Supplementary Information

Isolation, Total Synthesis and Structure Determination of Antifungal Macrocyclic Depsipeptide, Tetraselide

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1. General considerations

1-1. Solvents and Reagents

Unless otherwise noted, commercial reagents were purchased from Sigma Aldrich, Combi-blocks, TCI, Strem Chemicals, FUJIFILM Wako Pure Chemical Co., Watanabe Chemical Industries LTD., BLDPharm and/or Kanto Chemical Co., and used without additional purification. Solvents were purchased from Sigma Aldrich, TCI and/or Kanto Chemical Co, and used without additional purification (stored over molecular sieves). THF and DCM were sparged with argon and dried by passing through alumina columns using argon in a Glass Contour solvent purification system.

1-2. Experimental Procedures

Unless otherwise noted in the experimental procedures, reactions were carried out in flame- or oven-dried glassware under a positive pressure of N_2 in anhydrous solvents using standard Schlenk techniques. Reaction temperatures above room temperature (20–25 °C) were controlled by an IKA® temperature modulator or AS ONE Co. Oil Bath and monitored using liquid-in-glass thermometers. Reaction progress was monitored by thin-layer chromatography (TLC) on Sigma Aldrich/Millipore silica gel TLC plates (60 Å, F254 indicator). TLC plates were visualized by exposure to ultraviolet light (254 nm), and/or stained by submersion in *p*-anisaldehyde, ninhydrin, 2,4-dinitrophenyl hydrazine, or phosphomolybdic acid stains and heating with a heat gun or heating plate. Organic solutions were concentrated under reduced pressure on an EYELA temperature-controlled rotary evaporator equipped with a cooling condenser. Flash column chromatography was performed with glass columns using Kanto Chemical silica gel (60 N, spherical neutral, 40–50 μm particle size), using ACS grade solvents. All yields refer to spectroscopically (^{1}H and ^{13}C NMR) pure material.

1-3. Analytical Instrumentation

^{1}H NMR and ^{13}C NMR data were recorded on a JEOL JNM-ECA-500 (500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR) spectrometer or Agilent MR-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) using $CDCl_3$, CD_3OD, or $(CD_3)_2SO$ as a solvent, typically at 20–23 °C. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (δ 7.26 for ^{1}H NMR & δ 77.2 for ^{13}C NMR in $CDCl_3$, δ 3.31 for ^{1}H NMR & δ 49.0 for ^{13}C NMR in CD_3OD, and δ 2.49 for ^{1}H NMR & δ 39.5 for ^{13}C NMR in $(CD_3)_2SO$). Data for ^{1}H and ^{13}C spectroscopy are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent), coupling constant (Hz), integration. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-AX505HA, JEOL JMS-700 MStation and/or JEOL JMS-T100LP. Data acquisition and processing were performed using the Xcalibur™ software. Optical rotations were measured on a JASCO P-2020 polarimeter.

2. Isolation and Structure Elucidation of Tetraselide

2-1. Fermentation and Isolation

Strain *Trichoderma* sp. FKJ-0225 was grown and maintained on an agar slant containing 0.1% glycerol, 0.08% KH_2PO_4, 0.02% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% KCl, 0.2% $NaNO_3$, 0.02% yeast extract and 1.5% agar (adjusted to pH 6.0 before sterilization). A loopful of spores of the strain was inoculated into 100 mL of the seed medium containing 2.0% glucose, 0.5% Hipolypepton (Nihon Pharmaceutical Co. Ltd., Tokyo, Japan), 0.2% yeast extract, 0.2% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.1% agar (adjusted to pH 6.0 before sterilization) in twelve 500 mL-Erlenmeyer flasks. The flasks were incubated on a rotary shaker (210 rpm) at 27 °C for three days. The seed cultures (40 mL) were inoculated into each 30-culture bag (Ulpack 47, Hokken Co. Ltd., Tochigi, Japan) containing a production medium (1 kg of water-sodden rice and 10 g of seaweed tea powder (Ito-en Ltd., Tokyo, Japan)). Static fermentation was continued at 25 °C for 13 days.

Acetone (30 L) was added to the stationary cultured material (30 kg) as shown in Figure S1. After vacuum filtration and removal of acetone *in vacuo*, the resulting aqueous solution was filtered through a filter paper to obtain a water-insoluble material. The dried material (33.5 g) was dissolved in a small amount of $CHCl_3$/MeOH (100/10) and charged to a silica gel column (150 mL resin, 55 i.d. x 55 mm; Merck KgaA), and eluted stepwise with a solvent mixture of $CHCl_3$/MeOH (100/10, 100/20, 1/1, 0/100, 0/100+0.1% TFA, each 500 mL). The $CHCl_3$/MeOH = 1/1 fraction was dried *in vacuo*. The residue in the 1/1 fraction (6.52 g) was washed with $CHCl_3$ (20 mL) and MeOH (20 mL) to afford 870 mg as an insoluble material. A part of the insoluble material (750 mg) was subjected to reverse-phase HPLC using a Capcell Pak C18 column (20 i.d. x 250 mm; Osaka Soda Co. Ltd, Osaka, Japan) with an isocratic solvent system of MeCN/H_2O/TFA (45:55:0.1) at a flow rate of 7.0 mL/min detected by UV 210 nm. Because the sample had low solubility in the mobile phase, the injection sample was prepared as a 100 mg/mL DMSO solution, followed by an addition of the mobile phase at a ratio of 1:2 (DMSO solution: the mobile phase), and injected at 30 mg each in 25 portions. The fractions eluted in the retention time of 15–18 min were collected (Figure S1b). The solvent mixture was evaporated *in vacuo* to remove MeCN, and freeze-dried to afford the crude material (68.1 mg). A part of this material (26.2 mg) was subjected to reverse-phase HPLC using analogous conditions, in which changing solvent system MeCN/H_2O/trifluoroacetic acid (40:60:0.1) was used. The fractions eluted in the retention time of 31–34 min were collected (Figure

S1c). The solvent mixture was evaporated *in vacuo* to remove MeCN, and freeze-dried to afford tetraselide (**1**) (15.6 mg).

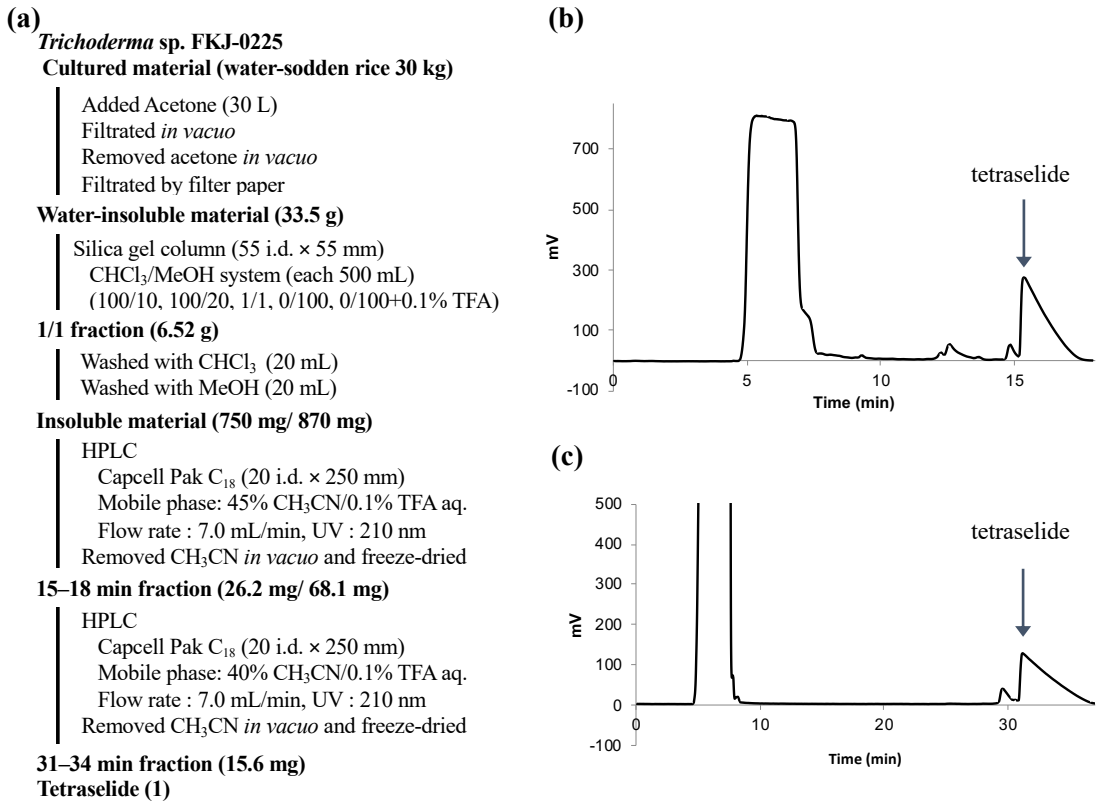

Figure S1. Isolation of tetraselide (**1**) from the cultured material of *Trichoderma* sp. FKJ-0225

(a) Isolation procedure of **1**

(b) First HPLC chart of insoluble material fraction,

(c) Second HPLC chart for isolation of **1**

2-2. Structure Elucidation of Tetraselide

Compound **1** was isolated as a colorless powder. The molecular formula of **1** was determined as $C_{41}H_{73}N_9O_{15}$ by HR-MS analysis (ESI, *m/z* 932.5301, calculated for $C_{41}H_{74}N_9O_{15}$ $[M+H]^+$ 932.5299), requiring 10 degrees of unsaturation (Figure S2). The typical ^1H and ^{13}C NMR signals and HSQC spectra of **1** in $(CD_3)_2SO$ (Table S1 and Figures S7–9) and IR absorption at 1655 cm^{-1} (Figure S4) indicated the presence of amide groups.

The ^1H-^1H COSY and HMBC spectra revealed the presence of eight α-amino acid residues (Orn, Thr, Ser, Ser, Ser, Ser, Ala, and Gly) and one β-hydroxy-γ-methyl-fatty acid (Figures S5, S10, and S11). Considering the fact that the molecular weight of **1** got increased by 18 (an addition of H_2O) under the simple basic hydrolysis conditions, we assumed that **1** was likely a cyclic depsipeptide. We could not assign the amino acid sequence by MS/MS fragment analysis of the basic hydrolysate of **1**. The linear peptide derivative was prepared by mono-acetylation of the ornithine residue, followed by macrolactone hydrolysis in **1** through LC-ESI-MS/MS analysis, which revealed the sequence of Orn-Ser-Ser-Ser-Ser-Thr-Gly (Figure S6). Finally, the overall planar structure was determined by HMBC cross-peaks from Ala H-1 (δ_H 4.18) to 3-hydroxy-4-methyl tetradecanoic acid (Hmta) C-1 (δ_C 169.5), from Orn NH (δ_H 8.10) to Ala C-2 (δ_C 172.5), from Ser-1 NH (δ_H 7.86) to Orn C-5 (δ_C 171.2), from Ser-2 H-1 (δ_H 4.09) to Ser-1 C-3 (δ_C 171.3), from Ser-3 NH (δ_H 7.71) to Ser-2 C-3 (δ_C 170.0), from Ser-4 NH (δ_H 7.53) to Ser-3 C-3 (δ_C 169.8), from Thr NH (δ_H 7.98) to Ser-4 C-3 (δ_C 170.3), from Gly H_2-1 (δ_H 3.85 and 3.77) to Thr C-3 (δ_C 170.2), and from Hmta H-3 (δ_H 5.04) to Gly C-2 (δ_C 168.8) as shown in Figure S5.

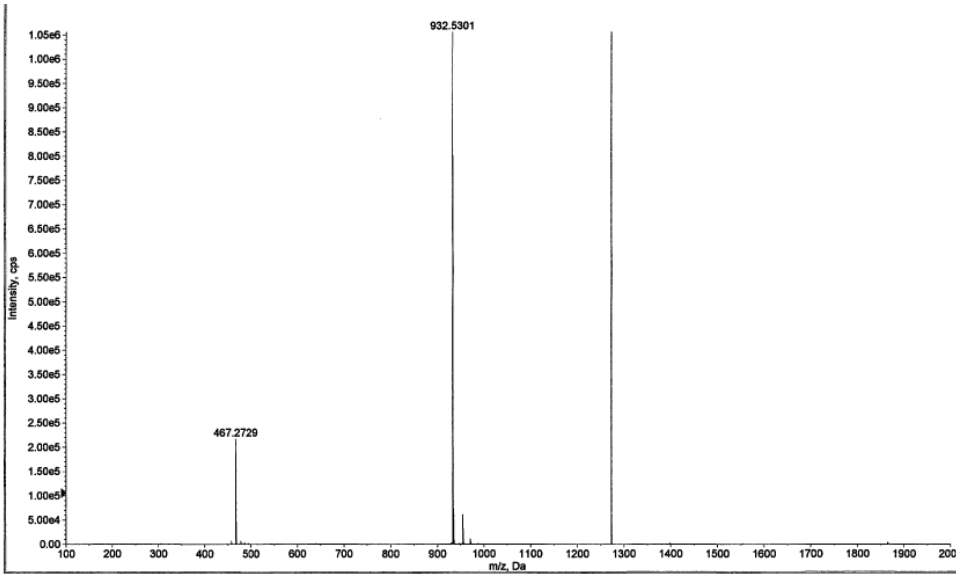

Figure S2. HR-ESI-MS data of **1**

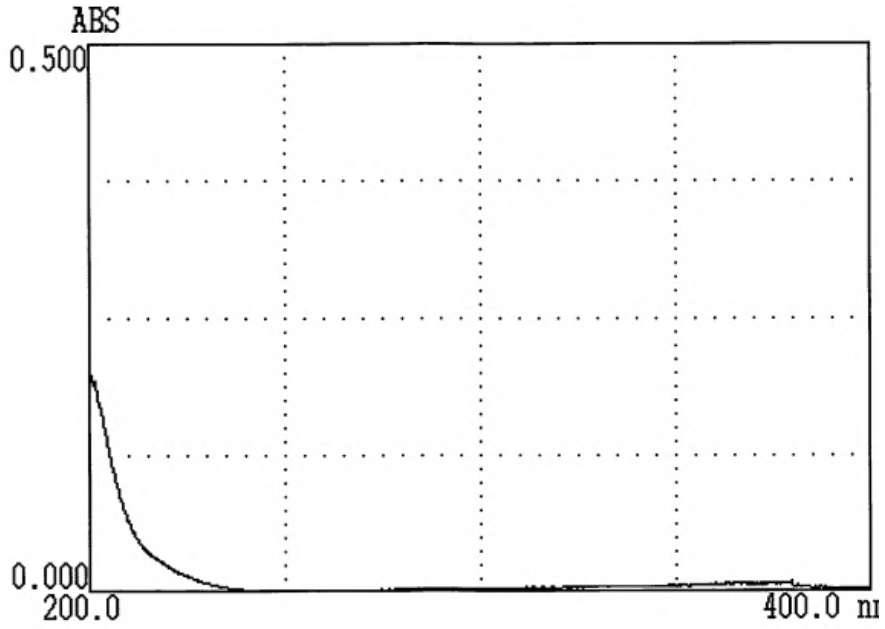

Figure S3. UV spectrum of **1** in MeOH

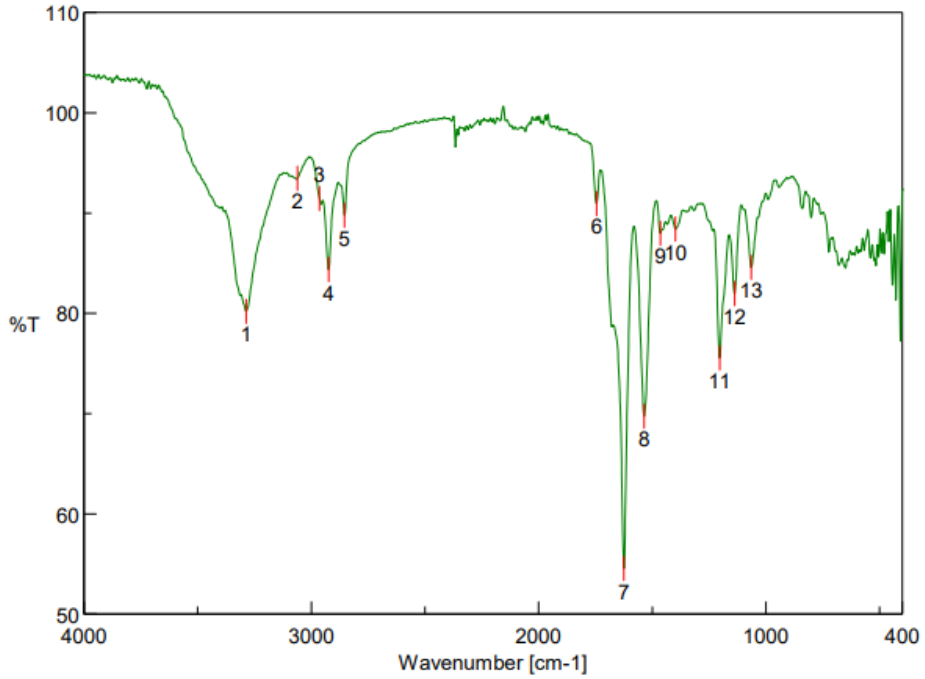

Figure S4. IR spectrum of **1** (ATR)

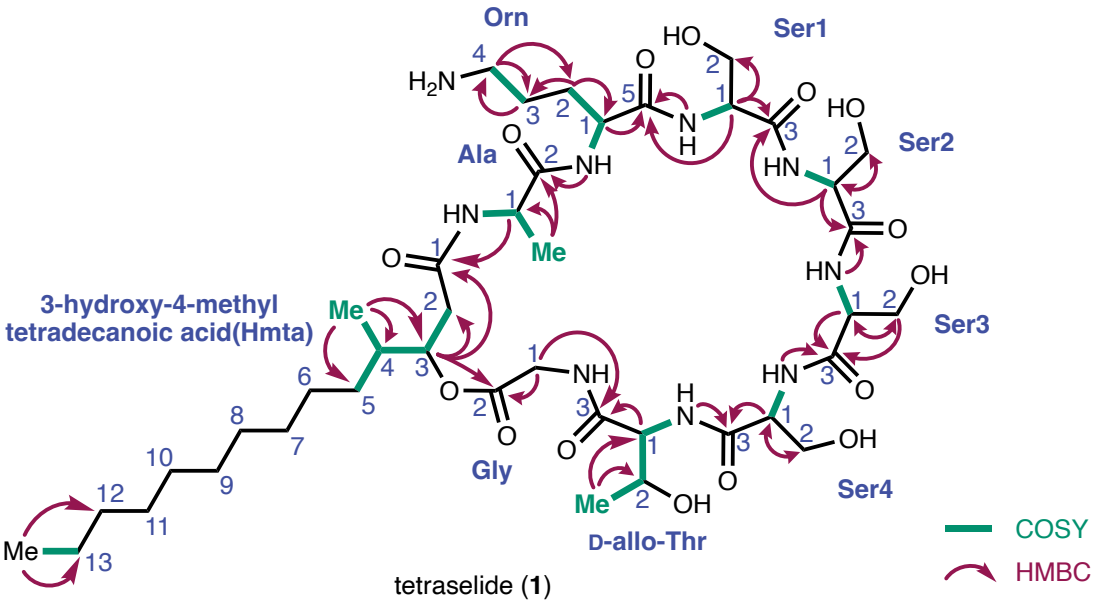

Figure S5. 2D NMR correlations of **1**

Table S1. NMR data of **1** in $(CD_3)_2SO$ (^1H: 400 MHz, ^{13}C: 100 MHz)

		Tetraselide (1)			
	position	δ_C	δ_H (int., mult., J in Hz)	COSY	HMBC
Ala	1	48.6	4.18 (1H, dq, 7.0, 7.0)	Ala NH, Ala 1-CH_3	Ala 1-CH_3, Ala-2, Hmta 1
	1-CH_3	17.7	1.18 (3H, d, 7.0)	Ala 1	Ala 1, Ala 2
	2	172.5	–		
	NH	–	7.99 (1H)*	Ala 1	
Orn	1	52.1	4.28 (1H, m)	Orn 2, Orn NH	Orn 5
	2	28.3	1.54 (1H, m)	Orn 1	Orn 1, Orn 3
			1.75 (1H, m)		Orn 3
	3	23.6	1.53 (2H, m)	Orn 4	Orn 4
	4	35.9	2.73 (2H, t, 7.2)	Orn 3	Orn 2, Orn 3
	5	171.2	–		
	NH	–	8.10 (1H, d, 8.0)	Orn 1	Ala 2
	4-NH_2	–	N.D.		
Ser-1	1	54.2	4.44 (1H, m)	Ser-1 2, Ser-1 NH	Orn 5, Ser-1 3, Ser-1 2
	2	62.2	3.82 (1H, m)		
			3.59 (1H, m)	Ser-1 1	Ser-1 1
	3	171.3	–		
	NH	–	7.86 (1H, d, 8.3)	Ser-1 1	Orn 5
	2-OH	–	N.D.		
Ser-2	1	57.2	4.09 (1H, m)	Ser-2 2, Ser-2 NH	Ser-1 3, Ser-2 2, Ser-2 3
	2	60.6	3.66 (2H, m)	Ser-2 1	Ser-2 1
	3	170.0	–		
	NH	–	8.43 (1H,br. s)	Ser-2 1	
	2-OH	–	N.D.		
Ser-3	1	55.9	4.24 (1H, m)	Ser-3 2, Ser-3 NH	Ser-3 2, Ser-3 3
	2	61.2	3.58 (2H, m)	Ser-3 1	Ser-3 1, Ser-3 3
	3	169.8	–		
	NH	–	7.71 (1H, d, 7.7)	Ser-3 1	Ser-2 3
	2-OH	–	N.D.		
Ser-4	1	54.9	4.39 (1H, m)	Ser-4 2, Ser-4 NH	Ser-4 2, Ser-4 3
	2	61.8	3.61 (2H, m)	Ser-4 1	Ser-4 1
	3	170.3	–		
	NH	–	7.53 (1H, d, 8.3)	Ser-4 1	Ser-3 3
	2-OH	–	N.D.		
Thr	1	58.4	4.26 (1H, m)	Thr 2	Thr 2, Thr 3
	2	66.6	3.88 (1H, m)	Thr 1, Thr 2-CH_3	Thr 1
	2-CH_3	19.8	1.05 (3H, d, 6.4)	Thr 2	Thr 1, Thr 2
	3	170.2	–		
	NH	–	7.98 (1H)*		Ser-4 3
	2-OH	–	N.D.		
Gly	1	41.1	3.85 (1H, dd, 16.6, 5.4)	Gly NH	Gly 2, Thr 3
			3.77 (1H, dd, 16.6, 5.6)		Gly 2, Thr 3
	2	168.8	–		
	NH	–	8.00 (1H)*	Gly 1	Thr 3
Hmta	1	169.5	–		
	2	36.9	2.31 (2H, br. d, 5.5)	Hmta 3	Hmta 1, Hmta 3, Hmta 4
	3	75.4	5.04 (1H, m)	Hmta 2, Hmta 4	Hmta 1, Hmta 2, Hmta 4, Hmta 4-CH_3, Hmta 5
	4	35.9	1.69 (1H, m)	Hmta 3, Hmta 4-CH_3, Hmta 5	
	4-CH_3	14.5	0.81 (3H, d, 4.0)	Hmta 4	Hmta 3, Hmta 4, Hmta 5
	5	31.8	1.31 (1H, m)		
			1.00 (1H, m)	Hmta 4	
	6–11	29.3			
		29.1			
		29.1	1.17–1.26 (12 H, overlapped)		
		29.1			
		28.8			
		26.5			
	12	31.3	1.22 (2H, m)		
	13	22.1	1.23 (2H, m)	Hmta 13-CH_3	
	13-CH_3	14.0	0.82 (3H, t, 6.7)	Hmta 13	Hmta 12, Hmta 13

N.D. Not detected * Overlapped

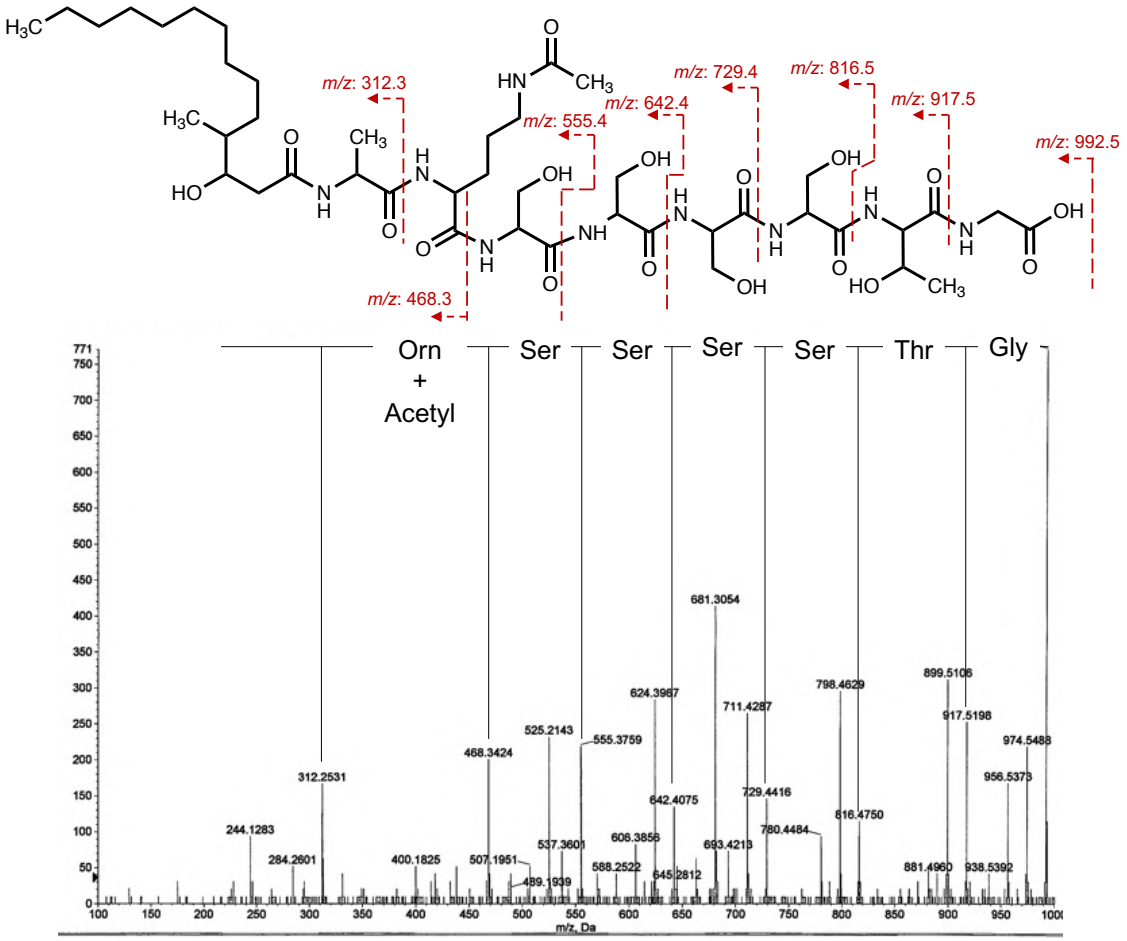

Figure S6. MS/MS fragmentation analysis of a linear-degraded compound from **1**

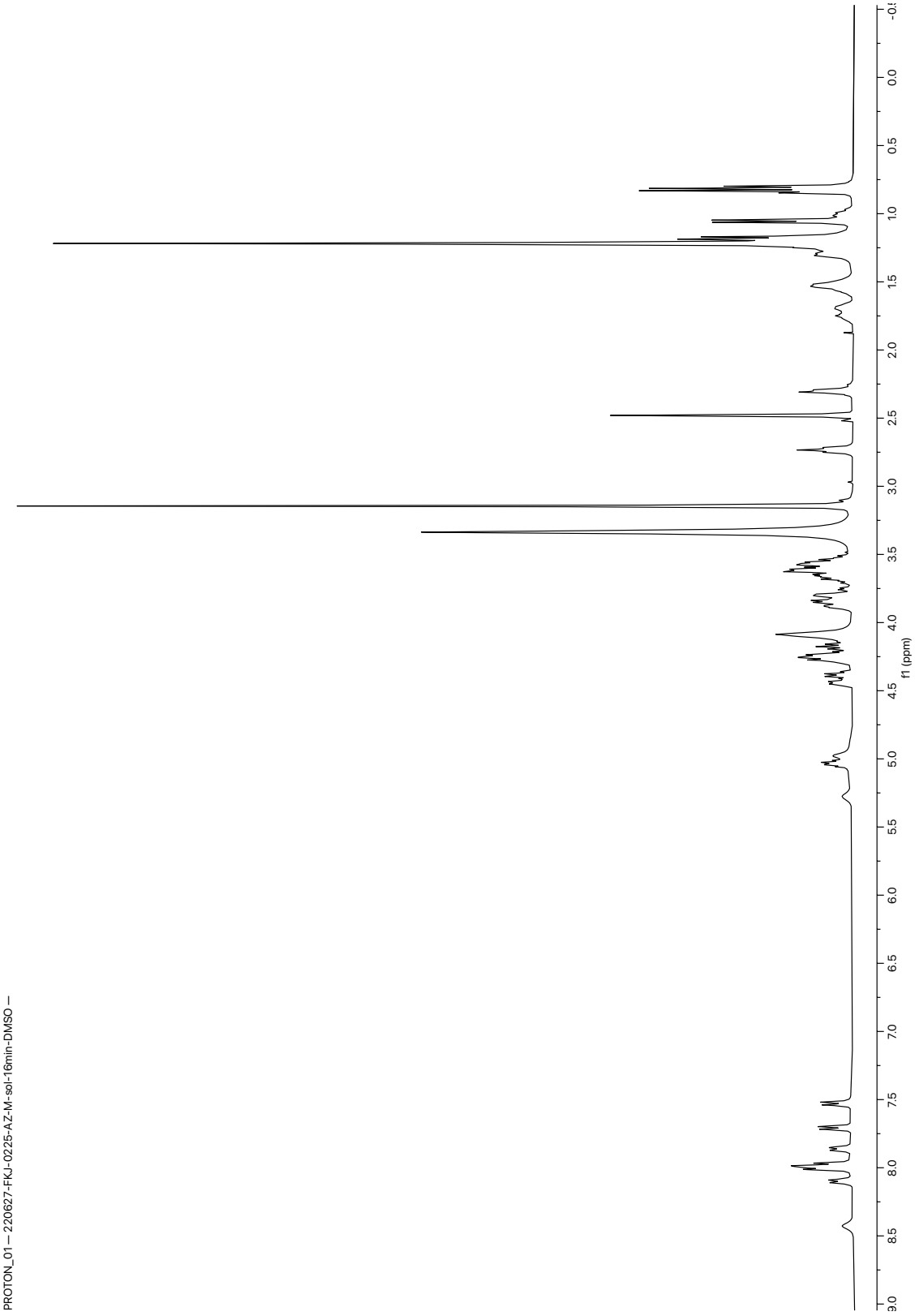

Figure S7. ^{1}H NMR data of **1** (400 MHz, $(CD_3)_2SO$)

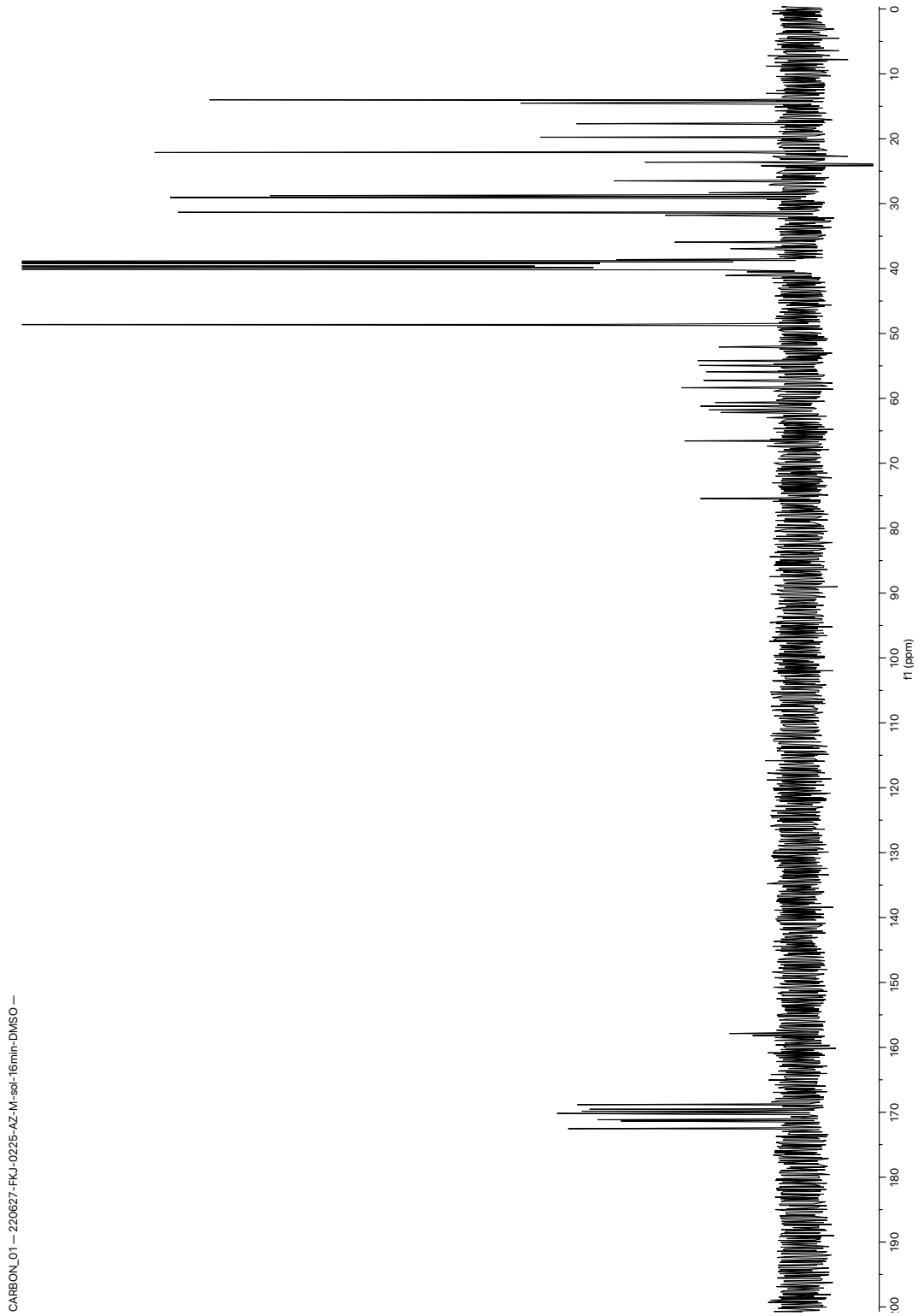

Figure S8. ^{13}C NMR data of **1** (100 MHz, $(CD_3)_2SO$)

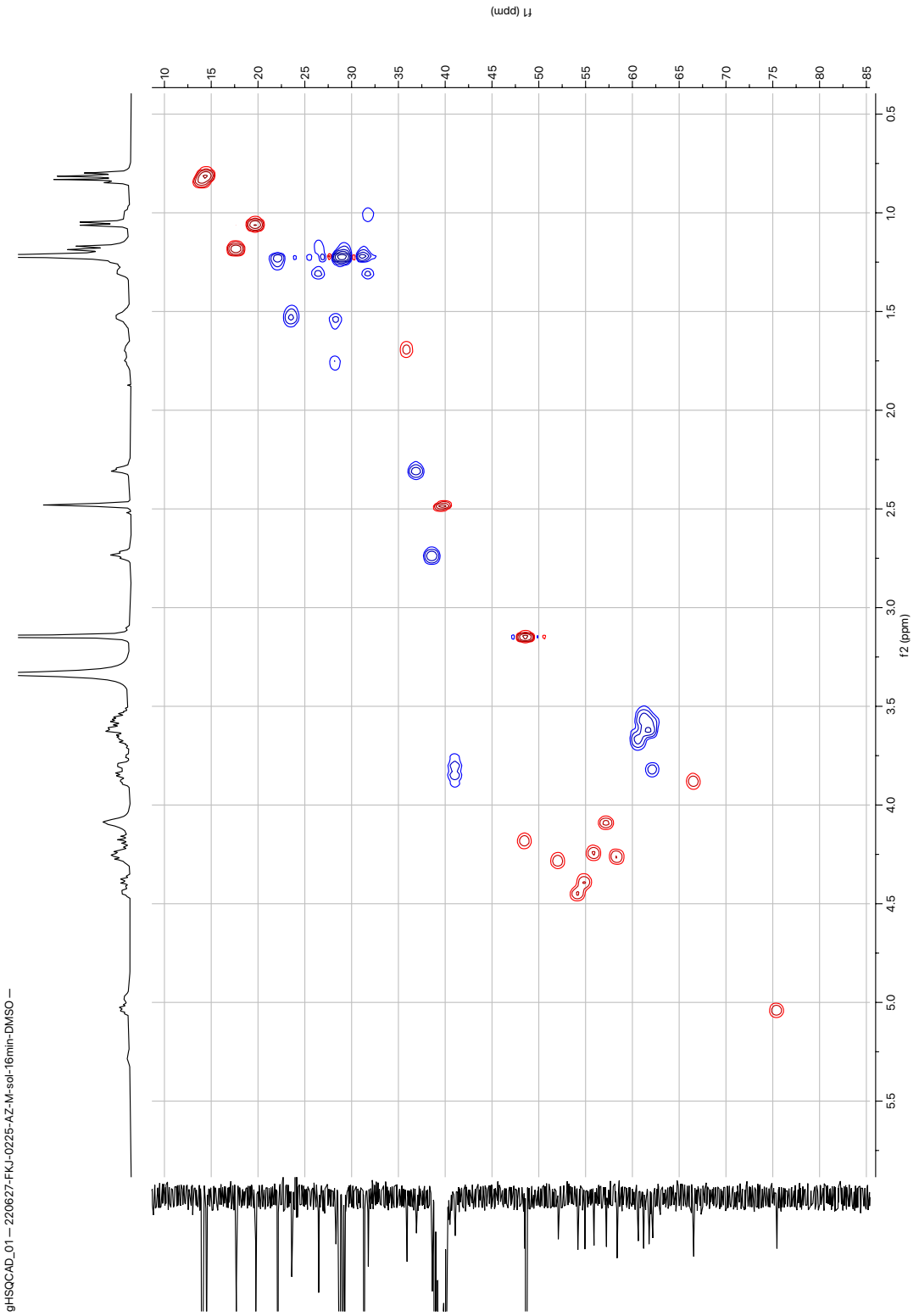

Figure S9. HSQC data of **1** (400 MHz, $(CD_3)_2SO$)

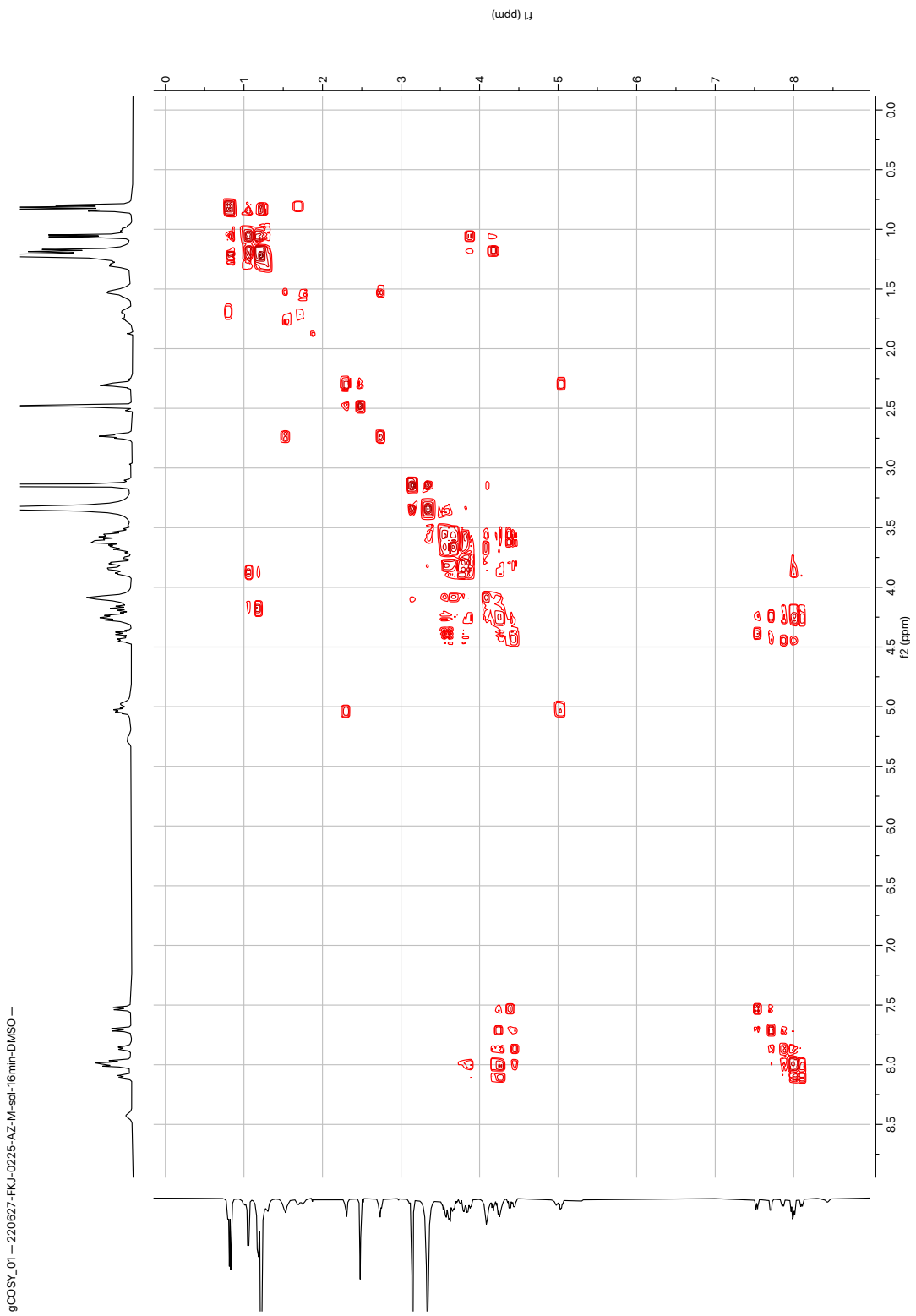

Figure S10. COSY data of **1** (400 MHz, $(CD_3)_2SO$)

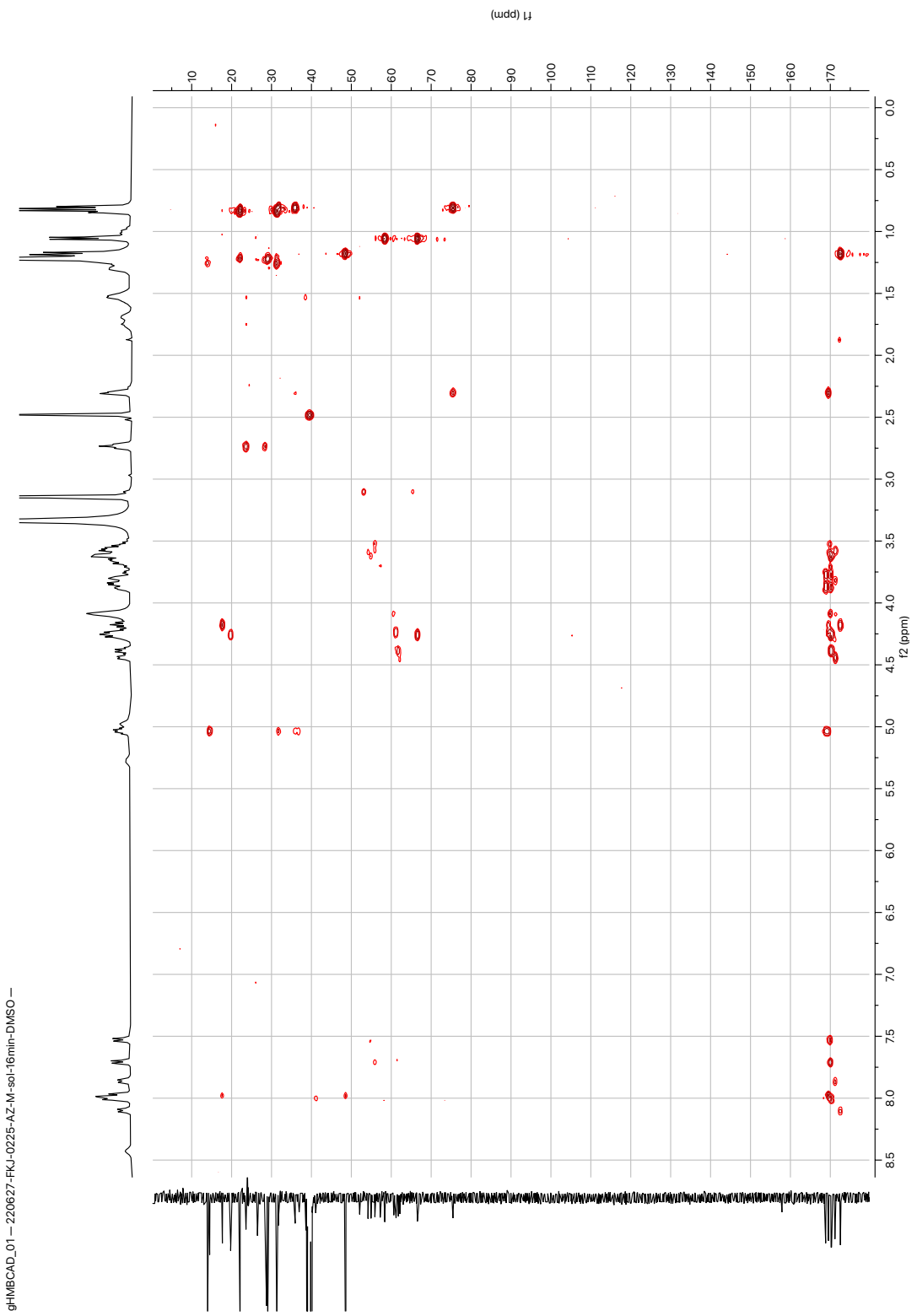

Figure S11. HMBC data of **1** (400 MHz, $(CD_3)_2SO$)

2-3. Determination of the Absolute Configuration of Amino Acid residues in Tetraselide

Attempts to determine the absolute configuration of amino acid residues in compound **1** were described as follows. The Ala, Orn, and Thr residues in **1** were determined as L-Ala, L-Orn, and D-*allo*-Thr by the advanced Marfey's method[1] (Figure S12). Similarly, the four Ser residues in **1** were detected as D-Ser:L-Ser = 1:3 although the order of the Ser residues remained ambiguous by this analysis. Therefore, we thought to undertake bioinformatic analyses to predict the absolute configurations at the C3 and C4 positions in Hmta and Ser-1–4 residues.

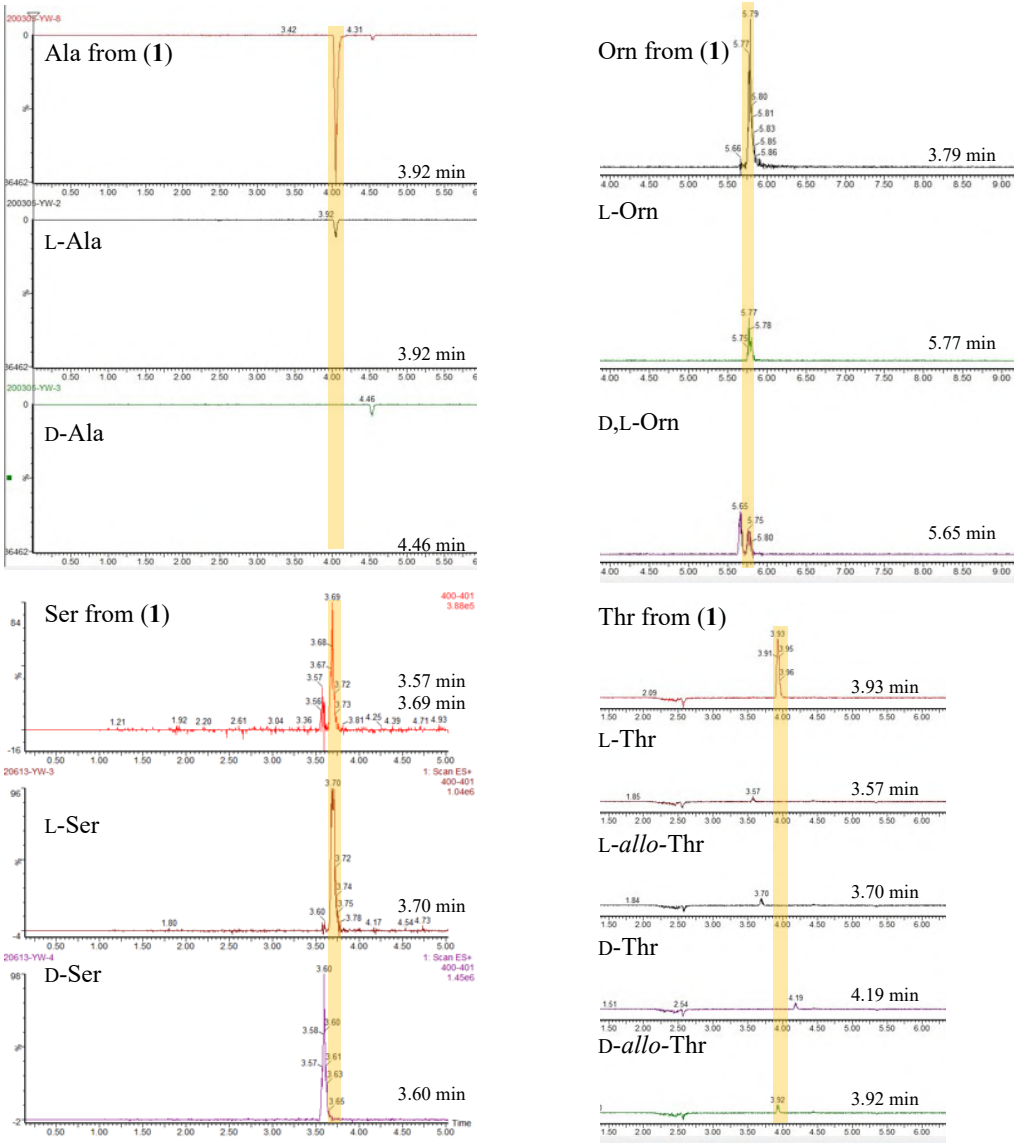

Figure S12. Extracted ion chromatogram profile of L-FDLA derivatives of alanine, ornithine, serine, threonine, and hydrolysates of **1**.

LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. x 50 mm)
Mobile phase A ; 10% CH_3CN aq. + 0.1% formic acid
Mobile phase B ; 100% CH_3CN aq. + 0.1% formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-10 min), A:B = 0:100 (10-15 min)
Flow rate ; 0.5 mL/min
UV ; PDA
MS ; ESI- MS, positive mode

Value of extracted ion, Ala; *m/z* = 384–385, Orn; *m/z* =721–722, Ser; *m/z* =400–401, and Thr; *m/z* =414–415.

Material and methods

The genomic DNA of strain FKJ-0225 was obtained from the mycelium. The strain FKJ-0225 was cultured for 3 days in 100 ml Potato dextrose broth at 27 °C. The myceliums were collected, washed and frozen at –80 °C. The frozen mycelium was ground into a powder and suspended in 10 mL of TE buffer (pH 8.0). To the resulting suspension was added 0.5 mL of 10% SDS and the mixture was incubated for 5 min on ice. After addition of 2.5 mL of 5 M KOAc, the mixture was incubated for 15 min on ice. To the resulting suspension was added 10 mL of a mixture of phenol:chloroform:isoamyl alchol (25:24:1). After adding EtOH and subsequent centrifugation, the DNA was obtained from the supernatant. The genomes were sequenced using the Pacbio RSII technology (Macrogen, Inc., Seoul, Republic of Korea). The obtained genomic sequence was analyzed using antiSMASH7.0 and 2ndFind. The amino acid sequences of polyketide synthase and nonribosomal peptide synthetase in tetraselide biosynthetic gene cluster are shown in bellow.

> polyketide synthase

MILDDDYVACVLYFNTLFPLVHSFFNTPKRKDLFNKMTADTMPEPIAVIGMSCRFPGDASEPSRLWDVLAAGKSAWSEVPEDRFNMK
AFYHGKDPHNTTTNTPGGHFLSTDVAAFDCSFFEIKPTEAQAVDPQQRITLELAYEAFENAGLTLPQLWGSSTSVYVGQWSSDYSEV
LARDPESQERYHTLGIGAAITSNRLSYFFNLRGPSFTVDTGCSASLVALHNAVQSLRSGESTMSLVGGVNILLDPQRFTYQSKLKMFS
PDGRCFSFDDRANGYGRGEGCGCVVLKPLSAAIRDGDRIRAVIRNSALNQDGRTPQGISVPSGEAQEELIRRAYAEVGLIPTETDYAE
AHGTGTAVGDPIEAEAIANVLARPRAPSQGPLLIGSMKGNIGHTESAAGIAGLIKAVLMLENQAIAPQVNYRRPNPKIPLDIWNLQIPLSF
EKKDIRRISVNSFGYGGTNAHVIVDRVEEHIRPQNGTNGVDDSHIEAERPRAFIVSGADEQSCHKNAERLAQYLIKSQDESVEDPNELL
DRLAITVNKRTVHEYNASVVAYDTEDLVEQLEVLQQTTIAVRTPTKGGARVGYVFGGQGAQYFNMGREMMKDWPVFRQSLDRANT
HLRTLGCKWDTLAELSHEKAEDSHVDEPEYGQTLSTVIQLALVDTLASLGLSPTSVVGHSSGEIAAAYAVGALSFEDSLTVAYHRGRL
TSKLIASGVSGGMLAVGSSPEVAQTYIDKVKATTSAELKIACYNSVGSVTLSGDNETISAVAALLQDNDVFHRKLKTQGAAYHSQMM
QAIEEEYRAALAHIKPRPVAGNMMSSLNGKKVSSELLLDGDYWARNLVSPVRFSGALRGMIVSEKQDGQHDLGVDILLEVGPHAQMQ
GAVKDTMKDSGYASRIQYLSCLKRKANAAENMLSTLADLFALGAPVSFHQANAGFTTKLPAMLNDVPPYAFNHDQRHWHESRISRE
YTHRQFLPHELLGNLSSDVNHTEPRWRRIMNLKEMPWLQHHMVQGQIVFPAAGYLAIAMEAMRRFAALKPENQSKATVGYSLSNCS
FVKALVLREGDTETEMCLSLRPETQSAKGSWQDWKEFRIFSISPGKGWSEHCRGRIRAVTDDGMLADRLNDTLDVKKSNLASIQHHV
TPIRFYSAARQIGMEWYAPFDNIVSLEALVDSSVATNKMPSLDAAAHPFGEECYVVHPGMLDSTLFHGICASLLVERESKSAMVPVFIE
QLTVSTALKVPEGTELRTYALAQDKGTSWSGQVESNDQAMMSFHGLRIAQLPGNDLSTAPRQLATSPEWVGHYPSMTREQIIANCM
NSLPAGSSRYRNIVLNADVRAHVERALKQVTPDQVATGFQQAWYKWMQSFVATKADSPELVAKARVAVASDPSISFEAVKRIGEDLE
NLLTGATNSLSILTLNDMLGKLYSEERNGRCYHQINEYCKTLGKYNPSLRIVEVGGGTASATLPLLEALTHHGKPLVAQYDFTDLSTAF
FPAARERLNQYSNVVNYKAFDLATSPDLQEFEPGTYDVVIACNVVHATPNIASSLENIHHLLRPGGVLILMEITNPEPYYQLIFGSLSGW
WLGVDEGRVSSAVLSVSDWKRELSKQGYESDPVIVSDYAEGEGETISVIFAKTPVAPQPDLSEVSLHFTNGGNLGKVAESLRQNTGL
GLRQITTGDLDSIPNIANTVLVIDPELSEALAGHMDASTWQKFQKCALSCKGLVLISRGATGDSPVPEGALTIGFARTLRLEQRGIRYVT

LDLDPDNSHGAIEQRLSQSLAKLLASEAFDLERDCIGLDFQFAERSGQLYVNRMFQNDQLDQHIVRSTARAKPEQTAFLQPSRPLKV
GLGVPGLMETFRWADDVEHARALAPDEIRIECRAASINFRDVLVASGELGASATMMNDCAGTVVEVGSNMKSRYSVGDRVCSYYAQ
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S

3. Biosynthetic Analysis

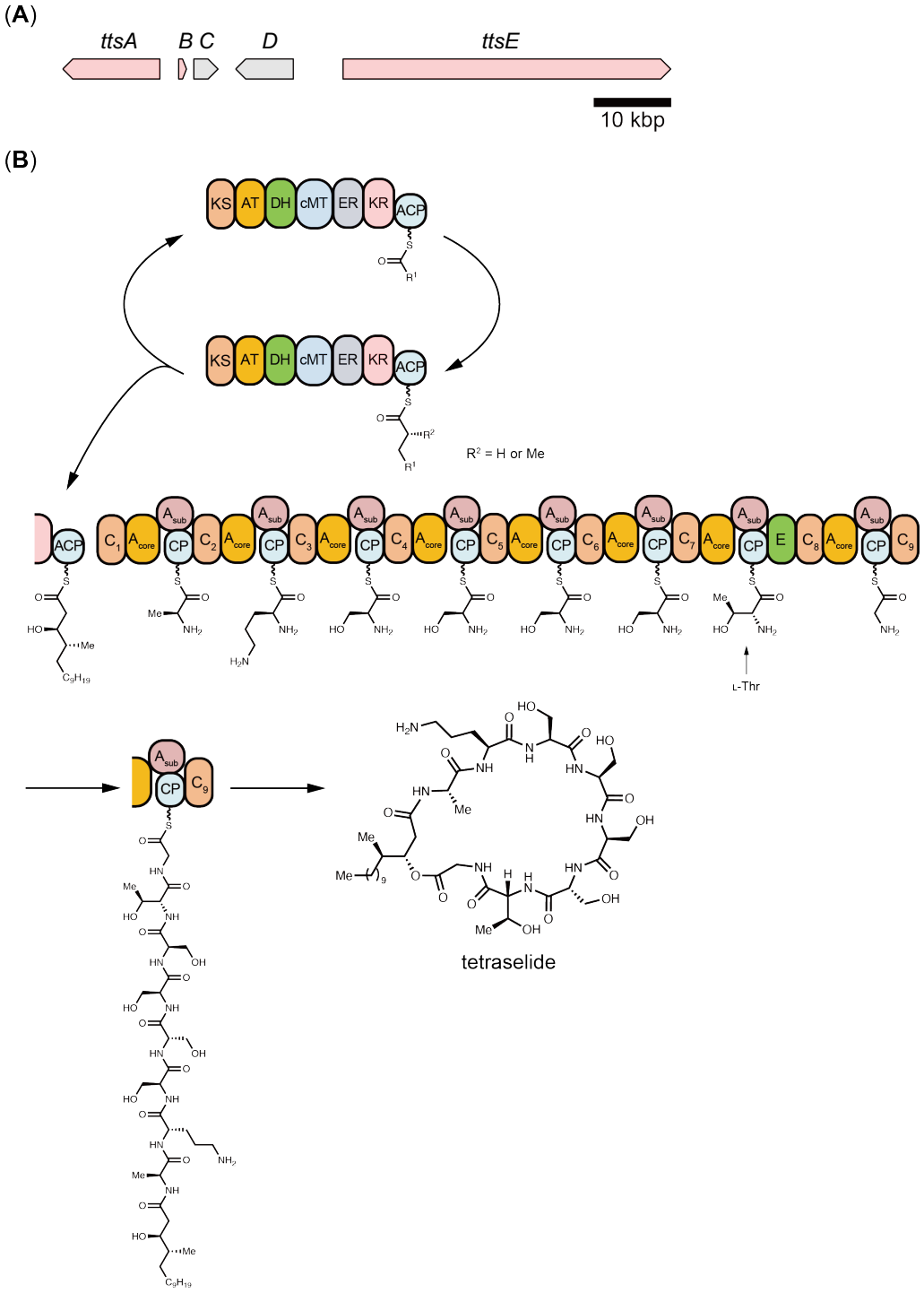


Figure S13. (**A**) Biosynthetic gene cluster of tetraselide; *ttsA*, *B*, *C*, *D* and *E* encoding polyketide synthase, thioesterase, transporter, transporter, and nonribosomal peptide synthetase, respectively. (**B**) Proposed biosynthetic pathway of tetraselide.

Domains recognizing serine were predicted by colabofold and named A_{Ser}1-4 from the N-terminal in NRPS. We identified the key residues to determine the substrate specificity by mesuaring 5 Å from docked L-Ser (Figure S13). Because all A_{Ser} domains have same key residues, it suggests that these domains likely recognize the same enantiomer of Ser.

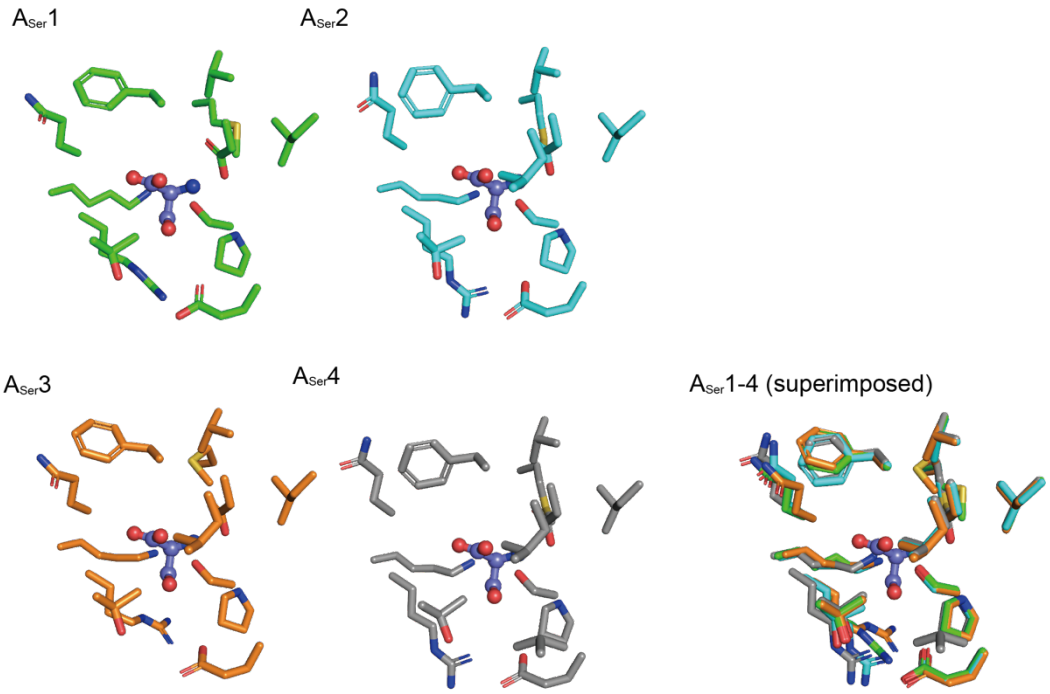


Figure S14. Comparison of substrate binding pockets in the corresponding adenylation domains predicted by Colabfold[2]

In general, phylogenetic analysis of condensation (C) domains can classify the corresponding C domains having similar functions (e.g. $_{L}C_{L}$ domain catalyzes condensation between two L-amino acids) into the same clade. Therefore, we conducted phylogenetic analysis of nine C domains in the tetraselide biosynthetic gene cluster and known C domains in cephamycin, cyclosporin, daptomycin, fumiquinazolines, cyclo-tetrapeptide, gramicidin, JBIR-34, penicillin, pyochelin, tyrocidine, vancomycin and echinocandin. As a result, we identified that C domains 2–6, 7–8 and 9 are different clade. C domains 1-5 are the same clade as ones that catalyze condensation of L-amino acids ($_{L}C_{L}$) in the biosynthesis of echinocandin[3]. C domains 6 and 7 are the same clade as ones that catalyze the condensation of an upstream D-amino acid and a downstream L-amino acid ($_{D}C_{L}$) in the biosynthesis of cyclic tetrapeptide biosynthesis[4] and fumiquinazoline biosynthesis[5]. Considering this phylogenetic analysis and the function of C domain 7 that catalyzes the condensation of D-Thr and Gly, we speculated that C domain 6 would deliver D-Ser as an upstream amino acid for the corresponding condensation reaction. This result implies that the C-terminal of the consecutive four Ser moiety in tetraselide might be in the D-configuration.

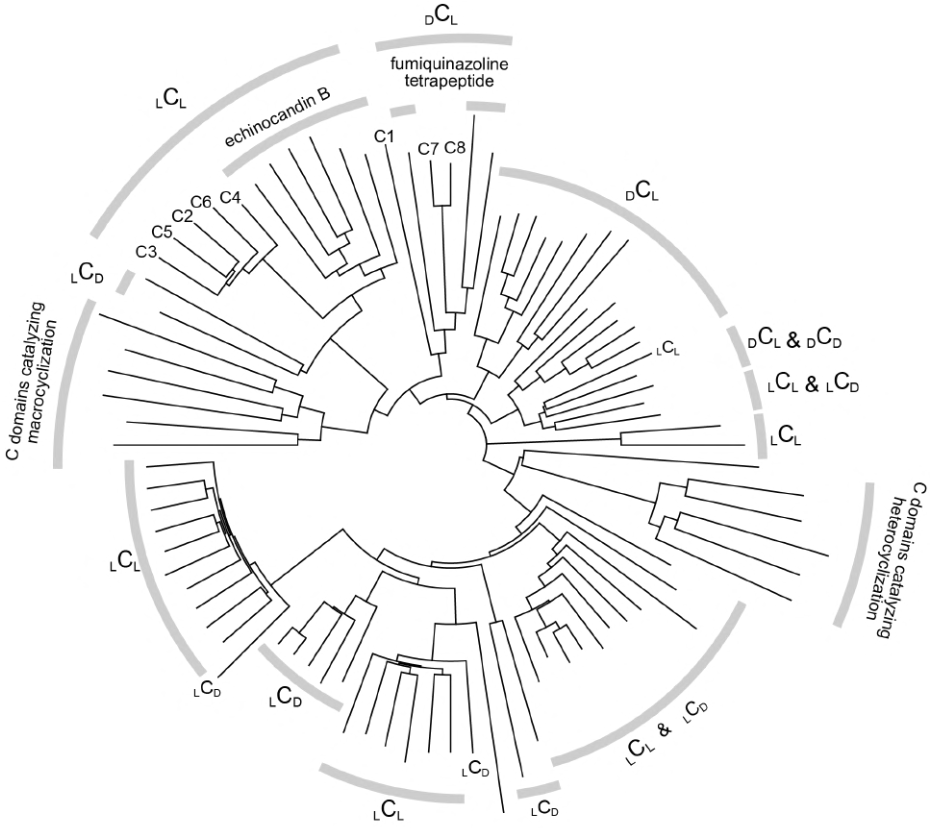


Figure S15. Phylogenetic analysis of C domains in the tetraselide biosynthetic gene cluster

Alignment of keto reductase (KR) and enoyl reductase (ER) domains shows that KR domain in this cluster is the B-type because it has a LDD motif (the second amino acid is changeable) which represents catalytically important residues to construct the corresponding stereocheistry. Alignment of ER domain also indicates that a D-methyl group should be formed in the growing polyketide chain because substitution of Tyr with Val/Leu in ER domain gives an α-substituent with D-configuration. Red bars and red asterisks in FIgure S16A show important residues for determination of the stereochemistry. The complete reaction sequence in this PKS is shown in Figure S16B.

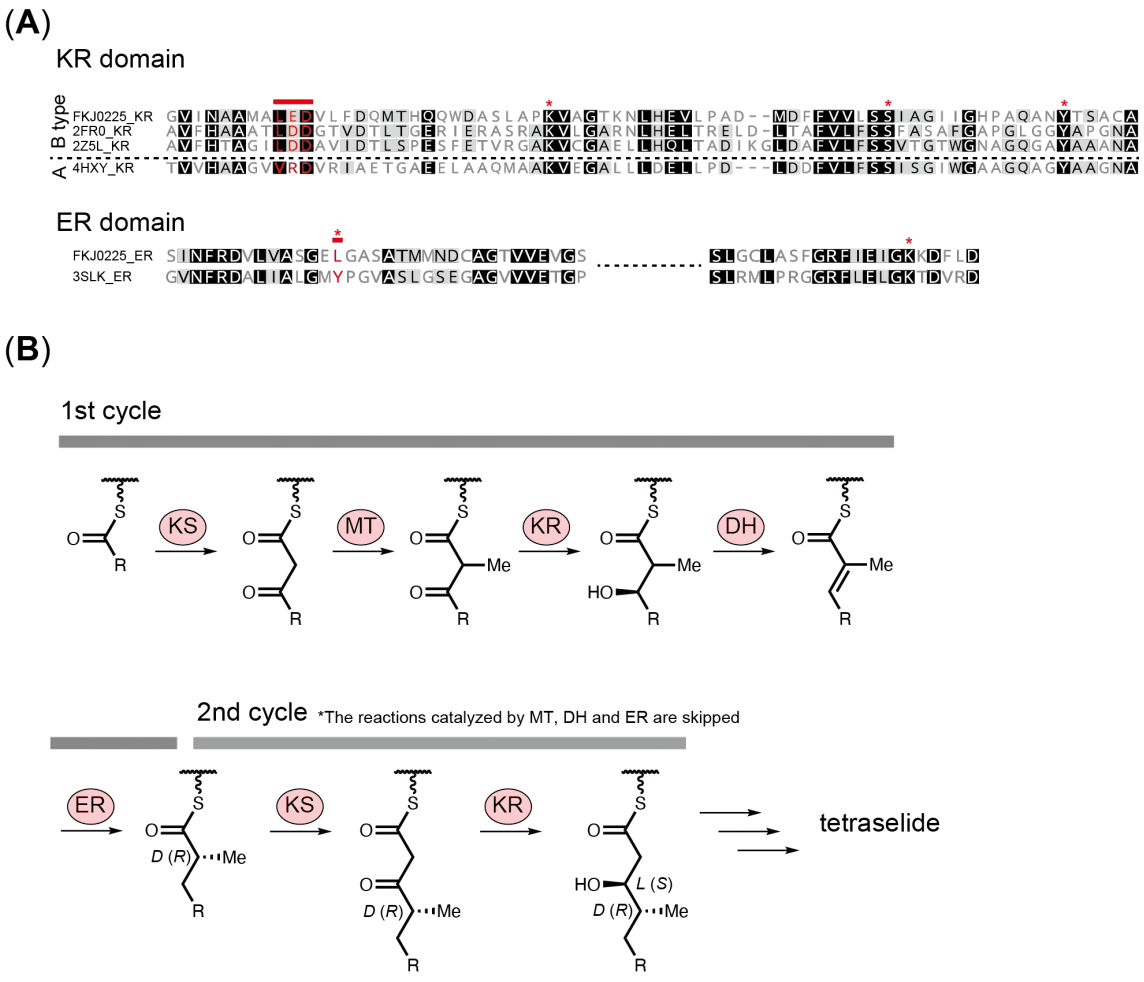
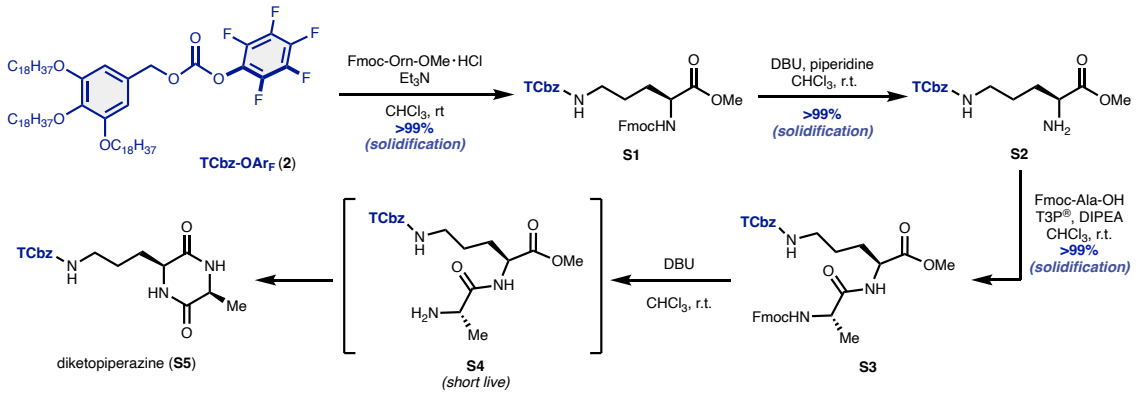


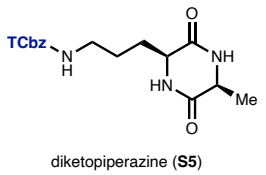
Figure S16. (**A**) Multiple sequence alignment of PKS domains. (**B**) Important reactions in PKS for construction of the stereochemistry.

4. Reaction Investigations

4-1. Initial attempts to synthesize dipeptides



We first attempted to synthesize a western fragment using Fmoc-Orn-OMe•HCl as a starting material. However, when we tried to remove the Fmoc group after condensation with Ala, the reaction gave a messy mixture, in which we detected the formation of diketopiperazine by FAB-MS. Therefore, we undertook a survey of the C-terminal protecting groups (**S6** to **S15**) in the Orn residue to suppress the problematic formation of the diketopiperazine (**Figure S17**).



HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{70}H_{129}O_7N_3Na$: 1146.9728, found: 1146.9750.

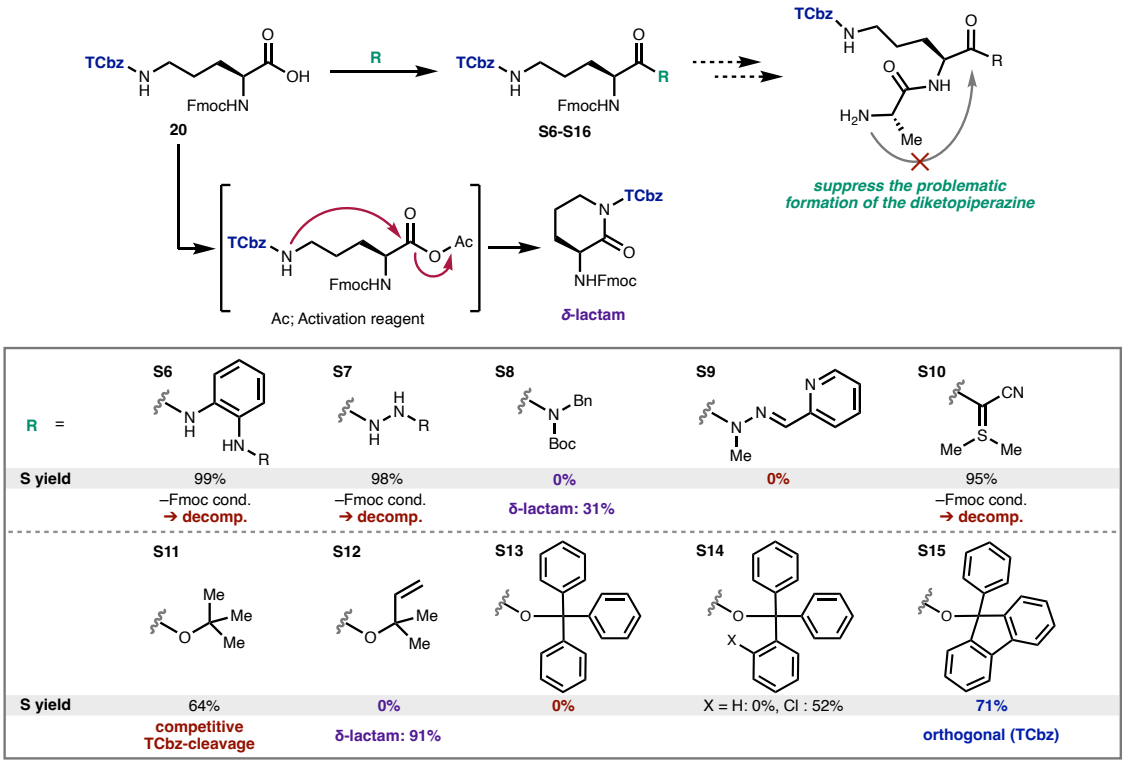
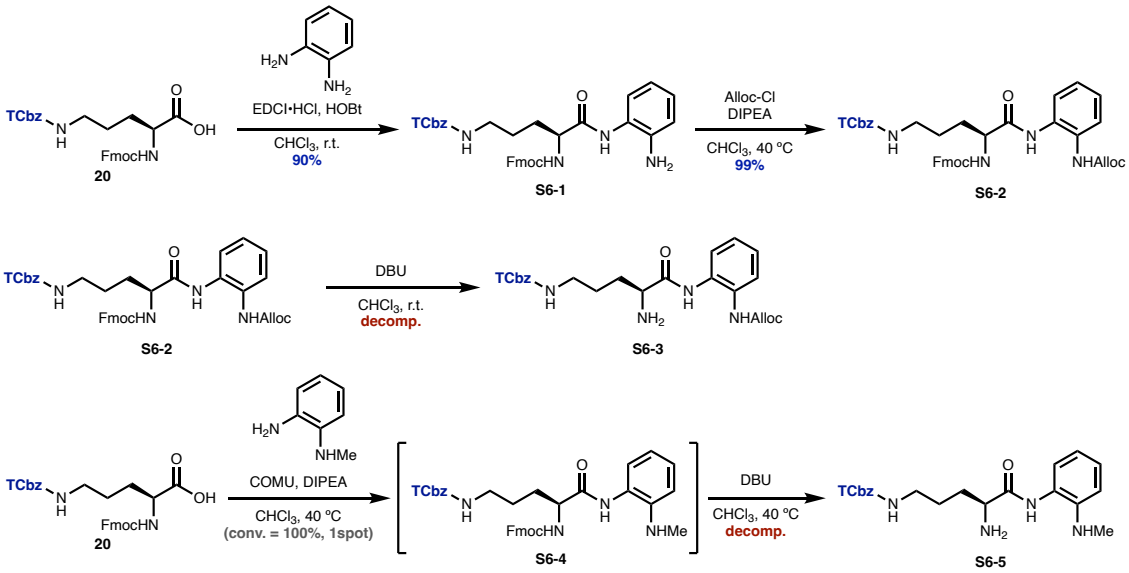
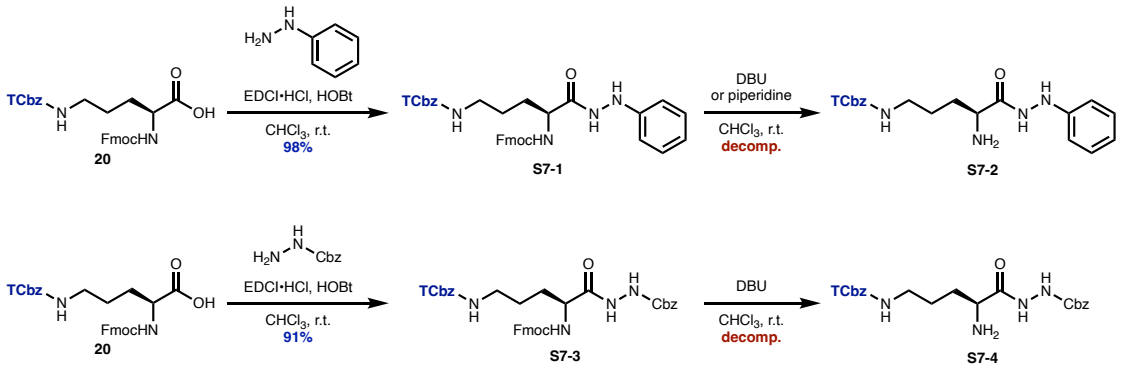


Figure S17. Investigation of the C-terminal protecting group to suppress a problematic diketopiperazine formation.

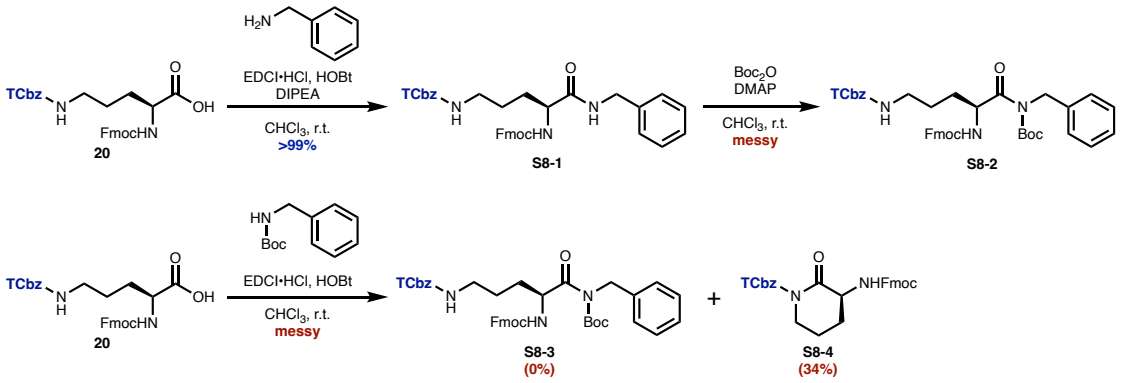
S6 (Reaction conditions were based on the known literature.[6])



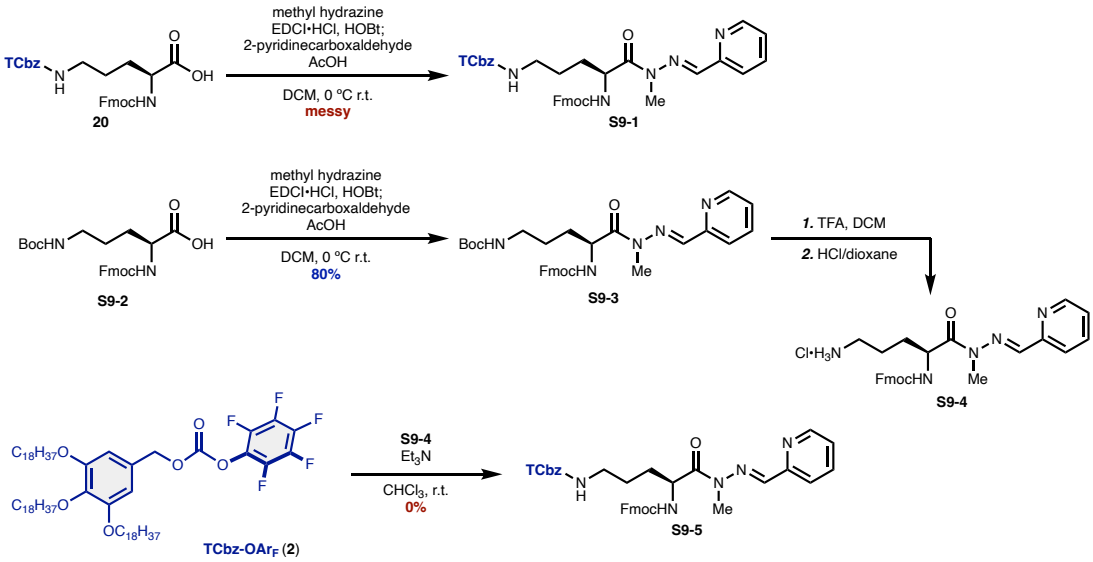
S7 (Reaction conditions were based on the known literature.[7])



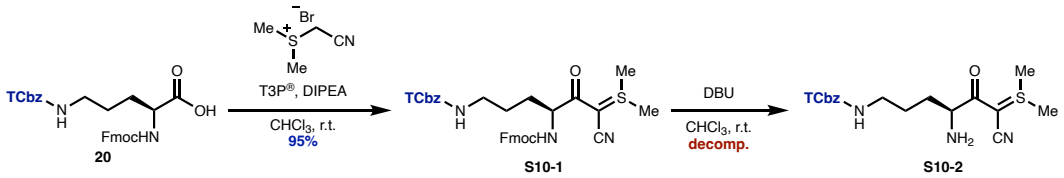
S8 (Reaction conditions were based on the known literature.[8])



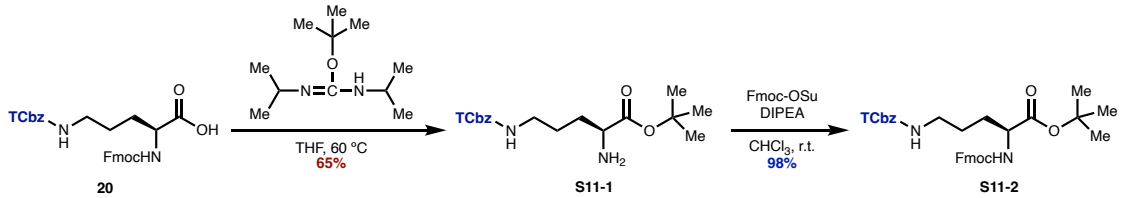
S9 (Reaction conditions were based on the known literature.[9])

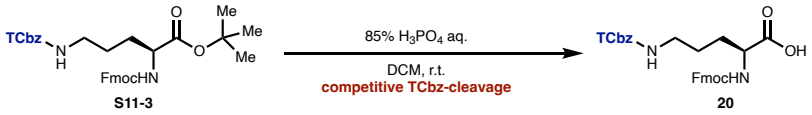


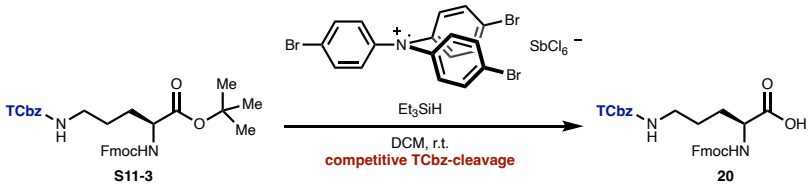
S10 (Reaction conditions were based on the known literature.[10])

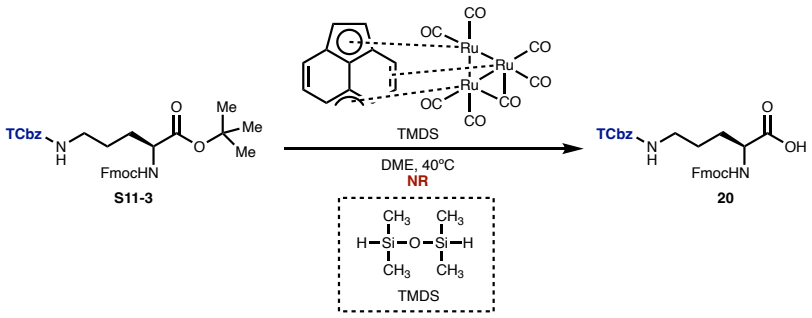


S11 (Reaction conditions were based on the known literature.[11])

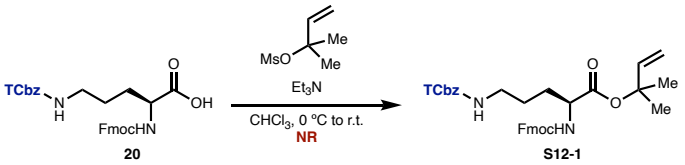


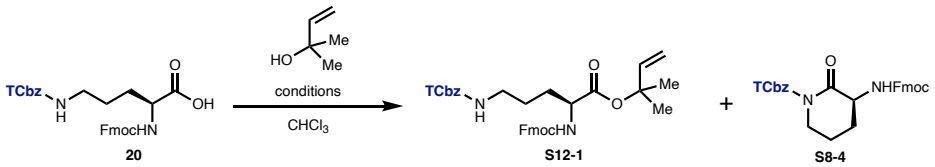




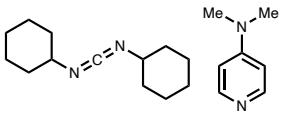


S12 (Reaction conditions were based on the known literature.[12])

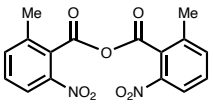




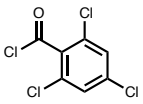
entry	conditions	temperture	time (h)	conv. (%)	yield (%)		results
					S12-1	S8-4	
1	DCC, DMAP, (±)-CSA	0 °C to r.t./40 °C	2.5/15.5	100	0	0	r.t. : no reaction
2	Shiina reagent, DMAP, DIPEA	r.t.	10	100	0	91	-
3	Yamaguchi reagent, DMAP, DIPEA	r.t./50°C	10/9	100	0	69	-
4	Mukaiyama reagent, Et_3N	r.t.	10	100	0	0	**messy**
5	Mandal reagent, DMAP, DIPEA	5	5	0	0	0	no reaction
6	Liu reagent, DIPEA	r.t.	10	100	0	31	**messy**



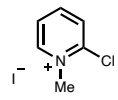
DCC, DMAP
(Steglich condition)



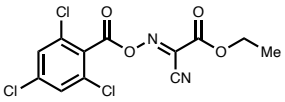
MNBA
(Shiina reagent)



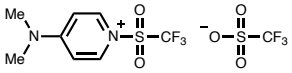
2,4,6-trichlorobenzoyl chloride
(Yamaguchi reagent)



2-Chloro-1-methylpyridinium Iodide
(Mukaiyama reagent)

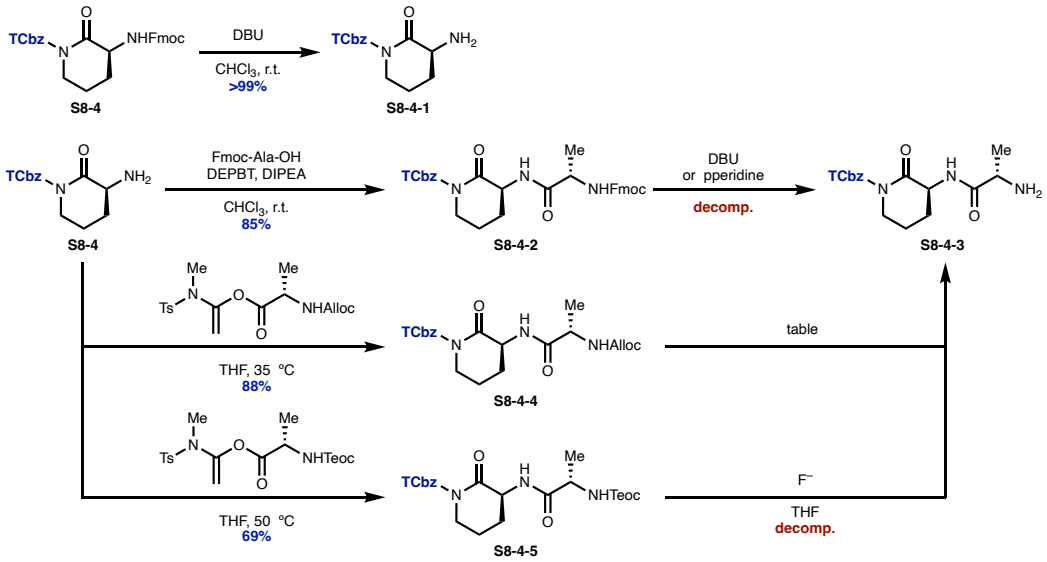


TCBOXY
(Mandal reagent)



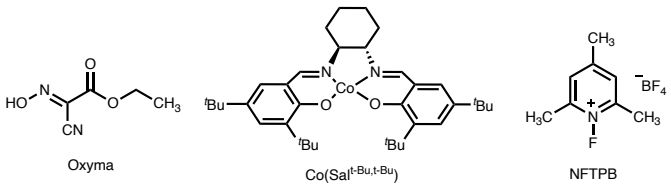
Tf-DMAP
(Liu reagent)

δ-lactam (Reaction conditions were based on the known literature.[13])



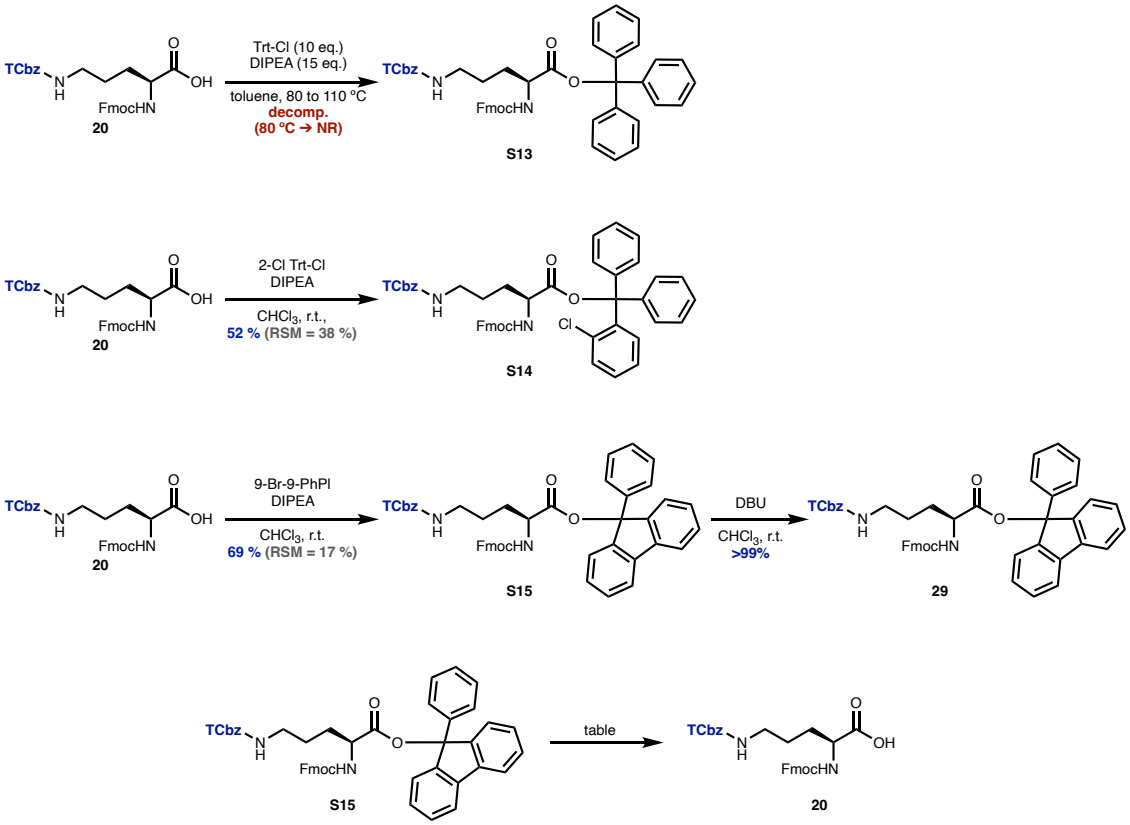
entry	conditions	additive	temp (°C)	time (h)	conv. (%)	yield (%)	note
1	$Pd(PPh_3)_4$ (10 mol%)	$PhSiH_3$ (5.0 eq.)	r.t.	0.5	100	12	lactam ring opning[a]
2	$Pd(PPh_3)_4$ (10 mol%)	Et_3SiH (5.0 eq +.5.0 eq.)	r.t.	16/22	incomplete	-	messy
3	$Pd(PPh_3)_4$ (10 mol%)	oxyma (5.0 eq.)	r.t./40	16/22	incomplete	-	trace
4	$Pd(PPh_3)_4$ (10 mol%)	imidazole (5.0 eq.)	r.t./40	8/24	incomplete	-	RSM: 24%
5	$Pd(PPh_3)_4$ (10 mol%)	N-Me imidazole (5.0 eq.)	r.t./40	5/24	incomplete	-	RSM: 48%
6	$Co(Sal^{t\text{-}Bu,t\text{-}Bu})$ (2.0 mol%)	$PhSiH_3$ (1.0 eq.), NFTPB (1.0 eq.)	r.t.	24	incomplete	16	lactam ring opning[a]
		EtOH (10%)[b]					+ RSM: 12%
7	$Pd(PPh_3)_4$ (100 mol%)	imidazole (5.0 eq.)	r.t.	5 (min)	100	5	lactam ring opning[a]

[a] accurate structure unidentified [b] added after 1 hour of stirring



Note: Attempts to use δ-lactam S8-4 as a C-terminal protective equivalent of the Orn residue failed to control the instability of the imide moiety.

S13, S14, S15

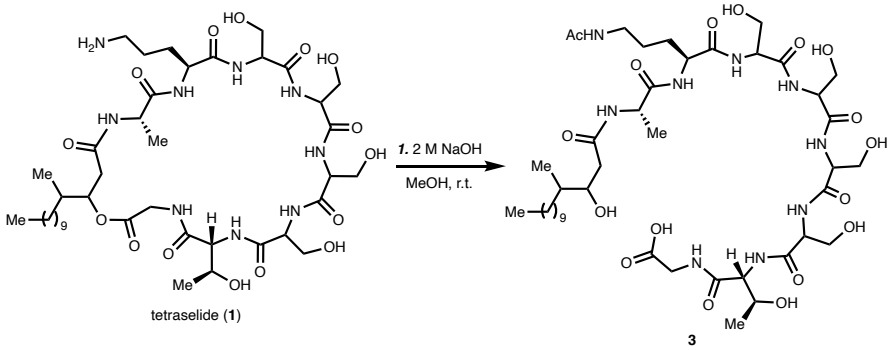


entry	conditions	additive	temp (°C)	time (h)	conv. (%)	yield (%)	note
1	10% HFIP/DCM(0.05 M)	-	r.t.	3		-	r.t. = prod observed
	to 67%HFIP/DCM (0.03 M)	-	r.t./40	2/3	100	70	+ 1 spot (above S.M)
2	50% AcOH/DCM(0.05 M)	-	r.t.	1	-	NR	-
3	50% TFE/DCM(0.05 M)	-	r.t.	10	-	trace	poor solubility
4	50% HFIP/DCM(0.05 M)	-	r.t.	30	-	-	incomplete
5	50% HFIP/DCM(0.05 M)	-	30	0.5	100	37	+ 1 spot (above S.M)
							→ 9-PhPI migration ? (^{1}H NMR)
6	50% HFIP/DCM(0.05 M)	$PhSiH_3$ (5.0 eq.)	30	2	100	>99	-

5. Experimental Procedures and Characterization Data

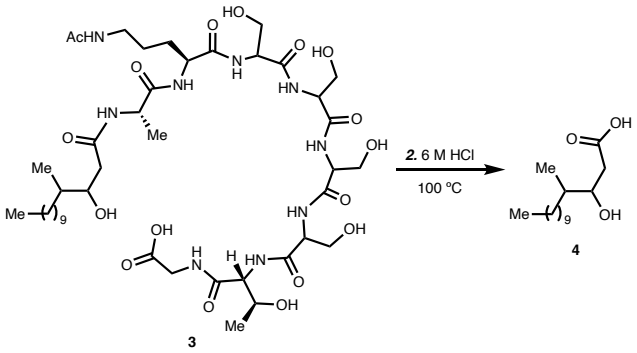
5-1. Degradation of Naturally Occurring Tetraselide

Linear peptide **3**



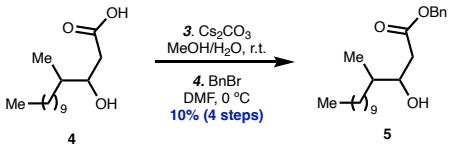
To a solution of tetraselide (**1**) (8.01 mg, 8.59 μmol) in MeOH (1.53 mL, 5.0 mM) was added 2 M NaOH aq. (170 μL) at room temperature. After stirring at room temperature for 3 h, to the reaction mixture was added Ac_2O (32.4 μL, 0.343 mmol, 40 equiv) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo.* This crude material was used in the next reaction without further purification.

Fatty acid **4**



To a glass vessel charged with the crude material (**3**) was added 6 M HCl aq. (1.00 mL, 8.6 mM) at room temperature. After stirring at 100 ºC for 24 h, the reaction mixture was extracted with hexane/EtOAc (1:1 v/v, 2 mL × 3). The combined organic layers were concentrated *in vacuo.* This crude material was used in the next reaction without further purification.

Benzyl ester derivative **5**



To a solution of the crude material (**4**) in MeOH (20 µL) was added 0.6 M Cs_2CO_3 aq. (10 µL) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was concentrated *in vacuo* and the resulting residue was azeotropically concentrated using toluene three times. The resulting residue was dissolved in DMF (20 µL) and cooled down to 0 °C under a N_2 atmosphere. To the resulting solution was added benzyl bromide (1.3 µL, 10.6 µmol) at 0 ºC. After stirring at room temperature for 23 h, the reaction mixture was quenched with H_2O at 0 ºC and extracted with hexane/EtOAc (1:1 v/v, 0.5 mL × 3). The combined organic layers were washed with brine and concentrated *in vacuo.* The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 4:1), yielding **5** (0.3 mg, 0.9 µmol, 10% over 4 steps) as a colorless oil.

Rf-value: 0.68 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{22}H_{26}O_3Na$: 371.2562, found: 371.2558.

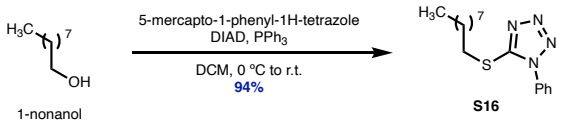
$\mathbf{[\alpha]_D^{24}}$ = –11.7 (c = 0.03, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39-7.31 (m, 5H), 5.15 (s, 2H), 3.89 (m, 1H), 2.85 (m, 1H), 2.53 (dd, *J* = 17.0, 2.5 Hz, 1H), 2.46 (dd, *J* = 17.0, 10.0 Hz, 1H), 1.45 (m, 1H), 1.36 (m, 1H), 1.37-1.25 (m, 16H), 1.11 (m, 1H), 0.88-0.86 (m, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.5, 135.6, 128.6 (2C), 128.4, 128.3 (2C), 71.8, 66.5, 38.1, 37.8, 32.3, 31.9, 29.9, 29.6, 29.6 (2C), 29.3, 27.1, 22.7, 14.8, 14.1

5-2. Fatty acid synthesis

Sulfide **S16**



To a solution of 1-nonanol (8.69 mL, 50.0 mmol), 5-mercapto-1-phenyl-1H-tetrazole (17.4 g, 97.5 mmol, 2.0 equiv) and PPh_3 (19.7g, 75.0 mmol, 1.5 equiv) in DCM (250 mL, 0.2 M) was added DIAD (1.9 M in toluene, 46.1 mL, 87.5 mmol, 1.8 equiv) dropwise at 0 ºC under a N_2 atmosphere. After stirring at room temperature for 15 h, the reaction mixture was concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 10:1 to 4:1), yielding **S16** (14.3 g, 47.0 mmol, 94%) as a colorless solid.

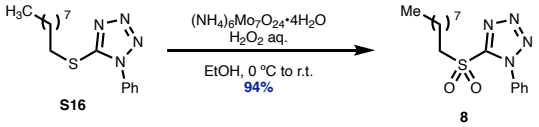
Rf-value: 0.74 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{16}H_{25}N_4S$: 305.1800, found: 305.1800.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.59-7.50 (m, 5H), 3.38 (t, J = 7.3 Hz, 2H), 1.80 (m, 2H), 1.43 (m, 2H), 1.34-1.25 (m, 10H), 0.86 (t, J = 7.3 Hz, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 154.5, 133.7, 130.0, 129.7 (2C), 123.8 (2C), 33.3, 31.8, 29.3, 29.2, 29.0, 29.0, 28.6, 22.6, 14.1

Sulfone **8**



To a solution of **S16** (14.3 g, 47.0 mmol) in EtOH (157 mL, 0.3 M) was added $(NH_4)_6Mo_7O_{24}$•$4H_2O$ (5.47 g, 4.70 mmol, 0.1 equiv) and H_2O_2 aq. (30.0-35.5 wt%, 70.3 mL) at 0 ºC. After stirring at room temperature for 6 h, the reaction mixture was quenched with sat. Na_2SO_3 aq. (250 mL) at 0 ºC and extracted with $CHCl_3$ (250 mL × 3). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 10:1), yielding **8** (14.3 g, 44.4 mmol, 94%) as a colorless solid.

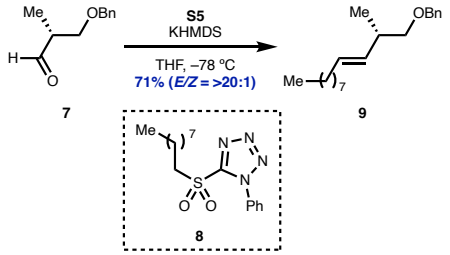
Rf-value: 0.60 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{16}H_{25}N_4O_2S$: 337.1698, found: 337.1698.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70-7.68 (m, 2H), 7.65-7.59 (m, 3H), 3.73 (t, *J* = 8.0 Hz, 2H), 1.95 (m, 2H), 1.49 (m, 2H), 1.34-1.27 (m, 10H), 0.88 (t, *J* = 7.0 Hz, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 153.5, 133.0, 131.4, 129.7 (2C), 125.0 (2C), 56.0, 31.7, 29.1, 29.1, 28.9, 28.1, 22.6, 21.9, 14.1

alkene **9**



To a solution of **8** (17.1 g, 53.0 mmol, 1.5 equiv) in THF (235 mL, 0.15 M) was added KHMDS (1.0 M in THF, 53.0 mL, 53.0 mmol, 1.5 equiv) dropwise at –78 ºC under a N_2 atmosphere. After stirring at –78 ºC for 1.5 h, to the reaction mixture was added **7**[14] (6.29 g, 35.3 mmol) in THF (118 mL) dropwise at –78 ºC. After stirring at –78 ºC for 1 h, the reaction mixture was warmed up to room temperature and stirred for an additional 37 h. The reaction mixture was quenched with sat. NH_4Cl aq. The resulting mixture was extracted with hexane/EtOAc (1:1 v/v, 353 mL × 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 10:1), yielding **9** (7.25 g, 25.2 mmol, 71%, *E*/*Z* = 20:1) as a colorless solid.

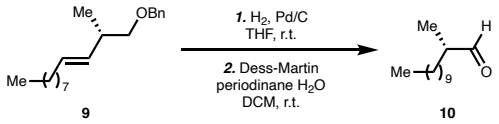
Rf-value: 0.64 (hexane/EtOAc = 5:1, stained with phosphomolybdic acid)

HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{20}H_{33}O_2$: 289.2531, found: 289.2545.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34-7.33 (m, 5H), 5.46 (m, 1H), 5.34 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.51 (s, 2H), 3.35 (dd, *J* = 9.1, 6.5 Hz, 1H), 3.26 (dd, *J* = 9.1, 6.5 Hz, 1H), 2.45 (m, 1H), 1.98 (m, 2H), 1.34-1.26 (m, 12H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 6.9 Hz, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 138.7, 132.5, 130.3, 128.3 (2C), 127.5 (2C), 127.4, 75.6, 72.8, 36.8, 32.7, 31.9, 29.5, 29.5, 29.3, 29.1, 22.7, 17.4, 14.1

Aldehyde **10**

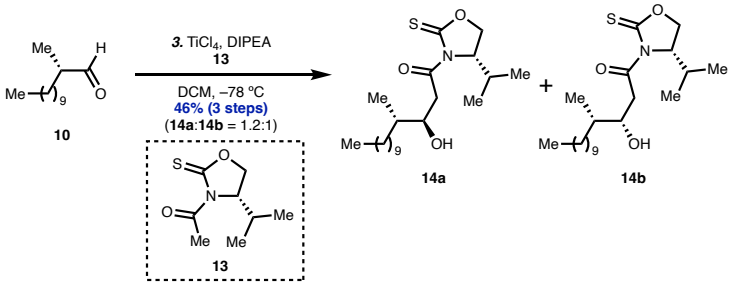


To a solution of **9** (1.44 g, 5.0 mmol) in THF (100 mL, 0.05 M) was added Pd/C (10 wt% loading, 1.62 g, 1.5 mmol, 30 mol%) at room temperature. The reaction vessel was carefully evacuated and backfilled with H_2 (balloon, 1 atm). After stirring at room temperature for 8 h under a H_2 atmosphere (1 atm), the reaction mixture was filtered through a pad of Celite (washed with $CHCl_3$) and concentrated *in vacuo.* This crude material was used in the next reaction without further purification.

**A portion was used from the crude material.*

To a solution of crude material * (120 mg, 0.60 mmol) in DCM (3.60 mL, 0.17 M) was Dess-Martin periodinane (469 mg, 1.11 mmol, 1.8 equiv) and H_2O (2 drops) at room temperature. After stirring at room temperature for 1.5 h, the reaction mixture was cooled down to 0 ºC and diluted with Et_2O (18.0 mL). After stirring at 0 ºC for 10 min, the reaction mixture was filtered through a pad of Celite (washed with Et_2O). The filtrate was washed with 10% $Na_2S_2O_3$ aq. (18.0 mL), sat. $NaHCO_3$ aq. (18.0 mL), brine (18.0 mL), and concentrated *in vacuo*. This crude material was used in the next reaction without further purification.

Oxazoline thione derivatives **14a** and **14b**

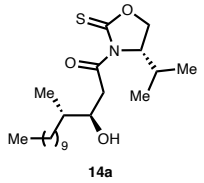


To a solution of **13**[15] (74.0 mg, 0.40 mmol) in DCM (2.60 mL, 0.15 M) was added $TiCl_4$ (1 M in DCM, 0.45 mL, 0.45 mmol, 1.2 equiv) dropwise and DIPEA (124 µL, 0.71 mmol, 1.80 equiv) dropwise at 0 ºC under a N_2 atmosphere. After stirring at 0 ºC for 30 min, the reaction mixture was cooled down to –78 ºC. To the reaction mixture was added aldehyde **10** (110 mg, theoretically 0.55 mmol, 1.4 equiv) in DCM (0.7 mL) dropwise at –78 ºC. After stirring at –78 ºC for 10 min, the reaction mixture was quenched with sodium phosphate buffer (pH = 7.0, 1.6 mL) and sat. NH_4Cl aq. (1.6 mL). The resulting mixture was extracted with DCM (3.3 mL × 3), dried over Na_2SO_4, filtered, and concentrated *in*

vacuo. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 50:1 to 10:1), yielding **14a** (38.1 mg, 98.8 μmol, 25%*, 3 steps) as a pale-yellow oil and **14b** (31.6 mg, 81.9 μmol, 21%*, 3 steps) as a pale-yellow oil.
*Yields were calculated from **9**.

*Note: The absolute configuration of the hydroxyl groups of **14a** and **14b** was determined by the Mosher method described below (**S17x** and **S17y**).*

14a (major diastereomer)



14a

Rf-value: 0.40 (hexane/EtOAc = 8:1, stained with phosphomolybdic acid)

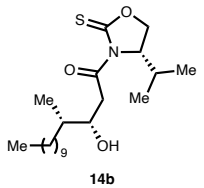
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{21}H_{40}NO_3S$: 386.2729, found: 386.2734.

$[\alpha]_D^{29}$ = +200.3 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.72 (m, 1H), 4.41 (app s, 1H), 4.39 (d, *J* = 3.0 Hz, 1H), 4.00 (ddd, *J* = 10.5, 6.5, 2.0 Hz, 1H), 3.60 (dd, J = 17.5, 2.0 Hz, 1H), 3.30 (dd, *J* = 17.5, 10.5 Hz, 1H), 2.47 (m, 1H), 2.36 (m, 1H), 1.67 (m, 1H), 1.50 (m, 1H), 1.39 (m, 1H), 1.32-1.21 (br-m, 16H), 1.16 (m, 1H), 0.97-0.92 (m, 6H), 0.89 (t, *J* = 7.8 Hz, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 186.1, 174.1, 71.6, 67.7, 63.2, 41.4, 38.2, 32.3, 31.9, 29.9, 29.6 (3C), 29.3, 29.0, 27.2, 22.7, 18.3, 15.0, 15.0, 14.1

14b (minor diastereomer)



14b

Rf-value: 0.48 (hexane/EtOAc = 8:1, stained with phosphomolybdic acid)

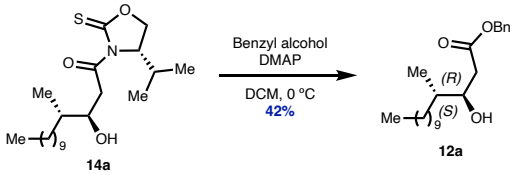
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{21}H_{40}NO_3S$: 386.2729, found: 386.2728.

$[\alpha]_D^{29}$ = +96.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.74 (m, 1H), 4.42-4.38 (m, 2H), 4.00 (ddd, *J* = 10.5, 4.5, 2.5 Hz, 1H), 3.61 (dd, *J* = 17.5, 10.5 Hz, 1H), 3.36 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.50-2.31 (m, 2H), 1.59 (m, 1H), 1.49 (m, 1H), 1.36 (m, 1H), 1.32-1.21 (m, 15H), 1.16 (m, 1H), 0.96-0.92 (m, 6H), 0.89 (t, *J* = 6.9 Hz, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 186.3, 174.2, 71.6, 67.8, 63.2, 42.0, 38.2, 32.8, 31.9, 29.9, 29.6 (3C), 29.3, 29.0, 27.3, 22.7, 18.2, 15.0, 14.4, 14.1

Benzyl ester **12a**



To a solution of **14a** (15.0 mg, 38.9 µmol) in DCM (0.2 mL, 0.19 M) was added benzyl alcohol (4.8 µL, 46.7 µmol, 1.2 equiv) and DMAP (0.95 mg, 7.8 µmol, 0.2 equiv) at 0 ºC. After stirring at room temperature for 10 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 4:1), yielding **12a** (5.7 mg, 16.4 µmol, 42%) as a colorless oil.

Rf-value: 0.72 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

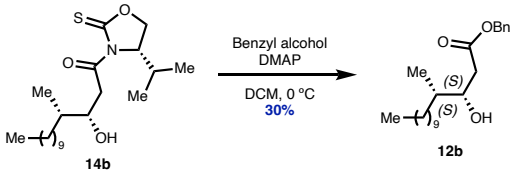
HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{22}H_{36}O_3Na$: 371.2562, found: 371.2554.

$[\alpha]_D^{26}$ = –7.2 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38-7.33 (m, 5H), 5.16 (s, 2H), 3.90 (br-m, 1H), 2.87 (br-d, *J* = 3.5 Hz, 1H), 2.53 (dd, *J* =17.0, 3.0 Hz, 1H), 2.46 (dd, *J* = 17.0, 10.0 Hz, 1H), 1.45 (m, 1H), 1.37 (br-m, 1H), 1.31-1.20 (m, 16H), 1.11 (m, 1H), 0.89-0.86 (m, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.4, 135.5, 128.6 (2C), 128.4, 128.3 (2C), 71.8, 66.5, 38.1, 37.8, 32.2, 31.9, 29.9, 29.6, 29.6 (2C), 29.3, 27.1, 22.7, 14.8, 14.1

Benzyl ester **12b**



To a solution of **14b** (4.5 mg, 11.7 µmol) in DCM (60.0 µL, 0.19 M) was added benzyl alcohol (1.4 µL, 14.0 µmol, 1.2 equiv) and DMAP (0.29 mg, 2.3 µmol, 0.2 equiv) at 0 ºC. After stirring at room temperature for 16 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 4:1), yielding **12b** (1.2 mg, 3.4 µmol, 30%) as a colorless oil.

Rf-value: 0.72 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

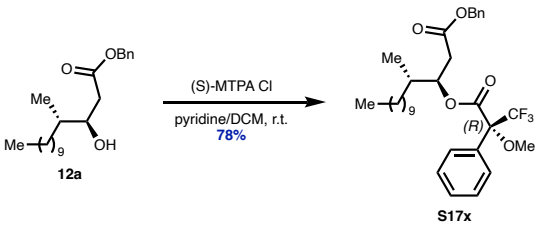
HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{22}H_{36}O_3Na$: 371.2562, found: 371.2556.

$\mathbf{[\alpha]_D^{26}}$ = –21.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39-7.33 (m, 5H), 5.16 (s, 2H), 3.96 (br-s, 1H), 2.74 (s, 1H), 2.56-2.48 (m, 2H), 1.51-1.43 (m, 2H), 1.33-1.18 (br-m, 16H), 1.11 (br-m, 1H), 0.91-0.87 (m, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.3, 135.6, 128.6 (2C), 128.3, 128.3 (2C), 71.2, 66.5, 38.8, 38.0, 32.7, 31.9, 29.9, 29.6, 29.6 (2C), 29.3, 27.2, 22.7, 14.2, 14.1

Mosher ester **S17x**



To a solution of **12a** (2.6 mg, 7.5 μmol) in pyridine/DCM (1:1 v/v, 150 μL, 0.05 M) was added (S)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl Chloride (2.9 μL, 15.5 μmol, 2.0 equiv) at room temperature under a N_2 atmosphere. After stirring at room temperature for 9 h, the reaction mixture was quenched with H_2O at 0 ºC. The resulting solution was extracted with DCM (1.0 mL × 3). The combined organic phase was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 4:1), yielding **S17x** (3.3 mg, 5.8 μmol, 78%) as a colorless oil.

Rf-value: 0.80 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

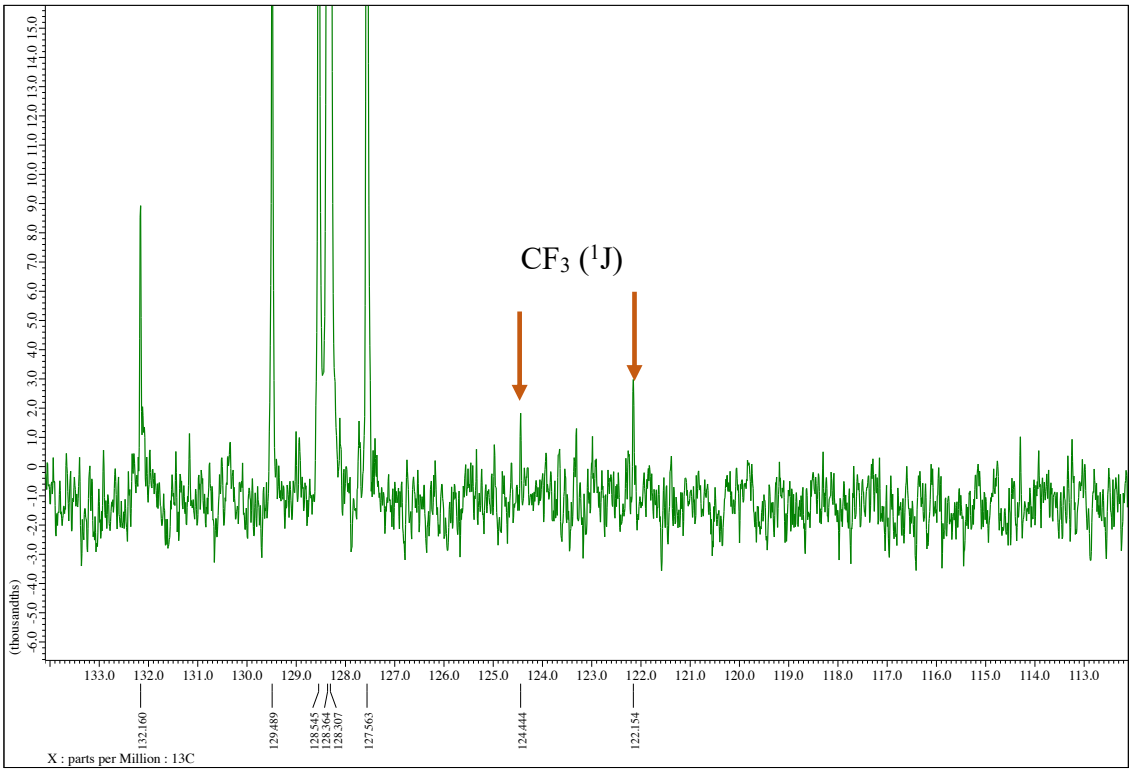
HRMS (*m/z*): ESI $[M+NH_4]^+$ calculated for $C_{32}H_{47}F_3NO_5$: 582.3406, found: 582.3407.

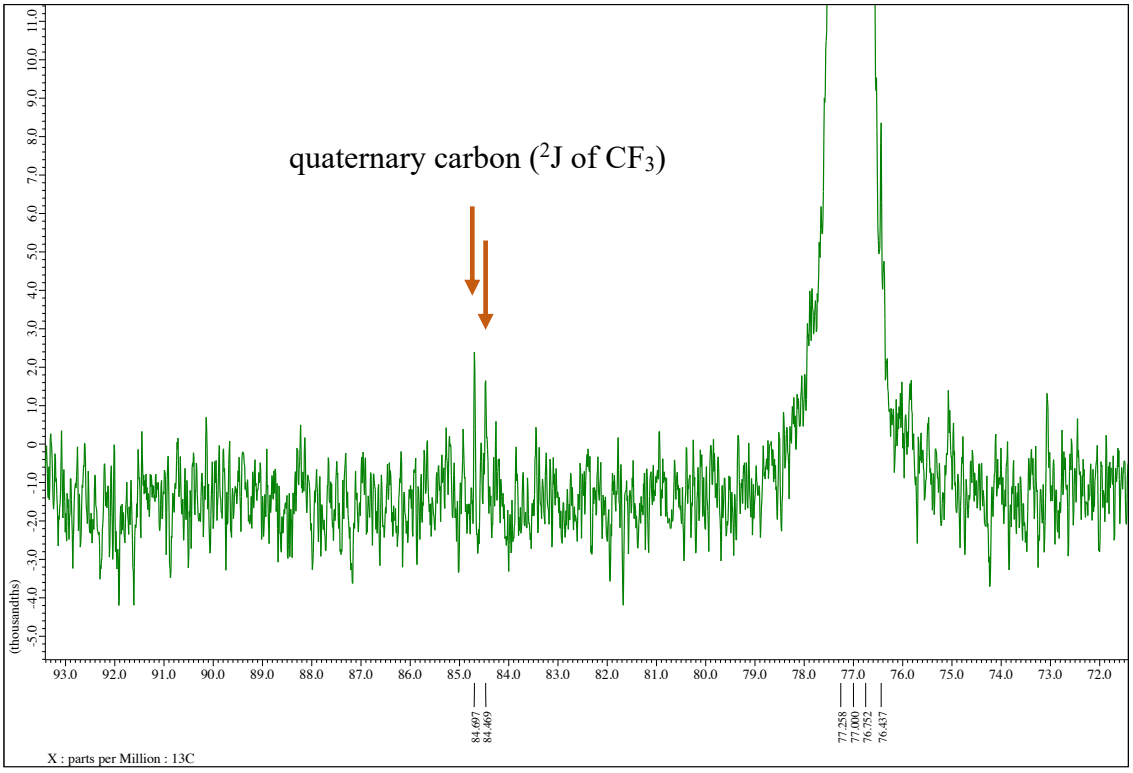
$[\alpha]_D^{29}$ = +59.7 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53-7.51 (m, 2H), 7.41-7.36 (m, 3H), 7.36-7.31 (m, 3H), 7.29-7.27 (m, 2H), 5.53 (m, 1H), 5.04-4.97 (m, 2H), 3.48 (s, 3H), 2.64 (dd, *J* = 16.0, 9.5 Hz, 1H), 2.55 (dd, *J* = 16.0, 3.5 Hz, 1H), 1.91 (m, 1H), 1.40-1.34 (m, 2H), 1.31-1.24 (m, 15H), 1.10 (m, 1H), 0.89 (m, 6H)

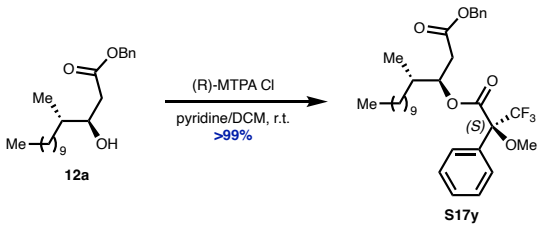
^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 165.8, 135.5, 132.2, 129.5 (2C), 128.5 (2C), 128.4, 128.3 (2C), 127.6 (3C), 124.4 (*CF_3, ^1J = 288.5 Hz), 122.2 (*CF_3, ^1J = 288.5 Hz), 84.7 (*CF_3, ^2J = 28.7 Hz), 84.7 (*CF_3, ^2J = 28.7 Hz), 76.4, 66.7, 55.3, 36.0, 34.7, 32.4, 31.9, 29.7, 29.6 (2C), 29.5, 29.3, 27.1, 22.7, 14.5, 14.1

Note: Only two peaks could be detected in ^{13}C NMR of CF_3 (^1J) and the neighboring quaternary carbon (^2J) (see below figure)


CF3 (1J)
(thousandths)
X : parts per Million : 13C


quaternary carbon (2J of CF3)
(thousandths)
X : parts per Million : 13C

Mosher ester **S17y**



To a solution of **12a** (2.6 mg, 7.5 μmol) in pyridine/DCM (1:1 v/v, 150 μL, 0.05 M) was added (*R*)-(–)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (1.0 M in DCM, 15.5 μL, 15.5 μmol, 2.0 equiv) at room temperature under a N_2 atmosphere. After stirring at room temperature for 9 h, the reaction mixture was quenched with H_2O at 0 ºC. The resulting solution was extracted with DCM (1.0 mL × 3). The combined organic phase was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 4:1), yielding **S17y** (4.2 mg, 7.5 μmol, >99%) as a colorless oil.

Rf-value: 0.80 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

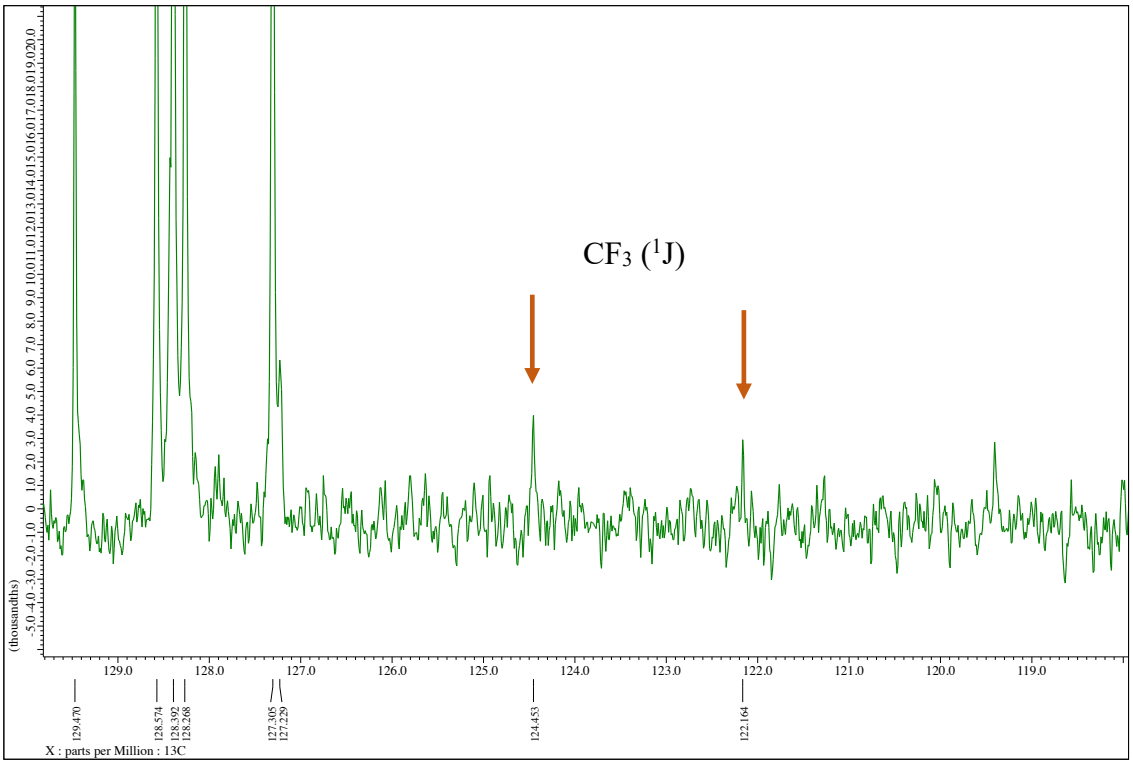
HRMS (*m/z*): ESI $[M+NH_4]^+$ calculated for $C_{32}H_{47}F_3NO_5$: 582.3406, found: 582.3411.

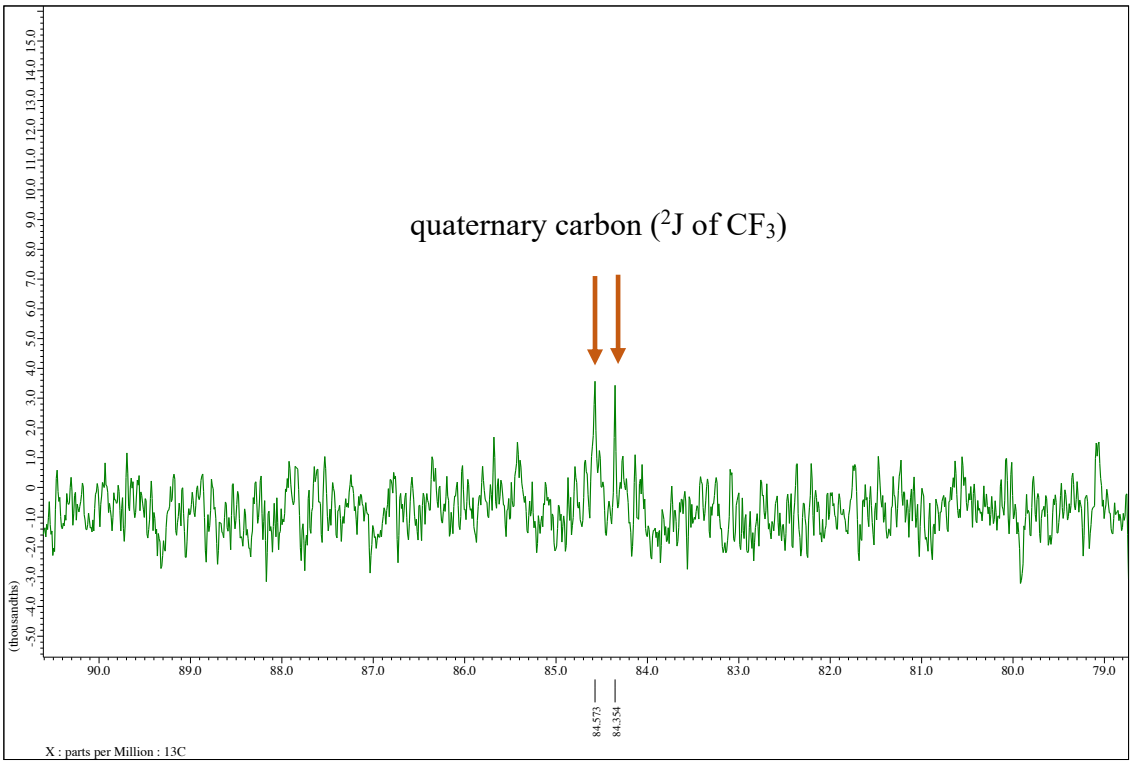
$[\alpha]_D^{29}$ = –33.5 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 (app d, *J* = 6.5 Hz, 2H), 7.39-7.31 (m, 8H), 5.50 (ddd, *J* = 10.0, 7.0, 3.5 Hz, 1H), 5.09 (s, 2H), 3.46 (s, 3H), 2.67 (dd, *J* = 17.0, 10.0 Hz, 1H), 2.58 (dd, *J* = 17.0, 3.5 Hz, 1H), 1.87 (m, 1H), 1.36-1.17 (m, 17H), 1.04 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 7.0 Hz, 3H)

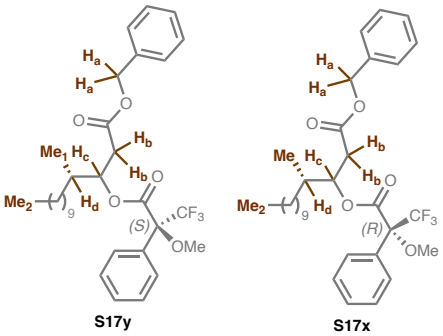
^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5, 165.8, 135.4, 132.3, 129.5 (2C), 128.6 (2C), 128.4, 128.3 (2C), 127.3 (2C), 127.2, 124.5 (*CF_3, ^1J = 288.4 Hz), 122.2 (*CF_3, ^1J = 288.4 Hz), 84.6 (*CF_3, ^2J = 27.6 Hz), 84.4 (*CF_3, ^2J = 27.6 Hz), 76.6, 66.7, 55.4, 35.7, 34.6, 32.3, 31.9, 29.7, 29.6 (2C), 29.5, 29.3, 27.0, 22.7, 14.1, 14.1

Note: Only two peaks could be detected in ^{13}C NMR of CF_3 (^1J) and the neighboring quaternary carbon (^2J) (see below figure)


CF3 (1J)
(thousandths)
129.0
128.0
127.0
126.0
125.0
124.0
123.0
122.0
121.0
120.0
119.0
129.470
128.574
128.392
128.268
127.305
127.229
124.453
122.164
X : parts per Million : 13C

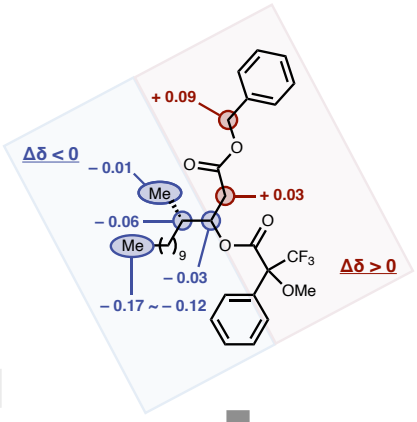

quaternary carbon (2J of CF3)
(thousandths)
90.0
89.0
88.0
87.0
86.0
85.0
84.0
83.0
82.0
81.0
80.0
79.0
84.573
84.354
X : parts per Million : 13C

5-3. Mosher ester analysis (Figure S18)

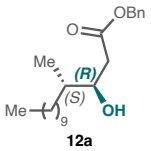


S17y

S17x



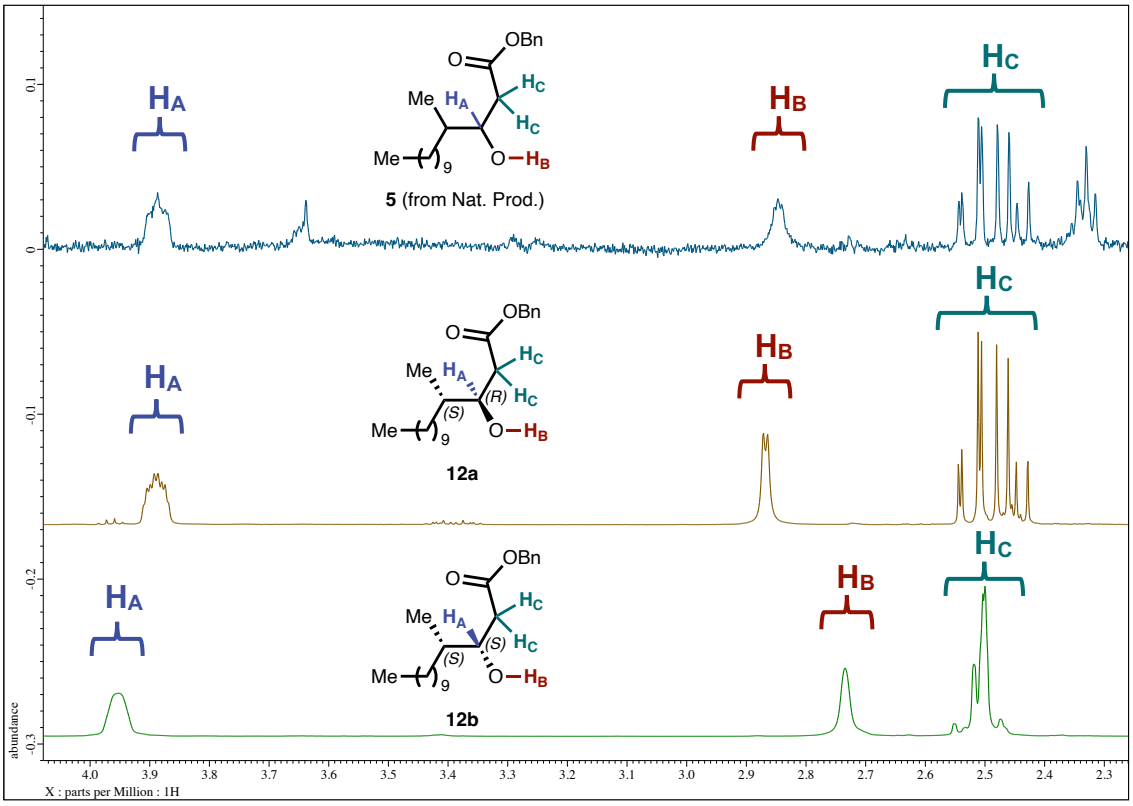
position	δ_H (**S17y**: δS)	δ_H (**S17x**: δR)	Δδ = δS – δR
H_a	5.09	5.00	**+ 0.09**
H_b	(2.67+2.58)÷2 = 2.63	(2.64+2.55)÷2 = 2.60	**+ 0.03**
H_c	5.50	5.53	**– 0.03**
H_d	(1.91+1.83)÷2 = 1.87	(1.94+1.91)÷2 = 1.93	**– 0.06**
Me_1	0.88	0.89	**– 0.01**
Me_2	0.74	0.86-0.91	**– 0.17 ~ – 0.12**

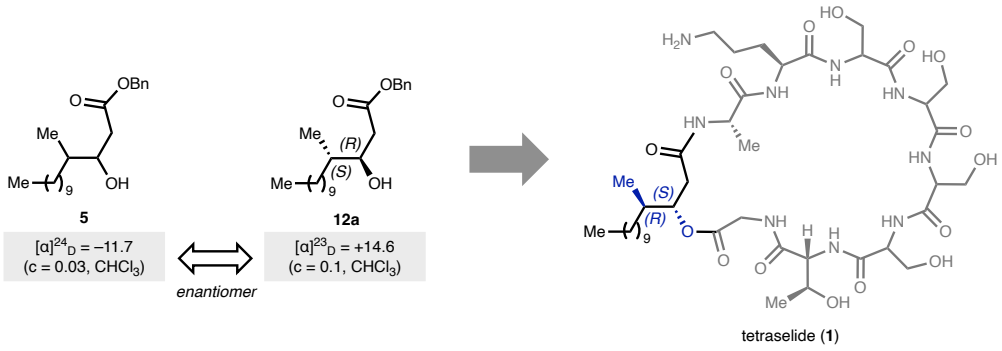


12a

5-4. Comparison of Natural and Synthetic *β*-hydroxy-*γ*-methyl fatty acids (Figure S19)

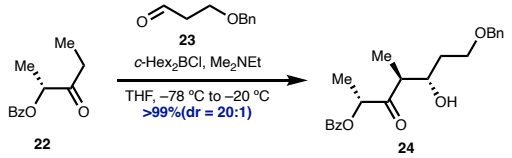
^{1}H NMR ($CDCl_3$)





5-5. (3S, 4R)-*β*-hydroxy-*γ*-methyl fatty acid synthesis

Benzoyl **24**



To a solution of *c*-Hex_2BCl (1.0 M in hexane 15.0 mL, 15.0 mmol, 1.5 equiv) was added Me_2NEt (1.94 mL, 18.0 mmol, 1.8 equiv) dropwise and **22**[16] (2.06 g, 10.0 mmol) in Et_2O (39.6 mL, 0.25 M) dropwise at –78 ºC under a N_2 atmosphere. After stirring at 0 ºC for 2 h, the reaction mixture was cooled down to –78 ºC. To the reaction mixture was added **23**[17] (2.46 g, 15.0 mmol, 1.5 equiv) in Et_2O (16.9 mL) dropwise at –78 ºC. After stirring at –78 ºC for 2 h, the reaction mixture was warmed up to –20 ºC and stirred for an additional 2 h. The reaction mixture was quenched with MeOH (28.3 mL), sodium phosphate buffer (pH = 7.0, 28.3 mL) and H_2O_2 aq. (30.0-35.5 wt%, 28.3 mL), and extracted with DCM (170 mL × 4). The combined organic phase was washed with sat. $NaHCO_3$ aq. and brine, and concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 4:1 to 1:1), yielding **24** (3.70 g, 10.0 mmol, >99%, dr = 20:1) as a colorless oil.

Rf-value: 0.23 (hexane/EtOAc = 4:1, stained with phosphomolybdic acid)

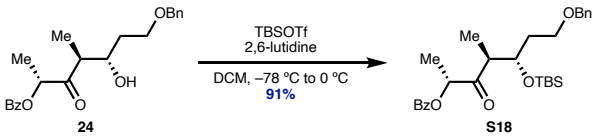
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{22}H_{27}O_5$: 371.1858, found: 371.1857.

$[\alpha]_D^{29}$ = –10.2 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08-8.07 (m, 2H), 7.58 (m, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.35-7.28 (m, 5H), 5.43 (q, *J* = 6.8 Hz, 1H), 4.50 (s, 2H), 4.00 (m, 1H), 3.73 (m, 1H), 3.66 (m, 1H), 3.24 (d, *J* = 4.0 Hz, 1H), 2.93 (m, 1H), 1.86 (m, 1H), 1.72 (m, 1H), 1.56 (d, *J* = 7.5 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 211.1, 165.8, 137.7, 133.2, 129.7 (2C), 129.4, 128.3 (4C), 127.6, 127.6 (2C), 74.8, 73.2, 70.2, 68.4, 48.2, 33.6, 15.5, 13.8

S18



To a solution of **24** (1.35 g, 3.64 mmol) in DCM (36.4 mL, 0.1 M) was added 2,6-lutidine (1.27 mL, 10.9 mmol, 3.0 equiv) and TBSOTf (1.68 mL, 7.29 mmol) dropwise at –78 ºC under a N_2 atmosphere. After stirring at –78 ºC for 2 h, the reaction mixture was warmed up to –10 ºC. After stirring at –10 ºC for 2 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (36.4 mL), and extracted with DCM (40 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 4:1), yielding **S18** (1.60 g, 3.32 mmol, 91%) as a pale-yellow oil.

Rf-value: 0.52 (hexane/EtOAc = 2:1, stained with phosphomolybdic acid)

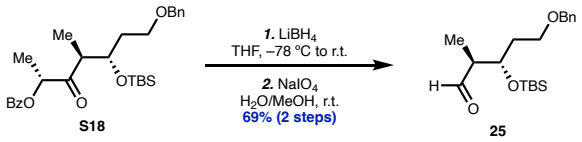
HRMS (*m/z*): ESI $[M+NH_4]^+$ calculated for $C_{28}H_{44}NO_5Si$: 502.2989, found: 502.2983.

$[\alpha]_D^{29}$ = +17.3 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (dd, *J* = 8.5, 1.0 Hz, 2H), 7.57 (m, 1H), 7.44 (t, *J* = 6.8 Hz, 2H), 7.34-7.28 (m, 5H), 5.40 (q, *J* = 6.8 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.18 (m, 1H), 3.62 (td, *J* = 9.0, 7.0 Hz, 1H), 3.56 (td, *J* = 9.0, 7.0 Hz, 1H), 3.09 (m, 7.1 Hz, 1H), 1.87-1.77 (m, 2H), 1.49 (d, *J* = 6.5 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 0.82 (s, 9H), 0.01 (s, 3H), -0.05 (s, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): 209.0, 165.7, 138.4, 133.2, 129.7 (2C), 129.5, 128.3 (2C), 128.2 (2C), 127.4 (2C), 127.4 (2C), 74.7, 72.9, 70.4, 65.6, 47.8, 32.9, 25.8 (3C), 15.4, 12.7, -4.8, -5.0

Aldehyde **25**



To a solution of **S18** (1.61 g, 3.32 mmol) in THF (32.5 mL, 0.1 M) was added $LiBH_4$ (4 M in THF, 14.9 mL, 59.7 mmol, 18.0 equiv) dropwise at –78 ºC under a N_2 atmosphere. After stirring at –78 ºC for 30 min, the reaction mixture was warmed up to room temperature. After stirring at room temperature for 37 h, the reaction mixture was quenched with H_2O (23.7 mL), sat. sodium potassium tartrate aq. (23.7 mL) and sat.

NH_4Cl aq., and extracted with DCM (40 mL × 4) and Et_2O (40 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This crude material was used in the next reaction without further purification.

To a solution of the crude material in H_2O/MeOH (3:5 v/v, 33.2 mL, 0.01 M) was added $NaIO_4$ (4.25 g, 19.9 mmol, 6.0 equiv) at room temperature. After stirring at room temperature for 3 h, the reaction mixture was quenched with H_2O (45 mL) and filtered through a pad of Celite (washed with Et_2O). The filtrate was extracted with Et_2O (45 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 10:1), yielding **25** (765 mg, 2.27 mmol, 69%) as a pale-yellow oil.

Rf-value: 0.61 (hexane/EtOAc = 10:1, stained with phosphomolybdic acid)

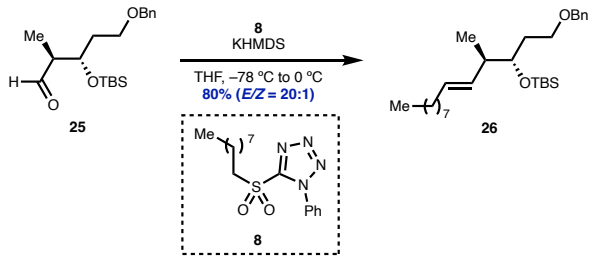
HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{19}H_{32}O_3SiNa$: 359.2018, found: 359.2015.

$[\alpha]_D^{29}$ = +2.7 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.72 (d, *J* = 2.5 Hz, 1H), 7.36-7.27 (m, 5H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H) 4.16 (dt, *J* = 6.5, 5.0 Hz, 1H), 3.55 (t, *J* = 6.8 Hz, 2H), 2.56-2.51 (m, 1H), 1.86 (m, 1H), 1.79 (m, 1H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.87 (s, 9H), 0.06 (s, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 204.7, 138.3, 128.4 (2C), 127.6 (3C), 73.0, 70.4, 66.3, 51.6, 34.7, 25.7 (3C), 18.0, 10.1, -4.5, -4.7

Alkene **26**



To a solution of **8** (1.10 g, 3.41 mmol, 1.5 equiv) in THF (15.1 mL) was added KHMDS (1.0 M in THF, 3.41 mL, 3.41 mmol, 1.5 equiv) dropwise at –78 °C under a N_2 atmosphere. After stirring at –78 °C for 1 h, to the reaction mixture was added **25** (765 mg, 2.27 mmol) in THF (7.57 mL) dropwise. After stirring at –78 °C for 1 h, the reaction mixture was warmed up to –40 °C and stirred for an additional 11 h. After stirring at 0 °C for an

additional 1 h, the reaction mixture was quenched with sat. NH_4Cl aq. (22.7 mL) and extracted with hexane (22.7 mL × 4). The combined organic phase was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 20:1), yielding **26** (809 mg, 1.81 mmol, 80%, *E*/*Z* = 20:1) as a yellow oil.

Rf-value: 0.70 (hexane/EtOAc = 20:1, stained with phosphomolybdic acid)

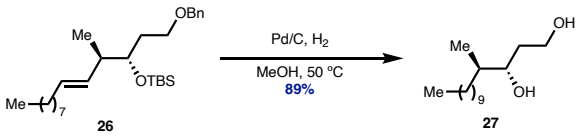
HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{28}H_{50}O_2SiNa$: 469.3478, found: 469.3470.

$[\alpha]_D^{29}$ = +5.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36-7.33 (m, 5H), 5.40-5.30 (m, 2H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 3.72 (m, 1H), 3.54-3.47 (m, 2H), 2.24 (m, 1H), 1.97 (dd, *J* = 13.0, 6.5 Hz, 2H), 1.75-1.62 (m, 2H), 1.33-1.26 (m, 12H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.89-0.86 (m, 12H), 0.04 (s, 3H), 0.03 (s, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 138.6, 132.0, 130.7, 128.3 (2C), 127.6 (2C), 127.4, 72.9, 72.8, 67.5, 42.3, 33.0, 32.7, 31.9, 29.5, 29.5, 29.3, 29.2, 25.9 (3C), 22.7, 18.1, 15.0, 14.1, -4.4, -4.6

Diol **27**



To a solution of **26** (265 mg, 0.594 mmol) in THF (11.9 mL, 0.05 M) was added Pd/C (10 wt% loading, 316 mg, 0.30 mmol, 50 mol%) at room temperature. The reaction vessel was carefully evacuated and backfilled with H_2 (balloon, 1 atm). After stirring at 50 ºC for 6 h under a H_2 atmosphere (1 atm), the reaction mixture was filtered through a pad of Celite (washed with $CHCl_3$) and concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 4:1 to $CHCl_3$/MeOH = 10:1), yielding **27** (129 mg, 0.529 mmol, 89%) as a colorless solid.

Rf-value: 0.53 ($CHCl_3$/MeOH = 10:1, stained with phosphomolybdic acid)

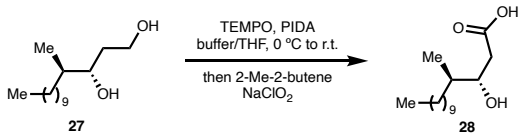
HRMS (*m/z*): ESI $[M+Na]^+$ calculated for $C_{15}H_{32}O_2Na$: 267.2300, found: 267.2301.

[α]$_D^{29}$ = +22.5 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.87 (m, 1H), 3.80 (m, 1H), 3.68 (m, 1H), 2.94 (br-s, 2H), 1.66-1.62 (m, 2H), 1.42 (m, 1H), 1.35 (m, 1H), 1.30-1.17 (m, 16H), 1.09 (m, 1H), 0.89 (s, 3H), 0.86 (s, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 76.3, 62.1, 39.1, 34.1, 32.2, 31.9, 29.9, 29.6 (3C), 29.3, 27.2, 22.7, 14.8, 14.1

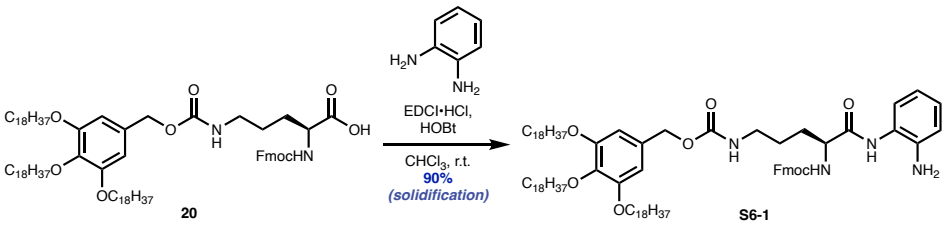
(3S, 4R)-*β*-hydroxy-*γ*-methyl fatty acid (**28**)



To a solution of **27** (86.9 mg, 0.356 mmol) in pH 7.0 sodium phosphate buffer/THF (1:4 v/v, 3.56 mL, 0.1 M) was added TEMPO (58.4 mg, 37.4 mmol, 1.1 equiv) and PIDA (241 mg, 2.1 equiv) at 0 ºC. After stirring at room temperature for 17 h, to the reaction mixture was added 2-methyl-2-butene (378 μL, 3.56 mmol, 10.0 equiv) and $NaClO_2$ (161 mg, 1.78 mmol, 5.0 equiv) at room temperature. After stirring at room temperature for 5 h, the reaction mixture was diluted with 1 M NaOH aq. (pH adjusted to 12). The aqueous phase was washed with DCM (3 times) and acidified with 1 M HCl aq. (pH adjusted to 2). The aqueous phase was extracted with EtOAc (Same amount as water layer × 4). The combined organic phase was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. This crude material was used in the next reaction without further purification.

5-6. Ornithine derivative synthesis

S6-1



To a solution of Fmoc-Orn(TCbz)-OH (**20**) [18] (38.8 mg, 30.0 µmol) in $CHCl_3$ (600 µL, 0.05 M) was added 1,2-phenylendiamine (4.5 mg, 42.0 µmol, 1.4 equiv), HOBt (1.2 mg, 9.0 µmol, 0.3 equiv) and EDCI•HCl (9.2 mg, 48.0 µmol, 1.6 equiv) at room temperature. After stirring at room temperature for 7 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (3.00 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **S6-1** (37.4 mg, 27.0 µmol, 90%) as a colorless powder.

Rf-value: 0.42 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

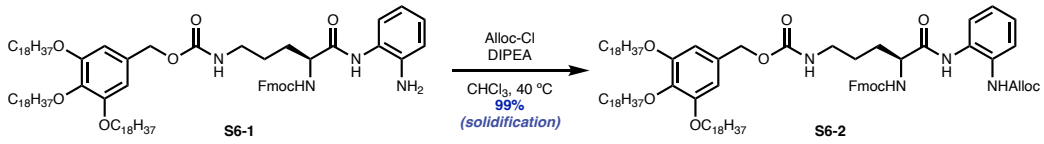
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (br-s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.61-7.59 (br-m, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.05-7.02 (m, 2H), 6.77 (t, *J* = 7.8 Hz, 2H), 6.54 (m, 1H), 6.50 (s, 2H), 5.67 (m, 1H) 5.02-4.92 (m, 2H), 4.59 (m, 1H), 4.45-4.40 (m, 2H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.97-3.87 (m, 6H), 3.55 (m, 1H), 3.19 (m, 1H), 1.99 (m, 1H) 1.80-1.69 (m, 7H), 1.45-1.41 (m, 6H), 1.34-1.11 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)
(*NH_2 proton was not detected.)

^{13}C NMR (126 MHz, $CDCl_3$, 45 ºC): δ 170.5, 157.3, 156.6, 153.3 (2C), 143.8, 143.7, 141.4 (2C), 140.5, 138.6, 131.2, 127.7 (2C), 127.1 (2C), 125.2, 125.0 (2C), 123.7, 120.0 (2C), 119.1, 117.6, 107.2 (2C), 73.5, 69.4 (2C), 67.4, 67.1, 54.3, 47.3, 39.7, 31.9 (3C), 30.4, 30.1, 29.7-29.3 (overlap, 39C), 26.5, 26.2 (3C), 22.7 (3C), 14.0 (3C)

*Note 1: Because compound **S6-1** has poor solubility in various solvents, the optical rotation could not be recorded.*

Note 2: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

S6-2



To a solution of **S6-1** (12.7 mg, 9.2 μmol) in $CHCl_3$ (459 μL, 0.02 M) was added DIPEA (2.6 μL, 14.7 μmol, 1.6 equiv) and Alloc-Cl (1.4 μl, 12.9 μmol, 1.4 equiv) at room temperature. After stirring at 40 ºC for 1 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (2.30 mL, 0.004 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **S6-2** (13.3 mg, 9.1 μmol, 99%) as a colorless powder.

Rf-value: 0.52 ($CHCl_3$/MeOH = 60:1, stained with phosphomolybdic acid)

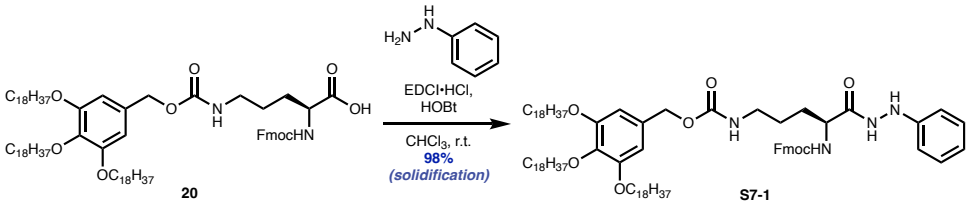
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.62 (br-s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (br-s, 3H), 7.43-7.37 (br-m, 2H), 7.33-7.28 (br-m, 2H), 7.21 (t, *J* = 7.3 Hz, 2H), 7.12 (t, *J* = 7.5 Hz, 2H), 6.54 (m, 1H), 6.51 (s, 2H), 5.89 (m, 1H), 5.67 (d, *J* = 5.5 Hz, 1H), 5.28 (d, *J* = 17.5 Hz, 1H), 5.17 (d, *J* = 10.5 Hz, 1H), 5.01 (d, *J* = 12.0 Hz, 1H) 4.92 (d, *J* = 12.0 Hz, 1H), 4.61-4.52 (m, 2H), 4.44 (br-d, *J* = 7.0 Hz, 1H), 4.23 (t, *J* = 6.8 Hz, 1H), 3.98-3.90 (m, 8H), 3.51 (br-s, 1H), 3.20 (br-s, 1H), 1.96 (br-m, 1H), 1.78-1.69 (m, 7H), 1.49-1.41 (m, 6H), 1.31-1.09 (m, 86H), 0.88 (t, *J* = 7.0 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$, 45 ºC): δ 166.8, 165.1, 157.2, 156.6, 153.3 (2C), 143.7 (2C), 141.4 (2C), 140.6, 138.5, 132.5, 131.1, 131.0, 127.7 (2C), 127.1 (2C), 126.6, 125.3, 125.0, 124.9, 123.2, 120.0 (2C), 118.8, 118.0, 117.1, 107.1 (2C), 73.5, 69.4 (2C), 67.3, 67.2, 66.1, 54.5, 47.3, 31.9 (3C), 30.4, 30.1, 29.7-29.3 (overlap, 39C), 26.3, 26.2 (2C), 22.7 (3C), 14.0 (3C)

*Note 1: Because compound **S6-2** has poor solubility in various solvents, the optical rotation could not be recorded.*

Note 2: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

S7-1



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (129 mg, 0.100 mmol) in $CHCl_3$ (2.00 mL, 0.05 M) was added phenylhydrazine (13.8 µL, 0.140 mmol, 1.4 equiv), EDCI•HCl (30.7 mg, 0.160 mmol, 1.6 equiv) and HOBt (4.1 mg, 30.0 µmol, 0.3 equiv) at room temperature. After stirring at room temperature for 9 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (10.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **S7-1** (135 mg, 97.7 µmol, 98%) as a pale-yellow powder.

Rf-value: 0.50 ($CHCl_3$/MeOH = 50:1, stained with phosphomolybdic acid)

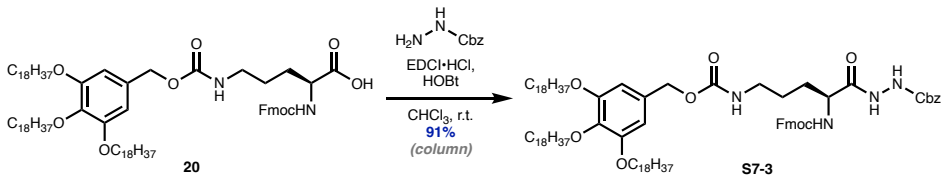
$[\alpha]_D^{29}$ = –6.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42 (d, *J* = 3.0 Hz, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.20 (t, *J* = 7.8 Hz, 2H), 6.87 (q, *J* = 7.5 Hz, 1H), 6.80 (br-d, *J* = 7.5 Hz, 2H), 6.56-6.52 (m, 1H), 6.50 (s, 2H), 6.03 (d, *J* = 3.5 Hz, 1H), 5.61 (d, *J* = 9.0 Hz, 1H), 5.00-4.95 (m, 2H), 4.90 (d, *J* = 12.0 Hz, 1H), 4.51 (br-s, 1H), 4.42 (app t, *J* = 6.5 Hz, 1H), 4.21 (t, *J* = 7.0 Hz, 1H), 3.98-3.90 (m, 6H), 3.51 (br-m, 1H), 3.16 (br-m, 1H), 1.86 (br-m, 1H), 1.79-1.71 (br-m, 6H), 1.55 (m, 1H), 1.46-1.37 (m, 6H), 1.37-1.12 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 157.2, 156.5, 153.2 (2C), 147.7, 146.7, 143.7, 143.6, 141.3 (2C), 138.1, 131.1, 129.5, 129.2, 127.7 (2C), 127.1 (2C), 125.0 (2C), 121.1, 120.0 (2C), 113.4, 106.7 (2C), 73.4, 69.1 (2C), 67.4, 67.0, 52.0, 47.1, 39.5, 31.9 (3C), 30.3, 29.8-29.7 (overlap, 32C), 29.4-29.4 (overlap, 7C), 26.3, 26.1 (3C), 22.7 (3C), 14.1 (3C)

Note: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

S7-3



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (129 mg, 0.100 mmol) in $CHCl_3$ (2.00 mL, 0.05 M) was added benzyl carbazate (23.3 mg, 0.140 mmol, 1.4 equiv), EDCI•HCl (30.7 mg, 0.160 mmol, 1.6 equiv) and HOBt (4.1 mg, 30.0 μmol, 0.3 equiv) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (10.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH = 50:1), yielding **S7-3** (132 mg, 91.3 μmol, 91%) as a pale-yellow powder.

Rf-value: 0.66 ($CHCl_3$/MeOH = 50:1, stained with phosphomolybdic acid)

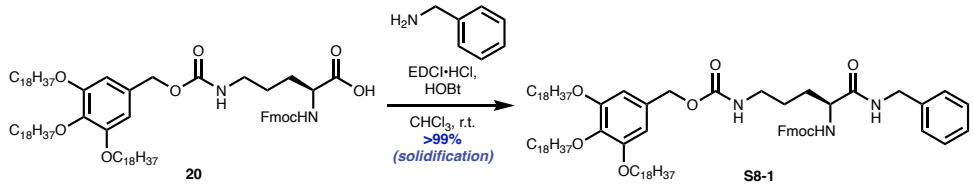
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{90}H_{144}O_{10}N_4Na$: 1464.0780, found: 1464.0787.

$[\alpha]_D^{29}$ = –7.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.60 (br-s, 1H), 7.74 (d, *J* = 7.0 Hz, 2H), 7.57 (br-s, 2H), 7.38 (t, *J* = 7.0 Hz, 2H), 7.51-7.27 (m, 7H), 6.84 (br-s, 1H), 6.56 (s, 1H), 6.53 (s, 2H), 5.67 (d, *J* = 7.5 Hz, 1H), 5.16-4.89 (br-m, 4H), 4.48-4.33 (br-m, 3H), 4.18 (br-s, 1H), 3.97-3.85 (m, 6H), 3.42 (br-s, 1H), 3.14 (br-s, 1H), 1.89 (br-s, 1H), 1.78-1.71 (m, 7H), 1.49- 1.39 (br-m, 6H), 1.38-1.10 (m, 86H), 0.88 (t, *J* = 6.0 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.2, 157.4, 156.6, 156.2, 153.3 (2C), 149.9, 143.9, 143.7, 141.4 (2C), 138.2, 135.5, 131.2, 128.6 (2C), 128.5 (2C), 128.3 (2C), 127.8 (2C), 127.2 (2C), 125.2, 120.1 (2C), 107.0, 73.5, 69.2 (2C), 67.9, 67.5, 67.2, 52.0, 47.2, 39.5, 32.0 (3C), 30.4, 30.1-29.7 (overlap, 33C), 29.6-29.5 (overlap, 7C), 26.2 (3C), 26.1, 22.8 (3C), 14.2 (3C)

S8-1



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (64.7 mg, 50.0 µmol) in $CHCl_3$ (1.00 mL, 0.05 M) was added benzyl amine (6.6 mg, 60.0 µmol, 1.2 equiv), EDCI•HCl (15.3 mg, 80.0 µmol, 1.6 equiv) and HOBt (2.0 mg, 15.0 µmol, 0.3 equiv) at room temperature. After stirring at room temperature for 10 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (10.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **S8-1** (68.9 mg, 49.8 µmol, >99%) as a colorless powder.

Rf-value: 0.75 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

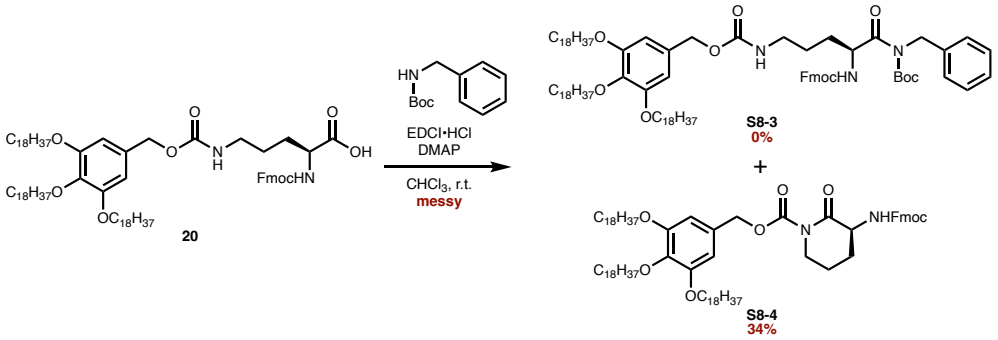
$[\alpha]_D^{29}$ = –7.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.0 Hz, 2H), 7.31-7.23 (m, 7H), 6.77 (br-s, 1H), 6.54 (m, 1H), 6.46 (s, 2H), 5.58 (d, *J* = 7.5 Hz, 1H), 4.86 (d, *J* = 11.0 Hz, 2H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.48-4.35 (m, 3H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.98-3.91 (m, 6H), 3.49 (br-s, 1H), 3.15 (br-s, 1H), 1.86 (br-m, 1H), 1.80-1.70 (m, 7H), 1.65 (br-m, 1H), 1.48-1.42 (m, 6H), 1.37-1.07 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.7, 157.1, 156.4, 153.1 (2C), 143.8, 143.7, 141.2 (2C), 138.0, 137.9, 131.1, 128.6 (2C), 127.7 (3C), 127.4, 127.0 (2C), 125.1 (2C), 119.9 (2C), 106.6 (2C), 73.4, 69.1 (2C), 67.1, 66.9, 53.3, 47.1, 43.5, 39.4, 31.9 (3C), 30.3, 29.7 (overlap, 33C), 29.4 (overlap, 7C), 26.4, 26.1 (3C), 22.7 (3C), 14.1 (3C)

Note: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

S8-4



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (12.9 mg, 10.0 μmol) in $CHCl_3$ (200 μL, 0.05 M) was added *N*-Boc benzyl amine (4.1 mg, 20.0 μmol, 2.0 equiv), EDCI•HCl (5.8 mg, 30.0 μmol, 3.0 equiv) and DMAP (0.4 mg, 3.00 μmol, 0.3 equiv) at room temperature. After stirring at room temperature for 19 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (1.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 2:1), yielding **S8-4** (4.3 mg, 3.37 μmol, 34%) as a colorless powder. The desire product **S8-3** wasn't obtained.

Rf-value: 0.55 (Hex/EtOAc = 2:1, stained with phosphomolybdic acid)

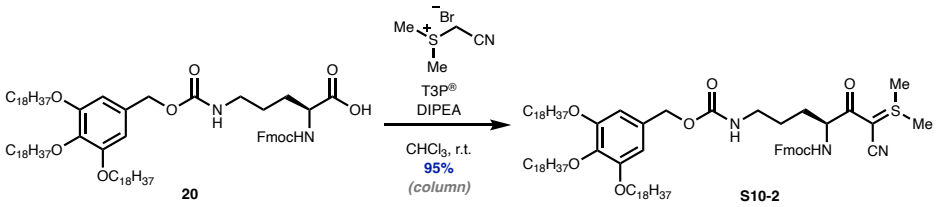
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{82}H_{134}O_8N_2Na$: 1298.0038, found: 1298.0022.

$[\alpha]_D^{29}$ = +2.7 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.64 (s, 2H), 5.79 (m, *J* = 5.0 Hz, 1H), 5.19 (s, 2H), 4.41-4.33 (m, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 4.08 (m, 1H), 3.99-3.92 (m, 6H), 3.63 (br-m, 1H), 2.60 (br-m, 1H), 1.96 (br-m, 1H), 1.81-1.72 (m, 6H), 1.60 (br-m, 1H), 1.49-1.41 (m, 6H), 1.40-1.07 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 170.8, 155.9, 153.3, 153.2, 143.8, 143.7, 141.3 (2C), 138.2, 129.9, 127.7 (2C), 127.0 (2C), 125.1 (2C), 119.9 (2C), 106.9 (2C), 73.4, 69.3, 69.1 (2C), 67.1, 53.0, 47.1, 44.3, 31.9 (3C), 30.3, 29.8-29.6 (overlap, 32C), 29.4-29.3 (overlap, 7C), 26.6, 26.1 (3C), 22.7 (3C), 20.3, 14.1 (3C)

S10-2



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (388 mg, 0.300 mmol) in $CHCl_3$ (6.00 mL, 0.05 M) was added T3P® (229 µg, 0.390 mmol, 1.3 equiv) and DIPEA (157 µL, 0.900 mmol, 3.0 equiv) at room temperature. After stirring at room temperature for 5 min, to the reaction mixture was added cyanosulfurylide (65.5 mg, 0.360 mmol, 1.2 equiv) at room temperature. After stirring at 40 ºC for 4 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (30.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH = 30:1), yielding **S10-2** (394 mg, 0.287 mmol, 95%) as a pale-yellow powder.

Rf-value: 0.58 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

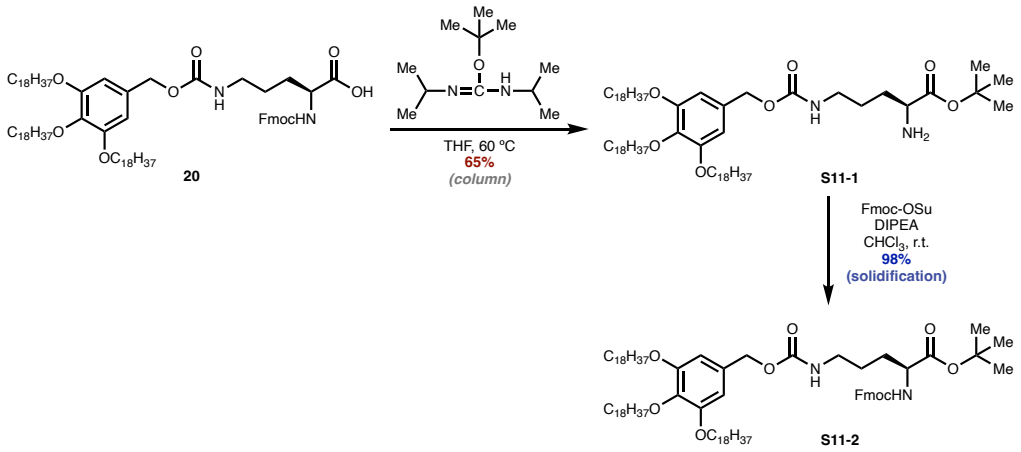
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{86}H_{141}O_8N_3SNa$: 1399.0337, found: 1399.0317.

$[\alpha]_D^{29}$ = +9.0 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.32-7.29 (m, 2H), 6.55 (s, 2H), 5.70 (d, *J* = 8.0 Hz, 1H), 5.45 (d, *J* = 8.0 Hz, 1H), 5.01-4.94 (m, 2H), 4.67 (m, 1H), 4.41-4.32 (m, 2H), 4.21 (t, *J* = 7.0 Hz, 1H), 3.97-3.91 (m, 6H), 3.28-3.15 (br-m, 2H), 2.86-2.72 (br-m, 6H), 1.87 (br-m, 1H), 1.80-1.70 (m, 6H), 1.62 (br-m, 1H), 1.50-1.44 (m, 6H), 1.37-1.13 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 189.7, 156.4, 155.8, 153.1 (2C), 144.0, 143.8, 141.3 (2C), 137.8, 131.5, 127.7, 127.6, 127.0 (2C), 125.2, 125.1, 119.9 (2C), 117.8, 106.6 (2C), 73.4, 69.0 (2C), 67.0, 66.8, 55.4, 47.2, 40.4, 31.9 (3C), 30.9, 30.3, 29.7 (overlap, 32C), 29.4-29.3 (overlap, 7C), 28.3, 28.2, 26.1 (3C), 25.5, 22.7 (3C), 14.1 (3C)

S11-2



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (129 mg, 0.100 mmol) in THF (1.00 mL, 0.10 M) was added *O*-tert-butyl-*N,N′*-diisopropylisourea (119 µg, 0.500 mmol, 5.0 equiv) at room temperature. After stirring at 60 ºC for 46 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (30.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography (hexane/EtOAc = 4:1 to $CHCl_3$/MeOH = 10:1), yielding **S11-1** (72.9 mg, 64.6 µmol, 65%) as a colorless powder.

Rf-value: 0.51 ($CHCl_3$/MeOH = 50:1, stained with phosphomolybdic acid)

To a solution of **S11-1** (72.9 mg, 64.6 µmol) in $CHCl_3$ (1.29 mL, 0.05 M) was added Fmoc-Osu (26.2 mg, 77.6 µmol, 1.2 equiv) and DIPEA (16.9 µL, 97.0 µmol, 1.5 equiv) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (6.45 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **S11-2** (85.8 mg, 63.6 µmol, 98%) as a colorless powder.

Rf-value: 0.62 (hexane/EtOAc = 2:1, stained with phosphomolybdic acid)

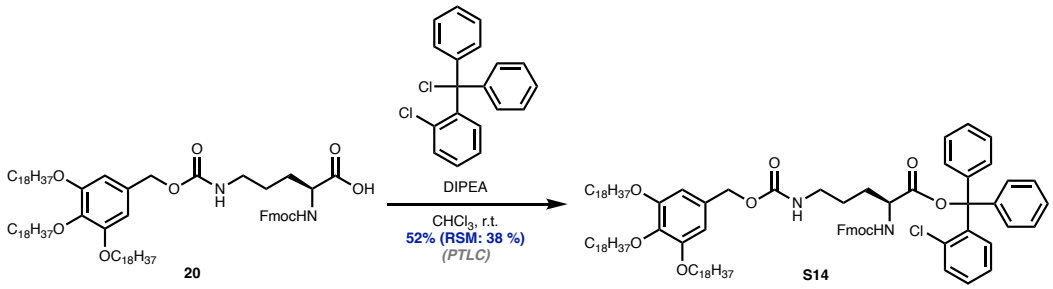
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{86}H_{144}O_9N_2Na$:1372.0770, found: 1372.0800.

$[\alpha]_D^{29}$ = +3.2 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.33-7.30 (m, 2H), 6.54 (s, 2H), 5.39 (d, *J* = 8.0 Hz, 1H), 4.97 (s, 2H), 4.81 (br-m, 1H), 4.43-4.36 (m, 2H), 4.28-4.17 (m, 2H), 3.96-3.91 (m, 6H), 3.22 (br-q, *J* = 6.2 Hz, 2H), 1.86 (br-m, 1H), 1.81-1.70 (m, 6H), 1.66 (br-m, 1H), 1.49-1.42 (m, 15H), 1.37-1.12 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3, 156.3, 155.9, 153.2 (2C), 143.9, 143.7, 141.3 (2C), 138.0, 131.3, 127.7 (2C), 127.0 (2C), 125.1, 125.0, 120.0 (2C), 106.8 (2C), 82.4, 73.4, 69.1 (2C), 67.1, 66.9, 53.9, 47.2, 40.6, 31.9 (3C), 30.3, 30.1, 30.0-29.7 (overlap, 31C), 29.4-29.4 (overlap, 7C), 28.0 (3C), 26.1 (3C), 25.7, 22.7 (3C), 14.1 (3C)

S14



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (25.9 mg, 20.0 μmol) in $CHCl_3$ (400 μL, 0.05 M) was added 2-chlorotrityl chloride (12.5 mg, 40.0 μmol, 2.0 equiv) and DIPEA (13.9 μL, 80.0 μmol, 4.0 equiv) at room temperature. After stirring at room temperature for 24 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (2.0 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC (hexane/EtOAc = 2:1), yielding **S14** (16.4 mg, 10.4 μmol, 52%) as a pale-yellow powder. (RSM: 9.9 mg, 38%)

Rf-value: 0.75 ($CHCl_3$/MeOH = 50:1, stained with phosphomolybdic acid)

$[\alpha]_D^{29}$ = +3.5 (c = 0.1, $CHCl_3$)

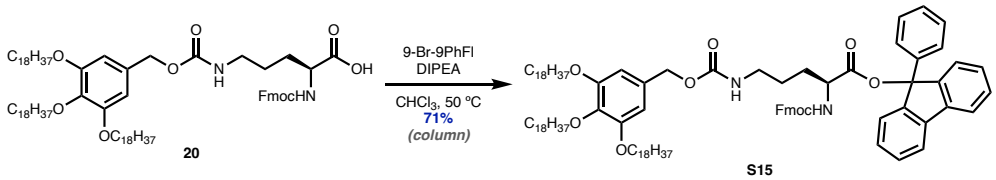
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74 (t, *J* = 5.8 Hz, 2H), 7.60-7.54 (br-m, 2H), 7.41-7.37 (m, 2H), 7.35-7.22 (m, 14H) 7.14-7.10 (td, *J* = 8.0, 1.5 Hz, 1H), 6.70 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.52 (s, 2H), 5.40 (d, *J* = 8.0 Hz, 1H), 5.02-4.95 (m, 2H), 4.72 (br-t, *J* = 7.5 Hz, 1H), 4.63 (m, 1H), 4.45-4.36 (m, 2H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.97-3.91 (m, 6H), 3.27-3.14 (br-m, 2H), 1.90 (br-m, 1H), 1.80-1.70 (m, 6H), 1.56 (br-m, 1H), 1.50-1.41 (m, 6H), 1.37-1.12 (m, 86H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 156.9, 153.2 (2C), 145.5, 143.8, 143.7, 141.3 (2C), 139.1, 138.0, 133.2, 131.8, 131.6, 131.5, 131.3, 131.2, 129.5, 129.1, 128.0 (overlap, 4C), 127.7 (overlap, 5C), 127.4 (2C), 127.0 (2C), 126.4, 126.0, 125.1, 125.1, 120.0 (2C), 106.8 (2C), 82.6, 73.4, 69.1, 69.0, 67.0, 47.1, 40.4, 31.9 (3C), 30.3, 29.9-29.7 (overlap, 33C), 29.5-29.4 (overlap, 7C), 26.1 (3C), 25.8, 22.7 (3C), 14.1 (3C)

Note: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

5-7. Western fragment synthesis

Fmoc-Orn(TCbz)-OFl (**S15**)



To a solution of Fmoc-Orn(TCbz)-OH (**20**) (64.7 mg, 50.0 μmol) in $CHCl_3$ (1.00 mL, 0.05 M) was added 9-bromo-9-phenylfluorene (24.2 mg, 75.0 μmol, 1.5 equiv) and DIPEA (34.8 μL, 0.200 mmol, 4.0 equiv) at room temperature. After stirring at 50 ºC for 10 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (5.00 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo.* The resulting residue was purified by silica gel flash column chromatography ($CHCl_3$ only), yielding Fmoc-Orn(TCbz)-OFl (**S15**) (54.4 mg, 35.5 μmol, 71%) as a pale-yellow powder. The notation "Fl" indicates the 9-phenylfluorene.

Rf-value: 0.50 (hexane/EtOAc = 3:1, stained with *p*-anisaldehyde)

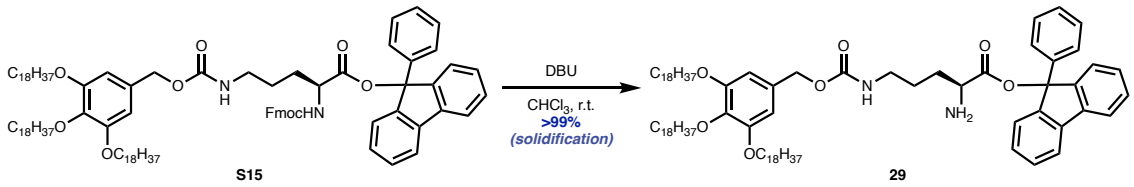
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{101}H_{148}O_9N_2Na$: 1556.1083, found: 1556.1082.

$[\alpha]_D^{29}$ = +4.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.53 (t, *J* = 6.5 Hz, 2H), 7.38 (dd, *J* = 15.5, 7.5 Hz, 4H), 7.31-7.22 (m, 11H), 6.54 (s, 2H), 5.30 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.77 (br-t, *J* = 5.8 Hz, 1H), 4.53 (br-dd, *J* = 12.5, 7.0 Hz, 1H), 4.38-4.30 (m, 2H), 4.15 (t, *J* = 7.0 Hz, 1H), 3.93 (td, *J* = 6.5, 4.0 Hz, 6H), 3.23 (br-dd, *J* = 13.5, 7.0 Hz, 2H), 2.00 (br-m, 1H), 1.80-1.70 (m, 6H), 1.54 (br-m, 1H), 1.49-1.41 (br-m, 6H), 1.32-1.21 (m, 86H), 0.88 (t, *J* = 7.3 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 169.7, 156.4, 155.7, 153.2 (2C), 146.0, 146.0, 143.7, 143.6, 141.2 (2C), 140.6 (2C), 140.4, 138.0, 131.2, 129.4, 129.4, 128.5 (2C), 128.3 (2C), 127.9, 127.6 (2C), 127.0 (2C), 125.1, 124.9 (3C), 124.5, 124.2, 120.2, 120.2, 119.9 (2C), 106.8 (2C), 89.8, 73.4, 69.0 (2C), 67.2, 66.9, 53.6, 47.0, 40.4, 31.9 (3C), 30.3, 29.9-29.6 (overlap, 32C), 29.4-29.3 (overlap, 7C), 26.1 (3C), 25.5, 22.7 (3C), 14.1 (3C)

Orn(TCbz)-OFl (**29**)



S15 29

To a solution of Fmoc-Orn(TCbz)-OFl (**S15**) (15.3 mg, 10.0 µmol) in $CHCl_3$ (200 µL, 0.05 M) was added DBU (4.0 µL, 26.8 µmol, 2.7 equiv) at room temperature. After stirring at room temperature until the consumption of the starting material (judged by TLC), the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.00 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding Orn(TCbz)-OFl (**29**) (13.1 mg, 10.0 µmol, >99%) as a pale-yellow powder.

Rf-value: 0.67 ($CHCl_3$/MeOH = 10:1, stained with phosphomolybdic acid)

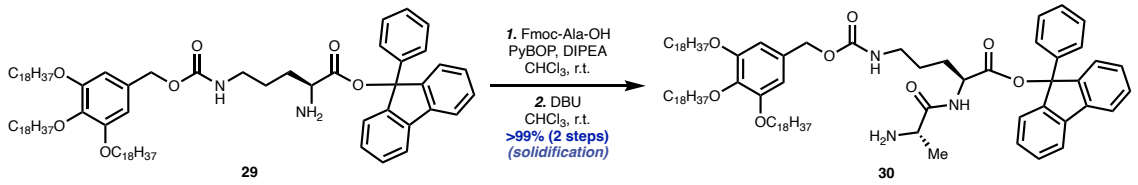
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{86}H_{138}O_7N_2Na$: 1334.0402, found: 1334.0396.

$[\alpha]_D^{29}$ = +5.2 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 (d, *J* = 6.0 Hz, 2H), 7.40-7.36 (m, 2H), 7.30-7.21 (m, 9H), 6.53 (s, 2H), 4.97 (s, 2H), 3.93 (m, 6H), 3.54 (br-s, 1H), 3.23 (m, 2H), 1.89 (br-m, 1H), 1.80-1.70 (m, 6H), 1.64 (br-m, 1H), 1.45 (br-m, *J* = 7.3 Hz, 6H), 1.37-1.20 (m, 85H), 1.14 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 9H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.4, 156.4, 153.1 (2C), 146.5, 146.4, 141.0, 140.5, 140.5, 138.0, 131.3, 129.3 (2C), 128.5 (2C), 128.2 (2C), 127.8, 124.9 (2C), 124.3, 124.1, 120.2 (2C), 106.8 (2C), 89.1, 73.4, 69.0 (2C), 67.1, 54.3, 40.7, 31.9 (3C), 30.3, 29.9-29.5 (overlap, 32C), 29.4-29.3 (overlap, 7C), 26.1 (3C), 26.0, 22.7 (3C), 14.1 (3C)

Dipeptide (**30**)



To a solution of Orn(TCbz)-OFl (**29**) (13.1 mg, 10.0 μmol) and Fmoc-Ala-OH (3.7 mg, 12.0 μmol, 1.2 equiv) in $CHCl_3$ (200 μL, 0.05 M) was added PyBOP (7.8 mg, 15.0 μmol, 1.5 equiv) and DIPEA (3.5 μL, 20.0 μmol, 2.0 equiv) at room temperature. After stirring at room temperature for 4 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.00 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum. This crude material was used in the next reaction without further purification.

To a solution of crude material in $CHCl_3$ (200 μL) was added DBU (4.0 μL, 26.8 μmol) at room temperature. After stirring at room temperature until the consumption of the starting material (judged by TLC), the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.00 mL). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding dipeptide (**30**) (13.8 mg, 10.0 μmol, >99%) as a pale-yellow powder.

Rf-value: 0.51 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

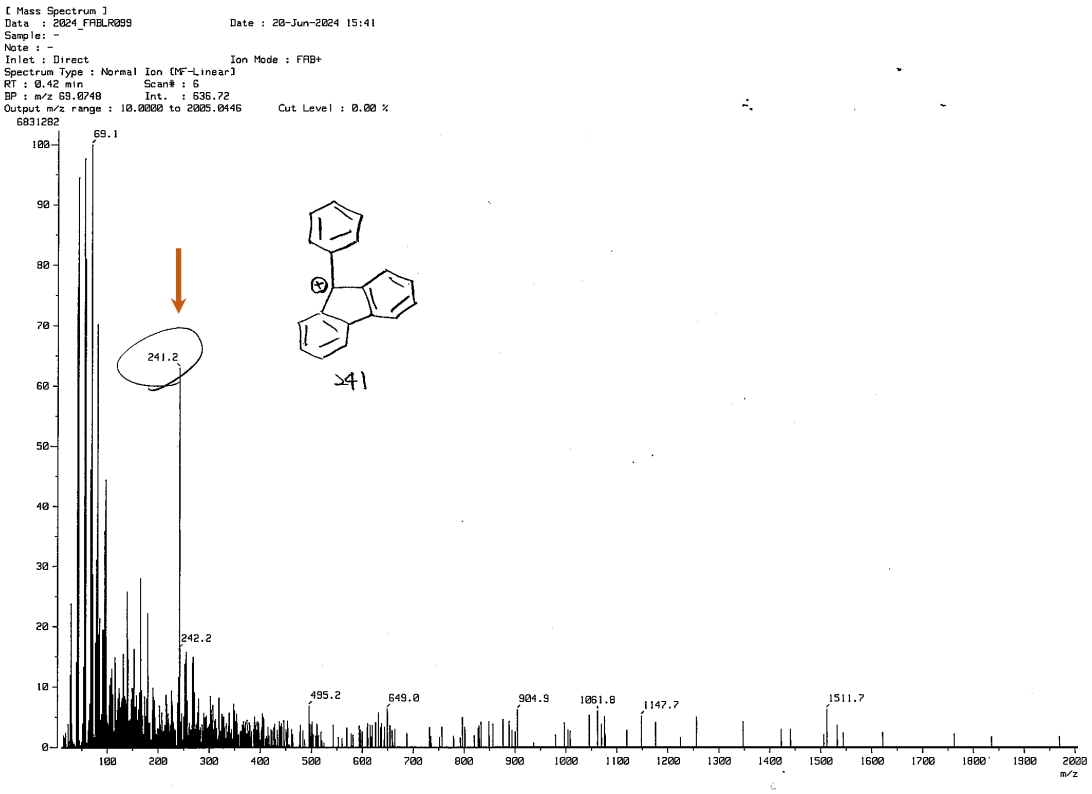
$\mathbf{[\alpha]_D^{29}}$ = +4.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71-7.69 (m, 2H), 7.39-7.35 (m, 2H), 7.31-7.20 (m, 9H), 6.53 (s, 2H), 4.97 (s, 2H), 4.87 (br-d, *J* = 5.0 Hz, 1H), 4.75 (br-q, *J* = 5.0 Hz, 1H), 3.93 (m, 6H), 3.44 (br-s, 1H), 3.22 (br-d, *J* = 6.0 Hz, 2H), 2.00 (br-m, 1H), 1.94 (s, 1H), 1.81-1.70 (m, 6H), 1.55 (br-m, 1H), 1.45 (br-m, 7.3 Hz, 6H), 1.30-1.14 (m, 89H), 0.88 (t, *J* = 7.0 Hz, 9H)
(*NH_2 proton was not detected.)

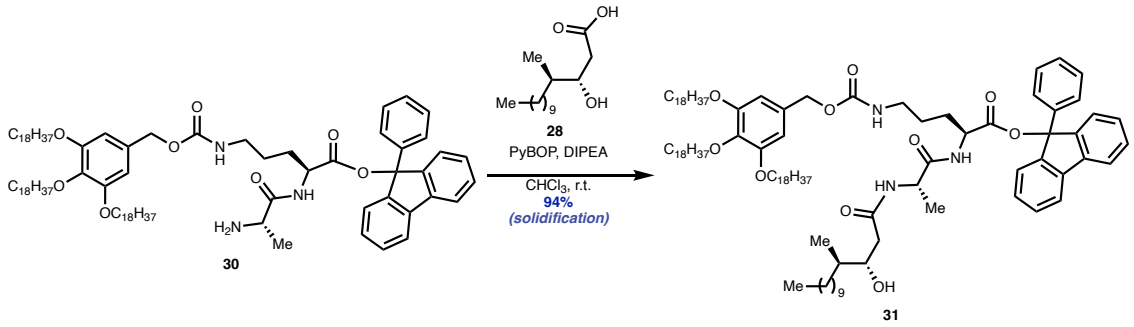
^{13}C NMR (126 MHz, $CDCl_3$): δ 180.2, 169.5, 156.3, 153.1 (2C), 146.2, 146.0, 140.8, 140.5, 140.3, 138.0, 131.3, 129.4, 129.3, 128.5 (2C), 128.3 (2C), 127.8, 125.0 (2C), 124.5,

124.2, 120.2, 120.2, 106.7 (2C), 89.6, 73.4, 69.0 (2C), 67.1, 51.4, 50.6, 40.5, 31.9 (3C), 30.3, 29.9, 29.7-29.5 (overlap, 31C), 29.5-29.4 (overlap, 7C), 26.1 (3C), 25.6, 22.7 (3C), 21.6, 14.1 (3C)

*Note: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted. Because we only observed the corresponding 9-phenyl fluorenyl cation including analysis of compounds, the products having a Fl group likely decomposed during the mass spectra measurement. Therefore, we provided HRMS data after cleavage of the Fl group and fully-characterized the product (**S19**) after condensation with phenol **35** (vide infra).*



Oligopeptide (**31**)



To a solution of dipeptide **30** (42.6 mg, 30.8 μmol) and **28** (9.6 mg, theoretically 37.0 μmol, 1.2 equiv) in $CHCl_3$ (616 μL, 0.05 M) was added PyBOP (24.0 mg, 46.2 μmol, 1.5 equiv) and DIPEA (10.7 μL, 61.6 μmol, 2.0 equiv) at room temperature. After stirring at room temperature for 5 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (3.08 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding dipeptide (**31**) (47.1 mg, 29.0 μmol, 94%) as a pale-yellow powder.

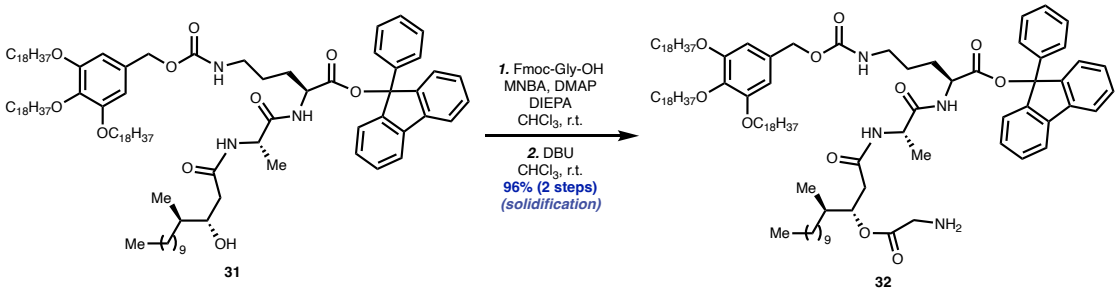
Rf-value: 0.55 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

$[\alpha]_D^{29}$ = –1.2 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 7.5, 3.0 Hz, 2H), 7.37 (app t, *J* = 7.5 Hz, 2H), 7.30-7.21 (m, 9H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.57 (d, *J* = 7.5 Hz, 1H), 6.53 (s, 2H), 5.05 (t, *J* = 6.0 Hz, 1H), 4.95 (app dd, *J* = 11.5 Hz, 2H), 4.70 (br-td, *J* = 7.5, 4.0 Hz, 1H), 4.37 (m, 1H), 3.93 (app dd, *J* = 11.0, 6.5 Hz, 6H), 3.72 (br-m, 1H), 3.54 (br-s, 1H), 3.15 (dd, *J* = 12.5, 6.5 Hz, 2H), 2.29 (dd, *J* = 15.0, 2.5 Hz, 1H), 2.24 (dd, *J* = 14.5, 9.5 Hz, 1H), 1.98 (br-m, 1H), 1.80-1.70 (m, 6H), 1.52 (m, 1H), 1.44 (m, 7H), 1.38-1.19 (m, 105H), 1.15 (m, 1H), 1.06 (m, 1H), 0.88 (app t, *J* = 7.3 Hz, 12H), 0.83 (d, *J* = 7.0 Hz, 3H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 171.8, 169.3, 156.6, 153.1 (2C), 146.0, 145.9, 140.6, 140.5, 140.3, 138.0, 131.2, 129.4, 129.3, 128.5 (2C), 128.3 (2C), 127.9, 125.0 (2C), 124.5, 124.2, 120.2, 120.2, 106.7 (2C), 89.8, 73.4, 72.4, 69.0 (2C), 67.2, 52.2, 48.7, 40.4, 38.9, 38.5, 32.2, 31.9 (4C), 30.3, 29.9, 29.7-29.5 (overlap, 35C), 29.4-29.3 (overlap, 8C), 27.1, 26.1 (3C), 25.5, 22.7 (4C), 17.6, 14.8, 14.1 (4C)

Oligopeptide (**32**)



To a solution of **31** (23.8 mg, 14.7 μmol) and Fmoc-Gly-OH (5.2 mg, 17.6 μmol, 1.2 equiv) in $CHCl_3$ (293 μL, 0.05 M) was added MNBA (7.6 mg, 22.0 μmol, 1.5 equiv), DMAP (0.5 mg, 4.4 μmol, 0.3 equiv) and DIPEA (5.1 μL, 29.3 μmol, 2.0 equiv) at room temperature. After stirring at room temperature for 5 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.47 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum. This crude material was used in the next reaction without further purification.

To a solution of crude material in $CHCl_3$ (293 μL) was added DBU (5.9 μL, 39.3 μmol) at room temperature. After stirring at room temperature until the consumption of the starting material (judged by TLC), the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.47 mL). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **32** (23.6 mg, 14.0 μmol, 96%) as a pale-yellow powder.

Rf-value: 0.47 ($CHCl_3$/MeOH = 30:1, stained with *p*-anisaldehyde)

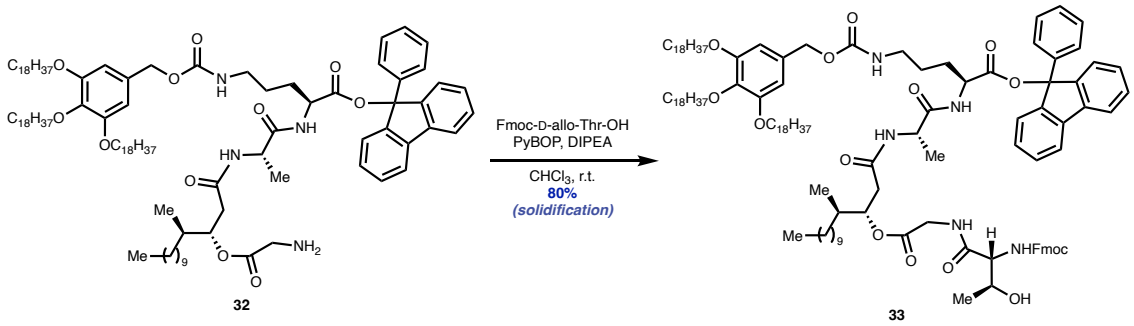
$[\alpha]_D^{29}$ = –5.4 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 7.5, 3.0 Hz, 2H), 7.37 (app t, *J* = 7.5 Hz, 2H), 7.30-7.21 (m, 9H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 7.5 Hz, 1H), 6.52 (s, 2H), 5.19 (br-t, *J* = 5.8 Hz, 1H), 5.14 (br-m, 1H), 4.95 (s, 2H), 4.71 (m, 1H), 4.35 (m, 1H), 3.93 (m, 6H), 3.33 (m, 2H), 3.23-3.15 (m, 2H), 2.40-2.33 (m, 2H), 2.02 (m, 1H), 1.80-

1.70 (m, 6H), 1.56 (m, 1H), 1.49-1.42 (m, 7H), 1.38-1.12 (m, 105H), 1.12-1.00 (m, 2H), 0.88 (app t, J = 6.8 Hz, 12H), 0.84 (d, J = 7.0 Hz, 3H)
(*NH_2 proton was not detected.)

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 171.9, 169.9, 169.3, 156.6, 153.1 (2C), 146.1, 146.0, 140.6, 140.5, 140.3, 138.0, 131.3, 129.4, 129.3, 128.5 (2C), 128.3 (2C), 127.8, 124.9 (2C), 124.5, 124.2, 120.2, 120.2, 106.8 (2C), 89.7, 75.2, 73.4, 69.1 (2C), 67.1, 52.2, 48.8, 43.8, 40.4, 37.9, 36.5, 32.1, 31.9 (4C), 30.3, 29.9-29.5 (overlap, 36C), 29.4-29.2 (overlap, 7C), 28.8, 27.0, 26.1 (3C), 25.5, 22.6 (4C), 18.1, 14.7, 14.1 (4C)

Oligopeptide (**33**)



To a solution of **32** (16.8 mg, 10.0 μmol) and Fmoc-D-allo-Thr-OH (4.1 mg, 12.0 μmol, 1.2 equiv) in $CHCl_3$ (200 μL, 0.05 M) was added HOBt (0.5 mg, 3.0 μmol, 0.3 equiv), EDCI•HCl (3.1 mg, 16.0 μmol, 1.6 quiv) and DIPEA (3.8 μL, 22.0 μmol, 2.2 equiv) at room temperature. After stirring at room temperature for 3 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (1.00 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **33** (16.1 mg, 8.0 μmol, 80%) as a pale-yellow powder.

Rf-value: 0.58 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

$[\alpha]_D^{29}$ = +4.6 (c = 0.1, $CHCl_3$)

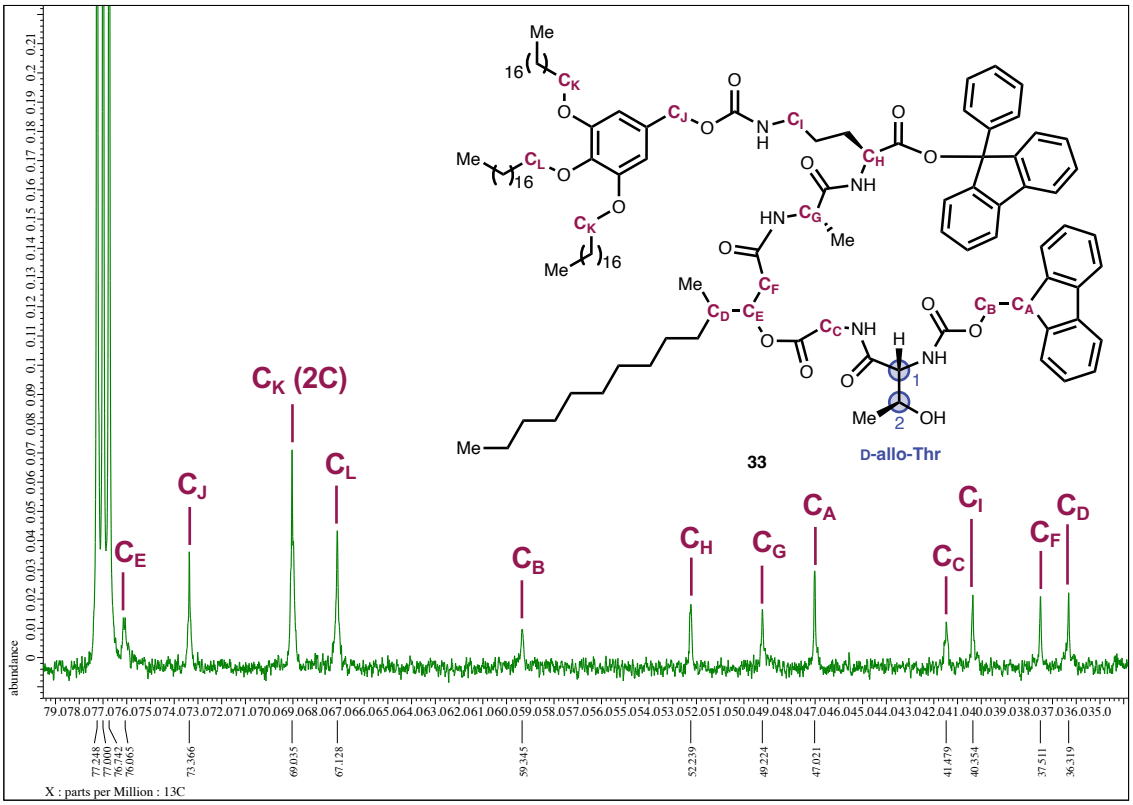
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, J = 7.5 Hz, 2H), 7.66 (dd, J = 7.5, 5.0 Hz, 2H), 7.57 (br-d, J = 7.0 Hz, 2H), 7.40-7.33 (m, 4H), 7.31-7.24 (m, 11H), 7.01 (br-s, 1H), 6.72 (br-d, J = 7.0 Hz, 1H), 6.63 (br-d, J = 6.0 Hz, 1H), 6.51 (s, 2H), 5.87 (br-d, J = 8.0 Hz, 1H), 5.15 (br-t, J = 5.3 Hz, 1H), 5.12-5.09 (br-m, 1H) 4.97-4.92 (m, 2H), 4.70 (br-dd, J

= 12.0, 6.5 Hz, 1H), 4.42 (app t, *J* = 8.8 Hz, 1H), 4.34 (app t, *J* = 8.8 Hz, 1H), 4.28 (m, 1H), 4.18 (t, *J* = 6.5 Hz, 1H), 4.04 (br-t, *J* = 6.5 Hz, 1H), 3.95-3.87 (m, 7H), 3.85 (br-d, *J* = 5.5 Hz, 1H), 3.81 (br-d, *J* = 5.5 Hz, 1H), 3.17 (app d, *J* = 6.0 Hz, 2H), 2.40-2.33 (m, 2H), 2.00 (m, 1H), 1.80-1.69 (m, 6H), 1.51 (m, 1H), 1.47-1.40 (m, 7H), 1.37-1.10 (m, 109H), 1.03 (m, 1H), 0.88 (app t, *J* = 6.8 Hz, 12H), 0.82 (d, *J* = 6.5 Hz, 3H)
(*OH proton was not detected.)

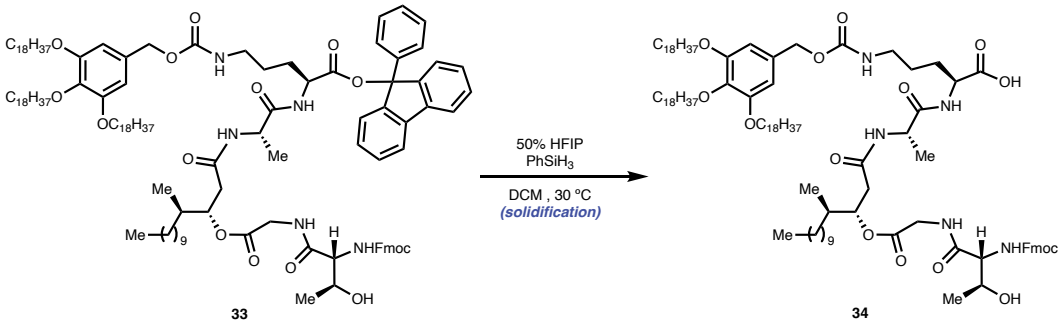
^{13}C NMR (126 MHz, $CDCl_3$): δ 172.2, 171.2, 170.1, 169.5 (2C), 156.6, 156.5, 153.1 (2C), 146.0, 145.9, 143.7, 143.6, 141.2 (2C), 140.6, 140.5, 140.2, 137.9, 131.3, 129.4, 129.3, 128.5 (2C), 128.3 (2C), 127.8, 127.7 (2C), 127.0 (2C), 125.0 (2C), 124.9 (2C), 124.5, 124.2, 120.2 (2C), 119.9 (2C), 106.8 (2C), 89.7, 76.1, 73.4, 69.0 (2C), 67.1, 59.3, 52.2, 49.2, 47.0, 41.5, 40.4, 37.5, 36.3, 32.1, 31.9 (4C), 30.3, 30.1-29.4 (overlap, 36C), 29.4-29.1 (overlap, 7C), 28.8, 26.9, 26.1 (3C), 25.4, 22.6 (4C), 19.6, 18.0, 14.5, 14.1 (4C)

D-allo-Thr carbon **1 and **2** were overlapped. See the ^{13}C NMR below.*

^{13}C NMR ($CDCl_3$)



Oligopeptide (**34**)

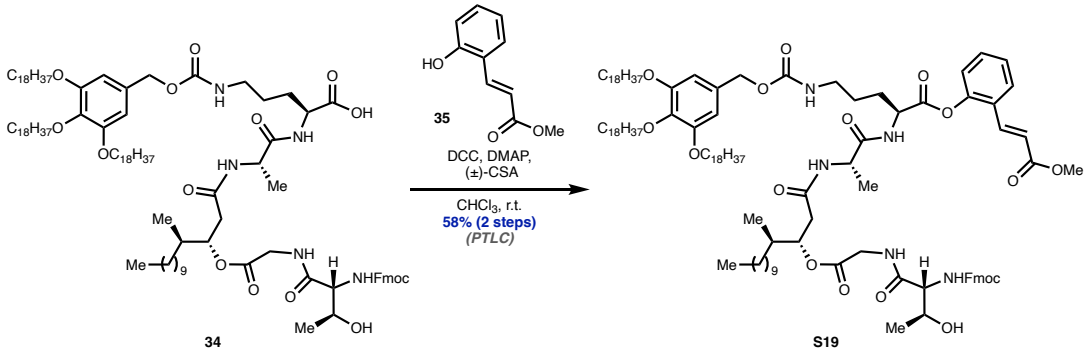


To a glass vessel charged with **33** (66.6 mg, 33.3 µmol) was added DCM/HFIP (1:1 v/v, 664 µL, 0.05 M) and phenylsilane (20.4 µL, 0.166 mmol, 5.0 equiv) at room temperature. After stirring at 30 ºC for 21 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (3.32 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum. This crude material **34** was used in the next reaction without further purification.

Rf-value: 0.40 ($CHCl_3$/MeOH/AcOH = 30:1:0.1, stained with phosphomolybdic acid)

HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{106}H_{179}O_{15}N_5Na$: 1785.3295, found: 1785.3292.

Oligopeptide (**S19**)



To a solution of crude material **34** and **35**[19] (7.1 mg, 39.9 µmol, 1.2 equiv) in $CHCl_3$ (665 µL, 0.05 M) was added DCC (15.1 mg, 73.1 µmol, 2.2 equiv), DMAP (1.2 mg, 10.0 µmol, 0.3 quiv) and (±)-CSA (2.3 mg, 10.0 µmol, 0.3 equiv) at room temperature. After stirring at room temperature for 11 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (13.33 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product

collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo*. The resulting residue was purified by thin-layer preparative TLC ($CHCl_3$/MeOH = 30:1), yielding **S19** (35.6 mg, 19.4 μmol, 58%, 2 steps) as a colorless powder.

Rf-value: 0.40 ($CHCl_3$/MeOH = 30:1, stained with phosphomolybdic acid)

HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{116}H_{187}O_{17}N_5Na$:1945.3820, found: 1945.3805.

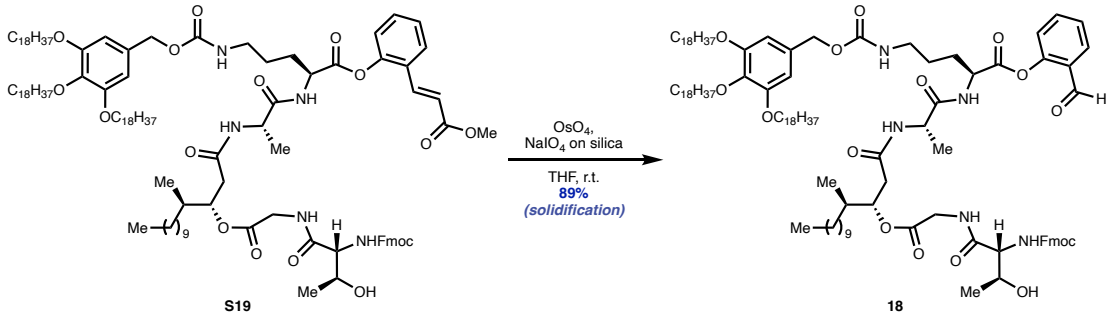
$[\alpha]_D^{29}$ = –26.5 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): 7.77-7.72 (m, 3H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.57 (br-s, 2H), 7.38 (app t, *J* = 7.3 Hz, 3H), 7.29-7.23 (m, 3H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.94 (br-d, 1H), 6.51 (s, 2H), 6.46 (br-d, *J* = 5.5 Hz, 1H), 6.41 (br-m, 1H), 5.98 (br-dd, *J* = 12.0, 8.5 Hz, 1H), 5.53 (br-m, 1H), 5.15 (br-m, 1H), 5.05 (m, 1H), 4.95 (app dd, *J* = 10.5 Hz, 2H), 4.80 (br-m, 1H), 4.57 (app t, *J* = 7.3 Hz, 1H), 4.51 (app t, *J* = 6.8 Hz, 1H), 4.41 (br-m, 1H), 4.32 (br-t, 8.5 Hz, 1H), 4.18 (t, *J* = 7.0 Hz, 1H), 4.14 (br-m, 1H), 4.06 (br-m, 1H), 3.95-3.90 (m, 8H), 3.79-3.70 (br-m, 3H), 3.28 (br-m, 1H), 2.48-2.42 (m, 2H), 2.12 (br-m, 1H), 1.97 (br-m, 1H), 1.79-1.63 (m, 6H), 1.48-1.38 (m, 8H), 1.35-1.10 (m, 108H), 1.09-0.95 (br-m, 1H), 0.88 (app t, *J* = 6.5 Hz, 15H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 172.9, 172.6, 171.2, 170.4, 169.5, 167.3, 156.8, 156.5, 153.1, 153.0, 149.0, 143.7, 143.6, 141.1, 138.2, 138.0, 137.9, 131.4, 127.6 (2C), 127.0 (3C), 126.5 (2C), 125.0 (2C), 122.9, 119.8 (2C), 119.6, 119.3, 106.7 (2C), 76.1, 73.3, 69.0 (2C), 67.1 (2C), 59.5, 52.7, 51.8, 48.9, 47.0, 41.6, 40.4, 37.4, 36.3, 32.2, 31.8 (4C), 30.3, 29.8-29.5 (overlap, 36 C), 29.4-29.3 (overlap, 7C), 28.4, 26.9, 26.1 (3C), 25.6, 22.6 (4C), 19.5, 18.1, 14.5, 14.0 (4C)

**A D-allo-Thr carbon peak was overlapped.*

Western fragment (**18**)



$NaIO_4$ on silica was prepared by following the literature procedure (The final concentration of silica supported $NaIO_4$ was calculated as 0.64 mmol/g).[20] To a solution of **S19** (98.8 mg, 51.4 μmol) in THF (2.05 mL, 0.025 M) was added OsO_4 (13.1 mg, 51.4 μmol, 1.0 equiv) and $NaIO_4$ on silica (1.4 mg, 5.8 μmol, 0.3 equiv) at room temperature. After stirring at room temperature for 10 h, the reaction mixture was cooled down to 0 °C and poured into cold MeOH (10.3 mL, 0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum, yielding **18** (85.0 mg, 45.5 μmol, 89%) as a colorless powder.

Rf-value: 0.52 ($CHCl_3$/MeOH/toluene = 30:1:5, stained with 2,4-dinitrophenyl hydrazine)

$[\alpha]_D^{29}$ = +1.8 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.02 (br-s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 7.5 Hz, 2H), 7.62-7.56 (m, 3H), 7.38 (m, 3H), 7.30-7.26 (m, 2H), 7.16 (t, *J* = 8.3 Hz, 1H), 6.75 (br-m, 1H), 6.53 (s, 2H), 5.97 (br-m, 1H), 5.24-5.13 (m, 2H), 5.04 (br-m, 1H), 4.96 (s, 2H), 4.82 (br-m, 1H), 4.50 (m, 1H), 4.41 (br-t, 8.8 Hz, 1H), 4.33 (br-m, 1H), 4.18 (t, *J* = 6.5 Hz, 1H), 4.13 (br-m, 1H), 4.08 (br-m, 1H), 4.02 (br-m, 1H), 3.98-3.89 (m, 8H), 3.27 (br-s, 2H), 2.48-2.37 (m, 2H), 2.15 (br-m, 1H), 1.95 (br-m, 1H), 1.77-1.60 (m, 6H), 1.47-1.37 (br-m, 8H), 1.29-1.08 (m, 108H), 1.03 (m, 1H), 0.88 (app t, 6.8 Hz, 15H)
(*NH proton was not detected.)

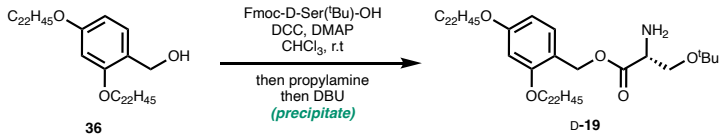
^{13}C NMR (126 MHz, $CDCl_3$): δ 189.1, 172.9, 171.3, 170.2, 169.5, 169.4, 156.8, 156.6, 153.2 (2C), 151.0, 143.8, 143.7, 141.3, 138.4, 135.3, 131.4, 128.1, 127.7 (2C), 127.0 (2C), 126.7, 125.0 (2C), 123.3, 119.9 (2C), 107.2 (2C), 76.2, 73.4, 69.3 (2C), 69.0, 67.3, 67.1, 59.6, 52.5, 49.2, 49.0, 47.2, 41.6, 40.5, 37.7, 36.5, 32.2, 31.9 (4C), 30.4, 30.0-29.4

(overlap, 40C), 29.3 (3C), 28.5, 27.0, 26.1 (3C), 25.6, 22.6 (4C), 19.6, 18.0, 14.6, 14.6, 14.0 (4C)

Note: HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted, presumably due to the instability at the C-terminal.

5-8. Serine fragments synthesis

D-Ser(ᵗBu)-OTag (D-**19**)



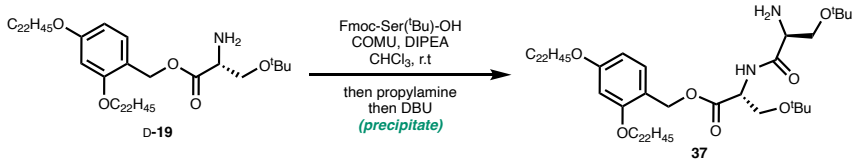
To a screw-cap centrifuge tube charged with **36**[21] (303 mg, 0.400 mmol) and Fmoc-D-Ser(ᵗBu)-OH (184 mg, 0.48o mmol, 1.2 equiv) was added $CHCl_3$ (8.00 mL, 0.05 M), DCC (182 mg, 0.880 mmol, 2.2 equiv) and DMAP (14.7 mg, 0.12 mmol, 0.3 equiv) at room temperature. After stirring at room temperature for 3 h, to the resulting mixture was added propylamine (240 μL, 2.92 mmol, 7.3 equiv) at room temperature. After stirring at room temperature for 30 min, to the resulting mixture was added DBU (240 μL, 1.61 mmol, 4.0 equiv) at room temperature. After stirring at room temperature for 1 h, to the reaction mixture was added a solution of HOBt in MeOH (10 mM, 40.0 mL, 0.01 M). The resulting heterogeneous solution was vortexed for 1 min. After centrifuging at 4×10^3 rpm for 5 min, the supernatant was removed by decantation. The resulting solid residue was washed once with a solution of HOBt in MeOH (10 mM, 40.0 mL, 0.01 M) and then twice with pure MeCN (40.0 mL, 0.01 M) by repeating the centrifuge separation described above (a total of 4 washes). The resulting precipitate was dried under high vacuum to afford D-Ser(ᵗBu)-OTag (D-**19**) as a colorless solid.

• General Procedure 1 for condensation of soluble hydrophobic tag-supported amines with an amino acid

To the centrifuge tube containing the residual tag-supported amine was added a solution of the corresponding Fmoc-amino acid (0.480 mmol, 1.2 equiv) in DCM (8.00 mL, 0.05 M) at room temperature. To the resulting mixture was added COMU (206 mg, 0.48 mmol, 1.2 equiv) and DIPEA (111 μL, 0.640 mmol, 1.6 equiv) at room temperature. After stirring at room temperature for 3h, to the resulting mixture was added propylamine (80.0 μL, 0.974 mmol, 2.4 equiv) at room temperature. After stirring at room temperature for 30 min, to the resulting mixture was added DBU (320 μL, 2.14 mmol, 5.4 equiv) at room temperature. After stirring at room temperature for 1 h, to the reaction mixture was added a solution of HOBt in MeCN (10 mM, 40.0 mL, 0.01 M). The resulting heterogeneous solution was vortexed for 1 min. After centrifuging at 4×10^3 rpm for 5 min, the supernatant was removed by decantation. The resulting solid residue was washed once

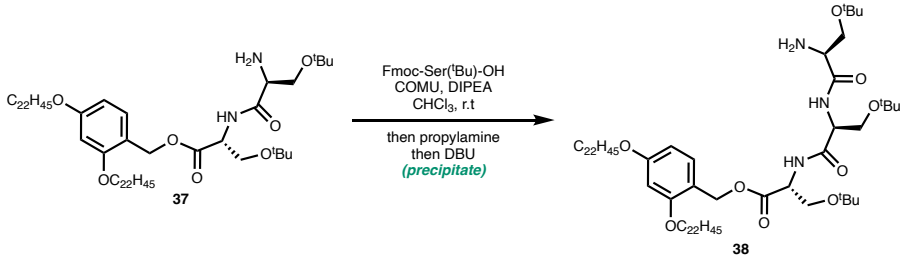
with a solution of HOBt in MeOH (10 mM, 40.0 mL, 0.01 M) and then twice with pure MeCN (40.0 mL, 0.01 M) by repeating the centrifuge separation described above (a total of 4 washes). The resulting precipitate was dried under high vacuum and used in the next reaction without further purification.

Dipeptide (**37**)



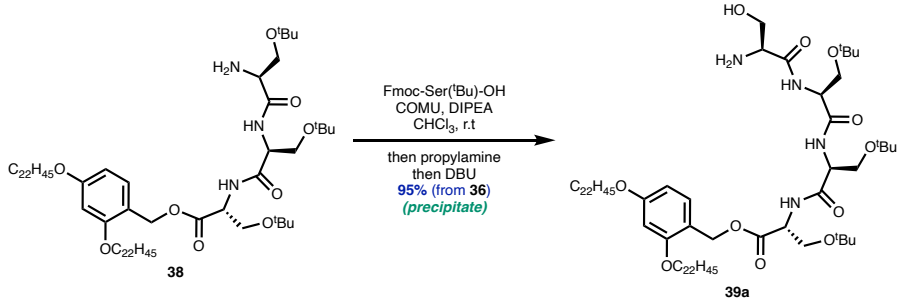
Prepared according to the General Procedure 1 from D-Ser(ᵗBu)-OTag (D-**19**) using Fmoc-Ser(ᵗBu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid.

Tripeptide (**38**)



Prepared according to the General Procedure 1 from dipeptide (**37**) using Fmoc-Ser(ᵗBu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid.

Tetrapeptide (**39a**)



Prepared according to the General Procedure 1 from tripeptide (**38**) using Fmoc-Ser-OH (157mg, 0.480 mmol, 1.2 equiv) as the amino acid. The resulting precipitate was concentrated *in vacuo* and dried under high vacuum, yielding **39a** (486 mg, 0.382 mmol, 95%) as a colorless powder.

Rf-value: 0.30 ($CHCl_3$/MeOH = 30:1 stained with phosphomolybdic acid)

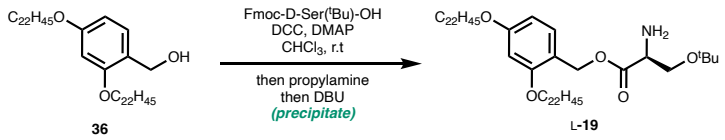
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{75}H_{140}O_{11}N_4Na$: 1296.0416, found: 1296.0406.

$[\alpha]_D^{29}$ = +8.1 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73 (d, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.32 (br-d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 9.0 Hz, 1H), 6.40-6.39 (m, 2H), 5.17 (d, *J* = 12.0 Hz, 1H), 5.13 (d, *J* = 12.0 Hz, 1H) 4.76 (br-s, 1H), 4.56 (td, *J* = 6.5, 3.5 Hz, 1H), 4.51 (br-m, 1H), 3.91 (t, *J* = 6.5 Hz, 4H), 3.84 (dd, *J* = 9.0, 3.0 Hz, 2H), 3.81-3.76 (m, 2H), 3.68 (dd, *J* = 10.5, 8.0 Hz, 1H), 3.53-3.45 (m, 3H), 3.31 (dd, *J* = 8.5, 6.5 Hz, 1H), 2.56 (br-s, 1H, * OH), 1.78-1.72 (m, 4H), 1.67 (br-s, 2H, *NH_2), 1.42-1.37 (br-m, 4H), 1.29-1.20 (br-m, 72H), 1.19-1.15 (m, 18H), 1.08-1.04 (m, 9H), 0.86 (t, *J* = 6.8 Hz, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.4, 170.6, 170.2, 169.8, 160.6, 158.1, 130.8, 116.1, 104.4, 99.4, 73.9 (2C), 73.3, 68.0 (2C), 65.5, 62.3, 61.9, 61.6, 61.3, 56.1, 53.5, 53.3, 53.1, 31.8 (2C), 29.6-29.0 (overlap, 34C), 27.3-27.1 (overlap, 9C), 26.0 (2C), 22.6 (2C), 14.0 (2C)

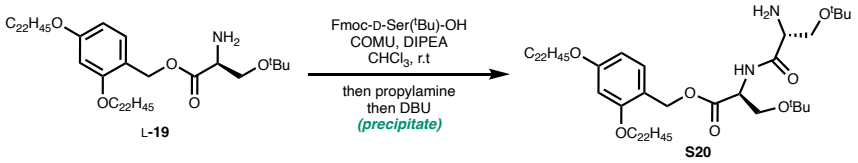
Ser(^{t}Bu)-OTag (L-**19**)



To a screw-cap centrifuge tube charged with **36**[21] (303 mg, 0.400 mmol) and Fmoc-Ser(^{t}Bu)-OH (184 mg, 0.480 mmol, 1.2 equiv) was added $CHCl_3$ (8.00 mL, 0.05 M), DCC (182 mg, 0.880 mmol, 2.2 equiv) and DMAP (14.7 mg, 0.120 mmol, 0.3 equiv) at room temperature. After stirring at room temperature for 3 h, to the resulting mixture was added propylamine (240 μL, 2.92 mmol, 7.3 equiv) at room temperature. After stirring at room temperature for 30 min, to the resulting mixture was added DBU (240 μL, 1.61 mmol, 4.0 equiv) at room temperature. After stirring at room temperature for 1 h, to the reaction mixture was added a solution of HOBt in MeOH (10 mM, 40.0 mL, 0.01 M). The resulting heterogeneous solution was vortexed for 1 min. After centrifuging at 4×10^3 rpm for 5 min, the supernatant was removed by decantation. The resulting solid residue was washed once with a solution of HOBt in MeOH (10 mM, 40.0 mL, 0.01 M) and then twice with pure MeCN (40.0 mL, 0.01 M) by repeating the centrifuge separation described above (a total

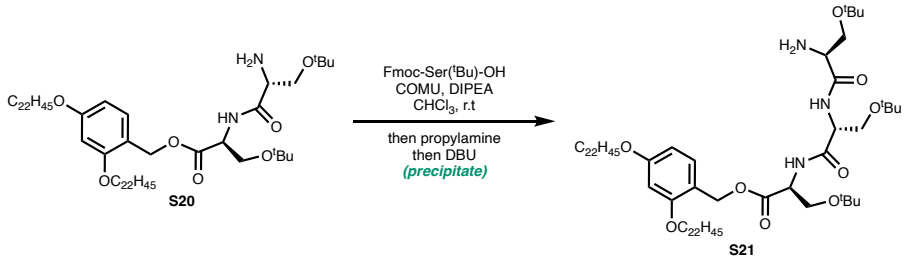
of 4 washes). The resulting precipitate was dried under high vacuum to afford Ser(ᵗBu)-OTag (L-**19**) as a colorless solid.

Dipeptide (**S20**)



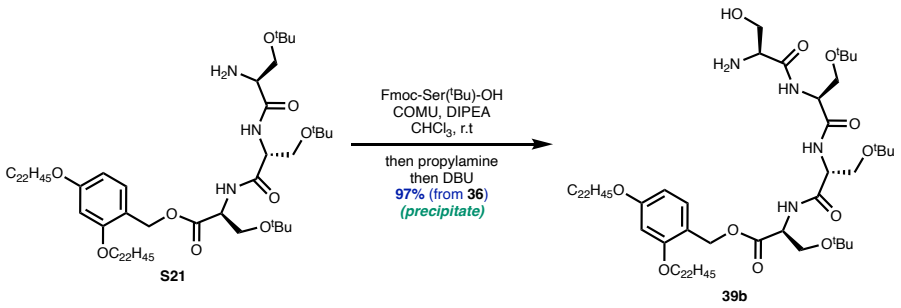
Prepared according to the General Procedure 1 from Ser(ᵗBu)-OTag (L-**19**) using Fmoc-D-Ser(ᵗBu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid. *The same reaction was performed using three tubes in parallel.

Tripeptide (**S21**)



Prepared according to the General Procedure 1 from dipeptide (**S20**) using Fmoc-Ser(ᵗBu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid.

Tetrapeptide (**39b**)



Prepared according to the General Procedure 1 from tripeptide (**S21**) using Fmoc-Ser-OH (157 mg, 0.480 mmol, 1.2 equiv) as the amino acid. The resulting precipitate was concentrated *in vacuo* and dried under high vacuum, yielding **39b** (492 mg, 0.386 mmol, 97%) as a colorless powder.

Rf-value: 0.31 ($CHCl_3$/MeOH = 30:1 stained with phosphomolybdic acid)

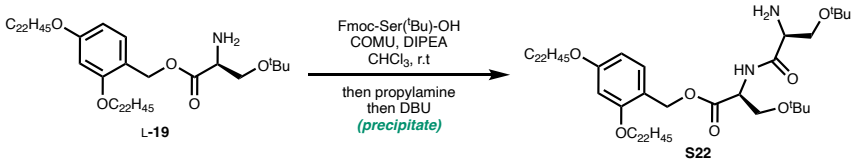
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{75}H_{140}O_{11}N_4Na$: 1296.0416, found: 1296.0397.

$[\alpha]_D^{29}$ = –4.8 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.5 Hz, 1H), 7.51-7.47 (m, 2H), 7.17 (d, *J* = 9.0 Hz, 1H), 6.39-6.36 (m, 2H), 5.16 (d, *J* = 12.5 Hz, 1H), 5.11 (d, *J* = 12.5 Hz, 1H), 4.69 (m, 1H), 4.49-4.47 (m, 2H), 3.91-3.86 (m, 4H), 3.85-3.75 (m, 3H), 3.66 (dd, *J* = 10.0, 6.0 Hz, 1H), 3.53 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.48 (dd, *J* = 8.5, 6.0 Hz, 2H), 3.32 (m, 1H), 3.26 (br-m, 1H), 2.52 (br-s, 1H, *OH), 1.77-1.70 (m, 4H), 1.65 (br-s, 2H, *NH_2), 1.44-1.37 (m, 4H), 1.30-1.22 (m, 72H), 1.19-1.14 (m, 18H), 1.05 (s, 9H), 0.85 (t, *J* = 7.0 Hz, 6H)

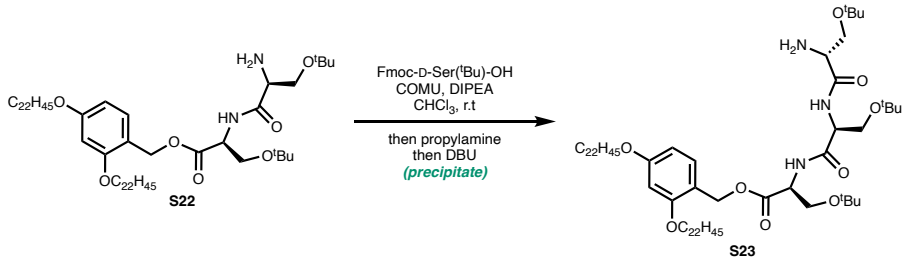
^{13}C NMR (126 MHz, $CDCl_3$): δ 174.2, 170.1, 169.9, 169.8, 160.5, 158.1, 130.8, 116.3, 104.4, 99.3, 74.0, 73.7, 73.2, 67.9 (2C), 65.2, 62.3, 61.8, 61.1, 61.0, 56.1, 53.3, 53.2 (2C), 31.8 (2C), 29.6-29.0 (overlap, 34C), 27.3-27.1 (overlap, 9C), 25.9 (2C), 22.6 (2C), 14.0 (2C)

Dipeptide (**S22**)



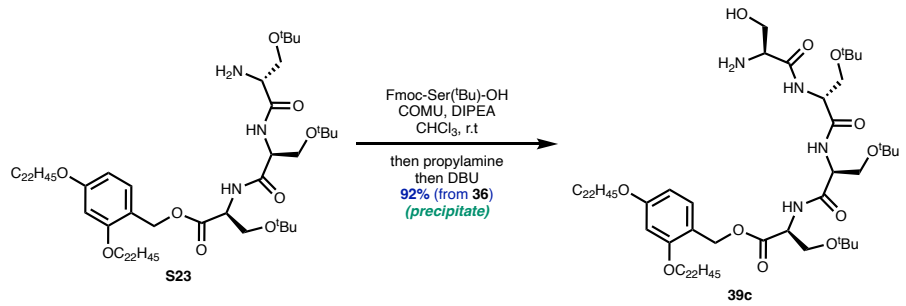
Prepared according to the General Procedure 1 from Ser(^{t}Bu)-OTag (L-**19**) using Fmoc-Ser(^{t}Bu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid. *The same reaction was performed using two tubes in parallel.

Tripeptide (**S23**)



Prepared according to the General Procedure 1 from dipeptide (**S22**) using Fmoc-D-Ser(^{t}Bu)-OH (184 mg, 0.480 mmol, 1.2 equiv) as the amino acid.

Tetrapeptide (**39c**)



Prepared according to the General Procedure 1 from tripeptide (**S23**) using Fmoc-Ser-OH (157mg, 0.480 mmol, 1.2 equiv) as the amino acid. The resulting precipitate was concentrated *in vacuo* and dried under high vacuum, yielding **39c** (467 mg, 0.368 mmol, 92%) as a colorless powder.

Rf-value: 0.29 ($CHCl_3$/MeOH = 30:1 stained with phosphomolybdic acid)

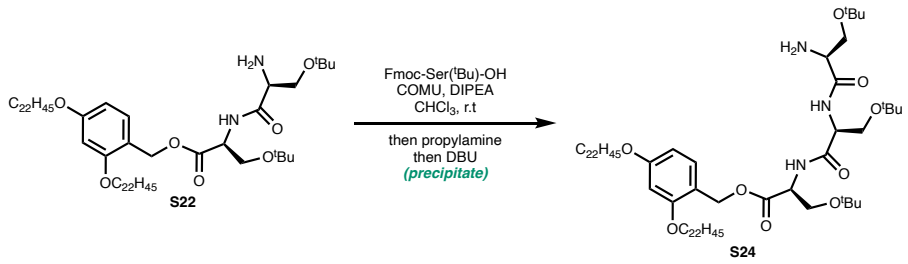
HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{75}H_{140}O_{11}N_4Na$: 1296.0416, found: 1296.0430.

$[\alpha]_D^{29}$ = +17.9 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (br-d, *J* = 7.0 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.55 (d, *J* = 7.0 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H), 6.40-6.39 (m, 2H), 5.16 (d, *J* = 12.0 Hz, 1H), 5.12 (d, *J* = 12.0 Hz, 1H), 4.70 (br-m, 1H), 4.52-4.45 (br-m, 2H), 4.06 (dd, *J* = 10.5, 3.0 Hz, 1H), 3.92-3.89 (m, 4H), 3.86 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.79 (dd, *J* = 8.5, 2.0 Hz, 1H), 3.74 (dd, *J* = 8.0, 3.0 Hz, 1H) 3.64 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.53-3.44 (m, 2H), 3.37 (app t, *J* = 8.8 Hz, 1H), 3.29 (m, 1H), 2.54 (br-s, 1H, *OH), 1.79-1.69 (m, 4H), 1.68 (br-s, 2H, *NH_2), 1.45-1.38 (m, 4H), 1.35-1.21 (br-m, 72H), 1.19-1.10 (m, 18H), 1.06-1.04 (m, 9H), 0.85 (t, *J* = 7.0 Hz, 6H)

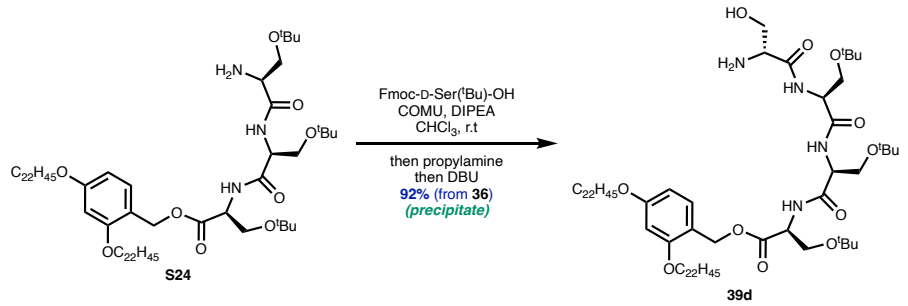
^{13}C NMR (126 MHz, $CDCl_3$): δ 174.5, 170.3, 170.0, 170.0, 160.5, 158.0, 130.7, 116.1, 104.4, 99.3, 74.2, 73.7, 73.3, 68.0 (2C), 65.2, 62.2, 61.9, 61.2, 60.4, 56.3, 53.0, 53.0, 52.8, 31.8 (2C), 29.6-29.0 (overlap, 34C), 27.3-27.2 (overlap, 9C), 26.0 (2C), 22.6 (2C), 14.0 (2C)

Tripeptide (**S24**)



Prepared according to the General Procedure 1 from dipeptide (**S22**) using Fmoc-Ser(ᵗBu)-OH (184mg, 0.480 mmol, 1.2 equiv) as the amino acid.

Tetrapeptide (**39d**)



Prepared according to the General Procedure 1 from tripeptide (**S24**) using Fmoc-D-Ser-OH (157mg, 0.480 mmol, 1.2 equiv) as the amino acid. The resulting precipitate was concentrated *in vacuo* and dried under high vacuum, yielding **39d** (468 mg, 0.367 mmol, 92%) as a colorless powder.

Rf-value: 0.30 ($CHCl_3$/MeOH = 30:1 stained with phosphomolybdic acid)

HRMS (*m/z*): FAB $[M+Na]^+$ calculated for $C_{75}H_{140}O_{11}N_4Na$: 1296.0416, found: 1296.0404.

$[\alpha]_D^{29}$ = +10.4 (c = 0.1, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (d, *J* = 7.5 Hz, 1H), 7.62 (br-d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.40-6.38 (m, 2H), 5.19-5.11 (m, 2H), 4.69 (br-m, 1H), 4.55 (m, 1H), 4.47 (br-m, 1H), 4.20 (dd, *J* = 10.5, 3.0 Hz, 1H), 3.92-3.87 (m, 4H), 3.79-3.72 (m, 2H), 3.62 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.56 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.52-3.45 (m, 2H), 3.33 (app t, *J* = 8.0 Hz, 1H), 3.27 (br-m, 1H), 2.48 (br-s, 1H, *OH), 1.77-1.72 (m, 4H), 1.65 (br-s, 2H *NH_2), 1.45-1.38 (m, 4H), 1.37-1.21 (br-m, 72H), 1.18-1.10 (m, 18H), 1.09-1.01 (m, 9H), 0.86 (t, *J* = 7.0 Hz, 6H)

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.2, 170.3, 169.9, 169.7, 160.5, 158.1, 130.9, 116.1, 104.4, 99.4, 73.9, 73.6, 73.6, 68.0 (2C), 65.1, 62.3, 62.0, 61.6, 61.2, 56.1, 53.6, 53.2, 52.7, 31.9 (2C), 29.6-29.0 (overlap, 34C), 27.3-27.1 (overlap, 9C), 26.0 (2C), 22.6 (2C), 14.1 (2C)

5-9. Cyclic depsipeptide synthesis

• General Procedure 2 for fragment coupling

To a glass vessel charged with Western fragment (**18**) (1.0 equiv) and Ser fragment **39x** (1.05 equiv) was added a solution of AcOH/pyridine = 1:1 mol in DCM (5:95 v/v, 0.03 M) at room temperature. After stirring at room temperature for 18 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN (0.006 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeCN). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography ($CHCl_3$;MeOH = 50:1), yielding oligopeptide **40x** as a powder.
(**x** = a,b,c,d)

• General Procedure 3 for cleavage of Fmoc groups and C-terminus soluble hydrophobic TAGs

To a solution of **40x** (1.0 equiv) in $CHCl_3$ (0.05 M) was added DBU (2.7 equiv) at room temperature. After stirring at room temperature until the consumption of the starting material (judged by TLC), the reaction mixture was cooled down to 0 °C and poured into cold MeCN (0.01 M). The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeOH). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum. This crude material was used in the next reaction without further purification.

To a solution of crude material in $CHCl_3$ was added HFIP (HFIP/$CHCl_3$ = 2:8 v/v, total 0.05 M) at room temperature. After stirring at room temperature until the consumption of the starting material (judged by TLC), the reaction mixture was concentrated *in vacuo* and dried under high vacuum. This crude material **42x** was used in the next reaction without further purification.
(**x** = a,b,c,d)

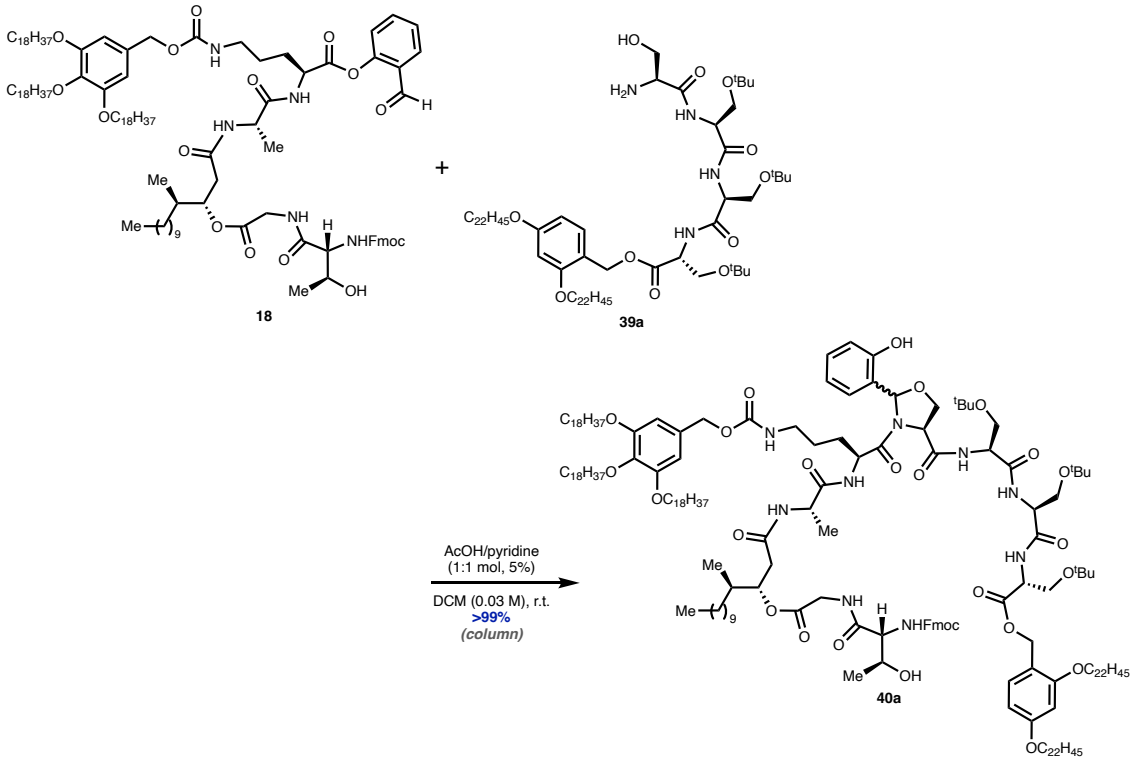
• General Procedure 4 for macrolactamization

To a solution of crude material **42x** in $CHCl_3$ (0.01 M) was added HATU (1.5 equiv) and DIPEA (2.0 equiv) at room temperature. After stirring at room temperature for 4 to14 h, the reaction mixture was cooled down to 0 °C and poured into cold MeCN. The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeCN). The product collected on the filter cake was dissolved in $CHCl_3$ and then eluted by suction filtration using excess $CHCl_3$. The resulting filtrate was concentrated *in vacuo* and dried under high vacuum. This crude material **43x** was used in the next reaction without further purification.
(**x** = a,b,c,d)

• General Procedure 5 for global deprotection

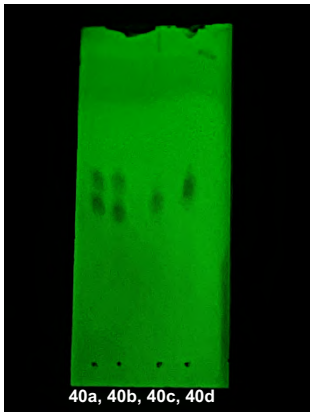
To a solution of crude material **43x** in $CHCl_3$ was added HFIP and TFA ($CHCl_3$/HFIP/TFA = 3:3:4 v/v, total 0.025 M) at room temperature. After stirring at room temperature for 3 h, the reaction mixture was cooled to –10 ºC and added MeCN (0.025 M) and NH_3 aq. (same amount as TFA). After stirring at –10 ºC for 1 h, the reaction mixture was poured into cold MeCN. The resulting suspension was stirred for 30 min at 0 ºC and filtered through a pad of Celite (washed with excess MeCN). The resulting filtrate was concentrated *in vacuo*. The resulting residue was dissolved in a solution of DMSO/MeCN/H_2O/TFA (60:16:24:0.04 v/v, 100 mg/mL) and purified by reverse-phase HPLC (Pegasil ODS SP100 (20 i.d. x 250 mm), isocratic 40% MeCN/H_2O with 0.1% TFA, 7 mL/min flow rate, UV = 210 nm detection), yielding **44x** as a colorless powder.
(**x** = a,b,c,d)

Oligopeptide **(40a)**

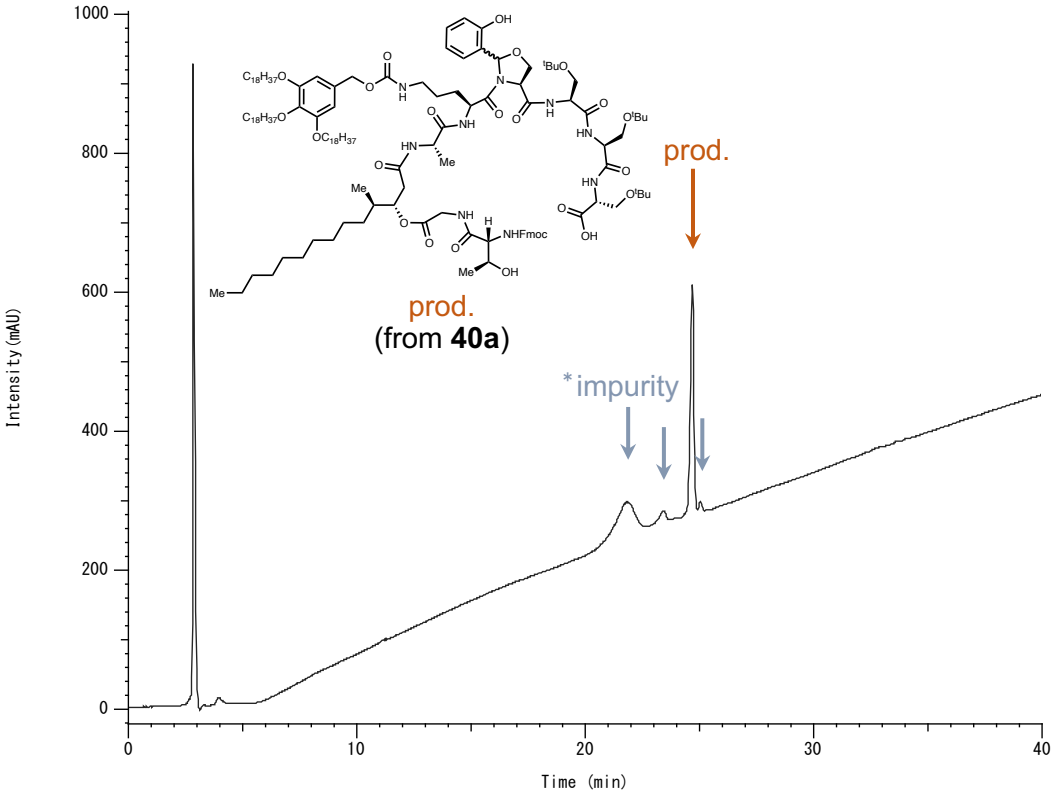


Prepared according to the General Procedure 2 from Western fragment **18** (43.3 mg, 23.2 μmol) using Ser fragment **39a** (31.0 mg, 24.2 μmol, 1.05 equiv), yielding **40a** (72.1 mg, 23.1 μmol, >99%) as a pale-yellow powder.

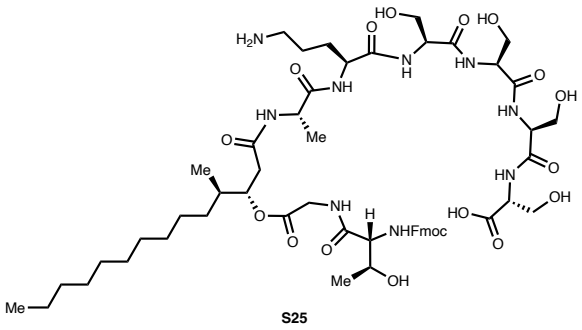
*Note: NMR spectra of the two-tagged compound (**40a**) was broad and most of the peaks were overlapped under the analysis conditions that we attempted using several solvents, temperatures and so on. In addition, HRMS using any routine conditions including Fast Atom Bombardment (FAB) could not be conducted because the molecular weight of **40a** is more than 3000. Therefore, we confirmed the compound after removal of a tag at the C-terminal by treatment with HFIP. The diastereomers (separable on TLC) were observed as a single peak on reversed-phase chromatography (see below).*



Rf-value: 0.55 and 0.53 (diastereomer)
($CHCl_3$/MeOH/toluene = 30:2:5, stained with 2,4-dinitrophenyl hydrazine)



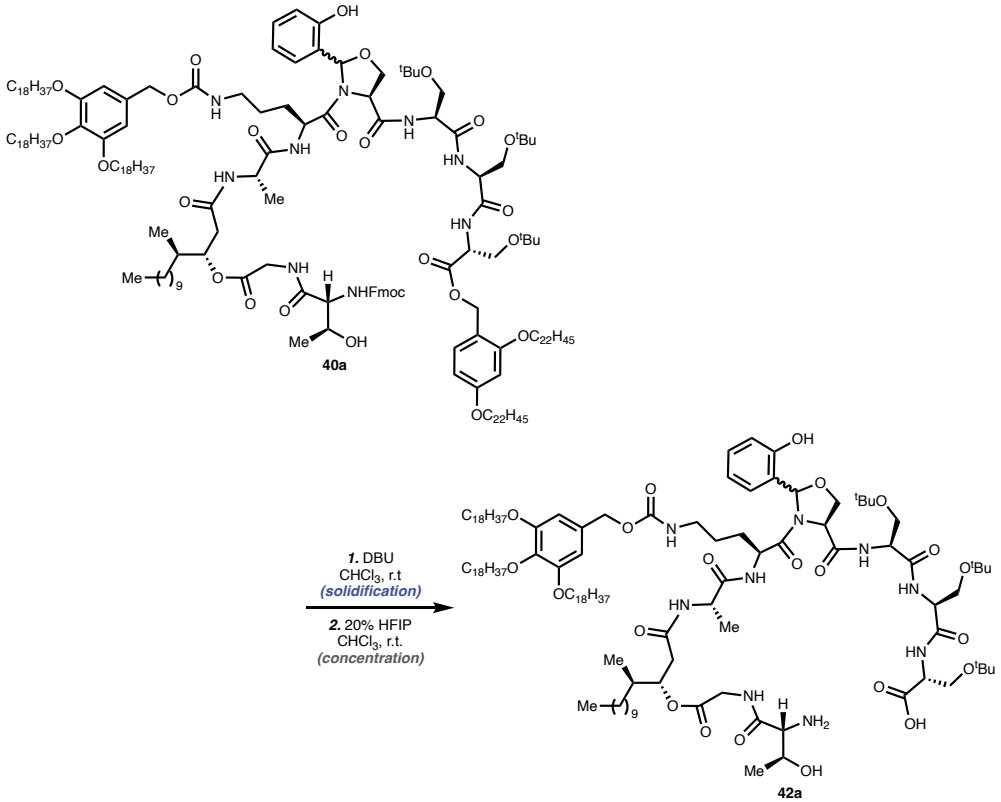
(GL Science, column: Inertsil ODS-4, 5 µm, 4.6 i.d. × 250 mm, flow rate: 1.0 mL/min, solvent condition: 20-80% MeCN/THF gradient, UV = 210 nm detection, injection solution: 0.2 mg/mL (100% THF), injection volume: 50.0 µL, retention time: 24.6 min [single peak] *Impurities could not be identified.)



HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{56}H_{86}O_{18}N_9$: 1172.6091, found: 1172.6071.

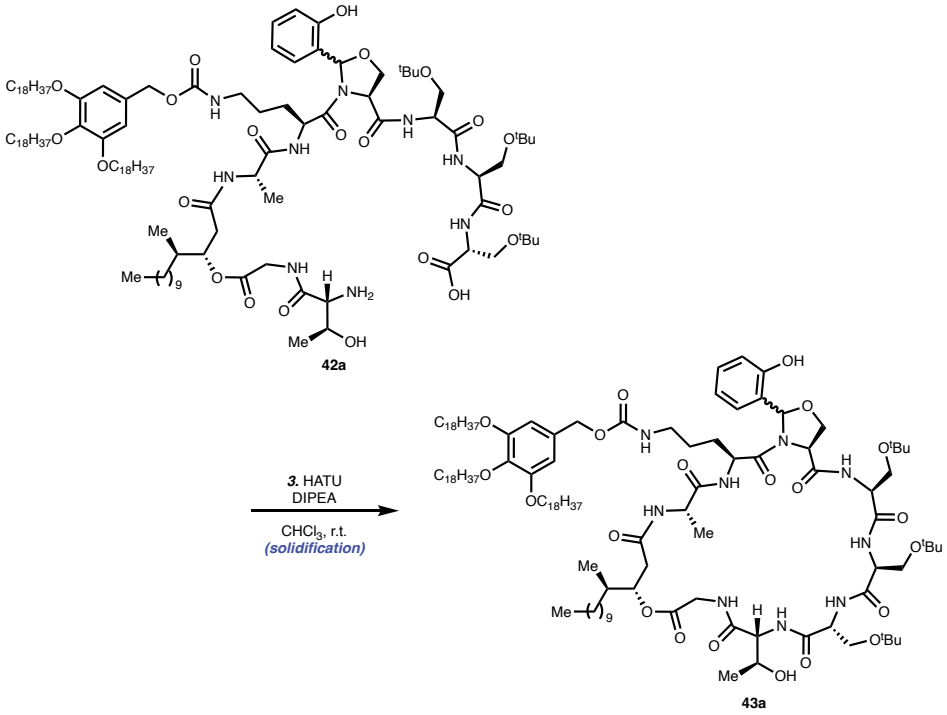
*Note: We identified the corresponding HRMS of compound **S25** after removal of the TCbz group at the side chain of the Orn residue using TFA.*

Cyclization precursor (**42a**)



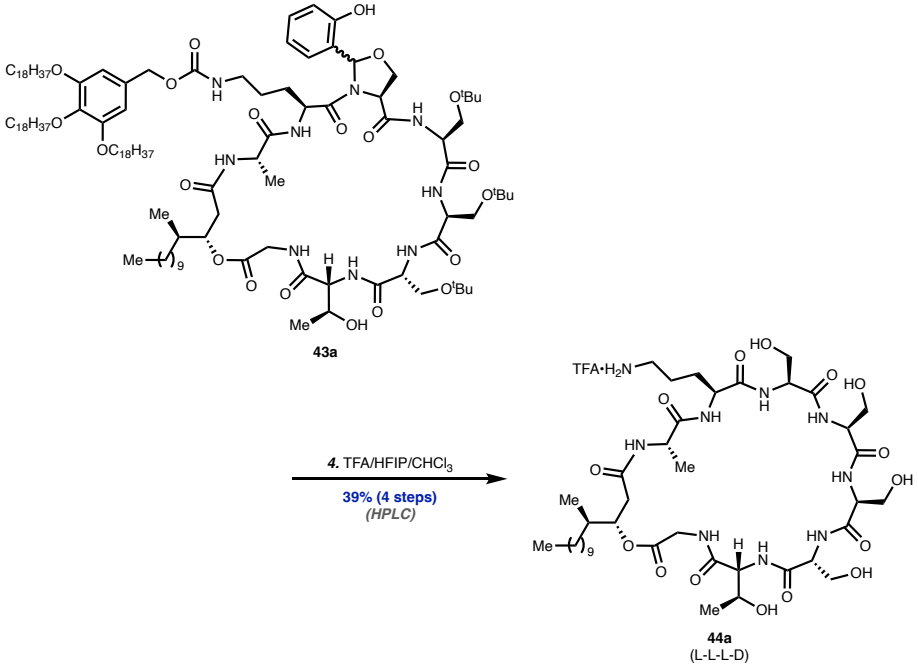
Prepared according to the General Procedure 3 from oligopeptide **40a** (62.5 mg, 20.0 μmol). The resulting crude material **42a** was used in the next reaction without further purification.

Tagged cyclic peptide (**43a**)



Prepared according to the General Procedure 4 from cyclic precursor **42a**. The resulting crude material **43a** was used in the next reaction without further purification.

Cyclic peptide (**44a**)



Prepared according to the General Procedure 5 from tagged cyclic peptide **43a**, yielding **44a** (7.2 mg, 7.7 µmol, 39% over 4 steps) as a colorless powder.

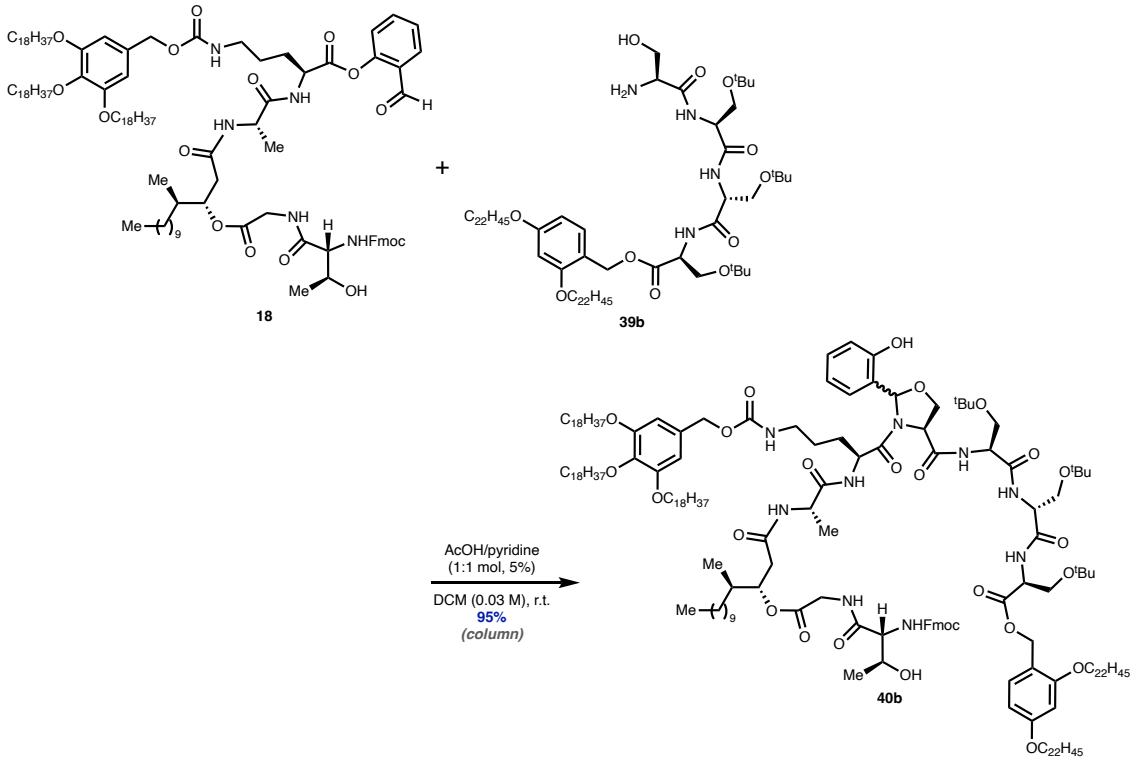
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{41}H_{74}O_{15}N_9$: 932.5304, found: 932.5308.

$[\alpha]_D^{26}$ = +16.1 (c = 0.05, pyridine)

^{1}H NMR (500 MHz, $(CD_3)_2SO$): 8.43 (d, *J* = 5.5 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.04-7.97 (m, 3H), 7.87 (d, *J* = 8.5, 1H), 7.71 (d, *J* = 7.5, 1H), 7.53 (d, *J* = 8.0 Hz, 1H,), 5.08-5.04 (m, 2H), 4.46 (br-dt, *J* = 9.5, 4.5 Hz, 1H), 4.40 (m, 1H), 4.29-4.26 (m, 3H), 4.19 (m, 1H), 4.09 (q, *J* = 5,2 Hz, 1H), 3.92-3.80 (m, 4H), 3.72-3.52 (m, 8H), 2.75 (br-s, 2H), 2.32-2.30 (m, 2H), 1.80-1.67 (br-m, 2H), 1.60-1.49 (br-m, 3H), 1.31 (br-m, 1H), 1.27–1.20 (m, 16H), 1.19 (d, *J* = 7.3 Hz, 3H), 1.07 (d, *J* = 6.0 Hz, 3H), 1.01 (br-m, 1H), 0.86-0.81 (m, 6H)

^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 172.6, 171.4, 171.2, 170.3, 170.2, 170.0, 169.9, 169.6, 168.9, 75.5, 66.6, 62.2, 61.8, 61.2, 60.7, 58.4, 57.3, 55.9, 55.0, 54.2, 52.1, 48.6, 41.1, 38.6, 36.9, 36.0, 31.8, 31.4, 29.3, 29.1, 29.1 (2C), 28.8, 28.3, 26.5, 23.5, 22.2, 19.8, 17.7, 14.5, 14.0.

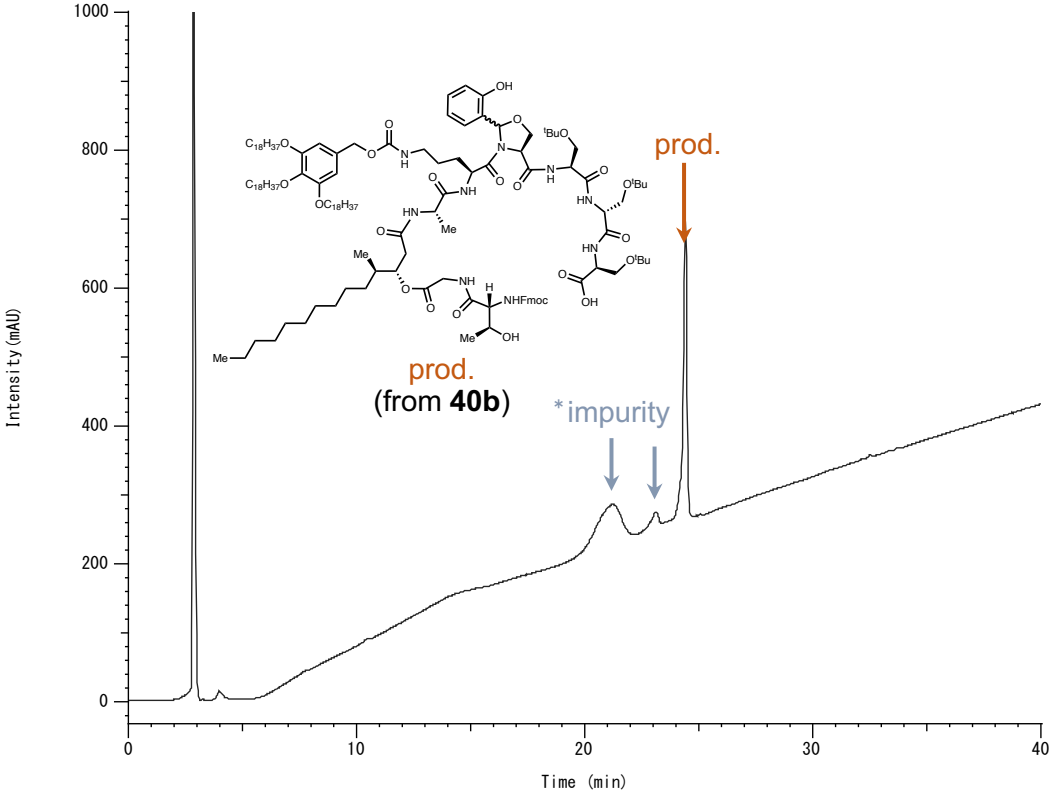
Oligopeptide **(40b)**



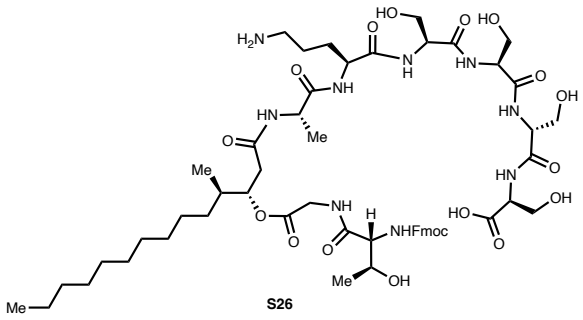
Prepared according to the General Procedure 2 from Western fragment **18** (93.4 mg, 50.0 μmol) using Ser fragment **39b** (66.9 mg, 52.5 μmol, 1.05 equiv), yielding **40b** (147.7 mg, 47.3 μmol, 95%) as a pale-yellow powder.

Note: NMR analysis of the two-tagged compound was difficult because many of the peaks were very broad and overlap, although the solvent, temperature, and other conditions were investigated. In addition, it was difficult to obtain Fast Atom Bombardment (FAB) mass spectra for a two-tagged compound with a molecular weight greater than 3000, so the two-tagged compound was identified using a compound with the serine C-terminal soluble hydrophobic tag removed using 1,1,1,3,3,3-Hexafluoropropan-2-ol.

Rf-value: 0.55 and 0.52 (diastereomer, second from left) ($CHCl_3$/MeOH/toluene = 30:2:5, stained with 2,4-Dinitro-phenylhydrazine)



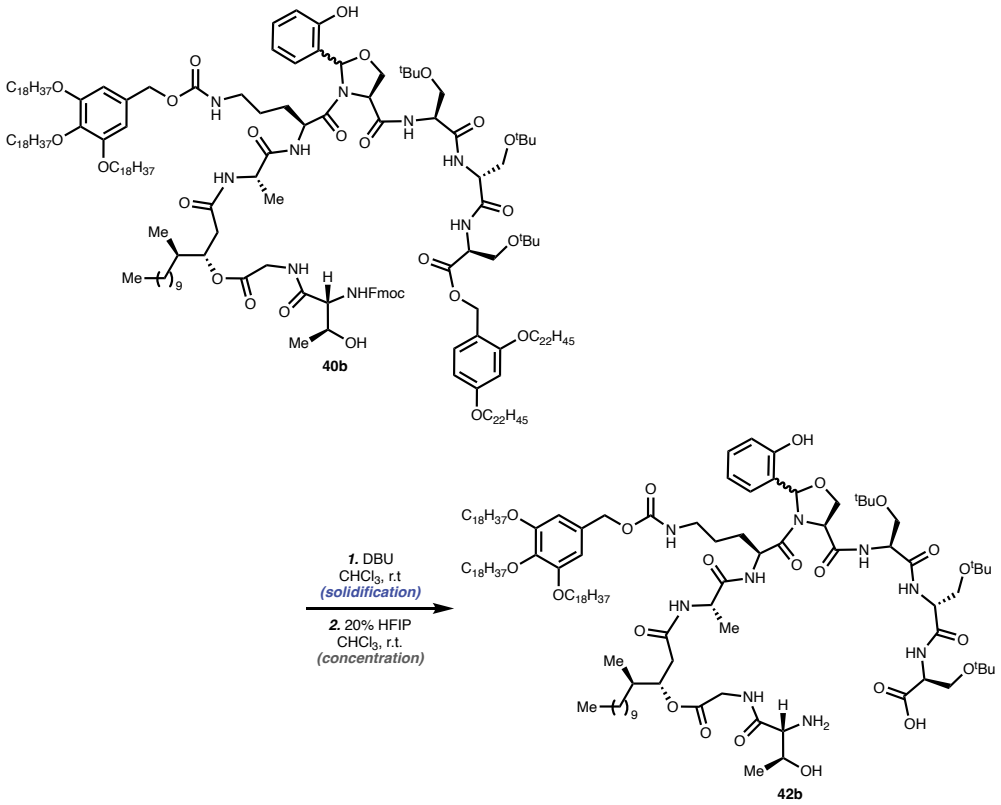
(GL Science, column: Inertsil ODS-4, 5 µm, 4.6 i.d. × 250 mm, flow rate: 1.0 mL/min, solvent condition: 20-80% MeCN/THF gradient, UV = 210 nm detection, injection solution: 0.2 mg/mL (100% THF), injection volume: 50.0 µL, retention time: 24.4 min [single peak] *Impurities could not be identified.)



HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{56}H_{86}O_{18}N_9$: 1172.6091, found: 1172.6080.

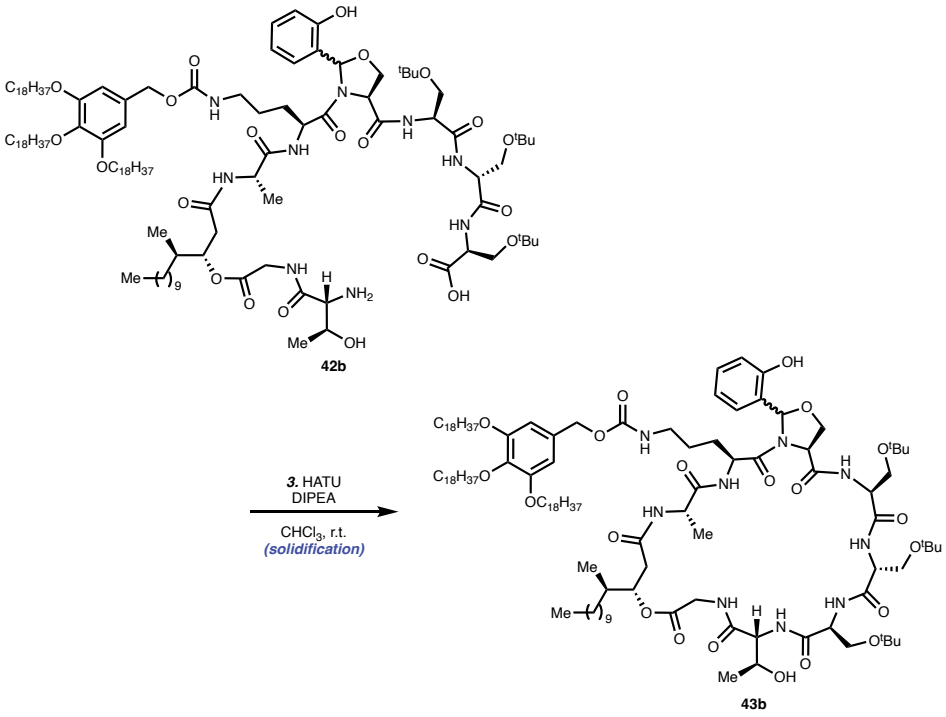
*Note: We identified the corresponding HRMS of compound **S26** after removal of the TCbz group at the side chain of the Orn residue using TFA.*

Cyclization precursor (**42b**)



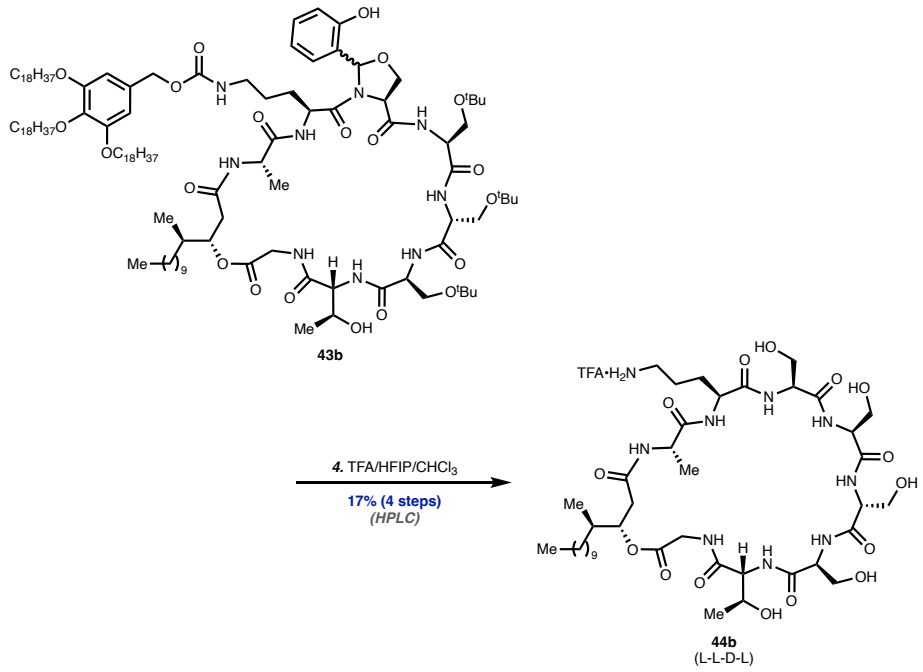
Prepared according to the General Procedure 3 from oligopeptide **40b** (147.7 mg, 47.3 μmol). The resulting crude material **42b** was used in the next reaction without further purification.

Tagged cyclic peptide (**43b**)



Prepared according to the General Procedure 4 from cyclic precursor **42b**. The resulting crude material **43b** was used in the next reaction without further purification.

Cyclic peptide (**44b**)



Prepared according to the General Procedure 5 from tagged cyclic peptide **43b**, yielding **44b** (7.6 mg, 8.2 µmol, 17%, 4 steps) as a colorless powder.

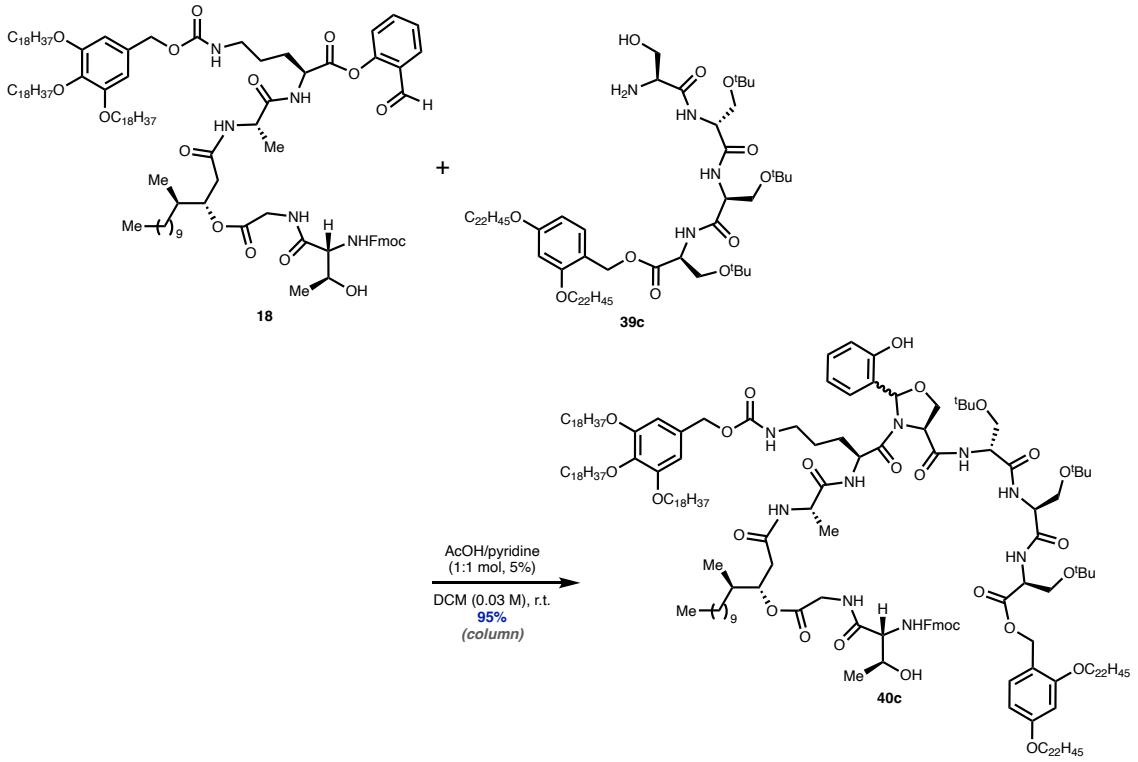
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{41}H_{74}O_{15}N_9$: 932.5304, found: 932.5301.

$[\alpha]_D^{27}$ = +8.04 (c = 0.05, pyridine)

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.31 (d, *J* = 7.6 Hz, 1H), 8.25-8.14 (m, 2H), 8.02 (d, *J* = 9.6 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.73-7.69 (br-m, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.56 (br-s, 1H), 5.60 (d, *J* = 8.0 Hz, 1H), 5.05-5.02 (m, 2H), 4.95 (br-s, 1H), 4.89 (br-s, 1H), 4.48 (dd, *J* = 13.6, 5. 2 Hz, 1H), 4.40-4.20 (m, 4H), 4.15-4.10 (m, 1H), 3.88-3.82 (m, 1H), 3.77 (d, *J* = 4.4 Hz, 1H), 3.73 (d, *J* = 4.0 Hz, 1H), 3.69-3.50 (m, 6H), 2.82-2.65 (br-m, 2H), 2.38-2.28 (m, 1H), 1.77-1.42 (br-m, 5H), 1.32 (br-s, 1H), 1.27-1.18 (m, 15H), 1.18-1.12 (m, 4H), 1.07-0.99 (m, 3H), 0.86-0.82 (m, 6H)
Note: Because many peaks broaden and overlap, some peaks were difficult to identify.

^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 172.2, 171.5, 170.6, 170.4, 170.3, 170.0, 169.9, 169.9, 169.2, 75.1, 66.7, 62.1, 61.6, 61.6, 61.4, 58.2, 55.8, 55.4, 55.3, 54.8, 51.7, 48.8, 47.5, 36.5, 36.2, 33.4, 31.6, 31.3, 29.3, 29.1, 29.0 (2C), 28.7 (2C), 26.6, 23.2, 22.1, 19.6, 17.2, 14.7, 14.0.

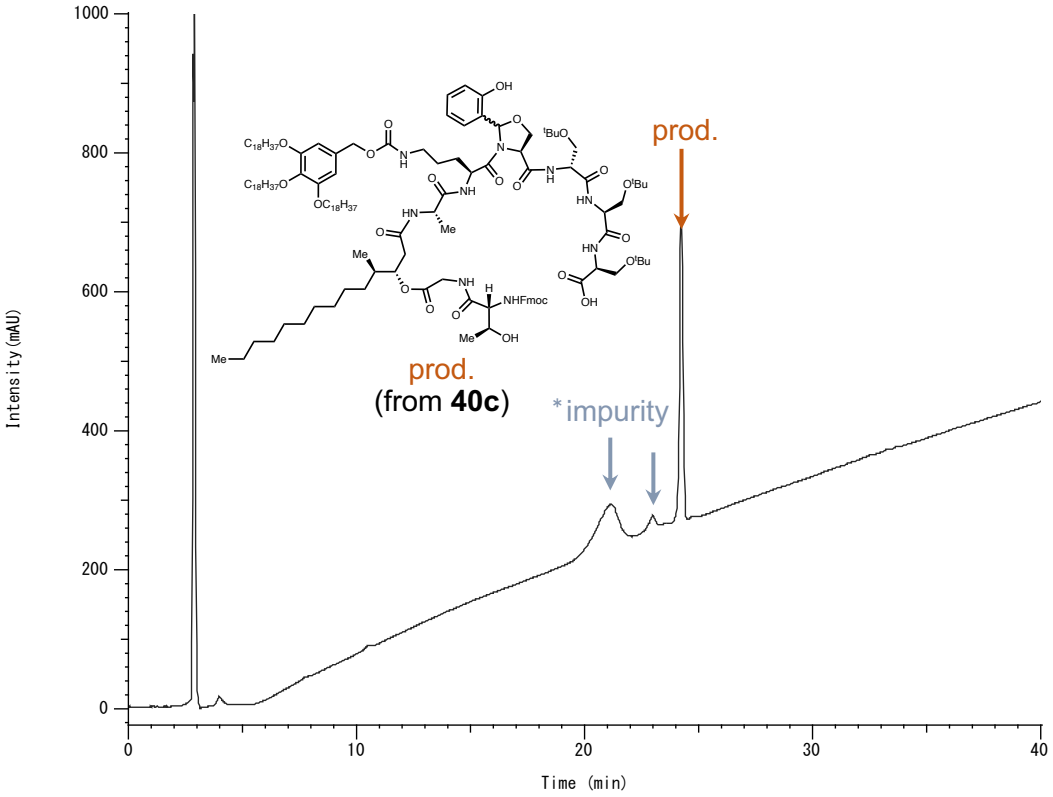
Oligopeptide **(40c)**



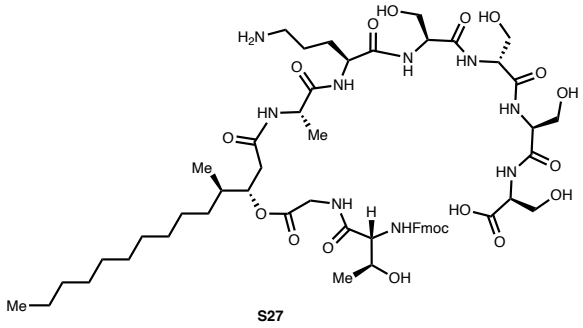
Prepared according to the General Procedure 2 from Western fragment **18** (37.4 mg, 20.0 μmol) using Ser fragment **39c** (26.8 mg, 21.0 μmol, 1.05 equiv), yielding **40c** (59.6 mg, 19.1 μmol, 95%) as a pale-yellow powder.

Note: NMR analysis of the two-tagged compound was difficult because many of the peaks were very broad and overlap, although the solvent, temperature, and other conditions were investigated. In addition, it was difficult to obtain Fast Atom Bombardment (FAB) mass spectra for a two-tagged compound with a molecular weight greater than 3000, so the two-tagged compound was identified using a compound with the serine C-terminal soluble hydrophobic tag removed using 1,1,1,3,3,3-Hexafluoropropan-2-ol.

Rf-value: 0.53 (one spot, third from left) ($CHCl_3$/MeOH/toluene = 30:2:5, stained with 2,4-Dinitro-phenylhydrazine)



(GL Science, column: Inertsil ODS-4, 5 μm, 4.6 i.d. × 250 mm, flow rate: 1.0 mL/min, solvent condition: 20-80% MeCN/THF gradient, UV = 210 nm detection, retention time: 24.3 min [single peak] *Impurities could not be identified.)

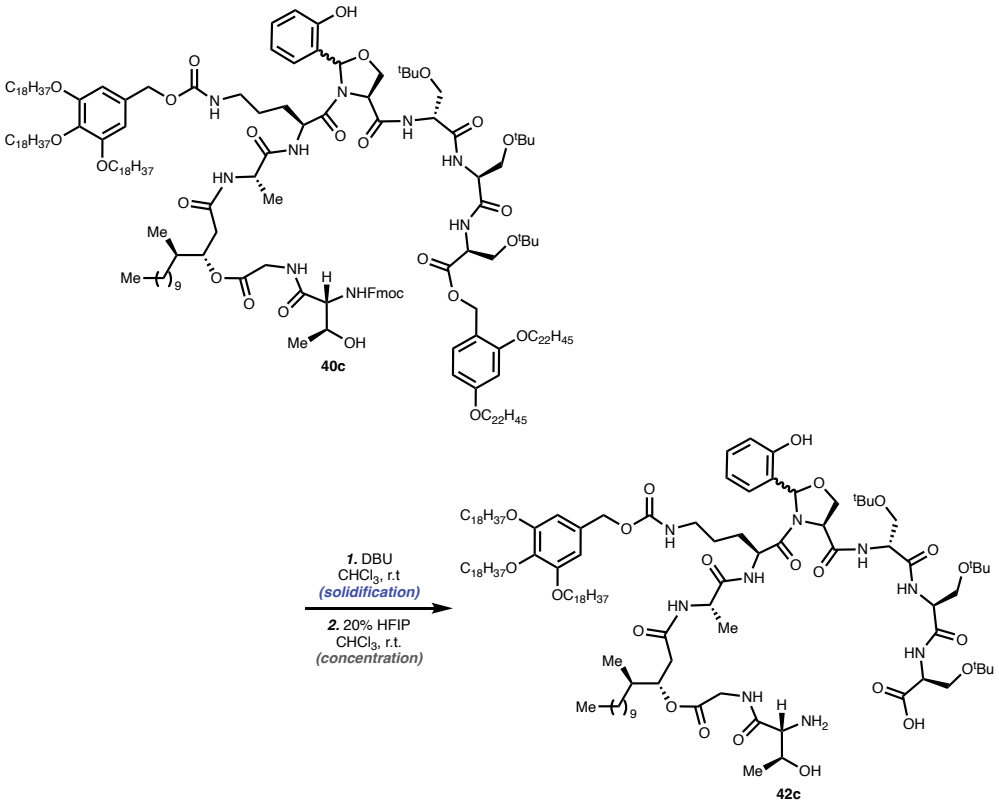


S27

HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{56}H_{86}O_{18}N_9$: 1172.6091, found: 1172.6086.

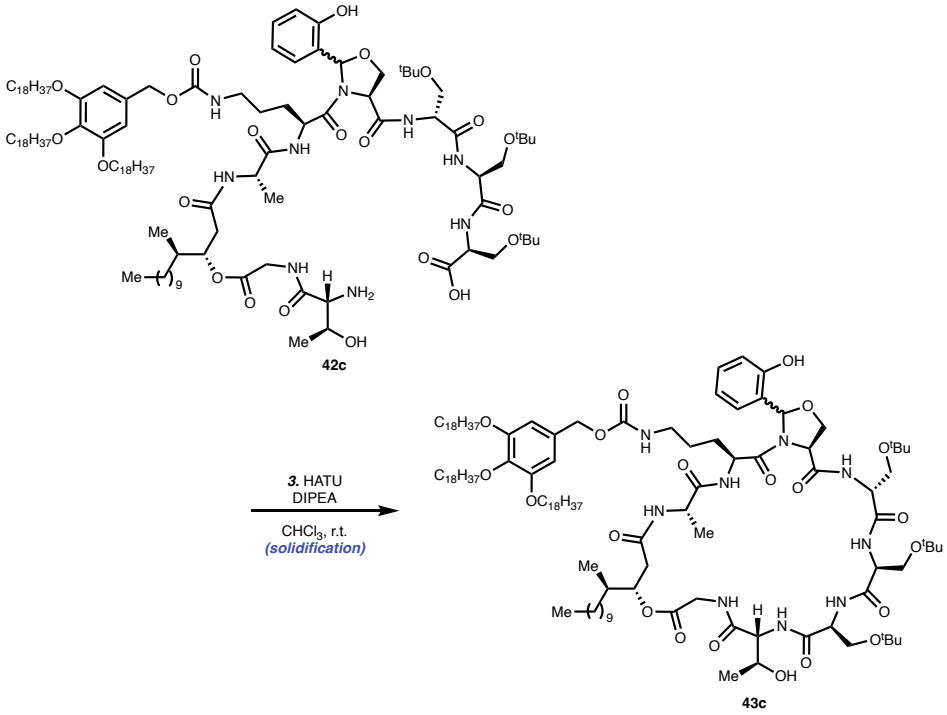
*Note: We identified the corresponding HRMS of compound **S27** after removal of the TCbz group at the side chain of the Orn residue using TFA.*

Cyclization precursor (**42c**)

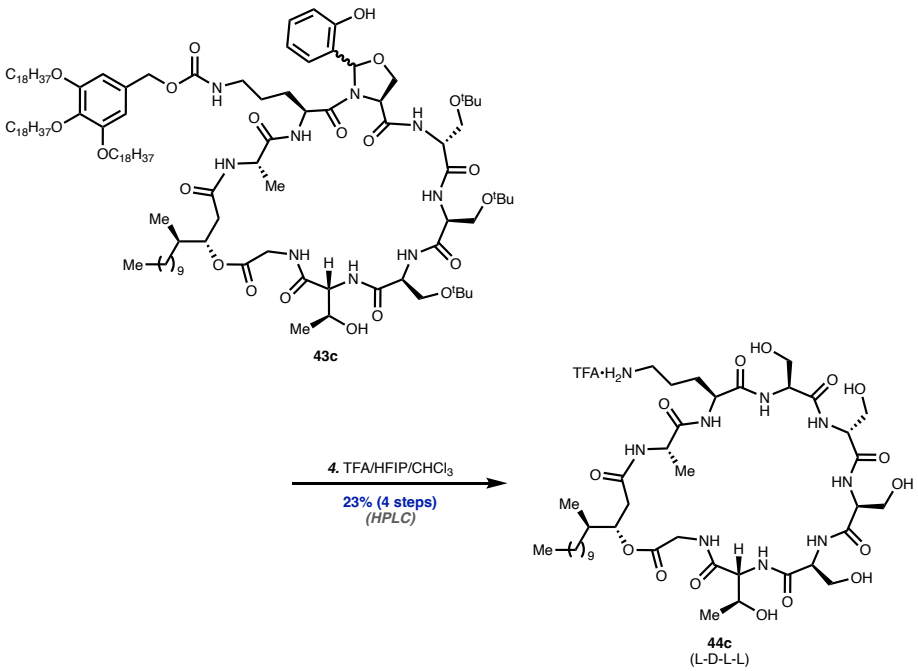


Prepared according to the General Procedure 3 from oligopeptide **40c** (46.9 mg, 15.0 μmol). The resulting crude material **42c** was used in the next reaction without further purification.

Tagged cyclic peptide (**43c**)



Prepared according to the General Procedure 4 from cyclic precursor **42c**. The resulting crude material **43c** was used in the next reaction without further purification.



Prepared according to the General Procedure 5 from tagged cyclic peptide **43c**, yielding **44c** (3.2 mg, 3.4 μmol, 23%, 4 steps) as a colorless powder.

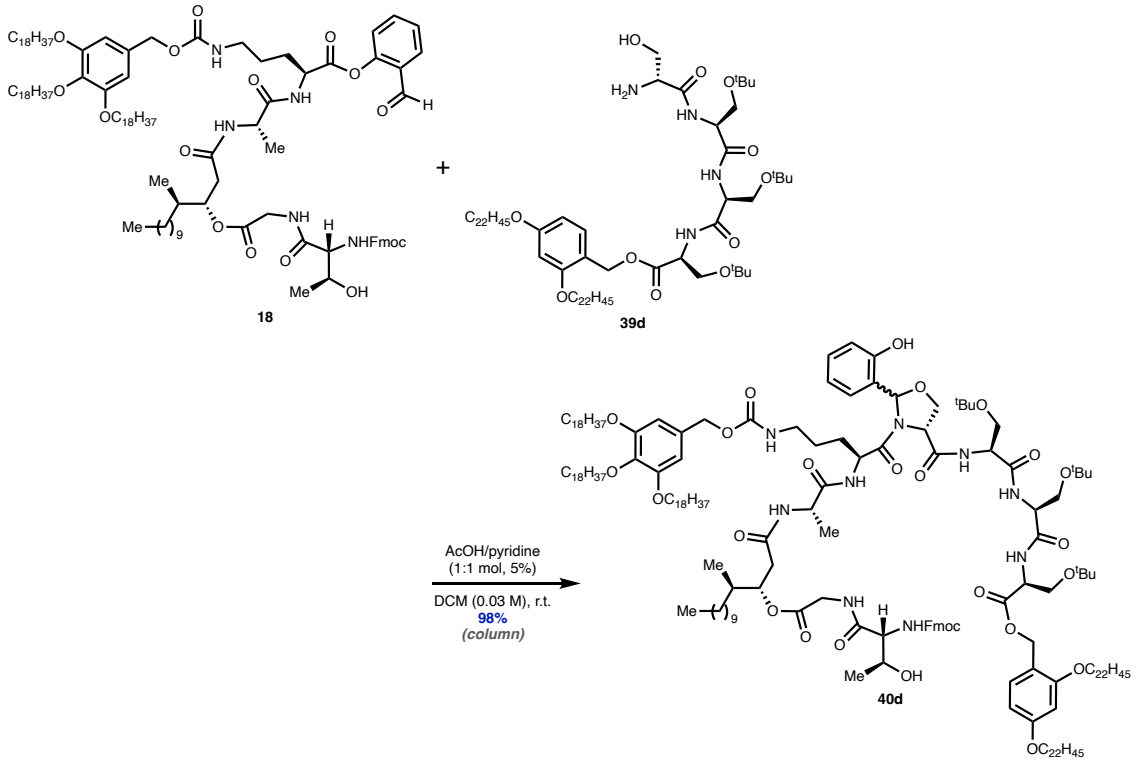
HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{41}H_{74}O_{15}N_9$: 932.5304, found: 932.5304.

$[\alpha]_D^{26}$ = +11.4 (c = 0.05, pyridine)

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.24 (br-s, 2H), 8.18 (d, *J* = 6.8 Hz, 1H), 8.08 (br-s, 1H), 8.02 (d, *J* = 6.8 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.72-7.58 (br-m, 4H), 5.14-5.10 (m, 1H), 4.98 (d, *J* = 4.8 Hz, 1H), 4.94-4.86 (m, 4H), 4.36-4.31 (m, 2H), 4.28-4.22 (m, 2H), 4.19-4.11 (m, 3H), 3.93-3.87 (m, 2H), 3.81 (d, *J* = 4.4 Hz, 1H), 3.77 (d, *J* = 4.8 Hz, 1H), 3.63 (br-d, *J* = 5.2 Hz, 6H), 2.77 (br-s, 2H), 2.37-2.33 (br-m, 2H), 1.79-1.71 (br-m, 1H), 1.69-1.64 (br-m, 1H), 1.61-1.52 (br-m, 3H), 1.34-1.28 (br-m, 2H), 1.26-1.14 (m, 19H), 1.03 (d, *J* = 6.0 Hz, 3H), 0.86-0.82 (m, 6H)
Note: Because many peaks broaden and overlap, some peaks were difficult to identify.

^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 172.9, 171.4, 171.2, 170.9, 170.6, 169.9, 169.9, 169.8, 169.4, 75.2, 66.8, 61.4, 61.2, 60.9, 60.8, 58.1, 56.3, 56.2, 56.2, 55.7, 54.9, 49.3, 41.2, 38.5, 37.2, 36.1, 31.6, 31.3, 29.3, 29.1, 29.0 (2C), 28.7, 28.6, 26.4, 23.5, 22.1, 19.7, 17.3, 14.7, 14.0.

Oligopeptide **(40d)**

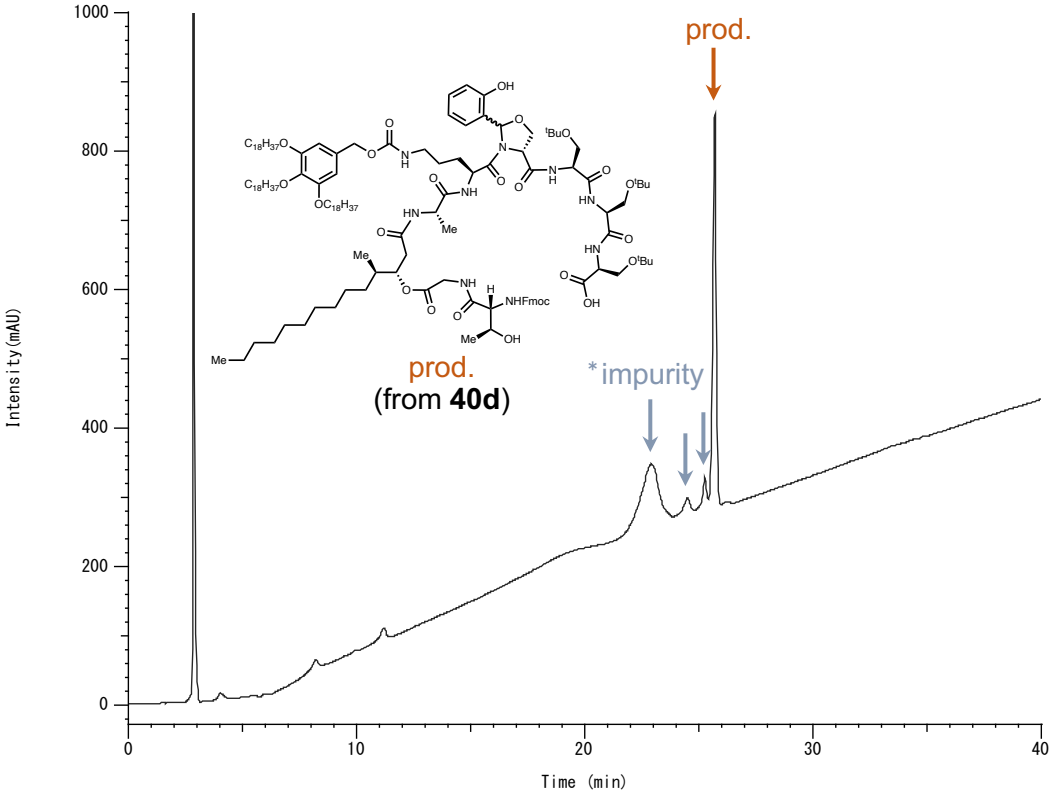


Prepared according to the General Procedure 2 from Western fragment **18** (121 mg, 65.0 μmol) using Ser fragment **39d** (86.9 mg, 68.3 μmol, 1.05 equiv), yielding **40d** (198.6 mg, 63.6 μmol, 98%) as a pale-yellow powder.

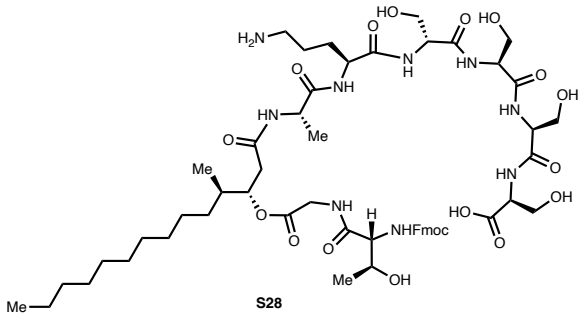
Note: NMR analysis of the two-tagged compound was difficult because many of the peaks were very broad and overlap, although the solvent, temperature, and other conditions were investigated. In addition, it was difficult to obtain Fast Atom Bombardment (FAB) mass spectra for a two-tagged compound with a molecular weight greater than 3000, so the two-tagged compound was identified using a compound with the serine C-terminal soluble hydrophobic tag removed using 1,1,1,3,3,3-Hexafluoropropan-2-ol.

Rf-value: 0.55 (broad spot, rightmost)

($CHCl_3$/MeOH/toluene = 30:2:5, stained with 2,4-Dinitro-phenylhydrazine)



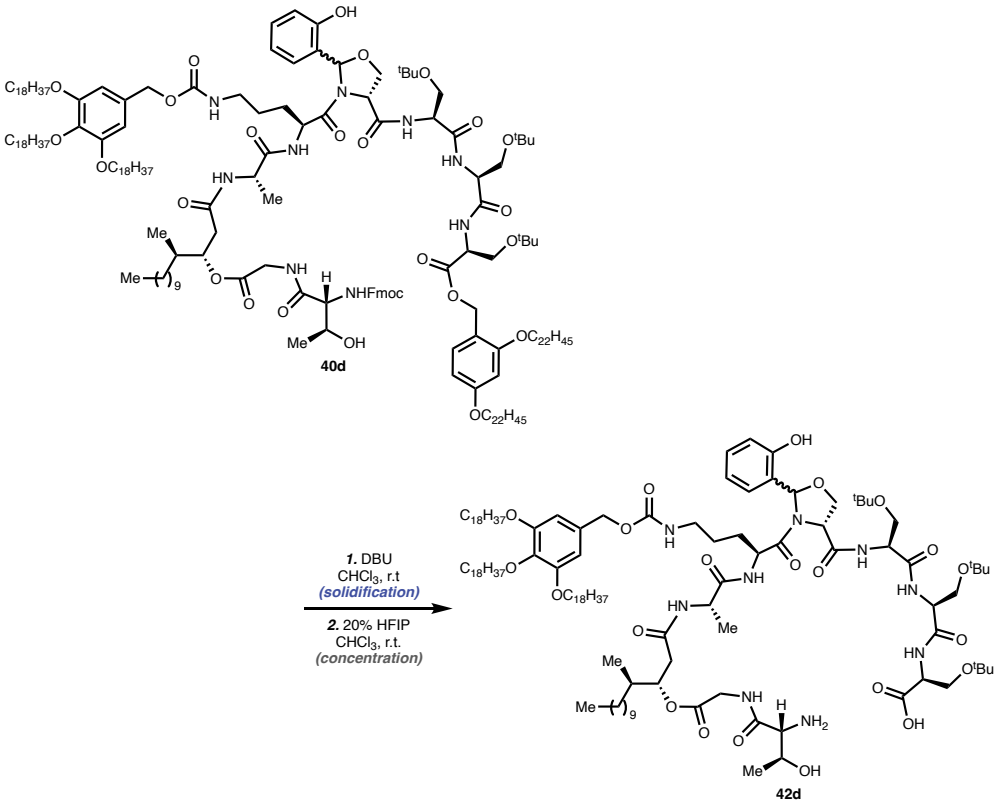
(GL Science, column: Inertsil ODS-4, 5 µm, 4.6 i.d. × 250 mm, flow rate: 1.0 mL/min, solvent condition: 20-80% MeCN/THF gradient, UV = 210 nm detection, retention time: 25.7 min [single peak] *Impurities could not be identified.)



HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{56}H_{86}O_{18}N_9$: 1172.6091, found: 1172.6090.

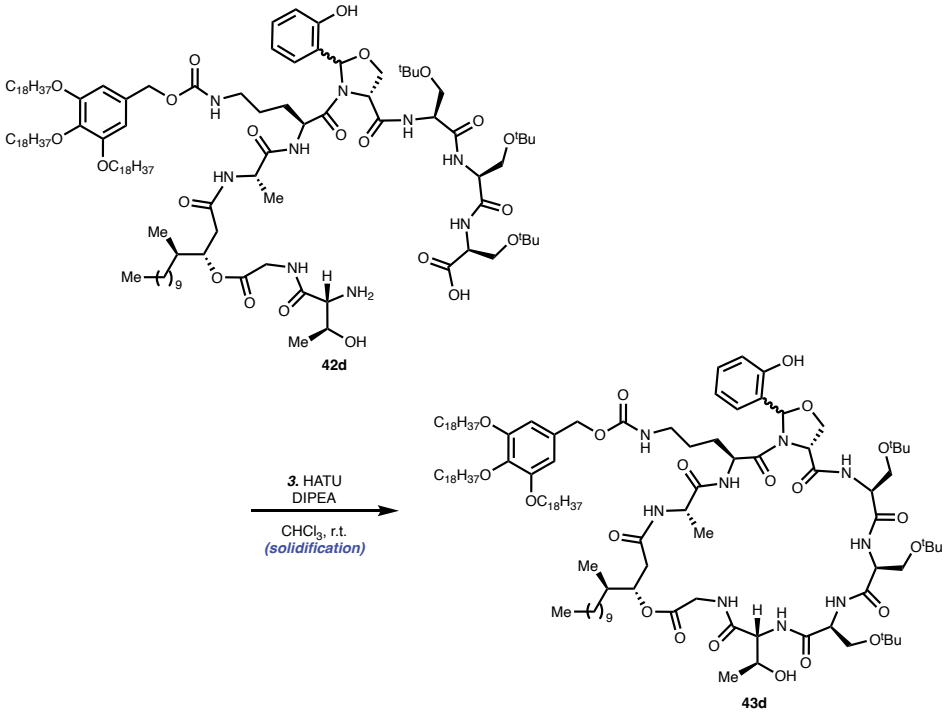
*Note: We identified the corresponding HRMS of compound **S28** after removal of the TCbz group at the side chain of the Orn residue using TFA.*

Cyclization precursor (**42d**)



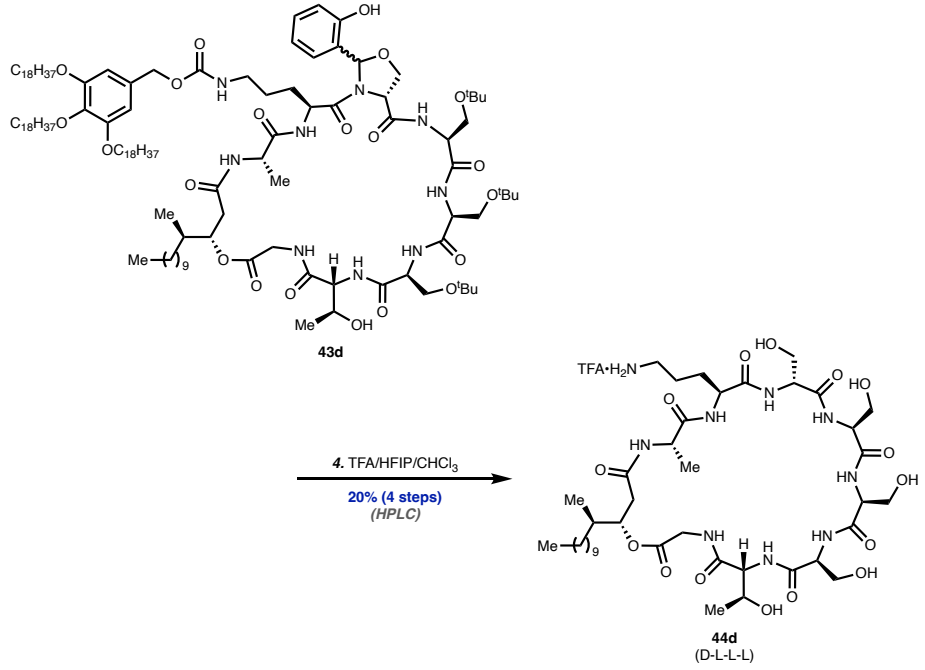
Prepared according to the General Procedure 3 from oligopeptide **40d** (198.6 mg, 63.6 μmol). The resulting crude material **42d** was used in the next reaction without further purification.

Tagged cyclic peptide (**43d**)



Prepared according to the General Procedure 4 from cyclic precursor **42d**. The resulting crude material **43d** was used in the next reaction without further purification.

Cyclic peptide (**44d**)



Prepared according to the General Procedure 5 from tagged cyclic peptide **43d**, yielding **44d** (11.6 mg, 12.5 µmol, 20%, 4 steps) as a colorless powder.

HRMS (*m/z*): ESI $[M+H]^+$ calculated for $C_{41}H_{74}O_{15}N_9$: 932.5304, found: 932.5303.

$[\alpha]_D^{26}$ = +12.9 (c = 0.05, pyridine)

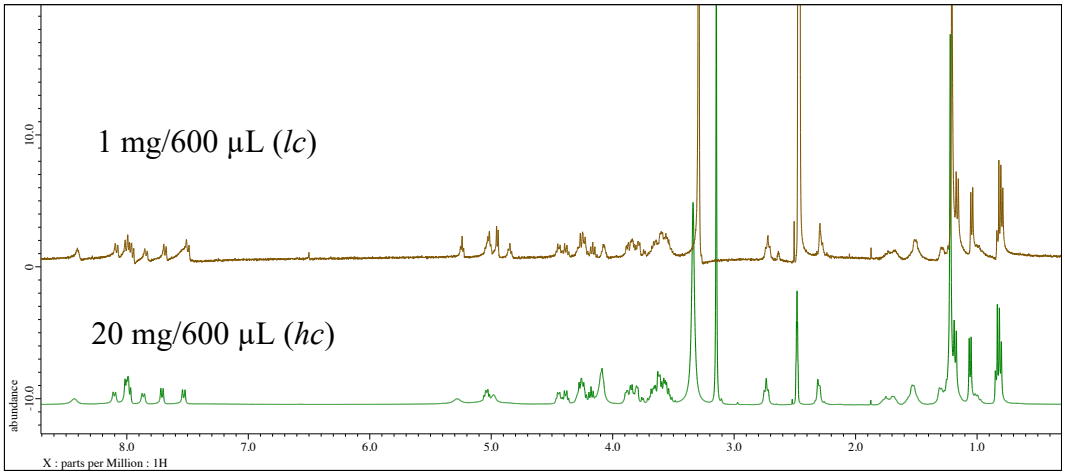
^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.39 (d, *J* = 6.8 Hz, 1H), 8.30 (d, *J* = 6.8 Hz, 1H), 8.20-8.15 (m, 2H), 7.86 (t, *J* = 8.6 Hz, 2H), 7.76 (br-d, *J* = 6.8 Hz, 4H), 7.67 (d, *J* = 7.6 Hz, 1H), 5.62 (d, *J* = 8.0 Hz, 1H), 5.16 (br-t, *J* = 5.0 Hz, 1H), 4.97 (br-d, *J* = 5.2 Hz, 4H), 4.91 (br-t, *J* = 5.0 Hz, 1H), 4.43-4.36 (m, 2H), 4.30-4.25 (m, 2H), 4.21-4.14 (m, 2H), 3.95-3.88 (m, 2H), 3.81 (dd, *J* = 17.2, 6.0 Hz, 1H), 3.73-3.52 (m, 8H), 2.80 (br-s, 2H), 2.33-2.23 (m, 2H), 1.78-1.48 (br-m, 6H), 1.30-1.18 (m, 18H), 1.10-1.04 (m, 5H), 0.88-0.78 (m, 6H)

^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 173.1, 171.8, 170.7, 170.4, 170.1 (2C), 169.7, 169.7, 168.7, 75.6, 66.6, 61.7, 61.6, 61.2, 61.0, 58.5, 56.4, 55.9, 55.0, 54.5, 52.5, 48.3, 47.5, 38.5, 36.7, 35.8,, 31.8, 31.3, 29.2, 29.1, 29.1, 29.0, 28.7, 27.7, 26.4, 23.6, 22.1, 20.1, 17.8, 14.3, 14.0

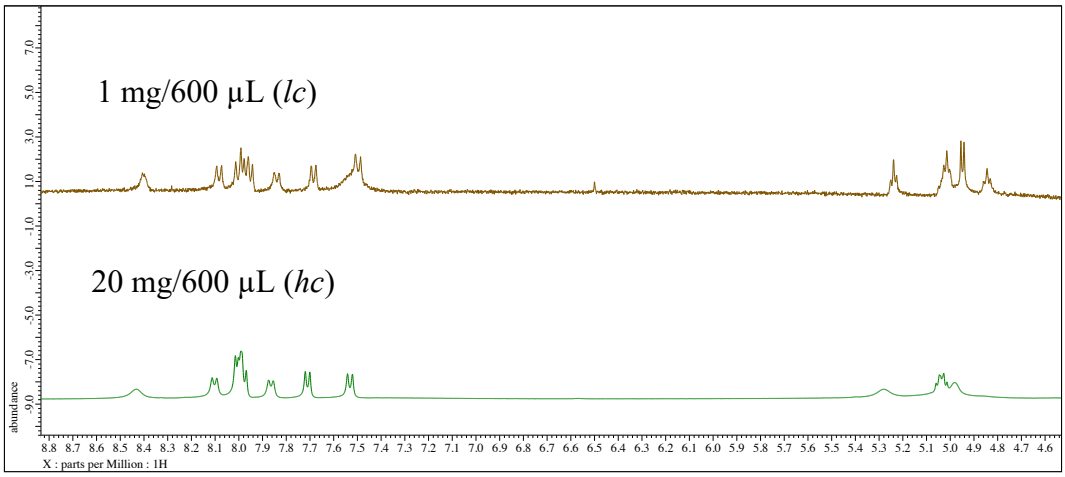
6. Spectral Data Comparison of Natural and Synthetic Tetraselide

6-1. Investigation of NMR measurement conditions of Authentic Tetraselide

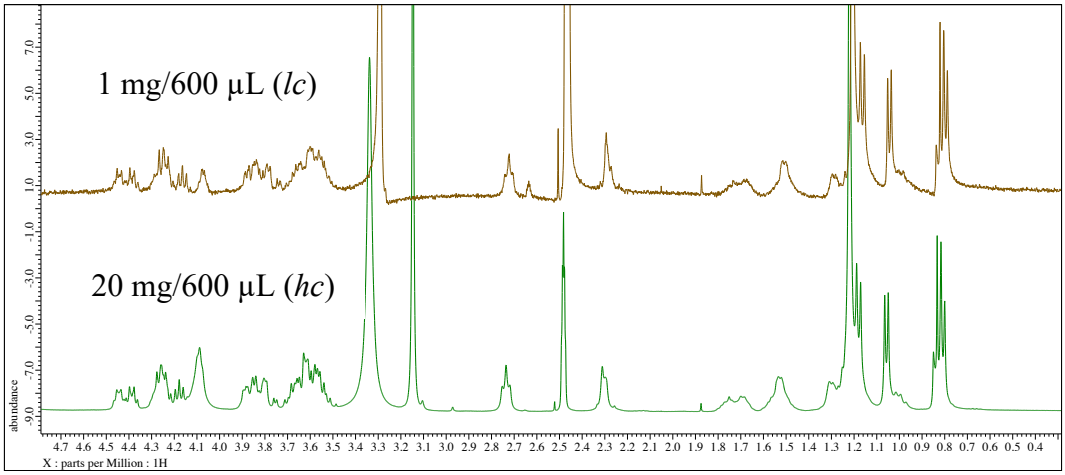
^{1}H NMR tetraselide (**1**): (400 MHz, $(CD_3)_2SO$)



Enlarged view: 8.8-4.5 ppm



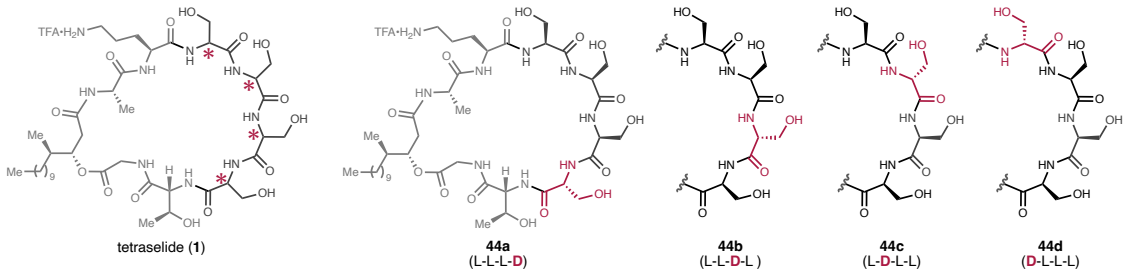
Enlarged view: 4.8-0.3 ppm



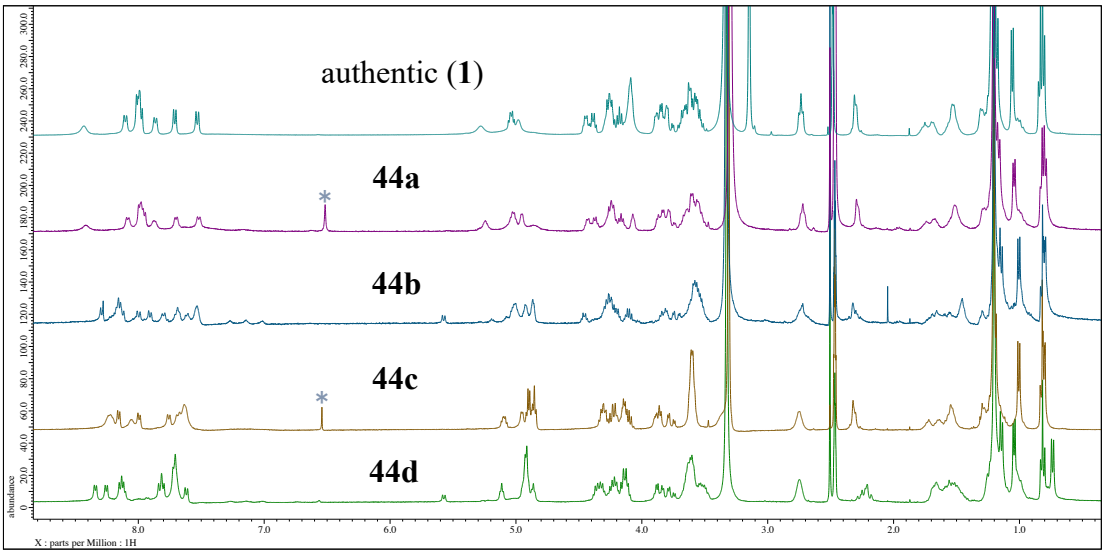
lc: low concentration, hc: high concentration

*Note: Because tetraselide (**1**) has a cyclic structure and a basic amino group, we investigated whether there is a difference in the NMR shift depending on the dissolution concentration of the substrate (see figure above). As a result, it was found that the shift of **1** doesn't differ significantly depending on the dissolution concentration of the substrate, so there is no need to specifically match the concentrations of the natural and synthetic samples for NMR measurements.*

6-2. Comparison of Spectral Data of Natural Tetraselide and Synthetic Compounds (40a–d)

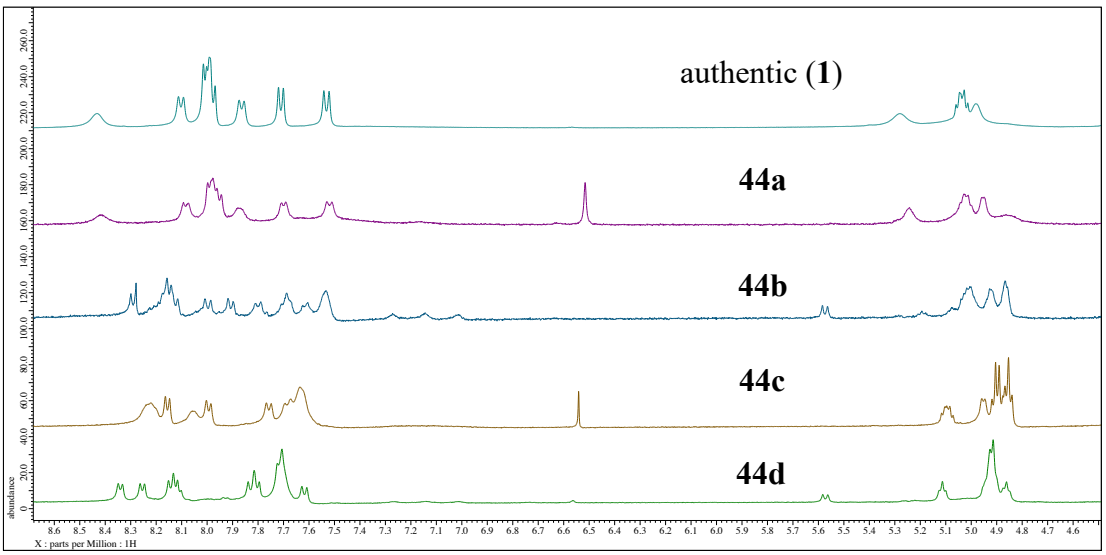


^{1}H NMR authentic (**1**): (400 MHz, $(CD_3)_2SO$), **44a**, **44b**, **44c**, **44d**: (400 MHz, $(CD_3)_2SO$)

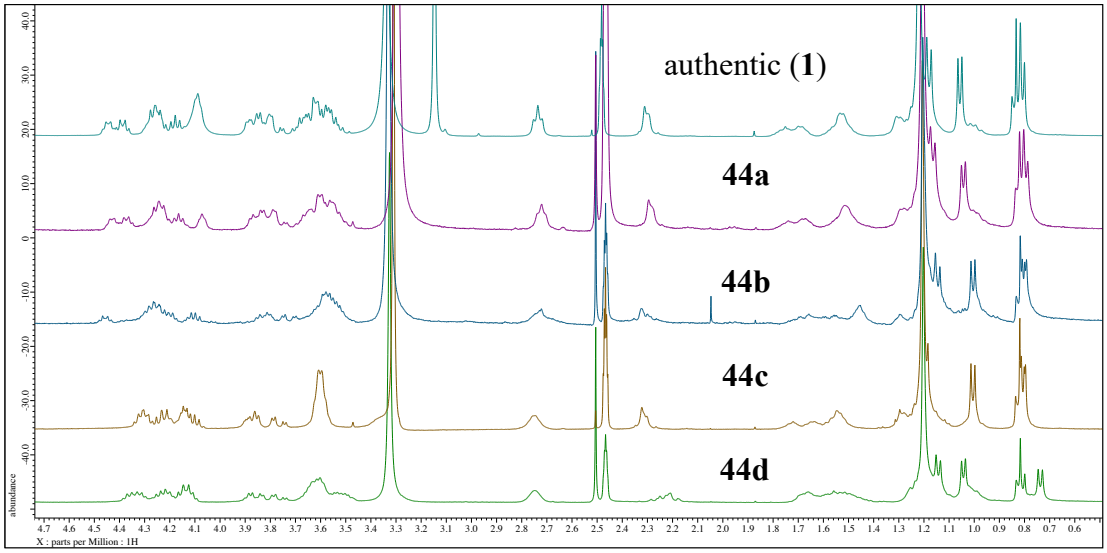


∗ : impurity

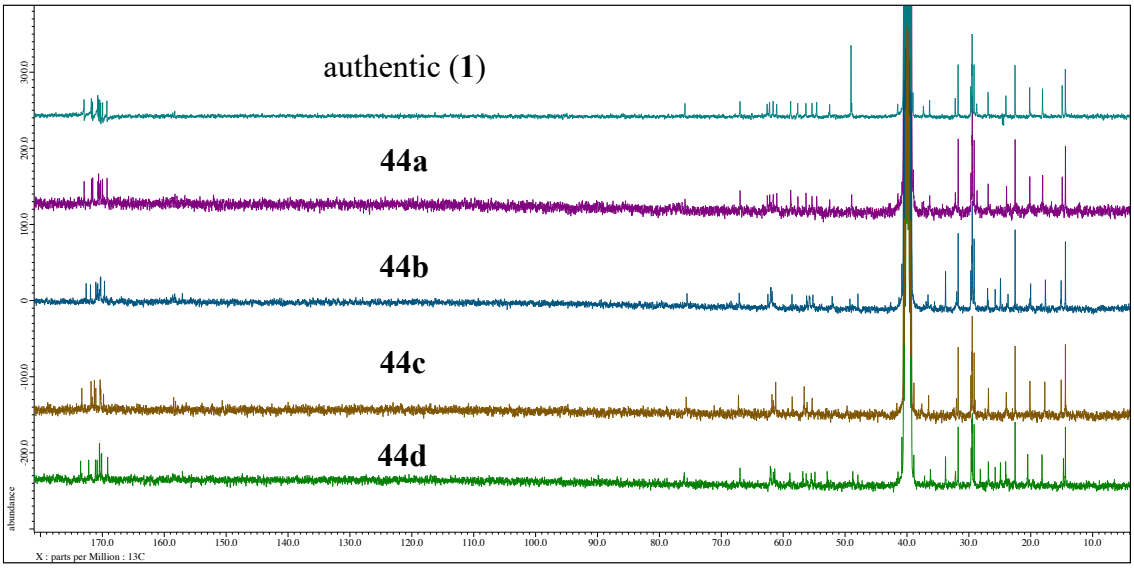
Enlarged view: 8.6-4.5 ppm



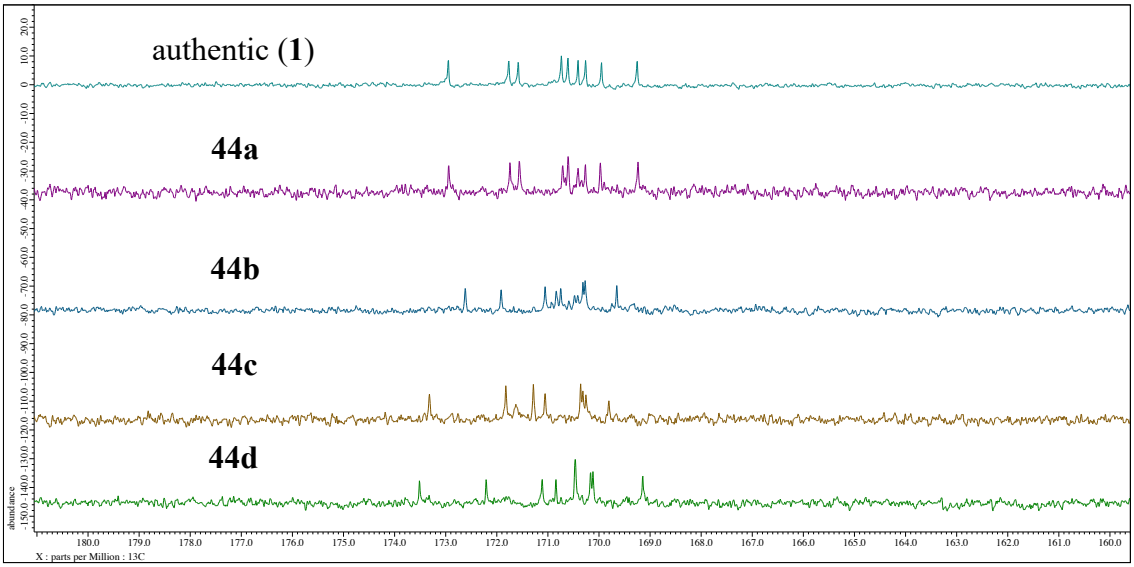
Enlarged view: 4.7-0.5 ppm



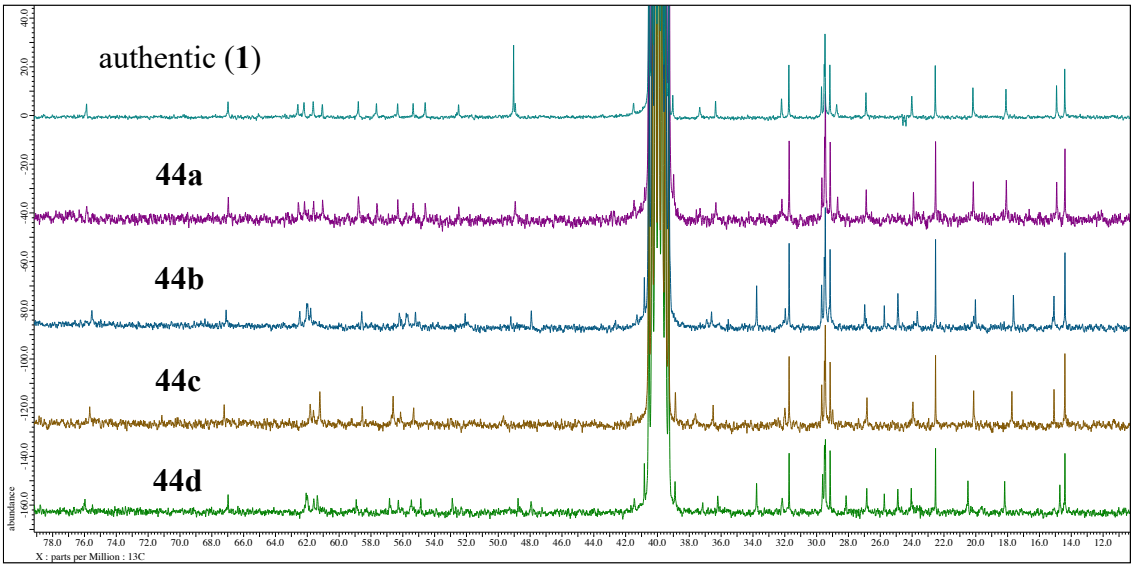
^{13}C NMR authentic (**1**): (100 MHz, $(CD_3)_2SO$), **44a**, **4b**, **44c**, **44d**: (100 MHz, $(CD_3)_2SO$)



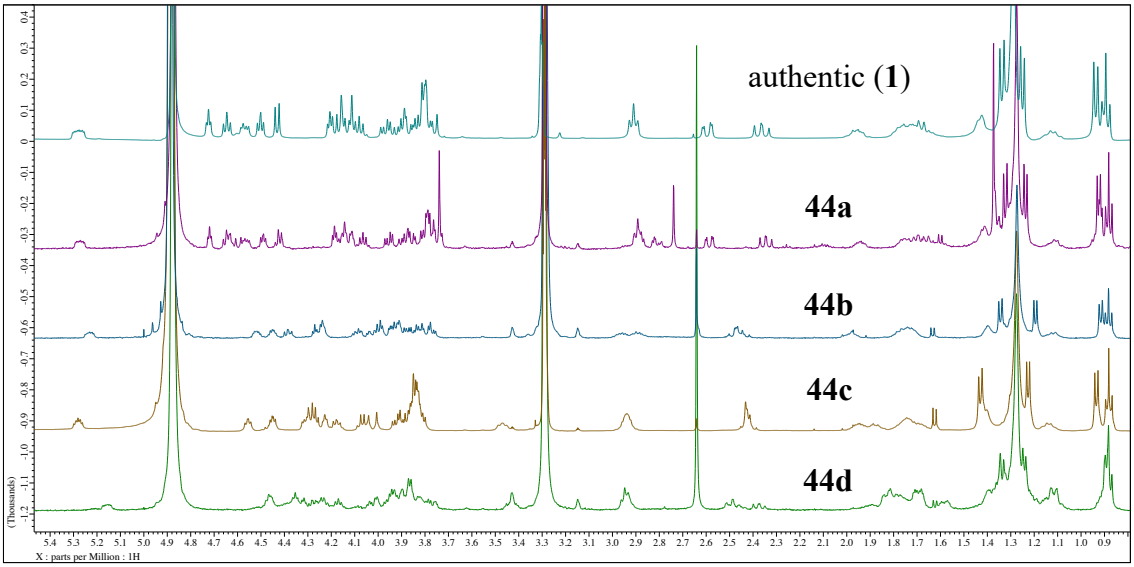
Enlarged view: 180-159 ppm



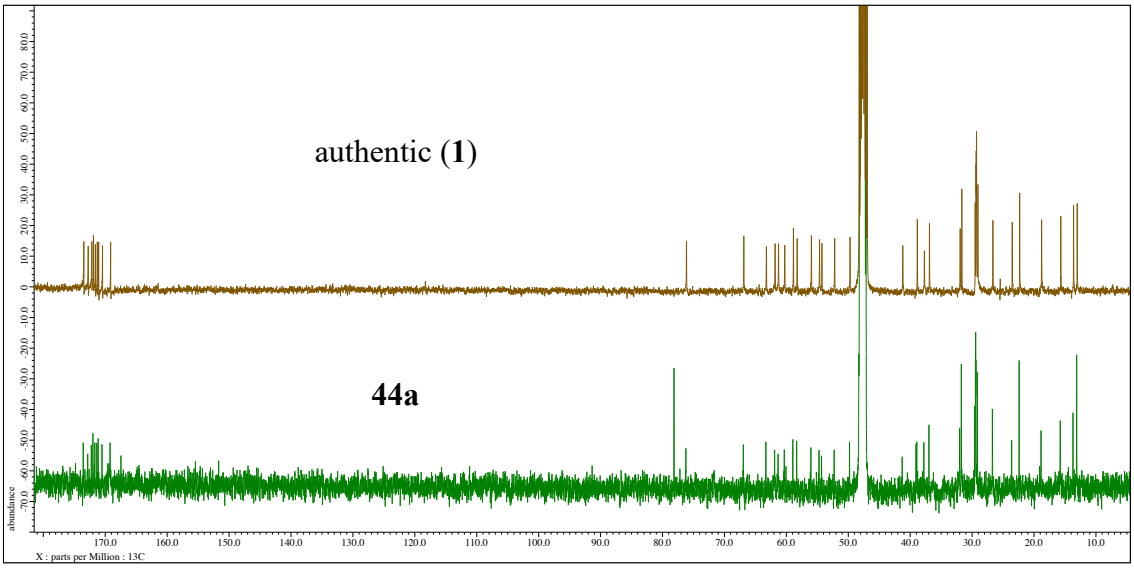
Enlarged view: 78-11 ppm



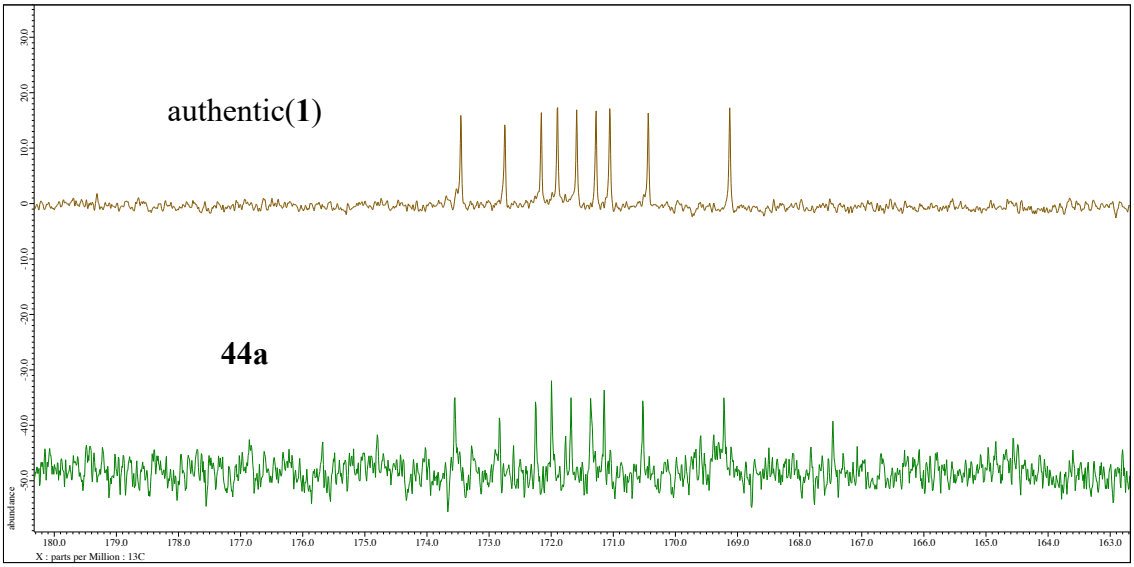
^{1}H NMR authentic (**1**): (400 MHz, CD_3OD)**,** **44a**, **44b**, **44c**, **44d**: (500 MHz, CD_3OD)



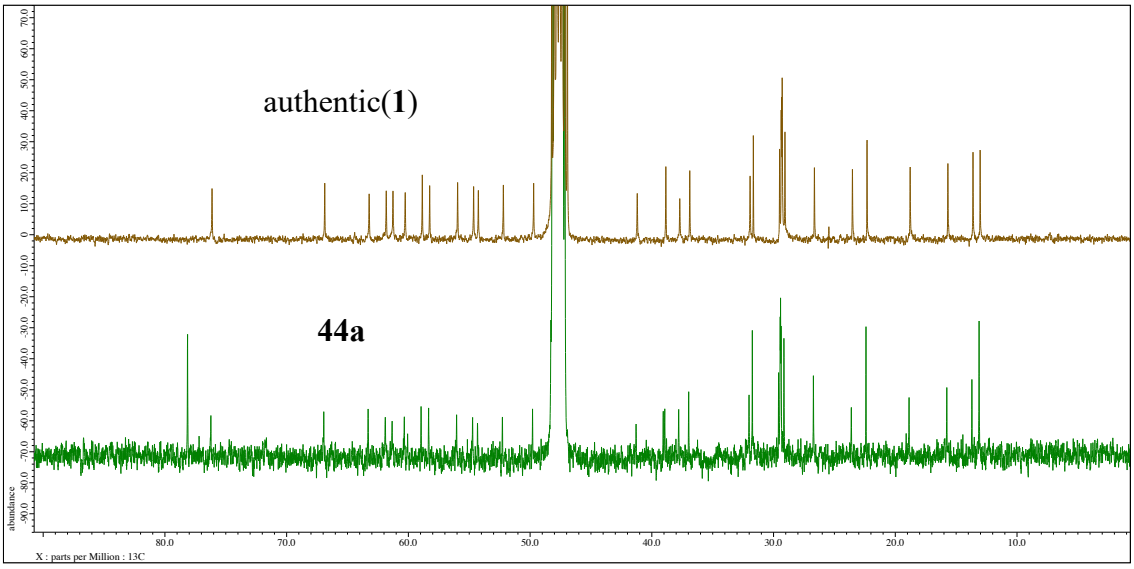
^{13}C NMR authentic (**1**): (400 MHz, CD_3OD), **44a**: 500 MHz, CD_3OD)

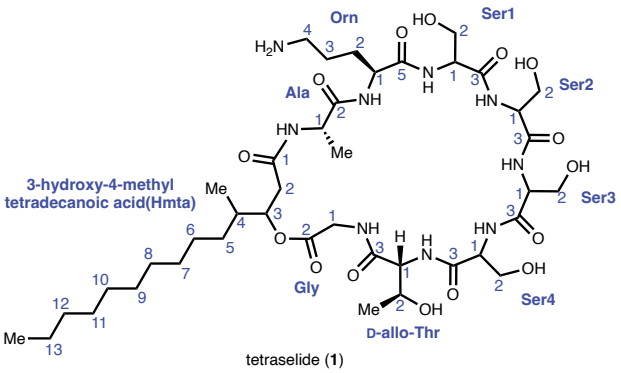


Enlarged view: 180-162 ppm



Enlarged view: 80-0 ppm





tetraselide (**1**)

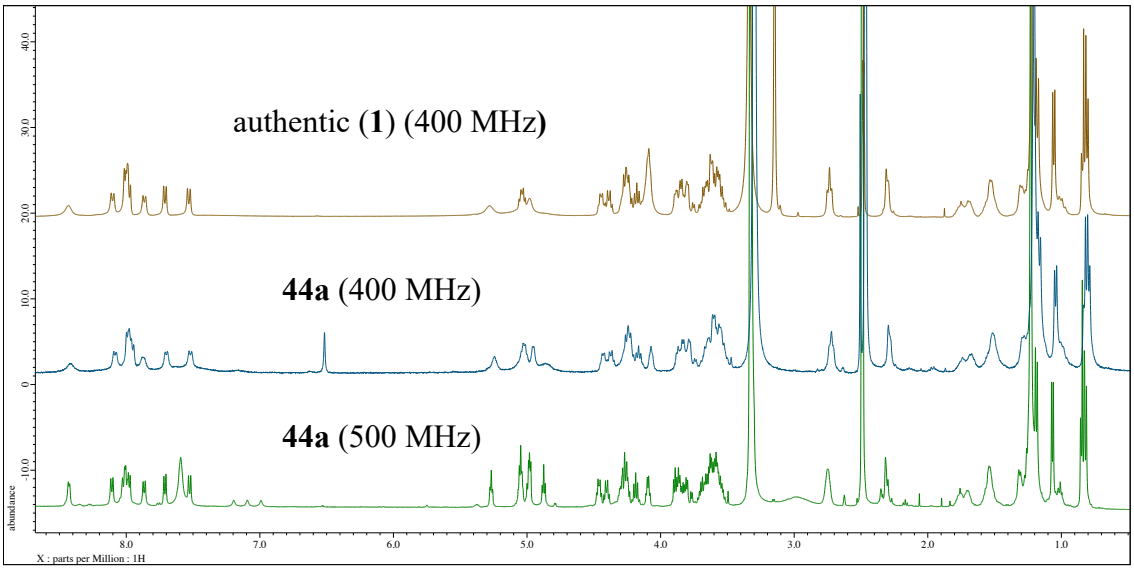
Table S2. ^{1}H and 13C data of **1** measured in $(CD_3)_2SO$

		tetraselide (1): natural product (δ_C: 100 MHz, δ_H:400 MHz)			S49 : synthetic product (δ_C: 100 MHz, δ_H:500 MHz)				
	position	δ_C	δ_H (mult., *J*, int.)		position	δ_C	δ_H (mult., *J*, int.)	$\Delta\delta_C$	$\Delta\delta_H$
Ala	1	48.5	4.19 (m, 1H)	**Ala**	1	48.5	4.19 (m, 1H)	0	0
	2	172.5			2	172.5		0	
	1-CH_3	17.7	1.19 (d, 7.2 Hz, 3H)		1-CH_3	17.7	1.19 (d, 7.3 Hz, 3H)	0	0
	NH		8.02-7.98 (overlap, m, 3H)		NH		8.04-7.97 (overlap, m, 3H)		–0.02-0.01
Orn	1	52.1	4.29-4.25 (overlap, m, 3H)	**Orn**	1	52.1	4.29-4.26 (overlap, m, 3H)	0	0- –0.01
	2	28.3	1.60-1.48 (overlap, br-m, 3H)		2	28.3	1.60-1.49 (overlap, br-m, 3H)	0	0- –0.01
			1.81-1.67 (overlap, br-m, 2H)				1.80-1.67 (overlap, br-m, 2H)		0.1-0
	3	23.6	1.60-1.48 (overlap, br-m, 3H)		3	23.5	1.60-1.49 (overlap, br-m, 3H)	0.1	0- –0.01
	4	38.6	2.74 (t, 6.8 Hz, 2H)		4	38.6	2.75 (app br-s, 2H)	0	–0.01
	5	171.2			5	171.2		0	
	NH		8.11 (d, 7.6 Hz, 1H)		NH		8.11 (d, 8.0 Hz, 1H)		0
	NH_2				NH_2				
Ser-1	1	54.2	4.45 (app-dd, 12.0, 4.4 Hz, 1H)	**Ser-1**	1	54.2	4.46 (br-dt, 9.5, 4.5 Hz, 1H)	0	–0.01
	2	62.2	3.72-3.52 (overlap, m, 7H)		2	62.2	3.72-3.52 (overlap, m, 8H)	0	0
	3	171.3			3	171.3		0	
	NH		7.87 (d, 7.6 Hz, 1H)		NH		7.87 (d, 8.5, 1H)		0
	2-OH				2-OH				
Ser-2	1	57.2	4.10 (overlap, app br-s, 4H)	**Ser-2**	1	57.2	4.09 (m, 1H)	0	0.01
	2	60.6	3.72-3.52 (overlap, m, 7H)		2	60.6	3.72-3.52 (overlap, m, 8H)	0	0
	3	170.0			3	170.0		0	
	NH		8.44 (app br-s, 1H)		NH		8.43 (d, 5.5 Hz, 1H)		0.01
	2-OH				2-OH				
Ser-3	1	55.9	4.29-4.25 (overlap, m, 3H)	**Ser-3**	1	55.9	4.29-4.26 (overlap, m, 3H)	0	0- –0.01
	2	61.2	3.72-3.52 (overlap, m, 7H)		2	61.2	3.72-3.52 (overlap, m, 8H)	0	0
	3	169.8			3	169.9		–0.1	
	NH		7.72 (d, 7.2, 1H)		NH		7.71 (d, 7.5, 1H)		0.01
	2-OH		–		2-OH				
Ser-4	1	54.9	4.40 (m, 1H)	**Ser-4**	1	55.0	4.40 (m, 1H)	–0.1	0
	2	61.8	3.72-3.52 (overlap, m, 7H)		2	61.8	3.72-3.52 (overlap, m, 8H)	0	0
	3	170.3	–		3	170.3	–	0	
	NH		7.54 (d, 8.0, 1H)		NH		7.53 (d, 1H, 8.0 HZ)		0.01
	OH				OH				
D-allo	1	58.4	4.29-4.25 (overlap, m, 3H)	**D-allo**	1	58.4	4.29-4.26 (overlap, m, 3H)	0	0- –0.01
-Thr	2	66.6	3.90-3.81 (overlap, m, 4H)	**-Thr**	2	66.6	3.92-3.80 (overlap, m, 4H)	0	–0.02- 0.01
	3	170.2			3	170.2		0	
	2-CH_3	19.8	1.07 (d, 6.4 Hz, 3H)		2-CH_3	19.8	1.07 (d, 6.0 Hz, 3H)	0	0
	2-OH				2-OH				
	NH		8.02-7.98 (overlap, m, 3H)		NH		8.04-7.97 (overlap, m, 3H)		–0.02- 0.01
Gly	1	41.1	3.90-3.81 (overlap, m, 4H)	**Gly**	1	41.1	3.92-3.80 (overlap, m, 4H)	0	–0.02- 0.01
	2	168.8			2	168.8		0	
	NH		8.02-7.98 (overlap, m, 3H)		NH		8.04-7.97 (overlap, m, 3H)		–0.02- 0.01
Hmta	1	169.5		**Hmta**	1	169.6		–0.1	
	2	36.9	2.31 (br-d, 5.2 Hz, 2H)		2	36.9	2.32-2.30 (m, 2H)	0	0
	3	75.4	5.05 (m, 1H)		3	75.5	5.08-5.04 (overlap, m, 2H)	–0.1	–0.01- 0.03
	4	35.9	1.81-1.67 (overlap, br-m, 2H)		4	35.9	1.80-1.67 (overlap, br-m, 2H)	0	0.01- 0
	4-CH_3	14.5	0.83 (overlapp, app dd, 13.6, 6.8 Hz, 6H)		4-CH_3	14.5	0.83 (overlap, app, dd, 14.5 7.0 Hz, 6H)	0	0
	5	31.8	1.31 (br-m, 1H)		5	31.8	1.31 (br-m, 1H)		0
			1.00 (br-m, 1H)				1.01 (br-m, 1H)		–0.01
	6–11	29.3, 29.1, 29.1, 29.1, 28.8, 26.5	1.26–1.20 (overlap, m, 16H)		6–11	29.3, 29.1, 29.1, 29.1, 28.7, 26.5	1.27–1.20 (overlap, m, 16H)	0, 0, 0, 0, 0.1, 0	–0.01-0
	12	31.3	1.26–1.20 (overlap, m, 16H)		12	31.3	1.27–1.20 (overlap, m, 16H)	0	–0.01-0
	13	22.1	1.26–1.20 (overlap, m, 16H)		13	22.1	1.27–1.20 (overlap, m, 16H)	0	–0.01-0
	13-CH_3	14.0	0.86-0.81 (m, 6H)		13-CH_3	14.0	0.86-0.81 (m, 6H)	0	0

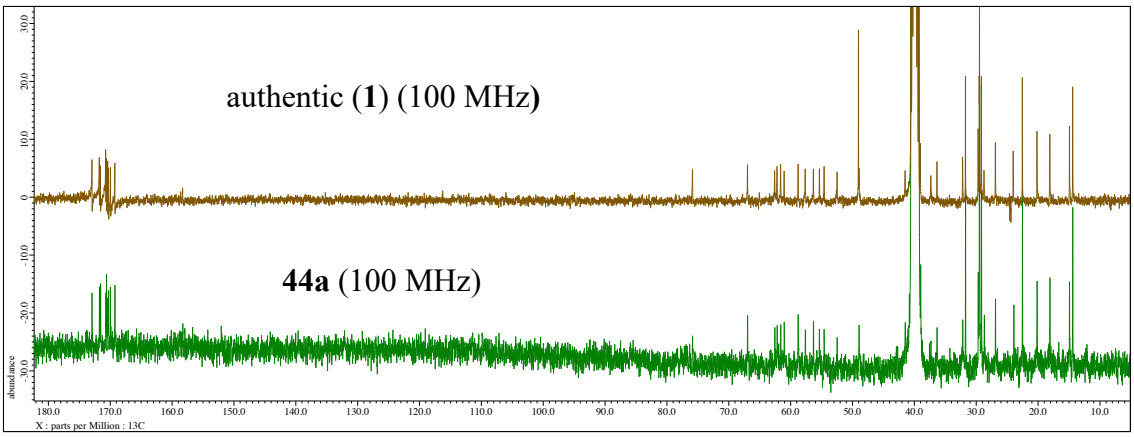
authentic (**1**): $[\alpha]_D^{26}$ = +16.16 (c = 0.05, pyridine)

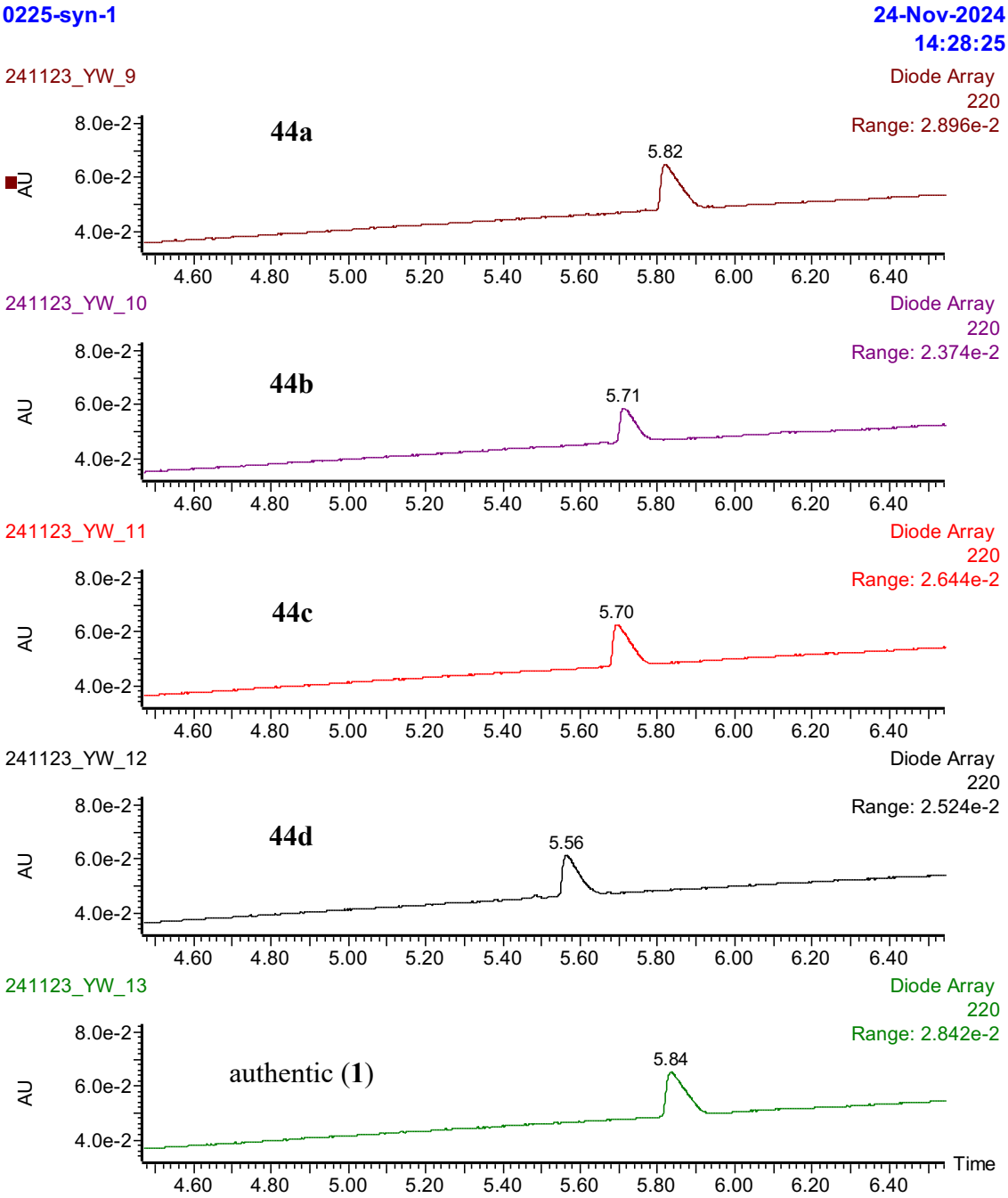
44a : $[\alpha]_D^{26}$ = +16.08 (c = 0.05, pyridine)

^{1}H NMR authentic (**1**) and **44a**: $(CD_3)_2SO$)



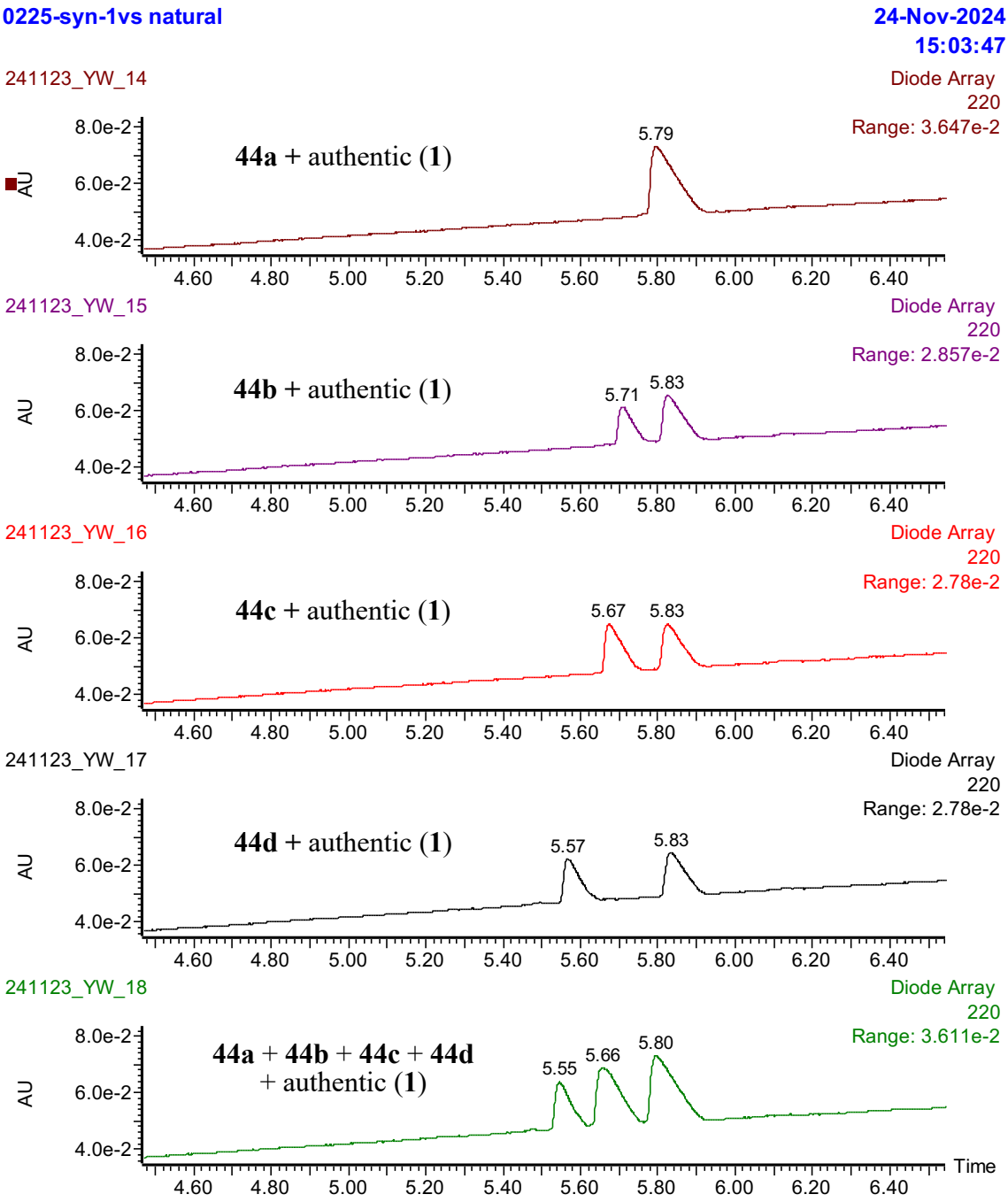
^{13}C NMR authentic (**1**) and **44a**: $(CD_3)_2SO$)





LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA

Figure S20. LC-MS analysis of **1**, **44a**, **44b**, **44c** and **44d**



LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA

Figure S21. LC-MS analysis (co-injection) of **1**, **44a**, **44b**, **44c** and **44d**

6-3. Antifungal Activity of the Natural and Synthetic Tetraselides using a Paper Disc Method

Natural **1**, synthesized **44a** and three constitutional isomers of the Ser moiety (**44b**: L-L-D-L, **44c**: L-D-L-L, and **44d**: D-L-L-L) were tested for antifungal activity using a paper disc method[22]. *Aspergillus fumigatus* IFM 61493, was provided by Medical Mycology Research Center, Chiba University through the National Bio-Resource Project, Japan. Only natural **1** and synthetic **44a** showed antifungal activity with the inhibition zone of 8 mm at 1 μg/6 mm disc. The other three stereoisomers showed no antifungal activity at 5 μg/8 mm disc. This result indicates that the configuration of the Ser moiety is important for antifungal activity (**Figure S22**).

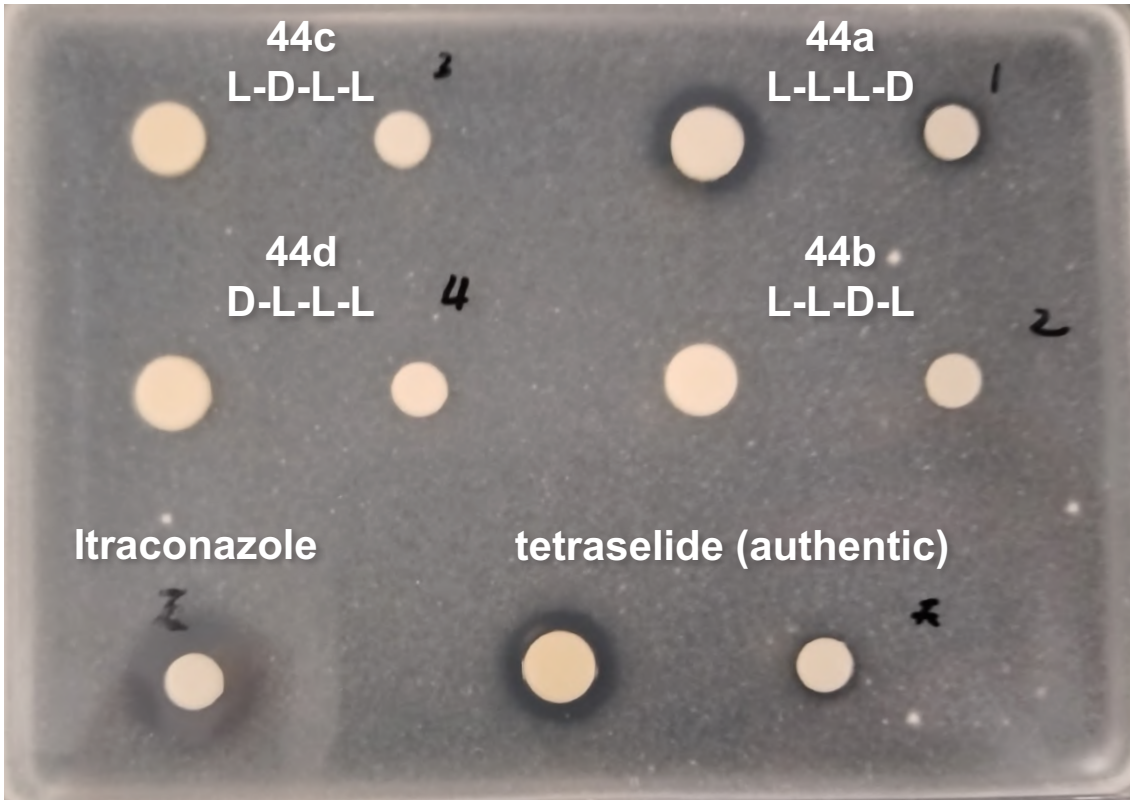


Figure S22. Antifungal activity against *Aspergillus fumigatus* IFM 61493

Test compounds were placed on an agar plate at 5 μg/8mm disc (left) and 1 μg/6mm disc (right). Itraconazole was placed on an agar plate at 2 μg/6mm disc.

7. References

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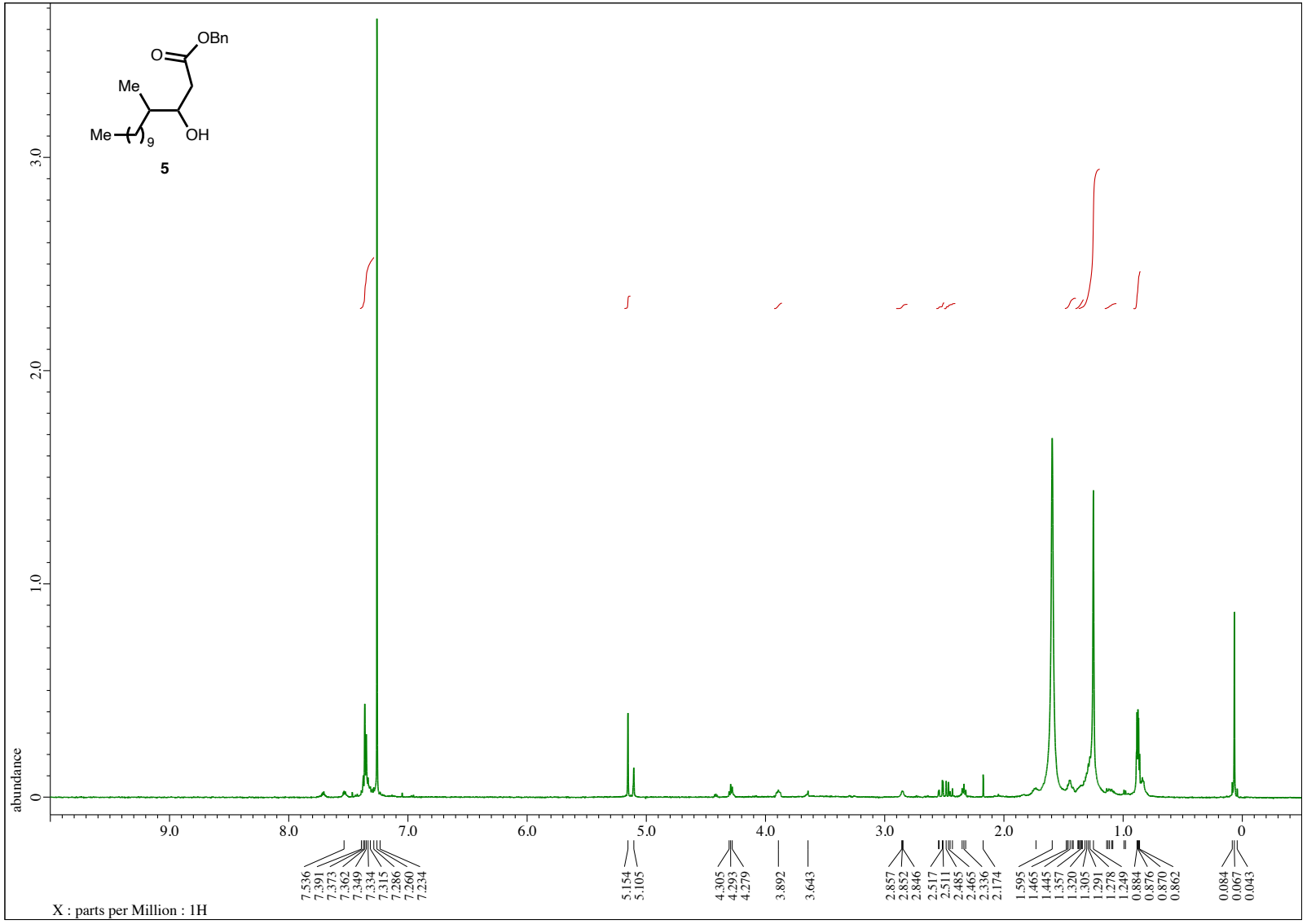
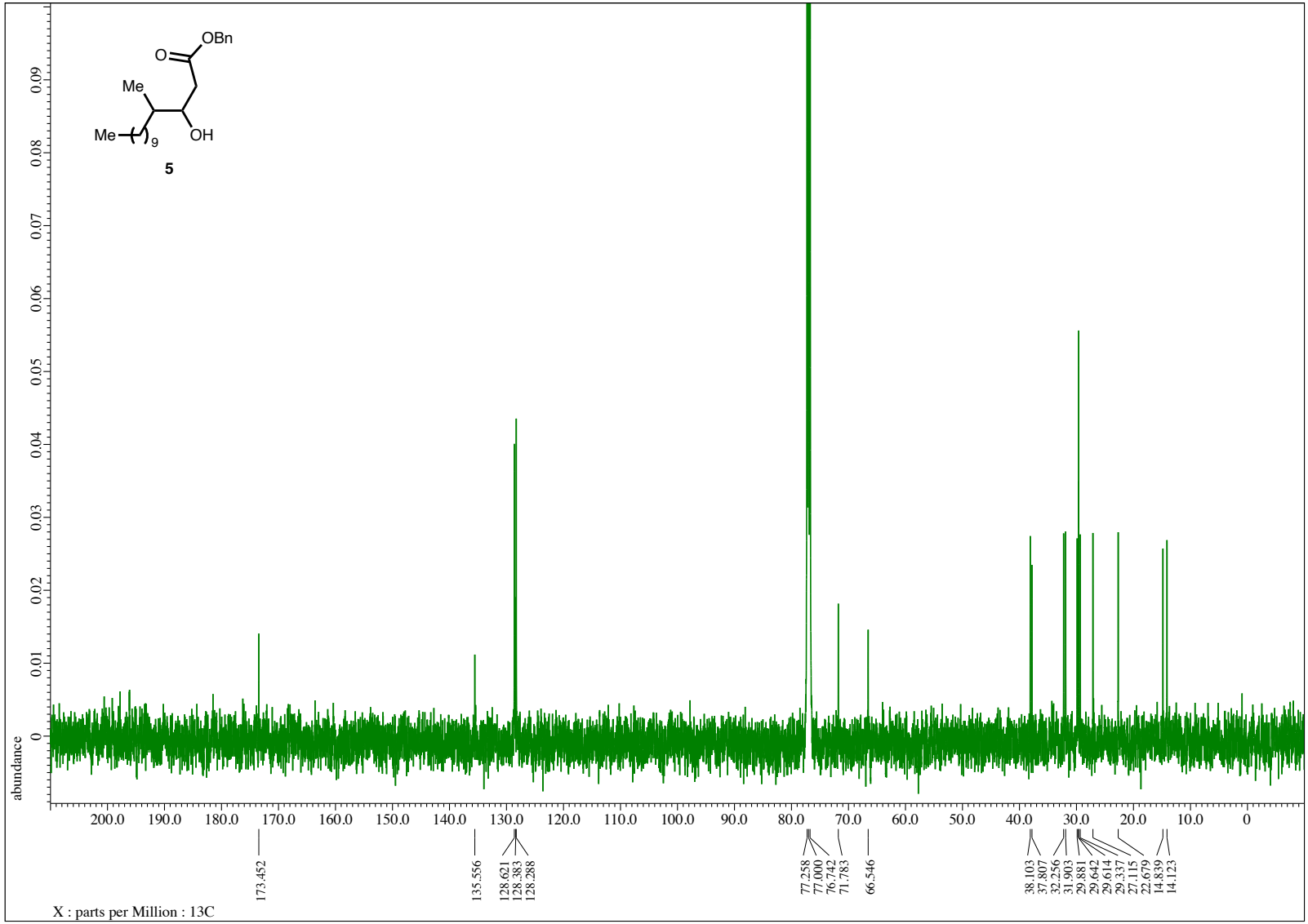
8. NMR NMR Spectra Chart




OBn
O
Me
Me
9
OH
5
abundance
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0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
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135.556
128.621
128.383
128.288
77.258
77.000
76.742
71.783
66.546
38.103
37.807
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29.337
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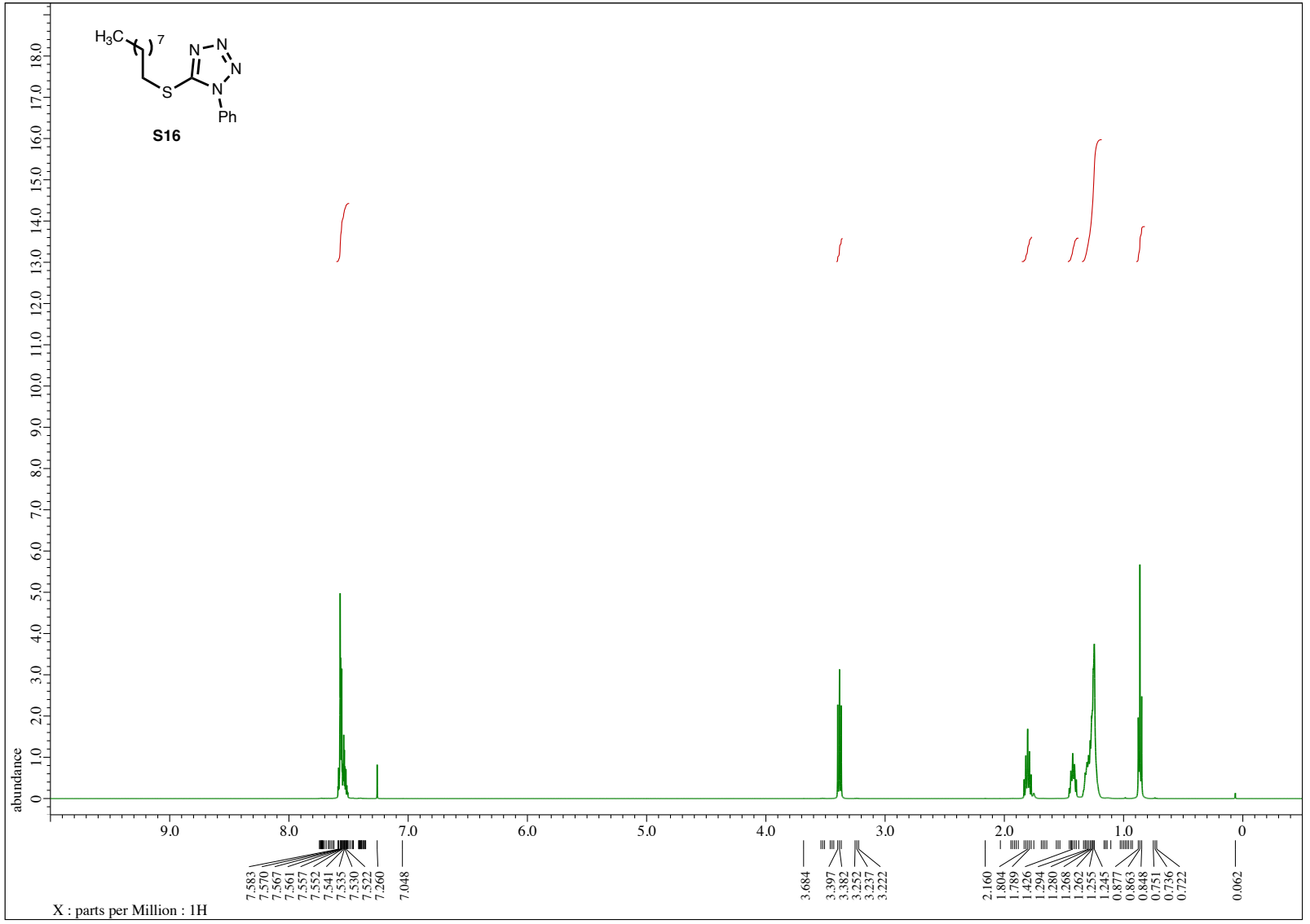
14.123
X : parts per Million : 13C


S16
abundance
X : parts per Million : 1H
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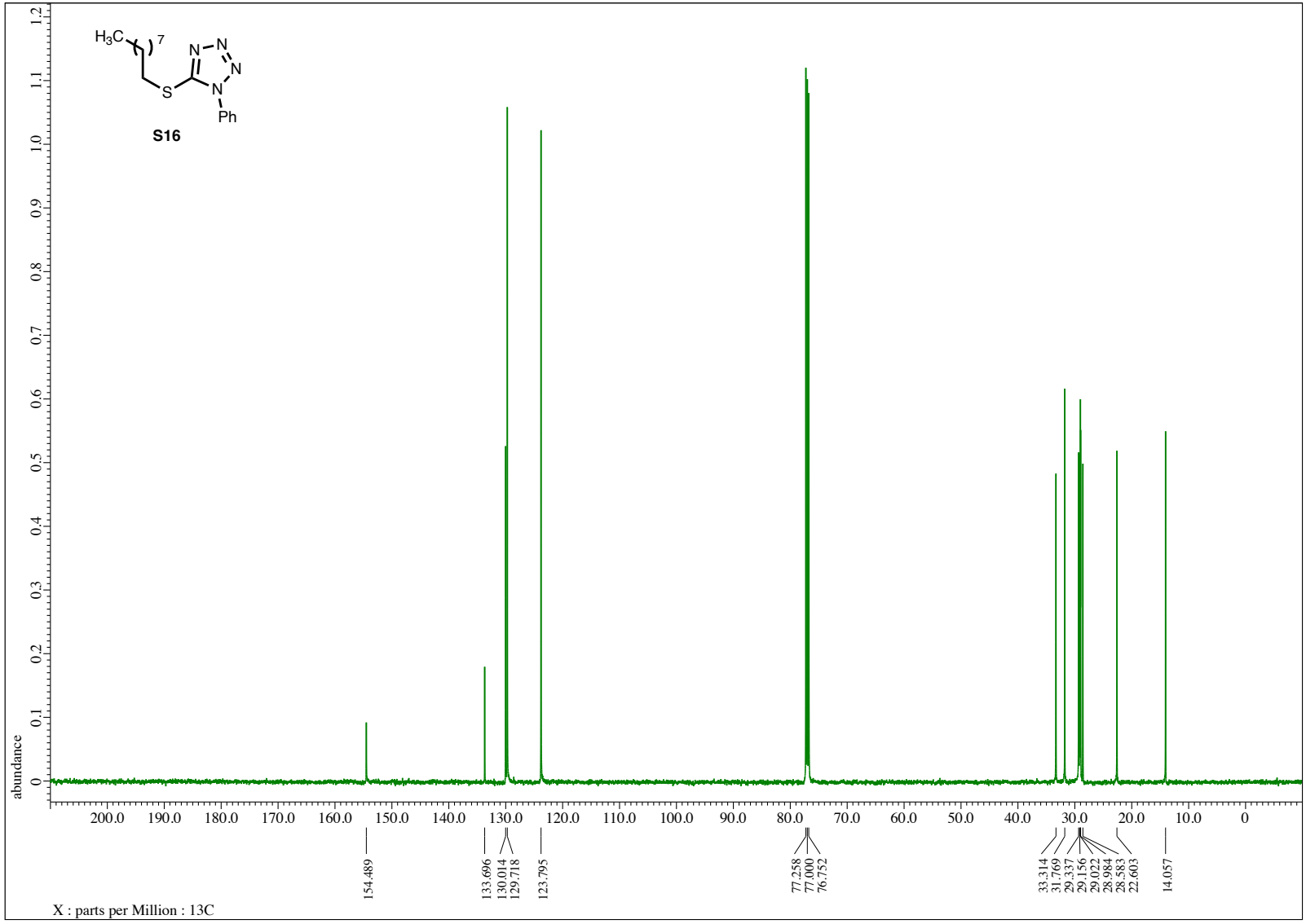
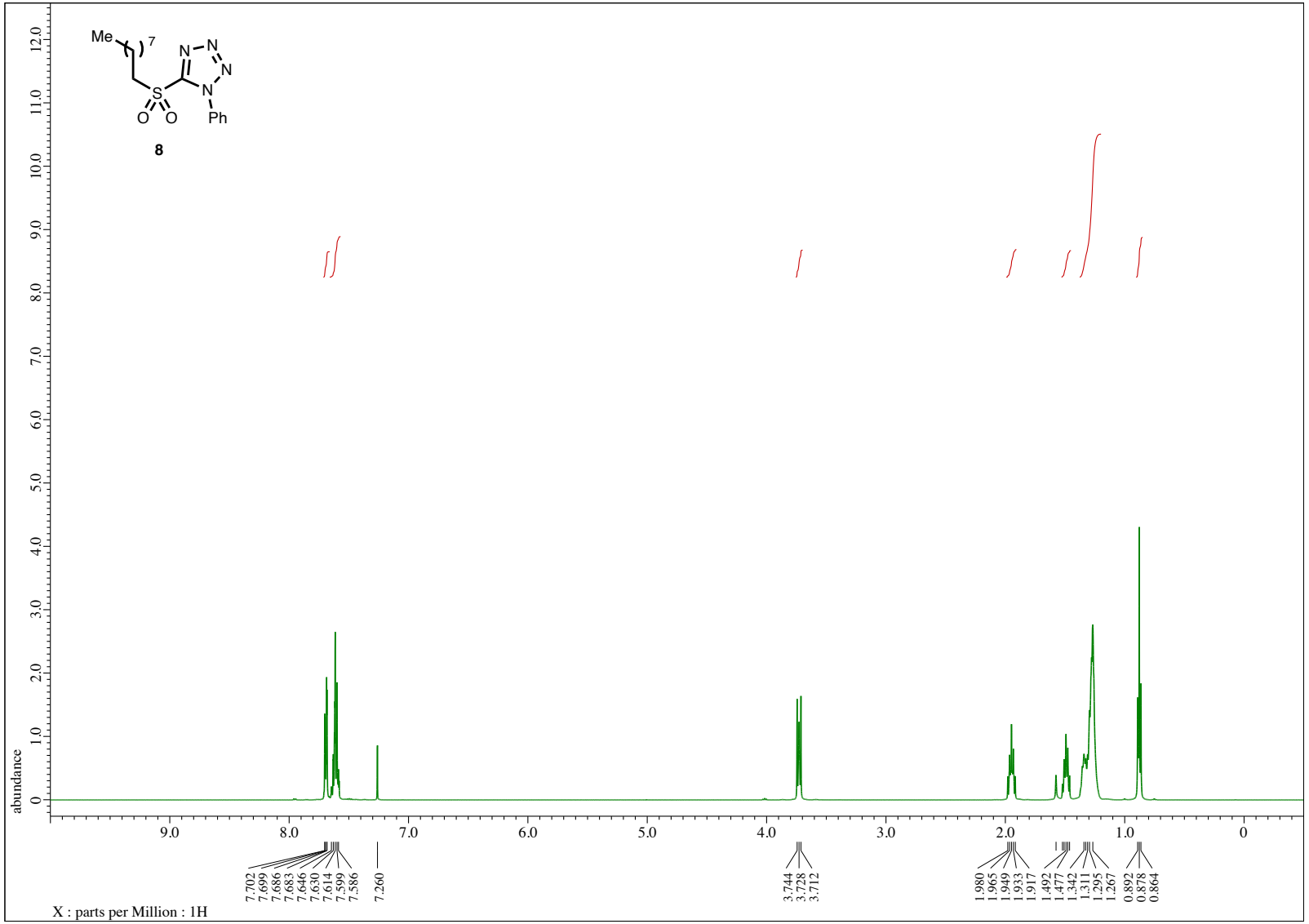
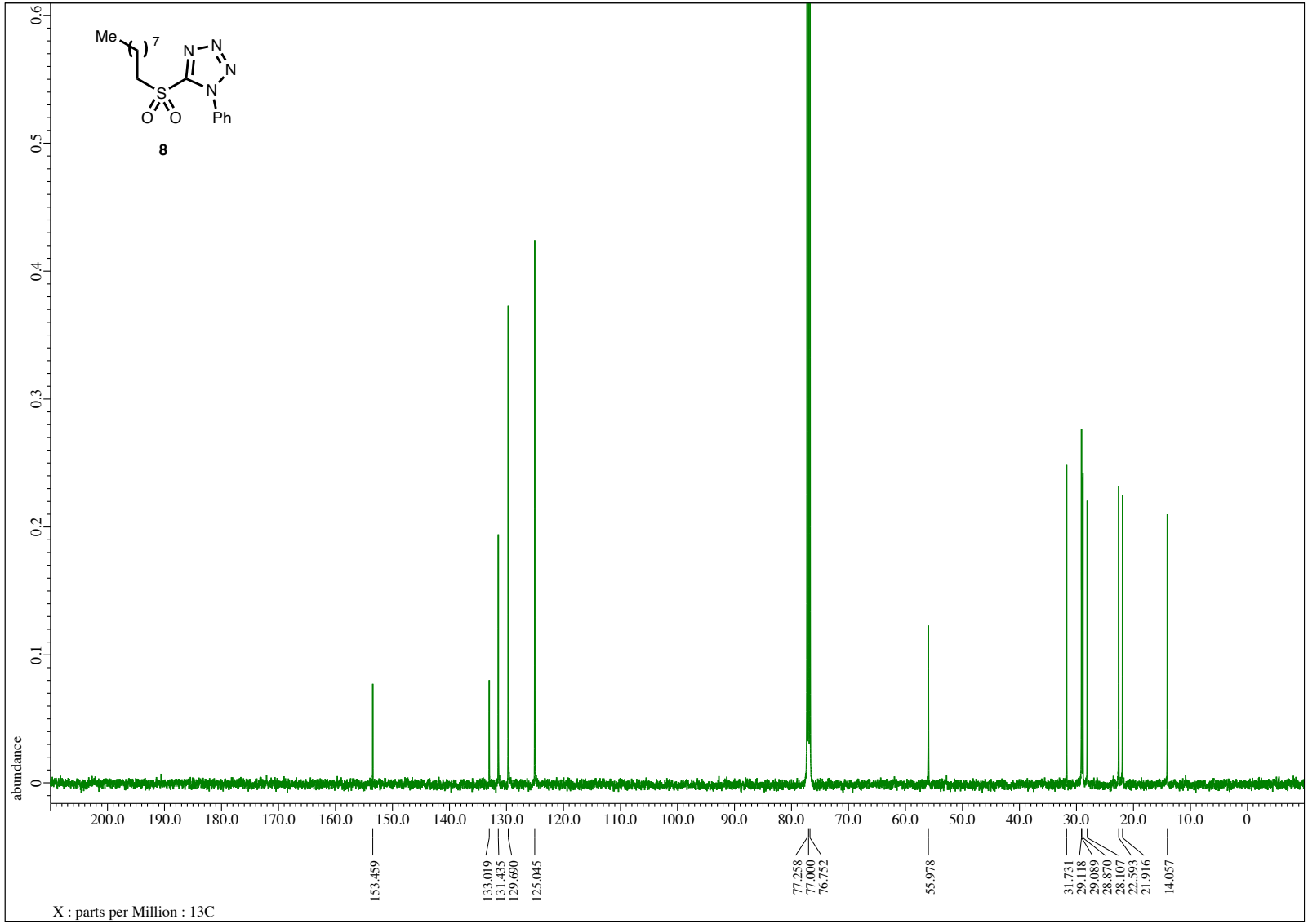
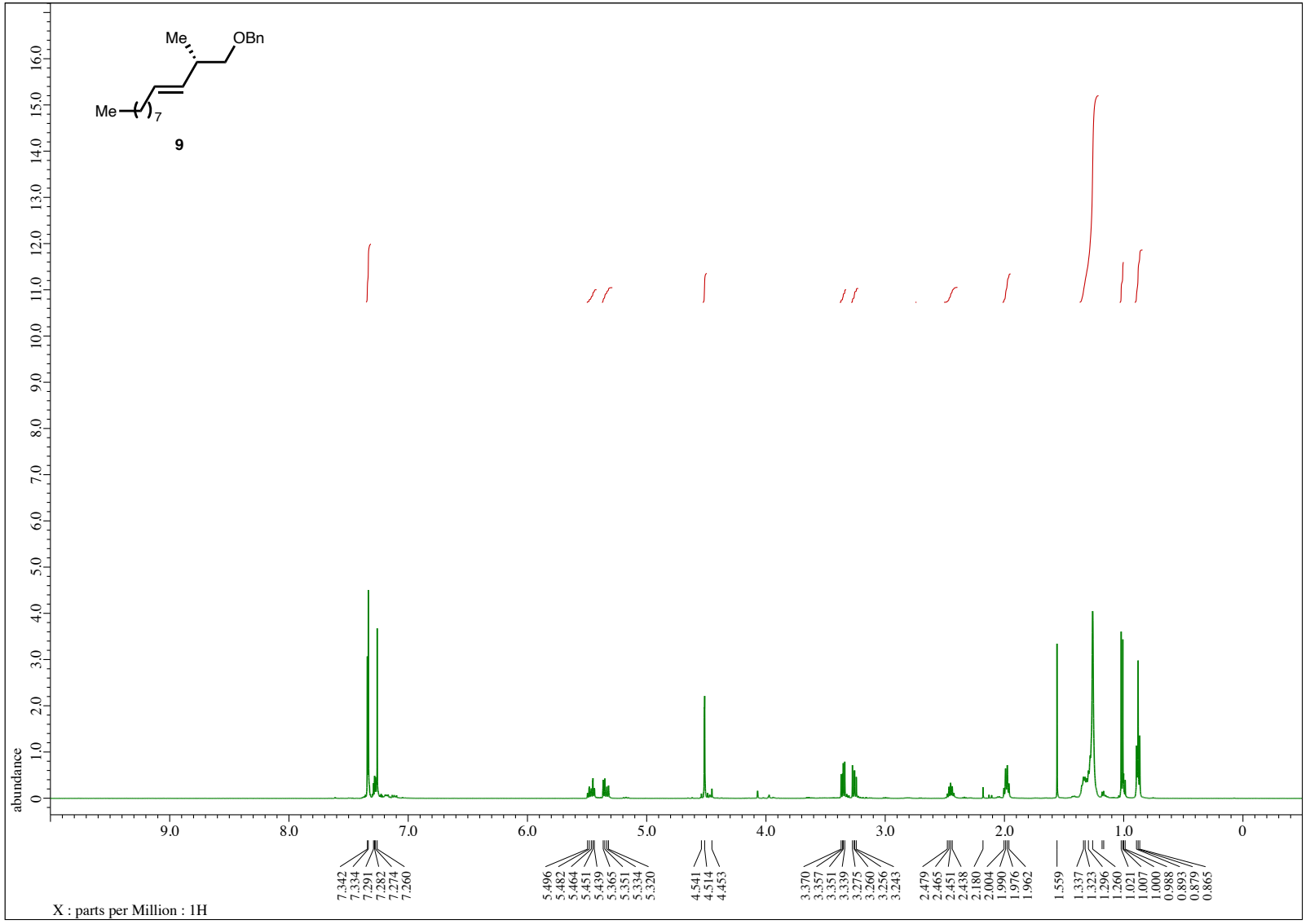
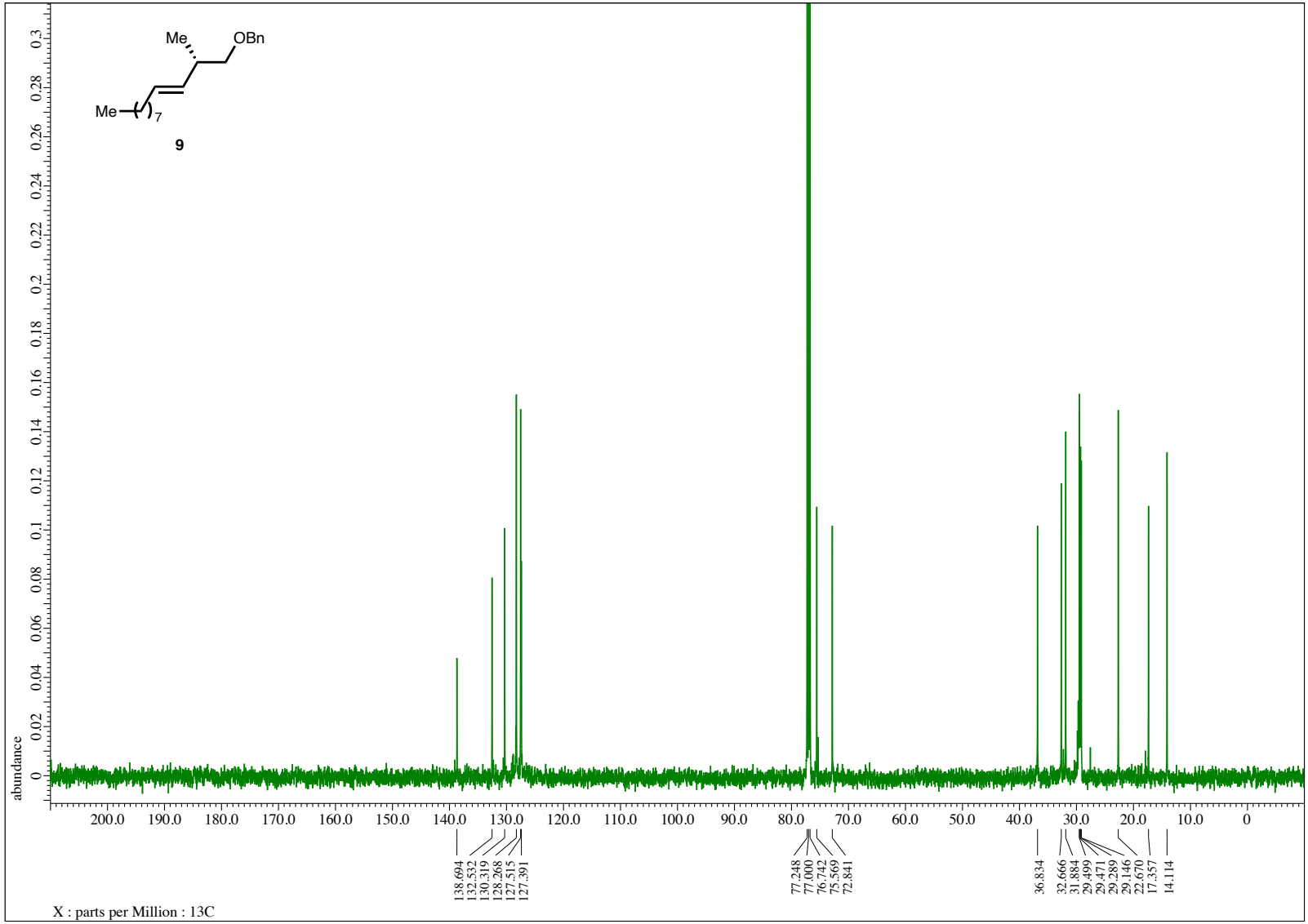
7.570
7.567
7.561
7.557
7.552
7.541
7.535
7.530
7.522
7.260
7.048
3.684
3.397
3.382
3.252
3.237
3.222
2.160
1.804
1.789
1.426
1.294
1.280
1.268
1.262
1.255
1.245
0.877
0.863
0.848
0.751
0.736
0.722
0.062

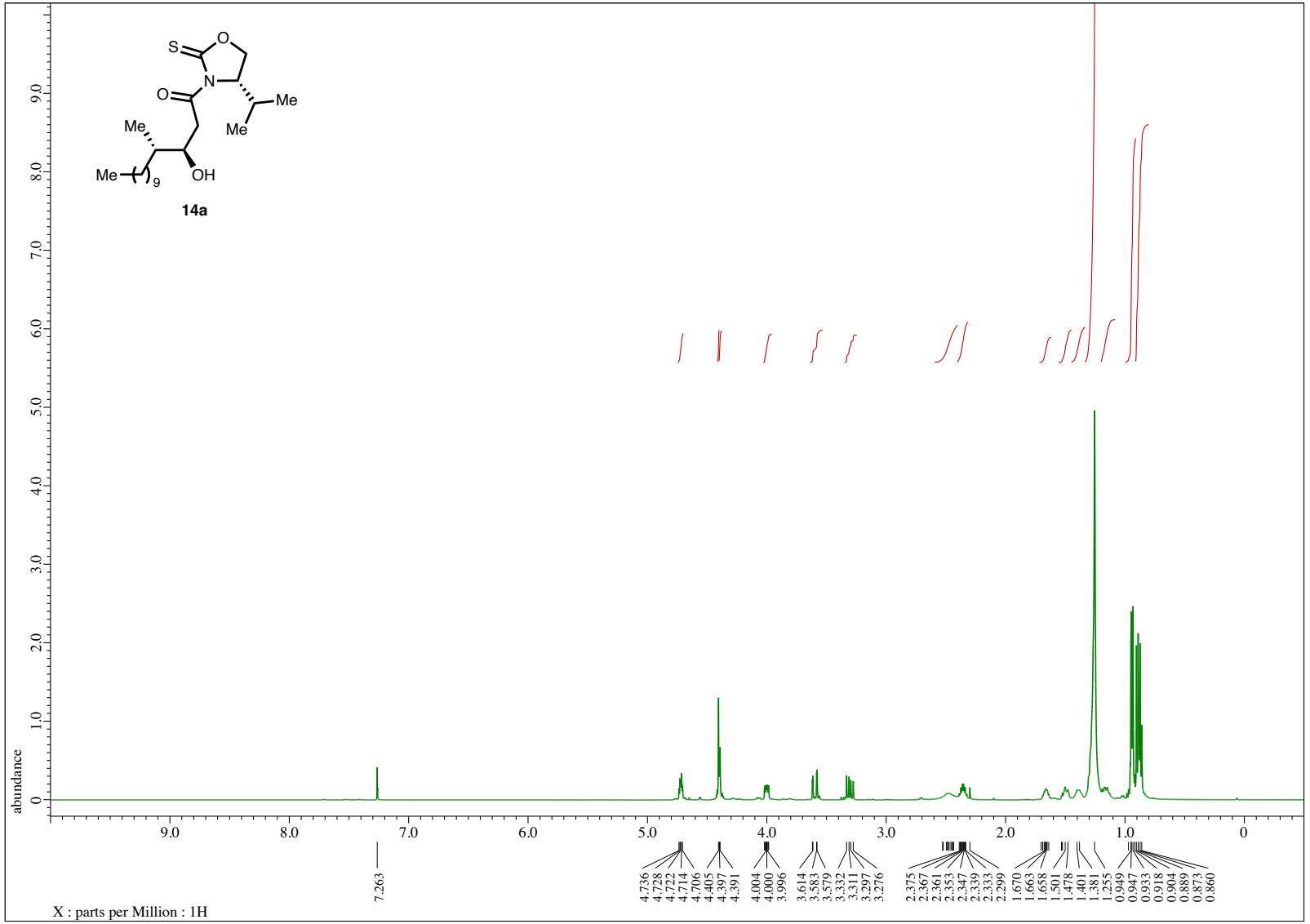

S16
abundance
X : parts per Million : 13C
154.489
133.696
130.014
129.718
123.795
77.258
77.000
76.752
33.314
31.769
29.337
29.156
29.022
28.984
28.583
22.603
14.057

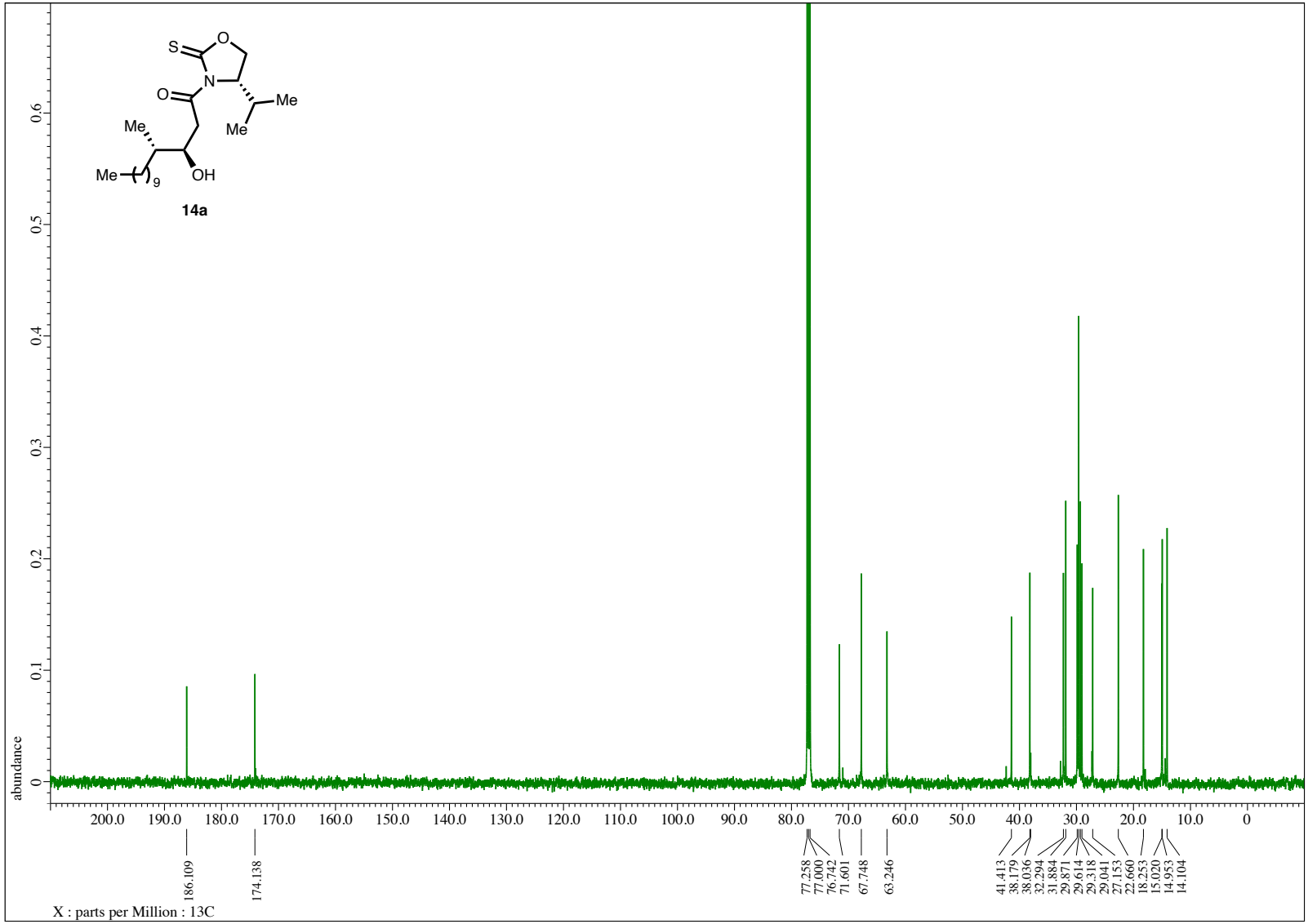

Me
7
N
N
N
S
O
O
N
Ph
8
abundance
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.702
7.699
7.686
7.683
7.646
7.630
7.614
7.599
7.586
7.260
3.744
3.728
3.712
1.980
1.965
1.949
1.933
1.917
1.492
1.477
1.342
1.311
1.295
1.267
0.892
0.878
0.864
X : parts per Million : 1H

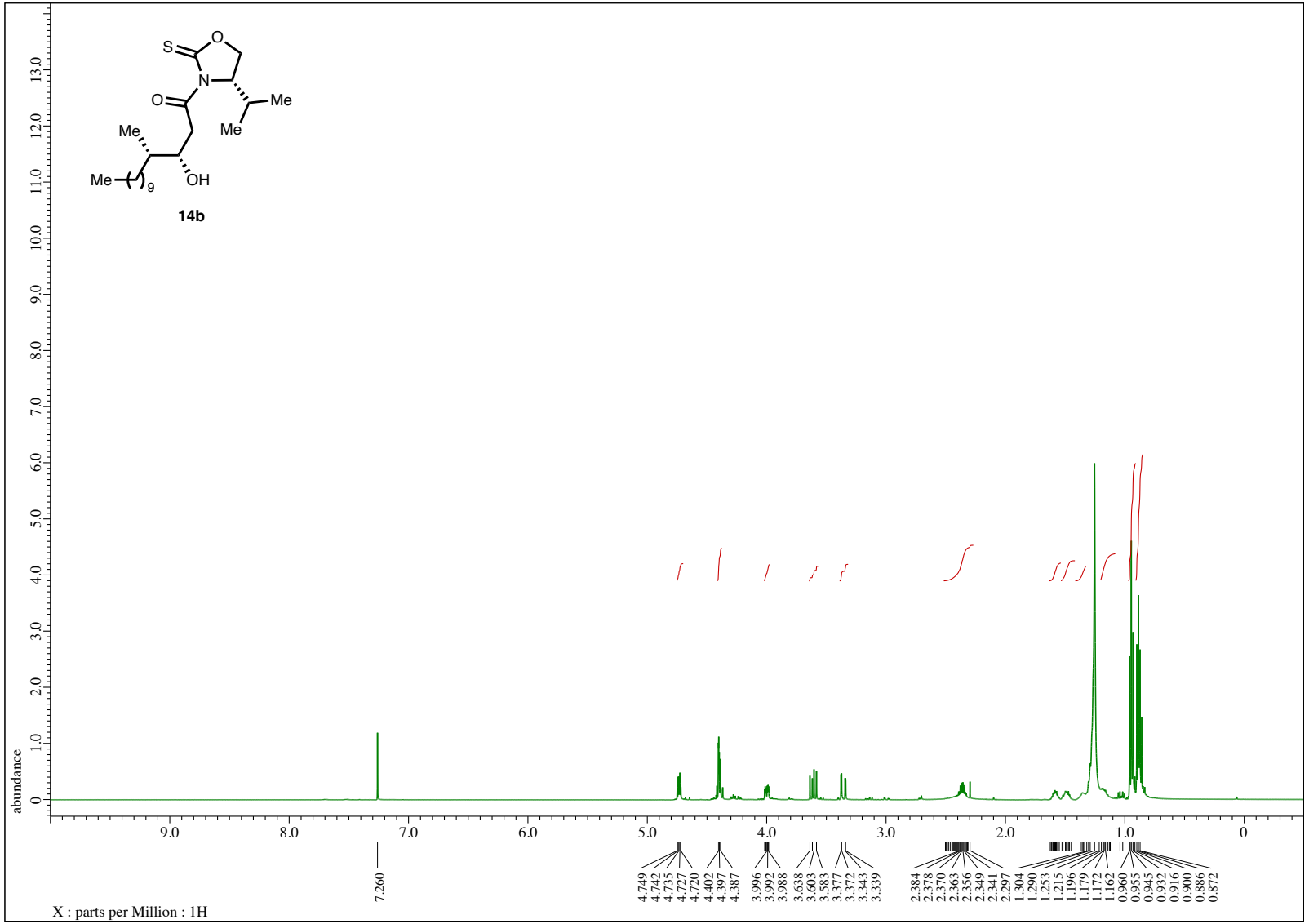

Me
7
N
N
N
S
O
O
N
Ph
8
abundance
0.6
0.5
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
153.459
133.019
131.435
129.690
125.045
77.258
77.000
76.752
55.978
31.731
29.118
29.089
28.870
28.107
22.593
21.916
14.057
X : parts per Million : 13C

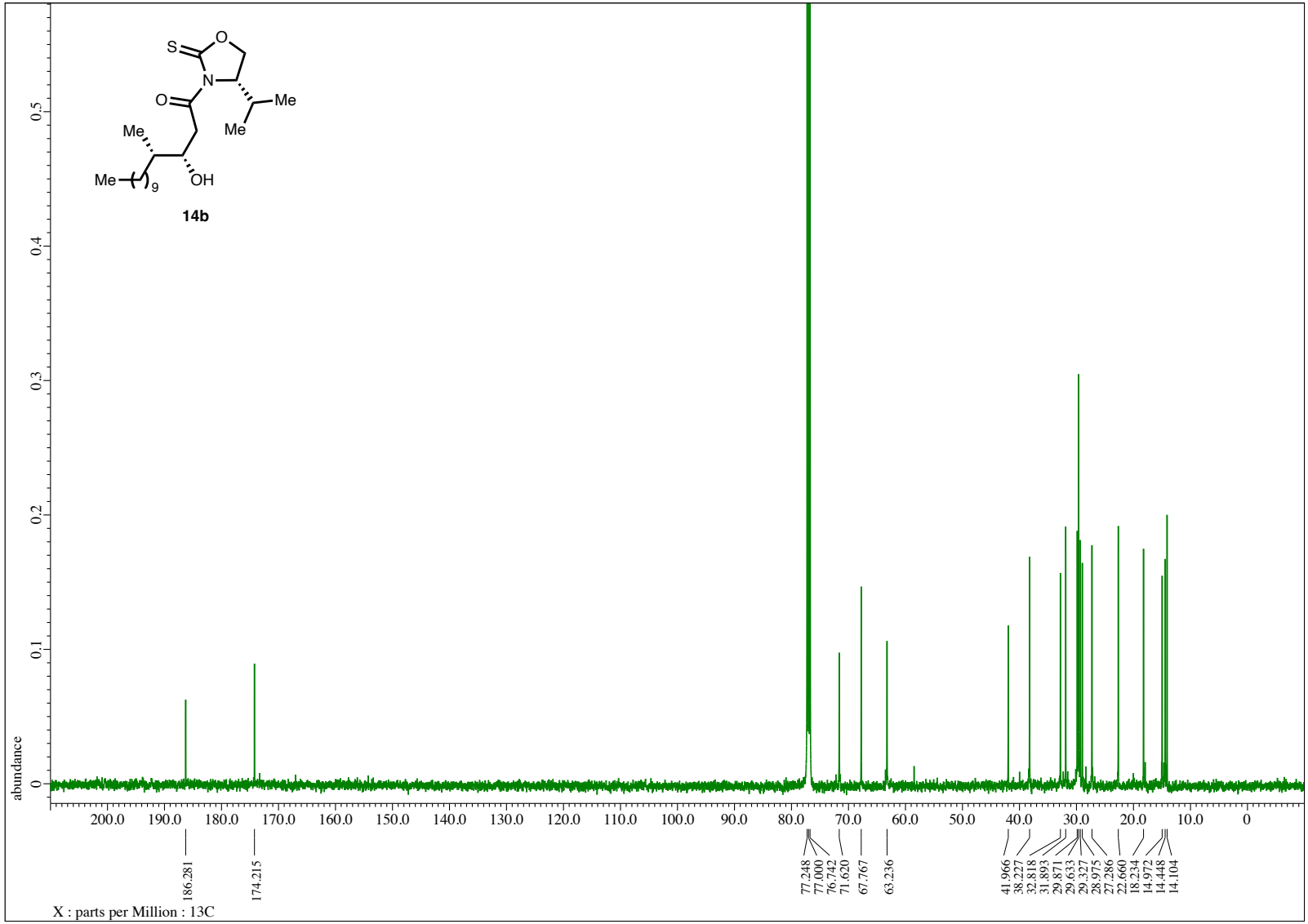

Me
OBn
Me
7
9
abundance
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.342
7.334
7.291
7.282
7.274
7.260
5.496
5.482
5.464
5.451
5.439
5.365
5.351
5.334
5.320
4.541
4.514
4.453
3.370
3.357
3.351
3.339
3.275
3.260
3.256
3.243
2.479
2.465
2.451
2.438
2.180
2.004
1.990
1.976
1.962
1.559
1.337
1.323
1.296
1.260
1.021
1.007
1.000
0.988
0.893
0.879
0.865
X : parts per Million : 1H

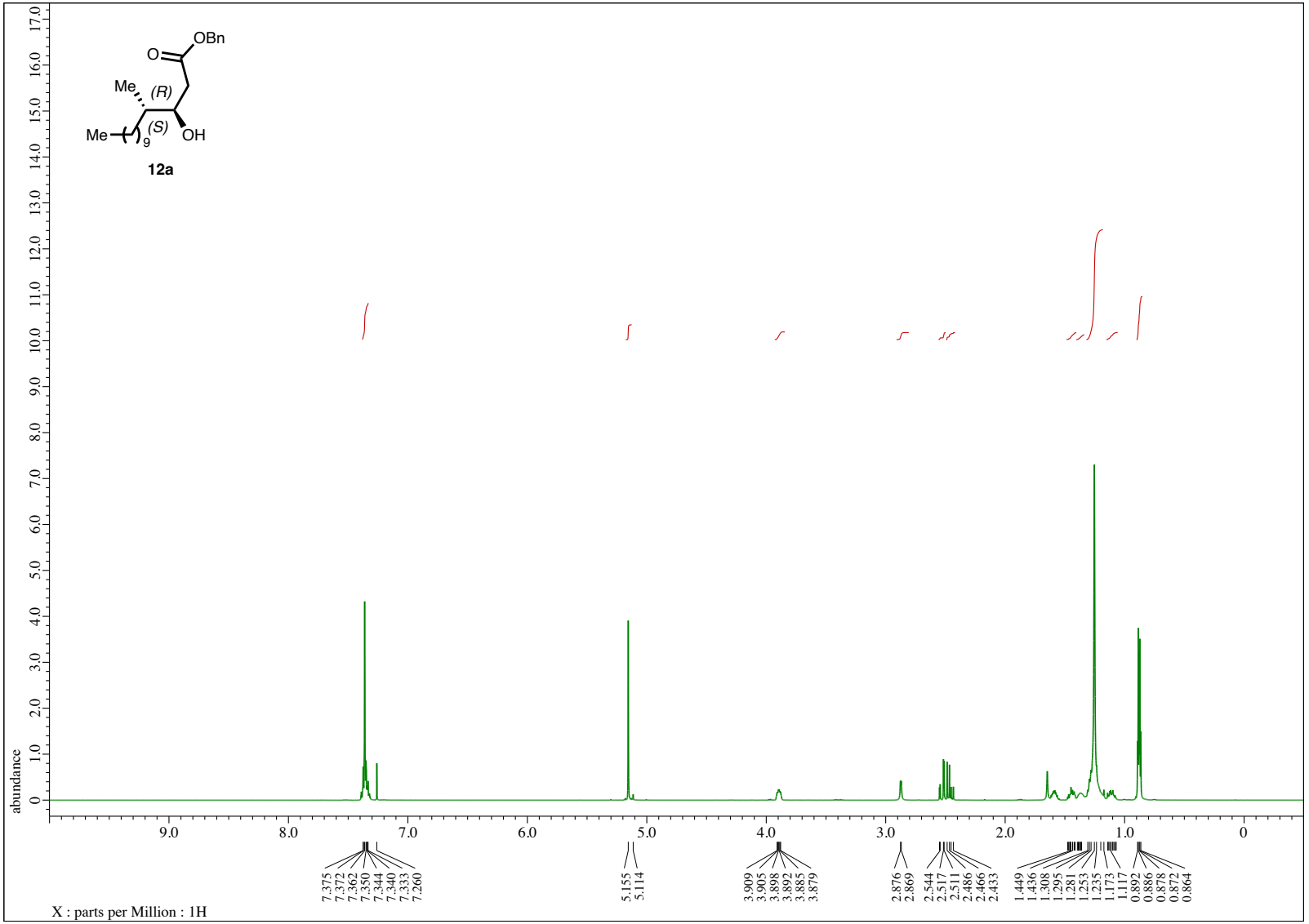

Me
OBn
Me
7
9
abundance
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
0.22
0.24
0.26
0.28
0.3
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
138.694
132.532
130.319
128.268
127.515
127.391
77.248
77.000
76.742
75.569
72.841
36.834
32.666
31.884
29.499
29.471
29.289
29.146
22.670
17.357
14.114
X : parts per Million : 13C

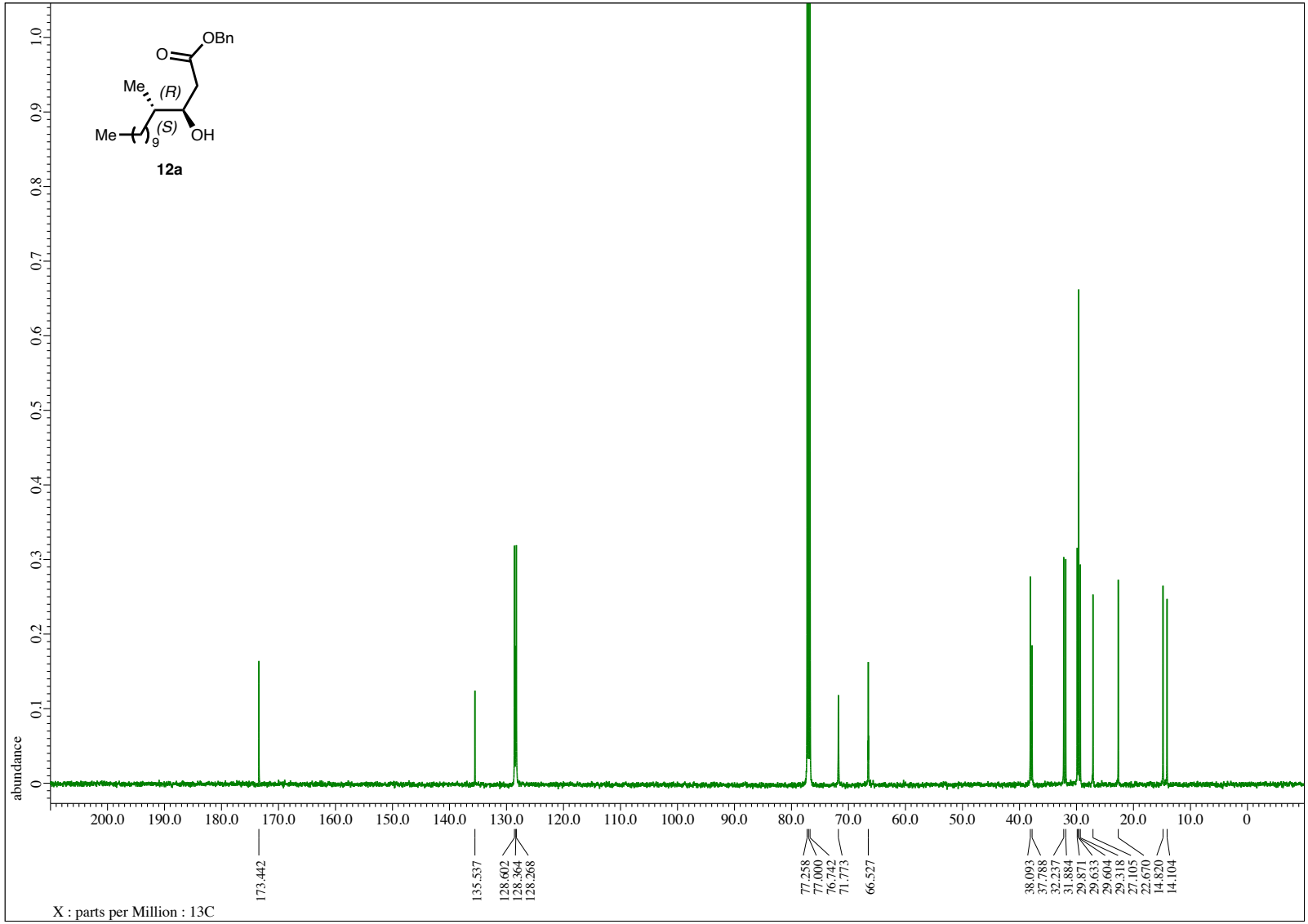

S
O
N
O
Me
Me
Me
Me
9
OH
14a
abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.263
4.736
4.728
4.722
4.714
4.706
4.405
4.397
4.391
4.004
4.000
3.996
3.614
3.583
3.579
3.332
3.311
3.297
3.276
2.375
2.367
2.361
2.353
2.347
2.339
2.333
2.299
1.670
1.663
1.658
1.501
1.478
1.401
1.381
1.255
0.949
0.947
0.933
0.918
0.904
0.889
0.873
0.860
X : parts per Million : 1H

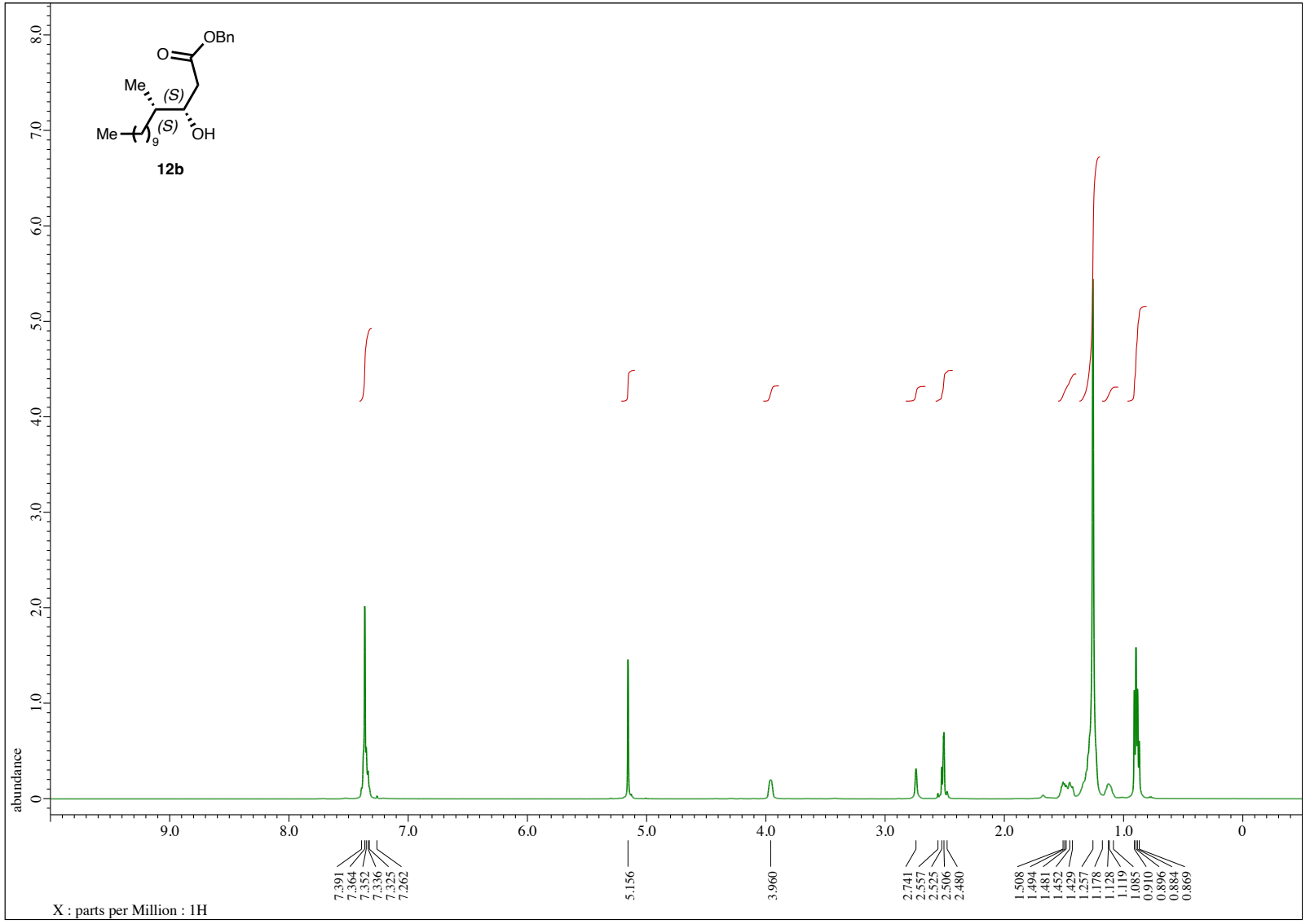

S
O
N
O
Me
Me
Me
Me
9
OH
14a
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
186.109
174.138
77.258
77.000
76.742
71.601
67.748
63.246
41.413
38.179
38.036
32.294
31.884
29.871
29.614
29.318
29.041
27.153
22.660
18.253
15.020
14.953
14.104
X : parts per Million : 13C

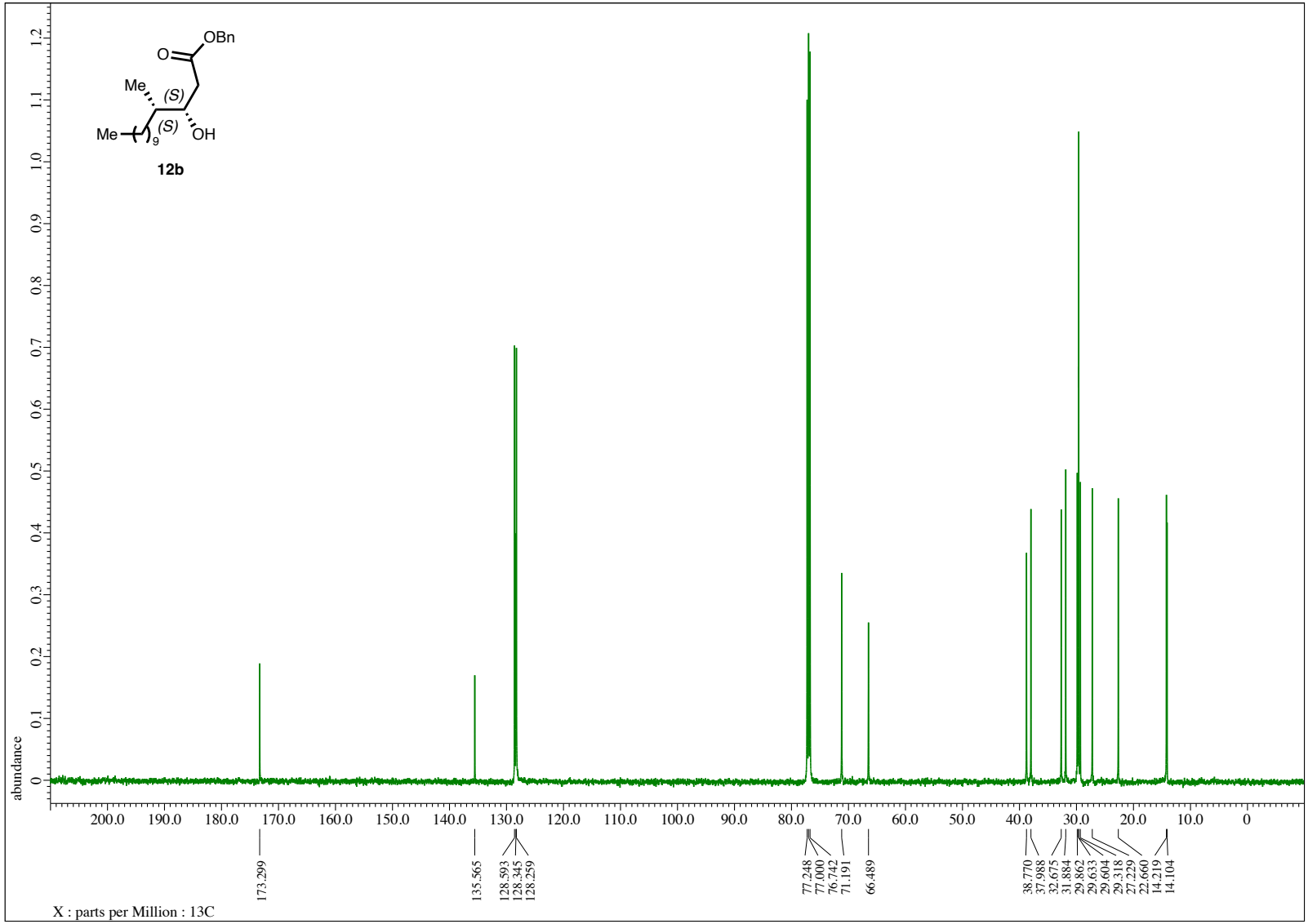

14b
abundance
X : parts per Million : 1H
7.260
4.749
4.742
4.735
4.727
4.720
4.402
4.397
4.387
3.996
3.992
3.988
3.638
3.603
3.583
3.377
3.372
3.343
3.339
2.384
2.378
2.370
2.363
2.356
2.349
2.341
2.297
1.304
1.290
1.253
1.215
1.196
1.179
1.172
1.162
0.960
0.955
0.945
0.932
0.916
0.900
0.886
0.872

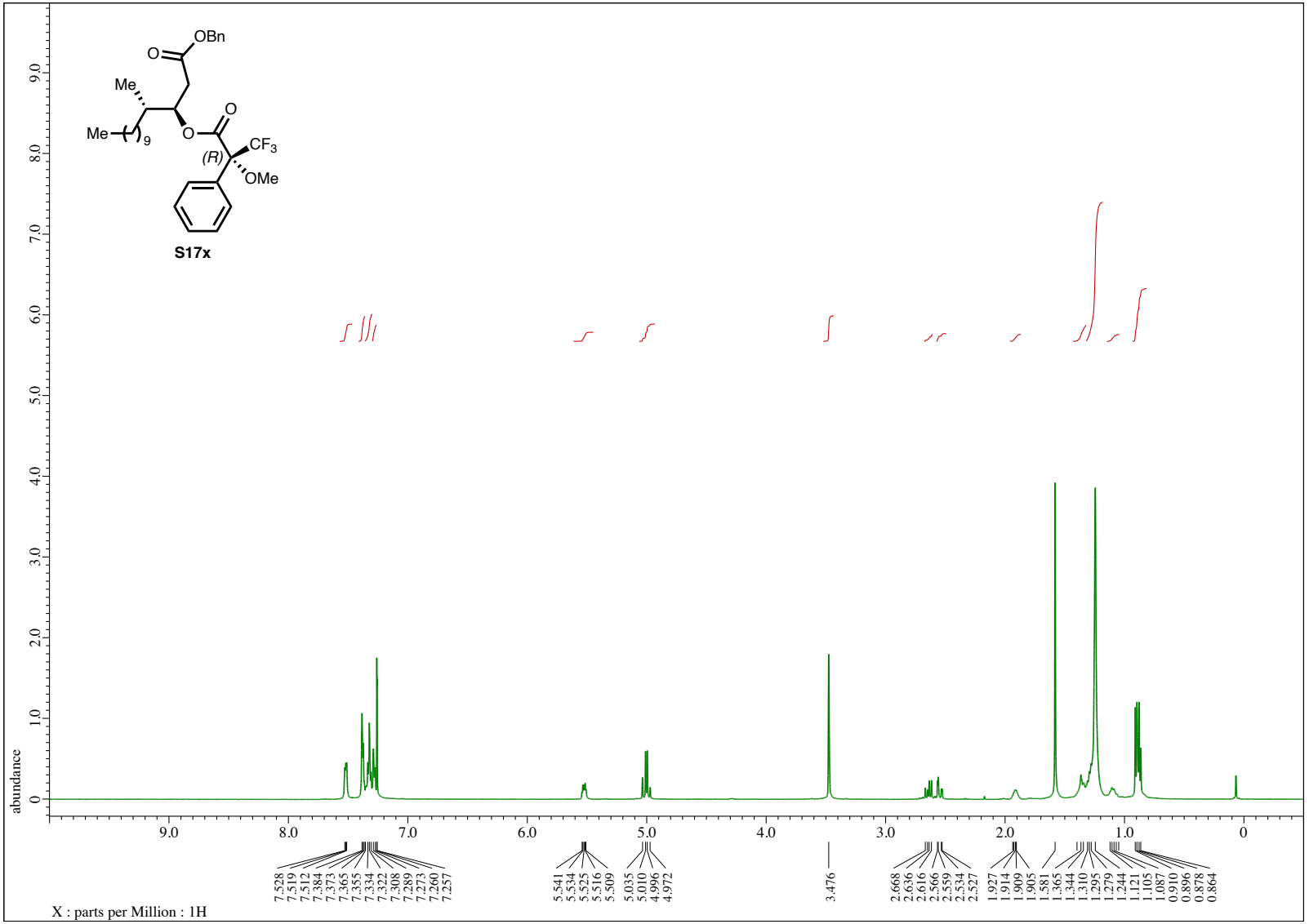

S
O
N
O
Me
Me
Me
Me
9
OH
14b
abundance
0
0.1
0.2
0.3
0.4
0.5
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
186.281
174.215
77.248
77.000
76.742
71.620
67.767
63.236
41.966
38.227
32.818
31.893
29.871
29.633
29.327
28.975
27.286
22.660
18.234
14.972
14.448
14.104
X : parts per Million : 13C

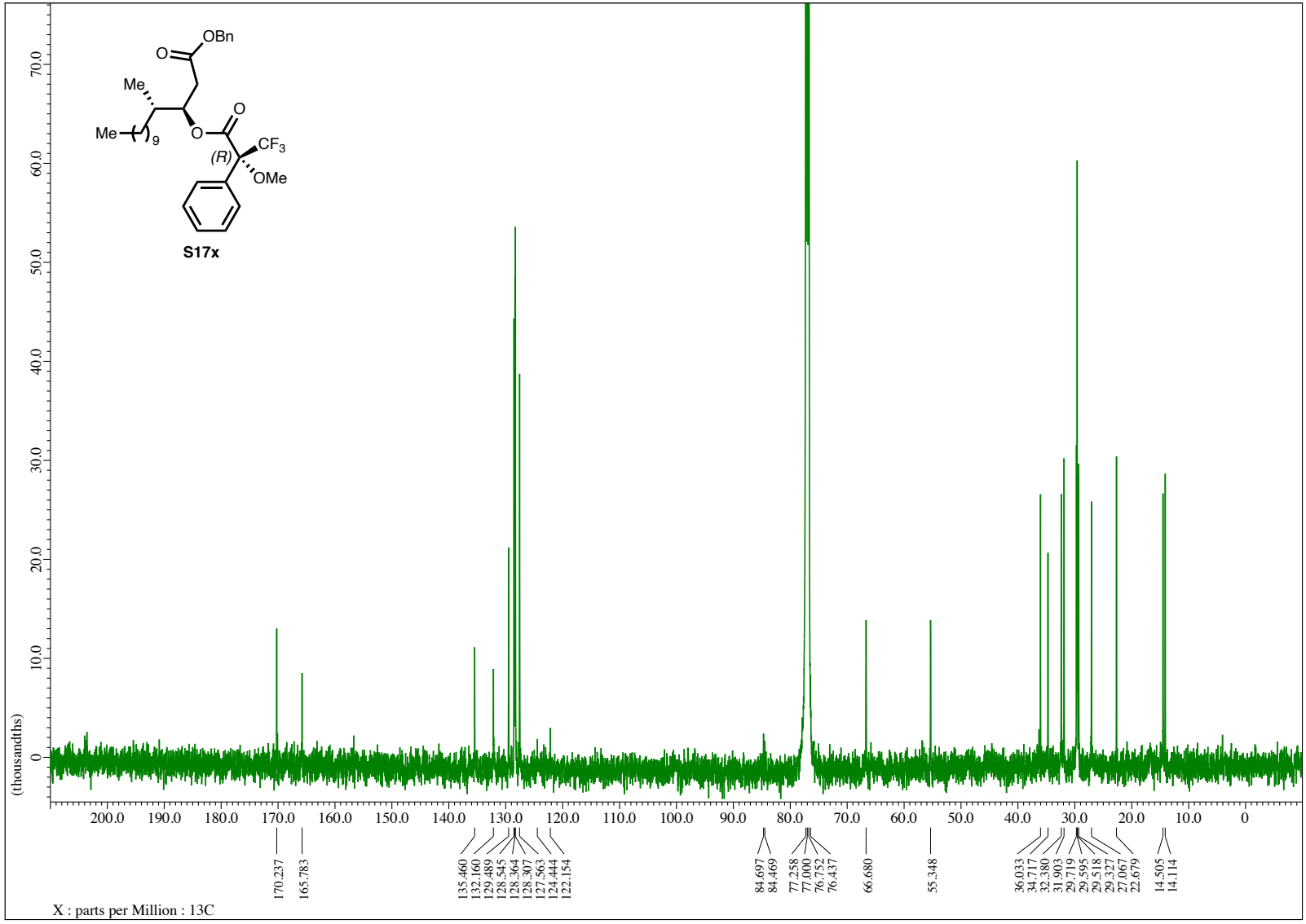

OBn
O
Me
(R)
(S)
OH
Me
9
12a
abundance
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.375
7.372
7.362
7.350
7.344
7.340
7.333
7.260
5.155
5.114
3.909
3.905
3.898
3.892
3.885
3.879
2.876
2.869
2.544
2.517
2.511
2.486
2.466
2.433
1.449
1.436
1.308
1.295
1.281
1.253
1.235
1.173
1.117
0.892
0.886
0.878
0.872
0.864
X : parts per Million : 1H

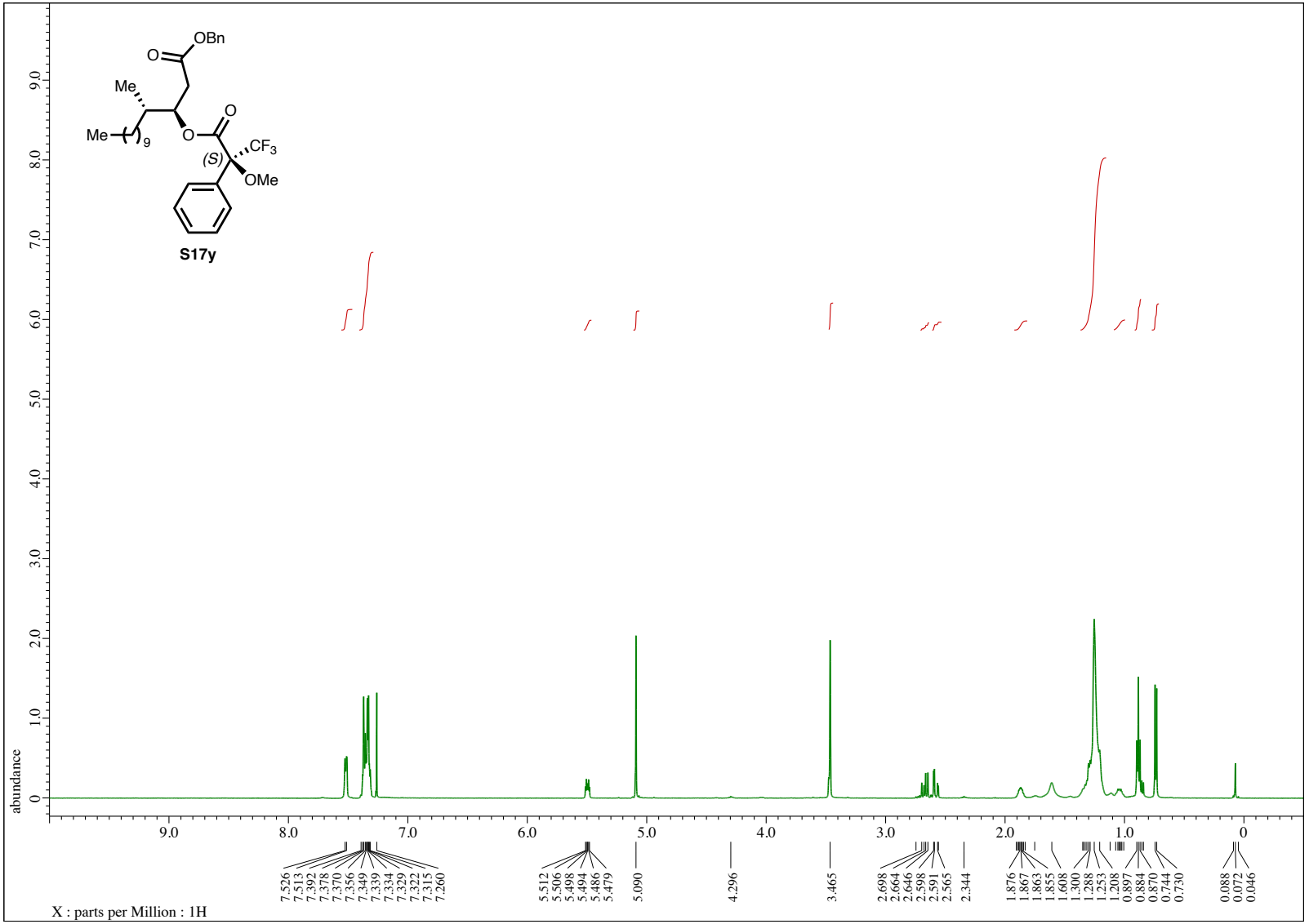

OBn
O
Me
(R)
(S)
Me
9
OH
12a
abundance
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
173.442
135.537
128.602
128.364
128.268
77.258
77.000
76.742
71.773
66.527
38.093
37.788
32.237
31.884
29.871
29.633
29.604
29.318
27.105
22.670
14.820
14.104
X : parts per Million : 13C

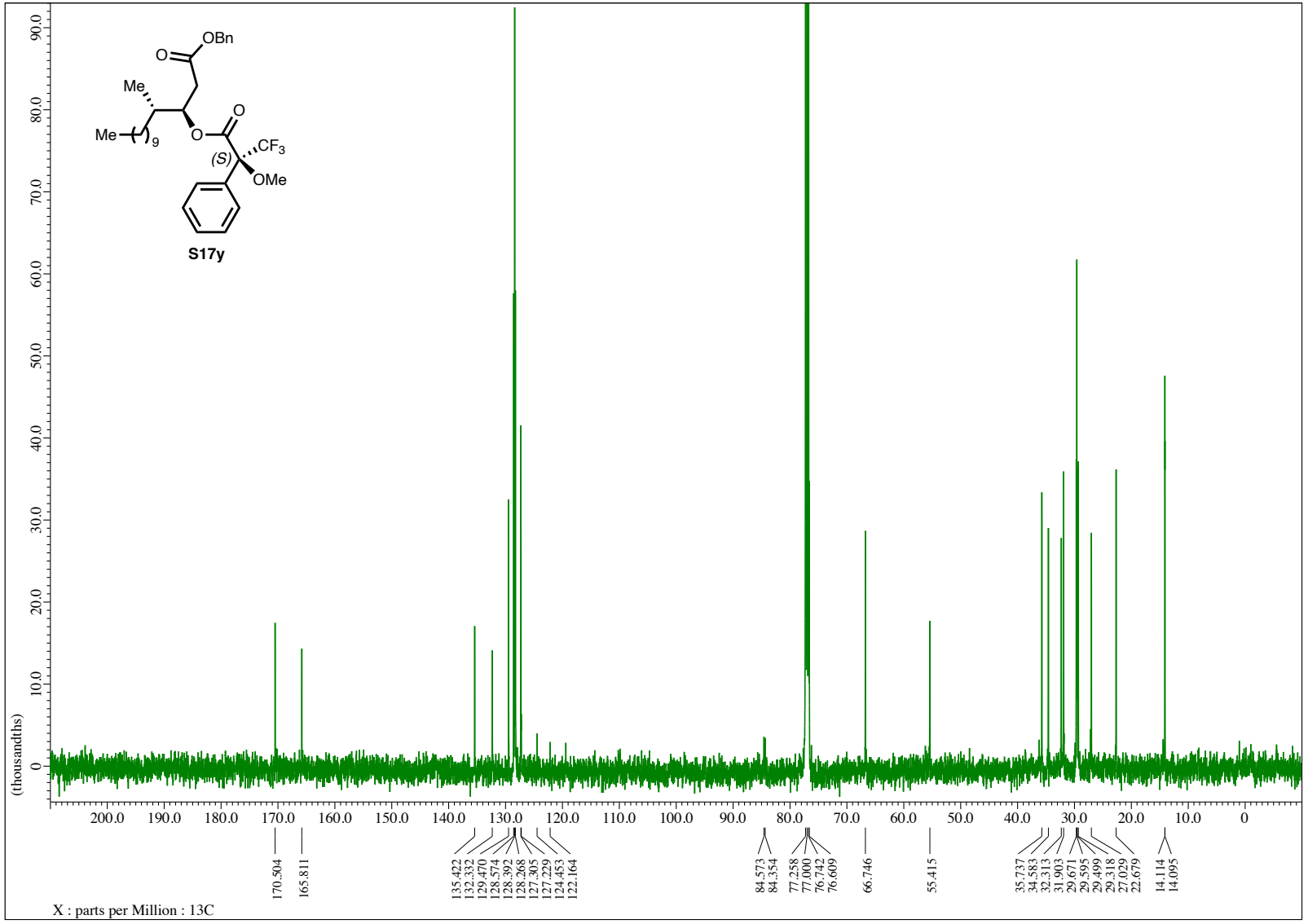

OBn
O
Me
(S)
(S)
Me
9
OH
12b
abundance
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.391
7.364
7.352
7.336
7.325
7.262
5.156
3.960
2.741
2.557
2.525
2.506
2.480
1.508
1.494
1.481
1.452
1.429
1.257
1.178
1.128
1.119
1.085
0.910
0.896
0.884
0.869
X : parts per Million : 1H

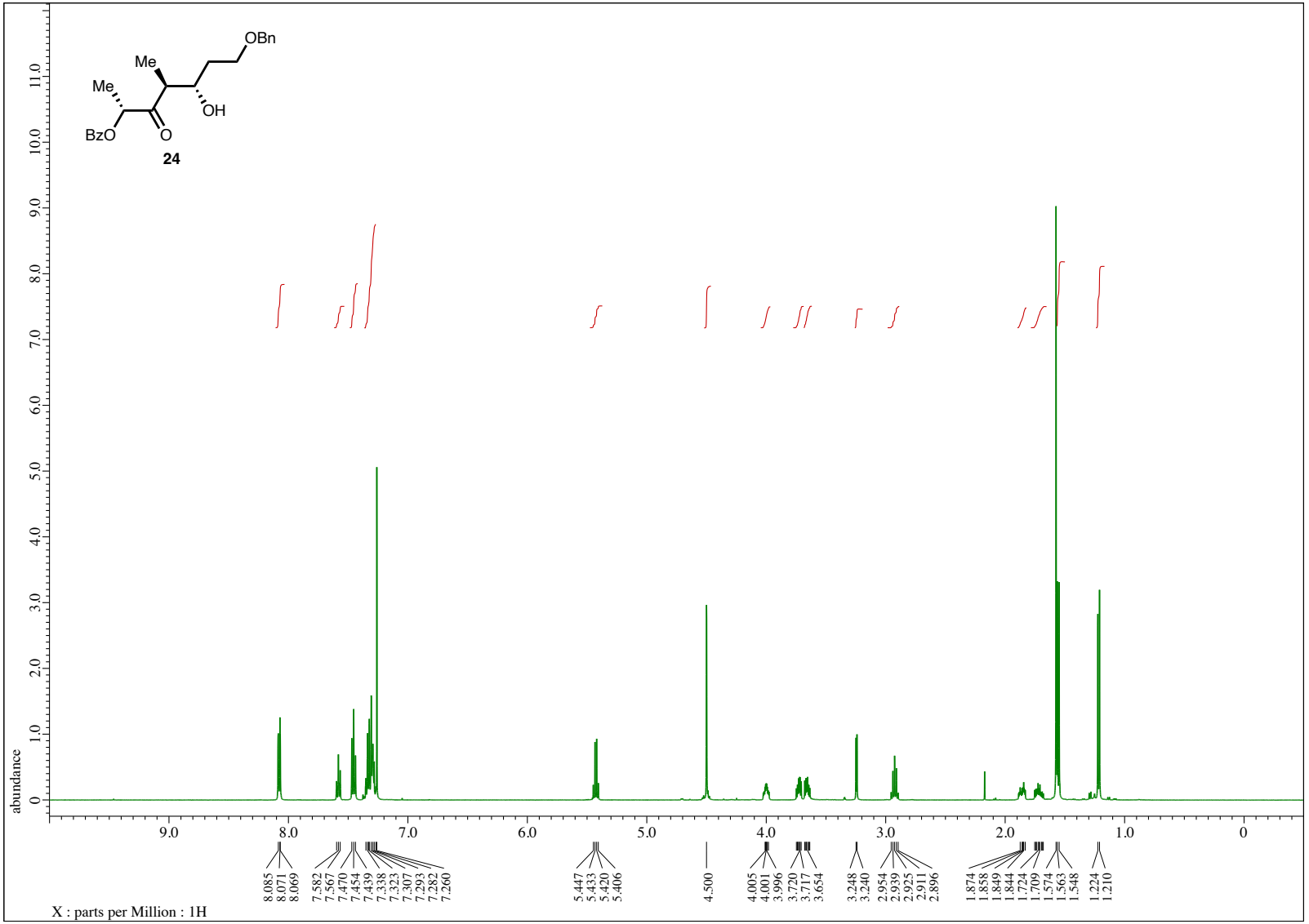

OBn
O
Me
(S)
Me
(S)
OH
9
12b
abundance
X : parts per Million : 13C
173.299
135.565
128.593
128.345
128.259
77.248
77.000
76.742
71.191
66.489
38.770
37.988
32.675
31.884
29.862
29.633
29.604
29.318
27.229
22.660
14.219
14.104

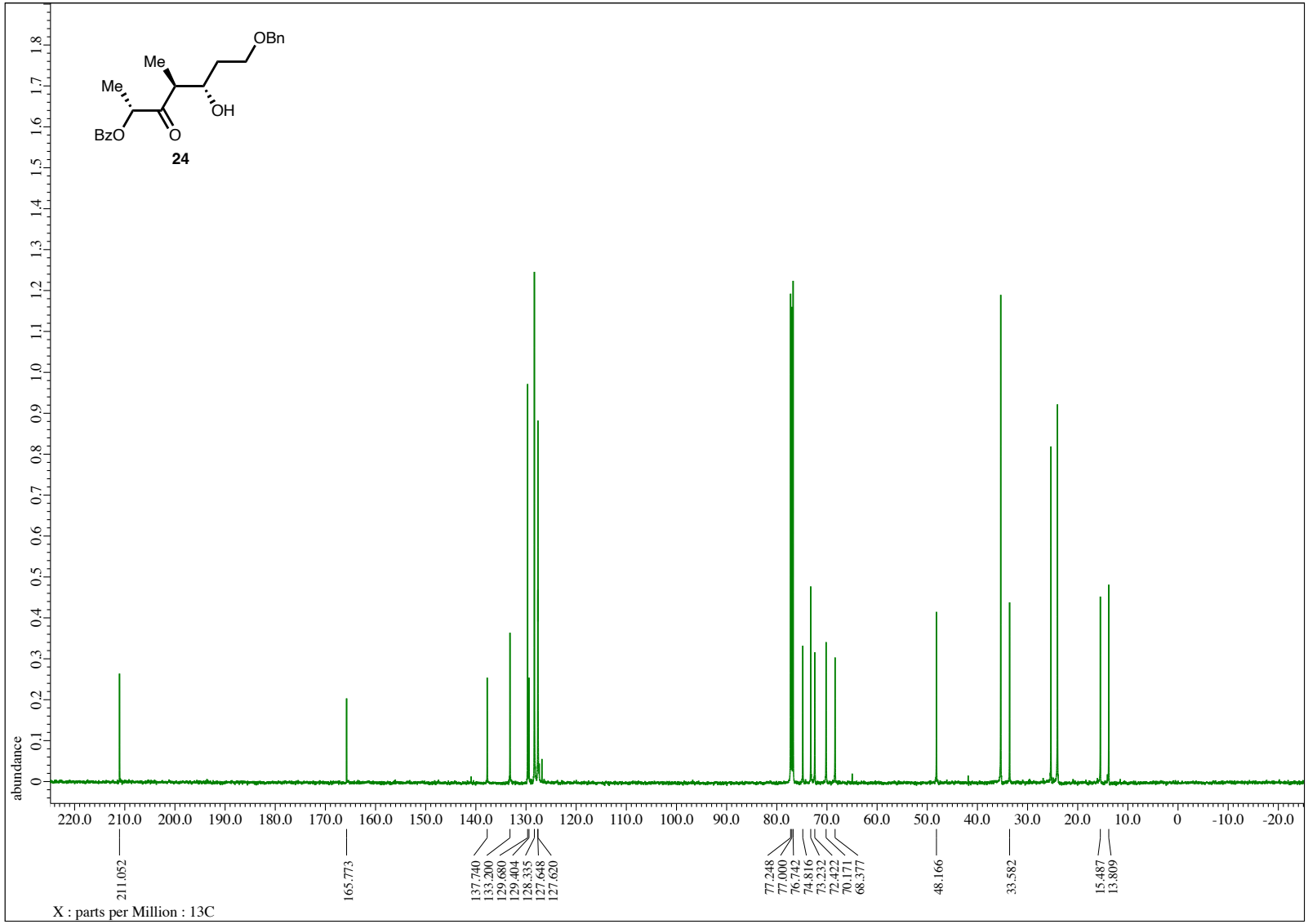

OBn
O
Me
O
Me
9
O
CF3
(R)
OMe
S17x
abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.528
7.519
7.512
7.384
7.373
7.365
7.355
7.334
7.322
7.308
7.289
7.273
7.260
7.257
5.541
5.534
5.525
5.516
5.509
5.035
5.010
4.996
4.972
3.476
2.668
2.636
2.616
2.566
2.559
2.534
2.527
1.927
1.914
1.909
1.905
1.581
1.365
1.344
1.310
1.295
1.279
1.244
1.121
1.105
1.087
0.910
0.896
0.878
0.864
X : parts per Million : 1H

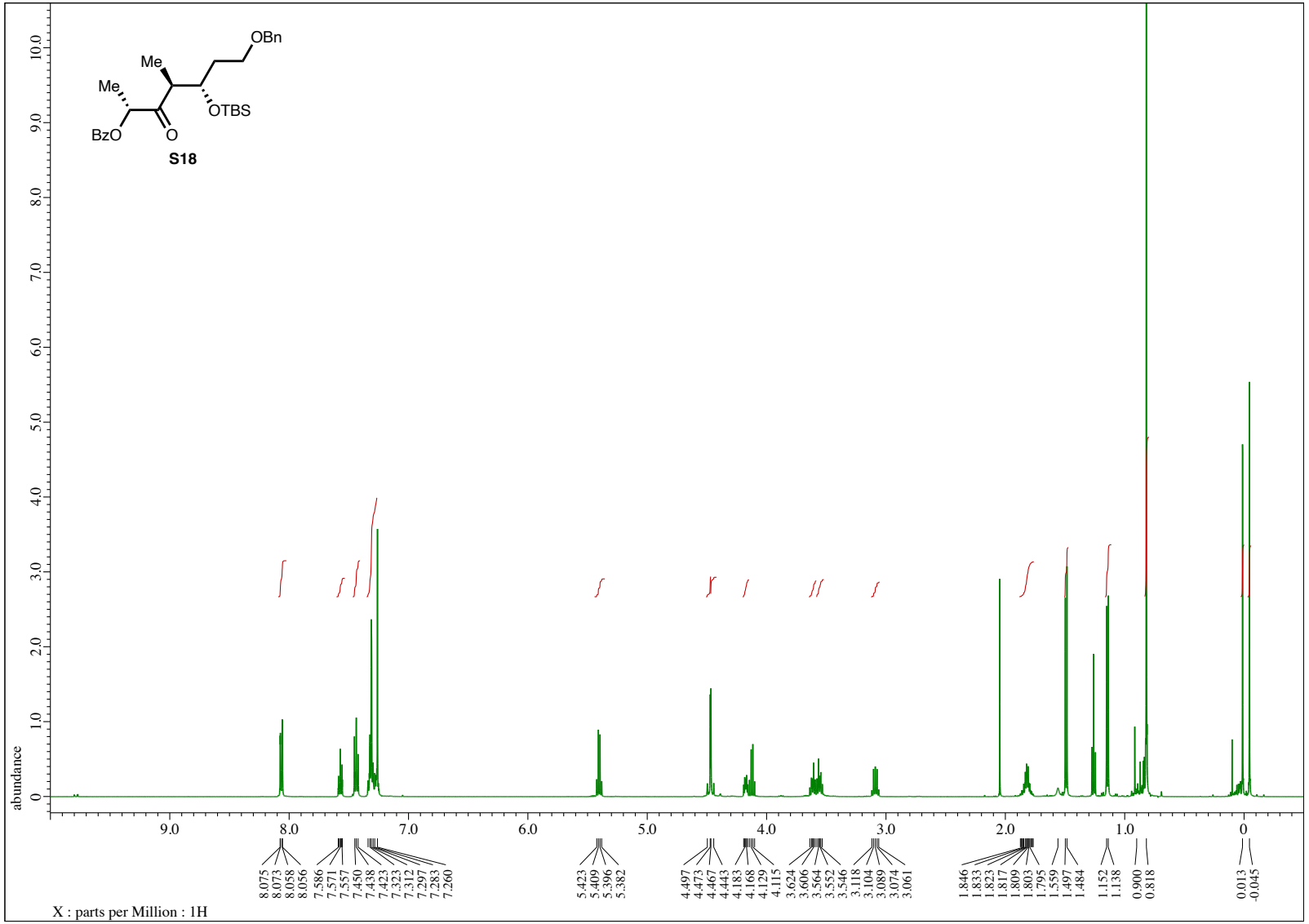

OBn
O
Me
O
Me
9
O
CF3
(R)
OMe
S17x
(thousandths)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
170.237
165.783
135.460
132.160
129.489
128.545
128.364
128.307
127.563
124.444
122.154
84.697
84.469
77.258
77.000
76.752
76.437
66.680
55.348
36.033
34.717
32.380
31.903
29.719
29.595
29.518
29.327
27.067
22.679
14.505
14.114
X : parts per Million : 13C

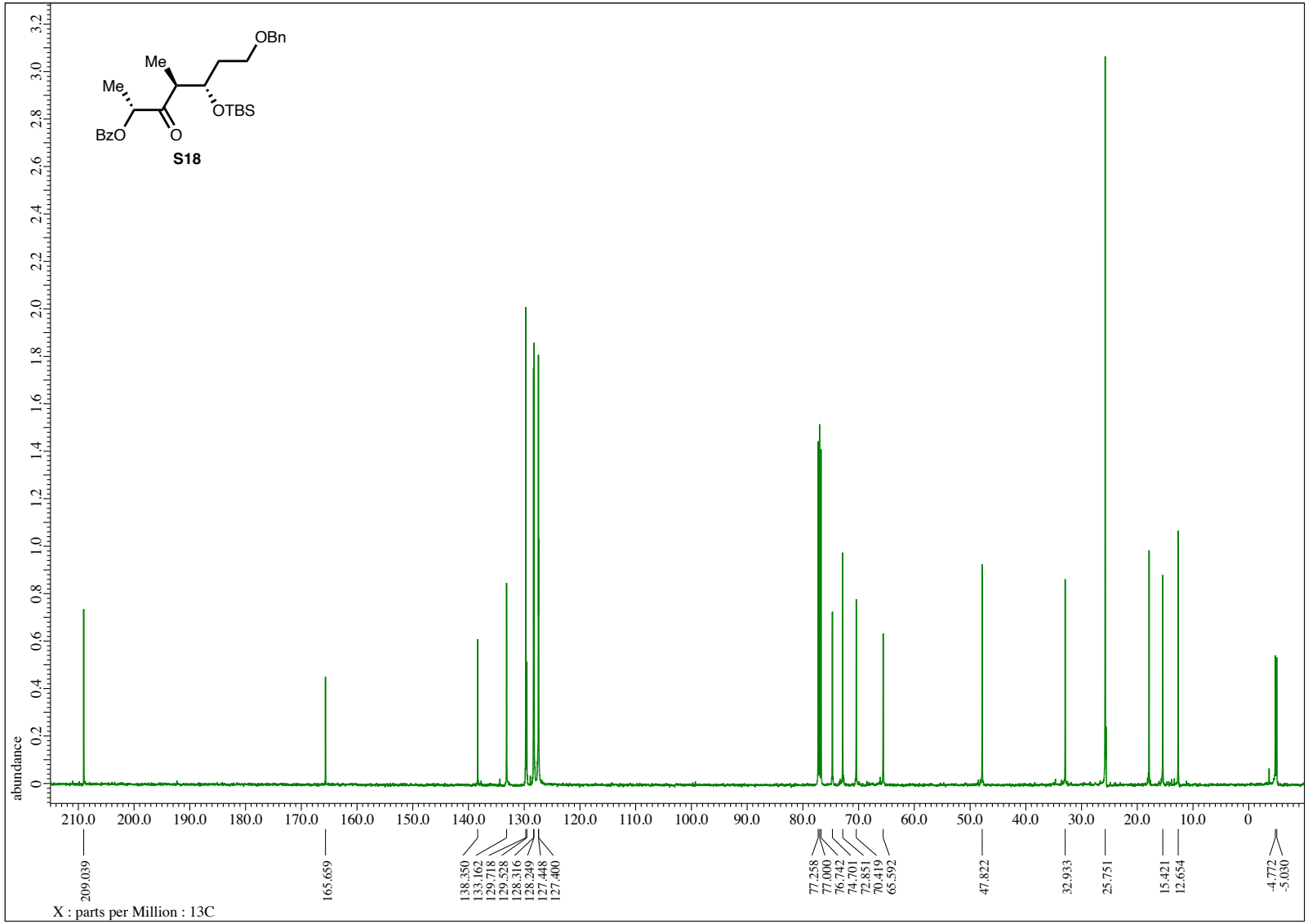

OBn
O
Me
O
Me
9
O
CF3
(S)
OMe
S17y
abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.526
7.513
7.392
7.378
7.370
7.356
7.349
7.339
7.334
7.329
7.322
7.315
7.260
5.512
5.506
5.498
5.494
5.486
5.479
5.090
4.296
3.465
2.698
2.664
2.646
2.598
2.591
2.565
2.344
1.876
1.867
1.863
1.855
1.608
1.300
1.288
1.253
1.208
0.897
0.884
0.870
0.744
0.730
0.088
0.072
0.046
X : parts per Million : 1H

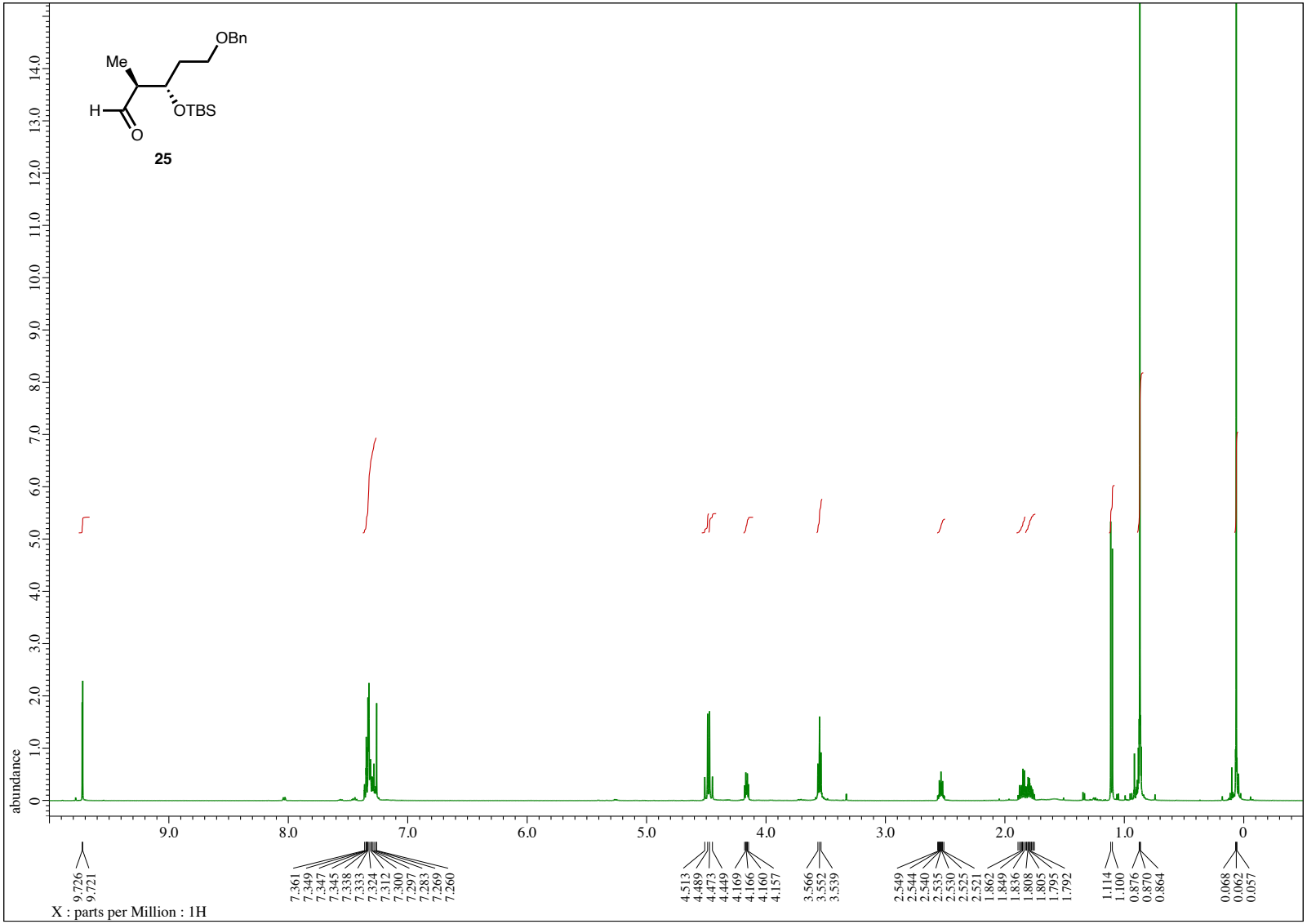

OBn
O
Me
O
Me
9
O
CF3
(S)
OMe
S17y
(thousandths)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
170.504
165.811
135.422
132.332
129.470
128.574
128.392
128.268
127.305
127.229
124.453
122.164
84.573
84.354
77.258
77.000
76.742
76.609
66.746
55.415
35.737
34.583
32.313
31.903
29.671
29.595
29.499
29.318
27.029
22.679
14.114
14.095
X : parts per Million : 13C

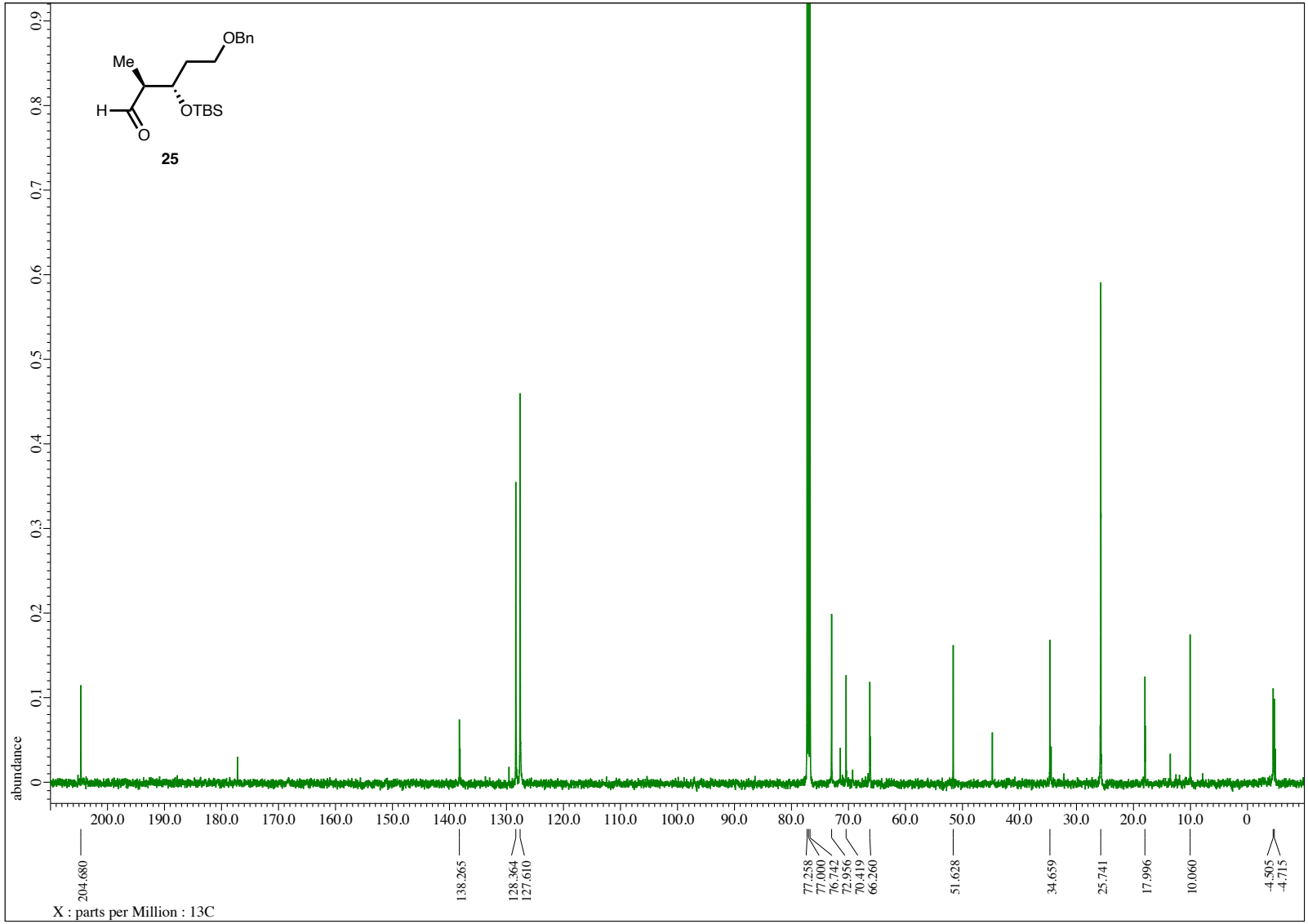

OBn
Me
Me
OH
BzO
O
24
abundance
X : parts per Million : 1H
8.085
8.071
8.069
7.582
7.567
7.470
7.454
7.439
7.338
7.323
7.307
7.293
7.282
7.260
5.447
5.433
5.420
5.406
4.500
4.005
4.001
3.996
3.720
3.717
3.654
3.248
3.240
2.954
2.939
2.925
2.911
2.896
1.874
1.858
1.849
1.844
1.724
1.709
1.574
1.563
1.548
1.224
1.210

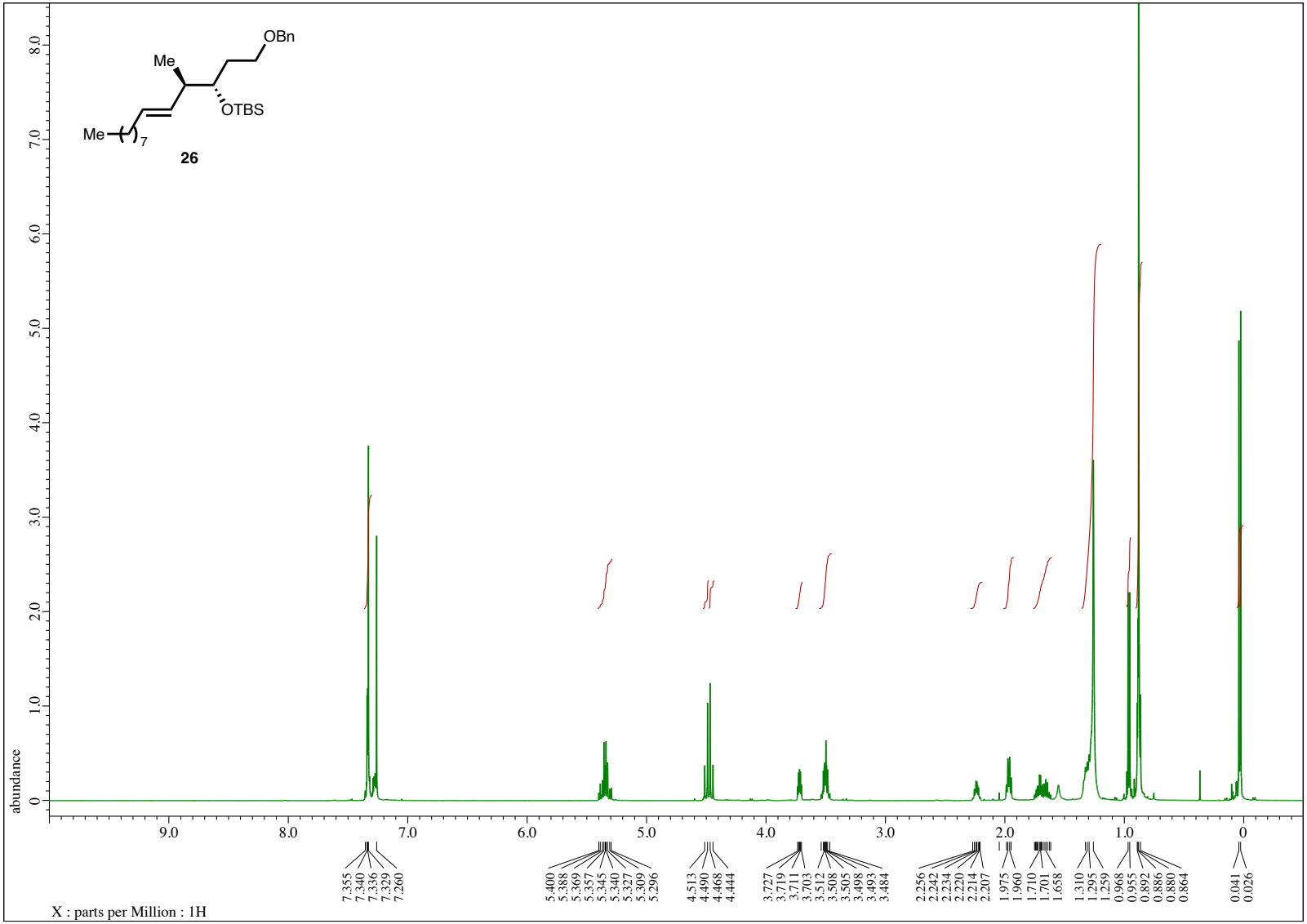

OBn
Me
Me
OH
BzO
O
24
abundance
X : parts per Million : 13C
211.052
165.773
137.740
133.200
129.680
129.404
128.335
127.648
127.620
77.248
77.000
76.742
74.816
73.232
72.422
70.171
68.377
48.166
33.582
15.487
13.809

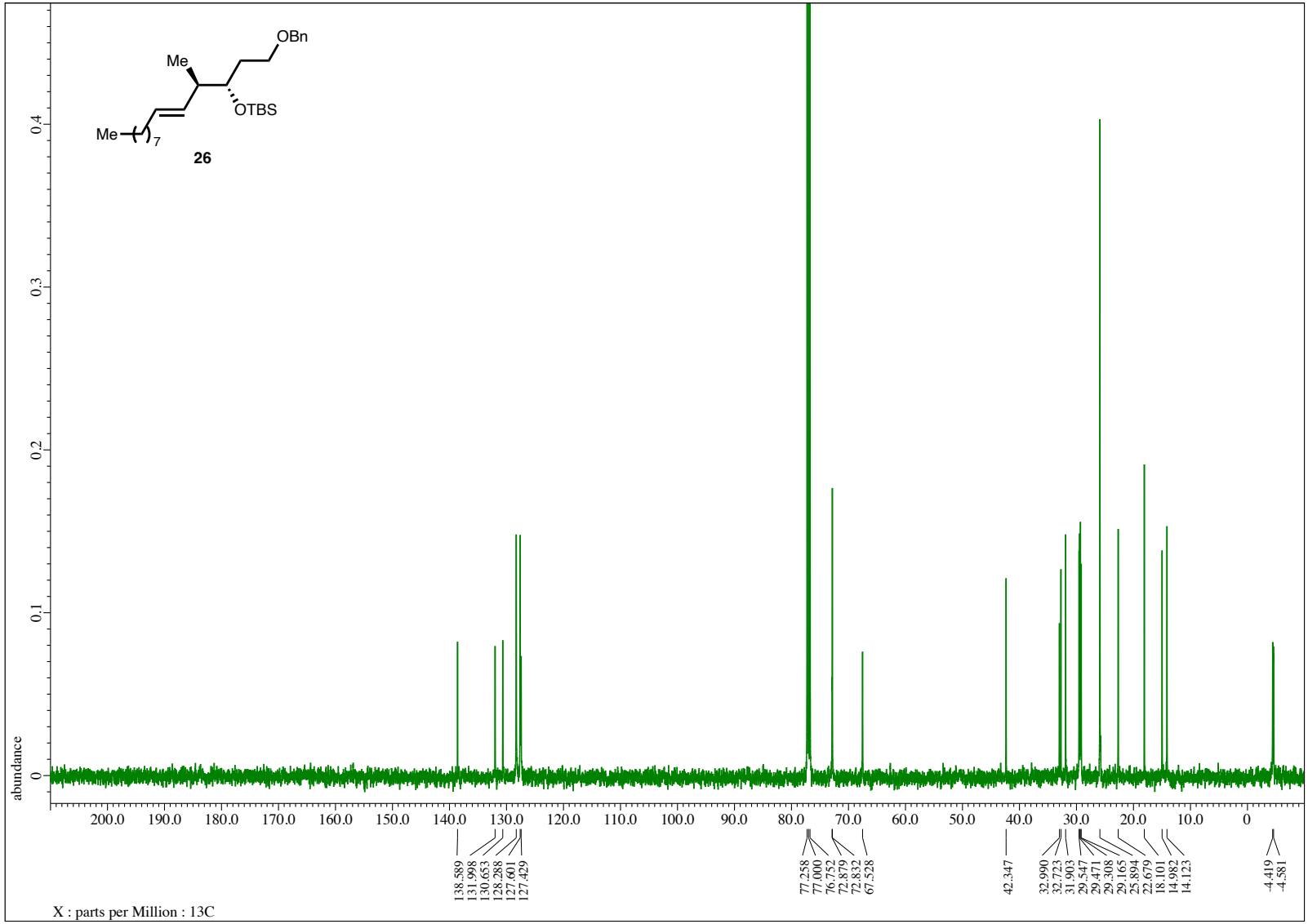

OBn
Me
Me
OTBS
BzO
O
S18
abundance
X : parts per Million : 1H
8.075
8.073
8.058
8.056
7.586
7.571
7.557
7.450
7.438
7.423
7.323
7.312
7.297
7.283
7.260
5.423
5.409
5.396
5.382
4.497
4.473
4.467
4.443
4.183
4.168
4.129
4.115
3.624
3.606
3.564
3.552
3.546
3.118
3.104
3.089
3.074
3.061
1.846
1.833
1.823
1.817
1.809
1.803
1.795
1.559
1.497
1.484
1.152
1.138
0.900
0.818
0.013
-0.045

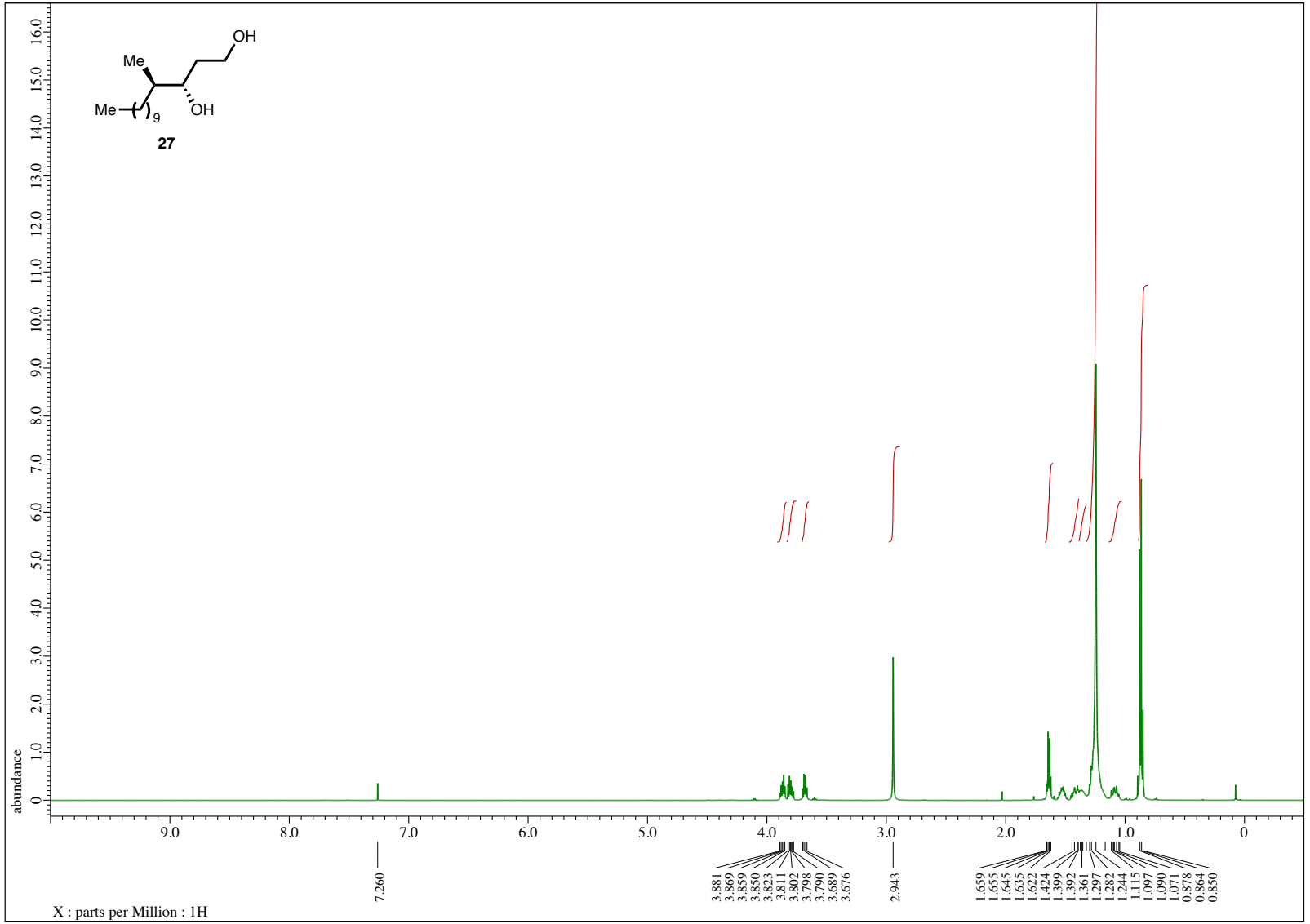

OBn
Me
Me
OTBS
BzO
O
S18
abundance
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
209.039
165.659
138.350
133.162
129.718
129.528
128.316
128.249
127.448
127.400
77.258
77.000
76.742
74.701
72.851
70.419
65.592
47.822
32.933
25.751
15.421
12.654
-4.772
-5.030
X : parts per Million : 13C

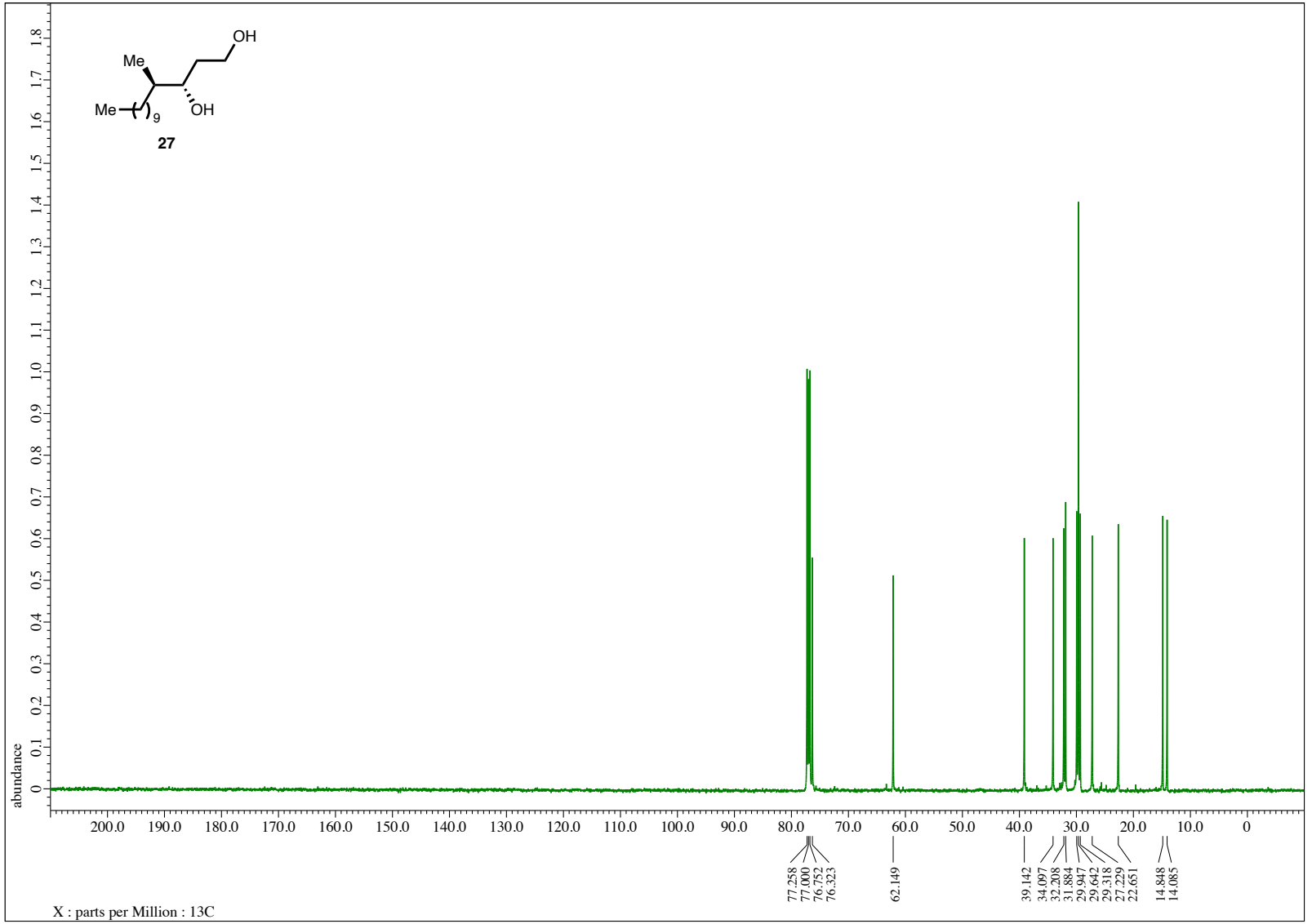

OBn
Me
H
OTBS
O
25
abundance
X : parts per Million : 1H

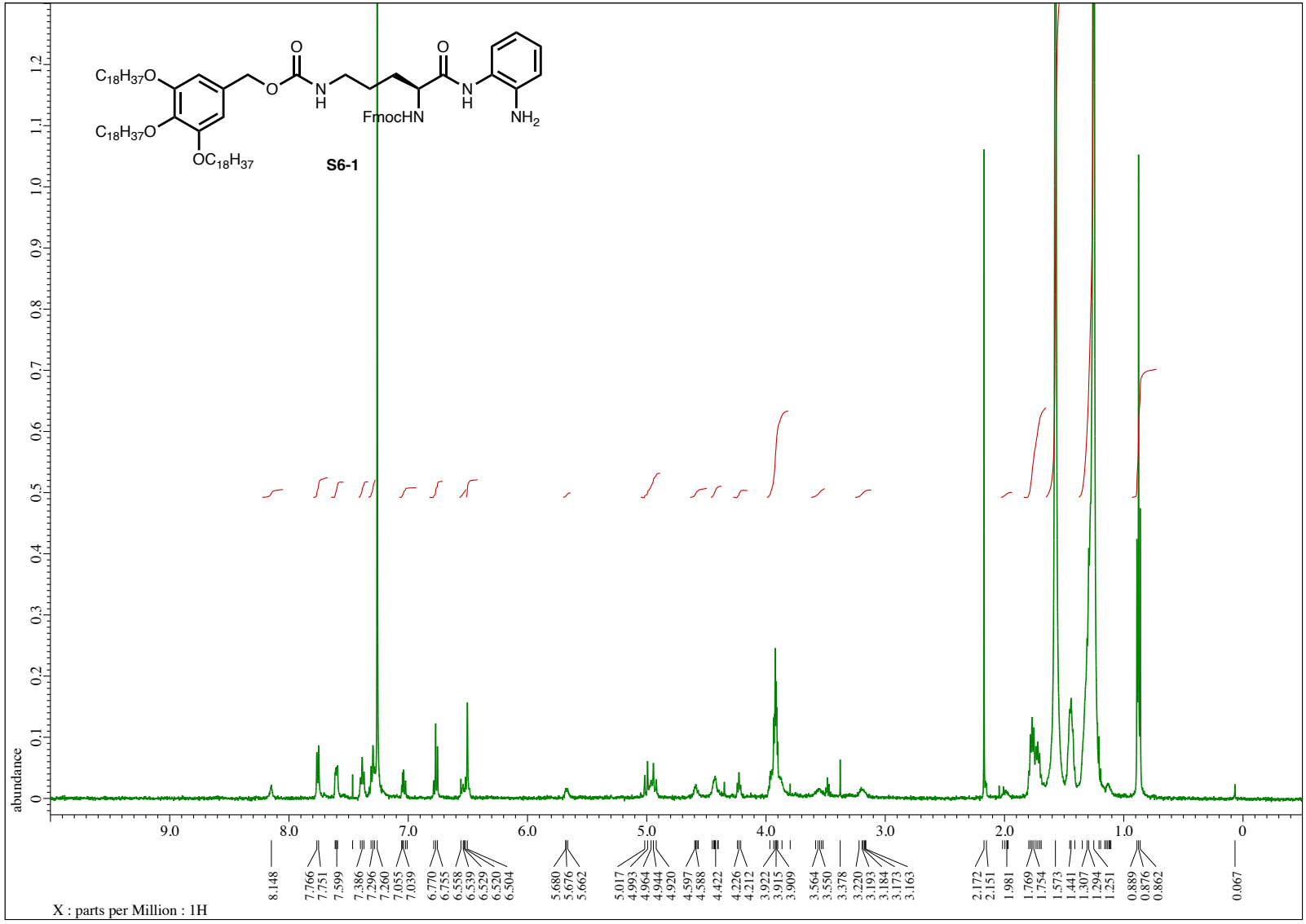

OBn
Me
H
OTBS
O
25
abundance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
204.680
138.265
128.364
127.610
77.258
77.000
76.742
72.956
70.419
66.260
51.628
34.659
25.741
17.996
10.060
-4.505
-4.715
X : parts per Million : 13C

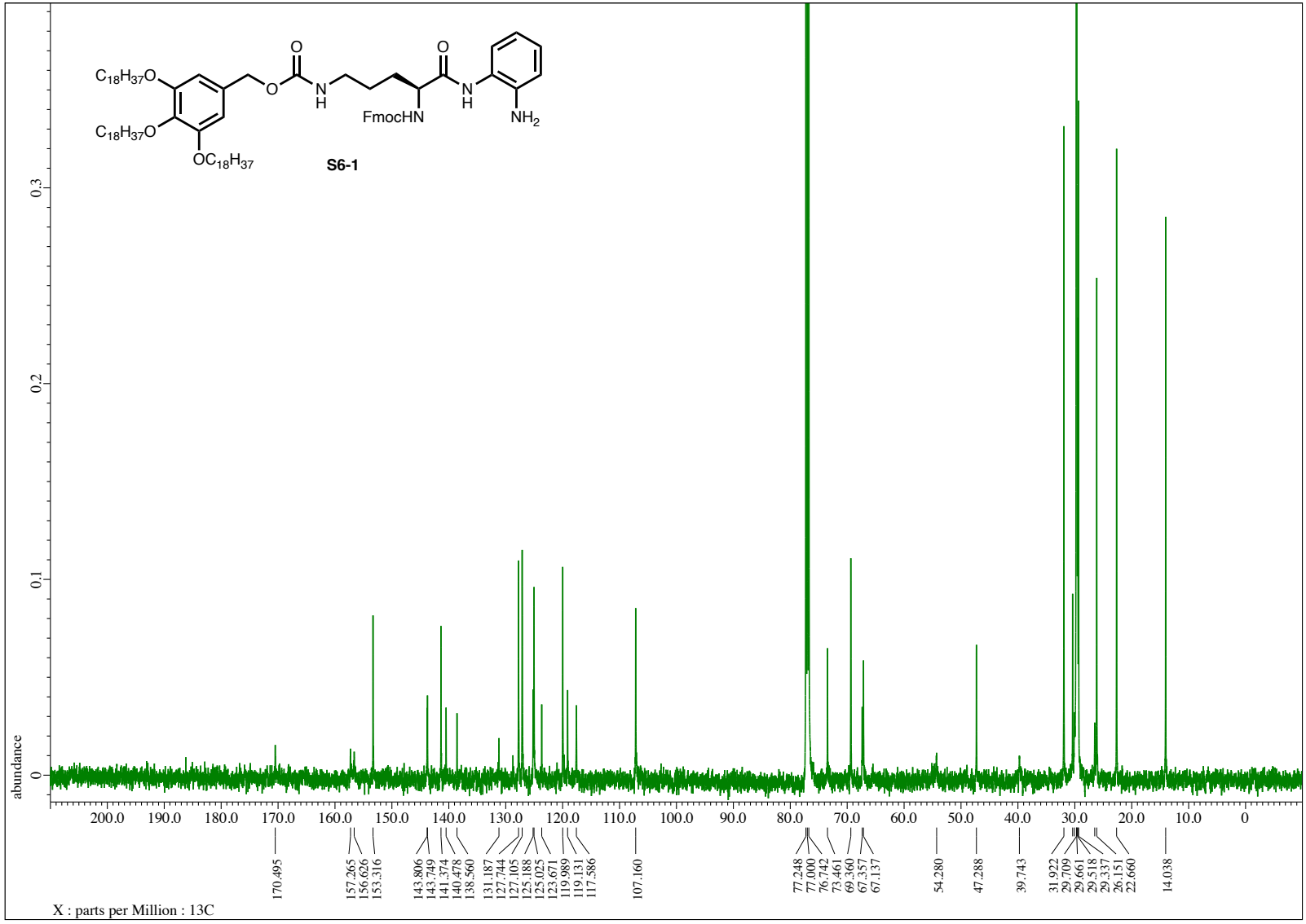

OBn
Me
OTBS
Me
7
26
abundance
X : parts per Million : 1H

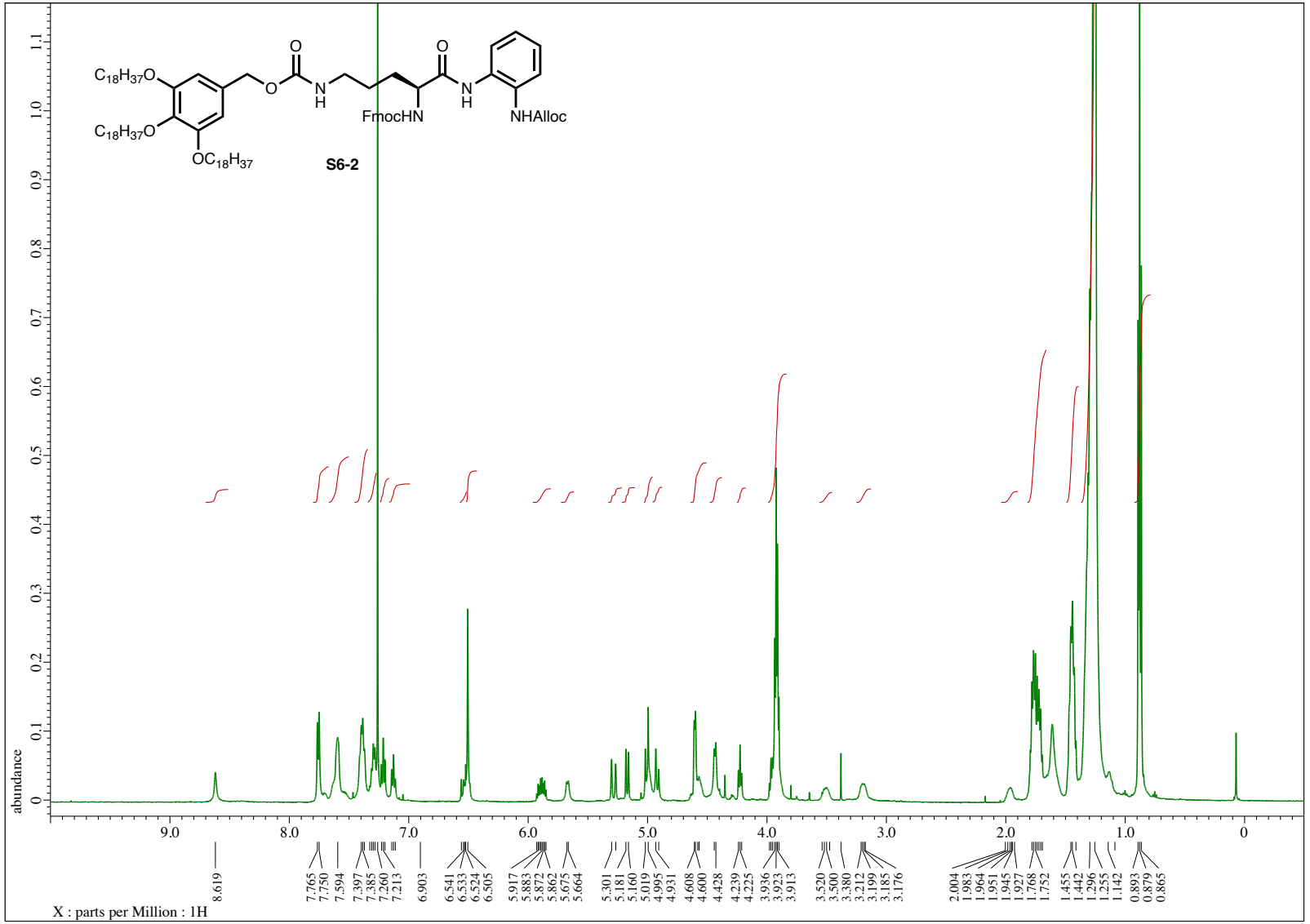

OBn
Me
OTBS
Me
7
26
abundance
0
0.1
0.2
0.3
0.4
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
138.589
131.998
130.653
128.288
127.601
127.429
77.258
77.000
76.752
72.879
72.832
67.528
42.347
32.990
32.723
31.903
29.547
29.471
29.308
29.165
25.894
22.679
18.101
14.982
14.123
-4.419
-4.581
X : parts per Million : 13C

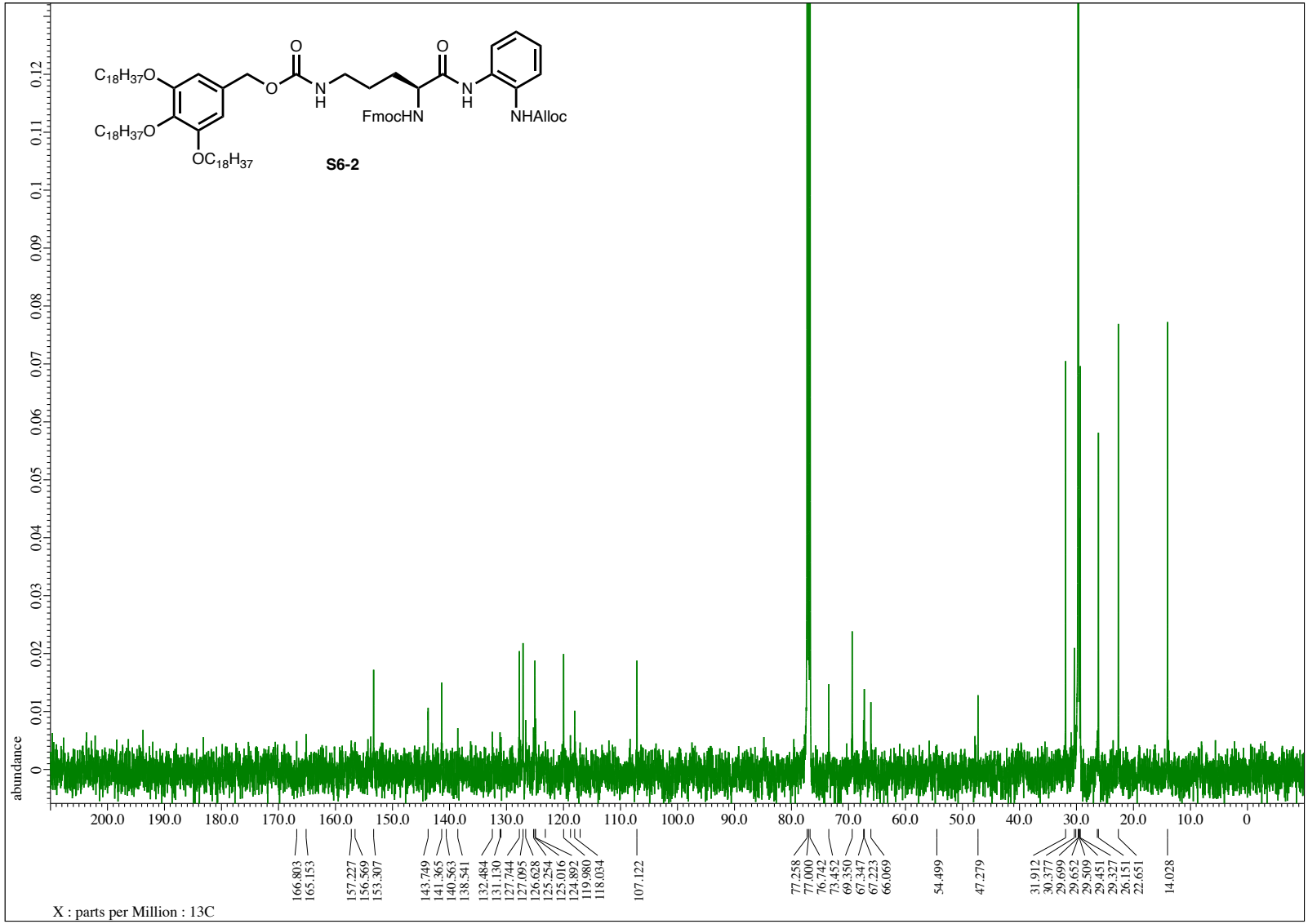

OH
Me
Me
9
OH
27
abundance
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.260
3.881
3.869
3.859
3.850
3.823
3.811
3.802
3.798
3.790
3.689
3.676
2.943
1.659
1.655
1.645
1.635
1.622
1.424
1.399
1.392
1.361
1.297
1.282
1.244
1.115
1.097
1.090
1.071
0.878
0.864
0.850
X : parts per Million : 1H

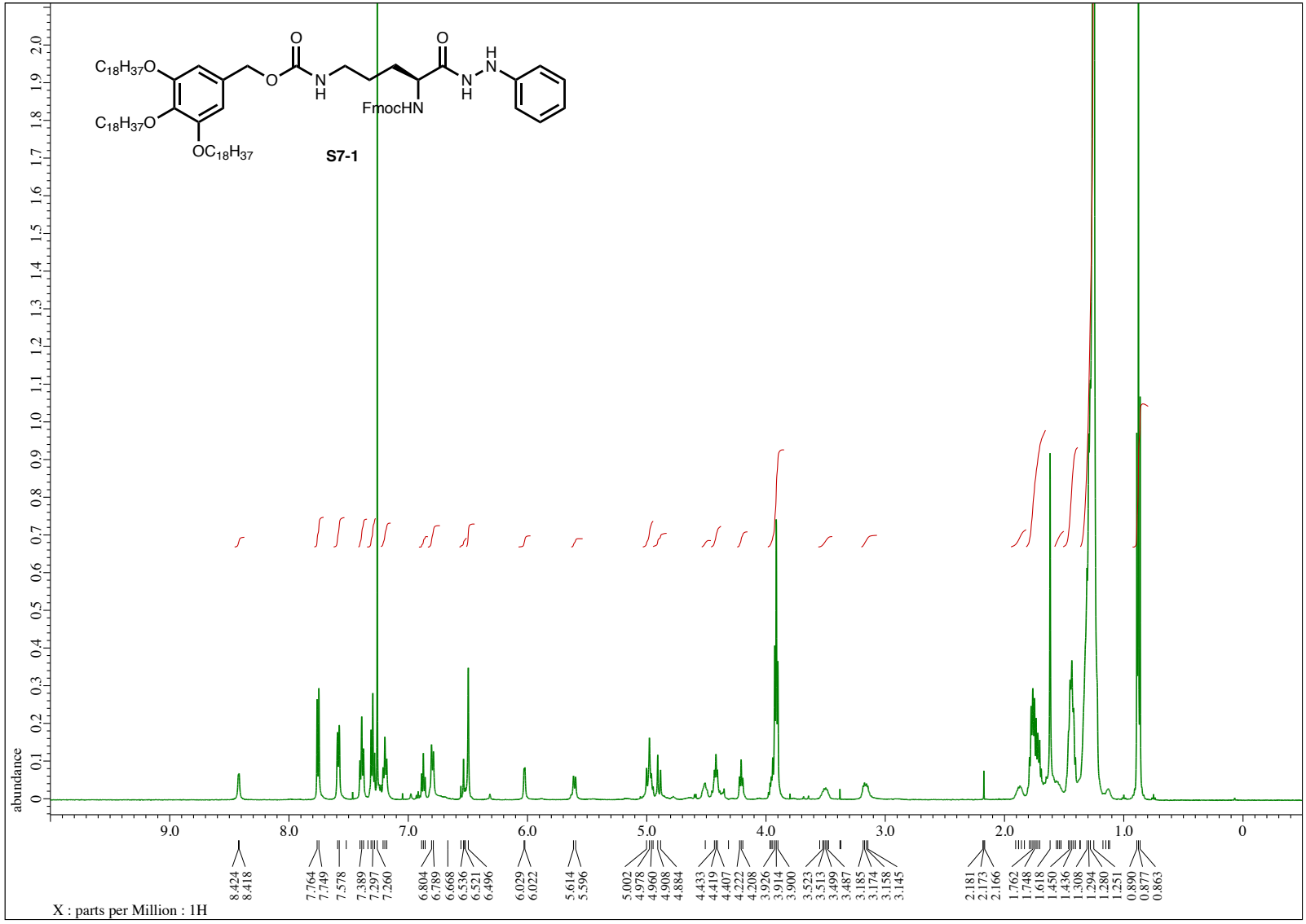

OH
Me
Me
9
OH
27
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
77.258
77.000
76.752
76.323
62.149
39.142
34.097
32.208
31.884
29.947
29.642
29.318
27.229
22.651
14.848
14.085
X : parts per Million : 13C

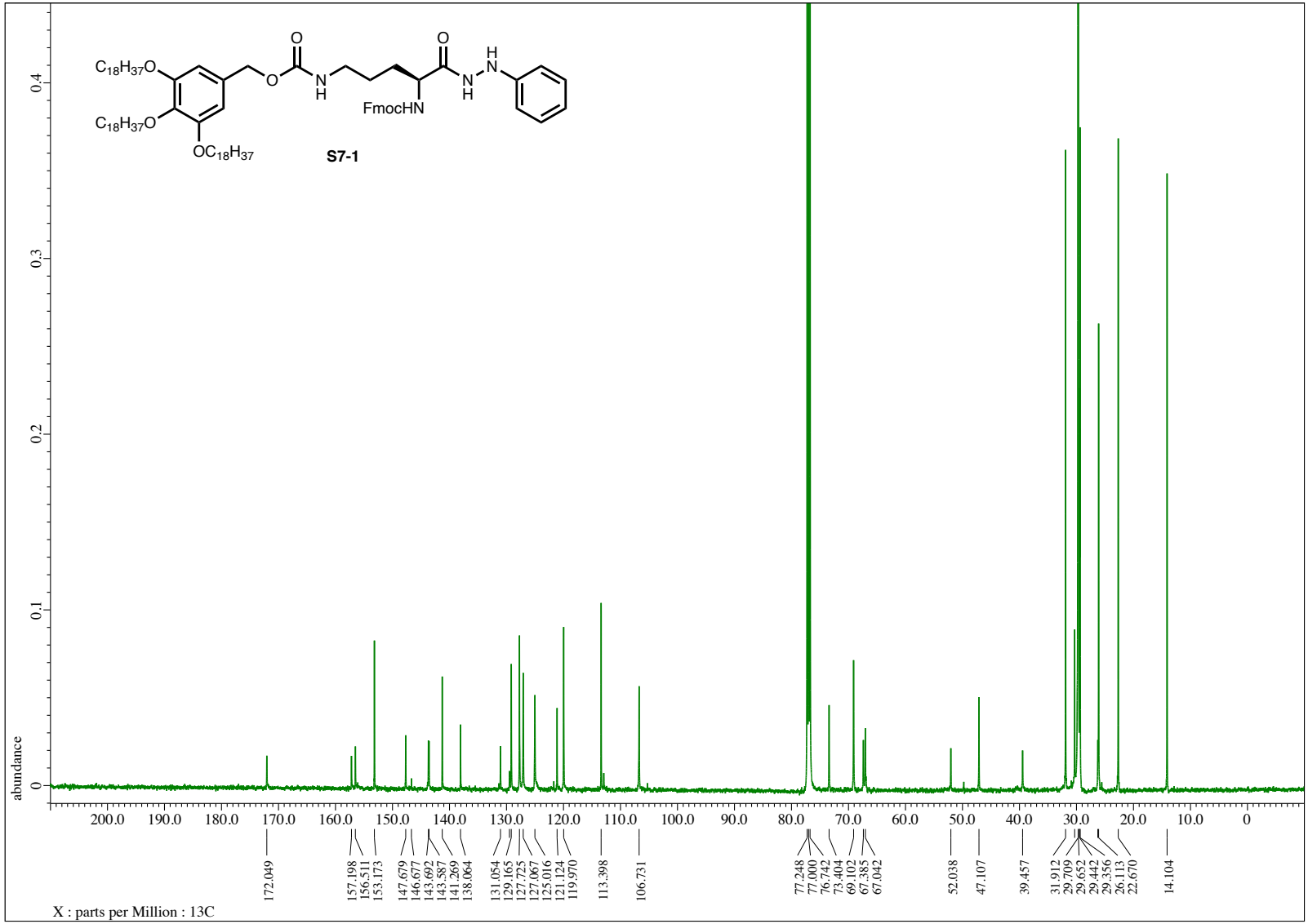

C18H37O
C18H37O
OC18H37
O
O
N
H
FmocHN
O
N
H
NH2
S6-1
abundance
X : parts per Million : 1H
8.148
7.766
7.751
7.599
7.386
7.296
7.260
7.055
7.039
6.770
6.755
6.558
6.539
6.529
6.520
6.504
5.680
5.676
5.662
5.017
4.993
4.964
4.944
4.920
4.597
4.588
4.422
4.226
4.212
3.922
3.915
3.909
3.564
3.550
3.378
3.220
3.193
3.184
3.173
3.163
2.172
2.151
1.981
1.769
1.754
1.573
1.441
1.307
1.294
1.251
0.889
0.876
0.862
0.067

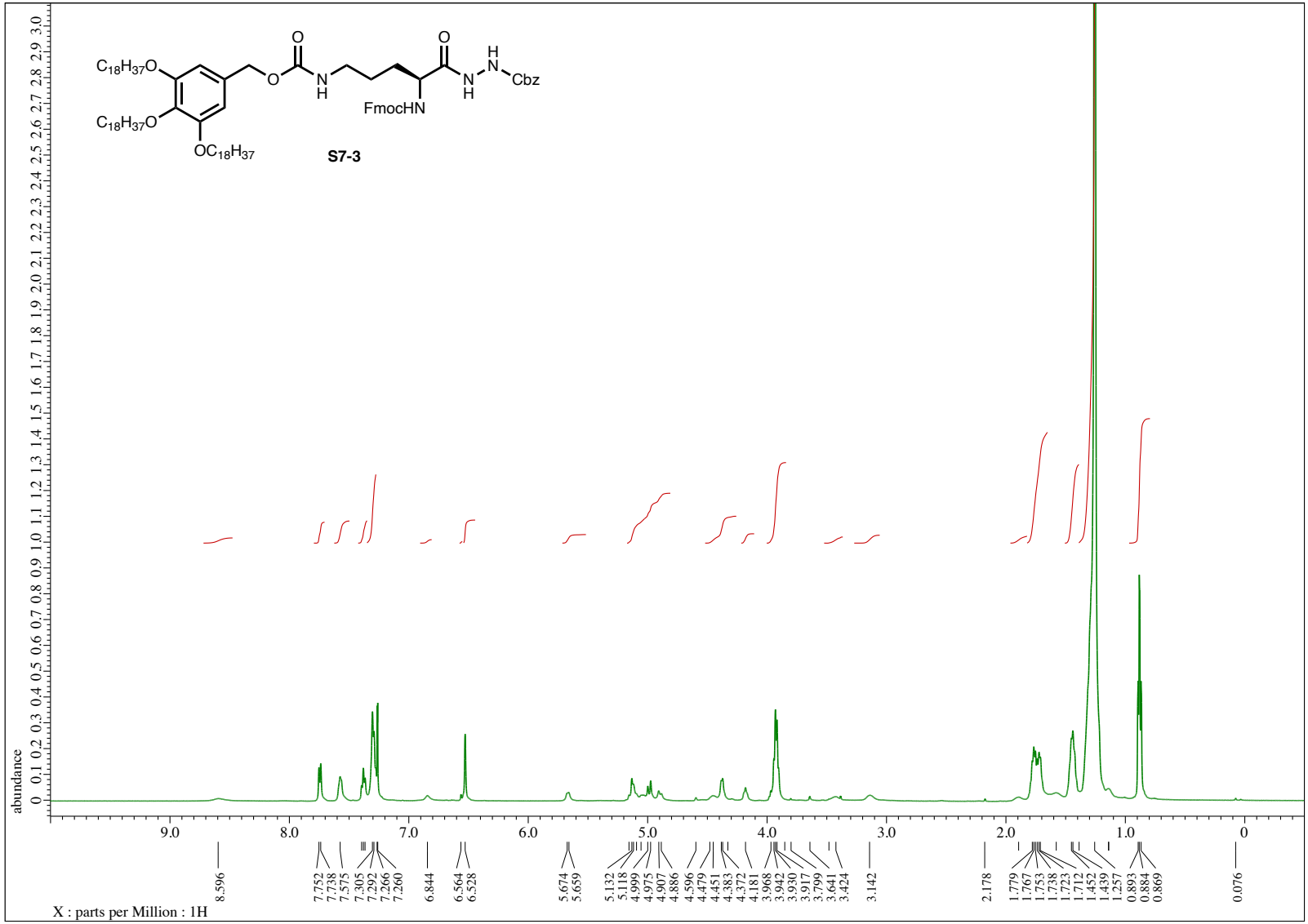

C18H37O
C18H37O
OC18H37
O
O
N
H
FmocHN
O
N
H
NH2
S6-1
abundance
0
0.1
0.2
0.3
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
170.495
157.265
156.626
153.316
143.806
143.749
141.374
140.478
138.560
131.187
127.744
127.105
125.188
125.025
123.671
119.989
119.131
117.586
107.160
77.248
77.000
76.742
73.461
69.360
67.357
67.137
54.280
47.288
39.743
31.922
29.709
29.661
29.518
29.337
26.151
22.660
14.038
X : parts per Million : 13C

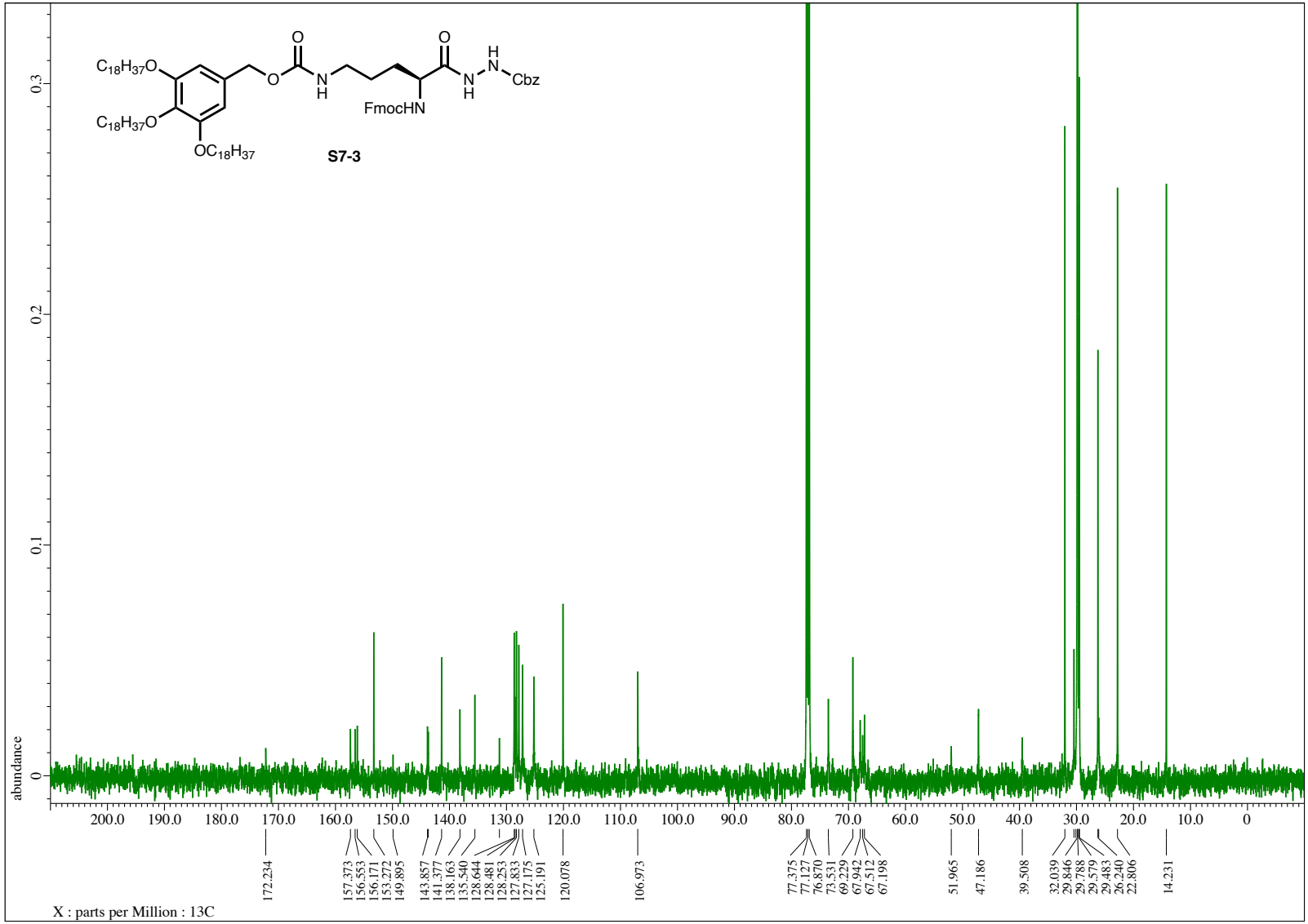

C18H37O
C18H37O
OC18H37
O
O
N
H
O
N
H
FmocHN
NHAlloc
S6-2
abundance
X : parts per Million : 1H
8.619
7.765
7.750
7.594
7.397
7.385
7.260
7.213
6.903
6.541
6.533
6.524
6.505
5.917
5.883
5.872
5.862
5.675
5.664
5.301
5.181
5.160
5.019
4.995
4.931
4.608
4.600
4.428
4.239
4.225
3.936
3.923
3.913
3.520
3.500
3.380
3.212
3.199
3.185
3.176
2.004
1.983
1.964
1.951
1.945
1.927
1.768
1.752
1.455
1.442
1.296
1.255
1.142
0.893
0.879
0.865

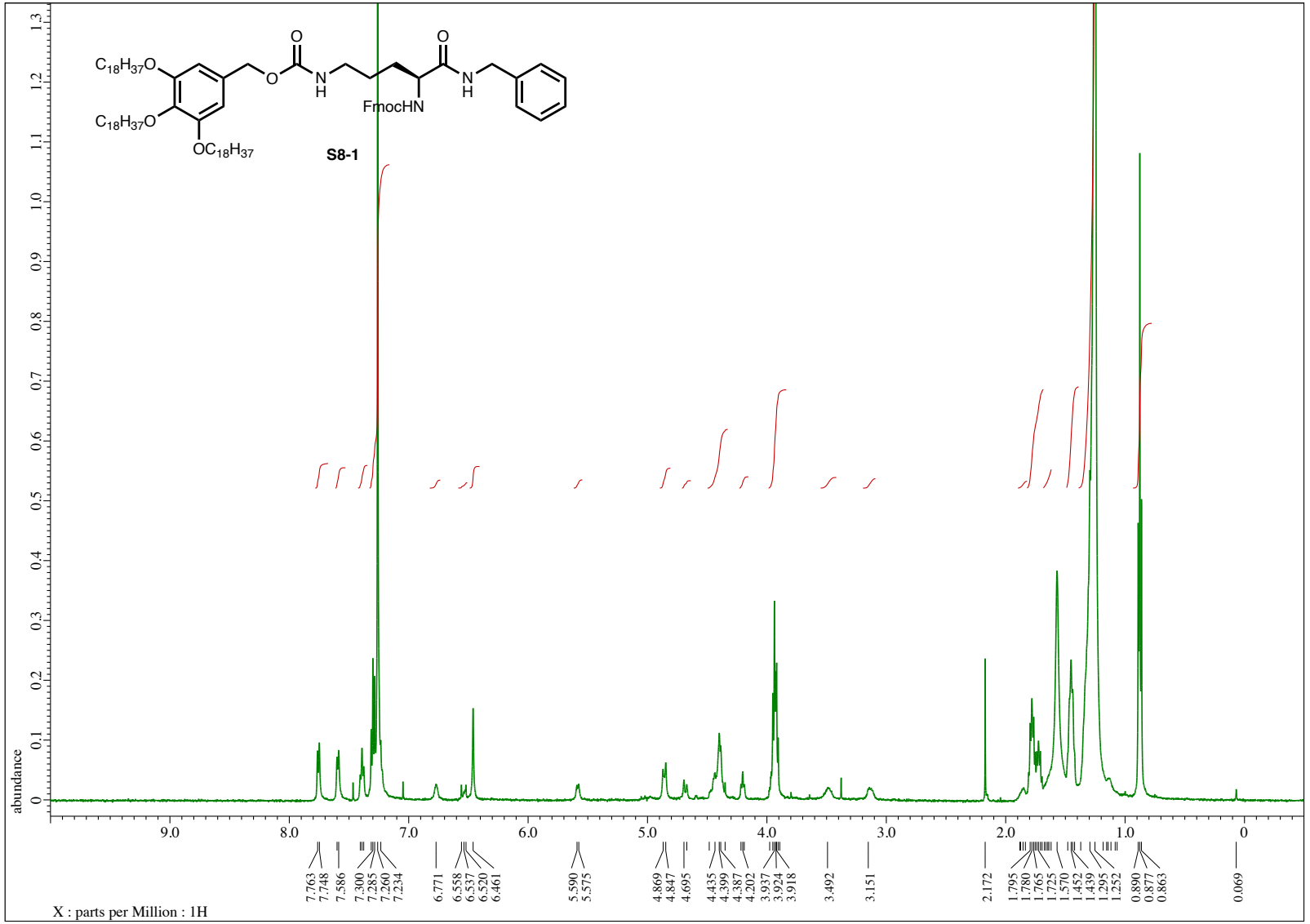

C18H37O
C18H37O
OC18H37
O
O
N
H
O
N
H
FmocHN
NHAlloc
S6-2
abundance
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
166.803
165.153
157.227
156.569
153.307
143.749
141.365
140.563
138.541
132.484
131.130
127.744
127.095
126.628
125.254
125.016
124.892
119.980
118.034
107.122
77.258
77.000
76.742
73.452
69.350
67.347
67.223
66.069
54.499
47.279
31.912
30.377
29.699
29.652
29.509
29.451
29.327
26.151
22.651
14.028
X : parts per Million : 13C

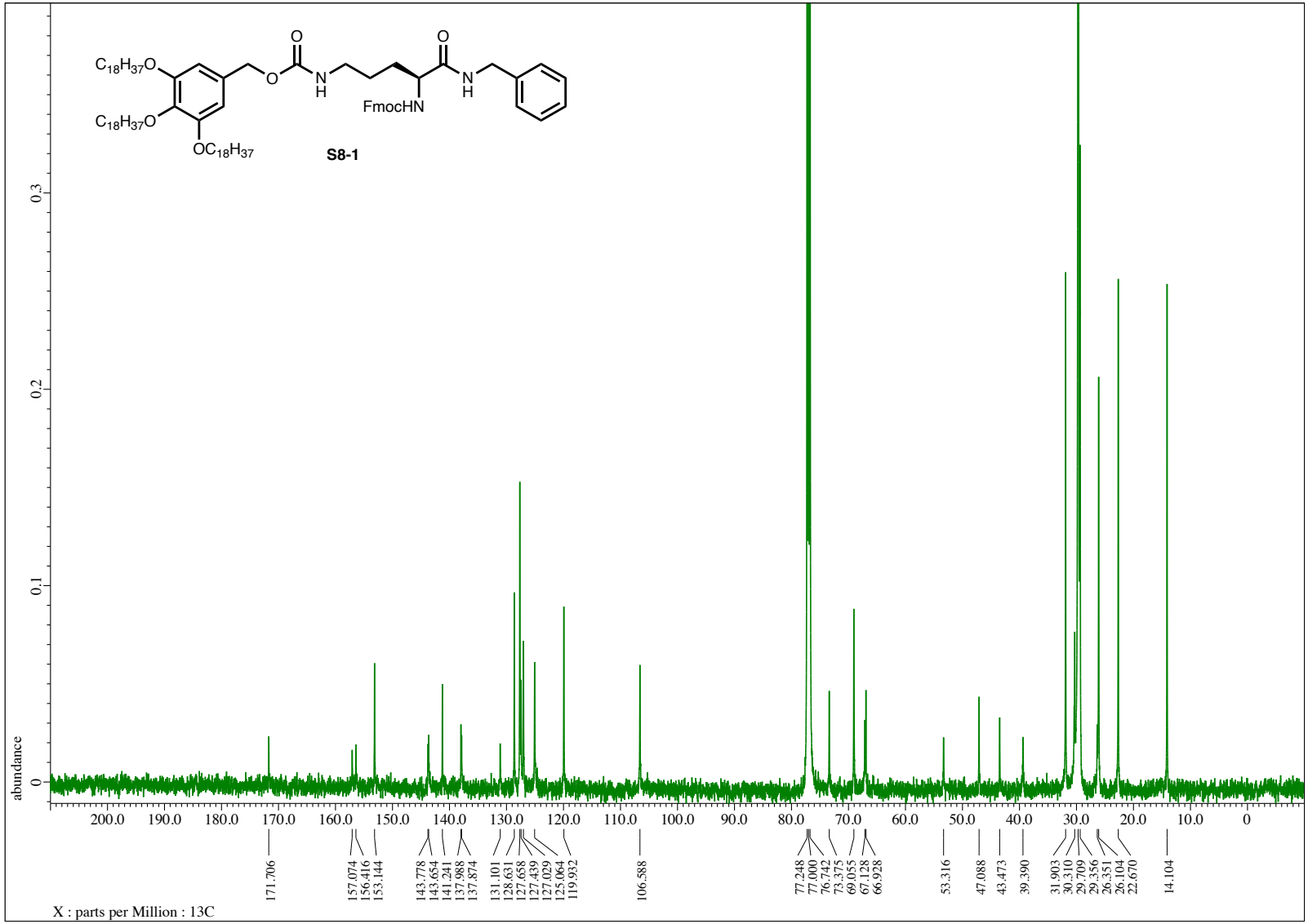

S7-1
FmocHN
abundance
X : parts per Million : 1H

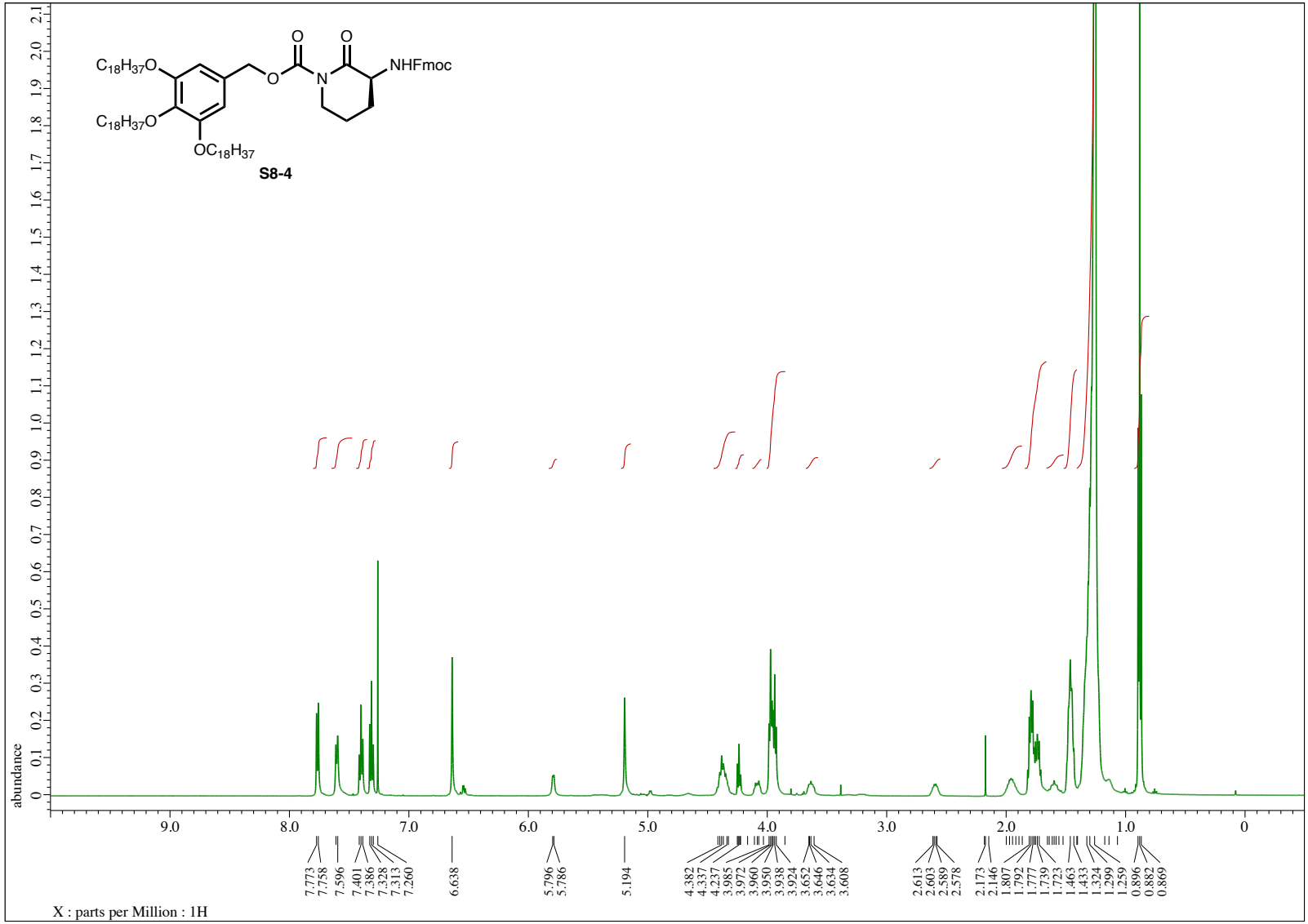

C18H37O
C18H37O
OC18H37
O
O
N
H
FmocHN
O
N
H
H
N
S7-1
abundance
0
0.1
0.2
0.3
0.4
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
172.049
157.198
156.511
153.173
147.679
146.677
143.692
143.587
141.269
138.064
131.054
129.165
127.725
127.067
125.016
121.124
119.970
113.398
106.731
77.248
77.000
76.742
73.404
69.102
67.385
67.042
52.038
47.107
39.457
31.912
29.709
29.652
29.442
29.356
26.113
22.670
14.104
X : parts per Million : 13C

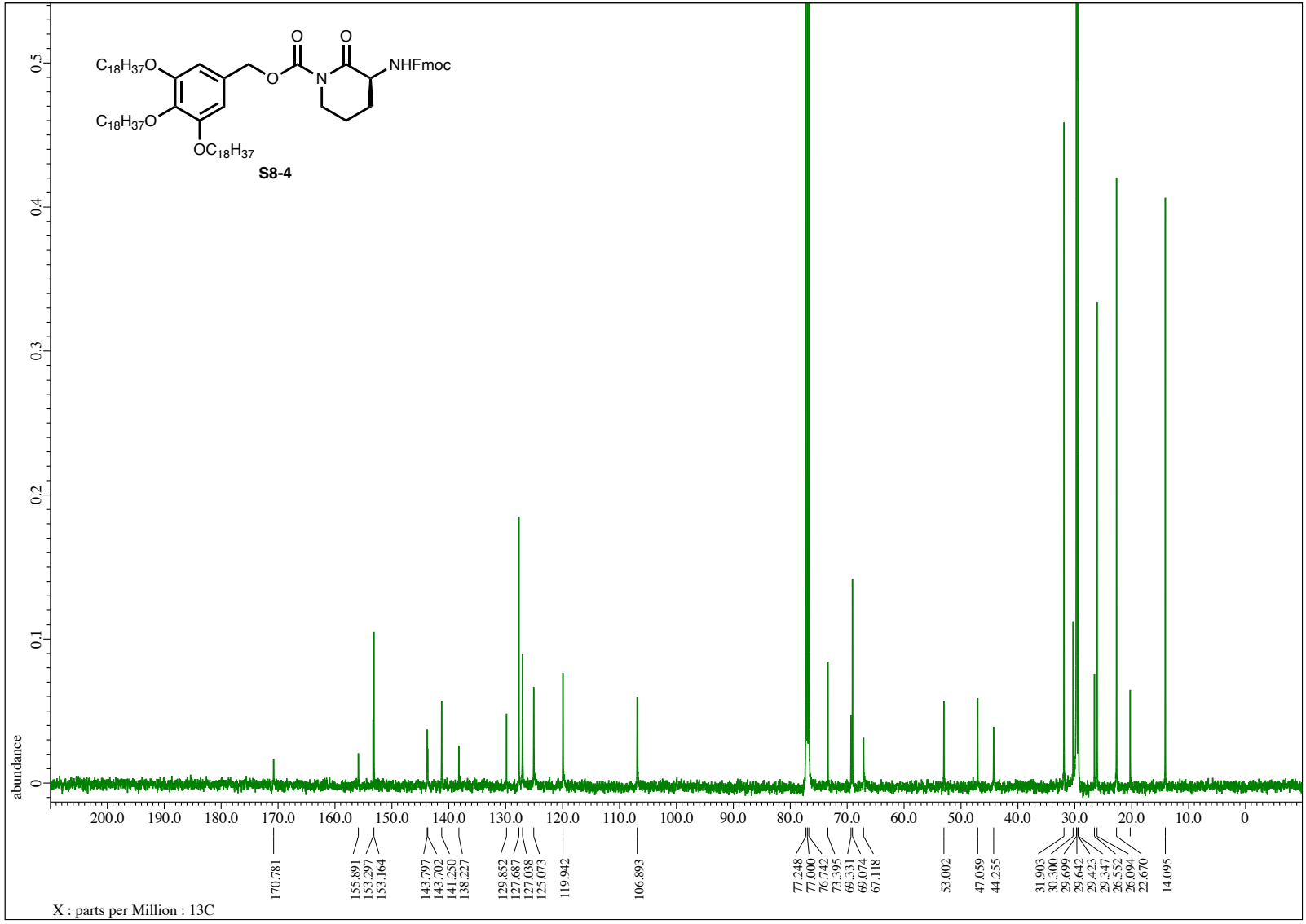

S7-3
FmocHN
Cbz
abundance
X : parts per Million : 1H
8.596
7.752
7.738
7.575
7.305
7.292
7.266
7.260
6.844
6.564
6.528
5.674
5.659
5.132
5.118
4.999
4.975
4.907
4.886
4.596
4.479
4.451
4.383
4.372
4.181
3.968
3.942
3.930
3.917
3.799
3.641
3.424
3.142
2.178
1.779
1.767
1.753
1.738
1.723
1.712
1.452
1.439
1.257
0.893
0.884
0.869
0.076

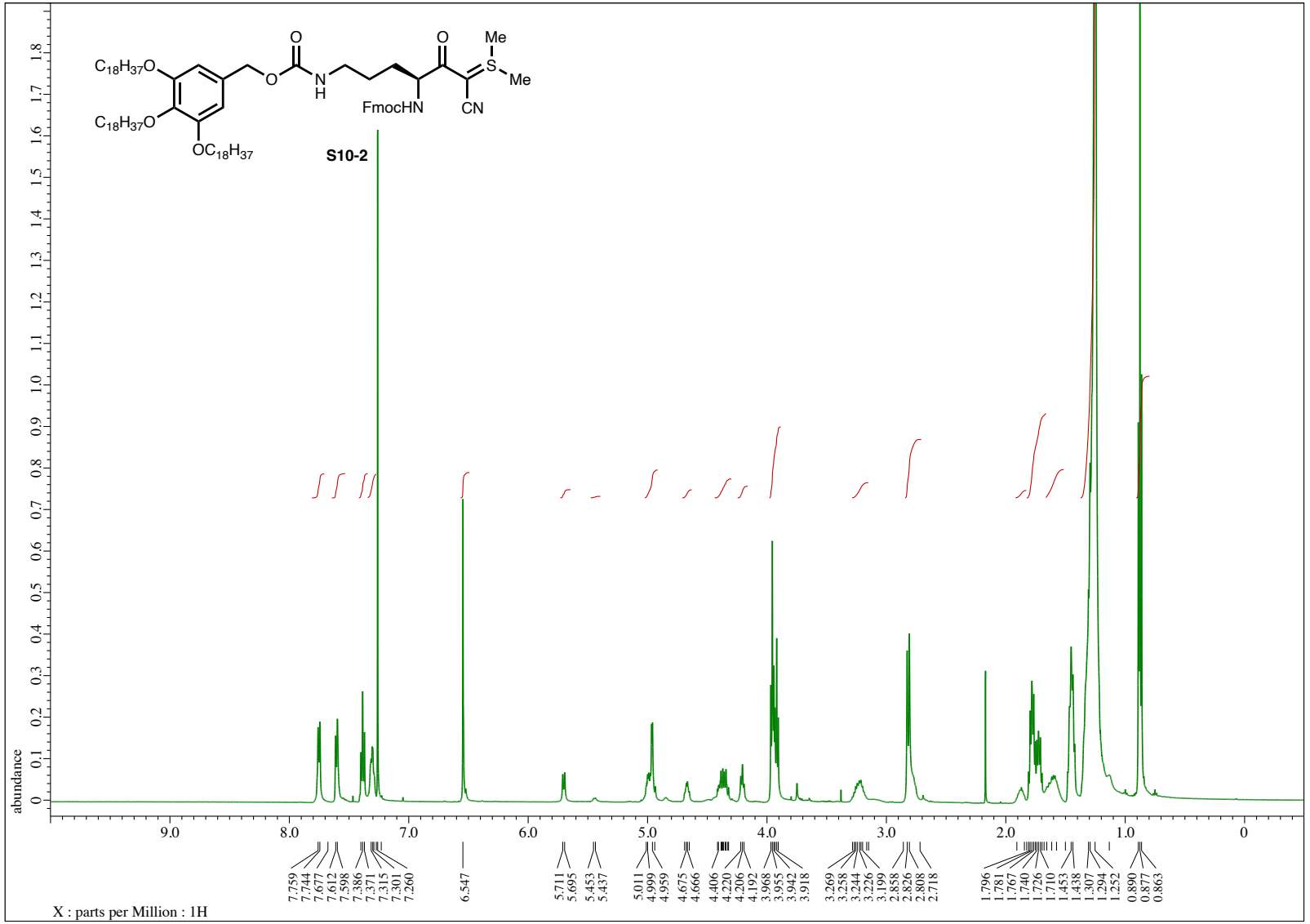

C18H37O
C18H37O
OC18H37
O
O
N
H
O
N
H
H
N
Cbz
FmocHN
S7-3
abundance
0.3
0.2
0.1
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
172.234
157.373
156.553
156.171
153.272
149.895
143.857
141.377
138.163
135.540
128.644
128.481
128.253
127.833
127.175
125.191
120.078
106.973
77.375
77.127
76.870
73.531
69.229
67.942
67.512
67.198
51.965
47.186
39.508
32.039
29.846
29.788
29.579
29.483
26.240
22.806
14.231
X : parts per Million : 13C

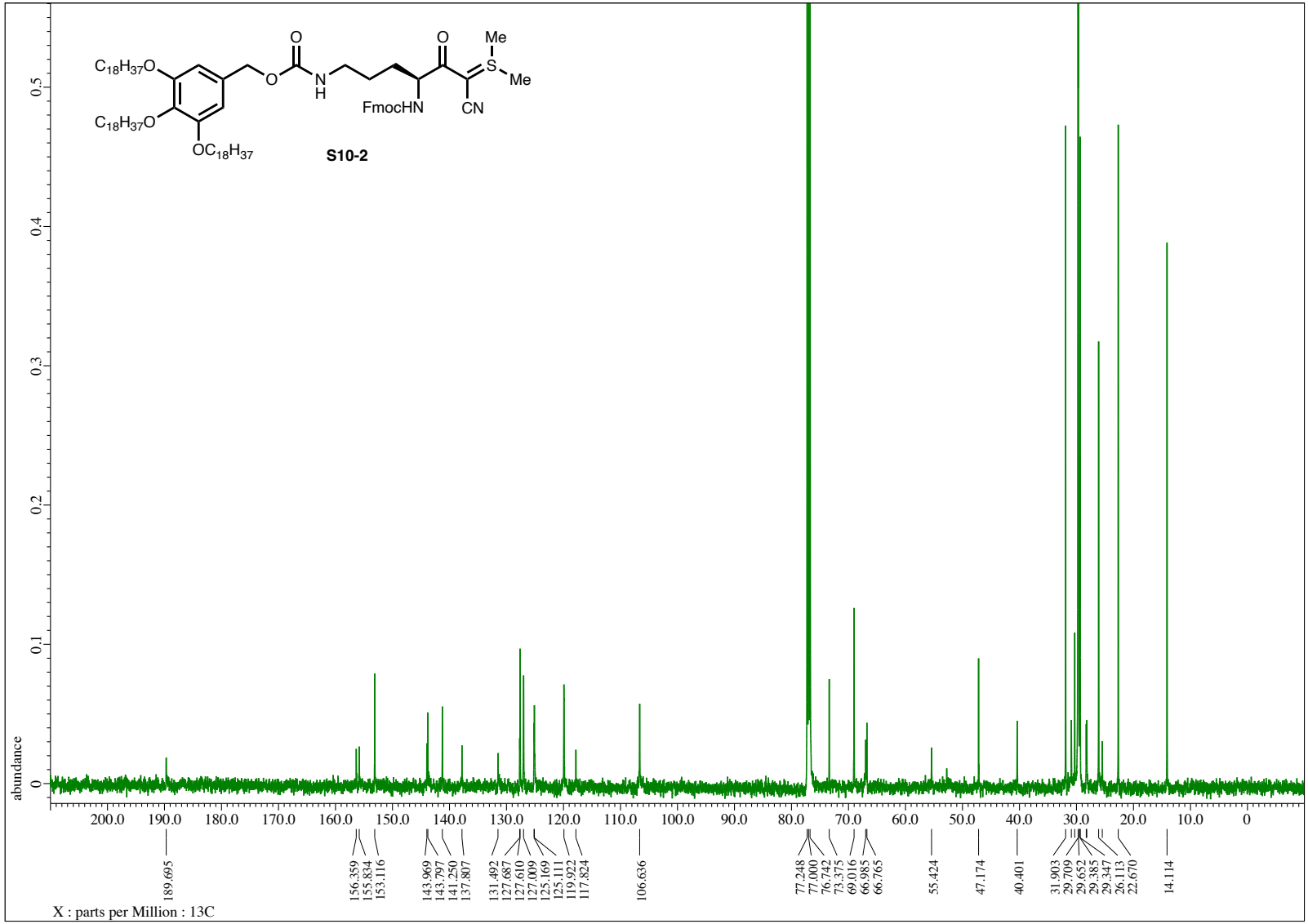

C18H37O
C18H37O
OC18H37
O
O
N
H
O
N
H
FmocHN
S8-1
abundance
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.763
7.748
7.586
7.300
7.285
7.260
7.234
6.771
6.558
6.537
6.520
6.461
5.590
5.575
4.869
4.847
4.695
4.435
4.399
4.387
4.202
3.937
3.924
3.918
3.492
3.151
2.172
1.795
1.780
1.765
1.725
1.570
1.452
1.439
1.295
1.252
0.890
0.877
0.863
0.069
X : parts per Million : 1H

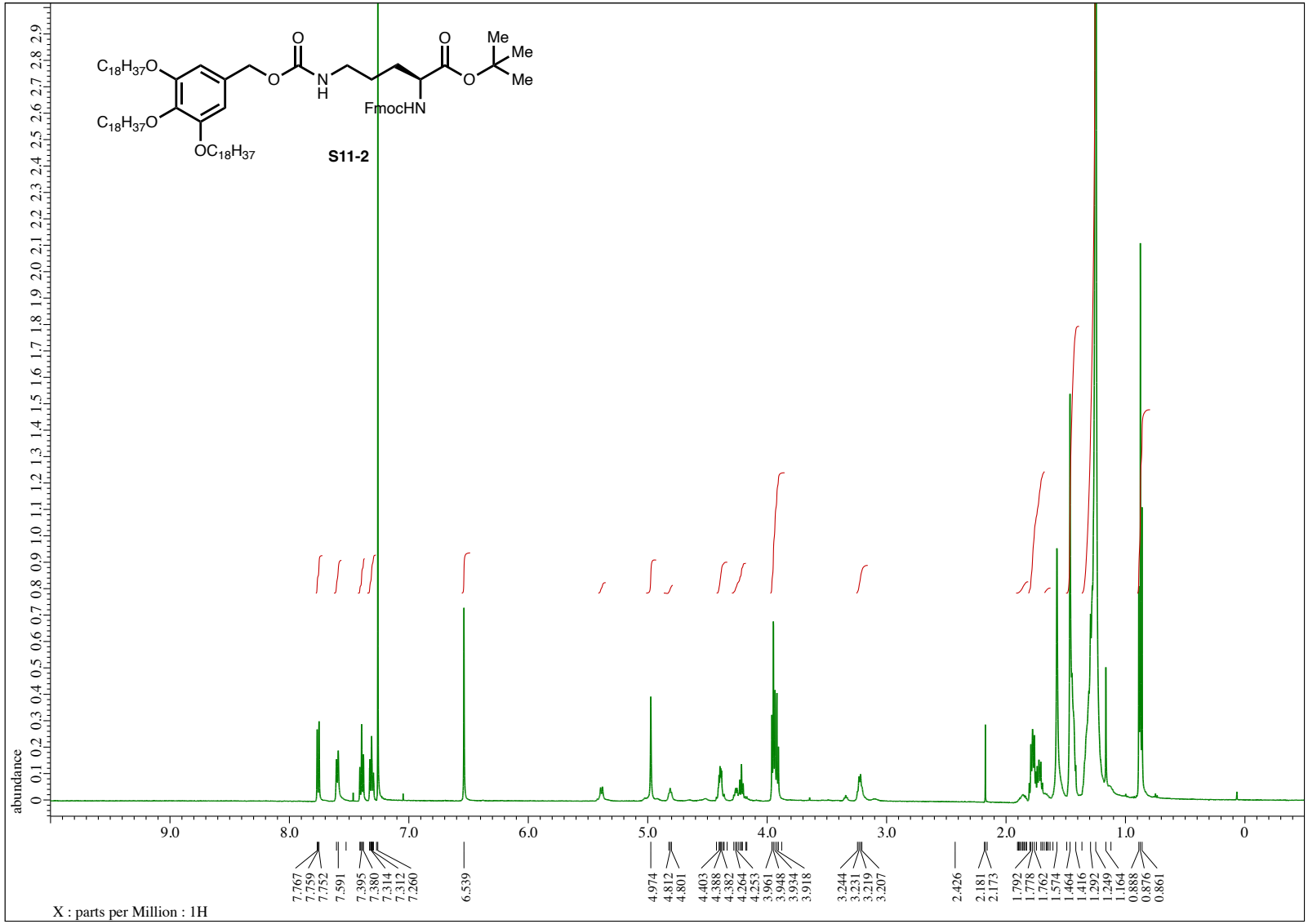

C18H37O
C18H37O
OC18H37
O
O
N
H
O
N
H
FmocHN
S8-1
abundance
0
0.1
0.2
0.3
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
171.706
157.074
156.416
153.144
143.778
143.654
141.241
137.988
137.874
131.101
128.631
127.658
127.439
127.029
125.064
119.932
106.588
77.248
77.000
76.742
73.375
69.055
67.128
66.928
53.316
47.088
43.473
39.390
31.903
30.310
29.709
29.356
26.351
26.104
22.670
14.104
X : parts per Million : 13C

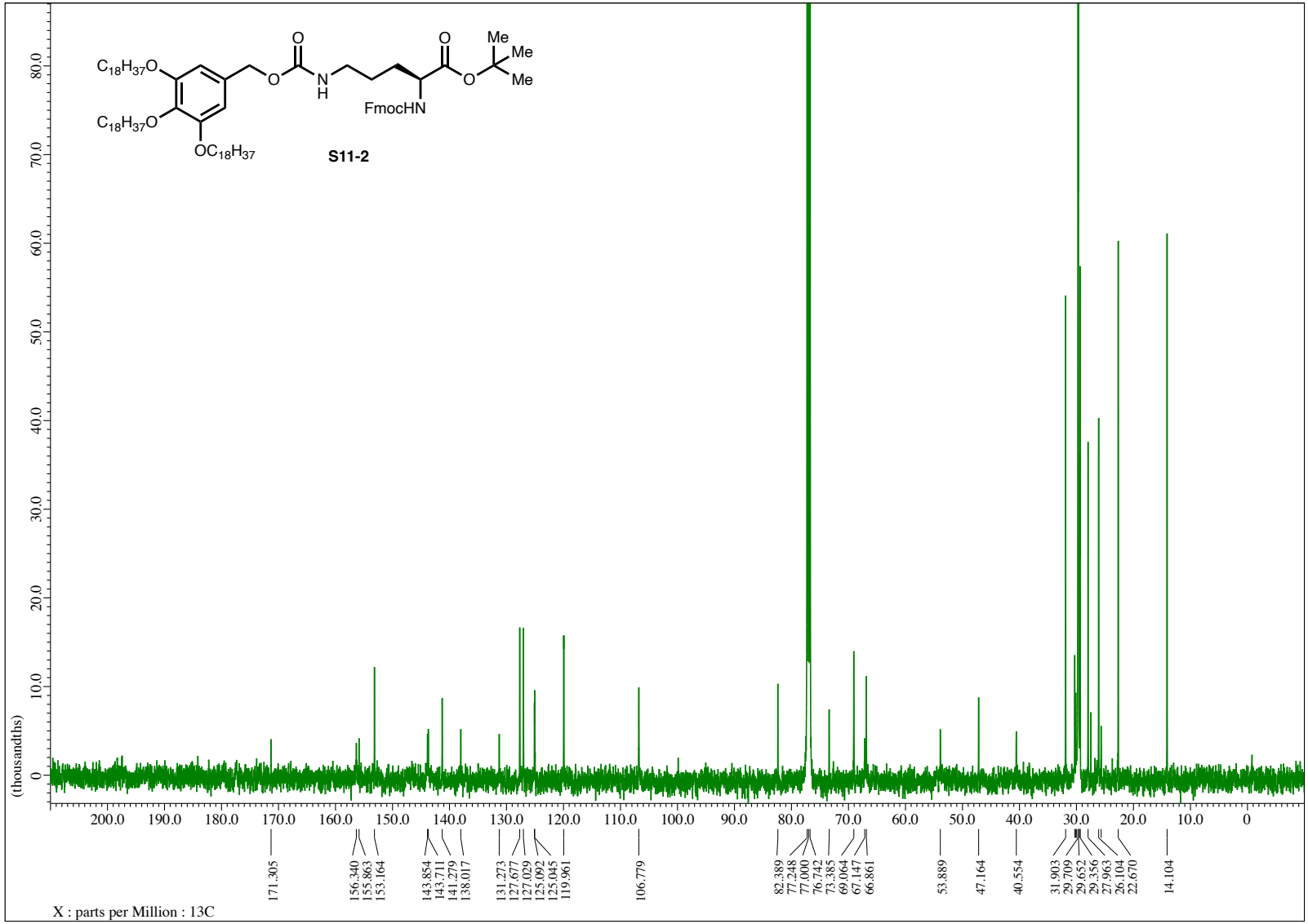

C18H37O
C18H37O
OC18H37
O
O
O
N
NHFmoc
S8-4
abundance
X : parts per Million : 1H
7.773
7.758
7.596
7.401
7.386
7.328
7.313
7.260
6.638
5.796
5.786
5.194
4.382
4.337
4.237
3.985
3.972
3.960
3.950
3.938
3.924
3.652
3.646
3.634
3.608
2.613
2.603
2.589
2.578
2.173
2.146
1.807
1.792
1.777
1.739
1.723
1.463
1.433
1.324
1.299
1.259
0.896
0.882
0.869

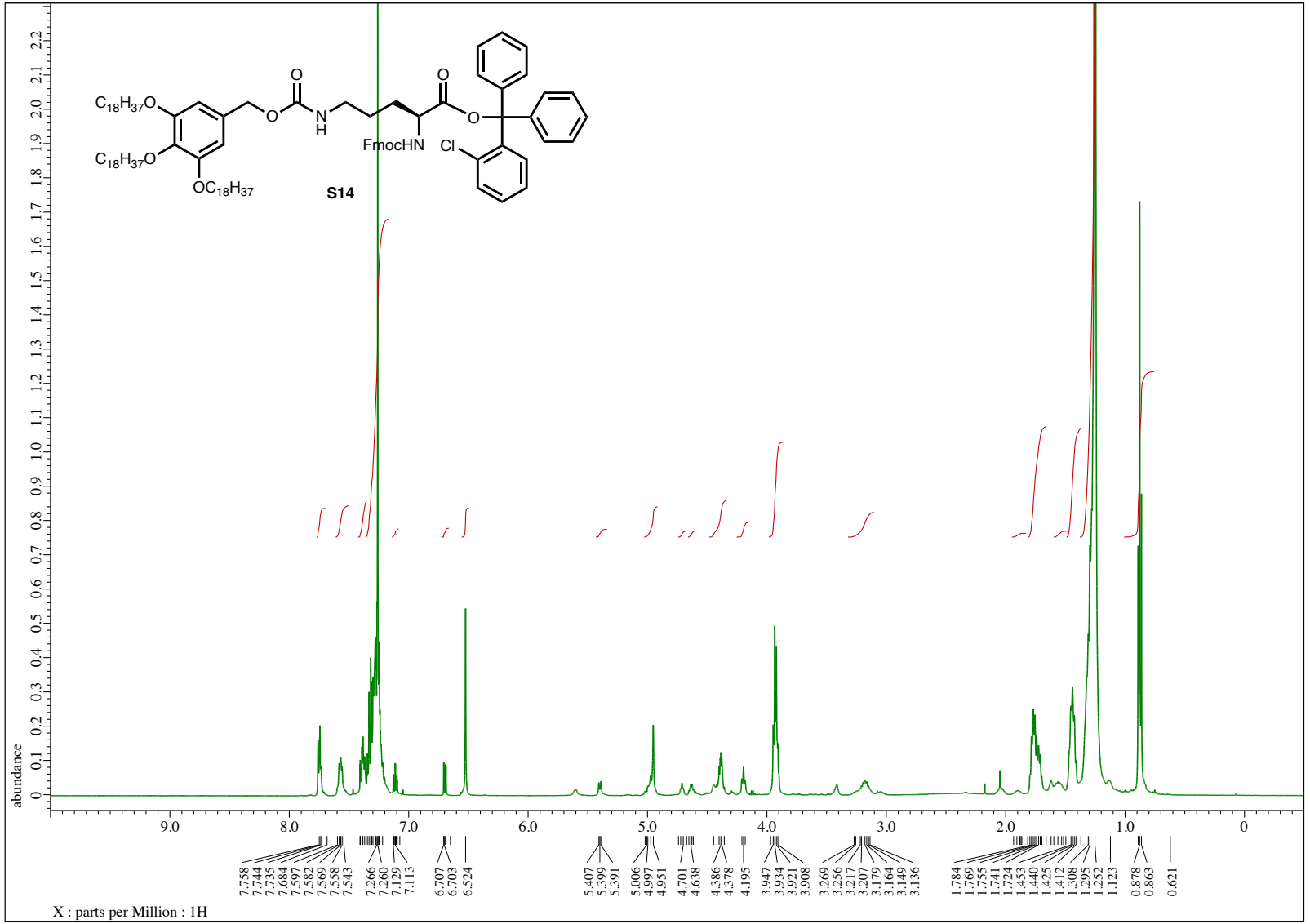

C18H37O
C18H37O
OC18H37
O
O
O
N
NHFmoc
S8-4
abundance
0
0.1
0.2
0.3
0.4
0.5
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
170.781
155.891
153.297
153.164
143.797
143.702
141.250
138.227
129.852
127.687
127.038
125.073
119.942
106.893
77.248
77.000
76.742
73.395
69.331
69.074
67.118
53.002
47.059
44.255
31.903
30.300
29.699
29.642
29.423
29.347
26.552
26.094
22.670
14.095
X : parts per Million : 13C

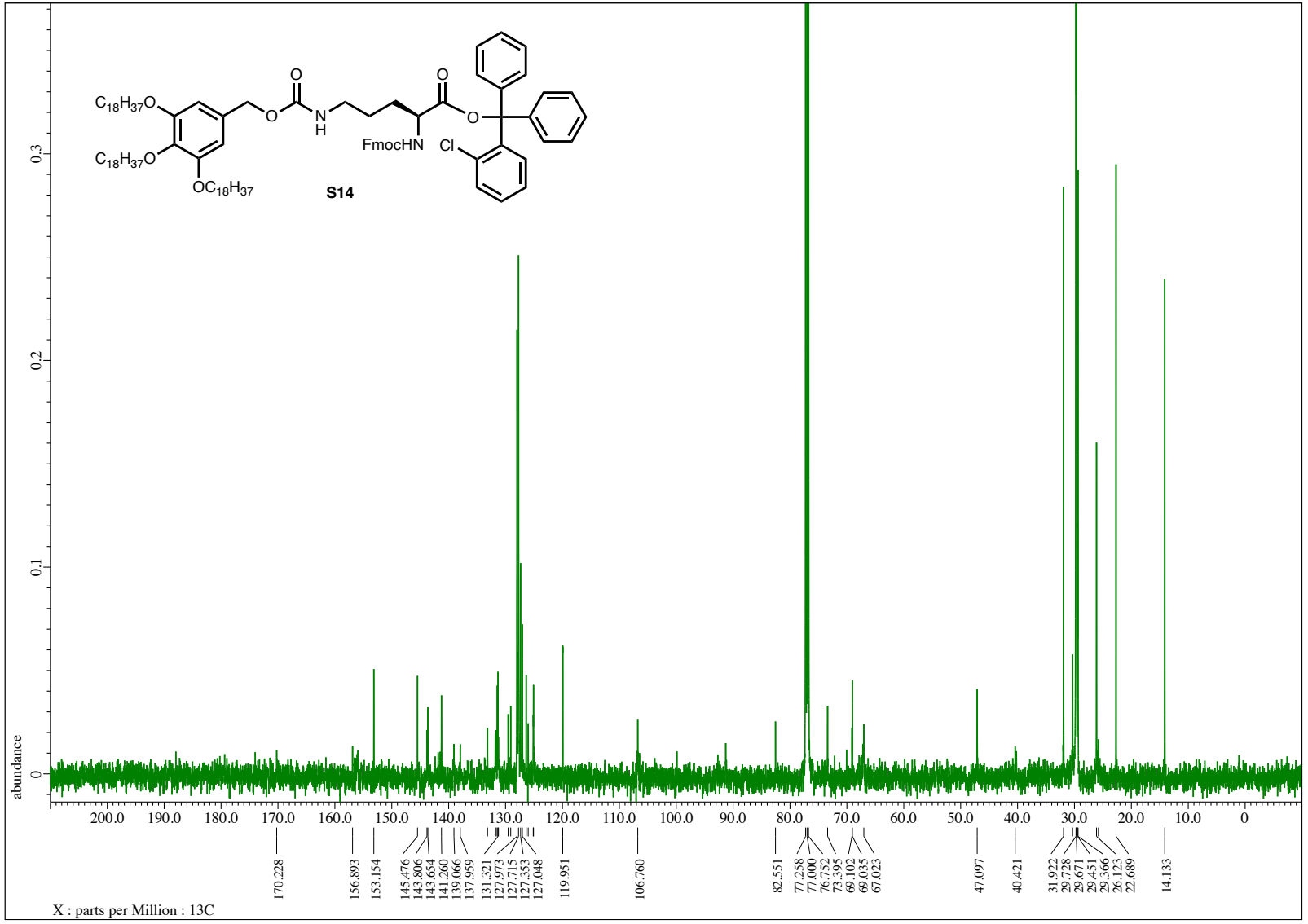

S10-2
abundance
X : parts per Million : 1H

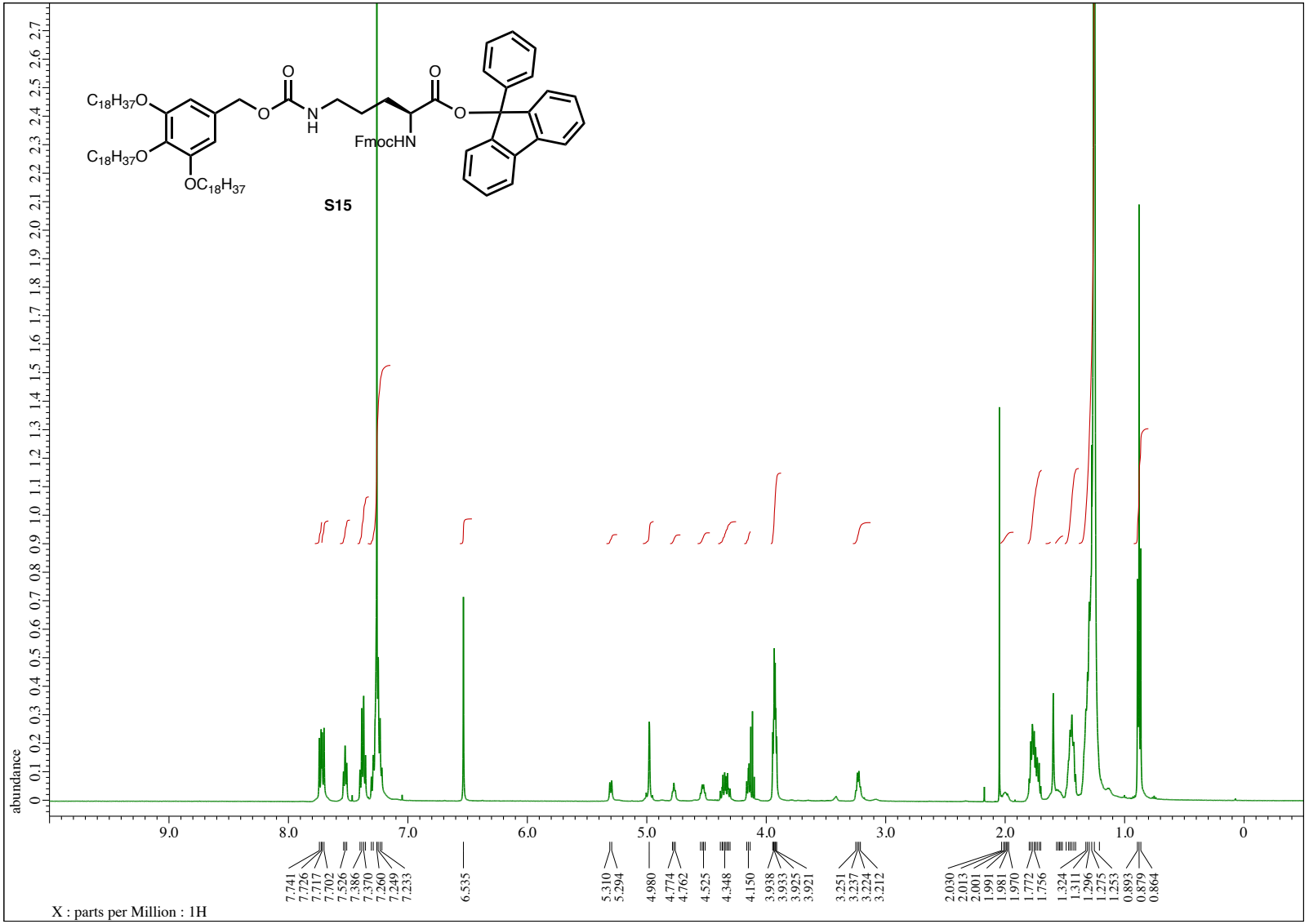

C18H37O
C18H37O
OC18H37
O
O
N
H
FmocHN
O
Me
S
Me
CN
S10-2
abundance
0.5
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
189.695
156.359
155.834
153.116
143.969
143.797
141.250
137.807
131.492
127.687
127.610
127.009
125.169
125.111
119.922
117.824
106.636
77.248
77.000
76.742
73.375
69.016
66.985
66.765
55.424
47.174
40.401
31.903
29.709
29.652
29.385
29.347
26.113
22.670
14.114
X : parts per Million : 13C

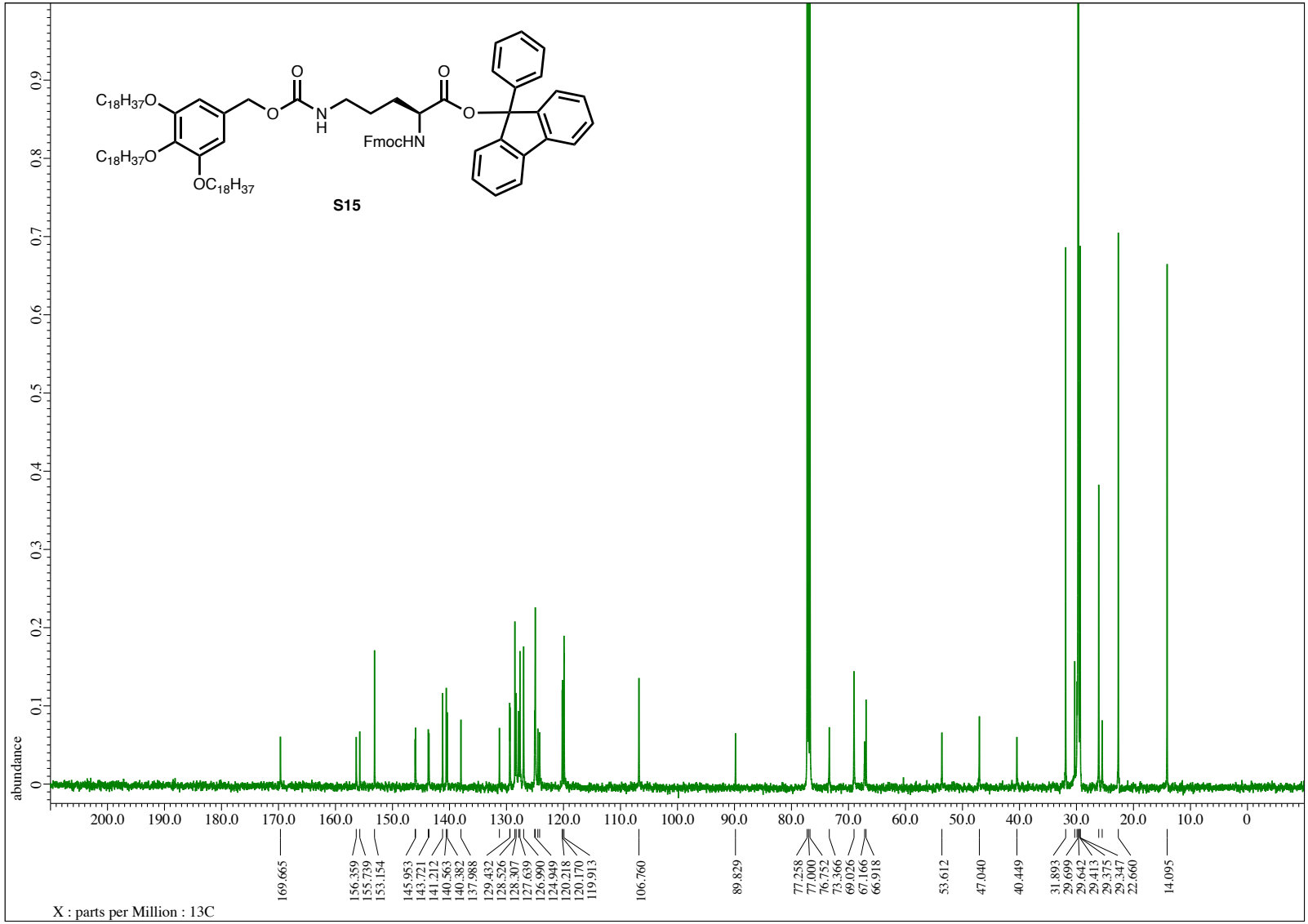

S11-2
abundance
X : parts per Million : 1H
7.767
7.759
7.752
7.591
7.395
7.380
7.314
7.312
7.260
6.539
4.974
4.812
4.801
4.403
4.388
4.382
4.264
4.253
3.961
3.948
3.934
3.918
3.244
3.231
3.219
3.207
2.426
2.181
2.173
1.792
1.778
1.762
1.574
1.464
1.416
1.292
1.249
1.164
0.888
0.876
0.861

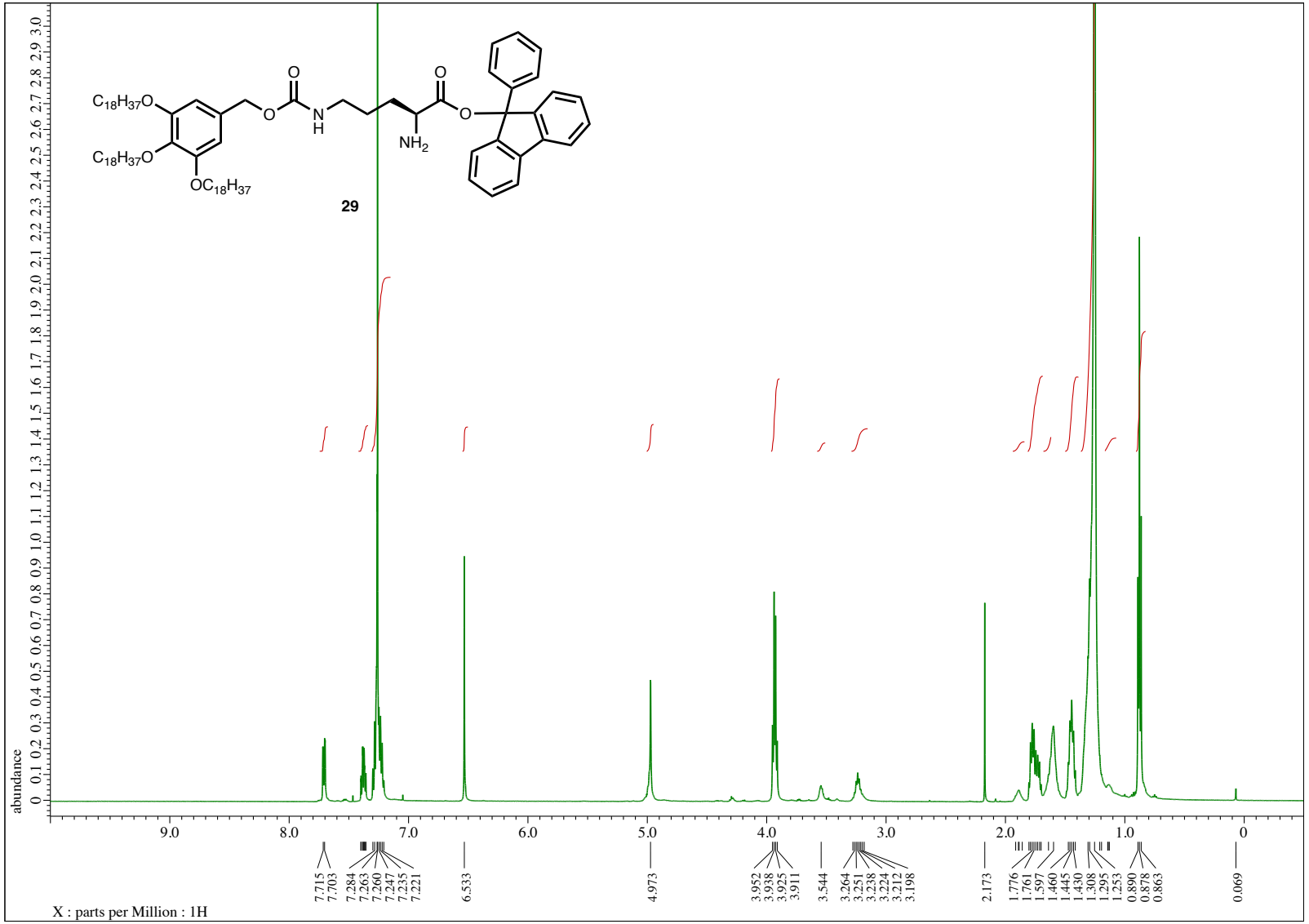

S11-2
(thousandths)
X : parts per Million : 13C
171.305
156.340
155.863
153.164
143.854
143.711
141.279
138.017
131.273
127.677
127.029
125.092
125.045
119.961
106.779
82.389
77.248
77.000
76.742
73.385
69.064
67.147
66.861
53.889
47.164
40.554
31.903
29.709
29.652
29.356
27.963
26.104
22.670
14.104

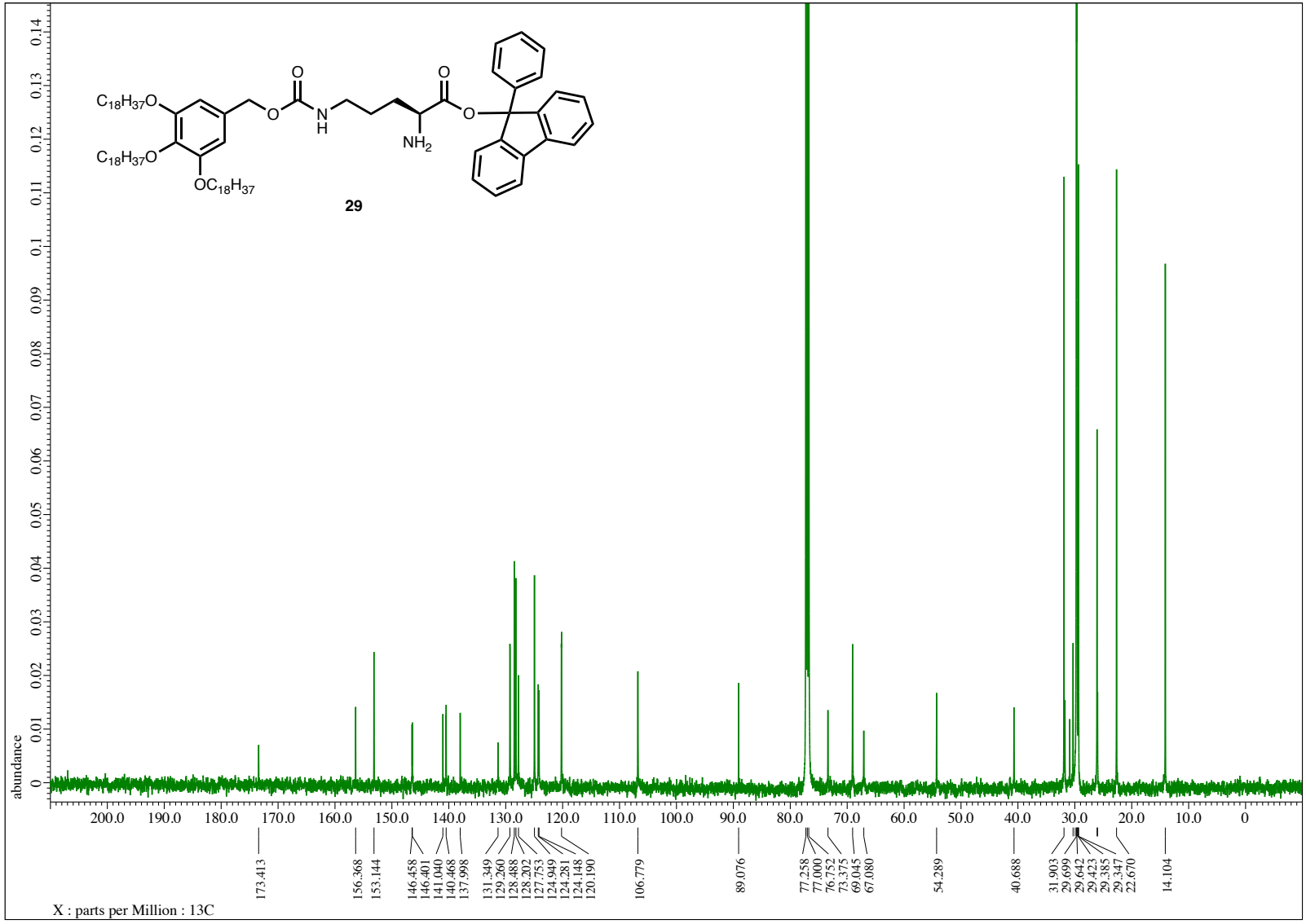

S14
abundance
X : parts per Million : 1H

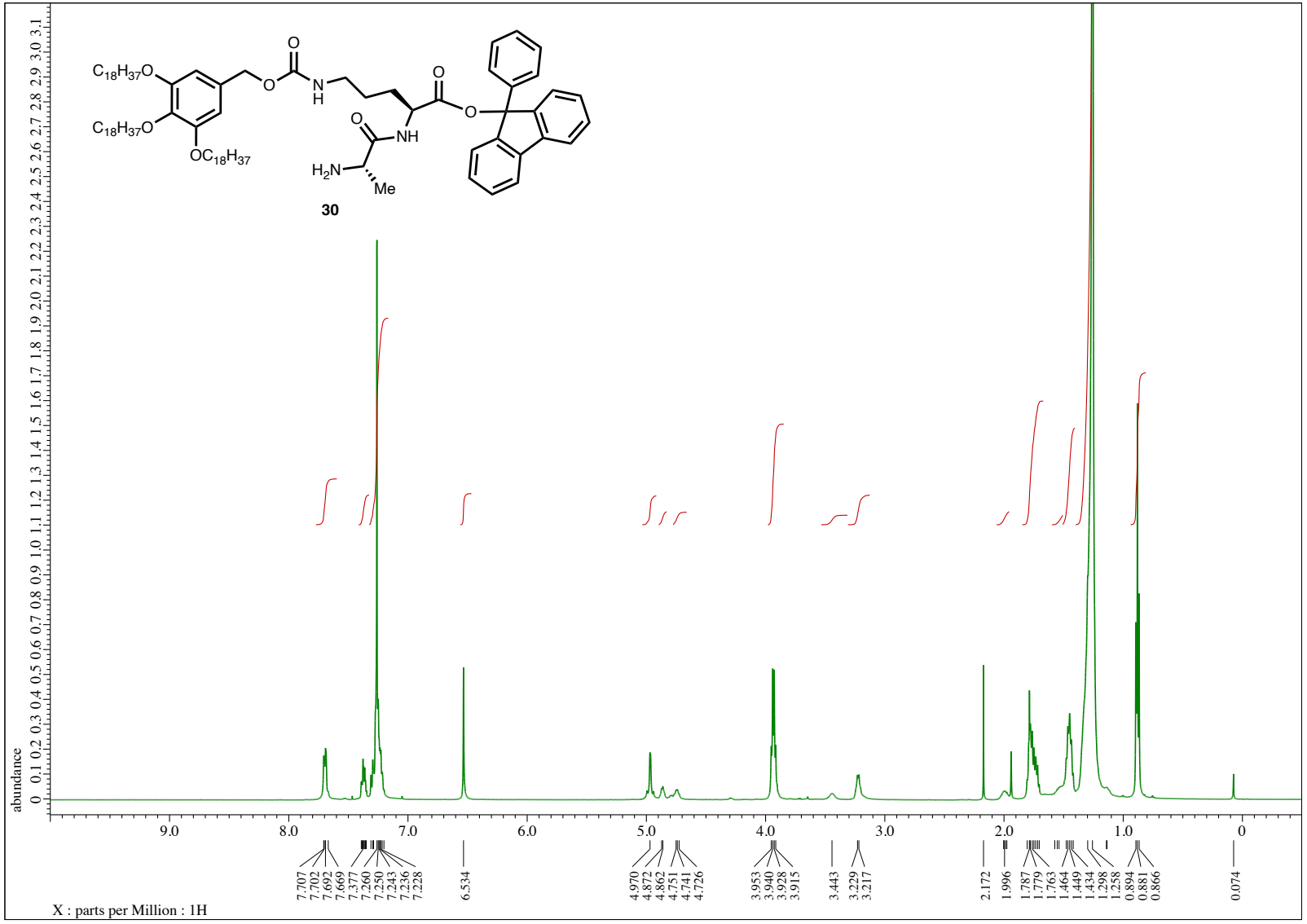

S14
abundance
X : parts per Million : 13C
170.228
156.893
153.154
145.476
143.806
143.654
141.260
139.066
137.959
131.321
127.973
127.715
127.353
127.048
119.951
106.760
82.551
77.258
77.000
76.752
73.395
69.102
69.035
67.023
47.097
40.421
31.922
29.728
29.671
29.451
29.366
26.123
22.689
14.133

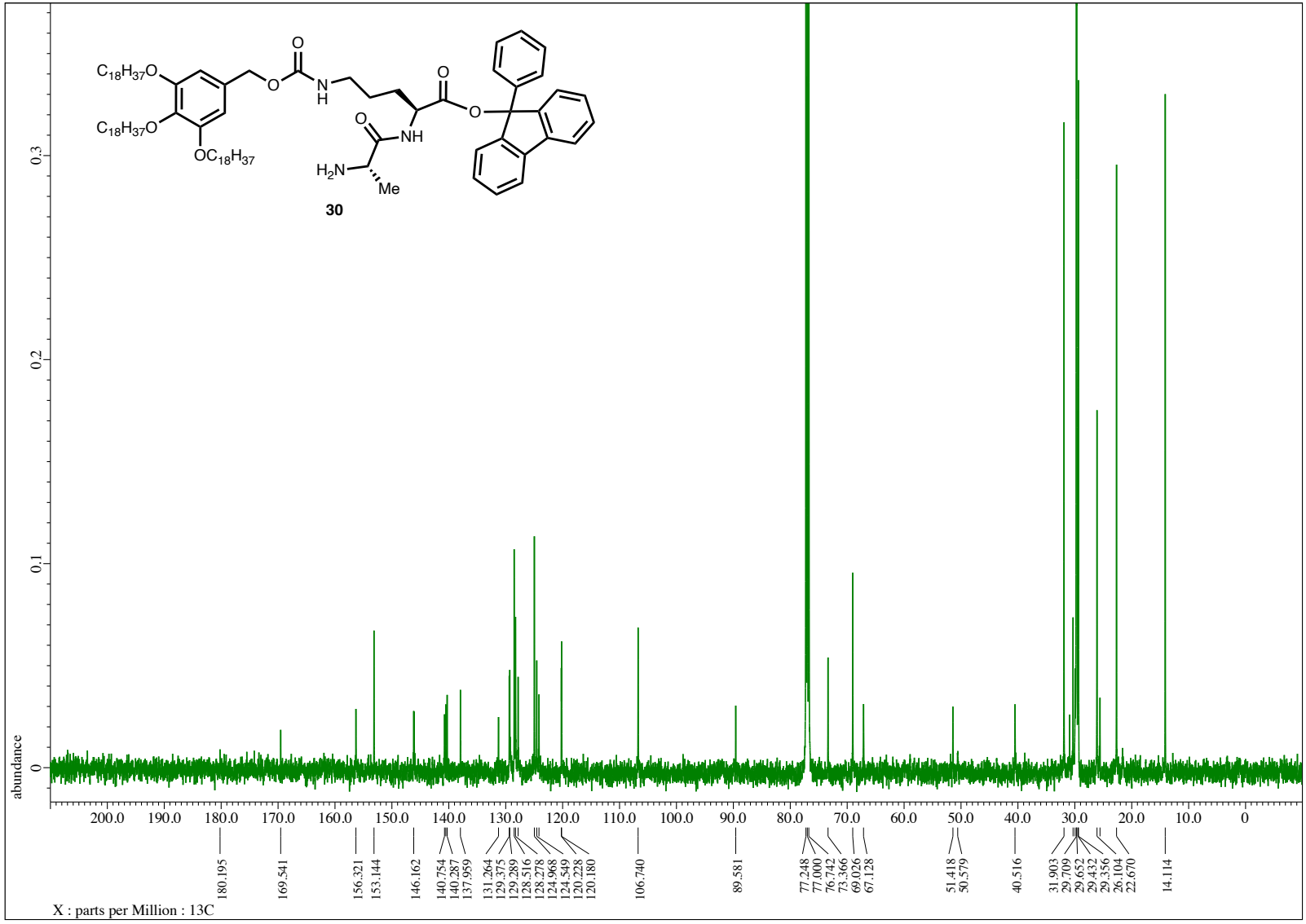

S15
abundance
X : parts per Million : 1H

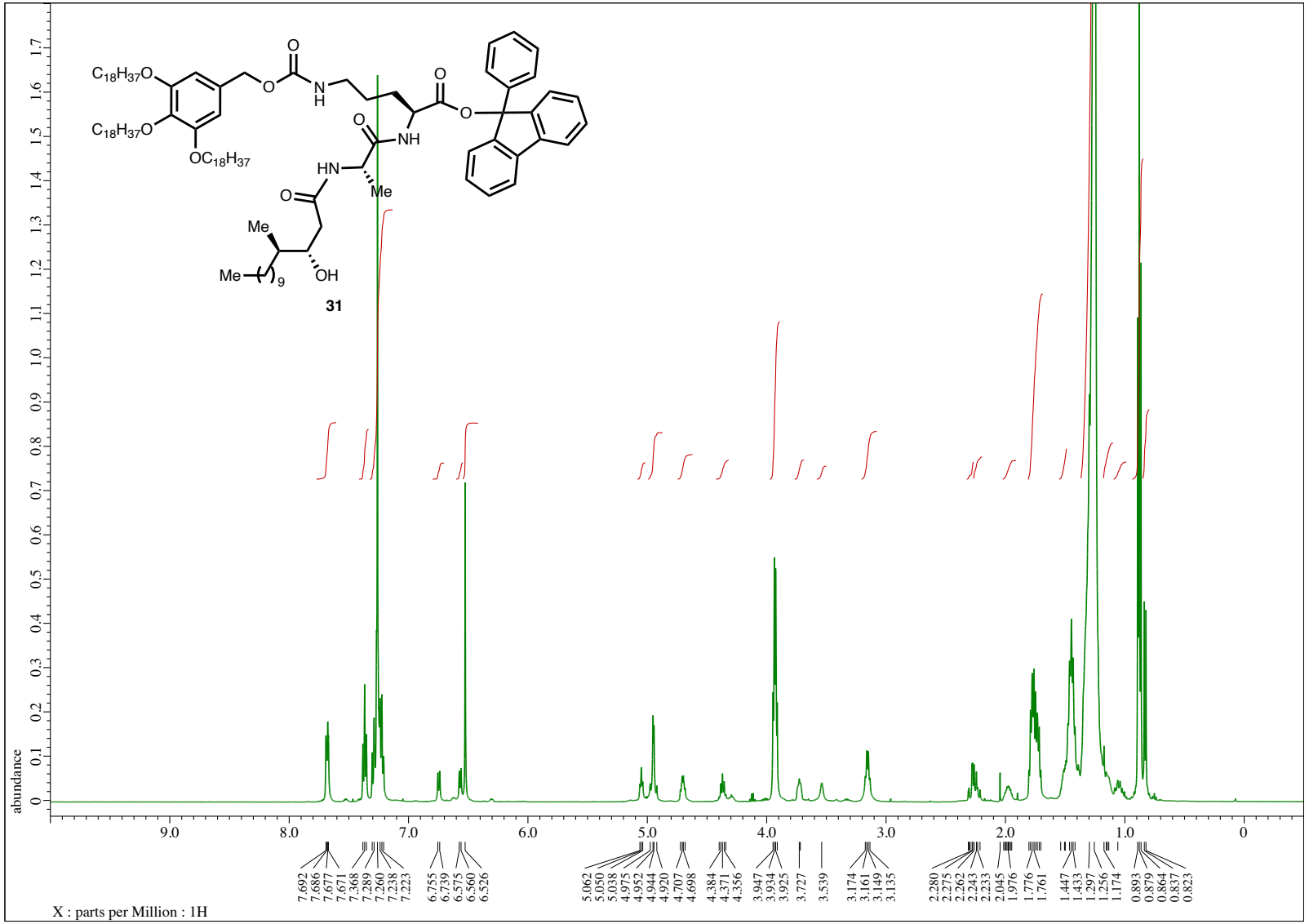

C18H37O
C18H37O
OC18H37
O
O
N
H
FmocHN
O
O
S15
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
169.665
156.359
155.739
153.154
145.953
143.721
141.212
140.563
140.382
137.988
129.432
128.526
128.307
127.639
126.990
124.949
120.218
120.170
119.913
106.760
89.829
77.258
77.000
76.752
73.366
69.026
67.166
66.918
53.612
47.040
40.449
31.893
29.699
29.642
29.413
29.375
29.347
22.660
14.095
X : parts per Million : 13C

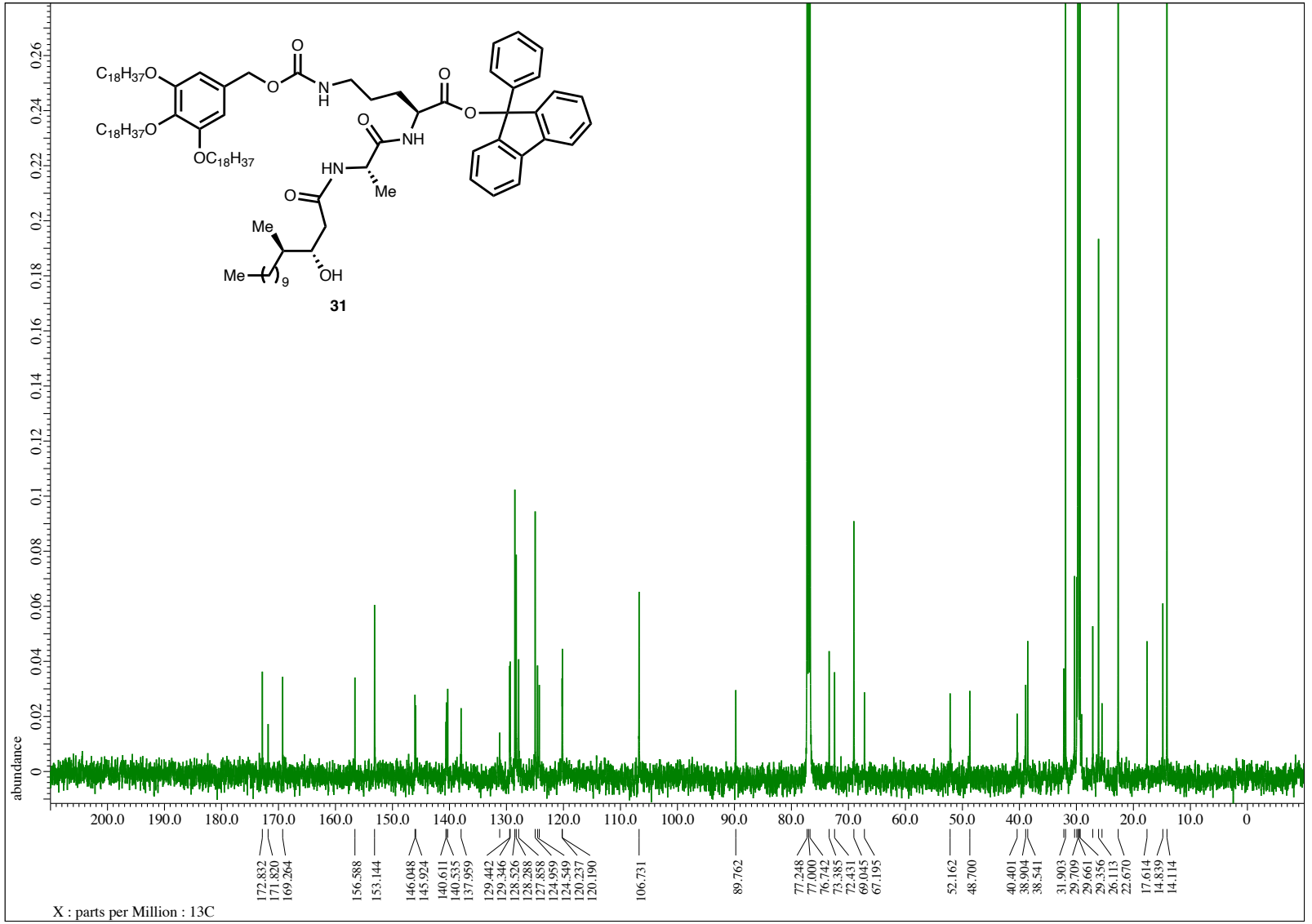

29
abundance
X : parts per Million : 1H
7.715
7.703
7.284
7.263
7.260
7.247
7.235
7.221
6.533
4.973
3.952
3.938
3.925
3.911
3.544
3.264
3.251
3.238
3.224
3.212
3.198
2.173
1.776
1.761
1.597
1.460
1.445
1.430
1.308
1.295
1.253
0.890
0.878
0.863
0.069


C18H37O
C18H37O
OC18H37
O
O
N
H
O
O
NH2
29
abundance
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
173.413
156.368
153.144
146.458
146.401
141.040
140.468
137.998
131.349
129.260
128.488
128.202
127.753
124.949
124.281
124.148
120.190
106.779
89.076
77.258
77.000
76.752
73.375
69.045
67.080
54.289
40.688
31.903
29.699
29.642
29.423
29.385
29.347
22.670
14.104
X : parts per Million : 13C

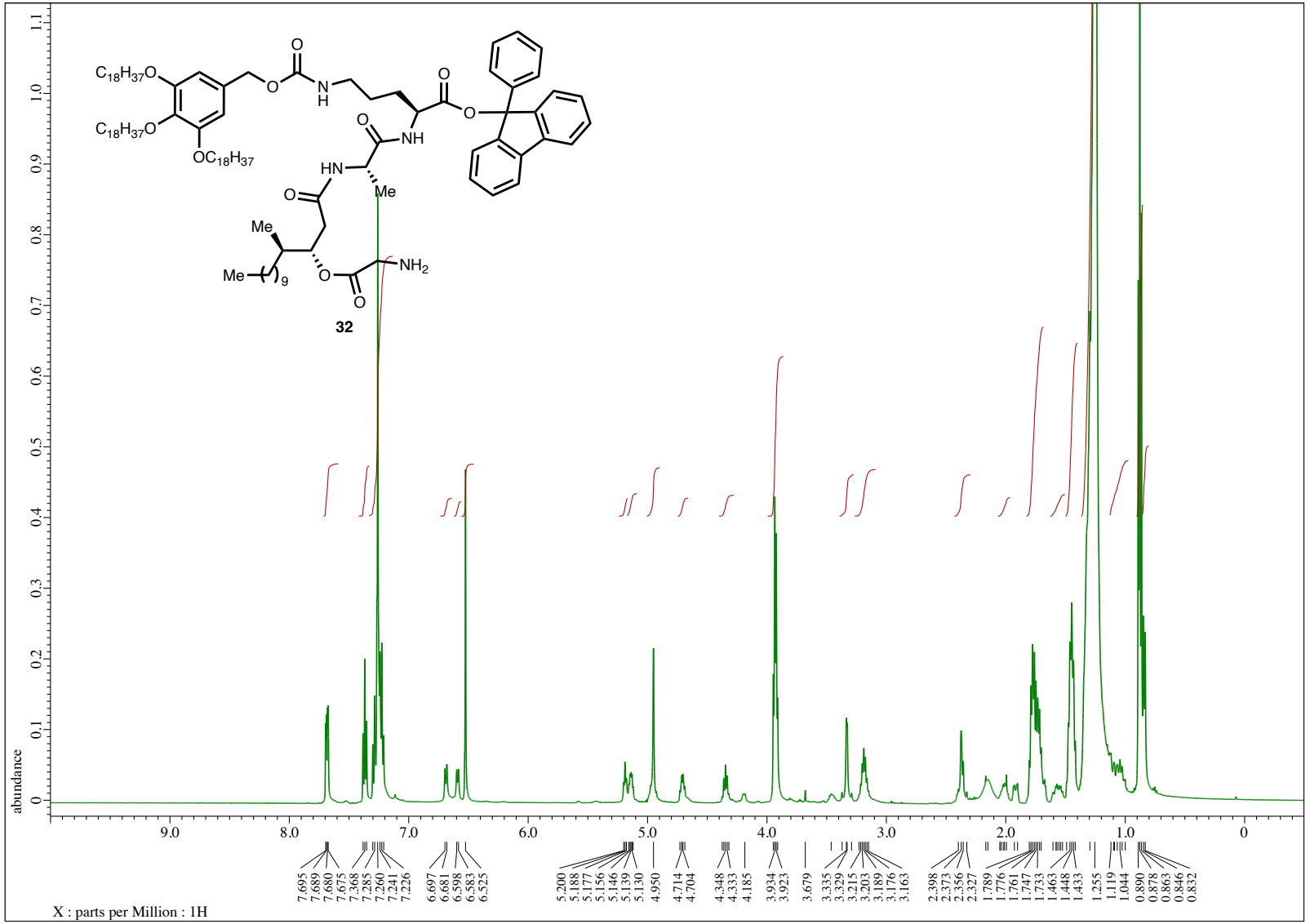

30
abundance
X : parts per Million : 1H
7.707
7.702
7.692
7.669
7.377
7.260
7.250
7.243
7.236
7.228
6.534
4.970
4.872
4.862
4.751
4.741
4.726
3.953
3.940
3.928
3.915
3.443
3.229
3.217
2.172
1.996
1.787
1.779
1.763
1.464
1.449
1.434
1.298
1.258
0.894
0.881
0.866
0.074

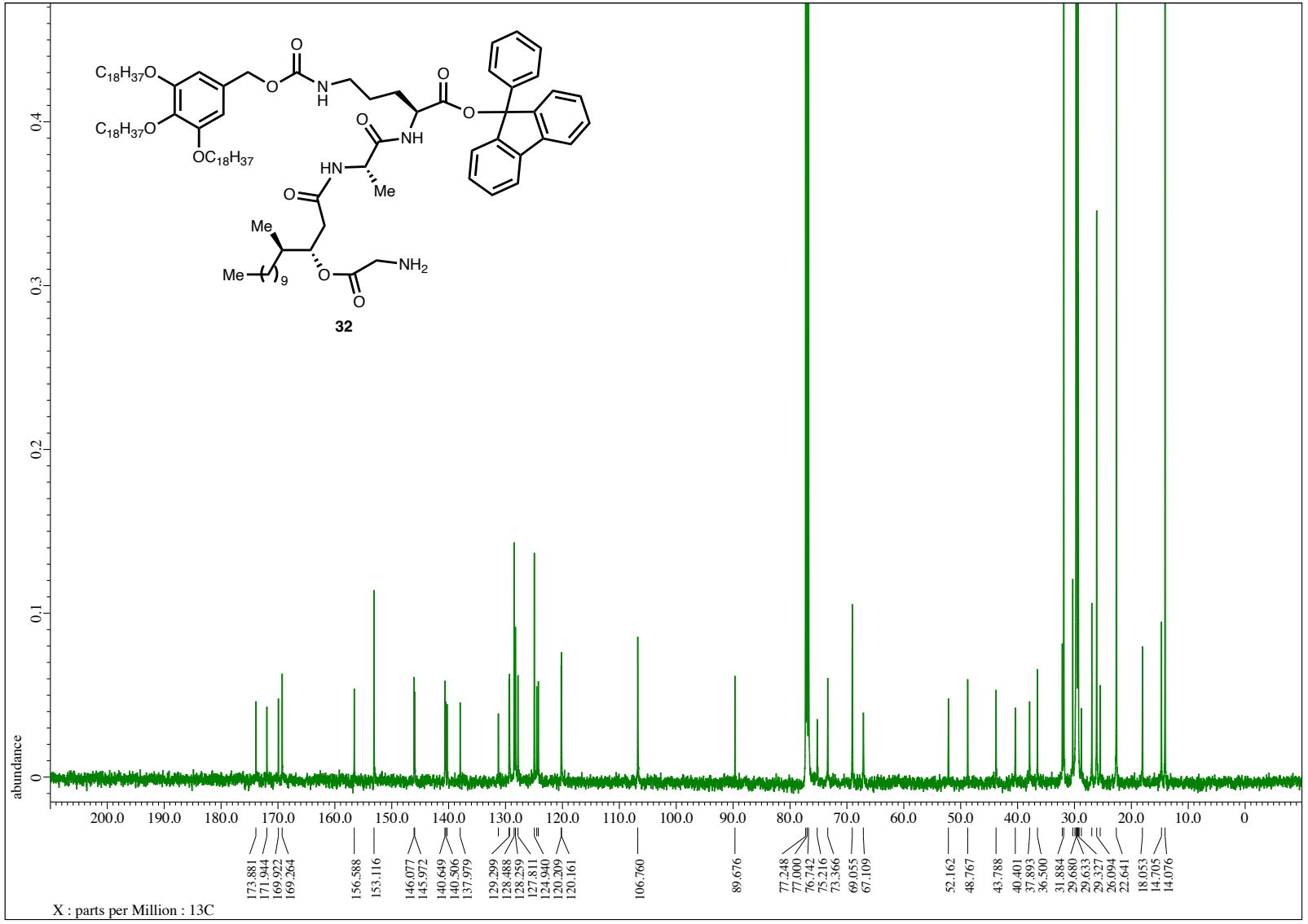

C18H37O
C18H37O
OC18H37
O
O
N
H
O
O
NH
H2N
Me
O
30
abundance
0
0.1
0.2
0.3
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
180.195
169.541
156.321
153.144
146.162
140.754
140.287
137.959
131.264
129.375
129.289
128.516
128.278
124.968
124.549
120.228
120.180
106.740
89.581
77.248
77.000
76.742
73.366
69.026
67.128
51.418
50.579
40.516
31.903
29.709
29.652
29.432
29.356
26.104
22.670
14.114
X : parts per Million : 13C

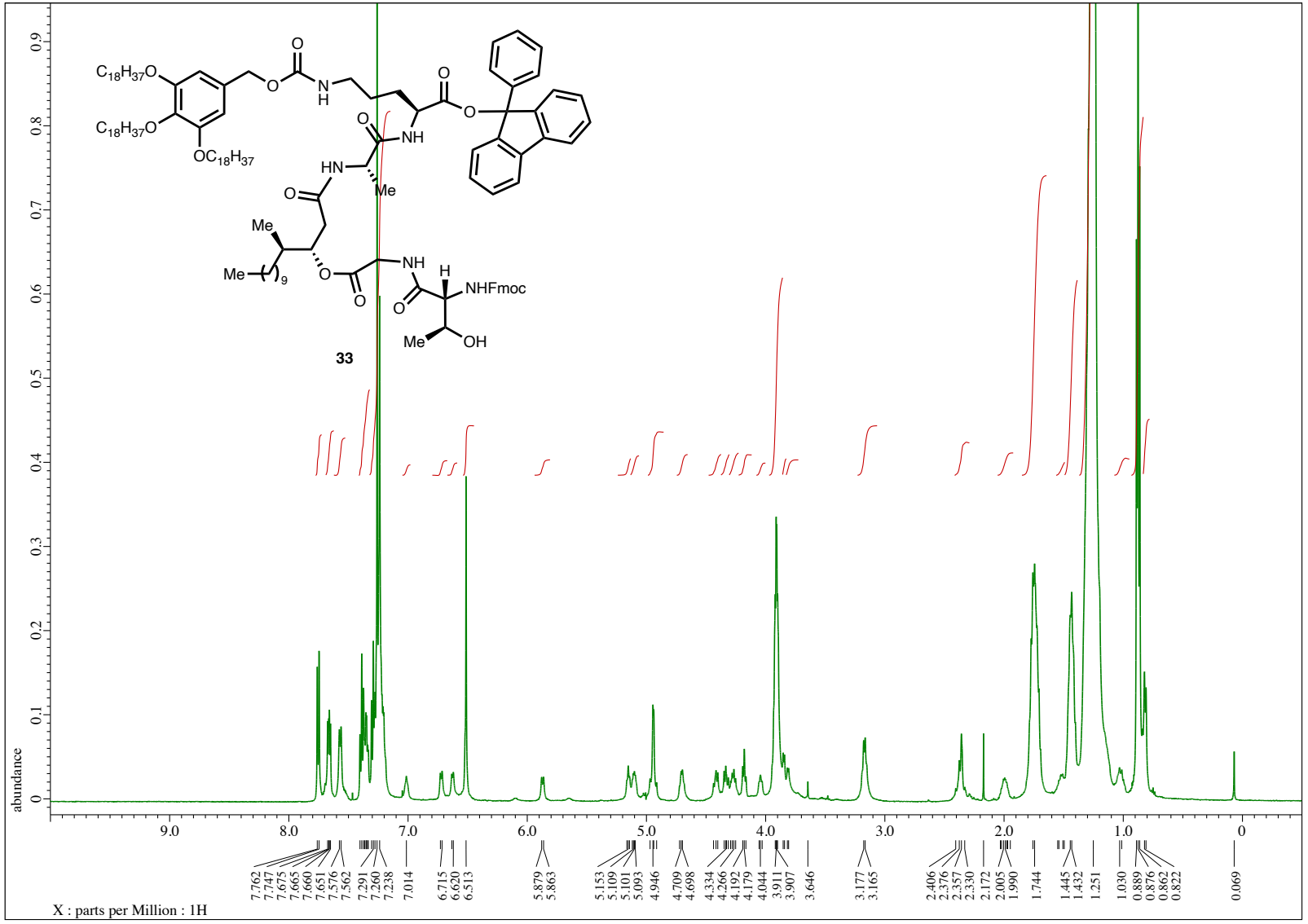

31
abundance
X : parts per Million : 1H

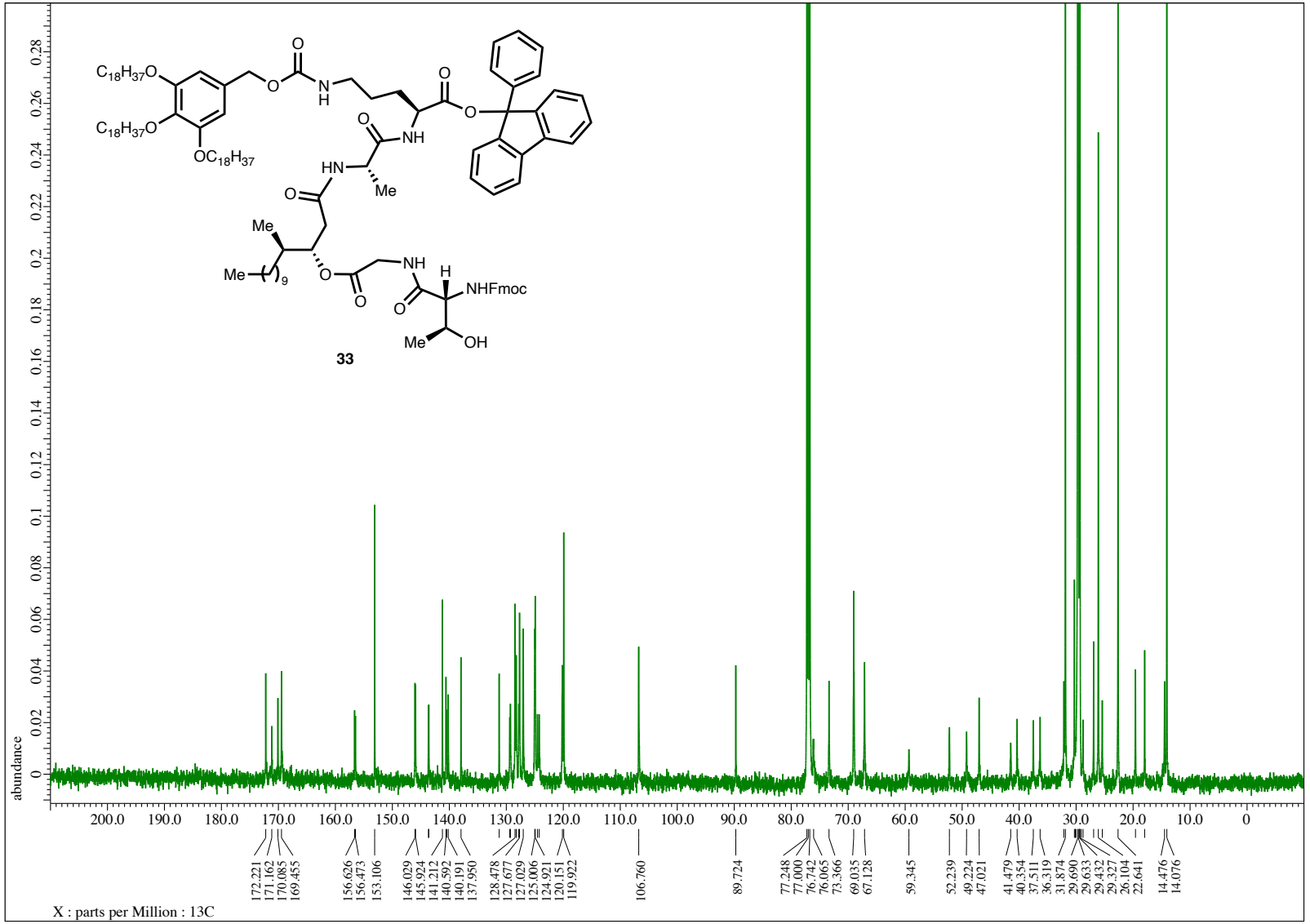

abundance
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
0.22
0.24
0.26
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
172.832
171.820
169.264
156.588
153.144
146.048
145.924
140.611
140.535
137.959
129.442
129.346
128.526
128.288
127.858
124.959
124.549
120.237
120.190
106.731
89.762
77.248
77.000
76.742
73.385
72.431
69.045
67.195
52.162
48.700
40.401
38.904
38.541
31.903
29.709
29.661
29.356
26.113
22.670
17.614
14.839
14.114
X : parts per Million : 13C

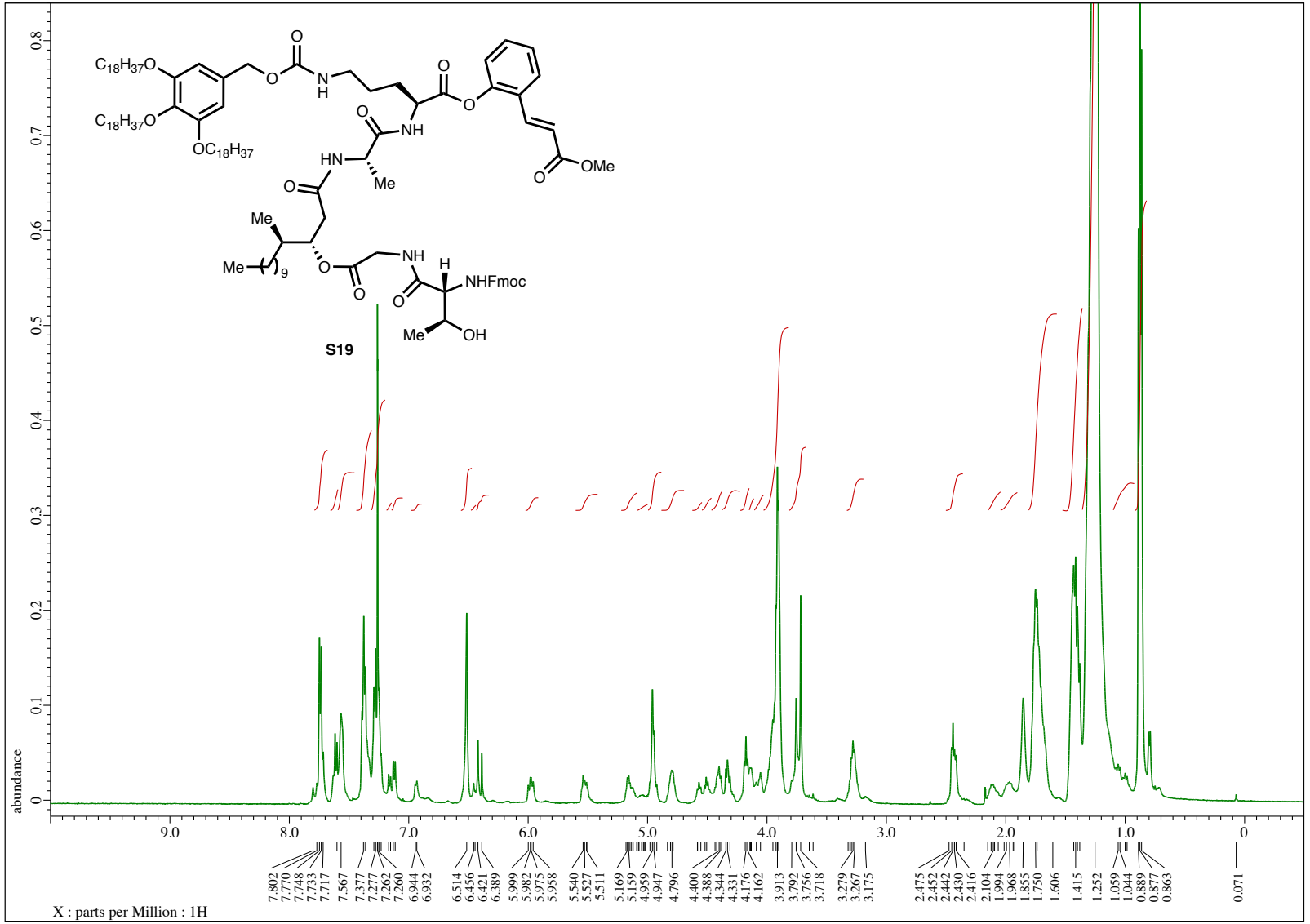
31

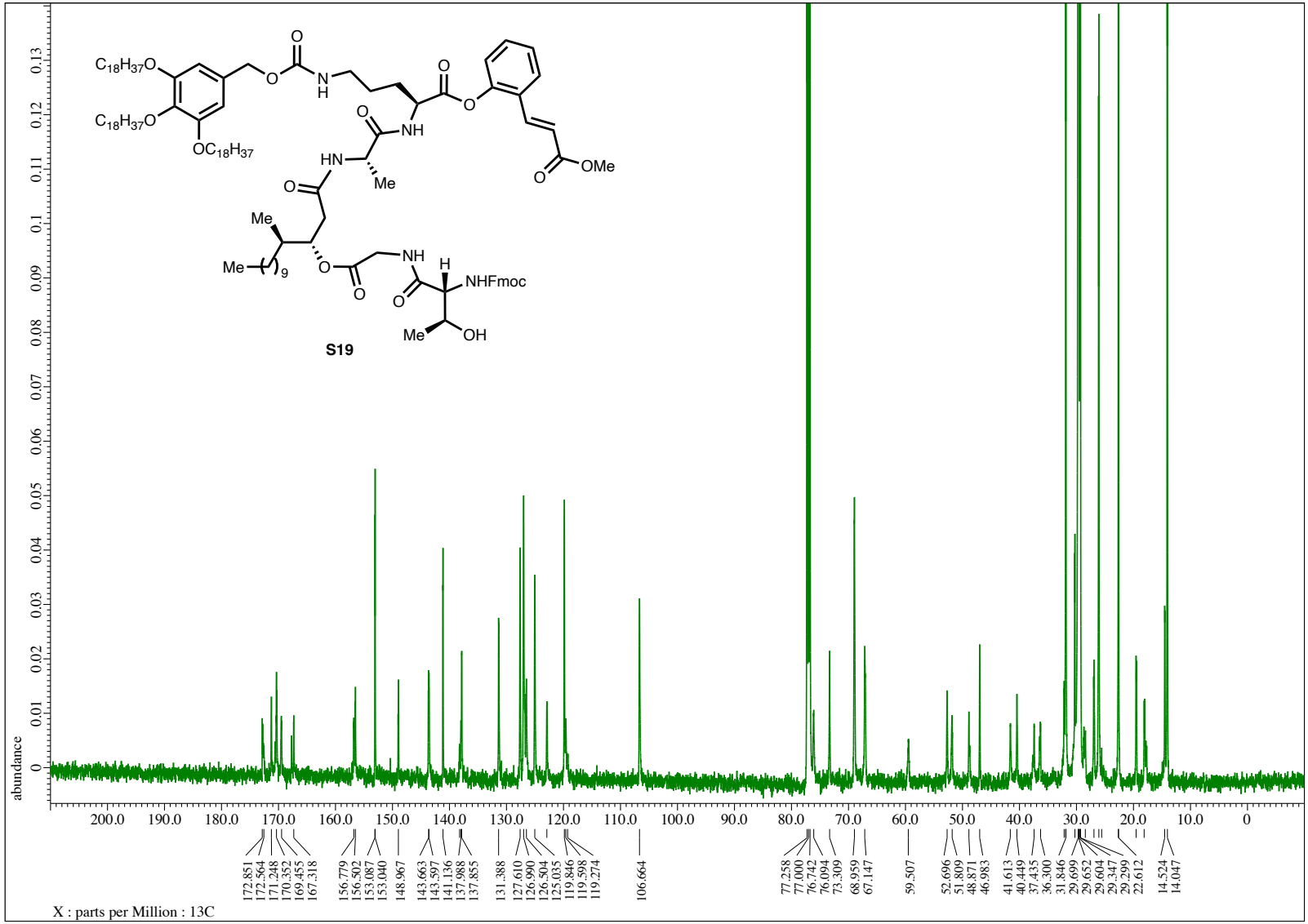

32
abundance
X : parts per Million : 1H

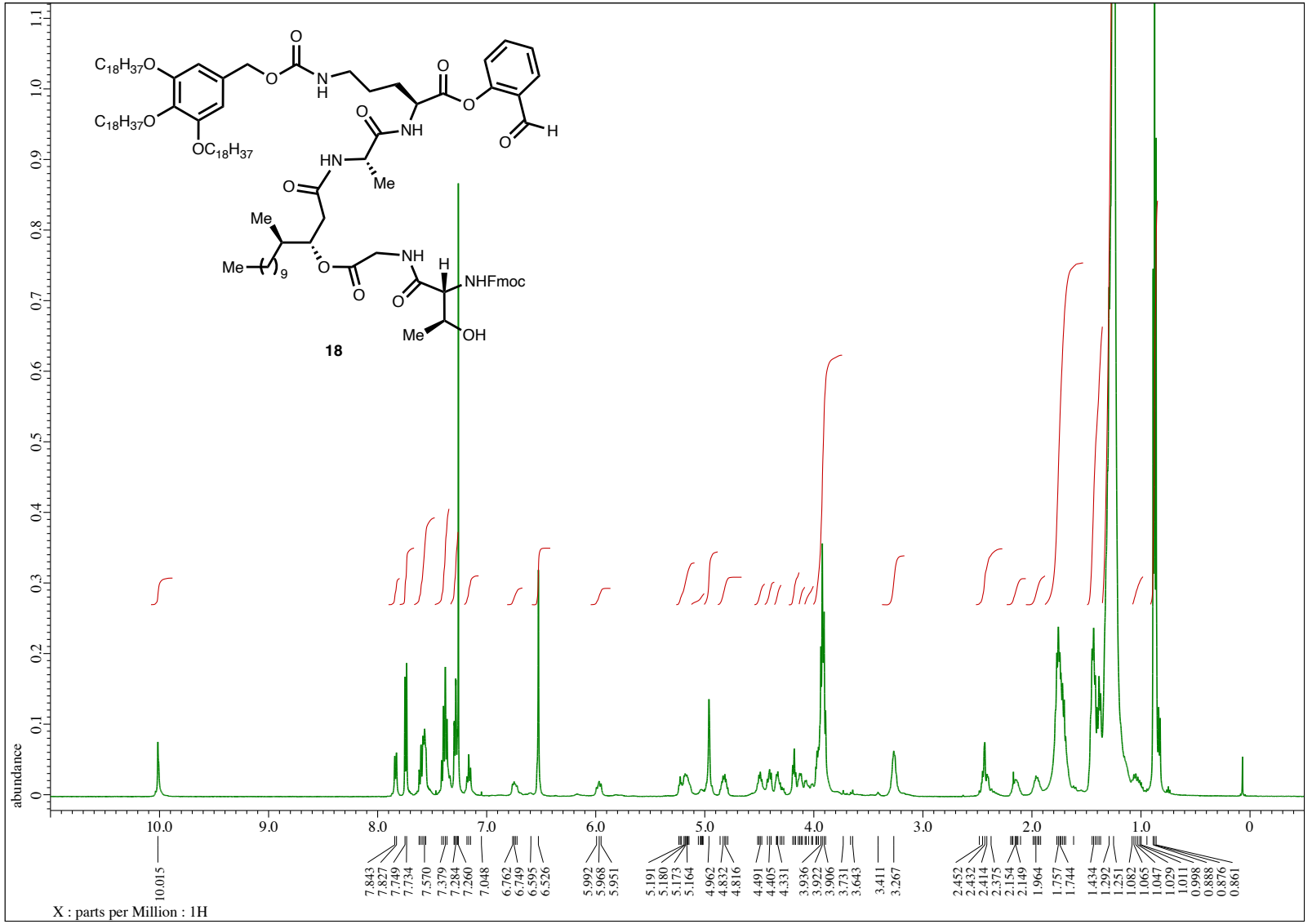

32
abundance
0
0.1
0.2
0.3
0.4
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
173.881
171.944
169.922
169.264
156.588
153.116
146.077
145.972
140.649
140.506
137.979
129.299
128.488
128.259
127.811
124.940
120.209
120.161
106.760
89.676
77.248
77.000
76.742
75.216
73.366
69.055
67.109
52.162
48.767
43.788
40.401
37.893
36.500
31.884
29.680
29.633
29.327
26.094
22.641
18.053
14.705
14.076
X : parts per Million : 13C

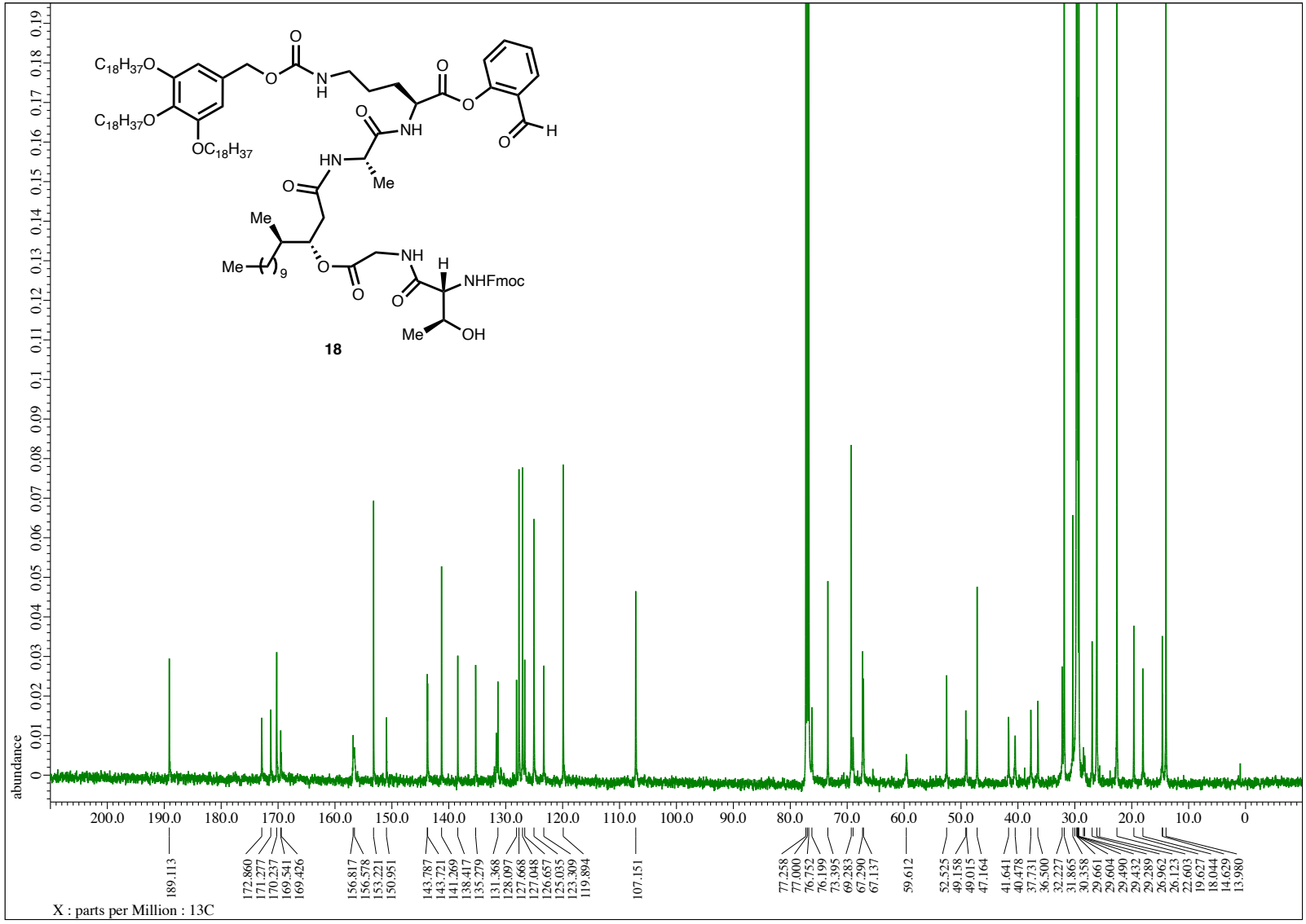

33
abundance
X : parts per Million : 1H
7.762
7.747
7.675
7.665
7.660
7.651
7.576
7.562
7.291
7.260
7.238
7.014
6.715
6.620
6.513
5.879
5.863
5.153
5.109
5.101
5.093
4.946
4.709
4.698
4.334
4.266
4.192
4.179
4.044
3.911
3.907
3.646
3.177
3.165
2.406
2.376
2.357
2.330
2.172
2.005
1.990
1.744
1.445
1.432
1.251
1.030
0.889
0.876
0.862
0.822
0.069


33
abundance
X : parts per Million : 13C
172.221
171.162
170.085
169.455
156.626
156.473
153.106
146.029
145.924
141.212
140.592
140.191
137.950
128.478
127.677
127.029
125.006
124.921
120.151
119.922
106.760
89.724
77.248
77.000
76.742
76.065
73.366
69.035
67.128
59.345
52.239
49.224
47.021
41.479
40.354
37.511
36.319
31.874
29.690
29.633
29.432
29.327
26.104
22.641
14.476
14.076

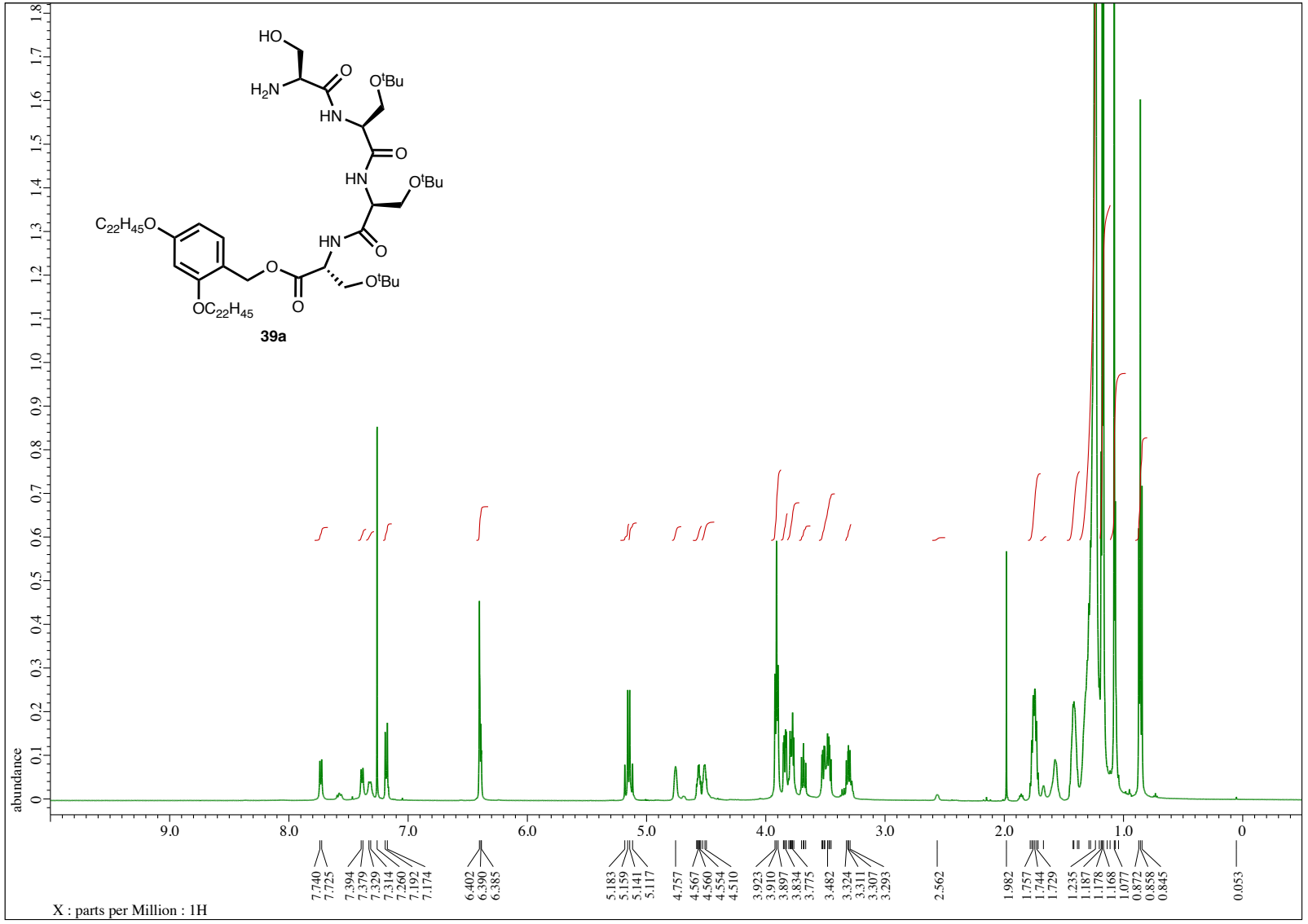

S19
abundance
X : parts per Million : 1H

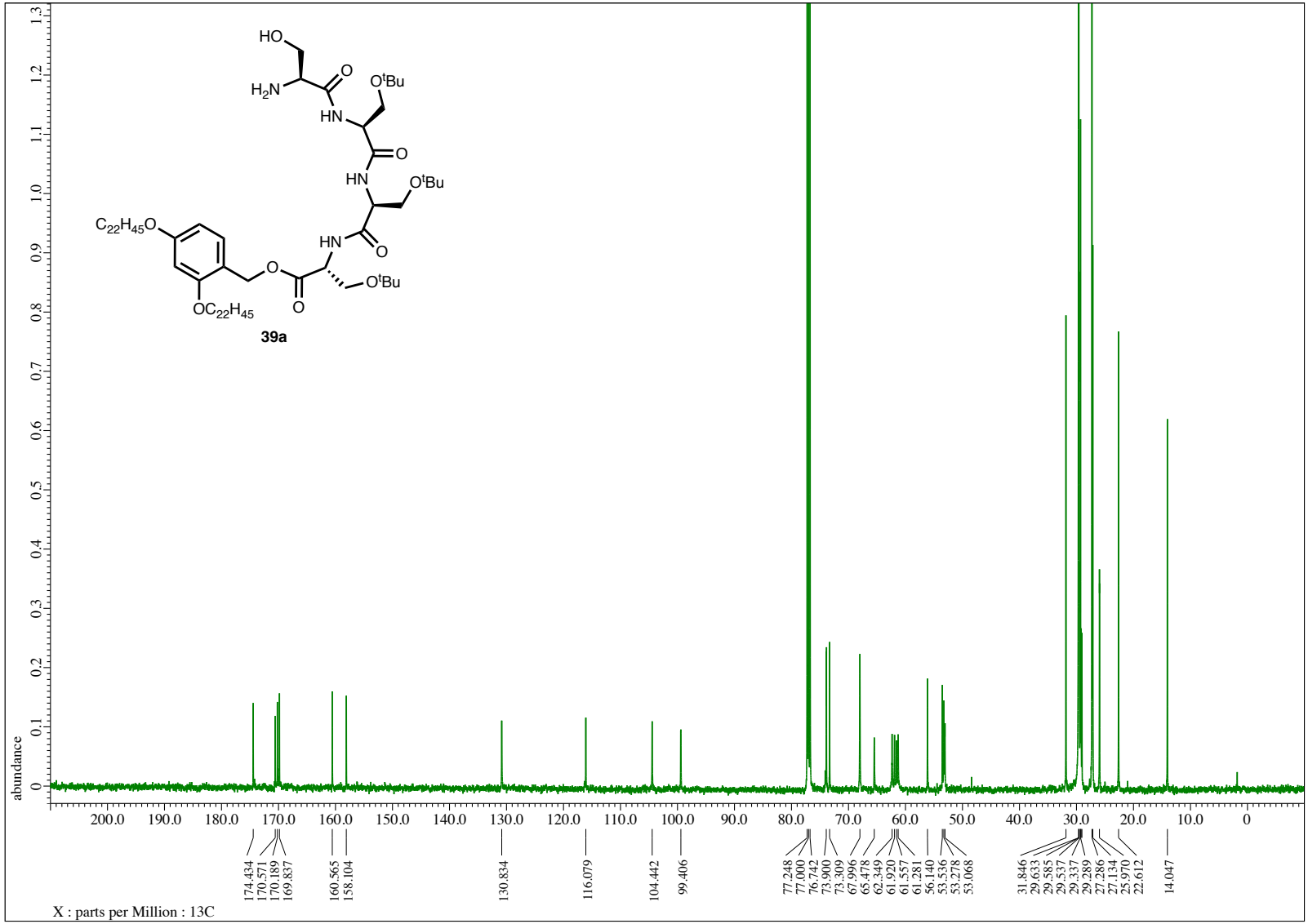

S19
abundance
X : parts per Million : 13C
172.851
172.564
171.248
170.352
169.455
167.318
156.779
156.502
153.087
153.040
148.967
143.663
143.597
141.136
137.988
137.855
131.388
127.610
126.990
126.504
125.035
119.846
119.598
119.274
106.664
77.258
77.000
76.742
76.094
73.309
68.959
67.147
59.507
52.696
51.809
48.871
46.983
41.613
40.449
37.435
36.300
31.846
29.699
29.652
29.604
29.347
29.299
22.612
14.524
14.047

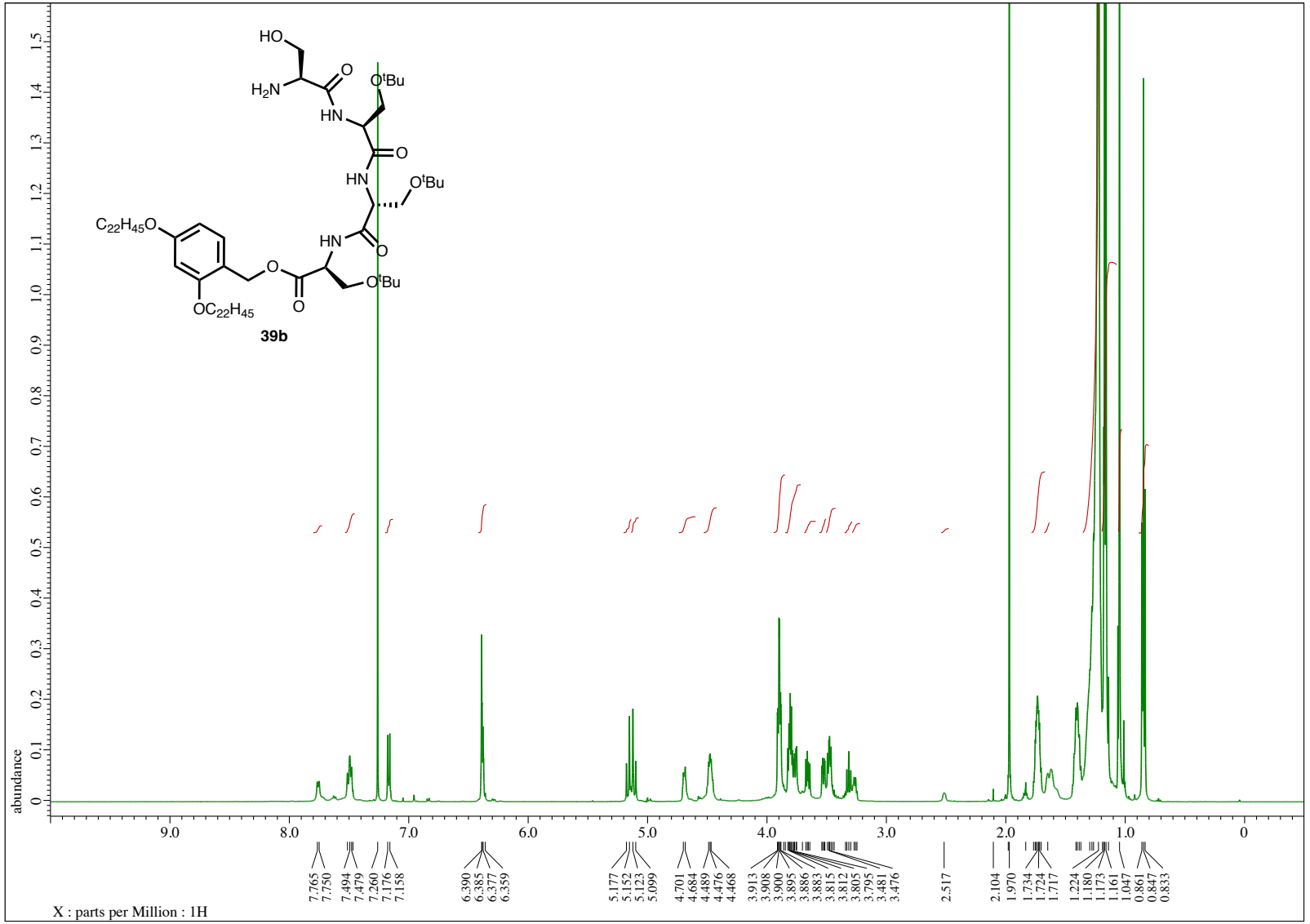

abundance
X : parts per Million : 1H
18
10.015
7.843
7.827
7.749
7.734
7.570
7.379
7.284
7.260
7.048
6.762
6.749
6.595
6.526
5.992
5.968
5.951
5.191
5.180
5.173
5.164
4.962
4.832
4.816
4.491
4.405
4.331
3.936
3.922
3.906
3.731
3.643
3.411
3.267
2.452
2.432
2.414
2.375
2.154
2.149
1.964
1.757
1.744
1.434
1.292
1.251
1.082
1.065
1.047
1.029
1.011
0.998
0.888
0.876
0.861

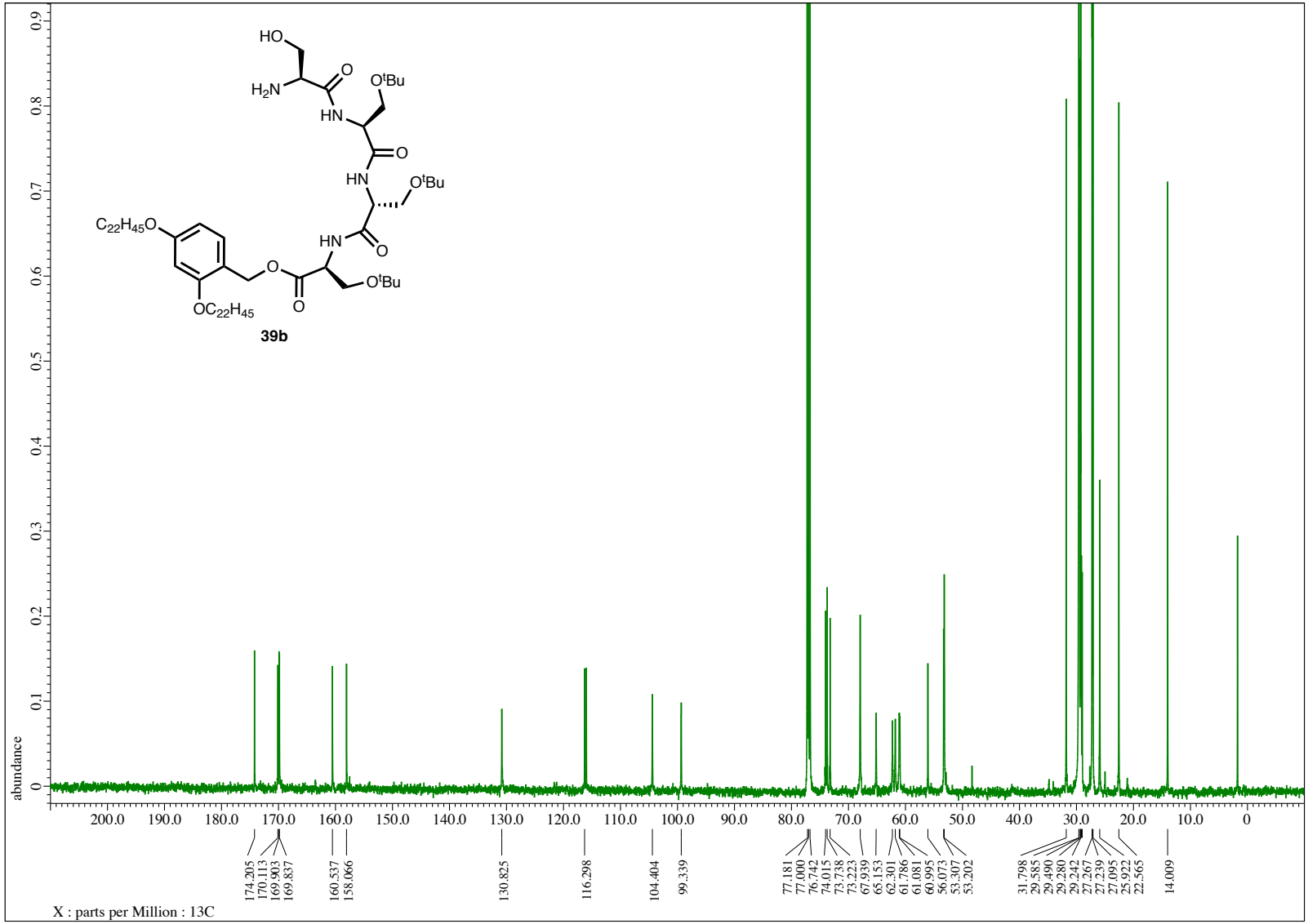

abundance
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.1 0.11 0.12 0.13 0.14 0.15 0.16 0.17 0.18 0.19
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
189.113
172.860 171.277 170.237 169.541 169.426
156.817 156.578 153.221 150.951
143.787 143.721 141.269 138.417 135.279
131.368 128.097 127.668 127.048 126.657 125.035 123.309 119.894
107.151
77.258 77.000 76.752 76.199 73.395 69.283 67.290 67.137
59.612
52.525 49.158 49.015 47.164
41.641 40.478 37.731 36.500
32.227 31.865 30.358 29.661 29.604 29.490 29.432 29.289 26.962 26.123 22.603 19.627 18.044 14.629 13.980
X : parts per Million : 13C

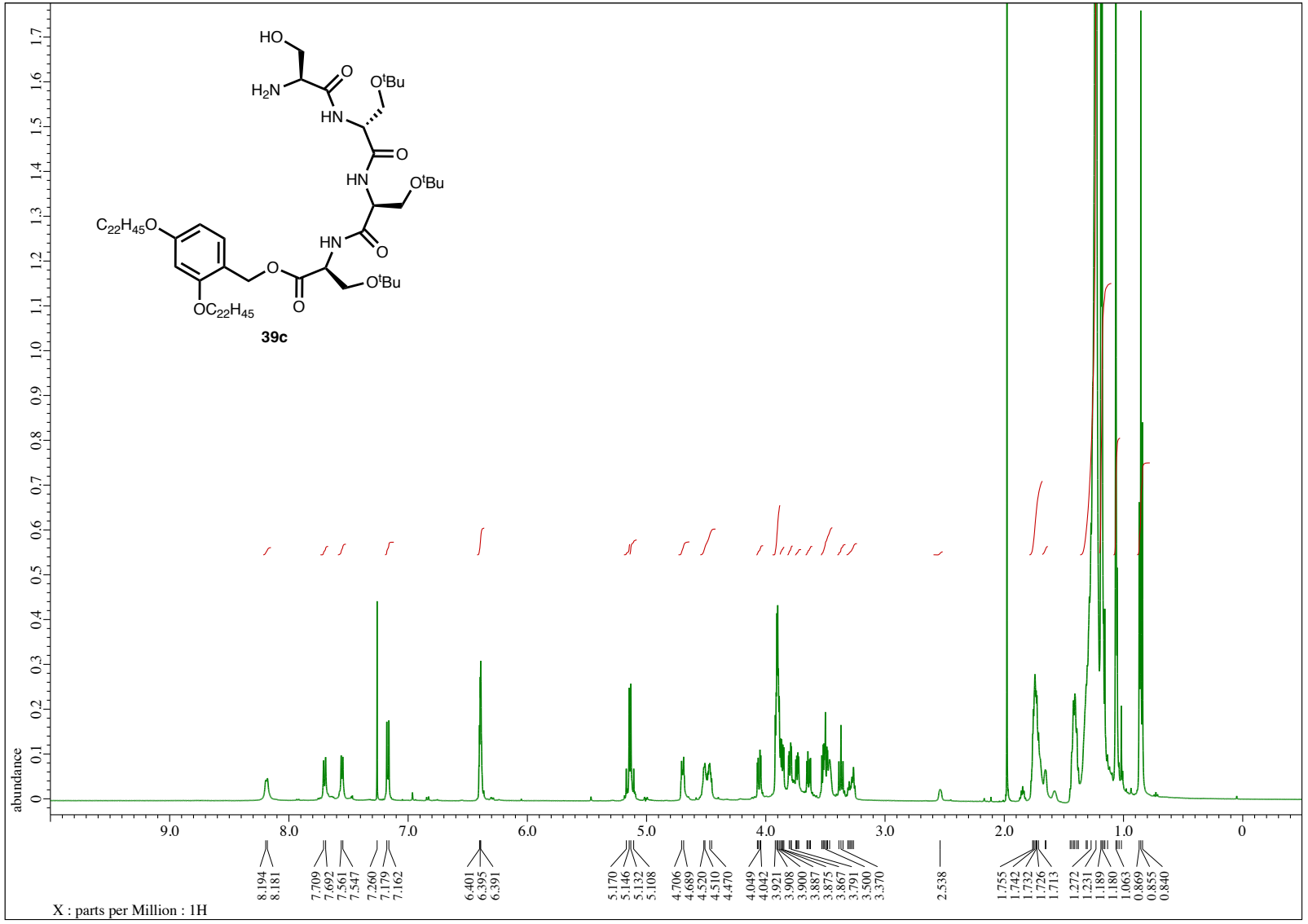
18

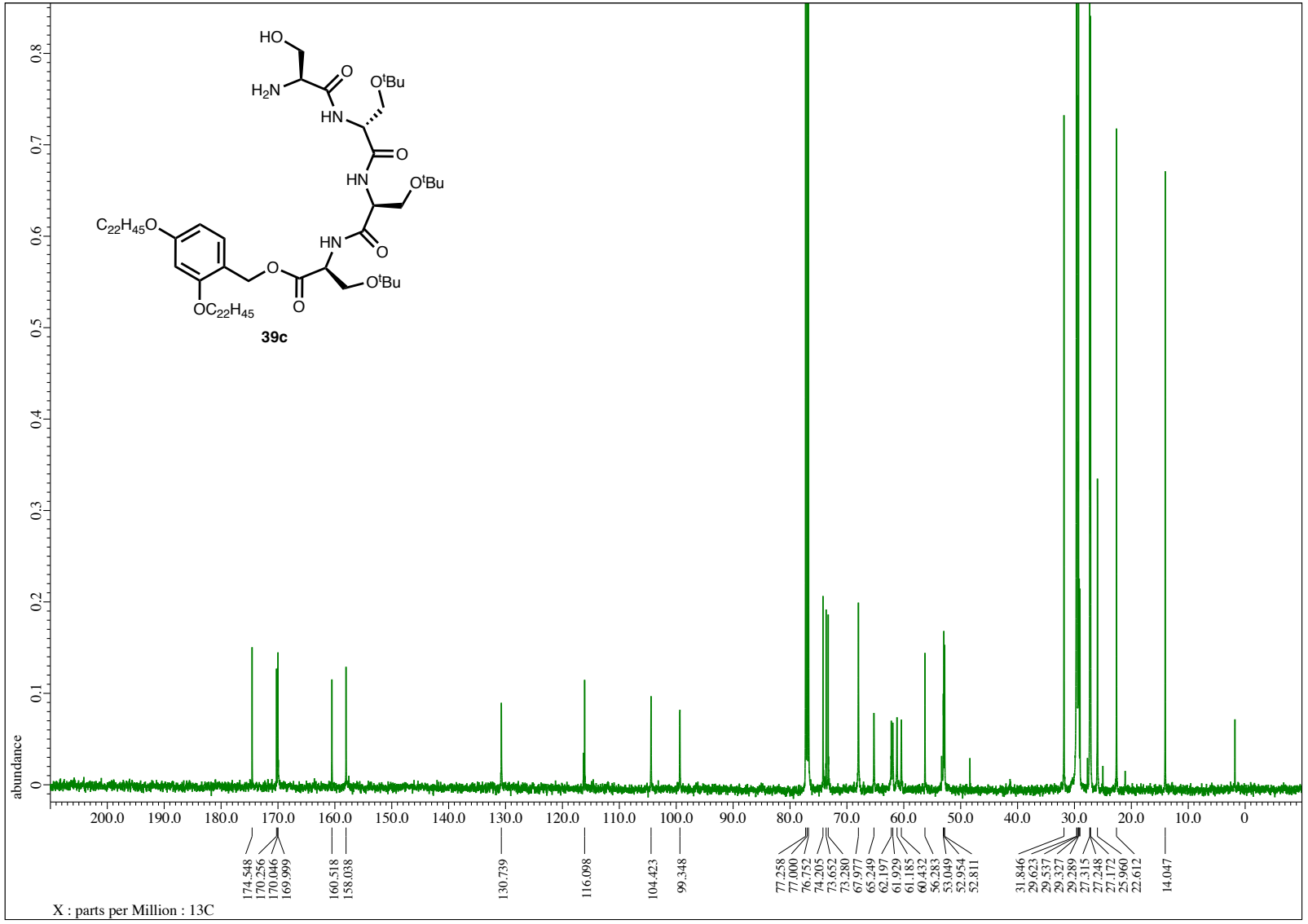

HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
O
OC22H45
O
39a
abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.740
7.725
7.394
7.379
7.329
7.314
7.260
7.192
7.174
6.402
6.390
6.385
5.183
5.159
5.141
5.117
4.757
4.567
4.560
4.554
4.510
3.923
3.910
3.897
3.834
3.775
3.482
3.324
3.311
3.307
3.293
2.562
1.982
1.757
1.744
1.729
1.235
1.187
1.178
1.168
1.077
0.872
0.858
0.845
0.053
X : parts per Million : 1H

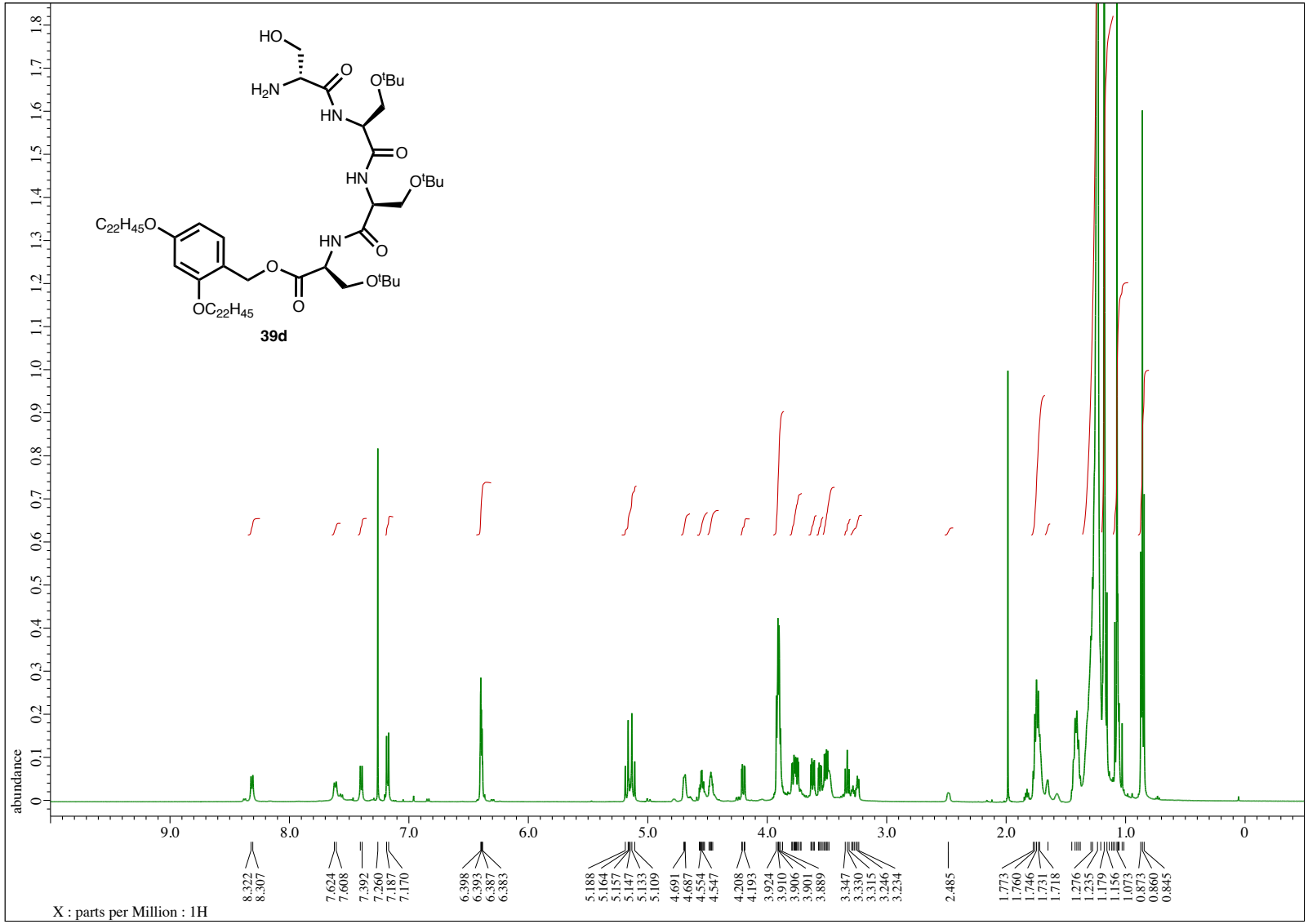

HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
O
OC22H45
39a
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
174.434
170.571
170.189
169.837
160.565
158.104
130.834
116.079
104.442
99.406
77.248
77.000
76.742
73.900
73.309
67.996
65.478
62.349
61.920
61.557
61.281
56.140
53.536
53.278
53.068
31.846
29.633
29.585
29.537
29.337
29.289
27.286
27.134
25.970
22.612
14.047
X : parts per Million : 13C

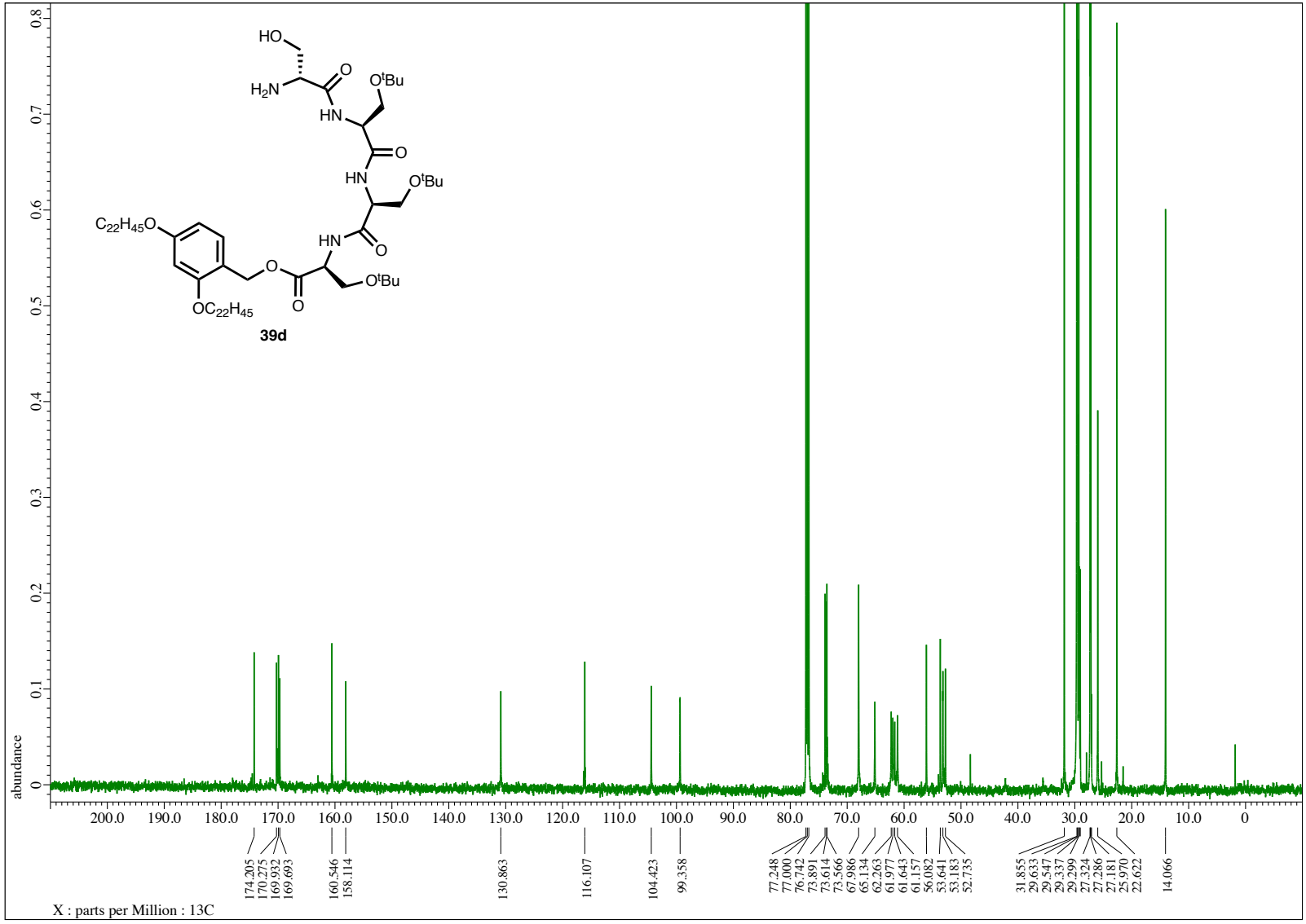

HO
H2N
O
HN
OtBu
O
HN
OtBu
C22H45O
HN
O
O
OtBu
O
OC22H45
O
39b
abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.765
7.750
7.494
7.479
7.260
7.176
7.158
6.390
6.385
6.377
6.359
5.177
5.152
5.123
5.099
4.701
4.684
4.489
4.476
4.468
3.913
3.908
3.900
3.895
3.886
3.883
3.815
3.812
3.805
3.795
3.481
3.476
2.517
2.104
1.970
1.734
1.724
1.717
1.224
1.180
1.173
1.161
1.047
0.861
0.847
0.833
X : parts per Million : 1H

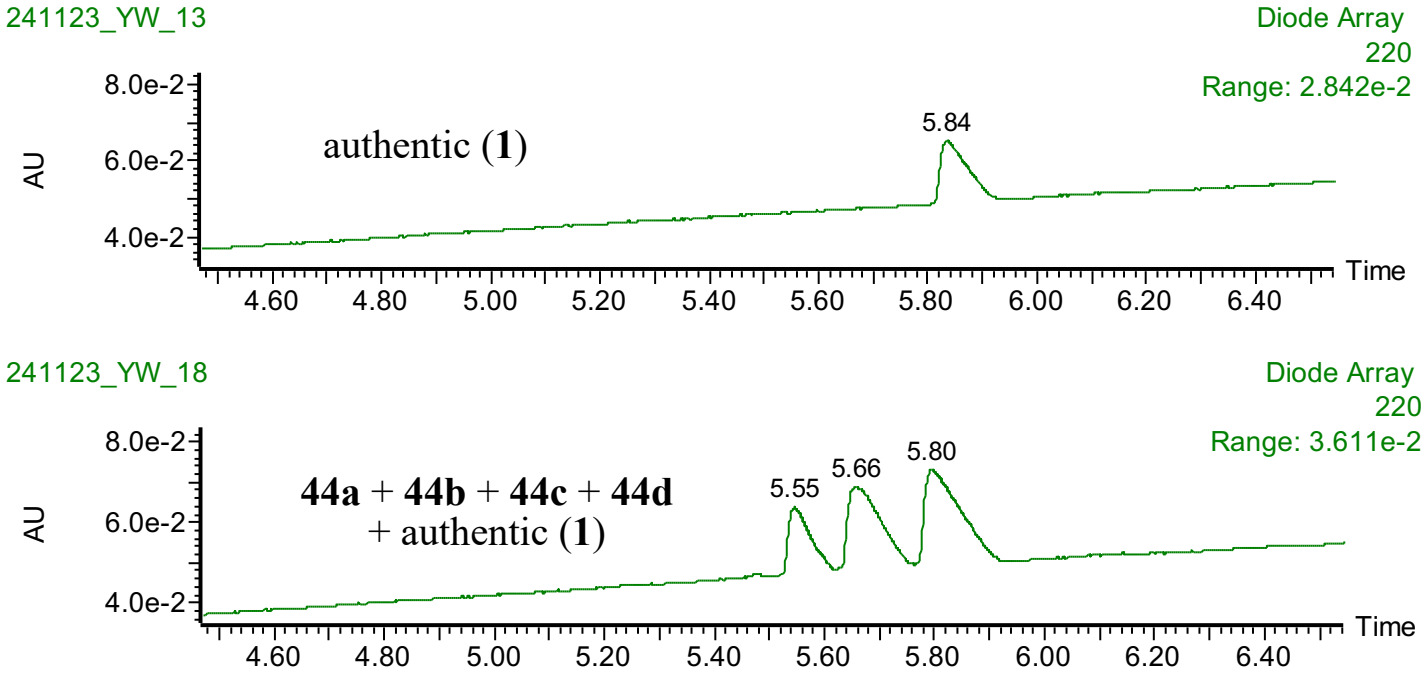

HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
O
OC22H45
39b
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
174.205
170.113
169.903
169.837
160.537
158.066
130.825
116.298
104.404
99.339
77.181
77.000
76.742
74.015
73.738
73.223
67.939
65.153
62.301
61.786
61.081
60.995
56.073
53.307
53.202
31.798
29.585
29.490
29.280
29.242
27.267
27.239
27.095
25.922
22.565
14.009
X : parts per Million : 13C


HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
OC22H45
O
39c
abundance
X : parts per Million : 1H

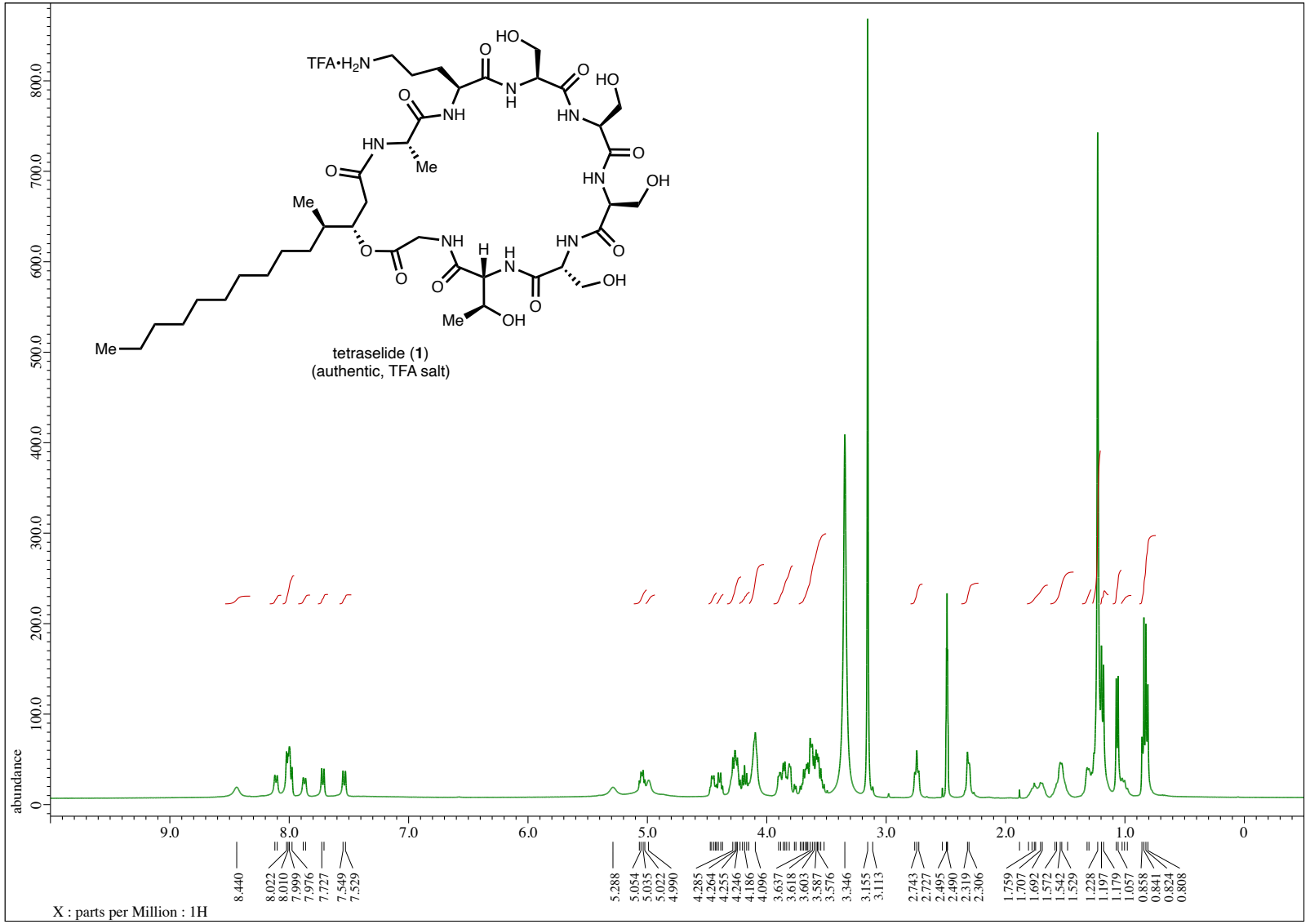

HO
H2N
O
OtBu
HN
O
HN
OtBu
HN
O
C22H45O
O
OtBu
OC22H45
O
39c
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
174.548
170.256
170.046
169.999
160.518
158.038
130.739
116.098
104.423
99.348
77.258
77.000
76.752
74.205
73.652
73.280
67.977
65.249
62.197
61.929
61.185
60.432
56.283
53.049
52.954
52.811
31.846
29.623
29.537
29.327
29.289
27.315
27.248
27.172
25.960
22.612
14.047
X : parts per Million : 13C

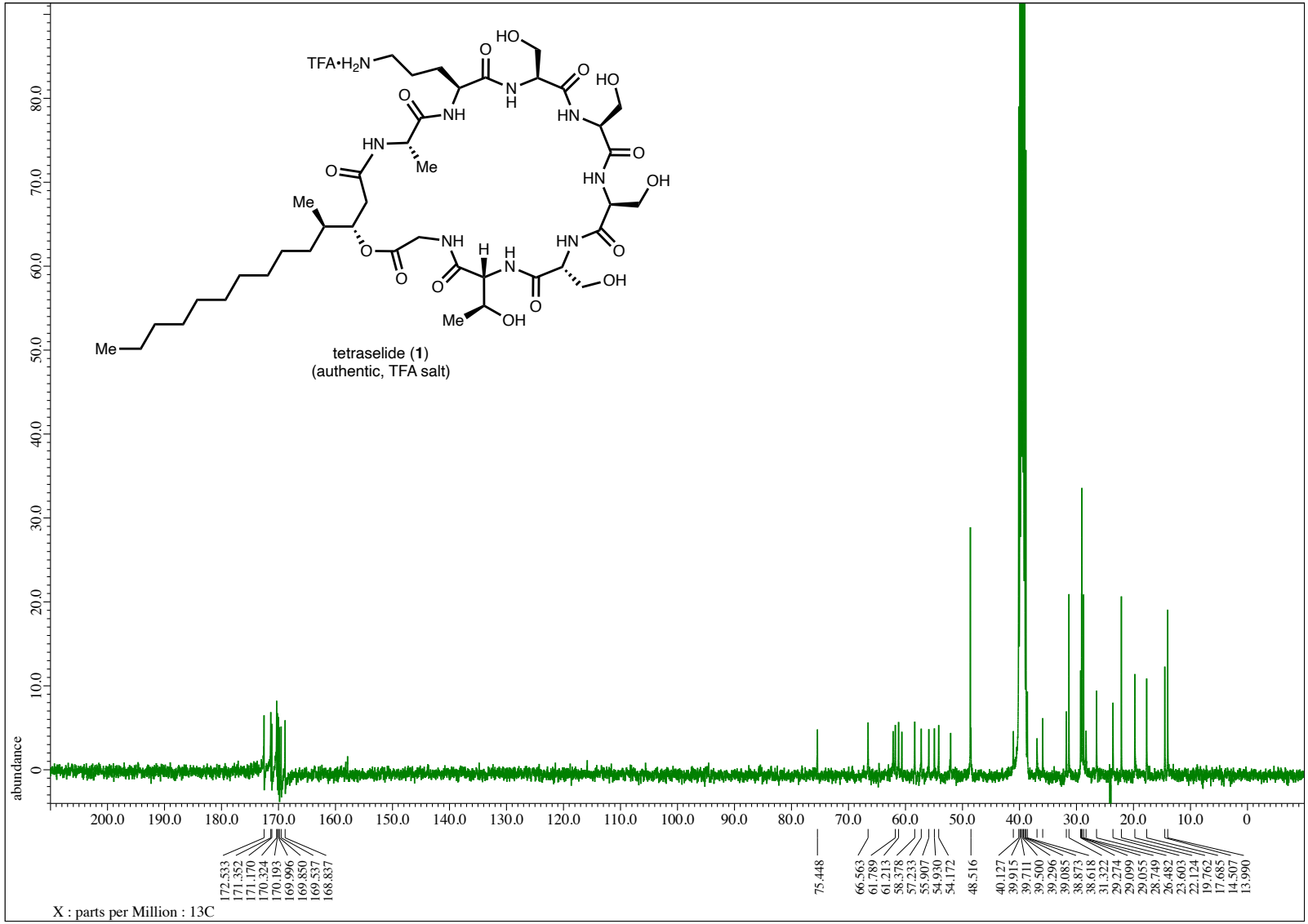

HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
OC22H45
O
39d
abundance
X : parts per Million : 1H
8.322
8.307
7.624
7.608
7.392
7.260
7.187
7.170
6.398
6.393
6.387
6.383
5.188
5.164
5.157
5.147
5.133
5.109
4.691
4.687
4.554
4.547
4.208
4.193
3.924
3.910
3.906
3.901
3.889
3.347
3.330
3.315
3.246
3.234
2.485
1.773
1.760
1.746
1.731
1.718
1.276
1.235
1.179
1.156
1.073
0.873
0.860
0.845

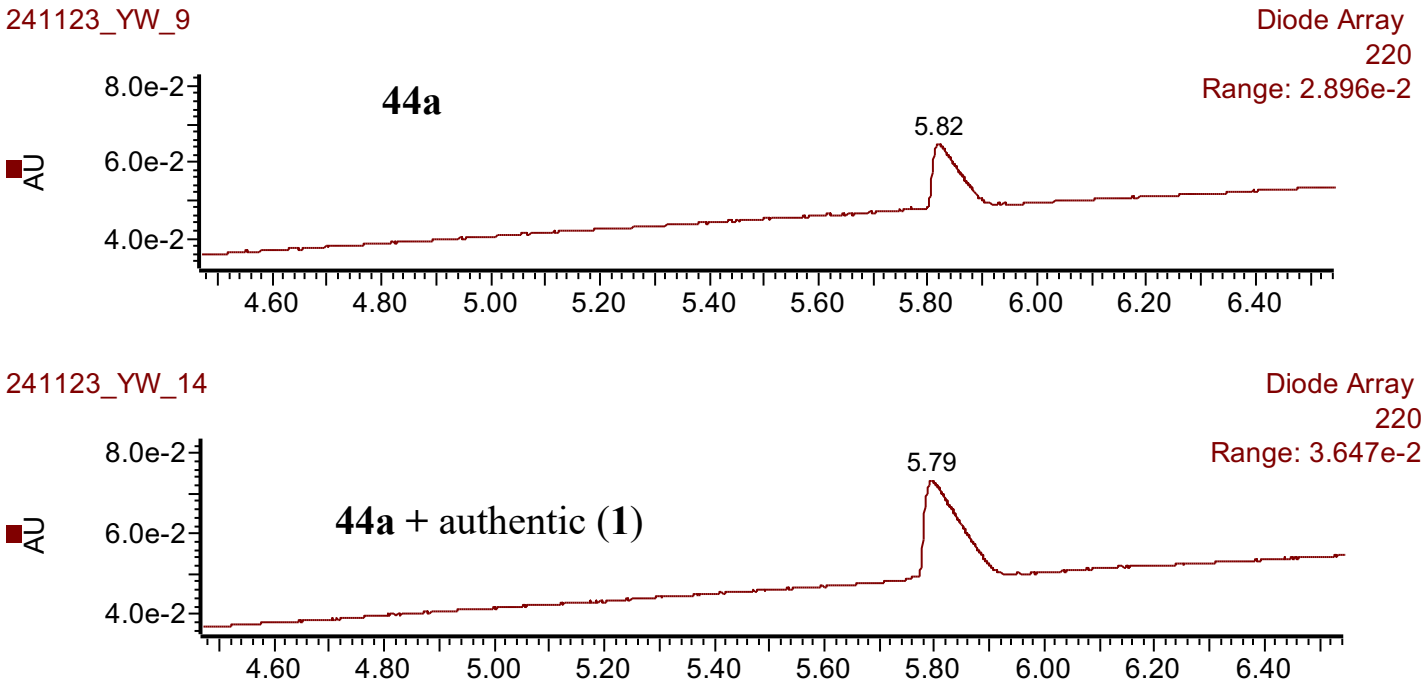

HO
H2N
O
OtBu
HN
O
HN
OtBu
C22H45O
HN
O
O
OtBu
OC22H45
O
39d
abundance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
174.205
170.275
169.932
169.693
160.546
158.114
130.863
116.107
104.423
99.358
77.248
77.000
76.742
73.891
73.614
73.566
67.986
65.134
62.263
61.977
61.643
61.157
56.082
53.641
53.183
52.735
31.855
29.633
29.547
29.337
29.299
27.324
27.286
27.181
25.970
22.622
14.066
X : parts per Million : 13C



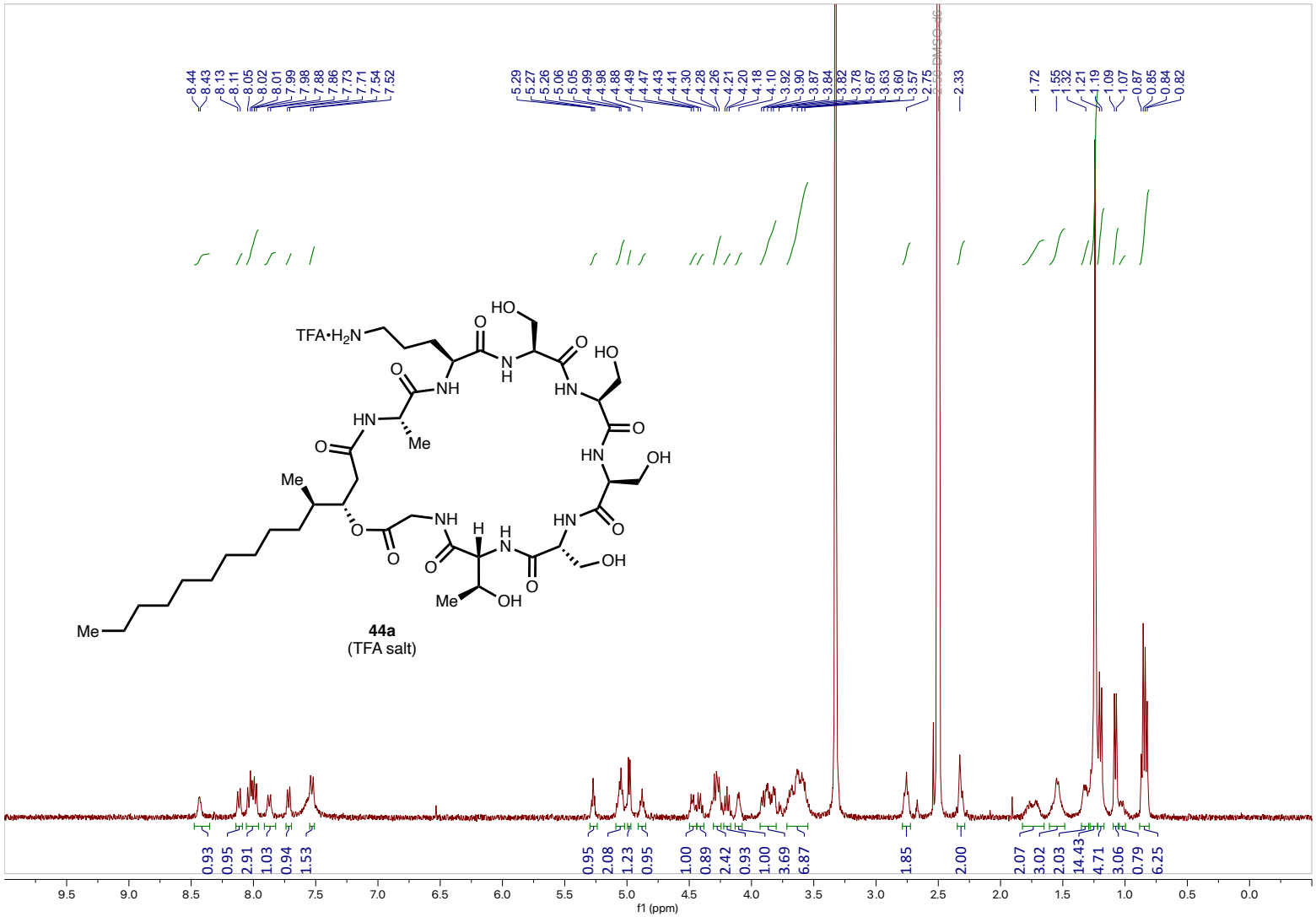
LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA


tetraselide (1)
(authentic, TFA salt)
abundance
X : parts per Million : 1H

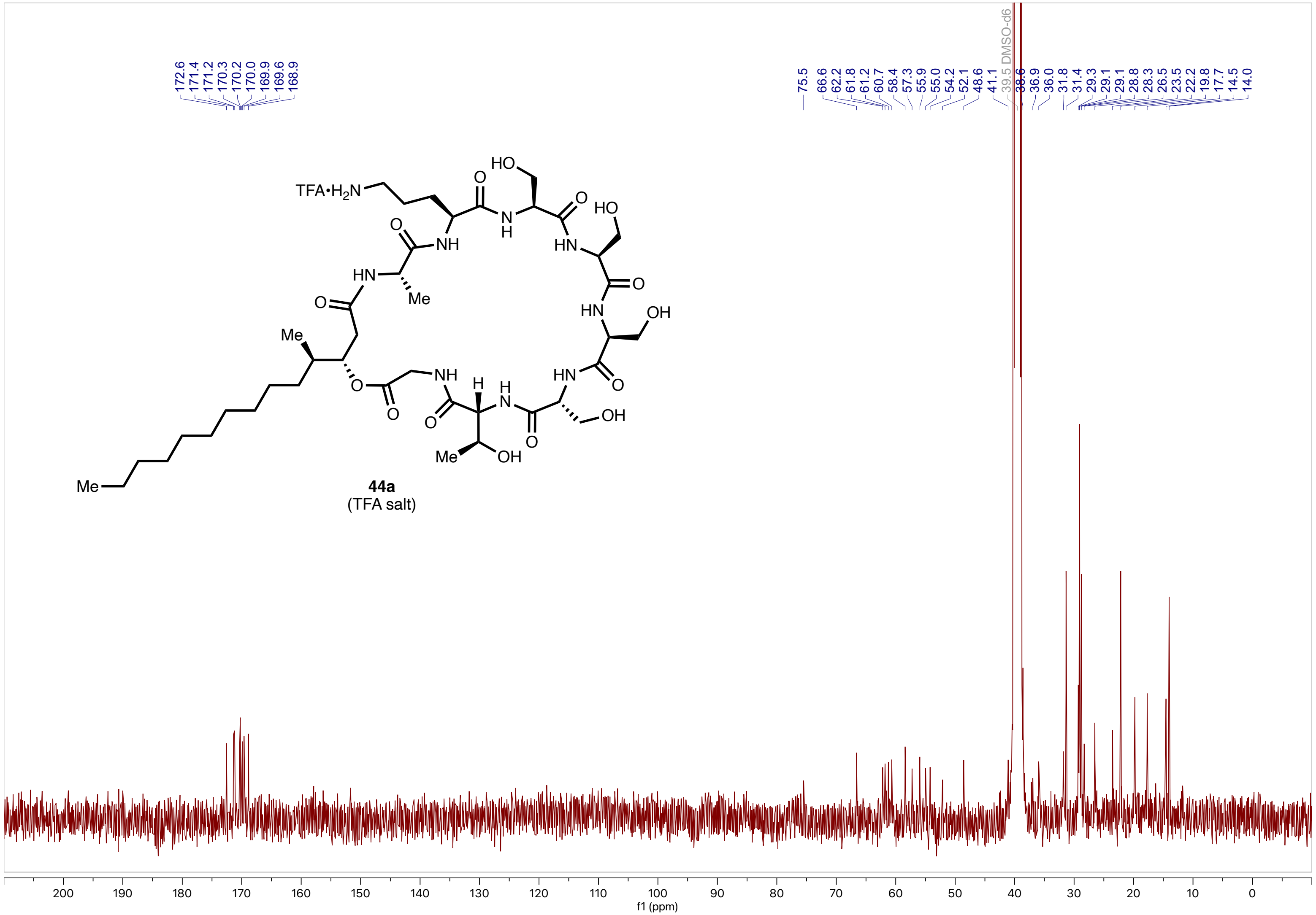

tetraselide (1)
(authentic, TFA salt)
abundance
X : parts per Million : 13C
172.533
171.352
171.170
170.324
170.193
169.996
169.850
169.537
168.837
75.448
66.563
61.789
61.213
58.378
57.233
55.907
54.930
54.172
48.516
40.127
39.915
39.711
39.500
39.296
39.085
38.873
38.618
31.322
29.274
29.099
29.055
28.749
26.482
23.603
22.124
19.762
17.685
14.507
13.990

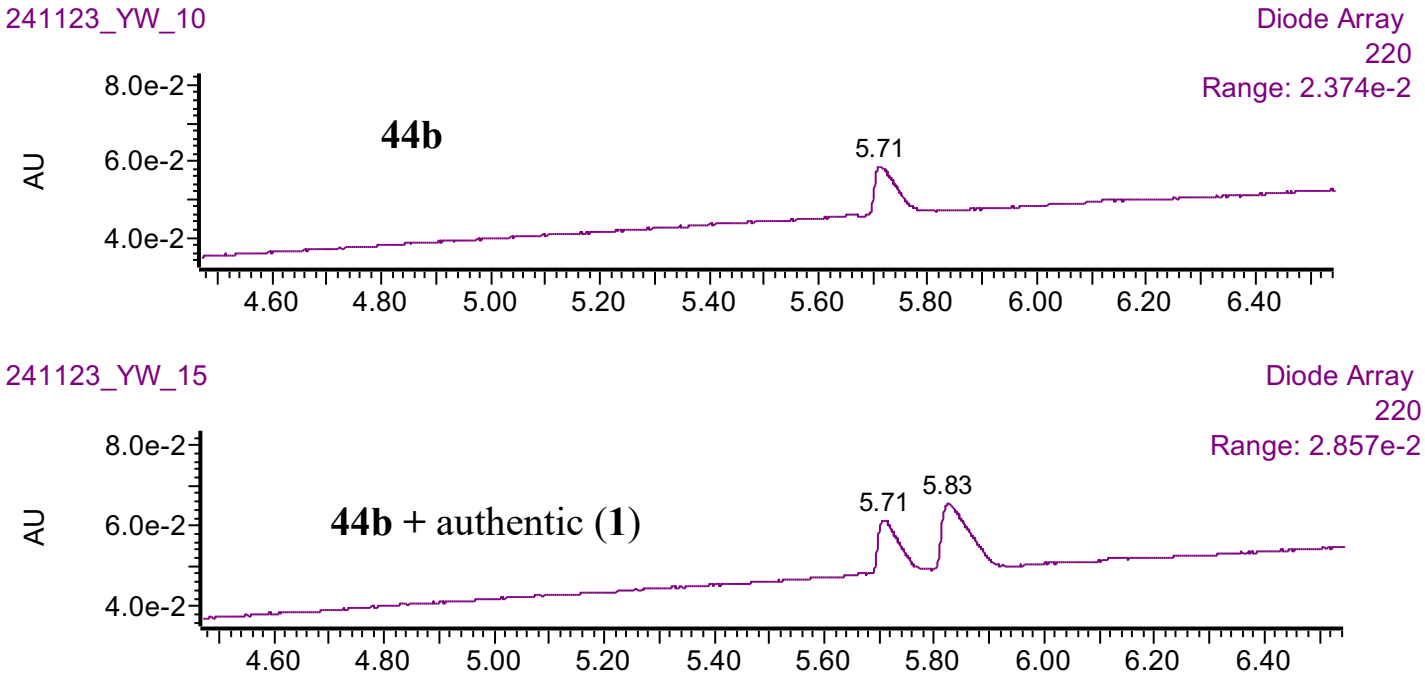


LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA

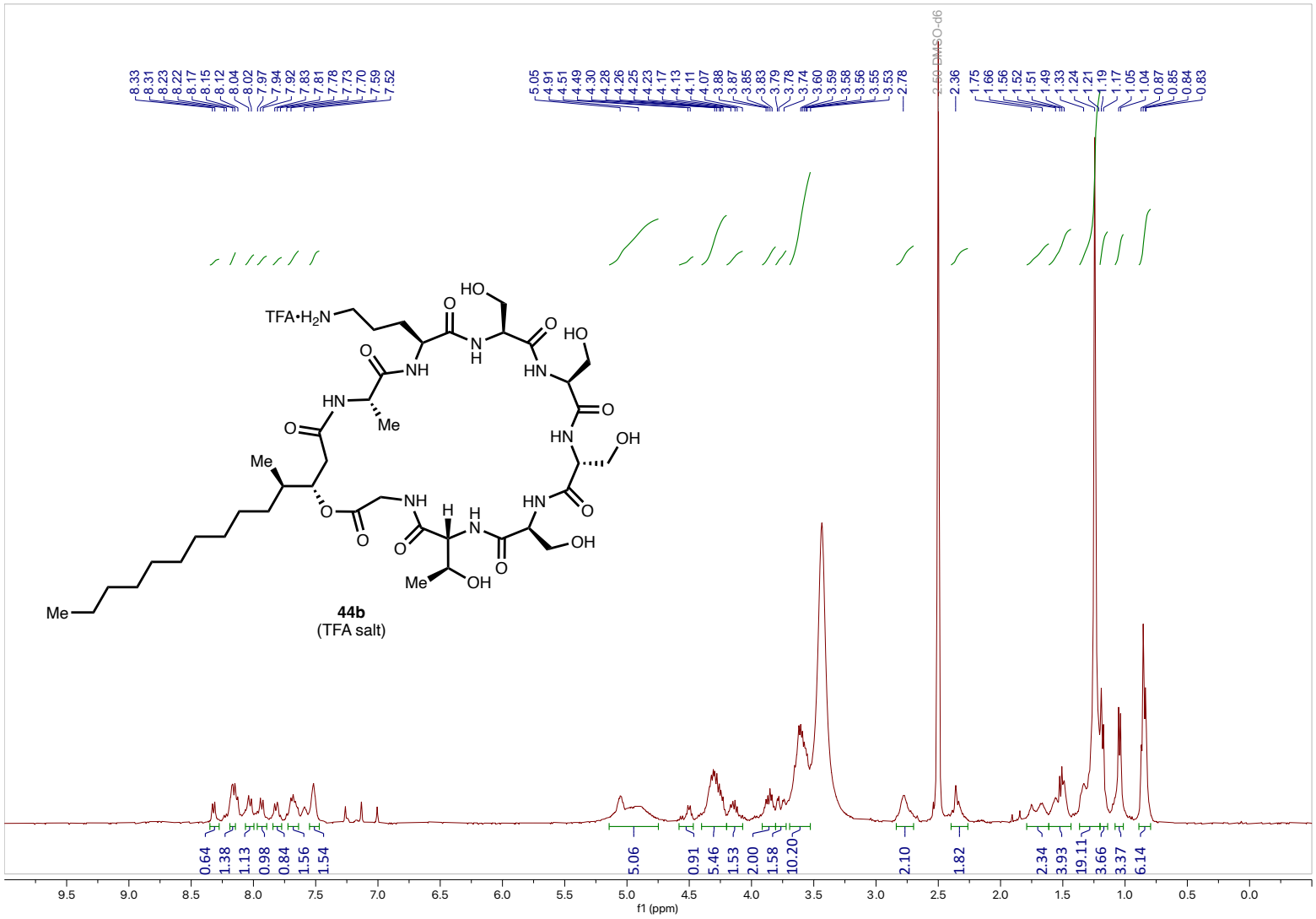

8.44
8.43
8.13
8.11
8.05
8.02
8.01
7.99
7.98
7.88
7.86
7.73
7.71
7.54
7.52
5.29
5.27
5.26
5.06
5.05
4.99
4.98
4.88
4.49
4.47
4.43
4.41
4.30
4.28
4.26
4.21
4.20
4.18
4.10
3.92
3.90
3.87
3.84
3.82
3.78
3.67
3.63
3.60
3.57
2.75
2.33
1.72
1.55
1.32
1.21
1.19
1.09
1.07
0.87
0.85
0.84
0.82
0.93
0.95
2.91
1.03
0.94
1.53
0.95
2.08
1.23
0.95
1.00
0.89
2.42
0.93
1.00
3.69
6.87
1.85
2.00
2.07
3.02
2.03
14.43
4.71
3.06
0.79
6.25
f1 (ppm)

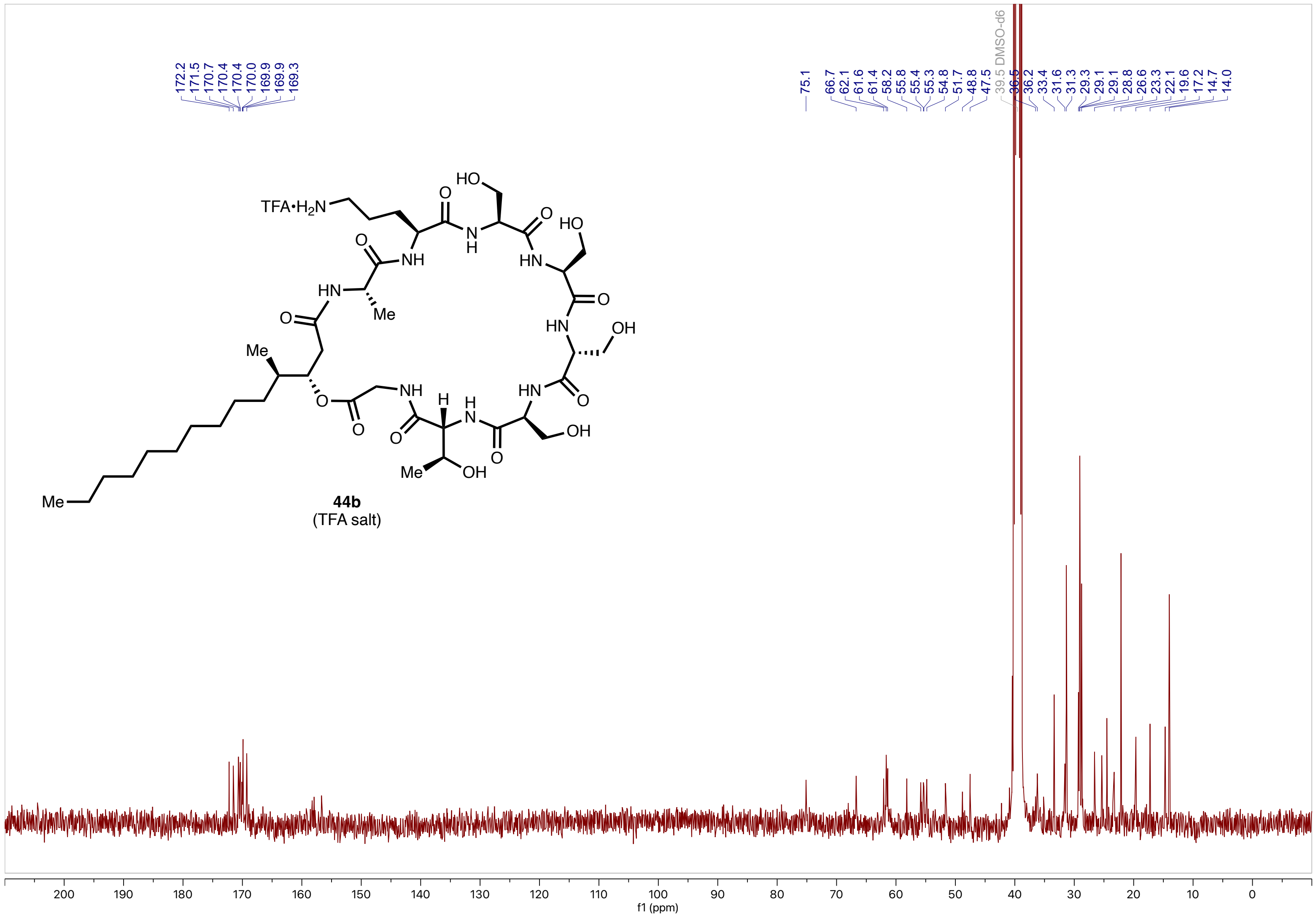
44a
(TFA salt)

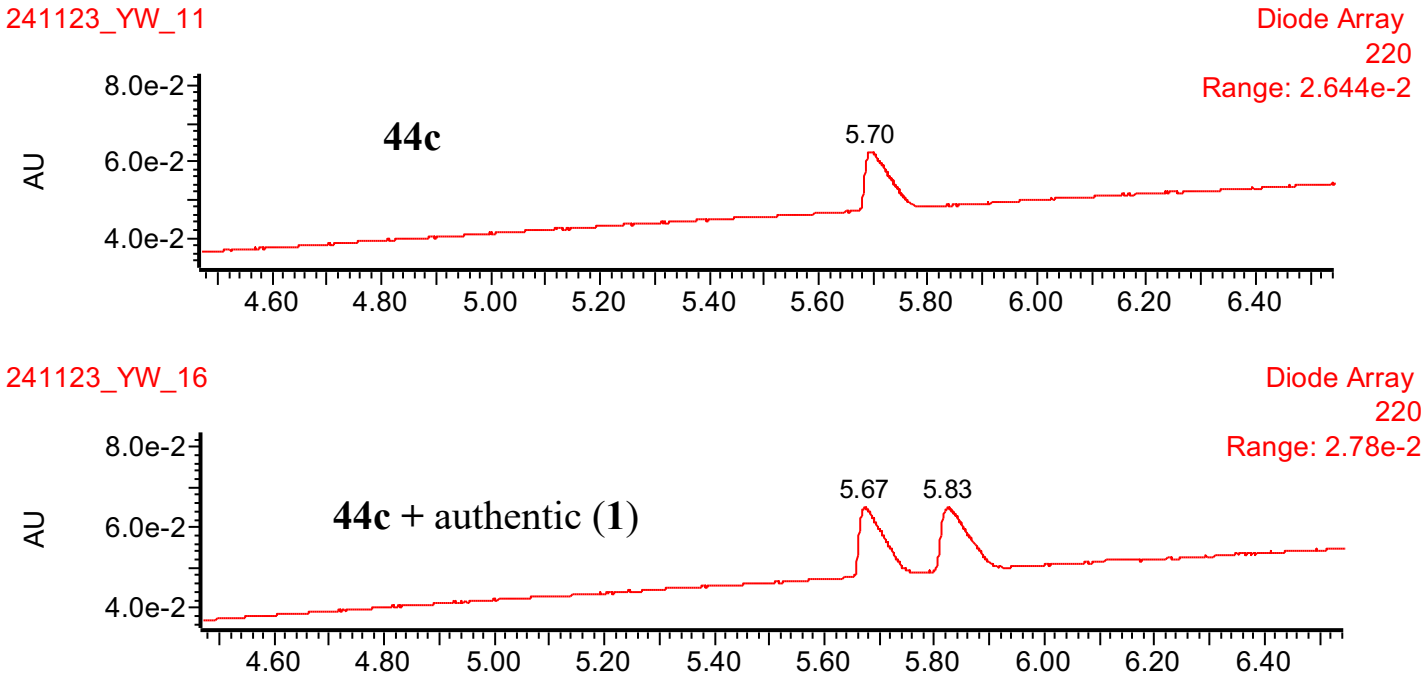

172.6
171.4
171.2
170.3
170.2
170.0
169.9
169.6
168.9
75.5
66.6
62.2
61.8
61.2
60.7
58.4
57.3
55.9
55.0
54.2
52.1
48.6
41.1
39.5 DMSO-d6
38.6
36.9
36.0
31.8
31.4
29.3
29.1
29.1
28.8
28.3
26.5
23.5
22.2
19.8
17.7
14.5
14.0
TFA•H2N
HO
O
HO
NH
N
H
HN
O
HN
OH
Me
Me
O
NH
H
H
N
OH
Me
OH
44a
(TFA salt)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



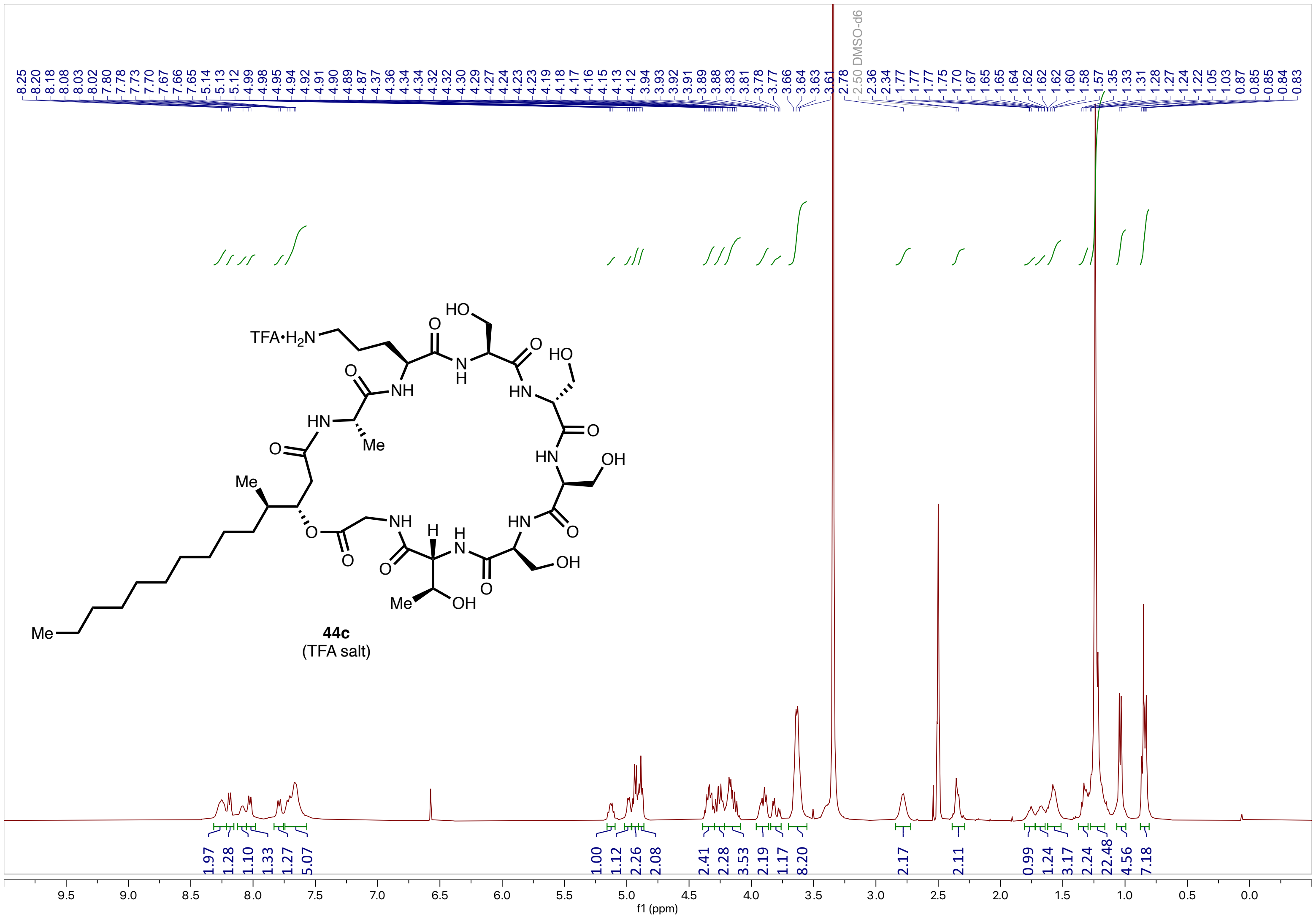
LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA

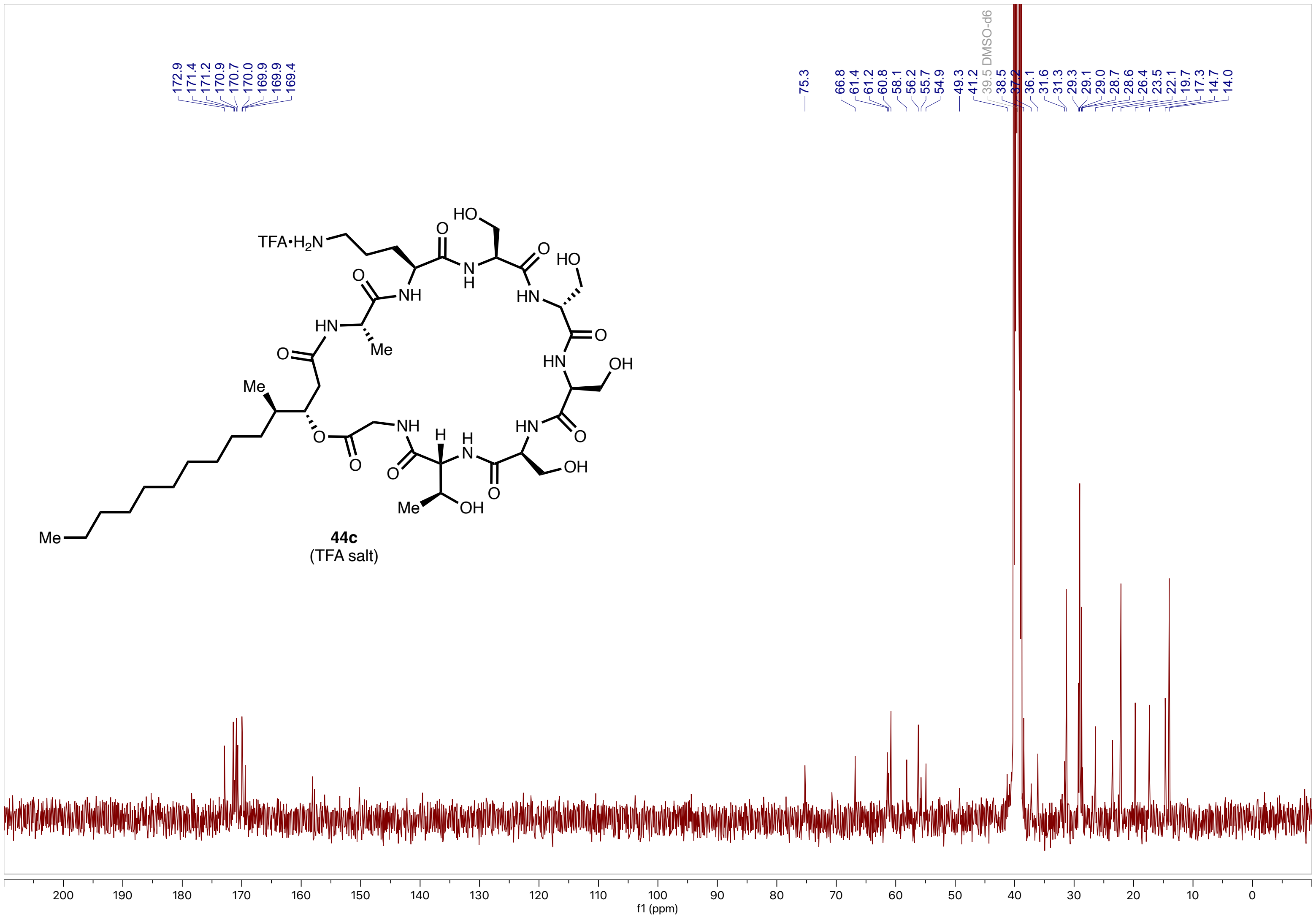

8.33
8.31
8.23
8.22
8.17
8.15
8.12
8.04
8.02
7.97
7.94
7.92
7.83
7.81
7.78
7.73
7.70
7.59
7.52
5.05
4.91
4.51
4.49
4.30
4.28
4.26
4.25
4.23
4.17
4.13
4.11
4.07
3.88
3.87
3.85
3.83
3.79
3.78
3.74
3.60
3.59
3.58
3.56
3.55
3.53
2.78
2.50 DMSO-d6
2.36
1.75
1.66
1.56
1.52
1.51
1.49
1.33
1.24
1.21
1.19
1.17
1.05
1.04
0.87
0.85
0.84
0.83
TFA•H2N
HO
HO
OH
OH
OH
Me
Me
Me
Me
44b
(TFA salt)
0.64
1.38
1.13
0.98
0.84
1.56
1.54
5.06
0.91
5.46
1.53
2.00
1.58
10.20
2.10
1.82
2.34
3.93
19.11
3.66
3.37
6.14
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
f1 (ppm)


172.2
171.5
170.7
170.4
170.4
170.0
169.9
169.9
169.3
75.1
66.7
62.1
61.6
61.4
58.2
55.8
55.4
55.3
54.8
51.7
48.8
47.5
39.5 DMSO-d6
36.2
33.4
31.6
31.3
29.3
29.1
28.8
26.6
23.3
22.1
19.6
17.2
14.7
14.0
TFA•H2N
HO
O
NH
H
HN
OH
Me
44b
(TFA salt)
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

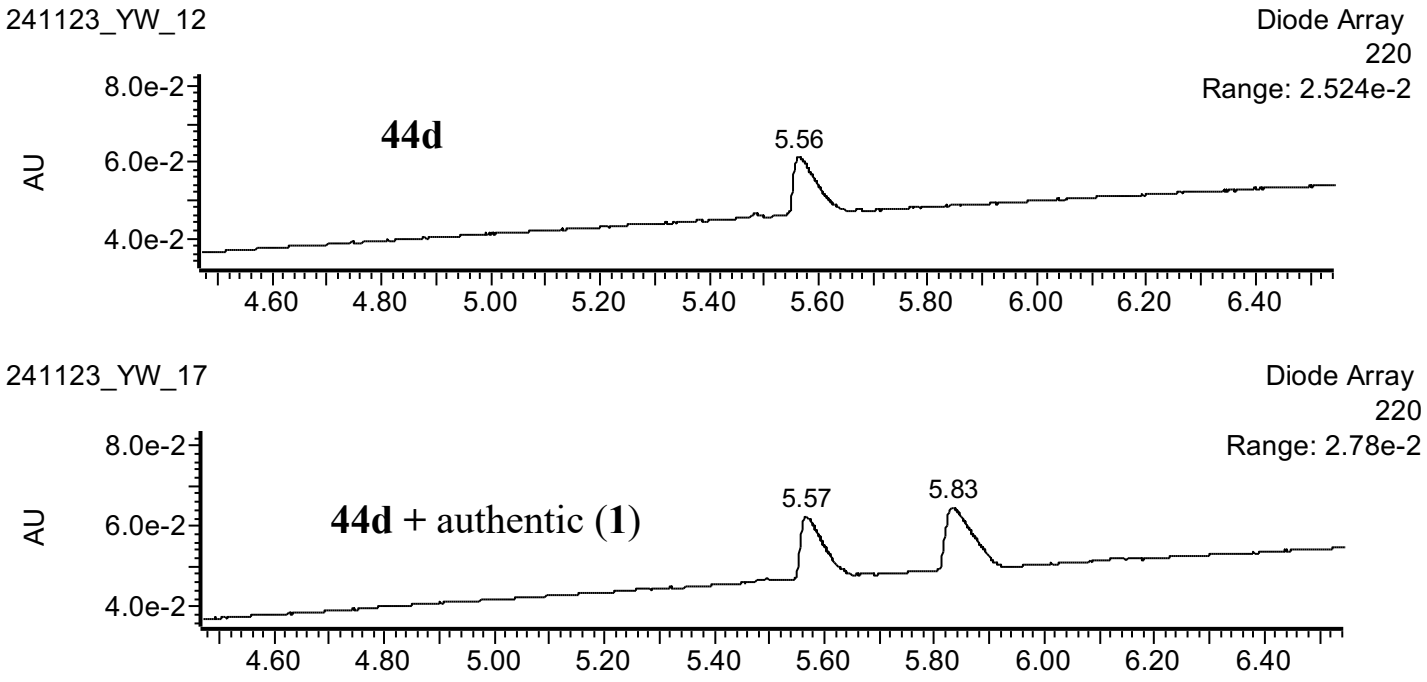


LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA

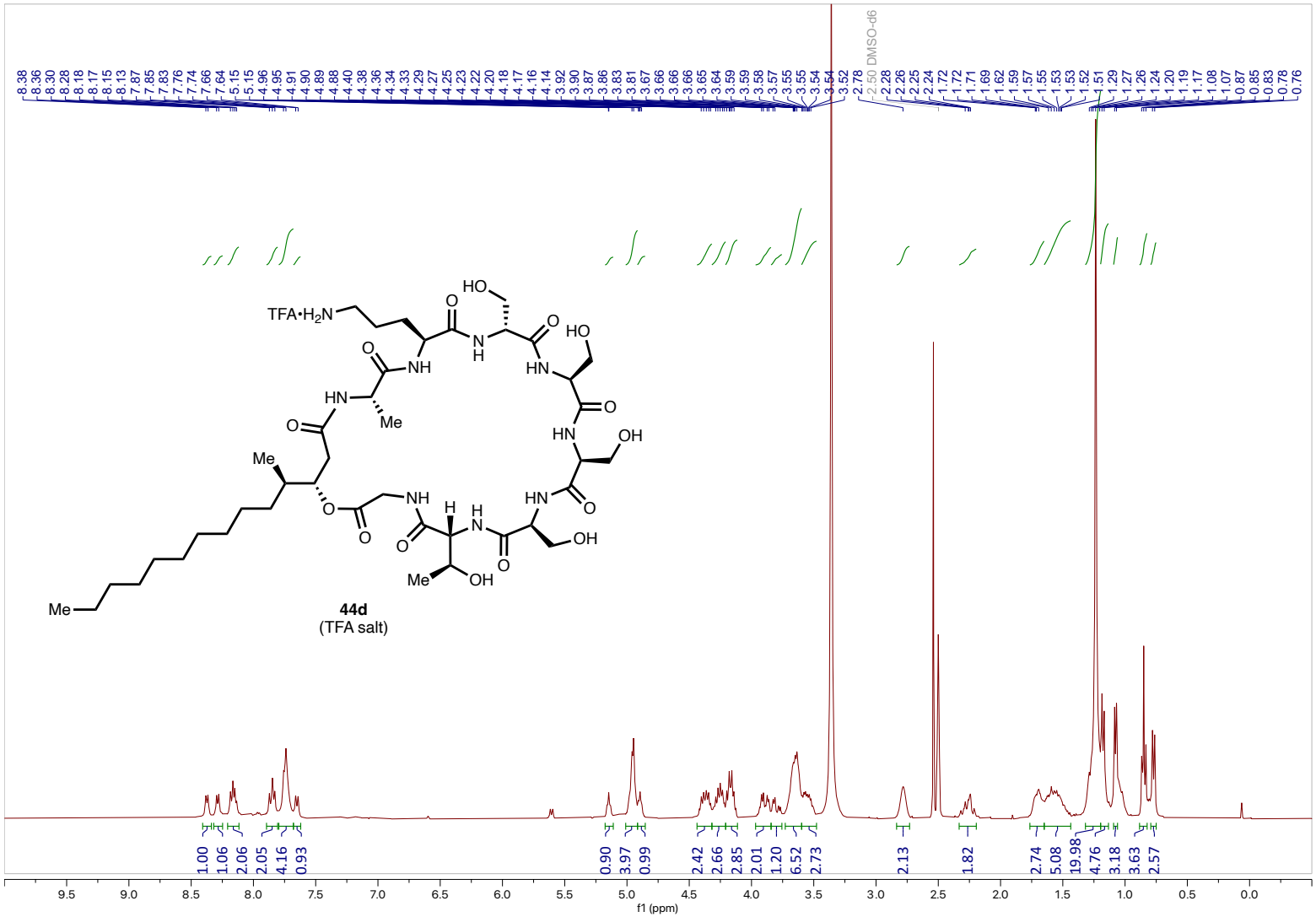

8.25 8.20 8.18 8.08 8.03 8.02 7.80 7.78 7.73 7.70 7.67 7.66 7.65 5.14 5.13 5.12 4.99 4.98 4.95 4.94 4.92 4.91 4.90 4.89 4.87 4.37 4.36 4.34 4.34 4.32 4.32 4.30 4.29 4.27 4.24 4.23 4.23 4.19 4.18 4.17 4.16 4.15 4.13 4.12 3.94 3.93 3.92 3.91 3.89 3.88 3.83 3.81 3.78 3.77 3.66 3.64 3.63 3.61 2.78
2.50 DMSO-d6
2.36 2.34 1.77 1.77 1.75 1.70 1.67 1.65 1.65 1.64 1.62 1.62 1.62 1.60 1.58 1.57 1.35 1.33 1.31 1.28 1.27 1.24 1.22 1.05 1.03 0.87 0.85 0.85 0.84 0.83
TFA•H2N
HO
OH
Me
44c
(TFA salt)
1.97 1.28 1.10 1.33 1.27 5.07 1.00 1.12 2.26 2.08 2.41 2.28 3.53 2.19 1.17 8.20 2.17 2.11 0.99 1.24 3.17 2.24 22.48 4.56 7.18
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
f1 (ppm)



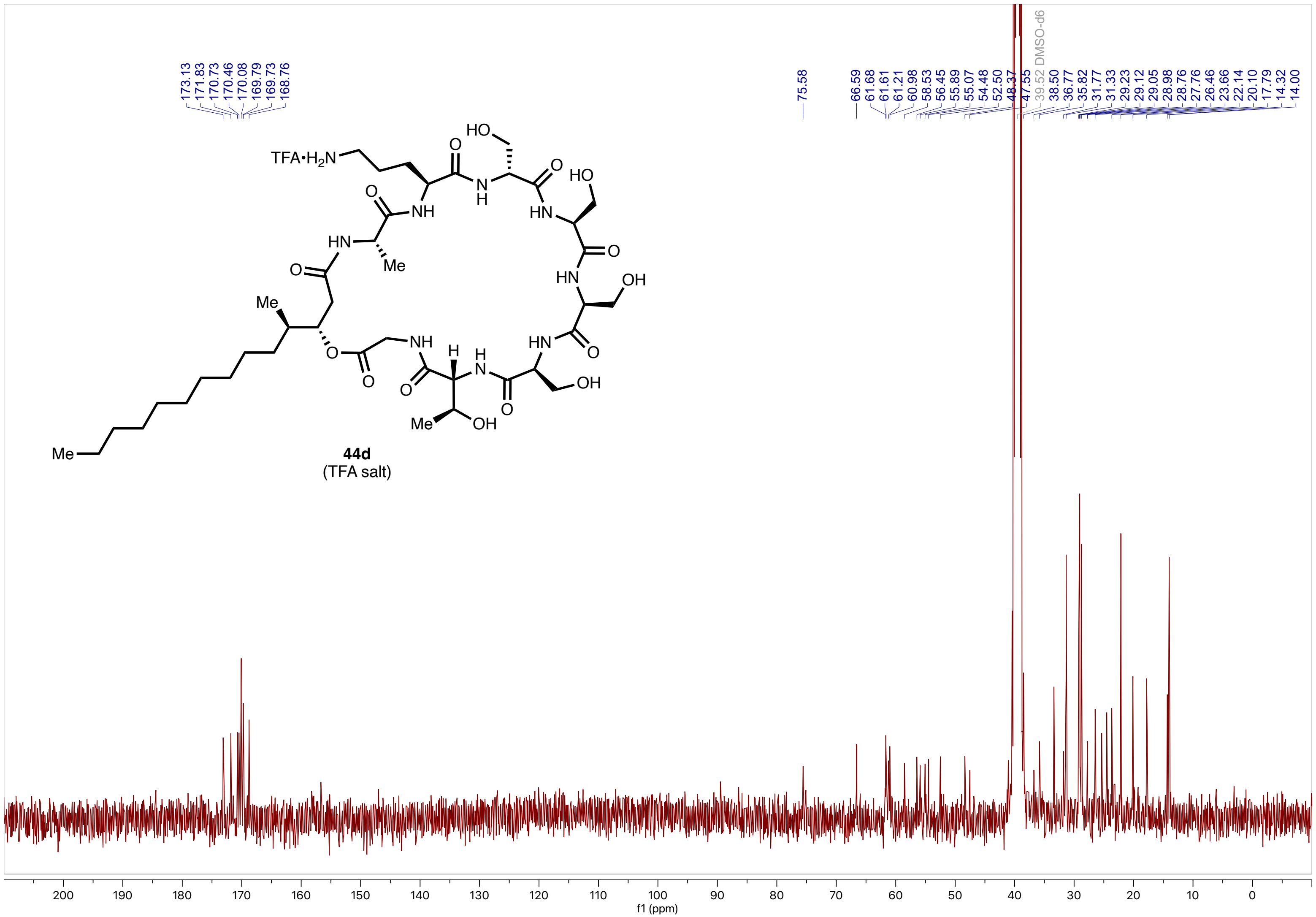
172.9
171.4
171.2
170.9
170.7
170.0
169.9
169.9
169.4
75.3
66.8
61.4
61.2
60.8
58.1
56.2
55.7
54.9
49.3
41.2
39.5 DMSO-d6
38.5
37.2
36.1
31.6
31.3
29.3
29.1
29.0
28.7
28.6
26.4
23.5
22.1
19.7
17.3
14.7
14.0
TFA•H2N
HO
O
NH
N
H
HO
HN
O
HN
Me
OH
Me
O
NH
H
HN
OH
Me
OH
44c
(TFA salt)
f1 (ppm)



LC-MS method
Column ; ACQUITY UPLC BEH C_{18} (2.1 i.d. × 50 mn)
Mobile phase A ; 10% CH_3CN aq. + 0.05 % formic acid
Mobile phase B ; 100 % CH_3CN + 0.05 % formic acid
Linear gradient ; A:B = 100:0 to A:B = 0:100 (0-14min)
Flow late ; 0.6 mL/min
UV ; PDA


8.38 8.36 8.30 8.28 8.18 8.17 8.15 8.13 7.87 7.85 7.83 7.76 7.74 7.66 7.64 5.15 5.15 4.96 4.95 4.91 4.90 4.89 4.88 4.40 4.38 4.36 4.34 4.33 4.29 4.27 4.25 4.23 4.22 4.20 4.18 4.17 4.16 4.14 3.92 3.90 3.87 3.86 3.83 3.81 3.67 3.66 3.66 3.65 3.64 3.59 3.59 3.58 3.57 3.55 3.55 3.54 3.54 3.52 2.78 2.50 DMSO-d6 2.28 2.26 2.25 2.24 1.72 1.71 1.69 1.62 1.59 1.57 1.55 1.53 1.53 1.52 1.51 1.29 1.27 1.26 1.24 1.20 1.19 1.17 1.08 1.07 0.87 0.85 0.83 0.78 0.76
1.00 1.06 2.06 2.05 4.16 0.93 0.90 3.97 0.99 2.42 2.66 2.85 2.01 1.20 6.52 2.73 2.13 1.82 2.74 5.08 19.98 4.76 3.18 3.63 2.57
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
f1 (ppm)

44d
(TFA salt)


173.13
171.83
170.73
170.46
170.08
169.79
169.73
168.76
75.58
66.59
61.68
61.61
61.21
60.98
58.53
56.45
55.89
55.07
54.48
52.50
48.37
47.55
39.52 DMSO-d6
38.50
36.77
35.82
31.77
31.33
29.23
29.12
29.05
28.98
28.76
27.76
26.46
23.66
22.14
20.10
17.79
14.32
14.00
TFA•H2N
44d
(TFA salt)
f1 (ppm)