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Optically pure β-substituted β-hydroxy aspartates as glutamate transporter blockers

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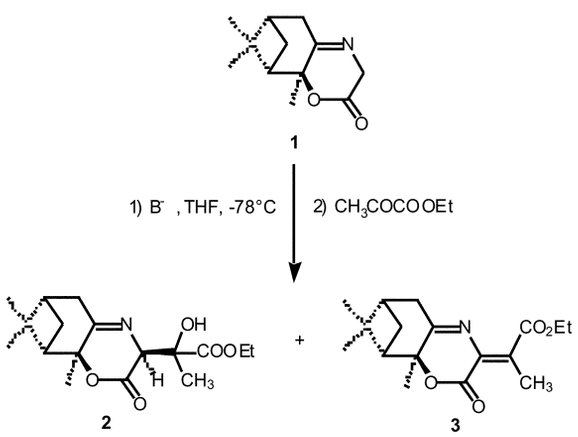
A short asymmetric synthesis of optically pure β-substituted β-hydroxy aspartates is described. The key step is an aldol reaction between a glycine enolate derived from an oxazinone intermediate used as chiral auxiliary and various α-keto esters. Excellent diastereomeric excesses are obtained.

Introduction

As the most predominant excitatory neurotransmitter,[1] glutamate has the potential to influence the function of most neuronal circuits in the central nervous system. To limit receptor activation and prevent overstimulation of glutamate receptors that can trigger excitotoxic mechanisms and cell death, extracellular concentrations of excitatory amino acids are tightly controlled by transport systems[2] on both neurons and glial cells. Five subtypes of excitatory amino acid transporters (EAATs) EAAT1–5 have been cloned from mammalian tissues. Imbalance of this amino acid has been implicated in central nervous system (CNS) disorders. A number of pharmacological agents[3] have been shown to inhibit glutamate transport but most of them act as competitive substrates. Therefore non transportable blockers are indispensable tools for the investigation of the physiological roles of glutamate transporters. It was reported that some derivatives of DL-*threo* β-hydroxyaspartate[4] acted as blockers; DL-*threo*-β-benzyloxyaspartate (DL-TBOA) was the most potent blocker for the human excitatory amino acid transporters, EAAT1 and EAAT2. To examine the precise interaction between a blocker and transporters, the same group[5] synthesized the optically pure isomers and they found that L-TBOA was the most potent blocker for the human (EAAT1–3), while D-TBOA revealed a difference in the pharmacophores between EAAT1 and EAAT3.

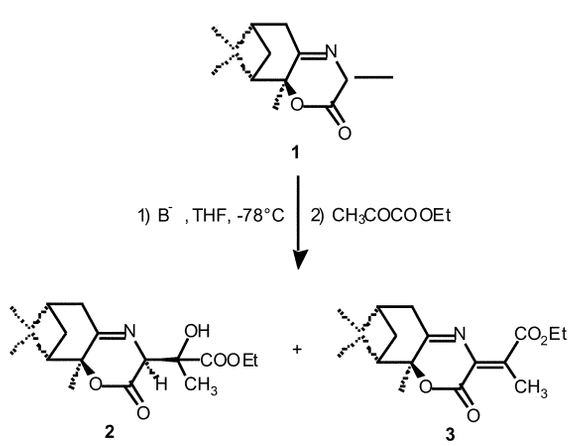
Results and discussion

We describe here the synthesis and characterization of new analogues of hydroxy aspartate, the optically pure β-substituted β-hydroxy aspartic acid derivatives. In this synthesis the key step is the reaction between glycine enolates and various activated ketones. The majority of strategies developed in stereoselective aldol methodology starting from glycine α-anion equivalents are suitable for aldehydes[6] but only a few reports concern prochiral ketones.[7] In 1989 Hayashi *et al.* described the asymmetric aldol reaction of α-keto esters with isocyanoacetate and isocyanoacetamide catalysed by a chiral ferrocenyl–phosphine–gold(I) complex. They obtained β-alkyl β-hydroxy aspartic derivatives *via* oxazolines of up to 90% ee.[8] In 1994 Sardina *et al.* realized the chirospecific and regioselective alkylation and hydroxylation of *N*-Pf-aspartates, using the 9-phenylfluoren-9-yl (Pf) group for protection of the amino group but they obtained only modest stereoselectivity.[9] A few years ago, we reported[10] that the potassium enolate obtained from the oxazinone **1** using t-BuOK as base reacted with aldehydes to yield directly the didehydro compound in good to excellent yields. We selected ethyl pyruvate (Scheme 1) to study the influence of the base on this reaction. Results are gathered in Table 1. Base (1.1 eq) treatment of the *S*-oxazinone **1** [easily prepared from (1*S*,2*S*,5*S*)-2-hydroxypinan-3-one and *Z*-glycine] at −78 °C in anhydrous THF under argon followed by addition of the electrophile (1.1 eq), the temperature being



Scheme 1

Table 1 Oxazinone aldol reaction: influence of the nature of the base



	Yield (%)		
Base	**2a**	**3a**	Starting material
t-BuOK	28–34	5–8	23–30
P4-t-Bu	16–18	6–10	15–33
BuLi	22.5	5–6	43
KHMDS	72		

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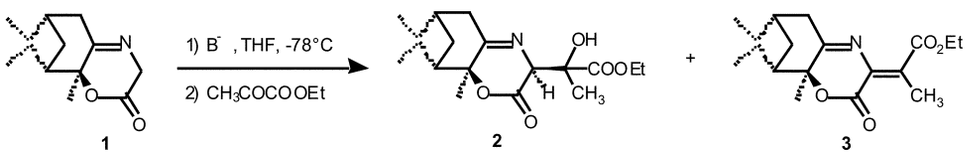
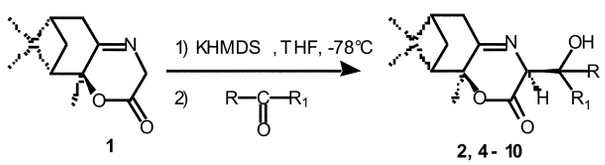
Table 2 Oxazinone aldol reaction using KHMDS as base: experimental conditions



α-Keto ester			KHMDS			Oxazinone		
R	Vol/cm^3	mmol	Vol/cm^3	mmol	Vol THF/cm^3	Quant/g	mmol	Reaction time/min
Me	0.4	3.73	7.4	3.7	17.5	0.7	0.7	55
iPr	0.38	3.72	5.3	2.6	12.5	0.5	0.5	40
$Ph(CH_2)_2$	0.7	3.72	7.4	3.7	17.5	0.7	0.7	40

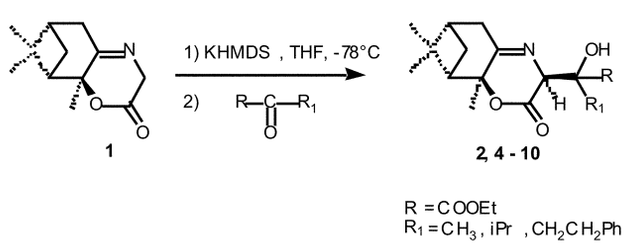
Table 3 Oxazinone aldol reaction: results



Entry	Compound	R	R_1	Yield (%)	Diastereoisomeric ratio **a** : **b**
1	**2**	CH_3	CO_2Et	72	70 : 30
2	**4**	CH_3	CO_2Me	54	55 : 45
3	**5**	CF_3	CO_2Me	74	52 : 48
4	**6**	iPr	CO_2Et	81	93 : 7
5	**7**	C_6H_5	CO_2Et	74	60 : 40
6	**8**	$PhCH_2CH_2$	CO_2Et	88	56 : 44
7	**9**	CH_3	COMe	51	60 : 40
8	**10**	$BrCH_2$	CO_2Et	52	69 : 31

maintained at −78 °C, afforded a mixture of the alcohol **2** and the didehydro compound **3**.

Using potassium hexamethyldisilazane only the alcohol **2** was obtained in 72% yield with a diastereoisomeric ratio (70 : 30) determined by ^{1}H NMR (Scheme 2, Table 2).



Scheme 2

Using potassium hexamethyldisilazane and the same experimental conditions, the reaction was studied with other electrophiles (Scheme 2, Table 3). In all cases only two diastereoisomers were detected (possibility of four isomers), showing that the electrophile approaches the enolate on the face opposite to the *gem* dimethyl group as expected.

With α-keto esters the yields were good to excellent. The nature of the ester has also an influence on the yield and on the diastereoisomeric ratio (see entries 1 and 2). The ethyl ester derivatives yielded the best results. When we used an electrophile containing CF_3 as an electron withdrawing group the yield was increased (compare entries 2 and 3).

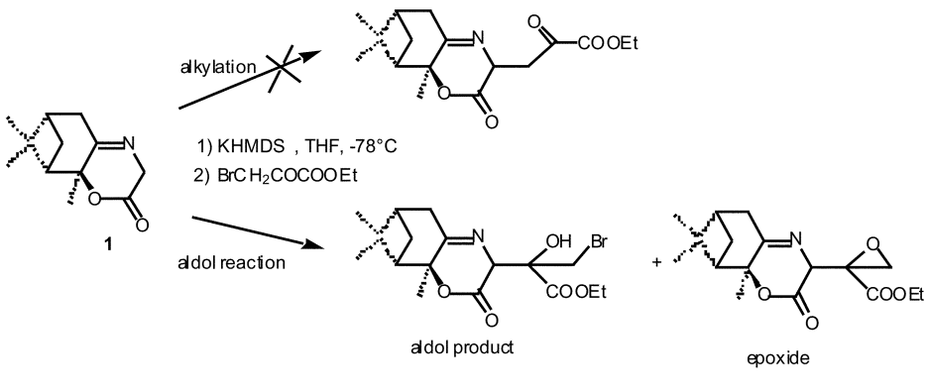
The reaction with butane-2,3-dione (entry 7) as electrophile afforded the corresponding alcohol **9** in 51% yield with a diastereoisomeric ratio of 60 : 40; in this case the two diastereomers were separated either by recrystallization or preparative HPLC. The major isomer **9a** crystallized and the X-ray study[11] allowed assignment of the configurations on the two carbons (2*S*,3*R*). The two diastereomers of **6** arising from the reaction with ethyl 3-methyl-2-oxobutyrate (entry 4) were also separated and the crystallized major isomer **6a** was suitable for X-ray study[12] which permitted assignment of the 2*S*,3*S* configuration.

It is well known that configuration and selectivity of the kinetically controlled aldol addition is dependent on the size of the substituents of the two reactants. The 2*S* configuration established for the major isomers of **6** and **9** confirmed that the electrophile approaches the *Re* face of the enolate (opposite to the *gem* dimethyl group). For carbon 3, the configuration depends on the electrophile; with butane-2,3-dione where unlike and like closed transition structures were equally possible, the 2*S*,3*R* major isomer arose from unlike approach (*Re*, *Si*). With ethyl 3-methyl-2-oxobutyrate the like approach (*Re*, *Re*) was favored due probably to the steric hindrance of the isopropyl group and afforded the 2*S*,3*S* major isomer.

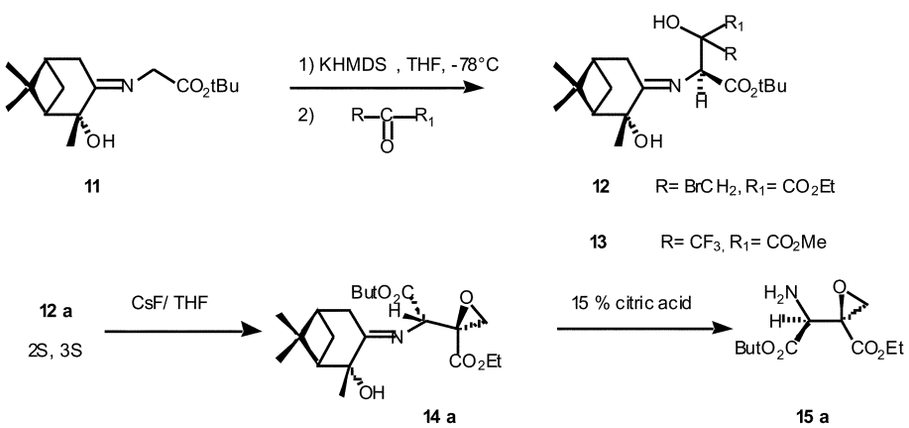
Regioselectivity of the reaction was shown using as electrophile ethyl bromopyruvate (entry 8). With ethyl bromopyruvate two reactions were in competition, alkylation and aldol reaction because of the strong electrophilic character of the ketone; no side product arising from the alkylation reaction was detected. On the other hand we observed intramolecular cyclisation giving unstable epoxide product in 25% yield (Scheme 3).

With this last electrophile the bad reproducibility of the reaction, ascribed to the instability of the adducts, prompted us to study the reaction starting from the enolate of the Schiff base **11** prepared from (1*R*,2*R*,5*R*) 2-hydroxypinan-3-one and *tert*-butyl glycinate (Scheme 4).

In this case also, among the different bases tested (LDA, LHMDS, KHMDS) KHMDS gave the best results and the reaction between the potassium Schiff base enolate and ethyl bromopyruvate afforded only the β-hydroxy compound **12** in 74% yield as a mixture of two diastereomers (dr = 88 : 12) which



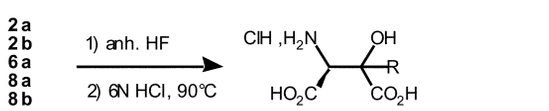
Scheme 3



Scheme 4

were separated by silica gel column chromatography. An X-ray study[13] of the crystallized major isomer **12a** permitted the assignment of the 2*S*,3*S* configuration. A similar reaction starting from methyl trifluoromethylpyruvate yielded **13** in 84% yield as a mixture of two diastereomers (dr = 59 : 41) which could not be separated by silica gel column chromatography or HPLC. **12a** was easily transformed into the epoxide **14a** by action of CsF in THF–CH_3CN (1 : 1) in 76% yield. After cleavage of the chiral auxiliary using 15% citric acid the amino ester **15a** was obtained in 50% yield. Epoxides are versatile intermediates[14] in organic synthesis and a large variety of reagents are known for the ring opening of these compounds; **14a** and (or) **15a** represent interesting precursors for the synthesis of diversely β-substituted β-hydroxy aspartic acid derivatives.

The next step was the cleavage of the oxazinone. It was performed on five compounds **2a**, **2b**, **6a**, **8a**, and **8b** (Scheme 5) selected to appreciate the influence on biological activity of the substituent and of chirality at carbon 3.



16 a, 16 b R= CH_3 (2S, 3R and 2S, 3S)
17 a R= iPr (2S, 3S)
18 a, 18 b R= CH_2CH_2Ph (2S, 3R and 2S, 3S)

Scheme 5

Treatment of **2a**, **2b**, **6a**, **8a** and **8b** with anhydrous HF at 0 °C in the presence of anisole provided the hydrofluoride salts in 71% to 90% yields. The synthesis was completed in a final step with the hydrolysis of the ethyl ester using 6 M HCl. **16a**, **16b**, **17a**, **18a** and **18b** were isolated as white powders in 72 to 90% yields. Their optical purities were proved by HPLC analysis and NMR spectra. These five compounds are under investigation in the laboratory of Cerebral Plasticity of our University. The first preliminary results showed that **16a** (R = CH_3, 2*S*,3*R* configuration) was a selective transporter blocker without any effect on glutamate receptors; other compounds were found to be inactive.

In summary we have prepared enantiomerically pure β-hydroxy β-substituted aspartic acid derivatives in four steps and in good overall yields; this strategy is general and can be applied to the synthesis of functionalized β substituted aspartic acid derivatives.

Concerning the biological activity, crowded substituents in the β position should be avoided and the configuration at position 3 of the carbon backbone seems to be critical.

Experimental

Melting points were obtained using a Büchi 510 capillary apparatus and are uncorrected.

Infrared spectra were recorded using a Perkin-Elmer Fourier transform spectrometer. ^{1}H NMR spectra were recorded at 250 MHz using a Brücker AC250 instrument. For ^{1}H NMR spectra recorded in $CDCl_3$ chemical shifts are quoted in parts per million and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are reported in Hertz (Hz). Diastereoisomeric ratio (dr) was determined by ^{1}H NMR on the crude product. Low resolution mass spectra were recorded on a micromass electrospray instrument with only the molecular ion and other major peaks being reported. Flash chromatography was carried out using E-Merck silica gel (Kieselgel 60, 230–400 mesh) as stationary phase. Thin layer chromatography was carried out on aluminium plates pre-coated with Merck silica gel 60F254 which were visualized by quenching of UV fluorescence or by staining with a 10% methanol phosphomolybdic acid solution followed by heating. Preparative HPLC was performed on a Waters delta 4000

apparatus equipped with a Delta-Pack C18 column (15 μm, 40 × 100 mm, porosity: 100×10^{-10} m), a delta-Pack pre-column (40 × 10 mm) and a UV detector, using a linear gradient of CH_3CN in H_2O with 0.1% TFA from 0 to 100% in 15 minutes. THF was distilled from sodium–benzophenone ketyl. Reagents were supplied from commercial sources (Aldrich, Fluka). The oxazinone **1** and the Schiff base **11** were prepared as previously described.[8]

General procedure for reactions of the oxazinone with carbonyl compounds

The oxazinone (0.2 g, 0.97 mmol) was added under argon, at −78 °C to a stirred solution of potassium bis(trimethylsilyl)amide (KHMDS), 0.5 M in toluene (2.12 cm^3, 1.06 mmol) in dry THF (5 cm^3). The mixture was stirred for 30 min at −78 °C. After addition of the carbonyl compound (1.06 mmol, 1.1 eq), the mixture was stirred at −78 °C and the reaction was followed by TLC. The mixture was poured into a solution of NH_4Cl (5 cm^3) and extracted with Et_2O (3 × 15 cm^3). The extracts were dried over $MgSO_4$. Filtration and removal of the solvent under reduced pressure followed by flash column chromatography or preparative HPLC afforded the desired product. The eluent conditions for separation techniques are given for each product. The quantities and respective yields are listed in Table 1 and Table 2.

2a (*S*,*R*) major, de 70 : 30: R_f = 0.4 (hexane–EtOAc 1 : 1) preparative HPLC (gradient: CH_3CN–H_2O 30 : 70, 35 min, flow: 50 ml min^{-1}): t_r = 10.95 min. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ_H: 1.01 (s, 3H), 1.1 (d, 1H, *J* = 11.1 Hz), 1.22 (t, 3H, *J* = 7.1 Hz), 1.35 (s, 3H), 1.59 (s, 3H), 1.68 (s, 3H), 2.12 (m, 2H), 2.33 (m, 1H), 2.74 (m, 2H), 4.15 (m, 3H). Elemental analysis. Found: C, 62.97; H, 8.1. Calc. for $C_{17}H_{26}NO_5$ (M^+ 324.41) C, 63.0; H, 8.0. MS (ES^+) *m*/*z* 325 $(M + H)^+$, 346 $(M + Na)^+$, 647.2 $(2M + H)^+$, 669.2 $(2M + Na)^+$. IR (film) ν_{max}/cm^{-1}: 3400 (OH), 2922 (CH), 1750 (CO), 1660 (CN).

2b (*S*,*S*) minor, de 70 : 30: R_f = 0.4 (hexane–EtOAc 1 : 1) preparative HPLC (gradient: CH_3CN–H_2O 30 : 70, 35 min, flow: 50 ml min^{-1}): t_r = 10.6 min. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ_H: 1.02 (s, 3H), 1.13 (d, 1H, *J* = 11.2 Hz), 1.32 (t, 3H, *J* = 7.2 Hz), 1.35 (s, 3H), 1.6 (s, 3H), 1.66 (s, 3H), 2.12 (m, 2H), 2.34 (m, 1H), 2.9 (m, 2H), 3.5 (s, 1H), 4.25 (q, 2H, *J* = 7.2 Hz), 4.40 (dd, 1H, *J* = 3.14 Hz, *J* = 3.22 Hz). MS (ES^+) *m*/*z* 324 $(M + H)^+$, 346 $(M + Na)^+$, 647.2 $(2M + H)^+$, 669.2 $(2M + Na)^+$. IR (film) ν_{max}/cm^{-1}: 3410 (OH), 2920 (CH), 1752 (CO), 1663 (CN).

6a (*S*,*R*) major de 93 : 7: R_f = 0.4 (hexane–EtOAc 1 : 1) preparative HPLC (gradient: CH_3CN–H_2O 45: 55, 35 min, flow: 50 ml min^{-1}): t_r = 12.73 min. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ_H: 0.9 (2d, 6H, *J* = 6.7 Hz), 1.08 (s, 3H), 1.12 (d, 1H *J* = 11.1 Hz), 1.22 (t, 3H, *J* = 7.1 Hz), 1.48 (s, 3H), 1.6 (s, 3H), 2.12 (m, 1H), 2.2 (t, 1H, *J* = 5.8 Hz), 2.35 (m, 3H), 2.8 (m, 1H), 3.69 (s, 1H), 4.05–4.31 (m, 3H). MS (ES^+) *m*/*z*: 352.1 $(M + H)^+$, 374 $(M + Na)^+$, 725.6 $(2M + Na)^+$. Elemental analysis. Found: C, 65.2; H, 7.9. Calc.for $C_{19}H_{29}NO_5$ (M^+ 351.1) C, 65.0; H, 8.0. IR (film) ν_{max}/cm^{-1}: 3435 (OH), 2950 (CH), 1740 (CO), 1660 (CN).

6b (*S*,*S*) minor de 93 : 7: R_f = 0.45 (hexane–EtOAc 1 : 1) preparative HPLC (gradient: CH_3CN–H_2O 45 : 55, 35 min, flow: 50 ml min^{-1}): t_r = 12.25 min. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ_H: 1.01 (2d, 6H, *J* = 7 Hz), 1.09 (s, 3H), 1.18 (d, 1H, *J* = 11.2 Hz), 1.32 (t, 3H, *J* = 7.1 Hz), 1.39 (s, 3H), 1.68 (s, 3H), 2.12 (m, 1H), 2.21 (t, 1H, *J* = 5.6 Hz), 2.36 (m, 1H), 2.75 (m, 1H), 2.88 (m, 2H), 3.5 (s, 1H), 4.32 (q, 2H, *J* = 7.1 Hz), 4.52 (t, 1H, *J* = 3.2 Hz). MS (ES^+) *m*/*z*: 352.1 $(M + H)^+$, 374 $(M + Na)^+$, 725.6 $(2M + Na)^+$. IR (film) ν_{max}/cm^{-1}: 3435 (OH), 2950 (CH), 1740 (CO), 1660 (CN).

8a (*S*,*R*) major de 56 : 44: R_f = 0.45 (hexane–EtOAc 6 : 4) preparative HPLC (gradient: CH_3CN–H_2O 48 : 52, 35 min, flow: 59 ml min^{-1}): t_r = 13.47 min. ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.62 (s, 3H), 1.18 (s, 3H), 1.19 (t, 3H, *J* = 7.1 Hz), 1.21 (d, 1H, *J* = 11.6 Hz), 1.22 (s, 3H), 1.62 (m, 1H), 1.93 (m, 2H), 2.15 (m, 2H), 2.85 (m, 2H), 3.33 (m, 2H), 3.59 (s, 1H), 4.33 (q, 2H, *J* = 7.1 Hz), 4.8 (dd, 1H, *J* = 2.28 Hz, *J* = 4 Hz), 6.9 (m, 5H). Elemental analysis. Found: C, 68.85; H, 7.85. Calc. for $C_{24}H_{32}NO_5$ (M^+ 415.52) C, 69.0; H, 8.0. MS (ES^+) *m*/*z*: 416 $(M + H)^+$, 438 $(M + Na)^+$, 829.6 $(2M + H)^+$, 851.8 $(2M + Na)^+$. IR (film) ν_{max}/cm^{-1}: 3300 (OH), 2933 (CH), 1736 (CO), 1636 (CN).

8b (*S*,*S*) minor de 56 : 44: R_f = 0.3 (hexane–EtOAc 6 : 4) HPLC (CH_3CN–H_2O 48 : 52) preparative HPLC (gradient: CH_3CN–H_2O 48 : 52, 35 min, flow: 50 ml min^{-1}): t_r = 13.74 min. ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.6 (s, 3H), 1.02 (s, 3H), 1.08 (t, 3H, *J* = 6.8 Hz), 1.15 (s, 3H), 1.25 (d, 1H, *J* = 10.94 Hz), 1.6 (m, 1H) 1.96 (m, 1H), 2.06 (m, 2H), 2.53 (m, 1H), 3.12 (m, 2H), 3.28 (m, 2H), 4.15 (q, 2H, *J* = 7.0 Hz), 4.4 (dd, 1H, *J* = 2.25 Hz, *J* = 4 Hz), 4.88 (s, 1H), 7.25 (m, 5H). MS (ES^+) *m*/*z*: 416 $(M + H)^+$, 438 $(M + Na)^+$, 829.6 $(2M + H)^+$, 851.8 $(2M + Na)^+$. IR (film) ν_{max}/cm^{-1}: 3300 (OH), 2933 (CH), 1736 (CO), 1636 (CN).

Synthesis of the β-hydroxy β-alkyl aspartic acid hydrochlorides **16a**, **16b**, **17a**, **18a**, **18b**

To compound **2a** (1 mmol) was added anisole (1 cm^3) at 0 °C followed by anhydrous HF (3 cm^3) and the mixture was magnetically stirred for 1 h at 0 °C. After evaporation, Et_2O was added to precipitate the amino ester hydrofluoride which was filtered (81% yield).

This compound was treated with a 6 M HCl solution (60 eq) and the mixture heated at 90 °C for 2 h. The solution was evaporated to afford the amino acid hydrochoride **16a** as a white powder in 75% yield.

Using the same experimental conditions **2b**, **6a**, **8a** and **8b** afforded the corresponding amino ester hydrofluorides in respectively 85%, 71%, 85% and 90% yields, which after treatment with 6 M HCl provided the amino acid hydrochlorides **16b**, **17a**, **18a** and **18b** in 84%, 72%, 90% and 91% yields (white powers).

16a (*S*,*R*) major: mp 171 °C. ^{1}H NMR (400 MHz, D_2O) δ_H: 1.45 (s, 3H), 4.3 (s, 1H). ^{13}C NMR (400 MHz, D_2O) δ_C: 21.61, 58.67, 73.94, 169.17, 175.45. MS (FAB^+) *m*/*z*: 164 $(M - HCl + H)^+$, 327 $(2M - HCl + H)^+$. HRMS (FAB^+) *m*/*z* calcd for $C_5H_{10}NO_5$·HCl 164.0559 found 164.0556. IR (film): 3000 (OH), 1734 (CO). $[\alpha]_D^{20}$: +9.8 (*c* 22.4 in D_2O).

16b (*S*,*S*) minor: mp 180 °C. ^{1}H NMR (400 MHz, D_2O) δ_H: 1.42 (s, 3H), 4.35 (s, 1H). ^{13}C NMR (400 MHz, D_2O) δ_C: 23.18, 59.36, 74.26, 169.16, 176.27. MS (FAB^+) *m*/*z*: 164 $(M - HCl + H)^+$, 327 $(2M - HCl + H)^+$. HRMS (FAB^+) *m*/*z* calcd for $C_5H_{10}NO_5$·HCl 164.0559 found 164.0556. IR (film) ν_{max}/cm^{-1}: 3000 (OH), 1734 (CO). $[\alpha]_D^{20}$: +2.8 (*c* 21.4 in D_2O).

17a (*S*,*R*) major: mp 87–89 °C. ^{1}H NMR (400 MHz, D_2O) δ_H: 0.9 (2d, 6H, *J* = 6.87 Hz, *J* = 6.66 Hz), 2.12 (m, 1H), 4.48 (m, 1H). ^{13}C NMR (400 MHz, D_2O) δ_C: 15.7, 16.99, 33.24, 56.63, 79.77, 169.95, 174.67. MS (FAB^+) *m*/*z*: 192 $(M + H)^+$, 383 $(2M + H)^+$. HRMS (FAB^+) *m*/*z* calcd for $C_7H_{14}NO_5$ 192.0872 found 192.0871. IR (film) ν_{max}/cm^{-1}: 3000 (OH), 1734 (CO). $[\alpha]_D^{20}$: +1.8 (*c* 20 in D_2O).

18a (*S*,*R*) major: mp 185–187 °C. ^{1}H NMR (400 MHz, D_2O) δ_H: 1.85 (m, 1H), 2.09 (m, 1H), 2.35 (m, 1H), 2.65 (m, 1H), 4.35 (s, 1H), 7.14 (m, 5H). ^{13}C NMR (400 MHz, CD_3 OD) δ_C: 28.18, 38.09, 59.57, 77.09, 126.76, 129.06, 141.03, 168.92, 174.64. MS (FAB^+) *m*/*z*: 253.1 $(M + H)^+$. HRMS (FAB^+) *m*/*z* calcd for $C_{12}H_{16}NO_5$ 254.1028 found 254.1003. IR (film) ν_{max}/cm^{-1}: 3000 (OH), 1734 (CO). $[\alpha]_D^{20}$: +9.2 (*c* 5 in 6 M HCl).

18b (*S*,*S*) minor: mp 197–199 °C. ^{1}H NMR (400 MHz, D_2O) δ: 2.65 (m, 2H), 3.11 (m, 1H), 3.27 (m, 1H), 4.85 (s, 1H), 7.8 (m, 5H). ^{13}C NMR (400 MHz, D_2O) δ_C: 30.13, 37.97, 59.42, 77.28, 127.34, 129.4, 129.73, 142.165, 170.21, 175.79. MS (FAB^+) *m*/*z*: 254 $(M + H)^+$. HRMS (FAB^+) *m*/*z* calcd for $C_7H_{16}NO_5$ 254.1028 found 254.1041. IR (film) ν_{max}/cm^{-1}: 3000 (OH), 1734 (CO). $[\alpha]_D^{20}$: +14 (*c* 10 in 6 M HCl).

Synthesis of 12 and 13

To a solution of the Schiff base **11** (4 g, 14.2 mmol) (prepared from (1*R*,2*R*,5*R*)-2-hydroxypinan-3-one and *tert*-butyl glycinate) in anhydrous THF (65 ml) was added CH_3MgBr (5.7 cm,[3] 17 mmol) followed after 15 min by addition of KHMDS 0.5 M in toluene (34 ml, 17 mmol) at −78 °C, under argon. After stirring for 20 min at the same temperature, the keto ester (17 mmol) (ethyl bromopyruvate or methyl trifluoromethylpyruvate) was added and the reaction followed by TLC. After treatment with a NH_4Cl saturated solution, the aqueous layer was extracted with Et_2O, the organic layer dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography and two diastereomers were obtained.

12: yield = 74%, dr = 88 : 12.

12a (*S*,*S*) major: R_f = 0.58 (EtOAc–petroleum ether 4 : 6) mp 92–93 °C. ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.7 (s, 3H), 1.05 (t, 3H, *J* = 7.1 Hz), 1.2 (s, 3H), 1.4 (s, 9H), 1.55 (s, 3H), 1.7–2.3 (m, 6H), 3.9–4.15 (m, 5H). MS (ES^+), *m*/*z*: 476–478 $(M + H)^+$.

12b (*S*,*R*), minor: R_f = 0.37 (EtOAc–petroleum ether 4 : 6). ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.7 (s, 3H), 1.05 (t, 3H, *J* = 7.1 Hz), 1.20 (s, 3H), 1.40 (s, 9H), 1.55 (s, 3H), 1.7–2.3 (m, 6H), 3.9–4.15 (m, 5H). MS (ES^+) *m*/*z*: 476–478 $(M + H)^+$.

13: yield = 74%, dr = 88 : 12.

13a: ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.6 (s, 3H), 1.0 (t, 3H, *J* = 7.13 Hz), 1.2 (s, 3H), 1.45 (s, 9H), 1.6 (s, 3H), 1.8–2.5 (m, 6H), 3.9 (m, 2H), 4.9 (s, 1H). MS (ESI) *m*/*z*: 452.2 $(M + H)^+$.

13b : ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.79 (s, 3H), 0.9 (t, 3H, *J* = 7.13 Hz), 1.2 (s, 3H), 1.4 (s, 9H), 1.67 (s, 3H), 1.8–2.5 (m, 6H), 4.2 (m, 2H), 5.3 (s, 1H).

Synthesis of 14a and 15a

To a solution of **12a** (2 g, 4.2 mmol) in a mixture of THF–CH_3CN 1 : 1 (84 cm^3) was added CsF (1.43 g, 9.45 mmol). The mixture was stirred at room temperature for 7 h. After addition of anhydrous $MgSO_4$, the mixture was filtered through Celite and the solid washed with EtOAc. The filtrate was concentrated under reduced pressure and the residue purified by silica gel column chromatography to afford **14a** in 74% yield. R_f = 0.5 (EtOAc–petroleum ether 4 : 6). ^{1}H NMR (250 MHz, C_6D_6) δ_H: 0.87 (s, 3H), 1.02 (t, 3H, *J* = 7.1 Hz), 1.2 (s, 3H), 1.51 (s, 3H), 1.52 (s, 9H), 1.7–2.3 (m, 6H), 3.4 (m, 2H), 4.0 (m, 2H), 5.22 (s, 1H). MS (ES^+) *m*/*z*: 396.2 $(M + H)^+$.

To a solution of **14a** (0.3 g, 0.76 mmol) in THF (1.7 ml) was added a 15% citric acid solution (1.66 ml). After stirring for 12 h, the THF was evaporated and the aqueous phase washed with Et_2O (3 × 2 ml). The aqueous phase was neutralized by addition of Na_2CO_3 and extracted with Et_2O. The organic phase was dried over $MgSO_4$ and evaporated to afford **15a** as an oil which was chromatographed (yield = 50%). R_f = 0.2 (Et_2O–petroleum ether 8 : 2). ^{1}H NMR (250 MHz, C_6D_6) δ_H: 1.07 (t, 3H, *J* = 7.1 Hz), 1.48 (s, 9H), 2.78 (d, 1H, *J* = 6.3 Hz), 3.05 (d, 1H, *J* = 6.3 Hz), 3.51 (s, 1H), 4.02 (m, 2H). MS (ES^+) *m*/*z*: 245.7 $(M + H)^+$ 189.7 $(M - tBu + H)^+$.

References

1 E. K. Michaelis, *Prog. Neurobiol. (Oxford)*, 1998, **54**, 369–415.
2 N. C. Danbolt, *Prog. Neurobiol. (Oxford)*, 2001, **65**, 1–105.
3 R. J. Bridges, M. P. Kawanaugh and A. R. Chamberlein, *Curr. Pharm. Des.*, 1999, **5**, 363–366.
4 (*a*) B. Lebrun, M. Sakaitani, K. Shimamoto, Y. Yasuda-Kamatani and T. Nakajima, *J. Biol. Chem.*, 1997, **272**, 20336–20340; (*b*) K. Shimamoto, B. Lebrun, Y. Yasuda-Kamatani, M. Sakaitani, Y. Shigeri, N. Yumoto and T. Nakajima, *Mol. Pharmacol.*, 1998, **53**, 195–197.
5 K. Shimanoto, Y. Shigeri, Y. Yasuda-Kamatani, B. Lebrun, N. Yumoto and T. Nakajima, *Biorg. Med. Chem. Lett.*, 2000, **10**, 2407–2410.
6 For recent reviews on asymmetric synthesis of α-amino acids: R. M. Williams, *Synthesis of optically active α-amino acids*, in Organic Chemistry Series, J. E. Baldwin, P. D. Magnus, Eds., Pergamon Press, Oxford, 1989, Vol. 7; R. P. Duthaler, *Tetrahedron*, 1994, **50**, 1539.
7 Y. Ito, M. Sawamura, H. Hamashima, T. Emura and T. Hayashi, *Tetrahedron Lett.*, 1989, **30**, 4681–4684.
8 E. Fernandez-Megia, M. M. Paz and F. J. Sardina, *J. Org. Chem.*, 1994, **59**, 7643–7652.
9 V. A. Soloshonok, A. D. Kacharov, D. V. Avilov, N. N. Ishikawa and T. Hayashi, *J. Org. Chem.*, 1997, **62**, 3470–3479.
10 El. Achqar, M. Boumzebra, M. L. Roumestant and Ph. Viallefont, *Tetrahedron*, 1988, **44**, 5319–5332.
11 T. Kassem, V. Rolland, J. Martinez and M. Rolland, *Acta Crystallogr., Sect. C*, 2000, **56**, 1037–1039.
12 T. Kassem, V. Rolland, M. Rolland and J. Martinez, *Acta Crystallogr., Sect. C*, 2001, **57**, 1415–1417.
13 M. Rolland, J. Wehbe, V. Rolland, J. Martinez, *Acta Crystallogr.*, submitted.
14 F. M. Hauser and S. R. Ellenberger, *J. Org. Chem.*, 1986, **51**, 50–57.