Synthesis of (S)-3,4-Diaminobutanenitriles as Precursors for 3-Amino-GABA Derivatives

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Starting from natural asparagine (1) a synthesis of the protected (S)-3,4-diaminobutanenitriles 5 and 8a-c via the β-homoserine derivative 2 is described. The amino function in position 4 was introduced by Mitsunobu-coupling or by reductive amination when a strange deformylation of the amino aldehyde 7 was observed as a side reaction. The Mitsunobu-product 5 was converted into the dibenzylamine substituted GABA 6b which was investigated for its affinity at the GABA-A receptor.

4-Aminobutyric acid (GABA) derivatives with substituents in position 3 have attracted major interest in recent years. Thus, 3-alkyl-4-aminobutyric acids have been described as the first anticonvulsants that activate L-glutamic acid decarboxylase. Furthermore, the 3-hydroxyl derivatives GABOB21 and carnitine31 are of considerable pharmacological and physiological relevance. The eumeridines A, B, C which show inhibitory activity on the long chain fatty acid oxidation, were identified as the acyl derivatives of (R)-3-amino-4-trimethylammonium butyric acid41. 3,4-Diaminobutyric acids51 are also of great importance as aspartic acid analogs in reduced peptide bond isosters and have been successfully employed in peptidomimetics of growth releasing-factor61, secretin71, cholecystokinin81, and tetragastrin91. On the other hand, 3,4-diaminobutanenitriles have been reported rarely10. To the best of our knowledge, syntheses of nonracemic members of this family of compounds have not yet been published.

We have recently shown that the β-homoserine derivative 2 which can be prepared from L-asparagine (1) in 61% overall yield, can serve as a valuable intermediate in the synthesis of enantiomERICALLY pure β-amino acids (3) (Scheme 1)11. For the construction of the respective side chains the hydroxyl function of 2 was activated by conversion into a methansulfonate and subsequently reacted with lower order organo cuprates or LiBH4. This strategy was also expected to provide a straightforward access to 3,4-diaminobutanenitriles and the respective amino acids. Employing amines as nucleophiles, however, did not give the projected displacement reaction. Instead, the aminobutenenitrile 4 was formed. We assume, that this is due to intramolecular attack of the sulfonate to give aziridinium intermediate followed by ring opening and deprotonation12.

To circumvent this side reaction we envisioned to introduce a phthalimido group as a precursor for a primary amine by Mitsunobu-coupling13 which usually works under very mild conditions. Thus, treatment of the β-homoserine derivative 2, prepared from L-asparagine (1)11,12, with phthalimide and PPh3/DEAD*) at room temp. afforded the substitution product 5 in 67% yield after flash chromatography (Scheme 2).

As an alternative for the synthesis of 3,4-diaminobutanenitrile derivatives, Swern oxidation of 2 followed by reductive amination was investigated. The projected N,N-dibenzylation aldehyde 714 could be prepared from 2 in 82% yield employing oxaly chloride, DMSO, and Et3N. Simple extraction of the reaction mixture afforded the analytically pure product. However, when 7 was stored at room temp. (or during our attempts to crystallize it) the elimination product 9 was isolated in almost quantitative yield. The
Experimental Part

General Remarks

THF was distilled from Na/benzophenone, and CH₂Cl₂ from CaH₂ immediately before use. All liquid reagents were also purified by distillation. Unless otherwise noted, reactions were conducted under dry N₂.

Evaporations of product solutions were done in vacuo with a rotary evaporator. Flash chromatography: 250-400 mesh silica gel. Melting points: Büchi melting point apparatus, uncorrected. IR spectra: Perkin Elmer 881 spectrometer. Mass spectra: Varian CH7 instrument. Methane was employed for CI-MS. NMR spectra: Jeol JNM-GX 400 spectrometer at 400 MHz, CDCl₃, tetramethylsilane as internal standard. Elemental analyses: Heraeus CHN Rapid instrument. trans-3-N,N-Dimethylamino-2-propenoneitrile was purchased from Aldrich Inc.

(S)-1-(2,2-N,N-Dibenzylamino-3-cyano)-propyl-N-phthalimide (5)

To a solution of 2 (1.02 g, 3.65 mmol), phthalimide (590 mg, 4.02 mmol), and PPh₃ (1.07 g, 4.02 mmol) in THF (70 ml) was added DEAD (1.82 ml, 4.02 mmol, 39% solution in toluene). After being stirred for 3 d at room temp, the solvent was evaporated and the residue was purified by flash chromatography (petrol ether - EtOAc 7:1). After being stirred for 3 d, the imido group remained untouched under the fairly harsh reaction conditions. Using 5 as an example, the cyano function was labelled with [3H]-GABA.

A mixture of 5 (440 mg, 1.07 mmol) in cone. aqueous HCl (40 ml) was stirred for 4 h at 80°C. After being cooled to room temp, the mixture was neutralized by NaHCO₃. After extraction with EtOAc the organic layer was washed with water, dried (MgSO₄) and evaporated. The residue was purified by flash chromatography (CH₂Cl₂ - MeOH 95:5) to give 6a (276 mg, 60%) as a colorless solid; mp. 161°C; [α]_D -16 (c = 0.127 ml, 2.57 mmol). After being stirred for 4 h at 80°C, the solvent was evaporated and the residue was purified by flash chromatography (petrol ether - EtOAc 7:1). To a solution of 2 (1.02 g, 3.65 mmol), phthalimide (590 mg, 4.02 mmol), and PPh₃ (1.07 g, 4.02 mmol) in THF (70 ml) was added DEAD (1.82 ml, 4.02 mmol, 39% solution in toluene). After being stirred for 3 d at room temp, the solvent was evaporated and the residue was purified by flash chromatography (petrol ether - EtOAc 7:1). The imido group remained untouched under the fairly harsh reaction conditions. Using 5 as an example, the cyano function was labelled with [3H]-GABA.

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Evaluation of the data did not show significant binding properties (IC₅₀ > 100 μM). However, further efforts are necessary to estimate the capability of 6b to interact with the GABA-B receptor (16), modulatory sites, the regulatory enzymes GABA aminotransferase, and glutamic acid decarboxylase (1) or transport proteins (17).

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Precursors of 3-Amino-GABA

(S)-3-NN-Dibenzylamino-3-formylpropane-1-nitrile (7)

To a solution of oxalyl chloride (0.20 ml, 2.36 mmol) in CH₂Cl₂ (2 ml) at -60°C was slowly added a solution of DMSO (0.34 ml, 4.74 mmol) in CH₂Cl₂ (2 ml). After being stirred for 15 min, a solution of 2 (600 mg, 4.74 mmol) was added at -60°C. 10 min later, H₂O (10 ml) was added and the pH was adjusted to 5 by aqueous citric acid (10%). Then, the mixture was extracted with CH₂Cl₂ and the org. layer was dried (MgSO₄). The pH was adjusted to 5 by aqueous citric acid (1%). Then, the mixture was brought to room temp. and stirring was continued for 20 h. The solvent was evaporated and Et₂O and satd. aqueous NaHCO₃ were added to the residue. The org. layer was dried (MgSO₄). To a solution of oxalyl chloride (0.20 ml, 2.36 mmol) in CH₂Cl₂ (2 ml) was added. Then the mixture was brought to room temp. and stirring was continued for 20 h. The solvent was evaporated and Et₂O and satd. aqueous NaHCO₃ were added to the residue. The org. layer was dried (MgSO₄).

(S)-3-NN-Dibenzylamino-4-(1-pyrrolidinyl)butane-1-nitrile (8a)

A mixture of 7 (80 mg, 0.29 mmol) in MeOH (10 ml) was added pyrrolidine - HCl. After being cooled to 0°C, NaCNBH₃ (32 mg, 0.46 mmol) was added. Then the mixture was brought to room temp. and stirring was continued for 20 h. The solvent was evaporated and Et₂O and satd. aqueous NaHCO₃ were added to the residue. The org. layer was dried (MgSO₄) and evaporated and the residue was purified by flash chromatography (n-hexane - acetone 85: 15) to give 8a (19 mg, 20%) as a colorless oil; [α]D₂⁰ -29 (c 0.18, CHCI₃). C₂₂H₂₇N₃ (333.5) Calcd. C 79.24 H 8.16 N 12.60 Found C 79.34 H 8.00 N 12.68; m/z-mass 334 (M + H)⁺ (EI-MS). IR (NaCl): 3340; 3030; 2930; 2240; 1730 cm⁻¹. -¹H-NMR (CDCl₃): δ (ppm) 1.22 (d, J = 13.5 Hz, 2H, CH₂N), 3.60-3.67 (m, 2H, 4-H), 3.66 (d, J = 13.5 Hz, 2H, PhCH₃N), 3.70-3.74 (m, 10 H arom.), 9.61 (s, 1H, CHO).

Bonding Experiments

The affinity of our compound for GABA-A receptors was determined according to standard radioligand binding assays¹⁸,¹⁹ which were slightly modified as described below:

Membrane preparation: Bovine brains were obtained from a local slaughter house. Cortices were dissected and homogenized with a Potter (PotterS Braun, 800 rpm, 8 up-and-down strokes) in 10 vol. of ice-cold 0.32 M sucrose and centrifuged at 1000xg for 10 min at 4°C. The supernatant was centrifuged at 48000xg for 60 min at 4°C. The resulting pellet was homogenized in 20 vol. aqua bident with a Polytron (Kinetica) and centrifuged at 48000xg for 30 min at 4°C. Osmotic shock and following centrifugation were repeated, the resulting pellet was centrifuged at 48000xg for 30 min at 4°C. The affinity of our compound for GABA-A receptors was determined according to standard radioligand binding assays¹⁸,¹⁹ which were slightly modified as described below:

₁⁻³H]-GABA binding: Homogenate (about 250 µg protein) was incubated in 500 µl of medium containing 50 mM Tris-citrate pH 7.1, about 3 nM ₁⁻³H]-GABA (DuPont NEN), and various concentrations of competing drugs for 15 min at 4°C in 1.5 ml Eppendorf caps. The samples were centrifuged at 20000 rpm (Sorvall RC 5C, SS34 rotor, adapter for Eppendorf caps) for 10 min at 4°C. The supernatants were discarded and the pellets were twice rinsed superficially with 1 ml cold buffer. The tips of the Eppendorf caps containing the rinsed pellets were cut off and put into scintillation vials which were filled with scintillation cocktail (rotiszint eco plus). Bound radioactivity was determined by liquid scintillation spectrometry (Canberra Packard TriCarb 1600) after 18 h. Non specific binding was defined using 100 µM GABA.

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For optical purity studies, the crude product (before flash chromatography) was investigated, compared to the 1:1 mixture of diastereomers obtained from 7 and rac: alanine ethyl ester - HCl [¹⁻³H]-NMR (CDCl₃): δ (ppm) = 1.23-1.30 (m, 12H, 3-H, CH₂CH₂), 2.48-2.55 (m, J = 11.7, 8.8, 6.6 Hz, 4H, 3'-H, 1'-H), 2.60 (dd, J = 16.9, 5.9 Hz, 1H, 3'-H), 2.71 (dd, J = 12.5, 7.3 Hz, 1H, 1'-H), 2.82 (dd, J = 12.5, 7.3 Hz, 1H, 1'-H), 2.97 (dd, J = 11.0, 7.3 Hz, 1H, 1'-H), 3.13-3.25 (m, J = 7.3, 6.6 Hz, 4H, 2'-H, 2'-H), 3.59 (d, J = 13.9 Hz, 2H, PhCH₃N), 3.68 (d, J = 14.7 Hz, 2H, PhCH₃N), 3.71 (d, J = 14.7 Hz, 2H, PhCH₃N), 3.78 (d, J = 13.9 Hz, 2H, PhCH₃N), 4.14-4.22 (m, 4H, CH₂O).
References

14. For review on the versatility of N,N-dibenzyamino aldehydes as synthetic intermediates, see: M.T. Reetz, Angew. Chem. 1991, 103, 1559-1573.
15. M.T. Reetz, private communication.