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(54) Title: METHOD FOR CLEAVING AMIDE BONDS

(57) Abstract: a) providing a molecule comprising an amide group; b) reacting the molecule comprising an amide group with a hydroxylamine salt to cleave the amide bond of the amide group.

METHOD FOR CLEAVING AMIDE BONDS

Technical field of the invention

The present invention relates to methods for cleaving amide bonds in organic molecules comprising amide groups. Such methods find use in various organic synthetic applications, e.g. for deacetylation.

Background of the invention

Traditional methods to cleave amide bonds via saponfication or hydrolysis are harsh processes requiring strongly basic conditions (i.e. concentrated NaOH or the like) or strongly acidic conditions (i.e. concentrated HCl or the like) at elevated temperatures for long periods of time. Because of the harsh conditions required, these methods have major chemical compatability issues with regard to protecting groups and to preservation of chiral centers during the reaction.

Among the more recent methods cited is hydrazinolysis. Hydrazine permits the cleavage of amide bonds under almost anhydrous conditions. Hydrazine is a powerful nucelophile thanks to the *alpha effect*, and its reduced basicity as compared to NaOH permits the cleavage of amide bonds in the presence of other protecting groups and the preservation of certain chiral centres. This was recently highlighted in two high profile publications from Ohshima *et al.* (Angew. Chem. Int. Ed. 2012, 51, 8564 –8567 and Chem. Commun., 2014, 50, 12623-12625). In these two communications Ohshima used ethylene diamine and hydrazine in the presence of ammonium salts, respectively, under microwave irradiation to cleave amide bonds while maintaining other sensitive protecting groups and chiral centers.

Even in the light of the recent findings of Ohshima *et al.* there is still a need in the field for improved and methods allowing for cleavage of amide bonds while preserving protecting groups and chiral centres.

Summary of the invention

It is an object of the present invention to provide a method for cleaving amide bonds which can alleviate at least some of the problems in the prior art.

It is an object of the present invention to provide a method for cleaving amide bonds which allows for cleavage of amide bonds while preserving protecting groups and/or chiral centres.

It is a further object of the present invention to provide a method for cleaving amide bonds which does not require strongly basic conditions (i.e. concentrated NaOH or the like) or strongly acidic conditions (i.e. concentrated HCl or the like) at elevated temperatures for long periods of time.

According to aspects illustrated herein, there is provided a method for cleaving amide bonds, comprising:

a) providing a molecule comprising an amide group;

b) reacting the molecule comprising an amide group with hydroxylamine (NH_2OH) or a salt thereof to cleave the amide bond of the amide group.

The method preferably involves the use of a hydroxylamine salt.

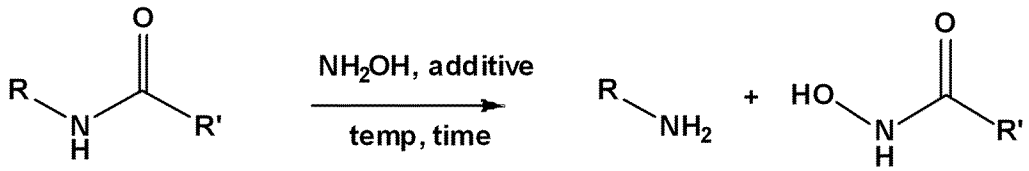
The inventors have surprisingly found that when a hydroxylamine salt is used instead of hydroxylamine itself, the same or even higher reaction rate can be achieved with a significantly lower molar concentration of the reagent and/or lower pH. This means that the hydroxylamine salts allow for the cleavage of amide bonds under less harsh reaction conditions than hydroxylamine itself,

resulting in less risks of degradation or undesired side reactions in the amide group containing compound. In practice, this allows for cleavage of different types of amide bonds, including amide bonds in amides that are typically difficult to cleave, such as in secondary amide groups. It also allows for cleavage of amide groups in various “sensitive” substrates, i.e. substrates including other bonds that would easily be cleaved under harsher reaction conditions. Examples include substrates comprising pH sensitive protecting groups or pH sensitive chiral centers.

An “amide group” as referred to herein, is a compound with the functional group $R_nE(O)_xNR'_2$ (wherein R and R′ refer to H or organic groups). Most common are carboxamides (organic amides) (n = 1, E = C, x = 1), but other important types of amides are known, including phosphoramides (n = 2, E = P, x = 1 and many related formulas) and sulfonamides (E = S, x = 2) (IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997)). The term amide refers both to classes of compounds and to the functional group $R_nE(O)_xNR'_2$ within those compounds. The bond between the nitrogen atom and the E in the amide group is referred to herein as the “amide bond”. Amides should not be confused with imides, which comprise two acyl groups bound to the same nitrogen atom, and which are comparatively easy to cleave.

Thus, in some embodiments the “amide group” is a carboxamide group, a sulfonamide group or a phosphoramide group. The inventive method has been found to be particularly useful for cleaving amides which are typically more difficult to cleave, such as carboxamides or sulfonamides. Accordingly, in some embodiments the “amide group” is a carboxamide group or a sulfonamide group. In a preferred embodiment, the “amide group” is a carboxamide group. Carboxamides are derived from a carboxylic acid and an amine.

The present invention is based on the inventive realization that hydroxylamine (NH_2OH) and especially salts thereof can advantageously be used for cleavage of amide bonds in molecules comprising amide groups under mild reaction conditions. This allows for cleavage of amide bonds while avoiding undesired degradation of the molecule and preserving protecting groups and/or chiral centres. The reaction is illustrated generally by reaction scheme I.



Reaction Scheme I

For example, polysaccharides, and particularly glycosaminoglycans such as hyaluronic acid, chondroitin and chondroitin sulfate, are often prone to degradation of the backbone under harsh reaction conditions (e.g. very high or low pH, or high temperatures). The inventive method is therefore especially useful for cleavage of amide bonds in such polysaccharides. The inventive method is for example useful for obtaining at least partially deacetylated glycosaminoglycans in which a significant portion of the molecular weight of the starting material is retained. Using hydroxylamine or salts thereof for deacetylation has been found to allow for N-deacetylation under mild conditions resulting in only minor degradation of the polymeric backbone of hyaluronic acid. Using hydroxylamine or salts thereof for deacetylation thus allows for production of deacetylated hyaluronic acid with retained high molecular weight. This is in contrast to previously known methods, such as deacetylation using hydrazine or NaOH as the deacetylating agent, where high degrees of deacetylation have been inevitably accompanied by severe degradation of the polymeric backbone.

The inventive method is useful for cleaving amide bonds in primary, secondary and tertiary amide groups. A primary amide refers to an amide in

which the amide nitrogen is bound to one carbon atom. A secondary amide refers to an amide in which the amide nitrogen is bound to two carbon atoms. A tertiary amide refers to an amide in which the amide nitrogen is bound to three carbon atoms. The method is particularly advantageous for cleaving amide bonds in more hindered amides, i.e. secondary and tertiary amide groups, since such bonds are typically more difficult to cleave using conventional methods. Thus, according to embodiments, the amide group is a primary, secondary or tertiary amide group, preferably a secondary amide group.

The inventive method may also be particularly useful for deacylation, particularly deacetylation, of molecules comprising acyl or acetyl groups. Thus, according to embodiments, the amide group is an N-acyl amide group, preferably an N-acetyl amide group.

According to embodiments, the molecule comprising an amide group further comprises a pH sensitive chiral center. Examples of molecules comprising an amide group and further comprising a pH sensitive chiral center include, but are not limited to, certain oligosaccharides, polysaccharides, and amino acids.

As evidenced by the attached Examples, cleavage of amide bonds in a molecule using hydroxylamine or salts thereof can also be achieved without cleaving common protecting groups present in the molecule.

According to embodiments, the molecule comprising an amide group further comprises a pH sensitive protecting group. By “pH sensitive protecting group”, we mean a protecting group which is cleaved off under high or low pH conditions. By high pH in this context we generally mean a pH of 12 or higher (e.g. concentrated NaOH or the like). By low pH in this context we generally mean a pH of 2 or lower (e.g. concentrated HCl or the like). Examples of pH sensitive protecting groups include, but are not limited to Boc (tert-

Butyloxycarbonyl), Fmoc (9-Fluorenylmethyloxycarbonyl), Cbz (Carbobenzyloxy), i-PrCO, t-BuCO and Tr (triphenylmethyl).

According to some embodiments, the molecule comprising an amide group is a biopolymer comprising acetyl groups. According to some embodiments, the biopolymer comprising acetyl groups is a glycosaminoglycan. According to some embodiments, the biopolymer comprising acetyl groups is selected from the group consisting of sulfated or non-sulfated glycosaminoglycans such as hyaluronan, chondroitin, chondroitin sulphate, heparan sulphate, heparosan, heparin, dermatan sulphate and keratan sulphate, preferably hyaluronic acid, chondroitin and chondroitin sulfate, and mixtures thereof. According to some embodiments, the biopolymer comprising acetyl groups is hyaluronic acid. In some embodiments, the biopolymer is a hyaluronic acid gel. In some embodiments, the biopolymer is a hyaluronic acid gel crosslinked by 1,4-butanediol diglycidyl ether (BDDE).

Hyaluronic acid is one of the most widely used biocompatible polymers for medical use. Hyaluronic acid and the other GAGs are negatively charged heteropolysaccharide chains which have a capacity to absorb large amounts of water. Hyaluronic acid and products derived from hyaluronic acid are widely used in the biomedical and cosmetic fields, for instance during viscosurgery and as a dermal filler.

According to embodiments, a product formed by the cleavage of the amide bond of step b) is an amine.

According to embodiments, the method further comprises the step

c) recovering a product, preferably an amine, formed by the reaction of step b).

The recovery in step c) may comprise any suitable organic synthetic work-up or purification technique or combination of techniques.

The reaction temperature in step b) is preferably selected so as not to cause excessive degradation of the molecule and so as to preserve protecting groups and/or chiral centres. The reaction may generally be performed at a temperature of 200 °C or less, such as a temperature of 150 °C or less. According to embodiments, the reaction in step b) comprises reacting the molecule comprising an amide group with the hydroxylamine or salt thereof at a temperature of 100 °C or less. According to embodiments, the reaction in step b) comprises reacting the molecule comprising an amide group with the hydroxylamine or salt thereof at a temperature in the range of 10-100 °C, preferably 20-90 °C, preferably 30-70 °C, preferably 30-50 °C. The temperature may for example be in the range of 70-90 °C, such as about 80 °C, or in the range of 30-50 °C, such as about 40 °C.

The reaction time in step b) depends on the desired degree of amide cleavage. The reaction time is preferably selected so as not to cause excessive degradation of the molecule and so as to preserve protecting groups and/or chiral centres, and is also dependent on the temperature and pH. The reaction time may generally be anywhere from 5 minutes to 200 hours or more. According to some embodiments, the reaction in step b) comprises reacting the molecule comprising an amide group with the hydroxylamine or salt thereof for 2-200 hours. According to some embodiments, the reaction in step b) comprises reacting the molecule comprising an amide group with the hydroxylamine or salt thereof for 2-150 hours, preferably 5-150 hours, preferably 5-100 hours. In other embodiments, e.g. where a higher temperature or pH is used, the reaction time can be much shorter, such as in the range of 5 minutes to 2 hours, in the range of 30 minutes to 2 hours, or in the range of 1-2 hours. Likewise, under otherwise mild reaction conditions, the reaction time can be much longer than 200 hours.

The cleavage of the amide bond is preferably achieved using a hydroxylamine salt. The hydroxylamine salt refers to a salt formed by hydroxylamine and an acid. The hydroxylamine salt may for example be a salt formed by hydroxylamine and an acid selected from the group consisting of mineral acids and organic acids or mixtures thereof.

According to embodiments, the hydroxylamine salt is a salt formed by hydroxylamine and a mineral acid. According to embodiments, the acid is selected from the group consisting of sulfuric acid, hydrochloric acid, hydroiodic acid, hydrobromic acid and phosphoric acid, and combinations thereof. Preferred mineral acids include hydrochloric acid, hydroiodic acid and hydrobromic acid. A particularly preferred mineral acid is hydroiodic acid.

According to embodiments, the hydroxylamine salt is a salt formed by hydroxylamine and an organic acid. According to embodiments, the acid is selected from the group consisting of acetic acid, propionic acid, pivalic acid, citric acid, oxalic acid, malonic acid, lactic acid, benzoic acid, and halogenated carboxylic acids, such as trifluoroacetic acid (TFA) and trichloroacetic acid, and combinations thereof.

According to embodiments, the acid is selected from the group consisting of acetic acid, propionic acid, pivalic acid, and a halogenated carboxylic acid, preferably trifluoroacetic acid, and combinations thereof. According to embodiments, the acid is a halogenated carboxylic acid, preferably trifluoroacetic acid.

According to embodiments, the hydroxylamine salt is a salt formed by hydroxylamine and an acid selected from the group consisting of hydrochloric acid, hydroiodic acid and hydrobromic acid, propionic acid, pivalic acid and trifluoroacetic acid.

The reaction in step b is preferably performed in a solvent capable of at least partially dissolving both the molecule comprising an amide group and the hydroxylamine or salt thereof. The solvent may for example be water or an organic solvent or a mixture thereof. Non-limiting examples of preferred solvents include water or a mixture of water and a lower alcohol, such as ethanol. However, may other solvents would be useful, depending on the particular molecule comprising the amide group to be cleaved, and the selection of hydroxylamine or salt thereof. One example of a useful organic solvent is tetrahydrofuran (THF).

According to embodiments, the reaction in step b) comprises reacting the molecule comprising an amide group with hydroxylamine in water.

The method may preferably be performed in water or aqueous solution, optionally further comprising another solvent, such as ethanol. Thus according to some embodiments, step b) comprises contacting a molecule comprising an amide group with hydroxylamine in water so that an aqueous mixture or solution of the molecule and the hydroxylamine is formed.

According to embodiments, the concentration of hydroxylamine in step b) is at least 10 % by weight, preferably at least 20 % by weight, preferably at least 30 % by weight. A higher concentration of hydroxylamine may increase the reaction rate.

Hydroxylamine is often provided in the form of an aqueous solution, typically at a concentration of 50% by weight. In some embodiments, the molecule comprising an amide group may be mixed and dissolved directly in the aqueous solution of hydroxylamine, optionally diluted. Alternatively, a solid salt of hydroxylamine, for example hydroxylamine hydrochloride or hydroxylamine sulfate, can be dissolved in an aqueous solution of the molecule comprising an amide group. Adding a salt of hydroxylamine, and converting the salt to hydroxylamine, may be done as an alternative or as a

complement to dissolving the molecule comprising an amide group in an aqueous solution of hydroxylamine.

The molar concentration of hydroxylamine in the reaction mixture is preferably in the range of 5-20 M. For example, a concentration of hydroxylamine of 50% by weight roughly corresponds to a molar concentration of 16 M.

The inventors have surprisingly found that when a hydroxylamine salt is used instead of hydroxylamine itself, the same reaction rate can be achieved with a significantly lower molar concentration. Thus, the molar concentration of hydroxylamine salt in the reaction mixture is preferably in the range of 0.01-10 M, preferably in the range of 0.1-5 M.

According to some embodiments, the molecule comprising an amide group is dissolved in an aqueous solution of hydroxylamine or salt thereof in step a). According to some embodiments, a salt of hydroxylamine is dissolved in an aqueous solution of a molecule comprising an amide group in step a). According to some embodiments, the molecule comprising an amide group is dissolved in an aqueous solution of hydroxylamine, and a salt of hydroxylamine is dissolved in the aqueous solution of the molecule comprising an amide group in hydroxylamine.

The pH in the reaction step b) is preferably selected so as not to cause excessive degradation of the molecule and so as to preserve protecting groups and/or chiral centres. The pH of a 50% v/v solution of hydroxylamine in water is 10.2. According to some embodiments, the reaction in step b) is performed at a pH value in the range of 4-12. According to some embodiments, the reaction in step b) is performed at a pH value in the range of 9-11. According to some embodiments, the reaction in step b) is performed at a pH value in the range of 4-9, preferably in the range of 6-8.

The inventors have found through extensive experimentation that addition of a pH reducing agent can significantly increase the reaction rate of the reaction in step b), particularly when hydroxylamine is used. This effect is both surprising and highly advantageous. It is noted that a corresponding addition of a pH reducing agent to a hydrazine amide cleavage reaction did not result in any increase of the reaction rate. A lower pH value during the reaction is also preferred in order to avoid excessive degradation of the molecule and so as to preserve protecting groups and/or chiral centres.

Thus, according to some embodiments, the pH of the reaction is lowered by addition of a pH reducing agent. According to some embodiments, the pH of the reaction is lowered to a value in the range of 4-9, preferably in the range of 6-8, by addition of a pH reducing agent. The pH reducing agent may for example be selected from the group consisting of mineral acids, organic acids and pH reducing salts, and combinations thereof.

According to embodiments, the pH reducing agent is selected from the group consisting of mineral acids, organic acids and pH reducing salts, and combinations thereof.

According to embodiments, the pH reducing agent is a mineral acid. According to embodiments, the pH reducing agent is selected from the group consisting of sulfuric acid, hydrochloric acid, hydroiodic acid, hydrobromic acid and phosphoric acid, and combinations thereof.

According to embodiments, the pH reducing agent is an organic acid. According to embodiments, the pH reducing agent is selected from the group consisting of acetic acid, propionic acid, pivalic acid, citric acid, oxalic acid, malonic acid, lactic acid, benzoic acid, and halogenated carboxylic acids, such as trifluoroacetic acid and trichloroacetic acid, and combinations thereof. According to embodiments, the pH reducing agent is selected from the group consisting of acetic acid, propionic acid, pivalic acid, and a halogenated

carboxylic acid, preferably trifluoroacetic acid, and combinations thereof. According to embodiments, the pH reducing agent is a halogenated carboxylic acid, preferably trifluoroacetic acid.

According to embodiments, the pH reducing agent is a pH reducing salt. According to embodiments, the pH reducing agent is selected from the group consisting of ammonium chloride, ammonium bromide, ammonium iodide, hydroxylamine hydrochloride and hydroxylamine sulfate, and combinations thereof. According to embodiments, the pH reducing agent is selected from the group consisting of hydroxylamine hydrochloride or hydroxylamine sulfate, preferably hydroxylamine hydrochloride.

According to some embodiments, the reaction in step b) is performed in inert atmosphere and/or in darkness.

The present invention is based on the inventive realization that hydroxylamine (NH_2OH) and particularly salts thereof can advantageously be used for cleaving an amide bond in a molecule comprising an amide group under mild reaction conditions. Thus according to other aspects illustrated herein, there is provided the use of a hydroxylamine salt for cleaving an amide bond in a molecule comprising an amide group.

The use may be further characterized as described above with reference to the method described above.

Other aspects and preferred embodiments of the present invention will be evident from the following Examples and the appended claims.

The term “molecular weight” as used herein in connection with various polymers, e.g. polysaccharides, refers to the weight average molecular weight, M_w, of the polymers, which is well defined in the scientific literature. The weight average molecular weight can be determined by, e.g., static light

scattering, small angle neutron scattering, X-ray scattering, and sedimentation velocity. The unit of the molecular weight is Da or g/mol.

The person skilled in the art realizes that the present invention by no means is limited to the preferred embodiments described herein. On the contrary, many modifications and variations are possible within the scope of the appended claims. Additionally, variations to the disclosed embodiments can be understood and effected by the skilled person in practicing the claimed invention, from a study of the drawings, the disclosure, and the appended claims. In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

EXAMPLES

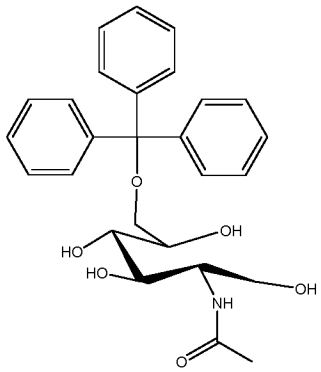
Without desiring to be limited thereto, the present invention will in the following be illustrated by way of examples.

Analysis methods

^{1}H NMR spectra were recorded on a BRUKER Biospin AVANCE 400 spectrometer. Chemical shifts are reported as δ values downfield from internal TMS in appropriate organic solutions. The purity and the structures of the products were confirmed by LCMS (254 nm) on a Waters 2690 photodiode array detector system using the following conditions: Column, Symmetry C-18; Solvent A, water 0.1% formic acid; Solvent B, CH_3CN; flow rate, 2.5 ml/min; run time, 4.5 min; gradient, from 0 to 100% solvent B; mass detector, micro mass ZMD. Purifications were carried out directly by mass-triggered preparative LCMS Waters X-Terra reverse-phase column (C-18, 5 microns silica, 19 mm diameter, 100 mm length, flow rate of 40 ml / minute) and

decreasingly polar mixtures of water (containing 0.1% formic acid) and acetonitrile as eluent. The fractions containing the desired compound were evaporated to dryness to afford the final compounds usually as solids.

Example 1 – Preparation of N-((2R,3R,4S)-1,3,4,5-tetrahydroxy-6-(trityloxy)hexan-2-yl)acetamide



A solution of *N*-((2R,3S,5S)-2,4,5-trihydroxy-6-trityloxymethyl-tetrahydro-pyran-3-yl)-acetamide (556 mg, 1.20 mol, 1.00 eq.) in a mixture of THF-H_2O (20 ml, 4:1) at r.t., was treated with solid sodium borohydride (49.92 mg, 1.32 mol, 1.10 eq.) [gas evolution]. The reaction mixture was stirred at r.t. for 2h, concentrated to dryness to afford *N*-((2*R*,3*R*,4*S*)-1,3,4,5-tetrahydroxy-6-(trityloxy)hexan-2-yl)acetamide (500 mg, 89.54 %) as a white solid that was used without further purification.

LCMS: t_R = 1.01 min., purity = 100%; ES+, 464.26 $(M-H)^-$.

Example 2 – Deacetylation of N-((2R,3R,4S)-1,3,4,5-tetrahydroxy-6-(trityloxy)hexan-2-yl)acetamide

A suspension of *N*-((2*R*,3*R*,4*S*)-1,3,4,5-tetrahydroxy-6-(trityloxy)hexan-2-yl)acetamide (1 eq) in hydroxylamine (10 volumes) was either treated with acid additives to lower the pH to 7 or not as set out in Table 1, Examples 1-10. The mixture was heated at 80 °C until full conversion of the deacetylation was reached. Deacetylation of *N*-((2*R*,3*R*,4*S*)-1,3,4,5-tetrahydroxy-6-(trityloxy)hexan-2-yl)acetamide with hydrazine (pH 13) under the same conditions as in Example 2-1 is also included as Example 2-10.

The results are displayed in Table 1. The results show that the deacetylation procedure proceeds considerably faster with hydroxylamine than with hydrazine, and is significantly by the addition of a pH reducing agent.

Table 1.

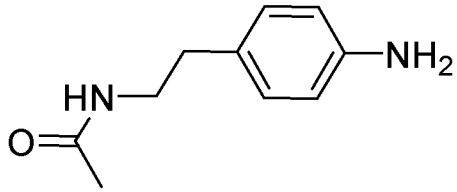
Example	**Solvent (50 vols)***	**Additive**	**pH**	**Time to reach 100% conversion**
2-1	50% NH_2OH (aq)	None	10.2	72 h
2-2	50% NH_2OH (aq)	HCl	7	12 h
2-3	50% NH_2OH (aq)	HBr	7	9 h
2-4	50% NH_2OH (aq)	HI	7	5 h
2-5	50% NH_2OH (aq)	H_2SO_4	7	29 h
2-6	50% NH_2OH (aq)	CH_3COOH	7	6 h
2-7	50% NH_2OH (aq)	TFA	7	4 h
2-8	50% NH_2OH (aq)	$(CH_3)_3COOH$	7	5 h
2-9	50% NH_2OH (aq)	CH_3CH_2COOH	7	8 h
2-10	$NH_2NH_2.H_2O$	None	13	120 h

The reaction mixtures were purified directly by Preparative LCMS to afford (2*R*,3*R*,4*S*)-2-amino-6-(trityloxy)hexane-1,3,4,5-tetraol as a white solid.

LCMS: t_R = 0.88 min., purity = 99%; ES+, 422.11 $(M-H)^-$.

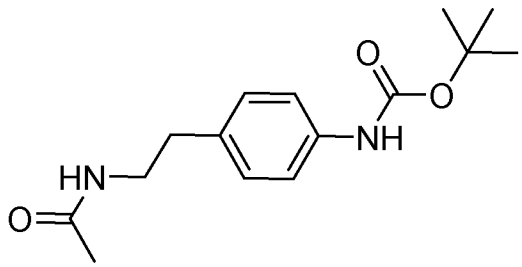
^1H NMR (DMSO-d_6) δ: 7.47 – 7.37 (m, 6H), 7.30 (dd, *J* = 8.3, 6.7 Hz, 6H), 7.26 – 7.15 (m, 3H), 3.92 (m, 1H), 3.83 – 3.74 (m, 1H), 3.62 – 3.53 (m, 1H), 3.52 – 3.41 (m, 1H), 3.34 – 3.27 (m, 1H), 3.22 – 3.16 (m, 1H), 3.13 – 3.04 (m, 1H), 3.01 – 2.91 (m, 1H).

Example 3 – Preparation of *N*-(4-aminophenethyl)acetamide



A 4-(2-aminoethyl)aniline (1.50 g; 11.01 mmol; 1.00 eq.) was added neat *p*-cresyl acetate (1.65 g, 11.0 mmol, 1.00 eq.) and the reaction mixture was stirred at room temperature for 30 h. The resulting orange solution was absorbed directly on silica gel and purified by flash chromatography (silica gel, DCM/MeOH 0-5%) to afford *N*-(4-aminophenethyl)acetamide (1.76 g, 89.7% yield). LCMS: t_R = 0.58 min., purity = 99.5%; ES+, 179.5 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.78 (s, 3H), 2.50 (m, 2H hidden by DMSO signal) 3.14 (m, 2H), 4.83 (s, 2H), 6.49 (d, J = 7.5 Hz, 2H), 6.84 (d, J = 7.5 Hz, 2H), 7.82 (s, 1H).

Example 4 – Preparation of *tert*-butyl (4-(2-acetamidoethyl)phenyl)carbamate



To a stirred solution of *N*-[2-(4-Amino-phenyl)-ethyl]-acetamide (500 mg, 2.81 mmol, 1.00 eq.) in DCM (20 ml) at r.t., was added triethylamine (0.51 ml, 3.65 mmol, 1.30 eq.) followed by di-*tert*-butyl dicarbonate (673.48 mg, 3.09 mmol, 1.10 eq.). The reaction mixture is stirred at r.t. for 1 h, washed with water (5 ml), a saturated solution of $NaHSO_4$ (aq) (5 ml) and water (3 x 5 ml), dried over $MgSO_4$ and concentrated to dryness to afford *tert*-butyl (4-(2-acetamidoethyl)phenyl)carbamate (496 mg, 63% yield) as a pale orange solid. LCMS: t_R = 1.11 min., purity = 100%; ES+, 279.5 (M+H). ^{1}H-NMR (DMSO-d_6) δ 1H NMR (400 MHz, DMSO-d6) δ 1.57 (s, 9H), 1.87 (s, 3H), 2.75 – 2.64 (m, 2H), 3.36 – 3.20 (m, 2H), 7.27 – 7.07 (m, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.94 (t, *J* = 5.6 Hz, 1H), 9.31 (s, 1H).

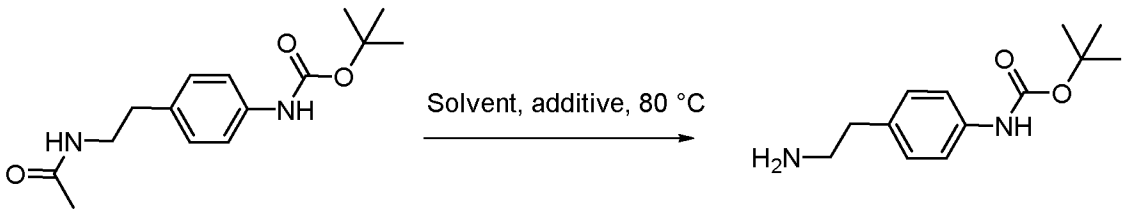
Example 5 – Preparation of $NH_2OH.HI$

To a stirred solution of 50% NH_2OH (aq) (9.28 ml, 0.15 mol, 1.00 eq) at 0 °C was added carefully dropwise 57% HI (aq) over a period of 5 minutes until a pH of 7 was achieved. A dense white crystalline solid formed that was collected by filtration, washed carefully with ice cold water to afford hydroxylamine hydrogen iodide (6.80 g, 28%).

Example 6 – Preparation of $NH_2OH.TFA$

To a stirred solution of 50% NH_2OH (aq) (9.28 ml, 0.15 mol, 1.00 eq) at 0 °C was added carefully dropwise TFA over a period of 5 minutes until a pH of 7 was achieved. The reaction mixture was concentrated under nitrogen sparging to afford hydroxylamine.trifluoroacetate (11.0 g, 98%) as clear colourless oil.

Example 7 – Comparative studies of NH_2OH and salts thereof versus commonly used transamidation agents such as $NH_2NH_2.H_2O$ and NaOH



To a stirred solution / suspension of *tert*-butyl (4-(2-acetamidoethyl)phenyl) carbamate (50 mg, 0.18 mmol) in the chosen solvent (5 volumes) was added the salt (5 eq) and the resulting mixture was heated at 80 °C for the time necessary to complete the reaction. The results are displayed in Table 2. The results show that the deacetylation procedure proceeds quickly with for example hydroxylamine hydrogen iodide (Example 7-3) or hydroxylamine trifluoroacetate (Example 7-9), even when the relative concentration of hydroxylamine in the salts is much lower than the concentration of hydroxylamine alone in Example 7-1. LCMS: t_R = 0.81 min., purity = 100%; ES+, 237.51$(M+H)^+$.

^{1}H-NMR (DMSO-d_6) δ 1H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.40 (s, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 2.89 (m, 2H), 2.80 – 2.63 (m, 2H), 1.47 (s, 9H) (isolated as formate salt).

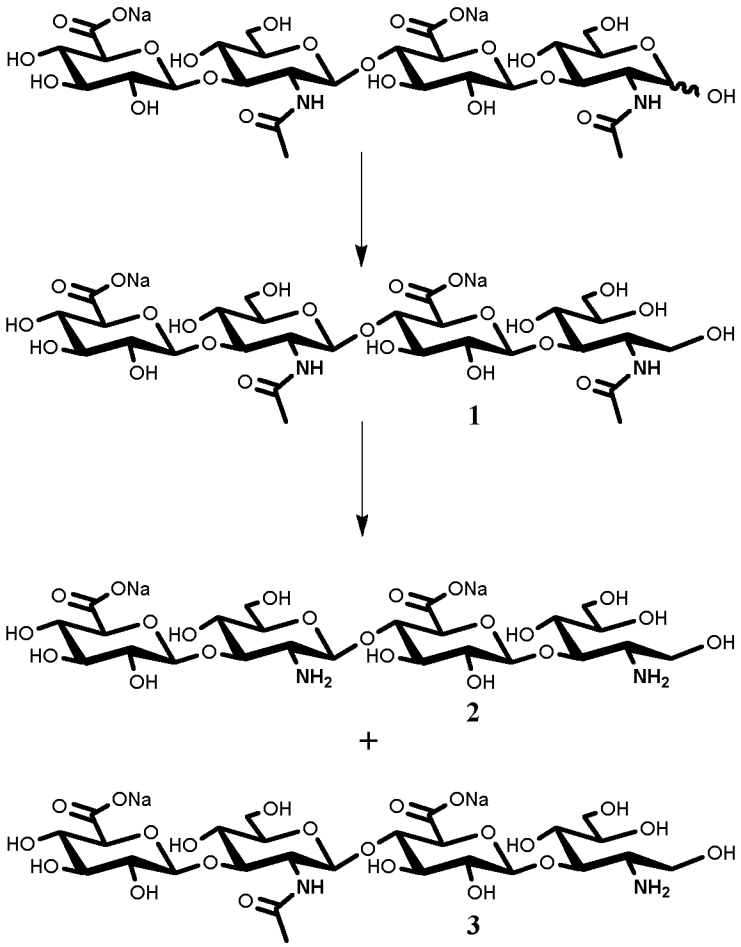
Table 2.

Example	**Solvent (5 vols)***	**Additive**	**pH**	**1 h (% conv.)**	**2 h (% conv.)**	**4 h (% conv.)**
7-1	50% NH_2OH (aq)	None	10.2	34.8	64.7	83.0
7-2	50% NH_2OH (aq)	5 eq $NH_2OH.HI$	9	48.6	83.5	97.0
7-3	EtOH / H_2O (4:1)	5 eq $NH_2OH.HI$	7	63.8	85.8	98.9
7-4	$NH_2NH_2.H_2O$	None	13	13.6	34.9	35.2
7-5	$NH_2NH_2.H_2O$	5 eq $NH_2OH.HI$	13	57.9	86.9	97.4
7-6	EtOH (4 vols)	4N NaOH (aq) (1 vol)	14	3.7	11.63	14.5
7-7	EtOH / H_2O (4:1)	5 eq $NH_2OH.HCl$	7	3.4	5.8	17.2
7-8	EtOH / H_2O (4:1)	5 eq $NH_2OH.H_2SO_4$	7	0	0.2	0.7
7-9	EtOH / H_2O (4:1)	5 eq $NH_2OH.TFA$	7	34.2	72.4	91.3
7-10	EtOH / H_2O (4:1)	5 eq NH_4I	7	0	0	0

**Volume = 1 g = 1 ml = 1 volume*

Example 8 – Preparation and deacetylation of reduced HA-4 by hydroxylamine

Diaminotetra-HA (DA-4HA) was synthesized according to the below scheme:



Step 1

A solution of HA-4 (500 mg, 0.61 mmol) in water (5 ml) at room temperature was treated with sodium borohyride (23.05 mg, 0.61 mmol) and the resulting solution was stirred for 3 h, concentrated to dryness to afford the reduced product **1** (532 mg, assumed 100%) as a white foam.

LCMS (t_r = 0.28 min., ES+ = 779.4 (M-2 Na + 2H)

Step 2

The reduced product **1** (532 mg) was dissolved in aqueous NH_2OH (5 ml, 50% v/v/) and solid NH_4I (100 mg) was added. The resulting suspension was

heated at 70 °C for 48 h, cooled to room temperature and concentrated to dryness to afford a residue. The residue was precipitated in neat EtOH and the resulting precipitate was collected by filtration and dried to a constant weight to afford the a 1:1 mixture of diamine **2** and mono-amine **3** in quantitative yield.

2 : LCMS (t_r = 0.16 min., ES+ = 695.36 (M-2 Na + 2H)
3: LCMS (t_r = 0.19 min., ES+ = 737.47 (M-2 Na + 2H)

Example 9 – Deacetylation of reduced HA-4 by $NH_2OH.HI$

Reduced HA-4 (532 mg) prepared as described in Step 1 of Example 26, is dissolved in EtOH-H_2O (2.5 ml, 1:1) and solid $NH_2OH.HI$ (491 mg, 3.05 mmol) is added. The resulting suspension is heated at 80 °C for 6 h, cooled to room temperature and the reaction mixture is purified by Preparative HILIC chromatography to afford deacetylated HA-4 as a white solid.
Deacetylated HA-4 : LCMS (t_r = 0.16 min., ES+ = 695.36 (M-2 Na + 2H)

Example 10 – Deacetylation of Hyaluronic Acid by hydroxylamine

0.2 g or 20 g of HA (Mw 2 500 kDa, DoA 100%) was solubilised in hydroxylamine (Sigma-Aldrich 50 vol% solution), or a mixture of hydroxylamine/water as set out in Table 3. The solution was incubated in darkness and under argon at 30 - 70 °C for 5 - 353 hours. After incubation, the mixture was precipitated by ethanol. The obtained precipitate was filtered, washed with ethanol and then re-dissolved in water. The solution was purified by ultrafiltration and subsequently lyophilized to obtain the deacetylated HA (de-Ac HA) as a white solid. Examples 10-1 to 10-14 were performed using approx. 0.2 g HA and examples 10-15 to 10-16 were performed using 20 g HA. The results are displayed in Table 3. Deacetylation by hydroxylaminolysis is more efficient, and conserves the Mw of the HA backbone better as compared to hydrazinolysis (Example 11) and alkaline methods (Examples 12-13).

Table 3.

Example	**Temp** (°C)	**Time** (h)	**pH**	**Conditions**	**Start Mw** (kDa)	**NMR DoA** (%)	**Mw** (kDa)
10-1	30	24	10	NH_2OH (50 wt% in water)	2500	99	970[a]
10-2	30	72	10	NH_2OH (50 wt% in water)	2500	98	1060 [a]
10-3	30	196	10	NH_2OH (50 wt% in water)	2500	95	1060 [a]
10-4	40	24	10	NH_2OH (50 wt% in water)	2500	98	1050 [a]
10-5	40	72	10	NH_2OH (50 wt% in water)	2500	95	980 [a]
10-6	40	353	10	NH_2OH (50 wt% in water)	2500	80	490 [a]
10-7	40	24	10	NH_2OH (35 wt% in water)	2500	99	1090 [a]
10-8	40	24	10	NH_2OH (20 wt% in water)	2500	100	1130 [a]
10-9	40	24	10	NH_2OH (50 wt% in water)	1000	98	670 [b]
10-10	55	5	10	NH_2OH (50 wt% in water)	2500	99	1010 [a]
10-11	55	72	10	NH_2OH (50 wt% in water)	2500	86	740 [a]

10-12	55	120	10	NH_2OH (50 wt% in water)	2500	78	400[b]
10-13	60	24	10	NH_2OH (50 wt% in water)	2500	92	930 [b]
10-14	70	24	10	NH_2OH (50 wt% in water)	2500	86	720 [b]
10-15	40	72	10	NH_2OH (50 wt% in water)	2500	95	1870 [b]
10-16	55	72	10	NH_2OH (50 wt% in water)	2500	89	1050 [b]

a: SEC-UV b: SEC-MALS

Example 11 – Deacetylation of Hyaluronic Acid by Hydrazinolysis – Comparative Example

0.2 g of HA (Mw 2 500 kDa, DoA 100%) was solubilised in 10 mL of a 1% solution of hydrazine sulphate in hydrazine monohydrate. The reaction took place in dark and under argon at 30-55 °C for 24-120 hours. The mixture was precipitated by ethanol. The precipitate obtained was filtered, washed with ethanol and then re-dissolved in water. The final deacetylated HA product was obtained after ultrafiltration, and freeze-dried. The results are displayed in Table 4. Deacetylation by hydrazinolysis gives more degradation of the HA backbone, i.e. lower Mw of the deacetylated product as compared to hydroxylaminolysis (Examples 10-1 to 10-16).

Table 4.

Example	**Temp** (°C)	**Time** (h)	**pH**	**Conditions**	**DoA** (%)	**Mw (SEC MALS)** (kDa)
11-1	30	24	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	100	220
11-2	30	120	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	96	320
11-3	40	48	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	96	260
11-4	40	120	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	92	170
11-5	55	24	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	93	60
11-6	55	48	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	89	70
11-7	55	72	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	83	40
11-8	55	120	13	NH_2NH_2 + $NH_2NH_2H_2SO_4$	77	50

Example 12 – Deacetylation of Hyaluronic Acid by Homogeneous Alkaline hydrolysis – Comparative Example

HA (1 000 kDa) was weighed to a reaction vessel, NaOH solution was added and the reaction was mixed until a homogenous solution was obtained. The mixture was incubated without stirring and subsequently diluted with water and EtOH. The mixture was neutralized by adding 1.2 M HCl, precipitated by

adding EtOH. The precipitate was washed with ethanol (70 w/w%) followed by ethanol and dried in vacuum over night to obtain a solid. The results are displayed in Table 5.

Deacetylation by homogenous alkaline hydrolysis gives more degradation of the HA backbone, i.e. lower Mw of the deacetylated product as compared to hydroxylaminolysis (Examples 10-1 to 10-16).

Table 5.

Example	**Temp** (°C)	**Time** (h)	**pH**	**Conditions**	**DoA** (%)	**Mw (SEC UV)** (kDa)
12	65	4	13	1 M NaOH (aq.)	99	10

Example 13 – Deacetylation of Hyaluronic Acid by Heterogeneous Alkaline hydrolysis – Comparative Example

HA (1 000 kDa) was weighed to a reaction vessel and NaOH in EtOH (70% w/w%) was added. The heterogeneous mixture was incubated and subsequently neutralized by addition of 1.2 M HCl. The precipitate was washed with ethanol (75 w/w%) followed by ethanol and dried in vacuum over night to obtain a solid. The results are displayed in Table 6.

Deacetylation by heterogeneous alkaline hydrolysis gives more degradation of the HA backbone, i.e. lower Mw of the deacetylated product as compared to hydroxylaminolysis (Examples 10-1 to 10-16).

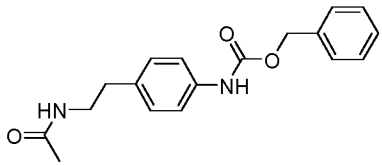
Table 6.

Example	Temp (°C)	Time (h)	Conditions	DoA (%)	Mw (SEC UV) (kDa)
13	35	24	1.0 M NaOH (70% EtOH)	99	60

Further examination of substrate scope and chemoselectivity

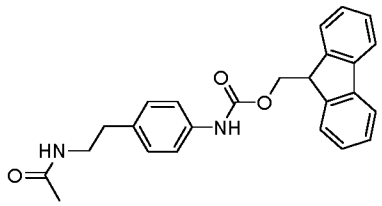
Protection of *N*-[2-(4-Amino-phenyl)-ethyl]-acetamide with a variety of widely used protecting groups.

Example 14 – Preparation of benzyl (4-(2-acetamidoethyl)phenyl)carbamate



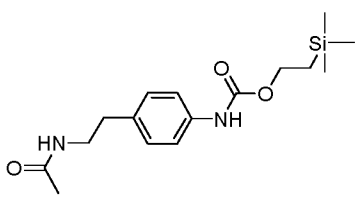
To a stirred solution of *N*-[2-(4-Amino-phenyl)-ethyl]-acetamide (150 mg, 0.84 mmol) in THF (2 ml) and water (0.5 ml), was added sodium hydrogencarbonate (91.9 mg, 1.09 mmol) followed by benzyl chloroformate (151 mg, 0.88 mmol) at 0 °C over 1 minute. The reaction mixture was stirred for 1 h, diluted with DCM (30 ml), washed with water (3 x 5 ml), dried ($MgSO_4$) to afford benzyl (4-(2-acetamidoethyl)phenyl)carbamate (224 mg, 85%) as a white solid: LCMS (t_r = 1.13 min., ES+ = 313.68 (M+H)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.51 – 7.27 (m, 7H), 7.17 – 7.02 (m, 2H), 5.14 (s, 2H), 3.29 – 3.11 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.4 Hz, 2H), 1.78 (s, 3H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.02, 153.43, 137.18, 136.74, 133.58, 128.90, 128.49, 128.11, 128.06, 118.33, 65.68, 39.07, 34.59, 22.65.

Example 15 – Preparation of (9H-fluoren-9-yl)methyl (4-(2-acetamidoethyl) phenyl)carbamate



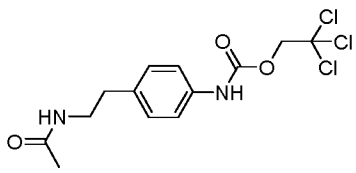
To a stirred solution of *N*-[2-(4-Amino-phenyl)-ethyl]-acetamide (150 mg, 0.84 mmol) in THF (2 ml) and water (0.5 ml) was added sodium hydrogencarbonate (91.9 mg, 1.09 mmol) followed by Fmoc-OSu (298 mg, 0.88 mmol) at 0 °C. The reaction mixture was stirred at r.t. for 1 h and the resulting suspension was diluted with water (2 ml) and precipitate collected by filtration washed with water (2 ml) and diethyl ether (5 ml) to afford (9*H*-fluoren-9-yl)methyl (4-(2-acetamidoethyl)phenyl)carbamate (316 mg, 94%) as a white solid: LCMS (t_r = 1.29 min., ES+ = 401.52 (M+H), 423.55 (M+Na)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 7.99 – 7.79 (m, 2H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.50 – 7.28 (m, 6H), 7.09 (d, *J* = 8.1 Hz, 2H), 4.83 (s, 1H), 4.48 (d, *J* = 6.7 Hz, 2H), 4.31 (t, *J* = 6.6 Hz, 1H), 3.27 – 3.07 (m, 2H), 2.63 (t, *J* = 7.4 Hz, 2H), 1.78 (s, 3H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 7.92 (d, *J* = 7.4 Hz, 2H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.44 (ddd, *J* = 8.1, 7.4, 1.2 Hz, 2H), 7.36 (td, *J* = 7.4, 1.2 Hz, 4H), 7.09 (d, *J* = 8.1 Hz, 2H), 4.48 (d, *J* = 6.7 Hz, 2H), 4.31 (t, *J* = 6.7 Hz, 1H), 3.26 – 3.12 (m, 2H), 2.68 – 2.58 (m, 2H), 1.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.02, 153.48, 143.85, 140.86, 137.13, 133.60, 128.97, 128.86, 127.34, 127.16, 125.17, 121.43, 65.54, 46.71, 40.78, 34.59, 22.66.

Example 16 – Preparation of 2-(trimethylsilyl)ethyl (4-(2-acetamidoethyl) phenyl)carbamate



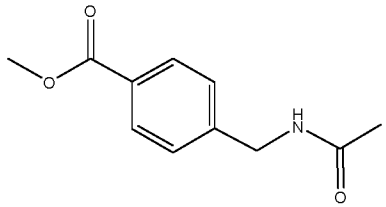
To a stirred solution of *N*-(4-aminophenethyl)acetamide (200 mg, 1.12 mmol, 1 eq) and TEA (in DCM (10 ml) at 0 °C, is added 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate (286 mg, 1.01 mmol) over a period of 5 minutes. The reaction mixture is stirred at room temperature overnight, diluted with DCM (10 ml), washed with a saturated aqueous solution of $NaHSO_4$ (5 ml), a saturated solution of $NaHCO_3$ (aq) (5 ml), water (3 x 5 ml), dried over $MgSO_4$ and concentrated to dryness to afford the title compound as a white solid: LCMS (t_r = 1.25 min., ES+ = 323.47 (M+H), 345.47 (M+Na)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 7.80 (t, *J* = 5.6 Hz, 1H), 7.42 – 7.23 (d, *J* = 8.3 Hz, 2H), 7.12 – 6.96 (d, *J* = 8.3 Hz, 2H), 4.17 – 3.97 (m, 2H), 3.22 – 3.06 (m, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 1.76 – 1.67 (m, 3H), 0.00 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.99, 153.70, 137.39, 133.35, 128.82, 118.34, 67.07, 40.31, 34.59, 25.40, 22.65, -1.52.

Example 17 – Preparation of 2,2,2-trichloroethyl (4-(2-acetamidoethyl) phenyl)carbamate



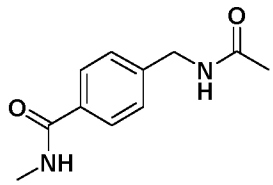
To a stirred solution of *N*-(4-aminophenethyl)acetamide (150 mg, 0.84 mmol) in THF (2 ml) and water (0.5 ml) at 0 °C, was added sodium hydrogencarbonate (91.9 mg, 1.09 mmol) followed by trichloromethyl chloroformate (175 mg, 0.88 mmol) (286 mg, 1.01 mmol) over a period of 1 minute. The reaction mixture is stirred at room temperature for 1 h, diluted with DCM (10 ml), washed with water (3 x 5 ml), dried over $MgSO_4$ and concentrated to dryness to afford the title compound (200 mg, 67%) as a white solid; LCMS (t_r = 1.17 min., ES+ = 353.35 (M+H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.19 – 7.02 (d, *J* = 8.1 Hz, 2H), 4.94 (s, 2H), 3.27 – 3.10 (m, 2H), 2.64 (t, *J* = 7.4 Hz, 2H), 1.78 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.03, 151.85, 136.52, 134.30, 129.00, 118.74, 96.05, 73.39, 39.99, 34.59, 22.65.

Example 18 – Preparation of methyl 4-(acetamidomethyl)benzoate



To a stirred suspension of methyl 4-(aminomethyl)benzoate hydrochloride (260 mg, 1.29 mmol) in DCM (5 mL) at r.t., were added triethylamine (0.39 ml, 2.84 mmol) followed by acetic anhydride (171 mg, 1.68 mmol) and the reaction mixture was stirred at r.t. for 1 h, diluted with DCM (10 mL) and washed with water (2 mL), a saturated aqueous solution of $NaHSO_4$ (2 mL) and water (2 x 2 mL). The organic extract was concentrated to dryness to afford methyl 4-(acetamidomethyl)benzoate (232 mg, 87%) as a white solid: LCMS (t_r = 0.92 min., ES+ = 208.42 (M+H)); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.42 (t, J = 6.0 Hz, 1H), 8.03 – 7.82 (d, J = 7.7 Hz, 2H), 7.49 – 7.24 (d, J = 7.7 Hz, 2H), 4.33 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 1.90 (s, 3H) ; ^{13}C NMR (DMSO-d_6) δ 169.40, 166.16, 145.42, 129.27, 127.10, 52.07, 41.94, 34,59, 22.68.

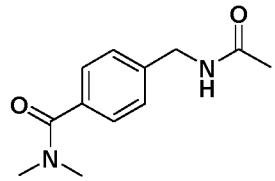
Example 19 – Preparation of 4-(Acetamidomethyl)-*N*-methylbenzamide



To a stirred suspension of 4-aminomethyl-*N*-methyl-benzamide (250 mg, 1.52 mmol) in DCM (20 ml) at r.t., were added triethylamine (274 µl, 1.98 mmol) followed by acetic anhydride (155 mg, 1.52 mmol). The resulting suspension was stirred for 16 h at r.t. and the precipitate was collected by filtration, washed with DCM (2 ml) and dried to a constant weight to afford 4-(acetamidomethyl)-*N*-methylbenzamide (301 mg, 94.07 %) as a white solid: LCMS (t_r = 0.66 min., ES+ = 207.40 (M+H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ

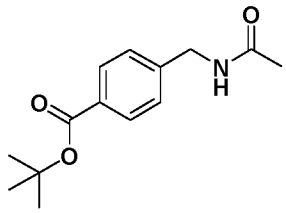
8.37 (t, *J* = 5.7 Hz, 1H), 7.85 – 7.71 (m, 2H), 7.37 – 7.23 (m, 2H), 4.29 (d, *J* = 6.0 Hz, 2H), 2.78 (d, *J* = 4.5 Hz, 3H), 1.89 (s, 3H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.27, 166.43, 142.78, 133.08, 127.11, 127.01, 41.86, 39.71, 26.25, 22.61.

Example 20 – Preparation of 4-(Acetamidomethyl)-*N,N*-dimethylbenzamide



To a stirred suspension of 4-aminomethyl-*N,N*-dimethyl-benzamide (250 mg, 1.40 mmol) in DCM (20 ml) at r.t., were added triethylamine (253 µl, 1.82 mmol) followed by acetic anhydride (143 mg; 1.40 mmol). The resulting solution was stirred for 16 h at r.t., washed with a saturated solution of $NaHSO_4$ (aq) and water. The organic phase was dried ($MgSO_4$) and concentrated to dryness to afford 4-(Acetamidomethyl)-*N,N*-dimethylbenzamide (265 mg, 86%) as a pale yellow oil; LCMS (t_r = 0.75 min., ES+ = 221.14 (M+H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.41 – 7.13 (m, 5H), 4.28 (d, *J* = 6.0 Hz, 2H), 3.10 – 2.77 (m, 6H), 1.89 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.06, 169.26, 140.9, 134.97, 127.07, 127.01, 41.87, (($\underline{C}H_3)_2$NC=O-) under DMSO peak at 40.28-38.97), 22.61.

Example 21 – Preparation of *tert*-Butyl 4-(acetamidomethyl)benzoate



To a stirred suspension of 4-aminomethyl-benzoic acid *tert*-butyl ester (250 mg, 1.21 mmol) in DCM (20 ml) at r.t., were added triethylamine (217 µl, 1.57 mmol) followed by acetic anhydride (123 mg; 1.21 mmol). The resulting solution was stirred for 16 h at r.t., washed with a saturated solution of $NaHSO_4$ (aq) and water. The organic phase was dried ($MgSO_4$) and

concentrated to dryness to afford *tert*-Butyl 4-(acetamidomethyl)benzoate (291 mg, 97%) as a clear, colourless oil: LCMS (t_r = 1.14 min., ES+ = 250.50 (M+H), 194.46 (M-^{t}Bu+H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (t, *J* = 6.0 Hz, 1H), 7.90 – 7.77 (m, 2H), 7.47 – 7.27 (m, 2H), 4.31 (d, *J* = 5.9 Hz, 2H), 1.89 (s, 3H), 1.55 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.32, 164.89, 144.94, 129.88, 129.10, 127.22, 80.59, 41.90, 27.86, 22.59.

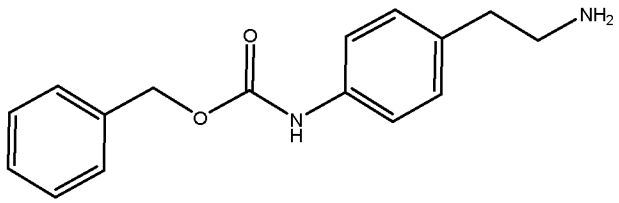
Example 22-30 Chemoselective cleavage of acetamides over common carbamate protecting groups esters and other amides

Example 22 – General deacetylation procedure

To a stirred solution of the 'acetamide' (50 mg, 1 eq) in EtOH-H_2O (4:1, 5 volumes) was added NH_2OH.TFA solution (50% v/v, 5 eq) and the suspension / solution was heated at 80 °C for 5 h, cooled to room temperature and purified directly by Mass Triggered Preparative LCMS to afford the desired compounds as their trifluoroacetate salts.

Example 23 – Deacetylation of benzyl (4-(2-acetamidoethyl)phenyl) carbamate (Example 14) using the general procedure of Example 22.

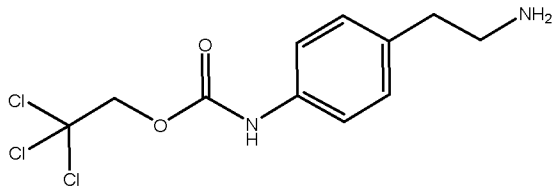
Benzyl (4-(2-aminoethyl)phenyl)carbamate was isolated as a white solid (41.2 mg, 67%) following the general procedure of Example 22.



LCMS (t_r= 0.51 min., ES+ 271.31 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 7.81 (s, 3H), 7.53 – 7.27 (m, 7H), 7.18 (d, *J* = 8.6 Hz, 2H), 5.15 (s, 2H), 3.01 (t, *J* = 7.9 Hz, 2H), 2.79 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.44, 137.84, 136.68, 131.15, 129.06, 128.50, 128.13, 128.10, 118.52, 65.75, 40.10, 32.45.

Example 24 – Deacetylation of 2,2,2-trichloroethyl (4-(2-acetamidoethyl) phenyl)carbamate (Example 17) using the general procedure of Example 22.

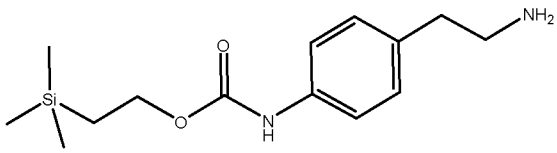
2,2,2-Trichloroethyl (4-(2-aminoethyl)phenyl)carbamate was isolated a white solid (45 mg, 75%) following the general procedure of Example 22.



LCMS (t_r= 0.56 min., ES+ 311.12, 313.15, 315.09 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 7.76 (s, 3H), 7.47 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 4.94 (s, 2H), 3.02 (dd, J = 9.2, 6.6 Hz, 2H), 2.81 (dd, J = 9.2, 6.6 Hz, 2H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.87, 137.18, 131.89, 129.16, 118.95, 96.01, 73.41, 40.11, 32.53.

Example 25 – Deacetylation of 2-(trimethylsilyl)ethyl (4-(2-acetamidoethyl) phenyl)carbamate (Example 16) using the general procedure of Example 22.

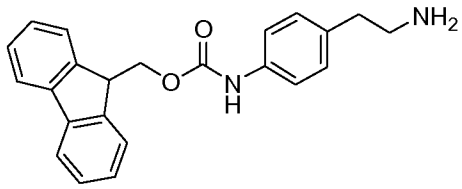
2-(Trimethylsilyl)ethyl (4-(2-aminoethyl)phenyl)carbamate (48 mg, 78%) was isolated as an off-white solid following the general procedure of Example 22.



LCMS (t_r= 0.93 min., ES+ 281 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 7.76 (s, 3H), 7.36 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 4.19 – 4.01 (m, 2H), 3.05 – 2.87 (m, 2H), 2.73 (dd, J = 9.3, 6.6 Hz, 2H), 1.08 – 0.82 (m, 2H), 0.00 (s, 9H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.69, 138.06, 130.92, 128.98, 118.50, 62.12, 40.12, 32.46, 17.40, -1.39.

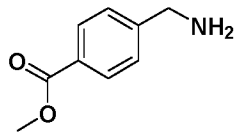
Example 26 – Deacetylation of (9H-fluoren-9-yl)methyl (4-(2-acetamidoethyl) phenyl)carbamate (Example 15) using the general procedure of Example 22.

(9H-Fluoren-9-yl)methyl (4-(2-aminoethyl)phenyl)carbamate (35 mg, 59%) was isolated as an off-white solid following the general procedure of Example 22.



LCMS (t_r= 0.99 min., ES+ 395.47 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.51 – 7.28 (overlapping signals, 9H), 7.16 (d, *J* = 8.2 Hz, 2H), 4.49 (d, *J* = 6.6 Hz, 2H), 4.31 (t, *J* = 6.6 Hz, 1H), 2.96 (m, 2H), 2.84 – 2.59 (m, 2H).

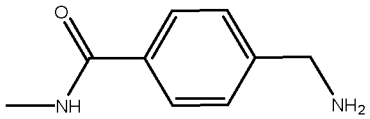
Example 27 – Deacetylation of 4-(acetamidomethyl)benzoate (Example 18).



To a stirred solution of 4-(acetylamino-methyl)-benzoic acid methyl ester (40.0 mg, 0.19 mmol) in THF (200 µL) was added $NH_2OH.TFA$ (85.15 mg, 0.58 mmol) and the solution was heated at 80 °C for 2 h, cooled to r.t. and purified by Prep LCMS pour donner methyl 4-(aminomethyl)benzoate (22.0 mg, 69%) as a trifluoroacetate salt: LCMS (t_r = 0.92 min., ES+ = 166.37 (M+H), 149.72 (M-NH_3)+H); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.32 (br s, 3H), 8.09 – 7.94 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.50 (d, *J* = 7.5 Hz, 2H), 4.14 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.93, 163.06, 139.34, 129.66, 129.43, 129.11, 52.31, 41.95.

Example 28 – Deacetylation of 4-(Acetamidomethyl)-*N*-methylbenzamide (Example 19) using the general procedure of Example 22.

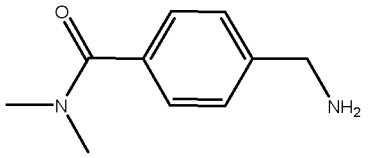
4-(Aminomethyl)-*N*-methylbenzamide (22 mg, 54%) was isolated as a white solid following the general procedure of Example 22.



LCMS (t_r= 0.15 min., ES+ 165 (M+H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, *J* = 4.5 Hz, 1H), 7.84 – 7.69 (d, 2H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.20 (s, 1H), 4.17 (d, *J* = 5.8 Hz, 2H), 2.78 (d, *J* = 4.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.55, 142.78, 133.08, 127.01, 126.70, 26.25.

Example 29 – Deacetylation of 4-(Acetamidomethyl)-*N,N*-dimethylbenzamide (Example 20) using the general procedure of Example 22.

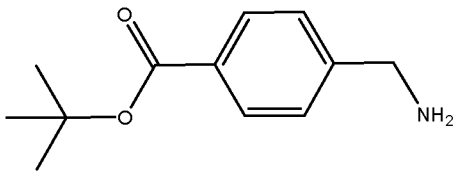
4-(Aminomethyl)-*N,N*-dimethylbenzamide (24 mg, 59%) was was isolated as a white solid following the general procedure of Example 22.



LCMS (t_r= 0.15 min., ES+ 179 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 – 7.22 (m, 4H), 3.75 (s, 1H), 2.94 (2 s, *rotmamers*, 6H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 166. 55, 145.40, 134.43, 127.10, 126.70, 45.70, 26.25.

Example 30 – Deacetylation of *tert*-Butyl 4-(acetamidomethyl)benzoate (Example 21) using the general procedure of Example 22.

Tert-Butyl 4-(aminomethyl)benzoate (29 mg, 70%) was isolated as a clear, colourless oil following the general procedure of Example 22.



LCMS (t_r= 0.79 min., ES+ 208 (M+H) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 – 7.78 (d, J = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 2H), 1.55 (s, 9H) ; ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.44, 144.13, 134.21, 129.03, 126.94, 80.53, 43.71, 27.88.

CLAIMS

1. A method for cleaving amide bonds, comprising:

a) providing a molecule comprising an amide group;

b) reacting the molecule comprising an amide group with a hydroxylamine salt to cleave the amide bond of the amide group.

2. The method according to claim 1, wherein the method further comprises:

c) recovering a product formed by the reaction of step b).

3. The method according to any one of the preceding claims, wherein the amide group is a primary, secondary or tertiary amide group, preferably a secondary amide group.

4. The method according to any one of the preceding claims, wherein the amide group is an N-acyl amide group, preferably an N-acetyl amide group.

5. The method according to any one of the preceding claims, wherein the molecule comprising an amide group further comprises a pH sensitive chiral center.

6. The method according to any one of the preceding claims, wherein the molecule comprising an amide group further comprises a pH sensitive protecting group.

7. The method according to any one of the preceding claims, wherein step b) comprises reacting the molecule comprising an amide group with the hydroxylamine salt at a temperature of 100 °C or less.

8. The method according to claim 7, wherein b) comprises reacting the molecule comprising an amide group with the hydroxylamine salt at a

temperature in the range of 10-90 °C, preferably 20-80 °C, preferably 30-70 °C, preferably 30-50 °C.

9. The method according to any one of the preceding claims, wherein step b) comprises reacting the molecule comprising an amide group with the hydroxylamine salt for 2-200 hours.

10. The method according to any one of the preceding claims, wherein step b) comprises reacting the molecule comprising an amide group with the hydroxylamine salt for 2-150 hours, preferably 5-150 hours, preferably 5-100 hours.

11. The method according to any one of the preceding claims, wherein the hydroxylamine salt is a salt formed by hydroxylamine and an acid selected from the group consisting of mineral acids and organic acids or mixtures thereof.

12. The method according to any one of the preceding claims, wherein the hydroxylamine salt is a salt formed by hydroxylamine and an acid selected from the group consisting of hydrochloric acid, hydroiodic acid, hydrobromic acid, acetic acid, propionic acid, pivalic acid, citric acid, oxalic acid, malonic acid, lactic acid, benzoic acid, and halogenated carboxylic acids, such as trifluoroacetic acid (TFA) and trichloroacetic acid or mixtures thereof.

13. The method according to any one of the preceding claims, wherein the hydroxylamine salt is a salt formed by hydroxylamine and an acid selected from the group consisting of hydrochloric acid, hydroiodic acid and hydrobromic acid, propionic acid, pivalic acid and trifluoroacetic acid or mixtures thereof.

14. The method according to any one of the preceding claims, wherein the hydroxylamine salt is a salt of hydroxylamine and hydroiodic acid or trifluoroacetic acid.

15. The method according to any one of the preceding claims, wherein the concentration of the hydroxylamine salt in step b) is in the range of 0.1-5 M.

16. The method according to any one of the preceding claims, wherein the reaction in step b) is performed in a solvent capable at least of at least partially dissolving the hydroxylamine salt.

17. The method according to any one of the preceding claims, wherein the reaction in step b) is performed in water or an aqueous solution.

18. The method according to any one of the preceding claims, wherein the reaction in step b) is performed at a pH value in the range of 4-12.

19. The method according to any one of the preceding claims, wherein the reaction in step b) is performed at a pH value in the range of 9-11.

20. The method according to claim 18, wherein the reaction in step b) is performed at a pH value in the range of 4-9, preferably in the range of 6-8.

21. The method according to claim 20, wherein the pH of the reaction is lowered to a value in the range of 4-9, preferably in the range of 6-8, by addition of a pH reducing agent.

22. The method according to claim 21, wherein the pH reducing agent is selected from the group consisting of mineral acids, organic acids and pH reducing salts, and combinations thereof.

23. The method according to claim 22, wherein the pH reducing agent is a mineral acid.

24. The method according to claim 23, wherein the pH reducing agent is selected from the group consisting of sulfuric acid, hydrochloric acid, hydroiodic acid, hydrobromic acid and phosphoric acid, and combinations thereof.

25. The method according to claim 22, wherein the pH reducing agent is an organic acid.

26. The method according to claim 25, wherein the pH reducing agent is selected from the group consisting of acetic acid, propionic acid, pivalic acid, citric acid, oxalic acid, malonic acid, lactic acid, benzoic acid, and halogenated carboxylic acids, such as trifluoroacetic acid and trichloroacetic acid, and combinations thereof.

27. The method according to claim 26, wherein the pH reducing agent is a halogenated carboxylic acid, preferably trifluoroacetic acid.

28. The method according to claim 22, wherein the pH reducing agent is a pH reducing salt.

29. The method according to claim 28, wherein the pH reducing agent is selected from the group consisting of ammonium chloride, ammonium bromide, ammonium iodide, hydroxylamine hydrochloride and hydroxylamine sulfate, and combinations thereof.

30. The method according to claim 29, wherein the pH reducing agent is selected from the group consisting of hydroxylamine hydrochloride or hydroxylamine sulfate, preferably hydroxylamine hydrochloride.

31. Use of a hydroxylamine salt for cleaving an amide bond in a molecule comprising an amide group.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/063029

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08B37/00 C08B37/08 C08L5/00 C08J3/075
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C08B C08L C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/081739 A2 (FRAUNHOFER GES FORSCHUNG [DE]; BREDEHORST REINHARD [DE]; HINTSCHE RAIN) 17 October 2002 (2002-10-17) the whole document in particular: abstract; page 5, line 13 - page 6, line 29; claims 1-38; figures 1-4; examples	1-31
X	US 2015/045573 A1 (CHENG WEIJUN [US] ET AL) 12 February 2015 (2015-02-12) the whole document in particular: abstract; [0008]-[0019]; claims 1-20; figures 1-13; examples	1-31

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2017	31/07/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ferreira, Roger

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/082078 A2 (FRAUNHOFER GES FORSCHUNG [DE]; BREDEHORST REINHARD [DE]; HINTSCHE RAIN) 17 October 2002 (2002-10-17) the whole document in particular: abstract; page 4, line 1 - page 7, line 9; claims 1-45; figures 1 and 2; examples	1-31
X	US 6 132 750 A (PERRIER ERIC [FR] ET AL) 17 October 2000 (2000-10-17) the whole document in particular: abstract, column 3, line 15 - column 11, line 10; claims 1-45; examples	1-31
X	WO 97/11958 A1 (SCRIPPS RESEARCH INST [US]; KENT STEPHEN B H [US]; MUIR TOM W [US]; DA) 3 April 1997 (1997-04-03) the whole document in particular: abstract; page 7, line 10 - page 14, line 20; claims 1-16; figures 1-33; examples	1-31
X	ZHU ET AL: "Selective cleavage of isoaspartyl peptide bonds by hydroxylamine after methyltransferase priming", ANALYTICAL BIOCHEMISTRY, ELSEVIER, AMSTERDAM, NL, vol. 364, no. 1, 31 March 2007 (2007-03-31), pages 1-7, XP022005959, ISSN: 0003-2697, DOI: 10.1016/J.AB.2007.02.013 the whole document in particular: abstract; materials and methods; results and discussion; figures 1-5; tables 1 and 2	1-31
X	E CANOVA-DAVIS ET AL: "Chemical heterogeneity as a result of hydroxylamine cleavage of a fusion protein of human insulin-like growth factor I", BIOCHEMICAL JOURNAL, vol. 285, no. 1, 1 July 1992 (1992-07-01), pages 207-213, XP055368110, GB ISSN: 0264-6021, DOI: 10.1042/bj2850207 the whole document in particular: abstract; materials and methods; results; figures 1-10; tables 1-3	1-31

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TSIGOS I ET AL: "Chitin deacetylases: new, versatile tools in biotechnology", TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 18, no. 7, 1 July 2000 (2000-07-01), pages 305-312, XP004908536, ISSN: 0167-7799, DOI: 10.1016/S0167-7799(00)01462-1 the whole document -----	1-30
A	EP 2 682 409 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 8 January 2014 (2014-01-08) the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/063029

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