Asymmetric Synthesis of a New 8-Hydroxyquinoline-Derived α-Amino Acid and Its Incorporation in a Peptidylsensor for Divalent Zinc

Nathalie Jotterand, Dierdre A. Pearce, and Barbara Imperiali*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

imper@mit.edu

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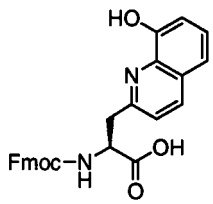
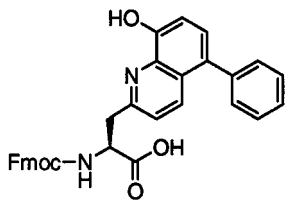
The need for sensors capable of monitoring real-time concentrations of free Zn(II) in a complex aqueous medium remains a technological challenge. Toward this goal, strategies for the development of sensitive fluorescent chemosensors with ideal photophysical properties are still needed. The approach taken by this group involves adapting the modular architecture of the polypeptide backbone for the construction of new and useful sensing molecules. The first generation of designs were based on the zinc finger domain.[1] These constructs demonstrated excellent selectivity toward Zn(II); however, the chemosensor molecules were large (>20 amino acid residues in length). Second generation designs provided smaller peptidyl fluorescent sensors (7 residues) in which the reporter functionality was a residue bearing the bidentate ligand 8-hydroxy-quinoline[2] (oxine) **1**, a chromophore known to produce a chelation enhanced fluorescence (CHEF) upon metal binding.[3] Although these last sensors have shown good selectivity for Zn(II), their use coupled with fiber optic devices is restricted because of the excitation wavelength of the oxine reporter at 355 nm, which is too close to the limit where the transmission of light through the optic fiber starts to become efficient.[4] Therefore, the focus has been to redesign the oxine fluorophore to shift the excitation wavelength of the quinoline reporter to longer, more practical wavelengths. In many conjugated systems this "red shift" can be achieved by extending the aromatic system, thereby reducing the π to π^* gap.[5] Consequently, emphasis has been made to substitute the quinoline moiety with different aromatic groups that include a more extended chromophore.

Herein we report the asymmetric synthesis of (*S*)-2-amino-N^{α}-9-fluorenylmethoxycarbonyl-3-(5-phenyl-8-hydroxyquinoline-2-yl) propionic acid **2** and its incorporation into a short model peptide (7 residues).[6] The synthesis is outlined in Scheme 1 and proceeds with two key processes starting from the commercially available 8-hydroxy-2-methylquinoline. The first process involves the attachment of a phenyl ring to the 5 position of the hydroxyquinoline via a Suzuki-type coupling; the second involves the construction of the α-amino acid strereocenter via an asymmetric alkylation.



Fmoc-2Oxn-OH

1



Fmoc-(5Ph)2Oxn-OH

2

8-Hydroxy-2-methyl-quinoline **3** was protected as the *tert*-butyldimethylsilyl ether and regioselectively brominated at the 5 position by treatment with bromine. The resulting bromide **4** was coupled with benzene boronic acid in the presence of $Pd(PPh_3)_4$ to afford the corresponding phenyl derivative **5**. Attempts to convert **5** directly to the bromomethyl **6** under free radical conditions (NBS, CCl_4)[2] led mainly to bromination of the phenyl ring. Additionally, replacement of the silyl ether by a benzenesulfonate ester[7] (HCl, 1 M, then Et_3N, $PhSO_2Cl$) led to the same observations. Finally, an alternative was found using a three-step procedure that included the oxidation of **5** by selenium dioxide, which provided the corresponding aldehyde, reduction with $NaBH_4$, and smooth conversion to the bromomethyl derivative, **6**, by treatment with a mixture of NBS and methyl sulfide. The overall yield is 67%, and only one chromatographic purification is necessary.

The asymmetric alkylation of commercially available *N*-(diphenylmethylene)glycine *tert*-butylester **7** with **6** was carried out using the Corey modification[8] of the O'Donnell method,[9] using the phase transfer catalyst (8*S*,9*R*)-*O*-(9)-allyl-*N*-9-anthracenyl methylcinchonidium bromide to favor the formation of the adduct possessing the L configuration.[10] Using a slight excess of bromide **6**

(1) (a) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053−3054. (b) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443−3450. A sensor incorporating a zinc finger structure has been independently reported: Godwin, H. A.; Berg, J. M. *J. Am. Chem. Soc.* **1996**, *118*, 6514−6515.

(2) Walkup, G. K.; Imperiali, B. *J. Org. Chem.* **1998**, *63*, 6727−6731.

(3) CHEF effect of oxine in the presence of Zn(II): Vogel, A. I. In *Vogel's Textbook of Quantitative Chemical Analysis*; Wiley: New York, 1989; Chapter 18, pp 731−740. CHEF effect of oxine in the presence of other metal ions such as Mg(II), Ca(II), Pb(II): see ref 5.

(4) (a) Thompson, R. B. *IEEE Circuit Device* **1994**, *10*, 14−21. (b) Signal attenuation in optic fibers results from scattering caused by defects in the fibers and becomes significant below 360 nm. The value of a fiber's attenuation is typically specified during manufacture.

(5) Seitz, W. R. *Critical Reviews in Analytical Chemistry*; CRC Press: Boca Raton, FL, 1980; pp 367−404.

(6) The asymmetric synthesis of (*S*)-2-Amino-N^{α}-(9-fluorenylmethyloxycarbonyl)-3-(8-hydroxyquinoline-2-yl) propanoic acid has been previously reported (see ref 2). However, the presence of a supplementary aromatic ring on the oxine moiety required the development of a new synthetic procedure.

(7) The silyl ether was hydrolyzed at 40 °C in dioxane/aqueous HCl, 1 M (1: 1). The mixture was neutralized (NaOH, 1 M) and extracted (CH_2Cl_2). The organic phase was dried (K_2CO_3) and evaporated. The residue was taken in dry THF, and the protection of the alcohol was carried out according to ref 2 to afford 8-benzenesulfonyloxy-2-methyl-5-phenylquinoline with an overall yield of 90%. R_f = 0.31 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) 8.06 (d, J = 8.5, 1H), 8.05 (d, J = 8.5, 1H), 8.02 (d, J = 8.8, 1H), 7.67 (d, J = 8.0, 1H), 7.61 (bt, J = 7.5, 1H), 7.52−7.39 (m, 8H), 7.15 (d, J = 8.5, 1H), 2.52 (s, 3H); ^{13}C NMR ($CDCl_3$) 160.0, 144.9, 141.6, 140.4, 139.4, 137.4, 135.2, 134.5, 130.6, 129.6, 129.3, 129.2, 128.6, 126.8, 126.5, 123.3, 25.8; HRMS (ES) calcd for $C_{22}H_{18}NO_3S$ $[M + H]^+$ 376.1007, found: 376.1054.

(8) (a) Corey, E. J.; Xu, F.; Noe, M. C. *J. Am. Chem. Soc.* **1997**, *119*, 12414−12415. (b) Corey, E. J.; Noe, M. C.; Xu. F. *Tetrahedron Lett.* **1998**, *39*, 5347−5350.

(9) O'Donnell, M. J.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353−2355.

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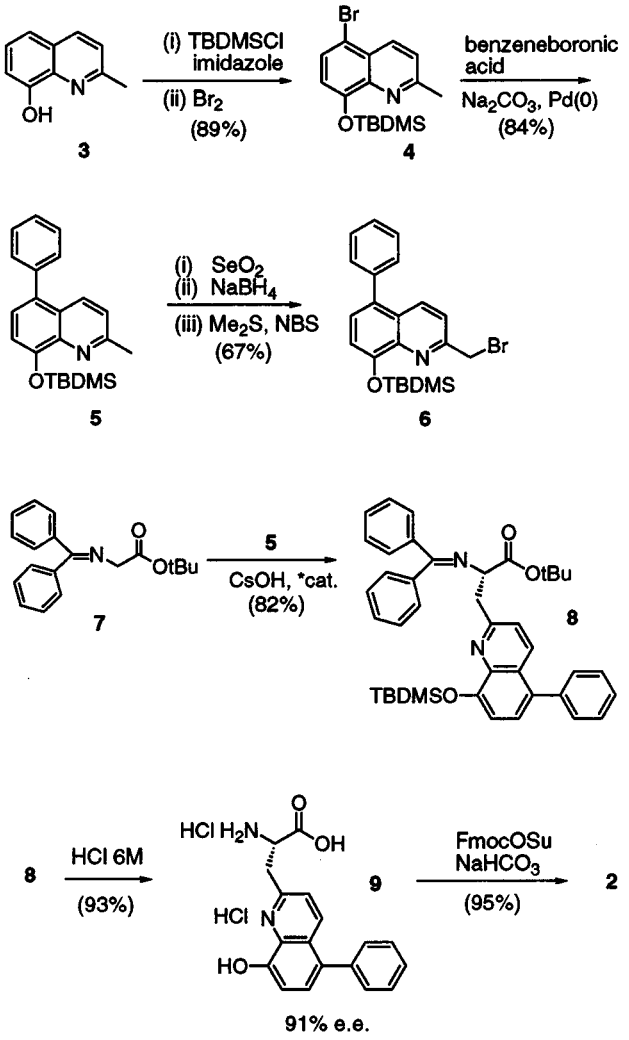
Scheme 1



(1.2 equiv), the alkylation adduct **8** was obtained with 82% yield. Simultaneous deprotection of the imine, ester, and phenol functionalities was carried out under acid hydrolysis (HCl, 6 M, reflux) to afford the corresponding amino acid as the hydrochloride salt form **9**. Derivatization of **9** with Marfey's reagent[11] (1-fluoro-2,4-dinitrophenyl-5-L-alanineamide) followed by HPLC analysis of the adducts revealed a 91% ee in favor of the L-amino acid.[12]

To incorporate **9** into a polypeptide sequence via solid-phase synthesis techniques,[13] the amino acid was converted to the corresponding 9-fluorenyl-methoxycarbonyl (Fmoc) derivative **2** by treatment with (9-fluorenyl-methoxycarbonyl)succinimidyl carbonate (Fmoc-Su) (88% yield from **7**).[14] The amino acid derivative **2** was successfully incorporated into the heptapeptide **P1**,[15] a sequence that contains the reverse turn promoting fragment -Val-Pro-DSer-Phe- and an additional "soft" thiolate-donor ligand (from cysteine) to improve the affinity of the ligand for Zn(II),[16] compared with the parent **2**. The major product, containing the L-amino acid was purified from the minor product, containing the D-amino acid, by HPLC.[17] For the purposes of comparison an analogous peptide **P2**, containing the parent oxine residue **1**, was also prepared.

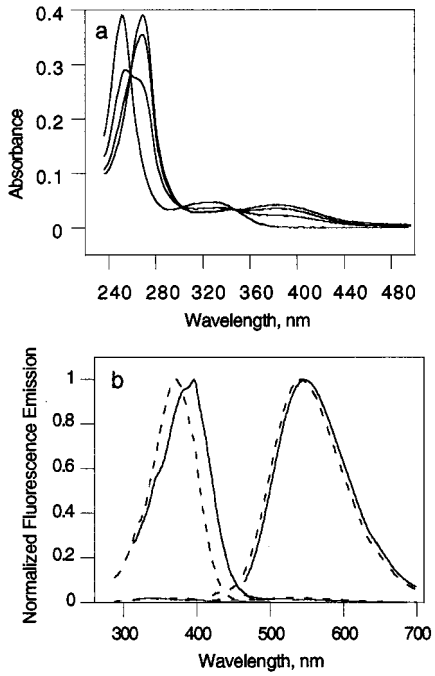


Figure 1. UV−visible absorption spectra and steady-state emission spectra of **P1** (10 μM) on addition of $ZnCl_2$ (to 100 μM). (a) UV−visible absorption spectra of **P1** in the presence of 0, 5, 10, and 100 μM $ZnCl_2$. (b) Comparison of fluorescence excitation and emission spectra of **P1** (solid line spectra) and **P2** (dashed line spectra). Baseline spectra were acquired in the absence of $ZnCl_2$; other spectra were acquired on addition of 100 μM $ZnCl_2$. Samples were prepared in 50 mM HEPES (pH 7.01), containing 150 mM NaCl. Spectra were acquired at 21 °C, and baseline were corrected using a sample of the buffer solution.

P1: Ac-5PhOxn-Val-Pro-DSer-Phe-Cys-Gly-NH_2

P2: Ac-Oxn-Val-Pro-DSer-Phe-Cys-Gly-NH_2

Addition of Zn(II) to **P1** was monitored by both UV−visible and steady-state fluorescence emission spectroscopy. Changes in the UV−visible absorption spectra of **P1** on addition of Zn(II) are summarized in Figure 1a. Absorbance bands at 251 and 357 nm in spectra of **P1** are replaced by bands at 269 and 391 nm on addition of Zn(II).[18] The absorbance bands at 357 and 391 nm are red shifted compared with the corresponding absorbance bands of spectra of free and Zn(II)-bound **P2** (308 and

(10) The stereochemical outcome of the Corey alkylation has proven to be predictable for a wide range of electrophiles. See ref 8 and references therein.

(11) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 3346−3352.

(12) The general and unambiguous applicability of the Marfey method has been well described. Thus, determination of the absolute configuration of an amino acid by HPLC analysis is feasible: the L-derivative elutes faster than the parent D-derivative. See ref 10 and references therein.

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

(14) The product **2** was stable on storage in the dark, at 5 °C. The internal, unprotected base is not strong enough to cause loss of the *N*-Fmoc protecting group under these conditions.

(15) The peptide containing the (D) enantiomer of **9** was readily separated by reversed phase HPLC purification.

(16) (a) Imperiali, B.; Fisher, S. L.; Moats, R. A.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3182−3188. (b) Imperiali, B.; Kapoor, T. M. *Tetrahedron* **1993**, *49*, 3501−3510. (c) Cheng, R. P.; Fisher, S. L.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 11349−11356.

(17) In the final synthetic cycle, the completed sequences were acetyl capped. A subsequent piperidine de-block cycle was performed. HPLC analysis of the completed peptides showed no trace of byproduct peptides, corresponding to esterification of the quinoline OH.

372 nm respectively, data not shown). Fluorescence spectra from Zn(II)-bound **P1** was compared with those from the analogous Zn(II)-bound **P2**. Neither species is fluorescent at pH 7.0 (50 mM HEPES, 150 mM NaCl) in the absence of the metal ion, and on addition of Zn(II) the fluorescence emission spectra of the two species almost overlap. Spectra of Zn(II)-bound **P1** and Zn(II)-bound **P2** exhibit emission maxima of 546 and 544 nm, respectively. However, while the emission from Zn(II)-bound **P2** reaches a maximum at $\lambda_{ex} = 372$ nm, emission from Zn(II)-bound **P1** reaches a maxima at $\lambda_{ex} = 396$ nm in the corresponding excitation spectra, representing a red shift of some 24 nm, Figure 1b. The quantum yield[19] of Zn(II)-bound **P1** was found to be 0.005, a value consistent with the modest quantum yields of metal complexes of other oxine derivatives.[20] A method for the systematic increase in quantum yield of Zn(II) oxine complexes has been recently reported[21] and will be investigated as means to improve the luminescence properties of derivatives such as **2**.

In conclusion, this note describes the asymmetric synthesis, peptide incorporation, and characterization of a new α−amino acid containing the bidentate ligand 8-hydroxyquinoline to which a phenyl ring has been attached to the 5 position. This modification results in significant red shifts in the excitation maxima of the ligand when complexed to Zn(II). Fluorescent sensors designed to be compatible with fiber optic technology must take into consideration the loss of transmitted light due to Rayleigh scattering and inhomogeneities in the fiber that are particularly significant in the UV region of the spectrum below 360 nm; beyond this point signal transmittance increases almost linearly to about 500 nm.[4a] The simple modification reported here produces a red shift in the excitation maxima of the derivative to almost 400 nm and represents a prototype for the systematic design of new sensors for the selective and sensitive analysis of trace Zn(II) through fiber optic devices. Further studies, to increase the quantum yield and spectral red shifts of such residues, are in progress.

Experimental Section

General Procedures. All reagents and solvents from commercial suppliers were used without further purification. *O*-(9)-Allyl-*N*-9-anthracenylmethylcinchonidium bromide was prepared according to the method developed by Corey.[8] Methylene chloride was distilled from calcium hydride. Anhydrous reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Thin-layer chromatography was carried out using TLC plates (silica gel IB-F) obtained from J. T. Baker. TLC plates were visualized by UV or 0.2% ninhydrin solution in ethanol followed by heat. Flash column chromatography was performed according to the procedure of Still[22] using 230−400 mesh silica gel. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz for proton frequency and 75 MHz for carbon frequency. Chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (external standard). Optical rotations were recorded at room temperature.

Peptide Synthesis. The peptides (0.0333 mmol) were synthesized using standard Fmoc amino acid protection chemistry on Fmoc-PAL-PEG-PS resin (0.170 mmol equiv). Residues 1−6 were synthesized on a Milligen series 9050 PepSynthesizer with a 4-fold excess of activated amino acid ester (prepared in situ using *N*-hydroxybenzotriazole/2-(benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HOBT/HBTU)). The final residue was added manually, using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) and diisopropylethylamine (DIEA) to generate the activated ester. In this case, coupling was carried out with a 2-fold excess of amino acid using the following conditions: removal of Fmoc protecting group (20% piperidine in DMF, 2 × 8 min), wash (DMF 2 × 2 min, MeOH 1 × 2 min, DMF 2 × 2 min); amino acid coupling (amino acid/PyBOP/DIEA 2:2:2), wash (DMF 5 × 2 min). The *N*-terminal Fmoc was removed as previously, and the *N*-terminal α-amine was acetylated with 2 mL (3 × 10 min) of a mixture consisting of DMF/CH_2Cl_2/Ac_2O/HOBT (90 mL:10 mL:2.5 mL:48 g). Any aryl ester formed was cleaved (20% piperidine in DMF, 2 × 8 min), and the resin washed (as above). After a final wash with MeOH, the resin was dried under vacuum and the peptide was cleaved from the resin (TFA, 5% v/v triisopropylsilane, 90 min). The resulting solution was concentrated, and the crude peptide was precipitated by addition of an ether/hexane 1:1 solution. The suspension was triturated using the same solution (3 × 5 mL). The pellet containing the peptide was dissolved in water, lyophilized from the aqueous eluent overnight, and purified to homogeneity (as evidenced by the appearance of a single peak in HPLC traces on re-injection of the purified sample) by reversed phase high-pressure liquid chromatography (HPLC, C_{18}).

8-*tert*-Butyldimethylsilyloxy-2-methylquinoline. A 100-mL flask was successively loaded with dry CH_2Cl_2 (50 mL), 8-hydroxy-2-methylquinoline (5.1 g, 32.037 mmol), imidazole (2.29 g, 33.639 mmol, 1.05 equiv), and *tert*-butyldimethylsilyl chloride (5.31 g, 35.24 mmol, 1.1 equiv). The solution was stirred at room temperature for 10 h, diluted with Et_2O (500 mL), washed with aqueous HCl (0.1 M, 50 mL), brine (100 mL), water (100 mL), dried over K_2CO_3 and evaporated under reduced pressure to afford 8.41 g (96%) of protected product as a colorless oil. $R_f = 0.58$ (EtOAc/hexane 5:95); $R_f = 0.35$ (Et_2O/hexane 2:98); ^{1}H NMR ($CDCl_3$) δ 7.98 (d, $J = 8.5$, 1H), 7.35 (ap. t, $J = 8.3$, 1H), 7.34 (d, $J = 8.3$, 1H), 7.24 (d, $J = 8.3$, 1H), 7.15 (bd, $J = 8.5$, 1H), 2.76 (s, 3H), 1.08 (s, 9H), 0.29 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 157.3, 152.4, 141.8, 136.0, 128.0, 126.1, 122.1, 120.3, 118.3, 26.3, 25.4, 19.3, −3.6; IR (thin film) 2927, 2888, 2855, 1599, 1566, 1504, 1470, 1432, 1326, 1276, 1247, 1237, 1104, 926, 833, 783, 616; HRMS (ES) calcd for $C_{16}H_{24}NOSi$ $[M + H]^+$ 274.1629, found 274.1645.

5-Bromo-8-*tert*-butyldimethylsilyloxy-2-methyl-quinoline (4). A solution of bromine (1.16 mL, 22.67 mmol, 1 equiv) in CH_2Cl_2 (20 mL) was added dropwise to a vigorously stirred solution of the silyl ether (6.20 g, 22.67 mmol) in CH_2Cl_2 (150 mL). The completion of the reaction was checked by TLC (UV detection). If necessary, more bromine solution was added. The solution was diluted with CH_2Cl_2 (1 L), washed with aqueous saturated $Na_2S_2O_4$ (50 mL), aqueous saturated $NaHCO_3$ (100 mL), brine (50 mL), and water (100 mL), dried over K_2CO_3, and evaporated under reduced pressure to afford 7.42 g (93%) of bromide **3** as a yellowish oil. An analytical sample was obtained by flash chromatography (UV detection, hexane/Et_2O 98:2). $R_f = 0.57$ (Et_2O/hexane 2:98); $R_f = 0.67$ (EtAc/hexane 10:90); ^{1}H NMR ($CDCl_3$) δ 8.32 (d, $J = 8.8$, 1H), 7.59 (d, $J = 8.3$, 1H), 7.33 (d, $J = 8.5$, 1H), 7.03 (d, $J = 8.3$, 1H), 2.75 (s, 3H), 1.07 (s, 9H), 0.27 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.0, 152.4, 142.3, 135.6, 129.6, 126.9, 123.3, 118.8, 112.5, 26.3, 25.1, 19.3, −3.5; IR (thin film) 2954, 2927, 2855, 1590, 1466, 1321, 1255, 1077, 840, 616; HRMS (ES) calcd for $C_{16}H_{23}NOSiBr$ $[M + H]^+$ 352.0733, found 352.0727.

8-*tert*-Butyldimethylsilyloxy-2-methyl-5-phenylquinoline (5). A 50-mL Schlenk flask containing bromide **4** (369 mg, 1.0473 mmol) was successively loaded with benzene (11 mL), aqueous Na_2CO_3 2 M (12.9 mL), ethanol (2.9 mL), and benzeneboronic acid (140 mg, 1.152 mmol, 1.1 equiv). The emulsion

(18) Addition of a single equivalent of Zn(II) almost saturated **P1** (10 μM); however, analysis of titration data was complicated by nonlinear behavior due to peptide aggregation in solution. The apparent dissociation constant was estimated to be 0.1−0.3 μM, using the method described in ref 16c.

(19) Determined with reference to quinine sulfate in 0.1 M H_2SO_4. Uncertainty estimated at 15%. Chen, R. F. *Anal. Chem.* **1967**, *19*, 374−387.

(20) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769−6770.

(21) Pearce, D. A.; Jotterand, N.; Carrico, I. S.; Imperiali, B. **2000**, submitted for publication.

(22) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

was degassed, and $Pd(PPh_3)_4$ (36.7 mg, 0.0317 mmol, 0.0303 equiv) was rapidly added under a stream of nitrogen. The flask was equipped with a condenser, and the mixture was refluxed with vigorous stirring until no more bromide **4** remained (ca. 48 h). The mixture was cooled, diluted with benzene (100 mL), washed with water (25 mL) and brine (25 mL), dried over K_2CO_3, and evaporated under reduced pressure. The crude residue was purified by flash column chromatography (Et_2O/hexane 2:98, UV detection) to afford 307 mg (84%) of phenyl **5** as a yellowish oil. $R_f = 0.57$ (EtOAc/hexane 5:95); $R_f = 0.41$ (Et_2O/hexane 2:98); ^{1}H NMR ($CDCl_3$) 8.07 (d, $J = 8.5$, 1H), 7.47−7.35 (m, 5H), 7.28 (ap. t, $J = 7.7$, 1H), 7.18 (d, $J = 8.8$, 1H), 2.73 (s, 3H), 1.09 (s, 9H), 0.31 (s, 6H); ^{13}C NMR ($CDCl_3$) 157.0, 151.9, 141.5, 140.1, 134.4, 133.0, 130.3, 128.6, 128.5, 127.2, 126.9, 126.0, 122.0, 117.7, 26.3, 25.1, −3.4; IR (thin film) 2927, 2854, 1570, 1467, 1320, 1247, 1099, 856, 620; HRMS (ES) calcd for $C_{22}H_{28}NOSi$ $[M + H]^+$ 350.1941, found 350.1913.

8-*tert*-Butyldimethylsilyloxy-5-phenylquinoline-2-carbaldehyde. Molecular sieves 4 Å (200 mg) then selenium dioxide (66 mg, 0.5952 mmol, 1.01 equiv) were added under nitrogen to a solution of **5** (206 mg, 0.5893 mmol) in dry 1,4-dioxane (4 mL). The mixture was stirred at 90 °C for 3 h, cooled, and filtered over a pad of Celite. The Celite was rinsed with dioxane (6 mL), and the solvent was removed under reduced pressure. The residue was taken up in Et_2O (100 mL), washed with brine (25 mL) and water (25 mL), dried over K_2CO_3, and evaporated under reduced pressure to afford 74 mg (81%) of the corresponding aldehyde as a yellowish oil. An analytical sample was obtained by flash chromatography (Et_2O/ hexane 2:98, UV detection). $R_f = 0.54$ (EtOAc/hexane 5:95); $R_f = 0.38$ (Et_2O/hexane 2:98); ^{1}H NMR ($CDCl_3$) δ 10.3 (s, 1H), 8.34 (d, $J = 8.4$, 1H), 7.94 (d, $J = 9.0$, 1H), 7.54−7.42 (m, 6H), 7.30 (d, $J = 7.8$, 1H), 1.14 (s, 9H), 0.35 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 193.7, 153.1, 151.2, 141.9, 139.2, 135.9, 135.5, 130.8, 130.3, 129.7, 128.8, 127.8, 118.7, 117.4, 26.2, 19.2, −3.4; IR (thin film) 2928, 2856, 1712, 1595, 1567, 1467, 1362, 1332, 1320, 1307, 1256, 1092, 1071, 906, 840, 784, 759, 703. HRMS (ES) calcd for $C_{22}H_{26}NOSi$ $[M + H]^+$ 364.1733, found 364.1712.

8-*tert*-Butyldimethylsilyloxy-5-phenylquinoline-2-methanol. A solution of the previous aldehyde (214 mg, 0.5893 mmol) in CH_2Cl_2 (2 mL) and absolute ethanol (2 mL) was added dropwise under nitrogen to an ice-cooled solution of $NaBH_4$ (22.3 mg, 0.5893 mmol, 1 equiv) in absolute ethanol (3 mL). The solution was stirred at 0 °C for 10 min, diluted with Et_2O (50 mL), washed with aqueous HCl 0.1 M (5 mL) and water (2 × 15 mL), dried over Na_2SO_4, and evaporated under reduced pressure to afford 200 mg (93%) of the corresponding alcohol as a yellowish oil. An analytical sample was obtained by flash chromatography (EtOAc/hexane 5:95, UV detection). $R_f = 0.14$ (EtOAc/hexane 5:95); $R_f = 0.12$ (Et_2O/hexane 2:98); ^{1}H NMR ($CDCl_3$) δ 8.19 (d, $J = 8.8$, 1H), 7.50−7.42 (m, 5H), 7.37 (d, $J = 8.0$, 1H), 7.21 (ap. t, $J = 8.0$, 1H), 4.93 (s, 2H), 1.15 (s, 9H), 0.31 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 157.3, 151.2, 140.7, 139.7, 135.5, 133.6, 130.3, 129.2, 128.7, 128.4, 127.5, 127.4, 118.5, 118.2, 64.4, 26.2, 18.9, −3.8; IR (thin film) 3386, 2928, 1711, 1471, 1254, 839, 762, 703; HRMS (ES) calcd for $C_{22}H_{28}NO_2Si$ $[M + H]^+$ 366.1890, found 366.1866.

2-Bromomethyl-8-*tert*-butyldimethylsilyloxy-5-phenylquinoline (6). To a solution of *N*-bromosuccinimide (487 mg, 2.737 mmol, 1.5 equiv) in anhydrous CH_2Cl_2 (8 mL) was added dry methyl sulfide (241 μL, 3.284 mmol, 1.8 equiv) dropwise at 0 °C under nitrogen. The yellow suspension was cooled to −15 °C, and a solution of the methyl alcohol oil (667 mg, 1.82465 mmol) freshly dried by azeotropic distillation with toluene (3 × 10 mL) in anhydrous CH_2Cl_2 (2.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 4 h, diluted with ether (200 mL), washed with water (50 mL) and brine (50 mL), dried over K_2CO_3, and evaporated under reduced pressure. The residue was purified by flash chromatography (Et_2O/hexane 2:98, UV detection) to afford 594 mg (89%) of bromide **6** as a colorless oil. $R_f = 0.64$ (EtOAc/hexane 5:95); $R_f = 0.50$ (Et_2O/hexane 2:98); ^{1}H NMR ($CDCl_3$) δ 8.22 (d, $J = 8.6$, 1H), 7.49−7.37 (m, 7H), 7.24 (d, $J = 7.8$, 1H), 4.75 (s, 2H), 1.10 (s, 9H), 0.31 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 155.0, 152.2, 141.3, 139.7, 135.8, 133.1, 130.3, 128.6, 128.4, 127.5, 127.0, 121.3, 118.4, 34.6, 26.3, 19.3, −3.5; IR (thin film) 2926, 2854, 1594, 1469, 1319, 1248, 1093, 839, 784, 615; HRMS (ES) calcd for $C_{22}H_{27}NOSiBr$ $[M + H]^+$ 428.1046, found 428.1026.

(*N*-Diphenylmethylene)-2-amino-3-(8-*tert*-butyldimethylsilyloxy-5-phenyl-quinoline-2-yl)-propanoic Acid *tert*-Butyl Ester (8). A 10-mL Schlenk flask was successively loaded with CH_2Cl_2 (0.5 mL), *tert*-butylglycinate benzophenone imine **7** (45.5 mg, 0.154 mmol, 1 equiv), *O*-(9)-allyl-*N*-9-anthracenylmethylcinchonidium bromide (9 mg, 0.0154 mmol, 0.1 equiv), and rapidly with CsOH monohydrate (258 mg, 1.54 mmol, 10 equiv). The flask was filled with nitrogen and cooled to −78 °C. A solution of bromide **5** (79.2 mg, 0.1848 mmol, 1.2 equiv) in CH_2Cl_2 (0.7 mL) was added dropwise and the suspension was vigorously stirred at −55 °C till the imine was completely consumed (ca. 23 h). The suspension was poured into Et_2O (50 mL), washed with water (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc/ hexane 5:95, UV detection) to afford 82 mg (82%) of adduct **7** as a yellow oil. $R_f = 0.15$ (EtOAc/hexane 5:95); $R_f = 0.27$ (Et_2O/hexane 10:90); ^{1}H NMR ($CDCl_3$) δ 8.03 (d, $J = 8.5$, 1H), 7.81 (d, $J = 7.7$, 1H), 7.59−7.15 (m, 16H), 6.65 (bd, $J = 7.2$, 1H), 4.54 (dd, $J = 9.3$, 4.1, 1H), 3.66 (dd, $J = 13.0$, 4.1, 1H), 3.56 (dd, $J = 13.0$, 9.3, 1H), 1.44 (s, 9H), 1.03 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.8, 157.5, 152.1, 141.7, 140.0, 139.7, 137.7, 136.3, 134.1, 132.9, 132.6, 130.3, 129.1, 129.0, 128.5, 128.4, 128.1, 127.7, 127.3, 127.2, 126.4, 122.8, 113.6, 81.4, 67.2, 43.0, 28.3, 26.3, 19.2, −3.3, −3.7; IR (thin film) 2929, 2856, 1570, 1466, 1367, 1279, 1249, 1150, 838, 782, 700; HRMS (ES) calcd for $C_{41}H_{47}N2O_3Si$ $[M + H]^+$ 643.3357, found 643.3350. $[\alpha]^{20}_D = -12.2°$ ($c = 3.33$, $CHCl_3$).

(*S*)-2-Amino-3-(5-phenyl-8-hydroxyquinoline-2-yl)propionic Acid Hydrochloride Salt (9). The *tert*-butyl ester **8** (82 mg, 0.1275 mmol) was refluxed in HCl 6 M (2 mL) under nitrogen until TLC analysis of the solution (*n*BuOH/AcOH/H_2O/EtOAc 1:1:1:1, UV and ninhydrin detection) showed complete deprotection (ca. 5 h). The hydrolyzed mixture was cooled, diluted with HCl 1 M (5 mL), extracted with EtOAc (2 × 10 mL), and concentrated to dryness under reduced pressure to afford 49 mg (93%) of the double hydrochloride salt **9** as a yellow solid: mp = 176−177 °C; $R_f = 0.16$ (CH_2Cl_2/MeOH/AcOH 85:15:3); $R_f = 0.61$ (*n*BuOH/H_2O/AcOH/EtOAc 1:1:1:1); ^{1}H NMR (CD_3OD) δ 8.76 (d, $J = 8.0$, 1H), 7.89 (d, $J = 8.0$, 1H), 7.67 (d, $J = 7.8$, 1H), 7.60−7.44 (m, 6H), 4.72 (dd, $J = 6.0$, 7.0, 1H), 3.99 (dd, $J = 16.0$, 7.0, 1H), 3.82 (dd, $J = 16.0$, 6.0, 1H); ^{13}C NMR (CD_3OD_3) δ 169.0, 153.9, 148.3, 143.5, 137.6, 132.0, 131.1, 130.6, 130.0, 128.8, 128.6, 128.1, 127.2, 123.3, 115.3, 51.7, 34.4; IR (thin film) 3388 (broad), 2934, 1732, 1633, 1594, 1542, 1514, 1380, 1314, 1222, 1072, 847, 765, 704; HRMS (ES) calcd for $C_{18}H_{17}N_2O_3$ $[M + H]^+$ 309.1240, found 309.1234. $[\alpha]^{20}_D = +2.4°$ ($c = 0.83$, MeOH/aqueous HCl, 1 M, 9:1).

Determination of the Enantiomeric Excess. The enantiomeric excess of the pure amino acid was evaluated by derivatization with 1-fluoro-2,4-(dinitrophenyl)-5-L-alanineamide (FDAA, Marfey's reagent) followed by reversed phase HPLC analysis of the adducts. The hydrochloride salt (1.3 mg, 0.00422 mmol) was dissolved in 0.1 M $NaHCO_3$ (370 μL), and the resulting solution was added to a freshly prepared solution of 1.49 mg (0.00548 mmol, 1.3 equiv) of Marfey's reagent in acetone (120 μL). The mixture was kept at 40 °C for 1 h with frequent vortexing/shaking. After cooling, the pH was adjusted to 4 by addition of HCl 1 M. The mixture was centrifuged, and 5 μL of the supernatant solution was analyzed by HPLC (C_{18} column; solvent A = H_2O, 0.1% v/v TFA; solvent B = MeCN, 0.1% v/v TFA; linear gradient 30−70% over 25 min). t_R: *S* derivative = 22.78 min, R derivative = 25.04 min. A 91% ee was calculated by comparison of peak areas. The compounds comprising the peaks were confirmed by mass spectroscopy. ESMS calcd for $C_{27}H_{25}O_8N_6$ $[M + H]^+$ 561.2, found, 561.3.

(*S*)-2-Amino-N^{α}-(9-fluorenylmethyloxycarbonyl)-3-(8-hydroxy-5-phenylquinoline-2-yl) Propanoic Acid (2). The previous hydrochloride salt (89 mg, 0.4957 mmol) was dissolved in aqueous Na_2CO_3 (1 M, 2.6 mL). (9-Fluorenylmethyl)succinimidyl carbonate (184 mg, 0.5453 mmol, 1.1 equiv) was dissolved in dioxane (6.6 mL) and added dropwise to the amino acid solution. The reaction mixture was stirred for 5 h. The dioxane was evaporated under reduced pressure at room temperature, and the aqueous phase was extracted with Et_2O (3 × 5 mL).

The aqueous phase was acidified to pH 3 with HCl (1 M) and extracted with EtOAc (4 × 20 mL). The combined EtOAc phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was taken in CH_2Cl_2 and crystallized by slow addition of chilled hexane to afford 276 mg (95%) of *N*-protected amino acid **2** as a yellow powder: mp 105−106 °C, R_f = 0.64 ($CHCl_3$/MeOH 4:1); R_f = 0.10 (EtAc); ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 8.9, 1H), 7.72 (bd, J = 7.3, 2H), 7.59−7.20 (m, 14H), 6.49 (d, J = 6.1, 1H), 4.73 (bs, 1H), 4.30 (d, J = 7.0, 2H), 4.14 (t, J = 7.0, 1H), 3.70 (bm, 2H); ^{13}C NMR ($CDCl_3$) δ 176.0, 173.9, 156.9, 155.6, 150.3, 144.5, 144.4, 141.9, 141.0, 138.8, 138.0, 133.7, 131.5, 130.7, 130.5, 129.4, 128.9, 128.4, 127.8, 126.9, 126.6, 125.9, 123.5, 120.6, 115.2, 67.8, 53.7, 47.7, 38.8; IR (thin film) 3332, 3058, 2924, 1702, 1596, 1509, 1449, 1380, 1220, 1079, 846, 761, 737, 703; 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) = 29.45 min; HRMS (ES) calcd for $C_{33}H_{28}N_2O_5$ $[M + H]^+$ 531.1920, found 531.1925; $[\alpha]^{20}_D = -32.3°$ (c = 1.5, MeOH/CH_2Cl_2/aqueous HCl 1 M 10:5:1).

Ac-L-2Oxn(5′-phenyl)-Val-Pro-DSer-Phe-Cys-Gly-NH$_2$ (P1). Yield, following HPLC purification, ∼20% from 16 chemical steps. 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) = 27.36 min; UV (0.1 M NaOH), λ_{max} = 369 nm. HRMS (ES) calcd for $C_{47}H_{58}O_{10}N_9S$ $[M + H]^+$ 940.4027, found 940.4022.[23]

Ac-L-2Oxn-Val-Pro-DSer-Phe-Cys-Gly-NH$_2$ (P2). The Fmoc-Oxn-OH amino acid, **1**, was prepared according to the literature method.[2] Yield of **P2**, following HPLC purification, ∼20% from 16 chemical steps. 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) = 25.77 min; UV (0.1 M NaOH), λ_{max} = 335 nm. MS (ES) calcd for $C_{41}H_{53}O_{10}N_9S$ $[M + H]^+$ 864.3714, found 864.3717.[23]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and relevant HPLC traces of **2**, Marfey derivatives of **9**, and peptides **P1** and **P2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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