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Total Synthesis of Two Photoactivatable Analogues of the Growth-Factor-Like Mediator Sphingosine 1-Phosphate: Differential Interaction with Protein Targets

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The first synthesis of two photoreactive analogues of the lipid mediator and second messenger sphingosine 1-phosphate (S1P), [^{32}P]-labeled (2*S*,3*R*)-14-*O*-(4′-benzoylphenyl)- and (2*S*,3*R*)-14-*O*-((4′-trifluoromethyldiazirinyl)phenyl)-(4*E*)-tetradecenyl-2-amino-3-hydroxy-1-phosphate, is described. The interactions of these probes with the S1P type-1 receptor ($S1P_1$) transfected into membranes of rat hepatoma cells and with plasma proteins were analyzed. The $S1P_1$ receptor interacted in a specific manner with the benzophenone-containing ligand ($K_D = 84 \pm 10$ nM vs K_D for S1P $= 36 \pm 2$ nM); in contrast, no saturable specific binding was found with the diazirine-containing ligand. However, the same pattern was found for labeling of plasma proteins by both probes, indicating that different parts of the S1P pharmacophore underlie the interaction of S1P with its receptor and plasma carrier proteins.

Introduction

Sphingosine 1-phosphate (S1P) is a member of the lysophospholipid growth factor family with diverse actions in almost every cell type.[1] S1P can act as an extracellular mediator through the activation of any of five G-protein-coupled plasma membrane receptors encoded by some of the endothelial differentiation gene (EDG) family, which are named S1P(1−5).[1c,2] Intracellularly, when generated from sphingosine by sphingosine kinases (SKs), S1P can also act as a second messenger through a set of targets that are not yet fully characterized.[3] Because of the many extra- and intracellular targets that bind to S1P, photoactivatable analogues of S1P would be of great practical importance provided that the photoreactive moiety does not compromise the specificity and often high-affinity interaction of the ligand with its binding protein. Ligand recognition of S1P by the $S1P_1$ receptor has been partially mapped to a cluster of three charged amino acids, but the position of the hydrophobic tail remains unknown.[4] Although residues involved in the catalytic activity of SKs have been identified, the binding of the substrate and the precise catalytic mechanism remain to be elucidated.[5] Elucidation of the S1P-mediated receptor activation or of the enzymatic mechanisms underlying S1P production are just two examples of important molecular events that may benefit from the availability of photoreactive S1P analogues. Binding of S1P to serum proteins has been shown to attenuate its biological activity.[6] Identification of S1P binding proteins in biological fluids and their binding domain could lead to the development of synthetic peptides that offer therapeutic applicability to block S1P-induced tumor angiogenesis.

The benzophenone and trifluoromethylphenyldiazirine chromophores have many useful features in common, including high chemical stability in subdued ambient light, photoactivation using near-UV light ($\lambda > 350$ nm), and the ability to insert randomly into accessible C−H bonds of amino acid residues to form photoadducts.[7] In the present study, we describe the total synthesis of two new S1P derivatives that bear a benzophenone or a (4-trifluoromethyl)phenoxydiazirine moiety in the sphingoid

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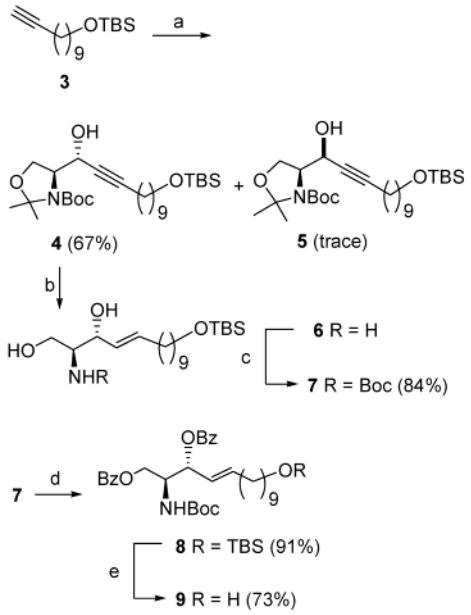
SCHEME 1. Synthesis of ω-Hydroxysphingosine 9[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, HMPA, (*S*)-Garner aldehyde, −78 °C; (b) Li, $EtNH_2$, THF, −78 °C; (c) Boc_2O, Et_3N, dioxane; (d) Bz_2O, DMAP, Py, rt; (e) TBAF, THF, rt.

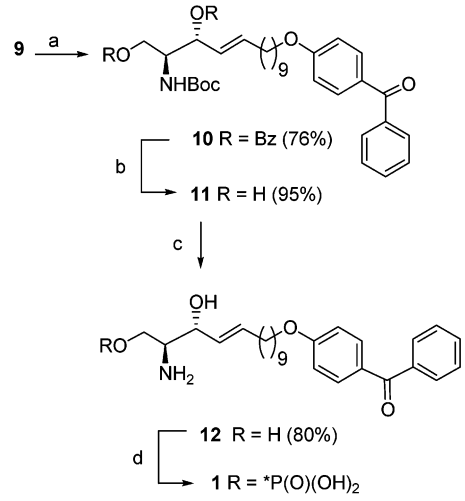
chain of S1P (compounds **1** and **2**, respectively) and the characterization of their interaction with the $S1P_1$ receptor and S1P-binding proteins present in rat plasma. These two new S1P analogues may facilitate the dissection of the molecular mechanisms involved in the specific lipid−protein interaction taking place between S1P and its protein targets.

Results and Discussion

Preparation of ω-Hydroxysphingosine Derivative 9 (Scheme 1). The hydroxy group of 10-undecyn-1-ol was protected as its TBS ether **3** with TBSCl (1.5 equiv) and imidazole (3 equiv) in CH_2Cl_2. The acetylide anion derived from **3** was coupled diastereoselectively with (*S*)-Garner aldehyde[8] in the presence of HMPA,[9] giving *erythro*-isomer **4** in 67% yield and, in some reactions, a trace amount of *threo*-isomer **5**. Birch reduction of e*rythro*-**4** afforded crude 2-amino-1,3-diol **6**. To introduce a benzophenone (Scheme 2) or (4-trifluoromethyl)phenoxydiazirinyl (Scheme 3) moiety at the terminal position of the long-chain base, the hydroxy and amino groups must be protected. As shown in Scheme 1, the amino group was protected as an *N*-BOC carbamate to afford **7** in 84% yield (for two steps from **4**). Then, the hydroxy groups of **7** were protected as benzoyl esters, affording **8** in 91% yield. The TBS group of **8** was cleaved (TBAF, THF) to give the ω-hydroxysphingosine intermediate **9** in 73% yield.

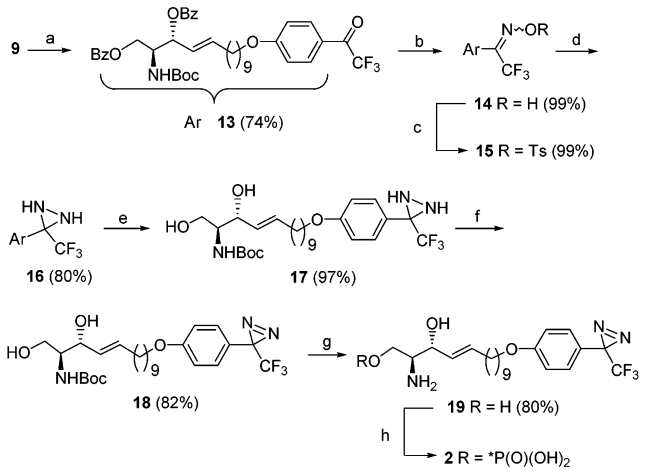
Synthesis of the Benzophenone S1P Analogue, Compound 1. Compound **1** was synthesized as shown in Scheme 2. The key step is the Mitsunobu reaction (DIAD/Ph_3P)[10] of 4-hydroxybenzophenone with intermediate **9**, which afforded coupling product **10** in 76% yield. After the benzoyl esters of **10** were hydrolyzed (1 M NaOH in MeOH) and the *N*-BOC group of **11** was removed by treatment with 4 M HCl in THF, sphingosine analogue **12** was obtained in 80% yield.

SCHEME 2. Synthesis of Compound 1[a]



[a] Reagents and conditions: (a) DIAD, PPh_3, 4-hydroxybenzophenone, CH_2Cl_2, 0 °C to rt; (b) 1 M NaOH, MeOH, rt; (c) 4 M HCl, THF, rt; (d) sphingosine kinase, [γ-^{32}P]ATP, pH 7.5, 37 °C, 1 h.

SCHEME 3. Synthesis of Compound 2[a]



[a] Reagents and conditions: (a) DIAD, PPh_3, 4-$CF_3COC_6H_4OH$ (**22**), CH_2Cl_2, rt; (b) NH_2OH, Py, EtOH, 50−60 °C; (c) *p*-TsCl, NEt_3, DMAP, CH_2Cl_2, 0 °C to rt; (d) liquid NH_3; (e) 1 N NaOH, MeOH, rt; (f) I_2, Et_3N, MeOH, rt; (g) 4 M HCl, THF, rt; (h) sphingosine kinase, [γ-^{32}P]ATP, pH 7.5, 37 °C, 1 h.

Synthesis of the (4-Trifluoromethyldiazirinyl)-phenoxy S1P Analogue, Compound 2. Compound **2** was prepared as shown in Scheme 3. Mitsunobu reaction (DIAD/Ph_3P) of 4-hydroxytrifluoroacetophenone (**22**) (prepared by trifluoroacetylation of the Grignard reagent of 4-bromoanisole with *N*-(trifluoroacetyl)piperidine (**20**), followed by *O*-demethylation of **21** with LiCl in DMF) with **9** gave trifluoroacetophenone ether **13** in 74% yield. The trifluoroacetyl group of **13** was converted to the (4-

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trifluoromethyl)phenoxydiazirinyl moiety by using standard procedures.[11] Briefly, a mixture of (*E*/*Z*)-oximes **14** derived from **13** was treated with *p*-TsCl and triethylamine in the presence of a catalytic amount of DMAP in CH_2Cl_2 to afford tosylates **15**. Tosylates **15** were converted to diaziridine **16** with liquid ammonia, and base-catalyzed hydrolysis of benzoyl ester **16** with 10% methanolic NaOH gave diol **17**. Oxidation of **17** (I_2, Et_3N, MeOH) gave the desired diazirine **18**. Finally, the *N*-BOC group of **18** was removed to give sphingosine analogue **19**.

Radiolabeling of S1P and the Sphingosine Photoactivatable Analogues. The reaction buffer contained 20 mM Tris, pH 7.5, 12 mM $MgCl_2$, 2 mM DTT, 0.25 mM EDTA, 5 mM NaF, 12 mM β-glycerophosphate, 1 mM sodium pyrophosphate, 5% glycerol, and 1% protease inhibitor cocktail. A 65 nmol sample of sphingosine or its analogue was incubated in 1 mL of reaction buffer containing 100 μg of protein from the cell lysate of RH7777 cells that had been transfected with SK and 250 μCi of [γ-^{32}P]ATP (30 Ci/mmol) at 37 °C for 1 h. The product was extracted with a mixture of 1.6 mL of $CHCl_3$/MeOH/HCl (100:200:1), 1 mL of 2 M KCl, and 1 mL of $CHCl_3$. The organic phase was extracted again with 2 mL of MeOH, 1 mL of $CHCl_3$, 2 mL of 2 M KCl, and 100 μL of NH_4OH. The aqueous phase was extracted with 3 mL of $CHCl_3$ and 200 μL of 14 N HCl. The organic extract was dried under a stream of N_2, and the residue was redissolved in 0.5 mL of PBS containing 1 mM BSA.

Radioligand Binding Assay. The rat hepatoma RH7777 cell line was obtained from ATCC commercially and was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). This cell line does not express endogenous $S1P_1$ and is nonresponsive to S1P.[4] Binding of ^{32}P-labeled S1P and the photoactivatable analogues to intact cells was carried out in subdued light after transient transfection of the $S1P_1$ receptor into RH7777 cells as described earlier.[12] Scatchard analysis of the binding curves was carried out using the Kaleidograph software. Each binding experiment was carried out on triplicate samples and was repeated at least three times using a new transfection of cells.

Photoaffinity Labeling of Plasma. Rat plasma diluted to 5% (v/v) in PBS was mixed with 5 nM photoaffinity analogue on ice. The sample was irradiated at 365 nm on ice for 10 min using a UV lamp. Aliquots of 10 μL were loaded on reducing 12.5% SDS−PAGE gels. The gels were silver stained using the SilverQuest kit and exposed to a phosphorimager screen for 24 h.

Ligand Binding Properties of Compounds 1 and 2 on $S1P_1$ Receptors. The RH7777 cell line does not express endogenous S1P receptors and has been used for the heterologous expression of the $S1P_1$ receptor.[4] Binding of the S1P photoactivatable analogues to $S1P_1$ was tested in the dark after transient transfection of the receptor into RH7777 cells. The dose−response curves of S1P (Figure 1A) and compound **1** (Figure 1B) showed saturable binding, whereas that of compound **2** did not (Figure 1C), even though both probes were tethered to the same position of the sphingoid base. Scatchard analysis yielded K_D values of 36 ± 2 and 84 ± 10 nM for S1P and compound **1**, respectively, indicating that compound **1** closely mimics the binding properties of the endogeneous ligand for $S1P_1$. Because of the lack of saturable binding, the K_D value could not be determined for compound **2**. These results indicate that compound **2** does not interact specifically with the high-affinity binding pocket of the $S1P_1$ receptor. These binding studies

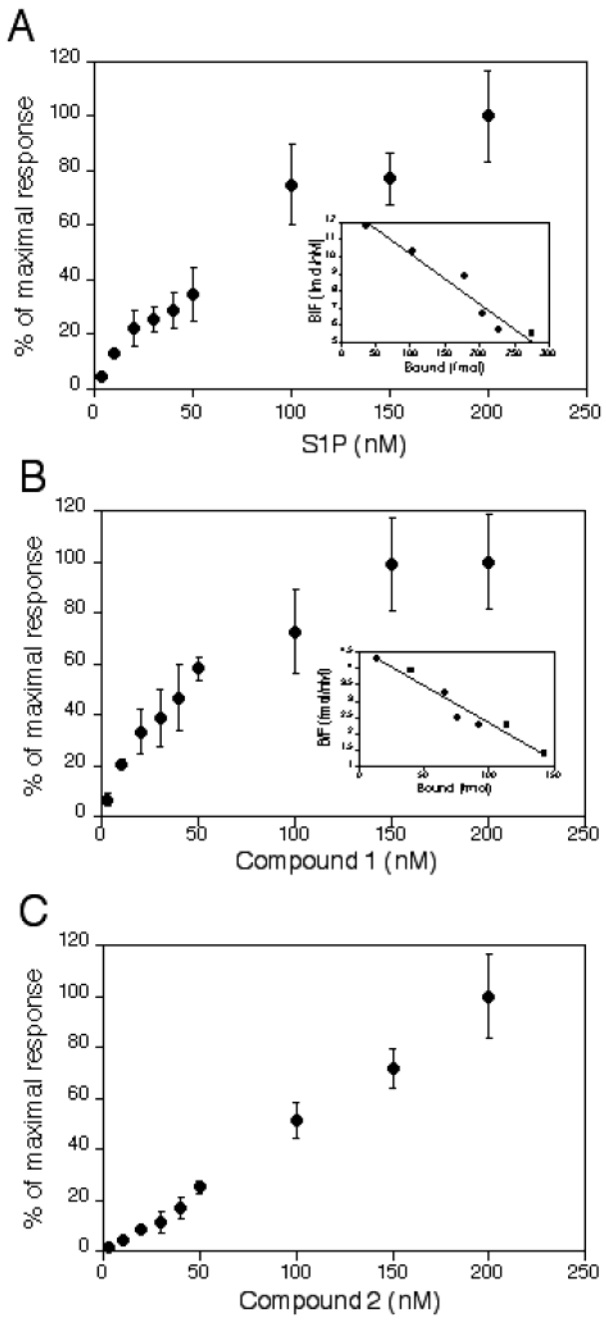

FIGURE 1. Binding of S1P and photoactivatable analogues to RH7777 cells transfected with $S1P_1$ receptor: (A) concentration dependence of specific binding of S1P (the inset shows the Scatchard plot); (B) concentration dependence of specific binding of compound **1** (the inset is the Scatchard plot); (C) concentration dependence of specific binding of compound **2**. Note that the binding of S1P and compound **1** shows saturation, whereas the binding of compound **2** does not.

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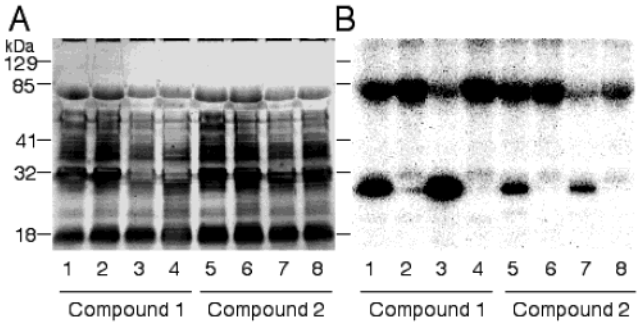

FIGURE 2. Photoaffinity labeling of normal Sprague−Dawley and analbuminemic Nagase rat plasma with compounds **1** and **2**. The labeled plasma was separated on a 12.5% SDS−PAGE gel, silver-stained (A), and exposed to a phosphorimager screen (B). Lanes 1 and 5: normal rat plasma. Lanes 2 and 6: normal rat plasma labeled in the presence of 20 μM S1P. Lanes 3 and 7: analbuminemic rat plasma. Lanes 4 and 8: analbuminemic rat plasma labeled in the presence of 20 μM S1P competitor.

suggest a highly restricted interaction between $S1P_1$ and its ligand that has been compromised for compound **2** but was relatively unaffected for compound **1**.

Photoaffinity Labeling of Rat Plasma with Compounds 1 and 2. To map the S1P interactions with plasma proteins, the photoactivatable analogues were used to label normal and analbuminemic rat plasma samples (Figure 2). The analbuminemic Nagase rats, which were derived from the Sprague−Dawley strain, contain reduced concentrations of albumin (19.4 mg/mL vs 0.4 mg/mL) because of a defect in albumin synthesis by the liver.[13] Both compounds gave an identical footprint of labeling of plasma proteins. Besides albumin, another protein with an apparent molecular weight of 25000 was labeled. The addition of S1P at 20 μM competed for the labeling of this protein, indicative of the specific nature of the interaction. Labeling of albumin was not affected by 20 μM S1P, possibly because of the high-affinity binding and the high concentration of albumin (~14.5 μM) in the plasma samples.

Conclusions

A convenient method has been demonstrated for the first synthesis of two photoactivatable S1P analogues. This method can be readily modified to prepare a range of compounds carrying linkers with different chain lengths to the photoreactive moiety at the terminal position of the long-chain base. The application of these photoactivatable S1P agents in the present study suggests that their interactions with the $S1P_1$ receptor, albumin, and an unidentified molecular weight 25000 novel plasma protein are affected by different parts of the S1P pharmacophore. With respect to $S1P_1$ recognition, we found that a benzophenone-containing derivative of S1P but not a diazirine analogue showed competitive displacement of S1P with nanomolar affinity from the ligand-binding site. In contrast, similar patterns of labeled proteins were obtained for both analogues in rat plasma, where albumin was the predominant carrier of the S1P analogues. The availability of these probes is likely to fuel future studies aimed at the identification of the binding sites of the biological targets of S1P at the molecular level, which will also provide important information for the synthesis of drugs selectively targeting proteins that bind S1P and regulate its biological activity.

Experimental Section

11-[(*tert*-Butyldimethylsilyl)oxy]-1-undecyne (3). This compound was prepared by a modification of a reported procedure.[14] To a solution of *tert*-butyldimethylsilyl chloride (4.52 g, 30 mmol) in dry CH_2Cl_2 (20 mL) was added imidazole (4.08 g, 60 mmol) at rt under a N_2 atmosphere. The mixture was stirred for 1 h before a solution of 10-undecyn-1-ol (3.36 g, 20 mmol) in dry CH_2Cl_2 (10 mL) was added. The mixture was stirred overnight, and then quenched with water (10 mL). After the mixture was extracted with CH_2Cl_2 (3 × 25 mL), the combined organic phases were washed with brine (40 mL) and dried ($MgSO_4$). The residue was purified by flash chromatography to afford **3** (5.53 g, 98%) as a colorless oil: R_f 0.95 (EtOAc/hexane, 1:3); ^{1}H NMR δ 0.01 (s, 6H), 0.85 (s, 9H), 1.23−1.53 (m, 14H), 1.87 (s, 1H), 2.12 (m, 2H), 3.55 (m, 2H); ^{13}C NMR δ −5.25, 18.2, 18.3, 25.6, 25.7, 28.4, 28.5, 28.7, 29.0, 29.1, 29.3, 29.4, 29.5, 32.8, 63.1, 68.0, 84.8.

***N*-*tert*-Butoxycarbonyl (4*S*,1′*R*)-2,2-Dimethyl-4-(1′-hydroxy-12′-*tert*-butyldimethylsilyloxy-2′-dodecynyl)oxazolidine (4).** To a solution of alkyne **3** (5.08 g, 18.0 mmol) in dry THF (150 mL) was added *n*-BuLi (2.5 M in hexane, 6.6 mL, 1.65 mmol) at −78 °C under a N_2 atmosphere. The mixture was stirred for 2 h before HMPA (116 mg, 114 μL, 0.65 mmol) was added. After the mixture was stirred for another 30 min, a solution of Garner aldehyde (3.45 g, 15.0 mmol) in 15 mL of dry THF was added slowly. The solution was stirred at −78 °C for 2.5 h before being quenched with aqueous saturated NH_4Cl solution (40 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography to afford **4** (4.34 g, 67%) as a colorless oil: $[\alpha]^{25}_D$ −33.2° (*c* 5.0, $CHCl_3$); R_f 0.61 (EtOAc/hexane, 1:3); ^{1}H NMR δ 0.01 (s, 6H), 0.85 (s, 9H), 1.23−1.53 (m, 29H), 2.15 (m, 2H), 3.54 (t, 2H, J = 6.4 Hz), 3.89 (s, 1H), 4.08 (m, 2H), 4.51 (s, 1H); ^{13}C NMR δ −5.25, 18.3, 18.8, 25.8, 28.3, 28.4, 28.5, 29.1, 29.4, 29.5, 32.9, 62.9, 63.3, 64.2, 65.1, 81.2, 86.6, 94.9, 99.4, 154.1; HR-MS [FAB, MNa^+] *m*/*z* calcd for $C_{28}H_{53}NO_5SiNa$ 534.3591, found 534.3561.

(2*S*,3*R*)-2-*N*-(*tert*-Butoxycarbonylamido)-14-*O*-(*tert*-butyldimethylsilyl)-(4*E*)-tetradecene-1,3,14-triol (7). To a blue solution prepared from lithium (1.40 g, 200 mmol) and dry $EtNH_2$ (50 mL) was added a solution of **4** (5.12 g, 10 mmol) in THF (10 mL) at −78 °C. The reaction mixture was stirred for 4 h at −78 °C and then quenched with solid NH_4Cl. After $EtNH_2$ was removed under reduced pressure, the aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated. To the residue were added dioxane (60 mL), H_2O (20 mL), Et_3N (5 mL), and Boc_2O (2.62 g, 12 mmol). The solution was stirred overnight at rt before being quenched with saturated aqueous NH_4Cl solution (15 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane, 1:1), providing **7** (3.97 g, 84%) as a colorless wax: $[\alpha]^{25}_D$ −1.33° (*c* 11.7, $CHCl_3$); R_f 0.43 (EtOAc/hexane, 1:1); ^{1}H NMR δ 0.00 (s, 6H), 0.85 (s, 9H), 1.12−1.47 (m, 23 H), 1.98 (q, 2H, J = 7.2 Hz), 3.55 (t, 2H, J = 6.4 Hz), 3.56−3.62 (m, 2H), 3.73 (m, 1H), 3.83 (m, 2H), 4.19 (m, 1H), 5.43 (t, 1H, J = 6.4 Hz), 5.47 (d, 1H, J = 6.4 Hz), 5.75 (m, 1H); ^{13}C NMR δ −5.26, 18.3, 25.8, 26.0, 28.4, 29.1, 29.4, 29.6,

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32.3, 32.8, 55.5, 62.3, 63.3, 74.1, 79.6, 129.2, 133.7, 156.3; MS (ESI) *m*/*z* 474.3 (MH^+).

(2*S*,3*R*)-1,3-*O*-Dibenzoyl-2-*N*-(*tert*-butoxycarbonylamido)-14-*O*-(*tert*-butyldimethylsilyl)-(4*E*)-tetradecene-1,3,-14-triol (8). (Dimethylamino)pyridine (137 mg, 0.55 mmol) was added to a solution of **7** (1.37 g, 2.89 mmol) and benzoic anhydride (2.61 g, 11.6 mmol) in pyridine (30 mL), and the mixture was stirred at rt overnight. Volatiles were evaporated under vacuum, and the residue was purified by chromatography (elution with EtOAc/hexane, 1:6), providing **8** (1.80 g, 91%) as a colorless oil: $[\alpha]^{25}_D$ −4.14° (*c* 5.6, $CHCl_3$); R_f 0.61 (EtOAc/hexane, 1:3); ^{1}H NMR δ 0.00 (s, 6H), 0.84 (s, 9H), 1.20−1.37 (m, 21H), 1.43 (m, 2H), 2.01 (q, 2H, J = 7.2 Hz), 3.54 (t, 2H, J = 6.8 Hz), 4.46 (m, 3H), 4.94 (d, 1H, J = 9.6 Hz), 5.54 (m, 1H), 5.63 (m, 1H), 5.88 (m, 1H), 7.37 (m, 4H), 7.50 (m, 2H), 7.99 (m, 4H); ^{13}C NMR δ −5.26, 18.4, 25.8, 26.0, 28.3, 28.7, 29.2, 29.4, 29.5, 32.3, 32.9, 52.3, 63.3, 63.6, 75.1, 79.8, 124.0, 128.3, 128.4, 129.5, 129.7, 130.0, 132.8, 133.1, 137.2, 155.3, 165.4, 166.4; MS (ESI) *m*/*z* 699.3 (MNH_4^+).

(2*S*,3*R*)-1,3-*O*-Dibenzoyl-2-*N*-(*tert*-butoxycarbonylamido)-(4*E*)-tetradecene-1,3,14-triol (9). A solution of TBAF (1 M, 7.2 mL) in THF (1.88 g, 7.20 mmol) was added to a solution of **8** (1.65 mg, 2.42 mmol) in THF (80 mL). After the mixture was stirred at rt overnight, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane, 1:6), providing **9** (1.00 g, 73%) as a colorless oil: $[\alpha]^{25}_D$ −5.85° (*c* 4.80, $CHCl_3$); R_f 0.39 (EtOAc/hexane, 1:1); ^{1}H NMR δ 1.20−1.58 (m, 23H), 2.04 (q, 2H, J = 7.2 Hz) 3.62 (t, 2H, J = 6.8 Hz), 4.46 (m, 3H), 4.90 (d, 1H, J = 9.2 Hz), 5.54 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (td, 1H, J = 6.4, 15.6 Hz), 7.41 (m, 4H), 7.57 (m, 2H), 8.03 (m, 4H); ^{13}C NMR δ 25.7, 28.3, 28.7, 29.0, 29.2, 29.3, 29.4, 32.3, 32.8, 52.4, 63.0, 63.6, 75.1, 79.9, 124.0, 128.4, 129.7, 129.8, 130.0, 133.1, 137.2, 155.3, 165.4, 166.5; HR-MS [FAB, MNa^+] *m*/*z* calcd for $C_{33}H_{45}NO_7Na$ 590.3094, found 590.3069.

(2*S*,3*R*)-1,3-*O*-Dibenzoyl-2-*N*-(*tert*-butoxycarbonylamido)-14-*O*-(4′-benzoylphenyl)-(4*E*)-tetradecene-1,3,14-triol (10). To a solution of DIAD (50 mg, 0.25 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added a solution of Ph_3P (70 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred for 10 min, a solution of 4-hydroxybenzophenone (56 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL) was added over a period of 20 min. The reaction mixture was stirred for another 10 min, and a solution of alcohol **9** (94 mg, 0.17 mmol) in dry CH_2Cl_2 (10 mL) was added over a period of 20 min. After the mixture was stirred for 10 min at 0 °C, the ice bath was removed, and the mixture was stirred at rt overnight. TLC indicated that **9** had disappeared. The organic solution was concentrated, and the resulting residue was purified by chromatography (elution with EtOAc/hexane, 1:6) to afford **10** (93 mg, 76%) as a white wax: $[\alpha]^{25}_D$ −2.71° (*c* 2.8, $CHCl_3$); R_f 0.42 (EtOAc/hexane, 1:3); ^{1}H NMR δ 1.27−1.58 (m, 21H), 1.77 (m, 2H), 2.04 (q, 2H, J = 7.2 Hz), 4.02 (t, 2H, J = 6.4 Hz), 4.42 (m, 1H), 4.50 (m, 2H), 4.87 (d, 1H, J = 9.2 Hz), 5.54 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (td, 1H, J = 6.4, 15.6 Hz), 6.93 (m, 2H), 7.45 (m, 6H), 7.57 (m, 3H), 7.75 (d, 2H, J = 7.2 Hz), 7.80 (d, 4H, J = 8.8 Hz), 8.04 (m, 4H); ^{13}C NMR δ 22.0, 26.0, 28.3, 28.7, 29.1, 29.3, 29.4, 30.9, 32.3, 40.4, 52.3, 63.6, 75.1, 79.9, 114.0, 124.1, 128.2, 128.4, 129.7, 129.9, 130.0, 131.8, 132.6, 133.2, 137.2, 138.4, 155.3, 162.9, 165.4, 166.5, 195.6.

(2*S*,3*R*)-2-*N*-(*tert*-Butoxycarbonylamido)-14-*O*-(4′-benzoylphenyl)-(4*E*)-tetradecene-1,3,14-triol (11). To a solution of 1 M NaOH (10 mL) in methanol was added **10** (57 mg, 0.076 mmol). After the solution was stirred overnight at rt, the volatiles were evaporated under vacuum. The residue was purified by chromatography (elution with EtOAc/hexane, 1:6), providing diol **11** (38.7 mg, 70%) as a white wax: $[\alpha]^{25}_D$ −1.21° (*c* 2.4, $CHCl_3$); R_f 0.38 (EtOAc/hexane, 1:1); ^{1}H NMR δ 1.26−1.50 (m, 21H), 1.79 (m, 2H), 2.04 (q, 2H, J = 7.2 Hz), 3.60 (m, 1H), 3.71 (dd, 1H, J = 3.6, 11.6 Hz), 3.92 (dd, 1H, J = 3.6, 11.6 Hz), 4.04 (t, 2H, J = 6.4 Hz), 4.31 (m, 1H), 5.34 (m, 1H), 5.51 (dd, 1H, J = 6.4, 15.6 Hz), 5.75 (dt, 1H, J = 8.0, 15.6 Hz), 6.93 (d, 2H, J = 7.2 Hz), 7.46 (t, 2H, J = 8.0 Hz), 7.57 (t, 1H, J = 7.2 Hz), 7.74 (d, 2H, J = 6.8 Hz), 7.80 (d, 2H, J = 7.6 Hz); ^{13}C NMR δ 24.0, 28.4, 29.0, 29.2, 29.3, 29.4, 29.5, 32.3, 55.4, 62.6, 68.3, 74.6, 79.7, 114.0, 124.0, 128.1, 129.0, 129.5, 129.7, 129.8, 131.9, 132.6, 133.8, 138.3, 156.3, 162.9, 195.7; MS (ESI) *m*/*z* 540.3 (MH^+).

(2*S*,3*R*)-2-Amino-14-*O*-(4′-benzoylphenyl)-(4*E*)-tetradecene-1,3,14-triol (12). To a solution of 4 M HCl (10 mL) and THF (10 mL) was added **11** (42 mg, 0.080 mmol). The solution was stirred at rt overnight and then neutralized with 4 M NaOH (10 mL). The product was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with brine and dried (Na_2SO_4). The volatiles were evaporated under vacuum, and the residue was purified by chromatography (elution with $CHCl_3$/MeOH/concd NH_4OH, 130:25:4), providing **12** (28 mg, 80%) as a white solid: mp 79.5−80.8 °C; $[\alpha]^{25}_D$ −6.24° (*c* 2.43, $CHCl_3$); R_f 0.30 ($CHCl_3$/MeOH/concd NH_4OH, 130:25:4); ^{1}H NMR δ 1.26−1.50 (m, 12H), 1.79 (m, 2H), 2.04 (q, 2H, J = 7.2 Hz), 3.02 (s, 1H), 3.45 (s, 4H), 3.71 (m, 2H), 4.02 (t, 2H, J = 6.4 Hz), 4.20 (m, 1H), 5.47 (dd, 1H, J = 6.4, 15.6 Hz), 5.78 (dt, 1H, J = 8.0, 15.6 Hz), 6.93 (d, 2H, J = 7.2 Hz), 7.46 (t, 2H, J = 8.0 Hz), 7.57 (t, 1H, J = 7.2 Hz), 7.74 (d, 2H, J = 6.8 Hz), 7.80 (d, 2H, J = 7.6 Hz); ^{13}C NMR δ 26.0, 29.1, 29.2, 29.3, 29.4, 29.5, 32.3, 56.4, 62.4, 68.3, 73.8, 114.0, 124.0, 128.1, 128.3, 129.7, 129.8, 131.9, 132.6, 134.7, 138.3, 162.9, 195.7; HR-MS [DCI, MH^+] *m*/*z* calcd for $C_{27}H_{38}NO_4$ 440.2801, found 440.2795.

(2*S*,3*R*)-1,3-*O*-Dibenzoyl-2-*N*-(*tert*-butoxycarbonylamido)-14-*O*-((4′-trifluoroacetyl)phenyl)-(4*E*)-tetradecene-1,3,14-triol (13). To a solution of DIAD (277 mg, 1.37 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added a solution of Ph_3P (395 mg, 1.51 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred for 10 min, a solution of 4-hydroxytrifluoroacetophenone (**22**) (260 mg, 1.37 mmol) in dry CH_2Cl_2 (5 mL) was added over a 20 min period. The reaction mixture was stirred for another 10 min, and a solution of **9** (521 mg, 0.92 mmol) in dry CH_2Cl_2 (10 mL) was added over a 20 min period. After the mixture was stirred for 10 min at 0 °C, the ice bath was removed, and the mixture was stirred at rt overnight. TLC (EtOAc/hexane, 1:6) indicated that **9** had disappeared. The organic solution was concentrated, and the residue was purified by chromatography (elution with EtOAc/hexane, 1:6), giving **13** (491 mg, 74%) as a colorless wax: $[\alpha]^{25}_D$ −2.82° (*c* 5.0, $CHCl_3$); R_f 0.41 (EtOAc/hexane, 1:3); ^{1}H NMR δ 1.27−1.58 (m, 21H), 1.79 (m, 2H), 2.04 (q, 2H, J = 7.2 Hz), 4.02 (t, 2H, J = 6.4 Hz), 4.42 (m, 1H), 4.50 (m, 2H), 4.87 (d, 1H, J = 9.2 Hz), 5.54 (dd, 1H, J = 7.2, 15.2 Hz), 5.67 (m, 1H), 5.89 (dt, 1H, J = 6.4, 15.6 Hz), 6.97 (d, 2H, J = 9.2 Hz), 7.43 (m, 4H), 7.57 (m, 2H), 8.02 (m, 6H); ^{13}C NMR δ 25.2, 27.5, 28.0, 28.2, 28.3, 28.6, 28.7, 31.6, 31.9, 38.9, 51.7, 62.9, 67.9, 74.5, 79.2, 114.2, 114.8 (q, J_{CF} = 290 Hz), 121.8, 123.4, 127.7, 129.0, 129.2, 132.0, 133.4, 136.5, 154.6, 164.4, 164.7, 165.7, 178.3 (q, J_{CF} = 34 Hz); MS (ESI) *m*/*z* 757.2 (MNH_4^+).

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Supporting Information Available: Preparation of compounds **14**−**22** and ^{1}H and ^{13}C NMR spectra for compounds **4** and **7**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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