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Structure-Based Design of Nonpeptide Inhibitors of Interleukin-1β Converting Enzyme (ICE, Caspase-1)

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Abstract—A novel class of reversible inhibitors of Interleukin-1β-converting enzyme (ICE, caspase-1) were discovered by iterative structure-based design. Guided by the X-ray crystal structure of analogues **1**, **7** and **10** bound to ICE, we have designed a non-peptide series of small molecule inhibitors. These compounds incorporate an arylsulfonamide moiety which replaces Val-His unit (P3-P2 residues) amino acids of the native substrate. The synthesis of the core structure, structure–activity relationships (SARs), and proposed binding orientation based on molecular modeling studies for this series of ICE inhibitors are described.

Interleukin-1 (IL-1) is the general term for two distinct proteins, IL-1α and IL-1β; the latter is the predominant species released by monocytes.[1] The mature cytokine IL-1β plays a key role in inflammation, septic shock, and in a variety of other physiological and pathological processes, including wound healing and the growth of certain leukemias. The cysteinyl protease interleukin-1β converting enzyme[2] (ICE) has been shown to cleave the 31 kDa IL-1β precursor (pro-IL-1β) at Asp116–Ala117 to generate the 17.5 kDa mature form of IL-1β.[3] Likewise the mature active form of human ICE is derived from a 404 amino acid precursor (p45) by proteolytic cleavage at Asp103, Asp119, Asp297 and Asp316. The active enzyme is a heterotetramer which consists of two subunits, p20 (residues 120–297) and p10 (residues 317–404), which are non-covalently bound. ICE has been characterized as a cysteine protease,[4] with cysteine285 of the p20 subunit serving as the catalytic residue.[4] The structure of ICE bound to a tetrapeptide aldehyde inhibitor (Ac-Tyr-Val-Ala-Asp-H) has been determined by X-ray diffraction.[5,6] ICE has the unique requirement for aspartic acid in the P1 position.[7,8] Exploiting this unusual specificity, two general inhibition strategies have been reported. The first utilizes peptidomimetic aldehydes, nitriles, and ketones, as covalent reversible inhibitors of ICE.[9,10] The second approach employs peptidomimetic α-substituted ketones of the general structure R-CO-CH_2-X, where X is a leaving group. These are covalent irreversible inhibitors.[9,10] Potent and selective tetrapeptide inhibitors have been reported that inhibit the production of IL-1β in monocytes.[4] Our goal is to employ structure-based design to identify low molecular weight, nonpeptidic, reversible inhibitors of ICE, as potential therapeutic agents for inflammatory diseases.

In an effort to develop more bioavailable, nonpeptidic inhibitors for assessing the effects of ICE inhibition in vivo, we simplified the known potent peptide inhibitor, Ac-Tyr-Val-Ala-Asp-CO–$(CH_2)_5$–Ph (**1**, $K_i = 19$ nM),[11] by replacing the Ac-Tyr-Val-Ala tripeptide with a benzyloxycarbonyl (CBZ) group providing **3** ($K_i = 119$ μM). Molecular modeling[12] of **3**, using the X-ray structure of **1** and manual docking, indicated that the CBZ would

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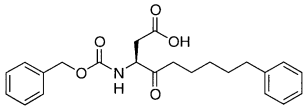
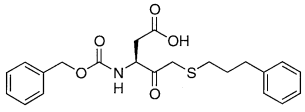
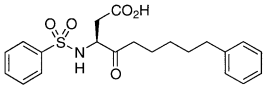
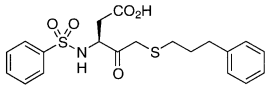
occupy the S2 site and the phenylpentyl group would be directed toward the prime side pocket. This prediction was based on the steric compatibility of the ligand with the enzyme binding region. In an attempt to understand the functionality contributing to the activity of **3**, with the aim of increasing the potency, analogues **5**, **7**, and **10** (Table 1, vide infra) were synthesized.[13] In designing our target molecule, we decided to replace the P1′ β-methylene in compound **3** with a sulfur atom, based on similar replacements in peptide ketone inhibitors,[14] to provide **5**. Such a replacement was expected to increase potency and indeed a 5-fold increase in potency compared to **3** was observed (**5**, $K_i = 25\,\mu M$). Replacement of the CBZ group in **3** with a functionality predicted to interact with ICE forming hydrogen bonds with the Arg341 side chain and backbone carbonyl led us to incorporate a phenyl sulfonamide moiety giving inhibitor **7**. The binding affinity of **7** was enhanced by 5-fold relative to **3** (**7**, $K_i = 24\,\mu M$). An attempt to further improve the potency of **7** by combining the potency enhancing thiomethylketone moiety with the phenyl sulfonamide to give **10**, surprisingly, resulted in no potency increase, as **10** was found to be equipotent with **5** and **7**.

In an effort to understand this lack of cooperative potency enhancement between the P side phenyl sulfonyl group and the prime side thiomethylketone functionality in **10**, the crystal structures of **7** and **10** were obtained (Fig. 1). As observed in the structure of **7** complexed with ICE, both oxygen atoms of the phenyl sulfonamide are hydrogen bonded to the protein. In this orientation, the sulfonamide N–H is hydrogen bonded to the carbonyl of Ser339 (2.83 Å), the in-plane sulfonyl oxygen atom is hydrogen bonded to the guanidino NH_2 of Arg341 (2.69 Å) while the other sulfonyl oxygen forms a hydrogen bond with the main chain amide of Arg341 (2.98 Å). The consequence of these interactions is that the phenyl ring of the arylsulfonamide group which points towards solvent is within hydrophobic contact of the Trp340 indole ring and the prime side polymethylene chain.

Table 1. Reversible inhibitors and their assay values tested against ICE PR in vitro[a]

Compd no.	Reversible inhibitors	K_i (μM)	IC_{50} (μM)
1	Ac-Tyr-Val-Ala-Asp-CO-$(CH_2)_5$-Ph	0.011	0.078
3		119	1034
5		25	141
7		24	166
10		24	176

[a]For a description of the assay, see ref 6. Values are the average of at least two determinations.

Examination of the X-ray crystal structure of **7** indicates that an *ortho* hydrogen atom of the phenylsulfonamide group is in close proximity to prime side methylene units of the phenylpentyl side chain extending from the aspartic acid-ketone. It is possible that this favorable binding mode is pre-organized by hydrophobic collapse. The X-ray crystal structure of **10** bound to ICE, indicates however, that the sulfur in the phenyl-thiomethyl side chain is oriented further away from the phenyl group than the corresponding methylene in **7**, perhaps to avoid a steric clash. An overlay of the bound crystal structures of **7** and **10** is shown in Figure 1.

On the basis of the X-ray structures of compounds **7** and **10**, it was surmised that if this C-terminal side chain in **10** was truncated to an aldehyde, then substitution of the *ortho* position of the phenyl sulfonamide group with a phenyl ring would provide an analogue with productive binding. The resulting *ortho* biphenyl sulfonamide was designed to provide a hydrophobic cap over the imidazole of the active site His237, partially blocking the oxyanion hole from bulk water while providing favorable van der Waals contact with the protein. We now describe the structure–activity relationship of these designed nonpeptide, sulfonamide-containing, aspartic acid aldehyde ICE inhibitors.

The preparation of a representative sulfonamide-containing aspartic acid aldehyde, **VII**, is shown in Scheme 1. We were gratified to find that the biphenyl sulfonamide aspartic acid aldehyde **12** was 15-fold more potent than the thioketone containing inhibitor **10** (**12**, $K_i = 1.6\,\mu M$). For comparison, the aldehyde derivative **11** which lacks the pendent *ortho* phenyl ring of **12**, is nearly 6-fold less potent than **12** (**11**, $K_i = 9.4\,\mu M$).

Compound **12**, was studied in its ICE bound state via X-ray crystallography to verify that the proposed binding mode of **12** was responsible for its potency increase (Fig. 2). This complex confirmed our original hypothesis regarding the mode of binding and the active site interactions of **12**. In this complex however, a second conformation of His237 side chain which was not predicted is populated. Specifically, His237 side chain has rotated from the previously observed +*gauche* orientation to a *trans* orientation creating a large pocket adjacent to the S1 site.[15]

The X-ray structure of compound **12** guided further design efforts to increase the binding affinity of this small nonpeptide inhibitor. We realized an improved potency by modifying the biphenyl ring system in the extended S1 site. Relative to the parent inhibitor **12**, two compounds were designed and synthesized to changed the torsion angle of the pendant aromatic ring: compound **13** with the methyl substituent on the phenyl ring at C-6 position and compound **14** with the methyl substituent on

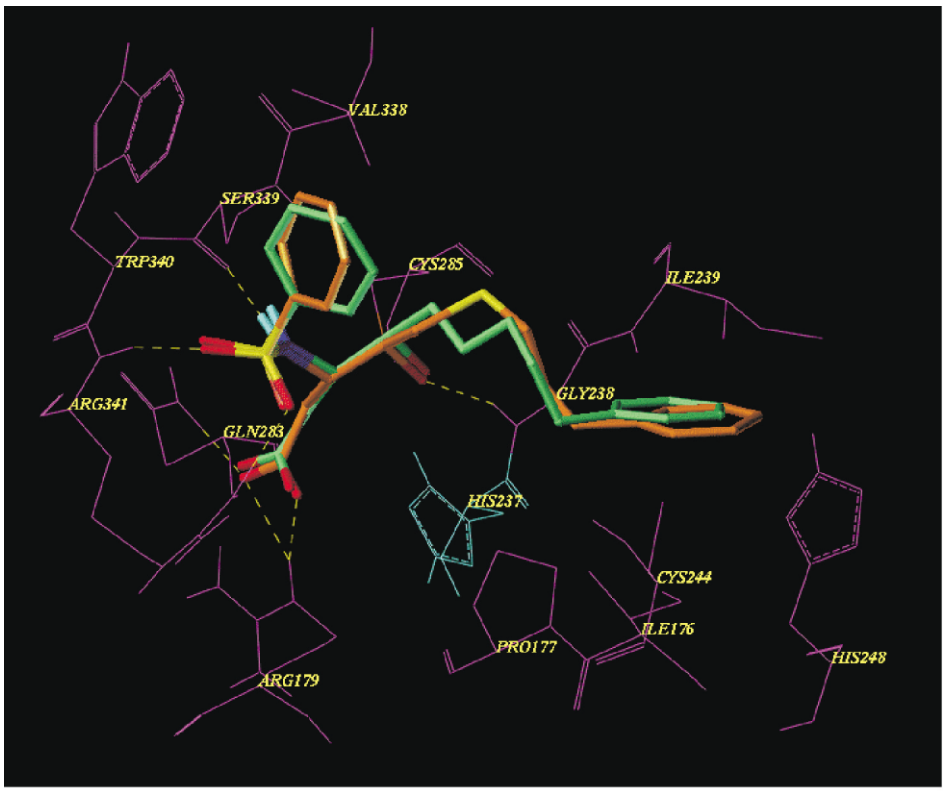
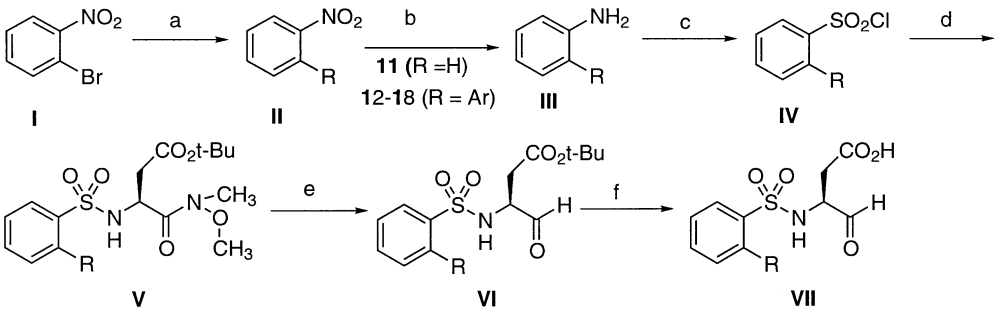


Figure 1. Overlay of the crystal structures of **7** (green thick lines) and **10** (orange thick lines) bound to the active site of ICE. The inhibitors are colored according to atom type [blue for nitrogens, red for oxygens, yellow for sulfurs, green (**7**) and orange (**10**) for carbons]. Enzyme residues are depicted in pink. The hydrogen bonds between the inhibitors sulfonamide (SO_2NH) and Arg341 (NH_2CNH) Ser339 (CO), and between the acid (CO_2) and Arg179 (NH_2CNH) are shown. His237 is shown in cyan.



Scheme 1. Preparation of sulfonamide-containing L-aspartic acid aldehyde. Reagents: (a) Ar-$B(OH)_2$, $Pd^0(Ph_3P)_4$, 2 N Na_2CO_3, DME, 100 °C, 12 h; (b) H_2 at 50 psi, Raney nickel, MeOH/THF (1:1), 6 h; (c) (i) HCl, H_2O, AcOH, (ii) 1.2 equiv $NaNO_2$, H_2O, −5 to 0 °C, (iii) 0.5 mol% CuCl, H_2O AcOH, SO_2, 50 °C, 12 h; (d) pyridine, CH_2Cl_2, Asp-(OtBu)–O–$CH_3N(OCH_3)$; (e) $LiAlH_4$, Et_2O, −65 °C, (f) 20% TFA/CH_2Cl_2.

the phenyl ring at C-2′ position. The designed inhibitors, **13** and **14**, were based on the expectation that the methyl groups on the phenyl rings would re-orient the distal phenyl ring toward Arg341. The constrained analogues of **12** showed a loss in binding affinity with respect to **12**, (**13**, $K_i = 1.9\,\mu M$ and **14**, $K_i = 6.8\,\mu M$).

The X-ray structure of the inhibitor **12** showed a major conformational change of His237 (vide supra), which created a large hydrophobic pocket adjacent to the S1 site. It was hoped that the substitutions appended from the phenylsulfonamide would serve to further fill this new pocket by employing methyl groups on the phenyl ring at C-3′ and C-4′ position, for example **15** ($K_i = 3.1\,\mu M$) and **16** ($K_i = 7.2\,\mu M$). Although these substitutions are tolerated, increases in potency were not observed.

Analysis of the X-ray structure (inhibitor **12**) indicated that replacing the distal phenyl ring in this series with a 1-naphthyl substituent was tolerated, although improved potency was not realized, as illustrated by **17** ($K_i = 6.9\,\mu M$). The X-ray crystallographic structure of **17** complexed with ICE was obtained, confirming that the His237 again moves from the standard +*gauche* conformation to a *trans* conformation. Subsequent molecular modeling indicated that replacing the second phenyl ring of the naphthyl group in **17** with an acetamide moiety could possibly provide a hydrogen bond donor to the Pro177(CO). This carbonyl normally forms

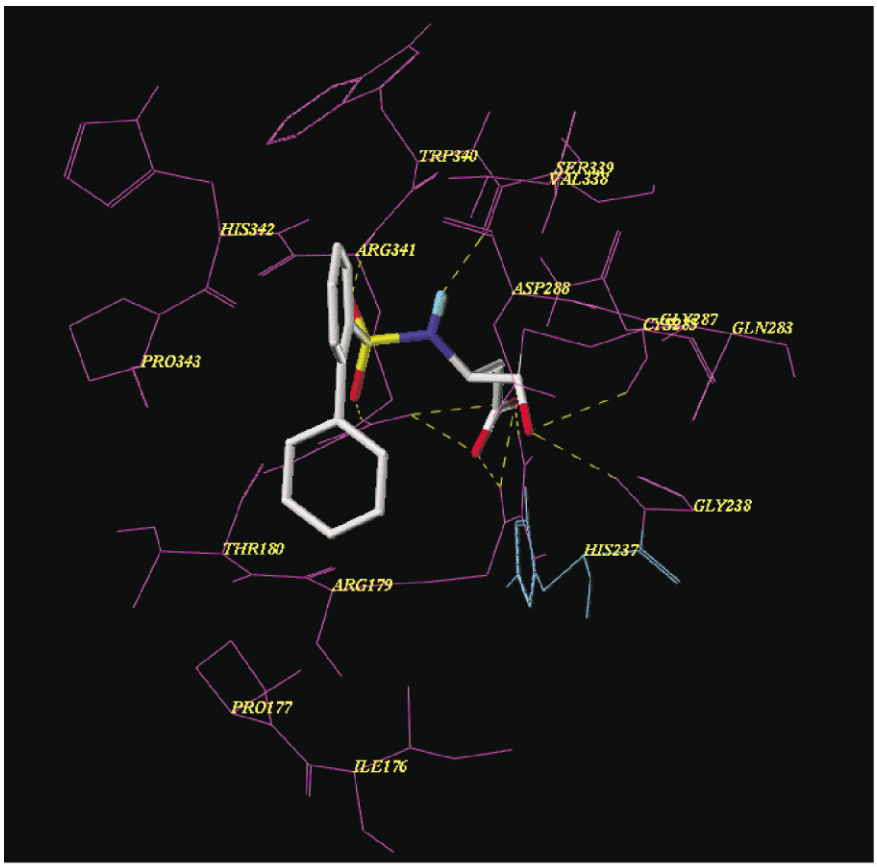


Figure 2. X-ray crystal structure of **12** in the ICE active site. The inhibitor is colored according to atom type (blue for nitrogens, red for oxygens, yellow for sulfurs, and white for carbons). The hydrogen bonds between the inhibitor sulfonamide (SO_2NH) and Arg341 (NH_2CNH) Ser339 (CO), and between the acid (CO_2) and Arg179 (NH_2CNH) are shown. His237 is shown in cyan.

a hydrogen bond with His237, when the His237 side chain is positioned in the standard +gauche orientation. This concept was tested by preparing an analogue containing a $NHCOCH_3$ substituent at the C-3′ position of the phenyl ring giving **18** ($K_i = 2.3\,\mu M$). Disappointingly, the resulting amide **18**, was 1.5 times less active relative to **12** (Table 2) perhaps due to desolvation penalty.

Conclusions

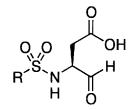
The X-ray crystal structure of **1** proved to be very useful in guiding the synthetic effort. Starting from a tetrapeptide (**1**) lead and a predicted mode of binding, followed by peptide-derived inhibitor structure–activity relationship analysis and molecular modeling, we have advanced a novel, low molecular weight class of inhibitors. The X-ray structure of **12** complexed with ICE has been determined. In this X-ray structure a major conformational change has been observed in which the catalytic His237 side chain has been rotated from a +*gauche* to a *trans* orientation creating a large hydrophobic pocket adjacent to the P1 site. Synthetic efforts are now directed toward increased potency in this series of non-peptidic ICE inhibitor. Taking advantage of the information gained with the X-ray structures, we have designed and synthesized more potent ICE inhibitors relative to our initial inhibitors **3**, **5**, **7**, and **10**. Compound **12** is the most potent inhibitor in this class and displays a $K_i = 1.6\,\mu M$ against human ICE. Further efforts aimed at improving compound potency as well as an investigation of their selectivity for specific members of the ICE family are under way and will be reported in due course.

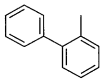
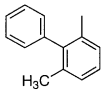
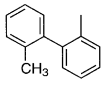
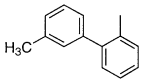
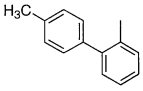
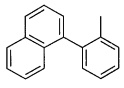
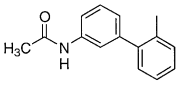
Experimental

General comments

Analytical data were recorded for the compounds described below using the following general procedures. Proton NMR spectra of the compounds were recorded on Varian, Gemini 2000 (300 MHz) or Unity (400 MHz); chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in deuteriochloroform or deuteriodimethyl sulfoxide or deuteriomethyl alcohol as specified below. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ, USA, and are within ±0.4% of the theoretical values. Positive and negative ion atmospheric pressure chemical ionization (APCI) mass spectra (MS) were recorded on a Micromass Platform LC mass spectrometer operating in open access mode. Samples were introduced by loop injection using a Gilson 215 autosampler into a mobile phase of 80:20 acetonitrile/water flowing at 200 μL/min delivered by a Hewlett-Packard HP1100 HPLC. The mass spectrometer source and probe temperatures were 150 and

Table 2. Evaluation of inhibitors against ICE

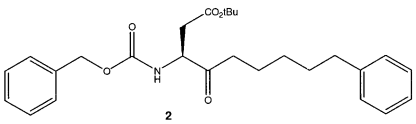


Compd no.	R	ICE	ICE
		K_i (μM)	IC_{50} (μM)
11		9.4	86.8
12		1.6	20.3
13		1.9	34.4
14		6.8	28.0
15		3.1	37.0
16		7.2	66.2
17		6.9	11.40
18		2.3	49.80

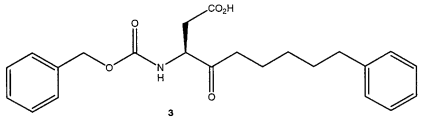
450 °C, respectively. The cone voltage was 15V while the corona pin was held at 3.5 kV in positive ion and 3.0 kV in negative ion mode.

Reagents were purchased from commercial sources. Chromatography was performed on silica gel using the solvent systems indicated below. For mixed solvent systems, the volume ratios are given. All reactions were performed under a nitrogen atmosphere using magnetic stirring. Commonly used abbreviations are: EtOAc (ethyl acetate), MeOH (methanol), DMF (*N*,*N*-dimethylformamide), DMAP (dimethylaminopyridine), HOAc (acetic acid), THF (tetrahydrofuran).

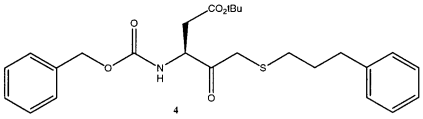
General procedures



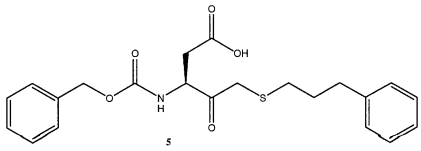
(*S*)-3-Benzyloxycarbonylamino-4-oxo-9-phenyl-nonanoic acid *tert*-butyl ester, 2. To a solution of (*S*)-3-Allyloxycarbonylamino-4-oxo-9-phenyl-nonanoic acid *tert*-butyl ester (0.36 g, 0.89 mmol) and bis(triphenylphosphine) palladium (II) chloride (0.03 g) in CH_2Cl_2/DMF (5 mL, 1:1) was added tri-*n*-butyltin hydride (97%, 0.4 g, 1.33 mmol) dropwise over 2 min at room temperature. After an additional 5 min and cooling to 0 °C benzyl chloroformate (0.23 mL 0.315 g, 1.8 mmol) and *N*-methylmorpholine (0.20 mL, 0.18 g, 1.8 mmol) were added simultaneously. The reaction mixture was stirred for 15 h at room temperature. The solvent was removed under reduced pressure and the residue was taken up in the ethyl acetate. The organic phase was washed successively with aqueous citric acid, aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue over silica (elution with dichloromethane/acetone 100:1) gave 0.125 g (31%) of **2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, 1H), 7.35–7.1 (m, 10H), 5.0 (s, 2H), 4.3 (dd, 1H), 2.65 (dd, 1H), 2.55–2.35 (m, 7H), 1.6–1.1 (m, 6H), 1.35(s, 9H).



(*S*)-3-Benzyloxycarbonylamino-4-oxo-9-phenyl-nonanoic acid, 3. A solution of **2** (0.11 g, 0.243 mmol) was treated with CH_2Cl_2/TFA (8 mL, 1:1) for 30 min at room temperature. Evaporation under reduced pressure followed by crystallization from dichloromethane/ether/hexane yielded 0.088 g (91%) of **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.8 (d, 1H), 7.4–7.15 (m, 10H), 5.05 (dd, 2H), 4.35 (dd, 1H), 2.7 (dd, 1H), 2.6–2.4 (m, 5H), 1.6–1.4 (m, 4H), 1.35–1.15 (m, 2H)

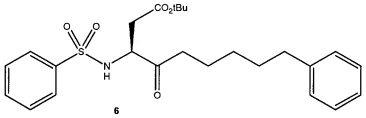


(*S*)-3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propyl-sulfanyl)-pentanoic acid *tert*-butyl ester, 4. A mixture of (*S*)-3-Benzyloxycarbonylamino-5-bromo-4-oxo-pentanoic *tert*-butyl ester (1.0 g, 2.5 mmol) and 3-phenylpropylmercaptan (0.38 g, 2.5 mmol) were dissolved in anhydrous DMF (1 mL). Potassium carbonate (0.173 g, 2.5 mmol) was added and the reaction was stirred at 25 °C under a nitrogen atmosphere for 16 h. The reaction was partitioned between EtOAc and H_2O. The organic layer was then washed with additional H_2O (3×50 mL), dried (Na_2SO_4), filtered and concentrated. The crude product was chromatographed on a silica gel column eluted with hexane/EtOAc/CH_2Cl_2 (7:1.5:1.5). The product **4** was isolated as yellow oil (0.89 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.23 (m, 3H), 7.18 (m, 3H), 5.82 (d, 1H), 5.10 (s, 2H), 4.75 (m, 1H), 3.38 (dd, 2H), 2.88 (dd, 1H), 2.70 (dd, 1H), 2.65 (t, 2H), 2.45 (t, 2H), 1.85 (q, 2H), 1.38 (s, 9H).



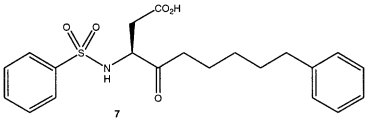
(*S*)-3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propyl-sulfanyl)-pentanoic acid, 5. To a solution of **4** (0.38 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was added TFA (2 mL). The reaction was stirred at 25 °C for 18 h and the solvent was removed in vacuo. The reaction mixture was

diluted with toluene (5 mL) and concentrated to eliminate the excess TFA. The crude product was chromatograph on a silica gel column and eluted with CH_2Cl_2/EtOAc (1:4) to produce the product as a semi-solid paste. The purified material was slurred in Et_2O to afford the product **5** as a beige solid (0.072 g, 22%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 4H), 7.20 (m, 3H), 7.12 (m, 3H), 5.80 (d, 1H), 5.05 (s, 2H), 4.98 (m, 1H), 3.32 (dd, 2H), 3.0 (dd, 1H), 2.82 (dd, 1H), 2.61 (t, 2H), 2.40 (t, 2H), 1.80 (q, 2H); MS (APCI) *m*/*z* 416.30 (M + 1). Anal. calcd for $C_{22}H_{25}N_1O_5S_1$ (M = 415.50): C 63.60; H 6.06; N 3.37. Found: C 63.20; H 6.06; N 3.26. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 6.25 min, 100% purity.



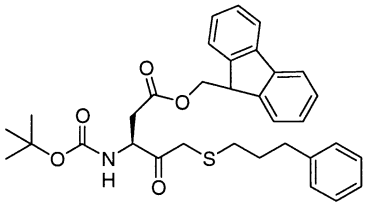
6

(*S*)-3-Benzenesulfonylamino-4-oxo-9-phenyl-nonanoic acid *tert*-butyl ester, 6. To a stirred solution of CH_2Cl_2/DMF (6.0 mL, 1:1) containing (*S*)-3-amino-4-oxo-9-phenyl-nonanoic acid *tert*-butyl ester (0.36 g, 0.89 mmol) prepared according to ref 11 and bis(triphenylphosphine) palladium(II) chloride (0.030 g) was added tri-*n*-butyltin hydride (97%, 0.40 g, 1.33 mmol) dropwise over 2 min at room temperature. After an additional 5 min and cooling to 0 °C benzensulfonyl chloride (0.23 mL, 0.315 g, 1.8 mmol) and *N*-methylmorpholine (0.20 mL, 1.80 mmol) were added simultaneously. The reaction was stirred for 15 h at room temperature. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate. The organic phase was washed successively with aqueous citric acid, aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue over silica (elution with CH_2Cl_2/acetone 100:1) gave 0.11 g (27%) of **6**, which was used without further purification for the next step.



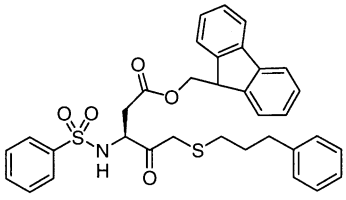
7

(*S*)-3-Benzenesulfonylamino-4-oxo-9-phenyl-nonanoic acid, 7. A solution of **6** (0.11 g, 0.24 mmol) was treated with CH_2Cl_2/TFA (10 mL, 1:1) for 30 min at room temperature. Evaporation under reduced pressure followed by crystallization from ether/hexane yielded 0.69 g (72%) of **7**. ^1H NMR (400 MHz, DMSO-d_6) δ 8.3 (bs, 1H), 7.8 (m, 2H), 7.65–7.55 (m, 3H), 7.3 (m, 2H), 7.15 (m, 3H), 4.05 (dd, 1H), 2.6-2.2 (m, 6H), 1.5 (m, 2H), 1.4 (m, 2H), 1.15 (m, 2H).



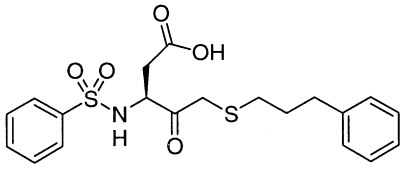
8

(*S*)-3-*tert*-Butoxycarbonylamino-4-oxo-5-(3-phenyl-propylsulfanyl)-pentanoic acid 9 *H*-fluoren-9-ylmethyl ester, 8. To a suspension of sodium hydride (60% suspension in oil, 4.5 mmol, 0.18 g) in DMF (2 mL) at 0 °C under an atmosphere of dry nitrogen was added dropwise 3-phenyl-propane-1-thiol (4.5 mmol, 0.68 g). The reaction mixture was stirred at 0 °C until bubbling had ceased and the resulting solution was added dropwise to a solution of (*S*)-3-*tert*-Butoxycarbonylamino-4-oxo-hexanoic acid 9*H*-fluoren-9-ylmethyl ester (4.6 mmol, 2.25 g) in DMF (5 mL) at 0 °C under an atmosphere of dry nitrogen. The reaction mixture was stirred at room temperature for 3 h and the DMF was removed under reduced pressure. The residue was taken up in ethyl acetate (50 mL) and washed with water (5×50 mL). The organic layer was evaporated and the residue purified by flash chromatography (silica gel) eluting with $CHCl_3$. Further purification was obtained by triturating the product with isopropyl ether/hexane until it solidified, and the product **8** (0.780 g, 31%) was collected by filtration. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, 2H), 7.53 (m, 2H), 7.38 (t, 2H), 7.30 (t, 2H), 7.22 (m, 3H), 7.12 (m, 2H), 5.46 (d, 1H), 4.65 (m, 1H), 4.36 (m, 2H), 4.18 (t, 1H), 3.52 (d, 1H), 3.28 (d, 1H), 3.05 (dd, 1H), 2.82 (d, 1H), 2.65 (t, 2H), 2.42 (t, 2H), 1.84 (q, 2H), 1.40 (s, 9H).



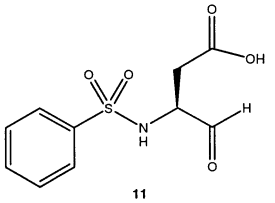
9

(*S*)-3-Benzenesulfonylamino-4-oxo-5-(3-phenyl-propylsulfanyl)-pentanoic acid 9*H*-fluoren-9-ylmethyl ester, 9. Compound **8** (0.350 g, 0.630 mmol) was dissolved in a solution of HCl (1 M) in ethyl acetate and the reaction mixture was allowed to stand at room temperature overnight. The solvent was evaporated. The residue was taken up in acetonitrile (35 mL), and treated dropwise with phenylsulfonyl chloride (0.120 g) in acetonitrile followed by dropwise treatment with a solution of DMAP (5 mg) and diisopropylethylamine (0.182 g). The reaction mixture was stirred at room temperature for 3 h and the solvent was evaporated. The residue was partitioned between ethyl acetate and water, and the aqueous layer was acidified to pH = 2 with 1 N HCl. The organic layer was collected, dried ($MgSO_4$) and evaporated. Purification by flash chromatography gave the desired product **9** (0.110 g, 30%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (m, 4H), 7.55 (m, 3H), 7.46 (m, 4H), 7.31 (m, 4H), 7.18 (m, 3H), 5.62 (d, 1H), 4.40 (m, 3H), 4.18 (t, 1H), 3.22 (d, 1H), 3.15 (d, 1H), 2.86 (dd, IH), 2.60 (m, 3H), 2.22 (t, 2H), 1.78 (q, 2H), 1.52 (s, 2H).



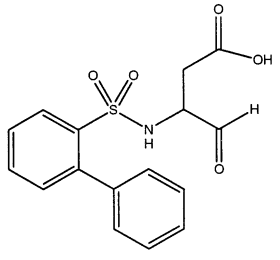
10

(*S*)-3-Benzenesulfonylamino-4-oxo-5-(3-phenyl-propylsulfanyl)-pentanoic acid, 10. A solution of **9** (0.084 g, 0.14 mmol) in dioxane (2 mL) and methanol (2 mL) was treated with a solution of 1.2 N NaOH (0.224 mL) diluted in 1 mL of methanol. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue triturated with hexane. The residue was taken up in water (0.5 mL), extracted with hexane (5 mL). The aqueous layer was acidified and the product extracted into ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (silica gel) eluting with $CHCl_3$/MeOH (95:5) followed by recrystalization from hexane/isopropyl ether) to afford **10** (0.019 g, 32%): mp 70–73 °C. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.88 (d, 2H), 7.60 (t, 2H), 7.58 (t, 2H), 7.24 (t, 2H), 7.17 (d, 2H), 5.50 (m, 1H), 3.42 (d, 2H), 2.62 (t, 2H), 2.52 (dd, 1H), 2.32 (t, 2H), 1.78 (q, 2H); MS (CI) *m*/*z* 422 (M + 1). Anal. calcd for $C_{20}H_{23}N_1O_5S_2 \cdot 0.38\ H_20$ (M = 423.145): C 56.77; H 5.52; N 3.31. Found: C 56.38; H 5.428; N 3.259.



11

(*S*)-3-Benzenesulfonylamino-4-oxo-butyric acid, 11. Benzenesulfonyl chloride was employed to acylate the amine of Asp(OtBu)–NMe(OMe), **23**, as described for the synthesis of **24**. From the resulting sulfonamide, the final product was obtained as a colorless foam employing the methods described for the synthesis of **13**. ^{1}H NMR (300 MHz, MeOH-d_4) as the hemiacetal δ 7.87 (m, 2H), 7.58 (m, 3H), 4.44, 4.38 (d, 1H), 3.74 (m, 1H); MS (APCI) *m*/*z* 240 $(M–OH)^+$. Anal. calcd for $C_{10}H_{11}NO_5S_1$ (M = 257.26): C 46.69; H 4.31; N 5.44. Found: C 46.74; H 4.18; N 5.10. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–66% over 22 min] t_R = 11.8 min, 100% purity.

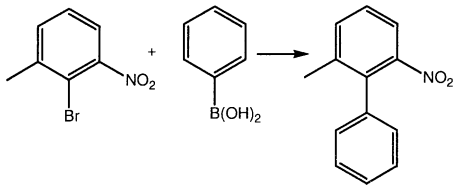


12

(*S*)-3-(Biphenyl-2-sulfonylamino)-4-oxobutryic acid, 12. Biphenyl-2-sulfonyl chloride[16] was employed to acylate the amine of Asp(OtBu)–NMe(OMe), **23**, as described for the synthesis of **24**. From the resulting sulfonamide, the final product was obtained as a colorless foam employing the methods described for the synthesis of **13**.

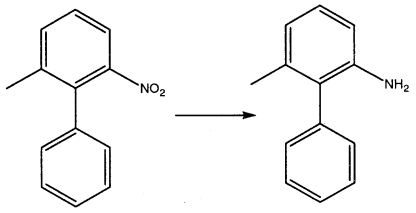
^{1}H NMR (300 MHz, MeOH-d_4) as the hemiacetal δ 8.18 (m, 1H), 7.62 (t, 1H), 7.53 (t, 1H), 7.40, (m, 5H), 7.30 (d, 1H), 4.43 (d, 1H), 3.82 (m, 1H), 2.53 (dt, 1H), 2.30 (m, 1H); MS (APCI) *m*/*z* 334 $(M + H)^+$. Anal. calcd for: $C_{16}H_{15}N_1O_5S_1$ (M = 333.36): C 57.92; H 4.50; N 4.19. Found: C 57.65, H 4.54, N 4.20.

General procedure for the synthesis of biphenyl-2-sulfonylamino)-4-oxo-butyric acids 13–18 (Table 2).



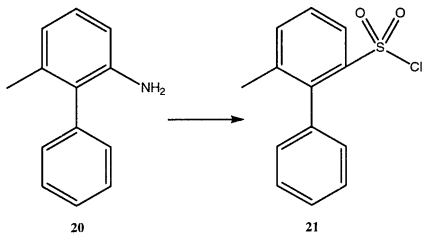
19

6-Methyl-2-nitro-biphenyl, 19. To a stirred solution of 2-bromo-3-nitrotoluene (5.0 g, 23.10 mmol) in toluene (70 mL), 2M aqueous sodium carbonate (23.0 mL, 46.20 mmol) and tetra-*n*-butylammonium bromide (0.37 g, 1.20 mmol) under nitrogen was added benzeneboronic acid (2.82 g, 23.10 mmol) in 70 mL of absolute ethanol via addition funnel. The reaction mixture was degassed for 30 min. Tetrakis(triphenylphosphine)palladium(0) (0.14 g, 5% by wt) was added. After 7 h at 100 °C, the reaction mixture was cooled, and ethyl acetate (200 mL) was added. The layers were separated, the aqueous layer was extracted with ethyl acetate (2×100 mL), and the combined organic phases were washed with water, brine, and dried with $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (elution with 10% EtOAc/hexanes) to give 4.80 g (97%) of **19** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, 1H, ArH), 7.50–7.35 (m, 5H, ArH), 7.18 (d, 2H, ArH), 2.14 (s, 3H).



19 20

6-Methyl-biphenyl-2-ylamine, 20. A solution of **19** (4.80 g, 22.5 0 mmol) in THF (100 mL) was shaken with Raney nickel (1.50 g) under H_2 (50 psi) for 3.5 h using Parr apparatus. The reaction mixture was filtered through Celite and concentrated. The crude product of **20** was obtained (4.00 g, 98%) as an off white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (t, 2H, ArH), 7.31 (t, 1H, ArH), 7.21 (d, 2H, ArH), 7.03 (t, 2H, ArH), 6.67 (d, 2H, ArH), 6.59 (d, 1H, ArH), 1.97 (s, 3H).

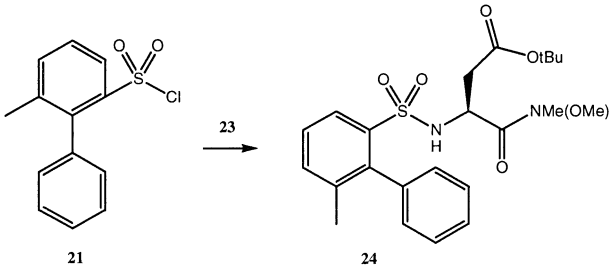


20 21

6-Methyl-biphenyl-2-sulfonyl chloride, 21. 6-Methyl-biphenyl-2-ylamine **20** (4.0 g, 21.17 mmol) was dissolved in glacial acetic acid (20 mL) and then added concd HCl (7 mL). The stirred solution was diazotised at −10 °C with $NaNO_2$ (1.95 g, 28.30 mmol) in water (5 mL). The solution was allowed to stirred at −10 °C for 30 min. The diazonium salt solution was poured into a mixture of 30% SO_2− glacial acetic acid (20 mL) and benzene (20 mL) in which $CuCl_2{\cdot}2H_2O$ (1.2 g, 6.5 mmol) had been suspended and which was at −5 °C initially. The reaction mixture was stirred at 0 °C for 2 h. The mixture was then heated to 50 °C using oil bath, and maintained at this temperature for 12 h. The mixture was then cooled and poured into water (200 mL) and the resultant oil was extracted into ethyl acetate (500 mL). The organic layer was washed with water (2×200 mL), saturated $NaHCO_3$, dried over $MgSO_4$ and the solvents were removed in vacuo. The crude oil was crystallized from hexanes/ether to give product **21** as yellow solid (0.750 g, 14%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 1H, ArH), 7.59 (d, 1H, ArH), 7.42–7.12 (m, 4H, ArH), 8.05 (d, 1H, ArH), 6.42 (d, 1H, ArH); MS (APCI) (M + 1) *m*/*z* 267.0.15

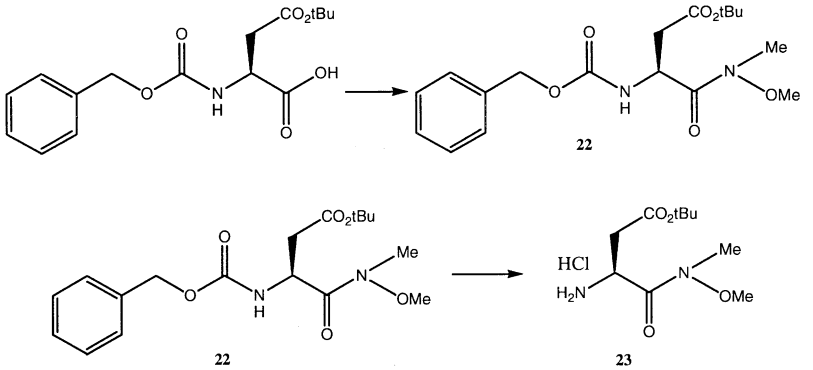
(*S*)-3-Benzyloxycarbonylamino-*N*-methoxy-*N*-methyl-succinamic acid *tert*-butyl ester, 22. To a solution of the acid (*S*)-2-benzyloxycarbonylamino-succinic acid 4-*tert*-butyl ester (50.0 g, 154.6 mmol) in dry dichloromethane (200 mL) and *N*-methylmorpholine (42.5 mL, 386.5 mmol) at −75 °C was added dropwise isobutyl-chloroformate (24.1 mL, 185.5 mmol) followed by stirring at −75 °C for 30 min. A solution (*N*,*O*-dimethylhydroxylamine) hydrochloride (18.10 g, 185.5 mmol) in dry dichloromethane (50 mL) and *N*-methylpiperidine (22.55 mL, 185.5 mmol) was added. The solution was allowed to warm to room temperature and stirred for 1 h, quenched by addition of 10% H_2SO_4 and extracted into ethyl acetate. The organic layer was washed sequentially with 10% H_2SO_4, H_2O, and brine. The solution was dried and the solvent removed. The resulting oil crystallized upon the addition of 20% ether/hexane and was collected and dried to give the corresponding amide **22** 45.0 g (80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (s, 5H), 5.62 (d,1H), 5.05 (s, 2H), 4.95 (m, 1H), 3.78 (s, 3H), 3.20 (s, 3H), 2.65 (dd, 1H), 2.55 (dd, 1H), 1.39 (s, 9H); MS (APCI) (M + 1) *m*/*z* 367; HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 18.2 min, 100% purity.

(*S*)-3-Amino-*N*-methoxy-*N*-methyl-succinamic acid *tert*-butyl ester, 23. To a solution of **22** (11.55 g, 31.50 mmol) in EtOH (100 mL) and concd HCl (3 mL) was added 20% Pd/C (0.50 g), and the mixture was stirred for 3 h under a hydrogen atmosphere at atmospheric pressure. The catalyst was filtered and the solvent removed. The resulting oil solidified upon the addition of ether. The solid was dried to give 8.40 g (99%) of **23** as an off-white solid. The solid was used immediately in the next step. It is important to solidify this intermediate before submitting to the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (br s, 1H), 4.68 (m, 1H), 3.84 (s, 3H), 3.28 (s, 3H), 3.05 (d, 2H), 1.25 (s, 9H); HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 10.80 min, 100% purity.

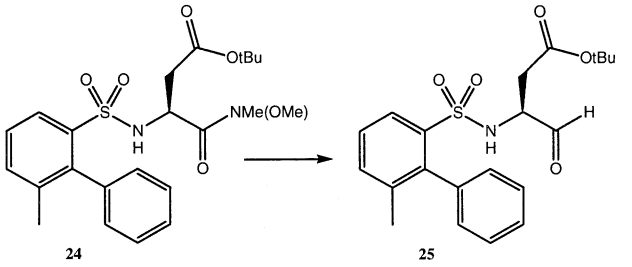


21 24

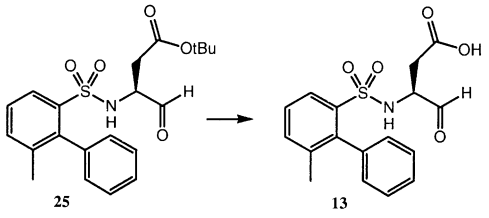
(*S*)-*N*-Methoxy-*N*-methyl-3-(6-methyl-biphenyl-2-sulfonylamino)-succinamic acid *tert*-butyl ester, 24. To a solution of **21** (0.75 g, 2.80 mmol) in dry dichloromethane (15 mL) and Asp(OtBu)–NMe(OMe) **23** (0.50 g, 1.90 mmol) was added pyridine (1.0 mL, 8.40 mmol). The resulting solution was stirred at room temperature for 12 h. The resulting solution was taken into ethyl acetate (100 mL), washed with 5% citric acid, saturated sodium bicarbonate, dried ($MgSO_4$), and the solvent was removed. The resulting oil was chromatograph (20% EtOAc/hexanes) to give 0.59 g (45%) of **24** as off white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H, ArH), 7.51–7.31 (m, 6H, ArH), 4.72 (br s, 2H), 3.65 (s, 3H), 3.05 (s, 3H), 2.58 (dd, 1H), 2.38 (dd, 1H), 1.98 (s, 3H), 1.38 (s, 9H); HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 21.60 min, 100% purity.



22 22 23

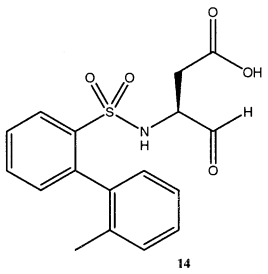


(*S*)-3-(6-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid *tert*-butyl ester, 25. To weinreb amide **24** (0.56 g, 1.10 mmol) solution in dry Et_2O (20 mL) at −65 °C was added $LiAlH_4$ (0.062 g, 1.6 mmol). The reaction mixture temperature was maintained for 2 h. The excess hydride was quenched by the addition of potassium hydrogen sulfate (2 equiv) dissolved in water. After warming to room temperature, Et_2O was added (100 mL) and the organic layer was washed with water and then with brine. The solvent was dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting crude product was purified on silica gel flash chromatography (15% EtOAc in hexanes) to give **25** as an off white foam (0.18 g, 40%). ^1H NMR (300 MHz, $CDCl_3$) δ 9.46 (s, 1H), 7.95 (t, 1H, ArH), 7.52–7.31 (m, 6H, ArH), 7.22 (d, 1H, ArH), 5.11 (d, 1H), 3.82 (m, 1H), 2.88 (dd, 1H), 2.62 (dd, 1H), 2.02 (s, 3H), 1.38 (s, 9H); HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], $t_R = 18.36$ min, 100% purity.



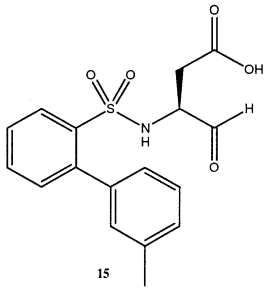
(*S*)-3-(6-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid, 13. A solution of **25** (0.18 g, 0.60 mmol) in TFA (2 mL) and CH_2Cl_2 (20 mL) was stirred for 3 h. After the solvent was removed under vacuum, the residue was subjected to preparative reverse-phase HPLC (Vydac, C18) using linear gradient of (A) water containing 0.1% TFA and (B) acetonitrile containing 0.1% TFA (10–45% B, in 120 min) at a flow rate of 20 mL/min. Fractions containing the major peak were pooled and lyophilized to yield 0.120 g (86%) of **13**. ^1H NMR (400 MHz, MeOH-d_4) δ 7.93 (dd, 1H, ArH), 7.55 (d, 1H, ArH), 7.42 (m, 4H, ArH), 7.32 (t, 1H, ArH), 7.21 (t, 1H, ArH), 4.46 (m, 1H), 3.65 (m, 1H), 2.52 (dd, 1H), 2.36 (dd, 1H), 1.98 (s, 3H); MS (APCI) m/z 348 (M + 1). Anal. calcd for $C_{17}H_{17}N_1O_5S_1$ (M = 347.393): C 58.78; H 4.93; N 4.03. Found: C 58.57; H 5.60; N 3.31. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], $t_R = 16.88$ min, 100% purity.

Compound **14** was synthesized using the method described for **13**.



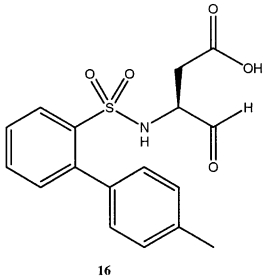
(*S*)-3-(2′-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid, 14. Yield 0.070 g (46%), ^1H NMR (400 MHz, MeOH-d_4) δ 8.12 (m, 1H, ArH), 7.62 (m, 1H, ArH), 7.58 (m, 1H, ArH), 7.28 (m, 5H, ArH), 4.58, 4.41 (dd, 1H), 3.71 (m, 1H), 2.58 (dd, 1H), 2.46 (dd, 1H), 2.05 (s, 3H). MS (APCI) m/z 348.4 (M + 1). Anal. calcd for $C_{17}H_{17}N_1O_5S_1$ (M = 347.393): C 58.78; H 4.93; N 4.03. Found: C 58.53; H 5.17; N 4.08. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], $t_R = 16.57$ min, 100% purity.

Compound **15** was synthesized using the method described for **13**.



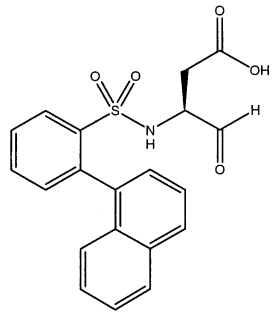
(*S*)-3-(3′-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid, 15. Yield 0.16 g (76%), ^1H NMR (400 MHz, MeOH-d_4) δ 8.06 (m, 1H, ArH), 7.60 (t, 1H, ArH), 7.52 (t, 1H, ArH), 7.25 (m, 5H, ArH), 4.42 (dd, 1H), 3.62 (m, 1H), 2.51 (dd, 1H), 2.39 (s, 3H), 2.34 (dd, 1H), MS (APCI) m/z 346.4 (M−1). Anal. calcd for $C_{17}H_{17}N_1O_5S_1$ (M = 347.393): C 58.78; H 4.93; N 4.03. Found: C 58.82; H 5.25; N 4.17. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], $t_R = 16.55$ min, 100% purity.

Compound **16** was synthesized using the method described for **13**.



(*S*)-3-(4′-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid, 16. Yield 0.073 g (70%), ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.07 (m, 1H, ArH), 7.60 (t, 1H, ArH), 7.28 (t, 3H, ArH), 7.22 (d, 2H, ArH), 4.41 (dd, 1H), 3.61 (m, 1H), 2.45 (dd, 1H), 2.41 (s, 3H), 2.28 (dd, 1H), MS (APCI) *m*/*z* 348.5 (M + 1). Anal. calcd for $C_{17}H_{17}N_1O_5S_1$ (M = 347.393): C 58.78; H 4.93; N 4.03. Found: C 58.88; H 5.37; N 4.28. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 20.12 min, 100% purity.

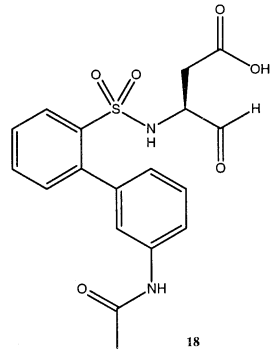
Compound **17** was synthesized using the method described for **13**.



17

(*S*)-3-(2-Naphthalen-1-yl-benzenesulfonylamino)-4-oxo-butyric acid, 17. Yield 0.110 g (35%), ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.19 (m, 1H, ArH), 7.92 (d, 2H, ArH), 7.66 (m, 2H, ArH), 7.50 (m, 3H, ArH), 7.32 (m, 3H, ArH), 4.41, 4.39 (dd, 1H), 3.65 (m, 1H), 3.31 (s, 3H), 2.50 (dd, 1H), 2.28 (dd, 1H), MS (APCI) *m*/*z* 384.4 (M + 1). Anal. calcd for $C_{20}H_{17}N_1O_5S_1$ (M = 383.426): C 62.65; H 4.47; N 3.65. Found: C 62.36; H 4.67; N 3.50. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 16.14 min, 100% purity.

Compound **18** was synthesized using the method described for **13**



18

(*S*)-3-(3′-Acetylamino-biphenyl-2-sulfonylamino)-4-oxo-butyric acid, 18. Yield 0.060 g (77%), ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.07 (m, 1H, ArH), 7.63 (m, 2H, ArH), 7.50 (m, 2H, ArH), 7.37 (d, 2H, ArH), 7.17 (d, 1H) 4.48 (dd, 1H), 3.71 (m, 1H), 2.55 (dd, 1H), 2.41 (dd, 1H), 2.12 (s, 3H), MS (APCI) *m*/*z* 390.8 (M + 1). Anal. calcd for $C_{18}H_{18}N_2O_6S_1$ (M = 390.418): C 55.38; H 4.65; N 7.18. Found: C 55.28; H 4.62; N 7.16. HPLC [Vydac C-18, 250×4.6 mm, acetonitrile in water (0.1% trifluoroacetic acid) at 1.50 mL/min 0–100% over 30 min], t_R = 13.01 min, 100% purity.

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