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Chemoselective Michael reactions on pyroglutamates. Expeditious synthesis of spiro-bis-γ-lactams as β-turn peptidomimetics

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Abstract—Starting from pyroglutamic acid, the synthesis of spiro-bis-γ-lactams, using as key step a chemoselective Michael reaction of pyroglutamates is reported. Thus, the reaction of *N*-BOC-L-methyl pyroglutamate with LiHMDS gives the enolates at C4 which react with several Michael acceptors. On the other hand, *N*-benzyl-L-methyl pyroglutamate reacts under the same conditions, to give the ester enolate which reacts with Michael acceptors leading to quaternized derivatives. The synthesis of the bicyclic spirolactams results from a reduction of the nitro group present in these derivatives which directly gives the spiro compounds. These final compounds may act as β-turn mimetics, as they have torsion angles which are in the range of β-turns of type II and II′.

1. Introduction

Pyroglutamic acid is a useful starting material for the synthesis of several natural products,[1] and has also been used as a chiral building block for the synthesis of proline derivatives[2] and glutamic acid analogs.[3] Due to its functionality, pyroglutamic acid has been modified many times to avoid racemization at C-2, and to direct the reactivity to either carbonyl group. Although many results have been reported about aldol condensations and alkylations,[4] we have found few studies on this type of Michael reactions.[5] We have previously reported that the nature of the nitrogen protecting group determines the chemoselectivity of the enolization.[6] The Michael addition of a 3-(β-nitrovinyl)-indole was used as a model system. In the present work, we report our studies on the scope of this reaction with other substrates. The results are used to effect an expeditious synthesis of bis-spiro-γ-lactams, which can be considered as β-turn mimetics.

The development of new compounds designed to mimic the main secondary structures of peptides is currently an important approach for the study of the bioactive conformations of a natural peptide. The β-turn is a secondary substructure related in many peptides to their biological activity. These turns are classified according to their torsion angles Ψ and Φ (Fig. 1). The most common β-turns are type II in which the ideal angles are −60, 120, 80 and 0° for Φ_2, Ψ_2, Φ_3, and Ψ_3, respectively.[7]

The synthesis of nonpeptidic mimetics of these secondary structures of peptides has received much attention in the past years.[8] Thus, we summarize in Fig. 2 some examples of structures described in the literature that share the characteristic of being spirolactams based on the proline and pyroglutamic rings. These spirocompounds are interesting peptidomimetics, since they have fixed angles that resemble some naturally abundant β-turns.[9] In addition, when they are introduced into peptides, the conformational constraints of these spirocyclic structures can lead to increased bioavailability.[10] In particular, compounds **1**–**3** are mimetics of type II β-turns with increasing constraints.[11] The synthesis of these spirolactams was carried out using a Mitsunobu reaction to close the spirocyclic system.[12] Other examples of type II β-turn mimetics are compound **4**,[13] and the very recently described spirolactams **5** and **6**.[14]

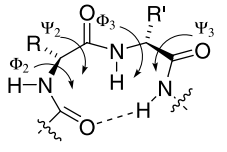

Figure 1. Typical β-turn with its angles.

Keywords: peptidomimetics; Michael reactions; aminoacids.

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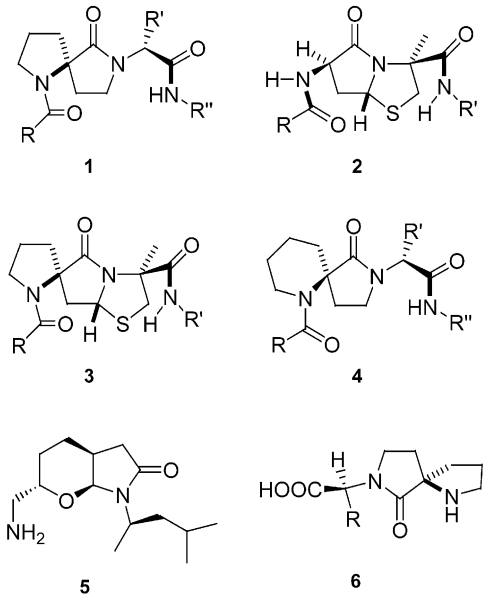
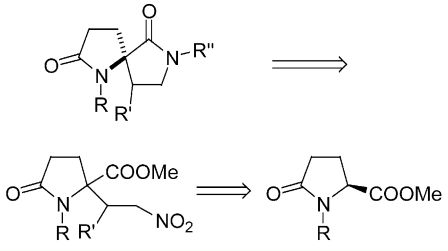


Figure 2. Some mimetics of β-turns.

Compound **5** was found to possess the same ability as PLG (L-prolyl-L-leucyl-glycinamide) to enhance the binding of [^{3}H]ADTN to dopamine receptors.[15]

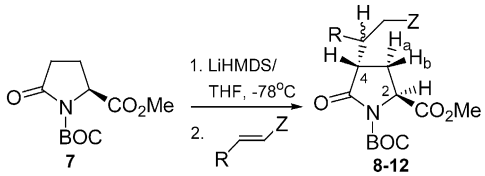
The conformational angles of these compounds depend on the substitution patterns they have. We believe that the introduction of different substituents in the spirolactam system may fix angles Φ and Ψ. Thus, starting from pyroglutamic acid, we report here the synthesis, in few steps, of spiro-bis-γ-lactams, using as key step a chemoselective Michael reaction of pyroglutamates (Scheme 1).



Scheme 1.

2. Results and discussion

We started this work with the study of the Michael reactions of L-methyl pyroglutamate. As expected from our previous experience for a similar system[6] and other earlier works that reported the results for related reactions,[4c] we found that the nature of the nitrogen protecting group directed the enolization. Thus, the reaction of *N*-BOC-L-methyl pyroglutamate, **7**, with LiHMDS at −78°C, gave the enolates at C4 which reacted with several Michael acceptors to give compounds **8**–**12** as mixtures of only two diastereomers (Scheme 2).



Scheme 2.

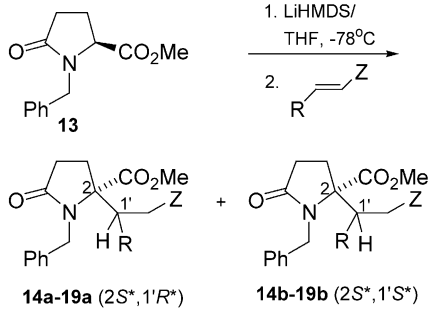
The nitrogen protecting group directs the deprotonation to each position of the pyroglutamic system due to electronic and chelation effects. In all these reactions, no multiple alkylation products were detected by NMR spectroscopy. The cleavage of the pyroglutamic ring was neither observed. Table 1 shows the results of these Michael reactions.

Functionalization of pyroglutamates at C4 by lithium enolate chemistry is well established for alkylations and for aldol reactions.[4] While alkylations are described to give exclusive formation of α products, aldol reactions are only totally diastereoselective when using titanium enolates.[16] Our results show exclusive formation of α products in Michael reactions. The relative stereochemistry of the pyroglutamic ring was assigned by n.O.e experiments and was *trans* in all cases.[17] Since in most cases conversion is not complete (entries 2, 3 and 5, Table 1) it is possible to recover unreacted starting materials. It is important to point out that besides total facial diastereoselectivity, with trans relative configuration between C2 and C4, in one case (entry 5, Table 1), only one product was obtained showing total

Table 1. Michael reactions at C4 of compound **7**

Entry	R	Z	Product	Ratio[a]	Yield
1	Ph	NO_2	**8**	85:15	40[b]
2	4-TBSOC_6H_4	NO_2	**9**	80:20	70:20[c]
3	3-(*N*-BOC-indolyl)	NO_2	**10**	60:40	60[b]
4	4-TBSOC_6H_4	CO_2Me	**11**	80:20	45[b]
5	3-(*N*-Tos-indolyl)	CO_2Me	**12**	>95:5[d]	50

[a] Calculated by integration of H2 in the ^{1}H NMR spectra of the crude mixture.
[b] In pure mixture of isomers. A small amount was separated by semi-preparative HPLC for characterization.
[c] Separated by column chromatography.
[d] Only one reaction product is detected by NMR.

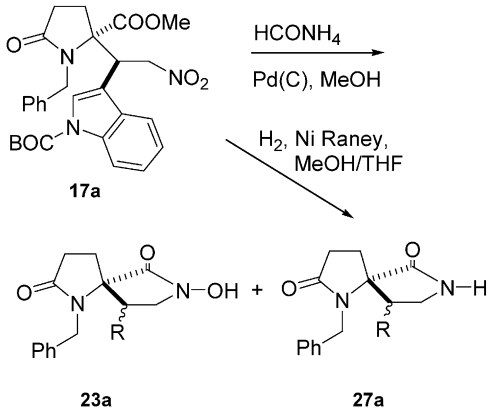


Scheme 3.

Table 2. Michael reactions of compound **13** at C2

Entry	R	Z	Products	Ratio[a]	Yield[b]
1	Ph	NO_2	**14a/14b**	1.5:1	35/20
2	2-$MeOC_6H_4$	NO_2	**15a/15b**	1:1	30/30
3	4-$TBSOC_6H_4$	NO_2	**16a/16b**	1.5:1	37/17
4	3-(*N*-BOC-indolyl)	NO_2	**17a/17b**	1:1.5	30/25
5	3,4,5-$(MeO)_3C_6H_3$	NO_2	**18a/18b**	1:1	30/30
6	4-TBSOC6H4	CO_2Me	**19a/19b**	1:1	25/35

[a] Calculated by integration of the signal of the benzylic methylenes in the ^1H NMR spectra of the crude mixture.
[b] In pure products separated by column chromatography.



Scheme 4.

diastereoselectivity of the Michael process. In all other cases, the two products obtained have opposite configuration at the stereogenic center located outside the ring, with one of them more abundant. This means that there must be an important difference in stability between the two possible transition states of the Michael reaction.

On the other hand, *N*-benzyl-L-methyl pyroglutamate **13** was prepared from pyroglutamic acid following literature procedures. The reaction with LiHMDS under the same conditions, gave the ester enolate which reacted with Michael acceptors. The final quenching with saturated ammonium chloride gave mixtures of diastereomeric racemic compounds, which were separated by column chromatography, or by semipreparative HLPC (Scheme 3, Table 2).[18] In this reaction, that involves the creation of a quaternary center, yields go from moderate to good.[19] Cleavage of the pyroglutamic ring or reaction at other positions was not observed in any case. On the other hand, Michael acceptors like cinnamonitrile and acrylonitrile failed to give any addition products under all tested conditions.

To identify each diastereomer, a single crystal of one of the isomers **14** was submitted to X-ray diffraction analysis, which showed that the relative stereochemistry corresponded to **14a** ($2S^*$,$1'R^*$).[20] The identification of the two diastereomeric mixtures obtained in the reactions of entries 2–5 in Table 2 was done by n.O.e. experiments carried out for spirolactams derived from these compounds (vide infra). Nevertheless, as compounds **19a** and **19b** (entry 6, Table 2) were not to be cyclised, we identified their stereochemistry by analogy of their NMR spectra with those of the rest of the series.

This procedure allows the preparation of glutamic derivatives with quaternary carbons. This is a remarkable feature, since the few examples reported in the literature, dealing with the preparation of these amino acid derivatives, usually involve the preparation of the pyroglutamic ring from previously α-substituted glutamic acids.[21]

The synthesis of the bicyclic spirolactams was planned by a reduction of the nitro group in compounds **14**–**18**, followed by aminolysis of the ester. Thus, compound **17a** was submitted to reduction with ammonium formate in the presence of Pd(C). We were very pleased to observe the direct cyclization of the intermediate to give two bicyclic compounds that turned out to be compounds **23a** and **27a** (Scheme 4).

The ^1H NMR spectra of these two compounds were very

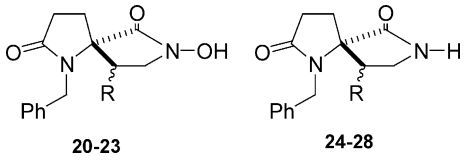
Table 3. The reduction reactions of compounds **13**–**18**

			Method A[a]			Method B[b]	
Entry	R	Substrate	Products	Ratio[c]	Yield[d]	Product	Yield[d]
1	Ph	**14a**	**20a/24a**	19:1	70/0	**24a**	72
2	Ph	**14b**	**20b/24b**	3:1	65/10	**24b**	70
3	2-MeOPh	**15a**	**21a/25a**	6:1	55/20	–	–
4	2-MeOPh	**15b**	**21b/25b**	3:1	60/25	–	–
5	4-TBSOPh	**16a**	**22a/26a**	6:1	70/15	**26a**	80
6	4-TBSOPh	**16b**	**22b/26b**	3:1	70/25	**26b**	90
7	3-(*N*-BOC-indolyl)	**17a**	**23a/27a**	4:1	70/25	**27a**	50
8	3-(*N*-BOC-indolyl)	**17b**	**23b/27b**	3:1	70/20	**27b**	50
9	3,4,5-$(MeO)_3$Ph	**18a**	–	–		**28a**	75
10	3,4,5-$(MeO)_3$Ph	**18b**	–	–		**28b**	70

[a] Ammonium formate/Pd(C) in MeOH.
[b] Hydrogen/Ni Raney at 45 psi in MeOH/THF.
[c] Calculated by integration of two well resolved signals in the ^1H NMR spectra of the crude mixture.
[d] In pure product with correct spectroscopic and analytical data.

similar, with the only remarkable difference of a signal at 10.38 ppm for compound **23a** that did not appear in **27a**. The presence of an OH band in the IR spectrum prompted us to propose the structure depicted in Scheme 1 for compound **23a**. Mass spectra of compounds **23a** and **27a** also supported this hypothesis. Compound **27a**, with M^+ at 459, shows a loss of the BOC fragment leading to a M+1−100 peak which is the base peak. For the *N*-hydroxylated compound **23a**, the M^+ peak could not be observed, but a peak corresponding also to the same BOC fragmentation was clear and indicated that the mass of this compound was 475, 16 amu more than **27a**. In order to confirm the structure of these compounds, we reduced **23a** with $TiCl_3$ in THF, following the conditions described by Miller for the reduction of *N*-hydroxy-β-lactams to *NH*-β-lactams.[22] This reaction led to **27a** with 82% yield. We can assume that a *N*-hydroxy intermediate is formed in the reduction of the nitro group which attacks the ester to give the cyclic hydroxamic acid.

Table 3 shows the results for the reaction of compounds **14**–**18** with ammonium formate and Pd(C). The reaction conditions were chosen to favor the formation of the hydroxamic acids as the major reaction products. The direct obtention of bis-γ-lactams such as **24**–**28** as the only reduction products was achieved by hydrogenation of the starting compounds **14**–**18** in the presence of Raney Nickel. The results are also included in Table 3 and show the possibility of the obtention of any of the two products just by changing the reduction conditions.



The configuration of these spirocompounds was assigned by n.O.e. experiments. The methodology used exemplified for compound **27** was as follows (Fig. 3). Proton at C9 was identified by means of an HETCOR experiment and irradiated observing a 6% n.O.e. effect in one of the protons situated at C4 only for isomer **27b**. The reverse n.O.e. effect was of 5%. These effects were not observed for the other diastereomer **27a**, confirming the relative stereochemistry depicted in Fig. 3.

The configuration of the rest of compounds shown in Table 3 was assigned the same way, resulting in a (5*S**,9*R**) configuration for compounds **20**–**28a** and compounds (5*S**,9*S**) for **20**–**28b**. These conclusions were later confirmed by an X-ray diffraction analysis of a single crystal of compound **24a** (Fig. 4).

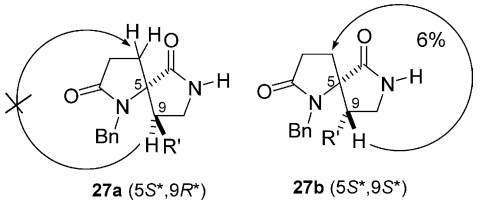


Figure 3. n.O.e. increments **27a**, **b**.

In order to effect the conformational study of these spirocompounds, we finally carried out the synthesis of two diastereomeric *N*-deprotected bis-γ-lactams, **32**, following Scheme 5. The PMB group was selected, since it was easier to remove than the benzyl group. Thus, starting from methyl *N*-(*p*-methoxybenzyl)pyroglutamate, **29**, we obtained the diastereomers **30a**, **b**, which were separated and treated with CAN before doing the reduction with hydrogen in the presence of Raney–Nickel. The spirolactams **32** were identified by means of n.O.e. experiments. The reactions proceeded with good yields in each step.

A Monte Carlo random variational conformational search using amber force field was performed on compounds **32** to locate water-solvated low energy minima, using the solution model GB/SA as implemented in Macromodel.[23–25] The lowest energy structures were subjected to a RHF/6-31G* geometry optimization using Gaussian 94.[26] According to these data, the torsion dihedral angles fit reasonably well with the ideal structural parameters of type-II β-turn. For compound **32a**, values of −37 and +136 for angles Φ_2 and Ψ_2, respectively, were found. These values are within the accepted interval (±30) to classify this compound as a β-turn type II mimetic. For compound **32b**, values of +35 and −106 for angles Φ_2 and Ψ_2, respectively, were obtained. These values lie within the interval to be considered as a type II′ β-turn mimetic. Optimized geometries are represented in Fig. 5.

In summary, Michael reactions of pyroglutamates can lead

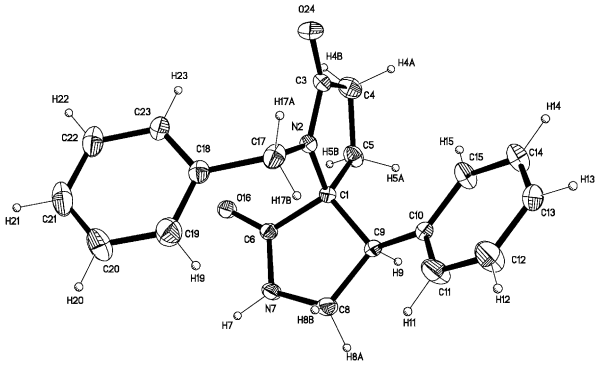
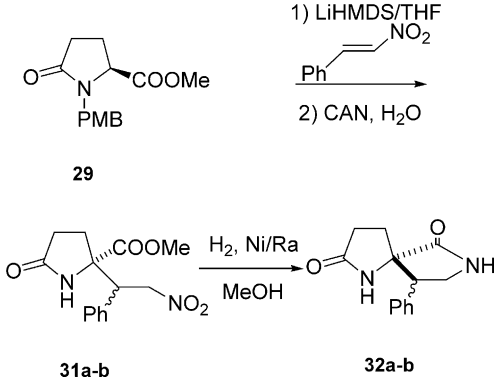


Figure 4. ORTEP drawing of compound **24a**.



Scheme 5. Synthesis of compounds **32a**, **b**.

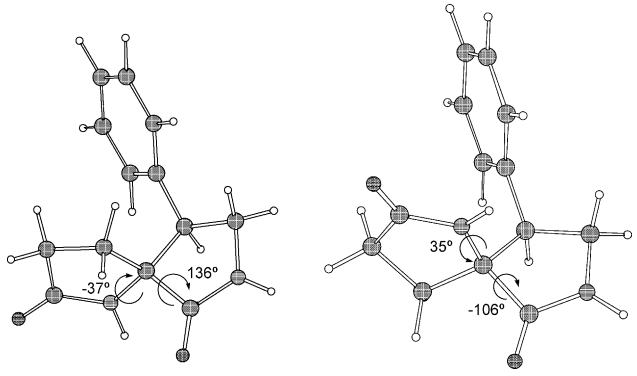


Figure 5. Optimized geometries for compounds **32a**, **b**.

to interesting compounds related to peptidomimetics. In the case of C4 enolizations, two new stereocenters are formed. Stereochemistry at C4 is exclusively α in all cases, showing total diastereofacial selectivity for this reaction. In addition, the stereogenic open chain carbon is formed with high stereoselectivity. For C2 enolization, interesting quaternizated aminoacid derivatives are obtained, that can be converted into spiro-bis-γ-lactams in only one step with good yields. These final compounds may act as β-turn mimetics, since they have torsion angles that are in the range of β-turns of type II and II′.

3. Experimental

3.1. General

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.43 MHz, respectively. NMR spectra were registered in $CDCl_3$ and chemical shifts are given in ppm relative to TMS (^{1}H, 0.00 ppm) or $CDCl_3$ (^{13}C, 77.00 ppm). Elemental analyses were preformed in the UCM Microanalysis Service (Facultad de Farmacia, Universidad Complutense de Madrid, Spain). For purification of crude reaction mixtures flash chromatography was applied in all cases. Silica gel (230–400 mesh) was used as the stationary phase. Analytical HPLC was with Hypersyl BDS C_{18} column (250×3 mm^2), monitoring at 220 nm and eluting with mixtures of CH_3CN/H_2O or $MeOH/H_2O$. Semipreparative HPLC was performed with precolumn and Zorbax ODS (250×9.4 mm^2) column.

3.2. Synthesis of 4-substituted pyroglutamates 8–12

General method. To a solution of ethyl (2*S*)-1-(*tert*-butoxycarbonyl)pyroglutamate **7** (1 mmol) in dry THF (5 ml) stirred at −78°C under argon atmosphere was added a 1 M solution of lithium hexamethyldisilazide in THF (1.2 ml, 1.2 mmol). The mixture was stirred at −78°C for 1 h.. The α,β-unsaturated compound (1.3 mmol) dissolved in dry THF (2 ml) was added at −78°C, and the mixture was then stirred for 3 h at this temperature. The reaction mixture was quenched with saturated solution of ammonium chloride at −78°C, and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated to dryness. The products were purified by silica gel chromatography, using hexane/EtOAc mixtures as eluent.

3.2.1. Methyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-[1′-phenyl-2′-nitroethyl]pyroglutamate, 8. This compound was obtained as a 1/4 mixture of diastereoisomers (440 mg, 40%). The isomers α and β were characterized from a small fraction of this mixture after separation by semipreparative HPLC. *Isomer α*: $[\alpha]_D=-48.8°$ (*c* 0.59, $CHCl_3$); IR ($CHCl_3$) 3010, 2980, 1790, 1745, 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.96–2.16 (m, 2H), 3.11–3.19 (m, 1H), 3.75–3.81 (m, 1H), 3.75 (s, 3H), 4.19 (dd, 1H, *J*=9.3 and 2.7 Hz), 5.16 (d, 2H, *J*=7.7 Hz), 7.24–7.25 (m, 2H), 7.30–7.35 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 172.8, 171.3, 149.1, 136.6, 129.2, 128.3, 127.9, 84.2, 77.9, 56.4, 52.7, 45.1, 43.2, 27.8, 27.4. *Isomer β*: $[\alpha]_D=-71.4°$ (*c* 0.07, $CHCl_3$); IR ($CHCl_3$) 3010, 2960, 1780, 1745, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.71–1.95 (m, 2H), 2.92–3.03 (m, 1H), 3.65–3.66 (m, 1H), 3.75 (s, 3H), 4.47 (dd, 1H, *J*=9.3 and 1.6 Hz), 4.74 (dd, 1H, *J*=12.6 and 10.4 Hz), 5.63 (dd, 1H, *J*=12.6 and 5.0 Hz), 7.16–7.22 (m, 2Hr), 7.29–7.37 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.4, 155.6, 149.1, 129.0, 128.9, 120.6, 84.2, 78.0, 56.4, 52.7, 44.3, 44.1, 27.8, 25.6.

3.2.2. Methyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-{2′-nitro-1′-[4-(*tert*-butyldimethylsilanyloxy)phenyl]ethyl}-pyro-glutamate, 9. This compound was obtained as a 4/1 mixture of diastereoisomers, which were separated by silica gel chromatography. *Isomer α*: white solid (350 mg, 71%); mp 57–60°C; $[\alpha]_D=+5.7°$ (*c* 0.70, $CHCl_3$); IR (KBr); 2950, 1785, 1745, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.18 (s, 6H), 0.96 (s, 9H), 1.49 (s, 9H), 1.75–1.95 (m, 2H), 2.88–2.98 (m, 1H), 3.62–3.70 (m, 1), 3.75 (s, 3H), 4.46 (dd, 1H, *J*=9.3 and 1.9 Hz), 4.69 (dd, 1H, *J*=13.2 and 10.4 Hz), 5.54 (dd, 1H, *J*=13.2 and 5.0 Hz), 6.78 (d, 2H, *J*=8.8 Hz), 7.02 (d, 2H, *J*=8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 172.9, 171.4, 155.6, 149.1, 129.0, 128.9, 120.6, 84.2, 78.0, 56.4, 52.7, 44.3, 43.4, 27.8, 27.3, 25.6, 18.1, −4.5. *Isomer β*: white solid (100 mg, 20%); mp 118–121°C; $[\alpha]_D=-77.8$ (*c* 0.80, $CHCl_3$); IR (KBr); 3010, 1785, 1745, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.19 (s, 6H, Me_2Si), 0.96 (s, 9H), 1.46 (s, 9H), 1.97–2.15 (m, 2H), 3.07–3.14 (m, 1H), 3.67–3.74 (m, 1H), 3.74 (s, 3H), 4.14 (dd, 1H, *J*=9.3 and 3.3 Hz), 5.12 (d, 2H, *J*=8.2 Hz), 6.79 (d, 2H, *J*=8.8 Hz), 7.11 (d, 2H, *J*=8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 172.9, 171.3, 155.8, 148.8, 129.9, 127.7, 120.6, 83.9, 77.0, 57.0, 52.6, 44.3, 43.6, 27.8, 25.6, 25.5, 18.1, −4.4.

3.2.3. Methyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-{1′-[(1-*tert*-butoxycarbonyl)indol-3-yl]-2′-nitroethyl}pyro-glutamate, 10. This compound was obtained as a 3/2 mixture of diastereoisomers (481 mg, 60%). The isomers were characterized from a small fraction of this mixture separated by semipreparative HPLC. *Isomer α*: oil; $[\alpha]_D=+18.9$ (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) 2985, 1785, 1735, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.68 (s, 9H), 1.88–2.09 (m, 2H), 3.11–3.21 (m, 1H), 3.74 (s, 3H), 3.97–4.05 (m, 1H), 4.48 (dd, 1H, *J*=9.3 and 1.6 Hz), 4.89 (dd, 1H, *J*=12.6 and 10.46 Hz), 5.59 (dd, 1H, *J*=12.6 and 15.0 Hz), 7.24–7.37 (m, 2H), 7.49 (s, 1H), 7.53 (d, 1H, *J*=7.7 Hz), 8.13 (d, 1H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 172.9, 171.3, 149.3, 149.1, 131.4, 125.1, 124.6, 124.5, 123.1, 118.7, 115.9, 115.7, 84.5,

84.2, 56.4, 52.7, 42.7, 36.9, 28.2, 27.9, 27.3. *Isomer* β: $[\alpha]_D=-58.4$ (*c* 0.15, $CHCl_3$); IR ($CHCl_3$) 3010, 2980, 1790, 1745, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.67 (s, 9H), 1.98–2.09 (m, 2H), 2.11–2.22 (m, 1H), 3.76 (s, 3H), 4.15–4.20 (m, 1H), 4.33 (d, 1H, *J*=8.2 Hz), 5.08 (dd, 1H, *J*=13.7 and 7.9 Hz), 5.26 (dd, 1H, *J*=13.7 and 7.2 Hz) 7.28–7.38 (m, 2H), 7.52 (d, 1H, *J*=7.7 Hz), 7.56 (s, 1H), 8.15 (d, 1H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.3, 149.2, 149.1, 130.5, 125.1, 124.5, 124.4, 123.1, 118.2, 115.5, 114.9, 84.3, 84.0, 56.7, 52.7, 43.8, 34.2, 28.2, 27.8, 25.9.

3.2.4. Methyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-{1′-[4-(*tert*-butyldimethylsilanyloxy)phenyl]-2′-(methoxycarbonyl)ethyl}pyroglutamate, 11. This compound was obtained as a 2/8 mixture of diastereoisomers (256 mg, 45%). The isomers were characterized from a small fraction of this mixture separated by semipreparative HPLC. *Isomer* α: oil; $[\alpha]_D=-28.6°$ (*c* 0.07, $CHCl_3$); IR ($CHCl_3$) 3010, 1780, 1840, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.18 (s, 6H), 0.97 (s, 9H), 1.45 (s, 9H), 1.97–2.13 (m, 2H), 2.87 (dd, 1H, *J*=15.9 and 8.2 Hz), 2.98–3.06 (m, 1H), 3.12 (dd, 1H, *J*=15.9 and 7.7 Hz), 3.45–3.51 (m, 1H), 3.58 (s, 3H), 3.72 (s, 3H), 4.11 (dd, 1H, *J*=9.1 and 1.4 Hz), 6.75 (d, 2H, *J*=8.2 Hz), 7.08 (d, 2H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 173.6, 172.5, 171.7, 155.6, 149.1, 132.3, 129.3, 120.1, 83.5, 56.8, 52.5, 51.6, 45.9, 41.3, 36.2, 27.9, 25.6, 25.4, 18.1, −4.4. *Isomer* β: oil; $[\alpha]_D=-15.5$ (*c* 0.20, $CHCl_3$); IR ($CHCl_3$) 3020, 1785, 1745, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.18 (s, 6H), 0.96 (s, 9H), 1.47 (s, 9H), 1.75–1.91 (m, 2H), 2.68 (dd, 1H, *J*=15.9 and 9.3 Hz), 2.83–2.93 (m, 1H), 3.31 (dd, 1H, *J*=15.9 and 6.0 Hz), 3.45–3.52 (m, 1H), 3.54 (s, 3H), 3.74 (s, 3H), 4.31 (dd, 1H, *J*=8.8 and 2.8 Hz), 6.75 (d, 2H, *J*=8.2 Hz), 7.03 (d, 2H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 173.6, 172.0, 171.7, 154.7, 149.2, 132.6, 129.1, 120.1, 83.6, 56.6, 52.5, 51.5, 45.7, 41.5, 38.5, 27.9, 26.3, 25.6, 18.1, −4.4.

3.2.5. Methyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-{2′-(methoxycarbonyl)-1′-[1-(toluene-4-sulfonyl)indol-3-yl]ethyl}pyroglutamate, 12. This compound was obtained as a single diastereoisomer (360 mg, 50%); $[\alpha]_D=-54.5°$ (*c* 0.30, $CHCl_3$); IR ($CHCl_3$) 3010, 1780, 1745, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (s, 9H, ^{t}Bu), 1.94–2.00 (m, 2H), 2.32 (s, 3H), 2.90 (dd, 1H, *J*=15.9 and 8.8 Hz), 3.07–3.20 (m, 2H), 3.53 (s, 3H), 3.71 (s, 3H), 3.88–3.94 (m, 1H), 4.33 (dd, 1H, *J*=7.7 and 3.3 Hz), 7.18–7.35 (m, 4H), 7.47 (s, 1H), 7.51 (d, 1H, *J*=7.2 Hz), 7.69 (d, 2H, *J*=8.2 Hz), 7.96 (d, 1H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 173.0, 172.0, 171.4, 149.2, 144.9, 138.2, 134.9, 130.4, 129.8, 126.8, 125.1, 124.0, 123.5, 121.8, 119.2, 113.8, 83.8, 56.5, 52.6, 51.7, 44.9, 35.5, 31.6, 27.9, 25.1, 21.5.

3.3. General procedure for the synthesis of 2-substituted pyroglutamates 14–19

To a solution of HMDS (1.1 mmol) in THF (2 ml) under argon atmosphere and at −78°C was added dropwise *n*-BuLi (1.05 mmol of 1.6 M hexane solution). After 30 min, a solution of Methyl (2*S*)-*N*-benzylpyroglutamate **13** (1 mmol) in THF (1 ml) was added at −78°C, and the solution stirred for 1 h. The α,β-unsaturated compound (1.2 ml) in THF (2 ml) was added also at −78°C, and the mixture was then stirred for 4 h. The reaction mixture was quenched with saturated solution of ammonium chloride at −25°C, and the solution allowed to warm to room temperature. The solution was extracted with Et_2O, and the combined organic phases dried (Na_2SO_4), and evaporated to dryness. The products were separated and purified by silica gel chromatography, using hexane/EtOAc as elluents.

3.3.1. Methyl 1-benzyl-2-(2′-nitro-1′-phenylethyl)pyroglutamate, 14. This compound was obtained as a 1.5:1 mixture of diastereoisomers. The isomers **14a** and **14b** were separated by a second flash chromatography, yielding 553 mg (35%) of **14a** and 316 mg (20%) of **14b**. *(2S*,1′R*)-**14a***: oil; IR ($CHCl_3$); 3010, 2960, 1740, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50–1.61 (m, 1H), 2.10–2.34 (m, 2H), 2.57–2.68 (m, 1H), 3.09 (s, 3H), 4.27 (d, 1H, *J*=15.4 Hz), 4.54 (dd, 1H, *J*=10.4 and 3.8 Hz), 4.72 (dd, 1H, *J*=13.7 and 3.8 Hz), 4.93 (dd, 1H, *J*=13.7 and 10.4 Hz), 5.07 (d, 1H, *J*=15.4 Hz), 7.17–7.36 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 175.3, 170.6, 136.1, 133.4, 129.2, 129.0, 128.5, 128.3, 127.5, 127.3, 75.4, 69.9, 52.6, 45.2, 44.6, 28.5, 23.6; MS *m*/*z* (%) 276 (12), 233 (15), 232 (63), 91 (100), 65 (11). *(2S*,1′S*)-**14b***: IR ($CHCl_3$); 3020, 2940, 1740, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02–2.13 (m, 1H), 2.38–2.46 (m, 1H), 2.57–2.68 (m, 2H), 3.33 (s, 3H), 4.30 (dd, 1H, *J*=13.2 and 3.3 Hz), 4.42 (dd, 1H, *J*=11.6 and 3.3 Hz), 4.66 (dd, 1H, *J*=13.2 and 11.6 Hz), 4.69 (d, 1H, *J*=15.4 Hz), 4.75 (d, 1H, *J*=15.4 Hz), 7.21–7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 176.1, 169.6, 137.0, 133.6, 129.2, 129.1, 128.8, 128.7, 127.9, 127.7, 75.3, 71.6, 52.3, 46.0, 44.9, 29.3, 25.4; MS *m*/*z* (%); 276 (10), 233 (12), 232 (62), 91 (100), 65 (12).

3.3.2. Methyl 1-benzyl-2-[1′-(2-methoxyphenyl)-2′-nitroethyl]pyroglutamate, 15. These compounds were obtained as a 1:1 mixture of diastereoisomers. The isomers **15a** and **15b** were separated by a second flash chromatography, yielding 483 mg (30%) of **15a** and 483 mg (30%) of **15b**. *(2S*,1′R*)-**15a***: white solid (MeOH/H_2O); mp 125–127°C; IR (KBr); 3000, 2960, 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.67–1.79 (m, 1H), 2.25–2.37 (m, 2H), 2.55–2.66 (m, 1H), 3.10 (s, 3H), 3.87 (s, 3H), 4.34 (d, 1H, *J*=15.9 Hz), 4.72 (dd, 1H, *J*=11.6 and 3.3 Hz), 4.91–5.00 (m, 3H), 6.88–6.97 (m, 2H), 7.13–7.33 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 175.4, 170.7, 158.0, 136.7, 130.1, 128.7, 128.3, 127.3, 122.1, 121.7, 111.5, 75.9, 70.3, 55.6, 52.5, 44.6, 39.5, 28.8, 24.2; Anal. calcd for $C_{22}H_{24}N_2O_6$: C, 64.07; H, 5.87; N, 6.79. Found: C, 64.14; H, 5.93; N, 7.01. *(2S*,1′S*)-**15b***: oil; IR ($CHCl_3$); 3000, 2940, 1735, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.14–2.24 (m, 1H), 2.28–2.38 (m, 1H), 2.42–2.53 (m, 1H), 2.58–2.66 (m, 1H), 3.31 (s, 3H), 3.83 (s, 3H), 4.36 (dd, 1H, *J*=13.2 and 3.3 Hz), 4.63 (d, 1H, *J*=15.4 Hz), 4.71 (d, 1H, *J*=15.4 Hz), 4.74 (dd, 1H, *J*=11.0 and 3.3 Hz), 4.91 (dd, 1H, *J*=13.2 and 11.0 Hz), 6.85–6.92 (m, 2H), 7.13–7.16 (m, 1H), 7.22–7.35 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 176.1, 170.5, 157.9, 137.4, 130.3, 129.9, 128.6, 127.9, 127.4, 122.3, 120.8, 111.4, 75.0, 72.2, 55.6, 52.2, 45.5, 41.6, 29.3, 27.1.

3.3.3. Methyl 1-benzyl-2-{1′-[(4-*tert*-butyldimethylsilanyloxy)phenyl]-2′-nitroethyl}pyroglutamate, 16. These compounds were obtained as a 1.5:1 mixture of diastereoisomers. The isomers **16a** and **16b** were separated

by a second flash chromatography, yielding 790 mg (37%) of **16a** and 362 mg (17%) of **16b**. *(2S*,1′R*>)-**16a***: solid (MeOH/H_2O); mp 102–104°C; IR (KBr); 3020, 2940, 1730, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.19 (s, 6H), 0.97 (s, 9H), 1.50–1.61 (m, 1H), 2.04–2.14 (m, 1H), 2.24–2.34 (m, 1H), 2.56–2.67 (m, 1H), 3.08 (s, 3H), 4.20 (d, 1H, J=15.4 Hz), 4.47 (dd, 1H, J=10.4 and 3.8 Hz), 4.68 (dd, 1H, J=13.2 and 3.8 Hz), 4.87 (dd, 1H, J=13.2 and 10.4 Hz), 5.07 (d, 1H, J=15.4 Hz), 6.81 (d, 2H, J=8.2 Hz), 7.10 (d, 2H, J=8.2 Hz), 7.17–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.3, 170.6, 156.2, 136.2, 129.5, 128.3, 127.4, 127.3, 125.5, 120.7, 75.5, 69.9, 52.6, 44.6, 44.5, 29.3, 25.5, 23.5, 18.1, −4.5; Anal. calcd for $C_{27}H_{36}N_2O_6Si$: C, 64.48; H, 5.99; N, 8.06. Found: C, 64.13; H, 5.74; N, 8.09. *(2S*,1′S*)-**16b***: solid (MeOH/H_2O); mp 110–112°C; IR (KBr); 3020, 2940, 1740, 1690, 1600, 1550, 1510 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.17 (s, 6H), 0.95 (s, 9H), 1.97–2.05 (m, 1H), 2.38–2.46 (m, 1H), 2.53–2.62 (m, 2H), 3.35 (s, 3H), 4.26 (dd, 1H, J=13.2 and 3.3 Hz), 4.35 (dd, 1H, J=11.5 and 3.3 Hz), 4.50 (dd, 1H, J=13.2 and 11.5 Hz), 4.69 (d, 1H, J=15.4 Hz), 4.73 (d, 1H, J=15.4 Hz), 6.73 (d, 1H, J=8.2 Hz), 7.07 (d, 1H, J=8.2 Hz), 7.24–7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 176.1, 169.7, 155.9, 137.1, 130.3, 128.8, 128.0, 127.7, 125.8, 120.3, 75,5, 71.7, 52.3, 45.4, 44.9, 29.3, 25.6, 25.5, 18.1, −4.4; Anal. calcd for $C_{27}H_{36}N_2O_6Si$: C, 64.48; H, 5.99; N, 8.06. Found: C, 64.39; H, 5.63; N, 7.95.

3.3.4. Methyl 1-benzyl-2-{1′-[(1-*tert*-butoxycarbonyl)-indol-3-yl]-2-nitroethyl}pyroglutamate, 17. This compound was obtained as a 1:1.5 mixture of diastereoisomers. This mixture was separated by a second flash chromatography, yielded 887 mg (25%) of **17a** and 1.06 g, (30%) of **17b**. *(2S*,1′R*)-**17a***: solid, mp 110–112°C (cyclohexane); IR (KBr); 3100–2990, 1730, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69 (s, 9H), 2.31–2.47 (m, 2H), 2.52–2.70 (m, 2H), 3.19 (s, 3H), 4.01 (d, 1H, J=15.4 Hz), 4.74 (d, 1H, J=15.4 Hz), 4.74–4.99 (m, 3H), 7.17–7.37 (m, 7H), 7.56 (s, 1H), 7.71–7.74 (m, 1H), 8.06 (d, 1H, J=6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 175.4 (C), 171.2 (C), 149.5 (C), 136.5 (C), 134.7 (C), 128.5 (C), 128.3 (CH), 128.0 (CH), 127.6 (CH), 125.2 (CH), 123.6 (CH), 123.2 (CH), 118.4 (CH), 115.6 (CH), 114.2 (C), 84.6 (C), 77.4 (CH_2), 69.7 (C), 52.6 (CH_3), 44.8 (CH_2), 37.6 (CH), 29.0 (CH_2), 28.2 (CH_3), 25.6 (CH_2); MS *m/z* (%); 375 (2), 315 (2), 301 (6), 232 (45), 188 (10), 174 (11), 143 (10), 130 (11), 115 (6), 91 (100), 57 (28); Anal. calcd for $C_{28}H_{31}N_3O_7$: C, 64.48; H, 5.99; N, 8.06. Found: C, 64.23; H, 5.69; N, 7.94. *(2S*,1′S*)-**17b***: solid mp 143–145°C (Tol/Hex); IR (KBr); 3100–2990, 1730, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.67 (s, 9H), 2.15–2.18 (m, 1H), 2.52–2.61 (m, 1H), 2.65–2.85 (m, 2H), 3.14 (s, 3H), 4.35 (dd, 1H, J=13.2 and 11.0 Hz), 4.55 (dd, 1H, J=11.0 and 3.3 Hz), 4.80 (s, 2H), 4.83 (dd, 1H, J=11.0 and 3.3 Hz), 7.20–7.38 (m, 7H), 7.52 (s, 1H), 7.66 (m, 1H), 8.02 (d, 1H, J=6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 176.1, 169.7, 149.3, 136.7, 134.5, 129.7, 128.9, 128.0, 127.9, 125.0, 123.9, 122.9, 119.3, 115.1, 114.7, 84.6, 76.5, 71.0, 52.4, 44.7, 36.5, 29.4, 28.1, 25.4;; MS *m/z* (%); 375 (1), 315 (1), 301 (4), 232 (43), 188 (6), 174 (8), 143 (6), 130 (7), 115 (6), 91 (100), 57 (27); Anal. calcd for $C_{28}H_{31}N_3O_7$: C, 64.48; H, 5.99; N, 8.06. Found: C, 64.32; H, 6.02; N, 8.00.

3.3.5. Methyl 1-benzyl-2-[2′-nitro-1′-(3,4,5-trimethoxyphenyl)ethyl]pyroglutamate, 18. This compound was obtained as a 1:1 mixture of diastereoisomers. This mixture was separated by a second flash chromatography yielding 590 mg (30%) of **18a** and 595 mg (30%) of **18b**. *(2S*,1′R*)-**18a***: solid; mp 72–75°C; IR (KBr); 3000, 2980, 1730, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57–1.69 (m, 1H), 2.05–2.14 (m, 1H), 2.29–2.39 (m, 1H), 2.56–2.67 (m, 1H), 3.13 (s, 3H), 3.84 (s, 9H), 4.28 (d, 1H, J=15.4 Hz), 4.42 (dd, 1H, J=11.0 and 3.8 Hz), 4.73 (dd, 1H, J=13.2 and 3.8 Hz), 4.89 (dd, 1H, J=13.2 and 11.0 Hz), 5.09 (d, 1H, J=15.4 Hz), 6.41 (s, 2H), 7.18–7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.5, 170.6, 153.5, 138.6, 136.0, 128.6, 128.4, 127.4, 127.3, 105.0, 75.5, 69.9, 61.0, 56.4, 52.7, 45.4, 44.7, 28.6, 24.2. Anal. calcd for $C_{24}H_{28}N_2O_8$: C, 61.01; H, 5.97; N, 5.93. Found: C, 58.01; H, 5.62; N, 5.73. *(2S*,1′S*)-**18b***: solid; mp 141.143°C; IR (KBr); 3000, 2980, 1725, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00–2.10 (m, 1H), 2.39–2.51 (m, 2H), 2.54–2.64 (m, 1H), 3.43 (s, 3H), 3.78 (s, 6H), 3.80 (s, 3H), 4.29–4.37 (m, 2H), 4.56–4.74 (m, 3H), 6.38 (s, 2H), 7.25–7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 176.2, 170.0, 153.2, 138.2, 137.0, 128.9, 128.0, 127.7, 127.6, 106.3, 74.6, 71.7, 60.8, 56.2, 52.5, 46.8, 45.1, 29.2, 26.3; Anal. calcd for $C_{24}H_{28}N_2O_8$: C, 61.01; H, 5.97; N, 5.93. Found: C, 61.26; H, 5.76; N, 6.00.

3.3.6. Methyl 1-benzyl-2-{1′-[4-(*tert*-butyldimethylsilanyloxy)phenyl]-2′-methoxycarbonylethyl}pyroglutamate, 19. This compound was obtained as a 1:1 mixture of diastereoisomers. This mixture was separated by a second flash chromatography, yielding 280 mg, (25%) of **19a** and 393 mg, (35%) of **19b**. *(2S*,1′R*)-**19a***: oil; IR ($CHCl_3$); 3010, 1740, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.16 (s, 6H), 0.95 (s, 9H), 2.08–2.16 (m, 1H), 2.32 (dd, 1H, J=15.9 and 2.8 Hz), 2.39–2.66 (m, 4H), 3.23 (s, 3H), 3.43 (s, 3H), 4.04 (dd, 1H, J=11.0 and 2.8 Hz), 4.62 (d, 1H, J=15.9 Hz), 4.79 (d, 1H, J=15.9 Hz), 6.71 (d, 2H, J=8.2 Hz), 7.08 (d, 2H, J=8.2 Hz), 7.20–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.6, 171.4, 170.8, 155.4, 136.8, 129.5, 128.2, 127.7, 128.0, 126.8, 120.2, 71.4, 52.3, 51.8, 44.5, 42.0, 34.6, 28.6, 25.6, 22.5, 18.1, −4.5. *(2R*,1′R*)-**19b***: oil; IR ($CHCl_3$); 3010, 2980, 1740, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.18 (s, 6H), 0.97 (s, 9H), 1.24–1.41 (m, 1H), 2.05–2.26 (m, 2H), 2.48 (dd, 1H, J=15.9 and 3.8 Hz), 2.69 (m, 1H), 2.84 (dd, 1H, J=15.9 and 11.0 Hz), 3.06 (s, 3H), 3.54 (s, 3H), 4.11 (dd, 1H, J=11.0 and 3.8 Hz), 4.34 (d, 1H, J=15.9 Hz), 5.10 (d, 1H, J=15.9 Hz), 6.79 (d, 2H, J=8.8 Hz), 7.08 (d, 2H, J=8.8 Hz), 7.13–7.23 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.6, 171.4, 170.8, 155.4, 136.8, 129.5, 128.2, 127.4, 127.3, 126.8, 120.2, 71.4, 52.3, 51.8, 44.5, 42.0, 34.6, 28.6, 25.6, 22.5, 18.1, −4.5.

3.4. General procedure for reduction of nitroethylpyroglutamates

Method A. To a stirred suspension of the nitrocompound **14**–**18** (1 mmol) and 10% Pd–C (150 mg) in dry methanol (20 ml), anhydrous ammonium formate (20 mmol) was added in a single portion. The resulting reaction mixture was stirred at room temperature for 10–30 min under argon. The catalyst was removed by filtration through a celite pad and washed with methanol. The filtrated was evaporated under reduced pressure and the resulting residue was triturated with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered,

and evaporated to dryness. The products were purified by flash chromatography ($CHCl_3$/MeOH mixtures).

Method B. A solution of the nitrocompound **14**–**18** (1 mmol) in 10 ml of a mixture MeOH/THF (2:1) was hydrogenated over 500 mg of Raney-Ni at 45 psi on a shaker at room temperature for 4 h. The catalyst was removed by filtration through a celite pad and washed with methanol. The filtrated was evaporated under reduced pressure. The resulting residue was triturated with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, evaporated to dryness and the product was purified by crystallization.

3.4.1. (5*S,9*R**)-1-Benzyl-7-hydroxy-9-phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 20a.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 100:1), 59 mg, (70%) were obtained as a white solid: mp 158–160°C (CH_2Cl_2/Et_2O). ^{1}H NMR ($CDCl_3$) δ 1.56–1.62 (m, 1H), 1.80–1.98 (m, 2H), 2.19–2.29 (m, 1H), 3.57 (t, 1H, *J*=8.8 Hz), 3.77 (dd, 1H, *J*=9.3 and 8.3 Hz), 3.94 (dd, 1H, *J*=9.9 and 8.3 Hz), 3.99 (d, 1H, *J*=14.8 Hz), 5.11 (d, 1H, *J*=14.8 Hz), 6.72–6.75 (m, 2H), 7.22–7.26 (m, 3H), 7.31–7.40 (m, 3H), 7.47–7.49 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.2, 168.0, 137.7, 134.0, 128.9, 128.8, 128.3, 128.2, 128.0, 127.9, 71.8, 49.5, 45.3, 43.1, 30.8, 28.6; IR (KBr) 3400, 2880, 1710, 1650 cm^{-1}; EM *m*/*z* (%) 320 (M^+-16, 26), 319 (17), 215 (59), 174 (8), 138 (8), 119 (12), 106 (13), 91 (100), 65 (18), 55 (18), 104 (9); Anal. calcd for $C_{20}H_{20}N_2O_3.H_2O$: C, 67.89; H, 6.26; N, 7.90. Found: C, 67.57; H, 6.03; N, 7.91.

3.4.2. (5*S,9*S**)-1-Benzyl-7-hydroxy-9-phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 20b.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 100:1), 52 mg, (65%) were obtained as a white solid: mp 152–155°C (CH_2Cl_2/Et_2O), together with 16 mg (10%) of **24b**. IR (KBr) 3250, 2720, 1690, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.14–2.18 (m, 1H), 2.34–2.61 (m, 3H), 3.54–3.59 (m, 2H), 3.84–3.90 (m, 1H), 3.96–4.07 (m, 2H), 7.17–7.21 (m, 7H), 7.33–7.34 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 175.7, 166.4, 136.1, 134.9, 129.0, 128.2, 127.9, 127.8, 127.7, 127.0, 71.1, 50.9, 46.7, 46.6, 30.2, 29.0; Anal. calcd for $C_{20}H_{20}N_20_3.H_2O$: C, 67.89; H, 6.26; N, 7.90. Found: C, 68.09; H, 5.76; N, 7.88.

3.4.3. (5*S,9*R**)-1-Benzyl-9-(2-methoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione 21a and (5*S**,9*R**)-1-benzyl-7-hydroxy-9-(2-methoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione 25a.** Following the method A, reduction of **15a** yielded, after purification by flash chromatography (CH_2Cl_2/MeOH, 100:1), 17 mg (21%) of **21a** as an oil, and 49 mg (56%)of **25a** as a white solid (CH_2Cl_2/Et_2O). *Compound **21a***: IR ($CHCl_3$) 3200, 1720, 1710, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.72–1.89 (m, 2H), 2.00–2.09 (m, 1H), 2.31–2.40 (m, 1H), 3.46 (dd, 1H, *J*=9.4 and 8.2 Hz), 3.64 (dd, 1H, *J*=9.4 and 8.2), 3.79 (s, 3H), 4.16 (t, 1H, *J*=8.2 Hz), 4.51 (d, 1H, *J*=15.4 Hz), 4.64 (d, 1H, *J*=15.4 Hz), 6.53 (sa, 1H), 6.86–6.96 (m, 3H), 7.24–7.35 (m, 4H), 7.43–7.45 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.1, 175.7, 157.6, 137.5, 129.1, 129.0, 128.3, 128.2, 127.2, 125.1, 120.7, 110.7, 71.7, 55.3, 44.8, 43.0, 40.3, 29.1, 25.4. *Compound **25a***: mp 164–167°C; IR (KBr) 3400, 1710, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.64–1.73 (m, 1H), 1.79–1.90 (m, 1H), 1.97–2.07 (m, 1H), 2.31–2.41 (m, 1H), 3.66–3.81 (m, 3H), 3.75 (s, 3H), 4.13 (d, 1H, *J*=15.4 Hz), 4.74 (d, 1H, *J*=15.4 Hz), 6.77–6.89 (m, 3H), 7.23–7.42 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 175.8, 167.4, 157.4, 137.3, 134.0, 128.9, 128.8, 128.3, 128.2, 128.0, 127.9, 71.5, 55.3, 50.6, 44.7, 38.2, 29.1, 25.3.

3.4.4. (5*S,9*S**)-1-Benzyl-9-(2-methoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione, 21b and (5*S**,9*S**)-1-benzyl-7-hydroxy-9-(2-methoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione 25b.** Following the method A, reduction of **15b** yielded, after purification by flash chromathography (CH_2Cl_2/MeOH, 100:1), 20 mg (24%) of **21b** as a white solid (Et_2O), and 50 mg (57%) of **25b** as a white solid (CH_2Cl_2/Et_2O). *Compound **21b***: mp 234–237°C; IR (KBr) 3360, 1710, 1680 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.27–2.32 (m, 1H), 2.37–2.52 (m, 3H), 3.42 (d, 1H, *J*=15.4 Hz), 3.59–3.69 (m, 2H), 3.80 (d, 1H, *J*=15.4 Hz), 3.82 (s, 3H), 4.21 (dd, 1H, *J*=7.1 and 4.4 Hz), 6.80 (sa, 1H), 6.87–6.97 (m, 2H), 7.17–7.33 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 175.8, 172.3, 157.5, 137.2, 129.2, 127.8, 127.7, 127.4, 126.6, 125.3, 120.7, 110.5, 72.7, 55.3, 46.7, 43.7, 41.8, 30.8, 29.5. *Compound **25***: mp 220–223°C; IR (KBr) 3250, 2720, 1690, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.00–2.06 (m, 1H), 2.19–2.40 (m, 2H), 2.48–2.59 (m, 1H), 3.21 (d, 1H; *J*=15.4 Hz), 3.50 (d, 1H; *J*=15.4 Hz), 3.69–3.75 (m, 1H), 3.80 (s, 3H), 3.86–3.92 (m, 1H), 4.00–4.03 (m, 1H), 6.86–6.91 (m, 2H), 7.18–7.30 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 175.5, 167.7, 157.5, 137.0, 129.4, 127.8, 127.7, 127.4, 126.7, 124.7, 120.7, 110.5, 72.0, 55.2, 50.6, 46.7, 38.6, 30.8, 29.2.

3.4.5. (5*S,9*R**)-1-Benzyl-7-hydroxy-9-[4-(*tert*-butyl-dimethylsilanyloxy)phenyl]-1,7-diaza-spiro[4.4]nonane-2,6-dione, 22a.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 75:1), 70 mg, (70%) were obtained as a white solid: mp 186–188°C (CH_2Cl_2/Et_2O), together with 14 mg (15%) of **26a**. IR ($CHCl_3$) 3500–3300, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.16 (s, 6H), 0.95 (s, 9H), 1.55–1.66 (m, 1H), 1.85–1.93 (m, 2H), 2.17–2.27 (m, 1H), 3.49 (t, 1H, *J*=8.8 Hz), 3.72–3.78 (m, 1H), 3.82–3.88 (m, 1H), 3.96 (d, 1H, *J*=15.4 Hz), 5.10 (d, 1H, *J*=14.8 Hz), 6.58 (d, 2H, *J*=8.2 Hz), 6.69 (d, 2H, *J*=8.2 Hz), 7.30–7.39 (m, 3H), 7.46–7.48 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.1, 168.2, 155.6, 137.8, 132.0, 129.3, 129.0, 128.8, 128.0, 126.2, 120.3, 72.0, 49.7, 45.4, 42.5, 29.7, 28.6, 25.6, 24.8, 18.1, −4.5; EM *m*/*z* (%)450 (47), 393 (30), 345 (50), 314 (13), 288 (15), 234 (10), 201 (10), 177 (23), 174 (27), 151 (7), 119 (11), 91 (100), 73 (32), 65 (8), 55 (10). Anal. calcd for $C_{26}H_{34}N_2O_4Si{\cdot}H_2O$: C, 64.43; H, 7.49; N, 5.78. Found: C, 64.80; H, 7.16; N, 5.46.

3.4.6. (5*S,9*S**)-1-Benzyl-7-hydroxy-9-[4-(*tert*-butyl-dimethylsilanyloxy)phenyl]-1,7-diazaspiro[4.4]nonane-2,6-dione, 22b.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 19:1), 65 mg, (70%) were obtained as a white solid: mp 192–195°C (CH_2Cl_2/Et_2O), together with 22 mg (25%) of **26b.** IR (KBr) 3500–3300, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.20 (s, 6H), 0.98 (s, 9H), 1.89–1.94 (m, 1H), 2.35–2.41 (m, 3H), 3.30 (t, 1H, *J*=7.2 Hz), 3.55 (d, 1H, *J*=15.4 Hz), 3.72–3.80 (m, 1H), 3.93–3.98 (m, 1H), 4.07 (d, 1H,

J=15.4 Hz), 6.76 (d, 2H, J=8.2 Hz), 6.92 (d, 2H, J=8.2 Hz), 7.16–7.26 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.4, 166.6, 155.7, 136.5, 129.0, 128.1, 127.9, 127.2, 127.0, 120.6, 70.9, 51.3, 46.6, 46.3, 30.1, 29.0, 25.6, 18.2, −4.4; Anal. calcd for $C_{26}H_{34}N_2O_4Si{\cdot}H_2O$: C, 64.43; H, 7.49; N, 5.7. Found: C, 64.17; H, 7.19; N, 5.42.

3.4.7. (5*S,9*R**)-1-Benzyl-7-hydroxy-9-[1-(*tert*-butoxycarbonyl)indol-3-yl]-1,7-diazaspiro[4.4]nonane-2,6-dione, 23a.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 19:1), 55 mg, (70%) were obtained as a white solid: mp 124–126°C (CH_2Cl_2/Et_2O), together with 47 mg (25%) of **27a**. IR (KBr) 3400, 1740, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.66 (s, 9H), 1.84–1.94 (m, 1H), 1.98–2.12 (m, 2H), 2.31–2.39 (m, 1H), 3.76–3.89 (m, 2H), 3.96–4.03 (m, 1H), 4.06 (d, 1H, J=15.4 Hz), 5.10 (d, 1H, J=15.4 Hz), 6.98 (d, 1H, J=7.7 Hz),7.09–7.14 (m, 2H), 7.29–7.40 (m, 4H), 7.49–7.51 (m, 2H), 8.10 (d, 1H, J=8.2 Hz). ^{13}C NMR ($CDCl_3$) δ 175.3 (C), 168.1 (C), 149.1 (C), 137.4 (C), 135.0 (C), 129.0 (CH), 128.9 (CH), 128.7 (CH), 128.0 (CH), 125.1 (CH), 124.8 (CH), 123.2 (CH), 119.2 (CH), 115.5 (CH), 114.9 (C), 84.4 (C), 71.8 (C), 49.9 (CH_2), 45.3 (CH_2), 35.9 (CH), 29.7 (CH_2), 28.1 (CH_3), 26.0 (CH_2); EM m/z (%) 375 (5), 359 (73), 357 (12), 302 (9), 254 (18), 174 (26), 158 (27), 143 (39), 130 (23), 117 (11), 115 (12), 91 (100), 65 (15), 56 (16); Anal. calcd for $C_{27}H_{29}N_3O_5{\cdot}H_2O$: C, 65.71; H, 6.33; N, 8.51. Found: C, 65.56; H, 6.25; N, 8.31.

3.4.8. (5*S,9*S**)-1-Benzyl-7-hydroxy-9-[1-(*tert*-buthoxycarbonyl)indol-3-yl]-1,7-diazaspiro[4.4]nonane-2,6-dione, 23b.** Following method A and after purification by flash chromathography ($CHCl_3$/MeOH, 19:1), 57 mg, (70%) were obtained as a white solid: mp 143–146°C (CH_2Cl_2/Et_2O), together with 15 mg (20%) of **27b**. IR (KBr) 3440, 1720, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 9H), 1.87–1.97 (m, 1H), 2.11–2.23 (m, 1H), 2.29–2.38 (m, 1H), 2.49–2.60 (m, 1H), 3.72 (t, 1H, J=8.9 Hz), 3.88–4.04 (m, 3H), 4.58 (d, 1H, J=15.4 Hz), 7.20–7.38 (m, 7H), 7.44–7.46 (m, 2H), 8.08 (d, 1H, J=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 175.2 (C), 166.3 (C), 149.2 (C), 136.4 (C), 135.1 (C), 129.8 (C), 128.1 (CH), 127.8 (CH), 127.4 (CH), 125.2 (CH), 124.1 (CH), 123.1 (CH), 117.9 (CH), 115.8 (CH), 113.5 (C), 84.6 (C), 70.4 (C), 51.9 (CH_2), 46.6 (CH_2), 39.4 (CH), 30.0 (CH_2), 29.0 (CH_2), 28.2 (CH_3); IR (KBr) 3440, 1720, 1700 cm^{-1}; EM m/z (%) 459 (1), 402 (3), 375 (5), 359 (69), 3.57 (10), 302 (6), 268 (3), 254 (13), 174 (19), 158 (17), 143 (27), 130 (17), 117 (8), 115 (10), 91 (100), 65 (17), 56 (51); Anal. calcd for $C_{27}H_{29}N_3O_5{\cdot}H_2O$: C, 65.71; H, 6.33; N, 8.51. Found: C, 65.60; H, 6.28; N, 8.21.

3.4.9. (5*S,9*R**)-1-Benzyl-9-phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 24a.** Following method B, 60 mg, (72%) were obtained as a white solid: mp 167–169°C (CH_2Cl_2/Et_2O). IR (KBr) 3200, 1720, 1710, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.58–1.69 (m, 1H), 1.94–1.99 (m, 2H), 2.20–2.31 (m, 1H), 3.48–3.57 (m, 1H), 3.62–3.71 (m, 2H), 4.10 (d, 1H, J=15.4 Hz), 5.17 (d, 1H, J=15.4 Hz), 6.69–6.72 (m, 2H), 7.16–7.23 (m, 3H), 7.32–7.40 (m, 3H), 7.52–7.54 (m, 2H), 7.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 176.6, 176.3, 138.1, 134.6, 130.2, 129.2, 128.6, 128.5, 128.1, 127.7, 127.6, 72.4, 46.3, 45.4, 42.2, 28.6, 24.6; MS m/z (%) 320 (M^+, 27), 216 (12), 215 (70), 174 (10), 138 (10), 119 (10), 106 (13), 91 (100), 65 (18), 55 (11), 104 (9); Anal. calcd for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.79. Found: C, 74.70; H, 6.28; N, 8.58.

3.4.10. (5*S,9*S**)-1-Benzyl-9-phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 24b.** Following method B, 58 mg, (70%) were obtained as a white solid: mp 162–164°C (CH_2Cl_2/Et_2O). IR (KBr) 3380, 1700, 1675 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.20–2.28 (m, 1H), 2.32–2.45 (m, 1H), 2.50–2.59 (m, 1H), 2.62–2.73 (m, 1H), 3.65–3.81 (m, 4H), 4.40 (d, 1H, J=15.9 Hz), 6.69 (sa, 1H), 7.21–7.25 (m, 7H), 7.35–7.38 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 175.6, 175.0, 136.4, 135.4, 129.0, 128.1, 127.9, 127.8, 127.7, 126.9, 70.9, 50.6, 46.4, 44.3, 30.0, 29.2; Anal. calcd for $C_{20}H_{20}N_2O_2$ C, 74.98; H, 6.29; N, 8.79. Found C, 74.75; H, 6.49; N, 8.78.

3.4.11. (5*S,9*R**)-1-Benzyl-9-[4-(*tert*-butyldimethylsilanyloxy)phenyl]-1,7-diaza-spiro[4.4]nonane-2,6-dione, 26a.** Following method B, 70 mg, (80%) were obtained as a white solid: mp 99–102°C (CH_2Cl_2/Et_2O). IR ($CHCl_3$) 3420, 1735, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.16 (s, 6H), 0.96 (s, 9H), 1.57–1.68 (m, 1H), 1.93–1.98 (m, 2H), 2.18–2.29 (m, 1H), 3.48–3.59 (m, 3H), 4.08 (d, 1H, J=14.8 Hz), 5.15 (d, 1H, J=14.8 Hz), 6.57 (d, 2H, J=8.8 Hz), 6.79 (d, 2H, J=8.8 Hz), 6.92 (s, 1H), 7.32–7.40 (m, 3H), 7.50–7.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.4, 176.0, 155.3, 138.2, 129.4, 129.2, 128.8, 127.1, 120.2, 72.3, 45.8, 45.4, 42.2, 28.6, 25.6, 24.7, 18.1, −4.5; EM m/z (%) 450 (M^+,50), 393 (27), 345 (53), 314 (13), 288 (16), 234 (10), 201 (9), 177 (25), 174 (30), 119 (11), 91 (100), 73 (32), 65 (10), 55 (10); Anal. calcd for $C_{26}H_{34}N_2O_3Si$: C, 69.30; H, 7.60; N, 6.22. Found: C, 69.05; H, 7.59; N, 6.29.

3.4.12. (5*S,9*S**)-1-Benzyl-9-[4-(*tert*-butyldimethylsilanyloxy)phenyl]-1,7-diaza-spiro[4.4]nonane-2,6-dione, 26b.** Following method B, 90 mg, (90%) were obtained as a white solid: mp 162–164°C (CH_2Cl_2/Et_2O). IR (KBr) 3450–3150, 1710, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.21 (s, 6H), 0.99 (s, 9H), 2.14–2.21 (m, 1H), 2.23–2.68 (m, 3H), 3.55–3.69 (m, 4H), 4.35 (d, 1H, J=15.9 Hz), 6.59 (s, 1H), 6.82 (d, 2H, J=8.8 Hz), 7.05 (d, 2H, J=8.8 Hz), 7.19–7.22 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.6, 174.8, 155.6, 136.5, 128.8, 128.1, 128.0, 127.8, 127.1, 120.7, 70.6, 49.8, 46.5, 44.4, 30.1, 29.3, 25.6, 18.2, −4.4; Anal. calcd for $C_{26}H_{34}N_2O_3Si$: C, 69.30; H, 7.60; N, 6.22. Found: C, 68.92; H, 7.49; N, 6.18.

3.4.13. (5*S,9*R**)-1-Benzyl-9-[1-(*tert*-buthoxycarbonyl)-indol-3-yl]-1,7-diaza-spiro[4.4]nonane-2,6-dione, 27a.** Following method B, 64 mg, (50%) were obtained as a white solid: mp 113–116°C (CH_2Cl_2/Et_2O). IR (KBr) 3400, 3250, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.67 (s, 9H), 1.89–1.94 (m, 1H), 2.09–2.2.14 (m, 2H), 2.31–2.42 (m, 1H), 3.60 (dd, 1H, J=9.9 and 8.8 Hz), 3.71–3.69 (dd, 1H, J=9.9 and 9.3 Hz), 3.91 (dd, 1H, J=9.3 and 8.8 Hz), 4.22 (d, 1H, J=15.4 Hz), 5.09 (d, 1H, J=15.4 Hz), 6.95 (d, 1H, J=7.7 Hz), 7.08–7.13 (m, 2H), 7.26–7.41 (m, 4H), 7.52–7.54 (m, 2H), 7.63 (s, 1H), 8.09 (d, 1H, J=8.2 Hz). ^{13}C NMR ($CDCl_3$) δ 176.3 (C), 176.0 (C), 149.4 (C), 137.8 (C), 134.9 (C), 129.4 (C), 129.0 (C), 128.7 (CH), 127.8 (CH), 124.9 (CH), 124.6 (CH), 122.9 (CH), 119.4 (CH), 115.6 (C), 115.4 (CH), 84.3 (C), 72.1 (C), 45.5 (CH_2),

42.9 (CH_2), 39.3 (CH), 29.1 (CH_2), 28.2 (CH_3), 26.0 (CH_2); EM *m/z* (%); 459 (1), 359 (80), 302 (6), 268 (3), 254 (21), 174 (32), 158 (29), 143 (42), 130 (21), 117 (10), 115 (11), 91 (100), 65 (13), 56 (14); Anal. calcd for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found: C, 70.56; H, 6.75; N, 9.46.

3.4.14. (5*S,9*S**)-1-Benzyl-9-[1-(*tert*-buthoxycarbonyl)-indol-3-yl]-1,7-diaza-spiro[4.4]nonane-2,6-dione, 27b.** Following method B, 52 mg, (50%) were obtained as a white solid: mp 110–113°C (CH_2Cl_2/Et_2O). IR (KBr) 3220, 2970, 1725, 1710, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70 (s, 9H), 2.16–2.29 (m, 2H), 2.35–2.46 (m, 1H), 2.69–2.80 (m, 1H), 3.53 (dd, 1H, *J*=9.9 and 8.3 Hz), 3.66 (dd, 1H, *J*=9.9 and 8.8 Hz), 3.86 (dd, 1H, *J*=8.8 and 8.3 Hz), 3.97 (d, 1H, *J*=15.4 Hz), 4.66 (d, 1H, *J*=15.4 Hz), 7.16–7.20 (m, 5H), 7.28–7.38 (m, 2H), 7.43 (s, 1H), 7.53 (d, 1H, *J*=7.2 Hz), 7.67 (s, 1H), 8.08 (d, 1H, *J*=7.9 Hz); ^{13}C NMR ($CDCl_3$) δ 175.8 (C), 174.9 (C), 149.3 (C), 136.2 (C), 135.0 (C), 130.0 (C), 127.9 (CH), 127.4 (CH), 127.0 (CH), 125.0 (CH), 123.8 (CH), 123.0 (CH), 117.9 (CH), 115.6 (CH), 114.6 (C), 84.4 (C), 70.4 (C), 46.4 (CH_2), 45.3 (CH_2), 42.7 (CH), 29.9 (CH_2), 29.1 (CH_2), 28.1 (CH_3); EM *m/z* (%) 459 (M^+, 3), 403 (3), 359 (80), 302 (4), 268 (3), 254 (16), 174 (23), 158 (21), 143 (27), 130 (17), 117 (8), 115 (9), 91 (100), 65 (17), 57 (28); Anal. calcd for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found: C, 70.17; H, 6.46; N, 8.67.

3.4.15. (5*S,9*R**)-1-Benzyl-9-(3,4,5-trimethoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione, 28a.** Following method B, 62 mg, (75%) were obtained as a white solid: mp 216–218°C (CH_2Cl_2/Et_2O). IR (KBr) 3410, 3250, 1710, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.64–1.75 (m, 1H), 1.95–2.00 (m, 2H), 2.21–2.31 (m, 1H), 3.55–3.72 (m, 2H), 3.68 (s, 6H), 3.78 (s, 3H), 3.78–3.86 (m, 1H), 4.02 (d, 1H, *J*=15.4 Hz), 5.21 (d, 1H, *J*=15.4 Hz), 5.99 (s, 2H), 7.28–7.89 (m, 3H), 7.49 (s, 1H), 7.59 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.6, 176.1, 153.2, 138.2, 137.6, 130.4, 129.6, 128.8, 127.8, 105.1, 72.4, 60.8, 56.3, 46.4, 45.7, 41.9, 28.8, 24.8; Anal. calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.45; H, 6.53; N, 6.72.

3.4.16. (5*S,9*S**)-1-Benzyl-9-(3,4,5-trimethoxyphenyl)-1,7-diaza-spiro[4.4]nonane-2,6-dione, 28b.** Following method B, 59 mg, (70%) were obtained as a white solid: mp 172–174°C (CH_2Cl_2/Et_2O). ^1H NMR ($CDCl_3$) δ 2.16–2.24 (m, 1H), 2.38 (m, 3H), 3.51–3.56 (m, 1H), 3.78–3.86 (m, 2H), 3.75 (d, 1H, *J*=15.4 Hz), 3.81 (s, 6H), 3.85 (s, 3H), 4.35 (d, 1H, *J*=15.4 Hz), 6.38 (s, 2H), 7.16–7.19 (m, 5H), 7.46 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 175.5, 174.9, 153.4, 137.8, 136.3, 131.2, 127.8, 127.4, 126.8, 105.1, 70.8, 60.9, 56.2, 50.4, 46.4, 44.6, 30.0, 29.2; IR (KBr); 3400, 3200, 3080, 1710, 1680 cm^{-1}; Anal. calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 66.98; H, 6.28; N, 6.67.

3.4.17. (5*S,9*R**)-9-Phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 32a.** Following method B, 42 mg, (70%) were obtained as a white solid: mp >226°C (dec) (CH_2Cl_2/MeOH). IR (KBr) 3320, 3200, 1720, 1680 cm^{-1}; ^1H NMR (MeOD-d_4) δ 1.28–1.43 (m, 1H), 1.86–2.02 (m, 2H), 2.04–2.17 (m, 1H), 3.55 (dd, 1H, *J*=9.2 and 7.3 Hz), 3.64–3.70 (m, 1H), 3.77–3.84 (m, 1H), 7.33–7.39 (m, 5H); ^{13}C NMR (MeOD-d_4) δ 181.2, 178.9, 136.7, 129.8, 129.4, 129.0, 69.0, 51.0, 42.3, 30.4, 26.2; IR (KBr) 3320, 3200, 1720, 1680 cm^{-1}; Anal. calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 68.02; H, 6.19; N, 11.94.

3.4.18. (5*S,9*S**)-9-Phenyl-1,7-diaza-spiro[4.4]nonane-2,6-dione, 32b.** Following method B, 45 mg, (75%) were obtained as a white solid: mp >230°C (dec) (CH_2Cl_2/MeOH). IR (KBr) 3220, 3100, 1710, 1690 cm^{-1}; ^1H NMR (MeOD-d_4) δ 1.92–2.04 (m, 1H), 2.11–2.20 (m, 1H), 2.25–2.35 (m, 1H), 2.45–2.55 (m, 1H), 3.53–3.71 (m, 3H), 7.32–7.36 (m, 5H); ^{13}C NMR (MeOD-d_4) δ 180.6, 178.0, 137.3, 130.2, 129.7, 128.9, 68.4, 52.2, 45.6, 30.7, 29.6; Anal. calcd for $C_{13}H_{14}N_2O_2$ C, 67.81; H, 6.13; N, 12.17. Found: C, 67.42; H, 6.29; N, 11.94.

3.4.19. Methyl 1-(4-methoxybenzyl)-2-(2′-nitro-1′-phenylethyl)pyroglutamate 30. Following the general method for Michael reactions at C2, this compound was prepared from methyl (2*S*)-1-(4-methoxibenzyl)pyroglutamate and *trans*-β-nitrostyrene, and obtained as a 1.5:1 mixture of diastereomers. Upon separation by flash column chromatography using Hex/AcOEt 2:1, 284 mg (39%) of **30a** and 210 mg (29%) of **30b** were obtained. *(2S*,1′R*)-**30a***: IR ($CHCl_3$); 3010, 2980, 1740, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00–2.09 (m, 1H), 2.35–2.43 (m, 1H), 2.50–2.62 (m, 2H), 3.38 (s, 3H), 3.76 (s, 3H), 4.29 (dd, 1H, *J*=13.2 Hz, *J*=2.8 Hz), 4.43 (dd, 1H, *J*=11.6 and 2.8 Hz), 4.61 (d, 1H, *J*=15.4 Hz), 4.66 (dd, 1H, *J*=13.2 and 11.6 Hz), 4.71 (d, 1H, *J*=15.4 Hz), 6.83 (d, 2H, *J*=8.2 Hz), 7.22–7.29 (m, 5H), 7.33 (d, 2H, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 176.0, 169.6, 159.0, 133.5, 129.4, 129.1, 129.0, 128.6, 128.5, 114.0, 75.2, 71.5, 55.1, 52.2, 45.8, 44.1, 29.2, 25.2. *(2S*,1′S*-**30b***: IR ($CHCl_3$); 3010, 2980, 1740, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52–1.64 (m, 1H), 2.11–2.34 (m, 2H), 2.54–2.64 (m, 1H), 3.16 (s, 3H), 3.68 (s, 3H), 4.13 (d, 1H, *J*=14.8 Hz), 4.54 (dd, 1H, *J*=10.4 and 3.8 Hz), 4.76 (dd, 1H, *J*=13.2 and 3.8 Hz), 4.92 (dd, 1H, *J*=13.2 and 10.4 Hz), 4.99 (d, 1H, *J*=14.8 Hz), 6.82 (d, 2H, *J*=8.8 Hz), 7.14 (d, 2H, *J*=8.8 Hz), 7.24–7.27 (m, 3H), 7.34–7.39 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.3, 170.8, 158.8, 133.4, 129.2, 129.1, 129.0, 128.5, 128.2, 113.8, 75.5, 69.8, 55.2, 52.8, 45.2, 44.0, 28.6, 23.7.

3.4.20. Methyl (2S*,1′R*)-2-(2′-nitro-1′-phenylethyl)-pyroglutamate, 31a. A solution of CAN (303 mg, 0.55 mmol) in H_2O (3 ml) was added to a solution of **30a** (73 mg, 0.18 mmol) in CH_3CN (2 ml) at −5°C. After the reaction mixture was stirred at 0°C for 25 min, the reaction mixture was diluted with H_2O, and extracted with EtOAc (2×20 ml). The combined organic phases were washed with 5% $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The product was purified by flash chromatography using CH_2Cl_2 as elluent, obtaining 32 mg (60%) of **31a** as a white solid, mp 201–204°C (CH_2Cl_2/MeOH); IR (KBr); 3400, 3180, 1725, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.66–1.77 (m, 1H), 2.12–2.32 (m, 2H), 2.36–2.48 (m, 1H), 3.80 (s, 3H), 3.96 (dd, 1H, *J*=10.4 and 4.4 Hz), 4.75 (dd, 1H, *J*=13.2 and 4.4 Hz), 4.98 (dd, 1H, *J*=13.2 and 10.4 Hz), 6.85 (s, 1H), 7.25–7.26 (m, 3H), 7.37–7.39 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.0, 169.2, 133.6, 129.2, 129.1, 128.9, 75.9, 67.3, 53.3, 51.2, 30.2, 29.3; Anal. calcd for $C_{14}H_{16}N_2O_5$: C, 57.43; H, 5.52, N, 9.58. Found: C, 57.49; H, 5.72, N, 9.48.

3.4.21. Methyl (2*S,1′*S**)-2-(2′-nitro-1′-phenylethyl)-pyroglutamate, 31b.** Following the same previous method, 38 mg, (65%) of **31b** were obtained as a white solid: mp >226°C (dec) (CH_2Cl_2/MeOH). (71 mg, 55%): mp 212–215°C; IR (KBr); 3400, 3200, 1730, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.25–2.39 (m, 3H), 2.50–2.59 (m, 1H), 3.63 (s, 3H), 4.02 (dd, 1H, J=9.9 and 4.9 Hz), 4.84–4.99 (m, 2H), 7.16–7.20 (m, 3H), 7.23 (s, 1H), 7.32–7.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 177.3, 171.9, 134.1, 129.1, 128.9, 128.3, 75.4, 67.9, 52.9, 50.6, 29.5, 28.6; Anal. calcd for $C_{14}H_{16}N_2O_5$: C, 57.43; H, 5.52, N, 9.58. Found: C, 57.37; H, 5.48, N, 9.28.

3.5. Molecular modeling

Conformational searches and energy minimizations were performed using Macromodel version 5.5.[23] The Macromodel implementation of the amber all atom force field was used (denoted AMBER*). All calculations were performed using the implicit water GB/SA solvation model of Still et al.[24] Conformational searches were performed using the Monte Carlo method of Goodman and Still.[25] For each search 1000 starting structures were generated and minimized to an energy convergence of 0.05 (kJ/mol)/Å using the Polak–Ribiere conjugate gradient minimization method implemented in Macromodel.[23] Duplicated structures and those greater than 50 kJ/mol above the global minimum were discarded. The lowest energy structures were subjected to a RHF/6-31G* geometry optimization using Gaussian 94.[26] The full geometries and energies of the ab initio optimized structures are given in the Supporting Information.

3.6. Supplementary material

ORTEP drawing for compound **14a**. Full geometries and energies for the optimized structures of **32a** and **32b**.

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