Supporting Information

for

5'- Vitamin B_{12} derivatives suitable for bioconjugation via the amide bond

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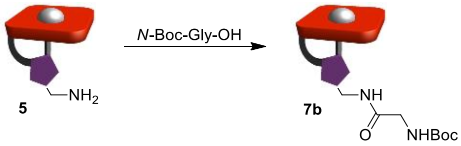
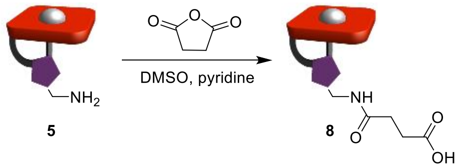
1. Optimization of the synthesis of amide **7b** with coupling reagent[a]



Entry	Coupling agent	Solvents	Base	Yield [%][b]
1	DCC	DMF/H_2O (1:1)	DIPEA	14
2	EDC	DMF/H_2O (1:1)	DIPEA	0
3	HBTU, HOBt	DMF/H_2O (1:1)	DIPEA	26
4	HBTU, HOBt	DMSO/H_2O (1:1)	DIPEA	31
5	HBTU, HOBt	DMF/H_2O/dioxane (2:1:2)	DIPEA	57
6	HBTU, HOBt	DMSO/H_2O/dioxane (2:1:2)	DIPEA	70
7	HBTU, HOBt	DMF/H_2O/DMSO (2:1:2)	DIPEA	82
8	**HBTU, HOBt**	**DMF/H_2O/DMSO (2:1:2)**	**DMAP**	**85**
9	HBTU, HOBt	DMF/H_2O/DMSO (2:1:2)	Et_3N	70
10[c]	HBTU, HOBt	DMF/H_2O/DMSO (2:1:2)	DMAP	88

[a]General conditions: amine **5** (15 mg, 10 µmol), *N*-Boc-Gly-OH (9 mg, 51 µmol), DCC (11 mg, 51 µmol), EDC (10 mg, 51 µmol), HBTU (19 mg, 51 µmol), HOBt (7 mg, 51 µmol), DIPEA (9 µL, 51 µmol), DMAP (6 mg, 51 µmol), Et_3N (7 µL, 51 µmol), total volume of solvents was 1.5 mL (v/v/v), r.t., 4 h; [b]Isolated yield; [c]Reaction performed with 102 µmol of *N*-Boc-Gly-OH, HBTU, HOBt and DMAP.

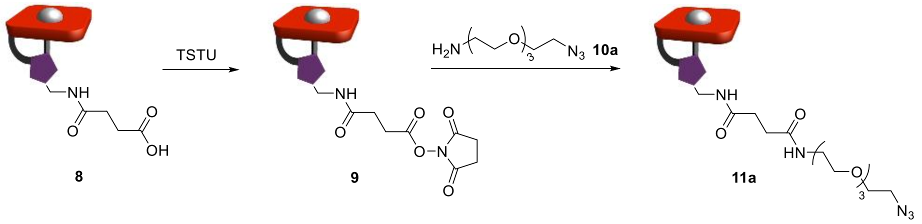
2. Optimization of the synthesis of acid **8**[a]



Entry	Succinic anhydride [equiv.]	Pyridine [equiv.]	Temperature [°C]	Time [h]	Yield [%][b]
1	5	5	r.t.	5	67
2	5	5	r.t.	17	91
3	10	10	r.t.	5	87
4	10	10	r.t.	17	96
5	5	5	40	5	81
6	5	5	40	17	96
7	**10**	**10**	**40**	**5**	**96**
8	10	10	40	17	96

[a]General conditions: amine **5** (5 mg, 3 µmol), DMSO (5 mL); [b]Yield estimated based on HPLC analysis.

3. Optimization of the synthesis of amide **11a** via active ester step[a]



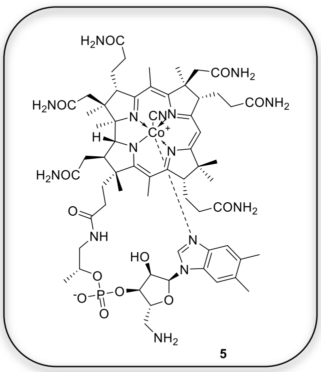
Entry	TSTU [equiv.]	DIPEA [equiv.]	Solvents	Yield [%][b]
1	2.0	2.0	DMF/H_2O/dioxane (2:1:2)	16
2	4.0	4.0	DMF/H_2O/dioxane (2:1:2)	30
3	**8.0**	**8.0**	**DMF/H_2O/dioxane (2:1:2)**	**54**
4	20.0	20.0	DMF/H_2O/dioxane (2:1:2)	traces
5	8.0	8.0	DMF/H_2O (2:1)	43
6	8.0	8.0	DMF/0.1 M $NaHCO_3$/dioxane (2:1:2)	15
7[c]	8.0	8.0	DMF/0.1 M $NaHCO_3$/dioxane (2:1:2)	9
8	8.0	8.0	MeCN/H_2O/dioxane (2:1:2)	41
9	8.0	8.0	NMP/H_2O/dioxane (2:1:2)	22

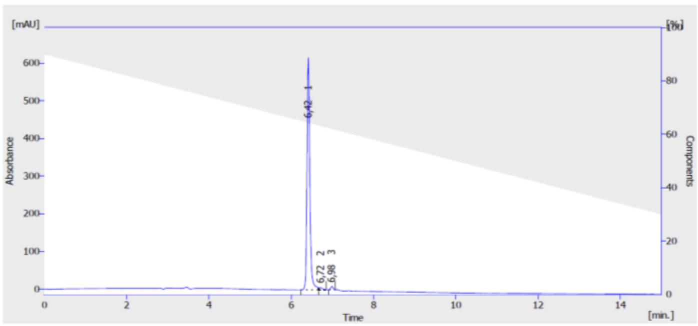
[a]General conditions: acid **8** (15 mg, 10 µmol), total volume of solvents was 1.5 mL (v/v/v), r.t., 20 h, amine **10a** (4.0 µL, 20 µmol), r.t., 75 min; [b]Isolated yield; [c]Without DIPEA.

4. HPLC chromatograms

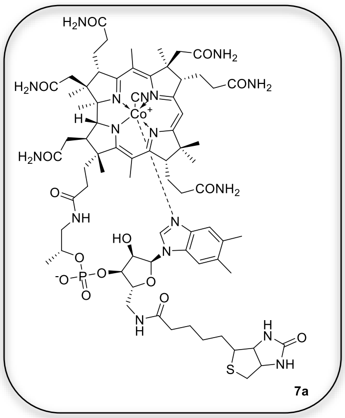
HPLC Method

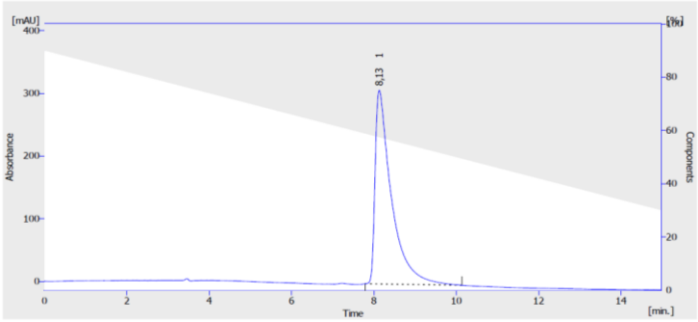
Time [min]	H_2O + 0.5‰ TFA [%]	MeCN [%]	λ [nm]
Initial	90	10	361
15	30	70	



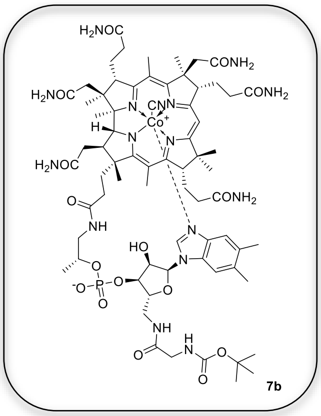


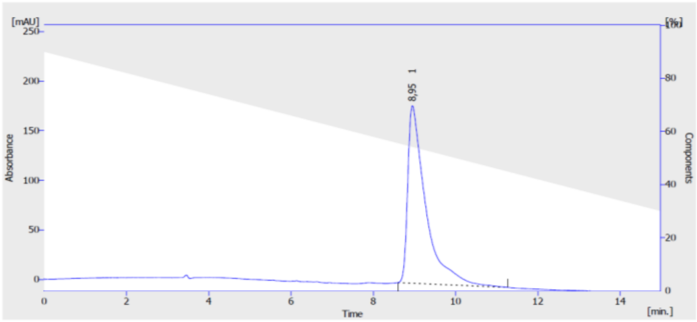
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,417	2931,116	615,739	97,5	97,6	0,08	748
2	6,717	37,950	6,176	1,3	1,0	0,10	980
3	6,983	35,703	9,028	1,2	1,4	0,08	934
	Total	3004,768	630,943	100,0	100,0		



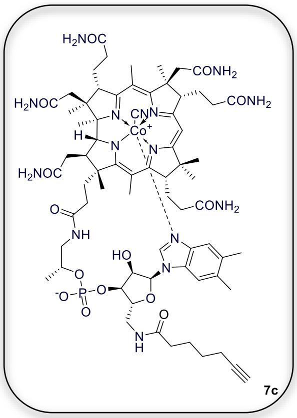


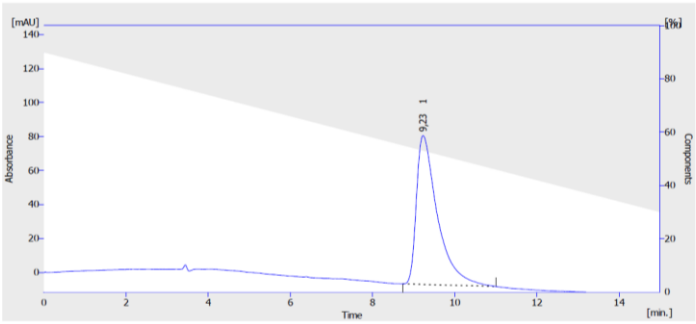
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	8,133	8531,444	309,029	100,0	100,0	0,38	628
	Total	8531,444	309,029	100,0	100,0		



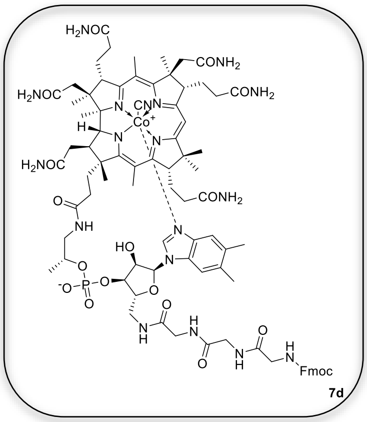


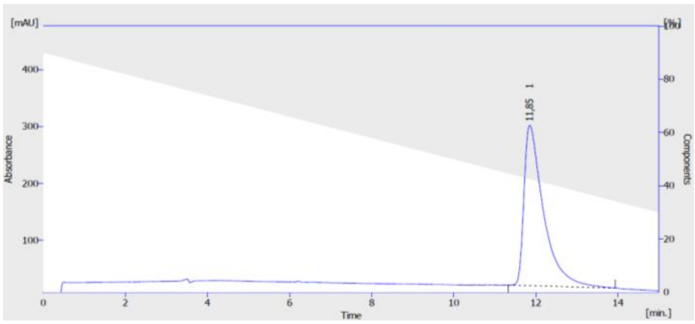
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	8,950	5427,793	179,252	100,0	100,0	0,43	702
	Total	5427,793	179,252	100,0	100,0		



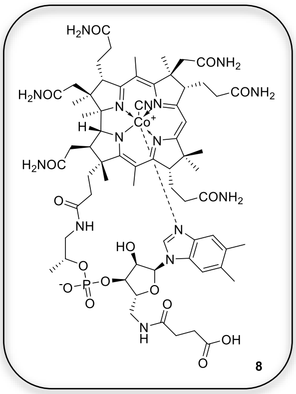


	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,233	3110,164	87,543	100,0	100,0	0,53	709
	Total	3110,164	87,543	100,0	100,0		

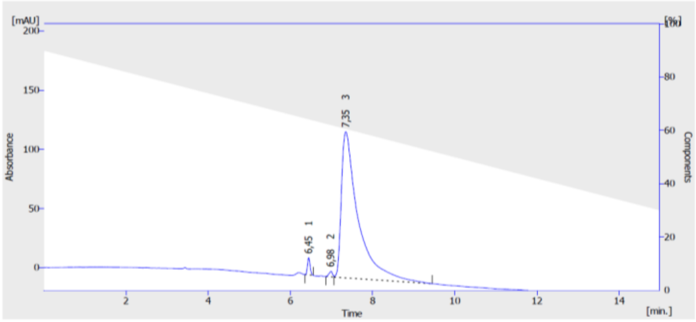




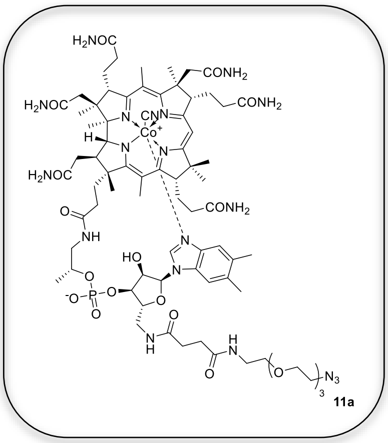
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,850	9307,431	281,781	100,0	100,0	0,48	552
	Total	9307,431	281,781	100,0	100,0		



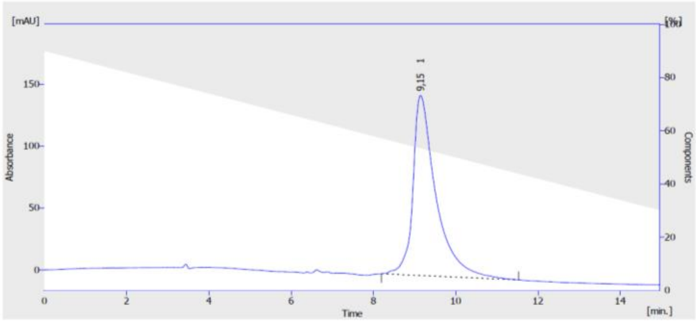
8



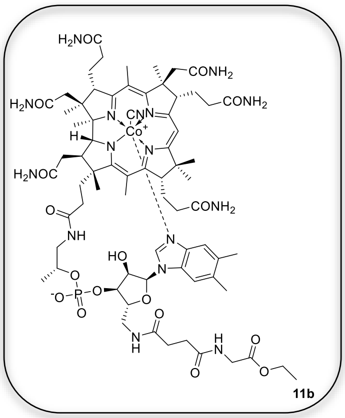
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,450	64,509	14,586	1,8	10,2	0,08	929
2	6,983	27,848	4,718	0,8	3,3	0,12	974
3	7,350	3505,415	123,476	97,4	86,5	0,38	744
	Total	3597,772	142,779	100,0	100,0		



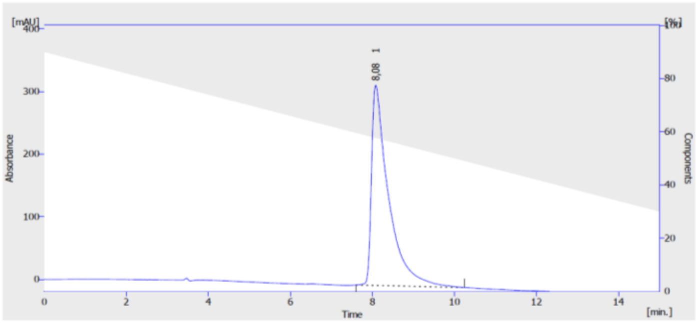
11a



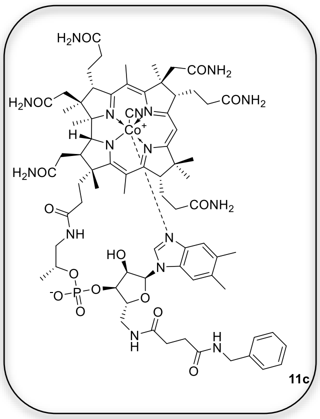
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,150	5667,932	145,556	100,0	100,0	0,53	688
	Total	5667,932	145,556	100,0	100,0		



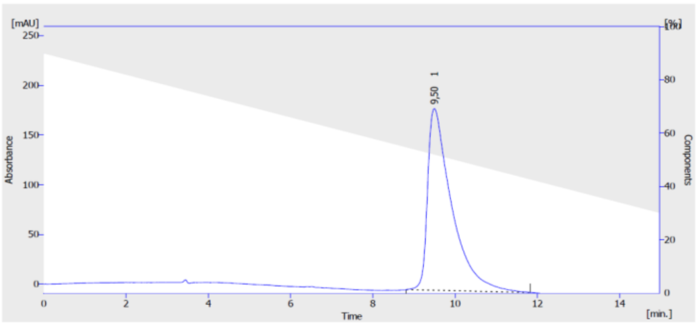
11b



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	8,083	9207,680	319,976	100,0	100,0	0,40	639
	Total	9207,680	319,976	100,0	100,0		

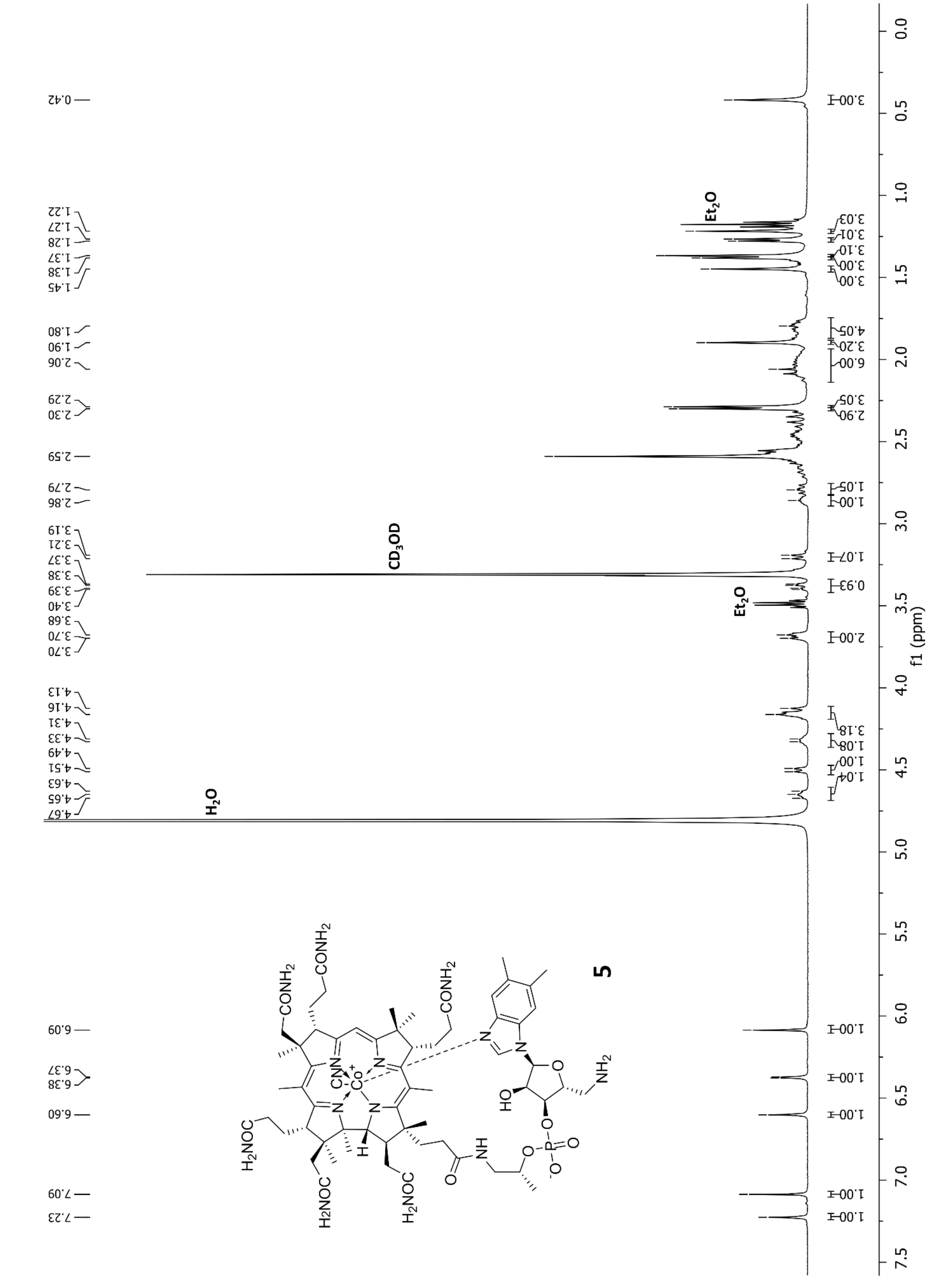


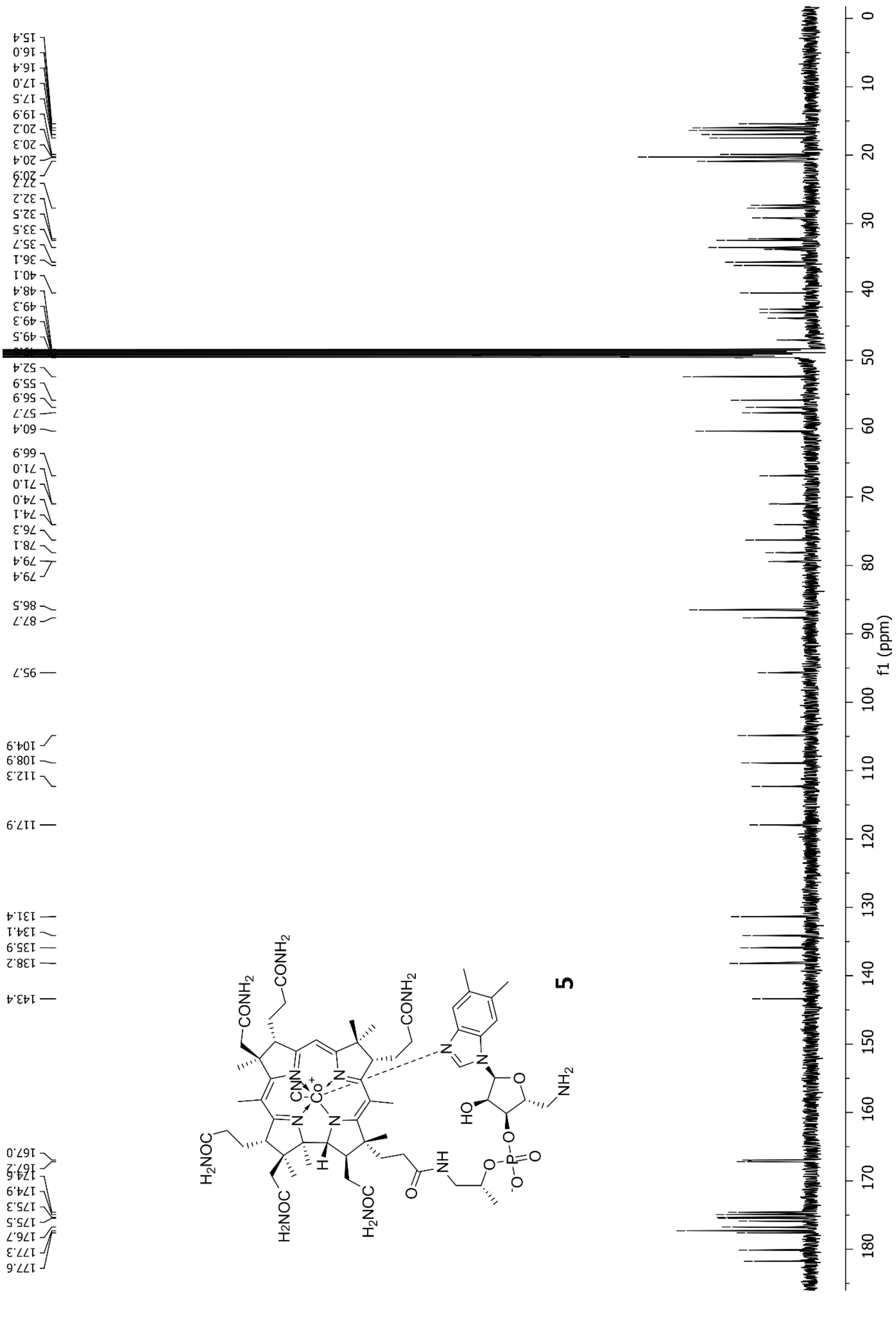
11c



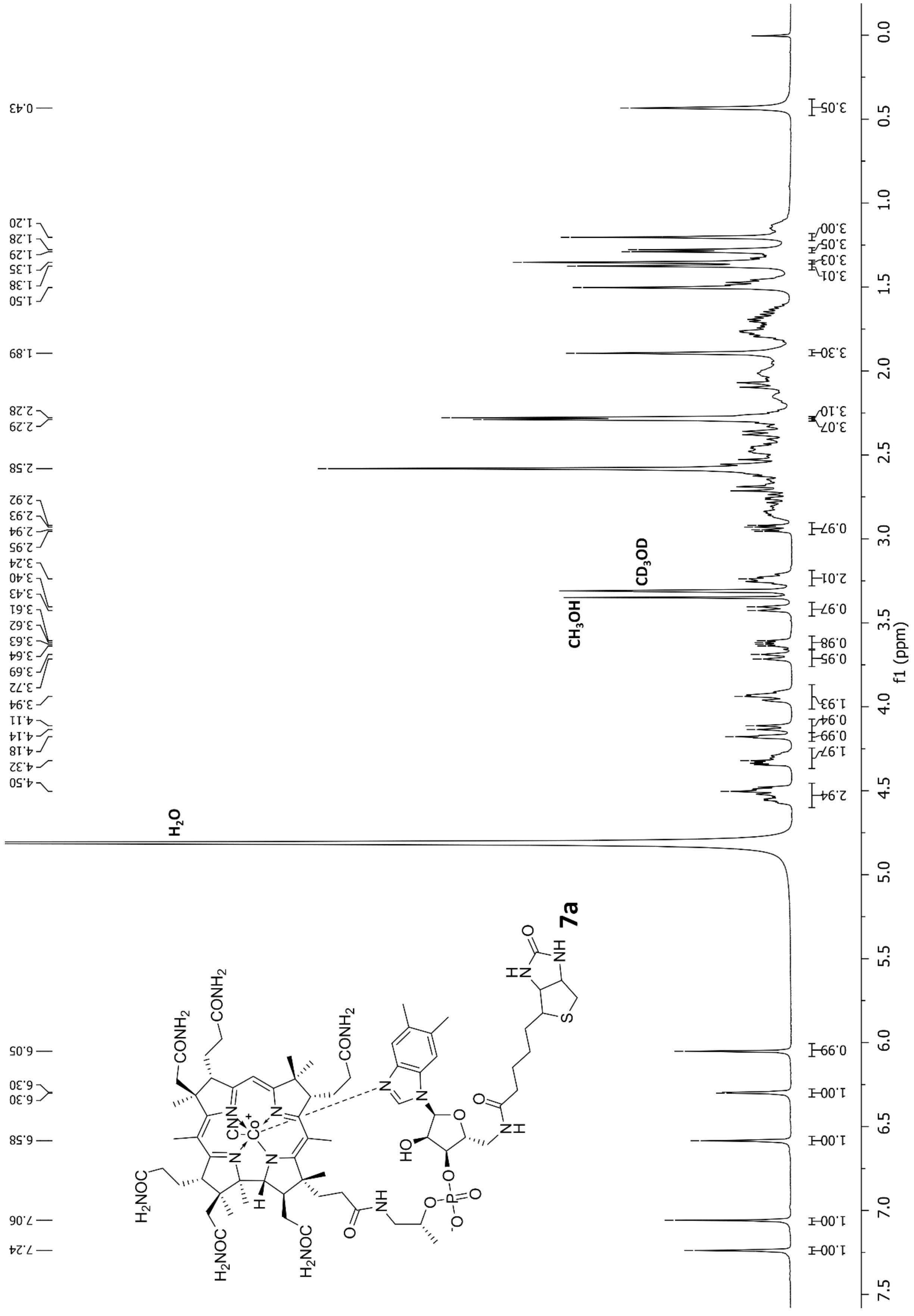
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,500	7414,466	182,862	100,0	100,0	0,58	680
	Total	7414,466	182,862	100,0	100,0		

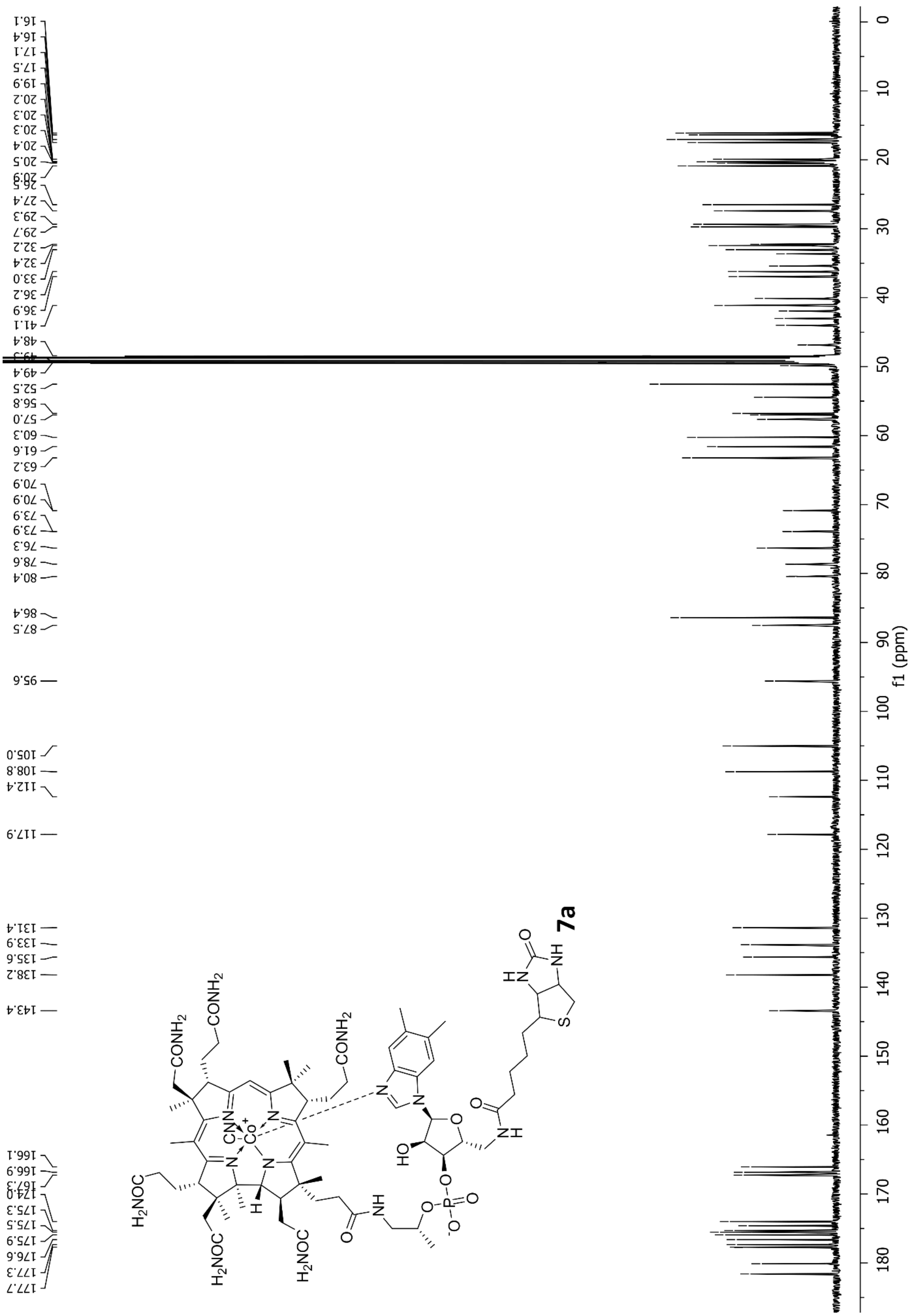
5. [1]H NMR (500 MHz) and [13]C NMR (126 MHz) of compound **5** in CD_3OD (for clarity only well isolated signals are integrated)



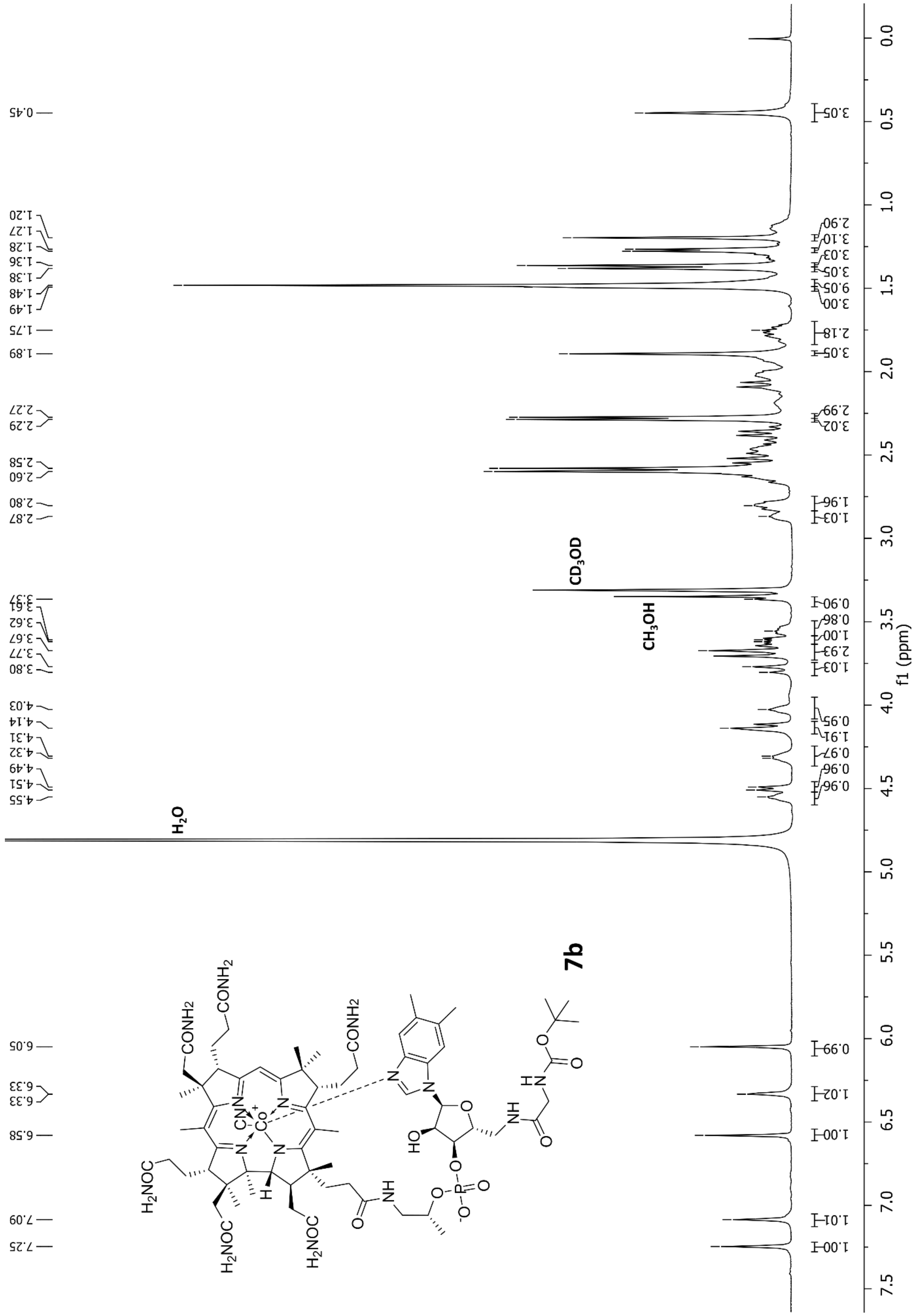

15.4
16.0
16.4
17.0
17.5
19.9
20.2
20.3
20.4
20.9
32.2
32.5
33.5
35.7
36.1
40.1
48.4
49.3
49.3
49.5
52.4
55.9
56.9
57.7
60.4
66.9
71.0
71.0
74.0
74.1
76.3
78.1
79.4
79.4
86.5
87.7
95.7
104.9
108.9
112.3
117.9
131.4
134.1
135.9
138.2
143.4
167.0
167.2
174.6
174.9
175.3
175.5
176.7
177.3
177.6
f1 (ppm)
5

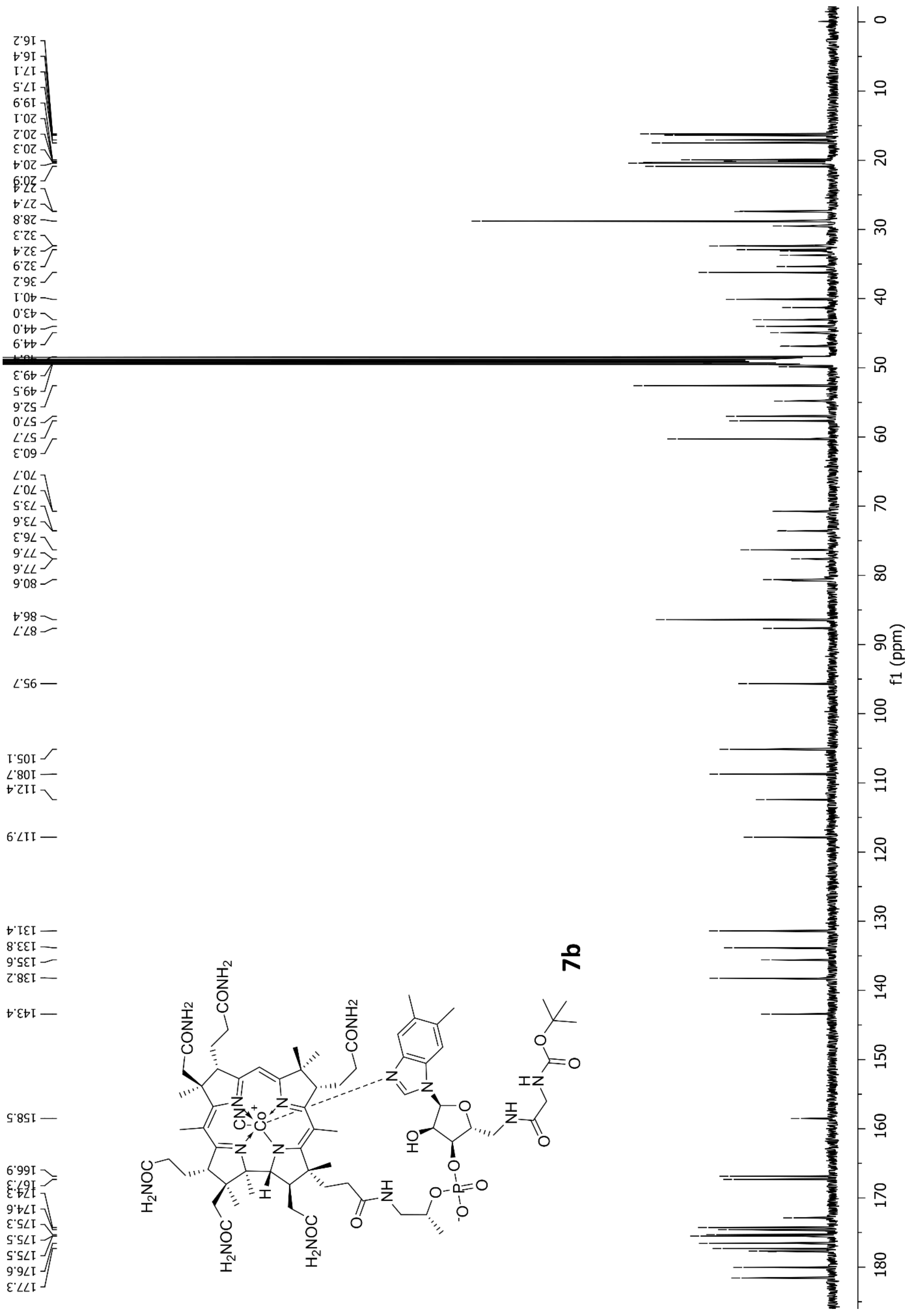
6. ^1H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **7a** in CD_3OD (for clarity only well isolated signals are integrated)



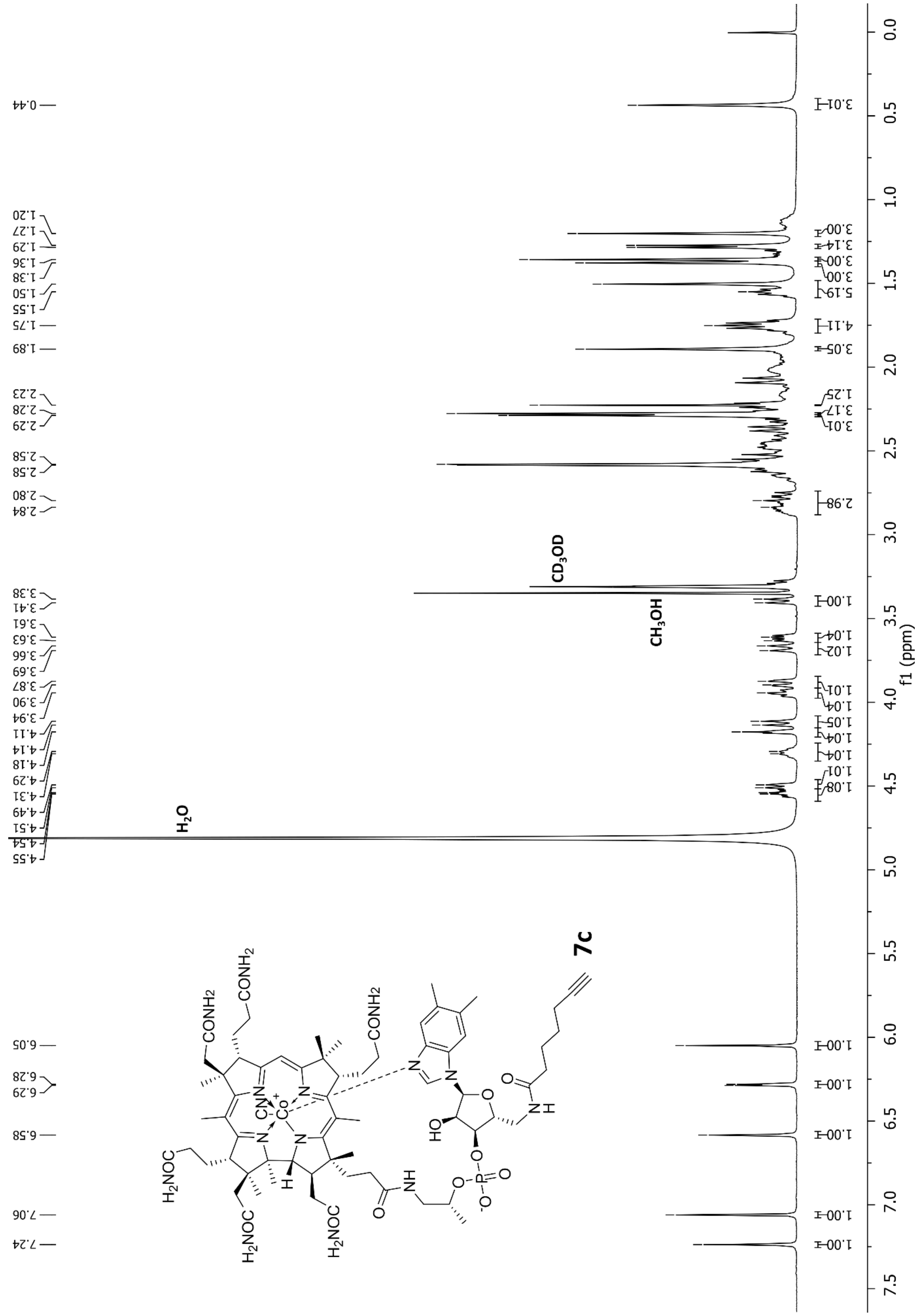

16.1
16.4
17.1
17.5
19.9
20.2
20.3
20.3
20.4
20.5
27.4
29.3
29.7
32.2
32.4
33.0
36.2
36.9
41.1
48.4
49.4
52.5
56.8
57.0
60.3
61.6
63.2
70.9
70.9
73.9
73.9
76.3
78.6
80.4
86.4
87.5
95.6
105.0
108.8
112.4
117.9
131.4
133.9
135.6
138.2
143.4
166.1
166.9
167.3
174.0
175.3
175.5
175.9
176.6
177.3
177.7
7a
f1 (ppm)

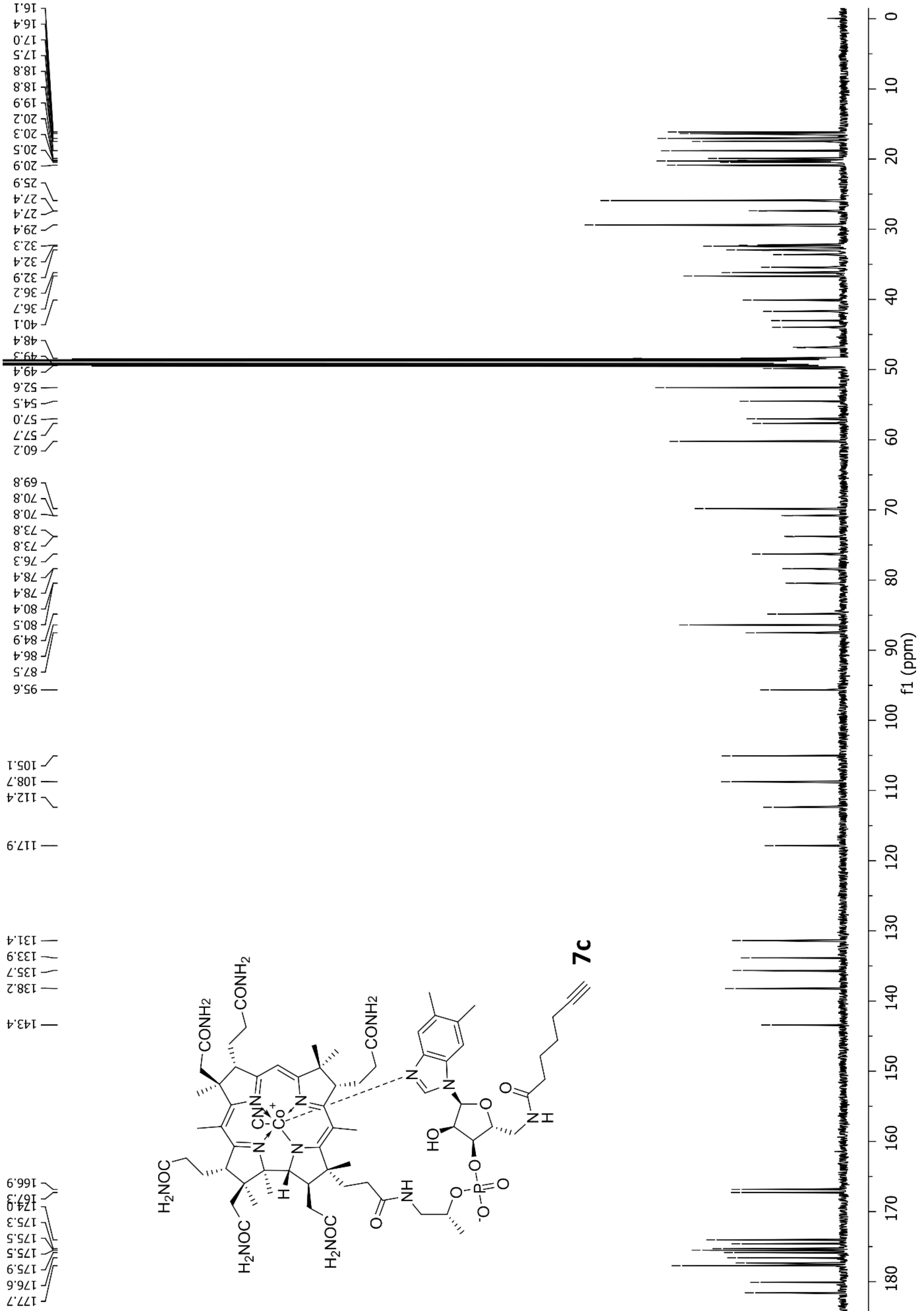
7. [1]H NMR (500 MHz) and [13]C NMR (126 MHz) of compound **7b** in CD_3OD (for clarity only well isolated signals are integrated)




16.2
16.4
17.1
17.5
19.9
20.1
20.2
20.3
20.4
20.9
27.4
28.8
32.3
32.4
32.9
36.2
40.1
43.0
44.0
44.9
49.3
49.5
52.6
57.0
57.7
60.3
70.7
70.7
73.5
73.6
76.3
77.6
77.6
80.6
86.4
87.7
95.7
105.1
108.7
112.4
117.9
131.4
133.8
135.6
138.2
143.4
158.5
166.9
167.3
174.3
174.6
175.3
175.5
175.5
176.6
177.3
f1 (ppm)
CONH2
H2NOC
NH
HO
CN
Co+
7b

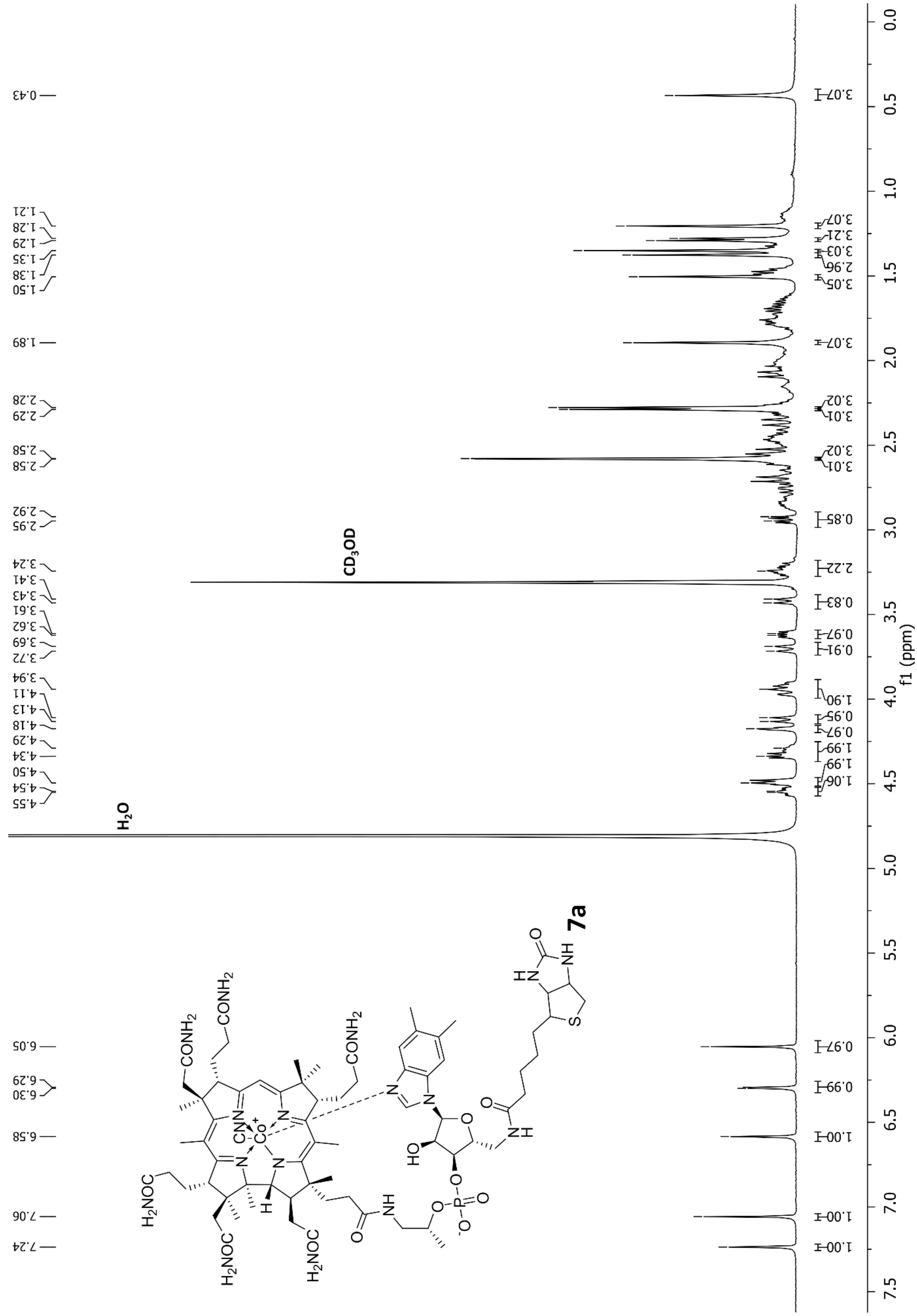
8. [1]H NMR (500 MHz) and [13]C NMR (126 MHz) of compound **7c** in CD_3OD (for clarity only well isolated signals are integrated)

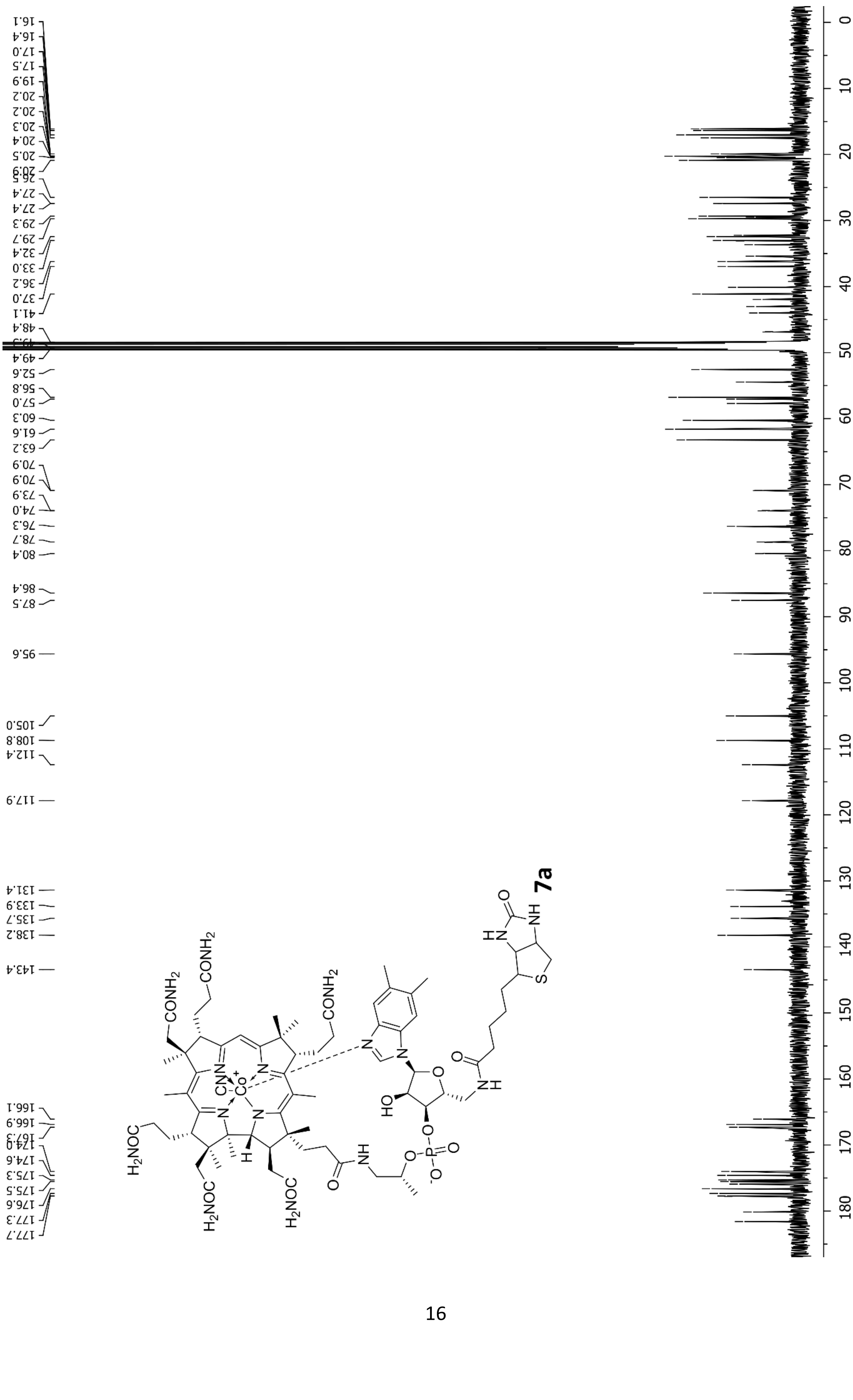




7c
f1 (ppm)
16.1
16.4
17.0
17.5
18.8
18.8
19.9
20.2
20.3
20.5
20.9
25.9
27.4
27.4
29.4
32.3
32.4
32.9
36.2
36.7
40.1
48.4
49.3
49.4
52.6
54.5
57.0
57.7
60.2
69.8
70.8
70.8
73.8
73.8
76.3
78.4
78.4
80.4
80.5
84.9
86.4
87.5
95.6
105.1
108.7
112.4
117.9
131.4
133.9
135.7
138.2
143.4
166.9
167.3
174.0
175.3
175.5
175.5
175.9
176.6
177.7

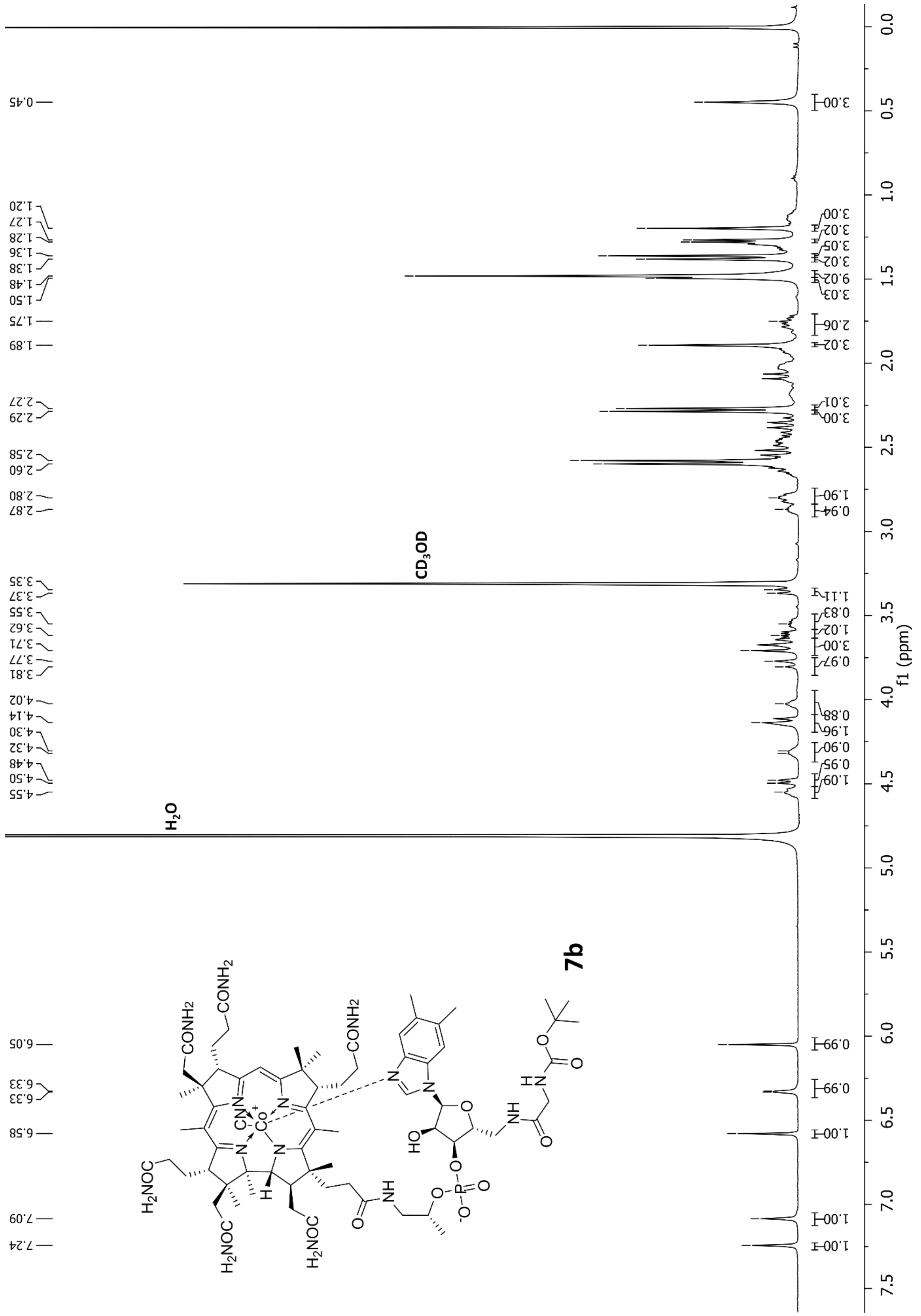
9. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **7a** (in physiological conditions) in CD_3OD (for clarity only well isolated signals are integrated)

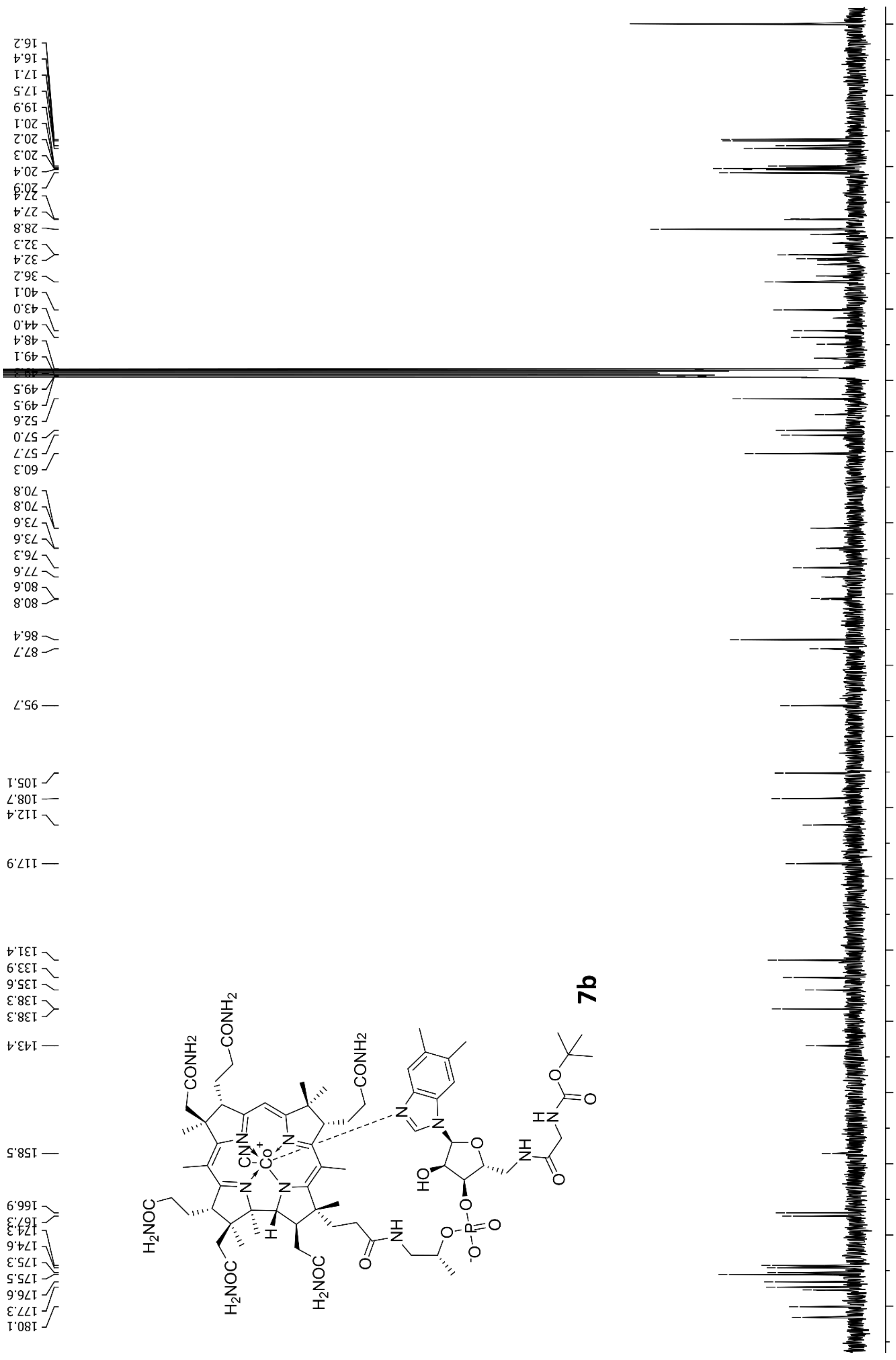




7a
f1 (ppm)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
16.1
16.4
17.0
17.5
19.9
20.2
20.2
20.3
20.4
20.5
26.5
27.4
27.4
29.3
29.7
32.4
33.0
36.2
37.0
41.1
48.4
49.4
52.6
56.8
57.0
60.3
61.6
63.2
70.9
70.9
73.9
74.0
76.3
78.7
80.4
86.4
87.5
95.6
105.0
108.8
112.4
117.9
131.4
133.9
135.7
138.2
143.4
166.1
166.9
174.6
175.3
175.5
176.6
177.3
177.7
CONH2
H2NOC
CN
Co+
HO
NH
O
S

10. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **7b** (with coupling reagent) in CD_3OD (for clarity only well isolated signals are integrated)

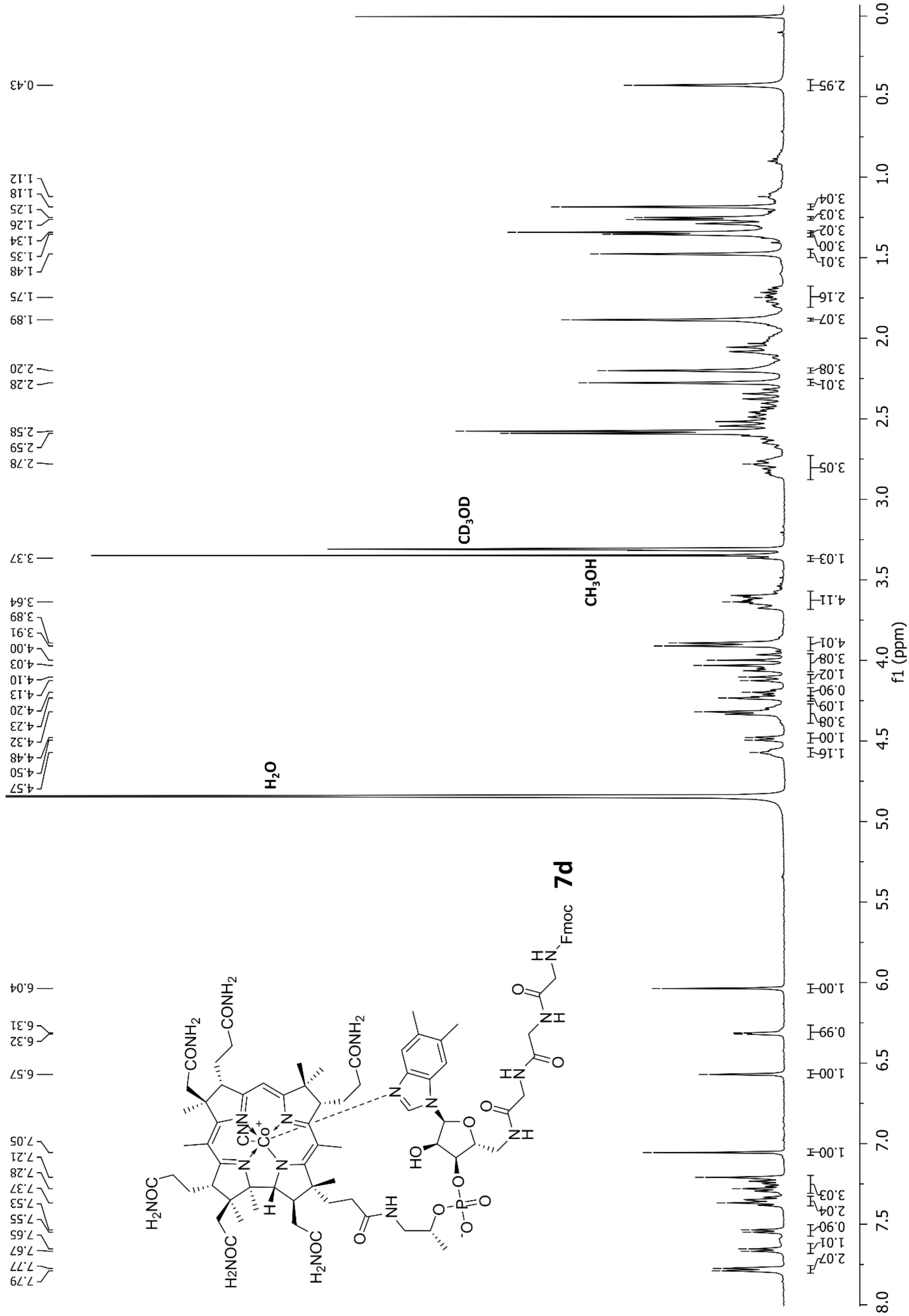


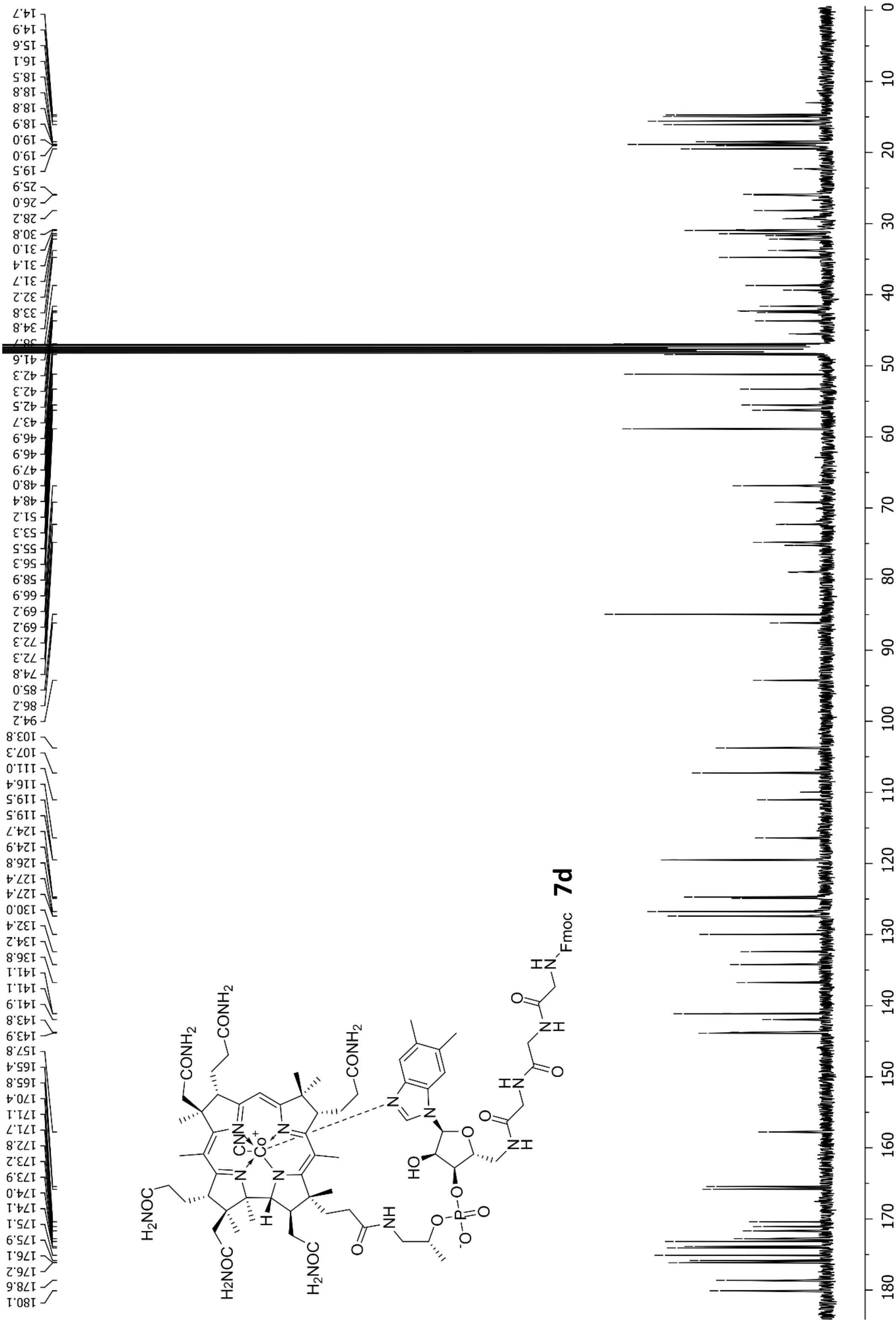


16.2
16.4
17.1
17.5
19.9
20.1
20.2
20.3
20.4
20.9
27.4
27.4
28.8
32.3
32.4
36.2
40.1
43.0
44.0
48.4
49.1
49.5
49.5
52.6
57.0
57.7
60.3
70.8
70.8
73.6
73.6
76.3
77.6
80.6
80.8
86.4
87.7
95.7
105.1
108.7
112.4
117.9
131.4
133.9
135.6
138.3
138.3
143.4
158.5
166.9
167.3
174.3
174.6
175.3
175.5
176.6
177.3
180.1
f1 (ppm)

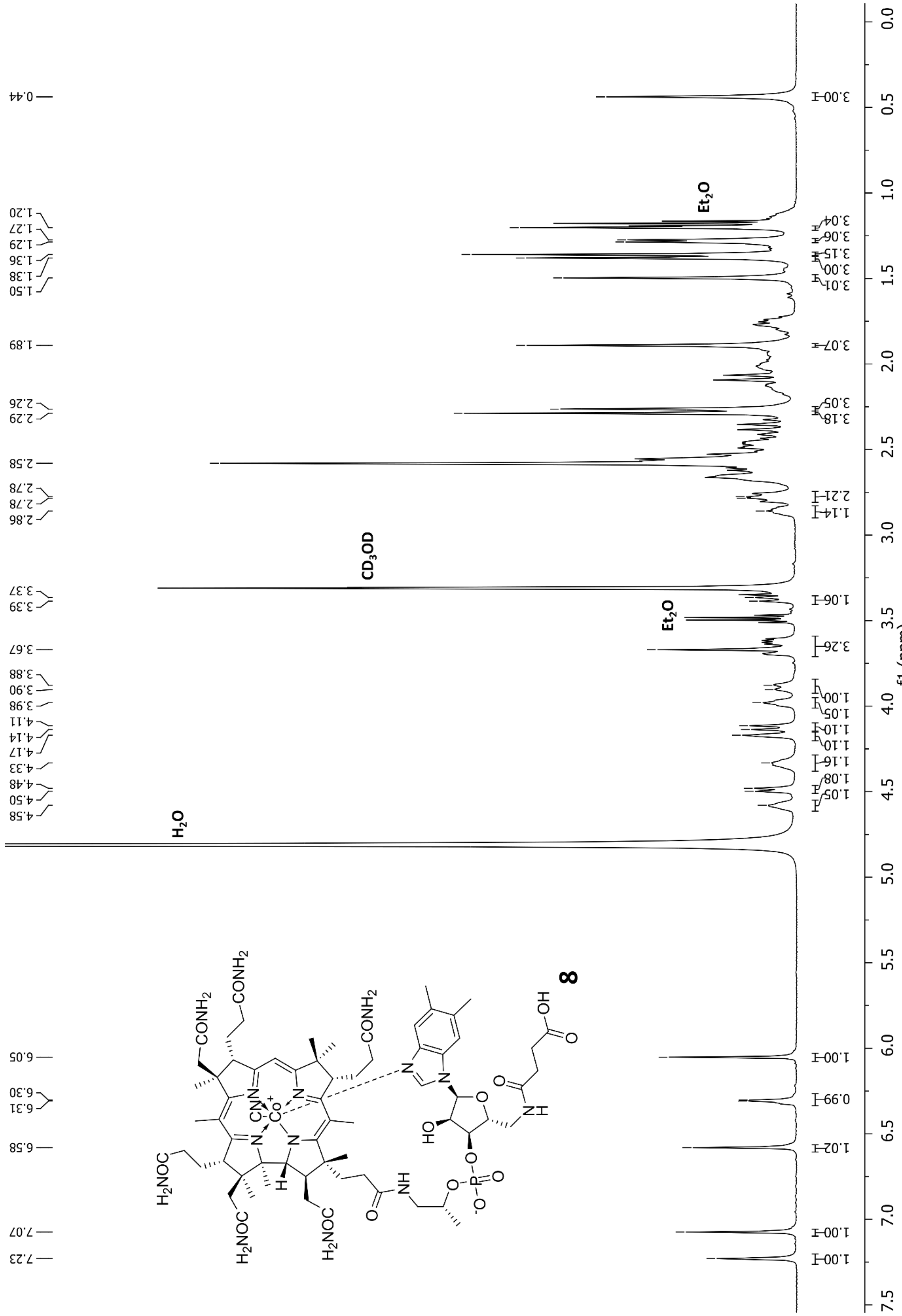
7b

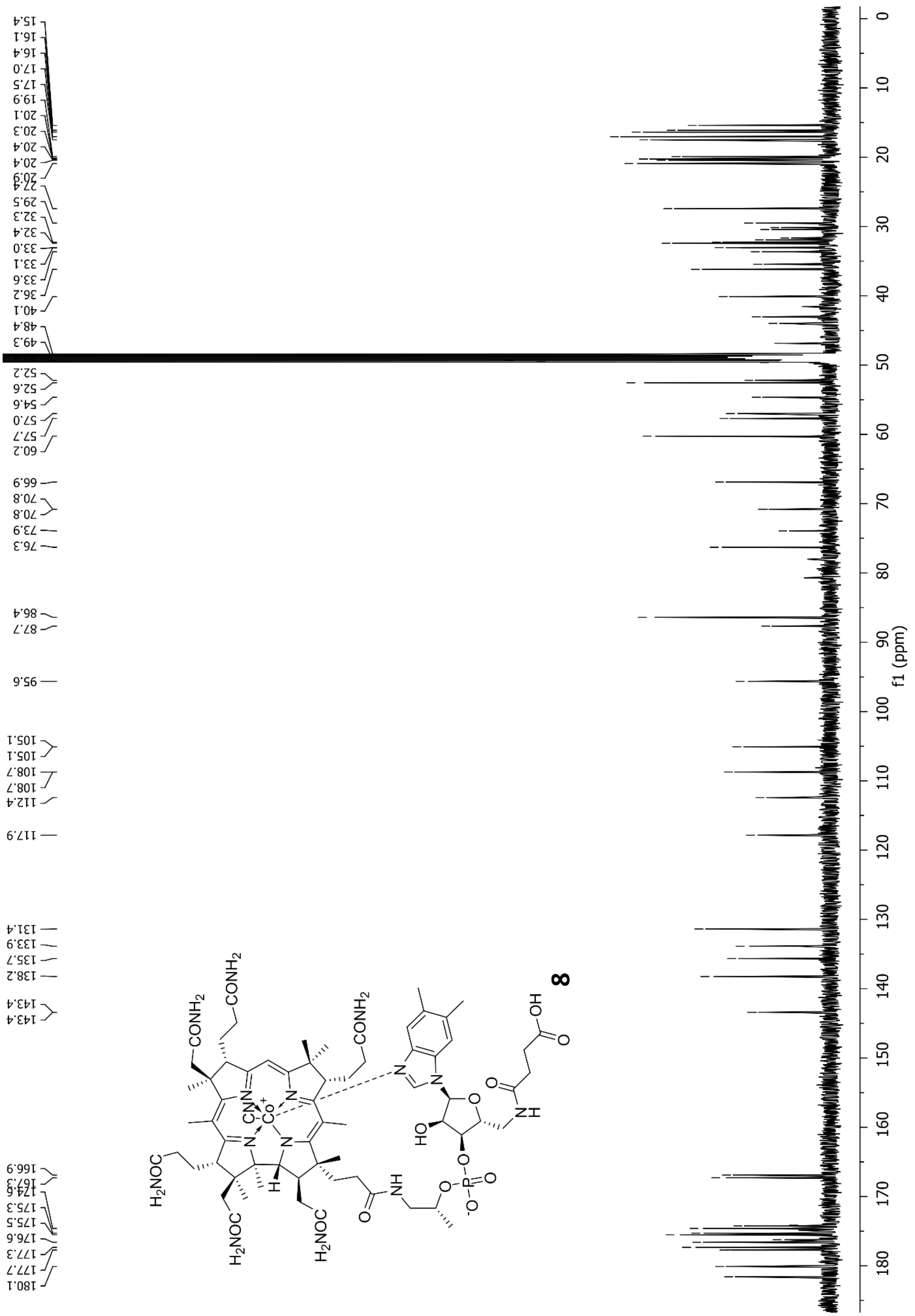
11. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **7d** (with coupling reagent) in CD_3OD (for clarity only well isolated signals are integrated)



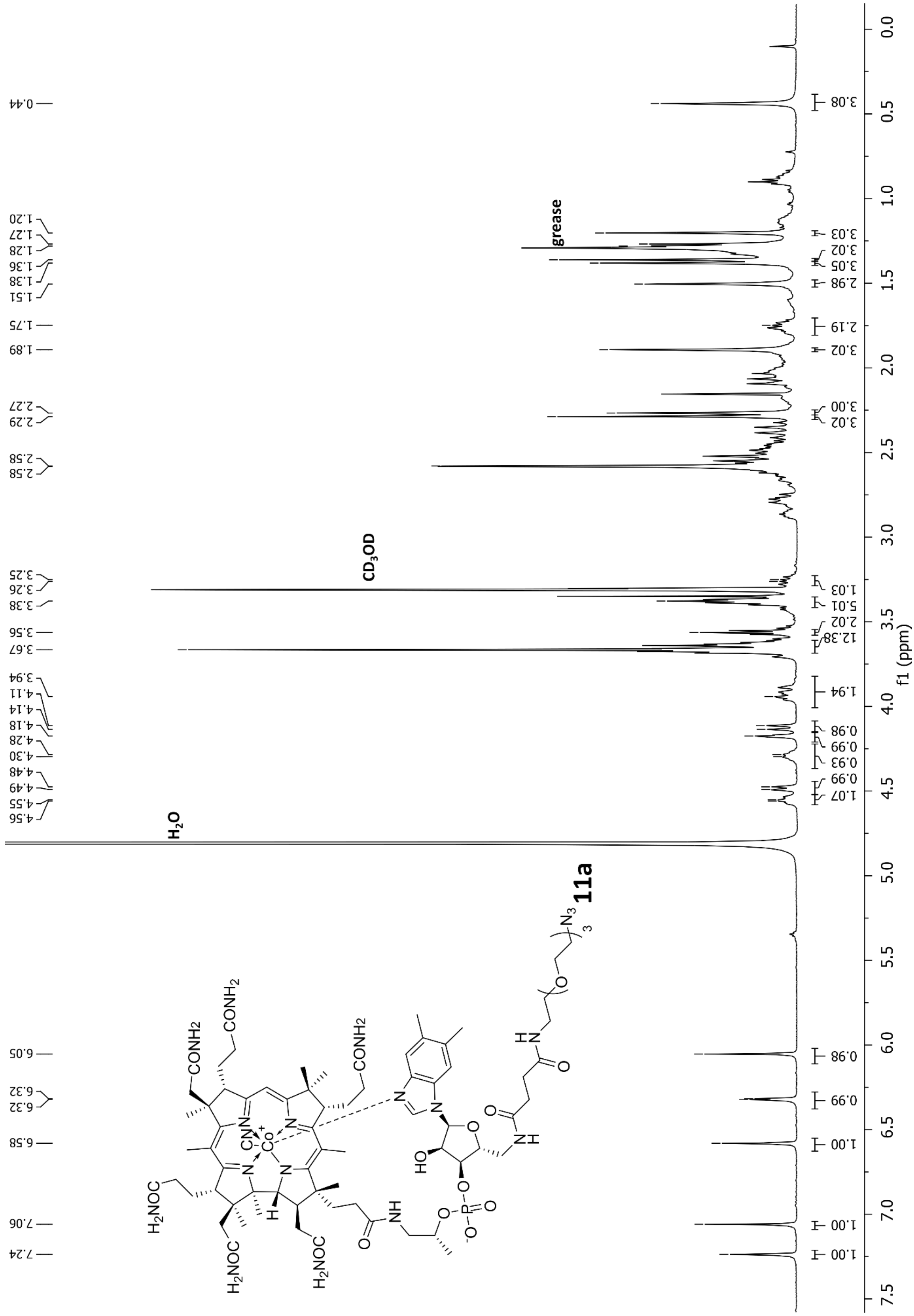

14.7
14.9
15.6
16.1
18.5
18.8
18.8
18.9
19.0
19.0
19.5
25.9
26.0
28.2
30.8
31.0
31.4
31.7
32.2
33.8
34.8
41.6
42.3
42.3
42.5
43.7
46.9
46.9
47.9
48.0
48.4
51.2
53.3
55.5
56.3
58.9
66.9
69.2
69.2
72.3
72.3
74.8
85.0
86.2
94.2
103.8
107.3
111.0
116.4
119.5
119.5
124.7
124.9
126.8
127.4
127.4
130.0
132.4
134.2
136.8
141.1
141.1
141.9
143.8
143.9
157.8
165.4
165.8
170.4
171.1
171.7
172.8
173.2
173.9
174.0
174.1
175.1
175.9
176.1
176.2
178.6
180.1
7d
f1 (ppm)

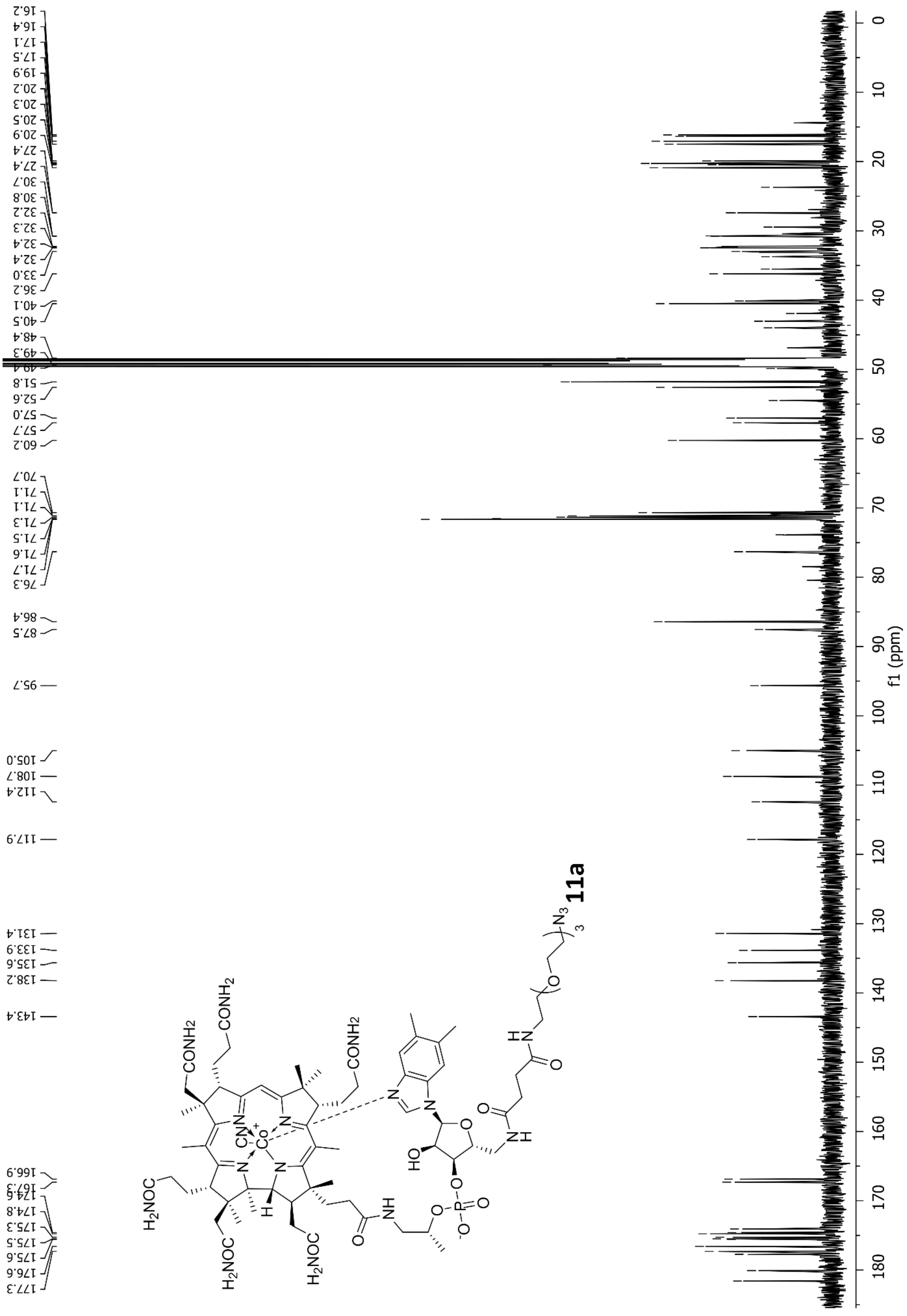
12. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **8** in CD_3OD (for clarity only well isolated signals are integrated)



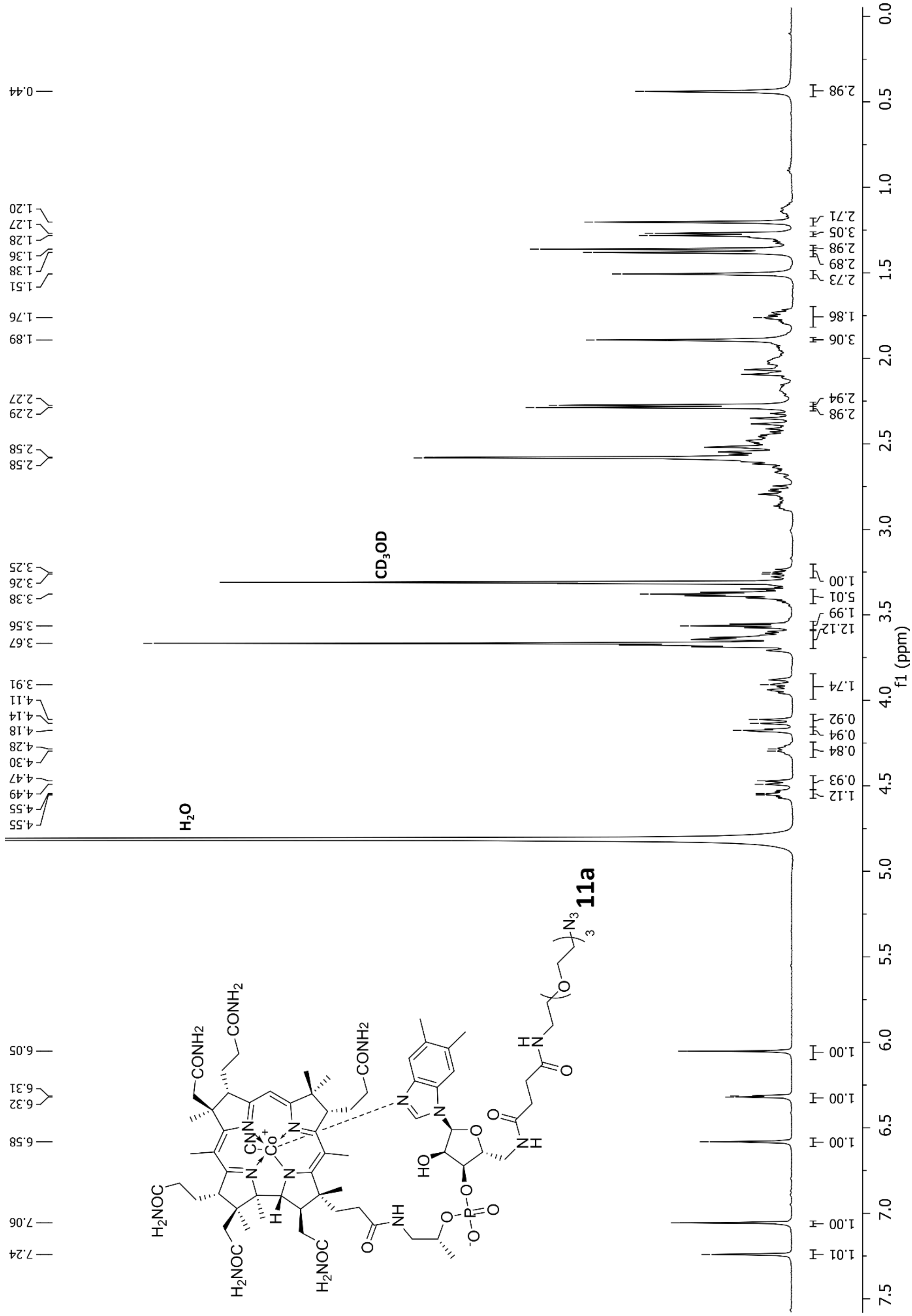

15.4
16.1
16.4
17.0
17.5
19.9
20.1
20.3
20.4
20.4
20.9
27.4
29.5
32.3
32.4
33.0
33.1
33.6
36.2
40.1
48.4
49.3
52.2
52.6
54.6
57.0
57.7
60.2
66.9
70.8
70.8
73.9
76.3
86.4
87.7
95.6
105.1
105.1
108.7
108.7
112.4
117.9
131.4
133.9
135.7
138.2
143.4
143.4
166.9
167.3
174.6
175.3
175.5
176.6
177.3
177.7
180.1
f1 (ppm)
8
HO
O
H
N
O
O
O
P
O-
OH
N
N
CONH2
H2NOC
CN
Co+
N
N
N
N
H
HN
O
CONH2
CONH2
H2NOC
H2NOC

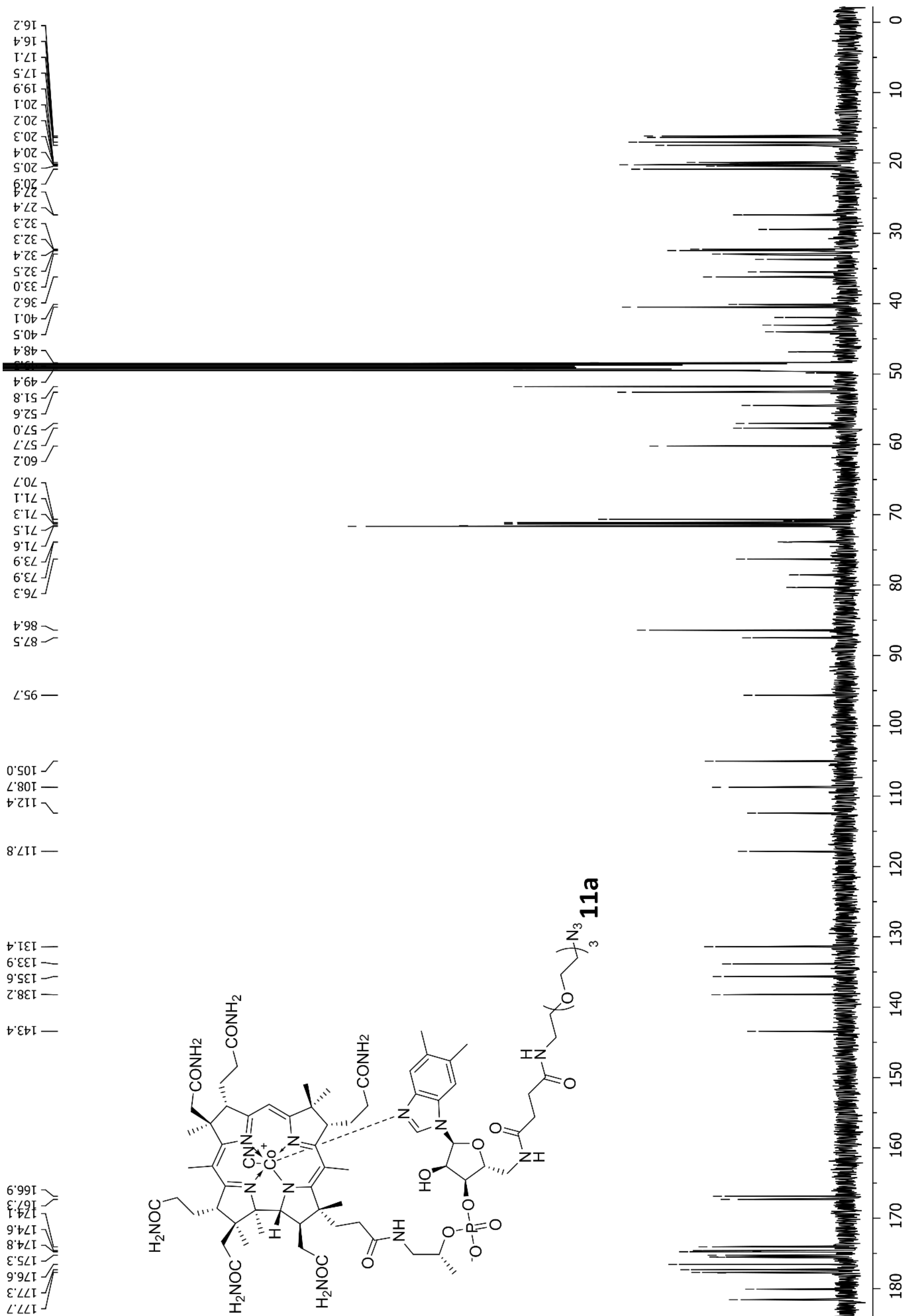
13. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **11a** (via active ester step) in CD_3OD (for clarity only well isolated signals are integrated)



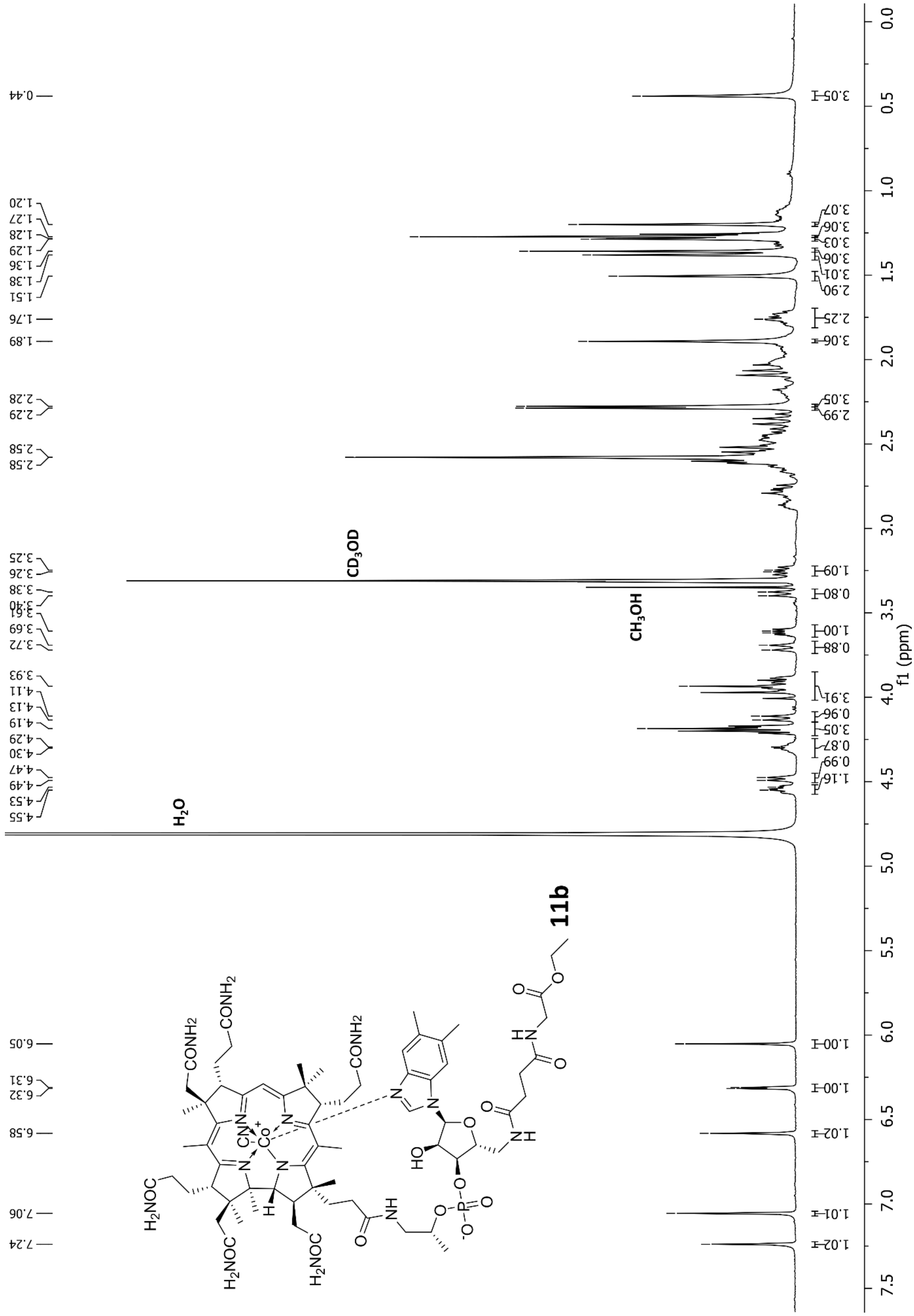

16.2
16.4
17.1
17.5
19.9
20.2
20.3
20.5
20.9
27.4
27.4
30.7
30.8
32.2
32.3
32.4
32.4
33.0
36.2
40.1
40.5
48.4
49.3
49.4
51.8
52.6
57.0
57.7
60.2
70.7
71.1
71.1
71.3
71.5
71.6
71.7
76.3
86.4
87.5
95.7
105.0
108.7
112.4
117.9
131.4
133.9
135.6
138.2
143.4
166.9
167.3
174.6
174.8
175.3
175.5
175.6
176.6
177.3
f1 (ppm)
11a

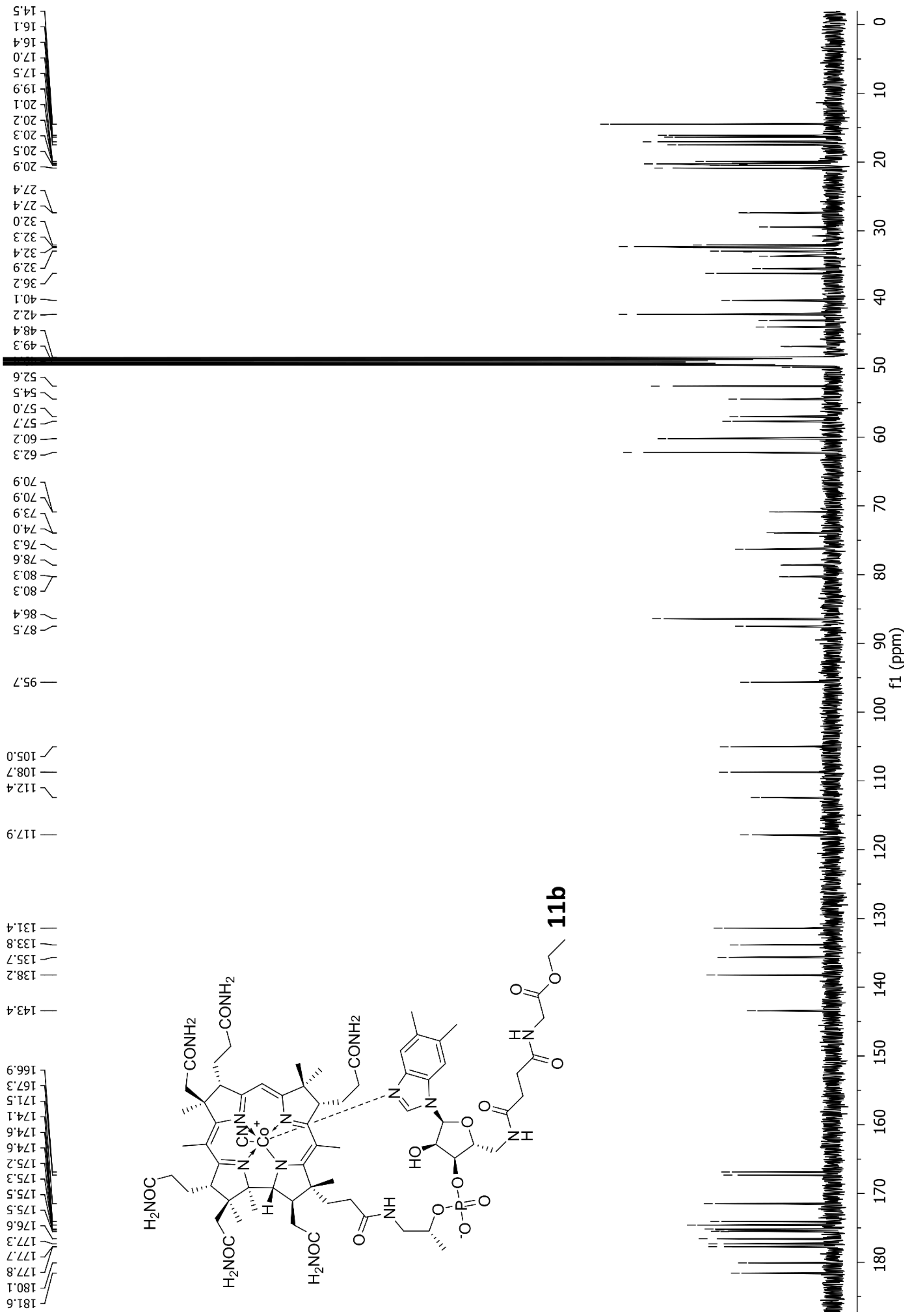
14. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **11a** in CD_3OD (for clarity only well isolated signals are integrated)



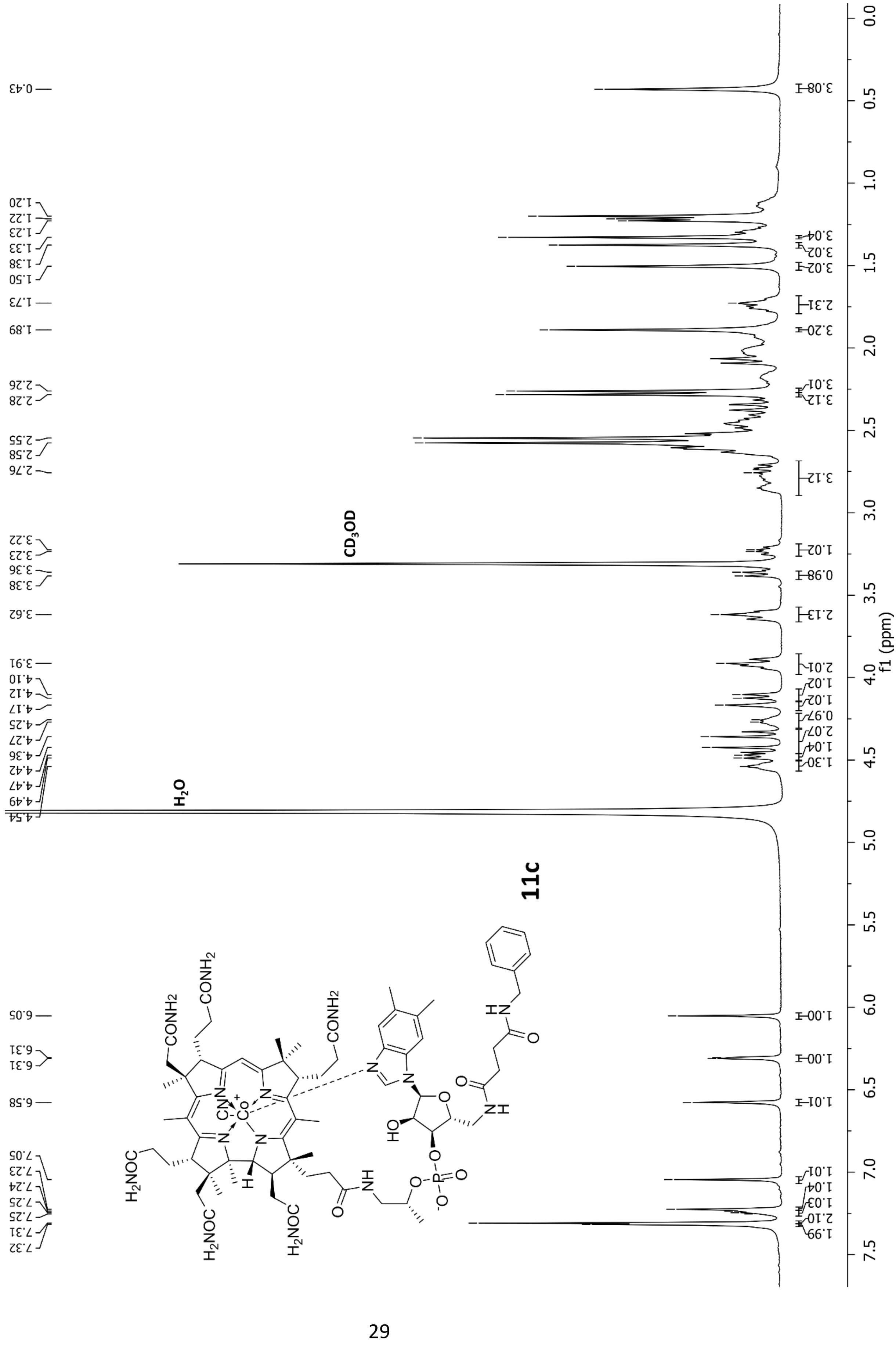

16.2
16.4
17.1
17.5
19.9
20.1
20.2
20.3
20.4
20.5
20.9
27.4
32.3
32.3
32.4
32.5
33.0
36.2
40.1
40.5
48.4
49.4
51.8
52.6
57.0
57.7
60.2
70.7
71.1
71.3
71.5
71.6
73.9
73.9
76.3
86.4
87.5
95.7
105.0
108.7
112.4
117.8
131.4
133.9
135.6
138.2
143.4
166.9
167.3
174.1
174.6
174.8
175.3
176.6
177.3
177.7
11a
f1 (ppm)

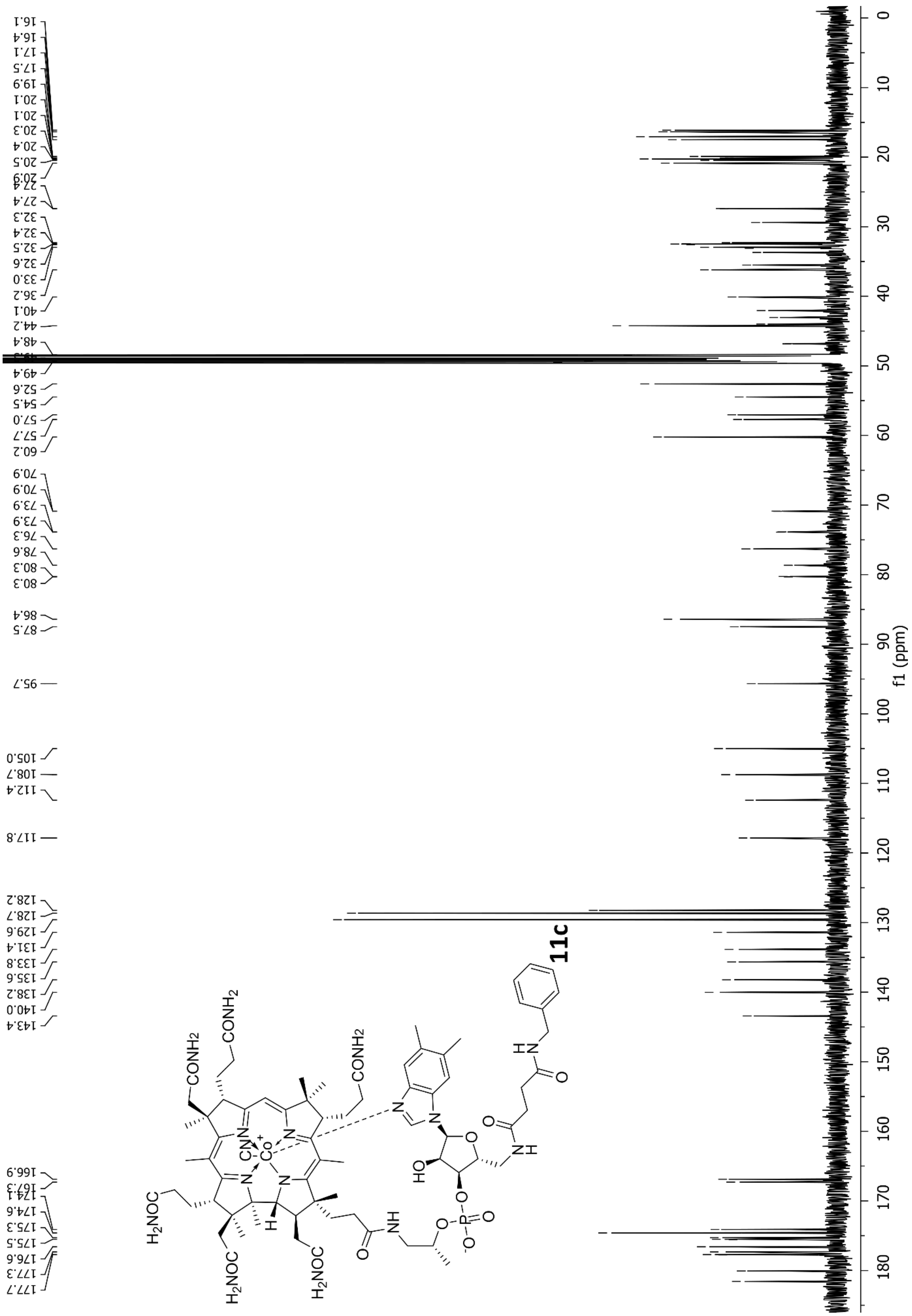
15. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) of compound **11b** in CD_3OD (for clarity only well isolated signals are integrated)




14.5
16.1
16.4
17.0
17.5
19.9
20.1
20.2
20.3
20.5
20.9
27.4
27.4
32.0
32.3
32.4
32.9
36.2
40.1
42.2
48.4
49.3
52.6
54.5
57.0
57.7
60.2
62.3
70.9
70.9
73.9
74.0
76.3
78.6
80.3
80.3
86.4
87.5
95.7
105.0
108.7
112.4
117.9
131.4
133.8
135.7
138.2
143.4
166.9
167.3
171.5
174.1
174.6
174.6
175.2
175.3
175.5
175.5
176.6
177.3
177.7
177.8
180.1
181.6
11b
f1 (ppm)

16. [1]H NMR (500 MHz) and [13]C NMR (126 MHz) of compound **11c** in CD_3OD (for clarity only well isolated signals are integrated)




16.1
16.4
17.1
17.5
19.9
20.1
20.1
20.3
20.4
20.5
20.9
27.4
27.4
32.3
32.4
32.5
32.6
33.0
36.2
40.1
44.2
48.4
49.4
52.6
54.5
57.0
57.7
60.2
70.9
70.9
73.9
73.9
76.3
78.6
80.3
80.3
86.4
87.5
95.7
105.0
108.7
112.4
117.8
128.2
128.7
129.6
131.4
133.8
135.6
138.2
140.0
143.4
166.9
167.3
174.1
174.6
175.3
175.5
176.6
177.3
177.7
f1 (ppm)
11c