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Enantioselective synthesis of new 4-substituted glutamic acid derivatives

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Abstract—Several new 4-substituted glutamic acid derivatives are described: 4-phosphinomethyl glutamic acid derivatives were prepared starting from (2*S*)-*N*-Boc-*tert*-butyl-4-methylene glutamate. The 4-carboxymethyl and 4-aminomethyl glutamic acid derivatives were obtained using asymmetric 1,4-addition conjugate of the enolate of Schiff base **1** to unsaturated esters.

1. Introduction

(*S*)-Glutamic acid is the main excitatory neurotransmitter in the mammalian central nervous system (CNS) and is involved in a variety of physiological functions including neuronal plasticity, memory, and learning. It also plays a role in both acute or chronic pathological processes such as brain ischemia, hypoxia, and neurodegenerative diseases. It is synthesized and stored in specialized glutamatergic neurones and released in the synaptic cleft following stimulation.

The different functions exerted by (*S*)-Glu are mediated by two main families of membrane receptors, ionotropic glutamate receptors (i-Glu Rs)[1–4] according to the selective agonists, NMDA, AMPA, and KA and the G-protein coupled metabotropic glutamate receptors (m-Glu Rs)[5,8,9] to phospholipase C (PLC)[5,6] or adenylate cyclase.[2,7] Each of these two families, in turn, is subdivided into a variety of receptor subtypes and isoforms; in the case of i-GluRs, they are known as GluR1–GluR6, KA1–KA2, NR1, and NR3 and in the case of m-Glu Rs molecular cloning,[10] it has been revealed the existence of at least eight subtypes: m-GluR1–m-GluR8. Removal of excess glutamic acid does not occur enzymatically but takes place through a family of high affinity sodium dependent transporter proteins.[11] These are named excitatory amino acid transporters (EAA T1-5). Small molecule inhibitors of these transporters have been postulated for use against CNS malfunctions.

The pharmacological characterization of this vast and complex receptor subtype array and of the transporter proteins has been made possible by the design and synthesis of (*S*)-Glu analogues.

Projects aiming at a structure-based design of new receptor ligands have been severely hampered by the, so far, unsuccessful attempts to crystallize entire i-Glu Rs or m-Glu Rs alone or with ligands bound to the receptor recognition or allosteric sites.

Engaged in the search for new molecules capable of activating, blocking, or modulating Glu receptor subtypes and (or) excitatory aminoacid transporters, we considered a few years ago the synthesis of various 4-substituted glutamate analogues: by Diels–Alder, 1–4 ionic and radical reactions performed starting from (2*S*)-4-methyleneglutamic acid: We obtained (2*S*)-4-(2-phtalimidoethyl)glutamic acid, (2*S*)-4-(4-phtalimidobutyl)glutamic acid, and 1-[(*S*)-2-amino-2-carboxyethyl]-3,4-dimethylcyclohex-3-ene-1-carboxylic acid, which presented moderate antagonist activities[12] on the preliminary pharmacological evaluation by measuring IP accumulation using rat forebrain synaptoneurosomes. Our group described the synthesis of various aromatic analogues of (*S*)-Glu applying a highly selective intermolecular Heck reaction for soluble polymer (PEG) supported synthesis.[13] More recently, Wehbe et al.[14] described a short four-step synthesis of (2*S*,3*R*) and

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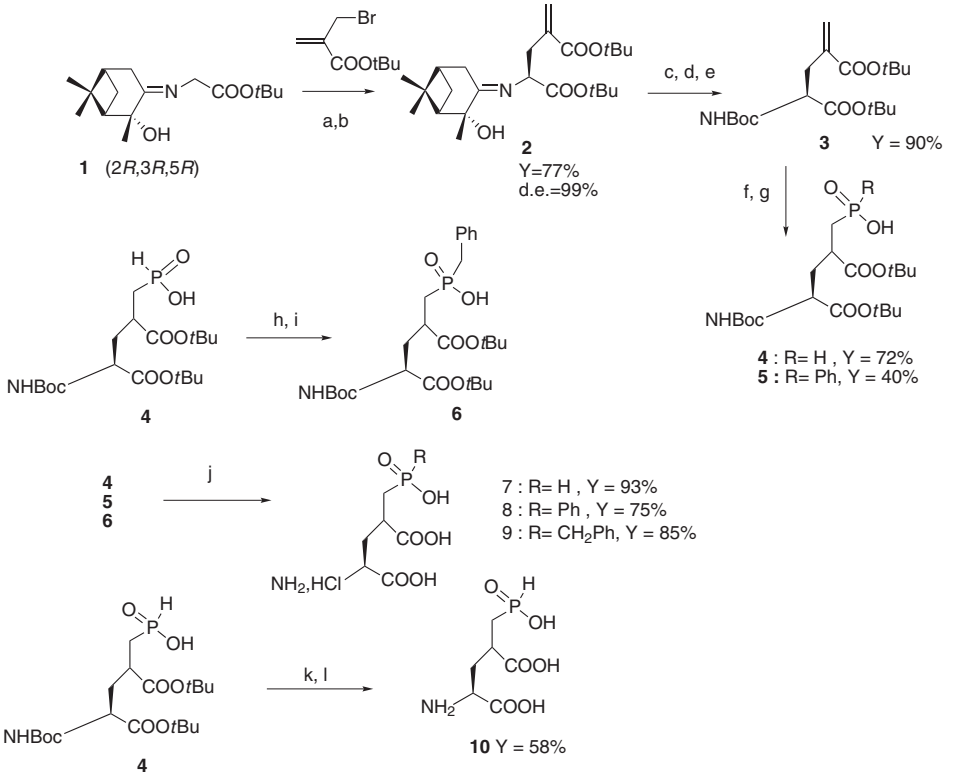
(2*S*,3*S*)-3-methyl glutamic acids as blocker of the glutamate transport by $EAAT_2$. The (2*S*,3*R*) isomer presented a significant effect on glutamate transport. The most studied inhibitor of the glutamate transport, 2S4RMG, (2*S*,3*R*)-4-methylglutamic acid[15] and the later studies from Vandenberg et al.[16] proved the real influence of the nature of the chemical group on the C_4. As the OH group and CH_3 group had already been tested, we chose to prepare various enantiomerically pure 4-substituted glutamic acid analogues with an acidic (carboxyclic or phosphinic acid) or basic (amine) function, which to our knowledge have not been described and would help explain the blocker effect on the glutamate transport.

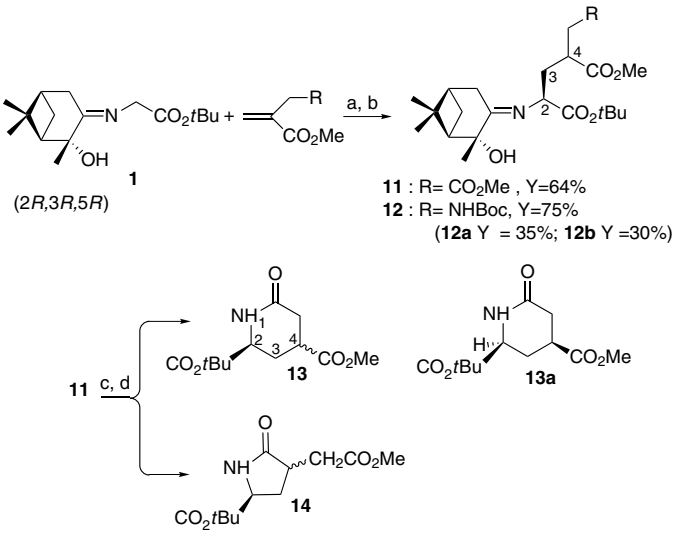
2. Results and discussion

The compounds with a phenylphosphinic acid group in their structure were described amongst other things as neurotransmitter isosteric analogues with $GABA_B$ antagonist activity.[17] The presented 4-phosphinomethyl glutamic acid derivatives were prepared starting from (2*S*)-*N*-Boc-*tert*-butyl-4-methylene glutamate **3** (Scheme 1). Compound **3** was easily obtained in a three short step synthesis, the key step being the alkylation of the chiral Schiff base **1** prepared from the commercially available chiral auxiliary (2*R*,3*R*,5*R*)-2-hydroxypinan-3-one[18,19] and *tert*-butyl glycinate, with *tert*-butyl-2-bromomethyl acrylate as electrophile to afford **2**. This reaction has already been reported[13,21] by our group using methyl-2-bromomethyl acrylate as the electrophile and benzophenoneimine of methyl glycinate with no diastereoselectivity. Wehbe et al.[14] described the alkylation of a chiral Schiff base **1** with ethyl crotonate and obtained two separated diastereomers with total retention of the (2*S*)-configuration. Having established that in alkylation reactions of such Schiff bases,[22] the nature of the ester was important to the diastereomeric ratio of the product, we replaced the methyl or ethyl ester with a bulky *tert*-butyl ester with **2** being obtained in good yield (65%) and excellent de (99%).

After hydrolysis of the imine function with 15% citric acid and Boc protection of the amine using Boc_2O, **3** was obtained in 90% yield and submitted to the addition of bis trimethylsilyl phosphonite[20] (generated in situ from sodium hypophosphite in the presence of triethyl amine and trimethylsilyl chloride) at 0 °C in CH_2Cl_2 to afford the phosphinic derivative **4** in 72% yield (d.r. = 77/23). Using the same experimental conditions, **3** was treated with bis trimethylsilyl phenyl phosphonite and yielded **5** (40%, d.r. = 78/22). Compound **6** was prepared from **4** via the action of benzyl bromide in CH_2Cl_2 in the presence of NEt_3 and TMSCl at 0 °C in 40% yield (d.r. = 70/30). To increase molecular diversity, various alkyl halides were used with the same experimental procedure. However, the two diastereomers obtained in each case could not be separated by silica gel



Scheme 1. Reagents and conditions: (a) 3 M CH_3MgBr; (b) LDA, THF, −90 °C, 4 h, then at 0 °C for 18 h, in a saturated NH_4Cl solution; (c) 15% citric acid, 4 days, rt; (d) Na_2CO_3; (e) $(Boc)_2O$, CH_2Cl_2, rt, 24 h; (f) Na-hypophosphite, Et_3N, TMSCl, 0 °C, 1 h; (g) **3** in CH_2Cl_2, 24 h; (h) 1 M HCl; (i) BnBr, CH_2Cl_2, 0 °C; (j) 3 M HCl; (k) propylene oxide, MeOH.



Scheme 2. Reagents and conditions: (a) 3 M CH_3MgBr in Et_2O, THF, −20 °C; (b) DBU, THF, −20 °C; (c) 15% citric acid, 4 days, rt; (d) Na_2CO_3.

chromatography or HPLC using different conditions. Cleavage of the protecting groups of **4**, **5**, and **6** was achieved using 3 M HCl at room temperature and afforded the amino acid hydrochlorides **7** (93% yield), **8** (75% yield), and **9** (85% yield) as diastereomers. Compound **7** was neutralized by propylene oxide in methanol to give the amino acid **10** in 58% yield.

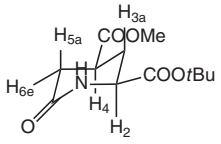
Studying the biological activity of **7** (R = H), **8** (R = Ph), and **9** ($R = CH_2Ph$), we obtained essential information on the effect of the chain length.

The 4-carboxymethyl and 4-aminomethyl glutamic acid derivatives were the analogues of 2S4RMG,[15] a very potent $EAAT_1$ and $EAAT_2$ blocker, with an amine or a carboxylic acid on the C_4 methylene group. These were prepared using an asymmetric 1,4-addition of the enolate prepared from Schiff base **1** to unsaturated esters (Scheme 2).

For the 4-carboxymethyl derivatives **11** and **12** preparation, several bases were tested such as LDA, KHMDS, DBU under different experimental conditions. In the reaction of Schiff base **1** and dimethyl itaconate, DBU was chosen because it did not give polycondensation products as the other chelating bases did.[14] With this base at −20 °C in THF, **11** was obtained in 64% yield as a mixture of two diastereomers (d.r. = 55/45), which could not be separated either by silica gel chromatography or by HPLC. Previous work by our group[18] and by other teams[19] showed that (2*R*,3*R*,5*R*)-2-hydroxypinan-3-one induced an (*S*)-configuration on the α carbon of α-aminoesters and allowed the attribution of the *S*-configuration on the α carbon of **11**. Acid treatment of **11** with 15% citric acid in THF at room temperature followed by neutralization with Na_2CO_3 yielded a cyclized product (74%); after recrystallization (hexane/ethyl acetate 8/2) the major product was obtained in 50% yield. The cyclization could produce a five membered ring **14** or a six membered ring **13**. Structural assignment was based mainly on ^1H NMR spectra and IR spectra. The IR spectra showed two C=O bands, one at 1727 cm^{-1} attributed to the esters and one at 1673 cm^{-1}, which seemed to demonstrate the presence of a six membered ring lactam.

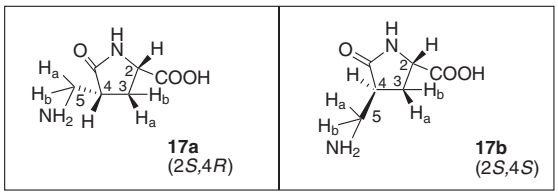
At first sight, the ^1H NMR spectra of the two structures could be similar as both possessed the same sequence –CH–CH_2–CH–CH_2– with six anisochronous hydrogen atoms. However, an iterative analysis of this six-spin system, performed with the gNMR program,[23] gave the chemical shifts and coupling constants gathered in Table 1. These values constitute a strong confirmation of the six membered ring structure **13**, especially the

Table 1. Chemical shifts (ppm/TMS) and coupling constants (Hz) of compound **13a** in $CDCl_3$



$\delta(H_2)$	4.000
$\delta(H_{3a})$	1.796
$\delta(H_{3e})$	2.596
$\delta(H_4)$	2.874
$\delta(H_{5a})$	2.526
$\delta(H_{5e})$	2.705
$\delta(CO_2Me)$	3.772
$\delta(CO_2t\text{-Bu})$	1.514
δ(NH)	6.285
$^3J(H_2,H_{3a})$	11.9
$^3J(H_2,H_{3e})$	4.3
$^2J(H_{3a},H_{3e})$	−13.2
$^3J(H_{3a},H_4)$	12.2
$^3J(H_{3e},H_4)$	3.1
$^4J(H_{3e},H_{5e})$	1.9
$^2J(H_4,H_{5a})$	12.0
$^3J(H_4,H_{5e})$	5.5
$^2J(H_{5a},H_{5e})$	−17.9

Table 2. Chemical shifts (ppm/TMS) and coupling constants (Hz) of compounds **17a** (2*S*,4*R*) and **17b** (2*S*,4*S*) in D_2O and CD_3OD



	D_2O	CD_3OD	Dihedral angle	J_{calc}	D_2O	CD_3OD	Dihedral angle	J_{calc}
$d(H_2)$	4.297	4.343			4.267	4.257		
$d(H_{3a})$	2.703	2.783			2.228	2.282		
$d(H_{3b})$	1.810	1.918			2.433	2.534		
$d(H_4)$	2.878	2.897			2.856	2.872		
$d(H_{5a})$	3.167	3.244			3.176	3.233		
$d(H_{5b})$	3.056	3.143			3.074	3.135		
$^3J(H_2,H_{3a})$	8.2	7.7	26	8.5	9.9	9.5	19	9.2
$^3J(H_2,H_{3b})$	8.1	8.3	145	8.2	2.2	1.7	101	1.6
$^4J(H_2,H_4)$	<0.5[a]	<0.5[a]			—	—		
$^2J(H_{3a},H_{3b})$	−13.2	−12.9			13.5	−13.3		
$^3J(H_{3a},H_4)$	9.1	8.6	31	7.9	10.0	10.5	145	8.6
$^3J(H_{3b},H_4)$	9.4	9.5	150	9.7	8.9	8.6	24	8.7
$^3J(H_4,H_{5a})$	7.4	8.1			7.6	8.3		
$^3J(H_4,H_{5b})$	7.5	6.9			7.2	6.9		
$^2J(H_{5a},H_{5b})$	−13.1	−12.8			−13.7	−12.9		

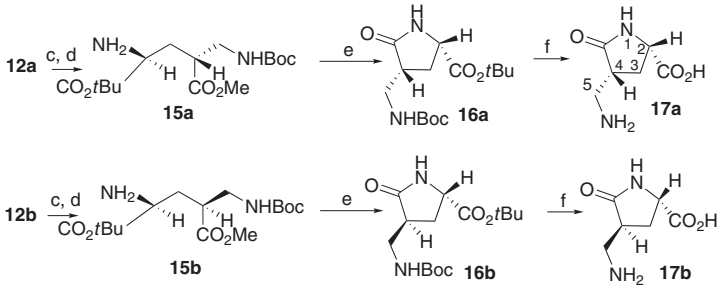
[a] Long-range coupling detected with a COSYLS experiment.

measurable ^4J coupling constant found between H_{3e} and H_{5e}, the value of which (1.9 Hz) is typical of cyclohexanic structures and would be smaller in structure **14** as observed in compound **17a** (see Table 2). At the same time, the axial position of H_4 demonstrated by the two axial–axial ^3J coupling constants (around 12 Hz) was proof of the (*S*)-configuration at C-4.

For the synthesis of 4-aminomethyl glutamic acid derivative **12**, the required alkylating agent methyl-2 (*tert*-butoxycarbonyl aminomethyl) acrylate[24] was prepared from commercially available methyl-2-bromomethyl acrylate by reaction with bis-*tert*-butyl iminodicarboxylate in CH_3CN in the presence of K_2CO_3, followed by treatment with a catalytic amount of Scandium triflate. The 1,4-addition reaction afforded **12** in 75% yield as a mixture of two diastereomers (d.r. = 55/45), which were separated by silica gel chromatography: **12a** (35%) and **12b** (30%).

As shown in Scheme 3, cleavage of the chiral auxiliary from **12a** and **12b** using 15% citric acid followed by Na_2CO_3 treatment yielded the aminoesters **15a** and **15b** in 85% yield, which at room temperature were transformed slowly and quantitatively into cyclized products **16a** and **16b**; after removal of *t*-Bu and Boc protecting groups by 2 M HCl hydrolysis, **17a** and **17b** were obtained in 95% yield. The stereochemical assignment (2*S*) was based on previous results [(2*R*,3*R*,5*R*)-2-hydroxypinan-3-one induced (2*S*) configuration].

Compounds **17a** and **17b** present the same –CH–CH_2–CH–CH_2– pattern as compound **13** but with five membered rings. These two diastereoisomers differed only with the configuration at C_4 whereas C_2 was *S* in both compounds. The results of the analyses of these six-spin systems are shown in Table 2. To assign each structure to its own spectrum, we determined some relevant dihedral angles measured on structures minimized using the MM+ force field of the HyperChem program.[25] The corresponding ^3J coupling constants were then calculated using the program published by Cerda-Garcia-Rojas et al.[26] based on the work of Haasnoot et al.[27]



Scheme 3. Reagents and conditions: (c) 15% citric acid; (d) Na_2CO_3 rt, 2 days; (e) rt; (f) 2 M HCl, rt, 2 h.

It is noteworthy that the assignment of H_{3a} and H_{3b} in compound **17a** could not be made on the basis of their coupling constants with H_2 and H_4 as these parameters were not significantly different. In fact, the signal at 2.703 ppm was assigned to H_{3a} because a NOEDIF experiment showed that its intensity increased when the H_2 signal at 4.297 ppm was saturated.

At the same time the signal of H_4 increased, which showed a dipolar interaction between H_2 and H_4. The later observation together with the detection of a small long-range coupling constant between H2 and H4, observed only in the spectrum of **17a**, is proof that **17a** and **17b** are the (2*S*,4*R*) and (2*S*,4*S*) stereoisomers, respectively.

3. Conclusion

Herein we have reported the synthesis of 4-phosphinomethyl, 4-carboxymethyl, and 4-aminomethyl glutamic acid derivatives to study the influence of an amine or an acidic function on the biological activity on the glutamate transport inhibition. The phosphinic derivative's synthesis was enantioselective. In the case of other derivatives we confirmed the stereochemistry of C_4 by IR and ^{1}H NMR studies in **17a** (2*S*,4*R*) and **17b** (2*S*,4*S*). These studies also confirmed the structures in the six membered ring of the final compound **13a** (2*S*,4*S*).

At this time the biological tests are currently underway in the Neuronal Plasticity Laboratory of Montpellier 2 University.

4. Experimental section

4.1. General remarks

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 Brüker AC250 instrument. For ^{1}H NMR or ^{13}C NMR spectra recorded in $CDCl_3$, D_2O, or MeOD; chemical shifts are quoted in parts per million and were 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 were reported in hertz (Hz). Diastereoisomeric ratios (d.r.) were determined by ^{1}H NMR on the crude product. Low resolution mass spectra were recorded on micromass electrospray instruments with only molecular ion and other major peaks being reported. Optical rotations were determined on a Perkin–Elmer 241 polarimeter at room temperature. Flash chromatography was carried out using E-Merck silica gel (Kieselgel 60, 230–400 mesh) as the stationary phase. Thin layer chromatography was carried out on aluminum plates pre-coated with Merck silica gel 60F254 and visualized by quenching of UV fluorescence, by iodine vapor or by ninhydrine spray. Preparative H.P.L.C. were performed on a Waters delta 4000 apparatus equipped with a delta-Pack C18 column (15 mm, 40×100 nm) and a UV detector, using a linear gradient of CH_3CN in H_2O with 0.1% TFA. THF was distilled from sodium/benzophenone ketyl. Reagents were supplied from commercial sources (ALDRICH, FLUKA). The Schiff base **1** was prepared as previously described.[17]

4.2. Synthesis of (2*S*)-*N*-Boc bis *tert*-butyl-4-methylene glutamate 3

4.2.1. Synthesis of the Schiff base of bis *tert*-butylmethylene glutamate: 2. At −10 °C, to a solution of chiral Schiff base **1** (0.46 g, 1.63 mmol) in anhydrous THF (5.75 mL) was added 3 M CH_3MgBr in ether (0.81 mL, 2.44 mmol). At −90 °C this solution was added to LDA prepared from 2.5 M BuLi in hexane (0.652 mL, 1.62 mmol) and diisopropyl amine (0.295 mL, 2.11 mmol) in anhydrous THF (9 mL). After stirring for 30 min, was added *tert*-butyl-2-bromomethyl acrylate (0.54 g, 2.42 mmol) with the temperature being maintained at −90 °C for 4 h, after which the temperature was allowed to rise to −30 °C in 18 h. The reaction was quenched with NH_4Cl saturated solution (5 mL) and the mixture extracted with Et_2O (2×10 mL). The organic phases were combined and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography using hexane/EtOAc (7/3) to yield **2** (72%) as a single diastereomer. R_f: 0.33 (hexane/EtOAc, 7/3). de = 99% ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.75 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.45 (s, 9H, *t*-Bu), 1.53 (s, 9H, *t*-Bu), 1.95 (m, 2H, HP), 2.21 (m, 1H, HP), 2.39 (m, 1H, HP), 2.59 (m, 2H, HP), 2.75 (s, 1H, OH), 2.9 (dd, 1H, $J = 8.7$ Hz, $J = 13.5$ Hz, CHβ), 2.98 (dd, 1H, $J = 5.1$ Hz, $J = 13.5$ Hz, CHβ), 4.35 (dd, 1H, $J = 4.4$ Hz, $J = 9.5$ Hz, CHα), 5.5 (d, 1H, $J = 1.8$ Hz, CH), 6 (d, 1H, $J = 1.8$ Hz, CH). MS (ESI) 422.21 ($M+H^+$).

4.2.2. Chiral Schiff base hydrolysis: (2*S*)-bis *tert*-butyl-4-methylene glutamate. Citric acid solution (15%, 2.2 mL, 1.05 mmol) was added to a solution of **2** (0.44 g, 1.05 mmol) in THF (2.5 mL) and the mixture then stirred for 4 days at room temperature. After evaporation of the solvent, H_2O (10 mL) was added to the residue and the solution then washed with Et_2O (3×15 mL). The aqueous layer was neutralized (pH = 7–8) using Na_2CO_3. The amino diester was extracted into Et_2O (3×5 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a yellow oil, which was purified by column chromatography yielding the aminodiester **2** in 77% (0.22 g). R_f: 0.53 (Et_2O). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.38 (s, 9H, *t*-Bu), 1.55 (s, 2H, NH_2), 2.35 (dd, 1H, $J = 8.47$ Hz, $J = 13.68$ Hz, CHβ), 2.6 (dd, 1H, $J = 5.93$ Hz, $J = 13.7$ Hz, CHβ), 3.55 (dd, 1H, $J = 5.9$ Hz, $J = 8.5$ Hz, CHα), 5.5 (s, 1H, CH), 6.25 (s, 1H, CH). MS (ES) 272.18 ($M+H^+$) 543.43 ($2M+H^+$).

4.2.3. Synthesis of (2*S*)-*N*-Boc bis *tert*-butyl-4-methylene glutamate 3. To a solution of the aminoester **2** (0.27 g, 1 mmol) in CH_2Cl_2 (8.3 mL) was added Boc_2O (0.22 g, 1 mmol). The mixture was stirred for 24 h at room temperature. The solvent was evaporated and chromatography of the residue using Et_2O/petroleum ether (1/2) afforded **3** as a white powder (0.33 g, 90%). Mp = 50–53 °C R_f: 0.7 (Et_2O/petroleum ether 1/2) ^1H NMR (250 MHz, $CDCl_3$): δ 1.4 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 1.49 (s, 9H, *t*-Bu), 2.51 (dd, 1H, $J = 8.6$ Hz, $J = 13.5$ Hz, CHβ), 2.65 (dd, 1H, $J = 5.6$ Hz, $J = 14$ Hz, CHβ), 4.27 (dd, 1H, $J = 5.9$ Hz, $J = 8.5$ Hz, CHα), 5.05 (d, 1H, $J = 8.32$ Hz, NH), 5.5 (s, 1H, CH), 6.25 (s, 1H, CH). MS (ES) 372.18 $(M+H^+)$, 271 $(M-Boc+H)^+$.

4.3. Synthesis of (2*S*)-4-phosphinomethyl glutamic acid 10

4.3.1. Synthesis of (2*S*)-*N*-Boc 4-phosphinic derivatives 4. Sodium hypophosphite (0.5 g, 5.6 mmol) was dissolved in NEt_3 (0.99 g, 9.8 mmol). After stirring for 15 min, TMSCl (1.22 mL, 9.6 mmol) was added at 0 °C and the mixture then stirred at this temperature for 1 h. *N*-Boc amino diester **3** (0.3 g, 0.8 mmol) dissolved in anhydrous CH_2Cl_2 (2 mL) was dropped slowly and the mixture then left at room temperature for 24 h. The mixture was filtered, the filtrate washed with 1 M HCl (10 mL), with H_2O (10 mL), and extracted into EtOAc (3 × 15 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated under reduced pressure. Purification on column chromatography (EtOAc/MeOH 8/2) afforded **4** as a white solid (0.2 g, 70%). Mp = 73 °C R_f: 0.45 (EtOAc/MeOH 8/2) d.r.: 78/22 (^1H NMR); de = 54% (HPLC) ^1H NMR (250 MHz, C_6D_6): δ 1.3 (s, 9H, *t*-Bu), 1.39 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 2.1 (m, 4H, CHβ, CHδ), 3 (m, 1H, CHγ), 4.5 (dd, 1H, $J = 3.3$ Hz, $J = 9.7$ Hz, CHα), 7.13 (d, 1H, $J = 560$ Hz, PH major), 7.17 (1H, d, $J = 559.1$ Hz, PH minor). MS (ES) m/z 338 $(M-Boc+H)^+$, 381.9 $(M-t\text{-}Bu)^+$, 438.3 $(M+H)^+$, 460.2 $(M+Na)^+$, 875.7 $(2M+H)^+$.

4.3.2. Synthesis of (2*S*)-*N*-Boc-4-phenylphosphinomethyl glutamic acid 5. Phenylphosphinic acid (0.07 g, 0.5 mmol) was dissolved in anhydrous DCM (1.1 mL) and the mixture was maintained at −7 °C under argon for the addition of Et_3N (0.18 mL, 1.05 mmol) and TMSCl (0.13 mL). Compound **3** (0.2 g, 0.54 mmol) was dissolved in anhydrous DCM (1 mL) separately and this solution then added when the first solution was at 0 °C. The reaction was then stirred at 0 °C overnight. The reaction was treated with 1 M HCl (2 mL) and extracted with DCM (3 × 5 mL). Organic layers were dried over $MgSO_4$ and the yellow oil obtained, was purified by silica gel chromatography with AcOEt/*i*-PrOH 9/1 v/v as eluent. Compound **5** was obtained as a colorless oil in 70% yield (0.18 g, 0.35 mmol). R_f: 0.26 (EtOAc/*i*-PrOH 9/1) d.r.: 67/33 (^1H NMR); de = 32% (HPLC) ^{31}P NMR (250 MHz, CD_3OD): δ 28.5, 28.79 ^1H NMR (250 MHz, CD_3OD): δ 1.32 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu), 1.89 (m, 4H, CHβ, CHδ), 2.57 (m, 1H, CHγ), 3.91 (m, 1H, CHα), 7.45 and 7.8 (2m, 5H, C_6H_5) MS (ES) m/z 457.9 $(M-t\text{-}Bu+H)^+$, 514.2 $(M+H)^+$.

4.3.3. Synthesis of (2*S*)-*N*-Boc-4-benzylphosphinomethyl glutamic acid 6. To a solution of **4** (0.1 g, 2.22 mmol) in anhydrous CH_2Cl_2 (2 mL), Et_3N (0.2 mL, 1.37 mmol), and TMSCl (0.14 g, 0.17 mL) were added. The mixture was stirred at 0 °C for 1 h and benzylbromide (0.054 mL, 0.66 mmol) then added. The reaction was stirred and the temperature raised to 20 °C in 4 h. The reaction was quenched (NH_4Cl) and extracted into CH_2Cl_2 (2 × 5 mL). The organic layers were then dried over $MgSO_4$ and DCM then evaporated under reduced pressure. The residue was purified on preparative HPLC using 0–90% CH_3CN in 15 min as eluent conditions. Product **5** was obtained as an oil in 40% yield. R_f: 0.35 (EtOAc/MeOH 8/2) d.r.: 70/30 (^1H NMR); de = 37% (HPLC) ^{31}P NMR (250 MHz, CD_3OD) δ 24, 25.2 ^1H NMR (250 MHz, $CDCl_3$): δ 1.4 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 1.85 (m, 4H, CHβ, CHδ), 2.7 (m, 1H, CHγ), 3.1 (d, 1H, $J = 17.2$ Hz, CH_2Ph), 4.1 (m, 1H, CHα), 5.2 (1H, m, NH), 7.3 (m, 5H, C_6H_5). MS (ES) m/z 428.1 $(M-Boc+H)^+$, 472 $(M-t\text{-}Bu+H^+)^+$, 528 $(M+H)^+$, 550 $(M+Na)^+$.

4.3.4. Synthesis of (2*S*)-4-phosphinic aminoacids 7–9. Compounds **4**, **5**, or **6** (0.45 mmol) in THF (3.6 mL) were treated with 3 M HCl (9 mL, 27.4 mmol). After stirring for 3 days at room temperature (reaction being monitored by TLC), and removal of the solvents under reduced pressure, amino acid hydrochlorides **7**, **8**, and **9** were obtained as white solids with 93%, 75%, and 85% yields, respectively.

4.3.4.1. 7: (2*S*)-4-Phosphinomethyl glutamic acid hydrochloride. Mp: 185–187 °C d.r.: 78/22 (^1H NMR) ^1H NMR (250 MHz, D_2O) δ2.2 (m, 4H CHβ and CHδ), 3.05 (m, 1H, CHγ), 4.15 (m, 1H, CHα), 7.2 (d, 1H, $J = 547.4$ Hz, P–H), ^{31}P NMR (250 MHz, D_2O) δ25.10, 29.86. MS (ES) m/z 225.7 $(M+H^+)$, 451.1 $(2M+H)^+$.

4.3.4.2. 8: (2*S*)-4-Phenylphosphinomethyl glutamic acid hydrochloride. Mp: 108–110 °C ^1H NMR (250 MHz, D_2O) $\delta = 2.37$ (m, 4H CHβ and CHδ), 2.95 (m, 1H, CHγ), 4.19 (m, 1H, CHα), 7.6 and 8.0 (m, 5H, C_6H_5) ^{31}P NMR (250 MHz, D_2O) $\delta = 39.65$, 39.86. MS (ES) m/z 302 $(M+H^+)$, 602.9 $(2M+H)^+$.

4.3.4.3. 9: (2*S*)-4-Benzylphosphinomethyl glutamic acid hydrochloride. Mp: 106–107 °C ^1H NMR (250 MHz, D_2O) $\delta = 2.17$ (m, 4H CHβ and CHδ), 3.06 (m, 1H, CHγ), 3.3 (d, 2H, $J = 16.8$ Hz, CH_2Ph), 4.1 (dd, 1H, $J = 7.55$ Hz and $J = 13.9$ Hz, CHα), 7.4 (m, 5H, C_6H_5) ^{31}P NMR (250 MHz, D_2O) $\delta = 48.19$, 48.43. MS (ES) m/z 315 $(M+H^+)$.

4.4. Synthesis of (2*S*)-4-phosphinomethyl glutamic acid 10

To amino acid hydrochloride **7** (0.14 g, 0.53 mmol) dissolved in MeOH, was added excess propylene oxide. A precipitate was formed, washed with Et_2O (3 × 10 mL) and dried under vacuum to provide **10** as a white solid

(0.078 g, 58%). Mp: 160–162 °C. d.r.: 78/22 (^{1}H NMR) ^{1}H NMR (250 MHz, D_2O): δ 2.1 (m, 4H HCβ and CHδ), 2.79 (m, 1H, CHγ), 3.79 (m, 1H, CHα), 7.01 (d, 1H, $J = 512.2$ Hz, P–H). ^{31}P NMR (250 MHz, D_2O) $\delta = 22.21$, 26.13. MS (ES) m/z 225.7 (M+H$^+$), 451.1 (2M+H)$^+$.

4.5. Synthesis of 11, 12a, and 12b

To Schiff base **1** (0.61 g, 2.19 mmol) dissolved in anhydrous THF (10 mL) was added at −20 °C 3 M CH_3MgBr (0.73 mL, 2.84 mmol). After stirring for 20 min, DBU (0.42 mL, 2.4 mmol) was added and the mixture then stirred for 30 min, after which the electrophile (2.5 mmol) was added and the reaction maintained at −20 °C for 3 h monitored by TLC. The reaction was then quenched with saturated NH_4Cl (10 mL) and the mixture extracted with EtOAc (3×10 mL); the organic layers were combined and dried over $MgSO_4$, evaporated and purified by silica gel column chromatography, to afford compounds **11** and **12**. From methyl itaconate as electrophile (Scheme 1, R = CO_2Me), compound **11** was obtained in 73% yield as a mixture of two unseparable isomers using the eluent EtOAc/hexane 7/3. R_f: 0.5 (EtOAc/hexane 7/3 for column chromatography) ^{1}H NMR (250 MHz, C_6D_6): δ 0.8 (s, 3H, CH_3 minor), 0.9 (s, 3H, CH_3 major), 1.21 (s, 3H, CH_3 minor), 1.25 (s, 3H, CH_3 major), 1.4 (2s, 2×9H, *t*-Bu), 1.71 (s, 3H, CH_3 minor), 1.78 (s, 3H, CH_3 major), 1.8–2.9 (m, 10H), 3.1 (m, 1H, CH_4 minor), 3.3 (m, 1H, CH_4 major), 3.4 (s, 3H, CO_2CH_3 major), 3.42 (s, 3H, CO_2CH_3 minor), 4.45 (s, 3H, CO_2CH_3 major), 3.48 (s, 3H, CO_2CH_3 minor), 4.32 (dd, 1H, $J = 8.3$ Hz, $J = 4.1$ Hz, H_2 minor), 4.45 (dd, 1H, $J = 10$ Hz, $J = 3.85$ Hz, H_2 major). MS (ES) m/z 440.3 (M+H)$^+$, 462.30 (M+Na)$^+$.

From methyl-2-(*tert*-butyloxycarbonyl aminomethyl) acrylate (Scheme 2—R = NHBoc) as the electrophile **12a** and **12b** were obtained in 75% yield as a mixture of two diastereomers, which were separated by column chromatography using as eluent CH_2Cl_2/Et_2O/petroleum ether (7/7/2). **12a** (2*S*,4*R*) yield = 35%. R_f: 0.5 (CH_2Cl_2/Et_2O/petroleum ether 7/7/2). ^{1}H NMR (250 MHz, C_6D_6): δ 0.9 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.49 (s, 9H, *t*-Bu), 1.56 (s, 9H, *t*-Bu), 1.77 (s, 3H, CH_3), 1.9–3.2 (s, 10H), 3.4 (m, 4H, CO_2CH_3+CH_4), 4.55 (dd, 1H, $J = 9.4$ Hz $J = 5.2$ Hz, CH_2), 5.45 (t, 1H, $J = 8.2$ Hz, NH). MS (ES) m/z 497.3 (M+H)$^+$, 519.3 (M+Na)$^+$ **12b** (2*S*,4*S*) yield = 30%. R_f: 0.41 (CH_2Cl_2 M/Et_2O/petroleum ether 7/7/2).^{1}H NMR (250 MHz, C_6D_6): δ 0.91 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.43 (s, 9H, *t*Bu), 1.51 (s, 9H, *t*Bu), 1.7 (s, 3H, CH_3), 1.9–3.2 (m, 10H), 3.41 (m, 4H, CO_2CH_3+H_4), 4.45 (dd, 1H, $J = 8.1$ Hz, $J = 4.7$ Hz, H_2), 4.9 (t, 1H, $J = 8.2$ Hz, NH). MS (ES) m/z 497.3 (M+H)$^+$, 519.3 (M+Na)$^+$.

4.6. Hydrolysis of the Schiff bases 11, 12a, and 12b

Using the experimental procedure described for compound **2**; **13**, **15a**, and **15b** were prepared. Compound **11** afforded **13** in 74% yield, as a white solid, which was a mixture of two cyclic aminoesters. After recrystallization (EtOAc/hexane 2/8), the major isomer **13a** (2*S*,4*S*) was isolated and characterized. Mp = 69–72 °C. IR: 1727 cm^{-1}, 1673 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) see Table 1. $[\alpha]_D = -4.2$ (*c* 5.7, C_6D_6). MS (ES) m/z 202 (M−*t*-Bu+H)$^+$, 258 (M+H)$^+$, 280 (M+Na)$^+$, 515.5 (2M+H)$^+$, 537.2 (2M+Na)$^+$.

12a provided **15a** in 85% yield as a yellow oil. R_f: 0.23 (CH_2Cl_2/Et_2O/petroleum ether 7/7/2). ^{1}H NMR (250 MHz, C_6D_6) δ 1.4 (s, 2×9H, *t*-Bu), 1.6 (s, 2H, NH_2), 1.7–1.9 (m, 2H, H_3), 2.8 (m, 1H, H_4), 3.35 (m, 3H, H_5+H_2), 3.7 (s, 3H, CH_3), 5.1 (m, 1H, NH). MS (ES) m/z 347.2 (M+H)$^+$.

12b afforded **15b** in 85% yield. R_f: 0.13 (CH_2Cl_2/Et_2O/petroleum ether 7/7/2). ^{1}H NMR (250 MHz, C_6D_6): δ 1.45 (s, 2×9H, *t*-Bu), 1.75 (s, 2H, NH_2), 2.21 (m, 2H, H_3), 2.85 (m, 1H, H_4), 3.4 (m, 3H, H_5+H_2), 3.72 (s, 3H, CH_3), 5.2 (m, 1H, NH). MS (ES) m/z 347.2 (M+H)$^+$.

15a and **15b** left at room temperature were quantitatively transformed into *tert*-butyl 4-(*tert*-butyloxycarbonylaminomethyl) pyroglutamates **16a** and **16b**.

16a (2*S*,4*R*) major isomer. R_f: 0.3 (CH_2Cl_2/Et_2O/petroleum ether 7/7/2). ^{1}H NMR (250 MHz, C_6D_6): δ 1.5 (s, 2×9H, *t*-Bu), 1.75 (m, 1H, H_3), 2.1 (m, 1H, H_3), 2.2 (m, 1H, H_5), 3.4 (m, 1H, H_4), 3.6 (m, 2H, H_2+H_5), 5.9 (m, 1H, NH). MS (ES) m/z 259 (M−*t*-Bu+H)$^+$, 315.1 (M+H)$^+$, 336.9 (M+Na)$^+$, 629 (2M+H)$^+$, 651.2 (2M+Na)$^+$ **16b** (2*S*,4*S*) minor isomer. R_f: 0.2 (CH_2Cl_2/Et_2O/petroleum ether 7/7/2). ^{1}H NMR (250 MHz, C_6D_6): δ 1.45 (s, 2×9H, *t*-Bu), 2.2 (m, 2H, H_3), 2.72 (m, 2H, H_5), 3.3 (m, 1H, H_4), 4.0 (m, 1H, H_2), 5.4 (m, 1H, NH). MS (ES) m/z 259 (M−*t*-Bu+H)$^+$, 315.1 (M+H)$^+$, 336.9 (M+Na)$^+$, 629 (2M+H)$^+$, 651.2 (2M+Na)$^+$.

4.7. Synthesis of 4-aminomethyl pyroglutamates 17a (2*S*,4*R*) and 17b (2*S*,4*S*)

To **16a** (or **16b**) (0.06 g, 0.17 mmol) dissolved in THF (1 mL) was added 2 M HCl (1 mL, 2 mmol) and the mixture stirred 2 h at room temperature. After evaporation of the solvent, the product was extracted into EtOAc (3×5mL). The organic layer was dried and evaporated under vacuum to afford **17a** (or **17b**) in 95% yield as a white solid. **17a** (2*S*,4*R*) ^{1}H NMR (400 MHz, D_2O or CD_3OD) see Table 2. Mp = 83–85 °C. ^{13}C NMR (400 MHz, D_2O): δ 29.08 (Cβ), 39.11 (Cγ), 40.40 (Cδ), 54.56 (Cα), 175.87 (CO), 178.88 (COOH). MS (FAB$^+$): $m/z = 159.1$ (M+H)$^+$, 383 (2M+H)$^+$ HRMS (FAB$^+$): m/z calcd for $C_6H_{12}NO_4$ (M+H)$^+$ 159.0767, found 159.0783. $[\alpha]_D = -11.2$ (*c* 13.2, CD_3OD).

17b (2*S*,4*S*) ^{1}H NMR (400 MHz, D_2O or CD_3OD) see Table 2. Mp = 65–67 °C. ^{13}C NMR (400 MHz, D_2O): δ 29.30 (Cβ), 37.80 (Cγ), 40.24 (Cδ), 54.35 (Cα), 176.46 (CO), 179.46 (COOH). MS (FAB$^+$): $m/z = 159.1$ (M+H)$^+$. HRMS (FAB$^+$): m/z calcd for $C_6H_{12}NO_4$ (M+H)$^+$ 159.0767, found 159.0765. $[\alpha]_D = +47.3$ (*c* 5.2, CD_3OD).

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