Bioorganic & Medicinal Chemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and SAR studies of GABA uptake inhibitors derived from 2-substituted pyrrolidine-2-yl-acetic acids

Tobias Steffan, Thejavathi Renukappa-Gutke, Georg Höfner, Klaus T. Wanner *

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

ARTICLE INFO

Article history:
Received 1 December 2014
Revised 20 January 2015
Accepted 21 January 2015
Available online xxxx

Keywords:
CNS
GABA uptake
SAR
2-Substituted pyrrolidine-2-yl-acetic acid
N-Acylpyrrolidinium ion

ABSTRACT

In this paper, we disclose the design and synthesis of a series of 2-substituted pyrrolidine-2-yl-acetic acid as core structures and the *N*-arylalkyl derivatives thereof as potential GABA transport inhibitors. The 2-position in the side chain of pyrrolidine-2-yl-acetic acid derivatives was substituted with alkyl, hydroxy and amino groups to modulate the activity and selectivity to mGAT1 and mGAT4 proteins. SAR studies of the compounds performed for the four mouse GABA transporter proteins (mGAT1–mGAT4) implied significant potencies and subtype selectivities for 2-hydroxy-2-pyrrolidine-2-yl-acetic acid derivatives. The racemate *rac*-(*u*)-**13c** exhibited the highest potency (pIC_{50} 5.67) at and selectivity for mGAT1 in GABA uptake assays. In fact, the potency of *rac*-(*u*)-**13c** at hGAT-1 (pIC_{50} 6.14) was even higher than its potency at mGAT1. These uptake results for *rac*-(*u*)-**13c** are in line with the binding affinities to the aforesaid proteins mGAT1 (pK_i 6.99) and hGAT-1 (pK_i 7.18) determined by MS Binding Assay based on NO711 as marker quantified by LC–ESI-MS–MS analysis. Interestingly, the 2-hydroxy-2-pyrrolidine-2-yl-acetic acid *rac*-(*u*)-**13d** containing 2-{[tris(4-methoxyphenyl)]methoxy} ethyl group at the nitrogen atom of the pyrrolidine ring showed high potency at mGAT4 and a comparatively better selectivity for this protein (>15 against mGAT3) than the well known mGAT4 uptake inhibitor (*S*)-SNAP-5114.

1. Introduction

GABA (γ-amino butyric acid, **1**, Fig. 1) is the most important inhibitory neurotransmitter in the central nervous system.[1–4] Attenuation in GABAergic neurotransmission plays an important role in the etiology of several neurological disorders including epilepsy,[2] Alzheimer's disease,[5] Huntington's chorea,[6] migraine,[7] Parkinson's disease,[8] neuropathic pain,[9] and depression.[10] Increase of GABAergic activity may be achieved for instance through direct agonism at the $GABA_A$ receptors, inhibition of enzymatic breakdown of GABA, or by inhibition of the GABA transport proteins (GATs).[11–13] The GABA transporters belong to a large family of membrane bound sodium and chloride ion dependent transporter proteins.[14] They mediate the cellular transport of GABA from the synaptic cleft into the presynaptic neurons and the surrounding glials. Hence, to inhibit the GATs results in a prolonged availability of the physiologically released GABA in the synaptic cleft and extrasynaptically that can restore the normal GABA neurotransmission.[13] This makes GATs attractive drug targets in brain disorders associated with decreased GABA activity. Molecular cloning experiments have revealed the existence of four distinct GABA transporters in humans and other species.[15,16] They are named differently depending on the species they originate from.[11,17] GATs are thus termed as GAT-1, BGT-1, GAT-2, and GAT-3 with a prefix 'r' or 'h', for example, rGAT-1 or hGAT-1 when cloned from rats or human cells, respectively.[11] Corresponding GABA transport proteins cloned from mouse cells are named as mGAT1–4.[11] Alternatively, the nomenclature given by the Human Genome Organisation (HUGO)[11,14b] to describe the human GABA transporters as GAT1 (slc6a1), BGT1 (slc6a12), GAT2 (slc6a13), and GAT3 (slc6a11) is also in use, which will be applied for universal description of the GABA transport proteins irrespective of the species they originate from in this study. For our biological assays, we have used GABA transporter proteins originating from both mouse and human cells, which are respectively termed as mGAT1–4 and hGAT-1.

The GATs differ in their distribution in the brain and the body.[15–19] Of the four GABA transporter subtypes, mGAT1 and mGAT4 are most prevalent in the brain. mGAT1 is observed in the cerebral cortex, cerebellum, hippocampus, basal ganglia, brain stem, spinal cord, retina, and olfactory bulb forming an important component of the GABAergic system whereas high expression of mGAT4 in the brain is found in the retina, olfactory bulb, thalamus, hypothalamus, the spinal cord and the brain stem. Furthermore,

* Corresponding author. Tel.: +49 (0) 89 2180 77249; fax: +49 (0) 89 2180 77247.
E-mail address: klaus.wanner@cup.uni-muenchen.de (K.T. Wanner).

http://dx.doi.org/10.1016/j.bmc.2015.01.035
0968-0896/

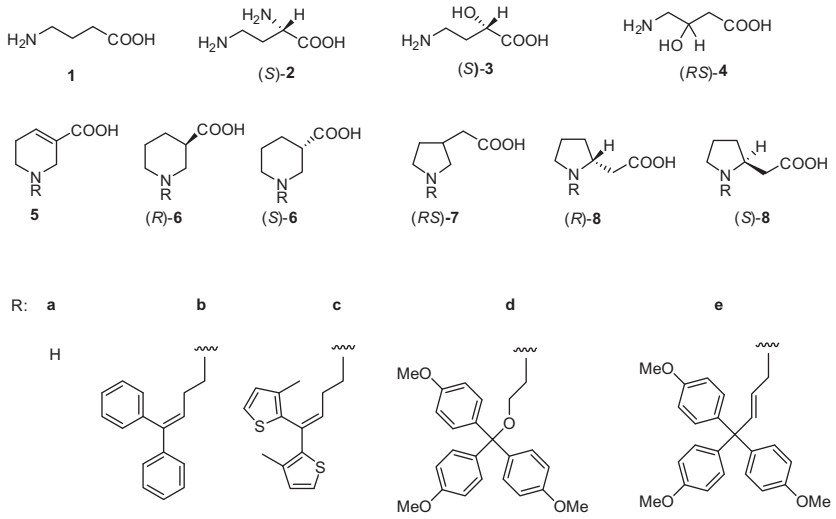


Figure 1. Examples of GABA uptake inhibitors.

mGAT1 is recognized as being responsible for neuronal uptake whereas mGAT4 predominantly takes care of the transport into the surrounding glial cells.[13] mGAT2 and mGAT3 have been observed in high concentrations in kidney and liver whereas their expression in the brain is mainly confined to the leptomeninges (mGAT2, mGAT3) and some cerebral blood vessels (mGAT3).[16,17,20,21] This in turn makes mGAT1 and mGAT4 the predominant drug targets for enhancing GABA neurotransmission by inhibiting its reuptake from the synaptic cleft.

In the past decades, intensive studies have given considerable insight with regard to molecular recognition features for the aforesaid targets.[22] Initial efforts led to the development of several GABA mimetics in the form of both acyclic[4,23] (e.g.: (*S*)-2,4-diaminobutyric acid [(*S*)-DABA] (*S*)-**2**, (*S*)-(−)-4-amino-2-hydroxybutyric acid (*S*)-**3**, (*RS*)-4-amino-3-hydroxybutyric acid (*RS*)-**4**, etc, see Fig. 1) and cyclic analogues[4] {e.g.: guvacine (**5a**), (*R*)- and (*S*)-nipecotic acid [(*R*)-**6a** and (*S*)-**6a**], (*RS*)-pyrrolidine-3-yl-acetic acid [(*RS*)-**7a**], see Fig. 1} of GABA. The introduction of diphenylbutenyl group as a lipophilic residue at the *N*-atom of the parent amino acids **5a**, (*R*)-**6a**, (*S*)-**6a**, and (*RS*)-**7a** made them blood brain barrier penetrable and increased their inhibitory activity in vivo.[24] Studies on the structural variations of these lipophilic residues led to the discovery of Tiagabine (Gabitril®) (*R*)-**6c**,[25] a highly potent and mGAT1 selective uptake inhibitor which has been in clinical use since 1997, for the treatment of epilepsy. The first mGAT3/mGAT4 selective compound (*S*)-SNAP-5114 (*S*)-**6d**,[26] containing a 2-{[tris(4-methoxyphenyl)]methoxy}ethyl group at the *N*-atom of the amino acid (*S*)-**6a** was discovered by Dhar et al. Recently, we reported on the design of DDPM-1457 (*S*)-**6e**,[27] as a chemically stable carba analogue of (*S*)-**6d** displaying an inhibitory potency at mGAT4 which is at least as high as that of (*S*)-**6d**, thus representing one of the two most potent mGAT4 inhibitor known to date. To further improve potency and subtype selectivity, the search for new GABA uptake inhibitors is still ongoing.[28–30]

Previously, we have reported a novel class of GAT inhibitors derived from pyrrolidine-2-yl-acetic acid [(*R*)-**8a–c**, (*S*)-**8a–c**], see Figure 1, in their enantiomerically pure form as analogues of GABA.[30] Compounds (*S*)-**8b** and (*S*)-**8c** exhibited high potencies at mGAT1 (IC_{50} = 0.40 μM and 0.34 μM, respectively) whereas derivative (*R*)-**8d** showed a reasonable potency at mGAT4 (IC_{50} = 3.1 μM) nearly equalling that of (*S*)-**6d**. Extending the scope of this project, we aimed at designing new parent compounds based on the amino acid core structure (*RS*)-**8a**.[30] Additionally, starting from the structures of the known GAT inhibitors (*S*)-**2**, (*S*)-**3**, and (*RS*)-**4**, it seemed interesting to study the influence of alkyl, hydroxy, and amino substituents in 2-position of (*RS*)-**8** on potency at and selectivity for the transporters mGAT1–4 of these compounds. Hence, compounds **9–14** designed by addition of OH, NH_2 and alkyl groups in 2-position of the aforementioned pyrrolidine-2-yl-acetic acid **8**[30] were studied for their inhibitory profile at mGAT1–4. Compounds exhibiting pronounced activity and subtype selectivity were further modified by introduction of residues like the 4,4-diphenylbut-3-enyl and 4,4-bis-(3-methylthiophene-2-yl)but-3-enyl moiety known to be favourable for enhanced potency at and selectivity for mGAT1 and by introduction of the 2-{[tris(4-methoxyphenyl)]methoxy}ethyl group being similarly beneficial for mGAT4 potency and selectivity. All compounds, from the individual diastereomers of the parent structures of **9**, **10**, **11**, **12**, **13a**, **14a**, and to those of the *N*-arylalkyl derivatives **13b–d** (only the structures and biological activity of compounds **13b** and **13c** have been previously published by us in the context of a novel MS Binding Assay using MALDI-MS-MS for detection and quantification of MS-marker[31]) and **14b–c** were, after having been synthesized, tested for their inhibition of mouse GABA transport

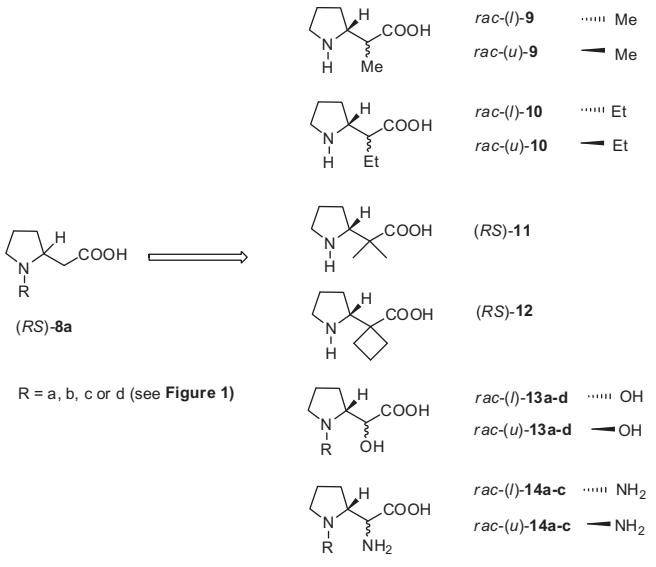


Figure 2. Target structures derived from pyrrolidine-2-yl-acetic acid.

proteins mGAT1–4, and in addition for hGAT-1 in case of *N*-arylalkyl derivatives **13b–d** and **14b–c** (see Fig. 2).

2. Results and discussion

2.1. Chemistry

Our general strategy for synthesizing 2-substituted pyrrolidine-2-yl-acetic acid derivatives was based on the well known electrophilic α-amidoalkylation reactions as a key step to introduce substituents in the α-position of the pyrrolidine core structure.[32–35] For the application of the aforementioned reaction to the preparation of our target compounds, we intended to react *N*-acylpyrrolidinium ions **17** generated from a suitable precursor **15** by Lewis acid activation with respective trialkylsilyl ketene acetals **16** (Scheme 1). Hence, using appropriately substituted acetic acid derivatives in form of their silyl ketene acetals **16** would enable the introduction of the desired substituents (alkyl, OH and NH_2) in the 2-position to the carboxylic group of the pyrrolidine-2-yl-acetic acid derivatives. The reagent to be employed for the generation of the *N*-acyliminium ions was envisaged to consist of a pyrrolidine ring with an acyl or alkoxycarbonyl group attached to nitrogen and to exhibit a leaving group such as an alkoxy moiety or a halogen in the α-position. Subsequent cleavage of the amide or carbamate moiety and of the ester function of the α-amidoalkylation product **18** would finally lead to the free amino acids **9–12**, **13a** and **14a**. For selected amino acids exhibiting high potencies as GABA uptake inhibitors, derivatives **13b–d** and **14b–c** displaying N-substituents common for GABA uptake inhibitors should be synthesized (Scheme 1).

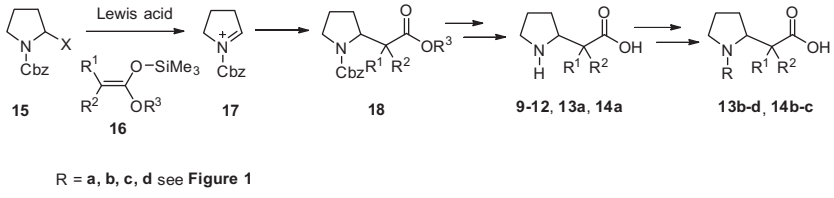
2.1.1. Preparation of parent amino acids

For the implementation of our synthetic strategy, pyrrolidine derivative (*RS*)-**21** was required as a starting compound. It was found that in addition to literature methods,[36,37] (*RS*)-**21** can be efficiently synthesized starting from the commercially available 4-aminobutanal diethyl acetal **19**. N-protection of **19** with benzyl chloroformate and subsequent cyclisation of **20**,[38] without prior purification, by means of catalytic amounts of $Sc(OTf)_3$ (1%) provided the α-amidoalkylation reagent (*RS*)-**21** in 90% yields over both steps (Scheme 2).

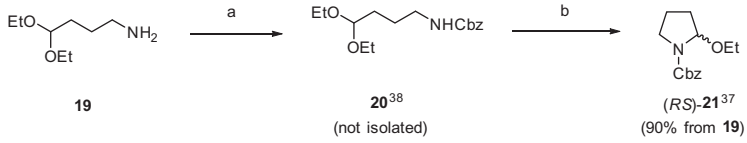
The preparation of the parent amino acids **9–12**, **13a** and **14a** by α-amidoalkylation reactions was accomplished as depicted in Table 1 and in Schemes 3 and 4. The α-amidoalkylation reactions were carried out according to a method developed by Kobayashi et al.[35] for the reaction of *N*-benzyloxycarbonyl-2-methoxypiperidine with silyl enol ethers mediated by Lewis acids. Thus, in the first step the pyrrolidine derivative (*RS*)-**21** was reacted with 1.7–2.0 equiv of the respective ketene silyl acetals **22–25**[39–45] prepared as described in literature[39,40] in presence of $Sc(OTf)_3$ as catalyst in acetonitrile at 0 °C (Table 1). The yields of the α-amidoalkylation products **28–31** amounted to 73–87% (Table 1, entries 1–4) whereby **28** and **29** were obtained as mixtures of diastereomers [*rac*-(*l*)-**28**[46]/*rac*-(*u*)-**28** = 55:45; *rac*-(*l*)-**29**/*rac*-(*u*)-**29** = 54:46] (Table 1). Interestingly, the diastereomeric composition of the α-amidoalkylation products **28** and **29** remained mostly unchanged when mixtures of isomeric silyl ketene acetals (*E*/*Z*)-**22**[39] and (*E*/*Z*)-**23**[41], respectively, of different composition were applied. Due to the difficulties encountered in the separation of the diastereomeric mixtures of **28** [*rac*-(*l*)-**28**/*rac*-(*u*)-**28**] and **29** [*rac*-(*l*)-**29**/*rac*-(*u*)-**29**], these were used as such for subsequent reactions. In the final steps compounds **28–31** were subjected to hydrolysis (KOH in MeOH/H_2O) and catalytic hydrogenolysis (H_2 over Pd/C in MeOH), which led to the carboxylic acids **34–37** and finally to the free amino acids **9–12** in total yields over both steps from 77–92%, (Scheme 3). The diastereomeric composition of the outlined amino acids **9** and **10** was identical or only marginally different from that of the starting compounds **28** and **29** amounting to 55:45 and 53:47 [*rac*-(*l*)-**9**/*rac*-(*u*)-**9**;[47] *rac*-(*l*)-**10**/*rac*-(*u*)-**10**], respectively. For the biological characterization the mixtures were used in this form.

The parent compounds *rac*-(*l*)-**13a**[48] and *rac*-(*u*)-**13a**[48] containing an OH group in α-position of the carboxylic acid group were prepared as laid out in Table 1 and Scheme 3. For the preparation of required silyl ketene acetals (*E*/*Z*)-**26** with a TBDMS protected OH group, the literature method[44] was slightly modified. Adding the ester to a solution of LDA in THF with chlorotrimethylsilane already present (instead of subsequently adding the reagent) increased the yield of (*E*/*Z*)-**26** from 34% to 55%. Reaction of silyl ketene acetals (*E*/*Z*)-**26**[44] with the α-amidoalkylation reagent (*RS*)-**21** (in MeCN at 0 °C) in the presence of 10 mol % of $Sc(OTf)_3$ gave a diastereomeric mixture of *rac*-(*l*)-**32**[49] and *rac*-(*u*)-**32** with a ratio of 45:55 (determined by ^1H NMR) and a total yield of 67% (Table 1, entry 5).[35] Subsequent deprotection of *rac*-(*l*)-**32**[49] and *rac*-(*u*)-**32** with TBAF in THF at 0 °C yielded the mixture of *rac*-(*l*)-**38**[50]/*rac*-(*u*)-**38** (95% isolated yield) which, upon repeated flash chromatography gave the pure diastereomers *rac*-(*l*)-**38**[50] and *rac*-(*u*)-**38** in 36% and 27% yield, respectively. Saponification of the pure diastereomers *rac*-(*l*)-**38**[50] and *rac*-(*u*)-**38** (0.1 M LiOH) and catalytic hydrogenolysis (H_2 over Pd/C in MeOH) yielded finally the free amino acids *rac*-(*l*)-**13a**[48] and *rac*-(*u*)-**13a**[48] (74% and 78%, for last two steps).

The synthesis of α-amino substituted pyrrolidine-2-yl-acetic acid derivatives (Table 1 and Scheme 4) was similarly accomplished utilizing an α-amidoalkylation reaction as key step.[35] Reaction of α-amidoalkylation reagent (*RS*)-**21** with a silyl ketene acetal

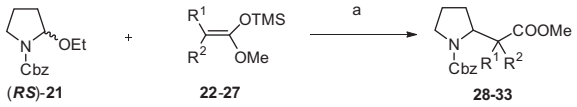


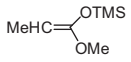
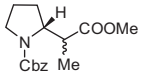
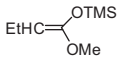
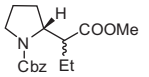
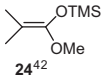
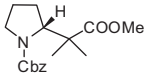
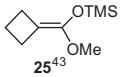
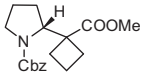
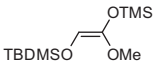
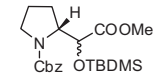
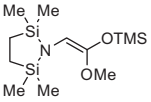
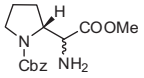
Scheme 1. Synthetic strategy.



Scheme 2. Synthesis of precursor (*RS*)-**21**.[37] Reagents and conditions: (a) Cbz-Cl, Na_2CO_3, CH_2Cl_2/H_2O, 0 °C, 3 h; (b) 1 mol % $Sc(OTf)_3$, heptane/EtOAc, rt, 3 h.

Table 1
α-Amidoalkylation reactions



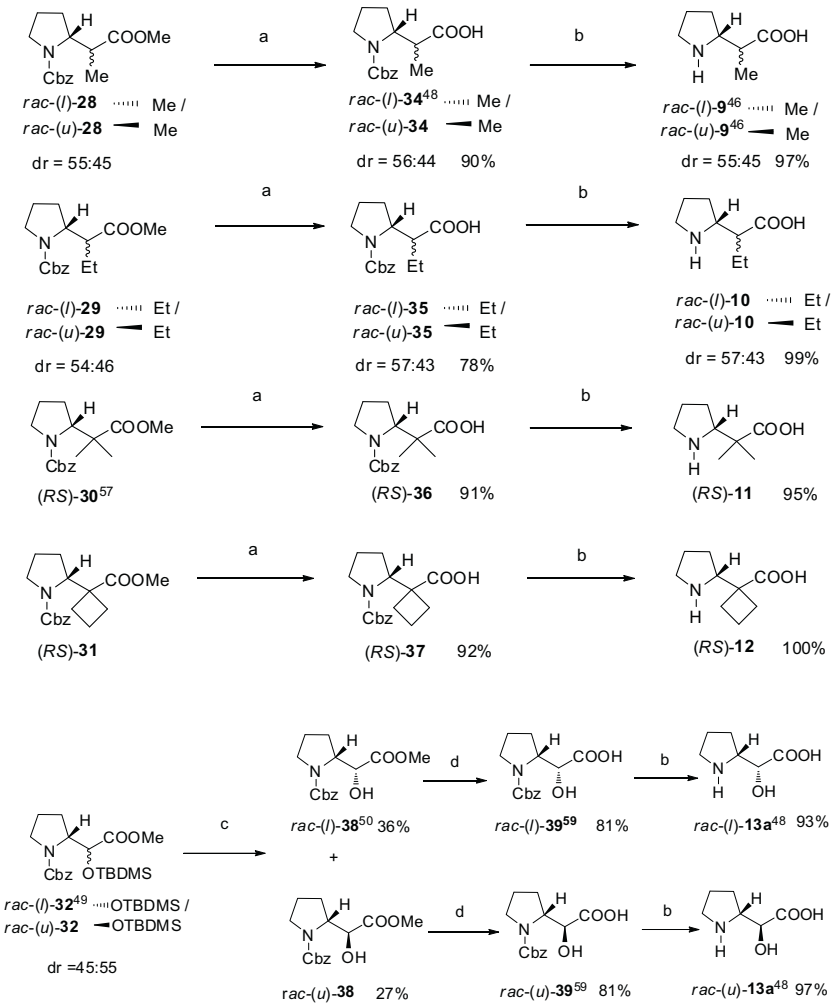
Entry	Silyl ketene acetals	*E*/*Z*	Time (h)	Products	Ratio[a] *l*/*u*	Yield (%)
1	 (*E*/*Z*)-**22**[39]	78/22	3	 *rac*-(*l*)-**28**[46] Me; *rac*-(*u*)-**28** Me	55:45	73
2	 (*E*/*Z*)-**23**[41]	89/11	1.5	 *rac*-(*l*)-**29** Et; *rac*-(*u*)-**29** Et	54:46	77
3	 **24**[42]	–	1.5	 (*RS*)-**30**[57]	–	87
4	 **25**[43]	–	4	 (*RS*)-**31**	–	73
5	 (*E*/*Z*)-**26**[44]	69/31	19	 *rac*-(*l*)-**32**[49] OTBDMS; *rac*-(*u*)-**32** OTBDMS	45:55	67
6	 (*E*)-**27**[45]	–	19	 rac-(*l*)-**33** NH_2; *rac*-(*u*)-**33**[50] NH_2	41:59	22(*l*) 32(*u*)

[a] Determined by ^{1}H NMR spectroscopy; (a) 10 mol % $Sc(OTf)_3$, CH_3CN, 0 °C.

with a stabase-protected amino group (*E*)-**27**[45,51] in MeCN at 0 °C in the presence of 10 mol % $Sc(OTf)_3$ for 19 h gave a diastereomeric mixture of the pyrrolidine derivative *rac*-(*l*)-**33**[50] and *rac*-(*u*)-**33**[50] with a ratio of 41:59 Table 1, entry 6. As expected the primary amino function of the silyl ketene acetal had been completely freed from the stabase protection group during the reaction. This, however, did not hamper performing the next steps. Thus, chromatographic separation of the mixture yielded 22% of *rac*-(*l*)-**33**[50] and 32% of *rac*-(*u*)-**33**.[50,52] To facilitate the isolation of the product resulting from ester hydrolysis, the primary amino groups in the diastereomers *rac*-(*l*)-**33** and *rac*-(*u*)-**33** were first *N*-Cbz protected. The respective compounds *rac*-(*l*)-**40** and *rac*-(*u*)-**40** were obtained in a yield of 96% and 87%, respectively. Hydrolysis (0.3 M LiOH in dioxane/water) of *rac*-(*l*)-**40** with LiOH (0.3 M) in dioxane/water yielded 95% of *rac*-(*l*)-**41** and subsequent, N-deprotection through catalytic hydrogenolysis (H_2 over Pd/C in MeOH) in the presence of trifluoroacetic acid yielded the free amino acid *rac*-(*l*)-**14a**·2TFA (81% total yield over both steps). This synthetic sequence turned out to effect epimerization when applied to the diastereomer *rac*-(*u*)-**40** (during the removal of the Cbz group by catalytic hydrogenolysis). Therefore, to obtain the pure diastereoisomer *rac*-(*u*)-**14a**·2TFA an alternative synthetic route was followed. In that case, the *N*-Cbz protecting groups of *rac*-(*u*)-**40** were both first replaced by *N*-Boc protecting groups. This was accomplished by catalytic hydrogenation (H_2 over Pd/C in MeOH) of *rac*-(*u*)-**40** to give *rac*-(*u*)-**42**·2TFA, (72%) and subsequent Boc-protection (Boc_2O, NEt_3, MeOH) that yielded *rac*-(*u*)-**43** (82%). Then, *rac*-(*u*)-**43** was transformed into the free acid *rac*-(*u*)-**44** in high yield (93%) by hydrolysis with 0.1 M LiOH in dioxane/water. Removal of the Boc-groups with trifluoroacetic acid in CH_2Cl_2 afforded finally the free amino acid *rac*-(*u*)-**14a** as its TFA salt in 99% yield without any detectable epimerization (Scheme 4).

2.1.2. Preparation of N-substituted derivatives

The ring nitrogen substituted hydroxypyrrolidine-2-yl-acetic acid derivatives **13b–d** and aminopyrrolidine-2-yl-acetic acid derivatives **14b–c** were synthesized as depicted in Scheme 5. For the preparation of the N-alkylated hydroxypyrrolydin-2-yl-acetic acid derivatives **13b–d**, the diastereomers *rac*-(*l*)-**38** and *rac*-(*u*)-**38** were subjected to catalytic hydrogenation over Pd/C in acetic acid



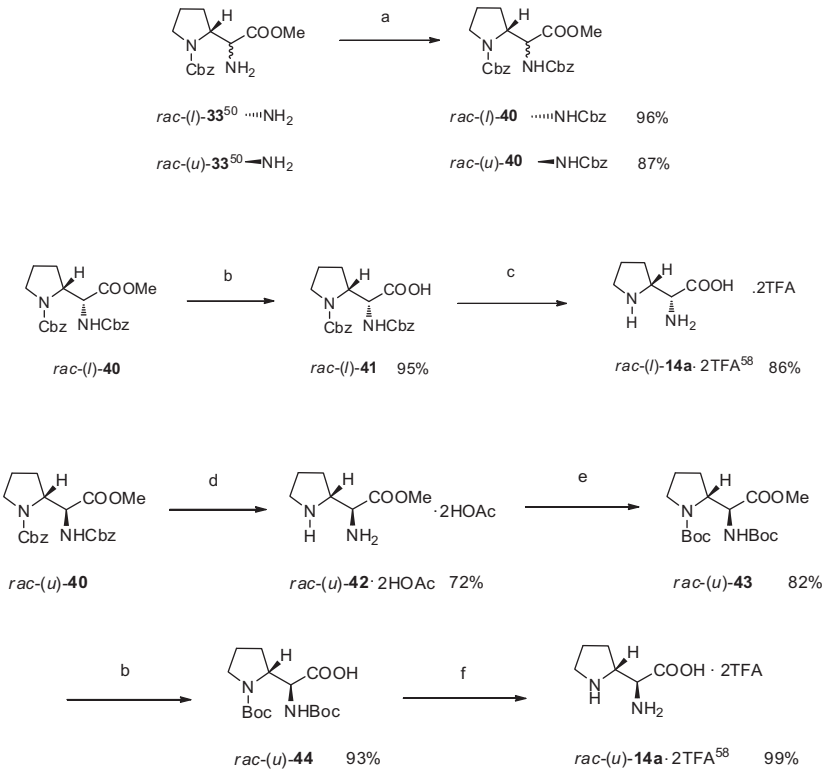
Scheme 3. Synthesis of 2-alkyl substituted pyrrolidine-2-yl-acetic acids **9**–**12** and synthesis of 2-hydroxy substituted pyrrolidine-2-yl-acetic acids **13a**. Reagents and Conditions: (a) KOH, MeOH/H_2O, reflux, 16–96 h; (b) H_2, Pd/C, MeOH, rt, 1–24 h; (c) TBAF, THF, rt, 1.5 h; (d) LiOH, dioxane/H_2O, rt, 1–2 h.

to remove the *N*-Cbz-protecting groups giving rise to acetic acid salts of the methyl esters *rac*-(*l*)-**45** and *rac*-(*u*)-**45**, respectively. Subsequent alkylation of the methyl esters *rac*-(*l*)-**45** and *rac*-(*u*)-**45** with the alkyl bromides (RBr) 4,4-diphenylbut-3-en-1-yl bromide[24] (R = b), 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide[25] (R = c) and 2-[tris(4-methoxyphenyl)methoxy]ethyl bromide[26] (R = d) in MeCN in the presence of K_2CO_3 and potassium iodide[30,53] provided the respective *N*-alkyl derivatives **46**–**48** in yields from 19–60%. Hydrolysis of the *N*-alkyl derivatives **46**–**48** with LiOH in dioxane/water finally afforded the target compounds **13b**–**d** in yields of 95–99%.

For the synthesis of the target compounds **14b**–**c**, the primary amino group present in the aminopyrrolidine-2-yl-acetic acid derivatives *rac*-(*l*)-**33** and *rac*-(*u*)-**33**[52] was protected to enable the selective alkylation of the secondary amino group of the pyrrolidine ring after its deprotection. This was accomplished by reacting the diastereoisomers *rac*-(*l*)-**33** and *rac*-(*u*)-**33** with Boc anhydride and triethylamine in dioxane at room temperature for 2.5 h to yield the corresponding Boc protected compounds *rac*-(*l*)-**49** and *rac*-(*u*)-**49**[52] in good yields (86% and 95%, respectively). Subsequent hydrogenolytic removal of the Cbz group (H_2, Pd/C, MeOH, and acetic acid) gave the acetic acid salts *rac*-(*l*)-**50** and *rac*-(*u*)-**50** in yields of 99% and 97%, respectively. Unfortunately, when N-alkylation of *rac*-(*l*)-**50** and *rac*-(*u*)-**50** was attempted with 4,4-diphenylbut-3-en-1-yl bromide (R = b) using K_2CO_3 as a base with KI in MeCN epimerization at the chiral center in 2-position of those compounds occurred leading to mixtures of diastereomers.[30,53] This inversion could be suppressed by carrying out the N-alkylation reaction of *rac*-(*l*)-**50** with 4,4-diphenylbut-3-en-1-yl bromide using $KHCO_3$ instead of K_2CO_3 as a base yielding 55% of *rac*-(*l*)-**51**. Similarly, the N-alkylated derivative *rac*-(*u*)-**51** was obtained in pure form in 18% yield by changing the base to DIPEA. The N-alkylation of *rac*-(*l*)-**50** with 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide in MeCN in the presence of KI and K_2CO_3 as base afforded *rac*-(*l*)-**52** in 49% yield. The alkylated derivative *rac*-(*u*)-**52** could be obtained without any epimerization only when instead of $KHCO_3$ triethylamine was used as a base (in CH_2Cl_2), in which case, however, the yield amounted to only 10%. Finally, saponification of the ester function of *rac*-**51** and *rac*-**52** with LiOH in a mixture of dioxane and water followed by deprotection of the primary amino group by treatment with TFA in CH_2Cl_2 gave the trifluoroacetic acid salts of the target compounds **14b**–**c** in 73–97% (Scheme 5).

2.2. Biological evaluation

The inhibitory potency of the synthesized amino acids and their subtype selectivity at mGAT1, mGAT2, mGAT3 and mGAT4 transport proteins were determined using well established [^{3}H]GABA uptake assays[54] based on mouse GABA transporters stably expressed in HEK cells.[54] The measurements were done in triplicates and the results are given as pIC_{50} ± SEM (n = 3). For the compounds that were unable to reduce the [^{3}H]GABA uptake to a value



Scheme 4. Synthesis of 2-amino substituted pyrrolidine-2-yl-acetic acids **14a**. Reagents and conditions: (a) Cbz-Cl, $NaHCO_3$, H_2O/THF, 0 °C to rt, 4 h; (b) LiOH, dioxane/H_2O, rt, 1–2 h; (c) H_2, Pd/C, MeOH, rt, 1 h, TFA; (d) H_2, Pd/C, MeOH, rt, 2 h, AcOH; (e) $(Boc)_2O$, NEt_3, MeOH, 50 °C, 30 min; (f) TFA, CH_2Cl_2, rt, 25 min.

of <50% at a specific concentration of 1 mM (pIC_{50} value is taken as $\leqslant 3$) or 100 μM (pIC_{50} value taken as $\leqslant 4$), the results are indicated as the percentage of the remaining [^{3}H]GABA uptake at this concentration of the test compound.

2.2.1. SAR of parent structures

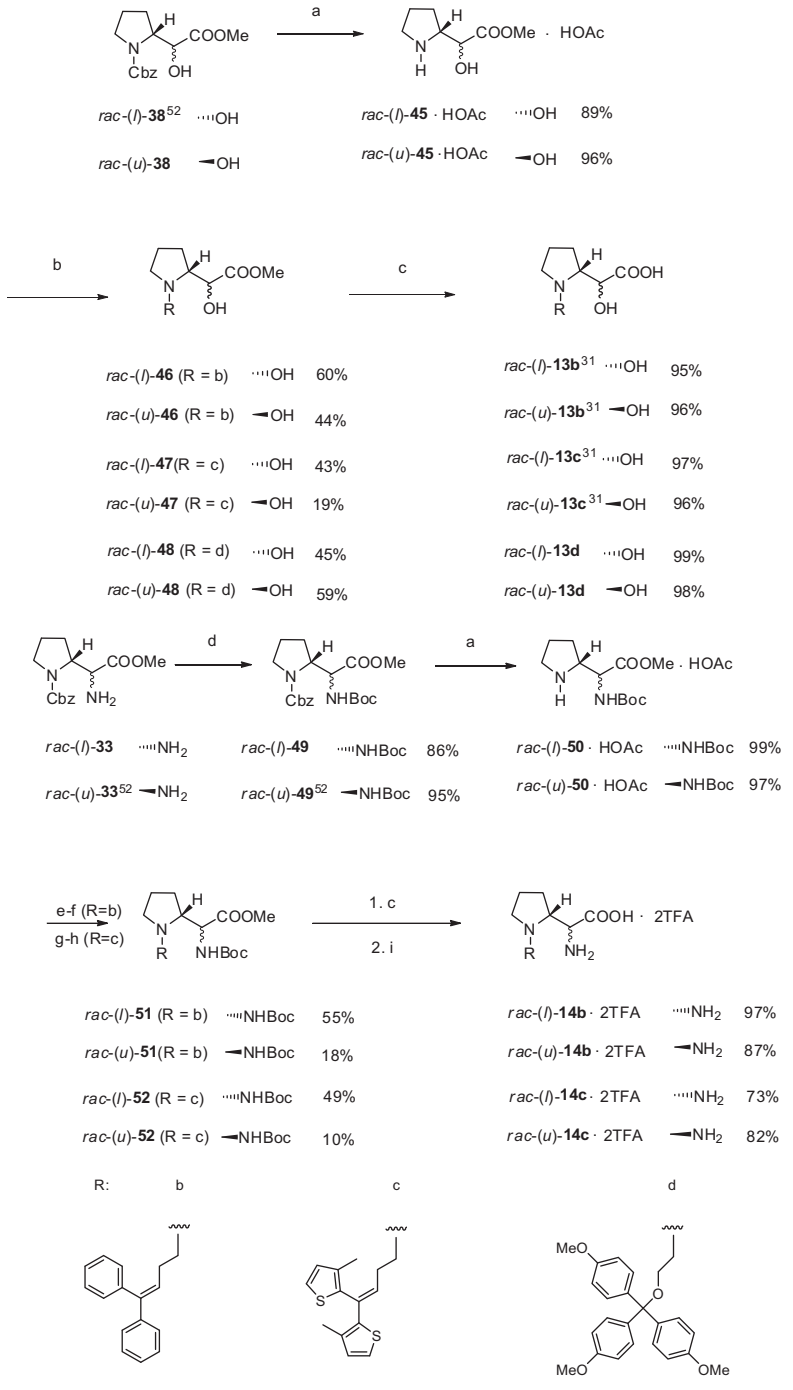
The pIC_{50} values of the unsubstituted pyrrolidine-2-yl-acetic acids (*R*)-**8a** and (*S*)-**8a** were used for reference. The parent amino acids (**9**–**12**, **13a**, **14a**) were all tested in their racemic form either as diastereomeric mixtures (roughly 1:1) or pure diastereomers and not as pure enantiomers like the reference compounds, the homoprolines (*R*)-**8a** and (*S*)-**8a**. This implies that some constituents of the mixture or the pure enantiomers may have potencies somewhat higher than the value reflected by the overall test result.

As shown in Table 2, among the parent amino acids substituted at the 2-position with alkyl groups (**9**–**12**, Table 2, entries 3–6), no reasonable improvement in the potency at and selectivity to GAT proteins was observed compared to the values obtained for the unsubstituted enantiomeric pure homoprolines (*R*)-**8a** and (*S*)-**8a** (Table 2, entries 1 and 2). The potencies of *rac*-(*l*)-**9**/*rac*-(*u*)-**9** (dr 55:45) at mGAT1–mGAT4 exhibiting a methyl group in the side chain of pyrrolidine-2-yl-acetic acid moiety were roughly the same as those found for (*R*)-**8a** and (*S*)-**8a**, except that the activity at mGAT1 appears to be somewhat lower (compare Table 2, entry 3 to entries 1 and 2). Further increase in the lipophilicity of the parent compounds by adding an ethyl [*rac*-(*l*)-**10**/*rac*-(*u*)-**10**, dr = 54:46] or two vicinal methyl groups [(*RS*)-**11**] or a 1,3-propandiyl moiety [(*RS*)-**12**] resulted in loss of activity and selectivity at GAT proteins. For the 2-hydroxy substituted pyrrolidine-2-yl-acetic acids *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**, *rac*-(*l*)-**13a** though still a racemic mixture showed better inhibitory potencies at the mGAT2 (pIC_{50} = 3.77), mGAT3 (pIC_{50} = 4.44), and mGAT4 (pIC_{50} = 4.85) proteins compared to both of the enantiomerically pure homoprolines (*R*)-**8a** and (*S*)-**8a** (compare Table 3, entry 7 to entry 1 and 2). Additionally, *rac*-(*l*)-**13a** exhibited a pronounced selectivity of >71 for mGAT4 against mGAT1 (Table 3) and >12 against mGAT2. In addition, this ligand also showed a considerable potency at mGAT3. In contrast, racemic *rac*-(*u*)-**13a** did not exhibit any significant potency at or selectivity to GAT proteins (Table 2, entry 8). Similar to what had been observed for the hydroxyl derivatives *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**, the results of the [^{3}H]GABA uptake assay for 2-amino-pyrrolidine-2-yl-acetic acids, the diastereomer *rac*-(*l*)-**14a** in comparison to the homoprolines (*R*)-**8a** and (*S*)-**8a** showed an improved binding potency at all four mouse GABA transport proteins with some selectivity for mGAT3 and mGAT4 proteins (see Table 2).

Based on these results, it is to be inferred that the addition of sterically demanding lipophilic groups to the 2-position of pyrrolidine-2-yl-acetic acid core structure is not tolerated by the GAT proteins. More importantly, it may be concluded that the presence of hydrophilic groups like a hydroxy or an amino moiety in the 2-position to the carboxyl group of homoproline with an appropriate stereochemical orientation appears to be well tolerated or to even enhance potency and to give rise to promising selectivities for mGAT3 and mGAT4 proteins.

2.2.2. SAR of the N-substituted derivatives

Unlike the parent compounds, the N-substituted derivatives **13b**–**c**, **14c** were also tested for their inhibitory activity at hGAT-1 in addition to the four mouse GABA transporters mGAT1–4. The [^{3}H]GABA uptake assay for hGAT-1 was identical to the one for mGAT1 except that membrane preparation of HEK293 cells expressing hGAT-1 have been employed.[54] The results of the GABA uptake experiments and subtype selectivities of the N-alkylated derivatives of 2-hydroxy-pyrrolidine-2-yl-acetic acid **13b**–**d** and the 2-amino-pyrrolidine-2-yl acetic acid **14b**–**c** are given in Table 3. The parent structures **13a** and **14a** (Table 2), Tiagabine (*R*)-**6c**, and (*S*)-SNAP-5114 (*S*)-**6d** (Table 3, entries 1 and 2) were used as

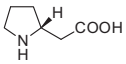
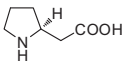
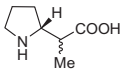
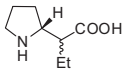
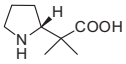
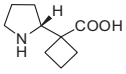
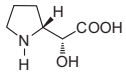
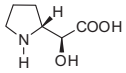
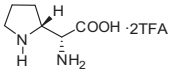
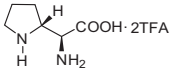


Scheme 5. Synthesis of *N*-arylalkyl substituted hydroxy- and aminopyrrolidine-2-yl-acetic acid derivatives **13b–d** and **14b–c**. Reagents and conditions: (a) H_2, Pd/C, HOAc, MeOH, rt, 1 h; (b) R-Br (R = b, c or d), K_2CO_3, KI, CH_3CN, reflux, 4–6 h; (c) LiOH, dioxane/H_2O, rt, 1–1.5 h followed by addition of Na_2HPO_4/NaH_2PO_4-buffer (pH = 7); (d) $(Boc)_2O$, NEt_3, dioxane, rt, 2.5 h; (e) R-Br (R = b), $KHCO_3$, KI, CH_3CN, reflux, 4 h; (f) R-Br (R = b), DIPEA, CH_3CN, rt, 72 h; (g) R-Br (R = c), K_2CO_3, KI, CH_3CN, reflux, 17 h; (h) R-Br (R = c), NEt_3, CH_2Cl_2, rt, 72 h; (i) TFA, CH_2Cl_2, rt, 25–30 min.

reference compounds. In a competitive MS Binding Assay developed by us,[55] the aforementioned compounds were also characterized for their binding affinities to the GABA transport proteins hGAT-1 and mGAT1. For this competitive assay, NO711[56] was used as unlabelled marker which was quantified by LC–ESI-MS–MS.[55] Affinity values obtained from the competitive MS Binding Assays are given as $pK_i \pm$ SEM (calculated from three independent experiments). Again, since the compounds have been tested as racemates, one of the pure enantiomers may possess better inhibitory properties or binding affinities than the racemic mixture.

By aiming most of all at compounds highly potent at and selective for mGAT1 and mGAT4 proteins, we first studied the influence of substituents known to enhance mGAT1 potency and selectivity like the 4,4-diphenylbut-3-enyl and the 4,4-bis(3-methylthiophen-2-yl)but-3-enyl group at the pyrrolidine ring nitrogen of both the parent structures **13a** and **14a**. Addition of a 4,4-diphenylbut-3-enyl residue to the pyrrolidine ring nitrogen of both racemic diastereomers of 2-hydroxypyrrolidine-2-yl-acetic acid derivatives, *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**, resulted in a significant increase of the inhibitory potency at mGAT1 (and similarly hGAT-1) of about

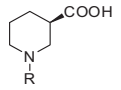
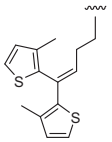
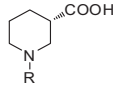
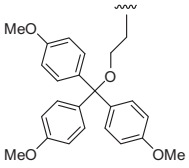
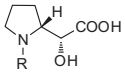
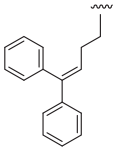
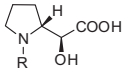
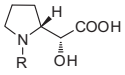
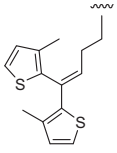
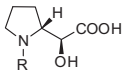
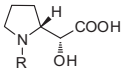
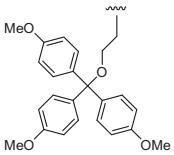
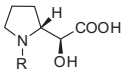
Table 2
Inhibitory potency of 2-substituted pyrrolidine-2-yl-acetic acid core structures and pyrrolidine-2-yl-acetic acid in [^{3}H]GABA uptake assays given as pIC_{50} values [mean ± SEM, n = 3] at mGAT1, mGAT2, mGAT3 and mGAT4 obtained from three independent experiments

Entry	Compounds	mGAT1	mGAT2	mGAT3	mGAT4
1		3.62 ± 0.05	1 mM (81%)	3.47 ± 0.04	2.95 ± 0.07
2	(*R*)-**8a** 	3.39 ± 0.10	1 mM (77%)	3.85 ± 0.01	3.76 ± 0.04
3	(*S*)-**8a** 	1 mM (83%)*	1 mM (59%)*	3.51 ± 0.06	3.47 ± 0.02
4	*rac*-(*l*)-**9**/*rac*-(*u*)-**9**[47] 55:45 	1 mM (78%)	1 mM (82%)*	1 mM (78%)*	1 mM (95%)*
5	*rac*-(*l*)-**10**/*rac*-(*u*)-**10** 54:46 	1 mM (83%)*	1 mM (108%)*	1 mM (93%)*	1 mM (95%)*
6	(*RS*)-**11** 	1 mM (92%)*	1 mM (91%)*	1 mM (73%)*	1 mM (110%)*
7	(*RS*)-**12** 	3.00	3.77 ± 0.02	4.44 ± 0.03	4.85 ± 0.03
8	*rac*-(*l*)-**13a**[48] 	1 mM (53%)*	1 mM (95%)*	3.57 ± 0.04	1 mM (61%)*
9	*rac*-(*u*)-**13a**[48] 	3.91 ± 0.05	3.80 ± 0.06	4.57 ± 0.03	4.67 ± 0.02
10	*rac*-(*l*)-**14a**·2TFA[58] 	1 mM (1%)*	1 mM (52%)*	1 mM (59%)*	3.43 ± 0.03
	rac-(*u*)-**14a**·2TFA[58]				

* Remaining [^{3}H]GABA uptake in the presence of 1 mM test substance as compared to control without inhibitor. Compounds that do not reduce at 1 mM the [^{3}H]GABA uptake to <50%, a pIC_{50} of ⩽3 is assumed to ease the comparitive analysis of the obtained results.

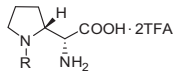
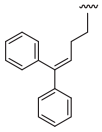
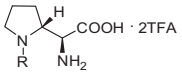
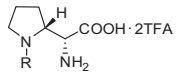
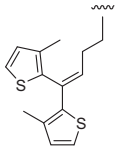
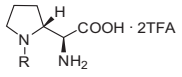
1.5 to 2 log units the pIC_{50} for *rac*-(*l*)-**13b** and *rac*-(*u*)-**13b** at mGAT1 amounting to 4.60 and 5.64, respectively. At the same time, the inhibitory potency at mGAT2–mGAT4 remained largely unchanged or became even lower, thus overall leading to an increased selectivity for mGAT1 (as compared to mGAT2–mGAT4, Table 3, entries 3 and 4). A strong increase in potency—this time for both diastereomers, *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**—was also effected by introduction of the 4,4-bis(3-methylthiophen-2-yl)but-3-enyl group. Here the compounds *rac*-(*l*)-**13c** and *rac*-(*u*)-**13c** exhibited pIC_{50} values of 5.47 and 5.67 at mGAT1 which means a substantial increase in activity up to two orders of magnitude compared to the core structures *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a** (compare Table 3, entries 5 and 6 to Table 2, entries 7 and 8). Whereas of the parent compounds *rac*-(*l*)-**13a** had better inhibitory potencies at mGAT3 and mGAT4 as compared to mGAT1 and mGAT2, the *N*-arylalkyl derivatives *rac*-(*l*)-**13c** and *rac*-(*u*)-**13c** exhibited selectivity in favour of mGAT1 [*rac*-(*l*)-**13c**: >29 for mGAT1 against mGAT2, mGAT3 and mGAT4; *rac*-(*u*)-**13c**: >47 for mGAT1 against mGAT2 and mGAT4, >20 against mGAT3]. With a pIC_{50} of 5.67, racemic *rac*-(*u*)-**13c** exhibited the nominally highest potency at

Table 3
Inhibitory potency of *N*-arylalkyl derivatives in [^{3}H]GABA uptake assays given as pIC_{50} values [mean ± SEM, $n = 3$] at hGAT-1, mGAT1, mGAT2, mGAT3 and mGAT4 obtained from three independent experiments

Entry	Compounds	R	hGAT-1	mGAT1	mGAT2	mGAT3	mGAT4
1	 Tiagabine (*R*) (*R*)-**6c**		**6.42 ± 0.01**	**6.88 ± 0.12**	100 μM (52%)*	100 μM (64%)*	100 μM (73%)
2	 (*S*)-SNAP-5114 (*S*)-**6d**		–	4.07 ± 0.05	100 μM (56%)*	**5.30 ± 0.02**	**5.81 ± 0.06**
3	 *rac*-(*l*)-**13b**[31]		4.86 ± 0.05	4.60 ± 0.04	100 μM (89%)*	100 μM (97%)*	100 μM (59%)*
4	 *rac*-(*u*)-**13b**[31]		**5.72 ± 0.03**	**5.64 ± 0.03**	100 μM (60%)*	4.00	100 μM (55%)*
5	 *rac*-(*l*)-**13c**[31]		**5.38 ± 0.05**	**5.47 ± 0.10**	100 μM (63%)*	100 μM (90%)*	100 μM (74%)*
6	 *rac*-(*u*)-**13c**[31]		**6.14 ± 0.02**	**5.67 ± 0.07**	100 μM (58%)*	4.37 ± 0.05	100 μM (59%)*
7	 *rac*-(*l*)-**13d**		–	100 μM (68%)*	100 μM (56%)*	**4.77 ± 0.05**	**5.24 ± 0.02**
8	 *rac*-(*u*)-**13d**		–	100 μM (80%)*	100 μM (64%)*	100 μM (70%)*	**5.18 ± 0.05**

(*continued on next page*)

Table 3 (*continued*)

Entry	Compounds	R	hGAT-1	mGAT1	mGAT2	mGAT3	mGAT4
9	 *rac*-(*l*)-**14b**·2TFA		–	4.31 ± 0.03	100 μM (66%)*	4.94	4.76 ± 0.04
10	 *rac*-(*u*)-**14b**·2TFA		–	4.46 ± 0.03	100 μM (61%)*	100 μM (71%)*	100 μM (83%)*
11	 *rac*-(*l*)-**14c**·2TFA		–	4.85 ± 0.04	4.37 ± 0.01	4.52 ± 0.03	4.50 ± 0.07
12	 *rac*-(*u*)-**14c**·2TFA		100 μM (57%)*	4.00	4.00	100 μM (63%)*	4.00

The bold values represent the most potent and highly affine compounds.

* Remaining [^{3}H]GABA uptake in the presence of 100 μM test substance as compared to control without inhibitor. Compounds that do not reduce at 100 μM the [^{3}H]GABA uptake to ⩽50%, a pIC_{50} of ⩽4 is assumed to ease the comparative analysis of the obtained results.

mGAT1. At hGAT-1, compounds *rac*-(*l*)-**13b**, *rac*-(*u*)-**13b**, *rac*-(*l*)-**13c** and *rac*-(*u*)-**13c** exhibited pIC_{50}s similar to those found for mGAT1 with one exception. In case of *rac*-(*u*)-**13c**, the pIC_{50} found for hGAT1 was almost 0.5 log units higher than that for mGAT1 (see Table 3, entry 6).

All of the 2-aminopyrrolidine-2-yl-acetic acid derivatives, that is, *rac*-(*l*)-**14b**, *rac*-(*u*)-**14b**, *rac*-(*l*)-**14c** and *rac*-(*u*)-**14c** showed only low to mediocre potencies. As far as there was any preference for one of the four transporters mGAT1–mGAT4, this was very small. This was also true for *rac*-(*l*)-**14b**. But this compound, *rac*-(*l*)-**14b** though exhibiting a diphenylbutenyl moiety favouring the inhibitory potency at mGAT1, showed its highest potency at mGAT3 (pIC_{50} = 4.94) followed by mGAT4 (pIC_{50} = 4.76; see Table 3, entry 9).

Finally, we also studied the influence of the 2-{[tris(4-methoxyphenyl)]methoxy}ethyl group (known to generally increase the potency at and selectivity for mGAT4)[26] on the inhibitory activity of the core structures of 2-hydroxypyrrolidine-2-yl-acetic acids, *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**, at mGAT1–mGAT4. The N-substituted compound *rac*-(*l*)-**13d** exhibited a pIC_{50} of 5.24 at mGAT4 with a selectivity of >17 for mGAT4 against mGAT1 and mGAT2 and also a slight selectivity against mGAT3 the pIC_{50} at which transporter amounted to 4.77 (see Table 3, entry 7). Though the inhibitory potency of *rac*-(*l*)-**13d** at mGAT4 is quite satisfying, the N-substitution has given rise to only a small increase of the former (compare Table 2, entry 7 with Table 3, entry 7). In case of *rac*-(*u*)-**13a**, the same N-substituent was far more effective causing an increase of the pIC_{50} for mGAT4 by more than two log units (compare Table 2, entry 8 to Table 3 entry 8). In addition *rac*-(*u*)-**13d** exhibited an improved selectivity of >15 for mGAT4 as compared to mGAT1, mGAT2 and mGAT3.

Compounds **13b–c** and **14c** have also been characterized with regard to their binding affinities to hGAT-1 and mGAT1 in the respective MS Binding Assays (Table 4). The binding affinities

Table 4
Affinities of selected *N*-arylalkyl derivatives toward HEK hGAT-1 and HEK mGAT1 membrane preparations given as pK_i values (mean ± SEM, n = 3) obtained from three independant experiments

Entry	Compounds	pK_i*	
		hGAT-1	mGAT1
1	Tiagabine (*R*)-**6c**	7.32 ± 0.11	7.43 ± 0.06
2	*rac*-(*l*)-**13b**	5.40 ± 0.01	5.41 ± 0.03
3	*rac*-(*u*)-**13b**	6.67 ± 0.01	6.84 ± 0.03
4	*rac*-(*l*)-**13c**	5.96 ± 0.07	5.97 ± 0.05
5	*rac*-(*u*)-**13c**	**7.18 ± 0.03**	**6.99 ± 0.04**
6	*rac*-(*l*)-**14c**	4.69 ± 0.03	4.76 ± 0.03
7	*rac*-(*u*)-**14c**	4.72 ± 0.03	4.43 ± 0.02

The bold values represent the most potent and highly affine compounds.

* K_i: Affinity constant measured with competitive MS Binding Assay[55] based on unlabelled NO711 as marker.

obtained for hGAT-1 and mGAT1 (given as pK_i in Table 4) were all very similar for the individual compounds and in good accord with those from the [^{3}H]GABA uptake assay for the same transporter (given as pIC_{50} in Table 3). The pK_i values were generally somewhat higher (~0.5–1.5 log units, see Table 4) than the corresponding pIC_{50} values which is a general phenomenon and thought to be due to the varying NaCl concentrations in the buffer system used in the uptake and binding assays as outlined previously.[55] Interestingly, the pK_i value of *rac*-(*u*)-**13c** for hGAT-1 (pK_i 7.18, Table 4, entry 5) is very close to the pK_i value of Tiagabine ((*R*)-**6c**, pK_i = 7.32, Table 4, entry 5), but markedly lower for mGAT1 (mGAT1: Tiagabine (*R*)-**6c**, pK_i = 7.43; *rac*-(*u*)-**13c**, pK_i = 6.99; see Table 4, entries 1 and 5). Similarly, *rac*-(*u*)-**13c** had reached a pIC_{50} value in the GABA uptake assay for hGAT-1 of 6.14 (Table 3, entry 6) and thus close to that for Tiagabine ((*R*)-**6c**) in the same test system (hGAT-1: pIC_{50} = 6.42), whereas for the mouse GABA transporter mGAT1 the potency of *rac*-(*u*)-**13c** was again distinctly

lower than that of Tiagabine ((*R*)-**6c**) (mGAT1: *rac*-(*u*)-**13c** pIC_{50} = 5.67, (*R*)-**6c** pIC_{50} = 6.88). This represents a clear example that test results obtained for mouse GAT1 do not necessarily correspond closely to those for the human protein.

To summarize, the N-substituted 2-hydroxy pyrrolidine-2-yl-acetic acids [*rac*-(*l*)-**13b**, *rac*-(*u*)-**13b**, *rac*-(*l*)-**13c** and *rac*-(*u*)-**13c**] are potent and selective inhibitors and binders at mGAT1 and hGAT-1, the (*u*)-configured diastereoisomers showing slightly higher affinity and activity than their (*l*)-configured counterparts. Remarkably, N-alkylation with groups known to enhance selectivity for mGAT1 gave rise to a shift in the selectivity of the parent compound (*rac*)-(*l*)-**13a** (pIC_{50} mGAT4/pIC_{50} mGAT1 >71) in favour of mGAT4 to a selectivity in favour of mGAT1 for the substituted compounds (see e.g., *rac*-(*l*)-**13c**, Table 3, entry 5). Above results also confirm the general ability of the 2-{[tris(4-methoxyphenyl)]methoxy}ethyl moiety to enhance inhibitory potency at mGAT4, the N-substituted compounds *rac*-(*l*)-**13d** and *rac*-(*u*)-**13d** showing higher pIC_{50} values for inhibition of mGAT4 than the parent compounds *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**.

3. Conclusions

In summary, a new series of core structures delineated from pyrrolidine-2-yl-acetic acid by substituting the 2-position with lipophilic groups like methyl, ethyl, vicinal dimethyl, 1,3-propandiyl and with hydrophilic moieties like the hydroxy and the amino groups has been studied for their potency as GABA uptake inhibitors. For the synthesis of these compounds a strategy comprising electrophilic α-amidoalkylation reaction based on *N*-acylpyrrolidinium ions as key steps was followed. Pharmacological evaluation of the activity of the parent compounds at mouse GABA uptake proteins mGAT1, mGAT2, mGAT3 and mGAT4 revealed that lipophilic groups like ethyl, vicinal methyl or the sterically more demanding 1,3-propandiyl group at the 2-position of the pyrrolidine-2-yl-acetic acids are not well tolerated. The amino acids *rac*-(*l*)-**13a** and *rac*-(*l*)-**14a** resulting from addition of hydrophilic moieties, that is, hydroxy or amino groups, respectively, exhibited an improved selectivity profile for mGAT3 and mGAT4 in GABA uptake assays associated with an increase in potencies for these proteins in comparison to the unsubstituted core amino acids, the homoprolines (*R*)-**8a** and (*S*)-**8a**.

The 2-hydroxypyrrolidine-2-yl-acetic acid derivatives *rac*-(*l*)-**13b**, *rac*-(*u*)-**13b**, *rac*-(*l*)-**13c** and *rac*-(*u*)-**13c** displaying a 4,4-diphenyl-3-butenyl group or the 4,4-bis(3-methylthiophen-2-yl) but-3-enyl group at the ring nitrogen exhibited high selectivity for mGAT1 in GABA uptake assays in addition to high potencies for this target in comparison to the core structures *rac*-(*l*)-**13a** and *rac*-(*u*)-**13a**. The 2-hydroxypyrrolidine-2-yl-acetic acid derivative *rac*-(*u*)-**13c** was the most potent GAT1 inhibitor with pIC_{50} values of 5.67 and 6.14 at mGAT1 and hGAT-1, respectively. In MS Binding Assays, this compound, *rac*-(*u*)-**13**, exhibited pK_i values of 6.99 and 7.18 for mGAT1 and hGAT-1, respectively, the value for the human GABA transporter being close to that observed for Tiagabine (*R*)-**6c** at the hGAT-1 protein (pK_i 7.32).

In addition, the N-alkylated (*u*)-configured diastereomers *rac*-(*u*)-**13b** and *rac*-(*u*)-**13c** exhibited higher potencies at and selectivities for mGAT1 than their corresponding (*l*)-configured counterparts in the GABA uptake assays. The N-substituted 2-aminopyrrolidine-2-yl-acetic acids showed negligible to no selectivity.

By introduction of 2-{[tris(4-methoxyphenyl)methoxy}ethyl residue known to enhance mGAT4 selectivity to the *N*-atom of the pyrrolidine ring of the two racemic diastereomers of 2-hydroxy-pyrrolidine-2-yl-acetic acid gave *rac*-(*l*)-**13d** and *rac*-(*u*)-**13d** with a reasonably high potency of 5.24 and 5.18 (pIC_{50} in GABA uptake assays), respectively, of which the latter showed also an acceptable selectivity of >15 for mGAT4 against mGAT1, mGAT2 and mGAT3.

Overall, the structure–activity relationships so far established for GAT inhibitors have been distinctly widened by the results presented in this study. As demonstrated the pyrrolidine-2-yl-acetic acid moieties with a hydroxy or an amino group in the 2-position represent novel and promising core structures for the development of new GAT inhibitors which may show altered physicochemical properties.

4. Experimental

4.1. Chemistry

The melting points were determined on a Büchi melting point apparatus no. 510 (Dr. Tottoli) and are uncorrected. IR spectra were recorded with a Perkin Elmer FT-IR spectrometer Paragon 1600. NMR spectra were obtained with JEOL JNMR-GX 400 (400 MHz), JNM-ECP 400 (400 MHz) or JNM-ECP 500 (500 MHz) spectrometers, respectively. Tetramethylsilane was used as internal standard and in exceptional cases the known chemical shift of solvent traces was used to nominate the spectra. The NMR spectra were recalculated with NUTS, 2D version 2002 from Acorn NMR. MS spectra were recorded on a Hewlett Packard 5989 A mass spectrometer with 59980 B particle beam LC/MS interface. LC–MS/MS-Mass spectrometer API 2000 from Applied Bio systems was used for the MS-binding assays. HRMS spectra were obtained with a JEOL JMS 700 and a JEOL JMS GC-Mate II mass spectrometer. CHN-analyses were determined an HERAEUS Rapid or an ELEMENTAR Vario EL elemental analysator. Column chromatography (CC) was performed as flash chromatography with silica gel 60 (Merck, 0.040–0.063 mm) in the normal phase and silanised silica gel 60 (Merck, 0.063–0.200 mm) in the reversed-phase chromatography. Analytical HPLC comprised of L-6200, L-7100 or L7100 pump, L-4000 or L-7400, UV/Vis. and Diode Array detector L-7450 and L-4000-UV, Software D-7000 HPLC-System-Manager (Merck-Hitachi), LiChroCart® with LiChroSpher® 100 RP-18 cartridge (5 μm, 250 × 4 mm with precolumn 4 × 4 mm) and LiChrosorb® Si 60 (5 μm, 250 × 4 mm with precolumn 4 × 4 mm) (Merck). Preparative HPLC comprised of L-7150 pump, UV–VIS-detector L-4000, integrator D-2000 (Merck–Hitachi), LiChrosorb® Si 60 (7 μm, 250 × 25 mm) and LiChrosorb® RP-18 (7 μm, 250 × 25 mm). All reactions were carried out in vacuum dried glassware under nitrogen atmosphere. All reagents were used as commercially available. Solvents were dried prior to use. DME, THF, Et_2O, NEt_3, diisopropylamine, DIPEA, 1,4-dioxane, toluene were freshly distilled from sodium metal/benzophenone ketyl, CH_2Cl_2, $CDCl_3$, DMF, CH_3CN from CaH_2 and the alcohols CH_3OH, C_2H_5OH from Mg prior to use. The silyl ketene acetals *E*/*Z*-**22**,[39] *E*/*Z*-**23**,[41] **24**,[42] **25**,[43] *E*/*Z*-**26**[44] and *E*-**27**[45] were prepared according to the literature.[39,40]

4.1.1. (2*RS*)-1-[(Benzyloxy)carbonyl]-2-ethoxypyrrolidine [(*RS*)-**21**][37]

A solution of Na_2CO_3 (5.81 g, 54.8 mmol, 3.3 equiv) in 50 ml of water was added to a solution of 4-aminobutyraldehyde diethyl acetal (2.64 g, 16.6 mmol) in 50 ml of CH_2Cl_2 and cooled to 0 °C. Benzyl chloroformate (3 g, 17.6 mmol, 2.5 ml, 1.07 equiv) was added dropwise and the reaction mixture was stirred at rt for 3 h, after which the organic phase was separated and evaporated to dryness in vacuum. The residue **20**[38] was cooled to 0 °C and stirred with a solution of $Sc(OTf)_3$ (81 mg, 0.17 mmol, 1 mol %) in 166 ml of heptane/EtOAc (70:30). After 45 min, the reaction was warmed to rt and stirred for further 105 min followed by removal of solvent in vacuum. The crude product on purification by CC

(SiO_2, Isohexane/EtOAc/EDMA = 95:5:3) afforded 3.67 g (90%) of (*RS*)-**21** as colourless oil.

Bp: 94–96 °C/2.0 mbar {Lit.[37] bp.: 83 °C, 2 mmHg}. IR (KBr): $\tilde{\nu}$ = 2976, 2894, 1708, 1498, 1455, 1407, 1359, 1325, 1287, 1211, 1179, 1092, 1075, 971, 918, 772, 754, 698, 603, 567 cm^{-1}. ^1H NMR (500 MHz, $C_2D_2Cl_4$, 90 °C): δ = 1.14 (m, 3H, CH_3), 1.75–1.98 (m, 3H, CH_2–CH_2), 2.04–2.17 (m, 1H, CH_2–CH_2), 3.39–3.66 (m, 4H, N–CH_2, O–CH_2), 5.18 (dd, J = 22.7/12.8 Hz, 2H, CH_2–Ar), 5.30 (s, 1H, CH–O), 7.31–7.42 (m, 5H, H_{ar}) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 23.3 °C, TMS): δ = 15.27 (CH_3), 15.36 (CH_3), 21.78 (N–CH_2–CH_2), 22.72 (N–CH_2–CH_2), 32.35 (CH–CH_2), 32.89 (CH–CH_2), 45.73 (N–CH_2), 45.90 (N–CH_2), 63.15 (O–CH_2–CH_3), 63.82 (O–CH_2–CH_3), 66.85 (CH_2–Ar), 67.08 (CH_2–Ar), 87.09 (CH), 87.73 (CH), 127.84 (CH_{ar}), 128.00 (CH_{ar}), 128.05 (CH_{ar}), 128.47 (CH_{ar}), 136.55 (C_{ar}), 136.72 (C_{ar}), 154.87 (O–CO–N), 155.76 (O–CO–N) ppm. MS (CI, CH_5^+); m/z (%) = 250 (5) $[M+H]^+$, 204 (100), 160 (54), 114 (25). Anal. calcd for $C_{14}H_{19}NO_3$ (249.31): C, 67.45; H, 7.68; N, 5.62. Found: C, 67.03; H, 7.64; N, 5.54.

The spectral data for the compound (*RS*)-**21** were not given in the literature.[37]

4.1.2. General procedure for the α-amidoalkylation of the precursor (*RS*)-21 (GP1):[35]

The reported procedure for the α-amidoalkylation was modified in that the given amount of the precursor (*RS*)-**21**[37] and 1.7–2 equiv of the respective silylketene acetal (**22**–**27**)[39–45] dissolved in MeCN (c = 1.0 M) was stirred with $Sc(OTf)_3$ (10 mol %) at 0 °C. After the given time, the reaction was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted several times with CH_2Cl_2, dried over $MgSO_4$ and evaporated to dryness in vacuum. The residue was then purified by CC.

4.1.2.1. (2*RS*)-2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl} propionic acid methyl ester [*rac*-(*l*)-28][46] and (2*RS*)-2-{(2*RS*)-1-[(benzyloxy)carbonyl]-pyrrolidine-2-yl}propionic acid methyl ester [*rac*-(*u*)-28].

According to GP1, (*RS*)-**21** (705 mg, 2.83 mmol), (*E*/*Z*)-**22**[37] (776 mg, 4.84 mmol), MeCN (28.3 ml) and $Sc(OTf)_3$ (139 mg, 0.28 mmol) for 5.5 h. CC (SiO_2, isohexane/EtOAc = 85:15) afforded 603 mg (73%) of [*rac*-(*l*)-**28**]/[*rac*-(*u*)-**28**] in a diastereomeric ratio of 55:45 (^1H NMR) as colourless oil.

IR (KBr): $\tilde{\nu}$ = 2952, 2881, 1734, 1702, 1456, 1412, 1360, 1339, 1262, 1206, 1103, 770, 698, 668 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25.6 °C, TMS): δ = 1.00–1.18 (m, 3H, CH_3), 1.73–2.03 (m, 4H, N–CH_2–CH_2–CH_2), 2.79–2.89 (m, 0.16 × 1H, C*H*–CH_3), 3.07–3.18 (m, 0.48 × 1H, C*H*–CH_3), 3.25–3.41 (m, 1.36H, N–CH_2, C*H*–CH_3), 3.48–3.69 (m, 4H, O–CH_3, N–CH_2), 4.08 (ddd, J = 7.9/5.8/4.3 Hz, 0.45 × 1H, N–CH), 4.27 (dt, J = 8.2/4.9 Hz, 0.55 × 1H, N–CH), 5.08–5.20 (m, 2H, CH_2–Ar), 7.28–7.40 (m, 5H, H_{ar}) ppm. Rotamers existed but the ratio could not be determined. ^{13}C NMR (500 MHz, $CDCl_3$, 25.2 °C, TMS): δ = 10.39 (CH–CH_3), 10.50 (CH–CH_3), 13.99 (CH–CH_3), 14.12 (CH–CH_3), 22.98 (CH_2), 23.68 (CH_2), 23.80 (CH_2), 24.27 (CH_2), 26.84 (CH_2), 27.42 (CH_2), 28.80 (CH_2), 29.72 (CH_2), 40.90 (*C*H–CH_3), 41.39 (*C*H–CH_3), 41.93 (*C*H–CH_3), 42.49 (*C*H–CH_3), 46.74 (N–CH_2), 47.00 (N–CH_2), 47.20 (N–CH_2), 47.67 (N–CH_2), 51.66 (O–CH_3), 58.32 (N–CH), 58.99 (N–CH), 59.63 (N–CH), 60.43 (N–CH), 66.64 (CH_2–Ar), 66.93 (CH_2–Ar), 127.74 (CH_{ar}), 127.81 (CH_{ar}), 127.93 (CH_{ar}), 128.46 (CH_{ar}), 128.48 (CH_{ar}), 136.78 (C_{ar}), 136.99 (C_{ar}), 137.13 (C_{ar}), 154.86 (O–CO–N), 155.23 (O–CO–N), 174.66 (COO), 174.91 (COO), 175.16 (COO), 175.21 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 292 (100) $[M+H]^+$, 260 (18), 248 (27), 204 (14), 184 (20), 160 (17), 156 (21). HRMS (70 eV): calcd for $C_{16}H_{21}NO_4$ $[M]^+$, 291.1471; found: 291.1470.

The analytical data of the *rac*-(*l*)-diastereomers (^1H NMR) were in accord with the values reported in the literature for a pure like enantiomer.[46]

4.1.2.2. (2*RS*)-2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl}butyric acid methyl ester [*rac*-(*l*)-29] and (2*SR*)-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}butyric acid methyl ester [*rac*-(*u*)-29].

According to GP1, (*RS*)-**21** (105 mg, 0.42 mmol), [(*E*/*Z*)-**23**][41] (146 mg, 0.84 mmol), MeCN (4.2 ml) and $Sc(OTf)_3$ (22 mg, 44 μmol) for 1.5 h. CC (SiO_2, pentane/EtOAc = 95:5) afforded 99 mg (77%) of [*rac*-(*l*)-**29**]/[*rac*-(*u*)-**29**] in a diastereomeric ratio of 54/46 (^1H NMR) as colourless oil.

IR (KBr): $\tilde{\nu}$ = 2965, 2878, 1732, 1702, 1498, 1410, 1359, 1200, 1103, 990, 920, 769, 752, 698, 605 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 24.8 °C, TMS): δ = 0.79–0.95 (m, 3H, CH_2–CH_3), 1.30–2.01 (m, 6H, CH_2–CH_3, N–CH–CH_2-CH_2), 2.70–2.77 (m, 0.46 × 0.39 × 1H, CH–COO), 2.79–2.87 (m, 0.54 × 0.42 × 1H, CH–COO), 2.89–2.97 (m, 0.54 × 0.58 × 1H, CH–COO), 2.97–3.03 (m, 0.46 × 0.61 × 1H, CH–COO), 3.22–3.39 (m, 1H, N–CH_2), 3.46–3.68 (m, 4H, O–CH_3, N–CH_2), 4.03–4.11 (m, 0.46 × 1H, N–CH), 4.15–4.21 (m, 0.54 × 1H, N–CH), 5.07–5.22 (m, 2H, CH_2–Ar), 7.28–7.42 (m, 5H, H_{ar}) ppm. Rotamer ratios of the individual diastereomer from [*rac*-(*l*)-**29**] and [*rac*-(*u*)-**29**] at 24.8 °C were 58:42 und 61:39, respectively. ^{13}C NMR (500 MHz, $CDCl_3$, 25.8 °C, TMS): δ = 12.20 (CH_2–CH_3), 12.59 (CH_2–CH_3), 19.30 (CH_2), 19.62 (CH_2), 22.66 (CH_2), 23.00 (CH_2), 23.48 (CH_2), 23.82 (CH_2), 24.13 (CH_2), 27.00 (CH_2), 27.44 (CH_2), 28.14 (CH_2), 46.54 (N–CH_2), 46.79 (N–CH_2), 46.99 (N–CH_2), 47.45 (N–CH_2), 49.08 (*C*H–COO), 49.77 (*C*H–COO), 50.13 (*C*H–COO), 50.52 (*C*H–COO), 51.49 (O–CH_3), 51.52 (O–CH_3), 58.56 (N–CH), 58.89 (N–CH), 59.22 (N–CH), 59.65 (N–CH), 66.60 (CH_2–Ar), 66.65 (CH_2–Ar), 66.85 (CH_2–Ar), 67.02 (CH_2–Ar), 127.73 (CH_{ar}), 127.83 (CH_{ar}), 127.90 (CH_{ar}), 128.04 (CH_{ar}), 128.45 (CH_{ar}), 136.76 (C_{ar}), 137.01 (C_{ar}), 137.12 (C_{ar}), 154.93 (N–CO–O), 155.18 (N–CO–O), 174.08 (COO), 174.35 (COO), 174.73 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 306 (100) $[M+H]^+$, 274 (27), 262 (30), 204 (19), 198 (22), 170 (20), 160 (24), 128 (7). HRMS (70 eV): calcd for $C_{17}H_{23}NO_4$ $[M]^+$, 305.1627; found: 305.1622.

4.1.2.3. 2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl]-2-methylpropionic acid methyl ester [(*RS*)-30][57].

According to GP1, (*RS*)-**21** (52 mg, 0.21 mmol), **24**[42] (64 mg, 0.37 mmol), MeCN (5.6 ml) and $Sc(OTf)_3$ (11 mg, 21 μmol) for 1 h 40 min. CC (SiO_2, isohexane/EtOAc = 80:20) afforded 55 mg (87%) of [(*RS*)-**30**] as colourless crystals.

Mp: 55 °C. IR (KBr): $\tilde{\nu}$ = 2976, 2950, 1731, 1702, 1455, 1404, 1349, 1266, 1193, 1134, 987, 699 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 23.4 °C, TMS): δ = 1.15 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.67–1.88 (m, 3H, N–CH_2–CH_2, CH_2–CH), 1.91–2.04 (m, 1H, CH_2–CH), 3.22–3.30 (m, 1H, N–CH_2), 3.54–3.88 (m, 4H, O–CH_3, N–CH_2), 4.31–4.36 (m, 1H, N–CH), 5.11 (br s, 2H, CH_2–Ar), 7.28–7.39 (m, 5H, H_{ar}) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 21.3 °C, TMS): δ = 21.45 (C–CH_3), 21.98 (C–CH_3), 22.37 (C–CH_3), 23.69 (N–CH_2–CH_2), 24.34 (N–CH_2–CH_2), 27.29 (N–CH–CH_2), 28.18 (N–CH–CH_2), 47.39 (N–CH_2), 47.88 (*C*–COO), 51.91 (O–CH_3), 62.94 (N–CH), 63.60 (N–CH), 66.93 (CH_2–Ar), 127.84 (CH_{ar}), 127.93 (CH_{ar}), 128.45 (CH_{ar}), 136.92 (C_{ar}), 156.27 (O–CO–N), 177.26 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 306 (100) $[M+H]^+$, 274 (60), 262 (22), 204 (16), 198 (19), 170 (9), 160 (18). Anal. calcd for $C_{17}H_{23}NO_4$ (305.38): C, 66.86; H, 7.59; N 4.59. Found: C, 66.85; H, 7.58; N, 4.67.

The spectral data for the compound [(*RS*)-**30**] were not given in the literature.[57]

4.1.2.4. 1-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl}cyclobutane-1-carboxylic acid methyl ester [(*RS*)-31].

According to GP1, (*RS*)-**21** (55 mg, 0.22 mmol), **25** (71 mg, 0.38 mmol), MeCN (2.2 ml) and $Sc(OTf)_3$ (11 mg, 21 μmol) for 4 h. CC (SiO_2, isohexane/EtOAc = 80:20) afforded 51 mg (73%) of [(*RS*)-**31**] as colourless oil.

IR (KBr): $\tilde{\nu}$ = 2950, 2891, 1727, 1702, 1498, 1447, 1405, 1354, 1280, 1243, 1211, 1154, 1114, 1029, 968, 918, 770, 753, 698,

606 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 24.7 °C, TMS): δ = 1.57–2.00 (m, 6H, C_q–CH_2–CH_2, N–CH_2–CH_2–CH_2), 2.05–2.42 (m, 4H, C_q–CH_2), 3.27–3.35 (m, 1H, N–CH_2), 3.56–3.78 (m, 4H, O–CH_3, N–CH_2), 4.22–4.46 (m, 0.42 × 1H, N–CH), 4.35–4.46 (m, 0.58 × 1H, N–CH), 5.06–5.24 (m, 2H, CH_2–Ar), 7.28–7.40 (m, 5H, H_{ar}) ppm. Rotamer ratio (24.7 °C) 58:42. ^{13}C NMR (500 MHz, $CDCl_3$, 25.6 °C, TMS): δ = 16.42 (CH_2), 23.40 (N–CH_2–CH_2), 24.06 (N–CH_2–CH_2), 27.70 (CH_2), 28.58 (CH_2), 29.22 (CH_2), 29.48 (CH_2), 47.73 (N–CH_2), 48.13 (N–CH_2), 51.99 (CH_3), 53.60 (*C*–COO), 53.78 (*C*–COO), 61.69 (N–CH), 62.23 (N–CH), 66.92 (CH_2–Ar), 127.82 (CH_{ar}), 127.93 (CH_{ar}), 128.14 (CH_{ar}), 128.20 (CH_{ar}), 128.44 (CH_{ar}), 136.61 (C_{ar}), 136.99 (C_{ar}), 155.95 (O–CO–N), 156.43 (O–CO–N), 176.72 (COO) ppm. MS (CI, CH_5^+); *m*/*z* (%) = 318 $[M+H]^+$ (100), 286 (50), 274 (23), 225 (8), 210 (20), 204 (14), 182 (11), 160 (16), 105 (13). MS (EI, 70 eV); *m*/*z* (%) = 317 (0) $[M]^+$, 204 (17), 182 (5), 160 (28), 91 (100), 65 (9). HRMS (70 eV): calcd for $C_{18}H_{23}NO_4$ $[M]^+$, 317.1627; found: 317.1617. Anal. calcd for $C_{18}H_{23}NO_4$ (317.39): C, 68.12; H, 7.30; N, 4.41. Found: C, 68.37; H, 6.85; N, 4.43.

4.1.2.5. (2*RS*)-2-[(*tert*-Butyldimethylsilanyl)oxy]-2-{(2*RS*)-1-(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-32][49] and (2*SR*)-2-[(*tert*-butyldimethyl-silanyl)oxy]-2-{(2*RS*)-1-(benzyloxy)carbonyl]pyrrolidine-2-yl}-acetic acid methyl ester [*rac*-(*u*)-32].

According to GP1, (*RS*)-**21** (1.58 g, 6.33 mmol), (*E*/*Z*)-**26**[44] (4.88 g, 12.7 mmol), MeCN (63 ml) and $Sc(OTf)_3$ (0.62 g, 1.26 mmol) for 19 h. CC (SiO_2, isohexane/EtOAc = 90:10) afforded 1.72 g (67%) of [*rac*-(*l*)-**32**][49]/[*rac*-(*u*)-**32**] in a diastereomeric ratio of 45/55 (^1H NMR).

In order to obtain the analytical data, the experiment was repeated and the crude product was subjected to repeated CC (SiO_2, isohexane/EtOAc = 70:30 followed by SiO_2, pentane/EtOAc = 95:5). This afforded 11% of [*rac*-(*l*)-**38**]/[*rac*-(*u*)-**38**], 15% of [*rac*-(*l*)-**32**][49] as colourless oil and 12% of [*rac*-(*u*)-**32**] also as colourless oil.

[*rac*-(*l*)-**32**]:[49] IR (KBr): $\tilde{\nu}$ = 2954, 2885, 2857, 1756, 1734, 1702, 1413, 1360, 1257, 1201, 1145, 1114, 1060, 1004, 913, 837, 779, 697, 605 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 22.1 °C): δ = −0.14 (s, 0.35 × 3H, Si–CH_3), −0.07 (s, 0.65 × 3H, Si–CH_3), −0.06 (s, 0.35 × 3H, Si–CH_3), 0.02 (s, 0.65 × 3H, Si–CH_3), 0.85 (s, 0.35 × 9H, C–CH_3), 0.87 (s, 0.65 × 9H, C–CH_3), 1.88–1.96 (m, 2H, CH_2), 1.93–2.07 (m, 2H, CH_2), 3.35–3.42 (m, 1H, N–CH_2), 3.46–3.58 (m, 1H, N–CH_2), 3.70 (s, 0.35 × 3H, O–CH_3), 3.71 (s, 0.65 × 3H, O–CH_3), 4.17–4.24 (m, 1H, N–CH), 4.66 (d, *J* = 2.7 Hz, 0.35 × 1H, CH–O), 4.93 (d, *J* = 2.7 Hz, 0.65 × 1H, CH–O), 5.08–5.24 (m, 2H, CH_2–Ar), 7.28–7.43 (m, 5H, H_{ar}) ppm. Rotamer ratio (22.1 °C) 65:35. ^{13}C NMR (500 MHz, $CDCl_3$, 25.4 °C): δ = −5.24 (Si–CH_3), −5.20 (Si–CH_3), −5.14 (Si–CH_3), −4.99 (Si–CH_3), 18.46 (C–CH_3), 24.19 (CH_2), 24.85 (CH_2), 25.71 (CH_2), 25.93 (C–CH_3), 26.00 (C–CH_3), 26.70 (CH_2), 47.58 (N–CH_2), 48.08 (N–CH_2), 52.09 (O–CH_3), 52.15 (O CH_3), 60.16 (N–CH), 60.72 (N–CH), 67.04 (CH_2–Ar), 67.14 (CH_2–Ar), 71.75 (CH–O), 73.23 (CH–O), 128.27 (CH_{ar}), 128.37 (CH_{ar}), 128.44 (CH_{ar}), 128.77 (CH_{ar}), 128.84 (CH_{ar}), 137.07 (C_{ar}), 137.24 (C_{ar}), 154.77 (O–CO–N), 154.96 (O–CO–N), 172.84 (COO), 173.04 (COO) ppm. MS (CI, CH_5^+); *m*/*z* (%) = 408 (100) [M+H]+, 392 (8), 364 (5), 350 (18), 300 (13), 204 (32), 160 (29). HRMS (70 eV): calcd for $C_{21}H_{33}NO_5Si$ $[M]^+$, 407.2128; found: 407.2167. Anal. calcd for $C_{21}H_{33}NO_5Si$ (407.59): C, 61.88; H, 8.16; N 3.44. Found: C, 62.16; H, 8.20; N 3.52.

The spectral data for the compound [*rac*-(*l*)-**32**] were not given in the literature.[49]

[*rac*-(*u*)-**32**]: IR (KBr): $\tilde{\nu}$ = 2952, 2930, 2886, 2857, 1752, 1705, 1412, 1360, 1339, 1253, 1207, 1114, 1006, 986, 837, 778, 697, 604 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 24 °C): δ = −0.10 (s, 0.45 × 3H, Si–CH_3), −0.05 (s, 0.45 × 3H, Si–CH_3), 0.08 (s, 0.55 × 3H, Si–CH_3), 0.08 (s, 0.55 × 3H, Si–CH_3), 0.84 (s, 0.45 × 9H, C–CH_3), 0.91 (s, 0.55 × 9H, C–CH_3), 1.65–1.84 (m, 2H, CH_2), 1.93–2.16 (m, 2H, CH_2), 3.30–3.38 (m, 1H, –CH_2), 3.44–3.57 (m, 1H, N–CH_2), 3.60 (s, 0.45 × 3H, O–CH_3), 3.67 (s, 0.55 × 3H, O–CH_3), 4.03–4.07 (m, 0.45 × 1H, N–CH), 4.14–4.19 (m, 0.55 × 1H, N–CH), 4.47 (d, *J* = 4.6 Hz, 0.45 × 1H, CH–O), 4.64 (d, *J* = 4.1 Hz, 0.55 × 1H, CH–O), 5.11–5.18 (m, 2H, CH_2–Ar), 7.27–7.40 (m, 5H, H_{ar}) ppm. Rotamers ratio (24 °C) 55:45. ^{13}C NMR (500 MHz, $CDCl_3$, 26 °C): δ = −5.15 (Si–CH_3), −4.93 (Si–CH_3), −4.77 (Si–CH_3), 18.46 (C–CH_3), 18.53 (C–CH_3), 23.16 (CH_2), 24.09 (CH_2), 25.95 (C–CH_3), 26.06 (C–CH_3), 27.05 (CH_2), 27.51 (CH_2), 47.41 (N–CH_2), 47.78 (N–CH_2), 60.59 (N–CH), 61.24 (N–CH), 66.93 (CH_2–Ar), 67.51 (CH_2–Ar), 72.05 (CH–O), 72.19 (CH–O), 127.91 (CH_{ar}), 128.15 (CH_{ar}), 128.42 (CH_{ar}), 128.68 (CH_{ar}), 128.77 (CH_{ar}), 128.84 (CH_{ar}), 136.94 (C_{ar}), 137.42 (C_{ar}), 155.08 (O–CO–N), 155.39 (O–CO–N), 172.87 (COO), 173.02 (COO) ppm. MS (CI, CH_5^+); *m*/*z* (%) = 408 (100) $[M+H]^+$, 392 (5), 364 (12), 350 (9), 300 (15), 204 (21), 160 (19). HRMS (70 eV): calcd for $C_{21}H_{33}NO_5Si$ $[M]^+$, 407.2128; found: 407.2140. Anal. calcd for $C_{21}H_{33}NO_5Si$ (407.59): C, 61.88; H, 8.16; N, 3.44. Found: C, 61.84; H, 8.21; N 3.42.

4.1.2.6. (2*RS*)-2-Amino-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-33][50] und (2*SR*)-amino-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-33][50].

According to GP1, (*RS*)-21 (830 mg, 3.33 mmol), (*E*)-27[45] (2.01 g, 6.6 mmol), MeCN (33 ml) and $Sc(OTf)_3$ (163 mg, 0.332 mmol) for 19 h. Purification and separation of diastereoisomers through multiple CC (SiO_2, pentane/EtOAc/EDMA = 50:50:2) afforded 217 mg (22%) of *rac*-(*l*)-33 and 310 mg (32%) of *rac*-(*u*)-33. The analytical data of both the diastereoisomers were in accord with the literature values.[50]

4.1.3. (2*RS*)-2-Hydroxy-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-38][50] and (2*SR*)-Hydroxy-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-38]

According to GP1, (*RS*)-**21** (1.86 g, 7.45 mmol), (*E*/*Z*)-**26**[43] (4.12 g, 14.9 mmol), MeCN (75 ml) and $Sc(OTf)_3$ (0.37 g, 0.75 mmol) for 19 h. After the usual work up, a solution of tetrabutylammonium fluoride trihydrate (5.92 g, 18.8 mmol) in THF (75 ml) was added to the product [*rac*-(*l*)-**32**]/[*rac*-(*u*)-**32**] and stirred at rt for 1 h. The reaction was quenched by adding 200 ml of CH_2Cl_2 and washed with NaOH (100 ml, 2 M). The organic phase was dried over $MgSO_4$ and concentrated to dryness. CC (SiO_2, isohexane/EtOAc = 60:40) of the crude product afforded 2.07 g (95%) of the diastereomeric mixture [*rac*-(*l*)-**38**]/[*rac*-(*u*)-**38**] with diastereomeric ratio being 39:61 (^1H NMR). Separation of the diastereomeric mixture by repeated CC (SiO_2, isohexane/EtOAc = 70:30) afforded 784 mg (36%) of [*rac*-(*l*)-**38**][50] as colourless oil, the analytical data of which were in accord with the reported values in the literature[50] and 592 mg (27%) of [*rac*-(*u*)-**38**] also a colourless oil.

[*rac*-(*u*)-**38**]: IR (KBr): $\tilde{\nu}$ = 3443, 3032, 2953, 2887, 1741, 1697, 1414, 1358, 1274, 1211, 1108, 1010, 766, 698 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 23.4 °C, TMS): δ = 1.77–2.16 (m, 4H, N–CH_2–CH_2–CH_2), 2.89 (br s, 0.23 × 1H, OH), 3.46–3.64 (m, 1.77H, N–CH_2, OH), 3.67 (m, 3H, O–CH_3), 4.16–4.30 (m, 2H, N–CH, CH–O), 4.95–5.28 (m, 2H, CH_2–Ar), 7.27–7.39 (m, 5H, H_{ar}) ppm. Rotamers ratio (23.4 °C) 77:23. ^{13}C NMR (400 MHz, $CDCl_3$, 20.7 °C): δ = 23.19 (N–CH_2–CH_2), 24.14 (N–CH_2–CH_2), 28.86 (N–CH–CH_2), 29.69 (N–CH–CH_2), 47.61 (N–CH_2), 48.06 (N–CH_2), 52.42 (O–CH_3), 52.73 (O–CH_3), 59.29 (N–CH), 60.30 (N–CH), 67.18 (CH_2–Ar), 72.58 (CH–OH), 73.74 (CH–OH), 127.89 (CH_{ar}), 128.09 (CH_{ar}), 128.56 (CH_{ar}), 136.64 (C_{ar}), 154.97 (O–CO–N), 156.40 (O–CO–N), 173.68 (COO), 173.91 (COO) ppm. MS (CI, CH_5^+); *m*/*z* (%) = 294 (93) $[M+H]^+$, 250 (79), 204 (11), 186 (100), 160 (16), 158 (10). HRMS (DEI+): calcd for $C_{15}H_{19}NO_5$ $[M]^+$, 293.1263; found: 293.1249.

4.1.4. General procedure for the preparation of Cbz-protected primary and secondary amines (GP2)

The given amount of amine together with $NaHCO_3$ (2.5 equiv) was dissolved in THF/H_2O-mixture (1:1, c = 0.1 M) and cooled to 0 °C. Benzyl chloroformate (1.1 equiv) was then added dropwise to the cooled solution. After the described reaction time, the mixture was extracted several times with a suitable organic solvent after which the organic phase was dried over $MgSO_4$, evaporated to dryness in vacuum followed by CC.

4.1.4.1. (2*RS*)-2-[(Benzyloxycarbonyl)amino]-2-{(2*RS*)-1-[(benzyloxy)carbonyl]-pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-40]. According to GP2, *rac*-(*l*)-**33** (51 mg, 0.17 mmol), $NaHCO_3$ (37 mg, 0.43 mmol), benzyl chloroformate (32 mg, 0.19 mmol, 33 µl), THF (0.9 ml), H_2O (0.9 ml) and the reaction time being 2.5 h. The extraction with CH_2Cl_2 (3 × 10 ml) and work up was done after acidifying the reaction mixture to pH = 1 with 2 M HCl. CC (SiO_2, isohexane/EtOAc = 75:25) of the crude product afforded 71 mg (96%) of *rac*-(*l*)-**40** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3367, 3032, 2953, 2888, 1725, 1702, 1528, 1454, 1415, 1358, 1338, 1212, 1100, 1040, 917, 740, 698, 603 cm^{-1}. ^1H NMR (400 MHz, $C_6D_5NO_2$, 140 °C): δ = 1.76–2.04 (m, 2H, N–CH_2–CH_2), 2.05–2.16 (m, 2H, N–CH–CH_2), 3.37–3.45 (m, 1H, N–CH_2), 3.62–3.70 (m, 1H, N–CH_2), 3.72 (s, 3H, O–CH_3), 4.35–4.41 (m, 1H, N–CH–CH_2), 4.98 (dd, J = 8.8/3.5 Hz, 1H, CH–COO), 5.13–5.30 (m, 4H, CH_2–Ar), 6.13 (br s, 1H, NH), 7.23–7.46 (m, 10H, H_{ar}) ppm. ^{13}C NMR (400 MHz, MeOD, 20.3 °C): δ = 24.38 (CH_2), 25.03 (CH_2), 28.41 (CH_2), 28.69 (CH_2), 48.17 (N–CH_2), 48.56 (N–CH_2), 53.11 (O–CH_3), 53.18 (O–CH_3), 57.21 (*C*H–COO), 57.33 (*C*H–COO), 60.04 (N–CH–CH_2), 60.74 (N–*C*H–CH_2), 67.96 (CH_2–Ar), 68.28 (CH_2–Ar), 68.80 (CH_2–Ar), 129.15 (CH_{ar}), 129.18 (CH_{ar}), 129.31 (CH_{ar}), 129.75 (CH_{ar}), 129.79 (CH_{ar}), 138.11 (C_{ar}), 138.48 (C_{ar}), 156.69 (O–CO–N), 157.08 (O–CO–N), 158.78 (O–CO–N), 158.92 (O–CO–N), 172.50 (COO), 172.77 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 427 (100) $[M+H]^+$, 383 (60), 319 (32), 275 (44), 204 (28), 185 (29), 160 (15). Anal. calcd for $C_{23}H_{26}N_2O_6$ (426.47): C, 64.78; H, 6.15; N, 6.57. Found: C, 64.83; H, 6.23; N, 6.49.

4.1.4.2. (2*SR*)-2-[(Benzyloxycarbonyl)amino]-2-{(2*RS*)-1-[(benzyloxy)carbonyl]-pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-40]. According to GP2, *rac*-(*u*)-**33** (151 mg, 0.35 mmol), $NaHCO_3$ (75 mg, 1.04 mmol), benzyl chloroformate (67 mg, 0.39 mmol, 67 µl), THF (1.8 ml), H_2O (1.8 ml) and the reaction time being 2 h. The extraction with CH_2Cl_2 (3 × 10 ml) and work up was done after acidifying the reaction mixture to pH = 1 with 2 M HCl. CC (SiO_2, isohexane/EtOAc = 70:30) of the crude product afforded 191 mg (87%) of *rac*-(*u*)-**40** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3420, 3032, 2953, 2888, 1704, 1519, 1454, 1413, 1358, 1337, 1212, 1102, 1059, 916, 740, 698, 604 cm^{-1}. ^1H NMR (400 MHz, $C_6D_5NO_2$, 140 °C): δ = 1.79–1.99 (m, 2H, N–CH_2–CH_2), 2.04–2.12 (m, 2H, N–CH–CH_2), 3.39–3.47 (m, 1H, N–CH_2), 3.59–3.67 (m, 1H, N–CH_2), 3.74 (s, 3H, O–CH_3), 4.44–4.50 (m, 1H, N–CH), 4.67 (dd, J = 8.1/6.2 Hz, 1H, CH–COO), 5.16–5.27 (m, 4H, CH_2–Ar), 5.94–6.06 (m, 1H, NH), 7.23–7.44 (m, 10H, H_{ar}) ppm. MS (CI, CH_5^+); m/z (%) = 427 (100) $[M+H]^+$, 383 (69), 319 (24), 293 (10), 275 (24), 204 (24), 185 (13). Anal. calcd for $C_{23}H_{26}N_2O_6$ (426.47): C, 64.78; H, 6.15; N, 6.57. Found: C, 64.78; H, 6.25; N, 6.54.

4.1.5. General procedure for the hydrogenolytic removal of Cbz-protecting groups (GP3)

The reactant was treated with 10% m/m of Pd/C in the given amount of solvent (0.03–0.1 M) and when required acid was added. The reaction mixture was hydrogenated at rt under the given pressure and duration, after which it was filtered several times and the solvent evaporated to dryness in vacuum.

4.1.5.1. (2*SR*)-2-Amino-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid methyl ester·2HOAc [*rac*-(*u*)-42·2HOAc]. According to GP3 (method C), *rac*-(*u*)-**40** (152 mg, 0.52 mmol), Pd/C (16.6 mg), HOAc (68.5 mg, 1.14 mmol, 65.5 µl), MeOH (10.4 ml) and the reaction time being 1 h. This afforded 104 mg (72%) of the acetic acid salt of the free amine *rac*-(*u*)-**42**·2HOAc as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3383, 2956, 1739, 1561, 1402, 1014, 651 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 24.1 °C, TMS): δ = 1.74–1.83 (m, 1H, N–CH–CH_2), 1.87–2.08 (m, 9H, N–CH–CH_2, N–CH_2–CH_2, CH_3–COO), 3.16 (t, J = 7.1 Hz, 2H, N–CH_2), 3.56–3.62 (m, 2H, N–*C*H–CH_2, CH–COO), 3.76 (s, 3H, O–CH_3), 6.00 (br s, 5H, NH) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 26.5 °C, TMS): δ = 22.81 (CH_3–COO), 24.71 (N–CH_2–CH_2), 28.38 (N–CH–CH_2), 45.12 (N–CH_2), 52.44 (O–CH_3), 56.27 (*C*H–COO), 60.79 (N–*C*H–CH_2), 173.56 (*C*H–COO), 177.16 (CH_3-*C*OO) ppm. MS (ESI); m/z (%) = 159 (100) $[M+H]^+$, 142 (46). HRMS (ESI): calcd for $C_7H_{15}N_2O_2$ $[M+H]^+$, 159.1134; found: 159.1130.

4.1.5.2. (2*RS*)-2-Hydroxy-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid methyl ester·HOAc [*rac*-(*l*)-45·HOAc]. According to GP3 with *rac*-(*l*)-**38** (40 mg, 0.14 mmol) in MeOH (2.8 ml), Pd/C (8 mg) and HOAc (9.0 µl, 0.15 mmol) at rt for 1 h afforded 26.8 mg (89%) of *rac*-(*l*)-**45**·HOAc as colourless crystals.

Mp: 81 °C. IR (KBr): $\tilde{\nu}$ = 3397, 2973, 2769, 1749, 1566, 1407, 1297, 1233, 1133, 1085, 1023, 930, 653 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 17.6 °C, TMS): δ = 1.83–2.09 (m, 7H, N–CH_2–*C*H$_2$–*C*H$_2$, CH_3–COO), 3.16–3.20 (m, 2H, N–CH_2), 3.78 (s, 3H, O–CH_3), 3.89 (td, J = 7.7/2.9 Hz, 1H, N–CH), 4.69 (d, J = 2.9 Hz, 1H, *C*H–OH) ppm. ^{13}C NMR (500 MHz, MeOD, 25.4 °C, TMS): δ = 23.80 (*C*H$_3$–COO), 25.24 (CH_2), 25.64 (CH_2), 47.38 (N–CH_2), 53.01 (O–CH_3), 61.99 (N–CH), 70.09 (CH–OH), 173.10 (CH–*C*OO), 179.96 (CH_3–*C*OO) ppm. MS (CI, CH_5^+); m/z (%) = 160 (100) $[M+H]^+$, 145 (11). HRMS (FAB, NBA): calcd for $C_7H_{14}NO_3$ $[M+H]^+$, 160.0974; found: 160.0980.

4.1.5.3. (2*SR*)-2-Hydroxy-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid methyl ester·HOAc [*rac*-(*u*)-45·HOAc]. According to GP3 with *rac*-(*u*)-**38** (101 mg, 0.34 mmol) in MeOH (5 ml), Pd/C (14 mg) and HOAc (22 µl, 0.38 mmol) at rt for 1 h to afford 72.3 mg (96%) of *rac*-(*u*)-**45**·HOAc as colourless crystals.

Mp: 83 °C. IR (KBr): $\tilde{\nu}$ = 2961, 1741, 1637, 1560, 1406, 1228, 1126, 1085, 652 cm^{-1}. ^1H NMR (500 MHz, MeOD, 24.3 °C): δ = 1.90 (CH_3–COO), 1.91–2.03 (m, 2H, N–CH_2–CH_2, N–CH–CH_2), 2.03–2.11 (m, 1H, N–CH_2–CH_2), 2.13–2.20 (m, 1H, N–CH–CH_2), 3.23–3.27 (m, 2H, N–CH_2), 3.79 (s, 3H, O–CH_3), 3.83 (ddd, J = 8.8/7.7/5.2 Hz, 1H, N–CH), 4.35 (d, J = 5.2 Hz, 1H, *C*H–OH) ppm. ^{13}C NMR (500 MHz, MeOD, 20.1 °C): δ = 23.93 (*C*H$_3$–COO), 25.33 (CH_2), 28.47 (CH_2), 47.18 (N–CH_2), 53.30 (O–CH_3), 62.88 (N–CH), 71.11 (CH–OH), 173.44 (*C*OOCH$_3$), 179.95 (CH_3–*C*OO) ppm. MS (CI, CH_5^+); m/z (%) = 160 (100) $[M+H]^+$. HRMS (FAB, NBA): calcd for $C_7H_{14}NO_3$ $[M+H]^+$, 160.0974; found: 160.0984.

4.1.5.4. (2*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid methyl ester·HOAc [*rac*-(*l*)-50·HOAc]. According to GP3 with *rac*-(*l*)-**49** (48 mg, 0.12 mmol), Pd/C (11.4 mg), HOAc (8 µl, 0.14 mmol) and MeOH (5 ml) at rt for 1 h afforded 38 mg (99%) of *rac*-(*l*)-**50**·HOAc as colourless crystals.

Mp: 98 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3378, 2978, 1742, 1713, 1393, 1367, 1290, 1252, 1168, 1020, 870 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 23.7 °C, TMS): δ = 1.45 (s, 9H, C–CH_3), 1.64–1.73 (m, 1H, N–CH–CH_2), 1.80–1.93 (m, 3H, N–CH–CH_2, N–CH_2–CH_2), 2.01 (s, 3H, CH_3–COO), 2.95–3.03 (m, 1H, N–CH_2), 3.03–3.12 (m, 1H, N–CH_2),

3.72–3.79 (m, 4H, N–C*H*–CH_2, O–CH_3), 4.48–4.54 (m, 1H, CH–COO), 5.72 (d, J = 5.5 Hz, 1H, NH), 6.82 (br s, 2H, NH_2^+) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 27 °C, $CDCl_3$): δ = 22.47 (*C*H_3–COO), 25.28 (CH_2), 27.15 (CH_2), 28.23 (C–*C*H_3), 46.25 (N–CH_2), 52.50 (O–CH_3), 56.17 (*C*H–COO), 60.06 (N–*C*H–CH_2), 80.38 (*C*–CH_3), 156.03 (N–CO–O), 171.58 (CH–*C*OO), 176.59 (CH_3–*C*OO) ppm. MS (ESI); m/z (%) = 259 (70) $[M+H]^+$, 245 (23), 217 (13), 203 (100), 189 (33), 159 (28), 142 (28). HRMS (FAB, NBA): calcd for $C_{12}H_{23}N_2O_4$ $[M+H]^+$, 259.1658; found: 259.1656.

4.1.5.5. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid methyl ester·HOAc [*rac*-(*u*)-50·HOAc]. According to GP3 with *rac*-(*u*)-**49** (194 mg, 0.49 mmol), Pd/C (21 mg), HOAc (31 µl, 0.54 mmol) and MeOH (5 ml) at rt for 1 h afforded 153 mg (97%) of *rac*-(*u*)-**50**·HOAc as colourless crystals.

Mp: 101 °C. IR (KBr): $\tilde{\nu}$ = 3397, 2979, 1740, 1702, 1527, 1367, 1255, 1165, 1017 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 20.7 °C, TMS): δ = 1.46 (s, 9H, C–CH_3), 1.78–2.14 (m, 7H, CH_3–COO, N–CH_2–*C*H_2–*C*H_2), 3.13–3.31 (m, 2H, N–CH_2), 3.76 (s, 3H, O–CH_3), 4.00 (td, J = 8.8/3.5 Hz, 1H, N–C*H*–CH_2), 4.61 (dd, J = 9.4/3.5 Hz, 1H, CH–COO), 7.12 (d, J = 9.4 Hz, 1H, NH), 9.16 (br s, 2H, NH_2^+) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 22.1 °C, $CDCl_3$): δ = 22.98 (*C*H_3–COO), 24.40 (CH_2), 27.60 (CH_2), 28.22 (C–*C*H_3), 45.70 (N–CH_2), 52.71 (O–CH_3), 54.10 (*C*H–COO), 59.32 (N–*C*H–CH_2), 80.08 (C_q), 156.45 (O–CO–N), 170.76 (CH–*C*OO), 177.42 (CH_3–*C*OO) ppm. MS (ESI); m/z (%) = 259 (100) $[M+H]^+$, 245 (17), 203 (87), 189 (25), 159 (71), 142 (20). HRMS (FAB, NBA): calcd for $C_{12}H_{23}N_2O_4$ $[M+H]^+$, 259.1658; found: 259.1684.

4.1.5.6. (2*RS*)-2-[(2*RS*)-Pyrrolidine-2-yl]propionic acid [*rac*-(*l*)-9] and (2*SR*)-2-[(2*RS*)-pyrrolidine-2-yl]propionic acid [*rac*-(*u*)-9][47]. According to GP3 with carboxylic acids [*rac*-(*l*)-**34**][46]/[*rac*-(*u*)-**34**] (419 mg, 1.51 mmol), Pd/C (43 mg), MeOH (15 ml) for 4 h afforded 210 mg (97%) of [*rac*-(*l*)-**9**]/[*rac*-(*u*)-**9**][47] as yellow crystals.

Mp: 170–176 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3425, 2972, 2879, 2759, 2567, 2465, 1572, 1458, 1400, 1366, 1287, 1197, 1145, 1100, 1059, 1033, 958, 875, 837, 786, 692, 644, 588, 506 cm^{-1}. ^{13}C NMR (400 MHz, D_2O, 21.5 °C, Acetone): δ = 15.57 (CH_3), 16.42 (CH_3), 23.80 (N–CH_2–*C*H_2), 28.35 (N–CH–*C*H_2), 28.98 (N–CH–*C*H_2), 44.04 (*C*H–COO), 44.24 (*C*H–COO), 45.75 (N–CH_2), 45.85 (N–CH_2), 63.50 (N–CH), 181.56 (COO), 182.37 (COO) ppm. HRMS (DEI+): calcd for $C_7H_{13}NO_2$ $[M]^+$, 143.0946; found: 143.0948.

The analytical data (^{1}H NMR and MS) of the diastereomeric mixture [*rac*-(*l*)-**9**]/[*rac*-(*u*)-**9**] were in accord with the values reported for the mixture in the literature.[47]

4.1.5.7. (2*RS*)-2-[(2*RS*)-Pyrrolidine-2-yl]butyric acid [*rac*-(*l*)-10] and (2*SR*)-2-[(2*RS*)-pyrrolidine-2-yl]butyric acid [*rac*-(*u*)-10]. According to GP3 with carboxylic acids *rac*-(*l*)-**35**/*rac*-(*u*)-**35** (26 mg, 1.51 mmol), Pd/C (4.5 mg), EtOAc (5 ml) for 24 h afforded 14 mg (99%) of *rac*-(*l*)-**10**/*rac*-(*u*)-**10** as colourless crystals.

Mp: 174–177 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3431, 2964, 2877, 1595, 1560, 1458, 1394, 1333, 1301, 1244, 1190, 804, 682 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 23.9 °C): δ = 0.93 (t, J = 7.4 Hz, 0.43 × 3H, CH_3), 0.93 (t, J = 7.4 Hz, 0.57 × 3H, CH_3), 1.56–1.74 (m, 3H, C*H*$_2$–CH_3, N–CH–*C*H_2), 1.91–2.11 (m, 2H, N–CH_2–*C*H_2), 2.18 (dtd, J = 13.2/7.1/3.8 Hz, 0.43 × 1H, N–CH–*C*H_2), 2.25 (dtd, J = 13.2/7.4/3.6 Hz, 0.57 × 1H, N–CH–*C*H_2), 2.41 (dt, J = 9.6/7.4 Hz, 0.43 × 1H, CH–COO), 2.47 (dt, J = 9.1/5.5 Hz, 0.57 × 1H, CH–COO), 3.28–3.37 (m, 2H, N–CH_2), 3.55 (td, J = 9.6/6.9 Hz, 0.43 × 1H, N–CH), 3.65 (dt, J = 10.2/6.5 Hz, 0.57 × 1H, N–CH) ppm, diastereomeric ratio dr = 57:43. ^{13}C NMR (500 MHz, 25.6 °C, D_2O, MeOH): δ = 11.05 (CH_3), 11.21 (CH_3), 23.29 (CH_2), 23.57 (CH_2), 23.68 (CH_2), 24.26 (CH_2), 28.65 (N–CH–*C*H_2), 28.74 (N–CH–*C*H_2), 45.47 (N–CH_2), 45.54 (N–CH_2), 50.59 (*C*H–COO), 52.72 (*C*H–COO), 61.71 (N–CH), 62.41 (N–CH), 180.10 (COO), 181.20 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 158 (100) $[M+H]^+$. HRMS (FAB, NBA): calcd for $C_8H_{16}NO_2$ $[M+H]^+$, 158.1181; found: 158.1199.

4.1.5.8. 2-Methyl-2-[(2*RS*)-pyrrolidine-2-yl]propionic acid [(*RS*)-11]. According to GP3 with carboxylic acid (*RS*)-**36** (171 mg, 0.59 mmol), Pd/C (18 mg), MeOH (5.8 ml) for 4 h afforded 87 mg (95%) of (*RS*)-**11** as colourless crystals.

Mp: 136 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3440, 2970, 1618, 1471, 1396, 1350, 1292, 1227, 1045, 941, 650, 576 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 24.7 °C): δ = 1.16 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.56–1.65 (m, 1H, C*H*$_2$–CH–N), 1.94–2.11 (m, 2H, C*H*$_2$–CH_2–N), 2.19–2.26 (m, 1H, C*H*$_2$–CH–N), 3.28–3.39 (m, 2H, CH_2–N), 3.46 (dd, J = 10.5/7.3 Hz, 1H, CH–N) ppm. ^{13}C NMR (400 MHz, D_2O, 21.3 °C, Acetone): δ = 23.26 (CH_3), 24.21 (N–CH_2–CH_2), 25.58 (CH_3), 27.42 (N–CH–*C*H_2), 44.07 (C_q), 45.81 (N–CH_2), 68.02 (N–CH), 184.04 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 158 (100) $[M+H]^+$. MS(DEI+); m/z (%) = 158 (2) $[M+H]^+$, 113 (13), 110 (19), 96 (82), 84 (50), 81 (58), 70 (100), 68 (48), 56 (35), 43 (60), 41 (67), 39 (25), 30 (53), 28 (54). HRMS (DEI+): calcd for $C_8H_{16}NO_2$ $[M+H]^+$, 158.1181; found: 158.1220.

4.1.5.9. 1-[(2*RS*)-Pyrrolidine-2-yl]cyclobutane-1-carboxylic acid [(*RS*)-12]. According to GP3 with carboxylic acid (*RS*)-**37** (502 mg, 1.65 mmol), Pd/C (51 mg), MeOH (16.5 ml) for 4 h afforded 282 mg (100%) of (*RS*)-**12** as colourless crystals.

Mp: 198 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3430, 2976, 2930, 2879, 2196, 1960, 1878, 1560, 1398, 1364, 1351, 1280, 1246, 1156, 1112, 1028, 1010, 995, 946, 856, 816, 802, 749, 688, 668, 630, 600 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 19.7 °C): δ = 1.28–1.42 (m, 1H, N–CH–C*H*$_2$), 1.61–1.97 (m, 6H, N–CH_2–*C*H_2, CH_2), 1.99–2.14 (m, 2H, N–CH–*C*H_2, N–CH_2–*C*H_2), 2.29–2.39 (m, 1H, CH_2), 3.07–3.20 (m, 2H, N–CH_2), 3.74–3.83 (m, 1H, N–CH) ppm. ^{13}C NMR (400 MHz, D_2O, 21.1 °C, Acetone): δ = 14.72 (CH_2), 24.15 (CH_2), 26.34 (N–CH–CH_2), 27.19 (CH_2), 30.08 (N–CH_2–CH_2), 45.88 (N–CH_2), 48.61 (C_q), 65.26 (N–CH), 182.93 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 170 (100) $[M+H]^+$, 152 (6), 126 (6). MS (DEI+); m/z (%) = 169 (2) $[M]^+$, 140 (7), 128 (5), 124 (10), 122 (8), 113 (8), 110 (15), 97 (68), 93 (19), 84 (51), 82 (12), 79 (19), 70 (100), 68 (30), 56 (17), 53 (13), 43 (31), 41 (36), 39 (20), 31 (30), 28 (35). HRMS (DEI+): calcd for $C_9H_{15}NO_2$ $[M]^+$, 169.1103; found: 169.1095.

4.1.5.10. (2*RS*)-2-Hydroxy-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid [*rac*-(*l*)-13a][48]. According to GP3 with carboxylic acid *rac*-(*l*)-**39** (132 mg, 0.47 mmol), Pd/C (13 mg), MeOH (4.7 ml) for 4 h afforded 63.3 mg (93%) of *rac*-(*l*)-**13a** as yellow crystals.

Mp: 190 °C (decomp.).

The analytical data of *rac*-(*l*)-**13a** were in accord with the literature values reported for one of the pure like enantiomer.[48]

4.1.5.11. (2*SR*)-2-Hydroxy-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid [*rac*-(*u*)-13a][48]. According to GP3 with carboxylic acid *rac*-(*u*)-**39** (41.6 mg, 0.15 mmol), Pd/C (4.2 mg), MeOH (1.5 ml) for 4 h afforded 20.5 mg (97%) of *rac*-(*u*)-**13a** as yellow crystals.

Mp: 224 °C (decomp.). The analytical data of *rac*-(*u*)-**13a** were in accord with the literature values reported for one of the pure unlike enantiomer.[48]

4.1.5.12. (2*RS*)-2-Amino-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid·2TFA [*rac*-(*l*)-14a·2TFA][58]. According to GP3 with carboxylic acid *rac*-(*l*)-**41** (24 mg, 59 µmol), Pd/C (2.4 mg), MeOH (5.8 ml), TFA (26 mg, 0.23 mmol, 17 µl) for 1 h afforded 19 mg (86%) of *rac*-(*l*)-**14a**·2TFA as a solid.

Mp: 185–188 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3431, 1674, 1522, 1437, 1207, 1136, 840, 802, 724, 520 cm^{-1}. ^1H NMR (500 MHz, D_2O, 23.9 °C, Dioxan): δ = 1.88–1.97 (m, 1H, N–CH–CH_2), 2.01–2.12 (m, 1H, N–CH_2–CH_2), 2.15–2.23 (m, 1H, N–CH_2–CH_2), 2.32–2.39 (m, 1H, N–CH–CH_2), 3.43 (dd, J = 8.5/6.3 Hz, 2H, N–CH_2), 3.89 (td, J = 9.6/6.9 Hz, 1H, N–C*H*–CH_2), 3.97 (d, J = 9.6 Hz, 1H, CH–COO) ppm. ^{13}C NMR (400 MHz, D_2O, 18.4 °C, Dioxan): δ = 23.42 (N–CH_2–CH_2), 28.96 (N–CH–CH_2), 46.31 (N–CH_2), 54.14 (C*H*–COO), 59.03 (N–CH–CH_2), 171.05 (CH–C*O*O) ppm. MS (ESI); m/z (%) = 167 (11) $[M+Na]^+$, 145 (100) $[M+H]^+$. HRMS (FAB, NBA): calcd for $C_6H_{13}N_2O_2$ $[M+H]^+$, 145.0977; found 145.0980.

The spectral data for the compound *rac*-(*l*)-**14a**·2TFA were not given in the literature.[58]

4.1.6. General procedure for the preparation of Boc-protected primary and secondary amines (GP4)

The given amount of amine together with di-*tert*-butyl carbonate (2 equiv per one amino function) was dissolved in dioxane (c = 0.1 M) and stirred with the required amount of NEt_3 (1.5–2 equiv per amino function) for 2.5 h at rt. After which the solvent was evaporated in vacuum followed by CC of the crude product.

4.1.6.1. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[(*tert*-butoxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-43]. According to GP4 with *rac*-(*u*)-**42**·2HOAc (152 mg, 0.55 mmol), di-*tert*-butyl carbonate (263 mg, 1.21 mmol), NEt_3 (0.18 g, 1.8 mmol, 0.25 ml) and dioxane (3 ml). CC (SiO_2, isohexane/EtOAc = 80:20) of the crude product afforded 161 mg (82%) of *rac*-(*u*)-**43** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3432, 2977, 1746, 1718, 1699, 1510, 1392, 1367, 1253, 1162, 1101, 1055, 1024, 869, 775 cm^{-1}. ^1H NMR (400 MHz, $C_2D_2Cl_4$, 120 °C): δ = 1.49 (s, 9H, C–CH_3), 1.53 (s, 9H, C–CH_3), 1.82–1.92 (m, 1H, N–CH_2–CH_2), 1.92–2.06 (m, 3H, N–CH_2–CH_2, N–CH–CH_2), 3.27–3.35 (m, 1H, N–CH_2), 3.44–3.53 (m, 1H, N–CH_2), 3.78 (s, 3H, O–CH_3), 4.19–4.25 (m, 1H, N–C*H*–CH_2), 4.30–4.36 (m, 1H, CH–COO), 5.47–5.57 (m, 1H, NH) ppm. ^{13}C NMR (400 MHz, $C_2D_2Cl_4$, 120 °C): δ = 23.35 (N–CH_2–CH_2), 28.42 (C–CH_3), 28.52 (C–CH_3), 28.95 (N–CH–CH_2), 46.92 (N–CH_2), 52.00 (O–CH_3), 57.74 (C*H*–COO), 58.31 (N–C*H*–CH_2), 79.70 (*C*–CH_3), 80.05 (*C*–CH_3), 155.21 (N–CO–O), 155.34 (N–CO–O), 171.62 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 359 (3) $[M+H]^+$, 303 (12), 247 (100), 229 (14), 203 (62), 185 (25), 170 (15), 114 (45). HRMS (FAB, NBA): calcd for $C_{17}H_{31}N_2O_6$ $[M+H]^+$, 359.2182; found: 359.2231.

4.1.6.2. (2*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-49]. According to GP4: *rac*-(*l*)-**33** (138 mg, 0.47 mmol) taken in dioxane (4.7 ml), di-*tert*-butyl dicarbonate (202 mg, 0.93 mmol) and NEt_3 (100 µl, 0.71 mmol). Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 75:25) afforded 158 mg (86%) of *rac*-(*l*)-**49** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3425, 2977, 1706, 1499, 1456, 1415, 1364, 1339, 1250, 1168, 1102, 1024, 770, 698 cm^{-1}. ^1H NMR (400 MHz, $C_6D_5NO_2$, 120 °C, $C_6D_5NO_2$): δ = 1.49–1.53 (m, 9H, C–CH_3), 1.78–1.89 (m, 1H, N–CH_2–CH_2), 1.95–2.14 (m, 3H, N–CH_2–CH_2, N–CH–CH_2), 3.39–3.49 (m, 1H, N–CH_2), 3.62–3.69 (m, 1H, N–CH_2), 3.70–3.74 (m, 3H, O–CH_3), 4.34–4.41 (m, 1H, N–CH), 4.94 (dd, J = 8.6/3.7 Hz, 1H, CH–COO), 5.21 (d, J = 12.5 Hz, 1H, CH_2–Ar), 5.30 (d, J = 12.5 Hz, 1H, CH_2–Ar), 5.94 (br s, 1H, NH), 7.24–7.47 (m, 5H, H_{ar}) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 20.9 °C, TMS): δ = 23.21 (N–CH_2–CH_2), 23.90 (N–CH_2–CH_2), 28.30 (C–CH_3, N–CH–CH_2), 46.99 (N–CH_2), 47.13 (N–CH_2), 52.37 (O–CH_3), 55.57 (CH–COO), 56.22 (C*H*–COO), 59.30 (N–C*H*–CH_2), 60.38 (N–CH–CH_2), 66.96 (CH_2–Ar), 67.27 (CH_2–Ar), 79.76 (*C*–CH_3), 80.05 (*C*–CH_3), 127.81 (CH_{ar}), 127.94 (CH_{ar}), 128.11 (CH_{ar}), 128.45 (CH_{ar}), 136.51 (C_{ar}), 136.78 (C_{ar}), 154.56 (O–CO–N), 155.31 (O–CO–N), 155.55 (O–CO–N), 155.71 (O–CO–N), 171.34 (COO) ppm. MS (ESI); m/z (%) = 431 (2) $[M^+ + K]$, 415 (12) $[M+Na]^+$, 393 (29) $[M+H]^+$, 337 (20), 293 (100), 249 (15). Anal. calcd for $C_{20}H_{28}N_2O_6$ (392.46): C, 61.21; H, 7.19; N, 7.14. Found: C, 61.13; H, 6.89; N, 7.18.

4.1.6.3. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-49][52]. According to GP4: *rac*-(*u*)-**33**[53] (752 mg, 2.57 mmol) taken in dioxane (17 ml), di-*tert*-butyl dicarbonate (1.17 g, 5.34 mmol) and NEt_3 (0.54 ml, 3.9 mmol). Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 70:30) afforded 957 mg (95%) of *rac*-(*u*)-**49** as colourless oil.

The analytical data of *rac*-(*u*)-**49** were in accord with the literature values reported for one of the pure unlike enantiomer.[52]

4.1.7. General procedure for the removal of Boc-protecting groups (GP5)

The given amount of the substance was dissolved in CH_2Cl_2 and treated with TFA. After stirring for 30 min at rt, the solvent was evaporated to obtain the respective trifluoro acetates.

4.1.7.1. 2-(*RS*)-2-Amino-2-[(2*RS*)-pyrrolidine-2-yl]acetic acid·2TFA [*rac*-(*u*)-14a·2TFA][58]. According to GP5 with carboxylic acid *rac*-(*u*)-**44** (11.6 mg, 32.3 µmol), CH_2Cl_2 (2 ml) and TFA (1.53 g, 13.5 mmol, 1 ml) afforded 12.4 mg (99%) of *rac*-(*u*)-**14a**·2TFA as colourless crystals.

Mp: 191–193 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3435, 3031, 1684, 1617, 1428, 1208, 1175, 1131, 837, 797, 723 cm^{-1}. ^1H NMR (500 MHz, D_2O, 24.6 °C, Dioxane): δ = 1.72–1.81 (m, 1H, N–CH–CH_2), 2.02–2.13 (m, 1H, N–CH_2–CH_2), 2.13–2.22 (m, 1H, N–CH_2–CH_2), 2.32 (dtd, J = 13.1/7.3/3.7 Hz, 1H, N–CH–CH_2), 3.39–3.49 (m, 2H, N–CH_2), 4.04 (ddd, J = 10.1/7.3/4.7 Hz, 1H, N–C*H*–CH_2), 4.40 (d, J = 4.7 Hz, 1H, CH–COO) ppm. ^{13}C NMR (400 MHz, D_2O, 21.8 °C, Dioxane): δ = 24.32 (N–CH_2–CH_2), 26.33 (N–CH–CH_2), 46.90 (N–CH_2), 52.38 (C*H*–COO), 58.35 (N–C*H*–CH_2), 116.93 (q, J = 291.3 Hz, CF_3), 162–156 (m, CF_3–C*O*O), 170.40 (CH–C*O*O) ppm. MS (CI, CH_5^+); m/z (%) = 145 (100) $[M+H]^+$, 128 (8), 115 (2). HRMS (FAB, NBA): calcd for $C_6H_{13}N_2O_2$ $[M+H]^+$, 145.0977; found: 145.0966.

The spectral data for the compound *rac*-(*u*)-**14a**·2TFA were not given in the literature.[58]

4.1.8. General procedure for the *N*-alkylation of the amino acid core structure (GP6)

The given amount of the reactant, the given base (1–3 equiv), KI (2.7 equiv) and the required amount of the alkyl halide (1.2 equiv) was dissolved in MeCN or CH_2Cl_2 and heated under reflux. Either the solvent was evaporated in vacuum followed by CC of the crude product or the reaction mixture twice in Et_2O followed by extraction in CH_2Cl_2 (3 × 10 ml), the unified organic phase was dried over $MgSO_4$, evaporated to dryness and subjected to CC.

4.1.8.1. (2*RS*)-2-Hydroxy-2-{[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-46]. According to GP6 with *rac*-(*l*)-**45**.AcOH (19 mg, 0.1 mmol) in MeCN (4.1 ml), K_2CO_3 (45 mg, 0.33 mmol), KI (63 mg, 0.38 mmol) and 4,4-diphenylbut-3-en-1-yl bromide (74 mg, 0.12 mmol) at 80 °C for 16 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 75:25) afforded 22.3 mg (60%) of *rac*-(*l*)-**46** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3442, 2965, 2802, 1755, 1733, 1494, 1443, 1361, 1221, 1128, 1086, 762, 701, 632 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 20.9 °C, TMS): δ = 1.62–1.76 (m, 4H, N–CH_2–CH_2–CH_2), 2.14–2.21

(m, 1H, N–CH_2–CH$_2$–CH$_2$), 2.31–2.36 (m, 2H, C=CH–CH_2), 2.45 (dt, J = 12.1/6.0 Hz, 1H, N–CH_2–CH$_2$–CH), 2.83–2.92 (m, 2H, N–CH_2–CH$_2$–CH, N–CH), 2.99–3.04 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 3.76 (s, 3H, OCH$_3$), 4.30 (d, J = 3.3 Hz, 1H, CH–OH), 6.06 (t, J = 7.7 Hz, 1H, C=CH), 7.15–7.40 (m, 10H, H$_{ar}$) ppm. ^{13}C NMR (500 MHz, CDCl$_3$, 20.9 °C, CDCl$_3$): δ = 23.40 (CH$_2$), 24.83 (CH$_2$), 28.97 (C=CH–CH_2), 52.00 (O–CH$_3$), 53.20 (N–CH_2–CH$_2$–CH), 53.48 (N–CH_2–CH$_2$–CH$_2$), 65.46 (N–CH), 69.18 (CH–OH), 126.71 (C=CH), 127.00 (CH$_{ar}$), 127.03 (CH$_{ar}$), 127.16 (CH$_{ar}$), 128.11 (CH$_{ar}$), 128.19 (CH$_{ar}$), 129.74 (CH$_{ar}$), 139.95 (C$_{ar}$), 142.33 (C$_{ar}$), 143.31 (C=CH), 172.79 (COO) ppm. MS (ESI); m/z (%) = 388 (9) [M+Na]$^+$, 366 (100) [M+H]$^+$, 276 (9), 129 (17). HRMS (FAB, NBA): calcd for $C_{23}H_{28}NO_3$ [M+H]$^+$, 366.2069; Found 366.2068.

4.1.8.2. (2*SR*)-2-Hydroxy-2-{[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)] pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-46]. According to GP6 with *rac*-(*u*)-**45**.HOAc (19 mg, 0.1 mmol) in MeCN (3.8 ml), K_2CO_3 (42 mg, 0.3 mmol), KI (44 mg, 0.26 mmol) and 4,4-diphenylbut-3-en-1-yl bromide (35 mg, 0.12 mmol) at 80 °C for 7 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 80:20) afforded 15.9 mg (44%) of *rac*-(*u*)-**46** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3429, 2924, 1748, 1494, 1443, 1269, 1201, 1116, 1073, 1017, 762, 701 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$, 18.3 °C, TMS): δ = 1.65–1.84 (m, 3H, N–CH$_2$–CH_2–CH$_2$, N–CH–CH_2), 2.03 (dq, J = 12.5/9.2 Hz, 1H, N–CH–CH_2), 2.17–2.35 (m, 3H, C=CH–CH_2, N–CH_2–CH$_2$–CH$_2$), 2.47 (ddd, J = 12.1/6.8/5.3 Hz, 1H, N–CH_2–CH$_2$–CH), 2.60 (dt, J = 12.1/8.1 Hz, 1H, N–CH_2–CH$_2$–CH), 2.94–3.00 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 3.09 (ddd, J = 9.2/4.0/2.0 Hz, 1H, N–CH), 3.73 (s, 3H, O–CH$_3$), 3.92 (d, J = 2.0 Hz, 1H, CH–OH), 6.07 (t, J = 7.5 Hz, 1H, C=CH), 7.15–7.41 (m, 10H, H$_{ar}$) ppm. ^{13}C NMR (500 MHz, CDCl$_3$, 25.8 °C, TMS): δ = 24.37 (N–CH$_2$–CH_2–CH$_2$), 29.02 (C=CH–CH_2), 30.36 (N–CH–CH_2), 52.03 (CH$_3$), 54.20 (N–CH_2–CH$_2$–CH$_2$), 55.85 (N–CH_2–CH$_2$–CH), 66.31 (N–CH), 72.19 (CH–OH), 126.38 (C=CH), 127.06 (CH$_{ar}$), 127.10 (CH$_{ar}$), 127.22 (CH$_{ar}$), 128.16 (CH$_{ar}$), 128.25 (CH$_{ar}$), 129.80 (CH$_{ar}$), 139.98 (C$_{ar}$), 142.37 (C$_{ar}$), 143.35 (C$_q$), 174.19 (COO) ppm. MS (CI, CH$_5^+$); m/z (%) = 366 (81) [M+H]$^+$, 306 (4), 276 (18), 193 (3), 172 (100). HRMS (FAB, NBA): calcd for $C_{23}H_{28}NO_3$ [M+H]$^+$, 366.2069; found: 366.2085.

4.1.8.3. (2*RS*)-2-Hydroxy-2-{(2*RS*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-47]. According to GP6 with *rac*-(*l*)-**45**.HOAc (23 mg, 0.1 mmol) in MeCN (4.1 ml), K_2CO_3 (47 mg, 0.34 mmol), KI (64 mg, 0.39 mmol) and 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide (133 mg, 0.41 mmol) at 80 °C for 17 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 80:20) afforded 18 mg (43%) of *rac*-(*l*)-**47** as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3444, 2950, 2923, 1756, 1733, 1438, 1265, 1211, 1126, 1008, 738, 712 cm^{-1}. ^{1}H NMR (400 MHz, CD$_2$Cl$_2$, 20.9 °C, TMS): δ = 1.63–1.70 (m, 4H, N–CH$_2$–CH_2–CH_2), 2.00 (s, 3H, Ar–CH$_3$), 2.05 (s, 3H, Ar–CH$_3$), 2.15–2.25 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 2.29–2.37 (m, 2H, N–CH$_2$–CH_2–CH), 2.41–2.49 (m, 1H, N–CH_2–CH$_2$–CH), 2.82–2.93 (m, 2H, N–CH, N–CH_2–CH$_2$–CH), 3.04–3.10 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 3.71 (s, 3H, O–CH$_3$), 4.26 (d, J = 3.3 Hz, 1H, CH–OH), 6.06 (t, J = 7.3 Hz, 1H, C=CH), 6.78 (d, J = 5.1 Hz, 1H, H$_{ar}$), 6.87 (d, J = 5.1 Hz, 1H, H$_{ar}$), 7.07 (d, J = 5.1 Hz, 1H, H$_{ar}$), 7.25 (d, J = 5.1 Hz, 1H, H$_{ar}$) ppm. ^{13}C NMR (500 MHz, CD$_2$Cl$_2$, 21.3 °C, CD$_2$Cl$_2$): δ = 14.21 (Ar–CH$_3$), 14.57 (Ar–CH$_3$), 23.58 (N–CH$_2$–CH_2–CH$_2$), 24.85 (N–CH–CH_2), 29.33 (N–CH$_2$–CH_2–CH), 51.73 (O–CH$_3$), 52.94 (N–CH_2–CH$_2$–CH), 53.51 (N–CH_2–CH$_2$–CH$_2$), 65.72 (N–CH), 69.31 (CH–OH), 122.78 (CH$_{ar}$), 124.40 (CH$_{ar}$), 128.59 (C$_{ar}$), 129.64 (CH$_{ar}$), 131.20 (CH$_{ar}$), 133.27 (C=CH), 133.78 (C$_{ar}$), 135.31 (C$_{ar}$), 135.58 (C$_{ar}$), 139.52 (C=CH), 172.70 (COO) ppm. MS (ESI); m/z (%) = 428 (32) [M+Na]$^+$, 406 (100) [M+H]$^+$, 316 (9), 308 (17), 247 (38), 149 (16). HRMS (FAB, NBA): calcd for $C_{21}H_{28}NO_3S_2$ [M+H]$^+$, 406.1511; found: 406.1519.

4.1.8.4. (2*SR*)-2-Hydroxy-2-{(2*RS*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-47]. According to GP6 with *rac*-(*u*)-**45**.HOAc (29.5 mg, 0.14 mmol) in MeCN (5.4 ml), K_2CO_3 (56 mg, 0.41 mmol), KI (61 mg, 0.37 mmol) and 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide (93 mg, 0.28 mmol) at 80 °C for 17 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 80:20) afforded 10 mg (19%) of *rac*-(*u*)-**47** as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3447, 2949, 1748, 1436, 1267, 1199, 1115, 1013, 967, 718 cm^{-1}. ^{1}H NMR (400 MHz, CD$_2$Cl$_2$, 17.9 °C, TMS): δ = 1.65–1.81 (m, 3H, N–CH$_2$N–CH$_2$–CH_2–CH$_2$, N–CH$_2$–CH$_2$–CH_2), 1.95–2.09 (m, 7H, Ar–CH$_3$, N–CH$_2$–CH$_2$–CH_2), 2.20–2.31 (m, 3H, N–CH_2–CH$_2$–CH$_2$, C=CH–CH_2), 2.41–2.51 (m, 1H, N–CH_2–CH$_2$–CH), 2.59 (dt, J = 11.9/8.1 Hz, 1H, N–CH_2–CH$_2$–CH), 3.01–3.10 (m, 2H, N–CH_2–CH$_2$–CH$_2$, N–CH), 3.69 (s, 3H, O–CH$_3$), 3.87 (d, J = 1.8 Hz, 1H, CH–OH), 6.04 (t, J = 7.3 Hz, 1H, C=CH), 6.78 (d, J = 5.1 Hz, 1H, H$_{ar}$), 6.88 (d, J = 5.1 Hz, 1H, H$_{ar}$), 7.08 (d, J = 5.1 Hz, 1H, H$_{ar}$), 7.26 (d, J = 5.1 Hz, 1H, H$_{ar}$) ppm. ^{13}C NMR (500 MHz, CD$_2$Cl$_2$, 23.9 °C, TMS): δ = 14.57 (Ar–CH$_3$), 14.91 (Ar–CH$_3$), 24.88 (N–CH$_2$–CH_2–CH$_2$), 29.94 (N–CH$_2$–CH_2–CH), 30.88 (N–CH$_2$–CH$_2$–CH_2), 52.11 (O–CH$_3$), 54.67 (N–CH_2–CH$_2$–CH$_2$), 55.59 (N–CH_2–CH$_2$–CH), 66.41 (N–CH), 72.73 (CH–OH), 123.11 (CH$_{ar}$), 124.71 (CH$_{ar}$), 128.75 (C=CH), 129.99 (CH$_{ar}$), 131.56 (CH$_{ar}$), 133.75 (C=CH), 134.13 (C$_{ar}$), 135.77 (C$_{ar}$), 135.90 (C$_{ar}$), 139.95 (C$_{ar}$), 174.84 (COO) ppm. MS (ESI); m/z (%) = 428 (54) [M+Na]$^+$, 406 (100) [M+H]$^+$, 316 (7), 308 (19), 247 (38), 149 (16). HRMS (FAB, NBA): calcd for $C_{21}H_{28}NO_3S_2$ [M+H]$^+$, 406.1511; found: 406.1490.

4.1.8.5. (2*RS*)-2-Hydroxy-2-[(2*RS*)-1-{2-[tris(4-methoxyphenyl) methoxy]ethyl}-pyrrolidine-2-yl]acetic acid methyl ester [*rac*-(*l*)-48]. According to GP6 with *rac*-(*l*)-**45**·HOAc (29 mg, 0.13 mmol) in MeCN (2.7 ml), K_2CO_3 (56 mg, 0.4 mmol), KI (60 mg, 0.36 mmol) and 2-{[tris(4-methoxyphenyl)]methoxy}ethyl bromide (74 mg, 0.16 mmol) at 80 °C for 16 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 60:40) afforded 32.1 mg (45%) of *rac*-(*l*)-**48** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3443, 2952, 2836, 1757, 1733, 1607, 1507, 1463, 1441, 1302, 1249, 1175, 1124, 1074, 1034, 827, 583 cm^{-1}. ^{1}H NMR (400 MHz, CD$_2$Cl$_2$, 18.4 °C, TMS): δ = 1.61–1.73 (m, 4H, N–CH$_2$–CH_2–CH_2), 2.23–2.32 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 2.53 (dt, J = 13.0/4.6 Hz, 1H, N–CH_2–CH$_2$–O), 2.89–3.00 (m, 2H, N–CH, N–CH_2–CH$_2$–O), 3.08–3.14 (m, 2H, N–CH_2–CH$_2$–CH$_2$, O–CH$_2$), 3.21 (ddd, J = 9.9/7.9/4.6 Hz, 1H, O–CH$_2$), 3.71 (s, 3H, COOCH$_3$), 3.77 (s, 9H, Ar–OCH$_3$), 4.28 (d, J = 3.5 Hz, 1H, CH–OH), 6.80–6.85 (m, 6H, H$_{ar}$), 7.30–7.35 (m, 6H, H$_{ar}$) ppm. ^{13}C NMR (400 MHz, CD$_2$Cl$_2$, 20 °C, TMS): δ = 24.04 (N–CH$_2$–CH_2–CH$_2$), 25.14 (N–CH$_2$–CH$_2$–CH_2), 52.06 (COOCH$_3$), 53.64 (N–CH$_2$–CH$_2$–O), 54.75 (N–CH_2–CH$_2$–CH$_2$), 55.59 (Ar–OCH$_3$), 62.71 (O–CH$_2$), 66.01 (N–CH), 69.67 (CH–OH), 86.19 (O–C$_q$), 113.38 (CH$_{ar}$), 130.07 (CH$_{ar}$), 137.27 (C$_{ar}$), 158.84 (C$_{ar}$), 173.10 (COO) ppm. MS (ESI); m/z (%) = 558 (7) [M+Na]$^+$, 536 (5) [M+H]$^+$, 333 (100). HRMS (DEI): calcd for $C_{31}H_{38}NO_7$ [M+H]$^+$, 536.2648; found: 536.2623.

4.1.8.6. (2*SR*)-2-Hydroxy-2-[(2*RS*)-1-{2-[tris(4-methoxyphenyl) methoxy]ethyl}-pyrrolidine-2-yl]acetic acid methyl ester [*rac*-(*u*)-48]. According to GP6 with *rac*-(*u*)-**45**·HOAc (71 mg, 0.32 mmol) in MeCN (6.4 ml), K_2CO_3 (133 mg, 0.96 mmol), KI (144 mg, 0.87 mmol) and 2-{[tris(4-methoxyphenyl)] methoxy}ethyl bromide (0.18 g, 0.39 mmol) at 80 °C for 16 h. Purification of the crude product by CC (SiO_2, isohexane/EtOAc = 70:30) afforded 102 mg (59%) of *rac*-(*u*)-**48** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3433, 2952, 2836, 1748, 1608, 1508, 1463, 1441, 1302, 1250, 1176, 1117, 1071, 1034, 828, 584 cm^{-1}. ^1H NMR (400 MHz, CD_2Cl_2, 20.4 °C, TMS): δ = 1.67–1.80 (m, 3H, N–CH_2–CH_2–CH_2, N–CH–CH_2), 1.93–2.06 (m, 1H, N–CH–CH_2), 2.29–2.38 (m, 1H, N–CH_2–CH_2–CH_2), 2.59 (dt, J = 13.2/5.1 Hz, 1H, N–CH_2–CH_2–O), 2.73–2.81 (m, 1H, N–CH_2–CH_2–O), 3.02–3.15 (m, 4H, N–CH, N–CH_2–CH_2–CH_2, O–CH_2), 3.70 (s, 3H, $COOCH_3$), 3.78 (s, 9H, O–CH_3), 3.87 (d, J = 2.9 Hz, 1H, CH–OH), 6.80–6.85 (m, 6H, H_{ar}), 7.30–7.35 (m, 6H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 25.2 °C, TMS): δ = 25.06 (N–CH_2–CH_2–CH_2), 30.58 (N–CH–CH_2), 52.67 ($COOCH_3$), 55.55 (N–CH_2–CH_2–CH_2), 55.60 (O–CH_3), 56.31 (N–CH_2–CH_2–O), 63.10 (CH_2–O), 66.67 (N–CH), 73.07 (CH–OH), 86.22 (O–C_q), 113.38 (CH_{ar}), 130.14 (CH_{ar}), 137.31 (C_{ar}), 158.87 (C_{ar}), 174.71 (COO) ppm. MS (ESI); m/z (%) = 558 (1) $[M+Na]^+$, 536 (15) $[M+H]^+$, 333 (100). HRMS (FAB, NBA): calcd for $C_{31}H_{38}NO_7$ $[M+H]^+$, 536.2648; found: 536.2693.

4.1.8.7. (2*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-51]. According to GP6 with *rac*-(*l*)-**50**·HOAc (11 mg, 36 μmol), $KHCO_3$ (7 mg, 71 μmol), KI (13 mg, 79 μmol) taken in CH_3CN (1.4 ml) with 4,4-diphenylbut-3-en-1-yl bromide (22 mg, 77 μmol) and heated to reflux for 4 h. The reaction mixture was extracted with ether (2 × 10 ml), CH_2Cl_2 (3 × 10 ml) and the unified organic phase dried over $MgSO_4$, concentrated in vacuum and purified by CC (SiO_2, isohexane/EtOAc = 95:5) to afford 9 mg (55%) of *rac*-(*l*)-**51** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3427, 2972, 1716, 1495, 1399, 1164, 1064, 1025, 762, 701 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 23 °C, TMS): δ = 1.41 (s, 9H, C–CH_3), 1.63–1.75 (m, 4H, N–CH_2–CH_2–CH_2, N–CH_2–CH_2–CH_2), 2.06–2.13 (m, 1H, N–CH_2–CH_2–CH_2), 2.21–2.34 (m, 3H, N–CH_2–CH_2–CH, N–CH_2–CH_2–CH), 2.74–2.82 (m, 1H, N–CH–CH_2), 2.82–2.90 (m, 1H, N–CH_2–CH_2–CH), 3.01–3.07 (m, 1H, N–CH_2–CH_2–CH_2), 3.69 (s, 3H, O–CH_3), 4.06–4.21 (m, 0.15 × 1H, CH–COO), 4.24–4.31 (m, 0.85 × 1H, CH–COO), 5.00 (br s, 0.15 × 1H, NH), 5.19 (s, 0.85 × 1H, NH), 6.10 (t, J = 6.9 Hz, 1H, C=CH), 7.15–7.40 (m, 10H, H_{ar}) ppm. Rotamer ratio (23 °C) 85:15. ^{13}C NMR (500 MHz, $CDCl_3$, 21.6 °C, TMS): δ = 22.49 (N–CH_2–CH_2–CH_2), 25.95 (N–CH_2–CH_2–CH_2), 28.30 (C_q–CH_3), 28.94 (N–CH_2–CH_2–CH), 52.07 (O–CH_3), 53.24 (N–CH_2–CH_2–CH_2), 53.69 (N–CH_2–CH_2–CH), 54.55 (CH–COO), 64.23 (N–CH–CH_2), 79.78 (C_q–CH_3), 126.89 (CH_{ar}), 126.96 (CH_{ar}), 127.14 (C=CH), 127.20 (CH_{ar}), 128.09 (CH_{ar}), 128.20 (CH_{ar}), 129.81 (CH_{ar}), 140.11 (C_{ar}), 142.52 (C_q), 142.57 (C_q), 155.94 (N–CO–O), 172.11 (COO) ppm. MS (ESI); m/z (%) = 487 (2) $[M+Na]^+$, 465 (71) $[M+H]^+$, 409 (100), 276 (20). HRMS (FAB, NBA): calcd for $C_{28}H_{37}N_2O_4$ $[M+H]^+$, 465.2753; found: 465.2741.

4.1.8.8. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{[(*RS*)-1-(4,4-diphenylbut-3-en-1-yl)]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-51]. According to GP6 with *rac*-(*u*)-**50**·HOAc (20 mg, 63 μmol) and 4,4-diphenylbut-3-en-1-yl bromide (38 mg, 0.13 mmol) in MeCN (1.3 ml) and DIPEA (81 mg, 0.63 mmol) at rt for 72 h. The reaction mixture was concentrated in vacuum and the crude product on CC (SiO_2, isohexane/EtOAc = 95:5) afforded 52 mg (18%) of *rac*-(*u*)-**51** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3423, 2972, 1750, 1716, 1490, 1365, 1321, 1258, 1204, 1164, 760, 702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 22.7 °C, TMS): δ = 1.40 (s, 0.88 × 9H, C–CH_3), 1.44 (s, 0.12 × 9H, C–CH_3), 1.58–1.71 (m, 4H, N–CH–CH_2, N–CH_2–CH_2–CH_2), 1.98 (dq, J = 12.4/8.5 Hz, N–CH–CH_2), 2.07 (q, J = 8.5 Hz, 1H, N–CH_2–CH_2–CH_2), 2.16–2.36 (m, 3H, N–CH_2–CH_2–CH, N–CH_2–CH_2–CH), 2.51–2.58 (m, 1H, N–CH_2–CH_2–CH), 2.82–2.88 (m, 0.88 × 1H, N–CH_2–CH_2–CH_2), 2.96–3.03 (m, 0.12 × 1H, N–CH_2–CH_2–CH_2), 3.07–3.17 (m, 1H, N–CH–CH_2), 3.68 (s, 0.12 × 3H, O–CH_3), 3.70 (s, 0.88 × 3H, O–CH_3), 3.95–4.00 (m, 0.12 × 1H, CH–COO), 4.15 (dd, J = 9.1/1.1 Hz, 0.88 × 1H, CH–COO), 5.35–5.41 (m, 0.12 × 1H, NH), 5.61 (d, J = 9.1 Hz, 0.88 × 1H, NH), 6.05 (t, J = 7.4 Hz, 1H, C=CH), 7.17–7.40 (m, 10H, H_{ar}) ppm. Rotamer ratio (22.7 °C) 88:12. ^{13}C NMR (500 MHz, $CDCl_3$, 22 °C, TMS): δ = 23.77 (N–CH_2–CH_2–CH_2), 28.31 (C–CH_3), 29.16 (N–CH_2–CH_2–CH), 29.99 (N–CH–CH_2), 52.06 (O–CH_3), 54.22 (N–CH_2), 54.87 (N–CH_2), 56.09 (CH–COO), 64.24 (N–CH–CH_2), 79.53 (C_q–CH_3), 126.85 (CH_{ar}), 126.92 (CH_{ar}), 127.26 (CH_{ar}), 127.50 (C=CH), 128.09 (CH_{ar}), 128.16 (CH_{ar}), 129.89 (CH_{ar}), 140.19 (CH_{ar}), 142.65 (C_{ar}), 142.72 (C_{ar}), 156.25 (O–CO–N), 172.94 (COO) ppm. MS (ESI); m/z (%) = 487 (4) $[M+Na]^+$, 465 (79) $[M+H]^+$, 409 (100), 276 (22). HRMS (FAB, NBA): calcd for $C_{28}H_{37}N_2O_4$ $[M+H]^+$, 465.2753; found: 465.2714.

4.1.8.9. (2*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[4,4-bis(3-methylthio-phen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*l*)-52]. According to the GP6 with *rac*-(*l*)-**50**.HOAc (19 mg, 59 μmol) taken in MeCN (2.4 ml), K_2CO_3 (28 mg, 0.2 mmol), KI (31 mg, 0.19 mmol) and 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide (58 mg, 0.18 mmol) at 80 °C for 17 h. Purification of the crude product by CC (SiO2, isohexane/EtOAc = 90:10) afforded 14.6 mg (49%) of *rac*-(*l*)-**52** as colourless oil

IR (KBr): $\tilde{\nu}$ = 3423, 2973, 1716, 1483, 1366, 1163, 1064, 1023, 711 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 50 °C, TMS): δ = 1.41 (s, 9H, C–CH_3), 1.62–1.76 (m, 4H, N–CH_2–CH_2–CH_2), 2.02 (s, 3H, Ar–CH_3), 2.04 (s, 3H, Ar–CH_3), 2.10–2.19 (m, 1H, N–CH_2–CH_2–CH_2), 2.24–2.38 (m, 3H, N–CH_2–CH_2–CH, N–CH_2–CH_2–CH), 2.77–2.84 (m, 1H, N–CH–CH_2), 2.84–2.92 (m, 1H, N–CH_2–CH_2–CH), 3.04–3.10 (m, 1H, N–CH_2–CH_2–CH_2), 3.70 (s, 3H, O–CH_3), 4.26 (br s, 1H, CH–COO), 5.10 (br s, 1H, NH), 6.03–6.06 (m, 1H, C=CH), 6.74 (d, J = 5.1 Hz, 1H, H_{ar}), 6.83 (d, J = 5.1 Hz, 1H, H_{ar}), 7.03 (d, J = 5.1 Hz, 1H, H_{ar}), 7.19 (d, J = 5.1 Hz, 1H, H_{ar}) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 22.2 °C): δ = 14.37 (Ar–CH_3), 14.79 (Ar–CH_3), 22.44 (N–CH_2–CH_2–CH_2), 25.78 (N–CH_2–CH_2–CH_2), 28.29 (C–CH_3), 29.02 (N–CH_2–CH_2–CH), 52.07 (O–CH_3), 53.01 (N–CH_2–CH_2–CH), 53.10 (N–CH_2–CH_2–CH_2), 54.36 (CH–COO), 64.09 (N–CH–CH_2), 79.77 (C_q–CH_3), 122.68 (CH_{ar}), 124.30 (CH_{ar}), 128.24 (C_{ar}), 129.53 (CH_{ar}), 131.10 (CH_{ar}), 133.23 (C=CH), 133.52 (C_{ar}), 135.31 (C_{ar}), 139.58 (C=CH), 155.97 (O–CO–N), 172.06 (COO) ppm. MS (ESI); m/z (%) = 527 (28) $[M+Na]^+$, 505 (97) $[M+H]^+$, 449 (100), 427 (11), 316 (19), 247 (16). HRMS (FAB, NBA): calcd for $C_{26}H_{37}N_2O_4S_2$ $[M+H]^+$, 505.2195; found: 505.2238.

4.1.8.10. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[4,4-bis(3-methylthio-phen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl}acetic acid methyl ester [*rac*-(*u*)-52]. According to GP6 with *rac*-(*u*)-**50**·HOAc (17 mg, 54 μmol) and 4,4-bis-(3-methylthien-2-yl)but-3-en-1-yl bromide (36 mg, 0.11 mmol) in CH_2Cl_2 (2 ml) with NEt_3 (10 mg, 14 μl, 0.1 mmol) stirred at rt for 72 h. Concentration of the crude product in vacuum followed by CC (SiO_2, isohexane/EtOAc = 95:5) afforded 2.3 mg (10%) of *rac*-(*u*)-**52** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3419, 2972, 1749, 1716, 1488, 1365, 1164, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 20.5 °C, TMS): δ = 1.45 (s, 9H, C–CH_3), 1.62–1.74 (m, 3H, N–CH–CH_2, N–CH_2–CH_2–CH_2), 1.94–2.06 (m, 7H, Ar–CH_3, N–CH–CH_2), 2.13 (t, J = 8.2 Hz, 1H, N–CH_2–CH_2–CH_2), 2.22–2.29 (m, 2H, N–CH_2–CH_2–CH), 2.29–2.36 (m, 1H, N–CH_2–CH_2–CH), 2.60 (dt, J = 11.0/7.7 Hz, 1H, N–CH_2–CH_2–CH), 2.99–3.04 (m, 1H, N–CH_2–CH_2–CH_2), 3.12–3.17 (m, 1H, N–CH–CH_2), 3.70 (s, 3H, O–CH_3), 3.98 (d, J = 7.7 Hz, 0.10 × 1H, CH–COO), 4.17 (dd, J = 9.3/1.6 Hz, 0.90 × 1H, CH–COO), 5.27–5.36 (m, 0.10 × 1H, NH), 5.54 (d, J = 9.3 Hz, 0.90 × 1H, NH), 6.03 (t, J = 7.1 Hz, 1H, C=CH), 6.76 (d, J = 4.9 Hz, 1H, H_{ar}), 6.85 (d,

J = 4.9 Hz, 1H, H_{ar}), 7.05 (d, J = 4.9 Hz, 1H, H_{ar}), 7.22 (d, J = 4.9 Hz, 1H, H_{ar}) ppm. Rotamer ratio (20.5 °C) 90:10. ^{13}C NMR (400 MHz, $CDCl_3$, 22.3 °C, $CDCl_3$): δ = 14.37 (CH_3–Ar), 14.79 (CH_3–Ar), 23.73 (N–CH_2–CH_2–CH_2), 28.35 (C–CH_3), 29.22 (C=CH–CH_2), 29.80 (N–CH–CH_2), 52.03 (O–CH_3), 54.11 (N–CH_2–CH_2–CH_2), 54.41 (N–CH_2–CH_2–CH), 56.06 (CH–COO), 64.42 (N–CH–CH_2), 79.54 (C_q–CH_3), 122.64 (CH_{ar}), 124.29 (CH_{ar}), 128.23 (C=CH), 129.51 (CH_{ar}), 131.15 (CH_{ar}), 133.28 (C=CH), 133.51 (C_{ar}), 135.34 (C_{ar}), 139.55 (C_{ar}), 156.27 (O–CO–N), 172.84 (COO) ppm. MS (ESI); m/z (%) = 505 (100) $[M+H]^+$, 449 (60). HRMS (FAB, NBA): calcd for $C_{26}H_{37}N_2O_4S_2$ $[M+H]^+$, 505.2195; found: 505.2187.

4.1.9. General procedure for the hydrolysis of methyl esters (GP7)

Method A: The ester was refluxed with the given amount of methanolic KOH (0.5 M) and H_2O, after which the MeOH was removed by distillation. The aqueous residue left behind was extracted thrice with CH_2Cl_2 and the extracts were discarded. After acidifying the aqueous phase with HCl (2 M) to pH = 1, it was again extracted thrice with CH_2Cl_2, then the unified organic phase was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by CC.

Method B: To the solution of the methyl ester in dioxane was added the given amount of aq LiOH (1.2 M) at rt. After stirring for the given time at rt, the aqueous phase was extracted with CH_2Cl_2 and the organic phase discarded. The aq phase was then acidified with 2 M HCl to pH = 1 and extracted thrice with CH_2Cl_2. Finally, the unified organic phase was dried over $MgSO_4$ and evaporated to dryness in vacuum.

Method C: Analogous to GP1, the methyl ester dissolved in dioxane was treated with aq LiOH. After stirring for 1.5 to 2 h at rt, the Na_2HPO_4/NaH_2PO_4-buffer pH = 7 (10 ml) was added to the reaction and extracted twice with CH_2Cl_2. The aqueous phase was acidified with 2 M HCl to pH = 6 and extracted twice with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated to dryness in vacuum. In exceptional cases, the crude product was purified by CC or HPLC.

4.1.9.1. (2*RS*)-2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl} propionic acid [*rac*-(*l*)-34][46] und (2*SR*)-2-{(2*RS*)-1-[(benzyloxy) carbonyl]pyrrolidine-2-yl}-propionic acid [*rac*-(*u*)-34].

According to GP7, method A with [*rac*-(*l*)-**28**][46]/[*rac*-(*u*)-**28**] (567 mg, 1.95 mmol), KOH (130 ml, 0.5 M in MeOH), H_2O (26 ml) heated for 30 h at 92 °C. This afforded 486 mg (90%) of the diastereomeric mixture [*rac*-(*l*)-**34**]/[*rac*-(*u*)-**34**] as colourless crystals.

Mp: 118 °C. IR (KBr): $\tilde{\nu}$ = 3090, 2974, 2880, 1724, 1668, 1585, 1500, 1458, 1429, 1361, 1344, 1316, 1286, 1267, 1205, 1166, 1137, 1109, 1029, 1006, 988, 960, 909, 802, 768, 748, 699, 630, 602, 476 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 19.9 °C, TMS): δ = 1.01–1.08 (m, 0.56 × 3H, CH_3), 1.11–1.19 (m, 0.44 × 3H, CH_3), 1.75–1.94 (m, 3H, CH_2–CH_2–N, CH_2–CH–N), 1.96–2.05 (m, 1H, CH_2–CH–N), 2.74–3.24 (m, 0.56 × 1H, CH–COO), 3.31–3.43 (m, 1.44H, N–CH_2, CH–COO), 3.51–3.69 (m, 1H, N–CH_2), 4.10–4.14 (m, 0.44 × 1H, CH–N), 4.29–4.34 (m, 0.56 × 1H, CH–N), 5.07–5.19 (m, 2H, CH_2–Ar), 7.28–7.38 (m, 5H, H_{ar}) ppm, diastereomeric ratio dr = 56:44 (*l*:*u*). MS (CI, CH_5^+); m/z (%) = 278 (52) $[M+H]^+$, 260 (10), 234 (100), 204 (9), 173 (5), 170 (24), 160 (12), 155 (6), 144 (46), 142 (18), 127 (18), 105 (9). MS (EI, 70 eV); m/z (%) = 277 (1) $[M]^+$, 209 (8), 204 (10), 160 (16), 142 (5), 111 (6), 105 (12), 97 (15), 94 (71), 91 (100), 85 (10), 83 (13), 81 (10), 79 (7), 77 (7), 73 (5), 71 (17), 70 (18), 69 (22), 67 (8), 65 (15), 57 (29), 55 (26). HRMS (70 eV): calcd for $C_{15}H_{19}NO_4$ $[M]^+$, 277.1314; Found 277.1314.

The analytical data of the *rac*-(*l*)-diastereomers (^{1}H NMR) were in accord with the values reported in the literature for a pure like enantiomer.[46]

4.1.9.2. (2*RS*)-2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl} butyric acid [*rac*-(*l*)-35] und (2*SR*)-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}butyric acid [*rac*-(*u*)-35].

According to GP7, method A with *rac*-(*l*)-**29**/*rac*-(*u*)-**28** (89 mg, 0.29 mmol), KOH (2.6 ml, 0.5 M in MeOH), H_2O (0.3 ml) and the reaction time was 16 h at 92 °C. Purification through CC (SiO_2, pentane/EtOAc/HOAc = 90:10:1) afforded 66 mg (78%) of the diastereomeric mixture *rac*-(*l*)-**35**/*rac*-(*u*)-**35** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 2967, 2879, 1731, 1698, 1498, 1455, 1416, 1359, 1259, 1197, 1114, 1029, 917, 876, 770, 698 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 20.2 °C, TMS): δ = 0.80–1.03 (m, 3H, CH_3), 1.30–2.01 (m, 6H, N–CH_2–CH_2–CH_2, CH_2–CH_3), 2.63–3.06 (m, 1H, CH–COO), 3.29–3.41 (m, 1H, N–CH_2), 3.47–3.68 (m, 1H, N–CH_2), 4.08–4.15 (m, 0.43 × 1H, N–CH), 4.21–4.28 (m, 0.57 × 1H, N–CH), 5.06–5.20 (m, 2H, CH_2–Ar), 7.27–7.41 (m, 5H, H_{ar}) ppm, diastereomeric ratio dr = 57:43. ^{13}C NMR (400 MHz, $CDCl_3$, 20.8 °C, TMS): δ = 12.20 (CH_3), 12.65 (CH_3), 18.86 (CH_2), 19.31 (CH_2), 23.09 (CH_2), 23.63 (CH_2), 23.83 (CH_2), 24.13 (CH_2), 27.48 (CH_2), 27.72 (CH_2), 28.00 (CH_2), 28.45 (CH_2), 46.81 (N–CH_2), 47.13 (N–CH_2), 47.62 (N–CH_2), 49.57 (CH–COO), 50.28 (CH–COO), 58.50 (N–CH), 58.87 (N–CH), 59.17 (N–CH), 59.51 (N–CH), 66.87 (CH_2–Ar), 127.85 (CH_{ar}), 127.97 (CH_{ar}), 128.48 (CH_{ar}), 136.73 (C_{ar}), 155.08 (O–CO–N), 155.47 (O–CO–N), 179.13 (COO), 179.57 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 292 (61) $[M+H]^+$, 274 (18), 248 (100), 204 (13), 184 (20), 160 (14). MS (EI, 70 eV); m/z (%) = 291 (0) $[M]^+$, 204 (13), 160 (24), 91 (100), 65 (9). Anal. calcd for $C_{16}H_{21}NO_4$ (291.1471): C, 65.96; H, 7.27; N, 4.81. Found: C, 65.79; H, 7.28; N 4.68.

4.1.9.3. 2-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl}-2-methylpropionic acid [(*RS*)-36].

According to GP7, method A, with (*RS*)-**30** (44 mg, 0.15 mmol), KOH (20 ml, 0.5 M in MeOH), H_2O (4 ml) and the reaction time at reflux was 96 h. This afforded 42 mg (91%) of (*RS*)-36 as colourless crystals.

Mp: 100 °C. IR (KBr): $\tilde{\nu}$ = 3436, 2976, 1701, 1458, 1420, 1362, 1137, 697, 668 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 24.5 °C, TMS): δ = 1.16 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.73–1.83 (m, 2H, N–CH–CH_2, N–CH_2–CH_2), 1.83–1.91 (m, 1H, N–CH_2–CH_2), 1.98–2.07 (m, 1H, N–CH–CH_2), 3.28 (dt, J = 11.2/7.1 Hz, 1H, N–CH_2), 3.72–3.81 (m, 1H, N–CH_2), 4.37 (dd, J = 8.2/3.9 Hz, 1H, N–CH), 5.07–5.14 (m, 2H, CH_2–Ar), 7.28–7.38 (m, 5H, H_{ar}) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 25.8 °C, TMS): δ = 21.50 (CH_3), 22.66 (CH_3), 24.13 (N–CH_2–CH_2), 27.82 (N–CH–CH_2), 47.39 (C–COO), 48.08 (N–CH_2), 63.25 (N–CH), 67.02 (CH_2–Ar), 127.98 (br s, 2 CH_{ar}), 128.47 (CH_{ar}), 136.72 (C_{ar}), 156.35 (N–CO–O), 182.61 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 292 (28) $[M+H]^+$, 274 (19), 248 (50), 195 (38), 184 (30), 158 (100), 145 (51), 127 (59). Anal. calcd for $C_{16}H_{21}NO_4$ (291.35): C, 65.96; H, 7.27; N, 4.81. Found: C, 65.68; H, 7.39; N, 4.58.

4.1.9.4. 1-{(2*RS*)-1-[(Benzyloxy)carbonyl]pyrrolidine-2-yl}cyclobutane-1-carboxylic acid [(*RS*)-37].

According to GP7. Method A with (*RS*)-**31** (753 mg, 2.37 mmol), KOH (132 ml, 0.5 M in MeOH), H_2O (26 ml) and the reaction time was 24 h at 92 °C. This afforded 664 mg (92%) of (*RS*)-**37** as colourless crystals.

Mp: 87 °C. IR (KBr): $\tilde{\nu}$ = 3422, 2952, 1700, 1419, 1354, 1281, 1194, 1116, 771, 735, 698, 606 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 25.3 °C, TMS): δ = 1.67–2.04 (m, 6H, N–CH_2–CH_2, N–CH–CH_2, CH_2), 2.16–2.30 (m, 3H, CH_2), 2.34–2.42 (m, 1H, CH_2), 3.36–3.43 (m, 1H, N–CH_2), 3.65–3.73 (m, 1H, N–CH_2), 4.34–4.38 (m, 1H, N–CH), 5.12–5.19 (m, 2H, CH_2–Ar), 7.28–7.39 (m, 5H, H_{ar}) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 21.2 °C, TMS): δ = 16.41 (N–CH–CH_2), 23.92 (N–CH_2–CH_2), 28.37 (CH_2), 29.29 (CH_2), 48.07 (N–CH_2), 53.56 (C_q), 61.72 (N–CH), 67.10 (CH_2–Ar), 127.99 (CH_{ar}), 128.47 (CH_{ar}), 136.65 (C_{ar}), 156.40 (O–CO–N), 181.84 (COOH) ppm. MS (CI, CH_5^+); m/z (%) = 304 (84) $[M+H]^+$, 286 (43), 260 (100), 210 (5), 204

(18), 196 (37), 170 (25), 168 (11), 160 (18), 123 (10). HRMS (70 eV): calcd for $C_{17}H_{21}NO_4$ $[M]^+$, 303.1471; found: 303.1469.

4.1.9.5. (2*RS*)-2-Hydroxy-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}acetic acid [*rac*-(*l*)-39][59]. According to GP7, method B with *rac*-(*l*)-**38** (283 mg, 0.96 mmol), LiOH (71 mg, 3 mmol, 2.4 ml, 1.2 M in H_2O), dioxane (21.7 ml) and the reaction time was 1.5 h. Unlike the GP 6, the extraction at pH 1 was first done with CH_2Cl_2 (3 × 50 ml) and later with EtOAc (2 × 50 ml). CC (SiO_2, pentane/EtOAc/HOAc = 20:80:2) of the crude product afforded 217 mg (81%) of *rac*-(*l*)-**39** as colourless oil.

TLC: R_f = 0.26 (SiO_2, Pentane/EtOAc/HOAc = 20:80:2). IR (KBr): $\tilde{\nu}$ = 3419, 2957, 1682, 1498, 1426, 1360, 1203, 1124, 1078, 1048, 1029, 911, 770, 736, 698, 669, 606, 552 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 19.6 °C, TMS): δ = 1.96–1.81 (m, 1H, N–CH_2–CH_2), 1.85–2.06 (m, 2H, N–CH_2–CH_2, N–CH–CH_2), 2.08–2.19 (m, 1H, N–CH–CH_2), 3.36–3.44 (m, 1H, N–CH_2), 3.50–3.59 (m, 1H, N–CH_2), 4.16–4.29 (m, 1H, N–CH), 4.57 (d, J = 1.8 Hz, 0.79 × 1H, CH–O), 4.68 (br s, 0.21 × 1H, CH–O), 5.06–5.22 (m, 2H, CH_2–Ar), 7.27–7.40 (m, 5H, H_{ar}) ppm. Rotamers ratio (19.6 °C) 79:21. ^{13}C NMR (400 MHz, $CDCl_3$, 20.4 °C, TMS): δ = 24.18 (N–CH_2–CH_2), 27.18 (N–CH–CH_2), 47.60 (N–CH_2), 61.09 (N–CH), 67.60 (CH_2–Ar), 72.32 (*C*H–COO), 127.85 (CH_{ar}), 128.19 (CH_{ar}), 128.55 (CH_{ar}), 136.10 (C_{ar}), 156.79 (O–CO–N), 175.07 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 280 (100) $[M+H]^+$, 236 (84), 204 (13), 172 (92), 160 (13), 146 (25), 144 (10), 123 (13), 108 (39). Anal. calcd for $C_{14}H_{17}NO_5$ (279.30): C, 61.42; H, 6.53; N, 4.78. Found: C, 61.18; H, 6.61; N, 4.76.

The ^1H NMR data was in accord with the literature values given for the diastereomeric mixture *rac*-(*l*)-**39**/*rac*-(*u*)-**39**.[59]

4.1.9.6. (2*SR*)-2-Hydroxy-2-{(2*RS*)-1-[(benzyloxy)carbonyl]pyrrolidine-2-yl}-acetic acid [*rac*-(*u*)-39][59]. According to GP7, method B with *rac*-(*u*)-**38** (280 mg, 0.96 mmol), LiOH (71 mg, 3 mmol, 2.4 ml, 1.2 M in H_2O), dioxane (21.5 ml) and the reaction time was 1.5 h. Unlike the GP6, the extraction at pH 1 was first done with CH_2Cl_2 (3 × 50 ml) and later with EtOAc (2 × 50 ml). CC (SiO_2, acetone/pentane/EtOAc/HOAc = 25:50:25:1) of the crude product afforded 269 mg (81%) of [*rac*-(*u*)-**39**] as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3441, 2960, 1680, 1426, 1359, 1197, 1121, 983, 913, 737, 698, 669, 603, 546 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 20.8 °C, TMS): δ = 1.76–1.89 (m, 1H, N–CH_2–CH_2), 1.93–2.10 (m, 2H, N–CH_2–CH_2, N–CH–CH_2), 2.09 (s, 0.58 × 1H, OH), 2.17 (s, 0.42 × 1H, OH), 2.18–2.31 (m, 1H, N–CH–CH_2), 3.40–3.48 (m, 1H, N–CH_2), 3.50–3.60 (m, 1H, N–CH_2), 4.10–4.16 (m, 1H, *CH*–OH), 4.16–4.23 (m, 1H, N–CH), 5.13 (br s, 2H, CH_2–Ar), 7.29–7.40 (m, 5H, H_{ar}) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, 21.4 °C, TMS): δ = 23.96 (CH_2), 29.02 (CH_2), 47.65 (N–CH_2), 60.43 (N–CH), 67.71 (CH_2–Ar), 73.69 (*C*H–COO), 127.93 (CH_{ar}), 128.18 (CH_{ar}), 128.53 (CH_{ar}), 136.05 (C_{ar}), 157.13 (O–CO–N), 174.88 (COO) ppm. MS (CI, CH_5^+); m/z (%) = 280 (4) $[M+H]^+$, 262 (6), 236 (6), 218 (6), 215 (17), 181 (6), 172 (100), 169 (9), 155 (8), 146 (10), 141 (10), 130 (31), 128 (71), 126 (9), 123 (15), 108 (41). HRMS (FAB, NBA): calcd for $C_{14}H_{18}NO_5$ $[M+H]^+$, 280.1185; Found: 280.1140.

The ^1H NMR data was in accord with the literature for the diastereomeric mixture *rac*-(*l*)-**39**/*rac*-(*u*)-**39**.[59]

4.1.9.7. (2*RS*)-2-[(Benzyloxycarbonyl)amino]-2-{(2*RS*)-1-[(benzyloxy)carbonyl]-pyrrolidine-2-yl}acetic acid [*rac*-(*l*)-41]. According to GP7, method B: *rac*-(*l*)-**40** (74 mg, 1.73 mmol) together with LiOH (57 mg, 2.4 mmol, 2.0 ml, 1.2 M in H_2O) and dioxane (6 ml) was stirred at rt for 2 h. The reaction mixture was acidified to pH = 2 with 2 M HCl and then extracted with CH_2Cl_2 (3 × 10 ml). The organic phase was pooled, dried over $MgSO_4$ and evaporated to dryness in vacuum to afford 67.6 mg (95%) of *rac*-(*l*)-**41** as colourless crystals.

Mp: 87 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3322, 3064, 3033, 2958, 1720, 1586, 1522, 1498, 1455, 1419, 1359, 1287, 1241, 1212, 1119, 1061, 1040, 1028, 982, 914, 824, 769, 738, 697, 603 cm^{-1}. ^1H NMR (400 MHz, $C_6D_5NO_2$, 140 °C): δ = 1.78–1.89 (m, 1H, N–CH_2–CH_2), 1.97–2.08 (m, 1H, N–CH_2–CH_2), 2.13–2.22 (m, 2H, N–CH–CH_2), 3.44–3.53 (m, 1H, N–CH_2), 3.64–3.73 (m, 1H, N–CH_2), 4.41–4.47 (m, 1H, N–CH), 5.08 (dd, J = 8.6/2.9 Hz, 1H, CH–COO), 5.14–5.30 (m, 4H, CH_2–Ar), 6.40 (br s, 1H, NH), 7.23–7.47 (m, 10H, H_{ar}) ppm. MS (ESI); m/z (%) = 435 (64) $[M+Na]^+$, 413 (86) $[M+H]^+$, 369 (100). Anal. calcd for $C_{22}H_{24}N_2O6$ (412.45): C, 64.07; H, 5.87; N, 6.79. Found: C, 63.82; H, 6.07; N, 6.60.

4.1.9.8. (2*SR*)-2-[(*tert*-Butoxycarbonyl)amino]-2-{(2*RS*)-1-[(*tert*-butoxy)carbonyl]pyrrolidine-2-yl}acetic acid [*rac*-(*u*)-44]. According to GP7, method B with *rac*-(*u*)-**43** (56 mg, 0.15 mmol), LiOH (29 mg, 1.2 mmol, 1.0 ml, 1.2 M in H_2O), dioxane (3 ml) and the reaction time was 1.5 h. This afforded 49.8 mg (93%) of *rac*-(*u*)-**44** as colourless oil.

IR (KBr): $\tilde{\nu}$ = 3441, 2979, 1718, 1508, 1395, 1367, 1255, 1165, 1054, 864, 774 cm^{-1}. ^1H NMR (400 MHz, $C_2D_2Cl_4$, 120 °C): δ = 1.50 (s, 9H, CH_3), 1.54 (s, 9H, CH_3), 1.83–2.05 (m, 3H, N–CH_2–CH_2, N–CH–CH_2), 2.06–2.18 (m, 1H, N–CH–CH_2), 3.31–3.40 (m, 1H, N–CH_2), 3.47–3.56 (m, 1H, N–CH_2), 4.25–4.34 (m, 2H, N–*CH*–CH_2, CH–COO), 5.92 (br s, 1H, NH) ppm. ^{13}C NMR (400 MHz, $C_2D_2Cl_4$, 120 °C): δ = 23.29 (N–CH_2–CH_2), 28.39 (CH_3), 28.47 (CH_3), 29.47 (N–CH–CH_2), 47.14 (N–CH_2), 57.83 (N–CH), 57.85 (N–CH), 80.52 (C_q), 80.67 (C_q), 155.80 (O–CO–N), 156.32 (O–CO–N), 171.88 (COO) ppm. MS (ESI); m/z (%) = 367 (100) $[M+Na]^+$, 345 (12) $[M+H]^+$. HRMS (FAB, NBA): calcd for $C_{16}H_{29}N_2O_6$ $[M+H]^+$, 345.2026; found: 345.1985.

4.1.9.9. (2*RS*)-2-Hydroxy-2-{[(2*RS*)(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)]pyrrolidine-2-yl}acetic acid [*rac*-(*l*)-13b]. According to GP7, method C: *rac*-(*l*)-**46** (22 mg, 60 µmol) in dioxane (6 ml) and aq LiOH (57 mg, 2.4 mmol) at rt for 2 h. This afforded 20 mg (95%) of *rac*-(*l*)-**13b** as colourless crystals. Mp: 75 °C. IR (KBr): $\tilde{\nu}$ = 3406, 3054, 3023, 2957, 2925, 1619, 1494, 1444, 1364, 1122, 1074, 1029, 763, 701, 632 cm^{-1}. ^1H NMR (400 MHz, CD_3OD, 20.9 °C): δ = 1.78–2.08 (m, 4H, N–CH_2–CH_2–CH_2), 2.60 (dt, J = 7.9/7.5 Hz, 2H, C=CH–CH_2), 2.97–3.08 (m, 1H, N–CH_2–CH_2–CH_2), 3.10–3.22 (m, 1H, N–CH_2–CH_2–CH), 3.44–3.59 (m, 2H, N–CH_2–CH_2–CH_2, N–CH_2–CH_2–CH), 3.65–3.75 (m, 1H, N–*CH*–CH_2), 4.22 (d, J = 3.3 Hz, 1H, *CH*–OH), 6.11 (t, J = 7.5 Hz, 1H, C=CH), 7.19–7.48 (m, 10H, H_{ar}) ppm. ^{13}C NMR (400 MHz, CD_3OD, 21.9 °C): δ = 22.77 (N–CH_2–CH_2–CH_2), 24.72 (N–CH–CH_2), 27.30 (C=CH–CH_2), 54.42 (N–CH_2), 55.04 (N–CH_2), 68.83 (CH–OH), 71.65 (N–CH), 123.33 (C=*C*H), 128.38 (CH_{ar}), 128.63 (CH_{ar}), 128.76 (CH_{ar}), 129.26 (CH_{ar}), 129.73 (CH_{ar}), 130.71 (CH_{ar}), 140.71 (C_q), 143.11 (C_q), 147.21 (C_q), 176.09 (COO) ppm. MS (ESI); m/z (%) = 374 (100) $[M+Na]^+$, 352 (75) $[M+H]^+$, 276 (9), 129 (17). HRMS (FAB, NBA): calcd for $C_{22}H_{26}NO_3$ $[M+H]^+$, 352.1913; found: 352.1901.

4.1.9.10. (2*SR*)-2-Hydroxy-2-{[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)]pyrrolidine-2-yl}acetic acid [*rac*-(*u*)-13b]. According to GP7, method C: *rac*-(*u*)-**46** (17 mg, 47 µmol) in dioxane (6 ml) and aq LiOH (57 mg, 2.4 mmol) at rt for 2 h. Recrystallisation of the crude product in hexane/CH_2Cl_2 afforded 15.8 mg (96%) of *rac*-(*u*)-**13b** as colourless crystals.

Mp: 44 °C. IR (KBr): $\tilde{\nu}$ = 3427, 2924, 1624, 764, 702 cm^{-1}. ^1H NMR (400 MHz, CD_2Cl_2, 17.7 °C, TMS): δ = 1.73–1.81 (m, 1H, N–CH–CH_2), 1.83–1.91 (m, 2H, N–CH_2–CH_2–CH_2), 2.04–2.13 (m, 1H, N–CH–CH_2), 2.52–2.58 (m, 3H, N–CH_2–CH_2–CH_2, C=CH–CH_2),

2.82 (dt, J = 12.6/6.0 Hz, 1H, N–CH_2–CH$_2$–CH), 3.26 (dt, J = 11.0/5.5 Hz, 1H, N–CH_2–CH$_2$–CH$_2$), 3.33 (dt, J = 11.0/5.5 Hz, 1H, N–CH_2–CH$_2$–CH), 3.40 (ddd, J = 8.8/7.1/5.5 Hz, 1H, N–CH), 4.11 (d, J = 5.5 Hz, 1H, CH–OH), 6.04 (t, J = 7.1 Hz, 1H, C=CH), 7.18–7.46 (m, 10H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 20.3 °C, TMS): δ = 23.33 (N–CH$_2$–CH_2–CH$_2$), 25.78 (N–CH–CH_2), 27.43 (N–CH$_2$–CH_2–CH), 53.72 (N–CH$_2$), 53.88 (N–CH$_2$), 66.51 (CH–OH), 67.52 (N–CH), 122.95 (C=CH), 127.78 (CH$_{ar}$), 127.97 (CH$_{ar}$), 128.01 (CH$_{ar}$), 128.59 (CH$_{ar}$), 128.94 (CH$_{ar}$), 129.98 (CH$_{ar}$), 139.69 (C_q), 142.17 (C_q), 146.40 (C_q), 175.56 (COO) ppm. MS (ESI); m/z (%) = 374 (9) [M+Na]$^+$, 352 (100) [M+H]$^+$. HRMS (FAB, NBA): calcd for $C_{22}H_{26}NO_3$ [M+H]$^+$, 352.1913; Found 352.1876.

4.1.9.11. (2*RS*)-2-Hydroxy-2-{(2*RS*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl}acetic acid [*rac*-(*l*)-13c]. According to GP7, method C: *rac*-(*l*)-**47** (13 mg, 31 μmol) in dioxane (6 ml) and aq LiOH (57 mg, 2.4 mmol) at rt for 2 h. Purification of the crude product by CC (RP2, H_2O/MeCN = 75:25) afforded 11.8 mg (97%) of *rac*-(*l*)-**13c** as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3397, 2923, 2854, 1618, 1457, 1380, 1124, 1010, 932, 712 cm^{-1}. ^{1}H NMR (500 MHz, CD_2Cl_2, 25.9 °C, TMS): δ = 2.01–2.21 (m, 4H, N–CH$_2$–CH_2–CH_2), 2.00 (s, 3H, CH$_3$), 2.04 (s, 3H, CH$_3$), 2.50–2.56 (m, 2H, C=CH–CH_2), 2.77–2..85 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 2.85–2.94 (m, 1H, N–CH_2–CH$_2$–CH), 3.09–3.16 (m, 1H, N–CH_2–CH$_2$–CH), 3.24 (br s, 1H, N–CH), 3.38–3.46 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 3.61–3.68 (m, 1H, CH–OH), 6.00 (t, J = 7.4 Hz, 1H, C=CH), 6.79 (d, J = 5.5 Hz, 1H, H_{ar}), 6.91 (d, J = 5.5 Hz, 1H, H_{ar}), 7.11 (d, J = 5.5 Hz, 1H, H_{ar}), 7.29 (d, J = 5.5 Hz, 1H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 22.4 °C, TMS): δ = 14.60 (Ar–CH$_3$), 15.08 (Ar–CH$_3$), 23.04 (N–CH$_2$–CH_2), 26.25 (N–CH–CH_2), 26.96 (C=CH–CH_2), 52.67 (N–CH_2–CH$_2$–CH, N–CH$_2$–CH$_2$–CH$_2$), 68.19 (N–CH, CH–O), 123.80 (C$_{ar}$), 125.22 (C$_{ar}$), 128.51 (C=CH), 130.41 (C$_{ar}$), 131.66 (C=CH), 131.82 (C$_{ar}$), 134.44 (C$_{ar}$), 134.96 (C$_{ar}$), 136.55 (C$_{ar}$), 138.90 (C$_{ar}$), 175.34 (COO) ppm. MS (ESI); m/z (%) = 414 (5) [M+Na]$^+$, 392 (100) [M+H]$^+$, 247 (15). HRMS (FAB, NBA): calcd for $C_{20}H_{26}NO_3S_2$ [M+H]$^+$, 392.1354; found: 392.1403.

4.1.9.12. (2*SR*)-2-Hydroxy-2-{(2*SR*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl} acetic acid [*rac*-(*u*)-13c]. According to GP7, method C: *rac*-(*u*)-**47** (22 mg, 60 μmol) in dioxane (6 ml) and aq LiOH (57 mg, 2.4 mmol) at rt for 2 h. This afforded 11.2 mg (96%) of *rac*-(*u*)-**13c** as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3421, 2922, 1618, 1106, 1007, 933, 714 cm^{-1}. ^{1}H NMR (500 MHz, CD_2Cl_2, 21.4 °C, TMS): δ = 1.77 (dq, J = 13.7/7.1 Hz, 1H, N–CH$_2$–CH$_2$–CH_2), 1.87–1.94 (m, 2H, N–CH$_2$–CH_2–CH$_2$), 2.00 (s, 3H, CH$_3$), 2.04–2.21 (m, 4H, CH$_3$, N–CH$_2$–CH$_2$–CH_2), 2.56 (q, J = 7.7 Hz, 2H, C=CH–CH_2), 2.67 (dt, J = 11.0/8.2 Hz, 1H, N–CH_2–CH$_2$–CH$_2$), 2.86 (dt, J = 12.6/7.1 Hz, 1H, N–CH_2–CH$_2$–CH), 3.30 (dt, J = 12.6/8.2 Hz, 1H, N–CH_2–CH$_2$–CH), 3.38–3.47 (m, 2H, N–CH, N–CH_2–CH$_2$–CH$_2$), 4.11 (d, J = 5.5 Hz, 1H, CH–OH), 6.02 (t, J = 7.7 Hz, 1H, C=CH), 6.80 (d, J = 5.5 Hz, 1H, H_{ar}), 6.91 (d, J = 5.5 Hz, 1H, H_{ar}), 7.12 (d, J = 5.5 Hz, 1H, H_{ar}), 7.30 (d, J = 5.5 Hz, 1H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 21.5 °C, TMS): δ = 14.59 (Ar–CH$_3$), 15.07 (Ar–CH$_3$), 23.46 (N–CH$_2$–CH_2–CH$_2$), 25.91 (N–CH$_2$–CH$_2$–CH_2), 27.72 (C=CH–CH_2), 53.62 (N–CH_2–CH$_2$–CH), 54.13 (N–CH_2–CH$_2$–CH$_2$), 66.80 (CH–OH), 67.36 (N–CH), 123.86 (CH$_{ar}$), 125.32 (CH$_{ar}$), 128.48 (C=CH), 130.37 (CH$_{ar}$), 131.84 (CH$_{ar}$), 131.87 (C=CH), 134.45 (C$_{ar}$), 135.01 (C$_{ar}$), 136.55 (C$_{ar}$), 138.83 (C$_{ar}$), 175.54 (COO) ppm. MS (ESI); m/z (%) = 414 (56) [M+Na]$^+$, 392 (100) [M+H]$^+$, 316 (4), 294 (5), 247 (17). HRMS (FAB, NBA): calcd for $C_{20}H_{26}NO_3S_2$ [M+H]$^+$, 392.1354; found: 392.1392.

4.1.9.13. (2*RS*)-2-Hydroxy-[(2*RS*)-1-{2-[tris(4-methoxyphenyl) methoxy]-ethyl}-pyrrolidine-2-yl]acetic acid [*rac*-(*l*)-13d]. According to GP7, method C: *rac*-(*l*)-**48** (23 mg, 44 μmol) in dioxane (6 ml) and aq LiOH (77 mg, 3.2 mmol) at rt for 2 h. Recrystallisation of the crude product in heptane/CH_2Cl_2 afforded 23 mg (99%) of *rac*-(*l*)-**13d** as colourless crystals.

Mp: 62 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3431, 2926, 2853, 1608, 1508, 1463, 1302, 1250, 1176, 1034, 827, 583 cm^{-1}. ^{1}H NMR (400 MHz, CH_2Cl_2, 19.9 °C, CD_2Cl_2): δ = 1.88–2.21 (m, 4H, N–CH$_2$–CH_2–CH_2), 2.81–3.00 (m, 2H, N–CH_2–CH$_2$–CH$_2$, N–CH_2–CH$_2$–O), 3.23–3.48 (m, 5H, N–CH, N–CH_2–CH$_2$–CH$_2$, N–CH_2–CH$_2$–O, O–CH$_2$), 3.74–3.79 (m, 10H, O–CH$_3$, CH–OH), 6.82–6.87 (m, 6H, H_{ar}), 7.31–7.36 (m, 6H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 25.8 °C, TMS): δ = 23.03 (N–CH$_2$–CH$_2$–CH$_2$), 30.11 (N–CH–CH_2), 52.24 (N–CH_2–CH$_2$–CH$_2$), 55.66 (N–CH$_2$–CH$_2$–O, O–CH$_3$), 59.43 (O–CH$_2$), 67.48 (N–CH), 68.09 (CH–O), 87.41 (C_q), 113.68 (CH$_{ar}$), 130.07 (CH$_{ar}$), 136.33 (C$_{ar}$), 159.11 (C$_{ar}$), 175.36 (COO) ppm. MS (ESI); m/z (%) = 544 (11) [M+Na]$^+$, 522 (2) [M+H]$^+$, 333 (100). HRMS (FAB, NBA): calcd for $C_{30}H_{36}NO_7$ [M+H]$^+$, 522.2492; Found 522.2509.

4.1.9.14. (2*SR*)-2-Hydroxy-[(2*RS*)-1-{2-[tris(4-methoxyphenyl) methoxy]-ethyl} pyrrolidine-2-yl]acetic acid [*rac*-(*u*)-13d]. According to GP7, method C: *rac*-(*u*)-**48** (101 mg, 0.19 mmol) in dioxane (6 ml) and aq LiOH (77 mg, 3.2 mmol) at rt for 2.5 h. This afforded 97 mg (98%) of *rac*-(*u*)-**13d** as colourless crystals.

Mp: 84 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3428, 2926, 1608, 1508, 1462, 1302, 1250, 1176, 1092, 1032, 828, 584 cm^{-1}. ^{1}H NMR (400 MHz, CD_2Cl_2, 20.1 °C, TMS): δ = 1.75–1.83 (m, 1H, N–CH–CH_2), 1.84–1.93 (m, 2H, N–CH$_2$–CH_2–CH$_2$), 2.08 (dq, J = 13.2/8.5 Hz, 1H, N–CH–CH_2), 2.62–2.69 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 2.75–2.83 (m, 1H, N–CH_2–CH$_2$–O), 3.29–3.46 (m, 5H, N–CH, N–CH_2–CH$_2$–CH$_2$, N–CH_2–CH$_2$–O, O–CH$_2$), 3.78 (s, 9H, O–CH$_3$), 4.11 (d, J = 5.5 Hz, 1H, CH–OH), 6.83–6.87 (m, 6H, H_{ar}), 7.32–7.36 (m, 6H, H_{ar}) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2, 23.9 °C, CD_2Cl_2): δ = 23.07 (N–CH$_2$–CH$_2$–CH$_2$), 25.41 (N–CH–CH$_2$), 53.56 (N–CH$_2$), 53.69 (N–CH$_2$), 55.26 (O–CH$_3$), 59.13 (CH$_2$–O), 66.33 (N–CH), 67.23 (CH–OH), 86.89 (O–C$_q$), 113.26 (CH$_{ar}$), 129.70 (CH$_{ar}$), 135.88 (C$_{ar}$), 158.72 (C$_{ar}$), 175.20 (COO) ppm. MS (FAB); m/z (%) = 522 (1) [M+H]$^+$, 391 (21), 333 (100). HRMS (FAB, NBA): calcd for $C_{30}H_{36}NO_7$ [M+H]$^+$, 522.2492; found: 522.2518.

4.1.9.15. (2*RS*)-2-Amino-2-{[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl)] pyrrolidine-2-yl}-acetic acid·2TFA [*rac*-(*l*)-14b·2TFA]. According to GP7, method C with *rac*-(*l*)-**51** (35.6 mg, 77 μmol) in dioxane (6 ml) and aq LiOH (77 mg, 3.2 mmol, 1.6 M) at rt for 2 h. Followed by the procedure according to GP5 in CH_2Cl_2 (2 ml) and TFA (6.1 g, 4 ml, 54 mmol) afforded 25.9 g (97%) *rac*-(*l*)-**14b**·2TFA as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3424, 2926, 1676, 1493, 1202, 1130, 763, 701 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 23.7 °C): δ = 1.90–2.00 (m, 1H, N–CH–CH_2), 2.00–2.12 (m, 2H, N–CH$_2$–CH_2–CH$_2$), 2.22–2.32 (m, 1H, N–CH–CH_2), 2.57–2.63 (m, 2H, N–CH$_2$–CH_2–CH), 2.97–3.06 (m, 1H, N–CH_2–CH$_2$–CH$_2$), 3.15–3.25 (m, 1H, N–CH_2–CH$_2$–CH), 3.36–3.51 (m, 2H, N–CH_2–CH$_2$–CH, N–CH_2–CH$_2$–CH$_2$), 3.76–3.83 (m, 1H, N–CH–CH$_2$), 3.87 (d, J = 8.8 Hz, 1H, CH–COO), 6.16 (t, J = 7.4 Hz, 1H, C=CH), 7.27–7.54 (m, 10H, H_{ar}) ppm. ^{13}C NMR (400 MHz, D_2O, 19.1 °C, MeOH): δ = 22.62 (CH$_2$), 23.01 (CH$_2$), 26.40 (C=CH–CH$_2$), 52.66 (CH–COO), 53.53 (N–CH$_2$), 56.20 (N–CH$_2$), 67.85 (N–CH–CH$_2$), 123.28 (C=CH), 127.91 (CH$_{ar}$), 128.47 (CH$_{ar}$), 128.58 (CH$_{ar}$), 129.23 (CH$_{ar}$), 129.38 (CH$_{ar}$), 130.34 (CH$_{ar}$), 139.77 (C$_{ar}$), 142.29 (C$_{ar}$), 146.20 (C=CH), 163.89 (COO) ppm. MS (ESI); m/z (%) = 373 (20) [M+Na]$^+$, 351 (100) [M+H]$^+$, 276 (29). HRMS (FAB, NBA): calcd for $C_{22}H_{27}N_2O_2$ [M+H]$^+$, 351.1994; found: 351.2033.

4.1.9.16. (2*SR*)-2-Amino-2-[(2*RS*)-1-(4,4-diphenylbut-3-en-1-yl) pyrrolidine-2-yl] acetic acid·2TFA [*rac*-(*u*)-14b·2TFA]. According to GP7, method C with *rac*-(*u*)-**51** (80 mg, 0.17 mmol) in dioxane (6 ml) and aq LiOH (77 mg, 3.2 mmol, 1.6 M) at rt for 2 h. Followed by the procedure according to GP5 in CH_2Cl_2 (2 ml) and TFA (6.1 g, 4 ml, 54 mmol). Here the reaction mixture was

extracted in CH_2Cl_2 (2 × 10 ml) at pH = 7 and pH = 6, the organic phase was dried over $MgSO_4$ and concentrated in vacuum to afford 52.5 mg (87%) of *rac*-(*u*)-**14b**·2TFA as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3442 cm^{-1}, 2924, 1683, 1635, 1205, 1130 cm^{-1}. ^1H NMR (400 MHz, D_2O, 20.8 °C): δ = 1.64–1.75 (m, 1H, N–CH–C*H*$_2$), 1.83–1.94 (m, 1H, N–CH$_2$–C*H*$_2$–CH$_2$), 1.94–2.06 (m, 1H, N–CH$_2$–C*H*$_2$–CH$_2$), 2.20–2.31 (m, 1H, N–CH–C*H*$_2$), 2.61–2.71 (m, 2H, N–CH$_2$–C*H*$_2$–CH), 2.95 (dt, *J* = 11.2/8.1 Hz, 1H, N–C*H*$_2$–CH$_2$–CH$_2$), 3.16–3.27 (m, 2H, N–C*H*$_2$–CH$_2$–CH, N–C*H*$_2$–CH$_2$–CH$_2$), 3.54–3.64 (m, 1H, N–C*H*$_2$–CH$_2$–CH), 3.69 (m, 1H, N–C*H*–CH$_2$), 4.10 (d, *J* = 3.7 Hz, 1H, CH–COO), 6.11 (t, *J* = 7.7 Hz, 1H, C=CH), 7.26–7.51 (m, 10H, H_{ar}) ppm. ^{13}C NMR (400 MHz, D_2O, 20.4 °C, dioxane): δ = 22.99 (N–CH$_2$–*C*H$_2$–CH$_2$), 26.38 (C=CH–*C*H$_2$), 26.48 (N–CH–*C*H$_2$), 52.34 (*C*H–COO), 53.97 (N–CH$_2$), 54.33 (N–CH$_2$), 68.25 (N–*C*H–CH$_2$), 123.66 (C=*C*H), 128.02 (CH_{ar}), 128.50 (CH_{ar}), 128.54 (CH_{ar}), 129.17 (CH_{ar}), 129.33 (CH_{ar}), 130.35 (CH_{ar}), 139.68 (C_{ar}), 142.41 (C_{ar}), 146.65 (*C*=CH), 176.00 (COO) ppm. MS (ESI); *m*/*z* (%) = 351 (100) $[M+H]^+$, 276 (29). HRMS (FAB, NBA): calcd for $C_{22}H_{27}N_2O_2$ $[M+H]^+$, 351.2073; found: 351.2045.

4.1.9.17. (2*RS*)-2-Amino-2-{(2*RS*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]-pyrrolidine-2-yl}acetic acid·2 TFA [*rac*-(*l*)-14c·2TFA]. According to GP7, method C with *rac*-(*l*)-**52** (20 mg, 39 µmol) taken in dioxane (6 ml) and aq LiOH (57 mg, 2.4 mmol, 1.2 M) at rt for 2 h. Followed by procedure according to GP5, in CH_2Cl_2 (2 ml) and TFA (6.1 g, 54 mmol, 4 ml) and on purification of the crude product by CC (RP2, H_2O/MeCN/TFA = 60:40:1) afforded 19.5 mg (73%) of *rac*-(*l*)-**14c**·2TFA as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3427, 2925, 1685, 1206, 1129, 834, 803, 721 cm^{-1}. ^1H NMR (500 MHz, D_2O, 21.8 °C): δ = 1.97–2.17 (m, 9H, C*H*$_3$, N–CH–C*H*$_2$, N–CH$_2$–C*H*$_2$), 2.30–2.38 (m, 1H, N–CH–C*H*$_2$), 2.58 (q, *J* = 7.1 Hz, 2H, N–CH$_2$–*C*H$_2$–CH), 3.13–3.21 (m, 1H, N–CH$_2$), 3.21–3.28 (m, 1H, N–C*H*$_2$–CH$_2$–CH), 3.39–3.48 (m, 1H, N–C*H*$_2$–CH$_2$–CH), 3.57–3.65 (m, 1H, N–CH$_2$), 3.84–3.90 (m, 1H, N–C*H*–CH$_2$), 3.92–3.95 (m, 1H, CH–COO), 6.08 (t, *J* = 7.1 Hz, 1H, C=CH), 6.88 (d, *J* = 5.2 Hz, 1H, H_{ar}), 7.00 (d, *J* = 5.2 Hz, 1H, H_{ar}), 7.26 (d, *J* = 5.2 Hz, 1H, H_{ar}), 7.42 (d, *J* = 5.2 Hz, 1H, H_{ar}) ppm. ^{13}C NMR (500 MHz, D_2O, 21.4 °C, MeOH): δ = 14.40 (CH_3), 14.89 (CH_3), 22.70 (N–CH$_2$–*C*H$_2$–CH$_2$), 26.62 (N–CH$_2$–CH$_2$–*C*H$_2$), 27.50 (N–CH$_2$–*C*H$_2$–CH), 52.48 (*C*H–COO), 53.91 (N–CH$_2$), 56.01 (N–CH$_2$), 67.87 (N–*C*H–CH$_2$), 117.05 (q, *J* = 291.7 Hz, *C*F$_3$), 124.58 (CH), 126.07 (CH), 128.56 (CH), 130.79 (CH), 131.67 (C_{ar}), 132.34 (CH), 134.61 (C_{ar}), 135.54 (C_{ar}), 137.14 (C_{ar}), 138.95 (*C*=CH), 163.52 (q, *J* = 35.5 Hz, CF$_3$–*C*OO), 170.62 (CH–*C*OO) ppm. MS (ESI); *m*/*z* (%) = 413 (13) $[M+Na]^+$, 391 (100) $[M+H]^+$, 330 (19), 316 (30), 247 (25). HRMS (FAB, NBA): $[M+H]^+$ required for $C_{20}H_{27}N_2O_2S_2$, 391.1514; found 391.1549.

4.1.9.18. (2*SR*)-2-Amino-2-{(2*RS*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-en-1-yl]pyrrolidine-2-yl} acetic acid·2TFA [*rac*-(*u*)-14c·2TFA]. According to GP7, method C with *rac*-(*u*)-**52** (17 mg, 33 µmol) in dioxane (6 ml) was added aq LiOH (57 mg, 2.4 mmol, 1.2 M) and stirred at rt for 2 h. Followed by procedure according to GP5, the residue taken in CH_2Cl_2 (2 ml) and TFA (6.1 g, 54 mmol, 4 ml). Here the reaction was worked up as in GP 6, method C, to afford 10 mg (82%) of *rac*-(*u*)-**14c**·2TFA as yellow oil.

IR (KBr): $\tilde{\nu}$ = 3433, 2924, 2854, 1629, 1459, 1202, 1175, 1130, 908, 734 cm^{-1}. ^1H NMR (400 MHz, D_2O, 18.4 °C, Dioxan): δ = 1.63–1.73 (m, 1H, N–CH–C*H*$_2$), 1.84–2.09 (m, 8H, CH$_3$, N–CH–CH$_2$–C*H*$_2$), 2.18–2.30 (m, 1H, N–CH–C*H*$_2$), 2.57–2.64 (m, 2H, N–CH$_2$–C*H*$_2$–CH), 2.99 (dt, *J* = 11.2/7.9 Hz, 1H, N–C*H*$_2$–CH$_2$–CH$_2$), 3.19 (dt, *J* = 12.7/6.2 Hz, 1H, N–C*H*$_2$–CH$_2$–CH), 3.37–3.45 (m, 1H, N–C*H*$_2$–CH$_2$–CH$_2$), 3.52 (dt, *J* = 12.7/7.5 Hz, 1H, N–C*H*$_2$–CH$_2$–CH), 3.65 (td, *J* = 8.6/4.2 Hz, 1H, N–C*H*–CH$_2$), 3.89 (d, *J* = 4.2 Hz, 1H, CH–COO), 6.10 (t, *J* = 7.5 Hz, 1H, C=CH), 6.87 (d, *J* = 5.3 Hz, 1H, H_{ar}), 6.89 (d, *J* = 5.3 Hz, 1H, H_{ar}), 7.24 (d, *J* = 5.3 Hz, 1H, H_{ar}), 7.41 (d, *J* = 5.3 Hz, 1H, H_{ar}) ppm. ^{13}C NMR (500 MHz, D_2O/MeOD = 1:1, 24.8 °C, MeOD): δ = 14.60 (CH_3), 14.95 (CH_3), 23.68 (N–CH$_2$–CH$_2$–*C*H$_2$), 27.80 (N–CH–*C*H$_2$), 28.00 (N–CH$_2$–*C*H$_2$–CH), 53.91 (CH–COO), 54.29 (N–*C*H$_2$–CH$_2$–CH), 54.79 (N–CH$_2$–*C*H$_2$–CH$_2$), 67.95 (N–*C*H–CH$_2$), 124.60 (CH_{ar}), 126.15 (CH_{ar}), 129.96 (C=CH), 131.03 (CH_{ar}), 132.10 (*C*=CH), 132.51 (CH_{ar}), 135.67 (C_{ar}), 137.30 (C_{ar}), 139.39 (C_{ar}), 135.29 (C_{ar}), 175.68 (COO) ppm. MS (ESI); *m*/*z* (%) = 413 (26) $[M+Na]^+$, 391 (100) $[M+H]^+$, 361 (19), 149 (25). HRMS (ESI): calcd for $C_{20}H_{27}N_2O_2S_2$ $[M+H]^+$, 391.1514; found: 391.1509.

4.2. Biological evaluation

4.2.1. GABA uptake assays

[^{3}H] GABA uptake assays with mGAT1, mGAT2, mGAT3, mGAT4 and hGAT-1 expressing HEK293 cells were performed as described earlier.[54] Stably hGAT-1 expressing HEK293 cells were obtained starting from the cDNA encoding hGAT-1 (kindly provided by Prof. N. Nelson) which was subcloned from pBluescript into the mammalian expression vector pcDNA3.1(+) (Invitrogen, Karlsruhe, Germany) using EcoR I and Xho I. Transfections with the linearised plasmid (Sca I) were performed as described earlier for mGAT1–4.[54]

4.2.2. MS-binding assays

MS-binding assays with mGAT1 obtained from a stable HEK293 cell line and NO 711[56] as a marker in competitive binding experiments were performed as described previously.[55] The hGAT-1 membrane preparation for the MS-binding assay was obtained in the same way as described for mGAT1.[55]

Acknowledgements

We would like to thank Prof. N. Nelson (University Tel Aviv) for kindly providing us the cDNA for hGAT-1. We also like to thank Katharina Heimberger for the editorial assistance and proof reading.

References and notes

1. Iversen, L. L.; Neal, M. J. *J. Neurochem.* **1968**, *15*, 1141.
2. Killam, K. F.; Bain, J. A. *J. Pharmacol. Exp. Ther.* **1957**, *119*, 255.
3. Czapinski, P.; Blaszczyk, B.; Czuczwar, S. J. *Curr. Top. Med. Chem.* **2005**, *5*, 3.
4. Hog, S.; Greenwood, J. R.; Madsen, K. B.; Larsson, O. M.; Frolund, B.; Schousboe, A.; Krogsgaard-Larsen, Povl.; Clausen, R. P. *Curr. Top. Med. Chem.* **2006**, *6*, 1861.
5. Lanctôt, K. L.; Herrmann, N.; Mazzotta, P.; Khan, L. R.; Ingber, N. *Can. J. Psychiatry* **2004**, *49*, 439.
6. Zadori, D.; Geisz, A.; Vamos, E.; Vecsei, L.; Klivenyi, P. *Pharmacol. Biochem. Behav.* **2009**, *94*, 148.
7. Mulleners, W. M.; Chronicle, E. P. *Cephalalgia* **2008**, *28*, 585.
8. Lloyd, K. G.; Shemen, L.; Hornykiewicz, O. *Brain Res.* **1977**, *127*, 269.
9. Jensen, T. S. *Eur. J. Pain* **2002**, *6*, 61.
10. Kalueff, A. N.; Nutt, D. J. *Depress. Anxiety* **2007**, *24*, 495.
11. Madsen, K. K.; White, H. S.; Schousboe, A. *Pharmacol. Ther.* **2010**, *125*, 394.
12. Bailer, M.; White, H. S. *Nat. Rev. Drug Disc.* **2010**, *9*, 68.
13. Krogsgaard-Larsen, Povl. *Med. Res. Rev.* **1988**, *8*, 27.
14. (a) Massen, J.; Sagné, C.; Hamon, M.; Mestikawy, S. E. *Pharmacol. Rev.* **1999**, *51*, 439; (b) Kirstensen, A. S.; Andersen, J.; Jorgensen, T. N.; Søvensen, L.; Eriksen, J.; Loland, C. J.; Strømgaard, K.; Gether, U. *Pharmacol. Rev.* **2011**, *63*, 585.
15. Dalby, N. O. *Eur. J. Pharmacol.* **2003**, *479*, 127.
16. Conti, F.; Minelli, A.; Melone, M. *Brain Res. Rev.* **2004**, *45*, 196.
17. Borden, L. A. *Neurochem. Int.* **1996**, *29*, 335.
18. Palacin, M.; Extevez, R.; Berthran, J.; Zorzano, A. *Physiol. Rev.* **1998**, *78*, 969.
19. Madsen, K. K.; Larson, O. M.; Schousboe, A. *Results Probl. Cell Differ.* **2007**, *44*, 201.
20. 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 Physiol.* **2012**, *302*, 316.
21. Zhou, Y.; Holmseth, S.; Guo, C.; Hassel, B.; Höffner, G.; Hultfeldt, S. H.; Wanner, K. T.; Danbolt, N. C. *J. Biol. Chem.* **2012**, *287*, 35733.
22. Kulig, K.; Szwaczkiewicz, M. *Mini-Rev. Med. Chem.* **2008**, *8*, 1214.
23. Kragler, A.; Höfner, G.; Wanner, K. T. *Eur. J. Pharmacol.* **2005**, *519*, 43.
24. Ali, F. E.; Bondinell, W. E.; Dandrige, P. A.; Frazee, J. S.; Garvey, E.; Girard, G. R.; Kaiser, C.; Ku, T. W.; Lafferty, J. J.; Moonsammy, G. I.; Oh, Hye-Ja.; Rush, J. A.;

Setler, P. E.; Stringer, O. D.; Venslavsky, J. W.; Volpe, B. W.; Yunger, L. M.; Zirkle, C. L. *J. Med. Chem.* **1985**, *28*, 653.
25. (a) Nielsen, E. B.; Suzdak, P. D.; Andersen, K. E.; Knutsen, J. S. L.; Sonnewald, U. *Eur. J. Pharmacol.* **1991**, *196*, 257; (b) Andersen, K. E.; Braestrup, C.; Gronwald, F. C.; Jorgensen, A. S.; Nielsen, E. B.; Sonnewald, U.; Sorensen, P. O.; Suzdak, P. D.; Knutsen, J. S. L. *J. Med. Chem.* **1993**, *36*, 1716.
26. 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.
27. Pabel, J.; Faust, M.; Prehn, C.; Wörlein, B.; Allmendinger, L.; Höfner, G.; Wanner, K. T. *ChemMedChem* **2012**, *7*, 1245.
28. Quandt, G.; Höfner, G.; Wanner, K. T. *Bioorg. Med. Chem.* **2013**, *21*, 3363.
29. Nakada, K.; Yoshikawa, M.; Ide, S.; Suemasa, A.; Kawamura, S.; Kobayashi, T.; Masuda, E.; Ito, Y.; Hayakawa, W.; Katayama, T.; Yamada, S.; Arisawa, M.; Minami, M.; Shuto, S. *Bioorg. Med. Chem.* **2013**, *21*, 4938.
30. Fülep, G. H.; Hoesel, C. E.; Höfner, G.; Wanner, K. T. *Eur. J. Med. Chem.* **2006**, *41*, 809.
31. Höfner, G.; Merkel, D.; Wanner, K. T. *ChemMedChem* **2009**, *4*, 1523.
32. Speckamp, W. N.; Moolenaar, M. J. *Tetrahedron* **2000**, *56*, 3817.
33. Maryanoff, B. E.; Zhang, H.-C.; Cohen, I. J.; Turchi, I. J.; Maryanoff, C. A. *Chem. Rev.* **2004**, *104*, 1431.
34. Dhimane, H.; Vanucci, G. L. *Tetrahedron Lett.* **1997**, *38*, 1415.
35. Okitsu, O.; Suzuki, R.; Kobayashi, S. *J. Org. Chem.* **2001**, *66*, 809.
36. Arns, S.; Barriault, L. *J. Org. Chem.* **2006**, *71*, 1809.
37. Nagasaka, T.; Tamano, H.; Hamaguchi, F. *Heterocycles* **1986**, *24*, 1231.
38. Macdonald, S. J. F.; Belton, D. J.; Buckley, D. M.; Spooner, J. E.; Anson, M. S.; Harrison, L. A.; Mills, K.; Upton, R. J.; Dowle, M. D.; Smith, R. A.; Molly, C. R.; Risley, C. *J. Med. Chem.* **1998**, *41*, 3919.
39. Ainsworth, C.; Chen, F.; Kuo, Y.-N. *J. Organomet. Chem.* **1972**, *46*, 59.
40. RajanBabu, T. V. *J. Org. Chem.* **1984**, *49*, 2083.
41. Emde, H.; Simchen, G. *Liebigs Ann. Chem.* **1983**, 816.
42. Revis, A.; Hilty, K. T. *J. Org. Chem.* **1990**, *55*, 2972.
43. Coppola, G. M.; Damon, R. E.; Eskesen, J. B.; France, D. S.; Paterniti, J. R., Jr. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2439.
44. Hattori, K.; Yamamoto, H. *Tetrahedron* **1994**, *50*, 3099.
45. Okano, K.; Morimoto, T.; Seliya, M. *Chem. Pharm. Bull.* **1985**, *33*, 2228.
46. Kawakami, T.; Ohtake, H.; Arakawa, H.; Okachi, T.; Imada, Y.; Murahashi, S.-I. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 2423.
47. Gilchrist, T. L.; Lemos, A.; Ottaway, C. J. *J. Chem. Soc. Perkin Trans. 1* **1997**, 3005.
48. Hiraga, Y.; Widianti, T.; Kunishi, T.; Abe, M. *Tetrahedron: Asymmetry* **2011**, *22*, 226.
49. Greco, M. N.; Zhong, H. M.; Maryanoff, B. E. *Tetrahedron Lett.* **1998**, *39*, 4959.
50. Hanessian, S.; Sharma, R. *Heterocycles* **2000**, *52*, 1231.
51. Cavelier-Frontin, F.; Jacquier, R.; Paladino, J.; Verducci, J. *Tetrahedron* **1991**, *47*, 9807.
52. Foresti, E.; Palmieri, G.; Petrini, M.; Profeta, R. *Org. Biomol. Chem.* **2003**, *1*, 4275.
53. Zhao, X.; Hoesl, C.; Hoefner, G. C.; Wanner, K. T. *Eur. J. Med. Chem.* **2005**, *40*, 231.
54. Kragler, A.; Höfner, G.; Wanner, K. T. *Eur. J. Med. Chem.* **2008**, *43*, 2404.
55. Zepperitz, C.; Höfner, G.; Wanner, K. T. *ChemMedChem* **2006**, *1*, 208.
56. Suzdak, P. D.; Frederiksen, K.; Andersen, K. E.; Serensen, P. O.; Knutsen, L. D. S.; Nielsen, E. B. *Eur. J. Pharmacol.* **1992**, *224*, 189.
57. Trachant, M.-J.; Moine, C.; Othmann, R. B.; Bousquest, T.; Othmann, M.; Dalla, V. *Tetrahedron Lett.* **2006**, *47*, 4477.
58. Zurawski Jr., V. R.; Stout, D. M.; Nitz, T. J. WO 2012/020389 A1.
59. Tsuda, M.; Muraoka, Y.; Someno, T.; Nagai, M.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1996**, *49*, 900.