Stereoselective Synthesis of Fluorescent α-Amino Acids Containing Oxine (8-Hydroxyquinoline) and Their Peptide Incorporation in Chemosensors for Divalent Zinc

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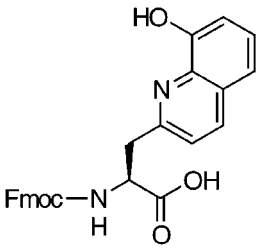
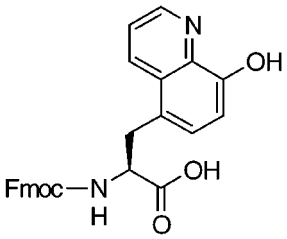
Received March 26, 1998

The development of fluorescent chemosensors for the detection of ionic species has been a focus for many researchers.[1] In the continuing search for new sensing molecules, engineering ligands which exhibit selective and avid divalent metal binding in aqueous solution is critical.[2,3] In light of these criteria, we have sought to harness within synthetic constructs the often unsurpassed metal-binding properties of naturally occurring peptides or proteins.[4] Highlighting the versatility of this approach, we have prepared selective chemosensors for both Zn(II) (based on the zinc finger domains)[5,6] and for Cu(II) (based on the amino-terminal Cu(II) and Ni(II) binding domains of the serum albumins).[7] More recently, we have begun to convert these and other solution-based chemosensing *reagents* to regenerable devices by attaching them to water-solvated resins. In this context, the relatively large size of our Zn(II) fluorosensors (25 residues) is prohibitive to the production of sensing materials with well-defined composition. Toward the goal of minimizing the size of the peptidyl component required for a successful Zn(II) sensor, we have developed new residues for solid-phase peptide synthesis that contain the high-affinity, bidentate metal-binding functionality of the well-known ligand oxine (8-hydroxyquinoline); this compound and its derivatives have historically found use as fluorometric indicators of Zn(II),[8] as well as several other cations.[9] We now report the syntheses of (*S*)-2-amino-N^{α}-9-fluorenylmethoxycarbonyl-3-(oxine-2-yl)propionic acid (Fmoc-2Oxn-OH, **1**) and (*S*)-2-amino-N^{α}-9-fluorenylmethoxycarbonyl-3-(oxine-5-yl)propionic acid (Fmoc-5Oxn-OH, **2**), as well as the incorporation of these residues in short peptides (7 residues) capable of reporting sub-micromolar levels of Zn(II).



1
Fmoc-2Oxn-OH



2
Fmoc-5Oxn-OH

The synthesis of **1**, employing the Myers pseudoephedrine glycinamide alkylation[10,11] in the key stereogenic step, is outlined in Scheme 1. Starting from commercially available 8-hydroxy-2-methylquinoline (**3**), bromide **4** was prepared in two steps via protection of the phenol as the benzenesulfonate ester, followed by free radical bromination. The (*R,R*)-(−) enantiomer of pseudoephedrine glycinamide (**5**) was chosen for alkylations in order to yield as the major diastereomer the α-amino acid derivative **6** possessing the L-configuration.[12] In general, 2 equiv of **5** per equiv of bromide **4** were used in order to drive the reactions to completion within 1−3 h when conducted at 0 °C. No attempt was made to determine the diastereoselectivity of the alkylation reaction, yet isolated yields of **6** as high as 89% were achieved, in >95% de as determined by ^{1}H NMR. Basic hydrolysis of **6** to free the α-amino acid from the benzenesulfonate ester and pseudoephedrine auxiliary, followed by protection of the newly liberated α-amine as the 9-fluorenylmethoxycarbonyl (Fmoc) derivative, provided **1** in 90% yield and >95% ee.[13]

The synthesis of **2**, outlined in Scheme 2, was performed using a strategy analogous to that used for the preparation of **1**. Methylquinoline **7** was prepared in two steps from oxine using literature methods.[14] Protection (as the benzensulfonate ester) and bromination to yield **8** proceeded smoothly in quantitative and moderate yields, respectively. As for the preparation of **6**, stereoselective alkylations of **8** (to yield product **9**) were performed with 2 equiv of **5** to drive the reactions to completion within 3 h at 0 °C. Finally, hydrolysis and Fmoc protection of **9** were performed to provide **2** in excellent yield with high optical purity (>99% ee).

To demonstrate the utility of these newly developed residues, **1** and **2** were used for the preparation of heptapeptides via standard Fmoc-based solid-phase peptide synthesis techniques.[15] Peptides **P1** and **P2** thus contain the oxine functionality formally attached to the

(1) For a recent review see: de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

(2) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423−428.

(3) Czarnik, A. W. Supramolecular Chemistry, Fluorescence, and Sensing. In *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993; pp 1−9.

(4) Glusker, J. P. *Adv. Protein Chem.* **1991**, *42*, 1−76.

(5) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053−3054.

(6) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443−3450.

(7) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 6609−6610.

(8) Vogel, A. I. Spectrofluorimetry. In *Vogel's Textbook of Quantative Chemical Analysis*; Wiley: New York, 1989; Chapter 18, pp 731−740.

(9) Ions that form fluorescent complexes with oxine include Mg(II), Ca(II), Pb(II), and Sr(II), among others. For a leading reference, see: Seitz, W. R. *Crit. Rev. Anal. Chem.* **1980**, *8*, 367−405.

(10) Myers, A. G.; Gleason, J. L.; Yoon, T. Y. *J. Am. Chem. Soc.* **1995**, *117*, 8488−8489.

(11) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656−673.

(12) The stereochemical outcome of the Myers alkylation has proven to be predictable for a wide variety of electrophiles. See ref 11 and references therein.

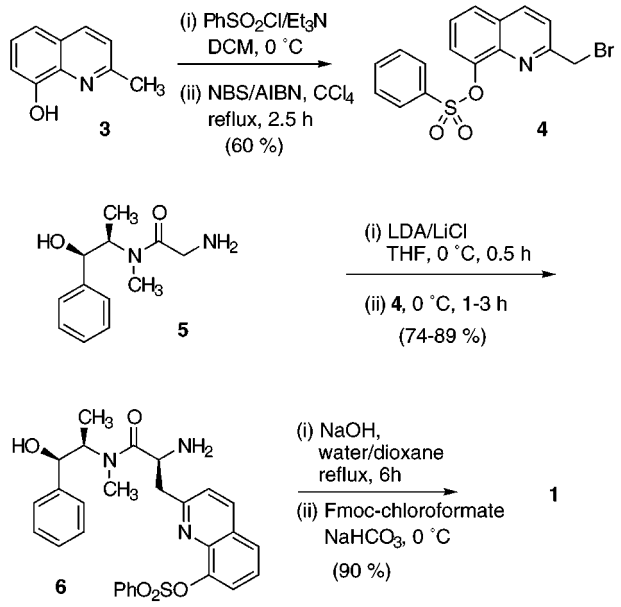
(13) The optical integrity of compounds **1** and **2** were verified by chiral HPLC analysis after removal of the N^{α}-Fmoc protecting group. See the Experimental Section.

(14) Burckhalter, J. H.; Leib, R. I. *J. Org. Chem.* **1961**, *26*, 4078−4083.

(15) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214.

S0022-3263(98)00563-5 CCC: $15.00
Published on Web 08/28/1998

Scheme 1



Scheme 2

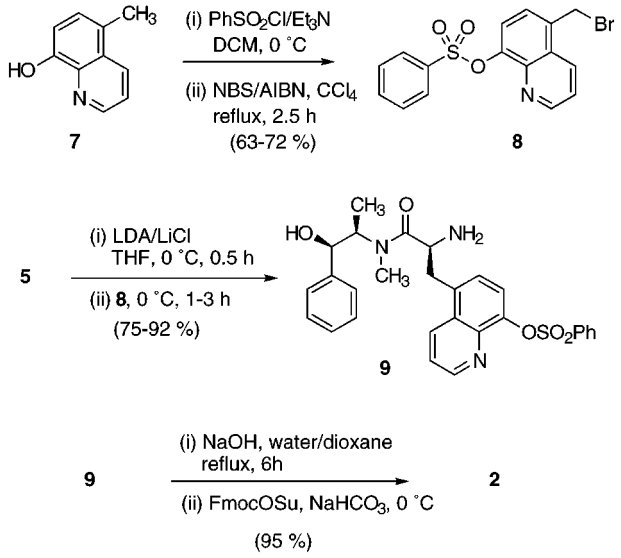


Chart 1

Ac - Xaa - Val - Pro - DSer - Phe - Cys - Ser - NH_2

P1: Xaa = 2Oxn
P2: Xaa = 5Oxn

side chain of an *N*-terminal alanine residue through the 2-position (2Oxn) and 5-position (5Oxn) of the heterocycle, respectively (Chart 1). Furthermore, the sequences of these peptides were designed to contain the internal sequence -Val-Pro-DSer-Phe- which has been demonstrated in prior studies to promote reverse-turn formation in aqueous solution.[16] The side chains of the flanking metal-binding residues are therefore placed in proximity (underlined in Chart 1); this arrangement has been observed to result in enhanced metal-binding properties.[17,18] To simultaneously enhance the metal-binding

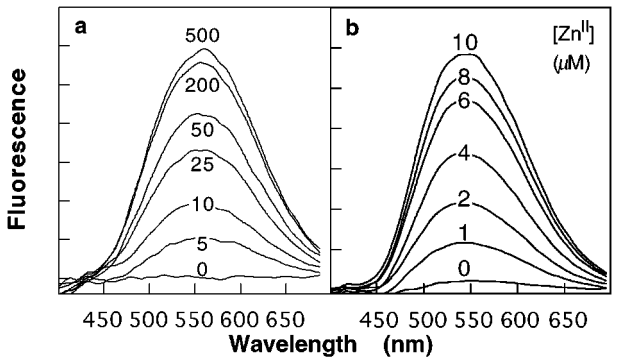


Figure 1. Fluorescence emission spectra of 10 μM solutions of **3** (panel a) and **P1** (panel b) taken after the addition of the indicated concentration of $ZnCl_2$ (in μM) with excitation at 350 nm. Spectra were taken at room temperature in 50 mM HEPES, pH 7.0, 150 mM NaCl and have been baseline subtracted against a sample containing buffer only.

selectivity of **P1** and **P2** for Zn(II), cysteine was incorporated as the additional intended ligand, which by virtue of its soft character may be expected to favor Zn(II) binding,[19] particularly in comparison to the hard ions such as Mg(II) and Ca(II) which also form fluorescent complexes with oxine.

Peptide **P2** was found to suffer from rapid oxidation to form the disulfide dimer under normal handling conditions (pH 7.0, rt, and exposure to air), thereby complicating the metal-binding analysis for this compound. However, **P1** exhibited avid Zn(II) complexation to form a fluorescent complex with 1:1 stoichiometry. Metal-binding titrations monitoring both UV−vis and fluorescence emission were performed at room temperature in pH 7.0 buffer (50 mM HEPES/150 mM NaCl) to determine the apparent dissociation constant of the **P1**−Zn(II) complex (100 nM) and that of the relevant model complex **3**−Zn(II) (17 μM, data not shown). This 170-fold enhancement in Zn(II) binding affinity suggests that intramolecular participation of the cysteine residue of **P1** assists in metal binding. This effect is highlighted by a comparison of the fluorescence response of 10 μM solutions of **3** and **P1** to Zn(II) (Figure 1). Furthermore, at lower concentrations of chemosensor, sub-micromolar concentrations of Zn(II) are clearly distinguishable, with the limit of detection less than 250 nM (Figure 2).

The response of peptide **P1** has also been studied in the presence of a number of competing metal ion species. At 10 μM **P1**, measurements made in the presence of 1 equiv of Zn(II) and 1 equiv of a competing metal ion reveal no significant competition from Mg(II), Ca(II), Mn(II), Fe(III), Co(II), or Ni(II) and only minor competition (approximately 10% binding) from Fe(II) and Cd(II). The only metal ion which shows competition is Cu(II); approximately 40% Cu(II) binding is observed at equivalent concentrations of the two metal ions, suggesting that the binding of **P1** to Zn(II) is slightly better compared to Cu(II).

In conclusion, this paper describes the stereoselective synthesis and peptide incorporation of two novel α-amino acids that contain the bidentate metal-binding functionality of oxine (8-hydroxyquinoline). These early results demonstrate that sensitive and selective reporting of Zn-

(16) Imperiali, B.; Fisher, S. L.; Moats, R. A.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3182−3188.
(17) Imperiali, B.; Kapoor, T. M. *Tetrahedron* **1993**, *49*, 3501−3510.
(18) Cheng, R. P.; Fisher, S. L.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 11349−11356.
(19) Fraústo da Silva, J. J. R.; Willams, R. J. P. *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*; Clarendon Press: New York, 1993; p 35.

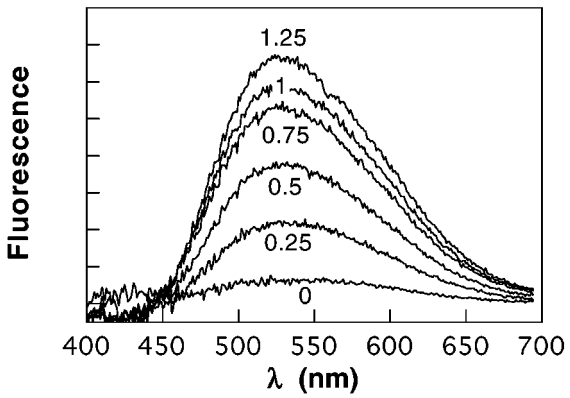


Figure 2. Fluorescence emission spectra of a 1 μM solution of **P1** taken after the addition of the indicated concentration of $ZnCl_2$ (in μM). Spectra were acquired using the same buffer and conditions described for Figure 1.

(II) is feasible within short peptides (7 residues). The new residues **1** and **2** will be useful tools in the continuing development of other peptidyl fluorescent chemosensors, a task which we are currently pursuing and will report on in due course.

Experimental Section

General Experimental Procedures. Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were of the highest possible purity and used without further purification. Tetrahydrofuran and diethyl ether were distilled from sodium benzophenone ketyl. Diisopropylamine, dichloromethane (DCM), 2-butanol, and toluene were distilled from calcium hydride. Lithium chloride was dried under vacuum at 150 °C for ≥12 h and flame dried immediately prior to use. *n*-Butyllithium was titrated with an analytically prepared solution of 2-butanol in toluene using 2,2′-bipyridyl in diethyl ether as indicator.[11,20] Anhydrous reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Thin-layer chromatography was performed using plates obtained from EM (SiO_2 60, F-254). Flash column chromatography was carried out according to the procedure of Still,[21] using 230−400 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$, unless otherwise noted. All melting points reported are uncorrected.

Chiral HPLC Analysis. Analyses were performed on aliquots of the intermediate α-amino acids taken from the hydrolyses of **6** and **9**, as well as of samples resulting from deprotection of the N^{α}-Fmoc-amino acids **1** and **2** by treatment with 20% piperidine in DMF. Optical purity was assessed using a Crown-Pak CR (+) analytical column (Daicel) [eluent 0.1 N aqueous $HClO_4$, flow rate 0.5 mL/min, UV detection (320 nm)]. Retention times: 2Oxn, 14.0 min (D), 15.0 min (L); 5Oxn, 10.1 min (D), 12.7 min (L).

Peptide Synthesis. Peptides were assembled on macro crown supports (Chiron, 6 μmol/crown) using standard Fmoc chemistry batch synthesis techniques. Iterative removal of N^{α}-Fmoc protecting groups was performed with piperidine (20% v/v in DMF) for 10 min, followed by washing with DMF (2 × 5 min) and MeOH (1 × 2 min, 2 × 5 min), allowing the support to dry for 5 min, and then reswelling the support in DMF (5 min). Coupling reactions were carried out using diisopropylcarbodiimide/1-hydroxybenzotriazole (DIPCDI/HOBT) activation chemistry using 0.1 M active ester in sufficient volume of DMF to provide a 6-fold excess of coupling reagent to support-bound amine for a minimum of 4 h. At the completion of the synthesis, the final Fmoc protecting group was removed, and the *N*-terminal α-amine acetylated with 1 mL of 0.1 M acetic anhydride/0.1 M diisopropylethylamine (DIEA) for 2 h. Following acetylation, an additional piperidine deblock cycle was performed to deprotect any aryl acetate ester that formed during the acetylation procedure. Final peptide deprotection was achieved with 5% v/v triisopropylsilane in TFA. The crude peptide was precipitated into 1:1 diethyl ether/hexanes, triturated using the same solvent (3 × 5 mL), and purified to homogeneity by reverse-phase (C_{18}) HPLC.

8-Benzenesulfonyloxy-2-methylquinoline. 8-Hydroxy-2-methylquinoline (**3**) (5.0 g, 31.4 mmol, 1 equiv) was dissolved in DCM (30 mL) and chilled to 0 °C with constant stirring. Triethylamine (5.29 mL, 37.7 mmol, 1.2 equiv) was added followed by the addition of benzenesulfonyl chloride (4.41 mL, 34.5 mmol, 1.1 equiv) dropwise over 15 min. After 0.5 h, the clear solution had become a white slurry, and the ice bath was removed. The reaction was allowed to warm to room temperature and was stirred for an additional 1.5 h. Water (10 mL) was added, and the biphasic reaction mixture was stirred vigorously an additional 0.5 h to quench any remaining benzenesulfonyl chloride. The slurry was transferred to a separatory funnel and the volume of DCM was increased to 100 mL. The organic layer was washed with 100 mL of saturated aqueous K_2CO_3. The layers were separated, and the aqueous layer was extracted with DCM (2 × 50 mL). The organic layers were combined, dried (K_2CO_3), and concentrated to afford the desired product as a white solid (9.33 g, 99%): mp 109−110 °C; R_f = 0.18 (75:25 hexanes/EtOAc), R_f = 0.33 (100:1 DCM/EtOAc); ^{1}H NMR δ 7.96 (m, 3H), 7.68 (dd, J = 1.2, 8.1 Hz, 1H), 7.64 (dd, J = 1.2, 7.8 Hz, 1H), 7.56 (tt, J = 2.1, 7.5 Hz, 1H), 7.35−7.45 (m, 3H), 7.19 (d, J = 8.4 Hz, 1H), 2.50 (s, 3H); ^{13}C NMR δ 25.1, 122.6, 123.0, 125.0, 126.7, 127.8, 128.4, 128.8, 133.6, 135.6, 136.7, 140.8, 145.0, 159.5; IR (thin film, cm^{-1}) 3064, 1605; HRMS (FAB) calcd for $C_{16}H_{14}NO_3S$ $[M + H]^+$ 300.0694, found 300.0691.

8-Benzenesulfonyloxy-2-bromomethylquinoline (4). 8-Benzenesulfonyloxy-2-methylquinoline (5.0 g, 16.6 mmol, 1.0 equiv) was placed in a round-bottom flask with deoxygenated CCl_4 (40 mL), the flask was fitted with a reflux condenser, and the mixture was heated to reflux to dissolve. *N*-Bromosuccinimide (4.14 g, 23.2 mmol, 1.4 equiv) was added, followed by 2,2′-azobis(2-methylpropiononitrile) (AIBN, 295 mg, 1.55 mmol, 0.1 equiv), and reflux was continued. Additional aliquots of AIBN (295 mg) were added at 0.5 h intervals until a total of 5 additions had been made. At 0.5 h after the final addition, the reaction flask was allowed to cool to room temperature, and the solvent was removed under reduced pressure. The resulting residue was suspended in EtOAc (400 mL), transferred to a separatory funnel, and washed with 1:1 saturated aqueous Na_2CO_3/Na_2SSO_3 (300 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (75 mL). The organic layers were combined, dried (K_2CO_3), and concentrated. The resulting residue was dissolved in a minimal quantity of chloroform and purified by silica flash chromatography (eluent 75:25 hexanes/EtOAc, R_f = 0.22). Both starting material (1.7 g) and the product were collected by concentration of the appropriate fractions. NMR analysis of the product revealed the presence of a contaminant (singlet at 1.73 ppm, $CDCl_3$) which was assigned to the radical termination product 2,3-dicyano-2,3-dimethylbutane. Recrystallization from hot EtOAc/hexanes provided the pure bromide **4** (3.25 g, 71% based on recovery of starting material) as a crystalline white solid: mp 138−139 °C; R_f = 0.40 (65:35 hexanes/EtOAc) R_f = 0.60 (100:1 DCM/EtOAc); ^{1}H NMR δ 8.12 (d, J = 8.4 Hz, 1H), 7.98 (dd, J = 8.4, 1.2 Hz, 2H), 7.74 (d, J = 8.1 Hz, 2H), 7.61 (m, 1H), 7.45−7.6 (m, 4H), 4.44 (s, 2H); ^{13}C NMR δ 33.9, 122.0, 123.8, 126.6, 126.7, 128.5, 128.7, 128.8, 134.0, 137.0, 137.1, 140.2, 145.3, 157.3; IR (thin film, cm^{-1}) 3605, 1600; HRMS (FAB) calcd for $C_{16}H_{13}NO_3SBr$ $[M + H]^+$ 377.9800, found 377.9797.

8-Benzenesulfonyloxy-5-methylquinoline. 8-Hydroxy-5-methylquinoline[14] (**7**) (6.23 g, 39.1 mmol, 1.0 equiv) was dissolved in DCM (90 mL) and chilled to 0 °C with constant stirring. Triethylamine (6.58 mL, 46.9 mmol, 1.2 equiv) was added, followed by the dropwise addition of benzenesulfonyl chloride (5.48 mL, 43.0 mmol, 1.1 equiv) over 15 min. After 0.5 h, the clear solution had become a white slurry, and stirring was continued overnight (10 h). Water (20 mL) was added, and the biphasic mixture was stirred vigorously for 1 h to ensure consumption of the remaining benzenesulfonyl chloride. The

(20) Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165−168.
(21) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

organics were removed under reduced pressure, and the aqueous phase was transferred to a separatory funnel. Saturated aqueous Na_2CO_3 (100 mL) was added, and the mixture was extracted with EtOAc (2 × 75 mL). The organic layers were combined, dried (K_2CO_3), and concentrated to afford a white solid. The solid was dissolved in a minimal quantity of $CHCl_3$ and loaded onto a silica flash column (eluent 65:35 hexanes/EtOAc, R_f = 0.21) to afford the desired product (10.95 g, 94%): mp 106−108 °C; R_f = 0.4 (1:1 hexanes/EtOAc); ^{1}H NMR δ 8.77 (dd, J = 1.5, 4.2 Hz, 1H), 8.26 (dd, J = 1.5, 8.7 Hz, 1H), 7.98 (dd, J = 0.6, 7.2 Hz, 2H), 7.59 (tt, J = 1.2, 7.5 Hz, 1H), 7.4−7.5 (m, 3H), 7.4 (dd, J = 4.2, 8.7 Hz, 1H), 7.32 (dd, J = 0.9, 7.8 Hz, 1H), 2.65 (s, 3H); ^{13}C NMR δ 18.3, 121.3, 122.1, 122.5, 126.1, 128.7, 132.3, 133.7, 134.1, 136.3, 138.6, 141.5, 143.9, 150.1; IR (thin film, cm^{-1}) 3062, 1598; HRMS (FAB) calcd for $C_{16}H_{14}NO_3S$ [M + H]$^+$ 300.0694, found 300.0697

8-Benzenesulfonyloxy-5-bromomethylquinoline (8). 8-(Benzenesulfonyloxy)-5-methylquinoline (1.28 g, 4.28 mmol, 1.0 equiv) was placed in a round-bottom flask with deoxygenated CCl_4 (30 mL), the flask was fitted with a reflux condenser, and the mixture was heated to reflux to dissolve. *N*-Bromosuccinimide (1.07 g, 6.0 mmol, 1.4 equiv) was added, followed by AIBN (70 mg, 0.43 mmol, 0.1 equiv), and reflux was continued. Additional aliquots of AIBN (70 mg) were added at 0.5 h intervals until a total of 5 additions had been made. At 0.5 h after the final addition, the reaction flask was allowed to cool to room temperature, and the solvent was removed under reduced pressure. The resulting residue was suspended in EtOAc (50 mL), transferred to a separatory funnel, and washed with 1:1 saturated Na_2CO_3/Na_2SSO_3 (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (50 mL). The organic layers were combined, dried (K_2CO_3), and concentrated. The resulting residue was dissolved in a minimal quantity of $CHCl_3$ and purified by silica flash chromatography (eluent 75:25 hexanes/EtOAc, R_f = 0.13) to yield **8** (1.02 g, 63%) as a white solid: mp 142−142 °C; R_f = 0.22 (65:35 hexanes/EtOAc), R_f = 0.60 (95:5 DCM/Et_2O); ^{1}H NMR δ 8.83 (dd, J = 1.8, 4.2 Hz, 1H), 8.44 (dd, J = 1.5, 8.7 Hz, 1H), 8.00 (dd, 1.2, 8.4 Hz, 2H), 7.63 (m, 1H), 7.56 (d, 4.2 Hz, 2H), 7.4−7.5 (m, 3H), 4.87 (s, 2H); ^{13}C NMR (125 MHz) δ 29.2, 121.9, 122.0, 127.4, 127.6, 128.7, 128.9, 132.1, 133.0, 134.0, 136.0, 142.0, 146.1, 150.8; IR (thin film, cm^{-1}) 3063, 1598; HRMS (FAB) calcd for $C_{16}H_{13}NO_3SBr$ [M + H]$^+$ 377.9800, found 377.9800.

[(*R*,*R*)-2*S*]-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methyl-2-amino-3-[2-(8-benzenesulfonyloxy)quinolinyl]-propionamide (6). A solution of *n*-butyllithium in hexanes (2.30 M, 1.70 mL, 3.90 mmol, 3.9 equiv) was added to a solution of diisopropylamine (560 μL, 4.0 mmol, 4.0 equiv) in deoxygenated THF (2 mL) at 0 °C. After 15 min, the resulting solution of lithium diisopropylamide (+ 2 mL wash) was transferred via cannula over 5 min to a stirred slurry of anhydrous (*R*,*R*)-(−)-pseudoephedrine glycinamide (454 mg, 2.0 mmol, 2.0 equiv) and flame-dried lithium chloride (509 mg, 12.0 mmol, 12.0 equiv) in deoxygenated THF (5 mL) at 0 °C. After 30 min at 0 °C, a solution of **4** (378 mg, 1.0 mmol, 1.0 equiv) in tetrahydrofuran (5 mL + 2 mL wash) at 0 °C was added slowly to the yellow enolate solution. The reaction mixture was stirred for 3 h at 0 °C. Water (30 mL) was added, the resulting biphasic mixture was warmed to room temperature, and the THF was removed under reduced pressure. The pH of the solution was made basic by the addition of aqueous NaOH (2 mL, 2 N), and the resulting aqueous phase was extracted with chloroform (3 × 20 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with DCM/MeOH/Et_3N (92:4:4). The *C*-alkylated diastereomers were separable under these conditions, and only those fractions containing the pure major diastereomer were collected. After concentration of the appropriate fractions, the product residue was concentrated from toluene (2 × 25 mL) and then chloroform (2 × 25 mL) to remove residual triethylamine. This afforded **6** as a pale yellow granular solid (0.47 g, 89%): mp 67−69 °C; R_f = 0.44 (90:5:5 $CHCl_3$/MeOH/TEA), R_f = 0.28 (95:5:1 $CHCl_3$/MeOH/NH_4OH); ^{1}H NMR (approximately 2:1 rotamer ratio, * denotes minor rotamer) δ 8.65* (d, J = 8.4 Hz, 0.33 H), 8.04 (d, J = 8.4 Hz, 0.67H), 7.9−8 (m, 2H), 7.2−7.8 (m, 12H), 4.74* (d, J = 8.1 Hz, 0.33H), 4.61 (d, J = 8.1 Hz, 0.67H), 4.4−4.6 (m, 2H), 3.46* (dd, J = 3.6, 14.7 Hz, 0.33H), 3.28* (dd, J = 8.1, 14.7 Hz, 0.33H), 3.09 (dd, J = 5.1, 14.7 Hz, 0.67H), 3.04 (s, 2H), 2.96* (s, 1H), 2.93 (dd, J = 8.4, 14.7 Hz, 0.67H), 1.5−2.5 (broad s, 3H), 1.04* (d, J = 6.6 Hz, 1H), 0.99 (d, J = 6.9 Hz, 2H); ^{13}C NMR δ 14.1, 15.5, 27.2, 31.3, 43.9, 51.2, 57.0, 57.7, 75.1, 75.4, 121.5, 122.0, 123.3, 123.5, 125.0, 125.1, 126.5, 126.6, 126.8, 127.1, 127.4, 127.8, 128.1, 128.2, 128.3, 128.4, 128.8, 128.9, 133.8, 135.8, 136.4, 140.9, 141.6, 142.0, 145.0, 159.9, 160.4, 174.8, 175.9; IR (thin film, cm^{-1}) 3360 (broad), 3062, 2982, 1625; HRMS (FAB) calcd for $C_{28}H_{30}N_3O_5S$ [M + H]$^+$ 520.1906, found 520.1909; [α]$^{20}_D$ = +61° (c = 1.0, $CHCl_3$).

[(*R*,*R*)-2*S*]-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methyl-2-amino-3-[5-(8-benzenesulfonyloxy)quinolinyl]-propionamide (9). A solution of *n*-butyllithium in hexanes (2.40 M, 1.63 mL, 3.90 mmol, 3.9 equiv) was added to a solution of diisopropylamine (560 μL, 4.0 mmol, 4.0 equiv) in deoxygenated THF (2 mL) at 0 °C. After 15 min, the resulting solution of lithium diisopropylamide (+ 2 mL wash) was transferred via cannula over 5 min to a stirred slurry of anhydrous (*R*,*R*)-(−)-pseudoephedrine glycinamide (454 mg, 2.0 mmol, 2.0 equiv) and flame-dried lithium chloride (509 mg, 12 mmol, 12 equiv) in deoxygenated THF (5 mL) at 0 °C. After 30 min of stirring at 0 °C, a solution of **8** (378 mg, 1.0 mmol, 1.0 equiv) in THF (3 mL + 2 mL wash) at 0 °C was added slowly to the yellow enolate solution. The mixture was stirred for 2 h at 0 °C. Water (30 mL) was added, the resulting biphasic mixture was warmed to room temperature, and the THF was removed under reduced pressure. The pH of the solution was made basic by the addition of NaOH (2 mL, 2 N), and the resulting aqueous phase was extracted with chloroform (3 × 25 mL). The combined organic layers were dried over anhydrous K_2CO_3, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel by gradient elution with DCM/MeOH/Et_3N (96.5:1.5:2 → 92:4:4). The *C*-alkylated diastereomers were separable under these conditions, and only those fractions containing the pure major diastereomer were collected. After concentration of the appropriate fractions, the product residue was concentrated from toluene (2 × 25 mL) and then chloroform (2 × 25 mL) to remove residual triethylamine. The product **9** was isolated as a pale yellow granular solid (482 mg, 92%): R_f = 0.55 (85:15:1 $CHCl_3$/MeOH/NH_4OH), R_f = 0.23 (90:5:5 $CHCl_3$/MeOH/Et_3N). ^{1}H NMR (1:2.3 rotameric forms, * denotes minor rotamer) δ 8.78* (d, J = 2.5 Hz, 0.3H), 8.74 (d, J = 2.5 Hz, 0.7H), 8.57* (d, J = 7.5 Hz, 0.3H), 8.39 (d, J = 7.5 Hz, 0.7H), 7.95−8.0 (m, 1.3H), 7.2−7.6 (m, 11.4H), 7.04* (d, J = 3.5 Hz, 0.3H), 4.60 (m, 0.7H), 4.43* (d, J = 8 Hz, 0.3H), 4.35 (d, J = 8 Hz, 0.7H), 4.2* (m, 0.3H), 4.14 (m, 0.7H), 4.01* (m, 0.3H), 3.66* (dd, J = 5 Hz, 14 Hz, 0.3H), 3.0−3.5 (m, 3.7H), 2.91* (s, 0.9H), 2.23 (s, 2.1H), 0.92* (d, J = 6 Hz, 0.9H), 0.46 (d, J = 6 Hz, 2.1H); ^{13}C NMR (contributions from both rotamers listed) δ 13.9, 14.0, 15.6, 27.2, 29.5, 37.6, 37.8, 51.8, 52.1, 55.2, 55.5, 58.0, 70.7, 71.2, 74.6, 75.2, 76.7, 121.95, 122.0, 126.7, 126.8, 127.3, 128.0, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 132.2, 132.6, 133.2, 133.9, 134.0, 136.1, 136.2, 141.3, 141.4, 141.5, 141.8, 144.4, 144.6, 150.2, 150.5, 174.9, 186.7; IR (thin film, cm^{-1}) 3350 (broad), 3062, 2980, 1626; HRMS (FAB) calcd for $C_{28}H_{30}N_3O_5S$ [M + H]$^+$ 520.1906, found 520.1906; [α]$^{20}_D$ = +12° (c = 1.0, $CHCl_3$).

(*S*)-2-Amino-*N*$^{\alpha}$-9-fluorenylmethoxycarbonyl-3-(oxine-2-yl)propionic Acid (1). Alkylated pseudoephedrine derivative **6** (100 mg, 0.192 mmol, 1.0 equiv) was dissolved in deoxygenated dioxane (1.5 mL), deoxygenated aqueous NaOH (0.5 M, 1.54 mL, 0.768 mmol, 4 equiv) was added, and the reaction mixture was heated to reflux. After 4 h, TLC analysis of the amber solution (*n*-BuOH/AcOH/H_2O/EtOAc 1:1:1:1) showed that only the deprotected amino acid (R_f = 0.23) remained as a UV-, ninhydrin-, and $FeCl_3$-active species. The solution was cooled, and the dioxane was removed under reduced pressure. The aqueous solution was washed with DCM (3 × 5 mL) to remove pseudoephedrine. A small aliquot of the aqueous phase (20 μL) was removed for chiral HPLC analysis, the remainder was chilled to 0 °C, and $NaHCO_3$ (64 mg, 0.768 mmol, 4.0 equiv) was added. A solution of 9-fluorenylmethoxychloroformate (60 mg, 0.23 mmol, 1.2 equiv) in dioxane (1.5 mL) was added, and the reaction mixture was stirred for 8 h. The dioxane was removed under reduced pressure, and HCl (1 N) was added dropwise until the solution was ca. pH 4. Water was added to increase the volume to ca. 10 mL, and the solution was extracted with DCM (3 × 10

mL). The organic layers were concentrated, and the resulting yellow residue was concentrated from toluene (2 × 25 mL) to remove traces of water. The residue was dissolved in $CHCl_3$ (2 mL) and precipitated by addition to a rapidly stirred solution of 1:1 hexanes/ether (25 mL). The yellow precipitate was filtered and dried under vacuum for 6 h to afford 81 mg (90%) of a pale yellow solid: $R_f = 0.70$ (85:15:3 $CHCl_3$/MeOH/AcOH); ^{1}H NMR (MeOH-d_4) δ 3.64 (t, $J = 9.9$ Hz, 1H), 3.7−4.5 (m, 3H), 4.81 (m, 1H), 7.0−8.0 (m, 12H), 8.9 (d, $J = 8.7$ Hz, 1H); ^{13}C NMR δ 38.1, 46.3, 47.0, 52.8, 67.1, 115.0, 117.3, 119.9, 122.7, 124.7, 127.0, 127.2, 127.6, 127.8, 129.4, 141.1, 141.7, 143.6, 143.8, 154.7, 156.2; IR (thin film, cm^{-1}) 3000 (broad), 2919, 1717, 1640, 1602; HRMS (FAB) calcd for $C_{27}H_{23}N_2O_5$ $[M + H]^+$ 455.1607, found 455.1605; $[\alpha]^{20}_D = -340°$ ($c = 0.5$, 9:1 MeOH/1.0 N aqueous HCl).

***(S)*-2-Amino-*N*$^\alpha$-9-fluorenylmethoxycarbonyl-3-(oxine-5-yl)propionic Acid (2).** Alkylated pseudoephedrine derivative **9** (100 mg, 0.192 mmol, 1.0 equiv) was dissolved in deoxygenated dioxane (1.5 mL), deoxygenated aqueous NaOH (0.5 M, 1.54 mL, 0.768 mmol, 4 equiv) was added, and the reaction mixture was heated to reflux. After 6 h, the solution was deep yellow, and analysis by TLC (1:1:1:1 *n*-BuOH/AcOH/water/EtOAc) showed that only the deprotected amino acid ($R_f = 0.23$) remained as a UV-, ninhydrin-, and $FeCl_3$-active species. The solution was cooled, and the dioxane was removed under reduced pressure. The aqueous solution was washed with DCM (3 × 5 mL) to remove pseudoephedrine. A small aliquot of the aqueous phase (20 μL) was removed for chiral HPLC analysis, the remainder was chilled to 0 °C, and $NaHCO_3$ (64 mg, 0.768 mmol, 4.0 equiv) was added. A solution of *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (78 mg, 0.23 mmol, 1.2 equiv) in dioxane (1.5 mL) was added, and the reaction mixture was stirred for 8 h. The dioxane was removed under reduced pressure, and HCl (1 N) was added dropwise until the solution was ca. pH 4. Water (5 mL) was added, and the solution was cooled to 4 °C and held at that temperature for 1 h, during which time a yellow precipitate formed. The solution was filtered and washed with cold water. The solid was transferred to a round-bottom flask and concentrated from toluene several times to remove traces of water. The resulting pale yellow solid was dried under vacuum for 6 h to afford 90 mg (95%) of **2**: $R_f = 0.37$ (85:15:3 $CHCl_3$/MeOH/AcOH); mp 114−116 °C; ^{1}H NMR (MeOH-d_4) δ 3.27 (dd, $J = 4.5$ Hz, 14.1 Hz, 1H), 3.65 (dd, $J = 4.5$ Hz, 14.1 Hz, 1H), 3.86 (d, $J = 6.9$ Hz, 2H), 4.01 (t, $J = 4.5$ Hz, 1H), 4.45 (m, 1H), 7.02 (d, $J = 7.8$ Hz, 1H), 7.1−7.8 (m, 10H), 8.61 (d, $J = 7.8$ Hz, 1H), 8.78 (d, $J = 4.2$ Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 32.3, 46.5, 55.0, 65.6, 114.3, 120.1, 122.0, 125.1, 125.2, 127.0, 127.1, 137.6, 127.8, 130.9, 140.6, 140.8, 143.6, 143.7, 144.6, 155.9, 172.9; IR (thin film, cm^{-1}) 3000 (broad), 3067, 2924, 1713, 1598; HRMS (FAB) calcd for $C_{27}H_{23}N_2O_5$ $[M + H]^+$ 455.1607, found 455.1599; $[\alpha]^{20}_D = -5°$ ($c = 0.5$, 9:1 MeOH/1.0 N aqueous HCl).

Ac-2Oxn-Val-Pro-D-Ser-Phe-Cys-Ser-NH$_2$ (P1). HPLC (C_{18}; solvent A = water, 0.1% v/v TFA; solvent B = MeCN, 0.1% v/v TFA), t_R (linear gradient 0−70% B over 25 min) = 18.5 min; UV (0.1 N NaOH), λ_{max} (nm) = 335; MS (electrospray) calcd for $C_{42}H_{56}N_9O_{11}S$ $[M + H]^+$ 894.3, found 894.3.

Ac-5Oxn-Val-Pro-D-Ser-Phe-Cys-Ser-NH$_2$ (P2). HPLC (C_{18}, solvent A = water, 0.1% v/v TFA; solvent B = MeCN, 0.1% v/v TFA), t_R (linear gradient 0−70% B over 25 min) = 17.9 min; UV (0.1 N NaOH), λ_{max} (nm) = 366; MS (electrospray) calcd for $C_{42}H_{56}N_9O_{11}S$ $[M + H]^+$ 894.3, found 894.3.

Acknowledgment. This research was supported by the NSF and the Gates Foundation. The award of a NIH predoctoral training grant in biology and chemistry to G.K.W. (GM07616) is also gratefully acknowledged.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra and chiral HPLC traces from the analysis of the optical purity of **1** and **2** (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO980563H