Resolution and Synthesis of the Individual Enantiomers of the Glycine Antagonist 3-Amino-1-hydroxypyrrolidin-2-one (HA-966)

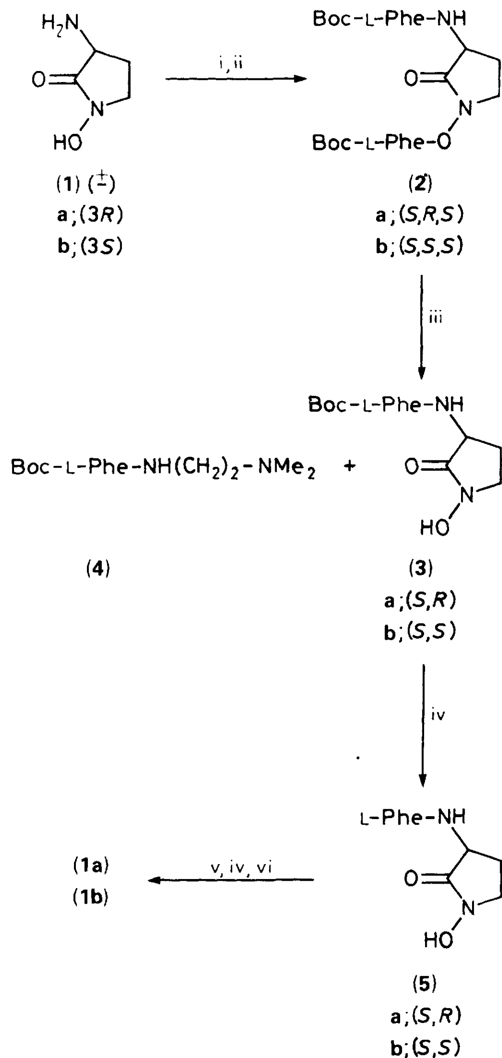
Brian J. Williams, Paul D. Leeson, Graeme Hannah, and Raymond Baker

Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K.

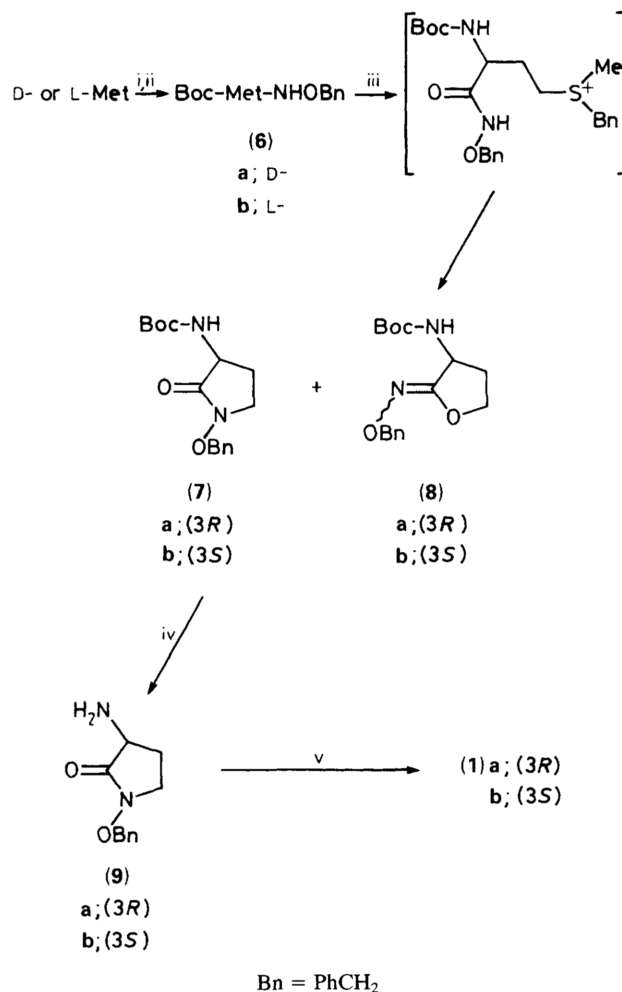
The individual enantiomers of the glycine antagonist HA-966 (**1**) have been obtained by resolution *via* the bis-Boc-L-Phe (Boc = t-butoxycarbonyl) derivatives (**2a** and **2b**) and by synthesis from D- and L-methionine.

Increases in extracellular concentrations of excitatory amino acids, notably glutamic acid, have been implicated in neuronal cell death following periods of anoxia such as stroke or cardiac arrest.[1] Potential for therapeutic intervention has been shown since protection against cell damage in animal models of cerebral ischaemia can be conferred by excitatory amino acid antagonists which are selective against the sub-type of post-synaptic receptor sensitive to *N*-methyl-D-aspartate (NMDA).[2] Opening of the cation-selective ion channel controlled by the NMDA receptor has been shown to be modulated by a glycine binding site[3] and antagonists which act at this site have neuroprotective properties.[4] The increasing importance of this area of neurobiology[5] has stimulated an intensive search for compounds having glycine antagonist activity.[6] Of these, 3-amino-1-hydroxypyrrolidin-2-one (HA-966, **1**)[6a] is probably of the greatest significance, since it has the highest activity *in vivo* as a result of its ability to penetrate the blood–brain barrier. However racemic (**1**) is known to display varied pharmacological actions[7] and consequently we undertook the synthesis of its individual enantiomers to allow separate evaluation of their biological properties.

The known synthetic procedure[8,9] can be used to provide substantial quantities of racemic HA-966 (**1**). α-Amino hydroxamates do not appear to have been resolved previously, and the use of classical derivatisation methods (acylation or sulphonylation) in the case of (**1**) is inappropriate since acidic hydrolysis to remove the chiral auxiliary would be accompanied by ring opening of the cyclic hydroxamate.[10] A



Scheme 1. *Reagents and conditions*: i, Boc-L-Phe-OH (2 equiv.)/DCC (2 equiv.); ii, separate diastereoisomers; iii, $Me_2NCH_2CH_2NH_2$/MeOH; iv, CF_3CO_2H; v, Ph–NCS/Et_3N; vi, Dowex (50W-X8, H^+).



Scheme 2. *Reagents*: i, Boc_2O/Na_2CO_3; ii, Bu^iOCOCl/*N*-methylmorpholine/$NH_2OBn{\cdot}HCl$; iii, BnBr/LiOH; iv, CF_33CO_2H; v: H_2/Pd–C.

modification which is likely to have growing importance for the resolution of hydrolytically unstable compounds is the use of Edman degradation methods.[11] This technique was applied successfully to the aminohydroxamate (**1**) (Scheme 1). Acylation of (**1**) using a two-fold excess of *N*-t-butoxycarbonyl-L-phenylalanine (Boc-L-Phe) and dicyclohexylcarbodiimide (DCC) gave the *N,O*-diacyl derivative as a mixture of the (*S,R,S*)- and (*S,S,S*)-diastereoisomers (**2a**) and (**2b**). Attempts to separate the mixture of (**2a**) and (**2b**) by chromatography were hampered by facile deacylation of the labile *O*-acylhydroxamate functionality, which gave inseparable mixtures of the *N*-acylated diastereoisomers (**3a**) and (**3b**). However, fractional crystallisation of the mixture of (**2a**) and (**2b**) from diethyl ether gave (**2a**)† (m.p. 174—175 °C) and (**2b**) in >95% diastereoisomeric excess (d.e.) as shown by ^1H NMR. Aminolysis of the separated diastereoisomers (**2a**) and (**2b**) with *N,N*-dimethyldiaminoethane smoothly gave (**3a**) (m.p. 155—156.5 °C) and (**3b**) (m.p. 185—186 °C) and allowed removal of the by-product (**4**) by extraction with aqueous citric acid. The conversion of (**3a**) and (**3b**) to (**1a**) and (**1b**), respectively, was accomplished by successive treatments with trifluoroacetic acid (TFA), phenyl isothiocyanate/triethylamine, and TFA. Purification of the intermediates was unnecessary and the pure enantiomers (**1a**) and (**1b**) were isolated by ion-exchange chromatography on Dowex 50W-X8 and crystallised from ethanol in overall yields [from (**1**)] of 38% ($[\alpha]_D$ +101°, *c* 1, H_2O) and 14% ($[\alpha]_D$ −106°, *c* 1, H_2O), respectively.

Comparison of the ^1H NMR spectral data of the intermediates (**5a**) and (**5b**) suggested[12] that the absolute configuration of these intermediates at the 3-position was (*R*) and (*S*), respectively. In order to confirm the stereochemical assignment unambiguously, a short route to (**1a**) and (**1b**) from enantiomerically pure starting materials was developed. Aldehydes derived from homoserine derivatives[10] have potential for the synthesis of (**1**) but preparation of the required D-precursors[13,14] makes the overall procedure lengthy. Alternative cyclisation reactions of readily available *S*-alkylated methionines and methionine amides have been shown to form γ-lactones[15] and, depending on the conditions employed, γ-lactams[16] or imino ethers.[17] Corresponding cyclisations of hydroxamate derivatives of methionine have not been investigated previously. Protection of both D- and L-methionine as the corresponding Boc derivatives (Scheme 2) followed by

† All new compounds exhibited spectral data consistent with their proposed structures and were characterised by elemental analyses and/or high resolution mass spectra.

mixed anhydride coupling with *O*-benzylhydroxylamine gave the protected hydroxamates (**6a**) {96.8%, m.p. 92—93 °C, $[\alpha]_D$ +38.6° (*c* 1, MeOH)}, and (**6b**) (96%). Ring closures of (**6a**) and (**6b**) by *in situ* *S*-alkylation with benzyl bromide in the presence of 1 mol. equiv. of lithium hydroxide gave mixtures of the *N*- and *O*-alkylated products (**7**) and (**8**), respectively, which were separated by chromatography. Use of alternative alkylating agents and bases did not improve the overall yield of (**7**), which was 30—40% after recrystallisation {(**7a**): m.p. 113—114 °C, $[\alpha]_D$ +56.8°, (*c* 1, MeOH); (**7b**): m.p. 113—114 °C, $[\alpha]_D$ −54.2° (*c* 1, MeOH)}. Deprotection with TFA gave (**9a**) and (**9b**) as their crystalline TFA salts, which upon catalytic hydrogenolysis gave (**1a**) {m.p. 166 °C, $[\alpha]_D$ +104.5° (*c* 1, H_2O)} and (**1b**) {m.p. 166 °C, $[\alpha]_D$ −106.1°, (*c* 1, H_2O)}, respectively in overall yields of 83 and 79%. The syntheses proceeded without significant racemisation, since the Moscher's amide derivatives of (**9a**) and (**9b**) were optically pure within the limits of NMR detection, and (**1a**) and (**1b**) were shown to >99.9% enantiomerically pure by chiral HPLC analysis. The described procedures not only allow a convenient and practical synthesis but also an unambiguous assignment of the absolute stereochemistry of (+)-HA-966 (**1a**) as (3*R*) and (−)-HA-966 (**1b**) as (3*S*).

The enantiomers of (**1**) proved to be crucial in separating the varied biological effects of racemic (**1**).[18] The glycine antagonist activity of HA-966 was shown to reside exclusively in the (+)-enantiomer (**1a**), which has the D-configuration in common with other glycine site ligands, for example D-serine, D-alanine,[6b] and D-cycloserine.[6h] Surprisingly, the (−)-enantiomer displays some of the *in vivo* biological effects of racemic (**1**).

We thank Dr. R. Herbert for NMR and mass spectra, Mr. R. Barnaby for chiral HPLC and our colleagues in the Biology Department at Terlings Park for permission to disclose the preliminary biological results, full details of which will be reported elsewhere.

Received, 14th August 1989; Com. 9/03470F

References

1 B. S. Meldrum, *Clin. Sci.*, 1985, **68**, 113.
2 A. C. Foster, R. Gill, and G. N. Woodruff, *J. Neurosci.*, 1988, **8**, 4745.
3 J. W. Johnson and P. Ascher, *Nature*, 1987, **325**, 529.
4 I. Shalaby, B. Chenard, and M. Prochniak, *Eur. J. Pharmacol.*, 1989, **160**, 309.
5 A. C. Foster and J. A. Kemp, *Nature*, 1989, **338**, 377; A. M. Thompson, *Trends Neurosci.*, 1989, **12**, 349.
6 (a) HA-966: E. J. Fletcher and D. Lodge, *Eur. J. Pharmacol.*, 1988, **151**, 165; A. C. Foster and J. A. Kemp, *J. Neurosci.*, 1989, **9**, 2191; (b) 2-carboxyquinolines: J. A. Kemp, A. C. Foster, P. D. Leeson, T. Priestley, R. Tridgett, L. L. Iversen, and G. N. Woodruff, *Proc. Natl. Acad. Sci. U.S.A.*, 1988, **85**, 6547; (c) 2-carboxyindoles: J. E. Huettner, *Science*, 1989, **243**, 1611; (d) quinoxaline-2,3-diones: P. J. Birch, C. J. Grossman, and A. G. Hayes, *Eur. J. Pharmacol.*, 1988, **156**, 177; (e) quinoxalic acids: M. Kessler, T. Terramani, G. Lynch, and M. Baudry, *J. Neurochem.*, 1989, **52**, 1319; P. Frey, D. Berney, P. L. Herrling, W. Mueller, and S. Urwyler, *Neurosci. Lett.*, 1988, **91**, 194; (f) 1-amino-1-carboxycyclobutane: W. F. Hood, E. T. Sun, R. P. Compton, and J. B. Monaghan, *Eur. J. Pharmacol.*, 1989, **161**, 281; (g) cycloleucine: L. D. Snell and K. M. Johnson, *Eur. J. Pharmacol.*, 1988, **151**, 165; (h) D-cycloserine: W. F. Hood, R. P. Compton, and J. B. Monaghan, *Neurosci. Lett.*, 1989, **98**, 91.
7 I. L. Bonta, C. J. De Vos, H. Grijsen, F. C. Hillen, E. L. Noach, and A. W. Sim, *Br. J. Pharmacol.*, 1971, **43**, 514; J. A. Van der Krogt, C. F. M. Van Valkenburg, R. D. M. Belfroid, and C. B. M. Heerkens, *Eur. J. Pharmacol.*, 1988, **158**, 29.
8 J. Smrt, J. Beranek, and M. Horak, *Collect. Czech. Chem. Commun.*, 1959, **24**, 1672.
9 Br. Pat. 1,041,861 (1966) to Organon Laboratories Ltd.
10 S. Fushiya, K. Maeda, T. Funayama, and S. Nozoe, *J. Med. Chem.*, 1988, **31**, 480.
11 K. E. Rittle, B. E. Evans, M. G. Bock, R. M. DiPardo, W. L. Whitter, C. F. Homnick, D. F. Veber, and R. M. Freidinger, *Tetrahedron Lett.*, 1987, **28**, 521.
12 H. Joshua and C. Deber, in 'Chemistry and Biology of Peptides: Proceedings of the 3rd American Peptide Symposium,' ed. J. Meienhofer, Ann Arbor Scientific Publications, Michigan, 1972, p. 67.
13 J. E. Baldwin, M. North, and A. Flinn, *Tetrahedron Lett.*, 1987, **28**, 3167.
14 R. D. Cooper, F. Jose, L. McShane, and K. A. Koppel, *Tetrahedron Lett.*, 1978, **19**, 2243.
15 J. E. Baldwin and A. Flinn, *Tetrahedron Lett.*, 1987, **28**, 3605.
16 R. M. Friedinger, D. S. Perlow, and D. F. Veber, *J. Org. Chem.*, 1982, **47**, 104.
17 E. Gross, in 'Methods in Enzymology,' ed. C. H. W. Hirs, Academic Press, New York, 1967, vol. 11, p. 238.
18 Eur. Pat. Appl. 0,318,091 A2 (1989) to Merck Sharp and Dohme Ltd.