Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIII, No. 6

CONVENIENT PREPARATION OF Boc-L-[4-^{15}N]ASPARAGINE AND -[5-^{15}N]GLUTAMINE FOR APPLICATION IN PEPTIDE SYNTHESIS

Leszek Lankiewicz*, Leif Grehn and Ulf Ragnarsson**

Department of Biochemistry, University of Uppsala, Biomedical Center, PO Box 576, S-751 23 Uppsala, Sweden

SUMMARY

The title compounds have been prepared by a simple and economical procedure from the corresponding Boc-protected α-benzyl esters and [^{15}N]ammonia with N,N'-carbonyldiimidazole as coupling agent. Catalytic hydrogenolysis furnished both labelled products in high overall yields suitable for synthetic work.

Keywords: [^{15}N]Amidation, Boc-L-[4-^{15}N]Asparagine, Boc-L-[5-^{15}N]Glutamine and N,N'-Carbonyldiimidazole.

INTRODUCTION

Whereas incorporation of ^{15}N and ^{13}C for assignment and interpretation of NMR spectra of proteins is fairly well established and can be accomplished biosynthetically (1), up to now much less work has been directed towards correspondingly labelled peptides. However, current methods of peptide synthesis (2) require access to suitably protected amino acid derivatives, which are not always readily available. As labelled peptides generally provide relevant structural information in addition to that afforded by unlabelled

*On leave from Dept. of Chemistry, University of Gdansk, Poland.

**To whom correspondence should be addressed. FAX +46 18 55 21 39.

0362-4803/93/060551-06$08.00

Received 5 February, 1993
Revised 18 February, 1993

species (3,4), rational synthetic routes to such amino acid derivatives are needed.

This paper describes a convenient procedure for the synthesis of Boc-L-[4-^{15}N]asparagine and -[5-^{15}N]glutamine. It is modelled on an older one for L-[4-^{15}N]asparagine starting from Z-L-Asp-OBzl (Z= benzyloxycarbonyl), which is first converted to its acid chloride (5). Fromageot *et al.* (6) also prepared L-[^{13}C(U)]asparagine from L-[^{13}C(U)]aspartic acid *via* the corresponding *p*-nitrobenzyl ester. We chose a route (Scheme 1) starting from Boc-L-Asp-OBzl (**1a**) and Boc-L-Glu-OBzl (**1b**) (Boc=*t*-butoxycarbonyl), but incorporating a simpler and more convenient amidation step involving *in situ* liberation of [^{15}N]ammonia from [^{15}N]ammonium chloride followed by coupling to the ω-carboxyl group using N,N'-carbonyldiimidazole (CDI) (7). We used this technique earlier for the synthesis of a set of [^{15}N]-labelled glycine amides (8). To the best of our knowledge, no similar work has been described with respect to L-[5-^{15}N]glutamine.

Boc-L-NHCH[$(CH_2)_n$COOH]CO-OBzl $\xrightarrow{i}$ Boc-L-NHCH[$(CH_2)_n$CO-$^{15}NH_2$]CO-OBzl (**1**) $\xrightarrow{ii}$ Boc-L-NHCH[$(CH_2)_n$CO-$^{15}NH_2$]COOH (**2**)

Scheme 1. **a**, n=1; **b**, n=2; i, CDI, $^{15}NH_4Cl$, NEt_3; ii, H_2/5% Pd/C.

MATERIALS AND METHODS

General Methods. M.p.s are uncorrected (Gallenkamp apparatus). DMF and MeOH were dried for several days over activated molecular sieves (4A). [^{15}N]NH_4Cl was obtained from Larodan, Malmö, Sweden (IN 5037) and Boc-L-Asp-OBzl and Boc-L-Glu-OBzl from Bachem, Bubendorf, Switzerland (A-1240 & A-1630). TLC analyses were performed on 0.25 mm thick precoated silica plates (Merck DC-Fertigplatten, Kieselgel 60 F_{254}) in (A) CH_2Cl_2-acetone-HOAc 40:10:1 (v/v) and (B) EtOAc-acetone-H_2O-HOAc 5:3:1:1 (v/v). Spots were visualized by inspection under UV-light and/or exposure to Cl_2 followed by dicarboxidine spray. Optical rotations were measured with a Perkin-Elmer

241 polarimeter and IR spectra (in KBr) with a Mattson Polaris spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 instrument at 270 and 67.9 MHz and ^{15}N spectra on a JEOL FX90Q at 9.03 MHz in $CDCl_3$ or DMSO-d_6. All shifts are given in ppm, the ^{15}N ones using $\delta_{HCO[N-15]NH_2}$=113.2 as a reference, and coupling constants in Hz.

Boc-L-[4-^{15}N]Asn-OBzl (**1a**) - Finely divided Boc-L-Asp-OBzl (1.62 g, 5 mmol) was dissolved in DMF (5 mL) and treated dropwise (15 min) at -30 °C under stirring with a solution of CDI (1.22 g, 7.5 mmol) in DMF (10 mL). After 1 h below -10 °C, $^{15}NH_4Cl$ (545 mg, 10 mmol) was added at -30 °C, followed by a solution of NEt_3 (1.02 g, 10 mmol) in DMF (5 mL), whereupon the turbid mixture was allowed to reach rm temp. and then left overnight. The DMF was stripped off (oil pump) and the residue partitioned between EtOAc and 1 M $NaHCO_3$ saturated with NaCl. The aq. phase was backwashed twice with EtOAc, the combined organic phases washed with 1 M $NaHCO_3$/NaCl, 1 M $KHSO_4$/NaCl and sat. NaCl and dried (Na_2SO_4). Evaporation gave a solid which was recrystallized from EtOAc-hexane; yield 1.31 g, (81%); m.p. 120.5-121.5 °C; pure by TLC (A); $[\alpha]^{25}_D$ -16.2° (c 1, DMF) [reported for unlabelled material: m.p. 120-122 °C, $[\alpha]^{25}_D$ -17.29° (c 1, DMF) (9)]; IR (cm^{-1}) 3385 (NH) and 1653 (CO_{amide}) (3399 and 1657 in unlabelled reference); δ_H ($CDCl_3$) 1.42 (9H, s, Me), 2.75 and 2.94 (2H, ABq, $J_{gem}\approx$16.2 further split by coupling to H_α, βCH_2), 4.56 (1H, m, CH), 5.18 (2H, s, Bzl), 5.65 and 5.68 (≈2H, 2xd, $J_{^{15}N,HN}\approx$88.9, $^{15}NH_2$), 5.76 (≈1H, d, $J_{\alpha CH,NH}\approx$8.7, NH), 7.34 (5H, s, Ar); δ_C 28.3 (Me_{Boc}), 37.4 (d, $^{2}J_{^{13}C,^{15}N}$=8.6, β-CH_2), 50.4 (CH), 67.4 (Bzl), 80.1 (CMe_3), 128.2, 128.4, 128.6, 135.4 (Ar), 155.7 (CO_{Boc}), 171.3 (CO_{ester}), 172.2 (d, $J_{^{15}N,^{13}C}\approx$14.6, CO_{amide}); δ_N 104.1.

Boc-L-[5-^{15}N]Gln-OBzl (**1b**) - This compound was prepared from Boc-L-Glu-OBzl (1.68 g, 5 mmol) and $^{15}NH_4Cl$ (545 mg, 10 mmol) according to the procedure given for **1a**. Recrystallization from EtOAc-hexane

furnished **1b** (0.90 g, 54%); m.p. 107-108.5 °C; pure by TLC (A); $[\alpha]^{25}_D$ -22.0° (*c* 1, DMF) [reported for unlabelled material: m.p. 108-110 °C, $[\alpha]^{25}_D$ -22.69° (*c* 1, DMF) (9)]; IR (cm^{-1}) 3178 (NH) and 1643 (CO_{amide}) (3185 and 1648 in unlabelled reference); δ_H ($CDCl_3$) 1.43 (9H, s, Me), 1.85-2.34 (4H, m, CH_2-CH_2), 4.36 (1H, m, $J_{\alpha CH,NH} \approx J_{\alpha CH,CH2} \approx 4.3$, CH), 5.15 and 5.20 (2H, ABq, J_{gem} 12.1, Bzl), 5.34 (≈1H, d, $J_{\alpha CH,NH} \approx 7.8$, NH), 5.45 and 6.04 (≈2H, 2xd, $J_{15N,NH} \approx 90.0$, $^{15}NH_2$), 7.36 (5H, s, Ar); δ_C 28.2 (Me_{Boc}), 28.9 (β-CH_2), 31.7 (d, $J_{13C,15N}$=8.5, γ-CH_2), 53.0 (CH), 67.3 (Bzl), 80.2 (CMe_3), 128.4, 128.5, 128.6. 135.2 (Ar), 155.8 (CO_{Boc}), 172.1 (CO_{ester}), 174.3 (d, $J_{13C,15N}$=14.6, CO_{amide}); δ_N 104.1.

Boc-L-[4-^{15}N]Asn (**2a**) - The benzyl ester **1a** (1.20 g, 3.7 mmol) was dissolved in MeOH (50 mL) and hydrogenolyzed with Pd catalyst (5% Pd/C, 100 mg). The reaction was carefully monitored by TLC (B) and, after completion, the catalyst was filtered off and the filtrate taken to dryness. The product was recrystallized from MeOH-EtOAc-hexane to give **2a** (0.83 g, 96%); m.p. 171-173 °C; pure by TLC (B); $[\alpha]^{25}_D$ -7.4° (*c* 1, DMF) [reported for unlabelled material: m.p. 178-179 °C, $[\alpha]^{25}_D$ -7.4° (*c* 1, DMF) (10)]; IR (cm^{-1})3402 (NH) and 1654 (CO_{amide}) (3418 and 1663 in unlabelled reference); δ_H (DMSO-d_6) 1.38 (9H, s, Me), 2.45 (2H, m, CH_2), 4.16 (minor conformer) and 4.23 (major one) (2H, 2xm, CH), 6.63 (minor conformer) and 6.90 (major one) (≈1H, 2xd $J_{\alpha CH,NH} \approx 8.4$, NH), 6.94 (dd, $J_{15N,HN}$= 87.4) and 7.33 (dd, $J_{15N,NH} \approx 88.8$, together ≈2H, $^{15}NH_2$), 12.54 (≈1H, s, COOH); δ_C 28.1 (Me_{Boc}), 36.7, (d, $J_{13C,15N}$=8.5, CH_2), 50.2 (CH), 78.1 (CMe_3), 155.1 (CO_{Boc}), 171.3 (d, $J_{15N,13C} \approx 15.9$, CO_{amide}), 173.4 (COOH); δ_N 110.5.

Boc-L-[5-^{15}N]Gln (**2b**) - This compound was prepared from **1b** (0.85 g, 2.5 mmol) by analogy with **2a**. Recrystallization from EtOAc-hexane afforded **2b** (0.56 g, 90%); m.p. 115.5-117 °C; pure by TLC (B); $[\alpha]^{25}_D$ -16.9° (*c* 1, DMF); [reported for unlabelled material: m.p. 116-119 °C, $[\alpha]^{25}_D$ -16° (*c* 1, DMF) (10)]; IR (cm^{-1}) 3330 (NH) and

1526 (amide-II) (3350 and 1536 in unlabelled reference); δ_H (DMSO-d_6) 1.38 (major conformer) and 1.34 (minor one) (together 9H, 2xs, Me), 1.70 and 1.89 (2H, 2xm, β-CH_2), 2.12 (2H, t, $J\approx7.6$, γ-CH_2), 3.78 (minor conformer) and 3.84 (major one) (1H, 2xm, CH), 6.74 (minor conformer) and 7.09 (major one) (≈1H, 2xd, $J_{\alpha CH,NH}\approx8.1$, NH), 6.78 (dd, $J_{15N,HN}=87.4$) and 7.29 (dd, $J_{15N,HN}=88.7$, together ≈2H, $^{15}NH_2$), 12.51 (1H, br. s, COOH); δ_C 26.5 (β-CH_2), 28.0 (minor conformer) 28.2 (major one, Me_{Boc}), 31.4, (d, $J_{13C,15N}=8.6$, γ-CH_2), 53.2 (α-CH), 78.0 (CMe_3), 155.6 (CO_{Boc}), 173.5 (d, $J_{15N,13C}\approx14.6$, CO_{amide}), 174.0 (CO_{acid}); δ_N 109.1.

COMMENTS

CDI has previously been extensively used for formation of amides and peptides (7), although other coupling agents are generally preferred nowadays for such reactions at chiral centra (2). Its major advantage in this context, apart from being highly reactive, is the simple workup required, leading directly to very pure products without contamination from the coupling agent. It is convenient to handle also on a small scale.

Recent synthetic work starting from labelled serine (11) has led to various specifically labelled amino acids, among which is aspartic acid (12). With such as starting material, the corresponding asparagine [4-^{15}N]isotopomers could easily be prepared as described in the present paper. In addition, this work also complements our efforts on ^{15}N-labelling of the α-amino group of amino acids (13).

Acknowledgements. This work is part of a project supported by grants from the *Swedish Natural Science Research Council* and the *National Swedish Board for Technical Development*. The purchase of isotopes was made possible by additional grants from the H. Ax:son Johnson and M. Bergvall Foundations. One of us (L.L.) is grateful to the *Swedish Institute* for a fellowship.

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