# Nematocidal Thiocyanatins from a Southern Australian Marine Sponge *Oceanapia* sp.[⊥]

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Investigations of a southern Australian marine sponge, *Oceanapia* sp., have yielded two new $\beta$ methyl branched bisthiocyanates, thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**), along with two new thiocarbamate thiocyanates, thiocyanatins $E_1$ (**4a**) and $E_2$ (**4b**). The new thiocyanatins belong to a rare class of bioactive marine metabolite previously only represented by thiocyanatins A−C (**1**, **2a**/**b**). Structures were assigned on the basis of detailed spectroscopic analysis, with comparisons to the known bisthiocyanate thiocyanatin A (**1**) and synthetic model compounds (**5**−**7**). The thiocyanatins exhibit potent nematocidal activity, and preliminary structure−activity relationship investigations have confirmed key characteristics of the thiocyanatin pharmacophore.

Recently we reported[1] the isolation, structure elucidation, synthesis, and biological activity of the first examples of a family of novel bisthiocyanate nematocides, thiocyanatins A−C (**1**−**2a**,**b**), isolated from a southern Australian marine sponge, *Oceanapia* sp. Marine natural products possessing a thiocyanate functionality are rare, with the earliest reports (1989 and 1992) by Faulkner et al. describing sesquiterpene monothiocyanates from a Palauan sponge, *Axinyssa* (= *Trachyopsis*) *aplysinoides*.[2,3] By comparison, the thiocyanatins are acyclic lipids with twin terminal thiocyanate moieties. The closest known natural analogues to the thiocyanatins are also marine metabolites, being a selection of bisisothiocyanates reported (1987) by Scheuer et al. from a Fijian sponge, *Pseudaxinyssa* sp.[4] Our ongoing interest in the thiocyanatins stems from the observation that thiocyanatin A (**1**) exhibits potent nematocidal activity against the commercial livestock parasite *Haemonchus contortus* ($LD_{99}$ = 1.3 $\mu$g/mL). Furthermore, the thiocyanatins feature a structure motif readily accessible by synthesis. In light of these properties we were keen to further explore our *Oceanapia* sp. extract as a possible source of new bioactive thiocyanatins, to extend the selection of available natural thiocyanatins and further define the pharmacophore. In this report we describe four new nematocidal thiocyanatins as pairs of inseparable isomers; the isomeric $\beta$ methyl branched bisthiocyanates, thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**), and the isomeric thiocyanate-thiocarbamates, thiocyanatins $E_1$ (**4a**) and $E_2$ (**4b**).

## Results and Discussion

As reported earlier,[1] the crude ethanolic extract from an *Oceanapia* sp. collected off southern Australia during scientific trawling operations exhibited significant nematocidal activity against the commercial livestock parasite *Haemonchus contortus* ($LD_{99}$ = 135 $\mu$g/mL). The extract was decanted, concentrated in vacuo, and triturated with $CH_2Cl_2$ to afford soluble material that displayed enhanced nematocidal activity. Further solvent triturations using hexane/diethyl ether mixtures followed by $C_{18}$ HPLC afforded the previously isolated metabolites thiocyanatins A−C (**1**−**2a/b**), as well as a series of new closely related analogues (**3a/b** and **4a/b**). The nematocidal activity was attributed to thiocyanatin A ($LD_{99}$ = 1.3 $\mu$g/mL), as well as inseparable mixtures of thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**) ($LD_{99}$ = 3.1 $\mu$g/mL) and thiocyanatins $E_1$ (**4a**) and $E_2$ (**4b**) ($LD_{99}$ = 8.3 $\mu$g/mL).

High-resolution (+)ESIMS analysis of the inseparable mixture **3a/b** yielded a pseudomolecular ion (M + Na, *m*/*z* 393.1995) consistent with a molecular formula ($C_{19}H_{34}S_2N_2O$, $\Delta$ mmu = −3.9) requiring 4 DBE. A strong and characteristic IR absorbance (2156 $cm^{-1}$) was consistent with incorporation of all 4 DBE and the N and S heteroatoms into two thiocyanate functionalities, an observation further supported by the appearance of two nonequivalent thiocyanate $^{13}C$ NMR resonances ($^{13}C$, 112.3 (C) and 112.8 (C) ppm). The NMR data for **3a/b** (see Table 1) also revealed a 2°-OH methine ($^1H$, $\delta$ 3.58 (m); $^{13}C$, 71.9 (CH)), comparable to that observed in thiocyanatin A, as well as a 2°-Me ($^1H$, $\delta$ 1.05 (CH); $^{13}C$, 18.6 ($CH_3$)) $\beta$ to a deshielded diastereotopic terminal methylene ($^1H$, $\delta$ 2.79 ($J$ = 12.6, 7.5 Hz) and 3.00 ($J$ = 12.6, 5.6 Hz); $^{13}C$, 41.4 ($CH_2$) ppm). Analysis of the 2D NMR COSY and gHMBC spectra for **3a/b** clearly supported the latter spin system (with correlations across the subunit −CH($CH_3$)$CH_2$SCN), as did $^1H$ NMR comparisons with the synthetic model compound (*S*)-2-methylbutyl thiocyanate (**5**)[6] (see Table 1). As with thiocyanatin A (**1**), NMR analysis of **3a/b** was unable to unambiguously position the 2°-OH on the carbon chain. Further to this, the $^{13}C$ NMR spectrum of **3a/b** revealed a doubling of selected resonances that was interpreted as being due to 2°-OH regioisomers brought about by the unsymmetrical nature of the termini (branched vs unbranched).

As was the case with thiocyanatin A (**1**), placement of the 2°-OH along the alkyl chain in **3a/b** was achieved by interpretation of key fragmentations in the EI mass

[⊥] Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

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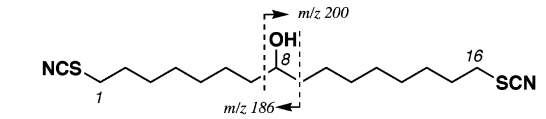
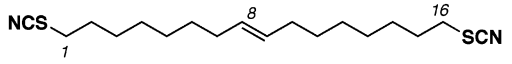
**Table 1.** $^1$H NMR (400 MHz, $CDCl_3$) Data for Thiocyanatins A (**1**), $D_{1/2}$ (**3a/b**), and $E_{1/2}$ (**4a/b**) and the Models **5** and **7**

| position | **1** | **3a/b** | **4a/b**[a] | **5**[b] | **7**[a] |
|---|---|---|---|---|---|
| $H_2$-1 | 2.95, t (7.4)[l] | 2.79, dd (7.5, 12.6)[c]<br>3.00, dd (5.6, 12.6)[c] | 2.81, t (7.2)[h] | 2.80, dd (7.6, 12.9)<br>3.01, dd (5.7, 12.9) | 2.81, t (7.2)[l] |
| H-2/$H_2$-2 | 1.85, m (7.4)[m] | 1.9, m[d] | 1.58, quin (7.1)[i] | 1.82, m | 1.58, quin (7.2)[m] |
| 2-Me | | 1.05, d (6.8)[e] | | 1.05, d (6.6) | |
| C*H*OH | 3.60, m | 3.58, m | 3.50, m | | 3.50, m |
| $H_2$-15 | *n* | 1.83, quin (7.4)[f] | 1.81, quin (7.3)[j] | | *p* |
| $H_2$-16 | *o* | 2.94, t (7.4)[g] | 3.02, t (7.2)[k] | | *q* |
| $CH_2$'s | 1.2−1.5, m | 1.2−1.7, m | 1.2−1.5, m | 1.2−1.6, m ($H_2$-3) | 1.2−1.7, m |

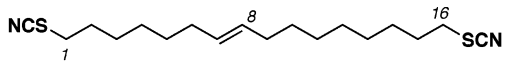
[a] 400 MHz, $d_4$-MeOH. [b] 300 MHz, $CDCl_3$. [c] **3b** $H_2$-16. [d] **3b** H-15. [e] **3b** 15-Me. [f] **3b** $H_2$-2. [g] **3b** $H_2$-1. [h] **4b** $H_2$-16. [i] **4b** $H_2$-15. [j] **4b** $H_2$-2. [k] **4b** $H_2$-1. [l] Also $H_2$-16. [m] Also $H_2$-15. [n] As for $H_2$-2. [o] As for $H_2$-1.



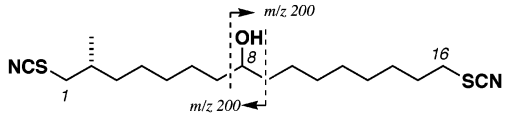

thiocyanatin A (**1**)



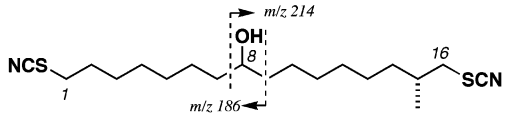

thiocyanatin B (**2a**)



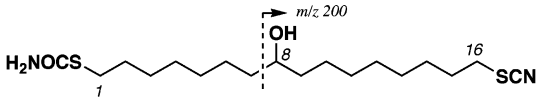

thiocyanatin C (**2b**)



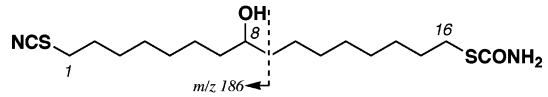

thiocyanatin $D_1$ (**3a**)



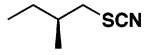

thiocyanatin $D_2$ (**3b**)



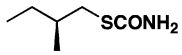

thiocyanatin $E_1$ (**4a**)



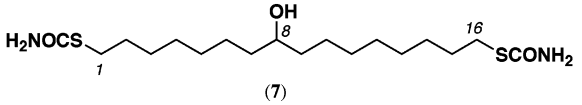

thiocyanatin $E_2$ (**4b**)




(**5**)




(**6**)




(**7**)

spectrum. Diagnostic C−C cleavage α to the 2°-OH in **3a/b** yielded ions of the general formula $[NCS(CH_2)_nHC{=}OH]^{\bullet+}$ at *m/z* 186, 200, and 214. The proposed structure for thiocyanatin $D_1$ (**3a**) is as indicated since this is the only 2°-OH regioisomer capable of giving rise to an ion at *m/z* 200 (as shown in the structure diagram). Similarly, the favored structure for **3b** as shown represents an alternate biosynthetic alkylation of thiocyanatin A (**1**). In this way thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**) can be viewed as two alternate biosynthetic β methyl branched analogues of thiocyanatin A (**1**), much the same as thiocyanatins B (**2a**) and C (**2b**) are alternate dehydration products of thiocyanatin A (**1**).

The inclusion of a β methyl branch in **3a/b** compared to thiocyanatin A (**1**) introduces a second asymmetric center. Whereas thiocyanatin A (**1**) possessed a single chiral center about the 2°-OH, the pseudo-symmetric nature of the molecule made it impossible to determine the enantio purity of **1**; thiocyanatin A (**1**) did not exhibit an optical rotation. It has been shown for a large number of labdane diterpenes in which the asymmetric ring system is separated by two methylene groups from an asymmetric center in the side chain that the molar rotation contributions for the two chiral subunits are additive.[7] We have successfully used this technique to assign absolute stereochemistry to a number of marine natural products.[8−14] Insofar as thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**) possess a "chiral" 2°-OH comparable to thiocyanatin A (**1**), it is reasonable to assume that this functionality does not contribute significantly to the optical properties of the **3a/b** mixture, requiring that the observed $[\Phi]_D = -13.7°$ be largely if not solely due to the β methyl branched chiral center. Comparison of this measurement with that reported for the known (*S*)-2-methylbutyl thiocyanate (**5**)[6] ($[\Phi]_D = +23.2°$) supports assignment of an *R* stereochemistry about the β methyl branch in both thiocyanatins $D_1$ (**3a**) and $D_2$ (**3b**).

High-resolution (+)ESIMS analysis of the inseparable mixture **4a/b** yielded a pseudomolecular ion (M + Na, *m/z* 397.1949) consistent with a molecular formula ($C_{18}H_{34}S_2N_2O_2$, Δ mmu = −2.7) requiring 3 DBE and leading to the initial impression that **4a/b** were monohydrolysis analogues of thiocyanatin A (**1**). The NMR data for **4a/b** (see Table 1) revealed discrete resonances for the two terminal methylene groups, one substituted by a thiocyanate ($^1$H, δ 3.02 (m); $^{13}$C, 34.9 ($CH_2$); gHMBC correlation C$H_2$−S*C*N (113.7 ppm)) and the other substituted by a thiocarbamate ($^1$H, δ 2.81 (m); $^{13}$C, 30.4 (t) ppm; gHMBC correlation C$H_2$−S*C*ONH$_2$ (172.0 ppm)). These observations, together with doubling of selected resonances in the $^{13}$C NMR spectrum, suggested that **4a/b** was a mixture of 2°-OH regioisomers of a mono-thiocarbamate analogue of thiocyanatin A (**1**). EI mass spectrometric analysis of **4a/b** revealed intense ions at *m/z* 186 and 200 of the general formula $[NCS(CH_2)_nHC{=}OH]^{\bullet+}$, consistent with the regioisomeric structures as shown, thiocyanatins $E_1$ (**4a**) and $E_2$ (**4b**). To confirm this assignment, the model bisthiocarbamate **7** was prepared (63%) from methanolic HCl hydrolysis of synthetic thiocyanatin A (**1**). It is noteworthy that the NMR data for the thiocarbamate terminus in the model compound **7** were in excellent agreement with that recorded for **4a/b** (see Table 1), and the EI mass spectrum for **7** did not reveal diagnostic C−C cleavages α to the 2°-OH. This latter observation is consistent with the interpretation of the diagnostic ions in the EI mass spectrum of **4a/b** and may reflect the suscep-

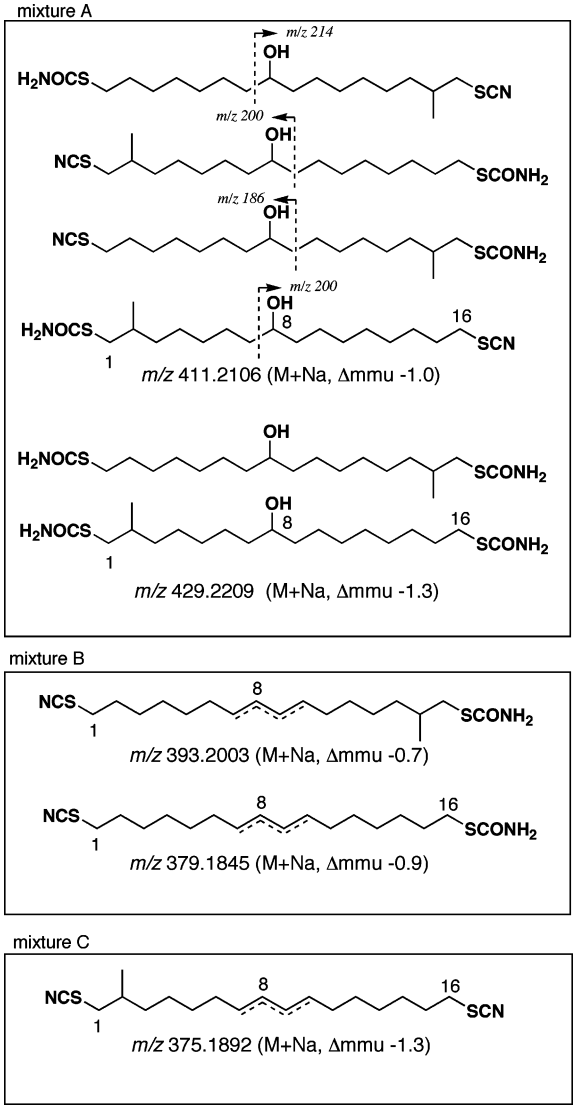



**Figure 1.** Minor thiocyanatins detected and *tentatively* identified in mixtures A−C.

tibility of the thiocarbamate functionality to EI fragmentation in advance of C−C cleavage α to the 2°-OH.

Our analysis of the *Oceanapia* extract also yielded three minor thiocyanatin fractions (mixture A (<0.5 mg), mixture B (<0.7 mg), and mixture C (<4 mg)) eluting as single peaks by $C_{18}$ HPLC.

$^1$H NMR and (+)ESI-HRMS analysis of mixture A suggested a selection of $\beta$ methyl branched and further hydrolyzed (SCN to $SCONH_2$) analogues of thiocyanatins $E_1/E_2$ (**3a/b**). More specifically, resonances in the $^1$H NMR ($CDCl_3$) spectrum for mixture A attributed to 2°-methyls ($\delta$ 1.05 and 0.95) and deshielded diastereotopic methylene protons ($\delta$ 2.68 and 2.78) were consistent with $\beta$ methyl branching adjacent to both SCN and $SCONH_2$ termini. This analysis was confirmed by direct spectroscopic comparison with authentic samples of both the *S* thiocyanate **6** and its thiocarbamate analogue **7**, which revealed a characteristic $^1$H NMR chemical shift difference between a 2°-Me $\beta$ to a SCN ($\sim\delta$ 1.05) versus a $SCONH_2$ ($\sim\delta$ 0.95). The remaining $^1$H NMR ($CDCl_3$) spectral data for mixture A were consistent with key resonances in thiocyanatins $E_1$/$E_2$ (**3a/b**), while the $^{13}$C NMR ($CDCl_3$) data revealed a resonance (20 ppm) consistent with the new $\beta$ branched methyl termini. A list of plausible mixture A components is shown in Figure 1. Although an inseparable mixture by $C_{18}$ HPLC, mixture A was resolved by GC into six components, four of which eluted early with similar retention times (42.3 (3%), 43.2 (18%), 43.6 (15%), and 43.8 (11%) min), while the remaining two eluted several minutes later, also with similar retention times (51.4 (28%) and 51.6 (24%) min). This GC analysis suggests that mixture A features most if not all the proposed structures shown in Figure 1.

Analysis of the $^1$H NMR and (+)ESI-HRMS data for mixture B suggested dehydration analogues along the lines of those indicated in Figure 1. The presence of only a single $^1$H NMR ($CDCl_3$) 2°-Me resonance ($\delta$ 0.95) was consistent with $\beta$ methyl branching adjacent to an $SCONH_2$ termini, while the remaining $^1$H NMR resonances compared well with thiocyanatins B and C (**2a/b**). On the basis of the hypothesis that the alkene thiocyanatins are biosynthetic dehydration products of the corresponding natural alcohols,[1] and given that the $SCONH_2$ functionality most likely arises from nonregiospecific hydrolysis of SCN moieties in suitable natural precursors, SCN and $SCONH_2$ asymmetrically terminated thiocyanatins would be expected to give rise to multiple alkene regioisomers (such as shown in Figure 1). GC analysis of mixture B revealed at least one major (95%) and one minor (5%) component (eluting at 50.7 and 51.0 min, respectively).

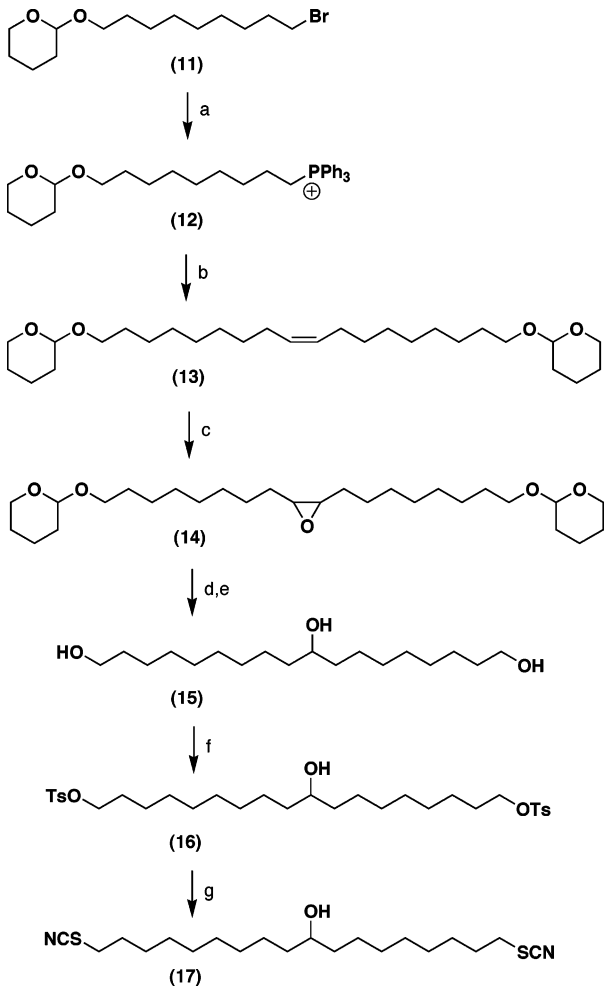
$^1$H NMR and (+)ESI-HRMS analyses of mixture C were consistent with $\beta$ methyl branched analogues of thiocyanatins B and C (**2a/b**), which based on arguments comparable to those detailed above indicated that mixture C may be comprised of a selection of alkene regioisomers (see Figure 1). GC analysis of mixture C revealed major components eluting at 43.2 (38%) and 51.2 (45%) min, with additional minor components eluting at 42.2 (8%) and 50.8 (9%) min, not inconsistent with the proposed series of regio- and stereoisomeric alkenes shown in Figure 1.

While we acknowledge that the complex status of mixtures A−C precludes unambiguous structure assignments, the observations summarized above and in Figure 1 are disclosed at this time as an illustration of the breadth of metabolic variety that *Oceanapia* assembles around the thiocyanatin molecular motif.

In summary, the *Oceanapia* sp. has yielded a remarkable new class of natural products, several examples of which display potent nematocidal activity. In addition to isolating and identifying thiocyanatins A (**1**), B (**2a**), C (**2b**), $D_1$ (**3a**), $D_2$ (**3b**), $E_1$ (**4a**), and $E_2$ (**4b**) and drawing attention to a series of minor metabolites (Figure 1), we have prepared the synthetic $\beta$ methyl branched thiocyanate **5** and thiocarbamate **6** and the bisthiocarbamate **7**. We have also prepared monothiocyanate (**8**) and trithiocyanate (**9**) analogues of thiocyanatin A (**1**), as well as the deoxy analogue **10**. Furthermore, as outlined in Scheme 1, we have prepared a chain elongated $C_{18}$ homologue **17** of thiocyanatin A (**1**). Together with natural thiocyanatins, these synthetic analogues provide some preliminary SAR observations, based around the most potent natural example of this structure class, thiocyanatin A (**1**). Some noteworthy analogues and their nematocidal activity ($LD_{99}$) are listed in Figure 2. Even this limited selection of analogues highlights the importance of both the 2°-alcohol and SCN functionalities and the influence of chain length on nematocidal activity.

While the nematocidal mode of action of the thiocyanatins has yet to be determined, it is interesting to note that synthetic thiocyanates attracted early attention as potential insecticides. Researchers in the mid to late 1900s observed that alkyl thiocyanates could under appropriate enzymatic conditions release HCN, and they went on to

**Scheme 1**[a]




[a] a, $PPh_3$; b, NaHMDS, $O_2$; c, mCPBA; d, LAH, e, $H^+$; f, TsCl; g, KSCN.

explore the agrochemical potential of this effect. For example, $C_{12}$ and $C_{13}$ *n*-alkyl and *sec*-alkyl thiocyanates were noted to be outstanding insecticides against adult female fruit-tree red spider mites,[15] while various other alkyl thiocyanates were described as lethal to mice and houseflies[16] and selected beetles[17] through the action of HCN. Although these early investigations focused on synthetic alkyl thiocyanates, it has long been known that certain plants and insects employ HCN as a defensive agent through the intermediary of cyanogenic glycosides. At this stage the chemical ecology of the thiocyanatins remains unknown, although it is tempting to speculate that naturally occurring alkyl thiocyanates such as the thiocyanatins may function as chemical defense agents, countering predatory attack through localized enzyme-mediated release of HCN.

## Experimental Section

**General Experimental Procedures.** See ref 1.

**Animal Material.** See ref 1.

**Extraction and Isolation.** The EtOH extract of the *Oceanapia* sp. was decanted and concentrated in vacuo (800 mg), and the nematocidal components were extracted by trituration with $CH_2Cl_2$ (329 mg), as detailed in an earlier report.[1] The $CH_2Cl_2$-soluble material was then subjected to a further series of triturations to give hexane (92.6 mg), 1:1 hexane/$Et_2O$ (103.1 mg), and $Et_2O$ soluble (73.5 mg) along with an insoluble (54.3 mg) fraction. The hexane-soluble material was treated with MeOH (0.5 mL), the precipitated fats and steroids (12.4 mg) were removed, and the supernatant was subjected to $C_{18}$ HPLC (2.0 mL/min 85−100% MeOH/water through a Zorbax Eclipse XDB 5 $\mu$m 250 × 9.4 mm $C_{18}$ column) to give in order of elution thiocyanatin A (**1**) (11.0 mg, 0.03%), an inseparable mixture of thiocyanatins $D_1$ and $D_2$ (**3a/b**) (4.6 mg, 0.013%), and an inseparable mixture of thiocyanatins B and C (**2a**,**b**) (23.2 mg, 0.066%). The 1:1 hexane/$Et_2O$-soluble material was treated in a similar fashion to give in order of elution an inseparable mixture of thiocyanatins $E_1$ and $E_2$ (**4a/b**) (5.4 mg, 0.015%), thiocyanatin A (**1**) (15.3 mg, 0.043%), an inseparable mixture of thiocyanatins $D_1$ and $D_2$ (**3a/b**) (3.8 mg, 0.011%), and an inseparable mixture of thiocyanatins B and C (**2a**,**b**) (1.6 mg, 0.004%). Also recovered were the HPLC inseparable mixtures A (<0.5 mg, <0.001%), B (<0.7 mg, <0.001%), and C (<4 mg, <0.01%). All reported yields are measured against the dry weight of the sponge sample. The known thiocyanatins A−C were identical in all respects to those previously reported.[1]

**Thiocyanatins $D_1$ and $D_2$ (3a/b):** clear viscous oil; $[\alpha]^{21}_D$ −3.7° (*c* 0.41, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3500 (br), 2156 $cm^{-1}$; $^1H$ NMR (see Table 1); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 18.6 (2-Me), 25.5, 25.6, 26.6, 27.86/27.91 (C-14), 28.78/28.82 (C-13), 29.3, 29.4, 29.5, 29.6, 29.9 (C-15), 33.8 (C-2), 34.1 (C-16), 35.2 (C-3), 37.4/37.5 (C-7/9 [**3a**], C-8/10 [**3b**]), 41.4 (C-1), 71.9 (C-8 [**3a**], C-9 [**3b**]), 112.3 (16-SCN), 112.8 (1-SCN); (+)ESIMS *m*/*z* 393 [$(M + Na)^+$], 353 [$(M + H)^+ - H_2O$]; (+)ESI-HRMS *m*/*z* 393.1995 [$(M + Na)^+$, calcd for $C_{19}H_{34}S_2N_2ONa$, 393.2010].

**Thiocyanatins $E_1$ and $E_2$ (4a/b):** clear viscous oil; $^1H$ NMR (see Table 1); $^{13}C$ NMR (100 MHz, $d_4$-MeOH) $\delta$ 26.6, 26.7, 28.9 (C-14), 29.7 (C-3), 29.9, 30.0, 30.2, 30.3, 30.4 (C-1), 30.5, 30.6, 30.69, 30.74, 31.1 (C-15), 31.6 (C-2), 34.9 (C-16), 38.4 (C-7/9 [**4a**], C-8/10 [**4b**]), 72.4 (C-8 [**4a**], C-9 [**4b**]), 113.7 (SCN), 172.0 ($SCONH_2$); (+)ESIMS *m*/*z* 397 [$(M + Na)^+$]; (+)ESI-HRMS *m*/*z* 397.1949 [$(M + Na)^+$, calcd for $C_{18}H_{34}S_2N_2O_2Na$, 397.1960].

***(S)*-2-Methylbutyl Thiocyanate (5).**[6] A mixture of $LiAlH_4$ (0.35 g, 9.18 mmol) and dry ether (15 mL) was added dropwise to a solution of (+)-(*S*)-2-methylbutyric acid (0.47 g, 4.59 mmol) in dry ether (10 mL) under gentle reflux. Stirring was continued at 40 °C for 16 h, after which the excess $LiAlH_4$ was filtered and the filtrate treated with EtOAc (20 mL) followed by 10% $HCl_{aq}$ (20 mL). The aqueous phase was extracted with ether (10 mL × 2) and the combined organic phase washed sequentially with 10% $HCl_{aq}$ (10 mL), $H_2O$ (10 mL), and brine (10 mL), then dried (anhydrous $MgSO_4$). The ether was removed under reduced pressure to give **(*S*)-2-methylbutanol** (0.17 g, 42%) as a pale yellow oil. The crude alcohol (0.17 g, 1.93 mmol) in dry $CH_2Cl_2$ (20 mL) was treated with $Et_3N$ (0.4 mL, 2.90 mmol) and the mixture chilled to −10 °C, at which point methansulfonyl chloride (0.15 mL, 2.04 mmol) was added dropwise and the reaction stirred at −10 °C for 1 h. The reaction mixture was poured onto ice and extracted with additional $CH_2Cl_2$ (10 mL × 2), after which the combined organic extract was washed sequentially with ice-cold water (10 mL × 2), 4% w/v aqueous oxalic acid (10 mL × 2), 2% $NaHCO_3$ (10 mL × 2), and brine (10 mL), then dried (anhydrous $Na_2SO_4$). The $CH_2Cl_2$ was removed under reduced pressure to give the **(*S*)-2-methylbutyl methanesulfonate** (0.16 g, 51%). A mixture of crude mesylate (0.16 mg, 0.96 mmol) and KSCN (93 mg, 0.96 mmol) in dry THF (20 mL) was stirred at reflux for 16 h. The reaction was cooled to RT and the solvent removed under reduced pressure. The residue was partitioned between $H_2O$ (10 mL) and ether (10 mL) and further extracted with ether (10 mL × 2), after which the combined organic phase was sequentially washed with $H_2O$ (10 mL) and brine (10 mL) and then dried (anhydrous $MgSO_4$). Evaporation of the ether followed by elution through a silica SPE cartridge (eluent 8% EtOAc/hexane) yielded the desired **(*S*)-2-methylbutyl thiocyanate** (**5**) (20 mg, 16%) as a pale yellow oil, along with unreacted mesylate (57 mg): $^1H$ NMR (see Table 1); $[\alpha]^{14}_D$ +18.8° (*c* 1.0, $CHCl_3$); IR (film) $\nu_{max}$ 2156 $cm^{-1}$; (+)ESIMS *m*/*z* 152 [$(M + Na)^+$].

***(S)*-2-Methylbutyl Thiocarbamate (6).** (*S*)-2-Methylbutyl thiocyanate (**5**) (8.4 mg, 0.065 mmol) was dissolved in MeOH (40 mL) and dry HCl gas passed through the solution for 2 h at 0 °C. The solution was then stirred at RT for 16 h before

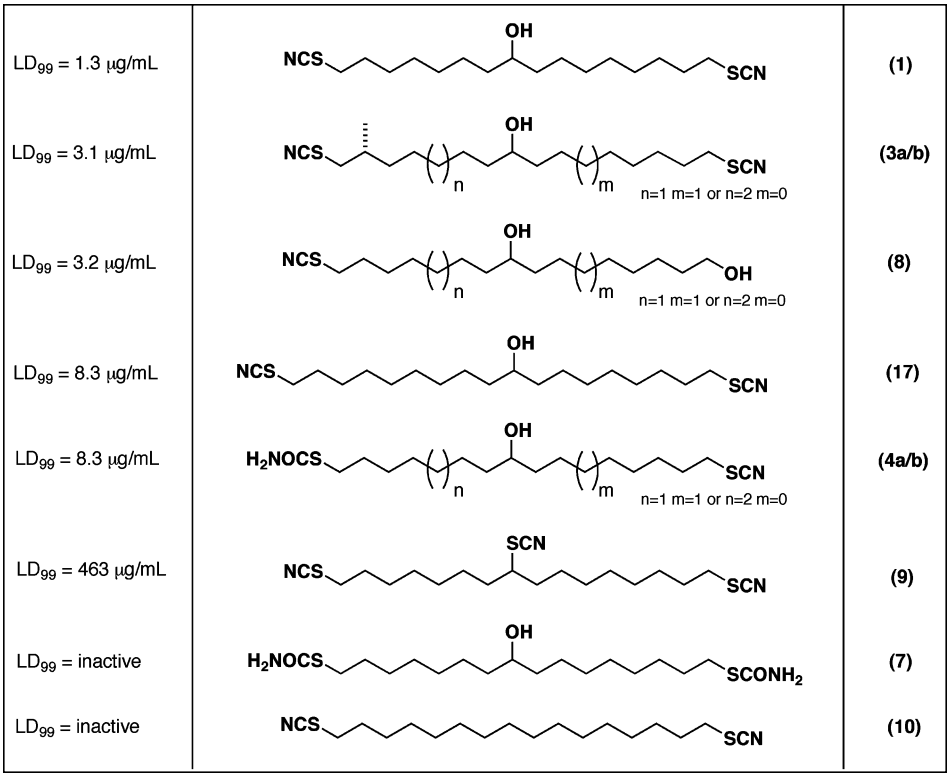



**Figure 2.** Selected SAR data on thiocyanatins.

removal of the solvent under reduced pressure to afford (*S*)-2-methylbutyl thiocarbamate (**6**) (6.8 mg, 71%) as a pale yellow oil: $[\alpha]^{18}_{D}$ +35° (*c* 0.5, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.90 (t, $J$ = 7.2 Hz, 4-$H_3$), 0.94 (d, $J$ = 6.8 Hz, 2-Me), 1.22 (m, 3-H), 1.46 (m, 3-H), 1.60 (m, 2-H), 2.78 (dd, $J$ = 13.2, 7.2 Hz, 1-H), 2.94 (dd, $J$ = 13.2, 5.6 Hz, 1-H); $^{13}$C NMR (100 MHz, $CD_3OD$) 11.3, 18.6, 28.5, 35.3, 36.8, 169.5; (+)ESIMS *m*/*z* 149 (M + $H_2$, 60); (+)ESI-HRMS *m*/*z* 170.0614 (M + Na) (calcd for $C_6H_{13}NOSNa$ 170.0616).

**Synthesis of 1,16-Dithiocarbamyl-8-hydroxyhexadecane (7).** Synthetic thiocyanatin A (**1**)[1] (43 mg, 0.12 mmol) was dissolved in MeOH (50 mL) and dry HCl gas passed through the solution for 2 h at 0 °C. The solution was then stirred at RT for 16 h before removal of the solvent under reduced pressure to afford 1,16-dithiocarbamyl-8-hydroxyhexadecane (**7**) (30.7 mg, 65%): IR (KBr) $\nu_{max}$ 3385 (br), 3182, 1651 cm$^{-1}$; $^1$H NMR (see Table 1); $^{13}$C NMR (100 MHz, $CD_3OD$) 26.6, 26.7 (C6, C10), 29.8 (C3, C14), 30.2, 30.3 (C1, C16), 30.4 (C4, C13), 30.6, 30.7, 30.8 (C5, C11, C12), 31.7 (C2, C15), 38.4 (C7, C9); (+)ESIMS *m*/*z* 415 (M + Na, 100); (+)ESI-HRMS *m*/*z* 415.2067 (M + Na) (calcd for $C_{18}H_{36}N_2O_3S_2Na$ 415.2065).

**1,8-Dihydroxy-16-thiocyanatohexadecane/8,16-Dihydroxy-1-thiocyanatohexadecane (8).** To a stirred suspension of 1,8,16-trihydroxyhexadecane[1] (0.28 g, 0.74 mmol), DMAP (6.5 mg, 0.053 mmol), and TsCl (0.28 g, 1.47 mmol) in dry $CH_2Cl_2$ (5.5 mL) was added $Et_3N$ (0.21 mL, 1.48 mmol) at 0 °C. After the mixture was stirred at RT for 16 h, additional TsCl (0.28 g, 1.47 mmol) and $Et_3N$ (0.21 mL, 1.48 mmol) were added and stirring continued for a further 16 h. The reaction mixture was concentrated under reduced pressure and the residue triturated with EtOAc. The EtOAc extract was concentrated under reduced pressured and subjected to silica column chromatography using 20−40% EtOAc/hexane as eluent to give a mixture containing the C-1 and C-16 monotosylates, the C-1 and C-16 ditosylates, and the tritosylate derivative. The mono-, di-, and tritosylates were separated by silica chromatography. As described in an earlier report,[1] the ditosylate was used to prepare synthetic thiocyanatin A (**1**). The mixed monotosylates (0.11 g, 0.26 mmol) were combined with KSCN (0.05 g, 0.52 mmol) in dry THF (5 mL) and stirred at reflux under $N_2$ for 16 h. The solvent was removed under reduced pressure and the residue partitioned between $H_2O$ (5 mL) and $Et_2O$ (5 mL). The aqueous phase was extracted with $Et_2O$ (3 × 5 mL) and the combined organic phase washed with brine (5 mL), dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 20% EtOAc/hexane as eluent yielded **8** (59 mg, 73%): IR $\nu_{max}$ (film) 3354, 2154 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.27−1.39 (m, methylene envelope), 1.49−1.54 (m, 2-$H_2$, 15-$H_2$), 2.91 (t, $J$ = 7.2 Hz, 16-$H_2$ (1-$H_2$)), 3.53 (m, 8-H), 3.58 (t, $J$ = 6.6 Hz, 1-$H_2$ (16-$H_2$)); $^{13}$C NMR (100 MHz, $CDCl_3$) 25.4, 25.5, 25.6, 27.8, 28.7, 29.2, 29.3, 29.4, 29.5, 29.7, 32.6, 33.9, 37.2, 37.3, 62.7, 71.7, 122.4; (+)ESIMS *m*/*z* 338 (M + Na, 100) 316 (M + H, 80); (+)ESI-HRMS *m*/z 338.2126 (M + Na) (calcd 338.2130).

**1,8,16-Trithiocyanatohexadecane (9).** A mixture of the tritosylate described above (0.70 g, 0.95 mmol) and KSCN (0.20 g, 2.09 mmol) in dry THF (25 mL) was stirred at reflux for 16 h, after which the solvent was removed and the residue partitioned between $H_2O$ (10 mL) and $Et_2O$ (10 mL). The aqueous phase was extracted with $Et_2O$ (3 × 10 mL), and the combined organic phase was washed with brine (20 mL), dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 10% EtOAc/hexane as eluent yielded **9** (0.31, 81%): IR $\nu_{max}$ (film) 2152 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.30−1.50 (m, methylene envelope), 1.72 (m, 3-$H_2$, 14-$H_2$), 1.80 (m, 2-$H_2$, 15-$H_2$), 2.91 (t, $J$ = 7.2 Hz, 1-$H_2$, 16-$H_2$), 3.02 (m, 8-H); $^{13}$C NMR (100 MHz, $CDCl_3$) 26.7, 26.8, 27.7, 27.8, 28.6, 28.7, 28.8, 28.9, 29.0, 29.7, 29.8, 33.9, 34.0, 35.2, 35.3, 51.6, 111.4, 112.4; (+)-ESIMS *m*/*z* 339 (M − SCN, 100), 420 (M + Na, 60); (+)ESI-HRMS *m*/*z* 420.1567 (M + Na) (calcd for $C_{19}H_{31}N_3S_3Na$ 420.1578).

**1,16-Dithiocyanatohexadecane (10).** A mixture of commercially available 1,16-hexadecanediol (0.25 g, 0.97 mmol), HBr (45%) (0.31 mL, 2.42 mmol), and concentrated $H_2SO_4$ (0.11 mL) was stirred at 40 °C for 5 h, then diluted with $H_2O$ (5 mL) and $Et_2O$ (5 mL). The aqueous phase was extracted with $Et_2O$ (2 × 5 mL), and the combined organic phase washed with $H_2O$ (2 × 5 mL), saturated $NaHCO_{3aq}$ (3 × 5 mL), and brine (5 mL), then dried (anhydrous $MgSO_4$). Filtration followed by concentration under reduced pressure gave 1,16-dibromohexadecane (0.18 g, 48%) as a pale yellow oil, which was then dissolved in dry THF (20 mL) and KSCN (0.11 g, 1.15 mmol)

was added. The mixture was stirred at reflux for 16 h, after which the solvent was removed under reduced pressure. The residue was partitioned between $H_2O$ (10 mL) and $Et_2O$ (10 mL), and the aqueous phase extracted with $Et_2O$ (3 × 10 mL). The combined organic phase was washed with brine (10 mL), dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica SPE cartridge using 5% EtOAc/hexane as eluent afforded the dithiocyanate **10** (0.13 g, 83%): IR $\nu_{max}$ (film) 2152 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.23−1.29 (m, methylene envelope), 1.43 (m, 3-$H_2$, 14-$H_2$), 1.82 (quin, $J$ = 7.5 Hz, 2-$H_2$, 15-$H_2$), 2.94 (t, $J$ = 7.2 Hz, 1-$H_2$, 16-$H_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$) 27.9, 28.8, 29.3, 29.4, 29.5, 29.6, 29.8, 34.0, 112.4; (+)ESIMS $m/z$ 363 (M + Na, 100), 341 (M + H, 60), 282 (M − SCN, 70); (+)ESI-HRMS $m/z$ 363.1902 (M + Na) (calcd for $C_{18}H_{32}N_2S_2Na$ 363.1905).

**1,9,18-Trihydroxyoctadecane (15).** A mixture of commercially available 9-bromo-1-nonanol (5 g, 22.4 mmol), dihydropyran (2.1 mL, 23.3 mmol), and concentrated HCl (22 $\mu$L) was stirred at RT for 18 h. The reaction mixture was diluted with $Et_2O$ (20 mL) and washed with $H_2O$ (20 mL), saturated $NaHCO_{3aq}$ (2 × 20 mL), and brine (20 mL), then dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 10% EtOAc/hexane as eluent yielded the THP-protected alcohol **11** (5.74 g, 84%). A mixture of **11** (5.74 g, 18.9 mmol) and $PPh_3$ (4.46 g, 18.9 mmol) in MeCN (45 mL) was stirred at reflux for 16 h, then concentrated under reduced pressure to give the corresponding Wittig salt **12** (10.6 g, 99%) as a colorless oil. To a stirred solution of **12** (4.89 g, 8.66 mmol) in dry THF (35 mL) and DMPU (11 mL) under $N_2$ at RT was added dropwise NaHMDS (8.66 mL, 8.66 mmol, 1 M solution in THF). Stirring was continued for 30 min, after which $O_2$ was passed into the reaction, and stirring continued at 55 °C for 16 h. Saturated $NH_4Cl_{aq}$ was added and the mixture poured into $H_2O$ (150 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phase washed with $H_2O$ (2 × 100 mL), brine (100 mL), then dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 5% EtOAc/hexane as eluent yielded the dimer alkene **13** (1.11 g, 61%) as a clear oil: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.29 (m, methylene envelope), 1.49−1.63 (m, 3′-$H_2$, 4′-$H_2$, 8′-$H_2$, 9′-$H_2$), 1.72 (m, 2′-$H_2$, 7′$H_2$), 1.82 (m, 7-$H_2$, 12-$H_2$), 2.01 (8-$H_2$, 11-$H_2$), 3.39 (dt, $J$ = 9.6, 6.6 Hz, 1-$H_2$, 16-$H_2$), 3.50 (m, 5′-$H_2$, 10′-$H_2$), 3.72 (dt, $J$ = 9.6, 6.9 Hz, 1-$H_2$, 16-$H_2$), 3.87 (m, 5′-$H_2$, 10′-$H_2$), 4.57 (t, $J$ = 4.5 Hz, 1′-$H_2$, 6′-$H_2$), 5.34 (m, 9-$H_2$, 10-$H_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 19.6, 25.5, 26.2, 27.2, 29.2, 29.4, 29.5, 29.7, 30.7, 62.3, 67.6, 98.8, 129.8; (+)ESIMS $m/z$ 285 (M − $(THP)_2$ + H, 100), 475 (M + Na, 50); (+)ESI-HRMS $m/z$ 475.3746 (M + Na) (calcd for $C_{28}H_{52}O_4Na$ 475.3763). A mixture of **13** (0.41 g, 0.91 mmol) and mCPBA (1.00 g, 5.3 mmol) in dry $CH_2Cl_2$ (80 mL) was stirred at RT under $N_2$ for 16 h. The reaction mixture was washed with saturated $NaHCO_{3aq}$ (3 × 80 mL), $H_2O$ (80 mL), and brine (80 mL) and dried (anhydrous $MgSO_4$), then filtered and concentrated under reduced pressure. Purification by silica column chromatography using 15% EtOAc/hexane as eluent afforded the corresponding epoxide **14** (0.31 g, 73%). To a stirred suspension of $LiAlH_4$ (0.13 g, 3.32 mmol) in dry $Et_2O$ (20 mL) under $N_2$ at RT was added dropwise a solution of **14** (0.31 g, 0.66 mmol) in dry $Et_2O$ (20 mL) maintaining a gentle reflux. Refluxing was continued for 18 h, and then the reaction was quenched with EtOAc followed by addition of 1 M $HCl_{aq}$ (40 mL) and $H_2O$ (40 mL). The aqueous phase was extracted with $Et_2O$ (3 × 40 mL) and the combined organic extract washed with 1 M $HCl_{aq}$ (3 × 40 mL), $H_2O$ (3 × 40 mL), and brine (40 mL), then dried (anhydrous $MgSO_4$) and filtered. Removal of the solvent under reduced pressure yielded a product (0.30 g, 0.63 mmol), which was taken up in MeOH (2 mL) and treated with added concentrated $H_2SO_4$ (0.1 mL) at RT for 16 h, then concentrated under reduced pressure. The residue was diluted with $Et_2O$ (25 mL) and washed with saturated $NaHCO_{3aq}$ (25 mL), $H_2O$ (25 mL), and brine (25 mL), then dried (anhydrous $Na_2SO_4$). The crude material was recrystallized from hexane/EtOAc to yield 1,9,18-trihydroxyoctadecane (**15**) (61 mg, 32%) as a white solid: mp 58−62 °C; IR $\nu_{max}$ (KBr) 3310 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.12−1.31 (m, methylene envelope), 1.36−1.45 (m, 2-$H_2$, 17-$H_2$), 3.42 (m, 9-$H_2$), 3.45 (t, $J$ = 6.6 Hz, 1-$H_2$, 18-$H_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$) 26.6, 26.7, 26.8, 26.9, 30.3, 30.4, 30.5, 30.6, 30.7, 30.8, 38.5, 64.1, 73.1; (+)ESIMS $m/z$ 325 (M + Na, 100), 341 (M + K, 10); (+)ESI-HRMS $m/z$ 325.2710 (M + Na) (calcd for $C_{18}H_{38}O_3Na$ 325.2719).

**9-Hydroxy-1,18-dithiocyanato-octadecane (17).** To a stirred suspension of **15** (61 mg, 0.20 mmol), DMAP (2.5 mg, 0.02 mmol), and *p*-TsCl (80.9 mg, 0.42 mmol) in dry $CH_2Cl_2$ (2 mL) was added $Et_3N$ (59 $\mu$L, 0.42 mmol) at 0 °C, and the mixture stirred at RT for 16 h, after which DMAP (1 mg, 8 $\mu$mol), *p*-TsCl (40 mg, 0.21 mmol), and $Et_3N$ (30 $\mu$L, 0.21 mmol) was added at 0 °C. The reaction was stirred at RT for a further 16 h, then concentrated under reduced pressure. The residue was purified by silica chromatography using 30% EtOAc/hexane as eluent to yield 1,16-ditosylate **16** (52 mg, 42%). A mixture of **16** (52 mg, 0.085 mmol) and KSCN (21 mg, 0.21 mmol) in dry THF (10 mL) was stirred at reflux for 20 h, after which solvent was removed under reduced pressure and the residue partitioned between $H_2O$ (10 mL) and $Et_2O$ (10 mL). The aqueous phase was extracted with $Et_2O$ (3 × 10 mL) and the combined organic phase washed with brine (10 mL), dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 20% EtOAc/hexane yielded 9-hydroxy-1,18-dithiocyanato-octadecane (**17**) (20 mg, 62%): IR $\nu_{max}$ (KBr) 3503, 2152 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.29−1.52 (m, methylene envelope), 1.82 (quin, $J$ = 7.8 Hz, 2-$H_2$, 15-$H_2$), 2.94 (t, $J$ = 6.9 Hz, 1-$H_2$, 16-$H_2$), 3.58 (m, 9-H); $^{13}C$ NMR (100 MHz, $CDCl_3$) 25.5, 25.6, 27.8, 27.9, 28.7, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8, 29.9, 34.0, 34.1, 37.4, 37.5, 71.9, 112.4; (+)ESIMS $m/z$ 407 (M + Na, 100), 423 (M + K, 80); (+)ESI-HRMS $m/z$ 407.2167 (M + Na) (calcd for $C_{20}H_{36}N_2OS_2Na$ 407.2167).

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