A Convenient and Efficient Synthesis of (2*S*,4*R*)- and (2*S*,4*S*)-4-Methylglutamic Acid

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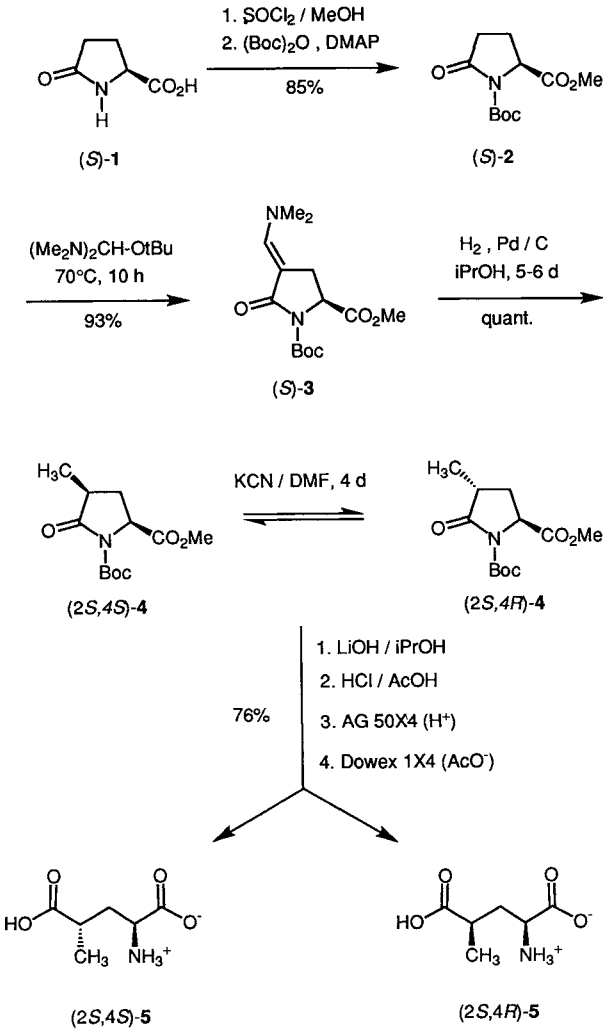
Both enantiomerically pure (2*S*,4*S*)- and (2*S*,4*R*)-4-methylglutamic acids have been prepared in an overall 60% yield, by a convenient 7-step synthesis based on C-4 alkylation/epimerization of readily available (*S*)-pyroglutamic acid.

Non-proteinogenic glutamic acid analogs have been widely used for investigation of biological systems in which glutamic acid plays a key role, such as glutamine synthetase,[1] vitamin K-dependent carboxylase,[2] D-glutamate ligase involved in the bacterial peptidoglycan biosynthesis,[3] folate-dependent enzymes[4,5] and glutamic acid receptors of the central nervous system.[6] Among the various analogs, diastereomeric 4-methylglutamic acids are of particular interest, either as free amino acids,[3,7-9] or incorporated into a peptide,[2] or as part of bioactive compounds.[10] It has been shown recently that the (2*S*,4*R*) isomer is a potent and selective agonist of kainate receptors[8] while the (2*S*,4*S*) epimer is a potent agonist of metabotropic receptors.[9] In order to be systematically used for *in vitro* pharmacological assays, a practical large scale synthesis of both epimers is needed. Moreover, when available on a multigram scale, such analogs could be incorporated in new peptide libraries[11] or used as chiral building blocks.

Enantiomerically pure epimeric 4-methylglutamic acids have been previously prepared by chemical[12,13] or enzymatic[7,14] resolutions, chemical[15,16] or enzymatic[17,18] asymmetric synthesis, and by alkylation of adequately protected glutamic acid[8,19-21] or pyroglutamic acid.[22-24] The latter syntheses took advantage of the chirality present in the starting glutamate (or pyroglutamate) and most approaches were focused on diastereoselective alkylation.[8,21-24] Among these, alkylation of protected pyroglutamate by Bredereck's reagent[25,26] is the most convenient and attractive route to (2*S*,4*S*)-4-methylglutamic acid.[23,24] Conversely the proposed syntheses of the epimeric (2*S*,4*R*) enantiomer, using either conjugated cuprate addition[24] or multistep synthesis,[22] are less obvious. As we had in hand a simple and efficient diastereomeric separation procedure,[17] we devised a synthetic scheme in which both epimers would be simultaneously obtained and then separated (Scheme).

All along this synthesis, special care for the measurement of diastereomeric and enantiomeric purities was taken. While purity of alkyl glutamates has up to now been determined by ^{1}H NMR techniques, we preferred to use a more sensitive analytical method such as gas chromatography (GC). A good separation of all stereoisomers was obtained for the methyl ester of pyroglutamate **1**, epimeric *N*-deprotected **4**, and epimeric *N*-trifluoroacetyl *O*,*O'*-diisopropyl esters of **5** on a Chirasil-Val column[27] (see experimental). Moreover such a sensitive method allowed us to detect a possible occurrence of contaminating glutamic acid.



Scheme

Commercial (*S*)-pyroglutamic acid[28] (**1**) of the best optical purity (99% ee[29]) was protected by esterification with thionyl chloride/MeOH, followed by *N*-*tert*-butyloxycarbonylation[30] giving **2**, without any change of the starting ee. It was then converted to enaminone **3** in 93% yield using Bredereck's reagent,[26] in optimized conditions for minimal C-2 epimerization (measured by GC at the next step) and maximal alkylation (10 h, 70°C). The absence of starting pyroglutamate **2** in recrystallized **3** was carefully checked by TLC to avoid elimination of contaminating glutamic acid at the last chromatographic purification. Catalytic hydrogenation of **3** proceeded slowly (about 5 days) but cleanly to afford (2*S*,4*S*)-4-methylpyroglutamate **4** with 98% de and 97.5% ee (measured by GC). The *cis*-relative stereochemistry of (2*S*,4*S*)-**4** was established after hydrolysis by GC com-

parison with an authentic (2*S*,4*S*)-4-methylglutamate sample.[17] Thus, hydrogenation occurred almost exclusively on the less hindered face of the pyroglutamate ring, confirming that a bulky ester group was not necessary to achieve diastereomeric selectivity.[23,31] Formally, epimerization at C-4 could have been achieved together with ring opening and deprotection by acidic hydrolysis,[17] but equilibration at C-4 was slow and required several days of heating in 6 N HCl solution, giving rise to undesired epimerization at C-2 [(2*S*,4*S*)-**5**/(2*S*,4*R*)-**5**: $dr^{32} = 51:49$; ee = 64% for (2*S*,4*S*)-**5** and 61% for (2*S*,4*R*)-**5**, after 126 h at 110°C].

Consequently we turned to milder previously described conditions.[31,33] Complete diastereomeric equilibration between (2*S*,4*S*)- and (2*S*,4*R*)-**4** (dr = 33:67) was achieved after 5 days in the presence of one equivalent of KCN at room temperature in DMF solution, with less than 1% concomitant epimerization at C-2. Ring opening, ester hydrolysis and *N*-deprotection were carried out by LiOH hydrolysis followed by acidic treatment with dry 2 N HCl in acetic acid. The mixture of the epimers **5** was first purified by cation exchange chromatography. The (2*S*,4*S*)- and (2*S*,4*R*)-4-methylglutamic acid epimers **5** were then completely separated on an anion exchange column eluted with 0.15–0.2 M acetic acid. Their diastereomeric excess (de > 99.8%) and enantiomeric excess (ee 96.6% and 97.4%, respectively) were evaluated by GC on OV-1701 and Chirasil-Val columns (after derivatization[27]), and by HPLC on a CR (+) Crownpack column. Since (*R*)-pyroglutamic acid is commercially available, (2*R*,4*R*)- and (2*R*,4*S*)-4-methylglutamic acids can be prepared following the same synthetic route.

In conclusion, a procedure for the preparation of (2*S*,4*S*)- and (2*S*,4*R*)-4-methylglutamic acids with high optical purity has been described. Such a synthesis presents several advantages compared to previous ones: it is suitable for large scale production, all reagents are of low cost, readily accessible and easy to handle, chiral integrity of compounds is accurately monitored and the overall yield (60%) starting from (*S*)-pyroglutamic acid is higher.

(*S*)-Pyroglutamic acid (er^{32} = L:D ≥ 99:1) and Bredereck's reagent [*tert*-butoxybis(dimethylamino)methane] were purchased from Fluka. Merck 60H silica gel (230–400 mesh) was used for flash chromatography. GC was performed on a Chirasil-Val capillary column (50 m × 0.32 mm, Alltech) or on a Flexibond™ OV-1701 capillary column (15 m × 0.25 mm, Pierce Chemical Co.). HPLC was carried out on a Crownpack CR (+) column (0.4 × 15 cm, Daicel) equilibrated with a pH 2 $HClO_4$ solution, at a 0.4 mL/min flow rate, with detection at 210 nm. ^1H (250.13 Mz) and ^{13}C (62.9 Mz) NMR were recorded on a ARX 250 Bruker spectrometer.

Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)pyroglutamate (2):[28]

$SOCl_2$ (4.06 mL, 56 mmol) was added dropwise to a cooled solution of 99% ee[29] (*S*)-pyroglutamic acid (3.61 g, 28 mmol) in MeOH (100 mL). The mixture was allowed to warm to r.t. After stirring for 2 h, the solution was evaporated and the residue dissolved in CH_2Cl_2 (200 mL), washed with aq $NaHCO_3$ (2 × 10 mL), brine (10 mL) and dried ($MgSO_4$). Evaporation of the organic phase gave methyl (*S*)-pyroglutamate as a crude oil (3.48 g, 87%, ee = 98.8%); TLC: CH_2Cl_2/MeOH/33% aq NH_4OH (70:26:4); R_f 0.71; GC (Chirasil-Val, 150°C): t_R (2*S*) 21.0 min, t_R (2*R*) 21.5 min.

A solution of this crude ester (3.48 g, 24.35 mmol), di-*tert*-butyl dicarbonate (6.71 mL, 29.22 mmol) and DMAP (4-*N*,*N*-dimethylaminopyridine) (0.298 g, 0.243 mmol) in MeCN (40 mL) was stirred for 1 h, and the residue obtained after removal of the solvent was purified by filtration on a short silica gel column (50 g) using a gradient of EtOAc (0–50%) in CH_2Cl_2. Fractions positively detected with TDM reagent[34] were collected, evaporated and gave **2** as an oil which solidified under vacuo (5.80 g, 98%); TLC: EtOAc/CH_2Cl_2 (8:2); R_f 0.45; GC (after *N*-deprotection, Chirasil-Val, 150°C): ee = 98.8%.

^1H NMR ($CDCl_3$): δ = 4.53 (dd, 1 H, NCH, *J* = 3.0, 9.0 Hz), 3.69 (s, 3 H, CO_2CH_3), 2.55–2.16 (m, 3 H, CH_2), 1.95 (m, 1 H, CH_2), 1.40 [s, 9 H, $(CH_3)_3CO$].

^{13}C NMR ($CDCl_3$): δ = 173.0, 171.6 (2 × CO), 149.0 (OCON), 83.3 [*C*$(CH_3)_3$], 58.6 (OCH_3), 52.3 (N*C*HCO$_2$), 30.9 (*C*H_2CO), 27.6 [C(*C*$H_3)_3$], 21.3 (*C*H_2CHN).

Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)-4-[(dimethylamino)methylidene]pyroglutamate (3):

A mixture of **2** (5.80 g, 23.85 mmol) in dimethoxyethane (21 mL) and *tert*-butoxybis(dimethylamino)methane (7.38 mL, 35.78 mmol) was heated at 68–70°C for 10 h. White crystals of **3** appeared in the red solution upon cooling to r.t. These were filtered and rinsed with cold Et_2O. Several other crops could be obtained and the absence of starting pyroglutamate was checked by TLC. Pure crystals of **3** were gathered (4.53 g, 64%). Purification of the mother liquor by flash chromatography [CH_2Cl_2/EtOAc (10–100% EtOAc)] afforded a last fraction of pure **3** (total yield: 93%); mp 129°C; TLC: EtOAc/CH_2Cl_2 (8:2); R_f 0.20.

^1H NMR ($CDCl_3$): δ = 7.08 (s, 1 H, C=CH), 4.51 (dd, 1 H, NCH, *J* = 3.8, 10.5 Hz), 3.71 (s, 3 H, CO_2CH_3), 3.2 (m, 1 H, NCH*C*H_2), 2.97 [s, 6 H, N$(CH_3)_2$], 2.84 (m, 1 H, NCH*C*H_2), 1.45 [s, 9 H, $(CH_3)_3CO$].

^{13}C NMR ($CDCl_3$): δ = 172.6, 169.4 (2 × CO), 150.5 (OCON), 146.4 (C=*C*H), 90.7 (*C*=CH), 82.2 [*C*$(CH_3)_3$], 55.9 (OCH_3), 52.3 (N*C*HCO$_2$), 42.0 [N$(CH_3)_2$], 28.0 [C(*C*$H_3)_3$], 26.2 (CH_2).

Anal. calc. for $C_{14}H_{22}N_2O_5$: C 56.36, H 7.43, N 9.39, found: C 56.41, H 7.26, N 9.25.

Methyl (2*S*,4*S*)- and (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-methylpyroglutamate (4):

A mixture of **3** (3.75 g, 12.53 mmol) and 10% Pd/C (0.72 g) in *i*-PrOH (400 mL) was stirred under H_2 (1 bar) for 5–6 d. The catalyst was filtered on a Celite pad and rinsed with EtOAc/*i*-PrOH (1:1). Evaporation of the filtrate afforded (2*S*,4*S*)-**4** as a colorless oil in quantitative yield with 98% de and 97.8% ee (GC, Chirasil-Val, 150°C); TLC: EtOAc/CH_2Cl_2 (8:2); R_f 0.52.

^1H NMR ($CDCl_3$): δ = 4.46 (m, 1 H, NCH), 3.74 (s, 3 H, CO_2CH_3), 2.54 (m, 2 H, CH_2), 1.61 (m, 1 H, NCOCH), 1.45 [s, 9 H, $(CH_3)_3CO$], 1.22 (d, 3 H, NCOCHC*H*$_3$), *J* = 6.8 Hz).

^{13}C NMR ($CDCl_3$): δ = 175.6, 172.0 (2 × CO), 149.4 (OCON), 83.6 [*C*$(CH_3)_3$], 57.3 (OCH_3), 52.4 (N*C*HCO$_2$), 37.5 (*C*HCON), 29.7 (CH_2), 27.8 [C(*C*$H_3)_3$], 16.1 (*C*H_3CHCO).

A mixture of (2*S*,4*S*)-**4** (3.22 g, 12.53 mmol) and KCN (0.816 g, 12.53 mmol) in DMF (100 mL) was stirred at r.t. for 4 d. The solution was evaporated under vacuo to afford a crude mixture of (2*S*,4*S*)-**4** and (2*S*,4*R*)-**4** (dr = 37:63; ee = 96.9% and 97.7% respectively) which was used without purification in the next step; GC (Chirasil-Val, 150°C): t_R (2*S*,4*S*) = 19.5 min, t_R (2*R*,4*R*) = 20.1 min, t_R (2*S*,4*R*) = 20.9 min, t_R (2*R*,4*S*) = 21.2 min.

(2*S*,4*S*)- and (2*S*,4*R*)-4-Methylglutamic Acid (5):

To the crude mixture of diastereoisomers **4** (3.22 g, 12.53 mmol) in *i*-PrOH (81 mL) was added 2.5 M aq LiOH (81 mL). The mixture was stirred overnight, concentrated and acidified to pH 3–4 with H_2SO_4 under a well ventilated hood. After stirring for 1 h, the *N*-protected diacids were extracted with EtOAc (4 × 120 mL). The organic phase was dried (Na_2SO_4) and evaporated to yield a foamy residue (2.76 g, 84%); TLC: CH_2Cl_2/MeOH/33% aq NH_4OH (65:35:10); R_f 0.35.

The crude residue was then dissolved in a solution of dry 2 N HCl in AcOH (8 mL) and stirred for 30 min. Removal of the solvent gave a crude mixture of (2*S*,4*S*)- and (2*S*,4*R*)-**5** as their hydrochlo-

ride salts, which was first purified on a AG 50X4 (H^+ form, 20–50 mesh, 3 × 15 cm) column by elution with 0.5 M NH_4OH solution. Evaporation of the ninhydrin reacting fractions gave a mixture of diastereoisomers **5** as their ammonium salts which was next separated on a Dowex 1X4 (AcO^- form, 200–400 mesh, 5.5 × 67 cm) column by elution with a gradient of AcOH (0.15 M–0.20 M, 8 L). Two sets of well separated ninhydrin reacting fractions were detected. Their evaporation afforded (2*S*,4*S*)-**5** (0.513 g, 3.19 mmol, 30%, de > 99.8%, ee = 96.6%) and (2*S*,4*R*)-**5** (1.015 g, 6.30 mmol, 60%, de > 99.8%, ee = 97.4%), respectively.

(2*S*,4*S*)-5:
Mp 166–167°C (Lit.[17] mp 170°C); GC of *N*-trifluoroacetyl-*O*,*O*'-diisopropyl ester:[27] (OV-1701, 160°C) t_R (2*SR*,4*SR*) = 6.7 min; (Chirasil-Val, 145°C) t_R (2*R*,4*R*) = 12.8 min, t_R (2*S*,4*S*) = 13.4 min; HPLC: t_R (2*R*,4*R*) = 5.3 min, t_R (2*S*,4*S*) = 12.7 min.

(2*S*,4*R*)-5:
Mp 174°C (Lit.[17] mp 177°C); GC of *N*-trifluoroacetyl-*O*,*O*'-diisopropyl ester:[27] (OV-1701, 160°C) t_R (2*SR*,4*RS*) = 7.2 min; (Chirasil-Val, 145°C) t_R (2*R*,4*S*) = 13.7 min, t_R (2*S*,4*R*) = 14.4 min; HPLC: t_R (2*R*,4*S*) = 4.9 min, t_R (2*S*,4*R*) = 8.8 min.

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