Protein Phosphatase Inhibitors Isolated from *Spongia irregularis* Collected in Papua New Guinea

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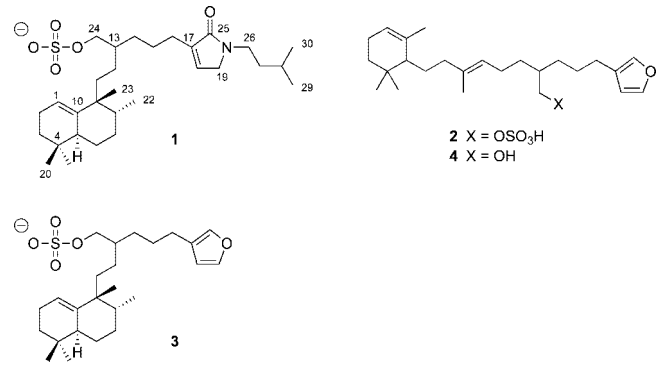
Irregularasulfate (**1**), a new nitrogen-containing sesterterpenoid, and the known sesterterpenoids hipposulfate C (**2**), halisulfate-7 (**3**), and igernellin (**4**), have been isolated from the marine sponge *Spongia irregularis* collected in Papua New Guinea. The structure of **1** was elucidated via analysis of its spectroscopic data. Sesterterpenoids **1**, **2**, and **3** are moderate inhibitors of the catalytic subunits of the mammalian Ser/Thr protein phosphatases calcineurin, PP-1, and PP-2A. The phosphate analogue of **3** and the thiophosphate analogue of **2** have been prepared from the corresponding natural products and evaluated for their ability to inhibit the phosphatase activity of calcineurin.

Calcineurin, a serine/threonine protein phosphatase, is the indirect cellular target of the two important immunosuppressive natural product drugs cyclosporine and FK506 (tacrolimus).[1] When receptors on the surface of T cells encounter antigens from foreign tissue associated with organ transplants, a calcium-dependent signal transduction cascade is activated. As part of this process, calcium levels in the cell increase due to stimulated release of calcium from intracellular stores in the endoplasmic reticulum and an influx of extracellular calcium through channels in the plasma membrane. The increased intracellular calcium binds to calmodulin, and the calcium/calmodulin complex in turn binds to calcineurin and activates its phosphatase activity against the principal substrate, multiply phosphorylated "nuclear factor of activated T cell" (NFAT) protein in the cytoplasm.[2] Once dephosphorylated, NFAT is translocated to the nucleus, where it enhances binding of transcription factors to genes encoding for cytokines such as interleukin 2 (IL-2). The upregulation of production of IL-2 and other cytokines is an important component of T cell activation and the immune response. Cyclosporin and FK506 inhibit calcineurin activity and, therefore, the immune response to foreign tissue in an indirect fashion. Cyclosporin binds to its direct molecular target cyclophilin, and the cyclosporine/cyclophilin complex then binds to calcineurin and inhibits its phosphatase activity. In a similar manner, FK506 binds to the 12 KDa FK506 binding protein (FKBP-12), and again this complex binds to calcineurin and inhibits its enzymatic activity. Even though cyclosporine and FK506 are important drugs that have revolutionized organ transplantation, they are not without side effects.[3,4] These include nephrotoxicity (kidney), hypertension, neurotoxicity, and diabetogenesis. Despite the obvious clinical importance of calcineurin inhibitors, there are few known potent and selective active-site inhibitors of this phosphatase.[5,6] This can be contrasted with the existence of okadaic acid,[7] motuporin,[8] spirastrellolide A,[9] and several other highly potent natural product inhibitors of the serine/threonine protein phosphatases PP-1 and PP-2A.

Recently, an assay suitable for detecting direct inhibitors of calcineurin has been developed in one of our laboratories.[10] This assay, based on the singly phosphorylated physiological calcineurin substrate inhibitor-1, was used to screen a library of marine invertebrate and microbial extracts, resulting in the discovery of several hits, including the MeOH extract from the marine sponge *Spongia irregularis.* Bioassay-guided fractionation of the *S. irregularis* extract led to the isolation of the new sesterterpenoid alkaloid irregularasulfate (**1**) and the known metabolites hipposulfate C (**2**)[11] and halisulfate-7 (**3**),[12] which all showed moderate calcineurin inhibition, along with igernellin (**4**),[13] which was inactive. The details of the isolation and structure elucidation of irregularasulfate (**1**), some SAR-driven chemical modifications of **3** and **4**, and the phosphatase inhibition activities of **1**, **2**, **3**, and their transformation products are presented below.



Specimens of *S. irregularis* (670 g wet wt) were collected by hand using scuba near Keviang, Papua New Guinea, frozen on site, and transported frozen to Vancouver. Thawed sponge tissue was exhaustively extracted with MeOH, which was filtered and concentrated *in vacuo* to give the crude extract. This extract was partitioned between H_2O and CH_2Cl_2, and the bioactive CH_2Cl_2-soluble portion was first fractionated on Si gel flash chromatography (step gradient elution: hexanes to EtOAc to MeOH). The active fraction, eluting with 10% MeOH in EtOAc, was subjected to reversed-phase flash chromatography (step gradient elution: H_2O to MeOH) to give a calcineurin inhibitory fraction (7:3 MeOH/H_2O) that was subjected to reversed-phase HPLC (7:3 MeOH/H_2O), yielding irregularasulfate (**1**, 1.6 mg) and the known compounds hipposulfate C (**2**, 20 mg)[11] and halisulfate-7 (**3**, 63 mg).[12] Igernellin (**4**)[13] was isolated from an inactive fraction by Si gel flash chromatography. The known compounds **2**, **3**, and **4** were identified by comparison of their spectroscopic data with the literature values.

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Table 1. ^{1}H and ^{13}C NMR Data for Irregularasulfate (**1**) and Halisulfate-7 (**3**) Recorded in $CDCl_3$ at 600 MHz

	irregularasulfate (**1**)			halisulfate 7 (**3**)		
position	δ_C	mult.	δ_H (J in Hz)	δ_C	mult.	δ_H (J in Hz)
1	117.0	CH	5.28 br, s	117.1	CH	5.29 br, s
2	23.5	CH_2	1.98, m; 2.02, m	23.4	CH_2	1.97, m; 2.02, m
3	31.5	CH_2	1.06, m; 1.36, m	31.5	CH_2	1.05, m; 1.34, m
4	31.5	C		31.5	C	
5	43.7	CH	1.51, m	43.8	CH	1.50, m
6	30.4	CH_2	1.06, m; 1.78, m	31.0	CH_2	1.06, m; 1.77, m
7	31.5	CH_2	1.43, m; 1.50, m	31.5	CH_2	1.43, m; 1.50, m
8	44.9	CH	1.23, m	44.9	CH	1.23, m
9	42.9	C		42.8	C	
10	146.4	C		146.3	C	
11	28.7	CH_2	1.56, m	28.6	CH_2	1.55, m
12	25.0	CH_2	1.05, m	24.6	CH_2	1.05, m
13	38.7	CH	1.53, m	38.7	CH	1.51, m
14	31.0	CH_2	1.25, m	30.4	CH_2	1.24, m
15	24.9	CH_2	1.49, m	26.9	CH_2	1.50, m
16	26.6	CH_2	2.23, m	25.1	CH_2	2.36, t (7.5)
17	140.6	C		125.2	C	
18	134.5	CH	6.62, s	111.3	CH	6.23 br, s
19	50.9	CH_2	3.80, s	142.8	CH	7.32 br, s
20	27.9	CH_3	0.83, s	27.9	CH_3	0.83, s
21	28.2	CH_3	0.84, s	28.2	CH_3	0.84, s
22	16.8	CH_3	0.81, d (7.0)	16.7	CH_3	0.82, d (7.2)
23	23.5	CH_3	0.95, s	23.4	CH_3	0.95, s
24	70.9	CH_2	3.87, t (8.6)	72.3	CH_2	3.86, t (8.3)
			3.97, dd (9.3, 3.7)			3.95, dd (9.1, 4.7)
25	172.0	C		139.1	CH	7.20, s
26	40.9	CH_2	3.43, t (7.5)			
27	37.6	CH_2	1.41, m			
28	26.0	CH	1.54, m			
29	22.8	CH_3	0.92, d (6.6)			
30	22.8	CH_3	0.92, d (6.6)			

Irregularasulfate (**1**) gave a $[M - H]^-$ ion at *m/z* 536.3407 in the negative ion HRESIMS appropriate for a molecular formula of $C_{30}H_{51}NO_5S$ (calcd for $C_{30}H_{50}NO_5S$, 536.3410), requiring six sites of unsaturation. It was possible to identify 30 carbon resonances ($5 \times C$, $6 \times CH$, $13 \times CH_2$, $6 \times CH_3$) and 50 protons attached to carbon from the 1D ^{13}C NMR, DEPT, and HSQC data (Table 1) in agreement with the HRESIMS measurement. Analysis of the COSY, HSQC, and HMBC data obtained for **1** and comparison with the same data set obtained for halisulfate-7 (**3**) showed that the two molecules were closely related. In particular, the ^{1}H and ^{13}C NMR resonances assigned to the C-1 to C-16 fragment including the attached C-20, C-21, C-22, C-23 methyl, and C-24 sulfated methyl branches in **3** (Table 1) and their COSY, HSQC, and HMBC correlations were nearly identical to the data for **1**, indicating that **1** and **3** contained identical C-1 to C-16 diterpenoid fragments.

The H-16 resonance at δ 2.23 in the ^{1}H NMR spectrum of **1** showed HMBC correlations to carbon resonances at δ 134.5 (C-18), 140.6 (C-17), and 172.0 (C-25), suggesting the attachment of C-16 to the α-carbon (C-17) of an α,β-unsaturated ester or amide. In agreement with this assignment, the olefinic resonance at δ 6.62 (H-18), which was correlated in the HSQC spectrum to δ 134.5 (C-18), showed HMBC correlations to carbon resonances at δ 172.0 (C-25), 140.6 (C-17), and 26.6 (C-16). The H-18 resonance (δ 6.62) was correlated in the COSY spectrum to a methylene resonance at δ 3.80 (H-19), which was in turn correlated to a carbon resonance at δ 50.9 (C-19) in the HSQC spectrum. The chemical shift of C-19 indicated that it was attached to nitrogen, and HMBC correlations from the H-19 resonance (δ 3.80) to the carbonyl resonance at δ 172.0 (C-25) showed that the nitrogen atom and carbonyl carbon were joined to form an α,β-unsaturated γ-lactam, accounting for the last site of unsaturation required by the molecular formula of **1**.

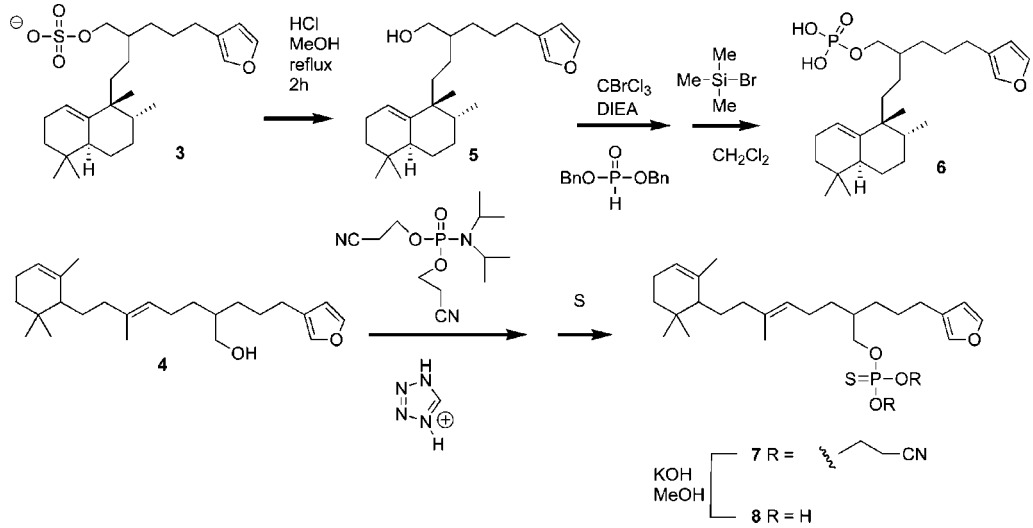
COSY and HMBC correlations routinely identified an isopentyl fragment (C-26−C-30) that accounted for the remaining atoms in **1** (Table 1). A proton resonance at δ 3.43 (t, $J = 7.5$ Hz; H-26), assigned to the methylene carbon terminus (C-26) of the isopentyl fragment, showed HMBC correlations to the lactam carbonyl (δ 172.0; C-25) and γ-methylene carbon (δ 50.9; C-19) resonances, demonstrating that the isopentyl moiety was attached to the lactam nitrogen. The near identity of the carbon and proton chemical shifts assigned to the decalin ring systems in **1** and **3** showed that the relative configurations in **1** at C-5, C-8, and C-9 were identical to those in **3**, as shown.[12b,14] The relative configuration at C-13 has not been assigned in either **1** or **3**.

Irregularasulfate (**1**) inhibited calcineurin *in vitro* with an IC_{50} of 59 μM, while hipposulfate C (**2**) and halisulfate 7 (**3**) showed calcineurin inhibition with IC_{50}'s of 66 and 69 μM, respectively. To test for selectivity, hipposulfate C (**2**) and halisulfate-7 (**3**) were also tested against pure preparations of the catalytic subunits of protein phosphatases PP-1 and PP-2A.[5] They both showed similar potency against PP-1 (IC_{50}'s: **2** 71 μM; **3** 64 μM) as they did against calcineurin, but were less active against PP-2A (IC_{50}'s: **2** 130 μM; **3** 140 μM). It seemed possible that the sulfated C-24 hydroxymethyl fragment in **1**, **2**, and **3** mimicked a phosphorylated serine residue in the natural substrates for calcineurin, PP-1, and PP-2A. In order to explore this further, phosphate and thiophosphate analogues of the major *S. irregularis* metabolites hipposulfate C (**2**) and halisulfate-7 (**3**) were prepared in an attempt to increase their potency as calcineurin inhibitors.

Halisulfate-7 (**3**) was first desulfated by treatment with refluxing HCl in MeOH to give the alcohol **5** (Scheme 1). Reaction of alcohol **5** with dibenzyl phosphite, $CBrCl_3$, and diisopropylethylamine gave the dibenzyl phosphate derivative of **5**.[15] Removal of the benzyl protecting groups by reaction with bromotrimethylsilane gave haliphosphate-7 (**6**). Igernellin (**4**) was reacted with bis(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite and tetrazole overnight at room temperature followed by addition of sulfur to give the bis(2-cyanoethyl)-protected thiophosphate **7**.[16] Removal of the protecting groups by treatment of **7** with KOH in MeOH gave the thiophosphate **8**.

As anticipated, the desulfated analogue **5** of halisulfate-7 (**3**), like igernellin (**4**), was completely inactive as a calcineurin inhibitor.

Scheme 1



The phosphate analogue **6** (IC_{50} 230 μM) was significantly less active than the sulfated natural product **3** (IC_{50} 66 μM) against calcineurin but was more active against PP-1c (IC_{50} 36 μM). One possible explanation for the lower potency of the phosphate analogue **6** against calcineurin was that it was acting as a substrate and was being converted to the inactive alcohol **5** during the assay. This prompted the preparation of the thiophosphate **8**, which should not be as good a substrate for calcineurin. The thiophosphate **8** had an IC_{50} of 70 μM against calcineurin, which was comparable to the IC_{50} for the corresponding sulfate **2**.

In summary, the new sesterterpenoid alkaloid irregularasulfate (**1**) and the known analogues hipposulfate C (**2**) and halisulfate-7 (**3**) have been isolated as the phosphatase inhibitory components of the crude MeOH extract from *S. irregularis* collected in Papua New Guinea. Chemical transformation of the natural product **3** to the alcohol **5** and the lack of activity observed for igernellin (**4**) showed that the sulfate functionality in the natural products is essential for phosphatase inhibition. The phosphate analogue **6** of halisulfate-7 was less active than the natural sulfate **3**, while the thiophosphate analogue **8** was comparable in activity to the natural product **2** against calcineurin. The phosphate and thiophosphate analogues of **2** and **3**, which might more closely resemble the natural substrates of calcineurin, were no better inhibitors than the natural sulfates. However, the phosphate analogue **6** of halisulfate-7 (**3**) (**6**: IC_{50} 36 μM) was slightly more active against PP-1c than the natural product (**3**: IC_{50} 64 μM), suggesting that it might be a substrate for calcineurin but not PP-1c. This is the first report of sulfated natural products as active site directed protein phosphatase inhibitors. Observed alterations in the selectivity of the chemically altered compounds toward either calcineurin, PP-1, and/or PP-2A may facilitate rational design of selective inhibitors of these phosphatases based on marine natural products as a starting template.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a JASCO P-1010 polarimeter equipped with a halogen lamp (589 nm) and a 10 mm microcell. NMR spectra were recorded on Bruker Avance 400 and Bruker Avance 600 (equipped with a cryoprobe) spectrometers at 400 and 600 MHz, respectively, in $CDCl_3$. Chemical shifts are given in δ (ppm) with the residual $CDCl_3$ solvent peak referenced to δ_H 7.24 and δ_C 77.23 as the internal reference. ESIMS spectra were obtained with Kratos MS-50, Micromass LCT, and Bruker Esquire-LC mass spectrometers. Si gel (Silicycle, 230–400 mesh) and Sephadex LH20 were used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm and Whatman, MKC18F 60 A) were used for TLC analysis. A Waters 1500 Series pump system equipped with a Waters 2487 dual λ absorbance detector and a CSC-Inertsil 150A/ODS2 column was used for HPLC.

Isolation of Sesterterpenoids. Specimens of *S. irregularis* (Ledenfeld) (670 g) were collected by hand using scuba near Keviang, Papua New Guinea (2°45.1′ S, 150°43′ E). A voucher sample has been deposited at the University of Amsterdam (ZMAPOR18522). The sponge was frozen on site and transported frozen to Vancouver. Thawed specimens were extracted exhaustively with MeOH, and the combined MeOH extracts were concentrated *in vacuo* to give the crude extract. The crude extract was partitioned between H_2O and CH_2Cl_2, and the CH_2Cl_2-soluble materials were subjected to Si gel chromatography (step gradient: hexanes to EtOAc to MeOH). The active fraction, eluting with 10% MeOH in EtOAc, was subjected to reversed-phase separation on a 10 g Waters C_{18} Sep-pak (step gradient: H_2O to MeOH). The fraction eluting with 70% MeOH/H_2O was subjected to isocratic reversed-phase HPLC (eluent: 70% MeOH/H_2O) to give irregularasulfate (**1**: 1.6 mg), hipposulfate C (**2**: 20 mg), and halisulfate-7 (**3**: 63 mg). Igernellin (**4**) was isolated from an inactive fraction from the first Si gel column by further isocratic flash Si gel chromatography (eluent: 10% EtOAc/hexanes).

Irregularasulfate (1): clear glass; $[\alpha]_D$ +39.8 (*c* 0.4 MeOH); ^{1}H NMR and ^{13}C NMR, see Table 1; HRESIMS(−) *m/z* 536.3407 (calcd for $C_{30}H_{50}NO_5S$, 536.3410).

Synthetic Transformations. Alcohol 5. Halisulfate-7 (**3**) was dissolved in 1 mL of MeOH, and 0.2 mL of 1 N HCl was added. The solution was stirred and heated to reflux for 2 h. The reaction mixture was cooled, and water (5 mL) was added before extraction with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 layers were dried over $MgSO_4$, filtered, concentrated *in vacuo*, and subjected to Si gel chromatography (eluent: 10% EtOAc/hexanes) to give alcohol **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (br. s, H-19), 7.18 (s, H-25), 6.24 (br s, H-18), 5.29 (br s, H-1), 3.50 (m, H_2-24), 2.39 (t, J = 5.0 Hz, H_2-16), 1.98–2.01 (m, H_2-2), 1.77 (m, H-6a), 1.45–1.60 (m, H-5, H_2-7, H_2-11, H-13, H_2-15), 1.20–1.40 (m, H-3b, H-8, H_2-14), 1.00–1.10 (m, H-3a, H-6b, H_2-12), 0.96 (s, H_3-23), 0.83 (s, H_3-20, H_3-21), 0.81 (d, J = 6.9 Hz, H_3-22); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.4 (C, C-10), 142.9 (CH, C-19), 139.0 (CH, C-25), 125.3 (C, C-17), 117.1 (CH, C-1), 111.1 (CH, C-18), 66.1 (CH_2, C-24), 44.9 (CH, C-8), 43.8 (CH, C-5), 42.8 (C, C-9), 41.5 (CH, C-13), 31.5 (CH_2, C-3), 31.5 (C, C-4), 31.5 (CH_2, C-7), 31.0 (CH_2, C-6), 30.4 (CH_2, C-14), 28.3 (CH_2, C-11), 28.2 (CH_3, C-21), 27.9 (CH_3, C-20), 27.6 (CH_2, C-15), 25.3 (CH_2, C-16), 24.7 (CH_2, C-12), 23.4 (CH_2, C-2), 23.4 (CH_3, C-23), 16.7 (CH_3, C-22); HRESIMS(+) *m/z* 395.2937 (calcd for $C_{25}H_{40}O_2Na$ 395.2926).

Phosphate 6. To a flask containing alcohol **5** (19.0 mg) dissolved in MeCN (5 mL), under N_2, at −10 °C, were added $CBrCl_3$ (0.1 mL) and DIEA (0.1 mL), followed by dibenzyl phosphite (0.3 mL, dropwise). The reaction was stirred at −10 °C for 1.5 h, at which point 0.5 M NaH_2PO_4 (5 mL) was added. The reaction mixture was then extracted with CH_2Cl_2 (3 × 5 mL), and the combined CH_2Cl_2 layers were dried over $MgSO_4$, filtered, concentrated, and subjected to Si gel chromatography (eluent: 5% EtOAc/hexanes) to give dibenzyl-protected

haliphosphate-7 (14.1 mg, 44%). To a flask containing dibenzyl-protected haliphosphate-7 (2.6 mg) under N_2 was added CH_2Cl_2 (1 mL), and the solution was cooled to 0 °C. Bromotrimethylsilane (0.05 mL) was added, and the reaction was stirred at 0 °C for 45 min before the solvent and reagent were removed *in vacuo* to give crude haliphosphate-7 (**6**). The crude phosphate **6** was dissolved in MeOH and purified via Sephadex LH-20 chromatography (eluent: MeOH) to give pure **6** (1.4 mg, 75%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (br s, H-19), 7.18 (s, H-25), 6.22 (br s, H-18), 5.29 (br s, H-1), 3.91 (m, H_2-24), 2.36 (m, H_2-16), 1.96–2.02 (m, H_2-2), 1.76 (m, H-6a), 1.45–1.55 (m, H-5, H_2-7, H_2-11, H-13, H_2-15), 1.20–1.40 (m, H-3b, H-8, H_2-14), 1.00–1.10 (m, H-3a, H-6b, H_2-12), 0.94 (s, H_3-23), 0.81 (s, H_3-20, H_3-21), 0.80 (d, J = 6.9 Hz, H_3-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.2 (C, C-10), 142.9 (CH, C-19), 139.0 (CH, C-25), 125.2 (C, C-17), 117.1 (CH, C-1), 111.2 (CH, C-18), 70.9 (CH_2, C-24), 44.9 (CH, C-8), 43.8 (CH, C-5), 42.8 (C, C-9), 39.5 (CH, C-13), 31.5 (CH_2, C-3), 31.5 (C, C-4), 31.5 (CH_2, C-7), 30.8 (CH_2, C-6), 30.4 (CH_2, C-14), 28.2 (CH_2, C-11), 28.1 (CH_3, C-21), 27.8 (CH_3, C-20), 27.2 (CH_2, C-15), 25.1 (CH_2, C-16), 24.3 (CH_2, C-12), 23.4 (CH_2, C-2), 23.4 (CH_3, C-23), 16.7 (CH_3, C-22); HRESIMS(−) *m/z* 451.2615 (calcd for $C_{25}H_{40}O_5P$ 451.2613).

Thiophosphate 8. Igernellin (**4**) (12 mg) was dissolved in CH_2Cl_2 (3 mL), and to this solution was added bis(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (0.1 mL) and tetrazole (0.7 mL) (1 M) in MeCN. The reaction mixture was stirred overnight at room temperature. Sulfur (75 mg) was added, and the reaction was allowed to stir for a further 4 h before being concentrated *in vacuo* and subjected to Si gel chromatography (eluent: step gradient hexanes to 25% EtOAc/hexanes) to give **7** (9.5 mg, 57%). Protected thiophosphate **7** (4.9 mg) was dissolved in 2 mL of 1 N KOH in MeOH, and the reaction was stirred for 2 h at room temperature. The solution was concentrated *in vacuo* and purified on a Waters 10 g reversed-phase Sep pak (eluent: step gradient H_2O to MeOH). The fraction eluting with 90% MeOH gave pure thiophosphate **8** (4.6 mg, 100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (br s, H-19), 7.26 (s, H-25), 6.21 (br s, H-18), 5.23 (br. s, H-1), 5.03 (br s, H-10), 3.74 (br s, H-24a), 3.65 (br s, H-24b), 2.34 (br s, H_2-16), 1.85–1.95 (m, H_2-2, H-8, H_2-11), 1.62 (s, H_3-22), 1.55 (m, H-13), 1.52 (s, H_3-23), 1.20–1.50 (m, H-3a, H-5, H_2-7, H_2-12, H_2-14, H_2-15), 1.07 (br d, J = 12.9 Hz, H-3b), 0.87 (s, H_3-20), 0.82 (s, H_3-21); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.9 (CH, C-19), 139.3 (CH, C-25), 136.9 (C, C-6), 136.1 (C, C-9), 125.4 (C, C-17), 124.6 (CH, C-10), 120.1 (CH, C-1), 111.5 (CH, C-18), 67.9 (CH_2, C-24), 49.3 (CH, C-5), 40.9 (CH_2, C-8), 38.6 (CH, C-13), 32.8 (C, C-4), 31.9 (CH_2, C-3), 31.0 (CH_2, C-7), 30.4 (CH_2, C-12), 30.1 (CH_2, C-14), 27.7 (CH_3, C-20), 27.7 (CH_3, C-21), 26.7 (CH_2, C-15), 25.4 (CH_2, C-11), 25.1 (CH_2, C-16), 23.7 (CH_3, C-22), 23.3 (CH_2, C-2), 16.3 (CH_3, C-23); HRESIMS(−) *m/z* 467.2387 (calcd for $C_{25}H_{40}O_4PS$ 467.2385).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for irregularasulfate (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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