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Synthesis and chromatographic resolution of conformationally constrained analogues of homotaurine

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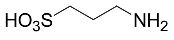
ABSTRACT

The first series of conformationally constrained analogues of homotaurine is reported. The partial constriction of the skeleton was realized through the insertion of a cyclopropyl ring, between the α,β- and β,γ-positions, thus affording, respectively, *trans*- and *cis*-2-aminomethylcyclopropane-1-sulfonic acids and *trans*- and *cis*-(2-aminocyclopropyl)methanesulfonic acids. The resolution of all four racemic mixtures was accomplished using HPLC system carrying the polysaccharide-based Chiralpak® IB® column as the chiral stationary phase. The coupling with an 'Evaporative Light Scattering Detector (ELSD)' has been particularly valuable during the chromatographic study.

1. Introduction

Homotaurine (3-aminopropane sulfonic acid, **1**) is a sulphur-containing amino acid that has attracted much attention in recent years, as a drug candidate for the treatment of Alzheimer's disease and haemorrhagic stroke (tramiprosate, Alzhemed™ and Cerebril™).[1] This small ionic compound binds to soluble amyloid β-peptide (Aβ) and decreases Aβ42-induced cell death in neuronal cell culture.[2] The reduction or inhibition of amyloid deposition in brain and cerebral vasculature is believed to be responsible for the beneficial effects observed in vivo studies.[1] Moreover acamprosate (Campral®, **2**), a synthetic compound structurally related to **1**, is one of the few medications currently approved, both in Europe and USA, for the prevention of alcohol relapse in detoxified alcohol-dependent patients.[3] Although its mechanism of action has been not yet fully defined, acamprosate (**2**) was originally proposed as a GABA analogue. Homotaurine (**1**), indeed, may well be considered as a bioisosteres of GABA. Whereas conformationally restricted GABA analogues,[4] such as *cis*-2-aminomethylcyclopropane-1-carboxylic acid [CAMP, (±)-**3**] and *trans*-2-aminomethylcyclopropane-1-carboxylic acid [TAMP, (±)-**4**] among others, have long been used as pharmacological tools to study GABA receptor subtypes,[5] conformationally constrained analogues of homotaurine (**1**) are so far unknown. Thus, as a part of our continuing interest in the synthesis and biological applications of conformationally constrained amino acids, we became interested in the preparation of homotaurine analogues incorporating the cyclopropane as the ring moiety to rigidify the carbon skeleton. The ring insertion was realized between the α,β- and β,γ-positions of **1**, thus affording, *trans*- and *cis*-2-aminomethylcyclopropane-1-sulfonic acids [*t*-AMCSA (±)-**5**, and *c*-AMCSA (±)-**6**] and *trans*- and *cis*-(2-aminocyclopropyl)-methanesulfonic acids [*t*-ACMSA, (±)-**7** and *c*-ACMSA, (±)-**8**], respectively.



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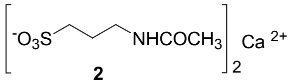

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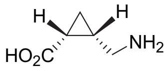
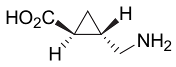
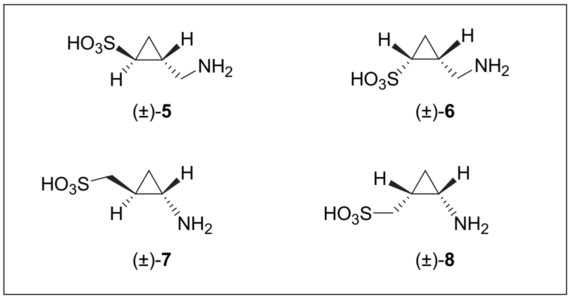


(±)-3



(±)-4



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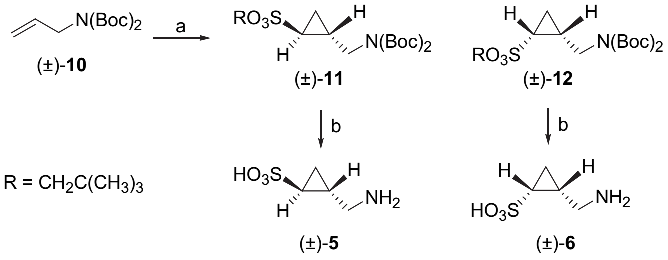
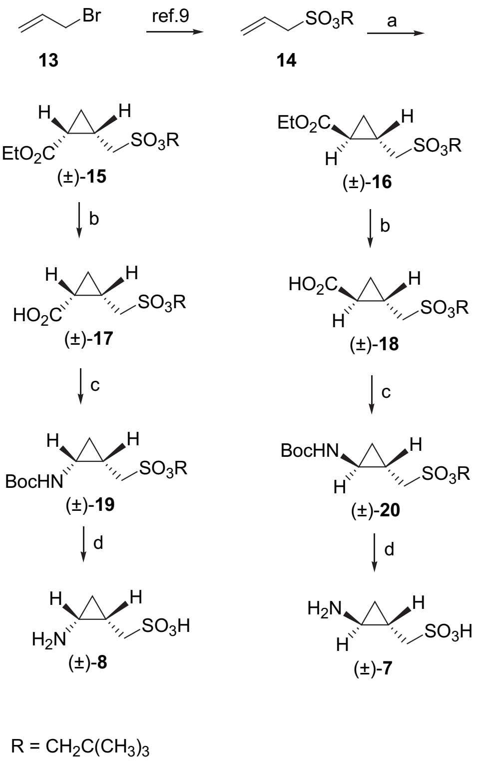
2. Results and discussion

The preparation of *t*-AMCSA [(±)-**5**] and *c*-AMCSA [(±)-**6**], as depicted in Scheme 1, involved as the key step the copper(II) acetylacetonate-catalyzed reaction of *neo*pentyl α-diazomethanesulfonate (DAMS)[6] with di(*tert*-butyl)allylimidodicarbonate (**10**), prepared starting from allylamine as described in the literature.[7] The cyclopropanation reaction proceeded with low conversion (30%) affording a mixture of the two possible diastereoisomers, in a 6:4 ratio (calculated by GC). Flash chromatography of the reaction mixture allowed us to recover the starting amine **10** (42%), followed by a less polar (±)-**11** (10%) and a more polar fraction (±)-**12** (7.5%), which were shown by spectroscopic analysis to be *trans*- and *cis*-cyclopropyl sulfonates. A proof of the spatial disposition between H-1 and H-2 is given by chemical shift values of H-2, which is more deshielded in the diastereoisomer (±)-**11** [1.99 ppm against 1.75 ppm for (±)-**12**] due to the proximity with the sulfonate moiety. On the other hand, in the diastereoisomer (±)-**12**, the deshielding effect of the sulfonate group is evident on the chemical shift of the α-aminomethylene [3.93 ppm against 3.75–3.84 ppm for (±)-**11**]. Also diagnostic, in this respect, were the chemical shifts of the C-1′ carbon atom, which was upfield by ca. 3 ppm in (±)-**12** [*vis-à-vis* (±)-**11**] due to γ-effect caused by the sulfonate group in *syn*-axial relationship.[8] The racemic mixture (±)-**11** was then submitted to acidic hydrolysis and ion-exchange resin chromatography to afford *t*-AMCSA [(±)-**5**] in 94% yield. By an analogous procedure, starting from the racemic mixture (±)-**12**, *c*-AMCSA [(±)-**6**] was obtained in 95% yield. The stereochemical assignments, described above, were supported by NOESY experiments performed on the final compounds: in the case of *t*-AMCSA [(±)-**5**], a strong NOE effect occurs between H-1 and the α-aminomethylene, whereas a NOE was observed between H-1 and H-2 in the spectrum of *c*-AMCSA [(±)-**6**].



Scheme 1. Reagents and conditions: (a) *i.* DAMS, $Cu(acac)_2$, CH_2Cl_2, 2 h; *ii.* Flash chromatography. (b) *i.* 6 N HCl, 95 °C, 18–24 h; *ii* Dowex 50WX2-200, H_2O.

The conformationally constrained analogues of homotaurine (**1**), characterized by the insertion of one methylene unit between the sulfonic acid moiety and the three-membered ring, namely *t*-ACMSA [(±)-**7**] and *c*-ACMSA [(±)-**8**], were prepared starting from *neo*pentyl prop-2-ene-1-sulfonate (**14**), in turn obtained from 3-bromopropene (**13**) using standard procedures[9] (Scheme 2). Thus, dirhodium tetraacetate-catalyzed cyclopropanation of **14** with ethyl diazoacetate (EDA) afforded the mixture of the two expected diastereoisomers, *cis*- and *trans*-ethyl 2-{[(*neo*pentyloxy)sulfonyl]-methyl}cyclopropane carboxylates [(±)-**15** and (±)-**16**] in a 3:7 ratio (calculated by GC).



Scheme 2. Reagents and conditions: (a) *i.* EDA, $[Rh(OAc)_2]_2$, CH_2Cl_2; *ii.* Flash chromatography. (b) 0.5 M aqueous NaOH, EtOH, 72 h; (c) *i.* EtOCOCl, Et_3N, THF, 0 °C, 30 min; *ii.* Aqueous NaN_3, 0 °C, 40 min; *iii.* Toluene, 90 °C, 40 min; *iv.* *t*-BuOH, 90 °C, 72 h; (d) *i.* 6 N HCl, 95 °C, 18–24 h; *ii* Dowex 50WX2-200, H_2O.

After flash chromatography, the desired cyclopropyl esters (±)-**15** and (±)-**16** were recovered in 34% and 61% yields, respectively. Also in this case the relative stereochemistries of the two racemic mixtures, (±)-**15** and (±)-**16**, were determined by spectroscopic analysis. In particular, clearly diagnostic was the strong γ-effect exerted by the carboxylate moiety on the C-1′ carbon atom of (±)-**15** [47.8 ppm against 54.2 ppm for (±)-**16**]. Partial hydrolysis of the *cis*-isomer (±)-**15** with aqueous NaOH furnished the carboxylic acid (±)-**17**, which was converted into the corresponding acyl azide via the mixed anhydride (ethyl chloroformate, triethylamine, then aqueous sodium azide). The crude acyl azide, thus obtained, was then refluxed in *tert*-butyl alcohol to give the *N*-Boc-protected amino sulfonate (±)-**19**, which was hydrolyzed under acidic conditions to afford, after purification by ion-exchange resin chromatography, *c*-ACMSA [(±)-**8**]. The synthetic protocol described above—[alkaline hydrolysis, Curtius rearrangement, acidic hydrolysis and purification]—was then applied to the *trans*-cyclopropyl isomer (±)-**16**, thus obtaining *t*-ACMSA [(±)-**7**]. The proposed stereochemistry was corroborated by NOESY correlations, performed on the derivatives obtained by the Curtius rearrangement, namely (±)-**19** and (±)-**20**; highly significant effects were observed between H-2 and the hydrogens in the α-position with respect to the sulfonate group in the *trans*-diastereoisomer (±)-**20**, whereas a NOE was observed between H-1 and H-2 in the case of the *cis*-derivative (±)-**19**.

It is well known that enantiomers of chiral compounds exert different pharmacological effects in a number of biological systems. Although it is rare that opposite effects are observed at a single target site, it occurs in the case of (±)-CAMP [(±)-**3**], with the enantiomers exhibiting opposite pharmacological activity at $GABA_C$ receptors.[10] Accordingly, in view of the necessity to obtain single enantiomers of the prepared racemic compounds, we devised an HPLC-based analytical strategy aimed at both separating and resolving the enantiomeric couples so as to be able to perform subsequent chromatographic preparative scale-up. The enantioseparation study was performed on the racemic compounds (±)-**11**, (±)-**12**, (±)-**19** and (±)-**20**. The presence of additional functionalities in the basic molecular structure is often exploited in chiral chromatography as an attempt to provide for efficient enantiodiscriminating interactions between the analyte and the chiral stationary phase (CSP). The engagement of the polysaccharide-based Chiralpak® IB® stationary phase was dictated by the huge number of works performed with this CSP, which emphasize this chiral stationary phase as the best choice when both analytical- and preparative-scale applications are planned. Additionally, the covalent linkage of the chiral selector expands its solvent compatibility range thus providing for new potential selectivity profiles.[11] Even though the possibility to work in a reversed-phase mode, the high solubility of our compounds in hexane/alcohol(s) mixtures led us to rely upon the conventional utilization of the normal phase for this cellulose-based CSP. The absence of relevant chromophoric moieties on the analytes, prompted us to adopt the 'mass sensitive' Evaporative Light Scattering Detector (ELSD). Moreover, the improved technology of our detector allows us to work at low nebulisation and evaporation temperatures (T_{neb} and T_{evap}, respectively), which are required in the presence of low-boiling point eluents usually employed in a normal phase environment. The possibility to fix the T_{neb} value and, in turn, to generate opportunely sized particles, facilitates the subsequent evaporation process thus providing for reduced baseline noise and higher sensitivity at once. The influence of the mobile phase composition as well as of all the detector tuneable parameters (T_{neb}, T_{evap}, *gain* factor, gas flow rate) upon the overall chromatographic performance were evaluated in the course of the enantiodiscrimination study. The best separation for (±)-**20** enantiomers was achieved with the mixture *n*-hexane/2-propanol (IPA)-95/5 (v/v) as the mobile phase. With a 15 °C column temperature and a flow rate of 0.8 mL/min, this eluent provided very appreciable enantioseparation (α=1.13) and resolution (R_S=3.41) factors (Fig. 1a). The optimal ELSD conditions, adopted for all analyses, were 25 °C as the T_{neb}, 25 °C as the T_{evap}, 1.0 L/min as the gas flow rate and 1.0 as the *gain* factor.

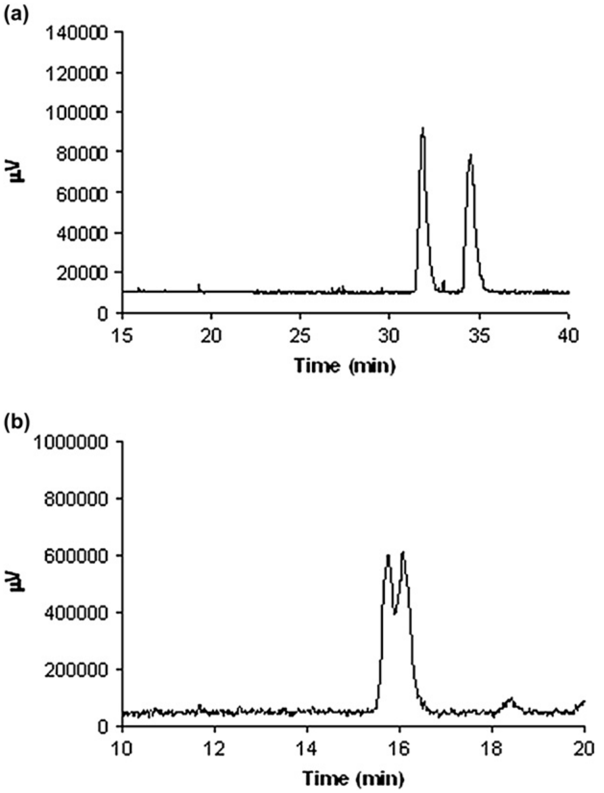


Figure 1. Chromatographic traces of (a) (±)-**20** and (b) (±)-**19** enantiomeric couples. ELSD conditions: T_{neb}=25 °C, T_{evap}=25 °C, gas flow rate=1.0 L/min, *gain* factor=1.0; mobile phase composition: *n*-hexane/IPA-95/5 (v/v); flow rate=0.8 mL/min.

As evident from Figure 1b, the same conditions were not as effective for the resolution of (±)-**19** (α=1.02, R_S=0.00). Consequently, further investigations on this couple were necessary to attain separation of the two enantiomers.

The chiral recognition accomplishment is accepted to be based on hydrogen bonding and dipole–dipole interactions with the polar carbamate moieties embedded in the chiral cavities of the helix. Steric and π-stacking interactions with the external aromatic rings can assist the resolution.[12] It is well known for helical-shaped CSPs that the enantiodiscrimination mechanism is crucially dependent on the analyte size and geometry, which result in a diverse steric fit inside the chiral grooves.[13] Moreover, the disturbance effect exerted by external portions of the polymer can play a relevant role. Because of the comparable dimension of *cis*- and *trans*-isomers, a different fit during the transient diastereomer formation can be most plausibly hypothesized in our study owing to the different shape of the molecules. This, in turn, produces a different retention and enantiodiscrimination as well. Although the interactions responsible for retention do not necessarily contribute to the level of enantiodiscrimination, their synergic effect can be here claimed. Indeed, besides a longer retention, (±)-**20** enantiomers underwent an improved separation when a less polar mobile phase was applied.

Accordingly, a reduction in the eluent polarity (2% instead of 5% IPA) was firstly attempted for the dedicated (±)-**19** enantioseparation study. As expected, an increase in the retention time resulted for both peaks owing to the diminished competition between the solute enantiomers and the eluent polar component towards several active sites on the stationary phase. Due to the non-stereoselective character of most of these sites, an increase in the retention time did not provide the desired results (α=1.03, R_S=0.99, Fig. 2a). A further improvement was reached through the simultaneous use of the two alcoholic modifiers IPA and ethanol (EtOH) as the minor components in the mobile phase (Fig. 2b). A worsening in the overall chromatographic performance was instead experienced by completely replacing IPA with the same content of the more polar EtOH.

It has been often observed that the polysaccharide type CSPs provide higher efficiencies in the presence of short linear alcohols. The variation in the chromatographic behaviour promoted by EtOH can be reasonably ascribed to an alteration of the steric environment of the chiral cavities.[14] The above morphologic alteration can help enantiomers to more easily access stereoselective contacts with the stationary phase. As a result, the two (±)-**19** components resulted in an almost baseline discrimination (α=1.65, R_S=1.04) with the ternary eluent *n*-hexane/IPA/EtOH-98/1/1 (v/v/v). This seems to corroborate what previously asserted about the different steric fit of (±)-**19** enantiomers inside the chiral grooves. As expected, owing to the higher polarity of EtOH (clogP value, −0.31) versus IPA (clogP value, 0.05), the retention of both enantiomers declined in the presence of the former in the eluent. This is consistent with the assumption that the polarity of the adopted mobile phase is the dominant factor in controlling the sample retention. The peak resolution was finally achieved by further enriching the eluent (0.2% in volume) with the most commonly used, basic additive diethylamine (DEA, Fig. 2c).[15] The use of amine additives is strongly suggested by some authors for its standardization effect, which is supposed to derive from the so called 'memory effect'[15], though clear evidence of this phenomenon is still lacking with the presently used CSP.

Despite the negligible variation in terms of enantiomer retention, appreciable separation (α=1.10) and resolution (R_S=1.80) factor values were computed with these new conditions. It is assumed the advantageous effect derives from a dual action by the amine molecules. In the first instance, these compounds tend to mask underivatized silanols, which are usually responsible for non-stereospecific interactions and hence for the 'peak tailing' phenomenon. Contemporarily, amine molecules bind to numerous other sites on the stationary phase thus providing for different CSP-

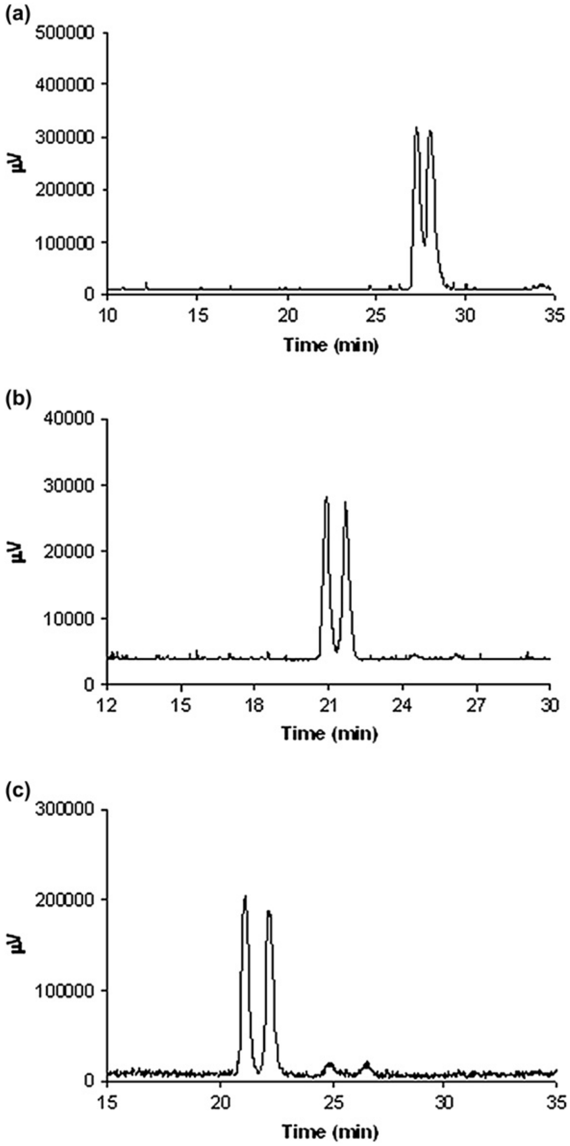


Figure 2. Chromatographic traces of (±)-**19**. ELSD conditions: T_{neb}=25 °C, T_{evap}=25 °C, gas flow rate=1.0 L/min, *gain* factor=1.0; mobile phase composition: (a) *n*-hexane/IPA-98/2 (v/v), (b) *n*-hexane/IPA/EtOH-98/1/1 (v/v/v), (c) *n*-hexane/IPA/EtOH/DEA-98/1/1/0.2 (v/v/v/v); flow rate=0.8 mL/min.

analyte interaction modalities, which often resolve into improved chromatographic performances. Worth emphasizing is the drawback of the high UV absorbance of DEA being completely overcome by making use of the ELSD instrument. Interestingly, a slight increase in terms of retention was observed for both (±)-**19** antipodes (k_1=7.36 and k_2=7.68 in the absence of DEA, k_1=7.45 and k_2=7.87 in the presence of DEA; the subscripts refer to the elution order) after incorporating the amine into the eluent. This was explained suggesting that amine molecules tend to hinder the access of the polar modifier to the stationary phase, thus limiting the displacement of the enantiomers.[15]

In analogy with before, a new enantioseparation attempt was carried out on the [(±)-**11**] and [(±)-**12**] racemic couples. Because of the excellent performances achieved for the (±)-**20** enantiomers with *n*-hexane/IPA-95/5 (v/v) mixture, the same mobile phase was selected as the starting eluent for these two new couples. With the same instrumental settings, a very appreciable enantiodiscrimination (α=1.29), accompanied with an excellent resolution (R_S=7.53) of the (±)-**12** enantiomers, was observed (Fig. 3a). Conversely, only a hint of separation (α=1.03, R_S=0.00) appeared for the (±)-**11** enantiomers (Fig. 3b).

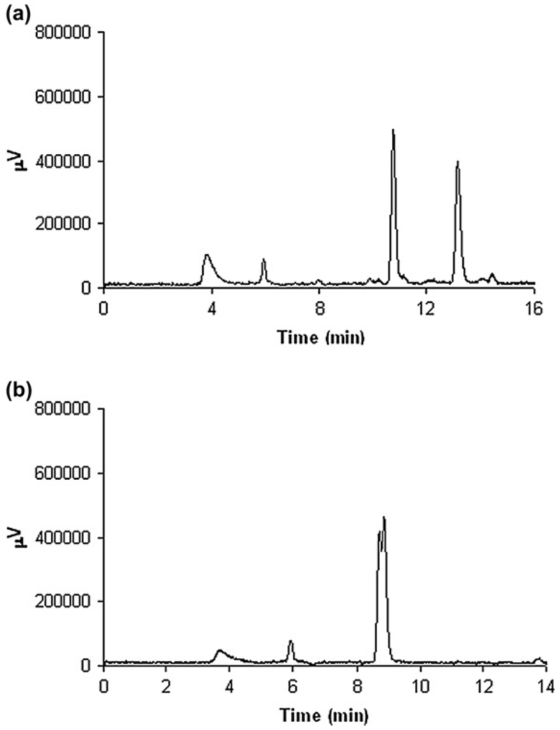


Figure 3. Chromatographic traces of (a) (±)-**12** and (b) (±)-**11** enantiomeric couples. ELSD conditions: T_{neb}=25 °C, T_{evap}=25 °C, gas flow rate=1.0 L/min, *gain* factor=1.0; mobile phase composition: *n*-hexane/IPA-95/5 (v/v), flow rate=0.8 mL/min.

The adoption of the previously defined best eluent conditions for the difficult-to-resolve (±)-**19** enantiomeric couple [*n*-hexane/IPA/EtOH/DEA-98/1/1/0.2 (v/v/v/v)] was no profitable for the (±)-**11** sample (α=1.04, R_S=0.00). The lack of success of the attempted analyses prompted us to investigate more fruitful eluents. Accordingly, after engaging a series of *n*-hexane/alcohol(s)/DEA mixtures by keeping constant the amine content, a reduction of the mobile phase polarity finally resulted in the (±)-**11** enantiomer resolution. Indeed, by means of a *n*-hexane/IPA/DEA-99/1/0.2 (v/v/v) solution as the eluent, enantioseparation and resolution factors of 1.06 and 1.88, respectively, were achieved (Fig. 4). Evidently, in this case, the EtOH behaves as a competitor towards stereoselective H-bond interactions thus diminishing the enantioselective fit of analytes into the chiral cavity. As expected, an increase in the enantiomeric retention was also observed.

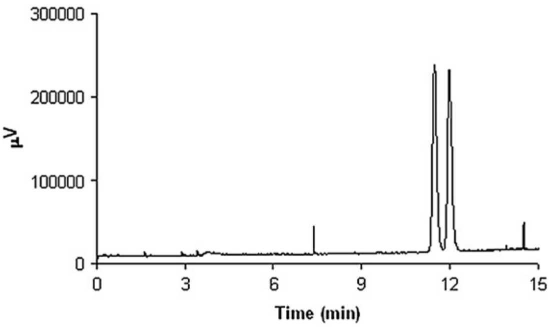


Figure 4. Chromatographic traces of (±)-**11**. ELSD conditions: T_{neb}=25 °C, T_{evap}=25 °C, gas flow rate=1.0 L/min, *gain* factor=1.0; mobile phase composition: *n*-hexane/IPA/DEA-99/1/0.2 (v/v/v), flow rate=0.8 mL/min.

3. Conclusions

In this paper we have reported the synthesis of the first series of conformationally constrained analogues of homotaurine (**1**), a sulphur-containing amino acid endowed with interesting biological activity. The partial reduction of the conformational flexibility of **1** was achieved by insertion of a cyclopropane ring between α,β- and β,γ-positions of the skeleton, thus affording *trans*- and *cis*-2-aminomethylcyclopropane-1-sulfonic acids [*t*-AMCSA (±)-**5**, and *c*-AMCSA (±)-**6**] and *trans*- and *cis*-(2-aminocyclopropyl)-methanesulfonic acids [*t*-ACMSA (±)-**7** and *c*-ACMSA (±)-**8**], respectively. All the compounds were prepared as racemic mixtures and then submitted to an HPLC-based analytical strategy aimed at both separating and resolving the enantiomeric couples. With the aim to avail of additional functionalities, the chromatographic enantioresolutions have been conducted on four specific precursors [namely (±)-**11**, (±)-**12**, (±)-**19** and (±)-**20**] of the final products. The excellent performances provided by the polysaccharide-based Chiralpak® IB® column can now be exploited to attempt preparative-scale applications.

4. Experimental

4.1. General methods

All reagents were commercially available unless otherwise noted. All reactions were carried out in dried glassware under a dry argon atmosphere. Dichloromethane was distilled over $LiAlH_4$ and then filtered through a short neutral aluminium oxide pad prior to use. Triethylamine was distilled over CaH_2 before use. Chromatographic purification of the products was accomplished on Merck silica gel (0.040–0.063 mm) using a Biotage SP1 HPCF separation module. 12 M (12 mm×15.0 cm, 9.0 g silica gel) cartridges were used. All new compounds were fully characterized by ^1H, and ^{13}C NMR. Nuclear magnetic resonance spectra were acquired on a Bruker AC 200 (200 MHz and 50.3 MHz for ^1H and ^{13}C, respectively) or on a Bruker AC 400 (400 MHz and 100 MHz for ^1H and ^{13}C, respectively) spectrometer at ambient temperature using $CDCl_3$ as solvent (otherwise indicated). Data for ^1H NMR are recorded as follows: chemical shift (δ in ppm), integration, multiplicity (s: singlet; d: doublet; dd: double doublet; ddd: double double doublet, dddd: double double double doublet, t: triplet, dt: double triplet, qt: quartet triplet, br s, broad signal), and coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ in ppm). GC–MS analyses were carried out with a HP-6850 gas chromatograph (dimethyl silicone column, 12.5 m) equipped with a HP-5975 mass-selective detector.

4.2. *trans*- and *cis*-*neo*Pentyl 2-{[bis(*tert*-butoxycarbonyl)-amino]methyl}cyclopropanesulfonates [(±)-11 and (±)-12]

A solution of *neo*pentyl α-diazomethanesulfonate[6] (0.51 g, 2.62 mmol) in dry dichloromethane (5.0 mL) was added by a syringe pump (0.6 mmol/h) to a magnetically stirred mixture of **10** (0.67 g, 2.62 mmol) and copper(II) acetylacetonate (0.013 g, 0.05 mmol) in dry dichloromethane (2.6 mL). After the addition was completed, the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was evaporated in vacuo and the residue, thus obtained, purified by flash chromatography. Elution with light petroleum/ethyl acetate (90:10) allowed us to recover 0.45 g of the starting amine **10**. Further elution with light petroleum/ethyl acetate (60:40) afforded *trans*-*neo*pentyl 2-{[bis(*tert*-butoxycarbonyl)amino]methyl}cyclopropanesulfonate as a colourless oil [(±)-**11**, 0.11 g, 10%]: δ_H (400 MHz) 3.84 (2H, s, $CH_2C(CH_3)_3$), 3.75 (1H, dd, *J* 5.7 and 14.5 Hz, $CH_aN(Boc)_2$), 3.45 (1H, dd, *J* 8.1 and 14.5 Hz, $CH_bN(Boc)_2$), 2.58 (1H, apparent quint, *J* 4.6 Hz, 1-CH), 2.00–1.98 (1H, m, 2-CH), 1.42 (18H, s, $N(Boc)_2$), 1.41 (1H, apparent q, *J* 5.3 Hz, 3-CH_a), 1.06 (1H, apparent dt, *J* 5.7 and 8.4 Hz, 3-CH_b), 0.95 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 153.2, 83.7, 80.0, 47.8, 33.0, 32.5, 28.79, 28.6, 26.8, 19.7, 11.6. Further elution with the same solvents gave *cis*-*neo*pentyl 2-{[bis(*tert*-butoxycarbonyl) amino]methyl}cyclopropanesulfonate as a colourless oil [(±)-**12**, 0.084 g, 7.5%]: δ_H (400 MHz) 3.95–3.90 (4H, m, $CH_2N(Boc)_2$ and $CH_2C(CH_3)_3$), 2.58 (1H, apparent dt, *J* 5.7 and 8.4 Hz, 1-CH), 1.80–1.70 (1H, m, 2-CH), 1.56–1.49 (10H, m, $N(Boc)_2$ and 3-CH_a), 1.40–1.36 (1H, m, 3-CH_b), 1.03 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 153.0, 83.5, 79.8, 44.8, 32.9, 32.4, 28.8, 26.8, 19.3, 13.1.

4.3. *cis*- and *trans*-Ethyl 2-{[(*neo*pentyloxy)-sulfonyl]methyl}cyclopropanecarboxylates [(±)-15 and (±)-16]

A solution of ethyl diazoacetate (4.93 g, 43.26 mmol) in dry dichloromethane (130 mL) was added by a syringe pump (2.0 mmol/h) to a magnetically stirred solution of **14**[9] (1.20 g, 5.76 mmol) and dirhodium(II) tetraacetate (0.474 g, 1.07 mmol) in dry dichloromethane (15 mL). After the addition was completed, the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated in vacuo and the residue, thus obtained, purified by flash chromatography. Elution with light petroleum/ethyl acetate (90:10) afforded *cis*-ethyl 2-{[(*neo*-pentyloxy)sulfonyl]methyl}cyclopropanecarboxylate as a colourless oil [(±)-**15**, 0.48 g, 34%]: δ_H (400 MHz) 4.20–4.16 (2H, m, $CO_2CH_2CH_3$), 3.88 (2H, d, *J* 3.0 Hz, 2H, $CH_2C(CH_3)_3$), 3.50 (2H, dd, *J* 0.5 and 6.8 Hz, $CH_2SO_3CH_2C(CH_3)_3$), 2.00–1.90 (1H, m, 1-CH), 1.78–1.71 (1H, m, 2-CH), 1.31–1.27 (4H, m, 3-CH_a and $CO_2CH_2CH_3$), 1.17–1.13 (1H, m, 3-CH_b), 0.99 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 172.2, 78.8, 61.0, 47.9, 31.7, 26.0, 17.5, 14.5, 14.2, 13.0; GC–MS (*m*/*z*): 278 (<1), 209 (7), 208 (29), 182 (15), 181 (5), 163 (8), 128 (69), 127 (40), 101 (8), 100 (20), 99 (76), 82 (8), 81 (15), 71 (100) 70 (10), 57 (47). Further elution with the same solvents gave *trans*-ethyl 2-{[(*neo*-pentyloxy)sulfonyl]methyl} cyclopropanecarboxylate as a colourless oil [(±)-**16**, 0.88 g, 61%]: (400 MHz) 4.16 (2H, q, *J* 7.1 Hz, $CO_2CH_2CH_3$), 3.92 (2H, s, $SO_3CH_2C(CH_3)_3$), 3.19 (1H, dd, *J* 7.1 and 14.6 Hz, $CH_aSO_3CH_2C(CH_3)_3$), 3.06 (1H, dd, *J* 7.5 and 14.6 Hz, $CH_bSO_3CH_2C(CH_3)_3$), 1.86–1.82 (1H, m, 2-CH), 1.73–1.69 (1H, m, 1-CH), 1.41 (1H, apparent dt *J* 5.0 and 8.8 Hz, 3-CH_a), 1.32–1.27 (4H, m, 3-CH_b and $CO_2CH_2CH_3$), 1.01 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 170.4, 79.6, 61.8, 54.2, 31.7, 26.8, 20.6, 16.1, 15.0, 14.9; GC–MS (*m*/*z*): 233 (7) [M−3(CH_3)], 209 (5), 208 (18), 181 (3), 180 (11), 163 (6), 128 (62), 127 (22), 101 (6), 100 (17), 99 (50), 85 (13), 81 (15), 71 (100) 70 (21), 57 (48).

4.4. *cis*-2-{[(*neo*Pentyloxy)sulfonyl]methyl}-cyclopropanecarboxylic acid [(±)-17]

A 0.5 M solution of NaOH in water (2.4 mL) was added to a magnetically stirred solution of (±)-**15** (0.33 g, 1.19 mmol) in ethanol (3.3 mL). After 72 h under stirring at room temperature, methanol was evaporated in vacuo. The residue was diluted with water (5 mL) and extracted with ethyl acetate (2×10 mL). The aqueous phase was acidified with 2 N hydrochloric acid and extracted with ethyl acetate (3×20 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give (±)-**17** as a pale yellow oil (0.140 g, 46%): δ_H (400 MHz) 3.89 (2H, d, *J* 2.4 Hz, $CH_2C(CH_3)_3$), 3.51 (2H, d, *J* 7.2 Hz, $CH_2SO_3CH_2C(CH_3)_3$), 1.98 (1H, apparent dt, *J* 5.7 and 8.3 Hz, 1-CH), 1.90–1.80 (1H, m, 2-CH), 1.38 (1H, apparent dt, *J* 5.1 and 8.5 Hz, 3-CH_a), 1.23 (1H, apparent q, *J* 5.7 Hz, 3-CH_b), 0.99 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 177.0, 79.6, 48.6, 32.5, 28.8, 17.7, 16.2, 14.7.

4.5. *trans*-2-{[(*neo*Pentyloxy)sulfonyl]methyl}-cyclopropanecarboxylic acid [(±)-18]

A 0.5 M solution of NaOH in water (7.0 mL) was added to a magnetically stirred solution of (±)-**16** (0.98 g, 3.52 mmol) in ethanol (9.8 mL). After 72 h under stirring at room temperature, methanol was evaporated in vacuo. The residue was diluted with water and extracted with ethyl acetate (2×20 mL). The aqueous phase was acidified with 2 N hydrochloric acid and extracted with ethyl acetate (3×30 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give (±)-**18** as a pale yellow oil (0.38 g, 42%): δ_H (400 MHz) 3.93 (2H, s, $CH_2C(CH_3)_3$), 3.19 (1H, dd, *J* 7.2 and 14.6 Hz, $CH_aSO_3CH_2C(CH_3)_3$), 3.09 (1H, dd, *J* 7.2 and 14.6 Hz, $CH_bSO_3CH_2C(CH_3)_3$), 1.97–1.91 (1H, m, 2-CH), 1.74 (1H, apparent quint, *J* 4.7 Hz, 1-CH), 1.48 (1H, apparent dt, *J* 5.0 and 9.4 Hz, 3-CH_a), 1.13 (1H, apparent dt, *J* 5.6 and 11.3 Hz, 3-CH_b), 1.01 (9H, s, $CH_2C(CH_3)_3$); δ_C (100 MHz) 179.1, 79.7, 54.0, 32.5, 26.8, 20.3, 17.0, 15.7.

4.6. *cis-neo*Pentyl {2-[(*tert*-butoxycarbonyl)amino] cyclopropyl}methanesulfonate [(±)-19]

Et_3N (0.50 g, 4.96 mmol) and ethyl chloroformate (0.538 g, 4.96 mmol) were added to a magnetically stirred solution of (±)-**17** (0.620 g, 2.49 mmol) in dry THF (7 mL) kept at 0 °C. After 35 min, a solution of NaN_3 (0.480 g, 7.44 mmol) in H_2O (7.6 mL) was added. The reaction mixture was stirred vigorously for 40 min at 0 °C and then 10 min at room temperature. The reaction mixture was diluted with H_2O (20 mL) and extracted with ethyl acetate (3×30 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue (ca. 2 mL) was diluted with toluene (30 mL) and the resulting solution was heated at 90 °C for 40 min. *tert*-Butanol (4.00 g, 52.00 mmol) was then added and the heating continued for 15 h. The volatiles were removed in vacuo to give a brownish solid, which was purified by flash chromatography. Elution with light petroleum/ethyl acetate (80:20) afforded (±)-**19** (0.486 g, 61%) as a white solid; mp 95–97 °C; υ_{max}(KBr) 2964, 2365, 1682, 1507; δ_H (400 MHz) 5.20 (1H, br s, $NHCOC(CH_3)_3$), 3.94 (2H, s, $CH_2C(CH_3)_3$), 3.33 (1H, dd, *J* 7.5 and 14.7 Hz, $CH_aSO_3CH_2C(CH_3)_3$), 3.15 (1H, dd, *J* 7.4 and 14.7 Hz, $CH_bSO_3CH_2C(CH_3)_3$), 2.73 (1H, apparent dt, *J* 4.5 and 7.1 Hz, 1-CH), 1.49 (9H, s, $NHCOC(CH_3)_3$), 1.36–1.32 (1H, m, 2-CH), 1.21–1.17 (1H, m, 3-CH_a), 1.00 (9H, s, $CH_2C(CH_3)_3$), 0.69 (1H, apparent q, *J* 5.8 Hz, 3-CH_b); δ_C (100 MHz) 157.5, 80.7, 79.6, 50.4, 32.5, 29.0, 27.5, 26.8, 13.3, 12.4.

4.7. *trans-neo*Pentyl {2-[(*tert*-butoxycarbonyl)amino] cyclopropyl}methanesulfonate [(±)-20]

Et_3N (0.720 g, 7.12 mmol) and ethyl chloroformate (0.772 g, 7.12 mmol) were added to a magnetically stirred solution of (±)-**18** (0.890 g, 3.56 mmol) in dry THF (10 mL) kept at 0 °C. After 35 min, a solution of NaN_3 (0.665 g, 10.68 mmol) in H_2O (11.0 mL) was added. The reaction mixture was stirred vigorously for 40 min at 0 °C and then 10 min at room temperature. The reaction mixture was diluted with H_2O (40 mL) and extracted with ethyl acetate (4×35 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue (ca. 3 mL) was diluted with toluene (30 mL) and the resulting solution was heated at 90 °C for 40 min. *tert*-Butanol (5.00 g, 67.00 mmol) was then added and the heating continued for 15 h. The volatiles were removed in vacuo to give a brownish solid, which was purified by flash chromatography. Elution with light petroleum/ethyl acetate (80:20) afforded (±)-**20** (0.610 g, 53%) as a white solid; mp 91–93 °C; δ_H (400 MHz) 4.80 (1H, br s, $NHCOC(CH_3)_3$), 3.90 (2H, s, $CH_2C(CH_3)_3$), 3.43 (1H, dd, *J* 6.0 and 14.5 Hz, $CH_aSO_3CH_2C(CH_3)_3$), 2.81 (1H, dd, *J* 8.8 and 14.6 Hz, $CH_bSO_3CH_2C(CH_3)_3$), 2.52 (1H, br s, 1-CH), 1.34–1.32 (2H, m, 3-CH_a and 2-CH), 1.20–1.00 (10H, m, 3-CH_b and $CH_2C(CH_3)_3$); δ_C (100 MHz) 156.9, 80.7, 79.5, 53.6, 32.5, 30.0, 29.0, 26.8, 15.2, 14.2.

4.8. General procedure for the final acidic hydrolysis

A magnetically stirred mixture of *N*-protected sulfonic ester (0.5 mmol) in 6 N hydrochloric acid (15 mL) was heated at 95 °C for 18–24 h. The reaction mixture was washed with CH_2Cl_2 (2×10 mL) and the aqueous phase evaporated to dryness. The residue was submitted to ion-exchange resin chromatography (Dowex 50WX2-200). Elution with water afforded the final compound.

*4.8.1. trans-2-(Aminomethyl)cyclopropanesulfonic acid [(±)-**5**].* The title compound was prepared from (±)-**11** in 94% yield as a white solid; mp 315 °C (dec); [found: C, 31.81; H, 5.99; N, 9.25. $C_4H_9NO_3S$ requires C, 31.78; H, 6.00; N, 9.26]; υ_{max}(KBr) 3040, 1710, 1641, 1540, 1494; δ_H (400 MHz) 2.89 (2H, d, *J* 8.00 Hz, CH_2NH_2), 2.37 (1H, apparent quint, *J* 4.42 Hz, 1-CH), 1.56–1.54 (1H, m, 2-CH), 1.15 (1H, apparent dt, *J* 5.43 and 9.13 Hz, 3-CH_a), 0.94 (1H, apparent dt, *J* 5.81 and 8.76 Hz, 3-CH_b); δ_C (100 MHz) 42.28, 34.87, 16.41, 10.95.

*4.8.2. cis-2-(Aminomethyl)cyclopropanesulfonic acid [(±)-**6**].* The title compound was prepared from (±)-**12** in 95% yield as a white solid; mp 310 °C (dec); [found: C, 31.85; H, 6.01; N, 9.24. $C_4H_9NO_3S$ requires C, 31.78; H, 6.00; N, 9.26]; υ_{max}(KBr) 3040, 1715, 1640, 1540, 1494; δ_H (400 MHz) 3.27–3.24 (2H, m, CH_2NH_2), 2.53 (1H, apparent q, *J* 7.33 Hz, 1-CH), 1.47–1.45 (1H, m, 2-CH), 1.25 (1H, apparent q, *J* 7.30 Hz, 3-CH_a), 1.02 (1H, apparent q, *J* 5.97 Hz, 3-CH_b); δ_C (100 MHz) 39.12, 34.53, 15.67, 11.60.

*4.8.3. cis-(2-Aminocyclopropyl)methanesulfonic acid [(±)-**8**].* The title compound was prepared from (±)-**20** in 86% yield as a white solid; mp 260 °C (dec); [found: C, 31.79; H, 6.01; N, 9.23. $C_4H_9NO_3S$ requires C, 31.78; H, 6.00; N, 9.26]; υ_{max}(KBr) 3045, 1734, 1615, 1540, 1455, 1428, 1343; δ_H (200 MHz) 3.10 (1H, dd, *J* 7.03 and 14.63 Hz, CH_aSO_3H), 2.91 (1H, dd, *J* 8.45 and 14.63 Hz, CH_bSO_3H), 2.73–2.70 (1H, m, 1-CH), 1.42–1.39 (1H, m, 2-CH), 1.14–1.12 (1H, m, 3-CH_a), 0.71 (1H, apparent dt, *J* 4.58 and 6.91 Hz, 3-CH_b); δ_C (100 MHz) 49.74, 27.02, 11.18, 9.98.

*4.8.4. trans-(2-Aminocyclopropyl)methanesulfonic acid [(±)-**7**].* The title compound was prepared from (±)-**19** in 75% yield as a white solid; mp 240 °C (dec); [found: C, 31.81; H, 6.01; N, 9.28. $C_4H_9NO_3S$ requires C, 31.78; H, 6.00; N, 9.26]; υ_{max}(KBr) 3048, 1734, 1614, 1544, 1456, 1428, 1343; δ_H (200 MHz) 2.85 (1H, dd, *J* 7.03 and 14.47 Hz, 1H, CH_aSO_3H), 2.78 (1H, dd, *J* 7.44 and 14.20 Hz, CH_bSO_3H), 2.80–2.60 (1H, m, 1-CH), 1.48–1.46 (1H, m, 2-CH), 1.10–1.07 (1H, m, 3-CH_a), 0.86 (1H, apparent q, *J* 6.89 Hz, 3-CH_b); δ_C (100 MHz) 52.78, 28.40, 12.97, 9.91.

5. Materials and methods for the enantioseparation study

5.1. Chemicals

Analytical grade ethanol (EtOH), *n*-hexane, 2-propanol (IPA) and diethylamine (DEA) were purchased from Sigma–Aldrich (Milano, Italy). All the employed mobile phases were filtered through a 0.22 μm Millipore filter (Bedford, MA, USA) and then degassed with 20 min sonication. Analytes to be injected were solubilized in the filtered selected mobile phase.

5.2. Instrumentation and procedures

All the HPLC experiments were carried out on a Shimadzu (Kyoto, Japan) Class-VP equipped with a EZ Start chromatography data software, a LC-10 AT_{VP} pump, a *SCL-10A$_{VP}$* system controller, a *FCV-10AL$_{VP}$* low pressure gradient formation unit, a *DGU-14A* on-line degasser and a Rheodyne 7725i injector (Rheodyne, Cotati, CA, USA) with a 20 μL stainless steel loop. A PL-ELS 2100 Ice (Polymer Laboratories Varian, Inc., Amherst, MA, USA) was utilized as the evaporative light scattering detector. A SS420X (Scientific Software, Inc., Pleasanton, CA, USA) interface has allowed the analogue-to-digital conversion of the output signal from the ELS detector.

The Chiralpak® IB® (250 mm×4.6 mm I.D., containing cellulose tris(3,5-dimethylphenylcarbamate) immobilized onto a 5 μm silica gel) was purchased from Chiral Technologies (West Chester, PA, USA) and used as the chiral column for all the analyses. Column temperature was controlled through a Grace (Sedriano, Italy) heater/chiller (Model 7956R) thermostat. The column was used after previous conditioning with the selected mobile phase at a 1.0 mL/min flow rate for at least 30 min.

In order to reduce as much as possible the baseline noise, the end of the liquid waste tube has been always kept not immersed into the discharged liquid.

A periodic cleaning of the detector components was made by pumping methanol into the instrument (without the column) at a 1.5 mL/min flow rate. A dry out of the detector chambers was done after the cleaning procedure by running the system at maximum temperatures, for about 30 min, without any eluent flow. The compressed air has been submitted to two filtration steps before entering the detector and used as the gas carrier.

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