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Ex-chiral pool synthesis and receptor binding studies of 4-substituted prolinol derivatives

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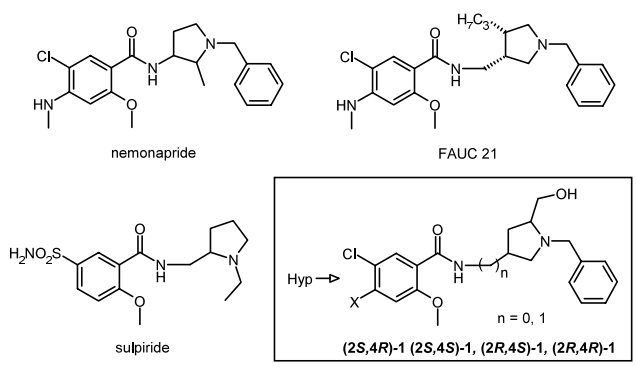
Abstract—Starting from natural 4-hydroxyproline, preparation of the four possible stereoisomers of 4-amino- and 4-aminomethyl-substituted prolinol derivatives, respectively, was accomplished by chemo- and regioselective functional group transformations at the 2- and 4-positions of the pyrrolidine moiety. These building blocks were used as valuable precursors for the preparation of new methoxybenzamide derivatives. Dopamine and serotonin binding studies involving the subtypes D1, $D2_{long}$, $D2_{short}$, D3 and D4 as well as 5-$HT1_A$ and 5-HT2, respectively, displayed interesting structure activity relationships, especially with respect to the absolute and relative configuration of the test compounds. As a complement to the D3 receptor preferring aminomethylpyrrolidine FAUC 21, the (2*R*,4*R*)-aminoprolinol derivative ***ent*-24** (FAUC 65) preferentially recognizing the D4 subtype was developed.

1. Introduction

2-Methoxybenzamide derivatives are known as highly active agents for the treatment of psychotic disorders including schizophrenia.[1] Unfortunately, their application is associated with extrapyramidal side-effects which are due to the affinity of these compounds to dopamine D2 receptors in striatal regions of the brain.[2] To decrease these negative side-effects, we were interested in the design of methoxybenzamide derived dopamine receptor antagonists with reduced D2 affinity and preference for the D3 and D4 subtype that seem to be especially involved in the symptoms of schizophrenia. In conjunction with this program, the correlation between the binding affinity and subtype selectivity on the one hand and the absolute and relative configuration on the other, as well as the spatial distance of the two nitrogen-atoms within the diaminopropane substructure, seemed to be of particular interest. Employing the antipsychotic agents sulpiride and nemonapride and our previously developed D3 ligand FAUC 21 as lead compounds,[3] structural hybridization led us to target compounds of type **1**. Starting from natural 4-hydroxyproline (Hyp), we herein describe an ex-chiral pool synthesis of the four possible stereoisomers of 4-amino and 4-aminomethyl substituted prolinol derivatives involving functional group transformations at the 2- and 4-positions of the pyrrolidine moiety. Coupling reactions of these compounds with pharmacophoric benzoic acids were performed leading to the test compounds of type **1** in all possible configurations (Scheme 1). Receptor binding investigations and subsequent SAR studies provided interesting results with respect to the substitution pattern and the stereochemistry.



Scheme 1.

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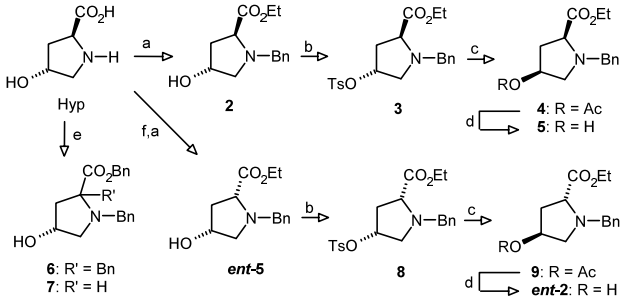
2. Synthesis

Recent SAR studies on nemonapride[3,4] and FAUC 21 derivatives led to the assumption that a benzyl substituent at the 1-position of the pyrrolidine moiety would induce high binding affinity. Thus, for the synthesis of the pyrrolidine moiety, we first reflected on the *N*-benzylated benzyl ester **7** as a suitable intermediate[5] giving access to further structural modifications at the 2- and 4-positions of the pyrrolidine moiety. Starting from natural 4-hydroxyproline, a one-step synthesis of **7** failed when the formation of 62% of the C-benzyl derivative **6** was observed as a 2:1 mixture of diastereomers (Scheme 2). Thus, we turned to a two-step synthesis of (2*S*,4*R*)-1-benzylproline ethyl ester **2** involving esterification and subsequent *N*-benzylation.[6,7] Interestingly, slight modification of the described conditions[6] gave an excellent yield (97%). The further three stereoisomeric hydroxyproline derivatives **5**, ***ent*-2** and ***ent*-5** with a (2*S*,4*S*)-, (2*R*,4*S*)- and a (2*R*,4*R*)-configuration, respectively, were synthesized by combinations of isomerizations at the 2- and 4-positions (Scheme 2).[8–16]

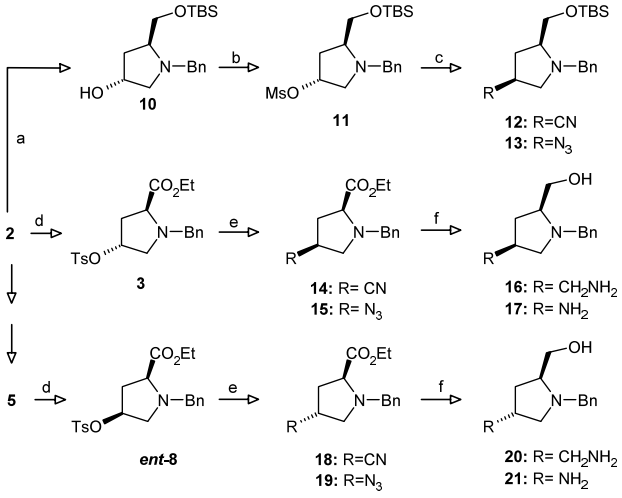


Scheme 2. *Reagents and conditions:* (a) 1. see Ref. 6 (**2**: 97%, ***ent*-5**: 98%); (b) TsCl, pyridine, 0°C to rt, 48 h (**3**: 67%, **8**: 89%); (c) tetrabutylammonium acetate, toluene, reflux, 4 h (**4**: 91%, **9**: 85%); (d) NaOEt, EtOH, 30 min, rt (**5**: 90%, ***ent*-2**: 81%); (e) aqueous K_2CO_3 (20%), BnBr, reflux, 3 h (**6**: 62%), 2:1 mixture of diastereomers; (f) see Refs. 12, 14, 15.

In detail, inversion at the 4-position was accomplished by *O*-sulfonylation of **2** (67% yield) and subsequent treatment of the tosylate **3** with tetrabutylammonium acetate. Selective cleavage of the thus formed acetoxy derivative **4** was performed with NaOEt giving access to the *cis*-hydroxyproline derivative **5** in 90% yield.[9–14] NOE experiments were performed, which clearly established the S_N2-type character of the nucleophilic displacement and thus complete inversion at the 4-position of the pyrrolidine moiety.

Epimerization of natural 4-hydroxyproline at the 2-position was done by subsequent treatment with acetic anhydride and 2 M HCl.[8,13–16] Esterification and *N*-benzylation of the thus produced *cis*-isomer afforded a 98% yield of the chiral building block ***ent*-5**.[6] The (2*R*,4*S*)-1-benzyl-4-hydroxyproline ethyl ester ***ent*-2** was synthesized in high overall yield by successive isomerization of both stereogenic centers.[8–16] Thus, *O*-activation of the protected cis-hydroxyproline derivative ***ent*-5** and subsequent nucleophilic displacement of the tosylate **8** employing tetrabutylammonium acetate resulted in formation of the *trans*-4-acetoxyproline derivative **9**. Finally, alcoholysis afforded the synthetic intermediate ***ent*-2**.

Selective functional group transformations at the 2- and 4-positions of the proline derivatives **2**, **5**, ***ent*-2** and ***ent*-5** gave access to the all possible stereoisomeres of 2-hydroxymethyl-4-aminomethylpyrrolidine[3] **16**, **20**, ***ent*-16** and ***ent*-20** and 2-hydroxymethyl-4-aminopyrrolidine[8,17–19] **17**, **21**, ***ent*-17** and ***ent*-21** as central intermediates for the preparation of the methoxybenzamides of type **1**. Two alternative synthetic pathways are also elaborated (Scheme 3).

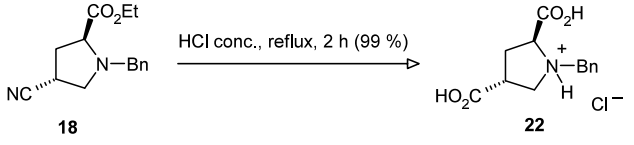


Scheme 3. *Reagents and conditions:* (a) 1. $LiAlH_4$, THF, 0°C, 1 h; 2. imidazole, TBSCl, DMF, 0°C, 1 h (**10**: 38%); (b) NEt_3, MsCl, $CHCl_3$, −10°C, 3 h (**11**: 80%); (c) NaCN, tetrabutylammonium cyanide, DMSO, 70°C, 24 h (**12**: 38%); or NaN_3, DMF, 60°C, 4 h (**13**: 53%); (d) TsCl, pyridine, 0°C to rt, 48 h (**3**: 67%, ***ent*-8**: 89%); (e) NaCN, DMSO, 60°C, 4 h (**14**: 90%, **18**: 98%); or NaN_3, DMF, 60°C, 7 h (**15**: 92%, **19**: 98%); (f) $LiAlH_4$, THF, 0°C, 1.5 h (**16**: 40%, **20**: 52%) or $LiAlH_4$, THF, 0°C, 1 h (**17**: 94%, **21**: 48%).

Following the first strategy, regioselective *O*-activation[8–14,20–23] at the 4-position of the pyrrolidine **2** was accomplished after $LiAlH_4$ reduction at the 2-position[6,7] and subsequent protection of the resulting primary alcohol using TBSCl in the presence of imidazole,[6,24,25] when the success of the selective silylation was strongly dependent on the reaction time due to the formation of the disilylated product[26] in case of prolonged treatment with TBSCl. Using MsCl in the presence of NEt_3, activation at the 4-position of the monosilylated hydroxyprolinol **10**, gave the sulfonate **11** in 80% yield,[8,18,22,23,27,28] which could then be readily transformed into the nitrile **12** upon nucleophilic displacement of the sulfonate leaving group with sodium cyanide in combination with tetrabutylammonium cyanide as a phase transfer catalyst.[10,20,21,29,30] Employing sodium azide as a nucleophilic agent for the displacement reaction, the formation of the azide **13** in 53% yield was observed.[8,18,21,28,31] Alternatively, we tried to

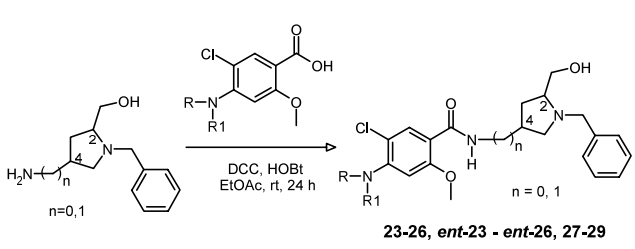
avoid the need of a regioselective protection by changing the order of reaction steps. Activation at the 4-position by TsCl was possible on the stage of the carboxylate **2** resulting in the formation of the tosylate **3** in 67% yield.[8–14,20,21] The corresponding mesylate could be prepared in 98% yield.[8,17,18] However, despite of the higher yield of the methane sulfonylation, tosylate **3** was used for further reactions because of its high stability, allowing for more convenient handling. Replacement of the leaving group by sodium cyanide[10,20,21] followed by simultaneous $LiAlH_4$ reduction of the nitrile function and the ethyl ester group of the intermediate **14** resulted in the formation of the primary amine **16**.[3] Synthesis of the aminopyrrolidine **17** was achieved by S_N2 displacement of the tosyl group with sodium azide and subsequent $LiAlH_4$ reduction of the azide **15**.[8,17–19,21,28,31] NOE experiments unambiguously established the *cis*-configuration indicating that the reaction proceeded under inversion. Neighboring-group participation of the pyrrolidine nitrogen facilitating an aziridiniumion formation and, as a consequence, a double inversion process with net retention of the configuration could not be observed.[17,18] The diastereomers **20** and **21** as well as the optical antipodes ***ent*-16**, ***ent*-17** and ***ent*-20**, ***ent*-21** could be readily synthesized along the same sequence starting from **5**, ***ent*-2** and ***ent*-5** in comparable yields.

As an extension of these studies, the *N*-benzyl protected 4-carboxyproline **22** as a conformationally constrained analogue of the excitatory neurotransmitter glutamic acid was prepared in an almost quantitative yield by refluxing the cyanoproline **18** in conc. HCl (Scheme 4).[10,32] The amino acid **22** can be applied as a useful building block for the synthesis of new peptidomimetics.



Scheme 4.

DCC/HOBt promoted coupling of the prepared amines **16**, **17**, **20** and **21** as well as their optical antipodes ***ent*-16**, ***ent*-17**, ***ent*-20**, ***ent*-21** to 5-chloro-2-methoxy-4-methylaminobenzoic acid gave the benzamides **23**–**26** and the optical antipodes ***ent*-23**–***ent*-26**, respectively (Scheme 5). Employing the diamines **16** and **17** as



Scheme 5.

representative examples, amide formation with 4-acetylamino- and 4-acetylmethylamino-substituted analogues was carried out to give the test compounds **27**, **28** and **29**.[3,8]

3. Pharmacology

The novel methoxybenzamide derivatives were evaluated in vitro with respect to their binding affinities and receptor subtype selectivities by radioligand binding assays for the dopamine receptors D1–D4[33–36] and for the serotonin receptors 5-$HT1_A$ and 5-HT2. Binding affinities for the D1 receptor were determined by employing bovine striatal membranes and the selective antagonist [^{3}H]SCH 23390 as a radioligand.[36] Binding studies on the subtypes of the D2 family were performed using membrane preparations of CHO cells stably expressing the human dopamine receptors $D2_{long}$, $D2_{short}$[33], D3[34] and D4.4[35] and [^{3}H]spiperone as the radioligand. Since both dopamine and serotonin receptors play an important part in psychotic disorders and some methoxybenzamides are known for their serotoninergic properties,[37] 5-HT binding was investigated employing porcine cortical membrane preparations and the radioligands [^{3}H]8-OH-DPAT and [^{3}H]ketanserin for 5-$HT1_A$ and 5-HT2, respectively. The antipsychotic drug sulpiride and the D3 preferring ligand FAUC 21 were utilized as reference compounds and the results are presented in Table 1. In particular, the aminopyrrolidine derivatives **23**, **24**, ***ent*-23** and ***ent*-24** displayed substantial affinities to the subtypes of the D2 family including $D2_{long}$, $D2_{short}$, D3 and D4. In contrast, the aminomethyl-substituted homologues showed only modest receptor binding indicating that the distance between the basic pyrrolidine nitrogen and the methoxybenzamide functionality as the major pharmacophoric elements is crucial for the receptor recognition. Within the aminopyrrolidine series, binding data gave interesting insights into the relationship of receptor binding and subtype selectivity and the stereochemical entities. Among the *cis*-isomers, ***ent*-24** (FAUC 65) displayed high binding affinity to the D4.4 receptor with a K_i value of 3.4 nM and a selectivity of 1800, 17, 5, 11, 850 and 1200 when compared to D1 ($K_i = 6000$ nM), $D2_{long}$ ($K_i = 59$ nM), $D2_{short}$ ($K_i = 17$ nM), D3 ($K_i = 37$ nM), 5-$HT1_A$ ($K_i = 2900$ nM) and 5-HT2 ($K_i = 4000$ nM). On the other hand the (2*S*,4*S*)-substituted antipode **24** as well as the *trans*-aminopyrrolidines **23** and ***ent*-23** showed comparable dopaminergic properties without a D4 preference. Interestingly, D3 affinities were significantly lower for the *trans*-diastereomers. Furthermore, it is worthy to note that the 5-$HT1_A$ binding of the (2*R*)-configured isomers **23** and **24** was substantially higher than those of their (2*S*)-enantiomers. Variations in the substitution pattern of the aromatic moiety resulted in a strongly decreased binding affinity of the test compounds **27**–**29**.[3]

In conclusion, starting form natural 4-hydroxyproline, practical and efficient methodology has been elaborated for the synthesis of all stereoisomeres of 4-amino- and 4-aminomethylprolinol in its *N*-benzyl protected form.

Table 1. Binding affinities of the methoxybenzamides **23**–**26**, ***ent*-23**–***ent*-26**, **27**–**29** and the reference compounds sulpiride and FAUC 21 to the bovine dopamine D1, the human $D2_{long}$, $D2_{short}$, D3 and D4.4 as well as to the porcine 5-$HT1_A$ and 5-HT2 receptors

Compound	R	R1	Pos. 2	Pos. 4	*n*	K_i values [nM][a]						
						[^{3}H]SCH23390		[^{3}H]Spiperone		[^{3}H]8-OH-DPAT		[^{3}H]Ketanserin
						D1	$D2_{long}$	$D2_{short}$	D3	D4.4	5-$HT1_A$	5-HT2
23	H	CH_3	*R*	*S*	0	29000	150	90	490	35	360	4300
***ent*-23**	H	CH_3	*S*	*R*	0	37000	160	110	2200	52	1600	2000
24	H	CH_3	*S*	*S*	0	21000	59	44	43	47	250	1200
***ent*-24**	H	CH_3	*R*	*R*	0	6000	59	17	37	3.4	2900	4000
25	H	CH_3	*S*	*R*	1	17000	330	310	1700	430	980	6700
***ent*-25**	H	CH_3	*R*	*S*	1	17000	290	430	510	210	2000	3600
26	H	CH_3	*R*	*R*	1	9900	280	220	1100	970	4800	4900
***ent*-26**	H	CH_3	*S*	*S*	1	17000	12000	6500	2600	680	1900	8800
27[b]	H	Ac	*S*	*S*	0	27%	9%	18%	45%	67%	17000	30000
28[b]	H	Ac	*S*	*R*	1	26%	8%	13%	31%	7%	34000	44000
29	CH_3	Ac	*S*	*S*	0	>100000	25000	22000	5800	5500	13000	14000
Sulpiride						50000	120	51	88	2100	9800	4300
FAUC 21						2800	190	190	31	200	N.d.	N.d.

[a] K_i value in [nM] are the means of two to three competition experiments each carried out in triplicate.
[b] Data in [%] show the ability of the test compound to displace the radioligand at a concentration of 10 μM.

The building blocks were employed for the preparation of biologically active methoxybenzamides. SAR studies led to the dopamine D4 receptor preferring nemonapride analogue FAUC 65.

4. Experimental

4.1. General procedures

Et_2O, THF and toluene were distilled from Na, $CHCl_3$, CH_2Cl_2 from CaH_2, and EtOH from Mg immediately before use. Dry DMF, DMSO and dry pyridine were bought from FLUKA. All liquid reagents were also purified by distillation. All reactions were conducted under anhydrous N_2. Evaporation of the final product solution was performed under vacuum with a rotatory evaporator. Flash chromatography was carried out with 230–400 mesh silica gel. Melting points: Büchi melting point apparatus, uncorrected. IR spectra: PERKIN-ELMER FT/IR 241 or Jasco FT/IR 410 spectrometer. Mass spectra: FINNIGAN MAT TSQ 70 instrument. High resolution mass spectrometry: FINNIGAN MAT 8200. ^{1}H and ^{13}C NMR spectra: BRUKER AM 360 spectrometer at 360, 90 and 62 MHz. Spectra were measured in $CDCl_3$ using TMS as the internal standard or in D_2O or DMSO. Optical rotations were measured at 23°C with a PERKIN-ELMER 241 polarimeter. Elementary analyses were performed by the Organic Chemistry Department of the Friedrich-Alexander-University Erlangen-Nürnberg or Beetz Microanalysis Laboratory, Kronach, Germany. For all new compounds satisfactory microanalysis obtained C±0.39, H±0.17, N±0.29, S±0.13.

4.2. Ethyl (2*S*,4*R*)-1-benzyl-4-hydroxyprolinate 2

First preparation of ethyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride **2a** was performed according to the literature[6] as follows.

To a solution of (2*S*,4*R*)-4-hydroxyproline (Hyp) (17.42 g, 133 mmol) in EtOH (174 mL) was added $SOCl_2$ (10.8 mL, 160 mmol) at 0°C. The mixture was refluxed for 6 h. After cooling to rt the mixture was diluted with Et_2O and the resulting precipitate collected by suction filtration. The filter cake was washed with ether and dried in vacuo to leave pure **2a** (25.48 g, 98%, lit.:[6] 86%), which was used without further purification.

According to the literature,[6] preparation of **2** was performed in a simply modified way as follows:

A solution of crude **2a** (10.04 g, 51.5 mmol), Et_3N (16.6 mL, 117 mmol) and benzyl bromide (6.48 mL, 54.7 mmol) in $CHCl_3$ (200 mL) was refluxed for 6 h. After cooling to rt, aq. 1 M NaOH (200 mL) was added and the mixture extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 3:7) to give **2** (12.40 g, 97%; lit.:[6] 70%) as a colorless oil: EI-MS $m/z=249$ [M^+]; TLC: $R_f=0.19$ (petroleum ether/EtOAc, 1:2); IR (film): ν 3417, 2935, 1731, 1450, 1187, 1033, 752 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.25 (t, 3H, $J=7.1$ Hz, $CO_2CH_2C\underline{H}_3$), 2.08 (ddd, 1H, $J=13.5$, 7.7, 2.5 Hz, H-3a), 2.26 (ddd, 1H, $J=13.5$, 7.7, 7.0 Hz, H-3b), 2.46 (dd, 1H, $J=10.0$, 3.8 Hz, H-5a), 3.32 (dd, 1H, $J=10.0$, 5.7 Hz, H-5b), 3.60 (dd, 1H, $J=7.7$, 7.7 Hz, H-2), 3.66

(d, 1H, $J=13.0$ Hz, NCH_2Ph), 3.93 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.13 (q, 2H, $J=7.1$ Hz, $CO_2C\underline{H}_2CH_3$), 4.45 (m, 1H, H-4), 7.18–7.36 (m, 5H, Ar); $[\alpha]_D^{20}=-56.3$ (*c* 1.0, $CHCl_3$), {Lit.[6]: $[\alpha]_D^{20}=-65.0$, (*c* 1.07, MeOH)}.

Preparation of ***ent*-2** was accomplished starting from **9** as follows.

To a solution of **9** (1.56 g, 5.35 mmol) in EtOH (25 mL) was added NaOEt (542 mg, 7.97 mmol) with the resulting mixture left to stir at rt for 30 min. Solid NH_4Cl was then added and the mixture stirred for another 10 min. The solvent was evaporated and the residue purified by flash chromatography (petroleum ether/EtOAc, 6:4) to give ***ent*-2** (1.1 g, 83%) as a colorless oil: $[\alpha]_D^{20}=+49.0$ (*c* 0.99, $CHCl_3$).

4.3. Ethyl (2*S*,4*R*)-1-benzyl-4-(4-methylphenylsulfonyl-oxy)prolinate 3

To a stirred solution of **2** (247 mg, 0.99 mmol) in dry pyridine (5 mL) was added *p*-toluenesulfonyl chloride (226.5 mg, 1.19 mmol) at 0°C. The solution was then allowed to warm up to rt. After 48 h of stirring, an aqueous solution of citric acid (40 g citric acid/250 mL H_2O) was added and the mixture extracted with Et_2O (3×15 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1) to give **3** (226 mg, 57%) as a slightly yellowish glutinous mass. Mp: ~24°C (rt). EI-MS $m/z=403.1$ [M^+]; TLC: $R_f=0.59$ (petroleum ether/EtOAc, 1:1); IR (film): *v* 2980, 1740, 1597, 1495, 1366, 1177, 893, 752 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.23 (t, 3H, $J=7.0$ Hz, $CO_2CH_2C\underline{H}_3$), 2.27 (m, 2H, H-3a/H-3b), 2.44 (s, 3H, $OSO_2C_6H_4C\underline{H}_3$), 2.63 (dd, 1H, $J=10.9$, 3.8 Hz, H-5a), 3.24 (dd, 1H, $J=10.9$, 5.7 Hz, H-5b), 3.54 (dd, 1H, $J=7.5$, 7.5 Hz, H-2), 3.59 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 3.87 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.11 (q, 2H, $J=7.0$ Hz, $CO_2C\underline{H}_2CH_3$), 4.98 (m, 1H, H-4), 7.24–7.33 (m, 7H, Ar/$OSO_2C_6\underline{H}_4CH_3$), 7.74 (m, 2H $OSO_2C_6\underline{H}_4CH_3$); Anal. calcd for $C_{21}H_{25}NO_5S$ (403.50): C, 62.51; H, 6.25; N, 3.74; S, 7.95, found: C, 62.54; H, 6.31; N, 3.48; S, 7.82; $[\alpha]_D^{20}=-13.8$ (*c* 0.99, $CHCl_3$). ***ent*-3** was prepared under the same reaction conditions, starting from ***ent*-2**: $[\alpha]_D^{20}=+14.1$ (*c* 1.0, $CHCl_3$).

4.4. Ethyl (2*S*,4*S*)-4-acetyloxy-1-benzylprolinate 4

To a stirred solution of **3** (23.5 g, 58.1 mmol) in toluene (250 mL) was added tetrabutylammonium acetate (22.78 g, 75.6 mmol) with the mixture allowed to reflux for 4 h. After cooling to rt the mixture was washed with water three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to give **4** as a yellow oil (15.46 g, 91%): EI-MS $m/z=291$ [M^+]; TLC: $R_f=0.13$ (petroleum ether/EtOAc, 8:2); IR (film): *v* 2981, 1737, 1369, 1243, 1027, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.22 (t, 3H, $J=7.2$ Hz, $CO_2CH_2C\underline{H}_3$), 1.95 (s, 3H, $OCOC\underline{H}_3$), 2.01 (ddd, 1H, $J=14.0$, 8.1, 3.4 Hz, H-3a), 2.51 (ddd, 1H, $J=14.0$, 8.1, 8.1 Hz, H-3b), 2.61 (dd, 1H, $J=11.1$, 6.2 Hz, H-5a), 3.00 (dd, 1H, $J=11.1$, 0.7 Hz, H-5b), 3.18 (dd, 1H, $J=8.1$, 8.1 Hz, H-2), 3.48 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 3.95 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.12 (m, 2H, $CO_2C\underline{H}_2CH_3$), 5.05 (m, 1H, H-4), 7.15–7.28 (m, 5H, Ar); Anal. calcd for $C_{16}H_{21}NO_4$ (291.35): C, 65.96; H, 7.27; N, 4.81, found: C, 66.04; H, 7.19; N, 4.70; $[\alpha]_D^{20}=-55.0$ (*c* 1.0, $CHCl_3$).

4.5. Ethyl (2*R*,4*R*)-1-benzyl-4-hydroxyprolinate ent-5

First preparation of (2*R*,4*R*)-4-hydroxyproline hydrochloride ***ent*-5a** was performed according to the literature[8,13–16] in a slightly modified way as follows.

A mixture of acetic anhydride (204 g, 2 mol) and glacial acetic acid (600 mL, 10.5 mol) was heated to 50°C at which point (2*S*,4*R*)-4-hydroxyproline (Hyp) (47.09 g, 360 mmol) was added in one portion. The mixture was refluxed for 5.5 h. After cooling to rt, the solvent was removed under reduced pressure. The residue was dissolved in 2 M HCl (650 mL) and refluxed for another 3 h. After cooling to rt the mixture was filtered through Celite and concentrated by rotary evaporation until white needles were formed. The precipitate was collected by suction filtration, washed with Et_2O and dried in vacuo to leave pure ***ent*-5a** (57.66 g, 96%, lit.:[13] 87%). Mp: 114–116°C; IR (KBr): *v* 3421–2507, 1708, 1581, 1373, 1272, 1087, 1064, 1025, 960, 902, 863, 829, 790, 763 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 2.33 (m, 1H, H-3a), 2.48 (ddd, 1H, $J=14.4$, 10.3, 4.2 Hz, H-3b), 3.40 (m, 2H, H-5a, H-5b), 4.45 (dd, 1H, $J=10.3$, 3.4 Hz, H-2), 4.58 (m, 1H, H-4); $[\alpha]_D^{20}=+6.5$ (*c* 1.0, MeOH).

Crude ***ent*-5a** was used for the preparation of ethyl (2*R*,4*R*)-4-hydroxyprolinate hydrochloride ***ent*-5b**. This compound is described in the literature.[6] Preparation was performed according to **2a** as follows.

To a solution of crude ***ent*-5a** (493.3 mg, 2.95 mmol) in EtOH (40.0 mL) was added $SOCl_2$ (0.31 mL, 4.51 mmol) at 0°C. Further treatment and work up was carried out as described for **2a** to give pure ***ent*-5b** (470 mg, 82%) as a white solid. Mp: 138–140°C; EI-MS $m/z=86$ (α-cleavage, $[M-CO_2C_2H_5]^+$); IR (KBr): *v* 3270, 2977, 1727, 1585, 1380, 1249, 1064, 651 cm^{-1}; ^1H NMR (360 MHz, DMSO): δ 1.24 (t, 3H, $J=7.1$ Hz, $CO_2CH_2C\underline{H}_3$), 2.14 (brd, 1H, $J=13.5$ Hz, H-3a), 2.31 (ddd, 1H, $J=13.5$, 9.6, 4.3 Hz, H-3b), 3.19 (m, 2H, H-5a/H-5b), 4.21 (m, 2H, $CO_2C\underline{H}_2CH_3$), 4.37 (m, 1H, H-4), 4.44 (dd, 1H, $J=9.6$, 3.9 Hz, H-2); $[\alpha]_D^{20}=+20.1$ (*c* 3.08, $CHCl_3$).

Preparation of ***ent*-5** was accomplished according to **2** as follows.

Crude ***ent*-5b** (238.4 mg, 1.22 mmol), Et_3N (0.39 mL, 2.8 mmol) and benzyl bromide (0.15 mL, 1.3 mmol) were dissolved in $CHCl_3$ (40 mL), reacted and worked up (petroleum ether/EtOAc, 1:1) as described for **2** to give ***ent*-5** (298 mg, 98%) as a colorless oil: EI-MS $m/z=249$ [M^+]; TLC: $R_f=0.16$ (petroleum ether/EtOAc, 1:1); IR (film): *v* 3412, 1732, 1454, 1375, 1198, 1028, 751, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.21 (t, 3H, $J=7.2$ Hz, $CO_2CH_2C\underline{H}_3$), 1.94 (brd, 1H, $J=14.2$ Hz, H-3a), 2.37 (ddd, 1H, $J=14.2$, 9.7, 5.7 Hz, H-3b), 2.64 (dd, 1H,

$J=9.7, 3.9$ Hz, H-5a), 3.02 (dd, 1H, $J=9.7, 1.4$ Hz, H-5b), 3.33 (dd, 1H, $J=9.7, 3.6$ Hz, H-2), 3.71 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 3.87 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.08 (m, 2H, $CO_2C\underline{H}_2CH_3$), 4.24 (m, 1H, H-4), 7.19–7.34 (m, 5H, Ar); $[\alpha]_D^{20}=+37.9$ (c 1.0, $CHCl_3$), {lit.:[6] $[\alpha]_D^{20}=+76.2$ (c 1.2, MeOH)}.

Preparation of **5** was accomplished starting from **4** as follows.

A mixture of **4** (3.04 g, 10.4 mmol) and NaOEt (1.05 g, 15.6 mmol) in EtOH (50 mL) was reacted and worked up as described for ***ent*-2** to give **5** as a colorless oil (2.33 g, 90%). $[\alpha]_D^{20}=-38.2$ (c 0.99, $CHCl_3$).

4.6. Benzyl (2*SR*,4*R*)-1,2-dibenzyl-4-hydroxyprolinate 6

To a suspension of (4*R*)-4-hydroxy-L-proline (Hyp) (109 mg, 0.83 mmol) in aqueous K_2CO_3 (6 mL, 20%) was added benzyl bromide (0.36 mL, 3.05 mmol) and the mixture refluxed for 3 h. After cooling to rt the mixture extracted with Et_2O, the organic layer dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8:2) to give **6** as a 2:1 diastereomeric mixture (207 mg, 62%) which could be isolated as a colorless oil: TLC*: $R_f=0.17$ (petroleum ether/EtOAc, 8:2); IR (film)*: ν 3360, 3086–2873, 1721, 1495, 1454, 1176, 1090, 1023, 736, 698 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$)*: δ 1.94 (brd, 1H, $J=14.1$ Hz, H-3a), 2.56 (dd, 1H, $J=14.1, 6.8$ Hz, H-3b), 2.77 (m, 2H, H-5a/H-5b), 3.12 (d, 1H, $J=13.8$ Hz, CCH_2Ph), 3.27 (m, 2H, CCH_2Ph/NCH_2Ph), 4.16 (m, 1H, H-4), 4.23 (d, 1H, $J=13.6$ Hz, NCH_2Ph), 5.19 (d, 1H, $J=12.2$ Hz, OCH_2Ph), 5.24 (d, 1H, $J=12.2$ Hz, OCH_2Ph), 7.11–7.48 (m, 15H, Ar); Anal. calcd for $C_{26}H_{27}NO_3$ (401.51): C, 77.78; H, 6.78; N, 3.49, found: C, 77.86; H, 6.75; N, 3.55.

*Data representing one isolated diastereomer.

4.7. Ethyl (2*R*,4*R*)-1-benzyl-4-(4-methylphenylsulfonyl-oxy)prolinate 8

A stirred solution of ***ent*-5** (17.08 g, 62.5 mmol) in dry pyridine (250 mL) and *p*-toluenesulfonyl chloride (15.7 mg, 82.2 mmol) were reacted and worked up (petroleum ether/EtOAc, 9:1) as described for **3** to give **8** (27.7 g, 100%) as slightly yellowish crystals. Mp: 47°C; EI-MS $m/z=403$ [M^+]; TLC: $R_f=0.53$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2981, 1742, 1362, 1176, 904, 702 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.26 (t, 3H, $J=7.0$ Hz, $CO_2CH_2C\underline{H}_3$), 2.21 (ddd, 1H, $J=14.5, 6.5, 2.9$ Hz, H-3a), 2.42 (s, 3H, $OSO_2C_6H_4C\underline{H}_3$), 2.48 (ddd, 1H, $J=14.5, 8.5, 7.9$ Hz, H-3b), 2.61 (dd, 1H, $J=11.1, 6.0$ Hz, H-5a), 3.07 (dd, 1H, $J=11.1, 1.9$ Hz, H-5b), 3.25 (brdd, 1H, $J=8.5, 6.5$ Hz, H-2), 3.51 (d, 1H, $J=13.2$ Hz, NCH_2Ph), 3.98 (d, 1H, $J=13.2$ Hz, NCH_2Ph), 4.15 (m, 2H, $CO_2C\underline{H}_2CH_3$), 4.98 (m, 1H, H-4), 7.21–7.32 (m, 7H, $Ar/OSO_2C_6\underline{H}_4CH_3$), 7.74 (m, 2H, $OSO_2C_6\underline{H}_4CH_3$); Anal. calcd for $C_{21}H_{25}NO_5S$ (403.50): C, 62.51; H, 6.25; N, 3.47; S, 7.95, found: C, 62.57; H, 6.20; N, 3.50; S, 7.92; $[\alpha]_D^{20}=+39.2$ (c 1.0, $CHCl_3$). ***ent*-8** was prepared under the same reaction conditions, starting from **5**; $[\alpha]_D^{20}=-39.7$ (c 1.0, $CHCl_3$).

4.8. Ethyl (2*R*,4*S*)-4-acetyloxy-1-benzylprolinate 9

A mixture of **8** (104 mg, 0.26 mmol) and tetrabutylammonium acetate (100 mg, 0.33 mmol) in toluene (10 mL) was reacted and worked up (petroleum ether/EtOAc, 8:2) as described for **4** to give **9** as a colorless oil (64 mg, 85%): EI-MS $m/z=291$ [M^+]; TLC: $R_f=0.62$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2981, 1740, 1242, 1029, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.26 (t, 3H, $J=7.0$ Hz, $CO_2CH_2C\underline{H}_3$), 2.02 (s, 3H, $OCOC\underline{H}_3$), 2.16 (ddd, 1H, $J=13.9, 7.6, 3.1$ Hz, H-3a), 2.35 (ddd, 1H, $J=13.9, 7.6, 7.4$ Hz, H-3b), 2.52 (dd, 1H, $J=11.0, 3.8$ Hz, H-5a), 3.43 (dd, 1H, $J=11.0, 6.2$ Hz, H-5b), 3.54 (dd, 1H, $J=7.6, 7.6$ Hz, H-2), 3.62 (d, 1H, $J=12.8$ Hz, NCH_2Ph), 3.93 (d, 1H, $J=12.8$ Hz, NCH_2Ph), 4.15 (m, 2H, $CO_2C\underline{H}_2CH_3$), 5.20 (m, 1H, H-4), 7.22–7.34 (m, 5H, Ar); Anal. calcd for $C_{16}H_{21}NO_4$ (291.35): C, 65.96; H, 7.27; N, 4.81, found: C, 65.66; H, 7.30; N, 4.77; $[\alpha]_D^{20}=+36.6$ (c 1.0, $CHCl_3$).

4.9. (3*R*,5*S*)-1-Benzyl-5-(*tert*-butyldimethylsilyloxy-methyl)pyrrolidin-3-ol 10

To a solution of (3*R*,5*S*)-1-benzyl-5-(hydroxymethyl)pyrrolidin-3-ol[6] (93.4 mg, 0.45 mmol) in DMF (5 mL) was added imidazole (222 mg, 1.8 mmol) and TBSCl (134 mg, 0.9 mmol) at 0°C. After stirring at 0°C for 1 h, an aqueous saturated solution of NH_4Cl was added and the mixture extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1) to give **10** (55.2 mg, 38%) as a colorless oil: EI-MS $m/z=321$ [M^+]; TLC: $R_f=0.2$ (petroleum ether/EtOAc, 1:1); IR (film): ν 3442, 2928, 1471, 1254, 1086, 835, 699 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 0.08 (s, 3H, $OSi(C\underline{H}_3)_2tBu$), 0.09 (s, 3H, $OSi(C\underline{H}_3)_2tBu$), 0.90 (s, 9H, $OSi(CH_3)_2t\underline{Bu}$), 1.90 (m, 2H, H-4a/H-4b), 2.31 (dd, 1H, $J=10.1, 5.0$ Hz, H-2a), 3.02 (m, 1H, H-5), 3.19 (dd, 1H, $J=10.1, 5.7$ Hz, H-2b), 3.52 (m, 2H, $C\underline{H}_2OSi(CH_3)_2tBu/NCH_2Ph$), 3.64 (dd, 1H, $J=10.3, 5.1$ Hz, $C\underline{H}_2OSi(CH_3)_2tBu$), 4.10 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.33 (m, 1H, H-3), 7.20–7.34 (m, 5H, Ar); $[\alpha]_D^{20}=-46.0$ (c 1.0, $CHCl_3$).

4.10. (3*R*,5*S*)-1-Benzyl-5-(*tert*-butyldimethylsilyloxy-methyl)pyrrolidin-3-yl methanesulfonate 11

To a solution of **10** (16.40 mg, 0.05 mmol) in $CHCl_3$ (1.5 mL) were added NEt_3 (0.033 mL, 0.23 mmol) and MsCl over a period of 60 min (0.028 mL, 0.20 mmol) at −10°C. After another 2 h of stirring the solvent was removed under reduced pressure while the reaction mixture was kept at −10°C. The residue was dissolved in CH_2Cl_2 and immediately purified by flash chromatography (petroleum ether/EtOAc, 8:2) to give **11** (16.2 mg, 80%) as a slightly yellowish liquid: EI-MS $m/z=399$ [M^+]; TLC: $R_f=0.63$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2929, 1359, 1255, 1176, 968, 836, 777, 700 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 0.05 (s, 3H, $OSi(C\underline{H}_3)_2tBu$), 0.06 (s, 3H, $OSi(C\underline{H}_3)_2tBu$), 0.89 (s, 9H, $OSi(CH_3)_2t\underline{Bu}$), 2.07 (ddd, 1H, $J=14.1, 7.2, 7.2$ Hz, H-4a), 2.22 (ddd, 1H, $J=14.1, 7.6, 3.5$ Hz, H-4b), 2.62 (dd, 1H, $J=10.2, 3.9$ Hz,

H-2a), 2.97 (s, 3H, OMs), 3.05 (m, 1H, H-5), 3.34 (dd, 1H, $J=10.2$, 5.5 Hz, H-2b), 3.55 (m, 2H, $C\underline{H}_2OSi(CH_3)_2tBu/NCH_2Ph$), 3.66 (dd, 1H, $J=10.1$, 4.6 Hz, $C\underline{H}_2OSi(CH_3)_2tBu$), 4.12 (d, 1H, $J=13.4$ Hz, NCH_2Ph), 5.10 (m, 1H, H-3), 7.19–7.38 (m, 5H, Ar); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 18.21 (C-Tbs), 25.87 (C-Tbs), 36.14 (C-4), 38.32 ($\underline{C}H_3$(Ms)), 58.87 ($N\underline{C}H_2Ph$), 59.49 (C-2), 63.44 (C-5), 65.69 ($\underline{C}H_2OTBS$), 79.34 (C-3), 127.17, 128.34, 128.72 (C-Ar); Anal. calcd for $C_{19}H_{33}NO_4SSi$ (399.63): C, 57.11; H, 8.32; N, 3.50; S, 8.02, found: C, 57.12; H, 8.37; N, 3.52; S, 8.09.

4.11. (3*S*,5*S*)-1-Benzyl-5-(*tert*-butyldimethylsilyloxymethyl)pyrrolidine-3-carbonitrile 12

To a solution of **11** (16.2 mg, 0.041 mmol) in DMSO (6 mL) was added NaCN (8.5 mg, 0.17 mmol) and tetrabutylammonium cyanide (10.99 mg, 0.041 mmol) under N_2 atmosphere in a glove box. The mixture was stirred at 70°C for 24 h. Saturated aqueous $NaHCO_3$ was then added and the mixture extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 96:4) to give **12** (5.1 mg, 38%) as a slightly yellowish oil: EI-MS $m/z=330$ [M^+]; TLC: $R_f=0.07$ (petroleum ether/EtOAc, 96:4); IR (film): ν 2928, 2240, 1471, 1256, 1107, 837, 699 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 0.05 (s, 6H, $OSi(C\underline{H}_3)_2tBu$), 0.07 (s, 9H, $OSi(CH_3)_2t\underline{Bu}$), 2.01 (ddd, 1H, $J=13.3$, 5.7, 5.7 Hz, H-4a), 2.34 (ddd, 1H, $J=13.3$, 8.6, 8.6 Hz, H-4b), 2.54 (dd, 1H, $J=9.6$, 6.9 Hz, H-2a), 2.81 (m, 1H, H-3), 2.92 (m, 1H, H-5), 3.16 (dd, 1H, $J=9.6$, 3.4 Hz, H-2b), 3.46 (d, 1H, $J=13.7$ Hz, NCH_2Ph), 3.61 (dd, 1H, $J=10.3$, 6.2 Hz, $C\underline{H}_2OSi(CH_3)_2tBu$), 3.73 (dd, 1H, $J=10.3$, 5.5 Hz, $C\underline{H}_2OSi(CH_3)_2tBu$), 4.12 (d, 1H, $J=$ 13.7 Hz, NCH_2Ph), 7.20–7.37 (m, 5H, Ar); Anal. calcd for $C_{19}H_{30}N_2OSi$ (330.55): C, 69.04; H, 9.15; N, 8.47, found: C, 69.09; H, 9.13; N, 8.41; $[\alpha]_D^{20}=-67.5$ (c 0.07, $CHCl_3$).

4.12. (2*S*,4*S*)-4-Azido-1-benzyl-2-(*tert*-butyldimethylsilyloxymethyl)pyrrolidine 13

Compound **11** (24.40 mg, 0.06 mmol) and NaN_3 (39.65 mg, 0.61 mmol) were dissolved in DMF (5 mL) and heated to 60°C. After stirring for 4 h and then cooling to rt water was added and the mixture extracted with Et_2O. The organic layer was separated, washed with water (2×5 mL), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 96:4) to give **13** (11.2 mg, 53%) as a colorless liquid: CI-MS m/z 347 [M^+]; TLC: $R_f=0.64$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2954, 2104, 1257, 1108, 837, 776, 698 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 0.07 (s, 6H, $OSi(C\underline{H}_3)_2tBu$), 0.90 (s, 9H, $OSi(CH_3)_2t\underline{Bu}$), 1.78 (m, 1H, H-3a), 2.34 (ddd, 1H, $J=14.8$, 8.3, 7.8 Hz, H-3b), 2.43 (dd, 1H, $J=10.6$, 5.1 Hz, H-5a), 2.74 (m, 1H, H-2), 2.99 (brd, 1H, $J=10.6$ Hz, H-5b), 3.38 (d, 1H, $J=13.4$ Hz, NCH_2Ph), 3.58 (dd, 1H, $J=9.9$, 6.5 Hz, $C\underline{H}_2OH$), 3.76 (m, 2H, $C\underline{H}_2OH$/H-4), 4.17 (d, 1H, $J=13.4$ Hz, NCH_2Ph), 7.21–7.37 (m, 5H, Ar); Anal. calcd for $C_{18}H_{30}N_4OSi$ (346.55): C, 62.39; H, 8.73; N, 16.17, found: C, 62.23; H, 8.61; N, 16.25; $[\alpha]_D^{20}=-79.0$ (c 0.3, $CHCl_3$).

4.13. Ethyl (2*S*,4*S*)-1-benzyl-4-cyanoprolinate 14

A mixture of **3** (275.7 mg, 0.68 mmol) and NaCN (50.1 mg, 1.02 mmol) in DMSO (10 mL) was stirred at 60°C for 4 h. After cooling to rt, saturated aqueous $NaHCO_3$ was added and the mixture extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 85:15) to give **14** (158.4 mg, 90%) as a colorless oil: EI-MS $m/z=258$ [M^+]; TLC: $R_f=0.51$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2981, 2241, 1730, 1454, 1186, 753, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.30 (t, 3H, $J=7.1$ Hz, $CO_2CH_2C\underline{H}_3$), 2.34 (ddd, 1H, $J=13.4$, 5.5, 5.4 Hz, H-3a), 2.53 (ddd, 1H, $J=13.4$, 9.6, 8.4 Hz, H-3b), 2.86 (dd, 1H, $J=9.4$, 7.7 Hz, H-5a), 3.05 (m, 1H, H-4), 3.24 (dd, 1H, $J=9.4$, 5.1 Hz, H-5b), 3.47 (dd, 1H, $J=8.4$, 5.4 Hz, H-2), 3.67 (d, 1H, $J=13.1$ Hz, NCH_2Ph), 3.97 (d, 1H, $J=13.1$ Hz, NCH_2Ph), 4.21 (q, 2H, $J=7.1$ Hz, $CO_2C\underline{H}_2CH_3$), 7.24–7.35 (m, 5H, Ar); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.17 ($CO_2CH_2\underline{C}H_3$), 25.90 (C-4), 33.48 (C-3), 55.01 (C-5), 56.35 ($N\underline{C}H_2Ph$), 60.96 ($CO_2\underline{C}H_2CH_3$), 63.41 (C-2), 121.22, 127.37, 128.63, 137.50, 171.94 (C-Ar); Anal. calcd for $C_{15}H_{18}N_2O_2$ (258.32): C, 69.74; H, 7.02; N, 10.84, found: C, 69.70; H, 7.13; N, 10.92; $[\alpha]_D^{20}=-17.9$ (c 1.0, $CHCl_3$); ***ent*-14** was prepared under the same reaction conditions, starting from ***ent*-3**: $[\alpha]_D^{20}=+17.9$ (c 1.0, $CHCl_3$).

4.14. Ethyl (2*S*,4*S*)-4-azido-1-benzylprolinate 15

A mixture of **3** (269 mg, 0.66 mmol) and NaN_3 (433 mg, 6.64 mmol) in DMF (20 mL) was stirred at 60°C for 7 h. After cooling to rt, saturated aqueous $NaHCO_3$ was added and the mixture extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 85:15) to give **15** (169 mg, 92%) as a slightly yellowish oil: EI-MS $m/z=274$ [M^+]; TLC: $R_f=0.67$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2984, 2105, 1740, 1265, 1182, 705 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.28 (t, 3H, $J=7.0$ Hz, $CO_2CH_2C\underline{H}_3$), 2.15 (ddd, 1H, $J=14.1$, 6.2, 3.3 Hz, H-3a), 2.51 (ddd, 1H, $J=14.1$, 9.3, 7.9 Hz, H-3b), 2.63 (dd, 1H, $J=10.3$, 5.8 Hz, H-5a), 3.06 (dd, 1H, $J=10.3$, 2.0 Hz, H-5b), 3.32 (dd, 1H, $J=9.3$, 6.2 Hz, H-2), 3.54 (d, 1H, $J=13.1$ Hz, NCH_2Ph), 3.88 (m, 1H, H-4), 4.05 (d, 1H, $J=13.1$ Hz, NCH_2Ph), 4.18 (q, 2H, $J=7.0$ Hz, $CO_2C\underline{H}_2CH_3$), 7.21–7.38 (m, 5H, Ar); Anal. calcd for $C_{14}H_{18}N_4O_2$ (274.33): C, 61.30; H, 6.61; N, 20.42, found: C, 61.37; H, 6.65; N, 20.71; $[\alpha]_D^{20}=-57.5$ (c 1.0, $CHCl_3$). ***ent*-15** was prepared under the same reaction conditions, starting from ***ent*-3**: $[\alpha]_D^{20}=+55.2$ (c 1.0, $CHCl_3$).

4.15. (2*S*,4*R*)-(4-Aminomethyl-1-benzylpyrrolidin-2-yl)methanol 16

To a stirred solution of **14** (281 mg, 1.08 mmol) in THF (10 mL) was added $LiAlH_4$ (2.18 mL, 1 M solution in THF) at 0°C. After 1.5 h the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was filtered through Celite and the filter cake extracted with

MeOH (3×10 mL). The filtrate was evaporated and the residue purified by flash chromatography (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/500 mL eluent) to give **16** (95.1 mg, 40%) as a yellow oil: EI-MS $m/z = 189$ (α-cleavage); TLC: $R_f = 0.17$ (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/500 mL eluent); IR (film): ν 3351, 2919, 1585, 1454, 1033, 744, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 2.07 (m, 3H, H-3a/H-3b/H-4), 2.47 (dd, 1H, $J = 9.8$, 7.5 Hz, H-5a), 2.70 (m, 4H, H-2/CH_2NH_2/CH_2NH_2/H-5b), 3.23 (d, 1H, $J = 13.2$ Hz, NCH_2Ph), 3.51 (dd, 1H, $J = 11.0$, 1.7 Hz, CH_2OH), 3.78 (dd, 1H, $J = 11.0$, 3.1 Hz, CH_2OH), 4.00 (d, 1H, $J = 13.2$ Hz, NCH_2Ph), 7.22–7.35 (m, 5H, Ar); HR-EI-MS for $C_{12}H_{17}N_2$ (M-): calcd 189.13918 found 189.13967, for $C_{12}H_{14}N$: calcd 172.11263 found 172.11293, for $C_{11}H_{12}N$: calcd 158.09697 found 158.09705; $[\alpha]_D^{20} = -30.7$ (c 0.27, $CHCl_3$). ***ent*-16** was prepared under the same reaction conditions, starting from ***ent*-14**: $[\alpha]_D^{20} = +29.3$ (c 0.04, $CHCl_3$).

4.16. (2*S*,4*S*)-(4-Amino-1-benzylpyrrolidin-2-yl)methanol 17

To a stirred solution of **15** (146 mg, 0.53 mmol) in THF (10 mL) was added $LiAlH_4$ (1.06 mL, 1 M solution in THF) at 0°C. After 1 h the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was filtered through Celite and the filter cake extracted with CH_2Cl_2. The filtrate was evaporated and the residue purified by flash chromatography (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/500 mL eluent) to give **17** (102 mg, 94%) as a colorless oil: EI-MS $m/z = 175$ (α-cleavage, $[M-CH_2OH]^+$); TLC: $R_f = 0.19$ (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/1000 mL eluent); IR (film): ν 3391, 2925, 1559, 1406, 1028, 745, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 2.01 (brd, 1H, $J = 14.1$ Hz, H-3a), 2.24 (ddd, 1H, $J = 14.1$, 10.1, 6.3 Hz, H-3b), 2.50 (dd, 1H, $J = 10.3$, 4.1 Hz, H-5a), 2.85 (m, 1H, H-2), 2.95 (brd, 1H, $J = 10.3$ Hz, H-5b), 3.46 (m, 2H, CH_2OH/NCH_2Ph), 3.59 (m, 1H, H-4), 3.68 (brd, 1H, $J = 10.0$ Hz, CH_2OH), 3.98 (d, 1H, $J = 13.4$ Hz, NCH_2Ph), 7.18–7.37 (m, 5H, Ar); HR-EIMS for $C_{11}H_{15}N_2$ ($[M-CH_2OH]^+$): calcd 175.12352, found 175.12326; $[\alpha]_D^{20} = -14.2$ (c 0.5, $CHCl_3$). ***ent*-17** was prepared under the same reaction conditions, starting from ***ent*-15**: $[\alpha]_D^{20} = +13.5$ (c 0.3, $CHCl_3$).

4.17. Ethyl (2*S*,4*R*)-1-benzyl-4-cyanoprolinate 18

A mixture of ***ent*-8** (130 mg, 0.32 mmol) and NaCN (23.6 mg, 0.48 mmol) in DMSO (10 mL) was reacted and worked up as described for **14** to give **18** (81.2 mg, 98%) as a colorless oil: EI-MS $m/z = 258$ [M^+]; TLC: $R_f = 0.48$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2913, 2240, 1730, 1450, 1189, 701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.21 (t, 3H, $J = 7.1$ Hz, $CO_2CH_2CH_3$), 2.35 (m, 2H, H-3a/H-3b), 2.70 (dd, 1H, $J = 8.5$, 7.9 Hz, H-5a), 3.09 (m, 1H, H-4), 3.23 (dd, 1H, $J = 8.5$, 8.2 Hz, H-5b), 3.46 (dd, 1H, $J = 8.2$, 6.2 Hz, H-2), 3.59 (d, 1H, $J = 13.0$ Hz, NCH_2Ph), 3.90 (d, 1H, $J = 13.0$ Hz, NCH_2Ph), 4.09 (q, 2H, $J = 7.1$ Hz, $CO_2CH_2CH_3$), 7.17–7.32 (m, 5H, Ar); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.17 ($CO_2CH_2CH_3$), 22.68 (C-4), 33.54 (C-3), 55.61 (C-5), 57.20 (NCH_2Ph), 60.99 ($CO_2CH_2CH_3$), 63.39 (C-2), 120.94, 127.50, 128.40, 128.82, 137.27, 171.95 (C-Ar); Anal. calcd for $C_{15}H_{18}N_2O_2$ (258.32): C, 69.74; H, 7.02; N, 10.84, found: C, 69.34; H, 6.98; N, 11.04; $[\alpha]_D^{20} = -64.8$ (c 1.0, $CHCl_3$). ***ent*-18** was prepared under the same reaction conditions, starting from **8**: $[\alpha]_D^{20} = +64.8$ (c 1.0, $CHCl_3$).

4.18. Ethyl (2*S*,4*R*)-4-azido-1-benzylprolinate 19

A mixture of ***ent*-8** (360 mg, 0.89 mmol) and NaN_3 (578 mg, 8.89 mmol) in DMF (20 mL) was reacted and worked up as described for **15** to give **19** (240.1 mg, 98%) as a colorless oil: EI-MS $m/z = 274$ [M^+]; TLC: $R_f = 0.65$ (petroleum ether/EtOAc, 1:1); IR (film): ν 2981, 2102, 1730, 1263, 1186, 700 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.26 (t, 3H, $J = 7.3$ Hz, $CO_2CH_2CH_3$), 2.16 (ddd, 1H, $J = 13.5$, 8.3, 4.8 Hz, H-3a), 2.33 (ddd, 1H, $J = 13.5$, 7.5, 7.0 Hz, H-3b), 2.53 (dd, 1H, $J = 10.1$, 5.0 Hz, H-5a), 3.30 (dd, 1H, $J = 10.1$, 6.4 Hz, H-5b), 3.54 (dd, 1H, $J = 8.3$, 7.0 Hz, H-2), 3.65 (d, 1H, $J = 13.0$ Hz, NCH_2Ph), 3.93 (d, 1H, $J = 13.0$ Hz, NCH_2Ph), 4.14 (m, 3H, H-4/$CO_2CH_2CH_3$), 7.23–7.36 (m, 5H, Ar); Anal. calcd for $C_{14}H_{18}N_4O_2$ (274.33): C, 61.30; H, 6.61; N, 20.42, found: C, 61.60; H, 6.69; N, 20.63; $[\alpha]_D^{20} = -53.2$ (c 1.0, $CHCl_3$). ***ent*-19** was prepared under the same reaction conditions, starting from **8**: $[\alpha]_D^{20} = +48.5$ (c 1.0, $CHCl_3$).

4.19. (2*S*,4*S*)-(4-Aminomethyl-1-benzylpyrrolidin-2-yl)-methanol 20

A mixture of **18** (48.9 mg, 0.19 mmol) in THF (5 mL) and $LiAlH_4$ (0.38 mL, 1 M solution in THF) was reacted and worked up (CH_2Cl_2/MeOH = 8:2+10 mL solution of MeOH saturated with NH_3/1000 mL eluent) as described for **16** to give **20** (21.8 mg, 52%) as a colorless oil: EI-MS $m/z = 189$ (α-cleavage, $[M-CH_2OH]^+$); TLC: $R_f = 0.08$ (CH_2Cl_2/MeOH = 8:2+10 mL solution of MeOH saturated with NH_3/1000 mL eluent); IR (film): ν 3355, 1604, 1454, 1376, 1033, 755, 701 cm^{-1}; $[\alpha]_D^{20} = +27.9$ (c 0.96, $CHCl_3$). ***ent*-20** was prepared under the same reaction conditions, starting from ***ent*-18**: $[\alpha]_D^{20} = -30.1$ (c 1.0, $CHCl_3$).

4.20. (2*S*,4*R*)-(4-Amino-1-benzylpyrrolidin-2-yl)-methanol 21

A mixture of **19** (31.0 mg, 0.11 mmol) in THF (5 mL) and $LiAlH_4$ (0.23 mL, 1 M solution in THF) was reacted and worked up (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/500 mL eluent) as described for **17** to give **21** (11.1 mg, 48%) as a colorless oil. ***ent*-21** was prepared under the same reaction conditions, starting from ***ent*-19**: EI-MS $m/z = 206$ [M^+]; TLC: $R_f = 0.33$ (CH_2Cl_2/MeOH = 8:2+6 mL solution of MeOH saturated with NH_3/500 mL eluent); IR (film): ν 3349, 2926, 1586, 1453, 1043, 746, 700 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.65 (ddd, 1H, $J = 12.9$, 9.4, 7.0 Hz, H-3a), 2.12 (dd, 1H, $J = 8.8$, 8.7 Hz, H-5a), 2.18 (ddd, 1H, $J = 12.9$, 7.4, 5.3 Hz, H-3b), 2.99 (m, 1H,

H-2), 3.18 (dd, 1H, $J=8.8$, 6.2 Hz, H-5b), 3.44 (m, 3H, H-4/CH_2OH/NCH_2Ph), 3.65 (dd, 1H, $J=11.0$, 3.1 Hz, CH_2OH), 3.96 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 7.21–7.34 (m, 5H, Ar); $[\alpha]_D^{20}=-65.8$ (c 1.0, $CHCl_3$); $[\alpha]_D^{20}=+74.7$ (c 1.0, $CHCl_3$).

4.21. (2*S*,4*R*)-1-Benzylpyrrolidine-2,4-dicarboxylic acid hydrochloride 22

A solution of **18** (12.7 mg, 0.05 mmol) in conc. HCl (15 mL) was refluxed for 2 h. After cooling to rt the solvent was removed under reduced pressure to give pure **22** (13.78 mg, 99%) as a white solid: Mp: 60–62°C; EI-MS $m/z=204$ (α-cleavage, ($[M-CO_2H]^+$); IR (KBr): ν 3378, 2923, 1646, 1403, 1249, 1157, 1045, 890, 790, 698 cm^{-1}; ^1H NMR (360 MHz, D_2O): δ 2.43 (ddd, 1H, $J=13.9$, 8.9, 8.9 Hz, H-3a), 2.75 (ddd, 1H, $J=13.9$, 8.9, 6.2 Hz, H-3b), 3.33 (m, 1H, H-4), 3.60 (dd, 1H, $J=12.0$, 8.5 Hz, H-5a), 3.79 (dd, 1H, $J=12.0$, 7.8 Hz, H-5b), 4.31 (dd, 1H, $J=8.9$, 8.9 Hz, H-2), 4.39 (d, 1H, $J=12.9$ Hz, NCH_2Ph), 4.54 (d, 1H, $J=12.9$ Hz, NCH_2Ph), 7.40–7.55 (m, 5H, Ar); HR-EIMS for $C_{13}H_{15}NO_4$: calcd 249.10011 found 249.10185, for $C_{12}H_{14}NO_2$: calcd 204.10245 found 204.10306 (Anal. calcd); $[\alpha]_D^{20}=+29.4$ (c 0.78, MeOH).

4.22. (3*S*,5*R*)-*N*-(1-Benzyl-5-hydroxymethyl-3-pyrrolidinyl)-5-chloro-2-methoxy-4-methylaminobenzamide 23

A suspension of 5-chloro-2-methoxy-4-methylaminobenzoic acid (34.85 mg, 0.16 mmol), HOBt (22.22 mg, 0.15 mmol) and DCC (30.3 mg, 0.15 mmol) in EtOAc (15 mL) was stirred at rt for 15 min. ***ent*-21** (27.6 mg, 0.13 mmol), dissolved in EtOAc, was added and the mixture stirred for another 24 h. The mixture was filtered through Celite and the filtrate evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to give **23** (32.7 mg, 60%) as a yellowish solid: Mp: 63–64°C; EI-MS $m/z=402$ [M^+], 404 [M^+]; TLC: $R_f=0.24$ (CH_2Cl_2/MeOH, 95:5); IR (film): ν 3388, 1602, 1519, 1282, 1248, 1036, 912, 732 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.91 (ddd, 1H, $J=13.2$, 9.1, 6.7 Hz, H-4a), 2.39 (m, 2H, H-4b/H-2a), 2.95 (d, 3H, $J=5.1$ Hz, $NHCH_3$), 3.10 (m, 1H, H-5), 3.49 (m, 3H, CH_2OH/NCH_2Ph/H-2b), 3.75 (dd, 1H, $J=11.1$, 3.3 Hz, CH_2OH), 3.95 (s, 3H, OCH_3), 4.09 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 4.46 (m, 1H, H-3), 4.73 (m, 1H, $NHCH_3$), 6.09 (s, 1H, $CHCOCH_3$), 7.21–7.34 (m, 5H, Ar), 7.69 (brd, 1H, $J=6.5$ Hz, NHCO), 8.06 (s, 1H, CHCCl); Anal. calcd for $C_{21}H_{26}ClN_3O_3$ (403.91): C, 62.45; H, 6.49; N, 10.40, found: C, 62.34; H, 6.42; N, 10.37; $[\alpha]_D^{20}=+64.4$ (c 0.15, $CHCl_3$).

***ent*-23** was prepared under the same reaction conditions, starting from **21**: $[\alpha]_D^{20}=-58.1$ (c 0.52, $CHCl_3$).

4.23. (3*S*,5*S*)-*N*-(1-Benzyl-5-hydroxymethyl-3-pyrrolidinyl)-5-chloro-2-methoxy-4-methylaminobenzamide 24

A suspension of 5-chloro-2-methoxy-4-(methylamino)benzoic acid (29.16 mg, 0.14 mmol), HOBt (18.63 mg, 0.12 mmol) and DCC (25.42 mg, 0.12 mmol) was reacted with **17** (23.1 mg, 0.11 mmol) and worked up (CH_2Cl_2/MeOH, 9:1) as described for **23** to give **24** (27.1 mg, 60%) as a yellowish solid. Mp: 127–129°C; EI-MS $m/z=372$ (α-cleavage, ($[M-CH_2OH]^+$), 198 (α-cleavage, ($[M-C_{12}H_{17}N_2O]^+$); TLC: $R_f=0.62$ (CH_2Cl_2/MeOH 9:1); IR (film): ν 3379, 1601, 1518, 1284, 1247, 1036, 700 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.80 (brdd, 1H, $J=14.1$, 5.8 Hz, H-4a), 2.48 (ddd, 1H, $J=14.1$, 9.9, 7.9 Hz, H-4b), 2.63 (dd, 1H, $J=9.9$, 5.1 Hz, H-2a), 2.85 (m, 1H, H-5), 2.95 (d, 3H, $J=5.1$ Hz, $NHCH_3$), 3.02 (brd, 1H, $J=9.9$ Hz, H-2b), 3.44 (m, 2H, CH_2OH/NCH_2Ph), 3.69 (dd, 1H, $J=11.0$, 3.1 Hz, CH_2OH), 3.96 (m, 4H, OCH_3/NCH_2Ph), 4.53 (m, 1H, H-3), 4.69 (m, 1H, $NHCH_3$), 6.10 (s, 1H, $CHCOCH_3$), 7.21–7.34 (m, 5H, Ar), 8.06 (s, 1H, CHCCl), 8.13 (brd, 1H, $J=7.2$ Hz, NHCO); ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 30.12 ($NHCH_3$), 34.87 (C-4), 47.68 (C-3), 55.79 (OCH_3), 56.95 (NCH_2Ph), 60.67, 60.71 (C-2/CH_2OH), 64.21 (C-5), 92.85 ($CHCOCH_3$), 127.83, 128.62, 129.16 (C-Ar), 132.10 (CHCCl), 148.13, 158.19, 164.18 (C-Ar), 170.15 (CONH); Anal. calcd for $C_{21}H_{26}ClN_3O_3$ (403.91): C, 62.45; H, 6.49; N, 10.40, found: C, 62.31; H, 6.53; N, 10.30; $[\alpha]_D^{20}=+42.3$ (c 1.0, $CHCl_3$).

***ent*-24** was synthesized under the same reaction conditions, starting from ***ent*-17**; $[\alpha]_D^{20}=-40.4$ (c 0.47, $CHCl_3$).

4.24. (3*R*,5*S*)-*N*-(1-Benzyl-5-hydroxymethyl-3-pyrrolidinylmethyl)-5-chloro-2-methoxy-4-methylaminobenzamide 25

A suspension of 5-chloro-2-methoxy-4-methylaminobenzoic acid (14.3 mg, 0.07 mmol), HOBt (9.07 mg, 0.06 mmol) and DCC (12.47 mg, 0.06 mmol) was reacted with **16** (12.1 mg, 0.06 mmol) and worked up (CH_2Cl_2/MeOH, 95:5) as described for **23** to give **25** (11.9 mg, 52%) as an opaque, yellowish solid. Mp: 85–90°C; EI-MS $m/z=386$ (α-cleavage, pyrrolidine moiety), 198 (aromatic moiety); TLC: $R_f=0.22$ (CH_2Cl_2/MeOH, 95:5); IR (film): ν 3400, 1602, 1519, 1281, 1246, 1036, 910, 731 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.69 (ddd, 1H, $J=13.2$, 8.1, 5.5 Hz, H-4a), 2.13 (ddd, 1H, $J=13.2$, 8.1, 8.1 Hz, H-4b), 2.53 (m, 2H, H-2a/H-3), 2.88 (m, 2H, H-5/H-2b), 2.95 (d, 3H, $J=5.1$ Hz, $NHCH_3$), 3.40 (m, 3H, CH_2NHCO/CH_2NHCO/NCH_2Ph), 3.54 (dd, 1H, $J=11.3$, 2.4 Hz, CH_2OH), 3.77 (dd, 1H, $J=11.3$, 3.4 Hz, CH_2OH), 3.86 (s, 3H, OCH_3), 4.05 (d, 1H, $J=13.4$ Hz, NCH_2Ph), 4.71 (m, 1H, $NHCH_3$), 6.08 (s, 1H, $CHCOCH_3$), 7.20–7.38 (m, 5H, Ar), 7.83 (m, 1H, NHCO), 8.07 (s, 1H, CHCCl); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 30.16 ($NHCH_3$), 29.69 (C-4), 35.43 (C-3), 43.65 (CH_2NH_2), 56.08 (OCH_3), 56.17 (C-2), 57.28 (NCH_2Ph), 60.54 (CH_2OH), 65.34 (C-5), 93.08 ($CHCOCH_3$), 127.34, 128.79 (C-Ar), 132.29 (CHCCl), 148.13, 158.20, 164.18 (C-Ar); Anal. calcd for $C_{22}H_{28}ClN_3O_3$ (417.94): C, 63.23; H, 6.75; N, 10.05, found: C, 63.05; H, 6.76; N, 10.15; $[\alpha]_D^{20}=-35.6$ (c 0.32, $CHCl_3$).

***ent*-25** was synthesized under the same reaction conditions, starting from ***ent*-16**; $[\alpha]_D^{20}=+32.8$ (c 0.41, $CHCl_3$).

4.25. (3*R*,5*R*)-*N*-(1-Benzyl-5-hydroxymethyl-3-pyrrolidinylmethyl)-5-chloro-2-methoxy-4-methylaminobenzamide 26

***ent*-20** was synthesized from ***ent*-18** (16.9 mg, 0.07 mmol) and was reacted with a suspension of 5-chloro-2-methoxy-4-methylaminobenzoic acid (17.0 mg, 0.07 mmol), HOBt (10.0 mg, 0.07 mmol) and DCC (14.0 mg, 0.07 mmol) as described for **23** and purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) and HPLC (column: RP 18, eluent: MeOH/H_2O, 95:5) to give **26** (12.6 mg, 46% over two steps) as a glutinous, colorless mass: EI-MS $m/z=404$ [M^+]; TLC: $R_f=0.35$ (CH_2Cl_2/MeOH, 9:1); IR (film): ν 3397, 1635, 1600, 1519, 1457, 1280, 1245, 1214, 1037, 809, 752 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.75 (ddd, 1H, $J=13.0$, 9.0, 9.0 Hz, H-4a), 2.05 (ddd, 1H, $J=13.0$, 8.3, 5.0 Hz, H-4b), 2.14 (dd, 1H, $J=9.8$, 9.0, H-2a), 2.38 (m, 1H, H-3), 2.87 (m, 1H, H-5), 2.95 (d, 3H, $J=5.0$ Hz, $NHCH_3$), 3.12 (dd, 1H, $J=9.8$, 6.7 Hz, H-2b), 3.41 (m, 4H, $CH_2NHCO/CH_2NHCO/NCH_2Ph/CH_2OH$), 3.66 (dd, 1H, $J=11.0$, 3.2 Hz, CH_2OH), 3.88 (s, 3H, OCH_3), 3.97 (d, 1H, $J=12.8$ Hz, NCH_2Ph), 4.71 (m, 1H, $NHCH_3$), 6.08 (s, 1H, $CHCOCH_3$), 7.14–7.43 (m, 5H, Ar), 7.63 (m, 1H, NHCO), 8.08 (s, 1H, CHCCl); HR-EI-MS: for $C_{22}H_{26}ClN_3O_2$: calcd 399.17136 found 399.17133, for $C_{21}H_{25}ClN_3O_2$: calcd 386.16354 found 386.16383, for $C_9H_9ClNO_2$: calcd 198.03218 found 198.03279; $[\alpha]_D^{20}=+30.1$ (c 0.05, $CHCl_3$).

***ent*-26** was synthesized under the same reaction conditions, starting from ***ent*-18**; $[\alpha]_D^{20}=-25.8$ (c 0.09, $CHCl_3$).

4.26. (3*S*,5*S*)-4-Acetylamino-*N*-(1-benzyl-5-hydroxymethyl-3-pyrrolidinyl)-5-chloro-2-methoxybenzamide 27

A suspension of 4-acetylamino-5-chloro-2-methoxybenzoic acid (39.88 mg, 0.2 mmol), HOBt (27.8 mg, 0.2 mmol) and DCC (37.9 mg, 0.2 mmol) was reacted with **17** (34.6 mg, 0.17 mmol) and worked up (CH_2Cl_2/MeOH, 95:5) as described for **23** to give **27** (72.3 mg, 88%) as a colorless, glutinous: EI-MS $m/z=432$ [M^+]; TLC: $R_f=0.51$ (CH_2Cl_2/MeOH, 9:1); IR (film): ν 3345, 1687, 1637, 1508, 1242, 1012, 742 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 2.08 (m, 1H, H-4a), 2.26 (s, 3H, $COCH_3$), 2.54 (m, 1H, H-4b), 3.10 (dd, 1H, $J=11.1$, 5.7 Hz, H-2a), 3.46 (m, 1H, H-2), 3.57 (brd, 1H, $J=11.1$ Hz, H-2b), 3.71 (dd, 1H, $J=12.3$, 3.1 Hz, CH_2OH), 3.82 (s, 3H,OCH_3), 3.97 (dd, 1H, $J=13.3$, 2.2 Hz, CH_2OH), 4.05 (d, 1H, $J=13.7$ Hz, NCH_2Ph), 4.37 (d, 1H, $J=13.7$ Hz, NCH_2Ph), 4.67 (m, 1H, H-3), 7.20–7.38 (m, 5H, Ar), 7.55 (d, 1H, $J=7.5$ Hz, NHCO), 7.70 (s, 1H, $NHCOCH_3$), 8.02/8.08 (s/s, 1H/1H, $CHCCl/CHCOCH_3$); Anal. calcd for $C_{22}H_{26}ClN_3O_4\times 0.5\ H_2O$: C, 60.55; H, 6.12, N; 9.24, found: C, 60.29; H, 5.98; N, 9.43; $[\alpha]_D^{20}=+60.1$ (c 0.23, $CHCl_3$).

4.27. (3*R*,5*S*)-4-Acetylamino-*N*-(1-benzyl-5-hydroxymethyl-3-pyrrolidinylmethyl)-5-chloro-2-methoxybenzamide 28

A suspension of 4-aetylamino-5-chloro-2-methoxybenzoic acid (30.72 mg, 0.15 mmol), HOBt (21.3 mg, 0.14 mmol) and DCC (29.06 mg, 0.14 mmol) was reacted with **16** (28.3 mg, 0.13 mmol) and worked up (CH_2Cl_2/MeOH, 95:5) as described for **23** to give **28** (16.9 mg, 29%) as a yellowish, opaque solid. Mp: 78–81°C. EI-MS $m/z=414$ (α-cleavage, [$M-CH_2OH$]$^+$); TLC: $R_f=0.29$ (CH_2Cl_2/MeOH, 9:1); IR (film): ν 3398, 1682, 1639, 1508, 1398, 1241, 729 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.67 (ddd, 1H, $J=13.2$, 8.1, 4.9 Hz, H-4a), 2.13 (ddd, 1H, $J=13.2$, 8.3, 7.9 Hz, H-4b), 2.27 (s, 3H, $COCH_3$), 2.47 (m, 2H, H-2a/H-3), 2.80 (m, 2H, H-5/H-2b), 3.44 (m, 4H, $CH_2NH/CH_2NH/CH_2OH/NCH_2Ph$), 3.76 (dd, 1H, $J=10.9$, 3.4 Hz, CH_2OH), 3.88 (s, 3H, OCH_3), 4.01 (d, 1H, $J=13.0$ Hz, NCH_2Ph), 7.22–7.36 (m, 5H, Ar), 7.77 (s, 1H, $NHCOCH_3$), 7.90 (m, 1H, CH_2NHCO), 8.18/8.30 (s/s, 1H/1H, CHCCl/$CHCOCH_3$); Anal. calcd for $C_{23}H_{28}ClN_3O_4$: C, 61.95; H, 6.33; N, 9.42, found: C, 62.08; H, 6.41; N, 9.32; $[\alpha]_D^{20}=-52.0$ (c 0.03, $CHCl_3$).

4.28. (3*S*,5*S*)-4-(*N*-Acetyl-*N*-methylamino)-*N*-(1-benzyl-5-hydroxymethyl-3-pyrrolidinyl)-5-chloro-2-methoxybenzamide 29

A suspension of 4-(*N*-acetyl-*N*-methylamino)-5-chloro-2-methoxybenzoic acid (31.34 mg, 0.12 mmol), HOBt (16.8 mg, 0.11 mmol) and DCC (22.8 mg, 0.11 mmol) in EtOAc (10 mL) was reacted with **17** (20.9 mg, 0.10 mmol) and worked up (CH_2Cl_2/MeOH, 95:5) as described for **23** to give **29** (15.0 mg, 50%) as a white, foamy mass. Mp: ~30°C. EI-MS $m/z=414$ (α-cleavage, ([$M-CH_2OH$]$^+$); TLC: $R_f=0.57$ (CH_2Cl_2/MeOH, 9:1); IR (film): ν 3384, 1651, 1531, 1486, 1232, 1036, 733 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ 1.82 (m, 4H, H-4a/$COCH_3$), 2.52 (ddd, 1H, $J=14.1$, 10.3, 7.9 Hz, H-4b), 2.65 (m, 1H, H-2a), 2.88 (m, 1H, H-5), 3.04 (brd, 1H, $J=9.9$ Hz, H-2b), 3.19 (s, 3H, NCH_3), 3.45 (m, 2H, CH_2OH/NCH_2Ph), 3.68 (brd, 1H, $J=11.3$ Hz, CH_2OH), 3.97 (m, 4H, OCH_3/NCH_2Ph), 4.53 (m, 1H, H-3), 6.86 (s, 1H, $CHCOCH_3$), 7.18–7.38 (m, 5H, Ar), 8.21 (d, 1H, $J=7.2$ Hz, NHCO), 8.27 (s, 1H, CHCCl); Anal. calcd for $C_{23}H_{28}ClN_3O_4\times 0.5\ H_2O$: C, 60.72; H, 6.42; N, 9.24, found: C, 60.66; H, 6.23; N, 9.16; $[\alpha]_D^{20}=+11.5$ (c 0.82, $CHCl_3$).

4.29. Dopamine receptor binding studies

Receptor binding studies were carried out as described in the literature.[36] In brief, the dopamine D1 receptor assay was done with bovine striatal membranes at a final protein concentration of 45 μg/assay tube and the radioligand [^3H]SCH 23390 at 0.3 nM ($K_d=0.35$–0.75 nM).

Competition experiments with the human $D2_{long}$, $D2_{short}$, D3 and D4.4 receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^3H]spiperone at a final concentration of 0.1 nM. The assays were carried out at a protein concentration of 5–25 μg/assay tube and K_d values of 0.10 nM for $D2_{long}$ and $D2_{short}$, 0.10–0.40 nM for D3 and 0.10–0.45 nM for D4.4.

Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[38]

4.30. 5-HT receptor binding studies

Receptor binding experiments were carried out with cortical homogenates prepared from porcine brain which was obtained from the local slaughterhouse. The cortex material was dissected and frozen at −80°C. Membranes were prepared by thawing, cutting up and homogenizing in an aqueous solution of sucrose (0.1 M). The suspension was washed by centrifugation at 2,500 g. The resulting supernatant was then pelleted by centrifugation at 80,000 g for 40 min. The pellet was re-suspended in Tris–EDTA buffer (50 mM Tris–HCl, 1 mM EDTA; pH 7.4), homogenized with a Potter–Elvehjam homogenizer and stored at −80°C in small aliquots.

For 5-HT1_A receptor binding assay porcine cortical membranes were diluted with binding buffer (50 mM Tris–HCl, 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 nM pargyline; pH 7.4) to a final concentration of 460 μg protein/assay tube (K_d values from 2.4–4.8 nM). Tubes were prepared with the radioligand [^{3}H]8-OH-DPAT (0.5 nM) (specific activity 135.0 Ci/mmol; PerkinElmer) and varying concentrations of test compounds (from 0.01–10,000 nM). Non-specific binding was determined in the presence of serotonine (10 μM). Incubation was started by adding membranes to the assay tube with a final volume of 800 μL and continued for 60 min at 37°C after which it was stopped by rapid filtration through GF/B filters precoated with 0.3% polyethylenimine, using an automated cell harvester (Inotech, CH). Filters were washed five times with ice-cold Tris–EDTA buffer, dried and counted in a MicroBeta Trilux (PerkinElmerWallac).

Binding assay with 5-HT2 receptors was done at 200 μg protein/assay tube with the radioligand [^{3}H]ketanserine (specific activity 63.3 Ci/mmol; PerkinElmer) at K_d values from 2.6–3.1 nM and methysergide (10 μM) for determination of non-specific binding. Incubation was carried out at a final volume of 500 μL for 60 min at 37°C and worked up as described above.

4.31. Data analysis

The resulting competition curves were analyzed by non-linear regression using the algorithms in PRISM (GraphPad Software, San Diego, CA). The data was initially fitted using a sigmoid model to provide an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. The IC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[39]

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