Side-Chain-Modified Sulfonic Analogues of Aspartic and Glutamic Acids: Synthesis, Protection, and Incorporation into Peptides

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There is considerable and still increasing interest in the synthesis of unnatural amino acids. These compounds can be incorporated into various peptide effectors, such as receptor ligands and enzyme inhibitors, to increase their affinity and/or selectivity. As part of our studies toward synthetic substrates and inhibitors of aminopeptidases,[1] we wish to report here our recent work concerning the synthesis of isosteric analogues of aspartic and glutamic acids, as orthogonally protected synthons suitable for peptide synthesis. In the latter compounds, the side-chain carboxylic acid of aspartic and glutamic acids was replaced by a sulfonate, leading to cysteic acid (also named sulfoalanine,[2] Sal) and homocysteic acid, respectively. These two acids are commercially available, as totally unprotected forms. Unfortunately, the high water solubility of the polar free sulfonate renders most organic transformations troublesome. Indeed, the presence of a free sulfonate considerably decreases the solubility in organic solvents and makes aqueous workup procedures impossible. Recently, an example of direct incorporation of *N*-Boc cysteic acid by SPPS was described with a fairly low yield.[2]

For these reasons, we have searched for a hydrophobic and stable protection for the sulfonic acid group. Sulfonamides are very readily accessible, but require drastic conditions to be deprotected, these methods being uncompatible with peptide synthesis. Moreover, the widely used reductive cleavage[3] of sulfonamides is aimed at yielding the amine portion of the sulfonamide and not the sulfonic acid. Sulfonate esters are generally so labile that they are employed as powerful alkylating reagents, such as methyl methanesulfonate. This problem can be overcome by the use of a bulkier sulfonate, provided that it is not derived from a tertiary alcohol which would be likely to result in elimination products. In the 1990s, an isopropyl sulfonate was reported[4] as a protecting group involved in the synthesis of modified carbohydrates. However, due to the mildness of its deprotection, i.e., NaI/acetone or ammonia/methanol treatments, we were interested in an even more stable sulfonic ester. Our attention was focused on the neopentyl sulfonate, which was first described by Truce et al.[5] and recently used as a protective group for arylsulfonic acids, in parallel with its neoN−B analog.[6]

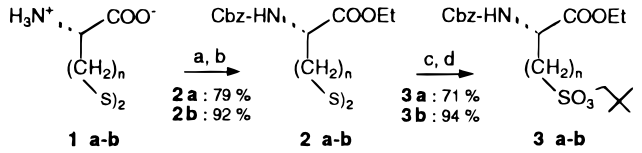

Figure 1. Completely protected cysteic and homocysteic acids. Reagents and conditions: (a) benzyl chloroformate, NaOH, dioxane/H_2O = 1:1, pH = 9; (b) EtOH, $SOCl_2$ (0.67 molar equiv), 0 °C to rt, overnight; (c) Cl_2, CCl_4/EtOH = 4:1; (d) neopentyl alcohol, Et_3N, CH_2Cl_2, 0 °C.

With this protective group in hand, we needed a straightforward method for its introduction. Direct introduction onto the poorly nucleophilic free sulfonate would not be advantageous, since it would require a powerful alkylating agent able to transfer the neopentyl group, such as neopentyl trifluoromethanesulfonate. Moreover, turning the sulfonic acid into its chloride would be effected using reagents unsuitable for peptide synthesis, e.g., PCl_5 or $SOCl_2$. Consequently, we chose to prepare these protected amino acids from cystine and homocystine, both commercially available as optically pure enantiomers.

Results and Discussion

The synthesis of totally and orthogonally protected cysteic and homocysteic acids was acomplished as depicted in Figure 1. Starting from commercially available L-cystine or L-homocystine **1a** and **1b**, the amine was protected by a Cbz group and the carboxylate esterified by ethanol with dropwise addition of $SOCl_2$ at 0 °C. The disulfide was then subjected to chlorine-mediated oxidative cleavage leading to the corresponding sulfonyl chloride. The latter was isolated but not purified and subsequently esterified by neopentyl alcohol in the presence of triethylamine or pyridine.

The totally protected synthons **3a** and **3b** were cleanly obtained on a multigram scale with overall yields of 71% and 94%, respectively. We then used various deprotection methods for the acid and the amine groups, as outlined in Figure 2. The deprotection of the acid was rendered difficult due to a possible racemization of the chiral center in alkaline medium,[7] the acidity of the α-proton being enhanced by the electron-withdrawing sulfonyl group. Moreover, when compound **3a** was treated at 0 °C with aqueous LiOH, unexpected degradation was observed. For these reasons, we used acidic conditions for the hydrolysis of the carboxylic ester of **3a** and **3b**. By treatment with 3 N HCl in dioxane/H_2O (1:1) at 50 °C, **4a** and **4b** were obtained in good yields. Under these conditions, the Cbz and neopentyl groups remained

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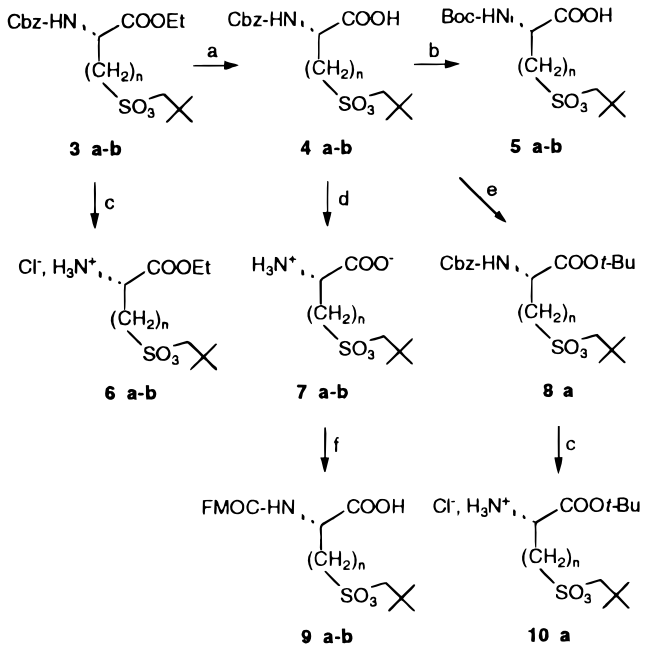

Figure 2. Orthogonal protections of amine and acid moieties of cysteic and homocysteic acids. Reagents and conditions: (a) 3 N HCl/dioxane:H_2O = 1:1, 97% (**4a**), 100% (**4b**); (b) H_2, Pd/C, Boc_2O, AcOEt, 92% (**5a**), 90% (**5b**); (c) H_2, Pd/C, HCl, EtOH, 100%; (d) H_2, Pd/C, 95% EtOH, 100% (**7a**), 99% (**7b**); (e) isobutylene, Et_2O, cat. H_2SO_4, 5 d, 33%; (f) Fmoc-OSu or Fmoc-Cl, Na_2CO_3, dioxane, H_2O, 95% (**9a**), 65% (**9b**).

unchanged, provided that the reaction time did not exceed 14 h. Indeed, further heating resulted in deprotection of both amine and sulfonic acid.

The free carboxylic acid could be reprotected as a *tert*-butyl ester (compound **8a**) by means of isobutene in ether with a catalytic amount of sulfuric acid.[8]

The Cbz group was removed by classical hydrogenolysis in the presence of H_2/Pd on charcoal, leading to **7a** and **7b**. No poisoning of the catalyst occurred, since the sulfur is in a high oxidation state. The free amine could be reprotected, affording the *N*-Boc and *N*-Fmoc derivatives, respectively **5a,b** and **9a,b** which are very useful N-protected amino acids for peptide synthesis. To shorten the synthesis time of the *N*-Fmoc derivative, the direct oxidation of (Fmoc-Cys-OH)$_2$ or (Fmoc-Cys-OEt)$_2$ by chlorine in CCl_4/H_2O or CCl_4/tBuOH was attempted but resulted in decomposition products, with loss of the Fmoc group.

All protections and deprotections described herein did not interfere with the sulfonate which remained unchanged during the course of the synthesis and did not induce any racemization of the chiral center.

As far as the sulfonic ester is concerned, its cleavage was recently described by Roberts et al.[6] in the presence of tetramethylammonium chloride in DMF at 160 °C. We first checked that these apparently harsh conditions did not result in degradation or racemization during the deprotection of the sulfonate moiety, when these sulfonate-bearing amino acids were incorporated into short peptides. Nevertheless, we examined this deprotection under milder conditions which could be more adapted to peptide synthesis. We observed (Figure 3) that both sulfonate and amino groups underwent a clean and

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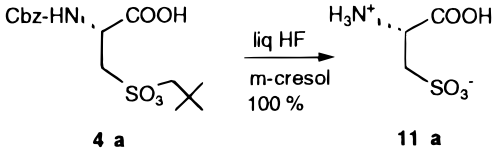

Figure 3. Deprotection of the sulfonate group of cysteic and homocysteic acids.

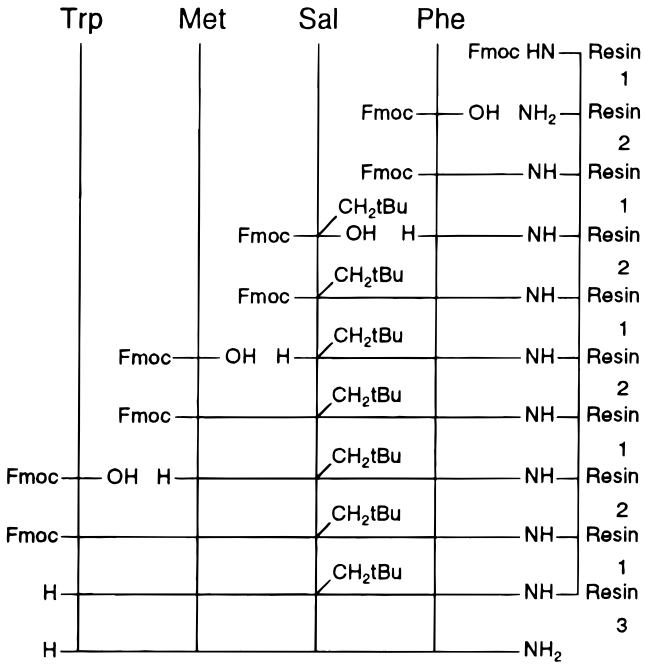

Figure 4. Solid-phase peptidic synthesis of CCK_4 sulfonic analogue. 1: piperidine, NMP. 2: DCC, HOBt, NMP. 3: HF, *m*-cresol, then TFA, Et_2O/*n*-heptane = 1:1.

complete deprotection, when compound **4a** was treated with liquid hydrogen fluoride (5 mL/100 mg) in the presence of *m*-cresol (250 μL/100 mg) as a scavenger at 0 °C to room temperature during 1 h. The deprotected amine was obtained as the hydrofluoride, which was displaced by TFA. We noticed that upon subsequent aqueous extraction and lyophylization compound **11a** was quantitatively obtained in the zwitterionnic form, with no trace of remaining sulfonic ester being observed.

As an illustration of the incorporation of sulfonate-protected cysteic acid into a peptide, the sulfonic analogue of CCK_4 (Trp-Met-Asp-Phe-NH_2, tetragastrin) was prepared, with Asp being replaced by cysteic acid. This synthesis (Figure 4) was performed either by means of liquid-phase synthesis (introduction as the *N*-Cbz compound, coupling steps using BOP/DIEA) or solid-phase peptidic synthesis (*N*-Fmoc derivative, Sieber-Amide resin, coupling steps with DCC/HOBT). In both cases, good yields of incorporation were obtained, both for the coupling of N-protected sulfoalanine and for the following amino acid (Met). In the last step, the sulfonic acid was deprotected by liquid HF. We noticed that the analogue was, as expected, much more hydrophilic (according to HPLC analysis) than CCK_4. Indeed, when the HPLC was run at 25% CH_3CN (C_{18} Kromasil column), CCK_4 exhibited a retention time of 13.3 min, versus 10 min for the sulfonic analogue. This synthesis is a useful example of an anionic isostere of a carboxylic moiety, aspartic acid being generally mimicked by serine *O*-sulfate. In biologi-

cal media, hydrolysis of the sulfate often readily takes place; this drawback is avoided when a sulfonate is used.

Conclusion

In summary, we have shown that the alkyl- and arylsulfonate-protecting neopentyl esters are compatible with amino acid synthesis and protection, as illustrated with cysteic and homocysteic acids. As both compounds are orthogonally protected, they can be used in further synthetic transformations as well as in peptide synthesis.

Experimental Section

General. NMR spectra were recorded on spectrometers operating at 270 and 400 MHz, in d_6-DMSO or $CDCl_3$. HPLC analyses were run on a C_{18} Kromasil reverse-phase column (5 μm, 100 Å), using CH_3CN/H_2O/TFA as the mobile phase. Cbz, Boc, and Fmoc groups were introduced by means of standard procedures described in the literature. Treatment with liquid HF was performed in a Teflon apparatus.

(Z-Cys-OEt)$_2$ (2a). Commercially available (H-Cys-OH)$_2$ purchased from ACROS Organics (14.2 g, 59 mmol) was solubilized in 220 mL of a 1:1 mixture of dioxane and water, and NaOH (4.73 g, 118 mmol) was added. At 0 °C, a solution of benzyl chloroformate (23.6 g, 138 mmol) in 40 mL of dioxane was added dropwise (2 h), as the pH was maintained around 9 by simultaneous addition of 1 N NaOH. After 1 h at this pH, the solution was washed with ether, acidified with 6 N HCl at 0 °C, and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. After concentration under reduced pressure, the oily residue was stirred in *n*-heptane overnight to allow the formation of white crystals which were filtered off and dried under vaccum (mp = 68 °C). They were stirred in ethanol with dropwise addition (3 drops/min of $SOCl_2$ at 0 °C, followed by stirring overnight at rt). After evaporation of the solvent, the residue was taken up in ethyl acetate, washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. After trituration in *n*-heptane, white crystals (26.5 g, 79%) were obtained: mp 83 °C; ^{1}H NMR (270 MHz, DMSO) 1.15 (3H, t, J = 7.2 Hz), 2.90 and 3.10 (2H, 2 dd, ABX), 4.08 (2H, q, J = 7.2 Hz), 4.25−4.34 (1H, m), 4.97 (2H, 2d, AB), 7.2−7.3 (5H, m); $[\alpha]^{20}_D$ = −67.5 (*c* 1.32, absolute EtOH).

(Z-Homocys-OEt)$_2$ (2b). According to the procedure described for **2a**, 4.1 g (92%) was obtained from 2.0 g (7.5 mmol) of commercially available (H-Homocys-OH)$_2$, purchased from Bachem. **2b**: ^{1}H NMR (270 MHz, DMSO) 1.14 (3H, t, J = 7.1 Hz), 1.84−2.1 (2H, m), 2.65−2.75 (2H, m), 3.96−4.16 (3H, m), 5.0 (2H, dd, AB), 7.2−7.38 (5H, m), 7.74 (1H, d, J = 8.2 Hz); TLC R_f = 0.67, eluent [AcOEt/cyclohexane = 1:1].

Ethyl 2(*S*)-2-[(Benzyloxycarbonyl)amino]-3-(neopentyloxysulfonyl)propanoate (3a). Compound **2a** (15.1 g, 27 mmol) was solubilized in CCl_4/EtOH = 4:1 (135 mL), and gaseous chlorine was bubbled at 0 °C through the solution during 40 min. The ice bath was removed, and stirring continued for an additional 20 min. Solvents were thoroughly evaporated, and the residue was dried under vaccum at 40 °C for 30 min. It was solubilized in dichloromethane (300 mL). At 0 °C neopentyl alcohol (5.91 g, 67 mmol) was added, followed by dropwise addition of Et_3N (10.3 g, 74 mmol). After 40 min of stirring at rt, the reaction mixture was diluted with ether, washed with 2 N HCl, $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel [AcOEt/cyclohexane = 1:4] afforded pure **3a** (15.2 g, 71%) as a pale yellow oil: ^{1}H NMR (270 MHz, DMSO) 0.85 (9H, s), 1.13 (3H, t, J = 7.4 Hz), 3.58−3.76 (2H, 2 dd, ABX spectrum), 3.85 (2H, dd, AB spectrum), 4.08 (2H, q, J = 7.4 Hz), 4.47 (1H, m), 5.00 (2H, s), 7.25−7.35 (5H, m), 7.95 (1H, d, J = 8.3 Hz); TLC R_f = 0.23, eluent [AcOEt/cyclohexane = 1:4]; HPLC [CH_3CN/H_2O/TFA = 50:50:0.05] t_R = 35.4 min; $[\alpha]^{20}_D$ = −20.8 (*c* 0.99, absolute EtOH). Anal. Calcd for $C_{18}H_{27}NO_7S$: C, 53.85; H, 6.78; N, 3.49. Found: C, 53.94; H, 6.86; N, 3.49.

Ethyl 2(*S*)-2-[(Benzyloxycarbonyl)amino]-3-(neopentyloxysulfonyl)butanoate (3b). Starting from 5.2 g (8.8 mmol) of compound **2b**, 6.6 g (16.4 mmol, 94%) of **3b** was obtained: ^{1}H NMR (270 MHz, DMSO) 0.88 (9H, s), 1.14 (3H, t, J = 7.2 Hz), 1.9−2.18 (2H, m), 3.16−3.48 (2H, m), 4.08 (2H, q, J = 7.2 Hz), 4.14−4.24 (1H, m), 5.0 (2H, s), 7.28−7.34 (5H, m), 7.82 (1H, d, J = 8 Hz); HPLC [CH_3CN/H_2O/TFA = 60:40:0.04] t_R = 14.6 min.

2(*S*)-2-[(Benzyloxycarbonyl)amino]-3-(neopentyloxysulfonyl)propanoic Acid (4a). To a solution of HCl (11.6 N, 52 mL), water (52 mL), and dioxane (104 mL) was added ester **3a** (10.6 g, 26.4 mmol), and the reaction mixture was stirred at 50 °C during 14 h. After evaporation to dryness and coevaporation with toluene (150 mL), compound **4a** (9.6 g, 97%) was cleanly obtained as a white powder: mp = 95 °C; ^{1}H NMR (270 MHz, DMSO) 0.86 (9H, s), 3.6 and 3.74 (2H, 2 dd, ABX), 3.84 (2H, 2 d, AB), 4.38−4.46 (1H, m), 5.02 (2H, s), 7.2−7.35 (5H, m), 7.8 (1H, d, J = 8 Hz); TLC R_f = 0.28, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; HPLC [CH_3CN/H_2O/TFA = 50:50:0.05] t_R = 12.3 min; $[\alpha]^{20}_D$ = −14.8 (*c* 1.08, absolute EtOH). Anal. Calcd for $C_{16}H_{23}NO_7S$: C, 51.46; H, 6.21; N, 3.75. Found: C, 51.35; H, 6.10; N, 3.89.

2(*S*)-2-[(Benzyloxycarbonyl)amino]-4-(neopentyloxysulfonyl)butanoic Acid (4b). According to the procedure described for **4a**, 5.5 g (100%) was obtained from 5.9 g (14.2 mmol) of **3b**: ^{1}H NMR (270 MHz, DMSO) 0.88 (9H, s), 1.92−2.16 (2H, m), 3.14−3.48 (2H, m), 3.82 (2H, s), 4.06−4.14 (1H, m), 5.0 (2H, s), 7.26−7.38 (5H, m), 7.54 (1H, d, J = 8 Hz); TLC R_f = 0.16, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; HPLC [CH_3CN/H_2O/TFA = 60:40:0.04] t_R = 6.6 min; MS (ESI) 388 (MH^+), 410 (MNa^+).

2(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(neopentyloxysulfonyl)propanoic Acid (5a). A suspension of compound **4a** (480 mg, 1.29 mmol), Et_3N (143 mg, 1.41 mmol), Boc_2O (282 mg, 1.29 mmol), and 10% Pd on carbon (137 mg, 0.13 mmol) in AcOEt (5 mL) was stirred for 24 h under H_2 (1 atm). After removal of the catalyst and washing with ethyl acetate, the filtrate was washed with 2 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford **5a** (400 mg, 92%) as a white powder: mp 115 °C; ^{1}H NMR (270 MHz, DMSO) 0.9 (9H, s), 1.35 (9H, s), 3.64 (2H, 2dd, ABX), 3.84 (2H, dd, AB), 4.28−4.36 (1H, m), 7.25 (1H, d, J = 8 Hz); TLC R_f = 0.27, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; HPLC [CH_3CN/H_2O/TFA = 60:40:0.04] t_R = 6.1 min. Anal. Calcd for $C_{13}H_{25}NO_7S$: C, 46.00; H, 7.42; N, 4.13. Found: C, 46.19; H, 7.37; N, 4.14.

2(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-4-(neopentyloxysulfonyl)butanoic Acid (5b). According to the procedure described for **5a** and starting from 184 mg (0.47 mmol) of **4b**, 152 mg (90%) of **5b** was obtained: ^{1}H NMR (270 MHz, DMSO) 0.9 (9H, s), 1.34 (9H, s), 1.9−2.15 (2H, m), 3.3−3.48 (2H, m), 3.82 (2H, s), 3.98−4.06 (1H, m), 7.16 (1H, d, J = 7.6 Hz); TLC R_f = 0.23, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; $[\alpha]^{20}_D$ = +1.7 (*c* 0.99, absolute EtOH). Anal. Calcd for $C_{14}H_{27}NO_7S$: C, 47.58; H, 7.70; N, 3.96. Found: C, 47.62; H, 7.79; N, 4.00.

Ethyl 2(*S*)-2-Amino-3-(neopentyloxysulfonyl)propanoate, Hydrochloride (6a). Compound **3a** (650 mg, 1.62 mmol) in absolute EtOH (15 mL) was stirred under 1 atm H_2 in the presence of 12 N HCl (0.2 mL, 2.4 mmol) during 2 h. The catalyst was filtered off and the filtrate evaporated to dryness and dried under vacuum to afford the hydrochloride **6a** (490 mg, 100%) as a white powder: ^{1}H NMR (270 MHz, DMSO) 0.9 (9H, s), 1.24 (3H, t, J = 7.2 Hz), 3.92−3.98 (4H, m), 4.18 (2H, t, J = 7.2 Hz), 4.42 (1H, t, J = 5.6 Hz), 8.88 (3H, br s); $[\alpha]^{20}_D$ = +7.8 (*c* 0.94, absolute EtOH); MS (ESI) 268 (M^+). Anal. Calcd for $C_{10}H_{22}NO_5SCl$: C, 39.54; H, 7.30; N, 4.61. Found: C, 38.82; H, 6.59; N, 4.69.

Ethyl 2(*S*)-2-Amino-4-(neopentyloxysulfonyl)butanoate, Hydrochloride (6b). According to the procedure described for **6a** and starting from 470 mg (1.17 mmol) of **3b**, 372 mg (100%) of **6b** was obtained: ^{1}H NMR (270 MHz, DMSO) 0.88 (9H, s), 1.18 (3H, t, J = 7.4 Hz), 2.15−2.25 (2H, m), 2.46−2.54 (2H, m), 3.85 (2H, s), 4.1−4.24 (3H, m), 8.58 (3H, br s).

2(*S*)-2-Amino-3-(neopentyloxysulfonyl)propanoic Acid, Hydrochloride (7a). Compound **4a** (665 mg, 1.78 mmol) in AcOEt (15 mL) was stirred under 1 atm H_2 during 36 h. The catalyst was filtered off and washed with 0.2 mL of concd HCl diluted in 20 mL of EtOH. The filtrate was evaporated to dryness and dried under vacuum to afford the hydrochloride **7a** (490 mg, 100%) as a white powder: mp 141 °C; ^{1}H NMR (270 MHz, DMSO) 0.9 (9H, s), 3.48−3.54 (1H, m), 3.92 (2H, 2d, AB); $[\alpha]^{20}_D$ = −2.4 (*c* 0.27, absolute EtOH); MS (ESI) 240 (M^+). Anal. Calcd

for $C_8H_{18}NO_5SCl$: C, 34.85; H, 6.21; N, 5.08. Found: C, 36.40; H, 6.67; N, 5.40.

2(*S*)-2-Amino-3-(neopentyloxysulfonyl)butanoic Acid (7b). According to the procedure described for **7a** except that no HCl was added, and starting from 834 mg (2.15 mmol) of **4b**, 540 mg (99%) of **7b** was obtained: mp 158 °C; ^{1}H NMR (270 MHz, DMSO + TFA) 0.87 (9H, s), 2.11−2.29 (2H, m), 3.36−3.56 (2H, m), 3.83 (2H, s), 3.98−4.04 (1H, m), 8.29 (3H, br s); $[\alpha]^{20}_D$ = +12.6 (*c* 0.53, absolute EtOH); MS (ESI) 254 (MH^+). Anal. Fits with $C_9H_{19}NO_5S\cdot{}^1/_2H_2O$: Calcd, C, 41.21; H, 7.68; N, 5.34. Found: C, 41.47; H, 7.28; N, 5.37.

***tert*-butyl 2(*S*)-2-[(Benzyloxycarbonyl)amino]-3-(neopentyloxysulfonyl)propanoate (8a).** Compound **4a** (1.87 g, 5.01 mmol) was placed into a high-pressure flask. Ether (30 mL) was added, followed by isobutylene (approximately 30 mL) at −78 °C and H_2SO_4 (0.2 mL). After 7 d at rt, the reaction mixture was cooled and cold water (5 mL) was added. Subsequent extractive workup followed by flash chromatography on silica gel (eluent cyclohexane/AcOEt = 7:3) afforded **8a** (704 mg, 33%) as a pale yellow oil: ^{1}H NMR (270 MHz, $CDCl_3$) 0.88 (9H, s), 1.42 (9H, s), 3.64−3.72 (2H, m), 3.80 (2H, dd, AB), 3.48−3.56 (1H, m), 5.06 (2H, s), 5.68 (1H, d, 8 Hz), 7.28−7.32 (5H, m); TLC R_f = 0.53, eluent [AcOEt/cyclohexane = 3:7]; $[\alpha]^{20}_D$ = −20.3 (*c* 0.91, absolute EtOH). Anal. Calcd for $C_{20}H_{31}NO_7S$: C, 55.93; H, 7.28; N, 3.26. Found: C, 56.06; H, 7.18; N, 3.19.

2(*S*)-2-[(9-Fluorenylmethyloxycarbonyl)amino]-3-(neopentyloxysulfonyl)propanoic Acid (9a). To a suspension of **7a** in the zwitterionic form (1.85 g, 7.73 mmol) in H_2O (55 mL) and dioxane (14 mL) was added at 0 °C $Na_2CO_3\cdot10H_2O$ (6.64 g) followed by a solution of Fmoc-Cl (2.20 g, 8.50 mmol) in dioxane (45 mL). After stirring overnight at rt, the reaction mixture was acidified with 2 N HCl and extracted with ethyl acetate. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure and then dried under vacuum to afford clean **9a** (3.38 g, 95%) as a white powder: mp 101 °C; ^{1}H NMR (270 MHz, DMSO) 0.86 (9H, s), 3.56 (2H, 2 dd, ABX), 3.85 (2H, dd, AB), 4.24 (2H, 2 dd, ABX), 4.36−4.46 (1H, m), 7.26 (2H, t, 7.8 Hz), 7.38 (2H, t, 7.8 Hz), 7.68 (2H, d, 7.8 Hz), 7.86 (2H, d, 7.8 Hz), 7.88 (1H, d, 8.0 Hz); TLC R_f = 0.29, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; HPLC [CH_3CN/H_2O/TFA = 50:50:0.05] t_R = 36.3 min; $[\alpha]^{20}_D$ = −13.4 (*c* 0.85, absolute EtOH). Anal. Calcd for $C_{23}H_{27}NO_7S$: C, 59.86; H, 5.90; N, 3.04. Found: C, 59.95; H, 5.79; N, 3.00.

2(*S*)-2-[(9-Fluorenylmethyloxycarbonyl)amino]-4-(neopentyloxysulfonyl)butanoic Acid (9b). According to the procedure described for **9a**, followed by flash chromatography through silica gel (eluent cyclohexane/Et_2O/HCOOH = 1:1:0.02), and starting from 197 mg (2.15 mmol) of **4b**, 240 mg (65%) of **9b** was obtained: mp 114 °C; ^{1}H NMR (270 MHz, DMSO + TFA) 0.88 (9H, s), 1.94−2.18 (2H, m), 3.14−3.44 (2H, m), 3.82 (2H, s), 4.06−4.20 (3H, m), 4.28 (2H, d, *J* = 7 Hz), 7.28 (2H, t, *J* = 7. 8 Hz), 7.36 (2H, t, *J* = 7. 8 Hz), 7.66 (2H, d, *J* = 7. 8 Hz), 7.82 (2H, d, *J* = 7. 8 Hz); TLC R_f = 0.24, eluent [Et_2O/cyclohexane/HCOOH = 1:1:0.02]; HPLC [CH_3CN/H_2O/TFA = 60:40:0.04] t_R = 14.9 min; $[\alpha]^{20}_D$ = −1.3 (*c* 1.07, absolute EtOH). Anal. Calcd for $C_{24}H_{29}NO_7S$: C, 60.62; H, 6.15; N, 2.95. Found: C, 60.47; H, 6.24; N, 2.94.

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Supporting Information Available: ^{1}H and ^{13}C spectra of most compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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