

SUPPORTING INFORMATION

Design and assessment of dimeric trehalose glycolipids as a strategy to enhance Mincle-mediated vaccine adjuvanticity

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^c Malaghan Institute of Medical Research, Wellington 6242, New Zealand, Wellington, New Zealand

[†] These authors contributed equally

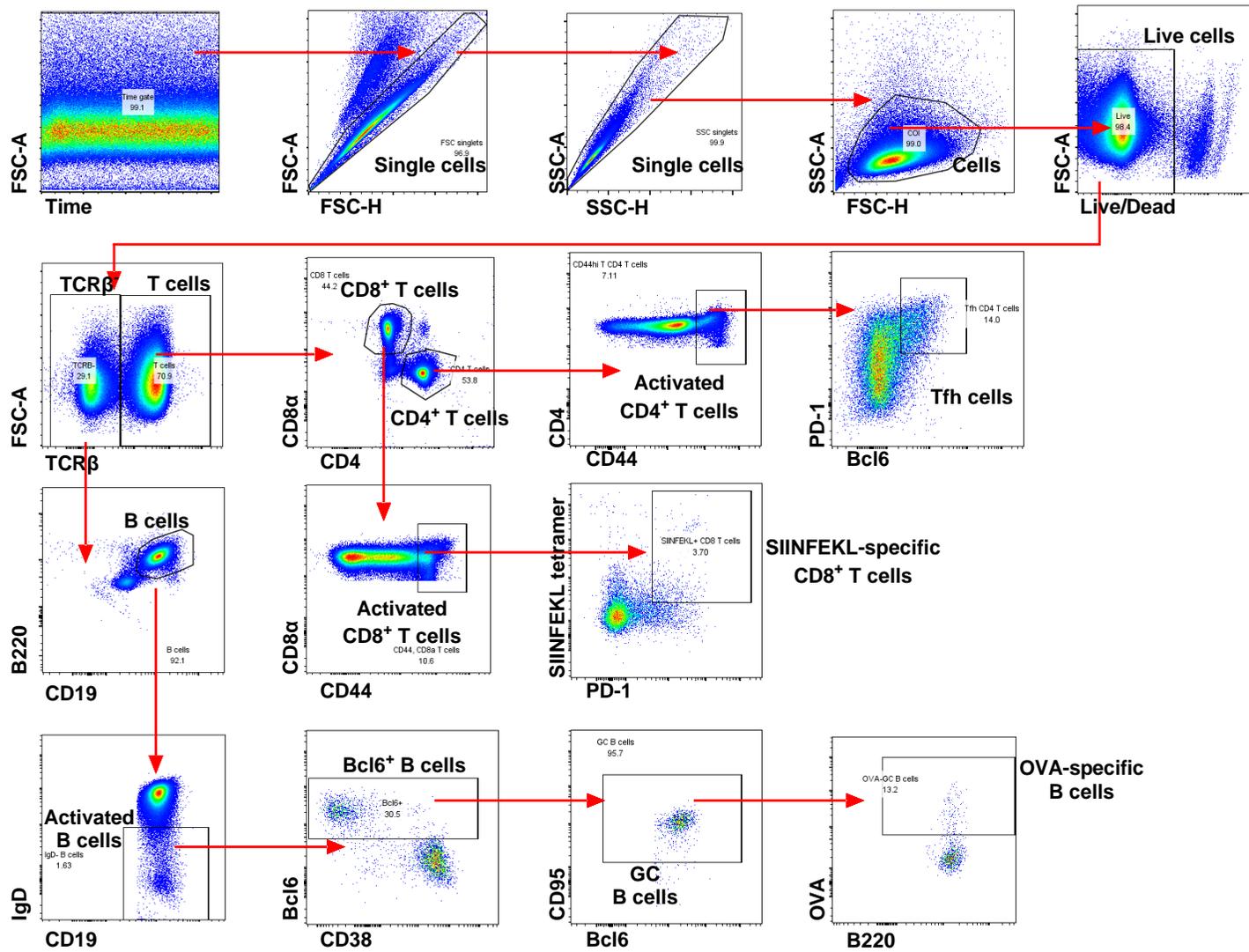
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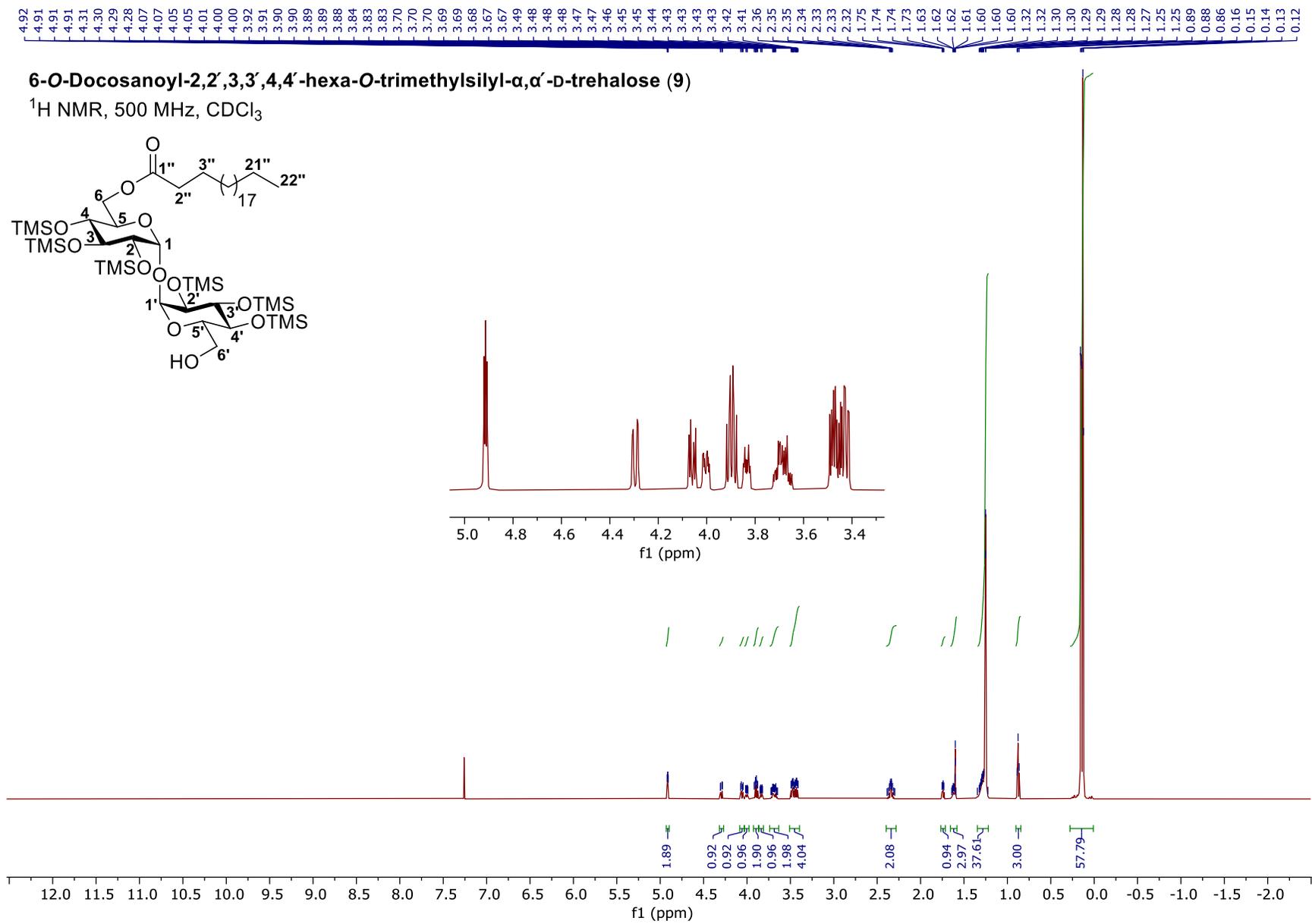
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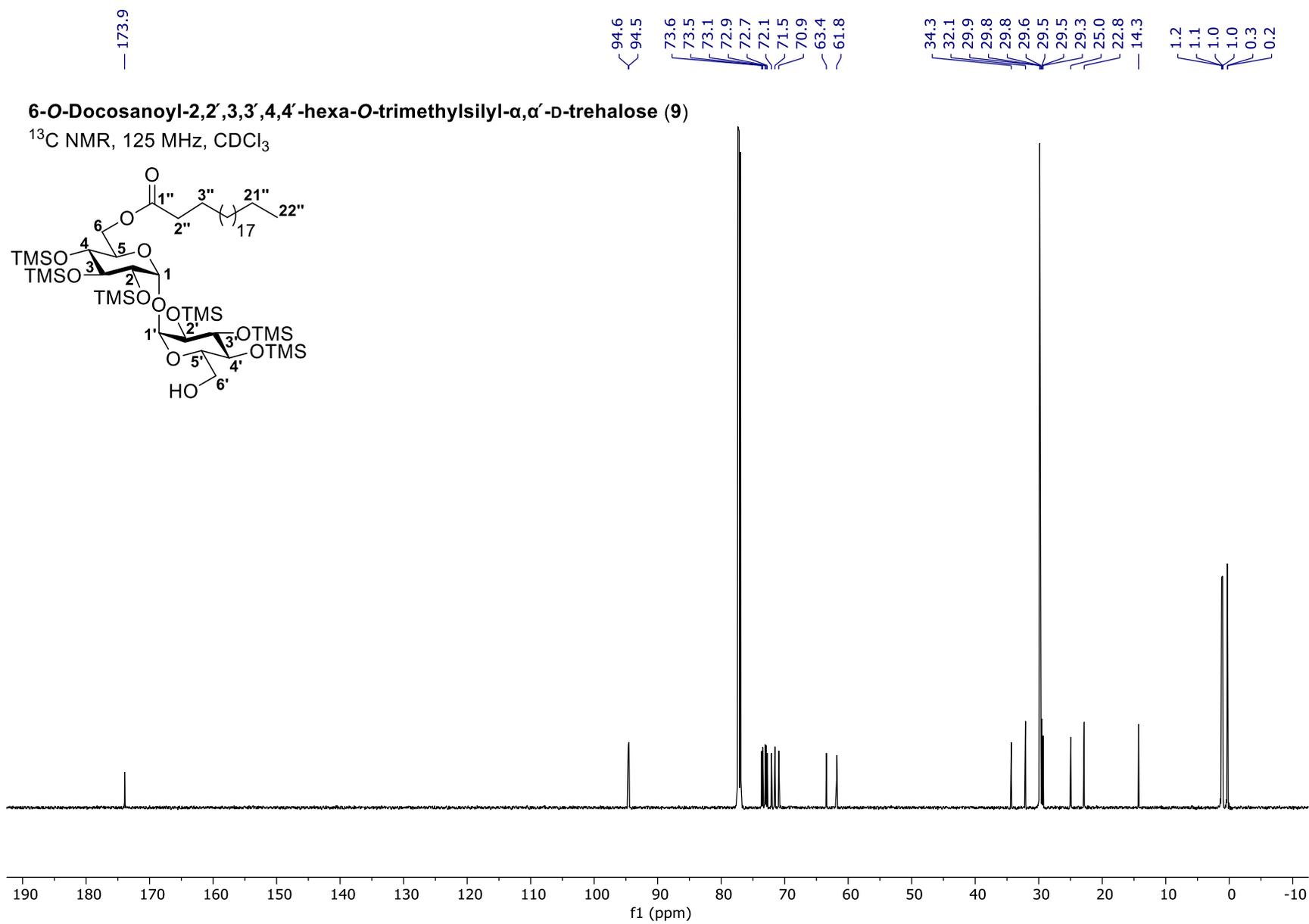
Flow cytometry fluorophores

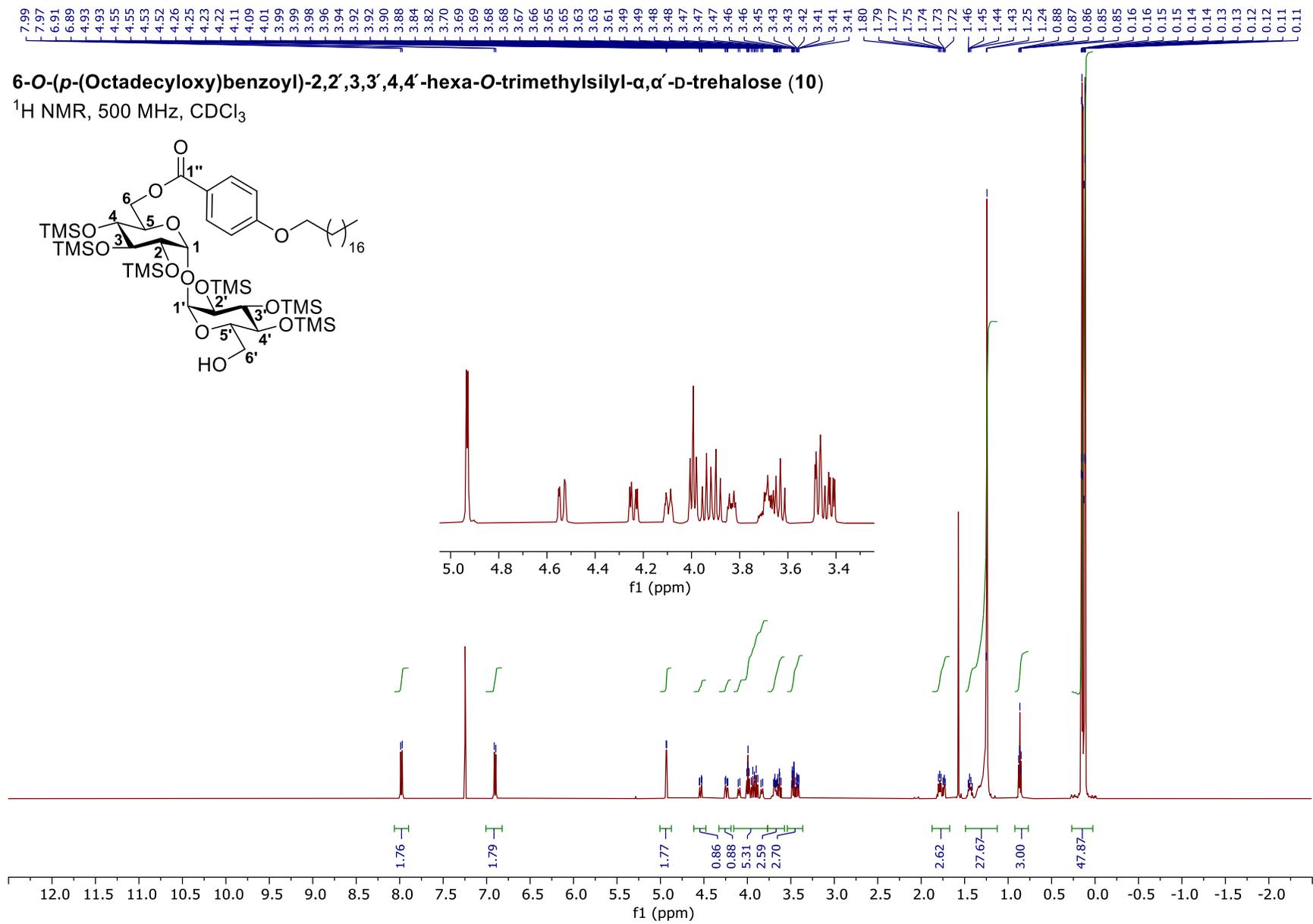
Specificity	Fluorophore/Tag	Dilution	Clone	Supplier
IgD	BUV395	1:200	11-26c.2a	BD Biosciences
CD44	BUV737	1:400	IM7	BD Biosciences
CD95	BUV805	1:200	Jo2	BD Biosciences
CD38	BV421	1:400	90	BioLegend
GL7	eFluor450	1:500	GL7	ThermoFisher
CD4	BV510	1:200	GK1.5	BioLegend
B220	BV570	1:400	RA3-6B2	BioLegend
CD8 α	BV605	1:200	53-6.7	BD BioSciences
TCR β	BV786	1:200	H57-597	BD BioSciences
CD19	AF488	1:600	6D5	BD BioSciences
OVA	Biotin	1:3000	-	Made in-house
SAv	PE CF594	1:1200	-	BD BioSciences
PD-1	PE Cy7	1:400	29F.1A12	BioLegend
Bcl6	AF647	1:50	K112-91	BD Biosciences
MHC-I:SIINFEKL tetramer	PE	1:800	-	NIH Tetramer Core Facility
Zombie NIR Live Dead	NIR	1:2000	-	BioLegend

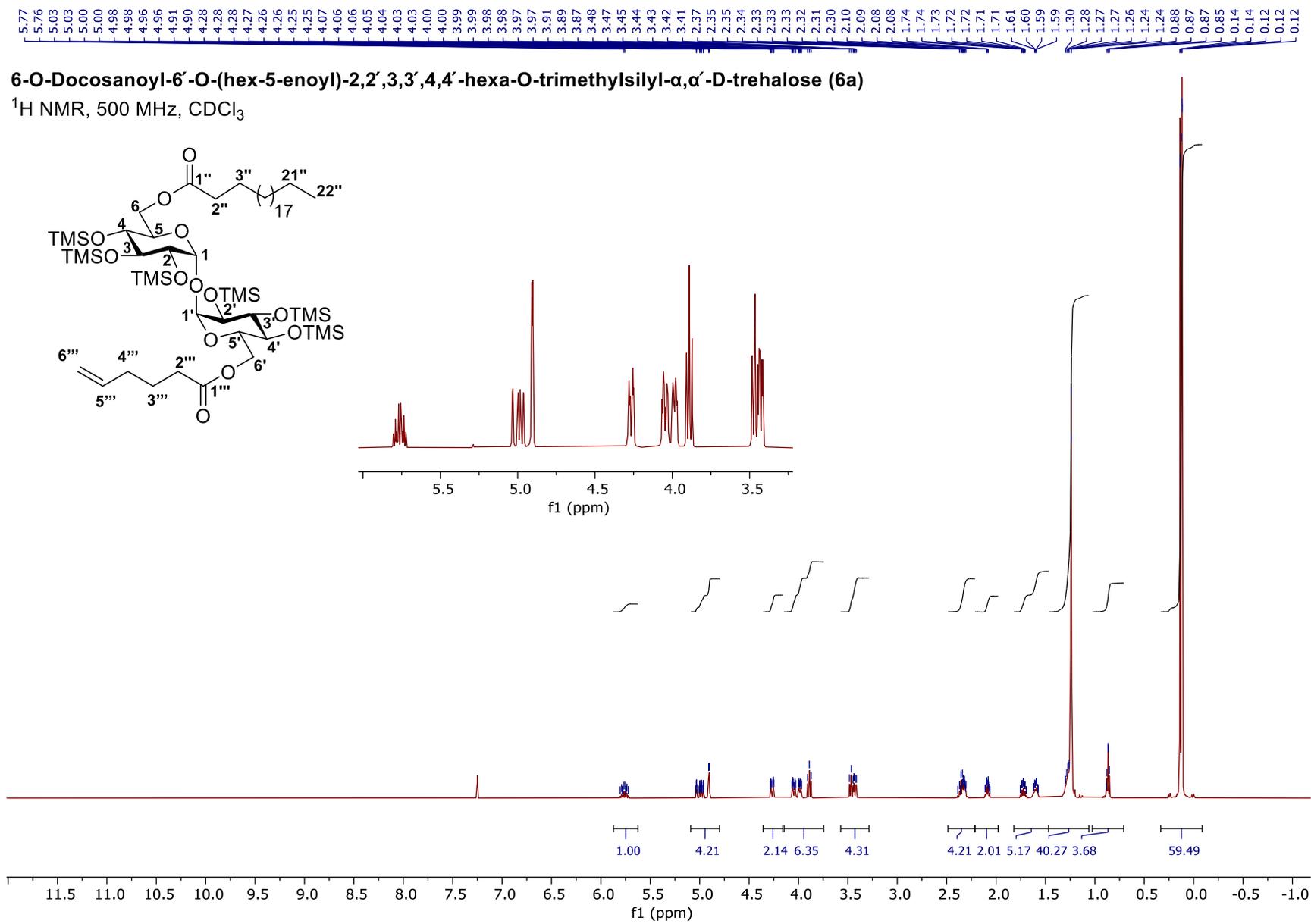
Flow Cytometry gating strategy

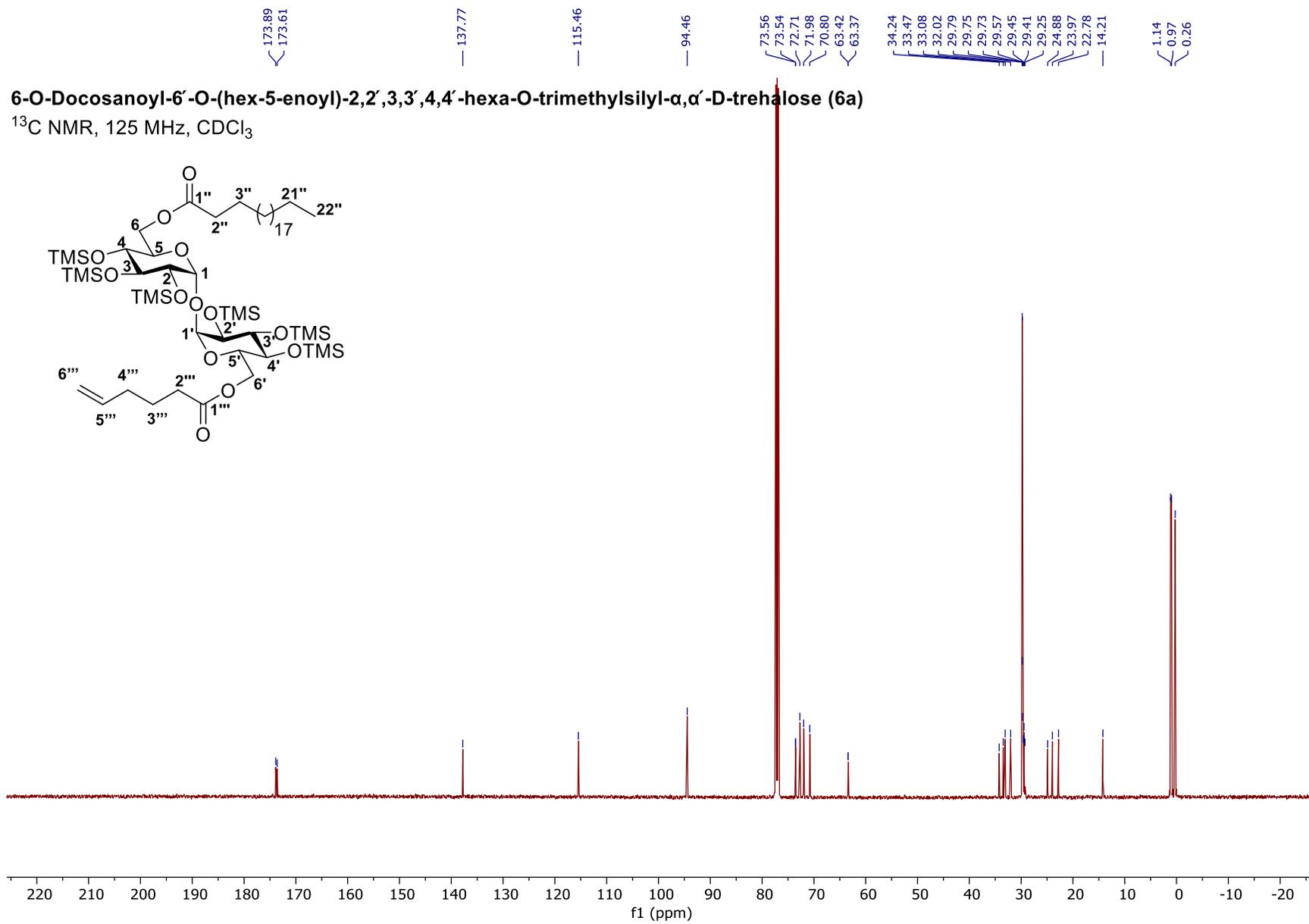








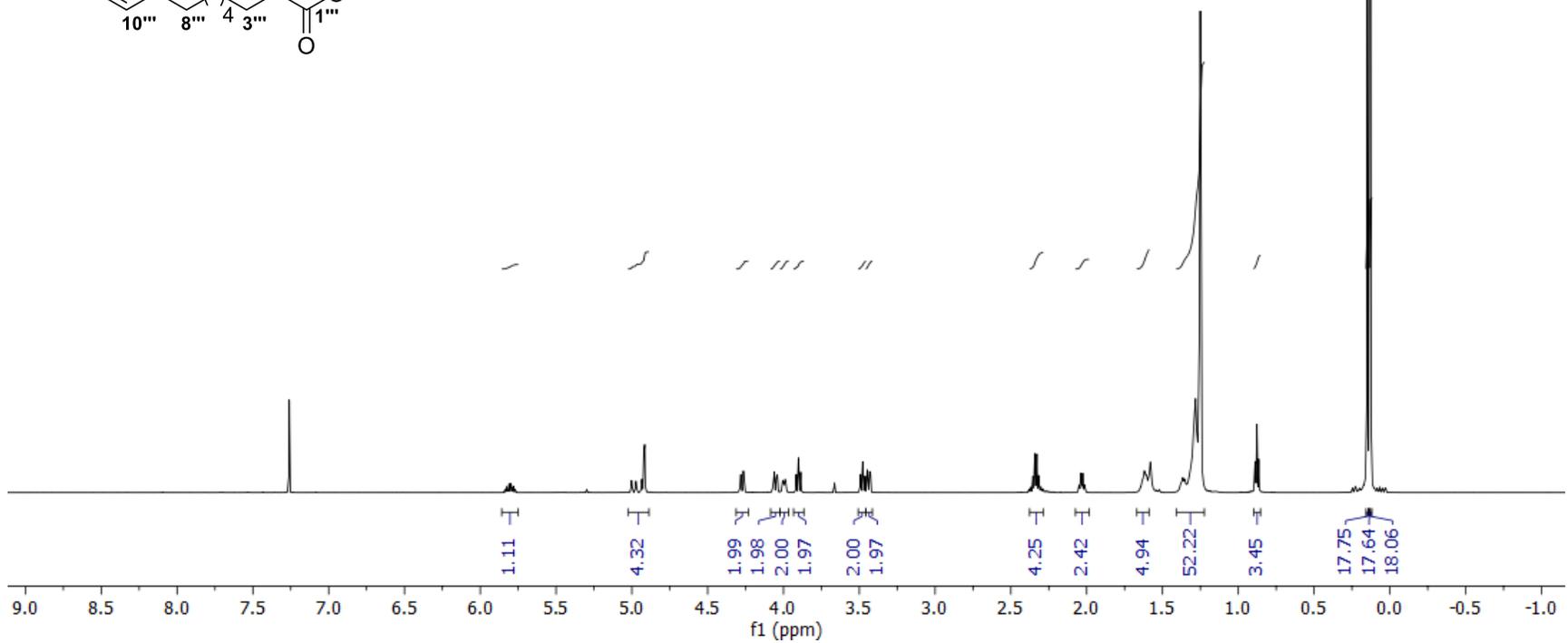
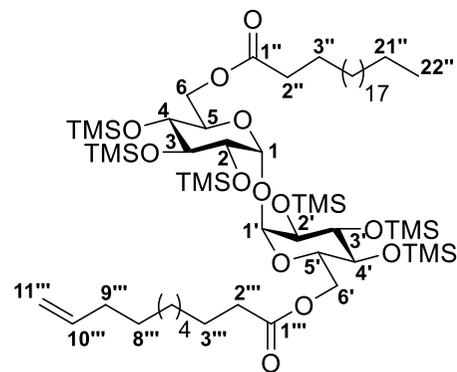


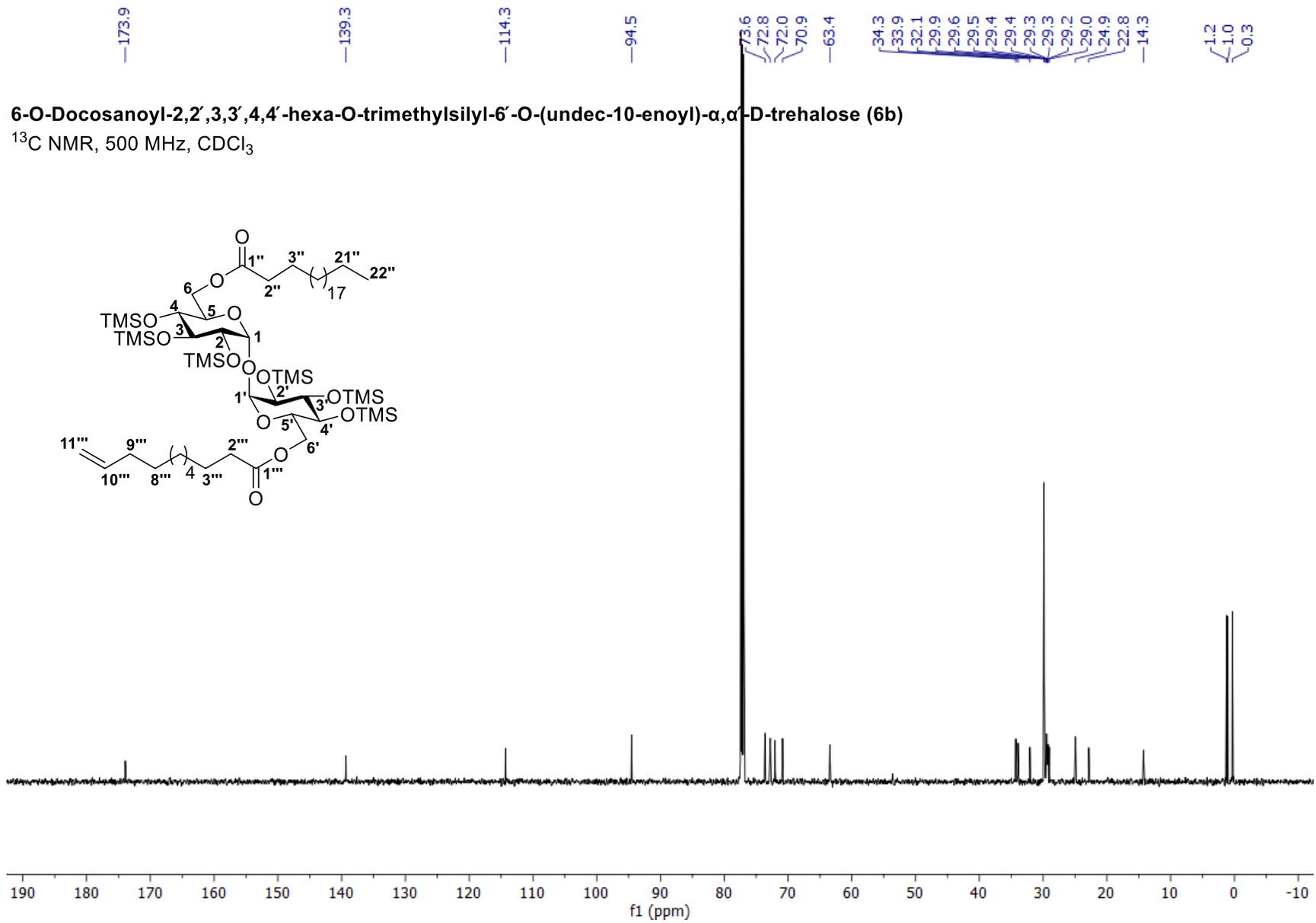


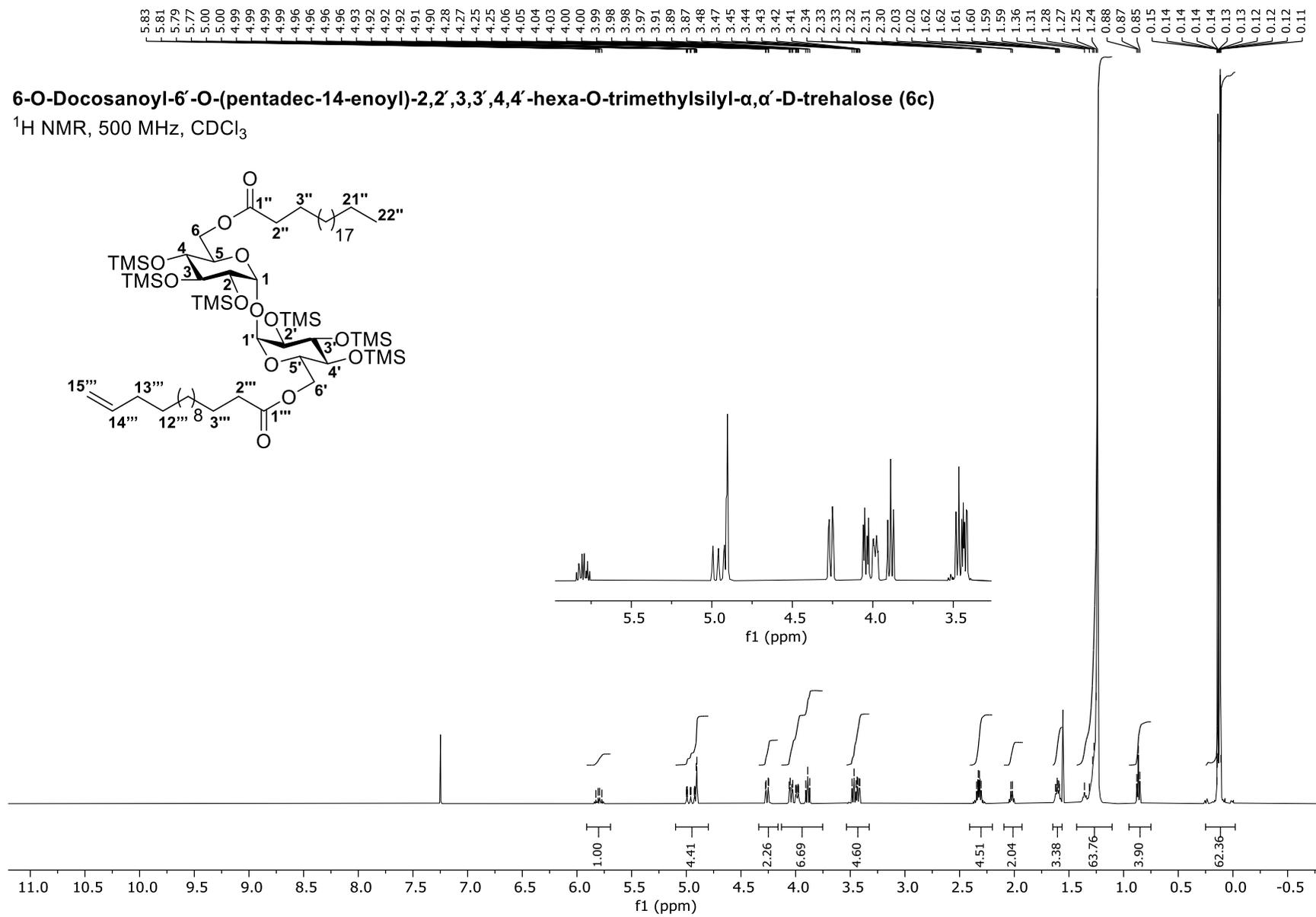
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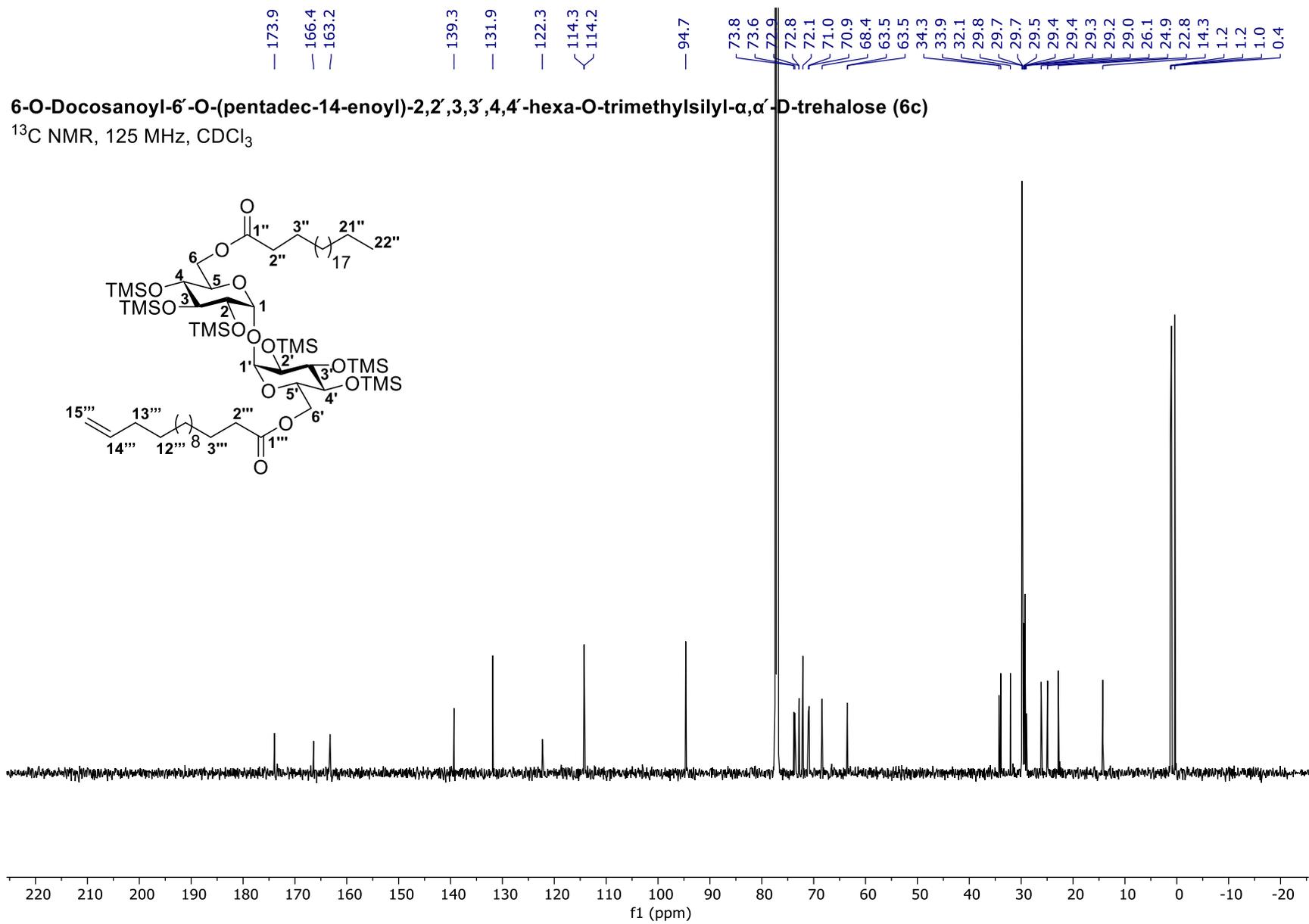
6-O-Docosanoyl-2,2',3,3',4,4'-hexa-O-trimethylsilyl-6'-O-(undec-10-enoyl)- α,α' -D-trehalose (6b)

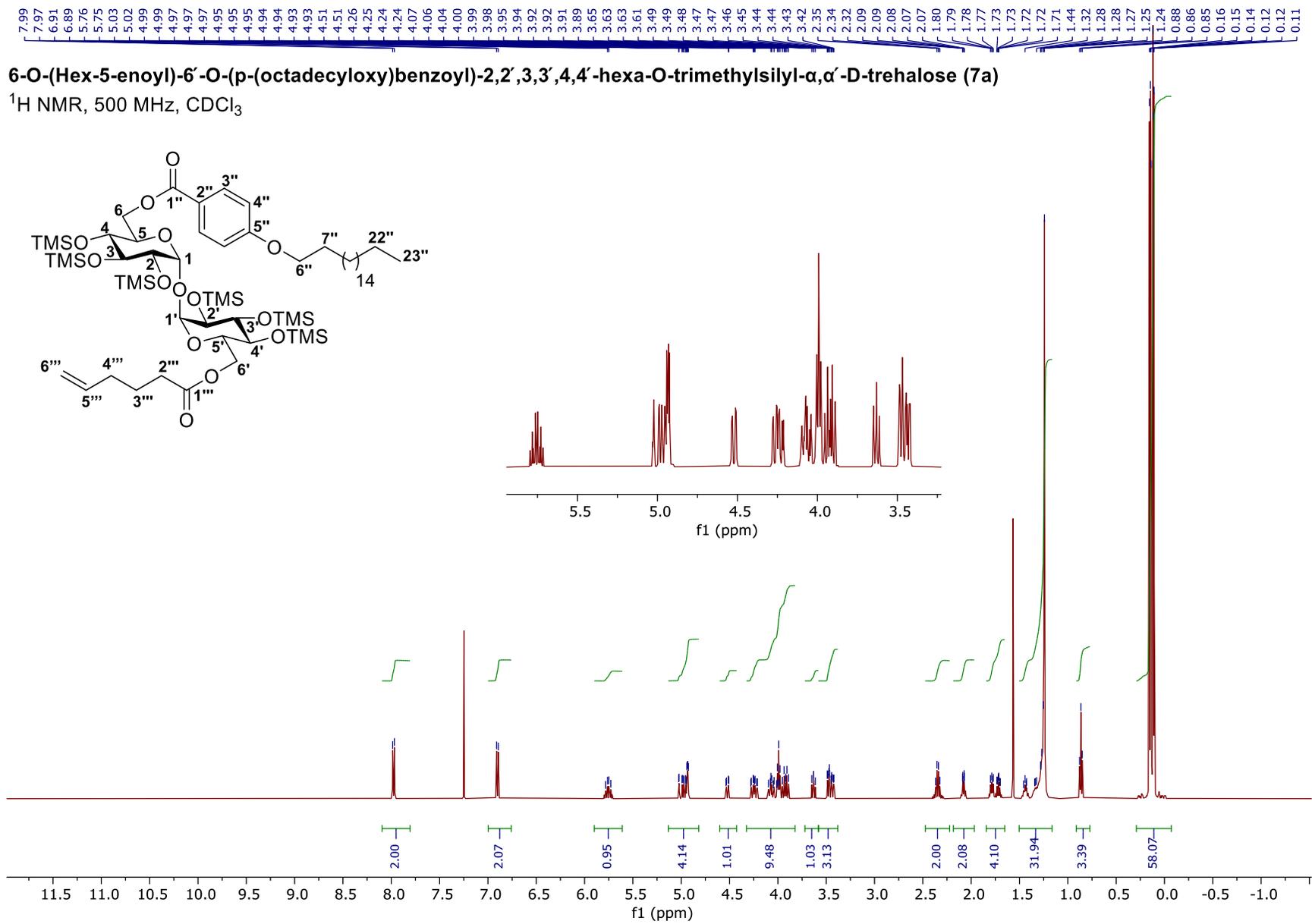
^1H NMR, 500 MHz, CDCl_3

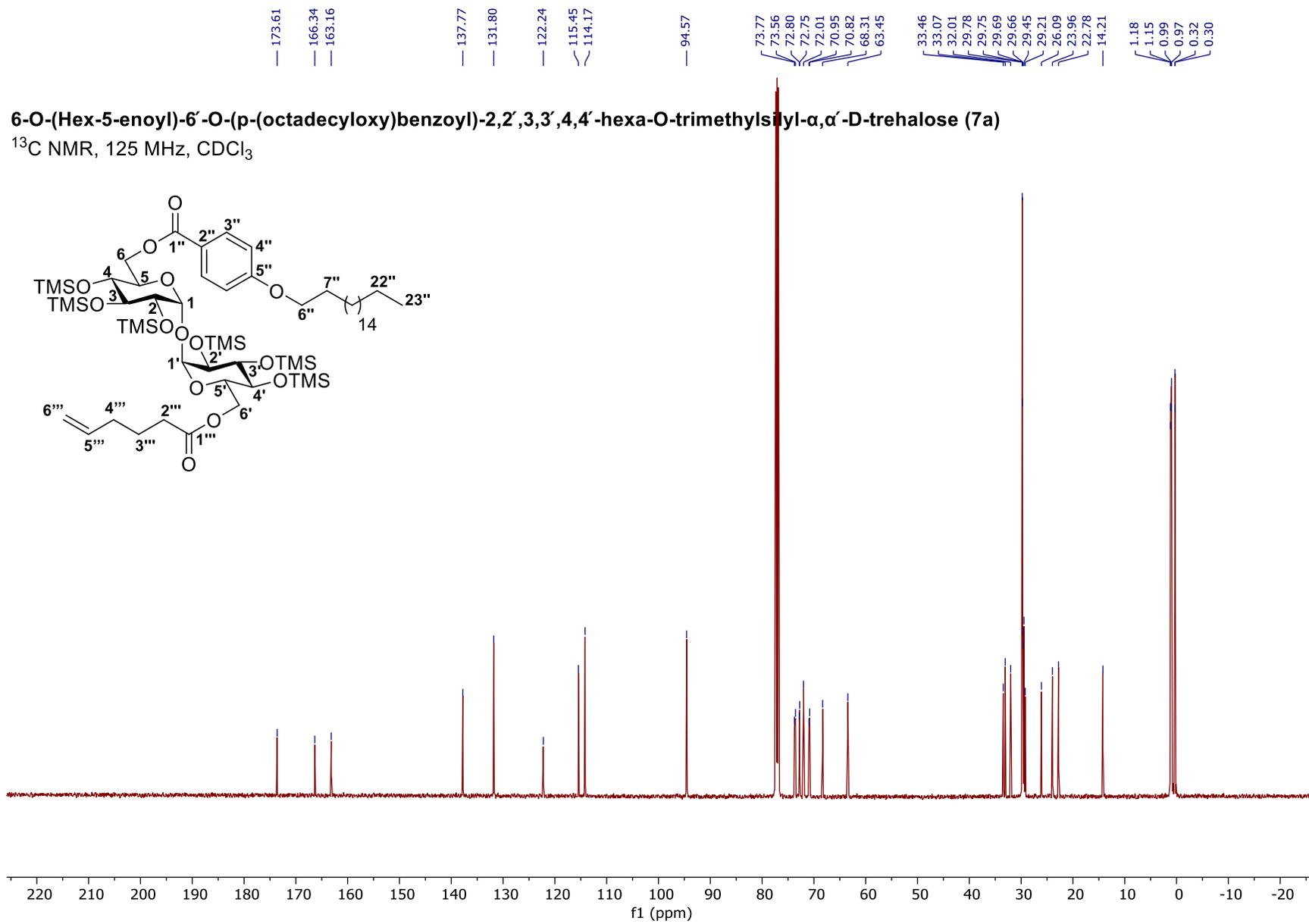








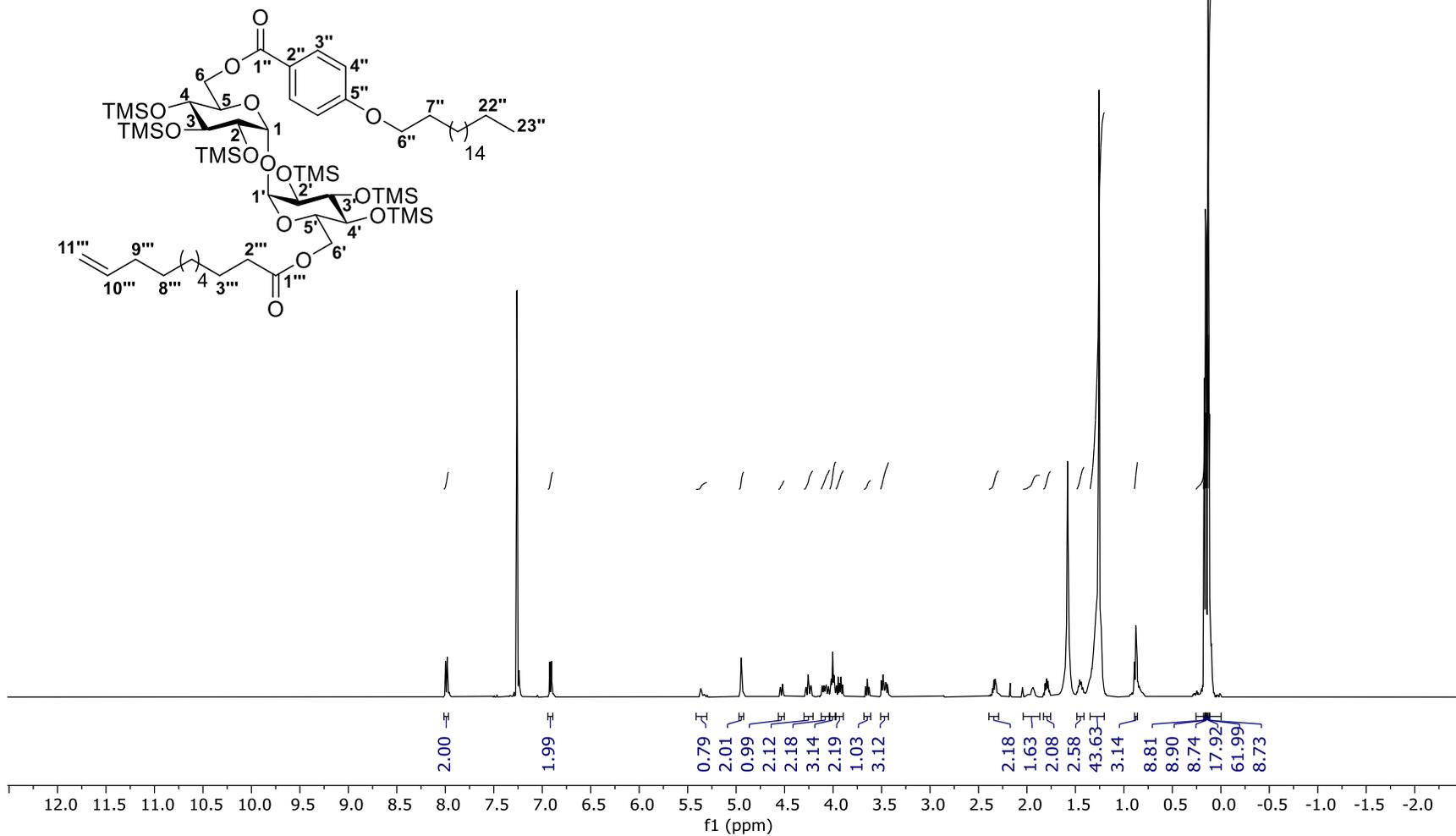


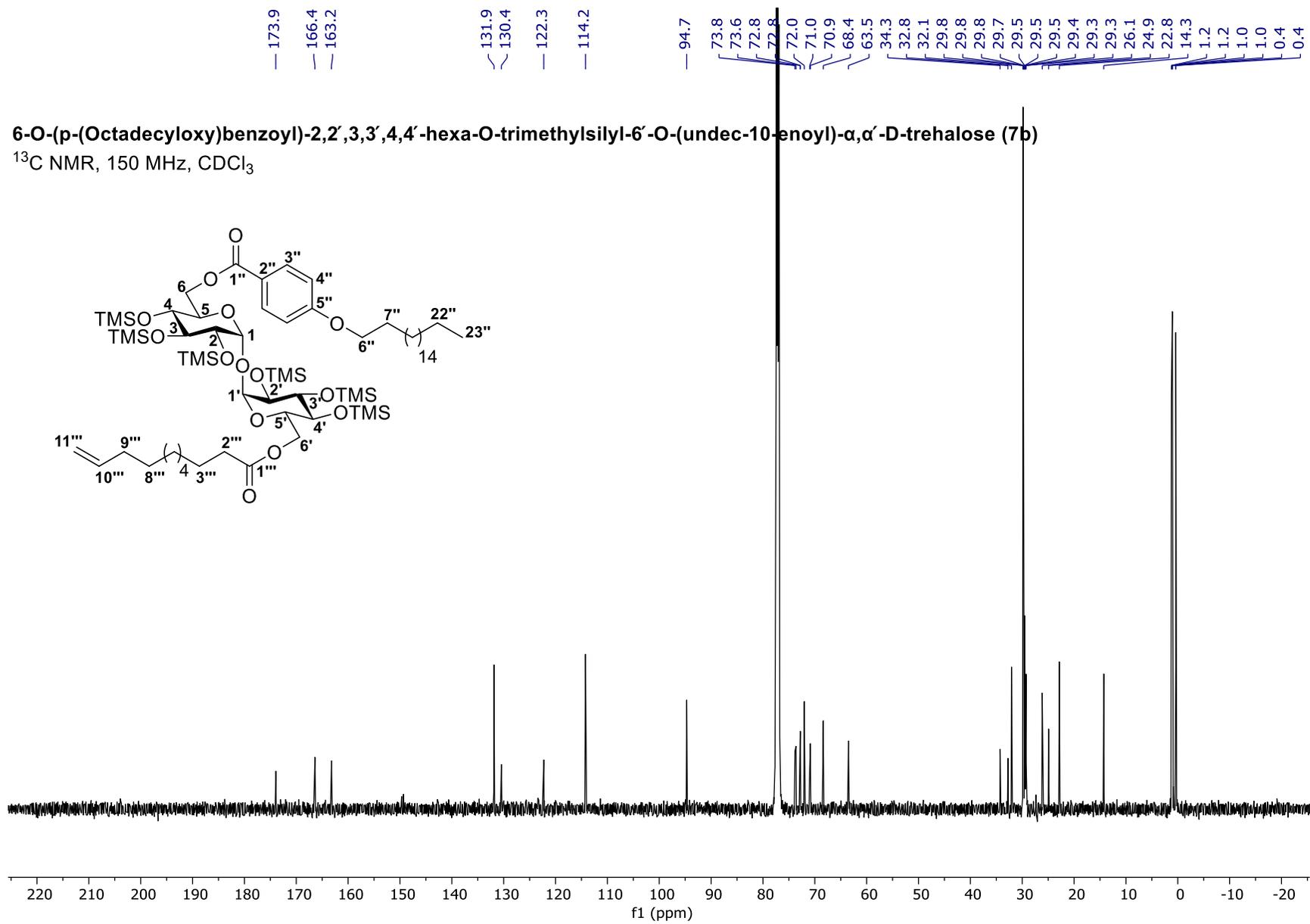


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6-O-(p-(Octadecyloxy)benzoyl)-2,2',3,3',4,4'-hexa-O-trimethylsilyl-6'-O-(undec-10-enoyl)- α,α' -D-trehalose (7b)

^1H NMR, 600 MHz, CDCl_3

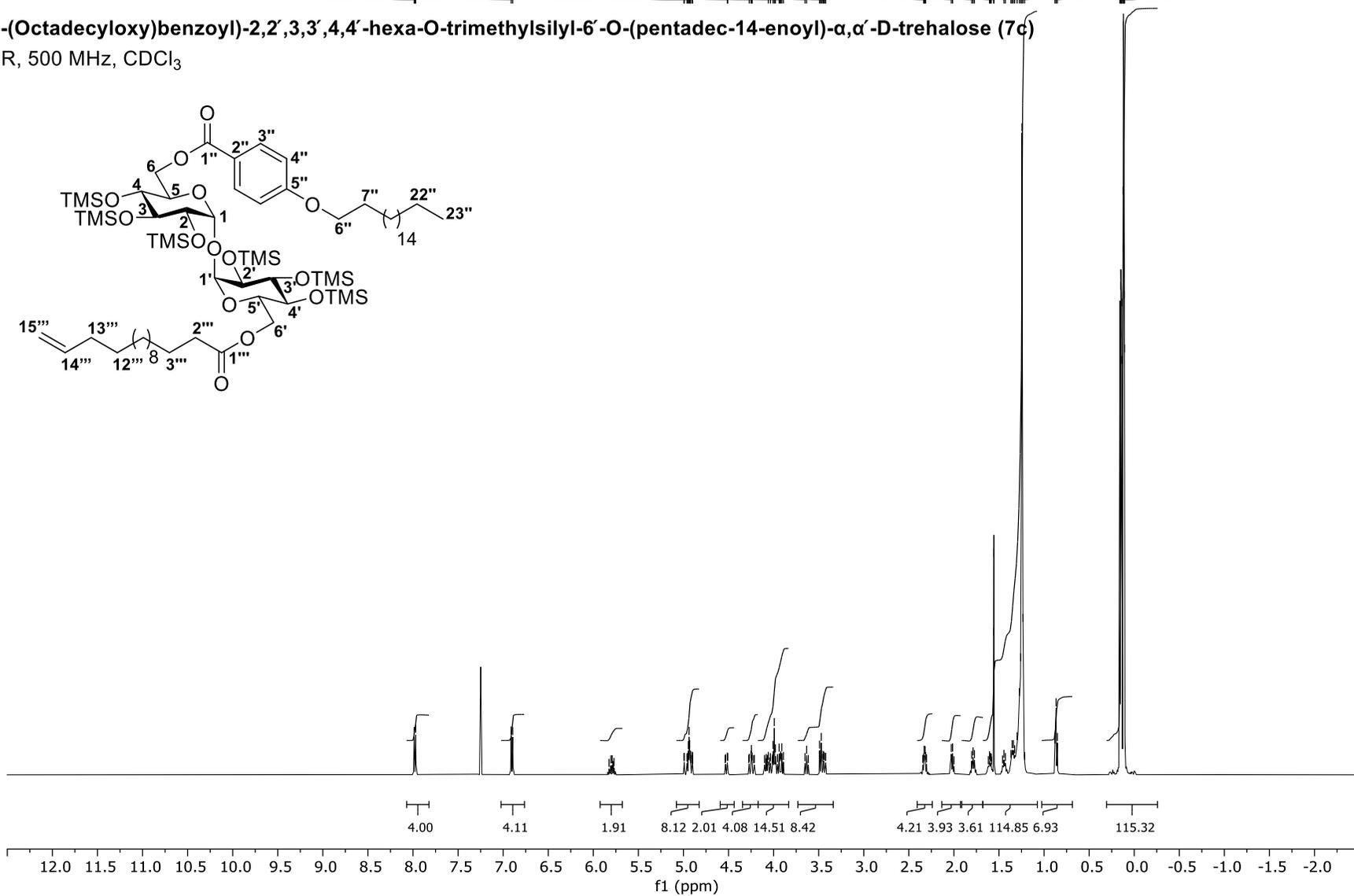
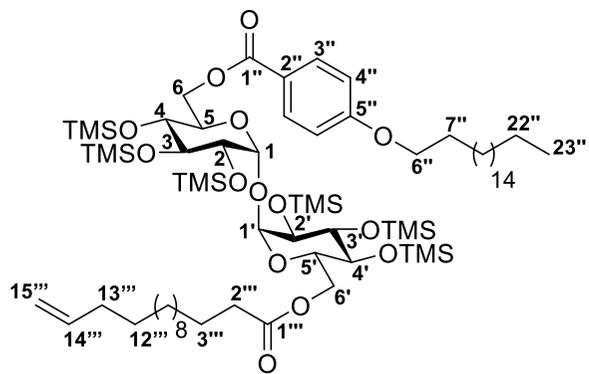


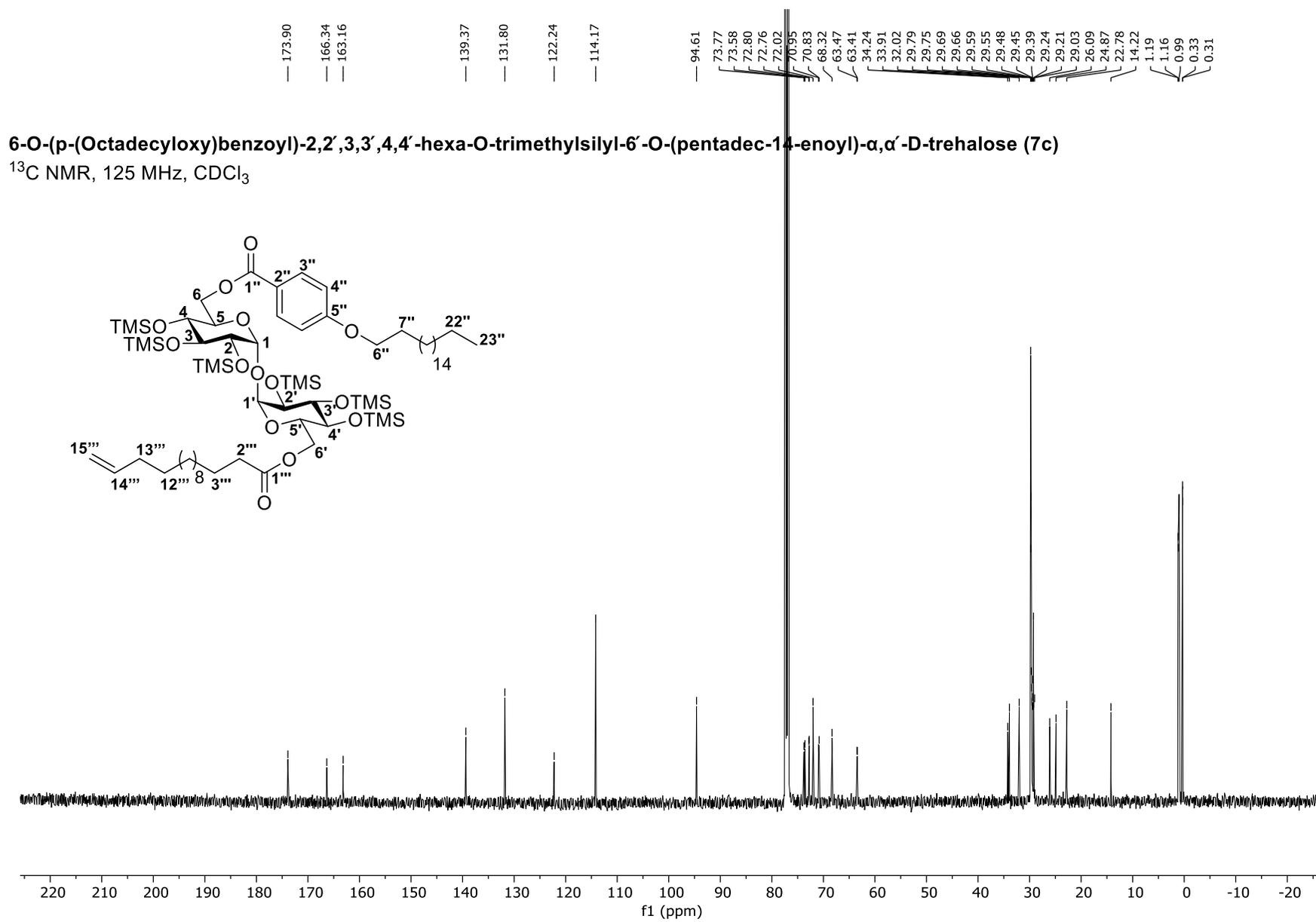


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6-O-(p-(Octadecyloxy)benzoyl)-2,2',3,3',4,4'-hexa-O-trimethylsilyl-6'-O-(pentadec-14-enoyl)- α,α' -D-trehalose (7c)

^1H NMR, 500 MHz, CDCl_3

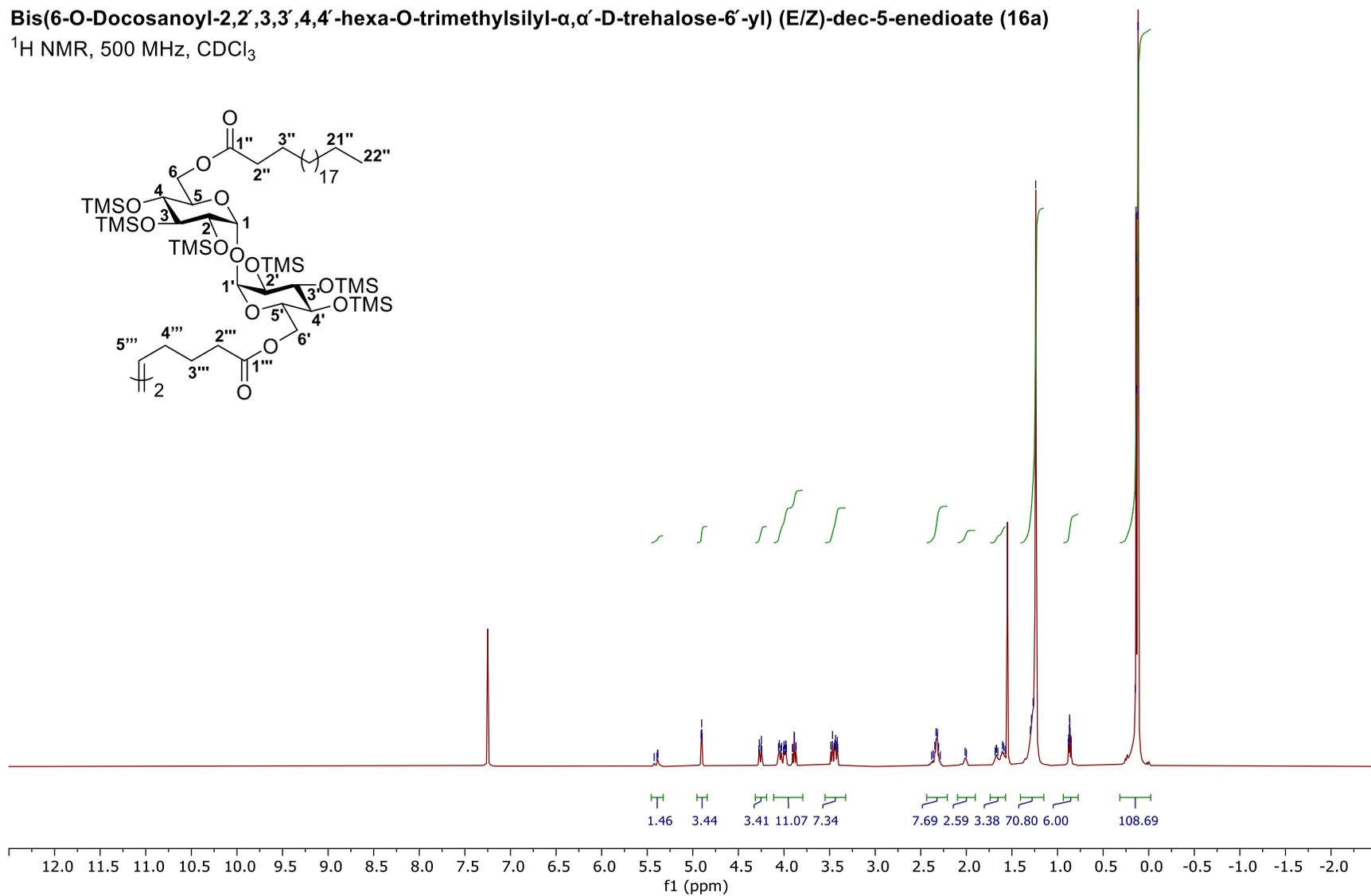
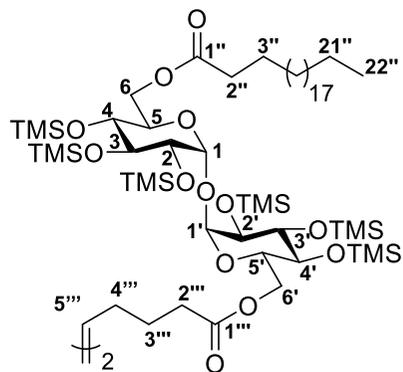


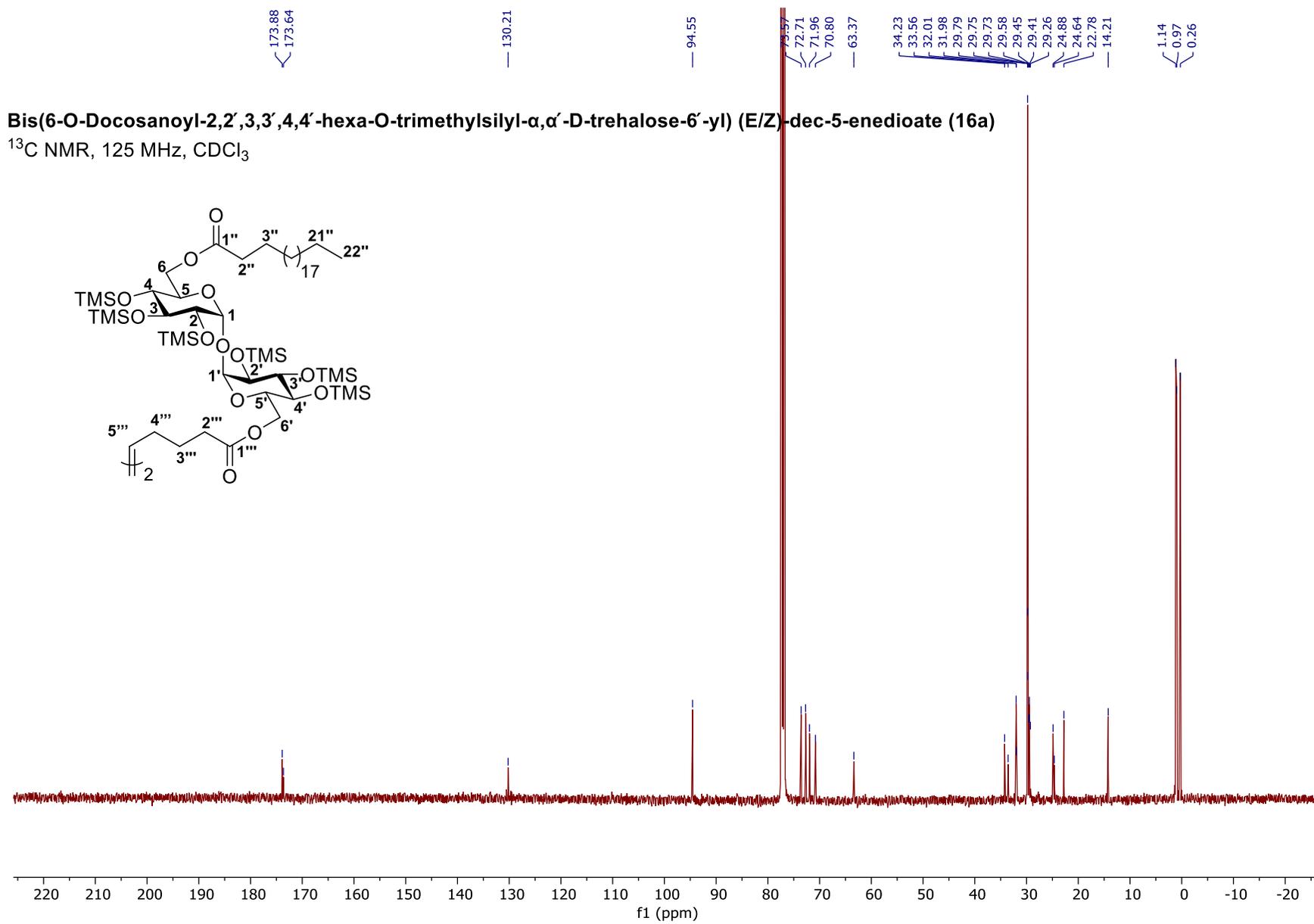


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Bis(6-O-Docosanoyl-2,2',3,3',4,4'-hexa-O-trimethylsilyl- α,α' -D-trehalose-6'-yl) (E/Z)-dec-5-enedioate (16a)

^1H NMR, 500 MHz, CDCl_3

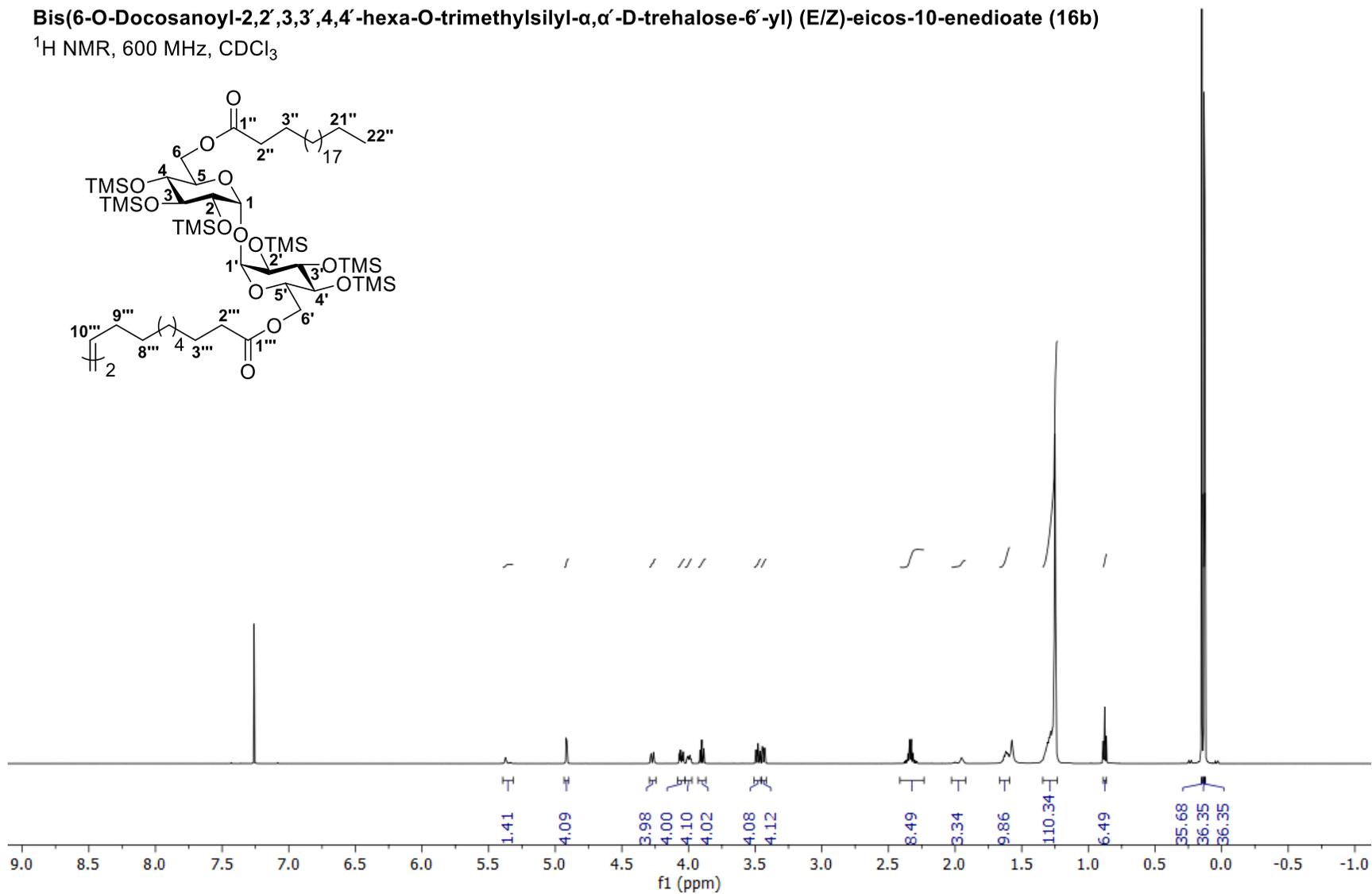
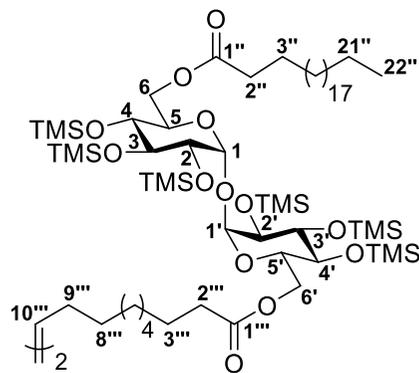


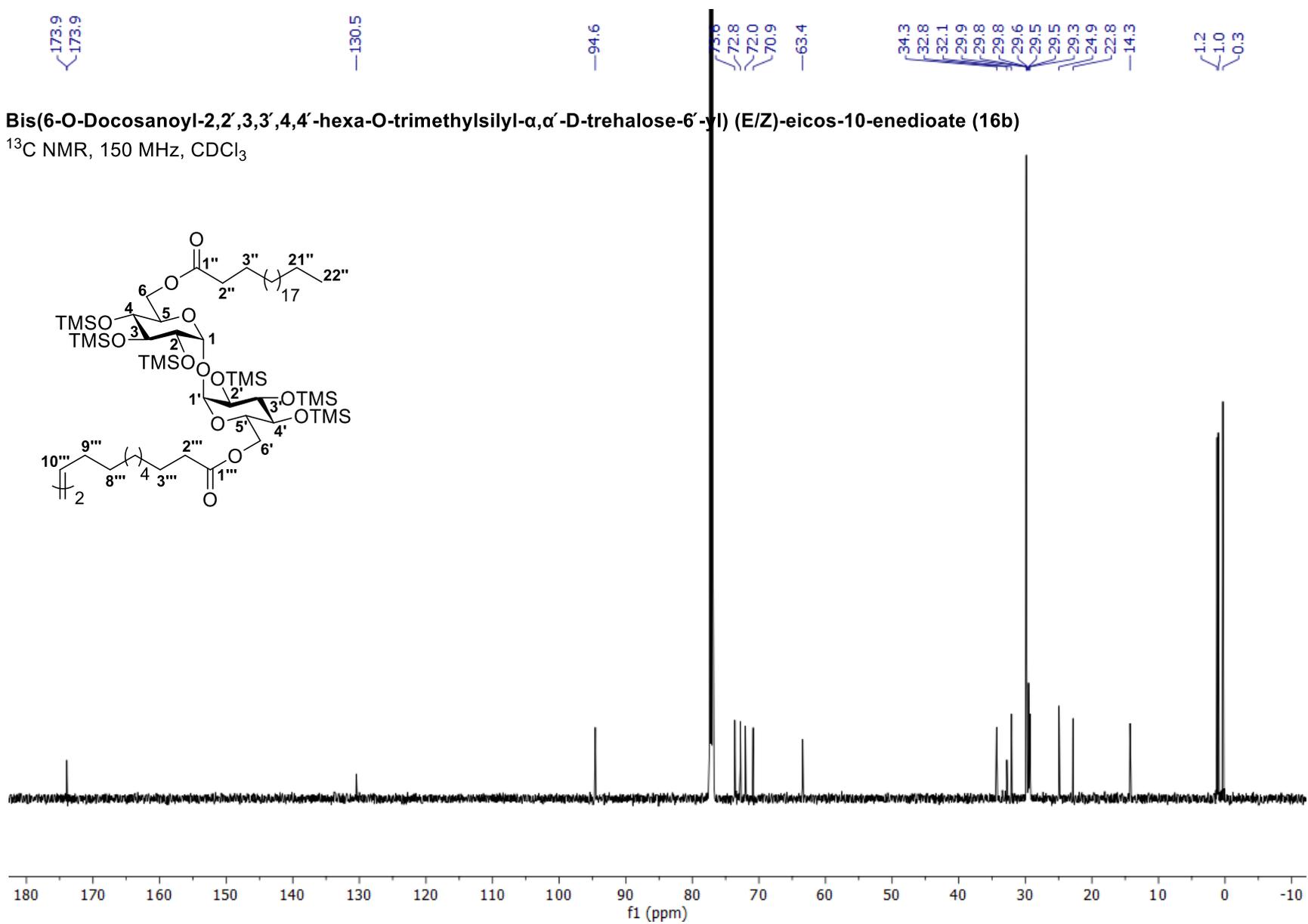


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Bis(6-O-Docosanoyl-2,2',3,3',4,4'-hexa-O-trimethylsilyl- α,α' -D-trehalose-6'-yl) (E/Z)-eicos-10-enedioate (16b)

^1H NMR, 600 MHz, CDCl_3

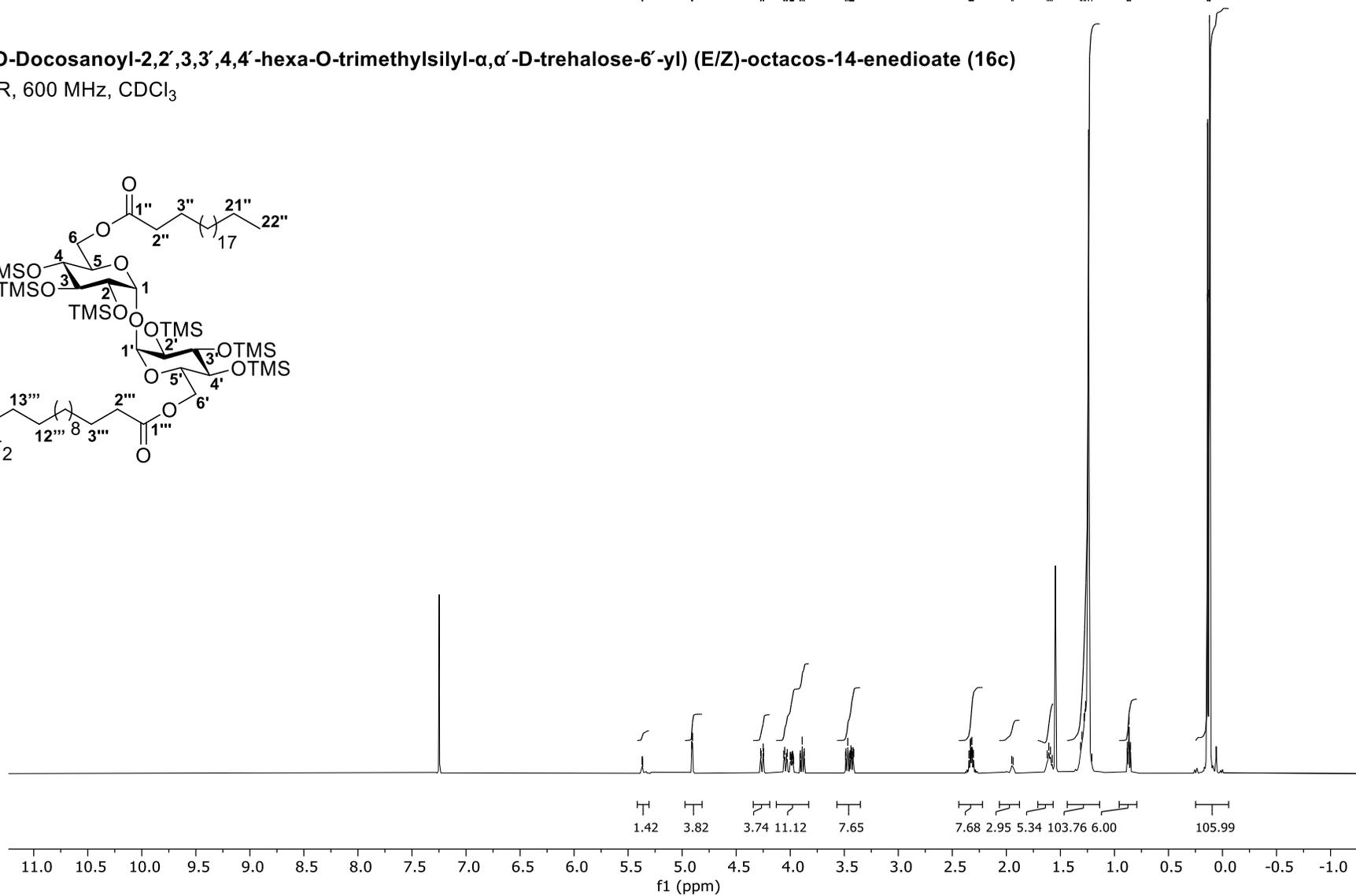
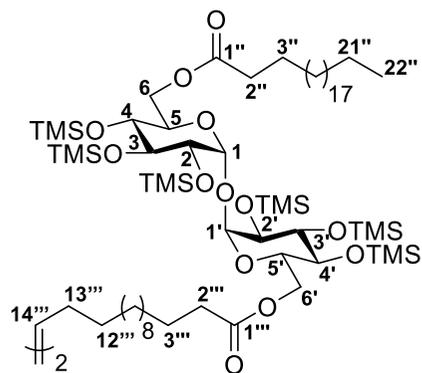


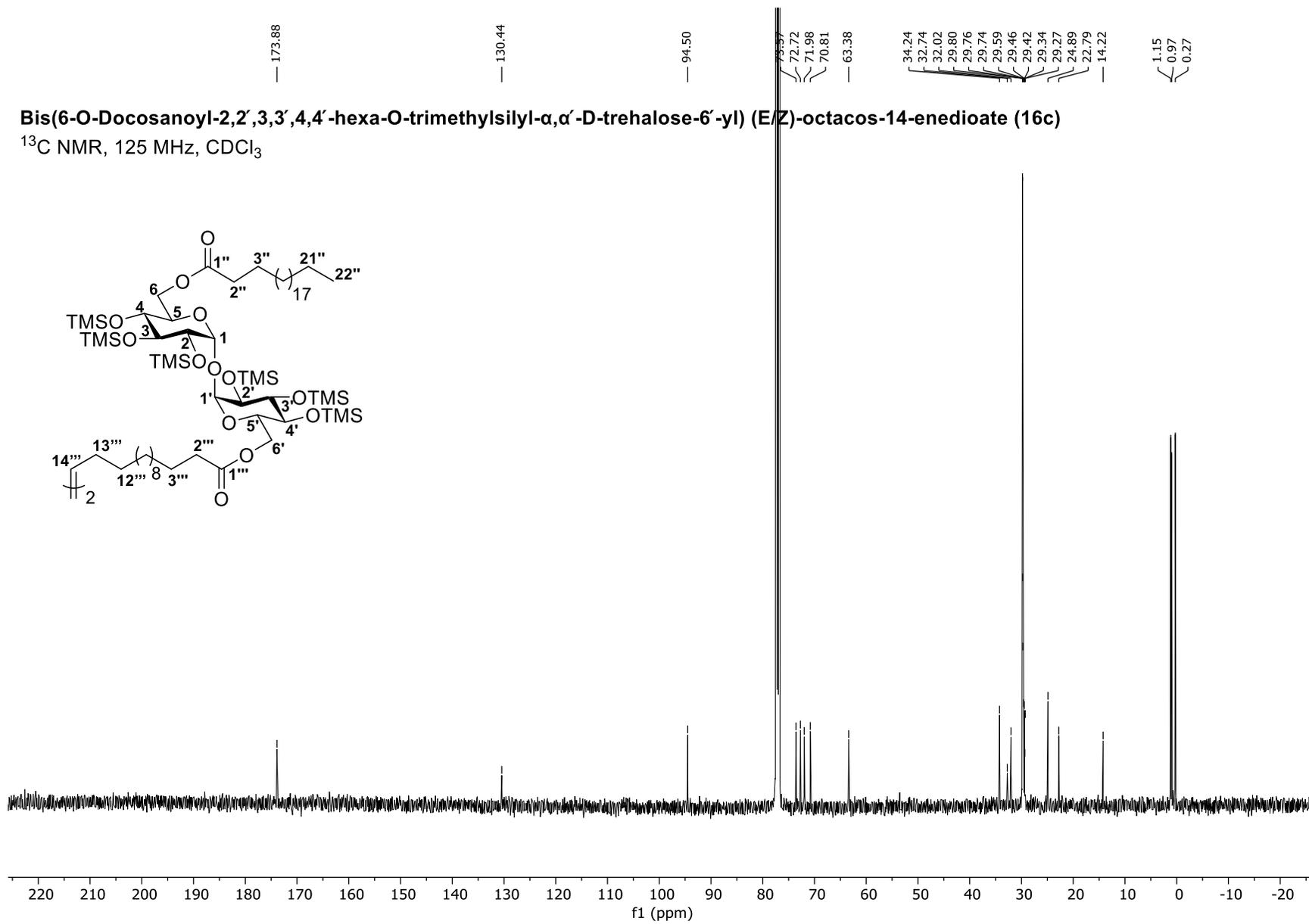


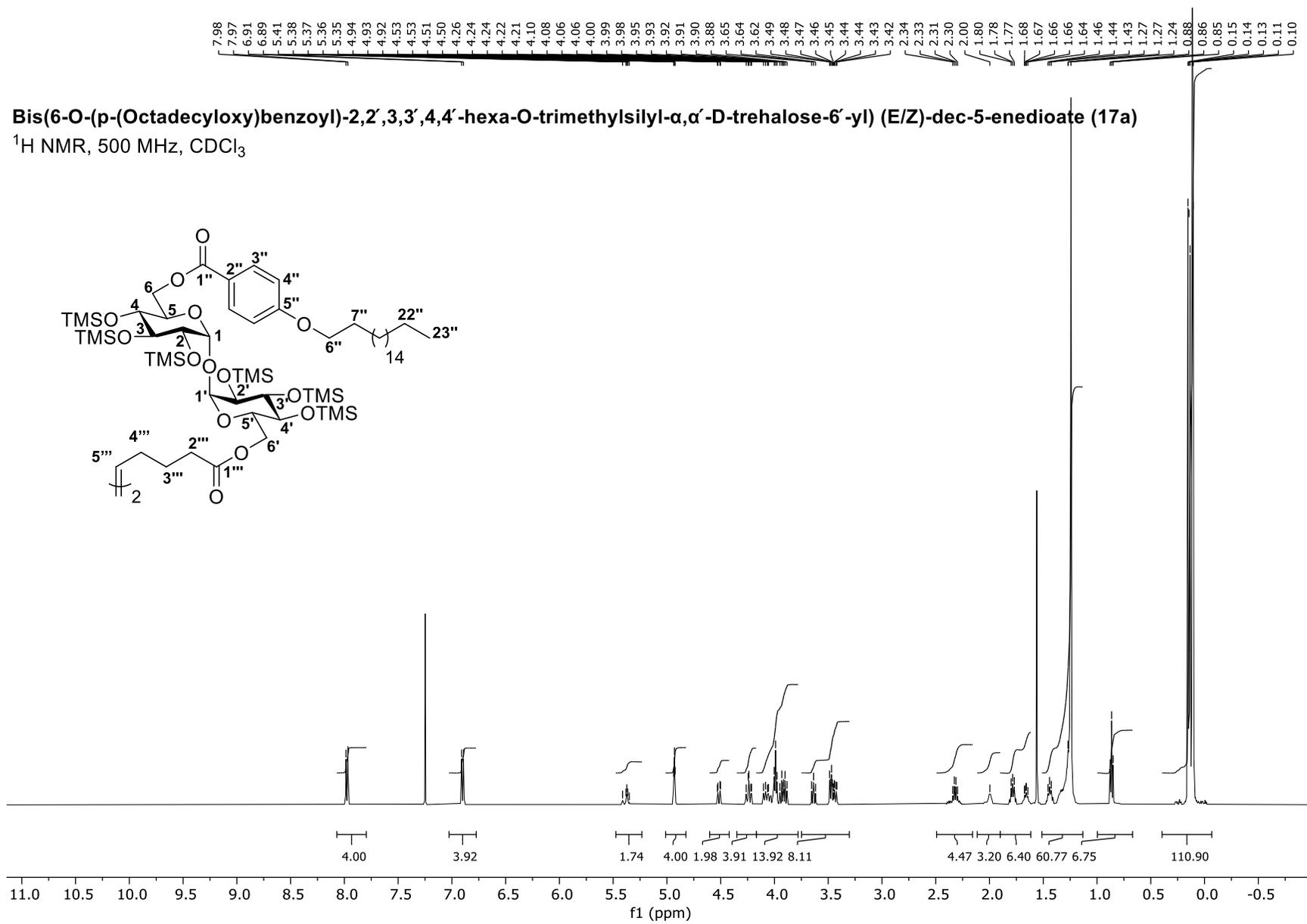
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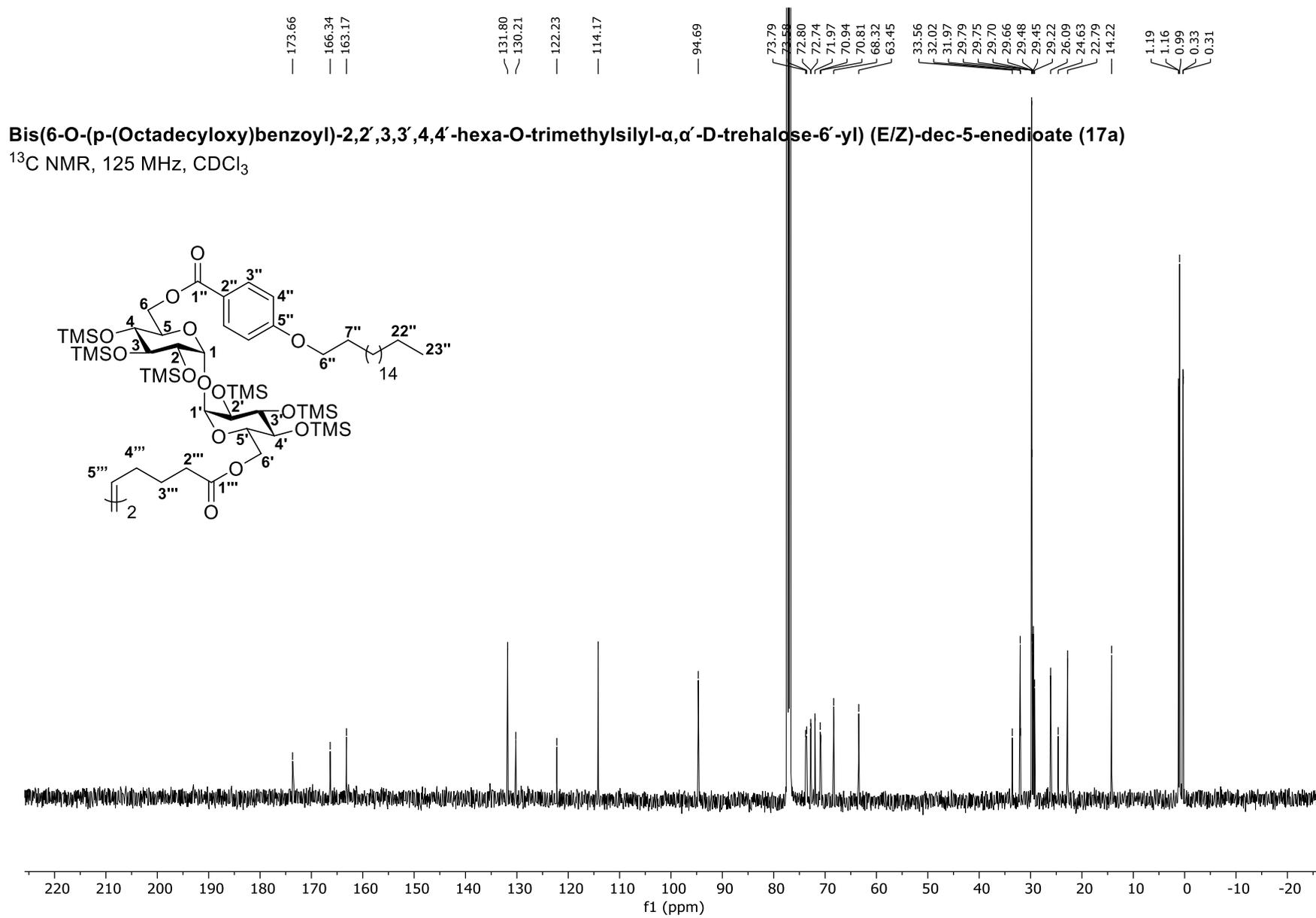
Bis(6-O-Docosanoyl-2,2',3,3',4,4'-hexa-O-trimethylsilyl- α,α' -D-trehalose-6'-yl) (E/Z)-octacos-14-enedioate (16c)

^1H NMR, 600 MHz, CDCl_3





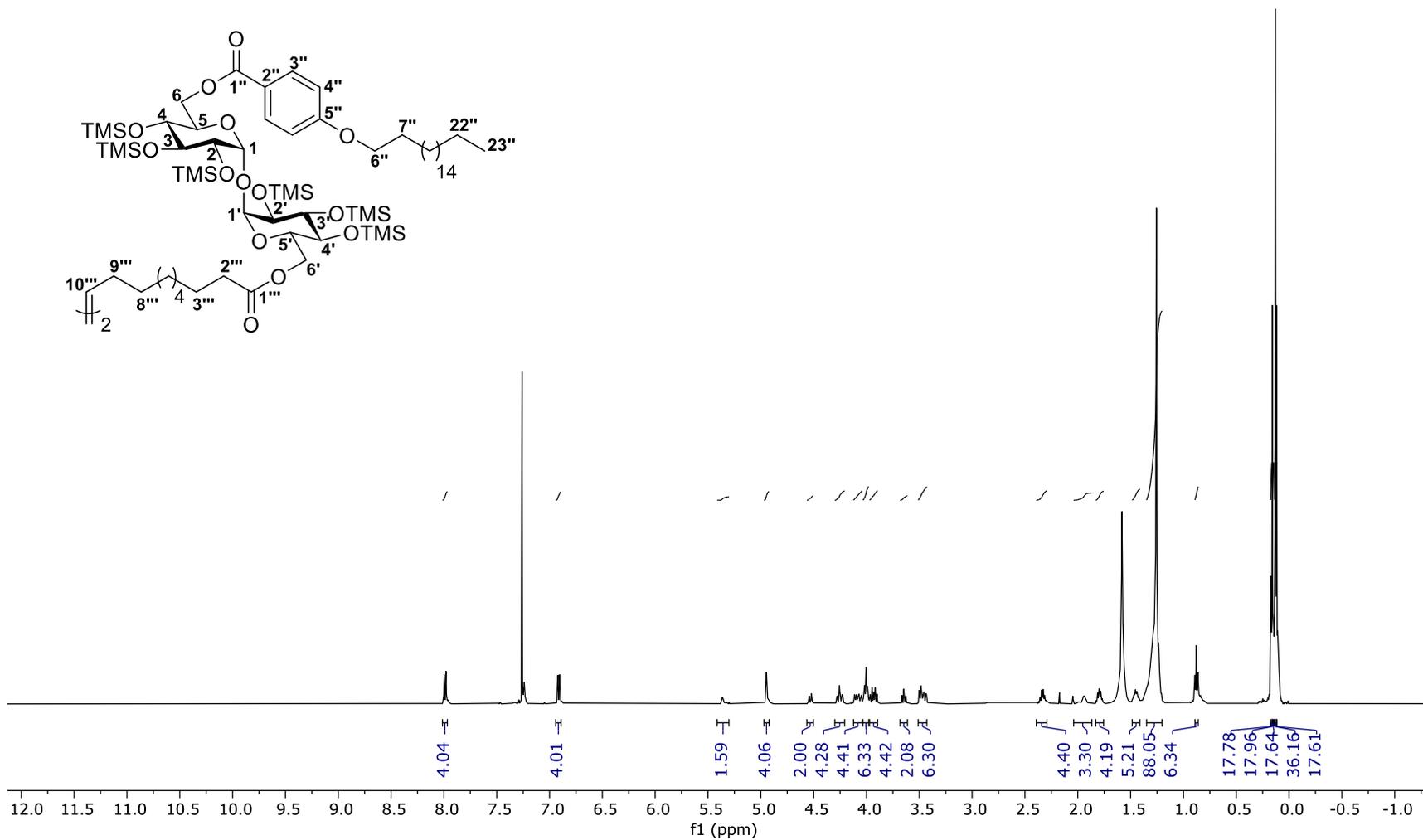
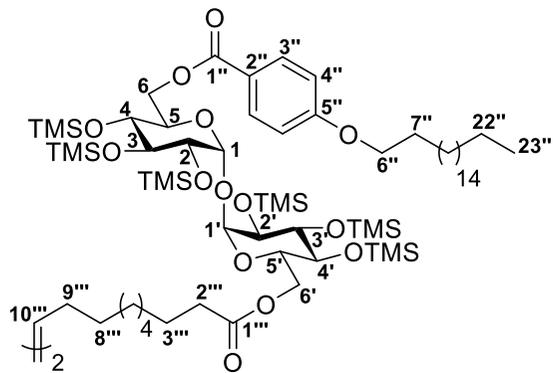


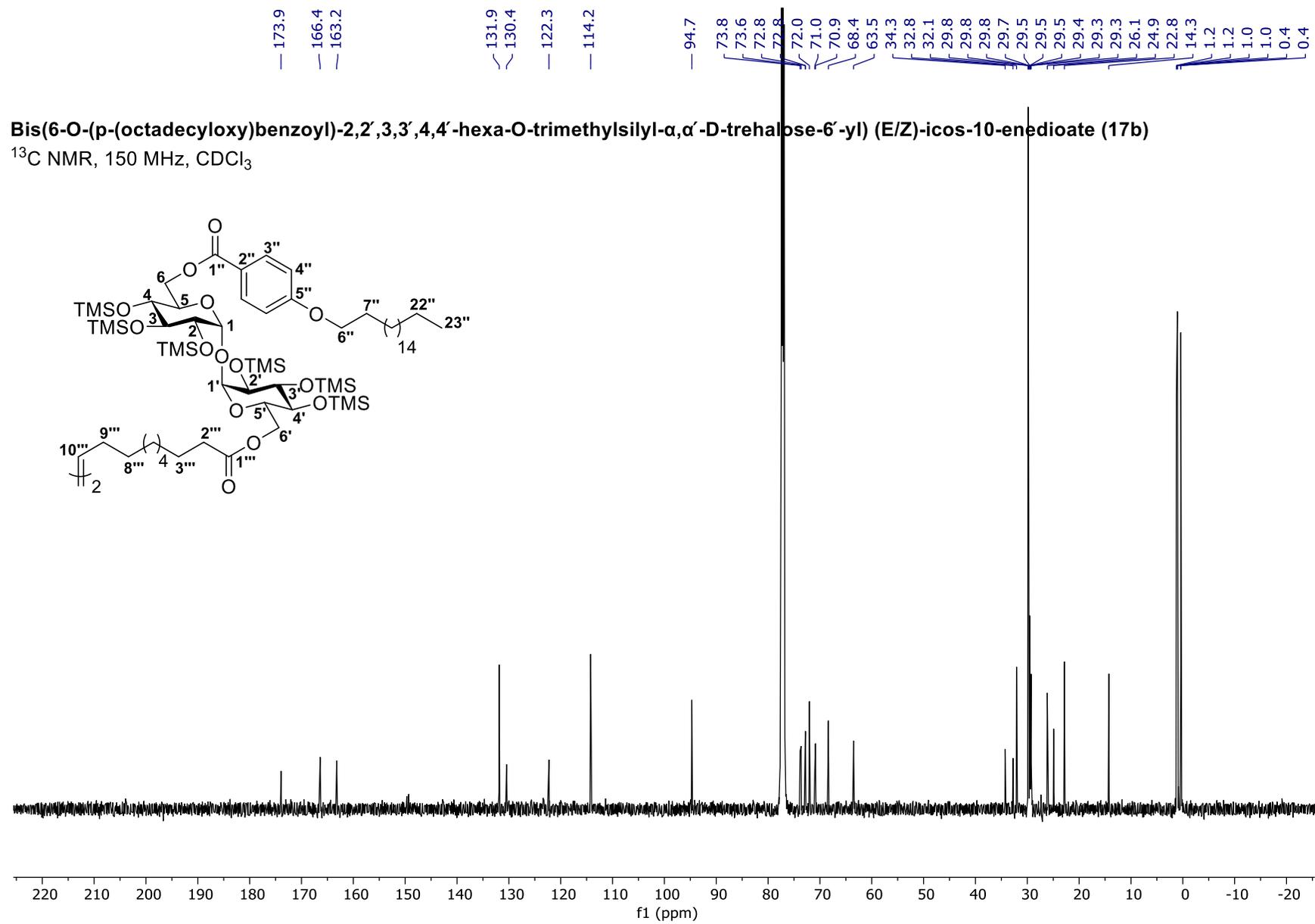


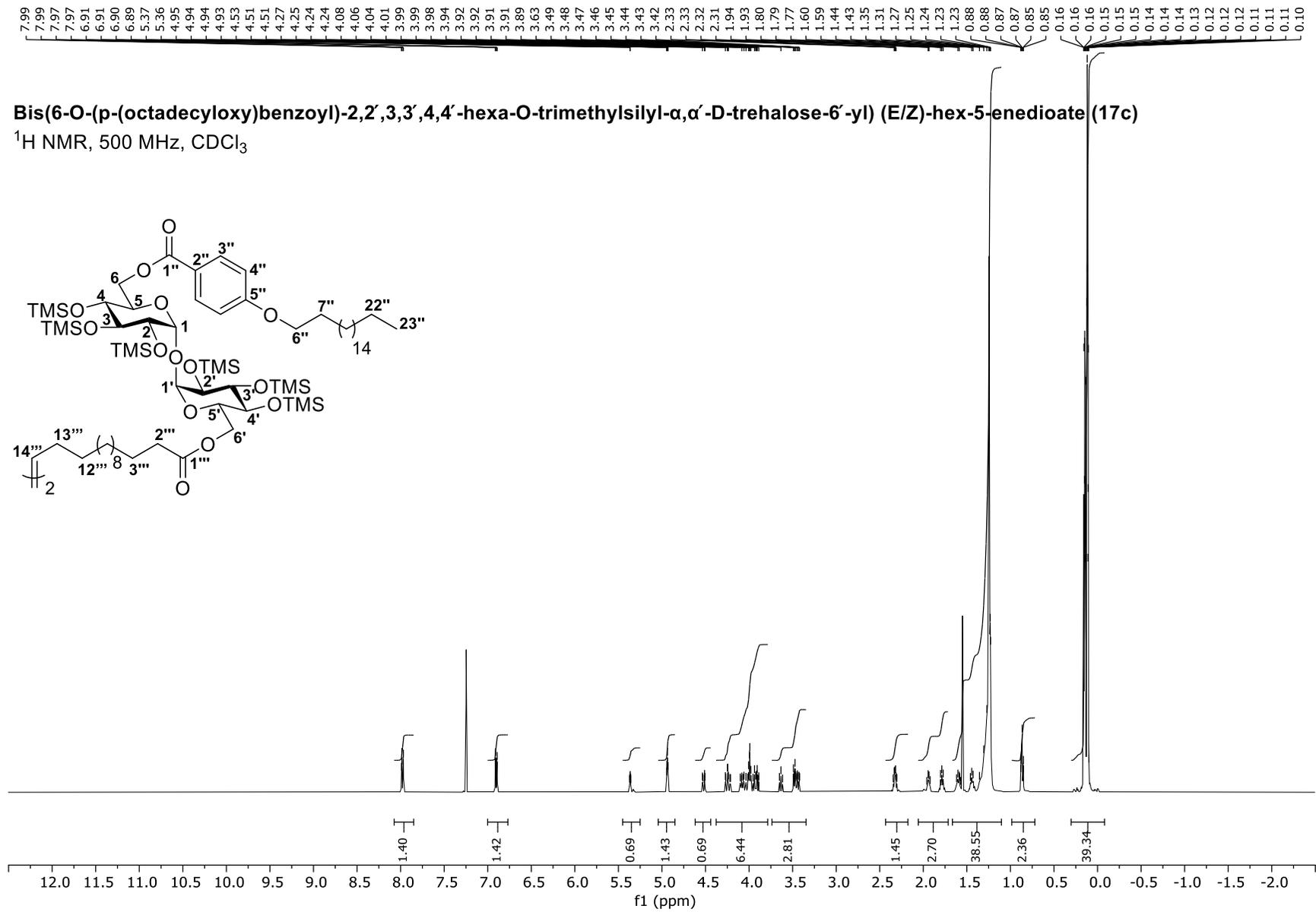
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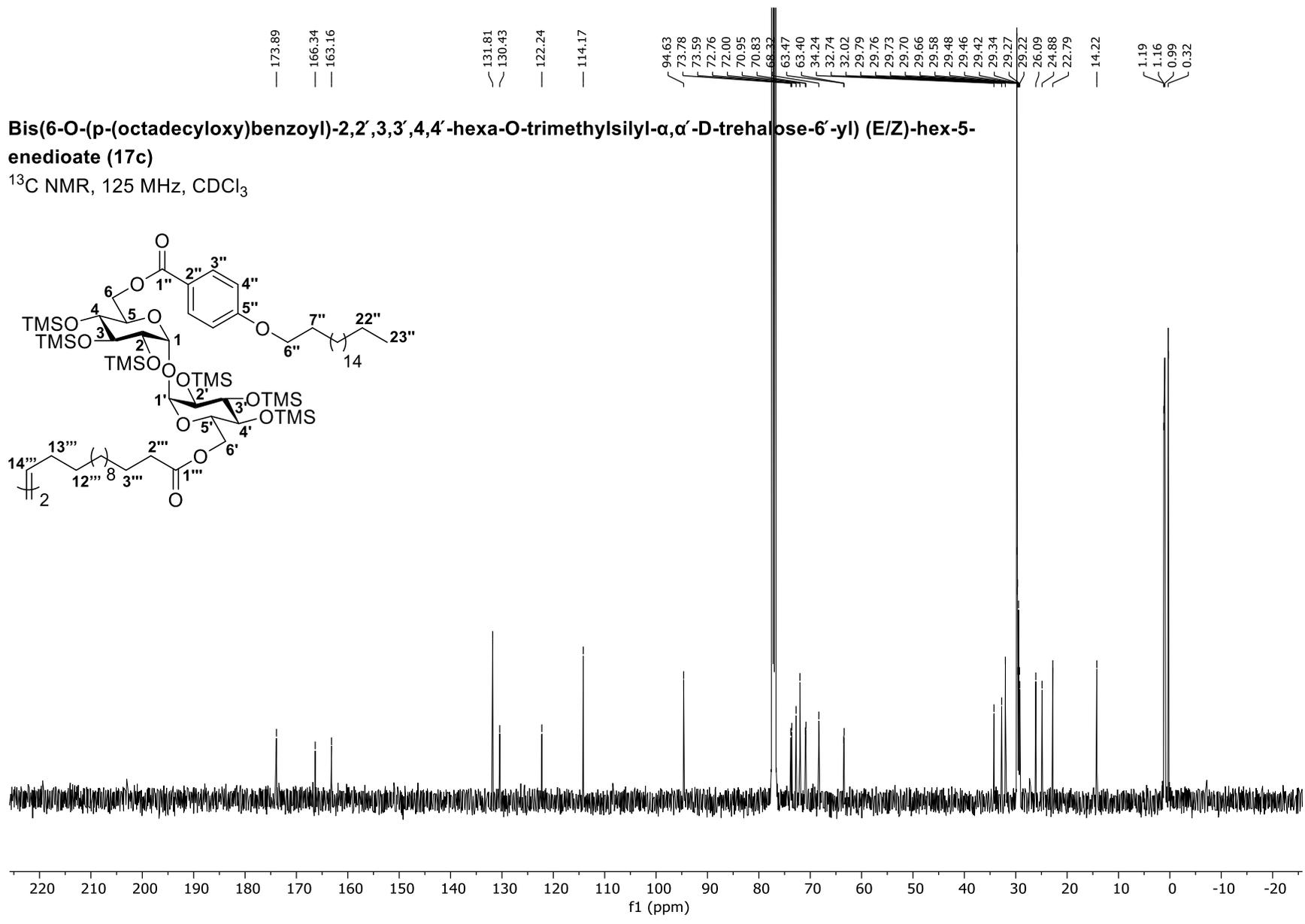
Bis(6-O-(p-(octadecyloxy)benzoyl)-2,2',3,3',4,4'-hexa-O-trimethylsilyl- α,α' -D-trehalose-6'-yl) (E/Z)-icos-10-enedioate (17b)

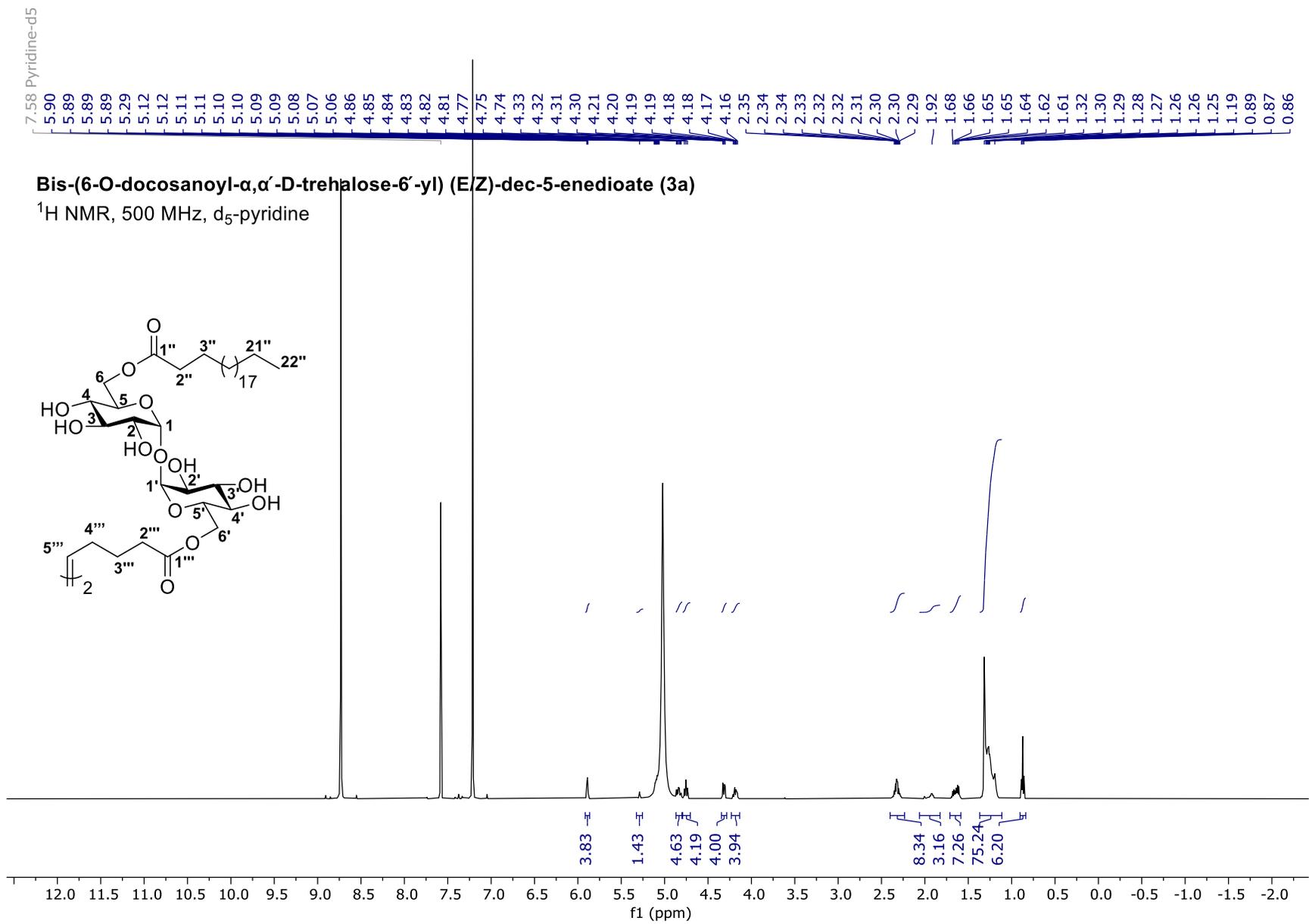
^1H NMR, 500 MHz, CDCl_3

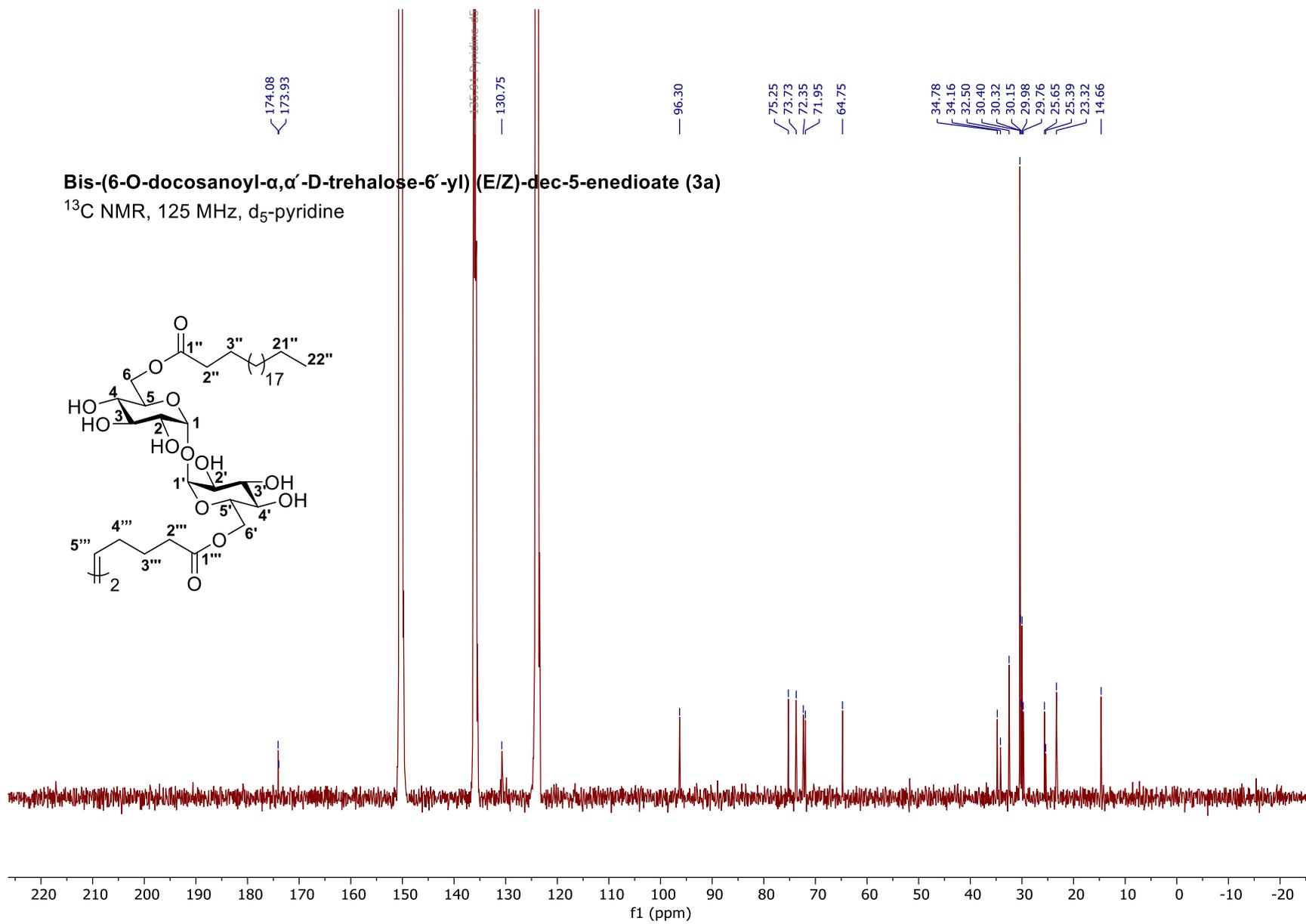


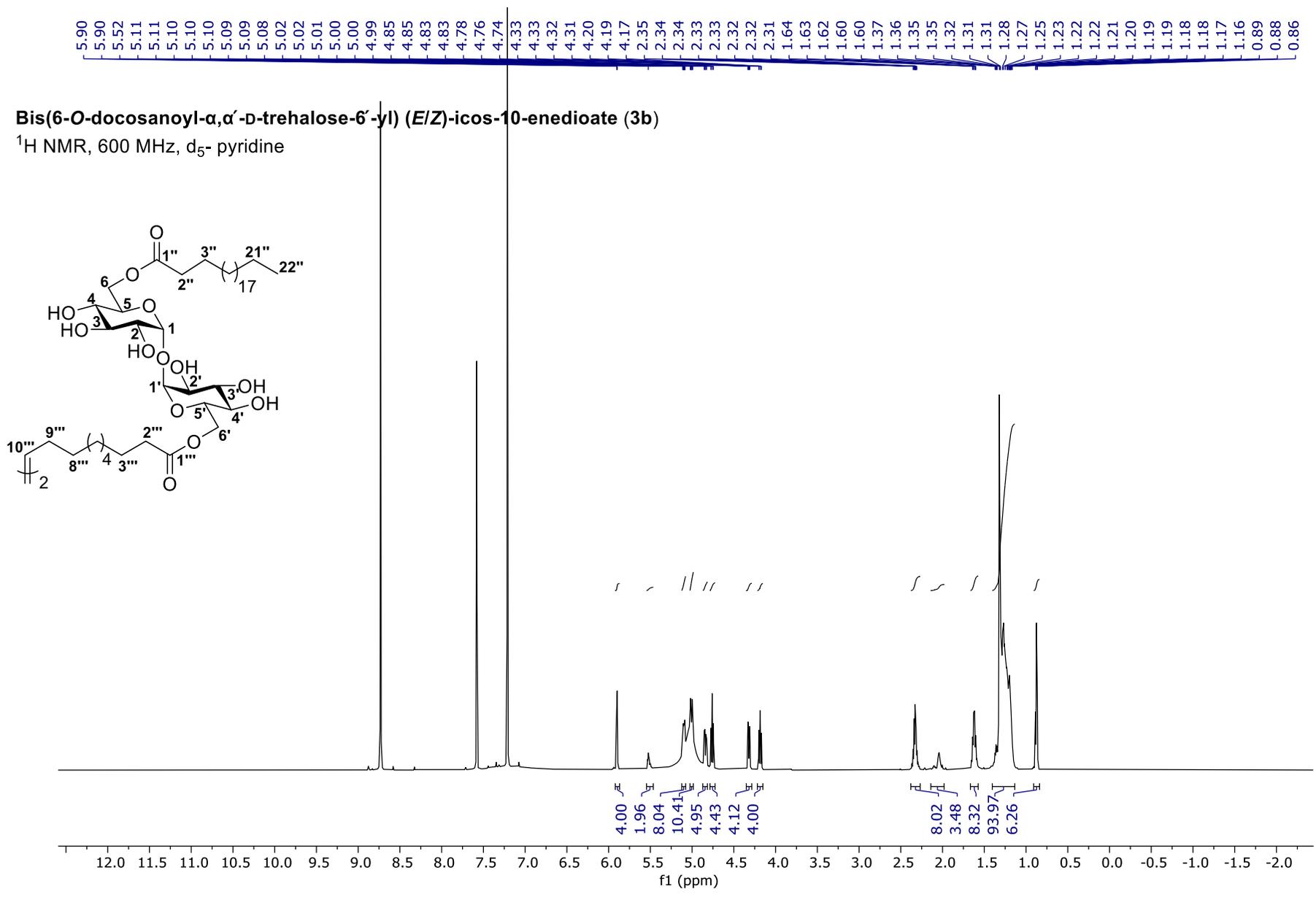


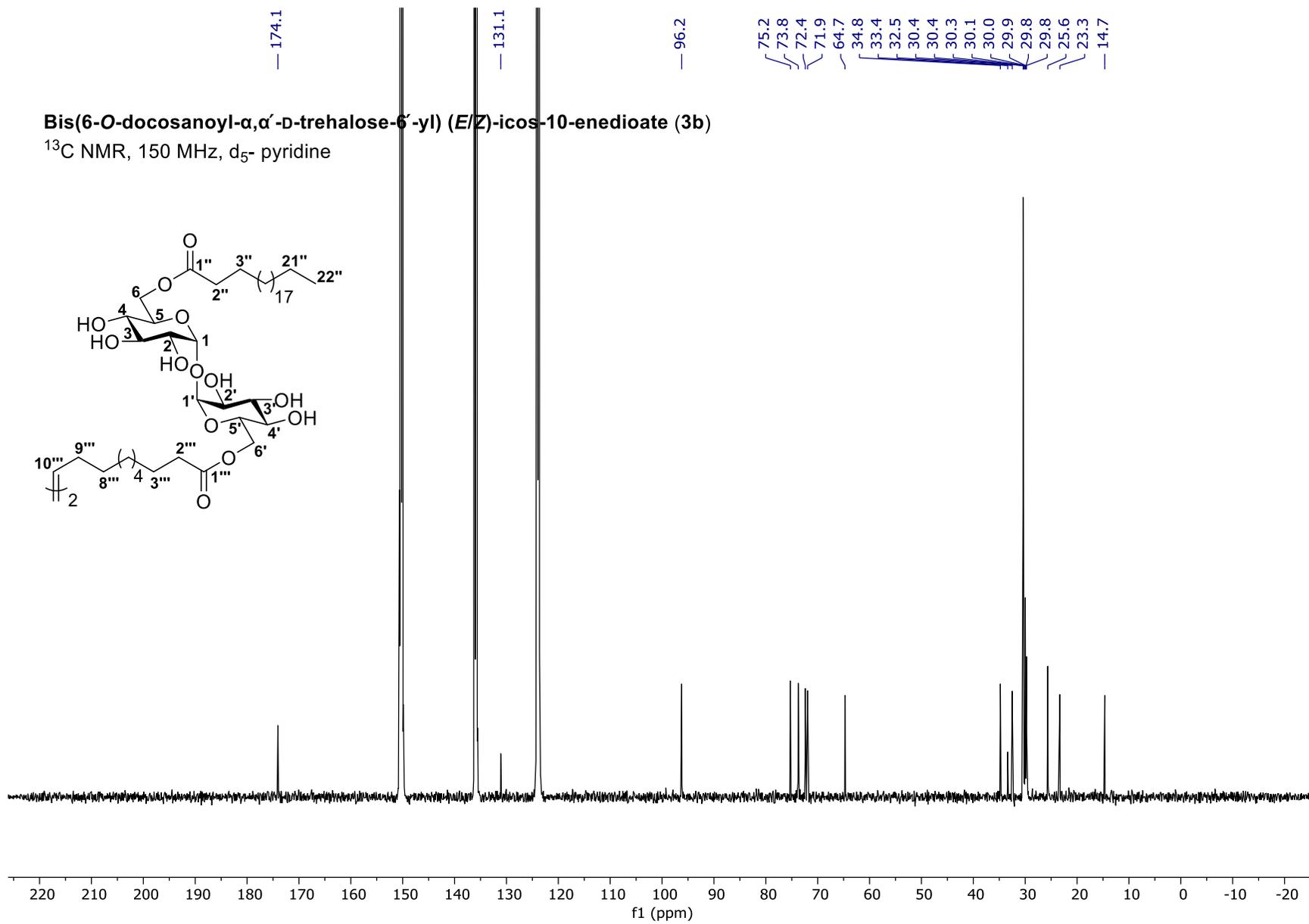






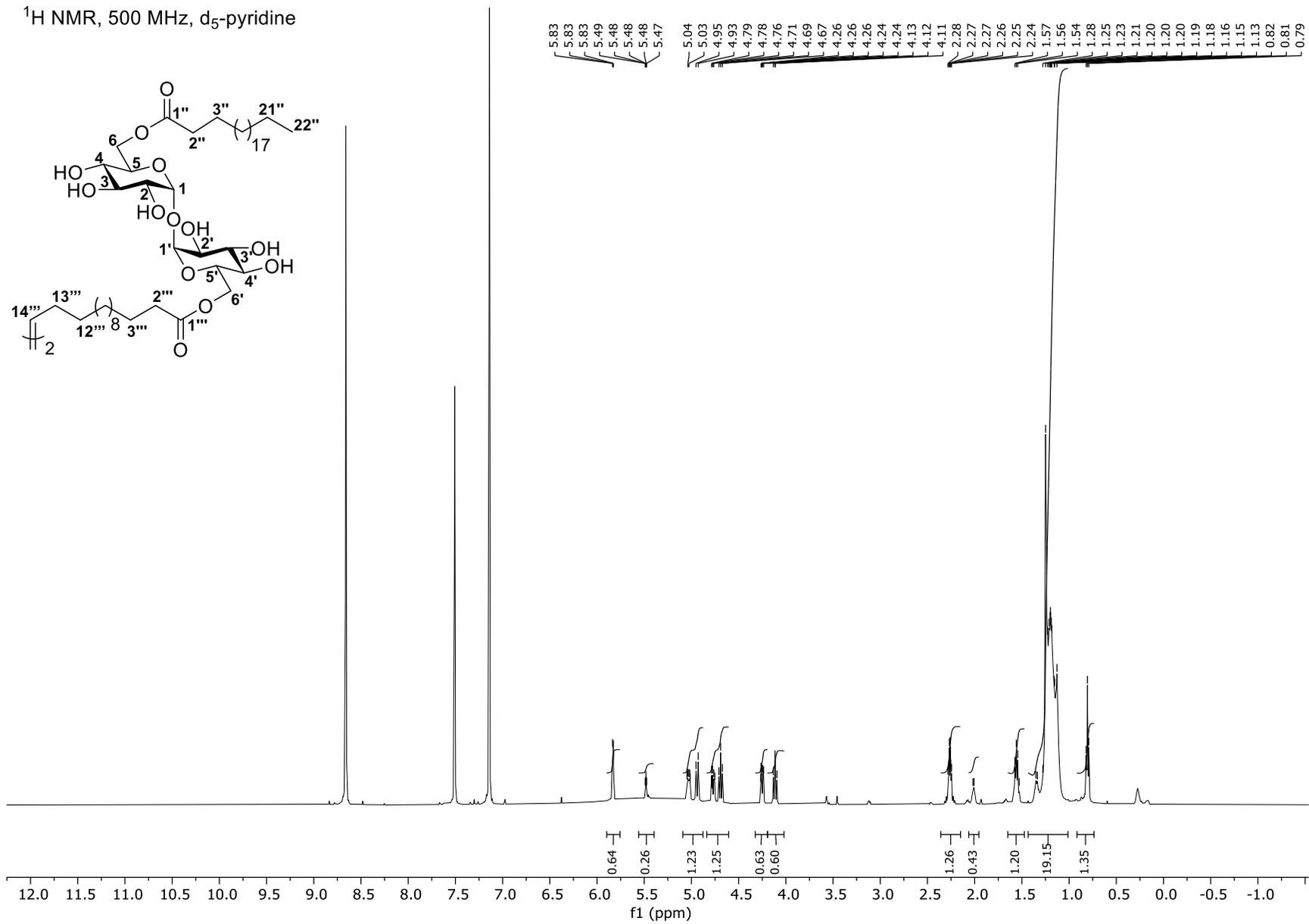






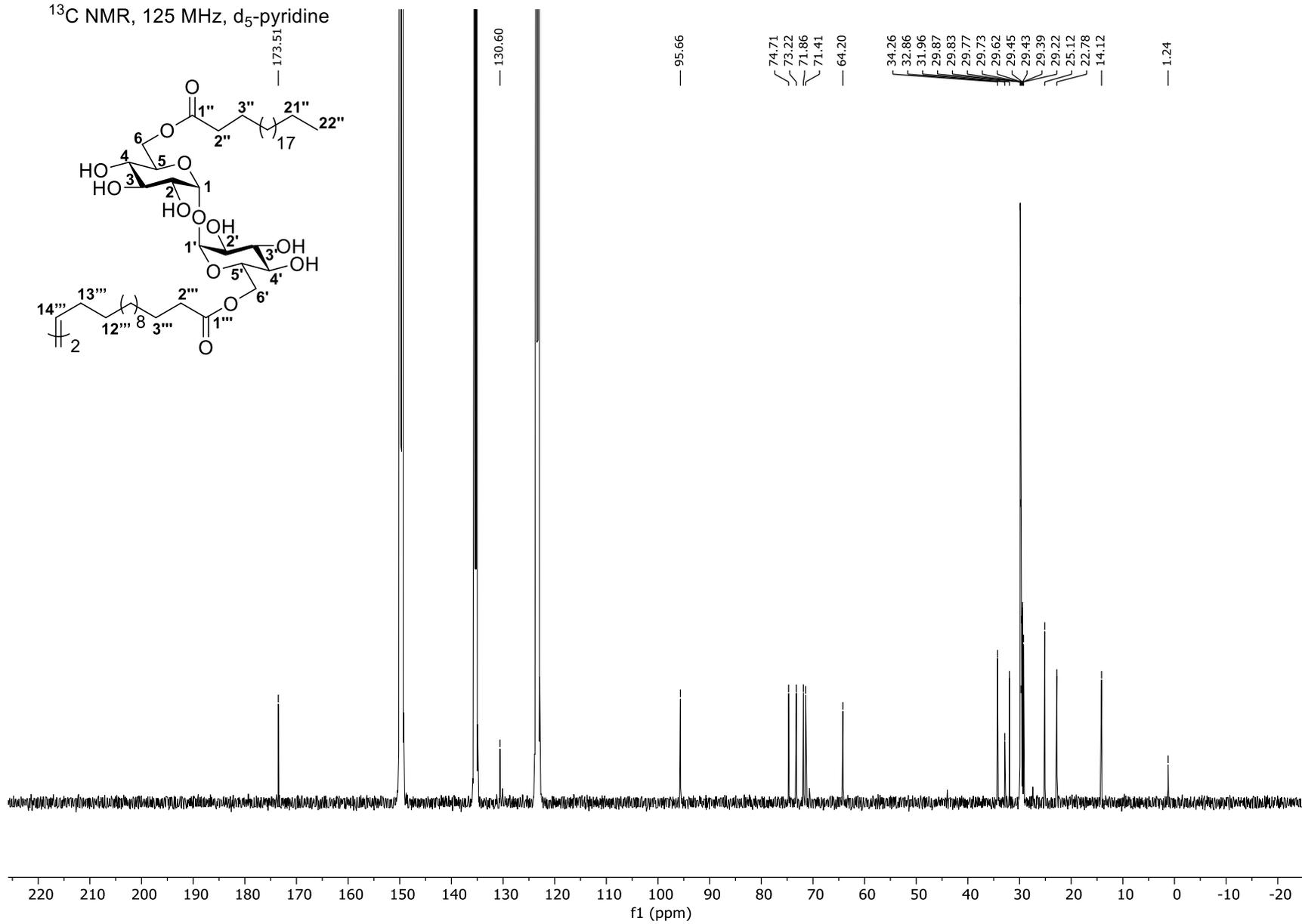
Bis(6-O-docosanoyl- α,α' -D-trehalose-6'-yl) (E/Z)-octacos-14-enedioate (3c)

^1H NMR, 500 MHz, d_5 -pyridine



Bis(6-O-docosanoyl- α,α' -D-trehalose-6'-yl) (E/Z)-octacos-14-enedioate (3c)

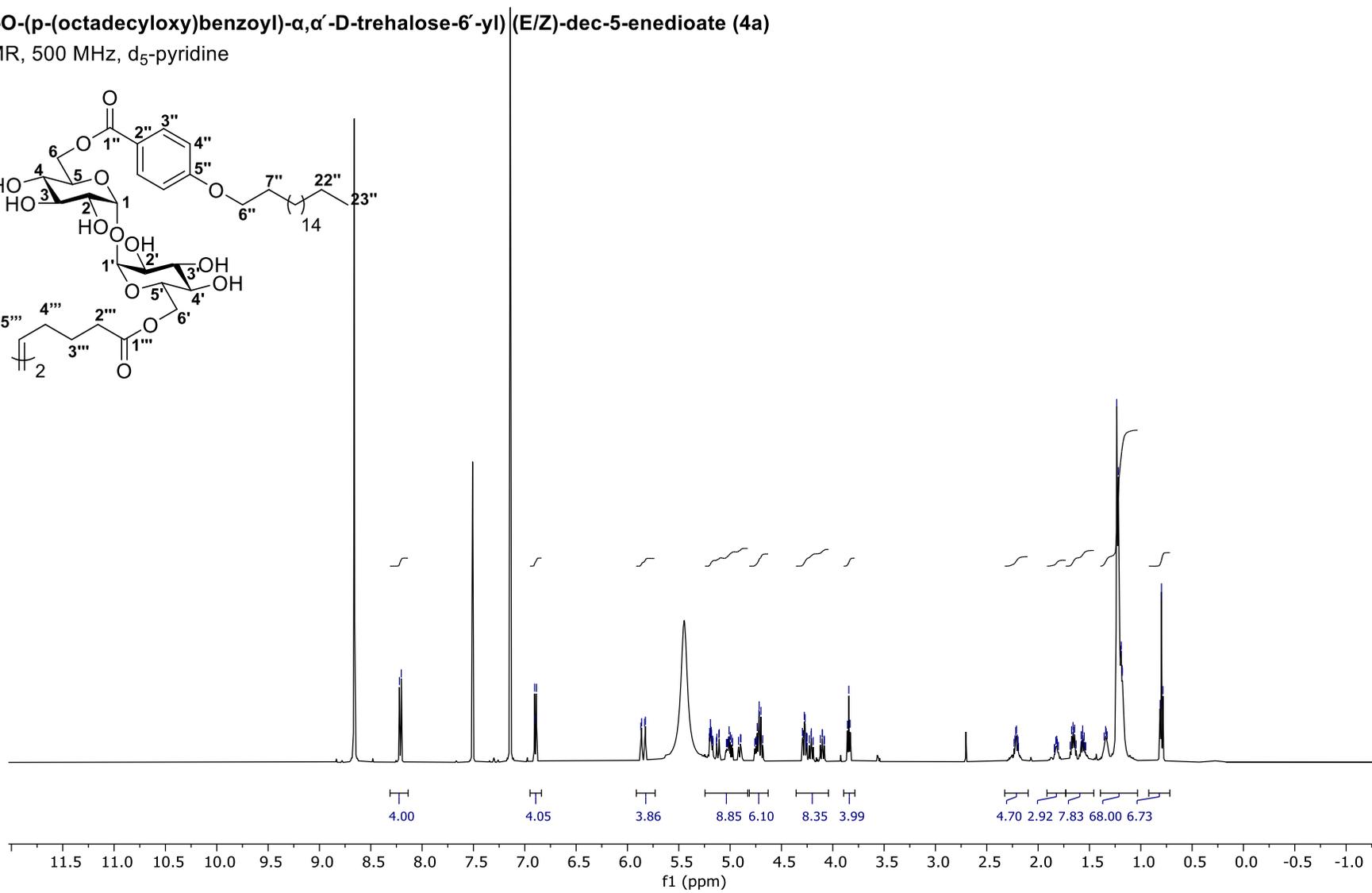
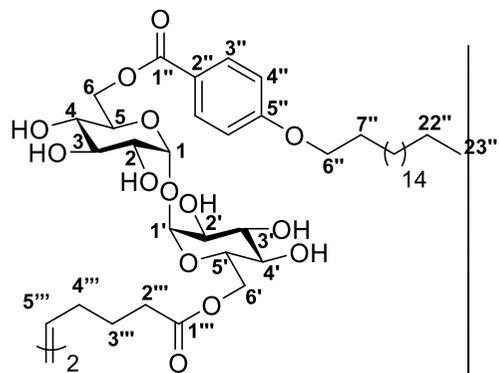
^{13}C NMR, 125 MHz, d_5 -pyridine

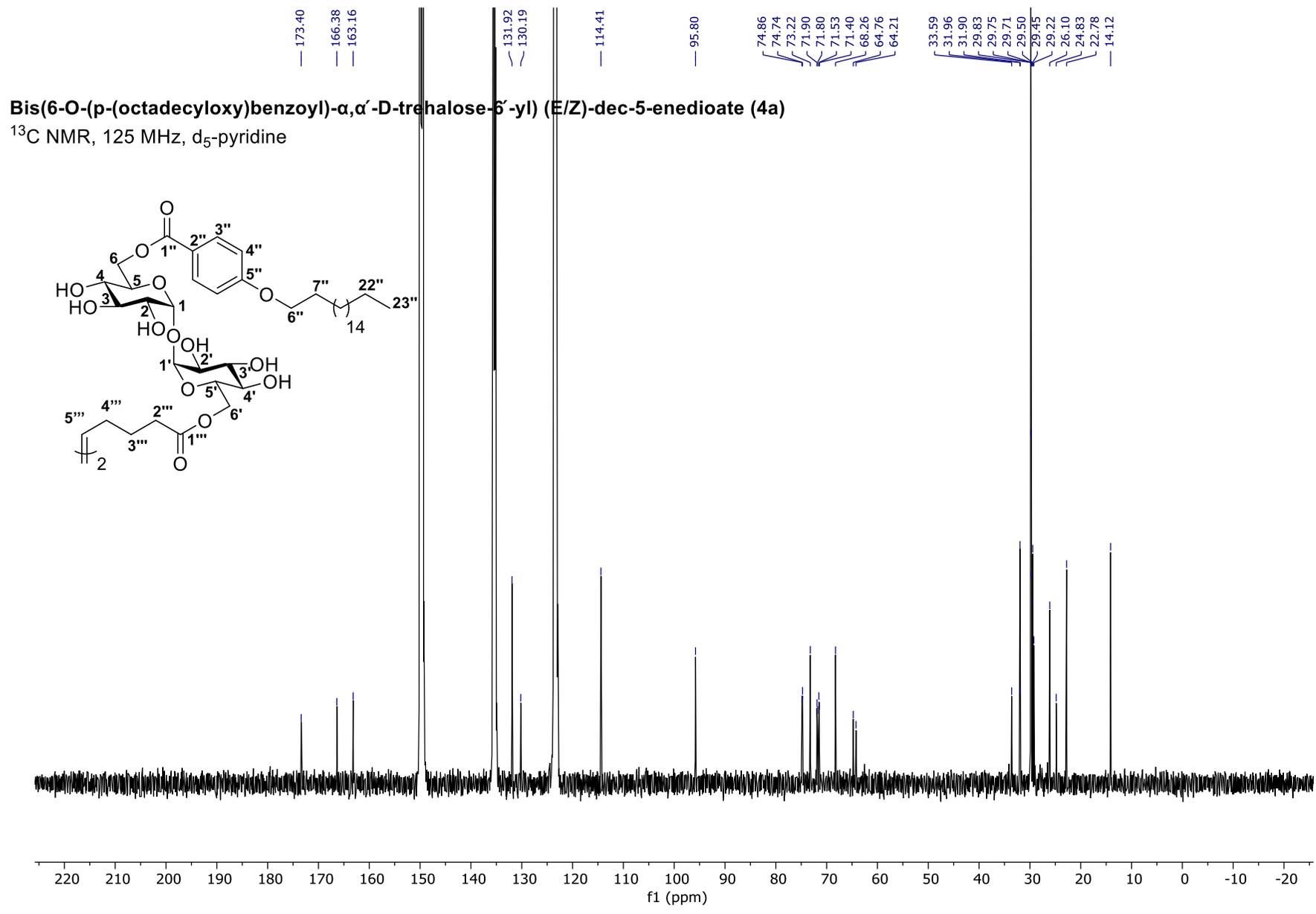


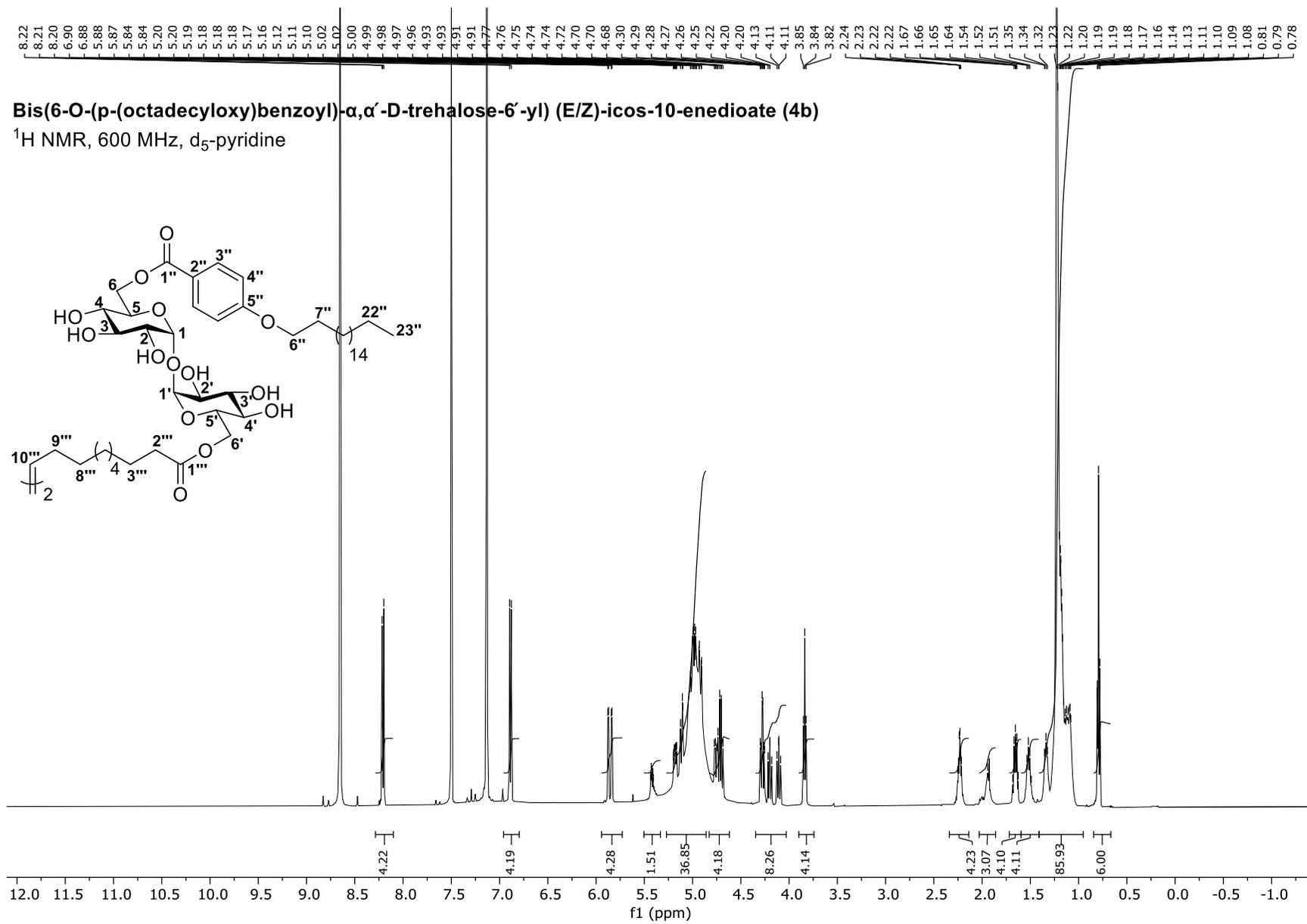
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2.20
2.20
2.20
1.83
1.82
1.67
1.66
1.66
1.64
1.58
1.57
1.55
1.55
1.36
1.34
1.34
1.33
1.23
1.22
1.19
1.19
1.18
1.18
0.81
0.80
0.80

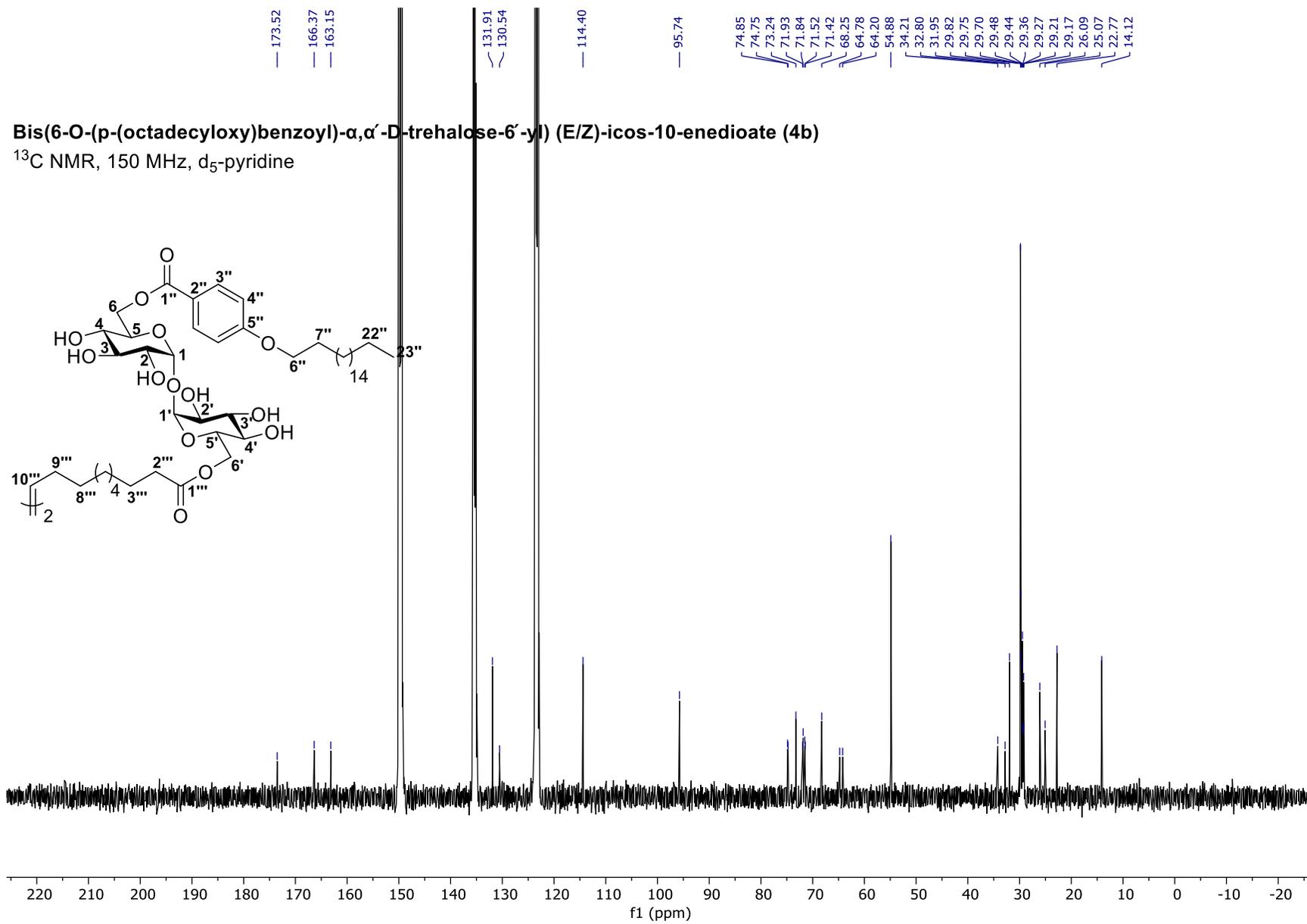
Bis(6-O-(p-(octadecyloxy)benzoyl)- α,α' -D-trehalose-6'-yl) (E/Z)-dec-5-enedioate (4a)

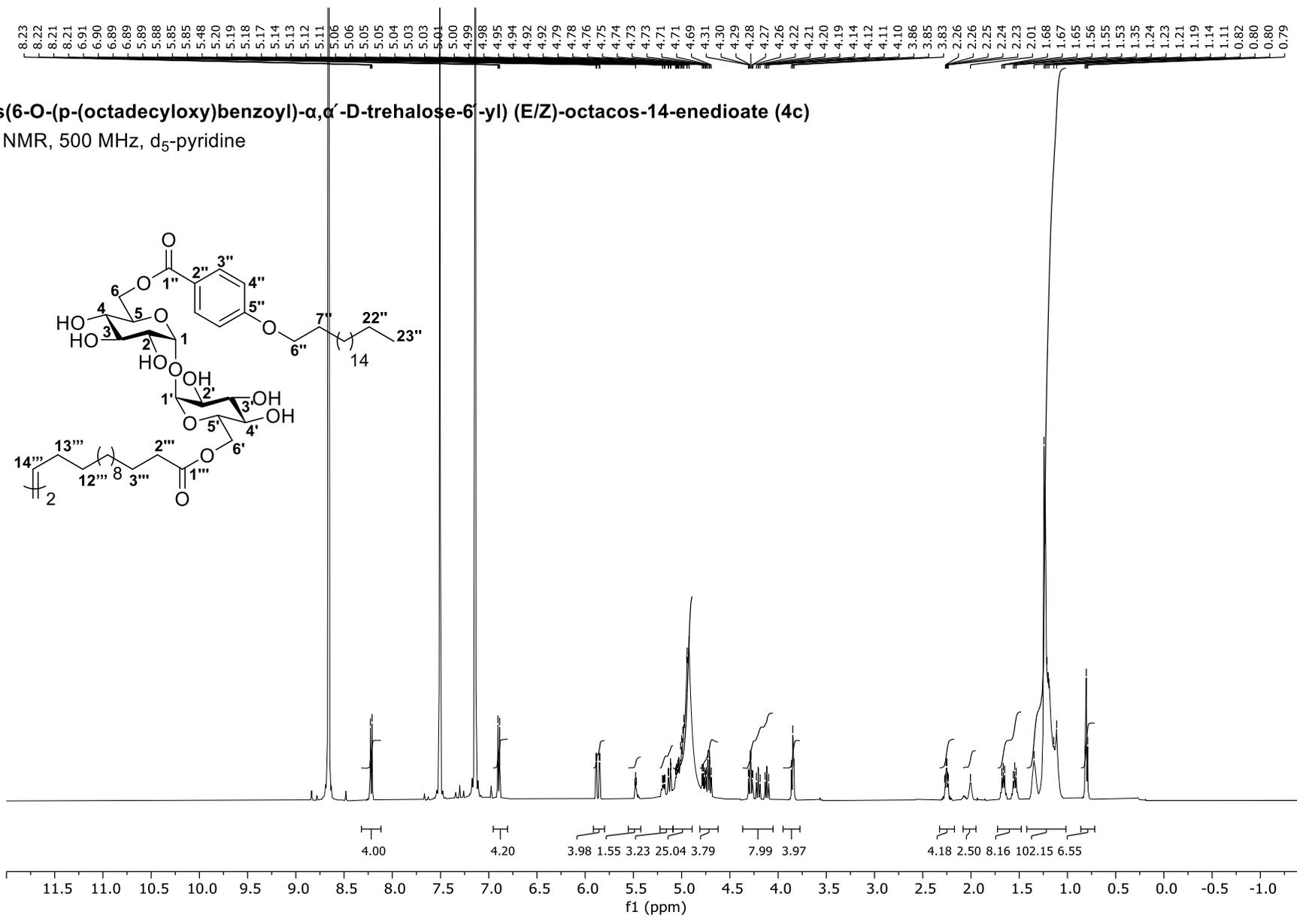
^1H NMR, 500 MHz, $\text{d}_5\text{-pyridine}$

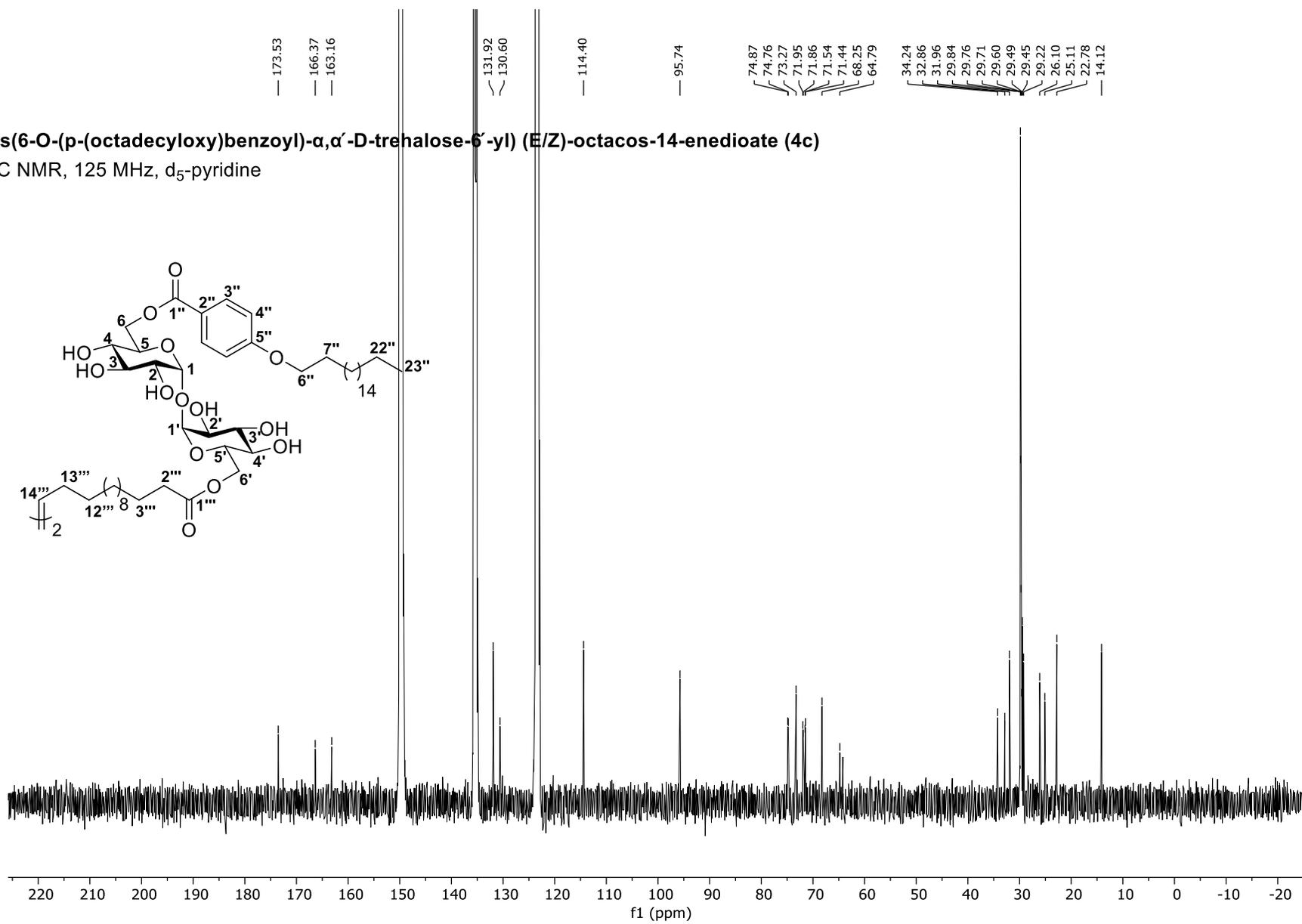


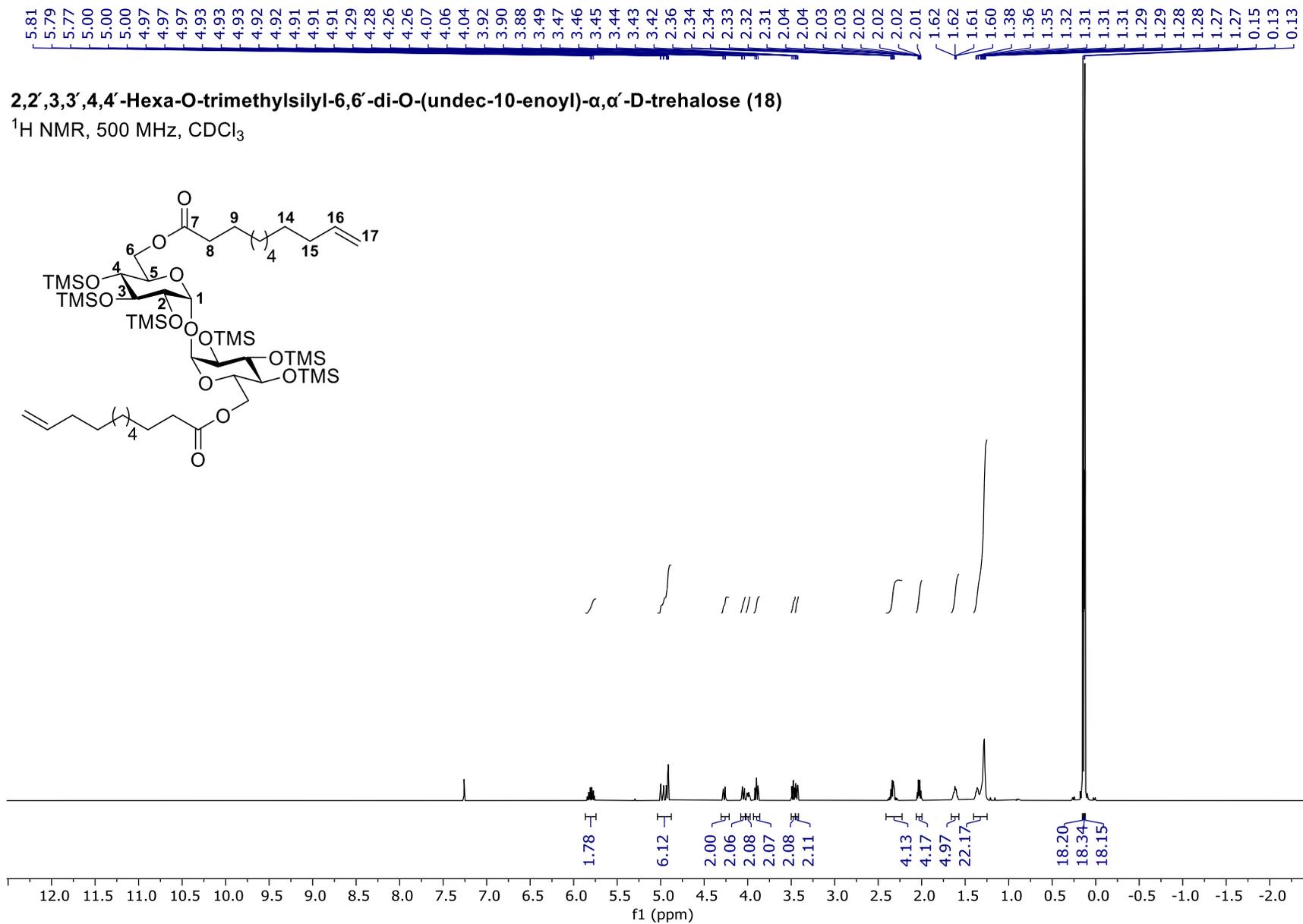






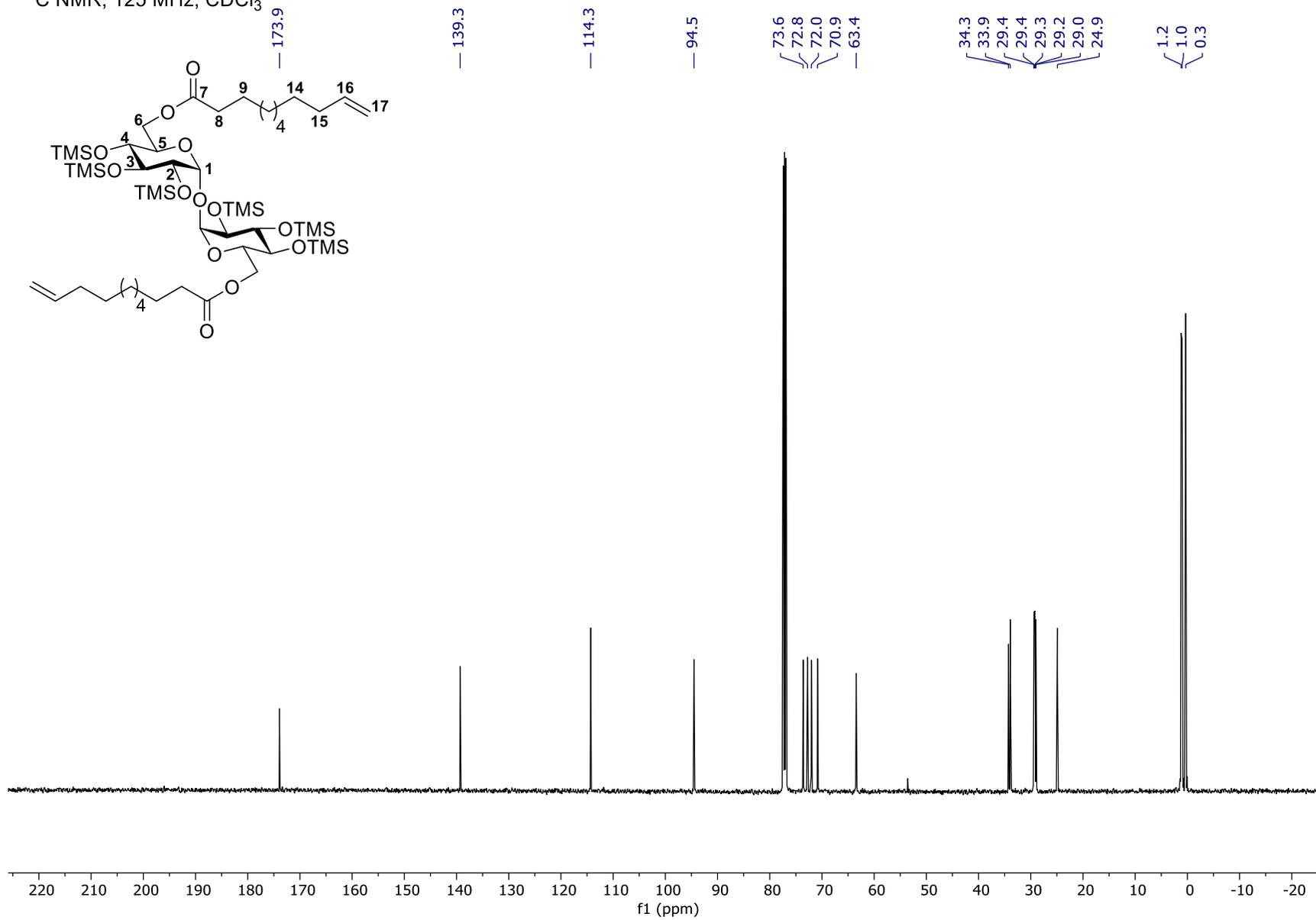






2,2',3,3',4,4'-Hexa-O-trimethylsilyl-6,6'-di-O-(undec-10-enoyl)- α,α' -D-trehalose (18)

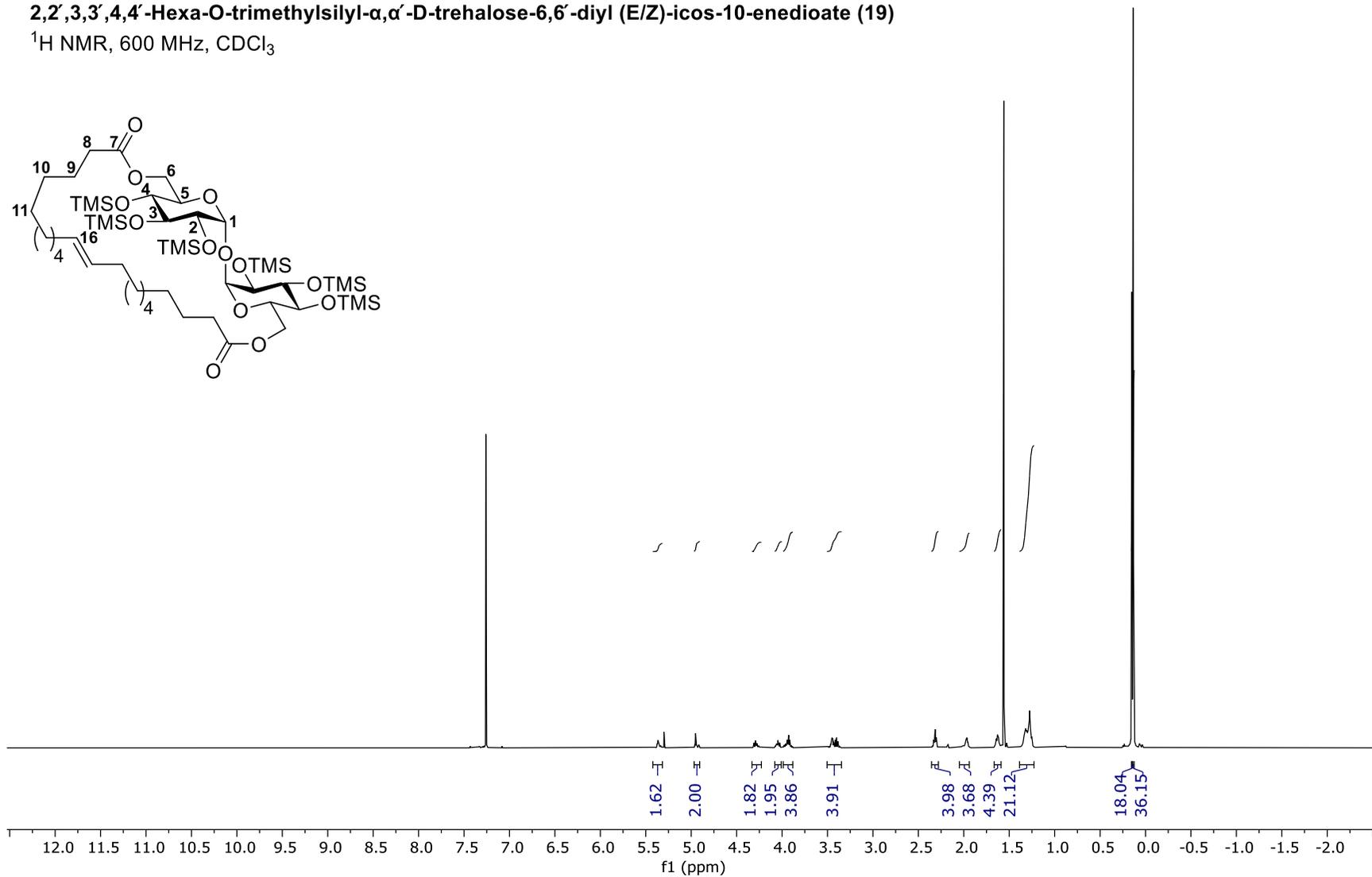
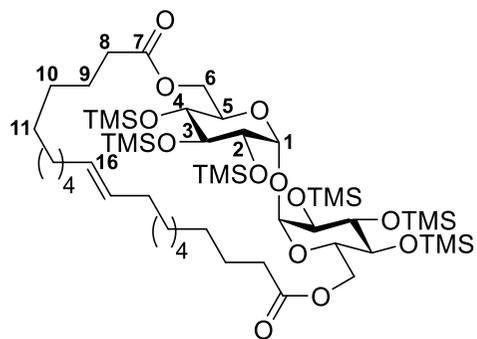
^{13}C NMR, 125 MHz, CDCl_3

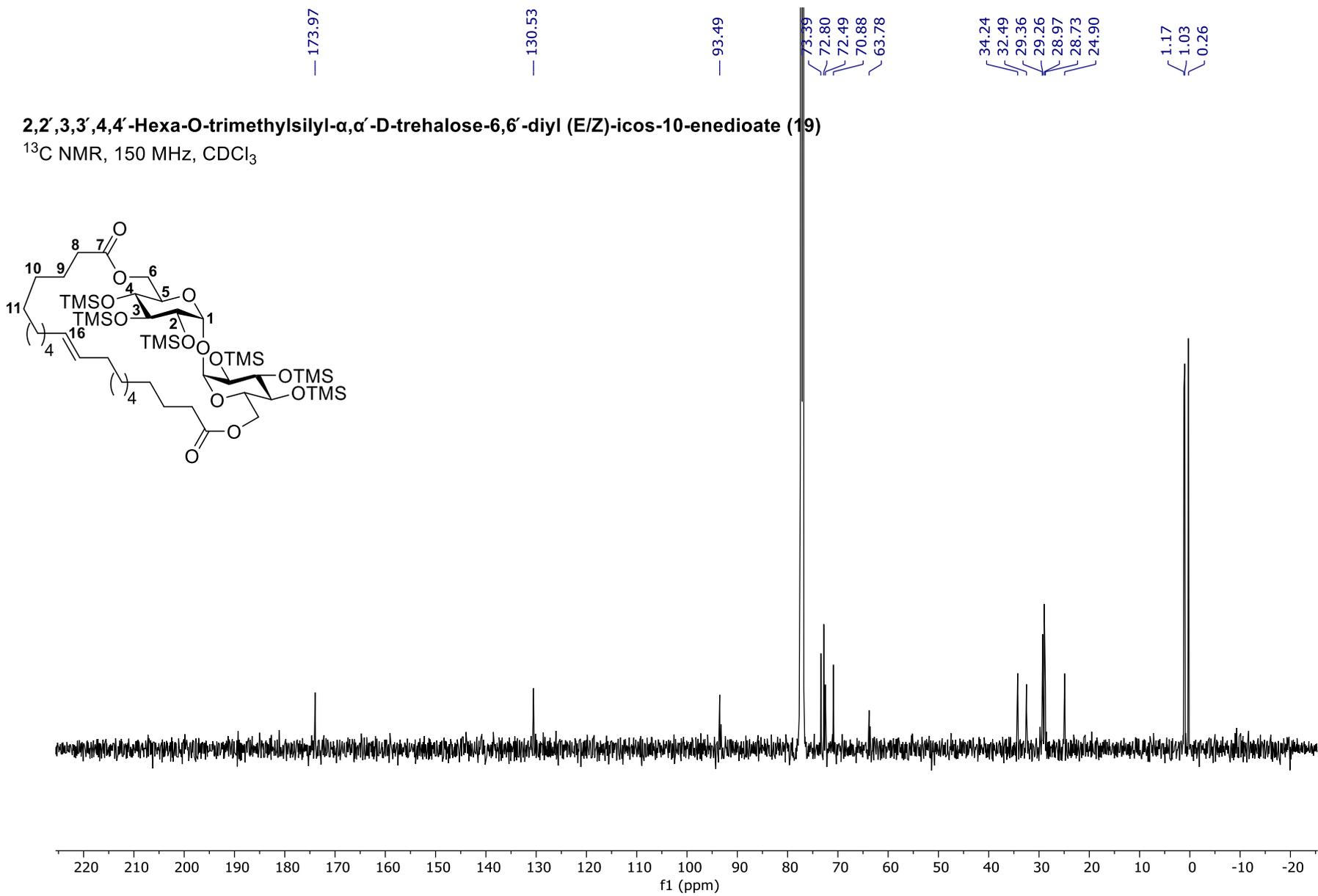


5.37
5.37
5.37
5.36
5.36
5.36
4.95
4.95
4.31
4.31
4.30
4.29
4.29
4.28
4.27
4.06
4.06
4.05
4.05
4.04
4.04
4.03
3.96
3.96
3.95
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3.94
3.94
3.93
3.93
3.92
3.91
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3.47
3.47
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2.31
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2.30
1.97
1.97
1.96
1.96
1.95
1.95
1.65
1.64
1.63
1.62
1.60
1.60
1.33
1.32
1.31
1.28
1.25
0.15
0.14

2,2',3,3',4,4'-Hexa-O-trimethylsilyl- α,α' -D-trehalose-6,6'-diyl (E/Z)-icos-10-enedioate (19)

^1H NMR, 600 MHz, CDCl_3





5.90
5.50
5.49
5.49
5.04
5.04
5.03
5.02
5.01
4.80
4.79
4.79
4.78
4.77
4.75
4.32
4.31
4.30
4.29
4.16
4.14
4.14
4.13
2.32
2.32
2.31
2.31
2.31
2.30
2.30
2.29
2.04
2.04
2.03
2.03
2.03
2.02
1.66
1.65
1.64
1.62
1.37
1.37
1.36
1.36
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1.34
1.34
1.27
1.26
1.26
1.25
1.25
1.23
1.23
1.22
1.21
1.20
1.19
1.19
1.18
1.17

α,α' -D-Trehalose-6,6'-diyl (E/Z)-icos-10-enedioate (5)

^1H NMR, 600 MHz, d_5 -pyridine

