Journal of Peptide Science
J. Peptide Sci. **5**: 56–58 (1999)

Short Communication

Efficient One-Pot Synthesis of N-Ethyl Amino Acids

THOMAS RÜCKLE, BENOIT DUBRAY, FRANCIS HUBLER and MANFRED MUTTER*

Institute of Organic Chemistry, University of Lausanne, Lausanne, Switzerland

Abstract: Mono-N-ethylated α-amino acid esters are obtained in high yields using reductive amination procedures. Formation of imine is achieved by excess of acetaldehyde, followed by removal of acetaldehyde and reduction by $NaBH(OAc)_3$. The elaborated one-pot synthesis allows for the efficient synthesis of side-chain protected amino acid derivatives. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: N-alkylation; amino acid derivatives; reductive amination; N-ethyl amino acids

N-alkyl amino acids are constituents of various naturally occurring peptides and depsipeptides such as cyclosporin, dolastatin and didemnin, isolated from marine species or microorganisms [1]. The common features of this interesting class of biomolecules are enhanced proteolytic stability, higher lipophilicity and unique conformational properties, resulting in pronounced biological effects, including antibiotic [2], anticancer [3], antiviral [4] and immunosuppressive activity [5]. Although a number of methods for the synthesis of N-methyl amino acids [6–12], as well as N-alkyl amino acids have been reported in the past [13,14], only a few examples for the synthesis of N-ethyl amino acid derivatives are described [15,16].

In general, classical procedures fail due to β-elimination of the ethyl derivative when applying an anionic mechanism [17]. N-ethylation via reductive amination appeared to be a more proposing approach [18]. The amine reacts with an aldehyde and the so formed imine is reduced by commonly available reducing agents like $NaBH_4$, $NaBH_3CN$, $NaBH(OAc)_3$.

Reductive amination is widely used in chemistry as well as in bioorganic chemistry to form N-alkyl derivatives, but overalkylation readily occurs and is of major concern [19]. This side reaction in the synthesis of mono-alkylated derivatives can be circumvented by using less than 1 equivalent of aldehyde relative to the amine [20], or by employing a dehydrating solvent like trimethyl orthoformate (TMOF). In the latter case the solvent forces the formation of imine intermediate [21], and only the monoalkylated product is formed [19].

However, N-ethylation of amino acid derivatives represents a major problem since neither an excess of amino acid nor the use of a dehydrating solvent result in the desired mono-ethylated amino acid as major product [19,20].

Here, we describe an efficient procedure for the synthesis of N-ethyl amino acid derivatives applying a one-pot synthesis.

In a typical protocol, amino acid ester (1 eq) and acetaldehyde (10 eq) are stirred in TMOF to form quantitatively the imine intermediate. Excess of aldehyde is readily removed under reduced pressure, followed by treatment with the weak reducing agent $NaBH(OAc)_3$ (5 eq). As a crucial step in this one-pot synthesis, the excess of aldehyde is removed completely *in vacuo* thus preventing formation of bis-alkylated product (Figure 1).

Optimized reaction conditions gave a ratio of non-, mono- and bis-alkylated product of 3:90:7, respectively. Applying this protocol, various amino acid derivatives have been N-ethylated in acceptable to high yields (Table 1). In general, $NaBH(OAc)_3$ gave more reproducible results than other commonly used reducing agents such as $NaBH_3CN$.

* Correspondence to: Institute of Organic Chemistry, University of Lausanne, BCH-Dorigny, CH-1015 Lausanne, Switzerland.

CCC 1075–2617/99/050056-03$17.50

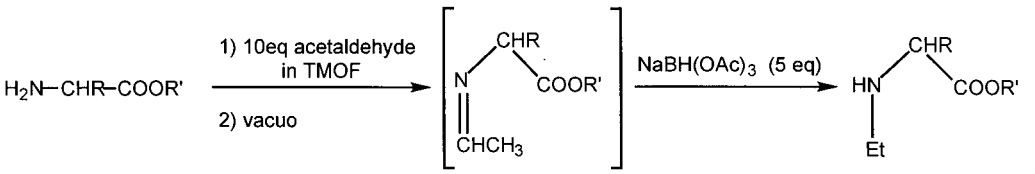

Figure 1 Reactions scheme for the synthesis of N-ethyl amino acids.

EXPERIMENTAL PART

Nuclear magnetic resonance spectra were recorded on a Brucker DPX-400 instrument, while the mass spectra were obtained on a Finnigan MAT SSQ 710 C spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter.

Thin layer chromatography was performed on Merck silica gel 60 F_{254} plates. The compounds were visualized by spray solution of ninhydrine reagent (0.1%, Merck) and heating. Analytical RP-HPLC was performed on Waters equipment using columns packed with Vydac Nucleosil 300 Å, 5 μm, C_{18} particles with a flow rate of 1 mL/min. The elution gradient comprised 100% A to 100% B over 30 min where solvent A consisted of 0.09% TFA in water and solvent B of 0.09% TFA in 90% acetonitrile. Flash chromatography was performed with Merck silica gel 60 (40–63 mesh).

Amino acids were purchased from Bachem (Bubendorf, Switzerland) and Novabiochem (Läufelfingen, Switzerland). The commercial source for reagents and solvents was Fluka (Buchs, Switzerland).

Table 1 Reductive Amination of Various Amino Acid Esters with Acetaldehyde and $NaBH(OAc)_3$ According to Figure 1

Amino acid ester	N-ethyl amino acid ester (% yield)
Val-O*t*Bu	85
Val-OMe	83
Leu-O*t*Bu	82
Phe-O*t*Bu	78
Lys(Z)-O*t*Bu	75
Thr-O*t*Bu	75
Thr-OBn	61
Thr(OBn)-OBn	87
Thr(O*t*Bu)-O*t*Bu	57

H-EtVal-O*t*Bu · HCl

Typically, 500 mg of H-Val-O*t*Bu · HCl were dissolved in 100 mL neat TMOF under Ar. The hydrochloride was neutralized with 1 eq of DIEA (0.42 mL). Under a weak argon stream 10 eq of acetaldehyde (1.34 mL) were added and the solution was allowed to stirr for 30 min at r.t. The excess of acetaldehyde was removed under reduced pressure during 1 h, followed by adding 5 eq of $NaBH(OAc)_3$ (2.5 g) in small portions under Ar. After 15 min the suspension was cooled to 0°C and quenched with 0.8% aqueous HCl (60 mL) to pH 4. The organic solvent was evaporated i.v. and the reaction mixture was diluted with 60 mL H_2O, and washed with ether (3×25 mL). The ether phase was extracted twice with 2% aqueous HCl, and the combined aqueous phases were cooled to 0°C and adjusted with 2 N NaOH to pH 10. The milky solution was extracted with ether (4×25 mL). The ether phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The liquid was purified by flash chromatography using silca gel 60 (0.04–0.063 mm) and ethylacetate/hexane 2:8 as eluent. The isolated monoalkylated product was dissolved in 30 mL dry ether and a solution of HCl in ether was added until pH 3, and kept in the cold overnight. The hydrochloride was collected by vacuum filtration, washed with ether and dried under reduced pressure to provide H-Et-Val-O*t*Bu·HCl as a colorless solid (480 mg, 85%).

R_f 0.40 (hexane/ethylacetate 8:2) (free amino acid); R_t 9.31 min (C_{18}-RP-HPLC); $[\alpha]_D^{25} = +33.0$, ($c = 0.2$, MeOH); m.p. = 183–185°C; ^{1}H-NMR (400 MHz, $CDCl_3$) (free amino acid) d: 2.85 (d, $J = 2.85$ Hz, 1H), 2.63 (m, 1H), 2.48 (m, 1H), 1.88 (m, 1H), 1.49 (s, 9H), 1.09 (t, $J = 7.2$ Hz, 3H), 0.92 (dd, $J = 4.4$, 6.8 Hz, 6H); ESI-MS: calc. for $C_{11}H_{23}NO_2$: 201.3; found 202.6 ($M + H^+$).

Acknowledgements

This work was supported by the *Swiss National Science Foundation*. T.R. is grateful for a grant from the *Deutsche Forschungsgemeinschaft*.

REFERENCES

1. J.M. Humphrey and A.R. Chamberlin (1997). Chemical synthesis of natural product peptides: coupling methods for the incorporation of noncoded amino acids into peptides. *Chem. Rev. 97*, 2243–2266 and references cited therein.
2. M. Ebata, Y. Takahashi and H. Otsuka (1966). The preparation and some properties of N-monomethylated L-amino acids. *Bull. Chem. Soc. Jpn. 39*, 2535–2538 and references cited therein.
3. G.R. Pettit, Y. Kamano, C.L. Herald, Y. Fujii, H. Kizu, M.R. Boyd, F.E. Boettner, D.L. Doubek, J.M. Schmidt, J.-C. Chapuis and C. Michel (1993). Isolation of dolastatins 10–15 from the marine mollux *Dolabella auricularia*. *Tetrahedron 49*, 9151–9170.
4. M. Thali (1995). Cyclosporins: imunosuppressive drugs with anti-HIV-1 activity. *Mol. Med. Today*, 287–291.
5. T. Oka and N. Yoshimura (1996). Immunosuppression in organ transplantation. *Jpn. J. Pharmacol. 71*, 89–100.
6. M. Goodman, G. Melancini and Y. Feng (1996). Collagen-like triple helices incorporating peptoïd residues. *J. Am. Chem. Soc. 118*, 10928–10929.
7. G. Melancini, Y. Feng and M. Goodman (1996). Collagen-based structures containing the peptoid residue N-isobutylglycine (Nleu). 6. Conformational analysis of Gly-Pro-Nleu sequences by ^{1}H-NMR, CD, and molecular modeling. *J. Am. Chem. Soc. 118*, 10725–10732.
8. J. Krujitzer and R. Liskamp (1995). Synthesis in solution of peptoids using Fmoc-protected N-substituted glycines. *Tetrahedron Lett. 36*, 6969–6972.
9. J. Reichwein and R. Liskamp (1998). Site-specific N-alkylation of peptides on the solid phase. *Tetrahedron Lett. 39*, 1243–1246.
10. J.J. Chruma, D. Sames and R. Polt (1997). General method for the synthesis of N-methyl amino acids and N-alkyl aminoesters from O'Donnell's Schiff Bases. *Tetrahedron Lett. 38*, 5085–5086.
11. J. Spengler and K. Burger (1998). An efficient synthesis of N-methylamino acids and some of their derivatives. *Synthesis*, 67–70.
12. G. Vidyasagar Reddy, G. Venkat Rao and D.S. Iyengar (1998). A practical approach for the optically pure N-methyl-α-amino acids. *Tetrahedron Lett. 39*, 1985–1986.
13. W.R. Bowman and D.R. Coghlan (1997). A facile method for the N-alkylation of α-amino esters. *Tetrahedron 53*, 15787–15798.
14. G. Bitan, D. Muller, R. Kasher, E.V. Gluhov and C. Gilon (1997). Builing units for N-backbone cyclic peptides. Part 4. Synthesis of protected N-α-functionalized alkyl acids by reductive alkylation of natural amino acids. *J. Chem. Soc. Perkin Trans. 1*, 1501–1510.
15. R.M. Freidinger, J.S. Hinkle, D.S. Perlow and B.H. Arison (1983). Synthesis of 9-fluorenylmethyloxycarbonyl-protected N-alkyl amino acids by reduction of oxazolidinones. *J. Org. Chem. 48*, 77–81.
16. F.M. Chen and N.L. Benoiton (1977). N-etylamino acid synthesis and N-acylamino acid cleavage using Meerwein's reagent. *Can. J. Chem. 55*, 1433–1435.
17. J.R. McDermott and N.L. Benoiton (1973). N-methylamino acids in peptide synthesis. II. New synthesis of N-benzyloxycarbonyl, N-methylamino acids. *Can. J. Chem. 51*, 1915–1919.
18. A.F. Abdel-Magid, K.G. Carson, B.D. Harris, C.A. Maryanoff and R.D. Shah (1996). Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedures. *J. Org. Chem. 61*, 3849–3862.
19. A.K. Szardenings, T.S. Burkoth, G.C. Look and D.A. Campbell (1996). A reductive alkylation procedure applicable to both solution and solid-phase syntheses of secondary amines. *J. Org. Chem. 61*, 6720–6722.
20. J.M. Ramanjulu and M.M. Joullié (1996). N-alkylation of amino-acid esters using sodium triacetoxyborohydride. *Synth. Commun. 26(7)*, 1379–1384.
21. G.C. Look, M.M. Murphy, D.A. Campbell and M.A. Gallop (1995). Trimethylorthoformate: a mild and effective dehydrating reagent for solution and solid phase imine formation. *Tetrahedron Lett. 36*, 2937–2940.

