Acta Chemica Scandinavica, 1995: 49: 149–151
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

Short Communication

Synthesis of ^{15}N-Labelled Leu-Enkephalins

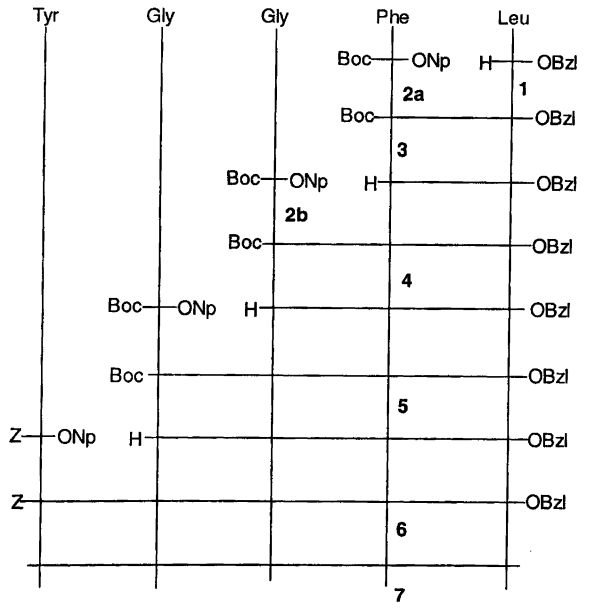
Fredrik Degerbeck, Bengt Fransson, Leif Grehn and Ulf Ragnarsson

Department of Biochemistry, University of Uppsala, Biomedical Center, P.O. Box 576, S-751 23 Uppsala, Sweden

Degerbeck, F., Fransson, B., Grehn, L. and Ragnarsson, U., 1995. Synthesis of ^{15}N-Labelled Leu-Enkephalins. – Acta Chem. Scand. 49: 149–151 © Acta Chemica Scandinavica 1995.

Recently we reported a convenient procedure for the preparation of ^{15}N-labelled Boc-amino acids[1,2] (Boc = *tert*-butoxycarbonyl) starting from triflates of α-hydroxy acids and exploiting di-*tert*-butyl [^{15}N]imidodicarbonate[3] as the nitrogen source. This reaction takes place with complete inversion of configuration. On the other hand, α-hydroxy acids can be obtained with total retention[2,4] directly from the corresponding α-amino acids and the latter approach becomes particularly attractive when the required enantiomer is commercially available. Recently, α-hydroxy acids have also been made by asymmetric synthesis,[5] thus further extending the scope of the aforementioned ^{15}N-labelling procedure.

This note reports on initial work related to the synthesis of ^{15}N-labelled peptides by use of such Boc-amino acids; namely, five Leu-enkephalin isotopomers (**7**). Leu-Enkephalin belongs to, nowadays, a well-known family of endogenous opiate peptides with a morphine-like activity in mammalian brain and many analogues have been prepared and studied, not least in attempts to characterize various opiate receptors.[6,7] One of these five isotopomers, highly enriched, has previously been prepared and studied,[8] thus allowing us to compare our results with those obtained earlier.



Scheme 1. Synthesis of Leu-enkephalin. For the isotopomers made see Table 1.

The isotopomers **7** were uniformly prepared by a stepwise procedure in solution starting from the *C*-terminus and using the correspondingly labelled or non-labelled Boc-amino acid *p*-nitrophenyl esters (Scheme 1). All protected intermediates were isolated and carefully characterized. Finally, after attaching the *N*-terminal tyrosine, the various products **6** were deprotected. Selected data, especially ^{15}N NMR shifts, are collected in Table 1.

Inspection of Table 1 shows that the ^{15}N shifts of the individual amino acid nitrogens fall in relatively narrow intervals of less than 3 ppm, although most of the data originate from spectra of protected peptides recorded in $CDCl_3$, whereas $(CD_3)_2SO$ was used for all pentapeptides **6** and **7**. More importantly, our data set provides independent, unambiguous assignments of all ^{15}N signals in the multiply labelled isotopomers and corroborates reported data for free Leu-enkephalin.[8] The earlier assignment was ultimately based on titration data for a triply ^{15}N-labelled tetrapeptide Tyr-Gly-Gly-Phe[12] and subsequent correlation of ^{15}N signals with those of the corresponding peptide protons by means of irradiation experiments. Complete assignment of the ^{1}H NMR spectrum of Leu-enkephalin has already been accomplished.[13]

Roques *et al.*[8] reported absolute values of 85.4, 83.0, 96.5 and 97.4 ppm, respectively, for the ^{15}N shifts of their quadruply ^{15}N-labelled Leu-enkephalin. To compensate for the different choice of reference substances, a correction factor obviously has to be applied. Addition of 22.3 ppm to the values above gives 107.7, 105.3, 118.8 and 119.7 ppm, respectively, for comparison with the corresponding values on the last line of Table 1. Recently, we have also prepared fully [^{13}C,^{15}N]backbone-labelled

Table 1. Selected data on ^{15}N-labelled Leu-enkephalins and intermediates.

Cpd.[a]	M.p./yield (°C/%)	$[\alpha]_D^{25}$ (c^b)	^{15}N shift[c, d]	$J_{^{13}C_i{}^{15}N_{i+1}}$[e] /Hz	$J_{^{15}N^1H}$ /Hz
2a	126–127[9]/80	−21.0[9] (2.01)	74.2		
2b	67–68[9]/~70		86.6		
3[f]	84–86[10]	−24.2[10] (1.01)			
3^{0}*	83.5–84.5/81	−23.8 (0.94)	117.6	15.8	91
3*^{0}	85–85.5/77	−24.0 (1.01)	88.6		89
3**	84–85.5/77	−24.1 (1.00)	88.6, 117.6	14.7	89, 91
4[f]	foam[10]	−24.4[10] (1.0)			
4^{00}*	foam/88	−23.1 (0.93)	118.8	15.9	92
4^{0}*^{0}	foam/90	−23.2 (1.00)	115.5	15.9	91
4*^{00}	foam/93	−24.2 (0.98)	76.2		92
4**	foam/~100	−23.4 (0.97)	76.2,115.2	14.6, 14.6	92, 92
			118.8		91
5[f]	161–162[10]	−16.8 (2.0)[10]			
5^{000}*	157–158/87	−15.9 (1.82)	118.8	15.9	
5^{00}*^{0}	161–162/87	−17.1 (1.98)	117.0	15.9	
5^{0}*^{00}	159.5–160.5/93	−16.5 (2.02)	103.6	—	
5*^{000}	162–164/92	−16.8 (1.79)	76.6		
5**	162–164/86	−16.9 (1.99)	76.5, 103.5	15.9, 15.9,	
			116.9, 118.6	14.6	
6[f]	159–162[11]				
6^{000}*	158–161/67[g]	−18.5 (1.0)	118.1	14.6	93
6^{00}*^{0}	161–164/80[g]	−18.6 (0.97)	116.7	14.7	92
6^{0}*^{00}	162–165/84[g]	−18.1 (1.0)	105.7	15.3	94
6*^{000}	159–162/69[g]	−18.8 (0.99)	106.3	14.7	93
6**	161–165/71[g]	−18.6 (0.99)	106.3, 105.6,	13.5, 13.5,	93, 93,
			116.7, 118.1	14.6, 13.5	92, 93
7^{000}*	—/~100	19.7 (0.49)	119.9	14.6	91
7^{00}*^{0}	—/~100	19.8 (0.49)	118.0	14.6	92
7^{0}*^{00}	—/~100	20.8 (0.50)	106.2	15.8	93
7*^{000}	—/95	20.6 (0.49)	107.5	15	93
7**	—/~100	20.9 (0.49)	107.7, 106.2,	14.6, 15,	~88, 92,
			118.0, 119.7	15, 14.6	92, 91

[a] Isotopomers are characterized with superscript combinations of * (labelled residue) and ^{0} (non-labelled residue), indicating positions of such counted from the *N*-termini, excluding tyrosine when present. [b] MeOH was used as the solvent in polarimetry except for **2** (DMF) and **7** (HOAc). [c] $HCO^{15}NH_2$ was used as a reference [$\delta(HCO^{15}NH_2) = 113.2$ ppm]; estimated error, due to minor variations in sample concentration, ±0.2 ppm. [d] $CDCl_3$ was used as solvent for **2**–**5**, otherwise DMSO-d_6. [e] Estimated error ±1 Hz. [f] Literature data refer to non-labelled compounds. [g] Yield after recrystallization.

Leu-enkephalin.[14] For this isotopomer we found the values 110.9, 106.6, 116.7 and 119.1 ppm. Minor deviations among and within the three series of data are ascribed primarily to instrumental factors and the presence of counter-ion(s) in the samples.

Experimental

TLC analyses and preparative chromatography were performed and NMR spectra were recorded as described previously.[1,2] Labelled (and non-labelled) *p*-nitrophenyl esters were synthesized essentially as described by Bodanszky and du Vigneaud.[15] Z-Tyr-ON*p* was purchased from Senn Chemicals AG, Switzerland. All coupling steps were monitored by TLC. In general, the most precious compound was used in a slight deficiency. The Boc-group was removed with TFA in dichloromethane and the free amine obtained by extractive work-up. All products were checked by TLC, characterized by ^1H, ^{13}C and ^{15}N NMR spectroscopy as well as amino acid analysis after hydrolysis and confirmed to be pure.

Boc-Phe-Leu-OBzl (**3**). A stirred solution of Leu-OBzl **1** in DMF (5–6 ml $mmol^{-1}$), was treated with solid Boc-Phe-ON*p* and left overnight. The solvent was evaporated off (oil pump) and the residue first partitioned between EtOAc and 1 M $KHSO_4$. The organic extract was then worked-up with 1 M Na_2CO_3 and brine (three times each) and finally dried (Na_2SO_4). The yellow semi-solid residue was triturated with light petroleum and collected as a crystalline fine-grained powder.

Boc-Gly-Phe-Leu-OBzl (**4**). The deprotected dipeptide was reacted as above with solid Boc-Gly-ON*p*. Treatment with 2-diethylaminoethylamine consumed the excess of active ester. Work-up as above followed by chromatography on silica afforded a foam.

Boc-Gly-Gly-Phe-Leu-OBzl (**5**). The deprotected tripeptide was again coupled with Boc-Gly-ON*p* as described for **3**. Work-up as above followed by treatment with diethyl ether provided a white, crystalline powder.

Z-Tyr-Gly-Gly-Phe-Leu-OBzl (**6**). The deprotected tetrapeptide was reacted with Z-Tyr-ON*p* (1.25 equiv.) after which the reaction mixture was worked-up as described above. The crude product was applied to a silica column (10 cm) in CH_2Cl_2–acetone 9:1, which was eluted with EtOAc and the material obtained was recrystallized from EtOH–heptane 1:4 to furnish a pale grey powder.

Tyr-Gly-Gly-Phe-Leu (**7**). The protected pentapeptide was dissolved in 80% HOAc (40 ml $mmol^{-1}$) and hydrogenated at 1 atm over Pd (5% on C). The reaction was monitored for completion (1–2 h) by TLC (EtOAc–acetone–HOAc–H_2O, 5:3:1:1). The catalyst was filtered off, the filtrate evaporated and the resulting viscous oil dissolved in 10% HOAc and lyophilized to give the title compound as a white fluffy powder. Its purity was confirmed by reversed-phase HPLC on a Spherisorb C_8 column (5 μm, 250 × 4.6 mm, mobile phase: 0.1 M phosphate buffer, pH = 3.0–EtOH, 80:20, 1.0 ml min^{-1}, detection at 210 nm, 0.1 AUFS).

Acknowledgements. This work was generously supported by grants from the National Swedish Board for Technical Development and the Swedish Natural Science Research Council (including a scholarship to F. D.).

References

1. Degerbeck, F., Fransson, B., Grehn, L. and Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1* (1992) 245.
2. Degerbeck, F., Fransson, B., Grehn, L. and Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1* (1993) 11.
3. Degerbeck, F., Grehn, L. and Ragnarsson, U. *Acta Chem. Scand. 47* (1993) 896.
4. Brewster, P., Hiron, F., Hughes, E. D., Ingold, C. K. and Rao, P. A. D. S. *Nature (London) 166* (1950) 179.
5. Corey, E. J. and Link, J. O. *Tetrahedron Lett. 33* (1992) 3431.
6. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. and Morris, H. W. *Nature (London) 258* (1975) 577.
7. Hruby, V. J. and Gehrig, C. A. *Med. Res. Rev. 9* (1989) 343.
8. Garbay-Jaureguiberry, C., Marion, D., Fellion, E. and Roques, B. P. *Int. J. Pept. Protein Res. 20* (1982) 443.
9. Sandrin, E. and Boissonnas, R. A. *Helv. Chim. Acta 46* (1963) 1637.
10. Clausen, K., Thorsen, M., Lawesson, S. O. and Spatola, A. F. *J. Chem. Soc., Perkin Trans. 1* (1984) 785.
11. Stimson, E. R., Meinwald, Y. C. and Scheraga, H. A. *Biochemistry 18* (1979) 1661.
12. Garbay-Jaureguiberry, C., Baudet, J., Florentin, D. and Roques, B. P. *FEBS Lett. 115* (1980) 315.
13. Garbay-Jaureguiberry, C., Roques, B. P., Oberlin, R., Anteunis, M., Combrisson, S. and Lallemand, J. Y. *FEBS Lett. 76* (1977) 93.
14. Nyasse, B., Grehn, L. and Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* (1994) 2005.
15. Bodanszky, M. and du Vigneaud, V. *Biochem. Prep. 9* (1962) 110.

Received August 23, 1994.