4-Substituted D-Glutamic Acid Analogues: The First Potent Inhibitors of Glutamate Racemase (MurI) Enzyme with Antibacterial Activity

Alfonso de Dios,*,† Lourdes Prieto,† Jose Alfredo Martín,† Almudena Rubio,†,‡ Jesus Ezquerra,† Mark Tebbe,§ Beatriz López de Uralde,† Justina Martín,|| Ana Sánchez,|| Deborah L. LeTourneau,§ James E. McGee,§ Carole Boylan,§ Thomas R. Parr, Jr.,§,⊥ and Michele C. Smith§

Eli Lilly and Co., Lilly S.A., Avenida de la Industria, 30, 28108 Alcobendas, Madrid, Spain, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, and Departamento de Química Orgánica, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain

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The first potent inhibitors of glutamate racemase (MurI) enzyme that show whole cell antibacterial activity are described. Optically pure 4-substituted D-glutamic acid analogues with (2*R*,4*S*) stereochemistry and bearing aryl-, heteroaryl-, cinnamyl-, or biaryl-methyl substituents represent a novel class of glutamate racemase inhibitors. Exploration of the D-Glu core led to the identification of lead compounds (−)-**8** and **10**. 2-Naphthylmethyl derivative **10** was found to be a potent competitive inhibitor of glutamate racemase activity (K_i = 16 nM, circular dichroism assay; IC_{50} = 0.1 μg/mL high-performance liquid chromatography (HPLC) assay). Thorough structure−activity relationship (SAR) studies led to benzothienyl derivatives such as **69** and **74** with increased potency (IC_{50} = 0.036 and 0.01 μg/mL, respectively, HPLC assay). These compounds showed potent whole cell antibacterial activity against *S. pneumoniae* PN−R6, and good correlation with the enzyme assay. Compounds **69**, **74** and biaryl derivative **52** showed efficacy in an in vivo murine thigh infection model against *Streptococcus pneumoniae*. Data described herein suggest that glutamate racemase may be a viable target for developing new antibacterial agents.

Introduction

An alarming increase in antimicrobial resistance[1,2] has motivated an increasingly active search for novel, viable targets that are essential for bacterial life.[3] Inhibition of the bacterial cell wall peptidoglycan biosynthesis is a very attractive target for the design and discovery of new antibacterial agents.[4−6] For example, the first total synthesis of the peptidoglycan Park Nucleotide[7] has been disclosed, which could lead the way to rational, structure-based design of new agents that inhibit the synthesis of the stem peptide portion of peptidoglycan precursors. Inhibitors of some of the ligases (adenosine 5′-triphosphate (ATP)-dependent amino acid-adding enzymes) required for synthesis of stem peptides such us MurC,[8] MurD,[9] and MurE[10] have recently been prepared. Agents that inhibit stem peptide biosynthesis have the potential for activity against all cell wall-containing bacteria and could show broad spectrum activity.

D-Glutamic acid (D-Glu) is a required biosynthetic building block added by MurD to the peptidoglycan intermediate uridine 5′-diphosphate (UDP)-*N*-acetylmuramoyl-alanine. The enzyme glutamate racemase (MurI) catalyzes the interconversion of glutamate enantiomers in a cofactor-independent fashion, providing bacteria with D-Glu.[11] The *mur*I gene is found in important pathogenic bacteria, including *Escherichia coli*[12] and staphylococci.[13] Recently, *mur*I gene expression[14] and glutamate racemase biosynthesis[15] in *Streptococcus pneumoniae* have been demonstrated. Genes for D-amino acid aminotransferase (D-AAT) have also been shown to be present in some bacteria.[13,16] It is not clear if this enzyme can serve as a substitute source of D-Glu. Significantly, mutagenesis knockout experiments in *S. pneumoniae* have shown that glutamate racemase is essential for the viability of this bacteria.[17]

In this context, seminal work by Tanner has led to the discovery of the first inhibitors of glutamate racemase, which remain the only ones described to date.[18,19] Although only modestly potent, these inhibitors interact with glutamate racemase by competitively interfering with the L-Glu/D-Glu transformation,[11] which mechanistically involves two active site cysteine residues.

We envisioned that D-Glu-based analogue design followed by a detailed structure−activity relationship (SAR) study could help to identify the first potent inhibitors of glutamate racemase and to better understand the potential utility of α-amino acids as antibacterials. Additionally, D-Glu-based agents might lack other biological effects associated with L-Glu (i.e., central nervous system (CNS) cross activity) since D-Glu is not naturally found in mammalian hosts.

In this paper, we describe the discovery of a new class of (2*R*,4*S*)-4-substituted D-Glu analogues represented by structure **1** (Figure 1). These compounds potently inhibit glutamate racemase and show promising whole cell activity against the PN-R6 strain of *S. pneumoniae*. This pathogen is one of the most frequent causes of bacterial

* To whom correspondence should be addressed. Tel: +34-91-6633412. Fax: +34-91-6633411. E-mail: de_dios_alfonso@lilly.com.
† Lilly S.A.
‡ Present address: Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.
§ Lilly Corporate Center.
|| Universidad de Alcalá.
⊥ Present address: Xenogen Corporation, 860 Atlantic Avenue, Alameda, CA 94501.

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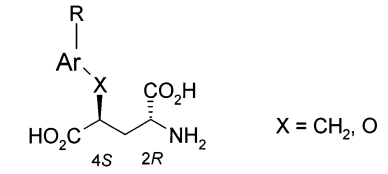

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Figure 1.

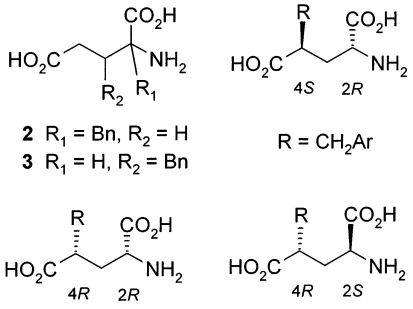


Figure 2.

respiratory infection and meningitis. In some communities, as many as 40% of all *S. pneumoniae* strains are resistant to certain drugs.[20] Exploration of the D-Glu scaffold, compound synthesis, glutamate racemase inhibition data, and antibacterial whole cell data as well as preliminary in vivo experiments are described.

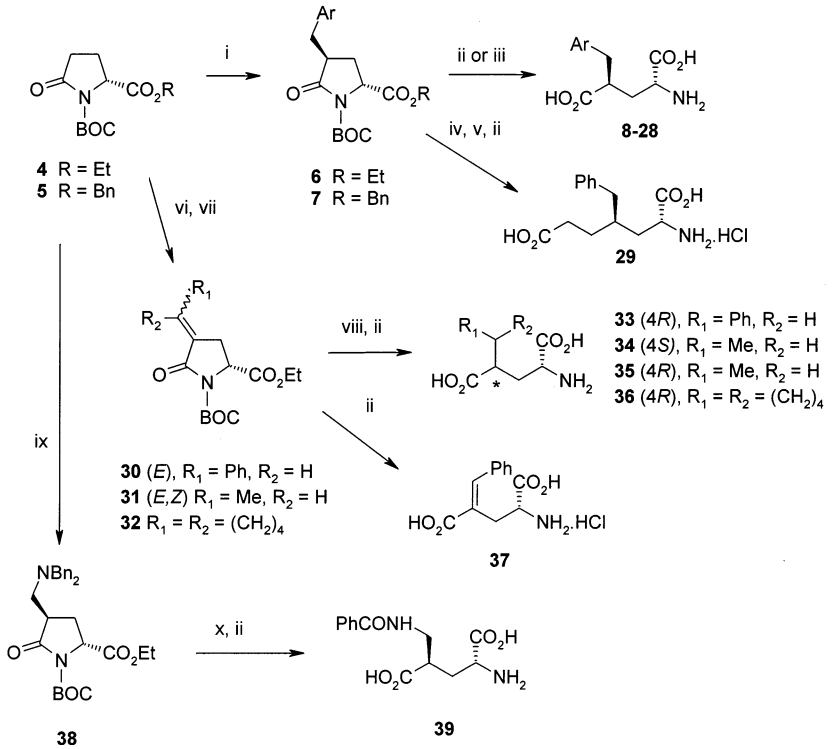
Chemistry

2-Benzyl glutamic acid **2**[21] and 3-benzyl glutamic acid **3**[22] (Figure 2) were prepared in racemic form and racemic mixture of diastereomers, respectively, using solid phase synthesis of unnatural amino acids. Optically pure 4-substituted glutamic acids with different substitutions and stereochemistry patterns (Figure 2) were prepared employing procedures that take advantage of well-described transformations starting from N-protected pyroglutamate derivatives.[23] These versatile building blocks were used in most of the synthetic routes described herein.

(2*R*,4*S*)-4-Substituted D-Glu analogues were obtained as previously described for the L series via selective C-4 lithiation of N-BOC-protected D-pyroglutamate esters **4** and **5** (Scheme 1) followed by electrophile addition.[24] This efficient procedure has proven to be highly regio- and stereoselective[24] leading to the trans isomers **6** and **7** when different benzyl and naphthylmethyl halides were used as electrophiles. A variety of final trans 2*R*,4*S* amino acids **8**−**28** were isolated using two alternative hydrolysis procedures.[25,26] Compounds were efficiently and conveniently obtained as pure enantiomers either as hydrochloride or zwitterion salts and showed the same biological activity for both salt forms.

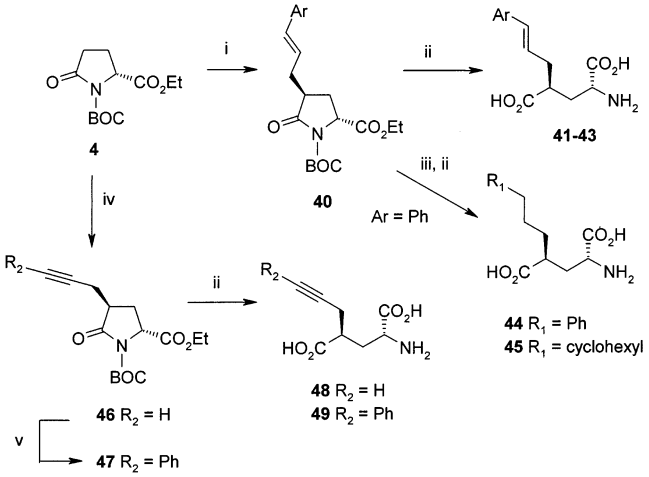
Chemoselective reduction of the lactam carbonyl in C-4 benzyl pyroglutamate **6** (Ar = Ph) using $LiEt_3BH$ was carried out as described for L pyroglutamates.[27] Horner−Emmons reaction of the resulting hemiaminal[28] followed by hydrogenation of the intermediate olefin led to **29**, where the glutamate backbone has been elongated with two additional atoms. Other D-glutamates with different C-4 substitution and stereochemistry were prepared from ethyl D-pyroglutamate **4**. Lewis acid-mediated aldol condensation of the lactam enolate of **4** with carbonyl compounds and mesylate elimination led to the olefins **30**−**32**.[29] While direct hydrolysis of **30*E*** exclusively yielded the 4*E*-benzylidenyl D-glutamate **37**,

Scheme 1[a]



[a] Conditions: (i) LHMDS, THF −78 °C and then $ArCH_2Br$. (ii) (a) 2.5 N aqueous LiOH, THF; (b) HCl(g), EtOAc; (c) propylene oxide, MeOH. (iii) 6 N aqueous HCl, reflux. (iv) (a) $LiBEt_3H$, THF −78 °C; (b) PPh_3PCHCO_2Et, dioxane, 110 °C. (v) H_2, PtO_2, EtOAc. (vi) LHMDS, THF, −78 °C and then R-CHO, $BF_3{\cdot}OEt_2$. (vii) MsCl, pyr. (viii) (a) H_2, PtO_2, EtOAc; (b) KCN, DMF; (c) diastereomers separation. (ix) LHMDS, THF, −78 °C and then $Bn_2N^+{=}CH_2I^-$. (x) (a) H_2, Pd/C, MeOH; (b) PhCOCl, Et_3N, DMAP, CH_2Cl_2.

Scheme 2[a]



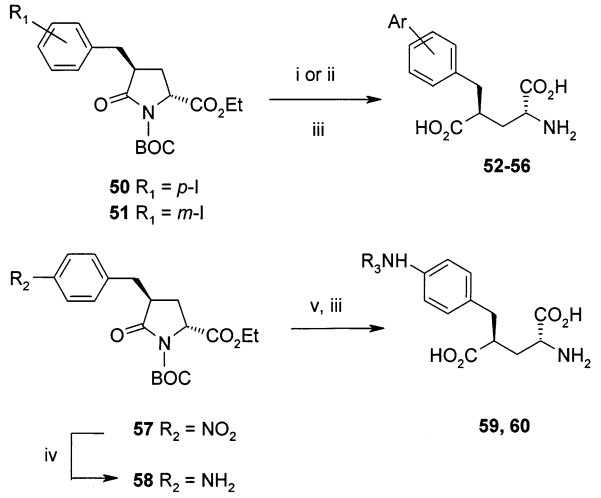
[a] Conditions: (i) LHMDS, THF −78 °C and then (*E*)-ArCH=CHCH$_2$Br. (ii) (a) 2.5 N aqueous LiOH, THF; (b) HCl(g), EtOAc; (c) propylene oxide, MeOH. (iii) H_2, PtO_2, EtOAc. (iv) LHMDS, THF, −78 °C and then propargyl bromide. (v) PhI, $PdCl_2(PPh_3)_2$, CuI, Et_3N.

hydrogenation of pyroglutamates **30**−**32** followed by hydrolysis selectively produced the cis 2*R*,4*R*-D-glutamic acids **33** and **35** along with the cyclopentyl analogue **36**. A previously described protocol utilizing a KCN-mediated equilibration of a mixture of trans/cis diastereomeric pyroglutamates was used as the key step to obtain (2*R*,4*S*)-4-ethyl D-Glu **34**.[29] Finally, highly functionalized side chains were introduced using a modified Eschenmosher's salt[25] as an electrophile followed by functional group transformations and hydrolysis to yield **39**.

Activated halides such as cinnamyl and propargyl bromides were also used as electrophiles in lithiation reactions of **4** leading to C-4-substituted trans pyroglutamates in a nonstereoselective fashion (Scheme 2).[24] Major diastereomers **40** and **46** were isolated from the mixtures, which after the usual hydrolysis led to cinnamyl[30] **41**−**43**- and propargyl[30] **46**-substituted derivatives, respectively. Similar analogues (**44**, **45**, and **49**) were obtained from hydrogenation of the cinnamyl side chain and Pd/CuI-catalyzed coupling reactions, respectively, followed by hydrolysis. To expand the scope of the benzyl substitutions at the C-4 position, Pd-catalyzed couplings were performed on *p*- and *m*-iodo benzyl pyroglutamic esters **50** and **51** (Scheme 3). Thus, a more convergent synthesis of a diverse set of biarylmethyl D-Glu analogues **52**−**56** was obtained. Alternatively, highly functionalized benzyl derivatives were synthesized from the nitro derivative **57** via reduction, amino group functionalization with $PhSO_2Cl$ or phenyl isocyantate, and hydrolysis to the sulfonamide and urea derivatives **59** and **60**.

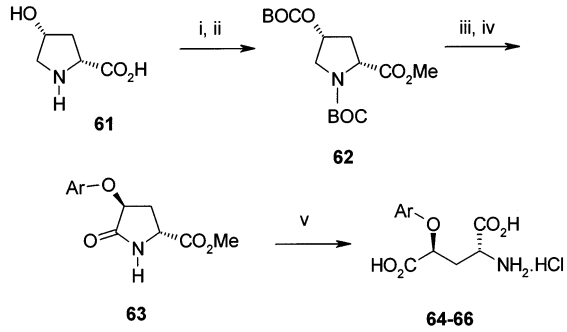
Introduction of a heteroatom into the C-4 side chain of the glutamic acid represents another important class of synthetic targets. Obviously, chemistry to replace the C-4 carbon substitution of benzyl analogues by oxygen required a nucleophilic displacement approach instead of the aforementioned electrophile addition to pyroglutamic ester enolates. Scheme 4 outlines the synthesis of (2*R*,4*S*)-4-aryloxy D-glutamic acids **64**−**66** starting from *cis*-4-hydroxy-D-proline where the key transformation was the Mitsunobu reaction of *cis*-4-hydroxy methyl pyroglutamate with different phenols. This nucleophilic displacement with inversion of the chiral center at C-4 led to the desired anti stereochemistry in **63** and to the final compounds after hydrolysis.

Scheme 3[a]



[a] Conditions: (i) $ArB(OH)_2$, Pd $(PPh_3)_4$, 2 N Na_2CO_3, Tol/EtOH, 20:1, reflux. (ii) $ArSnBu_3$, Pd_2dba_3, PPh_3, DMF, 60 °C. (iii) (a) 2.5 N aqueous LiOH, THF; (b) HCl(g), EtOAc; (c) propylene oxide, MeOH. (iv) $NH_4(CO_2H)$, Pd/C, EtOH, reflux. (v) Phenylsulfonyl chloride or phenylisocyanate, DMAP, Et_3N, CH_2Cl_2.

Scheme 4[a]

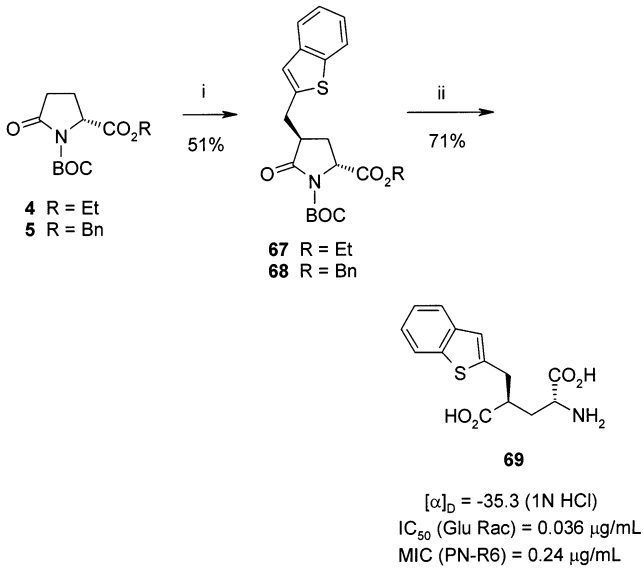


[a] Conditions: (i) HCl(g), MeOH. (ii) BOC_2O, Et_3N, DMAP, THF. (iii) (a) RuO_2, $NaIO_4$, EtOAc; (b) TFA, CH_2Cl_2. (iv) ArOH, DEAD, PPh_3, THF 0 °C. (v) (a) 2.5 N aqueous LiOH, THF; (b) HCl(g), EtOAc.

Finally, different heterocyclic methyl-substituted glutamic acids **69**−**75** were prepared in an analogous manner as described in Scheme 1 for **8**−**28**. Bioisosteric replacements of the aryl rings were efficiently prepared. Typically, the required heteroarylmethyl bromides were prepared in a straightforward synthesis that involved lithiation and formylation with *n*-BuLi/dimethylformamide (DMF), DIBAL-H reduction to the hydroxymethyl derivative, and then PBr_5- or HBr-mediated bromination. As an example, Scheme 5 shows the preparation of one of the most potent optically pure glutamic acids, **69**, that can be obtained in two steps starting from either ethyl or benzyl D-pyroglutamate **4** or **5** in quite good overall yield.

Diastereomeric purity for all of the compounds prepared was determined at the pyroglutamate stage as described for the L series,[24] and the optical rotations for several of the final D-glutamates matched those described for the optically pure L enantiomer.[29,30]

Scheme 5[a]

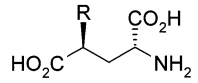


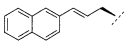
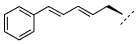
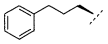
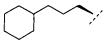
[a] Conditions: (i) LHMDS, THF −78 °C and then 2-(benzo[*b*]-thienyl)methyl bromide. (ii) (a) 2.5 N aqueous LiOH, THF; (b) HCl(g), EtOAc; (c) propylene oxide, MeOH.

Biological Results and Discussion

Lead Discovery. Recombinant glutamate racemase from *S. pneumoniae* expressed in *E. coli* was purified,[31] characterized, and used to test compounds as potential inhibitors. Both D and L series of glutamic acids could be of potential interest to explore the C-4 substitution since both L-Glu and D-Glu are substrates of glutamate racemase catalytic activity in a reversible "double base" racemization mechanism.[11] Different substitution patterns in the glutamic acid structure were initially explored to rapidly identify a potential lead where a more extensive SAR could be studied. Racemic 2-benzyl and 3-benzyl derivatives **2** and **3** were found to be completely inactive in the in vitro glutamate racemase enzyme assay. Interestingly, simple C-4 substitution of D-Glu showed important trends. Both enantiomers of 4-benzyl glutamic acid **8** were prepared. Trans D isomer (−)-**8** (Ar = Ph, Scheme 1) was found to be a fairly potent inhibitor (IC_{50} = 0.3 μg/mL, 1.26 μM) of glutamate racemase enzyme activity as determined by high-performance liquid chromatography (HPLC) separation and quantification of both enantiomers after derivatization with Marfey's reagent (HPLC method). On the other hand, the L isomer (+)-**8** did not show any significant activity. Additionally, (−)-**8** showed important whole cell antibacterial activity (minimum inhibitory concentration, MIC = 0.5 μg/mL) against *S. pneumoniae* PN-R6. Furthermore, it is well-known that several 4-substituted L-glutamic acids are important kainate[30] and metabotropic[32] glutamate receptor ligands. Therefore, SAR work around D-glutamate derivatives could avoid any potential CNS cross activity. With these data in hand, we decided to focus our SAR study on the D-Glu series. In sharp contrast to (−)-**8**, cis 2*R*,4*R* benzyl isomer **33** was shown to be 30 and 125-fold less potent in the enzyme and whole cell assays, respectively. The elongated analogue **29** was completely inactive indicating that the distance between carboxylic acids cannot be altered without losing activity. Simple alkyl substitution at C-4 in **34** (trans) led to a 10-fold decrease in activity as compared to **8** with almost a complete loss of whole cell effect. Once again, cis diastereomer **35** was basically inactive, as well as the branched alkyl (cyclopentyl) **36** (trans) and the *E*-benzylidenyl derivative **37**. These data clearly indicated very specific activity determinants for these derivatives. C-4 anti substitution (2*R*,4*S*) was essential for activity while 2*R*,4*R* diastereomers were much less potent and lacked cell-based activity. Aryl substitution at C-4 seemed to be clearly preferred over alkyl. Finally, elongation, branched alkyl, and exo olefin substitutions were not tolerated. On the basis of these data, expansion of the SAR study was carried out to further explore the C-4 substitution (Table 1).

Table 1. Initial Lead Identification; Differently Substituted (2*R*,4*S*)-D-Glutamic Acids

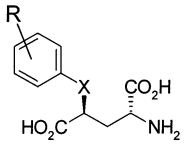


compd	R	Glu Rac IC_{50}[a]	*S. pneum.* MIC[b]
8		0.3	0.5
9		5.6	3.9
10		0.1	0.25
39		9.4	62.5
41		0.1	0.4
42		0.5	0.5
43		9.1	3.9
44		0.5	3.9
45		0.6	2.0
48		5.5	31.3
49		0.37	2.0
64		26.5	62.5

[a] In μg/mL, determined by HPLC method (see Experimental Section). [b] In μg/mL, in vitro whole cell growth inhibition against PN-R6 strain.

Interestingly, large aromatic groups were well-tolerated and retained or even increased activity (compare **8**, **10**, and **42**). However, a linear arrangement of aromatic rings seemed to be crucial for activity as the 1-naphthyl substitution **9** was 50-fold less potent than 2-naphthyl **10** (IC_{50} = 0.1 μg/mL, 0.31 μM). (*E*)-Cinnamyl and arylpropargyl derivatives were also quite potent (**41** and **49**) showing that elongation of the substitution in the right direction determined by C-4 stereochemistry was possible but clearly had a limit (**43**). A saturated alkyl linker between the D-Glu core and the aromatic ring also showed promising activity

Table 2. SAR for C-4 Benzyl-Substituted (2*R*,4*S*)-D-Glutamic Acids



compd	R	X	Glu Rac IC_{50}[a]	*S. pneumoniae* MIC[b]
8	H	C[c]	0.3	0.5
11	2-Cl	C	7.1	3.9
12	2-Br	C	6.3	31.3
13	2-Ph	C	>100	>250
14	3-Cl	C	3.4	1.0
15	3-Br	C	0.13	0.5
16	3-MeO	C	0.2	0.5
17	3-NO_2	C	5.2	2.0
18	3-Ph	C	0.7	0.5
19	3-CF_3	C	0.8	0.5
20	4-Cl	C	0.3	0.5
21	4-NO_2	C	5.2	2.0
22	4-CF_3	C	0.8	7.8
23	4-MeO	C	0.1	0.5
24	4-Ph	C	0.078	1.0
25	4-*t*-Bu	C	0.6	1.0
26	3,5-Cl_2	C	3.0	3.9
27	3,5-$(CF_3)_2$	C	>100	>250
28	3,4,5-$(MeO)_3$	C	81	>250
52	3-(2′-furyl)	C	0.5	0.24
53	3-(2′-benzothienyl)	C	0.3	0.5
54	4-(3′-thienyl)	C	0.32	0.24
55	4-(2′-benzothienyl)	C	0.65	3.9
56	4-(2′-naphthyl)	C	0.42	7.8
59	$PhSO_2NH$	C	10.2	>250
60	PhNHCONH	C	0.49	15.6
65	4-Ph	O	8.2	31.3
66	4-PhO	O	46.3	125

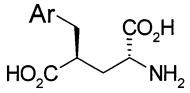
[a] In μg/mL, determined by HPLC method (see Experimental Section). [b] In μg/mL, in vitro whole cell growth inhibition against PN-R6 strain. [c] CH_2.

(**44**). Although a cyclohexyl derivative **45** retained activity, some sort of aromatic or lipophilic substitution in that position was necessary and heteroatoms were not well-tolerated in the linker (compare **48** and **49**, **39** and **44**, and **8** and **64**). Regarding antibacterial activity, linear arylmethyl groups were preferred, and for other kind of linkers, a large decrease in whole cell growth inhibition as compared to **10** was observed if the tether was not conformationally restricted. In most cases, a good correlation between enzyme inhibition and whole cell growth inhibition was observed.

Aryl-Substituted D-Glutamic Acids SAR. The 2-naphthyl analogue **10** was determined to be a potent, competitive inhibitor of glutamate racemase enzyme with a $K_i = 16$ nM. The kinetic characterization was performed using a CD assay,[33] which gave a K_m of 2.9 mM for D-Glu. The K_m for the reverse reaction using L-glutamic acid as the substrate was higher at 18 mM. We decided to further explore the SAR around the lead compounds **8** and **10** by screening differently substituted benzyl compounds (Table 2) and heterocyclic-substituted D-glutamic acids (Table 3), respectively.

A rapid survey of simple substitutions in the phenyl ring of **8** showed that *o*-substituents were quite detrimental for both enzyme and whole cell activity while m- and especially p-substituents were either well-tolerated or could potentially boost both activities (e.g., compare 2-, 3-, and 4-Cl, Br, and Ph derivatives **11**, **14**, and **20** and **13**, **18**, and **24**, respectively). This was in good agreement with previous observations for "nonlinear" side chains in **36** and **9**. Moving a chlorine atom around the aromatic ring led to a large increase in activity (from o to p, approximately 25-fold). Large lipophilic groups such as Br and Ph led to poorly active or inactive compounds at the ortho position, but quite potent compounds at m- and p-positions (see **15** and **24**) were obtained. With respect to possible electronic effects, several electron-withdrawing and electron-donating groups (compare **16** and **23**, **17** and **21**, for example) showed similar activity for the same substitutions and at both m- and p-positions. Polar nitro groups and highly functionalized groups such as sulfonamide **59** and urea **60** were considerably less active, and for these latter ones, the antibacterial activity was completely lost.

Table 3. SAR for C-4 Heteroarylmethyl-Substituted (2*R*,4*S*)-D-Glutamic Acids



compd	Ar	Glu Rac IC_{50}[a]	*S. pneumoniae* MIC[b]
10	2-naphthyl	0.1	0.25
69	2-benzothienyl	0.036	0.24
70	3- benzothienyl	1.7	7.8
71	2-benzofuryl	0.1	0.24
72	2-benzothiazolyl	0.38	0.24
73	2-indolyl	9.8	62.5
74	2-(3-Cl-benzothienyl)	0.01	0.5
75	2-thienyl	0.1	1.0

[a] In μg/mL, determined by HPLC method (see Experimental Section). [b] In μg/mL, in vitro whole cell growth inhibition against PN-R6 strain.

Regarding steric effects, a *t*-Bu group at C-4 (**25**) was well-tolerated. However, 3,5-disubstitutions of any kind (**26**−**28**) were found to be detrimental to enzyme inhibition. Interestingly, substitution with aryl groups retained enzyme activity, especially at the p-position (**52**−**56**). Smaller furyl **52** and thienyl **54** biaryls were found to be the most potent compounds in the whole cell assay in these series. Oxygen-linked biaryl glutamics **65** and **66** show quite weak activity as compared with the corresponding analogue **8**. In sharp contrast with the aryl side chain, changing the electronics in the D-Glu backbone was quite detrimental for glutamate racemase activity.

All of these observations were consistent with the hypothesis of a large and linear lipophilic binding pocket in the catalytic domain of glutamate racemase. The aromatic nonpolar substituents displayed in the right sterochemistry position may fit in this region when monosubstitution is present at the aryl ring at m- or p-positions. If these requirements are met, steric effects are not relevant (**55** and **56**). This very conservative domain seems to favor nonfunctionalized lipophilic side chains. MIC values for some derivatives described herein were lower relative to the corresponding IC_{50} for reasons that were not clear (an additional cytotoxic mode of action could be an explanation). Nevertheless, a consistent trend that allows correlating enzyme and bacterial inhibition was observed.

Heteroaryl-Substituted D-Glutamic Acids SAR. At this point, we moved on to study possible bioisosteric replacements for the aryl moiety with heterocycles and,

Table 4. Antibacterial Activity for Compounds **10** and **69**

	MIC (μg/mL)	
	10	**69**
S. pneumoniae R6	0.25	0.24
S. pneumoniae Park1	3.9	0.24
S. aureus 027	>250	>250
M. catharralis BC-1	62.5	>250

in particular, of the 2-naphthyl lead **10** (Table 3). Bicyclic heterocycles were found to be quite potent and to follow similar trends. 2-Benzothienyl **69** was almost three times more active than the lead compound while 3-benzothienyl **70** was approximately 20-fold less active with a very poor MIC value. This is not surprising since the 3-isomer correlated with the poor activity for the 1-naphthyl isomer **9** (Table 1). Once again, activity seemed to be quite sensitive to this type of ortho arrangement of aromatic rings. 2-Benzofuryl **71** and 2-benzothiazolyl **72** derivatives were less potent than **69**, but in all of these cases, whole cell growth inhibition was retained. More polar examples such as a 2-indolyl group showed a dramatic loss in activities, but the C-3′ benzothienyl chloride **74** was found to be the most potent glutamate racemase inhibitor described herein (IC_{50} = 0.01 μg/mL, 0.03 μM).

Obviously, *S. pneumoniae* growth inhibition also depended on the bacterial cell envelope permeability of the glutamate racemase inhibitors. Because of the polar nature of these series of amino acids, additional lipophilic and aryl groups could, in principle, afford better penetration to the bacterial cytoplasm. This effect could account for the different whole cell activity observed for some equally potent enzyme inhibitors.

Activity profiles against different bacteria for compounds **10** and **69** are presented in Table 4. Unfortunately, a lack of broad spectrum activity was observed in these series of D-glutamate derivatives. Although **69** showed good similar activity against the Park1 strain, both lead compounds were completely inactive against *Staphylococcus aureus*, *Moraxella catarrhalis*, and other pathogens. Low homology in the glutamate racemase active site with respect to *S. pneumoniae*, different bacterial permeability, and/or an alternative supply of D-Glu using the D-AAT pathway could account for this lack of whole cell activity.

In Vivo Efficacy Model. Compounds **69**, **52**, and **74** were selected for an in vivo study in a murine thigh infection model (Figure 3). The 0 h infection level serves as a reference point for quantitating the potential effect of test compounds on *S. pneumoniae* Park1 bacterial growth. Mice receiving no drug served as an infection control. A 2 log increase (from 0 h) in bacterial growth was observed at 7 h postinfection. Animals treated with one of the three test compounds (40 mg/kg × 2, ip) showed little if any increase in bacterial growth at 7 h postinfection. Treatment of compound **69** or **52** resulted in a lack of bacterial growth from 0 h infection control levels (bacterial stasis). Although all three compounds showed similar activity, **69** and **52** showed the most consistent effect on suppressing bacterial growth in this model. Vancomycin (10 mg/kg), as a positive control drug, resulted in a −1.5 log reduction in bacterial growth from the 0 h infection level. Overall, these compounds showed a clear reduction in infection as compared to the 7 h negative control.

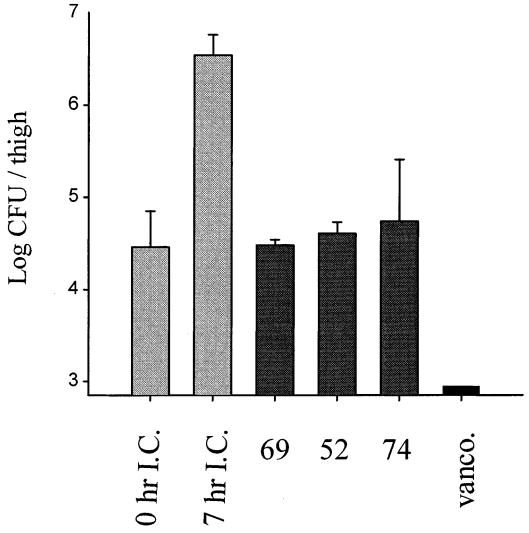


Figure 3. *S. pneumoniae* Park1 efficacy study in mouse thigh model. Mice were dosed ip with 40 mg/kg test compounds **69**, **72**, and **74** or 10 mg/kg vancomycin at 1 and 5 h postinfection. CFU were enumerated and expressed as log10 CFU per thigh. See Experimental Section.

Conclusions

The compounds described here represent a novel class of potent, competitive glutamate racemase inhibitors that for the first time, showed in vitro antibacterial activity. This SAR study has led to compounds with IC_{50} values as low as 10 ng/mL and good correlation with antibacterial activity. Following an exploration of the D-Glu core and lead identification, clear SAR trends have been described. Although restricted to *S. pneumoniae* strains due to lack of broad spectrum activity, this class of compounds shows inhibition of bacterial infection in a murine in vivo model. This new class of D-glutamates indicates that glutamate racemase (MurI) may be a viable target for developing new antibacterial agents. Work is in progress to use this class of compounds for better exploring and understanding this interesting bacterial cell wall biosynthetic enzyme.

Experimental Section

General Methods. All solvents and reagents were purchased from commercial sources, unless otherwise indicated. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl prior to use. All reactions were performed under positive pressure of nitrogen or argon. ^{1}H NMR and ^{13}C NMR data were recorded on a Bruker AC-200P or Bruker AC-300. Melting points were determined on a Buchi apparatus and are not corrected. Optical rotations were measured in a Perkin-Elmer 241 polarimeter. Analytical thin-layer chromatography (TLC) was performed on Merck TLC glass plates precoated with F254 silica gel 60 (UV, 254 nm and iodine). Chromatographic separations were performed by using 230−400 mesh silica gel (Merck). Elemental analyses were performed by the Universidad Complutense de Madrid. Starting N-BOC pyroglutamic acid esters were prepared as previously described.[34] Noncommercially available arylmethyl bromides were prepared via radical bromination (procedure A) or using a formylation/reduction/acidic bromination sequence (procedure B). 2-Bromomethyl benzothiazole[35] and *N*,*N*-dibenzyl-*N*-methylene ammonium iodide[36] were prepared as previously described.

General Procedures for the Preparation of Alkyl Bromides. Procedure A. To a solution of the starting material (8 mmol) in 25 mL of anhydrous CCl_4 under nitrogen, *N*-bromosuccinimide (NBS, 8 mmol, 1.0 equiv) and a catalytic amount of AIBN (5 mol %) were added and the mixture was refluxed for 4 h or until TLC showed complete conversion. The reaction mixture was cooled to 0 °C, filtered through a pad of Celite, and concentrated in vacuo to afford a crude oil, which was used as it was or recrystallyzed from mixtures of CH_2Cl_2/Hex at −10 °C when possible. For example, using this procedure, 2-thienylmethyl bromide (60%) and 3-benzo[*b*]thienylmethyl bromide (51%) were prepared.

Procedure B. To a solution of the heterocycle (20 mmol) in 120 mL of anhydrous THF under nitrogen and cooled to −78 °C, *n*-BuLi (1.1 equiv) was added dropwise, and the mixture was stirred for 1 h at −78 °C. Then, anhydrous DMF (3.0 equiv) was added, and the solution was stirred for 2 h while warming the reaction mixture to −10 °C. The reaction was hydrolyzed with saturated NH_4Cl, diluted with water, and extracted with Et_2O, and the organic layer was washed with water and brine. Drying over $MgSO_4$ and concentration in vacuo afforded a crude aryl-aldehyde that was used as it was. To a solution of the aldehyde (20 mmol) in 100 mL of anhydrous THF under N_2 and cooled to −78 °C, DIBAL-H (1 M solution in Hex, 1.05 equiv) was added dropwise, and the mixture was stirred for 1 h at −78 °C and then hydrolyzed with saturated NH_4Cl and diluted with Et_2O and 1 N HCl. The organic layer was washed with 1 N HCl, the aqueous phase was extracted with Et_2O, and the combined organic layers were washed with water and brine. Drying over $MgSO_4$ and concentration in vacuo afforded a crude aryl-methyl alcohol, which was used as it was or recrystallyzed from mixtures CH_2Cl_2/Hex at −10 °C when possible. To a solution of the starting alcohol (6.6 mmol) in 20 mL of anhydrous CH_2Cl_2 were added HBr (48% solution in water, 2.0 equiv) and a catalytic amount of concentrated H_2SO_4 (20 mol %). The mixture was stirred for 1.5 h or until TLC showed complete conversion and then neutralized by pouring into an iced-cooled solution of saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. Drying over Na_2SO_4 and concentration in vacuo afforded a crude arylmethylbromide that was used as it was; overall yields, 31−56%; three steps. For example, using this procedure, 2-benzo[*b*]thienylmethyl bromide, 2-benzo[*b*]furylmethyl bromide, and 2-(*N*-benzenesulfonyl)indolyl methyl bromide were prepared.

General Procedure for Alkylation Reactions of Pyroglutamate Esters. Procedure C. To a solution of (D)-*N*-BOC-pyroglutamate ester **4** or **5** (7.77 mmol) in anhydrous THF (40 mL) under nitrogen was added a 1 M solution of lithium hexamethyldisilazide (LHMDS) in anhydrous THF (8.55 mL) at −78 °C. After 1 h, a solution of the arylmethyl or cinnamyl bromide, 9.3 mmol, in anhydrous THF (20 mL) was added, and stirring was continued at −78 °C for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) at −78 °C and extracted with Et_2O (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to dryness obtaining a crude product, which was purified by column chromatography (mixtures Hex/EtOAc) to yield the pure trans isomer (2*R*,4*S*).

General Procedures for the Synthesis of Biarylmethyl Pyroglutamate Esters. Procedure D. The starting ethyl (2*R*,4*S*)-1-(*tert*-butoxycarbonyl)-*m*- or *p*-iodo-benzyl pyroglutamate **50** or **51** (1.5 mmol, prepared as described in procedure C) was dissolved in 30 mL of a mixture toluene/EtOH 20:1, and the solution was degassed for 15 min with N_2. Then, arylboronic acid (2.0 equiv) was added followed by 3 mL of 2 N aqueous solution of Na_2CO_3 and 10 mol % of $Pd(Ph_3P)_4$. The slurry mixture was vigorously stirred and heated at reflux for 4 h or until TLC of the mixture showed complete conversion of the starting pyroglutamate. The reaction mixture was then cooled to room temperature and diluted with water (30 mL) and Et_2O (40 mL), and the aqueous phase was extracted with Et_2O (4 × 10 mL). The combined organic layers were washed with water (15 mL) and brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. Silica gel chromatography using the appropriate eluent afforded pure biaryl coupling product.

Procedure E. To a solution of **51** (1.0 mmol) and aryl-tri-*n*-butylstannane (1.0 mmol) in 7 mL of anhydrous DMF under nitrogen, Ph_3P (8 mol %) was added followed by Pd_2dba_3 (2 mol %). The resulting solution was heated at 60 °C for 1.5 h and then cooled to room temperature and diluted with Et_2O and water. The aqueous layer was extracted with Et_2O, and the organic phase was washed with water, a saturated aqueous solution of KF, water, and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo to afford a crude product, which was purified by silica gel chromatography (in gradient, mixtures Hex/EtOAc) to afford pure biaryl coupling product.

General Procedure for the Hydrolysis of 4-Substituted Pyroglutamate Esters. Procedure F. To a solution of the corresponding 4-substituted pyroglutamate ester (2 mmol) in THF (15 mL) was added a 2.5 N aqueous solution of LiOH (14.4 mL, 36 mmol). The mixture was stirred at room temperature for 2 h and then acidified to pH 2 with 1 N HCl solution and thoroughly extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was diluted and stirred with 15 mL of a saturated dry HCl solution in ethyl acetate for 1 h at room temperature. The resulting white solid was filtered off, triturated with ethyl ether, and dried under high vacuum to afford the hydrochloride salt. Optionally, the compound was dissolved in the minimum amount of MeOH (approximately 5 mL) and treated with an excess of propylene oxide at room temperature until the zwitterion salt precipitated (1−16 h). The solids were filtered off and dried under high vacuum. In some cases, final compounds were directly obtained by refluxing a suspension of the starting material (1 mmol) in 6 N aqueous HCl (5 mL) for 16 h. The resulting solution was evaporated to dryness yielding a white solid, which was triturated with ethyl ether, filtered off, and dried under high vacuum.

(2*R*,4*S*)-2-Amino-4-benzyl Pentanedioic Acid, Hydrochloride ((−)-8). Alkylation of ethyl *N*-BOC-D-pyroglutamate **4** with benzyl bromide (procedure C) afforded ethyl (2*R*,4*S*)-*N*-BOC-4-benzyl pyroglutamate **6** (Ar = Ph, 60%) as previously described for the L enantiomer.[24] $[\alpha]_D = +37.7°$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): 7.40−7.10 (m, 5H), 4.43 (dd, *J* = 7.6, 3.7 Hz, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 3.25 (dd, *J* = 13.7, 4.0 Hz, 1H), 3.00−2.82 (m, 1H), 2.63 (dd, *J* = 13.7, 9.5 Hz, 1H), 2.05−1.95 (m, 2H), 1.47 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 3H). NMR and analytical data matched that described for the L form.[24] Hydrolysis (procedure F) afforded (−)-**8**. Both hydrochloride and zwitterion salt forms were obtained. Hydrochloride salt: 90%. Hygroscopic solid. $[\alpha]_D = -12.7°$ (*c* 1.0, MeOH). ^1H NMR (methanol-d_4/D_2O): 7.42−7.24 (m, 5H), 3.99 (dd, *J* = 7.5, 6.6 Hz, 1H), 3.03−2.92 (m, 3H), 2.37−2.23 (m, 1H), 2.05−1.94 (m, 1H). ^{13}C NMR (methanol-d_4/D_2O): 178.9, 172.7, 138.9, 130.1, 130.1, 129.7, 129.7, 127.9, 52.7, 44.6, 39.0, 32.3. Zwitterion, (−)-**8**: 70%; mp 184−185 °C. $[\alpha]_D = -35.5°$ (*c* 0.45, DMSO). ^1H NMR (D_2O/KOD): 7.18−7.09 (m, 5H), 3.03 (m, 1H), 2.63 (m, 2H), 2.46 (m, 1H), 1.77 (m, 1H), 1.39 (m, 1H). ^{13}C NMR (D_2O/KOD): 182.6, 181.8, 139.6, 127.9, 127.9, 127.4, 127.4, 125.1, 53.7, 47.2, 37.9, 37.5. Anal. ($C_{12}H_{15}NO_4$) C, H, N.

(2*S*,4*R*)-2-Amino-4-benzyl Pentanedioic Acid ((+)-8). This compound was prepared from ethyl *N*-BOC-L-pyroglutamate[32] as described above. Zwitterion. $[\alpha]_D = +33.8°$ (*c* 0.45, DMSO). Anal. ($C_{12}H_{15}NO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(1-naphthyl)methyl Pentanedioic Acid (9). Alkylation of ethyl *N*-BOC-D-pyroglutamate **4** with 1-naphthylmehthyl bromide (procedure C) afforded ethyl (2*R*,4*S*)-*N*-BOC-4-(1-naphthyl)methyl D-pyroglutamate **6** (Ar = 1-naphthyl, 47%). Hydrolysis (procedure F, 65%) afforded **9**; mp 144−146 °C. ^1H NMR (methanol-d_4/KOD): 8.19−8.07 (m, 1H), 7.89−7.55 (m, 2H), 7.53−7.31 (m, 4H), 3.85 (m, 1H), 3.49−3.05 (m, 2H), 2.95−2.72 (m, 1H), 2.23−2.05 (m, 1H), 1.52 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 184.0, 182.8, 136.0,

135.3, 133.5, 129.6, 128.1, 127.7, 126.8, 126.4, 125.2, 56.2, 40.8, 38.0. Anal. ($C_{16}H_{17}NO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(2-naphthyl)methyl Pentanedioic Acid, Hydrochloride (10). Alkylation of ethyl *N*-BOC-D-pyroglutamate **4** with 2-naphthylmehthyl bromide (procedure C) afforded ethyl (2*R*,4*S*)-*N*-BOC-4-(2-naphthyl)methyl D-pyroglutamate **6** (Ar = 2-naphthyl, 67%), as previously described for the L enantiomer.[24] NMR and analytical data matched that described for the L form.[24] Hydrolysis (procedure F, 75%) afforded **10**; mp 144 °C. $[\alpha]_D$= −21.4° (*c* 1.0, MeOH). ^{1}H NMR (methanol-d_4): 7.83−7.77 (m, 3H), 7.69 (s, 1H), 7.49−7.36 (m, 3H), 3.99 (dd, *J* = 8.5, 5.5 Hz, 1H), 3.26−3.19 (m, 1H), 3.09−2.97 (m, 2H), 2.43−2.28 (m, 1H), 2.00−1.86 (m, 1H). ^{13}C NMR (methanol-d_4): 177.3, 171.4, 137.8, 134.8, 133.7, 129.2, 128.7, 128.5 128.3, 127.0 126.6, 52.5, 44.4, 39.3, 32.8. Anal. ($C_{16}H_{18}ClNO_4$·1/2H_2O) C, H, N.

Compounds **11**−**28** were prepared in an analogous manner (yields for the last step).

(2*R*,4*S*)-2-Amino-4-(2-chloro)benzyl Pentanedioic Acid (11). 67%; mp 153 °C. ^{1}H NMR (methanol-d_4/KOD): 7.48−7.09 (m, 4H), 3.21 (dd, *J* = 10.1, 3.1 Hz, 1H), 3.11−2.62 (m, 3H), 2.18−2.01 (m, 1H), 1.55−1.48 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 177.1, 171.4, 137.0, 135.0, 132.5, 130.5, 129.3, 128.1, 52.5, 42.7, 36.8, 32.8. Anal. ($C_{12}H_{14}ClNO_4$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(2-bromo)benzyl Pentanedioic Acid (12). 48%. mp 163−164 °C. $[\alpha]_D$ = −9.2 (c 0.2, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.49 (d, *J* = 7.8 Hz, 1H), 7.35 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.05 (m, 1H), 3.21 (dd, *J* = 10.1, 2.9 Hz, 1H), 3.05 (m, 1H), 2.82−2.68 (m, 2H), 2.10 (m, 1H), 1.45 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 183.3, 182.8, 141.3, 133.6, 132.4 128.9, 128.5, 125.7, 56.3, 47.8, 41.0, 40.7. Anal. ($C_{12}H_{14}BrNO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(2-phenyl)benzyl Pentanedioic Acid (13). 78%; mp 144−145 °C. $[\alpha]_D$ = −33.0° (*c* 0.5, 2 N NaOH). ^{1}H NMR (methanol-d_4/KOD): 7.49−6.98 (m, 9H), 3.12−2.85 (m, 2H), 2.70−2.48 (m, 2H), 1.90−1.75 (m, 1H), 1.28−1.05 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 177.5, 175.4, 143.7, 142.5, 136.7 131.2, 130.6 130.3, 129.3, 128.6 128.0 127.6, 43.8, 36.1, 32.7, 20.9. Anal. ($C_{18}H_{19}NO_4$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-chloro)benzyl Pentanedioic Acid (14). 55%; $[\alpha]_D$ = −3.2° (*c* 0.31, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.30−7.01 (m, 4H), 3.19 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.00−2.85 (m, 1H), 2.71−2.50 (m, 2H), 2.05 (m, 1H), 1.45 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 177.0, 171.0, 141.8, 135.1, 130.9, 130.0, 128.4, 127.6, 51.6, 51.8, 44.3, 38.5, 32.9. Anal. ($C_{12}H_{14}ClNO_4$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-bromo)benzyl Pentanedioic Acid (15). 95%; mp 174−175 °C. $[\alpha]_D$ = −41.4° (*c* 0.5, 1 N HCl). ^{1}H NMR (D_2O/KOD): δ 7.41 (s, 1H), 7.30−7.13 (m, 3H), 3.23−3.18 (m, 1H), 2.95−2.87 (m, 1H), 2.66−2.61 (m, 2H), 2.01−1.96 (m, 1H), 1.51−1.46 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 183.4, 182.9, 144.9, 133.0, 131.0 129.9, 129.1, 123.1, 56.2, 49.5, 40.6. Anal. ($C_{12}H_{14}BrNO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-methoxy)benzyl Pentanedioic Acid (16). 78%; mp 148 °C. $[\alpha]_D$ = −37.2° (*c* 0.5, 1 N HCl). ^{1}H NMR (methanol-d_4/D_2O): 7.29 (t, *J* = 8.9 Hz, 1H), 7.11−6.91 (m, 3H), 3.81 (s, 3H), 3.73 (dd, *J* = 8.4, 5.4 Hz, 1H), 3.29−2.31 (m, 3H), 2.31−2.24 (m, 1H), 2.21−1.94 (m, 1H). ^{13}C NMR (methanol-d_4/D2O): 187.6, 187.0, 162.8, 146.7, 133.8, 126.0, 118.6, 116.0, 59.7, 58.9, 52.2, 43.1, 42.8. Anal. ($C_{13}H_{17}NO_5$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-nitro)benzyl Pentanedioic Acid (17). 50%; mp 169 °C. $[\alpha]_D$ = −10.3° (*c* 0.35, DMSO). ^{1}H NMR (methanol-d_4/KOD): 8.12 (s, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 3.22 (m, 1H), 3.01 (m, 1H), 2.90−2.59 (m, 2H), 2.09 (m, 1H), 1.51 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 183.0, 182.9, 149.4, 144.7, 137.0, 130.4, 124.8, 122.0, 56.3, 49.4, 40.7, 40.6. Anal. ($C_{12}H_{14}N_2O_6$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-phenyl)benzyl Pentanedioic Acid (18). 85%; mp 149−151 °C. $[\alpha]_D$ = −7.7° (*c* 0.4, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.61−7.12 (m, 9H), 3.29−2.90 (m, 2H), 2.79−2.55 (m, 2H), 2.05 (m, 1H), 1.50 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 184.0, 183.2, 142.7, 142.5, 142.2, 129.8, 129.2, 128.2, 128.1, 128.0, 125.6, 56.4, 41.2, 40.6. Anal. ($C_{18}H_{19}NO_4$·2.5H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-trifluoromethyl)benzyl Pentanedioic Acid (19). 50%; mp 153 °C. $[\alpha]_D$ = −15.4° (*c* 1.0, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.55 (m, 2H), 7.38 (m, 2H), 3.23 (dd, *J* = 9.8, 3.8 Hz, 1H), 3.10−2.89 (m, 1H), 2.82−2.59 (m, 2H), 2.05 (m, 1H), 1.48 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 183.2, 182.7, 142.6, 134.0, 131.0, (q, *J* = 32 Hz, 1C), 129.9, 126.7, 125.1 (q, *J* = 269 Hz, 1C), 126.6, 56.2, 40.7. Anal. ($C_{13}H_{14}F_3NO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-chloro)benzyl Pentanedioic Acid (20). 60%; mp 128−130 °C. $[\alpha]_D$ = −33.4° (*c* 0.5, 1 N HCl). ^{1}H NMR (methanol-d_4): 7.36−7.12 (m, 4H), 3.95 (dd, *J* = 8.7, 5.8 Hz, 1H), 3.12−2.70 (m, 3H), 2.40−2.12 (m, 1H), 1.98−1.75 (m, 1H). ^{13}C NMR (methanol-d_4): 176.9, 171.5, 138.5, 133.6, 131.6, 129.6, 52.0, 44.4, 38.4, 32.9. Anal. ($C_{12}H_{15}ClNO_4$·2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-nitro)benzyl Pentanedioic Acid, Hydrochloride (21). 45%; mp 159−161 °C. $[\alpha]_D$ = −32.4° (*c* 0.6, DMSO). ^{1}H NMR (methanol-d_4): 8.17 and 7.48 (AA′BB′ system, 4H), 4.03 (dd, *J* = 8.1, 5.9 Hz, 1H), 3.22−3.01 (m, 3H), 2.39−2.27 (m, 1H), 2.00−1.92 (m, 1H). ^{13}C NMR (methanol-d_4): 176.5, 171.3, 148.3, 147.8, 131.3, 124.5, 52.8, 44.1, 38.6, 33.1. Anal. ($C_{12}H_{15}ClN_2O_6$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-trifluoromethyl)benzyl Pentanedioic Acid (22). 90%; mp 109 °C. $[\alpha]_D$ = −13.2° (*c* 1.0, MeOH). ^{1}H NMR (methanol-d_4): 7.58 and 7.43 (AA′BB′ system, 4H), 3.97 (s, 1H), 3.21−3.01 (m, 3H), 2.40−2.31 (m, 1H), 1.94−1.80 (m, 1H). ^{13}C NMR (methanol-d_4): 176.9, 171.8, 144.4, 130.9, 129.9 (q, *J* = 32.0 Hz, 1C), 126.3 (q, *J* = 3.8 Hz, 2C), 125.7 (q, *J* = 270.2 Hz, 1C), 52.8, 44.3, 38.8, 32.2. Anal. ($C_{13}H_{14}F_3NO_4$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-methoxy)benzyl Pentanedioic Acid (23). 75%; mp 166−167 °C. $[\alpha]_D$ = −4.9° (*c* 0.39, DMSO). ^{1}H NMR (D_2O/KOD): 7.11 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 3.71 (s, 3H), 3.10 (dd, *J* = 9.5, 4.3 Hz, 1H), 2.71−2.54 (m, 2H), 2.53−2.45 (m, 1H), 1.89−1.79 (m, 1H), 1.48−1.39 (m, 1H). ^{13}C NMR (D_2O/KOD): 182.7, 181.8, 156.1, 132.2, 129.1, 129.1, 112.9, 112.9, 54.4, 53.7, 47.4, 37.4, 37.1. Anal. ($C_{13}H_{17}NO_5$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-phenyl)benzyl Pentanedioic Acid (24). 55%; mp 162−163 °C. ^{1}H NMR (methanol-d_4/KOD): 7.58−7.12 (m, 9H), 3.29−3.12 (m, 1H), 3.11−2.88 (m, 1H), 2.79−2.55 (m, 2H), 2.05 (m, 1H), 1.47 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 184.0, 183.3, 142.2, 143.3, 139.9, 132.1, 130.7, 129.8, 128.1, 127.7, 56.4, 40.7, 40.5. Anal. ($C_{18}H_{19}NO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(4-*tert*-butyl)benzyl Pentanedioic Acid (25). 57%; mp 150−152 °C. ^{1}H NMR (methanol-d_4/KOD): 7.21 and 7.16 (AA′BB′, 4H), 3.21 (dd, *J* = 9.8, 2.8 Hz, 1H), 2.90−2.87 (m, 1H), 2.62−2.54 (m, 2H), 2.08−2.02 (m, 1H), 1.53−1.40 (m, 1H), 1.24 (s, 9H). ^{13}C NMR (methanol-d_4/KOD): 184.1, 182.9, 149.5, 139.1, 129.8, 126.0, 56.3, 49.2, 40.5, 35.1, 31.9, 31.8. Anal. ($C_{16}H_{23}NO_4$·2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3,5-dichloro)benzyl Pentanedioic Acid (26). 80%; mp 144 °C. $[\alpha]_D$ = −27.4° (*c* 0.84, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.30−7.10 (m, 3H), 3.24 (m, 1H), 2.98−2.50 (m, 3H), 2.04 (m, 1H), 1.52 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 182.8, 146.6, 135.6, 128.9, 126.8, 56.2, 40.8, 40.2. Anal. ($C_{12}H_{13}Cl_2NO_4$·H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3,5-ditrifluoromethyl)benzyl Pentanedioic Acid (27). 65%; mp 152−153 °C. $[\alpha]_D$ = −11.9° (*c* 0.63, DMSO). ^{1}H NMR (methanol-d_4/KOD): 7.82 (s, 2H), 7.58 (s, 1H), 3.21 (dd, *J* = 8.5, 5.3 Hz, 1H), 3.20−2.95 (m, 1H), 2.94−2.52 (m, 2H), 2.05 (m, 1H), 1.50 (m, 1H). ^{13}C NMR (methanol-d_4/KOD): 183.0, 182.7, 145.7, 132.4 (q, *J* = 32.7 Hz), 130.7, 124.9 (q, *J* = 270 Hz), 120.5, 56.2, 49.2, 40.8, 40.4. Anal. ($C_{14}H_{13}F_6NO_4$·1/2H_2O) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3,4,5-trimethoxy)benzyl Pentanedioic Acid (28). 25%; mp 188−189 °C. $[\alpha]_D$ = −4.3° (*c* 0.4, DMSO). ^{1}H NMR (D_2O/KOD): 6.50 (brs, 2H), 3.71 (brs, 6H), 3.61 (s, 3H), 3.05 (m, 1H), 2.59 (m, 2H), 2.49 (m, 1H), 1.80 (m, 1H),

1.39 (m, 1H). ^{13}C NMR (D_2O/KOD): 183.3, 182.7, 151.9, 137.4, 106.2, 60.7, 55.9, 54.5, 48.0, 39.0, 38.4. Anal. ($C_{15}H_{21}NO_7 \cdot 2H_2O$) C, H, N.

(2*R*,4*S*,*E*)-2-Amino-4-(3-phenylprop-2-enyl) Pentanedioic Acid (41). Alkylation of **4** using cinnamyl bromide (procedure C) afforded ethyl (2*R*,4*S*,*E*)-*N*-BOC-4-cinnamyl D-pyroglutamate **40** (Ar = Ph, 52%) as described for the L isomer.[30] Hydrolysis (procedure F, 50%) afforded **41**. $[\alpha]_D = -23.7°$ (*c* 0.3, 1 N NaOH). Analytical and NMR data matched that described for the L enantiomer.[30]

(2*R*,4*S*,*E*)-2-Amino-4-[3-(2-naphthyl)prop-2-enyl]pentanedioic Acid (42). Alkylation of **4** using (2-naphthyl)-2*E*-propenyl bromide (procedure C) and hydrolysis (procedure F, 35%), afforded **42**. $[\alpha]_D = -4.8°$ (*c* 0.41, DMSO). Analytical and NMR data matched that described for the L enantiomer.[30]

(2*R*,4*S*)-2-Amino-4-(5-phenyl-2*E*,4*E*-pentadienyl)pentanedioic Acid (43). Alkylation of **4** using 5-phenyl-2*E*,4*E*-pentadienyl bromide (procedure C) and hydrolysis (procedure F, 25%) afforded **43**; mp 171−172 °C. $[\alpha]_D = -58.0°$ (*c* 1, 1 N HCl). ^{1}H NMR (D_2O/pyr-d_6): 7.30−6.90 (m, 5H), 6.46 (dd, *J* = 15.3, 10.3 Hz, 1H), 6.20−5.95 (m, 2H), 5.78 (m, 1H), 4.00 (m, 1H), 2.80−1.90 (m, 5H). ^{13}C NMR (D_2O/pyr-d_6): 184.2, 176.7, 139.2, 135.2, 134.4, 132.4, 131.4, 130.8, 129.5, 128.2, 55.9, 47.4, 38.7, 35.3. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(3-phenyl)propyl Pentanedioic Acid (44). Hydrogenation of **40** (Ar = Ph) with PtO_2 in EtOAc followed by hydrolysis (procedure F, 65%) afforded **44**, as previously described for the L enantiomer.[30] $[\alpha]_D = -20.1°$ (*c* 0.5, MeOH). Analytical and NMR data matched that described for the L enantiomer.

(2*R*,4*S*)-2-Amino-4-(3-cyclohexyl)propyl Pentanedioic Acid (45). Hydrogenation of **40** (Ar = Ph) with catalytic PtO_2 in EtOAc, for 3 days, followed by hydrolysis (procedure F, 75%) afforded **45**; mp 165−166 °C. ^{1}H NMR (methanol-d_4): 3.19 (dd, *J* = 9.5, 3.6 Hz, 1H), 2.34−2.28 (m, 1H), 1.72−1.05 (m, 15H), 0.98−0.75 (m, 4H). ^{13}C NMR (methanol-d_4): 184.9, 182.8, 58.3, 56.5, 41.2, 39.0, 35.6, 34.6, 27.8, 27.5, 26.4, 18.4. Anal. ($C_{14}H_{25}NO_4 \cdot 1/2H_2O$) C, H, N.

(2*R*,4*S*)-2-Amino-4-(prop-2-ynyl)pentanedioic Acid (48). Alkylation of **4** using propargyl bromide (procedure C) afforded ethyl (2*R*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(prop-2-ynyl) d-pyroglutamate **46** as previously described for the L enantiomer.[30] Hydrolysis (procedure F, 30%) afforded **48**. $[\alpha]_D = -15.8°$ (*c* 0.5, 1 N HCl). Anal. ($C_8H_{11}NO_4 \cdot 0.25H_2O$) C, H, N. NMR data matched that described for the L enantiomer.[30]

(2*R*,4*S*)-2-Amino-4-(3-phenyl)propargyl Pentanedioic Acid (49). Ethyl (2*R*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(prop-2-ynyl)pyroglutamate **46** (2.11 mmol) was dissolved in triethylamine (18 mL), and to this solution were added iodobenzene (2.81 mmol), $PdCl_2(PPh_3)_2$ (0.12 mmol), and CuI (0.06 mmol). The reaction mixture was stirred overnight under air atmosphere at room temperature. The yellow slurry that was formed was concentrated to dryness under reduced pressure, and the residue was purified by chromatography (Hex/EtOAc 4:1) to afford **47**, 71%. Hydrolysis (procedure F, 43%) afforded **49**; mp 157 °C. $[\alpha]_D^{20} = -39.0°$ (*c* 0.2, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.24−7.02 (m, 5H), 3.04−2.92 (m, 1H), 2.48−2.30 (m, 3H), 1.81−1.62 (m, 1H), 1.53−1.37 (m, 1H). ^{13}C NMR (D_2O/pyridine-d_5): 180.7, 173.8, 131.0, 127.9, 127.7, 122.2, 87.4, 81.6, 52.7, 43.4, 31.8, 22.3. Anal. ($C_{14}H_{15}NO_4 \cdot 0.35H_2O$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[3-(2-furyl)]benzyl Pentanedioic Acid (52). Alkylation of **4** with *m*-iodobenzyl bromide (procedure C) afforded ethyl (2*R*,4*R*)-*N*-BOC-4-(3-iodo)benzyl d-pyroglutamate **51**; mp 91−93 °C. $[\alpha]_D = +18.5°$ (*c* 0.74, $CHCl_3$). From **51**, Pd-catalyzed coupling using 2-furyl-tri-*n*-butyl stannane (procedure E) afforded the corresponding biaryl pyroglutamate. Hydrolysis (procedure F, 31%) afforded **52**; mp 134−135 °C. $[\alpha]_D = -38.7°$ (*c* 0.25, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.30 (m, 3H), 7.11 (brt, *J* = 7.5 Hz, 1H), 6.92 (brd, *J* = 6.8 Hz, 1H), 6.56 (brd, *J* = 3.3 Hz, 1H), 6.31 (dd, *J* = 3.3 and 1.6 Hz, 1H), 2.95 (brdd, *J* = 8.6 and 3.7 Hz, 1H), 2.59−1.74 (m, 3H), 1.72 (m, 1H), 1.30 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 184.5, 183.7, 154.6, 143.6, 142.2, 131.5, 130.0, 129.2, 125.1, 122.4, 112.9, 106.4, 55.8, 49.2, 40.0, 39.6. Anal. ($C_{16}H_{17}NO_5 \cdot H_2O$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[3-(2-benzo[*b*]thienyl)]benzyl Pentanedioic Acid (53). From **51**, Pd-catalyzed coupling using 2-benzothienyl boronic acid (procedure D) afforded the corresponding biaryl pyroglutamate. Hydrolysis (procedure F, 81%) afforded **53**; mp 222−223 °C. $[\alpha]_D = -10.6°$ (*c* 0.23, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.04−6.63 (m, 9H), 2.92 (m, 1H), 2.59−2.25 (m, 3H), 1.74 (m, 1H), 1.20 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 184.1, 183.5, 144.6, 142.2, 141.4, 139.9, 134.6, 134.6, 129.8, 127.4, 125.3, 125.2, 124.8, 124.5, 123.0, 120.4, 55.8, 49.1, 40.2, 39.9. Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[4-(3-thienyl)]benzyl Pentanedioic Acid, Hydrochloride (54). Alkylation of **4** with *p*-iodobenzyl bromide (procedure C) afforded ethyl (2*R*,4*R*)-*N*-BOC-4-(4-iodo)benzyl d-pyroglutamate **50**; mp 130−132 °C. $[\alpha]_D = +24.2°$ (*c* 1.72, $CHCl_3$). From **50**, Pd-catalyzed coupling using 3-thienyl boronic acid (procedure D) afforded the corresponding biaryl pyroglutamate. Hydrolysis (procedure F, 98%) afforded **54**; mp 186−187 °C. $[\alpha]_D = -32.5°$ (*c* 0.48, DMSO). ^{1}H NMR (methanol-d_4): 7.59−7.55 (m, 3H), 7.49−7.40 (m, 2H), 7.26 (brd, *J* = 8.2 Hz, 2H), 4.01 (dd, *J* = 7.8, 6.0 Hz, 1H), 2.97 (m, 3H), 2.30 (m, 1H), 2.02 (m, 1H). ^{13}C NMR (methanol-d_4): 178.0, 171.9, 142.8, 138.0, 137.8, 137.8, 135.4, 130.6, 127.6, 127.3, 126.9, 121.1, 52.4, 44.4, 38.6, 32.7. Anal. ($C_{16}H_{18}ClNO_4S$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[4-(2-benzo[*b*]thienyl)]benzyl Pentanedioic Acid, Hydrochloride (55). From **50**, Pd-catalyzed coupling using 2-benzothienyl boronic acid (procedure D) afforded the corresponding biaryl pyroglutamate. Hydrolysis (procedure F, 67%) afforded **55**; mp 250 °C (dec). $[\alpha]_D = -31.0°$ (*c* 0.51, DMSO). ^{1}H NMR (methanol-d_4): 7.82 (dt, *J* = 7.6, 1.4 Hz, 2H), 7.71 (dd, *J* = 8.1, 1.3 Hz, 2H), 7.64 (s, 1H), 7.34 (m, 4H), 4.07 (t, *J* = 6.1 Hz, 1H), 3.20−2.90 (m, 3H), 2.40 (m, 1H), 2.00 (m, 1H). ^{13}C NMR (methanol-d_4): 172.3, 171.1, 143.5, 140.9, 139.5, 138.8, 132.0, 130.2, 126.4, 125.2, 125.0, 124.0, 122.8, 120.0, 119.9, 51.1, 42.6, 37.4, 32.1. Anal. ($C_{20}H_{20}ClNO_4S$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[4-(2-naphthyl)]benzyl Pentanedioic Acid, Hydrochloride (56). From **50**, Pd-catalyzed coupling using 2-naphthyl boronic acid (procedure D) afforded the corresponding biaryl pyroglutamate. Hydrolysis (procedure F, 95%) afforded **56**; mp 175−176 °C. $[\alpha]_D = -32.0°$ (*c* 0.51, DMSO). ^{1}H NMR (methanol-d_4): 8.11 (s, 1H), 7.94 (m, 3H), 7.77 (t, *J* = 8.7 Hz, 3H), 7.52 (dd, *J* = 3.7, 1.3 Hz, 2H), 7.41 (d, *J* = 6.8 Hz, 2H), 4.61 (t, *J* = 6.2 Hz, 1H), 3.40−2.90 (m, 3H), 2.37 (m, 1H), 2.04 (m, 1H). ^{13}C NMR (methanol-d_4): 183.8, 182.7, 141.7, 139.7, 139.7, 135.2, 134.0, 130.8, 129.4, 129.1, 128.6, 128.0, 127.3, 126.8, 126.3, 126.1, 56.3, 49.6, 40.7, 40.5. Anal. ($C_{22}H_{22}ClNO_4$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[4-(*N*-benzenesulfonylamino)]benzyl Pentanedioic Acid, Hydrochloride (59). Alkylation of **4** using *p*-nitrobenzyl bromide afforded the corresponding pyroglutamate **57**. To a solution of **57** (4.30 mmol) in 90 mL of EtOH was added ammonium formate (NH_4CO_2H, 4 equiv) followed by 10% Pd/C (5 mol % of catalyst). The resulting mixture was heated to reflux for 2 h and then cooled to room temperature and filtered through a short pad of Celite, and the solvents were evaporated in vacuo to afford a crude oil. After it was purified by silica gel chromatography (Hex/EtOAc, 3:1), **58** was obtained in quantitative yield; mp 139−141 °C. $[\alpha]_D = +43.3°$ (*c* 0.32, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 6.95 (brd, *J* = 8.3 Hz, 2H), 6.61 (brd, *J* = 8.3 Hz, 2H), 4.44 (dd, *J* = 7.4, 4.0 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.49 (brs, 2H), 3.13 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.93−2.77 (m, 1H), 2.59 (dd, *J* = 13.8, 9.0 Hz, 1H), 2.05−1.96 (m, 2H), 1.49 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H). To a solution of **58** (0.83 mmol) in 10 mL of anhydrous CH_2Cl_2 and cooled to 0 °C was added benzenesulfonyl chloride (1.1 equiv) followed by Et_3N (2.0 equiv) and a catalytic amount of DMAP (10 mol %). The reaction mixture was stirred at room temperature for 45 min. Usual workup and silica gel chromatography (Hex/EtOAc, 3:1) afforded the benzenesulfonyl derivative, 96%. Then, hydrolysis (procedure F, 65%) afforded **59**; mp 113−114 °C. $[\alpha]_D = -14.0°$ (*c* 0.55, MeOH). ^{1}H NMR (methanol-d_4): 7.83−7.54 (m, 5H), 7.26 (brd, *J* = 8.2 Hz, 2H),

6.91 (brd, J = 6.9 Hz, 2H), 4.03 (brt, J = 7.1 Hz, 1H), 3.00 (m, 3H), 2.32 (m, 1H), 1.98 (m, 1H). ^{13}C NMR (methanol-d_4): 176.8, 171.4, 142.5, 140.5, 135.4, 133.9, 132.8, 131.0, 130.2, 129.5, 52.5, 44.2, 38.6, 33.1. Anal. ($C_{18}H_{21}ClN_2O_6S$) C, H, N.

(2*R*,4*S*)-2-Amino-4-[4-(*N*-phenylaminocarbonyl)amino]-benzyl Pentanedioic Acid, Hydrochloride (60). This compound was prepared as for **59** from **58** and phenyl isocyanate. Hydrolysis (procedure F, 38%) afforded **60**; mp 167−168 °C. $[\alpha]_D$ = −10.9° (*c* 1.1, MeOH). ^{1}H NMR (methanol-d_4): 7.43−6.94 (m, 9H), 3.94 (m, 1H), 2.77−3.05 (m, 3H), 2.27 (m, 1H), 1.87 (m, 1H). ^{13}C NMR (methanol-d_4): 177.2, 171.3, 155.5, 140.3, 138.9, 133.7, 130.5, 129.8, 123.7, 120.6, 120.3, 52.5, 44.4, 38.5, 32.6. Anal. ($C_{19}H_{22}ClN_3O_5$) C, H, N.

(2*R*,4*R*)-2-Amino-4-(2-benzo[*b*]thienyl)methyl Pentanedioic Acid (69). Alkylation of **4** with 2-(benzo[*b*]thienyl)-methyl bromide (procedure C) afforded ethyl (2*R*,4*R*)-*N*-BOC-4-(2-benzo[*b*]thienyl)methyl D-pyroglutamate **67**; 51%; mp 141−142 °C. $[\alpha]_D$ = +27.9° (*c* 0.31, $CHCl_3$).^{1}H NMR ($CDCl_3$): 7.76−7.56 (m, 2H), 7.29−7.14 (m, 2H), 6.98 (s, 1H), 4.44 (dd, J = 9.0, 1.9 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.41 (m, 1H), 2.96 (m, 2H), 2.20−1.92 (m, 2H), 1.42 (s, 9H), 1.19 (td, J = 7.1, 0.6 Hz, 3H). Hydrolysis (procedure F, 71%) afforded **69**; mp 167−168 °C. $[\alpha]_D$ = −35.3° (*c* 0.55, 1 N HCl).^{1}H NMR (D_2O/KOD): 7.53 (brd, J = 7.3 Hz, 1H), 7.43 (brd, J = 8.1 Hz, 1H), 7.02 (m, 2H), 6.80 (brs, 1H), 2.92 (dd, J = 8.5, 4.3 Hz, 1H), 2.84−2.65 (m, 2H), 2.44 (m, 1H), 1.69 (m, 1H), 1.30 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 183.9, 183.6, 145.2, 140.8, 140.0, 125.2, 124.6, 123.8, 123.1, 122.6, 55.5, 48.9, 39.5, 34.6. Anal. ($C_{14}H_{15}ClNO_4S$) C, H, N.

Compounds **70**−**73** were prepared in an analogous manner (yields for the last step).

(2*R*,4*S*)-2-Amino-4-(3-benzo[*b*]thienyl)methyl Pentanedioic Acid (70). 40%; mp 121−123 °C. $[\alpha]_D$ = −15.2° (*c* 0.61, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.82 (m, 2H), 7.31 (m, 2H), 7.17 (brs, 1H), 3.75 (dd, J = 9.1, 5.1 Hz, 1H), 2.95−2.69 (m, 3H), 1.89 (m, 1H), 1.50 (m, 1H). Anal. ($C_{14}H_{15}ClNO_4S$) C, H, N.

(2*R*,4*R*)-2-Amino-4-(2-benzo[*b*]furyl)methyl Pentanedioic Acid (71). 70%; mp 178−179 °C. $[\alpha]_D$ = −35.1° (*c* 0.50, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.36 (brd, J = 6.5 Hz, 1H), 7.29 (brd, J = 7.2 Hz, 1H), 7.05 (m, 1H), 6.36 (brs, 1H), 3.14 (m, 1H), 2.90−2.54 (m, 3H), 1.83 (m, 1H), 1.41 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 184.1, 183.7, 158.9, 155.4, 129.8, 124.6, 123.8, 121.7, 111.9, 104.0, 55.8, 46.0, 39.7, 32.7. Anal. ($C_{14}H_{15}NO_5$) C, H, N.

(2*R*,4*R*)-2-Amino-4-(2-benzo[*b*]thiazolyl)methyl Pentanedioic Acid, Dihydrochloride (72). Mixture of 4*R*/4*S* diastereomers; 98% overall; mp 131 °C. ^{1}H NMR (methanol-d_4): (data for the major isomer) 8.23 (d, J = 7.1 Hz, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.83−7.63 (m, 2H), 4.37−4.25 (m, 1H), 3.81 (d, J = 7.3 Hz, 1H), 3.79 (s, 1H), 3.64−3.34 (m, 1H), 2.66−2.13 (m, 2H). ^{13}C NMR (methanol-d_4): (data for the major isomer): 177.9, 174.8, 171.0, 144.1, 133.2, 130.1, 128.8, 124.7, 119.6, 52.2, 42.9, 34.7, 33.2. Anal. ($C_{13}H_{16}Cl_2N_2O_4S\cdot0.82H_2O$) C, H, N.

(2*R*,4*R*)-2-Amino-4-(2-indolyl)methyl Pentanedioic Acid, Hydrochloride (73). This compound was prepared using a modified procedure. Alkylation of **4** with 2-(*N*-benzenesulfonyl)indolyl methyl bromide afforded ethyl (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[2-(*N*-benzenesulfonyl) indolyl]methyl D-pyroglutamate. Aqueous 2 N NaOH (1 mL) was added to a solution of this compound (0.28 mmol) in 5 mL of MeOH. The reaction mixture was refluxed for 16 h and then cooled to room temperature and evaporated to dryness. The residue was diluted with water, acidified to pH 2 with 1 N HCl, extracted with Et_2O, and dried over Na_2SO_4 to afford, after concentration, a foamy solid, which was dissolved in a saturated solution of HCl in EtOAc. The mixture was stirred for 2 h, the solvent was evaporated, and the HCl salt was triturated with Et_2O and dried under high vacuum; 60%; mp 154−156 °C. $[\alpha]_D$ = −10.0° (*c* 0.6, MeOH). ^{1}H NMR (methanol-d_4): 7.48−6.90 (m, 5H), 4.01 (m, 1H), 3.09 (m, 3H), 2.41 (m, 1H), 1.93 (m, 1H). ^{13}C NMR (methanol-d_4): 176.8, 171.3, 134.0, 128.5, 123.3, 122.2, 121.0, 120.9, 119.0, 112.4, 52.5, 49.7, 42.7, 30.3. Anal. ($C_{14}H_{17}ClN_2O_4$) C, H, N.

(2*R*,4*R*)-2-Amino-4-[(3-chloro)-2-benzo[*b*]thienyl]methyl Pentanedioic Acid (74). Alkylation of D-ethyl *N*-BOC-pyroglutamate **4** with [(3-chloro)-2-benzo[*b*]thienyl]methyl bromide (procedure C) afforded ethyl (2*R*,4*R*)-*N*-BOC-4-[(3-chloro)-2-benzo[*b*]thienyl]methyl pyroglutamate; 30%. ^{1}H NMR ($CDCl_3$): 7.75 (dd, J = 7.8, 0.8 Hz, 2H), 7.47−7.32 (m, 2H), 4.53 (dd, J = 7.2, 0.8 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 3.55 (m, 1H), 3.17−3.02 (m, 2H), 2.14 (m, 2H), 1.51 (brs, 9H), 1.26 (td, J = 7.1, 0.7 Hz, 3H). Hydrolysis (procedure F, 43%) afforded **74**; mp 137−138 °C. $[\alpha]_D$ = −2.5° (*c* 0.45, DMSO). ^{1}H NMR (D_2O/KOD): 7.54 (dd, J = 6.5, 1.2 Hz, 1H), 7.44 (dd, J = 6.5, 1.4 Hz, 1H), 7.20−7.05 (m, 2H), 2.93 (dd, J = 9.3, 4.3 Hz, 1H), 2.80 (m, 2H), 2.47 (m, 1H), 1.73 (m, 1H), 1.31 (m, 1H). ^{13}C NMR (D_2O/KOD/methanol-d_4): 183.4, 183.4, 137.9, 137.5, 137.3, 126.1, 125.9, 123.6, 122.1, 118.6, 55.7, 48.0, 39.6, 32.5. Anal. ($C_{14}H_{14}ClNO_4S$) C, H, N.

(2*R*,4*R*)-2-Amino-4-(2-thienyl)methyl Pentanedioic Acid (75). 38%; mp 119−120 °C. $[\alpha]_D$ = −12.7° (*c* 0.36, 1 N HCl). ^{1}H NMR (D_2O/KOD): 7.13 (brd, J = 4.7 Hz, 1H), 6.85 (m, 1H), 6.78 (brs, 1H), 3.70 (dd, J = 9.1, 4.6 Hz, 1H), 2.97−2.77 (m, 3H), 1.87−1.77 (m, 1H), 1.51−1.41 (m, 1H). Anal. ($C_{10}H_{13}NO_4S$) C, H, N.

Purification of Glutamate Racemase. *E. coli* cell paste containing overexpressed *S. pneumoniae* glutamate racemase[15,16] was lysed with lysozyme and sonication. The supernatant was applied to a Ni(II) IMAC column and purified with an imidazole gradient, as described.[33] The pool of glutamate racemase-containing fractions was dialyzed against 100 mM Tris HCl, pH 7.8, buffer containing 20 mM β-mercaptoethanol to protect the active site cysteine residues from oxidation. The final dialysis buffer contained 5 mM BME.

Enzyme Assay. Glutamate racemase activity was determined under initial velocity conditions by HPLC separation following derivitization with Marfey's reagent. For a control assay, 6 μg mL^{-1} of glutamate racemase was incubated with L-glutamic acid (10 mM, dissolved in DMSO) for 15 min at room temperature (100 μL final volume). For the test assay in identical conditions, the inhibitor was dissolved in DMSO and mixed with L-glutamic acid such that the final percentage of DMSO present in both control samples and test samples was the same. The enzyme was then added to start the reaction. Both reactions were quenched by addition of 200 μL of 0.2% Marfey's reagent in acetone followed by addition of 40 μL of 1 M sodium bicarbonate. The mixtures were then incubated for 60 min at 40 °C followed by addition of 20 μL of 2 N HCL. The D- and L-isomers of glutamic acid were separated by C18 reverse phase chromatography using a linear gradient of 0.05 M triethylamine (pH 3.0) containing 25% (v/v) acetonitrile to 0.05 M triethylamine (pH 3.0) containing 75% acetonitrile over 20 min monitoring absorbance at A_{340}. Quantification of conversion was determined from integrated peak areas from the HPLC [(D-glutamate peak area)/(L-glutamate peak area + D-glutamate peak area)]. The test assay was repeated in decreasing concentrations of inhibitors in order to calculate the IC_{50} value for the test compound. Kinetic characterization was performed using circular dichroic spectra that were recorded at room temperature with a Jasco 700 Circular Dichroism System. A band width of 1 nm was used to record spectra at 210 nm of purified recombinant enzyme preparations at a 6 μg/mL in 10 mM phosphate buffer, pH 7.8.

***S. pneumoniae* PN-R6 Whole Cell Assay.** The compounds were evaluated for antimicrobial activity against *S. pneumoniae* PN-R6 using the microtiter broth dilution method of the National Committee for Clinical Laboratory Standards.[37] The test compounds were dissolved in DMSO and diluted using 2-fold serial dilution in Mueller−Hinton II broth (MHII, Difco Laboratories) supplemented with 5% lysed horse blood (LHB) to give a test concentration range of 250 to 0.25 μg/mL. The inoculum was prepared by diluting overnight cultures into MHII plus 5% LHB to give a final concentration of 5 × 10^5 colony-forming units (CFU)/mL in the test system. The final DMSO concentration was 2.5%, which did not affect bacterial

growth. Microtiter plates were incubated overnight at 35 °C. The MICs were determined as the lowest concentration that inhibited bacterial growth, as assessed by direct visualization. Further dilutions of test compounds were made as needed to determine the MIC.

Mouse Thigh Infection Model. *S. pneumoniae* Park1 used for in vivo studies had an MIC of 0.5 μg/mL for vancomycin. MICs were determined in BHI (Difco) broth with 5% sheep blood agar using a microtiter broth dilution method (NCCLS).[37] The bacteria were stored at −70 °C and propagated overnight on 5% sheep blood agar in the presence of 5% CO_2 atmosphere at 35 °C. Colonial growth was suspended in saline and adjusted to a density of approximately 10^8 cells/mL and then diluted 1:100 in BHI broth for inoculum. The mouse thigh infection model was adapted from Craig et al.[38] following approved animal care protocols. Six week old female ICR mice (Harlan Sprague−Dawley) were rendered neutropenic by intraperitoneal (ip) injection of cyclophosphamide (Sigma) at day −4 (150 mg/kg) and −1 (100 mg/kg) prior to infection. Mice were briefly anesthetized with isofluorane (Abbott), and thigh infections were initiated by injecting 0.1 mL of inoculum into each thigh (approximately 10^5 CFU/thigh). A clinical laboratory standard of vancomycin (Lilly) was used as a positive control drug and was prepared for ip administration immediately before each experiment. At 1 and 5 h postinfection, mice were dosed ip with 40 mg/kg test compound (2 mg/mL in 10% DMSO) or 10 mg/kg vancomycin (1 mg/mL in sterile water for injection). At 8 h postinfection, the mice were sacrificed using CO_2 asphyxiation. The entire thigh muscle mass was removed and homogenized (Polytron; Brinkman Instruments Inc.) decimally diluted in 9 mL of sterile 0.9% NaCl and 10 μL aliquots plated on BHI agar. Following overnight incubation at 35 °C with a 5% CO_2 atmosphere, CFU were enumerated and expressed as log10 CFU per thigh.

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Supporting Information Available: Biological data and experimental procedures for compounds **29**−**39** and **64**−**66** and kinetic characterization of compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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