Synthesis of the Four Stereoisomers of 1-Amino-2-(hydroxymethyl)-cyclobutanecarboxylic Acid and Their Biological Evaluation as Ligands for the Glycine Binding Site of the NMDA Receptor

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Dedicated with the best wishes to Prof. Gotthard Wurm on the occasion of his 65th birthday

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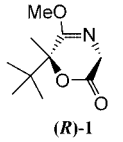
A synthesis of all four stereoisomers [(1*S*,2*S*)-, (1*R*,2*R*)-, (1*S*,2*R*)-, (1*R*,2*S*)-] of 1-amino-2-(hydroxymethyl)cyclobutanecarboxyclic acid is presented. The synthesis is based on the chiral glycine equivalent **1**, employed in both enantiomeric forms. The key step involves the cyclization of the silyl-protected iodohydrins **5a**–**d** to the corresponding *spiro* derivatives **6a**–**d** with the aid of the phosphazenic base *t*Bu-P_4. The final compounds were found to display moderate potency as ligands for the glycine binding site of the NMDA receptor.

1. Introduction

1-Aminocycloalkanecarboxylic acids, especially those with three-, four-, or five-membered rings, have attracted much attention, which may be regarded as a result of the particular conformational characteristics to which they give rise when incorporated in peptides.[1,2] Although numerous asymmetric syntheses of 1-aminocyclopropane- and 1-aminocyclopentanecarboxylic acids have been developed, no asymmetric syntheses of 1-aminocyclobutanecarboxyclic acids are yet known, according to a recent review and to the best of our knowledge.[3] Moreover, only a few racemic syntheses of 1-aminocyclobutanecarboxylic acids have been published,[4−6] this being indicative of the unfavorable situation in four-membered ring syntheses with respect both to small-angle strain and to entropy.

As part of a study directed towards the development of new ligands for the glycine site of the NMDA-receptor complex we have recently performed the asymmetric syntheses of 1-aminocyclopropanecarboxyclic acid derivatives, based on the chiral glycine equivalent **1** (Figure 1).[7] This compound is especially useful for the construction of α,α-disubstituted amino acids, as the chirality in **1** arises from a quaternary carbon and so the compound may be subjected repeatedly to deprotonation for alkylation reactions without the risk of racemization.



(*R*)-**1**

Figure 1. Chiral glycine equivalent for the synthesis of disubstituted amino acids

Together with 1-aminocyclopropanecarboxylic acid, 1-aminocyclobutanecarboxylic acid[8] (**9**) is also well known as a ligand for the glycine site of the NMDA receptor, so derivatives of this amino acid **9** were of great interest to us.

We now present an asymmetric synthesis of the four stereoisomeric 1-amino-2-(hydroxymethyl)cyclobutanecarboxylic acids **8a/b** and (***ent*)-8a/b** and the biological evaluation of these compounds as ligands for the glycine binding site of this receptor.

2. Results and Discussion

2.1 Synthesis of the Four Stereoisomeric 1-Amino-2-(hydroxymethyl)cyclobutanecarboxylic Acids 8a/b and (*ent*)-8a/b

Our synthetic approach commenced with the chiral glycine equivalent **1**, which is available in both enantiomeric

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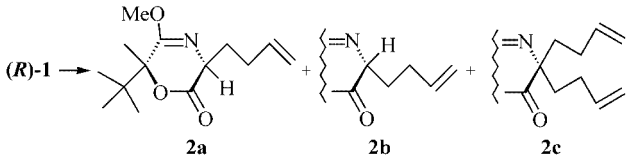
forms, (*R*)-**1** (Figure 1) and (*S*)-**1**. This is a major advantage, as it allows the synthesis of both enantiomers of a target compound simply by performing enantiomorphic synthetic sequences. This feature was also utilized in the current study, to accomplish the synthesis of the stereoisomers **8a/b** and of their enantiomers **(*ent*)-8a/b**.

Various synthetic concepts were used for the construction of the *spirocyclic* derivatives **7a−d**, which were intended to function as direct precursors for the desired amino acids. Treatment of **1** with 1,3-biselectrophiles to establish the desired ring system was considered to be one of the most direct approaches. The introduction of a but-3-enyl side chain in the 3-position of **1** also seemed quite promising, as a double bond would also easily allow a functional group suitable for ring closure to be established.

As various attempts at the double alkylation of (*R*)-**1** with appropriately substituted 1,3-biselectrophiles did not meet with any success, we turned our attention to the second route, based on the introduction of a butenyl side chain.

The first series of reactions was performed with 4-bromobut-1-ene as alkylating agent. When (*R*)-**1** was treated at −78 °C with *s*BuLi, followed by 4-bromobut-1-ene and NaI (to increase the reactivity), the desired product was obtained, but in poor yield (**2a** + **2b** = 15%, see Table 1) and with low diastereoselectivity (**2a/2b** = 75:25). Interestingly, significant amounts of the double alkylation product **2c** were also isolated, indicating that the intermediate lithium enolate had undergone some aggregate formation. Therefore, to attenuate this aggregate formation in further experiments, $LiN[Si(CH_3)_3]_2$, $NaN[Si(CH_3)_3]_2$ and $KN[Si(CH_3)_3]_2$} were employed in combination with appropriate crown ethers (see Table 1) for the deprotonation step.

When the thus generated enolates of (*R*)-**1** were subsequently treated with the electrophile, the results were again disappointing. The yield and the diastereoselectivity for **2a/2b** were still poor (see Table 1, yield ≈ 20%, *ds* ≈ 70:30) and, moreover, the amount of the undesired doubly alkylated product **2c** had even increased (13−29%). When the phosphazenic base *t*Bu-P_4 {*t*BuN=P[N=P(NMe_2)$_3$]$_3$} was used for deprotonation, however, a clear improvement in the results occurred.[9−11] The yield of **2a/2b** rose to 60% and the diastereoselectivity to 96.5:3.5 (*ds*). As a major drawback, though, the reproducibility of this reaction appeared to be poor and, furthermore, the side product **2c** was also found in all cases. Finally, the best results were obtained when *s*BuLi was used for the deprotonation and but-3-enyl triflate (generated in situ) was employed as alkylating agent. In this case the yield of **2a/2b** rose to almost 70%, the diastereoselectivity remained as high as that seen for the previously described reaction with *t*Bu-P_4 (*ds*, 95.5:4.5), and, most interestingly, the reaction product was now devoid of the undesired by-product **2c** (Scheme 1).



Scheme 1. Alkylation of (*R*)-**1**; reagents and conditions see Table 1

Next, the terminal double bond of the butenyl side chain in **2a/2b** had to be transformed into a functional unit suitable for the desired ring closure. To this end, the diastereomeric mixture **2a/2b** was treated with a catalytic amount of OsO_4 in combination with $ONMe_3$ as a co-oxidant to convert the double bond into a diol. Although the overall yield was good (76%), surprisingly, a mixture of the four diastereomeric dihydroxy derivatives **3a−3d** was formed in a ratio of ca. 4:4:1:1 (determined by ^{1}H NMR), indicating that epimerization at the 3-position of the oxazine ring must have occurred. The cause of this isomerization is most probably NMe_3 generated during the dihydroxylation reaction. As the mixture of diastereomers **3a−3d** appeared to be hardly separable by chromatography and as there was no need for the pure stereoisomers, the product was used unchanged for the subsequent reactions. In the next step the primary hydroxy group present in **3a−3d** was selectively protected[12,13] as a *tert*-butyldimethylsilyl ether by use of *t*$BuMe_2SiCl$, imidazole, and DMAP, producing **4a−4d** in a yield of 88%. Then, on treatment of **4a−4d** with PPh_3, iodine, and imidazole for selective replacement of the secondary hydroxy group with iodide,[14−16] the desired silylprotected iodohydrins **5a−5d** were obtained in high yield (90%). During these two steps (the syntheses of **4a−4d** and of **5a−5d**) the ratio of stereo-

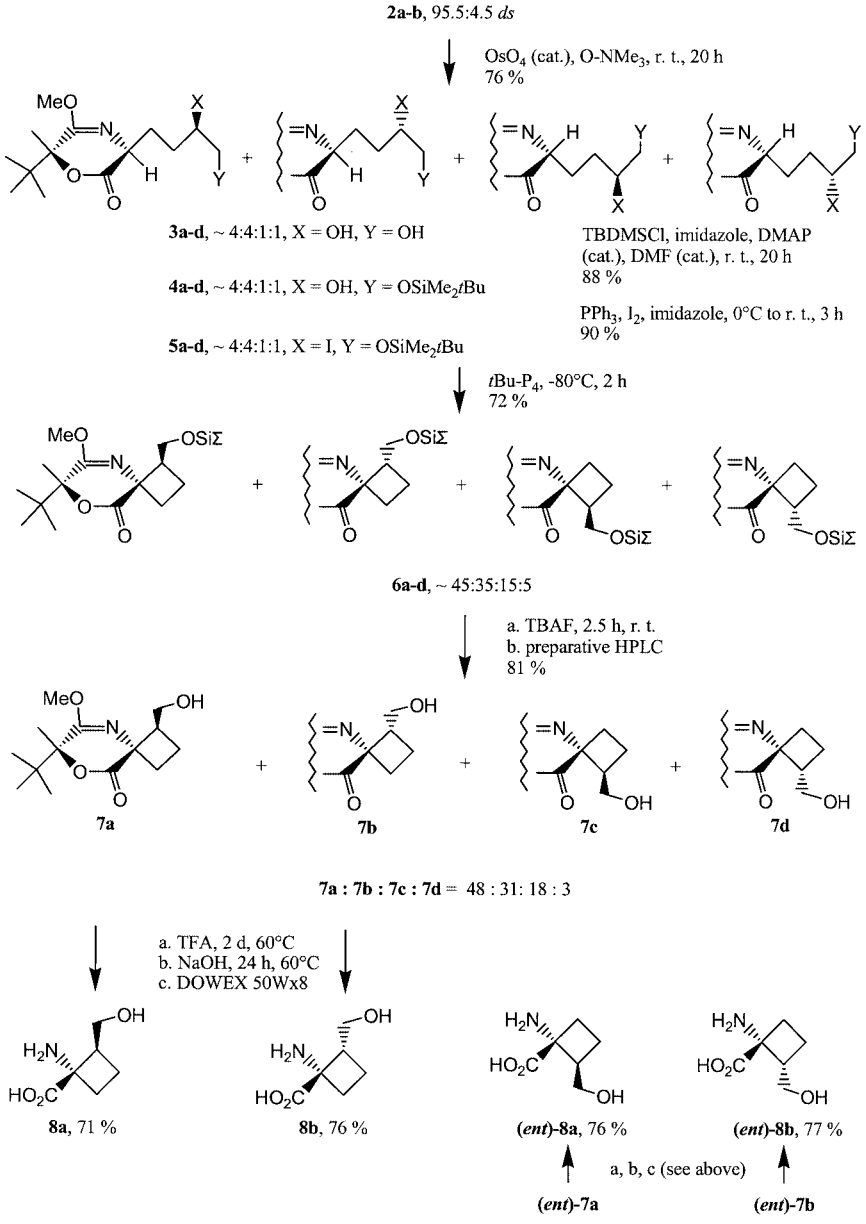
Table 1. Alkylation of (*R*)-**1**

Base	Electrophile	Additives	Temperature	Yield (%) **2a + 2b / 2c**	*ds* **2a / 2b**
*s*BuLi	$BrCH_2CH_2CH=CH_2$	NaI	−80 °C → 0 °C	15/7	≈ 75:25[b]
$LiN[Si(CH_3)_3]_2$	$BrCH_2CH_2CH=CH_2$	12-crown-4	−80 °C → 10 °C	23/29	≈ 70:30[b]
$NaN[Si(CH_3)_3]_2$	$BrCH_2CH_2CH=CH_2$[a]	15-crown-5	−50 °C	15/13	≈ 70:30[b]
$KN[Si(CH_3)_3]_2$	$BrCH_2CH_2CH=CH_2$	18-crown-6	−80 °C → −50 °C	13/17	≈ 70:30[b]
*t*Bu-P_4	$BrCH_2CH_2CH=CH_2$	−	−80 °C	60/13	96.5:3.5[c]
*s*BuLi	$TfOCH_2CH_2CH=CH_2$	−	−80 °C	69/−	95.5:4.5[c]

[a] DME was used as solvent instead of THF. [b] Selectivity determined by ^{1}H NMR spectroscopy. [c] Selectivity determined by analytical HPLC.

isomers remained unchanged (≈ 4:4:1:1, determined by ^{1}H NMR). Finally, as the crucial step of our synthesis, the cyclization of **5a**−**5d** to **6a**−**6d** had to be performed. This was expected to be tedious, as a sterically strongly encumbered secondary iodide − beside a CH_2OSiMe_2tBu group − was involved. In addition, to the best of our knowledge, no precedence for related cyclization reactions to cyclobutanes exists. In order to generate a highly reactive enolate, phosphazenic base $tBuP_4$ was chosen for the deprotonation reaction.[9−11] Upon treatment of **5a**−**5d** with this base (for three hours at −80 °C in DME/THF, 5:1) the desired cyclization indeed occurred and a mixture of the four diastereomeric *spirocyclic* compounds **6a**−**6d** was isolated. The ratio of the diastereomers amounted to ≈ 45:35:15:5 and the total yield to 72%.

A stereomodel for alkylation reactions of **1** has been proposed. According to this, the *tert*-butyl group, by shielding one face of the oxazine moiety, causes the electrophile to approach the molecule from the opposite side.[7] The results both for the alkylation reaction of (*R*)-**1** (→ **2a**/**2b**) and for the cyclization reaction of **5a**−**d** (→ **6a**−**d**) proceeded with the same sense of asymmetric induction and in line with the model described above. Furthermore, the diastereoselectivity found for the butenylation reaction was quite pleasing (> 95:5, see Table 1), whereas the selectivity for the cyclization reaction was far less satisfying (≈ 8:2, determined from the ratios observed for **6a**−**d** and **7a-d**). It seems likely that the stereoselectivity observed in the spirocyclization (stereochemistry at C-4) arises mainly from the asymmetric induction caused by the chiral auxiliary and that the chiral center



Scheme 2. Synthesis of the stereoisomeric 1-aminocyclobutanecarboxylic acids **8a/b** and (***ent***)-**8a/b**

located in the side chain of **5a−d** has only a minor influence.

The removal of the silyl protective group was accomplished by treatment of **6a−6d** with tetrabutylammonium fluoride. The resulting crude product was separated by preparative HPLC, providing **7a−7d** each as a single isomer in a ratio of 48:31:18:3 (**7a/7b/7c/7d**) and in a total yield of 81% (see Scheme 2).

For evaluation of the biological activity, the whole set of stereoisomeric amino acids **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b** was required. However, the minor diastereomers **7c−7d**, as precursors to **(*ent*)-8a** and **(*ent*)-8b**, were available only in small amounts. The whole synthetic sequence described so far was therefore also performed with the enantiomeric glycine equivalent (*S*)-**1**, to afford the enantiomeric spirocyclic compounds **(*ent*)-7a**, **(*ent*)-7b**, **(*ent*)-7c**, and **(*ent*)-7d**. The chemical yields and diastereoselectivities for each step in this sequence corresponded well to those described above.

In the final step for each of the two enantiomorphic series, the two major diastereomers [**7a** and **7b**, and **(*ent*)-7a** and **(*ent*)-7b**] had to be hydrolyzed to provide the free amino acids. This was accomplished in a two-step sequence,[7] by application first of aqueous TFA for the cleavage of the lactim ether function and then of a NaOH solution for the hydrolysis of the ester group. Thus, after purification by ion-exchange chromatography on an acidic ion-exchange resin (DOWEX 50Wx8), the four desired 1-aminocyclobutanecarboxylic acids **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b** were obtained in yields between 70 and 80% (Scheme 2).

2.2 Assignment of the Stereochemistry to 7a−7d, 8a, 8b, (*ent*)-8a, and (*ent*)-8b

The stereochemical assignment started with the *spirocyclic* derivative **7a**, which, having formed crystals, was subjected to an X-ray diffraction analysis.[17] From the results of this analysis and by taking into account the absolute configuration of the chiral auxiliary present in **7a** (exhibiting *R* configuration), the stereocenters at C-1 and C-4 of **7a** must be of *S* stereochemistry.

With the stereochemistry for **7a** being known, the absolute configurations of the remaining stereoisomers could be determined. This was accomplished mainly with the aid of NOE measurements carried out on **7a−7d**. Irradiation of the signal of the *tert*-butyl group ($\delta \approx 1.00$ ppm) of the diastereomers **7a−7b** resulted in each case in an enhancement of the signals arising from the protons in the 3-position (**7a**, 4.0 and 2.6%; **7b**, 3.4 and 1.5%; see Figure 3). Although this was to be expected for **7a**, because of the stereochemistry found by the X-ray analysis presented above, it also demonstrated that **7b** could differ from **7a** only in the stereochemistry at C-1 and so must therefore possess the stereochemistry shown. These results were fully supported by NOE measurements performed with the remaining diastereomers **7c−7d**. For these, upon activation of the *tert*-butyl group, enhancement of H-1 and of the protons of the exocyclic methylene group was seen in both cases (Figure 3).

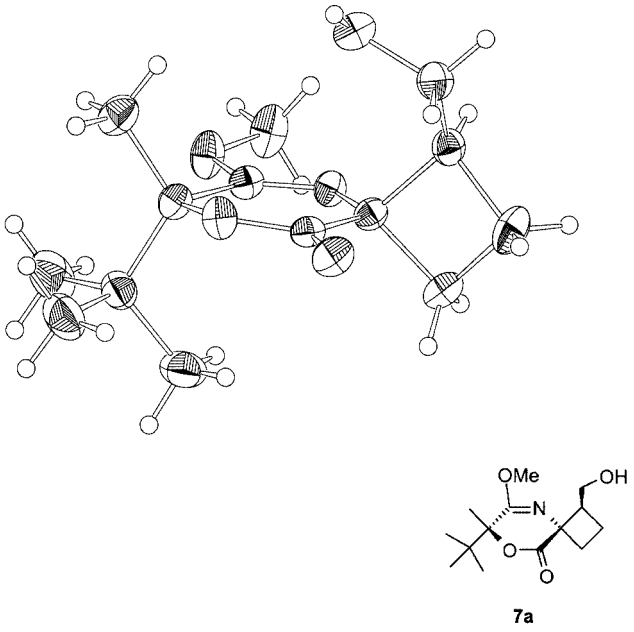


Figure 2. X-ray structure of **7a**

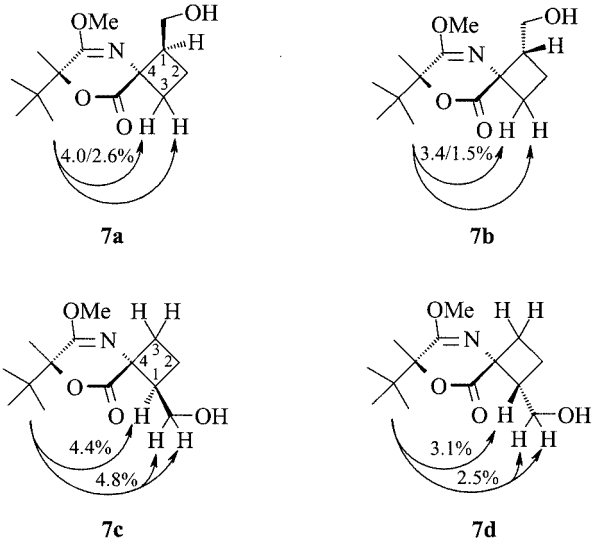
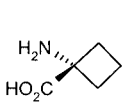
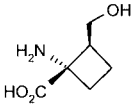
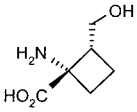
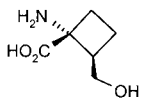
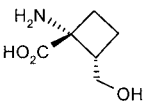


Figure 3. NOEs observed for the stereoisomers **7a−d**

As **7c** and **7d** were diastereomers of **7a** and **7b**, all originating from the same chiral glycine equivalent (*R*)-**1**, their stereochemistry at C-4 and at C-7 had to be as indicated, except that the configuration at C-1 was still unresolved. To provide this missing information, which was only a question of the relative stereochemistry, **7c** was hydrolyzed to the free amino acid **(*ent*)-8a**. The ^{1}H NMR spectrum of this amino acid **(*ent*)-8a** was identical with that of **8a** derived from **7a**. Therefore, the hydroxymethyl group and the imidate function in **7c**, and also in **(*ent*)-7c**, must be located *trans* to one another with respect to the cyclobutane ring. As a consequence, **7c** and **7d**, and the same is of course true for **(*ent*)-7c** and **(*ent*)-7d**, must have the stereochemistry as indi-

Table 2. Potency of **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b**, together with reference compound **9**, at the glycine binding site of the NMDA receptor

K_i [μM][a] – [^{3}H]MDL *105,519*				
				
9	**8a**	**8b**	**(*ent*)-8a**	**(*ent*)-8b**
45.2 ± 6.6	220 ± 10	260 ± 20	400 ± 60	340 ± 20

[a] Means ± standard error of the mean from three independent experiments each carried out in triplicate.

cated (see Scheme 2 and Figure 3). Of course, the stereochemistry of the final products, the 1-aminocyclobutanecarboxylic acids, had also become evident from this assignment.

3. Biological Test Results

The amino acids **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b** were evaluated for their in vitro activity on the glycine binding site of the NMDA receptor by a radioreceptor assay.[18] The binding affinities were determined on pig cortical brain membranes with [^{3}H]*MDL 105,519* as a specific ligand.

As a reference compound, 1-aminocyclobutanecarboxylic acid (**9**) was included in this process.[19] As depicted in Table 2, the derivatives **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b** appeared to be distinctly less potent than the parent compound **9**. Moreover, the potencies of compounds **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b** were only weakly influenced by their stereochemistry. It should be noted, though, that the two most potent stereoisomers − **8a** and **8b** − have the same configuration at C-1 of the cyclobutane ring. Accordingly, the stereochemistry at this stereocenter has a greater effect on the potencies of these compounds than that at C-2. Compounds **8b** and **(*ent*)-8b** were also evaluated for their ability to modulate [^{3}H]*MK-801* binding under non-equilibrium conditions. The two compounds **8b** and **(*ent*)-8b** gave rise to a distinct increase in binding of [^{3}H]*MK*-801, indicating that these derivatives are partial or possibly even full agonists.

4. Conclusions

In summary, an asymmetric synthesis of all four stereoisomers of 1-amino-2-(hydroxymethyl)cyclobutanecarboxylic acid, based on the chiral glycine equivalents (*R*)-**1** and (*S*)-**1**, has been presented. The cyclization of the silyl-protected iodohydrins **5a**−**5d**, derived from (*R*)-**1**, to the corresponding *spirocyclic* derivatives **6a**−**6d** with mediation by the phosphazenic base *t*Bu-P$_4$ should be considered the key step of the synthetic sequence.

The final compounds, amino acids **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b**, were found to be ligands for the glycine binding site of the NMDA receptor, but the potencies displayed by these derivatives were lower than the affinity exhibited by the parent compound **9**. So far this method has been used for the preparation of the free amino acids **8a**, **8b**, **(*ent*)-8a**, and **(*ent*)-8b**, but it should also easily provide access to related amino acids with different substituents in the 2-position of the cyclobutane ring. Further studies in this direction are underway.

5. Experimental Section

5.1 General Procedure

All experiments were carried out in oven-dried glassware under dry N_2 atmosphere. Standard vacuum techniques were used for the handling of air-sensitive materials. Solvents were dried and kept under N_2 and freshly distilled before use. Reagents were used as commercially available. Solvents used for HPLC were degassed prior to use. For the ion exchange chromatography doubly distilled H_2O was used; the NH_3 solution was freshly prepared prior to use by bubbling NH_3 through bidest H_2O. The phosphate buffer (pH = 7) was prepared by dissolving $NaH_2PO_4{\cdot}H_2O$ (12.17 g) and $Na_2HPO_4{\cdot}2\ H_2O$ (21.35 g) in H_2O (200 mL). M.p. (uncorrected values): Melting point apparatus Electrothermal IA9100. Optical rotation: Polarimeter 241 MC (Perkin−Elmer). ^{1}H NMR spectra: J NMR-GX 400 (400 MHz, Jeol) and J NMR-GX 500 (500 MHz, Jeol), chemical shifts (δ) in ppm, TMS as internal standard. IR: FT-IR spectrometer Paragon 1000 (Perkin−Elmer, Software Spectrum™), liquids were recorded as films, solids as KBr pellets. MS: 5989 A mass spectrometer with 59980 B particle beam LC/MS interface (Hewlett−Packard). Combustion analysis: Elementaranalysator Vario EL. Column chromatography (CC): Flash chromatography on silica gel (Merck Si60 0.040−0.063 mm). TLC: Merck Si60 F-254, detection with UV (λ = 254 nm) or with ammonium cerium(IV)heptamolybdate [5% $(NH_4)_xMo_7O_{24}$ and 0.2% $Ce(SO_4)_2$, dissolved in 400 mL of a 5% H_2SO_4 solution]. Analytical HPLC; Pump: L-6200 intelligent-pump (Merck−Hitachi) or L-7100 (Merck−Hitachi). UV/Vis detectors: L-7400 (Merck−Hitachi); Integrators: D-2500 (Merck−Hitachi) or D-7500 (Merck−Hitachi), Software Merck−Hitachi HSM D-7000 (PC); Columns: LiChroCart® with LiChrospher® Si 60 cartridge (5 μm, 250 × 4 mm with precolumn 4 × 4 mm, Merck). Preparative HPLC; Pump: L-6000 (Merck−Hitachi), UV/Vis detectors: L-4000 (Merck−Hitachi); Integrator: D-2000 Chromato integrator (Merck−Hitachi); Column: Hibar (prepacked) LiChrosorb® Si 60 (7 μm, 250 × 25 mm, Merck).

5.2 Experimental Data

(3*S*,6*R*)-3-But-3-enyl-6-*tert*-butyl-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (2a), (3*R*,6*R*)-3-But-3-enyl-6-*tert*-butyl-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (2b), and (6*R*)-3,3-Di(but-3-enyl)-6-*tert*-butyl-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (2c): A) Compound (*R*)-**1** (87 mg, 0.436 mmol) in THF (2.5 mL) was treated at −80 °C with *t*Bu-P_4 (458 μL, *c* = 1.0 M in *n*-hexane, 0.458 mmol, 1.05 equiv.). After 30 min, a precooled solution (−78 °C) of 4-bromobut-1-ene (177 mg, 1.31 mmol, 3 equiv.) in THF (0.8 mL) was added by cannula and the reaction mixture was stirred for 68 h. After addition of phosphate buffer (pH = 7, 3 mL), the reaction mixture was allowed to warm to room temperature. The organic phase was separated and the aqueous phase was extracted with Et_2O (4 × 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by CC (petroleum ether/ethyl acetate, 92:8, R_f = 0.28) to give **2a/2b** (66 mg, 60%) as a colorless oil, together with **2c** (R_f = 0.42, 17 mg, 13%), also as a colorless oil. The diastereoselectivity was determined on the crude product by analytical HPLC (*n*-heptane/ethyl acetate, 95:5; 1.50 mL/min): *ds* **2a/2b =** 96.5:3.5. **2a**: t_{Ret} = 12.57 min; **2b**: t_{Ret} = 14.69 min. For analytical purposes a sample of **2a/2b** was separated by preparative HPLC (*n*-heptane/ethyl acetate, 96:4; 15.0 mL/min): **2a**: t_{Ret} = 21.74 min; **2b**: t_{Ret} = 28.51 min.

Compound 2a: Colorless crystals, m.p. 37 °C. $[\alpha]_D^{20}$ = −76.1 (*c* = 0.85, $CHCl_3$). TLC: R_f = 0.31 (petroleum ether/ethyl acetate, 90:10). ^{1}H NMR ($CDCl_3$): δ = 1.01 [s, 9 H, C(CH_3)$_3$], 1.50 (s, 3 H, CH_3), 1.89−1.99 (m, 1 H, CH_2), 2.07−2.24 (m, 3 H, CH_2), 3.72 (s, 3 H, OCH_3), 4.13 (dd, *J* = 7.3/4.6 Hz, 1 H, CHCH$_2$), 4.96−5.09 (m, 2 H, CH=CH_2), 5.78−5.90 (m, 1 H, CH=CH$_2$). IR: ṽ = 2964 cm^{-1}, 1737, 1704, 1462, 1374, 1334, 1323, 1257, 1102, 989, 910. MS (CH_4, CI): *m*/*z* (%) = 254 (69) [M + H$^+$], 212 (23), 201 (13), 200 (100), 175 (14), 149 (23), 113 (15), 101 (23). $C_{14}H_{23}NO_3$ (253.3): calcd. C 66.37, H 9.15, N 5.53; found C 66.41, H 9.20, N 5.44.

Compound 2b: Colorless crystals, m.p. 35−36 °C. $[\alpha]_D^{20}$ = −3.8 (*c* = 0.26, $CHCl_3$). TLC: R_f = 0.31 (petroleum ether/ethyl acetate, 90:10). ^{1}H NMR ($CDCl_3$): δ = 1.04 [s, 9 H, C(CH_3)$_3$], 1.52 (s, 3 H, CH_3), 1.71−1.82 (m, 1 H, CH_2), 2.12−2.22 (m, 1 H, CH_2), 2.23−2.40 (m, 2 H, CH_2), 3.71 (s, 3 H, OCH_3), 4.15 (dd, *J* = 9.0/4.4 Hz, 1 H, CHCH$_2$), 4.98−5.12 (m, 2 H, CH=CH_2), 5.81−5.92 (m, 1 H, CH=CH$_2$). IR: ṽ = 2963 cm^{-1}, 1739, 1697, 1462, 1375, 1332, 1257, 1126, 1096. $C_{14}H_{23}NO_3$ (253.3). MS (CH_4, CI): *m*/*z* (%) = 254 (100) [M + H$^+$].

Compound 2c: Colorless oil. $[\alpha]_D^{20}$ = −19.4 (*c* = 1.22, $CHCl_3$). TLC: R_f = 0.42 (petroleum ether/ethyl acetate, 92:8). ^{1}H NMR ($CDCl_3$): δ = 1.04 [s, 9 H, C(CH_3)$_3$], 1.53 (s, 3 H, CH_3), 1.75−2.15 (m, 8 H, CH_2), 3.71 (s, 3 H, OCH_3), 4.91−5.07 (m, 4 H, CH=CH_2), 5.71−5.87 (m, 2 H, CH=CH$_2$). IR: ṽ = 3078 cm^{-1}, 2961, 1731, 1694, 1842, 1454, 1373, 1310, 1221, 1153, 1101, 994, 911− MS (CH_4, CI): *m*/*z* (%) = 308 (100) [M + H$^+$]. $C_{18}H_{29}NO_3$ (307.4): calcd. C 70.32, H 9.51, N 4.56; found C 70.45, H 9.72, N 4.34.

B) This was analogous to the synthesis of **(*ent*)-2a/b**, but starting from (1.18 g, 5.92 mmol) of (*R*)-**1**. Yield of **2a/b**: 1.04 g (69%), HPLC: *ds* **2a/2b =** 95.5:4.5).

(3*R*,6*S*)-3-But-3-enyl-6-*tert*-butyl-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-2a] and (3*S*,6*S*)-3-But-3-enyl-6-*tert*-butyl-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-2b]: *s*BuLi (1.3 M in cyclohexane, 24.0 mL, 31.18 mmol, 1.05 equiv.) was added at −10 °C to but-3-en-1-ol (2.14 g, 2.55 mL, 29.7 mmol) in THF (22 mL). After 15 min this solution was slowly (15 min) added by cannula at −78 °C to TfCl (5.0 g, 29.7 mmol) in THF (39 mL). After 30 min the resulting mixture was allowed to warm to −50 °C over 1 h. This solution was finally added over 20 min, by cannula, to another solution that had been prepared by treatment of (*S*)-**1** (1.186 g, 5.92 mmol) in THF (30 mL) at −80 °C with *s*BuLi (1.3 M in cyclohexane, 5.02 mL, 6.51 mmol, 1.1 equiv.) and had been allowed to react for 1 h. After 18 h at −80 °C, NEt_3 (9.86 mL, 7.17 g, 71.0 mmol, 12 equiv.) was added, followed after 3 h at −80 °C by phosphate buffer (pH = 7, 100 mL). After the mixture had warmed to room temperature, the organic phase was separated and the aqueous phase was extracted with Et_2O (4 × 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by CC (petroleum ether/ethyl acetate, 90:10; R_f = 0.27) to give **(*ent*)-2a/(*ent*)-2b** (884 mg, 59%) as a colorless oil. The diastereoselectivity was determined on the crude product by analytical HPLC (*n*-heptane/ethyl acetate, 95:5; 1.50 mL/min): *ds* **(*ent*)-2a/(*ent*)-2b =** 95:5. **(*ent*)-2a**: t_{Ret} = 12.78 min; **(*ent*)-2b**: t_{Ret} = 14.98 min. TLC: R_f = 0.31 (petroleum ether/ethyl acetate, 90:10). The ^{1}H NMR and IR data were in agreement with those described above for the pure isomers **2a** and **2b**.

(3*S*,6*R*)-6-*tert*-Butyl-3-[(*R*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (3a), (3*S*,6*R*)-6-*tert*-Butyl-3-[(*S*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (3b), (3*R*,6*R*)-6-*tert*-Butyl-3-[(*R*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (3c), and (3*R*,6*R*)-6-*tert*-Butyl-3-[(*S*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (3d): The synthesis was accomplished analogously to the preparation of **(*ent*)-3a−d**, by employment of OsO_4 (0.206 mmol) in H_2O (1.31 mL; 5 mol %), trimethylamine *N*-oxide (600 mg, 5.35 mmol, 1.3 equiv.), and **2a/2b** (95.5:4.5 mixture of diastereomers, 1.04 g, 4.10 mmol) in THF/H_2O (5:2, 39 mL). The crude product was purified by CC (petroleum ether/ethyl acetate, 10:90; R_f = 0.15) to give **3a−d** (897 mg, 76%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.15 (petroleum ether/ethyl acetate, 10:90). ^{1}H NMR, MS and IR as described for **(*ent*)-3a−d**. $C_{14}H_{25}NO_5$ (287.4): calcd. C 58.52, H 8.77, N 4.87; found C 58.06, H 8.88, N 4.71.

(3*R*,6*S*)-6-*tert*-Butyl-3-[(*S*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (*ent*)-3a, (3*R*,6*S*)-6-*tert*-Butyl-3-[(*R*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (*ent*)-3b, (3*S*,6*S*)-6-*tert*-Butyl-3-[(*R*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (*ent*)-3c, and (3*S*,6*S*)-6-*tert*-Butyl-3-[(*S*)-3,4-dihydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (*ent*)-3d: OsO_4 (0.168 mmol) in H_2O (1.03 mL; 5 mol %) and trimethylamine *N*-oxide (527 mg, 4.7 mmol, 1.4 equiv.) were added to **(*ent*)-2a/(*ent*)-2b** (95:5 mixture of diastereomers, 851 mg, 3.36 mmol) in THF/H_2O (5:2, 33 mL) and the mixture was stirred for 6 h at room temperature. Solid $Na_2S_2O_3$·5 H_2O (1 g) and phosphate buffer (pH = 7, 10 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (4 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by CC (ethyl acetate, R_f = 0.20) to give 800 mg (84%) of **(*ent*)-3a−d** as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.20 (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ = 1.02 (s, 0.8 × 9 H, C(CH_3)$_3$], 1.05 (s, 0.2 × 9 H, C(CH_3)$_3$], 1.51 (s, 0.4 × 3 H, CH_3), 1.52 (s, 0.4 × 3 H, CH_3), 1.53 (s, 0.1 × 3 H, CH_3),

1.54 (s, 0.1 × 3 H, CH_3), 1.57−2.02 (m, 3 H, CH_2), 2.22−2.50 (m, 1 H, CH_2), 3.45−3.54 (m, 1 H, C*H*OH or CH_2OH), 3.61−3.68 (m, 1 H, C*H*OH or CH_2OH), 3.73 (s, 0.1 × 3 H, OCH_3), 3.740 (s, 0.4 × 3 H, OCH_3), 3.748 (s, 0.1 × 3 H, OCH_3), 3.753 (s, 0.4 × 3 H, OCH_3), 3.70−3.85 (m, 1 H, C*H*OH or CH_2OH), 4.10 (dd, J = 8.5/3.3 Hz, 0.4 × 1 H, NC*H*), 4.15 (dd, J = 7.3/4.0 Hz, 0.4 × 1 H, NC*H*), 4.07−4.22 (m, 0.2 × 1 H, NC*H*). IR: $\tilde{\nu}$ = 3368 cm^{-1}, 2966, 1736, 1697, 1463, 1315, 1268, 1104. MS (CH_4, CI): m/z (%) = 288 (100) [M + H$^+$]. $C_{14}H_{25}NO_5$ (287.4): calcd. C 58.52, H 8.77, N 4.87; found C 58.38, H 8.59, N 4.69.

(3*S*,6*R*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (4a), (3*S*,6*R*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (4b), (3*R*,6*R*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (4c), and (3*R*,6*R*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (4d): DMF (600 μL), imidazole (468 mg, 6.85 mmol, 2.2 equiv.), DMAP (19 mg, 0.156 mmol), and ClSiMe$_2$*t*Bu (520 mg, 3.44 mmol, 1.1 equiv.) were added to **3a**−**d** (4:4:1:1 mixture of diastereomers, 891 mg, 3.10 mmol) in THF (31 mL). The reaction mixture was stirred for 20 h at room temperature before being hydrolyzed by addition of phosphate buffer (pH = 7, 4 mL). The mixture was concentrated in vacuo. CC (petroleum ether/ethyl acetate, 75:25; R_f = 0.25) yielded **4a**−**d** (1.09 g, 88%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.25 (petroleum ether/ethyl acetate, 75:25). ^{1}H NMR, MS and IR as described for **(*ent*)-4a**−**d**. $C_{20}H_{39}NO_5Si$ (401.6): calcd. C 59.81, H 9.79, N 3.49; found C 59.36, H 9.84, N 3.33.

(3*R*,6*S*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-4a], (3*R*,6*S*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-4b], (3*S*,6*S*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-4c], and (3*S*,6*S*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-hydroxybutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-4d]: DMF (270 μL), imidazole (397 mg, 5.83 mmol, 2.2 equiv.), DMAP (16 mg, 0.133 mmol), and ClSiMe$_2$*t*Bu (440 mg, 2.92 mmol, 1.1 equiv.) were added to **(*ent*)-3a**−**d** (4:4:1:1 mixture of diastereomers, 762 mg, 2.65 mmol) in THF (26 mL). The reaction mixture was stirred for 18 h at room temperature before being hydrolyzed with 10 mL of phosphate buffer (pH = 7). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (4 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by CC (petroleum ether/ethyl acetate, 70:30; R_f = 0.27) to give **(*ent*)-4a**−**d** (942 mg, 88%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.27 (petroleum ether/ethyl acetate, 70:30). ^{1}H NMR ($CDCl_3$): δ = 0.06 (s, 0.5 × 6 H, Si(CH_3)$_2$], 0.07 (s, 0.5 × 6 H, Si(CH_3)$_2$], 0.89 (s, 0.5 × 9 H, C(CH_3)$_3$], 0.90 (s, 0.5 × 9 H, C(CH_3)$_3$], 1.01 (s, 0.8 × 9 H, C(CH_3)$_3$], 1.04 (s, 0.2 × 9 H, C(CH_3)$_3$], 1.50 (s, 0.4 × 3 H, CH_3), 1.51 (s, 0.4 × 3 H, CH_3), 1.52 (s, 0.1 × 3 H, CH_3), 1.53 (s, 0.1 × 3 H, CH_3), 1.48−1.98 (m, 3.4 H, CH_2), 2.14−2.30 (m, 0.6 H, CH_2), 3.41−3.53 (m, 1 H, C*H*OH or CH_2OSi), 3.58−3.72 (m, 2 H, C*H*OH or CH_2OSi), 3.706 (s, 0.1 × 3 H, OCH_3), 3.712 (s, 0.1 × 3 H, OCH_3), 3.716 (s, 0.4 × 3 H, OCH_3), 3.720 (s, 0.4 × 3 H, OCH_3), 4.11 (dd, J = 7.8/4.2 Hz, 0.4 × 1 H, NC*H*), 4.17 (dd, J = 6.5/4.2 Hz, 0.4 × 1 H, NC*H*), 4.09−4.21 (m, 0.2 × 1 H, NC*H*). IR: $\tilde{\nu}$ = 3469 cm^{-1}, 2955, 2930, 2858, 1746, 1695, 1463, 1314, 1252, 1103, 838, 778. MS (CH_4, CI): m/z (%) = 402 (100) [M + H$^+$], 344 (48). $C_{20}H_{39}NO_5Si$ (401.6): calcd. C 59.81, H 9.79, N 3.49; found C 59.40, H 9.40, N 3.50.

(3*S*,6*R*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (5a), (3*S*,6*R*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (5b), (3*R*,6*R*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (5c), and (3*R*,6*R*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (5d): Imidazole (407 mg, 5.95 mmol, 2.2 equiv.), PPh_3 (782 mg, 2.98 mmol, 1.1 equiv.), and I_2 (792 mg, 3.12 mmol, 1.15 equiv.) were added at 0 °C to **4a**−**d** (4:4:1:1 mixture of diastereomers, 1.09 g, 2.71 mmol) in THF (27 mL). The reaction mixture was stirred for 1.5 h at 0 °C and for 1.5 h at room temperature, after which solid $Na_2S_2O_3$·5 H_2O (1 g) and phosphate buffer (pH = 7, 25 mL) were added. The workup was performed as described for **(*ent*)-5a**−**d**. CC (petroleum ether/ethyl acetate, 95:5; R_f = 0.32) yielded **5a**−**d** (1.25 g, 90%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.32 (petroleum ether/ethyl acetate; 95:5). ^{1}H NMR, MS and IR as described for **(*ent*)-5a**−**d**. $C_{20}H_{38}NO_4SiI$ (511.5): calcd. C 46.96, H 7.49, N 2.74; found C 47.22, H 7.49, N 2.27.

(3*R*,6*S*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-5a], (3*R*,6*S*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-5b], (3*S*,6*S*)-6-*tert*-Butyl-3-[(*R*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-5c], and (3*S*,6*S*)-6-*tert*-Butyl-3-[(*S*)-4-(*tert*-butyldimethylsilanyloxy)-3-iodobutyl]-5-methoxy-6-methyl-3,6-dihydro-2*H*-1,4-oxazin-2-one [(*ent*)-5d]: Imidazole (345 mg, 5.07 mmol, 2.2 equiv.), PPh_3 (663 mg, 2.53 mmol, 1.1 equiv.), and I_2 (677 mg, 2.67 mmol, 1.15 equiv.) were added at 0 °C to **(*ent*)-4a**−**d** (4:4:1:1 mixture of diastereomers, 929 mg, 2.31 mmol) in THF (23 mL), and the mixture was stirred for 1.5 h at 0 °C. The reaction mixture was then stirred for 1.5 h at room temperature, after which solid $Na_2S_2O_3$·5H_2O (1 g) and phosphate buffer (pH = 7, 5 mL) were added. The organic layer was separated and the aqueous layer was extracted with Et_2O (4 × 30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by CC (petroleum ether/ethyl acetate, 90:10; R_f = 0.62) to give **(*ent*)-5a**−**d** (1.067 g, 90%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 4:4:1:1, stereochemistry not assigned). TLC: R_f = 0.62 (petroleum ether/ethyl acetate, 90:10). ^{1}H NMR ($CDCl_3$): δ = 0.06−0.09 [m, 6 H, Si(CH_3)$_2$], 0.90 (s, 0.8 × 9 H, C(CH_3)$_3$], 0.91 (s, 0.2 × 9 H, C(CH_3)$_3$], 1.01 (s, 0.8 × 9 H, C(CH_3)$_3$], 1.04 (s, 0.2 × 9 H, C(CH_3)$_3$], 1.50 (s, 0.8 × 3 H, CH_3), 1.54 (s, 0.2 × 3 H, CH_3), 1.60−2.20 (m, 3.6 H, CH_2), 2.24−2.40 (m, 0.4 H, CH_2), 3.40−3.63 (m, 1 H, C*H*I or CH_2OSi), 3.68−3.80 (m, 1 H, C*H*I or CH_2OSi), 3.69 (s, 0.1 × 3 H, OCH_3), 3.70 (s, 0.1 × 3 H, OCH_3), 3.71 (s, 0.4 × 3 H, OCH_3), 3.72 (s, 0.4 × 3 H, OCH_3), 3.85−3.93 (m, 1 H, C*H*I or CH_2OSi), 4.05−4.28 (m, 1 H, NC*H*). IR: $\tilde{\nu}$ = 2955 cm^{-1}, 2930, 2857, 1746, 1694, 1462, 1312, 1253, 1104, 838, 778. MS (CH_4, CI): m/z (%) = 512 (22) [M + H$^+$], 380 (100). $C_{20}H_{38}NO_4SiI$ (511.5): calcd. C 46.96, H 7.49, N 2.74; found C 47.30, H 7.41, N 2.28.

(1*S*,4*S*,7*R*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (6a), (1*R*,4*S*,7*R*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (6b), (1*R*,4*R*,7*R*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (6c), and (1*S*,4*R*,7*R*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (6d): *t*Bu-P_4 (1.0 M in *n*-hexane, 2.68 mL, 2.68 mmol, 1.1 equiv.) was added at −80 °C to **5a−d** (4:4:1:1 mixture of diastereomers, 1.25 g, 2.44 mmol) in DME/THF (5:1, 59 mL). The reaction mixture was stirred for 2 h at −80 °C before being worked up as described for **(*ent*)-6a−d**. CC (petroleum ether/ethyl acetate, 90:10; R_f = 0.45) yielded **6a−d** (674 mg, 72%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 45:35:15:5, stereochemistry not assigned). TLC: R_f = 0.38 (petroleum ether/ethyl acetate, 95:5). ^{1}H NMR, MS and IR as described for **(*ent*)-6a−d**. $C_{20}H_{37}NO_4Si$ (383.6): calcd. C 62.62, H 9.72, N 3.65; found C 62.88, H 9.59, N 3.63.

(1*R*,4*R*,7*S*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-6a], (1*S*,4*R*,7*S*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-6b], (1*S*,4*S*,7*S*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-6c], and (1*R*,4*S*,7*S*)-7-*tert*-Butyl-1-(*tert*-butyldimethylsilanyloxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-6d]: *t*Bu-P_4 (1.0 M in *n*-hexane, 2.72 mL, 2.72 mmol, 1.1 equiv.) was added at −80 °C to **(*ent*)-5a−d** (4:4:1:1 mixture of diastereomers, 1.262 g, 2.47 mmol) in DME/THF (5:1, 59 mL), and the mixture was stirred at −80 °C for 2.5 h. Phosphate buffer (pH = 7, 20 mL) was then added. The organic layer was separated and the aqueous layer was extracted with Et_2O (4 × 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by CC (petroleum ether/ethyl acetate, 90:10; R_f = 0.45) to give **(*ent*)-6a−d** (813 mg, 86%) as a colorless oil (ratio of isomers determined by ^{1}H NMR: ≈ 45:35:15:5, stereochemistry not assigned). TLC: R_f = 0.38 (petroleum ether/ethyl acetate, 95:5). ^{1}H NMR ($CDCl_3$): δ = −0.03−0.03 [m, 6 H, Si(C*H*$_3$)$_2$], 0.83 (s, 0.35 × 9 H, C(C*H*$_3$)$_3$], 0.84 (s, 0.45 × 9 H, C(C*H*$_3$)$_3$], 0.85 (s, 0.05 × 9 H, C(C*H*$_3$)$_3$], 0.86 (s, 0.15 × 9 H, C(C*H*$_3$)$_3$], 0.96 (s, 0.8 × 9 H, C(C*H*$_3$)$_3$], 1.00 (s, 0.05 × 9 H, C(C*H*$_3$)$_3$], 1.01 (s, 0.15 × 9 H, C(C*H*$_3$)$_3$], 1.45 (s, 0.15 × 3 H, C*H*$_3$), 1.46 (s, 0.05 × 3 H, C*H*$_3$), 1.47 (s, 0.45 × 3 H, C*H*$_3$), 1.48 (s, 0.35 × 3 H, C*H*$_3$), 1.74−2.09 (m, 2.6 × 1 H), 2.12−2.31 (m, 0.8 × 1 H), 2.45−2.53 (m, 0.4 × 1 H), 2.60−2.73 (m, 0.6 × 1 H), 2.81−2.92 (m, 0.4 × 1 H), 3.06−3.24 (m, 0.2 × 1 H), 3.46−3.52 (m, 0.35 × 1 H, C*H*$_2$OSi), 3.57−3.69 (m, 0.50 × 1 H, C*H*$_2$OSi), 3.70 (s, 0.45 × 3 H, OC*H*$_3$), 3.72 (s, 0.15 × 3 H, OC*H*$_3$), 3.73 (s, 0.05 × 3 H, OC*H*$_3$), 3.76 (s, 0.35 × 3 H, OC*H*$_3$), 3.69−3.85 (m, 0.95 × 1 H, C*H*$_2$OSi), 3.88−3.94 (m, 0.20 × 1 H, C*H*$_2$OSi). IR: $\tilde{\nu}$ = 2950 cm^{-1}, 2857, 1738, 1732, 1694, 1471, 1463, 1315, 1255, 1106, 832, 777. MS (CH_4, CI): *m*/*z* (%) = 384 (100) [M + H$^+$]. $C_{20}H_{37}NO_4Si$ (383.6): calcd. C 62.62, H 9.72, N 3.65; found C 62.79, H 9.57, N 3.62.

(1*S*,4*S*,7*R*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (7a), (1*R*,4*S*,7*R*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (7b), (1*R*,4*R*,7*R*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (7c), and (1*S*,4*R*,7*R*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one (7d): Compounds **6a−d** (45:35:15:5 mixture of diastereomers, 634 mg, 1.64 mmol) were treated with $Bu_4N^+F^-$ (1.0 M in THF, 2.80 mL, 2.80 mmol, 1.7 equiv.). The reaction mixture was stirred for 2.5 h at room temperature. Phosphate buffer (pH = 7, 2 mL) was then added and the solvent was removed in vacuo. The residue was purified by CC (petroleum ether/ethyl acetate, 60:40; R_f = 0.22−0.30) to give **7a−d** (369 mg, 83%) as a mixture of diastereomers, which was separated by prep. HPLC (*n*-heptane/ethyl acetate, 70:30; 15 mL/min; *ds* = 48 (**7a**): 31 (**7b**): 18 (**7c**): 3 (**7d**); **7a**: t_R = 34.81 min; **7b**: t_R = 28.80 min; **7c**: t_R = 22.09 min; **7d**: t_R = 21.46 min). Thus 173 mg (39%) of **7a**, 111 mg (25%) of **7b**, 64 mg (14.5%) of **7c** and 10 mg (2.5%) of **7d** were obtained [total yield: 358 mg (81%)].

Compound 7a: Colorless crystals, m.p. 121−122 °C. $[\alpha]_D^{20}$ = −8.7 (*c* = 0.71, $CHCl_3$). ^{1}H NMR, MS and IR as described for **(*ent*)-7a**. $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.30, H 8.60, N 5.12.

Compound 7b: Colorless oil. $[\alpha]_D^{20}$ = −56.1 (*c* = 0.89, $CHCl_3$). ^{1}H NMR, MS and IR as described for **(*ent*)-7b.** $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.10, H 9.05, N 5.10.

Compound 7c: Colorless crystals, m.p. 81−83 °C. $[\alpha]_D^{20}$ = −70.8 (*c* = 0.59, $CHCl_3$). ^{1}H NMR, MS and IR as described for **(*ent*)-7c.** $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.49, H 8.61, N 5.03.

Compound 7d: Colorless crystals, m.p. 48 °C. $[\alpha]_D^{20}$ = −8.4 (*c* = 1.01, $CHCl_3$). ^{1}H NMR, MS and IR as described for **(*ent*)-7d.** $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.66, H 8.82, N 5.02.

(1*R*,4*R*,7*S*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-7a], (1*S*,4*R*,7*S*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-7b], (1*S*,4*S*,7*S*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-7c], and (1*R*,4*S*,7*S*)-7-*tert*-Butyl-1-(hydroxymethyl)-6-methoxy-7-methyl-8-oxa-5-azaspiro[3.5]non-5-en-9-one [(*ent*)-7d]: Compounds **(*ent*)-6a−d** (45:35:15:5 mixture of diastereomers, 747 mg, 1.95 mmol) were treated with $Bu_4N^+F^-$ (1.0 M in THF, 2.95 mL, 2.95 mmol, 1.5 equiv.). The mixture was stirred for 4 h at room temperature, and phosphate buffer (pH = 7, 3.5 mL) was then added. The solvent was removed in vacuo and the residue was purified by CC (petroleum ether/ethyl acetate, 60:40; R_f = 0.22−0.30) to give **(*ent*)-7a−d** (492 mg, 93%) as a mixture of diastereomers, which was separated by prep. HPLC (*n*-heptane/ethyl acetate, 70:30; 15 mL/min; *ds* = 48[**(*ent*)-7a**]:31[**(*ent*)-7b**]:18[**(*ent*)-7c**]:3[**(*ent*)-7d**]; **(*ent*)-7a**: t_{Ret} = 36.46 min; **(*ent*)-7b**: t_{Ret} = 29.86 min; **(*ent*)-7c**: t_{Ret} = 22.74 min; **(*ent*)-7d**: t_{Ret} = 21.89 min). Thus 233 mg (44.5%) of **(*ent*)-7a**, 152 mg (29%) of **(*ent*)-7b**, 85 mg (16%) of **(*ent*)-7c** and 13 mg (2.5%) of **(*ent*)-7d** were obtained [total yield: 483 mg (92%)].

Compound (*ent*)-7a: Colorless crystals, m.p. 118−120 °C. $[\alpha]_D^{20}$ = +3.5 (*c* = 0.20, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.97 [s, 9 H, C(C*H*$_3$)$_3$], 1.49 (s, 3 H, C*H*$_3$), 1.83 (br. s, 1 H, O*H*), 1.95−2.05 (m, 1 H, CHC*H*$_2$CH$_2$), 2.09−2.19 (m, 2 H, CHC*H*$_2$C*H*$_2$), 2.54−2.62 (m, 1 H, CHCH$_2$C*H*$_2$), 2.81−2.89 (m, 1 H, C*H*), 3.71−3.76 (m, 1 H, C*H*$_2$OH), 3.72 (s, 3 H, OC*H*$_3$), 3.78−3.85 (m, 1 H, C*H*$_2$OH). IR: $\tilde{\nu}$ = 3410 cm^{-1}, 2946, 1727, 1692, 1326, 1106. MS (CH_4, CI): *m*/*z* (%) = 270 (100) [M + H$^+$]. $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.72, H 8.54, N 5.06.

Compound (*ent*)-7b: Colorless oil. $[\alpha]_D^{20}$ = +54.4 (*c* = 1.70, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.97 [s, 9 H, C(C*H*$_3$)$_3$], 1.52 (s, 3 H, C*H*$_3$), 2.07−2.32 (m, 2 H, CHC*H*$_2$CH$_2$; 1 H, CHCH$_2$C*H*$_2$), 2.64−2.72

(m, 1 H, $CHCH_2CH_2$), 3.08−3.15 (m, 1 H, C*H*), 3.47 (br. s, 1 H, O*H*), 3.67 (dd, *J* = 12.0/5.3 Hz, 1 H, CH_2OH), 3.77 (s, 3 H, OCH_3), 3.78 (dd, *J* = 12.0/2.8 Hz, 1 H, CH_2OH). IR: $\tilde{\nu}$ = 3438 cm^{-1}, 2947, 1734, 1697, 1331, 1220, 1105. MS (CH_4, CI): *m*/*z* (%) = 270 (100) [M + H^+]. $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.56, H 8.84, N 5.11.

Compound (*ent*)-7c: Colorless crystals, m.p. 79−80 °C. $[\alpha]_D^{20}$ = +68.4 (*c* = 0.27, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 1.00 [s, 9 H, $C(CH_3)_3$], 1.49 (s, 3 H, CH_3), 1.98−2.09 (m, 2 H, $CHCH_2CH_2$), 2.33−2.42 (m, 1 H, $CHCH_2CH_2$), 2.58−2.65 (m, 1 H, C*H*), 2.67−2.76 (m, 1 H, $CHCH_2CH_2$), 3.74 (s, 3 H, OCH_3), 3.80−3.85 (m, 2 H, CH_2OH), OH not detectable. IR: $\tilde{\nu}$ = 3473 cm^{-1}, 2949, 2859, 1707, 1332, 1104. MS (CH_4, CI): *m*/*z* (%) = 270 (100) [M + H^+]. $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.70, H 8.79, N 5.00.

Compound (*ent*)-7d: Colorless crystals, m.p. 50−52 °C. $[\alpha]_D^{20}$ = +8.5 (*c* = 1.34, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 1.02 [s, 9 H, $C(CH_3)_3$], 1.50 (s, 3 H, CH_3), 2.17−2.34 (m, 3 H, $CHCH_2CH_2$), 2.61−2.68 (m, 1 H, $CHCH_2CH_2$), 3.00−3.06 (m, 1 H, C*H*), 3.47 (br. s, 1 H, O*H*), 3.66 (dd, *J* = 12.3/4.2 Hz, 1 H, CH_2OH), 3.76 (s, 3 H, OCH_3), 3.83 (dd, *J* = 12.3/2.8 Hz, 1 H, CH_2OH). IR: $\tilde{\nu}$ = 3457 cm^{-1}, 2961, 2850, 1716, 1694, 1336, 1100. MS (CH_4, CI): *m*/*z* (%) = 270 (100) [M + H^+]. $C_{14}H_{23}NO_4$ (269.3): calcd. C 62.43, H 8.61, N 5.20; found C 62.82, H 8.53, N 5.05.

General Procedure (GP) for the Preparation of the Free Amino Acids by Hydrolysis of 7a, 7b, (*ent*)-7a, and (*ent*)-7b: TFA (5 equiv.) was added to a 0.1 M solution of the respective starting material (**7a**, **7b**, **(*ent*)-7a**, or **(*ent*)-7b**) in H_2O/MeCN (9:1) and the reaction mixture was stirred for 48 h at 60 °C. The solvent was then removed in vacuo and the residue was treated with methanolic NaOH solution (0.2 M, 6 equiv.) and stirred at 60 °C for 24 h. The solvent was removed in vacuo, H_2O was added, and the alkaline solution was washed with Et_2O (2 × 5 mL), adjusted to pH 2 by addition of 2 N HCl, and again washed with Et_2O (2 × 5 mL). Ion-exchange chromatography of the aqueous phase with a DOWEX 50Wx8 resin and removal of the solvent yielded the free amino acid as colorless crystals.

(1*S*,2*S*)-1-Amino-2-(hydroxymethyl)cyclobutanecarboxylic Acid (8a): This compound was prepared by the **GP**, from **7a** (61 mg, 0.226 mmol). Yield: 24 mg (**8a**·0.25 H_2O, 71%). Colorless crystals, m.p. 179−180 °C (dec.). $[\alpha]_D^{20}$ = +50.8 (*c* = 1.05, H_2O). ^1H NMR, MS and IR as described for **(*ent*)-8a**. $C_6H_{11}NO_3$ (145.2); calcd. for $C_6H_{11}NO_3$·0.25 H_2O (149.7): calcd. C 48.15, H 7.74, N 9.36; found C 48.30, H 7.49, N 9.72.

(1*S*,2*R*)-1-Amino-2-(hydroxymethyl)cyclobutanecarboxylic Acid (8b): This compound was prepared by the **GP**, from **7b** (51 mg, 0.189 mmol). Yield: 21.5 mg (**8b**·0.25 H_2O, 76%). Colorless crystals, m.p. 186−188 °C (dec.). $[\alpha]_D^{20}$ = −36.0 (*c* = 0.41, H_2O). ^1H NMR, MS and IR as described for **(*ent*)-8b**. $C_6H_{11}NO_3$ (145.2); calcd. for $C_6H_{11}NO_3$·0.25 H_2O (149.7): calcd. C 48.15, H 7.74, N 9.36; found C 48.04, H 7.57, N 9.70.

(1*R*,2*R*)-1-Amino-2-(hydroxymethyl)cyclobutanecarboxylic Acid [(*ent*)-8a]: This compound was prepared by the **GP**, from **(*ent*)-7a** (61.2 mg, 0.227 mmol). Yield: 26 mg [**(*ent*)-8a**·0.25 H_2O, 76%]. Colorless crystals, m.p. 176−177 °C (dec.). $[\alpha]_D^{20}$ = −52.1 (*c* = 0.53, H_2O). ^1H NMR (D_2O): δ = 1.69−1.79 (m, 1 H, $CHCH_2$), 1.94−2.12 (m, 2 H, $CHCH_2CH_2$), 2.30−2.38 (m, 1 H, $CHCH_2CH_2$), 2.65−2.74 (m, 1 H, C*H*), 3.45 (dd, *J* = 11.2/7.5 Hz, 1 H, CH_2OH), 3.55 (dd, *J* = 11.2/7.2 Hz, 1 H, CH_2OH). IR: $\tilde{\nu}$ = 3415 cm^{-1}, 2999, 2951, 1601, 1401, 1278, 1031. MS (CH_4, CI): *m*/*z* (%) = 146 (100) [M + H^+], 128 (14). $C_6H_{11}NO_3$ (145.2); calcd. for $C_6H_{11}NO_3$·0.25 H_2O (149.7): calcd. C 48.15, H 7.74, N 9.36; found C 48.20, H 7.38, N 9.70.

(1*R*,2*S*)-1-Amino-2-(hydroxymethyl)cyclobutanecarboxylic Acid [(*ent*)-8b]: This compound was prepared by the **GP**, from **(*ent*)-7b** (61 mg, 0.226 mmol). Yield: 26 mg [**(*ent*)-8b**·0.25 H_2O, 77%]. Colorless crystals, m.p. 185−187 °C (dec.). $[\alpha]_D^{20}$ = +34.3 (*c* = 0.47, H_2O). ^1H NMR (D_2O): δ = 1.77−1.86 (m, 1 H, $CHCH_2$), 2.00−2.09 (m, 2 H, $CHCH_2CH_2$), 2.38−2.45 (m, 1 H, $CHCH_2CH_2$), 2.85−2.93 (m, 1 H, C*H*), 3.62 (dd, *J* = 12.0/7.0 Hz, 1 H, CH_2OH), 3.66 (dd, *J* = 12.0/5.5 Hz, 1 H, CH_2OH). IR: $\tilde{\nu}$ = 3241 cm^{-1}, 3067, 2946, 2924, 2849, 1594, 1403, 1258, 1072, 610. MS (CH_4, CI): *m*/*z* (%) = 146 (100) [M + H^+], 128 (15). $C_6H_{11}NO_3$ (145.2); calcd. for $C_6H_{11}NO_3$·0.25 H_2O (149.7): calcd. C 48.15, H 7.74, N 9.36; found C 48.15, H 7.56, N 9.60.

5.3 Radioreceptor Assays

[^{3} H] *MDL 105,519* binding to pig cortical brain membranes was performed as described previously.[18] Modulation of [^{3}H]*MK-801* binding to pig cortical brain membranes in the presence of L-glutamate (100 μM) under non-equilibrium conditions was studied as described previously.[20] K_i values for test compounds were calculated from competition experiments with at least six concentrations of test compounds, by the use of Prism 2.01 (GraphPad Software, San Diego, CA). If not stated otherwise, data are expressed as means ± standard error of the means (SEM) of three experiments, each carried out in triplicate.

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