Serinol-Derived Malyngamides from an Australian Cyanobacterium

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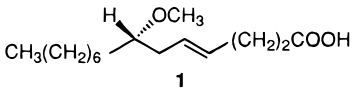
Serinol derivatives **4a** and **4b** were isolated from an Australian blue-green alga and characterized by spectroscopic means. Their absolute stereochemistry was established by chemical methods.

Cyanobacteria represent a prolific source of bioactive structurally diverse secondary metabolites.[1] The malyngamides, common metabolites of *Lyngbya majuscula*, are *N*-substituted amides of 7(*S*)-methoxytetradec-4(*E*)-enoic acid (**1**) or 7(*S*)-methoxy-9-methylhexadec-4(*E*)-enoic acid (**2**).[2] The amine portion of the malyngamides often bears a vinylic chloride and an oxygenated six-membered ring. Recently, malyngamides, one of which contained a benzene ring, were isolated for the first time from a red alga, *Gracilaria coronopifolia*.[3] The amine moiety of the malyngamides, though fairly complex and variable, is assumed to be of amino acid origin. We report here the isolation of two serinol amide derivatives of 7(*S*)-methoxyeicos-4(*E*)-enoic acid (**3**), a homologue of **1**, from a blue-green alga collected at the mouth of the King George River in northwestern Australia.[4] These compounds, **4a** and **4b**, display weak anti-HIV activity.



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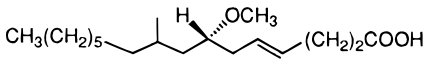

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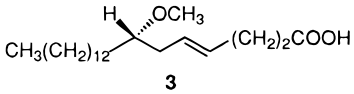

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The algal organic extract (CH_2Cl_2−MeOH) was partitioned between hexane and methanol, and then the methanol-soluble material was partitioned between methyl *tert*-butyl ether (MTBE)−hexane (9:1) and methanol−water (1:1). The hexane and hexane−MTBE extracts were subjected to vacuum liquid chromatography (VLC) on Si gel (petroleum ether−2-propanol, 30:1) to give **4a** (0.79%) and **4b** (0.41%) as colorless crystalline solids. Molecular formulas of $C_{27}H_{51}O_5N$ (**4a**) and $C_{25}H_{49}O_4N$ (**4b**) were established by HRMS. The ^1H and ^{13}C NMR spectra of **4a** (Table 1) showed the presence of a long methylene chain with proton absorption at δ 1.23−1.32 and carbon signals for 12 CH_2 groups between δ 22.7 and 33.3 (C-8−C-19). Methine absorptions at δ 130.6 and 127.6 and at δ 5.44 and 5.46 in the ^{13}C and ^1H NMR spectra, respectively, suggested one double bond. Two sharp singlets (3H each) at δ 3.31 and 3.29 implied that there were two methoxy groups present. A carbonyl carbon signal at δ 171.0 in the ^{13}C NMR spectrum and a sharp three-proton singlet at δ 2.04 in the ^1H NMR spectrum suggested an acetyl unit. Another carbonyl carbon signal at δ 172.2 and a broad one-proton doublet at δ 5.86 in the ^1H NMR spectrum indicated a secondary amide. IR spectroscopy supported these assignments with ester and amide carbonyl absorptions at 1738 and 1669 cm^{-1}, respectively, and an NH absorption at 3439 cm^{-1}.

Table 1. NMR Data for Compound **4a**

position	^{13}C[a]	^1H[b] (mult. *J*=Hz)	COSY	HMBC
1	172.2			2, NH
2	36.5	2.22 (m)	3, 4	4
3	28.6	2.30 (m)	4	4, 5
4	130.6	5.44 (m)	3	2
5	127.6	5.46 (m)	6	6, 7
6	36.3	2.16 (m)	5, 7	5, 7
7	80.7	3.13 (m)	6, 8	
8	33.3	1.32 (m)		7
9	25.3	1.42 (m)		7
10−15[c]	29.6	1.23 (m)		
16	29.7	1.23 (m)		
17[c]	29.4	1.23 (m)	18	
18	31.9	1.37 (m)	17	17, 20
19	22.7	1.23 (m)	20	20
20	14.1	0.84 (t, 6.2)	19	
1′a	63.2	4.10 (dd, 10.9, 5.9)	1′b, 2′	2′
1′b		4.13 (dd, 10.9, 5.9)	1′a, 2′	2′
2′	47.7	4.31 (m)	1′, 3′, NH	1′, NH
3′a	71.1	3.36 (dd, 3.4, 10.2)	2′, 3′b	1′
3′b		3.46 (dd, 3.4, 10.2)	2′, 3′a	1′
1″	171.0			1′
2″	20.8	2.04 (s)		
1‴	59.2	3.31 (s)		3′
1⁗	56.5	3.29 (s)		7
N		5.86 (d, 8.4)	2′	

[a] Recorded at 50 MHz, $CDCl_3$ as internal standard (δ 77.0); multiplicity is based on the JMODXH spectra. [b] Recorded at 500 MHz. [c] Assignments may be interchanged.

H−H correlations and C−H correlations within **4a** were established from ^1H−^1H COSY, TOCSY, HMQC, and HMBC spectra as illustrated in Table 1. The chemical shift values of δ 28.6 and 36.3 for allylic carbons C-3 and C-6, respectively, established the *trans* nature of the double bond. In the *cis* isomer these carbons would be expected to absorb 5 ppm further upfield.[5] Moreover, these chemical-shift values agree very well with the analogous carbons in the 7-methoxytetradec-4(*E*)-enoate moiety of the malyngamides,[2c,2d,3,6] as well as with the synthetic parent, 7-methoxytetradec-4(*E*)-enoic acid (**1**).[7]

The mass difference of 42 between **4a** and **4b** suggested that **4b** was the deacetylated version of **4a**. This was supported by the absence of the acetyl carbon signals (C-1″ at δ 171.0 and C-2″ at δ 20.8) in the ^{13}C NMR spectrum and confirmed by conversion of **4b** to **4a** by acetylation. The NMR data of **4b** is virtually identical to that of **4a** except for the serinol moiety. In **4b** the signals for the protons on C-1′ and C-2′ have shifted upfield, as expected.

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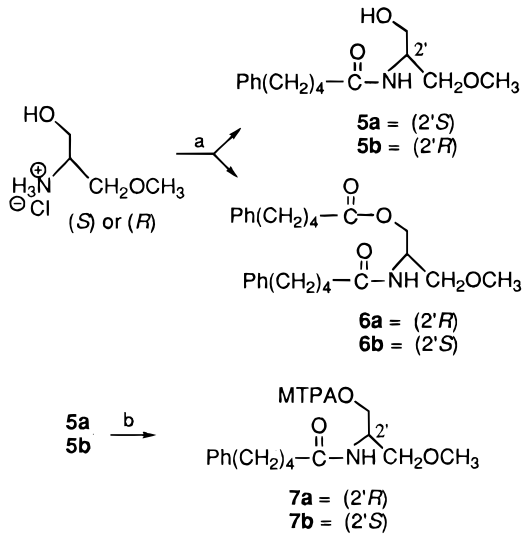
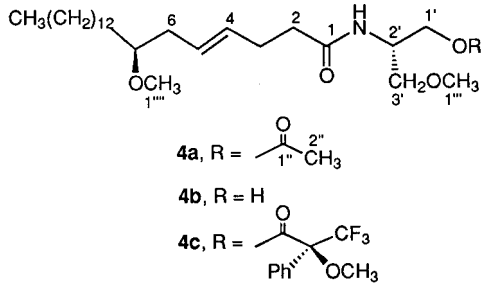
Scheme 1. Synthesis of **7a** and **7b**



[a]Key: (a) $Ph(CH_2)_4COOH$, DCC, Pyridine, 0°C; (b) (-)-MTPA, DCC, DMAP

The configuration of C-2′ in **4a** and **4b** was determined by esterification of **4b** with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)-α-phenylacetic acid (MTPA) to give **4c** for comparison with the corresponding MTPA esters of the synthetic analogues **5a** and **5b**. (*S*)- and (*R*)-2-Amino-3-methoxypropan-1-ol hydrochlorides were synthesized from the methyl ester of (*S*)-serine according to the method of Meyers and co-workers.[8] These were then converted to the 5-phenylpentylamides **5a** and **5b** following the procedure of Waki and Meienhofer.[9] Derivatization of the alcohol was also observed (Scheme 1). (2′*R*)−(−) MTPA ester **7a** and (2′*S*)−(−) MTPA ester **7b** were then prepared from the corresponding amido alcohols.



The C-3′ proton signals of (*R*)-isomer **7a** appeared as overlapping multiplets centered at δ 3.32 in the ^{1}H NMR spectrum. The C-3′ protons of (*S*)-isomer **7b** generated a different pattern of peaks, overlapping, but still identifiable as two doublets of doublets, one centered at δ 3.32 and the other centered at δ 3.41. A comparison of the ^{1}H NMR spectrum of (−)-MTPA ester **4c** with synthetic esters **7a** and **7b** clearly established **4c** as the (2′*S*)-isomer. The latter (**4c**) displayed two doublets of doublets at δ 3.31 and 3.42, as in **7b**, and lacked the absorptions characteristic of the **7a** isomer (Figure 1). Alcohol **4b** must then possess the 2′*R* configuration; and acetate **4a**, the 2′*S* configuration, allowing for the priority order change that occurs when the acyl group is replaced by a hydrogen.

To establish the absolute configuration at C-7, **4b** was hydrolyzed to give 7-methoxyeicos-4(*E*)-enoic acid (**3**), $[\alpha]^{20}_D$ −8.3° ($CHCl_3$). Although **3** has not been reported in the literature, its optical rotation compares favorably in both

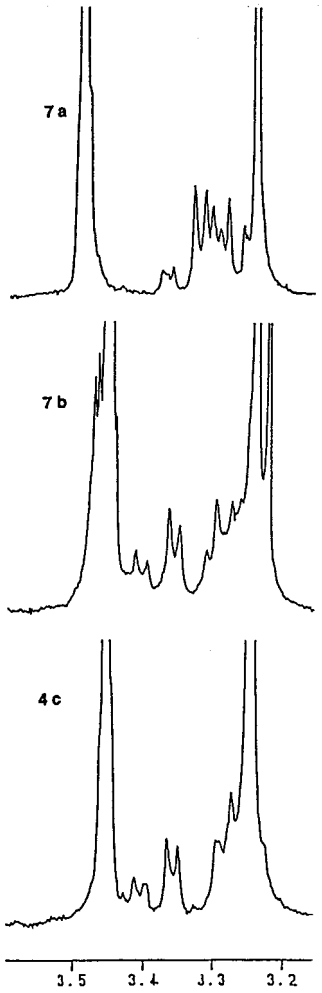


Figure 1. Portions of the ^{1}H NMR Spectra of **4c**, **7a**, and **7b**.

magnitude and sign with that of its C-14 analogue, 7(*S*)-methoxytetradec-4(*E*)-enoic acid (**1**) whose $[\alpha]^{20}_D$ is reported as −11.1° ($CHCl_3$)[5] and −11.3° ($CHCl_3$).[6] As the extra six carbons are not expected to substantially alter its rotation, we may conclude that **3** is also the *S* isomer.

We have classified compounds **4a** and **4b** as malyngamides on the basis of their fatty acid component, but their amine moiety, serinol, is far simpler than the amine moiety of previously described malyngamides. Its presumed derivation from serine is obvious, although we were unable to find other naturally occurring serinol amides in a search of the literature.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin−Elmer FT-IR spectrophotometer (PARAGON 500). Optical rotations were measured on a Rudolph Autopol II spectropolarimeter. ^{1}H and ^{13}CNMR spectra were obtained on Bruker AC 200 (200 MHz) and Varian Unity 500 (500 MHz) spectrometers. ^{1}H and ^{13}C NMR chemical shifts refer to $CDCl_3$ at 7.24 and 77.0 ppm, respectively. FABMS data were collected on a JEOL SX102 mass spectrometer operated at an accelerating voltage of 10 kV. Sephadex LH-20 was used for gel filtration, and Si gel 60H (7736 EM Sciences) was used for VLC. Preparative TLC was carried out on Sigma−Aldrich precoated Si gel $60F_{254}$ plates.

Plant Material. The organism was collected along the bank and at the head of the King George River, northwest-

ern Australia, on 21 August 1996. The alga grew as a thick mat of fine brown filaments on a mud substrate.[4]

Extraction and Isolation. The organism was ground in a Waring blender with dry ice, mixed with water, and centrifuged to give a water-first extract. The marc was freeze-dried and re-extracted with a mixture of CH_2Cl_2−MeOH (1:1 v/v) at 25 °C overnight. The crude organic extract was then concentrated to give a greenish-black sludge. A sample (2.05 g) of the crude extract was dissolved in 135 mL of MeOH, and the solution was extracted with hexane (3 × 150 mL). After separation of the layers, the hexane was evaporated to give 343 mg of oil. The MeOH layer was diluted with 150 mL of water and extracted with 9:1 MTBE−hexane (2 × 150 mL). The MTBE layer was dried and concentrated, affording 353 mg of oil. Each extract was passed through a Sephadex LH-20 column with hexane−CH_2Cl_2−MeOH (2:5:1) to give 12 fractions. Fraction 4 (17.5 mg), after repeated preparative TLC with hexanes−EtOAc (9:2) and then petroleum ether−2-propanol (8:1), afforded a total of 12.3 mg of **4a**. Fraction 5 from the Sephadex column (23.5 mg) was chromatographed by preparative TLC in hexanes−EtOAc (3:2) to yield a total of 8.0 mg of **4b**.

Compound 4a: colorless needles; mp 39−40.5 °C; $[\alpha]^{20}_D$ −6.1° (*c* 0.58, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3439, 2928, 2855, 1738, 1669, 1507, 1467, 1367, 1239, 1105, 1045, 971 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 470.3845 $[MH]^+$, (calcd for $C_{27}H_{51}O_5N$, 470.3848).

Compound 4b: colorless plates; mp 32−34 °C; $[\alpha]^{20}_D$ −3.0° (*c* 0.33, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3435, 3005, 2928, 2855, 1660, 1509, 1466, 1238, 1090, 971 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.85 (3H, t, J = 6.1 Hz, H-20), 1.23 (18 H, m, H-10-17,19), 1.37 (2H, m, H-18), 1.43 (2H, m, H-9), 2.17 (2H, m, H-6), 2.30 (4H, m, H-2,3), 3.14 (1H, m, H-7), 3.30 (3H, s, H-1‴), 3.34 (3H, s, H-1″), 3.53 (2H, m, H-3′a, H-3′b), 3.63 (1H, dd, J = 4.1, 11.2 Hz, H-1′a), 3.79 (1H, dd, J = 4.0, 11.3 Hz, H-1′b), 4.05 (1H, m, H-2′), 5.45 (1H, m, H-4), 5.47 (1H, m, H-5), 6.12 (1H, d, J = 7 Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 (C-20), 22.7 (C-19), 25.3 (C-9), 28.6 (C-3), 29.4 (C-17), 29.6 (C-10-15), 29.7 (C-16), 31.9 (C-18), 33.3 (C-8), 36.5 (C-2), 50.5 (C-2′), 56.4 (C-1‴), 59.3 (C-1‴), 64.2 (C-1′), 73.3 (C-3′), 80.7 (C-7), 127.7 (C-5), 130.6 (C-4), 173.0 (C-1); HRFABMS *m/z* 428.3741 [M + 1], (calcd for $C_{25}H_{49}O_4N$, 428.3742).

Acetylation of 4b. A mixture of 1.0 mg of **4b**, 0.1 mL of dry pyridine, and 0.2 mL of acetic anhydride was allowed to stand at 25 °C overnight. The reaction mixture was dissolved in ether (2 mL) and washed first with water until the pH was neutral, then with saturated $NaHCO_3$ solution. The organic layer was filtered through a short column of magnesium sulfate and concentrated. The residue displayed the same R_f values in petroleum ether−2-propanol (8:1), CH_2Cl_2−EtOAc (2:1), and hexanes−EtOAc (3:2) as the natural product **4a** as well as an identical ^1H NMR spectrum.

(*S*)- and (*R*)-2-Amino-3-methoxypropane-1-ol Hydrochlorides. (*S*)- and (*R*)-2-Amino-3-methoxypropan-1-ol hydrochlorides were synthesized from the methyl ester of (*S*)-serine by the method of Meyers and co-workers.[8] It should be noted that the (*S*)-enantiomer displayed an $[\alpha]^{20}_D$ of +3.1° (*c* 1.8, EtOH) and the (*R*)-enantiomer an $[\alpha]^{20}_D$ of −1.9° (*c* 2.8, EtOH), while Meyers reports an $[\alpha]_D$ of −1.9° (*c* 1.8, EtOH) for the (*S*)-enantiomer and does not report a rotation for the (*R*)-enantiomer.

Amides 5a and 6a. A 2M solution of 5-phenylvaleric acid (0.25 g, 1.40 mmol) in $CHCl_3$ (1.0 mL) was added dropwise to a solution of dicyclohexylcarbodiimide (0.33 g, 1.60 mmol) in $CHCl_3$ (1.75 mL) with stirring at 0 °C. The mixture was stirred for an additional 5 min and then added over a period of 30 min to an ice cold solution of (*S*)-2-amino-3-methoxypropan-1-ol hydrochloride (0.20 g, 1.40 mmol) in pyridine (3.5 mL). Stirring was continued for 4 h at 0 °C. Evaporation of the solvent, followed by addition of ether, precipitated the dicyclohexylurea, which was filtered and washed with ether. The filtrate was concentrated to give a yellow-brown oil. VLC with 30% EtOAc−hexane gave the **5a** (90 mg) and **6a** (28.4 mg).

Compound 5a: $[\alpha]^{20}_D$ +5.97° (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.66 (4H, m), 2.22 (2H, t, J = 7.0 Hz), 2.62 (2H, t, J = 6.9 Hz), 3.34 (3H, s), 3.49 (1H, dd, J = 4.3, 9.6 Hz), 3.57 (1H, dd, J = 4.1, 9.5 Hz), 3.62 (1H, dd, J = 4.2, 13.3 Hz), 3.78 (1H, dd, J = 4.2, 11.2 Hz), 4.05 (1H, m, J = 4.1 Hz), 6.22 (1H, d, J = 7.5 Hz, NH), 7.16 (2H m), 7.24−7.33 (3H, m); ^{13}C NMR ($CDCl_3$) δ 25.3 (CH_2), 30.9 (CH_2), 35.6 (CH_2), 36.4 (CH_2), 50.4 (CH), 59.1 (CH_3), 63.6 (CH_2), 72.7 (CH_2), 125.7 (CH), 128.2 (CH), 128.3 (CH), 142.1 (=C−), 173.4 (C=O).

Compound 6a: ^1H NMR ($CDCl_3$) δ 1.58−1.63 (6H, m), 2.14 (2H, t, J = 7.0 Hz), 2.28 (2H, t, J = 8.6 Hz), 2.13 (2H, t, J = 6.9 Hz), 2.53−2.58 (4H, m), 3.3 (3H, s), 3.32 (1H, dd, J = 4.5, 9.6 Hz), 3.42 (1H, dd, J = 3.5, 9.6 Hz), 4.07 (1H, dd, J = 11.0, 11.0 Hz), 4.10 (1H, dd, J = 11.0, 11.0 Hz), 4.27 (1H, m), 5.86 (1H, d, J = 8.3 Hz, NH), 7.09−7.13 (4H, m), 7.19−7.26 (6H, m); ^{13}C NMR δ ($CDCl_3$) 24.8 (CH_2), 25.5 (CH_2), 30.8 (CH_2), 30.9 (CH_2), 33.9 (CH_2),35.5 (CH_2), 35.6 (CH_2), 36.5 (CH_2), 47.8 (CH), 59.1 (CH_3), 63.0 (CH_2), 71.1 (CH_2), 125.7 (CH), 128.2 (CH), 128.3 (CH), 142.0 (=C−), 142.1 (=C−), 172.9 (C=O), 179.0 (C=O).

Amides 5b and 6b. The same procedure as for the synthesis of **5a** was carried out with (*R*)-2-amino-3-methoxypropane-1-ol hydrochloride to obtain 4.2 mg of **5b** and 30 mg of **6b**.

Compound 5b: $[\alpha]^{20}_D$ −3.57° (*c* 0.14, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.63 (4H, m), 2.19 (2H, J = 7.1 Hz), 2.58 (2H, t, J = 6.9 Hz), 3.30 (3H, s), 3.43 (1H, dd, J = 4.0, 9.6 Hz), 3.54 (1H, dd, J = 3.9, 9.59 Hz), 3.63 (1H, m), 3.77 (1H, dd, J = 4.3, 11.3 Hz), 4.01 (1H, m, J = 3.7 Hz), 6.09 (1H, d, J = 6.6 Hz, NH), 7.4 (2H, m), 7.20−7.26 (3H, m); ^{13}C NMR ($CDCl_3$) δ 25.3 (CH_2), 31.0 (CH_2), 35.6 (CH_2), 36.5 (CH_2), 50.4 (CH), 59.2 (CH_3), 64.3 (CH_2), 73.3 (CH_2), 125.7 (CH), 128.2 (CH), 128.3 (CH), 142.1 (=C−), 173.4 (C=O).

Compound 6b: ^1H NMR ($CDCl_3$) δ 1.60 (6H, m), 2.13 (2H, t, J = 6.8 Hz), 2.27 (4H, m), 2.55 (4H, m), 3.31 (1H, dd, J = 4.4, 10.1 Hz), 3.41 (1H, dd, J = 3.4, 9.7 Hz), 4.05 (1H, dd, J = 11.0, 10.9 Hz), 4.09 (1H, dd, J = 11.0, 10.9 Hz), 4.29 (1H, m, CH), 5.8 (1H, d, J = 8.4 Hz, NH), 7.08−7.12 (4H, m), 7.18−7.26 (6H, m); ^{13}C NMR ($CDCl_3$) δ 24.8 (CH), 25.2 (CH_2), 30.8 (CH_2), 30.9 (CH_2), 33.9 (CH_2), 35.5 (CH_2), 35.6 (CH_2), 36.5 (CH_2), 47.7 (CH), 59.1 (CH_3), 63.0 (CH_2), 71.0 (CH_2), 125.7 (CH), 128.3 (CH), 142.0 (=C−), 142.1 (=C−), 172.8 (C=O), 178.5 (C=O).

(−)-MTPA Derivative 7a. To a solution of alcohol **5a** (3.4 mg, 0.01 mmol), dicyclohexylcarbodiimide (DCC) (6.18 mg, 0.030 mmol), and dimethylaminopyridine (DMAP) (0.155 mg, 0.001 mmol) in dry CH_2Cl_2 (0.5 mL) was added 0.09 mL of a 0.2137 M stock solution of (−)-MTPA in CH_2Cl_2 (0.019 mmol). After the mixture was stirred at 25 °C overnight, the resulting white suspension was filtered to remove the *N*,*N*′-dicyclohexylurea and then partitioned between CH_2Cl_2 and water (2 mL). The organic layer was washed with 2 mL each of 1N HCl, water, saturated $NaHCO_3$ solution, and saturated NaCl solution, and then dried with $MgSO_4$, filtered, and concentrated. The residue

was purified by preparative TLC with EtOAc−CH_2Cl_2 (1:3 then 1:9) to yield 3.5 mg (66%) of **7a**.

Compound 7a: colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.60 (8H, m), 2.12 (2H, t, J = 7.0 Hz), 2.59 (2H, t, J = 7.1 Hz), 3.26 (3H, s), 3.32 (2H, m), 3.51 (3H, m), 4.30 (1H, m), 4.32 (1H, dd, J = 3.4, 10.6 Hz), 4.38 (1H, dd, J = 3.1, 10.6 Hz), 7.16 (2H, m), 7.22 (2H, m), 7.24−7.27 (3H, m), 7.27−7.49 (3H, m); ^{13}C NMR ($CDCl_3$) δ 25.1 (CH_2), 31.0 (CH_2), 35.6 (CH_2), 36.4 (CH_2), 47.2 (CH), 55.4 (CH_3), 59.1 (CH_3), 64.3 (CH_2), 70.7 (CH_2), 125.8 (CH), 127.3 (CH), 127.9 (q, J = 288.2 Hz, CF_3), 128.3 (CH), 128.5 (CH), 129.7 (CH), 142.1 (=C−), 166.3 (C=O), 172.5 (C=O).

(−)-MTPA Derivative 7b. The same procedure used for the synthesis of **7a** was carried out with **5b** to yield 4.5 mg of **7b** (72%).

Compound 7b: ^{1}H NMR ($CDCl_3$) δ 1.60 (8H, m), 2.12 (2H, t, J = 7.0 Hz), 2.59 (2H, t, J = 7.1 Hz), 3.26 (3H, s), 3.32 (1H, dd, J = 2.9, 7.4 Hz), 3.41 (1H, dd, J = 3.2, 15.0 Hz), 3.49 (3H, m), 4.33 (1H, dd, J = 2.9, 15.0 Hz), 4.34 (1H, m), 4.37 (1H, dd, J = 7.1, 8.8), 7.13 (2H, m), 7.21 (2H, m), 7.22−7.23 (3H, m), 7.33−7.46 (3H, m); ^{13}C NMR ($CDCl_3$) δ 25.1 (CH_2), 31.0 (CH_2), 35.6 (CH_2), 36.4 (CH_2), 47.2 (CH), 55.4 (CH_3), 59.1 (CH_3), 64.3 (CH_2), 70.7 (CH_2), 125.7 (CH), 127.4 (CH), 127.9 (q, J = 288.2 Hz, CF_3), 128.3 (CH), 128.5 (CH), 129.7 (CH), 142.1 (=C−), 166.2 (C=O), 172.5 (C=O).

(−)-MTPA Ester of 4b (4c). To a solution of **4b** (2.0 mg, 0.0047 mmol), CH_2Cl_2 (3.09 mg), and a catalytic amount of DMAP in dry CH_2Cl_2 (0.5 mL) was added 0.048 mL of a 0.2137 M stock solution of (−)-MTPA in dry CH_2Cl_2. After the mixture was stirred overnight, the resulting white suspension was filtered to removed the *N*,*N*′-dicyclohexylurea and then partitioned between EtOAc (1 mL) and water (0.5 mL). The organic layer was washed with 0.5 mL each of 1N HCl, water, saturated $NaHCO_3$ solution, and brine, then dried over $MgSO_4$, filtered, and concentrated to afford a colorless residue. The residue was subjected to repeated preparative TLC with EtOAc−CH_2Cl_2 (0.5:9.5) and then CH_2Cl_2−EtOAc (2:1) to afford the desired product (**4c**) as a colorless oil (2.5 mg, 83%): ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, t, J = 6.4), 1.21 (8H, m), 1.51 (6H, m) 2.12−2.26 (6H, m), 3.10 (1H, m, J = 5.6 Hz), 3.27 (3H, s), 3.31 (1H, m), 3.42 (1H, dd, J = 3.3, 9.1 Hz), 4.31 (1H, m), 4.35 (1H, dd, J = 7.6, 8.5 Hz), 4.36 (1H, dd, J = 7.1, 7.3 Hz), 5.40 (1H, d, J = 3.9 Hz), 5.41 (1H, d, J = 3.9 Hz), 5.68 (1H, d, J = 8.4 Hz, NH), 7.3 (2H, m), 7.36−7.47 (3H, m); ^{13}C NMR ($CDCl_3$) δ 28.5 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 33.3 (CH_2), 36.3 (CH_2), 47.2 (CH), 56.4 (OCH_3), 59.0 (CH_3), 64.2 (CH_2), 70.6 (CH_2), 80.6 (CH), 127.3 (CH), 127.6 (=C−), 128.4 (CH), 129.7 (CH), 130.4 (=C−).

7-Methoxyeicos-4(*E*)-enoic Acid (3). The amide, **4a** (10.0 mg), was hydrolyzed by refluxing with 2 mL of 10% w/w KOH−$HOCH_2CH_2OH$ for 4.5 h. After cooling, the solution was acidified with 3M HCl to pH 2. The cloudy solution was extracted three times with 5-mL portions of ether. The organic fraction was dried over $MgSO_4$ and concentrated to afford a colorless residue that was subjected to preparative layer chromatography with petroleum ether−EtOAc (1:1). The product **3** (5.5 mg, 76%) was obtained as a colorless oil: $[\alpha]^{20}_D$ −8.3° (*c* 0.18, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2928, 2855, 1712, 1601, 1467, 972; ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, t, J = 6.2 Hz), 1.21 (16H, m), 1.34−1.39 (2H, m), 1.99 (3H, s), 2.08 (2H, t, J = 7.7 Hz), 2.28−2.37 (4H, m), 3.10 (1H, m, J = 5.7 Hz), 3.27 (3H, s), 5.42 (1H, d, J = 3.6 Hz), 5.44 (1H, d, J = 4.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3), 22.7 (CH_2), 25.3 (CH_2), 27.7 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 33.3 (CH_2), 36.3 (CH_2), 56.5 (CH_3), 80.7 (CH_3), 127.8 (=C−), 130.1 (=C). The carbonyl carbon was too weak to be observed.

Bioassays. The anti-HIV assays were performed at the National Cancer Institute as described previously.[10]

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