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Spiro[2.2]pentane as a Dissymmetric Scaffold for Conformationally Constrained Analogues of Glutamic Acid: Focus on Racemic 1-Aminospiro[2.2]pentyl-1,4-dicarboxylic Acids

Roberto Pellicciari,*,† Maura Marinozzi,† Emidio Camaioni,† Maria del Carmen Nùnez,†,|| Gabriele Costantino,† Fabrizio Gasparini,‡ Gianluca Giorgi,§ Antonio Macchiarulo,† and Natarajan Subramanian‡

Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Via del Liceo, 1, 06123 Perugia Italy; Novartis Pharma, Basel, Switzerland; and Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Università di Siena, Via A. Moro, 53100 Siena, Italy

rp@unipg.it

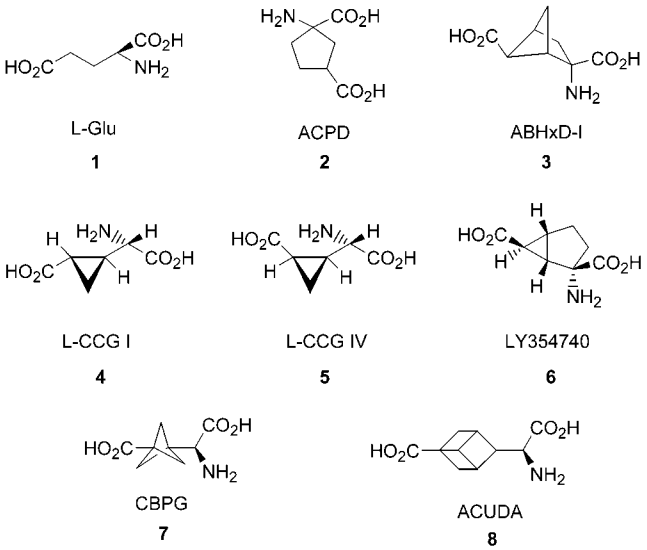
Received February 27, 2002

In search for novel conformationally constrained analogues of L-glutamic acid, a diastereodivergent synthesis of the four 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid racemic pairs is reported along with their stereochemical assignment, conformational analysis, and preliminary biological evaluation as potential glutamate (ionotropic and metabotropic) ligands.

Introduction

Several strategies aimed at the reduction or elimination of the conformational flexibility of biologically active substances have been developed.[1] Among these, rigidification through ring insertion,[2] induction of hydrophobic collapse,[3] and intramolecular hydrogen bond formation[4] are examples of "freezing" techniques that have productively been employed for the preparation of compounds endowed with improved properties such as potency, stability, biovailability, or selectivity. The quest for selectivity, in particular, is of special relevance because natural hormones, transmitters, and substrates usually interact in a specific way with heterogeneous populations of receptors by assuming a cluster of bioactive conformations. The case of L-glutamate (L-Glu, **1**, Chart 1) is a particularly relevant example. Indeed, L-Glu (**1**) is the principal excitatory neurotransmitter in the vertebrate CNS and is involved in a variety of physiological functions, including neuronal plasticity, long-term potentiation of synaptic activity, and memory, as well as in both acute or chronic pathological processes such as brain ischemia, hypoxia, and neurodegenerative diseases. The variety of pathophysiologal functions exerted by L-Glu (**1**) are mediated by two main families of membrane receptors, the integral membrane spanning ionotropic glutamate receptors (iGluRs)[5] and the G-protein coupled metabotropic glutamate receptors (mGluRs).[6] Each of these two families, in turn, is further subdivided in a multiple and heterogeneous variety of receptor subtypes and isoforms (known as GluR1−GluR6, KA1−KA2, NR1, NR3 in the case of iGluR's, and as mGluR1−mGluR8

CHART 1



* Tel: +39 075 585 5120. Fax: +39 075 585 5124.
† Università di Perugia.
‡ Novartis Pharma.
§ Università di Siena.
|| Present address: Departamento de Quimica Organica, Facultad de Farmacia, Campus de Cartuja s/n, 18071 Granada, Spain.

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10.1021/jo020138v CCC: $22.00
Published on Web 07/12/2002

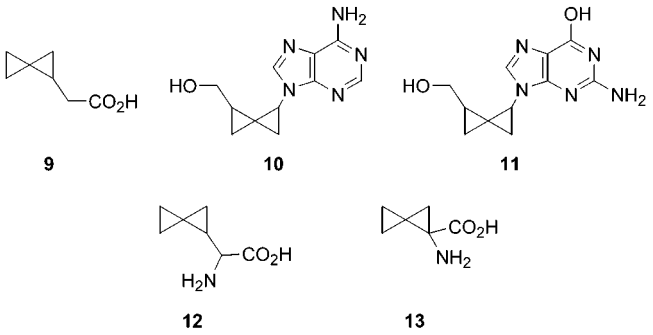
in the case of mGluR's) with several evidences indicating a peculiar physio-pathological role for each individual subtype. The pharmacological characterization of this vast and complex receptor subtype array has largely been made possible by the design and synthesis of conformationally constrained L-Glu (**1**) analogues.[7] We have been engaged for a long time in this endeavor,[8] mainly addressing our work to the preparation of non-proteinogenic, acidic amino acids endowed with potency and selectivity for L-Glu receptor subtypes of the two families. In this connection, ring insertion has been the strategy of choice to constrain the glutamate skeleton in search for its "frozen" subtype selective bioactive conformations; thus, cyclopentane (**2**),[9] cyclopropane (**4**, **5**),[10,7d] bicylo-[3.2.0]heptane (**3**),[7b] and bicylo[3.1.0]hexane (**6**)[7c] are relevant examples of ring moieties successfully used to conformationally rigidify L-Glu (**1**). More exotic scaffolds such as bicyclo[1.1.1]pentane (**7**)[8c] and the cubyl (**8**)[11] moiety have also been shown to be good surrogates of the phenyl ring as spacers able to constrain the pharmacophoric groups of L-Glu (**1**) into a coplanar disposition.

More recently, our attention was attracted by the possibility to employ the unusual spiro[2.2]pentane nucleus as a new scaffold for fully constrained L-Glu analogues. Indeed, the 1,4-functionalization of this nucleus gives rise to the corresponding 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acids (**22a**−**d**), conformationally blocked L-Glu analogues.

Few precedents of biologically active substances incorporating the spiropentane nucleus are known, and they include spiropentylacetic acid and spiropentyl-acetyl-CoA (**9**, Chart 2) as inhibitors of acycl-CoA dehydrogenases,[12] and spiropentane analogues of deoxyadenosine and deoxyguanosine (**10**, **11**, Chart 2) as inhibitors of human cytomegalovirus and Epstein−Barr virus.[13]

The following aspects make the spiro[2.2]pentane analogues of L-Glu (**1**) particularly attractive: (i) the unusual spiro junction between two cyclopropane rings forces the pseudotorsional angles into unnatural dispositions, whose possible biological significance is worthy of testing; (ii) the 1-4 functionalization of the spiro[2.2]-pentane nucleus to give the corresponding 1-aminospiro-[2.2]pentyl-1,4-dicarboxylic acid isomers generates three new chiral centers, so that four possible diastereoisomeric pairs are generated; (iii) an appropriate diastereodivergent synthesis would give rise to the availability of a stereolibrary of compounds in which differently oriented functional groups will mimic folded or extended conformations of L-Glu (**1**); (iv) the resulting libraries can be used to screen a variety of glutamate receptor subtypes toward a better definition of the conformation−activity relationships.

CHART 2



We report here the synthesis and the stereochemical characterization of the four pairs of 1-aminospiro[2.2]-pentyl-1,4-dicarboxylic acid (**22a**−**d**, ASPEDs) isomers.

Results

A number of entries into substituted [2.2]spiropentanes have been developed, mainly based on strategies involving the cyclopropanation of allenes or alkylidene cyclopropanes via a Simmons−Smith reaction[14,15] or diazoalkanes[12b,17] and diazoesters[15−17] addition. Dihalo- and bromospiro[2.2]pentane derivatives have been also prepared by addition of dihalocarbenes[18] or bromocarbenoid intermediates,[21] respectively, to 2-substituted methylenecyclopropanes. Trost et al.[20] have described the preparation of 1,2,4-trisubstituted spiropentanes by conjugate addition of phenylsulfonium cyclopropylidene to α,β-unsaturated carbonyl compounds. Recently, the synthesis of amino acids containing the spiropentyl unit such as (±)-spiropentylglycine (**12**) and (±)-1-amino spiropentane carboxylic acid (**13**) has been reported.[21] Thus, racemic **13** was prepared by the rhodium-catalyzed decomposition of dimethyl diazomalonate in the presence of methylenecyclopropane followed by Curtius rearrangement key

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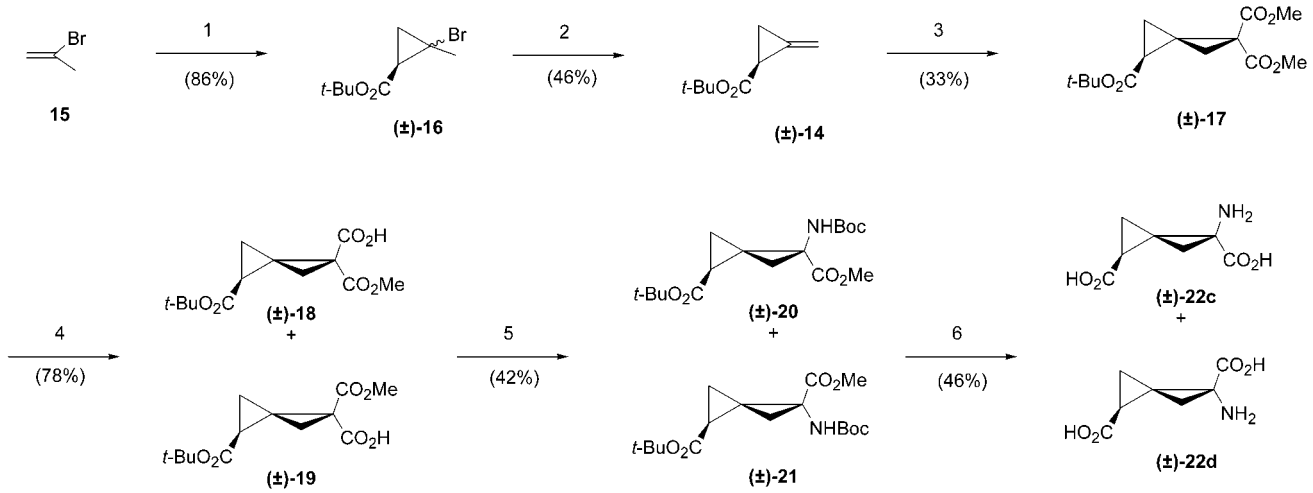
SCHEME 1[a]



[a] (1) tBuO$_2$CCHN$_2$, (Rh(OAc)$_2$)$_2$, CH$_2$Cl$_2$; (2) tBuOK, Et$_2$O; (3) (EtO$_2$C)$_2$CN$_2$, (Rh(OAc)$_2$)$_2$, CH$_2$Cl$_2$; (4) NaOH, MeOH; (5) (a) EtOCOCl, NaN$_3$, THF, (b) tBuOH; (6) (a) 8 N Jones reagent, (b) 6 N HCl, (c) Dowex, 10% pyr.

sequence.[21] Furthermore, the enantioselective synthesis of 1,1,4-trisubstituted spiropentanes by a dioxaborolane ligand controlled zinc-mediated cyclopropanation of hydroxymethylallenes has also been reported.[15]

Our initial approach to the synthesis of 1-amino-spiro-[2,2]pentyl-1,4-dicarboxylic acids (**22a**−**d**) began with the preparation of the key intermediate *tert*-butyl (methylenecyclopropyl)carboxylate[22] (±)-**14** from commercially available 2-bromopropene (**15**) and *tert*-butyl diazoacetate[23] by a dirhodium(II) tetraacetate catalyzed cyclopropanation as depicted in Scheme 1.

The bromide (±)-**16** thus obtained in 56% yield was submitted to a potassium *tert*-butoxide induced dehydrobromination in DMSO at room temperature to give (±)-**14** in 46% yield. A subsequent dirhodium(II) tetraacetate catalyzed cyclopropanation of (±)-**14** with a solution of dimethyl diazomalonate in CH_2Cl_2 afforded, surprisingly, only the distal isomer of the 1,1-dimethyl, 5-*tert*-butyl spiro[2.2]pentanetricarboxylate ((±)-**17**). No trace of the other isomer was detected by GC−MS analysis of the crude reaction mixture. Flash chromatography of the crude product led to the isolation of pure (±)-**17** with 33% yield. The stereochemistry of (±)-**17** was initially rationalized with the steric effect exerted by the bulky *tert*-butyl moiety of the olefin analogously with our previous reported result.[8d] Subsequently, this prediction was confirmed by comparison of the analytical data (^{1}H NMR, ^{13}C NMR, and HPLC) of the mixture of the final amino acids (**22c** + **22d**), obtained by this route with those of the single isomeric amino acids (**22a**−**d**) resulted from the alternative route below described.

Selective monohydrolysis of the malonate moiety of (±)-**17** with 1 equiv of aqueous NaOH furnished the mixture of the monoesters (±)-**18** and (±)-**19** (72%), which was converted into the corresponding acyl azides via the mixed anhydrides (ethyl chloroformate, 18-crown-6, potassium carbonate) followed by treatment with aqueous sodium azide. The crude acyl azides mixture was then refluxed overnight in *tert*-butyl alcohol to give the mixture of the *distal* and *medial-syn* isomeric *N*-Boc aminoesters (±)-**20** and (±)-**21** (42%). The unseparated mixture was hydrolyzed with 6 N HCl at 80 °C for 8 h to give, after purification by ion-exchange resin chromatography, the mixture of the 1-amino-spiro[2,2]pentyl-1,4-dicarboxylic acids ((±)-**22c** and (±)-**22d**, 46%).

To have access to all four possible racemic diastereomers of 1-amino-spiro[2,2]pentyl-1,4-dicarboxylic acids ((±)-**22a**−**d**) we decided to modify the above-described synthetic route in such a way as to decrease the steric bulk of the *tert*-butyl moiety on the olefin (Schemes 2 and 3).[24] Thus, ethyl methylenecyclopropanecarboxylate obtained from 2-bromopropene according to a known procedure[25] was submitted to $LiAlH_4$ reduction to afford the corresponding carbinol (±)-**23**,[13] which was protected as *tert*-butyldimethylsilyl ether using *tert*-butyldimethylsilyl chloride and 4-(dimethylamino)pyridine in CH_2Cl_2 to give the olefin (±)-**24** (55%). Subsequent slow addition of a solution of diethyl diazomalonate in CH_2Cl_2 to a refluxing solution of (±)-**24** and dirhodium(II) tetraacetate in the same solvent yielded a mixture of the two spiropentyl diastereoisomers (±)-**25** and (±)-**26** in a 1:5 ratio (GC−MS). Medium-pressure liquid chromatography (MPLC) of this mixture on silica gel (cyclohexanes−ethyl acetate, 95/5) afforded the less polar *proximal* isomer (±)-**25** and the more polar spiropentyl *distal* isomer (±)-**26** in 11% and 53% yield, respectively. Selective basic monohydrolysis of the malonate moiety of 4-(*tert*-butyldimethylsilyloxymethyl)-spiro[2.2]pentane-1,1-dicarboxylyc acid diethyl ester ((±)-**25**) with 0.5 N NaOH/EtOH at room temperature gave after careful neutralization (10% citric acid) a mixture of two *proximal* and *medial-anti* diastereoisomeric monoacids (±)-**27a** and (±)-**27b** (90%), since a new chiral center was introduced. The unresolved mixture of (±)-**27a** and (±)-**27b** was then transformed into the corresponding acyl azide by activation with ethyl chloroformate in the presence of 18-crown-6 and potas-

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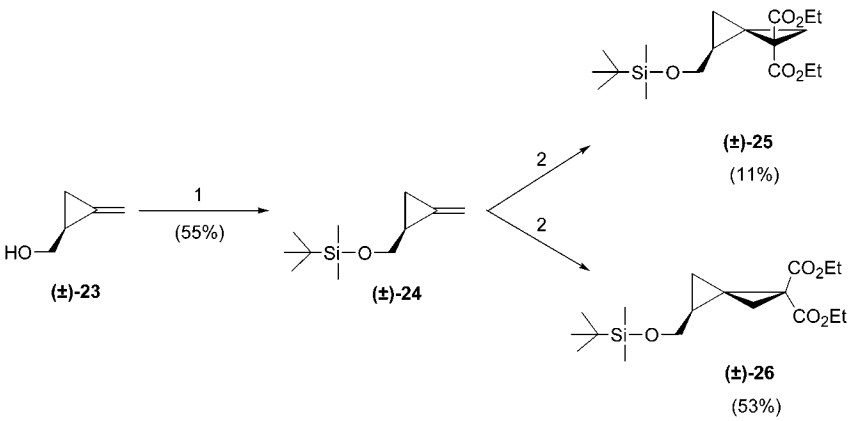
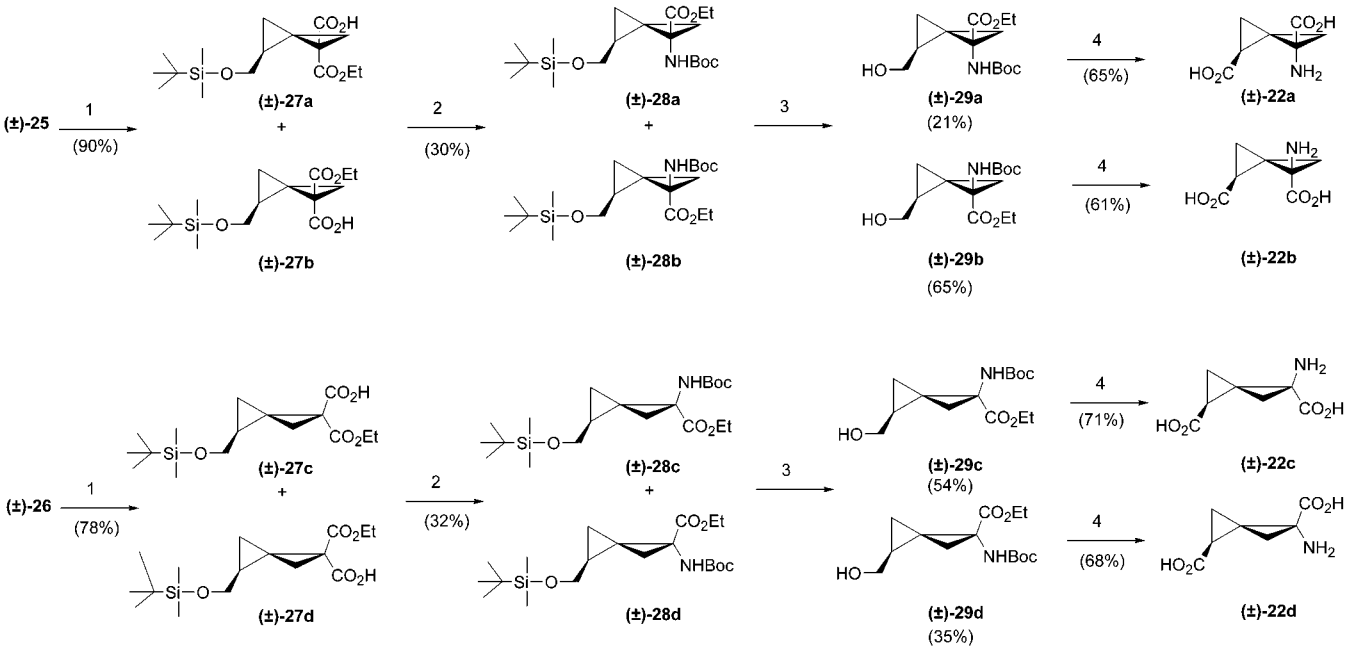
SCHEME 2[a]



[a] (1) TBDSCl, DMAP, CH_2Cl_2; (2) (a) $(EtO_2C)_2CN_2$, $(Rh(OAc)_2)_2$, CH_2Cl_2, (b) MPLC.

SCHEME 3[a]



[a](1) NaOH, EtOH; (2) (a) EtOCOCl, NaN_3, THF, (b) *t*BuOH; (3) (a) TBAF, THF, (b) mpic; (4) (a) BN Jones reagent, (b) 6 N HCl, (c) Dowex, 10% pyr.

sium carbonate and subsequent reaction with an aqueous solution of sodium azide at 0 °C.[26] Refluxing the crude acyl azide in the presence of *tert*-butyl alcohol afforded after flash chromatography (light petroleum−ethyl acetate, 90/10) the corresponding *proximal* and *medial-anti* *N*-Boc-protected aminospiropentanes (±)-**28a** and (±)-**28b** (30%), which could not be separated by silica gel chromatography. Nevertheless, after desilylation by Bu_4-NF in THF to the diastereisomeric *proximal* and *medial-anti* isomeric alcohols (±)-**29a** and (±)-**29b**, these were isolated individually by MPLC (light petroleum−ethyl acetate, 3/2) in 21% and 65% yields, respectively. By following an analogous procedure, selective basic monohydrolysis of the malonate moiety of isomer (±)-**26** afforded a mixture of the *distal* and *medial-syn* monoacids (±)-**27c** and (±)-**27d** (78%), which was converted into the mixture of the corresponding *N*-Boc amino *distal* and *medial-syn* derivatives (±)-**28c** and (±)-**28d** (32%). Removal of the silyl protecting group afforded *distal* and *medial-syn* isomers (±)-**29c** and (±)-**29d**, which were resolved by MPLC in 54% and 35% yields, respectively. Oxidation of the primary hydroxy group of the separated isomers (±)-**29a**−**d** with 8 N Jones's reagent in acetone at 0 °C and subsequent final acidic hydrolysis (6 N HCl, 90 °C, 16 h) furnished, after ion-exchange resin chromatography on Dowex 50X2-200 using 10% aqueous pyridine as eluent, the corresponding desired *proximal*, *medial-anti*, *distal*, and *medial-syn* spiropentyl amino acids (±)-**22a** (ASPED-A), (±)-**22b** (ASPED-B), (±)-**22c** (ASPED-C), and (±)-**22d** (ASPED-D) in 65%, 61%, 71%, and 68% yield, respectively.

Nomenclature and Stereochemical Assignment. Functionalization at 1−4 positions of the spiro[2.2]-

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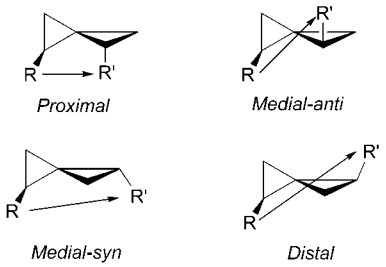
SCHEME 4



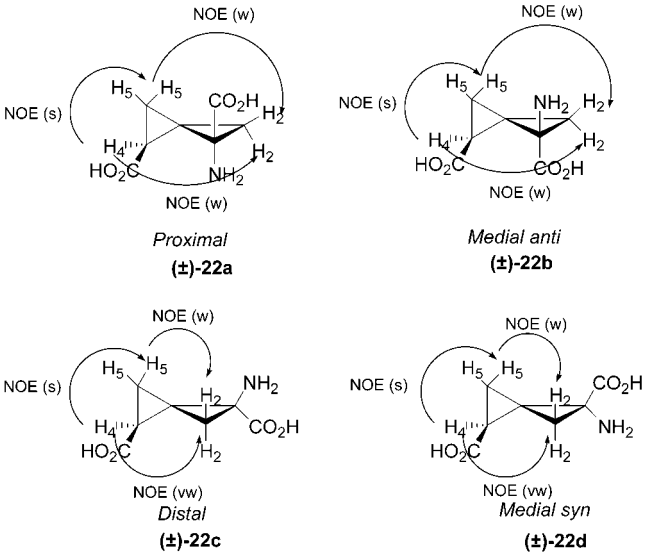
pentane moiety generates four pairs of enantiomers. To distinguish between these enantiomeric pairs it is convenient to adopt a trivial stereochemical nomenclature specifically suited for catching the spatial relationships between pharmacophoric groups. In 1970, Gayewski and Burka[16] assigned disubstituted spiropentanes with a stereochemical nomenclature based on the evaluation of (i) the linear distance between substituents and (ii) the relative orientation below and above a reference plane. Thus, as depicted in Scheme 4, the four possible diastereoisomeric disubstituted spiropentanes are classified as *proximal* (shortest distance between substituents), *distal* (longest distance between substituents), *medial-syn* (intermediate distance and syn disposition referring to the plane bearing the higher priority substituent), and *medial-anti* (intermediate distance and anti disposition referring to the plane bearing the higher priority substituent).

Although originally defined for disubstituted spiropentane derivatives, Gayewski and Burka's rules can be applied without ambiguity to our trisubstituted spiropentante derivatives **22a**−**d** if relative distances are considered between substituents with the higher IUPAC priority. Therefore, having defined this additional criterion, our racemic pair endowed with the largest distance between the amino group and the distal carboxylate will be named *distal*, the one with the shortest distance will be named *proximal*. The other two isomers will be assigned with the *medial-syn* and *medial-anti* nomenclature according to the orientation above or below the reference plane containing the highest priority substituent.

Once having defined these criteria, we faced the problem of the stereochemical assignment of the four racemic pairs **22a**−**d**. As a first attempt, we tried to identify diagnostic protons in bidimensional NMR experiments (Scheme 5).

The chemical shift of all protons was assigned by using (H,H)-COSY experiments. In particular, the downfield proton was assigned as H_4 (proton in α-position with respect to the distal carboxylate). Proton H_4 shows COSY effects with two upfield protons that were therefore assigned as H_5 (protons in the *β*-position with respect to the distal carboxylate). The remaining two protons are those in the 2-position of the spiropentane system. From the examination of NOESY data, NOE correlations were observed between proximal protons; thus a strong NOE effect between H_4 and both H_5 protons was observed in all isomers **22a**−**d**. This effect was therefore undiagnostic for the stereochemical assignment. Weak NOE correlations, furthermore, were observed in derivative **22a** and **22b** between H_4 and H_2 protons. This effect is present,

SCHEME 5



although much weaker, also in derivative **22c** and **22d**. As a result of the rigidity of the spiro[2.2]pentane moiety, this NOE effect must be ascribed to protons lying on the same face of the spiro[2.2]pentane system. Accordingly, **22a** and **22b** must be the *proximal* or the *medial-anti* isomers, whereas **22c** and **22d** must be the *distal* or *medial-syn* ones. No other diagnostic signals were identified, thus making impossible a further discrimination between the two diastereoisomeric pairs.

The definitive stereochemical assignment of the new derivatives was then based upon the single-crystal X-ray analysis carried out on selected spiropentane amino acids. Thus, the hydrobromic salts of the racemic spiropentane amino acids **22b**, **22c**, and **22d** were submitted to X-ray crystallography. Crystal data and relevant geometrical parameters are reported in Tables 1 and 2.

The solved crystal of the hydrobromic salt of derivative **22d** is endowed with a *S*-chirality at the amino acidic carbon atom and at the spiro carbon atom, while a *R*-chirality is observed at the carbon atom bearing the distal carboxylate at position 4 (see Figure S9, Supporting Information). Derivative **22d** belongs to a centric space group, thus confirming its racemic nature. Accordingly, the crystal structure of the hydrobromic salt of **22d** describes both (1*S*,3*S*,4*R*)-1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid and its (1*R*,3*R*,4*S*) enantiomer. In an analogous way, configuration (1*S*,3*R*,4*R*) and (1*R*,3*S*,4*S*) could be attributed to the racemic mixture **22c**, also belonging to a centric space group. Derivative **22b** was assigned with (1*S*,3*R*,4*S*) configuration, but in this case the compound crystallizes in a chiral space group ($Pna2_1$). Since the racemic mixture **22a** is chemically distinguishable from either **22b**, **22c**, or **22d** and possesses NOE properties similar to those of **22d**, it represents the fourth pair of diastereoisomeric 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acids and, hence, must be attributed the remaining (1*S*,3*S*,4*S*) and (1*R*,3*R*,4*R*) configurations.

The next step toward the stereochemical characterization of the four pairs **22a**−**d** was the attribution of the trivial nomenclature above-described. Thus, the spatial relationships between functional groups in the four pairs are summarized in Scheme 6 and, according to the chosen

TABLE 1. Crystal Data for Compounds 22b−d

	22d	22c	22b
formula	$C_7H_9NO_4 \cdot HBr$	$C_7H_9NO_4 \cdot HBr$	$C_7H_9NO_4 \cdot HBr \cdot H_2O$
M	252.07	252.07	270.09
crystal size (mm)	0.3 × 0.2 × 0.2	0.3 × 0.3 × 0.2	0.3 × 0.2 × 0.2
temperature (K)	293(2)	293(2)	293(2)
wavelength (Å)	0.71073	0.71073	0.71073
crystal system	monoclinic	monoclinic	orthorhombic
space group	$P2_1/c$ (No. 14)	$P2_1/c$ (No. 14)	$Pna2_1$ (No. 33)
a (Å)	6.922(1)	8.479(1)	9.051(1)
b (Å)	5.835(1)	10.238(1)	12.242(1)
c (Å)	24.653(3)	11.295(1)	9.123(1)
β (deg)	93.13(1)	99.11(1)	
U (Å^3)	994.2(3)	968.1(2)	1010.8(2)
Z	4	4	4
F(000)	504	504	544
D_c (g cm^{-3})	1.684	1.729	1.775
(Mo K) (mm^{-1})	4.118	4.229	4.064
scan mode	omega	omega	omega
scan range/°	2.9≤ Θ≤ 25.0	2.4≤ Θ≤ 25.0	2.8≤ Θ≤ 30.0
scan width/°	1.36	1.04	0.92
scan speed/° min^{-1}	3	3	3
reflections collected	2641	2320	3094
independent reflections	1738 (R_{int} = 0.02)	1705 (R_{int} = 0.05)	2924 (R_{int} = 0.02)
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2	full-matrix least-squares on F^2
no. of parameters refined	158	151	176
R_1 (all *data*)	0.0440	0.079	0.0728
wR_2 (all data)	0.0679	0.095	0.0953
goodness-of-fit on F^2	1.076	1.015	1.329

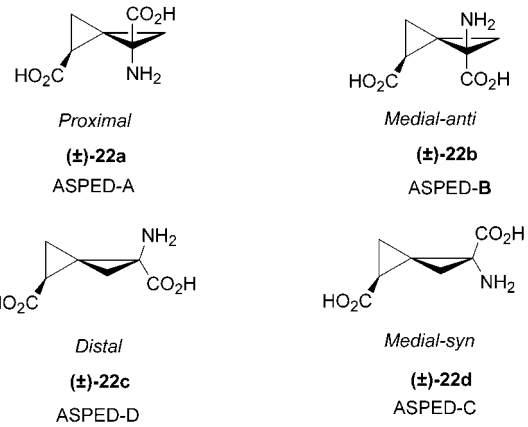
TABLE 2. Relevant Geometrical Parameters (Å, deg) for Non-Hydrogen Atoms of Compounds 22b−d

	22d	22c	22b
N(1)−C(1)	1.471(4)	1.468(6)	1.465(7)
O(1)−C(6)	1.322(4)	1.299(6)	1.329(7)
O(2)−C(6)	1.202(4)	1.215(6)	1.202(7)
O(3)−C(7)	1.271(4)	1.326(6)	1.324(8)
O(4)−C(7)	1.253(4)	1.209(7)	1.207(7)
C(1)−C(2)	1.538(5)	1.540(7)	1.550(8)
C(1)−C(3)	1.483(4)	1.477(7)	1.486(7)
C(1)−C(6)	1.482(4)	1.487(6)	1.482(7)
C(2)−C(3)	1.468(5)	1.464(7)	1.481(8)
C(3)−C(4)	1.456(5)	1.469(7)	1.461(9)
C(3)−C(5)	1.490(4)	1.483(7)	1.478(8)
C(4)−C(5)	1.532(5)	1.535(8)	1.524(12)
C(5)−C(7)	1.480(5)	1.481(8)	1.492(11)
N(1)−C(1)−C(2)	118.4(3)	118.7(4)	118.1(5)
C(2)−C(1)−C(3)	58.1(2)	58.0(3)	58.4(4)
C(3)−C(1)−C(6)	122.2(3)	122.4(4)	120.7(4)
C(1)−C(2)−C(3)	59.1(2)	58.8(3)	58.7(3)
C(1)−C(3)−C(2)	62.8(2)	63.1(3)	63.0(4)
C(1)−C(3)−C(4)	136.2(3)	135.0(4)	132.6(5)
C(2)−C(3)−C(5)	135.8(3)	138.6(4)	138.9(5)
C(4)−C(3)−C(5)	62.6(2)	62.6(4)	62.4(5)
C(3)−C(4)−C(5)	59.8(2)	59.1(3)	59.3(4)
C(3)−C(5)−C(4)	57.6(2)	58.2(3)	58.2(4)
C(3)−C(5)−C(7)	118.2(3)	119.4(5)	118.1(5)
O(1)−C(6)−O(2)	125.6(3)	125.9(5)	124.8(5)
O(3)−C(7)−O(4)	123.7(3)	123.6(5)	123.5(6)
C(5)−C(7)−O(4)	119.6(3)	123.5(5)	123.0(6)
C(5)−C(7)−O(3)	116.7(3)	112.8(5)	113.5(5)

criteria, **22a** is the *proximal*, **22b** the *medial-anti*, **22d** the *media- syn*, and **22c** the *distal* isomeric pair.

1-Aminospiro[2.2]pentyl-1,4-dicarboxylic Acids as Tools for Conformational Analysis. Although topologically equivalent to L-Glu (**1**), the 1-aminospiro[2.2]-pentyl-1,4-dicarboxylic acids (**22a**−**d**) have a three-dimensional structure that corresponds to high-energy conformers of the naturally occurring parent derivative. Indeed, X-ray crystallography confirms a perpendicular

SCHEME 6



arrangement of the two spirofused rings, with the substituents bisecting the corresponding angles of the ring. As expected, this perpendicular arrangement of the two cyclopropane rings forces the pseudo-torsional angles C1−C2−C3−C4 and C2−C3−C4−C5 to values that are attainable to L-Glu (**1**) only with a conspicuous energy expense. As an example, Table 3 reports the energy values, computed with the Universal Force Field by considering neutral zwitterionic species and by simulating a water environment with a dielectric constant of 80, necessary for L-Glu (**1**) to achieve the conformation mimicked by **22a**−**d**.

In alternative to dihedral angles, another parameter used as an indicator of the geometry of L-Glu analogues is the distance between functional groups.[27] According to this indicator, L-Glu (**1**) and L-Glu analogues are classified as extended or folded on the basis of the distances d_1 and d_2 between the distal carboxylate and the α-amino group or the α-carboxylate, respectively.

(27) Tellier, F.; Acher, F.; Brabet, I.; Pin, J. P.; Azerad, R. *Bioorg. Med. Chem.* **1998**, *6*, 195−208.

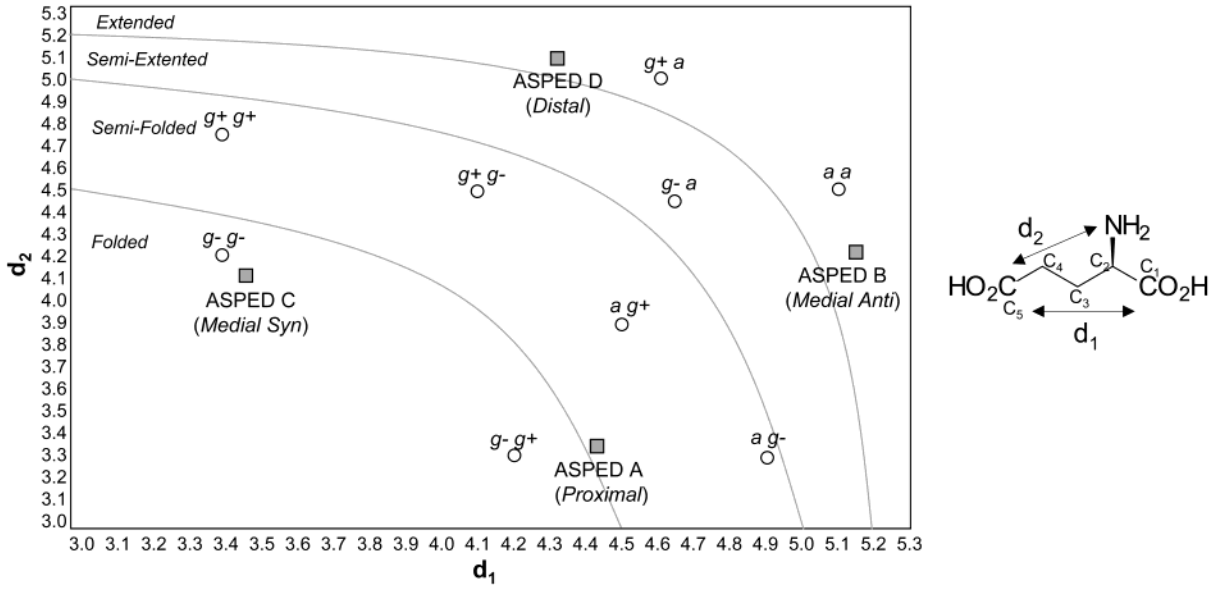


FIGURE 1. Projection of the four pairs of diastereoisomers of trisubstituted spiropentanes **22a**−**d** and of the nine staggered conformations of glutamate, into a bidimensional plot according to the d_1,d_2 distances; d_1 is defined as the distance between the carbons of the two carboxylate groups (C−C), and d_2 is defined as the distance between the carbon of the distal carboxylate group and the nitrogen of the amino acidic amino group.

TABLE 3. Energetic Penalties Required by Glutamate to Adopt Conformations Mimicked by 22a−d or Its Bioactive Conformations at iGluR's or mGluR's[a]

compound	energetic gap (kcal/mol)
ASPED A [**(±)-22a**]	21.39
ASPED B [**(±)-22b**]	13.01
ASPED C [**(±)-22d**]	12.38
ASPED D [**(±)-22c**]	10.20
AMPA-Glu	4.13
NMDA-Glu	0.80
$mGluR_1$-Glu	1.50

[a] Energetic penalties are calculated, by constraining the torsional bonds of glutamate to the values of the putative bioactive conformations or individual compounds **22a**−**d** and by calculating the difference in energy from the global minimum conformer. All of the species are considered in their zwitterionic forms

If the four pairs of ASPED diastereoisomers are plotted in a bidimensonal plot reporting the d_1 versus d_2 distances computed by using the Universal Force Field (see Experimental Section), a clear picture of the spatial relationship among functional groups is obtained. In particular, steroisomers with $(d_1 + d_2) \leq 7.5$ Å can be classified as folded isomers; those endowed with $7.5 \geq (d_1 + d_2) \leq 8.0$ Å can be classified as semifolded; those endowed with $8.0 \geq (d_1 + d_2) \leq 8.5$ Å can be named semiextended, and those with the $(d_1 + d_2) \geq 8.5$ Å are termed extended isomers. Clearly, because of the discrete nature of the torsional angles for rigid isomers, only singular points can be represented in the plot. It can be observed that, quite unexpectedly, the *medial-syn* 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22d**) falls into the region of folded conformation, whereas the *proximal* 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22a**) can rather be classified as a semifolded glutamate analogue. The other two pairs, the *medial-anti* **22b** and the *distal* **22c**, have a more extended disposition of functional groups and are projected between the semiextended and extended region. It is interesting to compare the position of the nine staggered conformations of glutamate when projected into the same plot (Figure 1). Thus, the *proximal* 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22a**) is endowed with distances close to the *g-g+* conformation of L-Glu (**1**). The distances d_1-d_2 in the *medial-syn* isomers **22d** are closely related to *the g-g-* conformation of L-Glu (**1**), whereas the *medial-anti* isomers **22b** resemble the *anti-anti* glutamic acid, and *the distal* isomers the *g+anti*. The bidimensional plot is also useful to compare the conformational profile of **22a**−**d** with respect to the parent carboxycyclopropylglycines (Figure 2). Thus, the introduction of the spiro-fused ring reduces the conformational degeneracy of CCGs from 12 possible conformations to only 4. The *proximal* isomers **22a** correspond to the *eclipsed-anti* conformation of L-CCG-IV (**5**), the *distal* pair corresponds to the *pseudoanti-g+* conformation of L-CCG-I (**4**), the *medial-syn* **22d** corresponds to the *eclipsed-g+* conformation of L-CCG-IV (**5**), and, finally the *medial-anti* pair **22b** corresponds to the *pseudoanti-anti* conformation of L-CCG-I (**4**). Of interest also is the possibility of projecting into the bidimensional plot the proposed bioactive conformation of L-Glu (**1**) experimentally determined (Figure 2). The proposed bioactive conformation of L-Glu (**1**) when acting at mGluR1 was extracted from the X-ray crystal structure (2.20 Å resolution) of the ligand binding domain of mGluR1[28] and shown to match the *pseudoanti-anti* conformation of L-CCG-I and the *medial-anti* isomer of 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22b**). The putative bioactive conformation of L-Glu (**1**) acting at the AMPA receptor was extracted from the X-ray structure (1.9 Å resolution) S1−S2 construct of the GluR2 receptor[29] and shown to match the *pseudoanti-g-* conformation of L-CCG-II (**4**) but none of the 1-aminospiro[2.2]-

(28) Kunishima, N.; Shimada, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature* **2000**, *407*, 971−977.

(29) Armstrong, N.; Gouaux, E. *Neuron* **2000**, *28*, 165−181.

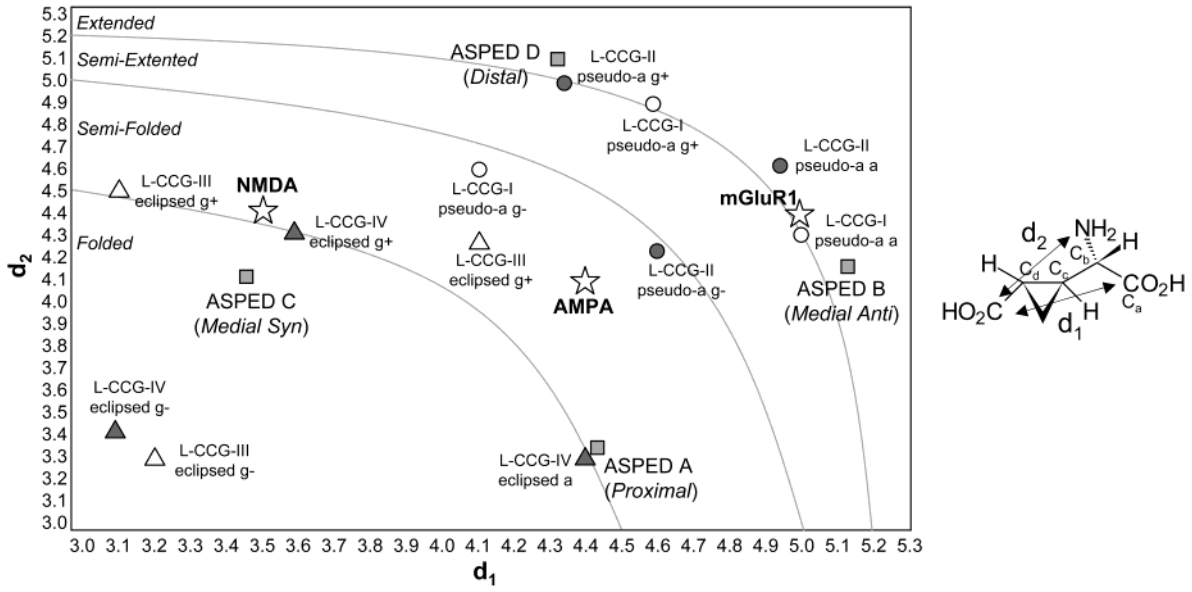


FIGURE 2. Comparison between **22a**−**d** and carboxycyclopropylglycine in the d_1-d_2 space. Again, d_1 is defined as the distance between the carbons of carboxylate groups (C−C), and d_2 is defined as the distance between the carbon of the distal carboxylate group and the nitrogen of the amino acidic amino group. Stars indicate the projection of the proposed bioactive conformations of glutamate when acting at selected iGluR's or mGluR's.

pentyl-1,4-dicarboxylic acid isomers (**22a**−**d**). Finally, the putative bioactive conformation of L-Glu (**1**) was inferred from a homology model of the NMDA-R2 receptor (unpublished result) and shown to correspond to the *eclipsed-g+* conformation of L-CCG-IV (**5**) and to *the media-syn* isomers of 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22d**). The analysis of this plot clearly confirms that the extended *pseudoanti-anti* conformation of L-CCG-I is the bioactive conformation at mGluR1, whereas the *eclipsed-g+* of L-CCG-IV (**5**) is the preferred one at the NMDA receptor. Most interestingly, the plot suggests the *medial-syn* isomers of 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22d**) as a potential NMDA-preferring ligand and the *medial-anti* 1-aminospiro[2.2]pentyl-1,4-dicarboxylic acid (**22b**) as a potential mimick of the mGluR1-bioactive conformation of L-Glu (**1**).

Biological Testing of 1-Aminospiro[2.2]pentyl-1,4-dicarboxylic Acids. The newly synthesized derivatives **22a**−**d** were preliminarily tested in a variety of glutamatergic receptor assays, including (i) binding to ionotropic glutamate receptors (NMDA, AMPA, and KA), (ii) evaluation of their ability to enhance or inhibiting mGluR1- or mGluR5-induced PI turnover, (iii) evaluation of their ability to enhance or inhibit the mGluR2-induced GTP(γ)-^{35}S stimulation. No agonistic or antagonistic activity was displayed up to a concentration of 50 μM at mGluR1 and mGluR5. On the other hand, two of the isomeric pairs significantly inhibit the binding of [^{3}H]-Glu to NMDA receptors: **22c** with an IC_{50} = 17.5 μM and **22b** with an IC_{50} = 39.8 μM.

The preliminary pharmacological characterization of the new derivatives (**22a**−**d**) did not reveal any particular activity at glutamate receptors, with exception of weak affinity of **22b** and **22c** to the NMDA receptor. It is worth analyzing these data having in mind the results reported in Figures 2 and 3. In particular, if the distances between functional (pharmacophoric) groups are taken as an indicator of the spatial arrangement of **22a**−**d**, thus using

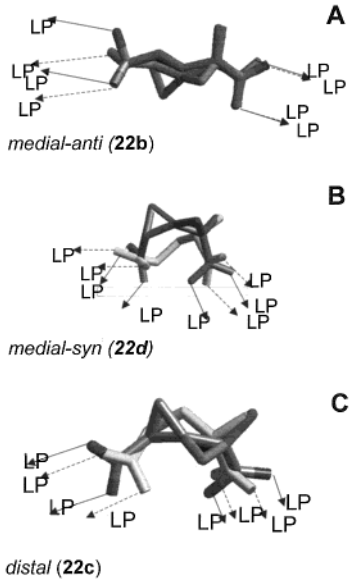


FIGURE 3. Superimposition of **22b**, **22c**, and **22d** on the proposed bioactive conformation of L-Glu (**1**) when acting at mGluR1, AMPA, and NMDA, respectively. The superimposition was accomplished by fitting the amino acidic moiety and the distal carboxylate oxygens. Only **22c** matches the lone pair projection of L-Glu (**1**).

the concept of folded and extended conformations, then one would have predicted the new derivatives more active than they actually are. In particular, the extended derivative **22b** could have been predicted as a mGluR1 ligand. The lack of activity can be attributed either to the presence of steric bumps or, more likely, to the peculiar orientation of the functional groups. Indeed, the visual comparison of the conformations of L-Glu (**1**) closer to the individual **22a**−**d** isomers allows verification that, despite a similarity in d_1-d_2 distances, the carboxylic groups have quite a different orientation, which results

in an unoptimized interaction of their electronic lone pairs with the receptorial counterpart (Figure 3).

Thus, our results indicate that the "folded-extended" concept, based on the distances between functional groups, although operatively useful and immediate, may not be appropriate when discussing structure−activity relationships.

Conclusion

A diastereoselective route to the synthesis of all four possible racemic isomers of 1-amino-spiro[2,2]pentyl-1,4-dicarboxylic acids (**22a**−**d**) has been reported along with their conformational characterization and preliminary biological evaluation as glutamate receptor ligands. The inclusion of the glutamate skeleton into a spiro[2.2]-pentane scaffold generated a library of molecules with interesting and quite unusual conformational properties. When tested on a series of glutamate receptors, only a moderate affinity to the NMDA receptor could be observed for two pairs (**21b** and **22c**), whereas no activity at metabotropic receptors was seen. Although these preliminary biological results are unsatisfactory, the availability of a synthetic route to this class of conformational analogues of L-Glu (**1**) can be of importance since the peculiar conformational properties of the new derivatives may be exploited in the design of novel peptidomimetics or in the incorporation of enzyme inhibitors.

Experimental Section

Chemistry. Melting points were determined by the capillary method and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with 200 MHz (unless noted) spectrometers. The abbreviations used are as follows: s, singlet; dd, double doublet; m, multiplet, p, pseudo. Bidimensional NOESY and COSY experiments were performed on 400 MHz spectrometer. TLC were carried out on precoated TLC plates with silica gel 60 F-254. For column chromatography, silica gel 60 (230−400 mesh) was used. GC−MS analyses were carried out with a gas chromatograph (12.5 m column) equipped with a mass-selective detector. GC analyses were performed on a gas chromatograph with a 30 m capillary column.

All final compounds **22a**−**d** displayed an HPLC purity > 95%. HPLC analyses were performed with a workstation equipped with a UV−vis detector using the following parameters: column, 2.5 × 25 mm, 0.5 μm RP-18; flow rate, 0.8 mL/min; detection at 210 nm; mobile phase, H_2O + 0.05%TFA.

(±)-*tert*-Butyl-2-methyl, 2-Bromo-cyclopropane-1-carboxylate (16). A solution of *tert*-butyl diazoacetate (58.4 g, 410 mol) in dry dichloromethane (500 mL) was added under argon via syringe pump to a magnetically stirred solution of 2-bromopropene (**15**) (38.5 g, 320 mol) and dirhodium(II) tetraacetate (1.35 g, 3.1 mmol) in dry dichloromethane (400 mL) over a period of 96 h. The reaction mixture was then filtered, and after evaporation of the solvent, the product **16** was isolated by distillation as a mixture of *cis* and *trans* isomers: bp 55−59 °C, 0.2 mbar (64.3 g, 86%); ^{1}H NMR ($CDCl_3$) δ 1.40 (dd, 1H, J = 6.7 and 13.6 Hz), 1.45−1.55 (m, 10 H), 1.87 (s, 3H), 2.25 (dd, 1H, J = 6.7 and 9.1 Hz).

(±)-*tert*-Butyl-2-methylene-cyclopropane-1-carboxylate (14). A solution of **16** (20.3 g, 86.3 mmol) in dry DMSO (11 mL) was added dropwise in 20 min to a magnetically stirred solution of potassium *tert*-butoxide (14.4 g, 128.3 mmol) in dry DMSO (87.5 mL) kept under argon at room temperature. The reaction mixture was stirred for 30 min at room temperature and then quenched with cold water (150 mL). Pentane was added (150 mL) and the organic phase was separated. The aqueous phase was extracted with pentane (3 × 70 mL). The combined organic phases were washed with water (1 × 70 mL) and dried (Na_2SO_4). The pentane solution was removed at atmospheric pressure using a 20-cm Vigreux column, and the product **14** was distilled: bp 69−70 °C, 19 mbar (6.1 g, 46%). Analytical data were identical with those previous reported.[30]

(±)-4-(*tert*-Butyl)-1,1-dimethyl Spiro[2.2]pentane-1,1,4-tricarboxylate (17). A solution of dimethyl diazomalonate (0.20 g, 1.25 mmol) in dry dichloromethane (5 mL) was added via syringe pump over a period of 3 h to a magnetically stirred solution of **14** (0.20 g, 1.3 mmol) and dirhodium(II) tetraacetate (0.02 g, 0.045 mmol) in dry dichloromethane (10 mL) kept under argon at reflux. The reaction mixture was then filtered, and after evaporation of the solvent, the residue was submitted to a flash chromatography. Elution with light petroleum−EtOAc (90:10) afforded **17** (0,121 g, 33%): ^{1}H NMR ($CDCl_3$) δ 1.45−1.70 (m, 11H), 1.90 (d, 1H, J = 4.8 Hz), 2.00−2.15 (m, 2H), 3.70 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 13.6, 20.0, 21.3, 27.3, 28.0, 52.3, 80.7, 168.5, 170.7.

(±)-4-(*tert*-Butyl)-1-methyl Spiro[2.2]pentane-1,1,4-tricarboxylates (18 + 19). A solution of NaOH (0.182 g, 4.55 mmol) in water (1 mL) was added to a magnetically stirred solution of **17** (1.29 g, 4.55 mmol) in methanol (1 mL). After 72 h of stirring methanol was evaporated in vacuo. The residual aqueous layer was diluted and extracted with ether (3 × 4 mL), acidified with a 10% citric acid solution, and extracted with ethyl acetate (4 × 10 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give the mixture of the two unseparated diastereoisomers **18** + **19** (0.89 g, 72%): ^{1}H NMR ($CDCl_3$) δ 1.30−1. 70 (m, 11H), 1.90−2.30 (m, 3H), 3.70 (s, 3H), 10.00 (brs, 1H).

(±)-4-(*tert*-Butyl) 1-Methyl-1-(*tert*-butoxycarbonyl)-amino Spiro[2.2]pentane-1,1,4-tricarboxylates (20 + 21). A magnetically stirred solution of **18** + **19** (0.220 g, 0.81 mmol) in dry THF (2.5 mL) was treated with potassium carbonate (0.226 g, 1.63 mmol) and 18-crown-6 (0.033 g, 0.12 mmol) at 0 °C under argon. A solution of ethyl chloroformate (0.098 g, 0.90 mmol) in dry THF (0.5 mL) was then added dropwise, and the mixture was stirred for 30 min at 0 °C and 90 min at room temperature. The solution was quickly filtered to remove solids (washed with 1.5 mL of THF). The solution was recooled to 0 °C, whereupon a solution of sodium azide (0.059 g, 0 0.90 mmol) in water (0.83 mL) was rapidly added. After stirring for 30 min, the mixture was poured into water (10 mL) and extracted with ether (3 × 10 mL). The combined ether extracts were washed with brine (12 mL), dried (Na_2SO_4), and evaporated in vacuo to give the intermediate acyl azide (0.183 g, 76%). The acyl azide was taken up in *tert*-butyl alcohol (5.0 mL) and heated to reflux for 17 h. The mixture was cooled and the solvent was removed in vacuo. The resulting residue was submitted to a flash chromatography. Elution with light petroleum−EtOAc (70:30) afforded an unseparated mixture of **20** + **21** (0.161 g, 58%): ^{1}H NMR ($CDCl_3$) δ 0.80−1.00 (m, 1H), 1.40−1.70 (m, 21H), 1.90−2.10 (m, 1H), 3.73 (s, 3H), 5.20 (brs, 1H).

(±)-*distal*- and (±)-*medial-syn*-1-Amino Spiro[2.2]pentane-1,4-dicarboxylic Acids (22c + 22d). A mixture of **20** + **21** (0.128 g, 0.37 mmol) and 6 N hydrochloric acid (13 mL) was heated at 80 °C for 8 h. The reaction mixture was evaporated to dryness, and the residue was submitted to ion exchange chromatography (Dowex 50WX2-200). Elution with 10% pyridine afforded an unseparated mixture of **22c** + **22d** (0.040 g, 63%): ^{1}H NMR (D_2O) δ 1.40−11.65 (m, 3H), 1.75−1.85 (2xd, 1H, J = 6.4 Hz), 2.00−2.20 (m, 1H); ^{13}C NMR (D_2O) δ 13.7, 14.9, 17.0, 20.0, 20.8, 27.7, 40.4, 172.8, 172.9, 176.8, 177.0. These spectral data are in agreement with those reported below for single isomers **22c** and **22d**.

(±)-(*tert*-Butyldimethylsilyloxymethyl)-2-methylenecyclopropane (24). A solution of methylenecyclopropylmethanol

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(**23**) (6.61 g, 78.6 mmol) in dry dichloromethane (200 mL) was treated with 4-(dimethylamino)pyridine (9.6 g, 78.6 mmol). After stirring for 10 min, this mixture was treated with *tert*-butyldimethylsilyl chloride (12.15 g, 80 mmol), and the resulting solution was stirred overnight at room temperature under argon. The reaction was quenched with a saturated solution of sodium bicarbonate. The aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined extracts were washed with brine (1 × 50 mL), dried (Na_2SO_4), and evaporated, and the mixture was purified by flash chromatography (*n*-hexane) to give **24** (8.5 g, 55%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.01 (6H, s,), 0.83 (9H, s), 1.20 (2H, m), 1.64 (1H, m), 3.36 (1H, dd, J = 11 and 7.5 Hz), 3.59 (1H, dd, J = 11 and 6.8 Hz), 5.3 (1H, m), 5.35 (1H, m).

(±)-(*proximal*)-4-(*tert*-Butyldimethylsilyloxymethyl)-spiro[2.2]pentane-1,1-dicarboxylic Acid Diethyl Ester (25) and (±)-(*distal*)-4-(*tert*-Butyldimethylsilyloxymethyl)-spiro[2.2]pentane-1,1-dicarboxylic Acid Diethyl Ester (26). A solution of diethyldiazomalonate (13.12 g, 70.4 mmol) in dry dichloromethane (600 mL) was added via syringe pump over 90 h to a magnetically stirred solution of the olefin **24** (3.5 g, 17.6 mmol) and dirhodium(II) tetraacetate (0.35 g, 0.78 mmol) in dry dichloromethane (200 mL) at reflux under argon. The reaction mixture was filtered and evaporated to dryness. The residue was submitted to flash chromatography; elution with light petroleum ether−EtOAc (98:2) gave a mixture of **25** and **26** as a colorless oil (**25**/**26** = 1/5, GC−MS). The mixture of diastereoisomers **25** and **26** was separated by MPLC. (cyclohexanes−EtOAc, 95:5) to give **25** (0.7 g, 11%) and **26** (3.3 g, 53%). **Data for 25**: ^{1}H NMR ($CDCl_3$) δ 0.10 (6H, bs), 0.94 (9H, bs), 1.14−1.17 (1H, m), 1.27−1.37 (6H, m), 1.73−1.90 (3H, m), 3.23 (1H, dd, J = 8.6 and 10.8 Hz),4.02 (1H, dd, J = 4.5 and 11.4 Hz), 4.19−4.30 (4H, m); ^{13}CNMR ($CDCl_3$) δ 5.25.2 10.114.0 14.218.4 20.6,21.3, 26.0 28.1 32.9, 61.2, 61.2, 64.4, 169.0, 169.4; GC-MS *m*/*z* (relative intensity) 355 (M − H$^+$, 1), 299 (100), 225 (32), 181 (16), 75 (38). **Data for 26**: ^{1}H NMR ($CDCl_3$) δ 0.01 (6H, bs), 0.85 (9H, bs),1.19−1.26 (7H, m), 1.51−1.98 (3H, 2m,), 3.43 (1H, dd, J = 2 6.6 and 10.6 Hz), 3.72 (1H, dd, J = 5.3 and 11.4 Hz), 4.06−4.19 (4H, m); ^{13}C NMR ($CDCl_3$) δ 3.9, 3.8, 11.8, 15.6, 19.7, 20.6, 21.2, 27.1, 29.4, 34.4, 62.5, 61.6, 65.7, 170.4, 170.5; GC−MS *m*/*z* (relative intensity) 355 (M − H$^+$, 1), 299 (100), 225 (33), 181 (67), 75 (83).

(±)-*proximal*- and (±)-*medial-anti-4-(tert*-Butyldimethylsilyloxymethyl)-spiro[2.2]pentane-1,1-dicarboxylic Acid Monoethyl Esters (27a + 27b). A solution of 0.5 M sodium hydroxyde (8.4 mL, 4.2 mmol) in water was added to a solution of **25** (1.5 g, 4.2 mmol) in 95% ethanol, and the resulting mixture was stirred at room temperature for 36 h. The reaction mixture was evaporated to dryness, water was added to the residue, and the mixture was neutralized with 10% citric acid solution and extracted with ethyl acetate (3 × 100 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give the mixture of products **27a** and **27b** (1.25 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, bs), 0.79 (9H, bs), 0.95−1.20 (1H, m), 1.27−1.37 (6H, m), 1.73−2.20 (3H, m), 3.50 (1H, m), 3.80 (1H, m), 4.07−4.30 (2H, m).

(±)-*distal*- and (±)-*medial-syn*-4-(*tert*-Butyldimethylsilyloxymethyl)-spiro[2.2]pentane-1,1-dicarboxylyc Acid Monoethyl Esters (27c + 27d). Monohydrolysis of **26** (4.6 g, 13.0 mmol) was performed according to the procedure described above for **25**. The mixture of the isomers **27c** + **27d** (3.3 g, 78%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.79 (9H, m), 1.05−1.20 (1H, m), 1.21−1.33 (4H, m), 1.53−1.75 (1H, m), 3.30−3.52 (1H, m), 3.70 (0.5H, dd, J = 8.6 and 10.8 Hz), 3.90 (0.5H, dd, J = 4.5 and 11.4 Hz), 4.10−4.30 (2H, m).

(±)-*proximal*- and (±)-*medial-anti*-4-(*tert*-Butyldimethylsilyloxymethyl)-1-*tert*-butoxycarbonylAminospiro[2.2]-pentane-1-carboxylic Acid Ethyl Esters (28a +28b). A stirred solution of **27a** + **27b** (1.24 g, 3.78 mmol) in dry tetrahydrofurane (30 mL) at 0 °C under argon was treated with potassium carbonate (1.04 g, 7.55 mmol), 18-crown-6 (0.10 g, 0.38 mmol), and freshly distilled ethylchloroformate (0.49 g, 4.54 mmol). The mixture was kept at 0 °C for 30 min and at room temperature for 90 min. The reaction mixture was filtered quickly under argon, and the remaining solid was washed with tetrahydrofurane (2 × 10 mL). A solution of sodium azide (0.29 g, 4.54 mmol) in water (5 mL) was added to the filtrate at 0 °C under argon. After 1 h, the reaction mixture was poured into cold water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined extracts were dried (Na_2SO_4) and evaporated. The residue was dissolved in *tert*-butyl alcohol (30 mL), and the solution was refluxed overnight under argon. The reaction mixture was concentrated under reduced pressure, and the residue was submitted to flash chromatography; elution with light petroleum ether−EtOAc (9:1) gave the product **28a** + **28b** (0.45 g, 30%) as a mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.78 (9H, s), 0.80−1.00 (1H, m), 1.18−1.28 (3H, m), 1.40 (9H, s), 1.50−1.70 (1H, m), 1.80−1.98 (1H, m), 3.18−3.32 (1H, m), 3.83−3.91 (1H, m), 3.98−4.26 (4H, m).

(±)-*distal*- and (±)-*medial-syn*-4-(*tert*-Butyldimethylsilyloxymethyl)-1-*tert*-butoxycarbonyl Aminospiro[2.2]-pentane-1-carboxylic Acid Ethyl Esters (28c + 28d). Starting from the mixture **18c** + **18d** (2.4 g, 7.32 mmol) the isomers **19c** + **19d** were obtained (0.94 g, 32%) according to the procedure described above for **18a** + **18b**: ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, m), 0.82 (9H, m), 1.05−1.35 (4H, m), 1.42 (9H, bs), 1.45−1.90 (3H, m), 3.38 (1H, dd, J = 6.6 and 10.6 Hz), 3.85 (1H, dd, J = 5.3 and 11.4 Hz), 4.08−4.22 (4H, m).

(±)-*proximal*-1-*tert*-Butoxycarbonylamino-4-hydroxymethylspiro[2.2]pentane-1-carboxylic Acid Ethyl Ester (29a) and (±)-*medial-anti*-1-*tert*-Butoxycarbonylamino-4-hydroxymethylspiro[2.2]pentane-1-carboxylic Acid Ethyl Ester (29b). A solution of **28a** + **28b** (0.44 g, 1.1 mmol) in distilled tetrahydrofurane (30 mL) was treated with tetra-*n*-butylammonium fluoride (0.44 g, 1.1 mmol). The solution was stirred at room temperature until no starting material was detected by TLC. Water (150 mL) was added to the mixture, and the aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give the mixture of derivatives **29a** and **29b,** which was separated by MPLC; elution with light petroleum ether−EtOAc (3:2) gave **29a** (0.065 g, 21%) and **29b** (0.202 g, 65%) as white solids. **Data for 29a**: mp 77−79 °C; ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, pt, J = 4.9 Hz) 1.05 (1H, dd, J = 5.0 and 8.2 Hz), 1.17 (3H, t, J = 7.1 Hz), 1.40 (9H, bs), 1.60−1.70 (1H, m), 2.01 (1H, d, J = 4.5 Hz), 3.22 (1H, pt, J = 10.8 Hz) 3.96−4.19 (3H, m); ^{13}C NMR ($CDCl_3$) δ 10.2, 14.3, 20.8, 23.3, 28.3, 61.1, 64.0, 79.8, 157.4, 172.0. **Data for 29b**: mp 89−81 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (2H, m), 1.18 (3H, t, J = 7.1 Hz), 1.37 (10H, m), 1.66−1.80 (1H, m), 1.85 (1H, d, J = 4.4 Hz), 3.40 (1H, pt, J = 8.2 Hz), 3.81 (1H, dd, J = 3.8 and 11.6 Hz), 4.02−4.22 (2H, m); ^{13}C NMR ($CDCl_3$) δ 7.7, 14.1, 22.4, 24.0, 28.2, 30.9, 38.7, 62.0, 63.6, 80.0, 155.9, 172.4.

(±)-*distal-1-tert*-Butoxycarbonylamino-4-hydroxymethylspiro[2.2]pentane-1-carboxylic Acid Ethyl Ester (29c) and (±)-*media-syn*-1-*tert*-Butoxycarbonylamino-4-hydroxymethylspiro[2.2]pentane-1-carboxylic Acid Ethyl Ester (29d). Both isomers were prepared as described above for spiropentanes **29a** and **29b** from a mixture of **19c** + **19d** (0.6 g, 2.33 mmol). MPLC in light petroleum ether−EtOAc (3:2) gave **29c** (360 mg, 54%) and **29d** (230 mg, 35%) as white solids. **Data for 29c**: mp 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (1H, t, J = 5.2 Hz), 1.20 (3H, t, J = 7.1 Hz), 1.35−1.50 (10H, m), 1.50−1.72 (1H, m), 1.93−2.10 (2H, 2m), 3.23 (1H, dd, J = 9.2 Hz, 10.6 Hz), 3.85 (1H, dd, J = 4.8 and 10.6 Hz), 4.11 (2H, q, J = 7.1 Hz); ^{13}C,NMR ($CDCl_3$) δ 11.3, 14.3, 19.5, 20.2, 20.8, 28.2, 29.2, 29.8, 38.9, 61.2, 65.3, 80.1, 156.1, 172.0. **Data for 29d**: mp 82−84 °C; ^{1}HNMR ($CDCl_3$) δ 0.78 (1H, t, J = 5.1 Hz), 1.10 (3H, t, J = 7.1 Hz), 1.35−1.50 (10H, m), 1.50−1.72 (1H, m), 1.93−2.10 (2H, 2m), 3.62 (2H, m), 4.17 (2H, m); ^{13}C NMR ($CDCl_3$) δ 10.4, 14.2, 20.6, 21.4, 28.2, 30.5, 38.6, 61.7, 65.1, 80.0, 155.9, 172.9.

(±)-1-Aminospiro[2.2]pentan-1,4-dicarboxylic Acids (22a−d). A magnetically stirred ice-cold solution of a single isomer of Boc-aminospiropentanes **29a**, **29b**, **29c**, or **29d** (0.06 g, 0.21 mmol) in acetone (10 mL) was treated with 8 N Jones' reagent (about 0.5 mL) until a persistent orange color was reached. The reaction mixture was stirred at room temperature for 1 h. 2-Propanol (1 mL) was added, and after 10 min, the mixture was filtered through Celite, washing with methanol. The solvent was removed under reduced pressure, and the residue was used for the next reaction without further purification. HCl (6 N, 20 mL) was added to a solution of crude mixture, and the reaction was stirred at 90 °C for 16 h. The solvent was removed under reduced pressure. The residue was submitted to ion-exchange resin chromatography (Dowex 50X2-200, 5% pyridine) to give the title compound **22a**, **22b**, **22c**, **or 22d**.

Data for *proximal* isomer 22a: 65% yield; mp 239−240 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.44 (1H, t, J = 4.7 Hz, 5-CH_a), 1.58−1.61 (2H, m, 2-CH_a and 5-CH_b); 1.80 (1H, d, J = 6.3 Hz, 2-CH_b); 2.30 (1H, dd, J = 4.8 and 8.3 Hz, 4-CH); ^{13}C NMR (D_2O) δ 15.5, 16.0, 21.3, 26.2, 40.0, 172.5, 176.7.

Data for *medial-anti* isomer 22b: 61% yield; mp 208−210 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.54−1.61 (2H, m, 5-CH_2), 1.65 (1H, d, J = 6.4 Hz, 2-CH_a), 1.88 (1H, d, J = 6.4 Hz, 2-CH_b), 2.22 (1H, dd, J = 4.9 and 7.8 Hz, 4-CH); ^{13}C NMR (D_2O) δ 13.2, 17.7, 21.0, 26.9, 39.3, 172.6, 176.3.

Data for *distal* isomer 22c: 71% yield; mp 210−212 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.58 (1H, t, J = 4.8 Hz, 5-CH_a), 1.65 (1H, dd, J = 5.0 and 8.4 Hz, 5-CH_b),1.71 (1H, d, J = 6.5 Hz, 2-CH_a), 1.86 (1H, d, J = 6.6 Hz, 2-CH_b), 2.14 (1H, dd, J = 5.0 and 8.2 Hz, 4-CH); ^{13}C NMR (D_2O) δ 13.6, 16.8, 20.7, 27.6, 40.1, 171.4, 177.0.

Data for *medial-syn* isomer 22d: 68% yield; mp 170−172 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.55−1.61 (2H, m, 5-CH_2), 1.64 (1H, d, J = 6.5 Hz, 2-CH_a), 1.94 (1H, d, J = 6.5 Hz, 2-CH_b), 2.23 (1H, dd, J = 4.8 and 8.2 Hz, 4-CH); ^{13}C NMR (D_2O) δ 14.9, 16.9, 19.9, 27.6, 40.2, 173.0, 177.1.

X-ray Analysis. Single crystals of **22b−d** were obtained by dissolution in water−48% HBr (10:1) and slow evaporation at room temperature. Colorless single crystals of each compound were submitted to X-ray data collection on a four-circle diffractometer with graphite monochromated Mo K radiation (λ = 0.71069 Å). Lattice parameters were determined by least-squares refinement on 40, 49, and 60 randomly selected and automatically centered reflections, respectively. The $\omega/2\theta$ scan technique was used in the data collection. Crystal details are given in Table 1. Absorption correction obtained by Ψ scans was applied to all the data collections. The structures were solved by direct methods implemented in the SHELX-97 program.[31] The refinements were carried out by full-matrix anisotropic least-squares on F^2 for all reflections for non-H atoms by using the SHELX-97 program.[31]

Computational Methods. All studied compounds were built using the Cerius2 libraries using molecular mechanics and semiempirical calculations. In particular, the structures of compounds **22a−d** were geometry optimized, in their neutral form, using the MOPAC-AM1 method. The geometries thus obtained for compounds **22b−d** were in good agreement with the experimentally determined ones. L-Glutamic acid (**1**) and the CCGs (**4**, **5**) were submitted to a conformational search using the grid search routine implemented in Cerius2. Two torsional angles for L-Glu (**1**) (t^1 defined as $C_1-C_2-C_3-C_4$, t^2 defined as $C_2-C_3-C_4-C_5$) and one torsional angle for the CCG **4** and **5** (t^1 defined as $C_a-C_b-C_c-C_d$) were analyzed in a range of 360° with a step increment of 1°. The energetical profiles were evaluated by using the Universal force field implemented in Cerius2 and by correcting the electrostatic contribution by setting a dielectric constant of 80. All conformers were minimized using the conjugate gradient until a convergence gradient of 0.001 was reached. After this protocol, the unique conformers were stored and used for further analysis. All calculations were carried out on a SGI O2 R5000 machine using the Cerius2 software package of Accelrys.

Biology. The preliminary biological screenings were performed as previously reported.[32]

Acknowledgment. Financial support of Italian Minister of University (MURST, COFIN2000) to R.P. is gratefully acknowledged.

Supporting Information Available: Characterization data and spectra for **22a−d**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO020138V

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