Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 4, pp. 731-744, 1994

0957-4166/94 $6.00+0.00

0957-4166(94)E0095-R

Resolution and Regioselective Protection of Glutamic Acid Analogues. I- Resolution of Diastereomeric α-Boroxazolidone Derivatives.

Francine Acher and Robert Azerad*

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Unité associée au CNRS N°400, Université René Descartes, 45 rue des Saints-Pères, 75270- Paris Cedex 06, France.

Abstract: Diastereomeric α-boroxazolidone γ-phenylethylamide (or γ-phenylethanolamide) derivatives of 2-, 3- or 4-substituted glutamic acid analogues have been separated by silicagel chromatography, resulting, after deprotection, in a practical method for the resolution of most of these unnatural amino acids.

INTRODUCTION

Among non-proteinogenic α-aminoacids, α,α'-dialkyl derivatives, and especially α-methyl substituted amino acids, have been the subject of numerous investigations over recent years, due to their considerable scientific and medical significance. Many of them have been employed as enzyme substrates or inhibitors [1]. Moreover, their incorporation into physiologically active peptides, instead of their natural amino acid counterparts, generally increases the resistance to hydrolysis by peptidases [2], and sometimes alters the conformational freedom of these peptides [3,4], promoting the tendancy toward helix formation [4,5], or locking active peptide conformations [6]. Quite recently, the successful incorporation of such amino acid residues into proteins and peptides, using *in vitro* protein synthesis systems, has been reported [7].

Both D- and L-enantiomers of glutamic acid (and glutamine) are key amino acids in various biological systems, either as free acids, or involved in a peptide structure: neurotransmitters in the mammalian central nervous system [8], substrates of several physiologically-important enzymic reactions [1,9,10], component of the folate group coenzymes [11] (L-enantiomer), component of the bacterial cell-wall peptidoglycane [12] (D-enantiomer). In several instances, enantiomerically pure glutamic acid (and glutamine) analogues have been prepared and used as essential tools for mechanistic and structure-activity relationship studies.

(R) or (S)-2-methylglutamic acids have been prepared, but in low yields, by asymmetric alkylation methods using appropriate chiral auxiliaries [13] or chiral precursors [14,15]. A resolution of the (RS)- amino acid by fractional crystallization of its brucine salt (4 and 14% yields for R- and S-enantiomers respectively) has been reported [16]. On the other hand, whereas enzymatic resolutions of several other 2-alkyl substituted amino acids have been described [17], this method does not seem to have been successfully applied to 2-methylglutamic acid derivatives, except for a small scale preparation using glutamine synthetase [18] or a minimally successfull two-steps chemoenzymatic process using the enzymatic kinetic resolution of a corresponding α-nitro α-methyl dicarboxylic acid ester [19].

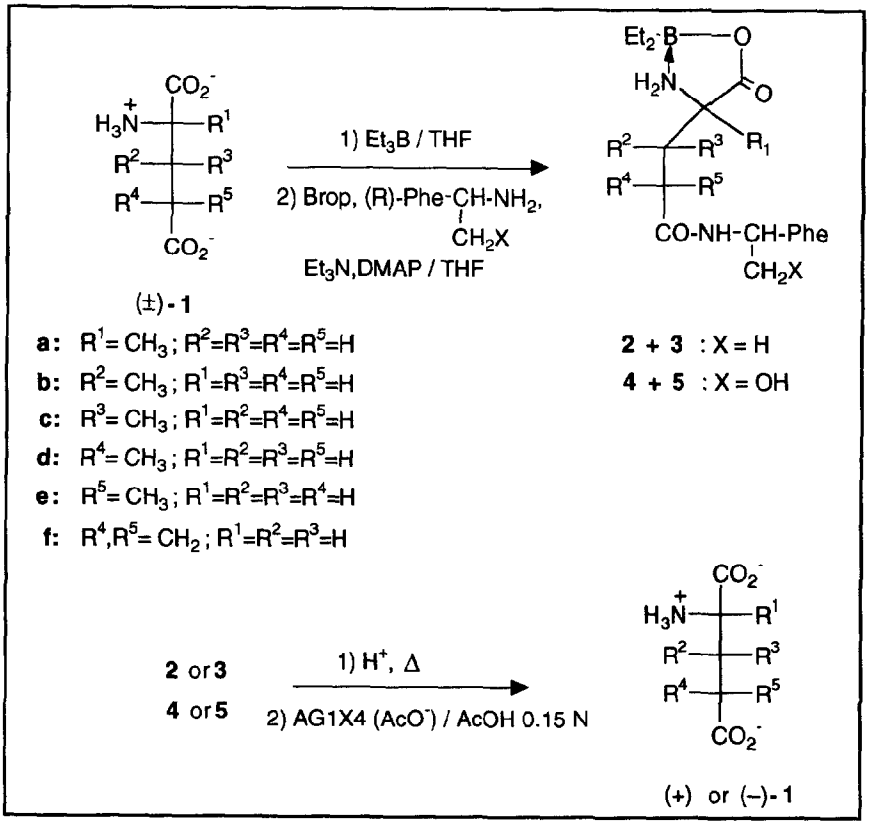
Various preparations of optically active acyclic, 3- or 4-alkyl (or alkylidene)-substituted glutamic acid isomers have been occasionally described, including chemical [20,21] or enzymatic [22,23] asymmetric synthesis methods, and chemical [24] or enzymatic [25-27] resolutions. Cycloglutamic acids (cyclic 2,4-dialkyl derivatives) enantiomers have been the subject of a pioneering work devoted to the description of conformational and steric requirements for glutamic acid binding at the active site of glutamine synthetase [25,28-30]. Asymmetric syntheses or resolutions of cyclic analogues of glutamic acid, corresponding to 2,3-, 2,4- or 3,4-dialkyl derivatives, have been also described in very active recent investigations, in connection with natural products structural assignment [31], studies of neurotransmisssion agonists [32-35] or search for inhibitors of the vitamin K-dependent carboxylations [36-38].

Although very attractive routes for the stereoselective asymmetric synthesis of some of these analogues have been reported in the last few years [39], we have preferred to develop, from well known synthetic procedures leading to racemic compounds, simple resolution methods [40] likely to produce in high yield both enantiomers. In addition, as we were dealing with trifunctional amino acids, we have privileged resolution methods which would possibly afford regioselectively α- (or γ-) protected derivatives as end-products, in the perspective of introducing these analogues into peptide structures by chemical synthetic methods.

Ten years ago, an elegant simultaneous protection method for the α-amino and α-carboxyl group of α-amino acids was described by Nefkens and Zwanenburg [41]. This method was based on the easy formation of boroxazolidone derivatives by reaction of triethyl borane with usual amino acids, including L-glutamic acid, the γ-carboxyl group of which could then be activated as a *p*-nitrophenyl ester, in order to obtain a suitable material for the synthesis of γ-glutamyl peptides [41]. We have recently and successfully used this method for the selective protection of 2-methylglutamic acid and 2,4-disubstituted cyclic analogues, their introduction into oligopeptides, and the synthesis of the corresponding γ-carboxamido compounds [38]. We describe hereafter another use of this selective protection to prepare diastereomeric γ-(R)-1-methylbenzylamide or γ-(R)-1-hydroxymethylbenzylamide derivatives of 2-methylglutamic acid and other substituted analogues, their chromatographic separation, and the subsequent resolution of these unnatural amino acids.

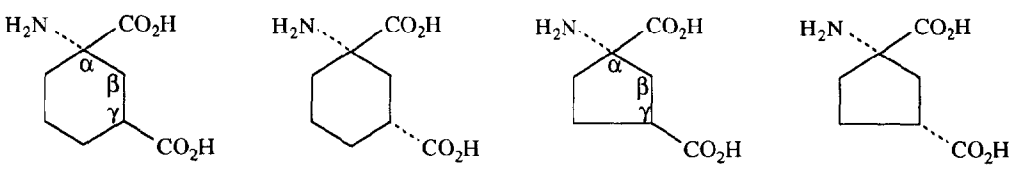
RESULTS AND DISCUSSION

(RS)-2-Methylglutamic acid **1a** (Scheme 1) was reacted with triethyl borane in THF to afford quantitatively a crystalline boroxazolidone derivative [38], which was difficultly coupled with R-(+)-1-phenylethylamine, using the mixed anhydride method [42] (50-60% yield). However, the corresponding diastereomeric amides **2a**+**3a** were obtained in 80% yield when coupling was effected with the Brop reagent [43], which has been shown to be particularly efficient in the difficult coupling of sterically hindered N-methyl or α,α'-dialkyl α-amino acids. The α,α'-protected diastereomeric amides **2a** and **3a** thus obtained were clearly separated by TLC ($\Delta Rf \geq 0.1$, see Table 1) and a transposition of the analytical separation to preparative column chromatography allowed a quantitative recovery of pure isomers. Their acidic hydrolysis, followed by anion exchange chromatography, afforded the pure enantiomeric 2-methylglutamic acids (>98% e.e. by HPLC [44]) in a final 78% yield (calculated from the racemic mixture) on a 10 mmoles scale. A similar, slightly better separation (Table 2), was obtained with the corresponding **4a** and **5a** amides deriving from R-(−)-phenylglycinol [45]. Unexpectedly, the order of elution of (R)-phenylethylamides (2R,1'R-**2a** before 2S,1'R-**3a**) was reversed for (R)-phenylethanolamides (2S,2'R-**5a** before 2R,2'R-**4a**), as shown after acidic hydrolysis to free (R)- and (S)-2-methylglutamic acid enantiomers.



Scheme 1

This method was extended to other (±)-methyl or methylene-substituted (**1b-f**), and to cyclic glutamic acid analogues (**6-9**). Variable TLC separations of the diastereomeric boroxazolidone (R)-phenylethylamide derivatives were noted (Table 1). In order to obtain a clear-cut separation on a preparative scale, the ΔR_f had to be ≥0.06: *threo*-3-methylglutamic acid (**1c**), *erythro*-4-methylglutamic acid (**1d**), 4-methylene-glutamic acid (**1f**), and *trans* 1-amino-cyclopentane-1,3-dicarboxylic acid (**9**) derivatives showed a satisfactory separation and were easily resolved, in 100 mg to 1 g amounts, by this method; *threo*-4-methylglutamic acid (**1e**), *erythro*-3-methylglutamic acid (**1b**) and the other cyclic analogues (**6-8**) were not sufficiently separated to obtain a quantitative resolution. In all successful separations, the 2R-enantiomer derivative (which also corresponded to a 3S- or 4S- configuration) was the first eluted.



Incidentally, when this method was applied to the resolution of 4-alkyl substituted (or 2,4-cyclic) analogues, a special care must be exercised in the final acidic hydrolysis step: an easy epimerization at C-4 [22,26] afforded new diastereomeric mixtures, requiring another ion-exchange chromatographic separation [46], as illustrated in the experimental part for the preparation of (2R,4S)-4-methylglutamic acid. This prompted us to systematically investigate the separation of phenylglycinol-derived amides **4** and **5** [45], prone to hydrolyze in milder acidic conditions through a preliminary intramolecular N- to O-acyl shift [47], and thus susceptible to minimize the epimerization of 4-alkyl substituted analogues. Table 2 shows that most diastereomeric separations were improved, as for example with *erythro*-3- and 4-methylglutamic acids (**1b** and **1d**), *threo*-4-methylglutamic acid (**1e**) and cyclic glutamic acid analogues amides; only *trans*-1-amino-1,3-cyclohexane-dicarboxylic acid (**7**) could not be separated in either derivatization method. Some of these separations were easily transposed to preparative column chromatography on a 1-5 mmoles (or larger) scale. However the stability of the boron complex was lower in some of these derivatives, especially **4b**, probably because of an intramolecular attack by the free hydroxyl group of the phenylglycinol residue; it was thus necessary to perform the chromatographic separation shortly after preparation in order to avoid an untimely decomplexation, resulting in lower yields.

Table 1: TLC separation of diastereomeric (R)-1-phenylethylamide derivatives of glutamic acid analogues (solvents: A, CH_2Cl_2-acetone 85:15; B, CH_2Cl_2-acetone 8:2; C, CH_2Cl_2-acetone 7:3; D, CH_2Cl_2-iPrOH 95:5)

	Solvent	Rf values (configuration)		Preparative separation
1a: 2-Methylglutamic acid	A	0.33 (2R)	0.23 (2S)	excellent
1b: *Erythro* -3-methylglutamic acid	A	0.26	0.22	poor
1c: *Threo* -3-methylglutamic acid	D	0.39 (2R,3S)	0.31 (2S,3R)	good
1d: *Erythro* -4-methylglutamic acid	A	0.27 (2R,4S)	0.19 (2S,4R)	good
1e: *Threo* -4-methylglutamic acid	B	0.27	0.27	no separation
1f: 4-methylene-glutamic acid	A	0.33 (2R)	0.21 (2S)	excellent
6: *Cis* 1-amino-1,3-cyclohexane-dicarboxylic acid	B	0.33	0.29	poor
7: *Trans* -1-amino-1,3-cyclohexane-dicarboxylic acid	A	0.38	0.38	no separation
8: *Cis* -1-amino-1,3-cyclopentane-dicarboxylic acid	C	0.29	0.29	no separation
9: *Trans* -1-amino-1,3-cyclopentane-dicarboxylic acid	A	0.35	0.28	good

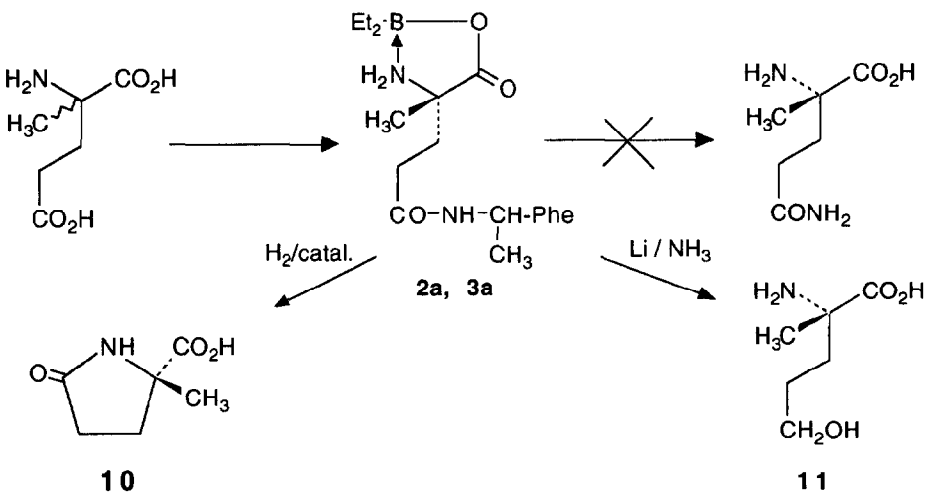
Table 2: TLC separation of diastereomeric (R)-phenylglycinol amide derivatives of glutamic acid analogues (solvents: A, CH_2Cl_2-acetone 6:4; B, CH_2Cl_2-acetone 4:6)

	Solvent	Rf values (configuration)		Preparative separation
1a: 2-Methylglutamic acid	A	0.25 (2S)	0.14 (2R)	excellent
1b: *Erythro* -3-methylglutamic acid	A	0.30 (2S,3S)	0.24 (2R,3R)	good
1c: *Threo* -3-methylglutamic acid	A	0.27 (2S,3R)	0.21 (2R,3S)	good
1d: *Erythro* -4-methylglutamic acid	A	0.35 (2S,4R)	0.20 (2R,4S)	excellent
1e: *Threo* -4-methylglutamic acid	A	0.33 (2R,4R)	0.24 (2S,4S)	excellent
6: *Cis* 1-amino-1,3-cyclohexane-dicarboxylic acid	A	0.34 (1R,3R)	0.21 (1S,3S)	excellent
7: *Trans* -1-amino-1,3-cyclohexane-dicarboxylic acid	A	0.35	0.35	no separation
8: *Cis* -1-amino-1,3-cyclopentane-dicarboxylic acid	B	0.37 (1R,3R)	0.29 (1S,3S)	good
9: *Trans* -1-amino-1,3-cyclopentane-dicarboxylic acid	A	0.32 (1S,3R)	0.24 (1R,3S)	good

For diastereomeric phenylglycinolamide derivatives substituted in α- or β-positions, the order of elution was related to the configuration at the C^{α}-carbon (S- before R-); on the contrary, with substitution in the γ-position, significant correlations with the C^{γ}-configuration (R- before S-) could be found, resulting in apparent reversed elution order of C^{α}-epimers for *threo*- and *erythro*-4-methylglutamic acids (Table 2, see **1d** and **1e**), or *cis*- and *trans*-cyclic analogues (**8** and **9**). It is not unexpected that the C^{γ}-asymmetric center, closer to the chiral auxiliary asymmetric center, does exert a larger effect on the separation, rather than the remote C^{α}- (or C^{β}-) center, if specific interactions of the hydroxyl group of the phenylglycinol residue are effectively involved in the binding to the silica adsorbent [45].

The hydrolysis of the phenylglycinol derived amides, which was easily achieved under relatively mild acidic conditions (2N HCl, 80°C, 12 h), afforded pure amino acid enantiomers, even in the case of 4-alkylated compounds, avoiding an additional separation step.

The use of γ-benzylamide derivatives raised the opportunity of preparing enantiomerically pure γ-glutamine analogues by hydrogenation of the N-benzyl bond. Current hydrogenation methods (catalytic hydrogenation in acetic acid [48] or in methanol, catalytic hydrogen transfer from formic acid [49] or ammonium formate in methanol [50]), experimented with the 2-methylglutamic phenylethylamide derivatives **2a** or **3a**, resulted in the expected decomplexation of the boron moiety, but essentially provided 2-methylpyroglutamic acid **10** (Scheme 2). A similar result was obtained by ammoniolysis (28% NH_4OH in water, 80°C, 24 h) of the corresponding phenylglycinolamide derivative. Simultaneous cleavage of the boroxazolidone protecting group and reduction of the γ-N-methylbenzyl group were expected from the treatment of diastereomeric phenylethylamides **2a** or **3a** with lithium in liquid ammonia [51]: the only isolated reduction products were the corresponding enantiomers of α-methyl δ-hydroxynorvaline **11**.



Scheme 2

The partial deprotection of such boroxazolidone benzylamide derivatives by methanol decomplexation of the boron moiety is easily realized. The resulting temporary blocking of the γ-amido group, which generally constitutes a major problem when introducing glutamine derivatives in peptide syntheses, might be a useful

feature if we consider that the favored pyroglutamate formation previously observed, when using hydrogenation methods for deprotection, may be minimized when the α-amino group is engaged in a N-acyl or N-carbamyl derivative.(±)-Boc-2-methyl glutamic acid γ-phenylethylamide was easily obtained, without pyroglutamate formation, by methanol decomplexation of **2a+3a**, followed by *t*-butyloxycarbonyl protection, but hydrogenation in various conditions (10% Pd/C with ammonium formate added [52], in methanol, ethanol, or water-AcOH, at room or reflux temperature) was unsuccessful and the starting material was recovered unchanged.

This method, particularly when using the phenylglycinolamide derivative, is highly efficient for the resolution of most of the tested glutamic acid analogues; it should probably be successfull with other new unnatural α-amino acids bearing an acidic side chain function. However, this method was uneffective to provide selectively γ-amido derivatives, through the attempted hydrogenation of the γ-phenylethylamido group. Enzymatic resolution methods, with adequately protected derivatives, are currently investigated, in order to provide selectively protected amino acid analogues enantiomers.

EXPERIMENTAL

R-(+)-1-phenylethylamine (≥ 99 % e.e.)was purchased from Fluka, R-(–)-2-phenylglycinol (99.5 % e.e.), 2-methylglutamic acid hemihydrate were from Janssen Chimica (France). Brop (bromo-tris[dimethylamino]-phosphonium hexafluorophosphate) [43] was a gift from Drs J.Coste and M.N.Dufour (Montpellier, France). (±)-4-Methyleneglutamic acid was synthetized as previously described [53]: m.p. 205-210°C dec (lit. [54] 197-210°C dec.). (±)-*Threo*- and *erythro*-3-methylglutamic acids were prepared by a modification of a published method [55,56]: the intermediate 4-methylpyrrolidone dicarboxyester was directly hydrolyzed (HCl 2N, 100°C, 6 h) to the diastereomeric amino acids (*threo/erythro*, 7:3), which were separated by anion exchange chromatography [46] (*erythro* first eluted). (±)-*Threo*- and *erythro*-4-methylglutamic acids were prepared according to [24,26,57] and separated by anion exchange chromatography [22,46]. (±)-*Cis*- and (±)-*trans*-1-amino-1,3-cyclohexanedicarboxylic acids [28] and (±)-*cis*- and (±)-*trans*-1-amino-1,3-cyclopentanedicarboxylic acids [30,33] were prepared according to published procedures, and separated by anion exchange chromatography [36]. Anion exchange chromatography was performed using AG1X4® resin (Biorad, Cl^-, 200-400 mesh) converted to the AcO^- form.

Melting points were determined with a Büchi capillary tube melting point apparatus and are uncorrected. Elemental analysis were carried out by the Service Central de Microanalyse du CNRS (Gif-s-Yvette, France). Optical rotations were determined in a 1 dm cell with a Perkin Elmer 241 spectropolarimeter using Na (589 nm) or Hg (578, 546, 436 and 365 nm) lines. ^1H (250.13 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on a WM 250FT Bruker Spectrometer; chemical shifts are given with reference to residual ^1H or ^{13}C in deuterated solvents (δ ppm): $CDCl_3$, 7.24, 77.00; acetone-D_6, 2.05, 20.60 and 208.00; DMSO-D_6, 2.49, 30.7. Multiplicities are reported as br. (broad), s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). MS (CI-NH_3) was performed on a Nermag R10-10 instrument. TLC was performed on Merck $60F_{254}$ precoated silicagel plates (0.2 mm thick, 10 cm migration) with the indicated solvent systems. Products were visualized by UV light (254 nm), 2% (w/v) ninhydrin in ethanol and TDM reagent [58]. Medium pressure flash chromatography was performed on Merck 60H silicagel (15-40 μm) on a Jobin et Yvon axial compression column (4 cm diameter), at 2-10 bars. HPLC was performed with an Altex Chromatem 380 pump, a Rheodyne 7125 (20μl loop), a Pye-Unicam LC-UV detector set at 210 nm, and a Shimadzu CR-3A integrator, using a Nucleosil $5C_{18}$ (10 cm x 4 mm I.D.) column with 0.1 M NaH_2PO_4 buffer pH 3.0 at a 0.3 $mL.min^{-1}$ flow rate. GC was performed either on an Flexibond™ OV-1701 (0.2 mm x 15 m) capillary column (Pierce Chem.Co) run at 150-160°C, or on a polysiloxane XE60-S-valine-S-phenylethylamide (0.25 mm. x 30 m) capillary column (Chrompack) run at 145°C, with helium (1 bar) as carrier gas. Purity of the resolved diastereoisomers was checked by TLC, careful examination of the 250 MHz ^1H-NMR spectra, and gas chromatography after hydrolysis to amino acids and derivatization to N-trifluoroacetyl-O-isopropyl esters (OV-1701): in most cases, only >98% pure fractions were considered for pooling. Enantiomeric purity of amino acids was checked, after derivatization, by HPLC [44] or GC [59].

Resolution of (±)-2-methylglutamic acid (1a) using R-(+)-1-phenylethylamine

1) mixed anhydride method: To a suspension of finely ground (±)-2-methylglutamic acid, 0.5 H_2O (0.340 g, 2 mmol) in dry THF (3 ml) under an argon atmosphere, was added a 1M solution of triethylboron in THF (3 ml). After overnight stirring, excess reagent was eliminated under a stream of argon and the solution cooled at - 15°C; triethylamine (0.3 ml, 2.16 mmol) then *i*-butylchloroformate (0.270 ml, 2.08 mmol) were added. After 5 minutes stirring at - 15°C, R-(+)-1-phenylethylamine (0.320 ml, 2.51 mmol) was added and stirring was continued overnight, with the temperature slowly arising to ambient. The solvent was evaporated and the residue dissolved in EtOAc (15 ml), washed with 0.1N HCl (15 ml), water (15 ml), saturated aqueous $NaHCO_3$ solution (15 ml), water (15 ml), dried over Na_2SO_4, then evaporated to a white solid (0.64 g). The diastereoisomeric boroxazolidone amides were separated by medium pressure chromatography on a silicagel column (90g). CH_2Cl_2/ acetone (85:15) eluted first the 2R,1'R-isomer (0.193 g, 0.58 mmol), a small amount of mixture (0.016 g) and the 2S,1'R-isomer (0.159 g, 0.48 mmol), corresponding to a total yield of 55% from (±)-2-methylglutamic acid. The same yield was obtained when the preparation was run on a 10 mmoles scale.

Analytical data for the 2R,1'R-diastereoisomer (**2a**): M.p.197°C (205°C after recrystallization in $CHCl_3$-pentane). Anal. calc.% for $C_{18}H_{29}N_2O_3B$: C 65.07, H 8.80, N 8.43, found % C 58.29, H 8.01, N 9.41 [60]. $[\alpha]_D^{21}$= +90.2 (c 2, acetone), $[\alpha]_{578}$= +93.8, $[\alpha]_{546}$= +108.1, $[\alpha]_{436}$= +196.2, $[\alpha]_{365}$= +336.5. ^1H NMR ($CDCl_3$) δ ppm, J Hz: 7.73 (d, 1H, N*H*, J = 12.1), 7.30 (m, 5H, Ar*H*), 6.89 (d, 1H, N*H*, J = 7.6), 5.01(dq, 1H, C*H*Ar, J = 7.1), 3.24 (d, 1H, N*H*, J = 12.2), 2.59 (ddd, 1H, γ-C*H*, J = 3.1, 6.8 and 17.8), 2.35 (ddd, 1H, γ-C*H*, J = 3.4, 8.3, and 17.8), 2.09 (m, 2H, β-CH_2), 1.52 (s, 3H, α-CH_3), 1.48 (d, 3H, Ar-CH-CH_3, J = 7.1), 0.79 and 0.78 (2 t, 6H, B[CH_2-CH_3]$_2$, J = 7.7), 0.43-0.17 (m, 4H, B[CH_2-CH_3]$_2$).

Analytical data for the 2S, 1'R-diastereoisomer (**3a**): M.p.185°C (unchanged after recrystallization in $CHCl_3$-pentane). Anal. calc.% for $C_{18}H_{29}N_2O_3B$: C 65.07, H 8.80, N 8.43, found % C 63.72, H 8.71, N 8.63 [60]. $[\alpha]_D^{21}$= +56.5 (c 2, acetone), $[\alpha]_{578}$= +58.8, $[\alpha]_{546}$= +68.2, $[\alpha]_{436}$= +128.5, $[\alpha]_{365}$= +230.4. ^1H NMR ($CDCl_3$) δ ppm, J Hz: 7.75 (d, 1H, N*H*, J = 11.8), 7.28 (m, 5 H, Ar*H*), 6.31 (d, 1H, N*H*, J = 7.5), 5.02 (dq, 1H, C*H*Ar, J = 7.1), 3.16 (d, 1H, N*H*, J = 11.7), 2.56 (ddd, 1H, γ-C*H*, J = 2.6, 7.5, and 17.7), 2.35 (ddd, 1H, γ-C*H*, J = 2.5, 9.0, and 17.7), 2.12 (m, 2H, β-CH_2), 1.54 (s, 3H, α-CH_3), 1.49 (d, 3 H, Ar-CH-CH_3, J = 7.15), 0.76 and 0.61 (2 t, 6H, B[CH_2-CH_3]$_2$, J = 7.7), 0.39-0.01 (m, 4H, B[CH_2-CH_3]$_2$).

2) Brop synthesis method: To the clear solution obtained from (±) 2-methylglutamic acid, 0.5 H_2O (**1a**) (0.296 g, 1.74 mmole) and triethylboron solution, as previously described, were added triethylamine (0.5 ml, 3.6 mmole), R-(+)-1-phenylethylamine (0.25 ml, 1.96 mmol), DMAP (0.213 g, 1.74 mmol) in THF (2 ml) and Brop (0.743g, 1.91 mmol) suspended in 10 ml THF. The mixture was stirred for 4 h at room temperature then evaporated. The residue was dissolved in EtOAc (35 ml), washed with aqueous $KHSO_4$ solution (up to pH 3, 35 ml), aqueous $NaHCO_3$ solution (35 ml), saturated NaCl solution (35 ml), dried over Na_2SO_4 and evaporated to yield a white solid (0.612 g) which was chromatographed on silicagel (40 g) and eluted with CH_2Cl_2-acetone (85:15, 800 ml) and CH_2Cl_2-acetone (82:18, 500 ml) to give the 2R,1'R-isomer **2a** (0.221 g, 0.666 mmol), m.p.197°C, a small amount of mixture (0.026 g, 0.079 mmol) and the 2S,1'R-isomer **3a** (0.229 g, 0.690 mmol), m.p.185°C (total yield: 82%).

(R)- and (S)-2-Methylglutamic acid (1a)

The separated boroxazolidone derivative [0.598 g (2R,1'R)-**2a**, 1.8 mmol] was suspended in 6N HCL (18ml) in a screw-capped bottle and heated at 110°C for 18 hours. The solution was evaporated and dried under vacuum over potassium hydroxyde. The residue was then dissolved in boiled water (270ml), adjusted to pH> 8 with 10M sodium hydroxyde, and added on the top of an AG1X4 (AcO^-) column (18 x 2 cm). After washing with boiled water, elution with 0.15M acetic acid, monitored by reverse-phase HPLC, yielded optically pure (R)-2-methylglutamic acid, which was dried by azeotropic evaporation with cyclohexane (0.278 g, 1.635 mmol, 91% yield); TLC in CH_2Cl_2-MeOH-28% aqueous NH_4OH (50:50:15 v/v): Rf. 0.23

Analytical data for the R-enantiomer: M.p.183-184°C (lit. [18] 169-172°C). $[\alpha]_D^{21}$ = –11.5 (c 4, 6N HCl) [lit. –12.1 (c 4, 6N HCl) [16]; –12.1 (c 3.24, 5N HCl) [14]], $[\alpha]_{578}$ = –12.0, $[\alpha]_{546}$= –13.6, $[\alpha]_{436}$= –23.3. E.e.≥ 97 % (by HPLC after derivatization [44]).

Analytical data for the S-enantiomer: M.p.183°C. $[\alpha]_D^{21}$ = +11.5 (c 4, 6N HCl) [lit. [16] +12.1 (c 4, 6N HCl)], $[\alpha]_{578}$ = +12.0, $[\alpha]_{546}$= +13.7, $[\alpha]_{436}$= +23.7, $[\alpha]_{365}$= +38.4 . E.e.≥ 97 % (by HPLC after derivatization [44])

Resolution of (±)-2-methylglutamic acid (1a) using R-(–)-2-amino-2-phenylethanol

The (±)-2-methylglutamic-boroxazolidone complex was coupled with R-(–)-2-phenylglycinol by the mixed anhydride or the Brop method to yield both diastereoisomers which were separated on a silicagel column (CH_2Cl_2-acetone, 6:4) as described for the 1-phenylethylamide derivatives. Hydrolysis was performed in 2N HCl at 80°C for 12 hours; pure amino acid and recovered (R)-phenylglycinol (m.p.76-77°C after crystallization

in ether-hexane) were separated by anion exchange chromatography (see above).

Analytical data for the 2R,2'R-diastereoisomer (**4a**): glassy solid. ^{1}H NMR (CD_3COCD_3) δ ppm, J Hz: 8.01 (d, 1H, N*H*, J = 7.6), 7.37-7.21 (m, 6H, Ar*H* + N*H*), 5.15 (d, 1H, N*H*, J= 12), 5.05 (m, 2H, C*H*-Ar), 3.76 (m, 2H, CH_2OH), 2.98 (br s, 1H, O*H*), 2.72 (ddd, 1H,CH_2, J = 4, 8, and 17.2), 2.48 (ddd, 1H, CH_2, J = 3.9, 8, and 17.1), 2.13-1.98 (m, 2H, CH_2), 1.49 (s, 3H, α-CH_3), 0.75 (t, 3H, B[CH_2-CH_3]$_2$, J = 7.7) ; 0.37-0.17 (m, 4H, B[CH_2-CH_3]$_2$). E.e. of (R)-2-methylglutamic acid, determined by HPLC [44] after acidic hydrolysis: 98.4 %.

Analytical data for the 2S,2'R-diastereoisomer (**5a**): M.p.166°C. ^{1}H NMR (CD_3COCD_3) δ ppm, J Hz: 8.04 (d, 1H, N*H*, J = 7.8), 7.45-7.24 (m, 6H, Ar*H* + N*H*), 5.10-4.98 (m, 2H, N*H* + C*H*-Ar), 3.77 (d, 2H,CH_2OH, J = 5.4), 3.00 (br s, 1H,O*H*), 2. 69 (ddd, 1H,C*H*, J = 3.5, 8.3, and 17.1) ; 2.52 (ddd, 1H, C*H*, J = 3.4, 5.5, and 17.1), 2.13-1.91 (m, 2H, CH_2) 1.46 (s, 3H, α-CH_3); 0.78 and 0.76 (2t, 6H, B[CH_2-CH_3]$_2$, J = 7.1) ; 0.41 - 0.25 (m, 4H, B[CH_2-CH_3]$_2$). E.e. of S-2-methylglutamic acid, determined by HPLC [44] after acidic hydrolysis: 98.8 %.

Resolution of (±)-4-methylene glutamic acid (1f) using R-(+)-1-phenylethylamine

The diastereoisomers mixture (**2f** and **3f**) was prepared from (±)-4-methyleneglutamic acid (**1f**) (0.890 g, 5.6 mmol), Et_3B and R-(+)-2-phenylethylamine by the mixed anhydride method, and separated by silica gel column chromatography (200 g, CH_2Cl_2-acetone 88:12, 2 L, and 85:15, 1 L). The 2R,1'R-isomer **2f** (0.485 g, 1.47 mmol) was eluted first, then the 2S,1'R-isomer **3f** (0.444 g, 1.345 mmol). Total yield from **1f**: 50%.

Analytical data for the 2R,1'R-diastereoisomer (**2f**): M.p. 153° C (157-158°C after recrystallization in CH_2Cl_2-hexane). Anal calc % for $C_{18}H_{27}N_2O_3B$: C 65.47, H 8.24, N 8.48; found C 64.79, H 8.21, N 8.26. $[\alpha]_D^{21}$= +53.4 (c 1, $CHCl_3$),$[\alpha]_{578}$= +56.0, $[\alpha]_{546}$= +63.0. $[\alpha]_D^{21}$= +35.0 (c 1, acetone), $[\alpha]_{578}$= +36.3, $[\alpha]_{546}$= +41.6, $[\alpha]_{436}$= +71.3, $[\alpha]_{365}$= +111.7 . ^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.38-7.27 (m, 5H, Ar*H*), 7.18 (m, 1H, N*H*), 6.34 (d, 1H, N*H*, J = 7.5), 5.77 and 5.76 (2s, 2H, = CH_2), 5.07 (dq, 1H, C*H*-Ar, J = 7.3), 3.75 (m, 2H, N*H*, + α-C*H*), 2.83 and 2.76 (2dd, 2H, CH_2, J = 5.6, 14.6, and 3.6), 1.53 (d, 3H, Ar-C-CH_3, J = 7.1), 0.76 and 0.74 (2t, 6H, B[CH_2-CH_3]$_2$, J = 7.6), 0.33 (m, 4 H, B[CH_2-CH_3]$_2$).

Analytical data for the 2S,1'R-diastereoisomer (**3f**): M.p. 173° C (unchanged after recrystallization in $CHCl_3$-hexane). Anal calc % for $C_{18}H_{27}N_2O_3B$: C 65.47, H 8.24, N 8.48; found C 64.89, H 8.19, N 8.43. $[\alpha]_D^{21}$= +17.9 (c 1, $CHCl_3$-EtOH, 9:1),$[\alpha]_{578}$= +19.6, $[\alpha]_{546}$= +23.4, $[\alpha]_{436}$= +54.0. $[\alpha]_D^{21}$= +5.0 (c 1, acetone), $[\alpha]_{578}$= +5.3, $[\alpha]_{546}$ = +6.7, $[\alpha]_{436}$ = +17.8, $[\alpha]_{365}$= +43.0 .
^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.46-7.18 (m, 6H, Ar*H* + N*H*), 6.27 (d, 1H, N*H*, J = 7.1), 5.79 and 5.74 (2s, 2H, =CH_2), 5.09 (dq, 1H, C*H*-Ar, J = 7.1), 3.80 (m, 2H, α-C*H* + N*H*), 2.87 and 2.79 (2dd, 2H, CH_2, J = 5.9, 14.6, and 4.4 Hz), 1.54 (d, 3H, Ar-C-CH_3, J = 6.9), 0.74 and 0.65 (2t, 6H, B[CH_2-CH_3]$_2$, J = 7.8), 0.38-0.19 (m, 4H, B[CH_2-CH_3]$_2$) .

The separated derivatives **2f** and **3f** were hydrolysed (6N HCl, 110°C, 18 hours) and (R)- and (S)-4-methyleneglutamic acids (**1f**) were purified as described previously.

Analytical data for the R-enantiomer (from **2f**, yield 66%): M.p. 188°C (lit. [61] 196-199°C). $[\alpha]_D^{21}$= –12.5 (c 1, 5 N HCl) [lit. [61] –7.9 (c 1, 3N HCl)], $[\alpha]_{578}$= –13.1, $[\alpha]_{546}$= –15.4, $[\alpha]_{436}$= –31.6, $[\alpha]_{365}$= –63.3. E.e. = 98 % (by GC after derivatization [59]).

Analytical data for the S-enantiomer (from **3f**, yield 71%): M.p. 196-197°C (lit. 196°C dec [57]; 195-197°C [62]; 192-195°C [63]; 188-190°C dec. [64]) $[\alpha]_D^{21}$= +12.8 (c 1, 5 N HCl) [lit. +12.8 (11% w/v HCl) [24]; +15.0 (c 1, 5N HCl) [27]; +13.2 (c 0.56, 5 N HCl) [63]; +14.6 (c 0.5, 5N HCl) [64]]. $[\alpha]_{578}$= +13.3, $[\alpha]_{546}$= +15.7, $[\alpha]_{436}$= +31.8, $[\alpha]_{365}$= +63.4. E.e. = 94 % (by GC after derivatization [59])

Resolution of (±)-*threo*-3-methylglutamic acid (1c) using R-(+)-1-phenylethylamine

The mixture of boroxazolidone diastereoisomers was prepared as previously described by the mixed anhydride method, on a 1 mmole scale. Separation was achieved on a silica gel (50 g) column eluting with CH_2Cl_2-acetone (85:15).The 2R,3S,1'R-isomer **2c** (0.0713 g, 0.21 mmole) was eluted first, then a mixed fraction (0.42 mmole), and last the 2S,3R,1'R-isomer **3c** (0.031 g, 0.093 mmole) with a total yield of 67% from 3-methylglutamic acid. Part of the mixture fraction (0.116 g, 0.35 mmole) was rechromatographed (25 g of silica gel, eluant CH_2Cl_2-*i*-PrOH 96:4, 250 ml, and 95:5, 200 ml) to give 0.027 g of the 2R,3S,1'R-isomer, 0.050 g of mixture and 0.023 g of the 2S,3R,1'R-isomer.

Analytical data for the 2R,3S,1'R-diastereoisomer (**2c**): M.p. 168°C. Anal calc % for $C_{18}H_{29}N_2O_3B$, C 65.07, H 8.80, N 8.43; found C 65.13, H 8.84, N 8.57. $[\alpha]_D^{21}$= +73.5 (c 1 acetone), $[\alpha]_{578}$= +76.7, $[\alpha]_{546}$= +88.8, $[\alpha]_{436}$= +162, $[\alpha]_{365}$= +281. ^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.35-7.21 (m, Ar*H*, 5H), 6.58 (d, 1H, N*H*, J = 7.6), 6.34 (br t, 1H, N*H*, J = 10.2), 5.01 (dq, 1H, C*H* -Ar, J = 7.1), 4.22 (br t, 1H, N*H*, J = 10.1), 3.65 (dt, 1H, α-C*H* , J = 2.2 and 8.8), 2.60-2.39 (m, 3H, C*H*-CH_2), 1.47 (d, 3H, Ar-C-CH_3, J =

7.1), 1.21 (d, 3H, β-CH_3, J = 6.6), 0.79 and 0.74 (2 t , 6H, B-[CH_2-CH_3]$_2$, J = 7.8), 0.41-0.26 (m, 4H, B-[CH_2-CH_3]$_2$).

Analytical data for the 2S,3R,1'R-diastereoisomer (**3c**): glass. Anal calc % for $C_{18}H_{29}N_2O_3B$, C 65.07, H 8.80, N 8.43; found %, C 64.34, H 8.80, N 8.43. $[\alpha]_D^{21}$= +40.7 (c 1, acetone), $[\alpha]_{578}$= +42.5, $[\alpha]_{546}$= +49.7, $[\alpha]_{436}$= +95.4, $[\alpha]_{365}$= +175.3. ^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.34-7.21 (m, Ar*H*, 5H), 6.48 (br s, 1H, N*H*), 5.91 (br t, 1H, N*H*, J = 10.2), 5.00 (dq, 1H, C*H*-Ar, J = 7.1), 4.37 (br m, 1H, N*H*), 3.73 (dt, 1H, α-C*H*, J = 2.3 and 8.6), 2.66-2.37 (m, 3H, C*H*-CH_2), 1.46 (d, 3H, Ar-C-CH_3, J = 7.1), 1.14 (d,3H, β-CH_3, J = 7.0), 0.74 and 0.66 (2 t, 6H, B-[CH_2-CH_3]$_2$), J = 7.8), 0.37-0.18 (m, 4H, B-[CH_2-CH_3]$_2$)

An analytical sample of each diastereoisomer was hydrolysed in 4N HCl for 18h at 100°C, dried and derivatized to determine enantiomeric excess by GC [59]: from isomer **2c** was obtained (2R,3S)-*threo*-3-methylglutamic acid, e.e. = 95.4 %; from isomer **3c**, (2S,3R)-*threo*-3-methylglutamic acid, e.e. = 93.4 % .

Resolution of (±)-*threo*-3-methylglutamic acid (1c) using R-(−)-2-amino-2-phenylethanol

The (±)-*threo*-3-methylglutamic-boroxazolidone complex was coupled on a 4 mmoles scale with R-(−)-2-phenylglycinol by the Brop method to yield diastereoisomers **4c** and **5c** (67 % yield from **1c**) which were separated (2S,3S,2'R first eluted) on a silicagel column eluted with CH_2Cl_2-acetone (65:35).

Analytical data for the 2R,3S,2'R-diastereoisomer (**4c**): glass. $[\alpha]_D^{21}$= −18.7 (c 1, acetone), $[\alpha]_{578}$= −19.7, $[\alpha]_{546}$= −23.1, $[\alpha]_{436}$= −46.3, $[\alpha]_{365}$= −88.1. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.74 (br.d, 1H, N*H*, J = 7.6), 7.38-7.22 (m, 5H, Ar*H*), 6.11 (m, 1H, N*H*), 5.67 (m, 1H, N*H*), 5.04 (br.q 1H, C*H*-Ar, J = 6.8), 3.90-3.74 (m, 3H, α-C*H*+CH_2OH), 2.80 (s, 1H, O*H*), 2.65-2.51 (m, 3H, β-C*H*+γ-CH_2), 1.15 (d,3H, β-CH_3, J = 6.8), 0.74 and 0.69 (2t, 6H, B-[CH_2 - CH_3]$_2$, J = 7.7), 0.34-0.21 (m, 4H, B-[CH_2 - CH_3]$_2$).

Analytical data for the 2S,3R,2'R-diastereoisomer (**5c**): glass. $[\alpha]_D^{21}$= −51.2 (c 1.1, acetone), $[\alpha]_{578}$= −53.5, $[\alpha]_{546}$= −61.9, $[\alpha]_{436}$= −113.1, $[\alpha]_{365}$= −194.3. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.76 (br.d, 1H, N*H*, J = 7.2), 7.38-7.23 (m, 5H, Ar*H*), 6.26 (m, 1H, N*H*), 5.62 (m, 1H, N*H*), 5.04 (br.q, 1H, C*H*-Ar, J = 6.6), 3.77 (m, 3H, α-C*H*+CH_2OH), 2.81 (s, 1H, O*H*), 2.73-2.48 (m, 3H, β-C*H*+γ-CH_2), 1.16 (d, 3H, β-CH_3, J = 6.8), 0.78 and 0.74 (2 t, 6H, B-[CH_2 - CH_3]$_2$, J = 7.8), 0.34 (m, 4H, B-[CH_2 - CH_3]$_2$).

Hydrolysis of **4c** and **5c** was performed in 2N HCl at 80°C for 12 hours; pure amino acids and recovered (R)-phenylglycinol were separated by anion exchange chromatography (see above).

Analytical data for the (2R,3S)-3-methylglutamic acid (from **4c**, yield: 72 %): M.p. 164°C. $[\alpha]_D^{21}$= −12.0 (c 1, H_2O), $[\alpha]_{578}$= −12.5, $[\alpha]_{546}$= −14.5, $[\alpha]_{436}$= −27.0, $[\alpha]_{365}$= −47.5. *Threo/erythro*, 99.6:0.4. E.e. = 97 % (by GC after derivatization [59]).

Analytical data for the (2S,3R)-3-methylglutamic acid (from **5c**, yield: 71 %): M.p. 163°C. $[\alpha]_D^{21}$= +14.4 (c 1, H_2O), $[\alpha]_{578}$= +15.0, $[\alpha]_{546}$= +17.0, $[\alpha]_{436}$= +30.7, $[\alpha]_{365}$= +53.9. *Threo/erythro*, 98:2. E.e. = 98 % (by GC after derivatization [59]). 2S,3R-monoammonium salt, m.p. 161°C; $[\alpha]_D^{21}$= +8.5 (c 1.1, H_2O) (lit.[21] $[\alpha]_D^{21}$= +6.0)

Resolution of (±)-*erythro*-3-methylglutamic acid (1b) using R-(−)-2-amino-2-phenylethanol

The (±)-*erythro*-3-methylglutamic-boroxazolidone complex was coupled on a 4 mmoles scale with R-(−)-2-phenylglycinol by the Brop method to yield diastereoisomers **4b** and **5b** (62 % yield from **1b**) which were separated (2S,3S,2'R first eluted) on a silicagel column (CH_2Cl_2-acetone, 65:35)

Analytical data for the 2R,3R,2'R-diastereoisomer (**4b**): M.p. 144°C. $[\alpha]_D^{21}$= −54.0 (c 0.5, MeOH), $[\alpha]_{578}$= −55.0, $[\alpha]_{546}$= −61.0, $[\alpha]_{436}$= −107.2, $[\alpha]_{365}$= −184.0. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.89 (m, 1H, N*H*), 7.33-7.23 (m, 5H, Ar*H*), 7.12 (br.s, 1H, N*H*), 5.64 (br s, 1H, N*H*), 5.04 (m, 1H, C*H*-Ar), 3.76 (m, 3H, α-C*H*+CH_2OH), 2.78 (s, 1H, O*H*), 2.77-2.30 (m, 3H, β-C*H*+γ-CH_2), 1.18 (d, 3H, β-CH_3, J = 6.4), 0.72 (m, 6H, B-[CH_2 - CH_3]$_2$), 0.32 (m, 4H, B-[CH_2 - CH_3]$_2$)

Analytical data for the 2S,3S,2'R-diastereoisomer (**5b**): M.p. 174°C. $[\alpha]_D^{21}$= −80.2 (c 1, acetone), $[\alpha]_{578}$= −83.5, $[\alpha]_{546}$= −96.0, $[\alpha]_{436}$= −172.2, $[\alpha]_{365}$= −289.4. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.98 (m, 1H, N*H*), 7.39-7.23 (m, 6H, Ar*H*+N*H*), 5.54 (br.s, 1H, N*H*), 5.02 (m, 1H, C*H*-Ar), 3.76-3.63 (m, 3H, α-C*H*+CH_2OH), 2.79 (s, 1H, O*H*), 2.78-2.44 (m, 3H, β-C*H*+γ-CH_2), 1.08 (d,3H, β-CH_3, J = 7.2), 0.79 and 0.74 (2t, 6H, B-[CH_2 - CH_3]$_2$, J = 7.6 and 8.0), 0.42-0.25 (m, 4H, B-[CH_2 - CH_3]$_2$)

Hydrolysis of **4b** and **5b** was performed in 2N HCl at 80°C for 12 hours; pure amino acids and recovered (R)-phenylglycinol were separated by anion exchange chromatography (see above).

Analytical data for (2R,3R)-3-methylglutamic acid (from **4b**, yield: 62 %): M.p. 170°C. $[\alpha]_D^{21}$= −15.6 (c 1, H_2O), $[\alpha]_{578}$= −16.3, $[\alpha]_{546}$= −18.7, $[\alpha]_{436}$= −34.1, $[\alpha]_{365}$= −59.2. No *threo* isomer detected. E.e. ≥ 99.5 % (by GC after derivatization [59]).

Analytical data for (2S,3S)-3-methylglutamic acid (from **5b**, yield: 65 %): M.p. 180°C. $[\alpha]_D^{21}$= +15.2 (c 1, H_2O), $[\alpha]_{578}$= +15.9, $[\alpha]_{546}$= +18.3, $[\alpha]_{436}$= +33.8, $[\alpha]_{365}$= +59.2. *Erythro/threo*, 99.4:0.6. E.e. ≥ 99.5 %

(by GC after derivatization [59])

Resolution of (±)-*erythro*-4-methylglutamic acid (1d) using R-(+)-1-phenylethylamine

The mixture of boroxazolidone diastereoisomers was prepared from (±)-*erythro*-4-methylglutamic acid (**1d**) by the mixed anhydride method,as previously described, on a 2 mmoles scale. Separation was achieved on a silica gel (50 g) column, eluting with CH_2Cl_2-acetone (86:14) the 2R,4S,1'R isomer **2d** (0.248 g, 0.75 mmole), then a mixed fraction (0.1 g, 0.3 mmole), and, with CH_2Cl_2-acetone (82:18), the 2S,4R,1'R isomer **3d** (0.192 g, 0.58 mmole). Total yield: 81% from **1d**.

Analytical data for the 2R,4S,1'R-diastereoisomer (**2d**): M.p. 185°C. $[\alpha]_D^{21}$= +92.9 (c 1, acetone), $[\alpha]_{578}$= +96.9, $[\alpha]_{546}$= +111.4, $[\alpha]_{436}$= +201, $[\alpha]_{365}$= +343. ^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.34-7.21 (m, 6H, Ar*H* + N*H*), 6.65 (br.s, 1H, N*H*), 6.17 (br.s, 1H, N*H*), 5.01 (dq, 1H, C*H*-Ar, J = 7.1), 3.80 (m, 1H, α-CH), 2.69 (m, 1H, γ-C*H*), 2.23-2.02 (m, 2H, β-C*H*$_2$), 1.44 (d, 3H, Ar-CH-C*H*$_3$, J = 6.9), 1.20 (d, 3H, γ-C*H*$_3$, J = 7.0), 0.76 and 0.71 (2 t, 6H, B-[CH$_2$-C*H*$_3$]$_2$, J = 7.7), 0.39-0.27 (m, 4 H, B-[C*H*$_2$-CH$_3$]$_2$).

Analytical data for the 2S,4R,1'R-diastereoisomer (**3d**): M.p. 76°C. $[\alpha]_D^{21}$= +28.6 (c 1 acetone), $[\alpha]_{578}$= +30.0, $[\alpha]_{546}$= +35.1, $[\alpha]_{436}$= +70, $[\alpha]_{365}$= +133. ^{1}H NMR ($CDCl_3$) δ ppm, J Hz: 7.33-7.20 (m, 6H, Ar*H* +N*H*), 6.34 (br.s, 1H, N*H*), 6.06 (br.s, 1H, N*H*), 4.99 (dq, 1H, C*H*-Ar, J = 7.0), 3.79 (m, 1H, α-C*H*), 2.60 (m, 1H, γ-C*H*), 2.18 (ddd, 1H, β-C*H*, J = 2.9, 8.8, and 15.3), 1.98 (ddd, 1H, β-C*H*, J = 2.1, 7.3, and 15.3), 1.44 (d, 3H, Ar-CH-C*H*$_3$, J = 7.0), 1.23 (d, 3H, γ-C*H*$_3$, J = 7.2), 0.70 and 0.64 (2t, 6H, B-[CH$_2$-C*H*$_3$]$_2$, J = 7.8), 0.32-0.14 (m, 4H, B-[C*H*$_2$-CH$_3$]$_2$.

2d (0.233g, 0.7 mmol) was refluxed in methanol (100 ml) for 8 h, then evaporated to dryness and heated in 4N HCl (40 ml) at 100°C for 15 h . The residue, containing a partially epimerized mixture [22] of *threo*- and *erythro* 4-methylglutamic acids (**1e** and **1d**, 22:78) was chromatographed on an AG-1X4 (AcO^-) column (2.5 x 12.5 cm). After washing with boiled water (200 ml), elution with 0.15 N acetic acid afforded the *threo* isomer (2R,4R)-**1e** (7.5 mg), a mixed fraction (27.4 mg) and 60 mg of pure *erythro* isomer (2R,4S)-**1d** (total yield: 88 %); e.e. (by GC after derivatization [59]) = 97 % . $[\alpha]_D^{21}$= –19.8 (c 1, 6N HCl) [lit.for the (2S,4R)-isomer [22] +23 (c 2.5, 6N HCl)], $[\alpha]_{578}$= –20.6, $[\alpha]_{546}$= –23.8, $[\alpha]_{436}$= –44.9, $[\alpha]_{365}$= –81.5.

Resolution of (±)-*erythro*-4-methylglutamic acid (1d) using R-(–)-2-amino-2-phenylethanol

The (±)-*erythro*-4-methylglutamic-boroxazolidone complex was coupled on a 2 mmoles scale with R-(–)-2-phenylglycinol by the Brop method to yield diastereoisomers **4d** and **5d** (51 % yield from **1d**) which were separated (2S,4R,2'R first eluted) on a silicagel column (CH_2Cl_2-acetone 6:4).

Analytical data for the 2R,4S,2'R-diastereoisomer (**4d**): M.p.190°C. $[\alpha]_D^{21}$= –15.0 (c 1, acetone-MeOH, 1:1), $[\alpha]_{578}$= –15.7, $[\alpha]_{546}$= –18.4, $[\alpha]_{436}$= –38.2. ^{1}H NMR (acetone-D$_6$) δ ppm, J Hz: 7.67 (br.s, 1H, N*H*), 7.38-7.22 (m, 5H, Ar*H*), 6.05 (br.s, 1H, N*H*), 5.72 (br.s, 1H, N*H*), 5.04 (br.q, 1H, C*H*-Ar, J = 6.6), 3.78-3.71 (m, 3H, α-C*H*+ C*H*$_2$OH), 2.79 (m+s, 2H, γ-C*H*+O*H*), 2.27 (ddd, 1H, β-C*H*, J = 3.4, 9.4, and 14.8), 1.83 (ddd, 1H, β-C*H*, J = 3.3, 4.9, and 14.9), 1.26 (d, 3H, γ-C*H*$_3$, J = 7.2), 0.74 and 0.67 (2t , 6H, B-[CH$_2$-C*H*$_3$]$_2$, J = 7.9 and 8.0), 0.25 (m, 4H, B-[C*H*$_2$-CH$_3$]$_2$).

Analytical data for the 2S,4R,2'R-diastereoisomer (**5d**): glassy solid. $[\alpha]_D^{21}$= –58.5 (c 1, acetone-MeOH, 9:1), $[\alpha]_{578}$= –61.3, $[\alpha]_{546}$= –70.4, $[\alpha]_{436}$= –126.9, $[\alpha]_{365}$= –215.3. ^{1}H NMR (acetone-D$_6$) δ ppm, J Hz: 7.78 (br.s, 1H, N*H*), 7.35-7.24 (m, 5H, Ar*H*), 6.00 (br.s, 1H, N*H*), 5.77 (br.s, 1H, N*H*), 5.07 (m, 1H, C*H*-Ar), 3.78 (m, 3H, α-C*H*+ C*H*$_2$OH), 2.90 (m+s, 2H, γ-C*H*+O*H*), 2.77 (m, 1H, β-C*H*), 1.90 (m, 1H, β-C*H*), 1.22 (d, 3H, γ-C*H*$_3$, J = 7.0), 0.80 and 0.77 (2 t , 6H, B-[CH$_2$-C*H*$_3$]$_2$, J = 7.0 and 7.6), 0.33 (m, 4H, B-[C*H*$_2$-CH$_3$]$_2$).

Hydrolysis of **4d** and **5d** was performed in 2N HCl at 80°C for 12 hours; pure amino acid and recovered (R)-phenylglycinol were separated by anion exchange chromatography (see above).

Analytical data for (2R,4S)-4-methylglutamic acid (from **4d**): M.p.169°C. $[\alpha]_D^{21}$= –20 (c 0.6, 6N HCl), $[\alpha]_{578}$= –20.7, $[\alpha]_{546}$= –24.5, $[\alpha]_{436}$= –47.2, $[\alpha]_{365}$= –87.2. No *threo* isomer detected. E.e = 95 % (by GC after derivatization [59]).

Analytical data for (2S,4R)-4-methylglutamic acid (from **5d**): M.p.160°C. $[\alpha]_D^{21}$= +19.9 (c 0.8, 6N HCl), $[\alpha]_{578}$= +20.8, $[\alpha]_{546}$= +23.9, $[\alpha]_{436}$= +44.9. *Erythro:threo*: 99.6:0.4. E.e = 96.2 % (by GC after derivatization [59]).

Resolution of (±)-*threo*-4-methylglutamic acid (1e) using R-(–)-2-amino-2-phenylethanol

The (±)-*threo*-4-methylglutamic-boroxazolidone complex was coupled on a 1 mmole scale with R-(–)-2-phenylglycinol by the Brop method to yield diastereoisomers **4e** and **5e** (62 % yield from **1e**) which were separated (2R,4R,2'R first eluted) on a silicagel column as described for the *erythro* derivatives.

Analytical data for the 2R,4R,2'R-diastereoisomer (**4e**): glass. $[\alpha]_D^{21}$= –62.5 (c 1.3, acetone), $[\alpha]_{578}$= –

65.5, $[\alpha]_{546}$= –75.7, $[\alpha]_{436}$= –139.8, $[\alpha]_{365}$= –244.5. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.89 (br.d, 1H, N*H*, J = 8.4), 7.39-7.24 (m, 5H, Ar*H*), 6.08 (m, 1H, N*H*), 5.16 (dt, 1H, C*H*-Ar, J = 4.6 and 8.6), 5.05 (m, 1H, N*H*), 3.84 (dd, 1H, C*H*OH, J = 4.5 and 10.9), 3.71(dd+m, 2H, C*H*OH+α-C*H*, J = 8.7 and 10.9), 2.85 (m+s, 2H, γ-C*H*+O*H*), 2.13-1.98 (m, 2H, β-CH_2), 1.16 (d, 3H, γ-CH_3, J = 7.0), 0.77 and 0.73 (2t , 6H, B-[CH_2-CH_3]$_2$, J = 8.0 and 7.8), 0.40-0.27 (m, 4H, B-[CH_2-CH_3]$_2$).

Analytical data for the 2S,4S,2'R- diastereoisomer (**5e**): glass. $[\alpha]_D^{21}$= –9.8 (c 1.3, acetone), $[\alpha]_{578}$= –10.4, $[\alpha]_{546}$= –12.2, $[\alpha]_{436}$= –24.5, $[\alpha]_{365}$= –46.4. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.67 (br.d, 1H, N*H*, J = 7.4), 7.65-7.24 (m, 5H, Ar*H*), 5.66 (m, 1H, N*H*), 5.37 (m, 1H, N*H*), 5.03 (m, 1H, C*H*-Ar), 3.80 (m, 2H, CH_2OH), 3.55 (m, 1H, α-C*H*), 2.96 (m, 1H, γ-C*H*), 2.85 (s, 1H, O*H*), 2.15-1.94 (m, 2H, β-CH_2), 1.23 (d, 3H, γ-CH_3, J = 7.0), 0.77 and 0.71 (2t , 6H, B-[CH_2-CH_3]$_2$, J = 7.6 and 7.8), 0.41-0.21 (m, 4H, B-[CH_2-CH_3]$_2$).

Hydrolysis of **4e** and **5e** was performed in 2N HCl at 80°C for 12 hours; pure amino acid and recovered (R)-phenylglycinol were separated by anion exchange chromatography.

Analytical data for (2R,4R)-4-methylglutamic acid (from **4e**): M.p.147°C. $[\alpha]_D^{21}$= –21.6 (c 0.5, H_2O), $[\alpha]_{578}$= –22.2, $[\alpha]_{546}$= –25.4, $[\alpha]_{436}$= –43.8, $[\alpha]_{365}$= –69.0. *Threo/erythro*, 98.7:1.3. E.e.= 98 % (by GC after derivatization [59]).

Analytical data for (2S,4S)-4-methylglutamic acid (from **5e**): M.p.151°C [lit. [22] 170°C]. $[\alpha]_D^{21}$= +22.8 (c 0.25, H_2O), [lit. +25 (c 1, H_2O) [22]], $[\alpha]_{578}$= +23.6, $[\alpha]_{546}$= +26.8, $[\alpha]_{436}$= +45.2, $[\alpha]_{365}$= +71.6. No *erythro* isomer detected. E.e.= 98 % (by GC after derivatization [59]).

Resolution of (±)-*cis*-1-amino-1,3-cyclohexanedicarboxylic acid (6) using R-(–)-2-amino-2-phenylethanol

The (±)-boroxazolidone complex [38] was coupled on a 0.1 mmole scale with R-(–)-2-phenylglycinol by the Brop method to yield diastereoisomers which were separated (1R,3R,2'R first eluted) on a silicagel column (CH_2Cl_2-acetone 6:4).

Analytical data for the 1R,3R,2'R-diastereoisomer: glass. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.34-7.20 (m, 6H, Ar*H*+N*H*), 5.40 (br.s, 2H, 2 N*H*), 4.98 (m, 1H, C*H*-Ar), 3.76 (m, 2H, CH_2OH), 2.91 (s, 1H, O*H*), 2.72 (m, 1H, C*H*-CO_2H), 2.25-1.80 (m, 7H, CH_2), 1.45 (m, 1H, CH_2), 0.75 and 0.74 (2 t , 6H, B-[CH_2-CH_3]$_2$, J = 7.6 and 7.8), 0.34 (m, 4H, B-[CH_2-CH_3]$_2$).

Analytical data for the 1S,3S,2'R-diastereoisomer: M.p.105°C. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.36-7.20 (m, 6H, Ar*H*+N*H*), 5.36 (br.s, 2H, 2 N*H*), 4.99 (m, 1H, C*H*-Ar), 3.76 (m, 2H, CH_2OH), 2.79 (s, 1H, O*H*), 2.78-2.60 (m, 1H, C*H*-CO_2H), 2.14-1.65 (m, 7H, CH_2), 1.65-1.46 (m, 1H, CH_2), 0.76 and 0.75 (2 t , 6H, B-[CH_2-CH_3]$_2$, J = 7.8), 0.34 (m, 4H, B-[CH_2-CH_3]$_2$).

Hydrolysis was performed in 2N HCl at 80°C for 12 hours. From the 1R,3R,2'R isomer was obtained (1R,3R)-**6** (e.e.= 98 %) and from the 1S,3S,2'R isomer, (1S,3S)-**6** (e.e.= 98 %, determined by HPLC after derivatization [44]).

Resolution of (±)-*cis*-1-amino-1,3-cyclopentanedicarboxylic acid (8) using R-(–)-2-amino-2-phenylethanol

The (±)-boroxazolidone complex [38] was coupled on a 0.1 mmole scale with R-(–)-2-phenylglycinol by the Brop method to yield diastereoisomers which were separated (1R,3R,2'R first eluted) on a silicagel column (CH_2Cl_2-acetone 1:1).

Analytical data for the 1R,3R,2'-diastereoisomer: glass. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.41-7.18 (m, 6H, Ar*H*+N*H*), 5.52 (br.s, 2H, 2 N*H*), 5.02 (m, 1H, C*H*-Ar), 3.75 (m, 2H, CH_2OH), 3.14 (m, 1H, C*H*-CO_2H), 2.78 (s, 1H, O*H*), 2.52 (m, 1H, CH_2), 2.34 (m, 2H, CH_2), 2.19 -2.02 (m, 3H, CH_2), 0.76 and 0.75 (2 t , 6H, B-[CH_2-CH_3]$_2$, J = 7.6), 0.34 (m, 4H, B-[CH_2-CH_3]$_2$).

Analytical data for the 1S,3S,2'R-diastereoisomer: glass. ^{1}H NMR (acetone-D_6) δ ppm, J Hz: 7.42 (br.d, 1H, N*H*, J = 7.6), 7.37-7.17 (m, 5H, Ar*H*), 5.52 (br.s, 2H, 2 N*H*), 5.02 (m, 1H, C*H*-Ar), 3.75 (m, 2H, CH_2OH), 3.17 (m, 1H, C*H*-CO_2H), 2.79 (s, 1H, O*H*), 2.59-2.41 (m, 1H, CH_2), 2.40-2.21 (m, 2H, CH_2), 2.18 -2.03 (m, 3H, CH_2), 0.75 (t , 6H, B-[CH_2-CH_3]$_2$, J = 7.6), 0.35 (m, 4H, B-[CH_2-CH_3]$_2$).

After hydrolysis in 2N HCl at 80°C for 12 hours, the 1R,3R,2'R isomer yielded (1R,3R)-**8** (e.e.= 96 %) and the 1S,3S,2'R isomer, (1S,3S)-**8** (e.e.= 95 %, determined by HPLC after derivatization [44]).

Resolution of (±)-*trans*-1-amino-1,3-cyclopentanedicarboxylic acid (9) using R-(–)-2-amino-2-phenylethanol

The (±)-boroxazolidone complex [38] was coupled on a 0.1 mmole scale with R-(–)-2-phenylglycinol by the Brop method to yield diastereoisomers which were separated (1S,3R,2'R first eluted) on a silicagel column

(CH_2Cl_2-acetone 6:4).

Analytical data for the 1R,3S,2'R-diastereoisomer: glass. ^1H NMR (acetone-D_6) δ ppm, J Hz: 7.96 (br.d, 1H, N*H*, J = 8.0), 7.42-7.20 (m, 5H, Ar*H*), 6.88 (br.d, 1H, N*H*, J = 11.2), 5.06-4.94 (m, 2H, C*H*-Ar+N*H*), 3.77 (m, 2H, CH_2OH), 3.35 (m, 1H, C*H*-CO_2H), 2.80 (s, 1H, O*H*), 2.44 (m, 1H, CH_2), 2.27-1.89 (m, 5H, CH_2), 0.75 (t , 6H, B-[CH_2-CH_3]$_2$, J = 7.6), 0.34 (m, 4H, B-[CH_2-CH_3]$_2$).

Analytical data for the 1S,3R,2'R-diastereoisomer: glass. ^1H NMR (acetone-D_6) δ ppm, J Hz: 8.03 (br.d, 1H, N*H*, J = 7.6), 7.39-7.23 (m, 5H, Ar*H*), 6.92 (br.d, 1H, N*H*, J = 11.2), 5.01 (m, 1H, C*H*-Ar), 4.90 (br.d, 1H, N*H*, J= 12.4), 3.75 (m, 2H, CH_2OH), 3.36 (m, 1H, C*H*-CO_2H), 2.82 (s, 1H, O*H*), 2.67-1.97 (m, 5H, CH_2), 1.76 (m, 1H, CH_2), 0.81 and 0.72 (2t , 6H, B-[CH_2-CH_3]$_2$, J = 7.8 and 7.6), 0.35 (m, 4H, B-[CH_2-CH_3]$_2$).

After hydrolysis in 2N HCl at 80°C for 12 hours, the 1S,3R,2'R isomer yielded (1S,3R)-**9** (e.e.≥ 99 %) and the 1R,3S,2'R isomer, (1R,3S)-**9** (e.e.= 88 %, determined by HPLC after derivatization [44]).

Hydrogenolysis of 2a.

a) catalytic hydrogenation : to (2R,1'R)-**2a** (0.209 g, 0.63 mmol) dissolved in methanol (5 ml) were added Pd/C (10%, 0.209 g) and ammonium formate (5 eq). After overnight stirring, only decomplexation was observed; the catalyst was filtered, formic acid (10 ml) was added to the evaporated filtrate, and the resulting solution was heated at 90°C for 8 hours. The single product formed was identified to 2-methylpyroglutamic acid **10** by comparison with an authentic sample: M.p.142°C (lit. [65] 140-143°C).

b) Li/NH3 reductive cleavage: to (2R,1'R)-**2a** (0.201 g, 0.6 mmol) dissolved in liquid ammonia (about 10 ml) was added lithium (50 mg) producing a blue color visible for 10-20 min. After evaporation of ammonia, the residue was purified by ion exchange chromatography, first on an AG50WX4 (H^+, 20-50 mesh) column, eluted with 0.5 M NH_4OH, then on an AG1X4 (AcO^-, 200-400 mesh) column, eluted with 0.05 N AcOH. Crystalline 5-hydroxynorvaline **11** (32.1 mg) was obtained, M.p.216-217°C (after recrystallisation in methanol-ether); this compound (R_f 0.13) slightly differed from 2-methylglutamine [18] (R_f 0.12) by TLC (CH_2Cl_2-MeOH-aqueous 28 % NH_4OH, 65:35:10) or reverse phase HPLC (Nucleosil $5C_{18}$ column, 0.4 x 30 cm, 0.1 M Na phosphate buffer, 0.5 ml/min): retention time, 7.0 min (6.8 min for 2-methylglutamine).

^1H NMR (DMSO-D_6) δ ppm, J Hz: 6.80 (br.s, 3H, NH_3), 3.32 (t, 2H, CH_2OH, J = 5.9), 3.15 (s, 1H, O*H*), 165-1.30 (m, 4H, 2 CH_2), 1.21 (s, 3H, α-CH_3). ^{13}C NMR (DMSO-D_6+D_2O) δ ppm: 175.59 (CO), 62.02 (CH_2OH), 61.58 (C-2), 35.21 and 27.65 (C-3 and C-4), 23.90 (α-CH_3). CIMS (NH_3): 148 $[M+1]^+$ (100), 130 (13), 128 (26), 102 $[M-CO_2H]^+$ (74), 88 (15), 85 (18), 84 (14).

References:

1. Jung, M. J. In *Chemistry and Biochemistry of the Amino Acids*, Barrett, G. C. Ed; Chapman and Hall: New York, 1985; pp. 227-245.
2. Sukling, C. J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 537-.
3. Wipf, P.; Heimgartner, H. *Helv. Chim. Acta* **1988**, *71*, 258-267.
4. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, M.; Cavicchioni, G.; Precigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061-1072.
5. Bardi, R.; Pizzesi, A. M.; Toniolo, C.; Sukumar, M.; AntonyRaj, P.; Balaram, P. *Int. J. Peptide Protein Res.* **1985**, *25*, 628-639. Toniolo, C.; Benedetti, E. *ISI Atlas of Sci: Biochem.* **1988**, 225-230. Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 487-491. Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747-6756. Altmann, K. H.; Altmann, E.; Mutter, M. *Helv. Chim. Acta* **1992**, *75*, 1198-1210.
6. Prasad, B. V. V.; Balaram, P. *Crit. Rev. Biochem.* **1984**, *16*, 307-348. Mapelli, C.; Kimura, H.; Stammer, C. H. *Int. J. Peptide Protein Res.* **1986**, *28*, 347-359.
7. Mendel, D.; Ellman, J.; Shultz, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 4359-4360 and references therein.
8. Johnson, R. L.; Koerner, J. F. *J. Med. Chem.* **1988**, *31*, 2057-2066. Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol.Toxicol.* **1989**, *29*, 365-402.Watkins, J. C.; Krogsgaard-Larsen, P.; Tage, H. *Trends Pharmacol. Sci.* **1990**, *11*, 25-33. Barnard, E. A.; Henley, J. M. *Trends Pharmacol. Sci.* **1990**, *11*, 500-507. Schoepp, D.; Bockaert, J ; Sladeczek, F. *Trends Pharmacol. Sci.* 1990, *11*, 508-515. Dingledine, R. *Trends Pharmacol. Sci.* **1991**, *12*, 360-362.
9. Meister, A. *Harvey Lect.* **1969**, *63*, 139-178.
10. Rich, D. H.; Lehrman, S. R.; Kawai, M.; Goodman, H. L.; Suttie, J. W. *J. Med. Chem.* **1981**, *24*, 706-711. Azerad, R.; Decottignies-LeMaréchal, P.; Ducrocq, C.; Righini-Tapie, A.; Vidal-Cros, A.; Bory, S.; Dubois, J.; Gaudry, M.; Marquet, A. In *Current Advances in Vitamin K Research*; Suttie, J. W. Ed; Elsevier: New york, 1988; pp. 17-24.

11. Rosowsky, A.; Bader, H.; Fresheim, J. H. *J. Med. Chem.* **1991**, *34*, 203-208. Abraham, A.; McGuire, J. J.; Galivan, J.; Nimec, Z.; Kisliuk, R. L.; Gaumont, Y.; Nair, M. G. *J. Med. Chem.* **1991**, *34*, 222-227.
12. Rogers, H. T.; Perkins, H. R.; Ward, J. B. *Microbial cell walls and membranes*; Chapman & Hall: London, 1980. Pratviel-Sosa, F.; Mengin-Lecreux, D.; VanHeijenoort, J. *Eur. J. Biochem.* **1991**, *202*, 1169-1176. Pratviel-Sosa, F.; Acher, F.; Trigalo, F.; Blanot, D.; Azerad, R.; VanHeijenoort, J. *FEMS Microbiol. Lett.* **1994**, *115*, 223-228.
13. Yamamoto, Y.; Kirihata, M.; Ichimoto, I.; Ueda, H. *Agric. Biol. Chem.* **1985**, *49*, 1761-1765.
14. Aebi, J. D.; Seebach, D. *Helv. Chim. Acta* **1985**, *68*, 1507-1518.
15. Altmann, E.; Nebel, K.; Mutter, M. *Helv.Chim.Acta* **1991**, *74*, 800-806.
16. Izumi, Y.; Tatsumi, S.; Imaida, M.; Fukuda, Y.; Akabori, S. *Bull. Soc. Chim. Jpn* **1965**, *38*, 1338-1340.
17. Baker, C. G.; Fu, S.-C. J.; Birnbaum, S. M.; Sober, H. A.; Greenstein, J. P. *J. Am. Chem. Soc.* **1952**, *74*, 4701-4702. Fu, S.-C. J.; Birnbaum, S. M. *J. Am. Chem. Soc.* **1953**, *75*, 918-920. Turk, J.; Panse, G. T.; Marshall, G. R. *J. Org. Chem.* **1975**, *40*, 953-955. Anantharamaiah, G. M.; Roeske, R. W. *Tetrahedron Lett.* **1982**, *23*, 3335-3336. Keller, J. W.; Hamilton, B. J. *Tetrahedron Lett.* **1986**, *27*, 1249-1250. Kruisinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. *J. Org.Chem.* **1988**, *53*, 1826-1827. Chenault, H. K.; Dahmer. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354-6364. Yee, C.; Blythe, T. A.; McNabb, T. J.; Walts, A. E. *J. Org. Chem.* **1992**, *57*, 3525-3527. Tian, Z.; Edwards, P.; Roeske, R. W. *Int. J. Peptide Protein Res.* **1992**, *40*, 119-126. Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Peters, P. J. H.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1113-1116.
18. Kagan, H. M.; Manning, L. R.; Meister, A. *Biochemistry* **1965**, *4*, 1063-1068.
19. Lalonde, J. J.; Bergbreiter, D. E.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 2323-2327.
20. Belokon, Y. N.; Bulychev, A. G.; Ryzhov, M. G.; Vitt, S. V.; Batsanov, A. S.; Struchkov, Y. T.; Bakhmutov, V. I.; Belikov, V. M. *J. Chem. Soc. Perkin Trans 1* **1986**, 1865-1872. Yanagida, M.; Hashimoto, K.; Ishida, M.; Shinozaki, H.; Shirahama, H. *Tetrahedron Lett.* **1989**, *30*, 3799-3802. Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1859-1866. Jako, I.; Uiber, P.; Mann, A.; Wermuth, C.-G.; Boulanger, T.; Norberg, B.; Evrard, G.; Durant, F. *J. Org. Chem.* **1991**, *56*, 5729-5733. Ouerfelli, O.; Ishida, M.; Shinozaki, H.; Nakanishi, K.; Ohfune, Y. *Synlett* **1993**, 409-410. Paz, M. M.; Sardina, F. J. *J. Org. Chem.* **1993**, *58*, 6990-6995. Moody, C. M.; Young, D. W. *Tetrahedron Lett.* **1993**, *34*, 4667-4670.
21. Suzuki, K.; Seebach, D. *Liebigs Ann.Chem.* **1992**, 51-61.
22. Righini-Tapie, A.; Azerad, R. *J. Appl. Biochem.* **1984**, *6*, 361-366.
23. Echalier, F.; Constant, O.; Bolte, J. *J. Org. Chem.* **1993**, *58*, 2747-2750.
24. Blake, J.; Fowden, L. *Biochem. J.* **1964**, *92*, 136-142.
25. Kagan, H. M.; Meister, A. *Biochemistry* **1966**, *5*, 2423.
26. Bory, S.; Dubois, J.; Gaudry, M.; Marquet, M.; Lacombe, L.; Weinstein, S. *J. Chem. Soc. Perkin Trans.1* **1984**, 475-480.
27. Guibé, E.; Decottignies-LeMaréchal, P.; LeMaréchal, P.; Azerad, R. *FEBS Lett.* **1984**, *177*, 265-268.
28. Gass, J. D.; Meister, A. *Biochemistry* **1970**, *9*, 842-846.
29. Gass, J. D.; Meister, A. *Biochemistry* **1970**, *9*, 1380-1389.
30. Stephani, R. A.; Rowe, W. B.; Gass, J. D.; Meister, A. *Biochemistry* **1972**, *11*, 4094-4100.
31. Stammer, C. H. *Tetrahedron* **1990**, *46*, 2231-2254, and references therein.
32. Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. *J. Org. Chem.* **1991**, *56*, 4167-4176.
33. Curry, K.; Peet, M. J.; Magnuson, D. S. K.; McLennan, H. *J. Med. Chem.* **1988**, *31*, 1076-1083.
34. Curry, K. *Can. J. Physiol. Pharmacol.* **1991**, *69*, 1076-1083.
35. Ohfune, Y.; Shimamoto, K.; Ishida, M.; Shinozaki, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 15-18.
36. Trigalo, F.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1988**, *29*, 6109-6112. Trigalo, F.; Buisson, D.; Acher, F.; Azerad, R. In *2nd Forum on Peptides*; Aubry, A.; Marraud, M. ; Vioux, B. Eds; Colloques INSERM/J.Libbey Eurotext Ltd: 1989; pp. 297-300.
37. Slama, J. T.; Satsangi, R. K.; Simmons, A.; Lynch, V.; Bolger, R. E.; Suttie, J. W. *J. Med. Chem.* **1990**, *33*, 824-832.
38. Acher, F.; Azerad, R. *Int. J. Peptide Protein Res.* **1991**, *37*, 210-219.
39. Williams, R. M. *Synthesis of optically active alpha–amino acids*; Pergamon: Oxford, 1989.
40. Bajgrowicz, J. A.; Cossec, B.; Pigière, C.; Jacquier, R.; Viallefont, P. *Tetrahedron Lett.* **1984**, *25*, 1789-1792. Obrecht, D.; Spiegler, C.; Schönholzer, P.; Müller, K.; Heimgartner, H. ; Stierli, F. *Helv. Chim. Acta* **1992**, *75*, 1666-1696.
41. Nefkens, G. H. L.; Zwanenburg, B. *Tetrahedron* **1983**, *39*, 2995-2998.
42. Meienhofer, J. In *The Peptides, analysis, synthesis, biology*, Gross, E. ; Meienhofer, J. Ed; Academic Press: New York, 1979; pp. 269-270.
43. Coste, J.; Dufour, M. N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669-672. Frerot, E.;

Coste, J.; Pantaloni, A.; Dufour, M. N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259-270. The Brop reagent generates a potential carcinogenic agent (HMPA); PyBrop (commercialized by Novabiochem, Meudon, France) may be alternatively used.
44. Maurs, M.; Trigalo, F.; Azerad, R. *J. Chromatogr.* **1988**, *440*, 209-215.
45. Helmchen, G.; Nill, G.; Flockerzi, P.; Schühle, W.; Youssef, M. S. K. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 62-63. Helmchen, G.; Nill, G.; Flockerzi, P.; Youssef, M. S. K. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 63-65.
46. Kristensen, E. P.; Larsen, L. M.; Olsen, O.; Sørensen, H. *Acta Chem. Scand. B* **1980**, *34*, 497-504.
47. Evans, D. A.; McGee, L. R. *J. Am. Chem. Soc.* **1982**, *103*, 2876-2878.
48. Giggand, R.; Conant, R. *Carbohyd. Res.* **1982**, *100*, C5?
49. Semple, J. E.; Wang, P.; Lysenko, Z.; Jouillé, M. *J. Am. Chem. Soc.* **1980**, *102*, 7505-7510.
50. Ram, S.; Spicer, L. D. *Tetrahedron Lett.* **1987**, *28*, 515-516.
51. Webster, F. X.; Millar, J. G.; Silverstein, R. M. *Tetrahedron Lett.* **1986**, *27*, 4941-4944.
52. Anwer, M. K.; Spatola, A. F.; Bossinger, C. D.; Flanigan, E.; Liu, R. C.; Olsen, D. B.; Stephenson, D. *J. Org. Chem.* **1983**, *48*, 3503-3507.
53. Trigalo, F.; Molliex, C.; Champion, B.; Azerad, R. *Tetrahedron Lett.* **1991**, *32*, 3049-3050.
54. Powell, G. K.; Dekker, E. E. *Prep. Biochem.* **1981**, *11*, 339-350.
55. Cocolas, G. H.; Hartung, W. H. *J. Am. Chem. Soc.* **1957**, *79*, 5203-5205.
56. Mauger, A. B. *J. Org. Chem.* **1981**, *46*, 1032-1035.
57. Done, J.; Fowden, L. *Biochem. J.* **1952**, *51*, 451-458.
58. VonArx, E.; Feyel, M.; Brugger, M. J. *J. Chromatogr.* **1976**, *120*, 224-228.
59. Maurs, M.; Ducrocq, C.; Righini-Tapie, A.; Azerad, R. *J. Chromatogr.* **1985**, *325*, 444-449.
60. Boroxazole derivatives are known to crystallize with all kind of molecules of solvents (DMF,DMSO, t-BuOMe, etc...) which were used for crystallization attempts (see ref. 41).
61. Boggs, T. I.; Bruton, H. D.; Craig, D. H.; Helpern, J. A.; Marsh, H. C.; Pegram, M. D.; Vandenbergh, D. J.; Koehler, K. A.; Hiskey, R. G. *J. Org. Chem.* **1982**, *47*, 1812-1816.
62. Kaneko, T.; Nakagawa, Y. *Nippon Kagaku Zasshi* **1957**, *78*, 1216 (*Chem. Abst.*, **54**, 1336f).
63. Baldwin, J. E.; Adlington, R. M.; Robinson, N. G. *J. Chem. Soc. Chem. Commun.* **1987**, 153-155.
64. Ouerfelli, O.; Ishida, M.; Shinozaki, H.; Nakanishi, K.; Ohfune, Y. *Synlett* **1993**, 409-410.
65. Bauce, L. G.; Goren, H. J. *Int. J. Peptide Protein Res.* **1979**, *14*, 216-226.

(*Received in UK* 17 *February* 1994)