Electronic Supplementary Material (ESI) for RSC Advances

Kinetic resolution of sulfoxides with high enantioselectivity by a new methionine sulfoxide reductase B

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1. Quantification of MsrB crude enzyme

The *li*MsrB protein was purified through the His_6-tag in the recombinant proteins using Ni-NTA affinity chromatography. Proteins were quantified by the method of BCA and then subjected to SDS-PAGE gel electrophoresis. Quantification of *li*MsrB was performed by analysis of the SDS-PAGE gel image through Gel-Pro software, using the pure *li*MsrB enzyme of known concentration as standards (Figure S1). The contents of recombinant *li*MsrB in the crude enzyme was 28%.

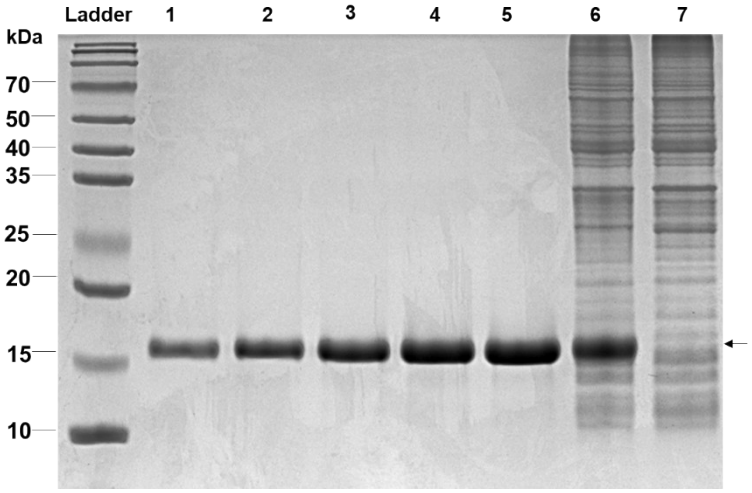
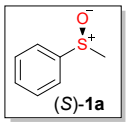
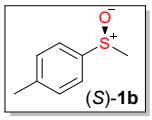


Figure S1. SDS-PAGE for *li*MsrB quantification. Lines 1-7 represents 2 μg of *li*MsrB, 4 μg of *li*MsrB, 6 μg of *li*MsrB, 8 μg of *li*MsrB, 10 μg of *li*MsrB, 20 μg of crude *li*MsrB enzyme and 20 μg of total proteins of the reference plasmid (pET-28a). The arrow indicates the recombinant proteins.

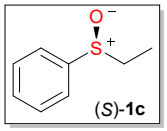
2. Spectral and *ee* data of 1a-1k after kinetic resolution and purification.



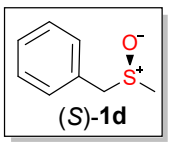
[α]D 25 = -128.9 (c=1.0, acetone) for (*S*), 95% *ee*. lit: [α]D 25 = -118.61 (c= 1.0, acetone) for (*S*), 92.8% *ee*.[1] Daicel OD-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 7% isopropyl alcohol / 93% n-hexane. Retention time: 15.7 min for (*R*)-**1a** and 21.8 min for (*S*)-**1a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66-7.60 (m, 2H), 7.51-7.49 (m, 3H), 2.71 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.43, 131.17, 129.43, 123.57, 43.87. Characterization data are consistent with previously reported values.[1]



[α]D 25 = -109.1 (c=1.0, acetone) for (*S*), 96% *ee*; lit: [α]D 25 =-113.52(c=1.0, acetone) for (*S*), 98.2% *ee*.[1] Daicel OD-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 7% isopropyl alcohol / 93% n-hexane. Retention time: 14.6 min for (*R*)-**1b** and 16.6 min for (*S*)-**1b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.51 (m, 2H), 7.31 (d, *J* = 8 Hz, 2H), 2.69 (s, 3H), 2.40 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 142.40, 141.65, 130.13, 123.63, 44.03, 21.49. Characterization data are consistent with previously reported values.[1]

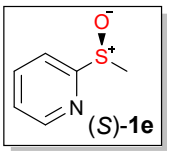


[α]D 25 = -159.2 (c=1.0, acetone) for (*S*), 98% *ee*; lit: [α]D 25 = -165.9 (*c*=0.5, acetone) for (*S*), 94.6% *ee*.[1] Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 25% isopropyl alcohol / 75% n-hexane. Retention time: 16.9 min for (*R*)-**1c** and 19.1 min for (*S*)-**1c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 – 7.62 (m, 2H), 7.58 – 7.55 (m, 3H), 3.05 – 2.96 (m, 1H), 2.79 – 2.70 (m, 1H), 1.01 (t, *J* = 8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 143.60, 130.65, 129.14, 124.12, 48.63, 5.29 Characterization data are consistent with previously reported values.[1]

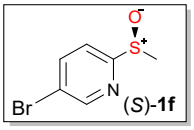


[α]D 25 = +101.2 (c=1.0, EtOH) for (*S*), 92% *ee*; lit: [α]D 25 = +97.9(c=0.25, EtOH) for (*S*), 87% *ee*.[2] Daicel OD-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 7% isopropyl alcohol /

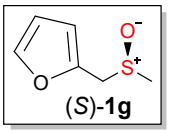
93% n-hexane. Retention time: 29.1 min for (*R*)-**1d** and 31.8 min for (*S*)-**1d**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 – 7.31 (m, 5H), 4.12 (d, *J* = 16 Hz, 1H), 3.94 (d, *J* = 12 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 131.41, 130.32, 128.46, 127.77, 58.51, 37.24. Characterization data are consistent with previously reported values.[2]



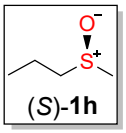
[α]D 25 = -62.1 (c=1, EtOH) for (*S*), 98% *ee*. lit: [α]D 25 = –10.5 (c=0.53, EtOH) for (*S*), 6% *ee*.[3] Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 30% isopropyl alcohol / 70% n-hexane. Retention time: 12.6 min for (*R*)-**1e** and 20.8 min for (*S*)-**1e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.00 (d, *J* = 8 Hz, 1H), 7.95 – 7.92 (m, 1H), 7.38 – 7.35 (m, 1H), 2.82 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 165.11, 148.57, 138.28, 125.21, 119.36, 40.49. Characterization data are consistent with previously reported values.[4]



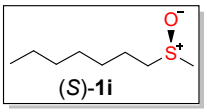
[α]D 25 = -54.3 (c=1.0, CH_3OH) for (*S*), 98% *ee*. Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 254 nm. The mobile phase used was 30% isopropyl alcohol / 70% n-hexane. Retention time: 14.0 min for (*R*)-**1f** and 26.3 min for (*S*)-**1f**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.38 (d, *J* = 8 Hz, 1H), 7.86 (d, *J* = 8 Hz, 1H), 2.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.84, 150.42, 141.25, 121.60, 120.99, 40.89. Characterization data are consistent with previously reported values.[4]



[α]D 25 = +35.1 (c=1.0, CH_3OH) for (*S*), 98% *ee*; lit: [α]D 25 = -51.6(c=1, CH_3OH) for (*R*), 99% *ee*.[5] Daicel OD-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 245 nm. The mobile phase used was 3% isopropyl alcohol / 97% n-hexane. Retention time: 51.0 min for (*R*)-**1g** and 55.9 min for (*S*)-**1g**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 1H), 6.36 – 6.33 (m, 2H), 4.15 (dd, *J* = 24, 12 Hz, 2H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.79, 143.54, 111.31, 111.17, 52.40, 37.86. Characterization data are consistent with previously reported values.[5]

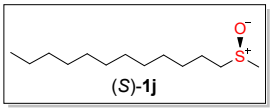


[α]D 25 = +95.10 (c=1.0, CH_3OH) for (*S*), 99% *ee*; lit: [α]D 25 = -89.1(c=1, CH_3OH) for (*R*), 99% *ee*.[5] Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 210 nm. The mobile phase used was 25% isopropyl alcohol / 75% n-hexane. Retention time: 23.9 min for (*R*)-**1h** and 27.7 min for (*S*)-**1h**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.74 – 2.67 (m, 1H), 2.59 – 2.56 (m, 1H), 2.52 (s, 3H), 1.77 – 1.72 (m, 2H), 1.03 (t, *J* = 8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 56.44, 38.34, 16.25, 13.33. Characterization data are consistent with previously reported values.[5]



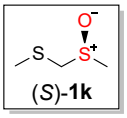
(*S*)-**1i**

[α]D 25 = +70.34 (c=1.0, CH_3OH) for (*S*), 99% *ee*. Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 210 nm. The mobile phase used was 25% isopropyl alcohol / 75% n-hexane. Retention time: 20.0 min for (*R*)-**1i** and 26.1 min for (*S*)-**1i**. ^{1}H NMR (400 MHz, $CDCl_3$) δ2.73 – 2.62 (m, 2H), 2.55 (s, 3H), 1.73 (t, *J* = 7.8 Hz, 2H), 1.46– 1.39 (m, 2H), 1.34 – 1.24 (m, 6H), 0.86 (t, *J* = 8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 54.80, 38.57, 31.62, 28.95, 28.83, 22.65, 22.63, 14.13. Characterization data are consistent with previously reported values.[6]



(*S*)-**1j**

[α]D 25 = +66.5 (c=1.0, acetone) for (*S*), 96% *ee*. lit: [α]D 25 = +61(c=1.166, acetone) for (*S*), 94% *ee*.[2] Daicel AS-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 228 nm. The mobile phase used was 25% isopropyl alcohol / 75% n-hexane. Retention time: 12.2 min for (*R*)-**1j** and 16.1 min for (*S*)-**1j**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 – 2.59 (m, 2H), 2.54 (s, 3H), 1.76 – 1.69 (m, 2H), 1.47 – 1.37 (m, 2H), 1.32 – 1.23 (m, 16H), 0.85 (t, *J* = 8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 54.85, 38.62, 31.98, 29.68, 29.61, 29.44, 29.42, 29.29, 28.88, 22.77, 22.65, 14.21. Characterization data are consistent with previously reported values.[2]

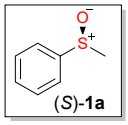


(*S*)-**1k**

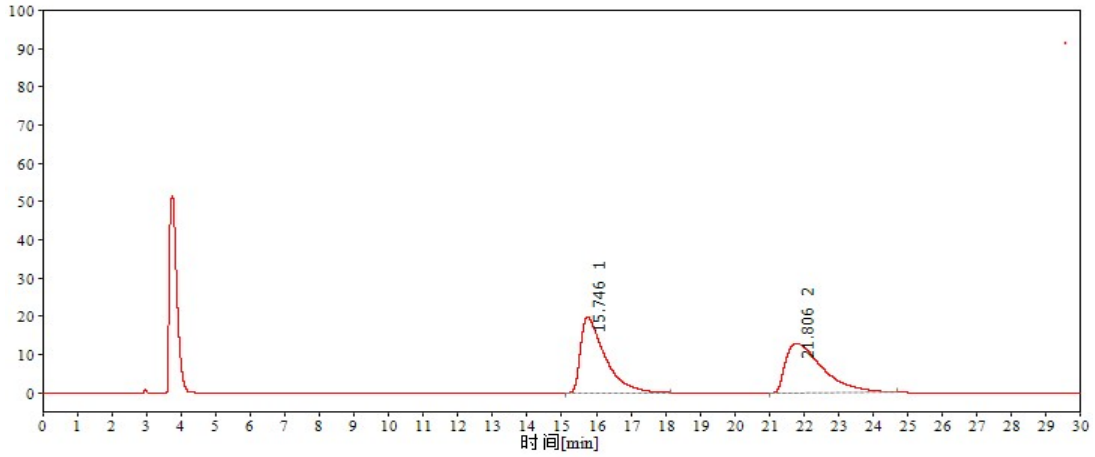
[α]D 25 = +98.94 (c=1.0, CH_3OH) for (*S*), 99% *ee*; lit: [α]D 25 = -108.2 (c=1.0, CH_3OH) for (R), 99% ee.[5] Daicel AD-H chiral column (250×4.6 mm, 5 mm) at 30 °C with a flow rate of 1 mL/min and UV detection at 210 nm. The mobile phase used was 10% isopropyl alcohol / 90% n-hexane. Retention time: 10.9 min for (*R*)-**1k** and 19.8 min for (*S*)-**1k**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (s, 2H), 2.63 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 56.55, 37.59, 17.25. Characterization data are consistent with previously reported values.[5]

3. HPLC and NMR spectrogram of 1a-1k after kinetic resolution and purification

3.1 HPLC and NMR spectra for compound (*S*)-1a

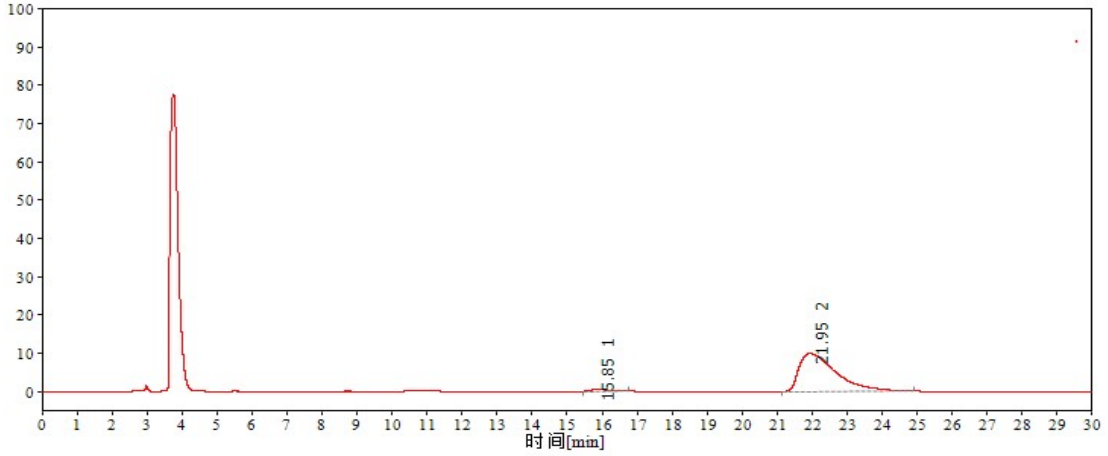


HPLC spectra for *rac*-**1a**



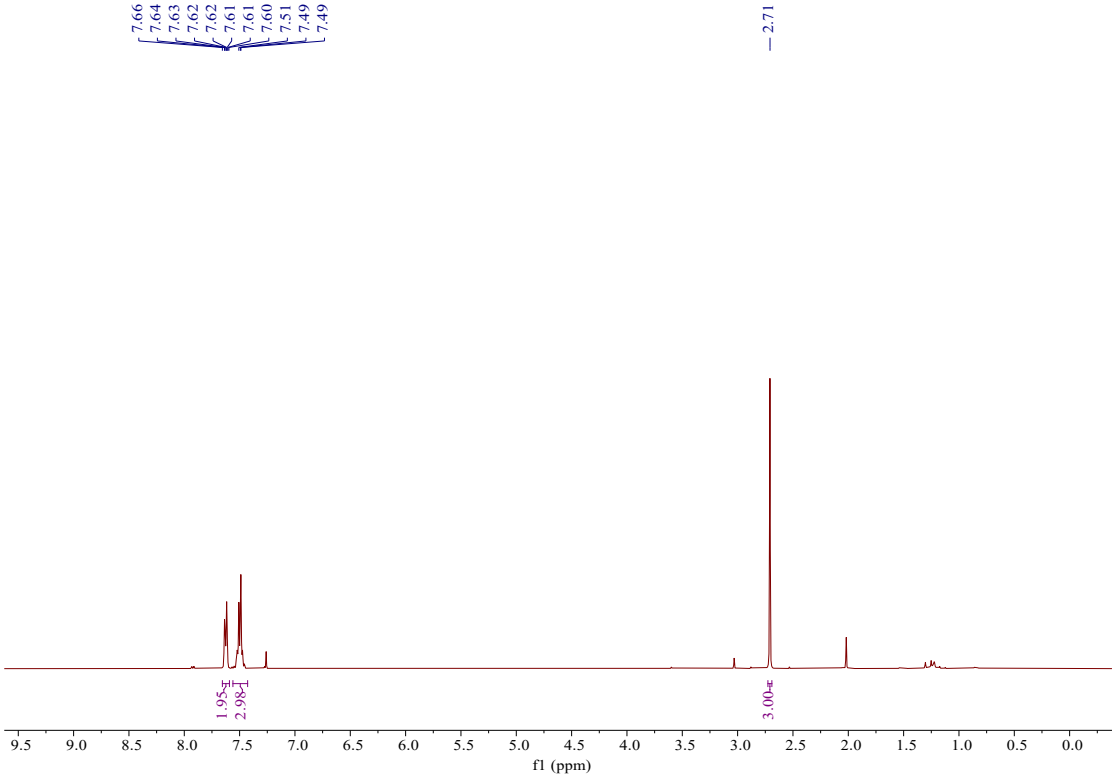
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	15.746	19.911	60.8	971.054	50.3	1.844	
2	21.806	12.816	39.2	959.952	49.7	2.637	
合计		32.727	100.0	1931.006	100.0		

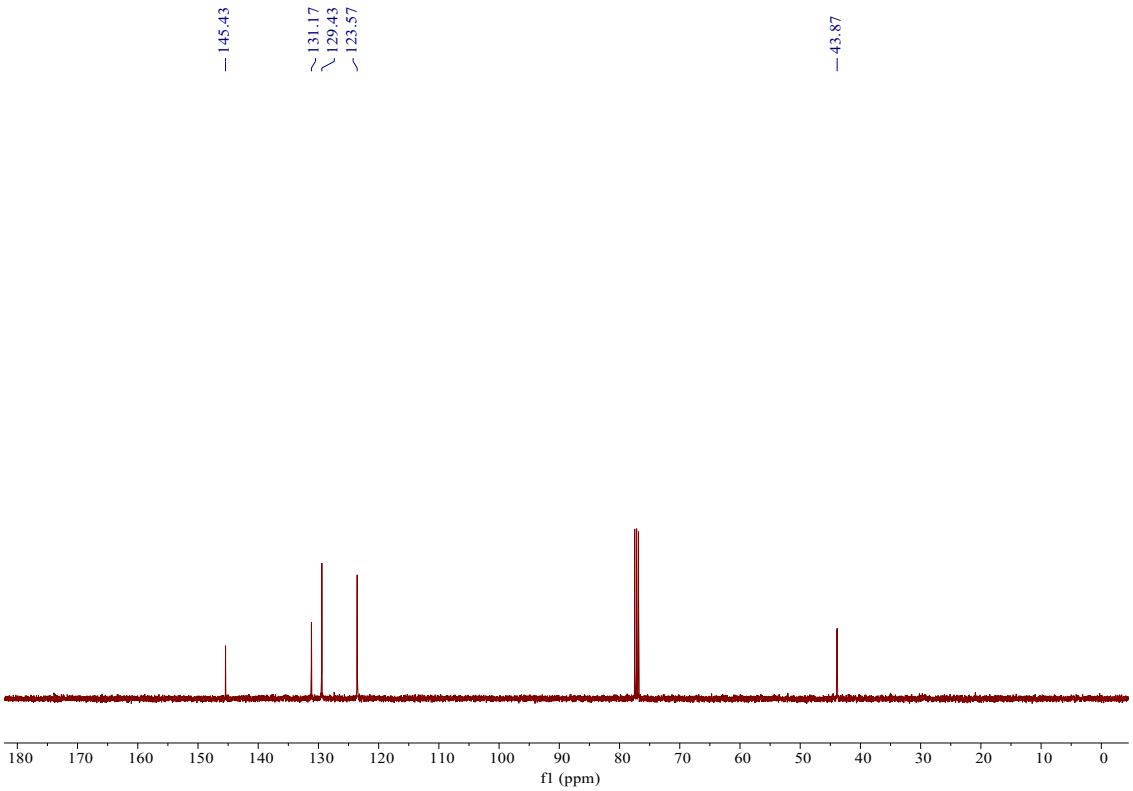
HPLC spectra for (*S*)-**1a**



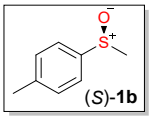
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	15.850	0.446	4.3	17.103	2.3	1.163	
2	21.950	9.888	95.7	730.203	97.7	2.646	
合计		10.334	100.0	747.306	100.0		

NMR spectra for (*S*)-**1a**

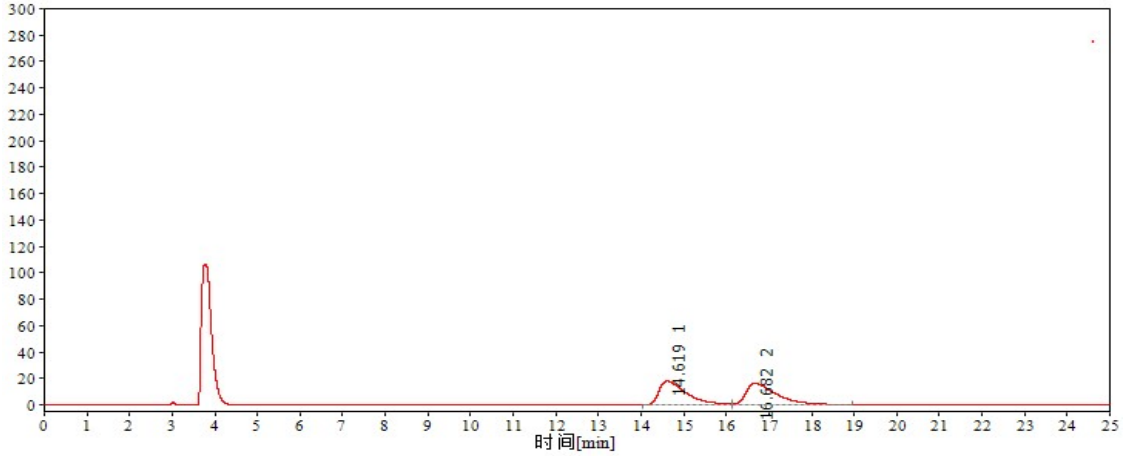




3.2 HPLC and NMR spectra data for compound (*S*)-1b

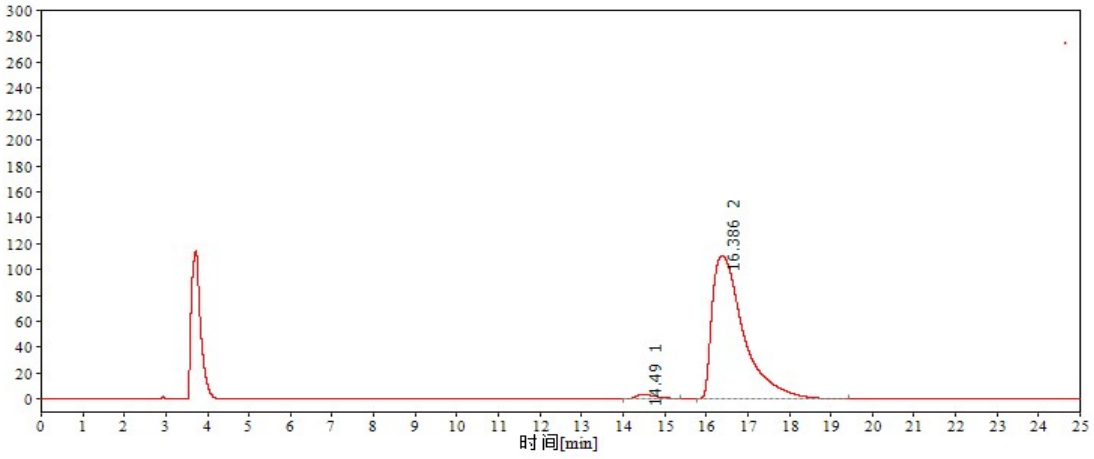


HPLC spectra for *rac*-**1b**



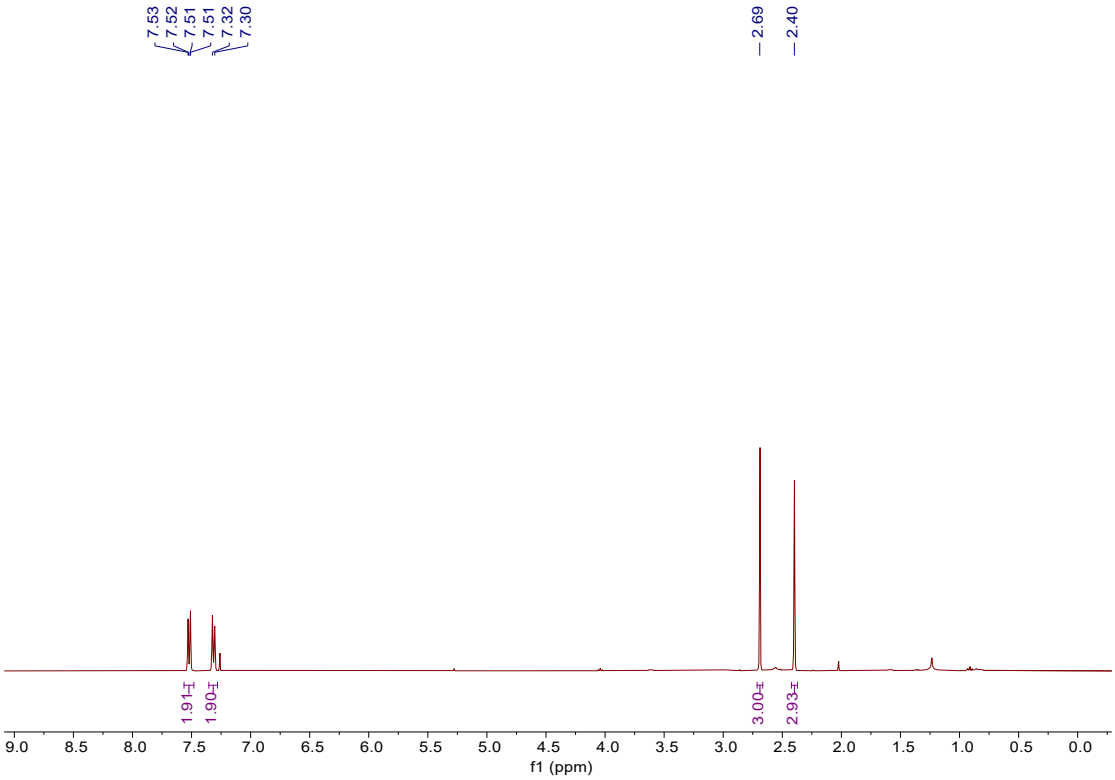
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	14.619	18.120	52.8	809.122	49.7	1.689	
2	16.682	16.177	47.2	820.455	50.3	1.922	
合计		34.297	100.0	1629.577	100.0		

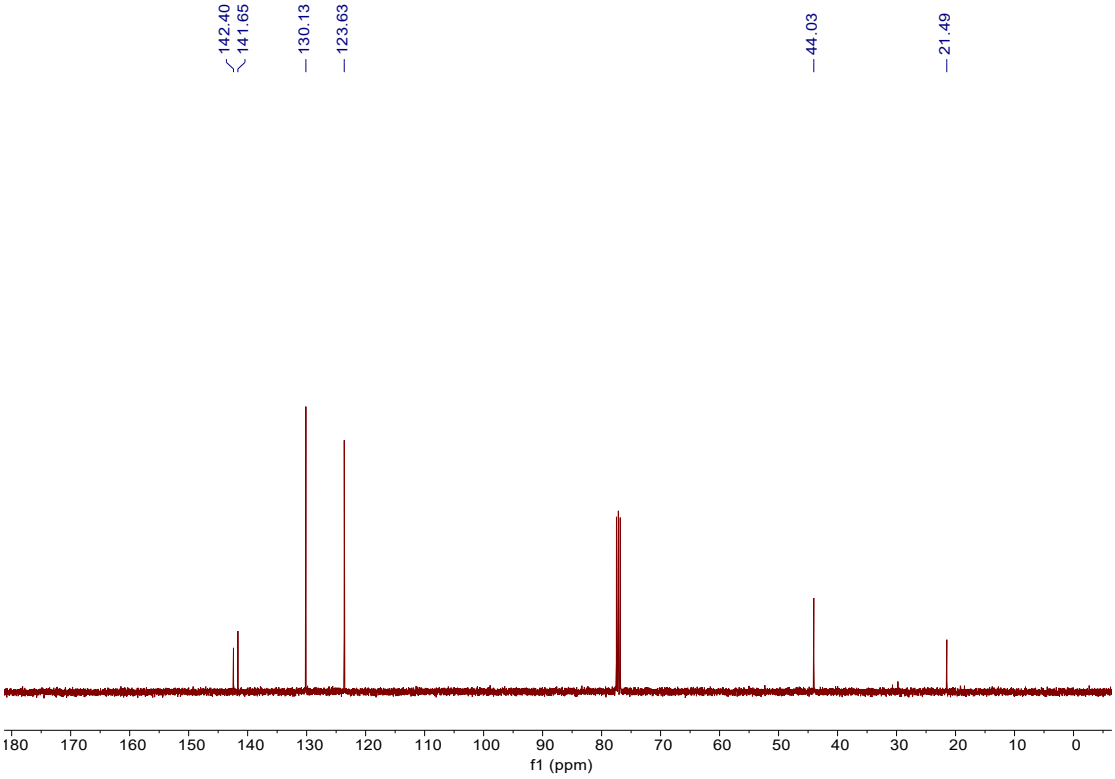
HPLC spectra for (*S*)-**1b**



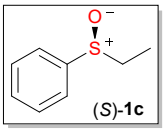
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	14.490	3.199	2.8	112.626	1.9	1.123	
2	16.386	110.141	97.2	5810.715	98.1	1.988	
合计		113.340	100.0	5923.341	100.0		

NMR spectra for (*S*)-**1b**

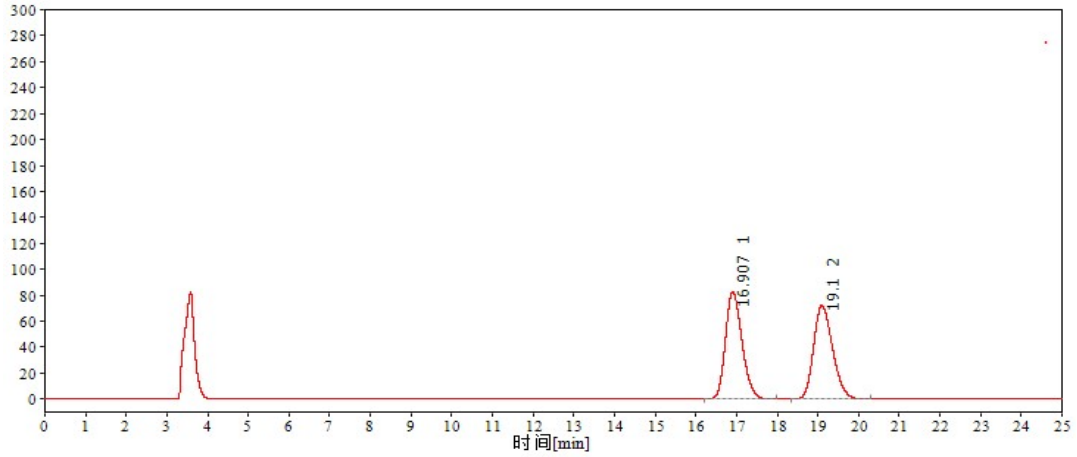




3.3 HPLC and NMR spectra data for compound (*S*)-1c

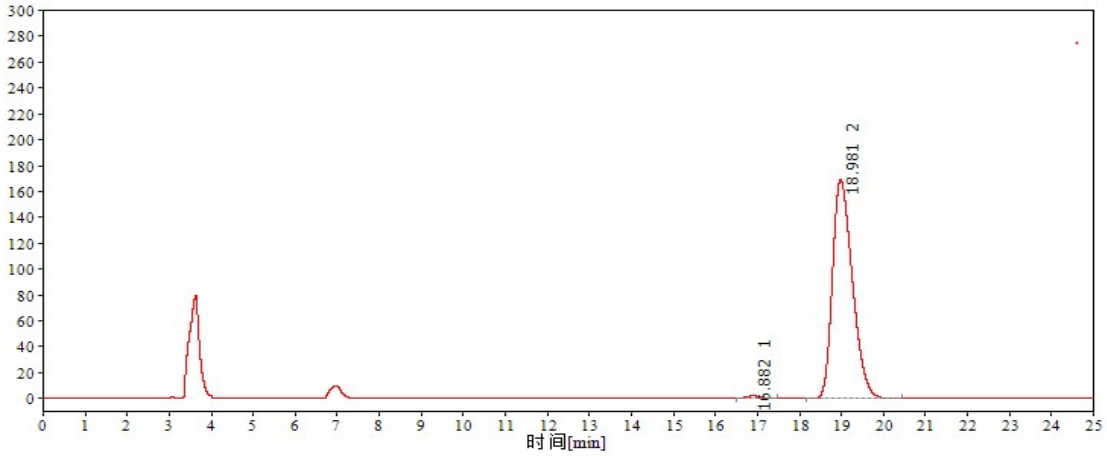


HPLC spectra for *rac*-**1c**



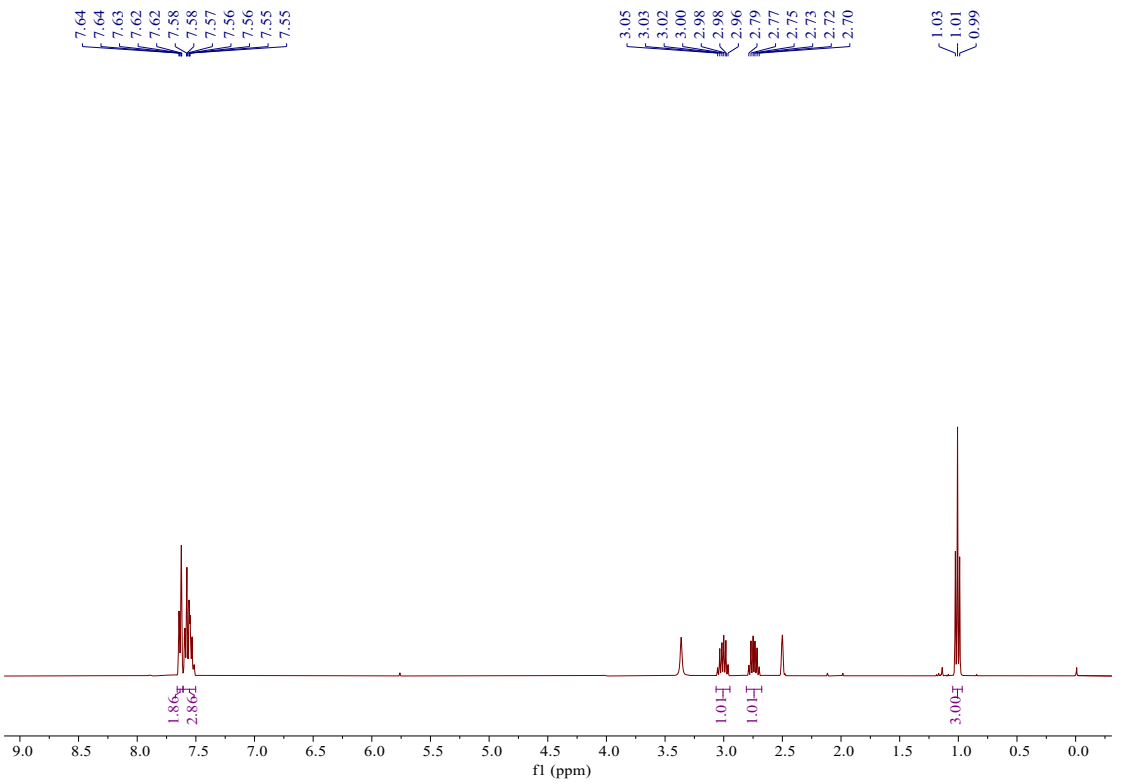
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	16.907	82.594	53.4	2345.454	50.0	0.945	
2	19.100	72.159	46.6	2345.421	50.0	1.079	
合计		154.753	100.0	4690.875	100.0		

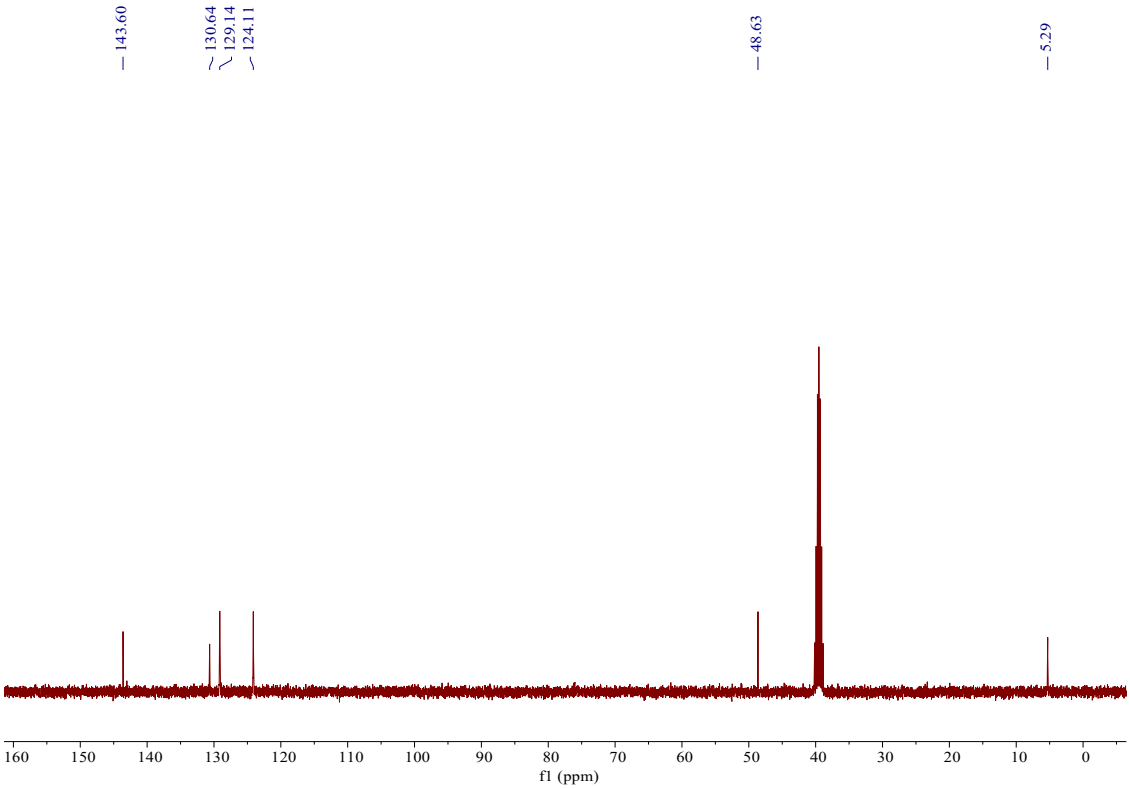
HPLC spectra for (*S*)-**1c**



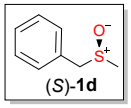
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	16.882	1.972	1.2	52.057	0.9	0.843	
2	18.981	168.948	98.8	5597.973	99.1	1.095	
合计		170.920	100.0	5650.030	100.0		

NMR spectra for (*S*)-**1c**

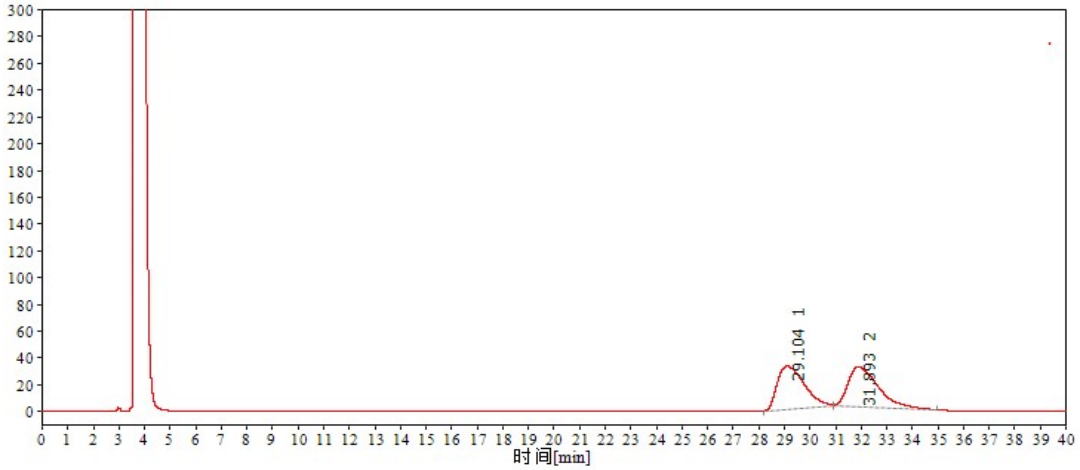




3.4 HPLC and NMR spectra for compound (*S*)-1d

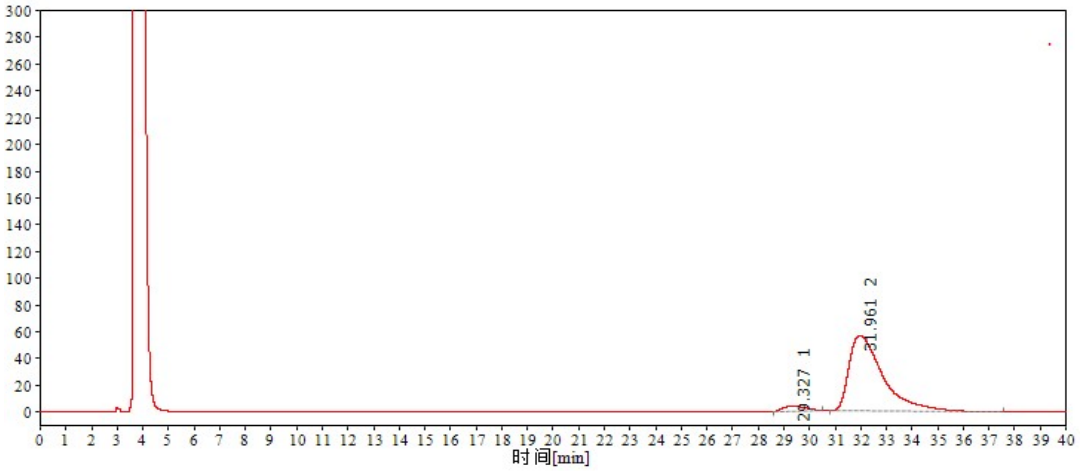


HPLC spectra for *rac*-**1d**



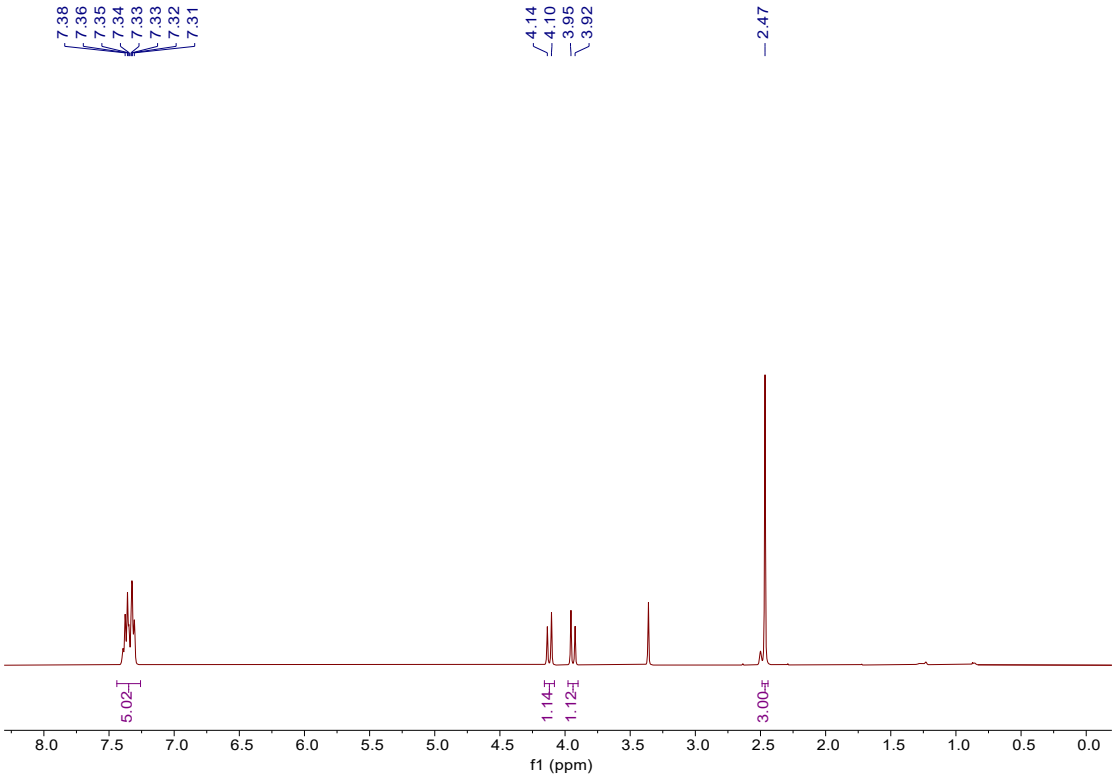
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	29.104	32.155	52.2	2336.020	49.5	2.227	
2	31.893	29.410	47.8	2385.627	50.5	3.122	
合计		61.565	100.0	4721.647	100.0		

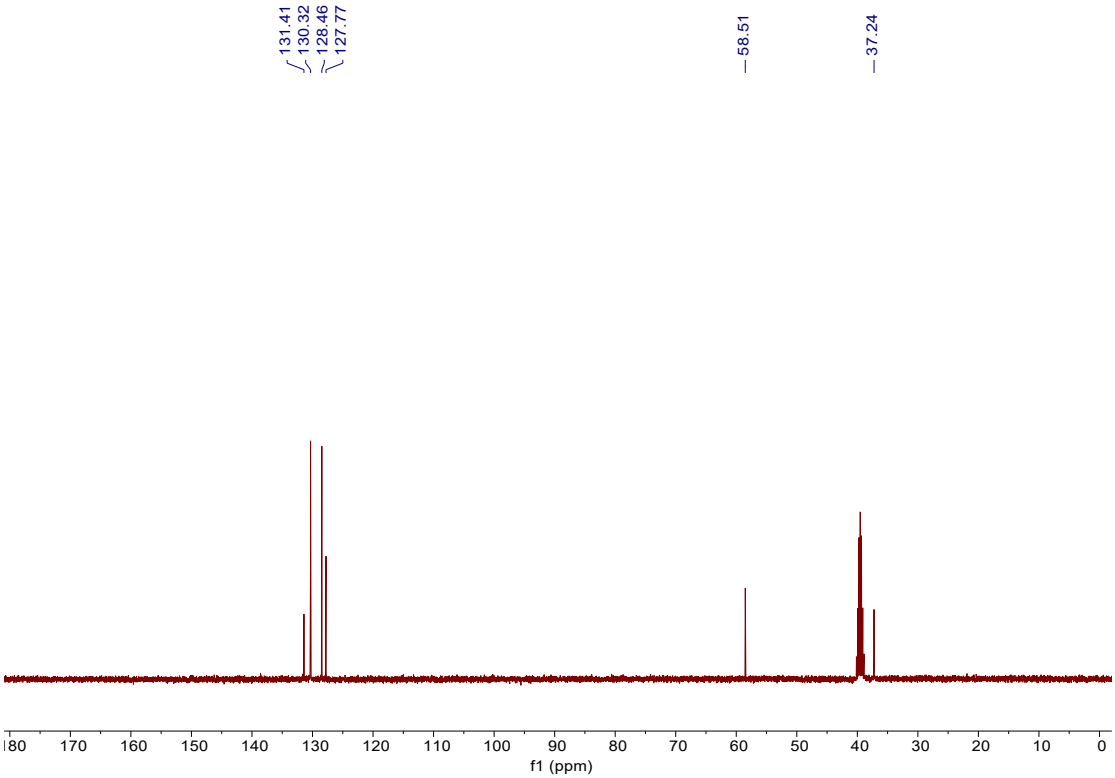
HPLC spectra for (*S*)-**1d**



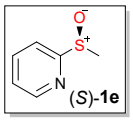
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	29.327	3.756	6.3	230.305	4.2	1.764	
2	31.961	56.321	93.7	5228.553	95.8	3.741	
合计		60.077	100.0	5458.858	100.0		

NMR spectra for (*S*)-**1d**

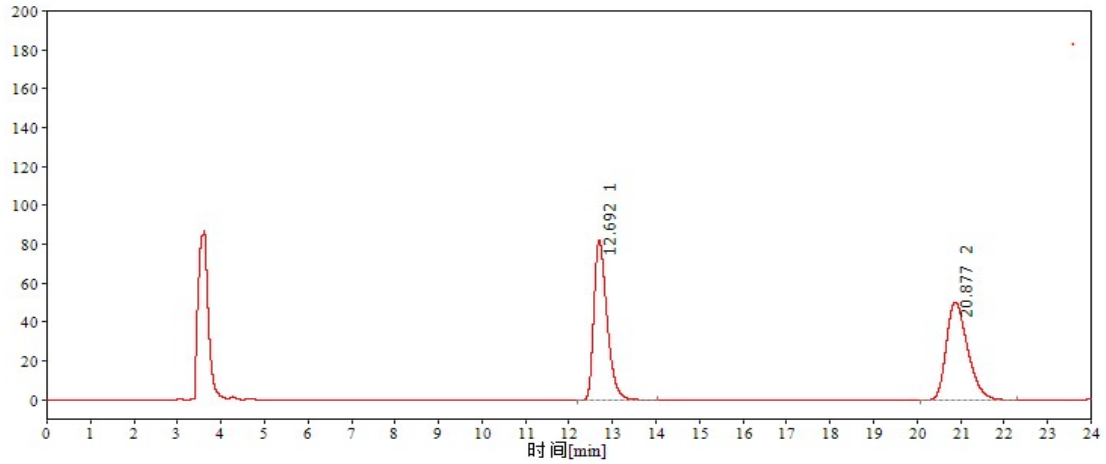




3.5 HPLC and NMR spectra data for compound (*S*)-1e

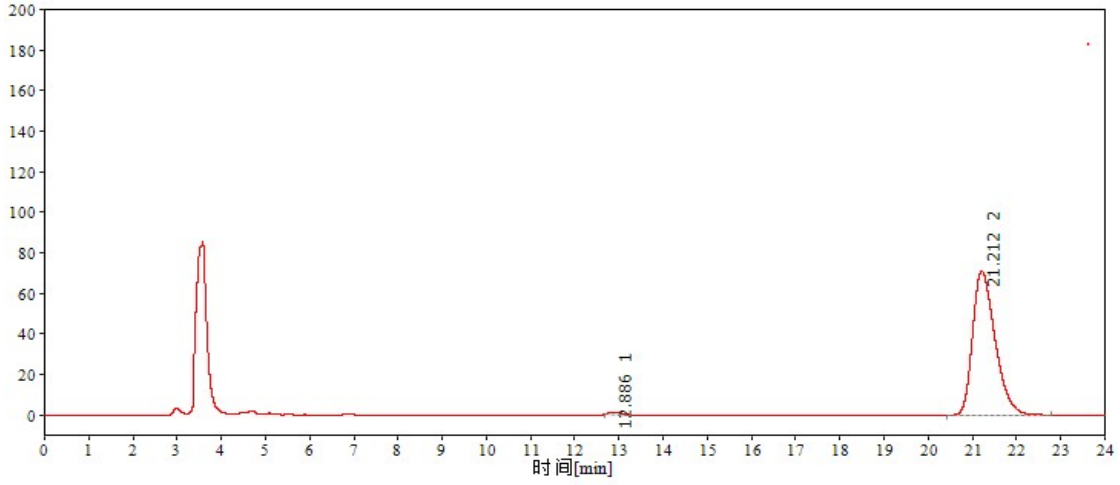


HPLC spectra for *rac*-**1e**



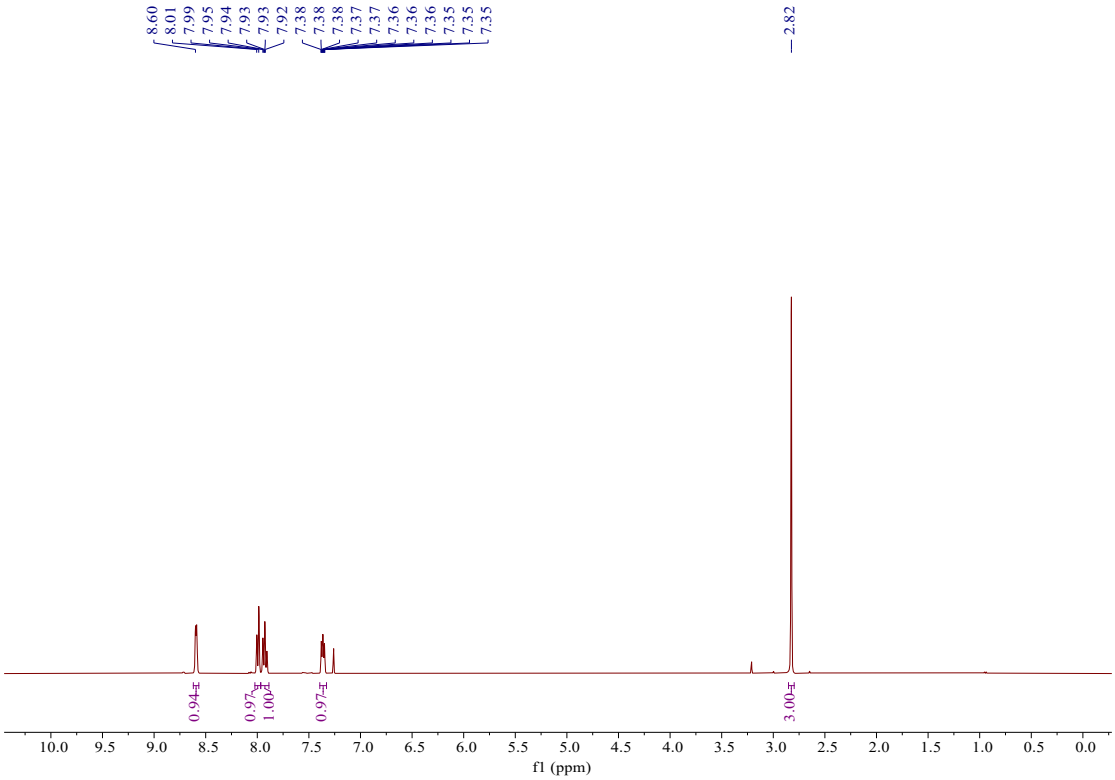
序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	12.692	82.512	62.0	1722.516	50.0	0.711	
2	20.877	50.480	38.0	1719.647	50.0	1.149	
合计		132.992	100.0	3442.163	100.0		

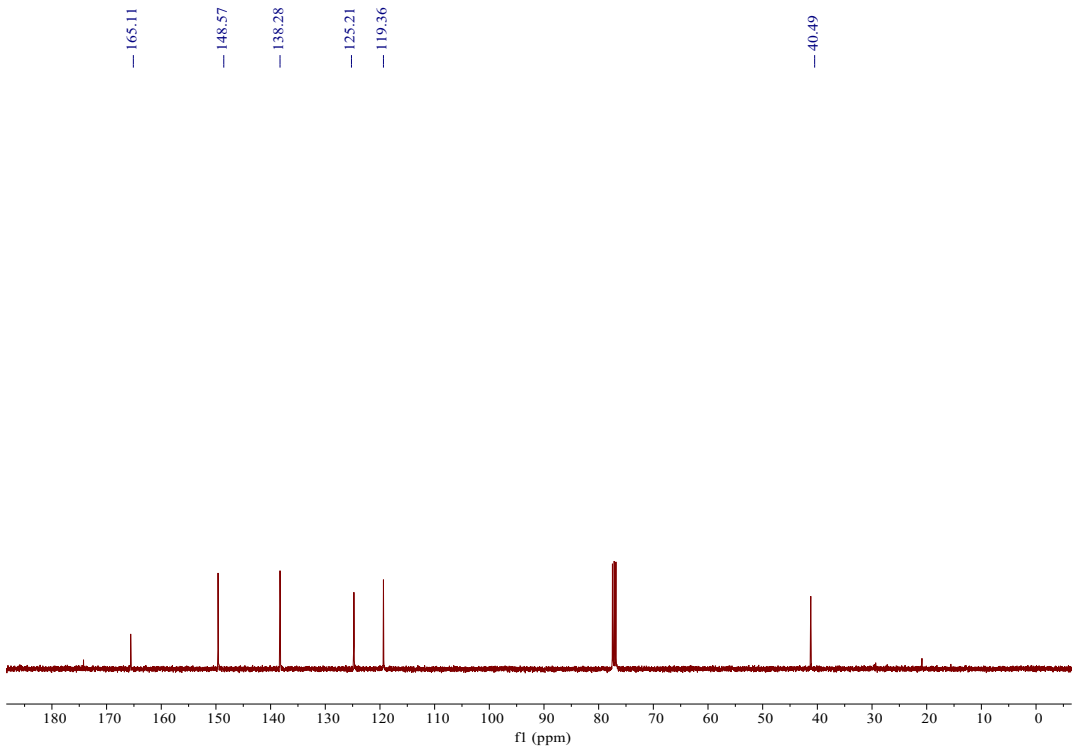
HPLC spectra for (*S*)-**1e**



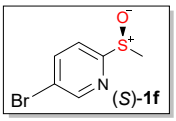
序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	12.886	1.180	1.6	20.498	0.8	0.537	
2	21.212	71.058	98.4	2508.016	99.2	1.188	
合计		72.238	100.0	2528.514	100.0		

NMR spectra for (*S*)-**1e**

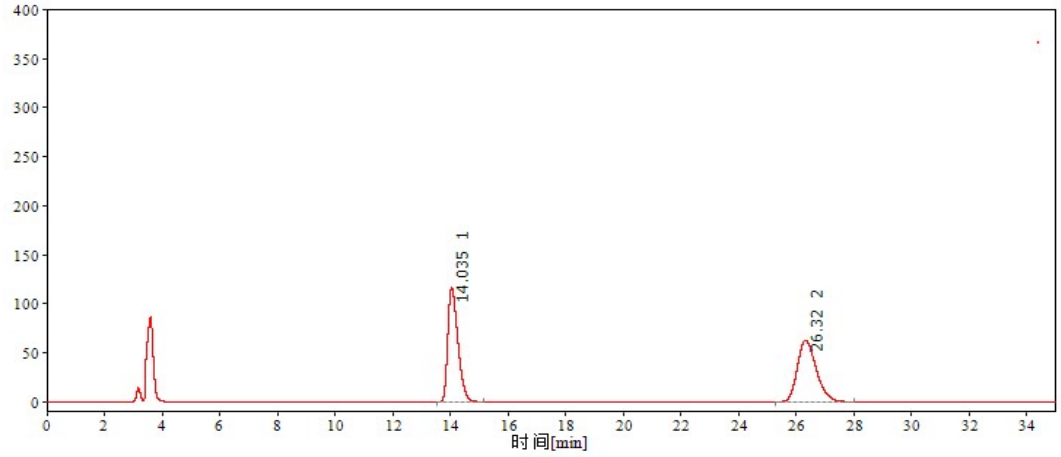




3.6 HPLC and NMR spectra data for compound (*S*)-1f

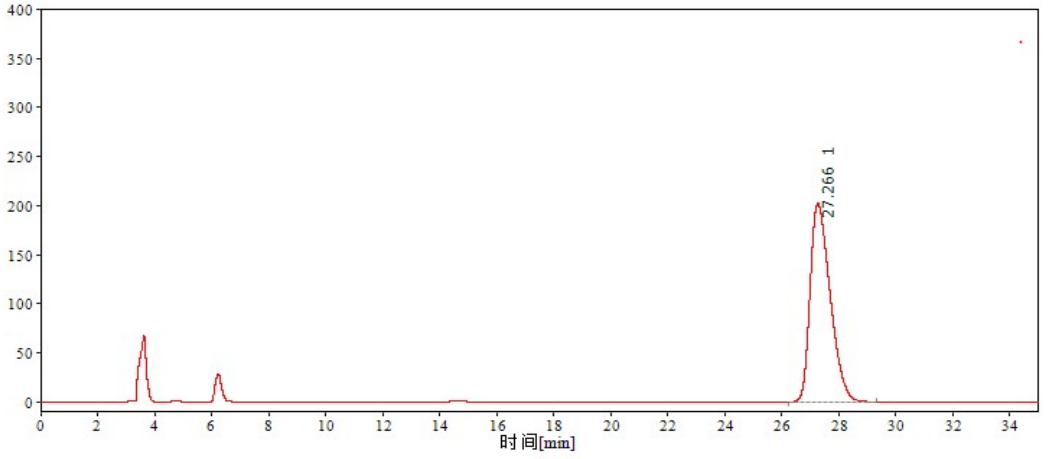


HPLC spectra for *rac*-**1f**



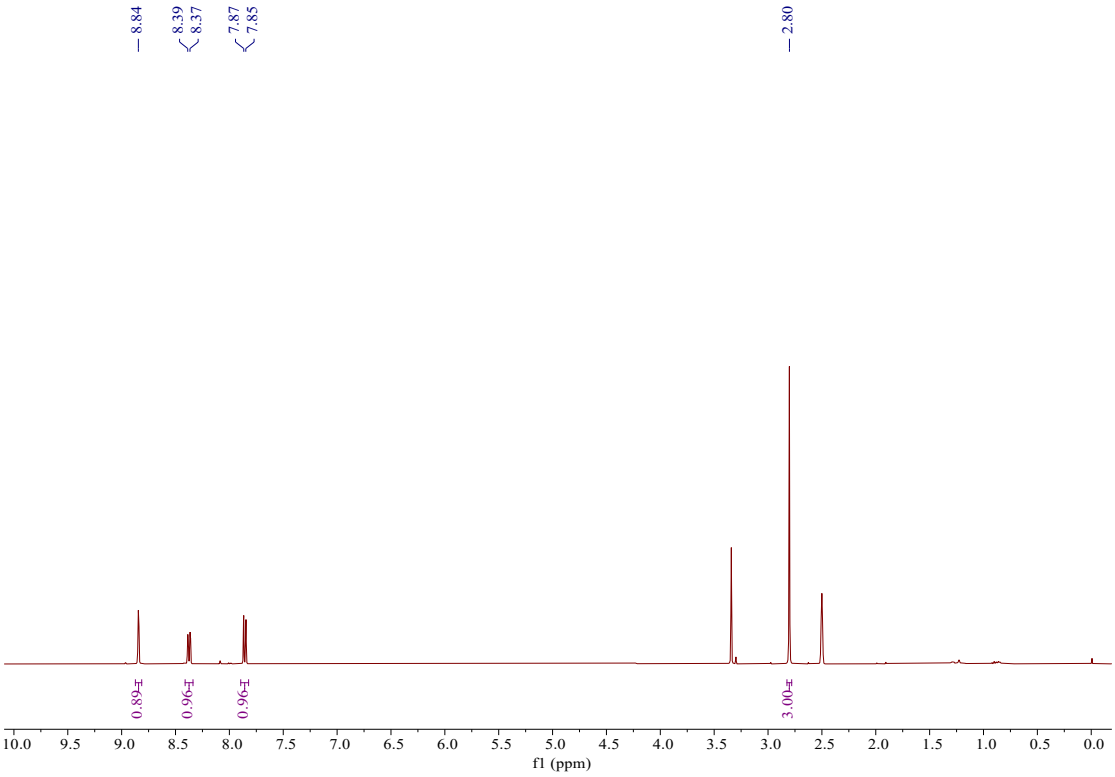
序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	14.035	116.683	65.0	2849.550	50.0	0.816	
2	26.320	62.862	35.0	2852.344	50.0	1.521	
合计		179.545	100.0	5701.894	100.0		

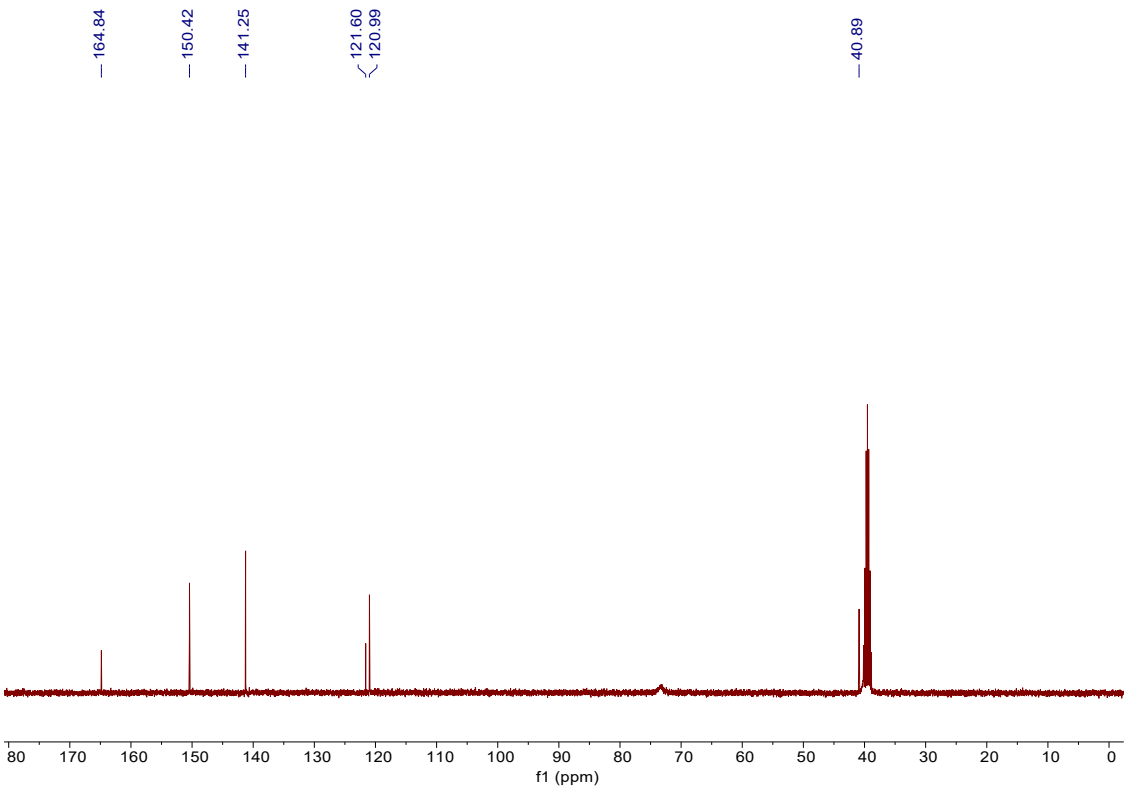
HPLC spectra for (*S*)-**1f**



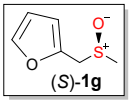
序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	27.266	202.016	100.0	9896.856	100.0	1.616	
合计		202.016	100.0	9896.856	100.0		

NMR spectra for (*S*)-**1f**

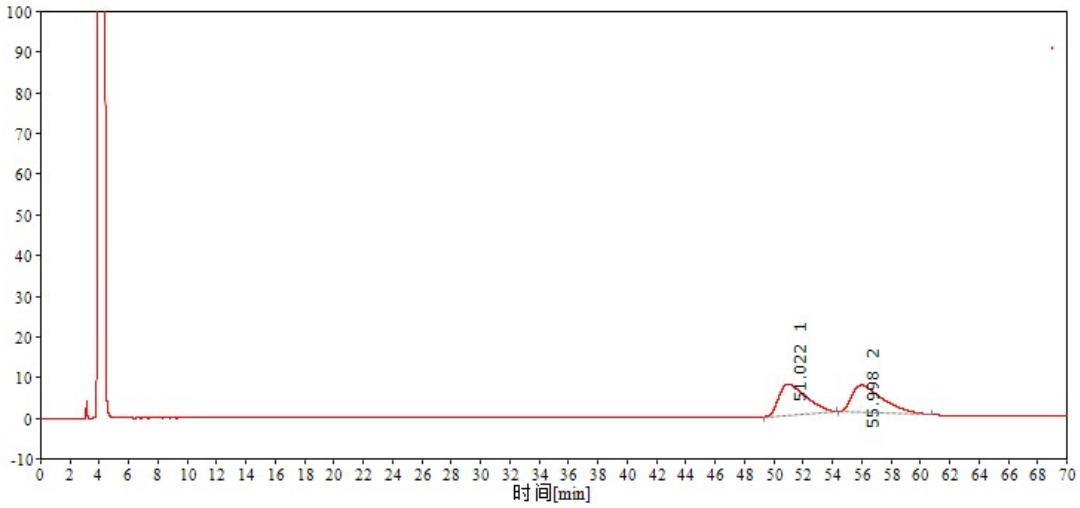




3.7 HPLC and NMR spectra for data compound (*S*)-1g

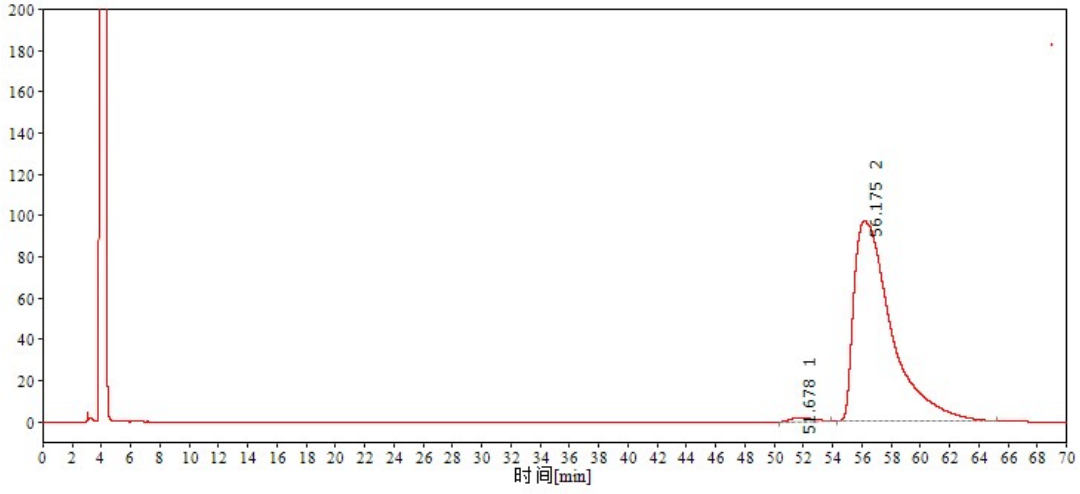


HPLC spectra for *rac*-**1g**



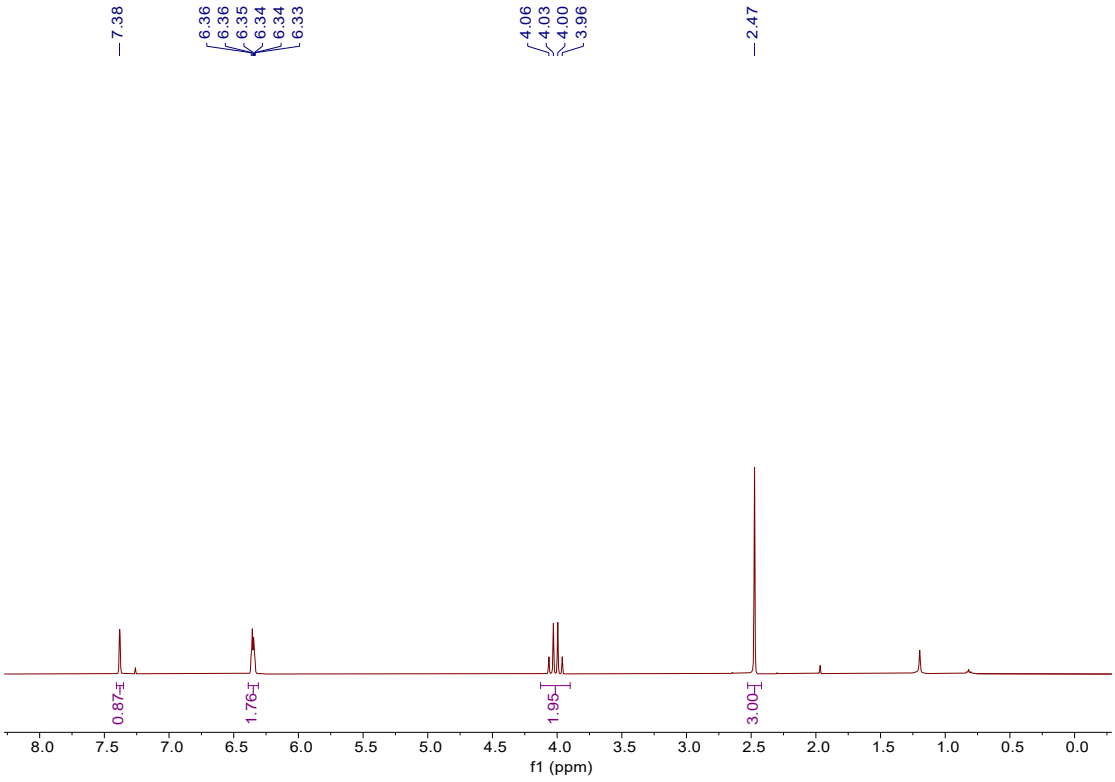
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	51.022	7.673	53.1	1011.845	50.2	4.181	
2	55.998	6.773	46.9	1003.080	49.8	5.313	
合计		14.446	100.0	2014.925	100.0		

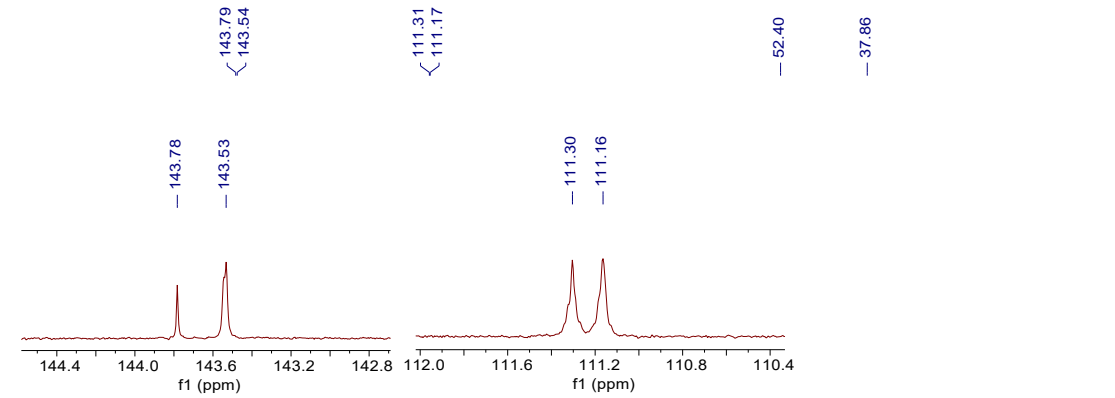
HPLC spectra for (*S*)-**1g**

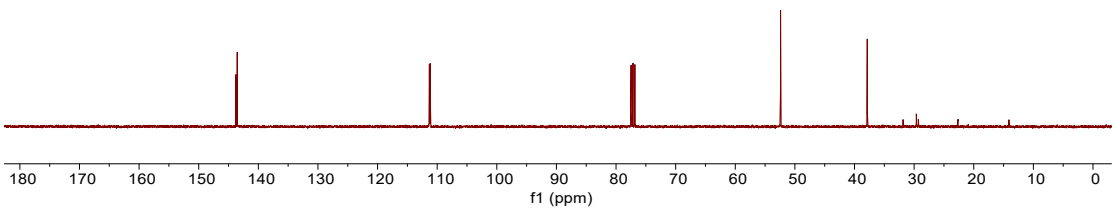


序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	51.678	1.870	1.9	197.862	1.1	3.173	
2	56.175	97.367	98.1	17344.651	98.9	6.856	
合计		99.237	100.0	17542.513	100.0		

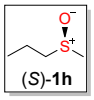
NMR spectra for (*S*)-**1g**



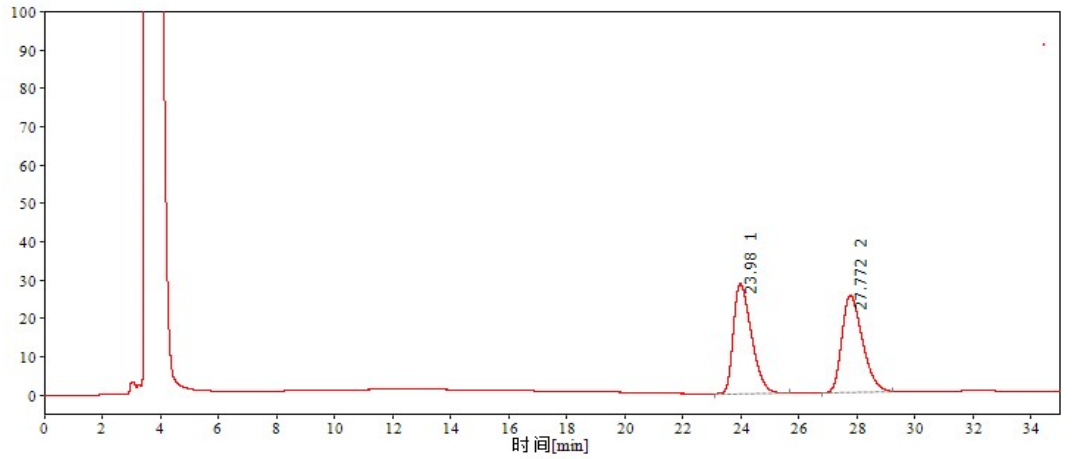




3.8 HPLC and NMR spectra data for compound (*S*)-1h

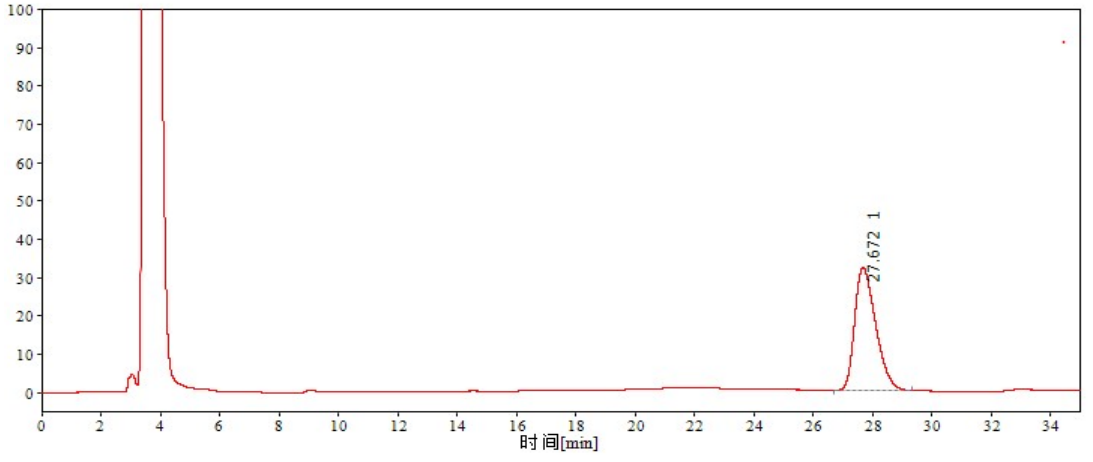


HPLC spectra for *rac*-**1h**



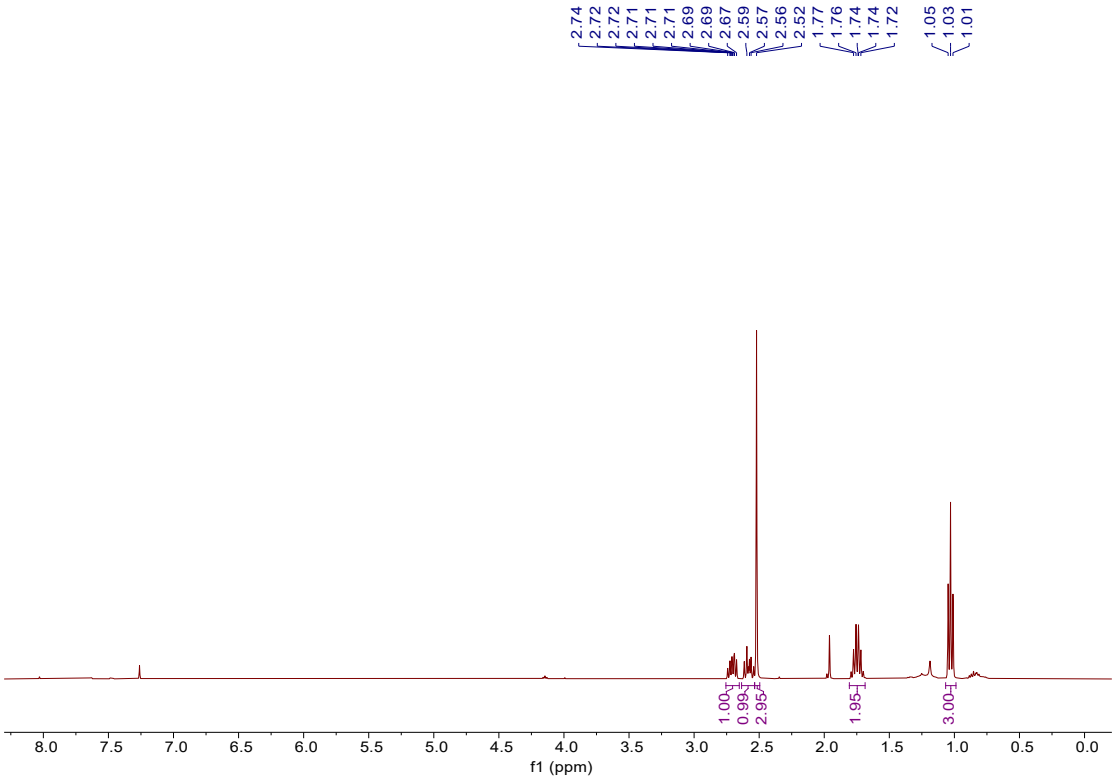
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	23.980	28.620	53.2	1242.098	50.0	1.435	
2	27.772	25.226	46.8	1240.186	50.0	1.624	
合计		53.846	100.0	2482.284	100.0		

HPLC spectra for (*S*)-**1h**

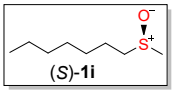


序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	27.672	32.080	100.0	1580.776	100.0	1.639	
合计		32.080	100.0	1580.776	100.0		

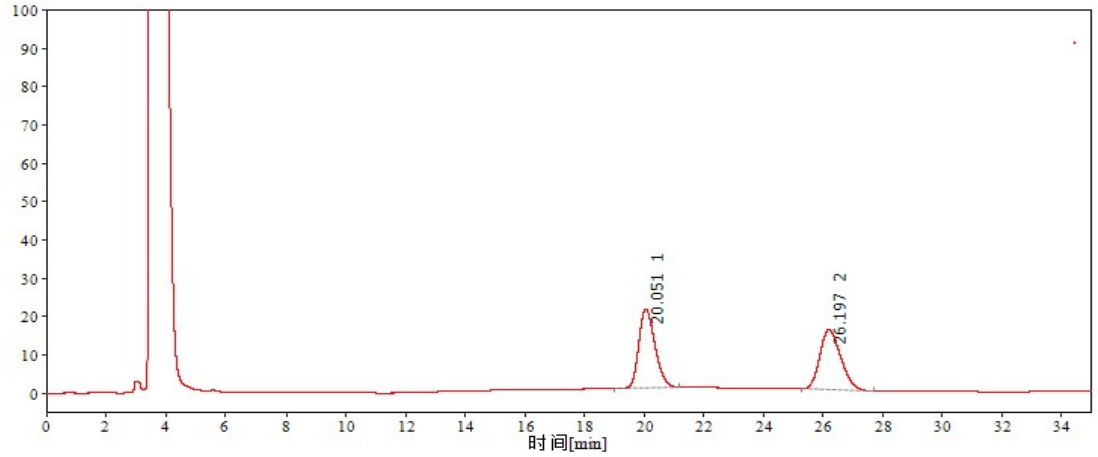
NMR spectra for (*S*)-**1h**



3.9 HPLC and NMR spectra data for compound (*S*)-1i

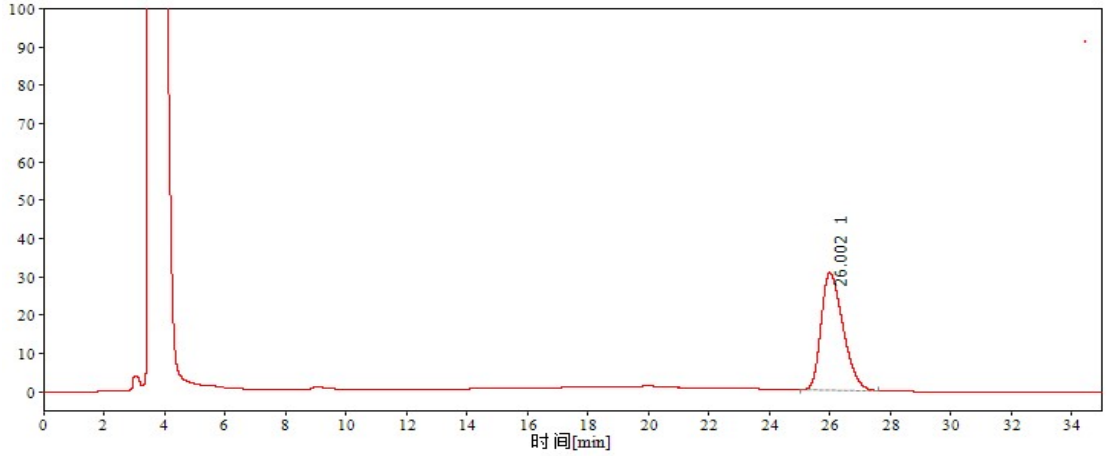


HPLC spectra for *rac*-**1i**



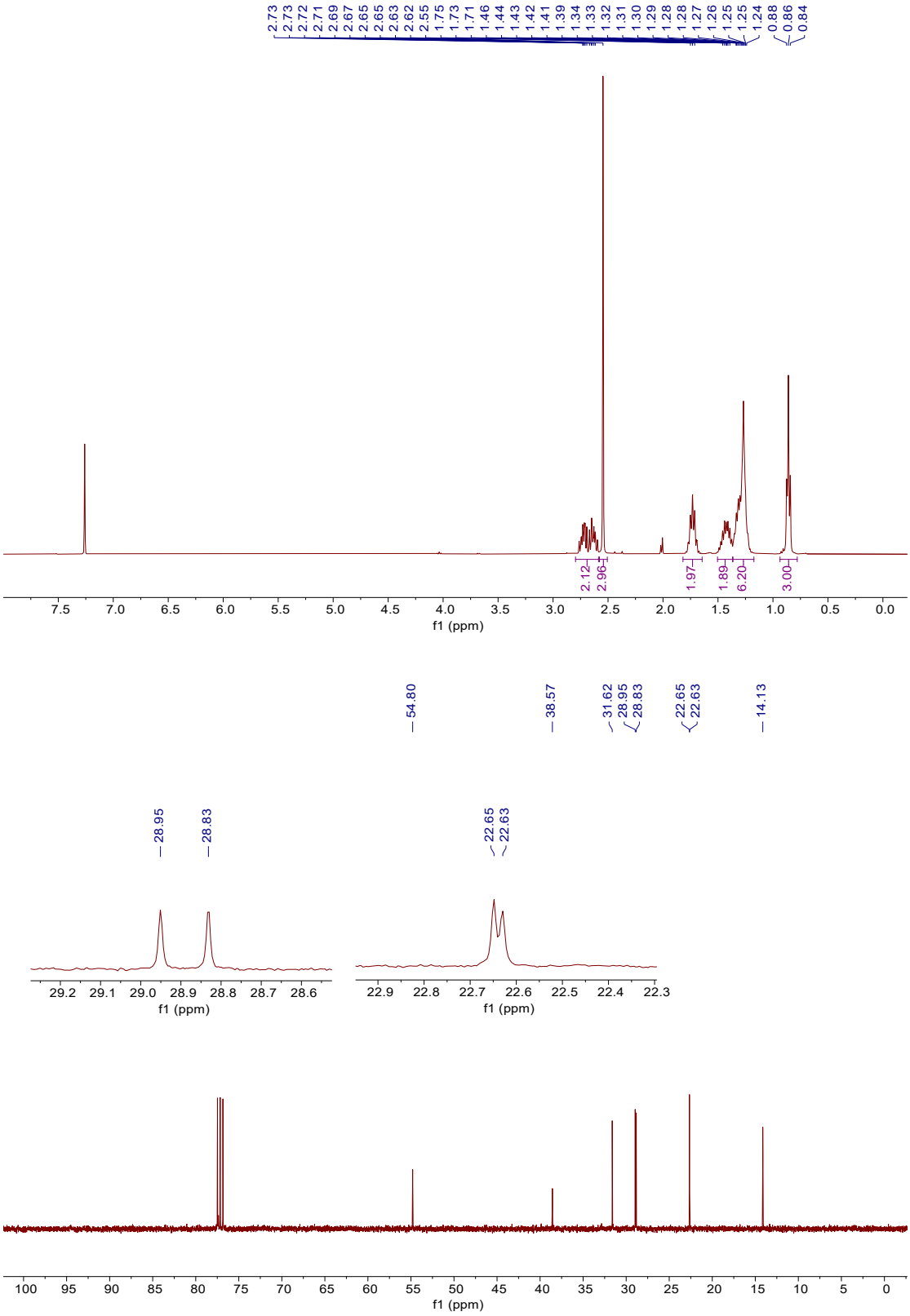
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	20.051	20.633	56.9	780.415	50.4	1.250	
2	26.197	15.643	43.1	767.004	49.6	1.635	
合计		36.276	100.0	1547.419	100.0		

HPLC spectra for (*S*)-**1i**

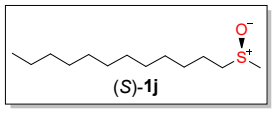


序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	26.002	30.668	100.0	1547.250	100.0	1.673	
合计		30.668	100.0	1547.250	100.0		

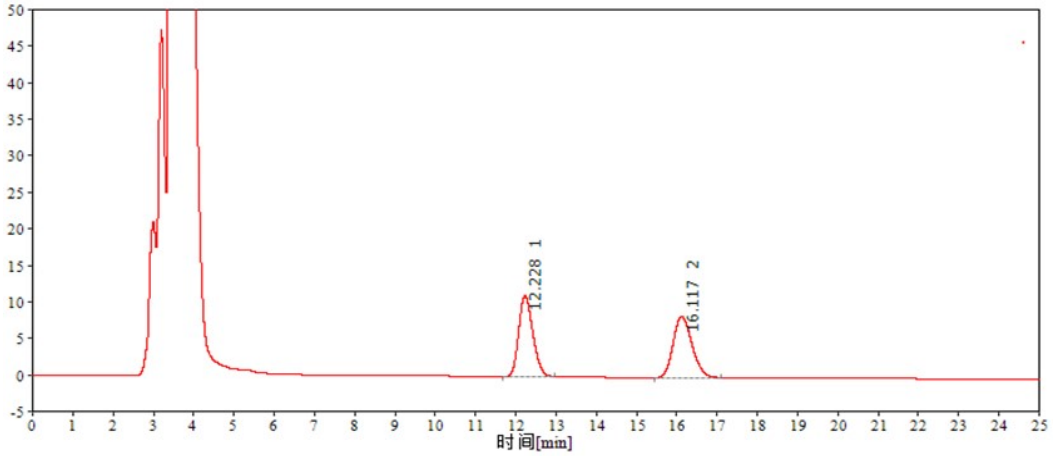
NMR spectra for (*S*)-**1i**



3.10 HPLC and NMR spectra data for compound (*S*)-1j

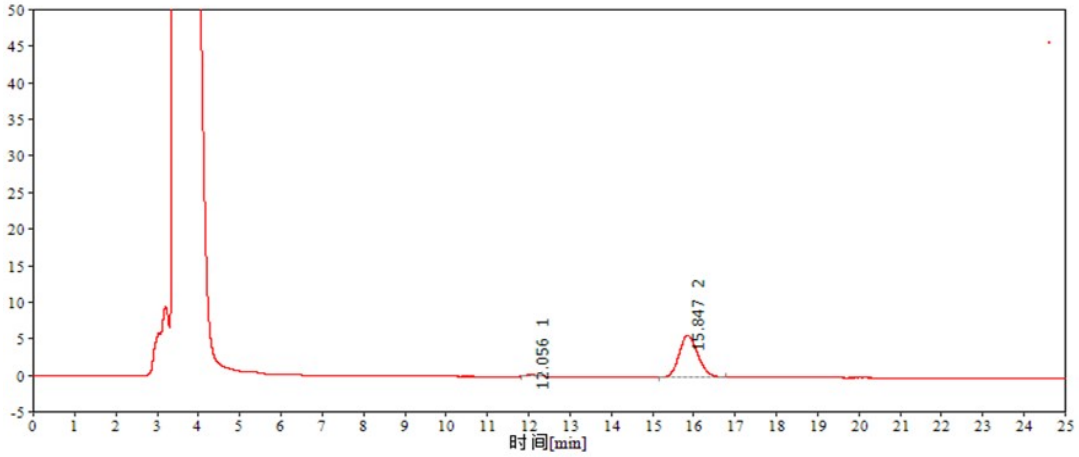


HPLC spectra for *rac*-**1j**



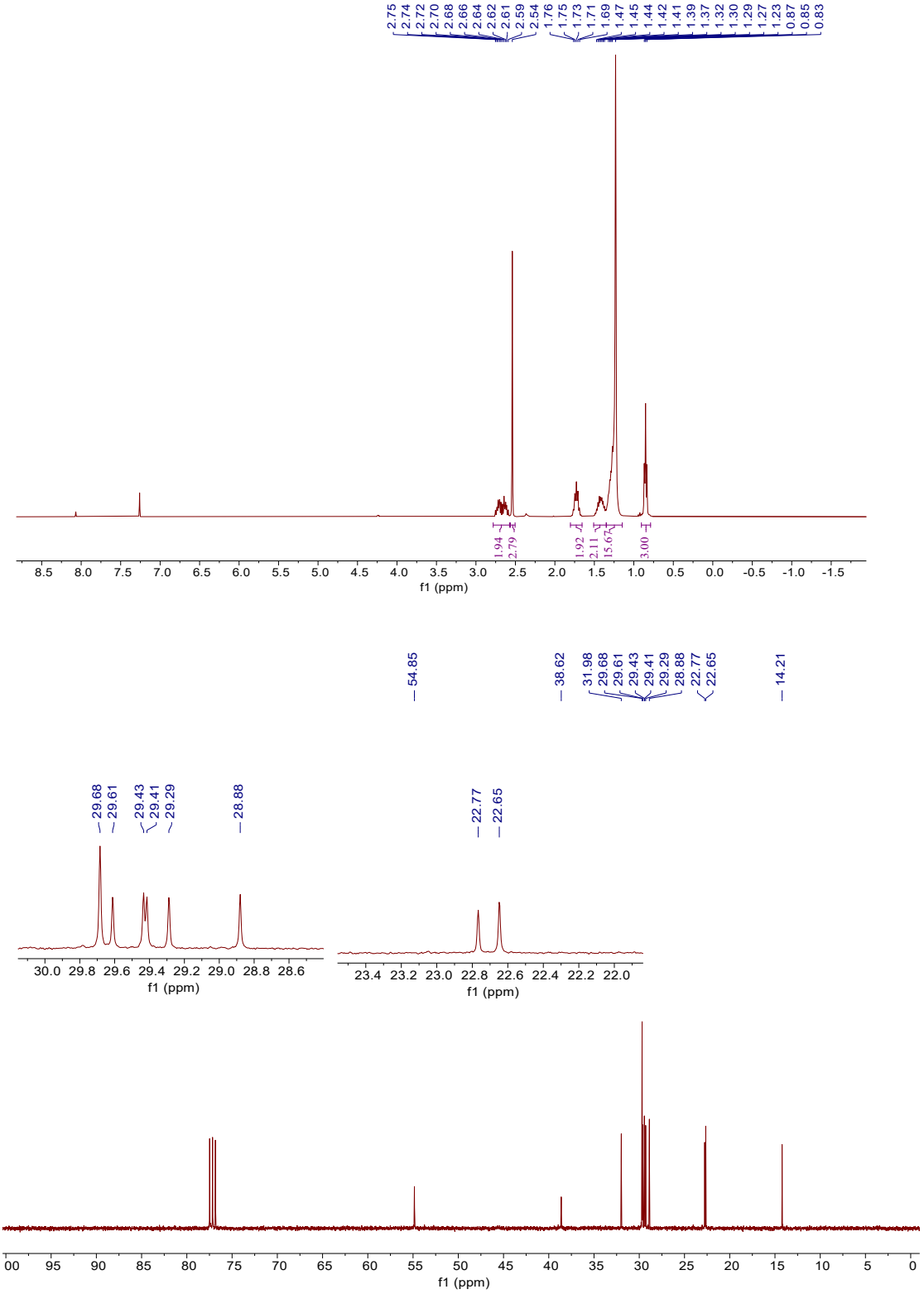
序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU. s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	12.228	11.073	56.9	271.605	49.8	0.814	
2	16.117	8.403	43.1	273.704	50.2	1.079	
合计		19.476	100.0	545.309	100.0		

HPLC spectra for (*S*)-**1j**

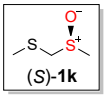


序号	保留时间[min]	峰高[mAU]	峰高[%]	峰面积[mAU. s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	12.056	0.193	3.3	3.318	1.8	0.477	
2	15.847	5.680	96.7	179.419	98.2	1.049	
合计		5.873	100.0	182.737	100.0		

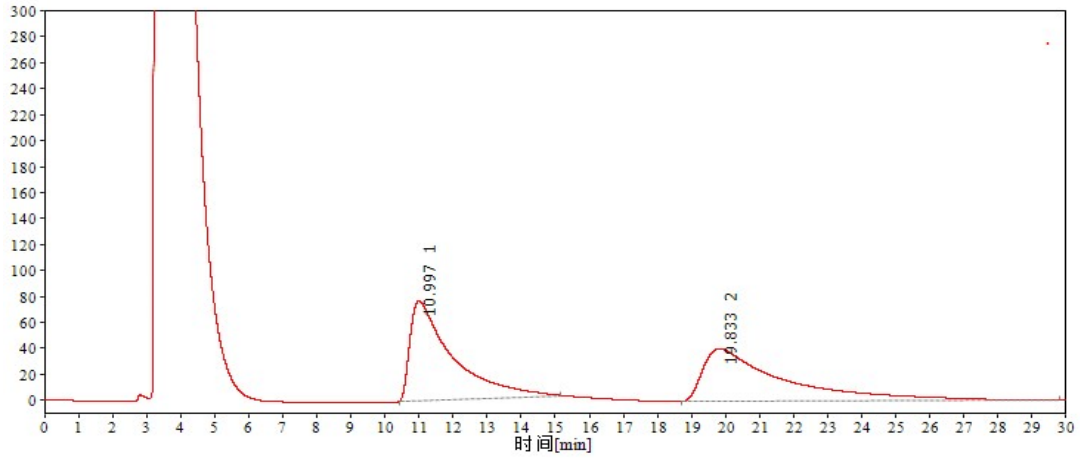
NMR spectra for (*S*)-**1j**



3.11 HPLC and NMR spectra data for compound (*S*)-1k

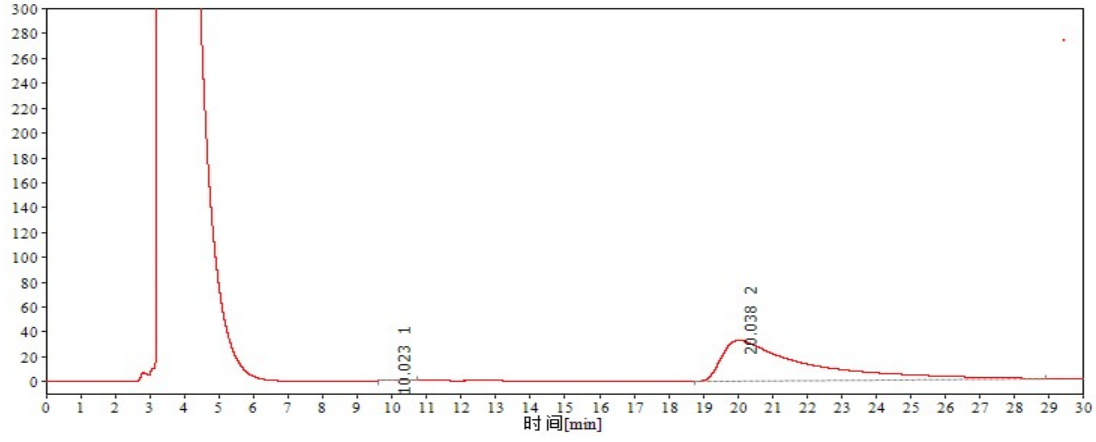


HPLC spectra for *rac*-**1k**



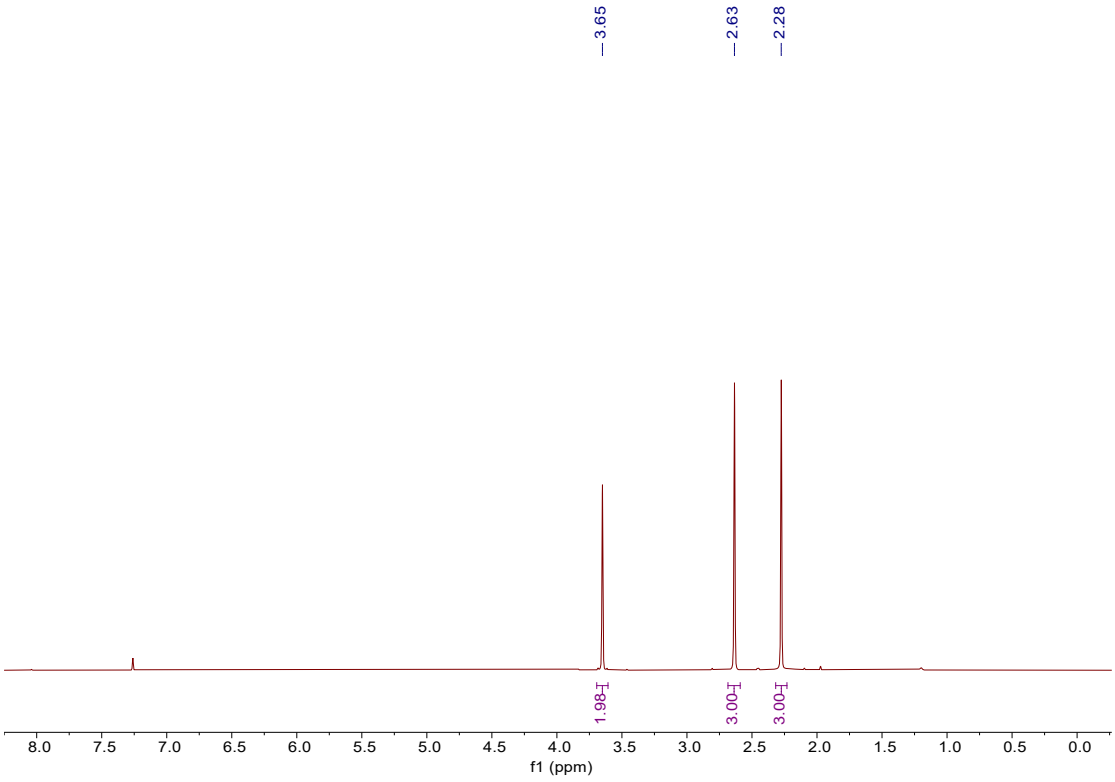
序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	10.997	76.941	65.7	6624.993	50.0	3.559	
2	19.833	40.249	34.3	6631.830	50.0	7.541	
合计		117.190	100.0	13256.823	100.0		

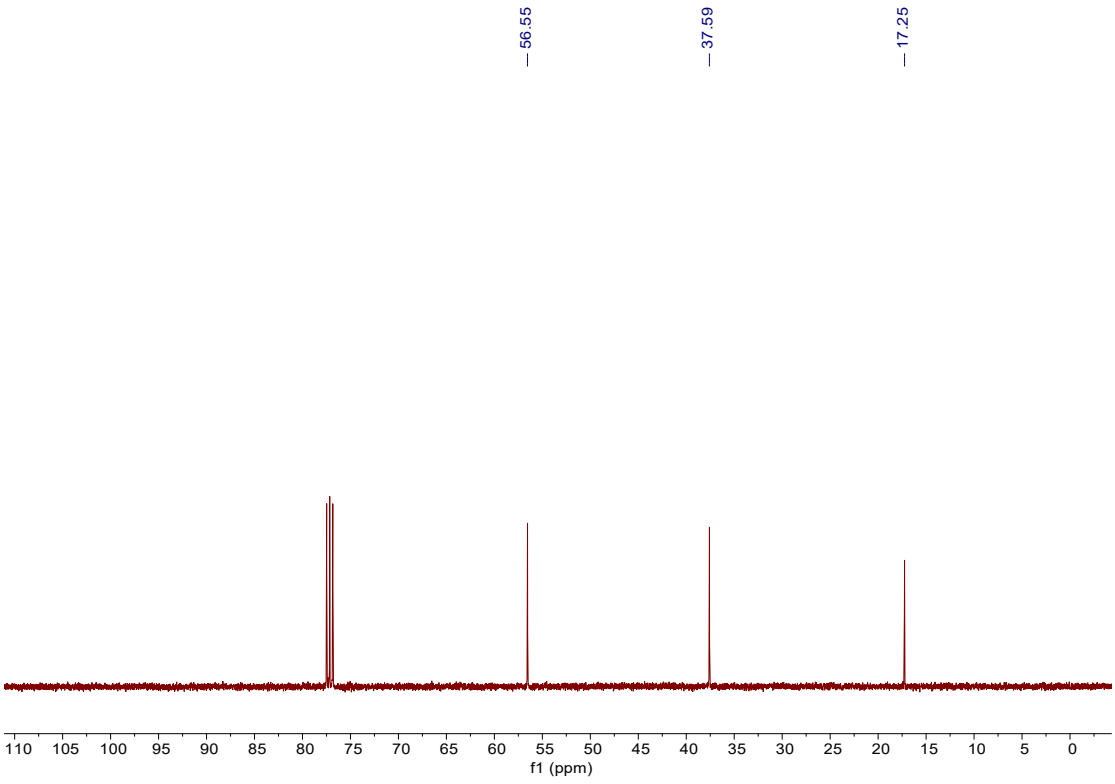
HPLC spectra for (*S*)-**1k**



序号	保留时间 [min]	峰高[mAU]	峰高[%]	峰面积 [mAU.s]	峰面积[%]	0.05峰高处峰宽[min]	化合物名称
1	10.023	0.599	1.8	21.033	0.4	1.025	
2	20.038	32.775	98.2	5462.526	99.6	7.196	
合计		33.374	100.0	5483.559	100.0		

NMR spectra for (*S*)-**1k**





References

1. Y. Wen, L. Peng, Y. Zhou, T. Peng, Y. Chen, X. Cheng, Y. Chen and J. Yang, *Catalysis Communications*, 2020, **136**, 105908.
2. H. Egami and T. Katsuki, *Journal of the American Chemical Society*, 2007, **129**, 8940-8941.
3. H. L. Holland, C. D. Turner, P. R. Andreana and D.Nguyen, Can. *J. Chem.* 1999, **77**, 463-471.
4. J. Yang , L. Wang, Y. Lv, N. Li, Y. An and S. Gao, *Tetrahedron Letters*, 2018, **59**, 156-159.
5. T. Peng, J. Tian, Y. Zhao, X. Jiang, X. Cheng, G. Deng, Q. Zhang, Z. Wang, J. Yang and Y. Chen, *Angew Chem Int Ed Engl*, 2022, **61**, e202209272.
6. S. Grabovskii, N. Andrijashina, A. Lobov, A. Antipin, R. Safiullin, *Synlett*, 2023,**34**. In press, DOI: 10.1055/a-2015-7526.