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The first synthesis and molecular docking studies of diastereomerically pure substituted 4-amino-7-hydroxyheptanoic acids

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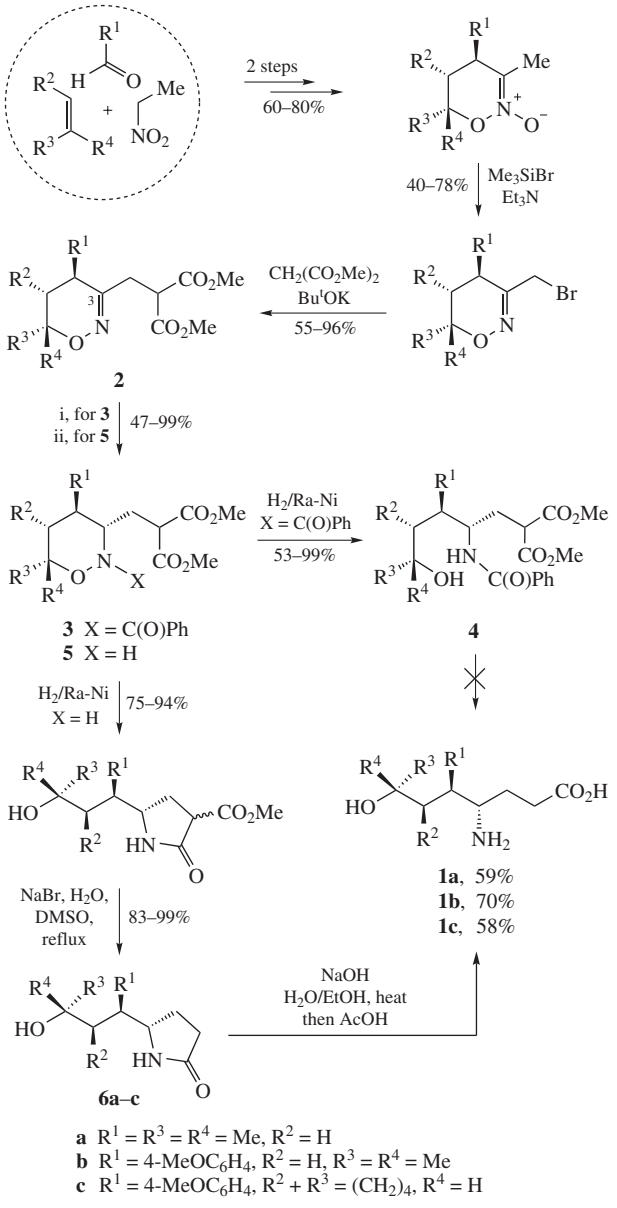
Diastereoselective synthesis of 4-amino-7-hydroxyheptanoic acids 1, new GABA analogues, was performed involving reduction of C-3 functionalized 5,6-dihydro-4*H*-1,2-oxazines available from nitroethane. Molecular docking studies showed that amino acids of type 1 may bind to GABA transaminase, however, no inhibition was observed in the experiments with the enzyme.

Homologues of α-amino acids attract considerable attention due to a unique role in living systems,[1] as building blocks for β- and γ-peptides with uncommon structure and biological activities,[2] as well as medically active substances.[1(a),3] The development of novel approaches to the stereoselective synthesis of natural and especially unnatural β-[4(a),(b)] and γ-amino acids[4(c)] for many years holds the place among the most challenging problems of organic synthesis.

Previously unknown 4-amino-7-hydroxyheptanoic acids **1** (Scheme 1) can be considered as γ-substituted analogues of γ-aminobutyric acid (GABA) which is the major inhibitory neurotransmitter in central nervous system (CNS).[1,5] The deficiency of GABA in brain is associated with such serious CNS disorders as epilepsy, Parkinson's disease, West's syndrome.[5(b),(c)] Treatment of these diseases using GABA itself is not efficient due its low lipophilicity and therefore poor ability to cross the blood-brain barrier.[5(d)] In this situation the synthesis of more lipophilic C-substituted analogues of GABA and GABA transaminase (the enzyme that degrades GABA) inhibitors becomes an important task of medicinal chemistry. In the recent years a number of such antiepileptic and anticonvulsant drugs has been introduced (Vigabatrin®, Baclon®, Pregabalin®) and commercialized in optically pure and racemic forms.[1,4(c),5(e)] The new class of γ-substituted GABAs **1** bearing a branched alkyl fragment with variable substituents and an additional hydroxyl group may give rise to a series of drug candidates with different combinations of lipophilicity and solubility in water. Therefore, the synthesis of 4-amino-7-hydroxyheptanoic acids **1** seems reasonable.

Recently we presented an original strategy for the stereoselective synthesis of γ-amino acids **1** containing several contiguous stereogenic centers (Scheme 1).[6(a),(b),†] This strategy employs the assembly of C-3 functionalized dihydrooxazines **2** from available nitroethane, aldehydes and olefins followed by their stereoselective reduction to N-benzoylated tetrahydro-2*H*-1,2-oxazines **3** and catalytic hydrogenation of the latter. However, unprotected 4-amino-7-hydroxyheptanoic acids **1** could not be synthesized because of difficulties with cleavage of benzamide group in intermediates **4** (Scheme 1).[6(a)] The introduction of benzoyl protective group at the nitrogen atom was necessary to prevent the lactamization of tetrahydro-2*H*-1,2-oxazines **5** under the reduction conditions of dihydrooxazines **2**.[6(a)]



Scheme 1 *Reagents and conditions*: i, $NaBH_3CN$, AcOH, ClC(O)Ph, 2 h; ii, $NaBH_3CN$, AcOH, 20 min.

† For other strategies of amino acids synthesis *via* dihydrooxazines, see refs. 6(*c*)–(*g*).

Here we report a protective group-free synthesis of diastereomerically pure 7-hydroxy-4-aminoheptanoic acids **1a–c** from dihydrooxazines **2a–c** (Scheme 1).‡

For this purpose the reduction procedure **2** → **5** was improved allowing to isolate individual tetrahydrooxazines **5** (procedure ii in Scheme 1).[7] The latter were then selectively converted into diastereomerically pure lactams of γ-amino acids **1** (products **6**) in high yields.[7] The hydrolysis of products **6a–c** with sodium hydroxide in refluxing aqueous ethanol[8] leads to a cleavage of the lactam ring furnishing target γ-amino acids **1a–c** in moderate to good yields (Scheme 1). As demonstrated on the example of **6b** the alternative procedure for the hydrolysis of lactams using aqueous hydrochloric acid[9] resulted in an unidentified mixture of compounds containing only trace amounts of the desired product **1b** (<5%).

After chromatography on silica gel or on ion-exchange resin DOWEX 50WX2 pure samples of γ-amino acids **1a–c** were obtained as individual diastereomers. Their structure was confirmed by ^{1}H, ^{13}C NMR, COSY, HSQC spectroscopy data, as well as by elemental analysis. The brutto-formula of amino acid **1c** was additionally confirmed by high resolution mass spectrometry data (micrOTOF).

Solid γ-amino acids **1a–c** are stable from room temperature to 100 °C. Upon melting (130–150 °C) γ-amino acids **1a–c** are converted into corresponding lactams **6a–c**. The amino acid **1b** in D_2O solution does not undergo lactamization below 50 °C. However, upon evaporation of the water solution *in vacuo* at 60–70 °C the formation of lactam **6b** is already noticeable. Therefore, all operations with solutions of γ-amino acids **1a–c** should be conducted at temperature not higher than 50 °C.

γ-Aminoheptanoic acids **1a–c** are soluble in water to the extent of 175 (**1a**), 26 (**1b**) and 4 (**1c**) mg cm^{-3} and have the calculated values of distribution coefficients log *D*: –2.39 (**1a**), –1.27 (**1b**), –0.66 (**1c**).§ For comparison the experimental and calculated (given in brackets) log *D* coefficients are for GABA: –3.09[10] (–3.14), for γ-vinyl-substituted GABA (vigabatrin): –2.16[11] (–2.60). Evidently, γ-aminoheptanoic acids **1a–c** should be more lipophilic than GABA. This may favor more efficient blood-brain barrier permeability and makes γ-aminoheptanoic acids **1a–c** perspective objects to study binding to GABA receptors as well as the inhibition of GABA transaminase (GABA-T).

Since the full atom model of GABA-T is available (in contrast to GABA receptors), molecular docking of amino acids **1** to this enzyme could be performed.¶ The cognate ligand (γ-ethynyl-GABA) of 1OHY crystal structure forms two covalent bonds (one with PLP cofactor and one with K357 protein residue) in the active site while GABA and amino acids **1** form only one covalent bond with PLP. Thus, it was anticipated that position of residue K357 in complexes with GABA and **1** should differ from that in complex with γ-ethynyl-GABA. Therefore, we modeled position of K357 sidechain by its covalent docking to the enzyme in order to determine its most realistic placement in complexes with inhibitors forming one covalent bond.

Structural analysis of GABA-T complex with its irreversible inhibitor γ-ethynyl-GABA revealed that a salt bridge between the enzyme's R220 residue and the ligand's carboxyl group was formed (Figure 1) so it was reasonable to expect that these inter-

‡ *General procedure for preparation of γ-amino acids* **1a–c**. A solution of 5-(3-hydroxypropyl)-2-pyrrolidone **6a–c**[7] (1.08 mmol) and NaOH (2.16 g, 54 mmol) in 7.6 ml of EtOH–H_2O (1:1) mixture was stirred under reflux for 2 h (for **6a**,**b**) or 3 h (for **6c**). After the consumption of starting material (TLC control), the solution was cooled to 0–5 °C and acetic acid (3.2 ml) was added dropwise to reach pH 7. The resulting mixture was concentrated *in vacuo* at 40–50 °C and dried. Then 150 ml of $CHCl_3$ (for **1a**,**b**) or THF (for **1c**) was added to the residual solid. The mixture was stirred at 40–50 °C for approximately 20 min, filtered and the filtrate was concentrated *in vacuo*. The residue was subjected to a flash-chromatography on silica gel [Chemapol, silica gel L 40/100, AcOEt–hexane (1:3) → AcOEt → AcOEt–MeOH (10:1 → 3:1 → 1:1)] to give NMR-pure samples of target amino acids as white solids (yields are summarized in Scheme 1). Ion-exchange chromatography on DOWEX 50WX2 (eluent H_2O–MeCN → 5% NH_3 in H_2O → 10% NH_3 in H_2O) provides γ-amino acids **1b**,**c** in similar yields. For analytical purposes products **1a–c** were recrystallized from CH_2Cl_2/MeOH and dried at 90–100 °C/0.2–0.5 Torr.

(4S,5R*)-4-Amino-7-hydroxy-5,7-dimethyloctanoic acid* **1a**. Yield 59%, mp 151–153 °C (with cyclization to **6a**). R_f 0.32 [EtOAc–MeOH (1:1), Alugram® Sil G/UV$_{254}$]. ^{1}H NMR (500 MHz, DMSO-d_6, COSY, HSQC) δ: 0.91 (d, 3H, Me, *J* 6.8 Hz), 1.11 (s, 6H, 2Me), 1.20 (dd, 1H, C*H*$_2$CMe$_2$OH, *J* 5.6 and 14.5 Hz), 1.42 (m, 1H, C*H*$_2$CH$_2$CO$_2$H), 1.46 (dd, 1H, C*H*$_2$CMe$_2$OH, *J* 5.1 and 14.5 Hz), 1.57 (m, 1H, C*H*$_2$CH$_2$CO$_2$H), 1.83 (m, 2H, C*H*Me, OH), 2.17 (m, 1H, CH$_2$C*H*$_2$CO$_2$H), 2.29 (m, 1H, CH$_2$C*H*$_2$CO$_2$H), 2.89 (m, 1H, C*H*NH$_2$), 6.24 (br., 3H, CO$_2$H, NH$_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 16.8 (*Me*CH), 25.1 (*C*H$_2$CH$_2$CO$_2$H), 29.1 and 30.5 (2Me), 32.6 (*C*HMe), 36.0 (CH$_2$*C*H$_2$CO$_2$H), 46.3 (*C*H$_2$CMe$_2$OH), 56.4 (*C*HNH$_2$), 69.0 (Me$_2$*C*OH), 175.6 (CO$_2$H). Found (%): C, 58.52; H, 10.74; N, 6.84. Calc. for $C_{10}H_{21}NO_3$ (%): C, 59.08; H, 10.41; N, 6.89.

(4S,5S*)-4-Amino-7-hydroxy-7-methyl-5-(4-methoxyphenyl)octanoic acid* **1b**. Yield 70%, mp 126–131 °C (with cyclization to **6b**). R_f 0.28 [EtOAc–MeOH (1:1), Alugram® Sil G/UV$_{254}$]. ^{1}H NMR (300 MHz, DMSO-d_6, COSY, HSQC) δ: 0.92 and 0.95 (2s, 6H, 2Me), 1.22 (m, 1H, C*H*$_2$CH$_2$CO$_2$H), 1.43 (m, 1H, C*H*$_2$CH$_2$CO$_2$H), 1.71 (dd, 1H, C*H*$_2$CMe$_2$OH, *J* 7.3 and 13.9 Hz), 1.92 (dd, 1H, C*H*$_2$CMe$_2$OH, *J* 2.2 and 13.9 Hz), 2.07–2.20 (m, 2H, CH$_2$C*H*$_2$CO$_2$H), 2.82 (m, 1H, C*H*C$_6$H$_4$OMe), 2.89 (m, 1H, C*H*NH$_2$), 3.71 (s, 3H, OMe), 6.30 (br., 4H, CO$_2$H, NH$_2$, OH), 6.84 (d, 2H, *o*-C$_6$*H*$_4$OMe, *J* 8.5 Hz), 7.11 (d, 2H, *m*-C$_6$*H*$_4$OMe, *J* 8.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 27.8 (*C*H$_2$CH$_2$CO$_2$H), 28.3 and 31.5 (2Me), 34.7 (CH$_2$*C*H$_2$CO$_2$H), 45.1 (*C*HC$_6$H$_4$OMe), 46.4 (*C*H$_2$CMe$_2$OH), 54.9 (OMe), 56.7 (CHNH$_2$), 69.0 (Me$_2$*C*OH), 113.7 (*o*-*C*$_6$H$_4$OMe), 129.3 (*m*-*C*$_6$H$_4$OMe), 135.7 (*i*-*C*$_6$H$_4$OMe), 157.6 (*C*OMe), 175.6 (CO$_2$H). Found (%): C, 65.07; H, 8.42; N, 4.77. Calc. for $C_{16}H_{25}NO_4$ (%): C, 65.06; H, 8.53; N, 4.74.

(4S,5S*)-4-Amino-5-[(1R,2R)-2-hydroxycyclohexyl]-5-(4-methoxyphenyl)pentanoic acid* **1c**. Yield 58%, mp 130–133 °C (with cyclization to **6c**). R_f 0.17 [EtOAc–MeOH (1:1), Alugram® Sil G/UV$_{254}$]. ^{1}H NMR (300 MHz, DMSO-d_6, COSY, HSQC) δ: 0.88 (m, 1H, CH$_2$ of cyclohexane ring), 0.99–1.11 (m, 2H, CH$_2$ of cyclohexane ring), 1.29 (m, 1H, CH$_2$ of cyclohexane ring), 1.36–1.55 (m, 3H, CH$_2$ of cyclohexane ring), 1.15 (m, 1H, C*H*$_2$CH$_2$CO$_2$H), 1.70–1.81 (m, 3H, C*H*$_2$CH$_2$CO$_2$H, C*H*CH$_2$ of cyclohexane ring, CH$_2$ of cyclohexane ring), 2.13–2.23 (m, 2H, CH$_2$C*H*$_2$CO$_2$H), 2.74 (dd, 1H, C*H*C$_6$H$_4$OMe, *J* 3.42 and 10.7 Hz), 3.39 (m, 1H, C*H*NH$_2$), 3.72 (s, 3H, OMe), 3.96 (m, 1H, C*H*OH), 5.58 (br., 4H, CO$_2$H, NH$_2$, OH), 6.86 (d, 2H, *o*-C$_6$*H*$_4$OMe, *J* 8.1 Hz), 7.08 (d, 2H, *m*-C$_6$*H*$_4$OMe, *J* 8.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.7 (CH$_2$ of cyclohexane ring), 25.5 and 25.6 (2CH$_2$ of cyclohexane ring, *C*H$_2$CH$_2$CO$_2$H), 33.4 (CH$_2$ of cyclohexane ring), 35.3 (CH$_2$*C*H$_2$CO$_2$H), 42.4 (*C*HCH$_2$ of cyclohexane ring), 50.5 (*C*HC$_6$H$_4$OMe), 51.4 (CHNH$_2$), 54.9 (OMe), 64.1 (CHOH), 113.6 (*o*-*C*$_6$H$_4$OMe), 130.7 (*m*-*C*$_6$H$_4$OMe), 130.8 (*i*-*C*$_6$H$_4$OMe), 157.9 (*C*OMe), 175.6 (CO$_2$H). HRMS (micrOTOF II, negative ion mode) *m*/*z*: 320.1869 (calc. for $C_{18}H_{26}NO_4$ [M – H]$^-$: 320.1856, Δ = 4.1 ppm). Found (%): C, 65.85; H, 8.57; N, 4.16. Calc. for $2C_{18}H_{27}NO_4 \cdot H_2O$ (%): C, 65.43; H, 8.54; N, 4.24.

§ Log *D* values were calculated using ACD Labs/LogD Software for standard conditions (25 °C and zero ionic strength) at pH 7.4.

¶ Full-atom model of GABA-T used in the current study was prepared from the raw PDB structure 1OHY by adding hydrogen atoms and assigning ionization states of amino acids with the use of the Model Builder program of the Lead Finder software package. To reconstruct the binding site geometry optimization of K357 was performed with the Lead Finder v.1.1.13 software[12,13] *via* covalent docking of lysine sidechain. Model was validated by covalent docking its cognate ligand (γ-ethynyl-GABA). Root mean square deviation (rmsd) of docked ligand pose from its crystallographic position was 0.29 Å.

Covalent docking of the sets of compounds **1** to the prepared model of GABA-T and binding energy calculations was performed with the Lead Finder v.1.1.13 software[12,13] under its default settings. The bond between ligand amino group and PLP carbonyl C atom was specified to enable covalent docking. Only the top-ranked poses were used for structural and energy analyses.

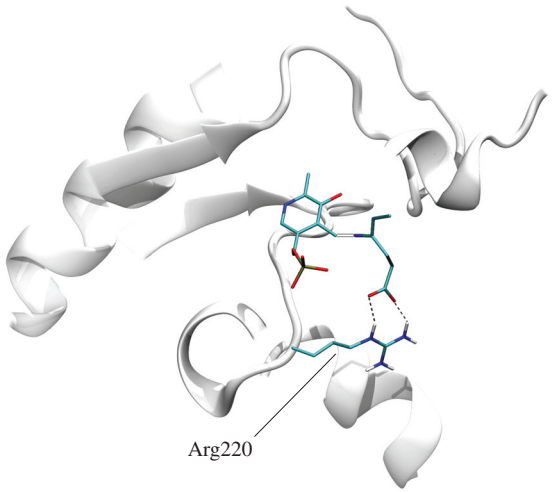


Figure 1 Binding of γ-ethynyl-GABA to GABA-T (PDB ID 1OHY).

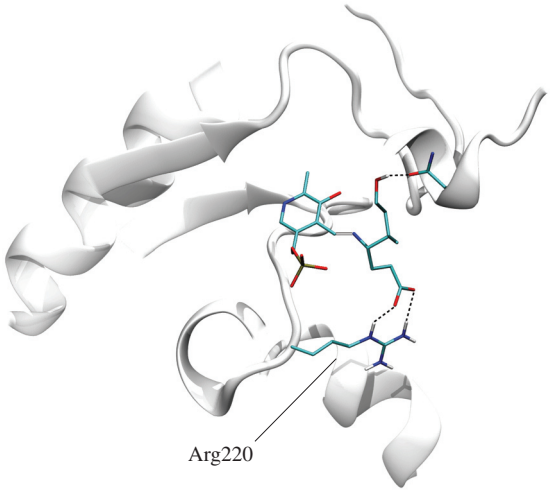


Figure 2 Binding of **1a** to GABA-T (as predicted by molecular docking).

actions were present in other enzyme–inhibitor complexes as well. Compounds of general formula **1** did form such salt bridges with R220. Visual analysis of the enzyme–ligand complexes revealed that the binding pocket was rather small to encompass large molecules. Thus, only the smallest derivative of amino acids **1** (product **1a**) could be successfully docked to GABA-T. This compound formed a salt bridge with R220 residue and, moreover, it formed an additional hydrogen bond with the backbone carbonyl oxygen of residue I100 (Figure 2).

However, inhibitory action of **1a** on GABA-T activity was not observed in an experiment [concentrations of **1a** and substrate (GABA) were *ca.* 10 mmol dm^{-3}].[††] Due to objective restrictions of the analytic technique used in that experiment we were not able to discriminate whether the absence of inhibitory activity of the ligand was due to a high saturating concentration of GABA, or the ligand indeed was less potent binder than the GABA itself. Anyway we can state that the described modification in the structure of ligand **1a** compared to GABA did not contribute to the potency of interaction with GABA-T. We may further suggest that this absence of inhibitory activity could be attributed to the reduction of amino group reactivity in **1a** as compared to GABA due to a more steric hindrance, rather than to its steric incomplementarity to GABA-T active site, as can be judged from our docking simulations. Further studies of the interaction of amino acids **1** with GABA receptors seem reasonable.

Thus, the first synthesis of diastereomerically pure 4-amino-7-hydroxyheptanoic acids **1** starting from nitroethane and available reagents has been developed. Preliminary studies of the biological activity of substituted GABA analogues **1** were conducted.

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[††] GABA-T inhibition assay was performed at Cerep, France. GABA-T from rat brain was incubated with testing compound, 9 mmol dm^{-3} GABA and 9 mmol dm^{-3} γ-ketoglutarate for 60 min at 37 °C. Fluorimetric quantification of succinic semialdehyde–3,5 diaminobenzoic acid adduct was used to estimate the enzyme activity.[14]