Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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SUPPORTING INFORMATION

for

Synthesis of functionalised HP-DO3A chelating agents for conjugation to biomolecules

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1.1. Analytical methods

HPLC System A:

Stationary phase:	Lichrosorb 60 RP-Select B 5 µm
	75 x 4 mm column packed by Merck KGaA
Temperature:	RT
Mobile phase:	gradient elution
	A = 0.01 M KH_2PO_4 and 0.017 M H_3PO_4 in water
	B = CH_3CN

Gradient timetable:

min	% A	% B
0	95	5
30	20	80
45	20	80

Flow rate:	1 mL/min
Detection (UV):	210 nm
Injection:	10 µL
Sample conc.:	1 mg/mL

HPLC System B:

Stationary phase:	Lichrosorb 60 RP-Select B 5 µm
	75 x 4 mm column packed by Merck KGaA
Temperature:	45 °C
Mobile phase:	gradient elution
	A = 0.01 M KH_2PO_4 and 0.017 M H_3PO_4 in water
	B = CH_3CN

Gradient timetable:

min	% A	% B
0	95	5
15	20	80
20	20	80

Flow rate:	1 mL/min
Detection (UV):	210 nm
Injection:	10 µL
Sample conc.:	1 mg/mL or 5 mg/mL

HPLC System C:

Stationary phase: Lichrosorb RP-Select B; 5 µm
250 x 4 mm column packed by Merck KGaA

Temperature: 45° C

Mobile phase: gradient elution
A = KH2PO4 0.01 M – H3PO4 0.017 M
B = CH3CN

Gradient timetable:

min	% A	% B
0	95	5
30	20	80
45	20	80

Flow rate: 1 mL/min

Detection (UV): 210 nm

Injection: 10 µl

Sample concentration: 1 mg/mL

HPLC System D:

Stationary phase: XTerra MS C18, 5 µm
4.6 × 50 mm column packed by Waters

Temperature: RT

Mobile phase: gradient elution
A = 0.1% TFA in water
B = 0.1% TFA in CH_3CN

Gradient timetable:

min	% A	% B
0	95	5
10	80	20

Flow rate: 3 mL/min

Detection (UV): 210 nm

Injection: 10 µL

Sample conc.: 1 mg/mL

HPLC System E:

Stationary phase:	Lichrosphere 100 RP18, 5 µm,
	4 × 125 mm column packed by Merck
Temperature:	RT
Mobile phase:	gradient elution
	A = 0.1% TFA in water
	B = 0.1% TFA in CH_3CN

Gradient timetable:

min	% A	% B
0	90	10
10	80	20

Flow rate:	1 mL/min
Detection (UV):	210 nm
Injection:	10 µL
Sample conc.:	1 mg/mL

1.2. Semi-preparative methods

HPLC system F:

Stationary phase:	XTerra PREP MS C18, 5 µm
	19 × 50 mm column packed by Waters
Temperature:	RT
Mobile phase:	gradient elution
	A = 0.1% TFA in water
	B = 0.1% TFA in CH_3CN

Gradient timetable:

min	% A	% B
0	97	3
16	60	40

Flow rate:	50 mL/min
Detection (UV):	210 nm

HPLC System G:

Stationary phase:	Lichrosorb C18, 7 µm
	25 × 250 mm column packed by Merck
Temperature:	RT
Mobile phase:	gradient elution
	A = 0.1% TFA in water
	B = 0.1% TFA in CH_3CN

Gradient timetable:

min	% A	% B
0	95	5
30	95	5
60	50	50
80	20	80
90	20	80

Flow rate:	20 mL/min
Detection (UV):	210 nm

1.3. FPLC method

Stationary phase:	Resource RPC 1 ml, 15 µm
	column packed by Amersham Pharmacia Biotech
Temperature:	RT
Mobile phase:	gradient elution
	A = 0.1% TFA in water
	B = CH_3OH

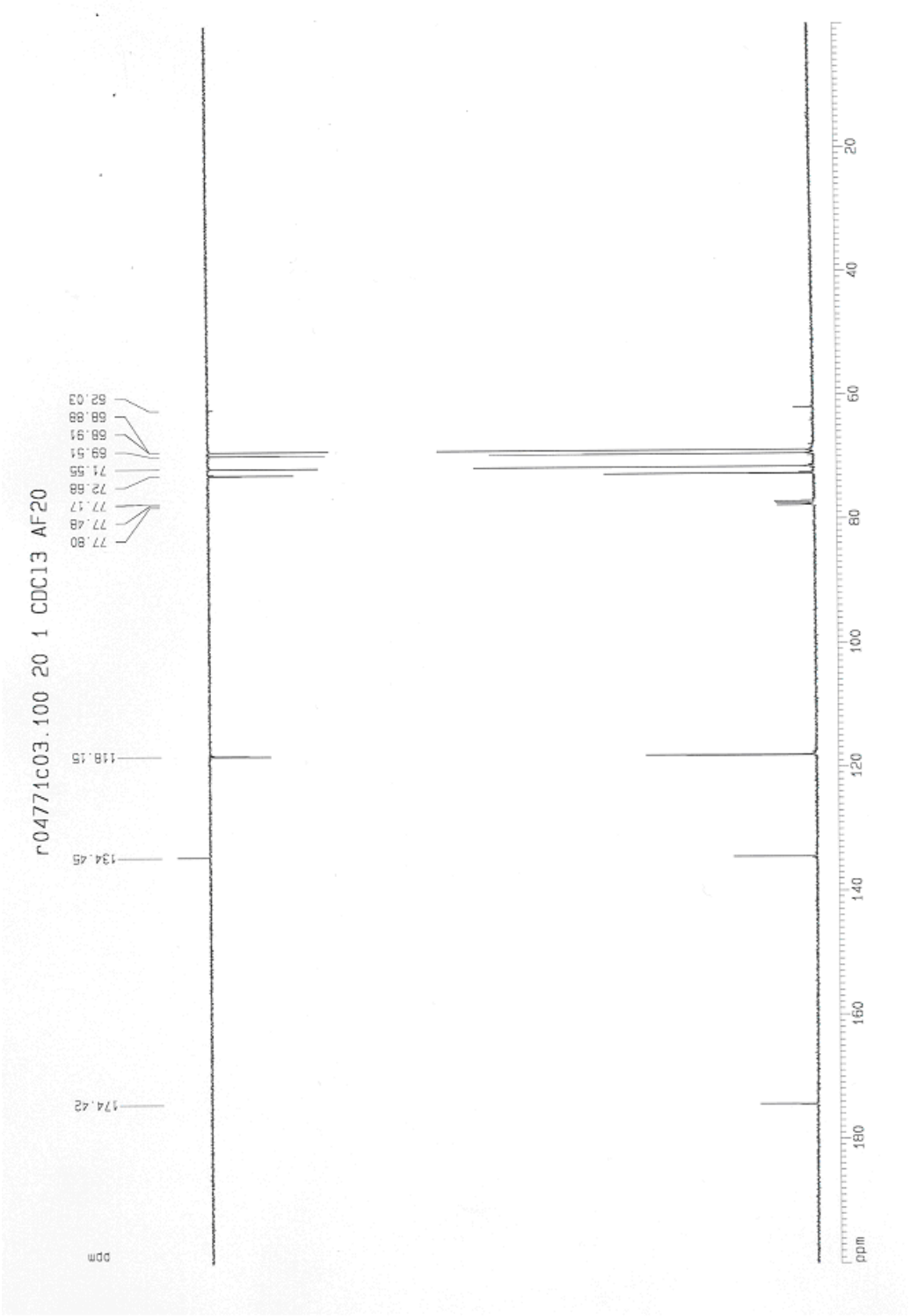
Gradient timetable:

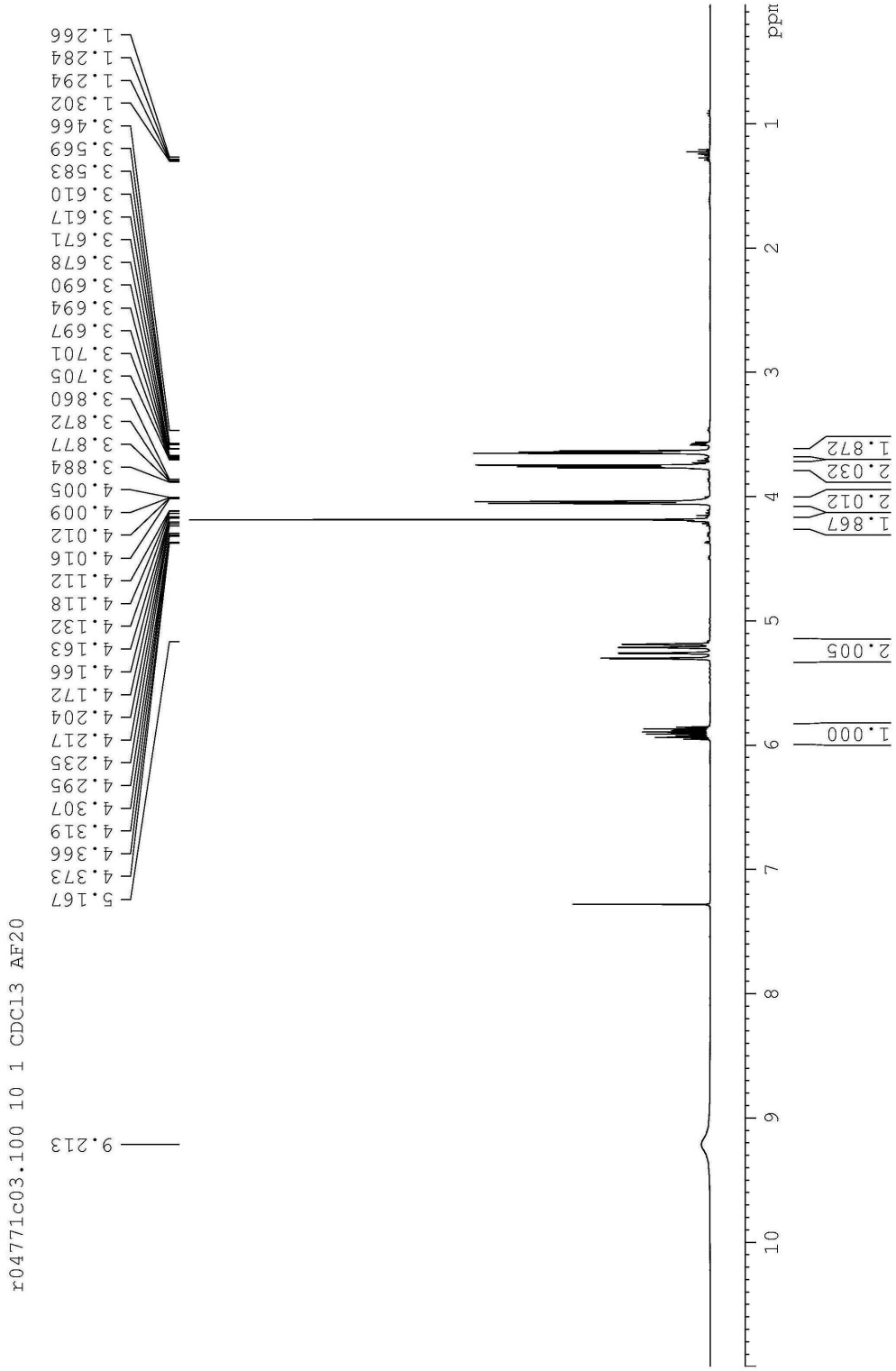
min	% A	% B
0	0	100
37	100	0

Flow rate:	4 mL/min
Detection:	UV 210 nm, radiometer

2. NMR Spectra

2-[2-(2-Propenyloxy)ethoxy]acetic acid (1)

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4.295
4.235
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4.204
4.172
4.166
4.163
4.132
4.118
4.112
4.016
4.012
4.009
4.005
3.884
3.877
3.872
3.860
3.705
3.701
3.697
3.694
3.690
3.678
3.671
3.617
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3.583
3.569
3.466
1.302
1.294
1.284
1.266
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2.012
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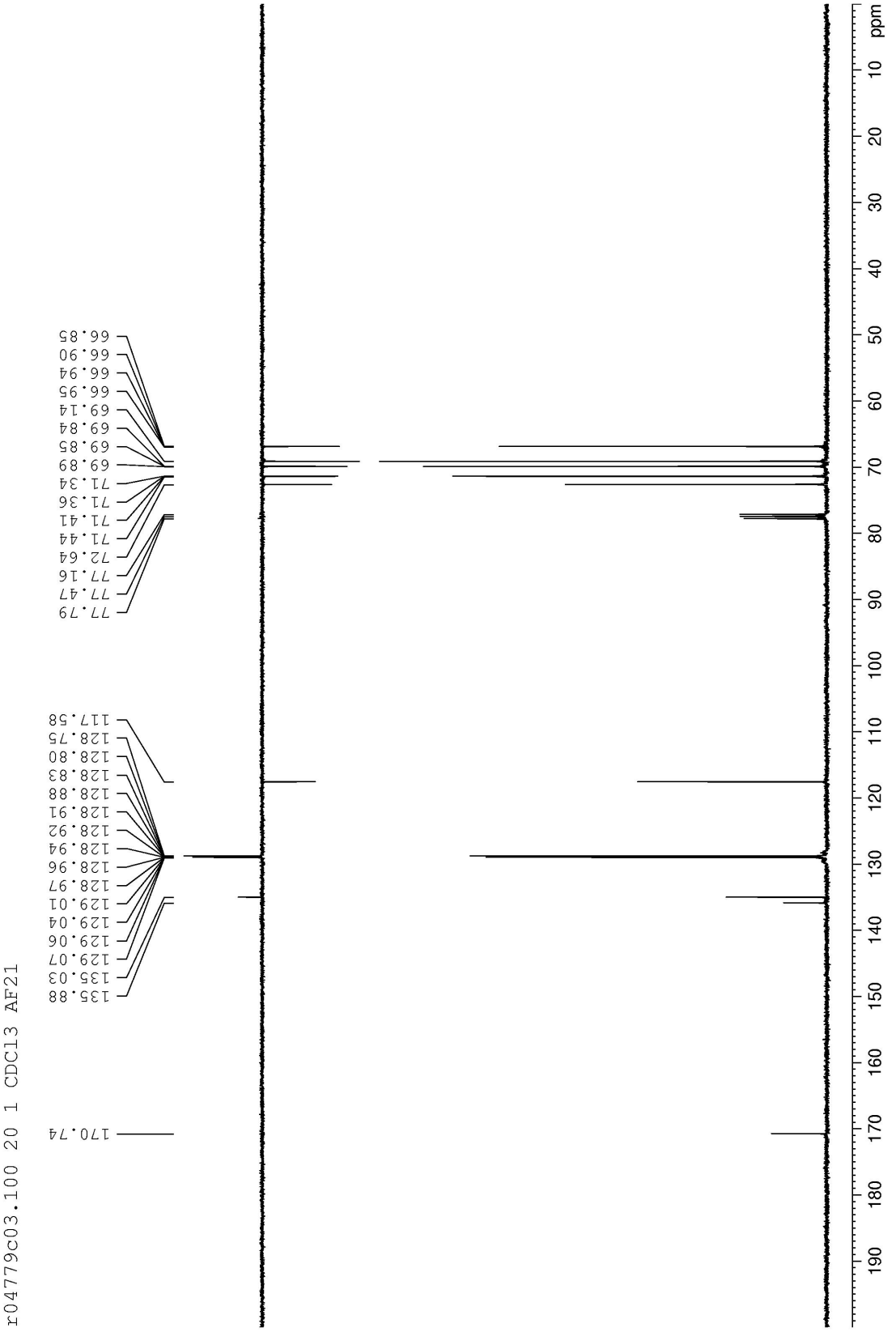
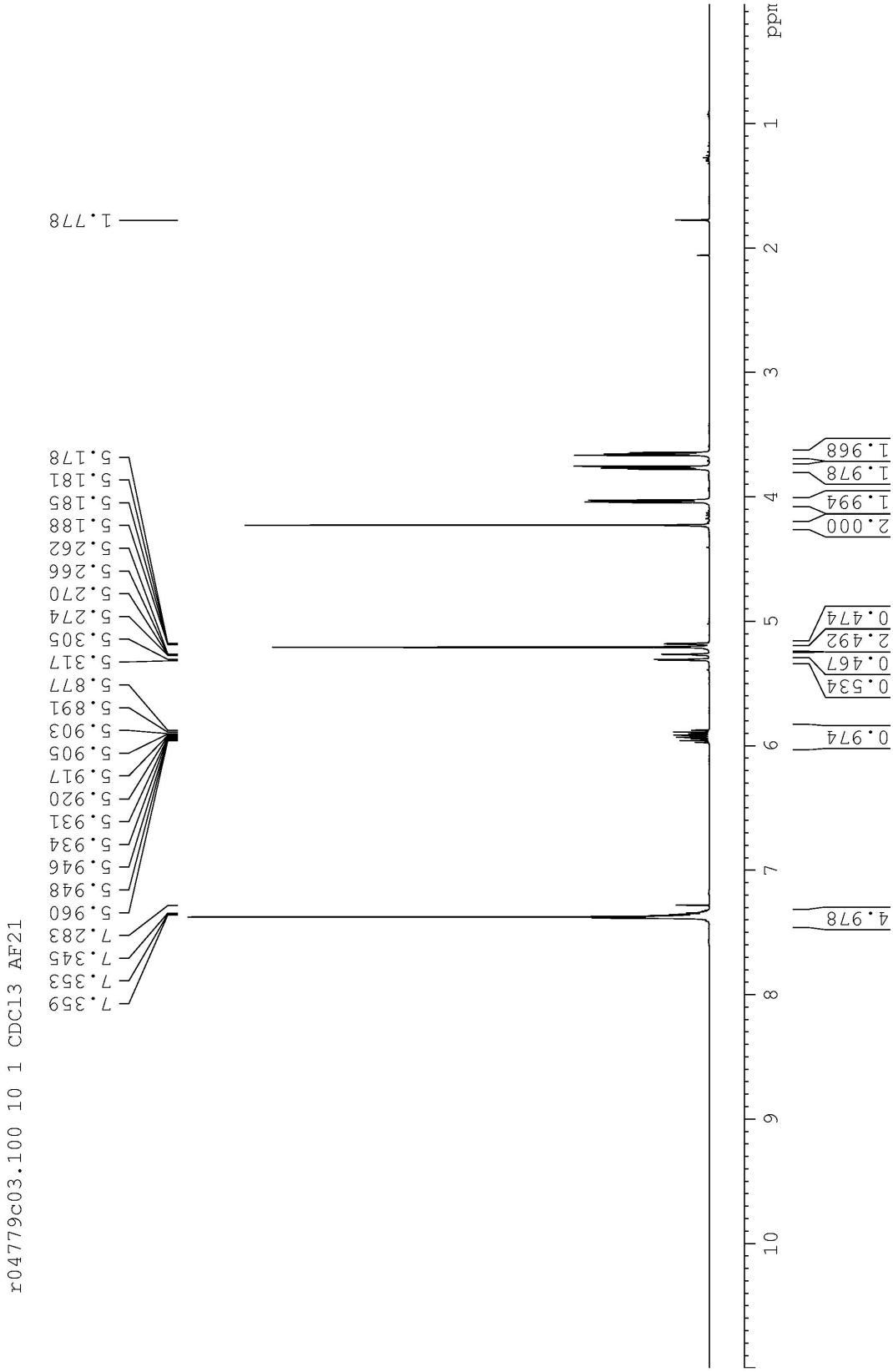
1.000
ppm

2-[2-(2-Propenyloxy)ethoxy]acetic acid benzyl ester (2)

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5.274
5.305
5.317
5.877
5.891
5.903
5.905
5.917
5.920
5.931
5.934
5.946
5.948
5.960
7.283
7.345
7.353
7.359
1.968
1.978
1.994
2.000
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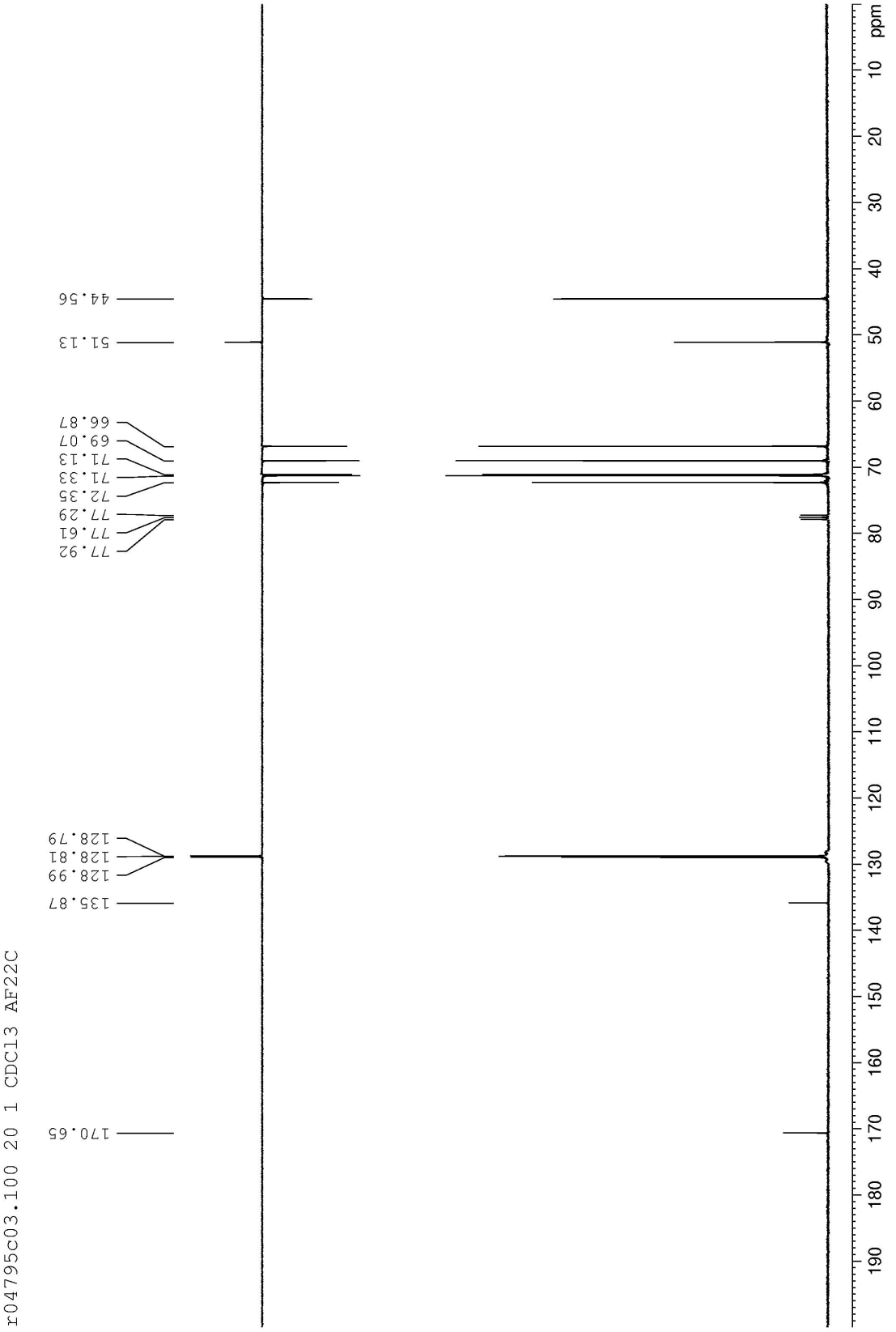
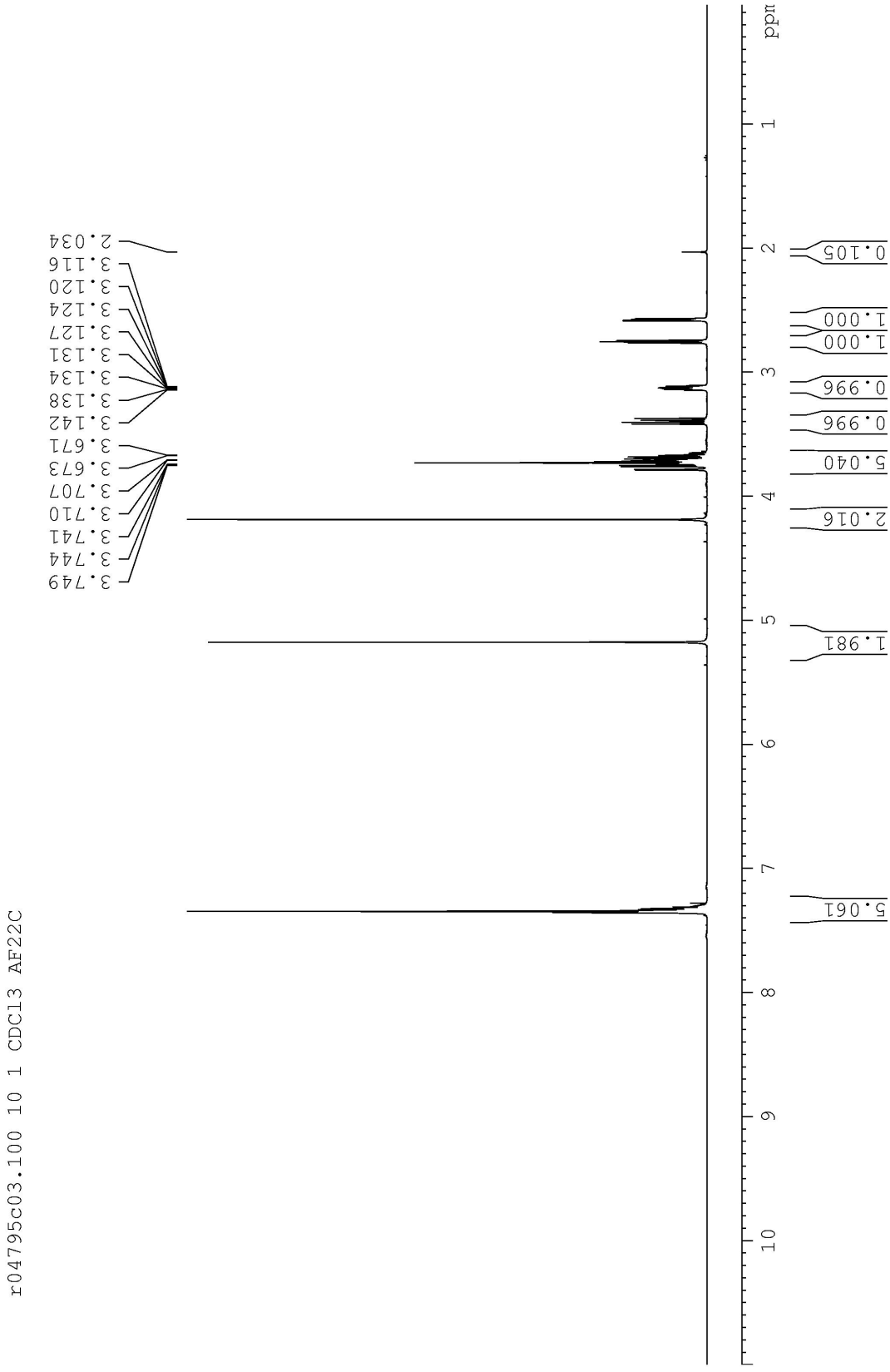
2.492
0.467
0.534
0.974
4.978

2-[2-(2-Oxiranylmethoxy)ethoxy]acetic acid phenylmethyl ester (3)

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1.000
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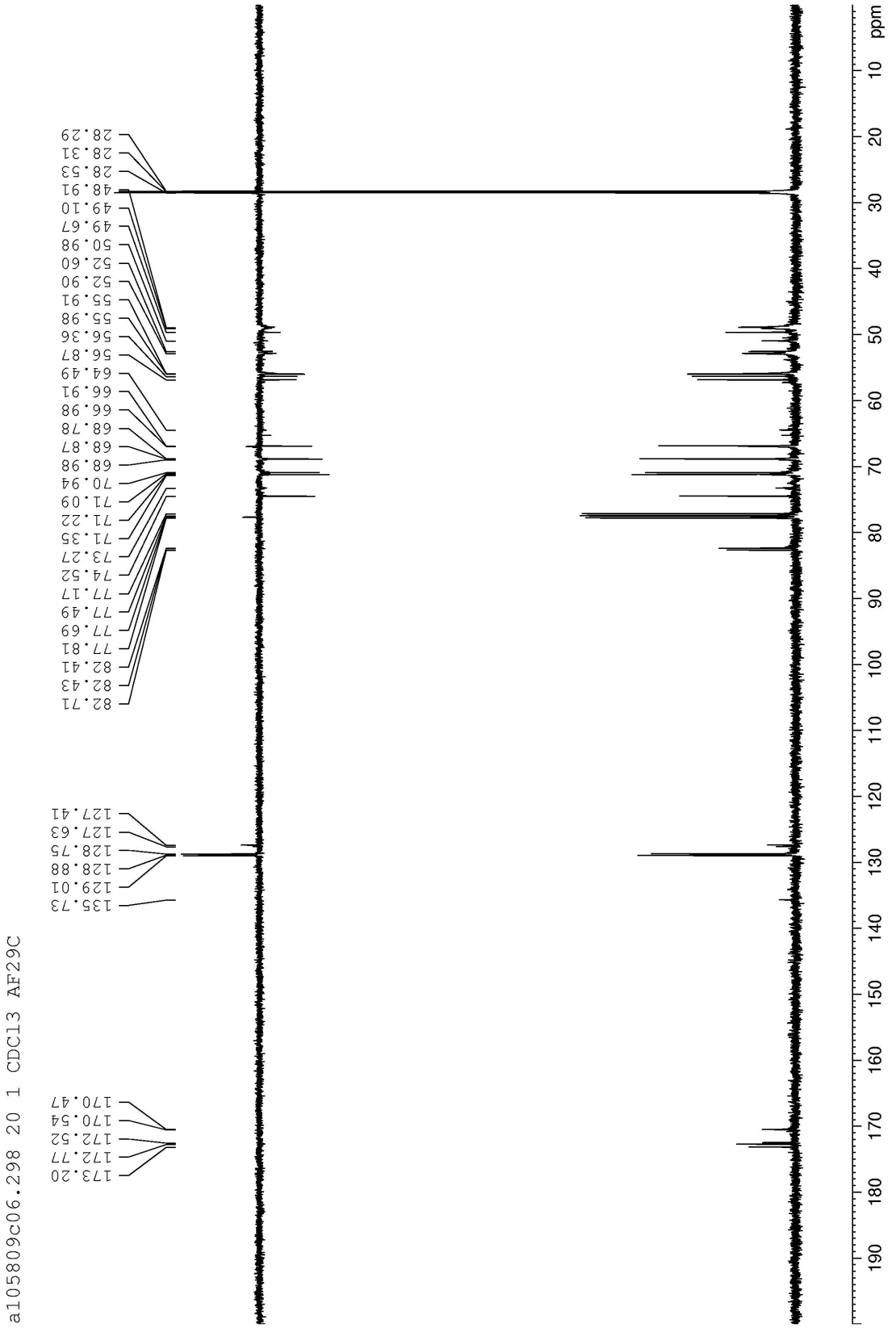
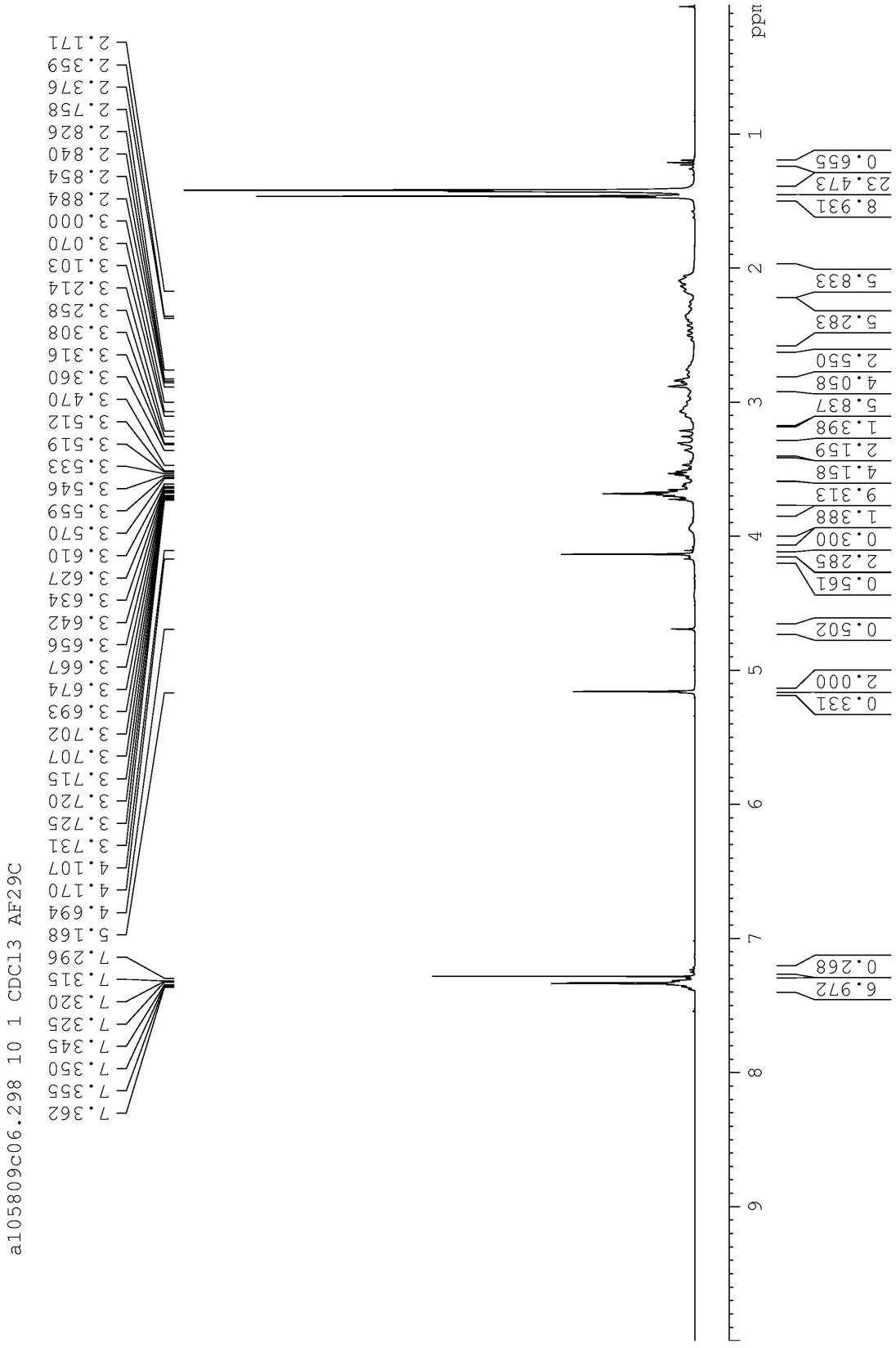
0.996
5.040
2.016
1.981
5.061
2.034
3.116
3.120
3.124
3.127
3.131
3.134
3.138
3.142
3.671
3.673
3.707
3.710
3.741
3.744
3.749

10-[2-Hydroxy-3-[2-[2-oxo-2-(phenylmethoxy)ethoxy]ethoxy]propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid 1,4,7-tris(1,1-dimethylethyl) ester (4)

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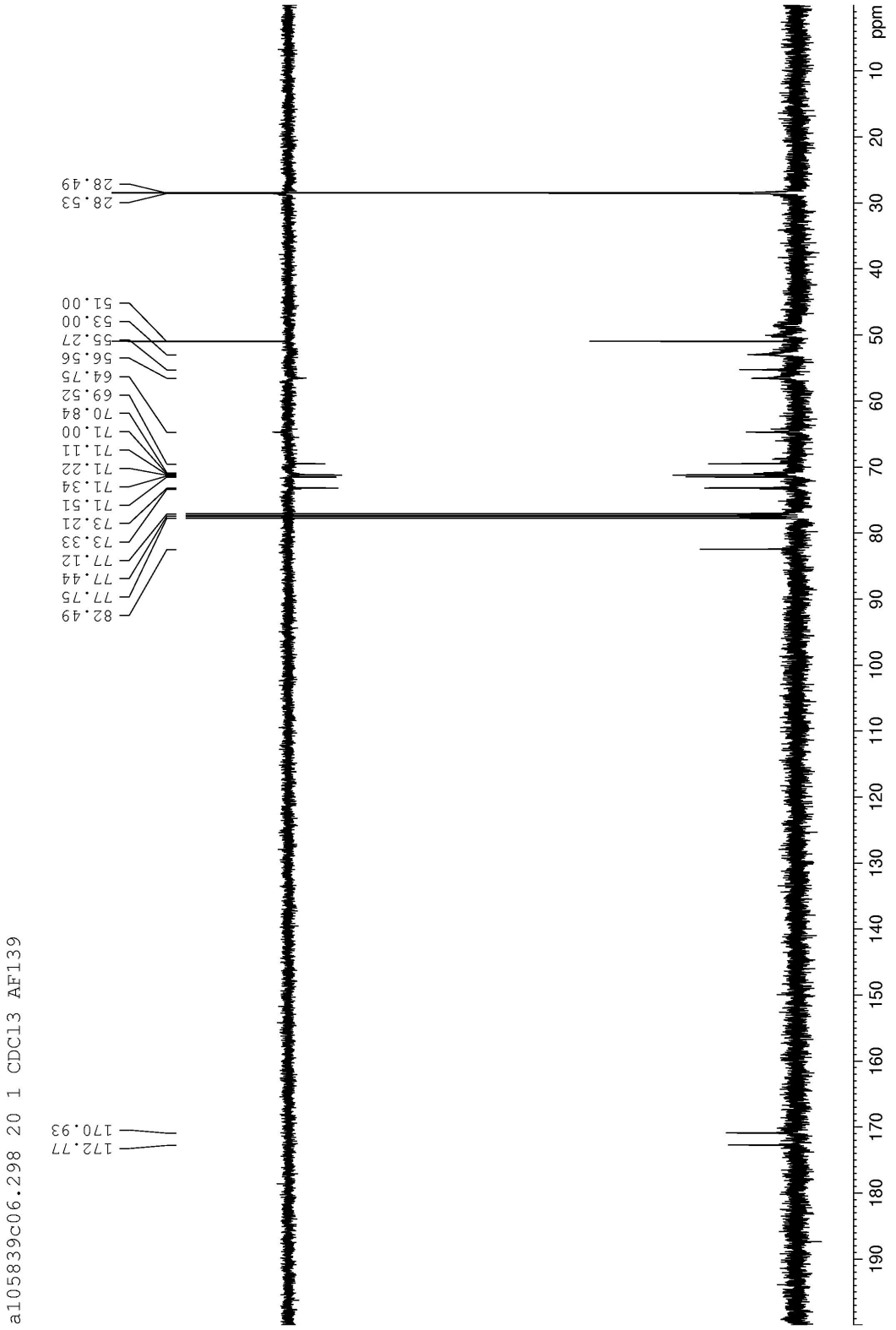
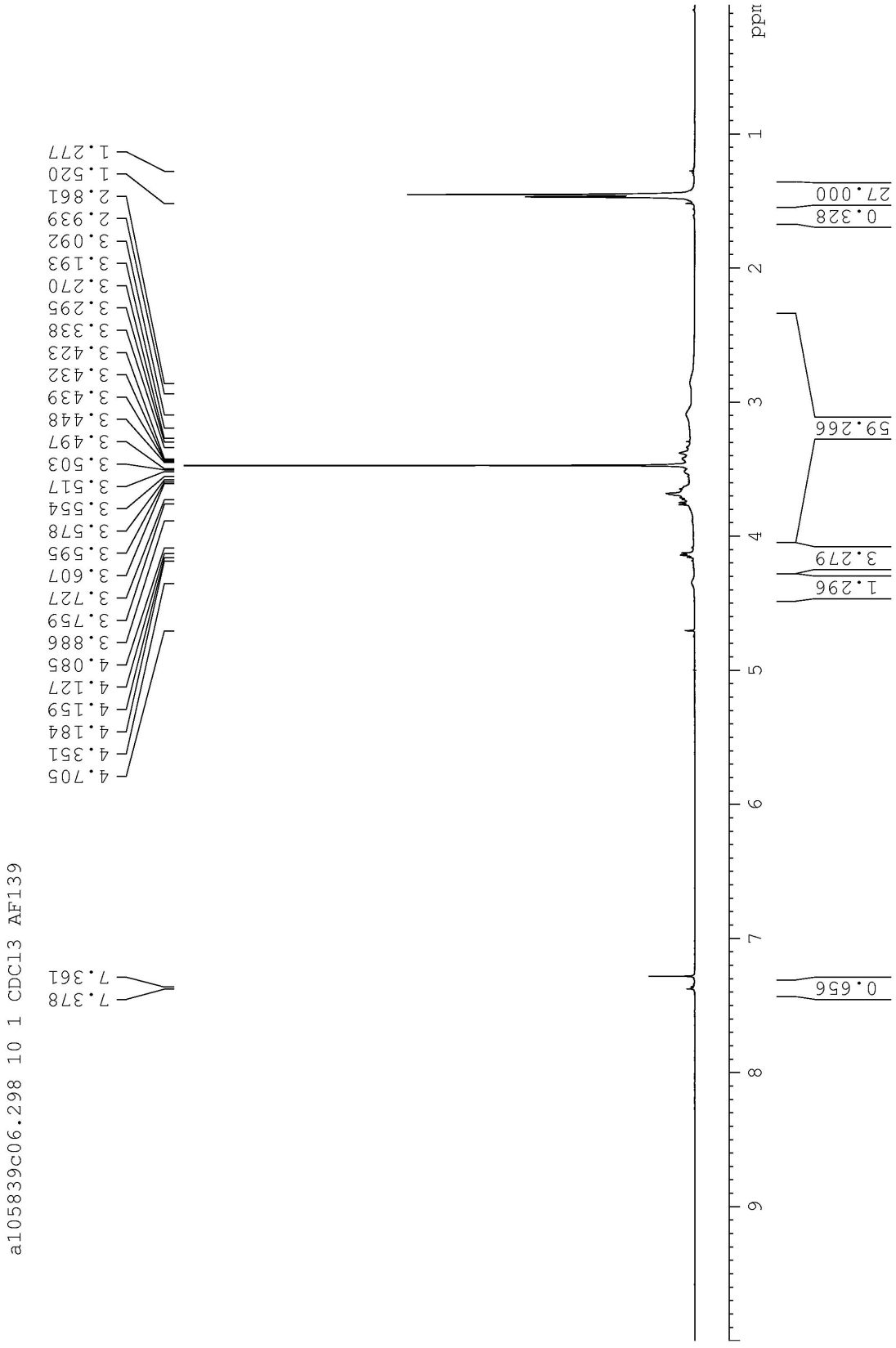
5.283
2.550
4.058
5.837
1.398
2.159
4.158
9.313
1.388
0.300
2.285
0.561
0.502
2.000
0.331
0.268
6.972

10-[3-[2-(Carboxymethoxy)ethoxy]-2-hydroxypropyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid 1,4,7-tris(1,1-dimethylethyl) ester (5)

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3.759
3.727
3.607
3.595
3.578
3.554
3.517
3.503
3.497
3.448
3.439
3.432
3.423
3.338
3.295
3.270
3.193
3.092
2.939
2.861
1.520
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ppm
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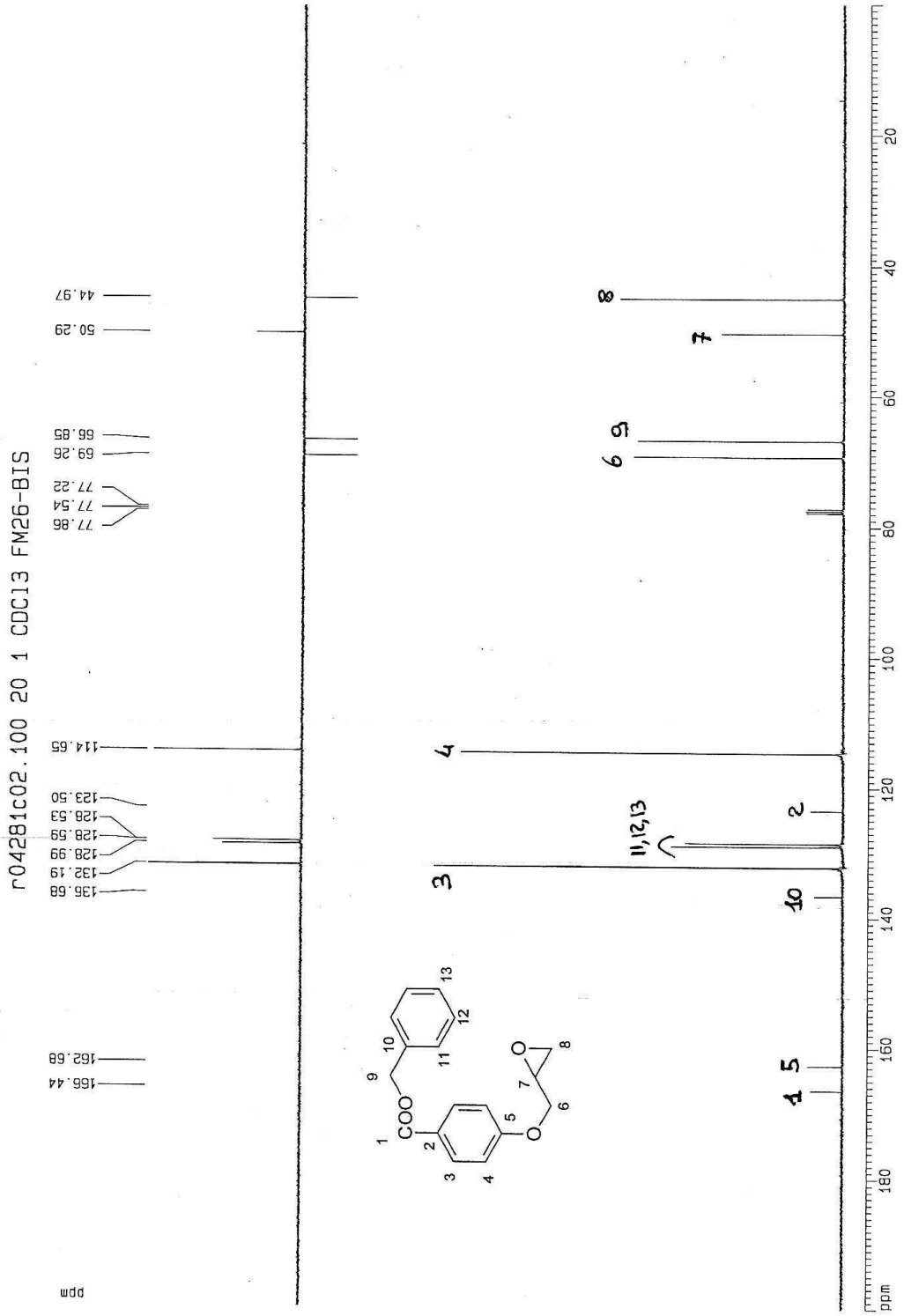
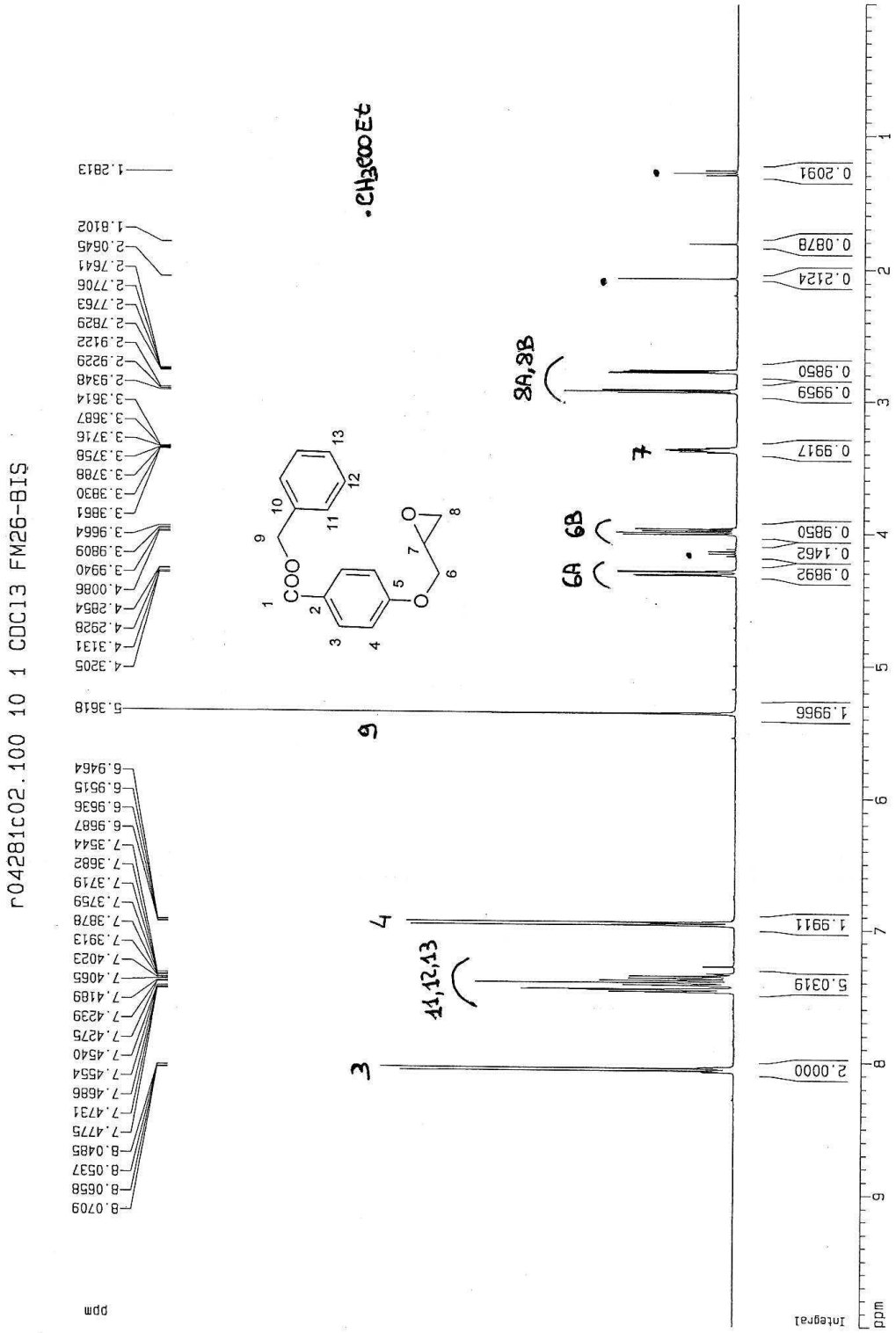
27.000

4-(Oxyranylmethoxy)benzoic acid phenylmethyl ester (6)

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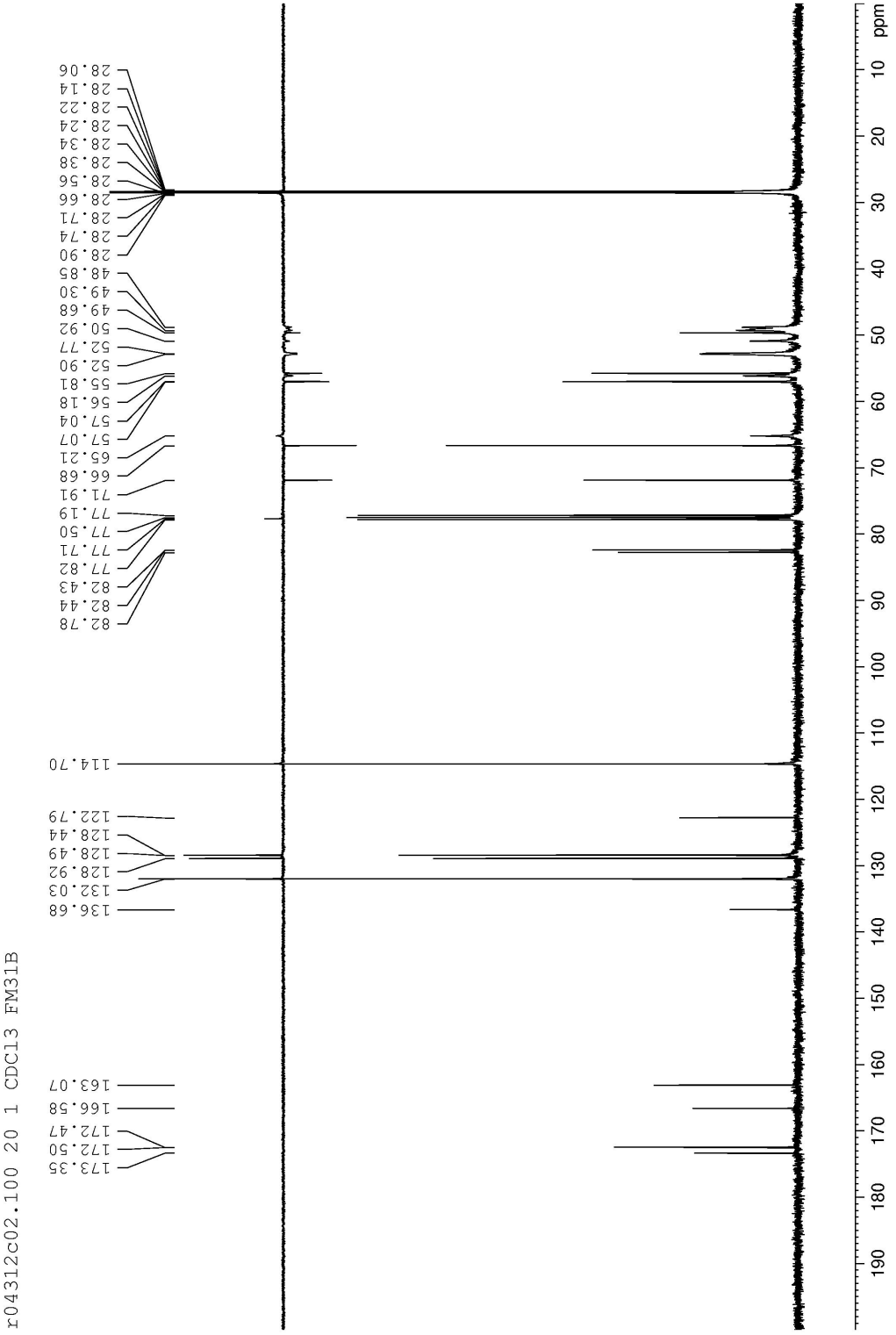
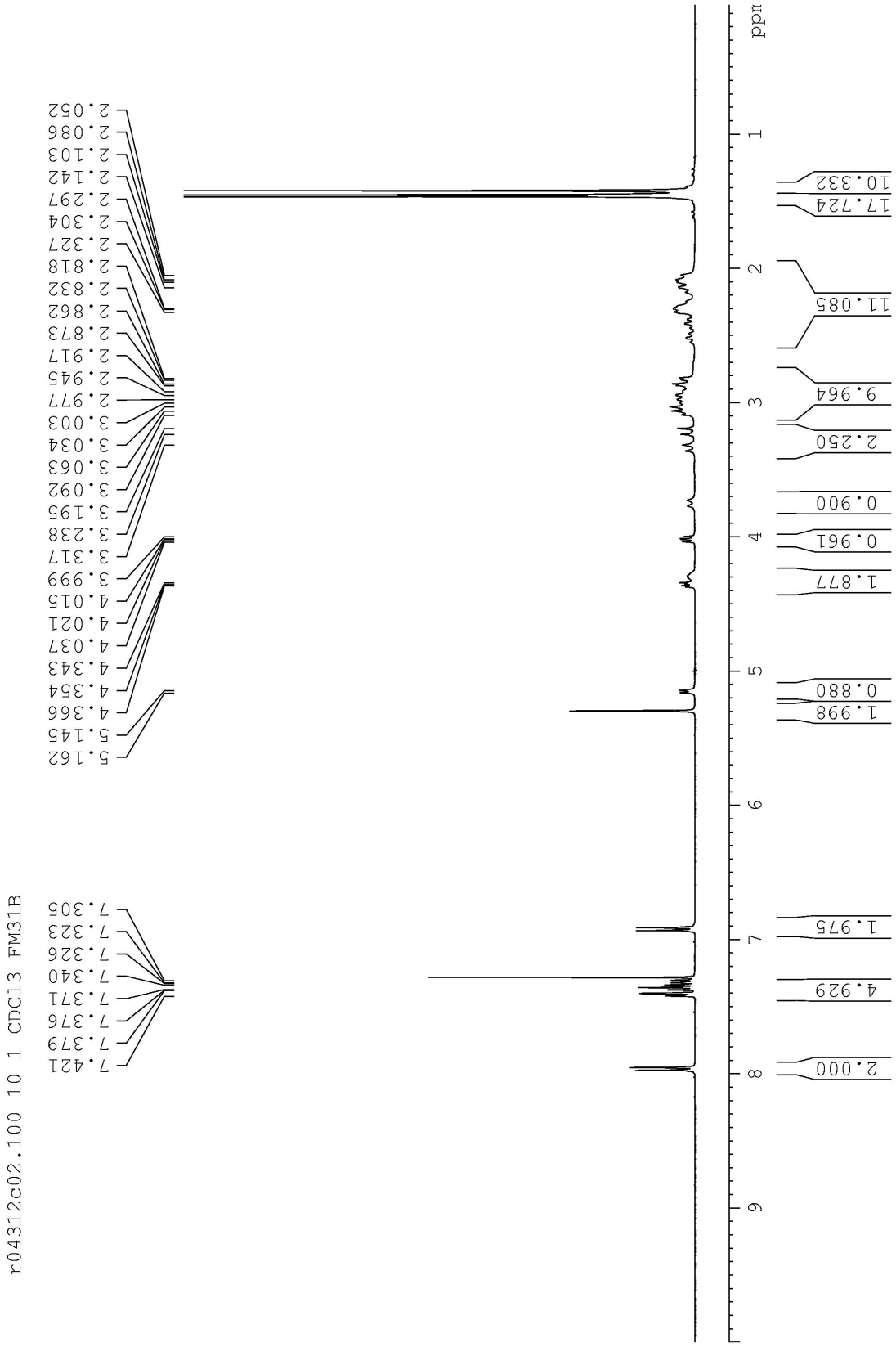
Integral
•CH3COOEt
COO
9
3
4
11,12,13
8A,8B
7
6A 6B

4-[2-Hydroxy-3-[4,7,10-tris[2-(1,1-dimethylethoxy)-2-oxoethyl]-1,4,7,10-tetrazacyclododec-1-yl]propoxy]benzoic acid phenylmethyl ester (7)

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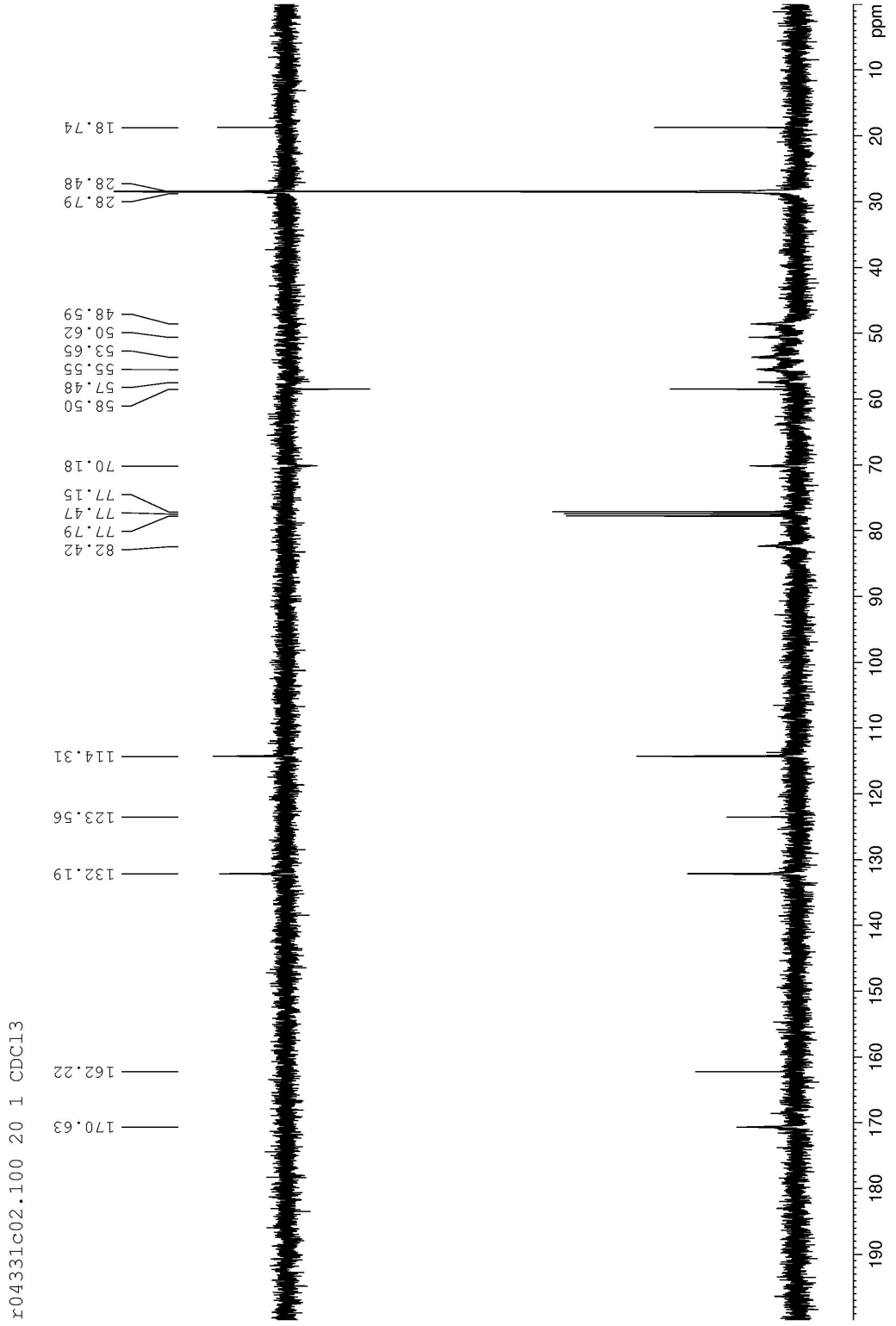
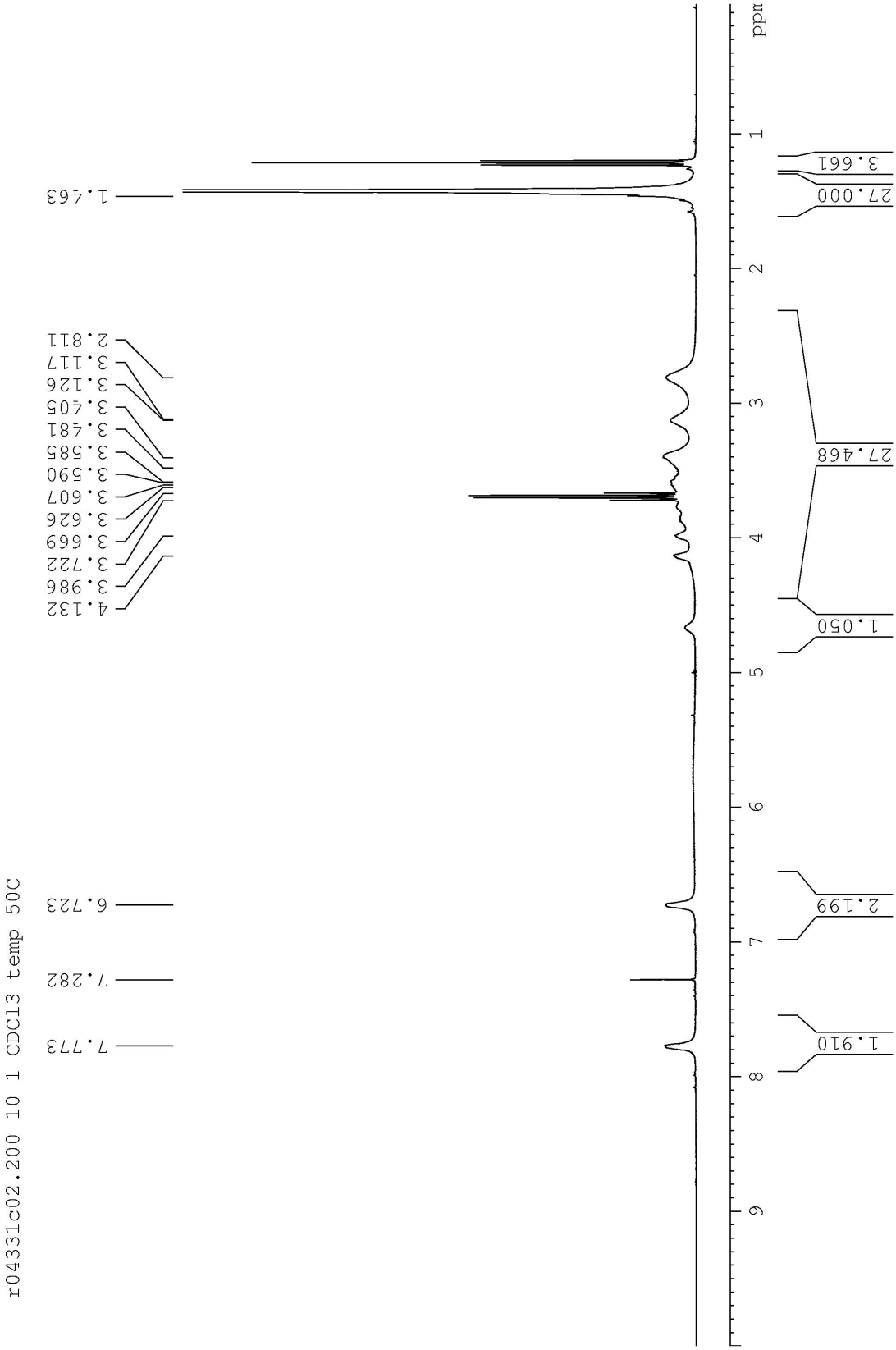
2.873
2.917
2.945
2.977
3.003
3.034
3.063
3.092
3.195
3.238
3.317
3.999
4.015
4.021
4.037
4.343
4.354
4.366
5.145
5.162
7.305
7.323
7.326
7.340
7.371
7.376
7.379
7.421
10.332
17.724
11.085
9.964
2.250
0.900
0.961
1.877
0.880
1.998
1.975
4.929
2.000

4-[2-Hydroxy-3-[4,7,10-tris[2-(1,1-dimethylethoxy)-2-oxoethyl]-1,4,7,10-tetrazacyclododec-1-yl]propoxy] benzoic acid (8)

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7.282
7.773
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