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Original article

New highly potent GABA uptake inhibitors selective for GAT-1 and GAT-3 derived from (*R*)- and (*S*)-proline and homologous pyrrolidine-2-alkanoic acids ☆

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Abstract

We synthesized proline and pyrrolidine-2-alkanoic acid derivatives in their enantiomerically pure form and evaluated them for their affinity to the GABA transport proteins GAT-1 and GAT-3. Among the compounds presented herein, (*R*)-pyrrolidine-2-acetic acid (*R*)-**4d** substituted with a 2-[tris(4-methoxyphenyl)methoxy]ethyl residue at the nitrogen atom showed the highest affinity at GAT-3 (IC_{50} = 3.1 μM) comparable with the well-known GAT-3 blocker (*S*)-SNAP-5114. Compound (*R*)-**4d** displayed excellent subtype selectivity for GAT-3 (GAT-3:GAT-1 = 20:1). (*S*)-2-pyrrolidineacetic acid derivatives (*S*)-**4b** provided with a 4,4-diphenylbut-3-en-1-yl moiety and (*S*)-**4c** substituted with a 4,4-[di(3-methylthiophen-2-yl)]phenylbut-3-en-1-yl residue at the nitrogen atom exhibited IC_{50} values of 0.396 μM and 0.343 μM at the GAT-1 protein, respectively.

Keywords: GABA uptake inhibitors; antiepileptic; GAT-1; GAT-3; pyrrolidines

1. Introduction

The search for antiepileptic compounds with more selective activity and lower toxicity continues to be an area of intensive investigation in medicinal chemistry. The amino acid γ-aminobutyric acid (GABA) as the major inhibitory neurotransmitter in the brain plays a crucial role in controlling neuronal excitability and information processing [1,2,3] network synchronization [4,5] and neuronal plasticity [6,7]. The GABAergic system is a main component of the pathology of epilepsy and constitutes an important target in the search for novel antiepileptic drugs. GABA interacts with three types of receptors: $GABA_A$, $GABA_B$, and $GABA_C$ [8]. Following binding of GABA to the ion channel $GABA_A$, which is associated with binding sites for benzodiazepines and barbiturates in the form of a receptor complex, channel opening occurs and chloride ions enter the neuron resulting in hyperpolarization [9,10]. The $GABA_B$ receptors are G-protein coupled receptors and exhibit their action via a cascade of second messengers. The role and mode of action of the $GABA_c$ receptor remains to be determined. The extracellular levels of GABA are regulated by specific high-affinity, Na^+/Cl^--dependent transport proteins [11,12]. Their action limits the overspill from the synaptic cleft and terminates GABA synaptic action thereby fine-tuning the GABAergic inhibitory tone in the central nervous system (CNS). Conversely, the inhibition of GABA transport proteins slows down the reuptake of synaptically released GABA and is an interesting concept to prolong inhibitory postsynaptic potentials for the prevention of epileptic seizures. Four distinct genes encoding GABA transporters (GATs), named GAT-1, GAT-2, GAT-3 and BGT-1 (nomenclature is differing for murine GABA transport proteins) have been identified using molecular cloning techniques. The different GABA transporters display diverse expression patterns with GAT-1 and GAT-3 being the most copiously expressed subtypes in the CNS [13,14,15,16].

Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]un-7-decene; CC, column chromatography; DIBAH, diisobutylaluminiumhydride; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; GP, general procedure; GABA, γ-aminobutyric acid; GAT, GABA transport protein; THF, tetrahydrofuran; TMEDA, *N*,*N*,*N*',*N*'-tetramethylethylendiamine; rt, room temperature.

☆ Dedicated to Prof. Eberhard Reimann on the occasion of his 70th birthday.

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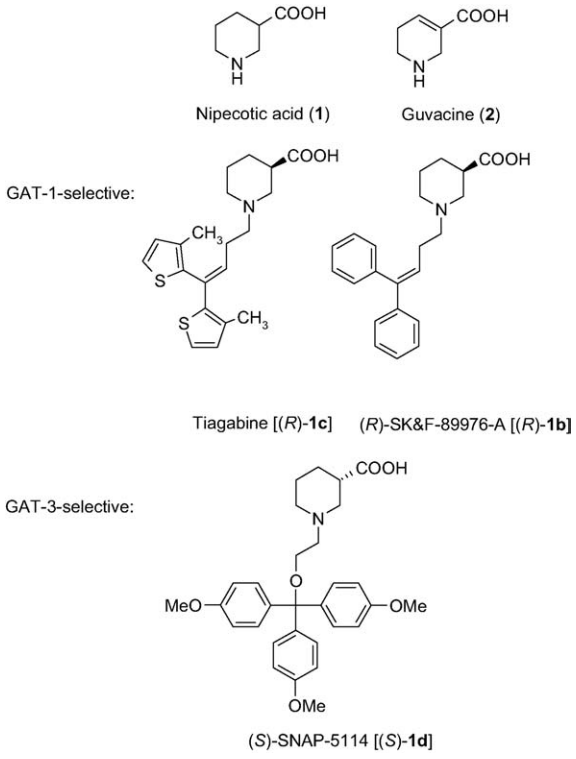


Fig. 1. Structure of GABA uptake inhibitors.

Selective targeting and modulation of GABA transporter subtypes is of therapeutical interest and additionally of fundamental importance for the elucidation of their specialized physiological function and individual structure. Since the initial discovery that nipecotic acid (**1**) and guvacine (**2**) are inhibitors of GABA transport with anticonvulsant activity [17,18] several potent GABA uptake inhibitors including tiagabine [(*R*)-**1c**] [19] and SK&F 89976-A [(*R*)-**1b**] (IC_{50} = 0.11 μM) [20] have been developed and found to be selective for GAT-1. Tiagabine (Gabitril®) is approved in combination with other medications for the treatment of epilepsy in humans [21]. In a small clinical study published most recently, tiagabine was also found to be effective in reducing pain due to complex regional pain syndrome type I [22,23]. The first inhibitor specifically blocking GAT-3 became available with the synthesis of (*S*)-SNAP-5114 [(*S*)-**1d**] (Fig. 1) exhibiting an IC_{50} value of 5 μM and a selectivity of 78:1 (GAT-3:GAT-1) [24].

As part of an ongoing research project aimed at the development, synthesis and optimization of lead structures and ligands for the binding areas of the GABA uptake proteins GAT-1 and GAT-3, we synthesized pyrrolidine derivatives **3**–**7** in their enantiomerically pure form (Fig. 2) as conformationally constrained analogues and homologues of GABA. In general, the structures of potent GABA uptake inhibitors contain a lipophilic side chain improving their potency and subtype selectivity besides enabling them to cross the blood-brain barrier. Thus, except for the parent compounds the derivatives **3**–**7** presented herein are substituted at the nitrogen atom with lipophilic groups which have been identified to be beneficiary for the binding to GAT-proteins. The synthesized compounds **3**–**7** were evaluated for their affinity to and selectivity for the GABA transport proteins GAT-1 and GAT-3.

2. Chemistry

2.1. Synthesis of pyrrolidine core structures

(*S*)- and (*R*)-proline [(*S*)-**3a** and (*R*)-**3a**] are commercially available. (*R*)-pyrrolidine-2-acetic acid (*R*)-**4a** was synthesized according to a four step procedure published by Cassal [25] starting from (*R*)-1-benzyloxycarbonylproline via Arndt–Eistert reaction, Wolff rearrangement reaction, hydrolysis of the methyl ester group and removal of the benzyloxycarbonyl protection group by catalytic hydrogenolysis (total yield: 34%). To access the corresponding enantiomer (*S*)-**4a** we used a method that had been developed in our group for the asymmetric

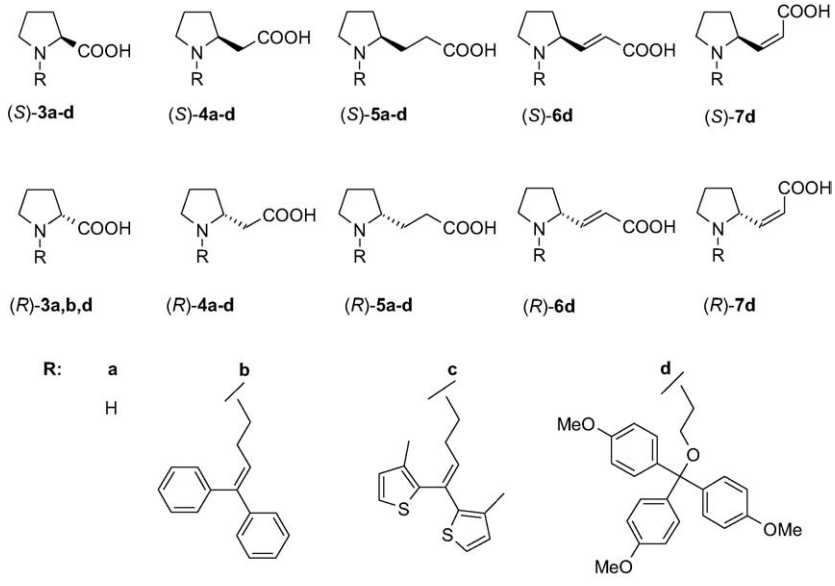


Fig. 2. Pyrrolidine target structures.

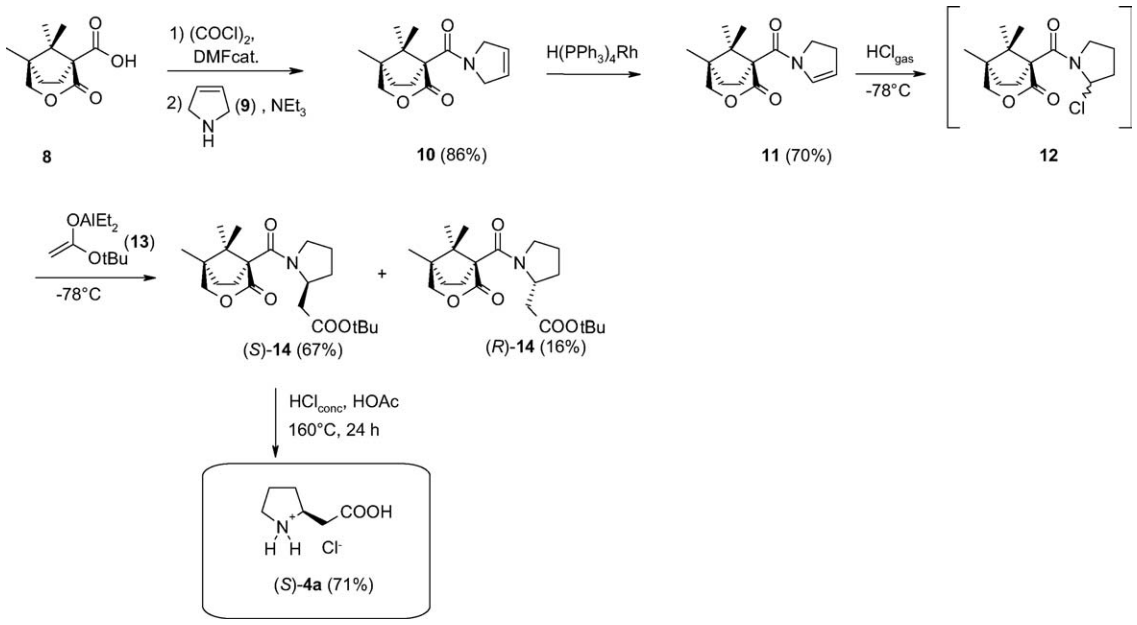


Fig. 3. Stereoselective synthesis of (*S*)-2-pyrrolidineacetic acid hydrochloride [(*S*)-**4a**].

synthesis of 2-substituted pyrrolidine derivatives [26,27]. This method is based on the enamide **11** as an asymmetric α-amidoalkylation reagent. Enamide **11** was accessible from allylamide **10** obtained by reaction of 2,5-dihydro-1*H*-pyrrole **9** with the carboxylic acid chloride of **8** by isomerization using hydridotetrakis(triphenylphosphine)rhodium as catalyst (Fig. 3). For the preparation of the desired β-amino acid (*S*)-**4a** enamide **11** was treated with HCl, which is likely to give α-chloramide **12**, followed by the aluminum enolate **13**. This led by electrophilic α-amidoalkylation reaction [28–34] to the pyrrolidine derivatives (*S*)-**14** and (*R*)-**14** with the diastereoselectivity amounting to 81.2:18.8 determined by analytical HPLC. Removal of the chiral auxiliary and the *t*Bu-protection group from (*S*)-**14** was achieved by hydrolysis with HCl/HOAc at 160 °C resulting in the target compound (*S*)-**4a** (overall yield starting from **8**: 29%). To verify that we obtained the correct configuration at the newly created stereocenter, we compared the specific optical rotation of our product $\{[\alpha]_D^{23} = +19.1$ ($c = 1.2$, H_2O)$\}$ with literature ($\{[\alpha]_D^{28} = +19.3$ ($c = 1.74$, H_2O)$\}$ [35] confirming that the (*S*)-configurated product had formed predominantly during the α-amidoalkylation step.

The synthesis of the 2-pyrrolidinepropionic acid hydrochlorides (*S*)-**5a** and (*R*)-**5a** was started from the Cbz-protected methyl ester (*S*)-**15** and (*R*)-**15** of (*S*)- and (*R*)-proline [(*S*)-**3a** and (*R*)-**3a**], respectively (Fig. 4) [36–39]. Reduction of the ester group using DIBAH gave the aldehydes (*S*)-**16** and (*R*)-**16**, which subsequently were subjected to an olefination reaction with trimethylphosphonoacetate (**17**) in the presence of LiCl and DIPEA leading to the E/Z isomeric mixtures of the acrylic acid derivatives (*S*)-**18** and (*R*)-**18**, respectively [40]. Following saponification of the methyl ester functionality, concurrent reduction of the double bond and removal of the Cbz-

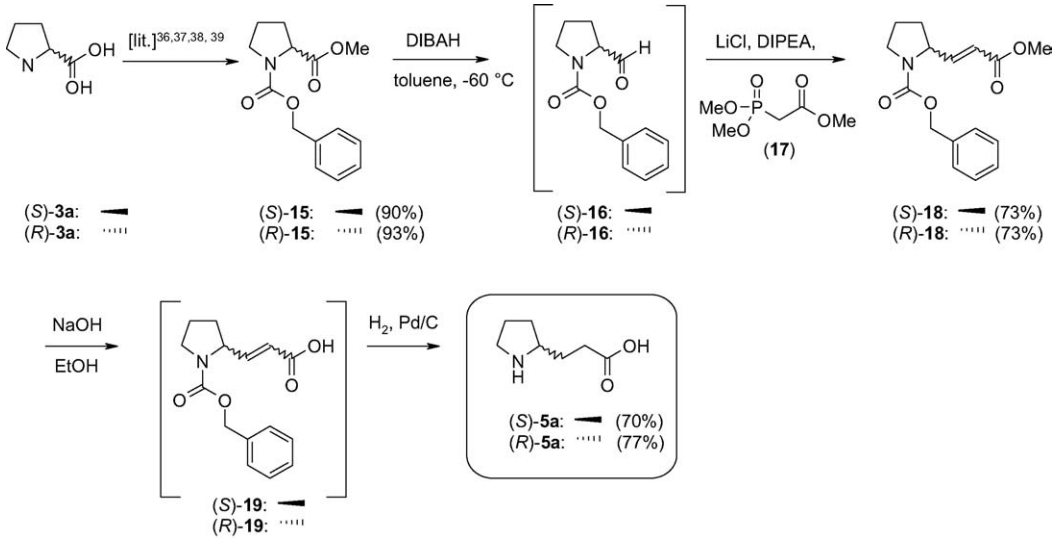


Fig. 4. Synthesis of pyrrolidinepropionic acids (*S*)-**5a** and (*R*)-**5a**.

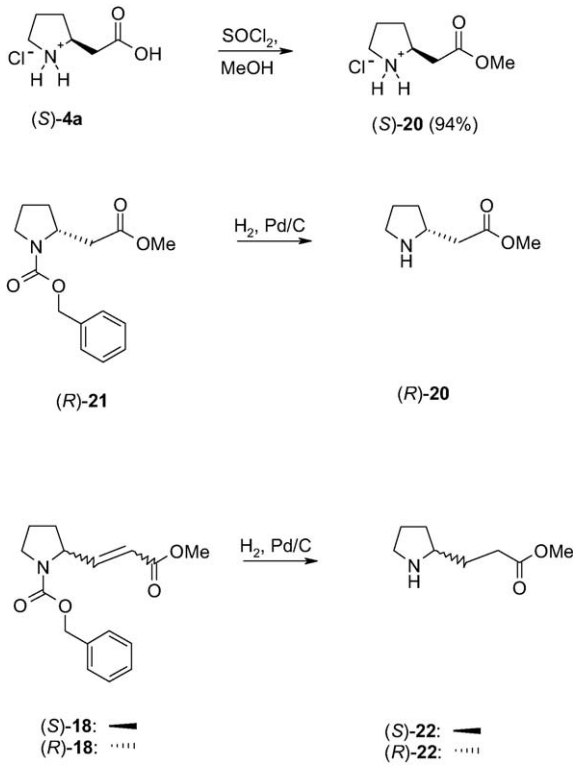


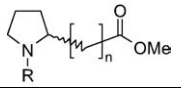
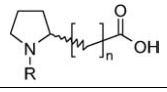
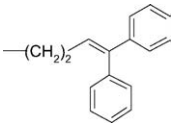
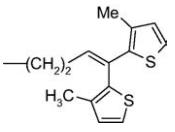
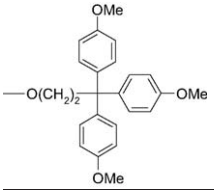
Fig. 5. Synthesis of methyl pyrrolidin-2-ylalkanoates.

protection group was realized by catalytic hydrogenation employing Pd/C. No epimerization has occurred throughout the reaction sequence as confirmed by comparison of the specific optical rotation of the products (*S*)-**5a** with literature {(*S*)-**5a**: $[\alpha]_D^{20} = -7.6$ ($c = 1.1$, 3.4 M HCl), lit.: [41] $[\alpha]_D^{20} = -7.5$ ($c = 1.0$, 3.4 M HCl)}. Correspondingly, for the enantiomer (*R*)-**5a** the specific optical rotation was determined to be $[\alpha]_D^{20} = +7.5$ ($c = 1.0$, 3.4 M HCl).

2.2. Synthesis of methyl pyrrolidin-2-yl-alkanoates

N-alkylation to access the lipophilic pyrrolidinealkanoic acid derivates **3b–d, 4b–d**, and **5a–d** required protection of the 2-pyrrolidine core structures at the carboxylic acid functionality. We decided to use the corresponding methyl esters. Compound (*S*)-**20** was easily obtainable by esterification of the (*S*)-pyrrolidine-2-acetic acid (*S*)-**4a** with thionyl chloride in MeOH. Catalytic hydrogenation of Cbz-protected methyl 2-pyrrolidineacetate (*R*)-**21** and methyl 2-pyrrolidineacrylates (*S*)-**18** and (*R*)-**18** led to the required methyl esters (*R*)-**20** [25] (*S*)-**22** and (*R*)-**22**, respectively, which were directly employed in the subsequent *N*-alkylation reaction (Fig. 5). The Cbz-protected methyl (*R*)-2-pyrrolidineacetate (*R*)-**21** was obtained according to literature [25]. (*S*)- and (*R*)-proline methyl ester hydrochloride (*S*)-**23** and (*R*)-**23** are commercially available.

Table 1

R	*N*		
	0	52% [(*S*)-**24b**]	82% [(*S*)-**3b**]
	0	48% [(*R*)-**24b**]	83% [(*R*)-**3b**]
	1	64% [(*S*)-**25b**]	83% [(*S*)-**4b**]
	1	61% [(*R*)-**25b**]	80% [(*R*)-**4b**]
	2	42% [(*S*)-**26b**]	75% [(*S*)-**5b**]
	2	45% [(*R*)-**26b**]	74% [(*R*)-**5b**]
	0	63% [(*S*)-**24c**]	93% [(*S*)-**3c**]
	1	41% [(*S*)-**25c**]	70% [(*S*)-**4c**]
	1	41% [(*R*)-**25c**]	68% [(*R*)-**4c**]
	2	41% [(*S*)-**26c**]	71% [(*S*)-**5c**]
	2	39% [(*R*)-**26c**]	68% [(*R*)-**5c**]
	0	37% [(*S*)-**24d**]	80% [(*S*)-**3d**]
	0	39% [(*R*)-**24d**]	79% [(*R*)-**3d**]
	1	35% [(*S*)-**25d**]	82% [(*S*)-**4d**]
	1	41% [(*R*)-**25d**]	82% [(*R*)-**4d**]
	2	22% [(*S*)-**26d**]	81% [(*S*)-**5d**]
	2	45% [(*R*)-**26d**]	83% [(*R*)-**5d**]

2.3. *N*-alkylation and saponification

N-alkylation of the respective methyl ester derivatives proceeded in good yields employing 4,4,-diphenylbut-3-en-1-yl bromide (R = **b**) [20] 4,4-bis-(3-methyl-thiophen-2-yl)-but-3-en-1-yl bromide (R = **c**) [19] and 2-[tris(4-methoxyphenyl) methoxy]ethyl bromide (R = **d**) [24]. Upon saponification using NaOH in EtOH, the target structures were obtained (Fig. 6). Table 1 summarizes the yields of the *N*-alkylation and saponification step.

2.4. Synthesis of *N*-arylalkyl-3-pyrrolidin-2-ylacrylic acid derivatives (*S*)-**6d**, (*S*)-**7d**, (*R*)-**6d**, and (*R*)-**7d**

To synthesize the *N*-arylalkyl-3-pyrrolidine-2-ylacrylic acid derivatives (*S*)-**6d**, (*S*)-**7d**, (*R*)-**6d**, and (*R*)-**7d** as pharmacologically interesting structural variations in the series of 3-(pyrrolidin-2-yl)-propionic acid derivatives, we started from the *N*-alkylated, enantiomerically pure compounds (*S*)-**24d** and (*R*)-

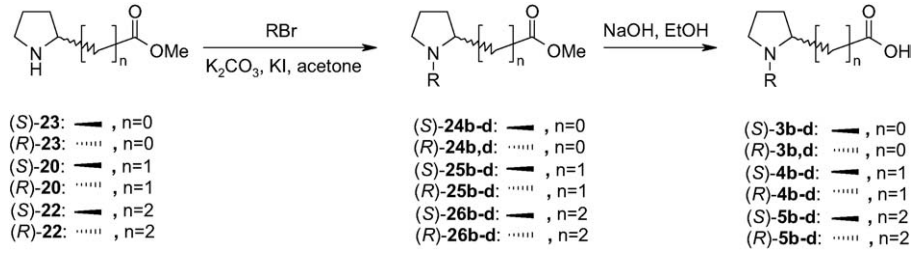


Fig. 6. Synthesis of pyrolidin-2-ylalkanoic acid.

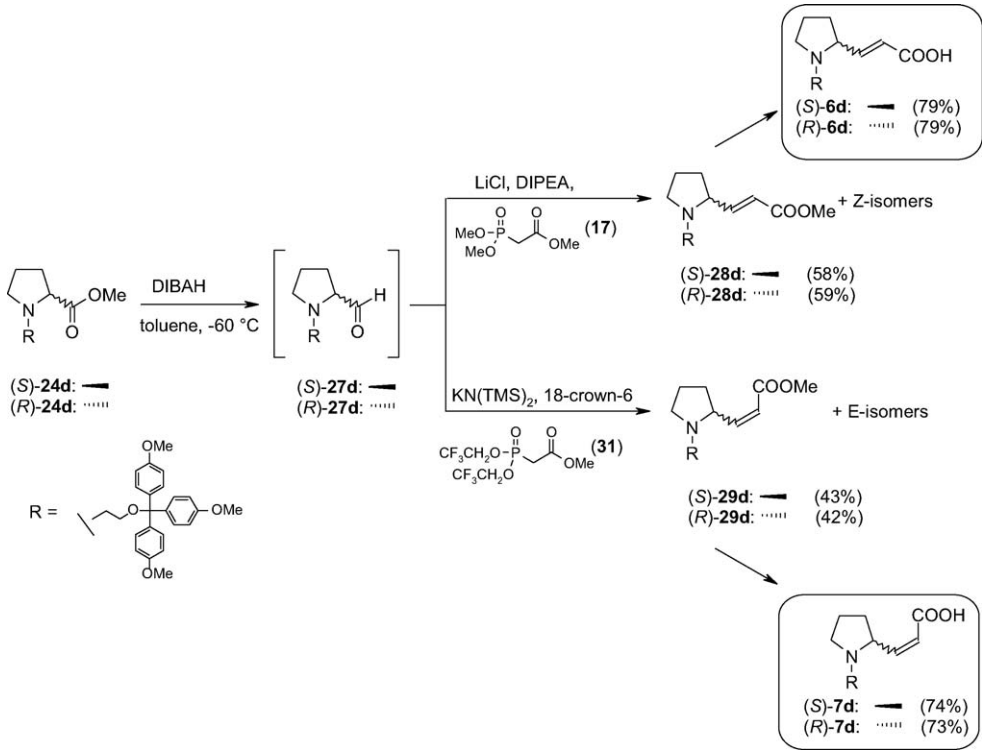


Fig. 7. Synthesis of 3-(pyrrolidin-2-yl)acrylic acid derivatives (*S*)-**6d,** (*S*)-**7d**, (*R*)-**6,** and (*S*)-**7d**.

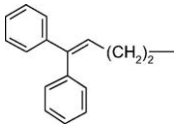
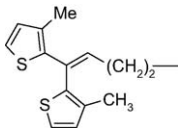
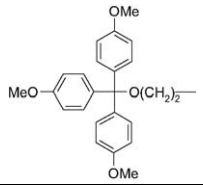
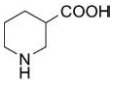
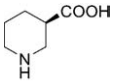
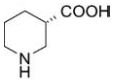
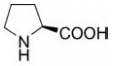
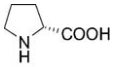
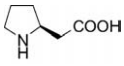
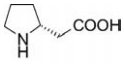
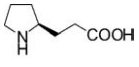
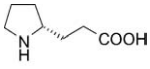
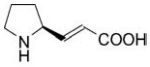
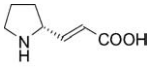
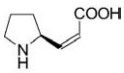
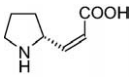
24d, respectively (Fig. 7). First, in analogy to the reaction sequence depicted in Fig. 7, we reduced the methyl ester group. To establish the desired unsaturated side chain the resulting aldehydes (*S*)-**27d** and (*R*)-**27d** were treated with trimethylphosphonoacetate (**17**), LiCl, and DIPEA. Following saponification of the ester functionality, analysis by NMR spectroscopy showed that the E-configurated acrylic acid derivatives (*S*)-**6d** and (*R*)-**6d** had been formed. To access the Z-isomers (*S*)-**7d** and (*R*)-**7d**, we performed the Horner-Emmons reaction under slightly modified reaction conditions according to a method by Still and Gennari [42]. In this case aldehydes (*S*)-**27d** and (*R*)-**27d**, respectively, were reacted with [bis-(2,2,2-trifluoroethoxy)-phosphoryl]-acetic acid methyl ester **31** and $KN(TMS)_2$ as a base in the presence of 18-crown-6. This resulted in a product mixture comprised of the Z- and E-isomer (**29d/28d**) in a ratio of approximately 3:1 as determined by NMR spectroscopy. Following purification by CC, the Z-isomers (*S*)-**29d** and (*R*)-**29d**, respectively, were obtained in good yields [(*S*)-**29d**: 43%, (*R*)-**29d**: 43%]. Saponification led to the target structures (*S*)-**7d** and (*R*)-**7d**.

3. Biological studies

The final enantiomerically pure compounds **3a–d, 4a–d, 5a–d, 6d**, and **7d** were tested for their inhibitory potency on GAT-1 and GAT-3 mediated GABA-uptake. To evaluate the affinity of the compounds to the GABA transport proteins, our group has developed an assay based on subcellular membrane fractions from frontal cortex ($bfcP_{2B}$) and brain stem ($bbsP_{2C}$) of bovine brain [43]. In general, most of the compounds showed moderate to very good inhibitory activity (Table 2). Among the series, the pyrrolidineacetic acid derivatives (*S*)-**4b** ($IC_{50} = 0.396 \pm 0.026$ μM) and (*S*)-**4c** ($IC_{50} = 0.343 \pm 0.044$ μM) were the most potent compounds at GAT-1 and their inhibitory potency was only slightly lower as compared to tiagabine [44]. The IC_{50} values of the (*S*)-configurated derivatives (*S*)-**4b** and (*S*)-**4c** were also found to be lower than those of their enantiomers (*R*)-**4b** and (*R*)-**4c**. This trend was also observed for the less potent, homologues pyrrolidinepropionic acid derivatives [(*S*)-**5b**: ($IC_{50} = 23.9 \pm 0.7$ μM and (*S*)-**5c**: $IC_{50} = 5.29 \pm 0.97$ μM versus (*R*)-**5b**: $IC_{50} = 109 \pm 11$ μM and (*R*)-**5c**: $IC_{50} = 34.7 \pm 11$ μM]. In contrast to the aforementioned compounds the proline derivatives (*S*)-**3b** and (*R*)-**3b** had quite similar IC_{50} values at GAT-1: 2.56 ± 0.29 and 2.97 ± 0.08 μM, respectively. Generally, amino acids provided with a 4,4-diphenylbut-3-en-1-yl or 4,4-bis(3-methylthiophen-2-yl)-but-3-en-1-yl moiety at their amino group are predominantly selective for the GAT-1 transport protein rather than GAT-3 [45]. Our results described above confirmed this rule again. In contrast, the 2-[tris(4-methoxyphenyl)methoxy]ethyl side chain is well known to be advantageous for binding to GAT-3 [46]. This rule, too, is verified by the results obtained in the present study.

All compounds of the series of homologous derivatives **3d-5d** provided with a 2-[tris(4-methoxyphenyl)methoxy]ethyl moiety displayed a higher potency at GAT-3 than at GAT-1. Even though this was true for both antipodes of a pair of enantiomers within this series, in every case the (*R*)-enantiomer showed the higher potency at and the higher selectivity for GAT-3. The most potent inhibitor at GAT-3 is also found

Table 2

	N-substituent: H		N-substituent: 		N-substituent: 		N-substituent: 	
	IC_{50} ± S.E.M. [μM]		IC_{50} ± S.E.M. [μM]		IC_{50} ± S.E.M. [μM]		IC_{50} ± S.E.M. [μM]	
	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3
	4.77 ± 1.17 (*rac*)-**1a**	17.3 ± 2.36 (*rac*)-**1a**	1.18 ± 0.24 (*rac*)-**1b**	290 ± 151 (*rac*)-**1b**	0.249 ± 0.064 (*rac*)-**1c**	186 ± 40 (*rac*)-**1c**	47.5 ± 0.6 (*rac*)-**1d**	1.89 ± 0.03 (*rac*)-**1d**
	2.15 ± 0.11 (*R*)-**1a**	18.5 ± 5.6 (*R*)-**1a**	–	–	0.159 ± 0.029 (*R*)-**1c**	483 ± 83 (*R*)-**1c**	–	–
	32.6 ± 6.5 (*S*)-**1a**	218 ± 54 (*S*)-**1a**	–	–	–	–	83.3 ± 15.7 (*S*)-**1d**	1.08 ± 0.24 (*S*)-**1d**
	875 ± 57 (*S*)-**3a**	1910 ± 290 (*S*)-**3a**	2.56 ± 0.29 (*S*)-**3b**	309 ± 29 (*S*)-**3b**	0.645 ± 0.055 (*S*)-**3c**	100 μM: 62.0% (*S*)-**3c**	123 ± 18 (*S*)-**3d**	57.7 ± 6.5 (*S*)-**3d**
	> 10 mM (*R*)-**3a**	6390 ± 180 (*R*)-**3a**	2.97 ± 0.08 (*R*)-**3b**	231 ± 24 (*R*)-**3b**	–	–	143 ± 39 (*R*)-**3d**	18.5 ± 4.0 (*R*)-**3d**
	482 ± 39 (*S*)-**4a**	74.2 ± 6.2 (*S*)-**4a**	**0.396 ± 0.026** (*S*)-**4b**	64.8 ± 12.1 (*S*)-**4b**	**0.343 ± 0.044** (*S*)-**4c**	26.6 ± 4.3 (*S*)-**4c**	35.4 ± 1.8 (*S*)-**4d**	28.7 ± 9.4 (*S*)-**4d**
	214 ± 16 (*R*)-**4a**	602 ± 59 (*R*)-**4a**	3.05 ± 0.47 (*R*)-**4b**	189 ± 22 (*R*)-**4b**	0.875 ± 0.067 (*R*)-**4c**	180 ± 23 (*R*)-**4c**	67.8 ± 19.0 (*R*)-**4d**	3.10 ± 0.45 (*R*)-**4d**
	1mM: 87.0%[a] (*S*)-**5a**	632 ± 75 (*S*)-**5a**	23.9 ± 0.7 (*S*)-**5b**	565 ± 32 (*S*)-**5b**	5.29 ± 0.97 (*S*)-**5c**	376 ± 24 (*S*)-**5c**	215 ± 6 (*S*)-**5d**	117 ± 41 (*S*)-**5d**
	1mM: 86.6%[b] (*R*)-**5a**	546 ± 96 (*R*)-**5a**	109 ± 11 (*R*)-**5b**	292 ± 19 (*R*)-**5b**	34.7 ± 11 (*R*)-**5c**	294 ± 44 (*R*)-**5c**	101 ± 9 (*R*)-**5d**	11.2 ± 4.2 (*R*)-**5d**
	–	–	–	–	–	–	105 ± 17 (*S*)-**6d**	75.1 ± 8.8 (*S*)-**6d**
	–	–	–	–	–	–	97.5 ± 12.3 (*R*)-**6d**	357 ± 100 (*R*)-**6d**
	–	–	–	–	–	–	369 ± 31 (*S*)-**7d**	449 ± 136 (*S*)-**7d**
	–	–	–	–	–	–	478 ± 129 (*R*)-**7d**	315 ± 186 (*R*)-**7d**

[a] All results were processed and evaluated in triplicate.

[b] Each IC_{50} was given as mean ± S.E.M. Whenever preliminary experiments indicated that the IC_{50} value is larger than 100 μM, no IC_{50} value was established.

among this subset of compounds: the pyrrolidineacetic acid derivative (*R*)-**4d**. The compound shows a remarkable affinity at GAT-3 of 3.10 ± 0.45 μM (IC_{50}) comparable to the well-known GAT-3 inhibitor (*S*)-SNAP-5114. The subtype selectivity of this compound for GAT-3 is quite satisfactory as well (GAT-3/GAT-1 = 20:1).

The 2-pyrrolidineacrylic acid derivatives **6d–7d** comprising a more rigidized side chain at position 2 due to the double bond were far less potent at GAT-3. Moreover, no clear selectivity in favor of GAT-3 could be detected for these compounds despite the presence of the 2-[tris(4-methoxyphenyl) methoxy]ethyl moiety which is known for enhancing GAT-3

potency. In short, compounds **6d–7d** showed only mediocre IC_{50} values for GAT-1 and GAT-3 ranging from ~75–500 μM.

Among the *N*-unsubstituted core structures **3a**, **4a**, and **5a**, the two enantiomers of 2-pyrrolidineacetic acid, (*R*)-**4a** and (*S*)-**4a**, appeared to be the most potent compounds, although their affinity is not very high. Interestingly, these two enantiomers display diametrically opposed subtype selectivities: enantiomer (*R*)-**4a** has a lower IC_{50} value at GAT-1 (214 ± 16 μM) than at GAT-3 (602 ± 59 μM), whereas (*S*)-**4a** is more potent at GAT-3 (IC_{50} 74.2 ± 6.2μM) than at GAT-1 (IC_{50} 482 ± 39 μM). However, only the *N*-unsubstituted pyrrolidineacetic acid derivatives of (*R*)-**4a** and (*S*)-**4a** display this characteristic that the highest potency at GAT-1 resides in the (*R*)- and at GAT-3 in the (*S*)-enantiomer. Interestingly, among the *N*-substituted derivatives **4b**-**4c** provided with a residue beneficial for improving the GAT-1 potency the most potent compounds at this transporter, (*S*)-**4b** and (*S*)-**4c,** are derived from the (*S*)-enantiomer (*S*)-**4a**. Whereas the most potent GAT-3 inhibitor based on a pyrrolidineacetic acid skeleton (*R*)-**4d** originated from the (*R*)-enantiomer (*R*)-**4a**.

4. Conclusion

We presented herein the synthesis of enantiomerically pure *N*-substituted proline, pyrrolidin-2-ylacetic acid, 2-pyrrolidinepropionic acid, and 2-pyrrolidineacrylic acid derivatives. The compounds were evaluated for their affinity at the GABA transport proteins GAT-1 and GAT-3 with a special emphasis on enantioselectivity of binding and subtype specificity. The two (*S*)-2-pyrrolidineacetic acid derivatives (*S*)-**4b** and (*S*)-**4c** substituted with a 4,4-diphenylbut-3-en-1-yl or a 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl) moiety at the nitrogen atom showed high affinity to the GAT-1 protein ((*S*)-**4b**: IC_{50} = 0.396 μM, (*S*)-**4c**: 0.343 μM). The (*R*)-2-pyrrolidineacetic acid derivative (*R*)-**4d** was found to display excellent inhibitory activity at GAT-3 with a high subtype selectivity (GAT-1/GAT-3 = 20:1).

5. Experimental section

5.1. Chemistry

5.1.1. General

Tetrahydrofuran, toluene and diisopropylamine were distilled from sodium under nitrogen. Other common solvents for recrystallization, column chromatography, analytical HPLC and preparative HPLC were distilled before 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 5% $(NH_4)_6Mo_7O_{24}$ $4H_2O$, 0.2% $Ce(SO_4)_2$ $4H_2O$ and 5% conc. H_2SO_4. If nothing else is stated, Merck silica gel (mesh 230–400) was used as stationary phase for flash chromatography (CC). Analytical HPLC: Column LiChrospher Si 60 (5 μm, 250 × 4 mm with precolumn 4 × 4 mm). Preparative HPLC: Column LiChrospher Si 60 (7 μm, 250 × 25 mm). Optical rotations: Polarimeter 241 MC at λ 589 cm^{-1}. Melting points: m.p. (uncorrected) were determined with a Büchi 510 Melting Point apparatus. Elementary analysis: Elementaranalysator Rapid (Heraeus). The results of elemental analyses for C, H and N were within ±0.4% of the theoretical values. IR spectroscopy: FT-IR Spectrometer 1600 and Paragon 1000 (Perkin Elmer), oils were measured as film, solid samples as KBr-pellets for measurements. Mass spectrometry: 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 MHz and 500 MHz) with TMS as internal standard and integrated with the program of NMR-software Nuts (2D Version 5.097, Acorn NMR, 1995). If not stated otherwise, measurements were performed at 400 MHz at room temperature.

5.1.2. General procedure for N-alkylation (GP1)

A: The respective alkyl bromide (1 equiv.) in acetone (1 ml $mmol^{-1}$) was added dropwise to a suspension of the corresponding hydrochloride amino acid ester (1 equiv.), KI (0.1 equiv.), and K_2CO_3 (2 equiv.) in acetone (1.5 ml $mmol^{-1}$). The reaction mixture was stirred at rt for the time given. Following the addition of water and CH_2Cl_2 and extraction with CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.

B: Pd/C was added to the respective Cbz-protected compound (1 equiv.) in MeOH (0.1 M) and the resulting mixture was subjected to hydrogen at rt for 1 h (1 bar). The mixture was filtrated to remove the catalyst and the filtrate was concentrated in vacuo. The resulting residue was dissolved in acetone (1.5 ml $mmol^{-1}$) followed by the addition of K_2CO_3 (1 equiv.) and KI (0.1 equiv.). The respective alkyl bromide (1 equiv.) in acetone (1 ml $mmol^{-1}$) was added dropwise and the reaction mixture was stirred at rt for the time given. Following the addition of water and CH_2Cl_2 and extraction with CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.

5.1.3. Saponification of the ester derivatives (GP2)

The respective ester derivative (1 equiv.) in EtOH (O.5 M) was cooled to 0 °C followed by the dropwise addition of aqueous NaOH (12 M, 2 equiv). The ice bath was removed and the reaction mixture was stirred at rt for the time given. The reaction mixture was again cooled to 0 °C and acidified (pH ≈ 6) using HCl (0.25 M). CH_2Cl_2 and H_2O were added followed by extraction with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.

5.1.4. (S)-1-(4,4-diphenylbut-3-en-1-yl-pyrrolidine-2-carboxylic acid hydrochloride [(S)-3b]

According to GP2 starting from compound (*S*)-**24b** (265 mg, 0.79 mmol) and NaOH (132 μl, 12 M); reaction time: 5 h. Differing from GP2, the crude product was acidified to pH ≈ 1 using 4 M HCl followed by extraction with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated

in vacuo. Purification by recrystallization in EtOH. Yield: 231 mg (82%); colorless crystals, m.p. 220 °C. $[\alpha]_D^{20} = -21.0$ ($c = 1.00$, CH_3OH). ^1H NMR (CD_3OD, 20 °C): δ = 1.88–2.00 (m, 1H, NCH_2CH_2), 2.08–2.21 (m, 2H, $NCH_2CH_2CH_2$), 2.43–2.53 (m, 1H, $NCHCH_2$), 2.54–2.62 (m, 2H, $=CHCH_2$), 3.05–3.14 (m, 1H, NCH_2), 3.20–3.30 (m, 1H, $=CHCH_2CH_2$), 3.45 (td, $J = 12.4$, 8.0 Hz, 1H, $=CHCH_2CH_2$), 3.62 (ddd, $J = 12.4$, 7.5, 4.0 Hz, 1H, NCH_2), 4.08 (dd, $J = 9.6$, 6.8 Hz, 1H, NCH), 6.11 (t, $J = 7.3$ Hz, 1H, =CH), 7.19–7.49 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3427$ cm^{-1}, 2926, 2854, 2602, 1728, 1630. MS (70 eV); *m/z* (%): 321 (1) $[M + 1]^+$, 276 (8), 239(6), 149 (85), 128 (100). $C_{21}H_{24}NO_2Cl$ (357.88).

5.1.5. (R)-1-(4,4-diphenylbut-3-en-1-yl)pyrrolidine-2-carboxylic acid hydrochloride [(R)-3b]

According to GP2 starting from compound (*R*)-**24b** (210 mg, 0.63 mmol) and NaOH (105 µl, 12 M); reaction time: 5 h. Differing from GP2, the crude product was acidified to pH ≈ 1 using 4 M HCl followed by extraction with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by recrystallization in EtOH. Yield: 184 mg (83%); colorless crystals, m.p. 218 °C. Analytical data (^1H NMR, IR, MS) were in accordance with the (*S*)-enantiomer (*S*)-**3b**. $[\alpha]_D^{20} = +21.9$ ($c = 0.71$, CH_3OH). $C_{21}H_{24}NO_2Cl$ (357.88).

5.1.6. (S)-1-[4,4-bis(4-methylthiophen-2-yl)but-3-en-1-yl] pyrrolidine-2-carboxylic acid [(S)-3c]

According to GP2 starting from compound (*S*)-**24c** (2.34 g, 6.23 mmol) and NaOH (1.04 ml, 12 M); reaction time 1 h. Purification by CC (CH_2Cl_2: EtOH = 8:2). Yield: 2.096 g (93%); colorless crystals, m.p. 128 °C (decomp.). $[\alpha]_D^{20} = -40.5$ ($c = 0.41$, $CHCl_3$, 500 MHz ^1H NMR ($CDCl_3$, 20 °C): δ = 1.85–1.90 (m, 1H, NCH_2CH_2), 1.96 (2, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.17–2.30 (m, 2H, $NCHCH_2$), 2.49–2.60 (m, 2H, $N=CHCH_2$), 2.60–2.68 (m, 1H, NCH_2), 2.90–3.00 (m, 1H, $=CHCH_2CH_2$), 3.12–3.23 (m, 1H, $=CHCH_2CH_2$), 3.50–3.59 (m, 1H, NCH), 3.59–3.70 (m, 1H, NCH_2), 5.97 (t, $J = 7.4$ Hz, 1H, =CH) 6.76 (d, $J = 5.1$ Hz, 1H, SC=CH), 6.86 (d, $J = 5.1$ Hz, 1H SC=CH), 7.07 (d, $J = 5.1$ Hz, 1H, SCH=), 7.23 (d, $J = 5.1$ Hz, 1H, SCH=). IR (KBr): $\tilde{\nu} = 3408$ cm^{-1}, 3042, 2967, 1627, 1381. MS (CI, CH_5^+); *m/z* (%): 362 (55) $[M + 1]^+$, 173 (56), 145 (89), 127 (10). $C_{19}H_{23}NO_2S_2$ (361.53).

5.1.7. (S)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidine-2-carboxylic acid [(S)-3d]

According to GP2 starting from compound (*S*)-**24d** (289 mg, 0.63 mmol) and NaOH (95 µl, 12 M); reaction time: 4 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 225 mg (80%); colorless crystals, m.p. 69–75 °C (decomp.). $[\alpha]_D^{20} = -8.2$ ($c = 4.92$, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.85–1.95 (m, 2H, NCH_2CH_2), 2.22 (q, $J = 7.3$ Hz, 2H, $NCHCH_2$), 2.77 (dt, $J = 9.8$, 8.8 Hz, 1H, NCH_2), 2.93–3.01 (m, 1H, OCH_2CH_2N), 3.27–3.36 (m, 2H, OCH_2CH_2N), 3.39–3.47 (m, 1H, OCH_2CH_2N), 3.54 (dt, $J = 9.8$, 5.5 Hz, 1H, NCH_2), 3.68–3.76 (m, 1H, NCH), 3.80 (s, 9H, OCH_3), 6.80–6.86 (m, 6H, $H_{aromat.}$), 7.28–7.33 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3420$ cm^{-1}, 3036, 2956, 2836, 1637, 1608, 1583. MS (CI, CH_5^+); *m/z* (%): 446 (1), 335 (11), 334 (48), 333 (45), 227 (21), 160 (100), 142 (50). MS (70 eV); *m/z* (%): 333 (100), 318 (13), 259 (5), 227 (6), 167 (4), 114 (4), 82 (8). $C_{29}H_{33}NO_6$ (491.59).

5.1.8. (R)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidine-2-carboxylic acid [(R)-3d]

According to GP2 starting from compound (*R*)-**24d** (243 mg, 0.48 mmol) and NaOH (80 µl, 12 M); reaction time: 4 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 186 mg (79%); colorless crystals, m.p. 69–75 °C (decomp.). Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**3d**. $[\alpha]_D^{20} = +8.1$ ($c = 2.73$, $CHCl_3$). $C_{29}H_{33}NO_6$ (491.59).

5.1.9. (S)-pyrrolidin-2-ylacetic acid hydrochloride [(S)-4a]

HCl_{conc} (6 ml) was added to a solution of compound (*S*)-**14** (325 mg, 0.87 mmol) in glacial acetic acid (4 ml) and the reaction mixture was stirred in a sealed tube at 160 °C for 24 h. The reaction mixture was poured with caution into ice water (15 ml) followed by extraction with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Recrystallization from acetone/MeOH/t_2O. Yield: 102 mg (71%); colorless crystals, m.p. 173–175 °C, $[\alpha]_D^{23} = +19.1$ ($c = 1.2$, H_2O) lit. [35] 175–176 °C. $[\alpha]_D^{28} = +19.3$ ($c = 1.74$, H_2O). ^1H NMR (CD_3OD, 20 °C): δ = 1.66–1.79 (m, 1H, $NCH_2CH_2CH_2$), 1.92–2.16 (m, 2H, $NCH_2CH_2CH_2$), 2.21–2.32 (m, 1H, $NCH_2CH_2CH_2$), 2.74–2.92 (m, 2H, CH_2COO), 3.26–3.34 (m, 2H, NCH_2), 3.79–3.89 (m, 1H, NCH).

5.1.10. (S)-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]acetic acid [(S)-4b]

According to GP2 starting from compound (*S*)-**25b** (139 mg, 0.398 mmol) and NaOH (66 µl, 12 M); reaction time: 5 h. Purification by CC (CH_2Cl_2/EtOH = 8:2). Yield: 110 mg (83%); colorless crystals, m.p. 130–137 °C (decomp.). $[\alpha]_D^{20} = -85.4$ ($c = 1.30$, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.67–1.94 (m, 3H, $NCH_2CH_2CH_2$), 2.05–2.15 (m, 1H, $NCHCH_2$), 2.35 (td, $J = 10.5$, 8.5 Hz, 1H, NCH_2), 2.42–2.54 (m, 4H, $= CCH_2CH_2N$, CH_2COO), 2.65 (dd, $J = 17.1$, 5.1 Hz, 1H, CH_2COO), 2.95–3.04 (m, 1H, NCH), 3.06–3.16 (m, 1H, $=CCH_2CH_2N$), 3.19 (ddd, $J = 10.5$, 7.1, 3.9 Hz, 1H, NCH_2), 6.02 (t, $J = 7.3$ Hz, 1H, =CH), 7.14–7.44 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3405$ cm^{-1}, 3053, 2958, 2570, 1578. MS (70 eV); *m/z* (%): 335 (1) $[M + 1]^+$, 295 (4), 276 (5), 247 (4), 191 (7),142 (100). $C_{22}H_{25}NO_2$ (335.45).

5.1.11. (R)-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]acetic acid [(R)-4b]

According to GP2 starting from compound (*R*)-**25b** (136 mg, 0.398 mmol) and NaOH (65 µl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 105 mg (80%); colorless crystals, m.p. 129–135 °C (decomp.). Analytical data (^1H

NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**4b** $[\alpha]_D^{20} = +86.5$ ($c = 0.47$, $CHCl_3$). $C_{22}H_{25}NO_2$ (335.45).

5.1.12. (S)-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl] pyrrolidin-2-yl}acetic acid [(S)-4c]

According to GP2 starting from compound (*S*)-**25c** (85 mg, 0.218 mmol) and NaOH (36 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 57 mg (70%); colorless oil. $[\alpha]_D^{20} = -64.9$ ($c = 0.85$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.66–1.79 (m, 1H, NCH_2CH_2), 1.79–1.95 (m, 2H, $NCH_2CH_2CH_2$), 1.98 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.04–2.17 (m, 1H, $NCHCH_2$), 2.38–2.55 (m, 5H, $=CCH_2CH_2N$, CH_2COO, NCH_2), 2.63 (dd, $J = 16.9$, 5.3 Hz, 1H, CH_2COO), 2.93–3.01 (m, 1H, NCH), 3.03–3.12 (m, 1H, $=CCH_2CH_2N$), 3.29 (ddd, $J = 11.0$, 7.5, 4.0 Hz, 1H, NCH_2), 6.01 (t, $J = 7.3$ Hz, 1H, =CH), 6.76 (d, $J = 5.1$ Hz, 1H, SC=CH), 6.87 (d, $J = 5.1$ Hz, 1H, SC=CH), 7.06 (d, $J = 5.1$ Hz, 1H, SCH=), 7.24 (d, $J = 5.1$ Hz, 1H, SCH=). IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 3100, 3057, 2952, 2868, 1594. MS (CI, NH_4^+); *m/z* (%): 376 (47) $[M + 1]^+$, 316 (14), 247 (9), 235 (5), 142 (500), 79 (100). $C_{20}H_{25}NO_2S_2$ (375.55).

5.1.13. (R)-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl] pyrrolidin-2-yl}acetic acid [(R)-4c]

According to GP2 starting from compound (*R*)-**25c** (105 mg, 0.27 mmol) and NaOH (45 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 69 mg (68%); colorless oil. Analytical data (^{1}H NMR, IR, MS) were in accordance with those of (*S*)-enantiomer (*S*)-**4c**. $[\alpha]_D^{20} = +65.2$ ($c = 1.02$, $CHCl_3$). $C_{20}H_{25}NO_2S_2$ (375.55).

5.1.14. (S)-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acetic acid [(S)-4d]

According to GP2 starting from compound (*S*)-**25d** (220 mg, 0.423 mmol) and NaOH (70 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH/*n*-pentane = 1:1). Yield: 176 mg (82%); colorless oil. m.p. 68–73 °C (decomp.). $[\alpha]_D^{20} = -32.0$ ($c = 0.66$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.69–1.97 (m, 3H, $NCH_2CH_2CH_2$), 2.04–2.15 (m, 1H, $NCHCH_2$), 2.44–2.59 (m, 3H, OCH_2CH_2N, NCH_2, CH_2COO), 2.68 (dd, $J = 17.2$, 4.3 Hz, 1H, CH_2COO), 2.98–3.07 (m, 1H, NCH), 3.07–3.17 (m, 1H, OCH_2CH_2N), 3.26–3.44 (m, 3 H, OCH_2CH_2N, NCH_2), 3.79 (s, 9 H, OCH_3), 6.84–6.86 (m, 6H, $H_{aromat.}$), 7.28–7.34 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 3036 cm^{-1}, 2931, 2835, 2958, 1607, 1508. MS (CI, NH_4^+); *m/z* (%): 506 (1) $[M + 1]^+$, 334 (19), 333 (36), 319 (1), 227 (13), 174 (100). $C_{30}H_{35}NO_6$ (505.62).

5.1.15. (R)-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acetic acid [(R)-4d]

According to GP2 starting from compound (*R*)-**25d** (135 mg, 0.26 mmol) and NaOH (43 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH/*n*-pentane = 1:1). Yield: 176 mg (82%); colorless oil. m.p. 68–73 °C (decomp.). Analytical data (^{1}H NMR, IR, MS) were in accordance with those of (*S*)-enantiomer (*S*)-**4d**. $[\alpha]_D^{20} = +32.7$ ($c = 1.02$, $CHCl_3$). $C_{30}H_{35}NO_6$ (505.62).

5.1.16. (S)-3-(pyrrolidin-2-yl)propionic acid [(S)-5a]

NaOH (66 μl, 12 M, 2 equiv.) was added to compound (*S*)-**18** (116 mg, 0.4 mmol) in EtOH (1.25 ml) at 0 °C. Following removal of the ice bath, the reaction mixture was stirred for 6 h at rt. The reaction mixture was again cooled to 0 °C and acidified (pH ≈ 6) using HCl (0.25 M). Following addition of water and extraction with CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was dissolved in MeOH (3 ml) followed by the addition of Pd/C (5 mg). The mixture was subjected to hydrogen at rt (1 bar) for 1 h. Following removal of the catalyst by filtration, the solution was concentrated in vacuo and the resulting crude product was recrystallized in Et_2O/EtOH. Yield: 40 mg (70%); colorless, hygroscopic crystals, m.p. 177 °C. $[\alpha]_D^{20} = -7.6$ ($c = 1.1$, 3.4 N HCl) [41] 179–180 °C; $[\alpha]_D^{20} = -7.5$ ($c = 1.0$, 3.4 M HCl)). ^{1}H NMR (CD_3OD, 20 °C): δ = 1.70 (dq, $J = 13.0$, 8.7 Hz, 1H, $NCH_2CH_2CH_2$), 1.90–2.13 (m, 4H, CH_2CH_2COO, $NCH_2CH_2CH_2$), 2.16–2.26 (m, 1H, $NCH_2CH_2CH_2$), 2.38–2.52 (m, 2H, CH_2COO), 3.26–3.33 (m, 2H, NCH_2), 3.54–3.64 (m, 1H, NCH).

5.1.17. (R)-3-(pyrrolidin-1-yl)propionic acid [(R)-5a]

According to the procedure described for compound (*S*)-**5a** starting from (*R*)-**18** (87 mg, 0.316 mmol). Yield: 35 mg (77%); colorless crystals, m.p. 176 °C. Analytical data (^{1}H NMR, IR, MS) were in accordance with those of (*S*)-enantiomer (*S*)-**5a**.

5.1.18. (S)-3-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl] propionic acid [(S)-5b]

According to GP2 starting from compound (*S*)-**26b** (122 mg, 0.336 mmol) and NaOH (56 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 88 mg (75%); colorless oil. $[\alpha]_D^{20} = -20.4$ ($c = 1.10$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.61–1.72 (m, 1H, NCH_2CH_2), 1.72–1.90 (m, 4H, CH_2CH_2COO, $NCH_2CH_2CH_2$), 1.90–2.02 (m, 1H, $NCHCH_2$), 2.32–2.59 (m, 6H, $=CCH_2CH_2N$, NCH_2, CH_2COO), 2.85–2.94 (m, 1H, NCH), 3.05 (td, J =11.0, 5.0 Hz, 1H, $=CCH_2CH_2N$), 3.18–3.25 (m, 1H, NCH_2), 6.03 (t, $J = 7.0$ Hz, 1H, =CH), 7.12–7.40 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3424$ cm^{-1}, 3053, 2958, 2788, 1700, 1576. MS (70 eV); *m/z* (%): 350 (1) $[M + 1]^+$, 276 (1), 206 (1), 156 (100), 126 (5). MS (CI, CH_5^+); *m/z* (%): 351 (26) [M + 1], 350 (100), 348 (4), 156 (51). $C_{23}H_{27}NO_2$ (349.48).

5.1.19. (R)-3-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl] propionic acid [(R)-5b]

According to GP2 starting from compound (*R*)-**26b** (107 mg, 0.336 mmol) and NaOH (49 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 76 mg (74%); colorless oil. Analytical data (^{1}H NMR, IR, MS) were in accordance

with those of the (*S*)-enantiomer (*S*)-**5b**. $[\alpha]_D^{20} = +19.5$ ($c = 0.87$, $CHCl_3$). $C_{23}H_{27}NO_2$ (349.48).

*5.1.20. (S)-3-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en1-1-yl] pyrrolidin-2-yl}propionic acid [(S)-**5c**]*

According to GP2 starting from compound (*S*)-**26c** (100 mg, 0.248 mmol) and NaOH (41 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 68 mg (71%); colorless oil. $[\alpha]_D^{20} = -17.0$ ($c = 0.73$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.70–1.85 (m, 2H, $NCH_2CH_2CH_2$), 1.85–2.10 (m, 4H, CH_2CH_2COO, $NCH_2CH_2CH_2$), 1.96 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.40–2.75 (m, 5H, $=CCH_2CH_2N$, NCH_2, CH_2COO), 2.69 (td, J =11.3, 5.0 Hz, 1H, $=CCH_2CH_2N$), 3.03–3.15 (m, 2H, $=CCH_2CH_2N$, NCHC), 3.28–3.38 (m, 1H, NCH_2), 5.96 (t, J = 7.3 Hz, 1H, =CH), 6.74 (d, J = 4.4 Hz, 1H, SC=CH), 6.85 (d, J = 4.4 Hz, 1H, SC=CH), 7.05 (d, J = 4.4 Hz, 1H, SCH=), 7.22 (d, J = 4.4 Hz, 1H, SCH=). IR (KBr): $\tilde{\nu}$ = 3423 cm^{-1}, 3057, 2953, 2868, 2788, 1711, 1577. MS (CI, CH_5^+); *m/z* (%): 390 (50) $[M + 1]^+$, 350 (7), 292 (1), 264 (7), 218 (2), 156 (100), 130 (14). $C_{21}H_{27}NO_2S_2$ (389.58).

*5.1.21. (R)-3-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl] pyrrolidin-2-yl}propionic acid [(R)-**5c**]*

According to GP2 starting from compound (*R*)-**26c** (170 mg, 0.421 mmol) and NaOH (70 μl, 12 M); reaction time: 5 h. Purification by CC (EtOH). Yield: 112 mg (68%); colorless oil. Analytical data (^{1}H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**5c**. $[\alpha]_D^{20} = +17.3$ ($c = 0.91$, $CHCl_3$). $C_{21}H_{27}NO_2S_2$ (389.58).

*5.1.22. (S)-3-(1-{2-tris-(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)propionic acid [(S)-**5d**]*

According to GP2 starting from compound (*S*)-**26d** (93 mg, 0.174 mmol) and NaOH (29 μl, 12 M); reaction time: 5 h. Purification by recrystallization in EtOH/*n*-pentane (1:1). Yield: 73 mg (81%); colorless crystals, m.p. 65–73 °C (decomp.). $[\alpha]_D^{20} = -4.4$ ($c = 0.51$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.73–2.07 (m, 6H, CH_2CH_2COO, $NCH_2CH_2CH_2$), 2.36–2.46 (m, 1H, CH_2COO), 2.50–2.60 (m, 1H, CH_2COO), 2.72–3.81 (m, 2H, OCH_2CH_2N, NCH_2), 3.03–3.12 (m, 1H, NCH), 3.18–3.28 (m, 1H, OCH_2CH_2N), 3.40–3.50 (m, 1H, NCH_2), 3.49–3.61 (m, 2H, OCH_2CH_2N), 3.70 (s, 9H, OCH_3), 6.78–6.86 (m, 6H, $H_{aromat.}$), 7.23–7.35 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1}, 3036, 1607, 1582, 1508. MS (70 eV); *m/z* (%): 334 (76), 333 (100), 318 (12), 303 (33), 227 (27), 156 (17), 114 (13). MS (CI, CH_5^+); *m/z* (%): 518 (1), 334 (38), 227 (82), 188 (100), 170 (30). $C_{31}H_{37}NO_6$ (519.64).

*5.1.23. (R)-3-(1-{2-tris-(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)propionic acid [(R)-**5d**]*

According to GP2 starting from compound (*R*)-**26c** (143 mg, 0.268 mmol) and NaOH (45 μl, 12 M); reaction time: 5 h. Purification by recrystallization in EtOH/*n*-pentane (1:1). Yield: 115 mg (83%) colorless crystals, m.p. 64–72 °C (decomp.). Analytical data (^{1}H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**5d**. $[\alpha]_D^{20} = +4.1$ ($c = 0.50$, $CHCl_3$). $C_{31}H_{37}NO_6$ (519.64).

*5.1.24. (E)-(S)-3-(1-{2-[tris-(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acrylic acid [(S)-**6d**]*

According to GP2 starting from compound (*S*)-**28d** (157 mg, 0.296 mmol) and NaOH (49 μl, 12 M); reaction time: 5 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 120 mg (79%); colorless crystals, m.p.: 78–86 °C (decomp.). $[\alpha]_D^{20} = -15.6$ ($c = 0.82$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.67–1.80 (m, 2H, $NCH_2CH_2CH_2$), 1.85–1.98 (m, 2H, $NCH_2CH_2CH_2$), 2.49–2.59 (m, 2H, OCH_2CH_2N, NCH_2), 3.04–3.18 (m, 2H, OCH_2CH_2N, NCH_2), 3.23–3.37 (m, 2H, OCH_2CH_2N), 3.60–3.68 (m, 1H, NCHC), 3.70 (s, 9H, OCH_3), 5.85 (d, J = 15.2 Hz, 1H, =CHCOO), 6.67 (dd, J = 15.2, 8.9 Hz, 1H, CH=CHCOO), 6.71–6.76 (m, 6H, $H_{aromat.}$), 7.21–7.25 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1}, 3036, 2953, 2835, 1705, 1658, 1607, 1582. MS (70 eV); *m/z* (%): 492 (1), 378 (1), 333 (100), 303 (43), 227 (53),182 (22), 149 (13). $C_{31}H_{35}NO_6$ (517.63).

*5.1.25. (E)-(R)-3-(1-{2-[tris-(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acrylic acid [(R)-**6d**]*

According to GP2 starting from compound (*R*)-**28d** (202 mg, 0.38 mmol) and NaOH (63 μl, 12 M); reaction time: 5 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 156 mg (79%); colorless crystals, m.p.: 78–86 °C (decomp.). Analytical data (^{1}H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**6d**. $[\alpha]_D^{20} = +16.2$ ($c = 3.31$, $CHCl_3$). $C_{31}H_{35}NO_6$ (517.63).

*5.1.26. (Z)-(S)-3(-1-{2-[tris-(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acrylic acid [(S)-**7d**]*

According to GP2 starting from compound (*S*)-**29** (85 mg, 0.16 mmol) and NaOH (27 μl, 12 M); reaction time: 23 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 61 mg (74%); colorless crystals, m.p.: 100–105 °C (decomp.). $[\alpha]_D^{20} = -12.7$ ($c = 1.30$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.82–1.95 (m, 2H, $NCH_2CH_2CH_2$), 1.98–2.10 (m, 1H, NCH_2CH_2), 2.18–2.27 (m, 1H, $NCHCH_2$), 2.59–2.71 (m, 2H, NCH_2, OCH_2CH_2N), 3.06 (dt, J = 13.2, 5.5 Hz, 1H, NCH_2), 3.39–3.50 (m, 4H, OCH_2CH_2N, NCH_2, NCH), 3.79 (s, 9H, OCH_3), 5.99–6.09 (m, 2H, CH=CH), 6.78–6.85 (m, 6H, $H_{aromat.}$), 7.24–7.32 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 3437 cm^{-1}, 3040, 2953, 2835, 1702, 1607, 1582. MS (CI, CH_5^+); *m/z* (%): 335 (17), 334 (44), 333 (32), 319 (8), 227 (100), 186 (56), 168 (37). $C_{31}H_{35}NO_6$ (517.63).

*5.1.27. (Z)-(R)-3-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidin-2-yl)acrylic acid [(R)-**7d**]*

According to GP2 starting from compound (*R*)-**29d** (91 mg, 0.17 mmol) and NaOH (29 μl, 12 M); reaction time: 23 h. Purification by recrystallization in Et_2O/*n*-pentane (1:1). Yield: 65 mg (73%); colorless crystals, m.p.: 100–105 °C (decomp.). $[\alpha]_D^{20} = +13.4$ ($c = 1.57$, $CHCl_3$). $C_{31}H_{35}NO_6$ (517.63).

*5.1.28. (1S,5R)-1-(2,5-dihydropyrrol-1-ylcarbonyl)-5,8,8-trimethyl-3-oxabicyclo[3.2.1]octan-2-one (**10**)*

A solution of carboxylic acid **8** (3.79 g, 14.4 mmol) in CH_2Cl_2 (60 ml) was cooled to 0 °C. Oxalyl chloride (1.78 g, 14.5 mmol) and DMF (15 μl) were added followed by the removal of the ice bath. After bubbling had stopped, the reaction mixture was flushed with nitrogen for one hour to remove excess of HCl. The reaction mixture was cooled to 0 °C followed by the addition of NEt_3 (3.54 g, 2.5 equiv.) and 3-pyrroline (9.1 g, 14.4 mmol). The reaction mixture was warmed to rt and stirred for 12 h. CH_2Cl_2 (30 ml) was added and the organic layers were extracted with HCl (0.5 M), dried ($MgSO_4$), and concentrated in vacuo. Purification by CC (petrolether/EtOAc = 7:3) and recrystallization from petrolether/EtOAc (7:3). Yield: 3.254 g (86%); colorless crystals, m.p. 108 °C. $[\alpha]_D^{20} = +97.9$ ($c = 0.65$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 0 °C): δ = 0.91 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.86–1.97 (m, 2H, CH_2CH_2), 2.27 (dt, $J = 11.0$, 5.1 Hz, 1H, CH_2CH_2), 2.43 (ddd, $J = 13.9$, 11.0, 5.9 Hz, 1H, CH_2CH_2), 3.94 (d, $J = 11.3$ Hz, 1H, CH_2O), 4.15 (dd, $J = 11.3$, 2.2 Hz, 1H, CH_2O), 4.12–4.18 (m, 1H, NCH_2), 4.23 (ddd, $J = 13.9$, 5, 2.2 Hz, 1H, NCH_2), 4.39 (ddd, $J = 13.9$, 5, 2.2 Hz, 1H, NCH_2), 4.56 (ddd, $J = 16.9$, 5, 2.2 Hz, 1H, NCH_2), 5.75 (ddd, $J = 6.6$, 5, 2.2 Hz, 1H, HC=), 5.85 (ddd, $J = 6.6$, 5, 2.2 Hz, 1H, HC=). IR (KBr): $\tilde{\nu} = 2960$ cm^{-1}, 2862, 1724, 1644, 1622. MS (70 eV); *m/z* (%): 264 (1) $[M + 1]^+$, 235 (1), 195 (1), 168 (1), 139 (3), 123 (3), 95 (4), 81 (11), 68 (100). $C_{15}H_{21}NO_3$ (263.34).

*5.1.29. (1S,5R)-1-(2,3-dihydropyrrol-1-ylcarbonyl)-5,8,8-trimethyl-3-oxabicyclo[3.2.1]octan-2-one (**11**)*

A mixture of compound **10** (1.0 g, 3.8 mmol) and hydridotetrakis(triphenylphosphine)rhodium(I) (15 mg) in xylene (4 ml) was stirred in a sealed tube at 140 °C for 44 h. The reaction mixture was filtrated and the filtrate was concentrated in vacuo. Purification by CC (petrolether/EtOAc/ethyldimethylamine = 80:20:1) and recrystallization from cyclohexene. Yield: 700 mg (70%); colorless crystals, m.p. 105 °C. $[\alpha]_D^{20} = +46.3$ ($c = 0.68$, $CHCl_3$). ^{1}H NMR (nitrobenzene-d_5, 130 °C): δ = 0.91 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.80–2.00 (m, 2H, CH_2CH_2), 2.18–2.33 (m, 1H, CH_2CH_2), 2.45–2.70 (m, 3H, NCH_2CH_2, CH_2CH_2), 3.89–3.96 (m, 2H, NCH_2), 3.99 (d, $J = 11.0$ Hz, 1H, CH_2O), 4.19 (dd, $J = 11.0$, 2.2 Hz, 1H, CH_2O), 5.11–5.20 (m, 1H, NCH=C*H*), 6.71–6.79 (m, 1H, NCH=). IR (KBr): $\tilde{\nu} = 2973$ cm^{-1}, 2917, 2890, 1731, 1633, 1612. MS (70 eV): *m/z* (%): 263 (40) $[M + 1]^+$, 247 (13), 195 (60), 167 (37), 139 (69), 121 (27), 81 (79), 67 (100). $C_{15}H_{21}NO_3$ (263.34).

*5.1.30. 1,1-Dimethylethyl {(2S)-1-[(1S,5R)-5,8,8-trimethyl-2-oxo-3-oxabicyclo[3.2.1]octan-1-ylcarbonyl]pyrrolidin-2-yl} acetate [(S)-**14**] and 1,1-dimethylethyl {(2R)-1-[(1S,5R)-5,8,8-trimethyl-2-oxo-3-oxabicyclo[3.2.1]octan-1-ylcarbonyl] pyrrolidin-2-yl}acetate} [(R)-**14**]*

Synthesis of the organometallic reagent (**13**): *n*-butyllithium (1.5 ml, 2.4 mmol, 1.6 M in hexane) was added to a solution of diisopropylamine (315 μl, 2.4 mmol) in THF (2.4 ml) at –78 °C. After 30 min *tert*-butyl acetate (320 μl, 2.4 mmol) was added and the reaction mixture was stirred for 40 min allowing it to warm to –30 °C. Following addition of diethylaluminum chloride (2.4 ml, 2.4 mmol, 1 M in hexane), the reaction mixture was stirred for additional 20 min.

Electrophilic α-amidoalkylation: Anhydrous CH_2Cl_2 (1 ml per 0.1 mmol of **11**) was saturated with gaseous HCl for 20 min at –85 °C using a gas-tight syringe. Enamide **11** (0.158 g, 0.6 mmol, 0.2 M in CH_2Cl_2) was added dropwise under vigorous stirring. Concurrently, the treatment of the solution with gaseous HCl was continued for another 10–20 min followed by the removal of excess of HCl in vacuo (for 1 h at –78 °C). The organometallic reagent **13** (6.6 ml, 4 equiv.) was added dropwise and the reaction mixture was stirred for 16 h. The reaction was quenched with water and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were extracted with brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (petrolether/EtOAc = 7:3). Yield: 203 mg (89%) (diastereomeric mixture of (*S*)-**14** and (*R*)-**14**). HPLC (*n*-heptane/EtOAc = 80:20; 1.5 ml min^{-1}); (*S*)-**14**: $t_R = 16.1$ min, 81.2%; (*R*)-**14**: $t_R = 20.4$ min, 18.8%. Separation of the diastereomers by prep. HPLC (*n*-heptane/EtOAc = 82:18; 13.5 ml min^{-1}; (*S*)-**14**: $t_R = 33.8$ min; (*R*)-**14**: $t_R = 43.6$ min).

(*S*)-**14**: Yield: 154 mg (67%); colorless crystals, m.p. 135 °C. $[\alpha]_D^{20} = +18.2$ ($c = 1.07$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 0.88 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.43 (s, 9H, $C(CH_3)_3$), 1.63–1.69 (m, 1H, $NCH_2CH_2CH_2$), 1.74–1.96 (m, 4H, $NCH_2CH_2CH_2$, CH_2CH_2), 2.14–2.24 (m, 2H, CH_2CH_2), 2.31–2.43 (m, 2H, CH_2CH_2, CH_2COO), 2.85 (dd, $J = 15.5$, 3.8 Hz, 1H, CH_2COO), 3.21 (td, $J = 9.5$, 6.5 Hz, 1H, NCH_2), 3.72 (ddd, $J = 9.5$, 7.3, 2.4 Hz, 1H, NCH_2), 3.91 (d, $J = 11.0$ Hz, 1H, $CH_2OC=O$), 4.12 (dd, $J = 11.0$, 2.2 Hz, 1H $CH_2OC=O$), 4.42 (qd, $J = 8.1$, 3.8 Hz, 1H, NCHC). IR (KBr): $\tilde{\nu} = 2980$ cm^{-1}, 2870, 1720, 1626. MS (CI, CH_5^+); *m/z* (%): 380 (10) $[M + 1]^+$, 360 (7), 324 (100), 184 (4), 128 (3). $C_{21}H_{33}NO_5$ (379.50).

(*R*)-**14**: Yield: 36 mg (16%); colorless crystals, m.p.: 89 °C. $[\alpha]_D^{20} = +56.6$ ($c = 1.1$, $CHCl_3$). ^{1}H NMR (nitrobenzene-d5, 140 °C): δ = 0.88 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.51 (s, 9H, $C(CH_3)_3$), 1.82–2.06 (m, 6H, $NCH_2CH_2CH_2$, CH_2CH_2), 2.26 (ddd, $J = 15.0$, 10.0, 5.6 Hz, 1H, CH_2CH_2), 2.35 (dd, $J = 15.6$, 9.3 Hz, 1H, CH_2COO), 2.61–2.72 (m, 1H, CH_2CH_2), 3.18 (dd, $J = 15.6$, 3.7 Hz, 1H, CH_2COO), 3.43–3.50 (m, 1H, NCH_2), 3.62 (dt, $J = 10.6$, 6.9 Hz, 1H, NCH_2), 3.92 (d, $J = 11.1$ Hz, 1H, $CH_2OC=O$), 4.17 (dd, $J = 11.1$, 2.0 Hz, 1H, $CH_2OC=O$), 4.60–4.67 (m, 1H, NCHC). IR (KBr): $\tilde{\nu} = 2976$ cm^{-1}, 2880, 1729, 1628. MS (CI, CH_5^+); *m/z* (%): 380 (10) $[M + 1]^+$, 360 (4), 324 (100), 184 (3), 128 (3). $C_{21}H_{33}NO_5$ (379.50).

*5.1.31. Methyl {(E)-(S)-3-[1-(benzyloxycarbonyl)pyrrolidin-2-yl]acrylate} and methyl {(Z)-(S)-3-[1-(benzyloxycarbonyl) pyrrolidin-2-yl]acrylate} [(S)-**18**]*

DIBAH (18 ml, 1 M in hexane) was added dropwise over 15 min to compound (*S*)-**15** (2.346 g, 8.92 mmol) in toluene

(50 ml) at –60 °C. After 1 h the reaction was quenched with CH_3OH (2 ml) and the mixture was allowed to warm to rt Following addition of 1 M HCl and Et_2O, the aqueous layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The resulting oily residue (2.07 g) was dissolved in CH_3CN (35 ml). Following addition of LiCl (454 mg, 10.7 mmol, 1.2 equiv.) and DIPEA (1.86 ml, 10.7 mmol, 1.2 equiv.), trimethylphosphonoacetate (**17**) (1.73 ml, 10.7 mmol, 1.2 equiv.) was added to the reaction mixture. After 16 h the organic solvent was removed in vacuo followed by the addition of Et_2O and water. The aqueous layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (petrolether/EtOAc = 7:3). Yield: 1.88 g (73%); colorless oil.

5.1.32. Methyl {(E)-(R)-3-[1-(benzyloxycarbonyl)pyrrolidin-2-yl]acrylate} and methyl {(Z)-(R)-3-[1-(benzyloxycarbonyl) pyrrolidin-2-yl]acrylate} [(R)-**18**]

According to the procedure described for compound (*S*)-**18** starting from compound (*R*)-**15** (1.85 mmol, 7.03 mmol). Yield: 1.48 g (73%); colorless oil.

5.1.33. (S)-2-pyrrolidin-2-ylacetic acid methyl ester hydrochloride [(S)-**20**]

Thionylchloride (0.3 ml, 4.2 mmol) was added dropwise to MeOH (1.2 ml) at 0 °C followed by the addition of (*S*)-**4a** (173 mg, 1.05 mmol). The reaction mixture was allowed to warm to rt and stirred for 24 h followed by concentration in vacuo. Yield: 176 mg (94%); colorless crystals. m.p. 53 °C. $[\alpha]_D^{20} = +3.3$ ($c = 1.2$, $CHCl_3$). MS (70 eV); *m*/*z* (%): 143 (33) $[M + 1]^+$, 128 (29), 115 (53), 110 (100).

5.1.34. Methyl (S)-1-(4,4-diphenylbut-3-en-1-yl)pyrrolidine-2-carboxylate [(S)-**24b**]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-carboxylate hydrochloride (*S*)-**23** (497 mg, 3 mmol), KI (49.8 mg, 0.3 mmol), K_2CO_3 (829 mg, 6 mmol), and diphenylbut-3-en-1-yl bromide [20] (861 mg, 3 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 527 mg (52%); colorless oil. $[\alpha]_D^{20} = -35.7$ ($c = 2.79$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.70–1.85 (m, 1H, NCH_2CH_2), 1.85–1.96 (m, 2H, $NCH_2CH_2CH_2$), 2.02–2.14 (m, 1H, $NCHCH_2$), 2.27–2.37 (m, 3H, NCH_2, $=CCH_2CH_2N$), 2.49–2.57 (m, 1H, $=CCH_2CH_2N$), 2.81 (dt, $J = 11.5$, 8.1 Hz, 1H, $=CCH_2CH_2N$), 3.11 (td, $J = 8.1$, 3.0 Hz, 1H, NCH_2), 3.16 (dd, $J = 8.7$, 5.9 Hz, 1H, NCHCOO), 3.68 (s, 3H, OCH_3), 6.08 (t, $J = 7.4$ Hz, 1H, =CH), 7.15–7.40 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 2950$ cm^{-1}, 2801, 1748, 1733, 1598. MS (70 eV); *m*/*z* (%): 335 (1), 276 (4), 142 (100), 114 (26). $C_{22}H_{25}NO_2$ (335.45).

5.1.35. Methyl (R)-1-(4,4-diphenylbut-3-en-1-yl)pyrrolidine-2-carboxylate [(R)-**24b**]

According to GP1 (A) starting from methyl (*R*)-pyrrolidine-2-carboxylate hydrochloride (*R*)-**23** (359 mg, 2.17 mmol), KI (36 mg, 0.217 mmol), K_2CO_3 (600 mg, 4.34 mmol), and diphenylbut-3-en-1-yl bromide [20] (623 mg, 2.17 mmol); reaction time: 48 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 350 mg (48%); colorless oil. Analytical data (^{1}H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**24b**. $[\alpha]_D^{20} = +34.9$ ($c = 1.72$, $CHCl_3$). $C_{22}H_{25}NO_2$ (335.45).

5.1.36. Methyl (S)-1-[4,4-bis(3-methylthiophen-2-yl] pyrrolidine-2-carboxylate [(S)-**24c**]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-carboxylate hydrochloride (*S*)-**23** (2.48, 15.0 mmol), KI (166 mg, 1.0 mmol), K_2CO_3 (5.18 g, 37.5 mmol), and 4,4-bis (3-methyl-2-thiophen-2-yl)-but-3-en-1-yl bromide (3.273 g, 10.0 mmol); reaction time: 7 d. Purification by CC (pentane/EtOAc = 8:2). Yield: 2.3 g (63%); colorless oil. $[\alpha]_D^{20} = -52.0$ ($c = 0.51$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.73–1.85 (m, 1H, NCH_2CH_2), 1.85–1.98 (m, 2H, $NCH_2CH_2CH_2$), 2.00 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.98–2.15 (m, 1H, $NCHCCH_2$), 2.28–2.40 (m, 3H, NCH_2, $=CCH_2CH_2N$), 2.47–2.55 (m, 1H,CCH_2CH_2N), 2.81 (ddd, $J = 11.6$, 8.8, 7.6 Hz, 1H, $=CCH_2CH_2N$), 3.08–3.17 (m, 2H, NCH_2), NCHCOO), 3.69 (s, 3H, OCH_3), 6.05 (t, $J = 7.3$ Hz, 1H, =CH), 6.75 (d, $J = 5.1$ Hz, 1H, SCH=CH), 6.83 (d, $J = 5.1$ Hz, SC=CH), 7.04 (d, $J = 5.1$ Hz, 1H, SCH =), 7.20 (d, $J = 5.1$ Hz, 1H, SCH=). IR (film): $\tilde{\nu} = 2950$ cm^{-1}, 2801, 1748, 1732, 1455, 1434. MS (CI, CH_5^+); *m*/*z* (%): 376 (23), $[M + 1]^+$, 142 (100). $C_{21}H_{25}NO_2S_2$ (375.56).

5.1.37. Methyl (S)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidine-2-carboxylate [(S)-**24d**]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-carboxylate hydrochloride (*S*)-**23** (248 mg, 1.5 mmol), KI (24.9 mg, 0.15 mmol), K_2CO_3 (415 mg, 3 mmol), and 2-[tris (4-methoxyphenyl)methoxy]ethyl bromide [24] (686 mg, 1.5 mmol); reaction time: 40 h. Purification by CC (Et_2O/petrolether = 7:3). Yield: 285 mg (38%); colorless crystals. $[\alpha]_D^{20} = -29.6$ ($c = 1.05$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.73–1.81 (m, 1H, NCH_2CH_2), 1.81–1.93 (m, 2H, $NCH_2CH_2CH_2$), 2.02–2.14 (m, 1H, $NCHCH_2$), 2.43 (q, $J = 8.7$ Hz, 1H, NCH_2), 2.73 (dt, $J = 12.6$, 6.3 Hz, 1H, OCH_2CH_2N), 2.95 (dt, $J = 12.6$, 6.3 Hz, 1H, OCH_2CH_2N), 3.09–3.16 (m, 1H, NCH_2), 3.21 (t, $J = 6.3$ Hz, 2H, OCH_2CH_2N), 3.26 (dd, $J = 8.9$, 5.8 Hz, 1H, NCHCOO), 3.65 (s, 3H, $COOCH_3$), 3.78 (s, 9H, OCH_3), 6.79–6.83 (m, 6H, $H_{aromat.}$), 7.29–7.34 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 2950$ cm^{-1}, 2835, 1733, 1608, 1582. MS (70 eV); *m*/*z* (%): 504 (1), 446 (2), 333 (100), 259 (2), 172 (12), 142 (93), 114 (14). MS (CI, CH_5^+); *m*/*z* (%): 446 (1), 335 (4), 334 (24), 333 (100), 227 (8), 174 (10), 142 (15). $C_{30}H_{35}NO_6$ (505.62).

5.1.38. Methyl (R)-1-{2-[tris(4-methoxyphenyl)methoxy]ethyl} pyrrolidine-2-carboxylate [(R)-**24d**]

According to GP1 (A) starting from methyl (*R*)-pyrrolidine-2-carboxylate hydrochloride (*R*)-**23** (331 mg, 2.0 mmol), KI (33.2 mg, 0.2 mmol), K_2CO_3 (553 mg, 4.0 mmol), and 2-[tris (4-methoxyphenyl)methoxy]ethyl bromide [24] (914 mg,

2.0 mmol); reaction time: 40 h. Purification by CC (Et_2O/petrolether = 7:3). Yield: 395 mg (39%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**24**. $[\alpha]_D^{20} = +30.5$ ($c = 1.75$, $CHCl_3$). $C_{30}H_{35}NO_6$ (505.62).

5.1.39. Methyl (S)-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]acetate [(S)-25b]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-acetate hydrochloride (*S*)-**20** (176 mg, 0.983 mmol), KI (16.6 mg, 0.1 mmol), K_2CO_3 (304 mg, 2.2 mmol), and 4,4-diphenylbut-3-en-1-yl bromide [20] (287 mg, 1.0 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 218 mg (64%); colorless oil. $[\alpha]_D^{20} = -62.5$ ($c = 3.35$, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.49–1.59 (m, 1H, NCHCH_2), 1.62–1.80 (m, 2H, NCH_2CH_2), 1.94–2.05 (m, 1H, NCHCH_2), 2.11 (dt, $J = 9$, 8.2 Hz, 1H, NCH_2), 2.26 (dd, $J = 14.9$, 9.0 Hz, 1H, CH_2COO), 2.28–2.35 (m, 3H, =CCH_2CH_2N), 2.60 (dd, $J = 14.9$, 4.3 Hz, 1H, CH_2COO), 2.70–2.79 (m, 1H, NCH), 2.80–2.88 (m, 1H, =CCH_2CH_2N), 3.02 (ddd, $J = 9$, 7.5, 3.1 Hz, 1H, NCH_2), 3.64 (s, 3H, OCH_3), 6.10 (t, $J = 7.0$ Hz, 1H, =CH), 7.16–7.40 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3022$ cm^{-1}, 2950, 2795, 1738, 1598. MS (70 eV); *m*/*z* (%): 348 (1), 292 (26), 276 (7), 247 (3), 156 (100). $C_{23}H_{27}NO_2$ (349.48).

5.1.40. Methyl (R)-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]acetate [(R)-25b]

According to GP1 (B) starting from methyl (*R*)-1-benzyloxycarbonylpyrrolidine-2-acetate (*R*)-**21** (227 mg, 0.82 mmol), KI (13.6 mg, 0.082 mmol), K_2CO_3 (113 mg, 0.82 mmol), and 4,4-diphenylbut-3-en-1-yl bromide [20] (235 mg, 0.82 mmol); reaction time: 47 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 175 mg (61%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**25**). $[\alpha]_D^{20} = +61.2$ ($c = 1.49$, $CHCl_3$). $C_{23}H_{27}NO_2$ (349.48).

5.1.41. Methyl (S)-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidin-2-yl}acetate [(S)-25c]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-acetate hydrochloride (*S*)-**20** (179 mg, 1.0 mmol), KI (16.6 mg, 0.1 mmol), K_2CO_3 (276 mg, 2.0 mmol), and 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl bromide [19] (327 mg, 1.0 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 159 mg (41%); colorless oil. $[\alpha]_D^{20} = -60.2$ ($c = 0.99$, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.49–1.57 (m, 1H, NCH_2CH_2), 1.67–1.76 (m, 2H, $NCH_2CH_2CH_2$), 1.96–2.03 (m, 1H, NCHCH_2), 2.02 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.13 (td, $J = 10$, 8.4 Hz, 1H, NCH_2), 2.25 (dd, $J = 14.8$, 8.8 Hz, 1H, CH_2COO), 2.28–2.36 (m, 3H, =CCH_2CH_2N), 2.62 (dd, $J = 14.8$, 4.4 Hz, 1H, CH_2COO), 2.70–2.78 (m, 1H, NCH), 2.81–2.89 (m, 1H, =CCH_2CH_2N), 3.04 (ddd, $J = 10$, 7.4, 3.3 Hz, 1H, NCH_2), 3.66 (s, 3H, OCH_3), 6.06 (t, $J = 7.0$ Hz, 1H, =CH), 6.77 (d, $J = 5.2$ Hz, 1H, SC=CH), 6.84 (d, $J = 5.2$ Hz, 1H, SC=CH), 7.06 (d, $J = 5.2$ Hz, 1H, SCH=), 7.21 (d, $J = 5.2$ Hz, 1H, SCH=). IR (KBr): $\tilde{\nu} = 2948$ cm^{-1}, 2794, 1737. MS (CI, CH_5^+); *m*/*z* (%): 390 (34) $[M + 1]^+$, 350 (11), 316 (16), 276 (5), 194 (5), 156 (100). $C_{21}H_{27}NO_2S_2$ (389.58).

5.1.42. Methyl (R)-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidin-2-yl}acetate [(R)-25c]

According to GP1 (B) starting from methyl (*R*)-1-benzyloxycarbonylpyrrolidine-2-acetate (*R*)-**21** (277 mg, 1.0 mmol), KI (16.6 mg, 1.0 mmol), K_2CO_3 (138 mg, 1.0 mmol), and 4,4-bis (3-methylthiophen-2-yl)but-3-en-1-yl bromide [19] (327 mg, 1.0 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 7:3). Yield: 161 mg (41.3%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**25c**. $[\alpha]_D^{20} = +61.3$ ($c = 1.04$, $CHCl_3$). $C_{21}H_{27}NO_2S_2$ (389.58).

5.1.43. Methyl (S)-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-yl)acetate [(S)-25d]

According to GP1 (A) starting from methyl (*S*)-pyrrolidine-2-acetate hydrochloride (*S*)-**20** (215 mg, 1.2 mmol), KI (19.9 mg, 0.12 mmol), K_2CO_3 (332 mg, 2.4 mmol), and 2-[tris(4-methoxyphenyl)methoxy]ethyl bromide [24] (548 mg, 1.0 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 20:80). Yield: 220 mg (35%); colorless oil. $[\alpha]_D^{20} = -27.6$ ($c = 1.77$, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.49–1.59 (m, 1H, NCH_2CH_2), 1.68–1.80 (m, 2H, $NCH_2CH_2CH_2$), 1.97–2.07 (m, 1H, NCHCH_2), 2.21–2.31 (m, 2H, CH_2COO, NCH_2), 2.51 (dt, $J = 12.5$, 6.5 Hz, 1H, OCH_2CH_2N), 2.65 (dd, $J = 15.1$, 4.0 Hz, 1H, CH_2COO), 2.79–2.87 (m, 1H, NCH), 2.97 (dt, $J = 12.5$, 6.5 Hz, 1H, OCH_2CH_2N), 3.06 (ddd, $J = 10.4$, 7.1, 3.5 Hz, 1H, NCH_2), 3.20 (m, 2H, OCH_2CH_2N), 3.67 (s, 3H, $COOCH_3$), 3.81 (s, 9H, OCH_3), 6.81–6.87 (m, 6H, $H_{aromat.}$), 7.34–7.39 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 2951$ cm^{-1}, 2835, 1737, 1608, 1508. MS (CI, CH_5^+); *m*/*z* (%): 520 (1) $[M + 1]^+$, 448 (1), 446 (4), 391 (2), 333 (100), 227 (11), 188 (17), 170 (12), 156 (25). $C_{31}H_{37}NO_6$ (519.64).

5.1.44. Methyl (R)-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-yl)acetate [(R)-25d]

According to GP1 (B) starting from methyl (*R*)-1-benzyloxycarbonylpyrrolidine-2-acetate (*R*)-**21** (227 mg, 0.82 mmol), KI (13.6 mg, 0.082 mmol), K_2CO_3 (113 mg, 0.82 mmol), and 2-[tris(4-methoxyphenyl)methoxy]ethyl bromide [24] (375 mg, 0.82 mmol); reaction time: 45 h. Purification by CC (petrolether/EtOAc = 20:80). Yield: 175 mg (41%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**25c**. $[\alpha]_D^{20} = +26.7$ ($c = 1.5$, $CHCl_3$). $C_{31}H_{37}NO_6$ (519.64).

5.1.45. Methyl (S)-3-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]propionate [(S)-26b]

According to GP1 (B) starting from compound (*S*)-**18** (269 mg, 0.93 mmol), KI (15.4 mg, 0.093 mmol), K_2CO_3 (128 mg, 0.93 mmol), and 4,4-diphenylbut-3-en-1-yl bromide [20] (267 mg, 0.93 mmol); reaction time: 48 h. Purification by

CC (*n*-hexane/Et_2O = 7:3). Yield: 142 mg (42%); colorless oil. $[\alpha]_D^{20}$ = −63.9 (*c* = 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.30–1.39 (m, 1H, NCHCH_2), 1.47–1.70 (m, 3H, CH_2CH_2COO, NCH_2CH_2), 1.75–1.83 (m, 1H, NCHCH_2), 1.83–1.93 (m, 1H, CH_2CH_2COO), 1.99 (td, *J* = 9.0, 8.2 Hz, 1H, NCH_2), 2.11–2.28 (m, 5H, =CCH_2CH_2N, CH_2COO, NCH), 2.33 (ddd, *J* = 15.6, 9.5, 5.9 Hz, 1H, CH_2COO), 2.84 (ddd, *J* = 15.3, 8.5, 6.1 Hz, 1H, =CCH_2CH_2N), 2.97 (ddd, *J* = 9.0, 7.4, 2.9 Hz, 1H, NCH_2), 3.59 (s, 3H, OCH_3), 6.05 (t, *J* = 7.3 Hz, 1H, =CH), 7.10–7.33 (m, 10H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 3019 cm^{-1}, 2953, 2789, 1737, 1598. MS (CI, NH_4^+); *m/z* (%): 364 (100) $[M + 1]^+$, 332 (1), 244 (1), 186 (8), 170 (42), 91 (4). $C_{24}H_{29}NO_2$ (363.50).

5.1.46. Methyl (R)-3-[1-(4,4-diphenylbut-3-en-1-yl)pyrrolidin-2-yl]propionate [(R)-**26b**]

According to GP1 (B) starting from compound (*R*)-**18** (231 mg, 0.8 mmol) KI (13.3 mg, 0.08 mmol) K_2CO_3 (111 mg, 0.8 mmol), and 4,4-diphenylbut-3-en-1-yl bromide [20] (230 mg, 0.8 mmol); reaction time: 44 h. Purification by CC (*n*-hexane/Et_2O = 7:3). Yield: 131 mg (45%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**26b**. $[\alpha]_D^{20}$ = +63.55 (*c* = 1.07, $CHCl_3$). $C_{24}H_{29}NO_2$ (363.50).

5.1.47. Methyl (S)-3-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidin-2-yl}propionate [(S)-**26c**]

According to GP1 (B) starting from compound (*S*)-**18** (289 mg, 1.0 mmol), KI (16.6 mg, 0.1 mmol), K_2CO_3 (138 mg, 1.0 mmol), and 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl bromide [19] (327 mg, 1.0 mmol); reaction time: 46 h. Purification by CC (petrolether/EtOAc = 5:5). Yield: 165 mg (41%); colorless oil. $[\alpha]_D^{20}$ = − 6.2 (*c* = 1.05, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.37–1.47 (m, 1H, NCHCCH_2), 1.53–1.78 (m, 3H, CH_2CH_2COO, NCH_2CH_2), 1.82–1.90 (m, 1H, NCHCH_2), 1.91–2.01 (m, 1H, CH_2CH_2COO), 2.03 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.07 (q, *J* = ~9 Hz, 1H, NCH_2), 2.18–2.35 (m, 5H, =CCH_2CH_2N, CH_2COO, NCH), 2.40 (ddd, *J* = 15.6, 9.5, 5.9 Hz, 1H, CH_2COO), 2.91 (dt, *J* = 11.4, 8.1 Hz, 1H, =CCH_2CH_2N), 3.07 (ddd, *J* = 9, 7.5, 3.0 Hz, 1H, NCH_2), 3.68 (s, 3H, OCH_3), 6.09 (t, *J* = 7.3 Hz, 1H, =CH), 6.77 (d, *J* = 5.2 Hz, 1H, SC=CH), 6.85 (d, *J* = 5.2 Hz, 1H, SC=CH), 7.06 (d, *J* = 5.2 Hz, 1H, SCH=), 7.21 (d, *J* = 5.2 Hz, 1H, SCH=). IR (KBr): $\tilde{\nu}$ = 2950 cm^{-1}, 2868, 2788, 1738. MS (CI, CH_5^+); *m/z* (%): 404 (45) $[M + 1]^+$, 364 (11), 290 (4), 194 (3), 170 (100). $C_{22}H_{29}NO_2S_2$ (403.61).

5.1.48. Methyl (R)-3-{1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidin-2-yl}propionate [(R)-**26c**]

According to GP1 (B) starting from compound (*R*)-**18** (474 mg, 1.64 mmol), KI (27.2 mg, 0.16 mmol), K_2CO_3 (227 mg, 1.64 mmol), and 4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl bromide [19] (536 mg, 1.64 mmol); reaction time: 47 h. Purification by CC (petrolether/EtOAc = 5:5). Yield: 260 mg (39%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**26c**. $[\alpha]_D^{20}$ = +6.3 (*c* = 1.18, $CHCl_3$). $C_{22}H_{29}NO_2S_2$ (403.61).

5.1.49. Methyl (S)-3-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-yl)propionate [(S)-**26d**]

According to GP1 (B) starting from compound (*S*)-**18** (289 mg, 1.0 mmol), KI (16.6 mg, 0.1 mmol), K_2CO_3 (138 mg, 1.0 mmol), and 2-[tris(4-methoxyphenyl)methoxy]ethyl bromide [24] (457 mg, 1.0 mmol); reaction time: 48 h. Purification by CC (petrolether/EtOAc = 2:8). Yield: 117 mg (22%); colorless oil. $[\alpha]_D^{20}$= –29.5 (*c* = 1.27, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.36–1.46 (m, 1H, NCH_2CH_2), 1.56–1.64 (m, 1H, CH_2CH_2COO), 1.64–1.73 (m, 2H, NCH_2CH_2CH_2), 1.81–1.89 (m, 1H, NCHCH_2), 1.90–2.00 (m, 1H, CH_2CH_2COO), 2.15 (q, *J* = 9.0 Hz, 1H, NCH_2), 2.21–2.47 (m, 4H, CH_2COO, OCH_2CH_2N, NCH), 2.98–3.10 (m, 2H, OCH_2CH_2N, NCH_2), 3.12–3.25 (m, 2H, OCH_2CH_2N), 3.67 (s, 3H, $COOCH_3$), 3.80 (s, 9H, OCH_3), 6.80–6.85 (m, 6H, $H_{aromat.}$), 7.33–7.37 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu}$ = 2951 cm^{-1}, 2948, 2873, 2835, 1736, 1608, 1583. MS (CI, NH_4^+); *m/z* (%): 534 (1) $[M + 1]^+$, 334 (25), 333 (100), 319 (1), 227 (9), 202 (9), 170 (13). $C_{32}H_{39}NO_6$ (533.67).

5.1.50. Methyl (R)-3-(1-{2-[tris(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-yl)propionate [(R)-**26d**]

According to GP1 (B) starting from compound (*R*)-**18** (289 mg, 1.0 mmol), KI (16.6 mg, 0.1 mmol), K_2CO_3 (138 mg, 1.0 mmol), and 2-[tris(4-methoxyphenyl)methoxy]ethyl bromide [24] (457 mg, 1.0 mmol); reaction time: 48 h. Purification by CC (petrolether/EtOAc = 2:8). Yield: 239 mg (45%); colorless oil. Analytical data (^1H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**26d**. $[\alpha]_D^{20}$ = +29.5 (*c* = 1.05, $CHCl_3$). $C_{32}H_{39}NO_6$ (533.67).

5.1.51. Methyl (E)-(S)-3-(1-{2-[tris-(4-methoxyphenyl)methoxy]ethyl}pyrrolidin-2-yl)acrylate [(S)-**28d**]

DIBAH (1.4 ml, 2.4 equiv., 1 M in *n*-hexane) was added dropwise over 10 min to compound (*S*)-**24d** (295 mg, 0.584 mmol) in toluene (4 ml) cooled to –60 °C. The reaction mixture was stirred for 2 h. The reaction was quenched by MeOH (0.5 ml) and warmed to rt followed by the addition of water and Et_2O. The aqueous layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The oily residue (275 mg) was dissolved in MeCN (2.5 ml) followed by the addition of LiCl (30 mg, 0.7 mmol, 1.2 equiv.) and DIPEA (123 μl, 0.7 mmol, 1.2 equiv.). Subsequently, trimethylphosphonoacetate (113 μl, 0.7 mmol, 1.2 equiv.) was added dropwise and the reaction mixture was stirred at rt for 16 h. The reaction mixture was concentrated in vacuo followed by the addition of Et_2O and water. The aqueous layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (*n*-hexane/Et_2O = 1:1). Yield: 180 mg (58%); colorless oil. $[\alpha]_D^{20}$ = –32.9 (*c* = 0.99, $CHCl_3$). ^1H NMR ($CDCl_3$, 20 °C): δ = 1.53–1.66 (m, 1H, NCHCH_2), 1.69–1.87 (m, 2H, NCH_2CH_2), 1.90–2.02 (m, 1H, NCHCH_2), 2.25 (q, *J* = 8.6 Hz, 1H, NCH_2), 2.41 (dt, *J* = 12.9,

5.8 Hz, 1H, OCH_2CH_2N), 2.86–3.02 (m, 2H, OCH_2CH_2N, NCHC), 3.07–3.24 (m, 3H, OCH_2CH_2N, NCH_2), 3.74 (s, 3H, $COOCH_3$), 3.78 (s, 9H, OCH_3), 5.98 (d, $J = 15.6$ Hz, 1H, =CHCOO), 6.78–6.87 (m, 7H, CH=, $H_{aromat.}$), 7.31–7.37 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3036$ cm^{-1}, 2997, 2950, 2873, 2834, 1722, 1655, 1608, 1582. MS (70 eV); *m/z* (%): 531 (1) [M^+], 334 (19), 333 (72), 198 (26), 168 (100). MS (CI, CH_5^+); *m/z* (%): 532 (1) $[M + 1]^+$, 500 (1), 361 (1), 333 (100), 227 (6), 200 (11), 168 (13). $C_{32}H_{37}NO_6$ (531.65).

5.1.52. Methyl (E)-(R)-3-(1-{2-[tris-(4-methoxyphenyl) methoxy]ethyl}pyrrolidin-2-yl)acrylate [(R)-**28d**]

As described for compound (*S*)-**28d** starting from (*R*)-**24d** (308 mg, 0.594 mmol), DIBAH (1.43 ml, 1 M in *n*-hexane), LiCl (30 mg, 0.7 mmol, 1.2 equiv.). DIPEA (125 μl, 0.71 mmol, 1.2 equiv.), trimethylphosphonoacetate (115 μl, 0.71 mmol, 1.2 equiv.). Yield: 181 mg (60%); colorless oil. Analytical data (^{1}H NMR, IR, MS) were in accordance with those of the (*S*)-enantiomer (*S*)-**28d**. $[\alpha]_D^{20} = +33.6$ ($c = 1.0$, $CHCl_3$). $C_{32}H_{37}NO_6$ (531.65).

5.1.53. Methyl (Z)-(S)-3-(1-{2-[tris-(4-methoxyphenyl) methoxy]ethyl}pyrrolidin-2-yl)acrylate [(S-**29d**]

Preparation of intermediate (*S*)-**27d** as described for compound (*S*)-**28d** starting from (*S*)-**24d** (437 mg, 0.865 mmol) in toluene (6 ml) and DIBAH (2.1 ml, 2.4 equiv., 1 M in *n*-hexane). The oily residue (410 mg) was dissolved in THF (5 ml). A solution of crown ether (18-crown-6) (1.143 g, 5 equiv.), methyl [bis(2,2,2-trifluoroethoxy)phosphoryl]acetic acid methyl ester (183 μl, 1 equiv.), and $KN(TMS)_2$ (1.15 ml, 1 equiv., 15% in toluene) in THF (5 ml) prepared at –78 °C was added. The reaction mixture was stirred for 1 h at rt. The reaction was quenched with NH_4Cl_{sat}. Following addition of CH_2Cl_2 and water and extraction with CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by CC (*n*-hexane/Et_2O = 1:1). Yield: 197 mg (43%); colorless oil. $[\alpha]_D^{20} = +9.6$ ($c = 0.54$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 20 °C): $\delta = 1.42$–1.55 (m, 1H, $NCHCH_2$), 1.73–1.88 (m, 2H, NCH_2CH_2), 2.05–2.16 (m, 1H, $NCHCH_2$), 2.25 (q, $J = 8.9$ Hz, 1H, NCH_2), 2.49 (dt, $J = 12.6$, 6.3 Hz, 1H, OCH_2CH_2N), 2.87 (dt, $J = 12.6$, 6.3 Hz, 1H, OCH_2CH_2N), 3.10–3.24 (m, 3H, OCH_2CH_2N, NCH_2), 3.70 (s, 3H, $COOCH_3$), 3.78 (s, 9H, OCH_3), 4.01 (q, $J = 8.0$ Hz, 1H, NCH), 5.80 (d, $J = 11.6$ Hz, 1H, =CHCOO), 6.18 (dd, $J = 11.6$, 8.0 Hz, 1H, CH=), 6.76–6.85 (m, 6H, $H_{aromat.}$), 7.29–7.38 (m, 6H, $H_{aromat.}$). IR (KBr): $\tilde{\nu} = 3035$ cm^{-1}, 2996, 2950, 2873, 2834, 1722, 1647, 1608, 1582. MS (70 eV); *m/z* (%): 334 (18), 333 (63), 198 (47), 168 (100), 136 (33). MS (CI, CH_5^+); *m/z* (%): 532 (1) $[M + 1]^+$, 334 (25), 333 (100), 227 (10), 200 (16), 198 (13), 168 (11). $C_{32}H_{37}NO_6$ (531.65).

5.1.54. Methyl (Z)-(R)-3-(1-{2-[tris(4-methoxyphenyl)methoxy] ethyl}pyrrolidin-2-yl)acrylate [(R)-**29d**]

Preparation of intermediate (*R*)-**27d** as described for compound (*S*)-**28d** starting from (*R*)-**24d** (259 mg, 0.513 mmol), DIBAH (1.23 ml, 1 M in *n*-hexane). Subsequent alkylidenation as described for (*S*)-**29d** with crown ether (16-crown-6) (678 mg, 5 equiv.), methyl [bis-(2,2,2-trifluoro-ethoxy)-phosphoryl]-acetic acid methyl ester (682 μl, 1 equiv.), and KN $(TMS)_2$ (682 μl, 1 equiv., 15% in toluene). Yield: 116 mg (42%); colorless oil. Analytical data (^{1}H NMR, IR, MS) in accordance with those of (*S*)-enantiomer (*S*)-**29d**. $[\alpha]_D^{20} = -9.1$ ($c = 0.50$, $CHCl_3$). $C_{32}H_{37}NO_6$ (531.65).

5.2. Biological test

5.2.1. Preparation of subcellular membrane suspensions

Two subcellular membrane pellets, termed $bfcP_{2B}$ (bovine frontal cortex) and $bbsP_{2C}$ (bovine brain stem), respectively, were prepared according to the recently described procedure [43]. Total protein was determined according to *Bradford* using BSA as standard [46].

5.2.2. Inhibition of GAT-1 and GAT-3 mediated GABA-uptake

The GABA uptake assays were performed as recently described [43]. Aliquots of the $bfcP_{2B}$ (GAT-1) or the $bbsP_{2C}$ membrane fraction (GAT-3), respectively, representing about 50–100 μg protein were preincubated with 10 μM aminooxyacetic acid and a test compound in 200 μl buffer (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. Subsequently, 25 μl of 12.5 nM [^{3}H] GABA (3 TBq mmol^{-1}, Amersham Biosciences, Freiburg, Germany) and 25 μl of 250 nM GABA (GAT-1) or 25 μl of 50 nM [^{3}H] GABA and 25 μl of 1 μM GABA (GAT-3) were added incubating the samples 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 3 ml of Rotiszint Eco Plus (Roth, Karlsruhe, Germany) 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.

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