Effects of Five-membered Ring Conformation on Bioreceptor Recognition: Identification of 3*R*-Amino-1-hydroxy-4*R*-methylpyrrolidin-2-one (L-687,414) as a Potent Glycine/*N*-Methyl-D-Aspartate Receptor Antagonist

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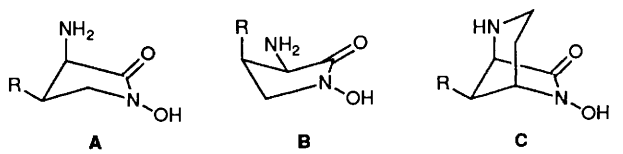
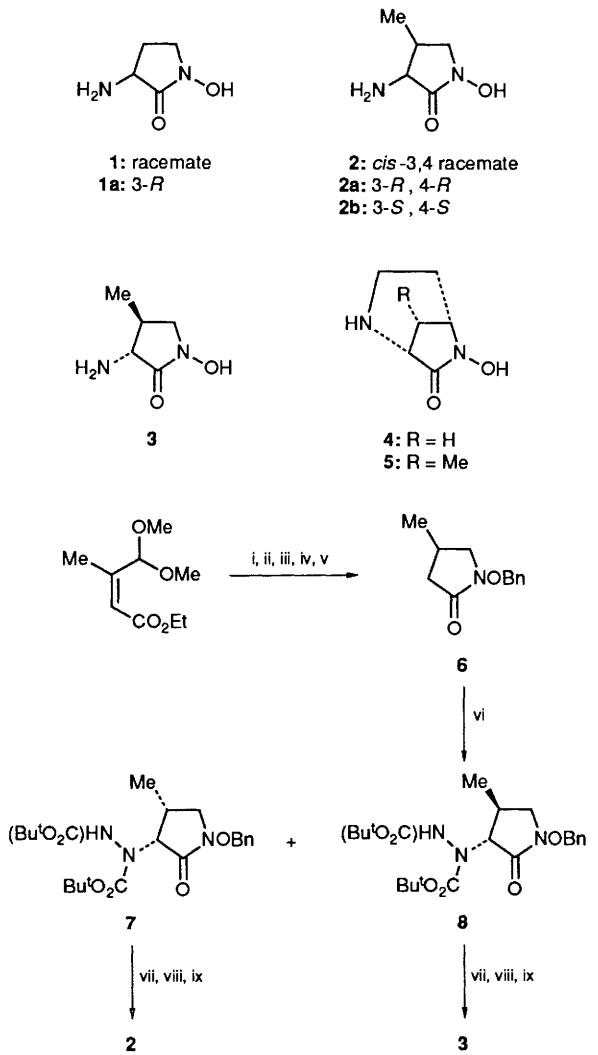
Syntheses of the 4-methyl (**2** and **3**) and [3.2.1]bicyclo (**4** and **5**) analogues of the glycine/*N*-methyl-D-aspartate (NMDA) antagonist 3-amino-1-hydroxypyrrolidin-2-one (HA-966, **1**) provide evidence that glycine receptor recognition requires the energetically less favoured 3-pseudoaxial conformation of the pyrrolidone ring, resulting in a 5–10 fold improvement in activity with the 3*R*-amino, 4*R*-methyl derivative (**2a**, L-687,414).

3-Amino-1-hydroxypyrrolidin-2-one (HA-966, **1**) has been shown to act as an antagonist at the glycine modulatory (or coagonist) site on the *N*-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptor.[1] Compound **1** has neuroprotective effects[2] and NMDA antagonists are currently being intensively evaluated for the treatment of cerebral ischemia.[3] Previously, we synthesised the individual enantiomers of **1**[4] and showed that the glycine antagonist activity resided in the 3*R*-(+) enantiomer **1a**.[5] In order to establish underlying structure–activity relationships and to identify more potent analogues of **1** for *in vivo* studies of the glycine site, we have synthesised substituted derivatives **2–5**. Two compounds with improved activity, the 3*R*-amino-4*R*-methyl derivative (**2a**, L-687,414) and the [3.2.1]bicyclic analogue (**4**), indicate that the energetically unfavourable conformation of the pyrrolidone ring, having the 3-amino group pseudoaxial, is required for recognition by the glycine receptor.

The 4-methyl derivatives (**2** and **3**) were synthesised using enolate amination methodology[6] (Scheme 1). Enolates derived from hydroxamates do not appear to have been reported previously, and initial attempts to deprotonate the 4-methylpyrrolidone **6**† with a variety of bases, followed by quenching with di-t-butyl azodicarboxylate (DBAD) resulted only in decomposition. However, addition of potassium hexamethyldisilazide to a solution of **6** and DBAD in tetrahydrofuran at −100 °C resulted in smooth *in situ* capture of the transient enolate to afford a 1 : 6 mixture of the *cis* and *trans* protected hydrazines (**7** and **8**) in high yield. Separation of **7** and **8** by chromatography, removal of the t-butoxycarbonyl groups, then debenzylation and hydrazine cleavage by sequential hydrogenolysis over Pd/C and Pt, afforded the 4-methyl diastereoisomers (**2**, m.p. 108–112 °C and **3**, m.p. 176 °C). The *cis* 4-methyl derivative **2** was found to be a more potent glycine/NMDA antagonist than **1**, but the *trans* isomer **3** was essentially inactive. The relative activities of **1–3** could be a consequence of specific hydrophobic and steric interactions at the glycine site, but the methyl groups in **2** and **3** will also influence the conformational mobility of the five-membered ring. The pyrrolidone ring can exist as two possible C-4 envelope conformers, where the amino group is either pseudoaxial **A** or pseudoequatorial **B**. In the solid state, **1** exists in conformation **B**[7] and the proton–proton coupling constants of **1–3**, determined from ^{1}H NMR spectra in D_2O, show that conformer **B** also predominates in solution for all three compounds. However, molecular mechanics calculations of **1–3** using OPTIMOL‡ indicate that the energy

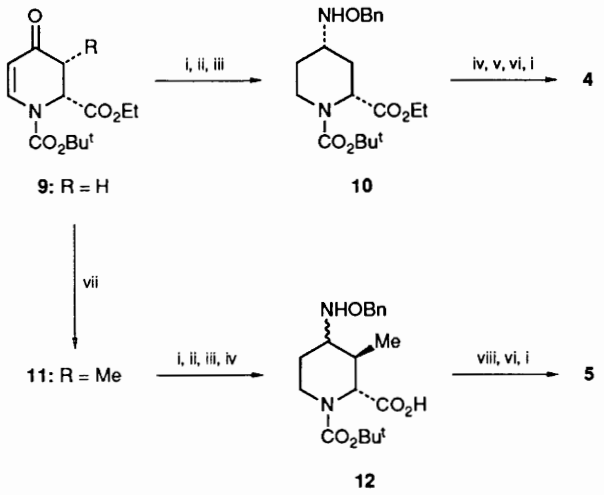




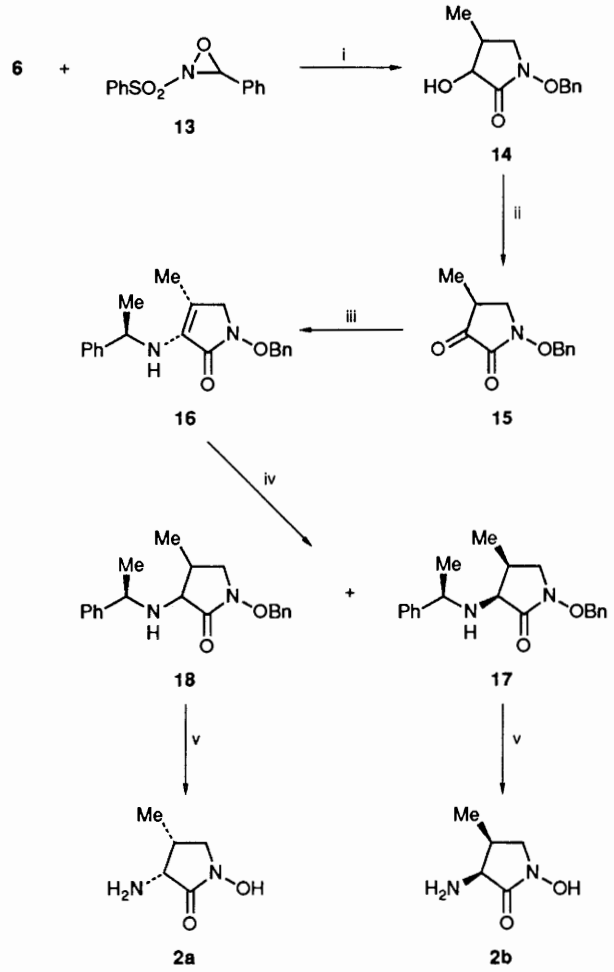
Scheme 1 *Reagents*: (i) H_2, Pd–C; (ii) Me_2CO, Dowex 50W-X8 (H^+ form); (iii) NH_2OBn, Et_3N; (iv) $NaCNBH_3$, HCl; (v) NaOMe, MeOH; (vi) $KN(SiMe_3)_2$, $Bu^tO_2C{-}N{=}N{-}CO_2Bu^t$; (vii) CF_3CO_2H; (viii) H_2, Pd–C; (ix) H_2,Pt (Bn = $PhCH_2$)

† All new compounds displayed spectral properties consistent with their proposed structures.

‡ Program written by Dr T. Halgren, Molecular Systems Group, MSDRL, Rahway, New Jersey.



Scheme 2 *Reagents*: (i) H_2, Pd–C; (ii) NH_2OBn, Et_3N; (iii) $NaCNBH_3$, H^+; (iv) NaOH; (v) cHex–N=C=N–cHex; (vi) CF_3CO_2H; (vii) (a) $KN(SiMe_3)_2$, (b) MeI; (viii) bis(2-oxo-3-oxazolidinyl)-phosphinic chloride, Et_3N



Scheme 3 *Reagents*: (i) $KN(SiMe_3)_2$, toluene, −90 °C; (ii) (a) Me_2SO $(CF_3CO)_2O$, (b) $NEt(Pr^i)_2$; (iii) *R*-PhCH(Me)NH_2, MeOH, 55 °C; (iv) (a) H_2, Pt, (b) $BnBr/K_2CO_3$; (v) H_2, $Pd(OH)_2$

difference between conformers **A** and **B** diminishes as activity increases, suggesting that the pseudoaxial conformer **A** may be recognised by the receptor. The bicyclic compounds **4** and **5** provide a test of this hypothesis, since they have rigid structures (conformation **C**) which mimic the axial conformers **A** of **1** and **2** respectively.

Piperidinone **9**[8] was hydrogenated, the resulting ketone converted to the *O*-benzyloxime, then reduction with sodium cyanoborohydride and separation of the resulting diastereoisomers gave the *cis* *O*-benzylhydroxylamine ester (**10**, Scheme 2). Ester hydrolysis, followed by cyclisation with dicyclohexylcarbodiimide and sequential deprotection by trifluoroacetic acid then hydrogenolysis, gave the [3.2.1]diazabicyclic analogue (**4**, m.p. 192 °C). Methylation of **9** gave the *cis* isomer **11** which was converted to a mixture of the 2,3-*trans* diastereoisomeric acids **12**, epimerisation occurring at the ester hydrolysis step. Cyclisation with bis(2-oxo-3-oxazolidinyl)phosphinic chloride in the presence of triethylamine, followed by deprotection, provided the *endo* methyl derivative (**5**, isolated as its tosylate salt, m.p. 226–228 °C). The structure **5** followed from ^1H NMR spectroscopy (D_2O) and was distinguished from the alternative *exo* isomer by the appearance of a selective nuclear Overhauser enhancement of the adjacent *exo* methylene proton (δ 2.17 ppm, =CH–CH_AH_B) after irradiation of the methyl group (δ 1.27 ppm). The bicyclic analogue **4** proved to be two-fold more potent than the monocycle **1**. In contrast to **2**, the presence of the methyl group in the rigid molecule **5** did not improve activity. The results imply that the bioactive conformers of **1** and **2** are pseudoaxial **A** and suggest that the enhanced activity of **2** may be attributable to an increase in the population of conformer **A** at the glycine site.

By analogy with **1a**,[4] the glycine antagonist activity of the racemic mixture **2** was predicted to be selectively associated with the 3*R*,4*R* enantiomer **2a**. This was confirmed by synthesis of the enantiomers **2a** and **2b**, using a route designed to introduce the required relative and absolute stereochemistries by reduction of a dehydro α-aminohydroxamic acid carrying a chiral auxiliary (**16**, Scheme 3). *In situ* capture of the enolate derived from **6** with the oxaziridine **13**[9] formed the alcohol **14**, which was oxidised to the ketone **15**. Treatment of **15** with *R*-α-methylbenzylamine§ in methanol resulted in formation of the endocyclic unsaturated aminohydroxamate **16**, which after reduction by hydrogen over Pt and rebenzylation, gave a 2:1 mixture of the diastereoisomers **17** and **18**. Separation of **17** and **18** by chromatography then hydrogenolysis using Pearlman's catalyst yielded the enantiomers of **2** {**2a**: $[\alpha]_D$ +16.5° (*c* = 0.48, MeOH); **2b**: $[\alpha]_D$ −15.0° (*c* = 0.31, MeOH)}, which were isolated as their D-(−) and L-(+) half tartrates respectively, and were >99% enantiomerically pure by chiral HPLC analysis. The absolute configurations of **2a** and **2b** were confirmed by X-ray crystallographic analysis of the hydrochloride salt of the *R*, 3*S*, 4*S* derivative **17**.

In established biological assays,[5] compound **2a** (L-687,414) is the most potent systematically active glycine/NMDA antagonist yet identified, being 5–10 fold more active than the desmethyl derivative **1a**, and is therefore a valuable tool for *in vivo* studies of this unique glycine site.

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§ Commercially available material (Aldrich Chemical Co.) had enantiometric excess (e.e.) 97% by chiral HLPC and was purified to e.e. >99.9% by crystallisation of the D-(−)-tartrate salt.

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