ELECTRONIC SUPPLEMENTARY INFORMATION

Fluorination as tool to improve bioanalytical sensitivity and COX-2-selective antitumor activity of cobalt alkyne complexes

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Synthesis and characterization of intermediate products

6-Fluorosalicylic acid (6F-SS)

From 5.00 g of 2,6-difluorobenzoic acid (31.6 mmol), 5.06 g of NaOH (127 mmol) and 62.9 ml of anhydrous DMSO as solvent (4 h, 130 °C). DMSO was removed under reduced pressure. The residue was dissolved in 250 ml of HCl solution (3%) and stirred at room temperature for 15 min. The product was obtained by filtration and washed with water, followed by drying in a desiccator.

Off-white powder; yield: 1.99 g (12.75 mmol, 40%); mp: 162 °C.

^{1}H NMR (200 MHz, d6-DMSO): δ 6.71-6.83 (m, 2H, ArH), 7.37-7.49 (m, 1H, ArH); IR ($\bar{\nu}$ cm^{-1}): 3026-2537 w (COOH), 1651 s (C=O), 1619 s (C=C), 1448 s, 1199 s, 1020 s, 810 s.

General procedure for the synthesis of the fluoroacetylsalicylic acids

2.0 g of fluorosalicylic acid (12.8 mmol) were dissolved in 10 ml of THF and added dropwise under cooling to a mixture of 10 ml of THF, 128 mmol acetic anhydride and 25.6 mmol of TEA. The mixture was stirred at room temperature for about 24 h. 40 ml of water was added and the reaction mixture was acidified with 1M HCl, then extracted three times with diethyl ether. The collected ether phases were washed with water and brine and were dried over sodium sulfate. The solvent was removed under reduced pressure. The product was recrystallized from toluene.

3-Fluoroacetylsalicylic acid (3F-ASS)

Reaction time: 26 h; colorless powder; yield: 1.81 g (9.14 mmol, 71%); mp: 124 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3), 7.25-7.48 (m, 2H, ArH-4, ArH-5), 7.88 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.0 Hz, 1H, ArH-6); IR ($\bar{\nu}$ cm^{-1}): 2968-2528 w (COOH), 1780 s (C=O), 1691 s (C=O), 1586 m (C=C), 1146 s, 900 s, 758 s.

4-Fluoroacetylsalicylic acid (4F-ASS)

Reaction time: 25.5 h; colorless powder; yield: 1.86 g (9.39 mmol, 73%); mp: 123 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 6.89 (dd, $^3J_{(H-F)}$ = 8.8 Hz, ^{4}J = 2.4 Hz, 1H, ArH-3), 7.02-7.12 (m, 1H, ArH-5), 8.16 (ddd, $^3J_{(H-F)}$ = 8.8 Hz, ^{3}J = 6.8 Hz, ^{4}J = 2.4 Hz, 1H, ArH-6); IR ($\bar{\nu}$ cm^{-1}): 3106-2647 w (COOH), 1763 s (C=O), 1690 s (C=O), 1605 m (C=C), 1207 s, 1082 s, 898 s, 857 s.

5-Fluoroacetylsalicylic acid (5F-ASS)

Reaction time: 22 h; colorless powder; yield: 1.91 g (9.64 mmol, 75%); mp: 135 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 7.21 (ddd, $^{3}J_{(H-F)}$ = 8.8 Hz, ^{3}J =4.8 Hz, ^{4}J = 3.2 Hz, 1H, ArH-4), 7. 82-7.38 (m, 1H, ArH-3), 7.80 (dd, $^{3}J_{(H-F)}$ = 8.8 Hz, ^{4}J = 3.2 Hz, 1H, ArH-6); IR ($\overline{\nu}$ cm^{-1}): 2998-2648 w (COOH), 1753 s (C=O), 1692 s (C=O), 1584 m (C=C), 1120 s, 891 s, 843 s.

6-Fluoroacetylsalicylic acid (6F-ASS)

Reaction time: 26.5 h; colorless powder; yield: 1.40 g (7.07 mmol, 55%); mp: 106 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 6.95-7.12 (m, 2H, ArH-3, ArH-5), 7.49-7.60 (m, 1H, ArH-4); IR ($\overline{\nu}$ cm^{-1}): 2937-2501 w (COOH), 1769 m (C=O), 1712 s (C=O), 1618 m (C=C), 1197 s, 1025 s, 871 s, 779 s.

Table S1: Graphite furnace time-temperature program for Co analysis by HR CS AAS

Step	Operation	Temperature	Heating rate	Hold time	Argon flow
1	Drying	80 °C	10 °C/s	10 s	Maximal
2	Drying	100 °C	10 °C/s	25 s	Maximal
3	Drying	120 °C	15 °C/s	10 s	Maximal
4	Pyrolysis	1100 °C	200 °C/s	20 s	Maximal
5	Auto-zero	1100 °C	0 °C/s	5 s	Stop
6	Atomization	2300 °C	1500 °C/s	5 s	Stop
7	Cleaning	2600 °C	500 °C/s	4 s	Maximal

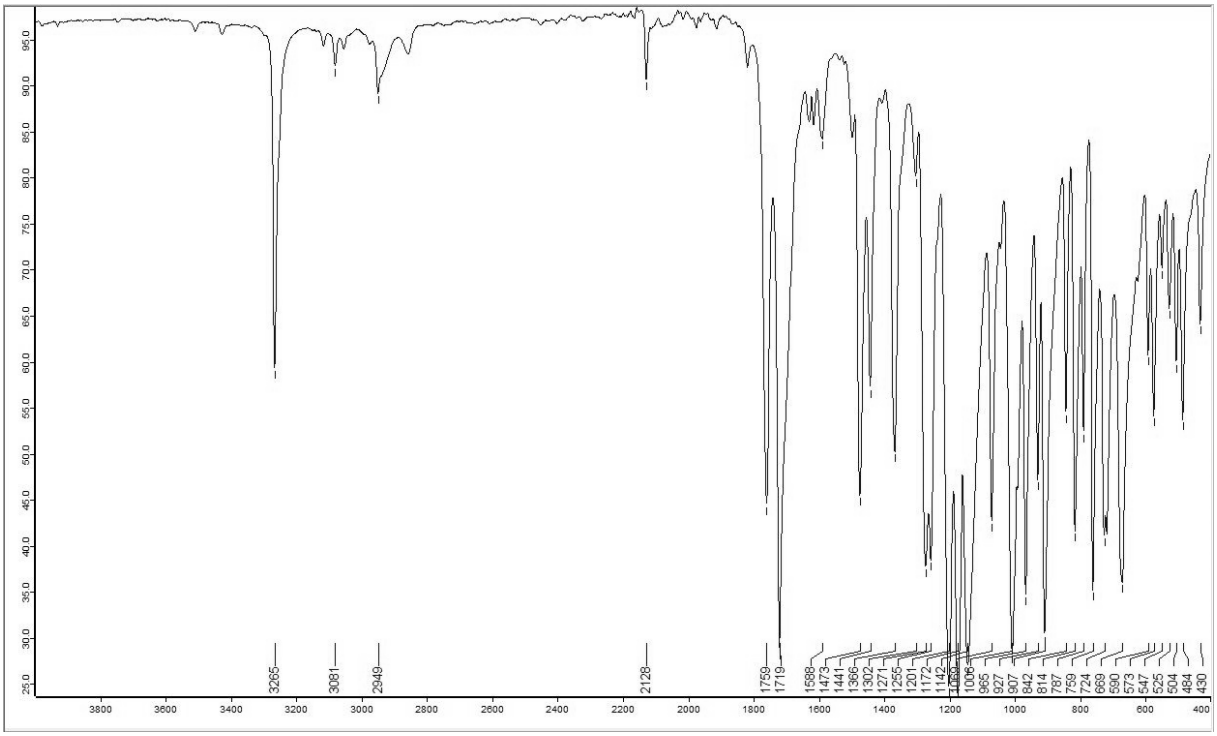
Table S2: Graphite furnace time-temperature program for F analysis by HR CS MAS

Step	Operation	Temperature	Heating rate	Hold time	Argon flow
1	Drying	120 °C	30 °C/s	5 s	Maximal
2	Cooling	100 °C	0 °C/s	1 s	Maximal
3	Drying	120 °C	2 °C/s	30 s	Maximal
4	Drying	300 °C	20 °C/s	3 s	Maximal
5	Pyrolysis	520 °C	200 °C/s	10 s	Maximal
6	Auto-zero	520 °C	0 °C/s	5 s	Stop
7	Vaporization	1400 °C	800 °C/s	7 s	Stop
8	Cleaning	2400 °C	1000 °C/s	4 s	Maximal

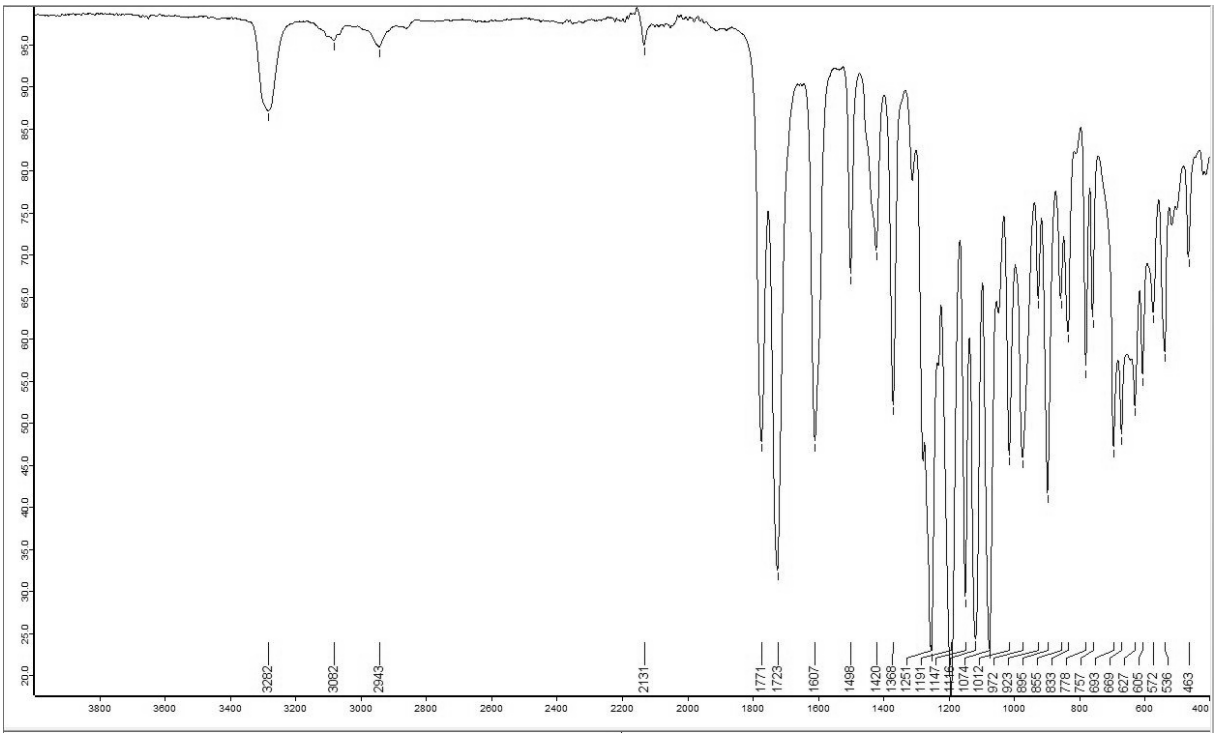
Table S3: Inhibition of COX-1/2

Compound	Concentration [µM]	Inhibition COX-1 [%]	Inhibition COX-2 [%]
ASS	10	28.80 ± 3.70	0.60 ± 2.80
ASS	100	73.30 ± 8.70	59.40 ± 8.70
3F-ASS	100	1.61 ± 1.53	47.42 ± 8.01
4F-ASS	100	85.42 ± 2.75	48.91 ± 1.14
5F-ASS	100	50.40 ± 1.33	42.95 ± 2.20
6F-ASS	100	40.49 ± 0.51	51.42 ± 1.34
Propargyl-ASS	100	25.41 ± 4.44	34.49 ± 4.21
Propargyl-3F-ASS	100	15.41 ± 4.06	44.75 ± 0.81
Propargyl-4F-ASS	100	30.35 ± 2.80	45.09 ± 0.84
Propargyl-5F-ASS	100	20.28 ± 3.34	50.19 ± 1.24
Propargyl-6F-ASS	100	25.41 ± 6.68	44.15 ± 0.78
Co-ASS	10	82.70 ± 3.20	78.50 ± 6.10
3F-Co-ASS	10	45.30 ± 3.90	67.70 ± 3.50
4F-Co-ASS	10	45.00 ± 5.40	73.90 ± 1.80
5F-Co-ASS	10	38.10 ± 4.90	68.00 ± 5.70
6F-Co-ASS	10	44.30 ± 3.10	66.80 ± 1.90

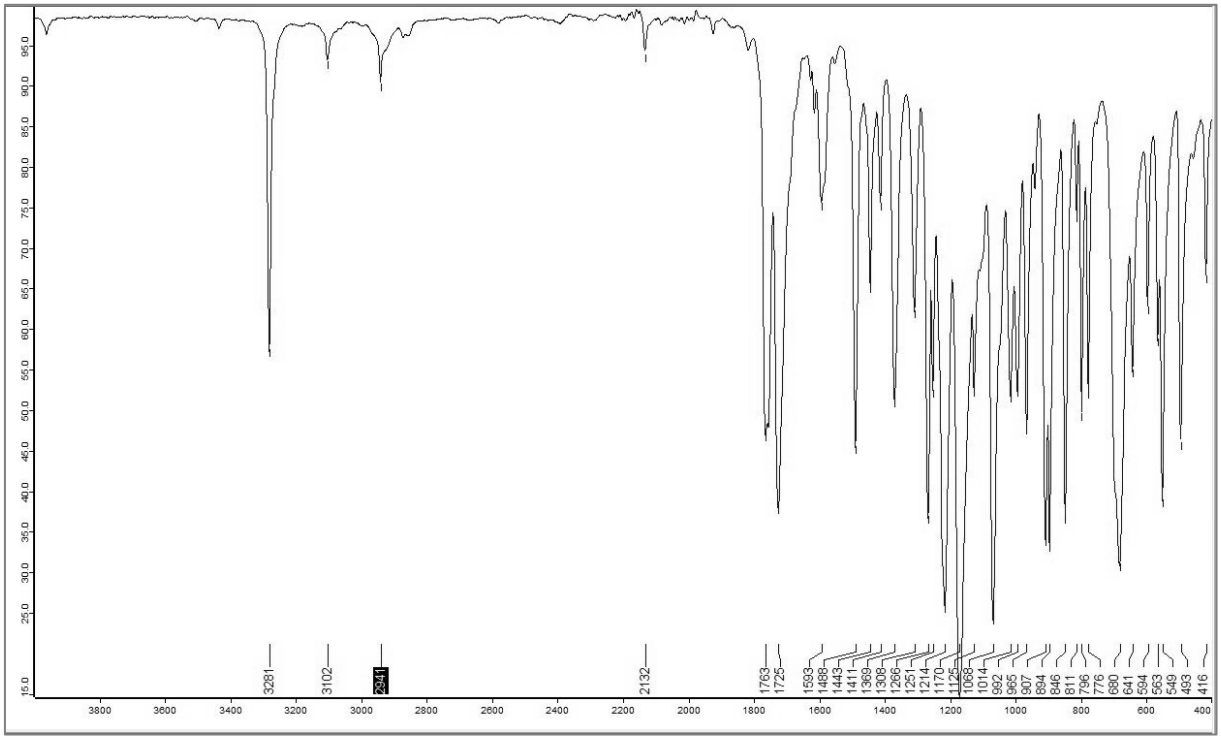
Supporting Figure S1: IR spectrum of propargyl-3F-ASS



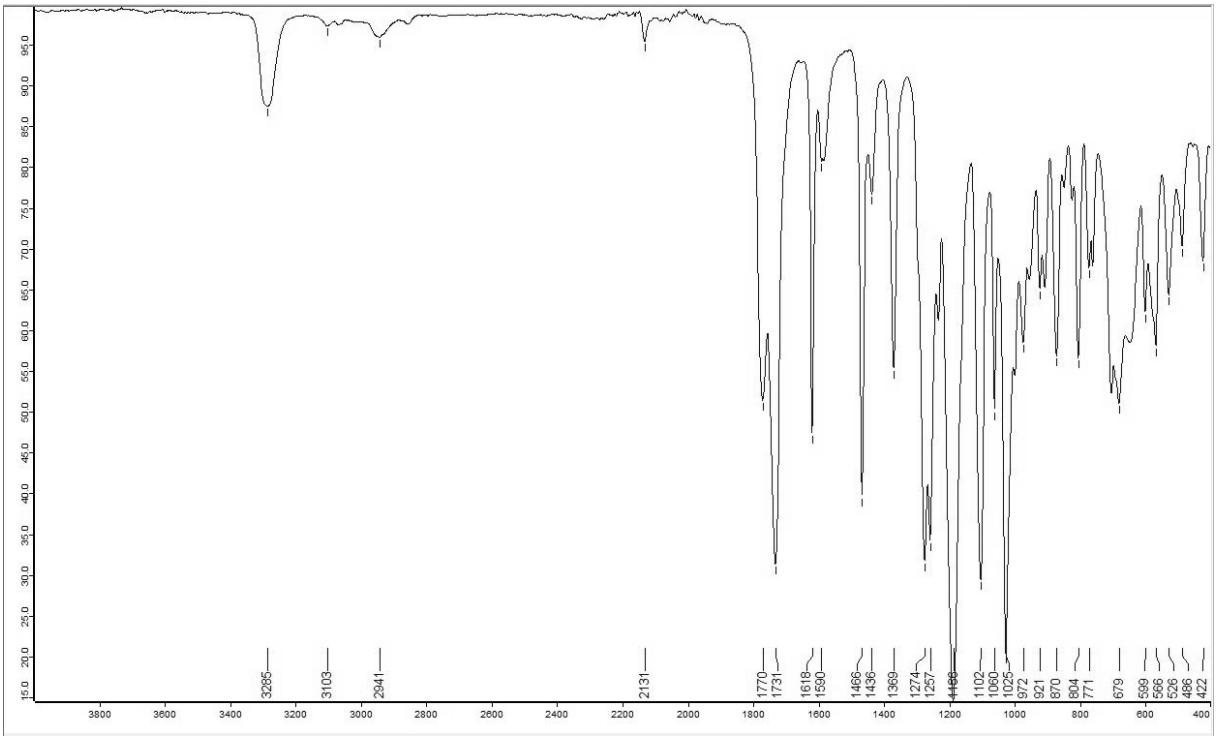
Supporting Figure S2: IR spectrum of propargyl-4F-ASS



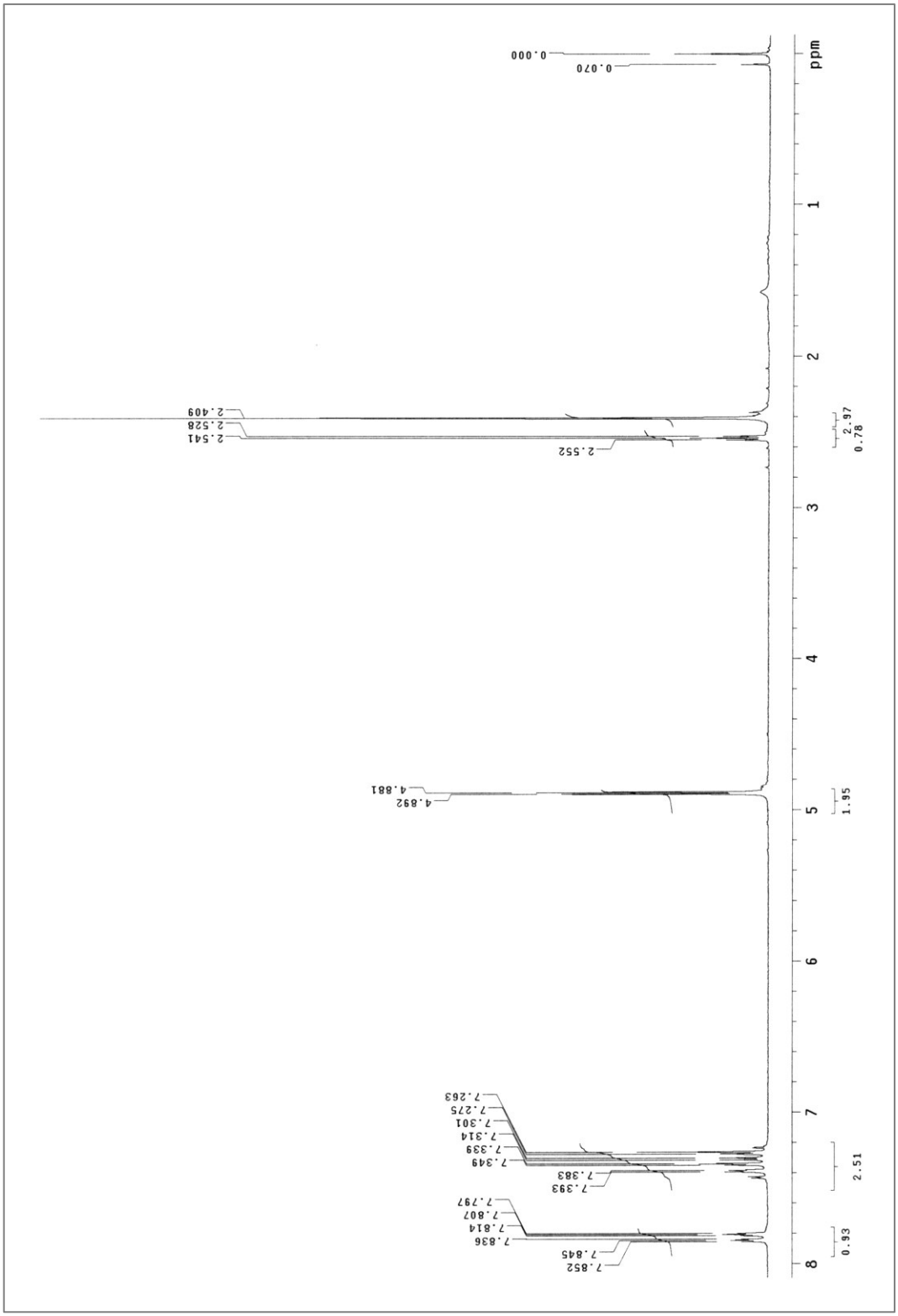
Supporting Figure S3: IR spectrum of propargyl-5F-ASS



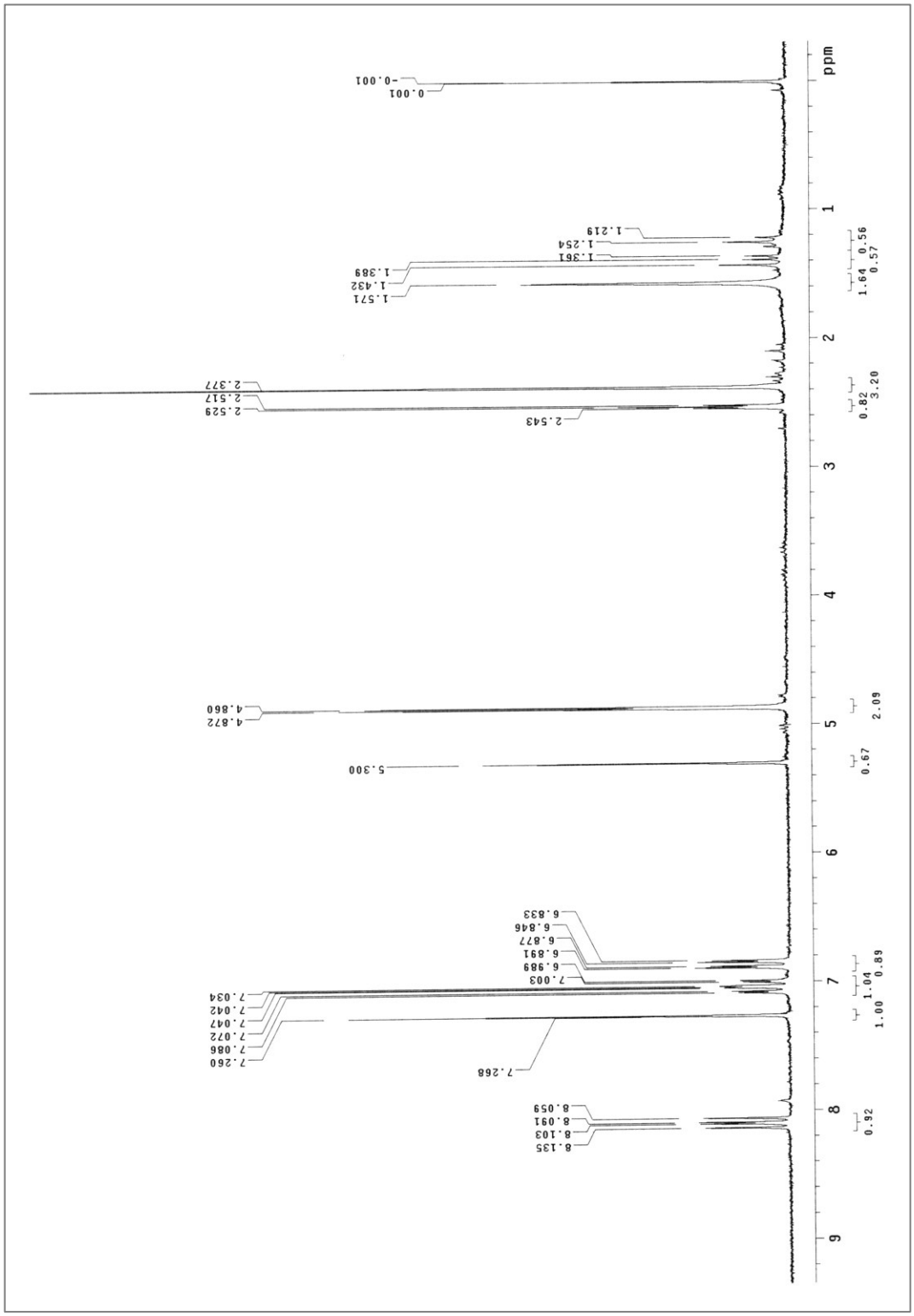
Supporting Figure S4: IR spectrum of propargyl-6F-ASS



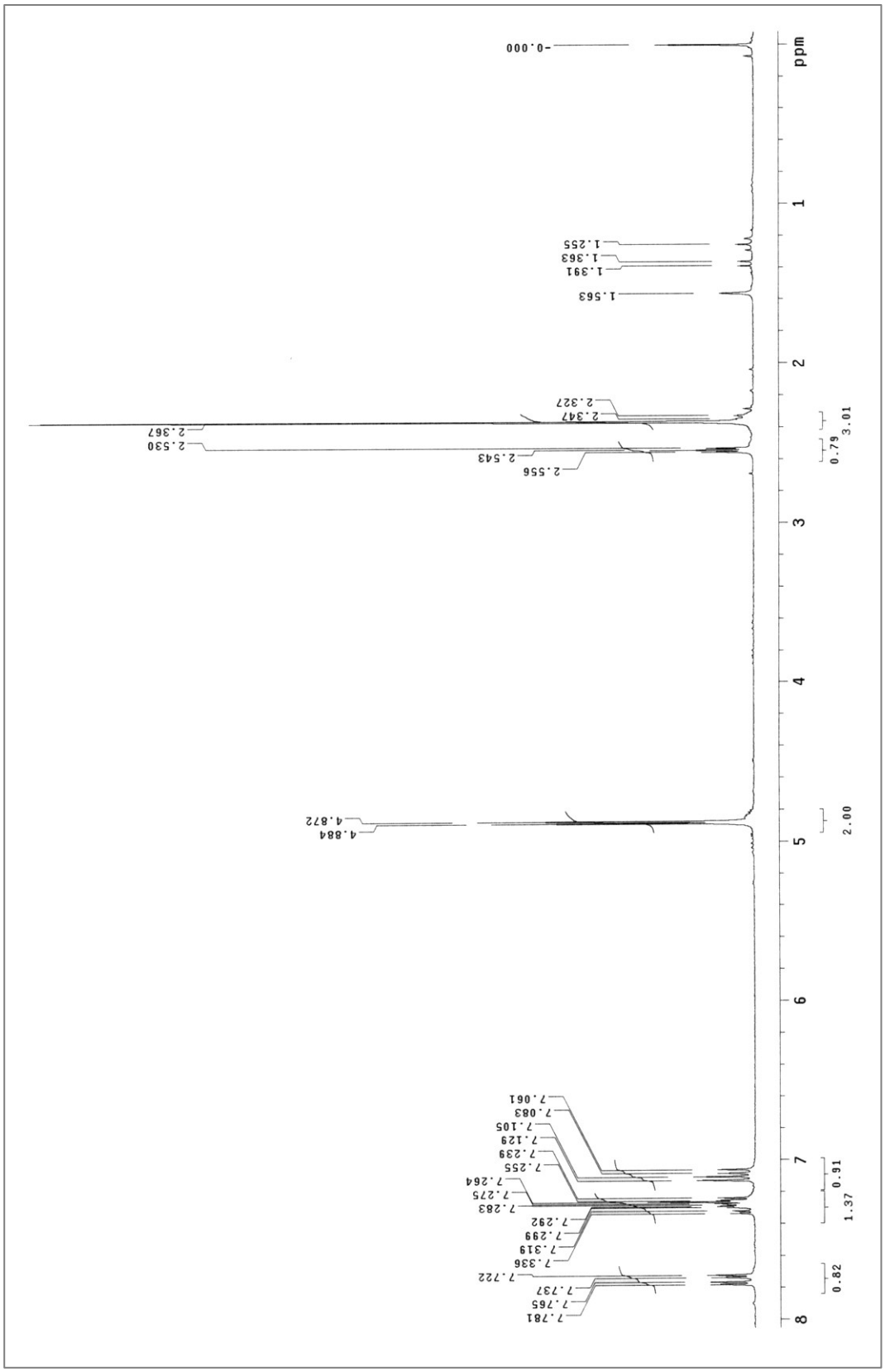
Supporting Figure S5: ^{1}H NMR spectrum of propargyl-3F-ASS



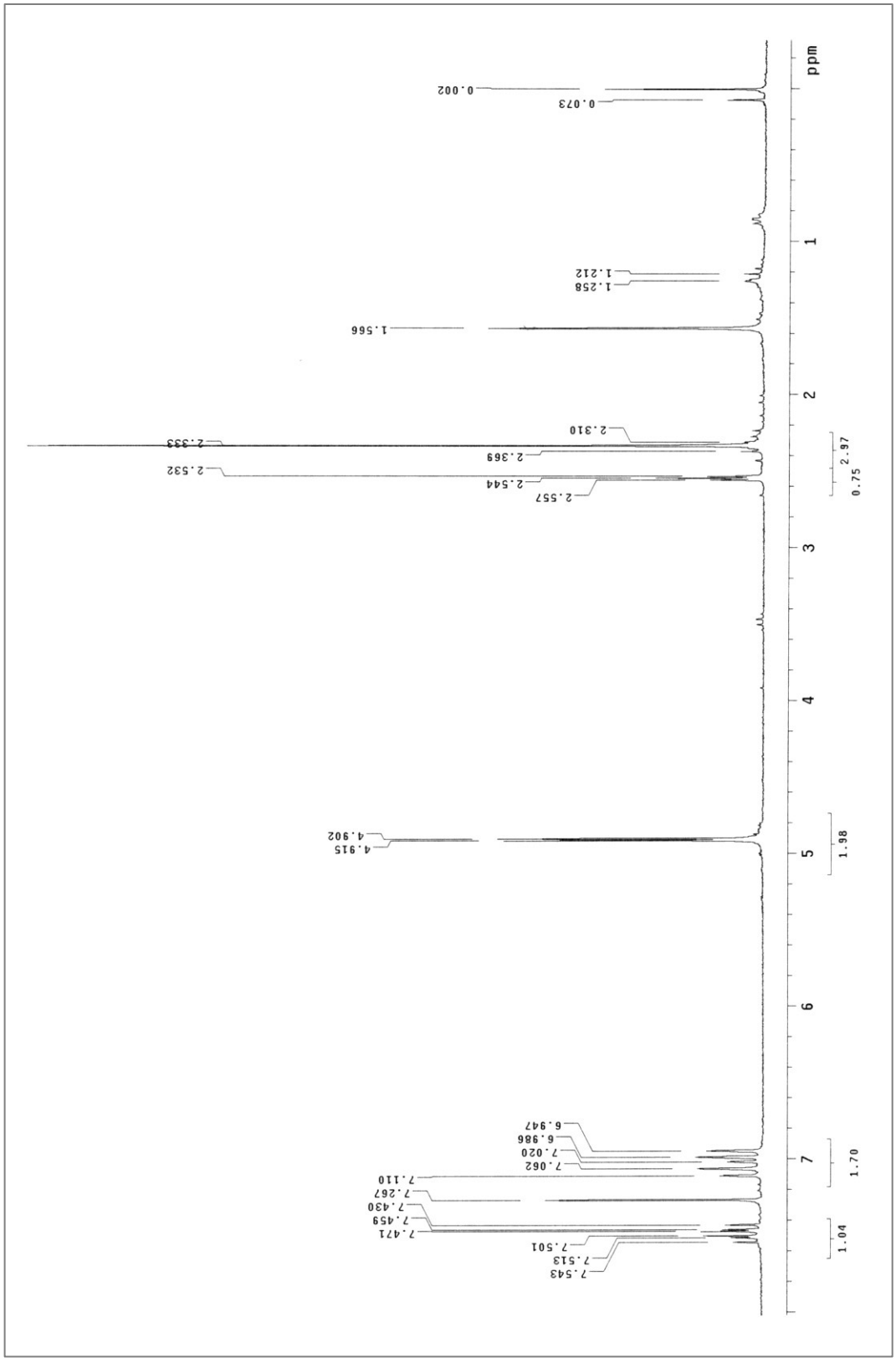
Supporting Figure S6: ^{1}H NMR spectrum of propargyl-4F-ASS



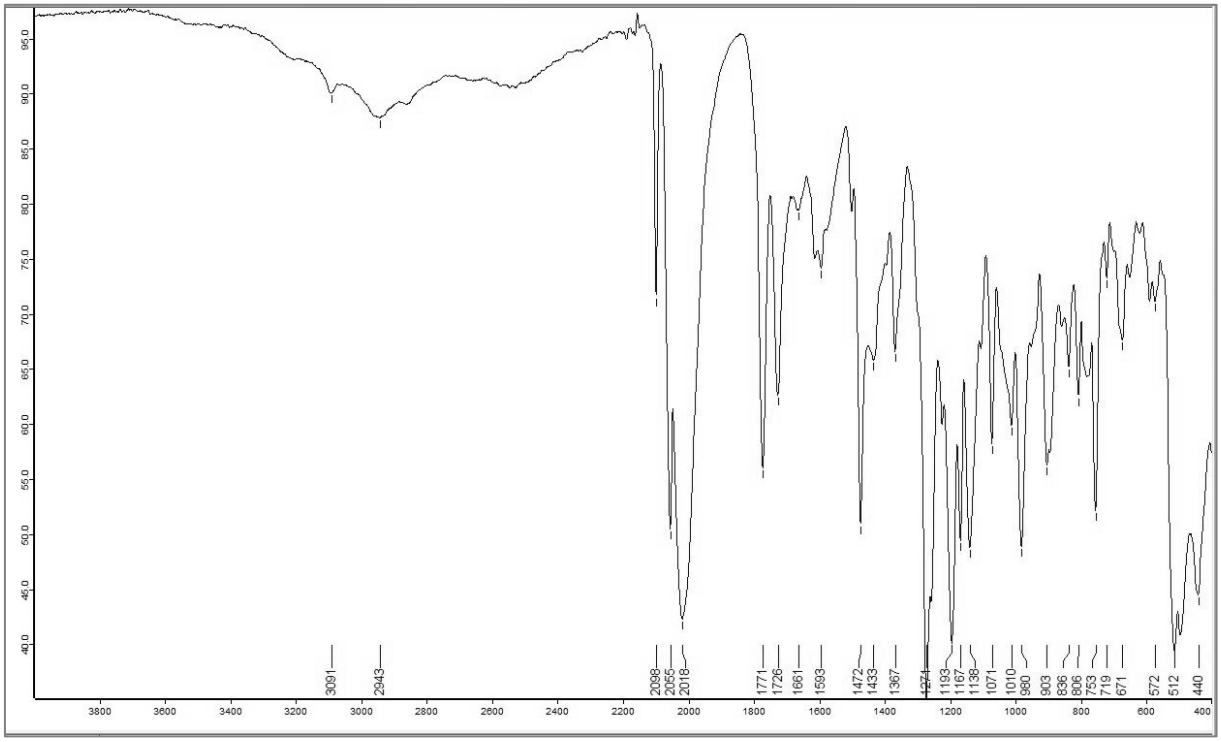
Supporting Figure S7: ^{1}H NMR spectrum of propargyl-5F-ASS



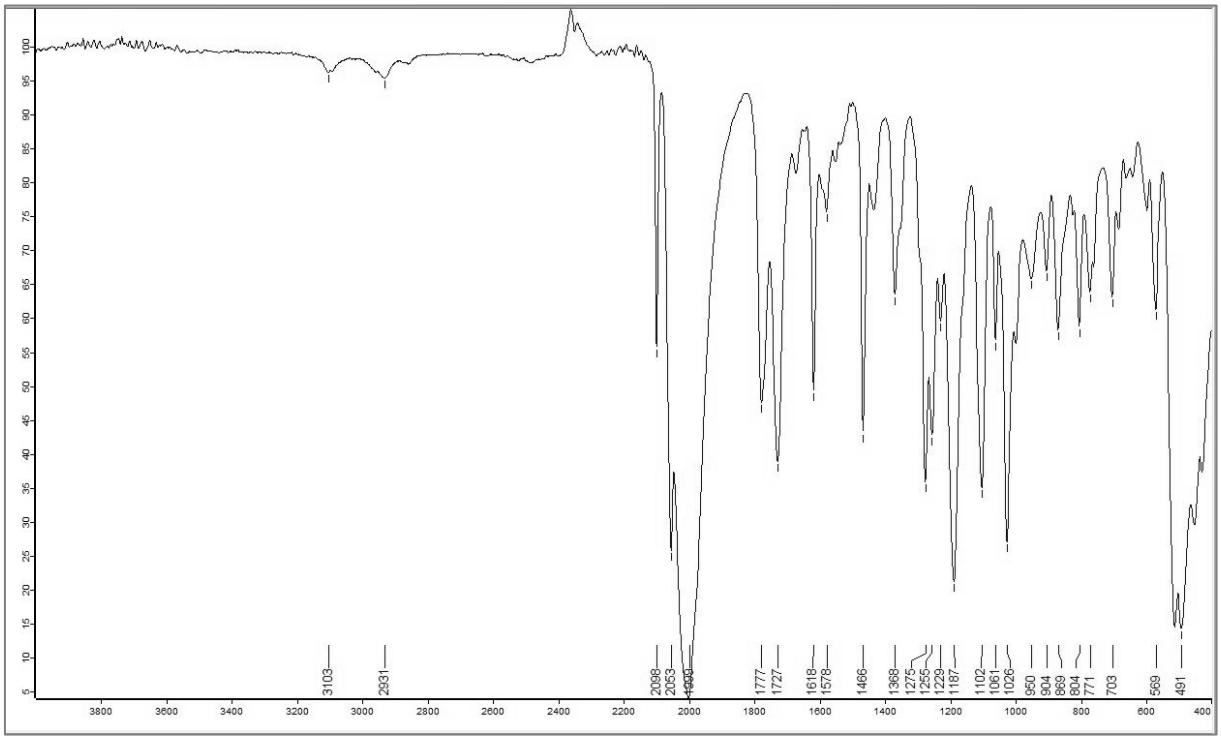
Supporting Figure S8: ^{1}H NMR spectrum of propargyl-6F-ASS



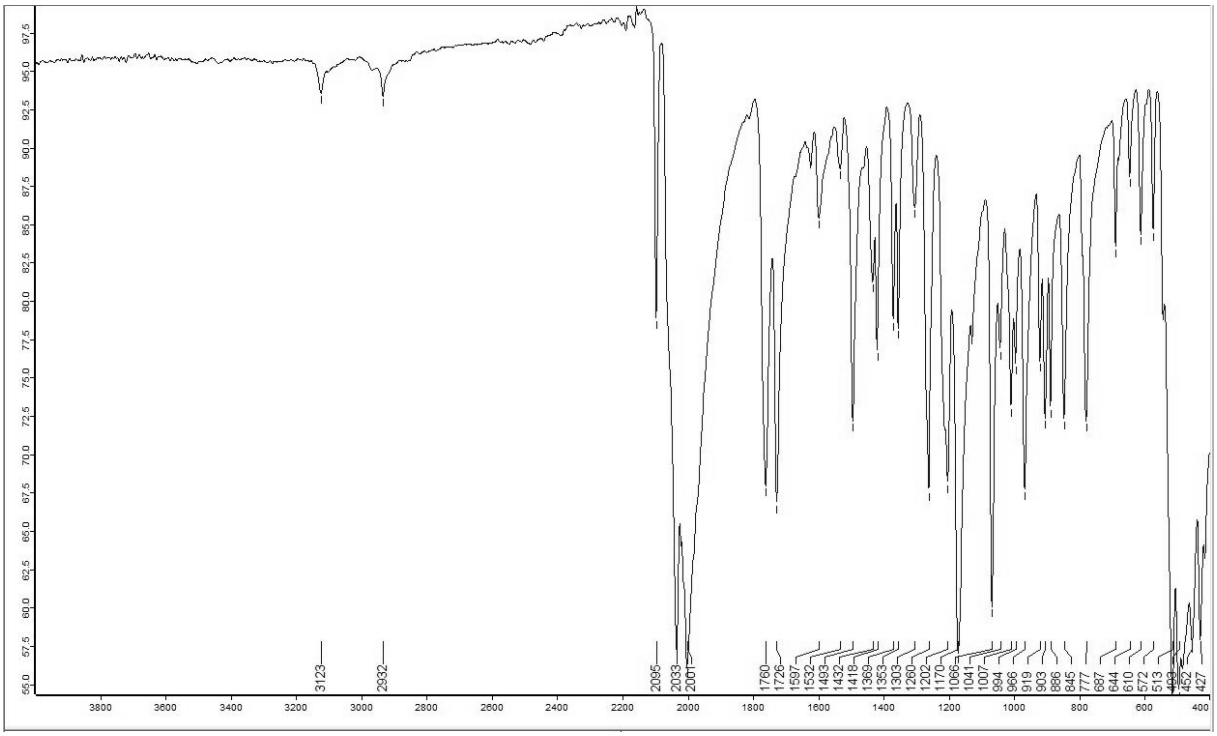
Supporting Figure S9: IR spectrum of 3F-Co-ASS



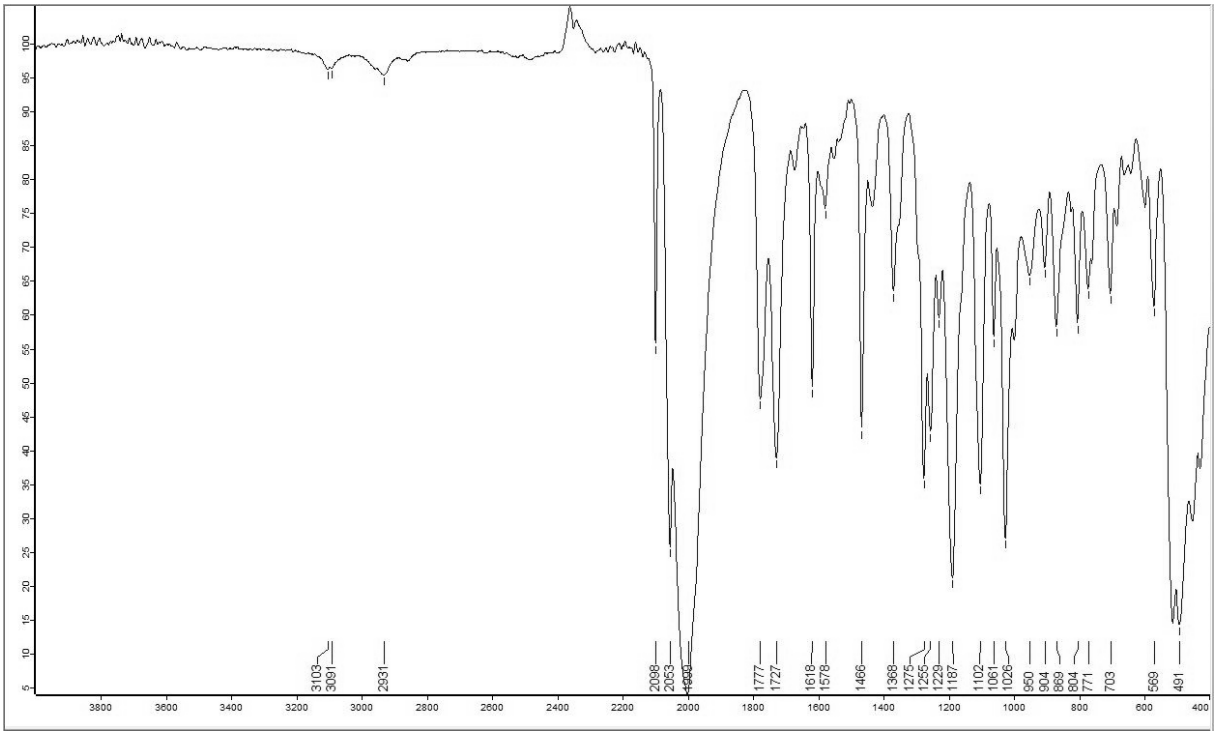
Supporting Figure S10: IR spectrum of 4F-Co-ASS



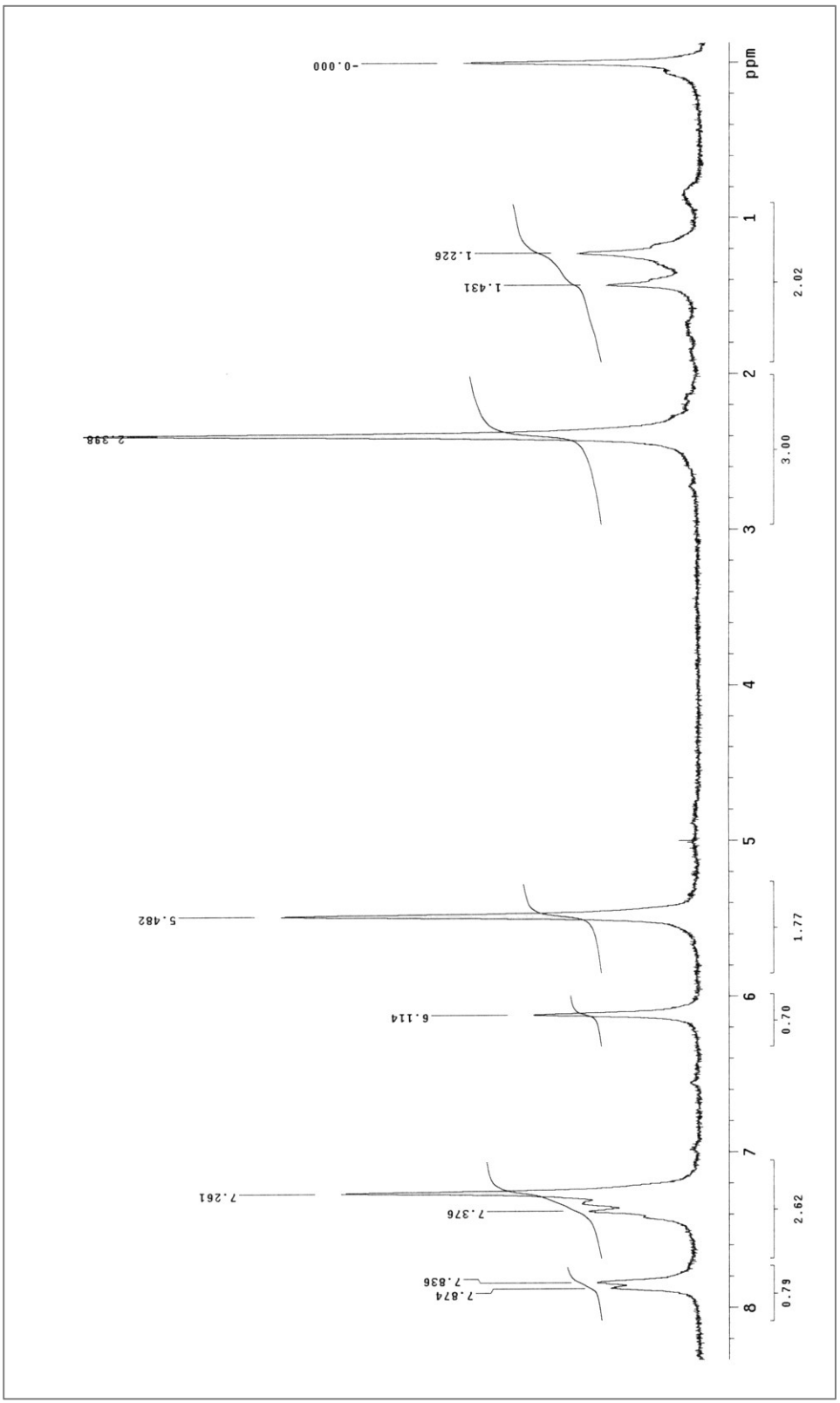
Supporting Figure S11: IR spectrum of 5F-Co-ASS



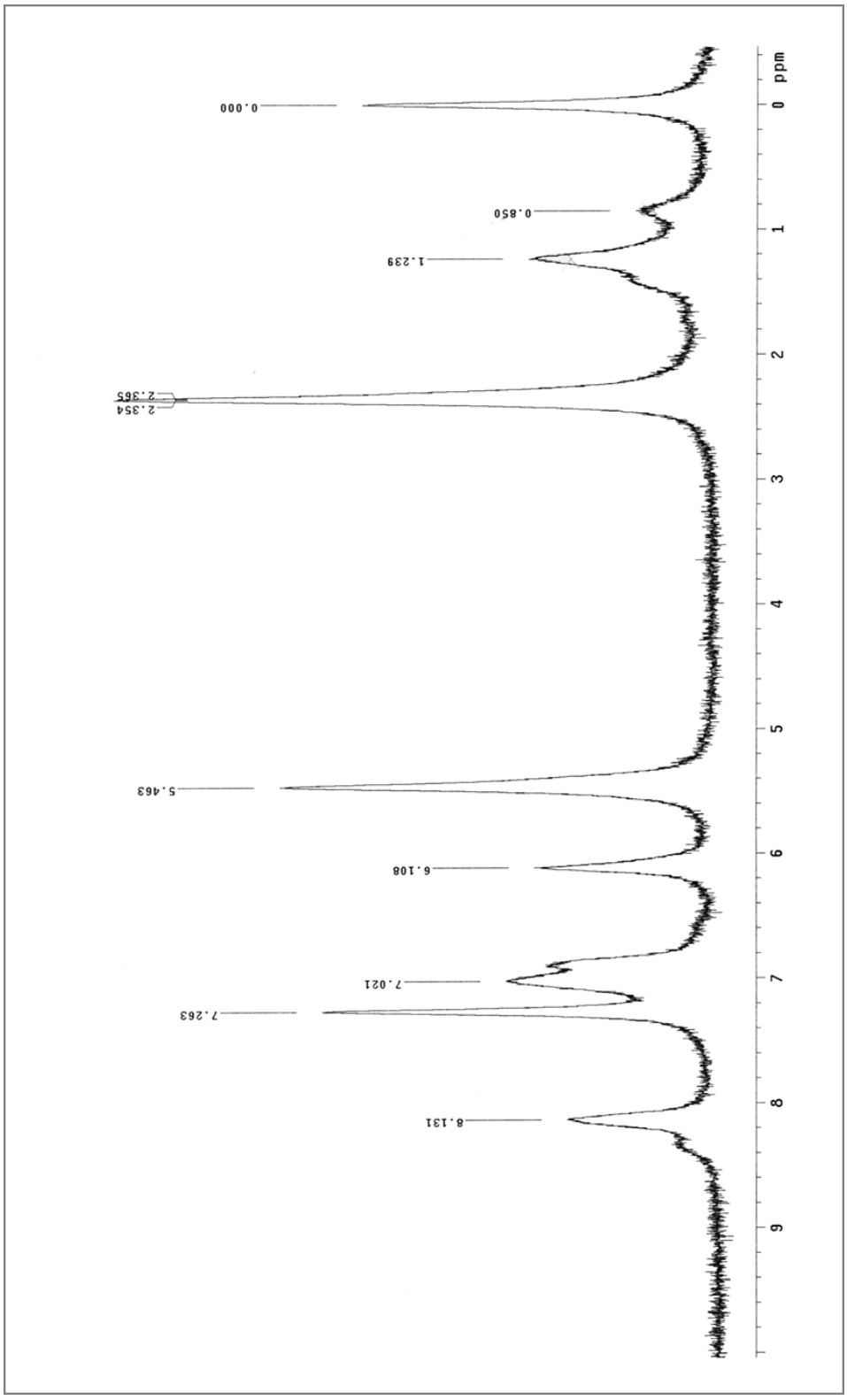
Supporting Figure S12: IR spectrum of 6F-Co-ASS



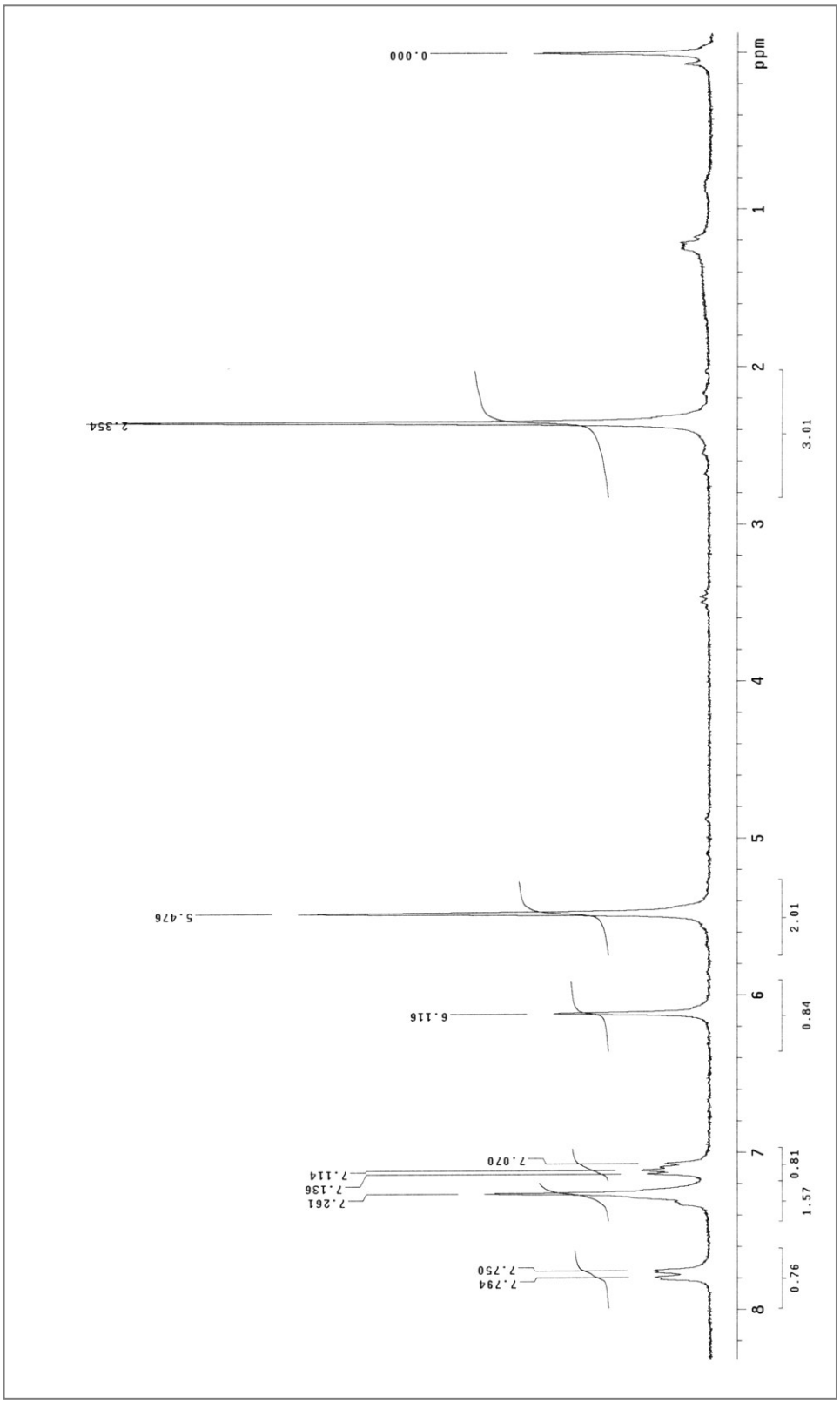
Supporting Figure S13: ^{1}H-NMR spectrum of 3F-Co-ASS



Supporting Figure S14: ^{1}H NMR spectrum of 4F-Co-ASS



Supporting Figure S15: ^{1}H NMR spectrum of 5F-Co-ASS



Supporting Figure S16: ^{1}H NMR spectrum of 6F-Co-ASS