Supporting Information

Studies on Tridecaptin B_1, a New Tridecaptin Analogue with Activity Against Multidrug Resistant Gram-Negative Bacteria

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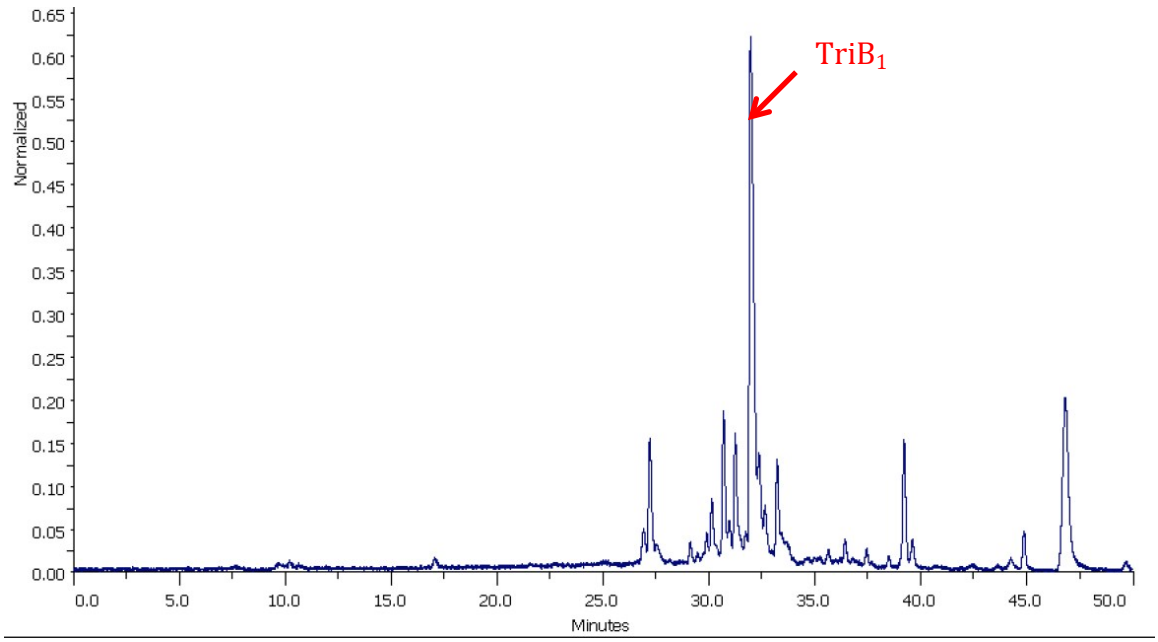
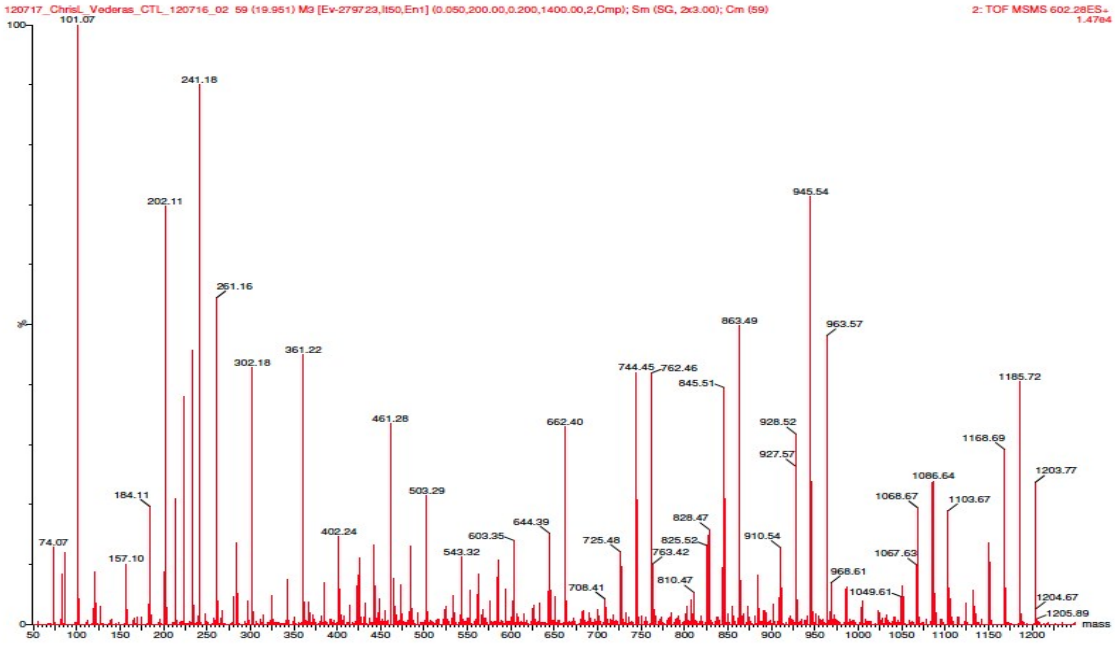
Supporting Information Contents:

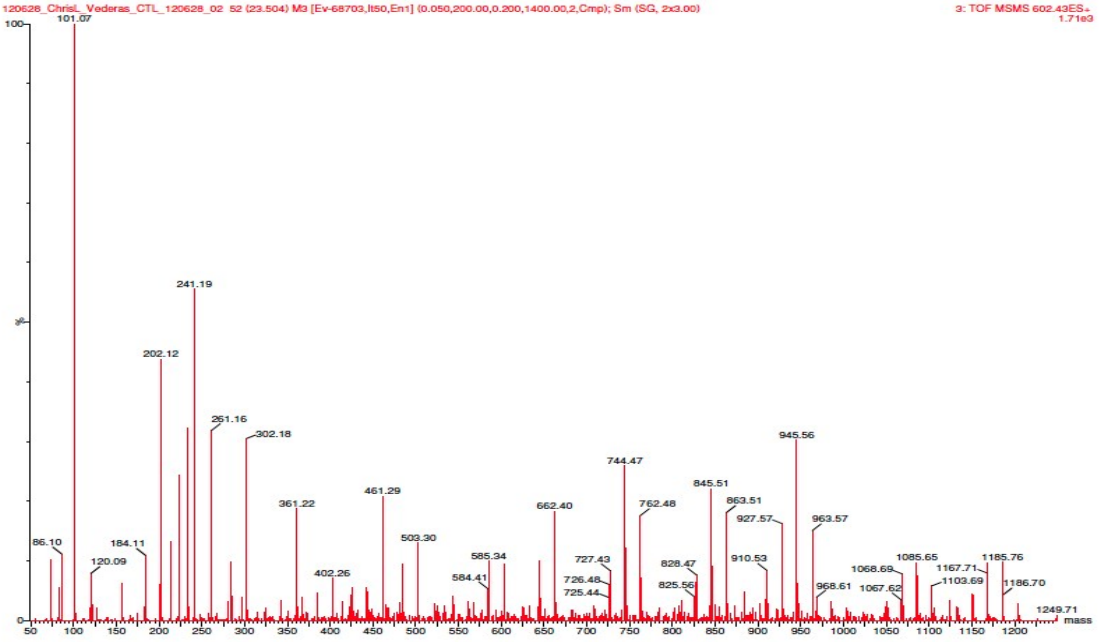
HPLC trace of crude tridecaptin B_1



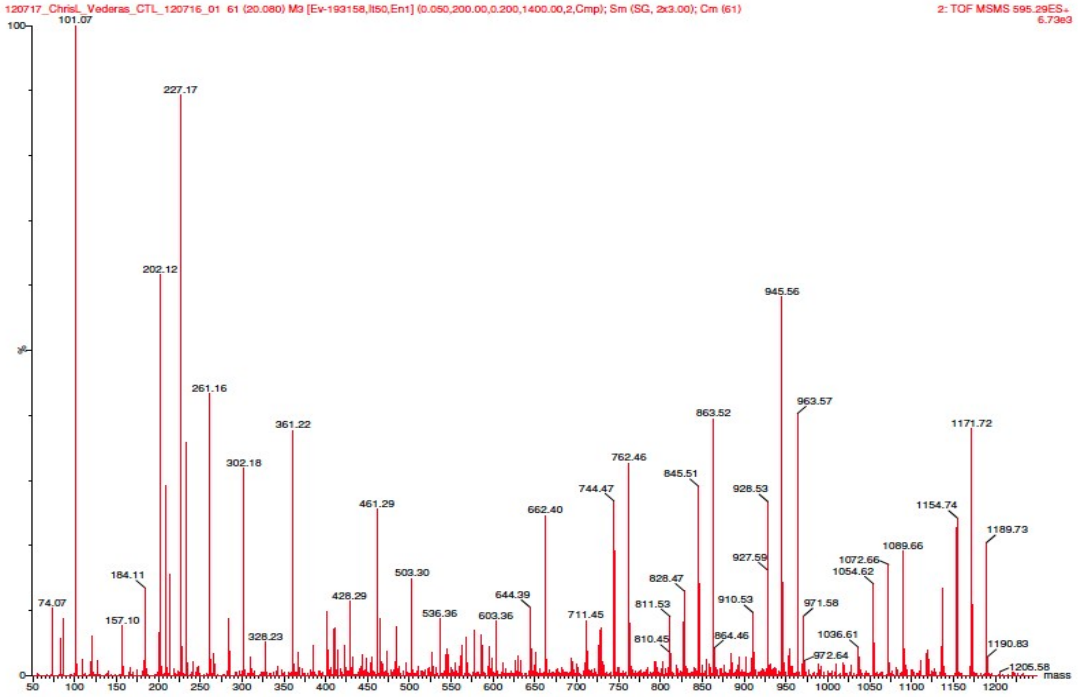
MS/MS spectrum of commercial polymyxin B_1



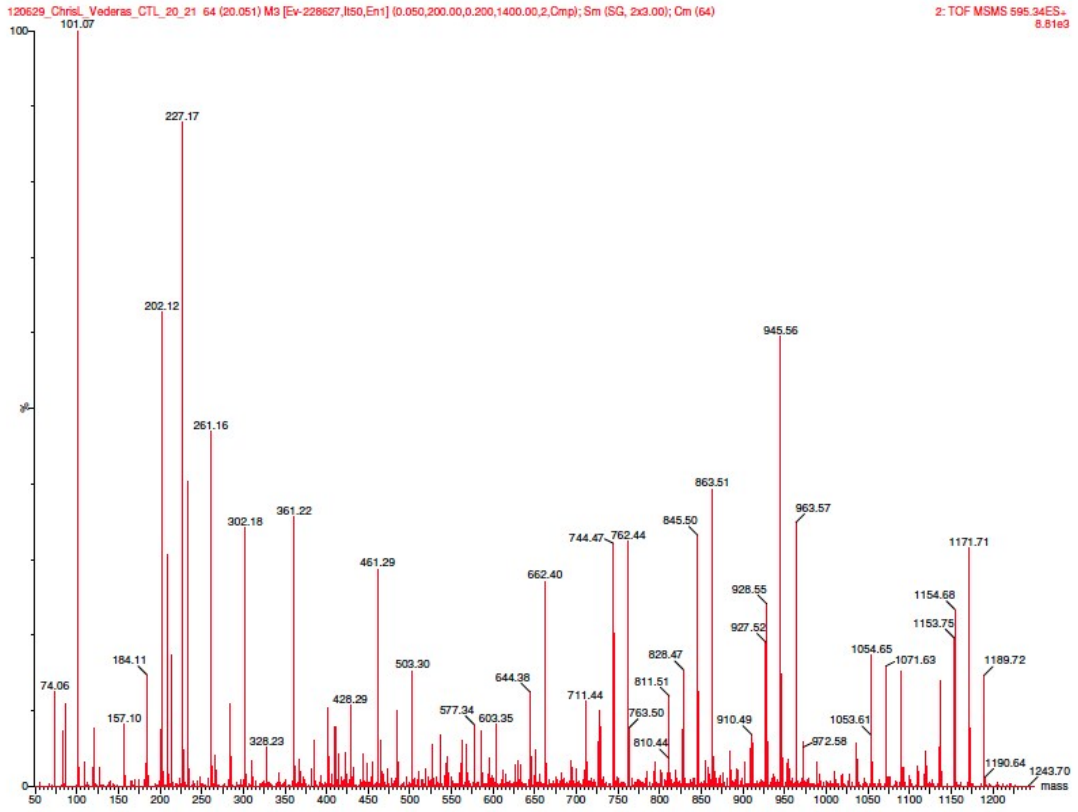
MS/MS spectrum of isolated polymyxin B_1



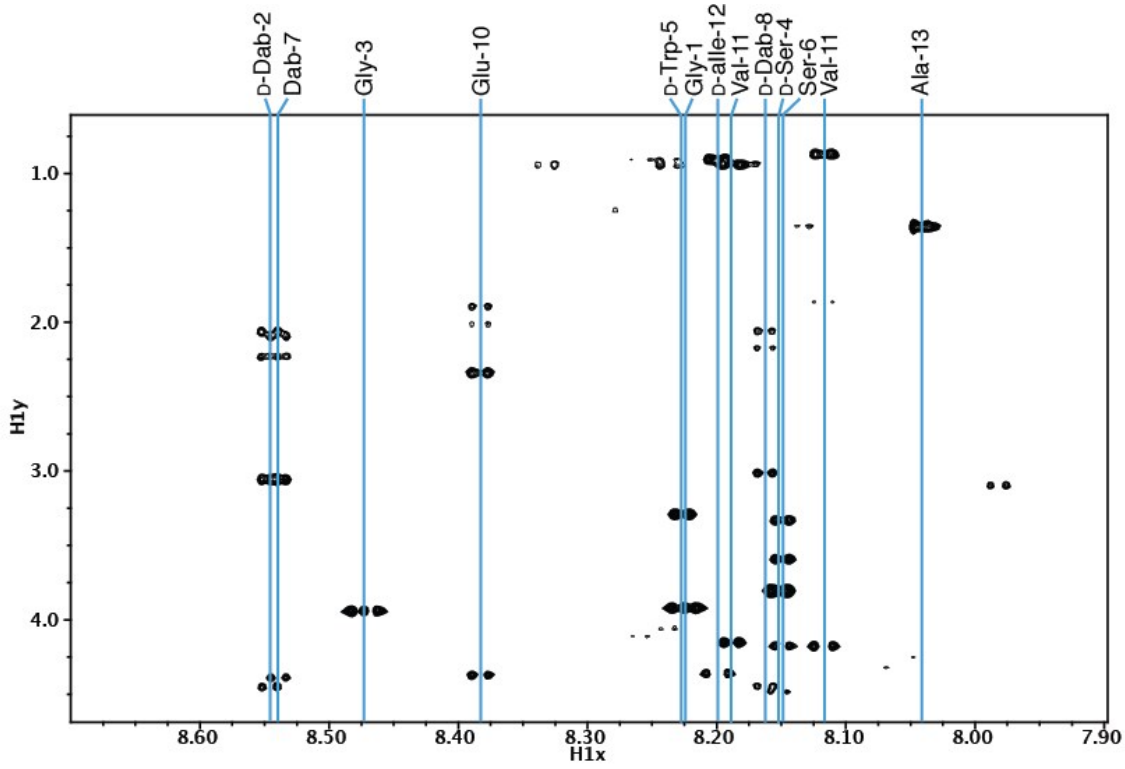
MS/MS spectrum of commercial polymyxin B_2



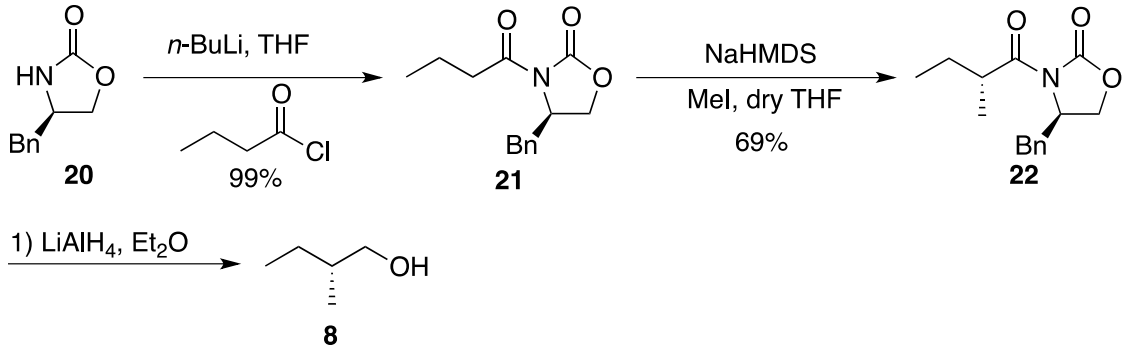
MS/MS spectrum of isolated polymyxin B_2



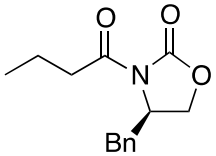
TOCSY spectrum of tridecaptin B_1



Synthesis of (2*R*)-Methylbutanol



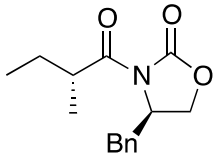
(*R*)-4-Benzyl-3-butyryloxazolidin-2-one (21)



21

(*R*)-4-benzyloxazolidin-2-one (**20**) (10.0 g, 56.4 mmol) was dissolved in dry THF (80 mL) and cooled to -78 °C under argon. 2.5 M *n*-BuLi (25.1 mL, 62.6 mmol) was added drop wise over 30 min and the reaction mixture stirred for a further 30 min. Butyryl chloride (7.1 mL, 81.3 mmol) was then added and the reaction stirred for 30 min at -78 °C and another 30 min at room temperature. The reaction was quenched by the addition of saturated NH_4Cl (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with 1 M NaOH (40 mL) and brine (40 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude oil was purified by flash column chromatography (SiO_2, 4:1 hexanes:EtOAc) to yield the product as a pale yellow oil (13.8 g, 99%). $[\alpha]_D^{25}$ = -56.4 (*c* = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast) 3029, 2965, 2934, 2876, 1783, 1701 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.34 (m, 2H, meta-ArH), 7.31-7.29 (m, 1H, para-ArH), 7.24-7.22 (m, 2H, ortho-ArH), 4.72-4.67 (m, 1H, NC<u>H</u>), 4.23-4.17 (m, 2H, C<u>H</u>$_2$Ph), 3.32 (dd, 1H, *J* = 13.4, 3.3 Hz, OC<u>H</u>H), 3.01-2.87 (m, 2H, NC(O)C<u>H</u>$_2$), 2.79 (dd, 1H, *J* = 13.4, 9.6 Hz, OCH<u>H</u>), 1.81-1.70 (m, 2H, C<u>H</u>$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.4 Hz, CH$_2$C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.2, 153.5, 135.4, 129.4, 129.0, 127.3, 66.2, 55.1, 38.0, 37.4, 17.7, 13.7; HRMS (ES) Calcd for $C_{14}H_{17}NO_3Na$ $[M+Na]^+$ 270.1101, found 270.1098.

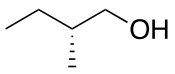
(*R*)-4-Benzyl-3-((*R*)-2-methylbutanoyl)oxazolidin-2-one (22)



22

Oxazolidinone **21** was dissolved in THF (80 mL) and cooled to -78 °C under argon. 1 M NaHMDS (67.0 mL, 67.0 mmol) was added via syringe over 5 min and the reaction stirred for 30 min. MeI (8.60 mL, 138 mmol) was then added and the reaction stirred for a further 3 h. Brine (50 mL) and CH_2Cl_2 (80 mL) were added and the reaction warmed to ambient temperature with stirring. The phases were separated and the aqueous layer was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column chromatography (SiO_2, 9:1 hexanes:EtOAc) to yield the product as a colourless oil (10.1 g, 69%). $[\alpha]_D^{25}$ = -69.1 (*c* = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast) 3030, 2969, 2934, 2877, 1781, 1698 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.34 (m, 2H, meta-ArH), 7.31-7.28 (m, 1H, para-ArH), 7.24-7.23 (m, 2H, ortho-ArH), 4.73-4.68 (m, 1H, NC<u>H</u>), 4.24-4.17 (m, 2H, C<u>H</u>$_2$Ph), 3.70-3.63 (m, 1H, NC(O)C<u>H</u>), 3.29 (dd, 1H, *J* = 13.4, 3.2 Hz, OC<u>H</u>H), 2.79 (dd, 1H, *J* = 13.4, 9.6 Hz, OCH<u>H</u>), 1.80 (ddt, 2H, *J* = 14.1, 7.1 Hz, CH_2C<u>H</u>H), 1.50 (ddt, 2H, *J* = 14.0, 7.0 Hz, CH_2C<u>H</u>H), 1.25 (d, 3H, *J* = 6.9 Hz, CHC<u>H</u>$_3$), 0.95 (t, 3H, *J* = 7.4 Hz, CH_2C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.2.2, 153.1, 135.4, 129.5, 129.0, 127.3, 66.0, 55.4, 39.2, 37.9, 26.4, 16.9, 11.7; HRMS (ES) Calcd for $C_{15}H_{19}NO_3Na$ $[M+Na]^+$ 284.1257, found 284.1259.

(2*R*)-Methylbutanol (6)



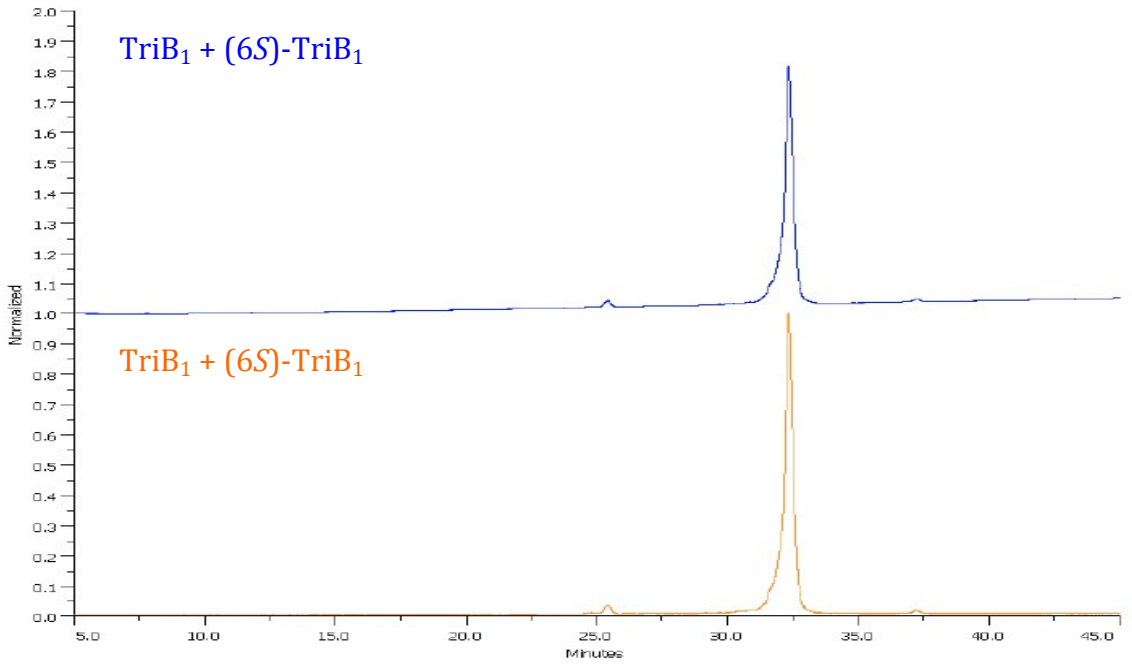
6

Oxazolidin-2-one **22** (10.1 g, 38.6 mmol) was dissolved in Et_2O (70 mL) and cooled to -20 °C. $LiAlH_4$ (4.4 g, 115.9 mmol) was added in parts and the reaction was stirred at 0 °C for 2 h. H_2O (10 mL) was added and the reaction mixture stirred at ambient temperature for 15 min. The mixture was then filtered through celite and the celite washed with Et_2O

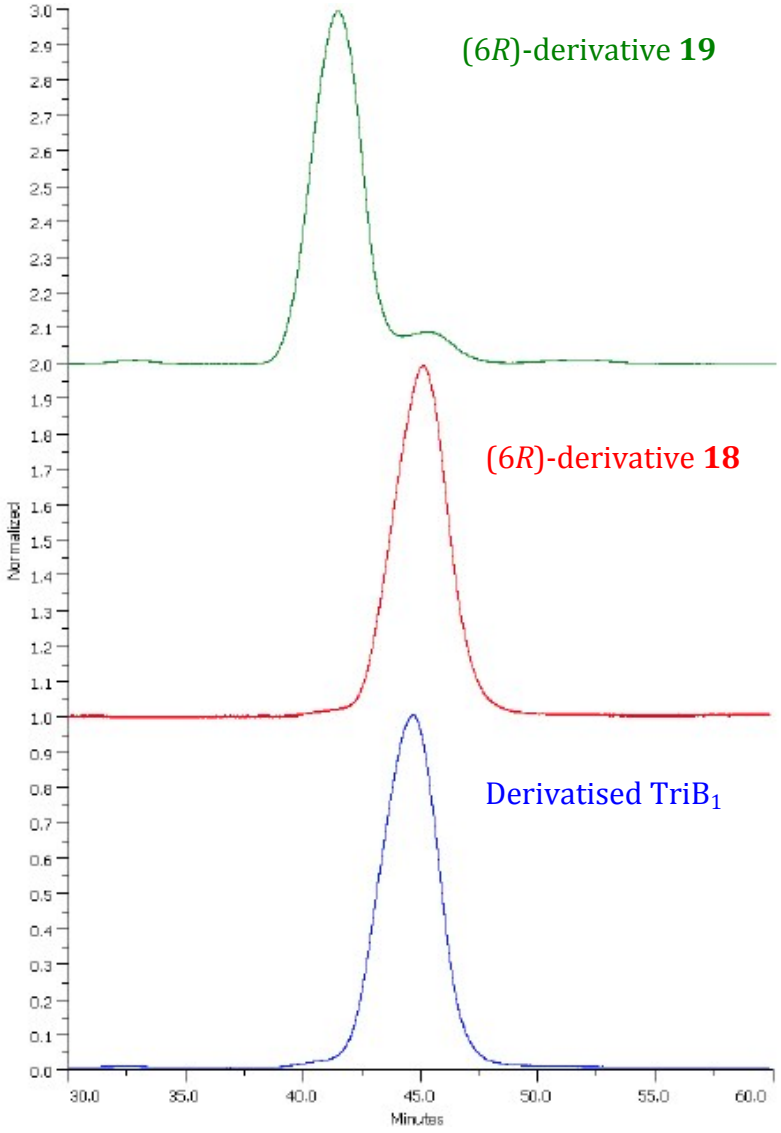
(4 x 100 mL). The combined organic extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated by rotary evaporator at 0 °C to approximately 10 mL. This crude alcohol was used in the next step without purification.

HPLC analysis of tridecaptin B_1 isomers

1 mM stock solutions of the natural peptide, (6*R*)-TriB and (6*S*)-TriB were prepared in MilliQ H_2O (1 mL). An aliquot of the natural peptide solution (50 µL) and the (6*S*)- TriB solution (50 µL) were mixed and analyzed by C_{18}-RP-HPLC: Vydac C_{18} column, flow rate 1 mL/min, detected at 220 nm. Gradient: starting from 20% MeCN (0.1% TFA) and 80% water (0.1% TFA) for 5 min, ramping up to 55% MeCN over 30 min, then ramping up to 95% MeCN over 3 min, staying at 95% MeCN for 3 min, ramping down to 20% MeCN over 2 min, then staying at 20% MeCN for 5 min. This procedure was also performed for a mixture of the natural peptide and (6*R*)-TriB. Both co-injections are shown below.



HPLC analysis of derivatised tridecaptin B_1 and synthetic standards



Synergy assay protocol

Muller Hinton (MH) broth (50 µL) was added to each well of a single row on a 96-well plate. 50 µL of a 100 µg/mL solution of rifampicin in MH broth was added to the first well and a serial dilution made across the row. A 25 µg/mL solution of H-TriB_1 in MH broth (50 µL) was added to all wells in the row G. Each well was inoculated with 5 µL of a suspension of *E. coli* ATCC 25922 cells (see CLSI guidelines) so that the final concentration in each well was 5 x 10^5 CFU/mL. The MIC of rifampicin without any

peptide was also determined for comparison. The MICs were taken as the lowest concentration in which no visible growth was observed.

Antibiotic	MIC (µg/mL)	Activity Increase
Rifampicin	6.25	N/A
H-TriB$_1$	>100	N/A
Rifampicin + 12.5 µg/mL H-TriB$_1$	0.0977	64-fold

* N/A = Not Applicable

Amino acids conferring specificity to TrbD and TrbE adenylation domains.
Specificity determinants and closest matches identified using NRPSPredictor.

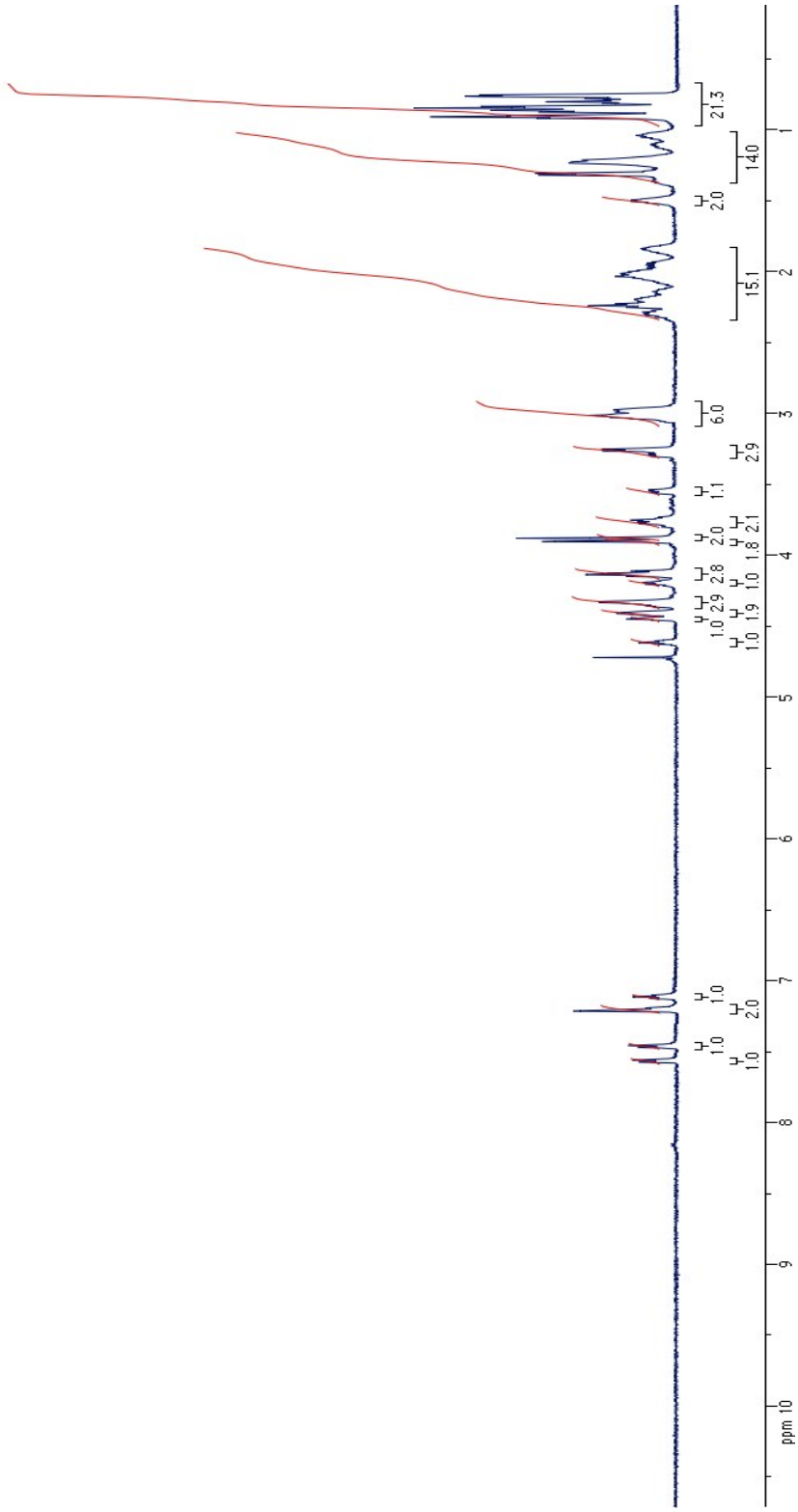
	Position of Residue within the Adenylation Domain										Amino Acid	
	235	236	239	278	299	301	322	330	331	517	Closest Match	Actual
1	D	A	F	W	L	G	G	T	F	K	Val	Gly
2	D	V	G	E	I	S	S	I	D	K	Orn	Dab
3	D	I	L	Q	M	G	M	V	W	K	Gly	Gly
4	D	V	W	H	F	S	L	V	D	K	Ser	Ser
5	D	A	W	A	F	A	G	V	A	K	Phe	Trp
6	D	V	W	H	F	S	L	V	D	K	Ser	Ser
7	D	V	G	E	I	S	S	I	D	K	Orn	Dab
8	D	V	G	E	I	S	S	I	D	K	Orn	Dab
9	D	A	W	A	F	A	G	V	A	K	Phe	Ile
10	D	A	K	D	L	G	V	V	D	K	Glu	Glu
11	D	A	F	W	L	G	G	T	F	K	Val	Val
12	D	A	F	F	L	G	I	T	F	K	Ile	Ile
13	D	V	F	W	L	G	G	T	F	K	Val	Ala

Comparison of adenylation domain active site signatures from tridecaptin NRPSs. Sequence alignment of A-domains 1 and 9 from TrbD (*P. polymyxa* NRRL B-30507) with corresponding domains from tridecaptin biosynthetic proteins (TriD) from *P. polymyxa* NRRL B-30509 and *P. terrae* NRRL B-30644. Conserved residues indicated with an asterisk, conservative substitutions with a colon, and residues with weakly similar properties with a period. Residues identified using NRPSPredictor.[1]

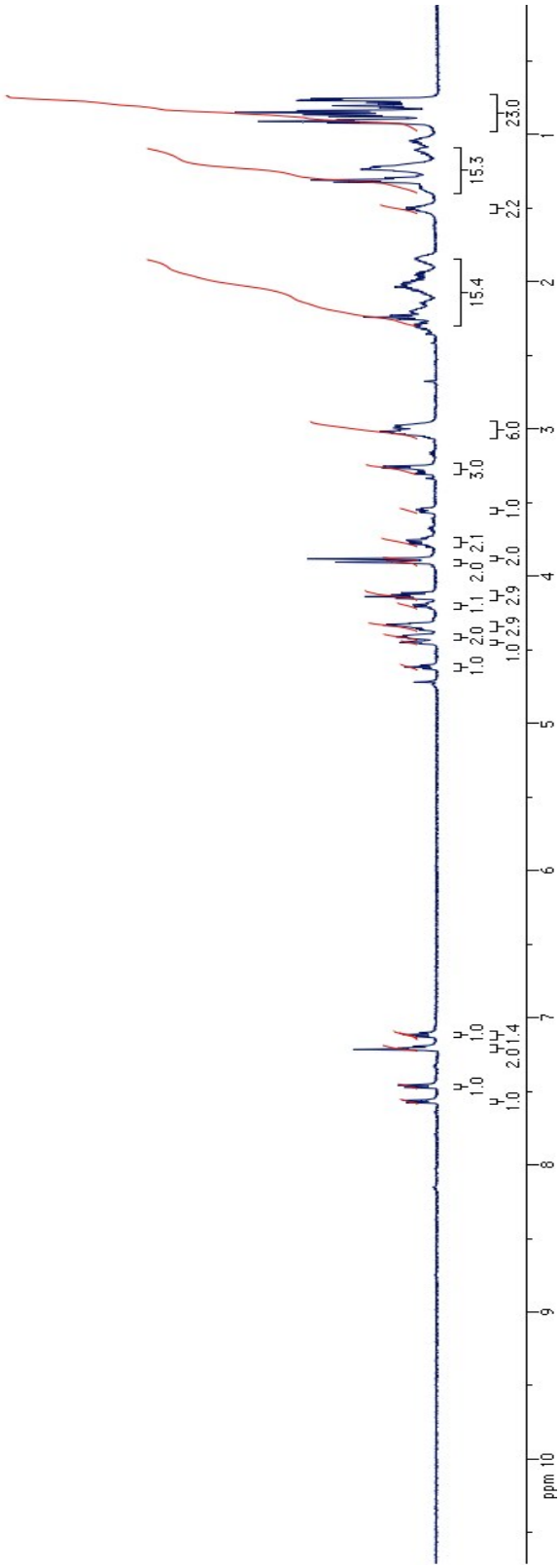
A-domain 1		
Specificity	**Strain**	**34 Residue Signature**
Gly	30507	LSTGFDASTFEGWLLVGGDINGYGPTENTTFSTT
Val	30509	LNTGFDASTFEGWLLVGGDINGYGPTENTTFSTT
Val	30644	LNTGFDASTFEGWLLVGGDINGYGPTENTTFSTT
	Alignment	*.*********************************
A-domain 9		
Specificity	**Strain**	**34 Residue Signature**
Ile	30507	LYFAFDAACWEQALFTAGSYNGYGPTENSVATSI
Phe	30509	LYFAFDAACWEQALVTAGSYNGYGPTENSVATSI
Phe	30644	LYFAFDAACWEQTLFTAGSYNGYGPTENSVATSV
	Alignment	***********:*.******************:

NMR Spectra

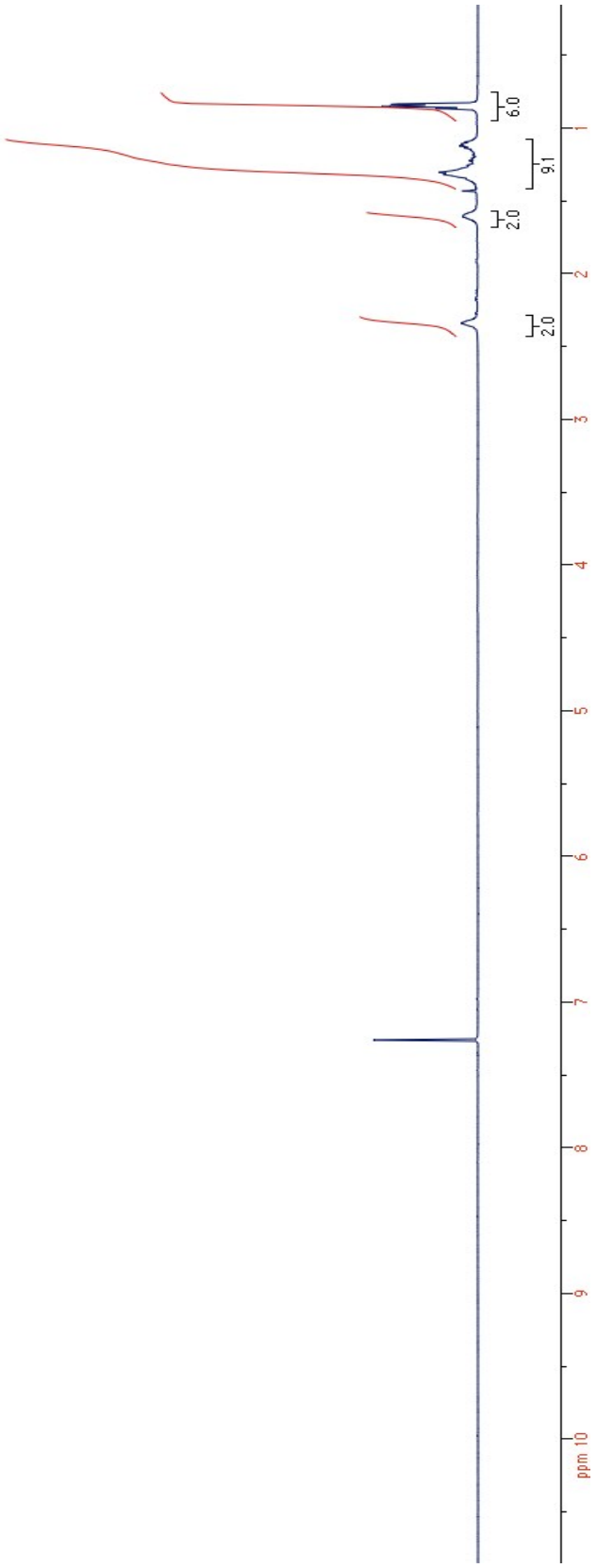
(6*S*)-TriB$_1$ (1)

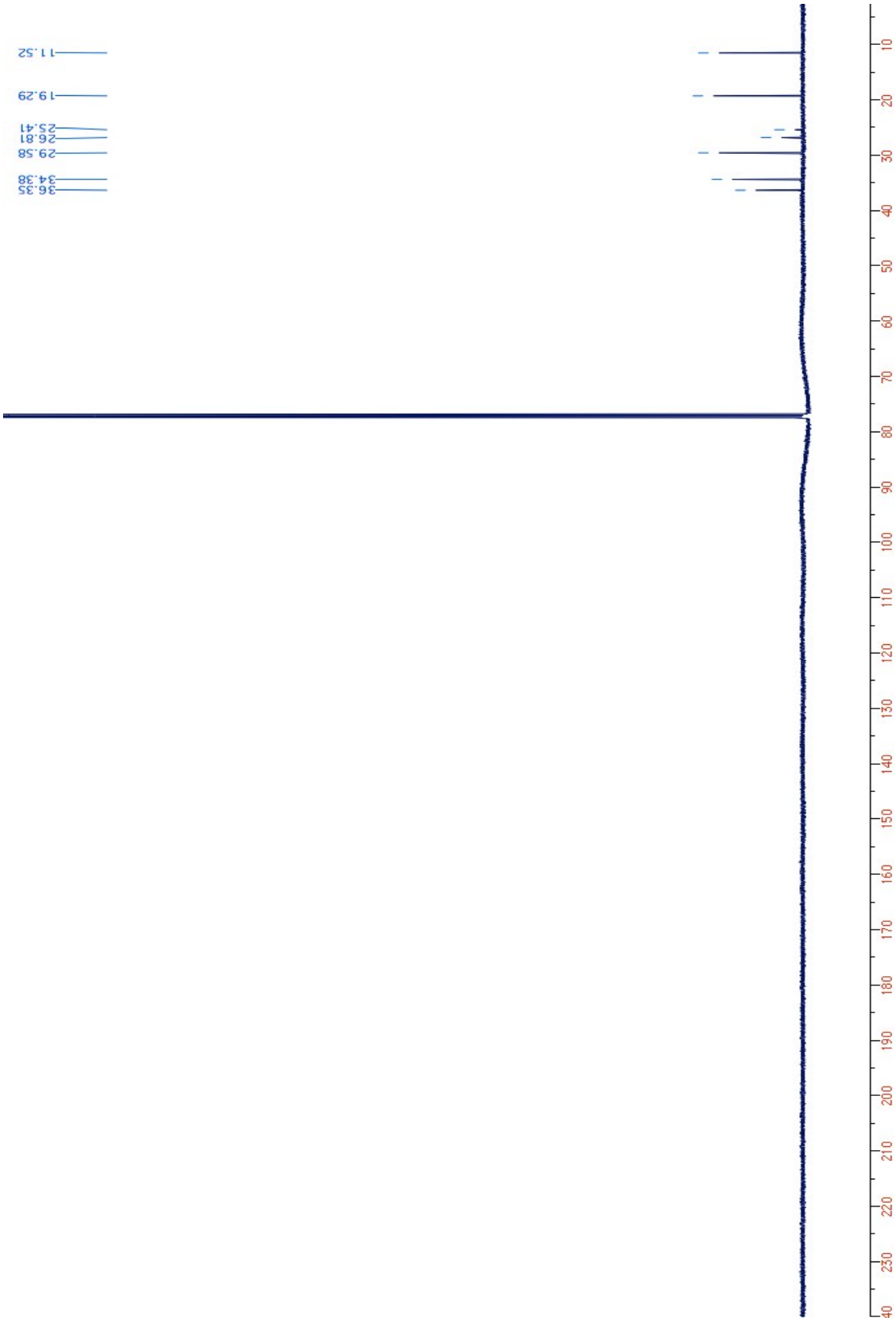


(6*R*)-TriB$_1$ (2)

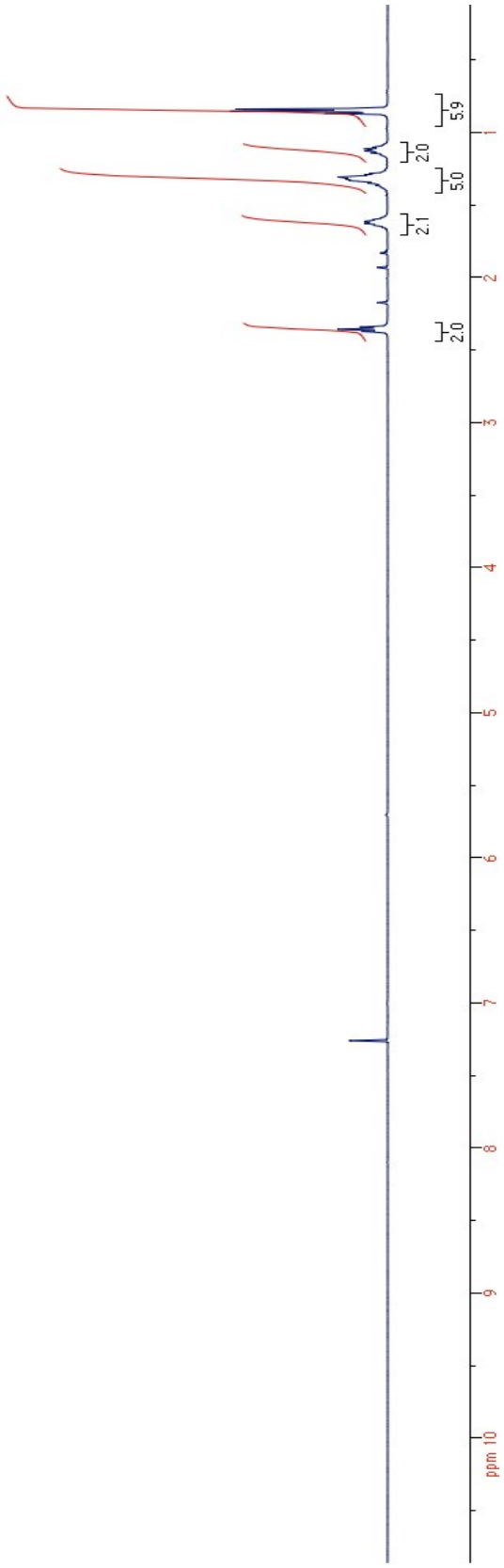

23.0
15.3
15.4
6.0
3.0
1.0
2.1
2.0
2.9
1.1
2.0
1.0
2.9
1.0
1.0
1.0
2.0
1.4
1.0
2.2
1
2
3
4
5
6
7
8
9
ppm 10

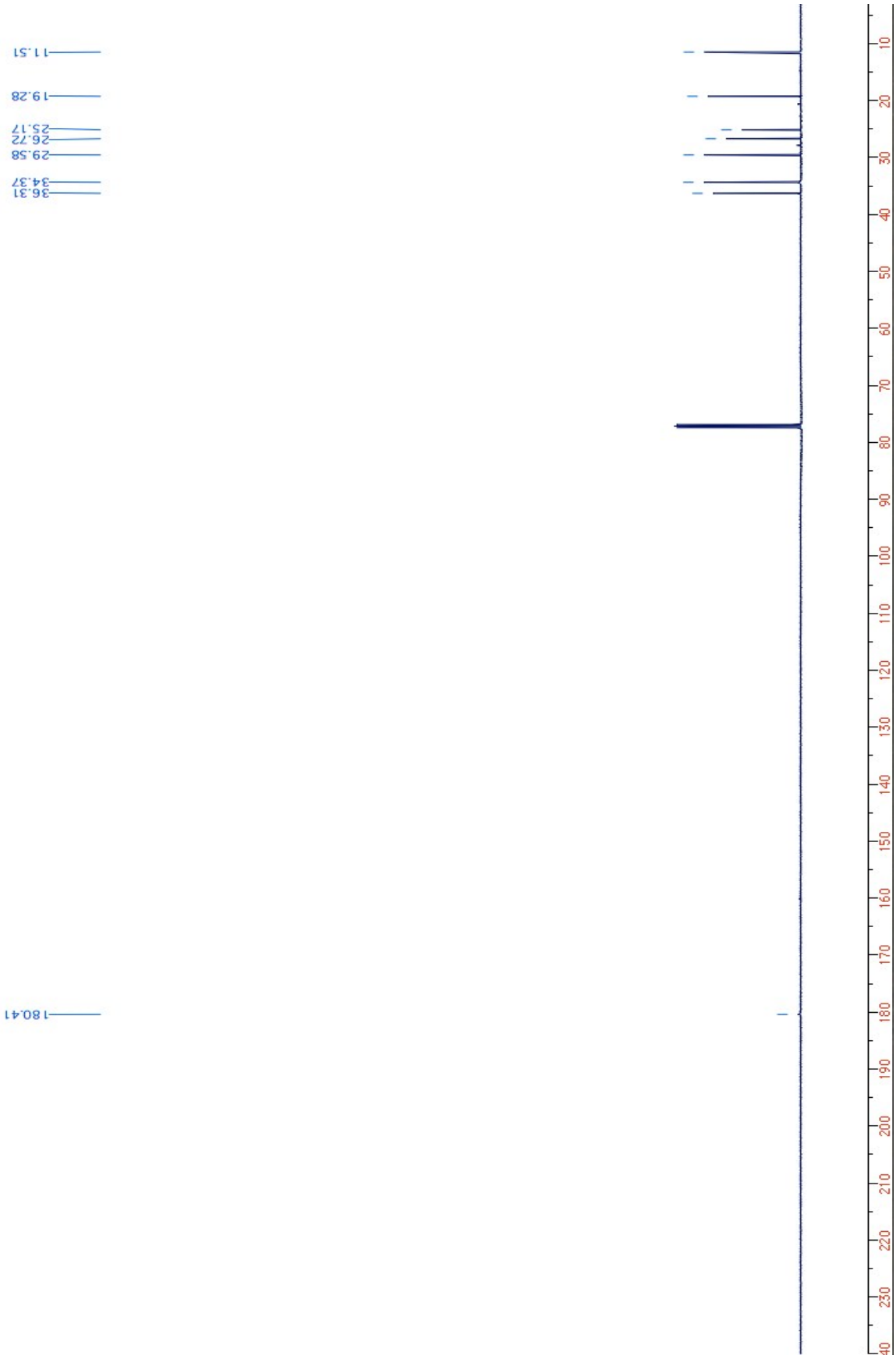
(6*S*)-methyloctanoic acid (3)



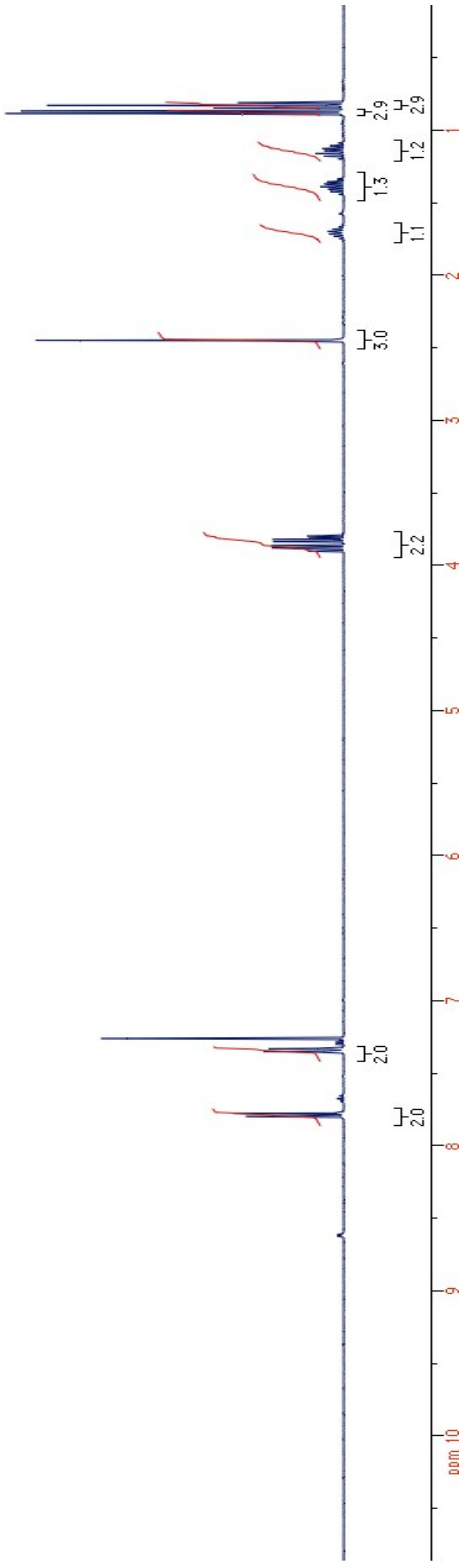

11.52
19.29
25.41
26.81
29.58
34.38
36.35

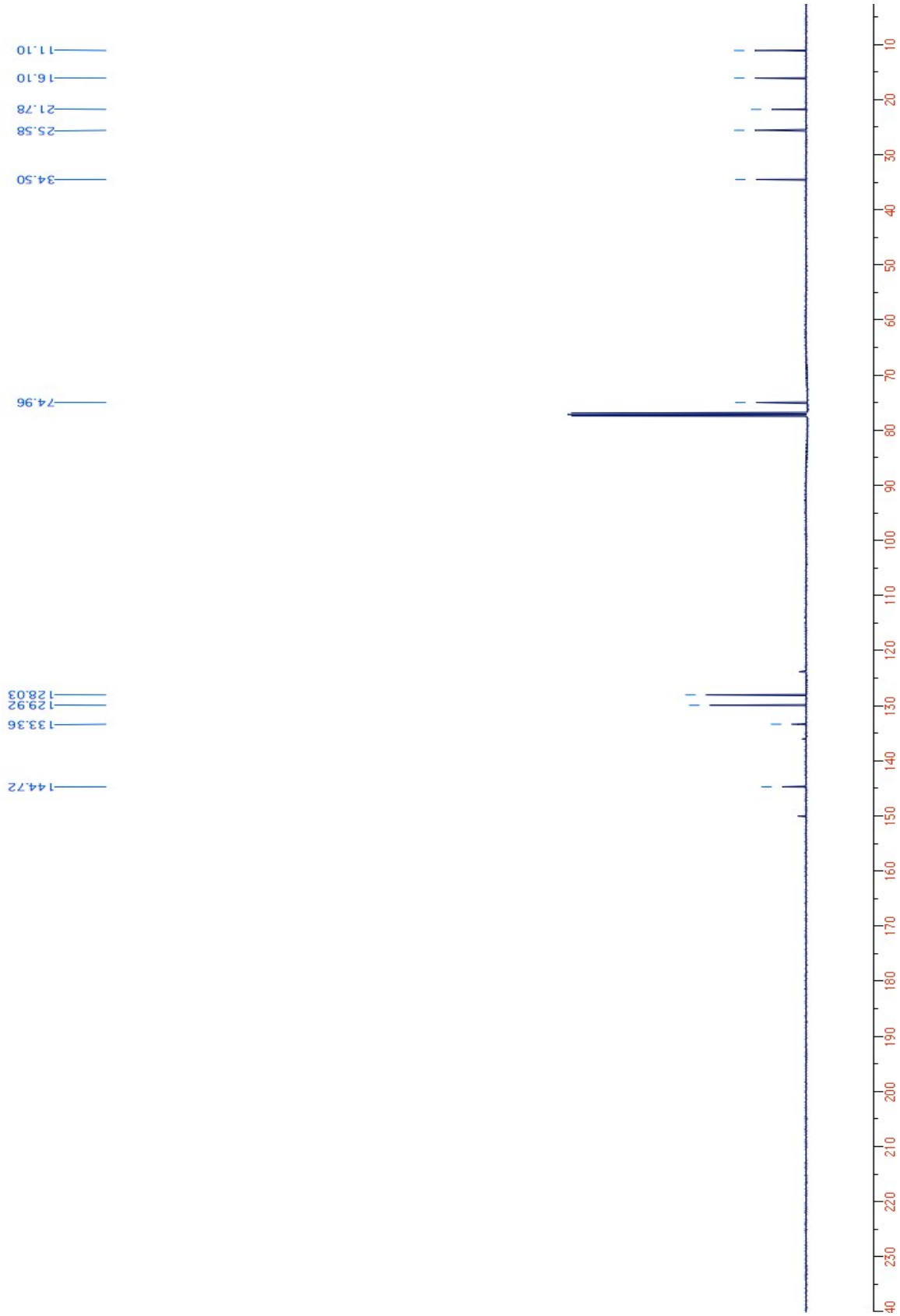
(6*R*)-methyloctanoic acid (4)



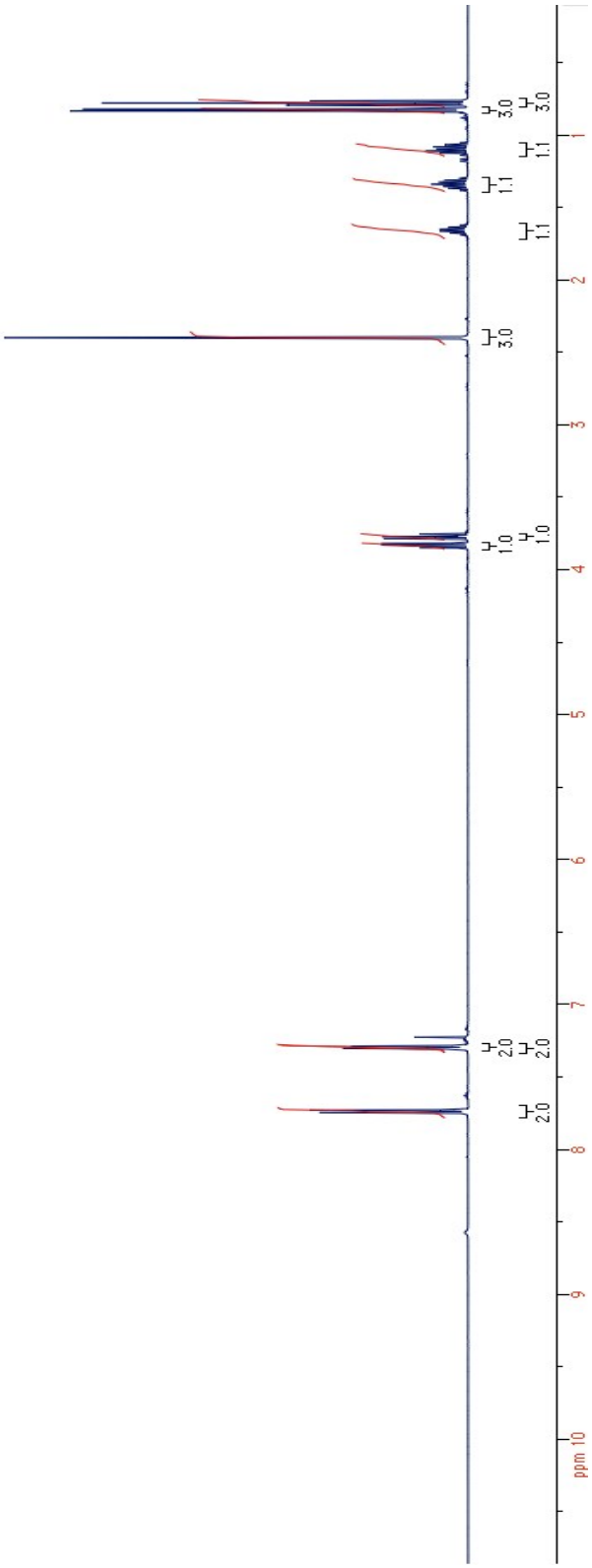

11.51
19.28
25.17
26.72
29.58
34.37
36.31
180.41

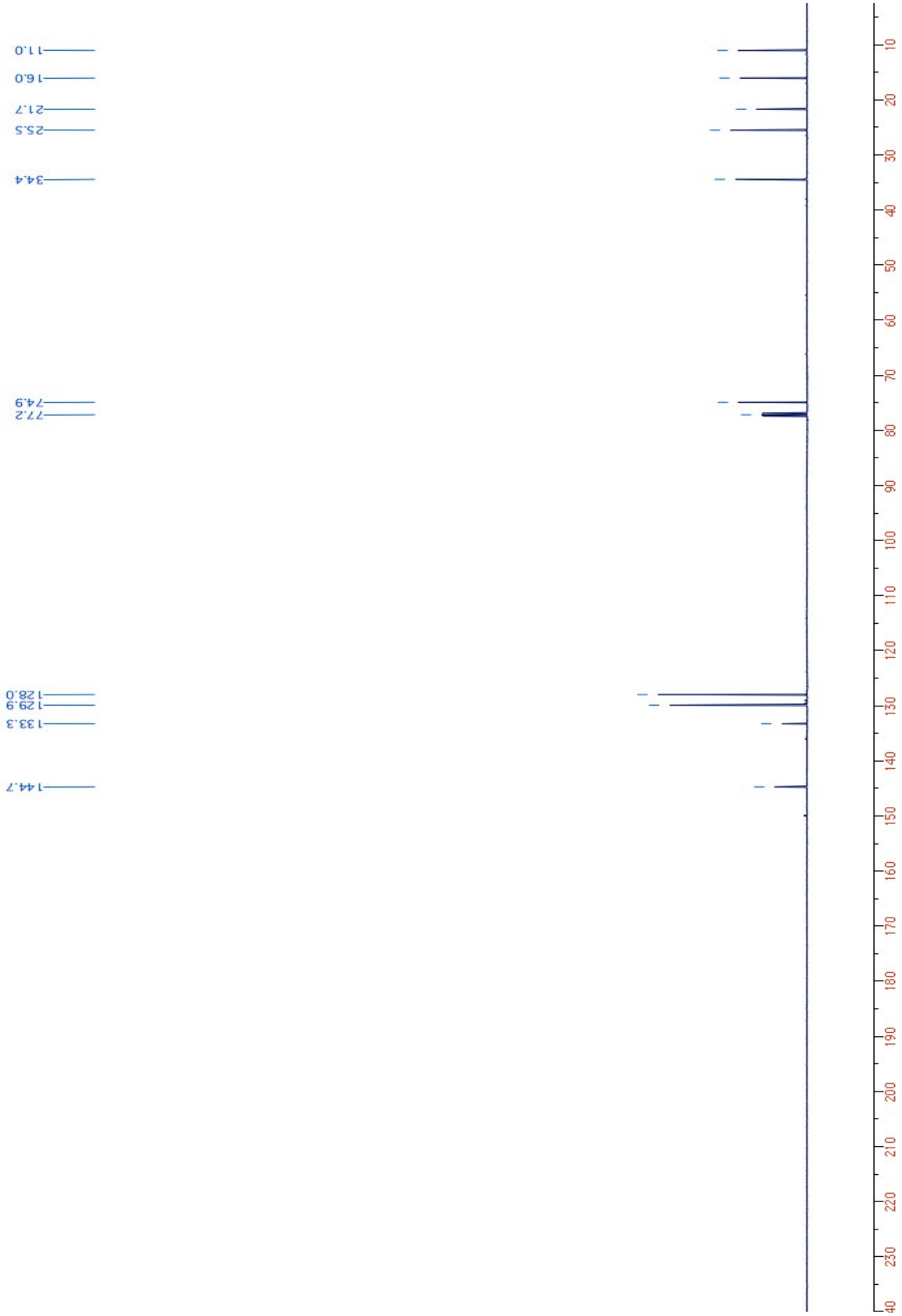
(2*S*)-methylbutyltosylate (7)



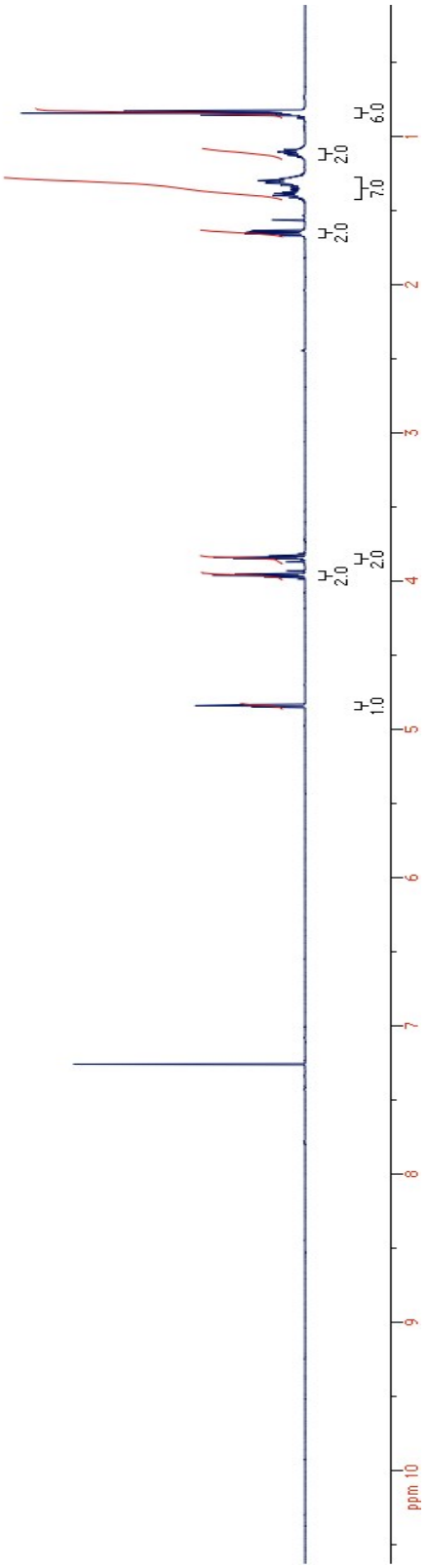

11.10
16.10
21.78
25.58
34.50
74.96
128.03
129.92
133.36
144.72

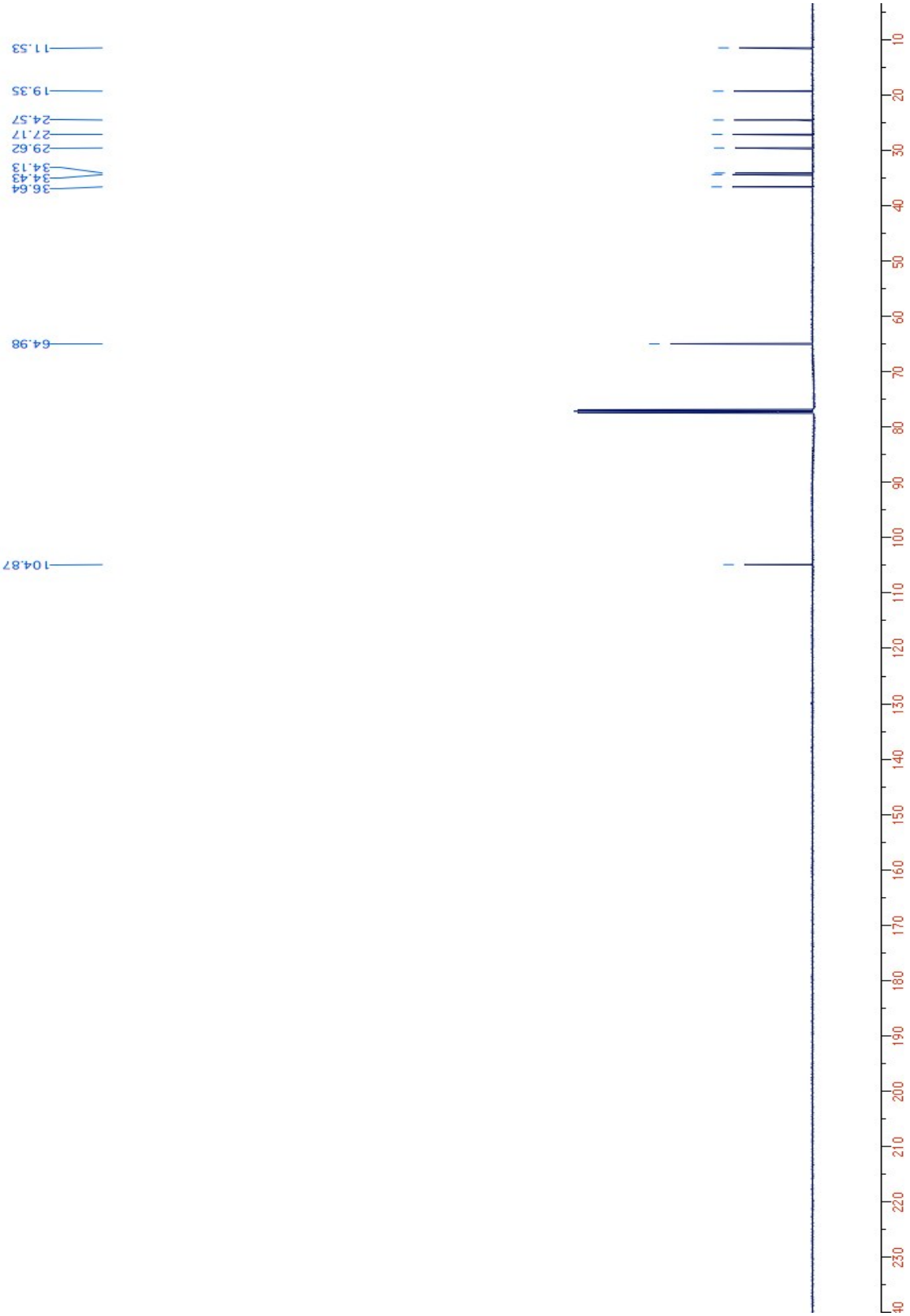
(2*R*)-methylbutyltosylate (8)



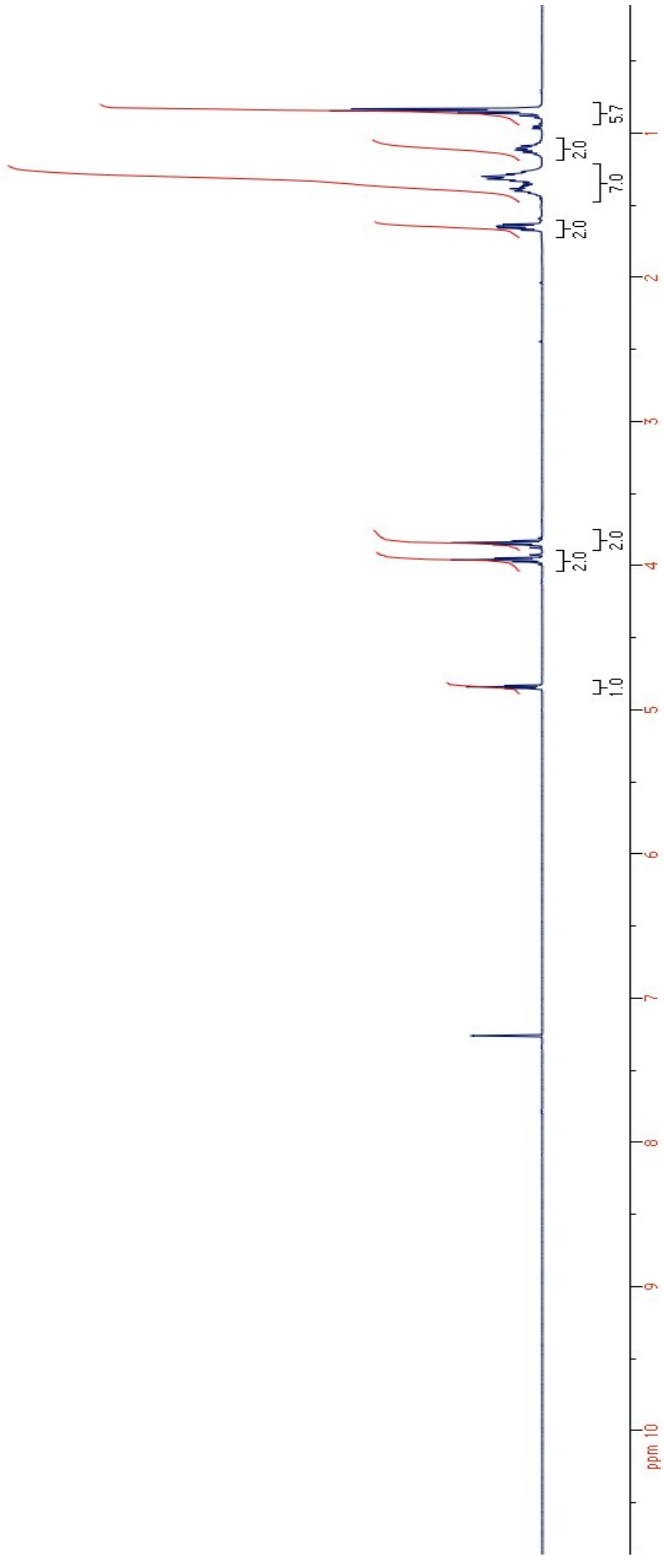

11.0
16.0
21.7
25.5
34.4
74.9
77.2
128.0
129.9
133.3
144.7

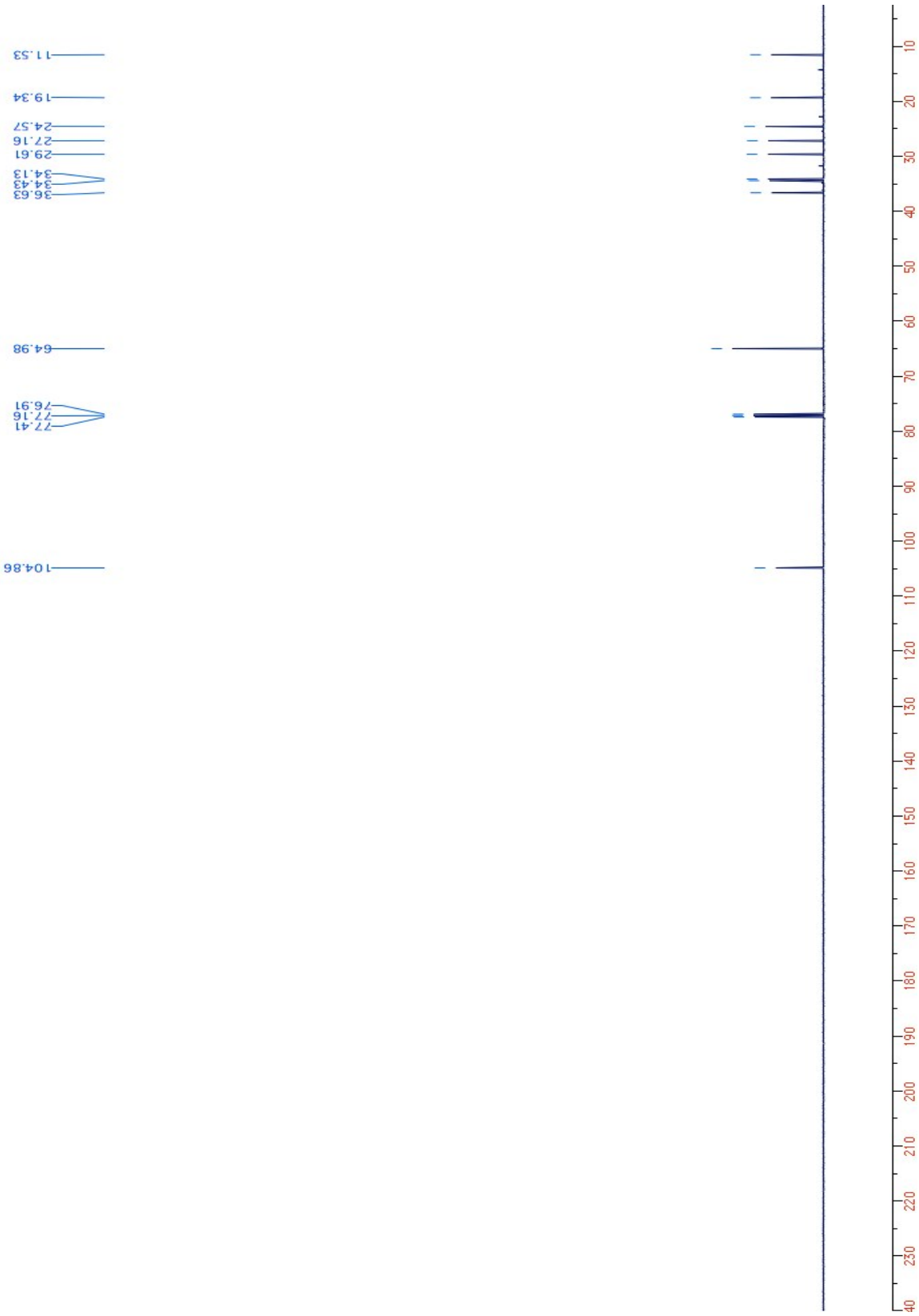
2-(1-(6*S*)-methyloctyl)-1,3-dioxolane (10)



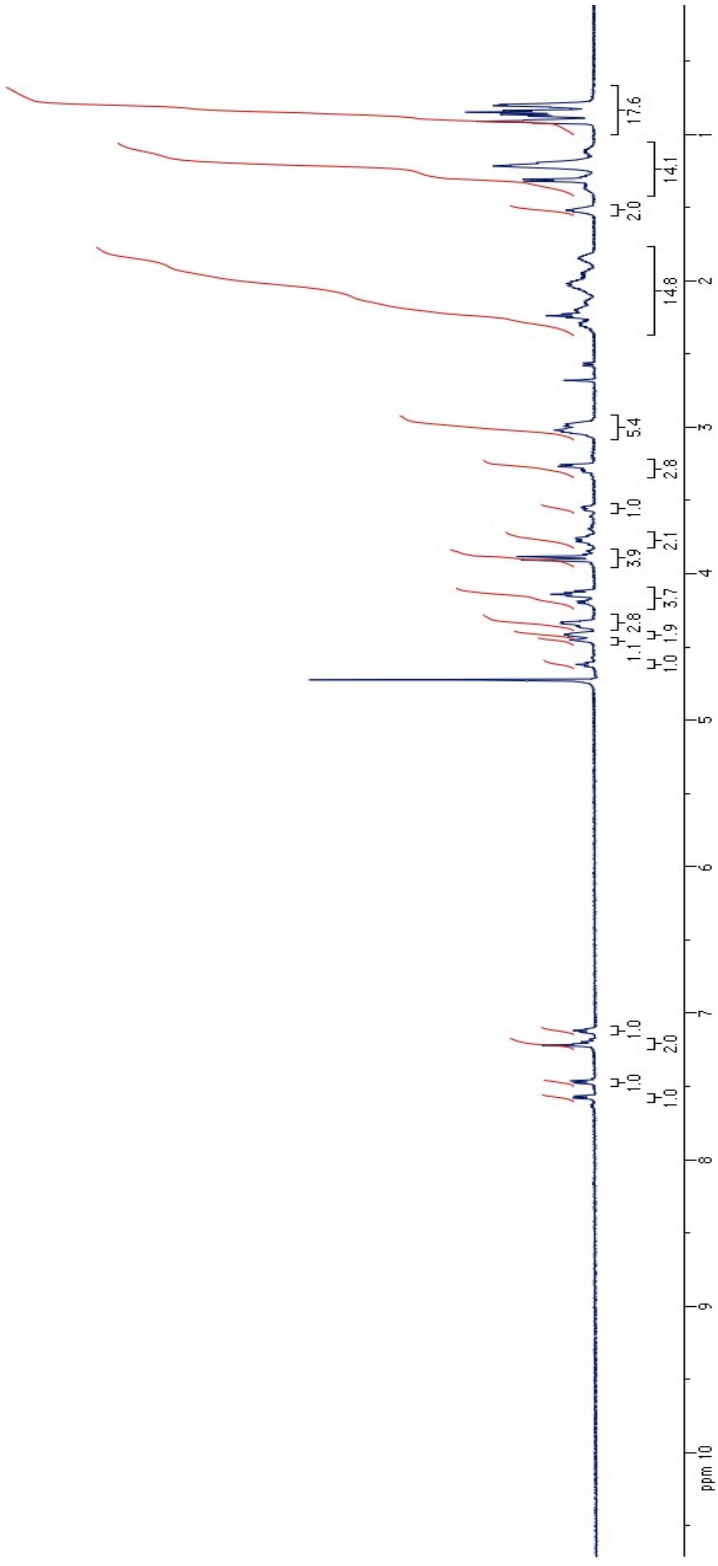

11.53
19.35
24.57
27.17
29.62
34.13
34.43
36.64
64.98
104.87

2-(1-(6*R*)-methyloctyl)-1,3-dioxolane (11)

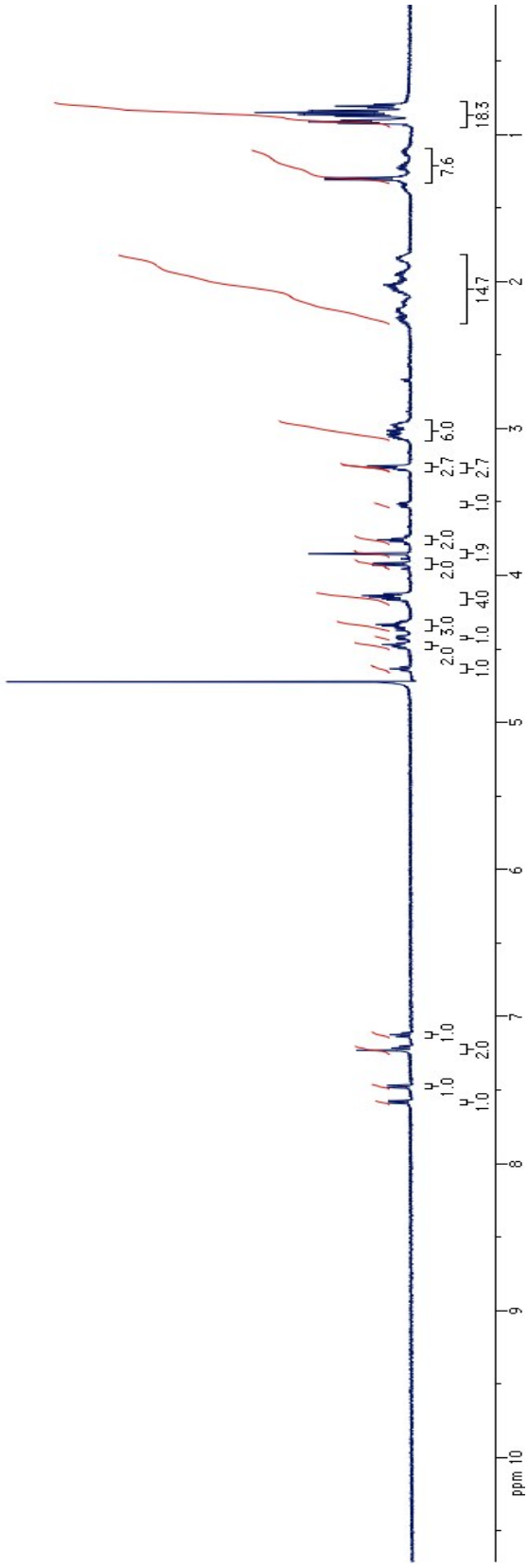



11.53
19.34
24.57
27.16
29.61
34.13
34.43
36.63
64.98
76.91
77.16
77.41
104.86
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240

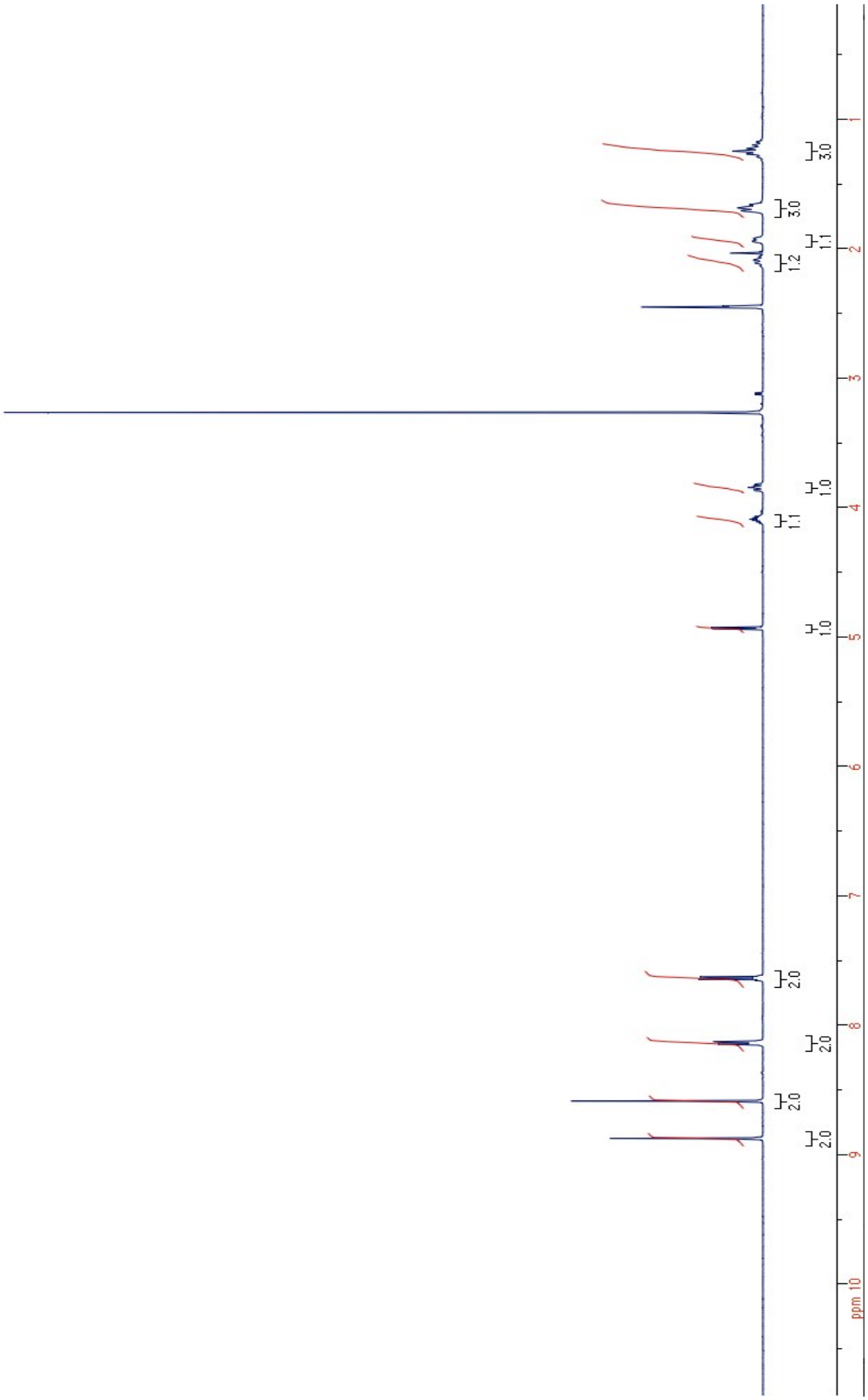
Oct-TriB_1 (13)

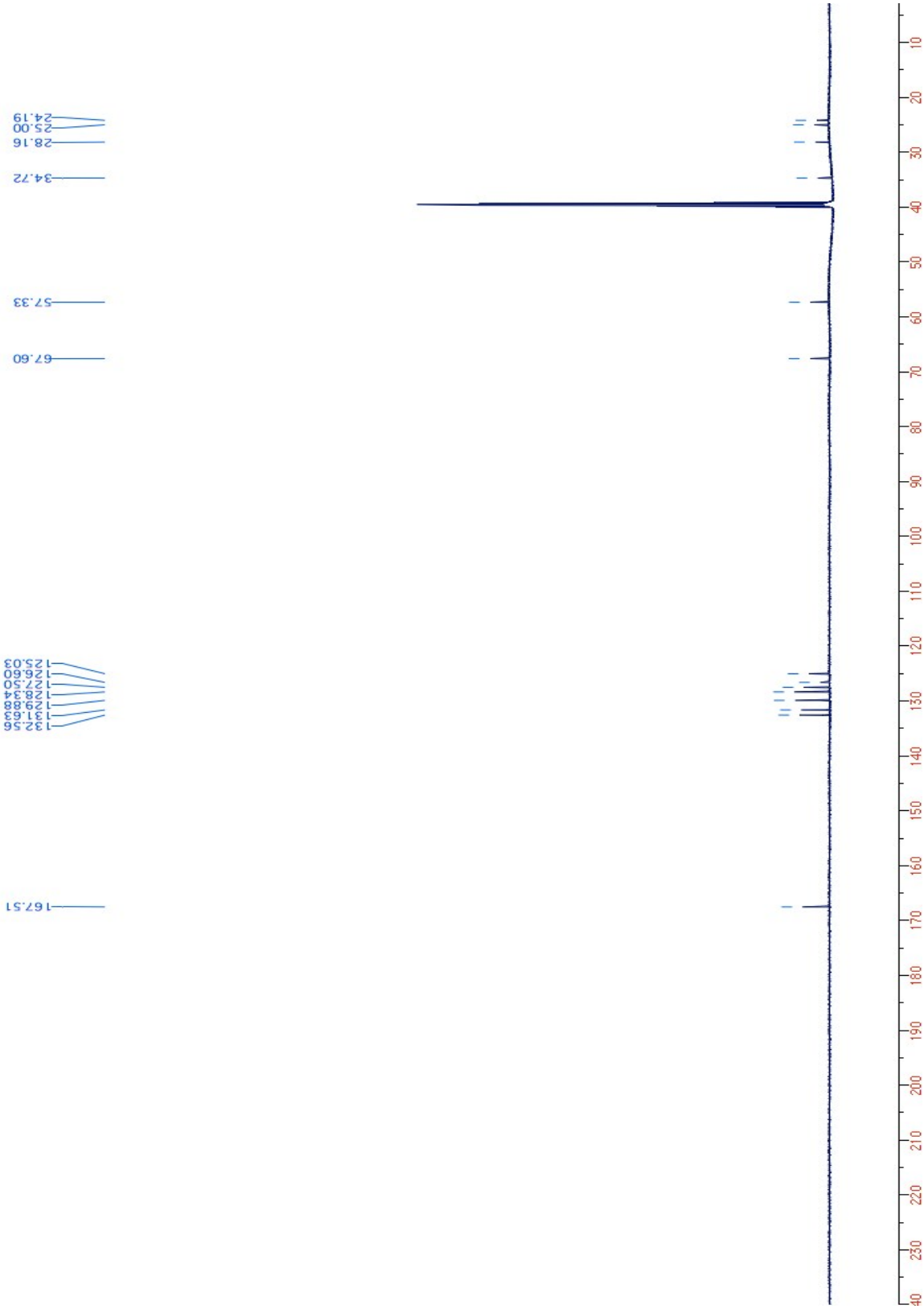


H-TriB$_1$ (14)

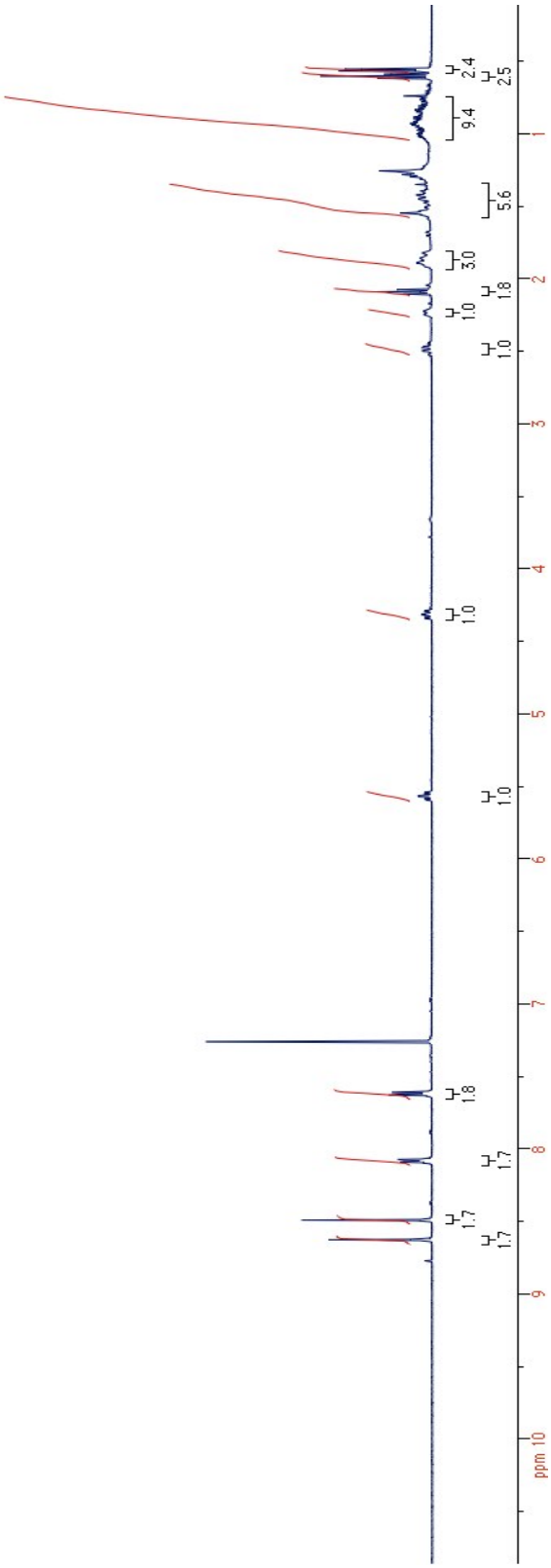


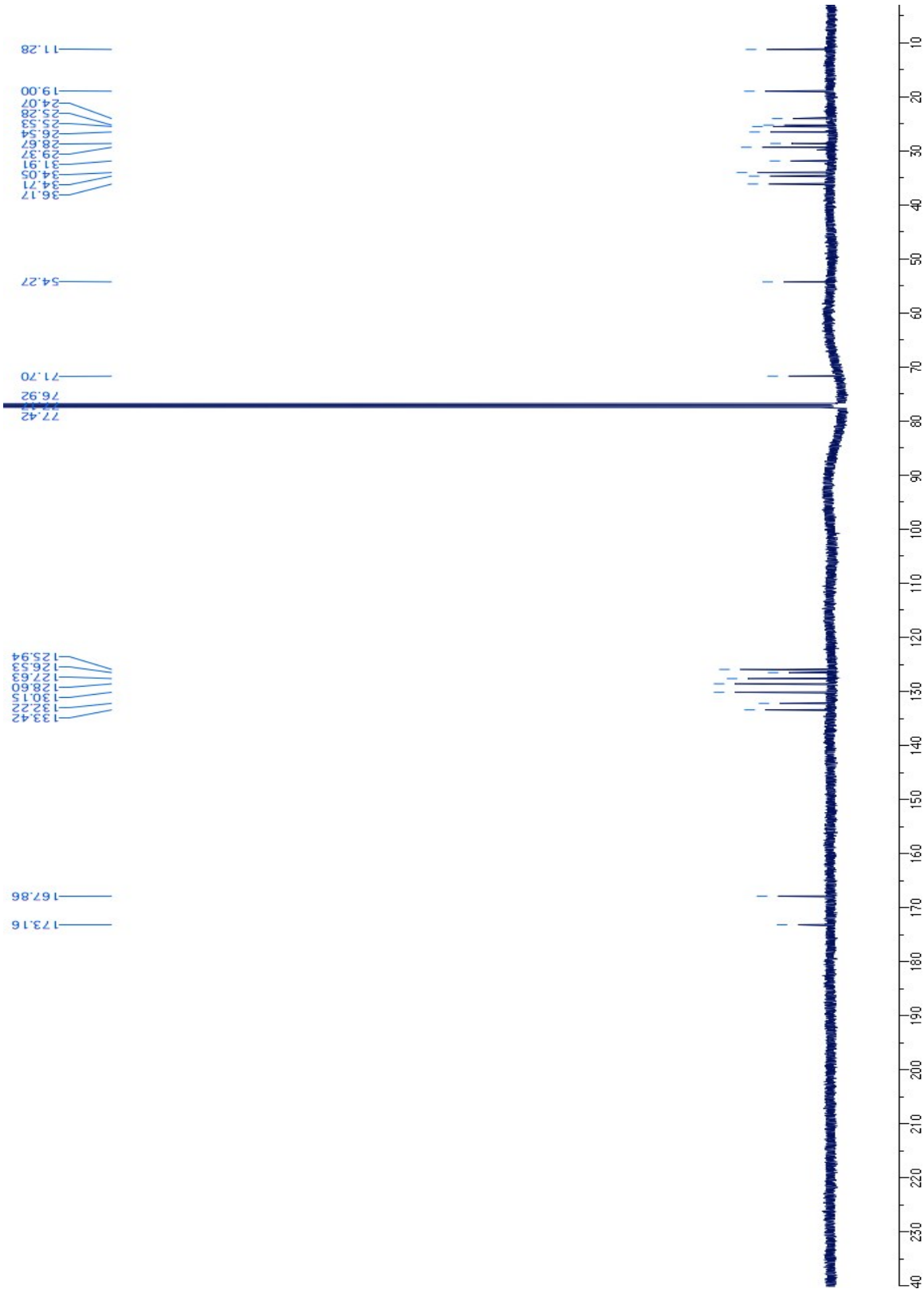
(1*R*, 2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanol (17)



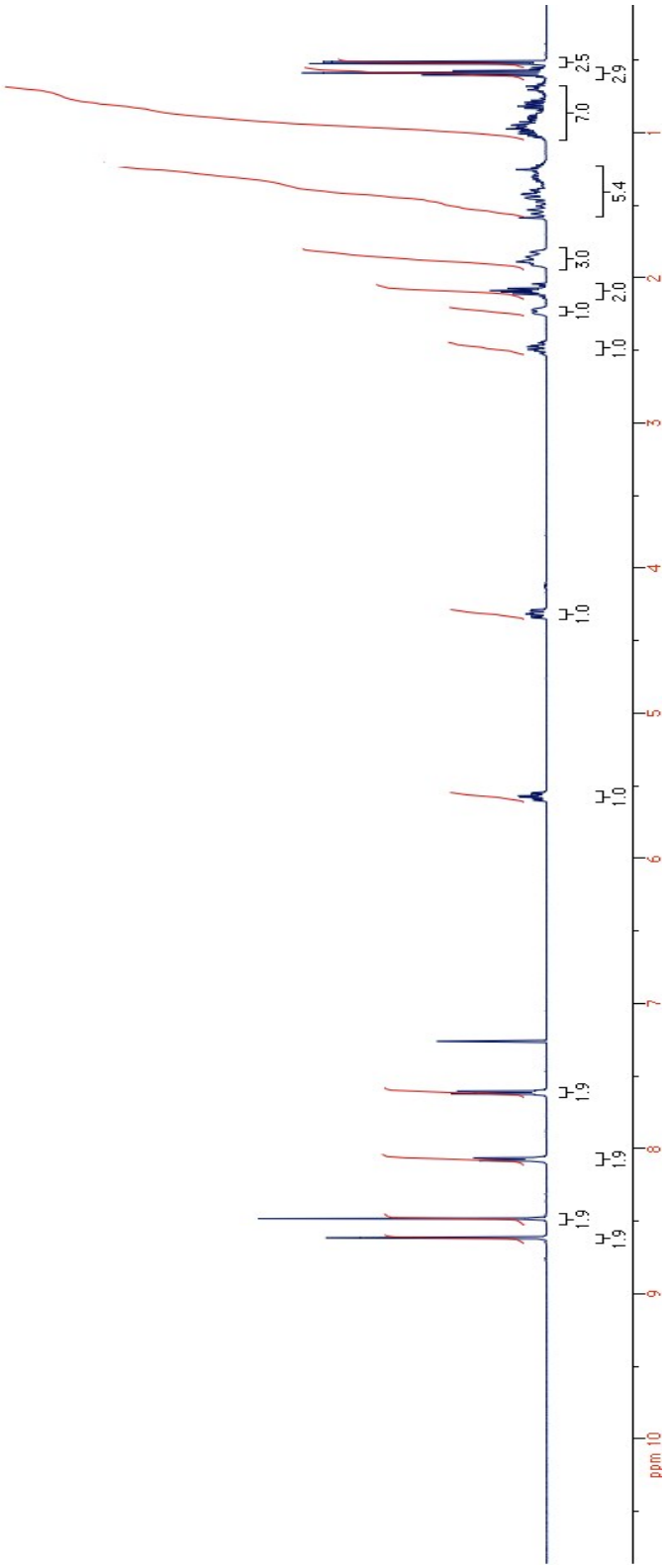

24.19
25.00
28.16
34.72
57.33
67.60
125.03
126.60
127.50
128.34
129.88
131.63
132.56
167.51

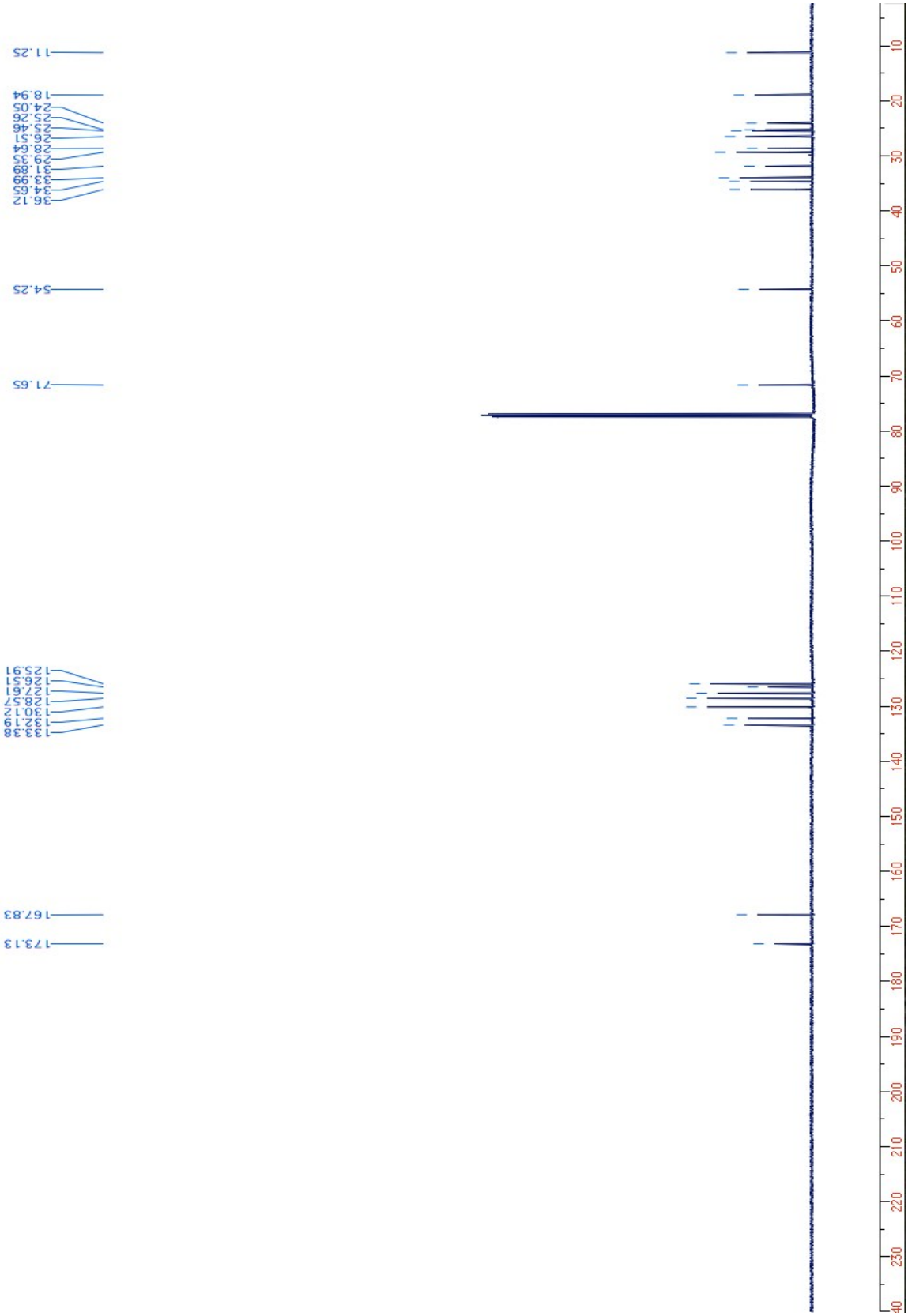
(1*R*, 2*R*)-1-((6*S*)-methyloctyl)-2-(2,3-anthracenedicarboximido) cyclohexanoate (18)



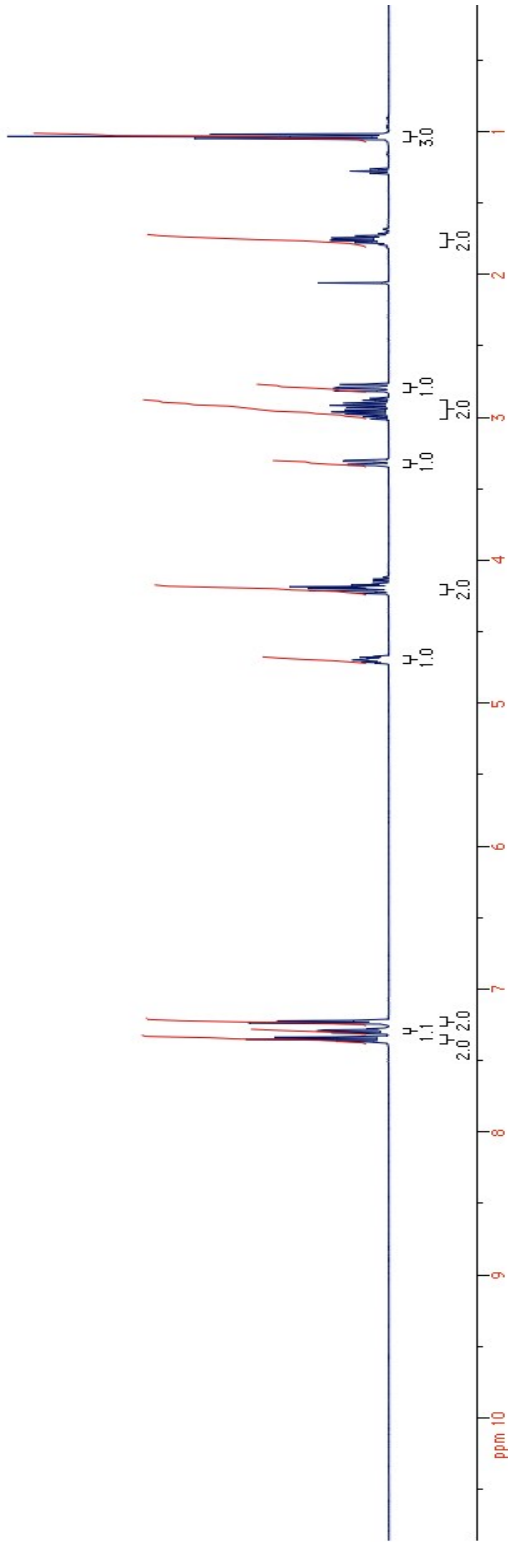

11.28
19.00
24.07
25.28
25.53
26.54
28.67
29.37
31.91
34.05
34.71
36.17
54.27
71.70
76.92
77.42
125.94
126.53
127.63
128.60
130.15
132.22
133.42
167.86
173.16
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240

(1*R*, 2*R*)-1-((6*R*)-methyloctyl)-2-(2,3-anthracenedicarboximido) cyclohexanoate (19)



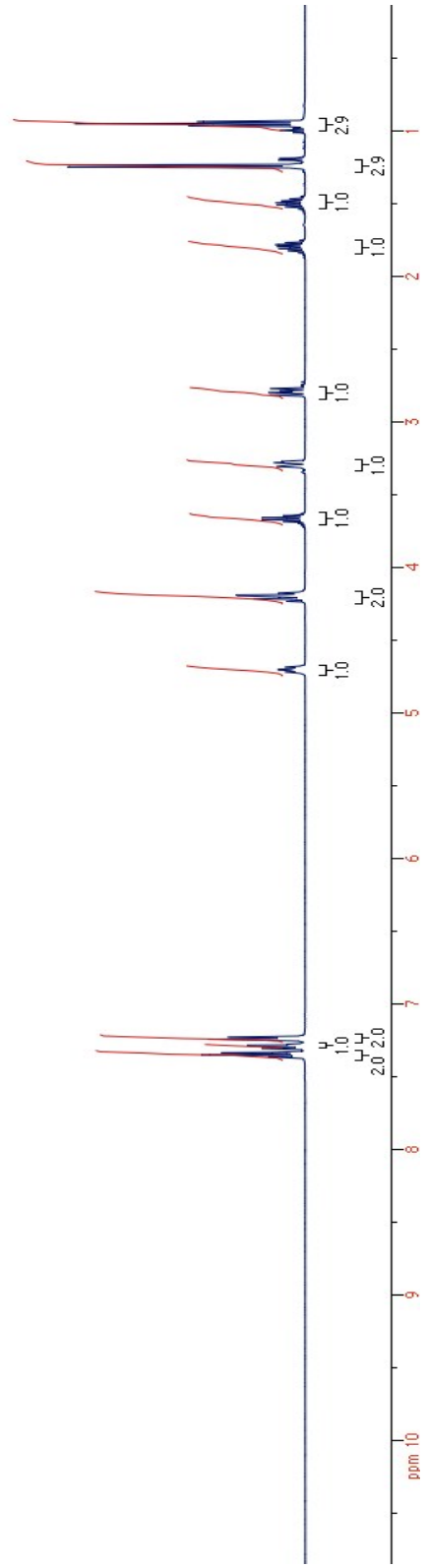

11.25
18.94
24.05
25.26
25.46
26.51
28.64
29.35
31.89
33.99
34.65
36.12
54.25
71.65
125.91
126.51
127.61
128.57
130.12
132.19
133.38
167.83
173.13
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240

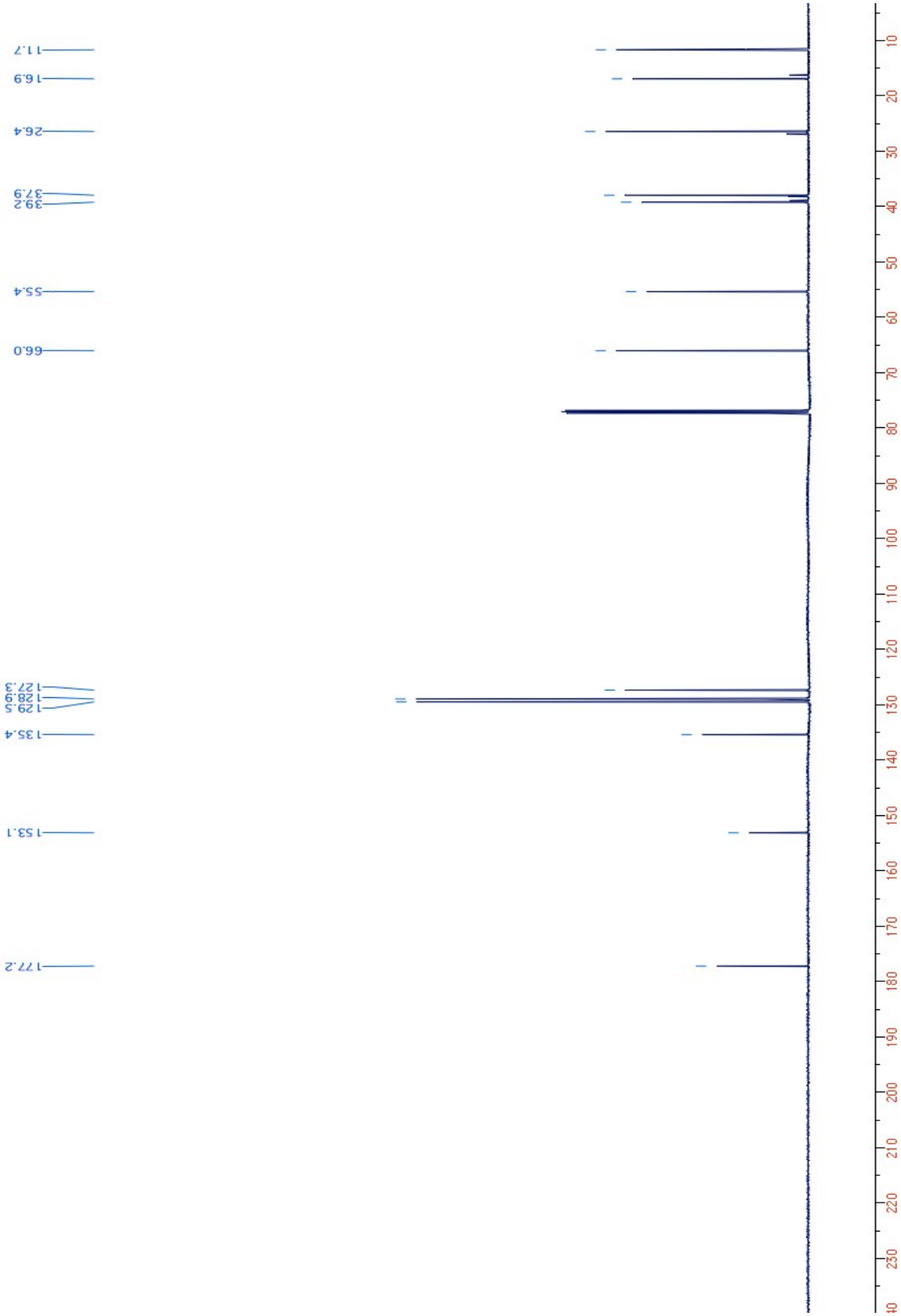
(*R*)-4-Benzyl-3-butyryloxazolidin-2-one (21)



13.7
17.7
38.0
37.4
55.1
66.2
77.3
77.1
76.8
127.3
129.0
129.4
135.4
153.5
173.2

(*R*)-4-Benzyl-3-((*R*)-2-methylbutanoyl)oxazolidin-2-one (22)




11.7
16.9
26.4
37.9
39.2
55.4
66.0
127.3
128.9
129.5
135.4
153.1
177.2

References

1. C. Rausch, T. Weber, O. Kohlbacher, W. Wohlleben, D. Huson, *Nucleic Acids Res*, 2005, **33**, 5799-5808.