Natural product-based synthesis of novel anti-infective isothiocyanate- and isoselenocyanate-functionalized amphilectane diterpenes

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Supplementary Data

Experimental Section

General Techniques. All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of argon. Column chromatography (CC) was performed on silica gel (35–75 μm); reactions were followed by TLC analysis using glass pre-coated silica gel plates with fluorescent indicator (254 nm) and visualized with a UV lamp or I_2 vapors. Semipreparative RP-HPLC was performed using a UV detector set at 254 nm and a column with 5 μm, 250 x 4.6 mm size with a flow rate of 1.0 mL/min. THF and commercially available reagents were purchased and used as received without further purification. Optical rotations were recorded with a polarimeter using a 0.5 mL capacity cell with 1 dm path length. Infrared spectra were recorded using thin films supported on NaCl discs. ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the specified field strength on a 700 MHz spectrometer. Spectra were obtained on $CDCl_3$ solutions in 5 mm diameter tubes, and chemical shifts are quoted in parts per million relative to the

residual signals of chloroform (δ_H = 7.26 ppm, δ_C = 77.0 ppm). Multiplicities in the ^{1}H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants are reported in hertz. High-resolution mass spectrometry (HRMS) was performed using a quadrupole mass analyzer, and the data are reported with ion mass/charge (*m/z*) ratios as values in atomic mass units. Yields shown are based on recovered starting material.

Animal Material. The Caribbean sponge *Svenzea flava* (phylum Porifera; class Demospongiae; order Halichondrida; family Dictyonellidae) was collected at a depth of 89 feet by scuba off Mona Island, Puerto Rico, in July 2006. A voucher specimen (no. IM06-04) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The species *Svenzea flava* was originally classified as *Pseudoaxinella flava*.[1] Despite lacking dark granulous cells that are a signature characteristic of other species within the genus *Svenzea*, it has been accepted as *Svenzea flava*.[2]

Isolation and Purification of (–)-8,15-Diisocyano-11(20)-amphilectene (1). The known sponge metabolite (–)-8,15-diisocyano-11(20)-amphilectene (**1**)[3] was obtained pure as white crystals (528 mg) from freshly collected sponge specimens as previously described (the sponge was originally reported by our group as *Hymeniacidon* sp.).[4] The structure characterization of **1** was established on the basis of IR, UV, $[\alpha]_D$, MS, and ^{1}H and ^{13}C NMR spectroscopic analyses.

Synthesis of Isothiocyanate-Containing Amphilectanes. To a 25° C solution of diisocyanide **1** (37.0 mg, 0.11 mmol) in dry THF (5.0 mL) was added selenium (0.9 mg, 0.01 mmol), sulfur (4.0 mg, 0.12 mmol), and TEA (76 μL, 0.5 mmol). After refluxing for 4 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo*. The crude oil obtained was purified by flash-silica gel CC with 100% hexane to afford unreacted **1** (23 mg) followed by another fraction consisting of a mixture of isothiocyanate-containing products that was purified by HPLC (MeOH/H_2O, 88/12). Retention times were 20.4 min for the more polar mixture of **3** and **4** (10 mg, 53%) and 23.8 min for the less polar compound **2** (3.0 mg, 18%).

*8,15-Diisothiocyano-11(20)-amphilectene (**2**)*: colorless oil; $[\alpha]^{20}_{D}$ -108.0 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (ε) 202 (19471) nm; IR (film) ν_{max} 2926, 2857, 2095, 1728, 1459, 1262 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 4.87 (br s, 1H, H-20β), 4.61 (br s, 1H, H-20α), 2.34 (m, 1H, H-9β), 2.26 (m, 2H, H-10), 2.16 (m, 1H, H-2β), 2.06 (dd, *J* = 1.3, 14.6 Hz, 1H, H-14β), 2.00 (m, 1H, H-5β), 1.89 (m, 1H, H-1), 1.75 (t, *J* = 10.8 Hz, 1H, H-12), 1.55 (m, 2H, H-6), 1.47 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.43–1.35 (br envelope, 2H, H-7, H-9α), 1.28 (m, 1H, H-14α), 1.11–1.00 (br envelope, 3H, H-3, H-4, H-13), 0.99 (d, *J* = 6.3 Hz, 3H, H-19), 0.94 (d, *J* = 6.1 Hz, 3H, H-18), 0.92–0.83 (br envelope, 2H, H-2α, H-5α); ^{13}C NMR (175 MHz, $CDCl_3$) δ 150.2 (C, C-11), 131.1 (C, C-21), 130.4 (C, C-22), 106.1 (CH_2, C-20), 69.6 (C, C-8), 60.6 (C, C-15), 56.9 (CH, C-13), 46.8 (CH_2, C-14), 46.7 (CH, C-12), 43.3 (CH, C-4), 42.5 (CH, C-7), 41.1 (CH_2, C-2), 40.1 (CH_2, C-9), 35.7 (CH, C-3), 34.1 (CH_2, C-10), 33.2 (CH, C-1), 31.9 (CH_3, C-17), 30.5 (CH_2, C-6), 29.9 (CH_2, C-5), 29.4 (CH_3, C-16), 19.9 (CH_3, C-18), 16.1 (CH_3, C-19); EI-LRMS *m/z* $[M]^+$ 388 (3), 330 (27), 329 (22), 271 (100), 255 (26), 215 (58), 201(30), 159 (31); EI-HRMS *m/z* calcd for $C_{22}H_{32}N_2S_2$ $[M]^+$ 388.2007, found 388.2007.

*8-Isothiocyano-15-isoselenocyano-11(20)-amphilectene (**3**) and 8-Isoselenocyano-15-isothiocyano-11(20)-amphilectene (**4**) (2:3 mixture of isomers)*: white solid; UV (MeOH) λ_{max} (ε) 192 (962), 202 (4457) nm; IR (film) ν_{max} 2920, 2870, 2255, 2096, 1453 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) (major isomer) δ 4.88 (br s, 1H, H-20β), 4.63 (br s, 1H, H-20α), 2.40–1.99 (br envelope, 6H, H-2β, H-5β, H-9β, H-10αβ, H-14β), 1.90 (m, 1H, H-1), 1.75 (m, 1H, H-12), 1.60–1.26 (br envelope, 5H, H-6αβ, H-7, H-9α, H-14α), 1.47 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.14–0.84 (br envelope, 5H, H-2α, H-3, H-4, H-5α, H-13), 1.00 (d, *J* = 6.4 Hz, 3H, H-19), 0.95 (d, *J* = 6.2 Hz, 3H, H-18); (minor isomer) δ 4.87 (br s, 1H, H-20β), 4.59 (br s, 1H, H-20α), 2.40–1.99 (br envelope, 6H, H-2β, H-5β, H-9β, H-10αβ, H-14β), 1.90 (m, 1H, H-1), 1.75 (m, 1H, H-12), 1.60–1.26 (br envelope, 5H, H-6αβ, H-7, H-9α, H-14α), 1.51 (s, 3H, H-17), 1.44 (s, 3H, H-16), 1.14–0.84 (br envelope, 5H, H-2α, H-3, H-4, H-5α, H-13), 0.99 (d, *J* = 6.3 Hz, 3H, H-19), 0.96 (d, *J* = 6.1 Hz, 3H, H-18); ^{13}C NMR (175 MHz,

$CDCl_3$) (major isomer) δ149.8 (C, C-11), 130.4 (C, C-22), 124.4 (C, C-21), 106.4 (CH_2, C-20), 71.0 (C, C-8), 60.6 (C, C-15), 56.7 (CH, C-13), 46.8 (CH_2, C-14), 46.6 (CH, C-12), 43.3 (CH, C-4), 42.3 (CH, C-7), 41.1 (CH_2, C-2), 39.8 (CH_2, C-9), 35.7 (CH, C-3), 33.9 (CH_2, C-10), 33.2 (CH, C-1), 31.9 (CH_3, C-17), 30.4 (CH_2, C-6), 29.8 (CH_2, C-5), 29.5 (CH_3, C-16), 19.8 (CH_3, C-18), 16.1 (CH_3, C-19); (minor isomer) δ 150.1 (C, C-11), 131.2 (C-21), 122.4 (C, C-22), 106.1 (CH_2, C-20), 69.6 (C, C-8), 61.3 (C, C-15), 56.9 (CH, C-13), 46.7 (CH, C-12), 46.5 (CH_2, C-14), 43.2 (CH, C-4), 42.5 (CH, C-7), 41.0 (CH_2, C-2), 40.1 (CH_2, C-9), 35.6 (CH, C-3), 34.0 (CH_2, C-10), 33.1 (CH, C-1), 31.6 (CH_3, C-17), 30.5 (CH_2, C-6), 29.9 (CH_2, C-5), 29.0 (CH_3, C-16), 19.9 (CH_3, C-18), 16.0 (CH_3, C-19); EI-LRMS *m/z* $[M]^+$ 436 (31), 330 (34), 329 (46), 271 (95), 270 (85), 255 (100), 215 (88), 201 (65), 159 (42); EI-HRMS *m/z* calcd for $C_{22}H_{32}N_2S^{80}Se$ $[M]^+$ 436.1451, found 436.1459. Multiple attempts to separate the mixture of regioisomers **3** and **4** by normal- and reversed-phase HPLC proved unsuccessful.

General Procedure for Synthesis of Isoselenocyanate-Containing Amphilectanes. *General Procedure A.* To a 25°C solution of diisocyanide **1** (12.0 mg, 0.04 mmol) in dry THF (2.0 mL) was added selenium (6.0 mg, 0.08 mmol) and TEA (0.07 mL, 0.5 mmol). After stirring for 24 h the reaction mixture was concentrated *in vacuo* and the crude oil obtained was purified by flash-silica gel CC using a 99:1 mixture of hexane/EtOAc to afford **5** as the sole product (14 mg, 78%).

General Procedure B. To a 25°C solution of diisocyanide **1** (16.0 mg, 0.05 mmol) in dry THF (2.0 mL) was added selenium (8.0 mg, 0.1 mmol) and TEA (0.1 mL, 0.7 mmol). After refluxing for 12 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo.* The crude oil obtained was purified by flash-silica gel CC using a 99:1 mixture of hexane/EtOAc to afford a fraction consisting of a mixture of two products that was subsequently purified by HPLC ($MeOH/H_2O$, 95/5). Retention times were 8.32 min for compound **5** (12.0 mg, 50%) and 9.29 min for compound **6** (6.0 mg, 33%).

*8,15-Diisoselenocyano-11(20)-amphilectene (**5**)*: colorless oil; $[\alpha]^{20}_{D}$ -139.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (ε) 202 (56143) nm; IR (film) ν_{max} 2921, 2870, 2258, 2110, 1455 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 4.89 (br s, 1H, H-20β), 4.60 (br s, 1H, H-20α), 2.38 (m, 1H, H-9β), 2.30 (m, 1H, H-10β), 2.24 (m, 1H, H-10α), 2.17 (m, 1H, H-2β), 2.10 (dd, *J* = 1.2, 14.7 Hz, 1H, H-14β), 2.02 (m, 1H, H-5β), 1.91 (m, 1H, H-1), 1.75 (t, *J* = 10.9 Hz, 1H, H-12), 1.52 (s, 3H, H-17), 1.49 (s, 3H, H-16), 1.48–1.32 (br envelope, 5H, H-6αβ, H-7, H-9α, H-14α), 1.14–1.02 (br envelope, 3H, H-3, H-4, H-13), 1.01 (d, *J* = 6.3 Hz, 3H, H-19), 0.96 (d, *J* = 6.3 Hz, 3H, H-18), 0.93–0.83 (br envelope, 2H, H-2α, H-5α); ^{13}C NMR (175 MHz, $CDCl_3$) δ 149.7 (C, C-11), 124.5 (C, C-21), 122.5 (C, C-22), 106.4 (CH_2, C-20), 70.9 (C, C-8), 61.3 (C, C-15), 56.6 (CH, C-13), 46.7(CH, C-12), 46.6 (CH_2, C-14), 43.3 (CH, C-4), 42.3 (CH, C-7), 41.0 (CH_2, C-2), 39.8 (CH_2, C-9), 35.6 (CH, C-3), 33.9 (CH_2, C-10), 33.2 (CH, C-1), 31.6 (CH_3, C-17), 30.4 (CH_2, C-6), 29.8 (CH_2, C-5), 29.1 (CH_3, C-16), 19.8 (CH_3, C-18), 16.1 (CH_3, C-19); EI-LRMS *m/z* $[M]^+$ 484 (28), 378 (23), 377 (21), 272 (45), 271 (100), 270 (86), 255 (97), 215 (94), 201 (82), 199 (65), 159 (68); EI-HRMS *m/z* calcd for $C_{22}H_{32}N_2{}^{80}Se_2$ $[M]^+$ 484.0896, found 484.0902.

*8-Isoselenocyanoamphilecta-11(20),15-diene (**6**)*: colorless oil; $[\alpha]^{20}_{D}$ + 70.0 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ε) 202 (9039) nm; IR (film) ν_{max} 2922, 2852, 2264, 2093, 1452 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 4.86 (s, 1H, H-20β), 4.76 (s, 1H, H-16β), 4.67 (s, 1H, H-16α), 4.66 (s, 1H, H-20α), 2.59 (d, *J* = 14.3 Hz, 1H, H-14β), 2.38 (m, 1H, H-9β), 2.29 (m, 2H, H-10), 2.01 (m, 1H, H-5β), 1.80 (m, 1H, H-2β), 1.76 (m, 2H, H-1, H-12), 1.72 (s, 3H, H-17), 1.54 (m, 3H, H-6αβ, H-14α), 1.41 (m, 2H, H-7, H-9α), 1.04 (m, 3H, H-3, H-4, H-13), 1.00 (d, *J* = 6.4 Hz, 3H, H-19), 0.90 (d, *J* = 5.9 Hz, 3H, H-18), 0.86 (m, 1H, 5α), 0.70 (m, 1H, 2α); ^{13}C NMR (175 MHz, $CDCl_3$) δ 149.7 (C, C-11), 144.2 (C, C-15), 123.8 (C, C-21), 111.3 (CH_2, C-16), 106.1 (CH_2, C-20), 70.9 (C, C-8), 56.7 (CH, C-13), 46.5 (CH, C-12), 43.6 (CH, C-4), 43.0 (CH_2, C-14), 42.3 (CH, C-7), 39.8 (CH_2, C-2), 39.6 (CH_2, C-9), 36.0 (CH, C-3), 33.9 (CH, C-1), 33.8 (CH_2, C-10), 30.5 (CH_2, C-6), 29.8 (CH_2, C-5), 22.6 (CH_3, C-17),

19.7 (CH_3, C-18), 16.1 (CH_3, C-19); EI-LRMS *m/z* $[M]^+$ 377 (17), 297 (7), 282 (18), 272 (42), 271 (100), 270 (83), 255 (94), 215 (81), 199 (60), 159 (55), 145 (48), 105 (52), 91 (59); EI-HRMS *m/z* calcd for $C_{21}H_{31}N^{80}Se$ $[M]^+$ 377.1622, found 377.1626.

Treatment of Diisoselenocyanate 5 with Elemental Sulfur to 8,15-Diisothiocyano-11(20)-amphilectene (2). To a 25° C solution of diisoselenocyanate **5** (7.0 mg, 0.01 mmol) in dry THF (2.0 mL) was added sulfur (0.9 mg, 0.03 mmol) and TEA (9.7 μL, 0.07 mmol). After refluxing for 4 h the reaction mixture was allowed to cool to 25° C and then concentrated *in vacuo*. The crude oil obtained was purified by flash-silica gel CC with a 98:2 mixture of hexane/EtOAc to afford **2** (4.0 mg, 71%) as the sole product.

Evaluation of Inhibition of *Plasmodium falciparum* Growth. The 3D7 and Dd2 strains of *P. falciparum* malaria (BEI Resources, MR4/ATCC, Manassas, VA) were cultured in human type O+ erythrocytes in complete medium consisting of RPMI 1640 (Cellgro), 0.043 mg/mL gentamicin (Gibco), 0.014 mg/mL hypoxanthine (Acros), 38.5 mM HEPES (Sigma), 0.18% sodium bicarbonate (Cellgro), 0.20% glucose (MP Biomedical), 0.003 mM NaOH (Sigma), 0.2% Albumax (Gibco), and 5% human serum as previously described.[5] Briefly, cultures were maintained in 25-cm^2 flasks (Corning) at a volume of 10 mL, gassed for 30 s with 3% CO_2, 1% O_2, and 96% N_2, and were finally incubated at 37°C. The antimalarial activity was determined with a SYBR Green based parasite proliferation assay as previously described.[6] After 72 h of incubation in the presence of serial dilutions of compounds, the increase of parasite DNA contained in human red blood cells was measured. The relative fluorescence values were measured using a Molecular Devices SpectraMAX Gemini EM fluorimeter (excitation 495 nm, and emission 525 nm). Data were analyzed using Microsoft Excel and were plotted using SigmaPlot 10 (Systat).

Evaluation of antitubercular activity. Antimycobacterial activity was determined against *Mtb* H_{37}Rv (ATCC 27 294) in the microplate Alamar blue assay (MABA) system as described previously.[7] The tuberculosis drug rifampicin (RMP) was used as a positive control in the assay.

Cytotoxicity Assay. The cytotoxic activity of tested compounds was determined with the Vero cell line ATCC CRL-1586 using an MTS assay as outlined previously.[8]

References

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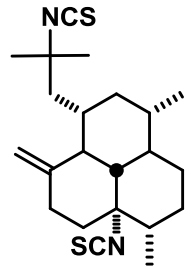
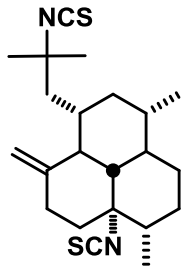
^{1}H NMR (700 MHz, $CDCl_3$)



2

^{13}C NMR (175 MHz, $CDCl_3$)



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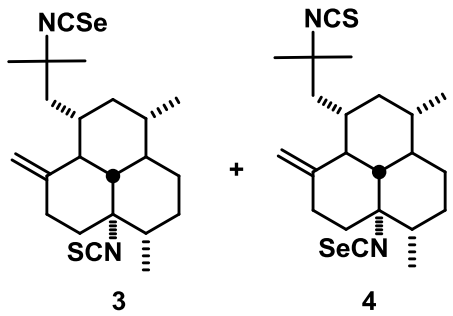

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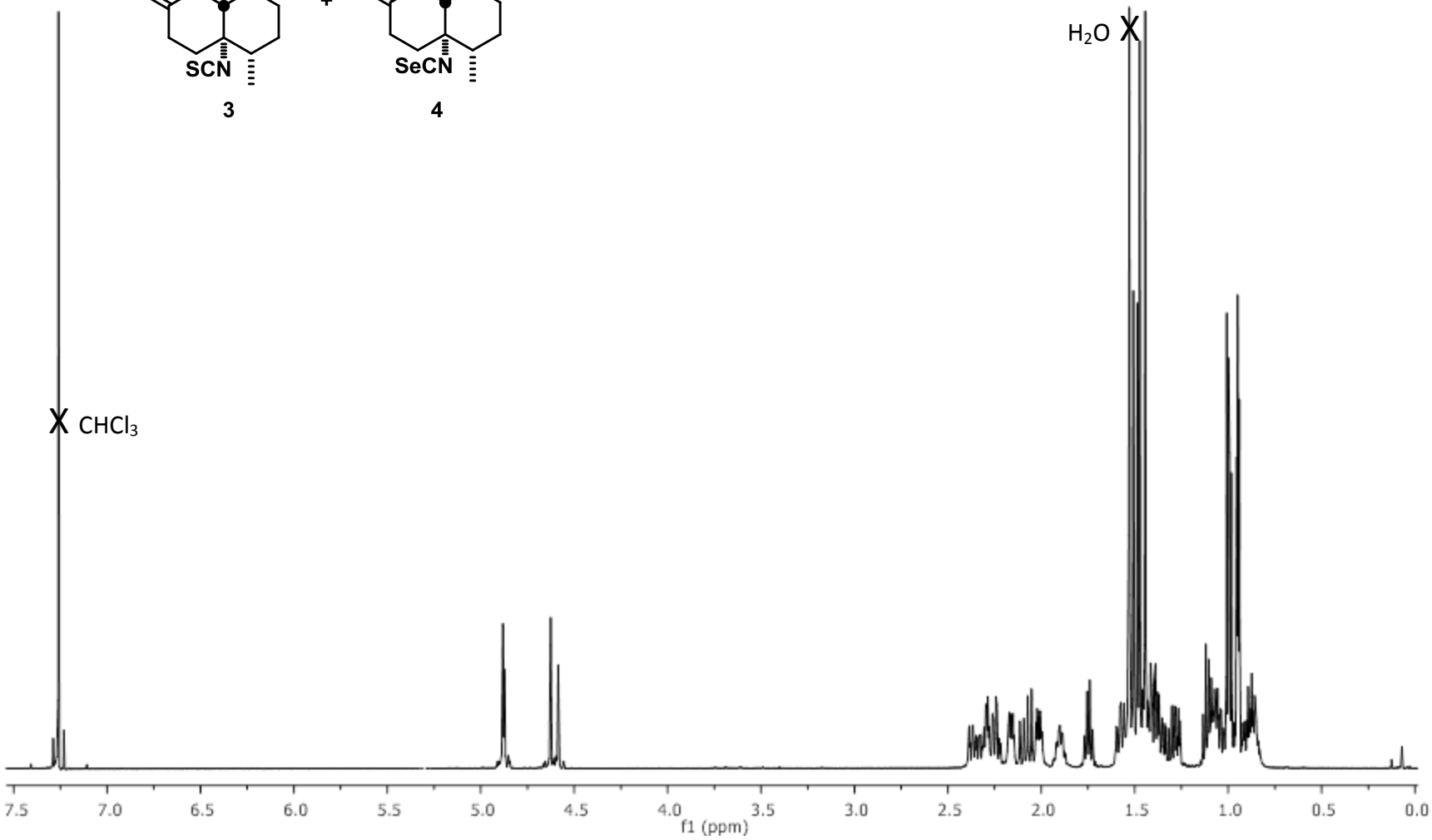
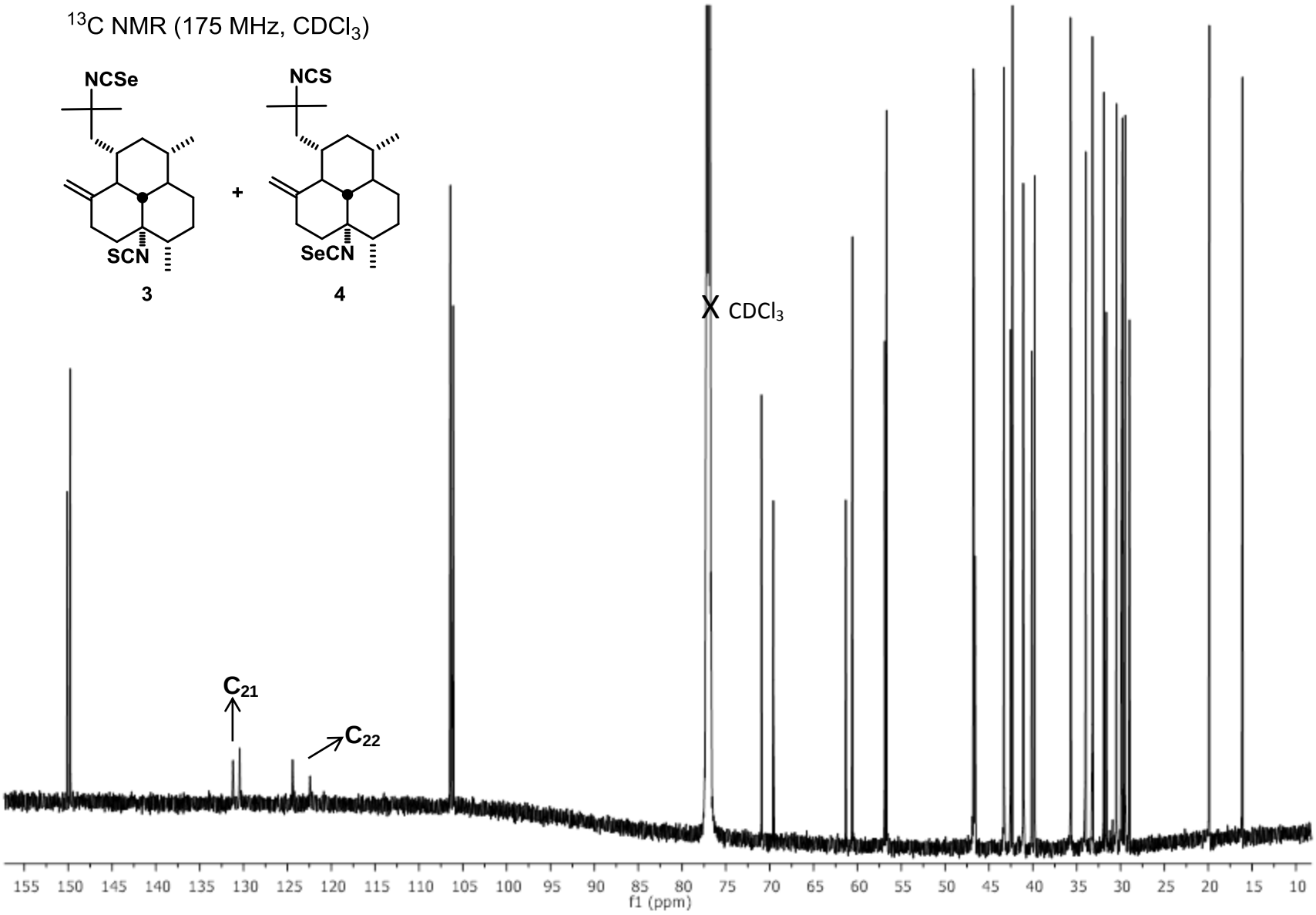
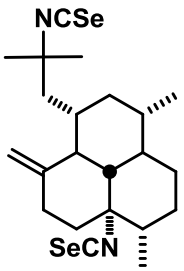



^{13}C NMR (175 MHz, $CDCl_3$)
NCSe
SCN
3
+
NCS
SeCN
4
X $CDCl_3$
C_{21}
C_{22}
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)

^{1}H NMR (700 MHz, $CDCl_3$)



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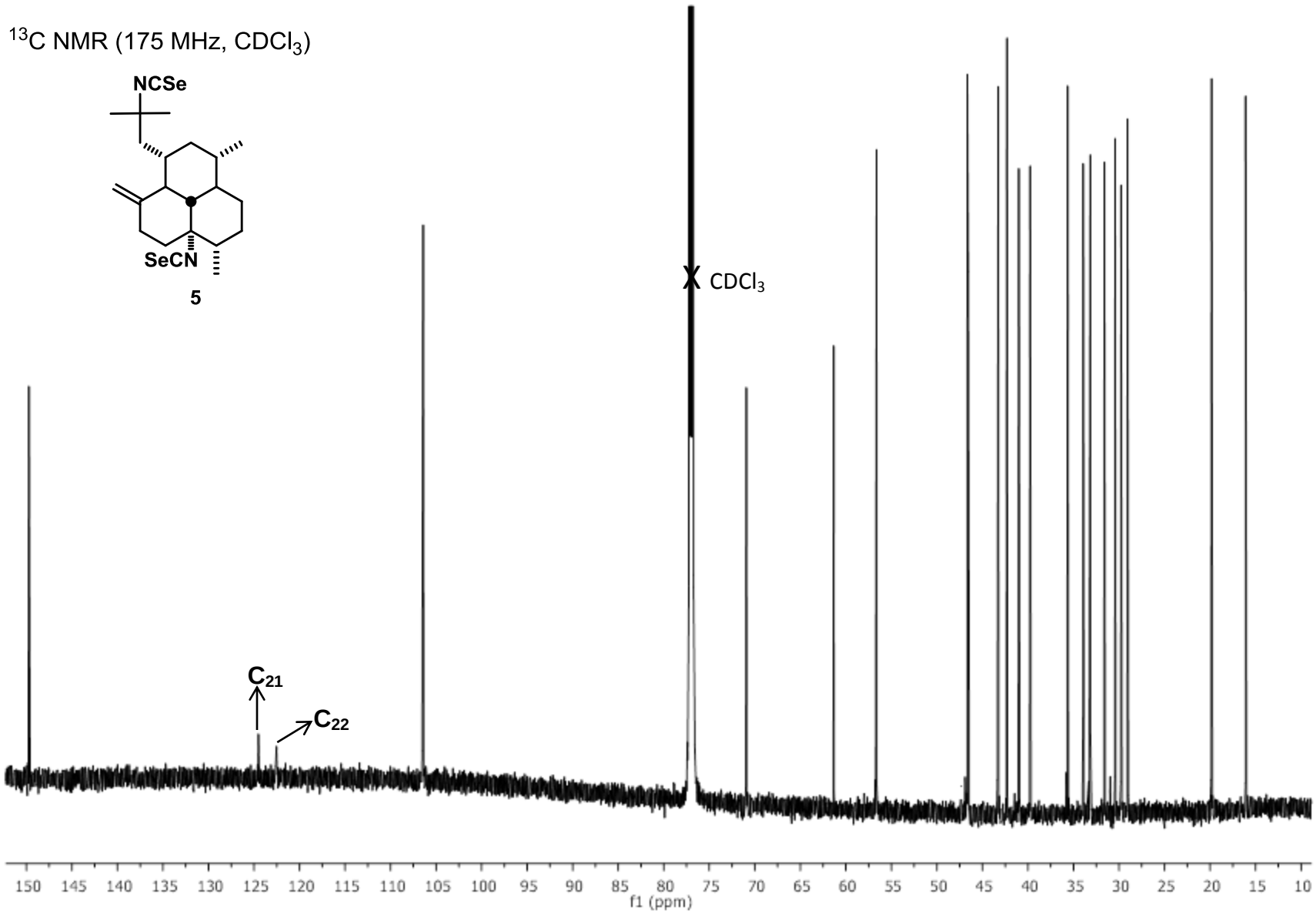
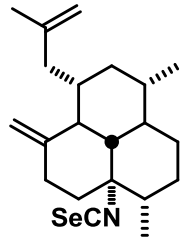

^{13}C NMR (175 MHz, $CDCl_3$)
NCSe
SeCN
5
$CDCl_3$
C_{21}
C_{22}
150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)

^{1}H NMR (700 MHz, $CDCl_3$)



6

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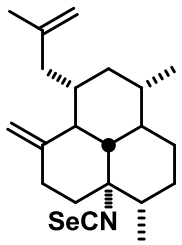

6

Elemental Composition Report: HREIMS

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
388.2007	388.2007	0.0	0.0	8.0	134.0	C22 H32 N2 S2[a]
436.1459	436.1451	0.8	1.8	9.0	9.4	C22 H32 N2 S 80Se[b]
484.0902	484.0896	0.6	1.2	10.0	1116.0	C22 H32 N2 80Se2[c]
377.1626	377.1622	0.4	1.1	8.0	58.8	C21 H31 N 80Se[d]

[a] Compound **2**, [b] Compounds **3:4**, [c] Compound **5**, [d] Compound **6**