Synthesis of N-urethane protected β-amino alcohols employing *N*-(protected-α-aminoacyl)benzotriazoles

Vommina V. Sureshbabu*, N.S. Sudarshan, L. Muralidhar and N. Narendra

Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr. B. R. Ambedkar Veedhi, Bangalore 560 001, India

A simple and racemisation-free synthesis of *N*-urethane protected α-amino/peptidyl alcohols by the reduction of the corresponding easily accessible *N*-acylbenzotriazoles is described. The method is practical, straightforward, fast and efficient for the synthesis of amino/peptidyl alcohols. All the alcohols made were isolated in high yields and purity.

Keywords: β-aminoalcohols, *N*-protecting groups, amino acids, benzotriazoles, sodium borohydride, reduction

N-Protected amino alcohols and peptidyl alcohols are important synthetic intermediates,[1] especially useful in the synthesis of amino/peptidyl aldehydes which have diverse synthetic as well as biological applications.[2,3] Several peptides including enkephalins have been shown to exhibit better biological activity upon reduction of the terminal carboxylic group into the corresponding alcohol.[4] The N-protected amino alcohols are also used in the synthesis of peptides possessing reduced peptide bonds[5] and in the preparation of stereochemically defined methylene-oxydipeptides.[6] They are also key intermediates in the synthesis of vicinal diamines, ureidopeptides,[7] oxazolidinones, peptidosulfinamides, and peptidosulfonamides.[8] Moreover, oligopeptidyl carbamates[9] as well as peptidyl carbonates are assembled starting from β-aminoalcohol building blocks.

The aminoalcohols are widely prepared by borane-mediated reduction of N-protected amino acids.[3] They are also synthesised by the reduction of the corresponding alkyl esters or active esters[10] with $NaBH_4$. Kokotos[11a] and Rodriguez *et al*.[11b] have reported the chemoselective reduction of mixed carboxylic anhydrides generated by the reaction of N-protected amino acid with ethyl chloroformate or isobutyl chloroformate in presence of a base. Similarly, urethane-protected *N*-carboxyanhydrides (UNCAs)[12] and various acid fluorides[13] are also reduced into alcohols using $NaBH_4$. On the other hand, there are several reports describing the use of reducing agents such as $LiAlH_4$,[14] DIBAL,[15] *etc.,* for such reductions.

Katritzky *et al*. have accomplished pioneering work on the synthesis of stable *N*-acylbenzotriazoles and they demonstrated their wide range of applications in organic synthesis.[16] In peptide chemistry, *N*-acylbenzotriazole derivatives of amino acids have been used to acylate unprotected amino acids under aqueous reaction conditions to obtain peptide acids,[17] prepare N-protected α-amino acid azides,[18] hydroxamic acids and peptide heterocycles such as oxadiazoles.[19] The utility of *N*-acylbenzotriazoles as activated precursors for the synthesis of aminoalcohols is to the best of our knowledge yet to be demonstrated.

The present work describes the simple and selective reduction of *N*-t-butoxycarbonyl (Boc)/benzyloxycarbonyl (Z)/9-fluorenylmethoxycarbonyl (Fmoc)/1,1-dioxobenzo-thiophen-2-ylmethyloxycarbonyl (Bsmoc) amino acid-derived acylbenzotriazoles using $NaBH_4$ to form the corresponding alcohols. During peptide synthesis, the amino group is usually protected with any one of the urethane type groups, Boc, Z, Fmoc and Bsmoc which differ from one another in their deprotection conditions. Boc and Z groups are deprotected using acidolysis whereas Fmoc and Bsmoc groups are deprotected using an organic base. We herein describe a general route for the reduction of α-amino acids carrying with all four commonly employed amine protecting groups.

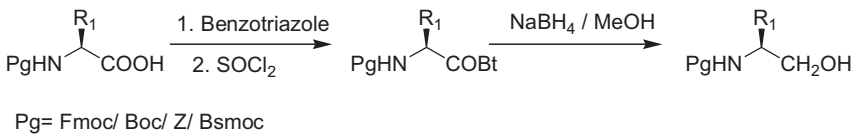
Results and discussion

N-Protected amino acylbenzotriazoles are accessible in excellent yields by reacting the corresponding amino acid or peptide acid with a solution of benzotriazole pretreated with $SOCl_2$.[17] In a typical procedure, the *N*-acylbenzotriazoles were treated with $NaBH_4$ in MeOH at room temperature to accomplish their conversion to the corresponding alcohol. The reduction was found to be very fast, being complete within 2 min. In many cases, the product separated out as a solid after the reaction. Consequently, the reaction mixture was diluted with water and the products were filtered and isolated. A regular aqueous workup method was also followed to isolate the products which could not be precipitated out from the reaction mixture (Scheme 1).

All the protected amino alcohols were obtained in excellent yield (95–99%). In the case of bifunctional amino acids the side chain protecting group such as tertiary butyl ester of Asp/Glu, a benzyl ether linkage on Ser/Thr remained unaffected during reduction. In order to demonstrate the versatility of this protocol, a series of Fmoc, Boc, Z and Bsmoc protected amino acids were converted into the corresponding alcohols (Table 1).

The protocol was then extended to the synthesis of N-protected peptide alcohols. The peptide acids were synthesised by coupling with *O,N*-bis-trimethylsilyl amino acid with mixed anhydride of N-protected amino acid.[20] They were then converted into the corresponding acylbenzotriazole derivatives and further reduced to the corresponding alcohols using $NaBH_4$ following the same procedure (Scheme 2).

The reaction was also tested for racemisation by recording the ^1H NMR spectra of compounds **4** and **5** (Table 1) synthesised via the present protocol. Compound **4** contained a doublet at δ 1.163, 1.180 while its epimer **5** showed the



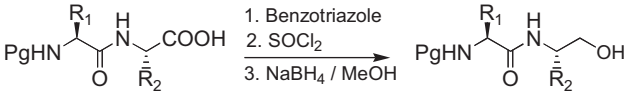
Pg= Fmoc/ Boc/ Z/ Bsmoc

Scheme 1 Synthesis of *N*-protected β-aminoalcohols from *N*-(Pg-α-aminoacyl)benzotriazoles.

* Correspondent. Email: haricch@rediffmail.com

Table 1 Preparation of *N*-protected amino and peptide alcohols

Compd	Protected amino or peptide alcohol	Yield/%	M.p./°C	$[\alpha]^{24}_D$ (c = 1, $CHCl_3$)	ES-MS $(M + 1)^+$
1	Fmoc-Leu-ol	94	131	–20.8	340
2	Fmoc-Asp(O^{t}Bu)-ol	90	97	–6.5	398
3	Fmoc-Phg-Phe-ol	91	137	–24.6	507
4	Fmoc-L-Phg-Ala-ol	89	126	–20.6	431
5	Fmoc-D-Phg-Ala-ol	91	128	+21.1	431
6	Boc-Phe-ol	89	94	–21.6	252
7	Boc-Thr(Bzl)-ol	90	oil	+12.0	308
8	Boc-Ser(Bzl)-ol	86	58	+12.1	296
9	Boc-Asp(OBzl)-Ala-ol	81	80	–11.6	381
10	Z-Phe-ol	94	88	–12.1	286
11	Z-Asp(ol)OBzl	82	gum	–29.1	344
12	Z-Glu(OMe)-ol	91	69	–18.2	282
13	Z-Ala-Ala-ol	86	144	–29.4	281
14	Bsmoc-Ala-ol	70	gum	–5.45	298
15	Bsmoc-Phe-ol	72	gum	–30.1	374



Pg= Fmoc / Boc / Z / Bsmoc

Scheme 2 Synthesis of alcohols from *N*-(Pg-α-aminopeptidyl)benzotriazoles.

peaks at δ 1.179, 1.196. The equimolar mixture of **4** and **5,** intentionally mixed**,** had two doublets at the different values δ 1.161, 1.174, 1.181, 1.194. This clearly demonstrated that the method described to make β-amino alcohols is racemisation-free.

In conclusion, we have developed an efficient method for the conversion of N-urethane protected amino acids/peptide acids into the corresponding β-amino alcohols using *N*-acylbenzotriazoles. The reduction is rapid, high yielding and proceeds with no side reactions or racemisation. Common side chain protecting groups remain unaffected. The present method is advantageous as it utilises acylbenzotriazoles which are easy to make and stable to store as C-activated precursors. Further, since the benzotriazoles of all common N-protecting groups can be prepared as shelf stable solids in good yield, the current method becomes a general protocol for reduction of N-urethane protected amino acids and peptide acids.

Experimental

Melting points were determined by the capillary method. IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer (KBr pellets). Specific rotations were recorded on a Rudolf Research Autopol IV automatic polarimeter. NMR spectra were measured on a Bruker AMX 400 MHz spectrometer. ES-MS spectra were obtained from an ES-MS (HP 1100 series, MSD single quadrupole) instrument. Elemental analyses were recorded using Perkin Elmer Analyser and the samples were dried under vacuum before analysis. The TLC analysis was carried out on precoated silica gel plates using the solvent system ethyl acetate: hexane (35: 65 v/v). All the solvents were freshly distilled prior to use.

General procedure for the preparation of N-Fmoc/Boc/Z/Bsmoc-β-amino alcohols

To the *N*-(Fmoc/Boc/Z/Bsmoc-α-aminoacyl)benzotriazole (10 mmol) in methanol was added 6.0 mmol (0.22 g) of $NaBH_4$ and the mixture was stirred at room temperature for 2–5 min. Upon completion of the reaction, as evident by TLC, the reaction mixture was diluted with excess of water. On precipitation of the product, which is common with Fmoc-protected alcohols, the product was filtered, washed with 10% citric acid, water and dried. In other cases, the product was extracted into ethyl acetate. The organic layer was washed with 10% citric acid followed by water and brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to obtain the compound as a white solid. The melting points of the products are listed in Table 1.

Fmoc-Leu-ol (**1**): NMR ($CDCl_3$): δ_H 0.93 (d, 6H, *J* = 5.4 Hz), 1.33 (m, 2H), 1.63 (m, 1H), 2.34 (br s, 1H), 3.55 (br m, 2H), 3.77 (m, 1H), 4.19 (t, 1H, *J* = 6.6 Hz), 4.41 (m, 2H), 5.08 (d, 1H, *J* = 8.8 Hz), 7.27–7.40 (m, 4H), 7.56 (d, 2H), 7.77 (d, 2H); δ_C 22.0, 23.0, 24.6, 40.3, 47.2, 51.2, 65.5, 66.4, 119.8, 124.9, 126.9, 127.6, 141.2, 143.8, 156.7. Anal. Calcd for $C_{21}H_{25}NO_3$: C, 74.31, H, 7.42, N, 4.13, Found, C, 74.30, H, 7.38, N, 4.09%.

Fmoc-Phg-Phe-ol (**3**): ^{1}H NMR (*δ,* $CDCl_3$): 2.07 (s, 1H), 2.87 (d, 2H, *J* = 6.4 Hz), 3.60 (m, 2H), 3.96 (m, 1H), 4.16 (t, 1H, *J* = 6.80 Hz), 4.4 (m, 3H), 5.91 (s, 1H), 7.10–7.40 (m, 14H), 7.56 (m, 2H), 7.77 (d, 2H, *J* = 7.6 Hz): δ_C 37.3, 47.2, 54.1, 64.0, 66.7, 68.5, 120.0, 124.7, 125.0, 126.2, 126.5, 126.9, 127.0, 127.2, 128.2, 130.8, 132.7, 139.1, 141.5, 143.9, 156.4, 166.7. Anal. Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87, H, 5.97, N, 5.53, Found, C, 75.81, H, 5.98, N, 5.50%.

Boc-Ser(Bzl)-ol (**8**): NMR ($CDCl_3$): δ_H 1.40 (s, 9H), 3.50–3.95 (m, 5H), 4.48 (s, 2H), 7.35 (s, 5H); δ_C 28.2, 59.4, 65.4, 67.1, 73.7, 81.1, 128.1, 128.0, 128.1, 128.3, 138.1, 156.1. Anal. Calcd for $C_{15}H_{23}NO_4$: C, 64.03, H, 8.24, N, 4.98, Found, C, 64.00, H, 8.30, N, 4.94%.

Z-Phe-ol (**10**): NMR ($CDCl_3$): δ_H 2.91 (d, 2H), 2.80 (br, H), 3.55 (m, 2H), 3.71 (br, 1H), 5.05 (s, 2H), 5.61 (s, 1H), 7.30 (s, 5H), 7.35 (s, 5H); δ_C 37.2, 54.1, 62.6, 64.0, 127.2, 127.57, 127.75, 128.10, 128.5, 136.5, 136.9, 156.6. Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.56, H, 6.71, N, 4.91, Found, C, 71.50, H, 6.77, N, 4.99%.

Bsmoc-Ala-ol (**14**): NMR ($CDCl_3$): δ_H 1.25 (3H, d, *J* = 7.2 Hz), 3.32 (1H, m), 3.71 (2H, d, *J* = 6.9 Hz), 5.10 (2H, s), 5.54 (1H, s), 7.15 (1H, s), 7.34 (1H, d, *J* = 7.0 Hz), 7.49 (2H, m), 7.71 (1H, d, *J* = 7.2 Hz); δ_C 17.4, 47.1, 66.3, 67.2, 121.3, 125.8, 126.3, 127.8, 130.6, 134.1, 137.0, 156.9. Anal. Calcd for $C_{13}H_{15}NO_5S$. C, 52.51, H, 5.09, N, 4.71, Found, C, 52.79, H, 5.10, N, 4.80%.

Bsmoc-Phe-ol (**15**): NMR ($CDCl_3$): δ_H 2.93 (2H, d, *J* = 4.9 Hz), 3.48 (2H, d(d)), 3.87 (1H, m), 5.1 (2H, s), 7.15 (1H, s), 7.33 (1H, d, *J* = 6.9 Hz), 7.49 (2H, m), 7.71 (1H, d, *J* = 7.1 Hz); δ_C 37.9, 47.3, 52.7, 66.9, 121.3, 125.8, 126.3, 127.8, 128.5, 128.6, 129.8, 130.6, 134.1, 137.0, 139.6, 140.6, 156.9. Anal. Calcd for $C_{19}H_{19}NO_5S$. C, 61.11, H, 5.13, N, 3.75, Found, C, 61.19, H, 5.18, N, 3.81%.

Received 27 August 2007; accepted 23 December 2007
Paper 07/4808 doi: 10.3184/030823407X272985

References

1 (a) J.-M. Cassal, A. Furst and W. Meier, *Helv. Chim. Acta,* 1976, **59**, 1917; (b) S. Thaisrivongs, D.T. Pals, W.M. Kati, S.R. Turner, L.M. Thomosco and W. Watt, *J. Med. Chem.,* 1986, **29**, 2080; (c) R.H. Schlessinger and E.J. Iwanowicz, *Tetrahedron Lett.,* 1987, **28**, 2083.
2 (a) R. Thompson, *Methods Enzymol.,* 1977, **46**, 220; (b) R.P. Sharma, M.G. Gore and M. Akhtar, *J. Chem. Soc., Chem. Commun,* 1979, 875.
3 C.F. Stanfield, J.E. Parker and P. Kanellis, *J. Org. Chem.,* 1981, **46**, 4797.
4 J. Pless, W. Bauer, F. Cordinaux, A. Closse, D. Hauser, R. Huguenia, D. Roemer, H.-H. Buescher and R.C. Hill, *Helv. Chim. Acta,* 1979, **62**, 398.
5 J. Martinez, J.P. Bali, M. Rodriguez, B. Castro, R. Magous, J. Laur and M.F. Lignon, *J. Med. Chem.,* 1985, **28**, 1874.
6 R.E. Ten Brink, *J. Org. Chem.,* 1987, **52**, 418.
7 (a) A. Boeijen, J.V. Ameijde and R.M.J. Liskamp, *J. Org. Chem.,* 2001, **66**, 8454; (b) G. Guichard, V. Sometey, C. Didierjean, A. Aubry, J.P. Briand and M. Rodriguez, *J. Org. Chem.,*1999, **64**, 8702.
8 M.C.F. Monnee, M.F. Marijne, A.J. Brouwer and R.M.J. Liskamp, *Tetrahedron Lett.,* 2000, **41**, 7991.

9 C.Y. Cho, R.S. Youngquist, S.J. Paikoff, M.H. Beresini, A.R. Hebert, L.T. Berleau, C.W. Liu, D.E. Wenner, T. Keough and P.G. Schultz, *J. Am. Chem. Soc.,* 1998, **120**, 7706.
10 (a) K. Soai, H. Oyamada and M. Takase, *Bull. Chem. Soc. Jpn,* 1984, **57**, 2327; (b) Y. Hamada, M. Shibata, T. Sugiura, S. Kato and T. Shioiri, *J. Org. Chem.,* 1987, **52**, 1252; (c) J. Nitakawa and T. Shiba, *Chem.. Lett.,* 1979, 981.
11 (a) G. Kokotos, *Synthesis*, 1990, 299; (b) M. Rodriguez, M. Llinares, S. Doulut, A. Heitz and J. Martinez, *Tetrahedron Lett.,* 1991, **32**, 923.
12 J.-A. Fehrentz, J.-C. Califano, M. Amblard, A. Lottet and J. Martinez, *Tetrahedron Lett.,* 1994, **35**, 569.
13 G. Kokotos and C. Noula, *J. Org. Chem.*, 1996, **61**, 6994.
14 P. Karrer and M. Suter, *Helv. Chim. Acta,* 1948, **32**, 1156.
15 A. Ito, R. Takahashi and Y. Baba, *Chem. Pharm. Bull.,* 1975, **23**, 3081.
16 A.R. Katritzky, X. Lan, J.Z. Yang and O.V. Denisko, *Chem. Rev.*, 1998, **98**, 409.
17 A.R. Katritzky, P. Angrish, D. Hur and K. Suzuki, *Synthesis*, 2005, **3**, 397.
18 A.R. Katritzky, K. Widyan and K. Kirichenko, *J. Org. Chem.,* 2007, **72**, 5802.
19 (a) A.R. Katritzky, N. Kirichenko and B.U. Rogovoy, *Synthesis*, 2003, 2777; (b) A.R. Katritzky, A.A. Shestopalov and K. Suzuki, *Arkivoc* 2005, vii, 36.
20S.J. Tantry and V.V. Sureshbabu, *Indian J. Chem.,* 2004, **43B**, 1282