Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 7057–7060

Controlling the outcome of overacylation of N-protected aminooxyacetic acid during the synthesis of an aminooxy-peptide for chemical ligation

Isidore P. Decostaire, Dominique Lelièvre, Haihui Zhang and Agnès F. Delmas*

Centre de biophysique moléculaire, UPR 4301 CNRS,† rue Charles Sadron, 45071 Orléans Cedex 2, France

Received 16 June 2006; revised 18 July 2006; accepted 20 July 2006
Available online 14 August 2006

Abstract—An aminooxy-containing peptide, the nucleophile partner for oxime ligations, is usually grafted on a NH_2-peptide resin by activating a protected aminooxyacetic acid as an active ester. Here, we have shown that its subsequent coupling to NH_2-peptide resin competes with the overacylation of the –NH–O– nitrogen and that the overacylation level increases with the basicity of the reaction mixture. Moreover, we found that overacylation is prevented when the COOH of the Aoa-derivatives is engaged in an amide bond.

Oxime ether chemistry has recently attracted attention as a convenient approach to the rapid formation of combinatorial libraries,[1] as well as to the chemoselective ligation of protein-like polypeptides as developed by Rose.[2] The oxime bond is generated by an addition–elimination between an electrophile carbonyl-containing compound such as aldehyde or ketone and an aminooxy nucleophile.[3] In chemical peptide and protein syntheses, the oximation reaction is chemoselective as aminooxy derivatives are weak bases although they are reactive nucleophiles toward carbonyl groups due to the α-effect of the neighboring heteroatom. The reaction proceeds under mild acidic conditions[4] at which the basic side chain of lysine, arginine and α-NH_2 are protonated. The oximation reaction has proven to be very efficient for the synthesis of compounds such as the following: polypeptides,[5] multimer compound for receptor targeting,[6] or vaccine design,[7] cyclic peptides,[8] glycopeptides,[9] and oligonucleotide–peptide conjugates.[10] It is also efficient for grafting a probe to microarray,[11] and for introducing a prosthetic group to a peptide.[12] Oximes are stable in a wide range around the physiological pH.[13] As the formation of oxime bonds in chemoselective ligation procedures has become a most efficient tool, the insertion of appropriate functionalities in each partner has been steadily growing. The introduction of an aldehyde and a ketone group has recently been reviewed.[14] The introduction of an aminooxy group is usually carried out using the commercially available *N*-Boc protected aminooxy acetic acid[15] (Aoa) and a few syntheses of a modified amino acid bearing an aminooxy group on its side chain.[16] However, side reactions such as overacylation may occur on the –NH–O– nitrogen leading to an Aoa(Aoa)-peptide as a by-product.[8a,17] The use of diprotected Aoa derivatives such as *N*,*N*-di-Boc protected Aoa (Boc_2–Aoa–OH)[17] and phthaloyl protected Aoa[18] prevents overacylation from occurring. Moreover, the use of a hindered and nonelectron withdrawing group such as trityl (Trt)[8a] versus an electron withdrawing group such as carbamate (Boc) as *N*-Aoa protecting group is also useful to prevent overacylation. Throughout these reports, the coupling step was performed using various coupling reagents.

Abbreviations: Aloc, allyloxycarbonyl; Aoa, aminooxyacetic acid; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, 2-(1*H*-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HCTU, 5-chloro-2-(-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

Keywords: Aminooxy group; Overacylation; Coupling reagent; Peptide synthesis; Chemoselective ligation.

† Affiliated with the University of Orléans and INSERM.
* Corresponding author. Tel.: +33 2 38 25 55 77; fax: +33 2 38 63 15 17; e-mail: delmas@cnrs-orleans.fr

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.07.092

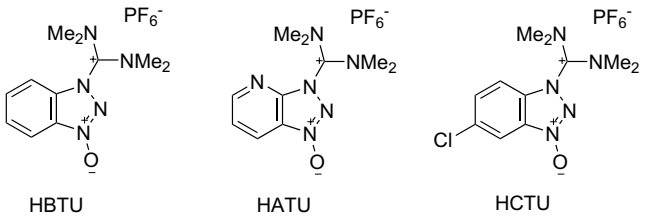
Reasoning that the α-effect of the oxygen atom not only affects the nucleophile but also the basic character of the neighboring amino-group with an increase in its acid character, we envisioned that the basic conditions during the coupling step would enhance the overacylation process. This hypothesis was considered using an Aoa derivative protected with a nonhindered withdrawing group such as allyloxycarbonyl (Aloc) by which overacylation should be maximized. To introduce Aloc–Aoa–OH, protocols were carried out using several coupling agents under different conditions such as concentration, reaction time, and presence of base.

Aloc–Aoa–OH[19] was coupled to the α-NH_2 of the peptide resin resulting from the elongation of the model peptide AGAG using a Wang resin and the Fmoc/*t*-Bu strategy (Table 1). The peptide was released with TFA, H_2O, 95/5. After TFA evaporation under vacuum, the peptide was solubilized in H_2O (1 mg/ml) and analyzed by HPLC and mass spectrometry. The purity of crude peptides was estimated by integration of the HPLC profiles.

Using HBTU[20] (4 equiv) as coupling reagent with DIEA (10 equiv), that is, a 2.5-fold excess of base, the introduction of Aloc–Aoa–OH led to a quantitative acylation over 30 min but with 40% of the overacylated form (Table 1, entry 1). With a lower excess of DIEA (1.25-fold), the overacylated form was only 24% (Table 1, entry 2). To further demonstrate the effect of the base, HBTU was used with the hindered base collidine (2,4,6-trimethylpyridine, pK_a 7.4), a weaker base than DIEA (pK_a 10.1). Whatever the excess of collidine used, the formation of the overacylated product was prevented (Table 1, entries 3 and 4). However, the acylation reaction was less effective than when DIEA was used (Table 1, entries 4 vs 1 and entries 3 vs 2). This observation is in agreement with the aminium/uronium salt activation of a hindered amino acid, which is optimal in the presence of DIEA versus collidine.[21]

In order to determine when overacylation occurs, that is, on the Aoa derivative in solution or on the Aoa derivative already grafted to the peptide resin, a double coupling was performed (Table 1, entry 5). No increase in the overacylation level was observed under these conditions. This suggests that the acylation of NH_2-peptide resin competes with the acylation of –NH–O– of the Aoa-derivative. In other words, the overacylated compound Aloc–Aoa(Aloc–Aoa)–OBt must be first formed in solution and then transferred on the H_2N-peptide resin before acylation is completed. Despite the well-known lability of OBt esters, we were pleased to characterize Aloc–Aoa(Aloc–Aoa)–OBt in the activation mixture (see Supplementary data). Consequently, the nucleophilicity of the nitrogen of the Aloc–Aoa–OH species changes and depends on the bond in which the carbonyl is engaged, the amide bond preventing the overacylation to occur. This might explain why the overacylation process has not been observed with amino acids that bear the oxyamine on the side chain.[16] During the activation of the COOH, the proton of the –NH–O– of the Aoa derivative becomes more labile, to the point of being abstracted by increasing the basicity of the reaction mixture. Hence, two ways can be pursued to reduce the overacylation process, that is, driving the coupling reaction as rapidly as possible to the formation of the Aloc–Aoa-peptide resin and/or diminishing the efficient basicity of the reaction mixture.

To increase the rate of the coupling reaction, HATU,[22] the more powerful aminium/uronium-based coupling reagent, was used to activate Aloc–Aoa–OH as well as the new HCTU[23] (Scheme 1, Table 2) whose HOBt-derivative moiety is as acidic as that of HATU (pK_a: 3.28 and 3.35 for HOAt and Cl-HOBt, respectively, vs 4.60 for HOBt). The use of HCTU led to 35% of the overacylation level (Table 2, entry 1) whereas the use of HATU led to 19% (Table 2, entry 2). This indicates that the neighboring group effect of the N of the pyridine ring of OAt active ester[22] does intervene in the coupling reaction affording more rapidly the amide form to the detriment of Aloc–Aoa(Aloc–Aoa)–OBt. Best results were obtained with HATU and a 1.25-fold excess of collidine, conditions under which no overacylation



Scheme 1.

Table 1. Acylation of N-terminal-amine of *H*-AGAG-Wang-resin with Aloc–Aoa–OH using HBTU as coupling reagent

	Aloc–Aoa–OH	Coupling reagent	Base	Time (min)	H_2N~~ (%)[a]	Aloc–Aoa–NH~~ (%)[a]	Aloc–Aoa(Aloc–Aoa)–NH~~ (%)[a]
Single coupling							
1	4 equiv	HBTU 4 equiv	DIEA 10 equiv	30	0	60	40
2	4 equiv	HBTU 4 equiv	DIEA 5 equiv	30	5	71	24
3	4 equiv	HBTU 4 equiv	Collidine 5 equiv	30	17	83	0
4	4 equiv	HBTU 4 equiv	Collidine 10 equiv	30	6	94	0
Double coupling							
5	4 equiv	HBTU 4 equiv	DIEA 10 equiv	30	0	58	42
	+4 equiv	+HBTU 4 equiv	+DIEA 10 equiv	30	0	60	40

[a] Estimated by integration of the HPLC peaks at 214 nm.

Table 2. Acylation of N-terminal amine of *H*-AGAG-Wang-resin with Aloc-Aoa-OH using HATU and HCTU as coupling reagents

	Aloc–Aoa–OH	Coupling reagent	Base	Time (min)	H_2N~ (%)[a]	Aloc–Aoa–NH~ (%)[a]	Aloc–Aoa(Aloc–Aoa)–NH~ (%)[a]
1	4 equiv	HCTU 4 equiv	DIEA 10 equiv	30	0	65	35
2	4 equiv	HATU 4 equiv	DIEA 10 equiv	30	0	81	19
3	4 equiv	HATU 4 equiv	Collidine 5 equiv	30	4	96	0
4	4 equiv	HATU 4 equiv	Collidine 10 equiv	30	5	95	0
5	4 equiv	HATU 4 equiv	Collidine 5 equiv	60	3	97	0

[a] Estimated by integration of the HPLC peaks at 214 nm.

Table 3. Acylation of N-terminal amine of *H*-AGAG-Wang-resin with Aloc–Aoa–OH using a carbodiimide as coupling reagent

	Aloc–Aoa–OH	Coupling reagent	Time (min)	H_2N~ (%)[a]	Aloc–Aoa–NH~ (%)[a]	Aloc–Aoa(Aloc–Aoa)–NH~ (%)[a]
Single coupling						
1	5 equiv	DCC/HOBt 5 equiv	60	24	73	3
2	5 equiv	DCC/HOBt 5 equiv	120	2	94	4
3	5 equiv	DCC/HOBt 5 equiv	150	1	94	5
4	4 equiv	DCC/HOBt 4 equiv	120	4	96	0
5	10 equiv	DCC/HOBt 10 equiv	120	2	94	4
Double coupling						
6	5 equiv	DCC/HOBt 5 equiv	60	24	73	3
	+3 equiv	+DCC/HOBt 3 equiv	+60	4	93	3

[a] Estimated by integration of the HPLC peaks at 214 nm.

was observed and only 4% of the nonacylated form (Table 2, entry 3). This incomplete acylation is quite acceptable since the acylation yield was good and no by-product could react with an aldehyde-containing peptide during the oximation reaction. No overacylation was observed even when a 2.5-fold excess of collidine or an increased reaction time was used (Table 2, entries 4 and 5).

Since the reactivity of –NH–O– toward overacylation increases as the basicity of the amine added to the aminium/uronium coupling reagent increases, it would be interesting to activate Aloc–Aoa–OH with dicylohexylcarbodiimide (DCC), a coupling reagent, which does not require the presence of an additional tertiary base to activate the carboxylic acid.[24] In this work, DCC was combined with HOBt,[25] an acidic nucleophile additive (Table 3). Provided that the reaction time and the equivalents of the coupling reagent were optimized, acylation was very efficient with a minimal level of overacylation. The best compromise was obtained using 4 equiv of DCC/HOBt over 120 min (Table 3, entry 4), which afforded no significant overacylated peptide (<1%) and less than 5% of the nonacylated peptide.

In summary, the conditions for coupling Aoa-derivatives were investigated in order to avoid overacylation of the NH–O group. We found that overacylation is prevented when the COOH of Aoa-derivatives is engaged in an amide bond. Coupling of aminooxyacetic acid protected with a nonhindered carbamate-based group is effective without the overacylation side reaction when using dicyclohexylcarbodiimide as the coupling reagent and HOBt as an acidic additive. Overacylation, favored by the use of uronium/aminium coupling reagents in the presence of DIEA, can be completely circumvented using a weaker base such as collidine. These data highlight the relationship between basic properties of the reaction mixture and the overacylation process of the *N*-Aloc protected aminooxyacetic acid.

Acknowledgments

This work was supported by a grant from the Ligue Nationale contre le cancer (section Centre). We are also grateful to Dr. Corinne Buré and Mr. Rémi Puppo for recording most of the MS spectra.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.07.092.

References and notes

1. (a) Nazarpack-Kandlousy, N.; Nelen, M. I.; Goral, V.; Eliseev, A. V. *J. Org. Chem.* **2002**, *67*, 59–65; (b) Renaudet, O.; Reymond, J. L. *Org. Lett.* **2003**, *5*, 4693–4696.
2. Rose, K. *J. Am. Chem. Soc.* **1994**, *116*, 30–33.
3. Jencks, W. P. *J. Am. Chem. Soc.* **1959**, *81*, 475–480.
4. Sayer, J. M.; Pinsky, B.; Schonbrunn, A.; Washtien, W. *J. Am. Chem. Soc.* **1974**, *96*, 7998–8009.
5. Canne, L. E.; Ferré d'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *117*, 2998–3007.
6. (a) Thumshirn, G.; Hersel, U.; Goodman, S. L.; Kessler, H. *Chem. Eur. J.* **2003**, *9*, 2717–2725; (b) Boturyn, D.; Coll, J. L.; Garanger, E.; Favrot, M. C.; Dumy, P. *J. Am. Chem. Soc.* **2004**, *126*, 5730–5739.

7. (a) Nardin, E. H.; Calvo-Calle, J. M.; Oliveira, G. A.; Nussenzweig, R. S.; Schneider, M.; Tiercy, J. M.; Loutan, L.; Hochstrasser, D.; Rose, K. *J. Immunol.* **2001**, *166*, 481–489; (b) Cremer, G. A.; Bureaud, N.; Lelièvre, D.; Piller, V.; Piller, F.; Delmas, A. *Chem. Eur. J.* **2004**, *10*, 6353–6360.
8. (a) Wahl, F.; Mutter, M. *Tetrahedron Lett.* **1996**, *37*, 6861–6864; (b) Roberts, K. D.; Lambert, J. N.; Ede, N. J.; Bray, A. M. *J. Pept. Sci.* **2004**, *10*, 659–665.
9. Hang, H. C.; Bertozzi, C. R. *Acc. Chem. Res.* **2001**, 727–736.
10. Zatsepin, T. S.; Stetsenko, D. A.; Gait, M. J.; Oretskaya, T. S. *Bioconjugate Chem.* **2005**, *16*, 471–489.
11. Salisbury, C. M.; Maly, D. J.; Ellman, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 14868–14870.
12. Marcaurelle, L. A.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 7279–7282.
13. (a) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893–3899; (b) Rose, K.; Zeng, W.; Regamey, P. O.; Chernushevich, I. V.; Standing, K. G.; Gaertner, H. F. *Bioconjugate Chem.* **1996**, *7*, 552–556.
14. Melnyk, O.; Fehrentz, J. A.; Martinez, J.; Gras-Masse, H. *Biopolymers* **2000**, *55*, 165–186.
15. Offord, R. E.; Gaertner, H. F.; Wells, T. N.; Proudfoot, A. E. *Methods Enzymol.* **1997**, *287*, 348–369.
16. (a) Lang, I.; Donzé, N.; Garrouste, P.; Dumy, P.; Mutter, M. *J. Pept. Sci.* **1998**, *4*, 72–80; (b) Spetzler, J. C.; Hoeg-Jensen, T. *J. Pept. Sci.* **1999**, *5*, 582–592.
17. Brask, J.; Jensen, K. J. *J. Pept. Sci.* **2000**, *6*, 290–299.
18. (a) Yang, D.; Li, B.; Ng, F.-F.; Yan, Y.-L.; Qu, J.; Wu, Y.-D. *J. Org. Chem.* **2001**, *66*, 7303–7312; (b) Leu, M.-R.; Lee, J.; Baek, B.-H.; Shin, I. *Synlett* **2003**, *3*, 325–328.
19. To a stirred solution of Aoa (2 g, 18.3 mmol) in 0.85 M Na_2CO_3 (43 ml) at 0 °C was added AlocOSu (3.09 g, 15.5 mmol) in DMF (100 ml) in one portion. Mixing was continued at room temperature for 30 min. H_2O was added until dissolution of the precipitate. The solution was then extracted with diethylether (3×) and ethyl acetate (3×). The aqueous phase was cooled, acidified at pH 2 with 5% citric acid and extracted with ethyl acetate (3×). This organic phase was washed with brine (3×), H_2O (3×), dried with Na_2SO_4, and evaporated to dryness. TLC (silica gel, $CHCl_3$/EtOH/AcOH 85:10:5, rf: 0.5). The residue was purified by semi-preparative HPLC. Overall yield: 55% Analytical HPLC (column C18, 300 Å, 4.6×250) Tr = 13.2 min (3–30% B in 30 min with A = H_2O/0.1% TFA and B = MeCN/0.1% TFA) NMR DMSO-d_6: δ (ppm): 12.85 (s, 1H, COO*H*); 10.55 (s, 1H, CO-N*H*–O); 5.9 (m, 1H, $H_2C{=}CH{-}CH_2$); 5.2–5.3 (dd, 2H, $J_{trans} = 17.22$ Hz, $^2J = 1.5$ Hz, $J_{cis} = 10{,}49$ Hz; $H_2C{=}CH{-}CH_2$–O); 4.55 (d, 2H, $J = 5.35$ Hz, $H_2C{=}CH{-}CH_2$–O); 4.3 (s, 2H, O–CH_2–COOH). ESI, MH^+: 176.0, calcd for $C_6H_{10}O_1N_5$ 176.14.
20. Dourtouglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, *7*, 572–574.
21. Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. *J. Org. Chem.* **1998**, *63*, 9678–9683.
22. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
23. (a) Israeli Patent Application No: IL141804; (b) Marder, O.; Shvo, Y.; Albericio, F. *Chim. Oggi* **2002**, *20*, 37–41.
24. DeTar, D. F.; Silverstein, R. *J. Am. Chem. Soc.* **1966**, *88*, 1020–1023.
25. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.