoniac gave diol **XX**, which was next benzylated into the 1,2-orthoacetate **XX** (XX% from **XX***MP et ???*). Isomerisation of the latter to the corresponding glycoside in the presence of TMSOTf, analogously to that described in the mannose series, [30, 31] gave the fully protected **XX** (XX%, *GL*, *MP*) together with the β-anomer **XX** (XX%, *GL, MP*). Zemplén deacetylation of the former gave **XX** quantitatively. Besides, **XX** is a convenient intermediate to the 2-*O*-acetylated donor **XX**.

*Synthesis of the pentasaccharide **1**:* The known allyl glycoside **XX**, acting as an **EC** acceptor, temporarily protected at the anomeric position and having a participating group at position 2_C, was prepared as described in 63% yield from allyl 2,3-*O*-ispropylidene-α-L-rhamnopyranoside. [21] Its condensation with the trichloroacetimidate donor **XX**, performed in the presence of a catalytic amount of TMSOTf, afforded the fully protected trisaccharide **XX** (XX%, ALG reproduire), and subsequently the known **B(E)C** acceptor **XX**[24] upon selective

removal of the *O*-levulinoyl group with hydrazine hydrate (XX%, ALG reproduire). Starting from **XX**, this two-step process was repeated to give first the fully protected **XX** (XX%), then the known **AB(E)C** acceptor **XX**[24] in XX% yield. According to this strategy, **XX** was obtained in XX% overall yield from the key disaccharide **XX**, which compares favourably with the 62% yield obtained in the previously described strategy involving the 2-*O*-acetylated trichloroacetimidate donor **XX**. [24] Besides, considering that selective deblocking at positions 2_B and 2_A was completed in overnight runs instead of the 5 days required for each corresponding chemoselective *O*-deacetylation steps, the use of the 2-*O*-levulinoyl donor appeared as a suitable alternative to that of **XX**, although its preparation, *may be somewhat lower-yielding (XX% instead of XX% from* **XX**, ALG/GL). Using a mixture of NIS and triflic acid as the promoter, condensation of the tetrasaccharide acceptor **XX** with the thioglycoside donor **XX** gave the key intermediate **XX** in 58% yield. Although alternative conditions in terms of promoters and solvents (not described) were tested, this rather low yield could not be improved. Radical dechlorination of **XX** using Bu_3SnH and a catalytic amount of AIBN readily afforded the corresponding acetamido key intermediate **1** (74%). *(attention schema)* On one hand, compound **1** may be efficiently converted to the acceptor building block **2** under Zemplén conditions. On the other hand, it was smoothly deallylated into the hemiacetal **XX**, following a two-step process as described above. Next, treatment of **XX** with trichloroacetonitrile and DBU allowed its conversion to the building block **3** (82% from **XX**).

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REFERENCES

[1] Almh, *Part 13 of the series Synthesis of ligands related to the O-specific polysaccharides of Shigella flexneri serotype 2a and Shigella flexneri serotype 5a. For part 12, see ref. XX.* **2003**.

[2] K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, M. M. Levine, *Bull. WHO* **1999**, *77*, 651.
[3] S. Ashkenazi, M. May-Zahav, J. Sulkes, Z. Samra, *Antimicrob. Agents Chemother.* **1995**, *39*, 819.
[4] World, Health, Organisation, *WHO Weekly Epidemiol. Rec.* **1997**, *72*, 73.
[5] D. Cohen, M. S. Green, C. Block, T. Rouach, I. Ofek, *J. Infect. Dis.* **1988**, *157*, 1068.
[6] D. Cohen, M. S. Green, C. Block, R. Slepon, I. Ofek, *J. Clin. Microbiol.* **1991**, *29*, 386.
[7] J. B. Robbins, C. Chu, R. Schneerson, *Clin. Infect. Dis.* **1992**, *15*, 346.
[8] D. N. Taylor, A. C. Trofa, J. Sadoff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson, J. B. Robbins, *Infect. Immun.* **1993**, *61*, 3678.
[9] J. H. Passwell, E. Harlev, S. Ashkenazi, C. Chu, D. Miron, R. Ramon, N. Farzan, J. Shiloach, D. A. Bryla, F. Majadly, R. Roberson, J. B. Robbins, R. Schneerson, *Infect. Immun.* **2001**, *69*, 1351.
[10] D. Cohen, S. Ashkenazi, M. S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, I. Ashkenazi, J. Shemer, D. N. Taylor, T. L. Hale, J. C. Sadoff, D. Pavliovka, R. Schneerson, J. B. Robbins, *The Lancet* **1997**, *349*, 155.
[11] V. Pozsgay, in *Adv. Carbohydr. Chem. Biochem., Vol. 56* (Ed.: D. Horton), Academic Press, San Diego, **2000**, pp. 153.
[12] V. Pozsgay, C. Chu, L. Panell, J. Wolfe, J. B. Robbins, R. Schneerson, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194.
[13] B. Benaissa-Trouw, D. J. Lefeber, J. P. Kamerling, J. F. G. Vliegenthart, K. Kraaijeveld, H. Snippe, *Infect. Immun.* **2001**, *69*, 4698.
[14] F. Mawas, J. Niggemann, C. Jones, M. J. Corbet, J. P. Kamerling, J. F. G. Vliegenthart, *Infect. Immun.* **2002**, *70*, 5107.
[15] L. A. Mulard, F. Nato, V. Marcel, A. Thuizat, P. Sansonetti, A. Phalipon, *in preparation* **2003**.
[16] oligosac, *Eur. J. Biochem.* **1982**, *126*, 433.
[17] D. A. R. Simmons, *Bacteriol. Reviews* **1971**, *35*, 117.
[18] A. A. Lindberg, A. Karnell, A. Weintraub, *Rev. Infect. Dis.* **1991**, *13*, S279.
[19] L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849.
[20] C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131.
[21] F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 000.
[22] L. Mulard, C. Guerreiro, C. Costachel, A. Phalipon, *in preparation* **2003**.
[23] F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, 000.
[24] F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2003**, *sumitted*.
[25] H. Dohi, Y. Nishida, T.Takeda, K. Kobayashi, *Carbohydr. Res.* **2002**, *337*, 983.
[26] G. Blatter, J.-M. Beau, J.-C. Jacquinet, *Carbohydr. Res.* **1994**, *260*, 189.
[27] P. Westerduin, P. E. d. Haan, M. J. Dees, J. H. v. Boom, *Carbohydr. Res.* **1988**, *180*, 195.
[28] J. J. Oltvoort, C. A. A. v. Boeckel, J. H. d. Koning, J. v. Boom, *Synthesis* **1981**, 305.
[29] M. A. Nashed, L. Anderson, *J. Chem. Soc. Chem. Commun.* **1982**, 1274.
[30] T. Ogawa, K. Beppu, S. Nakabayashi, *Carbohydr. Res.* **1981**, *93*, C6.
[31] T. K. Lindhorst, *J. Carbohydr. Chem.* **1997**, *162*, 237.

LMPP13-exp-brevet-pentablock

General methods

Optical rotations were measured for $CHCl_3$ solutions at 25°C, expect where indicated otherwise, with a Perkin-Elmer automatic polarimeter, Model 241 MC. TLC were performed on precoated slides of Silica Gel 60 F_{254} (Merck). Detection was effected when applicable, with UV light, and/or by charring in 5% sulfuric acid in ethanol.

Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 0.040-0.063 mm). For all compounds the NMR spectra were recorded at 25°C for solutions in $CDCl_3$, on a Bruker AM 400 spectometer (400 MHz for ^1H, 100 MHz for ^{13}C). External references : for solutions in $CDCl_3$, TMS (0.00 ppm for both ^1H and ^{13}C). Proton-signal assignements were made by first-order analysis of the spectra, as well as analysis of 2D ^1H-^1H correlation maps (COSY) and selective TOCSY experiments. Of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. The ^{13}C NMR assignments were supported by 2D ^{13}C-^1H correlations maps (HETCOR). Interchangeable assignments are marked with an asterisk in the listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the O-SP and identified by a subscript in the listing of signal assignments. Fast atom bombardment mass spectra (FAB-MS) were recorded in the positive-ion mode using dithioerythridol/dithio-L-threitol (4 :1, MB) as the matrix, in the presence of NaI, and Xenon as the gas. Anhydrous DCM, 1,2-DCE and Et_2O, sold on molecular sieves were used as such. 4 Å powder molecular sieves was kept at 100°C and activated before use by pumping under heating at 250°C.

Dodecyl 3,4,6-tri-*O*-acétyl-2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranoside (5).

A mixture of the peracetylated **4** (6.2 g, 12.5 μmol) and dodecanthiol (2.5 mL, 94 μmol), 4Å molecular sieves and dry 1,2-DCE (90 mL) was stirred for 1 h then cooled to 0°C. $BF_3.Et_2O$ (1.57 mL, 12.5 μmol) was added. The stirred mixture was allowed to reach rt in 2h30. Et_3N was added until neutral pH and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc to give **5** as a white solid (7.5 g, 93 %); $[\alpha]_D$ -20° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 6.82 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH), 5.31 (dd,

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1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.15 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.68 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 4.28 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b}$ = 2.3 Hz, H-6b), 4.11 (dd, 1H, H-2), 3.75 (m, 1H, H-5), 2.70 (m, 2H, $SCH_2(CH_2)_{10}CH_3$), 2.10, 2.05, 2.04 (3s, 9H, OAc), 1.65-1.20 (m, 20H, $SCH_2(CH_2)_{10}CH_3$), 0.90 (t, 3H, $SCH_2(CH_2)_{10}CH_3$). ^{13}C NMR ($CDCl_3$):δ 171.0, 170.7, 169.3 (C=O), 161.9 (*C*=$OCCl_3$), 92.3 (CCl_3), 84.2 (C-1), 76.5 (C-5), 73.4 (C-3), 68.6 (C-4), 62.6 (C-6), 55.2 (C-2), 32.3, 30.6, 30.0-29.1, 14.5 ($S(CH_2)_{11}CH_3$), 21.1, 21.0, 20.9 (OAc). FABMS of $C_{26}H_{42}Cl_3NO_8S$ (M, 635.0), *m/z* 658.1 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{42}Cl_3NO_8S$, C: 49.17, H: 6.67, N: 2.21. Found C: 49.16, H: 6.71, N: 2.13.

Dodecyl 2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside (7).

A mixture of **5** (5 g, 7.87 mmol) in MeOH (15 mL) was deacetylated by MeONa overnight. The solution was neutralized by IR 120 (H^+) and Filtrated. After concentration in vacuo, the residue **6** was treated by 2,2-dimethoxypropane (70 mL, 546 mmol) and APTS (148 mg, 0.94 mmol) in DMF (20 mL). After stirring overnight, the mixture was neutralized with Et_3N and concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane-EtOAc to give **7** as a white solid (3.45 g, 80 %); $[\alpha]_D$ -35° (*c* 1, $CHCl_3$).

^1H NMR ($CDCl_3$):δ 6.92 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH), 4.77 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 3.98 (m, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 3.88 (dd, 1H, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.70 (dd, 1H, $J_{5,6b}$ = 0.5 Hz, H-6b), 3.63 (m, 1H, H-2), 3.53 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 3.29 (m, 1H, H-5), 2.98 (s, 1H, OH), 2.60 (m, 2H, $SCH_2(CH_2)_{10}CH_3$), 1.60-1.10 (m, 20H, $SCH_2(CH_2)_{10}CH_3$), 1.45, 1.35 (2 s, 6H, $C(CH_3)_2$), 0.80 (t, 3H, $SCH_2(CH_2)_{10}CH_3$). ^{13}C NMR ($CDCl_3$):δ 162.5 (*C*=OCCl3), 100.3 ($C(CH_3)_2$), 92.8 (CCl_3), 84.0 (C-1), 74.6 (C-4), 72.3 (C-3), 71.7 (C-5), 62.2 (C-6), 58.3 (C-2), 29.3, 19.5 ($C(CH_3)_2$), 32.3, 30.8, 30.1-29.5, 29.1, 14.5

($SCH_2(CH_2)_{10}CH_3$). FABMS of $C_{23}H_{40}Cl_3NO_5S$ (M, 548.9), *m/z* 572.2 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{40}Cl_3NO_5S$, C: 50.32, H: 7.34, N: 2.55. Found C: 50.30, H: 7.40, N: 2.36.

Dodecyl 3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside (8).

A mixture of **7** (1.07 g, 1.94 mmol) in pyridine (10 mL) was cooled to 0°C. Ac_2O (5 mL) was added and the solution was allowed to reach rt in 2 h. The mixture was then concentrated and the pyridine coevaporated with toluene. The residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc with 0.2% of Et_3N to give **8** as a white solid (1.12 g, 97 %), $[\alpha]_D$ -62° (*c* 1, $CHCl_3$)

^1H NMR ($CDCl_3$):δ 7.51 (d, 1H, $J_{2,NH}$ = 9.7 Hz, NH), 5.40 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 4.62 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 4.20 (m, 1H, H-2), 4.01 (dd, 1H, $J_{5,6a}$ = 5.2 Hz, $J_{6a,6b}$ = 10.7 Hz, H-6a), 3.84 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.70 (m, 2H, H-5, H-6b), 2.68 (m, 2H, $SCH_2(CH_2)_{10}CH_3$), 2.09 (s, 3H, OAc), 1.60-1.20 (m, 20H, $SCH_2(CH_2)_{10}CH_3$), 1.52, 1.38 (2 s, 6H, $C(CH_3)_2$), 0.90 (t, 3H, $SCH_2(CH_2)_{10}CH_3$). ^{13}C NMR ($CDCl_3$):δ 171.4 (C=OCH_3), 161.8 (C=OCCl3), 99.5 ($C(CH_3)_2$), 92.3 (CCl_3), 84.6 (C-1), 73.6 (C-3), 72.0 (C-4), 71.9 (C-5), 62.2 (C-6), 55.0 (C-2), 29.1, 19.3 ($C(CH_3)_2$), 32.3, 30.7, 30.0-29.0, 14.5 ($SCH_2(CH_2)_{10}CH_3$). FABMS of $C_{25}H_{42}Cl_3NO_6S$ (M, 591.0), *m/z* 614.1 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{42}Cl_3NO_6S$, C: 50.80, H: 7.16, N: 2.37. Found C: 50.67, H: 7.32, N: 2.24.

3,4-Di-*O*-acetyl-1,2-*O*-allyloxyethylidene-β-L-rhamnopyranose (12). A mixture of L-rhamnose monohydrate (50 g, 274 mmol) in pyridine (410 mL) was cooled to 0°C. Ac_2O (170 mL) was added and the solution was allowed to reach rt overnight. MeOH (100 mL) was added and the solution concentrated. The resulting suspension was taken up in DCM, washed with water, satd aq $NaHCO_3$, water, and satd aq NaCl, successively. The organic layer was

dried and concentrated to give the crude peracetylated rhamnose (quant.) as a slightly yellow oil. A solution of latter (21.15 g, 63.7 mmol) in acetic acid (38 mL) and acetic anhydride (6.7 mL) was treated by a 33% solution of HBr in AcOH (86 mL), then stirred for 15 h at rt. The mixture was concentrated by repeated coevaporation with cyclohexane. The resulting suspension was taken up in DCM, washed with satd aq $NaHCO_3$ and water. The organic layer was dried and concentrated to give **11** (quant.) as a brown oil. A solution of the crude **11** (22.29 g) in anhydrous 2,6-lutidine (37 mL) was treated by AllOH (9.6 mL, 142 mmol) at rt. The solution was stirred overnight, then filtered and the solids were washed with EtOAc. The liquid layer was concentrated and the residue was taken up in DCM, washed with 1M HCl cold solution, water and satd aq NaCl. The organic layer was dried and concentrated by coevaporation with toluene. Chromatography of the crude residue (toluene:acetone, 49:1 containing 0.1% Et_3N) gave orthoester **12** (18.5 g, 88%) as a slightly yellow oil which crystallized on standing. An analytical sample was recristallized from isopropyl ether:petroleum ether; mp XX°C, $[\alpha]_D$ -XX° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$):δ 5.88 (m, 1H, All), 5.42 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1), 5.25-5.40 (m, 2H, All), 5.10 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-3), 5.05 (dd, 1H, $J_{4,5}$ = 6.3 Hz, H-4), 4.60 (dd, 1H, H-2), 4.05 (m, 2H, All), 3.50 (qd, 1H, $J_{5,6}$ = 6.2 Hz, H-5), 2.12, 2.06 (2s, 6H, OAc), 1.76 (s, 3H, CH_3), 1.23 (d, 3H, H-6); *^{13}C NMR ($CDCl_3$):δ 171.4 ($C{=}OCH_3$), 161.8 (C=OCCl3), 99.5 ($C(CH_3)_2$), 92.3 (CCl_3), 84.6 (C-1), 73.6 (C-3), 72.0 (C-4), 71.9 (C-5), 62.2 (C-6), 55.0 (C-2), 29.1, 19.3 ($C(CH_3)_2$), 32.3, 30.7, 30.0-29.0, 14.5 ($SCH_2(CH_2)_{10}CH_3$). FABMS of $C_{25}H_{42}Cl_3NO_6S$ (M, 591.0) m/z 614.1 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{42}Cl_3NO_6S$, C: 50.80, H: 7.16, N: 2.37. Found C: 50.67, H: 7.32, N: 2.24.*

3,4-Di-*O*-benzyl-1,2-*O*-allyloxyethylidene-β-L-rhamnopyranose (14). A solution of the crude peracetylated rhamnose (9.0 g, 27 mmol) was processed as described for the preparation

of **12**. A solution of the crude **12** thus obtained in MeOH (65 mL) was cooled to 0°C and treated with NH_3 until saturation. The solution was stirred for 6 h at rt, then concentrated by co-evaporation with toluene to give **13**. Column chromatography (DCM:MeOH, 49:1) gave pure **13** as a white solid. ^{1}H NMR ($CDCl_3$):δ 5.75 (m, 1H, All), 5.22 (d, 1H, H-1), 5.00-5.10 (m, 2H, All), 4.60 (dd, 1H, H-2), 4.30 (d, 1H, H-3), 3.80 (m, 2H, All), 3.50 (m, 1H, H-5), 3.20 (t, 1H, H-4), 1.80 (s, 3H, CH_3), 1.20 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6).

A solution of crude **13** in anhydrous DMF (90 mL) was cooled to 0°C. NaH (4.32 g, 108 mmol) was added in 30 min then BnBr (8.5 mL, 71 mmol) was added dropwise at 0°C. The solution was stirred overnight at rt, then MeOH (20 mL) was added dropwise at 0°C. The solution was allowed to reach rt in 2 h, then concentrated. The residue was taken up in DCM, washed with satd aq $NaHCO_3$ until neutral pH, water and satd aq NaCl. The organic layer was dried and concentrated. After evaporation, the residue was eluted from a column of silica gel with 9:1 cyclohexane-EtOAc and 0.2 % of Et_3N to give **14** as a white solid (8 g, 70%).

Crystallization of an analytical sample from isopropyl ether:petroleum ether gave **13** as white crystals; mp XX°C, $[\alpha]_D$ XX° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$):δ 7.35 (m, 10H, Ph), 5.90 (m, 1H, All), 5.30 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1), 5.28-5.43 (m, 2H, All), 4.95-4.65 (m, 4H, CH_2Ph), 4.40 (dd, 1H, $J_{2,3}$ = 4.0 Hz, H-2), 4.10 (m, 2H, All), 3.70 (d, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.50 (t, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 3.35 (m, 1H, $J_{5,6}$ = 6.2 Hz, H-5), 1.77 (s, 3H, CH_3), 1.33 (d, 3H, H-6); *^{13}C NMR ($CDCl_3$):δ 171.4 ($C{=}OCH_3$), 161.8 ($C{=}OCCl3$), 99.5 ($C(CH_3)_2$), 92.3 (CCl_3), 84.6 (C-1), 73.6 (C-3), 72.0 (C-4), 71.9 (C-5), 62.2 (C-6), 55.0 (C-2), 29.1, 19.3 ($C(CH_3)_2$), 32.3, 30.7, 30.0-29.0, 14.5 ($SCH_2(CH_2)_{10}CH_3$). FABMS of $C_{25}H_{42}Cl_3NO_6S$ (M, 591.0) m/z 614.1 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{42}Cl_3NO_6S$, C: 50.80, H: 7.16, N: 2.37. Found C: 50.67, H: 7.32, N: 2.24*

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Allyl 2-*O*-acétyl 3,4-Di-*O*-benzyl-β-L-rhamnopyranoside (15). *A mixture of the orthoester **14** (470 mg, 1.1 mmol), 4Å molecular sieves and dry DCE (6.1 mL) was stirred for 15 min. TMSOTf (72 µL, 0.4 mmol) was added at rt. The solution was stirred for 4 h at rt and the mixture filtered. The liquid layer was washed with satd aq $NaHCO_3$ and water. The organic layer was dried and concentrated. After evaporation, the residue was eluted from a column of silica gel with 95:5 toluene-EtOAc to give **15** as a white foam (238 mg, 51 %). 1H NMR analysis showed that 15 was mixture of anomers in a XX:XX ratio.*

Allyl 3,4-di-*O*-benzyl -β-L-rhamnopyranose (16). Compound **15** (194 mg, 0.4 mmol) was dissolved in DCM (4 mL). MeONa was added until pH=9. The mixture was stirred for 2 h then treated by IR 120 (H^+) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane-AcOEt to give **16** as a colorless oil (145 mg, 95 %); $[\alpha]_D$ ° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$):δ 7.35 (m, 10H, Ph), 5.90 (m, 1H, All), 5.30 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 5.05 (m, 2H, All), 4.90-4.65 (m, 4H, CH_2Ph), 4.05 (m, 1H, $J_{2,3}$ = 3.5 Hz, H-2), 3.95-4.15 (m, 2H, All), 3.90 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.75 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.47 (t, 1H, H-4), 2.50 (s, 1H, OH), 1.32 (d, 3H, H-6).

Allyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranoside (17). *A mixture of **16α** (2.0 g, 5.2 mmol) in THF (85 mL) was treated with DCC (?), levulinic acid (?) and DMAP (?) at rt. The solution was stirred during 15 h. The solution was concentrated. The residue was eluted from a column of silica gel with 99.5:0.5 to 98:2 DCM-AcOEt to give **17** (?g, ? %) as a foam.* ^{1}H NMR ($CDCl_3$): δ 7.3-7.4 (m, 10H, Ph), 5.90 (m, 1H, All), 5.40 (dq, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.4 Hz, H-2), 5.28 (m, 1H, All), 5.20 (m, 1H, All), 4.93 (d, 1H, CH_2Ph), 4.78 (d, 1H, $J_{1,2}$ =

1.6 Hz, H-1), 4.78 (d, 1H, CH_2Ph), 4.63 (d, 1H, CH_2Ph), 4.51 (d, 1H, CH_2Ph), 4.17 (m, 1H, All), 3.99 (m, 1H, All), 3.99 (m, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 3.78 (dq, 1H, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, H-5), 3.43 (pt, 1H, H-4), 2.80 (m, 4H, lev), 2.19 (s, 3H, Ac), 1.37 (d, 3H, H-6).^{13}C NMR ($CDCl_3$): δ 124.0-125.1 (Ph), 118.0 (All), 97.0 (C-1), 80.2 (C-4), 78.5 (C-3), 75.2 (CH_2Ph), 72.0 (CH_2Ph), 70.2 (C-2), 68.5 (All), 68.3 (C-5), 38.5 (Lev), 31.5 (Ac), 28.5 (lev), 20.1 (C-6).

3,4-Di-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranose (18). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg, 20 μmol) was dissolved THF (? mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **17** (1.4 g, 3.12 mmol) in tetrahydrofuran (? mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated to dryness. The residue was dissolved in a solution of I_2 (1.37 g, 5.4 mmol) in 30 mL of THF/H_2O (15:4). The mixture was stirred at rt for 1 h and THF was evaporated. The resulting suspension was taken up in DCM, washed twice with water, satd aq NaHSO3, water, satd aq NaHCO3, water and satd aq NaCl, successively. The organic layer was dried and concentrated. The residue was eluted from a column of silica gel with 7:3 to 6:4 cyclohexane-EtOAc to give the corresponding hemiacetal **18** (1.3 g, 93 %). ^{1}H NMR ($CDCl_3$): ^{1}H δ 7.3-7.4 (m, 10H, Ph), 5.40 (dq, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.4 Hz, H-2), 4.93 (d, 1H, CH_2Ph), 4.78 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.78 (d, 1H, CH_2Ph), 4.63 (d, 1H, CH_2Ph), 4.51 (d, 1H, CH_2Ph), 3.99 (m, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 3.78 (dq, 1H, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, H-5), 3.43 (pt, 1H, H-4), 2.80 (m, 4H, lev), 2.19 (s, 3H, Ac), 1.37 (d, 3H, H-6).

3,4-Di-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl trichloroacetimidate (19).

LMPP13-exp-brevet-pentablock

Trichloroacetonitrile (1.3 mL, 13 mmol) and DBU (51 μL, 0.3 mmol) were added to a solution of the residue **18** (1.0 g, 2.3 mmol) in anhydrous DCM (6 mL) at 0°C. After 2 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane-EtOAc and 0.2 % Et_3N to give **19** as a white foam (1.0 g, 95 %); $[\alpha]_D$ XX° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): ^1H δ 8.67 (s, 1H, NH), 7.3-7.4 (m, 10H, Ph), 6.19 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.48 (dd, 1H, $J_{1,2}$ = 2.0, $J_{2,3}$ = 3.3 Hz, H-2), 4.95 (d, 1H, CH_2Ph), 4.73 (d, 1H, CH_2Ph), 4.66 (d, 1H, CH_2Ph), 4.58 (d, 1H, CH_2Ph), 4.51 (d, 1H, CH_2Ph), 4.00 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 3.95 (dq, 1H, $J_{4,5}$ = 9.6, $J_{5,6}$ = 6.3 Hz, H-5), 3.52 (pt, 1H, H-4), 2.80 (m, 4H, lev), 2.20 (s, 3H, Ac), 1.36 (d, 3H, H-6).

Allyl (2-*O*-levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (22). A mixture of alcohol 21 (300 mg, 0.36 mmol) and imidate 19 (320 mg, 0.54 mmol) in anhydrous Et_2O (20 mL) was stirred for 15 min under dry Ar. After cooling at –75°C, Me_3SiOTf (13 μL, 70 μmol) was added dropwise and the mixture was stirred 3 h. Triethylamine (60 μL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 9:1 cyclohexane-EtOAc to give 22 (440 mg, 92 %) as a colorless foam; $[\alpha]_D$ XX° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.1-8.1 (m, 35H, Ph), 5.95 (m, 1H, All), 5.73 (dd, 1H, $J_{1,2}$ = 2.2, $J_{2,3}$ = 2.3 Hz, H-2_B), 5.43 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.30 (m, 2H, All), 5.08 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.03 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_B), 4.97 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1_C), 4.30-5.00 (m, 12H, CH_2Ph), 4.20 (m, 2H, All, H-3_C), 4.05 (m, 3H, All, H-3_E, 5_E), 3.98 (m, 1H, H-$6a_E$), 3.81 (m, 5H, H-3_B, 4_C, 4_E, 5_C, 6_E), 3.69 (dq, 1H, $J_{4,5}$ = 9.3, $J_{5,6}$ = 6.0 Hz, H-5_B), 3.52 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.29 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_B), 2.71 (m, 4H, Lev), (s, 3H, Ac), 1.40 (d, 3H, H-6_C), 1.01 (d, 3H, H-6_B).

LMPP13-exp-brevet-pentablock

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (23). The trisaccharide **22** (200 mg, 0.16 mmol) was treated with 0.4 mL of a solution 1M of hydrazine (100 mg) diluted in a mixture of pyridine (1.6 mL) and acetic acid (0.4 mL) at rt. The solution was stirred during 20 min. Acetone (1.2 mL) was added and the solution was concentrated. The residue was eluted from a column of silica gel with 98.5:1.5 Dichloromethane-AcOEt to give **23** (174 mg, 92 %) as a foam; $[\alpha]_D$ +14° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$):δ 7.05-8.10 (m, 35H, Ph), 5.82 (m, 1H, All), 5.25 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.19 (m, 2H, All), 5.00 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.87 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_B), 4.81 (d, 1H, H-1_C), 4.35-4.90 (m, 12H, CH_2Ph), 4.00-4.20 (m, 2H, All), 4.10 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3_C), 4.09 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 3.95 (m, 1H, $J_{4,5}$ = 9.5 Hz, H-5_E), 3.92 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3_E), 3.78 (m, 1H, $J_{5,6}$ = 6.0 Hz, H-5_C), 3.70 (m, 1H, H-4_C), 3.58-3.62 (m, 2H, H-$6a_E$, $6b_E$), 3.59 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5_B), 3.54 (dd, 1H, H-4_E), 3.48 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3_B), 3.45 (dd, 1H, H-2_E), 3.31 (dd, 1H, H-4_B), 2.68 (d, 1H, $J_{2,OH}$ = 2.3 Hz, O-H), 1.29 (d, 3H, H-6_C), 1.09 (d, 3H, H-6_B). ^{13}C NMR ($CDCl_3$):δ 166.2 (C=O), 118.2-137.5 (Ph, All), 103.1 (C-1_B), 98.5 (C-1_E), 96.6 (C-1_C), 82.1 (C-3_E), 81.4 (C-2_E), 80.4 (C-4_B), 79.7 (C-3_B), 79.4 (C-4_C), 78.9 (C-3_C), 78.1 (C-4_E), 76.0, 75.5, 74.5, 74.2, 73.6, 72.1 (*C*H_2Ph), 73.7 (C-2_C), 68.9 (C-6_E), 68.8 (C-5_B), 68.7 (All, C-5_E), 68.1 (C-5_C), 19.1 (C-6_C), 18.2 (C-6_B). FABMS of $C_{70}H_{76}O_{15}$ (M, 1156.5), *m/z* 1179.5 ([M+Na]$^+$). Anal. Calcd for $C_{70}H_{76}O_{15}$: C, 72.64; H, 6.62. Found C, 72.49; H, 6.80.

Allyl (3-*O*-acetyl-4,6-O-isopropylidene-2-trichloroacetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-

benzoyl-α-L-rhamnopyranoside (26). A mixture of the donor **8** (294 mg, 357 μmol) and the acceptor **25** (313 mg, 211 μmol), 4Å molecular sieves and dry DCM (4 mL) was stirred for 1.5 h then cooled to –15°C. NIS (94 mg, 0.42 mmol) and Triflic acid (8 μL, 0.1 mmol) were successively added. The stirred mixture was allowed to reach 0°C in 1.5 h. Et_3N (25 μL) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc and 0.5 % of Et_3N to give **26** as a white foam (232 mg, 58 %); $[\alpha]_D$ -2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): ^1H δ 7.00-8.00 (m, 45H, Ph), 6.81 (d, 1H, $J_{2,NH}$ = 9.0 Hz, NH_D), 5.82 (m, 1H, All), 5.30 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.10-5.23 (m, 2H, All), 4.96 (bs, 1H, H-1_A), 4.91 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.87 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.84 (bs, 1H, H-1_C), 4.79 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0Hz, H-3_D), 4.35 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.00 (dd, 1H, H-2_A), 3.90 (dd, 1H, H-2_D), 2.90-4.10 (m, 22H, All, H-2_E, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.93 (s, 3H, OAc), 1.2-0.9 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C NMR ($CDCl_3$):δ 170.7, 165.5, 161.7 (C=O), 138.4-117.3 (Ph, All), 101.7 (C-1_D), 100.8 (C-1_B), 100.6 (C-1_A), 99.5 ($C(CH_3)_2$), 97.9 (C-1_E), 95.7 (C-1_C), 92.0 (CCl_3), 82.2, 81.7, 81.6, 80.3, 79.9, 78.8, 77.9, 77.9, 76.6, 76.0, 75.8, 75.4, 75.1, 74.7, 74.3, 74.1, 73.3, 72.8, 72.6, 71.9, 71.5, 70.8, 69.0, 68.8, 68.5, 68.0, 67.8, 62.0, 56.7 (C-2_D), 28.6 ($C(CH_3)_2$), 21.3 (OAc), 19.4 ($C(CH_3)_2$), 19.0, 18.5, 18.4 (3C, C-6_A, 6_B, 6_C). FABMS of $C_{103}H_{114}Cl_3NO_{25}$ (M, 1872.3), *m/z* 1894.6 $[M+Na]^+$. Anal. Calcd for $C_{103}H_{114}Cl_3NO_{25}$, C: 66.07, H: 6.14, N: 0.75. Found C: 66.08, H: 6.09, N: 0.81.

Allyl (2-acetamido-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside

(2). The pentasaccharide **X** (2.65 g, 1.47 mmol) was dissolved in MeOH (20 mL). MeONa was added until pH=10. The mixture was stirred for 25 min then treated by IR 120 (H^+) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 9 :1 DCM-MeOH to give the expected triol which was then treated by 2,2-dimethoxypropane (11 mL, 0.1 mol) and APTS (20 mg, 0.17 mmol) in DMF (20 mL) overnight. Et_3N was added and the solution evaporated. The residue was eluted from a column of silica gel with 1:1 Cyclohexane-AcOEt and 0.2 % of Et_3N to give **2** as a white foam (2.05 g, 81 % from **X**); $[\alpha]_D$ +3° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$) : δ 6.98-8.00 (m, 45H, Ph), 6.17 (bs, 1H, NH_D), 5.82 (m, 1H, All), 5.30 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.11-5.25 (m, 2H, All), 5.06 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.88 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.84 (bs, 1H, H-1_C), 4.35 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.05 (dd, 1H, H-2_A), 3.36 (dd, 1H, H-2_E), 2.90-4.10 (m, 22H, All, H-2_D, 3_A, 3_B, 3_C, 3_D, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.5 (s, 3H, AcNH), 1.2-0.9 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C NMR: δ 172.7 (C=O), 164.9 (C=O), 137.7-116.7 (Ph, All), 102.3 (C-1_D), 100.2 (C-1_B), 100.0 (C-1_A), 98.9 ($C(CH_3)_2$), 97.2 (C-1_E), 95.1 (C-1_C), 82.1, 82.0, 81.8, 81.6, 80.6, 80.3, 79.0, 78.8, 78.3, 77.8, 77.6, 75.7, 75.6, 75.0, 74.3, 72.8, 71.8, 71.6, 70.8, 70.3, 69.0, 68.5, 67.8, 67.4, 61.9, 60.8, 60.5, 29.4 ($C(CH_3)_2$), 22.7 (AcNH), 19.0 ($C(CH_3)_2$), 18.9, 18.4, 18.2 (3C, C-$6_{A, B, C}$). FAB-MS for $C_{101}H_{115}NO_{24}$ (M = 1726.9) *m/z* 1749.7 [M + Na]$^+$. Anal. Calcd. for $C_{101}H_{115}NO_{24}.H_2O$: C, 69.52 ; H, 6.76 ; N, 0.80. Found C, 69.59; H 6.71 ; N, 0.57.

Allyl (2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (1). (a) A mixture of **2** (2.05 g, 1.19 mmol) in Pyridine (60 mL) was

cooled to 0°C. Ac_2O (20 mL) was added and the solution was stirred 2.5 h. The solution was concentrated and coevaporated with toluene. The residue was eluted from a column of silica gel with 2:1 Cyclohexane-AcOEt and 0.2 % of Et_3N to give **1** as a white foam (1.99 g, 94 %); $[\alpha]_D$ +1° (*c* 1, $CHCl_3$).

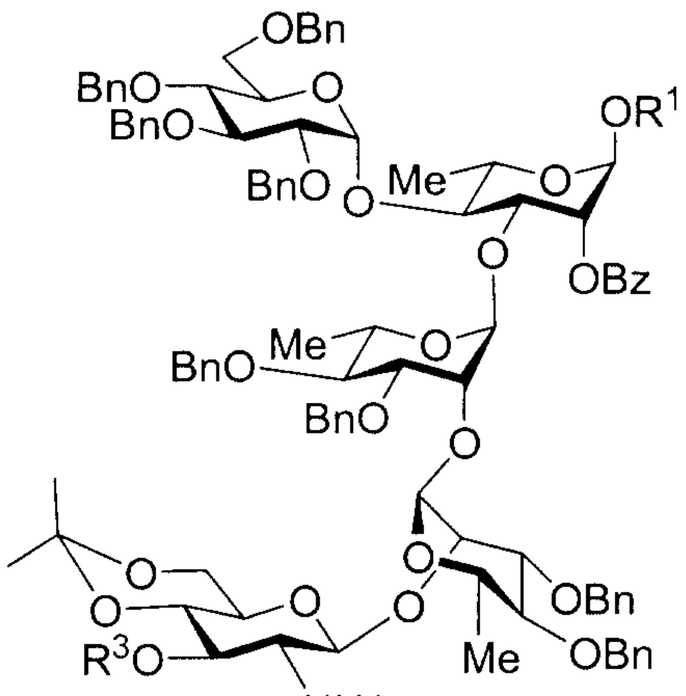
(b) A mixture of **26** (144 mg, 0.06 mmol), Bu_3SnH (0.1 mL, 0.37 mmol) and AIBN (10 mg) in dry toluene (3 mL) was stirred for 1 h at rt under a stream of dry Ar, then was heated for 1.5 h at 90°C, cooled and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc and 0.2 % of Et_3N to give **1** (100 mg, 74 %). ^1H NMR ($CDCl_3$): δ 6.95-8.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.46 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH_D), 5.29 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.11-5.25 (m, 2H, All), 5.00 (bs, 1H, H-1_A), 4.90 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.85 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.83 (bs, 1H, H-1_C), 4.70 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.37 (dd, 1H, H-2_E), 2.90-4.10 (m, 21H, All, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.92 (s, 3H, OAc), 1.57 (s, 3H, AcNH), 1.27-0.90 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ 171.3, 170.3, 166.2 (C=O), 138.7-117.9 (Ph, All), 103.9 (C-1_D), 101.5 (C-1_B), 101.4 (C-1_A), 99.9 ($C(CH_3)_2$), 98.5 (C-1_E), 96.3 (C-1_C), 82.1, 81.7, 81.6, 80.3, 80.1, 78.8, 78.1, 77.8, 76.0, 75.8, 75.3, 75.1, 74.7, 74.2, 73.6, 73.3, 72.7, 71.9, 71.4, 70.8, 69.0, 68.8, 68.7, 68.4, 68.1, 67.8, 62.1, 55.0 (C-2_D), 30.0 ($C(CH_3)_2$), 23.5 (AcNH), 21.6 (OAc), 19.2 ($C(CH_3)_2$), 19.0, 18.3, 18.2 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{103}H_{117}NO_{25}$ (M = 1769.0) *m/z* 1791.9 $[M + Na]^+$. Anal. Calcd. for $C_{103}H_{117}NO_{25}$: C, 69.93 ; H, 6.67 ; N, 0.79. Found C, 69.77; H, 6.84; N, 0.72.

(2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→

3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (3). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 58 μmol) was dissolved tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **1** (1.8 g, 1.02 mmol) in tetrahydrofuran (20 mL) was degassed and added. The mixture was stirred at rt overnight then concentrated to dryness. The residue was dissolved in acetone (9 mL), then water (2 mL), mercuric chloride (236 mg) and mercuric oxide (200 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and Satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give the corresponding hemiacetal **27**. Trichloroacetonitrile (2.4 mL) and DBU (72 μL) were added to a solution of the residue in anhydrous dichloromethane (24 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give **3** as a colorless oil (1.58 g, 82 %); $[\alpha]_D$ +2° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$) : δ 8.62 (s, 1H, C=NH), 6.95-8.00 (m, 45H, Ph), 6.24 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1_C), 5.48 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.41 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH_D), 4.99 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.88 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.69 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.38 (dd, 1H, H-2_E), 2.90-4.10 (m, 19H, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.95 (s, 3H, OAc), 1.55 (s, 3H, AcNH), 1.30-0.85 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ 172.4, 171.4, 166.9 (C=O), 140.2-128.9 (Ph), 104.2 (C-1_D), 101.4 (2C, C-1_A, 1_B), 101.1

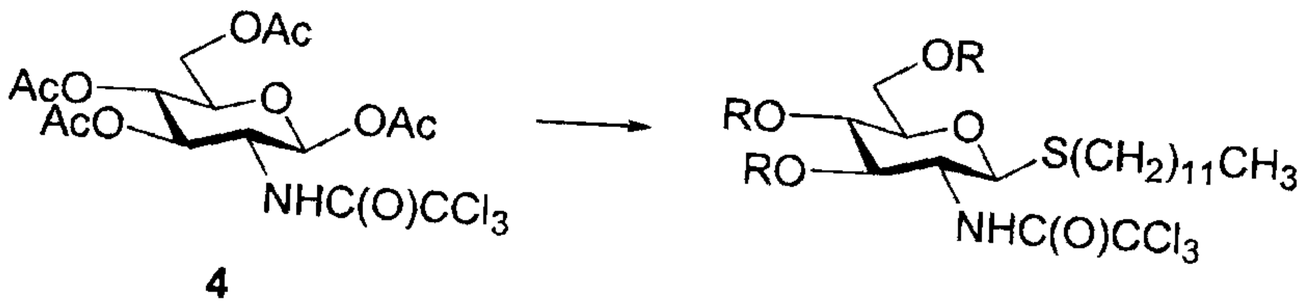
(*C*(CH_3)$_2$), 98.0 (C-1_E), 94.8 (C-1_C), 92.4 (CCl_3), 82.1, 81.5, 80.2, 80.1, 78.6, 78.1, 77.8, 77.6, 76.0, 75.8, 75.5, 75.0, 74.3, 74.2, 73.5 (C-3_D), 73.4, 71.9, 71.4, 71.0, 70.5, 69.2, 68.8, 68.3, 68.1, 62.1, 54.9 (C-2_D), 29.3 (C(*C*H_3)$_2$), 23.4 (AcNH), 21.4 (OAc), 19.2 (C(*C*H_3)$_2$), 19.0, 18.2, 18.1 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{102}H_{113}Cl_3N_2O_{25}$ (M = 1873.3) *m/z* 1896.3 [M + Na]$^+$. Anal. Calcd. for $C_{102}H_{113}Cl_3N_2O_{25}$: C, 65.40 ; H, 6.08 ; N, 1.50. Found C, 65.26; H, 6.02; N, 1.31.

LMPP13-Schemes-brevet-pentablock



	R^1	R^3
1	All	Ac
2	All	H
3	TCA	Ac

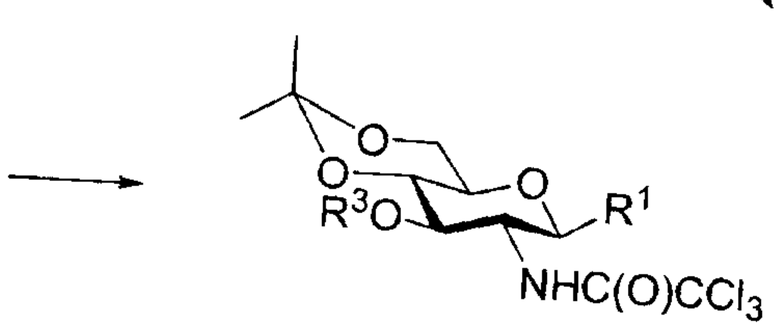
LMPP13-Schemes-brevet-pentablock



4

	R
5	Ac
6	H

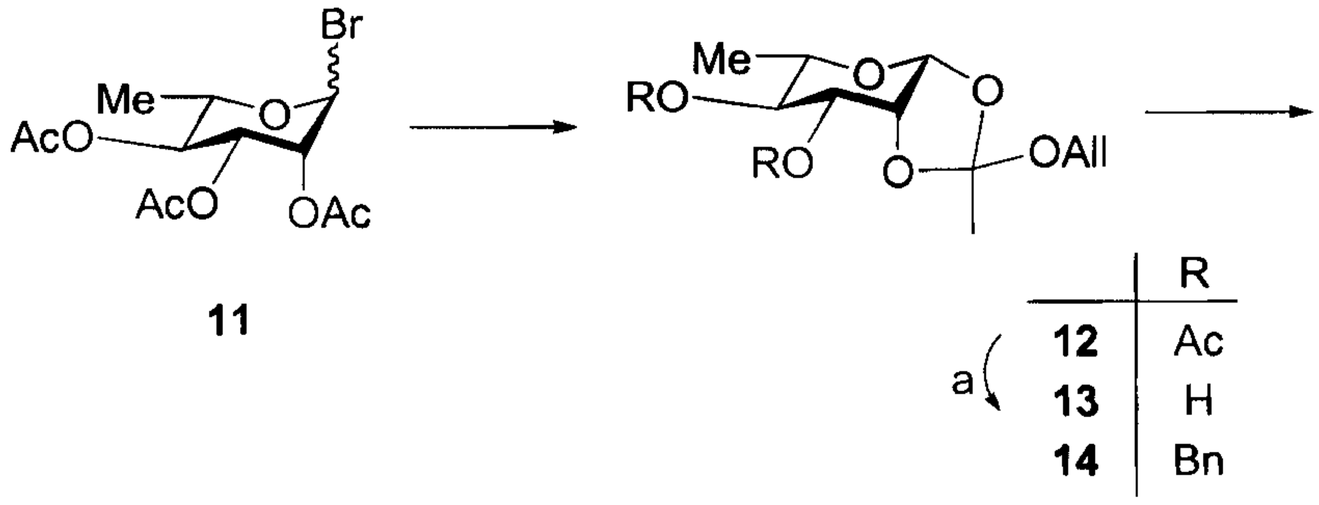
a



	R^1	R^3
7	$S(CH_2)_{11}CH_3$	H
8	$S(CH_2)_{11}CH_3$	Ac
9	OH	Ac
10	OTCA	Ac

a

LMPP13-Schemes-brevet-pentablock



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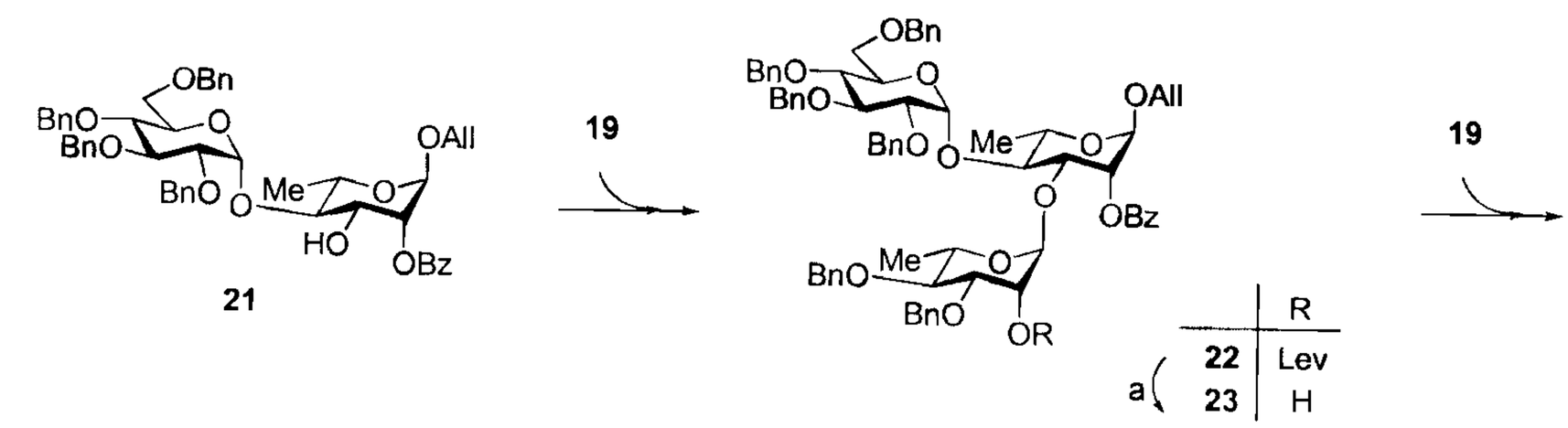

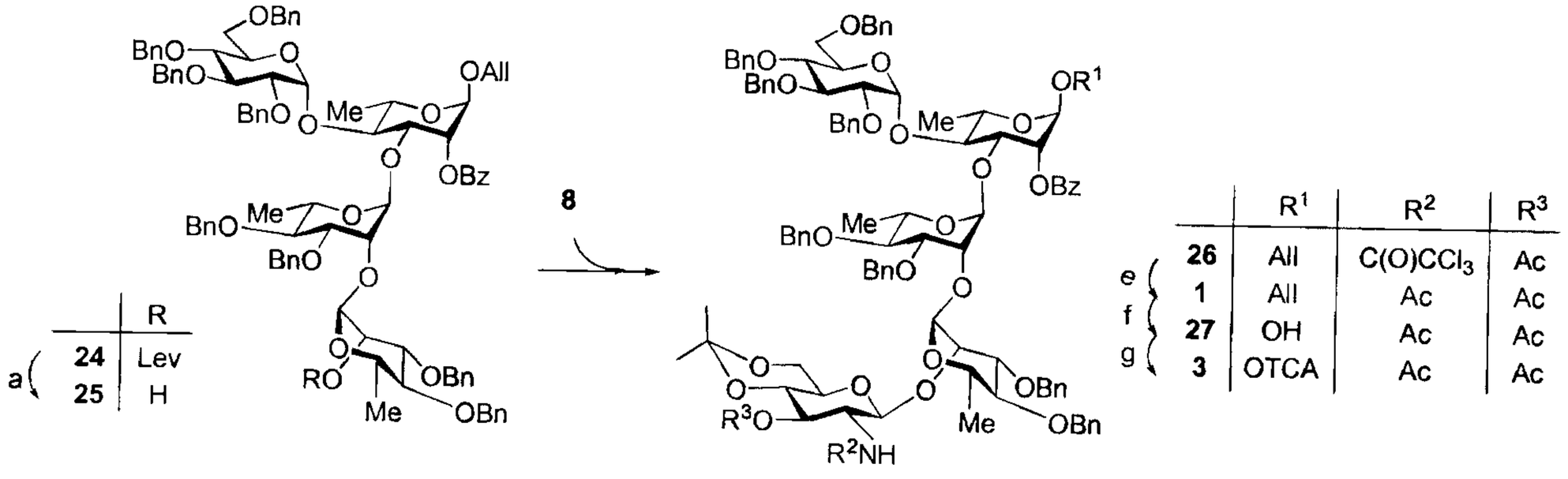

LMPP14theo-brevet-synlongs

Synthesis of spacer-armed hexa-, deca-, and pentasaccharide haptens representative of the O-specific polysaccharide of *Shigella flexneri* serotype 2a[1]

Abstract

INTRODUCTION

Shigellosis or bacillary dysentery is a serious infectious disease, responsible for some 200 million episodes annually, mostly in children and immunocompromised individuals living in areas were sanitary conditions are insufficient. [2] *Of the four species of Shigellae, Shigella flexneri is the major responsible of the endemic form of the disease, with serotype 2a being the most prevalent. Due to increasing resistance of all groups of Shigellae to antibiotics,* [3] *the development of a vaccine against shigellosis is of high priority as stated by the World Health Organization in its program against enteric diseases.* [4] *However, there are yet no licensed vaccines for shigellosis.*

As for other Gram negative bacteria, *Shigella*'s lipopolysaccharide (LPS) is a major surface antigen of the bacterium. The corresponding O-specific polysaccharide (O-SP), a polymer of less than 30 kDa, defines the serogroup and serotype of the bacteria. Besides, it is both an essential virulence factor and the target of the infected host's protective immune response. [5,6] However, O-SPs are T-cell independent antigens, [7,8] which are not immunogenic by themselves. Nevertheless, benefiting from the successful conversion of bacterial capsular polysaccharides from T-independent antigens to T-dependent ones through their covalent coupling to a protein carrier, it was shown that O-SPs could be turned into immunogens. Indeed, based on the former hypothesis that serum IgG anti-LPS antibodies may confer

specific protection against shigellosis, [9] several polysaccharide-proteine conjugates, targeting either *Shigella sonnei*, *Shigella dysenteriae* 1 or *S. flexneri* serotype 2a, were shown to be safe and immunogenic in humans. [10,11] In the case of *S. sonnei*, recent field trials allowed J.B. Robbins and co-workers to demonstrate the efficacy of a vaccine made of the corresponding detoxified LPS covalently linked to recombinant exoprotein A. [12] Even though efficient, polysaccharide-protein conjugate vaccines remain highly complex structures, whose immunogenicity depends of several parameters amongst which, the length and nature of the saccharide component as well as its loading on the protein. It is reasonably admitted that control of these parameters, and indeed standardization, are somewhat difficult when dealing with polysaccharides purified from bacterial cell cultures, or fragments thereof resulting from their partial hydrolysis. Mixture are often obtained, which may become a real drawback in terms of analysis of the products, particularly when multivalent vaccines are needed, as in the case of shigellosis. It may be assumed that the use of well-defined synthetic oligosaccharides suitable for single-point attachment on to the carrier would allow a better control, and consequently the optimisation, of the above mentioned parameters. That low molecular weight oligosaccharides mimicking antigenic determinants were immunogenic when conjugated onto a protein carrier was demonstrated in the late 30s, [13,14] and since then exploited on several occasions. [15] Indeed, available data on *S. dysenteriae* type 1 indicate that neoglycoconjugates incorporating di-, tri- or tetramers of the O-SP repeating unit were more immunogenic than a detoxified LPS-human serum albumin conjugate of reference. [16] In the case of heteropolysaccharides, oligosaccharides made of at least two contiguous repeating units were originally considered to be necessary for the corresponding oligosaccharide-protein conjugates to induce anti-polysaccharide antibodies. [17] However, more recent data demonstrated that neoglycoproteins incorporating oligosaccharides comprising one repeating unit or smaller fragments were immunogenic in mice. [18,19] Along this line, we recently reported the synthesis of three fully synthetic glycopeptides as potential vaccines against *Shigella flexneri* 2a infection. [20] These incorporated short oligosaccharide haptens, representative either of part or of the whole repeating unit of the O-SP of *S. flexneri* serotype 2a. Preliminary data indicate that two out of the three conjugates are immunogenic in mice.(Phalipon et al, unpublished results) Besides, we found that the corresponding neoglycoproteins consisting of the oligosaccharides covalently linked to tetanus toxoid via single-point attachment were also immunogenic in mice.(Phalipon et al, unpublished results)

Parallel studies on the recognition of synthetic fragments of the O-SP by protective homologous monoclonal antibodies suggested that sequences larger than one repeating unit

were more antigenic, thus probably better mimicking the natural polysaccharide than shorter ones. [21] Indeed, it is anticipated that better mimics of the O-SP, in terms of both antigenicity and conformation, would lead to conjugates of higher immunogenicity. For that reason, the preparation of oligo- or polysaccharides[22] made of two repeating units or more, in a form suitable for conjugation onto a carrier, was undertaken.

RESULTS AND DISCUSSION

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

I

The O-SP of *S. flexneri* 2a is a branched heteropolysaccharide defined by the pentasaccharide repeating unit **I**. [23,24] It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-SPs and comprises a *N*-acetyl glucosamine (**D**) and three rhamnose residues (**A**, **B**, **C**). The specificity of the serotype is associated to the α-D-glucopyranose residue linked to position 4 of rhamnose **C**.

Evaluation of the antigenicity of a panel of di- to pentasaccharides representative of frame-shifted fragments of **I**, had pointed out that the **ECD** portion was the minimal sequence required for binding, and that the **B(E)C** ramification had a great impact on the recognition process. [25] Based on theses data, we described recently the synthesis of the **ECD**, **B(E)CD** and **AB(E)CD** fragments functionalized with an aminoethyl spacer at their reducing end, and demonstrated that the later could serve as a suitable anchoring point. [20] As stated above, subsequent work outlined the impact of chain elongation on the recognition process. Taking both sets of data into account, we report herein on the synthesis of the 2-aminoethyl glycosides of a deca- (**1**) and a pentadecasaccharide (**2**), corresponding to sequences [**AB(E)CD**]$_2$ and [**AB(E)CD**]$_3$, respectively. The corresponding **D'AB(E)CD** hexasaccharide (**3**) was used as a model.

Considering the target **1** and **2**, a disconnection at the **D-A** linkage would appear most appropriate. However, others have shown that such a disconnection strategy was not suitable even when involving di- or trisaccharide building blocks, [26,27] and this route was avoided. More recently, disconnections at the **A-B**, **B-C** and **C-D** linkages were evaluated in this laboratory when synthesizing successfully the methyl glycoside of the frame-shifted decasaccharide **D'A'B'(E')C'DAB(E)C** by condensing a chain terminator pentasaccharide

donor and a methyl glycoside pentasaccharide acceptor. [28] It was demonstrated on that occasion that disconnection at the **C-D** linkage was indeed appropriate for the construction of large fragments of the *S. flexneri* 2a O-SP. Based on our experience in the field, a blockwise strategy to targets **1** and **2**, implicating a **DAB(E)C** potential acceptor acting as a donor, an **AB(E)C** tetrasaccharide donor, and the recently disclosed acceptor **XX**[20] as a precursor to the spacer-armed **D** residue (Scheme 1). Although permanent blocking of OH-4_D and OH-6_D with an isopropylidene acetal may appear somewhat unusual, this choice was a key feature of the strategy. It was based on former observations in the methyl glycoside series, demonstrating that its use could overcome some of the known drawbacks of the corresponding benzylidene acetal, [29,30] including its poor solubility. Compound **XX** was readily obtained from the known triacetate **XX**[31] (81%), by transesterification and subsequent treatment with 2,2-dimethoxypropane.

*Synthesis of the hexasaccharide **3** (Scheme 2):* In a preliminary study towards the target **3**, the **DAB(E)C** building block bearing the required acetamido function at position 2_D was used as the donor. It was obtained from the recently described precursor **XX**. [28] Indeed, reductive free-radical dechlorination of **XX** using Bu_3SnH in the presence of catalytic AIBN allowed the conversion of the *N*-trichloroacetyl moiety into *N*-acetyl, to give **XX** (68%). The latter was converted to the hemiacetal **XX** following a two-step process including Iridium complex promoted isomerisation of the allyl moiety into the propen-1-yl, [32] and hydrolysis of the latter upon treatment with aqueous iodine. [33] Subsequent reaction of **XX** with trichloroacetonitrile in the presence of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) cleanly gave the trichloroacetimidate donor **XX** (85% from **XX**). Previous glycosidation attempts in the series indicated that when run at low temperature or room temperature, reactions using the **D** acceptor **XX** occasionally resulted in a rather poor yield of the condensation product. This was tentatively explained by the still rather poor solubility of the acceptor **XX**. When using 1,2-dichloroethane (DCE) as the solvent, the condensation could be performed at higher temperature, which proved rewarding. Indeed, optimized coupling conditions relied on the concomitant use of a catalytic amount of triflic acid in the presence of 4Å molecular sieves as the promoter and DCE as the solvent, while the condensation was performed at 80°C. The fully protected hexasaccharide **XX** was isolated in a satisfactory 78% yield. That the hemiacetal **XX**, resulting from the hydrolysis of the excess donor could be recovered was of great advantage is one considers scaling up the process (not described). Acidic hydrolysis of the isopropylidene acetal smoothly converted **XX** into the corresponding

diol **XX** (94%). Resistance of isolated benzoyl groups to Zemplén transesterification has been reported. [34-36] It was also observed previously in the series, upon attempted removal of a benzoyl group located at position 2_C. [28] Again, the 2_C-*O*-benzoyl group in **XX** was particularly resistant to Zemplén de-*O*-acylation, and in that case, successful transesterification required a week. In that case, heating was avoided in order to prevent any potential migration of the acyl group which would lead to the *N*-deacylated product. Conversion of the hexaol **XX** into the target **3** was successfully accomplished upon concomitant hydrogenolysis of the remaining benzyl protecting group and reduction of the azido moiety into the corresponding amine. As observed earlier, [20,37] the latter was best performed under acidic conditions. The target **3** was isolated in 77% yield after reverse-phase chromatography.

*Synthesis of the decasaccharide **1** (Scheme 3):* Having the fully protected hexasaccharide **XX** in hands, we reasoned that a convenient access to **1** could involve the condensation of an **AB(E)C** tetrasaccharide donor and a **DAB(E)CD** hexasaccharide acceptor prepared from **XX**. Preparation of the former was conveniently achieved from the previously described tetrasaccharide **XX**. [28] Removal of the anomeric allyl protecting group involved a two-step process as described above for the preparation of **XX**. The hemiacetal was readily converted into the trichloroacetimidate donor **XX**, which was isolated in an unoptimized yield of 56% over the two steps. Taking advantage of the stability of the 2_C-*O*-benzoyl group under Zemplén conditions, selective chemical modification at the **D** residue of **XX** was anticipated to give easy access to the selected acceptor **XX**. Indeed, transesterification of the acetyl groups in **XX** gave the expected triol **XX**, which was further regioselectively protected at the 4_D and 6_D hydroxyl groups when treated with 2,2-dimethoxypropane. However, the key acceptor **XX** was isolated in 50% yield only. Condensation of the latter and **XX** was performed in DCE using triflic acid as the promoter. One may note that although the condensation involves the construction of the **C**-**D** linkage, thus somewhat resembling the preparation of the hexasaccharide **XX**, heating was not required and the glycosylation went smoothly at low temperature to give the fully protected decasaccharide **XX** (82%). Acidic hydrolysis of the acetals gave the tetraol **XX** (75%). Transesterification of the acyl groups was best performed by overnight heating of **XX** in methanolic sodium methoxide. Final hydrogenolysis of the benzyl groups and concomitant conversion of the azido group into the corresponding amine gave the target **1** (71% from **XX**).

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Synthesis of the pentadecasaccharide ***2****:* If the synthesis of 2 was to mimic that of **1**, the transformation of the non reducing 3,4,6-tri-*O*-acetyl **D** residue into the corresponding 4,6-*O*-isopropylidene one was to be performed twice. Considering that besides being rather low, the yield of the transformation of **XX** into **XX** was also poorly reproducible, considerable loss of two costly intermediate, namely first the hexasaccharide **XX**, then the undecasaccharide **XX**, was to be expected. The use of a pre-functionalized **DAB(E)C** building block, that could act both as a donor and an acceptor based on appropriate orthogonal protection, was considered as an attractive alternative. Such an intermediate (**XX**) was recently prepared in the laboratory by condensation of an **AB(E)C** tetrasaccharide acceptor (**XX**) [38] to a fully functionalized **D** thioglycoside donor (**XX**), and subsequent free-radical conversion of the *N*-trichloroacetyl into the corresponding acetamide (Scheme 4). [38] Since the condensation of **XX** and **XX** was somewhat low-yielding, another route to **XX** is disclosed herein. It takes advantage of the high-yielding condensation of the tetrasaccharide acceptor **XX** with the known trichloroacetimidate donor **XX**, [39] giving access to the fully protected **XX** (98%), [28] and subsequently to the corresponding acetamido derivative **XX** as described above. Controlled de-*O*-acetylation of **XX** under Zemplén conditions gave the triol **XX**, which was next converted to the corresponding alcohol **XX** upon reaction with 2,2-dimethoxypropane (81% from **XX**). Conventional acetylation at position 3_D then gave the key intermediate **XX** (94%). Transformation of the latter into the trichloroacetimidate donor **XX** (82%) was performed as described for the preparation of **XX** via the hemiacetal intermediate **XX**.

The rather satisfactory yields obtained all along the synthesis of the building block **XX** allowed the targeting of larger sequences. Indeed, when the newly formed pentasaccharide donor **XX** and the spacer-armed **D** acceptor **XX** were heated in DCE in the presence of triflic acid and 4Å molecular sieves as described for the preparation of **XX**, the condensation product was isolated in 78%. The resistance of the two isopropylidene acetals to the harsh acidic conditions of the glycosidation reaction is noteworthy. Selective deacetylation at the 3-OH of the non reducing residue, then gave the **D'AB(E)CD** acceptor **XX** in a yield of 76%, confirming indeed than this route to **XX** was more appropriate than that described above. This two-step glycosidation/deacetylation process was repeated. However, whereas the above mentioned glycosidations required heating, condensation of the hexasaccharide acceptor **XX** and the pentasaccharide donor **XX** in the presence of triflic acid was run at low temperature. Under such conditions, the fully protected undecasaccharide **XX** was isolated in an excellent yield of 90%. Zemplén transesterification at the non reducing 3_D-OH of the latter proved as efficient, and gave the required acceptor **XX** (91%). Condensation of this key intermediate

with the tetrasaccharide trichloroacetimidate donor **XX** was again performed at low temperature, using triflic acid as the promoter. The fully protected pentadecasaccharide **XX** was isolated in a satisfactory yield of 82%. Conversion of **XX** to the target **2** was performed according to the stepwise sequence described for the preparation of **3**. Acidic hydrolysis of the isopropylidene groups afforded the hexaol **XX** (83%). Again, running the transesterification step at high temperature allowed to overcome the resistance of benzoyl groups to Zemplén conditions. Conventional hydrogenolysis of the intermediate **XX**, finally gave the pentadecasaccharide hapten **3** (65% from **XX**).

CONCLUSION

The synthesis of the O-SP of *S. flexneri* Y by way of polycondensation of a tritylated cyanoethylidene tetrasaccharide was reported by others. [40] However, this is to our knowledge the first report on the total synthesis of fully defined oligomeric repeating unit glycosides mimicking the branched bacterial O-SPs in the *S. flexneri* series. The strategy disclosed herein gives access to extended fragments of the O-SP of *S. flexneri* serotype 2a in a spacer-armed form suitable for immunological studies. Indeed, amounts required for the synthesis of fully synthetic oligosaccharide conjugates as potential vaccines targeting *S. flexneri* 2a infection were made available. The preparation of such conjugates is in progress in the laboratory.

ACKNOWLEDGEMENTS

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REFERENCES

(1) Almi *Part 14 of the series Synthesis of ligands related to the O-specific polysaccharides of Shigella flexneri serotype 2a and Shigella flexneri serotype 5a. For part 13, see ref. XX.* **2003**.

(2) Kotloff, K. L.; Winickoff, J. P.; Ivanoff, B.; Clemens, J. D.; Swerdlow, D. L.; Sansonetti, P. J.; Adak, G. K.; Levine, M. M. *Bull. WHO* **1999**, *77*, 651-666.

(3) Ashkenazi, S.; May-Zahav, M.; Sulkes, J.; Samra, Z. *Antimicrob. Agents Chemother.* **1995**, *39*, 819-823.

(4) World; Health; Organisation *WHO Weekly Epidemiol. Rec.* **1997**, *72*, 73-80.

(5) Cohen, D.; Green, M. S.; Block, C.; Rouach, T.; Ofek, I. *J. Infect. Dis.* **1988**, *157*, 1068-1071.

(6) Cohen, D.; Green, M. S.; Block, C.; Slepon, R.; Ofek, I. *J. Clin. Microbiol.* **1991**, *29*, 386-389.

(7) Harding, C. V.; Kihlberg, J.; Elofsson, M.; Magnusson, G.; Unanue, E. R. *J. Immunol.* **1993**, *151*, 2419.

(8) Ishioka, G. Y.; Lamont, A. G.; Thomson, D.; Bulbow, N.; Gaeta, F. C. A.; Sette, A.; Grey, H. M. *J. Immunol.* **1992**, *148*, 2446.

(9) Robbins, J. B.; Chu, C.; Schneerson, R. *Clin. Infect. Dis.* **1992**, *15*, 346-361.

(10) Taylor, D. N.; Trofa, A. C.; Sadoff, J.; Chu, C.; Bryla, D.; Shiloach, J.; Cohen, D.; Ashkenazi, S.; Lerman, Y.; Egan, W.; Schneerson, R.; Robbins, J. B. *Infect. Immun.* **1993**, *61*, 3678-3687.

(11) Passwell, J. H.; Harlev, E.; Ashkenazi, S.; Chu, C.; Miron, D.; Ramon, R.; Farzan, N.; Shiloach, J.; Bryla, D. A.; Majadly, F.; Roberson, R.; Robbins, J. B.; Schneerson, R. *Infect. Immun.* **2001**, *69*, 1351-1357.

(12) Cohen, D.; Ashkenazi, S.; Green, M. S.; Gdalevich, M.; Robin, G.; Slepon, R.; Yavzori, M.; Orr, N.; Block, C.; Ashkenazi, I.; Shemer, J.; Taylor, D. N.; Hale, T. L.; Sadoff, J. C.; Pavliovka, D.; Schneerson, R.; Robbins, J. B. *The Lancet* **1997**, *349*, 155-159.

(13) Goebel, W. F. *J. Exp. Med.* **1940**, *72*, 33.

(14) Goebel, W. F. *J. Exp. Med.* **1939**, *69*, 353.

(15) Pozsgay, V. In *Adv. Carbohydr. Chem. Biochem.*; Horton, D., Ed.; Academic Press: San Diego, 2000; Vol. 56, pp 153-199.

(16) Pozsgay, V.; Chu, C.; Panell, L.; Wolfe, J.; Robbins, J. B.; Schneerson, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194-5197.

(17) Peeters, C. C. A. M.; Lagerman, P. R.; Weers, O. d.; Ooemn, L. A.; Hoogerhout, P.; Beurret, M.; Poolman, J. T. In *Vaccine Protocols*; Robinson, A., Farrar, G., Wiblin, C., Eds.; Humana Press Inc.: Totowa N. J., 1996, pp 111-133.

(18) Benaissa-Trouw, B.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Kraaijeveld, K.; Snippe, H. *Infect. Immun.* **2001**, *69*, 4698-4701.

(19) Mawas, F.; Niggemann, J.; Jones, C.; Corbet, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Infect. Immun.* **2002**, *70*, 5107-5114.

(20) Wright, K. **2003**, *submitted*.

(21) Mulard, L. A.; Nato, F.; Marcel, V.; Thuizat, A.; Sansonetti, P.; Phalipon, A. *in preparation* **2003**.

(22) oligosac *Eur. J. Biochem.* **1982**, *126*, 433.

(23) Simmons, D. A. R. *Bacteriol. Reviews* **1971**, *35*, 117-148.

(24) Lindberg, A. A.; Karnell, A.; Weintraub, A. *Rev. Infect. Dis.* **1991**, *13*, S279-S284.

(25) Mulard, L.; Guerreiro, C.; Costachel, C.; Phalipon, A. *in preparation* **2003**.

(26) Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650-2656.

(27) Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293-299.

(28) Bélot, F.; Wright, K.; Costachel, C.; Phalipon, A.; Mulard, L. A. *J. Org. Chem.* **2003**, *sumitted*.

(29) Bundle, D. R.; Josephson, S. *Can. J. Chem.* **1979**, *57*, 662-668.

(30) Mulard, L. A.; Costachel, C.; Sansonetti, P. J. *J. Carbohydr. Chem.* **2000**, *19*, 849-877.

(31) Hasegawa, A.; Terada, T.; Ogawa, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 319-???

(32) Oltvoort, J. J.; Boeckel, C. A. A. v.; Koning, J. H. d.; Boom, J. v. *Synthesis* **1981**, 305-308.

(33) Nashed, M. A.; Anderson, L. *J. Chem. Soc. Chem. Commun.* **1982**, 1274-1282.

(34) Liptak, A.; Szurmai, Z.; Nanasi, P.; Neszmelyi, A. *Carbohydr. Res.* **1982**, *99*.

(35) Szurmai, Z.; Kerékgyarto, J.; Harangi, J.; Liptak, A. *Carbohydr. Res.* **1987**, *174*, 313-325.

(36) Szurmai, Z.; Liptak, A.; Snatzke, G. *Carbohydr. Res.* **1990**, *200*, 201-208.

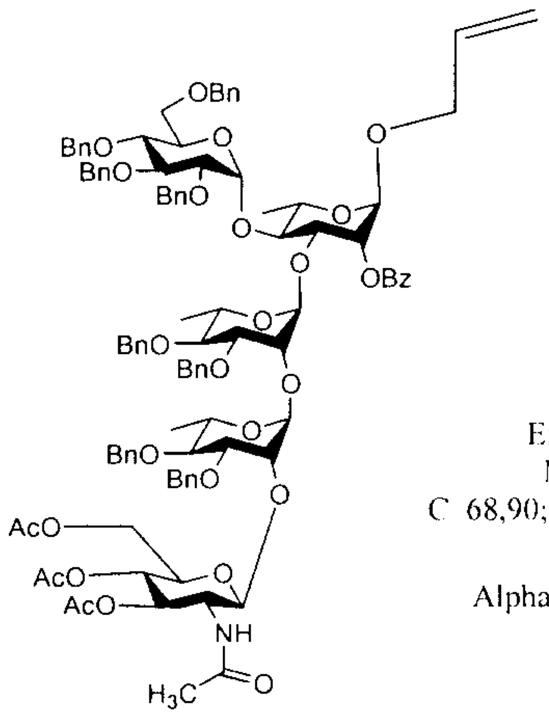
(37) Li, Q.; Li, H.; Lou, Q.-H.; Su, B.; Cai, M.-S.; Li, Z.-J. *Carbohydr. Res.* **2002**, *337*, 1929-1934.

(38) Mulard, L. A.; Bélot, F. *submitted* **2003**.

(39) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189-202.

(40) Kochetkov, N. K.; Byramova, N. E.; Tsvetkov, Y. E.; Backinovsky, L. V. *Tetrahedron* **1985**, *41*, 3363-3375.

LMPP14-exp-brevet-synlongs



$C_{104}H_{117}NO_{27}$
Exact Mass: 1811,78
Mol. Wt.: 1813,03
C 68,90; H 6,50; N 0,77; O 23,83

AlphaD=+3°, C=1, CHCl3

Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (X).

A mixture of **X** (3.14 g, 1.6 mmol), Bu_3SnH (2.5 mL, 9.3 mmol) and AIBN (240 mg) in dry toluene (40 mL) was stirred for 30 min at rt under a stream of dry Ar, then was heated for 1 h at 100°C, cooled and concentrated. The residue was eluted from a column of silica gel with 3:2 petroleum ether-EtOAc to give **X** as a white foam (2.0 g, 68 %); $[\alpha]_D$ +3° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$):δ 8.00-7.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.58 (d, 1H, $J_{2,NH}$ = 8.0 Hz, N-H_D), 5.35 (dd, 1H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.3 Hz, H-2_C), 5.19 (m, 2H, All), 5.10 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 4.92 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 10.5 Hz, H-3_D), 4.92 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 4.90 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_B), 4.89 (d, 1H, H-1_C), 4.88 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4_D), 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 4.90-4.35 (m, 16H, CH_2Ph), 4.40 (m, 1H, H-2_B), 4.10-4.00 (m, 2H, All), 4.08 (dd, 1H, $J_{2,3}$ = 2.4 Hz, H-2_A), 4.02 (dd, 1H, H-3_C), 3.91 (m, 1H, H-2_D), 3.90-3.70 (m, 11H, H-4_C, 5_C, 3_A, 5_A, $6a_D$, $6b_D$, 3_E, 4_E, 5_E, $6a_E$, $6b_E$), 3.61 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3_B), 3.55 (m, 1H, H-5_B), 3.41-3.40 (m, 3H, H-4_A, 5_D, 2_E), 3.47 (m, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.1 Hz, H-5_B), 3.35-3.33 (m, 3H, H-4_A, 5_D, 2_E), 3.25 (dd, 1H, H-4_B), 1.95, 1.70 (3s, 9H,

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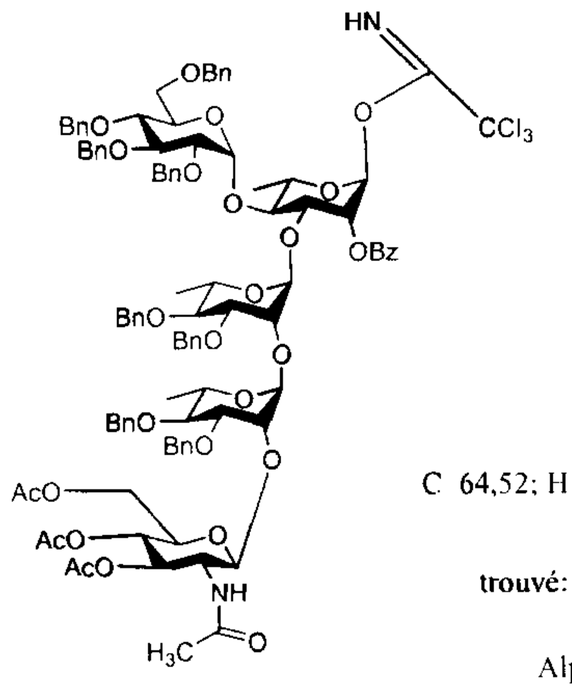
$CH_3C=O$), 1.65 (s, 3H, $CH_3C=ONH$), 1.32 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_A), 1.30 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_C), 0.97 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_B). ^{13}C NMR ($CDCl_3$):δ 171.1, 170.8, 170.2, 169.6, 166.2 (5C, C=O), 138.2-118.5 (Ph, All), 103.1 (C-1_D), 101.4 (C-1_B), 101.2 (C-1_A), 98.5 (C-1_E), 96.4 (C-1_C), 82.2 (C-3_E), 81.7 (C-2_E), 81.7 (C-4_A), 80.4 (C-4_B), 80.2 (C-3_C), 79.0 (C-3_A), 78.6 (C-3_B), 78.1 (C-2_A), 77.8 (C-4_C), 77.6 (C-4_E), 76.0, 75.8, 75.4, 74.7, 74.3, 74.2, 73.3, 70.5 (8C, CH_2Ph), 74.9 (C-2_B), 72.7 (C-2_C), 72.6 (C-3_D), 71.9 (2C, C-5_E, 5_D), 69.1 (C-5_B), 68.9 (2C, All, C-5_A), 68.3 (C-6_E), 67.8 (C-5_C), 62.3 (C-6_D), 54.6 (C-2_D), 23.5 (1C, $NHC=OCH_3$), 21.1, 21.0, 20.8 (3C, $C=OCH_3$), 19.0 (C-6_C), 18.4 (C-6_A), 18.2 (C-6_B). FABMS of $C_{104}H_{117}NO_{27}$ (M, 1913.1), *m/z* 1936.2 $[M+Na]^+$. Anal. Calcd. for $C_{104}H_{117}NO_{27}$: C, 68.90 ; H, 6.50 ; N, 0.77. Found C, 68.64 ; H, 6.66 ; N, 1.05.

LMPP14-exp-brevet-synlongs



$C_{103}H_{113}Cl_3N_2O_{27}$
Exact Mass: 1914,66
Mol. Wt.: 1917,35
C 64,52; H 5,94; Cl 5,55; N 1,46; O 22,53

trouvé: C:64.38%, H:6.10%, N:1.45%

AlphaD=+10°, c=1, CHCl3

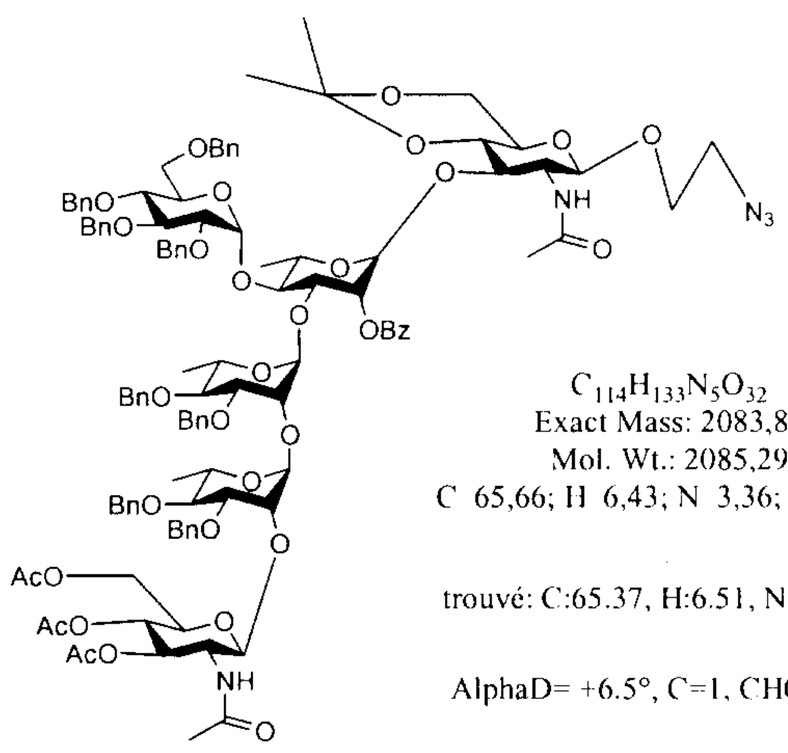
(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (X).

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg, 29 μ mol) was dissolved tetrahydrofuran (5 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **7** (1.0 g, 0.55 mmol) in tetrahydrofuran (10 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated to dryness. The residue was dissolved in acetone (5 mL), then water (1 mL), mercuric chloride (140 mg) and mercuric oxide (120 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 2:1 petroleum ether-EtOAc to give the corresponding hemiacetal. Trichloroacetonitrile (2.5 mL) and DBU (37 μL) were added to a solution of the residue in

anhydrous dichloromethane (12.5 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 5:4 cyclohexane-EtOAc and 0.2 % Et_3N to give **X** as a white foam (0.9 g, 85 %); $[\alpha]_D$ +10° (*c* 1, $CHCl_3$).

^1H NMR ($CDCl_3$):δ 8.70 (s, 1H, C=NH), 8.00-7.00 (m, 45H, Ph), 6.36 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1_C), 5.59 (m, 2H, N-H_D, H-2_C), 5.13 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 5.01-4.98 (m, 2H, H-1_E, 1_B), 4.92 (dd, 1H, H-3_D), 4.90 (dd, 1H, H-4_D), 4.68 (d, 1H, H-1_D), 5.00-4.02 (m, 19H, 8 CH_2Ph, H-3_C, 2_A, 2_B), 4.01 (dd, 1H, H-2_E), 4.00-3.20 (m, 16H, H-3_E, 4_E, 5_E, $6a_E$, $6b_E$, 4_C, 5_C, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A, 5_D, $6a_D$, $6b_D$), 2.02, 2.00, 1.75, 1.65 (4s, 12H, C=OCH_3), 1.40, 1.32 and 1.00 (3d, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR (partial) ($CDCl_3$):δ 170.2, 169.9, 169.3, 168.7, 164.9 (6C, C=O, C=N), 103.2 (C-1_D), 101.4 (2C, C-1_A, 1_B), 99.0 (C-1_E), 94.8 (C-1_C), 21.1, 20.9, 20.8 (3C, CH_3C=O), 19.1, 18.2 (3C, C-6_A, 6_B, 6_C). FABMS of $C_{103}H_{113}Cl_3N_2O_{27}$ (M, 1917.4), *m/z* 1930.9 $[M+Na]^+$. Anal. Calcd. for $C_{103}H_{113}Cl_3N_2O_{27}$: C, 64.52 ; H, 5.94 ; N, 1.46. Found C, 64.47 ; H, 5.99 ; N, 1.45.

LMPP14-exp-brevet-synlongs



$C_{114}H_{133}N_5O_{32}$
Exact Mass: 2083,89
Mol. Wt.: 2085,29
C 65,66; H 6,43; N 3,36; O 24,55

trouvé: C:65.37, H:6.51, N:3.18

AlphaD= +6.5°, C=1, CHCl3

2-Azidoethyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

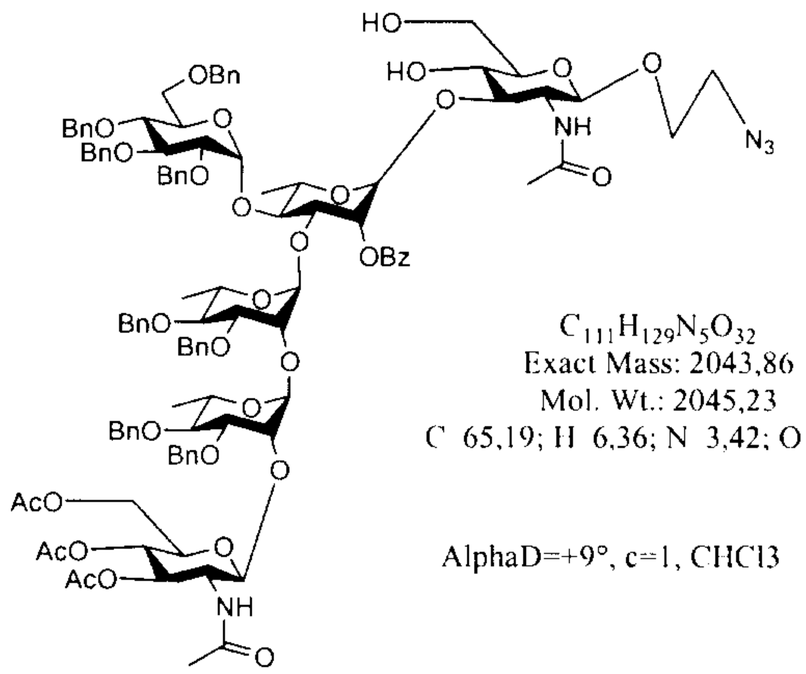
A mixture of alcohol **X** (110 mg, 330 μmol), imidate **X** (720 mg, 376 μmol) and 4Å molecular sieves in anhydrous DCE (6 mL) was stirred for 1 h under dry Ar. After cooling at 0°C, TfOH (16 μL, 180 μmol) was added dropwise and the mixture was stirred at 80°C for 2.5 h. Triethylamine (60 μL) was added and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 3:4 cyclohexane-EtOAc and Et_3N (0.2 %) to give **X** as a colorless oil (540 mg, 78 %); $[\alpha]_D$ +6.5° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$):δ 8.00-7.00 (m, 45H, Ph), 5.95 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH_D), 5.51 (d, 1H, $J_{2,NH}$ = 8.1 Hz, $NH_{D'}$), 5.20 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.08 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 5.05 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.93 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.87 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_B), 4.82 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_C), 4.80 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-$4_{D'}$), 4.76 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-$3_{D'}$), 4.75-4.30 (m, 16H, CH_2Ph), 4.57 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-

$1_{D'}$), 4.35 (dd, 1H, H-2_B), 4.30 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.6 Hz, H-3_D), 4.02 (dd, 1H, $J_{2,3}$ = 2.0 Hz, H-2_A), 4.00-3.60 (m, 16H, H-$6a_D$, $6b_D$, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_C, 4_C, 5_C, 3_B, 3_A, 5_A, $2_{D'}$, $6a_{D'}$, $6b_{D'}$), 3.48 (m, 1H, $J_{4,5}$ = 9.5 Hz, H-5_B), 3.46 (dd, 1H, H-4_D), 3.40 (m, 1H, H-5_D), 3.36 (dd, 1H, H-2_E), 3.35, 3.19 (m, 4H, $OCH_2CH_2N_3$), 3.30 (dd, 1H, H-4_A), 3.19 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-4_B), 3.17 (m, 1H, H-5_D), 3.02 (m, 1H, H-2_D), 1.90-1.60 (6s, 18H, CH_3C=O), 1.33, 1.26 (2s, 6H, $C(CH_3)_2$), 1.27 (d, 1H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.18 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_C), 0.90 (d, 3H, $J_{5,6}$ = 6.1Hz, H-6_B). ^{13}C NMR ($CDCl_3$):δ 172.1, 171.1, 170.8, 170.1, 169.6, 166.2 (6C, C=O), 139.2-127.1 (Ph), 103.05 (C-$1_{D'}$), 101.6 (C-1_B), 101.0 (C-1_A), 100.0 (C-1_D), 98.1 (C-1_E), 97.8 (C-1_C), 82.0 (C-2_E), 81.7, 81.5, 80.2, 78.6, 78.4, 77.9, 77.9 (8C, C-3_E, 4_E, 3_C, 4_C, 3_B, 4_B, 3_A, 4_A), 77.8 (C-2_A), 76.0, 74.6 (2C, C-3_D, $3_{D'}$), 74.0 (C-2_B), 73.4 (C-4_D), 73.3 (C-2_C), 72.2, 71.9 (2C, C-5_D, $5_{D'}$), 68.9, 68.8, 67.7 (3C, C-5_A, 5_B, 5_E), 68.6 (C-$4_{D'}$), 68.5 (C-6_E), 67.5 (C-5_C), 62.6, 62.2 (2C, C-6_D, $6_{D'}$), 59.7 (C-2_D), 54.6 (C-$2_{D'}$), 51.0 (CH_2N_3), 29.5 ($C(CH_3)_2$), 23.9, 23.5, 21.1, 20.9, 20.7 (C=OCH_3), 19.6 ($C(CH_3)_2$), 18.9 (C-6_C), 18.4 (C-6_A), 18.2 (C-6_B).

FABMS of $C_{114}H_{133}N_5O_{32}$ (M, 2085.3), *m/z* 2107.9 $[M+Na]^+$

LMPP14-exp-brevet-synlongs



$C_{111}H_{129}N_5O_{32}$
Exact Mass: 2043,86
Mol. Wt.: 2045,23
C 65,19; H 6,36; N 3,42; O 25,03

AlphaD=+9°, c=1, CHCl3

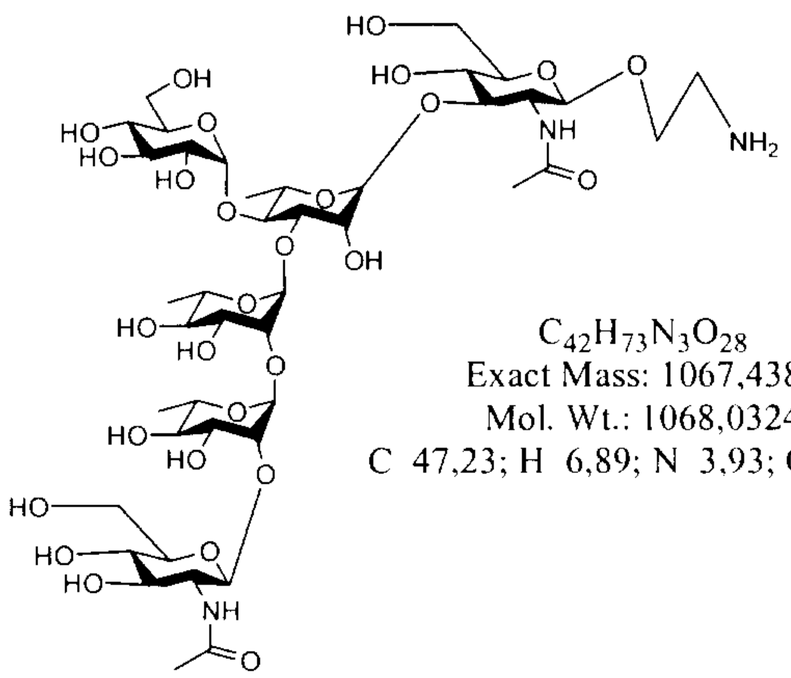
2-Azidoethyl (2,3,4-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

To a solution of **X** (503 mg, 241 μmol) in AcOH (6 mL) was added dropwise, water (1.5 mL) at rt. The mixture was stirred for 1 h at 60°C then concentrated by successive coevaporation with water and toluene. The residue was eluted from a column of silica gel with 1:4 Cyclohexane-EtOAc to give **X** as a white foam (463 mg, 94 %); $[\alpha]_D$ +9° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$):δ 8.00-7.00 (m, 45H, Ph), 5.70 (d, 1H, NH_D), 5.46 (d, 1H, $J_{2,NH}$ = 8.0 Hz, $NH_{D'}$), 5.25 (dd, 1H, H-2_C), 5.05 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1_D), 5.00 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 4.86 (m, 3H, H-1_C, $3_{D'}$, $4_{D'}$), 4.84 (m, 2H, H-1_B, 1_E), 4.56 (d, 1H, H-$1_{D'}$), 4.40 (dd, 1H, H-3_E), 4.35 (dd, 1H, H-2_B), 4.15 (dd, 1H, H-3_D), 4.80-4.00 (m, 16H, CH_2Ph), 4.03 (dd, 1H, H-2_A), 4.00-3.00 (m, 26H, H-4_D, 5_D, $6a_D$, $6b_D$, 2_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_C, 4_C, 5_C, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A, $2_{D'}$, $5_{D'}$, $6a_{D'}$, $6b_{D'}$, $OCH_2CH_2N_3$), 2.99 (m, 1H, H-2_D), 1.85-1.60 (5s, 15H, CH_3C=O), 1.25 and 0.85 (3d, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR (partial) ($CDCl_3$):δ 171.6, 171.4, 170.8, 170.1, 169.6 (C=O), 140.0-127.1 (Ph), 103.1 (C-$1_{D'}$), 101.2 (C-1_A), 99.6 (2C, C-1_E, 1_B), 99.4 (C-1_D), 99.0 (C-1_C), 23.8, 23.5 (2C, NHC=OCH_3), 21.1, 20.9, 20.8 (3 CH_3C=O), 19.1, 18.5, 18.2 (C-

6_A, 6_B, 6_C). FABMS of $C_{111}H_{129}N_5O_{32}$ (M, 2045.2), *m/z* 2067.9 $[M+Na]^+$. Anal. Calcd for $C_{111}H_{129}N_5O_{32}$ C: 65.19, H: 6.36, N: 3.42. Found C: 65.12, H: 6.51, N: 3.41.

LMPP14-exp-brevet-synlongs

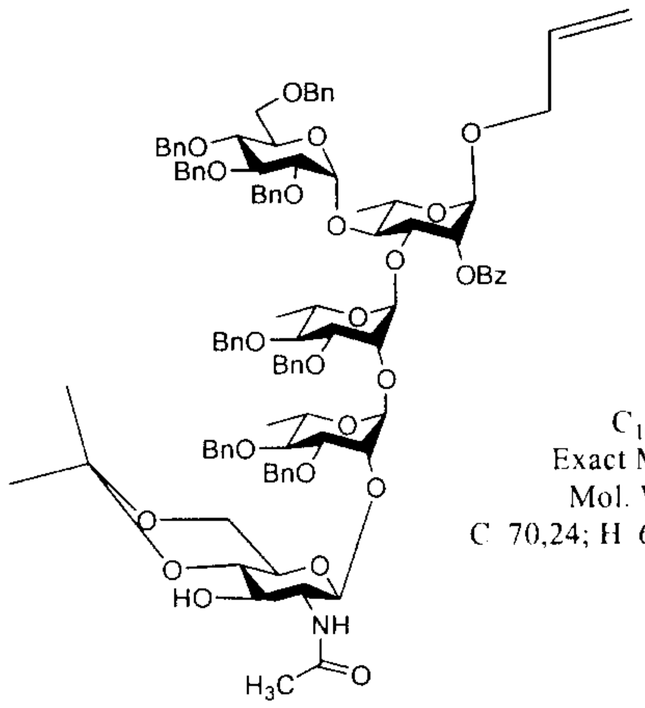


$C_{42}H_{73}N_3O_{28}$
Exact Mass: 1067,4381
Mol. Wt.: 1068,0324
C 47,23; H 6,89; N 3,93; O 41,94

2-Aminoethyl (2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

A mixture of **X** (207 mg, 101 μmol) in MeOH (5 mL) was treated by MeONa until pH=9. The mixture was stirred 1 week at rt. IR 120 (H^+) was added until neutral pH and the solution was filtered and concentrated. The residue was eluted from a column of silica gel with 20:1 to 15:1 DCM-MeOH to give an amorphous residue. A solution of this residue in EtOH (2.2 mL), EtOAc (220 μL), 1M HCl (172 μL, 2 eq) was hydrogenated in the presence of Pd/C (180 mg) for 72 h at rt. The mixture was filtered and concentrated , then was eluted from a column of C-18 with wated and freeze-dried to afford amorphous **X** (81 mg, 77 %); $[\alpha]_D$ -10° (*c* 1, H_2O). ^1H NMR partial (D_2O):δ 5.12 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1_E), 5.07 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_{Rha}), 4.94 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_{Rha}), 4.75 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_{Rha}), 4.63 (d, 1H, $J_{1,2}$ = 8.35 Hz, H-1_{GlcNac}), 4.54 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_{GlcNac}), 1.98 and 1.96 (2s, 6H, 2 CH_3C=ONH), 1.28-1.20 (m, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR partial (D_2O):δ 175.2, 174.8 (C=O), 103.1 (C-$1_{D'}$), 101.6, 101.4 (3C, C-1_A, 1_B, 1_C) 100.8 (C-1_D), 97.9 (C-1_E), 56.2, 55.4 (2C, C-2_D, $2_{D'}$), 22.7, 22.6 (2 NHC=OCH_3), 18.2, 17.2, 17.0 (3C, C-6_A, 6_B, 6_C). HRMS: calculated for $C_{42}H_{73}N_3O_{28}$+Na: 1090.4278. Found 1090.4286.

LMPP14-exp-brevet-synlongs



$C_{101}H_{115}NO_{24}$
Exact Mass: 1725,7809
Mol. Wt.: 1726,9861
C 70,24; H 6,71; N 0,81; O 22,23

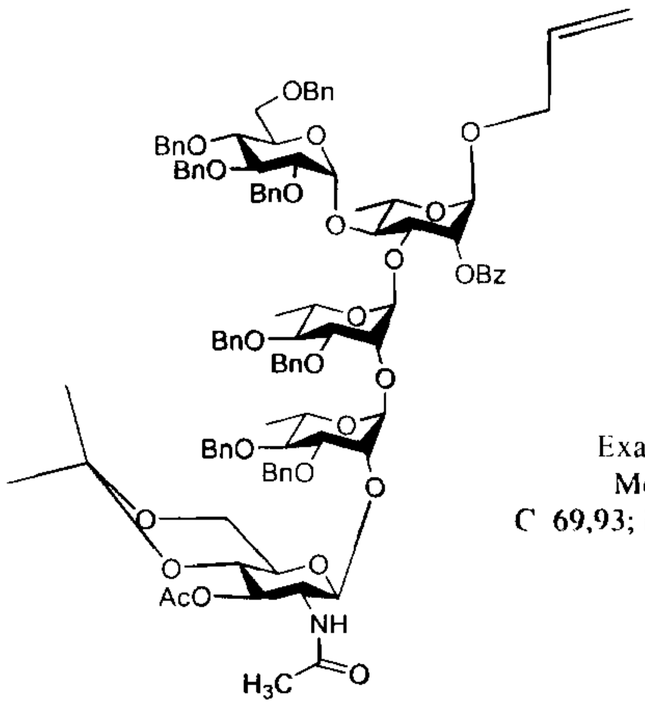
Allyl (2-acetamido-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (X).

The pentasaccharide **X** (2.65 g, 1.47 mmol) was dissolved in MeOH (20 mL). MeONa was added until pH=10. The mixture was stirred for 25 min then treated by IR 120 (H^+) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 9 :1 DCM-MeOH to give the expected triol which was then treated by 2,2-dimethoxypropane (11 mL, 0.1 mol) and APTS (20 mg, 0.17 mmol) in DMF (20 mL) overnight. Et_3N was added and the solution evaporated. The residue was eluted from a column of silica gel with 1:1 Cyclohexane-AcOEt and 0.2 % of Et_3N to give **X** as a white foam (2.05 g, 81 % from **X**); $[\alpha]_D$ +3° (*c* 1, $CHCl_3$).

NMR ($CDCl_3$) : ^1H δ 6.98-8.00 (m, 45H, Ph), 6.17 (bs, 1H, NH_D), 5.82 (m, 1H, All), 5.30 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.11-5.25 (m, 2H, All), 5.06 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.88 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.84 (bs, 1H, H-1_C), 4.35 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.05 (dd, 1H, H-2_A), 3.36 (dd, 1H, H-2_E), 2.90-4.10 (m, 22H, All, H-2_D, 3_A, 3_B, 3_C, 3_D, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.5 (s, 3H, AcNH), 1.2-0.9 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ : 172.7 (C=O),

164.9 (C=O), 137.7-116.7 (Ph, All), 102.3 (C-1_D), 100.2 (C-1_B), 100.0 (C-1_A), 98.9 ($C(CH_3)_2$), 97.2 (C-1_E), 95.1 (C-1_C), 82.1, 82.0, 81.8, 81.6, 80.6, 80.3, 79.0, 78.8, 78.3, 77.8, 77.6, 75.7, 75.6, 75.0, 74.3, 72.8, 71.8, 71.6, 70.8, 70.3, 69.0, 68.5, 67.8, 67.4, 61.9, 60.8, 60.5, 29.4 ($C(CH_3)_2$), 22.7 (AcNH), 19.0 ($C(CH_3)_2$), 18.9, 18.4, 18.2 (3C, C-$6_{A, 6B, 6C}$). FAB-MS for $C_{101}H_{115}NO_{24}$ (M = 1726.9) *m/z* 1749.7 $[M + Na]^+$. Anal. Calcd. for $C_{101}H_{115}NO_{24}.H_2O$: C, 69.52 ; H, 6.76 ; N, 0.80. Found C, 69.59; H 6.71 ; N, 0.57.

LMPP14-exp-brevet-synlongs



$C_{103}H_{117}NO_{25}$
Exact Mass: 1767,7915
Mol. Wt.: 1769,0228
C 69,93; H 6,67; N 0,79; O 22,61

Allyl (2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranoside (X).

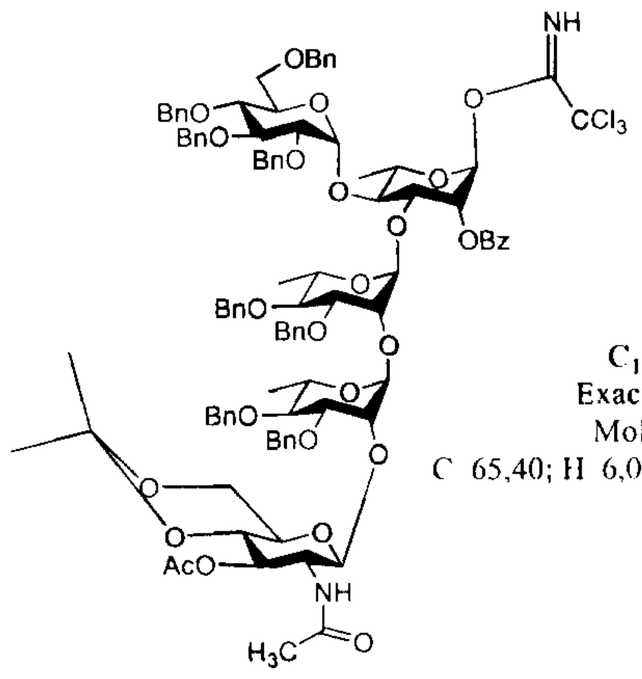
a) A mixture of **X** (2.05 g, 1.19 mmol) in Pyridine (60 mL) was cooled to 0°C. Ac_2O (20 mL) was added and the solution was stirred 2.5 h. The solution was concentrated and coevaporated with toluene. The residue was eluted from a column of silica gel with 2:1 Cyclohexane-AcOEt and 0.2 % of Et_3N to give **X** as a white foam (1.99 g, 94 %); $[\alpha]_D$ +1° (*c* 1, $CHCl_3$).

b) A mixture of **X** (144 mg, 0.06 mmol), Bu_3SnH (0.1 mL, 0.37 mmol) and AIBN (10 mg) in dry toluene (3 mL) was stirred for 1 h at rt under a stream of dry Ar, then was heated for 1.5 h at 90°C, cooled and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc and 0.2 % of Et_3N to give **X** (100 mg, 74 %).

NMR ($CDCl_3$) : ^1H δ 6.95-8.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.46 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH_D), 5.29 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.11-5.25 (m, 2H, All), 5.00 (bs, 1H, H-1_A), 4.90 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.85 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.83 (bs, 1H, H-1_C), 4.70 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.37 (dd, 1H, H-2_E), 2.90-4.10 (m, 21H, All, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.92 (s, 3H, OAc), 1.57 (s,

3H, AcNH), 1.27-0.90 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ 171.3, 170.3, 166.2 (C=O), 138.7-117.9 (Ph, All), 103.9 (C-1_D), 101.5 (C-1_B), 101.4 (C-1_A), 99.9 ($C(CH_3)_2$), 98.5 (C-1_E), 96.3 (C-1_C), 82.1, 81.7, 81.6, 80.3, 80.1, 78.8, 78.1, 77.8, 76.0, 75.8, 75.3, 75.1, 74.7, 74.2, 73.6, 73.3, 72.7, 71.9, 71.4, 70.8, 69.0, 68.8, 68.7, 68.4, 68.1, 67.8, 62.1, 55.0 (C-2_D), 30.0 ($C(CH_3)_2$), 23.5 (AcNH), 21.6 (OAc), 19.2 ($C(CH_3)_2$), 19.0, 18.3, 18.2 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{103}H_{117}NO_{25}$ (M = 1769.0) *m/z* 1791.9 $[M + Na]^+$. Anal. Calcd. for $C_{103}H_{117}NO_{25}$: C, 69.93 ; H, 6.67 ; N, 0.79. Found C, 69.77; H, 6.84; N, 0.72.

LMPP14-exp-brevet-synlongs



$C_{102}H_{113}Cl_3N_2O_{25}$
Exact Mass: 1870,6698
Mol. Wt.: 1873,3452
C 65,40; H 6,08; Cl 5,68; N 1,50; O 21,35

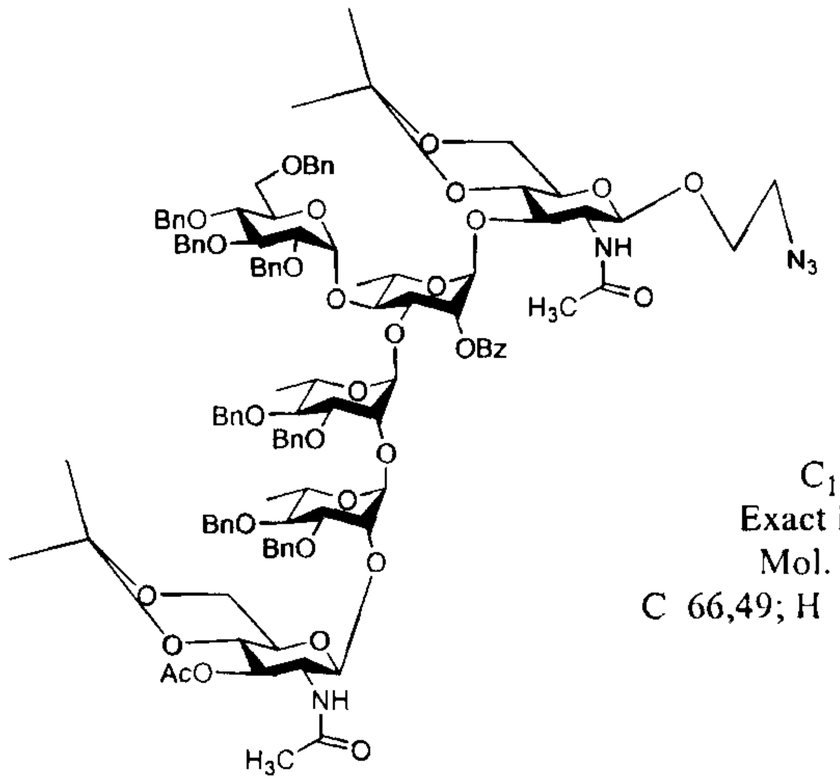
(2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (X).

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 58 μ mol) was dissolved tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **X** (1.8 g, 1.02 mmol) in tetrahydrofuran (20 mL) was degassed and added. The mixture was stirred at rt overnight then concentrated to dryness. The residue was dissolved in acetone (9 mL), then water (2 mL), mercuric chloride (236 mg) and mercuric oxide (200 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and Satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give the corresponding hemiacetal. Trichloroacetonitrile (2.4 mL) and DBU (72 μL) were added to a solution of the residue in anhydrous dichloromethane (24 mL) at 0°C. After 1 h, the mixture was concentrated. The

residue was eluted from a column of silica gel with 3:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give **X** as a colorless oil (1.58 g, 82 %); $[\alpha]_D$ +2° (*c* 1, $CHCl_3$).

NMR ($CDCl_3$) : ^1H δ 8.62 (s, 1H, C=NH), 6.95-8.00 (m, 45H, Ph), 6.24 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1_C), 5.48 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.41 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH_D), 4.99 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.88 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.69 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.38 (dd, 1H, H-2_E), 2.90-4.10 (m, 19H, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.95 (s, 3H, OAc), 1.55 (s, 3H, AcNH), 1.30-0.85 (m, 15H, $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ 172.4, 171.4, 166.9 (C=O), 140.2-128.9 (Ph), 104.2 (C-1_D), 101.4 (2C, C-1_A, 1_B), 101.1 ($C(CH_3)_2$), 98.0 (C-1_E), 94.8 (C-1_C), 92.4 (CCl_3), 82.1, 81.5, 80.2, 80.1, 78.6, 78.1, 77.8, 77.6, 76.0, 75.8, 75.5, 75.0, 74.3, 74.2, 73.5 (C-3_D), 73.4, 71.9, 71.4, 71.0, 70.5, 69.2, 68.8, 68.3, 68.1, 62.1, 54.9 (C-2_D), 29.3 ($C(CH_3)_2$), 23.4 (AcNH), 21.4 (OAc), 19.2 ($C(CH_3)_2$), 19.0, 18.2, 18.1 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{102}H_{113}Cl_3N_2O_{25}$ (M = 1873.3) *m/z* 1896.3 $[M + Na]^+$. Anal. Calcd. for $C_{102}H_{113}Cl_3N_2O_{25}$: C, 65.40 ; H, 6.08 ; N, 1.50. Found C, 65.26; H, 6.02; N, 1.31.

LMPP14-exp-brevet-synlongs



$C_{113}H_{133}N_5O_{30}$
Exact Mass: 2039,9035
Mol. Wt.: 2041,2808
C 66,49; H 6,57; N 3,43; O 23,51

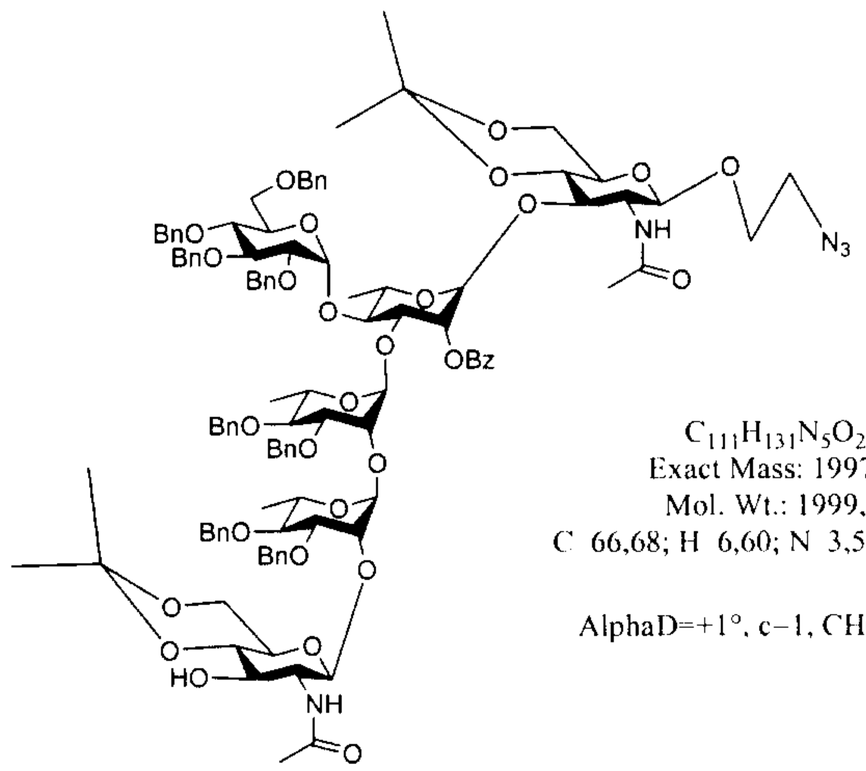
2-Azidoethyl (2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

A mixture of donor **X** (745 mg, 0.4 mmol) and acceptor **X** (170 mg, 0.51 mmol), 4 Å molecular sieves and dry 1,2-DCE (12 mL), was stirred for 1 h then cooled to 0°C. Triflic acid (25 µL) was added. The stirred mixture was allowed to reach rt in 10 min then stirred again for 2.5 h at 75°C. After cooling to rt, Et_3N (100 µL) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 1:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give **X** as a white foam (615 mg, 76 %); $[\alpha]_D$ +0° (*c* 1, $CHCl_3$).

NMR ($CDCl_3$) : ^1H δ 6.95-7.90 (m, 45H, Ph), 6.02 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH_D), 5.46 (d, 1H, $J_{2,NH}$ = 8.6 Hz, $NH_{D'}$), 5.20 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.03 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1_D), 5.02 (bs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.85 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.82 (bs, 1H, H-1_C), 4.70 (dd, 1H, H-$3_{D'}$), 4.44 (d, 1H, H-$1_{D'}$), 4.30 (dd, 1H, H-2_B), 4.20-4.80 (m, 16H, CH_2Ph), 3.99 (dd, 1H, H-2_A), 3.37 (dd, 1H, H-2_E), 2.90-3.95 (m, 29H, H-2_D, $2_{D'}$, 3_A, 3_B, 3_C, 3_D, 3_E, 4_A, 4_B, 4_C, 4_D, $4_{D'}$, 4_E, 5_A, 5_B, 5_C, 5_D, $5_{D'}$, 5_E, $6a_D$, $6b_D$, $6a_{D'}$, $6b_{D'}$, $6a_E$, $6b_E$, $OCH_2CH_2N_3$), 2.00 (s, 3H, AcNH), 1.92 (s, 3H, OAc), 1.57 (s, 3H, AcNH), 1.27-0.90 (m,

21H, 2 $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C δ 172.1, 171.5, 170.3, 166.2 (C=O), 139.0-127.7 (Ph), 103.9 (C-$1_{D'}$), 101.7 (C-1_B), 101.2 (C-1_A), 100.0 (C-1_D), 99.9, 99.8 (2C, $C(CH_3)_2$), 98.3 (C-1_E), 97.8 (C-1_C), 82.0, 81.7, 81.5, 80.8, 80.2, 80.1, 78.9, 78.6, 78.0, 77.9, 76.0, 75.9, 75.8, 75.3, 74.8, 74.6, 74.2, 74.0, 73.6, 73.5, 73.4, 73.0, 71.9, 71.4, 70.8, 69.1, 69.0, 68.8, 68.6, 68.0, 67.7, 67.6, 62.6, 62.1, 60.8, 59.7 (C-2_D), 55.0 (C-$2_{D'}$), 51.1 ($O(CH_2)_2N_3$), 29.5 ($C(CH_3)_2$), 29.3 ($C(CH_3)_2$), 23.9 (AcNH), 23.5 (AcNH), 21.3 (OAc), 19.7 ($C(CH_3)_2$), 19.2 ($C(CH_3)_2$), 18.8, 18.4, 18.2 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{113}H_{133}N_5O_{30}$ (M = 2041.3) *m/z* 2064.2 $[M + Na]^+$. Anal. Calcd. for $C_{113}H_{133}N_5O_{30}$: C, 66.49 ; H, 6.57 ; N, 3.43. Found C, 65.93; H, 6.57; N, 2.61.

LMPP14-exp-brevet-synlongs



$C_{111}H_{131}N_5O_{29}$
Exact Mass: 1997,89
Mol. Wt.: 1999,24
C 66,68; H 6,60; N 3,50; O 23,21

AlphaD=+1°, c=1, CHCl3

2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

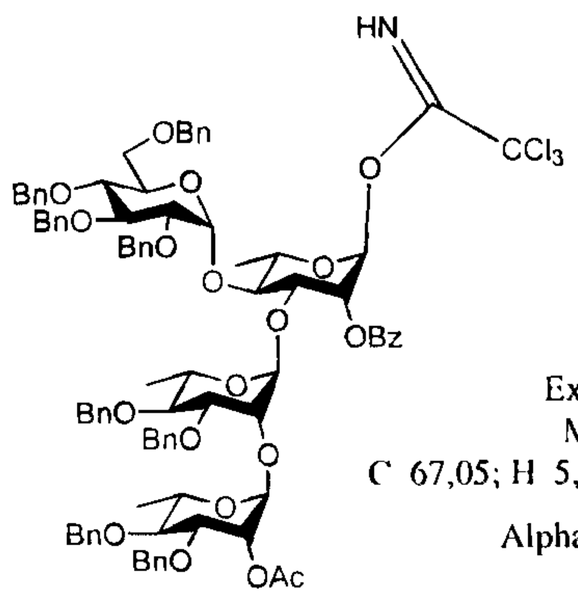
a) The hexasaccharide **X** (615 mg, 0.30 mmol) was dissolved in MeOH (8 mL). MeONa was added until pH=9. The mixture was stirred for 3 h then treated by IR 120 (H^+) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 25:1 DCM-MeOH and 0.2 % of Et_3N to give **X** as a white foam (590 mg, 97 %); $[\alpha]_D$ +1° (*c* 1, $CHCl_3$).

b) To a mixture of **X** (770 mg, 370 μmol) in MeOH (5 mL) was added MeONa until pH=9. The solution was stirred for 40 min. Amberlite IR 120 (H^+) was added until neutral pH and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 20:1 DCM-MeOH and Et_3N to give a residue which was dissolved in DMF (2 mL). The mixture was treated by 2-methoxypropene (200 μL, 2.1 mmol) and CSA (20 mg) at rt. After 1 h, more 2-methoxypropene (200 μL) was added and the mixture was stirred 1 h. Et_3N (160

μL) was added and the solution was concentrated. The residue was eluted from a column of silica gel with 2:3 toluene-EtOAc and Et_3N (0.2 %) to give **X** (400 mg, 54%).

^1H NMR ($CDCl_3$):δ 8.00-7.00 (m, 45H, Ph), 6.10 (d, 1H, $NH_{D'}$), 6.05 (d, 1H, $J_{2,NH}$ = 7.4 Hz, NH_D), 5.20 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.10 (d, 1H, $J_{1,2}$ = 1.0Hz, H-1_A), 4.99 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.96 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.90 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_B), 4.86 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_C), 4.52 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-$1_{D'}$), 4.37 (dd, 1H, H-2_B), 4.22 (dd, 1H, H-3_D), 4.02 (dd, 1H, H-2_A), 4.80-4.00 (m, 16H, CH_2Ph), 4.00-2.95 (m, 30H, H-2_D, 4_D, 5_D, $6a_D$, $6b_D$, 2_E, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_C, 4_C, 5_C, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A, $2_{D'}$, $3_{D'}$, $4_{D'}$, $5_{D'}$, $6a_{D'}$, $6b_{D'}$, $OCH_2CH_2N_3$), 2.00-0.92 (6s, 3d, 27H, 2 CH_3C=O, 2 $C(CH_3)_2$, H-6_A, 6_B, 6_C). ^{13}C NMR ($CDCl_3$) partial: δ 173.9, 172.1, 166.3 (C=O), 140.0-125.0 (Ph), 103.6 (C-$1_{D'}$), 101.7 (C-1_B), 101.2 (C-1_A), 100.2 ($C(CH_3)_2$), 100.2 (C-1_D), 99.9 ($C(CH_3)_2$), 98.2 (C-1_E), 97.8 (C-1_C), 29.4, 29.3, 23.9, 22.8, 19.6, 19.2, 18.9, 18.4, 18.2 (C-6_A, 6_B, 6_C, 2 CH_3C=O, 2 $C(CH_3)_2$). FAB-MS for $C_{111}H_{131}N_5O_{29}$ (M = 1999.2) *m/z* 2021.8 $[M + Na]^+$. Anal. Calcd. for $C_{111}H_{131}N_5O_{29}$: C, 66.68 ; H, 6.60 ; N, 3.50. Found C, 66.63 ; H, 6.78 ; N, 3.32.

LMPP14-exp-brevet-synlongs



$C_{91}H_{96}Cl_3NO_{20}$
Exact Mass: 1627,56
Mol. Wt.: 1630,09
C 67,05; H 5,94; Cl 6,52; N 0,86; O 19,63

AlphaD=+22° C=1, CHCl3

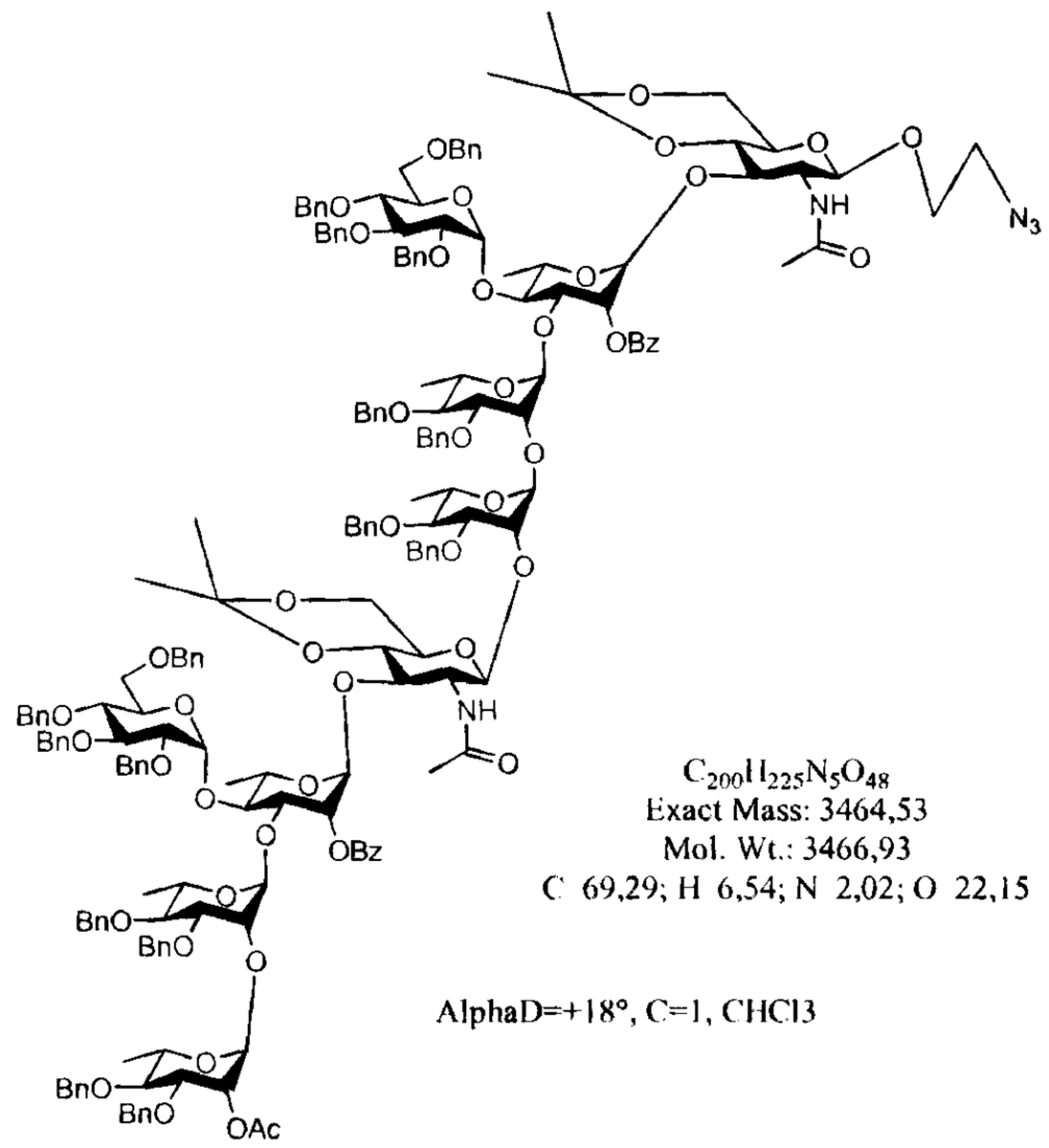
(2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (X).

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (80 mg, 93 μ mol) was dissolved tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **X** (2.55 g, 1.67 mmol) in tetrahydrofuran (20 mL) was degassed and added. The mixture was stirred at rt overnight then concentrated to dryness. The residue was dissolved in acetone (15 mL), then water (3 mL), mercuric chloride (380 mg) and mercuric oxide (320 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:1 petroleum ether-EtOAc to give the corresponding hemiacetal. Trichloroacetonitrile (2.0 mL) and DBU (25 μL) were added to a solution of the residue in anhydrous dichloromethane (15 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:1 petroleum ether-EtOAc and 0.2 %

Et_3N to give **X** as a white foam (1.5 g, 56 %); $[\alpha]_D$ +22° (*c* 1, $CHCl_3$).

^1H NMR ($CDCl_3$):δ 8.72 (s, 1H, C=NH), 8.00-7.00 (m, 45H, Ph), 6.39 (d, 1H, $J_{1,2}$ = 2.5 Hz, H-1_C), 5.60 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.58 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.0 Hz, H-2_A), 5.12 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.08 (m, 2H, H-1_A, 1_B), 5.00-4.00 (m, 16H, CH_2Ph), 4.20 (dd, 1H, H-3_C), 4.05 (dd, 1H, H-3_E), 4.00-3.35 (m, 14H, H-2_E, 4_E, 5_E, $6a_E$, $6b_E$, 4_C, 5_C, 2_B, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A), 2.05 (s, 3H, C=OCH_3), 1.42, 1.36 and 1.00 (3d, 9H, H-6_A, 6_B, 6_C). ^{13}C NMR ($CDCl_3$):δ 170.3, 165.8 (C=O), 138-127 (Ph), 99.9 (2C, C-1_A, 1_B), 98.5 (C-1_E), 94.7 (C-1_C), 82.1, 81.2, 80.4, 80.0, 79.1, 78.1, 78.0, 75.2, 71.7, 71.2, 70.7, 69.5, 69.4, 68.7 (16C, C-2_A, 3_A, 4_A, 5_A, 2_B, 3_B, 4_B, 5_B, 2_C, 3_C, 4_C, 5_C, 2_E, 3_E, 4_E, 5_E), 76.0, 75.7, 75.5, 75.1, 74.3, 73.3, 72.2, 71.2 (8C, PhCH_2), 68.5 (C-6_E), 21.4 (C=OCH_3), 19.2, 18.5, 18.1 (C-6_A, 6_B, 6_C). Anal. Calcd. for $C_{91}H_{96}Cl_3NO_{20}$: C, 67.05 ; H, 5.94 ; N, 0.86. Found C, 66.44 ; H, 6.21 ; N, 0.93.

LMPP14-exp-brevet-synlongs



$C_{200}H_{225}N_5O_{48}$
Exact Mass: 3464,53
Mol. Wt.: 3466,93
C 69,29; H 6,54; N 2,02; O 22,15

AlphaD=+18°, C=1, CHCl3

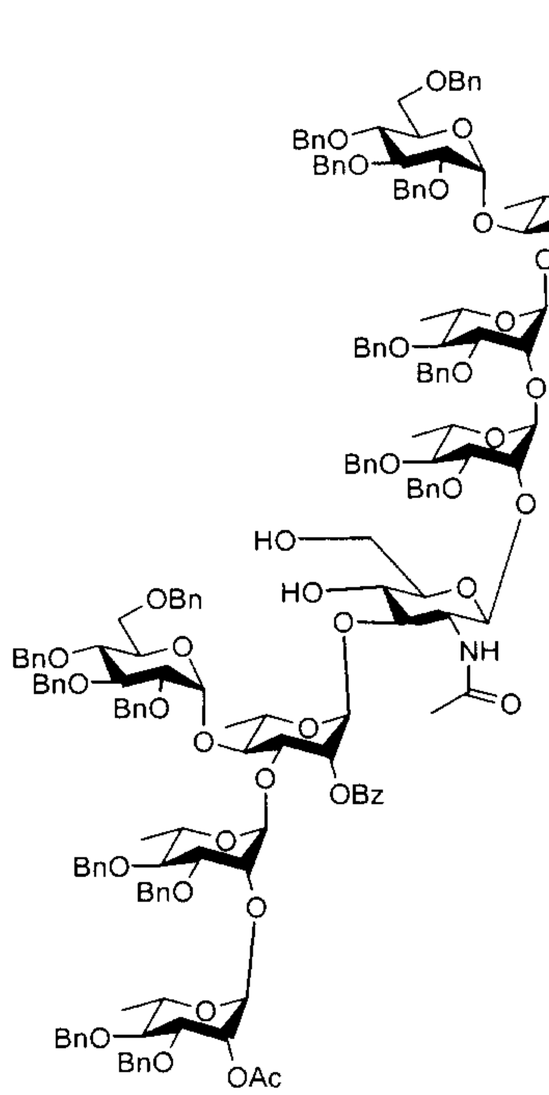
2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

A mixture of alcohol **X** (110 mg, 55 μmol), imidate **X** (179 mg, 110 μmol) and 4Å molecular sieves in anhydrous DCE (2.5 mL) was stirred for 1 h under dry Ar. After cooling at -35°C, TfOH (5 μL, 50 μmol) was added dropwise and the mixture was stirred for 2.5 h and allowed to reach 10°C. Et_3N (25 μL) was added and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 4:1 to 3:1 toluene-EtOAc and Et_3N (0.2 %) to give **X** as a white foam (158 mg, 82 %); $[\alpha]_D$ +18° (*c* 1, $CHCl_3$).

LMPP14-exp-brevet-synlongs

^{1}H NMR ($CDCl_3$):δ 8.00-6.90 (90H, m, Ph), 5.90 (d, 1H, $J_{2,NH}$ = 7.0 Hz, N-H_D), 5.58 (d, 1H, $J_{2,NH}$ = 7.5 Hz, N-$H_{D'}$), 5.45, 5.22 (m, 2H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.0 Hz, H-2_C, $2_{C'}$), 5.12 (dd, 1H, H-$2_{A'}$), 5.11 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.05 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_A), 5.01 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.96 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1_C), 4.94 (m, 2H, H-1_E, 1_B), 4.86 (d, 1H, H-1_B), 4.82 (d, 1H, H-1_C), 4.72 (d, 1H, H-$1_{D'}$), 4.70 (d, 1H, H-$1_{A'}$), 4.90-4.20 (m, 36H, 16 OCH_2Ph, H-2_B, $2_{B'}$, 3_D, $3_{D'}$), 4.00-2.90 (m, 45H, H-2_D, 4_D, 5_D, $6a_D$, $6b_D$, 3_C, 4_C, 5_C, 2_E, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_B, 4_B, 5_B, 2_A, 3_A, 4_A, 5_A, $2_{D'}$, $4_{D'}$, $5_{D'}$, $6a_{D'}$, $6b_{D'}$, $3_{C'}$, $4_{C'}$, $5_{C'}$, $2_{E'}$, $3_{E'}$, $4_{E'}$, $5_{E'}$, $6a_{E'}$, $6b_{E'}$, $3_{B'}$, $4_{B'}$, $5_{B'}$, $3_{A'}$, $4_{A'}$, $5_{A'}$, $OCH_2CH_2N_3$), 2.00 (s, 3H, AcNH), 1.88 (s, 3H, OAc), 1.86 (s, 3H, AcNH), 1.40-0.82 (m, 30H, 6 H-6_{Rha}, 2 C(CH_3)$_2$). ^{13}C NMR (partial) ($CDCl_3$):δ 172.1, 171.4, 170.2, 166.2, 165.9 (C=O), 102.7 (C-$1_{D'}$), 101.6, 101.2 (2C, C-1_B, $1_{B'}$), 101.1 (C-1_A), 99.8 (C-1_D), 99.7 (C-1_C), 98.2 (2C, C-1_E, $1_{A'}$), 97.2 (2C, C-1_C, 1_E), 63.3, 62.6 (2C, C-6_E, $6_{E'}$), 60.0, 57.8 (2C, C-2_D, $2_{D'}$), 51.0 ($OCH_2CH_2N_3$), 29.5, 29.4 (2C, C(CH_3)$_2$), 24.0 (2C, 2 AcNH), 21.3 (AcO), 19.6, 19.5 (2C, C(CH_3)$_2$), 19.1, 18.9, 18.8, 18.5, 18.2, 18.1 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$). FABMS of $C_{200}H_{225}N_5O_{48}$ (M, 3446.9), *m/z* 3489.5 ([M+Na]$^+$). Anal. Calcd for $C_{200}H_{225}N_5O_{48}$ +5H_2O, C: 67.47, H: 6.65, N: 1.96. Found C: 67.40, H: 6.57, N: 1.72.

LMPP14-exp-brevet-synlongs



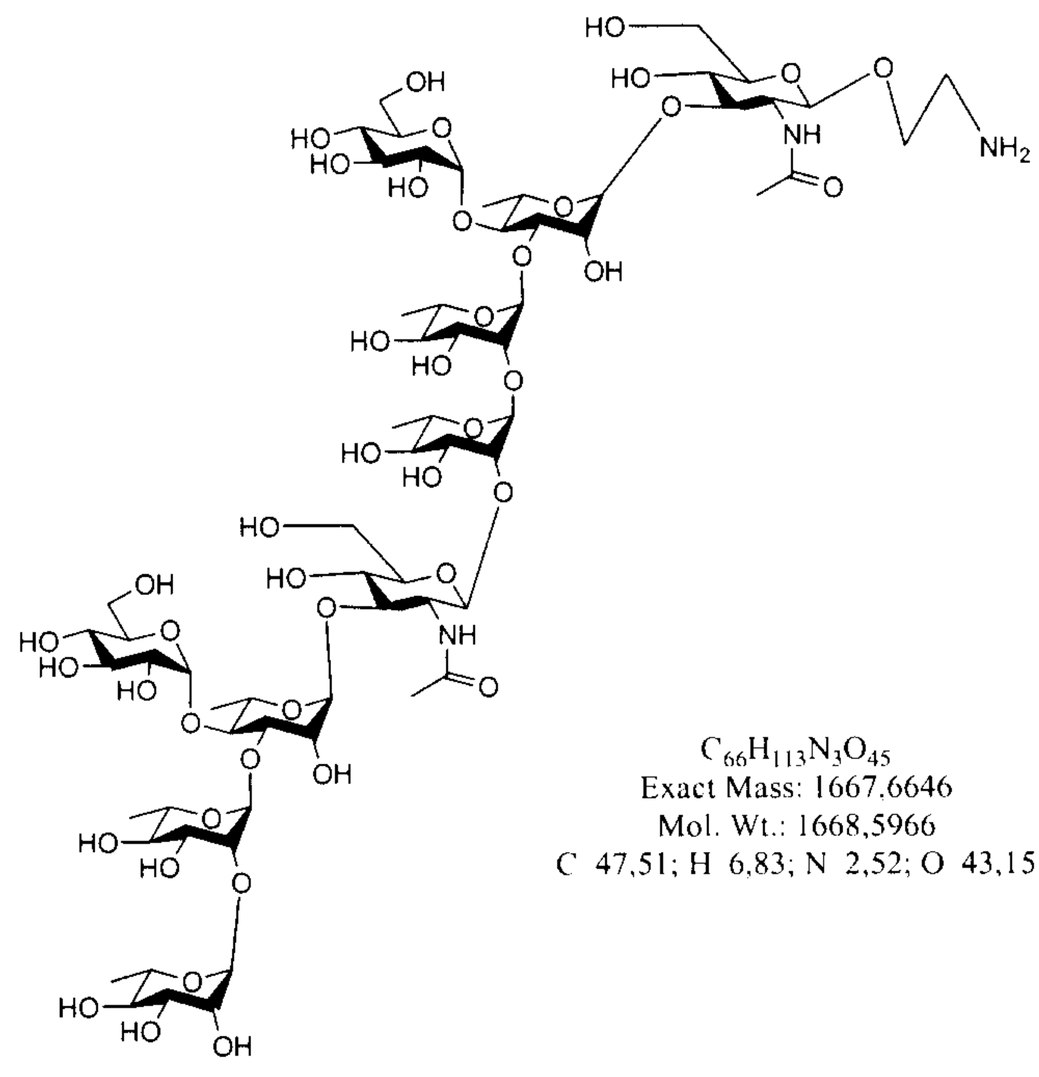
$C_{194}H_{217}N_5O_{48}$
Exact Mass: 3384,47
Mol. Wt.: 3386,80
C 68,80; H 6,46; N 2,07; O 22,68

AlphaD=+9°, C=1 CHCl3

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

To a solution of **X** (630 mg, 181 μmol) in DCM (12 mL) was added dropwise, at 0°C, a solution of TFA (2 mL) and water (2 mL). The mixture was stirred for 3 h then concentrated by coevaporation with successively water and toluene. The residue was eluted from a column of silica gel with 1:1 toluene-EtOAc to give **X** as a white foam (460 mg, 75 %); $[\alpha]_D$ +9° (*c* 1, $CHCl_3$). FABMS of $C_{194}H_{217}N_5O_{48}$ (M, 3386.8), *m/z* 3409.2 ($[M+Na]^+$). Anal. Calcd for $C_{194}H_{217}N_5O_{48}$ +H_2O, C: 68.43, H: 6.45, N: 2.06. Found C: 68.40, H: 7.02, N: 1.61.

LMPP14-exp-brevet-synlongs



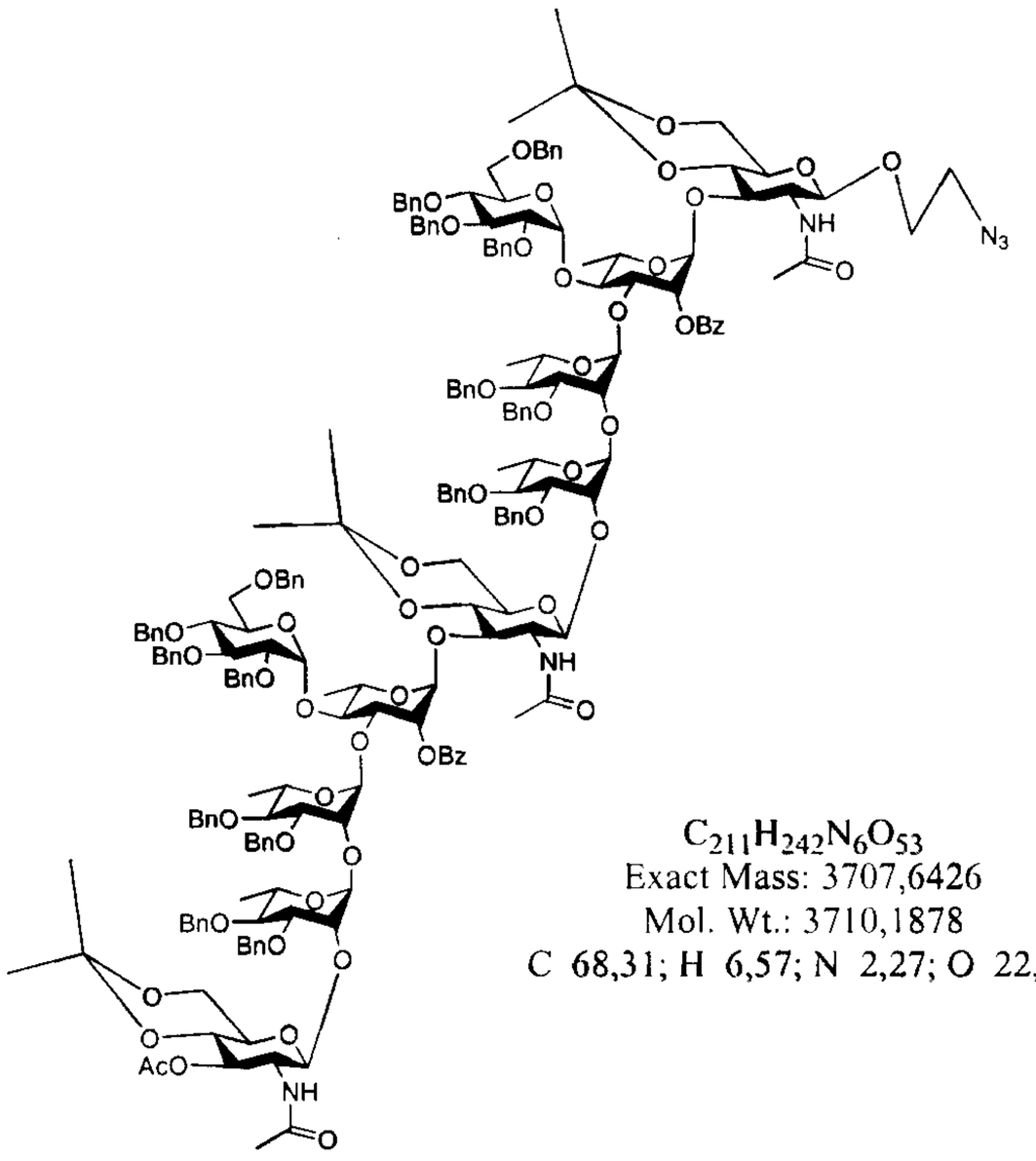
$C_{66}H_{113}N_3O_{45}$
Exact Mass: 1667,6646
Mol. Wt.: 1668,5966
C 47,51; H 6,83; N 2,52; O 43,15

2-Aminoethyl (α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

A mixture of **X** (130 mg, 38 μmol) in MeOH (4 mL) was treated by MeONa until pH=9. The mixture was stirred 1 h at rt, then heated at 55°C and stirred overnight. After cooling at rt, IR 120 (H^+) was added until neutral pH and the solution was filtered and concentrated. The residue was eluted from a column of silica gel with 25:1 to 20:1 DCM-MeOH to give an amorphous residue. A solution of this residue in EtOH (1.5 mL), EtOAc (150 μL), 1M HCl (66 μL, 2 eq) was hydrogenated in the presence of Pd/C (100 mg) for 72 h at rt. The mixture

was filtered and concentrated , then was eluted from a column of C-18 with wated and freeze-dried to afford amorphous **X** as a white foam (41 mg, 71 %); $[\alpha]_D$ -7° (*c* 1, H_2O). ^1H NMR partial (D_2O):δ 4.90 (m, 2H, $J_{1,2}$ = 3.5 Hz, 2 H-1_E), 4.82 (bs, 1H, H-1_{Rha}), 4.76 (bs, 1H, H-1_{Rha}), 4.72 (bs, 1H, H-1_{Rha}), 4.67 (bs, 1H, H-1_{Rha}), 4.52 (bs, 1H, H-1_{Rha}), 4.51 (bs, 1H, H-1_{Rha}), 4.41 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_{GlcNac}), 4.29 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_{GlcNac}), 1.77 (s, 6H, 2 CH_3C=ONH), 1.15-0.96 (m, 18H, H-6_{Rha}). ^{13}C NMR partial (D_2O):δ 174.8, 174.7 (C=O), 102.9 (C-1_{Rha}), 102.6 (C-1_{GlcNac}), 101.8 (2C, 2 C-1_{Rha}), 101.6 (C-1_{Rha}), 101.4 (C-1_{Rha}), 101.3 (C-1_{Rha}), 100.8 (C-1_{GlcNac}), 97.9 (2C, 2 C-1_{Glc}), 56.0, 56.4 (2 C, 2 C_{GlcNac}), 22.7, 22.6 (2 NHC=OCH_3), 18.2, 17.2, 17.0, 16.9 (6C, 6 C-6_{Rha}). HRMS: calculated for $C_{66}H_{113}N_5O_{45}$+Na: 1690.6544. Found 1690.6537.

LMPP14-exp-brevet-synlongs



$C_{211}H_{242}N_6O_{53}$
Exact Mass: 3707,6426
Mol. Wt.: 3710,1878
C 68,31; H 6,57; N 2,27; O 22,86

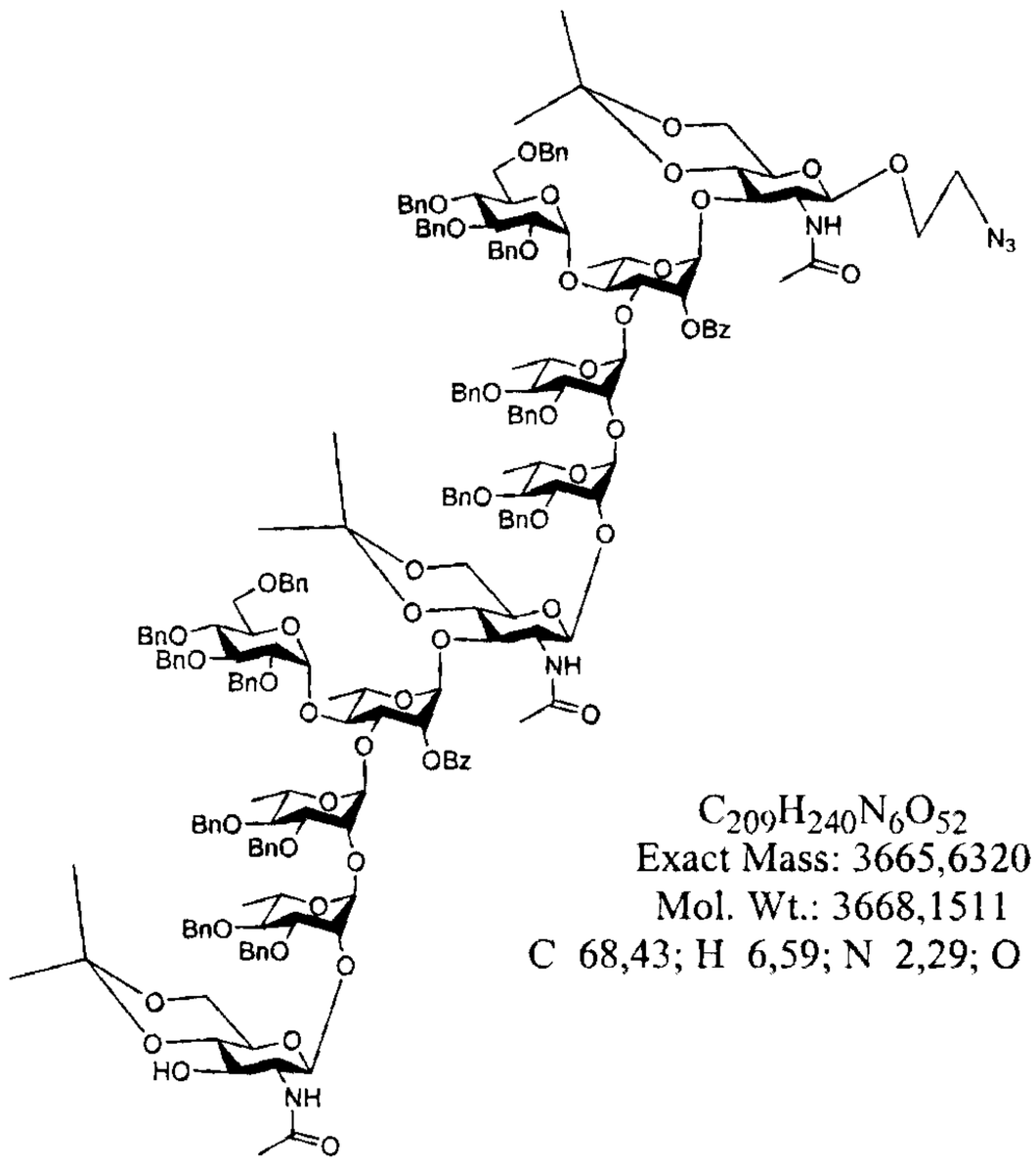
2-Azidoethyl (2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

A mixture of donor **X** (835 mg, 0.44 mmol) and acceptor **X** (590 mg, 0.3 mmol), 4 Å molecular sieves and dry 1,2-DCE (12 mL), was stirred for 1 h then cooled to -30°C. Triflic acid (35 μL) was added. The stirred mixture was allowed to reach 5°C in 2.5 h. Et_3N (150 μL) was added and the mixture filtered. After evaporation, the residue was eluted from a column

of silica gel with 1:2 Cyclohexane-AcOEt and 0.2 % Et_3N to give **X** as a white foam (990 mg, 90 %); $[\alpha]_D$ +10° (*c* 1, $CHCl_3$).

^1H NMR ($CDCl_3$): partial δ 6.95-7.90 (m, 90H, Ph), 5.98 (d, 1H, $J_{2,NH}$ = 6.9 Hz, NH_D), 5.60 (d, 1H, $J_{2,NH}$ = 7.5 Hz, NH_D), 5.45 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH_D), 5.22 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.13 (dd, 1H, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.08 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.07 (bs, 1H, H-1_A), 5.04 (bs, 1H, H-1_A), 4.97 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1_E), 4.94 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1_E), 4.90 (bs, 1H, H-1_B), 4.86 (bs, 1H, H-1_B), 4.82 (bs, 1H, H-1_C), 4.73 (d, 1H, H-1_D), 4.70 (bs, 1H, H-1_C), 4.43 (d, 1H, H-1_D), 4.20-4.80 (m, 16H, CH_2Ph), 2.00, 1.85, 1.58 (3s, 9H, AcNH), 1.95 (s, 3H, OAc), 1.37-0.85 (m, 36H, 3 $C(CH_3)_2$, 2H-6_A, 2H-6_B, 2H-6_C). ^{13}C NMR ($CDCl_3$) partial: δ 171.7, 170.8, 169.8, 165.8, 165.4 (C=O), 139.0-127.7 (Ph), 103.9 (C-1_D), 102.8 (C-1_D), 101.5 (2C, C-1_B), 101.3 (C-1_A), 101.1 (C-1_A), 100.0 (C-1_D), 99.5, 99.3 (3 $C(CH_3)_2$), 98.3 (C-1_E), 98.1 (2C, C-1_C, 1_E), 97.8 (C-1_C), 82.0, 81.7, 81.6, 81.4, 80.3, 80.2, 80.1, 79.5, 79.2, 78.9, 78.7, 78.4, 78.1, 77.9, 77.8, 77.6, 76.0, 75.8, 75.3, 75.2, 74.7, 74.4, 74.1, 74.0, 73.6, 73.5, 73.4, 73.3, 73.0, 72.7, 71.9, 71.4, 70.9, 70.8, 69.1, 69.0, 68.9, 68.7, 68.6, 68.5, 68.1, 67.8, 67.7, 67.5, 62.6, 62.3, 62.1, 60.8, 59.9 (C-2_D), 57.9 (C-2_D), 55.0 (C-2_D), 51.1 ($O(CH_2)_2N_3$), 29.5, 29.4, 29.3 (3 $C(CH_3)_2$), 24.0, 23.9, 23.5 (3 AcNH), 21.3 (OAc), 19.7, 19.6, 19.2 (3 $C(CH_3)_2$), 18.9, 18.8, 18.6, 18.5, 18.2, 18.1 (6C, 2 C-6_A, 6_B, 6_C). FAB-MS for $C_{211}H_{242}N_6O_{53}$ (M = 3710.2) *m/z* 3733.3 $[M + Na]^+$. Anal. Calcd. for $C_{211}H_{242}N_6O_{53}$: C, 68.31 ; H, 6.57 ; N, 2.27. Found C, 68.17; H, 6.74; N, 2.12.

LMPP14-exp-brevet-synlongs



$C_{209}H_{240}N_6O_{52}$
Exact Mass: 3665,6320
Mol. Wt.: 3668,1511
C 68,43; H 6,59; N 2,29; O 22,68

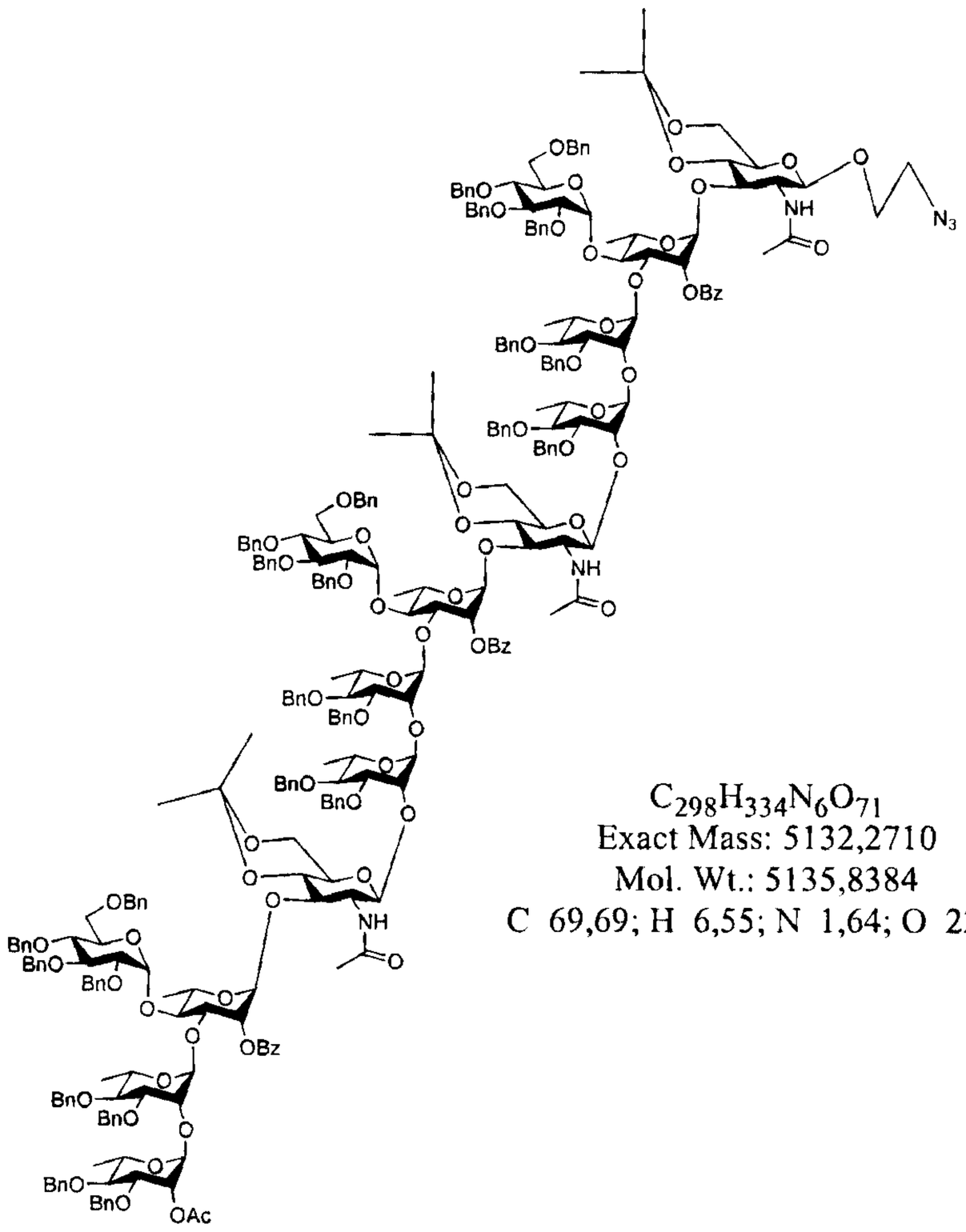
2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (X).

The undecasaccharide **X** (990 mg, 0.27 mmol) was dissolved in MeOH (30 mL). MeONa was added until pH=9. The mixture was stirred for 3 h then treated by IR 120 (H^+) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 1:1 toluene-AcOEt and 0.2 % of Et_3N to give **X** as a white foam (900 mg, 91 %); $[\alpha]_D$ +15° (*c* 1, $CHCl_3$).

LMPP14-exp-brevet-synlongs

^{1}H NMR ($CDCl_3$): partial δ 6.95-8.00 (m, 90H, Ph), 6.19 (bs, 1H, NH_D), 5.96 (d, 1H, $J_{2,NH}$ = 6.8 Hz, NH_D), 5.57 (d, 1H, $J_{2,NH}$ = 6.8 Hz, NH_D), 5.22 (dd, 1H, H-2_C), 5.13 (dd, 1H, H-2_C), 5.10 (d, 1H, H-1_D), 5.07 (bs, 1H, H-1_A), 5.04 (bs, 1H, H-1_A), 4.96 (d, 1H, H-1_E), 4.94 (d, 1H, H-1_E), 4.85 (bs, 1H, H-1_B), 4.84 (bs, 1H, H-1_B), 4.82 (bs, 1H, H-1_C), 4.70 (d, 1H, H-1_C), 4.67 (bs, 1H, H-1_D), 4.44 (d, 1H, H-1_D), 4.20-4.80 (m, 16H, CH_2Ph), 2.00, 1.85, 1.58 (3s, 9H, AcNH), 1.37-0.80 (m, 36H, 3 $C(CH_3)_2$, 2H-6_A, 2H-6_B, 2H-6_C). ^{13}C NMR ($CDCl_3$) partial: δ 172.8, 170.9, 170.3, 165.1, 164.7 (C=O), 139.0-127.7 (Ph), 103.5 (C-1_D), 103.1 (C-1_D), 101.5 (2C, C-1_B), 101.2 (C-1_A), 101.1 (C-1_A), 99.9 (C-1_D), 99.0, 98.8, 98.7 (3 $C(CH_3)_2$), 98.3 (C-1_E), 98.1 (2C, C-1_C, 1_E), 97.8 (C-1_C), 82.1, 82.0, 81.9, 81.7, 81.6, 81.5, 80.6, 80.3, 80.2, 80.1, 79.7, 79.1, 78.9, 78.5, 77.9, 77.6, 75.7, 74.9, 74.6, 74.3, 73.3, 73.0, 72.7, 71.9, 71.8, 69.1, 68.9, 68.7, 68.5, 68.0, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 61.9, 60.5, 59.9 (C-2_D), 57.4 (C-2_D), 55.0 (C-2_D), 51.0 ($O(CH_2)_2N_3$), 29.51, 29.47, 29.3 (3 $C(CH_3)_2$), 24.0, 23.9, 22.7 (3 AcNH), 19.7, 19.6, 19.3 (3 $C(CH_3)_2$), 19.0, 18.9, 18.6, 18.5, 18.2, 18.1 (6C, 2 C-6_A, 6_B, 6_C). FAB-MS for $C_{209}H_{240}N_6O_{52}$ (M = 3668.1) *m/z* 3690.8 $[M + Na]^+$. Anal. Calcd. for $C_{211}H_{242}N_6O_{53}$: C, 68.43 ; H, 6.59 ; N, 2.29. Found C, 68.28; H, 6.72; N, 2.11.

LMPP14-exp-brevet-synlongs



$C_{298}H_{334}N_6O_{71}$
Exact Mass: 5132,2710
Mol. Wt.: 5135,8384
C 69,69; H 6,55; N 1,64; O 22,12

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-

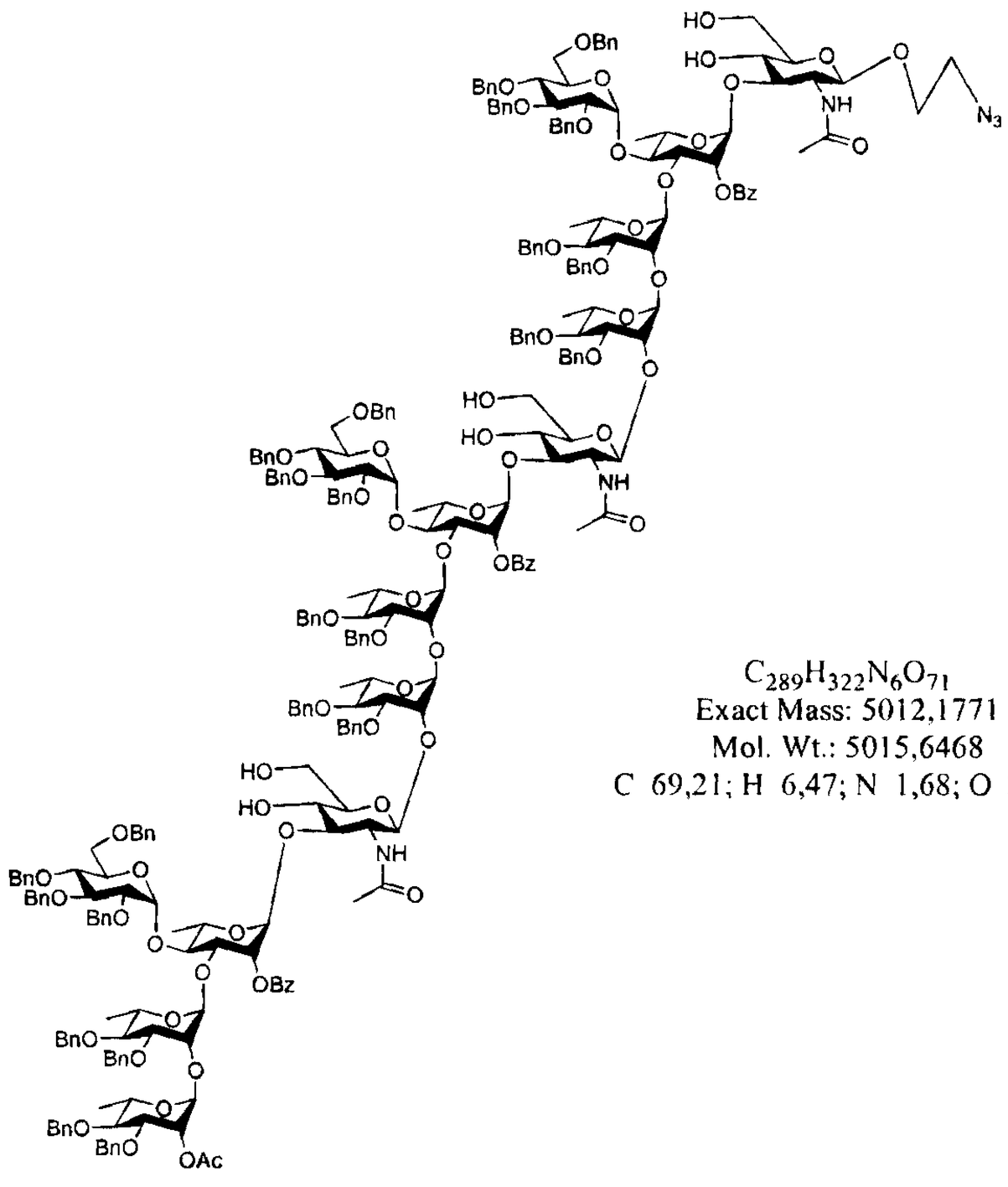
LMPP14-exp-brevet-synlongs

_O_-benzyl-α-D-glucopyranosyl-(1→4)]-(2-_O_-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-_O_-isopropylidene-β-D-glucopyranoside (X).

A mixture of donor **X** (377 mg, 0.230 mmol) and acceptor **X** (427 mg, 0.115 mmol), 4 Å molecular sieves and dry 1,2-DCE (10 mL), was stirred for 1 h then cooled to -30°C. Triflic acid (20 μL) was added. The stirred mixture was allowed to reach 5°C in 2.5 h. Et_3N (150 μL) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 3:1 toluene-AcOEt and 0.2 % Et_3N to give **X** as a foam (490 mg, 82 %); [α]$_D$ +20° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): partial δ 6.90-8.00 (m, 135H, Ph), 5.95 (d, 1H, $J_{2,NH}$ = 6.6 Hz, NH_D), 5.60 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH_D), 5.59 (d, 1H, $J_{2,NH}$ = 7.5 Hz, NH_D), 5.44 (dd, 1H, H-2$_C$), 5.22 (dd, 1H, H-2$_C$), 5.10 (dd, 1H, H-2$_C$), 2.20 (s, 3H, OAc), 2.00, 1.85, 1.84 (3s, 9H, AcNH), 1.40-0.80 (m, 45H, 3 $C(CH_3)_2$, 3H-6$_A$, 3H-6$_B$, 3H-6$_C$). ^{13}C NMR ($CDCl_3$) partial: δ 173.2, 172.6, 172.5, 171.3, 167.4, 167.0, 166.9 (C=O), 140.2-126.8 (Ph), 102.8, 102.7, 101.5, 101.3, 101.1, 99.9, 99.8, 98.1, 97.8, 82.0, 81.7, 81.5, 81.4, 80.2, 80.1, 79.6, 79.4, 78.9, 78.6, 78.0, 77.9, 77.6, 75.5, 73.4, 73.3, 73.0, 72.8, 71.9, 71.6, 69.4, 69.1, 69.0, 68.6, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 60.0, 57.9, 57.7, 51.0 ($OCH_2CH_2N_3$), 30.5 (3C, $C(CH_3)_2$), 25.0, 22.4 (3C, AcNH), 22.9 (OAc), 20.7, 20.6, 20.2 (3C, $C(CH_3)_2$), 20.0, 19.9, 19.8, 19.7, 19.6, 19.3, 19.2, 19.1 (9C, 3 C-6$_A$, 6$_B$, 6$_C$). FAB-MS for $C_{298}H_{334}N_6O_{71}$ (M = 5135.8) *m/z* 5159.3 $[M + Na]^+$. Anal. Calcd. for $C_{298}H_{334}N_6O_{71}$: C, 69.69 ; H, 6.55 ; N, 1.64. Found C, 69.74; H, 6.72; N, 1.49.

LMPP14-exp-brevet-synlongs



$C_{289}H_{322}N_6O_{71}$
Exact Mass: 5012,1771
Mol. Wt.: 5015,6468
C 69,21; H 6,47; N 1,68; O 22,65

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

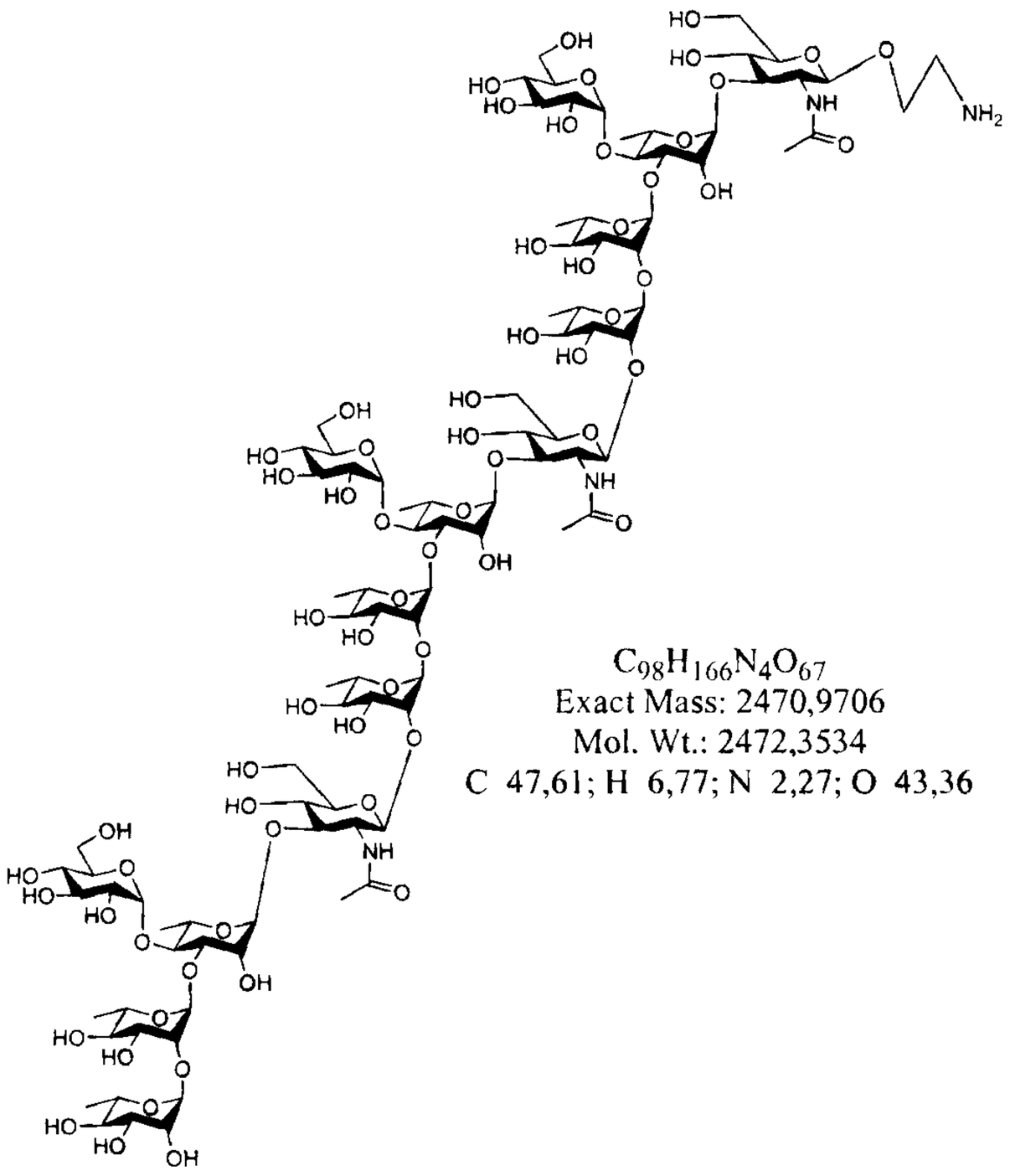
To a solution of the pentadecasaccharide **X** (480 mg, 93 μmol) in DCM (14 mL) was added dropwise at 0°C, a solution of TFA (1.75 mL) and water (1.75 mL). The mixture was stirred

for 3 h then concentrated by coevaporation with successively water and toluene. The residue was eluted from a column of silica gel with 1:1 toluene-AcOEt to give **X** as a white foam (390 mg, 83 %); $[\alpha]_D$ +12° (*c* 1, $CHCl_3$).

FAB-MS for $C_{289}H_{322}N_6O_{71}$ (M = 5015.6) *m/z* 5037.2 $[M + Na]^+$.

Anal. Calcd. for $C_{289}H_{322}N_6O_{71}.8H_2O$: C, 67.27 ; H, 6.60 ; N, 1.63. Found C, 67.31; H, 6.45; N, 1.64.

I.MPP14-exp-brevet-synlongs

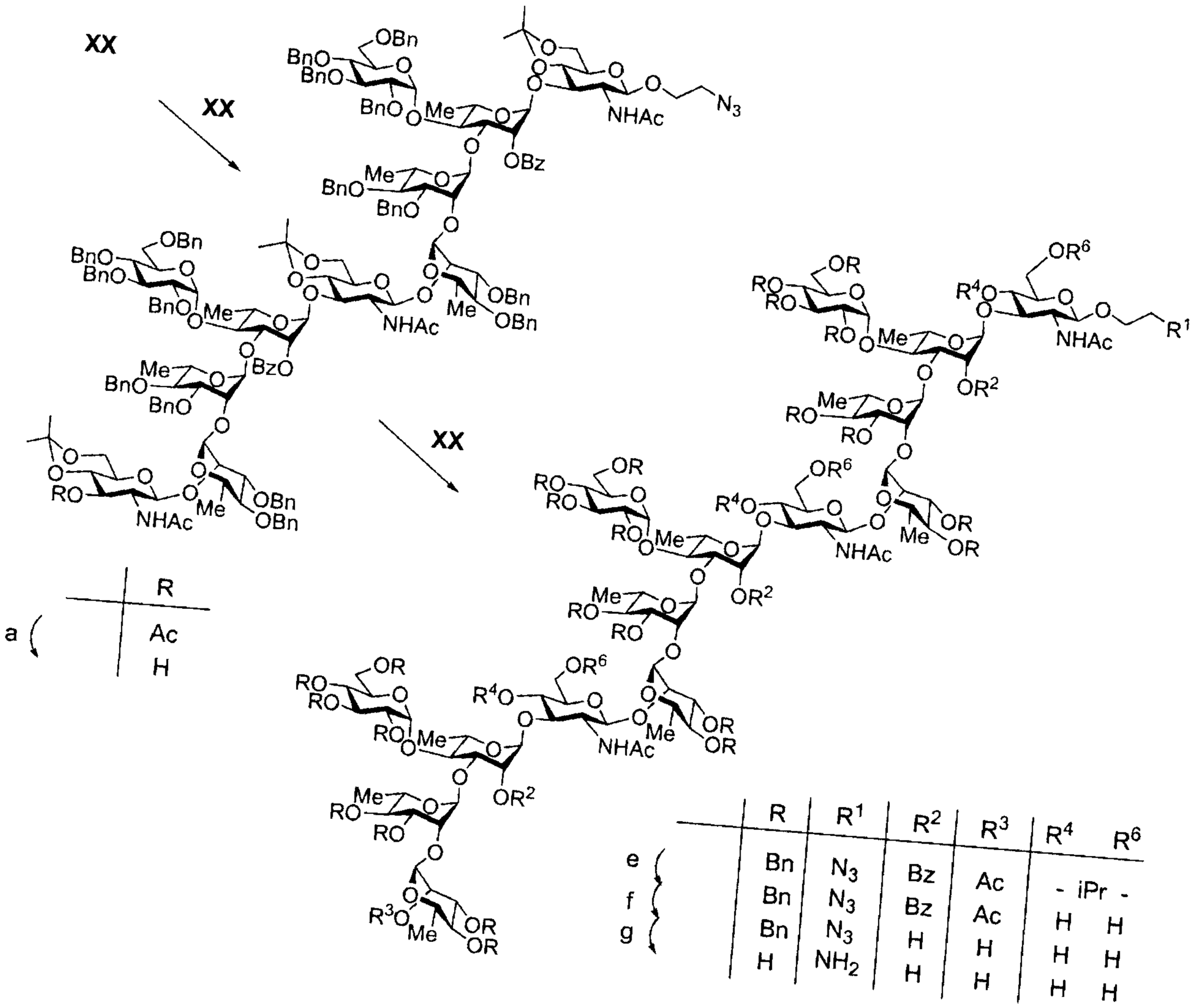


$C_{98}H_{166}N_4O_{67}$
Exact Mass: 2470,9706
Mol. Wt.: 2472,3534
C 47,61; H 6,77; N 2,27; O 43,36

2-Aminoethyl (α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-(α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (X).

A solution of the partially deprotected pentadecasaccharide **X** (390 mg, 77 μmol) in MeOH (10 mL) was treated by MeONa until pH=10. The mixture was stirred overnight at 55°C. After cooling at rt, IR 120 (H^+) was added until neutral pH and the solution was filtered and concentrated, then was eluted from a column of silica gel with 20:1 DCM-MeOH to give the benzylated residue (252 mg). A solution of this residue in EtOH (3 mL), AcOEt (250 μL) and

1M HCl (106 μL) was hydrogenated in the presence of Pd/C (300 mg) for 48 h at rt. The mixture was filtered and concentrated, then was eluted from a column of C-18 with water/CH_3CN and freeze-dried to afford amorphous **X** (127 mg, 65 %); $[\alpha]_D$ -5° (*c* 1, H_2O). ^1H NMR (D_2O): partial δ 5.13 (m, 3H, 3 H-1_E), 5.07 (m, 2H, H-1_{Rha}), 4.99 (bs, 1H, H-1_{Rha}), 4.95 (m, 2H, H-1_{Rha}), 4.90 (m, 1H, H-1_{Rha}), 4.75 (m, 3H, H-1_{Rha}), 4.63 (d, 2H, $J_{1,2}$ = 8.5 Hz, 2 H-1_D), 4.51 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1_D), 2.00 (s, 9H, 3 AcNH), 1.30-1.18 (m, 27H, 3H-6_A, 3H-6_B, 3H-6_C). ^{13}C NMR ($CDCl_3$): δ 174.8, 174.7 (3C, C=O), 102.9, 102.6, 101.7, 101.3, 100.8, 97.9, 81.8, 81.7, 79.6, 79.0, 76.3, 76.2, 73.0, 72.7, 72.4, 72.1, 71.6, 70.5, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 68.6, 66.0, 61.0, 56.0, 55.4, 39.8, 22.7, 22.6 (AcNH), 18.2, 17.2, 17.0, 16.9 (9C, 3 C-6_A, 6_B, 6_C). FAB-MS for $C_{98}H_{166}N_4O_{67}$ (M = 2470.9706) *m/z* 2493.9660[M + Na]$^+$.


XX
XX
XX
XX
a
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Ac
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Bn N3 Bz Ac - iPr -
Bn N3 Bz Ac H H
Bn N3 H H H H
H NH2 H H H H

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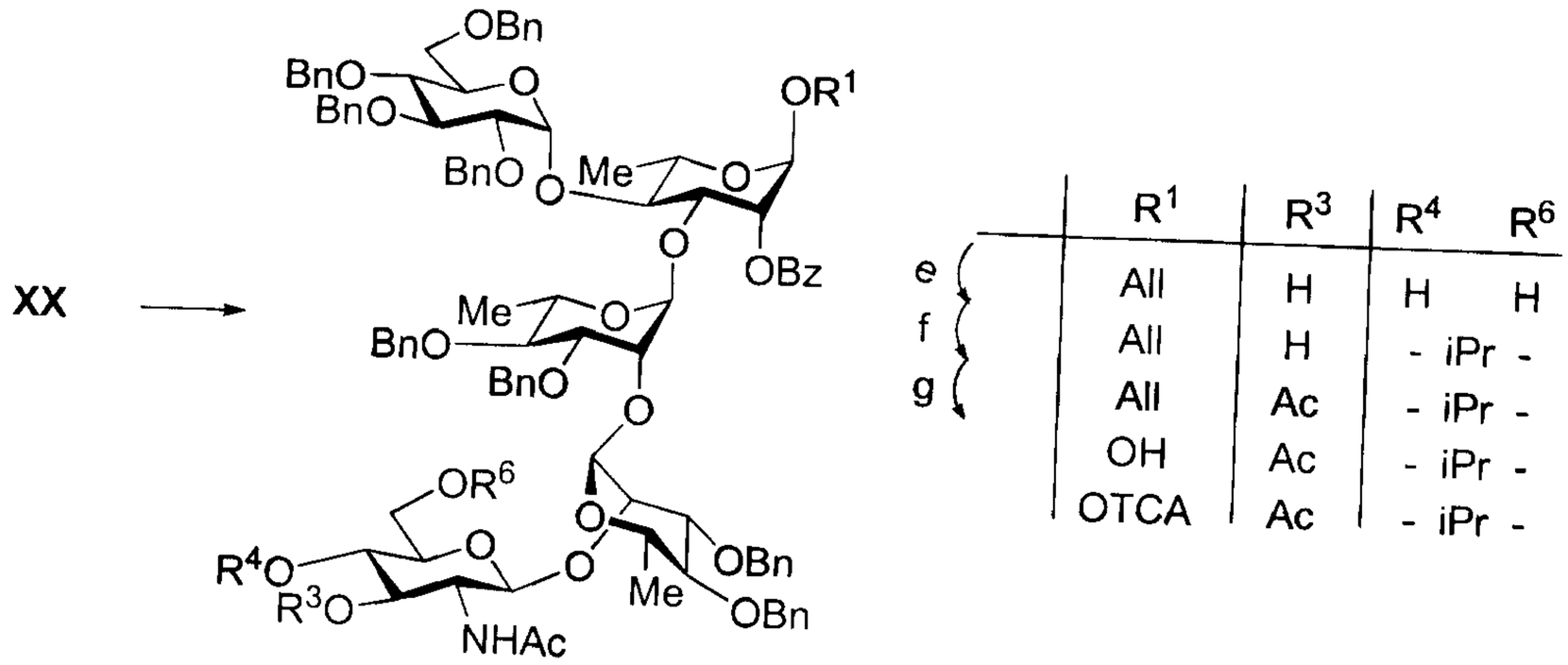

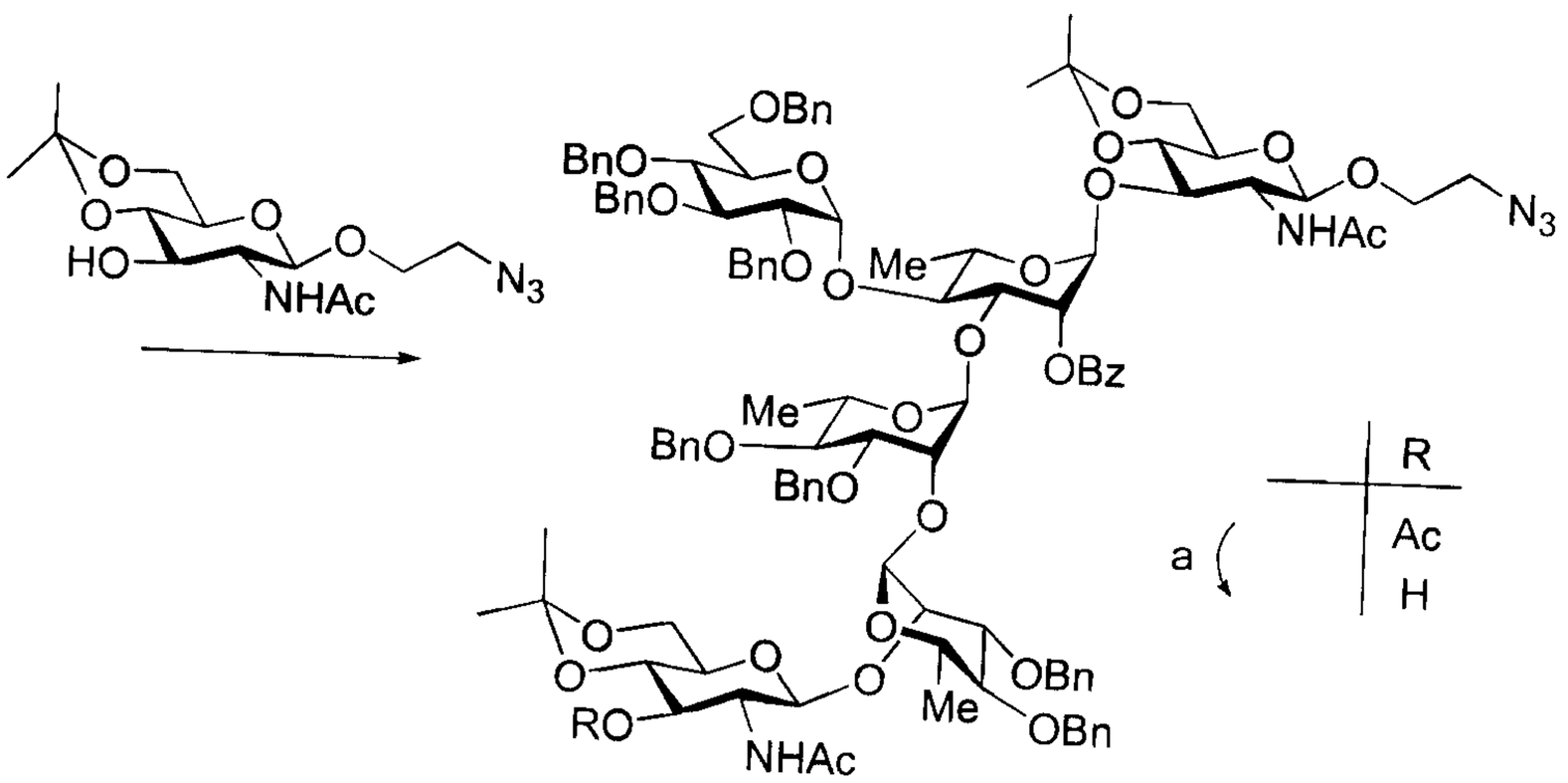

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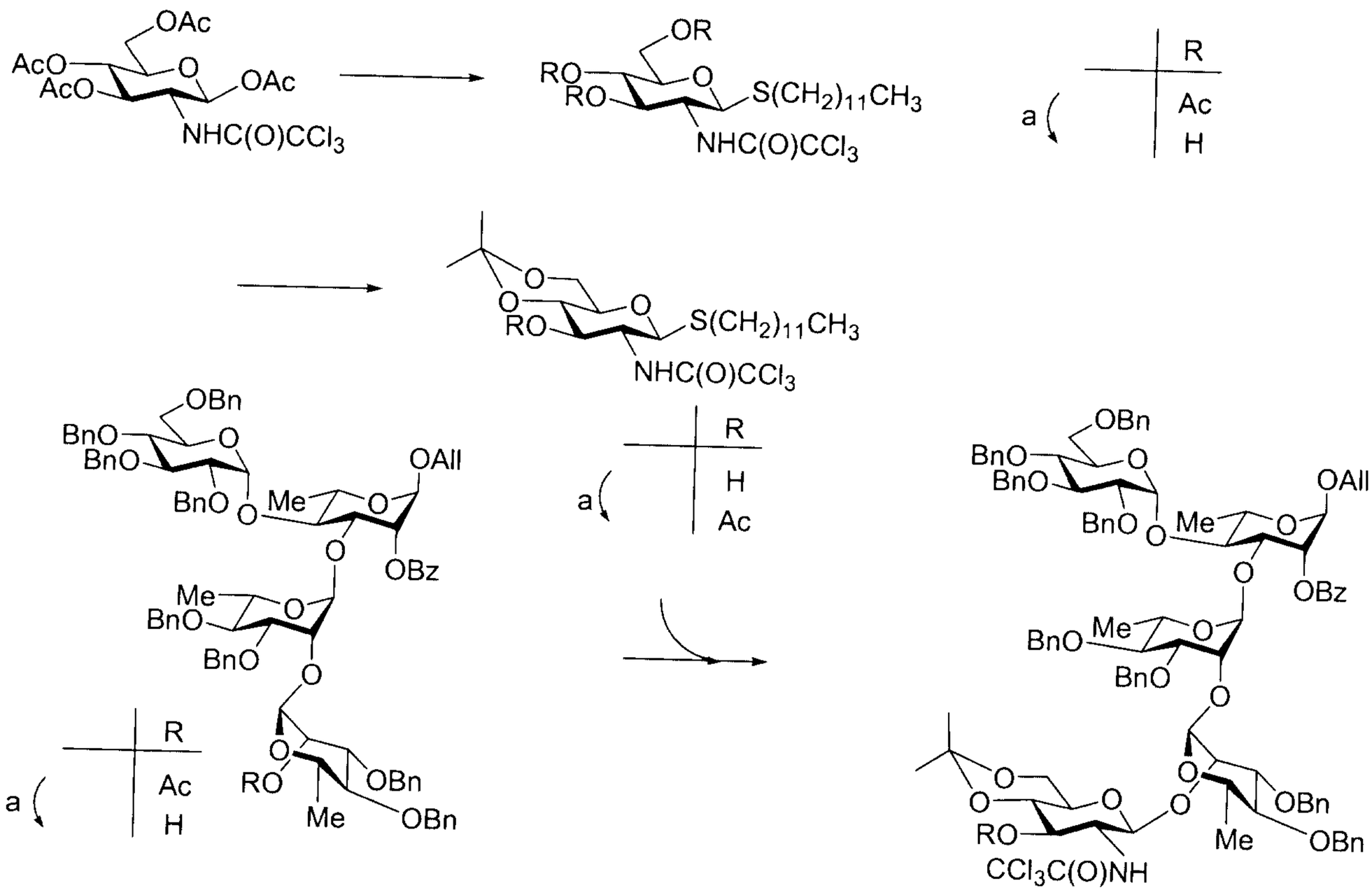

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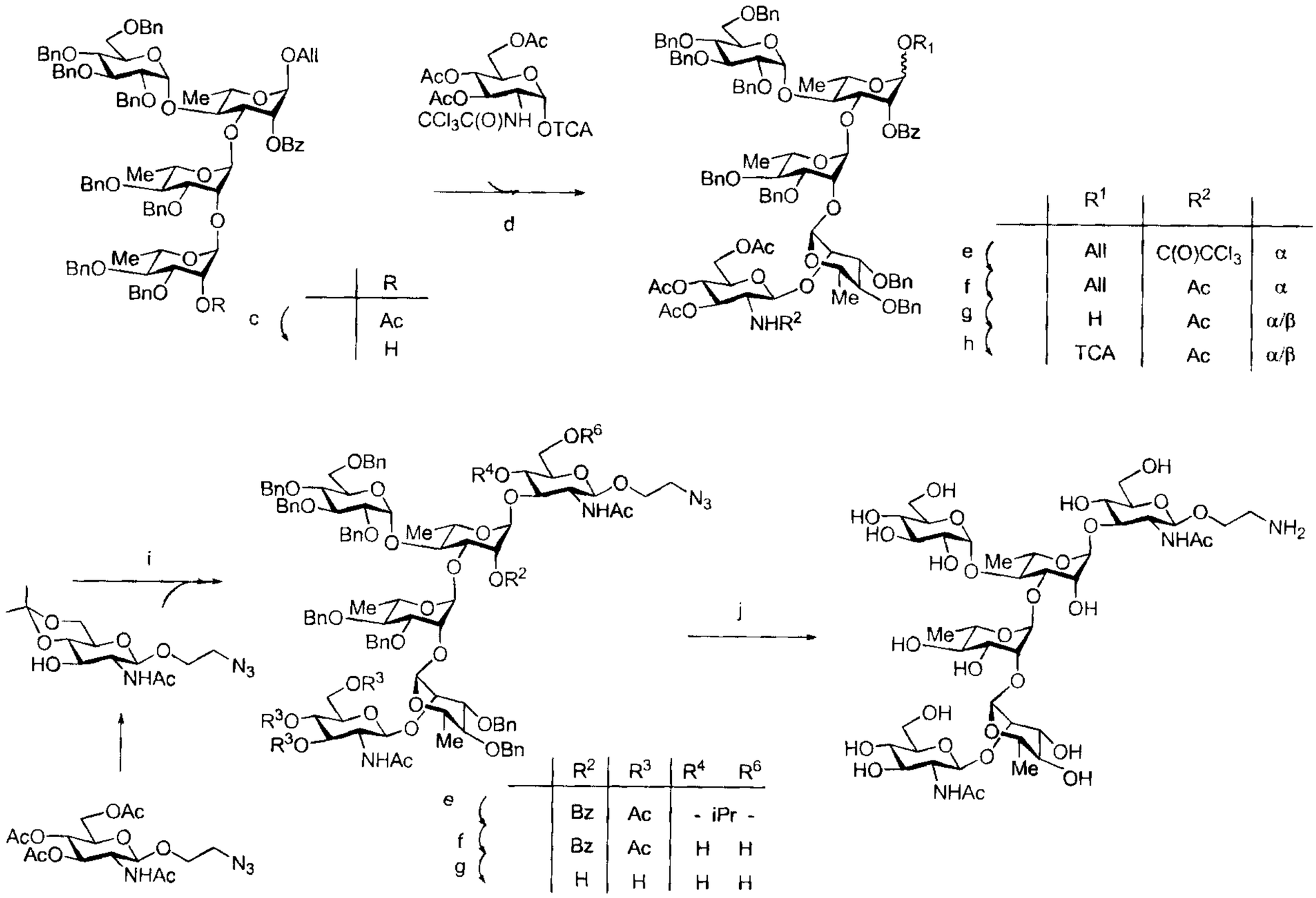

The serum immunoglobulin G-mediated response to serotype-specific determinants of *Shigella flexneri* lipopolysaccharide protects against experimental shigellosis

Abstract

Both intestinal secretory IgA (SIgA) and serum IgG specific for the O-antigen (O-Ag), the polysaccharide part of the bacterial lipopolysaccharide (LPS) are elicited upon *Shigella* infection, the causative agent of bacillary dysentery. We have addressed here the protective role of the anti-LPS IgG response, using the murine model of pulmonary infection. Upon intraperitoneal (i.p.) immunization with killed *Shigella flexneri* 2a bacteria, mice were shown to elicit a serum, but not a local, anti-LPS IgG response that conferred only partial protection against intranasal (i.n.) challenge with the homologous virulent strain. However, mice intranasally administered with, prior to i.n. challenge, an anti-LPS IgG polyclonal serum from i.p. immunized mice, showed a significant antibody dose-dependent decrease of the lung-bacterial load in comparison to mice that received preimmune serum. Using murine monoclonal antibodies (mAbs) of the G isotype (mIgG) representative of the different IgG subclasses and specific for serotype-specific determinants on the O-Ag, we showed that each IgG subclass exhibited a similar serotype-specific protective capacity, with significant reduction of the lung-bacterial load and of subsequent inflammation and tissue destruction. In contrast, different subclasses of mIgG specific for the invasins IpaB or IpaC did not confer protection. In conclusion, the IgG-mediated systemic response to serotype-specific determinants contributes to protection against homologous *Shigella* infection, if the effectors are present locally at the time of mucosal infection.

Introduction

Shigellosis is a major cause of infant morbidity and mortality in developing countries but an increasing number of cases in industrialized countries has been recently reported (33). *Shigella*, the causative agent of bacillary dysentery, invades the human colonic epithelial cells by manipulating processes that control the host cytoskeletal dynamics (8).

Host response to bacterial infection is characterized by the development of an acute inflammation which is responsible for the destruction of the colonic mucosa and accounts for the symptoms observed at the early stage of the disease (53). Acquired humoral immunity induced upon primary infection confers protection against re-infection, although the duration of the disease-induced immunity seems to be limited. Antibody-mediated protection is species- and serotype-specific, pointing out LPS as the major protective antigen (19, 22, 38). In fact, species and serotypes among a given species are defined by the structure of the repeated saccharide unit that forms the O-Ag polysaccharide part of LPS (35). Other bacterial antigens, as for example the invasins IpaB and IpaC, are recognized by sera from convalescent patients (18, 45, 46, 63), but their contribution to protective immunity is poorly documented.

Both intestinal SIgA and serum IgG directed against the O-Ag are elicited (13, 28, 31, 69). However, the respective protective roles of local and systemic humoral immunity remain unclear. The ineffectiveness of parenterally injected inactivated whole-cell vaccines in inducing protection, despite the high level of anti-LPS serum IgG antibodies raised, has led to the belief that serum antibodies do not confer protection (21, 25). However, several indirect pieces of evidence suggest that anti-O-Ag serum IgG may confer protection during natural infection. A correlation was found between the level of anti-LPS IgG antibodies and resistance to shigellosis among Israeli soldiers (14, 15), and an inverse relationship exists between the age of incidence of shigellosis and the presence of IgG antibodies to *Shigella*

LPS (47, 63). In addition, a detoxified LPS-based conjugate vaccine administered parenterally and eliciting mainly, if not only, serum antibodies has been shown to induce protective immunity (16).

The use of experimental models of shigellosis has allowed the study of, at least in part, *Shigella* specific humoral immunity. The rabbit ileal loop model has been used to assess SIgA-mediated antibody response (31, 32), and more recently, the mouse model of pulmonary infection has been developed (51, 66). Following i.n. administration of bacteria, mice develop an acute broncho-pneumonia leading to massive destruction of the lung tissues. This response mimics the acute inflammation developed in intestinal tissues in the course of shigellosis. This model has been used to assess the immunogenicity and protective capacity of different *Shigella* vaccine candidates, either live attenuated strains administered i.n., or subunit vaccines administered parenterally (3, 34, 36, 65). Using this model, we have demonstrated that the IgA-mediated immune response specific for a serotype-specific determinant is sufficient to confer protection, (51), with an improved protective capacity of the IgA when bound to secretory component (52). In the current study, using the same experimental model and specific polyclonal serum or mIgG, we have addressed the protective role of serum IgG recognizing serotype-specific LPS determinants or peptide epitopes on the invasins IpaB and IpaC.

Materials and Methods

Bacterial strains

M90T, an invasive isolate of *S. flexneri* serotype 5a, and 454, an invasive isolate of *S. flexneri* serotype 2a, were the virulent strains of reference. For i.n. infection, bacteria were routinely grown on Luria Bertoni agar plates at 37°C. They were recovered from plates and bacterial dilutions were performed in 0.9% NaCl with the consideration that, for an optical density of 1 at 600 nm, the bacterial concentration was 5 x 10^8 c.f.u./ml. Killed bacteria for systemic immunizations were prepared from bacterial cultures at stationary phase, diluted to 5 x 10^8 c.f.u. /ml in 0.9% NaCl, and then incubated at 100°C for 1h. They were then kept at –20°C in aliquots.

Production and characterization of mAbs specific for S. flexneri LPS

BALB/c mice were immunized i.p. with 10^7 c.f.u. of killed *S. flexneri* 5a or *S. flexneri* 2a bacteria three times at 3 week-intervals. Mice eliciting the highest anti-LPS antibody response were given an intravenous booster injection 3 days before being sacrificed for splenic B cell fusion according to Kohler and Milstein (30). Hybridoma culture supernatants were screened for antibody production by ELISA using purified *S. flexneri* 5a or 2a LPS. We selected only the hybridoma cells secreting mIgG reacting specifically with LPS homologous to the strain used for immunization, *i. e.* recognizing serotype-specific determinants on the LPS O-Ag. Those selected were then cloned by limiting dilution, and injected i. p. into histocompatible mice for ascitis production. mIgG were precipitated with 50% ammonium sulfate from ascitis fluid, centrifuged, and dialysed against PBS before being purified using ion-exchange chromatography as previously described (2, 50). The avidity of anti-LPS mIgG was determined as follows: various concentrations of LPS were incubated in solution overnight at 4°C with a defined amount of a given mIgG until equilibrium was reached. Each mixture was

then transferred to a microtiter plate previously coated with homologous purified LPS. Bound antibodies were detected by using peroxidase-conjugated anti-mouse immunoglobulins specific for IgG subclasses. IC_{50} was defined as the concentration of LPS required to inhibit 50% of mIgG binding.

Active and passive immunization of mice

To obtain polyclonal serum, mice were immunized i.p. with 5 x 10^7 killed bacteria, three times at 3 week-intervals. After bleeding, anti-LPS antibody titer in the polyclonal sera was measured by ELISA, as described below, and those ranging from low (1/4,000) to high titer (1/64,000) were used for i.n. passive transfer. Purified mAbs (20 or 2 μg) were also administered intranasally. All i.n. administrations were performed using a volume of 20μl and mice previously anesthesized via the intramuscular route with 50μl of a mixture of 12.5% ketamine (Merial , Lyon, France) and 12.5% acepromazine (Vetoquinol, Lure, France). Each experiment was performed using 10 mice per group and was repeated three times.

Protection experiments

Intranasal challenge was performed using either 10^9 live virulent bacteria when protection was assessed by mortality assay or 10^8 bacteria when protection was assessed by measurement of the lung-bacterial load. Naive mice were used as controls in each experiment. Mice immunized i.p. were challenged i.n. with virulent bacteria, 3 weeks after the last immunization. Mice passively transferred i.n. with polyclonal sera or with purified mAbs were challenged 1h after administration of the mAbs. Measurement of lung-bacterial load was performed at 24h post infection as follows. Mice were sacrificed by cervical dislocation and lungs were removed « *en bloc* » and ground in 10 ml sterile PBS (Ultra Turrax T25 apparatus, Janke and Kunkel IKA Labortechnik GmbH, Staufen, Germany). Dilutions were then plated on Trypticase Soy Broth plates for c.f.u. enumeration.

ELISA

Hybridoma culture supernatants were tested by ELISA for the presence of anti-LPS antibodies as previously described (2, 50) except that LPS purified according to Westphal (67) was used at a concentration of 5µg/ml in PBS. As secondary antibodies, anti-mouse IgG- or IgM- or IgA-alkaline phosphatase-labeled conjugate (Sigma) were used at a dilution of 1:5,000. To measure the anti-LPS antibody titer in polyclonal serum, biotin-labeled Abs to IgG and its different subclasses (IgG1,-2a, -2b, -3) (Pharmingen) and avidin conjugated with alkaline phosphatase (Sigma) were used at a dilution of 1:5,000. Antibody titers were defined as the last dilution of the sample giving an OD at least twice that of the control.

Histopathological studies

Mice were anesthesized, their trachea catheterized, and 4% formalin injected in order to fill the bronchoalveolar space. Lungs were then removed and fixed in 4% formalin before being processed for histopathological studies. Ten-micrometer paraffin sections were stained with Hematoxiline and Eosin (HE), and observed with a BX50 Olympus microscope (Olympus Optical, Europa, GmbH, Hamburg, Germany).

Statistical analysis

Significant differences were compared using the Student's test. Probability values < 0.05 were considered significant.

Results

1) Protection conferred upon systemic immunization or intranasal administration of specific immune serum.

Firstly, to address the role of the systemic anti-LPS IgG antibody response in protection against the mucosal infection, we assessed the protection conferred against i.n. challenge with a lethal dose of *S. flexneri* 2a bacteria in mice immunized i.p. with the homologous killed bacteria. Antibodies induced upon such an immunization were mainly anti-LPS IgG antibodies (data not shown) with all the IgG subclasses similarly elicited (Figure 1A). No mucosal response was elicited, as reflected by the absence of anti-LPS antibody response detectable in the bronchoalveolar lavage of immunized mice. Only 40% of the immunized mice survived the i.n. challenge, whereas 100% of naive mice succumbed. The low efficacy of systemic immunization in inducing protection could be due to either the inability of anti-LPS IgG to be protective or the absence of the protective antibodies (or their presence but in insufficient amount) in the mucosal compartment at the time of i.n. challenge.

We, therefore, tested whether the anti-LPS IgG antibodies may confer protection if present locally prior to mucosal challenge. Polyclonal sera exhibiting different anti-LPS antibody titers were intranasally administered to naive mice 1h prior to i.n. infection with a sublethal dose of *S. flexneri* 2a bacteria. Protection was assessed by the reduction of the lung-bacterial load in comparison to control mice and mice receiving preimmune serum. In contrast to control mice and mice receiving preimmune serum, naive mice receiving anti-LPS IgG serum showed a significant decrease of the lung-bacterial load. The reduction was dependent on the amount of anti-LPS IgG antibodies administered as reflected by the anti-LPS antibody titer of the immune serum used for passive transfer. Thus, the highest reduction was obtained with serum having the highest anti-LPS antibody titer (1/64,000) (Figure 1B, c) ($p=5 \times 10^{-6}$ in comparison to mice receiving preimmune serum). However, in mice receiving immune serum

with lower anti-LPS antibody titer (1/16,000 and 1/4,000) (Fig. 1B, a and b), even if less efficient, the decrease of the bacterial load was still significant in comparison to mice receiving preimmune serum (p = 0, 027 and 0, 015, respectively).

These results demonstrated that, if present locally at the time of mucosal challenge, the anti-LPS IgG antibodies were protective, thus limiting bacterial invasion.

2) Protective capacity of mAbs specific for *S. flexneri* 2a serotype determinants and representative of the different IgG subclasses

Depending of the infecting strain, different subclasses of IgG specific for LPS are induced following natural *Shigella* infection (28). To test whether the different anti-LPS IgG subclasses exhibit similar protective capacity, murine mIgG specific for serotype determinants on the O-Ag and, representative of each of the four murine IgG subclasses were obtained. We selected 5 mIgG specific for *S. flexneri* 2a LPS : mIgG F22 (IgG1), mIgG D15 (IgG1), mIgG A2 (IgG2a), mIgG E4 (IgG2b) and mIgG C1 (IgG3). The avidity of each mIgG for LPS, defined by IC_{50}, ranged from 2 to 20 ng/ml. To analyse the protective capacity of the selected mAbs, naive mice were administered i.n. with each of the purified mIgG prior to i.n. challenge with a *S. flexneri* sublethal dose. Upon challenge, lung-bacterial load in mice passively administered with 20 µg of each of the mIgG specific for *S. flexneri* 2a LPS was significantly reduced in comparison to mice receiving PBS (Fig. 2A). Upon passive transfer using 2µg of mIgG, only mIgG D15, A2 and E4 were shown to significantly reduce the lung-bacterial load in comparison to control mice, but with much less efficiency than that observed using 20µg (Fig. 2A). As shown in Figure 2B, reduction of lung-bacterial load in mice receiving 20 µg of mIgG was accompanied by a reduction of inflammation and therefore of subsequent tissue destruction. In comparison to control mice showing an acute broncho-alveolitis with diffuse and intense polymorphonuclear cell infiltration (Figure 2B, *a*, *b*)

associated with tissular dissemination of bacteria (Figure 2B, *c*), only restricted areas of inflammation were observed, essentially at the intra- and peribronchial level (Figure 2B, *d*, *e*), where bacteria localized (Figure 2B, *f*). Following passive administration with 2μg of mIgG, inflammation resembled that of the control mice with a similar pattern of PMN infiltration and tissue destruction, in accordance with the very low, if any, reduction in lung-bacterial load (data not shown).

3) Serotype-specific protection induced by the anti-LPS mIgG

Antibodies specific for epitopes common to several serotypes of a given species as well as serotype-specific antibodies are elicited upon natural or experimental infection (58, 64). However, the serotype-specific protection observed following natural or experimental infection suggests that the antibodies directed against serotype determinants play a major protective role (19, 38). For instance, mIgA specific for *S. flexneri* serotype 5a has been shown to protect only against homologous challenge (51). We, therefore, tested whether the protection observed with the anti-LPS mIgG obtained in this study was also serotype-specific. Mice passively administered with 20 μg of mIgG C1 specific for *S. flexneri* 2a were protected against homologous challenge, but not upon heterologous challenge with *S. flexneri* 5a bacteria (Fig. 3A). Similarly, mice receiving 20 μg of mIgG C20, a mAb specific for *S. flexneri* serotype 5a and, of the same isotype than mIgG C1, *i.e.* IgG3, showed a significant reduction of lung-bacterial load upon i.n. challenge with *S. flexneri* 5a, but not with *S. flexneri* 2a (Figure 3A). In mice protected against homologous challenge, inflammation was dramatically reduced with a slight intra- and peribronchial PMN infiltrate remaining present (Figure 3B, *b* and *c*). In contrast, in mice not protected upon heterologous challenge (Figure 3B, *a* and *d*), inflammation and tissue destruction were similar to those observed in control mice (Figure 2B, *a* and *b*).

4) Protective capacity of mIgG specific for *S. flexneri* invasins

The invasins IpaB and IpaC are essential to the expression of the *Shigella* invasive phenotype (39). Moreover, they are targets for the humoral response since antibodies specific for both proteins are detected in sera of patients convalescent from shigellosis (18, 45, 46, 63). To assess whether the anti-invasin antibody response may contribute to protection, in addition to the anti-LPS antibody response, we used mIgG recognizing different epitopes on IpaB or IpaC (2, 50). Whatever the dose used, in contrast to mIgG C20, no reduction in lung-bacterial load was measured upon challenge in mice treated with mIgG H16 and mIgG H4 recognizing distinct epitopes in the central region of IpaB or with mIgG J22 and mIgG K24 recognizing the N- and the C-termini domain of IpaC, respectively (Figure 5). Protection was also not observed upon combining anti-IpaB and anti-IpaC mIgG (data not shown).

Discussion

To date, the respective roles of local and systemic humoral immune responses specific for LPS O-Ag in protection against *Shigella* infection remained unclear, although this question is crucial for the design of accurate vaccine candidates. Indirect evidence has suggested a protective role for anti-LPS IgG (14, 15, 16, 34, 47, 63). We demonstrate here for the first time, using polyclonal serum and specific mAbs, that the systemic IgG-mediated response specific for serotype determinants carried by LPS O-Ag confers protection against mucosal infection, if present locally at the time of bacterial challenge.

LPS has been recognized for a long time as the major protective antigen (19, 22, 38). However, the question of the protective role of the antibody response to bacterial proteins remains unanswered. Among the proteins recognized by sera from patients convalescent from shigellosis, IpaB and IpaC, the invasins involved in the entry of bacteria into enterocytes, are two major antigens. Only indirect evidence suggested that the systemic response to these two virulence factors was not essential for protection (16). We show here that mIgG specific for IpaB or IpaC are not protective despite the fact that they are directed against epitopes located in different regions of these proteins (2, 50) and that they have been shown to interfere with their functional properties in *in vitro* studies (4, 40). The most likely explanation is that these invasins, that are secreted through the type III secretion apparatus, are injected straight into the host cell, upon contact of the bacterium with the cell membrane (6, 41). Therefore, there is probably very limited access, if any, for specific antibodies to interact with their targets. Although not tested, it is unlikely that the local SIgA-mediated response to these proteins will be protective.

In the past, several sets of mAbs of M or G isotype specific for *Shigella* species have been produced. They are directed against the O-Ag of *S. sonnei* (1), of *S. dysenteriae* (20, 56, 60) and, of *S. flexneri* (9, 10 11, 24, 26, 60). However, as the goal was to develop diagnostic tests

for *Shigella* identification (12, 27), their protective properties have not been investigated. Except for a few (42, 43), the sequence of the V_H and V_L genes is unknown. Similarly, the oligosaccharide determinants they recognize have not been characterized, except for 2 mAbs specific for *S. dysenteriae* 1 (43, 49). Thus, for a better understanding of carbohydrate antigen/antibody interactions, we are currently characterising the fine specificities of recognition between the mAbs obtained in this study and the O-Ag they recognize.

To obtain mIgG, hybridoma cells were selected, upon cell fusion, on the basis of their secretion of mAb recognizing determinants specific for the *S. flexneri* serotype used for immunization, *i.e.* serotype 2a and serotype 5a, respectively. During the screening, we observed that most of the hybridoma cells tested (about 90%) were secreting serotype-specific mAbs. This result slightly differs from previous reports showing the obtention of mAbs directed to determinants common to several *S. flexneri* serotypes including 2a and 5a (11, 24), However, it may be explained by recent new insights on bacterial O-Ag conformation. For instance, in the case of *S. dysenteriae* 1, the α-L-Rhap-(1-->2)- α-D-Galp disaccharide represents the major antigenic epitope on the O-Ag. Interestingly, in the proposed conformational model of *S. dysenteriae* 1 O-Ag, which is a left-handed helical structure, the galactose residues protrude radially at the helix surface, therefore resulting in a pronounced exposure of both the galactose and the adjacent rhamnose of each repeating unit (44). A similar result has been obtained in our hands with the O-Ag of *S. flexneri* 5a. In that case, the branched glucosyl residue specifying this serotype and required for recognition by serotype-specific antibodies (L. Mulard and A. Phalipon, personal communication) points out of the surface of the helix, which exhibits a right-handed three-fold helical structure (M. J. Clement and M. Delepierre, personal communication). Therefore, we may reasonably hypothesize that these peculiar sugar residues repeatedly exposed at the O-Ag surface, and therefore at the

bacterial surface, preferentially trigger B cell receptor-mediated recognition, thus leading to the induction of a predominant anti-serotype specific antibody response.

In humans, depending on the infecting strain, different subclasses of IgG specific for LPS are induced following natural *Shigella* infection (28). For instance, *S. flexneri* 2a and *S. dysenteriae* 1 preferentially induce IgG2, whereas *S. sonnei* mainly induces IgG1. Similarly, upon vaccination with glycoconjugate vaccines using detoxified LPS from *S. flexneri* 2a and *S. sonnei*, the same pattern is observed, IgG2 and IgG1, respectively. These antibodies may confer protection by different pathways involving or not the complement cascade. In the present study, all the different murine IgG subclasses were shown to be protective, suggesting that depending on the subclass, different mechanisms may be involved in IgG-mediated protection. Whereas antibody-dependant cellular cytotoxicity (ADCC) has been reported for *Shigella*-specific secretory IgA and lymphocytes from the gut-associated lymphoid tissues (61), *Shigella* IgG-mediated ADCC occurs *in vitro* with splenic T cells but not with T lymphocytes from the GALT (62). Further studies using mice deficient for T cells or for proteins of the complement cascade will be required to analyze the IgG-mediated protective mechanisms *in vivo*.

The protective role of the serotype-specific antibody response has been firstly emphasized in a study using a monoclonal dimeric IgA (mIgA) specific for a *S. flexneri* serotype 5a determinant (51). Here, we demonstrate that mIgGs specific for *S. flexneri* serotype 2a or serotype 5a also confer serotype-specific protection. It seems that whatever the antibody isotype and the bacterial strain, the serotype-specific antibody response is protective against homologous bacterial challenge. It should be noted that using the same amount of mIgA and mIgG specific for *S. flexneri* 5a, both exhibiting a similar IC_{50} for LPS, reduction in lung-bacterial load was much more efficient with mIgA. Actually, in contrast to mIgG, protection was observed in the presence of 2μg of mIgA. The discrepancy between the two isotypes may

be due to the dimeric/polymeric (d/p) form of mIgA, which mimicks the IgA response at the mucosal surface. In contrast to monomeric IgG, interaction of d/p IgA exhibiting at least four antigen-binding sites with a specific determinant highly repeated on the bacterial O-Ag surface may lead to the formation of aggregates that are efficiently removed by local physical mechanisms (17). Also, quantitative assessment of IgG and IgA subclass producing cells in the rectal mucosa during shigellosis in humans has revealed the predominance of the IgA response. The IgG response which is about 50 times lower than the IgA response is mainly IgG2 and correlates with the presence of specific IgG2 in serum. This correlation suggests that the majority of the *Shigella* specific serum antibodies are derived from the rectal mucosa (29). Together, these results suggest that in the situation where both local and systemic anti-LPS antibody responses are induced, as for example upon natural infection, the local SIgA-mediated response will be the major protective response, with the IgG-mediated response possibly contributing to a lesser extent to local protection.

On the other hand, our data suggest that in the absence of local SIgA-mediated response, as for example upon vaccination via the systemic route using glycoconjugate vaccines, the systemic anti-O-Ag response induced is effective in protecting against homologous *Shigella* infection, if the effectors are present locally. Previous reports have shown that serum IgGs may protect from gastrointestinal infections (7, 54). Therefore, it should be admitted that serum IgG efficiently gain access to the intestinal barrier in order to prevent bacterial invasion and dissemination. How IgG crosses the epithelial barrier to function in mucosal immunity remains unclear. One possible pathway is passive transudation from serum to intestinal secretions (5, 37, 67). After its passage of the intestinal barrier through M cells and its interaction with resident macrophages and epithelial cells, *Shigella* initiates an inflammatory response leading to infiltration of the infected tissues with polymorphonuclear cells (53). We may therefore reasonably envision that specific serum IgGs

transudate to the intestinal tissue during this inflammatory process that occurs very soon after bacterial translocation. Another explanation could be the involvement of the FcRn receptor in IgG transport. FcRn was firstly identified as the Fc receptor responsible for transferring maternal IgGs from mother's milk across the intestinal EC of the neonatal gut of rodents. Much evidence supports the concept that FcRn is ubiquitously expressed in adult tissues and plays a role in IgG homeostasis, dealing with IgG half-life (23). It has been recently reported that this receptor is expressed by enterocytes in human adults and mediates transcytosis of IgG in both direction across the intestinal epithelial monolayer (57). Further investigation is required to improve our knowledge on the role played by FcRn in IgG-mediated protection of the intestinal barrier against enteropathogens. Nevertheless, the existence of such a pathway already enlarges the current view of the humoral response at mucosal surfaces.

To conclude, our data are in favor of the hypothetical concept proposed by Robbins et al. stating that protection against bacterial enteric diseases may be conferred by serum IgG antibodies to the O-Ag of their bacterial LPS (59). The demonstration of the protective role of anti-LPS IgG-mediated systemic response against *Shigella* infection supports vaccine approaches based on detoxified LPS/protein glycoconjugate vaccines administered parenterally (47). In addition, the serotype-specific protection suggests that, upon their characterisation, the protective serotype-specific determinants for prevalent *Shigella* strains could be suitably combined in order to develop a multivalent synthetic vaccine for parenteral vaccination, since promising results have been recently obtained with synthetic oligosaccharides as immunogenic conjugates (55).

References

1. Archi-Berglund R., and A. A. Lindberg. 1996. Rapid and sensitive detection of *Shigella sonnei* in feces by the use of an O-antigen-specific monoclonal antibody in a combined immunomagnetic separation-polymerase chain reaction assay. Clin. Microbiol. Infect. **2** : 55-58.

2. Barzu, S., F. T. Nato, S. Rouyre, J. C. Mazie, P. J. Sansonetti, and A. Phalipon. 1993. Characterization of B-cell epitopes on IpaB, an invasion-associated antigen of *Shigella flexneri*: identification of an immunodominant domain recognized during natural infection. Infect. Immun. **61**:3825-31.

3. Barzu, S., A. Fontaine, P. J. Sansonetti, and A. Phalipon. 1996. Induction of local anti-IpaC antibody response in mice by use of a *Shigella flexneri* 2a candidate vaccine : implications for use of IpaC as a protein carrier. Infect. Immun. **64** : 1190-1196.

4. Barzu, S., Z. Benjelloun-Touimi, A. Phalipon, P. J. Sansonetti, and C. Parsot. 1998. Functional analysis of the *Shigella flexneri* IpaC invasin by insertional mutagenesis. Infect. Immun. **65** : 1599-1605.

5. Batty, I., and J. J. Bullen. 1961. The permeability of the sheep and rabbit intestinal wall to antitoxin present in the circulation. J. Pathol. Bacteriol. **81** : 447-458.

6. Blocker, A., N. Jouihri, E. Larquet, P. Gounon, F. Ebel, C. Parsot, P. J. Sansonetti, and A. Allaoui. 2001. Structure and composition of the *Shigella flexneri* « needle complex », a part of its type III secreton. Mol. Microbiol. **39** : 652-663.

7. Bougoudogo, F., F. Vely, F. Nato, A. Boutonnier, P. Gounon, J. C. Mazié, and J. M. Fournier. 1995. Protective activities of serum immunoglobulin G on the mucosal surface to *Vibrio cholerae* 01. Bull. Inst. Past. **93** : 273-283.

8. Bourdet-Sicard, R., E. Egile, P. J. Sansonetti, and G. Tran Van Nhieu. 2000. Diversion of cytoskeletal processes by *Shigella* during invasion of epithelial cells. Microbes Infect. **2**: 813.

9. Carlin, N. I., and A. A. Lindberg. 1983. Monoclonal antibodies specific for O-antigenic polysaccharides of *Shigella flexneri* : clones binding to II, II: 3,4 and 7, 8 epitopes. J. Clin. Microbiol. **18** : 1183-1189.

10. Carlin, N. I., M. A. Gidney, A. A. Lindberg, and D. R. Bundle. 1986. Characterization of *Shigella flexneri*-specific murine monoclonal antibodies by chemically defined glycoconjugates. J. Immunol. **137** : 2361-2366.

11. Carlin, N. I., and A. A. Lindberg. 1987. Monoclonal antibodies specific for *Shigella flexneri* lipopolysaccharides : clones binding to type IV, V, and VI antigens, group 3, 4 antigen, and an epitope common to all *Shigella flexneri* and *Shigella dysenteriae* type 1 strains. Infect. Immun. **55** : 1412-1420.

12. Carlin, N. I., M. Rahman, D. A. Sack, A. Zaman, B. Kay, and A. A. Lindberg. 1989. Use of monoclonal antibodies to *Shigella flexneri* in Bangladesh. J. Clin. Microbiol. **27** : 1163-1166.

13. Cleary, T. G., D. K. Winsor, D. Reich, G. Ruiz-Palacios, and J. J. Calva. 1989. Human milk immunoglobulin A antibodies to *Shigella* virulence determinants. Infect. Immun. **57** : 1675-1679.

14. Cohen, D., M. S. Green, C. Block, T. Rouach, and T. Ofek. 1988. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J. Inf. Dis. **157** : 1068.

15. Cohen D., M. S. Green, C. Block, R. Slepon, I. Ofek. 1991. A prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. J. Clin. Microbiol. **29** : 386.

16. Cohen D., S. Ashkenazi, M. S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, I. Ashkenazi, J. Shemer, D. N. Taylor, T. L. Hale, J. C. Sadoff, D. Pavliakova, R. Schneerson, and J. B. Robbins. 1997. Double-blind vaccine-controlled randomized efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. Lancet **349** : 155.

17. Corthésy, B., and J. P. Kraehenbuhl. 1999. Antibody-mediated protection of mucosal surfaces. Curr. Top. Microbiol. Immunol. **236** : 93-111.

18. Dam Cam, P. T., T. Pal, and A. A. Lindberg. 1993. Immune response gainst lipopolysaccharide and invasion plasmid-encoded antigens of shigellae in Vietnamese and Swedish dysenteric patients. J. Clin. Microbiol. **31** : 454-457.

19. DuPont H. L., R. B. Hornick, M. J. Synder, J. P. Libonati, S. B. Formal, and E. J. Gangarosa. 1972. Immunity to shigellosis. II. Protection induced by oral live vaccine or primary infection. J. Infect. Dis. **125** : 12.

20. Falt, I. C., and A. A. Lindberg. 1994. Epitope mapping of six monoclonal antibodies recognizing the *Shigella dysenteriae* type 1 O-antigenic repeating unit expressed in *Escherichia coli* K-12. Microbiol. Pathog. **16** : 27-41.

21. Formal S. B., R. M. Maenza, S. Austin, and E. H. LaBrec. 1967. Failure of parenteral vaccines to protect monkeys against experimental shigellosis. Proc. Soc. Exp. Biol. Med. **125** : 347.

22. Formal, S. B., E. V. Oaks, R. E. Olsen, M. Wingfield-Eggleston, P. J. Snoy, and J. P. Cogan. 1991. Effect of prior infection with virulent *Shigella flexneri* 2a on the resistance of monkeys to subsequent infection with *Shigella sonnei*. J. Inf. Dis. **164** : 533-537.

23. Ghetie, V., and E. S. Ward. 2000. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Ann. Rev. Immunol. **18** : 739-766.

24. Hartman, A. B., L. L. Van de Verg, C. R. Mainhart, B. D. Tall, and S. J. Smith-Gill. 1996. Specificity of monoclonal antibodies elicited by mucosal infection of BALB/c mice with virulent *Shigella flexneri* 2a. Clin. Diagn. La. Immunol. **3** : 584-589.

25. Higgins, A. R., T. M. Floyd, and M. A. Kader. 1955. Studies in shigellosis. III. A controlled evaluation of a monovalent *Shigella* vaccine in a highly endemic environment. Am. J. Trop. Med. **4** : 281-288.

26. Islam, M. S., and W. H. Stimson. 1989. Production and characterization of monoclonal antibodies with diagnotic potential against *Shigella flexneri*. J. Clin. Lab. Microbiol. **29** : 199-206.

27. Islam, D., S. Tzipori, M. Islam, and A. A. Lindberg. 1993. Rapid detection of *Shigella dysenteriae* and *Shigella flexneri* in faeces by an immunomagnetic assay with monoclonal antibodies. Eur. J. Clin. Microbiol. Infect. Dis. **12** : 25-32.

28. Islam D., B.Wretlin, M. Ryd, A. A. Lindberg, and B. Christensson. 1995. Immunoglobulin subclass distribution and dynamics of *Shigella*-specific antibody responses in serum and stool samples in shigellosis. Infect. Immun. **63** : 2045-2061.

29. Islam, D., B. Veress, P. K. Bardhan, A. A. Lindberg, and B. Christensson. 1997. Quantitative assessment of IgG and Ig subclass producing cells in rectal mucosa during shigellosis. J. Clin. Pathol. **50** : 513-520.

30. Köhler, G., and E. Milstein. 1976. Derivation of specific antibody tissue culture lines by cell fusion. Eur. J. Immunol. **6**: 511-519.

31. Keren, D. F., S. E. Kern, D. H. Bauer, P. J. Scott, and P. Porter. 1982. Direct demonstration in intestinal secretions of an IgA memory response to orally administered *Shigella flexneri* antigens. J. Immunol. **128** : 475.

32. Keren, D. F., J. E. Brown, R. A. McDonald and J. S. Wassef. 1989. Secretory immunoglobulin A response to Shiga toxin in rabbits : kinetics of the initial mucosal immune response and inhibition of toxicity *in vitro* and *in vivo*. Infect. Immun. **57** : 1885-1889.

33. Kotloff, K. L., J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, and M. M. Levine.1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 77:651-66.

34. Levenson, V. J., C. P. Mallett, and T. L. Hale. 1995. Protection against local *Shigella sonnei* infection in mice by parenteral immunization with a nucleoprotein subcellular vaccine. Infect. Immun. **63** : 2762-2765.

35. Lindberg, A. A., A. Karnell, and A. Weintraub. 1991. The lipopolysaccharide of *Shigella* bacteria as a virulence factor. Rev. Infect. Dis. **13** : S279-284.

36. Mallett, C. P., T. L. Hale, R. W. Kaminski, T. Larsen, N. Orr, D. Cohen, and G. H. Lowell. 1995. Intranasal or intragastric immunization with proteosome-*Shigella* lipopolysaccharide vaccines protects against lethal pneumonia in a murine model of *Shigella* infection. Infect. Immun. **63** : 2382-2386.

37. McCleery, J. L., S. C. Kraft, and R. Rothberg. 1970. Demonstration of antibody in rabbit feces after active or passive parenteral immunization. Digestion **3** : 213-221.

38. Mel, D. M., B. L. Arsic, B. D. Nikolic, and M. L. Radovanovic. 1968. Studies on vaccination against bacillary dysentery. -IV. Oral immunization with live monotypic and combined vaccines. Bull. W. H. O. **39** : 375-380.

39. Ménard, R., P . J. Sansonetti, and C. Parsot. 1993. Nonpolar mutagenesis of the ipa genes defines IpaB, IpC and IpaD as effectors of *Shigella flexneri* entry into epithelial cells. J. Bacteriol. **175** : 5899-58906.

40. Ménard, R., P. J. Sansonetti, C. Parsot, and T. Vasselon. 1994a. Extracellular association and partitioning of the IpaB and IpaC invasins of *Shigella flexneri*. Cell. **79** : 515-525.

41. Ménard, R., P. J. Sansonetti, and C. Parsot.1994b. The secretion of the *Shigella flexneri* Ipa invasins is induced by the epithelial cell and controlled by IpaB and ipaD. EMBO J. **13** : 5293-5302.

42. Miller, C. E., K. Huppi, D. Siwarski, A. Karpas, A. Newman, C. Mainhart, and C. P. J. Glaudemans. 1995. A murine antibody to *Shigella dysenteriae* type 1 employs V-genes that contain a rearranged codon for the lambda light chain. Mol. Immunol. **9** : 679-682.

43. Miller, C. E., A. Karpas, R. Schneerson, K. Huppi, P. Kovac, V. Pozsgay, and C. P. J. Glaudemans. 1996. Of four murine, anti-*Shigella dysenteriae* type 1 O-polysaccharide antibodies, three employ V-genes that differ extensively from those of the fourth. Mol. Immunol. 33 : 1217-1222.

44. Nyholm, P.G., L. A. Mulard, C. E. Miller, T. Lew, R. Olin, and C. P. Glaudemans. 2001. Conformation of the O-specific polysaccharide of *Shigella dysenteriae* type 1: molecular modeling shows a helical structure with efficient exposure of the antigenic determinant alpha-L-Rhap-(1-->2)-alpha-D-Galp. Glycobiology. **11**:945-55.

45. Oaks, E. V., T. L. Hale, and S. B. Formal. 1986. Serum immune response to *Shigella* protein antigens in Rhesus monkeys and humans infected with *Shigella* spp. Infect. Immun. **53** : 57-63.

46. Oberhelman, R. A., D. J. Kopecko, E. Salazar-Lindo, E. Gotuzzo, J. M. Buysse, M. M. Venkatesan, A. Yi, C. Fernandez-Prada, M. Guzman, R. Leon-Barua, and R. B. Sack. 1991. Prospective study of systemic and mucosal immune responses in dysenteric patients to specific *Shigella* invasion plasmid antigens and lipopolysaccharide. Infect. Immun. **59** : 2341-2350 .

47. Passwell, J. H., S. Freier, R. Shor, N. Farzam, C. Block, M. Lison, E. Shiff, and S. Ashkenazi. 1995. *Shigella* lipopolysaccharide antibodies in pediatric populations. Pediatr. Infect. Dis. J. **14** : 859.

48. Passwell J. H., E. Harlev, S. Ashkenazi, C. Chu, D. Miron, R. Ramon, N. Farzan, J. Shiloach, D. A. Bryla, F. Majadly, R. Roberson, J. B. Robbins, and R. Schneerson. 2001. Safety and immunogenicity of improved *Shigella* O-specific polysacchairide-protein conjuguate vaccines in adults in Israel. Infect. Immun. **69** : 1351.

49. Pavliak, V., E. M. Nashed, V. Pozsgay, P. Kovac, A. Karpas, C. Chu, R. Schneerson, J. B. Robbins, and C. P. J. Glaudemans. 1993. Binding of the O-antigen of *Shigella dysenteriae* type 1 and 26 related synthetic fragments to a monoclonal IgM antibody. J. Biol. Chem. **268** : 25797-25802.

50. Phalipon, A., J. Arondel , F. T. Nato, S. Rouyre, J. M. Mazie, and P. J. Sansonetti. 1992. Identification and characterization of B-cell epitopes of IpaC, an invasion-associated protein of *Shigella flexneri*. Infect. Immun. **60**:1919-26.

51. Phalipon, A., M. Kaufmann, P. Michetti, J. M. Cavaillon, M. Huerre, P. J. Sansonetti, and J. P. Kraehenbuhl. 1995. Monoclonal immunoglobulin A antibody directed against serotype-specific epitope of *Shigella flexneri* lipopolysaccharide protects against murine experimental shigellosis. J. Exp. Med. **182** : 769.

52. Phalipon, A., A. Cardona, J. P. Kraehenbuhl, L. Edelman, P. J. Sansonetti, and B. Corthésy. 2002. Secretory component : a new role in secretory IgA-mediated immune exclusion *in vivo*. Immunity **17 :** 107-115.

53. Philpott, D. J., J. D. Edgeworth, P. J. Sansonetti. 2000. The pathogenesis of *Shigella flexneri* infection: lessons from *in vitro* and *in vivo* studies. Philos Trans R Soc Lond B Biol Sci. **29**(355):575-86.

54. Pier, G. B., G. Meluleni, and J. B. Goldberg. 1995. Clearance of *Pseudomonas aeruginosa* from the murine gastrointestinal tract is effectively mediated by O-antigen-specific circulating antibodies. Infect. Immun. **63** : 2818-2825.

55. Pozsgay, V, C. Chu , L. Pannell , J. Wolfe, J. B. Robbins , and R. Schneerson . 1999. Protein conjugates of synthetic saccharides elicit higher levels of serum IgG lipopolysaccharide antibodies in mice than do those of the O-specific polysaccharide from *Shigella dysenteriae* type 1. Proc. Natl. Acad. Sci. U S A. **96**(9):5194-5197.

56. Qatri, F., T. Azim, A. Hossain, A. Chowdhury, and M. J. Albert. 1994. Monoclonal antibodies specific for *Shigella dysenteriae* serotype 13. Production, characterization, and diagnostic application. Diagn. Microbiol. Infect. Dis. **18** : 145-149.

57. Ramaligan, T. S., S. A. Detmer, W. L. Martin, P. J. Bjorkman. 1997. IgG transcytosis and reclycling by FcRn expressed in MDCK cells reveals ligand-induced redistribution. EMBO J. **21** : 590-601.

58. Rasolofo-Razanamparany, V., A. M. Cassel-Béraud, J. Roux, P. J. Sansonetti, and A. Phalipon. 2001. Predominance of serotype-specific mucosal antibody response in *Shigella flexneri*-infected humans living in an area of endemicity. Infect. Immun. **69** : 5230-5234.

59. Robbins, J. B., C. Chu, and R. Schneerson. 1992. Hypothesis for vaccine development : protective immunity to enteric diseases caused by non typhoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharide. Clin. Infect. Dis. **15** : 346-361.

60. Suzuki, K., and T. Takeda. 1989. Monoclonal atibodies against the surface antigens of *Shigella flexneri* serotype 1b and *Shigella dysenteriae* serotype 1. Microbiol. Immunol. **33**: 897-906.

61. Tagliabue, A ., L. Necioni, L. Villa, D. F. Keren, G. H. Lowell, and D. Boraschi. 1983. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. Nature **306** : 184-186.

62. Tagliabue, A., D. Boraschi, L. Villa, D. F. Keren, G. H. Lowell, R. Rappuoli, and L. Nencioni. 1984. IgA-dependent cell-mediated activity against enteropathogenic bacteira : distribution, specificity, and characterization of the effector cells. J. Immunol. **133** : 988-992.

63. Van de Verg, L., D. A. Herrington, J. Boslego, A. A. Lindberg, and M. M. Levine. 1992. Age-specific prevalence of serum antibodies to the invasion plasmid and polysaccharide of *Shigella* species in chilean and north-american populations. J. Infect. Dis. **166** : 158-161.

64. Van de Verg, L. L., N. O. Bendiuk, K. kotloff, M. M. Marsh, J. L. Ruckert, J. L. Puryear, D. N. Taylor, and A. B. Hartman. 1996. Cross-reactivity of *Shigella flexneri* serotype 2a O antigen antibodies following immunization or infection. Vaccine **14** : 1062-1068.

65. Vecino, W. H., P. M. Morin, R. Agha, W. R. Jacobs, and G. J. Fennelly. 2002. Mucosal DNA vaccination with highly attenuted *Shigella* is superior to attenuated *Salmonella* and comparable to intramuscular DNA vaccination for T cells against HIV. Immunol. Lett. 82 : 197-204.

66. Voino-Yasenetsky, M. V., and M. K. Voino-Yasenetskaya. 1961. Experimental pneumonia caused by bacteria of the shigella group. Acta. Morpho. **XI** : 440.

67. Wernet, P., H. Breu, J. Knop, and D. Rowley. 1971. Antibacterial action of specific IgA and transport of IgM, IgA and IgG from serum into the small intestine. J. Infect. Dis. **124** : 223-226.

68. Westphal, O., and J. Jann. 1965. Bacterial lipopolysaccharides : extraction with phenol-water and further application of the procedures. Methods Carbohydr. Chem. **5** : 83-91.

69. Winsor, D. K., J. J. Mathewson, and H. L. DuPont. 1988. Comparison of serum and fecal antibody responses of patients with naturally acquired *Shigella sonnei* infection. J. Infect. Dis. **158** : 1108-1112.

Legends of figures

Figure 1 : Protection conferred by immune serum specific for *S.flexneri* 2a LPS intranasally administered prior to i.n. challenge.

A) Serum IgG subclasses elicited in mice upon i.p. immunization with killed *S. flexneri* 2a bacteria. ▬ represents the mean value of the antibody titer (n=10 mice).

B) Protection assessed by reduction of lung-bacterial load in mice receiving anti-*S.flexneri* 2a LPS immune serum raised upon i.p. immunization, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* 2a bacteria. a, b, c, correspond to immune sera exhibiting an anti-*S. flexneri* 2a LPS IgG antibody titer of 1/4,000, 1/16,000 and 1/64,000, respectively. Standard deviation is indicated (n=10 mice per group).

Figure 2 : Protection conferred by different subclasses of mIgG specific for *S. flexneri* 2a serotype determinants.

A : mice receiving intranasally 20µg and 2µg of purified mIgG, respectively, 1h prior to i .n. challenge with a sublethal dose of *S. flexneri* 2a. Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group).

B : Histopathological study of mouse lungs. Upper row : control mice. Lower row : mice receiving mIgG. HE staining : *a* and *d* magnification x 40 ; *b* and *e* magnification x 100. Immunostaining using an anti-LPS antibody specific for *S. flexneri* serotype 2a : *c* and *f* magnification x100.

Figure 3: Serotype-specific protection conferred by the anti-LPS mIgG.

A : Mice were receiving i.n. 20µg of each of the purified mIgG, C20 and C1, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* serotype 2a (A) or serotype 5a (B) bacteria.

Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group).

B : Histopathological study of mouse lungs. *a* and *b* : mice receiving mIgGC20 specific for *S. flexneri* serotype 5a and challenged with *S. flexneri* serotype 2a and 5a, respectively. *c* and *d* : mice receiving mIgGC1 specific for *S. flexneri* 2a prior to challenge with *S. flexneri* serotype 2a and 5a, respectively. HE staining, magnification x 100.

Figure 4 : Protection conferred by mIgG specific for *S. flexneri* IpaB or IpaC invasins.

Mice were receiving i.n. 20µg of each of the purified mIgG, H4, H16, J22, K24, and C20, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* serotype 5a. Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group).

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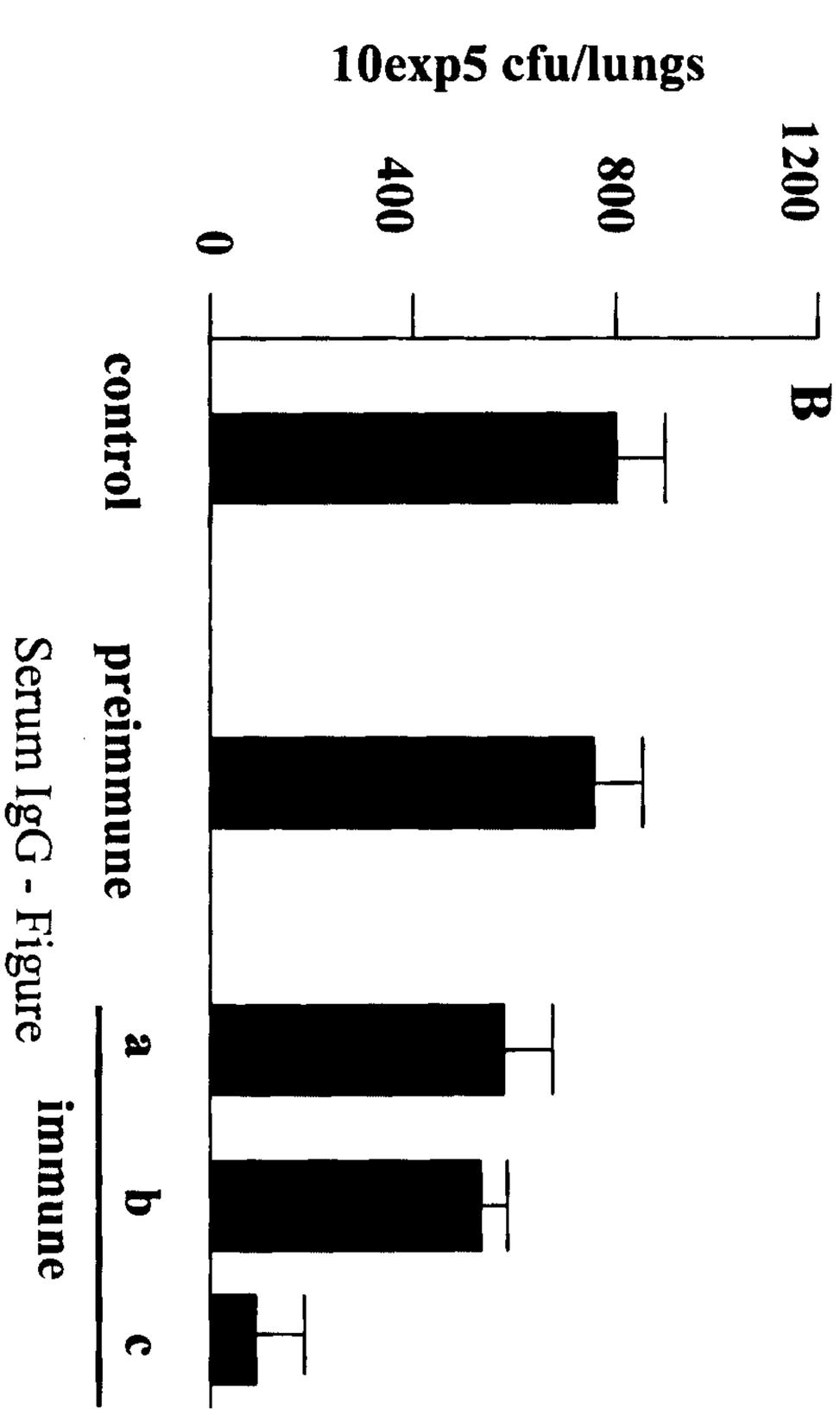
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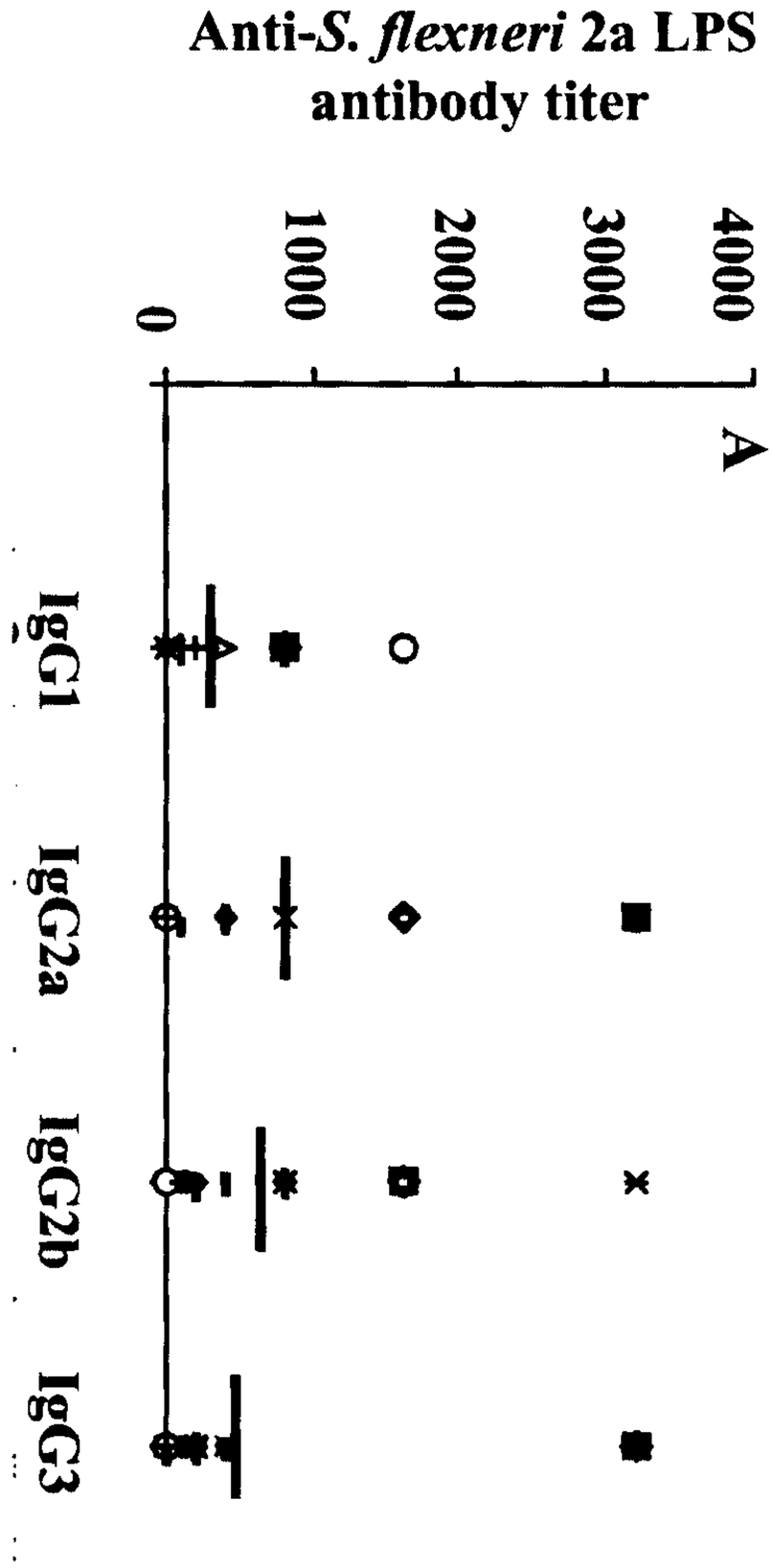
Serum IgG - Figure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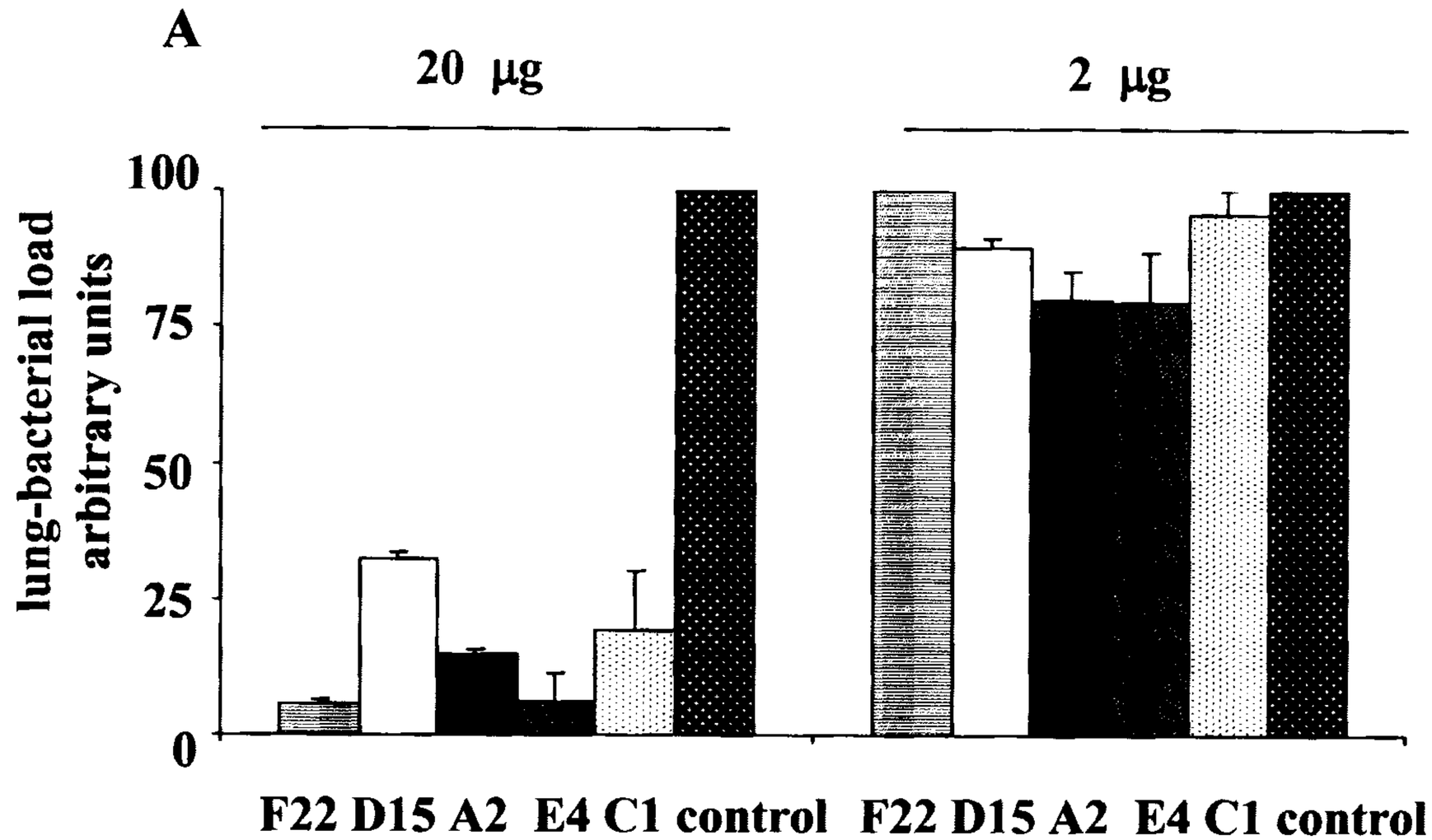
IgG3

A



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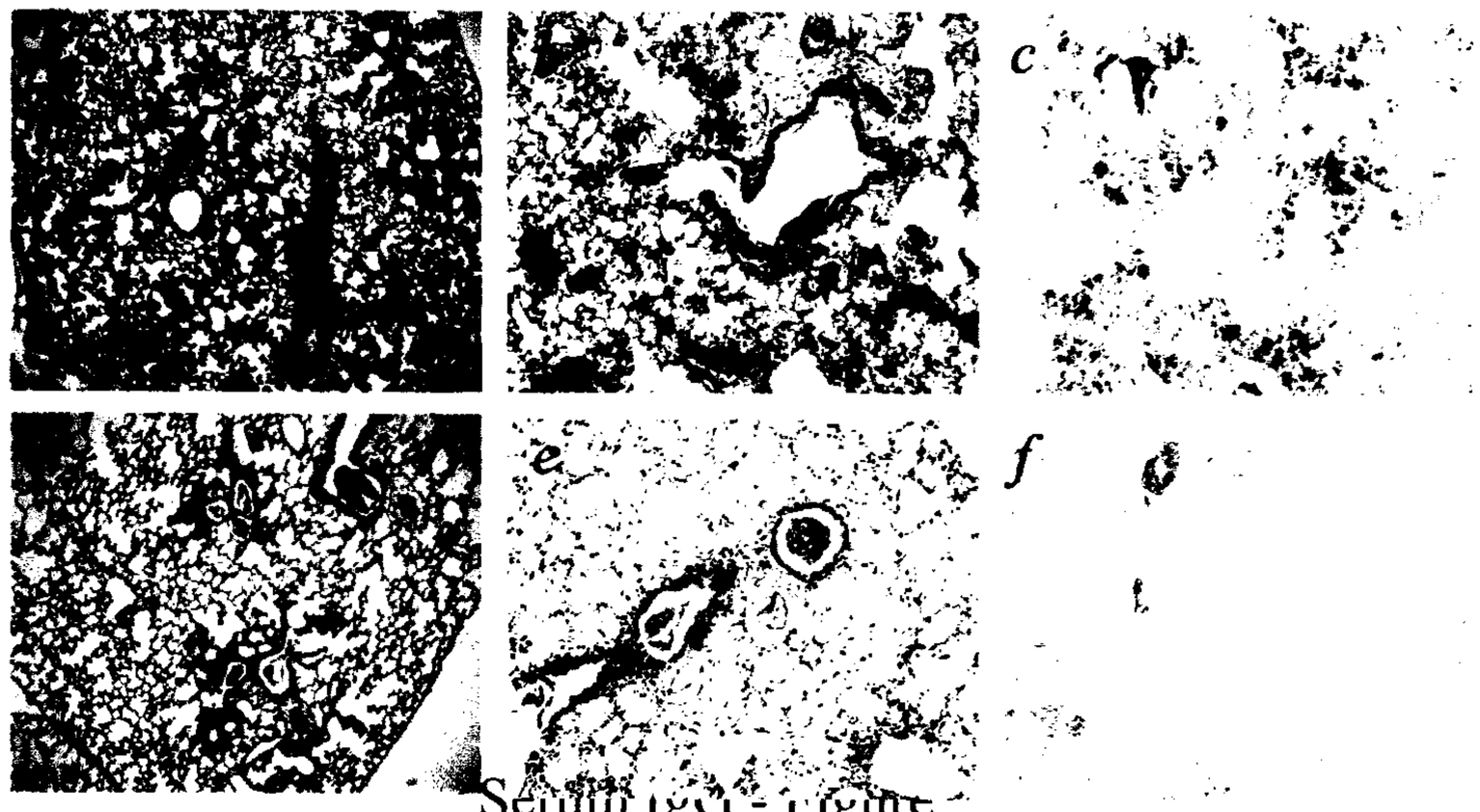
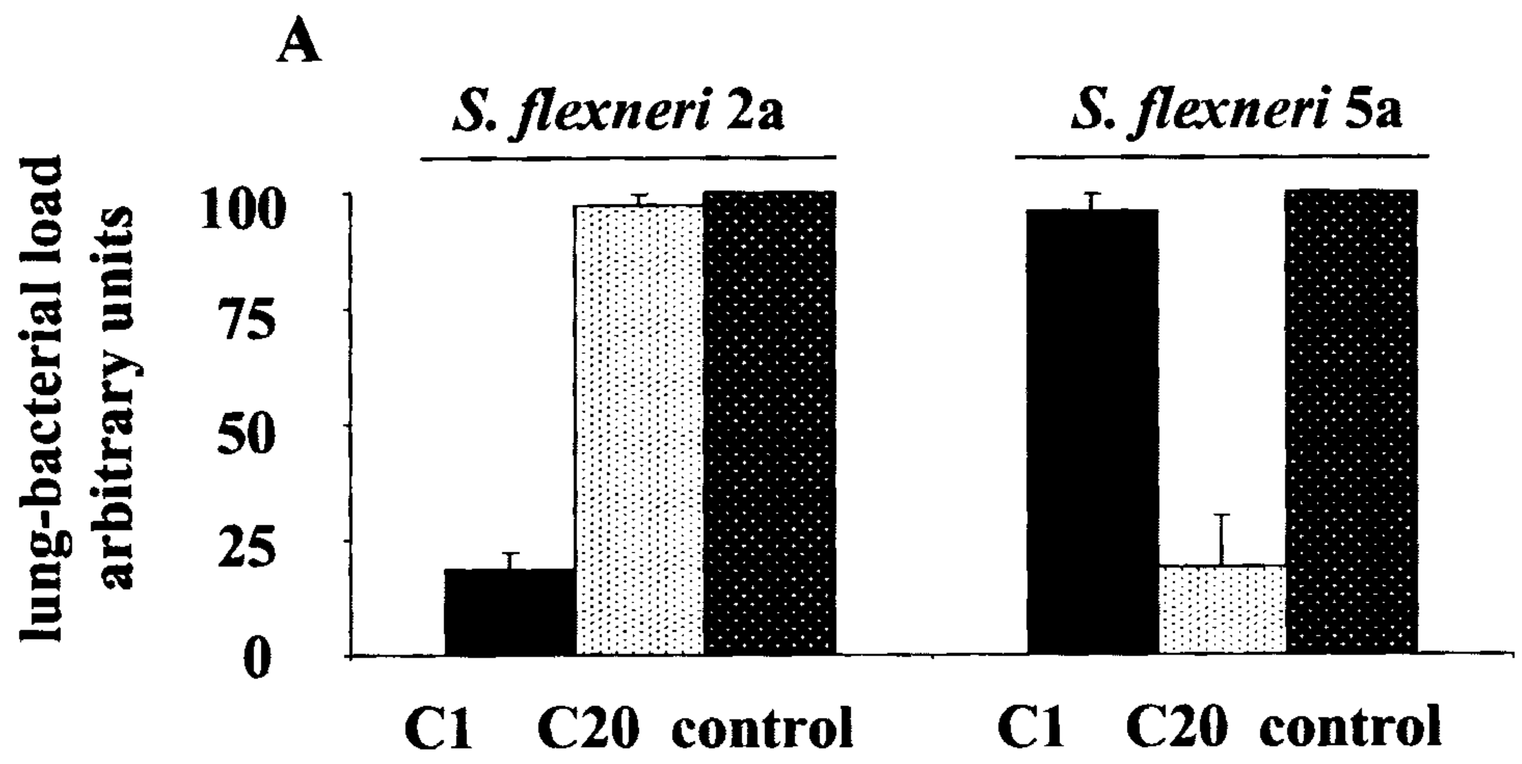


Serum IgG - Figure 2

A



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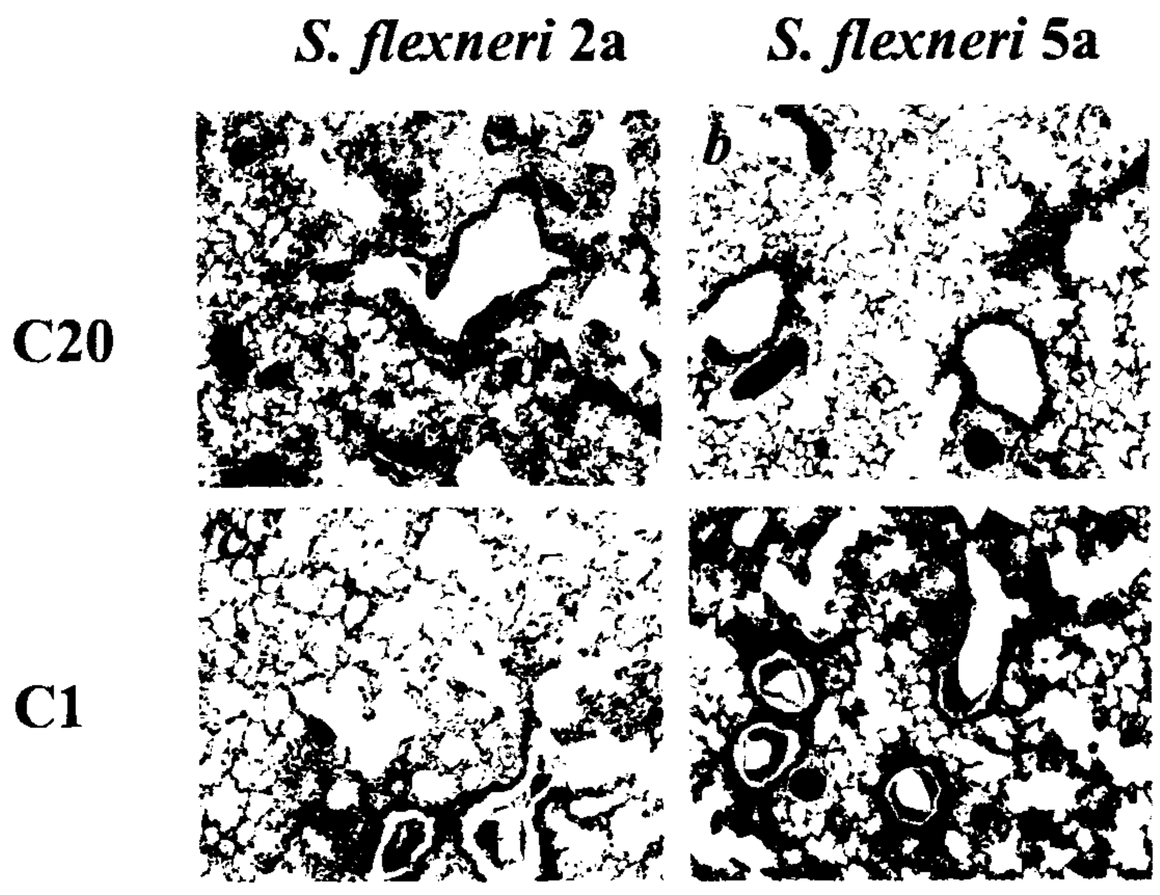
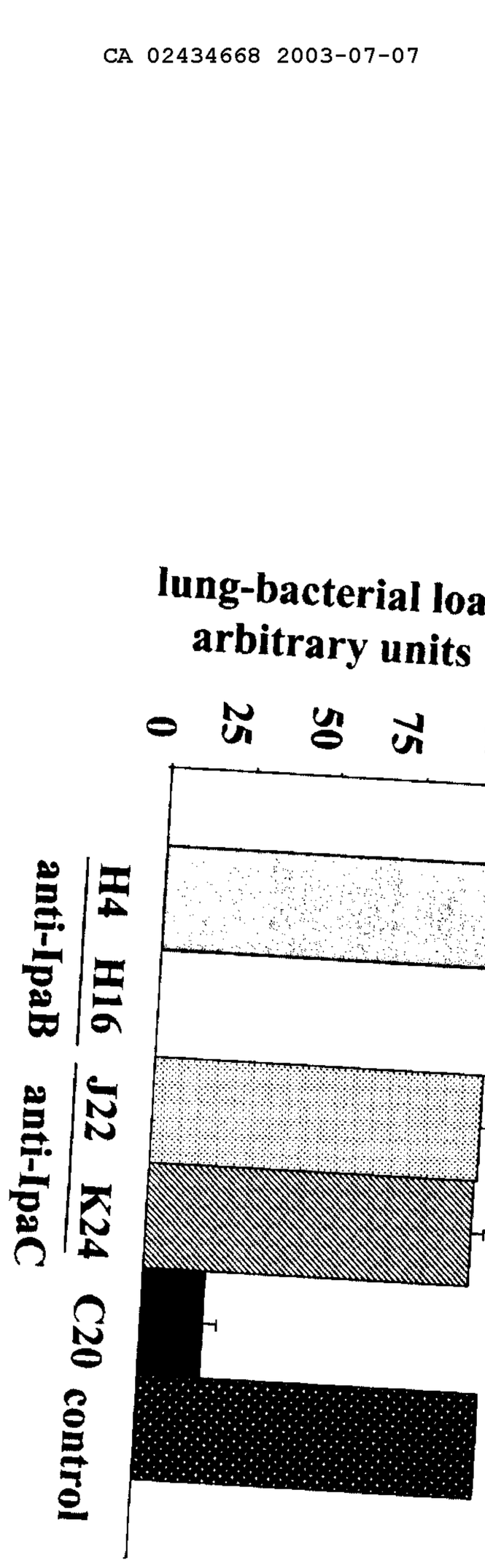


Serum IgG - Figure



Serum IgG - Figure

4

Sflex5a-NMR-IC50protocol-brevet

Conformational studies of the *O*-specific polysaccharide of *Shigella flexneri* 5a and of four related synthetic pentasaccharide fragments using NMR and molecular modeling.

Streptococcus pneumoniae, *Neisseria meningitidis* or *Salmonella typhi* are commercially available (2). However, as they were ineffective in infants, polysaccharide:protein conjugate vaccines were then successfully developed, as illustrated by *Haemophilus influenzae* b and *Neisseria meningitidis* Group C vaccines recently made available (3,4). Glycoconjugate vaccines derived from detoxified LPSs are under study in several laboratories, since protein conjugates of the polysaccharide moiety of LPSs were shown to be safe and immunogenic in humans (5). However, improving the immunogenicity of such conjugates is still of high interest, although it may be impaired by a poor knowledge of the critical parameters. A possible alternative to polysaccharide conjugate vaccines may derive from the use of accurate synthetic mimics of the bacterial polysaccharide.

The development of such an alternative, targeting a synthetic vaccine against *Shigella flexneri* infections, is ongoing in the laboratory. *S. flexneri* is a Gram-negative bacillus responsible for the endemic form of shigellosis, a dysenteric syndrome. The disease, characterized by bacterial invasion of the human colonic mucosa (6), causes a high rate of mortality among infants, particularly in developing countries (7). It has been demonstrated that the *O*-specific polysaccharide moiety (O-SP) of the LPS is the major target of the protective immune response. Indeed, protein conjugates of the polysaccharide moiety of *S. flexneri* serotype 2a appear as promising vaccine candidates (8).

The intimate relationship between polysaccharide shape and biological function is now well-established and it is assumed that knowledge of the conformational behaviour in solution of the bacterial surface polysaccharide may help the mimic approach. Indeed, following the pioneering work by Lemieux and his co-workers (9), and taking advantage of the development of powerful new methods for conformational analysis, both in NMR and Molecular Modeling, increasing knowledge of the conformation of bacterial polysaccharide has been made available

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(10). The study presented herein deals with the model bacterium *S. flexneri* 5a, whose specificity is defined by the structure of the repeating unit **II** of its O-SP (11).

A **B** **C** **D**

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*–(1→3)-β-D-GlcNAc*p*-(1→

(1↑3)

E α-D-Glc*p* **(II)**

This branched pentasaccharide comprises the linear tetrasaccharide **I**, which is made of three α-L-rhamnopyranosyl (α-L-Rha) and one 2-acetamido-2-deoxy-β-D-glucopyranosyl (β-D-GlcNAc*p*) residues and is common to the repeating units of the LPSs of all *S. flexneri* serotypes (12).

A **B** **C** **D**

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*–(1→3)-β-D-GlcNAc*p*-(1→ **(I)**

The specificity of serotype 5a is associated to the presence of an α-D-glucopyranosyl (α-D-Glc*p*) residue (E) at position 3 of residue B (Figure 1) (11).

(Figure 1)

Frame-shifted di-, tri-, tetra- and pentasaccharide fragments of the **O-SP 5a**, all bearing the characteristic **EB** segment, have been recently synthesized as their methyl glycosides with the natural anomeric configuration at their reducing end (13-17). In order to define if short fragments can express conformational features of the **O-SP 5a**, we carried out a comparative study of the conformational behavior of the four possible pentasaccharides **1**, **2**, **3**, **4** specific for the **O-SP 5a** (Figure 1) and that of the native O-SP. Given the putative flexible nature of oligosaccharides and polysaccharides, a combination of NMR and molecular modeling analysis was undertaken to accurately deduce the conformational properties of these molecules. Moreover, the affinity of a

protective monoclonal antibody specific for *Shigella flexneri* 5a for these pentasaccharides has been investigated by inhibition enzyme-linked immunosorbent assay (ELISA). Immunochemical and structural data will allow correlating the inhibitor activity of pentasaccharides with their conformational features.

The NMR conformational study lies in the analysis of ^{1}H and ^{13}C chemical shifts, the evaluation of inter-residue distances as well as heteronuclear $^{3}J_{C,H}$ coupling constants across the glycosidic linkages which are related to Φ and Ψ torsion angles in a Karplus-type relationship (18). The $^{3}J_{C,H}$ coupling constants have been determined using two different techniques, *ex*citation-*s*culptured *i*ndirect-*d*etection *e*xperiments (EXSIDE) (19) and gradient-selected *J*-HMBC experiments (20). The molecular modeling of the pentasaccharides consists in a complete conformational search with the CICADA algorithm (21) interfaced with MM3 force field (22,23) which has proven to be very efficient for exploring the conformational space of flexible molecules (21), including oligosaccharides (24-26). Boltzman-averaged inter-residue distances and $^{3}J_{C,H}$ coupling constants were calculated on the generated conformers and compared with NMR data for all frame-shifted pentasaccharides. As the regular helical conformation of polysaccharides that is observed in their solid state (27) can be used as starting point for studying their solution behavior, the possible helical shapes of the **O-SP 5a** have been determined by means of a molecular builder, POLYS (28), which combines a database of monosaccharide structures with the conformational information of disaccharide fragments. Based on the comparison of the NMR and molecular modeling data described herein with those obtained for the *S. flexneri* variant Y polysaccharide (29) (**O-SP Y**), whose repeating unit is the tetrasaccharide **I**, particular attention was paid to the influence of the branched glucosyl residue E on the conformational properties of **O-SP 5a.**

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EXPERIMENTAL PROCEDURES

Material. Solution concentrations of the pentasaccharides **1**, **2**, **3** and **4** in 600 µl deuterium oxide were 28 mM, 30 mM, 24 mM and 12.7 mM respectively. The **O-SP 5a** was prepared by acid hydrolysis of the LPS as described earlier (30). Mass spectrometry analysis showed that the purified O-SP is composed of an average of 15 repeating units. About 10 mg of **O-SP 5a** were dissolved in 400 µl of deuterium oxide.

Nomenclature. The two torsion angles describing a glycosidic linkage are defined as $\Phi = O_5\text{-}C_1\text{-}O_1\text{-}C'_X$ and $\Psi = C_1\text{-}O_1\text{-}C'_X\text{-}C'_{X+1}$ with the primed atoms belonging to the reducing side and the sign being in agreement with IUPAC nomenclature (31).

NMR spectroscopy. NMR experiments were recorded on Varian Unity and Inova spectrometers, operating at ^1H frequency of 500 MHz and 600 MHz respectively, equipped with a triple resonance pulsed field gradient probe with an actively shielded *z* gradient. Chemical shifts are given relative to an external standard of sodium 2,2-dimethyl-2-silapentane-5 sulfonate (DSS) at 0 ppm for both ^{13}C and ^1H chemical shifts. DQF-COSY (32), TOCSY (33), off-resonance ROESY (34), gHSQC (35), gHSQC-TOCSY (35,36), and gHMBC (35) experiments were performed as described previously (16,17), at 35°C or 38°C for the pentasaccharides and at 50°C for the **O-SP 5a**. The $^3J_{H,H}$ coupling constants were obtained from one-dimensional spectrum with a digital resolution of 0.1 Hz/point, or from the DQF-COSY experiment with a digital resolution of 0.5 Hz/point. The $^1J_{C1,H1}$ coupling constants were measured from the gHMBC spectrum with a digital resolution of 0.5 Hz/point.

The NOESY experiments were carried out at 10°C with mixing periods of 100, 200, 400 and 600 ms for the pentasaccharides, and at 50°C with mixing periods of 80, 100, 200, 300 ms for the O-SP to obtain build-up curves.

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For the measurement of long-range heteronuclear $^{3}J_{C,H}$ coupling constants, two different methods were used, *ex*citation-*s*culptured *i*ndirect-*d*etection *e*xperiments (EXSIDE) (19) and gradient-selected *J*-HMBC experiments (gs-*J*-HMBC) (20). Concerning the first one, all proton band-selective π pulses were Gaussian cascade Q3 pulses (37) with phase modulation to achieve off-resonance inversion. They were generated using the Pandora's Box pulse-shaping program available in Varian software. Sixteen scans of 2048 complex points were collected for each of the 512 t_1 increments. A *J*-scaling factor (N) of 15 was applied. A recovery delay of 2.5 s was used prior to each scan. The spectra were transformed after zero filling to 2048 □ 4096 complex points using unshifted Gaussian window function along the F_1 and F_2 dimensions. In EXSIDE spectra, the $^{3}J_{C,H}$ coupling constants were measured in F_1 dimension with a digital resolution of 0.3 Hz/point. For the gs-*J*-HMBC experiments, nine 2D spectra were acquired with 16 and 96 scans per increment (356 t_1 increments) for the pentasaccharides and the **O-SP 5a** respectively. A constant time delay τ_{max} of 230 ms and τ values from 50 to 220 ms were used. The $^{3}J_{C,H}$ coupling constants values were obtained by measuring cross peak intensities as a function of τ and fitting them to an equation of the form

$$y = A \sin (\pi\, {}^{n}J_{C,H}\, \tau)$$

Indeed, the intensity, $s(t_1,t_2)$, of a particular correlation in a HMBC spectrum depends on the amplitude of the long range $J_{C,H}$ and can be described by the following equation (20,38) :

$$s(t_1,t_2) = \exp [-(\Delta + \tau_{max} + t_1 + t_2) / T_2] \sin (\pi\, {}^{n}J_{CH}\, t_2) \prod [\cos [\pi\, J_{Hk}(\Delta + \tau_{max} + t_1 + t_2)] \sin (\pi\, {}^{n}J_{CH}\, \tau)$$

with $\Delta = (2\, {}^{1}J_{C,H})^{-1}$.

The ^{13}C spin-lattice relaxation time measurements of the **O-SP 5a** were carried out at 125 MHz by means of 2D-double-INEPT type inverse-detected experiments with suppression of cross-

correlation (39). Eight experiments were performed with relaxation delays, τ, ranging from 0.005 to 1 s. T_1 values were deduced by measuring the cross peak intensities as a function of T, and fitting the volumes to an equation of the form

$$y = A \exp(-\tau / T_1)$$

Error on data points was estimated as 5 noise rsmds.

All 2D data, except for EXSIDE and HMBC experiments, were collected in the phase sensitive mode using the States-Haberkorn method (40).

Inter-proton distances from cross-peak volumes. The cross-peak volumes from off-resonance ROESY (400 ms mixing time) and NOESY experiments were measured with the VNMR software. The proton-proton distances were calculated using the usual $1/r^6$ NOE/distance relationship (41). NOE-derived distances were obtained from initial NOE build-up rates which are calculated thanks to the fitting of NOE volumes at the different mixing times. The intra-residue distance of 2.52 Å between H-1 and H-2 protons of the α-rhamnose unit B was used as reference for distance calibration.

Energy calculations. All geometry optimizations were performed using the molecular mechanics program MM3 (22,23). The block-diagonal minimization method was used for geometry optimization, with the default energy-convergence criterion (0.00008*n kcal/mol per five iteration, n = number of atom). The dielectric constant was set to 78 in order to attenuate the influence of hydrogen bonding on the potential energy surface.

Starting models. All the disaccharides and oligosaccharides were built using MONOBANK, a database of three-dimensional structures of monosaccharides (42).

Relaxed energy maps of the disaccharides. The five disaccharides constituting the **O-SP 5a** have been the subject of a systematic grid search study for the conformation of the glycosidic

linkage. The calculations were performed on disaccharide methyl glycosides. Starting from minimized disaccharides, the Φ and Ψ torsion angles were driven by steps of 20° over the whole angular range while the molecular mechanics program, MM3, provided full geometry relaxation. Several maps were calculated for each disaccharide to take into account several possible orientations of the primary and secondary hydroxyl groups. At most, 24 starting geometries were needed to take into account the two most stable staggered orientations of the hydroxymethyl groups, referred as gg and gt, and the two possible networks of secondary hydroxyl groups, around each ring referred as clockwise and counterclockwise. For each disaccharide, the results of these calculations are projected on a so-called 'adiabatic' map where only the conformer with the lowest energy for each (Φ,Ψ) value is considered. Iso-energy contours were then plotted by interpolation of 1 kcal/mol within an 8 kcal/mol window.

CICADA calculations. Exploration of the potential energy surface of the pentasaccharides was performed with the CICADA program (43). Input for the CICADA program, which is an interface to the MM3 force field, consists mainly of one or a few conformers in MM3 format and a file containing the list of torsion angles to be driven and/or monitored. During the CICADA calculations, each selected torsion angle is driven one after the other in each direction from the initial conformation at a given increment. For the pentasaccharides, the driven torsion angles were Φ and Ψ at each linkage and the torsion angle of each hydroxymethyl group (O_5-C_5-C_6-O_6), leading to a dimensionality of 10 for the potential energy surface to explore. The torsion angle of *N*-acetyl group (C_1-C_2-N-C_8) and the torsion angles of all the secondary hydroxyl groups were monitored but not driven. The increment step was set to 20° and two conformations were considered to be different when one of the driven or monitored angles differed by at least 30°. A relative energy cut-off of 50 kcal/mol was applied for exploring the potential energy surface. The

search was stopped when no new conformer with energy lower than 5 kcal/mol could be detected.

Analysis of the potential energy surface. The conformations and transition states found by CICADA were analyzed by the PANIC program (44), which explores the paths along the potential energy surface. Conformations were clustered into families within an energy window of 5 kcal/mol with the FAMILY program (45). In the present study, a conformer belongs to a conformational family if at least one of its torsion angles differs by less than 10° to at least one of the conformers of the family. As for the relative importance of each family, their population is calculated. The relative population of the *i*th conformational state, *P*i, with energy, *E*i, is dictated by the Boltzmann distribution

$$P\mathrm{i} = \exp(-E\mathrm{i} / k\mathrm{T}) / \sum \exp(-E\mathrm{i} / k\mathrm{T})$$

Calculations of theoretical distances and coupling constants. For each pentasaccharide, Boltzmann-averaged interproton distance $\langle r^{-6} \rangle$ matrices and $\langle {}^3J_{C,H} \rangle$ coupling constants were calculated for the two lowest energy conformational families and for the average of all conformational families with population of more than 1% at 298 K.

The ${}^3J_{C,H}$ coupling constants were calculated by using a Karplus-type equation for C-O-C-H segment (18).

$${}^3J_{C,H} = 5.7 \cos^2 \theta_H - 0.6 \cos \theta_H + 0.5$$

Then, using the fractional population (*P*i) for each conformational microstate, the average interproton distances and coupling constants were computed from

$$\langle r \rangle = (\sum P\mathrm{i} \, . r^{-6})^{-1/6}$$

$$\text{and } \langle {}^3J_{C,H} \rangle = \sum P\mathrm{i} . {}^3J_{C,H}$$

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Construction of the O-SP 5a. Possible conformations of the **O-SP 5a** were modeled using the molecular builder POLYS (28). Different polysaccharide fragments made of six repeating units were constructed using all the combinations of the energy-minima obtained from adiabatic energy maps calculated on the disaccharides constituting it. The models that did not present serious steric conflicts were submitted to a POLYS procedure that optimized the values of Φ and Ψ at each glycosidic linkage in order to attain the closest regular fold helical symmetry.

Inhibition ELISA. Fisrt of all, a standard curve was established with IgGC20, a protective monoclonal antibody specific for *S. flexneri* 5a (A. Phalipon, personal communication). Different concentrations of the antibody were incubated at 4°C overnight and then incubated on microtiter plates coated with purified *S. flexneri* 5a LPS at a concentration of 5 µg/ml in carbonate buffer at pH 9.6, and previously incubated with PBS/BSA 1% for 30 min at 4°C. After washing with PBS-Tween 20 (0.05%), alkaline phosphatase-conjugated anti-mouse IgG was added at a dilution of 1:5000 (Sigma Chemical CO.) for 1h at 37°C. After washing with PBS-Tween 2à (0.05%), the substrate was added (12 mg of p-nitrophenylphosphate in 1.2 ml of Tris, HCl buffer pH 8.8 and 10.8 ml of NaCl 5M). Once the color developed, the plate was read at 405 nm (Dinatech MR 4000 microplate reader). A standard curve OD = f(antibody concentration) was fitted to the quadratic equation $Y = aX^2+bX+c$ where Y is the OD and X is the antibody concentration. Correlation factor (r^2) of 0.99 were routinely obtained.

Then, the amount of oligosaccharides giving 50% inhibition of IgGC20 binding to LPS (IC50) was determined as follows. IgGC20 at a given concentration (chosen as the minimal concentration of antibody giving the maximal OD on the standard curve) was incubated overnight at 4°C with various concentrations of each of the oligosaccharides to be tested, in PBS/BSA 1%. Measurement of unbound IgGC20 was performed as described above using microtiter plates

coated with purified LPS from *S. flexneri* 5a and the antibody concentration was deduced from the standard curve. Then, IC50 were determined.

RESULTS AND DISCUSSION

NMR spectroscopy. Protons and carbons of the methyl glycosides **3** and **4** as those of the **O-SP 5a** have been assigned as described previously for pentasaccharides **1** (16) and **2** (17) using one- and two-dimensional NMR spectra such as DQF-COSY, TOCSY, gHSQC, gHMBC and gHSQC-TOCSY, crucial to solving the final ambiguities. Taking into account that different references were used, the ^{1}H and ^{13}C chemical shifts of the **O-SP 5a** determined here are in good agreement with those previously published (46,47).

Comparison of ^{1}H and ^{13}C chemical shifts of pentasaccharides with those of the **O-SP 5a** shows that the lack of third branching sugar on residue B for pentasaccharides **1** and **3** induces differences not only on residue B but also on internal residues (See supplemental material Tables S1-S2). For example, the chemical shifts of the H-2_C, H-3_C and C-3_C of **1**, and the H-4_A, H-5_A and C-5_A of **3** do not coincide with those of corresponding atoms in the native O-SP. Moreover, all ^{1}H and ^{13}C chemical shifts of internal residues of pentasaccharide **4** are similar to those of the **O-SP 5a**, except for protons H-1_B and H-5_B (Table 1 and 2). For pentasaccharide **2**, many more differences are observed for protons as well as carbons (See supplemental material Tables S1-S2).

(Table 1)

(Table 2)

As chemical shifts are extremely sensitive to conformation, one can state that pentasaccharide **4** is the fragment whose conformation more closely mimics that of the native O-SP. The large differences observed for residues at the reducing and non-reducing ends are due to glycosylation

effects or to the presence of the methyl aglycone. Comparison of ^{1}H and ^{13}C chemical shifts of **O-SP 5a** with those of **O-SP Y** (29) (Table 1 and 2) shows that the presence of the glucosyl residue E at position 3 of rhamnosyl residue B in **O-SP 5a** introduces significant changes into the chemical shifts of the backbone residues. The major differences in chemical shifts are obviously observed for residue B. Additionally, minor differences are observed for residues A and C, the former being involved in a 2,3-*cis* vicinal pattern with residue E. Long range effects of residue E are also observed on chemical shifts of residue D. These observations point out the critical influence of the branched glucopyranoses E on the overall solution conformation of the **O-SP 5a** backbone.

The vicinal coupling constants $^{3}J_{H,H}$ of **3** and **4** are fully consistent with a $^{1}C_4$ conformation for the L-rhamnopyranoses (A, B and C) and a $^{4}C_1$ conformation for the D-glucose and the *N*-acetyl D-glucosamine residues (E and D) (See supplemental material Table S3) as it was previously observed for the frame-shifted pentasaccharides **1** (16) and **2** (17).

As expected, the heteronuclear one-bound $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants measured in gHMBC experiment for each pentasaccharide and **O-SP 5a** are in agreement with an α configuration for the L-rhamnopyranose and D-glucose residues and with a β configuration for *N*-acetyl D-glucosamine residue (Table 2 and supplemental material Table S4) (48). The $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants of the rhamnose residues A and B in the native O-SP are slightly larger than expected for such residues (173 Hz vs 170 Hz). Interestingly, amongst the synthetic pentasaccharides, only compound **4**, presents this increase for both residues A and B. The larger values measured for $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants may be explained by an increase of steric constraint surrounding C-1 and C-2 of these residues (49). This argument is corroborated by the values of ^{13}C spin-lattice relaxation times (T_1) (Table 2) determined for the **O-SP 5a**. Indeed, the C-1_A and C-2_A present

^{13}C T_1 values that reflect some rigidity relative to the other carbon atoms of its cycle (300 ms vs 350 ms). The C-1_B and C-2_B present the lowest ^{13}C T_1 values ($\approx$270 ms). On the contrary, the high ^{13}C T_1 values measured for the branched glucosyl residue E may be interpreted as an indication of greater mobility.

The conformations of the four pentasaccharides and that of **O-SP 5a** were examined in more details using the inter-residue ^1H-^1H distances obtained from NOESY and off-resonance ROESY experiments. The latter experiment allows to avoid spin diffusion effect as well as Hartmann-Hahn artifacts (34). Averaged inter-residue ^1H-^1H distances deduced from ROE and NOE volumes are in agreement (Table 3 and supplemental material Table S5). Moreover, no significant difference is observed between the inter-residue ^1H-^1H distances of the four pentasaccharides and those of the native O-SP. Generally, NOE or ROE cross-peaks across glycosidic linkages can fit to many different conformations. Here, the large number of connectivities observed in addition to NOEs and ROEs across glycosidic linkages between anomeric and aglyconic protons are useful for the definition of a single conformation. Futhermore, the presence of long-range NOE and ROE connectivities such as H-6_A/Nac_D in **2** and H-6_B/N-Ac_D in **1**, **2** (See supplemental material Table S5) and **O-SP 5a**, is an indication of the folded nature of the backbone of these molecules.

(Table 3)

In order to complete this conformational analysis, long range $^3J_{C,H}$ coupling constants across the glycosidic linkages, which are related to Φ and Ψ torsion angles in a Karplus relationship, were determined from two different NMR experiments, EXSIDE and gradient-selected *J*-HMBC (Table 4).

(Table 4)

The former experiment, EXSIDE, is a semiselective version of the gradient-selected HSQC sequence (50,51), that provides cross-peaks *J*-scaled in the carbon dimension. The $^{3}J_{C,H}$ coupling constants are measured directly on the spectrum (Figure 2) with no interference from any homonuclear couplings due to a proton band selection based on the excitation-sculpting technique (52). The unique chemical-shift region of anomeric protons in oligosaccharides makes this method easily applicable for a rapid obtaining of coupling constants involving H-1 protons.

(Figure 2)

Nevertheless, a difficulty exists for the measurement of some $^{3}J_{C\text{-}1,H\text{-}X}$ coupling constants due to the proton spectral overlap rendering measurement without interference from homonuclear couplings impossible. In order to obtain the missing $^{3}J_{C\text{-}1,H\text{-}X}$ coupling constants, gs-*J*-HMBC experiments were performed, although in that case obtaining $^{3}J_{C,H}$ coupling constants is much more time consuming than the EXSIDE experiment. Indeed, several 2D spectra must be acquired with different evolution time τ. Then, the signals being amplitude modulated by sin ($\pi J_{C,H}$ τ), the heteronuclear long-range $^{3}J_{C,H}$ coupling constants are obtained by fitting a sine curve to the experimental data. According to the $^{3}J_{C,H}$ values measured (Table 4 and supplemental material Table S6), both approaches lead to identical results with the same precision (± 0.3 Hz). The pentasaccharide $^{3}J_{C,H}$ coupling constants are rather similar to those of the **O-SP 5a** except for the $^{3}J_{C\text{-}1B,H\text{-}3C}$ of **1** which is very different (6.1 Hz vs 3.7 Hz), as could be expected considering that the pentasaccharide **1** lacks the critical A(E)B branching point.

Molecular Modeling of the pentasaccharides. Five different glycosidic linkages A-B, B-C, C-D, D-A and E-B are needed to build the **O-SP 5a**. Each of the corresponding disaccharides was studied by a systematic grid search approach using MM3 program and the resulting adiabatic energy maps are displayed in Figure 3. Each of the disaccharides can access a large number of

conformational states and therefore have high potential flexibility. The linkages with the α orientation are quite restricted for the rotations about the Φ torsion angle with values centered at about -80° for L configuration and about 80° for the D configuration as dictated by the exo-anomeric effect (53). Much more flexibility is exhibited by the Ψ torsion angles which can adopt two or three different low energy values and which can span the entire angular range within an energy barrier of 8 kcal/mol. The disaccharides A-B and B-C present two main energy minima, at Φ/Ψ ≈ -80°/200° (**I**) and at Φ/Ψ ≈ -80°/260° (**II**), separated by a very low barrier. The disaccharide C-D shows a global minimum at Φ/Ψ ≈ -80°/140° (**I**) and a secondary minimum at Φ/Ψ ≈ -95°/80° (**II**). The disaccharide E-B has its global minimum at Φ/Ψ ≈ 80°/200° (**I**) and a secondary minimum at Φ/Ψ ≈ 95°/280° (**II**). The only glycosidic linkage with β anomerie, D-A, is not as strictly limited as for the Φ angle but nevertheless presents the same main energy minima as A-B and B-C.

(Figure 3)

The conformational behavior of the four pentasaccharides **1**, **2**, **3** and **4** has been fully investigated with the CICADA algorithm (21) interfaced with MM3 force field, starting from the geometries of energy minima of each glycosidic linkage. After about 10,000 energy minimizations, the CICADA calculations led to a total of about 3000 or 4000 energy minima on the potential energy surface of each pentasaccharide. Since the conformational analysis was performed in conformational spaces having ten dimensions, it is not straightforward to describe the results. Projection of the calculated conformations on the energy map of each glycosidic linkage allows to see that the conformational space has been well explored (See supplemental material Figures). The complete ensemble of conformations resulting from the CICADA analysis

has been clustered into different conformational families within an energy window of 5 kcal/mol above the global minimum (See supplemental material Tables S7-8).

The Φ torsion angles always display similar value for all conformational families of all pentasaccharides. The occurrence of the different families depends essentially on the $\Psi_{C\text{-}D}$ and $\Psi_{D\text{-}A}$ torsion angles. Indeed, the conformers of the major families can adopt the low energy conformation **I** or **II** for both C-D and D-A glycosidic linkages whereas they always adopt the energy minimum conformation **I** for A-B, B-C and E-B glycosidic linkages. For pentasaccharide **4**, the unique difference between the lowest energy conformations of the two major conformational families is the $\Psi_{D\text{-}A}$ torsion angle which adopts the low energy conformation **I** or **II** (Table 5).

(Table 5)

These lowest energy conformations are energetically equivalent, they belong to a very flat plateau which allows easy interconversions. Figure 4 gives a graphical representation of the two lowest energy families of pentasaccharide **4**.

(Figure 4)

Combination of NMR and Modeling Data. Average-weighted inter-proton distances and heteronuclear $^{3}J_{C,H}$ coupling constants were calculated for the two lowest energy families, Fam. 1 and Fam. 2, and for the average of all families with energy-weighted population more than 1% at 298 K to compare with the NMR data (See supplemental material Table S9-10). The analysis of calculated and experimental distances shows that experimental values are rather in agreement with conformations adopted in Fam.1 for the pentasaccharide **1** and with those of Fam.2 for pentasaccharides **2**, **3** and **4** (See supplemental material Table S9). Indeed, according to Table 3, the distances measured between residue A and D for pentasaccharide **4** are almost in agreement

with the conformations of Fam.2 in which the $\Psi_{D\text{-}A}$ torsion angle adopts the low energy conformation **II**. This is substantiated by the comparison of experimental and calculated $^{3}J_{C,H}$ coupling constants and particularly those of C-1_D/H-2_A atom pairs (Table 4). To sum up, NMR data of all pentasaccharides are in agreement with the conformers that adopt the low energy conformation **I** for the A-B, B-C, C-D and E-B glycosidic linkages and the low energy conformation **II** for the D-A glycosidic linkage.

Immunochemical Properties of Oligosaccharide Fragments of O-SP 5a. Inhibition ELISA have been performed in order to evaluate the affinity of a protective monoclonal antibody specific for *S. flexneri* 5a, IgGC20, for different frame-shifted tri-, tetra- and pentasaccharide fragments of **O-SP 5a**, with or without residue E. The results show that residue E is essential for recognition. Moreover, the position of residues C and D in the different fragments seem to be also important (Table 6). Indeed, if a residue C is added to the trisaccharide A(E)B-OMe at the reducing extremity, the affinity is 10-fold higher. On the other hand, the addition of residue D at the reducing extremity of tetrasaccharide A(E)BC-OMe does not change the affinity. Similarly, the addition of residue D at the non reducing extremity of trisaccharide A(E)B-OMe improves the affinity whereas this does not change when adding residue C at the non reducing extremity of tetrasaccharide DA(E)B-OMe. Eventually, IgGC20 shows the best affinity when residues C and D are placed on either side of trisaccharide A(E)B-OMe i.e. for pentasaccharide DA(E)BC-OMe. This latter corresponds precisely to the pentasaccharide fragment which, according to the structural analysis, more closely mimics the conformational features of the **O-SP 5a**.

(Table 6)

Molecular Modeling of the O-SP. Possible models of **O-SP 5a** have been built by means of the POLYS program using an approximation of independent neighboring glycosidic linkages

(28). Different fragments composed of six repeating units were constructed using all combinations of the energy minima of disaccharide energy maps. Interestingly, the sole combination that gave a structure devoid of steric clashes corresponds to the energy minima adopted by all pentasaccharide fragments i.e. energy minimum **I** for A-B, B-C, C-D and E-B glycosidic linkages and energy minimum **II** for D-A glycosidic linkages. This combination leads to a conformation that is close to a right-handed helical structure with a three-fold symmetry. Refinement of the helical parameters was then conducted as a function of small variations of the Φ and Ψ torsion angles at each glycosidic linkage around the different energy minima. Several three-fold right-handed regular helices, with similar overall shape, could be built using conformations in this particular low-energy region. Figure 5 shows two possible helical structures of the **O-SP 5a**, **O-SP 5a** (1) and **O-SP 5a** (2), characterized by the torsion angles listed in Table 6.

(Figure 5)

(Table 7)

The two models differ mainly by the extension of the helices. The **O-SP 5a** (1) structure has a pitch of 19.4 Å and a diameter of about 15 Å whereas the **O-SP 5a** (2) structure has a pitch of 23.2 Å and a diameter of about 14 Å. The **O-SP 5a** (1) structure presents hydrogen bonds between $HN\text{-}_D$ and $HO\text{-}3_A$ that can contribute to the stabilization of the helical structure. Interestingly, for both models, the glucosyl residue E protudes at the helix surface and is well exposed to the solvent, consistent with the long T_1 values measured for the carbons of these residues. The ability of **O-SP 5a** to form different helices with the same shape but different extensions, based on minor fluctuations in the Φ/Ψ torsion angles, was observed for other bacterial PSs and may be correlated to their biological properties as was hypothesized in the case

of Type III Group B *Streptococcus* CPS (GBSP III) (54). If **O-SP 5a** presents different conformations in solution, the NOE's will be averaged over all the conformations. According to Table 3, the inter-residue distances measured for the two possible structures are almost in agreement with the NMR data, demonstrating thus that the predominant solution conformation of **O-SP 5a** might be close to the regular three-fold shape of **O-SP 5a**. The few small differences observed in relation to NMR data do not permit to really discriminate between the two models. The role of residue glucosyl E in the helix formation was deduced from the comparison of our model of **O-SP 5a** with that of **O-SP Y** constructed by means of the hard-sphere *exo*-anomeric (HSEA) approach (29). The model of **O-SP Y** (Figure 6), which is in agreement with NMR data, is characterized by the Φ and Ψ torsion angles given in Table 6. According to the Φ /Ψ values, residue E seems to have an influence only upon $\Psi_{A\text{-}B}$ and $\Psi_{B\text{-}C}$ torsion angles. Indeed, these angles adopt the low-energy conformation **I** in **O-SP 5a** and the low energy conformation **II** in **O-SP Y**. The **O-SP Y** model consists in a random coil compact chain. Thus, the helical shape found for the **O-SP 5a** structure is likely due to the presence of its branched residues E. Interestingly, analogous influence of side-chain residues has been also demonstrated for the **GBSP III** CP whose branched sialic acids were found to exert control over the conformational features of the polysaccharide backbone (54).

(Figure 6)

Often, conformational analysis of bacterial polysaccharides are discussed in terms of the size and shape of antigenic determinants. In relation to vaccine development, the immunological function of these PSs may be looked at as the specific interaction of carbohydrate epitopes and antibody binding sites. Often comprising 2 to 4 sugar residues, carbohydrate epitopes may be compact with a relatively rigid conformation even though they pertain to a rather flexible

polysaccharide (55) or they may be flexible, thus allowing antibody binding to a conformation selected from several preferred ones. Besides, extensive analysis of the *meningococcal* Group B CP (56) and of the type III Group B Streptococcal CPS (54) has led to the concept of conformational epitope as introduced by Jennings and his co-workers, thus outlining a key feature in the design of functional mimics of such bacterial PS. Overall, the 3D-conformation of biologically active epitopes, isolated either as short oligosaccharides or mimics thereof, may not be identical to that of the corresponding fragment in the native polymer, emphasizing the need for comparative conformational studies .

Herein, The combination of NMR and molecular modeling analysis showed that all pentasaccharide fragments of the O-SP of *S. flexneri* 5a present rather similar conformational behaviors like the native O-SP. Indeed, the pentasaccharides and **O-SP 5a** adopt the same low energy conformations. However, according to ^{1}H and ^{13}C chemical shifts, $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants, the pentasaccharide **4**, **DA(E)BC-OMe**, appears to mimic the conformational features of the **O-SP 5a** with high accuracy. Moreover, inhibition ELISA showed that the protective monoclonal antibody, IgGC20, specific for **O-SP 5a** displays a better affinity for this pentasaccharide as compared to the others. Thus, NMR and immunochemical (n'est ce pas affinity) data seem to suggest that the pentasaccharide **4** is likely to induce polysaccharide-specific antibodies effective against *S. flexneri* 5a and then could be used in a conjugate vaccines approach against this pathogen.

Both NMR and molecular modeling data of **O-SP 5a** are consistent with a right-handed three-fold helical structure with the glucosyl residue E, pointing out of the helix surface. Comparison of this structure with a model of the O-SP of *S. flexneri* Y revealed that the branched glucosyl residue E, which constitutes the structural specificity of **O-SP 5a**, is responsible for the helical shape of the latter. The inhibition ELISA realized with oligosaccharide fragments of **O-SP 5a**

showed that residue E is essential for recognition by antibodies. Interestingly, according to our models of **O-SP 5a**, it is ideally located for interaction with antibodies. Analogously to data previously obtained on the model bacterium *S. dysenteriae* type 1 (57), it is likely to be part of the epitopes recognized by protective monoclonal antibodies targeting *S. flexneri* 5a infection. In order to confirm (test c'est mieux cela ne laisse pas présager du résultat) this hypothesis, structural studies of the interaction of the pentasaccharide fragments of the **O-SP 5a** with such antibodies (58) are in progress in our laboratory.

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REFERENCES

1. MacLeod, C. M., Hodges, R. G., Heidelberg, M., and Bernhard, W. G. (1945) *J. Exp. Med.* **82**, 445-465
2. Cadoz, M. (1998) *Vaccine* **16**, 1391-1395
3. Wenger, J. D., Booy, R., Health, P. T., and Moxon, R. (1997) in *New Generation Vaccines* (Levine, M. M., Woodrow, G. C., Kaper, J. B., and Cobon, G. S., eds), Second edition, Revised and Expanded Ed., pp. 489-502, Marcel Dekker, Inc, New York

4. MacLennan, J. M., Shackley, F., Heath, P. T., Deeks, J. J., Flamank, C., Herbert, M., Griffith, H., Hatzmann, E., Goilav, C., and Moxon, E. R. (2000) *JAMA* **283**, 2795-2801

5. Robbins, J. B., Schneerson, R., and Szu, S. C. (1997) in *New Generation Vaccines* (Levine, M. M., Woodrow, G. C., Kaper, J. B., and Cobon, G. S., eds), pp. 803-815, Marcel Dekker

6. Sansonetti, P. (1991) *Rev. Infect. Dis.* **13**, S285

7. Kotloff, K. L., Winickoff, J. P., Ivanoff, B., Clemens, J. D., Swedlow, P. J., Sansonetti, P. J., Adak, G. K., and Levine, M. M. (1999) *Bull. World Health Organ.* **77**, 651-666

8. Passwell, J. H., Harlev, E., Ashkenazi, S., Chu, C., Miron, D., Ramon, R., Farzan, N., Shiloach, J., Bryla, D. A., Majadly, F., Roberson, R., Robbins, J. B., and Schneerson, R. (2001) *Infect. Immun.* **69**, 1351-1357

9. Lemieux, R. U., Bock, K., Delbaere, L. T. J., Koto, S., and Rao, V. S. (1980) *Can. J. Chem.* **58**, 631-653

10. Bush, C. A., Martin-Pastor, M., and Imberty, A. (1999) *Annu. Rev. Biophys. Biomol. Struct.* **28**, 269-293

11. Simmons, D. A. R. (1971) *Bacteriol. Reviews* **35**, 117

12. Kenne, L., Lindberg, B., Petersson, K., and Romanowska, E. (1977) *Carbohydr. Res.* **56**, 363-370

13. Mulard, L. A., and Ughetto-Monfrin, J. (2000) *J. Carbohydr. Chem.*, 193-220

14. Mulard, L. A., and Ughetto-Monfrin, J. (1999) *J. Carbohydr. Chem.* **18**, 721-753

15. Mulard, L. A., and Ughetto-Monfrin, J. (2000) *J. Carbohydr. Chem.* **19**, 503-526

16. Mulard, L. A., Clément, M. J., Ségat-Dioury, F., and Delepierre, M. (2002) *Tetrahedron* **58**, 2593-2604

17. Mulard, L. A., Clément, M. J., Imberty, A., and Delepierre, M. (2002) *Eur. J. Org. Chem.* **15**, 2486-2498

18. Tvaroska, I., Hricovini, M., and Petrakova, E. (1989) *Carbohydr Res* **189**, 359-362

19. Krishnamurthy, V. V. (1996) *J Magn Reson* **121**, 33-41

20. Willker, W., and Leibfritz, D. (1995) *Magn. Reson. Chem.* **33**, 632-638

21. Koca, J. (1998) *Progr Biophys Mol Biol* **70**, 137-173

22. Allinger, N. L., Yuh, Y. H., and Lii, J. H. (1989) *J. Am. Chem. Soc.* **111**, 8551-8556

23. Allinger, N. L., Rahman, M., and Lii, J. H. (1990) *J. Am. Chem. Soc.* **112**, 8293-8307

24. Koca, J., Pérez, S., and Imberty, A. (1995) *J Comp Chem* **16**, 296-310

25. Imberty, A., Mikros, E., Koca, J., Mollicone, R., Oriol, R., and Pérez, S. (1995) *Glycoconj J* **12**, 331-349

26. Pérez, S., Imberty, A., Engelsen, S. B., Gruza, J., Mazeau, K., Jimenez-Barbero, J., Poveda, A., Espinosa, J. F., van Eyck, B. P., Johnson, G., French, A. D., Kouwijzer, M. L., Grootenius, P., Bernardi, A., Rainomdi, L., Senderowitz, H., Durier, V., Vergoten, G., and Rasmussen, K. (1998) *Carbohydr Res* **314**, 141-155

27. Chandrasekaran, R. (1997) *Adv. Carbohydr. Chem. Biochem.* **52**, 311-439

28. Engelsen, S. B., Cros, S., Mackie, W., and Pérez, S. (1996) *Biopolymers* **39**, 417-433

29. Bock, K., Josephson, S., and Bundle, D. R. (1982) *J. Chem. Soc., Perkins Trans. 2*, 59-70

30. Taylor, D. N., Trofa, A. C., Sadoff, J., Chu, C., Bryla, D., Shiloach, J., Cohen, D., Ashkenazi, S., Lerman, Y., Egan, W., Schneerson, R., and Robbins, J. B. (1993) *Infect. Immun.* **61**, 3678-3687

31. IUPAC-IUB. (1971) *Arch. Biochem. Biophys.* **145**, 405-621

32. Rance, M., Sorensen, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R., and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.* **117**, 479

33. Griesinger, C., Otting, G., Wütrich, K., and Ernst, R. R. (1988) *J. Am. Chem. Soc.* **110**, 7870

34. Desvaux, H., Berthault, P., Birlirakis, N., and Goldman, M. (1994) *J. Magn. Reson.* **108**, 219- 229

35. Willer, W., Leibfritz, D., Kerssebaum, R., and Bermel, W. (1993) *Magn. Reson. Chem.* **31**, 287

36. Delay, C., Gavin, J. A., Aumelas, A., Bonnet, P. A., and Roumestand, C. (1997) *Carbohydr Res* **302**, 67-78

37. Emsley, L., and Bodenhausen, G. (1992) *J Magn Reson* **97**, 135-148

38. Clore, G. M., Bax, A., Wingfield, P., and Gronenborn, A. M. (1988) *FEBS Lett.* **238**, 17-21

39. Kay, L. E., Nicholson, L. K., Delaglio, F., Bax, A., and Torchia, D. A. (1992) *J Magn Reson* **97**, 359-375

40. States, D. J., Haberkorn, R. A., and Ruben, D. J. (1982) *J. Magn. Reson.* **48**, 286

41. Baleja, J., Moult, J., and Sykes, B. D. (1990) *J. Magn. Reson.* **87**, 375

42. Pérez, S., and Delage, M. M. (1991) *Carbohydr Res* **212**, 523-529

43. Koca, J. (1994) *J Mol Struct (Theochem)* **308**, 13-24

44. Koca, J. (1993) *J Mol Struct* **291**, 255-269

45. Imberty, A., and Pérez, S. (1994) *Glycobiology* **4**, 351-366

46. Jansson, P. E., Kenne, L., and Wehler, T. (1987) *Carbohydr Res* **166**, 271-282

47. Jansson, P. E., Kenne, L., and Wehler, T. (1988) *Carbohydr Res* **179**, 359-368

48. Bock, K., and Pedersen, C. (1974) *J. Chem. Soc. Perkin Trans 2*, 293-297

49. Pozsgay, V., Coxon, B., and Yeh, H. (1993) *Biooorganic and Medicinal Chemistry* **1**, 237-257

50. Davis, A. L., Keeler, J., Laue, E. D., and Moskau, D. (1992) *J Magn Reson* **98**, 207

51. Boyd, J., Soffe, N., John, B. K., Plant, D., and Hurd, R. (1992) *J Magn Reson* **98**, 660

52. Scott, K., Stonehouse, J., Keeler, J., Hwang, T., and Shaka, A. J. (1995) *J Am Chem Soc* **117**, 4199-4200

53. Lemieux, R. U., Koto, S., and Voisin, D. (1979) *In Anomeric Effect, Origin and Consequences ACS Symposium Series Vol 87 (Szarek A, Horton D, eds) pp. 17-29. Washington DC: American Chemical Society*

54. Brisson, J.-R., Uhrinova, S., Woods, R. J., van der Zwan, M., Jarrell, H. C., Paoletti, L. C., Kasper, D. L., and Jennings, H. J. (1997) *Biochemistry* **36**, 3278-3292

55. Gunawardena, S., Fiore, C. R., Johnson, J. A., and Bush, C. A. (1999) *Biochemistry* **38**, 12062-12071

56. Zou, W., Mackenzie, R., Thérien, L., Hirama, T., Yang, Q., Gidney, M. A., and Jennings, H. J. (1999) *J. Immunol.* **163**, 820-825

57. Nyholm, P. G., Mulard, L. A., Miller, C. E., Lew, T., Olin, R., and Glaudemans, C. J. P. (2001) *Glycobiology* **11**, 945-955

58. Phalipon, A., Kauffmann, M., Michetti, P., Cavaillon, J.-M., Huerre, M., Sansonetti, P., and Krahenbuhl, J.-P. (1995) *J. Exp. Med.* **182**, 769-778

FIGURE LEGENDS

Figure 1. Structure of the repeating unit of the O-SP of *S. flexneri* 5a. Residues are labelled A-E. Structures of the synthetic methyl glycosides representative of all possible frame-shifted pentasaccharides of the O-SP of *S. flexneri* 5a are surrounded.

Figure 2. Anomeric region of the EXSIDE spectrum of the pentasaccharide **4** in D_20 at 38°. The $^3J_{C,H}$ coupling constants were measured along F_1 (carbon) dimension with a *J*-scaling factor of 15 and a digital resolution of 0.3 Hz/point.

Figure 3. Adiabatic relaxed energy maps of all constituting-disaccharides of the pentasaccharides as a function of the Φ and Ψ torsion angles. Energy contours have been drawn by step of 1 kcal/mol steps, with an outer limit of 8 kcal/mol. Principal energy minima have been indicated by a label.

Figure 4. Graphical representation of the two lowest energy conformation families, Fam. 1 and Fam. 2, of **4** determined with the CICADA method. For each family, the lowest energy conformation has been drawn in thick lines. The conformations with the largest difference in Φ and Ψ torsion angles within an energy window of 5 kcal mol^{-1} are drawn in thin lines. All conformations were fitted using the ring atoms of residue B in order to give an idea of the global flexibility.

Sflex5a-NMR-IC50protocol-brevet

Figure 5. Two possible right-handed three-fold helical structures of the **O-SP 5a**, **O-SP 5a (1)** and **O-SP 5a (2)**, obtained with POLYS. a) and b): Side view ; c) and d): Axial view.

Figure 6. Models of three and four repeating units of **O-SP Y** and **O-SP 5a** respectively. The model of **O-SP Y** has been built on the basis of Φ and Ψ values of the reference 29.

Sflex5a-NMR-IC50protocol-brevet

Table 1. ^{1}H NMR chemical shifts [a]

H - atoms	Compound		
	4	**O-SP 5a**	**O-SP Y** [b]
H-1_A	5.11	5.10	**5.12**
H-2_A	4.12	4.12	4.13
H-3_A	3.86	3.86	**3.84**
H-4_A	3.31	3.30	3.31
H-5_A	3.66	3.66	3.67
H-6_A	1.23	1.22	1.22
H-1_B	**5.23**	5.20	**5.11**
H-2_B	4.25	4.24	**4.04**
H-3_B	3.99	3.97	**3.91**
H-4_B	3.58	3.58	**3.46**
H-5_B	**3.80**	3.76	**3.72**
H-6_B	1.30	1.31	**1.29**
H-1_C	**4.66**	4.84	4.84
H-2_C	**3.97**	3.83	**3.85**
H-3_C	3.75	3.76	**3.74**
H-4_C	3.54	3.53	3.53
H-5_C	**3.69**	3.98	**4.00**
H-6_C	**1.28**	1.21	1.22
H-1_D	**4.72**	4.75	**4.71**
H-2_D	**3.68**	3.80	**3.82**
H-3_D	**3.55**	3.62	**3.64**
H-4_D	**3.39**	3.46	**3.50**
H-5_D	3.44	3.46	**3.43**
H-6_D	3.91	3.92	**3.90**
H-$6'_D$	3.71	3.72	**3.74**
N-Ac_D	2.04	2.04	
H-1_E	5.07	5.06	
H-2_E	3.58	3.57	
H-3_E	3.77	3.77	
H-4_E	3.43	3.42	
H-5_E	3.90	3.90	
H-6_E	3.82	3.81	
H-$6'_E$	3.76	3.75	

[a] Chemical shifts measured in ppm with an accuracy of ± 0.01 ppm are referenced to external DSS (δ 0.00). Chemical shifts that present significant differences ($\geq$|0.02| ppm) with those of the **O-SP 5a** are in bold.

[b] Data taken from reference 29.

Table 2. ^{13}C NMR data.[a, b]

H- atoms	Compound					
	4		**O-SP 5a** [c]		**O-SP Y** [d]	
C-1_A	103.6	*172.6*	103.6	(303 ± 6)	*173.2*	103.7
C-2_A	81.2		81.1	(297 ± 6)		**81.4**
C-3_A	72.6		72.6	(355 ± 15)		72.5
C-4_A	74.9		75.1	(358 ± 17)		75.0
C-5_A	71.9		71.9	(349 ± 16)		71.8
C-6_A	19.1		19.1			**19.4**
C-1_B	103.2	*173.2*	103.2	(265 ± 7)	*173.5*	**103.5**
C-2_B	77.2		77.1	(278 ± 6)		**80.8**
C-3_B	76.1		76.4	(284 ± 8)		**72.7**
C-4_B	73.0		73.2	(284 ± 12)		**74.8**
C-5_B	71.6		71.7	(303 ± 10)		**71.0**
C-6_B	19.3		19.3			19.4
C-1_C	103.4	*170*	103.8	(296 ± 7)	*169.9*	103.9
C-2_C	**72.4**		73.2	(309 ± 5)		73.3
C-3_C	79.8		79.8	(303 ± 9)		80.0
C-4_C	**74.4**		74.3	(304 ± 8)		74.3
C-5_C	71.2		71.6	(317 ± 8)		71.7
C-6_C	19.1		19.1			19.1
C-1_D	105.0	*162.6*	104.6	(328 ± 9)	*162.7*	104.8
C-2_D	58.6		58.3	(315 ± 13)		58.3
C-3_D	**76.3**		84.1	(320 ± 15)		84.2
C-4_D	**72.6**		71.2	(304 ± 7)		**71.8**
C-5_D	78.4		78.6	(322 ± 7)		78.6
C-6_D	63.5		63.6			63.4
CO_D	**177.3**		176.8			
N-Ac_D	24.9		24.9			
C-1_E	97.1	*168.9*	97.3	(336 ± 6)	*168.9*	
C-2_E	73.7		73.8	(366 ± 9)		
C-3_E	75.6		75.7	(365 ± 9)		
C-4_E	72.1		72.3	(370 ± 7)		
C-5_E	74.4		74.4	(364 ± 8)		
C-6_E	63.2		63.3			

[a] Chemical shifts measured in ppm with an accuracy of ± 0.2 ppm are referenced to external DSS (δ_C 0.0). Pentasaccharide chemical shifts that present significant differences (≥|0.3| ppm) with those of the **O-SP 5a** are in bold.
[b] Data in italic are experimental $^1J_{C\text{-}1,H\text{-}1}$ (in Hz ± 0.5Hz) coupling constants
[c] Data in parenthesis are the ^{13}C T_1 values (in ms) measured for the **O-SP 5a**.
[d] Data taken from reference 29.

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Table 3. Inter-residue ^{1}H-^{1}H distances (Å).

Proton Pairs	Compound				
	4		**O-SP 5a**		
	NMR data [a]	Molecular modeling data [b]	NMR data [a]	Molecular modeling data	
				(1)	**(2)**
$H\text{-}1_A$ / $H\text{-}1_B$	3.3 / 3.2	2.7 / 2.8 / 2.8	3.1 / 3.0	2.5	3.0
$H\text{-}1_A$ / $H\text{-}2_B$	2.2 / 2.2	2.2 / 2.2 / 2.2	2.1 / 2.1	2.1	2.0
$H\text{-}1_A$ / $H\text{-}4_B$	4.7 / [c]	4.6 / 4.7 / 4.6	4.7 / 4.6	4.7	4.7
$H\text{-}1_A$ / $H\text{-}1_D$	**3.7 / 3.9**	**2.8 / 4.1 / 3.1**	[e] / 3.5	4.1	3.8
$H\text{-}1_A$ / $H\text{-}2_D$	4.4 / [d]	4.5 / 4.5 / 4.5	4.5 / [d]	4.7	4.5
$H\text{-}1_A$ / $H\text{-}5_D$	[c]	**3.2 / 5.0 / 3.6**	[d]	5.3	4.7
$H\text{-}1_A$ / $H\text{-}1_E$	3.3 / [d]	3.5 / 3.5 / 3.3	[d]	3.8	3.5
$H\text{-}2_A$ / $H\text{-}1_B$	4.8 / 4.8	4.8 / 4.8 / 4.8	[c] / 4.7	4.7	4.9
$H\text{-}2_A$ / $H\text{-}1_D$	2.2 / 2.3	2.2 / 2.3 / 2.3	2.0 / 2.1	2.1	2.1
$H\text{-}2_A$ / $H\text{-}2_D$	4.4 / [d]	4.5 / 4.4 / 4.4	4.5 / 4.5	4.4	4.3
$H\text{-}2_A$ / $H\text{-}5_D$	[d]	4.2 / 3.7 / 3.9	4.1 / 3.8	3.7	3.6
$H\text{-}2_A$ / $H\text{-}1_E$	4.0 / [d]	4.1 / 4.0 / 4.1	4.1 / 4.1	4.2	4.3
$H\text{-}4_A$ / $H\text{-}1_D$	4.2 / 4.3	4.7 / 4.8 / 4.8	4.2 / 4.3	4.0	4.3
$H\text{-}4_A$ / $N\text{-}Ac_D$	**3.4 / 3.6**	**5.3 / 3.4 / 3.8**	3.4 / 3.3	3.1	3.9
$H\text{-}5_A$ / $H\text{-}1_B$	2.4 / 2.5	3.0 / 3.0 / 2.9	2.4 / 2.6	3.2	2.7
$H\text{-}5_A$ / $H\text{-}2_B$	4.3 / 4.4	4.3 / 4.3 / 4.3	4.6 / 4.3	4.3	4.1
$H\text{-}6_A$ / $H\text{-}1_B$	3.4 / 3.4	3.5 / 3.6 / 3.6	3.5 / 3.1	3.8	3.2
$H\text{-}6_A$ / $N\text{-}Ac_D$	[c]	8.3 / 5.5 / 6.2	[c]	5.5	6.2
$H\text{-}1_B$ / $H\text{-}2_C$	[d]	2.7 / 2.7 / 2.8	3.4 / 2.9	3.0	2.9
$H\text{-}1_B$ / $H\text{-}3_C$	2.3 / 2.3	2.2 / 2.2 / 2.2	2.1 / 2.1	2.1	2.0
$H\text{-}1_B$ / $H\text{-}4_C$	4.3 / 4.2	4.5 / 4.5 / 4.2	4.3 / 4.4	4.4	4.4
$H\text{-}2_B$ / $H\text{-}4_C$	4.6 / 4.5	4.8 / 4.8 / 4.5	4.6 / 4.8	4.8	4.8
$H\text{-}2_B$ / $H\text{-}1_E$	2.3 / [d]	2.2 / 2.2 / 2.2	2.3 / 2.4	2.1	2.1
$H\text{-}3_B$ / $H\text{-}1_E$	2.5 / 2.5	2.6 / 2.6 / 2.6	2.5 / 2.2	2.5	2.5
$H\text{-}3_B$ / $H\text{-}5_E$	3.3 / 3.2	3.2 / 3.1 / 3.2	3.0 / [d]	3.2	3.2
$H\text{-}6_B$ / $H\text{-}2_C$	3.6 / 3.5	3.9 / 3.9 / 3.9	3.2 / 3.1	3.3	3.4

[a] The two values correspond to the distances calculated from ROE and NOE volumes respectively. The errors on distance values are ~ 10%.

[b] The three values correspond to the averaged-weighted distances calculated for the family Fam. 1, for the family Fam. 2 and for the average of all families respectively. Distances permitting to discriminate between Fam. 1, Fam. 2 and the average of all families are in bold.

[c] Distance not determined, probably corresponding to long distance (≥5 Å).

[d] Distance not determined due to superposition of peaks.

[e] NOE peak not observed.

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Table 4. Values of $^{3}J_{C,H}$ coupling constants (Hz) for pentasaccharide **4** and the **O-SP 5a**.

Atom pairs	**Compound**		
	4		**O-SP 5a**
	NMR data [a]	Molecular modeling data [b]	NMR data [c]
$H\text{-}1_A / C\text{-}2_B$	4.3 / 4.1	4.1 / 4.0 / 4.1	4.0
$C\text{-}1_A / H\text{-}2_B$	3.9 / 3.9	3.1 / 3.1 / 3.3	4.0
$H\text{-}1_B / C\text{-}3_C$	3.6 / 3.9	4.3 / 4.2 / 4.1	4.0
$C\text{-}1_B / H\text{-}3_C$	nd / 3.7	2.8 / 2.8 / 3.2	3.7
$H\text{-}1_C / C\text{-}3_D$	-	-	4.4
$C\text{-}1_C / H\text{-}3_D$	-	-	4.8
$H\text{-}1_D / C\text{-}2_A$	4.2 / 4.3	3.9 / 3.5 / 3.6	4.1
$C\text{-}1_D / H\text{-}2_A$	5.3 / 5.1	2.6 / 5.0 / 4.0	5.2
$H\text{-}1_E / C\text{-}3_B$	2.9 / 3.3	3.3 / 3.2 / 3.2	2.9
$C\text{-}1_E / H\text{-}3_B$	nd / 3.3	3.3 / 3.2 / 3.2	3.0

[a] The two values correspond to the $^{3}J_{C,H}$ obtained from EXSIDE and gs-*J*-HMBC experiments respectively, with an accuracy of ± 0.3 Hz; nd : not determined.

[b] The three values correspond to the weighted average $^{3}J_{C,H}$ coupling constants calculated for the family Fam. 1, for the family Fam. 2 and for all families respectively.

[c] For the **O-SP 5a**, the $^{3}J_{C,H}$ coupling constants have been obtained only with the gs-*J*-HMBC experiments.

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Table 5. Characteristics of lowest energy conformations of the major families of the pentasaccharide **4**.[a, b]

Compound	Families	Φ_{A-B} / Ψ_{A-B}	Φ_{B-C} / Ψ_{B-C}	Φ_{C-D} / Ψ_{C-D}	Φ_{D-A} / Ψ_{D-A}	Φ_{E-B} / Ψ_{E-B}	E_{rel} [c]	% [d]
4	Fam. 1	270 / 195 **(I)**	266 / 191 **(I)**	-	268 / 186 **(I)**	76 / 194 **(I)**	0.00	31.8
	Fam. 2	276 / 205 **(I)**	270 / 192 **(I)**	-	273 / 262 **(II)**	78 / 204 **(I)**	0.34	26.4

[a] Only the conformations having an energy-weighted population of more than 10% have been listed.
[b] The number in parenthesis indicates the energy minimum of the disaccharide energy maps.
[c] Energy relative.
[d] Energy-weighted population.

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Table 6. Inhibition by synthetic oligosaccharides of binding of monoclonal antibodies IgGC20 to *Shigella flexneri* 5a LPS.

oligosaccharides	**IC_{50} (μM)**	**rmsd**
A(E)B-OMe	>1000	
A(E)BC-OMe	208	+/- 108
A(E)BCD-OMe (**2**)	389	+/- 84
DA(E)B-OMe	242	+/- 25
CDA(E)B-OMe (**3**)	268	+/- 180
DA(E)BC-OMe (**4**)	39	+/- 19
EBCDA-OMe (**1**)	> 1000	

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Table 7. Torsion angles (°) in two possible models of *S. flexneri* 5a O-SP obtained with POLYS and in the model of the O-SP of *S. flexneri Y* determined with HSEA method.[a]

Torsion angles	**O-SP 5a (1)**	**O-SP 5a (2)**	**O-SP Y**
$\Phi_{\tilde{D}\square}$ / $\Psi_{\tilde{D}\square}$	269 / 189 (**I**)	278 / 202 (**I**)	284 / 256 (**II**)
$\Phi_{\grave{B}C}$ / $\Psi_{\grave{B}C}$	279 / 200 (**I**)	276 / 201 (**I**)	290 / 256 (**II**)
$\Phi_{\tilde{C}D}$ / $\Psi_{\tilde{C}D}$	274 / 140 (**I**)	273 / 146 (**I**)	280 / 135 (**I**)
$\Phi_{\grave{D}A}$ / $\Psi_{\grave{D}A}$	271 / 261 (**II**)	281 / 240 (**II**)	289 / 248 (**II**)
$\Phi_{\grave{E}B}$ / $\Psi_{\grave{E}\square}$	80 / 200 (**I**)	80 / 200 (**I**)	

[a] The number in parenthesis indicates the energy minimum of the disaccharide energy maps.

INVENTION MULARD L. et al.+

INFORMAL PATENT APPLICATION

PROVISIONAL CLAIMS, TO BE ADDED TO THE FILED TEXT

1) A glycopeptide comprising an immunogenic carrier compound congugated to a synthetic oligosaccharide derived from the O-specific polysaccharide of *Shigella flexneri* selected among the group consisting of:

{ABC}n
{BCD}n
{CDA}n
{DAB}n

{B(E)C}n
{(E)CD}n

{AB(E)C}n
{B(E)CD}n
{(E)CDA}n

{DAB(E)C}n
{B(E)CDA}n
{(E)CDAB}n
{AB(E)CD}n

{B(E)CDAB(E)C}n
{DAB(E)CDAB(E)C}n

wherein :
A is an alphaLRha*p*-(1,2) residue
B is an alphaRha*p*-(1,3) residue
C is an alphaLRha*p*-(1,3) residue
E is an [alphaDGlc*p*-(1,4)] residue
D is a betaDGlcNacp-(1, residue

and wherein n is an integer comprised between 1 and 10 and preferably between 2 and 6.

2) A glycoconjugate according to the claim 1 wherein the synthetic olygosaccharide is a derived Omethyl derivative.

3) A glycoconjugate according to the claim 1 wherein the immunogenic carrier compound is selected among an immunogenic protein, an immunogenic peptide or a derivative thereof.

4) A glycoconjugate according to claim 3, wherein the immunogenic carrier is the peptide PADRE.

5) A glycoconjugate according to claim 3, wherein the immunogenic carrier compound is the Tetanus toxine.

6) A glycoconjugate according to anyone claims 1 to 5 wherein the oligosaccharide is directly coupled to the immunogenic carrier compound.

7) A glycoconjugate according to anyone claims 1 to 5 wherein the oligosaccharide is coupled to the immunogenic carrier compound via an arm spacer.

8) A glycoconjugate according to claim 7 wherein the arm spacer is an alanine derivative.

10) A glycoconjugate according to the claim 1 wherein the synthetic olygosaccharide is a selected among the hexasaccharide, the decasaccharide and the pentasaccharide depicted in Figure 1

11) Composition useful to induce an immune response against Shigella comprising an efficient amount of a glycoconjugate according to any claims 1 to 8.

Obviously also methods to obtain the oligosaccharides, the oligosaccharide derivatives to be conjugated to the immunogenic carrier and the glycopeptides must be claimed.

Application number / numéro de demande: 2434668

Figures: 4, 6

Pages:

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
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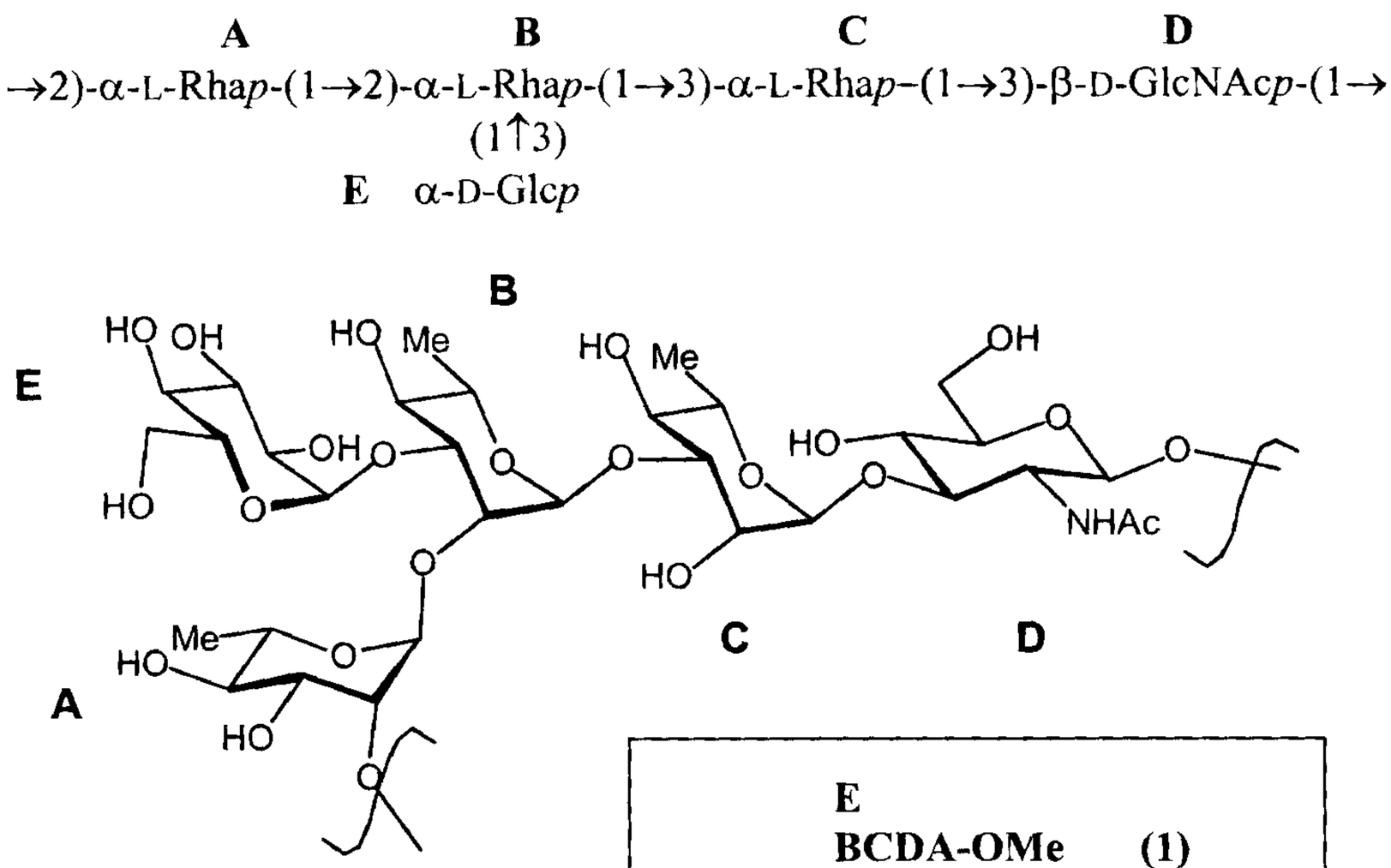

E **BCDA-OMe**	**(1)**
E **ABCD-OMe**	**(2)**
E **CDAB-OMe**	**(3)**
E **DABC-OMe**	**(4)**

Figure 1

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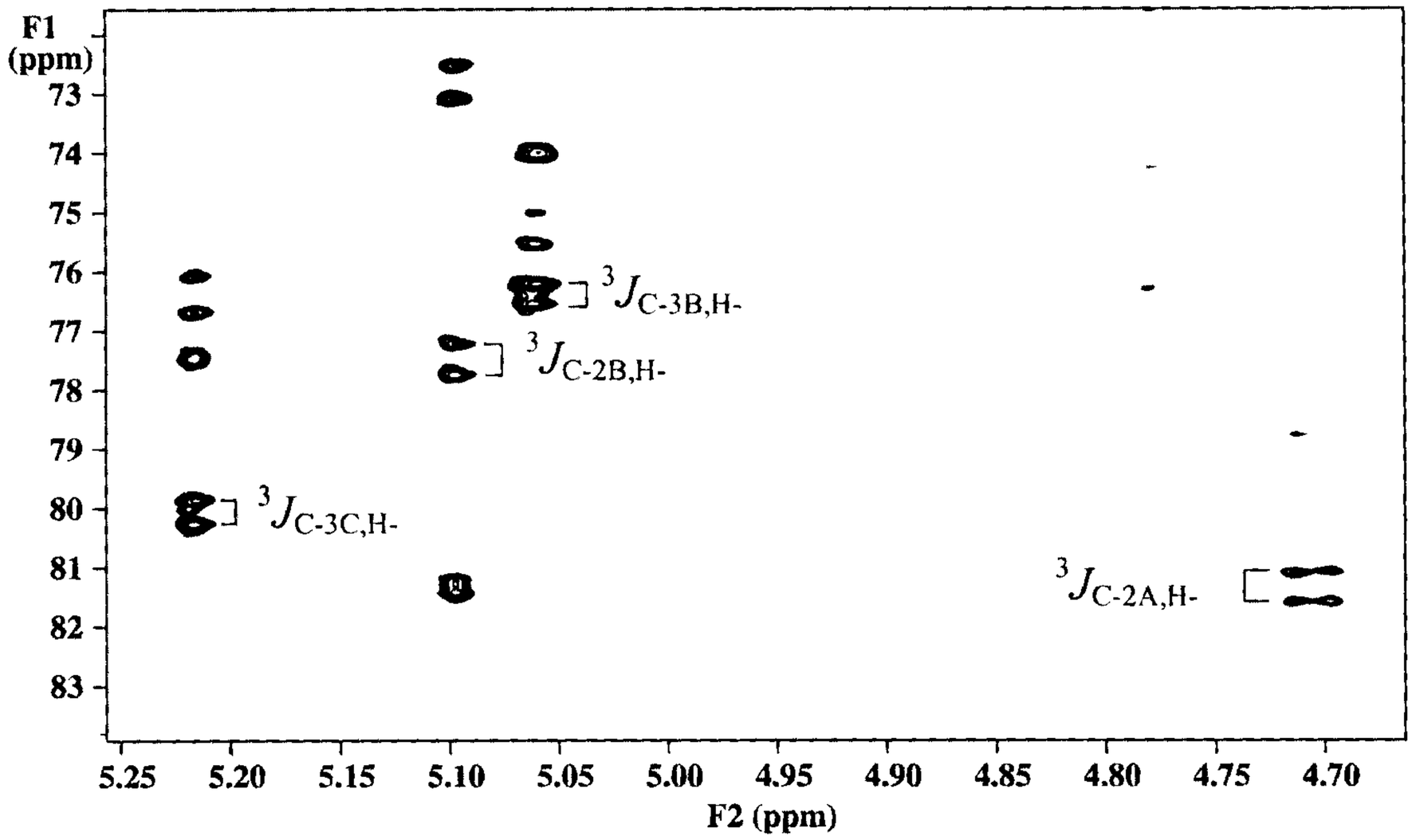

Figure 2

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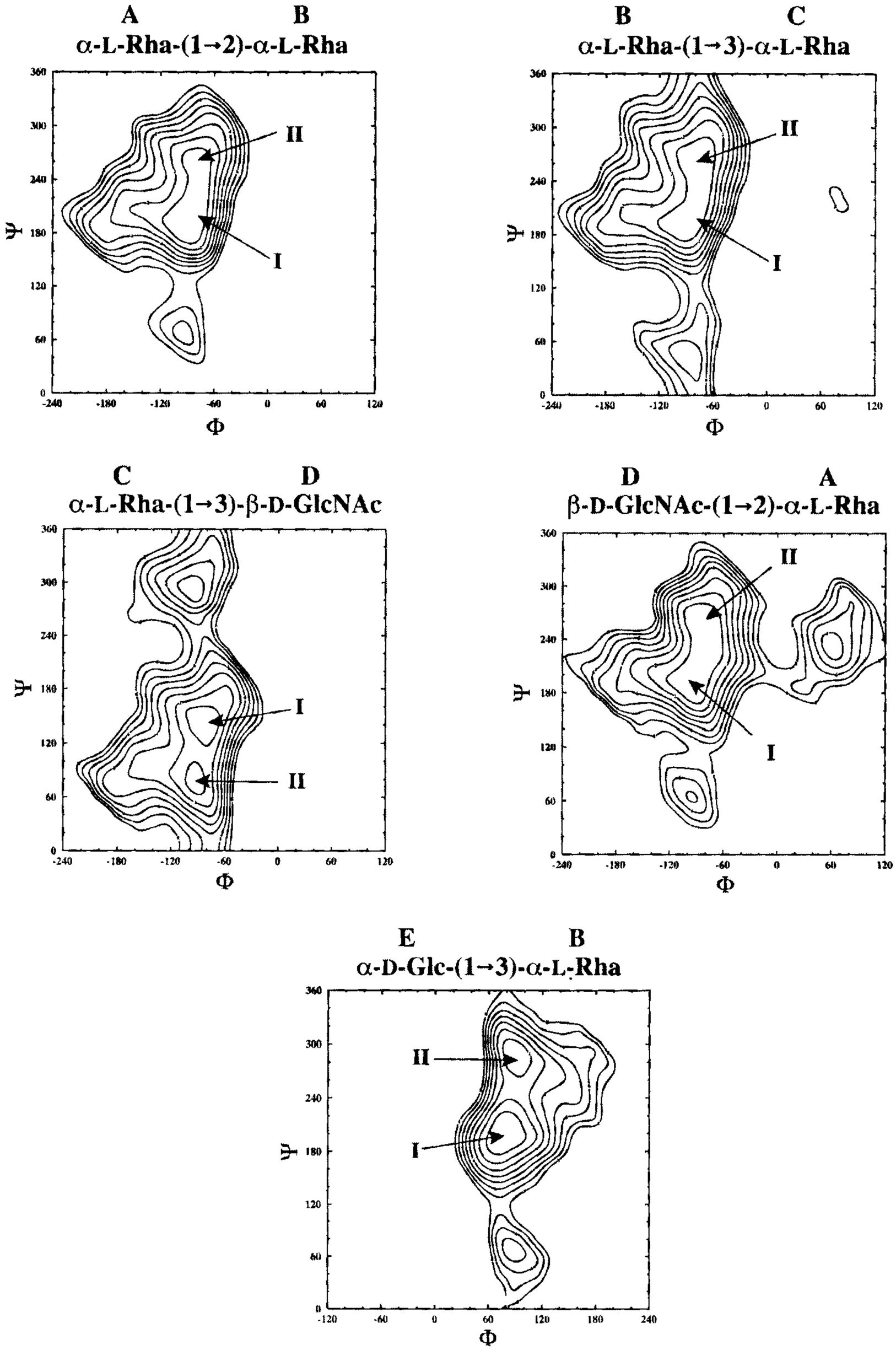

Figure 3

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