Supporting Materials

On the Importance of the Thiazole Nitrogen in Epothilones: Semisynthesis and Microtubule-Binding Affinity of Deaza-Epothilone C

Adriana Edenharter, Lucie Ryckewaert, Daniela Cintulová, Juan Estévez-Gallego, José Fernando Díaz, and Karl-Heinz Altmann*

Synthesis protocols and analytical data.

Stability assessment of deaza-Epo C (5) in cell culture medium.

Synthesis protocols and analytical data

General Methods

All non-aqueous reactions were performed under an argon atmosphere using flame-dried glassware and standard syringe/septa techniques.

Dichlormethane (DCM), tetrahydrofuran (THF) and diethylether (Et_2O) used for reactions were distilled under argon prior to use (DCM from CaH_2, THF and Et_2O from Na/benzophenone). All other absolute solvents were purchased as anhydrous grade from Fluka (puriss.; dried over molecular sieves; H_2O <0.005%) and used without further purification unless otherwise stated. Solvents for extractions, flash column chromatography and thin layer chromatography (TLC) were purchased as commercial grade and distilled prior to use. All other commercially available reagents were used without further purification unless otherwise stated. Reactions were magnetically stirred and monitored by TLC performed on Merck TLC aluminum sheets (silica gel 60 F254). Spots were visualized with UV light (λ = 254 nm, rarely λ = 366 nm) or through staining with $KMnO_4/K_2CO_3$, rarely with $Ce_2(SO_4)_3$/phosphomolybdic acid/H_2SO_4 (CPS) or anisaldehyde/AcOH/H_2SO_4/EtOH. Chromatographic purification of products was performed using Fluka silica gel 60 or Silicycle Silia Flash® for preparative column chromatography (particle size 40-63 µm).

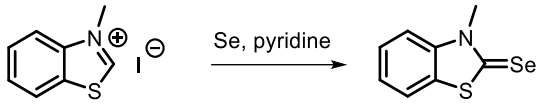
^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AV-400 400 MHz or on a Bruker AV-500 500 MHz spectrometer at room temperature. Chemical shifts (δ) are reported in ppm and are referenced to residual solvents peaks in deuterated chloroform (δ 7.26 ppm for ^{1}H, δ 77.0 ppm for ^{13}C) or DMSO-d_6 (δ 2.50 ppm for ^{1}H, δ 39.5 ppm for ^{13}C). All ^{13}C-NMR spectra were measured with complete proton decoupling. Data for NMR spectra are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintett, m = multiplet, J = coupling constant in Hz.

Infrared spectra (IR) were recorded on a Jasco FT/IR-6200 instrument. Resonance frequencies are given as wavenumbers in cm^{-1}. Data are reported as follows: w=weak, m=middle, s=strong.

Optical rotations were measured on a Jasco P-1020 polarimeter or on an Anton-Paar MCP 300 at the sodium D line with a 10 or 100 mm path length cell in $CHCl_3$ unless otherwise noted and are reported as follows: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: (concentration (g/100 mL)).

High resolution mass spectra (HRMS) were recorded on a Bruker maXis (ESI) or on Waters' AutoSpec Ultima spectrometer (EI), respectively, by the ETH Zürich MS service (Louis Bertschi, Rolf Häfliger and Oswald Greter under the direction of Dr. Xiangyang Zhang).

Preparative HPLC was performed on a device by Gilson equipped with a Waters SymmetryPrep C18 column (5 µm, 19x100 mm) at room temperature.



10a **10**

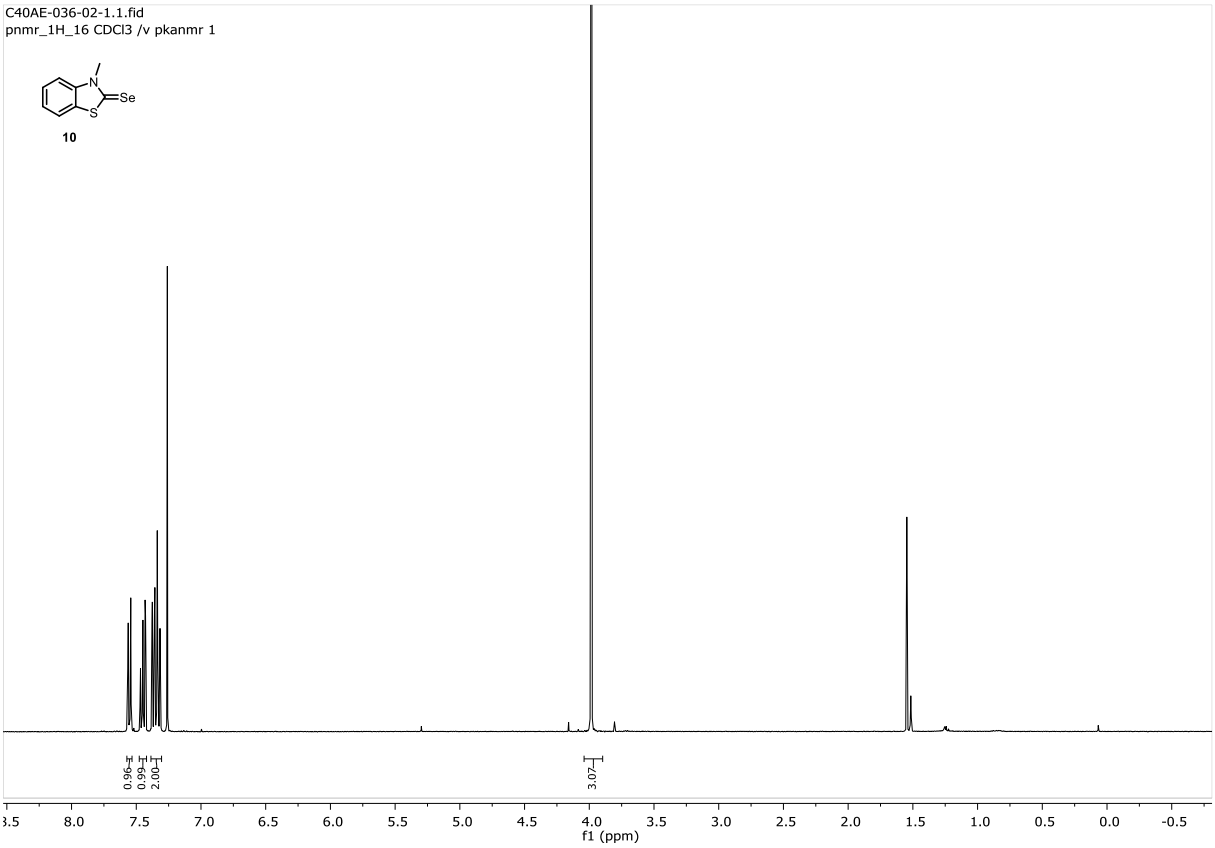
3-Methylbenzo[d]thiazole-2(3H)-selenone (10): A brown suspension of **10a** (3.00 g, 10.8 mmol, 1 eq) and selenium (855 mg, 10.8 mmol, 1 eq) in dry pyridine (10 mL) was refluxed (oil bath temperature: 140 °C) for 2.5 h. The reaction mixture was cooled to RT and then filtered to remove unreacted selenium. The mixture was poured into cold water (50 mL), forming an orange precipitate which was filtered and recrystallized in EtOH (70 mL) to obtain 1.73 g of **10** of yellow crystals.

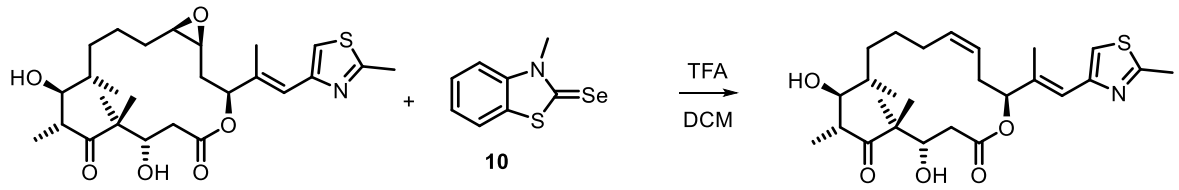
Yield: 1.91 g (77%);

R_f = 0.34 (DCM/acetone = 9:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.55 (ddd, *J* = 7.8, 1.3, 0.6 Hz, 1H), 7.45 (ddd, *J* = 8.1, 7.4, 1.3 Hz, 1H), 7.40 – 7.29 (m, 2H), 3.99 (s, 3H).

This compound has been described in Calo, V.; Lopez, L.; Mincuzzi, A.; Pesce, G. *Synthesis* **1976**, 200.


C40AE-036-02-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 1
N
Se
S
10
0.96
0.99
2.00
3.07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



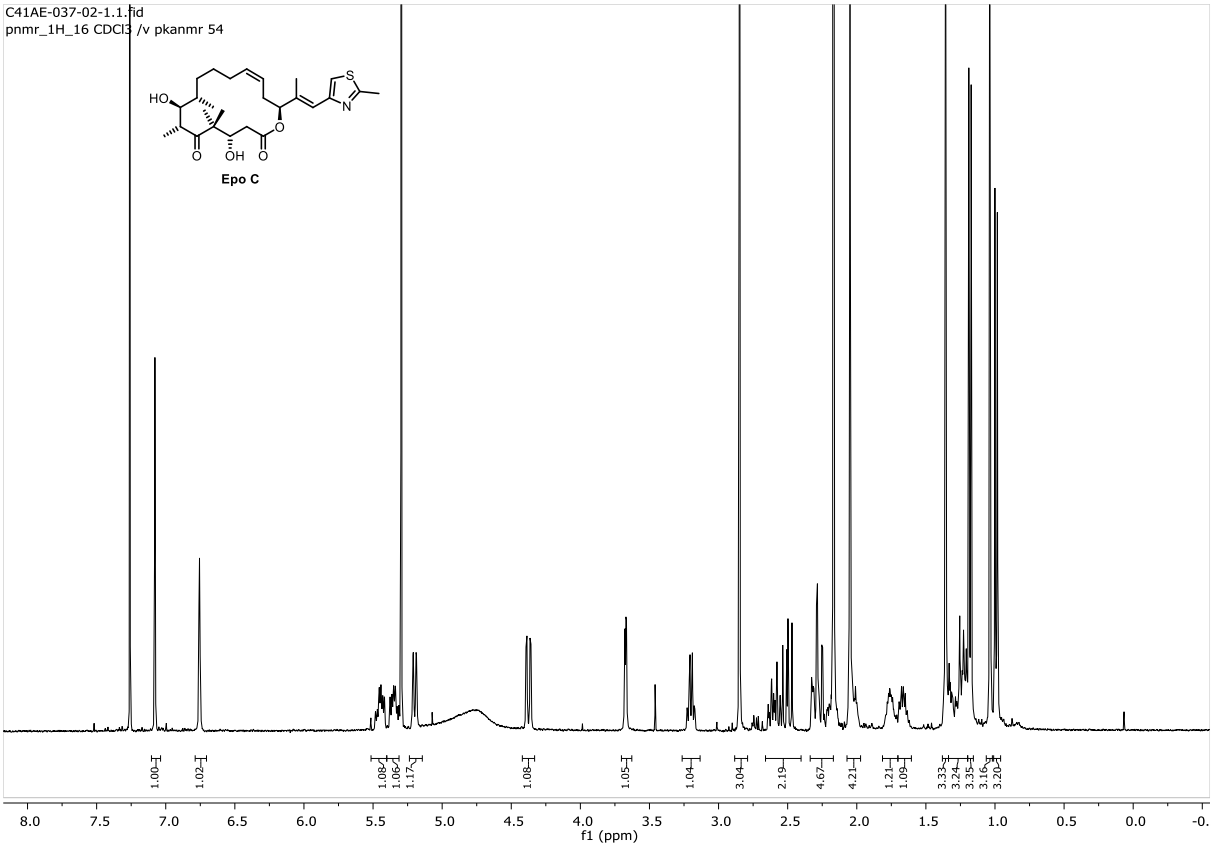
(4S,7R,8S,9S,16S,Z)-4,8-Dihydroxy-5,5,7,9-tetramethyl-16-((E)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl)oxacyclohexadec-13-ene-2,6-dione (Epo C): An orange solution of **Epo A** (1.5 g, 3.04 mmol, 1.0 eq), **10** (693 mg, 3.04 mmol, 1.0 eq) and TFA (1.37 mL, 9.12 mmol, 3.0 eq) in DCM (7.5 mL) was stirred at RT for 3 h. First a brown suspension was formed, which turned redder over time. TLC (DCM/acetone = 4:1) of the red-brown suspension indicated that there was **Epo A** left. Therefore, **10** (150 mg, 0.657 mmol, 0.22 eq) was added. 2 h later TLC still indicated no complete conversion, so TFA (0.5 mL, 3.32 mmol, 1.1 eq) was added once more. Another hour later TLC looked the same so the reaction was stopped. The reaction mixture was concentrated under reduced pressure. The crude was purified by flash column chromatography (DCM/acetone = 9:1 to 4:1, 60 g, 3 cm, 30 mL) yielding 628 mg (43%) of **Epo C** as a slightly yellow oil and 707 mg a 1:1 mixture of **Epo A** and **Epo C** as a slightly yellow solid.

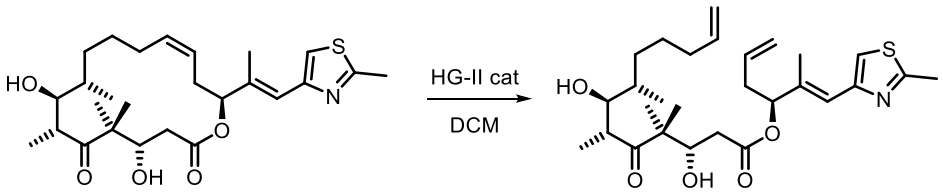
Yield: 628 mg (43%);

R_f = 0.62 (DCM/acetone = 4:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.08 (s, 1H), 6.76 (s, 1H), 5.45 (td, *J* = 10.4, 5.0 Hz, 1H), 5.34 (td, *J* = 10.1, 4.9 Hz, 1H), 5.20 (d, *J* = 9.1 Hz, 1H), 4.38 (dd, *J* = 11.6, 2.8 Hz, 1H), 3.67 (dd, *J* = 4.1, 1.7 Hz, 1H), 3.20 (qd, *J* = 6.8, 1.7 Hz, 1H), 2.85 (s, 3H), 2.66 – 2.54 (m, 1H), 2.50 (dd, *J* = 15.2, 11.6 Hz, 1H), 2.34 – 2.13 (m, 3H), 2.07 – 1.97 (m, 4H), 1.81 – 1.71 (m, 1H), 1.71 – 1.61 (m, 1H), 1.36 (s, 3H), 1.34 – 1.20 (m, 3H), 1.18 (d, *J* = 6.8 Hz, 3H), 1.04 (s, 3H), 0.99 (d, *J* = 7.1 Hz, 3H).

This compound has been described in: Altmann, K.-H.; Bold, G.; Caravatti, G.; End, N.; Flörsheimer, A.; Guagnano, V.; O'Reilly, T.; Wartmann, M. *Chimia* **2000**, *54*, 612.


C41AE-037-02-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 54
HO
O
OH
O
O
S
N
Epo C
1.00
1.02
1.08
1.06
1.17
1.08
1.05
1.04
3.04
2.19
4.67
4.21
1.21
1.09
3.33
3.24
3.35
3.16
3.20
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.
f1 (ppm)



Epo C **9**

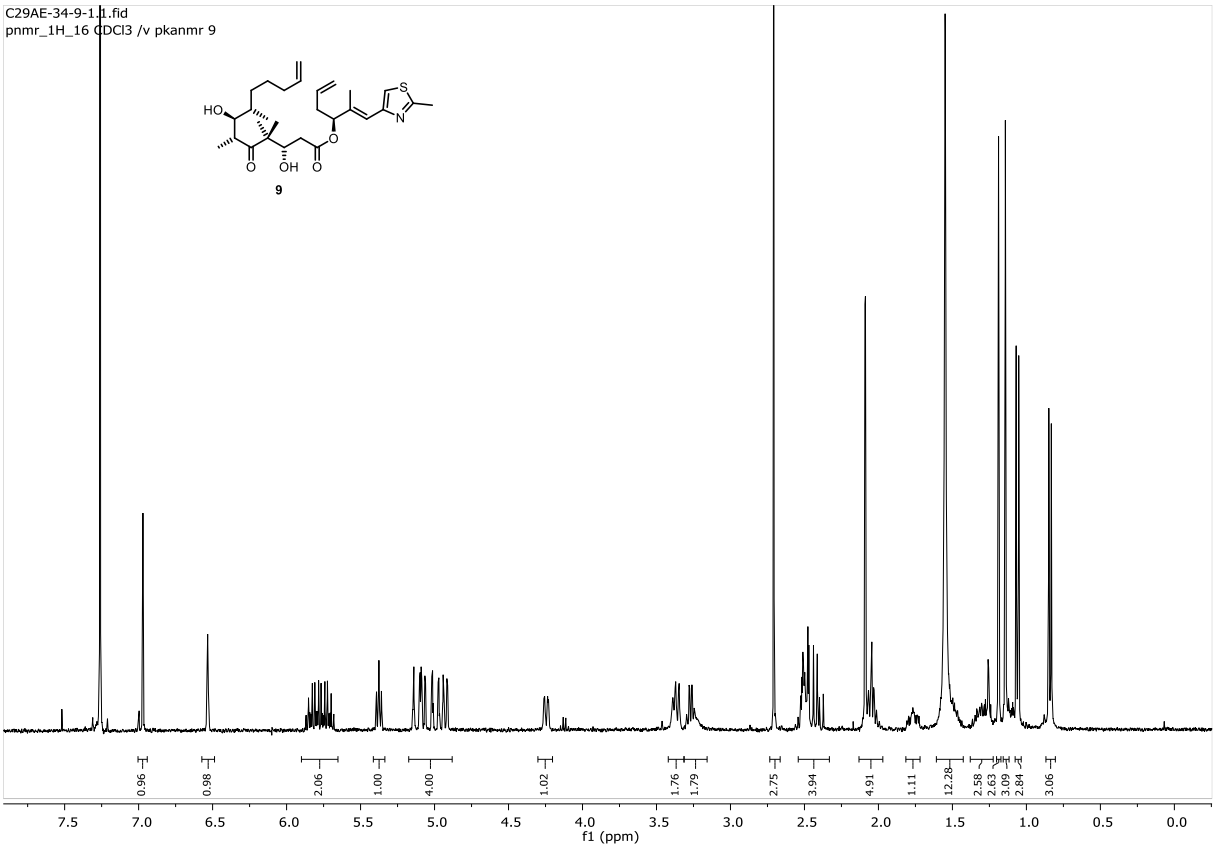
(3S,6R,7S,8S)-(S,E)-2-Methyl-1-(2-methylthiazol-4-yl)hexa-1,5-dien-3-yl 3,7-dihydroxy-4,4,6,8-tetra-methyl-5-oxotridec-12-enoate (**9**): Ethylene was bubbled through a green solution of **Epo C** (628 mg, 1.31 mmol, 1.0 eq) and Grubbs-Hoveyda (90.6 mg, 0.145 mmol, 0.11 eq) in DCM (300 mL) with a balloon for about 5 min, then the mixture was stirred under an ethylene atmosphere. After about 10 min the green solution turned to a brownish green. After stirring for 19 h at RT, TLC (DCM/acetone = 9:1) indicated almost no starting material anymore. The mixture was concentrated under reduced pressure. Purification by flash column chromatography (DCM/acetone = 20:1 to 9:1, 40 g, 2 cm, 30 mL) yielded 408.6 mg (61%) of **9** as a brown oil.

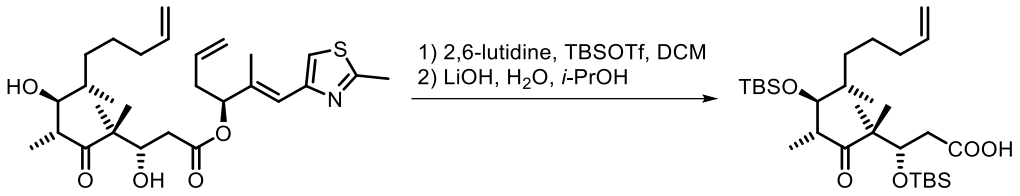
Yield: 409 mg (61%);

R_f = 0.55 (DCM/acetone = 9:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.97 (s, 1H), 6.56 – 6.50 (m, 1H), 5.77 (ddt, *J* = 16.9, 10.3, 6.6 Hz, 2H), 5.37 (ddd, *J* = 7.2, 6.3, 1.0 Hz, 1H), 5.17 – 4.88 (m, 4H), 4.24 (dd, *J* = 10.1, 2.4 Hz, 1H), 3.41 – 3.21 (m, 3H), 2.70 (s, 3H), 2.55 – 2.34 (m, 4H), 2.12 – 1.97 (m, 5H), 1.83 – 1.70 (m, 1H), 1.59 – 1.42 (m, 3H), 1.39 – 1.26 (m, 1H), 1.19 (s, 3H), 1.14 (s, 3H), 1.06 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H).

This compound has been described in: Karama, U.; Höfle, G. *Eur. J. Org. Chem.* **2003**, 1042.


C29AE-34-9-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 9
HO
O
OH
O
O
S
N
9
0.96
0.98
2.06
1.00
4.00
1.02
1.76
1.79
2.75
3.94
4.91
1.11
12.28
2.58
2.63
3.09
2.84
3.06
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



9 **8**

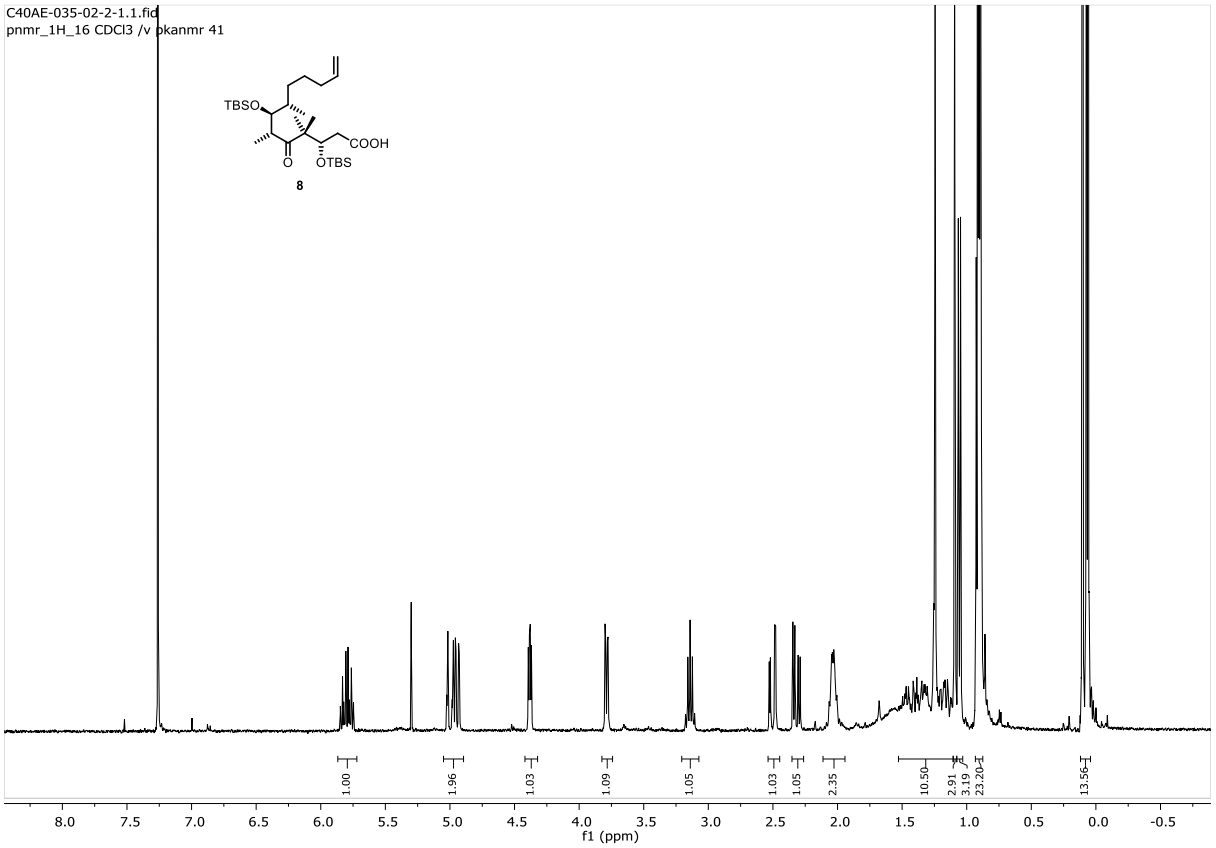
(3S,6R,7S,8S)-3,7-Bis((tert-butyldimethylsilyl)oxy)-4,4,6,8-tetramethyl-5-oxotridec-12-enoic acid (8): A slightly brown solution of **9** (40.3 mg, 0.0797 mmol, 1.0 eq) and 2,6-lutidine (176 µL, 1.20 mmol, 15 eq) in DCM (1.5 mL) was stirred in a microwave vial for 10 min, then TBSOTf (137 µL, 0.598 mmol, 7.5 eq) was added and the mixture was irradiated at 110 °C for 5 min (30 s pre-stirring, absorption high). TLC (DCM/acetone = 9:1) of the slightly brown solution indicated the consumption of **9**. The mixture was evaporated under reduced pressure. The crude was purified by flash column chromatography (DCM/acetone = 9:1, 5 g, 1 cm, 6 mL) yielding the TBS protected acid as a colourless oil, which was then stirred in a microwave vial with $LiOH \cdot H_2O$ (76.9 mg, 1.83 mmol, 23 eq) in water (0.9 mL) and isopropanol (0.6 mL) for 5 min at 150 °C (30 s pre-stirring, adsorption very high) to deprotect the acid. The yellow emulsion was diluted with saturated aqueous NH_4Cl solution (20 mL) and EtOAc (20 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were concentrated under reduced pressure. The crude was purified by flash column chromatography (DCM/MeOH = 97:3, 6 g, 1 cm, 3 mL) yielding 24.6 mg of **8** as a yellow oil.

Yield: 24.6 mg (57%);

R_f = 0.33 (DCM/MeOH = 94:6);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.80 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.05 – 4.91 (m, 2H), 4.38 (dd, *J* = 6.1, 3.4 Hz, 1H), 3.79 (dd, *J* = 7.3, 2.0 Hz, 1H), 3.14 (quint, *J* = 6.9 Hz, 1H), 2.53 – 2.45 (m, 1H), 2.31 (dd, *J* = 16.5, 6.2 Hz, 1H), 2.09 – 1.98 (m, 2H), 1.51 – 1.26 (m, 3H), 1.25 (s, 3H), 1.23 – 1.11 (m, 2H), 1.09 (s, 3H), 1.06 (d, *J* = 6.9 Hz, 3H), 0.95 – 0.83 (m, 21H), 0.16 – 0.03 (m, 12H).

This compound has been described in: Karama, U.; Höfle, G. *Eur. J. Org. Chem.* **2003**, 1042.


C40AE-035-02-2-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 41
TBSO
COOH
O
OTBS
8
1.00
1.96
1.03
1.09
1.05
1.03
1.05
2.35
10.50
2.91
3.19
23.20
13.56
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

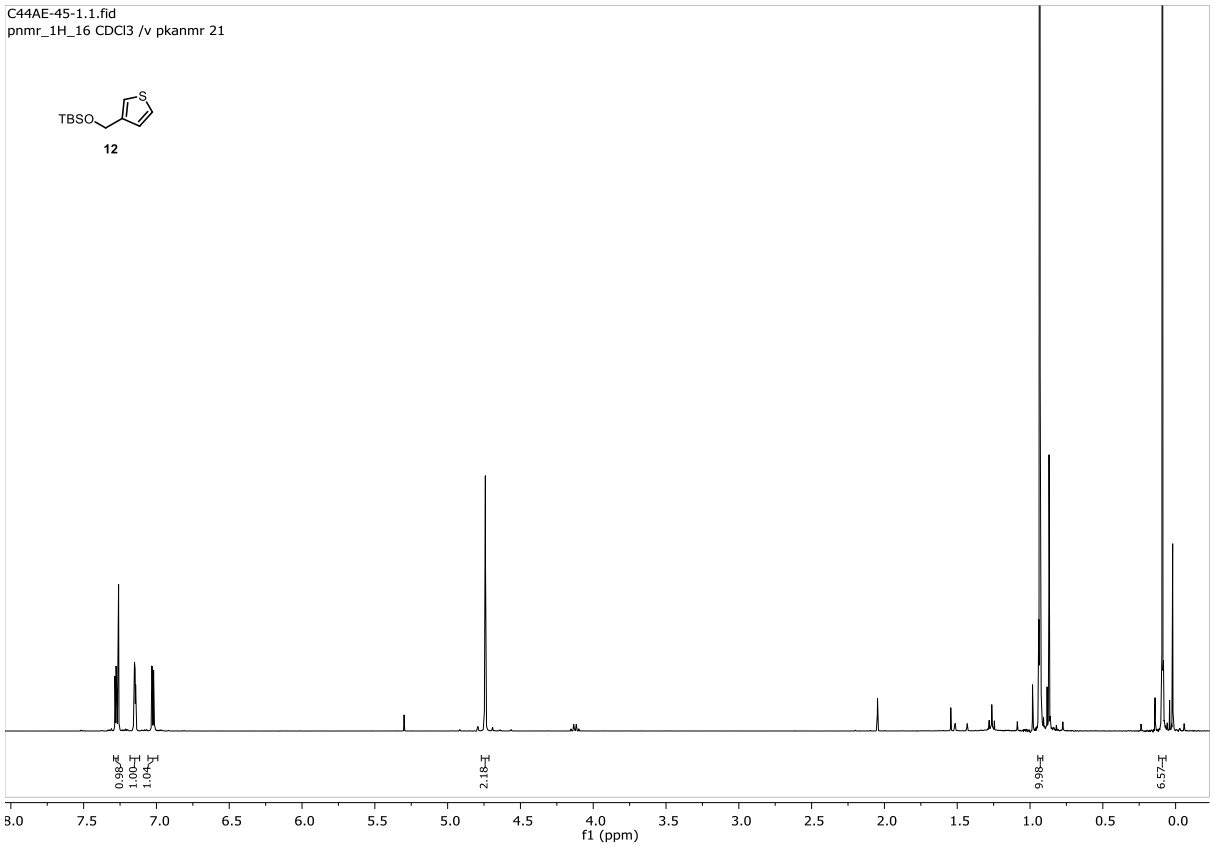
11 **12**

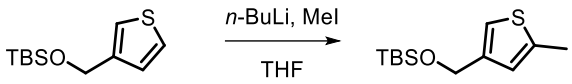
tert-Butyldimethyl(thiophen-3-ylmethoxy)silane (12): A yellow solution of thiophene-3-methanol (**11**) (5.0 g, 43.8 mmol, 1.0 eq), TBSCl (9.90 g, 65.7 mmol, 1.5 eq) and imidazole (8.94 g, 131 mmol, 3.0 eq) in DMF (60 mL) was stirred for 1 h at RT and for 5 h at 45 °C. TLC (hexane/Et_2O = 4:1) indicated the consumption of **11**. The brown solution was diluted with Et2O (150 mL) and washed with water (150 mL). The aqueous layer was extracted with Et_2O (3 x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/Et_2O = 9:1, 200 g, 5 cm, 50 mL) to obtain 11.2 g of **12** as a slightly yellow liquid that was slightly contaminated with residual TBS-OH.

Yield: 11.2 g (quant);

R_f = 0.85 (Hex/AcOEt = 4:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.27 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.16 – 7.14 (m, 1H), 7.02 (dd, *J* = 5.0, 1.2 Hz, 1H), 4.74 (d, *J* = 1.1 Hz, 2H), 0.93 (s, 9H), 0.09 (s, 6H).


C44AE-45-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 21
TBSO
S
12
0.98
1.00
1.04
2.18
9.98
6.57
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



12 **13**

tert-Butyldimethyl((5-methylthiophen-3-yl)methoxy)silane (13): n-BuLi (1.6 M in hexane, 55.2 mL, 88.83 mmol) was added dropwise over 75 min to a solution of **12** (18.29 g, 80.18 mmol) in THF (395 mL) (temperature between -35 °C and -30 °C). 30 min after completion of the n-BuLi addition, iodomethane (22.70 g, 160 mmol) was added to the solution dropwise over a period of 45 min; the mixture was then allowed to warm to rt overnight. 200 ml of water and 300 ml of ether were added with ice-cooling, the phases were separated and the aqueous phase was washed with 200 ml of ether. The combined organic phases were washed with 200 ml of water and the aqueous washing was re-extracted with ether. The combined organic phases were dried over $MgSO_4$, filtered, and the filtrate evaporated. Purification of the residue by flash chromatography gave 12.05 g (62 %) of the desired isomer **13** and 1.81 g (9%) of its 2-methyl isomer.

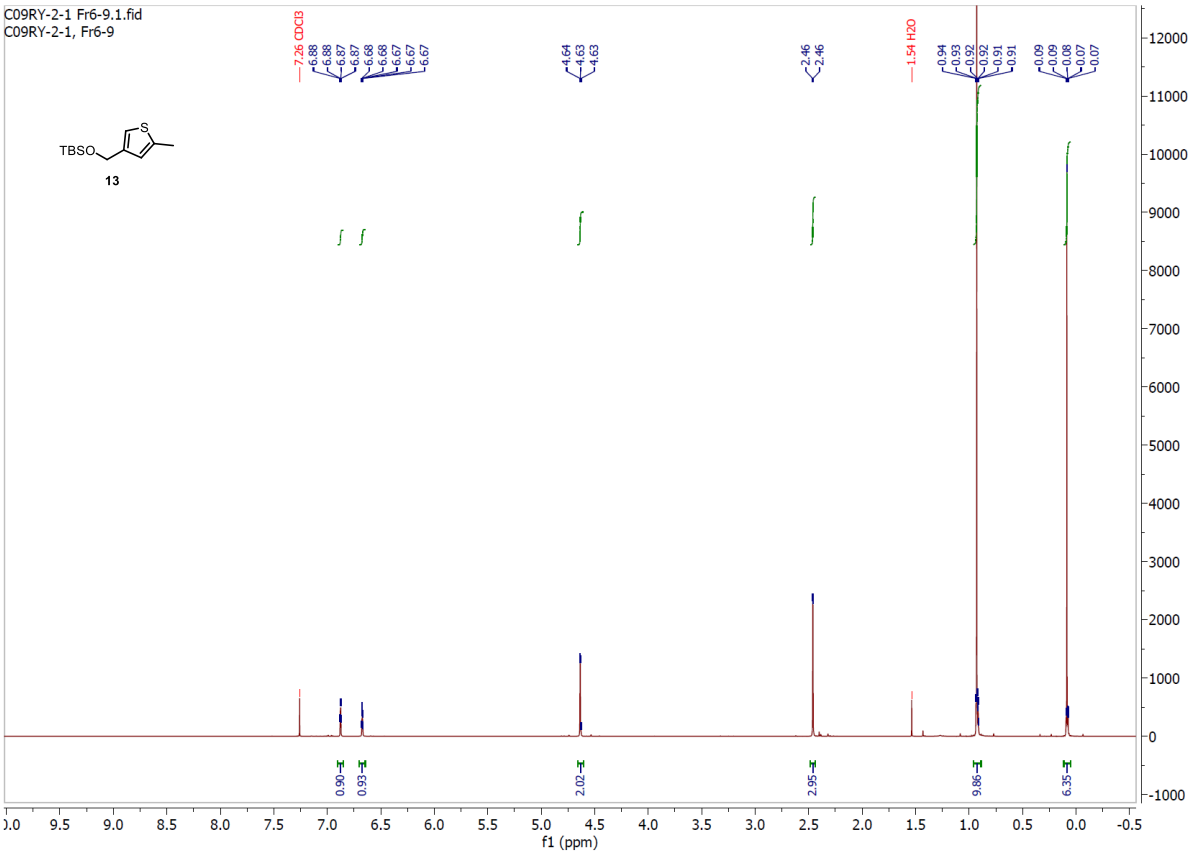
Yield: 12.05 g (62%);

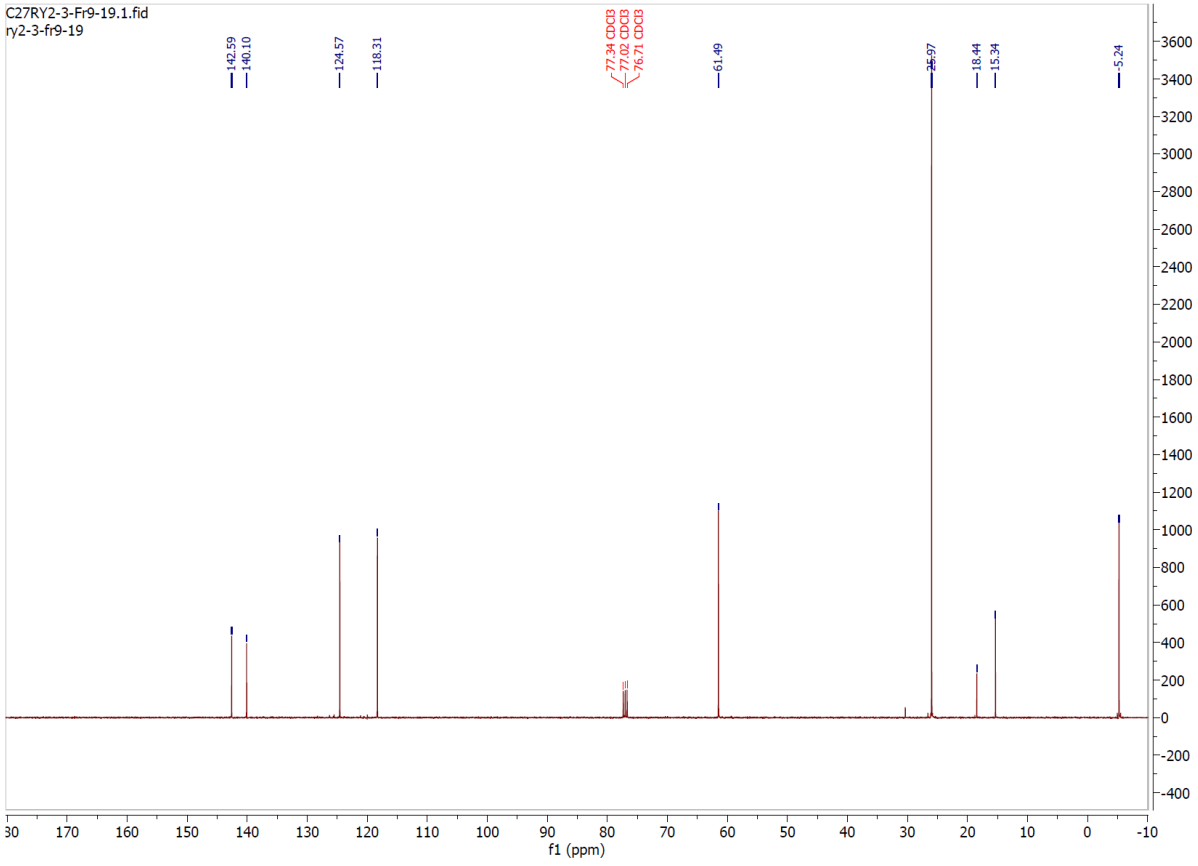
R_f = 0.87 (Hex/AcOEt = 4:1);

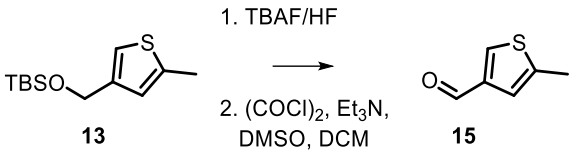
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.88 (q, *J* = 1.2 Hz, 1H), 6.67 (quint, *J* = 1.1 Hz, 1H), 4.64 (d, *J* = 1.1 Hz, 2H), 2.46 (d, *J* = 1.1 Hz, 3H), 0.92 (s, 9H), 0.08 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 142.59, 140.10, 124.57, 118.31, 61.49, 25.97, 18.44, 15.34, -5.24.

MS (ESI^+): m/z= 250.0 $[M+Li]^+$, m/z= 266.1 $[M+Na]^+$


C09RY-2-1 Fr6-9.1.fid
C09RY-2-1, Fr6-9
TBSO
S
13
7.26 CDCl3
6.88
6.87
6.68
6.67
4.64
4.63
2.46
1.54 H2O
0.94
0.93
0.92
0.91
0.09
0.08
0.07
0.90
0.93
2.02
2.95
9.86
6.35
f1 (ppm)


C27RY2-3-Fr9-19.1.fid
ry2-3-fr9-19
142.59
140.10
124.57
118.31
77.34 CDCl3
77.02 CDCl3
76.71 CDCl3
61.49
25.97
18.44
15.34
-5.24
f1 (ppm)



13 **15**

5-Methylthiophene-3-carbaldehyde (15):

A solution of **13** (4.15 g, 17.14 mmol) and TBAF (5.40 g, 17.14 mmol) in THF (50 mL) was stirred at RT for 21 h. 160 ml of sat. aqu. NH_4Cl and 320 ml of ether were added, the layers were separated and the aqueous phase was extracted with 320 ml of ether. The combined organic phases were washed with 160 ml of sat. aqu. $NaHCO_3$ and the aqueous layer was re-extracted with Et_2O (2 x 50 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH 95:5) to yield 1.95 g of **15**. Mixed fraction were re-chromatographed with DCM/MeOH 97:3 to yield additional 290 mg of material that was slightly contaminated with TBS-residues.

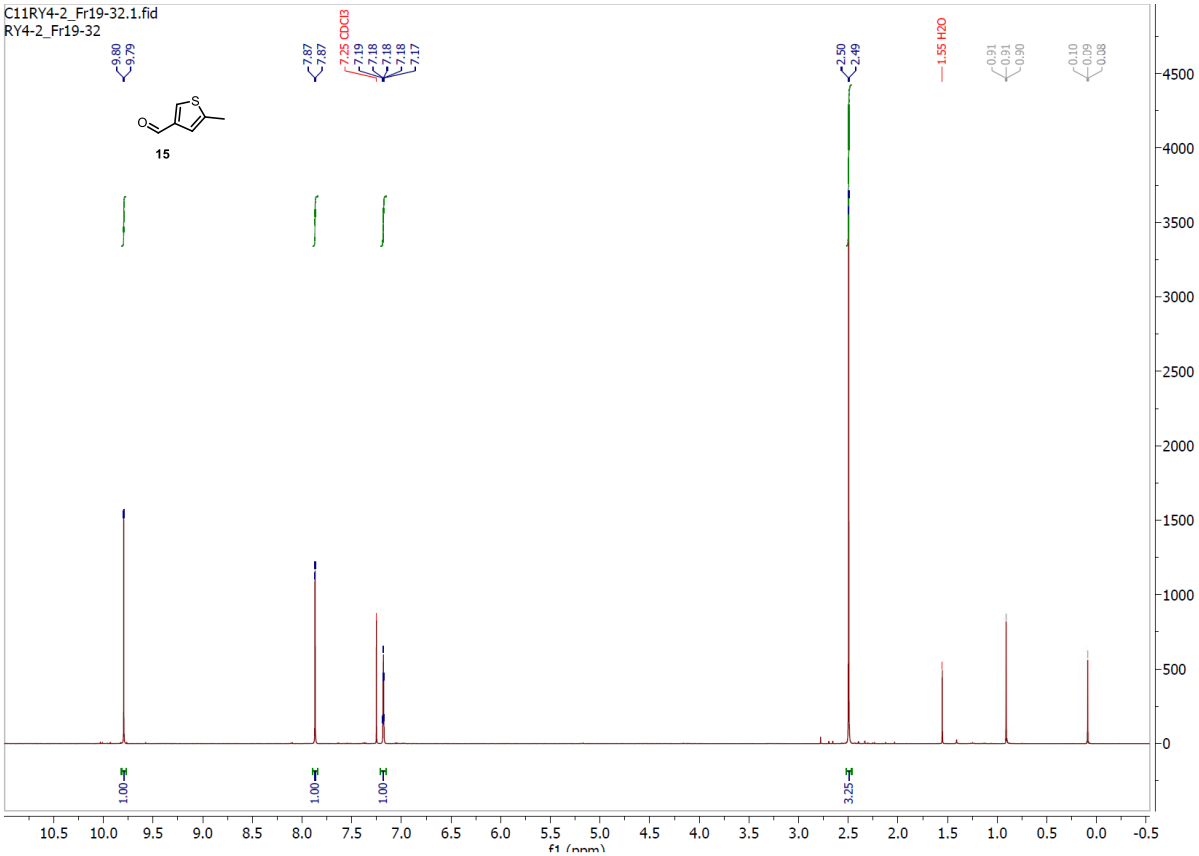
A solution of 3.08 g (33.58 mmol) of oxalylchloride in 57 ml of DCM was cooled to -73 °C and a solution of 3.47 ml of DMSO (48.92 mmol) in 10 ml of DCM (+2 mL for rinsing) was added dropwise over a period of 35 min. After 15 more min, a solution of the combined material from the above chromatographic runs (2.24 g, 17.47 mmol) in 73 ml of DCM was added dropwise at a temperature between -66 °C and -74 °C. After 2 h at -73 °C, 16.4 ml (117.92 mmmol) of triethylamine were added dropwise. 10 min after the end of the addition, the temperature was increased to -10 °C and 120 ml of water and 150 mL of DCM were added. The phases were separated and the organic phase was washed with 200 ml of brine, while the aqueous phase was extracted with 150 ml of DCM. The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated. Purification of the residue by flash chromatography (hexane/EtOAc 9:1) yielded 1.57 g (71 %) of aldehyde **15**.

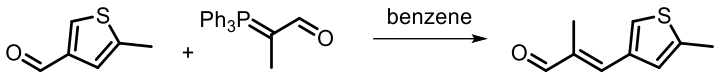
Yield: 1.57 g (71%)

R_f = 0.48 (Hex/AcOEt = 4:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.80 (d, J= 0.4 Hz, 1H), 7.87 (d, *J* = 1.4 Hz, 1H), 7.18 (quint, *J* = 1.3 Hz, 1H), 2.50 (d, *J* = 1.1 Hz, 3H).

The data are in full agreement with those reported by Spring and co-workers, who prepared **15** from 4-bromo-2-methyl thiophene: Strizhak, A. V., Sharma, K., Babii, O., Afonin, S., Ulrich, A. S., Komarov, I. V., Spring, D. R. *Org. Biomol. Chem.* **2018**, *16*, 8559.


C11RY4-2_Fr19-32.1.fid
RY4-2_Fr19-32
9.80
9.79
7.87
7.87
7.25 CDCl3
7.19
7.18
7.18
7.18
7.17
2.50
2.49
1.55 H2O
0.91
0.91
0.90
0.10
0.09
0.08
O
S
15
1.00
1.00
1.00
3.25
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



15 **16** **17**

(E)-2-Methyl-3-(5-methylthiophen-3-yl)acrylaldehyde (17): A yellow suspension of aldehyde **15** (1.64 g, 13 mmol, 1.0 eq) and 2-(triphenyl-phosphanylidene)propionaldehyde (**16**) (4.14 g, 13 mmol, 1.0 eq) in benzene (40 mL) was heated to reflux (bath temperature 103 °C) for 20 h. The mixture was then concentrated under reduced pressure and the residue was directly submitted to flash chromatography (hexane/Et_2O 6:1) to yield 1.53 g of material that still contained substantial amounts of starting aldehyde. The mixture was thus resubmitted to Wittig reaction with 1.76 g (5.52 mmol) of 2-(triphenyl-phosphanylidene)propionaldehyde (**16**) in benzene (28 mL). After 20 h at reflux the mixture was worked up as above to yield 1.53 g (71%) of aldehyde **17** as a yellow solid (after flash column chromatography).

Yield: 1.53 g (71%);

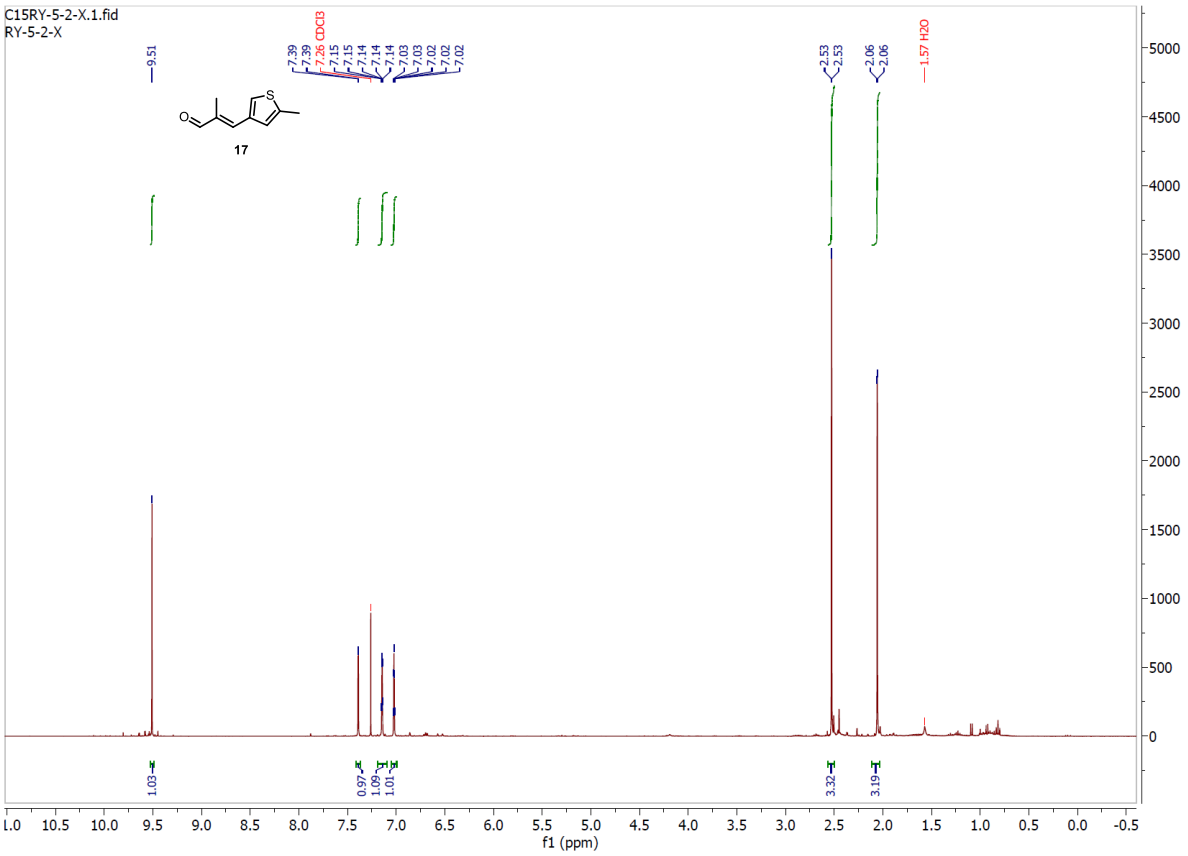
R_f = 0.49 (Hex/AcOEt = 4:1);

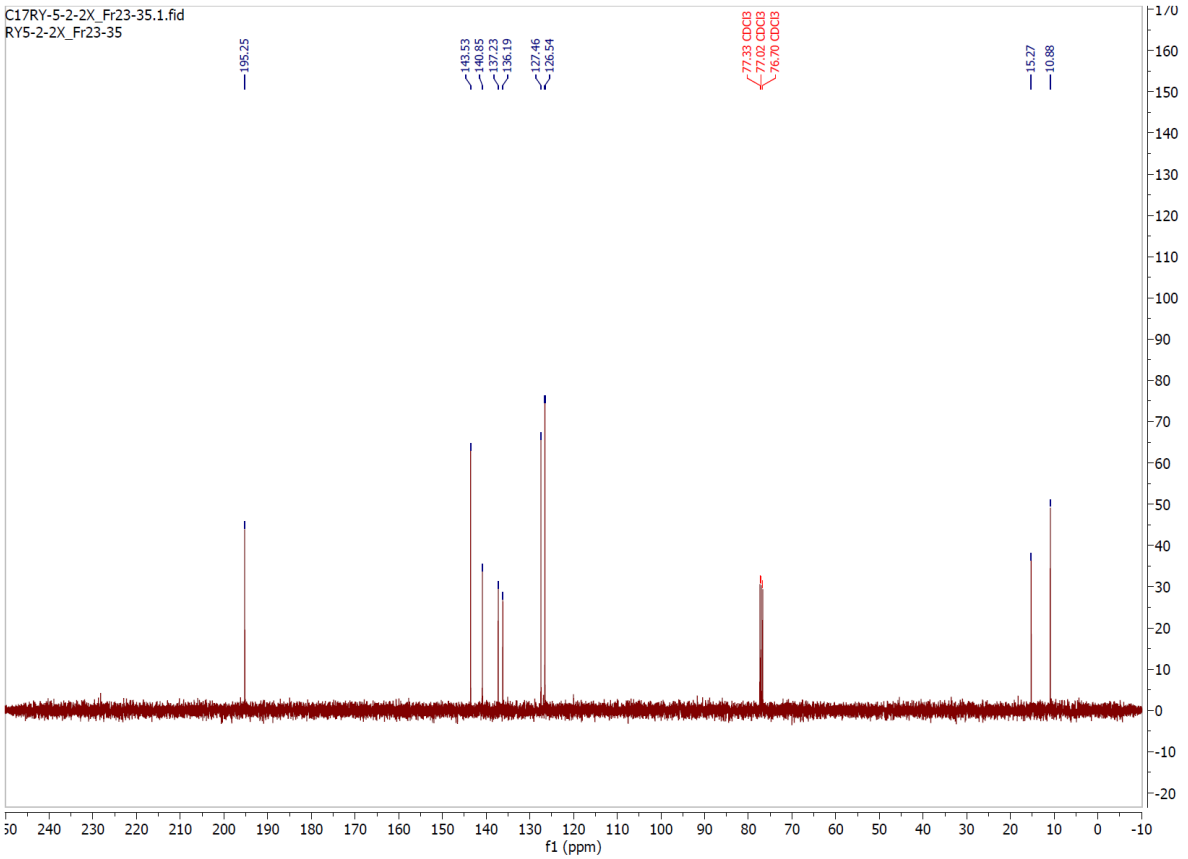
M.p.: 41-42 °C;

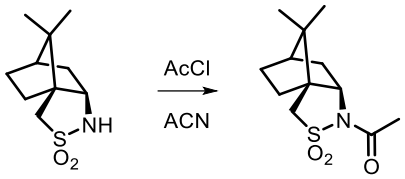
^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.51 (s, 1H), 7.39 (d, *J* = 1.4 Hz, 1H), 7.14 (dd, 1H, J= 1.7, 1.0 Hz), 7.02 (quint, *J* = 1.2 Hz, 1H), 2.53 (d, *J* = 1.1 Hz, 3H), 2.06 (d, *J* = 1.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 195.25, 143.53, 140.85, 137.23, 136.19, 127.46, 126.54, 15.27, 10.88.

HRMS (ESI-TOF) *m/z* calcd. for $C_9H_{10}OS$ $[M]^+$ 166.0447, found 166.0449.


C15RY-5-2-X.1.fid
RY-5-2-X
9.51
7.39
7.39
7.26 CDCl3
7.15
7.15
7.14
7.14
7.14
7.03
7.03
7.02
7.02
7.02
2.53
2.53
2.06
2.06
1.57 H2O
17
1.03
0.97
1.09
1.01
3.32
3.19
f1 (ppm)


C17RY-5-2-2X_Fr23-35.1.fid
RY5-2-2X_Fr23-35
195.25
143.53
140.85
137.23
136.19
127.46
126.54
77.33 CDCl3
77.02 CDCl3
76.70 CDCl3
15.27
10.88
f1 (ppm)



18a **18**

1-((3aR,6R,7aS)-8,8-Dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)ethanone (18): A yellow solution of (-)-D-2,10-Camphorsultam (**18a**) (10 g, 46.4 mmol, 1.0 eq) and acetyl chloride (8.25 mL, 92.9 mmol, 2.0 eq) in acetonitrile (200 mL) was stirred for 17 h at reflux (oil bath 105 °C). K_2CO_3 (12.8 g, 116 mmol, 2.5 eq) was added and the mixture was stirred for 2 h at RT. The yellow suspension was concentrated and then diluted with EtOAc (200 mL) and water (250 mL). The aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was recrystallized from EtOH (40 mL) yielding 10.1 g of white crystals of **18**. The mother liquor was evaporated and the residue was recrystallized again from EtOH (5 mL) yielding another 657 mg of white crystals of **18**.

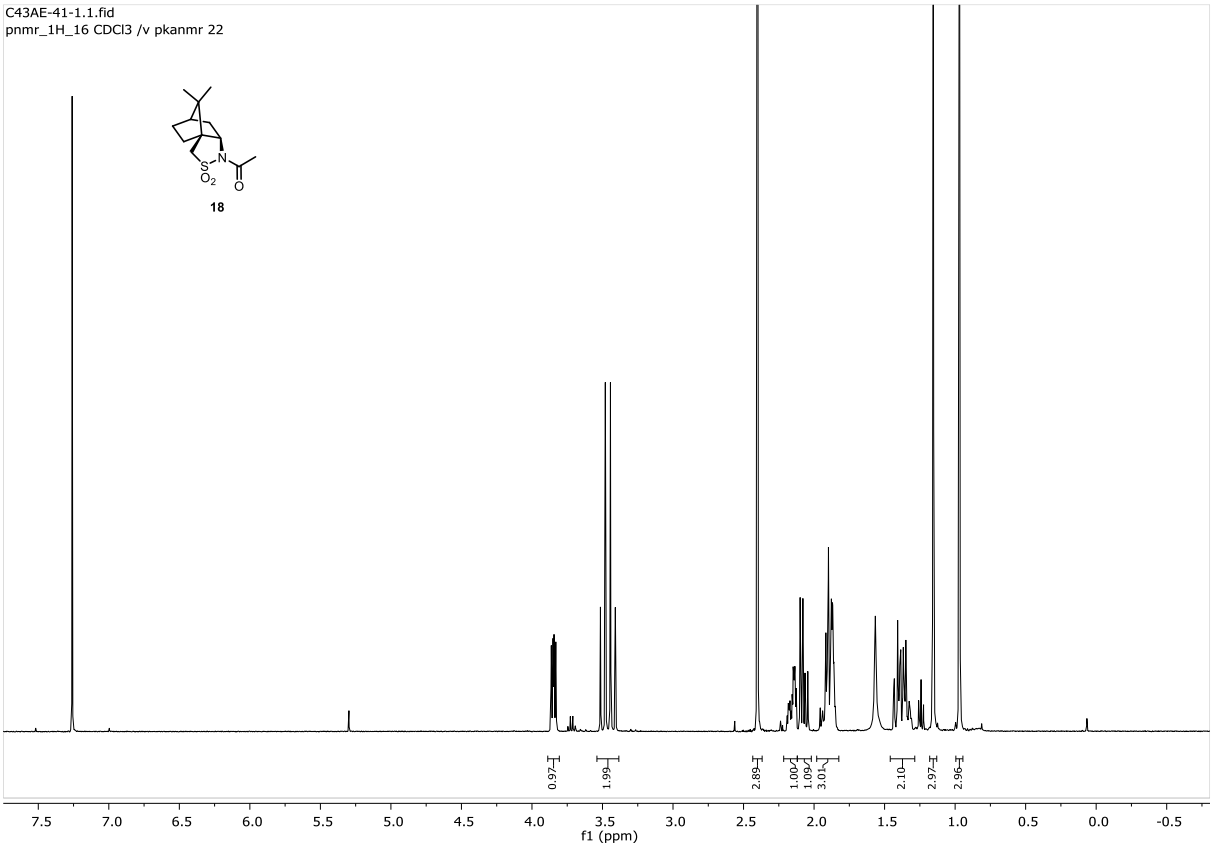
Yield: 10.1 g (90%);

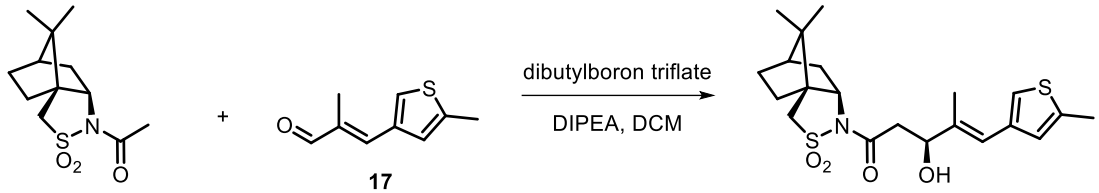
R_f = 0.41 (Hex/AcOEt = 4:1);

M.p.: 137-138 °C (lit. 134-135 °C)[1];

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.85 (dd, *J* = 7.8, 5.0 Hz, 1H), 3.46 (q, *J* = 13.8 Hz, 2H), 2.40 (s, 3H), 2.22 – 2.12 (m, 1H), 2.07 (dd, *J* = 13.9, 7.8 Hz, 1H), 1.98 – 1.82 (m, 3H), 1.46 – 1.29 (m, 2H), 1.16 (s, 3H), 0.97 (s, 3H).

[1]This compound has been described, for example, in: Oppolzer, W.; Starkemann, C. *Tetrahedron Lett.* **1992**, *33*, 2439-2443. NMR-specroscopic data for *ent*-**18** can be found in: Bond, S.; Perlmutter, P. N- *J. Org. Chem.* **1997**, *62*, 6397-6400.


C43AE-41-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 22
18
0.97
1.99
2.89
1.00
1.09
3.01
2.10
2.97
2.96
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



(S,E)-1-((3aR,6R,7aS)-8,8-Dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)-3-hydroxy-4-methyl-5-(5-methylthiophen-3-yl)pent-4-en-1-one (19): A colourless solution of **18** (3.41 g, 13.2 mmol, 1.1 eq) in DCM (30 mL) was added dropwise over 15 min to a yellow solution of dibutylboron triflate (1 M in DCM, 13.2 mL, 13.2 mmol, 1.1 eq) cooled to 0 °C giving a brown solution. 5 min later DIPEA (2.46 mL, 14.4 mmol, 1.2 eq) was added dropwise over 12 min first discolouring the mixture and then giving a slightly yellow solution. The mixture was cooled to -78 °C and **17** (2.0 g, 12.0 mmol, 1.0 eq) in DCM (30 mL) was added slowly over 40 min giving a bright yellow solution. The mixture was stirred at -78 °C for 3 h. TLC (hexane/Et_2O = 4:1) indicated the consumption of the starting material and the formation of 2 new spots. The orange solution was quenched at -78 °C with aqueous PH 7 buffer (5 mL), followed by MeOH (25 mL) and finally H_2O_2 (aqueous, 30%, 5 mL). The crude was allowed to warm up to RT and stirred at RT for another 30 min. Then the emulsion was diluted with water (100 mL) and DCM (20 mL). The aqueous layer was extracted with DCM (2 x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/Et_2O = 4:1 to 2:1, 200 g, 5 cm, 50 mL) to furnish a mixture of two stereoisomers. The mixture was re-chromatographed (hexane/Et_2O = 5:1 to 2:1, 200 g, 5 cm, 50 mL) to give 2.61 g of pure **19** as a colourless liquid and 1.12 g of a mixture of **19** and its C15 diastereoisomer (epothilone numbering) *dia*-**19**.

Yield: 2.61 g (51%);

R_f = 0.23 (Hex/AcOEt = 2:1);

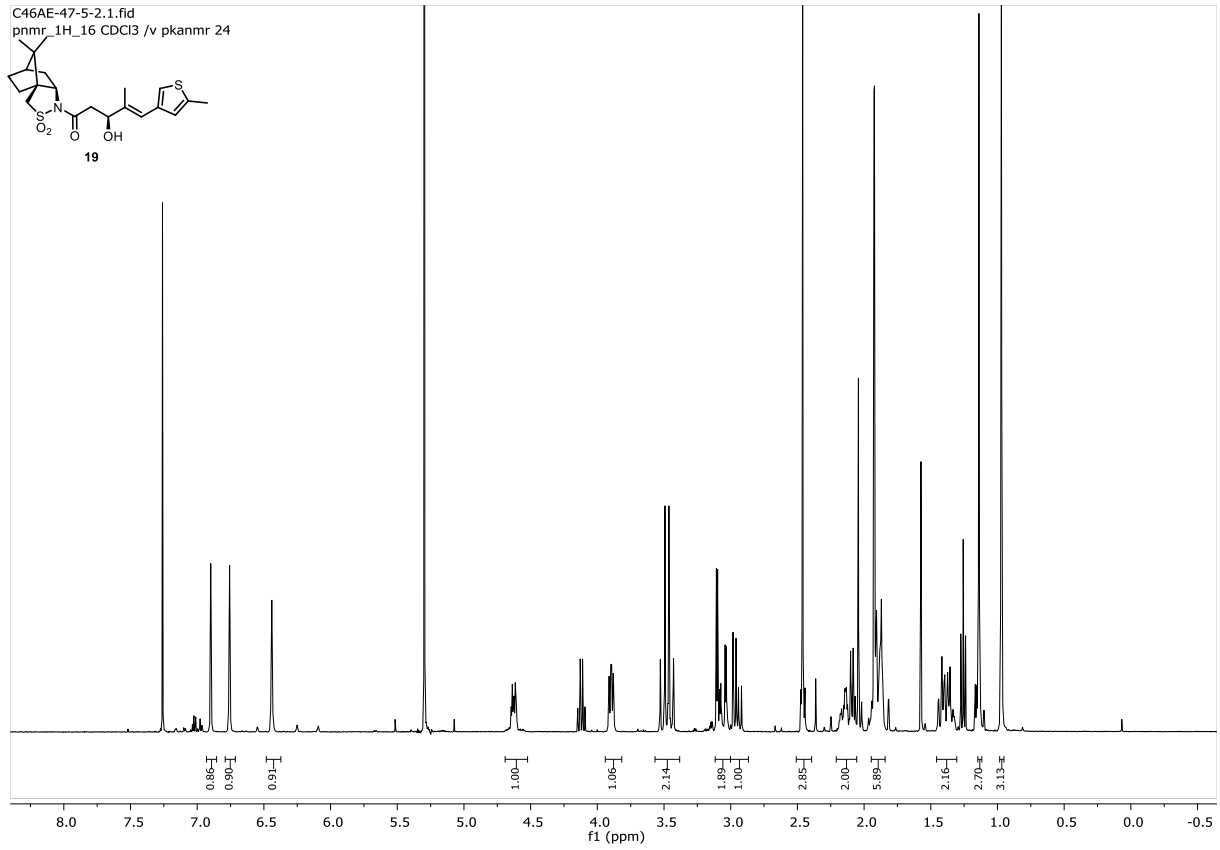
$[\alpha]_D^{23}$: -21.95 (c 0.15, EtOAc);

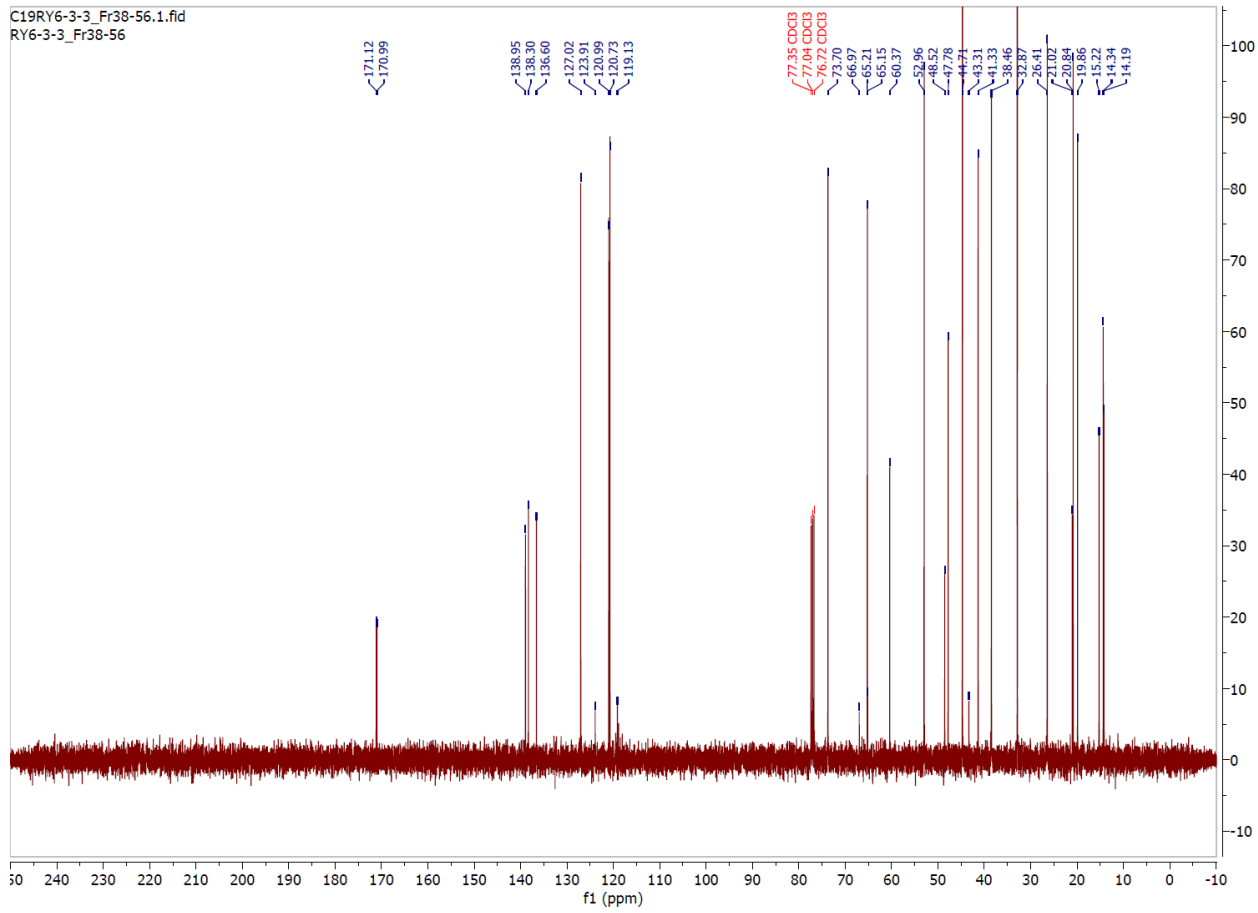
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.90 (s, 1H), 6.76 (quint, *J* = 1.1 Hz, 1H), 6.44 (q, *J* = 1.2 Hz, 1H), 4.65 – 4.58 (m, 1H), 3.90 (dd, *J* = 7.8, 5.0 Hz, 1H), 3.48 (q, *J* = 13.9 Hz, 2H), 3.12 – 2.95 (m, 3H), 2.46 (d, *J* = 1.1 Hz, 3H), 2.23 – 1.99 (m, 2H), 1.95 – 1.79 (m, 6H), 1.47 – 1.31 (m, 2H), 1.14 (s, 3H), 0.97 (s, 3H).

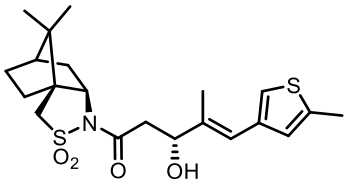
^{13}C NMR (400 MHz, $CDCl_3$) δ = 171.12, 170.99, 138.95, 138.30, 136.60, 127.02, 123.91, 120.99, 120.73, 119.13, 73.79, 66.97, 65.21, 65.15, 60.37, 52.96, 48.52, 47.78, 44.71, 43.31, 41.33, 38.46, 32.87, 26.41, 21.02, 20.84, 19.86, 15.22, 14.34, 14.19.

Note: While the material was chromatographically homogenous, the number of signals ins the carbon spectrum of **19** by far exceeds the number of carbons in the molecule. We ascribe this to the presence of rotamers about the (O=)C-NSO_2 bond. Interestingly, no doubling of signals was observed in the spectrum of the corresponding silyl ether **20** (*vide infra*).

HRMS (ESI-TOF) *m/z* calcd. for $C_{21}H_{29}NO_4S_2Na$ $[M+Na]^+$ 446.1430, found 446.1432.


C46AE-47-5-2.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 24
19
0.86
0.90
0.91
1.00
1.06
2.14
1.89
1.00
2.85
2.00
5.89
2.16
2.70
3.13
f1 (ppm)


C19RY6-3-3_Fr38-56.1.fid
RY6-3-3_Fr38-56
171.12
170.99
138.95
138.30
136.60
127.02
123.91
120.99
120.73
119.13
77.35 CDCl3
77.04 CDCl3
76.72 CDCl3
73.70
66.97
65.21
65.15
60.37
52.96
48.52
47.78
44.71
43.31
41.33
38.46
32.87
26.41
21.02
20.84
19.86
15.22
14.34
14.19
f1 (ppm)

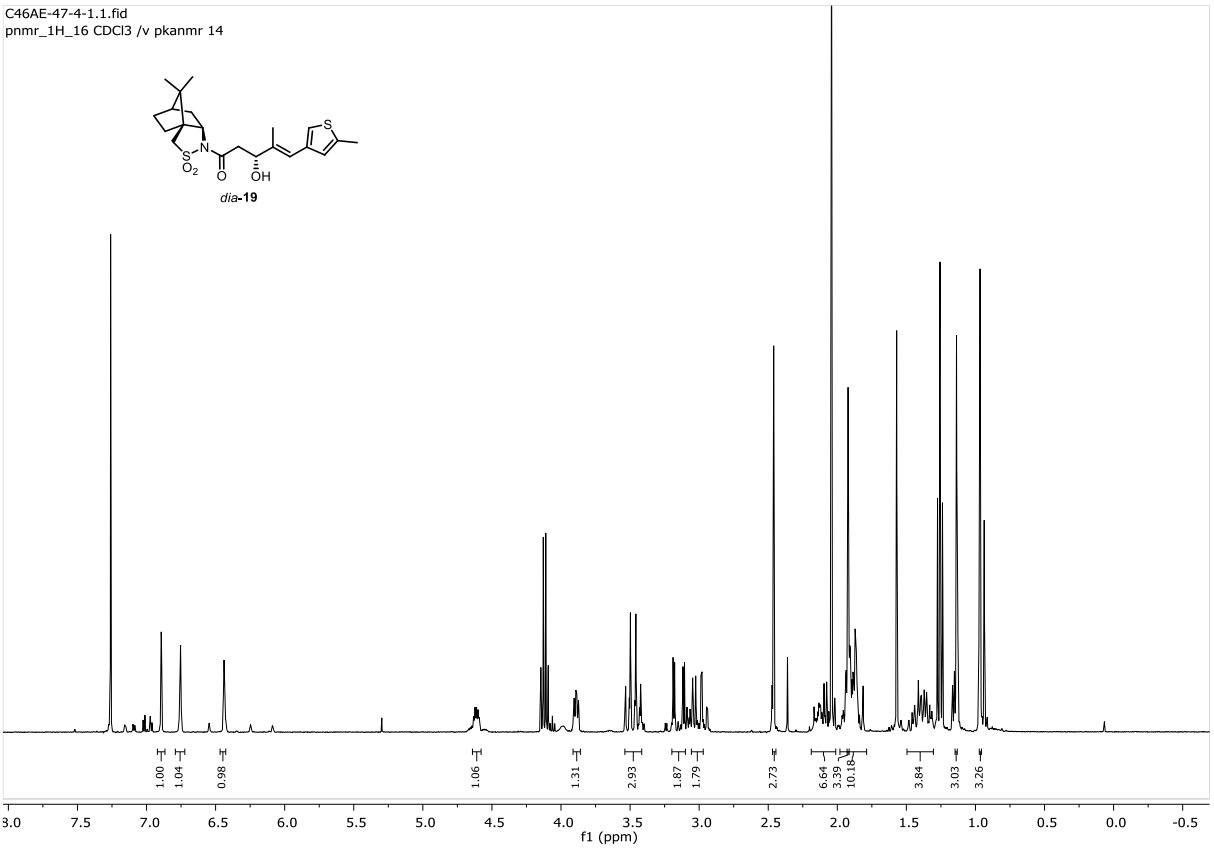


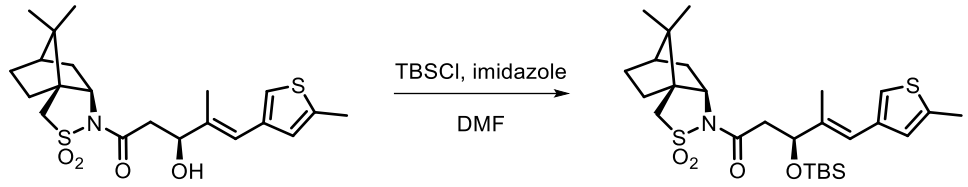
dia-**19**

(R,E)-1-((3aR,6R,7aS)-8,8-Dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)-3-hydroxy-4-methyl-5-(5-methylthiophen-3-yl)pent-4-en-1-one (dia-19)

R_f = 0.30 (Hex/AcOEt = 2:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.89 (s, 1H), 6.75 (quint, *J* = 1.2 Hz, 1H), 6.44 (q, *J* = 1.2 Hz, 1H), 4.64 – 4.58 (m, 1H), 3.89 (dd, *J* = 7.8, 4.9 Hz, 1H), 3.56 – 3.39 (m, 2H), 3.21 – 2.93 (m, 3H), 2.46 (d, *J* = 1.0 Hz, 3H), 2.19 – 2.01 (m, 2H), 1.98 – 1.79 (m, 6H), 1.50 – 1.30 (m, 2H), 1.14 (s, 3H), 0.97 (s, 3H).


C46AE-47-4-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 14
S
N
S
O2
O
OH
dia-19
1.00
1.04
0.98
1.06
1.31
2.93
1.87
1.79
2.73
6.64
3.39
10.18
3.84
3.03
3.26
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



19 **20**

(S,E)-3-((tert-Butyldimethylsilyl)oxy)-1-((3aR,6R,7aS)-8,8-dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)-4-methyl-5-(5-methylthiophen-3-yl)pent-4-en-1-one (20): A colourless solution of **19** (2.61 g, 6.16 mmol, 1.0 eq), imidazole (1.26 g, 18.5 mmol, 3.0 eq) and TBSCl (1.11 g,7.39 mmol, 1.2 eq) in DMF (20 mL) was stirred at 45°C for 2 h. TLC (hexane/AcOEt = 4:1) indicated the consumption of the starting material. The yellow solution was diluted with sat. aq. $NaHCO_3$ solution (40 mL) and EtOAc (40 mL). The organic layer was extracted with H_2O (2 x 30 mL). The combined aqueous layers were extracted with EtOAc (30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/AcOEt = 10:1 to 4:1, 40 g, 3 cm, 30 mL) to obtain the product as a white solid. Recrystallization from hexane gave 2.62 g of **20** as white needles.

Yield: 2.62 g (79%);

R_f = 0.40 (Hex/AcOEt = 4:1);

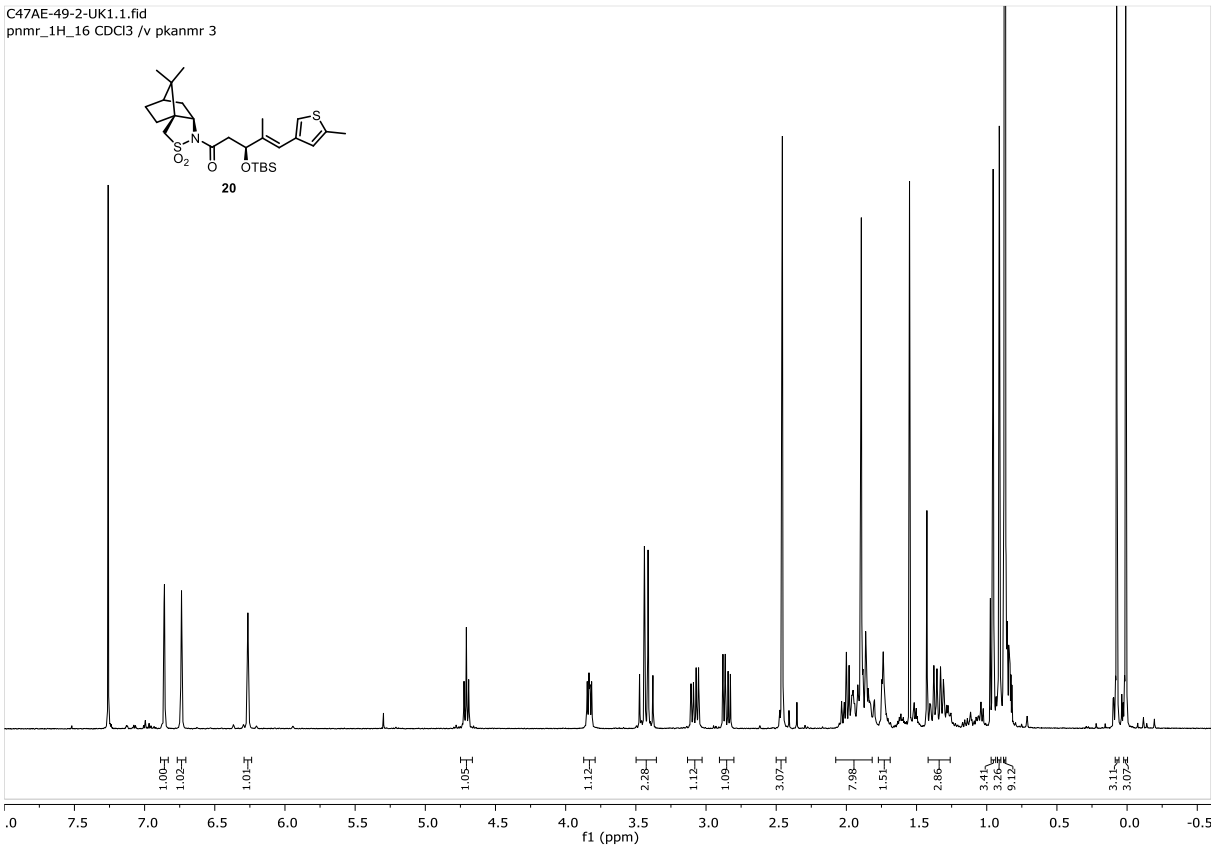
M.p.: 109-110 °C;

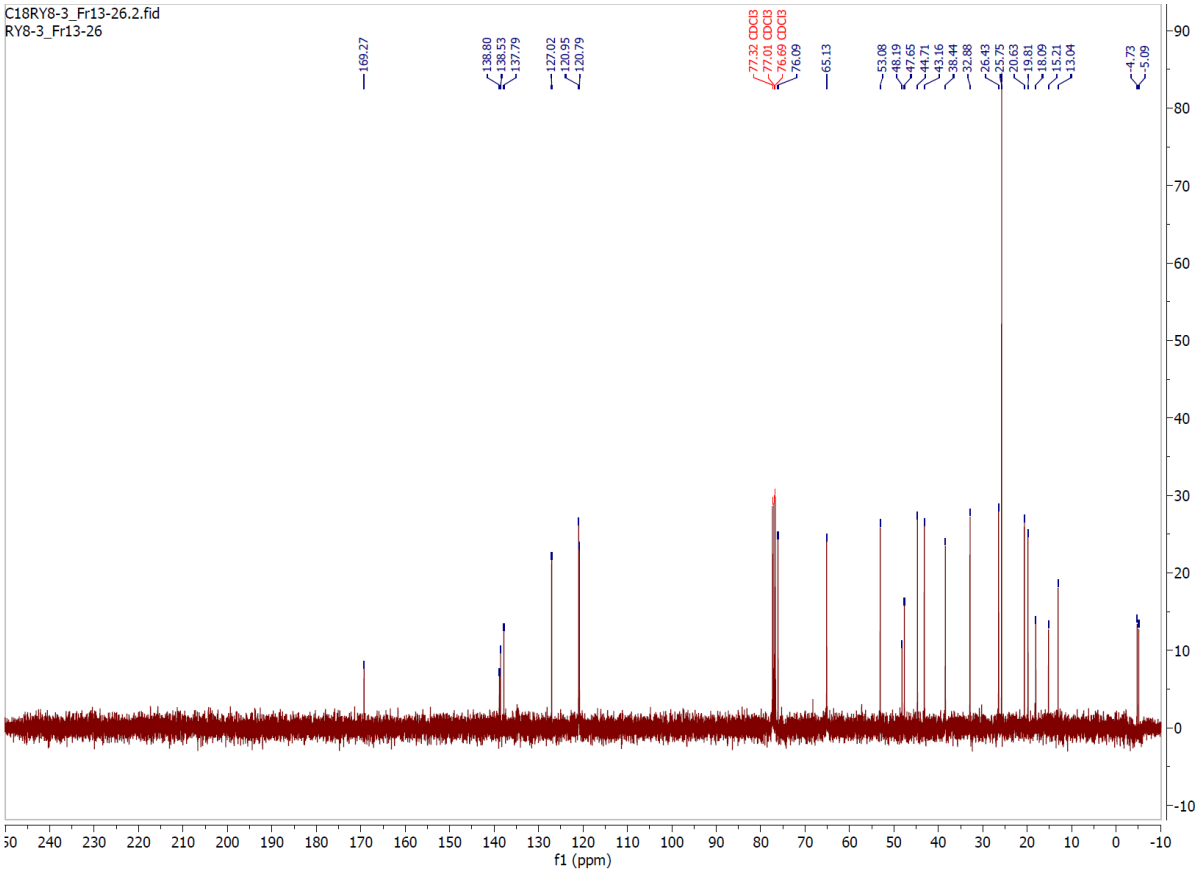
$[\alpha]_D^{23}$: -21.95 (c 0.15, EtOAc)

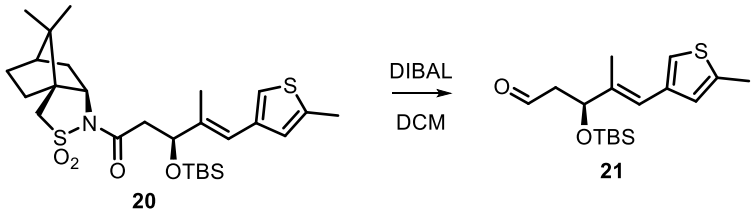
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.86 (s, 1H), 6.74 (quint, *J* = 1.0 Hz, 1H), 6.26 (s, 1H), 4.70 (t, *J* = 6.7 Hz, 1H), 3.83 (dd, *J* = 7.6, 5.0 Hz, 1H), 3.43 (q, *J* = 13.8, 2H), 3.08 (dd, *J* = 14.7, 6.9 Hz, 1H), 2.85 (dd, *J* = 14.7, 6.5 Hz, 1H), 2.46 (d, *J* = 1.1 Hz, 3H), 2.08 – 1.82 (m, 7H), 1.74 (t, *J* = 3.8 Hz, 1H), 1.42 – 1.26 (m, 2H), 0.95 (s, 3H), 0.91 (s, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 138.80, 138.53, 173.79, 127.02, 120.95, 120.79, 76.09, 65.13, 53.98, 48.19, 47.65, 44.71, 43.16, 38.44, 32.88, 26.43, 25.75, 20.63, 19.81, 18.09, 15.21, 13.04, -4.73, -5.09.

HRMS (ESI-TOF) *m/z* calcd. for $C_{27}H_{43}NO_4S_2SiNa$ $[M+Na]^+$ 560.2295, found 560.2286.


C47AE-49-2-UK1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 3
20
1.00
1.02
1.01
1.05
1.12
2.28
1.12
1.09
3.07
7.98
1.51
2.86
3.41
3.26
9.12
3.11
3.07
f1 (ppm)


C18RY8-3_Fr13-26.2.fid
RY8-3_Fr13-26
169.27
138.80
138.53
137.79
127.02
120.95
120.79
77.32 CDCl3
77.01 CDCl3
76.69 CDCl3
76.09
65.13
53.08
48.19
47.65
44.71
43.16
38.44
32.88
26.43
25.75
20.63
19.81
18.09
15.21
13.04
-4.73
-5.09
f1 (ppm)



(S,E)-3-((tert-Butyldimethylsilyl)oxy)-4-methyl-5-(5-methylthiophen-3-yl)pent-4-enal (21): DIBAL-H (1 M in DCM, 5.72 mL, 5.72 mmol, 2.5 eq) was added dropwise over 15 min to a colourless solution of **20** (1.23 g, 2.29 mmol, 1.0 eq) in DCM (10 mL) cooled to -78 °C. The mixture was stirred for 2 h at -78 °C. TLC (hexane/Et_2O = 4:1) indicated the consumption of **20**. The reaction was quenched with aqueous Na/K tartrate solution (20 mL) at -78 °C. The mixture was allowed to warm to RT and stirred for 30 min at RT. Water (20 mL) and DCM (40 mL) were added. The aqueous layer was extracted with DCM (3 x 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/EtOAc = 10:1 to 5:1, 80 g, 4 cm, 30 mL) to obtain 593 mg of **21** as a slightly yellow liquid.

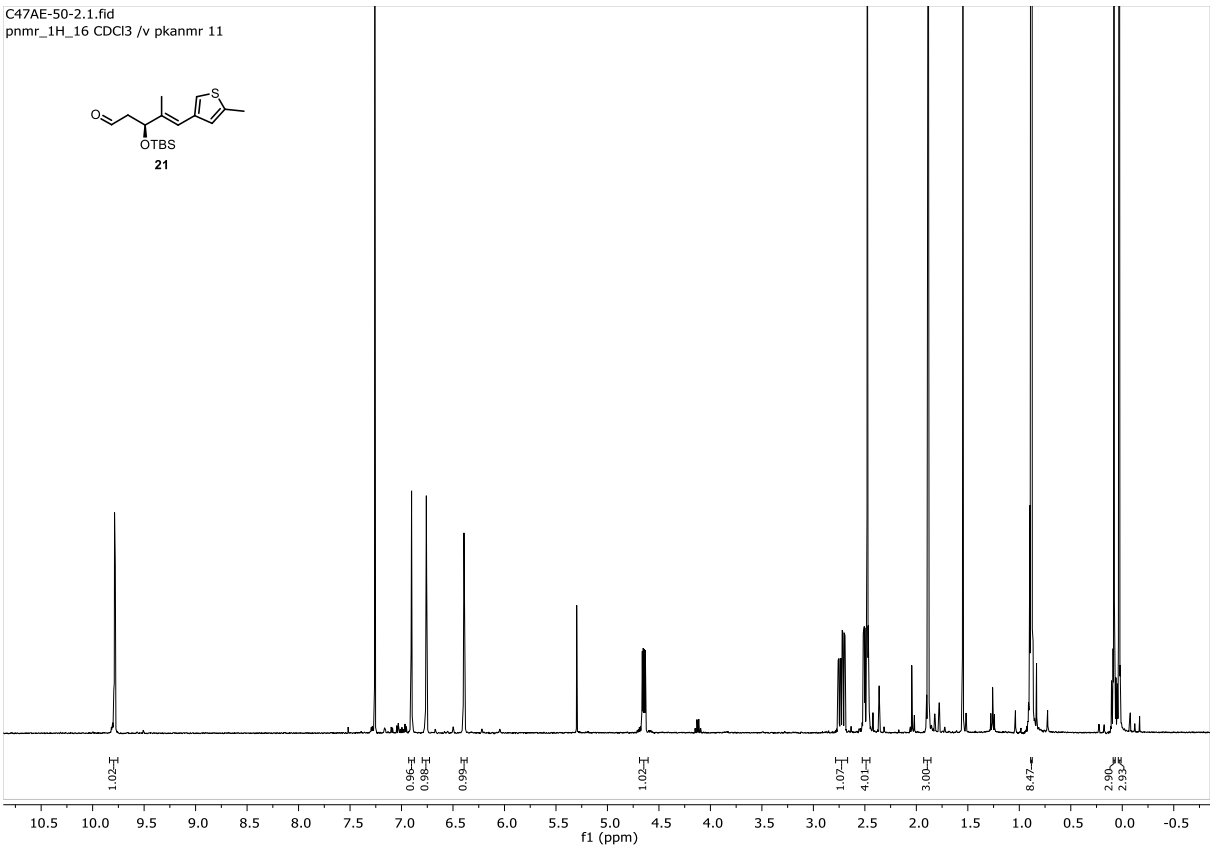
Yield: 593 mg (80%);

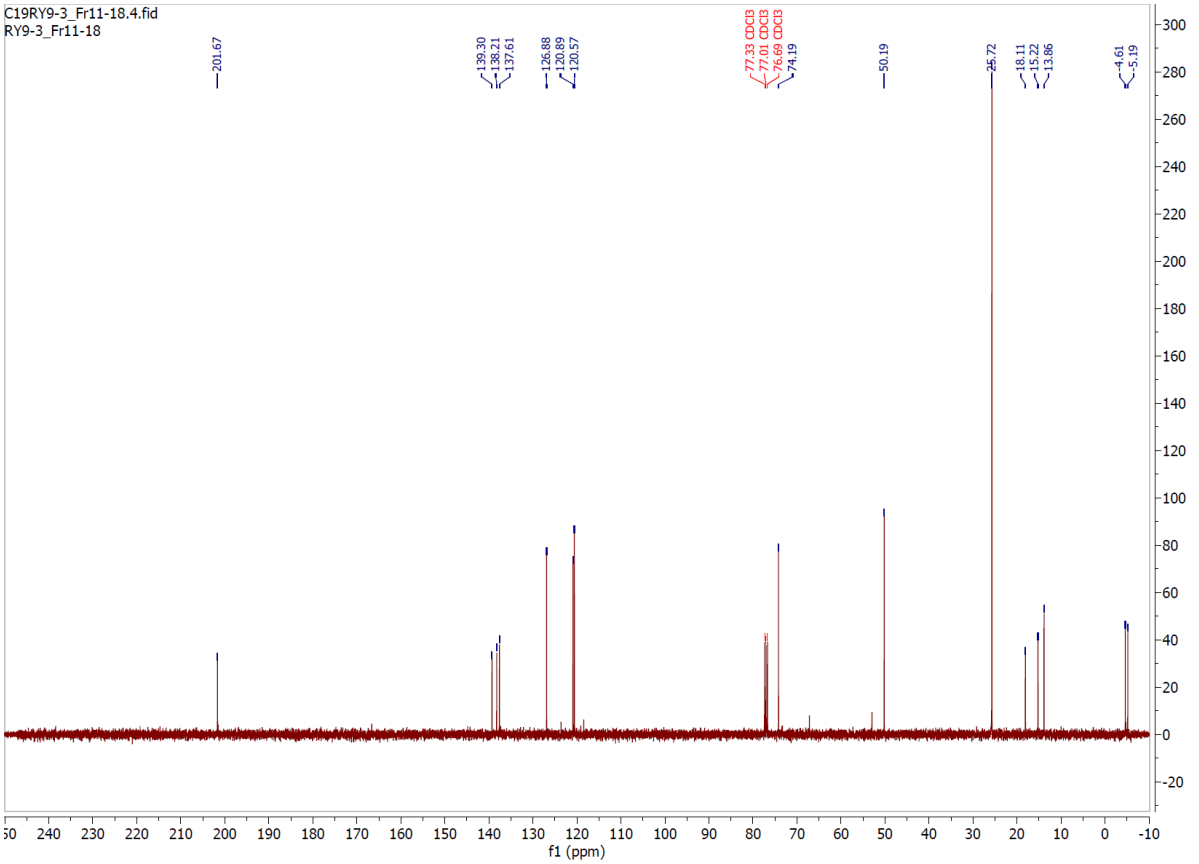
R_f = 0.60 (Hex/AcOEt = 4:1);

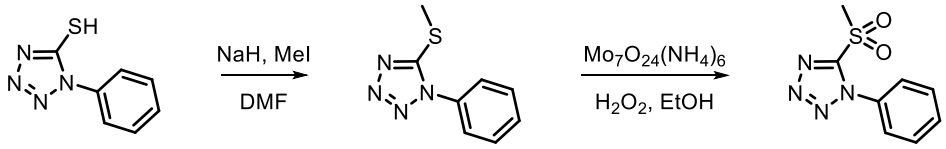
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{23}}$: -21.95 (c 0.14, EtOAc);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.79 (dd, *J* = 2.9, 2.1 Hz, 1H), 6.90 (s, 1H), 6.76 (quint, *J* = 1.1 Hz, 1H), 6.39 (q, *J* = 1.1 Hz, 1H), 4.65 (ddd, *J* = 8.3, 4.1, 1.0 Hz, 1H), 2.73 (ddd, *J* = 15.4, 8.3, 2.9 Hz, 1H), 2.53 – 2.45 (m, 4H), 1.88 (d, *J* = 1.3 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 201.67, 139.30, 138.21, 137.61, 126.88, 120.89, 120.57, 74.19, 50.19, 25.72, 18.11, 15.22, 13.86, -4.61, -5.19.


C47AE-50-2.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 11
O
OTBS
S
21
1.02
0.96
0.98
0.99
1.02
1.07
4.01
3.00
8.47
2.90
2.93
f1 (ppm)


C19RY9-3_Fr11-18.4.fid
RY9-3_Fr11-18
201.67
139.30
138.21
137.61
126.88
120.89
120.57
77.33 CDCl3
77.01 CDCl3
76.69 CDCl3
74.19
50.19
25.72
18.11
15.22
13.86
-4.61
-5.19
f1 (ppm)



22a **22b** **22**

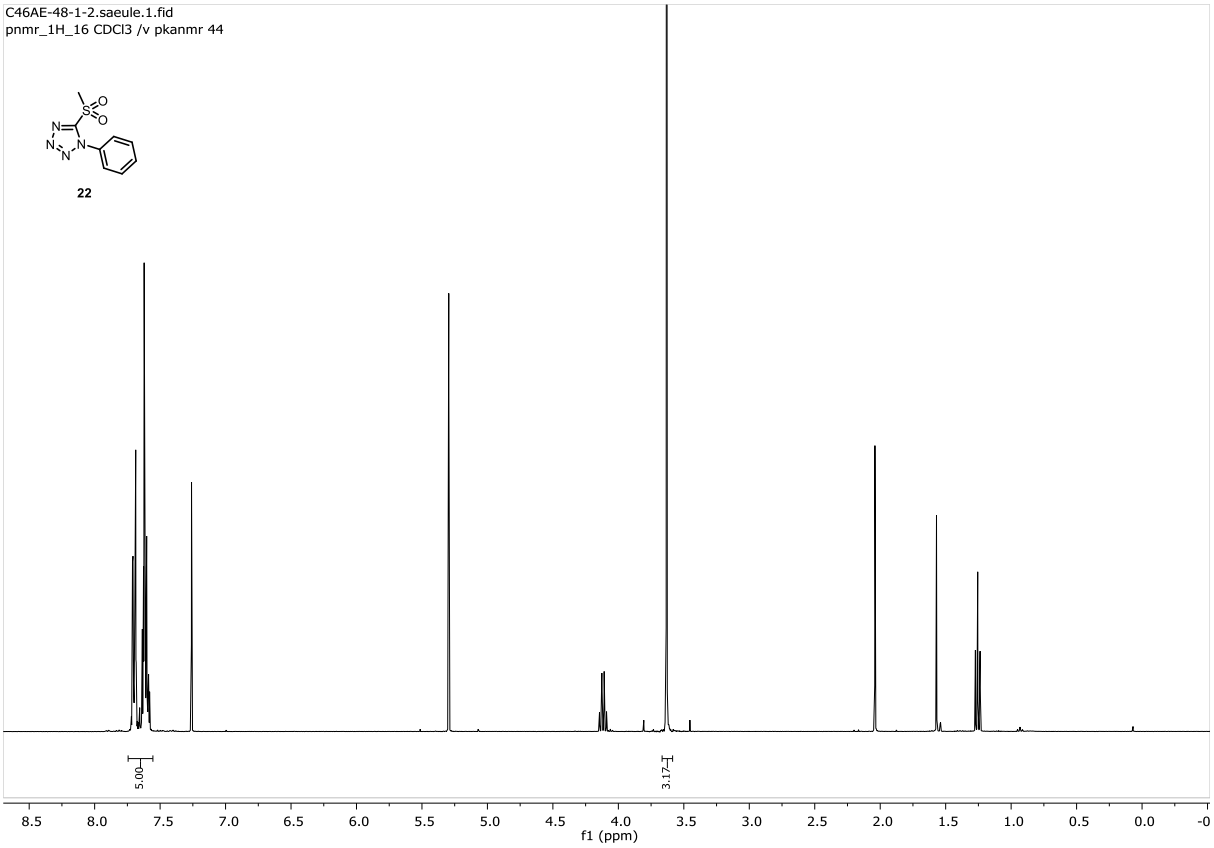
5-(Methylsulfonyl)-1-phenyl-1H-tetrazole (22): NaH (1.38 g, 35.1 mmol, 1.25 eq) was added to a colourless solution of 5-mercapto-1-phenyl-1H-tetrazole (**22a**) (5.0 g, 28.1 mmol, 1.0 eq) in DMF (120 mL) cooled to 0 °C. The mixture was stirred for 20 min at 0 °C. MeI (1.75 mL, 28.1 mmol, 1.0 eq) was added over 5 min. The mixture was allowed to warm to RT after the addition and the slightly yellow suspension was stirred at RT for 3 h. TLC (hexane/Et_2O = 2:1) indicated the consumption of **22a**. The mixture was quenched with saturated aqueous NH_4Cl solution (100 mL) and EtOAc (200 mL). The organic layer was washed with water (2 x 100 mL). The combined aqueous layers were extracted with EtOAc (50 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give crude **22b**.

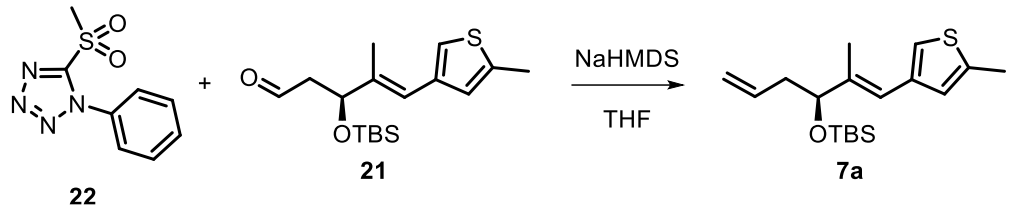
$Mo_7O_{24}(NH_4)_6 \cdot 4\ H_2O$ (17.3 g, 14.0 mmol, 0.5 eq) was added to a solution of crude **22b** in EtOH (120 mL) cooled to 0 °C. Then H_2O_2 (aqueous 30%, 12.9 mL, 126 mmol, 4.5 eq) was added to the slightly yellow suspension over 5 min giving a brown-red suspension. After 30 min at 0 °C the reaction was allowed to warm to RT and was stirred overnight for 24 h. The bright yellow suspension was cooled to 0 °C and then quenched with saturated aqueous $Na_2S_2O_3$ solution (50 mL). The mixture was diluted with water (200 mL) and EtOAc (200 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/EtOAc = 4:1 to 2:1, 150 g, 5 cm, 50 mL) to obtain 4.88 g of **22** as a white solid.

Yield: 4.88 g (77%);

R_f = 0.33 (Hex/AcOEt = 2:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 – 7.56 (m, 5H), 3.63 (s, 3H).


C46AE-48-1-2.saeule.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 44
22
5.00
3.17
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)



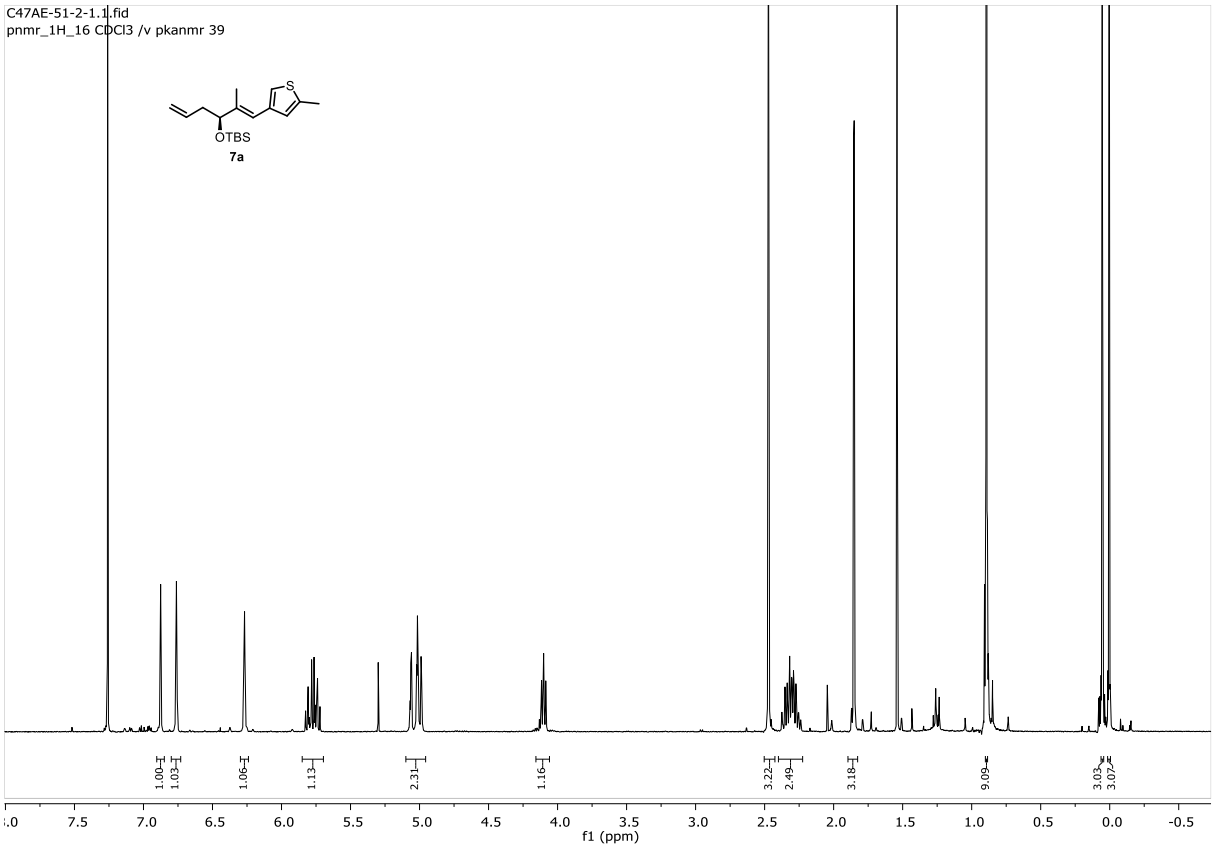
22 **21** **7a**

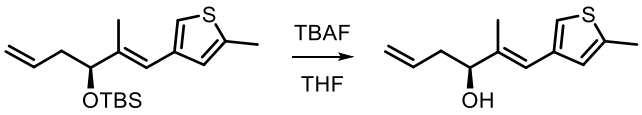
(S,E)-tert-Butyldimethyl((2-methyl-1-(5-methylthiophen-3-yl)hexa-1,5-dien-3-yl)oxy)silane (7a): NaHDMS (1 M in THF, 2.18 mL, 2.18 mmol, 1.2 eq) was added to a colourless solution of **21** (590 mg, 1.82 mmol, 1.0 eq) and **22** (489 mg, 2.18 mmol, 1.2 eq) in THF (15 mL) cooled to -78 °C over 2 min. The mixture was stirred at -78 °C for 2 h. TLC (hexane/Et_2O = 4:1) indicated the consumption of **21**. The slightly yellow solution was quenched at -78 °C with aqueous pH 7 buffer solution (3 mL). The mixture was allowed to warm to RT. EtOAc (40 mL) and water (40 mL) were added. The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/Et_2O = 10:1, 100 g, 4 cm, 30 mL) to obtain 517 mg of **7a** as a colourless liquid.

Yield: 517 mg (88%);

R_f = 0.88 (Hex/AcOEt = 4:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.88 (s, 1H), 6.76 (quint, *J* = 1.1 Hz, 1H), 6.27 (q, *J* = 1.1 Hz, 1H), 5.77 (ddt, *J* = 17.2, 10.1, 7.1 Hz, 1H), 5.10 – 4.96 (m, 2H), 4.13 – 4.07 (m, 1H), 2.47 (d, *J* = 1.1 Hz, 3H), 2.40 – 2.22 (m, 2H), 1.85 (d, *J* = 1.3 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.00 (s, 3H).


C47AE-51-2-1.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 39
S
OTBS
7a
1.00
1.03
1.06
1.13
2.31
1.16
3.22
2.49
3.18
9.09
3.03
3.07
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



7a **7**

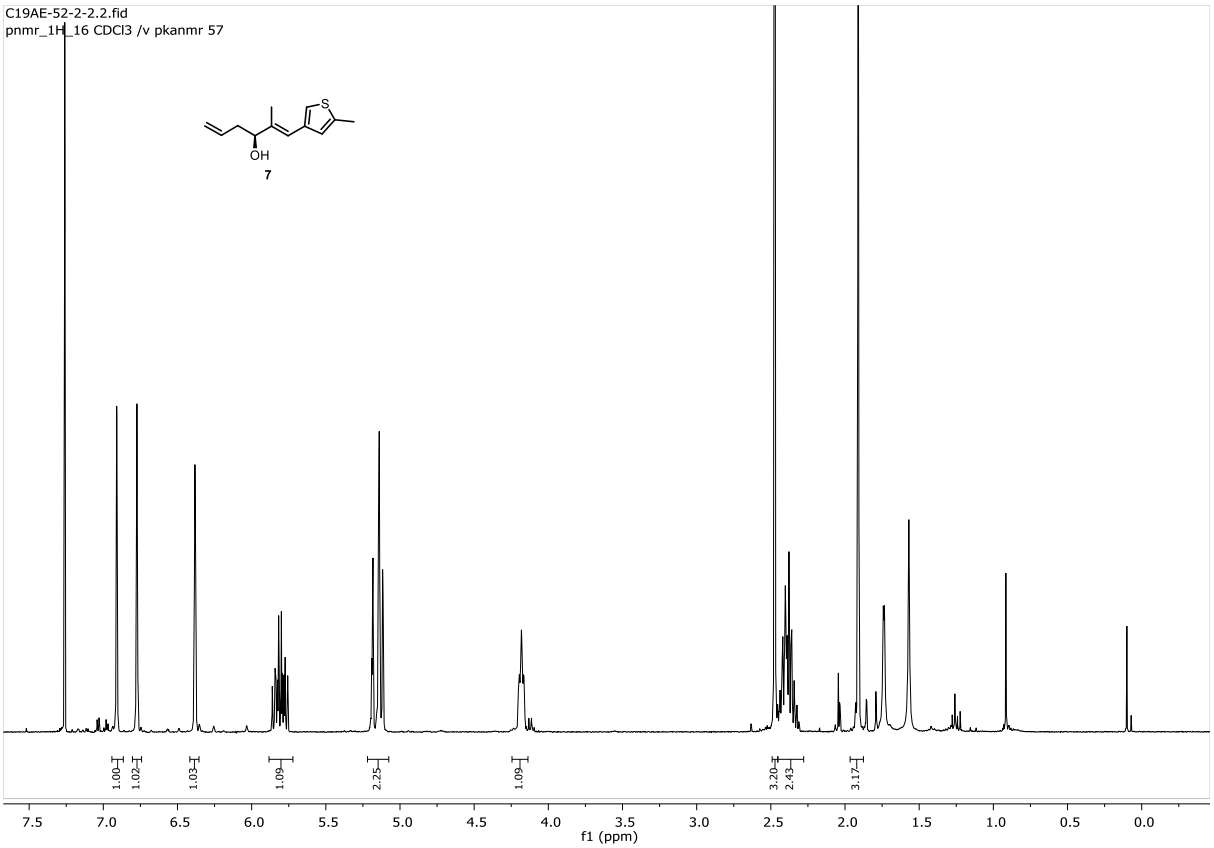
(S,E)-2-methyl-1-(5-methylthiophen-3-yl)hexa-1,5-dien-3-ol (7): TBAF (1 M in THF, 3.95 mL, 3.95 mmol, 2.5 eq) was added to a colourless solution of **7a** (510 mg, 1.58 mmol, 1.0 eq) in THF (15 mL) at 0 °C over 5 min. The slightly yellow solution was stirred at 0 °C for 10 min and was then stirred at RT for 4.5 h. TLC (hexane/Et_2O = 4:1) indicated the consumption of **7a**. The mixture was quenched with saturated aqueous $NaHCO_3$ solution (40 mL) and diluted with EtOAc (40 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/EtOAc = 8:1, 30 g, 2 cm, 20 mL) to obtain 309 mg of **7** as a colourless oil.

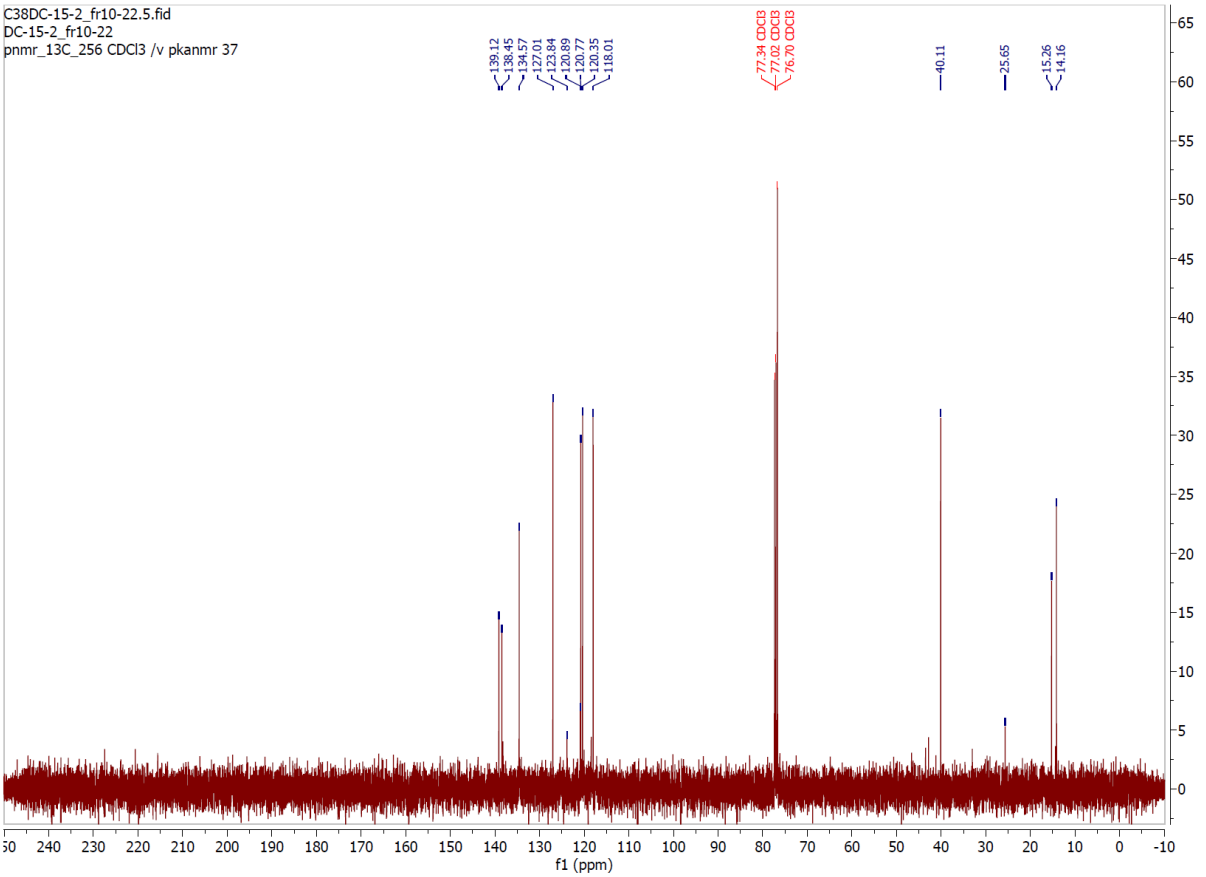
Yield: 309 mg (94%);

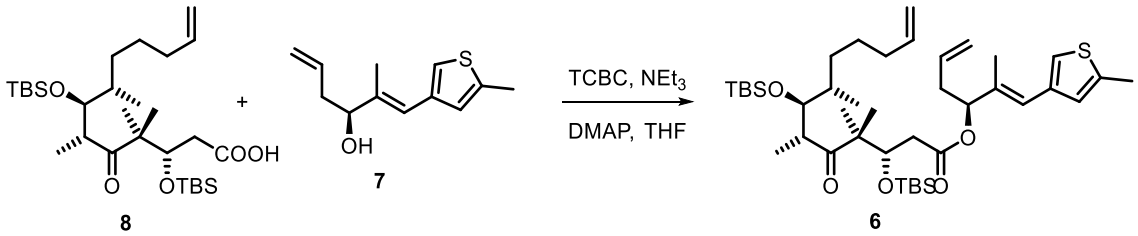
R_f = 0.46 (Hex/AcOEt = 2:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.91 (s, 1H), 6.77 (quint, *J* = 1.2 Hz, 1H), 6.39 – 6.36 (m, 1H), 5.89 – 5.73 (m, 1H), 5.23 – 5.08 (m, 2H), 4.22 – 4.15 (m, 1H), 2.47 (d, *J* = 1.1 Hz, 3H), 2.46 – 2.30 (m, 2H), 1.91 (d, *J* = 1.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 139.12, 138.45, 134.57, 127.01, 123.84, 120.89, 120.77, 120.35, 118.01, 40.11, 15.26, 14.16.


C19AE-52-2-2.2.fid
pnmr_1H_16 CDCl3 /v pkanmr 57
S
OH
7
1.00
1.02
1.03
1.09
2.25
1.09
3.20
2.43
3.17
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


C38DC-15-2_fr10-22.5.fid
DC-15-2_fr10-22
pnmr_13C_256 CDCl3 /v pkanmr 37
139.12
138.45
134.57
127.01
123.84
120.89
120.77
120.35
118.01
77.34 CDCl3
77.02 CDCl3
76.70 CDCl3
40.11
25.65
15.26
14.16
65
60
55
50
45
40
35
30
25
20
15
10
5
0
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



(3S,6R,7S,8S)-(S,E)-2-methyl-1-(5-methylthiophen-3-yl)hexa-1,5-dien-3-yl 3,7-bis((tert-butyl-dimethylsilyl)oxy)-4,4,6,8-tetramethyl-5-oxotridec-12-enoate (6): Triethylamine (41.1 µL, 0.295 mmol, 4.0 eq) followed by 2,4,6-trichlorobenzoyl chloride (TCBC) (17.3 µL, 0.111 mmol, 1.5 eq) were added to a brown solution of **8** (40.1 mg, 0.0739 mmol, 1.0 eq) in benzene (1 mL). The slightly brown solution was stirred at RT for 45 min. TLC (DCM/acetone = 9:1) indicated the consumption of **8**. Alcohol **7** (16.9 mg, 0.0812 mmol, 1.1 eq) in benzene (1 mL) was added over 2 min, followed by DMAP (11.7 mg, 0.0960 mmol, 1.3 eq) in benzene (1 mL), which was also added over 2 min. The orange suspension was stirred for 30 min. TLC (Hex/Et_2O = 4:1) indicated the consumption of **7** and the formation of 2 new spots. The yellow suspension was diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Two runs of flash chromatography (hexane/EtOAc = 50:1, 6 g, 1 cm, 6 mL) gave 35.2 mg of **6** as a colourless liquid.

Yield: 35.2 mg (65%);

R_f = 0.81 (Hex/AcOEt = 4:1);

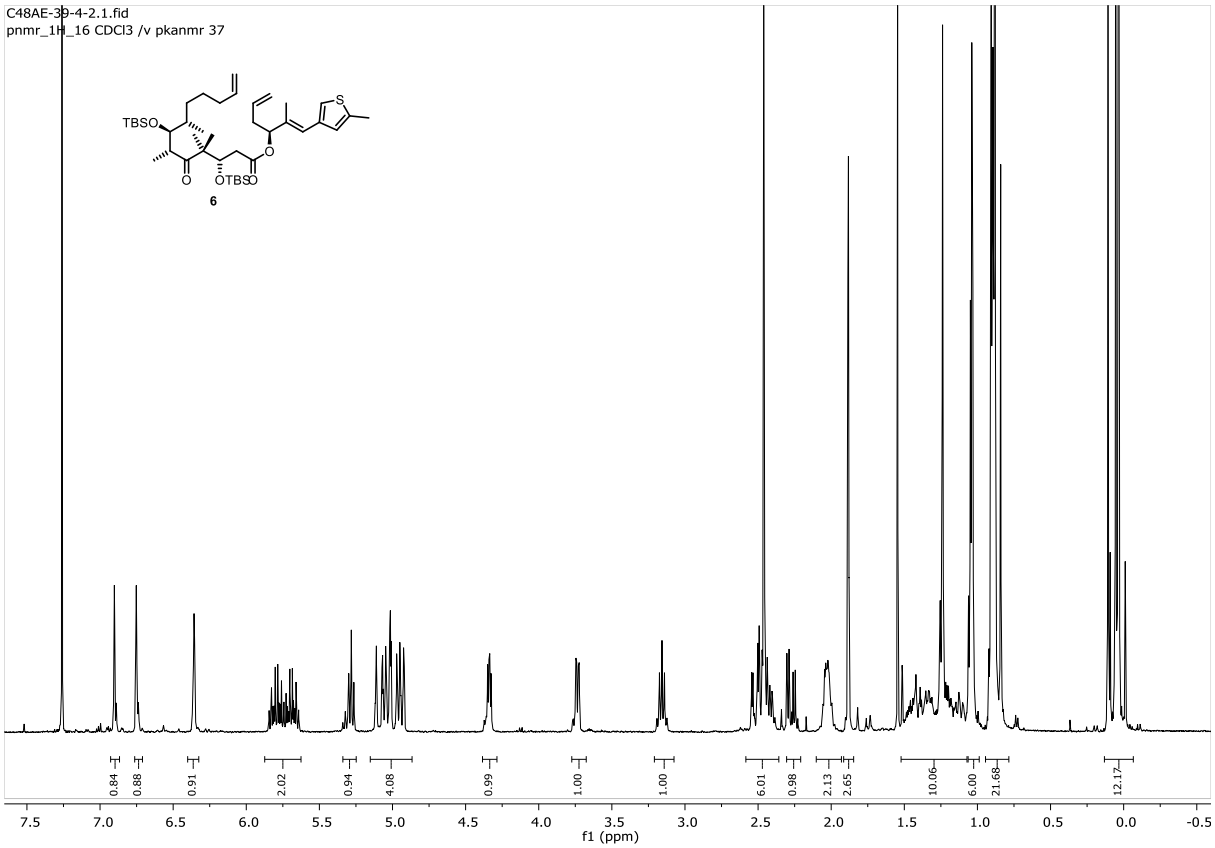
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$**:** -17.12 (c = 0.375, $CHCl_3$);

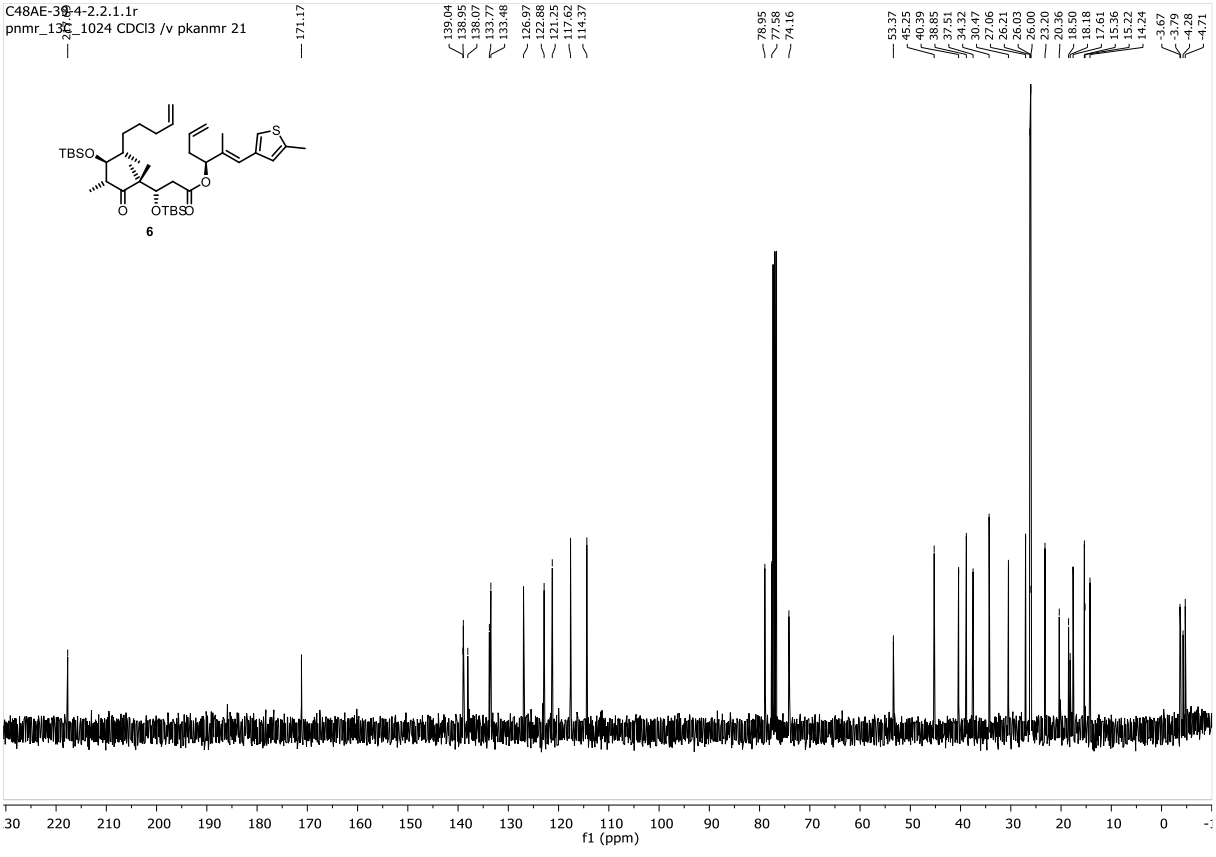
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.90 (s, 1H), 6.75 (t, *J* = 1.1 Hz, 1H), 6.36 (s, 1H), 5.85 - 5.63 (m, 2H), 5.28 (t, *J* = 7.0 Hz, 1H), 5.13 - 4.91 (m, 4H), 4.34 (dd, *J* = 3.7, 5.9 Hz, 1H), 3.73 (dd, *J* = 2.2, 6.8 Hz, 1H), 3.16 (t, *J* = 6.9 Hz, 1H), 2.55 – 2.37 (m, 6H), 2.27 (dd, *J* = 5.9, 16.9 Hz, 1H), 2.06 – 1.99 (m, 2H), 1.88 (d, *J* = 1.3 Hz, 3H), 1.50 - 1.08 (m, 8H), 1.06 – 1.02 (m, 3H), 0.91 – 0.87 (m, 24H), 0.11 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H);

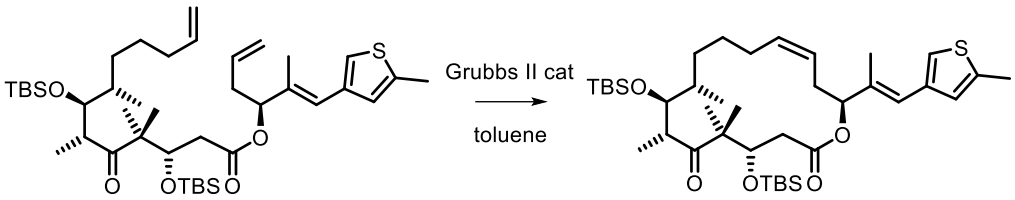
^{13}C NMR (101 MHz, $CDCl_3$) δ = 217.7, 171.2, 139.1, 139.0, 138.1, 133.8, 133.5, 127.0, 122.9, 121.3, 117.6, 114.4, 79.0, 77.6, 74.2, 53.4, 45.3, 40.4, 38.9, 37.5, 34.3, 30.5, 27.1, 26.2, 26.0, 26.0, 23.2, 20.4, 18.5, 18.2, 17.6, 15.4, 15.2, 14.2, -3.7, -3.8, -4.3, -4.7;

IR (film): ν = 2954m, 2930m, 2857m, 1738m, 1695w, 1468w, 1381w, 1292w, 1254m, 1173w, 1086m, 987m, 835s, 776s;

HRMS (ESI-TOF) *m/z* calcd. for $C_{41}H_{72}NaO_5SSi_2$ $[M+Na]^+$ 755.4531, found 755.4528.


C48AE-39-4-2.1.fid
pnmr_1H_16 CDCl3 /v pkanmr 37
TBSO
O
OTBSO
O
S
6
0.84
0.88
0.91
2.02
0.94
4.08
0.99
1.00
1.00
6.01
0.98
2.13
2.65
10.06
6.00
21.68
12.17
f1 (ppm)


C48AE-39-4-2.2.1.1r
pnmr_13C_1024 CDCl3 /v pkanmr 21
171.17
139.04
138.95
138.07
133.77
133.48
126.97
122.88
121.25
117.62
114.37
78.95
77.58
74.16
53.37
45.25
40.39
38.85
37.51
34.32
30.47
27.06
26.21
26.03
26.00
23.20
20.36
18.50
18.18
17.61
15.36
15.22
14.24
-3.67
-3.79
-4.28
-4.71
TBSO
O
OTBSO
O
S
6
f1 (ppm)



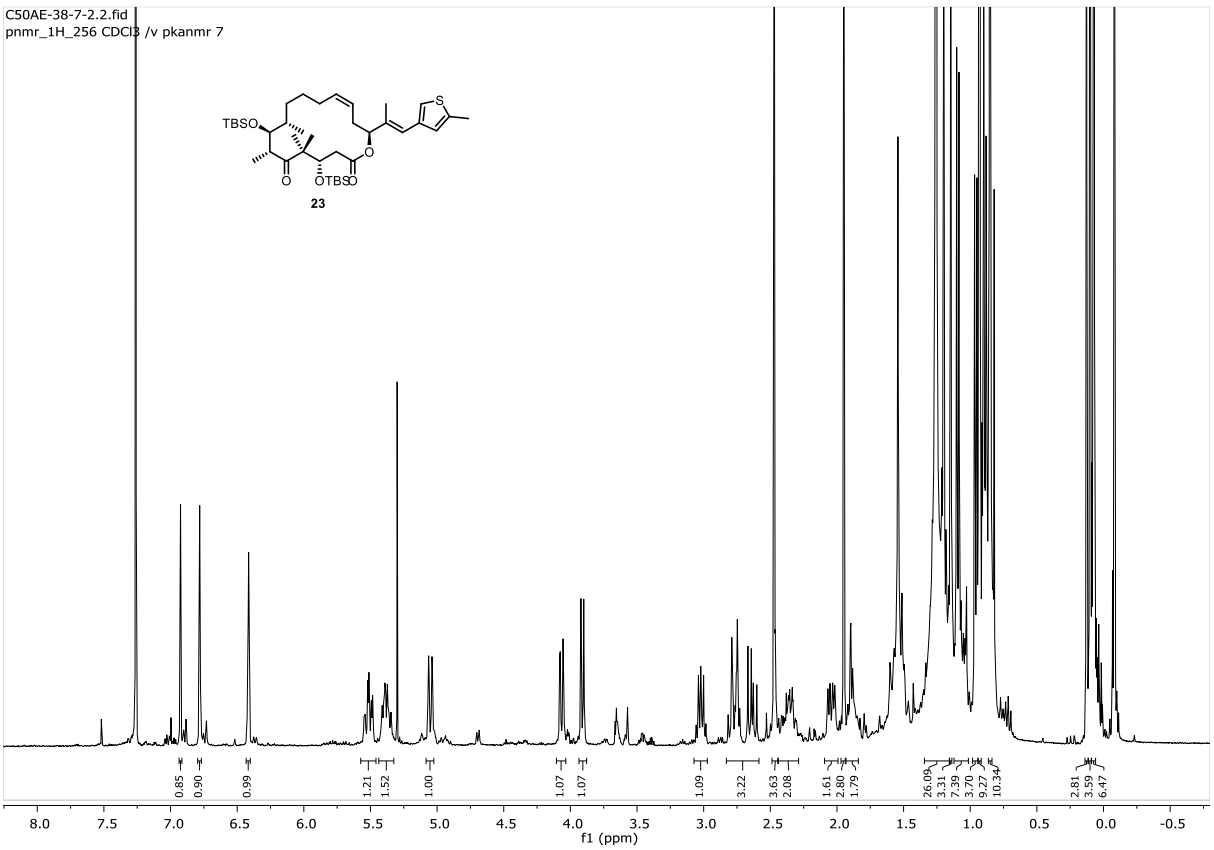
6 **23**

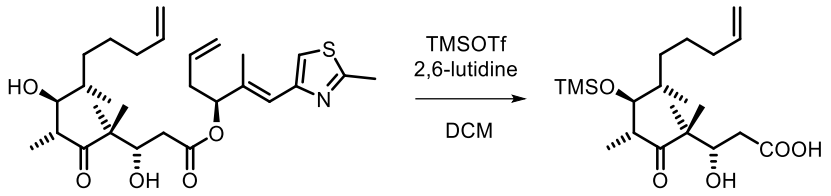
(4S,7R,8S,9S,16S,Z)-4,8-bis((tert-butyldimethylsilyl)oxy)-5,5,7,9-tetramethyl-16-((E)-1-(5-methylthiophen-3-yl)prop-1-en-2-yl)oxacyclohexadec-13-ene-2,6-dione (23): A slightly red solution of **6** (20.0 mg, 27.3 µmol, 1.0 eq) and Grubbs 2nd generation catalyst (2.32 mg, 2.73 µmol, 0.1 eq) in toluene (40 mL) was stirred for 2 h at 40 °C. TLC (hexane/ Et_2O = 19:1, anisaldehyde stain) of the yellow solution indicated that the reaction was not finished yet, nevertheless it was quenched with 2 mL of wet MeOH. The mixture was filtered over silica and the pad was flushed with hexane. The filtrate was concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/Et_2O = 50:1 to 20:1, 2 g, 1 cm, 6 mL) to obtain 3.5 mg of impure **23**. Attempts at further purification of this material by preparative TLC were unsuccessful.

Yield: 3.5 mg (17%);

R_f = 0.46 (hexane/Et_2O = 19:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.93 (s, 1H), 6.78 (t, *J* = 1.2 Hz, 1H), 6.41 (s, 1H), 5.52 (td, *J* = 11.0, 3.8 Hz, 1H), 5.44 – 5.32 (m, 1H), 5.05 (d, *J* = 10.0 Hz, 1H), 4.07 (dd, *J* = 10.2, 1.8 Hz, 1H), 3.91 (d, *J* = 8.7 Hz, 1H), 3.07 – 2.97 (m, 1H), 2.82 – 2.72 (m, 2H), 2.63 (dd, *J* = 16.4, 10.1 Hz, 1H), 2.47 (d, *J* = 1.1 Hz, 3H), 2.46 – 2.29 (m, 1H), 2.04 (dd, *J* = 14.4, 6.4 Hz, 1H), 1.95 (d, *J* = 1.3 Hz, 3H), 1.93 – 1.84 (m, 1H), 1.23 (d, *J* = 23.0 Hz, 7H), 1.15 (s, 3H), 1.12 – 1.01 (m, 4H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.93 (s, 9H), 0.85 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H), 0.07 (s, 3H), 0.07 (s, 3H).


C50AE-38-7-2.2.fid
pnmr_1H_256 CDCl3 /v pkanmr 7
TBSO
O
OTBSO
O
S
23
0.85
0.90
0.99
1.21
1.52
1.00
1.07
1.07
1.09
3.22
3.63
2.08
1.61
2.80
1.79
26.09
3.31
7.39
3.70
9.27
10.34
2.81
3.59
6.47
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)



9 **25**

(3S,6R,7S,8S)-3-Hydroxy-4,4,6,8-tetramethyl-5-oxo-7-((trimethylsilyl)oxy)tridec-12-enoic acid (25): 2,6-Lutidine (2.33 mL, 15.8 mmol, 50 eq) was added to a yellow solution of TMSOTf (1.44 mL, 7.91 mmol, 25 eq) in DCM (3 mL) at 0 °C. This yellow solution was then added to a slightly brown solution of **9** (160.0 mg, 316 µmol, 1.0 eq) in DCM (5 mL) over 10 min at 0 °C. The mixture was stirred for 15 min at 0 °C and for 1 h at RT. MS and TLC (hexane/EtOAc = 2:1 + 2% AcOH) indicated the consumption of **9**, the formation of **25** and a non-polar spot, corresponding to the eliminated thiazole fragment, could be seen on TLC. The mixture was diluted with EtOAc (30 mL) and added dropwise into a beaker filled with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with EtOAc (4 x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (hexane/EtOAc = 2:1 + 1% AcOH, 5 g, 1 cm, 6 mL) to yield 128 mg of **25** containing 1 equiv. of AcOH and 1.2 equiv. of 2,6-lutidine.

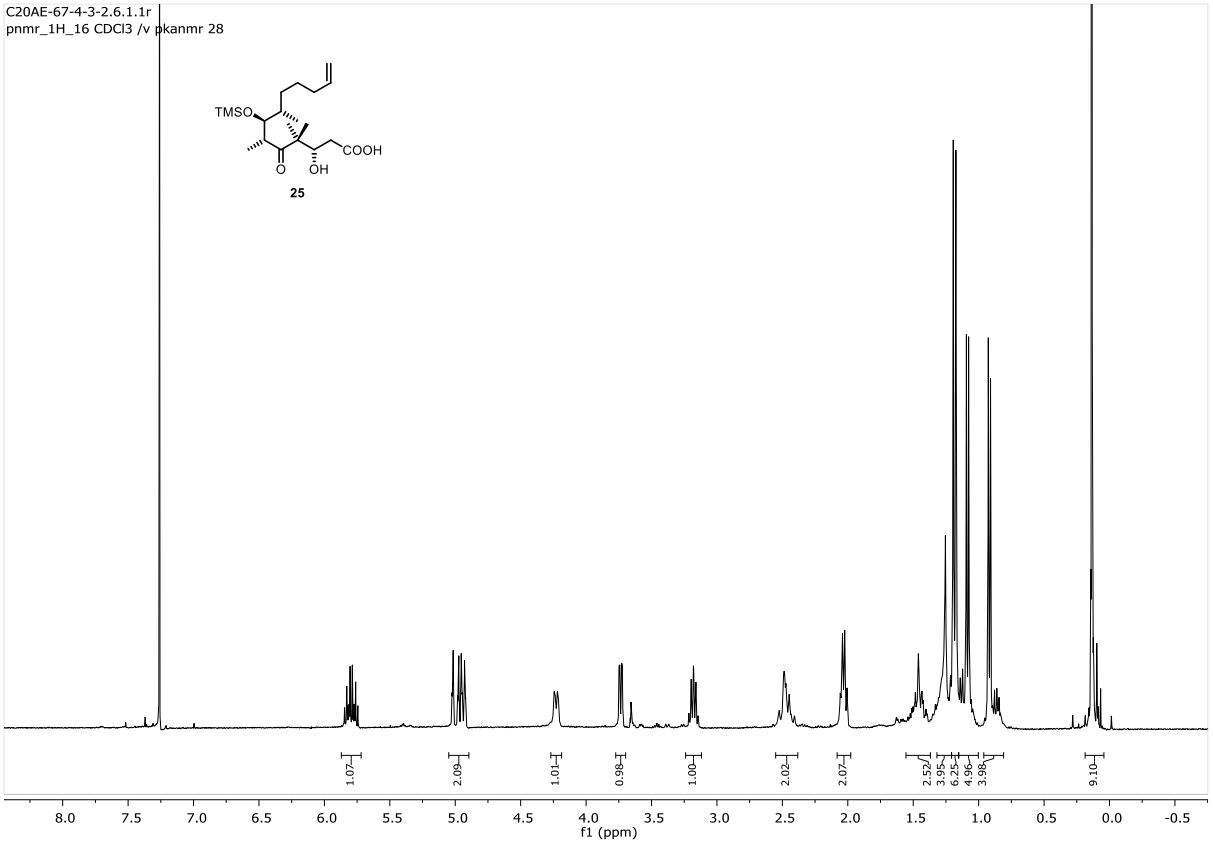
Yield: 128 mg (53%; corrected for residual AcOH and 2,6-lutidine);

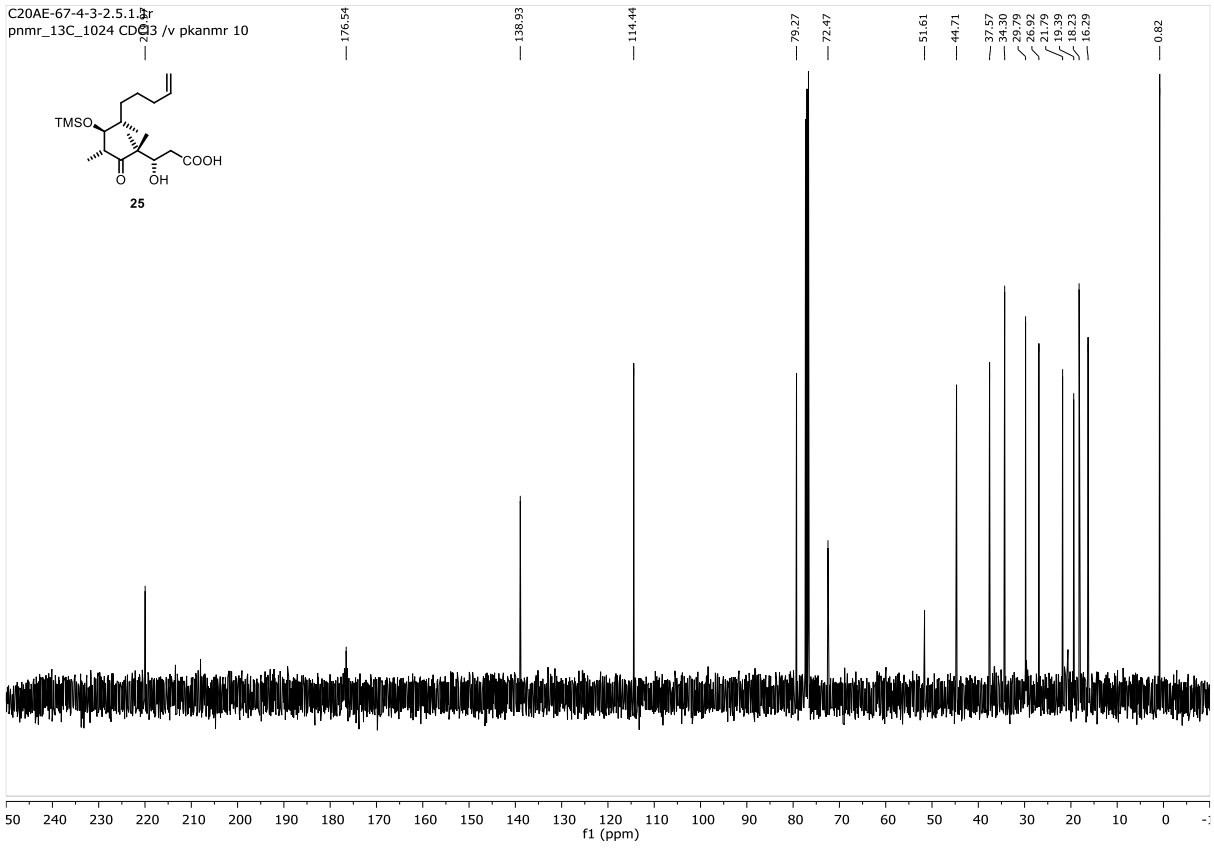
R_f = 0.57 (hexane/AcOEt = 2:1 + 2% AcOH, without 2,6-lutidine), 0.29 (hexane/AcOEt = 2:1 + 2% AcOH, with 2,6-lutidine); 0.12 (hexane/AcOEt = 2:1);

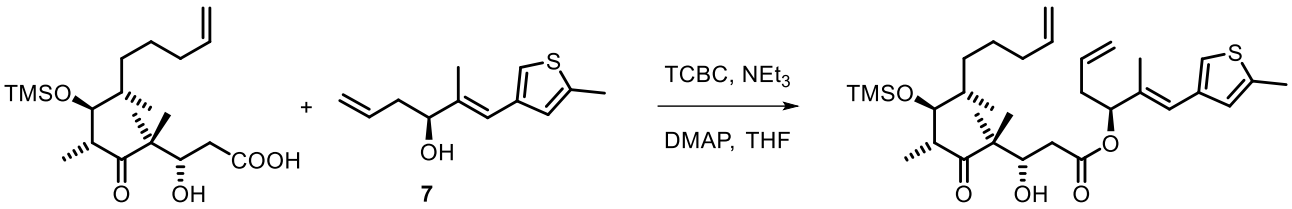
^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.80 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 1H), 5.05 – 4.89 (m, 2H), 4.23 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.74 (dd, *J* = 7.8, 2.5 Hz, 1H), 3.23 – 3.14 (m, 1H), 2.52 – 2.39 (m, 2H), 2.07 – 1.98 (m, 2H), 1.56 – 1.39 (m, 2H), 1.35 – 1.24 (m, 2H), 1.23 – 1.15 (m, 6H), 1.15 – 1.06 (m, 4H), 0.95 – 0.89 (m, 3H), 0.13 (d, *J* = 3.3 Hz, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ = 220.0, 176.3 (HMBC), 138.9, 114.4, 79.3, 72.5, 51.6, 44.7, 37.6, 36.4 (HSQC), 34.3, 29.8, 26.9, 21.8, 19.4, 18.2, 16.3, 0.8;

MS (ESI) *m/z* 409.2 $[M+Na]^+$, 385.5 $[M-H]^-$.


C20AE-67-4-3-2.6.1.1r
pnmr_1H_16 CDCl3 /v pkanmr 28
TMSO
COOH
O
OH
25
1.07
2.09
1.01
0.98
1.00
2.02
2.07
2.52
3.95
6.25
4.96
3.98
9.10
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


C20AE-67-4-3-2.5.1.1r
pnmr_13C_1024 CDCl3 /v pkanmr 10
176.54
138.93
114.44
79.27
72.47
51.61
44.71
37.57
34.30
29.79
26.92
21.79
19.39
18.23
16.29
0.82
TMSO
COOH
O
OH
25
50
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



(3S,6R,7S,8S)-(S,E)-2-Methyl-1-(5-methylthiophen-3-yl)hexa-1,5-dien-3-yl 3-hydroxy-4,4,6,8-tetra-methyl-5-oxo-7-((trimethylsilyl)oxy)tridec-12-enoate (26): Triethylamine (102 µL, 737 µmol, 3.0 eq) followed by DMAP (6.0 mg, 49.1 µmol, 0.2 eq) in THF (1 mL) was added to the colourless solution of 2,4,6-trichlorobenzoyl chloride (TCBC) (42.3 µL, 270 µmol, 1.1 eq), **25** (127 mg, 24.6 µmol (corrected for residual AcOH and 2,6-lutidine, see above), 1.0 eq) and **7** (71.7 mg, 34.4 µmol, 1.4 eq) in THF (4 mL) at 0 °C over 3 min. The mixture was stirred for 2 h at RT. TLC (hexane/EtOAc = 2:1 + 2% AcOH) indicated the consumption of **25** and the formation of several new spots. MS indicated the formation of **26** and **27**. The slightly yellow suspension was diluted with EtOAc (30 mL) and quenched with saturated aqueous $NaHCO_3$ solution (40 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of this material by two runs of flash column chromatography (hexane/EtOAc = 50:1 to 4:1, 6 g, 1 cm, 12 mL and hexane/EtOAc = 10:1, 6 g, 1 cm, 6 mL) gave 67.3 mg of a *ca*. 2:1 (molar) mixture of **26** (*ca*. 37.0 mg, 26%) and **27** (*ca*. 30.3 mg, 13%) together with 28.2 mg (39%) of recovered **25**.

26 could be separated from **27** and purified to near homogeneity by further chromatography, but only at the expense of large losses of material.

Ester 26:

R_f = 0.13 (hexane/AcOEt = 10:1), 0.48 (hexane/EtOAc = 4:1);

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.94 – 6.91 (m, 1H), 6.80 – 6.75 (m, 1H), 6.41 – 6.37 (m, 1H), 5.88 – 5.64 (m, 2H), 5.36 (t, *J* = 6.9 Hz, 1H), 5.16 – 4.91 (m, 4H), 4.24 (ddd, *J* = 9.8, 4.0, 2.8 Hz, 1H), 3.75 (dd, *J* = 7.8, 2.7 Hz, 1H), 3.26 – 3.12 (m, 1H), 2.58 – 2.34 (m, 7H), 2.08 – 1.98 (m, 2H), 1.92 (d, *J* = 1.4 Hz, 3H), 1.52 – 1.40 (m, 2H), 1.35 – 1.22 (m, 3H), 1.17 (d, *J* = 2.9 Hz, 6H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.14 (s, 9H);

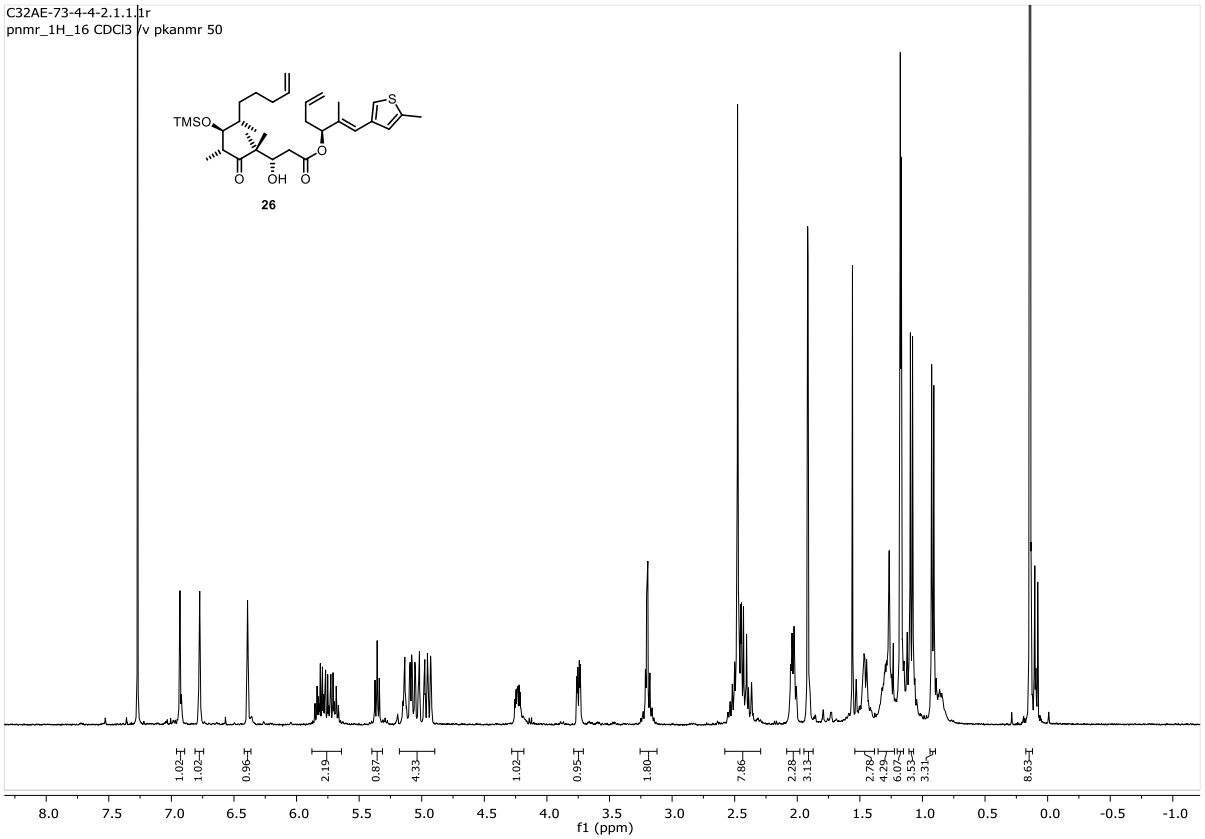
^{13}C NMR (101 MHz, $CDCl_3$) δ = 219.1, 172.1, 139.2, 139.0, 137.9, 133.6, 133.3, 126.9, 123.0, 121.4, 117.8, 114.4, 79.3, 79.3, 72.5, 51.7, 44.7, 37.5, 37.5, 36.9, 34.3, 29.8, 26.9, 21.7, 19.7, 18.2, 16.1, 15.2, 14.2, 0.8;

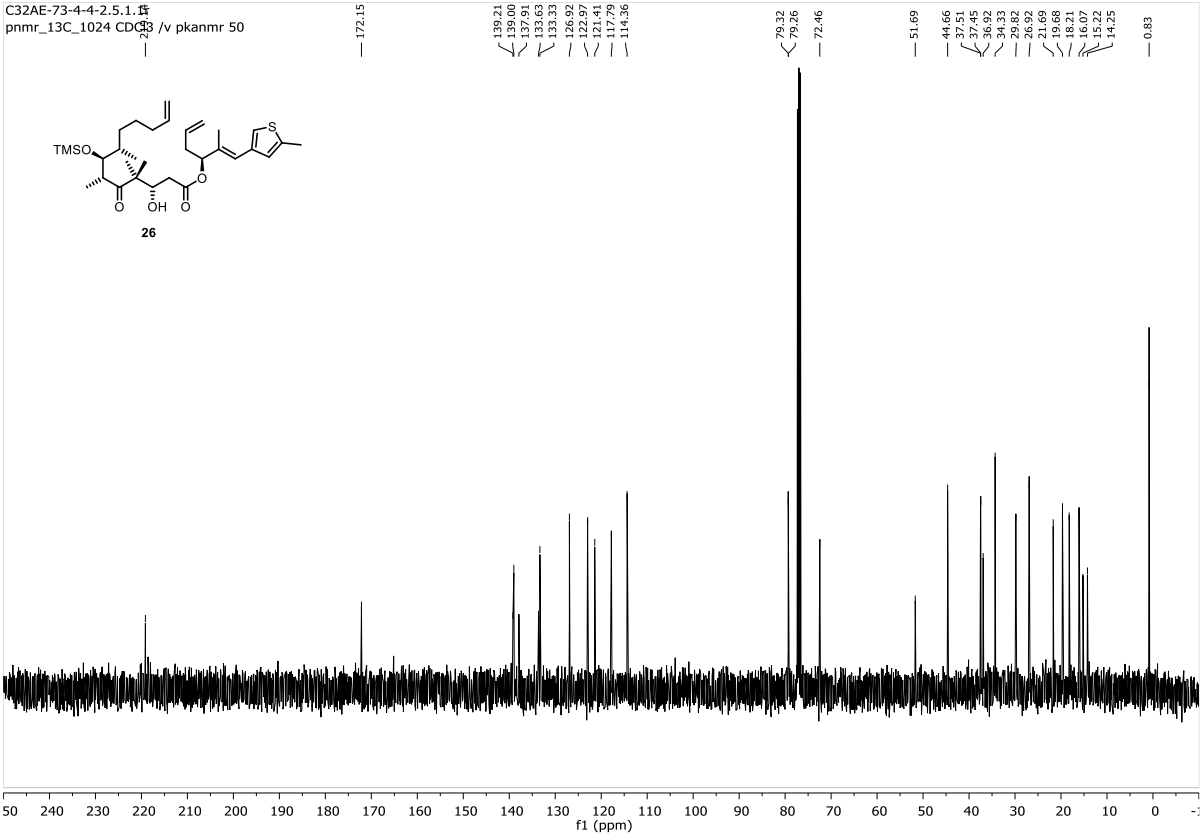
IR (film): ν = 3518br, 3077w, 2959m, 2929m, 2861w, 2371w, 2353w, 1731m, 1696m, 1642w, 1461w, 1371w, 1297w, 1251s, 1173m, 1119w, 1076w, 1044m, 1011m, 987s, 912m, 886s, 839s, 750w;

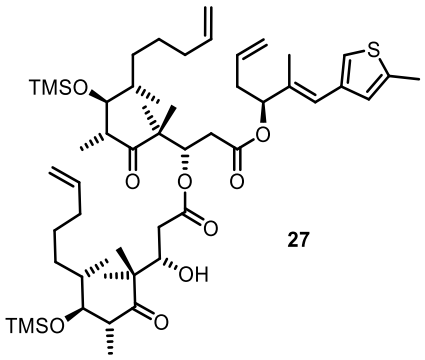
HRMS (ESI-TOF) *m/z* calcd. for $C_{32}H_{52}NaO_5SSi$ $[M+Na]^+$ 599.3197, found 599.3196.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{24}}$= -45.25 (c = 0.304, $CHCl_3$)

HRMS *m/z* (ESI) calcd. for $C_{32}H_{52}NaO_5SSi$ $[M+Na]^+$ 599.3197, found 599.3196.


C32AE-73-4-4-2.1.1.1r
pnmr_1H_16 CDCl3 /v pkanmr 50
TMSO
S
O
OH
O
26
1.02
1.02
0.96
2.19
0.87
4.33
1.02
0.95
1.80
7.86
2.28
3.13
2.78
4.29
6.07
3.53
3.31
8.63
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)


C32AE-73-4-4-2.5.1.1r
pnmr_13C_1024 CDCl3 /v pkanmr 50
172.15
139.21
139.00
137.91
133.63
133.33
126.92
122.97
121.41
117.79
114.36
79.32
79.26
72.46
51.69
44.66
37.51
37.45
36.92
34.33
29.82
26.92
21.69
19.68
18.21
16.07
15.22
14.25
0.83
TMSO
S
O
OH
O
26
50
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

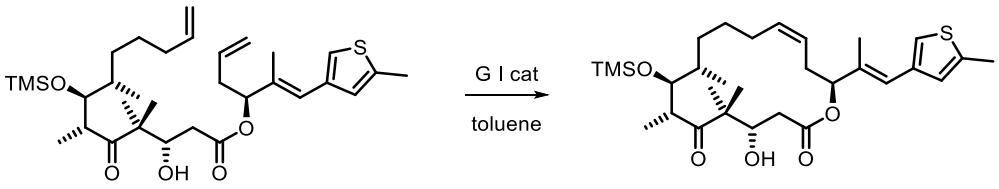


27

(3S,6R,7S,8S)-(S,E)-2-Methyl-1-(5-methylthiophen-3-yl)hexa-1,5-dien-3-yl 3-(((3S,6R,7S,8S)-3-hydroxy-4,4,6,8-tetramethyl-5-oxo-7-((trimethylsilyl)oxy)tridec-12-enoyl)oxy)-4,4,6,8-tetramethyl-5-oxo-7-((trimethylsilyl)oxy)tridec-12-enoate (27)

R_f = 0.13 (hexane/EtOAc = 10:1), 0.48 (hexane/EtOAc = 4:1)

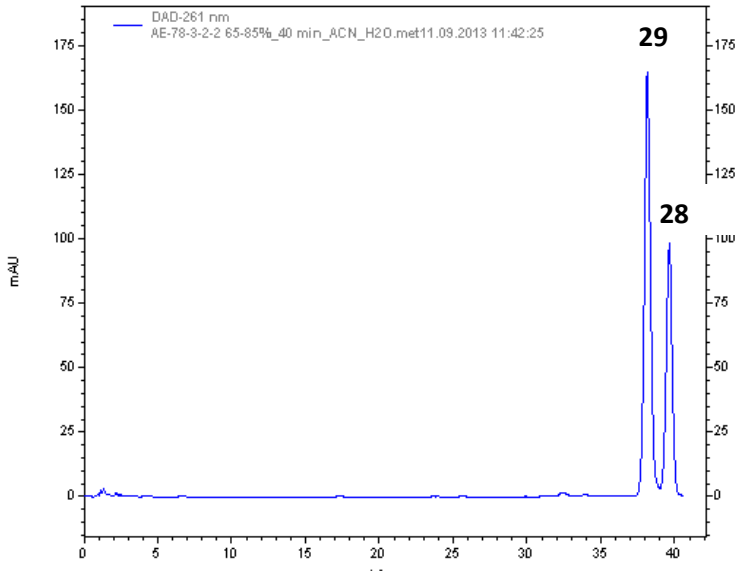
MS (ESI) *m/z* 967.6 $[M+Na]^+$.



26 **28**

(4S,7R,8S,9S,16S,Z)-4-Hydroxy-5,5,7,9-tetramethyl-16-((E)-1-(5-methylthiophen-3-yl)prop-1-en-2-yl)-8-((trimethylsilyl)oxy)oxacyclohexadec-13-ene-2,6-dione (28): A slightly violet solution of a mixture of **26** and **27** in toluene (75 mL), containing *ca*. 37 mg of **26** (64.1 µmol, 1.0 eq), was stirred for 16 h at RT in the presence of Grubbs 1st generation catalyst (8.6 mg, 10.4 µmol, 0.1 eq). MS and TLC (hexane/EtOAc = 4:1) indicated the disappearance of **26**. Ethylvinylether was added and after 30 min of stirring the mixture was flushed through a pad of silica to remove the catalyst and the filtrate was then concentrated under reduced pressure. The residue was purified by RP-HPLC (Symmetry C18 5 µm, 19 x 100 mm, 70:30 to 85:15 ACN/H_2O within 40 min to pure ACN for 20 min) to yield 14.3 mg of a mixture of **28** and its 12,13-*trans* isomer **29** together with 6.9 mg (19%) of **26** and 36.4 mg of what we tentatively assign as dimeric side products that were not characterized. The mixture of **28** and **29** was submitted to a second RP-HPLC run (Symmetry C18 5 µm, 19 x 100 mm, 70:30 to 85:15 ACN/H_2O within 40 min) to furnish 4.5 mg (13%) of **28** (peak 2) and 6.6 mg (19%) of **29**. (Yields are based on the amount of **26** present in the **26**/**27** mixture at the beginning the reaction).

Separation of **28** and **29** by RP-HPLC (second run)



28:

Yield: 4.5 mg (13%);

R_f = 0.44 (hexane/AcOEt = 4:1), 0.74 (hexane/EtOAc = 2:1);

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$**:** -7.24 (c = 0.225 g/100 mL, $CHCl_3$);

^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.13 (d, *J* = 0.8 Hz, 1H), 6.86 (t, *J* = 1.0 Hz, 1H), 6.42 (s, 1H), 5.50 – 5.32 (m, 2H), 5.16 – 5.07 (m, 2H), 4.06 (ddd, *J* = 10.1, 6.6, 3.2 Hz, 1H), 3.71 (dd, *J* = 6.9, 1.7 Hz, 1H), 3.12 (quint, *J* = 6.8 Hz, 1H), 2.66 (dt, *J* = 14.5, 9.4 Hz, 1H), 2.48 – 2.30 (m, 5H), 2.27 – 2.17 (m, 1H), 2.14 (dd,

J = 14.3, 5.2 Hz, 1H), 1. 96-1.85 (m, 4H), 1.61 – 1.47 (m, 1H), 1.44 – 1.33 (m, 2H), 1.19 (s, 3H), 1.09-0.99 (m, 5H), 0.92 (s, 3H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.10 (s, 9H);

^{1}H NMR (500 MHz, DMSO-d_6, irradiated at 2.203 ppm, C12&13) δ = 5.48 (d, *J* = 10.8 Hz, 1H), 5.42 (dd, *J* = 10.9, 7.2 Hz, 1H);

^{1}H NMR (500 MHz, DMSO-d_6, irradiated at 2.651 ppm, C12&13) δ = 5.52 – 5.45 (m, 1H), 5.42 (d, *J* = 11.0 Hz, 1H);

^{13}C NMR (126 MHz, DMSO-d_6) δ = 217.1, 170.1, 138.5, 137.9, 135.0, 133.1, 127.1, 124.6, 121.4, 119.9, 78.3, 78.2, 71.1, 53.2, 44.1, 38.7, 37.9, 31.2, 30.6, 27.6, 27.5, 22.4, 20.7, 17.3, 16.5, 14.9, 14.8, 0.8;

IR (film): ν = 3483br, 3357br, 2958w, 2929w, 2879w, 1731m, 1694m, 1461w, 1369w, 1249m, 1154w, 1114w, 1046m, 1008m, 987m, 886s, 837s, 754m, 687w, 631w;

HRMS (ESI-TOF) *m/z* calcd. for $C_{30}H_{49}O_5SSi$ $[M+H]^+$ 549.3064, found 549.3055.

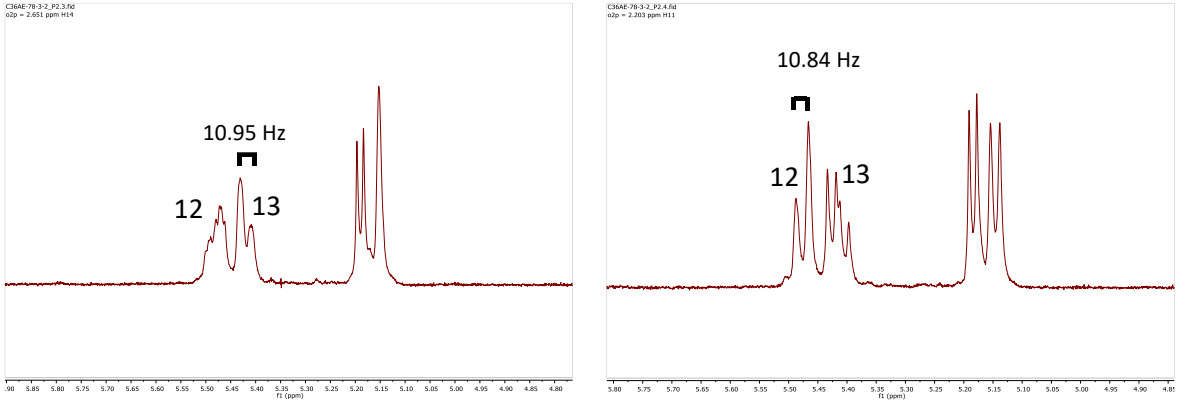
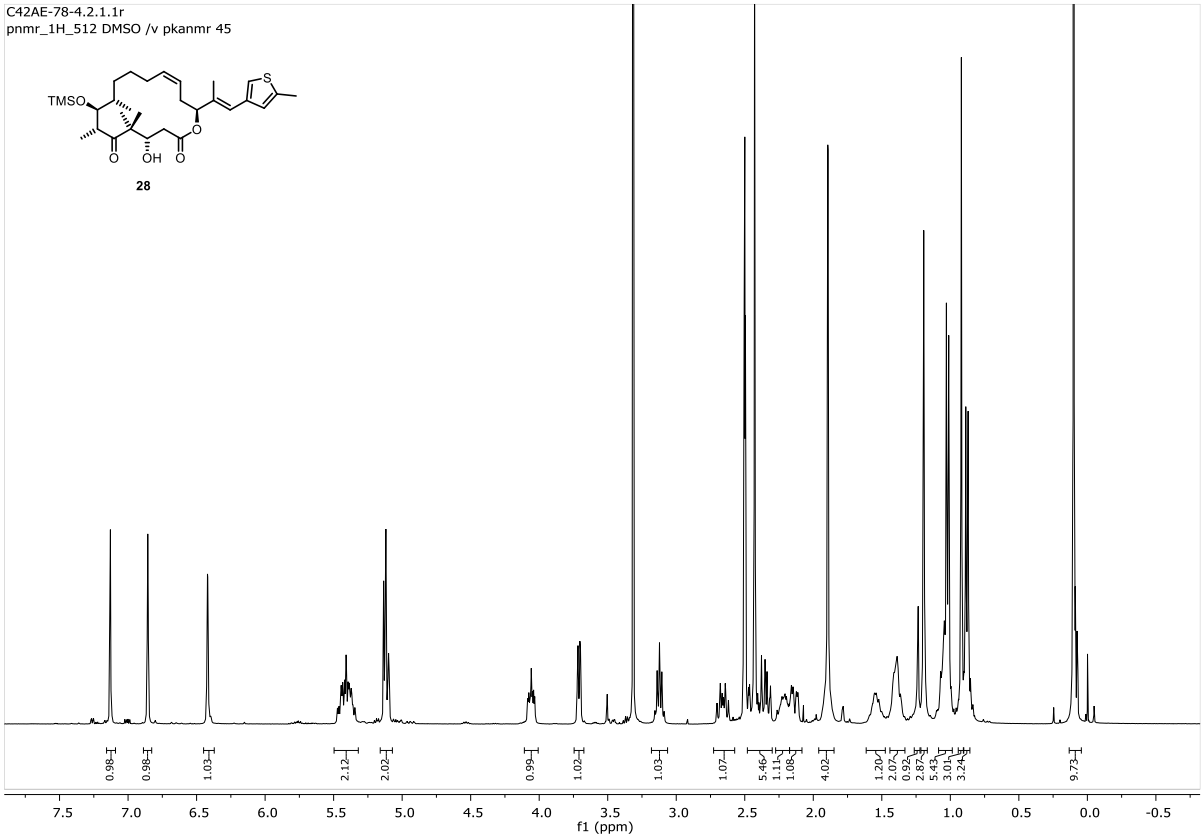
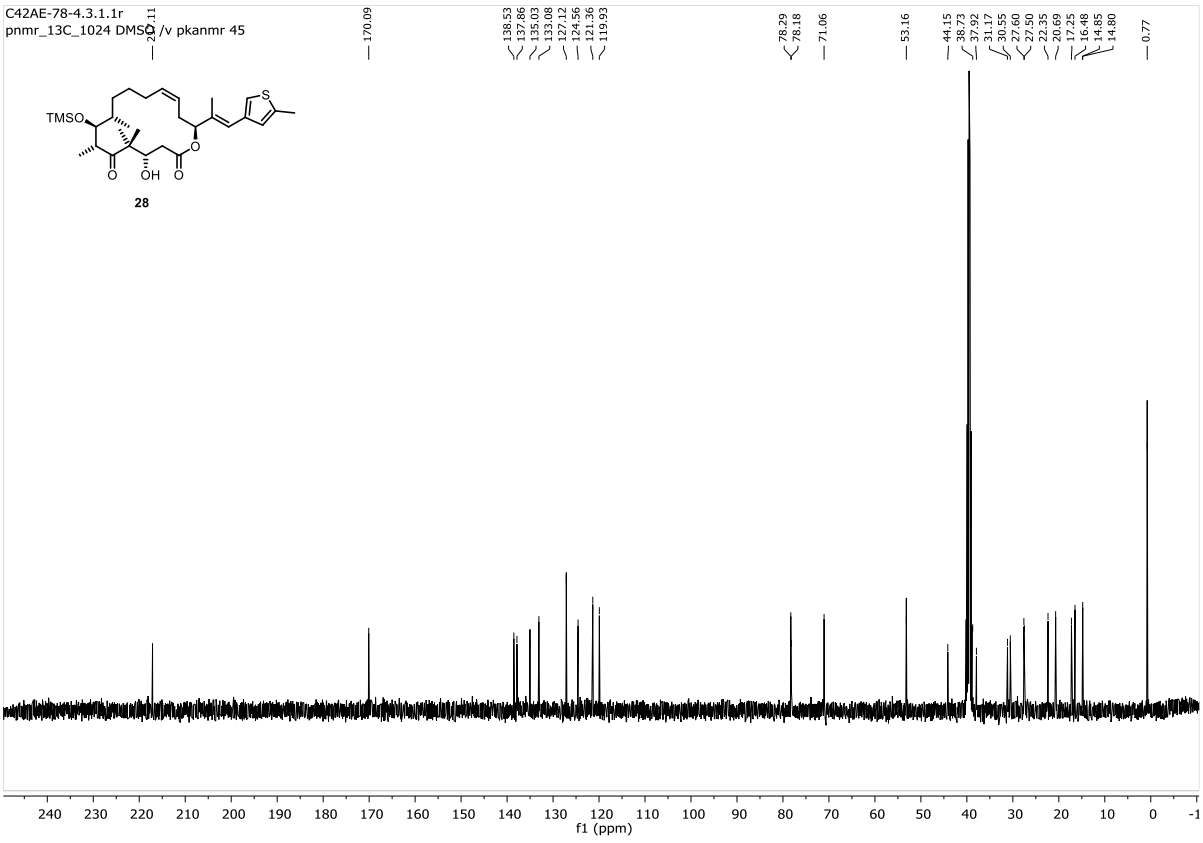
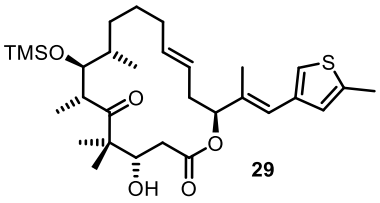


Figure S1: Decoupling experiments with **28**. Irraditation of a C14 proton (2.65 ppm, left) and of a C11 proton (2.20 ppm, right).


C42AE-78-4.2.1.1r
pnmr_1H_512 DMSO /v pkanmr 45
TMSO
O
OH
O
O
S
28
0.98
0.98
1.03
2.12
2.02
0.99
1.02
1.03
1.07
5.46
1.11
1.08
4.02
1.20
2.07
0.92
2.87
5.43
3.01
3.24
9.73
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


C42AE-78-4.3.1.1r
pnmr_13C_1024 DMSO /v pkanmr 45
TMSO
O
OH
O
O
S
28
170.09
138.53
137.86
133.03
133.08
127.12
124.56
121.36
119.93
78.29
78.18
71.06
53.16
44.15
38.73
37.92
31.17
30.55
27.60
27.50
22.35
20.69
17.25
16.48
14.85
14.80
0.77
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)



29

(4S,7R,8S,9S,16S,E)-4-Hydroxy-5,5,7,9-tetramethyl-16-((E)-1-(5-methylthiophen-3-yl)prop-1-en-2-yl)-8-((trimethylsilyl)oxy)oxacyclohexadec-13-ene-2,6-dione (29)

Yield: 6.6 mg (16%);

R_f = 0.41 (hexane/EtOAc = 4:1), 0.68 (hexane/EtOAc = 2:1);

^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.11 (s, 1H), 6.86 (s, 1H), 6.41 (s, 1H), 5.42 – 5.26 (m, 2H), 5.25 – 5.18 (m, 2H), 4.53 (q, *J* = 6.3 Hz, 1H), 3.70 (d, *J* = 8.7 Hz, 1H), 3.33 – 3.25 (m, 1H), 2.61 – 2.49 (m, 2H), 2.45 – 2.32 (m, 5H), 2.16 (d, *J* = 13.4 Hz, 1H), 1.90 (d, *J* = 1.4 Hz, 3H), 1.78 (quint, *J* = 9.1 Hz, 1H), 1.56 (d, *J* = 8.6 Hz, 2H), 1.45 (dq, *J* = 12.2, 6.9, 5.0 Hz, 1H), 1.17 – 1.09 (m, 1H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.00 (s, 4H), 0.90 (s, 3H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.10 (s, 9H);

^{1}H NMR (500 MHz, DMSO-d_6, irradiated at 1.778 ppm, C12&13) δ = 5.40 (dd, *J* = 15.4, 3.9 Hz, 0.5H), 5.34 (dt, *J* = 15.5, 5.9 Hz, 0.5H), 5.28 – 5.22 (m, 1H);

^{1}H NMR (500 MHz, DMSO-d_6, irradiated at 2.566 ppm, C12&13) δ = 5.46 – 5.37 (m, 0.5H), 5.34 (d, *J* = 15.2 Hz, 0.5H), 5.28 – 5.22 (m, 1H);

^{13}C NMR (126 MHz, DMSO-d_6) δ = 217.7, 170.0, 138.8, 138.0, 134.1, 133.1, 127.0, 125.6, 121.3, 119.4, 79.5, 76.5, 70.2, 53.1, 43.9, 39.2, 37.3, 35.2, 32.0, 27.5, 25.9, 21.6, 18.6, 18.3, 16.9, 15.6, 14.9, 0.9.

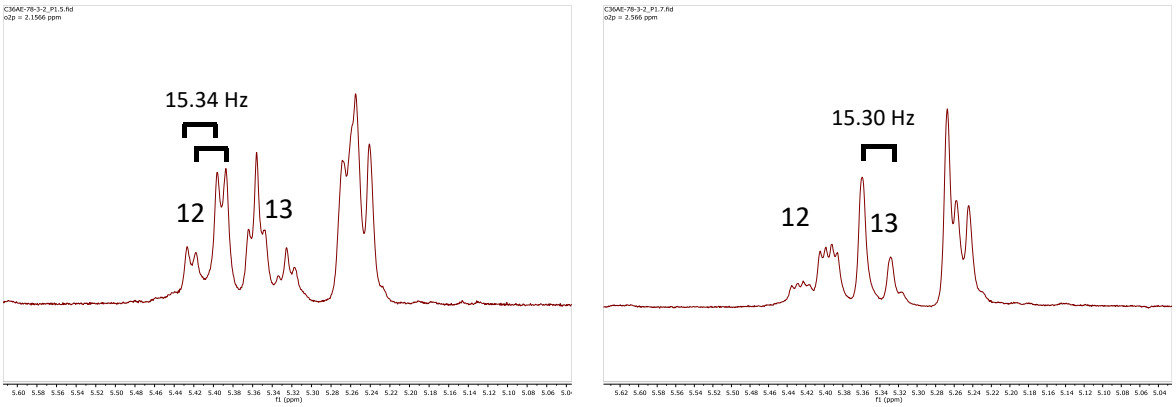
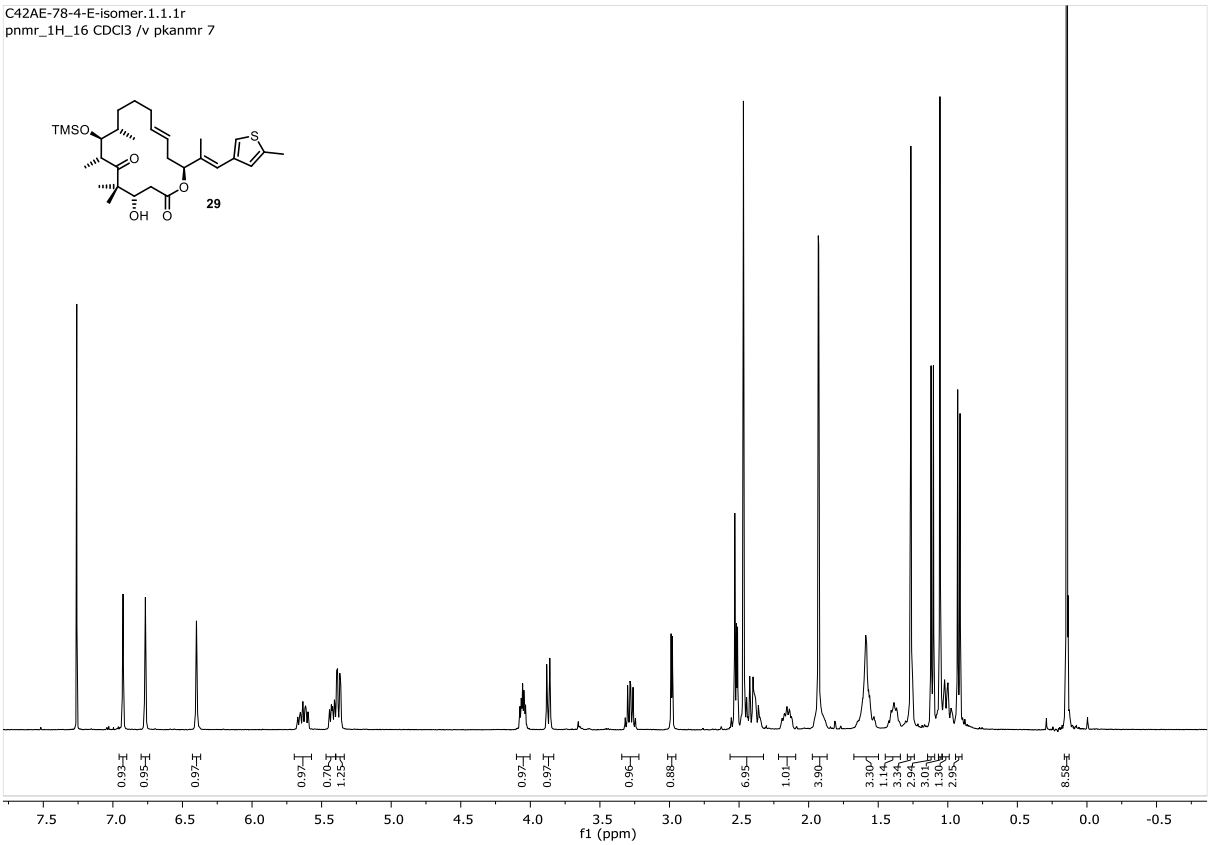
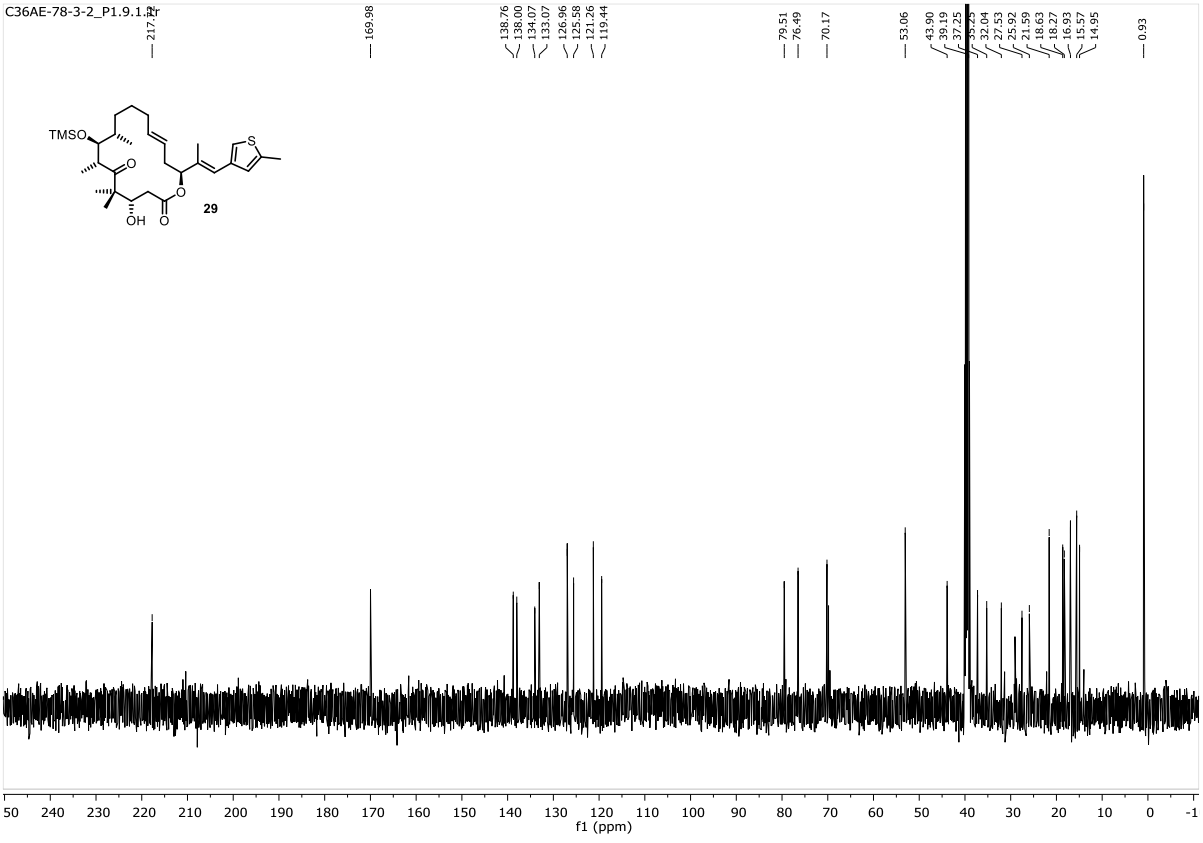
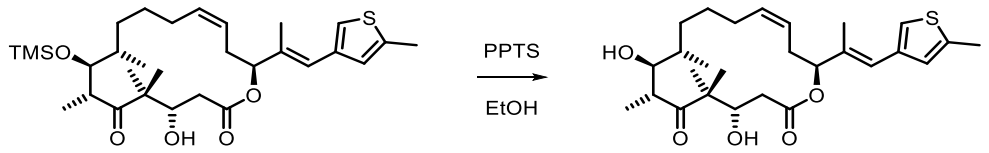


Figure S2: Decoupling experiments with **29**. Irraditation of a C11 proton (2.16 ppm, left) and of a C14 proton (2.20 ppm, right).


C42AE-78-4-E-isomer.1.1.1r
pnmr_1H_16 CDCl3 /v pkanmr 7
TMSO
S
O
O
OH
O
29
0.93
0.95
0.97
0.97
0.70
1.25
0.97
0.97
0.96
0.88
6.95
1.01
3.90
3.30
1.14
3.34
2.94
3.01
1.30
2.95
8.58
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


C36AE-78-3-2_P1.9.1.1r
217.42
169.98
138.76
138.00
134.07
133.07
126.96
125.58
121.26
119.44
79.51
76.49
70.17
53.06
43.90
39.19
37.25
35.23
32.04
27.53
25.92
21.59
18.63
18.27
16.93
15.57
14.95
0.93
TMSO
S
O
O
OH
O
29
50
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)



28 **5**

(4S,7R,8S,9S,16S,Z)-4,8-Dihydroxy-5,5,7,9-tetramethyl-16-((E)-1-(5-methylthiophen-3-yl)prop-1-en-2-yl)oxacyclohexadec-13-ene-2,6-dione (5): PPTS (2.31 mg, 9.18 µmol, 1.2 eq) was added to a colourless solution of **28** (4.2 mg, 7.65 µmol, 1.0 eq) in EtOH (3 mL) at 0 °C. The colourless solution was stirred at for 1.5 h at 0 °C. TLC (hexane/EtOAc = 4:1) indicated only traces of remaining **28**. The colourless solution was diluted with EtOAc (10 mL) and quenched with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude (3.8 mg) was purified by reversed phase prep column (Symmetry C18 5 µm, 19 x 100 mm, 40:60 to 75:25 to 100:0 ACN/H_2O within 25 min) to obtain 2.43 mg (67%) of **28** as a white solid.

Yield: 2.43 mg (67%);

R_f = 0.16 (hexane/AcOEt = 4:1), 0.44 (hexane/EtOAc = 2:1);

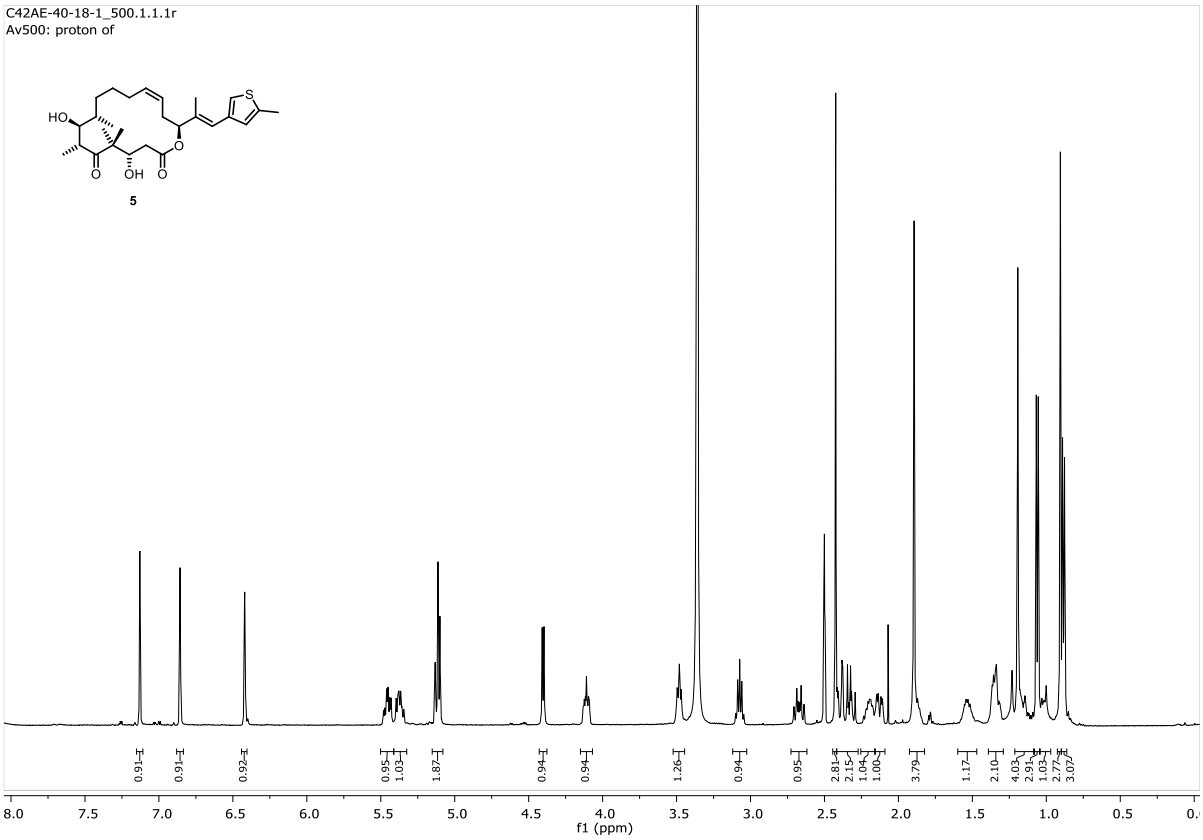
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$**:** -16.67 (c = 0.059, $CHCl_3$);

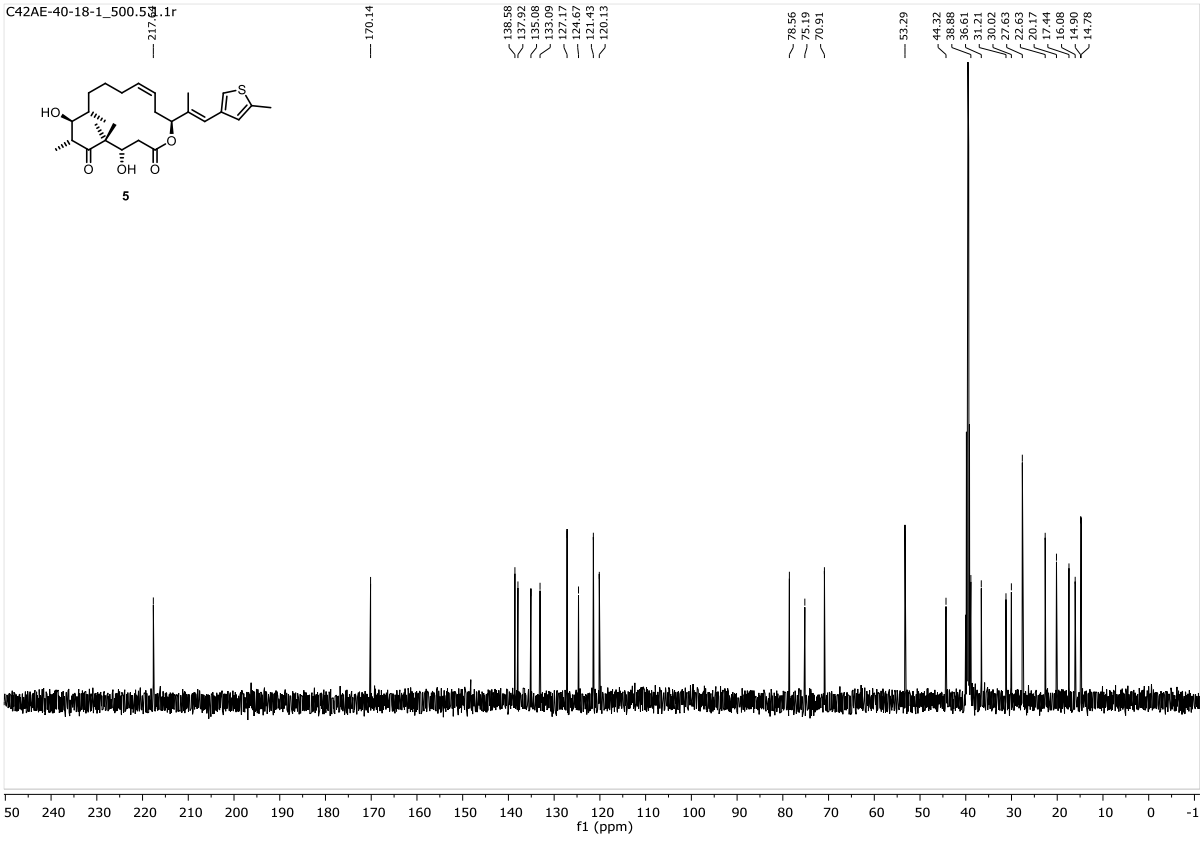
^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.13 (s, 1H), 6.86 (s, 1H), 6.42 (s, 1H), 5.46 (td, *J* = 10.6, 4.7 Hz, 1H), 5.37 (td, *J* = 10.0, 5.7 Hz, 1H), 5.15 – 5.08 (m, 2H), 4.40 (d, *J* = 5.9 Hz, 1H), 4.11 (ddd, *J* = 10.3, 6.7, 3.3 Hz, 1H), 3.52 – 3.45 (m, 1H), 3.07 (dq, *J* = 6.7 Hz, 1H), 2.67 (ddd, *J* = 14.6, 9.6 Hz, 1H), 2.42 (d, *J* = 1.1 Hz, 3H), 2.42 – 2.27 (m, 2H), 2.25 – 2.16 (m, 1H), 2.13 (dd, *J* = 14.6, 5.8 Hz, 1H), 1.93 – 1.82 (m, 4H), 1.60 – 1.47 (m, 1H), 1.39 – 1.29 (m, 2H), 1.21 – 1.09 (m, 4H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.04 – 0.97 (m, 1H), 0.90 (s, 3H), 0.88 (d, *J* = 6.7 Hz, 3H);

^{13}C NMR (126 MHz, DMSO-d_6) δ = 217.6, 170.1, 138.6, 137.9, 135.1, 133.1, 127.2, 124.7, 121.4, 120.1, 78.6, 75.2, 70.9, 53.3, 44.3, 38.9, 36.6, 31.2, 30.0, 27.6, 22.6, 20.2, 17.4, 16.1, 14.9, 14.8;

IR (film): ν = 3449br, 3354br, 2958w, 2925m, 2855w, 1729m, 1685m, 1460w, 1375w, 1334w, 1259m, 1148w, 1090m, 1023s, 1010s, 981m, 878w, 801s, 758m, 700w, 663w;

HRMS (ESI-TOF) *m/z* calcd. for $C_{27}H_{41}O_5S$ $[M+H]^+$ 477.2669, found 477.2667.


C42AE-40-18-1_500.1.1.1r
Av500: proton of
HO
O
OH
O
O
S
5
0.91
0.91
0.92
0.95
1.03
1.87
0.94
0.94
1.26
0.94
0.95
2.81
2.15
1.04
1.00
3.79
1.17
2.10
4.03
2.91
1.03
2.77
3.07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


C42AE-40-18-1_500.5.1.1r
217.11
170.14
138.58
137.92
135.08
133.09
127.17
124.67
121.43
120.13
78.56
75.19
70.91
53.29
44.32
38.88
36.61
31.21
30.02
27.63
22.63
20.17
17.44
16.08
14.90
14.78
HO
O
OH
O
O
S
5
50
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

Stability assessment of deaza-Epo C (5) in cell culture medium

The stability of deaza-Epo C (**5**) in the cell culture media used for the cell proliferation experiments was determined by RP-HPLC. The media investigated were Flak (Ham's F-12K 21127) supplemented with 10% fetal bovine serum and 1% antibodies (used for experiments with A 549 cells) and DMEM (high glucose 41965) with 10% fetal bovine serum, 1% antibodies and 1% L-glutamine (used for experiments with MCF-7 cells). The compound was dissolved in the respective cell culture medium containing 1% DMSO at a concentration of 100 µM and the solution was incubated at 40 °C. Samples were removed at different time points and analyzed by RP-HPLC. Due to the limited sensitivity of the detection method (UV absorption at 250 nm), the stability studies were conducted at a concentration 10 times higher than the highest concentration applied in the proliferation experiments.

As illustrated in Fig. S3, deaza-Epo C (**5**) decomposed in DMEM medium with a half-life of *ca*. 2 h, as indicated by the diminishing intensity of the peak for the parent compound. After 24 h, **5** had completely disappeared without any distinctive decomposition products being detectable at 250 nm (Fig. S4). Similar results were obtained with FLAK medium (data not shown).

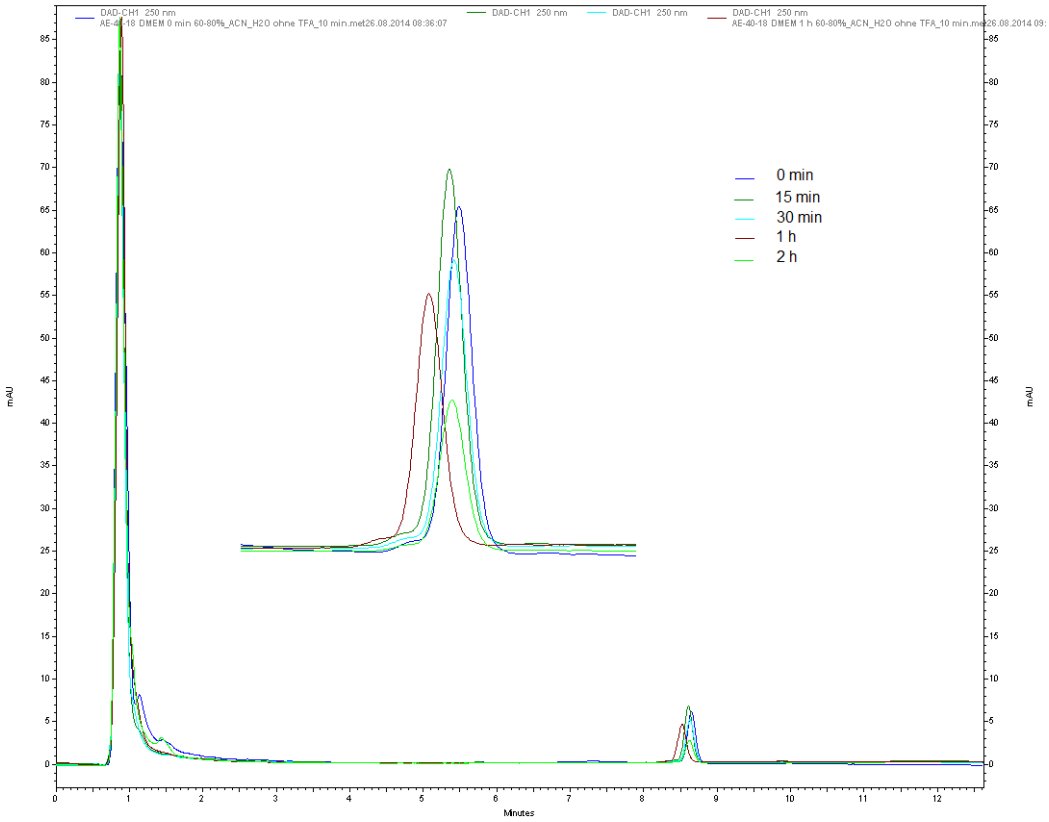


Figure S3: Stability of deaza-Epo C (**5**) in DMEM over a 2 h time period. HPLC traces obtained with a Waters Symmetry C18 3.5 µm, 4.6x100 mm column. Elution method: Gradient from 60:40 to 80:20 ACN/H_2O within 12 min, flow rate: 1 mL/min. Detection at 250 nm.

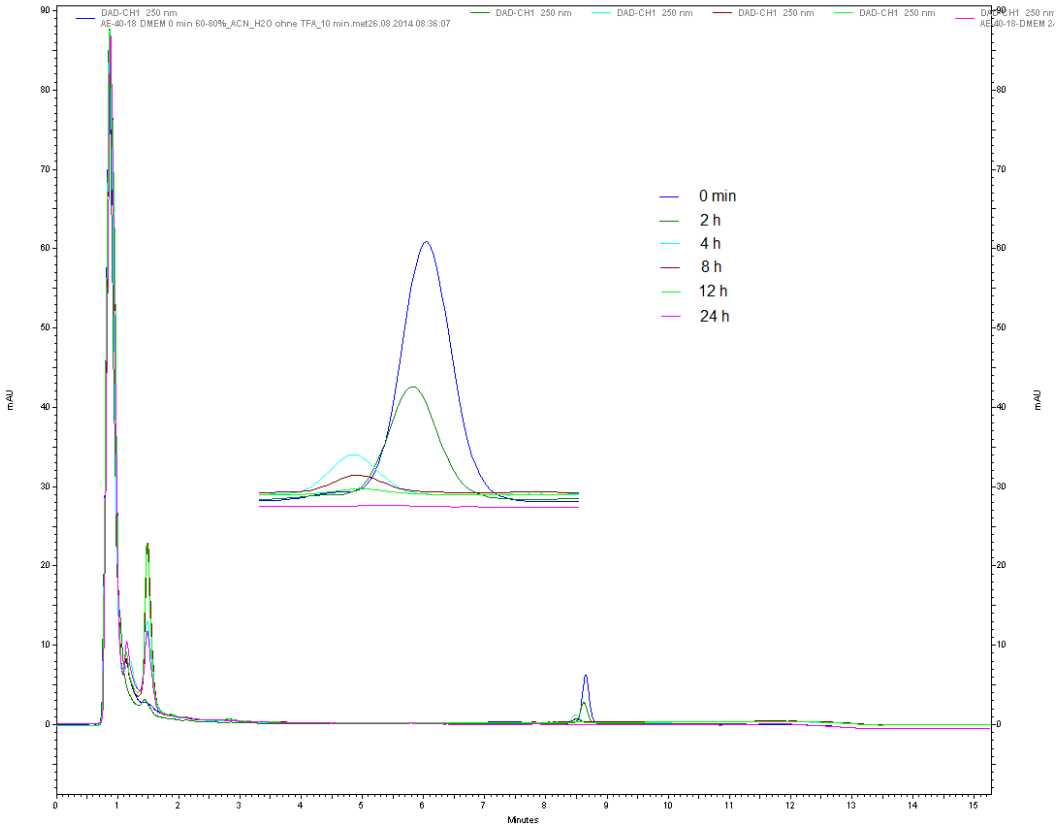


Figure S4: Stability of deaza-Epo C (**5**) in DMEM over a 24 h time period. HPLC traces obtained with a Waters Symmetry C18 3.5 µm, 4.6x100 mm column. Elution method: Gradient from 60:40 to 80:20 ACN/H_2O within 12 min, flow rate: 1 mL/min. Detection at 250 nm.

Experiments in DMEM were also carried out with Epo C, which was found to be significantly more stable (Figs. S5 and S6).

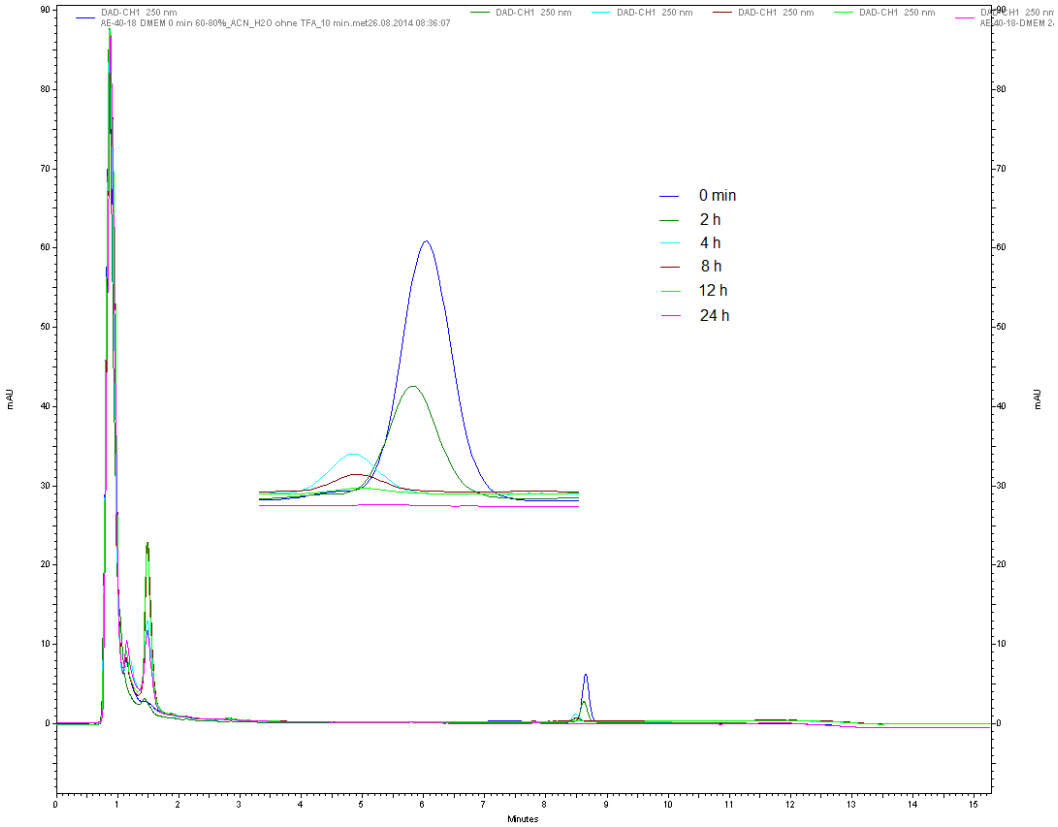


Figure S5: Stability of **Epo C** in DMEM over a 2 h time period. HPLC traces obtained with a Waters Symmetry C18 3.5 µm, 4.6x100 mm column. Elution method: Gradient from 60:40 to 80:20 ACN/H_2O within 12 min, flow rate: 1 mL/min. Detection at 250 nm.

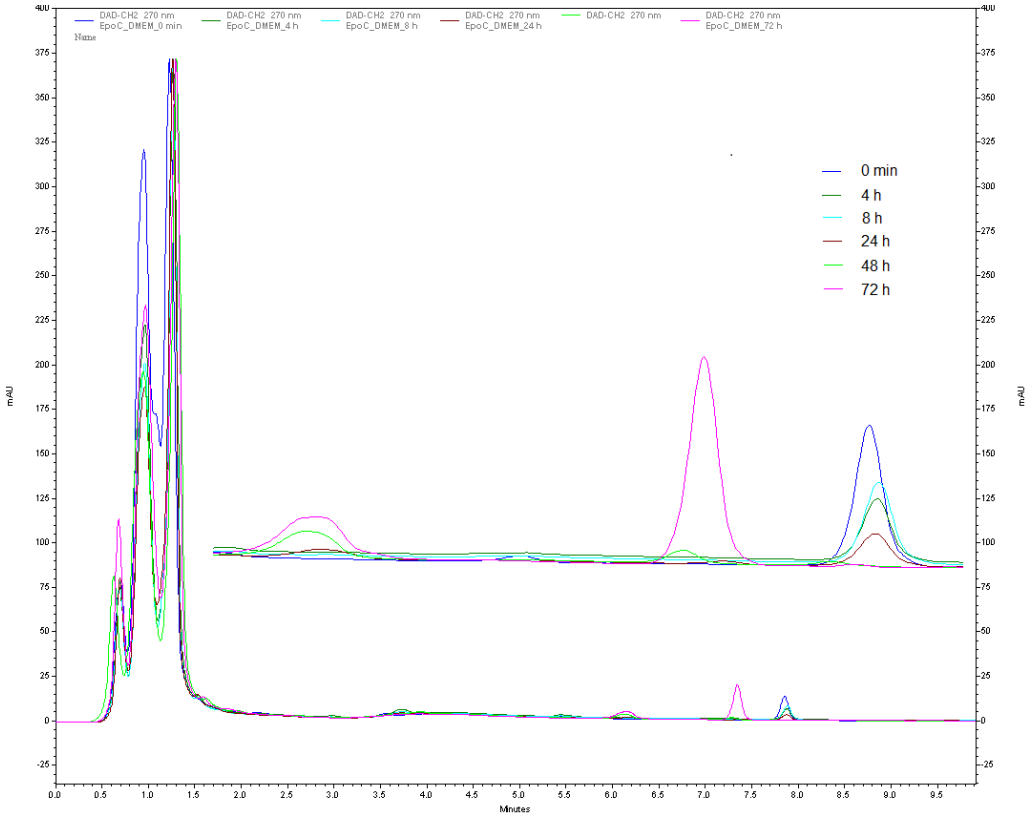


Figure S6: Stability of **Epo C** in DMEM over a 24 h time period. HPLC traces obtained with a Waters Symmetry C18 3.5 µm, 4.6x100 mm column. Elution method: Gradient from 60:40 to 80:20 ACN/H_2O within 12 min, flow rate: 1 mL/min. Detection at 250 nm.