Biosynthesis of Cell Envelope-Associated Phenolic Glycolipids in *Mycobacterium marinum*

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This paper is dedicated to the memory of our wonderful colleague, Dr. Clifford E. Soll, who recently passed away.

Supplemental Material

TABLE S1. Bacterial strains

Strain	Characteristics	Source or Reference
E. coli DH5α	Cloning host	Invitrogen
E. coli BAP1	Recombinant protein expression host; contains a genome-integrated copy of *sfp*, encoding the phosphopantetheinyl transferase Sfp from *Bacillus subtilis*	(1, 2)
E. coli BL21(DE3)	Recombinant protein expression host	Stratagene
M. marinum strain M	Wild-type	American Type Culture Collection
M. marinum Δ*fadD22*	Carries an in-frame, unmarked *fadD22* deletion	This study
M. marinum Δ*fadD26*	Carries an in-frame, unmarked *fadD26* deletion	This study
M. marinum Δ*fadD28*	Carries an in-frame, unmarked *fadD28* deletion	This study
M. marinum Δ*fadD29*	Carries an in-frame, unmarked *fadD29* deletion	This study
M. marinum $ppsA_{S\text{-}A}$	Carries a *ppsA* mutated allele with a Ser-to-Ala substitution disrupting the phosphopantetheine attachment site of the ACP_L domain	This study

TABLE S2. Plasmids

Plasmid	Characteristics	Source or Reference
pCR2.1-TOPO	Cloning vector, kanamycin and ampicillin resistance	Invitrogen
p2NIL	Vector with kanamycin resistance gene and OriE	(3)
pGOAL19	Vector with *sacB-lacZ Pac*I cassette, hygromycin resistance, OriE	(3)
p2NILGOALc-Δ*fadD22*c	*fadD22* deletion cassette delivery vector	This study
p2NILGOALc-Δ*fadD26*c	*fadD26* deletion cassette delivery vector	This study
p2NILGOALc-Δ*fadD28*c	*fadD28* deletion cassette delivery vector	This study
p2NILGOALc-Δ*fadD29*c	*fadD29* deletion cassette delivery vector	This study
p2NILGOALc-*ppsA*c	*ppsA* mutagenesis cassette delivery vector	This study
pCP0	Mycobacterial expression vector, kanamycin resistance	(4)
pCP0-FadD22	pCP0 derivative expressing FadD22	This study
pCP0-FadD26	pCP0 derivative expressing FadD26	This study
pCP0-FadD28	pCP0 derivative expressing FadD28	This study
pCP0-FadD29	pCP0 derivative expressing FadD29	This study
pCP0-PpsA	pCP0 derivative expressing PpsA	This study
pCOLADuet-1	*E. coli* expression vector, kanamycin resistance	Novagen
pCOLADuet-FadD29	pCOLADuet-1 derivative expressing His_6-FadD29	This study
pETDuet-1	*E. coli* expression vector, ampicillin resistance	Novagen
pETDuet-PpsA	pETDuet-1 derivative expressing His_6-PpsA	This study

TABLE S3. PCR primer pairs and amplicon information used in mutant screening and verification

Mutant	Primer name	Primer sequence (5'-3')	Amplicon (bp)	
			Mutant strain	Wild-type strain
Δ*fadD22*	SalIFadD22OF	GTCGACTCACCATCGTGGAGTTCGCCAGCTGG	2,016	4,110
	NotIFadD22OR	GCGGCCGCACGCGCTGGGCGAGCCCATT		
	HindIIIFadD22For	AAGCTTGCTAGGGCGGTCATCCAATAATGC	0	2,156
	HpaIFadD22Rev	GTTAACCTCAGTTGCTCCTGTTCATGATTC		
Δ*fadD26*	HindIIIMarFadD26OF	AAGCTTATGAAGAATTTCGAGTGGGAGTCCTGCCGACATT	1,930	3,658
	KpnIMarFadD26OR	GGTACCTCCGCGAAACACCGCCGGAGACAACAGCAAATT		
	pCP0ForMarFadD26	GAATTCAATGGGAGTGTAGTGCGATGCCGG	0	1,794
	pCP0RevMarFadD26	AAGCTTATGCTCGGTGTCATACCGTCACGT		

TABLE S3. Continuation

Δ*fadD28*	BamHIMarFadD28OF	GGATCCACCCGCTTCACCTCTTCGAGGAAAACTGGAAA	1,928	3,639
	NotIMarFadD28OR	GCGGCCGCTTCGGGCCAGCAGTAGCGGGGCGGTGAGTAT		
	pCP0ForMarFadD28	AAGCTTCGGTAACGTGATGCCCATGAGTGT	0	1,779
	pCP0RevMarFadD28	GCTAGCTTGACACGCTAGACGTCCAGGCGG		
Δ*fadD29*	HindIIIMarFadD29OF	AAGCTTACCACGGTCTGAGGTATCTGCTGGTGATCAAT	1,966	3,804
	PmlIMarFadD29OR	CACGTGAAAACCTGCACCAGCTCCGGCGAATCCGGCATA		
	pCP0ForMarFadD29	GAATTCTCTTCCGTCGAGAGCAAGGATGAT	0	1,919
	pCP0RevMarFadD29	AAGCTTTAAAGCCGCCAACATGCCGTCATG		
$\Delta ppsA_{S\text{-}A}$	ppsAACPMutOF	AAGCTTCGCTCCCTTCAACCTCAGCCCCGCTGCG	1,832 (split by BssHII)	1,832 (no split by BssHII)
	ppsAACPMutOR	CCGGCACCTTCACCACGGACGAAACCGTCG		
	pm7	CGATCGAGGACTTGCGGTCACAG	526 (Ser-to-Ala mutations)	526 (wild-type sequence)
	pm8	GGTGGCCGACGTCGTGCTGGTAC		

TABLE S4. Primers and amplicons pertaining to construction of mutagenesis cassettes & expression plasmids

Amplicon (bp)	Primer name	Primer sequence (5'-3')	Comments
Δ*fadD22*c 5' arm (1,025)	SalIFadD22OF	GTCGACTCACCATCGTGGAGTTCGCCAGCTGG	Full-length cloned into p2NIL as a SalI-NotI fragment
	FadD22IR	TCCTGTTCATGATTCCCGCATTATTGGATGACCGCCCTA	
Δ*fadD22*c 3' arm (1,018)	FadD22IF	ATCCAATAATGCGGGAATCATGAACAGGAGCAACTGAG	
	NotIFadD22OR	GCGGCCGCACGCGCTGGGCGAGCCCATT	
Δ*fadD22*c full-length (2,016)	SalIFadD22OF	GTCGACTCACCATCGTGGAGTTCGCCAGCTGG	
	NotIFadD22OR	GCGGCCGCACGCGCTGGGCGAGCCCATT	

TABLE S4. Continuation

Δ*fadD26*c 5’ arm (1,029)	HindIIIMarFadD26OF	AAGCTTATGAAGAATTTCGAGTGGGAGTCCTGCCGACATT	Full-length cloned into p2NIL as a HindIII-KpnI fragment
	FadD26IR	TCATACCGTCACGTCGGTCACCGGCATCGCACTACACTCCCATTC	
Δ*fadD26*c 3’ arm (958)	FadD26IF	ATGCCGGTGACCGACGTGACGGTATGACACCGAGCATCGGTG	
	KpnIMarFadD26OR	GGTACCTCCGCGAAACACCGCCGGAGACAACAGCAAATT	
Δ*fadD26*c full-length (1,952)	HindIIIMarFadD26OF	AAGCTTATGAAGAATTTCGAGTGGGAGTCCTGCCGACATT	
	KpnIMarFadD26OR	GGTACCTCCGCGAAACACCGCCGGAGACAACAGCAAATT	

TABLE S4. Continuation

Δ*fadD28*c 5’ arm (983)	BamHIMarFadD28OF	GGATCCACCCGCTTCACCTCTTCGAGGAAAACTGGAAA	Full-length cloned into p2NIL as a BamHI-NotI fragment
	FadD28IR	CTAGACGTCCAGGCGGGGGAACGCACACTCATGGGCATCACGTTA	
Δ*fadD28*c 3’ arm (972)	FadD28IF	ATGAGTGTGCGTTCCCCCGCCTGGACGTCTAGCGTGTCAACCGAC	
	NotIMarFadD28OR	GCGGCCGCTTCGGGCCAGCAGTAGCGGGGCGGTGAGTAT	
Δ*fadD28*c full-length (1,922)	BamHIMarFadD28OF	GGATCCACCCGCTTCACCTCTTCGAGGAAAACTGGAAA	
	NotIMarFadD28OR	GCGGCCGCTTCGGGCCAGCAGTAGCGGGGCGGTGAGTAT	

TABLE S4. Continuation

Δ*fadD29*c 5’ arm (982)	HindIIIMarFadD29OF	AAGCTTACCACGGTCTGAGGTATCTGCTGGTGATCAAT	Full-length cloned into p2NIL as a HindIII-PmlI fragment
	MarFadD29IR	ATGCGGACCGGTCCAAAGTCCATGATCATCCTTGCTCTCGACGGAA	
Δ*fadD29*c 3’ arm (1,015)	MarFadD29IF	AGGATGATCATGGACATTTGGACCGGTCCGCATGACGGCATGTT	
	PmlIMarFadD29OR	CACGTGAAAACCTGCACCAGCTCCGGCGAATCCGGCATA	
Δ*fadD29*c full-length (1,966)	HindIIIMarFadD29OF	AAGCTTACCACGGTCTGAGGTATCTGCTGGTGATCAAT	
	PmlIMarFadD29OR	CACGTGAAAACCTGCACCAGCTCCGGCGAATCCGGCATA	

TABLE S4. Continuation

Δ*ppsA*c 5’ arm (950)	ppsAACPMutOF	AAGCTTCGCTCCCTTCAACCTCAGCCCCGCTGCG	Full-length cloned into p2NIL as a as HindIII-HpaII insert
	ppsAACPMutIR	AGCACAACGGCGTCGCGCGCGGCCACGCCGAGGTCA	
Δ*ppsA*c 3’ arm (912)	ppsAACPMutIF	TGACCTCGGCGTGGCCGCGCGCGACGCCGTTGTGCT	
	ppsAACPMutOR	CCGGCACCTTCACCACGGACGAAACCGTCG	
Δ*ppsA*c full-length (1,832)	ppsAACPMutOF	AAGCTTCGCTCCCTTCAACCTCAGCCCCGCTGCG	
	ppsAACPMutOR	CCGGCACCTTCACCACGGACGAAACCGTCG	
RBS-*fadD22* (2,156)	HindIIIFadD22For	AAGCTTGCTAGGGCGGTCATCCAATAATGC	Cloned into pCP0 as a HindIII-HpaI insert
	HpaIFadD22Rev	GTTAACCTCAGTTGCTCCTGTTCATGATTC	

TABLE S4. Continuation

RBS-*fadD26* (1,794)	pCP0ForMarFadD26	GAATTCAATGGGAGTGTAGTGCGATGCCGG	Cloned into pCP0 as an EcoRI-HindIII insert
	pCP0RevMarFadD26	AAGCTTATGCTCGGTGTCATACCGTCACGT	
RBS-*fadD28* (1,779)	pCP0ForMarFadD28	AAGCTTCGGTAACGTGATGCCCATGAGTGT	Cloned into pCP0 as a NheI-HindIII insert
	pCP0RevMarFadD28	GCTAGCTTGACACGCTAGACGTCCAGGCGG	
RBS-*fadD29* (1,919)	pCP0ForMarFadD29	GAATTCTCTTCCGTCGAGAGCAAGGATGAT	Cloned into pCP0 as an EcoRI-HindIII insert
	pCP0RevMarFadD29	AAGCTTTAAAGCCGCCAACATGCCGTCATG	
RBS-*ppsA* (4,853)	pm25	GCCAGTTAACATTCAATCGGAGGAAAGTGACGG TATGACACCGAGCATCGGTGGAGAAGCCGA	Cloned into pCP0 as a HpaI-NheI insert
	pm26	GATGGCTAGCTTACCCAGGGCCCTCCGGGAGCA GTGAA	

TABLE S4. Continuation

fadD29 (1,881)	MarfadD29NTFor	GAATTCTATCATGGACACCAACGCCGTTTCG	Cloned into pCOLADuet as an EcoRI-NotI insert
	MarfadD29NTRev	GCGGCCGCTCATGCGGACCGGTCCAAACGGGT	
ppsA NT1 (2,100)	MarppsANT1For	GGATCCTACACCGAGCATCGGTGGAGAAGC	NT1+NT2+NT3 cloned (multistep) into pETDuet
	MarppsANT1Rev	GGCGCGCCGAGCTGCATGCCGATCAGACCTAGCT	
ppsA NT2 (2,000)	MarppsANT2For	GCCGAACTGGAACCGGTATTCCTGGCCGAA	
	MarppsANT2Rev	GCCGCTGGTGGCGGTGTAGTGGGCGAGCCA	
ppsA NT3 (809)	MarppsANT3For	GTGCGCGCCATGTTTGCACCCAAACTCGAC	
	MarppsANT3Rev	AAGCTTTTACCCAGGGCCCTCCGGGAGCAG	

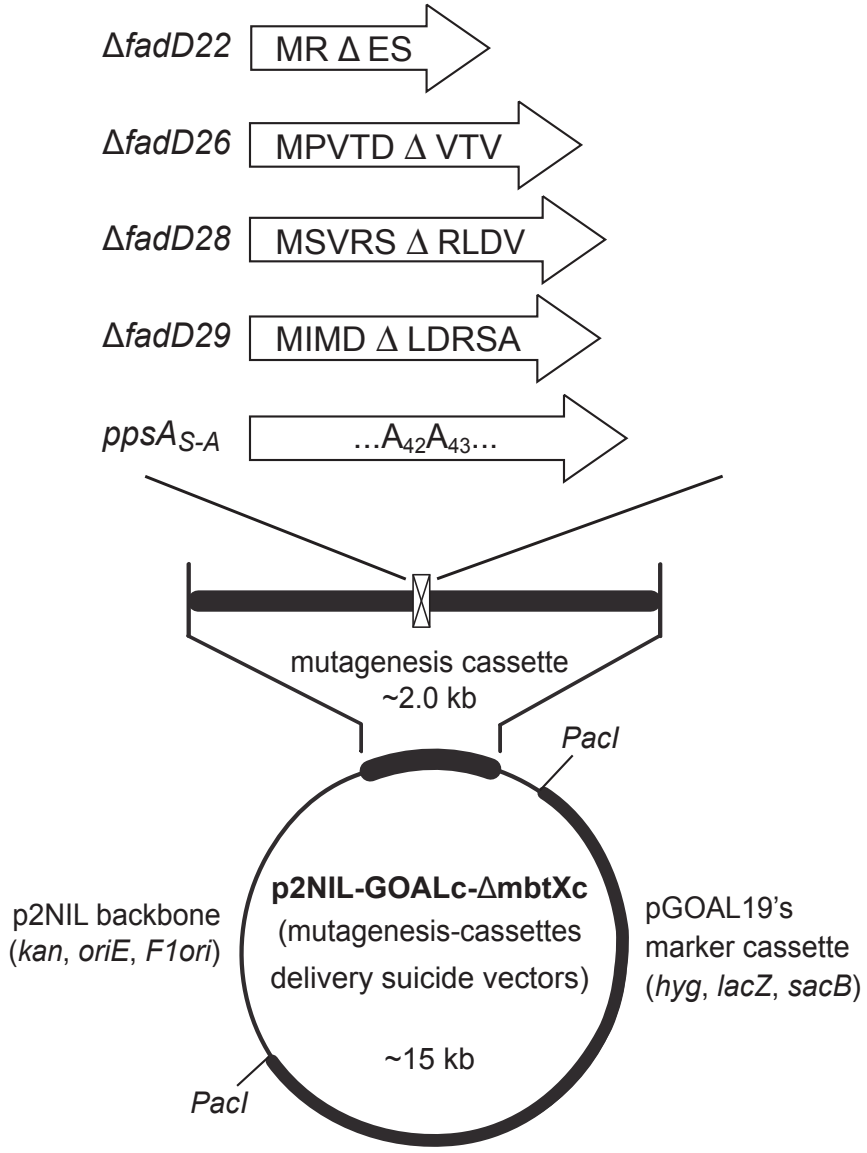
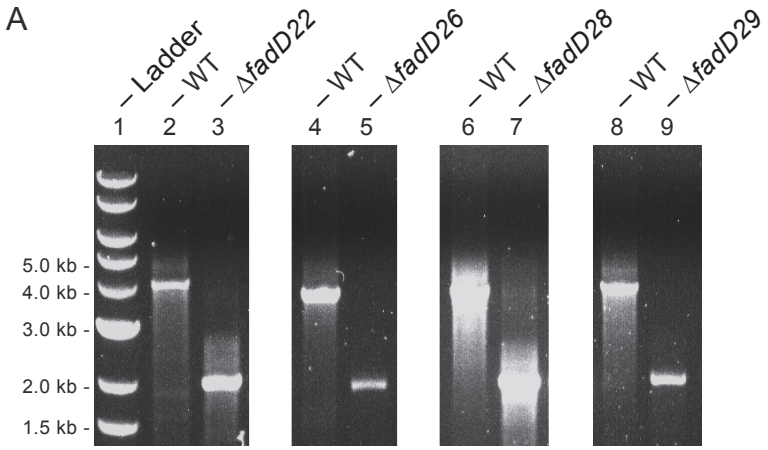
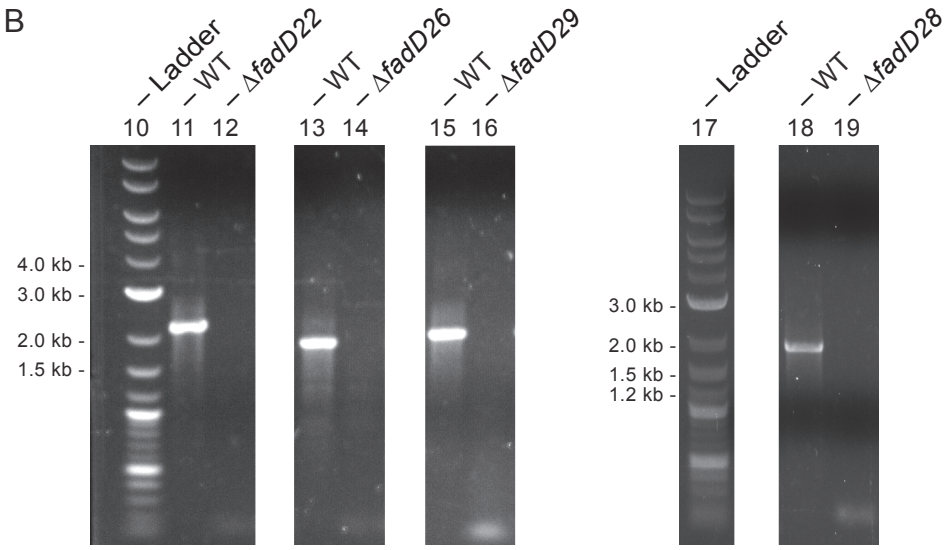


Fig. S1. Mutagenesis cassette-delivery suicide vectors used for construction of *M. marinum* mutants. The predicted translational products of the gene remnants or the amino acid substitutions in the mutagenesis cassette-delivery vectors are shown. The deletion/mutation point is flanked by *ca*. 1.0 kb of downstream and upstream wild-type sequence for homologous recombination with the chromosome.


A
– Ladder
– WT
– ΔfadD22
– WT
– ΔfadD26
– WT
– ΔfadD28
– WT
– ΔfadD29
1 2 3 4 5 6 7 8 9
5.0 kb -
4.0 kb -
3.0 kb -
2.0 kb -
1.5 kb -


B
– Ladder
– WT
– ΔfadD22
– WT
– ΔfadD26
– WT
– ΔfadD29
– Ladder
– WT
– ΔfadD28
10 11 12 13 14 15 16 17 18 19
4.0 kb -
3.0 kb -
2.0 kb -
1.5 kb -
3.0 kb -
2.0 kb -
1.5 kb -
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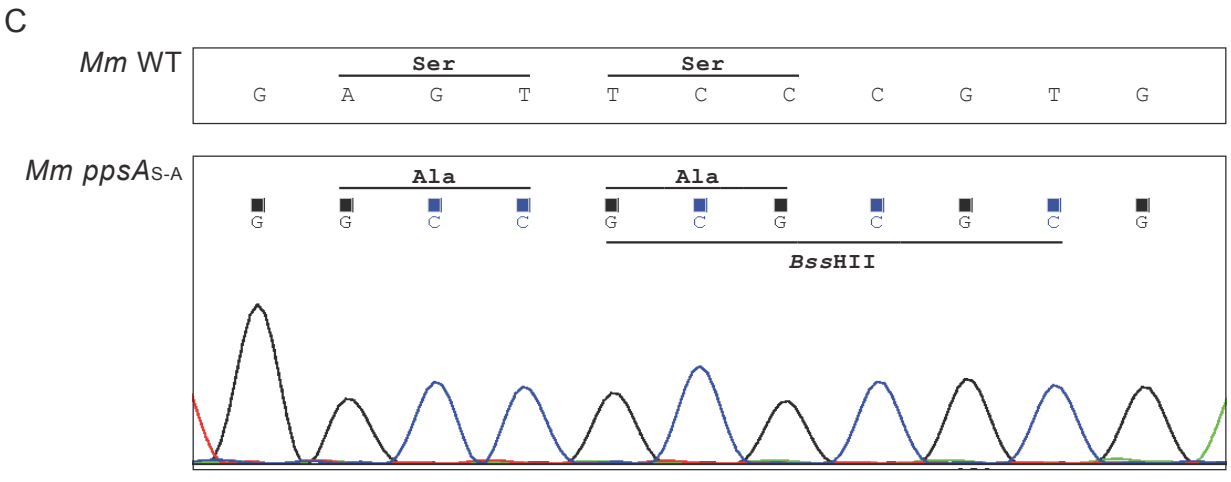

C
Mm WT
Ser
Ser
G A G T T C C C G T G
Mm ppsAS-A
Ala
Ala
G G C C G C G C G C G
BssHII

Fig. S2. Mutant verification analysis. Each chromosomal deletion was confirmed by PCR using two independent primer pairs. One primer pair was used to verify the expected ~2-kb deletion at the deletion site (A). A second primer pair was used to confirm the elimination of the target gene from the chromosome (B). The nucleotide substitutions in *Mm ppsA*$_{S\text{-}A}$ introducing Ser-to-Ala substitutions and a BssHII restriction site were confirmed by sequence analysis (C). Amplicon information for the agarose gel electrophoresis image shown in panel A: lane 2, *Mm* WT (4,110-bp amplicon expected with primers SalIFadD22OF and NotIFadD22OR); lane 3, *Mm* Δ*fadD22* (2,016-bp amplicon expected with SalIFadD22OF and NotIFadD22OR); lane 4, *Mm* WT (3,658-bp amplicon expected with primers HindIIIMarFadD26OF and KpnIMarFadD26OR); lane 5, *Mm* Δ*fadD26* (1,930-bp amplicon expected with HindIIIMarFadD26OF and KpnIMarFadD26OR); lane 6, *Mm* WT (3,639-bp amplicon expected with primers BamHIMarFadD28OF and NotIMarFadD28OR); lane 7, *Mm* Δ*fadD28* (1,928-bp amplicon expected with BamHIMarFadD28OF and NotIMarFadD28OR); lane 8, *Mm* WT (3,804-bp amplicon expected with primers BamHIMarFadD28OF and NotIMarFadD28OR); lane 9, *Mm* Δ*fadD29* (1,966-bp amplicon expected with HindIIIMarFadD29OF and PmlIMarFadD29OR). Amplicon information for the agarose gel electrophoresis image shown in panel B: lane 11, *Mm* WT, 2,156-bp amplicon expected with primers HindIIIFadD22For and HpaIFadD22Rev; lane 12, *Mm* Δ*fadD22*, no amplicon expected with HindIIIFadD22For and HpaIFadD22Rev; lane 13, *Mm* WT, 1,794-bp amplicon expected with primers pCP0ForMarFadD26 and pCP0RevMarFadD26; lane 14, *Mm* Δ*fadD26*, no amplicon expected with pCP0ForMarFadD26 and pCP0RevMarFadD26; lane 15, *Mm* WT, 1,919-bp amplicon expected with primers pCP0ForMarFadD29 and pCP0RevMarFadD29; lane 16, *Mm* Δ*fadD29*, no amplicon expected with pCP0ForMarFadD29 and pCP0RevMarFadD29; lane 18, *Mm* WT, 1,779-bp amplicon expected with primers pCP0ForMarFadD28 and pCP0RevMarFadD28; lane 19, *Mm* Δ*fadD28*, no amplicon expected with pCP0ForMarFadD28 and pCP0RevMarFadD28. DNA ladder marker: lanes 1, 10, and 17. The sizes of relevant DNA makers flanking the PCR product of interests are indicated. WT, wild-type. *Mm*, *M. marinum*. See Table S3 for primer information.

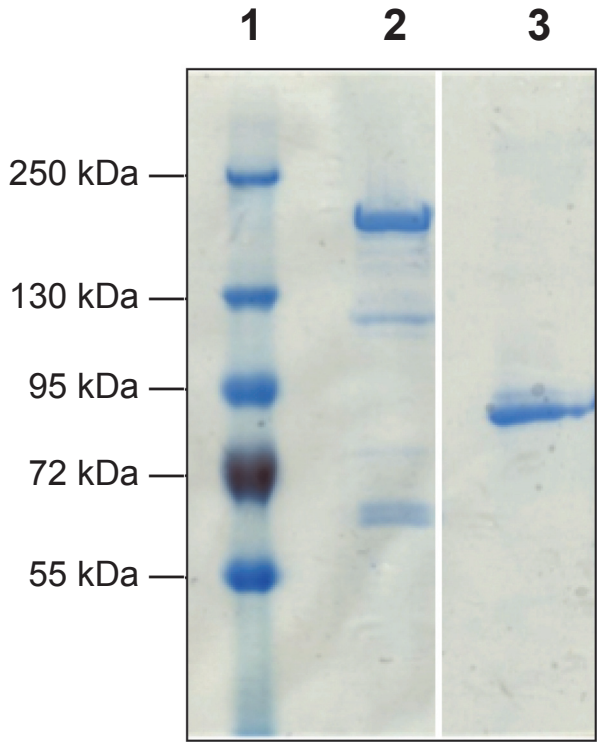


Fig. S3. Recombinant PpsA (lane 2) and FadD29 (lane 3) proteins. Protein samples were resolved by SDS-PAGE and stained with GelCode Blue Stain (Pierce). The positions of molecular weight markers (lane 1) are indicated.

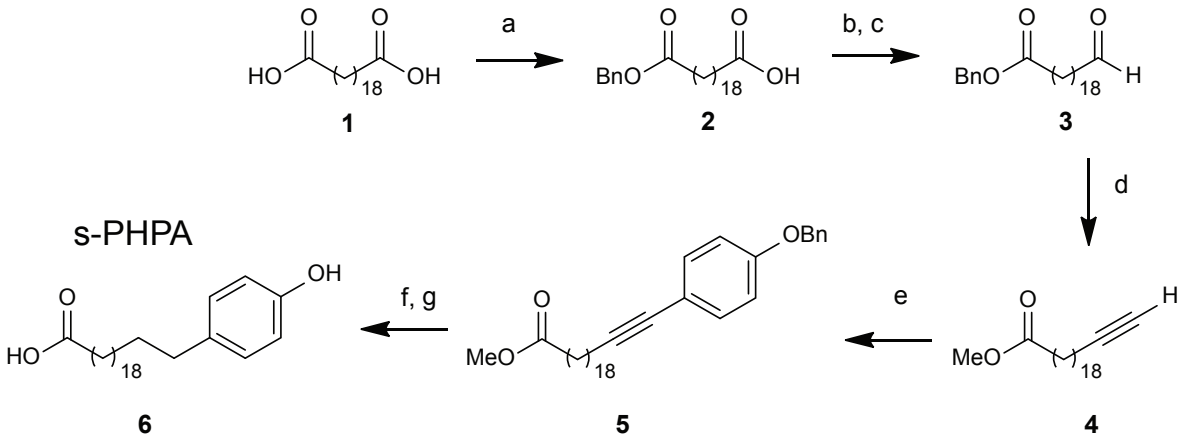


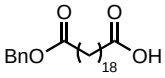
Fig. S4. s-PHPA synthesis outline. Conditions: (a) tetrabutylammonium fluoride, benzyl bromide (44%); (b) BH_3·dimethylsulfide (99%); (c) oxalyl chloride, dimethylsulfoxide, triethylamine; (d) dimethyl (1-diazo-2-oxopropyl)phosphonate, K_2CO_3 (88%, 2 steps); (e) 1-(benzyloxy)-4-iodobenzene, $PdCl_2(PPh_3)_2$, CuI, triethylamine (73%); (f) LiOH (95%); (g) Palladium/activated Carbon (10 mol %), H_2 (98%).

Synthesis of s-PHPA

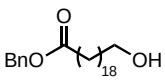
1) General experimental procedures

Unless otherwise stated, all commercially available materials were purchased from Aldrich, TCI America, or Alfa Aesar and used without any subsequent purification. When necessary, solvents and reagents were dried prior to use, using standard protocols. All non-aqueous reactions were carried out in oven-dried glassware under an atmosphere of Argon. Flash column chromatography was conducted following the protocol reported by Still and co-workers (5), using ICN Silted 32–63 D 60 Å silica gel. Analytical thin layer chromatography (TLC) was performed employing Merck 250 micron 60F-254 silica plates. The plates were visualized either by exposure to UV light, staining with iodine impregnated silica gel, or by staining with ceric ammonium molybdate (CAM). Mass spectra were recorded on a Waters Micromass SQD spectrometer by electrospray (ESI) ionization in negative ionization mode. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker DRX-500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS), using either TMS or the solvent resonance as an internal standard (TMS, ^{1}H: 0 ppm; chloroform, ^{13}C: 77.0 ppm; MeOH-d_4, ^{1}H: 3.31 ppm; ^{13}C: 49.0 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), integration, and coupling constant. Supporting NMR spectra are presented below, after Experimental Procedures.

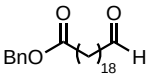
2) Experimental Procedures



20-(Benzyloxy)-20-oxoicosanoic acid (2). To a 35˚C solution of icosanedioic acid (**1**, 10 g, 29.2 mmol) in tetrahydrofuran (THF) (400 ml) was added tetrabutylammonium fluoride (TBAF) (1.0 M in THF, 29.2 ml, 29.2 mmol) dropwise (6). The resulting mixture stirred for 30 min at which point a solution of benzyl bromide (3.47 ml, 29.2 mmol) in THF (50 ml) was added via addition funnel over 3 h. Upon complete addition, the reaction stirred an additional 14 h at 35 ˚C, was cooled to rt and slowly diluted with aqueous HCl (1 M, 400 ml), then extracted with ethyl acetate (EtOAc) (4 × 100 ml). The combined organic layers were washed with aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo* to provide the crude product as a white powder. Purification by flash chromatography (0–10% EtOAc in dichloromethane (DCM)) yielded the title compound as a white, powdery solid (5.51 g, 44%). ^{1}H NMR ($CDCl_3$): δ 7.40–7.28 (m, 5H), 5.11 (s, 2H), 2.35 (t, *J* = 7.6 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.67–1.59 (m, 4H), 1.36–1.22 (brm, 28H); ^{13}C NMR ($CDCl_3$): δ 179.6, 173.9, 136.3, 128.7, 128.3, 66.2, 34.5, 34.1, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 25.1, 24.8.

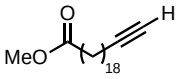


Benzyl 20-hydroxyicosanoate. To a stirred 0˚C solution of 20-(benzyloxy)-20-oxoicosanoic acid (**2**, 5.51 g, 12.7 mmol) in THF (200 ml) was added borane dimethyl sulfide complex (1.8 ml, 1.45 g, 19.1 mmol). The reaction was slowly warmed to ambient temperature over the course of 90 min, where it stirred an additional 6 h. At that point, the mixture was cooled to 0˚C and slowly quenched by the addition of methanol (100 ml) before warming again to ambient temperature. Removal of the solvent *in vacuo* yielded the title compound as a white solid (5.31 g, 99%). This material was essentially pure by NMR and was used without subsequent purification. ^{1}H NMR ($CDCl_3$): δ 7.40–7.30 (m, 5H), 5.11 (s, 2H), 3.64 (t, *J* = 6.7 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.67–1.60 (m, 2H), 1.60–1.53 (m, 2H), 1.36–1.23 (brm, 30H); ^{13}C NMR ($CDCl_3$): δ173.9, 136.3, 128.7, 128.3, 66.2, 63.2, 34.5, 33.0, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 25.9, 25.1.



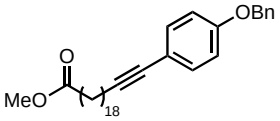
Benzyl 20-oxoicosanoate (3). To a stirred –78˚C solution of oxalyl chloride (0.8 ml, 1.2 g, 9.8 mmol) in DCM (50 ml) was added dimethylsulfoxide (DMSO) (1.36 ml, 1.5 g, 19.2 mmol) dropwise. The mixture was stirred at –78 ˚C for 30 minutes, until the observed bubbling had stopped. To this solution was then added a solution of benzyl 20-hydroxyicosanate (2.0 g, 4.8 mmol) in DCM (50 ml). The reaction was stirred at –78 ˚C for 90 min, during which time it became cloudy. At that point, triethylamine (Et_3T) (6.64 ml, 4.84 g, 47.6 mmol) was added and the reaction continued to stir for 1 h, then was warmed to 0˚C over the course of 1 h. The mixture was then poured into brine

(200 ml) and extracted with DCM (3 × 100 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to provide the title compound as a light yellow solid. The material was unstable at room temperature, so was either stored at –80˚C for short periods of time or used immediately.



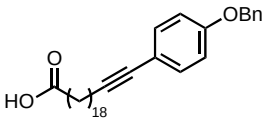
Methyl henicos-20-ynoate (4). Potassium carbonate (736 mg, 5.3 mmol) was added to freshly distilled methanol (30 ml) and the resulting suspension stirred 12 h, until the solution was clear. It was then cooled to 0˚C and dimethyl (1-diazo-2-oxopropyl)phosphonate (550 μl, 700 mg, 3.7 mmol) was added dropwise, followed by a solution of benzyl 20-oxoicosanoate (**3**, 634 mg 1.5 mmol) in methanol (5 ml) and THF (30 ml) (7). The reaction continued stirring at 0˚C until bubbles ceased evolving (40 min), at which point it was warmed to ambient temperature and stirred an additional 5 h. Brine (50 ml) was added to quench the reaction, then all solvents were removed *in vacuo* and the resulting residue was taken up in water (50 ml), then extracted with DCM (4 × 50 ml). The combined organic layers were dried over $MgSO_4$ and concentrated to provide the crude product as a yellow oil. Purification by flash chromatography (5% EtOAc in hexanes) gave the title product as a white powder (556 mg, 88%). NMR analysis confirmed the complete transposition of the benzyl ester to the methyl ester under the reaction conditions. ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 3H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.16 (td, *J* = 7.2, 2.6 Hz, 2H), 1.92 (t, *J* = 2.6 Hz, 1H), 1.66–1.57 (m, 2H), 1.55–1.47 (m,

2H), 1.41–1.34 (m, 2H), 1.32–1.16 (brm, 26H); ^{13}C NMR ($CDCl_3$): δ 174.4, 84.9, 68.1, 51.5, 34.2, 29.8, 29.8, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 28.9, 28.6, 25.1, 18.5.

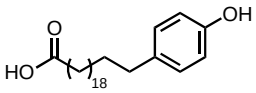


Methyl 21-(4-(benzyloxy)phenyl)henicos-20-ynoate (5). To a well-stirred, degassed solution of 1-benzyloxy-4-iodobenzene (693 mg, 2.24 mg) in THF (10 ml) at ambient temperature was added bis(triphenylphosphine)palladium(II) dichloride (63 mg, 0.09 mmol), followed by copper (I) iodide (51 mg, 0.27 mmol) and Et_3N (3.7 ml, 2.72 g, 26.8 mmol). The reaction vessel was covered in aluminum foil and stirred at ambient temperature for 1 h. At that point a solution of methyl henicos-20-ynoate (**4**, 602 mg, 1.79 mmol) in THF (5 ml) was slowly added and the resulting reaction stirred at ambient temperature for 3 h before being quenched by the addition of a saturated aqueous solution of ammonium chloride (20 ml). The quenched reaction stirred at ambient temperature for 1 h before being extracted with EtOAc (4 × 20 ml). The combined organic layers were washed with a saturated aqueous solution of ammonium chloride (4 × 20 ml), then dried over $MgSO_4$ and concentrated to provide the crude product as an orange solid. Purification by column chromatography (33% DCM in hexanes $\rightarrow$ 100% hexanes $\rightarrow$ 5% EtOAc in hexanes $\rightarrow$ 10% EtOAc in hexanes) furnished the title compound as a white powdery solid (676 mg, 73%). ^{1}H NMR ($CDCl_3$): 7.47–7.30 (m, 7H), 6.89 (d, *J* = 8.4 Hz, 2H), 5.04 (s, 2H), 3.67 (s, 3H), 2.39 (t, *J* = 7.1 Hz, 2H), 2.32 (t, *J* = 7.5 Hz, 2H), 1.68–1.57 (m, 4H), 1.50–1.43 (m, 2H), 1.38–1.23 (brm, 26H); ^{13}C NMR ($CDCl_3$): δ 174.2, 158.2, 136.8, 132.9, 132.9, 128.6, 128.0, 127.5, 127.4, 116.7, 114.7,

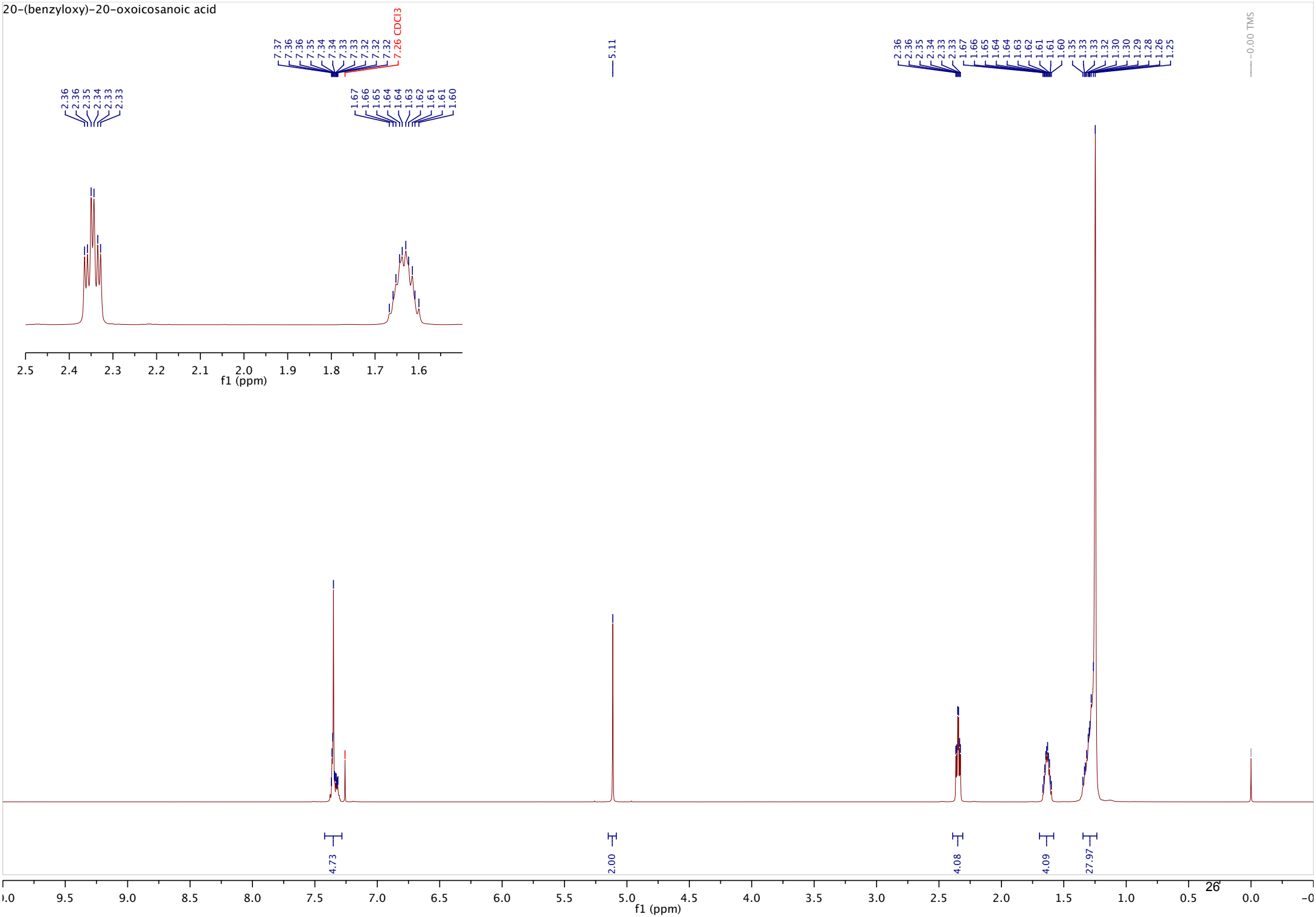
114.7, 88.9, 80.3, 70.0, 51.4, 34.1, 29.8, 29.7, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 29.0, 29.0, 25.0, 19.5.



21-(4-(Benzyloxy)phenyl)henicos-20-ynoic acid. To a stirred solution of methyl 21-(4-(benzyloxy)phenyl)henicos-20-ynoate (**5**, 676 mg, 1.3 mmol) in THF (20 ml) was added lithium hydroxide (2 M solution in water, 14 ml). The reaction was heated to 40˚C and stirred for 1 d, then cooled to ambient temperature and diluted with an aqueous solution of sodium bisulfate (10% wt/wt, 20 ml) (B. Iliev, A. Linden, R. Kunz, and H. Heimgartner, Tetrahedron 62:1079-1094, 2006). The resulting mixture was vigorously stirred for 5 min at which point a pH measurement indicated 1.5. The solution was extracted with EtOAc (4 × 25 ml) and the combined organic layers were dried (MgSO4) then concentrated *in vacuo* to provide the title compound as a white powder (623 mg, 95%). NMR analysis indicated the product was effectively pure, so it was carried on without further purification. ^{1}H NMR ($CDCl_3$): δ 7.47–7.29 (m, 7H), 6.89 (d, *J* = 8.3 Hz, 2H), 5.06 (s, 2H), 2.39 (t, *J* = 7.1 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.70–1.55 (m, 4H), 1.49–1.41 (m, 2H), 1.40–1.23 (brm, 26H); ^{13}C NMR ($CDCl_3$): δ 179.8, 158.4, 137.0, 133.0, 128.7, 128.1, 127.6, 116.9, 115.0, 89.1, 80.4, 70.2, 34.2, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1, 24.9, 19.6.



21-(4-hydroxyphenyl)henicosanoic acid (s-PHPA, 6). A dry three-necked flask was charged with 10% palladium on activated carbon (100 mg) and two of the necks were sealed with a rubber septum. The third was sealed with a fritted glass vacuum adapter and a vacuum was applied. The flask was back-filled with argon and the process repeated (3×). At that point, EtOAc (75 ml) and a solution of 21-(4-(benzyloxy)phenyl)henicos-20-ynoic acid (623 mg, 1.2 mmol) in EtOAc (25 ml) were added. A balloon filled with H_2 gas was attached and the flask purged using the vacuum-fill method as described above (4×). A fresh H_2 balloon was attached and the reaction heated to 40˚C under vigorous stirring. It was kept at this temperature for 3 h and then warmed to 50˚C, where it stirred an additional 20 h before being cooled to ambient temperature and filtered through a pad of Celite. The residue was washed with EtOAc (3 × 25 ml) and the combined filtrate and washings were concentrated *in vacuo* to provide the title compound as a white solid (504 mg, 98%). NMR analysis indicated no purification was necessary. ^{1}H NMR (DMSO-d_6): δ 11.96 (s, 1H), 9.08 (s, 1H), 6.95 (d, *J* = 8.0 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 2H), 2.43 (t, *J* = 7.7 Hz, 2H), 2.18 (t, *J* = 7.3 Hz, 2H), 1.53–1.43 (m, 4H), 1.28–1.18 (brm, 32H); ^{13}C NMR (DMSO-d_6): δ 174.5, 155.2, 132.3, 129.0, 114.9, 34.3, 33.7, 31.3, 29.0, 28.9, 28.9, 28.7, 28.6, 28.


20-(benzyloxy)-20-oxoicosanoic acid
7.37
7.36
7.36
7.35
7.34
7.34
7.33
7.33
7.32
7.32
7.32
7.26 CDCl3
5.11
2.36
2.36
2.35
2.34
2.33
2.33
1.67
1.66
1.65
1.64
1.64
1.63
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1.61
1.60
2.36
2.36
2.35
2.34
2.33
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1.64
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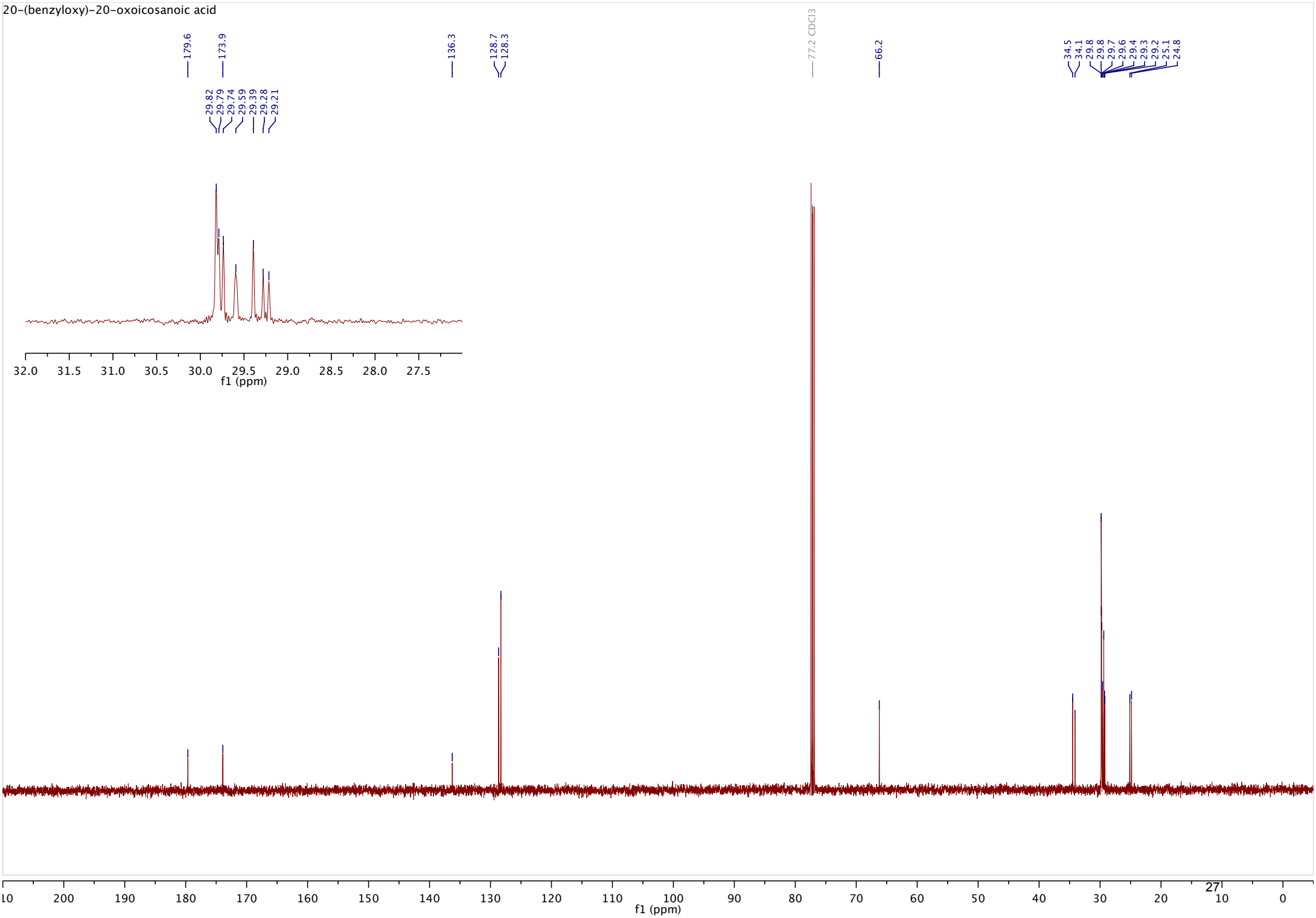
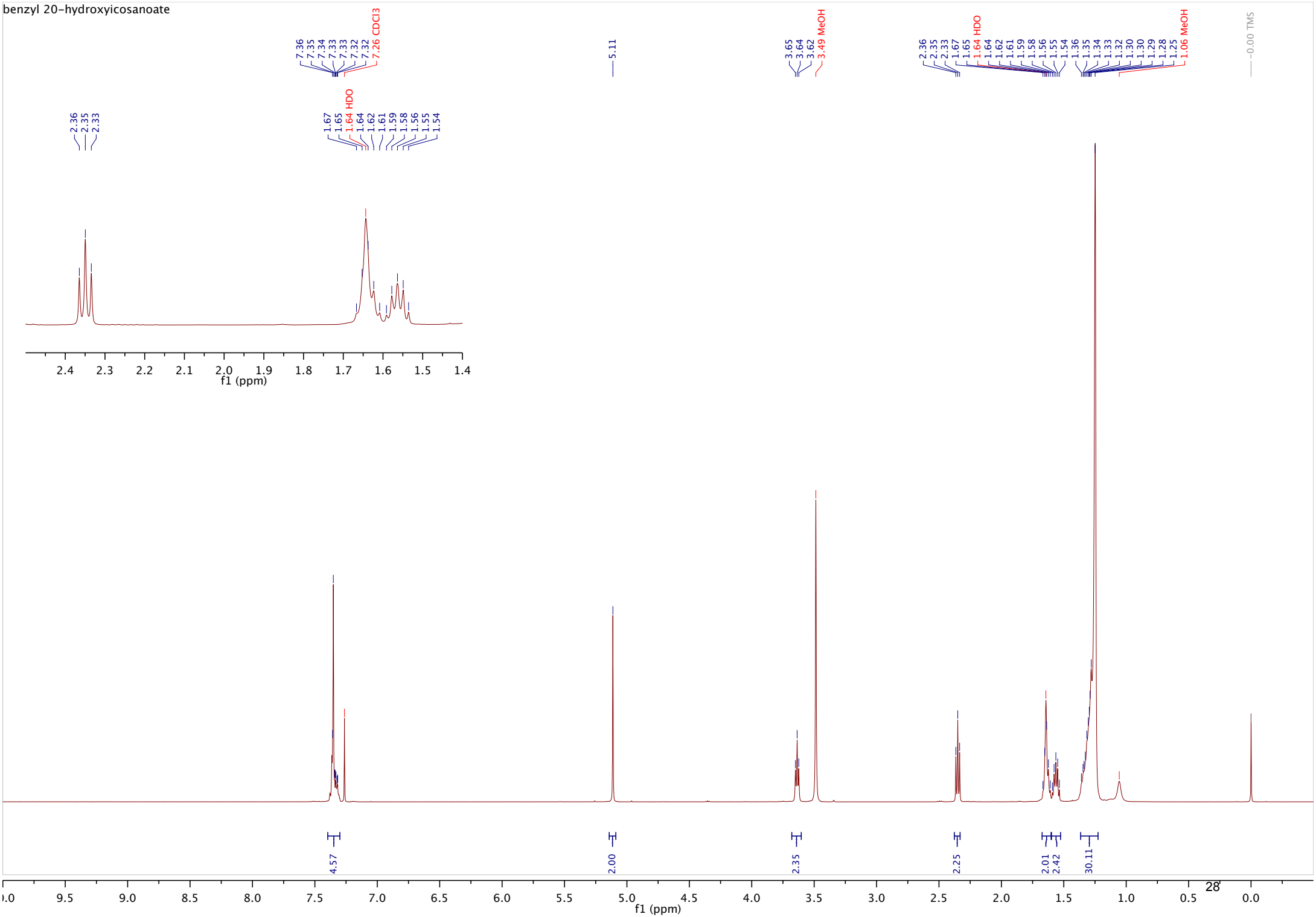
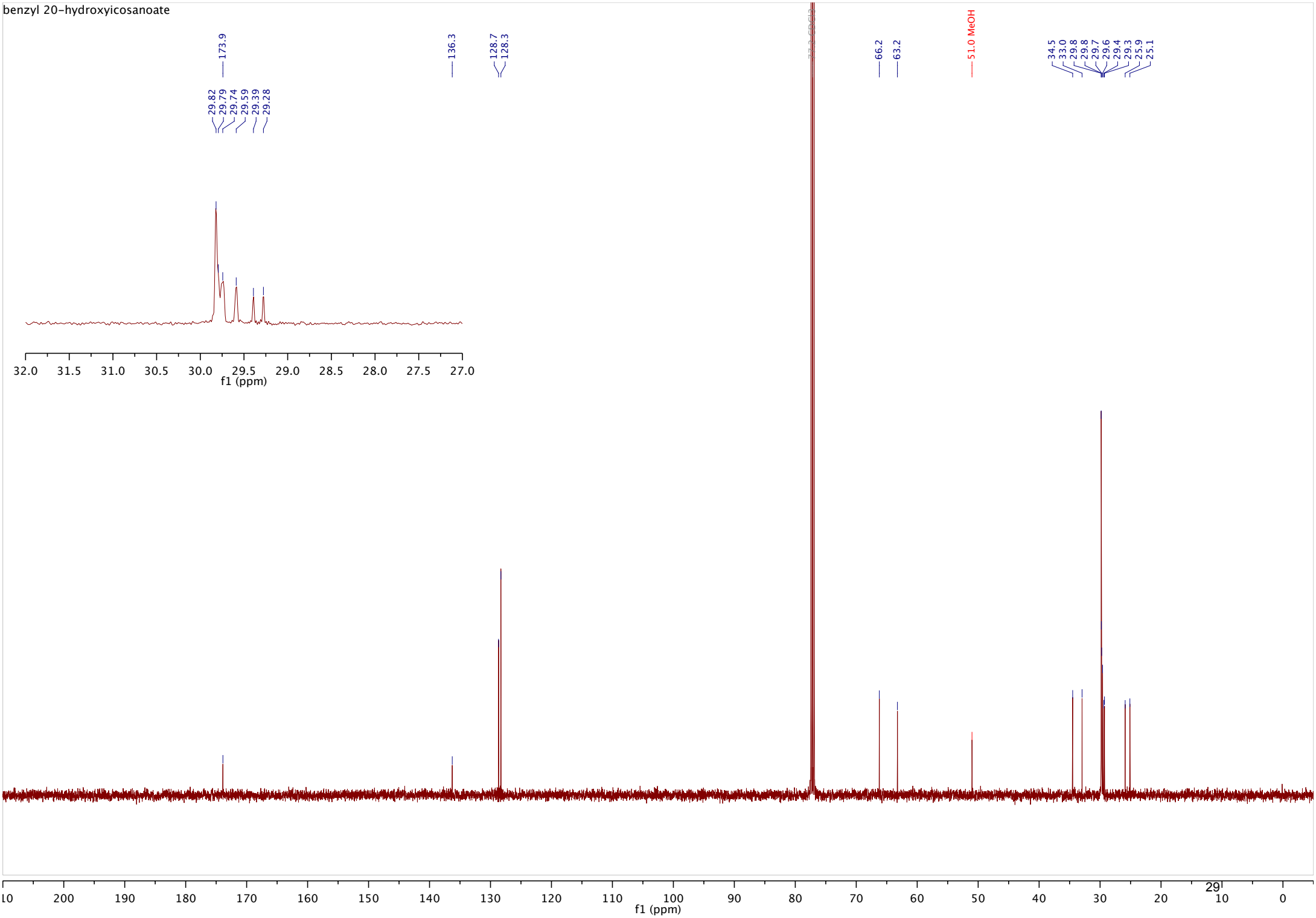
1.30
1.29
1.28
1.26
1.25
-0.00 TMS
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
f1 (ppm)
4.73
2.00
4.08
4.09
27.97
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

20-(benzyloxy)-20-oxoicosanoic acid



benzyl 20-hydroxyicosanoate




benzyl 20-hydroxyicosanoate
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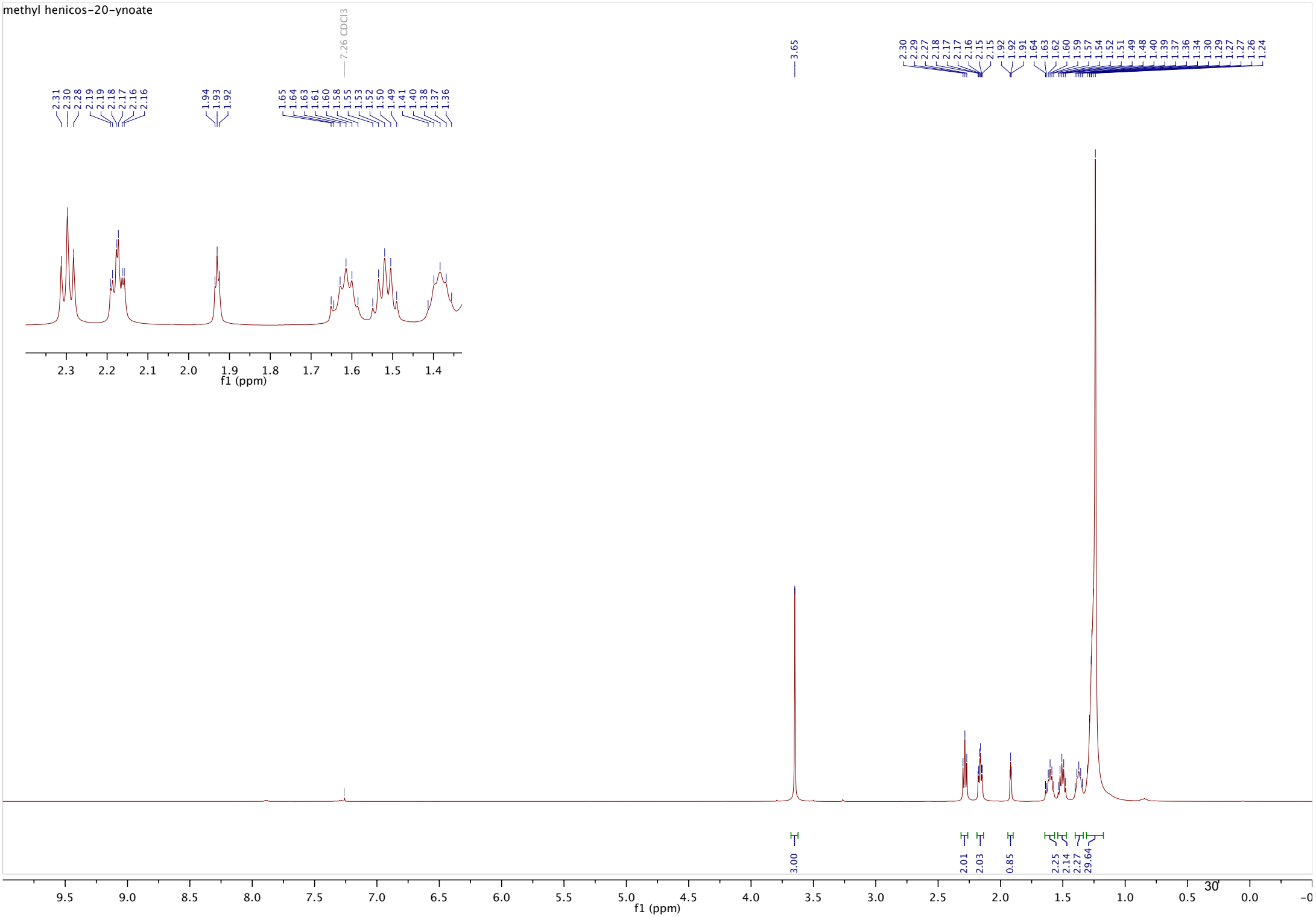
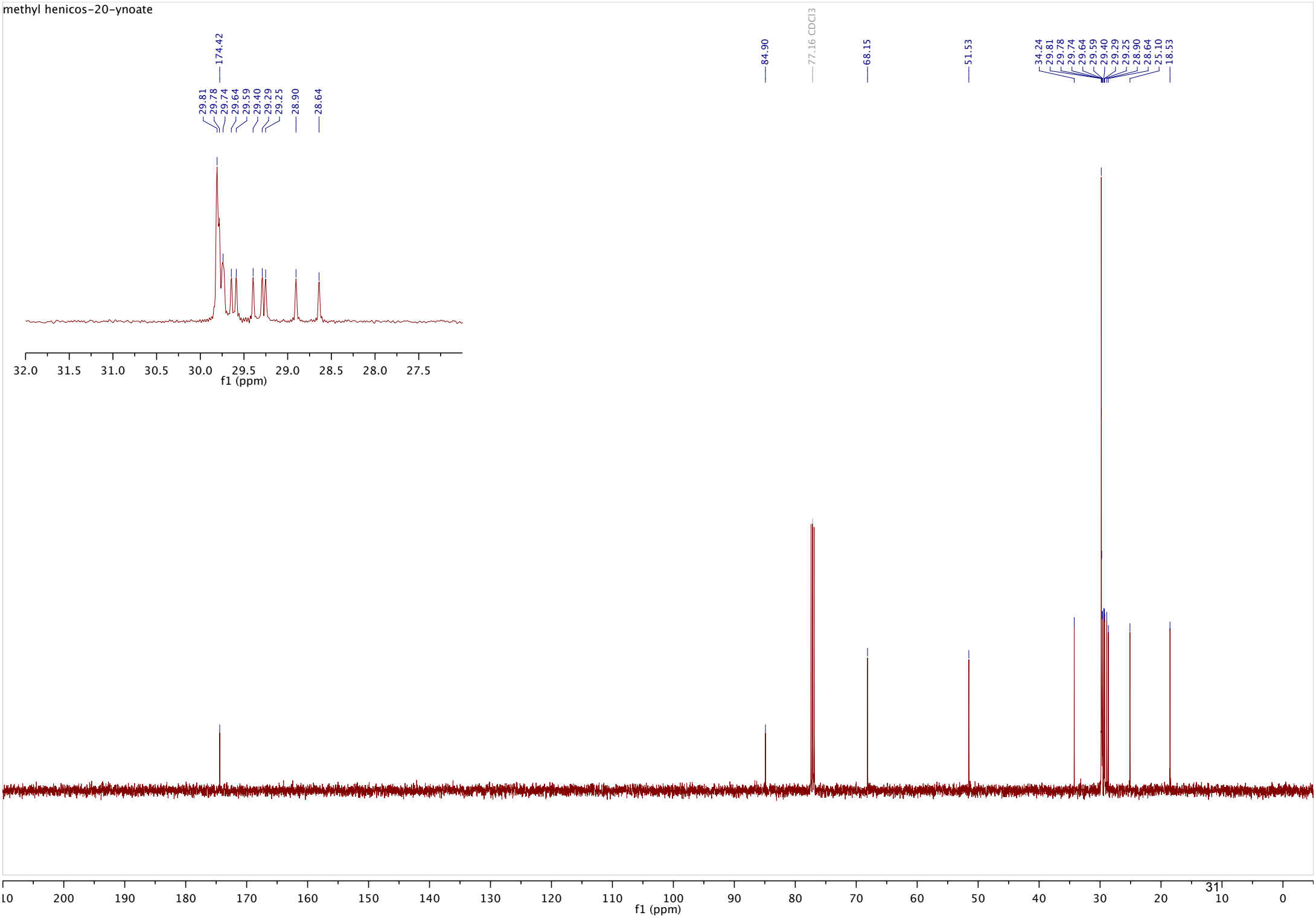
136.3
128.7
128.3
77.3 CDCl3
66.2
63.2
51.0 MeOH
34.5
33.0
29.8
29.8
29.7
29.6
29.4
29.3
25.9
25.1
29.82
29.79
29.74
29.59
29.39
29.28
32.0 31.5 31.0 30.5 30.0 29.5 29.0 28.5 28.0 27.5 27.0
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

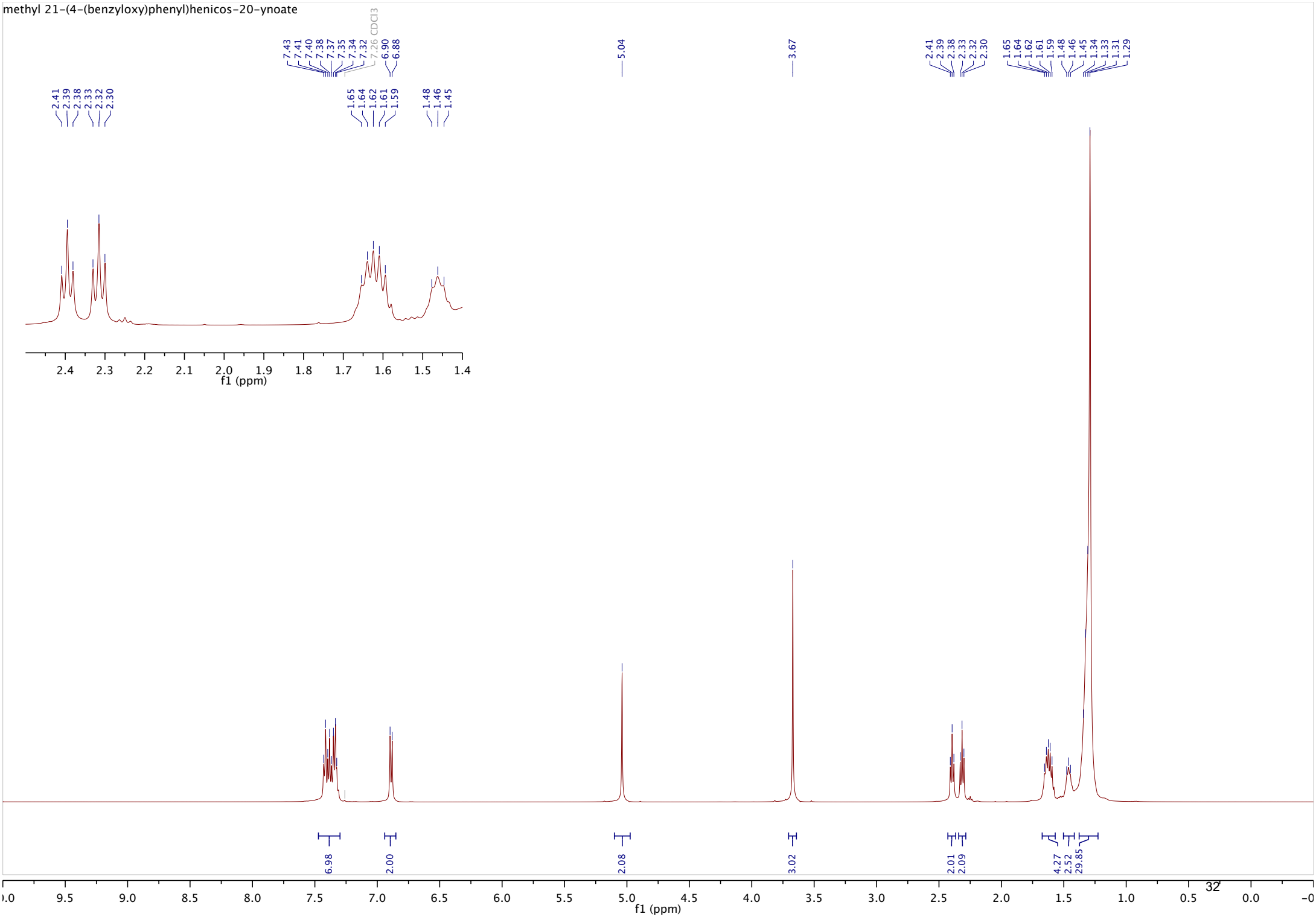
methyl henicos-20-ynoate



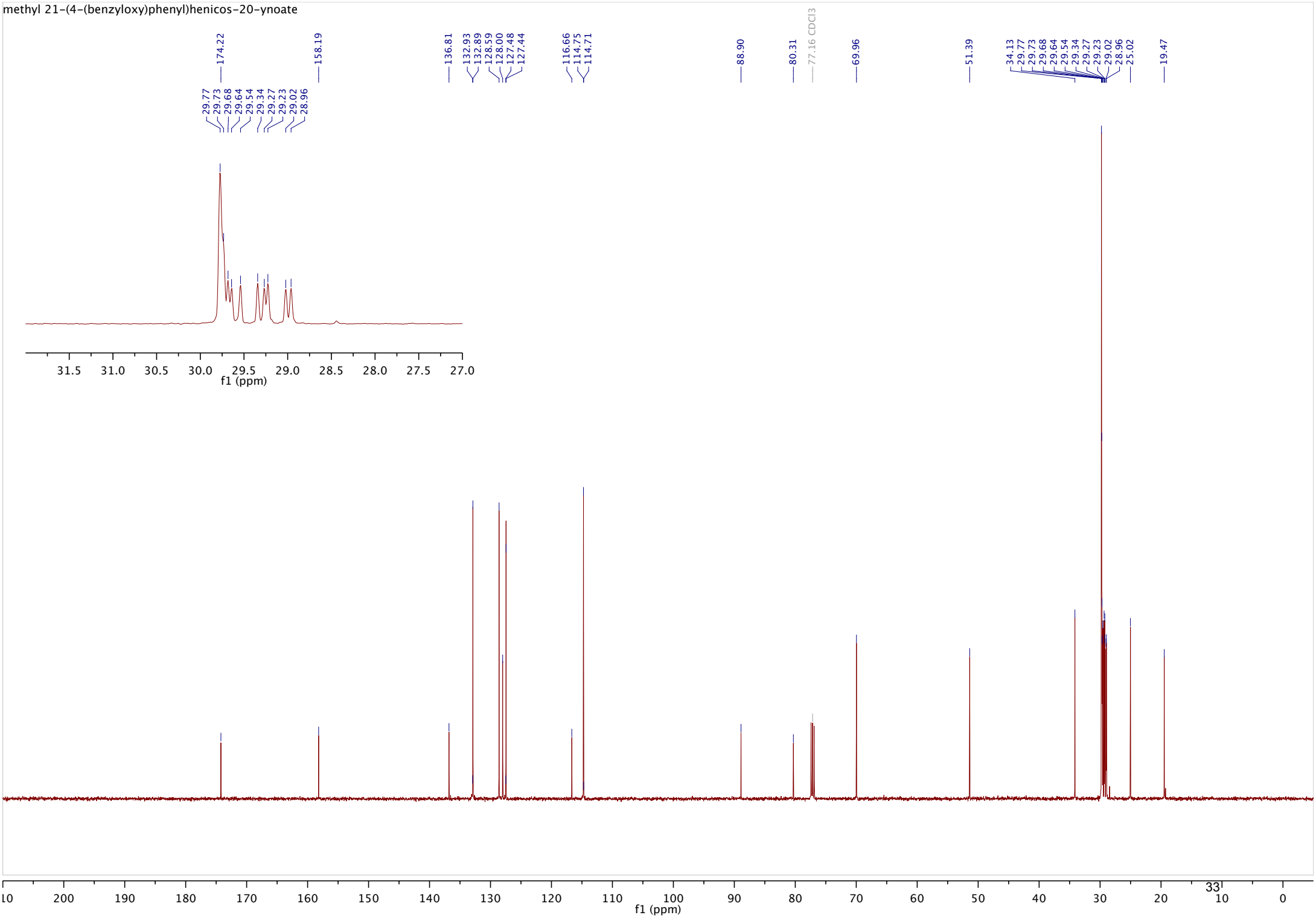
methyl henicos-20-ynoate



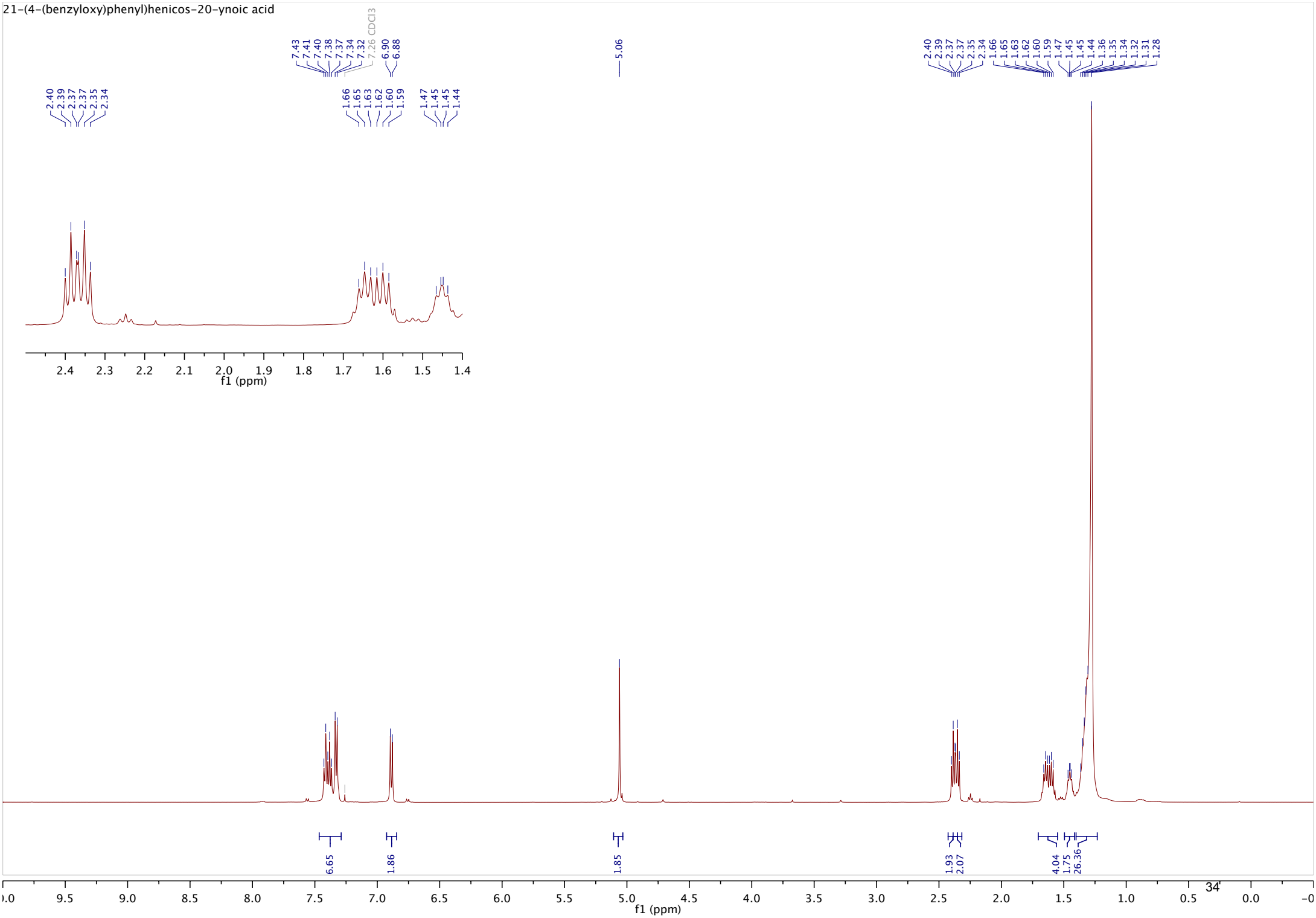
methyl 21-(4-(benzyloxy)phenyl)henicos-20-ynoate



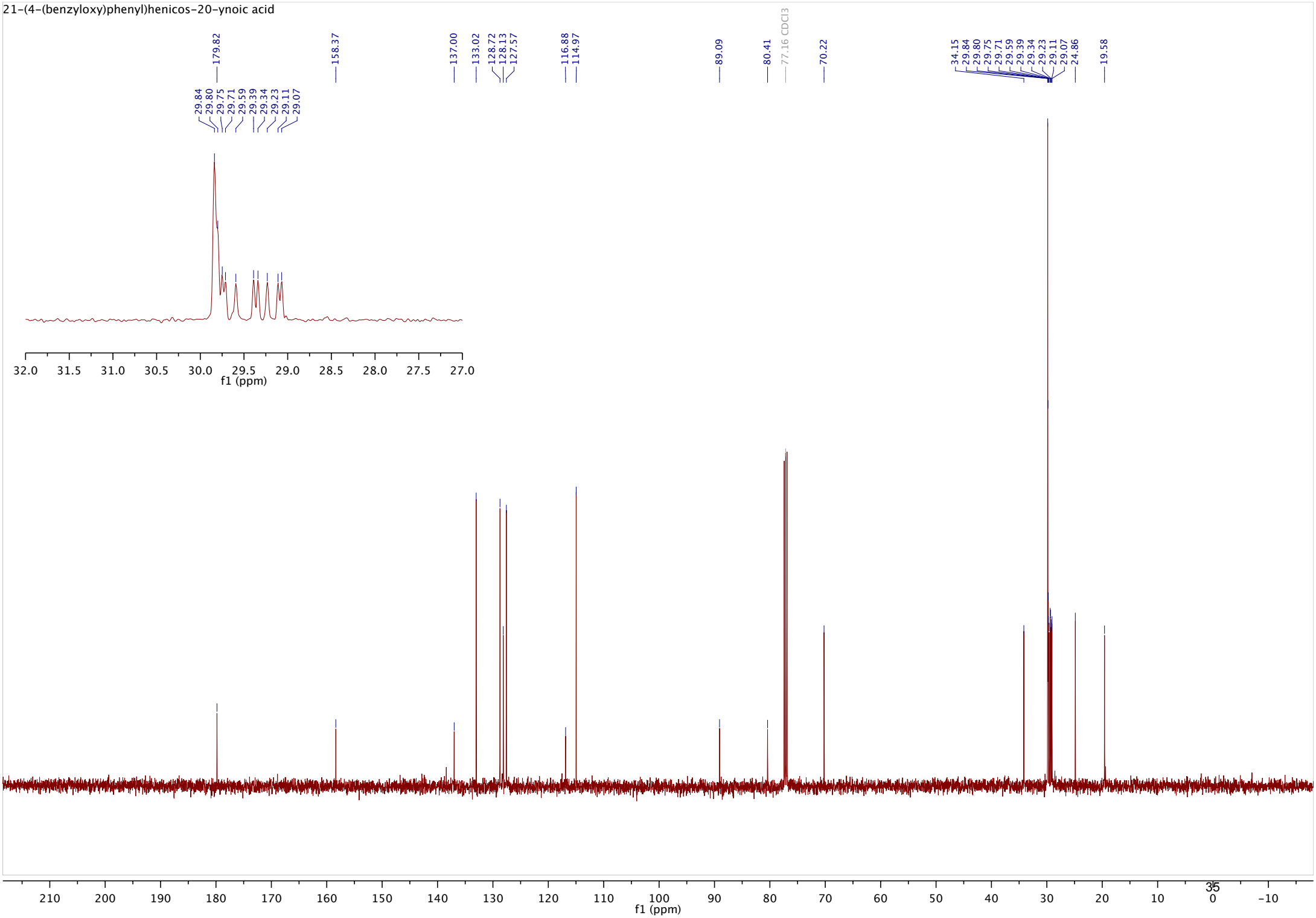
methyl 21-(4-(benzyloxy)phenyl)henicos-20-ynoate



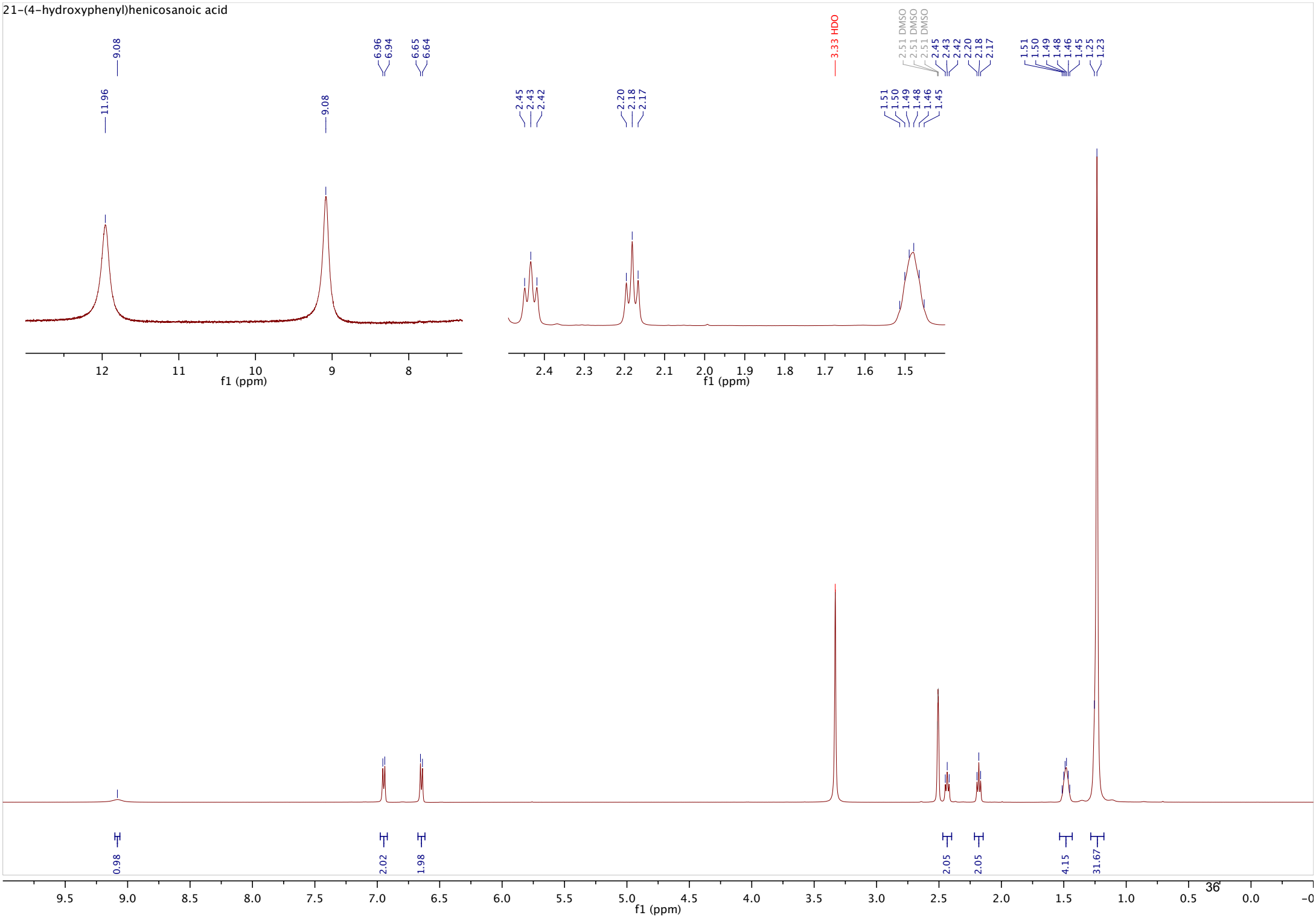
21-(4-(benzyloxy)phenyl)henicos-20-ynoic acid



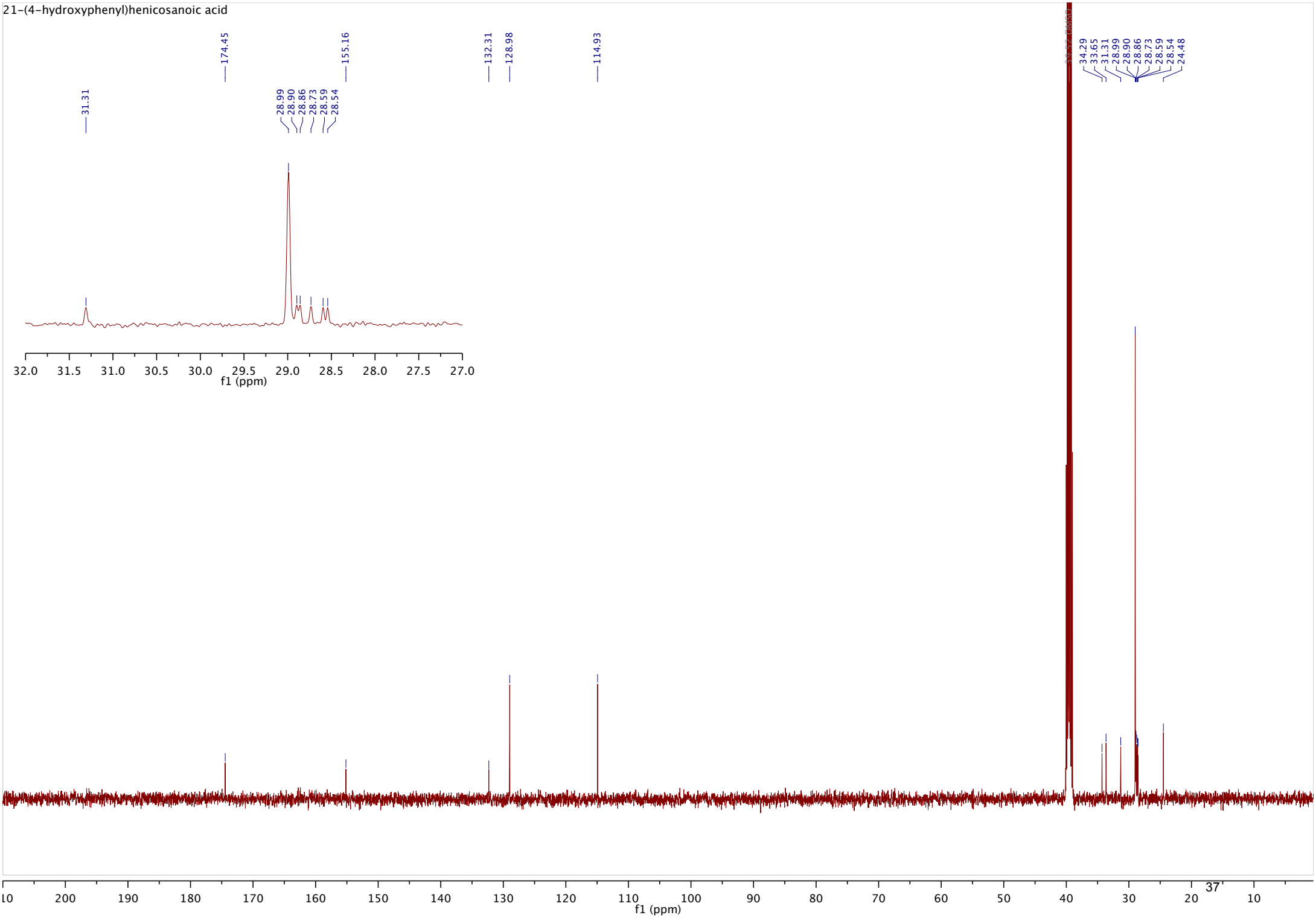
21-(4-(benzyloxy)phenyl)henicos-20-ynoic acid



21-(4-hydroxyphenyl)henicosanoic acid



21-(4-hydroxyphenyl)henicosanoic acid



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