Design and Synthesis of 13,14-Dihydro Prostaglandin $F_{1\alpha}$ Analogues as Potent and Selective Ligands for the Human FP Receptor

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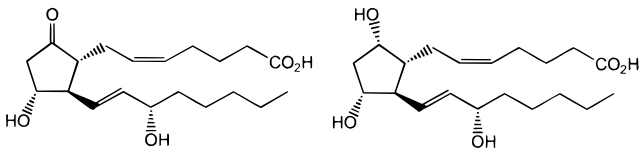
The in vitro evaluation of a new class of potential bone anabolic agents for the treatment of osteoporosis is described. These compounds are potent and selective ligands for the human prostaglandin F receptor (hFP receptor). The compounds lack the olefin unsaturation required for potency in the natural ligand $PGF_{2\alpha}$ yet retain binding affinity for the hFP receptor in the nanomolar to micromolar range. Removal of the alkenes also results in a better selectivity ratio for the hFP receptor over the other prostaglandin receptors tested. A rationale for the selectivity differences of various analogues, based on ligand docking experiments to a putative hFP receptor model, is also described.

Introduction

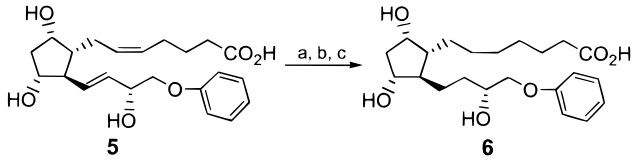
It is known within the prostaglandin family that members of the PGE class and $PGF_{2\alpha}$ produce a bone anabolic response in several animal models.[1] There remains, however, considerable speculation as to the exact pathway(s) and receptors responsible for the anabolic response seen in various models. Naturally occurring prostaglandins are characterized by their activity against a particular prostaglandin receptor (i.e. for $PGF_{2\alpha}$, the primary mediator of its activity is the prostanoid receptor type F, the FP receptor, following the standard nomenclature)[2] but generally are not specific for any one receptor.[3] In addition, species-to-species variations within receptor subtypes can greatly affect the binding affinity of specific ligands.[4]



PGE_2 $PGF_{2\alpha}$

Recently, the cloning and expression of the various prostaglandin receptors have greatly clarified the relationship between prostaglandin structure and function[5] and have raised speculation that highly receptor-selective ligands could be designed which would eliminate the deleterious side effects associated with indiscriminate binding to multiple receptors. As an example, $PGF_{2\alpha}$ and selective analogues thereof have been studied for inducing and synchronizing estrus in the field of animal husbandry[6] and for glaucoma management.[7] However, little data is available on the specific binding affinity for these and similar ligands to individual human receptors, raising questions as to the receptor(s) responsible for the pharmacological effects resulting from these compounds. In addition, it remains unclear as to which characteristics of these ligands are essential for binding and efficacy. In the course of our work in identifying various prostaglandins as potential bone anabolic agents for the treatment of osteoporosis, we now wish to disclose the synthesis of and structure−activity relationship for a novel series of PGF-type ligands which show a high degree of potency and specificity for the hFP receptor and, thus, may possess an enhanced efficacy and a more manageable side effect profile relative to current prostaglandin treatments being evaluated.

Scheme 1



Key: a) CH_2N_2, Et_2O, 80 % b) Pd/C, H_2, EtOAc, 74 %
c) LiOH, THF/H_2O, 85 %

Chemistry

The initial features of the prostaglandin skeleton which were examined for contribution to the binding activity of these molecules were the alkenes at positions C_5−C_6 and C_{13}−C_{14}. A series of known hFP receptor ligands were thus converted to their corresponding saturated counterparts. The synthesis of the saturated PGF analogues was completed in one of two ways. For analogues for which the starting material was commercially available, a three-step sequence using esterification, followed by hydrogenation and deprotection, was performed as shown in Scheme 1. The formation of the methyl ester in step a in Scheme 1 increased the solubility of the prostaglandin, which allowed for the use of ethyl acetate as solvent in the hydrogenation step. The use of ethyl acetate resulted in a much cleaner reduction than hydrogenation of the parent prostaglandin in polar, protic solvents (MeOH, EtOH), resulting in a more efficient process overall. A simple saponification completed the synthesis. Compounds **2**, **4**, **6**, and **14** were synthesized in this manner.

For compounds incorporating a sulfur or nitrogen atom at the 17-position, the synthetic sequence outlined

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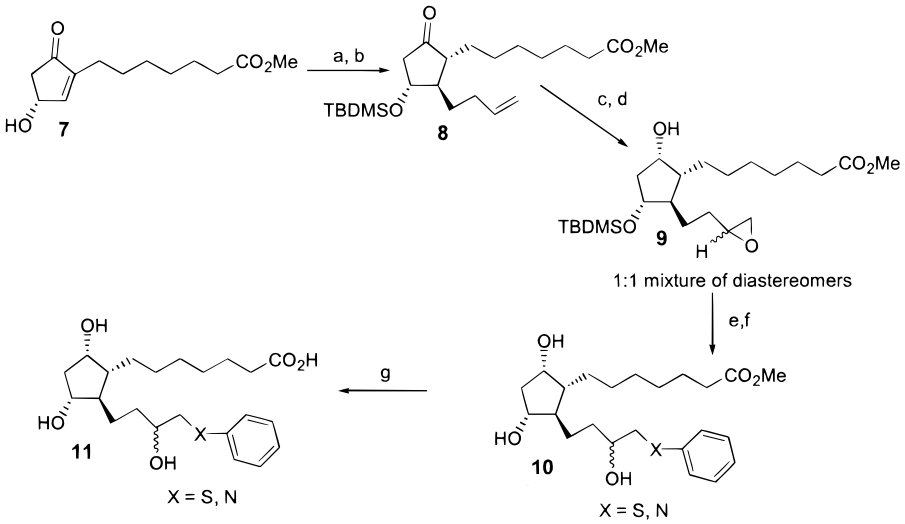
Scheme 2



Key:

a) TBDMSOTf, 2,6 Lutidine, 80 % b) Mg^{o},CuBr·DMS, 4-bromo-1-butene, 70 %

c) $NaBH_4$, MeOH, 60 %, 4:1 (α/β) d) *m*-CPBA, CH_2Cl_2, 75 %, 1:1 at C_{15}

e) Et_3N, thiophenol or $Mg(ClO_4)_2$, aniline f) HF/pyridine, 60 % for two steps

g) LiOH, THF/H_2O, 75 %

in Scheme 2 was utilized. The commercially available methyl 7-[3(*R*)-hydroxy-5-oxo-1-cyclopenten-1-yl]heptanoate (**7**)[8] was silylated using standard conditions[9] and then was treated with the cuprate reagent derived from 4-bromo-1-butene[10] to provide the alkene **8**. The stereoselective reduction of the C_9 carbonyl (prostaglandin numbering) proceeded to provide the C_9 α-alcohol as the major isomer (4:1 α/β), which was easily purified away from the β-isomer using conventional flash silica gel chromatography. The alcohol was subjected to epoxidation using *m*-chloroperbenzoic acid, which provided the epoxide intermediate **9** as an inseparable mixture of diastereomers at C_{15}.

A regiospecific ring-opening of the epoxide either with aromatic thiols using triethylamine or with anilines using magnesium perchlorate provided, after desilylation, the triol intermediate **10**.[11] Final deprotection with aqueous lithium hydroxide provided the carboxylic acid product **11**. The product in all cases proved difficult to separate by conventional silica gel chromatography (either normal or reverse phase)[12] and was subsequently tested as the isomeric mixture. Compounds **12**, **13**, and **15**−**20** were assembled in this fashion.

Biological Results and Discussion

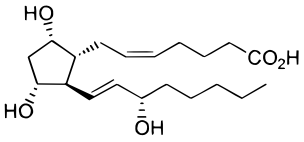
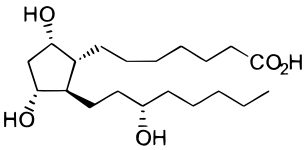
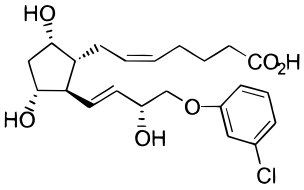
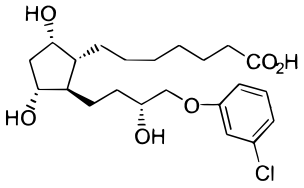
Initially, $PGF_{2\alpha}$ (**1**) and Cloprostenol (**3**) were chosen in order to study the effects of saturation on the SAR of the PGF backbone. The results of introducing saturation are shown in Table 1. While the effect of removing the C_5−C_6 and C_{13}−C_{14} olefins in $PGF_{2\alpha}$ was a 70× *decrease* in binding affinity for the hFP receptor (**1** vs **2**), the saturated version of Cloprostenol (**4**) maintained an equivalent binding affinity of approximately 2 nM for the hFP receptor when compared to the parent compound (**3**). In addition, the binding affinity for the other prostaglandin receptors tested (hEP_1, hEP_2, hEP_3) *decreased* with removal of the olefins (**4** vs **3**), suggesting that selectivity for the hFP receptor could be *increased* by removal of the C_5−C_6 and C_{13}−C_{14} olefins. It should be noted that Cloprostenol (**3**) appeared to be more selective for the hFP receptor than $PGF_{2\alpha}$ (**1**), suggesting that the aromatic ring may also play a role in enhancing receptor selectivity. These ideas were subsequently incorporated into further analogue design.

We subsequently concentrated our effort on generating additional SAR for 13,14-dihydro $PGF_{1\alpha}$ analogues which possessed aromatic substituents in the C_{17}−C_{20} region of the prostaglandin skeleton. We initially synthesized a series of 13,14-dihydro $PGF_{1\alpha}$ analogues with various heteroaromatic linkages at the C_{17} position in order to explore the effect of different heteroatoms at this position on binding affinity and selectivity. The results are shown in Table 2.

The results of this SAR study suggest that the substitution at the C_{17} position plays an important role in the in vitro profile of these compounds. For example, the 17-S linkage proved to be the most potent in the series, based on the compound **12**, with a binding affinity at the hFP receptor of approximately 2.5 nM and a good selectivity profile for hFP relative to the other receptors tested (hEP_1/hFP > 100/1). In addition, although the 17-O compound **6** appears approximately 5× more potent than the 17-N compound **13** (10 nM vs 50 nM at hFP), the latter compound appears to be more selective for the hFP receptor with a hEP_1/hFP ratio of 100, versus approximately 70 for compound **6**. The 17-C linked analogue **14** possessed the poorest affinity for the hFP receptor (570 nM), with only modest receptor selectivity (hEP_2/hFP ∼ 4.3). Overall, the data suggest that the sulfur linkage offers the best combination of receptor potency and selectivity.

We subsequently explored the effect of various meta-substituents on the sulfur ring in a direct comparison to compound **4**. The results are shown in Table 3. The results of this study suggest that both a steric and an electronic requirement appear necessary in order to

Table 1. Comparison of hFP Binding Data for PGF$_{2\alpha}$, Cloprostenol, and Saturated Analogues

Entry	Compound	hFP	hEP1	hEP2	hEP3	hEP4	hTP	hDP	hIP
		IC$_{50}$ (nM)[a]							
1		3-7	217-380	>10^4	110-322	4200	>10^4	4500	>10^4
2		350-522	>10^4	4900	>10^4	>10^4	>10^4	>10^4	>10^4
3		1-2	496	3700	635	>10^4	>10^4	>10^4	>10^4
4		1-2.6	1360	4300 - 6700	799 - 3100	>10^4	>10^4	>10^4	>10^4

[a] See Experimental Section for details of the receptor binding assay.

achieve optimal potency and selectivity. The unsubstituted thiophenol **12** and the thiophene **19** were the most potent compounds tested at 2−6.5 nM. The electron-deficient *m*-fluoro- and *m*-chloro-substituted thiophenols **17** and **18** are approximately 3× less potent than either unsubstituted aromatic ring; however, they are approximately 9× more potent than the tolyl analogue **15**. Also of note is the marked lack of binding affinity for the more polar thiazole **20** in which a nitrogen has been introduced into the thiophene ring. In general, aromatic rings with electron-withdrawing substituents appear to provide enhanced receptor binding affinity relative to electron-donating substituents.

We have attempted to understand the differences in the binding affinity of analogues based on a hFP receptor homology model constructed from the known human FP receptor amino acid sequencing data[13] and site-directed mutagenesis studies.[14] The computer model was created by mapping the transmembrane sections of the human FP receptor from the primary amino acid sequence onto the rhodopsin template of the seven-transmembrane domain receptors available from the Swiss Institute.[15] Individual ligands were then minimized within the putative docking site of the receptor. As shown in Figure 1, the docking of compound **12** into the putative receptor site indicates that the extended top, or α, chain lies in a narrow hydrophobic cleft formed

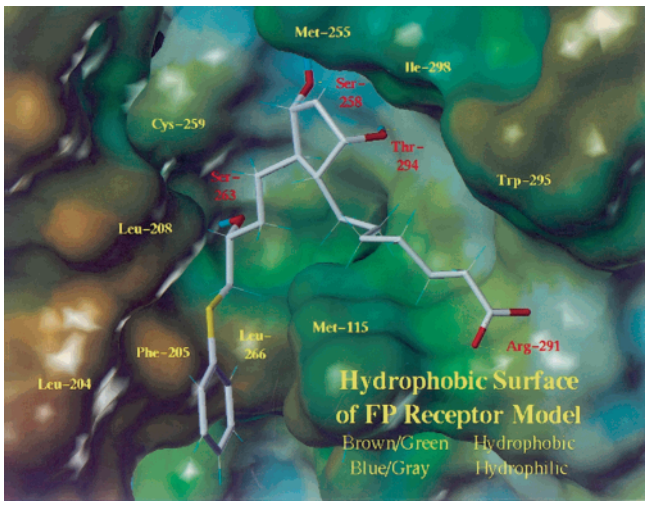
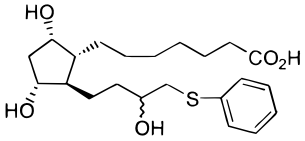
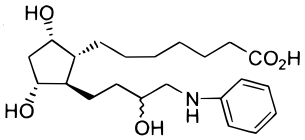
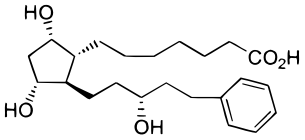
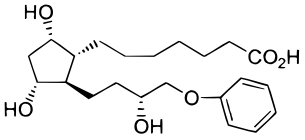


Figure 1. Receptor model with docked ligand **12**.

by Arg-291, Thr-294, Trp-295, and Ile-298 of transmembrane (TM) helix 7 and Met-115 of TM3. At one end of the cleft, a favorable salt bridge forms between the C_1 carboxylate of the ligand and the guanidinium group in Arg-291. It is hypothesized that the methyl group on Thr-294 creates a steric perturbation in the cleft and influences the conformation of the α chain. This preferred conformation orients the cyclopentyl ring of FP-

Table 2. SAR of Unsubstituted 17-Aryl-13,14-dihydro $PGF_{1\alpha}$ Analogues

		IC_{50} (nM)[a]							
Entry	Compound	hFP	hEP1	hEP2	hEP3	hEP4	hTP	hDP	hIP
12[b]		2-3	274-912	>10⁴	>10⁴	>10⁴	>10⁴	>10⁴	>10⁴
13[b]		20 - 90	5000	>10⁴	>10⁴	>10⁴	>10⁴	>10⁴	>10⁴
14		570	5600	2500	>10⁴	>10⁴	>10⁴	>10⁴	>10⁴
6		8 -12	675-713	>10⁴	1600 - 5900	>10⁴	5885 - 6909	3100	>10⁴

[a] See Experimental Section for details of the receptor binding assay. [b] Tested as a 1:1 mixture of diastereomers at C_{15} (prostaglandin numbering).

type ligands so the C_9 sp^3 hydroxyl can favorably hydrogen bond with the hydroxyl on Thr-294 while the C_{11} hydroxyl can form a hydrogen bond with Ser-258. The receptor site model also suggests that the ω chain ($C_{17}-C_{20}$ region) lies in an adjacent hydrophobic pocket formed by residues Leu-204, Phe-205, and Leu-208 of TM5, Cys-259 and Leu-266 of TM6, and Met-115 of TM3. Hydrophobic interactions position the C_{15} hydroxyl within hydrogen-bonding distance of Ser-263 on TM6. The low binding of saturated aliphatic compounds is perhaps rationalized by an increase in the number of degrees of freedom allowed to assume unproductive conformers. Within the aromatic series, it is plausible that the increased binding affinity of compounds such as **4**, **12**, and **19** may be the result of conformational compensation from the aryl "tail" of the ligand via a favorable π-stacking interaction with aromatic amino acid residues (i.e. Phe-205) at the receptor surface.[16] This phenomenon may also enhance receptor selectivity, in addition to selectivity derived from the area occupied by the aromatic substitutions near Val-164 on TM4 and Val-116 on TM3 in the hFP receptor.

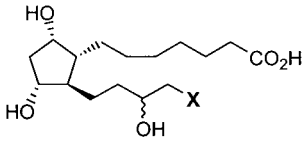
Conclusion

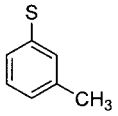
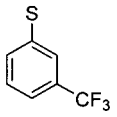
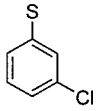
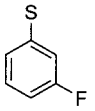
The results from these studies demonstrate that unsaturation of the top and bottom chains in the PGF series is not necessarily critical for maintaining binding affinity for the hFP receptor. The 13,14-dihydro $PGF_{1\alpha}$ derivatives which contain aromatic substitution at the $C_{17}-C_{20}$ region, e.g., 16-phenoxy-16-tetranor analogue **6**, maintain nanomolar binding affinity and selectivity for the hFP receptor. However, saturated derivatives of PGF-type prostaglandins which contain the acyclic $C_{17}-C_{20}$ ω chain, e.g., $PGF_{2\alpha}$, were found to possess weak binding affinity for the receptor. On the basis of these initial observations, we have designed a series of novel saturated PGF analogues which possess good binding affinity and selectivity for the human FP receptor. In addition, preliminary results of these compounds in vivo demonstrate that they are bone anabolic agents in the OVX rat model.[17] It should be noted that the ability to design receptor-selective prostaglandin analogues has potential application in a number of areas where prostanoids are currently being developed for and/or used as therapeutic agents.

Experimental Section

General Methods. ^1H NMR spectra were recorded on a Varian Unity Plus 300 MHz spectrometer and are referenced to either the deuteriochloroform singlet at 7.27 ppm or deuteriomethanol singlet at 4.87 ppm. ^{13}C NMR spectra were obtained on a Varian Unity Plus 300 MHz spectrometer and are referenced at either the center line of the deuteriochloroform triplet at 77.0 ppm or the deuteriomethanol heptet at 49.15 ppm. Infrared absorption spectra were obtained on a Perkin-Elmer model 197 spectrophotometer and are referenced to polystyrene (1601 cm^{-1}). Mass spectra were obtained on either a Fison Platform-II Quadrupole mass spectrometer or a Fison Trio2000 Quadrupole mass spectrometer. High-resolution mass spectra were obtained from the Procter & Gamble

Table 3. SAR of Various 3-Substituted-17-phenylthio-13,14-dihydro PGF$_{1\alpha}$ Analogues



		IC$_{50}$ (nM)[a]							
Entry	X	hFP	hEP1	hEP2	hEP3	hEP4	hTP	hDP	hIP
15[b]		63	4600	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
16[b]		208 - 320	2700	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
17[b]		17	>10^4	2400	>10^4	>10^4	>10^4	>10^4	>10^4
18[b]		7-29	10 - 364	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
19[b]		2-11	1100	1300	1000	>10^4	>10^4	>10^4	>10^4
20[b]		1200 - 1424	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4

[a] See Experimental Section for details of the receptor binding assay. [b] Tested as a 1:1 mixture of diastereomers at C_{15} (prostaglandin numbering).

CRD Mass Spectrometry Lab (M. Lacey). Elemental analyses were obtained from the Procter & Gamble EA Lab in Norwich, NY. Melting points were determined in open Pyrex capillary tubes on a Thomas-Hover Unimelt apparatus. Melting points and boiling points are uncorrected. All solvents were purchased anhydrous (Aldrich Chemical) and used without further purification. PGF$_{2\alpha}$, 13,14-dihydro PGF$_{1\alpha}$, Cloprostenol, 16-phenoxy PGF$_{2\alpha}$, and 17-phenyl PGF$_{2\alpha}$ were purchased from Cayman Chemical and used without further purification. Methyl 7-[3(*R*)-hydroxy-5-oxo-1-cyclopenten-1-yl]heptanoate was purchased from Sumitomo Chemical and used without purification. All air-sensitive reactions were performed under an anhydrous nitrogen atmosphere. Flash chromatography was performed on silica gel (70−230 mesh, Aldrich; or 230−400 mesh, Merck) as appropriate. Thin-layer chromatography analysis was performed on glass mounted silica gel plates (200−300 mesh; Baker) and visualized using UV, 5% phosphomolybdic acid in EtOH, or ammonium molybdate/cerric sulfate in 10% aqueous H_2SO_4.

Radioligand Binding Assay. COS-7 cells were transiently transfected with a hFP recombinant plasmid using LipofectAMINE reagent. 48 h later, the transfected cells were washed with Hank's balanced salt solution (HBSS, without $CaCl_2$, $MgCl_2$, $MgSO_4$, or phenol red). The cells were detached with versene, and HBSS was added. The mixture was centrifuged at 200*g* for 10 min, at 4 °C to pellet the cells. The pellet was resuspended in phosphate-buffered saline−EDTA buffer (PBS, 1 mM EDTA, pH 7.4, 4 °C). The cells were disrupted by nitrogen cavitation (Parr model 4639), at 800 psi, for 15 min at 4 °C. The mixture was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was centrifuged at 100000*g* for 60 min at 4 °C. The pellet was resuspended to 1 mg protein/mL TME buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 6.0, 4 °C) based on protein levels measured using the Pierce BCA protein assay kit. The homogenate was mixed using a Kinematica polytron for 10 s. The membrane preparations were then stored at −80 °C, until thawed for assay use.

The receptor competition binding assays were developed in a 96-well format. Each well contained 100 μg of hFP membrane, 5 nM [^{3}H]PGF$_{2\alpha}$, and the various competing compounds in a total volume of 200 μL. The plates were incubated at 23 °C for 1 h. The incubation was terminated by rapid filtration using the Packard Filtermate 196 harvester through Packard UniFilter GF/B filters that were prewetted with TME buffer. The filter was washed four times with TME buffer. Packard Microscint 20, a high-efficiency liquid scintillation cocktail, was

added to the filter plate wells and the plates remained at room temperature for 3 h prior to counting. The plates were read on the Packard TopCount microplate scintillation counter.

The hEP_1 receptor was transiently expressed in COS-7 cells and followed the above-mentioned transient transfection protocol. Five of the other receptors, hEP_2, hEP_3, hEP_4, hTP, and hIP, were stably expressed in the CHO cell line. The hDP receptor was stably expressed in the HEK-293 cell line and required a TME buffer pH of 7.4. The CHO cells, and HEK-293 cells expressing the receptors were collected 4−5 days after seeding, washed with PBS, and detached with versene. Cells were centrifuged at 100*g* for 10 min at 4 °C. Cells were resuspended in lysis buffer (10 mM Tris-HCl, 5 mM EDTA, 10 μg/mL leupeptin and benzamidine, 2 μg/mL aprotinin) and the homogenate was centrifuged at 200*g* for 10 min at 4 °C. The supernatant was centrifuged at 100000*g* for 60 min at 4 °C. The pellet was resuspended to 1 mg protein/mL TME buffer based on measured protein levels. The homogenate was mixed using a Kinematica polytron for 10 s. The membrane preparations were then stored at −80 °C, until thawed for assay use. The radiolabeled ligands used for the competition binding assays of the other seven receptors were as follows: the EP_1, EP_2, EP_3, and EP_4 assays used [^{3}H]PGE_2, the DP assay used [^{3}H]PGD_2, the IP assay used [^{3}H]Iloprost, and the TP assay used [^{3}H]SQ-29548. All of the radiolabeled ligands were run at 5 nM in the experiment and were purchased from DuPont NEN and Amersham Life Science.

Methyl 7-(5-But-3-enyl-2-hydroxy-4-(1,1,2,2-tetramethyl-1-silapropoxy)cyclopentyl)heptanoate (8). To a solution of methyl 7-[3(*R*)-hydroxy-5-oxo-1-cyclopenten-1-yl]heptanoate **(7)** (10.0 g, 41.66 mmol, 1 equiv) in CH_2Cl_2 (200 mL) at −78 °C was added 2,6-lutidine (6.31 mL, 54.16 mmol, 1.3 equiv) dropwise over 15 min. The solution was kept at −78 °C, and TBDMS triflate (11.46 mL, 49.92 mmol, 1.2 equiv) in CH_2Cl_2 (50 mL) was added dropwise over 15 min. The reaction was warmed gradually to rt and stirred at rt for 15 h. Aqueous 10% HCl was added and the layers were separated. The water layer was extracted with 200 mL CH_2Cl_2 and the organic layers were combined. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was distilled under vacuum (house vacuum, 10 mmHg) to provide 13.3 g (89%) of the silyl ether as a yellow liquid: bp 175−180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (br s, 1H), 4.85 (m, 1H), 3.65 (s, 3H), 2.65 (dd, J = 6.0, 18.0 Hz, 1H), 2.10−1.95 (m, 5H), 1.65−1.10 (m, 8H), 0.83 (s, 9H), 0.04 (d, J = 3.3 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.1, 174.4, 158.6, 147.3, 69.2, 51.6, 45.7, 45.1, 34.2, 29.2, 29.0, 27.4, 26.0, 25.6, 25.0, 24.6, 18.3, −3.4, −4.5; MS *m*/*z* 354 $(M + H)^+$ 355.2, $(M + NH_4)^+$ 372.2; HRMS calcd for $(C_{19}H_{34}O_4Si + H)^+$ 355.2305, found 355.2304.

To a slurry of Mg^0 powder (1.34 g, 55.86 mmol, 2 equiv) in THF (75 mL) at rt were added one crystal I_2 and 4-bromo-1-butene (5.67 mL, 55.86 mmol, 2 equiv) dropwise over 10 min. The reaction proceeded to exotherm as the addition continued. After the addition was complete, the reaction was refluxed for 3 h and cooled to rt. The Grignard was diluted with 50 mL THF and added via cannula to a three-necked flask, equipped with mechanical stirring, and charged with CuBr·DMS (11.48 g, 55.86 mmol, 2 equiv) in 100 mL of a 1:1 solution of THF/DMS at −78 °C. After the addition of the Grignard (∼20 min), the reaction was stirred 1 h at −78 °C. The color of the reaction was dark red at this point. A solution of the silyl ether (10.0 g, 27.93 mmol, 1 equiv) in THF (25 mL) was then added dropwise over 25 min. The reaction was stirred at −78 °C for 15 min, then allowed to warm slowly to rt over 2 h. The reaction was quenched with aq NH_4Cl and the excess DMS allowed to evaporate overnight. The reaction was partitioned between brine/CH_2Cl_2 and the layers separated. The aqueous layer was back-extracted with 3 × 200 mL CH_2Cl_2 and the organic layers were combined and dried (Na_2SO_4). The solvent was removed in vacuo and the residue chromatographed on silica gel (10% hexane/EtOAc) to give 8.13 g (71%) of the ketone **8** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (dq, J = 6.0, 16.6 Hz, 1H), 4.94 (overlapping dd, J = 1.8, 11.4 Hz and 3.3, 11.4 Hz, 2H), 4.01 (dd, J = 5.7, 6.0 Hz, 1H), 3.61 (s, 3H), 2.52 (dd, J = 5.0, 11.1 Hz, 1H), 2.24 (t, J = 7.5 Hz, 2H), 2.15−1.10 (m, 17H), 0.83 (s, 9H), 0.03 (d, J = 8.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.1, 174.5, 138.5, 115.1, 73.7, 53.5, 51.7, 48.9, 47.8, 34.3, 31.9, 29.6, 27.1, 25.9, 25.1, 18.1, −4.3, −4.6; MS *m*/*z* 410 $(M + NH_4)^+$ 428.5. Anal. ($C_{23}H_{42}O_4Si$) C, H.

Methyl 7-(2-Hydroxy-5-(2-(2-oxiranyl)ethyl)-4-(1,1,2,2-tetramethyl-1-silapropoxy)cyclopentyl)heptanoate (9). The ketone **8** (11.0 g, 26.8 mmol, 1 equiv) was dissolved in MeOH (30 mL) and cooled to −40 °C. Sodium borohydride (912 mg, 24.12 mmol, 0.9 equiv) was added portionwise over 10 min. After the addition was completed, the reaction was stirred for 13 h at −40 °C and then 12 h at −78 °C. The reaction was quenched with water, partitioned between brine and CH_2Cl_2 and the layers separated. The aqueous layer was back-extracted with CH_2Cl_2 (3 × 200 mL) and the organic layers combined and dried (Na_2SO_4). The solvent was removed in vacuo and the residue chromatographed on silica gel (30% EtOAc/hexanes) to give 8.2 g (61%) of the alcohol as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80 (dq, J = 6.0, 16.6 Hz, 1H), 4.94 (overlapping dd, J = 1.8, 11.4 Hz and 3.3, 11.4 Hz, 2H), 4.09 (m 1H), 4.00 (d, J = 4.5 Hz, 1H), 3.60 (s, 3H), 2.95 (br s, OH, 1H), 2.30 (t, J = 7.5 Hz, 1H), 2.10−1.05 (m, 18 H), 0.85 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 138.7, 114.8, 80.1, 75.4, 53.4, 52.2, 51.6, 42.4, 34.4, 34.2, 32.5, 30.4, 29.9, 29.4, 28.6, 25.9, 25.2, 17.9, −4.5; MS *m*/*z* 412 $(M + H)^+$ 413.4. Anal. ($C_{23}H_{44}O_4Si$) C, H.

The alcohol (4.05 g, 9.83 mmol, 1 equiv) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. Sodium bicarbonate (100 mg) was added, followed by *m*-CPBA (57−85% purity) (5.10 g, 29.5 mmol, 3 equiv) portionwise over 15 min. After the addition was completed, the reaction was stirred for 20 h at rt. The reaction was poured onto water, partitioned between brine and CH_2Cl_2 and the layers separated. The aqueous layer was back-extracted with CH_2Cl_2 (3 × 200 mL) and the organic layers combined, washed with satd $Na_2S_2O_3$ (4 × 250 mL), satd $NaHCO_3$ (3 × 250 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the resulting oil can be used crude or can be purified by chromatography on silica gel (20% EtOAc/hexanes) to provide 3.0 g (73%) of the epoxide diastereomers **9** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.09 (t, J = 4.8 Hz, 1H), 3.96 (d, J = 4.2 Hz, 1H), 3.65 (s, 2H), 2.90 (m, 1H), 2.75 (m, 1H), 2.48 (m, 1H), 2.30 (t, J = 7.5 Hz, 2H), 1.90−1.29 (m, 20 H), 0.80 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 80.0, 79.9, 75.2, 52.1, 52.0, 51.6, 47.3, 47.2, 42.4, 34.3, 31.3, 31.2, 30.8, 30.4, 30.3, 29.8, 28.5, 25.9, 17.9, −4.5; MS *m*/*z* 428 $(M + H)^+$ 429.4. Anal. ($C_{23}H_{44}O_5Si$) C, H.

General Procedure for Synthesis of 13,14-Dihydro-16-phenylthio-16-tetranor $PGF_{1\alpha}$ Methyl Esters. In a 5 mL round-bottom flask, 0.2 g of epoxide **9** (0.47 mmol) and dry benzene (100 uL) were added. The flask was cooled at 0 °C, then treated with the appropriate thiol (0.56 mmol, 1.2 equiv) and triethylamine (0.56 mmol, 1.2 equiv). The ice bath was removed and the reaction was stirred at room temperature under nitrogen for 20 h. An excess amount of thiophenol was added as necessary. The reaction was quenched with brine, and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed three times with 1 N HCl, brine, dried (Na_2SO_4), and concentrated. Without further purification to this crude reaction mixture, 3 mL of CH_3CN and 0.5 mL of HF/pyridine were added while the flask was kept at 0 °C. After 3 h at 0 °C, the reaction was quenched with satd NaCl. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined and washed three time with 1 N HCl, brine, and dried (Na_2SO_4) to provide the crude product. Silica gel chromatography purification (7:3 hexanes/ethyl acetate) provided pure thiol methyl ester.

General Procedure for Synthesis of 13,14-Dihydro-16-phenylamino-16-tetranor $PGF_{1\alpha}$ Methyl Esters To a 10 mL round-bottom flask were added THF (2 mL), 0.36 g of epoxide **9** (1.26 mmol), aniline (1.26 mmol, 1.5 equiv), and 10 mg of magnesium perchlorate. The reaction was refluxed under nitrogen for 3 h, cooled to room temperature, and the solvent removed in vacuo. Without further purification to the crude reaction mixture, 3 mL of CH_3CN and 0.5 mL of HF/pyridine

were added while the flask was kept at 0 °C. After 5 h at 0 °C, the reaction was quenched with satd NaCl. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined and washed three time with satd $NaHCO_3$, brine, dried (Na_2SO_4), and the resulting oil purified by silica gel chromatography (95% CH_2Cl_2, 5% MeOH) to provide 0.182 g (35%) of the methyl ester of **13** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32−1.75 (m, 20H), 1.87 (br s, 2H), 2.27−2.33 (t, J = 7.5 Hz, 2H), 2.27 (s, 3H), 2.97−3.04 (m, 1H), 3.19−3.24 (dd, J = 2.7, 13.6 Hz, 1H), 3.66 (s, 3H), 3.84 (s, 1H), 3.91 (s, 1H), 4.167 (s, 1H), 6.45−6.47 (m, 2H), 6.54−6.56 (dd, J = 7.5 Hz, 1H), 7−7.08 (dd, J = 7.8, 8.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.8, 25.0, 28.3, 28.8, 28.9, 29.2, 29.7, 30.0, 33.2, 33.764, 34.2, 42.4, 42.5, 50.5, 51.7, 51.8, 52.2, 52.9, 70.5, 70.6, 74.3, 74.4, 78.2, 78.7, 110.7, 114.3, 118.9, 129.3, 139.1, 148.5, 174.7; MS m/z 408 (M + H)$^+$ 408.3.

General Procedure for Saponification of Methyl Ester to Free Acid. To a 5 mL round-bottom flask was added the appropriate PGF$_{1\alpha}$ methyl ester (0.012 mmol.). A mixture of THF/water solution (4 mL; 3:1 THF:H_2O) was added, and the flask was cooled to 0 °C. An excess amount of lithium hydroxide (2.5 equiv) was added, the ice bath was removed, and the reaction stirred at room temperature overnight. The reaction was diluted with CH_2Cl_2 and satd citric acid and the layers separated. The aqueous layer was washed with CH_2Cl_2 (3 × 10 mL) and the organic layers were combined and washed with brine, dried (Na_2SO_4) and concentrated. Column chromatography on silica gel (CH_2Cl_2/CH_3OH/AcOH, 9.6/0.3/0.1) provided the desired free acid.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-(phenylamino)butyl)-cyclopentyl)heptanoic acid (13): 57% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 7.12 (dd, J = 7.2, 8.4 Hz, 2H), 6.67 (m, 3H), 4.12 (br s, 1H), 3.89 (q, J = 3.6, 7.2 Hz, 1H), 3.64 (br s, 1H), 3.20 (dd, J = 4.5, 12.9 Hz, 1H), 3.05 (dd, J = 7.5, 12.9 Hz, 1H), 2.31 (t, J = 7.2 Hz, 2H), 2.10 (m, 1H), 1.77−1.36 (m, 22H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 179.0, 150.1, 130.1, 118.3, 114.4, 78.5, 73.8, 71.5, 71.3, 52.7, 52.6, 43.8, 43.7, 35.6, 34.1, 34.0, 30.9, 30.4, 30.1, 29.6, 29.5, 29.1, 26.3; MS m/z 393 (M + H)$^+$ 394.3; HRMS calcd for ($C_{22}H_{35}O_5N$ + H)$^+$ 394.2593, found 394.2585. Anal. ($C_{22}H_{35}O_5N$·1H_2O) C, N; H: calcd, 9.06; found, 8.32.

7-(5-(4-(3-Chlorophenoxy)-3-hydroxybutyl)-2,4-dihydroxycyclopentyl)heptanoic acid (4):[18] 47% yield; IR (thin film) 3378, 2928, 2859, 1707, 1594, 1479, 1248 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.28 (m, 1H), 6.95 (m, 3H), 4.12 (br s, 1H), 3.95 (m, 5H), 3.35 (m, 2H), 2.05 (m, 1H), 1.10−1.80 (m, 20H); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 24.9, 27.9, 28.2, 28.6, 29.1, 29.7, 31.2, 33.9, 42.8, 50.1, 51.1, 69.8, 72.4, 77.3, 113.0, 114.9, 120.7, 130.4, 134.7, 160.2, 176.6; MS (+ES) m/z (relative intensity) 446.1 (M + NH_4^+, 50), 429.1 (M + H^+, 100), 393.1 (M − 2H_2O, 25); HRMS (FAB$^+$) calcd for ($C_{22}H_{33}O_6Cl$ + Na)$^+$ 451.1863, found 451.1880.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-phenoxybutyl)cyclopentyl)heptanoic acid (6):[18] 67% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 7.30 (t, J = 7.5 Hz, 2H), 6.95 (br d, J = 8.1 Hz, 3H), 4.15 (br s, 1H), 3.95 (m, 5H), 3.35 (m, 2H), 2.10 (t, J = 7.0 Hz, 2H), 1.15−1.90 (m, 19 H); MS ESI m/e 395 (M^+).

7-(2,4-Dihydroxy-5-(3-hydroxy-5-phenylpentyl)cyclopentyl)heptanoic acid (14):[18] 62% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 7.30 (t, J = 7.5 Hz, 2H), 6.95 (br d, J = 8.1 Hz, 3H), 4.15 (br s, 1H), 3.95 (m, 5H), 3.35 (m, 2H), 2.10 (t, J = 7.0 Hz, 2H), 1.15−1.90 (m, 19 H); MS ESI m/e 395 (M^+).

7-(2,4-Dihydroxy-5-(3-hydroxy-4-phenylthiobutyl)cyclopentyl)heptanoic acid (12): 63% yield; IR (thin film) 3388, 2927, 2954, 1708, 1583, 1438, 1113, 1024, 739, 691 cm^{-1}; ^{1}H NMR (300 Hz, $CDCl_3$) δ 1.28−1.75 (m, 19H), 1.86 (br s, 2H), 2.30−2.35 (t, J = 7.2 Hz, 2H), 2.87−2.95 (dd, J = 8.4, 13.5 Hz, 1H), 3.10−3.16 (dd, J = 3.3, 13.5 Hz, 1H), 3.69−3.71 (m, 1H), 3.95 (s, 1H), 4.17 (s, 1H), 5.20 (br s, 1H), 7.18−7.23 (m, 1H), 7.27−7.32 (overlapping dd, J = 7.2, 7.8 Hz, 2H), 7.37−7.40 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.5, 25.7, 26.5, 26.7, 27.2, 27.4, 28.0, 32.2, 39.8, 40.0, 40.3, 40.4, 48.4, 49.5, 50.4, 50.7, 67.6, 67.9, 72.3, 76.2, 76.4, 124.5, 127.1, 127.8, 133.3, 176.4; MS m/z 411 (M + H)$^+$ 411.4; HRMS calcd for ($C_{22}H_{34}O_5S$ + H)$^+$ 411.2205, found 411.2197.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-(3-methylphenylthio)-butyl)cyclopentyl)heptanoic acid (15): 49% yield; IR (neat) 3391, 2927, 2855, 1709, 1592, 1415, 1217, 1079, 774, 689 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25−1.74 (m, 19H), 1.86 (br s, 2H), 2.29−2.37 (m, 5H), 2.86−2.93 (dd, J = 8.4, 13.5 Hz, 1H), 3.08−3.14 (dd, J = 3.7, 13.5 Hz, 1H), 3.68−3.71 (m, 1H), 3.95 (s, 1H), 4.10−4.17 (s, 1H), 5.30−5.31 (br s, 1H), 7.00−7.03 (dd, J = 7.5, 3.3 Hz, 1H), 7.17 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 24.7, 28.0, 28.8, 28.9, 29.5, 29.6, 34.4, 41.9, 42.1, 42.5, 42.6, 51.6, 51.7, 52.6, 52.8, 69.8, 70.2, 74.4, 78.4, 78.8, 127.1, 127.6, 129.1, 130.7, 135.3, 139.1, 178.6; MS m/z 425 (M + H)$^+$ 425.4; HRMS calcd for ($C_{23}H_{36}O_5S$ + H)$^+$ 425.2331, found 425.2336. Anal. ($C_{23}H_{36}O_5S$·1H_2O) C, H.

7-(5-(4-(3-Fluorophenylthio)-3-hydroxybutyl)-2,4-dihydroxycyclopentyl)heptanoic acid (18): 89% yield; IR (neat) 3369, 2928, 2855, 1708, 1599, 1578, 1474, 1427, 1264, 1215, 1066, 881, 775, 730, 678 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.79 (m, 19H), 1.88 (br s, 2H), 2.31−2.36 (t, J = 7.2 Hz, 2H), 2.93−3.00 (dd, J = 7.8, 13.5 Hz, 1H), 3.11−3.15 (m, 1H), 3.76−3.77 (m, 1H), 3.97 (s, 1H), 4.19 (s, 1H), 5.21 (br s, 1H), 6.85−6.92 (dd, J = 2.4, 8.4 Hz, 1H), 7.05−7.14 (m, 2H), 7.18−7.30 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.6, 27.9, 28.8, 29.3, 29.7, 30.5, 34.0, 34.6, 41.7, 41.9, 42.6, 42.8, 51.7, 51.9, 53.0, 53.2, 69.7, 70.2, 74.7, 78.7, 78.8, 113.4, 113.7, 116.1, 116.4, 125.1, 128.4, 130.5, 130.6, 125.1, 128.4, 130.5, 130.6, 138.2, 138.5, 161.9, 165.4, 178.3; ^{19}F NMR ($CDCl_3$) δ 54.87, 54.85, 54.87, 54.90, 54.93; MS m/z 429 (M + H)$^+$ 429.3; HRMS calcd for ($C_{22}H_{33}O_5SF$ + H)$^+$ 429.211, found 429.2096. Anal. ($C_{22}H_{33}O_5$-SF·1.5H_2O) H; C: calcd, 58.00; found, 58.57.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-(3-trifluoromethylphenylthio)butyl)cyclopentyl)heptanoic acid (16): 84% yield; IR (neat) 3369, 2929, 1708, 1421, 1323, 1166, 1125, 1073, 696 cm^{-1}; ^{1}H NMR (300, MHz, $CDCl_3$) δ 1.20−1.95 (m, 21H), 2.29−2.34 (t, J = 7.2 Hz, 2H), 2.97−3.05 (dd, J = 7.8, 13.5 Hz, 1H), 3.13−3.19 (dt, J = 3.3, 13.5 Hz, 1H), 3.76−3.79 (m, 1H), 3.97 (s, 1H), 4.18 (s, 1H), 5.820 (br s, 1H), 7.29−7.43 (m, 2H), 7.51−7.53 (d, J = 6.9 Hz, 1H), 7.583 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7, 27.8, 28.8, 29.4, 30.0, 34.5, 34.7,-41.2, 41.4, 42.4, 51.6, 52.3, 52.6,70.0, 70.4, 74.3, 78.3, 78.6, 122.1, 122.9, 125.5, 125.8, 129.5, 130.8, 131.2, 131.7, 132.2 (d, J = 128.7 Hz), 138.0, 179.0; ^{19}F NMR ($CDCl_3$) 103.9; MS m/z 448 (M + H)$^+$ 479.3; HRMS calcd for ($C_{23}H_{33}O_5SF_3$ + H)$^+$ 479.2079, found 479.2076. Anal. ($C_{23}H_{33}O_5SF_3$·0.5H_2O) C, H.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-(2,5-thiazolylthio)-butyl)cyclopentyl)heptanoic acid (20): 62% yield; IR (neat) 3368, 2925, 2853, 1710, 1384, 1302, 1026, 912, 730 cm^{-1}; $_1$H NMR (300 MHz, $CDCl_3$) δ 1.35−1.74 (m, 19H), 1.90 (s, 2H), 2.32−2.37 (t, J = 7.2 Hz, 2H), 3.19−3.27 (ddd, J = 1.8, 7.2, 14.4 Hz, 1H), 3.35−3.42 (br d, J = 2.7, 14.4 Hz, 1H), 4.01 (br s, 2H), 4.20 (s, 1H), 5.21 (br s, 1H), 7.24−7.25 (d, J = 3.6 Hz, 1H), 7.64−7.65 (d, J = 3.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7, 27.9, 28.7, 29.3, 29.8, 30.3, 41.9, 42.0, 42.6, 42.6, 51.7, 51.7, 52.8, 53.0, 71.4, 71.7, 74.6, 78.6, 78.7, 119.8, 142.3, 165.8, 178.3; MS m/z 417 (M + H)$^+$ 418.1; HRMS calcd for ($C_{19}H_{31}O_5S_2N$ + H)$^+$ 418.1722, found 418.1726.

7-(5-(4-(3-Chlorophenylthio)-3-hydroxybutyl)-2,4-dihydroxycyclopentyl)heptanoic acid (17): 89% yield; IR (neat) 3369, 2926, 1707, 1577, 1461, 1072, 777, 678 cm^{-1}; ^{1}H NMR (300 Hz, $CDCl_3$) δ 1.20−1.73 (m, 19H), 1.88 (br s, 2H), 2.31−2.36 (t, J = 7.2 Hz, 2H), 2.91−2.99 (dd, J = 13.5, 8.1 Hz,1H), 3.10−3.17 (ddd, J = 13.5, 3.6, 2.7 Hz, 1H), 3.73−3.76 (m, 1H), 3.97 (s, 1H), 4.19 (s, 1H), 4.68 (br s, 1H), 7.15−7.20 (m, 1H), 7.22−7.27 (m, 2H), 7.35 (br s, 1H); ^{13}C NMR (75.5 Hz, $CDCl_3$) δ 24.7, 27.9, 28.8, 29.4, 29.6, 30.2, 34.2, 34.5, 41.6, 41.8, 42.6, 51.8, 52.6, 52.9, 69.9, 70.3, 74.5, 78.5, 78.7, 126.7, 127.6, 129.1, 130.3, 135.0, 138.1, 178.6; MS m/z 444 (M + Na)$^+$ 467.0; HRMS calcd for ($C_{22}H_{33}O_5SCl$ + Na)$^+$ 467.1635, found 467.1654. Anal. ($C_{22}H_{33}O_5SCl$·0.75H_2O) C, H.

7-(2,4-Dihydroxy-5-(3-hydroxy-4-(2-thienylthio)butyl)-cyclopentyl)heptanoic acid (19): 75% yield; IR (neat) 3369, 2926, 2851, 1708, 1407, 1213, 1109, 1072, 1022, 985, 840 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.87 (m, 19H), 1.87 (br s,

2H), 2.31−2.36 (t, J = 7.2 Hz, 2H), 2.75−2.82 (dd, J = 13.5, 8.4 Hz, 1H), 2.94−3.00 (ddd, J = 13.5, 3.6, 1.8 Hz, 1H), 3.71−3.74 (m, 1H), 3.96 (s, 1H), 4.18 (s, 1H), 4.92 (br s, 1H), 6.97−7.00 (dd, J = 5.4, 3.6 Hz, 1H), 7.15−7.17 (dd, J = 3.6, 1.2 Hz, 1H), 7.35−7.37 (dd, J = 5.4, 1.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7, 28.0, 28.8, 28.9, 29.5, 29.7, 30.2, 34.2, 42.0, 42.7, 46.6, 46.8, 51.6, 51.7, 52.6, 53.0, 69.8, 70.1, 74.5, 78.5, 78.7, 127.9, 129.8, 133.6, 134.2, 178.5; MS m/z 416 $(M + H)^+$ 417.2. Anal. ($C_{20}H_{32}O_5S_2 \cdot 0.5CH_3OH$) C, H.

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