

Supporting Information for

Microfluidics Biocatalysis System Applied for the Synthesis of N-substituted Benzimidazole Derivatives by aza-Michael Addition

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Materials

All chemicals in this study were obtained from commercial sources and did not require further purification. Lipzyme TL IM from *Thermomyces lanuginosus* was purchased from Novo Nordisk (Copenhagen, Denmark). Benzimidazole, 2-chlorobenzimidazole, 2-methylbenzimidazole, 2-chloroacrylonitrile, acrylonitrile, methyl acrylate, methyl methacrylate, phenyl vinyl sulfone were purchased from Aladdin (Shanghai, China). Harvard Instrument PHD 2000 syringe pump was purchased from Harvard University (Holliston, Massachusetts, USA). The flow reactor and Y-mixer were purchased from Beijing Haigui Medical Engineering Design Co., Ltd (Beijing China). A 400 MHz NMR spectrometer (Billerica, MA, USA) were also used in this study.

Purification of the product

When the conversion of the benzimidazole derivative reaches a maximum (determined by TLC), the reaction is terminated by filtering the enzyme, and the methanolic solvent is rotary evaporated under

reduced pressure. The product is separated by silica gel chromatography (mobile phase dichloromethane/methanol, 60/1 to 40/1). Purification was monitored by TLC. The graded fractions containing the major product were combined, the solvent evaporated and the residue analyzed by ^1H NMR, ^{13}C NMR.

Experimental setup

A continuous-flow protocol for the aza-Michael addition of benzimidazoles to α , β -unsaturated compounds in microreactors is described in Figure 6. The experimental setup consists of two syringe pumps, coil reactor 1 and coil reactor 2, Y-shaped mixers ($\varphi = 1.8$ mm). Syringe pumps (Harvard apparatus PHD 2000) were used to introduce separate feed streams to 3.1 mL PFA coil reactors (2.0 mm I.D.). Silica gel tubes were filled with lipase TL IM and immersed in a constant temperature water bath to control the temperature. 5 mmol of benzimidazoles were dissolved in 10 mL of methanol (feed 1), and 30 mmol of α , β -unsaturated compounds were dissolved in 10 mL of methanol (feed 2). Feeds 1 and 2 were placed in separate 10 mL feeders and mixed at a flow rate of $8.91 \mu\text{L min}^{-1}$ in a Y-mixer at 45°C . The resulting stream ($17.8 \mu\text{L min}^{-1}$) was connected to a sample vial for collection of the final mixture. The final mixture was then evaporated and the residue was separated by silica gel chromatography (200-300 mesh). Grades containing the major product were combined and the solvent was evaporated. The main products were determined by ^1H NMR and ^{13}C NMR.



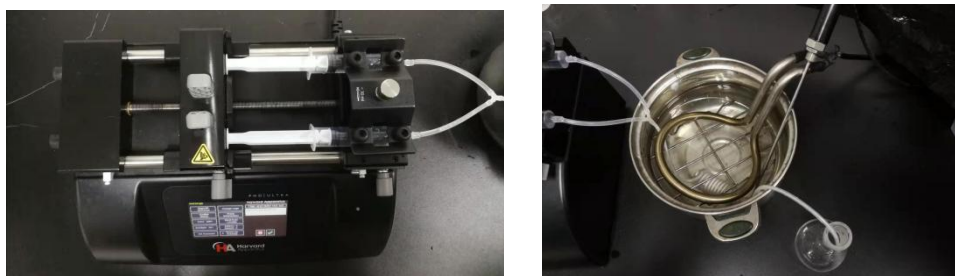


Figure S1 Continuous flow microreactor

Thin-Layer Chromatography

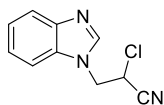
TLC analysis with methanol/dichloromethane 1/20 (v/v) as the eluent. The results were detected by UV irradiation at 254 nm.

Experiments

General procedure for the synthesis of N-substituted benzimidazole derivatives in a continuous flow microreactor

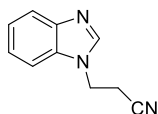
Silica gel tubes were filled with lipase TL IM and immersed in a constant temperature water bath to control the temperature. 5 mmol of the benzimidazole derivative was dissolved in 10 mL of methanol (feed 1) and 30 mmol of the unsaturated olefin was dissolved in 10 mL of methanol (feed 2). Feeds 1 and 2 were placed in separate 10 mL feeders and mixed together at a flow rate of $8.91 \mu\text{L min}^{-1}$ in a Y-mixer at 45°C . The resulting stream ($17.8 \mu\text{L min}^{-1}$) was connected to a sample vial for collection of the final mixture. The final mixture was then evaporated and the residue separated by silica gel chromatography (200-300 mesh). Grades containing the major product were combined and the solvent was evaporated. The main products were determined by ^1H NMR, ^{13}C NMR and ESI-MS.

Experimental data of products

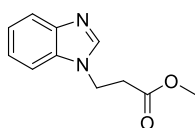


3-(1H-benzo[d]imidazol-1-yl)-2-chloropropanenitrile (3a). White solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.30 (s, 1H), 7.80 (dt, $J = 8.1, 1.0$ Hz, 1H), 7.72 – 7.65 (m, 1H), 7.27 (dddd, $J = 21.6, 8.4, 7.2, 1.2$ Hz, 2H), 5.77 (t, $J = 6.2$ Hz, 1H), 5.04 (dd, $J = 6.2, 1.4$ Hz, 2H). ^{13}C NMR (101 MHz,

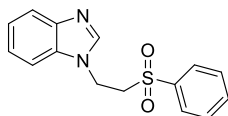
DMSO-*d*₆) δ 144.64, 143.13, 133.75, 122.93, 122.29, 119.63, 116.64, 111.17, 46.91, 42.68. HRMS (ESI): calculated for C₁₀H₉ClN₃ [M + H]⁺: 206.0480, found 206.0474.



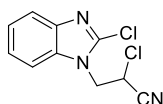
3-(1H-benzo[d]imidazol-1-yl)propanenitrile (3b)[1]. White solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.28 (s, 1H), 7.69 (ddt, *J* = 16.9, 7.8, 0.9 Hz, 2H), 7.26 (dddd, *J* = 25.2, 8.3, 7.2, 1.2 Hz, 2H), 4.58 (t, *J* = 6.5 Hz, 2H), 3.12 (t, *J* = 6.5 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 144.06, 143.38, 133.44, 122.68, 121.97, 119.59, 118.64, 110.62, 39.91, 18.56. HRMS (ESI): calculated for C₁₀H₁₀N₃ [M + H]⁺: 172.0869, found 172.0863.



methyl 3-(1H-benzo[d]imidazol-1-yl)propanoate (3c)[2]. Transparent liquid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.21 (s, 1H), 7.69 – 7.59 (m, 2H), 7.23 (ddd, *J* = 14.6, 7.8, 1.3 Hz, 2H), 4.50 (t, *J* = 6.7 Hz, 2H), 3.56 (s, 3H), 2.93 (t, *J* = 6.7 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 171.28, 144.21, 143.42, 133.57, 122.39, 121.59, 119.50, 110.43, 51.61, 39.99, 33.83. HRMS (ESI): calculated for C₁₁H₁₂N₂O₂ [M + H]⁺: 205.0982, found 205.0974.

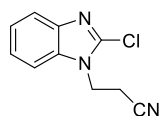


1-(2-(phenylsulfonyl)ethyl)-1H-benzo[d]imidazole (3e)[3]. White solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (s, 1H), 7.85 – 7.79 (m, 2H), 7.70 – 7.63 (m, 1H), 7.60 – 7.51 (m, 3H), 7.45 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.26 – 7.14 (m, 2H), 4.59 (t, *J* = 6.6 Hz, 2H), 4.00 (t, *J* = 6.6 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 144.12, 143.29, 138.76, 133.96, 133.27, 129.36, 127.41, 122.40, 121.66, 119.44, 110.27, 53.54, 38.19. HRMS (ESI): calculated for C₁₅H₁₅N₂O₂S [M + H]⁺: 287.0849, found 287.0836.

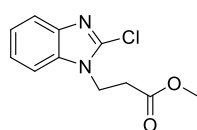


2-chloro-3-(2-chloro-1H-benzo[d]imidazol-1-yl)propanenitrile (3f). Transparent liquid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.86 – 7.80 (m, 1H), 7.64 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.32 (dtd, *J* = 21.4, 7.5, 1.3 Hz, 2H), 5.78 (dd, *J* = 7.3, 6.0 Hz, 1H), 5.03 (qd, *J* = 15.5, 6.6 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 140.98, 140.42, 134.81, 123.39, 123.09, 118.75, 116.28, 111.40, 46.47, 41.66.

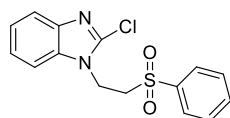
HRMS (ESI): calculated for $C_{10}H_8Cl_2N_3$ $[M + H]^+$: 240.0090, found 240.0088.



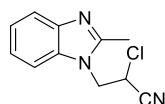
3-(2-chloro-1H-benzo[d]imidazol-1-yl)propanenitrile (3g). White solid. 1H NMR (400 MHz, $DMSO-d_6$) δ 7.75 – 7.68 (m, 1H), 7.66 – 7.59 (m, 1H), 7.31 (dtd, J = 21.2, 7.4, 1.3 Hz, 2H), 4.59 (t, J = 6.4 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 141.19, 140.03, 134.69, 123.37, 122.94, 118.84, 118.36, 110.94, 48.73, 17.75. HRMS (ESI): calculated for $C_{10}H_9ClN_3$ $[M + H]^+$: 206.0480, found 206.0474.



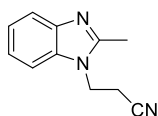
methyl 3-(2-chloro-1H-benzo[d]imidazol-1-yl)propanoate (3h). Transparent liquid. 1H NMR (400 MHz, $DMSO-d_6$) δ 7.72 – 7.54 (m, 2H), 7.27 (dtd, J = 23.4, 7.3, 1.3 Hz, 2H), 4.51 (t, J = 6.9 Hz, 2H), 3.37 (d, J = 1.4 Hz, 3H), 2.87 (t, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 170.79, 141.13, 139.90, 134.73, 123.03, 122.49, 118.65, 110.75, 51.66, 39.95, 33.13. HRMS (ESI): calculated for $C_{11}H_{12}ClN_2O_2$ $[M + H]^+$: 239.0582, found 239.0577.



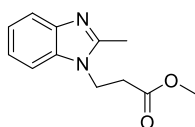
2-chloro-1-(2-(phenylsulfonyl)ethyl)-1H-benzo[d]imidazole (3j). White solid. 1H NMR (400 MHz, $DMSO-d_6$) δ 7.86 – 7.79 (m, 2H), 7.72 – 7.63 (m, 1H), 7.59 – 7.46 (m, 4H), 7.25 (dtd, J = 19.2, 7.4, 1.3 Hz, 2H), 4.58 (t, J = 6.7 Hz, 2H), 3.99 (t, J = 6.7 Hz, 2H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 141.01, 139.73, 138.59, 134.44, 134.01, 129.35, 127.30, 123.01, 122.58, 118.59, 110.71, 52.49, 38.02. HRMS (ESI): calculated for $C_{15}H_{14}ClN_2O_2S$ $[M + H]^+$: 321.0459, found 321.0456.



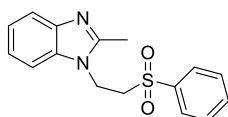
2-chloro-3-(2-methyl-1H-benzo[d]imidazol-1-yl)propanenitrile (3k). White solid. 1H NMR (400 MHz, $DMSO-d_6$) δ 7.75 – 7.65 (m, 1H), 7.59 – 7.48 (m, 1H), 7.25 – 7.13 (m, 2H), 5.78 – 5.70 (m, 1H), 5.02 (dd, J = 15.4, 6.2 Hz, 1H), 4.93 (dd, J = 15.4, 7.7 Hz, 1H), 2.61 (s, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 152.29, 142.20, 134.95, 121.88, 121.85, 118.30, 116.69, 110.70, 45.81, 42.01, 13.77. HRMS (ESI): calculated for $C_{11}H_{11}ClN_3$ $[M + H]^+$: 220.0636, found 220.0634.



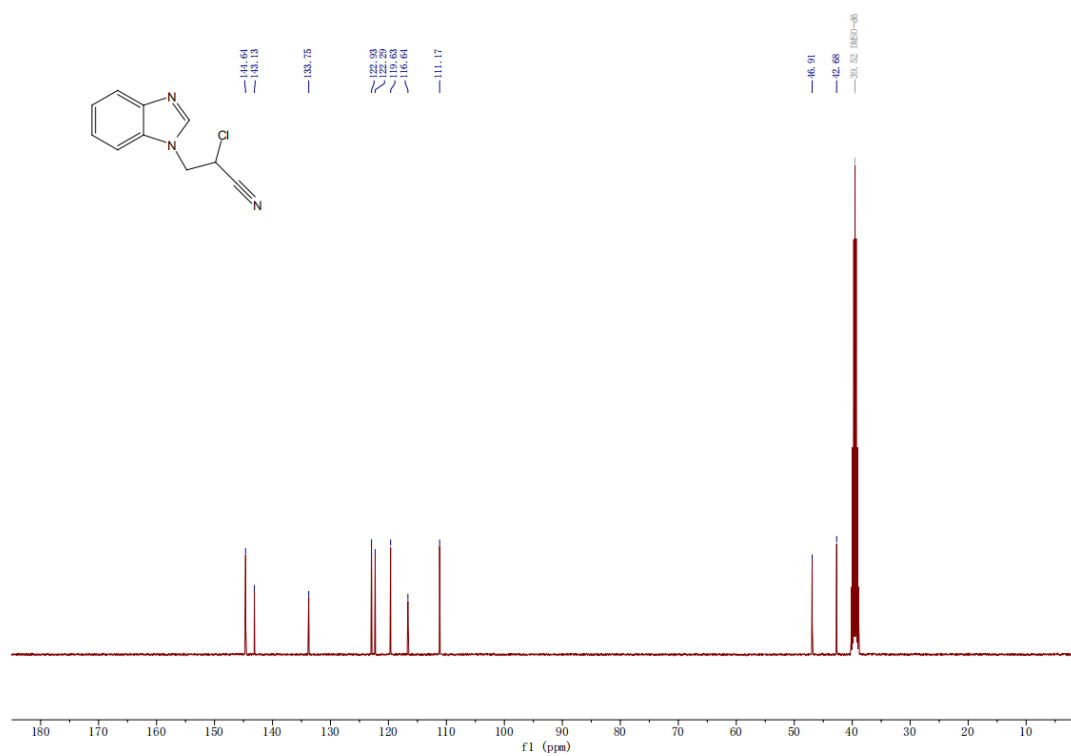
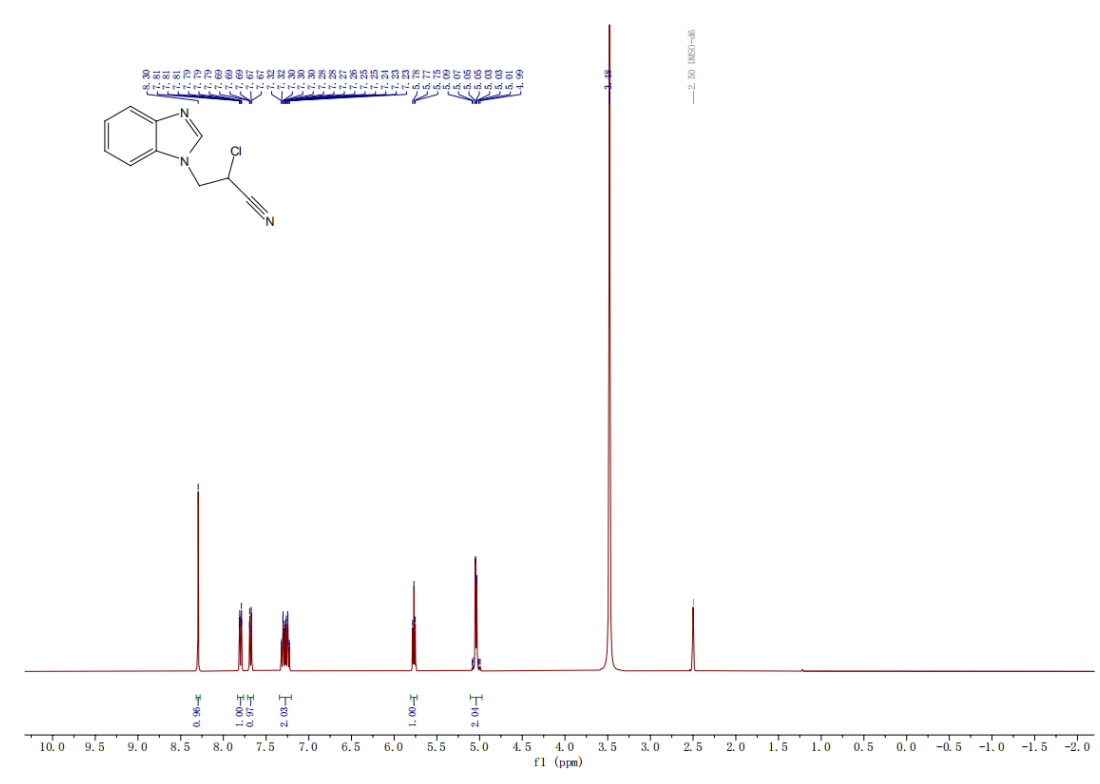
3-(2-methyl-1H-benzo[d]imidazol-1-yl)propanenitrile (3l). White solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.65 – 7.58 (m, 1H), 7.58 – 7.49 (m, 1H), 7.18 (pd, J = 7.2, 1.4 Hz, 2H), 4.52 (t, J = 6.6 Hz, 2H), 3.05 (t, J = 6.6 Hz, 2H), 2.59 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 151.84, 142.36, 134.70, 121.69, 121.57, 118.80, 118.31, 110.04, 38.72, 17.87, 13.50. HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{12}\text{N}_3$ $[\text{M} + \text{H}]^+$: 186.1026, found 186.1020.



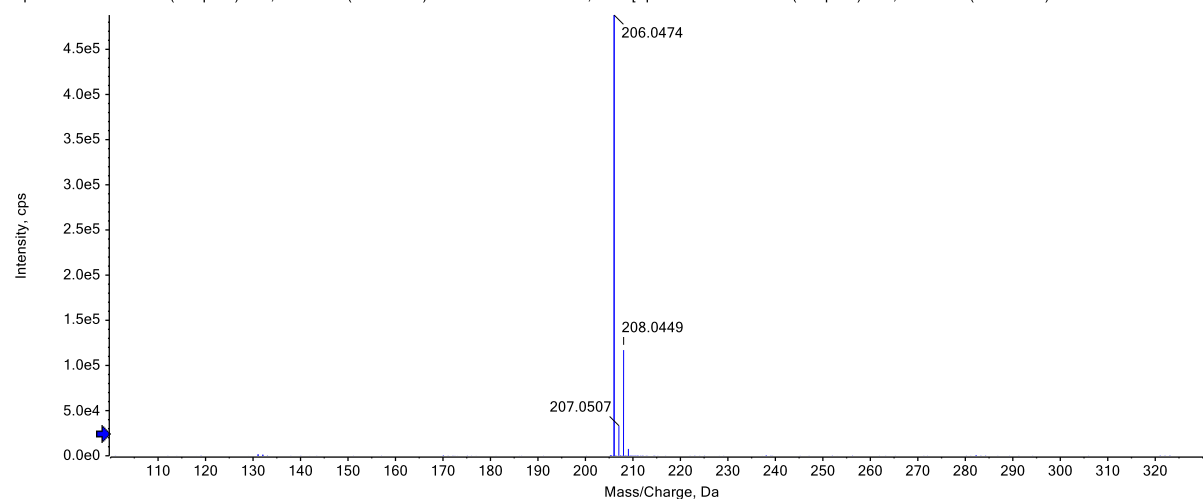
methyl 3-(2-methyl-1H-benzo[d]imidazol-1-yl)propanoate (3m). Transparent liquid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.58 – 7.45 (m, 2H), 7.16 (pd, J = 7.2, 1.4 Hz, 2H), 4.43 (t, J = 7.0 Hz, 2H), 3.56 (s, 3H), 2.85 (t, J = 6.9 Hz, 2H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 171.24, 151.81, 142.43, 134.75, 121.48, 121.25, 118.24, 109.86, 51.56, 38.99, 33.35, 13.40. HRMS (ESI): calculated for $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2$ $[\text{M} + \text{H}]^+$: 219.1129, found 219.1118.



2-methyl-1-(2-(phenylsulfonyl)ethyl)-1H-benzo[d]imidazole (3o)[4]. White solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.90 – 7.82 (m, 2H), 7.74 – 7.65 (m, 1H), 7.57 (t, J = 7.8 Hz, 2H), 7.48 – 7.40 (m, 1H), 7.33 – 7.25 (m, 1H), 7.17 – 7.06 (m, 2H), 4.49 (t, J = 6.8 Hz, 2H), 3.93 (t, J = 6.8 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 151.68, 142.28, 138.76, 134.49, 133.99, 129.38, 127.35, 121.53, 121.38, 118.20, 109.68, 52.95, 36.97, 13.36. HRMS (EI): calculated for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$ $[\text{M}]^+$: 300.0932, found 300.0903.

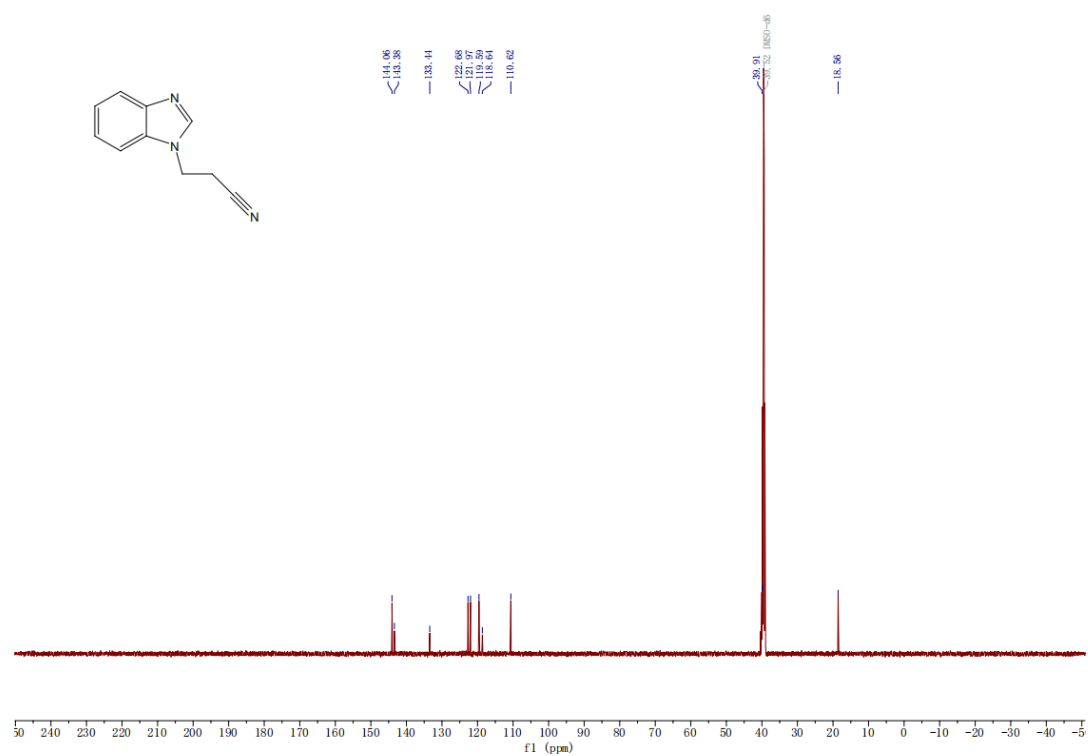
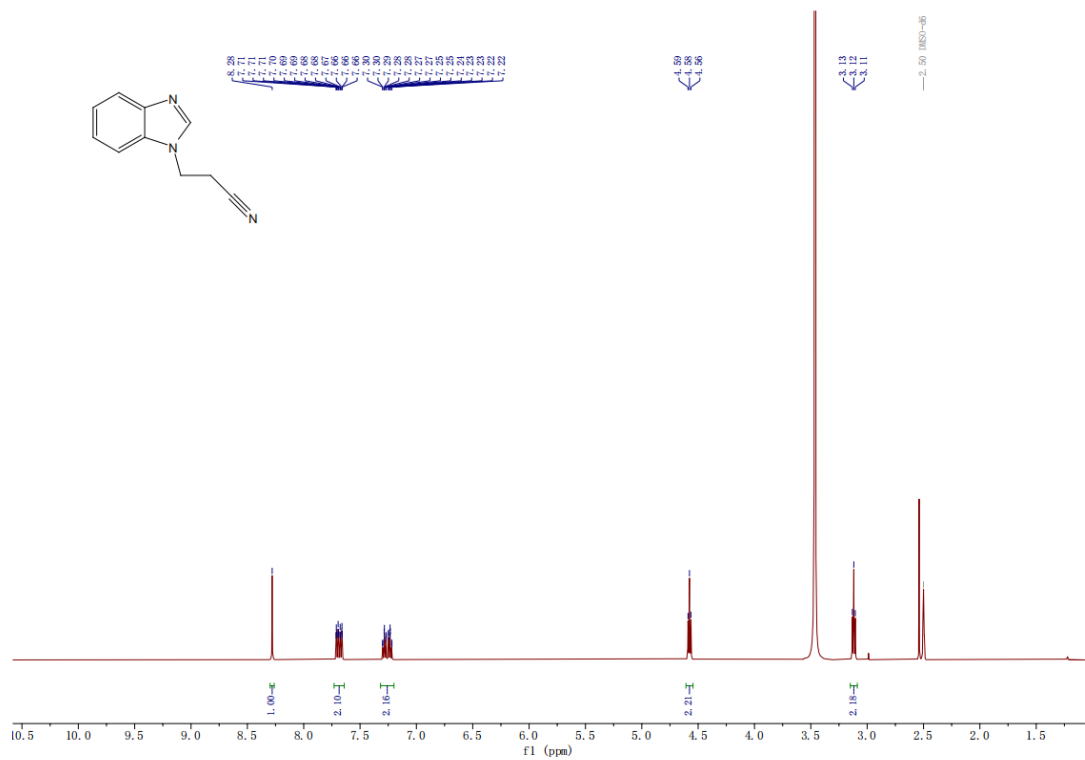


Spectrum from 05.wiff2 (sample 2) - BL, +TOF MS (100 - 1000) from 0.258 to 0.277 min, su...: [Spectrum from 05.wiff2 (sample 2) - BL, +TOF MS (100 - 1000) from 0.443 to 0.966 min]

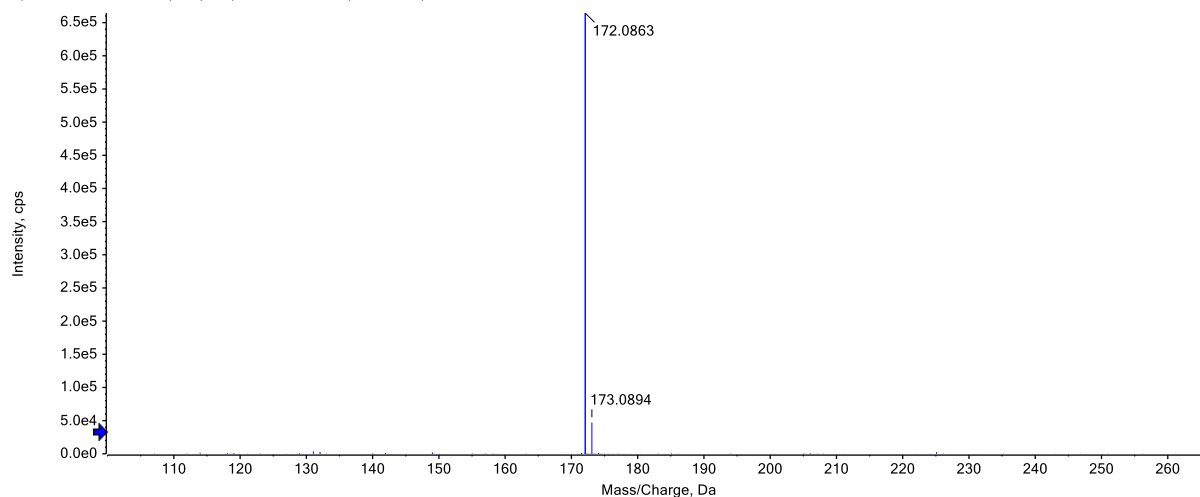


Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{10}H_9ClN_3$	206.04795	-2.8	-0.57	8.0

3b

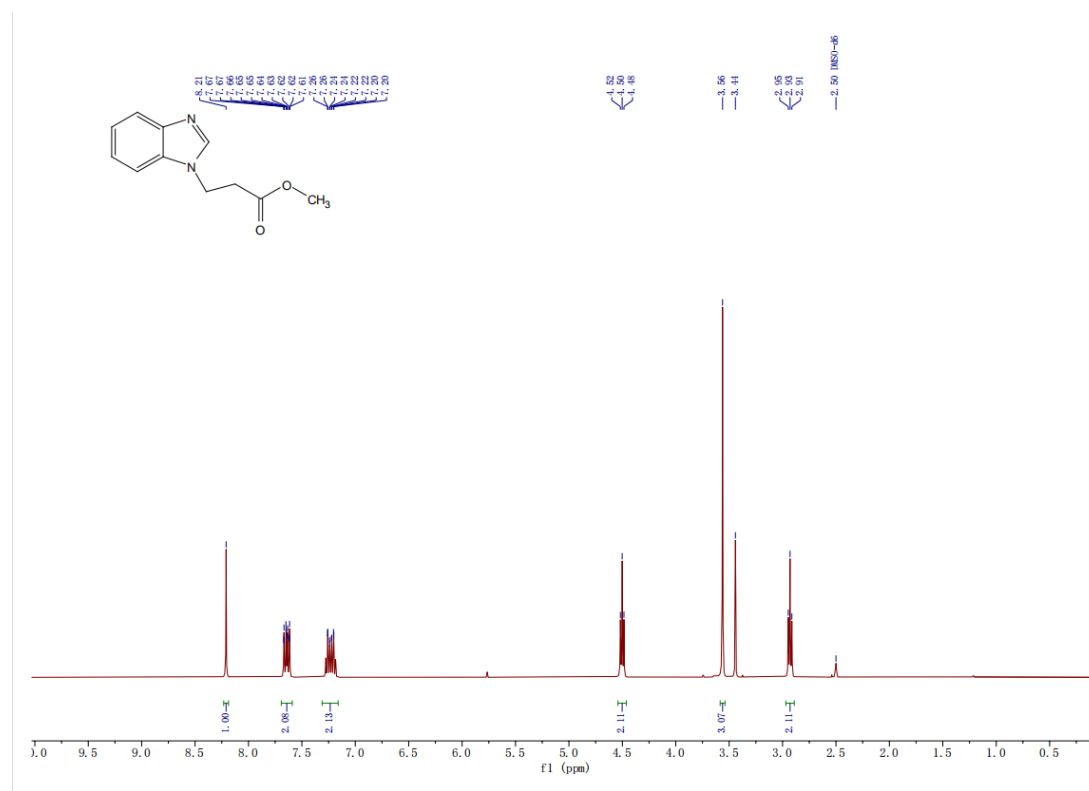


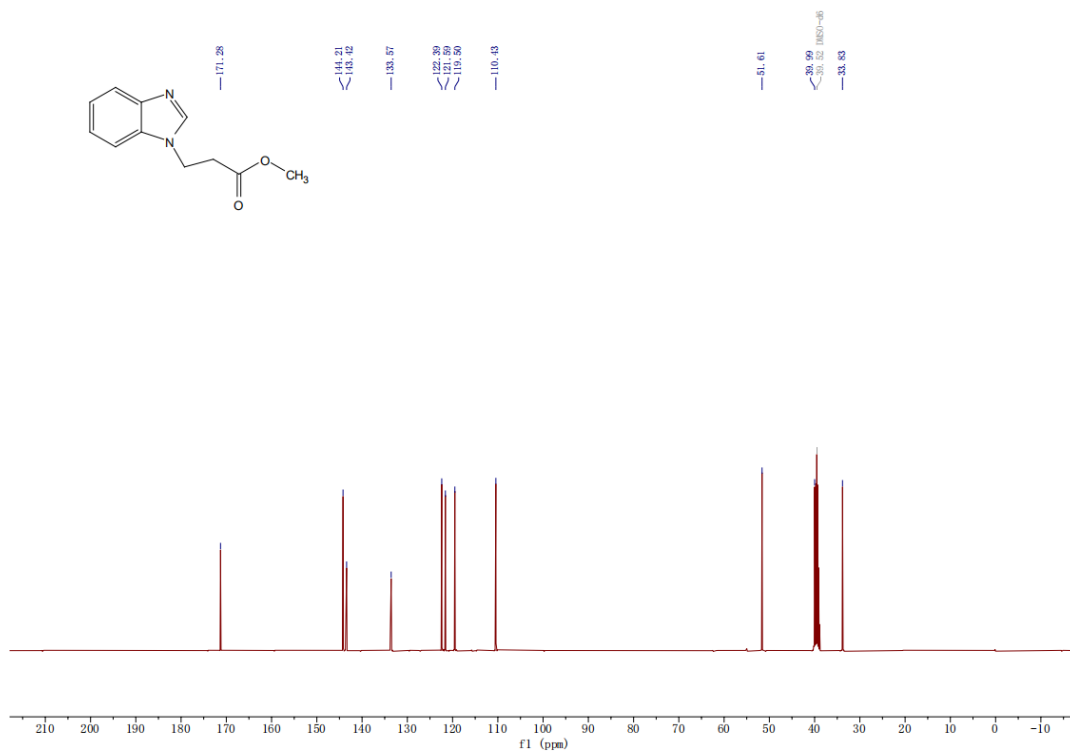
Spectrum from 05.wiff2 (sample 4) - BB, +TOF MS (100 - 1000) from 0.258 to 0.277 min



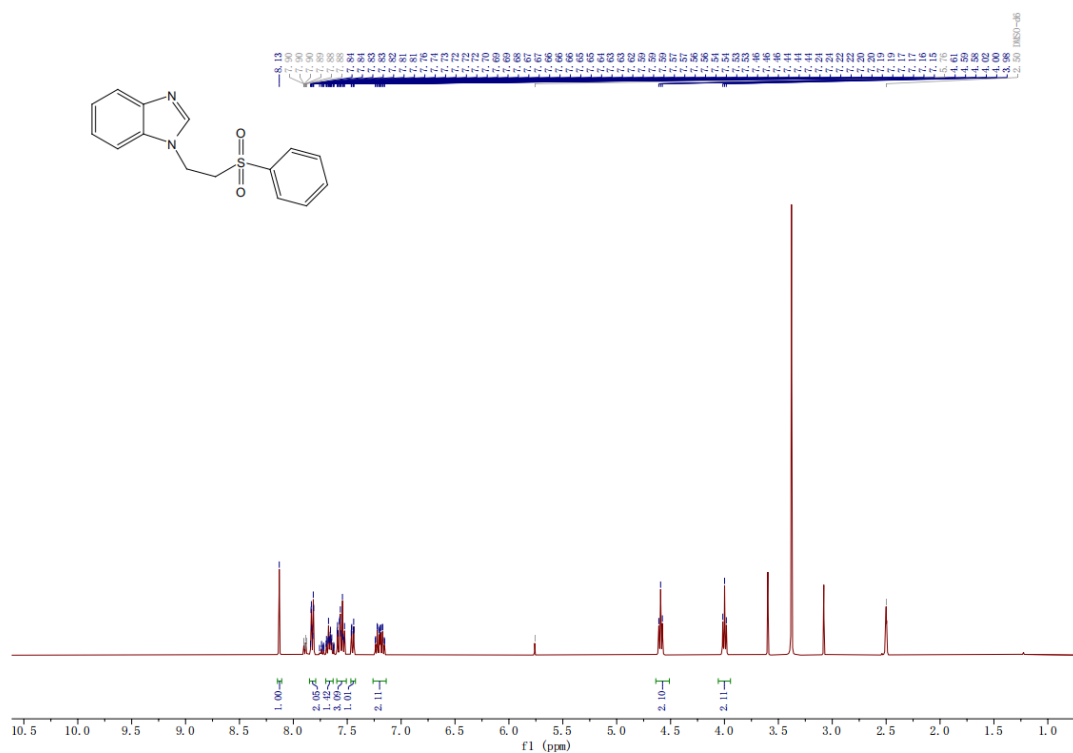
Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{10}H_{10}N_3$	172.08692	-3.7	-0.64	8.0

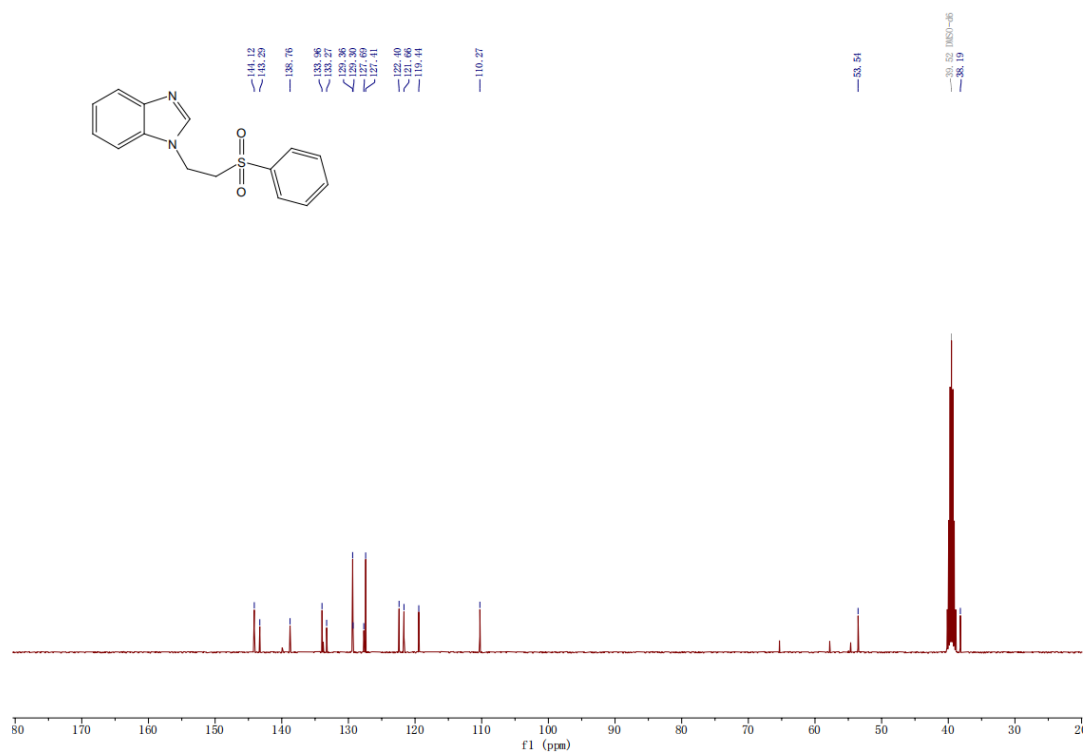
3c



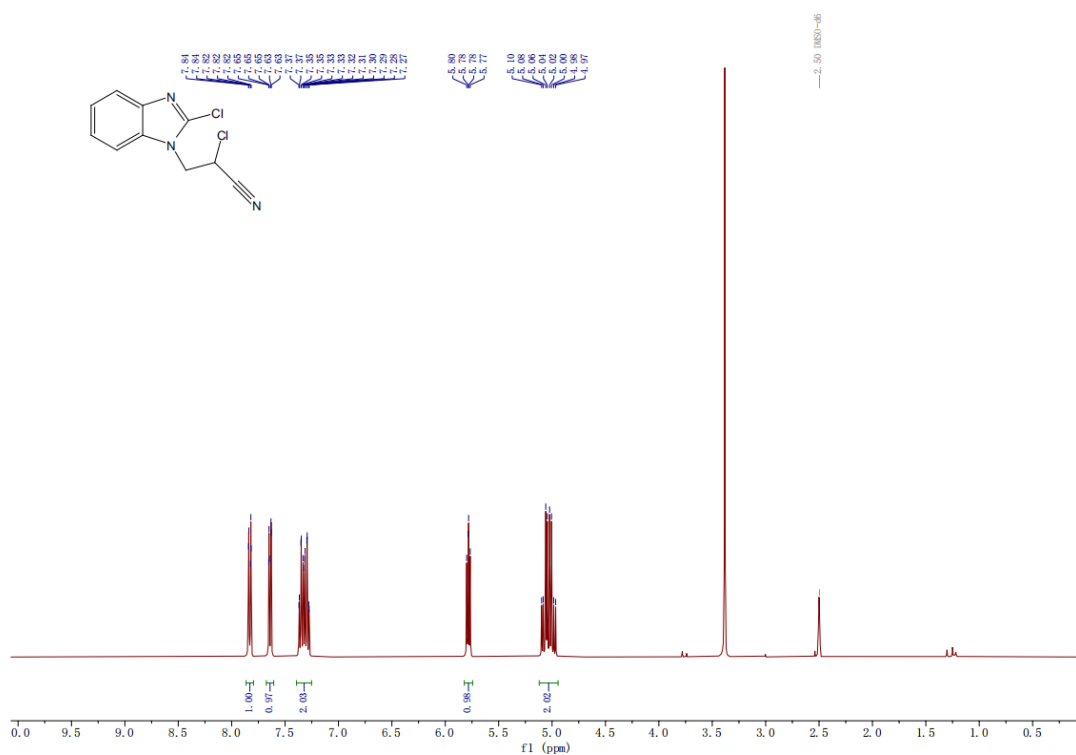


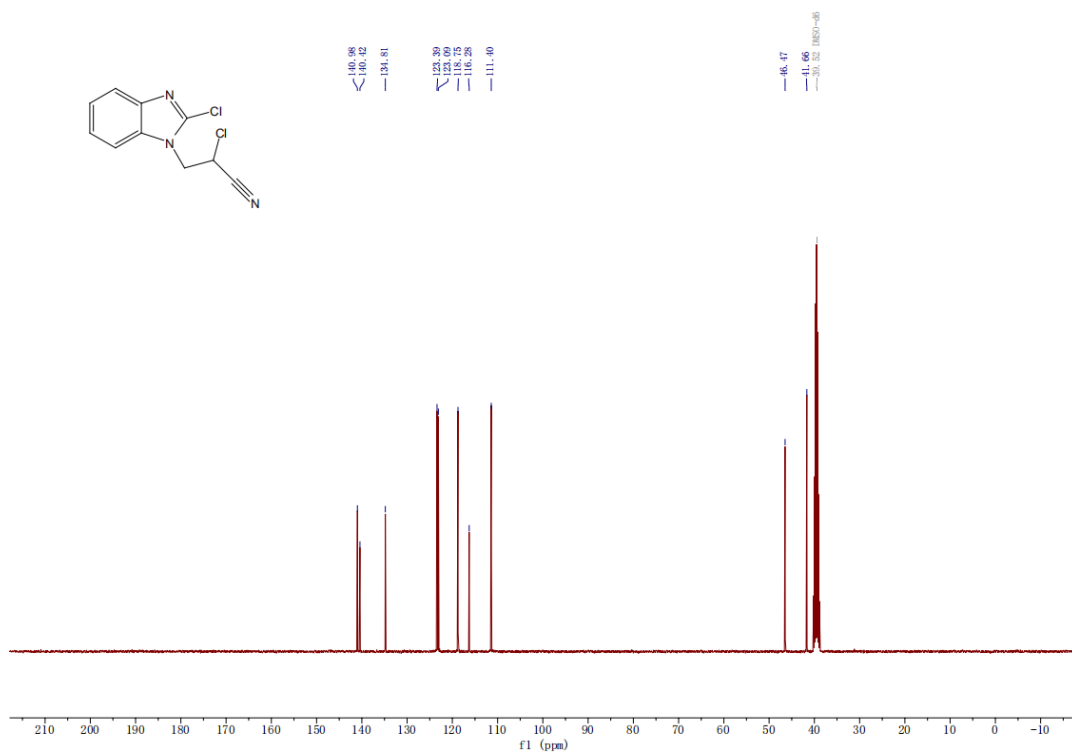
3e



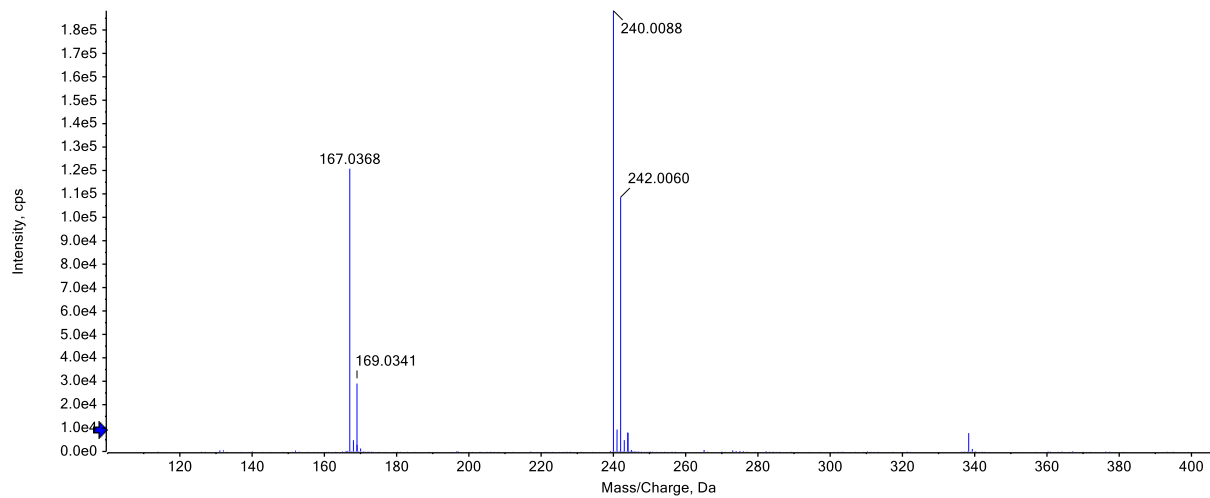


3f



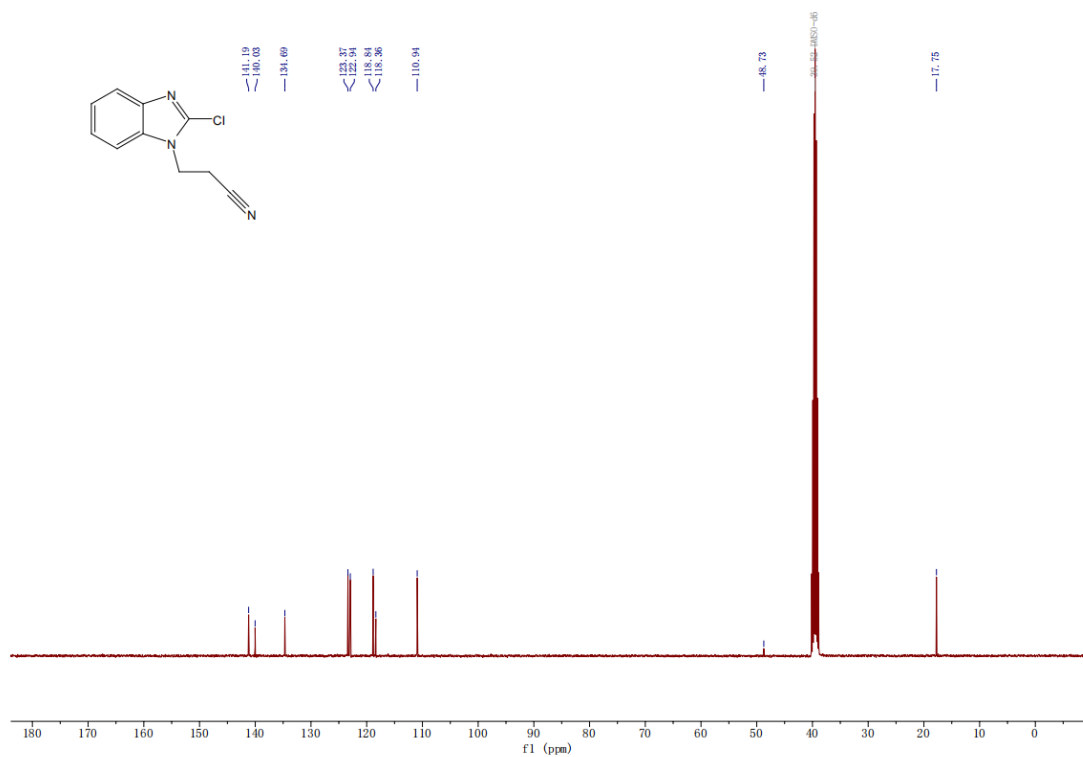
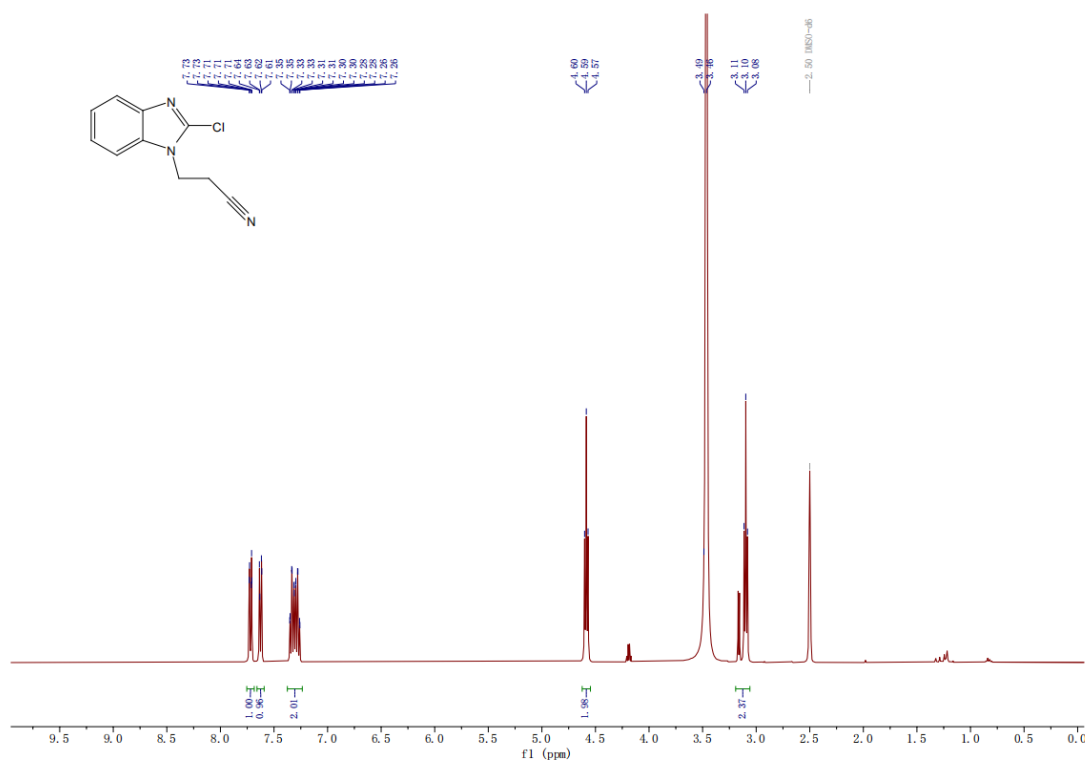


Spectrum from 05.wiff2 (sample 7) - L+L, +TOF MS (100 - 1000) from 0.221 to 0.240 min, s... [Spectrum from 05.wiff2 (sample 7) - L+L, +TOF MS (100 - 1000) from 0.443 to 0.966 min]

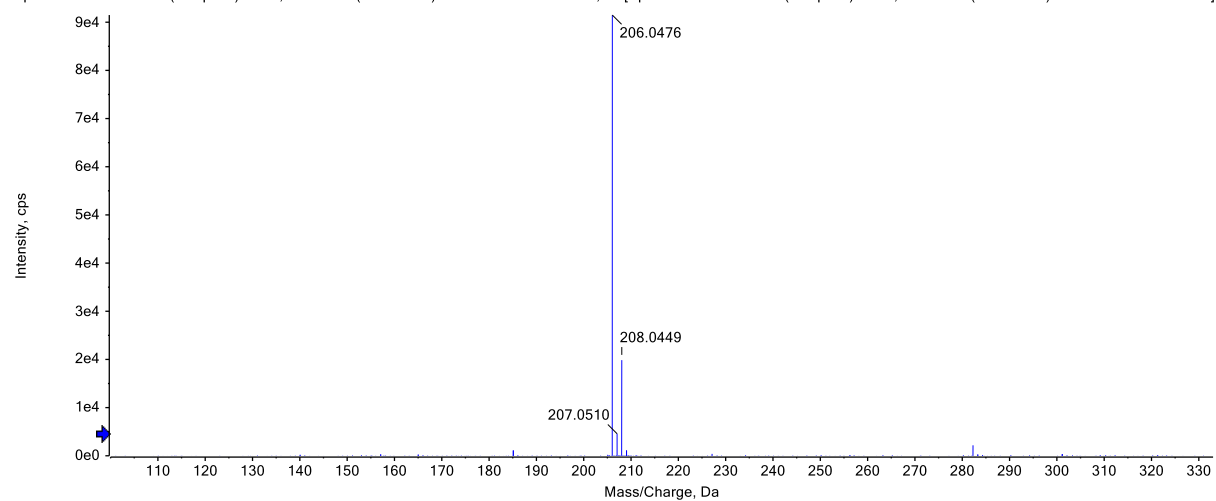


Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
C ₁₀ H ₈ Cl ₂ N ₃	240.00898	-0.7	-0.16	8.0

3g

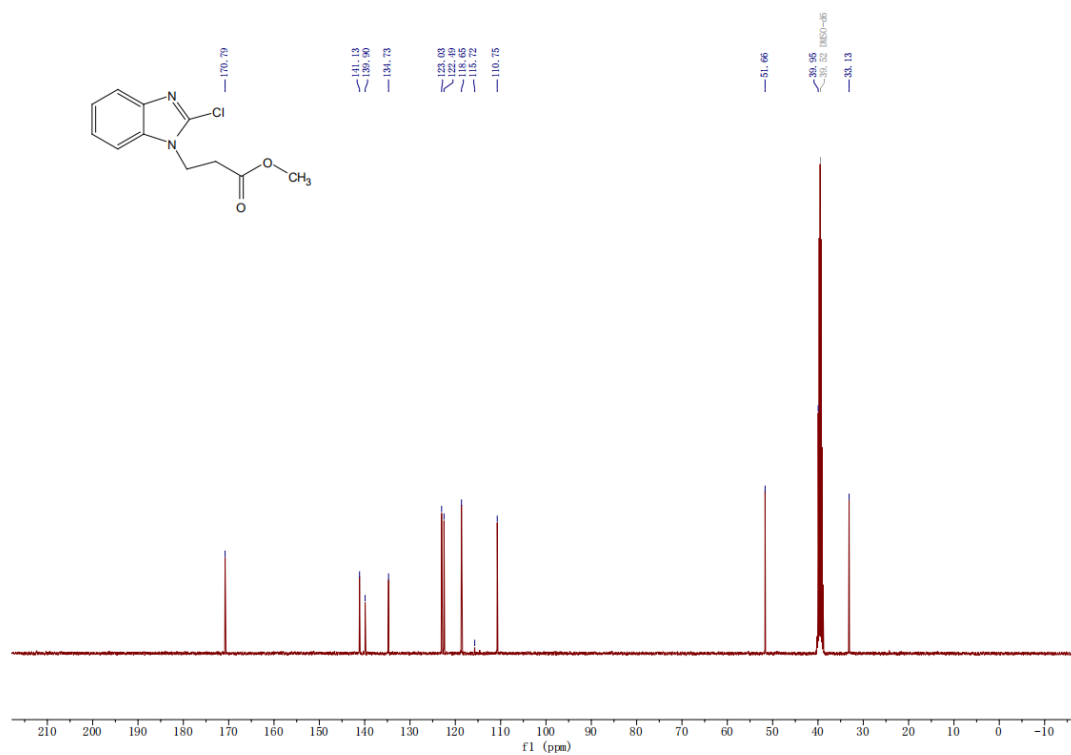
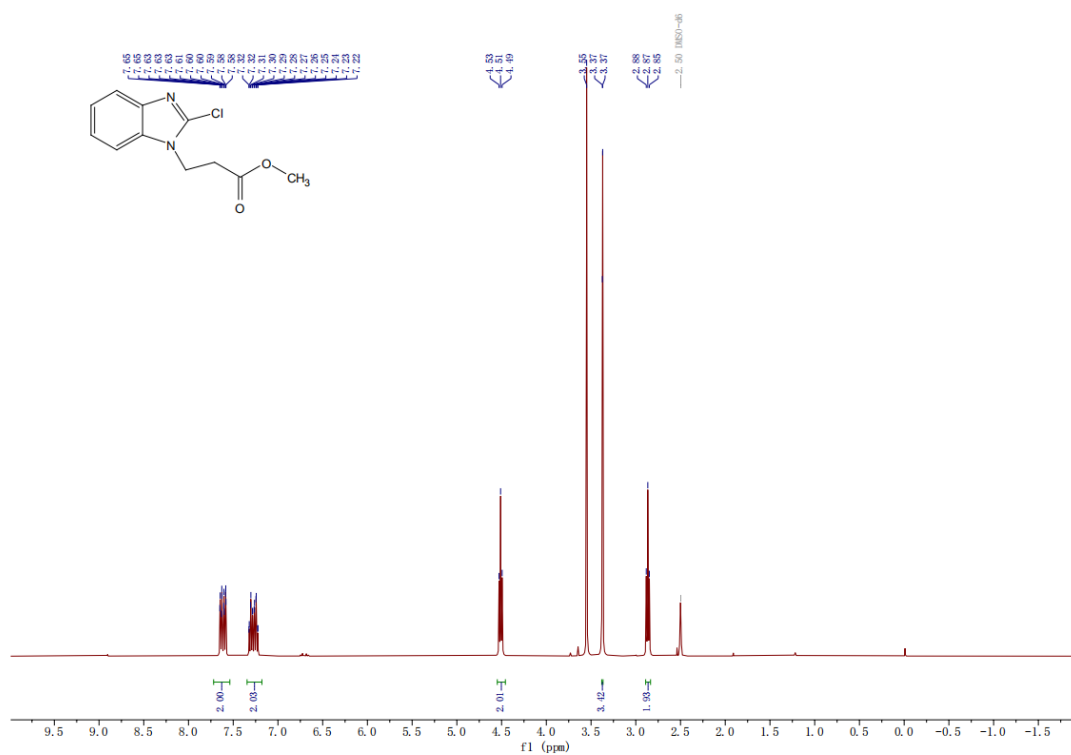


Spectrum from 05.wiff2 (sample 6) - L+B, +TOF MS (100 - 1000) from 0.194 to 0.212 min, s...[Spectrum from 05.wiff2 (sample 6) - L+B, +TOF MS (100 - 1000) from 0.443 to 0.966 min]

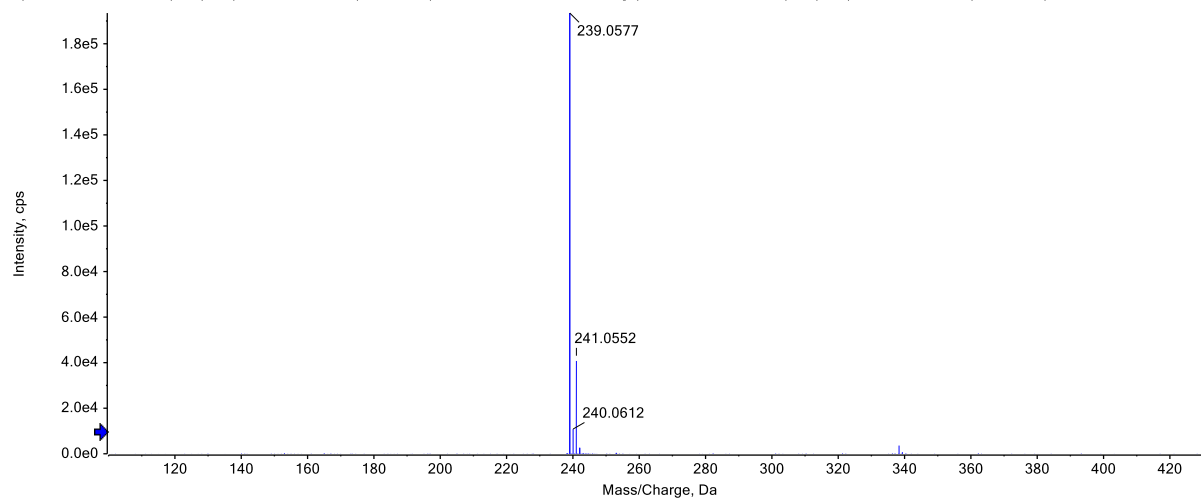


Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{10}H_9ClN_3$	206.04795	-1.6	-0.32	8.0

3h

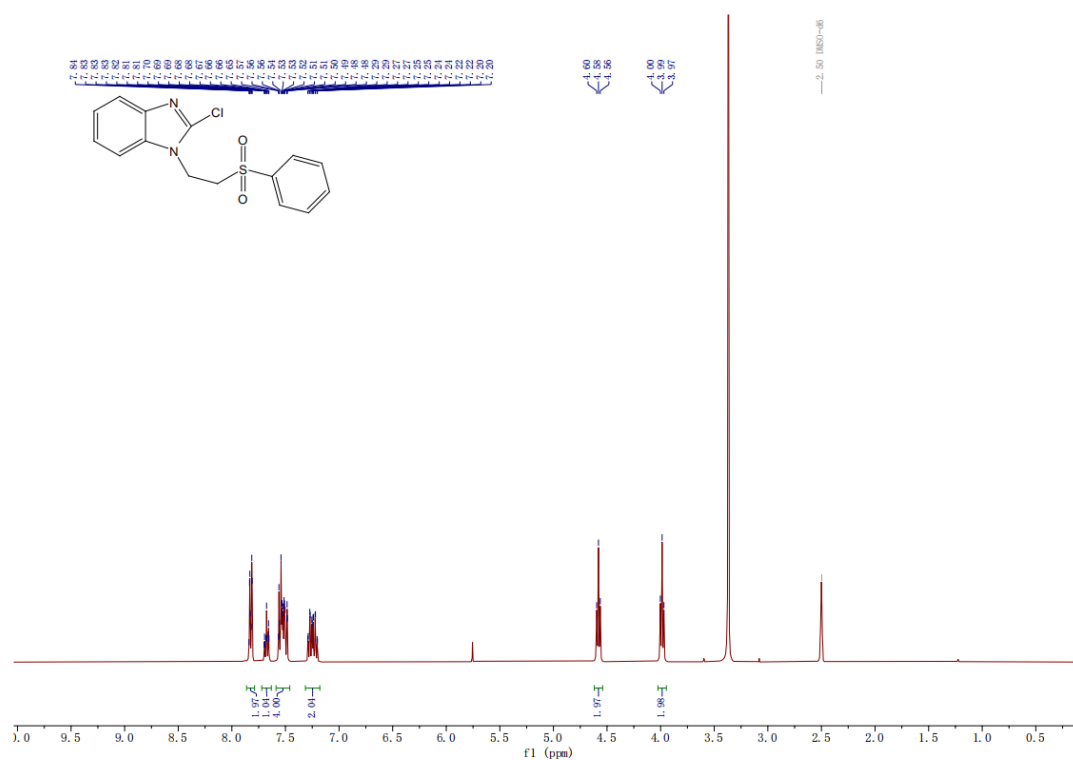


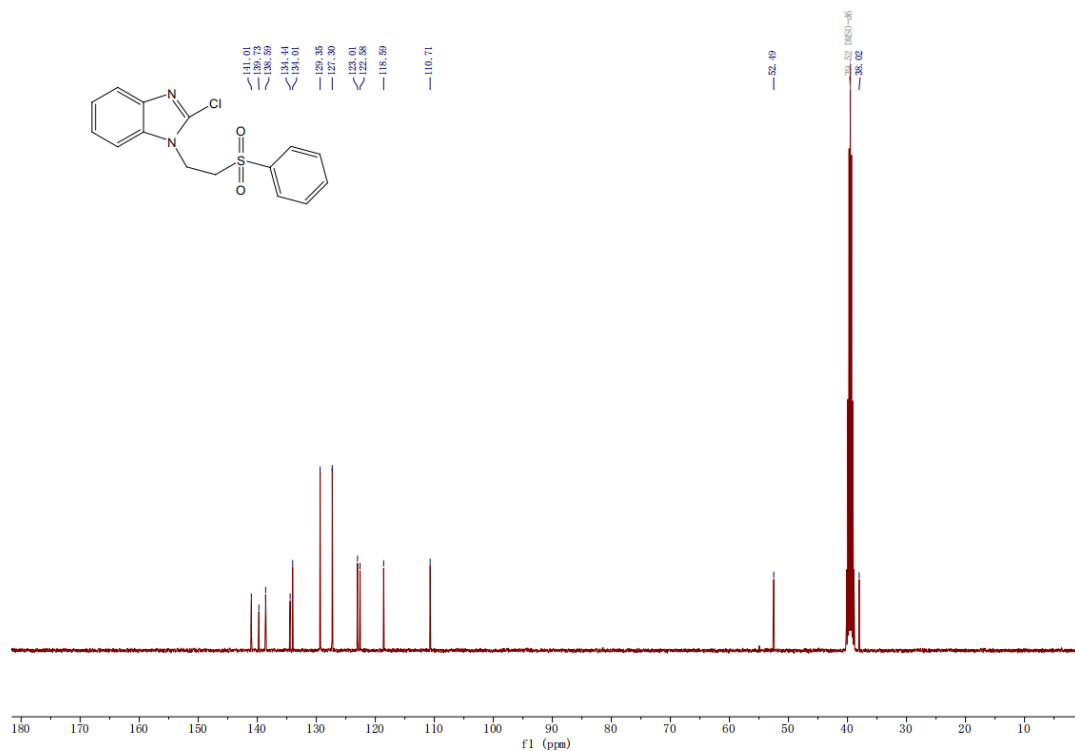
Spectrum from 05.wiff2 (sample 8) - L+S, +TOF MS (100 - 1000) from 0.217 to 0.235 min, s...[Spectrum from 05.wiff2 (sample 8) - L+S, +TOF MS (100 - 1000) from 0.443 to 0.966 min]



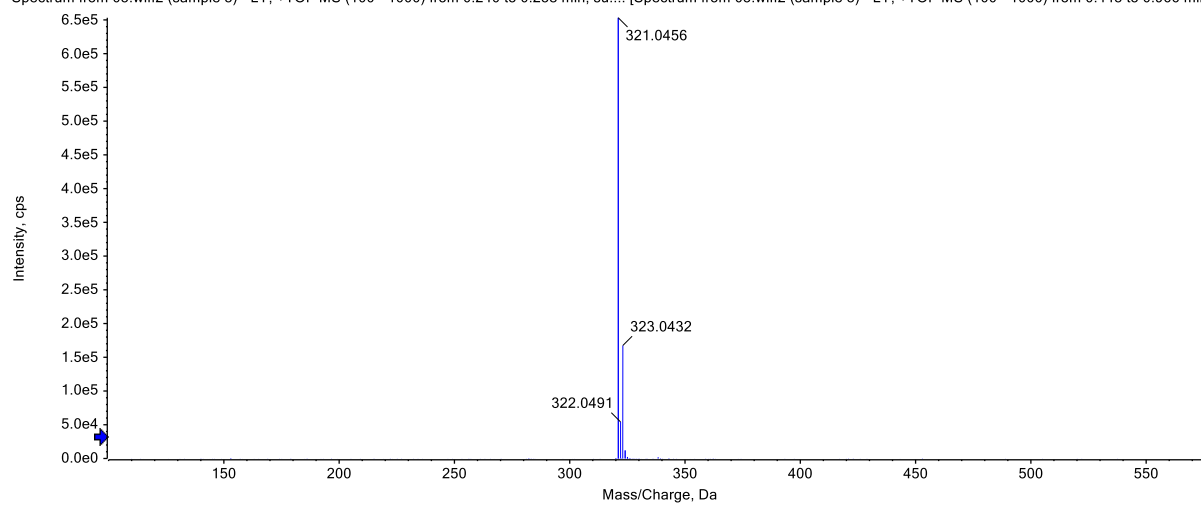
Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{11}H_{12}ClN_2O_2$	239.05818	-2.0	-0.47	7.0

3j

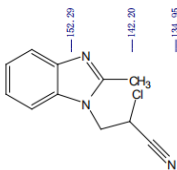
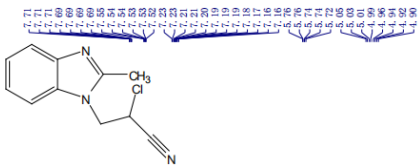




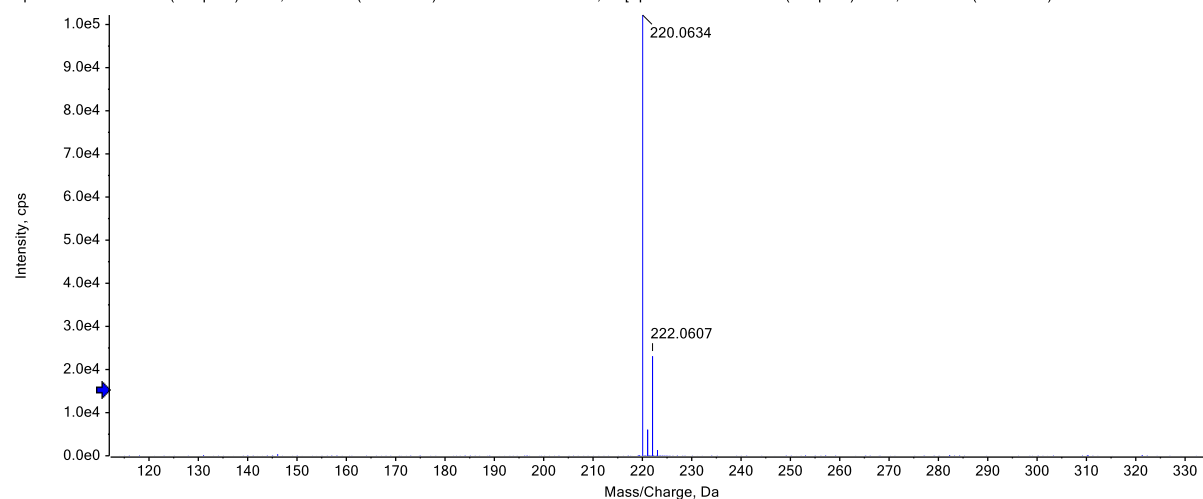
Spectrum from 05.wiff2 (sample 3) - LY, +TOF MS (100 - 1000) from 0.240 to 0.258 min, su...: [Spectrum from 05.wiff2 (sample 3) - LY, +TOF MS (100 - 1000) from 0.443 to 0.966 min]



Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{15}H_{14}ClN_2O_2S$	321.04590	-1.0	-0.32	10.0

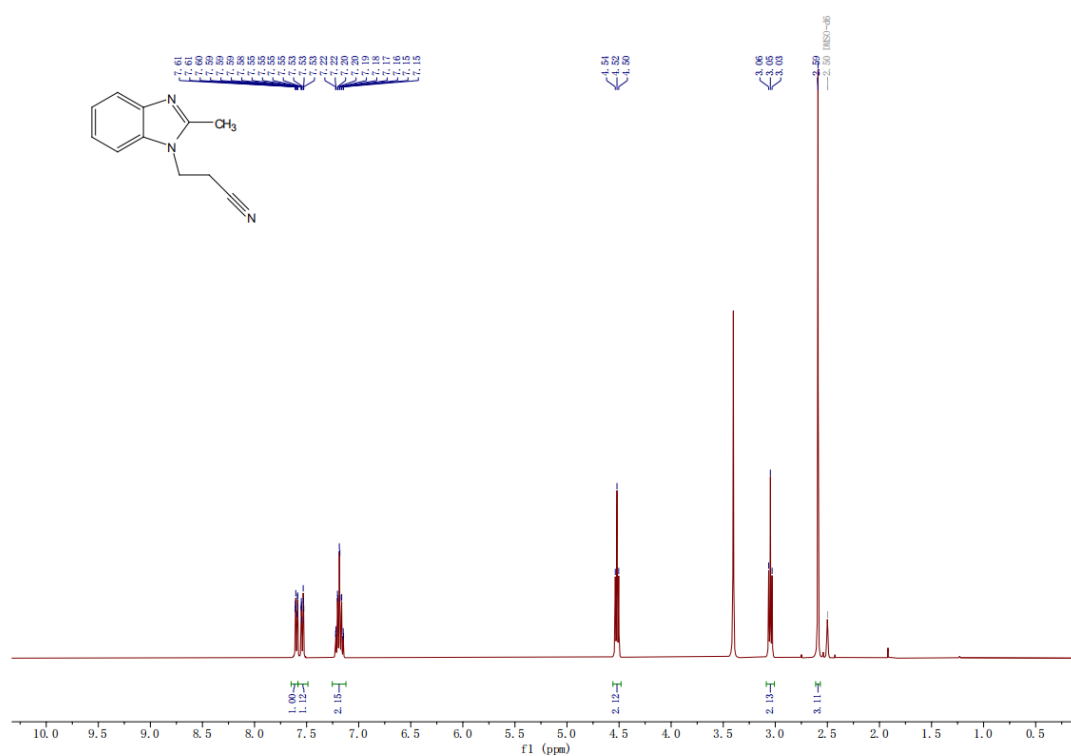


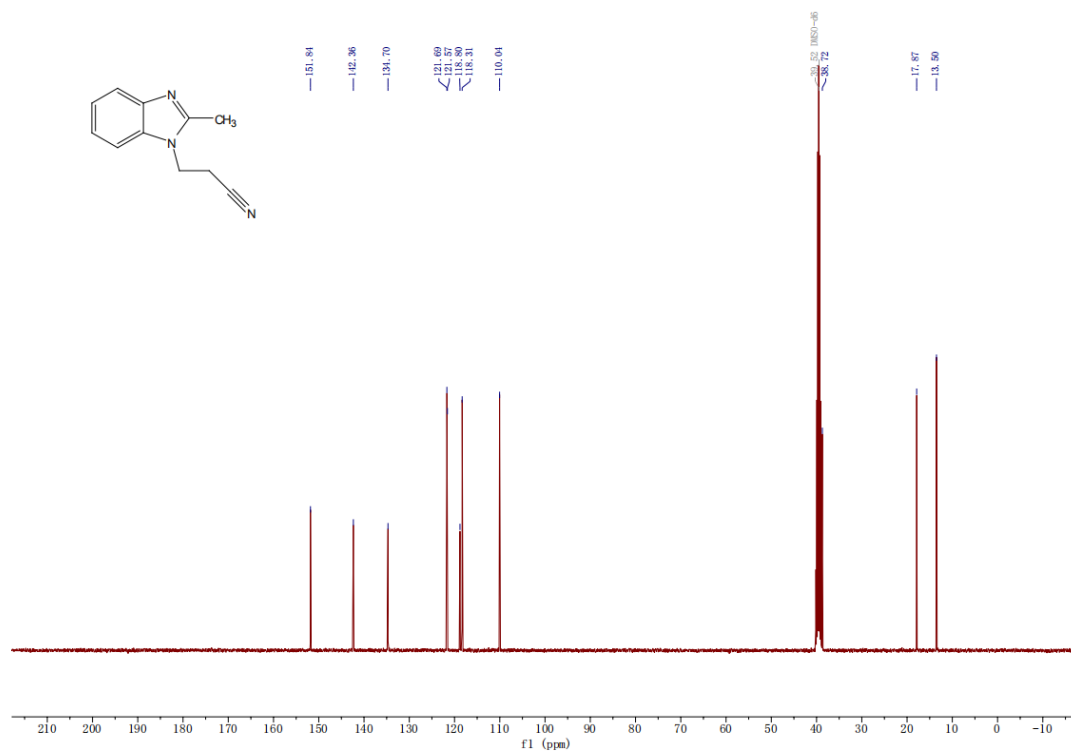
Spectrum from 05.wiff2 (sample 9) - C+L, +TOF MS (100 - 1000) from 0.217 to 0.235 min, s...[Spectrum from 05.wiff2 (sample 9) - C+L, +TOF MS (100 - 1000) from 0.443 to 0.966 min]



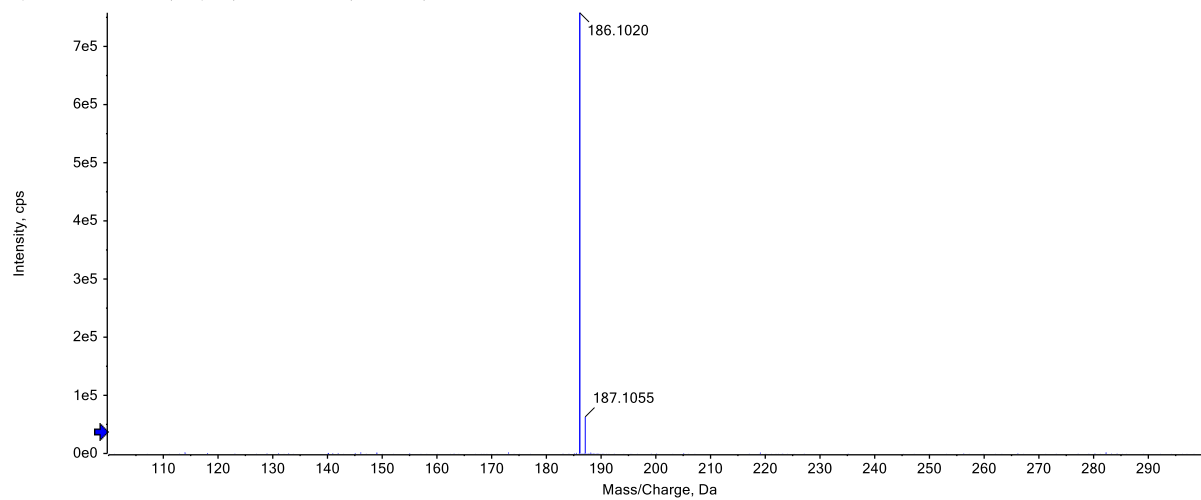
Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
$C_{11}H_{11}ClN_3$	220.06360	-1.1	-0.23	8.0

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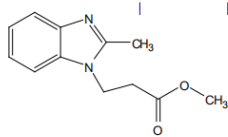
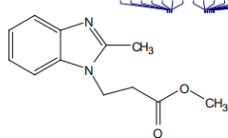


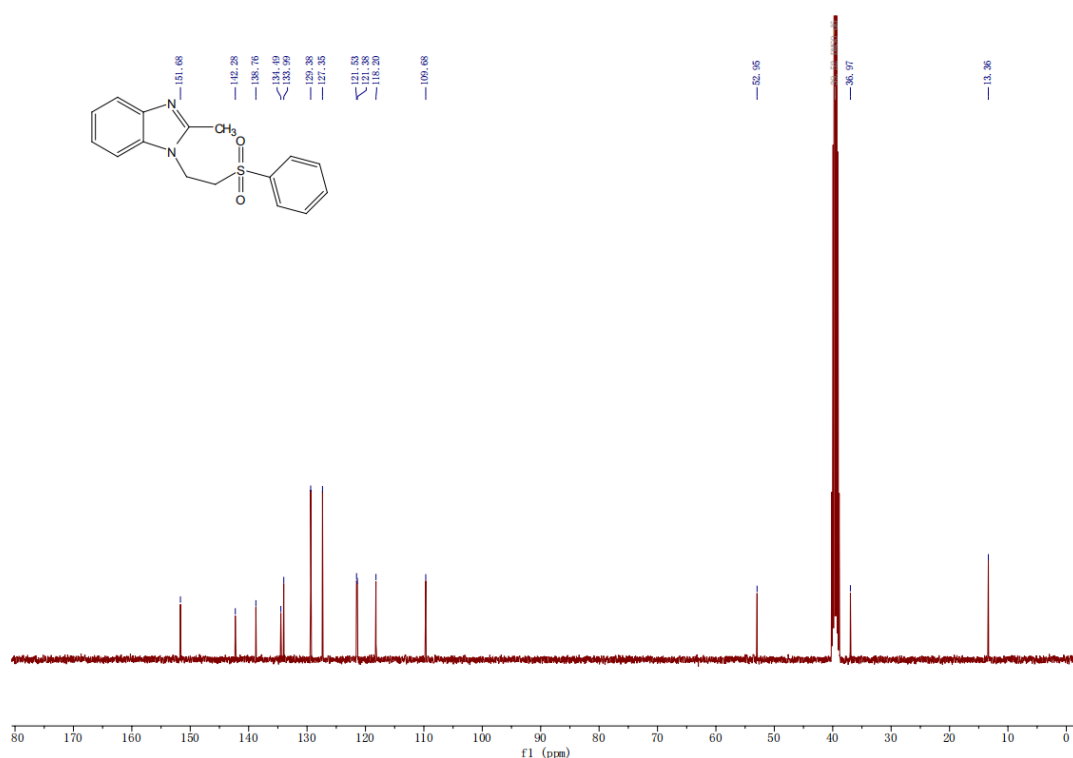
Spectrum from 05.wiff2 (sample 1) - CB, +TOF MS (100 - 1000) from 0.240 to 0.258 min



Ion Formula	Calculated m/z	Error (ppm)	Error (mmu)	RDB
C ₁₁ H ₁₂ N ₃	186.10257	-3.0	-0.56	8.0

3m





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