Bioorganic & Medicinal Chemistry 21 (2013) 470–484

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of 4-hydroxy-4-(4-methoxyphenyl)-substituted proline and pyrrolidin-2-ylacetic acid derivatives as GABA uptake inhibitors

Xueqing Zhao [†], Jörg Pabel, Georg C. Höfner, Klaus T. Wanner *

Department für Pharmazie-Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 Munich, Germany

ARTICLE INFO

Article history:
Received 19 July 2012
Revised 7 November 2012
Accepted 8 November 2012
Available online 27 November 2012

Dedicated with warmest wishes to Wolfgang Beck on the occasion of his 80th birthday

Keywords:
GABA uptake inhibitors
GAT1
GAT3
Antiepileptic
Pyrrolidines

ABSTRACT

A series of enantiomerically pure 4-hydroxy-4-(4-methoxyphenyl)-substituted proline and pyrrolidin-2-ylacetic acid derivatives have been synthesized starting from the respective N-protected 4-hydroxy derivatives via oxidation to the corresponding 4-oxo compounds, subsequent addition of organometallic reagents, final hydrolysis and deprotection. The major diastereoisomers obtained by the addition of the Grignard reagents were found to have opposite stereoconfigurations depending on whether cerium trichloride was present or absent as an additive. The final compounds were evaluated for their capability to inhibit the GABA transport proteins GAT1 and GAT3. 4-Hydroxyproline derivatives substituted with a tris(4-methoxyphenyl)methyloxyethyl residue at the nitrogen and a 4-methoxyphenyl group in 4-position showed, with the exception of the (2*R*,4*R*)-diastereomer, an improved inhibition at GAT3 compared to the derivatives missing the 4-methoxyphenyl group in 4-position. This may imply that an appropriate lipophilic group at the C-4 position of the proline moiety is beneficial for potent inhibition at GAT3.

1. Introduction

γ-Aminobutyric acid (GABA) is the dominant inhibitory neurotransmitter in the brain. GABAergic dysfunction has been found to be involved in a number of CNS disorders, including epilepsy,[1] Huntington's chorea,[2] migraine,[3] Morbus Parkinson,[4] and depression.[5–7] Enhancement of GABA function can be achieved through several mechanisms, such as direct receptor agonism[8] or inhibition of enzymatic breakdown of GABA.[9] But long-term administration is often limited by rapid development of tolerance.[10–12] Since the discovery of GABA transport proteins (GAT), the inhibition of these GABA uptake proteins has become a new and efficient approach to palliate GABA deficiency.[13] Four distinct GABA transporters have been characterized over the years. Following a species independent nomenclature, also used by the Humane Genome Organization (HUGO), these transporters are termed GAT1 (slc6a1), GAT2 (slc6a13), GAT3 (slc6a11), and BGT-1 (slc6a12),[14–20] respectively (for a detailed discussion see Ref. 21). The four subtypes differ in their distribution and their pharmacological role. GAT1 and GAT3 are almost exclusively located in the central nervous system (CNS)[22] and were found in close proximity to the synapse or in the synapse itself. On the other hand, BGT-1 and GAT2 are found mainly in peripheral organs, that is, in the liver and in the kidneys, whereas in the brain significant concentrations are restricted to the leptomeninges (GAT2 and BGT-1) and to cerebral blood vessels (GAT2), indicating that these transporters are unlikely to play an important role for inactivation of the neurotransmitter GABA.[23,24]

Many potent uptake inhibitors selective for GAT1 like (*R*)-SK&F-89976-A (**1**), SK&F-100591-A (2[‡]),[25] and Tiagabine (**3**) have been discovered and investigated for their pharmacological properties since 1985. As a GAT1 inhibitor, Tiagabine (Gabitril®) was firstly marketed for add-on treatment of epilepsy in 1997, new indications for the treatment of diabetic neuropathy and migraine are under clinical trials.[26] However, until now the number of inhibitors with high affinity for the other GAT subtypes is still small, which is especially regrettable for GAT3, which, in addition, to GAT1 possesses great importance for GABA inactivation in the brain.[27–29] (*S*)-SNAP-5114 (**4**)[30,31] and NNC-05-2045 (**5**, Fig. 3)[32,33] range among the most potent GAT3 inhibitors known, but their potencies and subtype selectivities are still relatively low. Therefore, there is still a great need for more selective and more potent GAT3 inhibitors.

* Corresponding author. Tel.: +49 89 2180 77249; fax: +49 89 2180 77247.
E-mail address: Klaus.Wanner@cup.uni-muenchen.de (K.T. Wanner).
[†] Present Address: Synthetic Group, Fujian Institute of Microbiology, Jinbu Road 25, Fuzhou 350007, PR China.

[‡] Mixture of racemic diastereomers.

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.11.015

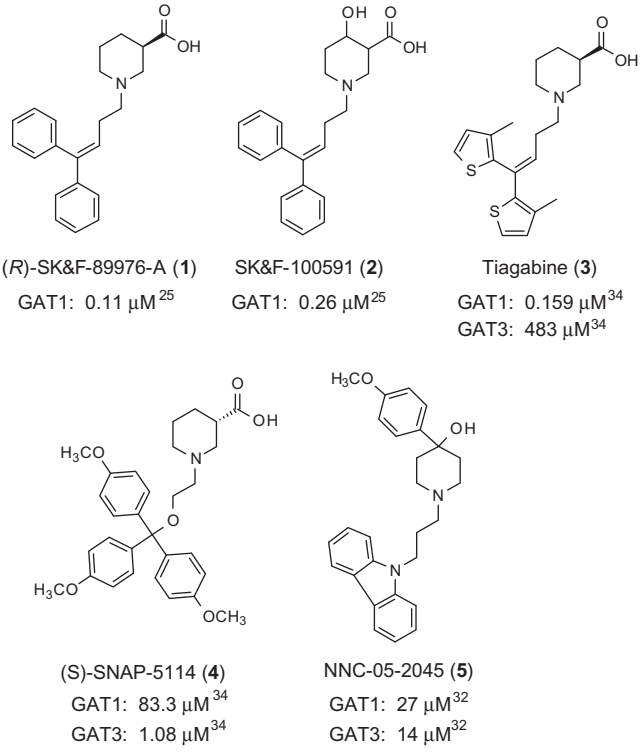


Figure 1. Representative known GABA uptake inhibitors (potencies are given as IC_{50}).

Previously, we reported the synthesis and biological evaluation of all stereoisomers of the pyrrolidine derivatives **6 to 9b–d** (Figs. 2 and 4) as potential GABA uptake inhibitors.[34,35] The compounds **6** and **7** which in contrast to **8** and **9** (Fig. 4) are devoid of a substituent in 4-position exhibited high selectivity for and potency at GAT1 and GAT3 depending on their stereo configuration and structure of their N-substituent (Fig. 2; best compounds at GAT1: (*S*)-**6b**, (*S*)-**7c**; best compounds at GAT3: (*R*)-**6d**, (*R*)-**7d**). Introduction of a hydroxy group into the 4-position of the pyrrolidine ring resulting in compounds **8** and **9** (Fig. 4), however, was found to significantly decrease the inhibitory potency even for the most potent stereoisomers and at both, GAT1 and GAT3 (Figs. 2 and 4; (2*R*,4*R*)-**8b**: 9.4 μM at GAT1; (2*S*,4*R*)-**9c**: 3.15 μM at GAT1; (2*R*,4*S*)-**9d**: 19.9 μM at GAT3).

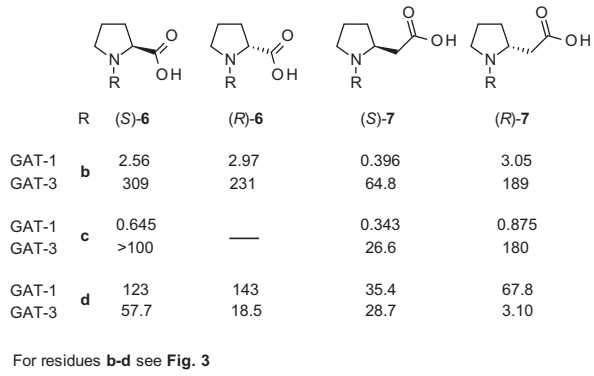


Figure 2. Structures of GABA uptake inhibitors with a pyrrolidine moiety. Structures of R are given in Figure 3 (IC_{50} unit: μM).

Herein, we present the synthesis and biological evaluation of new pyrrolidine derivatives with further structural modifications. Motivated by the structural characteristics of NNC-05-2045 (**5**), compound series **10a**,**b**,**d** and **11a**,**c**,**d** (Fig. 3) bearing a 4-methoxyphenyl group at the 4-position of the pyrrolidine ring were prepared in a stereoselective pure form. Since our previous investigations had shown that 4-hydroxypyrrolidine derivatives substituted with the 3-(carbazol-9-yl)propyl residue of NNC-05-2045 (**5**, Fig. 1) exhibited only weak GAT inhibition,[35] we decided to employ only the lipophilic N-substituents **b** and **c** which are typical for GAT1 inhibitors (Fig. 3). Residue **d** was chosen (Fig. 3) as it is known to enhance GAT3 activity, as for example in the case of (*S*)-SNAP-5114 (**4**).

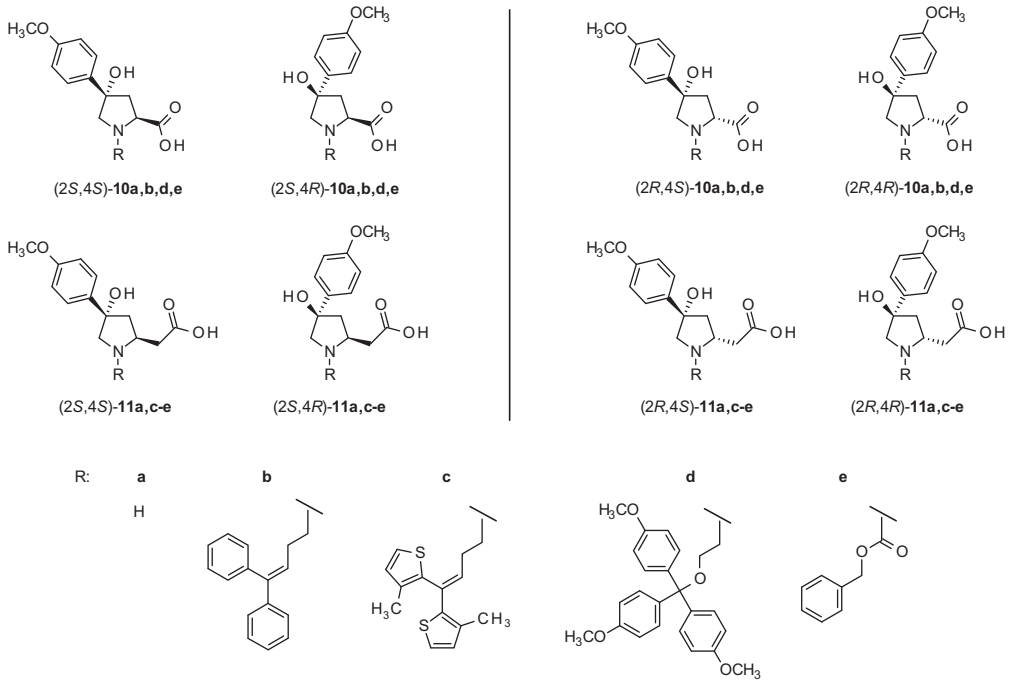


Figure 3. Structures of the target compounds.

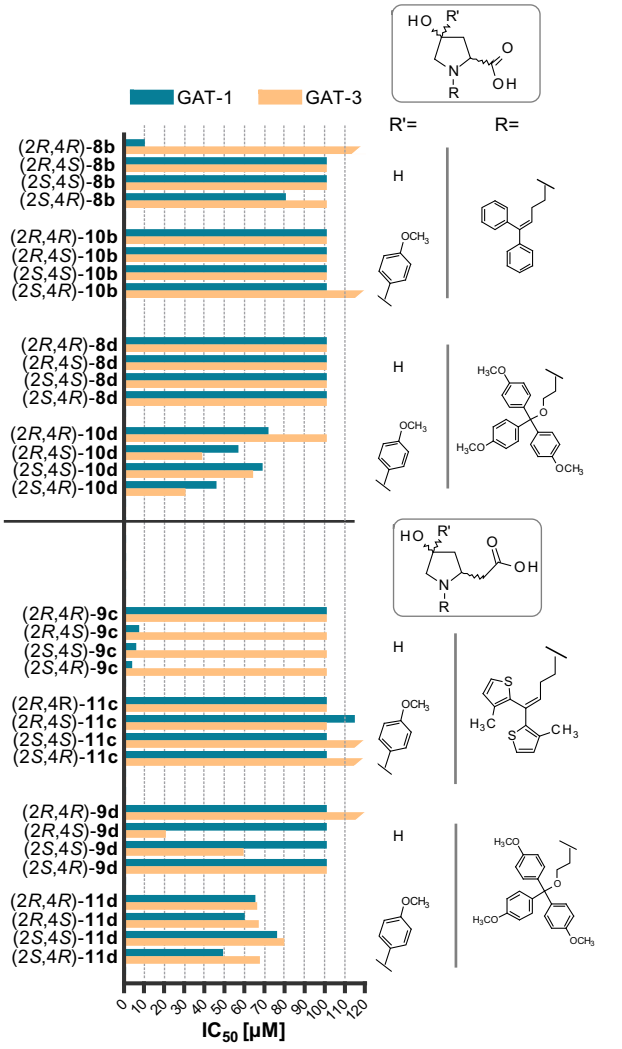


Figure 4. Diagram with IC_{50} values of the target compounds **10** and **11** compared to the values of compounds **8** and **9**[35] with no aryl substituent in 4-position. Values of >100 μM are given as 100 μM.

2. Chemistry

The proline and pyrrolidine derivatives **12b,d,e** and **15c–e** (Schemes 2 and 3), the syntheses of which had been described previously,[35–37] were chosen as starting materials to prepare the target compounds.

The reaction routes to all of the target compounds are outlined in Schemes 2 and 3. They comprise the following steps: (1) oxidation of the 4-hydroxy group, (2) subsequent addition of an organometallic reagent to the resulting keto group, (3) basic hydrolysis, and (4), in the case of Cbz-protected compounds, additionally reductive removal of the Cbz group.

2.1. Oxidation

Swern oxidation[38] of the compounds (2*S*,4*R*)-**12b,d** and (2*S*,4*R*)-**15c,d** provided the corresponding 4-oxoproline derivatives (*S*)-**13b,d** as well as the 2-(4-oxopyrrolidin-2-yl)acetic acid derivatives (*S*)-**16c,d** in 83–89% yields (Schemes 2 and 3). The C–O bond present in the lipophilic side chain **d** was not affected under these reaction conditions, even though it is highly sensitive to acids. The Cbz-protected derivatives (*S*)-**13e** and (*S*)-**16e**, which were required to access the corresponding amino acids lacking a substituent at the amino nitrogen, were obtained only in very low yields (10–27%) by Swern oxidation of (2*S*,4*R*)-**12e** and (2*S*,4*R*)-**15e**, respectively. Thus, compounds (*S*)-**13e** and (*S*)-**16e** were finally prepared by Jones oxidation according to literature procedures.[36,37]

Similar to the aforementioned syntheses, the enantiomers (*R*)-**13b,d,e** and (*R*)-**16c–e** were obtained from diastereomers (2*R*,4*R*)-**12b,d,e** and (2*R*,4*R*)-**15c–e**, respectively (Schemes 2 and 3).

2.2. Organometallic addition reactions

For the preparation of the 4-methoxyphenyl substituted derivatives (2*S*,4*R*)-**14b,d** and (2*S*,4*S*)-**14b,d** as well as of (2*S*,4*R*)-**17c,d** and (2*S*,4*S*)-**17c,d**, (4-$MeOC_6H_4$)MgBr was added to the 4-oxoproline derivatives (*S*)-**13b,d** and 4-oxopyrrolidin-2-ylacetic acid derivatives (*S*)-**16c,d** yielding in each case the (2*S*,4*S*)-configured diastereomers (2*S*,4*S*)-**14b,d** and (2*S*,4*S*)-**17c,d** as major products (Schemes 2 and 3). With ratios of 89:11 [(2*S*,4*S*)-**14b**/(2*S*,4*R*)-**14b**], 85:15 [(2*S*,4*S*)-**14d**/(2*S*,4*R*)-**14d**], 87:13 [(2*S*,4*S*)-**17c**/(2*S*,4*R*)-**17c**] and 81:19 [(2*S*,4*S*)-**17d**/(2*S*,4*R*)-**17d**], respectively, the diastereoselectivities were quite pleasing, but the yields of the addition reaction were with 45–62% only moderate.[39]

Numerous examples exist, according to which the presence of cerium trichloride may significantly alter the stereoselectivity of Grignard addition reactions to ketones.[40–43] Therefore,, a mixture of 4-$MeOC_6H_4MgBr$ and $CeCl_3$ was added to (*S*)-**13b,d** and (*S*)-**16c,d** in order to reverse the asymmetric induction (general procedure 3A in Section 6.3). This time, indeed, the diastereomers (2*S*,4*R*)-**14b,d** (Scheme 2) and (2*S*,4*R*)-**17c,d**, (Scheme 3) to which the nucleophile had added from the side opposite to the ester function were formed as major products (*ds* 52:48–17:83).

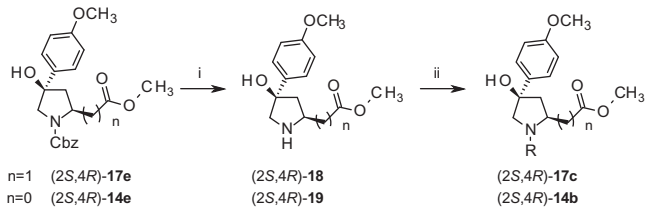
Addition reactions to the N-Cbz-protected compounds (*S*)-**13e** and (*S*)-**16e** showed compared to the *N*-alkyl substituted compounds (*S*)-**13b,d** and (*S*)-**16c,d** a different outcome concerning asymmetric induction. Here addition of (4-$MeOC_6H_4$)MgBr yielded only the (2*S*,4*R*)-configured diastereomers (2*S*,4*R*)-**14e** and (2*S*,4*R*)-**17e** (Scheme 2 and 3) as single diastereomers (*ds* >2:98) which could be easily isolated in pure form (yield 38–56%). The sense of asymmetric induction remained this time unchanged when $CeCl_3$ was used as additive in the addition reaction.

For the preparation of the enantiomeric reaction products required for the biological testing an identical reaction sequence was followed, in which, however, the starting materials had been replaced by their enantiomeric counter parts, (*R*)-**13b,d,e** and (*R*)-**16c–e**. The results obtained for these transformations were similar to those of the original sequence.

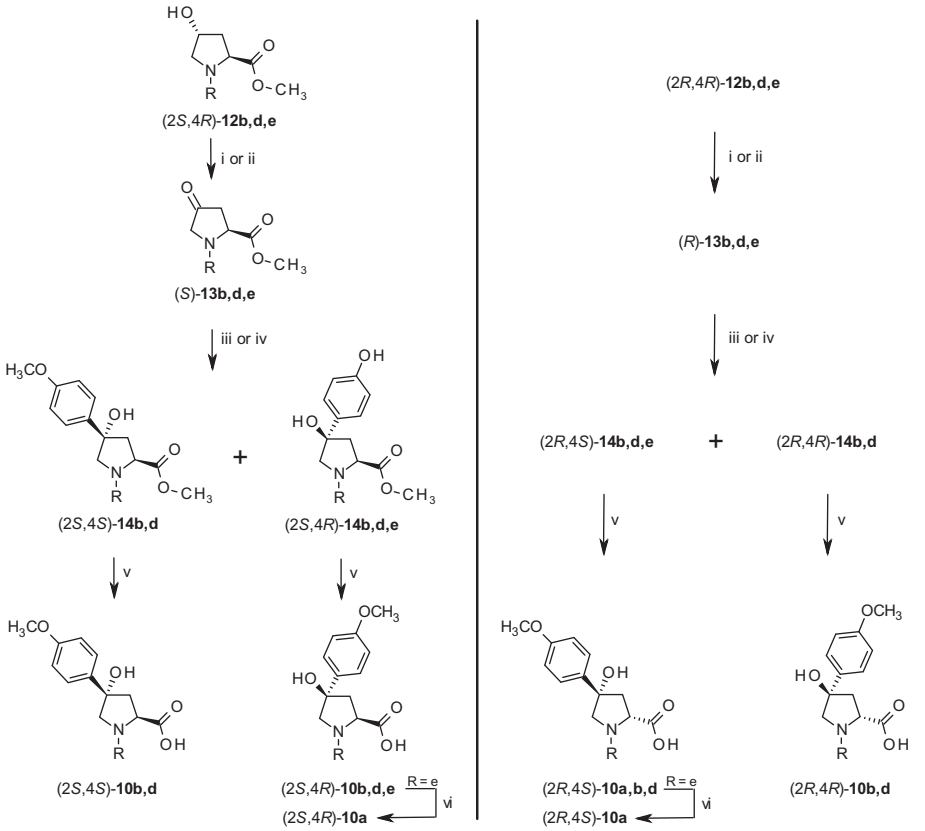
The determination of the configuration of all of the newly generated compounds is described in Section 2.5.

2.3. Hydrolysis

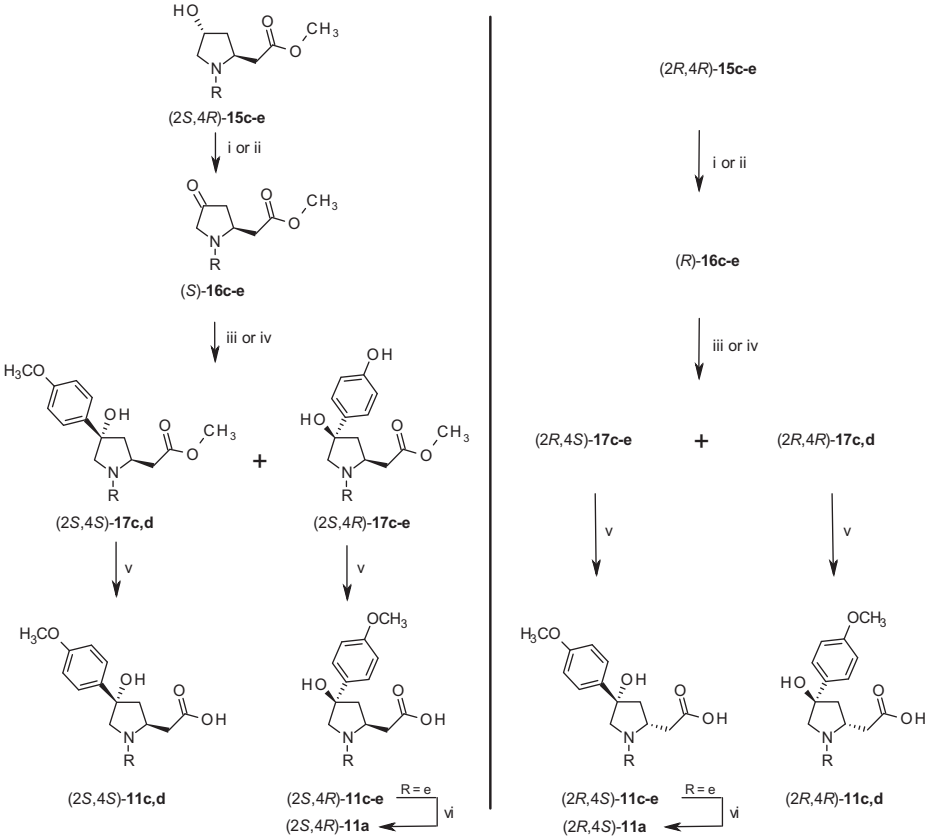
Each of the diastereomerically and enantiomerically pure products **14b,d** and **17c,d** was subjected to an alkaline hydrolysis in methanol or ethanol, to give all stereoisomeres of the target compounds **10b,d** and **11c,d** in 70–98% yield. Besides, also the N-Cbz-protected carboxylic acids (2*S*,4*R*)-**10e**, (2*S*,4*R*)-**11e** and



Scheme 1. (i) H_2/10%Pd-C, TEA, MeOH, rt; (ii) K_2CO_3, KI, RBr (R = **c** in the case of (2*S*,4*R*)-**18** and R = **b** in the case of (2*S*,4*R*)-**19**).



Scheme 2. Scheme for the synthesis of the compound series **10**. (i) Swern oxidation (81–92%); (ii) Jones oxidation (71–77%); (iii) 4-$MeOC_6H_4MgBr/CeCl_3$, −60 to −78 °C, THF (32–56%); (iv) 4-$MeOC_6H_4MgBr$, −78 °C, Et_2O (37–65%); (v) 1.0 M NaOH (2–3 equiv), MeOH or EtOH, rt (73–98%); (vi) H_2/10% Pd-C, TEA, MeOH, rt (91–100%).



Scheme 3. Scheme for the synthesis of the compound series **11.** For reaction conditions see Scheme 1.

their enantiomers were obtained by alkaline hydrolysis from compounds (2*S*,4*R*)-**14e** and (2*R*,4*S*)-**14e**, (2*S*,4*R*)-**17e** and (2*R*,4*S*)-**17e**, with yields in the range of 80–91%.

2.4. Hydrogenolysis

For the preparation of the N-unsubstitued amino acids (2*S*,4*R*)-**10a** and (2*R*,4*S*)-**10a**, (2*S*,4*R*)-**11a** and (2*R*,4*S*)-**11a** the N-Cbz-protected amino acids (2*S*,4*R*)-**10e** and (2*R*,4*S*)-**10e**, (2*S*,4*R*)-**11e**, and (2*R*,4*S*)-**11e** were subjected to catalytic hydrogenation over Pd-C in the presence of TEA, affording the target compounds in high yields (92–100%).

2.5. Stereochemical assignment for the compounds obtained by organometallic additions

NOE experiments performed on the sodium salt of (2*S*,4*R*)-**11a** (Scheme 3)[44] revealed the aromatic ring in 4-position being *trans* to the carboxylic acid ester function. Given that the stereocenter in 2-position must be *S*-configured, resulting from the stereochemistry of the starting material (2*S*,4*R*)-**15e** used, the 4-position must possess *R*-configuration. Taking into account that (2*S*,4*R*)-**11e** and (2*S*,4*R*)-**17e** are synthetic precursors of (2*S*,4*R*)-**11a** and (2*R*,4*S*)-**11a**, (2*R*,4*S*)-**11e** and (2*R*,4*S*)-**17e** are their enantiomeric counter parts led to the stereochemistry of these compounds to be either identical with the stereochemistry of (2*S*,4*R*)-**11a** or opposite to it. In the next step, the N-Cbz protected product (2*S*,4*R*)-**17e** with now known stereochemistry was transformed into (2*S*,4*R*)-**17c** displaying a 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl moiety by N-deprotection and subsequent N-alkylation (Scheme 1). The thus obtained compound (2*S*,4*R*)-**17c** was identical with the major isomer formed upon addition of 4-$MeOC_6H_4MgBr/CeCl_3$ to (*S*)-**16c**. By comparison of the ^1H NMR spectra of (2*S*,4*R*)-**17c** and (2*S*,4*R*)-**17d** the stereochemistry of the latter compound could be identified to be identical to that of the former compound. The stereochemistry of the remaining stereoisomers of **17c**, **17d**, **11c**, and **11d** could, finally, be assigned considering their stereochemical relation to (2*S*,4*R*)-**17c** and (2*S*,4*R*)-**17d**, being either diastereomers or enantiomers of these compound depending on their syntheses.

Following the same strategy, the stereochemistry could also be assigned to the individual stereoisomers of **10** and **14** (Scheme 2). This time, however, the NOE experiments for the determination of the relative configuration were performed on the N-deprotected compound (2*S*,4*R*)-**19**. Taking into account the results of these NOE experiments together with the fact that (2*S*,4*R*)-**19** had been delineated from (2*S*,4*R*)-**12e** exhibiting (2*R*)-configuration the (2*S*,4*R*)-configuration of (2*S*,4*R*)-**19** became apparent. Serving as a precursor in the synthesis of (2*S*,4*R*)-**19** the same absolute configuration had to be assigned to (2*S*,4*R*)-**14e**. The stereochemical relation between the isomer (2*S*,4*R*)-**14e** exhibiting a N-Cbz protective group and the *N*-alkyl derivatives **14b**,**d** and thus also of the free amino acids **10b**,**d** was established via the 4-bromo-1,1-diphenylbutenyl substituted compound (2*S*,4*R*)-**14b** (see Scheme 1).

3. Biological tests

The final compounds **10a**,**b**,**d** and **11a**,**c**,**d** were tested for their inhibitory potency at GAT1 and GAT3. For the evaluation of the activity of the test compounds at the GABA transport proteins, an assay developed in our group has been employed that is based on subcellular membrane fractions from frontal cortex (bfcP2B) and brain stem (bbsP2C) of bovine brain.[45] Due to the emergence of bovine spongiform encephalitis in Germany, calf brain (cfcP2B, cbsP2C) and finally porcine brain material (pfcP2B, pbsP2C) was temporarily used for this test system instead of the bovine brain material. The reliability of the newly developed assay systems was evaluated using a series of reference compounds. Results were found to be in accordance with both, the previously used assay based on bovine brain material as well as the alternative assay systems published by other research groups.

4. Results and discussion

The parent compounds (2*S*,4*R*)-**10a** and (2*R*,4*S*)-**10a**, (2*S*,4*R*)-**11a** and (2*R*,4*S*)-**11a** with no lipophilic residue at the nitrogen were found to be devoid of significant inhibitory potency at GAT1 as well as GAT3, even when tested at a concentration of 100 μM (see Tables 1 and 2).

To allow a better comparison of the biological data from Tables 1 and 2 with the already published[35] affinities of compounds **8** and **9** bearing no aryl substituent in 4-position, these data are quoted in this manuscript, too (Fig. 4). The bar diagrams (Fig. 4) display the effects on the selectivities for and the potencies at GAT1 and GAT3, when a 4-methoxyphenyl group is present in 4-position of the 4-hydroxypyrrolidine and 4-hydroxyproline derivatives as compared to compounds **8** and **9**. For compounds which at a concentration of 100 μM in preliminary GABA uptake experiments remaining GABA uptake has been reduced to no less than 50% no IC_{50} values have been determined (in Tables 1 and 2 listed as IC_{50} >100 μM). For the sake of simplicity, these compounds are listed in Figure 4 with IC_{50}s = 100 μM.

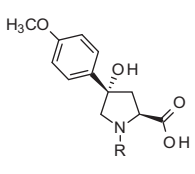
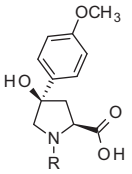
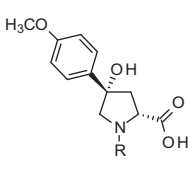
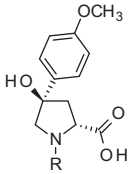
For the 4,4-diphenylbut-3-en-1-yl substituted 4-hydroxyprolines **8b** and the 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl (**c**) substituted 4-hydroxypyrrolidin-2-ylacetic acids **9c** (Fig. 4) with N-residues typical for GAT1 inhibitors, introduction of a 4-methoxyphenyl group resulting in **10b** and **11c**, respectively, led either to no enhancement or even a significant decrease in the inhibitory effect on GAT1 and GAT3 (IC_{50} >100 μM for all isomers of **10b** and **11c** at GAT1 and GAT3; Tables 1 and 2, Fig. 4). Especially the good potencies at and selectivities for GAT1 of (2*S*,4*R*)-**9c** of series **9c** (Fig. 4) was not reflected in any of the 4-aryl substituted analogues of **11c** (Table 2).

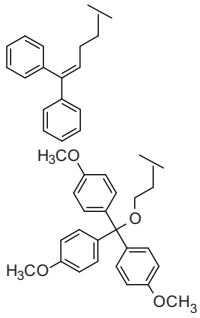
A somewhat different picture emerged for compounds **8d** and **9d** exhibiting a tris(4-methoxyphenyl)methoxyethyl moiety (**d**) known from (*S*)-SNAP-5114 and analogous compounds to enhance GAT3 potency. Upon introduction of a 4-(4-methoxyphenyl) residue in the pyrrolidine ring the inhibitory potency at GAT1 proteins increases, all isomers of **10d** and **11d** being more potent than their analogues **8d** and **9d** devoid of the aforementioned 4-substituent (Fig. 4).

Furthermore, all isomers of series **10d** with exception of (2*R*,4*R*)-**10d** showed also improved activity at GAT3 as compared to compounds **8d** (Fig. 4). Thereby, the *cis*-isomers (regarding the 4-hydroxy and 2-carboxy function) (2*R*,4*S*)-**10d** (IC_{50} at GAT3: 38.0 μM) and (2*S*,4*R*)-**10d** (IC_{50} at GAT3: 29.7 μM) are stronger inhibitors, at GAT3, than the corresponding *trans*-isomers (2*R*,4*R*)-**10d** and (2*S*,4*S*)-**10d** (IC_{50} = 63.3 μM and >100 μM, respectively). Of this series of compounds (2*S*,4*R*)-**10d** showed the best uptake inhibitory potency, but not only at GAT3 (IC_{50} = 29.7 μM), but also at GAT1 (IC_{50} = 45.1 μM).

Whereas for all isomers of **9d** with no aryl group in 4-position the inhibitory potency at GAT1 increases when switching to the 4-aryl-substituted compounds **11d**, the situation is less consistent for GAT3. Thus, the potencies of the isomers (2*R*,4*S*)-**11d** and (2*S*,4*S*)-**11d** are worse at GAT3 than those of the enantiomers **9d** lacking a 4-aryl-substituent. In contrast, the potencies of (2*S*,4*R*)-**11d** and (2*R*,4*R*)-**11d** are better at GAT3. Interestingly, albeit bearing a lipophilic residue typical for GAT3 selective inhibitors, all compounds

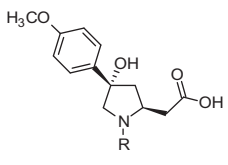
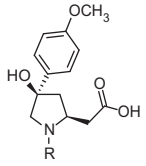
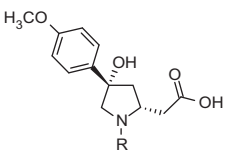
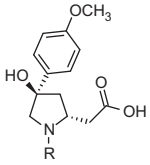
Table 1
Results of the biological testing of the compound series **10**

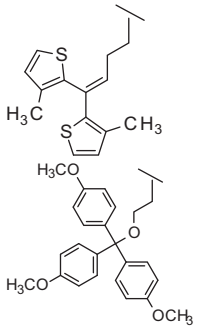





R		IC_{50} ± SEM (μM)			
H	GAT1 GAT3	–	>100[c] >100[d] (2*S*,4*R*)-**10a**	>100[c] >100[d] (2*R*,4*S*)-**10a**	–
	GAT1 GAT3	>100[a] >100[b] (2*S*,4*S*)-**10b**	>100[a] 237 ± 160[b] (2*S*,4*R*)-**10b**	>100[e] >100[f] (2*R*,4*S*)-**10b**	>100[e] >100[f] (2*R*,4*R*)-**10b**
	GAT1 GAT3	68.1 ± 4.4[a] 63.3 ± 5.8[b] (2*S*,4*S*)-**10d**	45.1 ± 2.7[a] 29.7 ± 0.8[b] (2*S*,4*R*)-**10d**	56 ± 4.3[c] 38.0 ± 5.8[d] (2*R*,4*S*)-**10d**	71 ± 1.4[c] >100[b] (2*R*,4*R*)-**10d**

Footnotes a–f specify the used biological materials: [a]bfcP2B, [b]bbsP2C, [c]pfcP2B, [d]pbsP2C, [e]cfcP2B, [f]cbsP2C. All the results of the measurements were processed and evaluated in triplicate unless otherwise stated. Each IC_{50} was given as mean ± SEM. For compounds where at a concentration of 100 μM in preliminary GABA uptake experiments remaining GABA uptake has been reduced to no less than 50% no IC_{50} values have been determined (IC_{50} >100 μM).

Table 2
Results of the biological testing of the compound series **10**

R		IC_{50} ± SEM (μM)			
H	GAT1 GAT3	–	>100[c] >100[d] (2*S*,4*R*)-**11a**	>100[c] >100[d] (2*R*,4*S*)-**11a**	–
	GAT1 GAT3	>100[c] 122 ± 9[d] (2*S*,4*S*)-**11c**	>100[c] 124 ± 15[d] (2*S*,4*R*)-**11c**	114 ± 12.3[c] >100[d] (2*R*,4*S*)-**11c**	>100[c] >100[d] (2*R*,4*R*)-**11c**
	GAT1 GAT3	75.2 ± 8.8[c] 78.9 ± 18.5[d] (2*S*,4*S*)-**11d**	48.4 ± 1.6[c] 66.7 ± 11.2[d] (2*S*,4*R*)-**11d**	59.2 ± 4.5[c] 66.1 ± 3.5[d] (2*R*,4*S*)-**11d**	64.5 ± 3.0[c] 65.4 ± 5.1[d] (2*R*,4*R*)-**11d**

Footnotes a–f specify the used biological materials: [a]bfcP2B, [b]bbsP2C, [c]pfcP2B, [d]pbsP2C, [e]cfcP2B, [f]cbsP2C. All the results of the measurements were processed and evaluated in triplicate unless otherwise stated. Each IC_{50} was given as mean ± SEM. For compounds where at a concentration of 100 μM in preliminary GABA uptake experiments remaining GABA uptake has been reduced to no less than 50% no IC_{50} values have been determined (IC_{50} >100 μM)

of series **11d** show only marginal to small differences with regard to their potencies at both transporters (GAT1 and GAT3).

Besides, it should be noted that the differences of the proline derivatives **10d** and their homologues **11d** with respect to their inhibitory potencies at GAT1 and GAT3 are not very large either.

5. Conclusion

Herein, we reported the synthesis and biological evaluation of optically pure 4-(4-methoxyphenyl) substituted 4-hydoxyproline and 4-hydroxypyrrolidin-2-ylacetic acid derivatives. The results of the biological tests showed that none of the tested compounds

exhibits very strong inhibitory activity at GAT1 or GAT3. The presence of a 4-methoxyphenyl unit in 4-position is clearly detrimental for stereoisomers of series **10b** and **11c** as the inhibitory potency at both GAT1 and GAT3 is far lower than that of the analogous compounds **8b** and **9c** lacking this substituent. However, for the stereoisomers of the proline derivatives **10d** (except stereoisomer (2*R*,4*R*)-**10d**), the presence of this moiety in 4-position is beneficial as it gives rise to improved inhibitory potencies at GAT3 and at GAT1 as compared to compounds of series **8d**. In most cases, this is also true for series **11d** compared to **9d**. Especially for GAT3 for which potent uptake inhibitors are still missing, appropriately 4-substituted proline and pyrrolidin-2-ylacetic acid derivatives might be a promising starting point for the search of new and highly potent GAT3 inhibitors.

6. Experimental part

In general, tetrahydrofuran, ether and diisopropylamine were distilled from sodium under nitrogen. Triethylamine (TEA) was refluxed with benzoyl chloride, followed by distillation under nitrogen. Other common solvents for recrystallization, column chromatography, analytical HPLC, and preparative HPLC were distilled prior to use. Purchased chemical reagents were used without further purification. TLC plates were made from silica gel 60 F_{254} on aluminum sheets (Merck). Compounds were stained with a solution of 5% $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.2% $Ce(SO_4)_2{\cdot}4H_2O$, and 5% concd H_2SO_4. If not stated otherwise, Merck silica gel (mesh 230–400) was used as stationary phase for flash chromatography. Analytical HPLC: Column LiChrospher Si 60 (5 μm, 250 × 4 mm with precolumn 4 × 4 mm). Prep. (preparative) HPLC: Column LiChrospher Si 60 (7 μm, 250 × 25 mm). Optical rotations: Polarimeter 241 MC at λ 589 cm^{-1}. Melting points (uncorrected) were determined on a Büchi 510 Melting Point apparatus. Elementary analysis: Elementaranalysator Rapid (Heraeus). IR spectroscopy: FT-IR Spectrometer 1600 and Paragon 1000 (Perkin Elmer), oils as film, solid samples as pellets for measurements. Mass spectroscopy: Mass Spectrometer 5989 A with 59980 B particle beam LC/MS interface (Hewlett Packard). NMR spectroscopy: NMR spectra were recorded on JNMR-GX (Jeol, 400 and 500 MHz) with TMS as internal standard and integrated with the program of NMR-software Nuts (2D Version 5.097, Acorn NMR, 1995).

6.1. General procedure 1 for the preparation of the 4-oxopyrrolidine derivatives (2*S*)-13b,d, (2*R*)-13b,d, (2*R*)-16c,d and (2*R*)-16c,d by Swern oxidation

To a solution of $(COCl)_2$ (1.5 equiv) in CH_2Cl_2 at −78 °C, DMSO (3 equiv) was added dropwise to generate an oxidizing reagent. After 15 min, the respective 4-hydroxypyrrolidine derivative of (2*S*,4*R*)-**12b,d**, (2*R*,4*R*)-**12b,d**, (2*S*,4*R*)-**15c,d**, and (2*R*,4*R*)-**15c,d** (1 equiv) in CH_2Cl_2 was added to the oxidizing solution at −78 °C. After 15 min (10 min for the derivatives with side chain **b**) between −78 °C and –70 °C, TEA (3.2 equiv) was added. The stirring was continued at rt for 15 min (10 min for the derivative with side chain **b**). After adding water and aq KOH or NaOH (3.2 equiv), the organic layer was isolated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried ($MgSO_4$), and concentrated in vacuum. The residual oil was purified by CC (column chromatography).

6.1.1. Methyl (2*S*)-1-(4,4-diphenylbut-3-en-1-yl)-4-oxopyrrolidine-2-carboxylate [(2*S*)-13b]

From DMSO (227 mg, 2.91 mmol) and $(COCl)_2$ (193 mg, 1.46 mmol) in CH_2Cl_2 (4.5 mL), (2*S*,4*R*)-**12b** (357 mg, 0.970 mmol) in CH_2Cl_2 (1.5 mL); TEA (304 mg, 3.00 mmol), aq 0.85 M KOH (3.7 mL, 3.2 mmol); purification by CC (*n*-heptane/acetone, 4:1). Yield: 312 mg (87%) as a colorless oil. $[\alpha]_D^{25}$ −32.4 (*c* 1.26, EtOH). IR: $\tilde{\nu}$ = 1761, 1737 cm^{-1}; MS (*m*/*z*): 350 $[M+1]^+$. ^1H NMR ($CDCl_3$) δ: 2.26 (q, 2H, *J* = 7.4 Hz, NCH_2CH_2), 2.43 (dd, 1H, *J* = 18.0, 5.5 Hz, $NCHCH_2$), 2.54–2.62 (m, 2H, $NCHCH_2$ and NCH_2CH_2), 2.77 (dt, 1H, *J* = 12.1, 7.4 Hz, NCH_2CH_2), 2.91 (d, 1H, *J* = 17.2 Hz, NCH_2CO), 3.29 (d, 1H, *J* = 17.2 Hz, NCH_2CO), 3.65 (s, 3H, OCH_3), 3.70 (dd, 1H, *J* = 7.8, 5.5 Hz, NCH), 6.03 (t, 1H, *J* = 7.4 Hz, $=CHCH_2$), 7.07–7.34 (m, 10H, H_{aromat}). Calcd for $C_{22}H_{23}NO_3$ (349.43): C, 75.62; H, 6.64; N, 4.01. Found: C, 75.79; H, 6.75; N, 3.73.

6.1.2. Methyl (2*R*)-1-(4,4-diphenylbut-3-en-1-yl)-4-oxopyrrolidine-2-carboxylate [(2*R*)-13b]

From DMSO (62 mg, 0.79 mmol) and $(COCl)_2$ (50 mg, 0.40 mmol) in CH_2Cl_2 (2 mL), (2*R*,4*R*)-**12b** (92 mg, 0.26 mmol) in CH_2Cl_2 (2 mL); TEA (83 mg, 0.82 mmol), aq 1.0 M NaOH (0.80 mL, 0.80 mmol); purification by CC (*n*-heptane/EtOAc, 4:1). Yield: 78 mg (85%) as a colorless oil. $[\alpha]_D^{20}$ +33.2 (*c* 0.60, EtOH). The spectra (^1H NMR, IR, and MS) were identical with those of (2*S*)-**13b**. Calcd for $C_{22}H_{23}NO_3$ (349.43): C, 75.62; H, 6.64; N, 4.01. Found: C, 75.51; H, 6.66; N, 3.87.

6.1.3. Methyl (2*S*)-4-oxo-1-{2-[tris(4-methoxyphenyl)methoxy]-1-ethyl}pyrrolidine-2-carboxylate [(2*S*)-13d]

From DMSO (72 mg, 0.92 mmol) and $(COCl)_2$ (61 mg, 0.46 mmol) in CH_2Cl_2 (2 mL), (2*S*,4*R*)-**12d** (160 mg, 0.307 mmol) in of CH_2Cl_2 (1.5 mL), TEA (108 mg, 1.07 mmol), aq 0.85 M KOH (1.2 mL, 1.0 mmol); purification by CC (Al_2O_3, pH 7.5 ± 0.5; *n*-heptane/EtOAc, 1:1). Yield: 132 mg (83%) as a colorless viscous oil. $[\alpha]_D^{29}$ −6.4 (*c* 1.83, EtOAc). IR: $\tilde{\nu}$ = 1754, 1604 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^1H NMR ($CDCl_3$) δ: 2.48 (dd, 1H, *J* = 18.0, 5.5 Hz, $NCHCH_2$), 2.65 (dd, 1H, *J* = 18.0, 7.8 Hz, $NCHCH_2$), 2.83 (dt, 1H, *J* = 13.0, 5.7 Hz, NCH_2CH_2), 2.94 (dt, 1H, *J* = 13.0, 5.7 Hz, NCH_2CH_2), 3.13 (d, 1H, *J* = 17.4 Hz, NCH_2CO), 3.24 (t, 2H, *J* = 5.7 Hz, NCH_2CH_2), 3.44 (d, 1H, *J* = 17.4 Hz, NCH_2CO), 3.72 (s, 3H, $COOCH_3$), 3.78 (s, 9H, $ArOCH_3$), 3.84 (dd, 1H, *J* = 7.8, 5.5 Hz, NCH), 6.79–6.83 (m, 6H, H_{aromat}), 7.29–7.32 (m, 6H, H_{aromat}). Calcd for $C_{30}H_{33}NO_7$ (519.59): C, 69.35; H, 6.40; N, 2.70. Found: C, 69.53; H, 6.59; N, 2.33.

6.1.4. Methyl (2*R*)-4-oxo-1-{2-[tris(4-methoxyphenyl)methoxy]-1-ethyl}pyrrolidine-2-carboxylate [(2*R*)-13d]

From DMSO (93 mg, 1.2 mmol) and $(COCl)_2$ (75 mg, 0.59 mmol) in CH_2Cl_2 (5 mL), (2*R*,4*R*)-**12d** (206 mg, 0.395 mmol) in CH_2Cl_2 (5 mL); TEA (132 mg, 1.30 mmol), aq 1.0 M NaOH (1.2 mL, 1.2 mmol); purification (*n*-heptane/acetone = 3:1). Yield: 183 mg (89%) as a colorless oil. $[alpha]_D^{20}$ +4.3 (*c* 1.5, EtOAc). The spectra (^1H NMR, IR, and MS) were identical with those of (2*S*)-**13d**. Calcd for $C_{30}H_{33}NO_7$ (519.59): C, 69.35; H, 6.40; N, 2.70. Found: C, 69.42; H, 6.75; N, 2.82.

6.1.5. Methyl (2*S*)-4-oxo-1-{2-[tris(4-methoxyphenyl)methoxy]-1-ethyl}pyrrolidin-2-acetate [(2*S*)-16d]

From $(COCl)_2$ (65 mg, 0.51 mmol) and DMSO (80 mg, 1.0 mmol) in CH_2Cl_2 (3 mL), (2*S*,4*R*)-**15d** (183 mg, 0.342 mmol) in CH_2Cl_2 (3 mL); TEA (114 mg, 1.14 mmol), aq 1.0 M NaOH (1.1 mL, 1.1 mmol); purification by CC (*n*-heptane/acetone = 3:2). Yield: 148 mg (81%) as a colorless oil. $[\alpha]_D^{20}$ −45.2 (*c* 0.95, EtOAc). IR: $\tilde{\nu}$ = 1756, 1738 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^1H NMR ($CDCl_3$) δ: 2.23 (dd, 1H, *J* = 18.4, 9.3 Hz, CH_2COO), 2.41 (dd, 1H, *J* = 15.5, 8.8 Hz, CH_2CHCH_2COO), 2.49 (dt, 1H, *J* = 12.7, 5.7 Hz, NCH_2CH_2), 2.60 (dd, 1H, *J* = 18.4, 6.7 Hz, CH_2COO), 2.79 (d, 1H, *J* = 17.7 Hz, NCH_2CO), 2.82 (dd, 1H, *J* = 15.5, 3.8 Hz, CH_2CHCH_2COO), 3.01–3.07 (m, 1H, NCH_2CH_2), 3.12–3.28 (m, 3H, NCH and 2H of NCH_2CH_2), 3.45 (d, 1H, *J* = 17.7 Hz, NCH_2CO), 3.65 (s, 3H, $COOCH_3$), 3.77 (s, 9H, $ArOCH_3$), 6.78–6.84 (m, 6H, H_{aromat}), 7.28–7.32 (m, 6H,

H_{aromat}). Calcd for $C_{31}H_{35}NO_7$ (533.62): C, 69.78; H, 6.61; N, 2.62. Found: C, 69.29; H, 6.75; N, 2.54.

6.1.6. Methyl (2*S*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-oxopyrrolidin-2-acetate [(2*S*)-16c]

From $(COCl)_2$ (56 mg, 0.44 mmol) and DMSO (69 mg, 0.87 mmol) in CH_2Cl_2 (2.5 mL), (2*S*,4*R*)-**15c** (118 mg, 0.291 mmol) in CH_2Cl_2 (2.5 mL); TEA (98 mg, 0.96 mmol), aq 1.0 M NaOH (0.92 mL, 0.92 mmol); purification by CC (*n*-heptane/acetone, 3:1). Yield: 108 mg (92%) as a slightly yellow oil. $[\alpha]_D^{20}$ +68.3 (*c* 0.75, EtOAc). IR: $\tilde{\nu}$ = 1760, 1732 cm^{-1}. MS (*m*/*z*): 404 $[M+1]^+$. ^1H NMR ($CDCl_3$) δ: 2.00 (s, 3H, thienyl-CH_3), 2.02 (s, 3H, thienyl-CH_3), 2.23–2.43 (m, 5H, CH_2COO, N*C*H_2CH_2, *C*H_2CHCH_2COO and 2H of NCH_2CH_2), 2.59–2.66 (m, 2H, N*C*H_2CO and CH_2COO), 2.80 (dd, 1H, *J* = 15.4, 3.9 Hz, *C*H_2CHCH_2COO), 2.99 (dt, 1H, *J* = 11.6, 7.9 Hz, N*C*H_2CH_2), 3.17–3.23 (m, 1H, NCH), 3.35 (d, 1H, *J* = 17.5 Hz, NCH_2CO), 3.67 (s, 3H, OCH_3), 6.05 (t, 1H, *J* = 7.3 Hz, =*C*HCH_2), 6.76 (d, 1H, *J* = 5.2 Hz, SCH), 6.84 (d, 1H, *J* = 5.2 Hz, SCH), 7.05 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.21 (d, 1H, *J* = 5.2 Hz, SCH=C*H*). Calcd for $C_{21}H_{25}NO_3S_2$ (403.560): C, 62.50; H, 6.24; N, 3.47. Found: C, 62.25; H, 5.97; N 3.40.

6.1.7. Ethyl (2*R*)-4-oxo-1-{2-[tris(4-methoxyphenyl)methoxy]-1-ethyl}pyrrolidin-2-acetate [(2*R*)-16d]

From $(COCl)_2$ (69 mg, 0.54 mmol) and DMSO (85 mg, 1.1 mmol) in CH_2Cl_2 (2 mL), (2*R*,4*R*)-**15d** (149 mg, 0.271 mmol) in CH_2Cl_2 (2.5 mL); TEA (121 mg, 1.19 mmol), aq 1.0 M NaOH (1.1 mL, 1.1 mmol); purification by CC (*n*-heptane/acetone, 3:2). Yield: 130 mg (87%) as a pale yellow oil. $[\alpha]_{578}^{20}$: +56.9 (*c* 1.53, EtOAc). IR: $\tilde{\nu}$ = 1760, 1732, 1608 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^1H NMR ($CDCl_3$) δ: 1.23 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.23 (dd, 1H, *J* = 18.4, 9.3 Hz, CH_2COO), 2.40 (dd, 1H, *J* = 15.5, 8.8 Hz, *C*H_2CHCH_2COO), 2.48 (dt, 1H, *J* = 12.7, 5.7 Hz, N*C*H_2CH_2), 2.60 (dd, 1H, *J* = 18.4, 6.7 Hz, CH_2COO), 2.79 (d, 1H, *J* = 17.7 Hz, NCH_2CO), 2.81 (dd, 1H, *J* =15.5, 3.8 Hz, *C*H_2CHCH_2COO), 3.02–3.08 (m, 1H, N*C*H_2CH_2), 3.13–3.29 (m, 3H, N*C*H and 2H of NCH_2CH_2), 3.45 (d, 1H, *J* = 17.7 Hz, NCH_2CO), 3.77 (s, 9H, $ArOCH_3$), 4.06–4.17 (m, 2H, *C*H_2CH_3), 6.79–6.82 (m, 6H, H_{aromat}), 7.29–7.23 (m, 6H, H_{aromat}). Calcd for $C_{32}H_{37}NO_7$ (547.65): C, 70.18; H, 6.81; N, 2.56. Found: C, 69.98; H, 6.79; N 2.43.

6.1.8. Ethyl (2*R*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-oxopyrrolidin-2-acetate [(2*R*)-16c]

From $(COCl)_2$ (140 mg, 1.10 mmol) and DMSO (173 mg, 2.20 mmol) in CH_2Cl_2 (3 mL), (2*R*,4*R*)-**15c** (230 mg, 0.548 mmol) in CH_2Cl_2 (3 mL); TEA (246 mg, 2.42 mmol), aq 1.0 M NaOH (2.3 mL, 2.3 mmol); purification by CC (*n*-heptane/acetone, 3:1). Yield: 201 mg (88%) as a yellow oil. $[\alpha]_D^{20}$ −59.4 (*c* 2.00, EtOAc). IR: $\tilde{\nu}$ = 1759, 1732 cm^{-1}. MS (*m*/*z*): 418 $[M+1]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.25 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.00 (s, 3H, thienyl-CH_3), 2.02 (s, 3H, thienyl-CH_3), 2.22–2.42 (m, 5H, CH_2COO, N*C*H_2CH_2, *C*H_2CHCH_2COO and 2H of NCH_2CH_2), 2.59–2.66 (m, 2H, N*C*H_2CO and CH_2COO), 2.80 (dd, 1H, *J* = 15.4, 3.9 Hz, *C*H_2CHCH_2COO), 2.99 (dt, 1H, *J* = 11.6, 7.9 Hz, N*C*H_2CH_2), 3.17–3.23 (m, 1H, NCH), 3.35 (d, 1H, *J* = 17.5 Hz, NCH_2CO), 4.09–4.18 (m, 2H, *C*H_2CH_3), 6.05 (t, 1H, *J* = 7.3 Hz, =*C*HCH_2), 6.76 (d, 1H, *J* = 5.2 Hz, SCH), 6.84 (d, 1H, *J* = 5.2 Hz, SCH), 7.05 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.21 (d, 1H, *J* = 5.2 Hz, SCH=C*H*). Calcd for $C_{22}H_{27}NO_3S_2$ (417.59): C, 63.28; H, 6.52; N, 3.35; S 15.35. Found: C, 63.20; H, 6.53; N, 3.20; S 14.92.

6.2. General procedure 2 for the preparation of the Cbz-protected 4-oxopyrrolidine derivatives (2*S*)-13e, (2*R*)-13e, (2*S*)-16e and (2*R*)-16e by Jones oxidation

To a solution of the respective N-protected 4-hydroxypyrrolidine derivative of (2*S*,4*R*)-**12e**, (2*R*,4*R*)-**12e**, (2*S*,4*R*)-**15e**, and (2*R*,4*R*)-**15e** (1 equiv) in acetone (15 mL), chromic acid in aq H_2SO_4 (2.67 M, 8.50 equiv) was added at rt for 5 min. The brown mixture was stirred at rt for 30 min followed by the addition of MeOH (9 equiv) to destroy excessive oxidizing agent. The supernatant was decanted, diluted by CH_2Cl_2 (70 mL), washed with sat. aq NH_4Cl, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was purified by CC (*n*-heptane/acetone, 3:1).

6.2.1. Methyl (2*S*)-1-benzyloxycarbonyl-4-oxopyrrolidine-2-carboxylate [(2*S*)-13e]

From (2*S*,4*R*)-**12e** (500 mg, 1.79 mmol) in acetone (28 mL), 2.67 M chromic acid in aq H_2SO_4 (1.90 mL, 15.2 mmol). Yield: 354 mg (71%); colorless oil. $[\alpha]_D^{20}$ +17.8 (*c* 1.10, $CHCl_3$) {Lit.[30b]: $[\alpha]_D^{20}$ +18.5 (*c* 1.0, $CHCl_3$)}.

6.2.2. Methyl (2*R*)-1-benzyloxycarbonyl-4-oxopyrrolidine-2-carboxylate [(2*R*)-13e]

From (2*R*,4*R*)-**12e** (800 mg, 2.87 mmol), 2.67 M chromic acid in aq H_2SO_4 (3.04 mL, 24.3 mmol). Yield: 623 mg (78%); colorless oil. $[\alpha]_D^{20}$ −18.8 (*c* 1.31, $CHCl_3$) {lit.[30b]: $[\alpha]_D^{20}$ −19.4 (*c* 1.0, $CHCl_3$)}.

6.2.3. Methyl (2*S*)-1-benzyloxycarbonyl-4-oxopyrrolidin-2-acetate [(2*S*)-16e]

From (2*S*,4*R*)-**15e** (300 mg, 1.02 mmol) in acetone (16 mL), 2.67 M chromic acid in aq H_2SO_4 (1.08 mL, 8.7 mmol). Yield: 225 mg (76%) as colorless crystals. mp 53–54 °C. $[\alpha]_D^{20}$: +10.7 (*c* 1.06, $CHCl_3$). IR: $\tilde{\nu}$ = 1765, 1732 cm^{-1}. MS (*m*/*z*): 292 $[M+1]^+$. ^1H NMR ($C_6D_5NO_2$, 120 °C) δ: 2.59 (d, 1H, *J* = 18.4 Hz, CH_2COO), 2.83–2.99 (m, 3H, CH_2COO and 2H of *C*H_2CHCH_2COO), 3.63 (s, 3H, OCH_3), 3.88 (d, 1H, *J* = 18.1 Hz, NCH_2), 4.02 (d, 1H, *J* = 18.1 Hz, NCH_2), 4.75–4.82 (m, 1H, NCH), 5.27 (s, 2H, *C*H_2Ph), 7.27–7.44 (m, 5H, H_{aromat}). Calcd for $C_{15}H_{17}NO_5$ (291.31): C, 61.85; H, 5.88; N, 4.81. Found: C, 61.77; H, 6.07; N 4.74.

6.2.4. Ethyl (2*R*)-1-benzyloxycarbonyl-4-oxopyrrolidin-2-acetate [(2*R*)-16e]

From (2*R*,4*R*)-**15e** (192 mg, 0.625 mmol), 2.67 M chromic acid in aq H_2SO_4 (0.69 mL, 5.5 mmol). Yield: 147 mg (77%) as a colorless oil. $[\alpha]_D^{20}$ −11.3 (*c* 1.38, $CHCl_3$). IR: $\tilde{\nu}$ = 1766, 1732 cm^{-1}. MS (*m*/*z*): 306 $[M+1]^+$. ^1H NMR ($CDCl_3$) δ: 1.66–1.70 (m, 3H, CH_2CH_3), 3.06–3.12 (m, 1H, CH_2COO), 3.31–3.37 (m, 1H, CH_2COO), 3.39–3.47 (m, 2H, *C*H_2CHCH_2COO), 4.36 (d, 1H, *J* = 18.6 Hz, NCH_2), 4.50 (d, 1H, *J* = 18.6 Hz, NCH_2), 4.58–4.64 (m, 2H, *C*H_2CH_3), 5.23–5.29 (m, 1H, NCH), 5.74 (s, 2H, *C*H_2Ph), 7.72–7.84 (m, 5H, H_{aromat}). Calcd for $C_{16}H_{19}NO_5$ (305.34): C, 62.94; H, 6.27; N, 4.59. Found: C, 62.76; H, 6.28; N, 4.55.

6.3. General procedure 3 (GP3) for the preparation of the optically pure 4-hydroxy-4-(4-methoxyphenyl)pyrrolidine derivatives 14b,d and 17c,d by organometallic addition

Method A: To a mixture of Mg (266 mg, 11.1 mmol) and THF (22 mL), 4-$MeOC_6H_5Br$ (1.43 mL, 2.08 g, 11.1 mmol) was added for 40 min to yield 4-$MeOC_6H_4MgBr$ (0.48 M in THF). The resulting anhydrous $CeCl_3$[46] (0.3–2.0 equiv) was dried in vacuo at elevated temperature for 15 min before use and then cooled to 0 °C. THF (10–20 mL $mmol^{-1}$) and 4-$MeOC_6H_4MgBr$ (1.0–2.1 equiv, 0.48 M in THF) were added. The resulting white suspension was stirred for 1 h at 0 °C and then cooled to the temperature given. A solution of the respective 4-oxopyrrolidine (1 equiv) in THF (10–20 mL $mmol^{-1}$) was added. Stirring was continued for the time given. The reaction was quenched with aq sat. NH_4Cl. The organic layer was separated and the aqueous layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residual oil was subjected to analytical

HPLC, purified by CC and by prep. (preparative) HPLC. Unconsumed 4-oxopyrrolidine derivative was recovered.

Method B: 4-$MeOC_6H_4Br$ (1.43 mL, 2.08 g, 11.1 mmol) was added for 40 min to a mixture of Mg (266 mg, 11.1 mmol) in Et_2O (25 mL). The resulting solution of 4-$MeOC_6H_4MgBr$ (0.44 M in Et_2O, 1.80 equiv) was added dropwise to a solution of the respective 4-oxopyrrolidine (1 equiv) in Et_2O (35 mL mmol^{-1}) at the temperature given and stirring was continued for the time given. Workup and purification were similar to GP3A.

6.3.1. Methyl (2*S*,4*S*)-1-(4,4-diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*S*,4*S*)-14b] and methyl (2*S*,4*R*)-1-(4,4-diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*S*,4*R*)-14b]

According to GP3A: from $CeCl_3$ (148 mg, 0.601 mmol) and 4-$MeOC_6H_4MgBr$ (1.25 mL, 0.48 M in THF, 0.60 mmol) in THF (8 mL); (2*S*)-**13b** (150 mg, 0.429 mmol) in THF (7 mL); reaction: at −78 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc/iso-propanol, 88:12:0.77; 1.3 mL min^{-1}) *ds*: (2*S*,4*R*)-**14b** /(2*S*,4*S*)-**14b** = 70:30 [(2*S*,4*R*)-**14b** t_R = 22.8 min, (2*S*,4*S*)-**14b** t_R = 28.4 min]; prep. HPLC (*n*-heptane/EtOAc/isopropanol, 90:10:1.0; 12 mL min^{-1}) yielded (2*S*,4*R*)-**14b** (t_R = 39.7 min) and (2*S*,4*S*)-**14b** (t_R = 49.5 min).

(2*S*,4*R*)-**14b**: 54 mg (28%) as colorless crystals. mp 105–106 °C (*i*Pr_2O). $[\alpha]_D^{20}$ −49.4 (*c* 0.815, $CHCl_3$). IR: $\tilde{\nu}$ = 3471, 1720 cm^{-1}. MS (*m*/*z*): 458 [M+1]$^+$. ^{1}H NMR ($CDCl_3$) δ: 2.15 (ddd, 1H, *J* = 13.9, 3.2, 2.0 Hz, NCHC*H*$_2$), 2.25 (q, 2H, *J* = 7.5 Hz, NCH$_2$C*H*$_2$), 2.49 (dd, 1H, *J* = 13.9, 10.7 Hz, NCHC*H*$_2$), 2.63–2.70 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$COH), 2.80 (dt, 1H, *J* = 12.1, 7.5 Hz, NC*H*$_2$CH$_2$), 3.09 (dd, 1H, *J* = 9.2, 2.0 Hz, NC*H*$_2$COH), 3.38 (dd, 1H, *J* = 10.7, 3.2 Hz, NCH), 3.66 (s, 3H, $COOCH_3$), 3.72 (s, 3H, $ArOCH_3$), 3.92 (s, 1H, OH), 6.05 (t, 1H, *J* = 7.5 Hz, =C*H*CH$_2$), 6.78–6.81 (m, 2H, H$_{aromat.}$), 7.09–7.33 (m, 12H, H$_{aromat.}$). Calcd for $C_{29}H_{31}NO_4$ (457.57): C, 76.12; H, 6.83; N, 3.06. Found: C, 76.03; H, 6.89; N, 3.09.

(2*S*,4*S*)-**14b**: 24 mg (12%) as colorless crystals. mp 103–104 °C (*i*Pr_2O). $[\alpha]_D^{20}$ −1.8 (*c* 1.00, $CHCl_3$). IR: $\tilde{\nu}$ = 3424, 1742 cm^{-1}. MS (*m*/*z*): 458 [M+1]$^+$. ^{1}H NMR ($CDCl_3$): δ 2.27 (q, 2H, *J* = 7.5 Hz, NCH$_2$C*H*$_2$), 2.34 (d, 2H, *J* = 7.7 Hz, NCHC*H*$_2$), 2.67–2.75 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$COH), 2.85 (dt, 1H, *J* = 12.0, 7.5 Hz, NC*H*$_2$CH$_2$), 3.35 (d, 1H, *J* = 10.3 Hz, NC*H*$_2$COH), 3.63 (s, 3H, $COOCH_3$), 3.71 (s, 3H, $ArOCH_3$), 3.78 (t, 1H, *J* = 7.7 Hz, NCH), 6.05 (t, 1H, *J* = 7.5 Hz, =C*H*CH$_2$), 6.76–6.81 (m, 2H, H$_{aromat.}$), 7.09–7.37 (m, 12H, H$_{aromat.}$). Calcd for $C_{29}H_{31}NO_4$ (457.57): C, 76.12; H, 6.83; N, 3.06. Found: C, 76.23; H, 6.86; N 2.92.

According to GP3B: from (2*S*)-**13b** (150 mg, 0.429 mmol) in Et_2O (15 mL), 4-$MeOC_6H_4MgBr$ (1.76 mL, 0.44 M in Et_2O, 0.77 mmol); reaction: at −78 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc/iso-propanol, 88:12:0.77; 1.3 mL min^{-1}), *ds*: (2*S*,4*R*)-**14b** /(2*S*,4*S*)-**14b** = 11:89 [(2*S*,4*R*) t_R = 23.1 min, (2*S*,4*S*) t_R = 28.4 min]; prep. HPLC (*n*-heptane/EtOAc/isopropanol, 90:10:1.0; 12 mL min^{-1}) yielded (2*S*,4*R*)-**13b** (12 mg, yield: 6%; t_R = 39.0 min) and (2*S*,4*S*)-**13b** (109 mg, yield: 56%; t_R = 53.6 min).

6.3.2. Methyl (2*R*,4*S*)-1-(4,4-diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*R*,4*S*)-14b] and methyl (2*R*,4*R*)-1-(4,4-diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*R*,4*R*)-14b]

According to GP3A from $CeCl_3$ (315 mg, 1.28 mmol) and 4-$MeOC_6H_4MgBr$ (2.67 ml, 0.48 M in THF, 1.3 mmol) in THF (14 mL); (2*R*)-**13b** (320 mg, 0.915 mmol) in THF (10 mL); reaction: at −60 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc/*iso*-propanol, 88:12:0.77), *ds*: (2*R*,4*S*)-**14b** /(2*R*,4*R*)-**14b** = 58:42 [(2*R*,4*S*)-**14b** t_R = 21.8 min, (2*R*,4*R*)-**14b** t_R = 27.2 min]. Purification by CC (*n*-heptane/EtOAc, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 86:14:0.9) yielded (2*R*,4*S*)-**14b** (t_R = 26.0 min) and (2*R*,4*R*)-**14b** (t_R = 37.1 min); (2*R*)-**13b** (25 mg, 7.8%) was recovered.

(2*R*,4*S*)-**14b**: 116 mg (28%) as colorless crystals. mp 105–107 °C (*i*Pr_2O). $[\alpha]_D^{20}$ +47.9 (*c* 0.90, $CHCl_3$). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**14b**. Calcd for $C_{29}H_{31}NO_4$ (457.57): C, 76.12; H, 6.83; N, 3.06. Found: C, 76.03; H, 6.86; N, 3.02.

(2*R*,4*R*)-**14b**: 82 mg (20%) as colorless crystals. mp 103–104 °C (*i*Pr_2O). $[\alpha]_D^{20}$ +1.6 (*c* 0.80, $CHCl_3$). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*S*)-**14b**. Calcd for $C_{29}H_{31}NO_4$ (457.57): C, 76.12; H, 6.83; N, 3.06. Found: C, 76.07; H, 6.83; N, 3.09.

6.3.3. Methyl (2*S*,4*S*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylate [(2*S*,4*S*)-14d] and methyl (2*S*,4*R*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylate [(2*S*,4*R*)-14d]

According to GP3A from $CeCl_3$ (130 mg, 0.527 mmol) and 4-$MeOC_6H_4MgBr$ (0.80 mL, 0.67 M in THF, 0.53 mmol) in THF (5 mL); (2*S*)-**13d** (196 mg, 0.377 mmol) in THF (6 mL); reaction: at −60 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc, 50:50), *ds*: (2*S*,4*S*)-**14d**/(2*S*,4*R*)-**14d** = 48:52 [(2*S*,4*S*)-**14d** t_R = 7.6 min, (2*S*,4*R*)-**14d** t_R = 11.9 min]. Purification by CC (*n*-heptane/EtOAc, 3:2) and separation by prep. HPLC (*n*-heptane/EtOAc, 55:45) afforded (2*S*,4*S*)-**14d** (t_R = 19.7 min) and (2*S*,4*R*)-**14d** (t_R = 24.6 min).

(2*S*,4*S*)-**14d**: 57 mg (24%) as a colorless viscous oil. $[\alpha]_D^{20}$ −7.1 (*c* 1.10, acetone). IR: $\tilde{\nu}$ = 3478, 1738 cm^{-1}. MS (*m*/*z*): 333 [Ar_3C]$^+$. ^{1}H NMR ($CDCl_3$) δ: 2.34–2.44 (m, 2H, NCHC*H*$_2$), 2.54 (s, 1H, OH), 2.94–3.00 (m, 2H, NC*H*$_2$COH and NC*H*$_2$CH$_2$), 3.04–3.10 (m, 1H, NC*H*$_2$CH$_2$), 3.17–3.26 (m, 2H, NCH$_2$C*H*$_2$), 3.49 (d, 1H, *J* = 10.7 Hz, NC*H*$_2$COH), 3.69 (s, 3H, $COOCH_3$), 3.78 (s, 9H, $ArOCH_3$), 3.79 (s, 3H, Ar'OCH_3), 3.94 (t, 1H, *J* = 7.6 Hz, NCH), 6.79–6.83 (m, 6H, H$_{aromat}$), 6.85–6.88 (m, 2H, H$_{aromat}$), 7.31–7.34 (m, 6H, H$_{aromat}$), 7.38–7.42 (m, 2H, H$_{aromat}$). Calcd for $C_{37}H_{41}NO_8$ (627.73): C, 70.80; H, 6.58; N, 2.23. Found: C, 70.33; H, 7.07; N, 2.22.

(2*S*,4*R*)-**14d**: 62 mg (26%) as a colorless viscous oil. $[\alpha]_D^{20}$ −22.7 (*c* 1.66, acetone). IR: $\tilde{\nu}$ = 3476, 1733 cm^{-1}. MS (*m*/*z*): 333 [Ar_3C]$^+$. ^{1}H NMR ($CDCl_3$) δ: 2.23 (dt, 1H, *J* = 13.9, 3.0 Hz, NCHC*H*$_2$), 2.56 (dd, 1H, *J* = 13.9, 10.7 Hz, NCHC*H*$_2$), 2.87–2.95 (m, 2H, NC*H*$_2$COH and NC*H*$_2$CH$_2$), 2.98–3.05 (m, 1H, NC*H*$_2$CH$_2$), 3.19–3.24 (m, 3H, NC*H*$_2$COH and 2H of NCH$_2$C*H*$_2$), 3.63 (dd, 1H, *J* = 10.7, 3.0 Hz, NCH), 3.71 (s, 3H, $COOCH_3$), 3.79 (s, 9H, $ArOCH_3$), 3.81 (s, 3H, Ar'-OCH_3), 6.81–6.84 (m, 6H, H$_{aromat}$), 6.86–6.90 (m, 2H, H$_{aromat}$), 7.31–7.35 (m, 6H, H$_{aromat}$), 7.37–7.40 (m, 2H, H$_{aromat}$). Calcd for $C_{37}H_{41}NO_8$ (627.73): C, 70.80; H, 6.58; N, 2.23. Found: C, 70.51; H, 6.62; N, 2.15.

6.3.4. Methyl (2*R*,4*S*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylate [(2*R*,4*S*)-14d] and methyl (2*R*,4*R*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylate [(2*R*,4*R*)-14d]

According to GP3A from $CeCl_3$ (165 mg, 0.669 mmol) and 4-$MeOC_6H_4MgBr$ (1.40 mL, 0.48 M in THF, 0.67 mmol) in THF (10 mL); (2*R*)-**13d** (248 mg, 0.478 mmol) in THF (10 mL); reaction: at −60 °C for 20 h. Analytical HPLC (*n*-heptane/EtOAc/*iso*-propanol, 70:30:1.0; 1.3 mL min^{-1}), *ds*: (2*R*,4*R*)-**14d**/(2*R*,4*S*)-**14d** = 45:55 [(2*R*,4*R*)-**14d** t_R = 17.5 min, (2*R*,4*S*)-**14d** t_R = 22.9 min]. Purification by CC (*n*-heptane/acetone, 2:1) and separation by prep. HPLC (*n*-heptane/EtOAc, 60:40; 12 mL min^{-1}) yielded (2*R*,4*R*)-**14d** (t_R = 29.3 min) and (2*R*,4*S*)-**14d** (t_R = 43.7 min); (2*R*)-**13d** (19 mg, 7.7%) was recovered.

(2*R*,4*R*)-**14d**: 57 mg (19%) as a colorless viscous oil. $[\alpha]_D^{23}$ +7.6 (*c* 0.93, acetone). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*S*)-**14d**. Calcd for $C_{37}H_{41}NO_8$ (627.73): C, 70.80; H, 6.58; N, 2.23. Found: C, 70.47; H, 6.62; N, 2.13.

(2*R*,4*S*)-**14d**: 63 mg (21%) as a colorless oil. $[\alpha]_D^{20}{}_D{}^{24}$: +22.9 (*c* 1.12, acetone). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**14d**. Calcd for $C_{37}H_{41}NO_8$ (627.73): C, 70.80; H, 6.58; N, 2.23. Found: C, 70.61; H, 6.58; N, 2.15.

6.3.5. Methyl (2*S*,4*R*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetate [(2*S*,4*R*)-17d] and methyl (2*S*,4*S*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetate [(2*S*,4*S*)-17d]

According to GP3A from $CeCl_3$ (110 mg, 0.45 mmol) and 4-$MeOC_6H_4MgBr$ (0.93 mL, 0.48 M in THF, 0.45 mmol) in THF (8 mL); (2*S*)-**16d** (170 mg, 0.320 mmol) in THF (4 mL); reaction: at −78 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc/*iso*-propanol, 54:45:1.2; 1.3 mL min^{-1}) *ds*: (2*S*,4*S*)-**17d**/(2*S*,4*R*)-**17d** = 17:83 [(2*S*,4*S*)-**17d** t_R = 10.0 min, (2*S*,4*R*)-**17d** t_R = 14.1 min]; purification by CC (*n*-heptane/acetone, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/isopropanol, 54:45:1.2; 12 mL min^{-1}) yielded (2*S*,4*S*)-**17d** (t_R = 20.4 min) and (2*S*,4*R*)-**17d** (t_R = 28.0 min). (2*S*)-16**b** (35 mg, 21%) was recovered.

(2*S*,4*S*)-**17d**: 11 mg (5%) as a colorless oil. $[\alpha]_D^{20}$ −14.4 (*c* 0.95, acetone). IR: $\tilde{\nu}$ = 3462, 1732, 1608 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.05 (dd, 1H, *J* = 13.0, 9.6 Hz, C*H*$_2$CHCH$_2$COO), 2.28–2.37 (m, 2H, C*H*$_2$CHCH$_2$COO and CH$_2$COO), 2.69–2.77 (m, 2H, NC*H*$_2$CH$_2$ and CH$_2$COO), 2.82 (d, 1H, *J* = 10.9 Hz, NC*H*$_2$COH), 2.99–3.06 (m, 1H, NC*H*$_2$CH$_2$), 3.10–3.20 (m, 2H, NCH$_2$C*H*$_2$), 3.43 (d, 1H, *J* = 10.9 Hz, NC*H*$_2$COH), 3.44–3.51 (m, 1H, NCH), 3.63 (s, 3H, COOCH$_3$), 3.77 (s, 9H, Ar'OCH$_3$), 3.79 (s, 3H, ArOCH$_3$), 6.78–6.82 (m, 6H, H_{aromat}), 6.83–6.86 (m, 2H, H_{aromat}), 7.31–7.35 (m, 6H, H_{aromat}), 7.36–7.40 (m, 2H, H_{aromat}). Calcd for $C_{38}H_{43}NO_8$ (641.77): C, 71.12; H, 6.75; N, 2.18. Found: C, 71.10; H, 6.86; N, 2.11.

(2*S*,4*R*)-**17d**: 88 mg (43%) as a colorless oil. $[\alpha]_D^{20}$ −36.8 (*c* 0.88, acetone). IR: $\tilde{\nu}$ = 3481, 1732, 1607 cm^{-1}. MS; (*m*/*z*): 333 $[Ar_3C]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.08 (ddd, 1H, *J* = 14.1, 5.2, 1.8 Hz, C*H*$_2$CHCH$_2$COO), 2.51–2.61 (m, 4 H, C*H*$_2$CHCH$_2$COO, CH$_2$COO, NC*H*$_2$CH$_2$ and NC*H*$_2$COH), 2.72 (dd, 1H, *J* = 16.0, 3.5 Hz, CH$_2$COO), 2.99–3.08 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*), 3.12–3.25 (m, 3H, NC*H*$_2$COH and 2H of NCH$_2$C*H*$_2$), 3.55 (s, 1H, OH), 3.64 (s, 3H, COOCH$_3$), 3.77 (s, 9H, Ar'OCH$_3$), 3.79 (s, 3H, ArOCH$_3$), 6.80–6.88 (m, 8H, H_{aromat}), 7.30–7.34 (m, 6H, H_{aromat}), 7.35–7.39 (m, 2H, H_{aromat}). Calcd for $C_{38}H_{43}NO_8$ (641.77): C, 71.12; H, 6.75; N, 2.18. Found: C, 71.06; H, 7.08; N, 2.14.

6.3.6. Methyl (2*S*,4*R*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*S*,4*R*)-17c] and methyl (2*S*,4*S*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*S*,4*S*)-17c]

According to GP3A from $CeCl_3$ (127 mg, 0.521 mmol) and 4-$MeOC_6H_4MgBr$ (1.1 mL, 0.48 M in THF, 0.52 mmol) in THF (8 mL), (2*S*)-**16c** (150 mg, 0.372 mml) in THF (7 mL); reaction: at −78 °C for 20 h; analytical HPLC (*n*-heptane/EtOAc/*iso*-propanol, 75:25:0.5; 1.3 mL min^{-1}) *ds*: (2*S*,4*S*)-**17c**/(2*S*,4*R*)-**17c** = 21:79 [(2*S*,4*S*)-**17c** t_R = 16.7 min, (2*S*,4*R*)-**17c** t_R 19.6 min;]. Purification by CC (*n*-heptane/acetone, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 75:25:0.5; 12 mL min^{-1}) yielded (2*S*,4*S*)-**17c** (t_R = 39.1 min) and (2*S*,4*R*)-**17c** (t_R = 46.2 min). (2*S*)-**16c** (52 mg, 35%) was recovered.

(2*S*,4*S*)-**17c**: 16 mg (8%) as a colorless oil. $[\alpha]_D^{20}$ −50.4 (*c* 1.09, $CHCl_3$). IR: $\tilde{\nu}$ = 3493, 1732, 1613 cm^{-1}. MS (*m*/*z*): 512 $[M+1]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.00 (s, 3H, thienyl-CH$_3$), 2.02 (s, 3H, thienyl-CH$_3$), 2.04 (dd, 1H, *J* = 13.2, 9.5 Hz, C*H*$_2$CHCH$_2$COO), 2.27–2.35 (m, 4H, C*H*$_2$CHCH$_2$COO, CH$_2$COO and 2H of NCH$_2$C*H*$_2$), 2.54–2.61 (m, 1H, NC*H*$_2$CH$_2$), 2.67–2.72 (m, 2H, CH$_2$COO and NC*H*$_2$COH), 2.90 (dt, 1H, *J* = 12.0, 8.0 Hz, NC*H*$_2$CH$_2$), 3.35–3.42 (m, 2H, NC*H*$_2$COH and NC*H*), 3.64 (s, 3 H, COOCH$_3$), 3.78 (s, 3H, ArOCH$_3$), 6.09 (t, 1H, *J* = 7.2 Hz, =C*H*CH$_2$), 6.76 (d, 1H, *J* = 5.2 Hz, SCH), 6.82–6.86 (m, 3H, SC*H* and 2 H of H_{aromat}), 7.05 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.19 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.37–7.41 (m, 2H, H_{aromat}). Calcd for $C_{28}H_{33}NO_4S_2$ (511.70): C, 65.72; H, 6.50; N, 2.74; S, 12.53. Found: C, 65.97; H, 6.84; N, 2.56; S, 12.35.

(2*S*,4*R*)-**17c**: 45 mg (24%) as colorless crystals. mp 99–100 °C (*i*Pr$_2$O). $[\alpha]_D^{20}$ +73.1 (*c* 1.00, $CHCl_3$). IR: $\tilde{\nu}$ = 3523, 1731 cm^{-1}. MS (*m*/*z*): 512 $[M+1]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.00 (s, 3H, thienyl-CH$_3$), 2.03 (s, 3H, thienyl-CH$_3$), 2.09 (ddd, 1H, *J* = 14.2, 5.2, 2.0 Hz, C*H*$_2$CHCH$_2$COO), 2.29–2.37 (m, 2H, NCH$_2$C*H*$_2$), 2.39–2.46 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*$_2$COH), 2.50–2.60 (m, 2H, C*H*$_2$CHCH$_2$COO and CH$_2$COO), 2.73 (dd, 1H, *J* = 15.7/3.5 Hz, CH$_2$COO), 2.90–3.02 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*), 3.13 (dd, 1H, *J* = 9.4, 2.0 Hz, NC*H*$_2$COH), 3.42 (s, 1H, OH), 3.66 (s, 3H, COOCH$_3$), 3.79 (s, 3H, ArOCH$_3$), 6.08 (t, 1H, *J* = 7.2 Hz, =C*H*CH$_2$), 6.76 (d, 1H, *J* = 5.2 Hz, SCH), 6.83 (d, 1H, *J* = 5.2 Hz, SCH), 6.84–6.88 (m, 2H, H_{aromat}), 7.05 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.20 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.36–7.40 (m, 2H, H_{aromat}). Calcd for $C_{28}H_{33}NO_4S_2$ (511.70): C, 65.72; H, 6.50; N, 2.74; S, 12.53. Found: C, 66.07; H, 6.70; N, 2.70; S, 12.48.

6.3.7. Ethyl (2*R*,4*R*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetate [(2*R*,4*R*)-17d] and ethyl (2*R*,4*S*)-4-hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetate [(2*R*,4*S*)-17d]

According to GP3A from $CeCl_3$ (76 mg, 0.31 mmol) and 4-$MeOC_6H_4MgBr$ (0.63 mL, 0.48 M in THF, 0.30 mmol) in THF (5 mL), (2*R*)-**16d** (119 mg, 0.217 mmol) in THF (4 mL); reaction: at −78 °C for 20 h. Purification by CC (*iso*-hexane/acetone, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 60:40:0.88; 12 mL min^{-1}.) yielded (2*R*,4*R*)-**17d** (t_R = 23.0 min) and (2*R*,4*S*)-**17d** (t_R = 30.4 min). (2*R*)-**16d** (20 mg, 17%) was recovered.

(2*R*,4*R*)-**17d**: 2 mg (1%) as a colorless oil. $[\alpha]_D^{20}$ +14.6 (*c* 2.18, EtOAc). IR: $\tilde{\nu}$ = 3492, 1729, 1608 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H, *J* = 7.1 Hz, CH$_2$C*H*$_3$), 2.10 (br. s, 1H, OH), 2.05 (dd, 1H, *J* = 13.1, 9.7 Hz, C*H*$_2$CHCH$_2$COO), 2.28–2.35 (m, 2H, C*H*$_2$CHCH$_2$COO and CH$_2$COO), 2.67–2.77 (m, 2H, NC*H*$_2$CH$_2$ and CH$_2$COO), 2.82 (d, 1H, *J* = 10.8 Hz, NC*H*$_2$COH), 3.00–3.07 (m, 1H, NC*H*$_2$CH$_2$), 3.10–3.20 (m, 2H, NCH$_2$C*H*$_2$), 3.42 (d, 1H, *J* = 10.8 Hz, NC*H*$_2$COH), 3.44–3.51 (m, 1H, NCH), 3.77 (s, 9H, Ar'-OCH$_3$), 3.79 (s, 3H, ArOCH$_3$), 4.05–4.13 (m, 2H, C*H*$_2$CH$_3$), 6.77–6.81 (m, 6H, H_{aromat}), 6.82–6.86 (m, 2H, H_{aromat}), 7.31–7.35 (m, 6H, H_{aromat}), 7.36–7.40 (m, 2H, H_{aromat}). Calcd for $C_{39}H_{45}NO_8$ (655.80): C 71.43, H 6.92, N 2.14. Found: C 71.43, H 7.17, N 2.05.

(2*R*,4*S*)-**17d**: 63 mg (44%) as a colorless oil. $[\alpha]_D^{20}$ +36.5 (*c* 0.85, EtOAc). IR: $\tilde{\nu}$ = 3462, 1728, 1608 cm^{-1}. MS (*m*/*z*): 333 $[Ar_3C]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, 3H, *J* = 7.2 Hz, CH$_2$C*H*$_3$), 2.08 (dd, 1H, *J* = 14.1, 5.2, 1.8 Hz, C*H*$_2$CHCH$_2$COO), 2.51–2.60 (m, 4H, C*H*$_2$CHCH$_2$COO, CH$_2$COO, NC*H*$_2$CH$_2$ and NC*H*$_2$COH), 2.72 (dd, 1H, *J* = 15.9, 3.3 Hz, CH$_2$COO), 2.99–3.08 (m, 2H, NC*H*$_2$CH$_2$ and NC*H*), 3.12–3.25 (m, 3H, NC*H*$_2$COH and 2H of NCH$_2$C*H*$_2$), 3.54 (s, 1H, OH), 3.77 (s, 9H, Ar'OCH$_3$), 3.79 (s, 3H, ArOCH$_3$), 4.05–4.16 (m, 2H, C*H*$_2$CH$_3$), 6.80–6.88 (m, 8H, H_{aromat}), 7.30–7.34 (m, 6H, H_{aromat}), 7.35–7.39 (m, 2H, H_{aromat}). Calcd for $C_{39}H_{45}NO_8$ (655.80): C, 71.43; H, 6.92; N, 2.14. Found: C, 71.43; H, 7.17; N, 2.05.

6.3.8. Ethyl (2*R*,4*S*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*R*,4*S*)-17c] and ethyl (2*R*,4*R*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*R*,4*R*)-17c]

According to GP3A from $CeCl_3$ (146 mg, 0.601 mmol) and 4-$MeOC_6H_4MgBr$ (1.30 mL, 0.48 M in THF, 0.60 mmol) in THF (12 mL), (2*R*)-**16c** (176 mg, 0.421 mmol) in THF (6 mL); reaction: at −78 °C for 20 h; analytical HPLC *ds*: (2*R*,4*R*)-**17c**/(2*R*,4*S*)-**17c** = 19:81

[(2*R*,4*R*)-**17c** t_R = 13.9 min, (2*R*,4*S*)-**17c** t_R = 17.7 min; *n*-heptane/EtOAc/*iso*-propanol, 75:25:0.5; 1.3 mL min^{-1}]. Purification by CC (*iso*-hexane/acetone, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 80:20:0.5, 12 mL min^{-1}) yielded (2*R*,4*R*)-**17c** (t_R = 40.5 min) and (2*R*,4*S*)-**17c** (t_R = 45.6 min). (2*R*)-**16c** (46 mg, 26%) was recovered.

(2*R*,4*R*)-**17c**: 16 mg (7%) as a colorless oil. $[\alpha]_D^{20}$ +51.0 (*c* 1.07, $CHCl_3$). IR: $\tilde{\nu}$ = 3482, 1732 cm^{-1}. MS (*m*/*z*): 526 [M+1]$^+$. ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, 3H, *J* = 7.1 Hz, CH_2CH_3), 2.00 (s, 3H, thienyl-CH_3), 2.02 (s, 3H, thienyl-CH_3), 2.00–2.07 (m, 1H, CH_2CHCH_2COO), 2.26–2.34 (m, 4H, CH_2COO, CH_2CHCH_2COO and 2H of NCH_2CH_2), 2.53–2.61 (m, 1H, NCH_2CH_2), 2.65–2.72 (m, 2H, CH_2COO and NCH_2COH), 2.91 (dt, 1H, *J* = 12.0, 8.0 Hz, NCH_2CH_2), 3.34–3.42 (m, 2H, NCH_2COH and NC*H*), 3.78 (s, 3H, $ArOCH_3$), 4.11 (q, 2H, *J* = 7.1 Hz, CH_2CH_3), 6.09 (t, 1H, *J* = 7.2 Hz, $=CHCH_2$), 6.76 (d, 1H, *J* = 5.2 Hz, SCH), 6.82–6.87 (m, 3H, SC*H* and H_{aromat}), 7.05 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.19 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.37–7.41 (m, 2 H, H_{aromat}). Calcd for $C_{29}H_{35}NO_4S_2$ (525.73): C, 66.25; H, 6.71; N, 2.66; S, 12.20. Found: C, 66.50; H, 6.79; N, 2.51; S, 11.89.

(2*R*,4*S*)-**17c**: 55 mg (25%) as a colorless oil. $[\alpha]_D^{20}$ −77.9 (*c* 0.67, $CHCl_3$). IR: $\tilde{\nu}$ = 3468, 1713 cm^{-1}. MS (*m*/*z*): 527.1 [M+1]$^+$. ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, 3H, *J* = 7.3 Hz, CH_2CH_3), 1.99 (s, 3H, thienyl-CH_3), 2.03 (s, 3H, thienyl-CH_3), 2.09 (ddd, 1H, *J* = 14.2, 5.2, 2.0 Hz, CH_2CHCH_2COO), 2.30–2.37 (m, 2H, NCH_2CH_2), 2.39–2.46 (m, 2H, NCH_2CH_2 and NCH_2COH), 2.50–2.60 (m, 2H, CH_2CHCH_2COO and CH_2COO), 2.73 (dd, 1H, *J* = 15.7, 3.5 Hz, CH_2COO), 2.90–3.02 (m, 2H, NCH_2CH_2 and NC*H*), 3.12 (dd, 1H, *J* = 9.4, 2.0 Hz, NCH_2COH), 3.43 (s, 1H, OH), 3.79 (s, 3H, $ArOCH_3$), 4.09–4.17 (m, 2H, CH_2CH_3), 6.07 (t, 1H, *J* = 7.2 Hz, $=CHCH_2$), 6.76 (d, 1H, *J* = 5.0 Hz, SCH), 6.82–6.88 (m, 3H, SC*H* and 2 H of H_{aromat}), 7.05 (d, 1H, *J* = 5.0 Hz, SCH=C*H*), 7.20 (d, 1H, *J* = 5.0 Hz, SCH=C*H*), 7.36–7.40 (m, 2H, H_{aromat}). Calcd for $C_{29}H_{35}NO_4S_2$ (525.73): C, 66.25; H, 6.71; N, 2.66; S, 12.20. Found: C, 66.28; H, 6.86; N, 2.56; S, 11.99.

6.3.9. Methyl (2*S*,4*R*)-1-benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*S*,4*R*)-14e]

According to GP3A from $CeCl_3$ (330 mg, 1.34 mmol) and 4-$MeOC_6H_4MgBr$ (2.80 mL, 0.48 M in THF, 1.34 mmol) in THF (20 mL), (2*S*)-**14e** (354 mg, 1.28 mmol) in THF (5 mL); reaction: at −60 °C for 4.5 h; purification by CC (*n*-heptane/acetone, 3:1). (2*S*)-**14e** (61 mg, 17%) was recovered. Yield: 276 mg (56%) as a colorless oil. $[\alpha]_D^{20}$ −23.5 (*c* 1.36, EtOAc). IR: $\tilde{\nu}$ = 3438, 1756, 1706 cm^{-1}. MS (*m*/*z*): 385 [M+1]$^+$. ^{1}H NMR ($C_6D_5NO_2$, 120 °C) δ: 2.51 (dd, 1H, *J* = 13.6, 1.8 Hz, $NCHCH_2$), 2.83 (ddd, 1H, *J* = 13.6, 9.7, 1.5 Hz, $NCHCH_2$), 3.78–3.81 (m, 6H, $COOCH_3$ and $ArOCH_3$), 3.93 (dd, 1H, *J* = 11.4, 1.5 Hz, NCH_2), 4.07 (dd, 1H, *J* = 11.4, 1.3 Hz, NCH_2), 4.77 (dd, 1H, *J* = 9.7, 1.3 Hz, NCH), 5.23 (d, 1H, *J* = 12.6 Hz, $PhCH_2$), 5.32 (d, 1H, *J* = 12.6 Hz, $PhCH_2$), 6.89–6.94 (m, 2H, H_{aromat}), 7.27–7.50 (m, 7H, H_{aromat}). Calcd for $C_{21}H_{23}NO_6$ (385.42): C, 65.44; H, 6.02; N, 3.63. Found: C, 65.11; H, 6.19; N, 3.70.

6.3.10. Methyl (2*R*,4*S*)-1-benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*R*,4*S*)-14e]

According to GP3A from $CeCl_3$ (399 mg, 1.62 mmol) and 4-$MeOC_6H_4MgBr$ (3.38 mL, 0.48 M in THF, 1.62 mmol) in THF (25 mL), (2*R*)-**14e** (427 mg, 1.54 mmol); reaction: at −60 °C for 5.5 h; purification by CC (*n*-heptane/acetone, 3:1). (2*R*)-**14e** (84 mg, 20%) was recovered. Yield: 265 mg (45%) as a colorless oil. $[\alpha]_D^{20}$ +24.8 (*c* 1.08, EtOAc). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**14e**. Calcd for $C_{21}H_{23}NO_6$ (385.42): C, 65.44; H, 6.02; N, 3.63. Found: C, 65.48; H, 6.16; N, 3.57.

6.3.11. Methyl (2*S*,4*R*)-1-benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*S*,4*R*)-17e]

According to GP3B from (2*S*)-**16e** (168 mg, 0.611 mmol) in Et_2O (30 ml), 4-$MeOC_6H_4MgBr$ (1.53 mL, 0.44 M in Et_2O, 0.67 mmol); reaction: at −78 °C for 4 h; purification by CC (*n*-heptane/acetone, 80:20) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 78:22:0.5; t_R = 64.2 min; 12 mL min^{-1}). (2*S*)-**16e** (91 mg, 54%) was recovered. Yield: 92 mg (38%) as a colorless oil. $[\alpha]_D^{20}$ −29.1 (*c* 0.95, $CHCl_3$). IR: $\tilde{\nu}$ = 3440, 1734, 1700 cm^{-1}. MS (*m*/*z*): 382 [M+1]$^+$. ^{1}H NMR ($C_6D_5NO_2$, 120 °C) δ: 2.40 (d, 1H, *J* = 13.7 Hz, CH_2CHCH_2COO), 2.68 (dd, 1H, *J* = 13.7, 9.1 Hz, CH_2CHCH_2COO), 2.78 (s, 1H, OH), 3.17 (dd, 1H, *J* = 15.4, 3.3 Hz, CH_2COO), 3.32 (dd, 1H, *J* = 15.4, 3.3 Hz, CH_2COO), 3.70 (s, 3H, $ArOCH_3$), 3.76 (s, 3H, $COOCH_3$), 3.90–4.00 (m, 1H, NCH), 4.56–4.64 (m, 2H, NCH_2), 5.29, 5.34 (2*s*, 2H, CH_2Ph), 6.86–6.91 (m, 2H, H_{aromat}), 7.25–7.48 (m, 7H, H_{aromat}). Calcd for $C_{22}H_{25}NO_6$ (399.45): C, 66.15; H, 3.51; N, 6.31. Found: C, 66.45; H, 3.44; N, 6.67.

6.3.12. Ethyl (2*R*,4*S*)-1-benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetate [(2*R*,4*S*)-17e]

According to GP3B from (2*R*)-**16e** (120 mg, 0.393 mmol) and 4-$MeOC_6H_4MgBr$ (0.94 mL, 0.44 M in Et_2O, 0.41 mmol) in Et_2O (20 mL); reaction: at −78 °C for 20 h; purification by CC (*n*-heptane/acetone, 3:1) and separation by prep. HPLC (*n*-heptane/EtOAc/*iso*-propanol, 78:22:0.5; t_R = 47.4 min; 12 mL min^{-1}). (2*R*)-**16e** (54 mg, 45%) was recovered. Yield: 60 mg (37%) as a colorless oil. $[\alpha]_D^{20}$ +26.1 (*c* 1.05, $CHCl_3$). IR: $\tilde{\nu}$ = 3440, 1730, 1700 cm^{-1}. MS (*m*/*z*): 396 [M+1]$^+$. ^{1}H NMR ($C_6D_5NO_2$, 120 °C) δ: 1.22–1.29 (m, 3H, CH_2CH_3), 2.43 (d, 1H, *J* = 13.7 Hz, CH_2CHCH_2COO), 2.65–2.72 (m, 1H, CH_2CHCH_2COO), 2.80 (s, 1H, OH), 3.18 (dd, 1H, *J* = 15.4, 3.3 Hz, CH_2COO), 3.27–3.34 (m, 1H, CH_2COO), 3.76, 3.78 (2*s*, 3H, $ArOCH_3$), 3.91–4.00 (m, 1H, NCH), 4.17–4.24 (m, 2H, CH_2CH_3), 4.57–4.65 (m, 2H, NCH_2), 5.30, 5.34 (2*s*, 2H, CH_2Ph), 6.86–6.90 (m, 2H, H_{aromat}), 7.26–7.48 (m, 7H, H_{aromat}). Calcd for $C_{23}H_{27}NO_6$ (413.48): C, 66.81; H, 6.58; N, 3.39. Found: C, 66.95; H, 6.77; N, 3.20.

6.4. General procedure 4 for the preparation of the N-alkylated 4-hydroxy-4-(4-methoxyphenyl)-substituted proline and pyrrolidin-2-acetic acid derivatives (10b,d and 11c,d)

The respective ester of **14b,d** and **17c,d** (1 equiv) was hydrolyzed in EtOH or MeOH (10–50 mL mmol^{-1}) at rt with aq 0.85 M KOH or aq 1.0 M NaOH (3 equiv) and for the time given. The mixture was neutralized with aq 1.0 M HCl to pH 7 and followed by addition of buffer (pH 5.5–6.6). The mixture was concentrated in vacuo and the residue was purified by CC and recrystallization.

6.4.1. (2*S*,4*S*)-1-(4,4-Diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*S*,4*S*)-10b]

From (2*S*,4*S*)-**14b** (50 mg, 0.11 mmol), aq 0.85 M KOH (0.39 mL, 0.33 mmol) in EtOH (1.7 mL); reaction time: 1 h; purification by CC (gradient elution, *i*Pr_2O→EtOH). Yield: 36 mg (74%) as colorless crystals. mp 173–175 °C (MeOH). $[\alpha]_D^{20}$ −28.0 (*c* 0.64, MeOH). IR: $\tilde{\nu}$ = 3254, 1622 cm^{-1}. MS (*m*/*z*): 444 [M+1]$^+$. ^{1}H NMR (CD_3OD) δ: 2.42 (dd, 1H, *J* = 13.3, 12.0 Hz, $NCHCH_2$), 2.57 (q, 2H, *J* = 7.6 Hz, NCH_2CH_2), 2.65 (ddd, 1H, *J* = 13.3, 6.7, 2.0 Hz, $NCHCH_2$), 3.26–3.33 (m, 1H, NCH_2COH), 3.36–3.40 (m, 1H, NCH_2CH_2), 3.55 (dt, 1H, *J* = 12.2, 7.6 Hz, NCH_2CH_2), 3.68 (d, 1H, *J* = 12.3 Hz, NCH_2COH), 3.78 (s, 3H, $ArOCH_3$), 4.25 (dd, 1H, *J* = 12.0, 6.7 Hz, NCH), 6.12 (t, 1H, *J* = 7.6 Hz, $=CHCH_2$), 6.90–6.93 (m, 2H, H_{aromat}), 7.17–7.44 (m, 12H, H_{aromat}). Calcd for $C_{28}H_{29}NO_4$ (443.54): C, 75.82; H, 6.59; N, 3.16. Found: C, 76.04; H, 6.84; N, 3.06.

6.4.2. (2*R*,4*R*)-1-(4,4-Diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*R*,4*R*)-10b]

From (2*R*,4*R*)-**14b** (62 mg, 0.14 mmol), aq 1 M NaOH (0.42 mL, 0.42 mmol) in MeOH (3.6 mL); reaction time: 12 h; purification by recrystallization from MeOH. Yield: 56 mg (94%) as colorless crystals. mp 179–181 °C (MeOH). $[\alpha]_D^{20}$ +28.0 (*c* 0.50, MeOH). The

spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*S*)-**10b**. Calcd for $C_{28}H_{29}NO_4$ (443.54)·1.0H_2O: C, 72.86; H, 6.77; N, 3.03. Found: C, 72.77; H, 6.57; N, 2.95.

6.4.3. (2*S*,4*R*)-1-(4,4-Diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*S*,4*R*)-10b]

From (2*S*,4*R*)-**14b** (20 mg, 0.044 mmol), aq 0.85 M KOH (0.15 mL, 0.13 mmol) in EtOH (1 mL); reaction time: 1 h; purification by CC (gradient elution *i*Pr_2O→EtOH). Yield: 17 mg (88%) as colorless crystals. mp 168–173 °C. $[\alpha]_D^{20}$ −39.3 (*c* 0.81, MeOH). IR: $\tilde{\nu}$ = 3431, 1612 cm^{-1}. MS (*m*/*z*): 444 [M+1]$^+$. ^{1}H NMR (CD_3OD) δ: 2.54–2.71 (m, 3H, NCHCH_2 and 2H of NCH_2CH_2), 2.92 (dd, 1H, *J* = 13.5, 11.4 Hz, NCHCH_2), 3.33–3.53 (m, 3H, NCH_2COH and 2H of NCH_2CH_2), 3.62 (dd, 1H, *J* = 11.2, 1.8 Hz, NCH_2COH), 3.78 (s, 3H, $ArOCH_3$), 4.39 (dd, 1H, *J* = 11.4, 1.8 Hz, NCH), 6.09 (t, 1H, *J* = 7.5 Hz, =C*H*CH_2), 6.90–6.95 (m, 2H, H_{aromat}), 7.16–7.45 (m, 12H, H_{aromat}). Calcd for $C_{28}H_{29}NO_4$ (443.54): C, 75.82; H, 6.59; N, 3.16. Found: C, 75.71; H, 6.82; N, 2.96.

6.4.4. (2*R*,4*S*)-1-(4,4-Diphenylbut-3-en-1-yl)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*R*,4*S*)-10b]

From (2*R*,4*S*)-**14b** (88 mg, 0.19 mmol), aq 1 M NaOH (0.57 mL, 0.57 mmol) in MeOH (6 mL); reaction time: 12 h; purification by recrystallization from MeOH/*i*Pr_2O. Yield: 72 mg (84%) as colorless crystals. mp 165–170 °C (MeOH/*i*Pr_2O). $[\alpha]_D^{20}$ +40.4 (*c* 1.55, MeOH). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**10b**. Calcd for $C_{28}H_{29}NO_4$ (443.54)·0.4H_2O: C, 74.56; H, 6.62; N, 2.92. Found: C, 74.61; H, 6.66; N, 3.11.

6.4.5. (2*S*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylic acid [(2*S*,4*S*)-10d]

From (2*S*,4*S*)-**14d** (30 mg, 0.048 mmol), aq 0.85 M KOH (0.39 mL, 0.33 mmol) in EtOH (1 mL); reaction time: 1.75 h; buffer (1 mL, pH 6.6). Yield: 26 mg (89%) as colorless crystals. mp 138–139 °C (EtOH/H_2O). $[\alpha]_D^{20}$ −3.9 (*c* 0.85, MeOH). IR: $\tilde{\nu}$ = 3425, 1630 cm^{-1}. MS (ESI$^+$) (*m*/*z*): 614 [M+1]$^+$. ^{1}H NMR (CD_3OD) δ: 2.32 (dd, 1H, *J* = 13.3, 11.6 Hz, NCHCH_2), 2.57 (ddd, 1H, *J* = 13.3, 7.1, 1.5 Hz, NCHCH_2), 3.14 (dd, 1H, *J* = 12.5, 2.1 Hz, NCH_2COH), 3.30–3.51 (m, 5H, NCH_2COH and 2H of NCH_2CH_2 and 2H of NCH_2CH_2), 3.65 (s, 9H, $ArOCH_3$), 3.69 (s, 3H, $ArOCH_3$), 4.29 (dd, 1H, *J* = 11.6, 7.1 Hz, NCH), 6.73–6.76 (m, 6H, H_{aromat}), 6.78–6.81 (m, 2H, H_{aromat}), 7.23–7.27 (m, 8H, H_{aromat}). Calcd for $C_{36}H_{39}NO_8$ (613.71): C, 70.46; H, 6.41; N, 2.28. Found: C, 70.18; H, 6.62; N, 2.35.

6.4.6. (2*R*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylic acid [(2*R*,4*R*)-10d]

From (2*R*,4*R*)-**14d** (56 mg, 0.089 mmol), aq 1.0 M NaOH (0.27 mL, 0.27 mmol) in MeOH (3 mL); reaction time: 5 h; buffer (0.5 mL, pH 6.6). Yield: 48 mg (88%) as colorless crystals. mp 158–160 °C (MeOH/H_2O). $[\alpha]_D^{20}$ +3.6 (*c* 0.50, MeOH). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*S*)-**10d**. Calcd for $C_{36}H_{39}NO_8$ (613.71)·0.3H_2O: C, 69.84; H, 6.45; N, 2.26. Found: C, 69.76; H, 6.67; N, 2.15.

6.4.7. (2*S*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylic acid [(2*S*,4*R*)-10d]

From (2*S*,4*R*)-**14d** (62 mg, 0.099 mmol), aq 0.85 M KOH (0.47 mL, 0.40 mmol) in EtOH (2 mL); reaction time: 3.5 h; buffer (2.0 mL, pH 6.6). Yield: 54 mg (89%) as colorless crystals. mp 105–108 °C. $[\alpha]_D^{20}$ +1.5 (*c* 1.15, MeOH). IR: $\tilde{\nu}$ = 3421, 1637 cm^{-1}. MS (ESI$^+$) (*m*/*z*): 614 [M+1]$^+$. ^{1}H NMR (CD_3OD) δ: 2.48 (dd, 1H, *J* = 13.5, 2.1 Hz, NCHCH_2), 2.75 (dd, 1H, *J* = 13.5, 11.5 Hz, NCHCH_2), 3.16–3.22 (m, 2H, NCH_2COH), 3.25–3.32 (m, 2H, NCH_2CH_2), 3.49–3.62 (m, 2H, NCH_2CH_2), 3.67 (s, 9H, Ar'OCH_3), 3.70 (s, 3H, $ArOCH_3$), 4.06 (dd, 1H, *J* = 11.5, 2.1 Hz, NCH), 6.77–6.84 (m, 8H, H_{aromat}), 7.22–7.25 (m, 2H, H_{aromat}), 7.28–7.32 (m, 6H, H_{aromat}). Calcd for $C_{36}H_{39}NO_8$ (613.71)·1.0H_2O: C, 68.45; H, 6.54; N, 2.22. Found: C, 68.62; H, 6.40; N, 2.18.

6.4.8. (2*R*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidine-2-carboxylic acid [(2*R*,4*S*)-10d]

From (2*R*,4*S*)-**14d** (60 mg, 0.096 mmol), aq 1.0 M NaOH (0.29 mL, 0.29 mmol) in MeOH (3 mL); reaction time: 3 h; buffer (0.5 mL, pH 6.6). Yield: 52 mg (89%) as colorless crystals. mp 158–160 °C (MeOH/H_2O). $[\alpha]_D^{20}$ −1.6 (*c* 0.75, MeOH). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**10d**. Calcd for $C_{36}H_{39}NO_8$ (613.71)·0.5H_2O: C, 69.44; H, 6.68; N, 2.25. Found: C, 69.31; H, 6.51; N, 2.17.

6.4.9. (2*S*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetic acid [(2*S*,4*S*)-11d]

From (2*S*,4*S*)-**17d** (70 mg, 0.11 mmol), aq 1.0 M NaOH (0.33 mL, 0.33 mmol) in MeOH (2.7 mL); reaction time: 2 days; purification by CC (MeOH-H_2O, 4:1) and recrystallization from C_6H_6/*i*Pr_2O. Yield: 50 mg (73%) as a colorless powder. mp 117–121 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ −9.4 (*c* 0.50, MeOH). IR: $\tilde{\nu}$ = 3422, 1608 cm^{-1}. MS (ESI$^+$) (*m*/*z*): 628 [M+1]$^+$. ^{1}H NMR (CD_3OD) δ: 2.28–2.42 (m, 2H, CH_2CHCH_2COO), 2.46 (dd, 1H, *J* = 16.9, 1.5 Hz, CH_2COO), 2.85 (dd, 1H, *J* = 16.9, 5.1 Hz, CH_2COO), 3.23 (dd, 1H, *J* = 12.4, 1.5 Hz, NCH_2COH), 3.30–3.34 (m, 1H, NCH_2CH_2), 3.38 (d, 1H, *J* = 12.4 Hz, NCH_2COH), 3.43–3.68 (m, 3H, NCH_2CH_2 and 2H of NCH_2CH_2), 3.74 (s, 9H, Ar'OCH_3), 3.79 (s, 3H, $ArOCH_3$), 4.05–4.12 (m, 1H, NCH), 6.84–6.88 (m, 6H, H_{aromat}), 6.90–6.94 (m, 2H, H_{aromat}), 7.31–7.37 (m, 8H, H_{aromat}). Calcd for $C_{37}H_{41}NO_8$ (627.74)·0.8 H_2O: C, 69.21; H, 6.69; N, 2.18. Found: C, 69.22; H, 6.78; N, 2.02.

6.4.10. (2*R*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetic acid [(2*R*,4*R*)-11d]

From (2*R*,4*R*)-**17d** (42 mg, 0.065 mmol), aq 1.0 M NaOH (0.20 mL, 0.20 mmol) in MeOH (3 mL); reaction time: 2 days; purification by recrystallization from C_6H_6/*i*Pr_2O. Yield: 38 mg (93%) as a colorless powder. mp 110–117 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ +9.5 (*c* 0.62, MeOH). The spectra [^{1}H NMR (500 MHz), IR, and MS] were identical with those of (2*S*,4*S*)-**11d**. Calcd for $C_{37}H_{41}NO_8$ (627.74)·0.9 H_2O: C, 69.01; H, 6.67; N, 2.18. Found: C, 68.85; H, 6.56; N, 2.21.

6.4.11. (2*S*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetic acid [(2*S*,4*R*)-11d]

From (2*S*,4*R*)-**17d** (115 mg, 0.179 mmol), aq 1.0 M NaOH (0.54 mL, 0.54 mmol) in MeOH (4.5 mL); reaction time: 2 days; purification by recrystallization from C_6H_6/*i*Pr_2O. Yield: 97 mg (86%) as a colorless powder. mp 106–111 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ −20.5 (*c* 0.81, MeOH). IR: $\tilde{\nu}$ = 3421, 1608 cm^{-1}. MS (ESI$^+$, *m*/*z*): 628 (M+1)$^+$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 2.19 (ddd, 1H, *J* = 14.1, 6.0, 1.4 Hz, CH_2CHCH_2COO), 2.70 (dd, 1H, *J* = 16.5, 4.1 Hz, CH_2COO), 2.74–2.82 (m, 2H, CH_2CHCH_2COO and CH_2COO), 3.19–3.25 (m, 1H, NCH_2CH_2), 3.33 (d, 1H, *J* = 11.1 Hz, NCH_2COH), 3.44–3.49 (m, 3H, NCH_2COH and 2 H of NCH_2CH_2), 3.62–3.67 (m, 1H, NCH_2CH_2), 3.74 (s, 9H, Ar'OCH_3), 3.78 (s, 3H, $ArOCH_3$), 3.87–3.93 (m, 1H, NCH), 6.83–6.86 (m, 6H, H_{aromat}), 6.89–6.92 (m, 2H, H_{aromat}), 7.31–7.38 (m, 8H, H_{aromat}). Calcd for $C_{37}H_{41}NO_8$ (627.74)·0.7 H_2O: C, 69.40; H, 6.67; N, 2.19. Found: C, 69.45; H, 6.94; N, 2.19.

6.4.12. (2*R*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-acetic acid [(2*R*,4*S*)-11d]

From (2*R*,4*S*)-**17d** (125 mg, 0.191 mmol), aq 1.0 M NaOH (0.58 mL, 0.58 mmol) in MeOH (6.4 mL); reaction time: 2 days; purification by CC (MeOH-H_2O, 4:1) and recrystallization from C_6H_6/*i*Pr_2O. Yield: 107 mg (89%) as colorless crystals. mp 116–122 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ +20.3 (*c* 0.69, MeOH). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**11d**. Calcd for $C_{37}H_{41}NO_8$ (627.74)·0.6 H_2O: C, 69.60; H, 6.66; N, 2.19. Found: C, 69.50; H, 6.75; N, 2.08.

6.4.13. (2*S*,4*R*)-1-[4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*S*,4*R*)-11c]

From (2*S*,4*R*)-**17c** (83 mg, 0.16 mmol), aq 1.0 M NaOH (0.49 mL, 0.49 mmol) in MeOH (4.5 mL); reaction time: 3 days; purification by CC (MeOH) and recrystallization from C_6H_6/*i*Pr_2O. Yield: 56 mg (70%) as a colorless powder. mp 73–76 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ −37.9 (*c* 0.61, MeOH). IR: $\tilde{\nu}$ = 3385, 1609 cm^{-1}. MS (*m*/*z*): 498 [M+1]$^+$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 1.96 (s, 3H, thienyl-CH_3), 2.02 (s, 3H, thienyl-CH_3), 2.13–2.18 (m, 1H, CH_2CHCH$_2$COO), 2.60 (q, 2H, *J* = 7.4 Hz, NCH$_2$CH_2), 2.69–2.81 (m, 3H, CH_2CHCH$_2$COO and 2H of CH_2COO), 3.08–3.12 (m, 1H, NCH_2CH$_2$), 3.18–3.22 (m, 1H, NCH_2COH), 3.54–3.62 (m, 2H, NCH_2CH$_2$ and NCH_2COH), 3.77 (s, 3H, ArOCH_3), 3.86–3.90 (m, 1H, NCH), 6.04 (t, 1H, *J* = 7.4 Hz, =C*H*CH$_2$), 6.78 (d, 1H, *J* = 5.2 Hz, SCH), 6.88–6.93 (m, 3H, SCH and 2 H of H_{aromat}), 7.16 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.34 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.38–7.40 (m, 2H, H_{aromat}). Calcd for $C_{27}H_{31}NO_4S_2$ (497.67)·0.5 $H_2$0: C, 64.00; H, 6.37; N, 2.76. Found: C, 63.84; H, 6.67; N, 2.54.

6.4.14. (2*R*,4*S*)-1-[4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*R*,4*S*)-11c]

From (2*R*,4*S*)-**17c** (87 mg, 0.17 mmol), aq 1.0 M NaOH (0.50 mL, 0.50 mmol) in MeOH (4.5 mL); reaction time: 2 days; purification by recrystallization from C_6H_6/*i*Pr_2O. Yield: 68 mg (82%) as a slightly yellow powder. mp 69–73 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ +34.0 (*c* 0.85, MeOH). The spectra [^{1}H NMR (500 MHz), IR, and MS] were identical with those of (2*S*,4*R*)-**11c**. Calcd for $C_{27}H_{31}NO_4S_2$ (497.67)·0.6 H_2O: C, 63.78; H, 6.38; N, 2.75; S, 12.61. Found: C, 63.60; H, 6.38; N, 2.67; S, 12.43.

6.4.15. (2*S*,4*S*)-1-[4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*S*,4*S*)-11c]

From (2*S*,4*S*)-**17c** (60 mg, 0.12 mmol), aq 1.0 M NaOH (0.35 mL, 0.35 mmol) in MeOH (3.6 mL); reaction time: 3 days; purification by recrystallization from C_6H_6/*i*Pr_2O. Yield: 57 mg (98%) as a colorless powder. mp 73–76 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ −38.6 (*c* 0.70, MeOH). IR: $\tilde{\nu}$ = 3420, 1610 cm^{-1}. MS (*m*/*z*): 498 [M+1]$^+$. ^{1}H NMR (CD_3OD, 500 MHz) δ: 1.98 (s, 3H, thienyl-CH_3), 2.03 (s, 3H, thienyl-CH_3), 2.24–2.31 (m, 1H, CH_2CHCH$_2$COO), 2.39 (ddd, 1H, *J* = 13.3, 6.0, 1.9 Hz, CH_2CHCH$_2$COO), 2.51 (dd, 1H, *J* = 16.7, 2.3 Hz, CH_2COO), 2.61 (q, 2H, *J* = 7.4 Hz, NCH$_2$CH_2), 2.79 (dd, 1H, *J* = 16.7, 5.8 Hz, CH_2COO), 3.25–3.31 (m, 2H, NCH_2CH$_2$ and NCH_2COH), 3.52–3.61 (m, 1H, NCH_2CH$_2$), 3.64 (d, 1H, *J* = 12.4 Hz, NCH_2COH), 3.77 (s, 3H, ArOCH_3), 4.05–4.12 (m, 1H, NCH), 6.07 (t, 1H, *J* = 7.4 Hz, =C*H*CH$_2$), 6.77 (d, 1H, *J* = 5.2 Hz, SCH), 6.89–6.92 (m, 3H, SCH and 2H of H_{aromat}), 7.15 (d, 1H, *J* = 5.2 Hz, SCH=C*H*), 7.34–7.39 (m, 3H, SCH=C*H* and 2H of H_{aromat}). Calcd for $C_{27}H_{31}NO_4S_2$ (497.67)·0.5 $H_2$0: C, 64.00; H, 6.37; N, 2.76. Found: C, 64.27; H, 6.59; N, 2.47.

6.4.16. (2*R*,4*R*)-1-[4,4-Bis(3-methylthiophen-2-yl)but-3-en-1-yl]-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*R*,4*R*)-11c]

From (2*R*,4*R*)-**17c** (61 mg, 0.12 mmol), aq 1.0 M NaOH (0.35 mL, 0.35 mmol) in MeOH (4 mL); reaction time: 2 days; purification by recrystallization from C_6H_6/*i*Pr_2O. Yield: 55 mg (95%) as a slight yellow powder. mp 70–74 °C (C_6H_6/*i*Pr_2O). $[\alpha]_D^{20}$ +40.1 (*c* 0.7, MeOH). The spectra [^{1}H NMR (500 MHz), IR, and MS] were identical with those of (2*S*,4*S*)-**11c**. Calcd for $C_{27}H_{31}NO_4S_2$ (497.67)·0.9H_2O: C, 65.11; H, 6.43; N, 2.73. Found: C, 65.09; H, 6.24; N, 2.64.

6.5. General procedure 5 for the preparation of the N-Cbz-protected 4-hydroxy-4-(4-methoxyphenyl)-substituted prolines [10e] and pyrrolidin-2-acetic acids [11e]

The respective ester of **14e** and **17e** (1 equiv) was hydrolyzed with 1.0 M NaOH (2.0–3.0 equiv) in MeOH (10–50 mL mmol^{-1}) at rt for the time given. After MeOH had been removed in vacuo, the residue was dissolved in a small amount of water and acidified to pH 1–2 with aq 1.0 M HCl. The mixture was extracted with CH_2Cl_2 and the combined extracts were dried (Na_2SO_4) and concentrated in vacuo to yield the carboxylic acid.

6.5.1. (2*S*,4*R*)-1-Benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*S*,4*R*)-10e]

From (2*S*,4*R*)-**14e** (131 mg, 0.340 mmol), aq 1.0 M NaOH (1.02 mL, 1.02 mmol) in MeOH (4 mL); reaction time: 3 h. Yield: 111 mg (88%) as a colorless foam. mp 62–65 °C. $[\alpha]_D^{20}$ −25.0 (*c* 1.00, MeOH). IR:$\tilde{\nu}$ = 3420, 1705, 1611 cm^{-1}. MS (*m*/*z*): 370 [M-1]$^+$. ^{1}H NMR ($C_6D_5NO_2$, 120 °C) δ: 2.74–2.89 (m, 2H, NCHCH_2), 3.77 (s, 3H, ArOCH_3), 3.91 (d, 1H, *J* = 11.0 Hz, NCH$_2$), 4.09 (d, 1H, *J* = 11.0 Hz, NCH$_2$), 4.88 (d, 1H, *J* = 9.4 Hz, NCH), 5.29–5.36 (m, 2H, CH_2Ph), 6.89–6.93 (m, 2H, H_{aromt}), 7.26–7.52 (m, 7H, H_{aromat}). Calcd for $C_{20}H_{21}NO_6$ (371.40): C, 64.68; H, 5.70; N, 3.77. Found: C, 64.85; H, 5.42; N, 3.53.

6.5.2. (2*R*,4*S*)-1-Benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*R*,4*S*)-10e]

From (2*R*,4*S*)-**14e** (107 mg, 0.278 mmol), aq 1.0 M NaOH (0.83 mL, 0.83 mmol) in MeOH (4.2 mL); reaction time: 4 h. Yield: 83 mg (80%); colorless foam. mp 58–62 °C. $[\alpha]_D^{20}$ +25.8 (*c* 0.64, MeOH). The spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**10e**. Calcd for $C_{20}H_{21}NO_6$ (371.40): C, 64.68; H, 5.70; N, 3.77. Found: C, 64.74; H, 5.93; N, 3.56.

6.5.3. (2*S*,4*R*)-1-Benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*S*,4*R*)-11e]

From (2*S*,4*R*)-**17e** (93 mg, 0.23 mmol) and aq 1.0 M NaOH (0.48 mL, 0.48 mmol) in MeOH (4.5 mL); reaction time: 4 days. Yield: 73 mg (81%) as a colorless foam. $[\alpha]_D^{20}$ −30.0 (*c* 0.80, MeOH). ^{1}H NMR ($C_6D_5NO_2$, 120 °C) δ: 2.40–2.51 (m, 1H, CH_2CHCH$_2$COO), 2.68–2.74 (m, 1H, CH_2CHCH$_2$COO), 3.27 (dd, 1H, *J* = 16.0, 8.9 Hz, CH_2COO), 3.41 (dd, 1H, *J* = 16.0, 3.8 Hz, CH_2COO), 3.77 (s, 3H, ArOCH_3), 3.93 (d, 1H, *J* = 11.5 Hz, NCH$_2$), 4.00 (d, 1H, *J* = 11.5 Hz, NCH$_2$), 4.59–4.68 (m, 1H, NCH), 5.26–5.33 (m, 2H, CH_2Ph), 6.85–6.91 (m, 2H, H_{aromat}), 7.25–7.51 (m, 7H, H_{aromat}). IR: $\tilde{\nu}$ = 3424, 1702, 1677 cm^{-1}. MS (*m*/*z*): 368 [M-OH]$^+$. Calcd for $C_{21}H_{23}NO_6$ (385.42): C, 65.44; H, 6.02; N, 3.63. Found: C, 65.22; H, 6.14; N, 3.40.

6.5.4. (2*R*,4*S*)-1-Benzyloxycarbonyl-4-hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*R*,4*S*)-11e]

From (2*R*,4*S*)-**17e** (40 mg, 0.097 mmol) and aq 1.0 M NaOH (0.20 mL, 0.20 mmol) in MeOH (2.8 mL); reaction: 4 days. Yield: 34 mg (91%) as a colorless foam. $[\alpha]_D^{20}$ +29.2 (*c* 0.49, MeOH). The

spectra (^{1}H NMR, IR, and MS) were identical with those of (2*S*,4*R*)-**11e**. Calcd for $C_{21}H_{23}NO_6$ (385.42): C, 65.44; H, 6.02; N, 3.63. Found: C, 65.56; H, 6.32; N, 3.37.

6.6. General procedure 6 for the preparation of the 4-hydroxy-4-(4-methoxyphenyl)-substituted proline [10a] and pyrrolidin-2-acetic acid [11a]

To a solution of the respective N-protected pyrrolidine of **10e** and **11e** (1 equiv) and TEA (0.33–20 equiv) in MeOH or EtOAc (40–50 mL mmol^{-1})), 10% Pd-C was added. This mixture was subjected to hydrogen at rt under ambient pressure for the time given. The reaction mixture was filtrated and concentrated in vacuo to give the respective N-deprotected amine.

6.6.1. (2*S*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*S*,4*R*)-10a]

From (2*S*,4*R*)-**10e** (140 mg, 0.377 mmol) and TEA (12 mg, 0.12 mmol) in MeOH (17 mL), 10% Pd-C (70 mg, 0.066 mmol); reaction time: 1.5 h. Yield: 82 mg (92%) as colorless crystals. mp 244–247 °C (MeOH, decomp.). $[\alpha]_D^{20}$ +0.7 (*c* 0.45, H_2O). IR: $\tilde{\nu}$ = 3383, 1614 cm^{-1}. MS (*m*/*z*): 237 M$^+$. ^{1}H NMR (D_2O) δ: 2.47 (dt, 1H, *J* = 14.0, 2.2 Hz, NCHCH_2), 2.63 (dd, 1H, *J* = 14.0, 11.0 Hz, NCHCH_2), 3.40 (d, 1H, *J* = 12.2, NCH_2), 3.55 (dd, 1H, *J* = 12.2, 2.2 Hz, NCH_2), 3.67 (s, 3H, $ArOCH_3$), 4.23 (dd, 1H, *J* = 11.0, 2.2 Hz, NCH), 6.85–6.89 (m, 2H, H_{aromat}), 7.26–7.30 (m, 2H, H_{aromat}). Calcd for $C_{12}H_{15}NO_4$ (237.25)·0.2MeOH: C, 60.13; H, 6.53; N, 5.74. Found: C, 60.00; H, 6.47; N, 5.76.

6.6.2. (2*R*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylic acid [(2*R*,4*S*)-10a]

From (2*R*,4*S*)-**11e** (66 mg, 0.17 mmol) and TEA (5.8 mg, 0.056 mmol) in EtOAc (8 mL), 10% Pd-C (33 mg, 0.031 mmol); reaction time: 4 h. Yield: 38 mg (96%) as colorless crystals. mp 244–246 °C (MeOH, decomp.). $[\alpha]_D^{20}$ −0.9 (*c* 0.76, H_2O). The spectra (^{1}H NMR (500 MHz), IR, and MS) were identical with those of (2*S*,4*R*)-**10a**. Calcd for $C_{12}H_{15}NO_4$ (237.25)·0.4MeOH: C, 59.56; H, 6.69; N, 5.60. Found: C, 59.32; H, 6.54; N, 5.68.

6.6.3. (2*S*,4*R*)-4-Hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*S*,4*R*)-11a]

From (2*S*,4*R*)-**11e** (59 mg, 0.15 mmol), TEA (0.20 mL, 1.4 mmol) in MeOH (5 mL), 10% Pd-C (40 mg, 0.038 mmol); reaction time: 3 h. Yield: 38 mg (100%) as colorless crystals. mp 261–262 °C (MeOH, decomp.). $[\alpha]_D^{20}$ +5.6 (*c* 0.50, 0.05 M NaOH in MeOH). IR: $\tilde{\nu}$ = 3424, 1648, 1610 cm^{-1}. MS (*m*/*z*): 252 [M+1]$^+$. ^{1}H NMR (its sodium salt in CD_3OD, 500 MHz; NOE) δ: 1.93 (ddd, 1H, *J* = 13.9, 6.4, 1.8 Hz, CH_2CHCH_2COO), 2.43 (dd, 1H, *J* = 13.9, 9.3 Hz, CH_2CHCH_2COO), 2.51 (dd, 1H, *J* = 15.4, 6.8 Hz, CH_2COO), 2.60 (dd, 1H, *J* = 15.4, 5.4 Hz, CH_2COO), 2.90 (d, 1H, *J* = 12.0 Hz, NCH_2), 3.04 (dd, 1H, *J* = 12.0, 1.8 Hz, NCH_2), 3.54–3.61 (m, 1H, NCH), 3.77 (s, 3H, $ArOCH_3$), 6.85–6.89 (m, 2H, H_{aromat}), 7.38–7.41 (m, 2H, H_{aromat}). Calcd for $C_{13}H_{17}NO_4$ 251.4191 HRMS (DEI$^+$): M$^+$ 251.4235.

6.6.4. (2*R*,4*S*)-4-Hydroxy-4-(4-methoxyphenyl)pyrrolidin-2-acetic acid [(2*R*,4*S*)-11a]

From (2*R*,4*S*)-**11e** (73 mg, 0.19 mmol) and TEA (0.50 mL, 3.5 mmol) in MeOH (10 mL), 10% Pd-C (38 mg, 0.036 mmol); reaction time: 1 h. Yield: 45 mg (95%) as colorless crystals. mp 251–253 °C (MeOH, decomp.). $[\alpha]_D^{20}$ −5.1 (*c* 0.685, 0.04 M NaOH in MeOH). The spectra [^{1}H NMR (its sodium salt), IR, and MS] were identical with those of (2*S*,4*R*)-**11a**. Calcd for $C_{13}H_{17}NO_4$ (251.29): C, 62.14; H, 6.82; N, 5.57. Found: C, 62.11; H, 7.08; N, 5.31.

6.6.5. Methyl (2*S*,4*R*)-4-hydroxy-4-(4-methoxyphenyl)pyrrolidine-2-carboxylate [(2*S*,4*R*)-19]

From (2*S*,4*R*)-**14e** (144 mg, 0.374 mmol) and TEA (11 mg, 0.11 mg) in EtOAc (15 mL), 10% Pd-C (74 mg, 0.070 mmol); reaction time: 6 h. Yield: 94 mg (100%) as colorless crystals. mp 96–97 °C (EtOAc). $[\alpha]_D^{20}$ +27.3 (*c* 1.00, $CHCl_3$). IR: $\tilde{\nu}$ = 3303, 1740, 1612 cm^{-1}. MS (*m*/*z*): 252 [M+1]$^+$. ^{1}H NMR ($CDCl_3$, NOE for the determination of the C-4 configuration) δ: 2.35 (dt, 1H, *J* = 13.7, 2.5 Hz, NCHCH_2), 2.51 (dd, 1H, *J* = 13.7, 10.0 Hz, NCHCH_2), 3.07 (br. s, 1H, OH), 3.10 (d, 1H, *J* = 11.8 Hz, NCH_2), 3.27 (dd, 1H, *J* = 11.8, 2.1 Hz, NCH_2), 3.77 (s, 3H, $COOCH_3$), 3.79 (s, 3H, $ArOCH_3$), 3.99 (dd, 1H, *J* = 10.0, 2.5 Hz, NCH), 6.85–6.88 (m, 2H, H_{aromat}), 7.33–7.37 (m, 2H, H_{aromat}). Calcd for $C_{13}H_{17}NO_4$ (251.29): C, 62.14; H, 6.82; N, 5.57. Found: C, 62.11; H, 6.97; N, 5.53.

6.7. Biological test

6.7.1. Preparation of subcellular membrane suspensions

Two subcellular membrane pellets, termed bfcP2B (a) (from bovine frontal cortex) and bbsP2C (b) (from bovine brain stem), respectively, were prepared according to literature.[37] Their suspensions were prepared and measured as described by *Bradford*[47] pfcP2B (c) (from porcine frontal cortex) and pbsP2C (d) (from porcine brain stem), cfcP2B (e) (from calf frontal cortex) and cbsP2C (f) (from calf brain stem), were applied alternatively instead of bfcP2B and bbsP2C, respectively.

6.7.2. Inhibition of GAT1 mediated GABA-uptake

Aliquots of about bfcP2B protein (50–100 μg, alternatively cfcP2B or pfcP2B) were preincubated with 10 μM aminooxyacetic acid and a test compound in the buffer (200 μL, a solution of 119 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 11 mM Glucose and 25 mM Tris HCl pH 7.2) for 10 min at 37 °C. Following the addition of 12.5 nM [^{3}H] GABA (25 μL) and 250 nM GABA (25 μL), the sample was incubated at 37 °C for 4 min. The incubation was terminated by filtration in a Brandel M-24R Harvester through Whatman GF/C filters, which had been immersed in 0.9% NaCl for 1 h. The filters were washed with 0.9% NaCl (4 × 2 mL) and then measured in Rotiszint Eco Plus (3 mL) by the use of a Packard TriCarb 1600 Counter. Specific uptake was defined as difference between entire uptake and non-specific uptake, which was determined with identical samples lacking NaCl.

6.7.3. Inhibition of GAT3 mediated GABA-uptake

Aliquots of about bbsP2C protein (50–100 μg, alternatively cbsP2C or pbsP2C) were preincubated with 10 μM aminooxyacetic acid, 10 μM NNC-711 and a test compound in the buffer (200 μL, a solution of 119 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 11 mM Glucose and 25 mM Tris HCl pH 7.2) for 10 min at 37 °C. The additions of 50 nM [^{3}H] GABA (25 μL) and 1 μM GABA (25 μL) were followed.

Acknowledgment

Financial support of this work by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the BMBF are gratefully acknowledged.

References and notes

1. Lippa, A. S.; Coupet, J.; Greenblatt, E. N.; Klepner, C. A.; Beer, B. *Pharmacol. Biochem. Behav.* **1979**, *11*, 99.
2. *Huntington's Disease*; Chase, T. N., Wexler, N. S., Barbeau, A., Eds.; Ravan Press: New York, 1979.
3. Welch, K. M.; Chabi, E.; Bartosh, K.; Achar, V. S.; Meyer, J. S. *Br. Med. J.* **1975**, *3*, 516.

4. Collelo, G. D.; Hockenberry, D. M.; Bosmann, H. B.; Fuchs, S.; Folkers, K. *Proc. Natl. Acad Sci. U.S.A.* **1978**, *75*, 6319.
5. (a) Pilc, A.; Lloyd, K. G. *Life Sci.* **1984**, *35*, 2149; (b) Lloyd, K. G.; Morselli, P.; Bartholin, G. *Med. Biol.* **1987**, *65*, 159.
6. Lloyd, K. G.; Thuret, F.; Pile, A. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 191.
7. Pratt, G. D.; Bowery, N. G. In *GABAB Receptors in Mammalian Function*; Bowery, N. G., Bittiger, H. R., Olpe, P. P., Eds.; Wiley: Chichester, United Kingdom, 1990; p 319.
8. White, H. S.; Brown, S. D.; Woodhead, J. H.; Skeen, G. A.; Wolf, H. H. *Epilepsy Res.* **1997**, *28*, 167.
9. Lippert, B.; Metcalf, B. W.; June, M. G.; Casara, P. *Eur. J. Biochem.* **1977**, *74*, 441.
10. Haigh, J. R. M.; Feely, M. *Trends Pharmacol. Sci.* **1988**, *9*, 361.
11. Schmidt, D. In *Antiepileptic Drugs*; Levy, R. H., Mattson, R. H., Meldrum, B. S., Eds., 4th ed.; Raven Press: New York, 1995; p 705.
12. Devinsky, O. *Epilepsia* **1995**, *36*, S46.
13. Schousboe, A.; Kanner, B. I. In *Glutamate and GABA Receptors and Transporters: Structure, Function and Pharmacology*; Egebjerg, J., Schousboe, A., Krogsgaard-Larsen, P., Eds.; Francis & Taylor: London, 2002; p 337.
14. GABA transporters originated from human and rat are specified by addition of the appropriate prefix, 'h' or 'r' in addition to a dash preceding the subtype number (e.g., hGAT-1), whereas in the literature for mouse the following nomenclature is used: mGAT1 (slc6a1), mGAT2 (slc6a12), mGAT3 (slc6a13) and mGAT4 (slc6a11), respectively.
15. Kristensen, A. S.; Andersen, J.; Jørgensen, T. N.; Sørensen, L.; Eriksen, J.; Loland, C. J.; Strømgaard, K.; Gether, U. *Pharmacol. Rev.* **2011**, *63*, 585–640.
16. Guastella, J.; Nelson, N.; Nelson, H.; Czyzyk, L.; Keynan, S.; Miedel, M.; Davidson, N.; Lester, H.; Kanner, B. *Science* **1990**, *249*, 1303.
17. Lopez-Corcuera, B.; Liu, Q.; Mandiyan, S.; Nelson, H.; Nelson, N. *J. Biol. Chem.* **1992**, *267*, 17491.
18. Niu, Y.; Lopez-Corcuera, B.; Mandiyan, S.; Nelson, H.; Nelson, N. *J. Biol. Chem.* **1993**, *268*, 2106.
19. Liu, Q.; Mandiyan, S.; Nelson, H.; Nelson, N. *Proc. Natl. Acad Sci. U.S.A.* **1992**, *89*, 6639.
20. Yamauchi, A.; Uchida, S.; Kwon, H. M.; Preston, A. S.; Robey, R. B.; Garcia-Perez, A.; Burg, M. B.; Handler, J. S. *J. Biol. Chem.* **1992**, *267*, 649.
21. Madsen, K. K.; Clausen, R. P.; Larsson, O. M.; Krogsgaard-Larsen, P.; Schousboe, A.; White, H. S. *J. Neurochem.* **2009**, *109*(Suppl 1), 139–144.
22. Dalby, N. O. *Eur. J. Pharmacol.* **2003**, *479*, 127–137.
23. Zhou, Y.; Holmseth, S.; Hua, R.; Lehre, A. C.; Olofsson, A. M.; Poblete-Naredo, I.; Kempson, S. A.; Danbolt, N. C. *Am. J. Physiol. Renal.* **2012**, *302*, F316.
24. Zhou, Y.; Holmseth, S.; Guo, C.; Hassel, B.; Höfner, G.; Huitfeldt, H. S.; Wanner, K. T.; Danbolt, N. C. *J. Biol. Chem.* **2012**, *287*, 35733–35746.
25. Bondinell, F.; Ali, W.; Dandridge, P.; Frazee, J.; Garvey, E.; Girard, G.; Kaiser, C.; Ku, T.; Lafferty, J.; Moonsammy, G.; Oh, H.-J.; Rush, J.; Setler, P.; Stringer, O.; Venslavsky, J.; Volpe, B.; Yunger, L.; Zirkle, C. *J. Med. Chem.* **1985**, *28*, 653.
26. Collins, S. D.; Deaton, R. L.; Giardina, W. J.; Gillbert, A. L. Abbott Laboratories, U.S. Patent 6,872,734 B2, 2005.
27. Rasmus, C. P.; Moltzen, E. K.; Perregaard, J.; Lenz, S. M.; Sanchez, C.; Falch, E.; Frølund, B.; Bolvig, T.; Sarup, A.; Larsson, O.; Schousboe, A.; Krogsgaard-Larsen, P. *Bioorg. Med. Chem.* **2005**, *13*, 895.
28. Cohen-Kfir, E.; Lee, W.; Eskandari, S.; Nelson, N. *Proc. Natl. Acad Sci. U.S.A.* **2005**, *102*, 6154.
29. Karakossian, M. H.; Spencer, S. R.; Gomez, A. Q.; Padilla, O. R.; Sacher, A.; Loo, D. D. F.; Nelson, N.; Eskandari, S. *J. Membr. Biol.* **2005**, *203*, 65.
30. Dhar, T. G. M.; Borden, L. A.; Tyagarajan, S.; Smith, K. E.; Branchek, T. A.; Weinshank, R. L.; Gluchowski, C. *J. Med. Chem.* **1994**, *37*, 2334.
31. Dalby, N. O.; Nielsen, E. B. *Epilepsy Res.* **1997**, *28*, 63.
32. Thomsen, C.; Sørensen, P. O.; Egebjerg, J. *Br. J. Pharmacol.* **1996**, *120*, 983.
33. Cosford, N. D. P.; McDonald, L. A.; Schweiger, E. J. *Annu. Rep. Med. Chem.* **1998**, *33*, 60.
34. Fuelep, G. H.; Hoesl, C. E.; Hoefner, G.; Wanner, K. T. *Eur. J. Med. Chem.* **2006**, *41*, 809.
35. Zhao, X.; Hoesl, C. E.; Hoefner, G. C.; Wanner, K. T. *Eur. J. Med. Chem.* **2005**, *40*, 231.
36. Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.
37. Beeli, R.; Steger, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 2235.
38. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
39. Only one experimental sample is given in Section 6.3.1.
40. Imamoto, T.; Takiyama, N.; Nakamura, K.; Hatajima, T.; Kamiya, Y. *J. Am. Chem. Soc* **1989**, *111*, 4392.
41. Liu, H.-J.; Shia, K.-S.; Shang, X.; Zhu, B.-Y. *Tetrahedron* **1999**, *55*, 3803–3830.
42. Imamoto, T.; Sugiura, Y.; Takiyama, N. *Tetrahedron Lett.* **1984**, *25*, 4233.
43. Imamoto, T.; Takiyama, N.; Nakamura, K. *Tetrahedron Lett.* **1985**, *26*, 4763.
44. The solubility of (2*S*,4*R*)-**41** was very poor in common ^{1}H NMR solvents ($CDCl_3$, CD_3OD or DMSO-d_6).
45. Dodd, P. R.; Hardy, J. A.; Oakley, A. E.; Edwardson, J. A.; Perry, E. K.; Delaunoy, J.-P. *Brain Res.* **1981**, *226*, 107.
46. $CeCl_3{\cdot}7H_2O$ was heated at 140 °C under vacuum for 3.5 h and stored in a desiccator.
47. Bradford, M. *Anal. Biochem.* **1976**, *72*, 248.