Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Benzimidazolium salts prevent and disrupt methicillin-resistant *Staphylococcus aureus* biofilms

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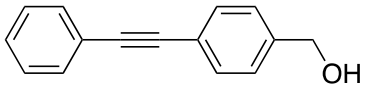
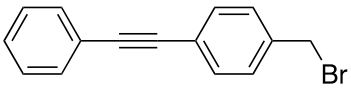
Contents

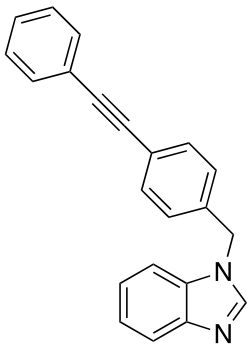
Synthesis.



(4-phenylethynyl)benzyl alcohol (2). To a carefully degassed solution of 4-iodobenzyl alcohol (4.0 g, 17.1 mmol), PPh_3 (90 mg, 0.343 mmol), and $PdCl_2(PPh_3)_2$ (120 mg, 0.171 mmol) in 50 mL of dry THF was added CuI (65 mg, 0.343 mmol) and Et_3N (19.1 mL, 137 mmol). The mixture was degassed for another 5 min before phenylacetylene (1.98 mL, 18 mmol) was added dropwise. The reaction was stirred overnight at 50 °C under nitrogen atmosphere. Then, 50 mL of water was added and the aqueous layer was extracted three times with EtOAc. The combined organic fractions were wash with $NH_4Cl_{(sat)}$ where a blue aqueous phase was observed, brine, and dried over Na_2SO_4. The solvent is removed under reduced pressure and the resulting brownish solid was triturated with hexane until no more apolar (solvent front) product was observed by TLC (40 % EtOAc/hexane). Filtration of the resulting solid afforded **(4-phenylethynyl)benzyl alcohol** (**2**) as a white solid in 90 % isolated yield (3.21 g, 15.4 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ7.54-7.50 (m, 4H), 7.37-7.24 (m, 5H), 4.66 (s, 2H), 2.00 (b, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ140.9, 131.7, 131.5, 128.3, 128.2, 126.7, 123.1, 122.3, 89.3, 89.1, 64.8. HRMS (ESI) calcd for $C_{15}H_{13}O^+[M+H]^+$: 209.0961, found 209.0969.



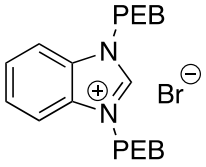
(4-phenylethynyl)benzyl bromide (3). (4-Phenylethynyl) benzyl alcohol (**2**) (1.0 g, 4.8 mmol) was dissolved in 20 mL of dry CH_2Cl_2. The mixture was put at 0 °C and phosphorus tribromide (0.68 mL, 7.20 mmol) was added dropwise. Then the mixture was stirred 2 hours at 0 °C then water was added and the aqueous phase was extracted 3 times with CH_2Cl_2. Then, silica gel was added before the solvent was removed under reduced pressure. The product was purified by filtration on silica gel (Hexane 100 %) to afford **(4-phenylethynyl)benzyl bromide** (**3**) as a white solid in 100 % isolated yield (1.3 g, 4.8 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ7.54-7.48 (m, 4H), 7.37-7.24 (m, 5H), 4.48 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ137.6, 131.9, 131.5, 129.0, 128.3, 128.2, 123.3, 122.9, 90.2, 88.8, 32.9. HRMS (ESI) calcd for $C_{15}H_{12}Br^+[M+H]^+$: 271.0117, found 271.0109.



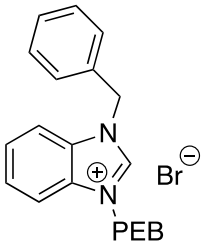
(4-phenylethynyl)benzyl benzimidazole (5). To a solution of benzimidazole (869 mg, 7.36 mmol) in THF (30.0 mL) at 0 °C was added sodium hydride (336 mg, 8.4 mmol) portion wise. After stirring for 15 minutes, (**4-phenylethynyl)benzyl bromide** (**3**) (1.9 g, 7.00 mmol) was slowly added. The reaction is allowed to warm up to R.T. and stirred until no more starting benzimidazole was observed by TLC (100% EtOAc) (2-3 hours). The reaction was then quenched with water (200 mL) and the resulting precipitate was filtered and washed with water, EtOAc, and Hexane to afford **(4-phenylethynyl)benzyl benzimidazole** (**5**) as a white solid in 93 % yield (2.0 g, 6.5 mmol). ^{1}H NMR (500 MHz, DMSO-d^6) δ 7.66 –

7.51 (m, 7H), 7.44 – 7.38 (m, 3H), 7.33 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 6.2 Hz, 2H), 5.56 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d^6) δ 137.6, 131.7, 131.5, 131.4, 131.3, 128.8, 128.8, 128.8, 127.7, 122.5, 122.1, 121.6, 89.5, 88.9, 47.5. HRMS (ESI) calcd for $C_{22}H_{16}N_2$ $[M+H]^+$: 309.1386, found 309.1380.

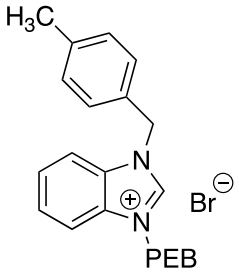
General procedure for 1st and 2nd generation benzimidazolium salt formation (6-21). To a solution of **(4-phenylethynyl)benzyl benzimidazole** (**5**) (135 mg, 0.438 mmol) in MeCN (2 mL) was added the corresponding alkyl or benzyl bromide (1.31 mmol, 3 eq.). The reaction is stirred until LCMS analysis show completion of the reaction (24-48h). MeCN was evaporated and the resulting solid is triturated with EtOAc to remove the excess alkyl or benzyl bromide. The resulting white solid was filtered, washed with hexane, and dried to afford the corresponding benzimidazolium salt (**6-21**) as a white powder.



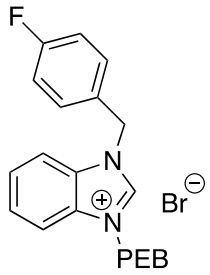
1,3-bis(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (6). Yield 95 %. Purity 98.99 %. ^{1}H NMR (300 MHz, $CDCl_3$): δppm = 9.97 (s, 1 H), 7.90 -7.96 (m, 1 H), 7.52 (m, 14 H), 7.38 -7.45 (m, 7 H), 5.81 (s, 4 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.5, 137.3, 131.7, 131.5, 131.4, 131.3, 128.8, 127.6, 122.5, 122.1, 121.1, 89.5, 88.9, 47.5. HRMS (ESI): m/z Calcd for $C_{37}H_{27}N_2$[M]+: 499.2169, found 499.2175.



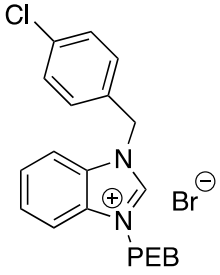
3-benzyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (7). Yield 65 %. Purity 100 %. ^{1}H NMR (400 MHz, DMSO-d^6) δ 10.11 (s, 1H), 7.98 (m, J = 8.8, 6.4, 3.0 Hz, 3H), 7.75 – 7.51 (m, 12H), 7.50 – 7.34 (m, 8H), 5.84 (d, J = 15.6 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d^6) δ 143.3, 134.9, 134.4, 132.4, 131.9, 131.6, 129.5, 129.3, 129.2, 129.2, 128.8, 127.3, 123.1, 122.4, 114.5, 114.5, 90.6, 89.2, 50.5, 50.2. HRMS (ESI): m/z Calcd for $C_{29}H_{23}N_2$[M]$^+$: 399.1856, found 399.1856.



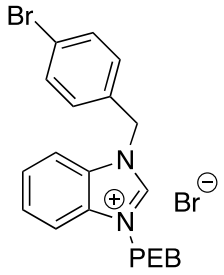
3-(4-methylbenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (8). Yield 65 %. Purity 99.48 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.03 (s, 1H), 7.96 (m, J = 12.2, 6.8, 3.1 Hz, 2H), 7.69 – 7.60 (m, 4H), 7.60 – 7.52 (m, 4H), 7.48 – 7.40 (m, 5H), 7.24 (d, J = 7.9 Hz, 2H), 5.83 (s, 2H), 5.74 (s, 2H), 2.30 (s, 3H).^{13}C NMR (126 MHz, DMSO-d^{6}) δ 142.7, 138.3, 134.5, 131.9, 131.4, 131.1, 130.8, 129.6, 129.0, 128.8, 128.7, 128.4, 126.9, 122.7, 122.0, 114.1, 114.0, 90.1, 88.7, 49.9, 49.7, 20.7. HRMS (ESI): m/z Calcd for $C_{30}H_{25}N_2$[M]+: 413.2001, found 413.2012.



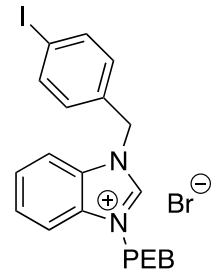
3-(4-fluorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (9). Yield 79%. Purity 98.65 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.07 (s, 1H), 8.08 – 7.90 (m, 2H), 7.68 – 7.49 (m, 10H), 7.46 – 7.39 (m, 3H), 7.29 (t, J = 8.9 Hz, 2H), 5.82 (d, J = 17.6 Hz, 4H).^{13}C NMR (126 MHz, DMSO-d^{6}) δ 163.7, 161.7, 143.3, 134.9, 132.4, 131.9, 131.6, 131.5, 131.4, 131.3, 130.6, 129.5, 129.3, 129.2, 127.3, 123.1, 122.4, 116.5, 116.3, 114.5, 114.5, 90.6, 89.2, 65.4, 62.5, 50.2, 49.8. ^{19}F NMR (471 MHz, DMSO-d^{6}) δ -114.73. HRMS (ESI): m/z Calcd for $C_{29}H_{22}FN_2$[M]$^{+}$: 417.1762, found 417.1760.



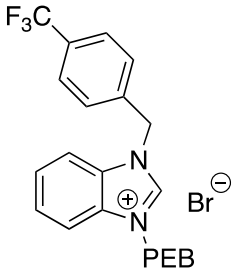
3-(4-chlorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (10). Yield 75%. Purity 96.89 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.07 (s, 1H), 8.04 – 7.90 (m, 2H), 7.76 – 7.49 (m, 12H), 7.47 – 7.35 (m, 3H), 5.83 (d, J = 12.9 Hz, 4H).^{13}C NMR (126 MHz, DMSO) δ 143.0, 134.4, 133.5, 132.8, 131.9, 131.4, 131.1, 131.0, 130.4, 129.0, 128.8, 128.7, 126.9, 122.6, 122.0, 114.0, 90.1, 88.7, 49.7, 49.3. HRMS (ESI): m/z Calcd for $C_{29}H_{22}ClN_2$[M]$^{+}$: 433.1466, found 433.1456.



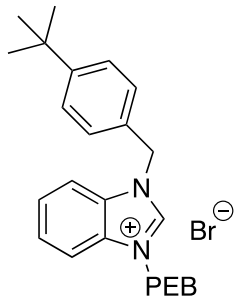
3-(4-bromobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (11). Yield 80%. Purity 99.53 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.09 (s, 1H), 7.97 (s, 2H), 7.72 – 7.48 (m, 12H), 7.43 (s, 3H), 5.83 (d, J = 19.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 143.0, 134.4, 133.2, 131.9, 131.4, 131.1, 131.0, 130.7, 129.0, 128.8, 128.7, 126.9, 122.6, 122.1, 122.0, 114.0, 90.1, 88.7, 49.7, 49.4. HRMS (ESI): m/z Calcd for $C_{29}H_{22}CBrN_2$[M]+: 477.0961, found 477.0959.



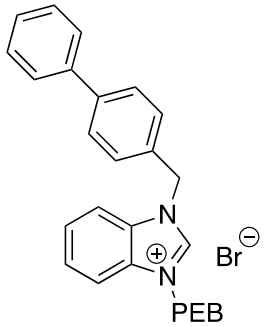
3-(4-iodobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (12). Yield 99%. Purity 100 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.04 (s, 1H), 7.95 (s, 2H), 7.81 (m, J = 6.9 Hz, 2H), 7.70 – 7.51 (m, 8H), 7.49 – 7.24 (m, 5H), 5.80 (d, J = 28.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 142.9, 137.8, 134.4, 133.5, 131.9, 131.4, 131.1, 131.1, 130.7, 129.0, 128.8, 128.7, 126.9, 122.6, 122.0, 114.0, 95.5, 90.1, 88.7, 49.7, 49.5. HRMS (ESI): m/z Calcd for $C_{29}H_{22}IN_2$[M]+: 525.0822, found 525.0804.



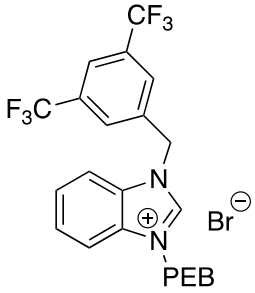
1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (13). Yield 65%. Purity 99.57 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 10.11 (s, 1H), 8.09 – 7.90 (m, 2H), 7.83 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.71 – 7.55 (m, 6H), 7.55 (m, 2H), 7.48 – 7.37 (m, 3H), 5.91 (d, J = 39.2 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 143.4, 138.7, 134.5, 132.1, 131.6, 131.3, 129.3, 129.2, 129.0, 128.9, 127.1, 127.1, 126.1, 126.0, 122.8, 122.1, 114.2, 114.1, 90.2, 88.8, 49.9, 49.6. ^{19}F NMR (471 MHz, DMSO) δ -63.71. HRMS (ESI): m/z Calcd for $C_{30}H_{22}F_3N_2$[M]$^{+}$: 467.1729, found 467.1727.



3-(4-(*tert*-butyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (14). Yield 62%. Purity 99.52 %. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.09 (s, 1H), 8.06 – 8.02 (m, 1H), 7.98 – 7.93 (m, 1H), 7.68 – 7.52 (m, 8H), 7.50 – 7.41 (m, 7H), 5.80 (d, J = 40.9 Hz, 4H), 2.08 (s, 1H), 1.26 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 151.3, 142.7, 134.5, 131.9, 131.4, 131.1, 131.1, 131.0, 129.0, 128.8, 128.2, 126.8, 125.8, 122.6, 122.0, 114.1, 114.0, 90.1, 88.7, 49.7, 34.4, 31.0. HRMS (ESI): m/z Calcd for $C_{33}H_{31}N_2[M]^+$: 455.2482, found 455.2483.

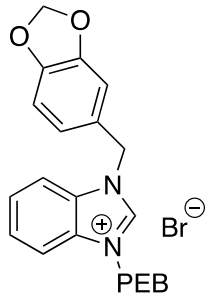


3-([1,1'-biphenyl]-4-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (15). Yield 69%. Purity 100 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 10.09 (s, 1H), 8.09 – 8.03 (m, 1H), 8.00 – 7.95 (m, 1H), 7.79 – 7.71 (m, 2H), 7.71 – 7.52 (m, 12H), 7.51 – 7.32 (m, 6H), 5.85 (s, 4H).^{13}C NMR (126 MHz, DMSO) δ 142.9, 140.6, 139.3, 134.4, 133.0, 131.9, 131.4, 131.1, 129.0, 129.0, 128.8, 128.7, 127.8, 127.2, 126.9, 126.7, 122.7, 122.0, 114.1, 114.0, 90.1, 88.7, 49.7. HRMS (ESI): m/z Calcd for $C_{35}H_{27}N_2[M]$+: 475.2169, found 455.2159.

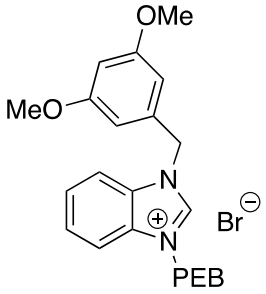


3-(3,5-bis(trifluoromethyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (16). Yield 68%. Purity 97.62 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 10.03 (s, 1H), 8.35 (s, 2H), 8.19 (s, 1H), 8.07 – 8.01 (m, 1H), 7.99 – 7.91 (m, 1H), 7.74 – 7.51 (m, 8H), 7.48 – 7.39 (m, 3H),

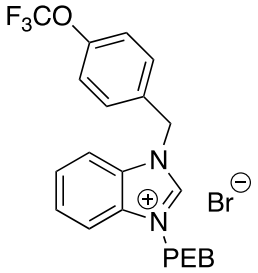
5.97 (s, 2H), 5.84 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 143.5, 137.0, 134.4, 131.8, 131.4, 131.1, 131.1, 130.8, 130.6, 129.8, 129.0, 128.8, 128.7, 127.0, 126.9, 122.6, 122.0, 114.1, 113.8, 90.1, 88.7, 49.8, 48.9. ^{19}F NMR (471 MHz, DMSO) δ -62.79. HRMS (ESI): m/z Calcd for $C_{31}H_{21}F_6N_2$[M]+: 535.1603, found 535.1609.



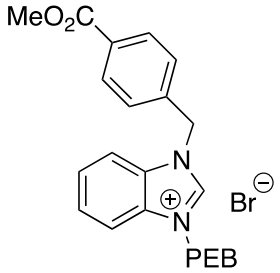
3-(benzo[*d*][1,3]dioxol-5-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (17). Yield 87%. Purity 98.85 %. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.07 (s, 1H), 8.05 – 7.99 (m, 1H), 7.99 – 7.88 (m, 1H), 7.70 – 7.49 (m, 8H), 7.47 – 7.36 (m, 3H), 7.20 (d, J = 1.7 Hz, 1H), 7.13 (dd, J = 8.0, 1.8 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.04 (s, 2H), 5.84 (s, 2H), 5.69 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 147.7, 147.7, 142.7, 134.5, 131.9, 131.4, 131.1, 131.0, 129.0, 128.8, 128.7, 127.2, 126.8, 126.8, 122.7, 122.6, 122.0, 114.1, 113.9, 109.0, 108.6, 101.4, 90.1, 88.7, 50.0, 49.7. HRMS (ESI): m/z Calcd for $C_{30}H_{23}N_2O_2$[M]+: 443.1754, found 443.1756.



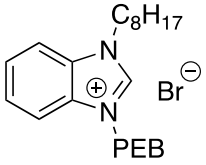
3-(3,5-dimethoxybenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (18). Yield 87%. Purity 99.09 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.15 (s, 1H), 8.15 – 7.86 (m, 2H), 7.76 – 7.51 (m, 8H), 7.49 – 7.41 (m, 3H), 6.75 (s, 2H), 6.51 (s, 1H), 5.87 (s, 2H), 5.72 (s, 2H), 3.74 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 160.9, 142.9, 135.9, 134.5, 131.9, 131.4, 131.1, 131.0, 129.0, 128.8, 128.7, 126.8, 122.6, 122.0, 114.1, 114.0, 106.7, 100.0, 90.1, 88.7, 55.4, 50.1, 49.7. HRMS (ESI): m/z Calcd for $C_{31}H_{27}N_2O_2$[M]+: 459.2067, found 459.2078.



1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethoxy)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (19). Yield 75%. Purity 99.63 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.09 (s, 1H), 8.06 – 7.89 (m, 2H), 7.72 – 7.51 (m, 10H), 7.48 – 7.38 (m, 5H), 5.85 (d, J = 2.7 Hz, 4H). ^{13}C NMR (126 MHz, CDCl3) δ 148.3, 142.9, 134.2, 133.2, 131.7, 131.2, 130.9, 130.9, 130.4, 128.8, 128.6, 128.6, 126.7, 122.5, 121.8, 121.4, 113.9, 113.8, 89.9, 88.5, 49.6, 49.0. ^{19}F NMR (471 MHz, CDCl3) δ -52.03. HRMS (ESI): m/z Calcd for $C_{30}H_{22}F_3N_2O$[M]+: 483.1679, found 483.1688.

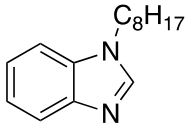


3-(4-(methoxycarbonyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (20). Yield 84%. Purity 100 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.19 (s, 1H), 8.14 – 7.85 (m, 4H), 7.78 – 7.49 (m, 10H), 7.49 – 7.25 (m, 3H), 5.92 (d, J = 23.2 Hz, 4H), 3.85 (s, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 165.6, 143.0, 139.0, 134.2, 131.7, 131.2, 130.9, 129.6, 129.5, 128.8, 128.6, 128.4, 126.7, 122.5, 121.8, 113.9, 113.8, 89.9, 88.5, 52.1, 49.6, 49.5. HRMS (ESI): m/z Calcd for $C_{31}H_{25}N_2O_2$[M]+: 457.1911, found 457.1912.

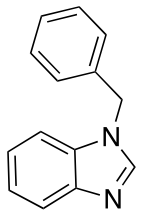


3-octyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (21). Yield 66%. Purity 99.06 %. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.97 (s, 1H), 8.27 – 8.07 (m, 1H), 7.94 (dd, J = 7.3, 1.2 Hz, 1H), 7.76 – 7.50 (m, 8H), 7.43 (d, J = 2.2 Hz, 3H), 5.82 (s, 2H), 4.51 (t, J = 7.3 Hz, 2H), 2.10 – 1.73 (m, 2H), 1.41 – 1.09 (m, 10H), 0.84 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 142.4, 134.5, 131.8, 131.3, 131.2, 130.7, 128.9, 128.7, 128.5, 126.7, 126.6, 122.5, 121.8, 113.8, 113.7, 90.0, 88.5, 49.5, 46.8, 31.0, 28.4, 28.3, 25.7, 22.0, 13.8. HRMS (ESI): m/z Calcd for $C_{30}H_{33}N_2$[M]+: 421.2638, found 421.2645.

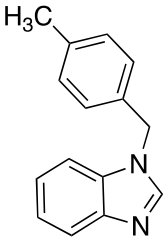
General procedure for benzimidazole alkylation (23-32). To a solution of benzimidazole (**4**) (1.00 g, 8.46 mmol) in THF (25.0 mL) at 0 °C was added sodium hydride (406 mg, 10.2 mmol) portion wise. After stirring for 15 minutes, a solution of the corresponding benzyl or alkyl bromide (8.89 mmol, 1.05 eq.) in THF (5 mL) was slowly added. The reaction is allowed to warm up to R.T. and stirred until no more starting benzimidazole is observed by TLC (100% EtOAc) (2-3 hours). The reaction is then quenched with water and the aqueous layer is extracted three times with EtOAc, dried over Na_2SO_4 and purified using silica gel chromatography (100% EtOAc) to afford the corresponding alkylated benzimidazole (**23-32**).



1-octyl-1*H*-benzo[*d*]imidazole (23). Yield 96%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.76-7.71 (m, 1H), 7.35-7.31 (m, 1H), 7.25-7.20 (m, 2H), 4.17 (d, 6.3 Hz, 2H), 1.86 (m, 2H), 1.19-1.13 (m, 10H), 0.80 (t, 1.8 Hz, 3H) ^{13}C NMR (75 MHz,$CDCl_3$) δ 143.1, 142.6, 133.8, 122.7, 121.2, 120.2, 109.8, 45.3, 31.6, 29.2, 29.3, 26.7, 22.5, 14.0. HRMS (ESI): m/z Calcd for $C_{15}H_{22}N_2[M+H]^+$: 231.1863, found 231.1867.

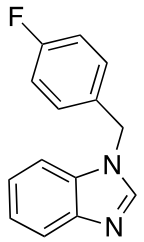


1-benzyl-1*H*-benzo[*d*]imidazole (24). Yield 91%, ^{1}H NMR (500 MHz, DMSO-d^6) δ 8.41 (s, 1H), 7.68 – 7.64 (m, 1H), 7.53 – 7.49 (m, 1H), 7.35 – 7.25 (m, 5H), 7.23 – 7.16 (m, 2H), 5.50 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 144.25, 143.58, 136.98, 133.67, 128.71, 127.75, 127.40, 122.41, 121.60, 119.51, 110.73, 47.64. HRMS (ESI): m/z Calcd for $C_{14}H_{13}N_2[M+H]^+$: 209.1073, found 209.1079.

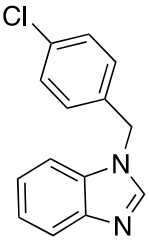


1-(4-methylbenzyl)-1*H*-benzo[*d*]imidazole (25). Yield 40%, ^{1}H NMR (500 MHz, DMSO-d^6) δ 8.39 (s, 1H), 7.70 – 7.60 (m, 1H), 7.55 – 7.40 (m, 1H), 7.27 – 7.04 (m, 6H), 5.44 (s, 2H), 2.24 (s, 3H).^{13}C

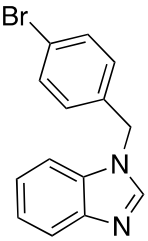
NMR (126 MHz, DMSO) δ 144.2, 143.6, 137.0, 133.9, 133.6, 129.2, 127.4, 122.3, 121.5, 119.5, 110.7, 47.4, 20.6. HRMS (ESI): m/z Calcd for $C_{15}H_{15}N_2[M+H]^+$: 223.1230, found 223.1232.



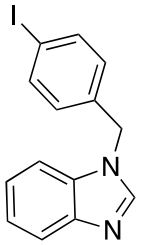
1-(4-fluorobenzyl)-1*H*-benzo[*d*]imidazole (26). Yield 97%, ^{1}H NMR (500 MHz, DMSO-d^6) δ 8.44 (s, 1H), 7.71 – 7.66 (m, 1H), 7.57 – 7.51 (m, 1H), 7.43 – 7.36 (m, 2H), 7.27 – 7.13 (m, 4H), 5.50 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 162.59, 160.65, 144.15, 143.62, 133.55, 133.20, 133.17, 129.62, 129.56, 122.42, 121.61, 119.53, 115.58, 115.40, 110.66, 46.87. ^{19}F NMR (471 MHz, DMSO) δ -114.63. HRMS (ESI): m/z Calcd for $C_{14}H_{12}FN_2[M+H]+$: 227.0979, found 227.0973.



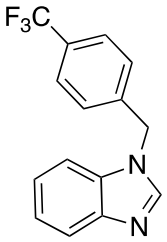
1-(4-chlorobenzyl)-1*H*-benzo[*d*]imidazole (27). Yield 92%, ^{1}H NMR (500 MHz, DMSO-d6) δ 8.42 (s, 1H), 7.73 – 7.62 (m, 1H), 7.55 – 7.47 (m, 1H), 7.43 – 7.36 (m, 2H), 7.36 – 7.26 (m, 2H), 7.25 – 7.15 (m, 2H), 5.50 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 144.23, 143.59, 135.98, 133.55, 132.41, 129.30, 128.70, 122.50, 121.69, 119.56, 110.66, 46.90. HRMS (ESI): m/z Calcd for $C_{14}H_{12}ClN_2[M+H]+$: 243.0684, found 243.0680.



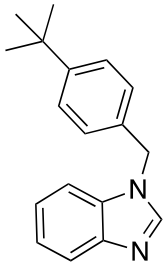
1-(4-bromobenzyl)-1*H*-benzo[*d*]imidazole (28). Yield 62%, ^{1}H NMR (500 MHz, DMSO-d^6) δ 8.42 (s, 1H), 7.71 – 7.63 (m, 1H), 7.57 – 7.46 (m, 3H), 7.29 – 7.24 (m, 2H), 7.24 – 7.15 (m, 2H), 5.49 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 144.2, 143.6, 136.4, 133.6, 131.6, 129.6, 122.5, 121.7, 120.9, 119.6, 110.7, 46.9. HRMS (ESI): m/z Calcd for $C_{14}H_{12}BrN_2[M+H]+$: 287.0178, found 287.0173.



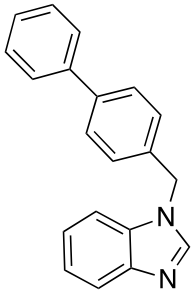
1-(4-iodobenzyl)-1*H*-benzo[*d*]imidazole (29). Yield 71%, ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 8.40 (s, 1H), 7.77 – 7.56 (m, 3H), 7.48 (d, J = 5.7 Hz, 1H), 7.29 – 6.92 (m, 4H), 5.47 (s, 2H). ^{1}3C NMR (126 MHz, DMSO) δ 143.7, 143.1, 135.9, 133.0, 131.1, 129.1, 122.0, 121.2, 120.4, 119.1, 110.2, 46.5. HRMS (ESI): m/z Calcd for $C_{14}H_{12}IN_2$[M+H]+: 335.0040, found 335.0041.



1-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazole (30). Yield 96%, ^{1}H NMR (500 MHz, DMSO-d6) δ 8.45 (s, 1H), 7.79 – 7.60 (m, 3H), 7.56 – 7.43 (m, 3H), 7.28 – 7.09 (m, 2H), 5.63 (s, 2H).^{13}C NMR (126 MHz, DMSO) δ 144.3, 143.6, 141.8, 133.6, 128.0, 125.6, 125.6, 125.6, 122.6, 121.7, 119.6, 110.6, 47.0. ^{19}F NMR (471 MHz, DMSO) δ -64.06. HRMS (ESI): m/z Calcd for $C_{15}H_{12}F_3N_2$[M+H]+: 277.0947, found 277.0942.

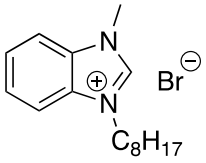


1-(4-(*tert*-butyl)benzyl)-1*H*-benzo[*d*]imidazole (31). Yield 84%, ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 8.41 (s, 1H), 7.66 (d, J = 7.3 Hz, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.26 – 7.16 (m, 4H), 5.45 (s, 2H), 1.22 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 150.1, 134.0, 127.1, 125.4, 122.3, 121.5, 119.5, 110.7, 47.2, 34.2, 31.0. HRMS (ESI): m/z Calcd for $C_{18}H_{21}N_2$[M+H]+: 265.1699, found 265.1697.



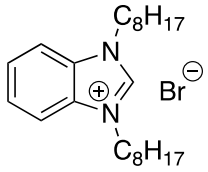
1-([1,1'-biphenyl]-4-ylmethyl)-1*H*-benzo[*d*]imidazole (32). Yield 44%, ^{1}H NMR (500 MHz, DMSO-d6) δ 8.45 (s, 1H), 7.70 – 7.54 (m, 6H), 7.47 – 7.32 (m, 5H), 7.26 – 7.11 (m, 2H), 5.55 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 144.4, 143.8, 139.8, 136.3, 133.8, 129.1, 128.1, 127.7, 127.2, 126.8, 122.6, 121.8, 119.7, 110.9, 47.4. HRMS (ESI): m/z Calcd for $C_{20}H_{17}N_2[M+H]^+$: 285.1386, found 285.1386.

General procedure for 3rd generation benzimidazolium salt formation (33-43). To a solution of corresponding alkylated benzimidazole (**22-32**) (0.438 mmol) in MeCN (2 mL) is added octyl bromide (1.31 mmol, 3 eq.). The reaction is stirred until LCMS analysis show completion of the reaction (24-48h). Another 3 equivalent of octyl bromide is added if the reaction is not completed after 48h and stirred for an additional 24h. MeCN is then evaporated and the resulting solid is triturated with EtOAc to remove the excess reagents. The resulting white solid is filtered, washed with hexane, and dried to afford the corresponding benzimidazolium salt (**33-43**) as a white powder.

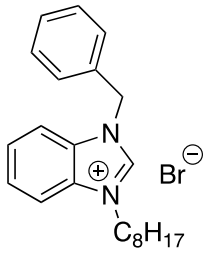


1-methyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (33). Yield 94%. Purity 99.36 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.20 (s, 1H), 7.77 – 7.73 (m, 1H), 7.71 – 7.66 (m, 1H), 7.64 – 7.59 (m, 2H),

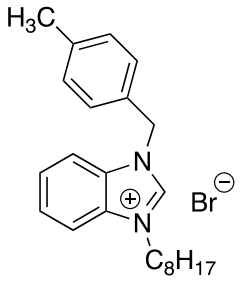
4.55 – 4.48 (m, 2H), 4.26 (s, 3H), 1.98 (q, J = 7.6 Hz, 2H), 1.38 – 1.31 (m, 2H), 1.31 – 1.22 (m, 2H), 1.22 – 1.11 (m, 6H), 0.76 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 142.72, 131.97, 131.02, 127.28, 127.25, 113.10, 112.90, 47.68, 33.86, 31.58, 29.46, 28.92, 28.91, 26.49, 22.47, 13.97. HRMS (ESI): m/z Calcd for $C_{16}H_{25}N_2$[M]+: 245.2012, found 245.2012.



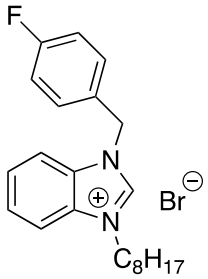
1,3-dioctyl-1*H*-benzo[*d*]imidazol-3-ium bromide (34). Yield 81%. Purity 95.46 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 9.88 (s, 1H), 8.11 (dd, J = 6.2, 3.1 Hz, 2H), 7.69 (dd, J = 6.3, 3.1 Hz, 2H), 4.49 (t, J = 7.1 Hz, 4H), 1.98 – 1.78 (m, 4H), 1.47 – 1.07 (m, 20H), 0.83 (t, J = 6.7 Hz, 6H).^{13}C NMR (126 MHz, DMSO) δ 142.07, 131.10, 126.55, 113.74, 46.67, 39.52, 31.14, 28.50, 28.45, 28.39, 25.73, 22.04, 13.93. HRMS (ESI): m/z Calcd for $C_{23}H_{39}N_2$[M]+: 343.3113, found 343.3115.



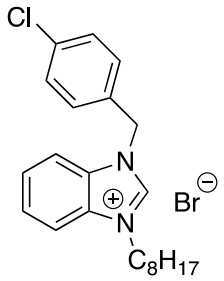
1-benzyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (35). Yield 67%. Purity 95.83 %. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.03 (s, 1H), 8.13 (dd, J = 7.2, 1.3 Hz, 1H), 7.98 (dd, J = 7.1, 1.3 Hz, 1H), 7.77 – 7.61 (m, 2H), 7.58 – 7.48 (m, 2H), 7.47 – 7.34 (m, 3H), 5.80 (s, 2H), 4.53 (t, J = 7.3 Hz, 2H), 1.98 – 1.90 (m, 2H), 1.37 – 1.17 (m, 10H), 0.91 – 0.82 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 142.4, 134.1, 131.3, 130.8, 129.0, 128.7, 128.2, 126.7, 126.6, 113.9, 113.9, 49.8, 46.8, 31.1, 28.5, 28.4, 28.4, 25.8, 22.0, 13.9. HRMS (ESI): m/z Calcd for $C_{22}H_{29}N_2$[M]+: 321.2325, found 321.2326.



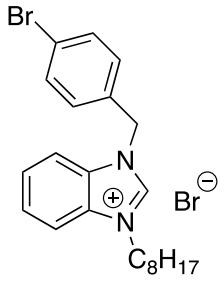
1-(4-methylbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (36). Yield 99%. Purity 99.69 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.13 (s, 1H), 8.12 (d, J = 6.9 Hz, 1H), 8.04 – 7.94 (m, 1H), 7.65 (m, 2H), 7.44 (d, J = 7.9 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H), 5.76 (s, 2H), 4.53 (t, J = 7.2 Hz, 2H), 2.27 (s, 3H), 1.92 (m, 2H), 1.38 – 1.13 (m, 10H), 0.83 (t, J = 6.7 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 142.2, 138.1, 131.3, 131.1, 130.7, 129.4, 128.3, 126.6, 126.6, 113.9, 113.9, 49.6, 46.8, 31.1, 28.5, 28.4, 28.4, 25.7, 22.0, 20.7, 13.9. HRMS (ESI): m/z Calcd for $C_{23}H_{31}N_2$[M]+: 335.2482, found 335.2473.



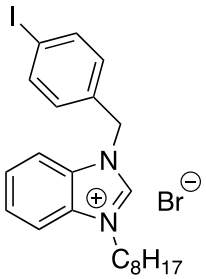
1-(4-fluorolbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (37). Yield 81%. Purity 100 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 10.00 (s, 1H), 8.12 (dd, J = 7.0, 1.7 Hz, 1H), 8.06 – 7.94 (m, 1H), 7.74 – 7.57 (m, 4H), 7.35 – 7.22 (m, 2H), 5.77 (s, 2H), 4.51 (t, J = 7.3 Hz, 2H), 1.93 (p, J = 7.6 Hz, 2H), 1.43 – 1.18 (m, 10H), 0.84 (t, J = 6.7 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 206.5, 163.1, 161.2, 142.4, 131.3, 130.8, 130.7, 130.3, 130.3, 126.7, 126.7, 115.9, 115.8, 113.9, 113.8, 49.1, 46.8, 31.1, 30.7, 28.5, 28.4, 25.8, 22.0, 13.9. ^{19}F NMR (471 MHz, DMSO) δ -114.68. HRMS (ESI): m/z Calcd for $C_{22}H_{28}FN_2$[M]+: 339.2231, found 339.2232



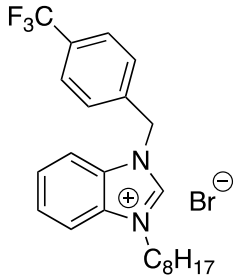
1-(4-chlorobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (38). Yield 88% Purity 100 %. ^{1}H NMR (500 MHz, DMSO-^{d6}) δ 9.99 (s, 1H), 8.21 – 8.05 (m, 1H), 8.05 – 7.93 (m, 1H), 7.75 – 7.64 (m, 2H), 7.57 (d, J = 8.5 Hz, 2H), 7.53 – 7.39 (m, 2H), 5.79 (s, 2H), 4.51 (t, J = 7.3 Hz, 2H), 1.96 – 1.89 (m, 2H), 1.37 – 1.20 (m, 10H), 0.84 (t, J = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 142.9, 133.9, 133.5, 131.8, 131.3, 130.8, 129.4, 127.2, 127.1, 114.4, 114.3, 49.6, 47.3, 31.6, 29.0, 28.9, 26.2, 22.5, 14.4. HRMS (ESI): m/z Calcd for $C_{22}H_{28}ClN_2$[M]+: 355.1936, found 355.1941.



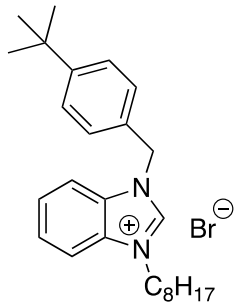
1-(4-bromobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (39). Yield 93%. Purity 100 %. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.88 (s, 1H), 8.12 (dd, J = 7.1, 1.3 Hz, 1H), 7.95 (dd, J = 7.0, 1.3 Hz, 1H), 7.75 – 7.54 (m, 4H), 7.48 (d, J = 8.5 Hz, 2H), 5.74 (s, 2H), 4.50 (t, J = 7.3 Hz, 2H), 1.92 (d, J = 7.1 Hz, 2H), 1.40 – 1.15 (m, 10H), 0.85 (t, J = 6.9 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 142.9, 133.9, 132.3, 131.8, 131.2, 131.1, 127.2, 127.1, 122.5, 114.4, 114.3, 49.6, 47.3, 31.6, 29.0, 28.9, 26.2, 22.5, 14.4. HRMS (ESI): m/z Calcd for $C_{22}H_{28}BrN_2$[M]+: 399.1430, found 399.1424.



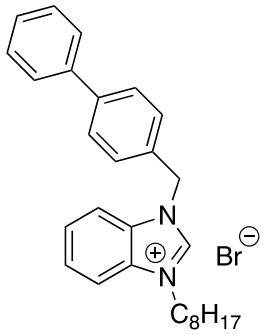
1-(4-iodobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (40). Yield 46%. Purity 97.73 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 9.89 (s, 1H), 8.11 (dd, J = 7.3, 1.3 Hz, 1H), 7.94 (dd, J = 7.2, 1.2 Hz, 1H), 7.86 – 7.73 (m, 2H), 7.73 – 7.55 (m, 2H), 7.32 (d, J = 8.4 Hz, 2H), 5.72 (s, 2H), 4.50 (t, J = 7.3 Hz, 2H), 1.98 – 1.84 (m, 2H), 1.40 – 1.14 (m, 10H), 0.84 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 142.5, 133.4, 131.8, 131.3, 130.8, 130.6, 126.7, 126.6, 122.0, 113.9, 113.8, 49.1, 46.8, 31.1, 28.5, 28.4, 25.8, 22.0, 13.9. HRMS (ESI): m/z Calcd for $C_{22}H_{28}IN_2$[M]+: 447.1292, found 447.1281.



3-octyl-1-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (41). Yield 71%. Purity 100 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.05 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 7.97 (d, J = 7.5 Hz, 1H), 7.83 – 7.58 (m, 6H), 5.92 (s, 2H), 4.52 (t, J = 7.2 Hz, 2H), 2.10 – 1.81 (m, 2H), 1.27 (d, J = 35.6 Hz, 10H), 0.84 (t, J = 6.6 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 143.1, 139.2, 131.8, 131.3, 129.5, 127.3, 127.2, 126.3, 126.3, 114.4, 114.2, 49.7, 47.4, 31.6, 29.0, 28.9, 26.2, 22.5, 14.4. ^{19}F NMR (471 MHz, DMSO) δ -63.87. HRMS (ESI): m/z Calcd for $C_{23}H_{28}F_3N_2$[M]+: 389.2199, found 389.2206.



1-(4-(*tert*-butyl)benzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (42). Yield 83%. Purity 98.83 %. ^{1}H NMR (400 MHz, DMSO-d^{6}) δ 10.00 (s, 1H), 8.18 – 8.08 (m, 1H), 8.08 – 7.96 (m, 1H), 7.74 – 7.62 (m, 2H), 7.54 – 7.37 (m, 4H), 5.73 (s, 2H), 4.51 (t, J = 7.2 Hz, 2H), 1.96 – 1.76 (m, 2H), 1.24 (s, 19H), 0.96 – 0.67 (m, 3H).^{13}C NMR (126 MHz, DMSO) δ 151.2, 142.3, 131.3, 131.2, 130.9, 128.0, 126.7, 126.6, 125.7, 113.9, 49.5, 46.8, 34.4, 31.1, 31.0, 28.5, 28.4, 25.8, 22.0, 13.9. HRMS (ESI): m/z Calcd for $C_{26}H_{37}N_2$[M]+: 377.2951, found 377.2954.



1-([1,1'-biphenyl]-4-ylmethyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (43). Yield 97%. Purity 98.66 %. ^{1}H NMR (500 MHz, DMSO-d^{6}) δ 10.02 (s, 1H), 8.12 (dd, J = 6.5, 2.4 Hz, 1H), 8.03 (dd, J = 6.5, 2.4 Hz, 1H), 7.74 – 7.60 (m, 8H), 7.46 (t, J = 7.6 Hz, 2H), 7.41 – 7.32 (m, 1H), 5.82 (s, 2H), 4.52 (t, J = 7.3 Hz, 2H), 2.02 – 1.83 (m, 2H), 1.47 – 1.18 (m, 10H), 0.91 – 0.66 (m, 3H).^{13}C NMR (126 MHz, DMSO) δ 142.9, 141.0, 139.8, 133.7, 131.8, 131.4, 129.5, 129.4, 128.2, 127.7, 127.2, 127.2, 114.4, 50.0, 47.3, 31.6, 29.0, 28.9, 26.3, 22.5, 14.4. HRMS (ESI): m/z Calcd for $C_{28}H_{33}N_2$[M]+: 397.2638, found 397.2638.

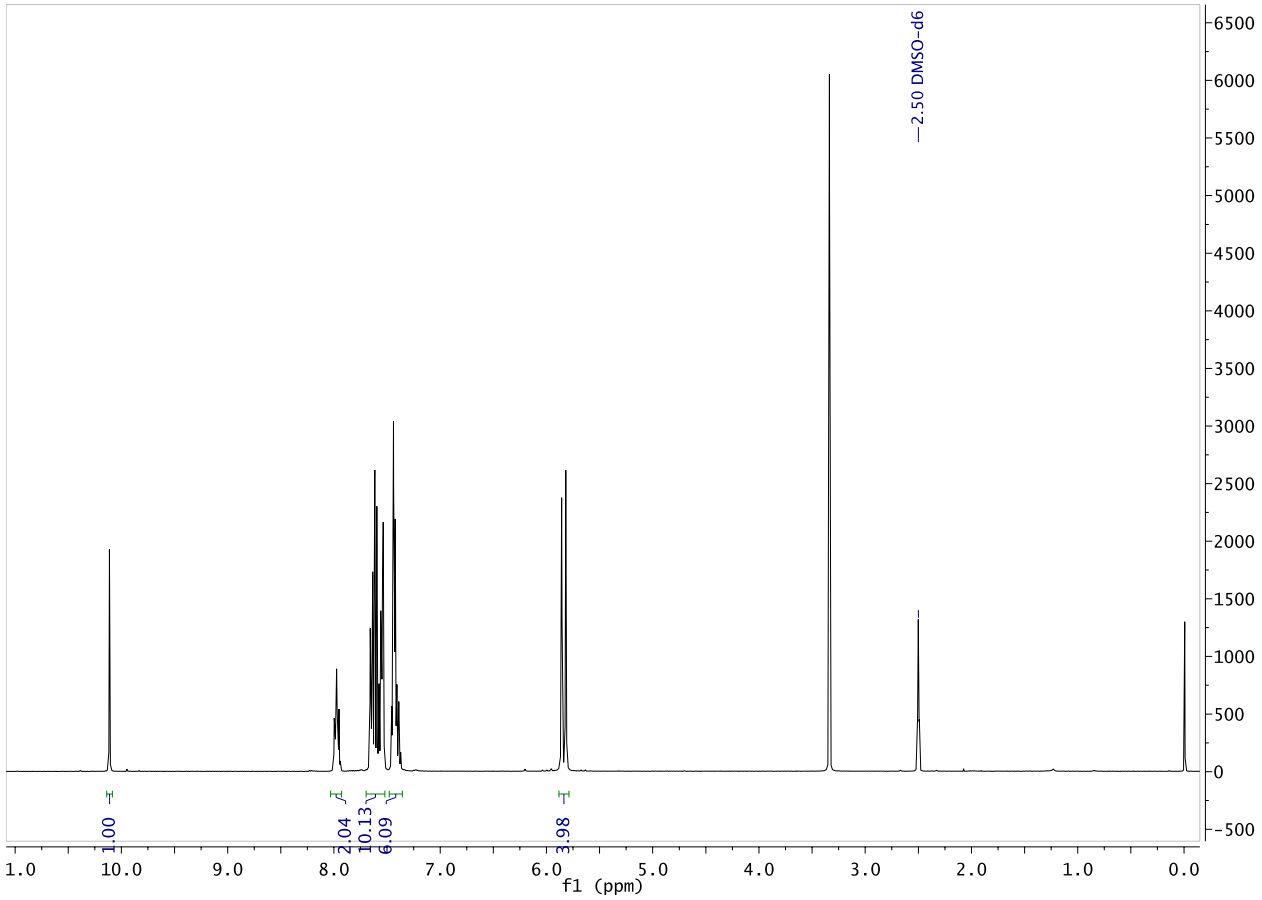
Hemolytic assay. Red blood cells in Alserver's solution were centrifuged 10 min. at 300 x g, washed 3 times with PBS buffer and resuspended in PBS at 2% v/v. In a 96 wells plate was added 195 µL of red blood cells solution and 5 µL of compound in DMSO, and the plate was incubated with light agitation for 1 h at 37 °C. The plate was then centrifuged for 10 min. at 300 x g and 50 µL of the supernatant of each well was transferred to another plate. Absorbance was measured at λ = 405 nm.

Biofilm inhibition assay. *S. aureus* cells were incubated in LB medium at 37 °C for 5 h and rediluted in LB medium to the desired final concentration (OD_{600nm}= 0.1-0.15). *S. Aureus* biofilms were labeled with *Live/Dead* stain after 12 h incubation with antibiotics in growth media (LB broth). Negative Control: DMSO (final concentration not exceeding 10 % volume). Positive Control: 70 % ethanol.

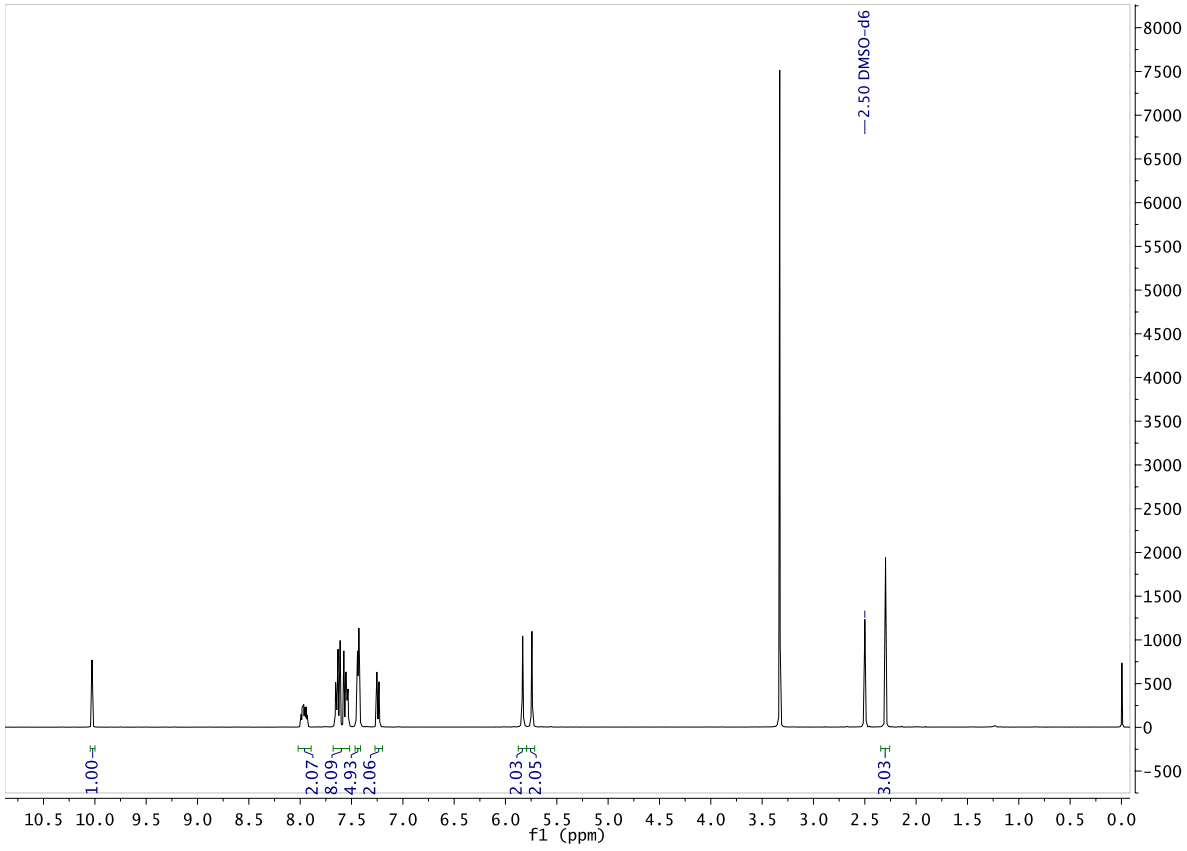
Biofilm staining and confocal laser scanning microscopy (LSM) analysis. Biofilms stained with FilmTracer™ LIVE/DEAD® Biofilm viability kit (Molecular Probes, Life Technologies Ltd.). Briefly, a working solution of fluorescent stains was prepared by adding 1 µL of SYTO® 9 stain and 1 µL of PI stain to 1 mL of filter-sterilized water. Two hundred µL of staining solution were deposited on each well of a chambered coverglass (8-well), after 15 min at room temperature in the dark, samples were washed with sterile saline (0.9% NaCl) from base of the support material. Then, biofilms were examined with a confocal laser microscope (Leica model TCS SP5; Leica Microsystems CMS GmbH, Mannheim, Germany) using a 20x dry objective (HC PL FLUOTAR 20.0 x 0.50 DRY). A 488 nm laser line was used to excite SYTO® 9, while the fluorescent emission was detected from 500 to 540 nm. PI was sequentially excited with 561 nm laser line and its fluorescent emission was detected from 600 to 695 nm.

Scanning electron microscopy. Bacterial samples were applied to titanium surfaces for 12 hours in order to form biofilms, following by a fixation for 1h at 4 °C in 4% Paraformaldehyde and 0,1% glutaraldehyde in 0.1M phosphate buffer (PB), pH 7.3, and subsequently rinsed three times with PB. Following fixation, samples were incubated for 1 hour in 1% osmium tetroxide at RT and then dehydrated through an ethanol series (30%, 50%, 70%, 90%, 95% and two times 100%) followed by drying using a Critical Point Drier CPD300 (Leica Biosystems, Concord, ON, Canada). A JEOL JSM-7400F (JEOL Ltd, Tokyo, Japan) field-emission scanning electron microscope (FE-SEM) operated at 1.5 kV was used to image the samples.

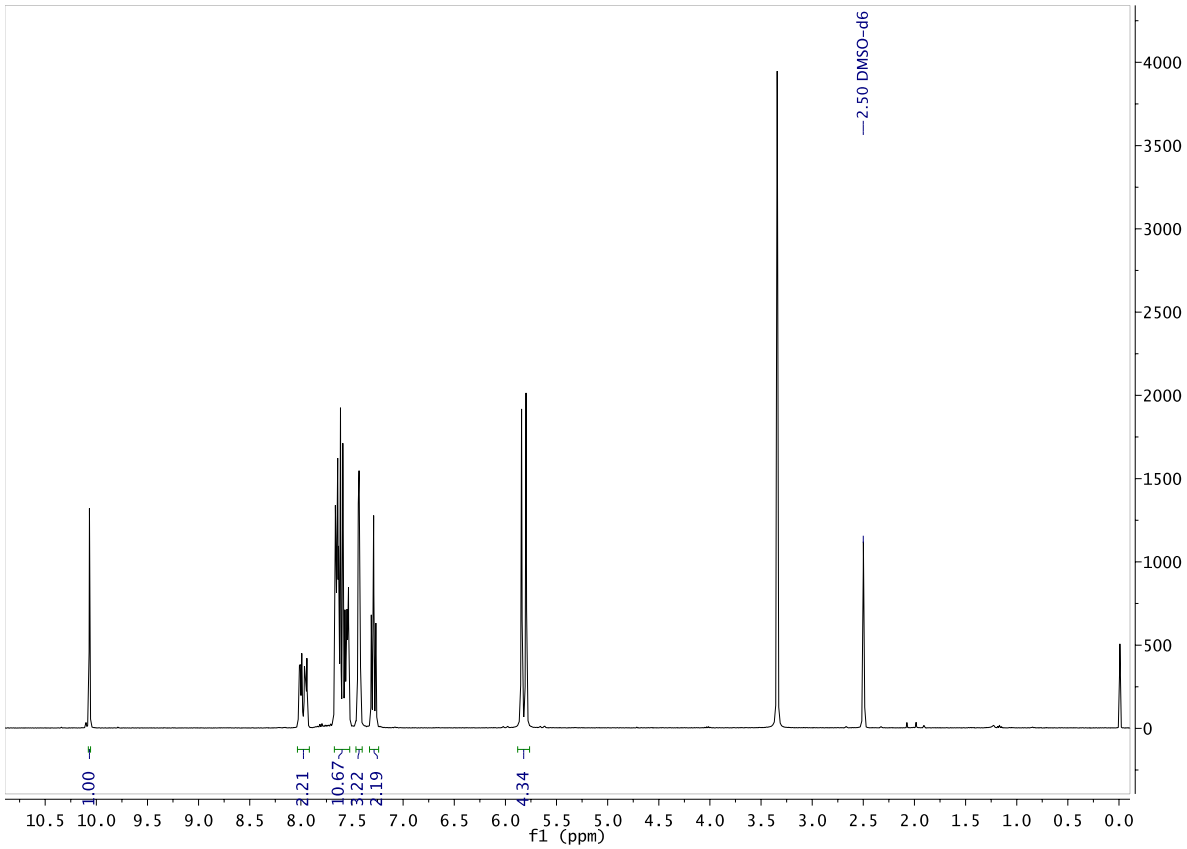
3-benzyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (7).



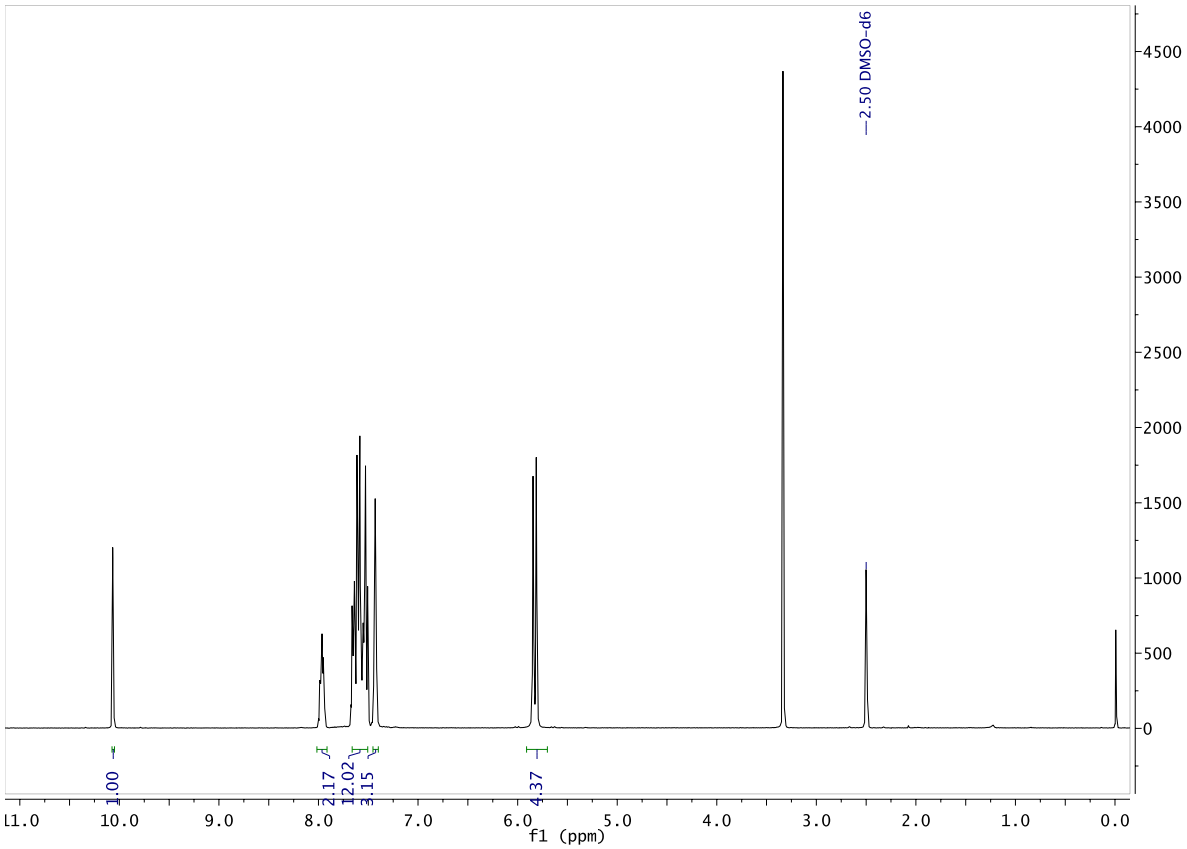
3-(4-methylbenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (8).



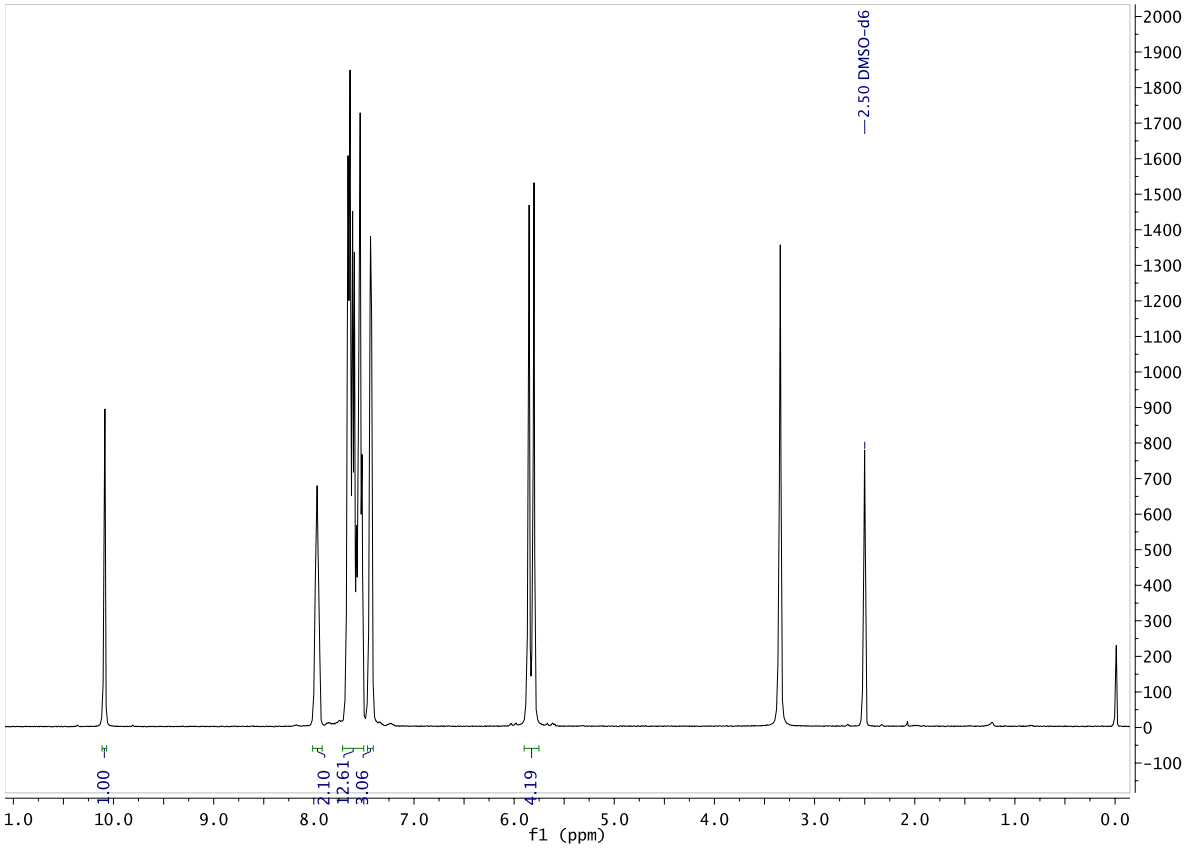
3-(4-fluorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (9).



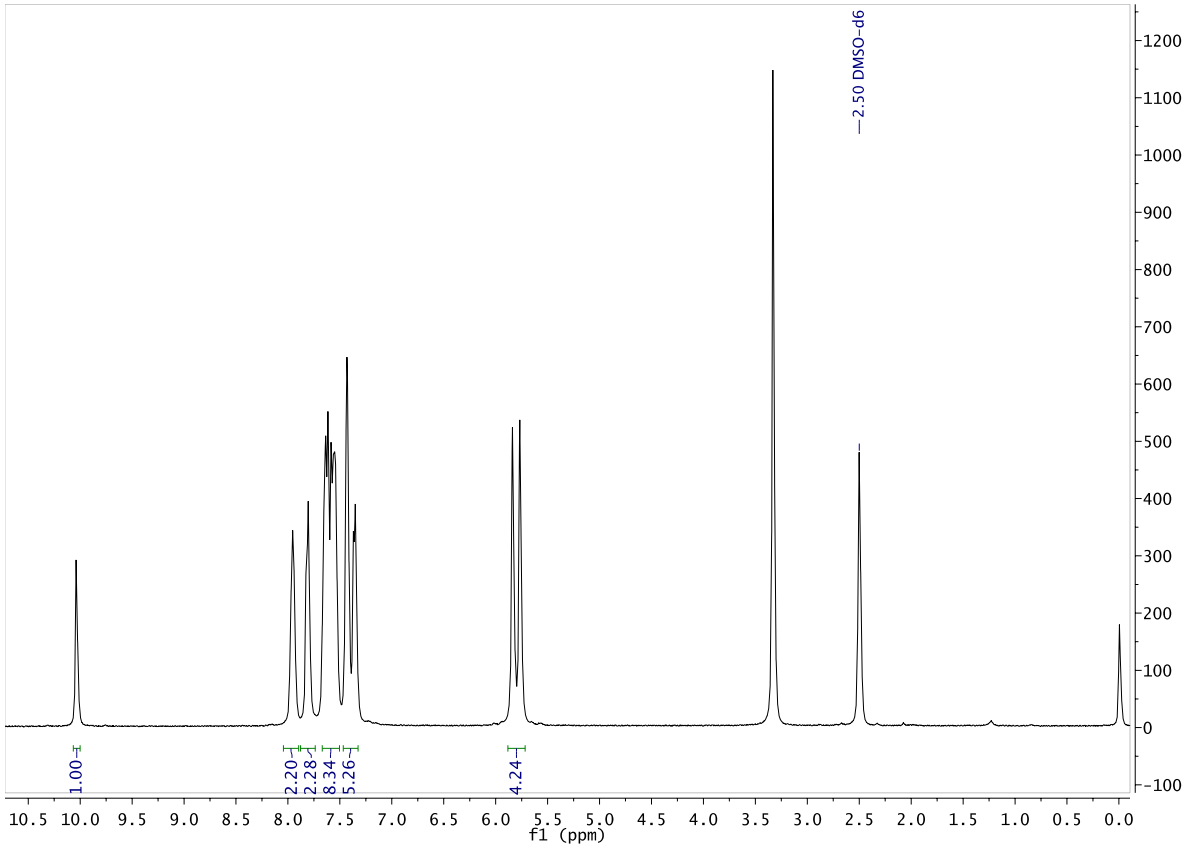
3-(4-chlorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (10).



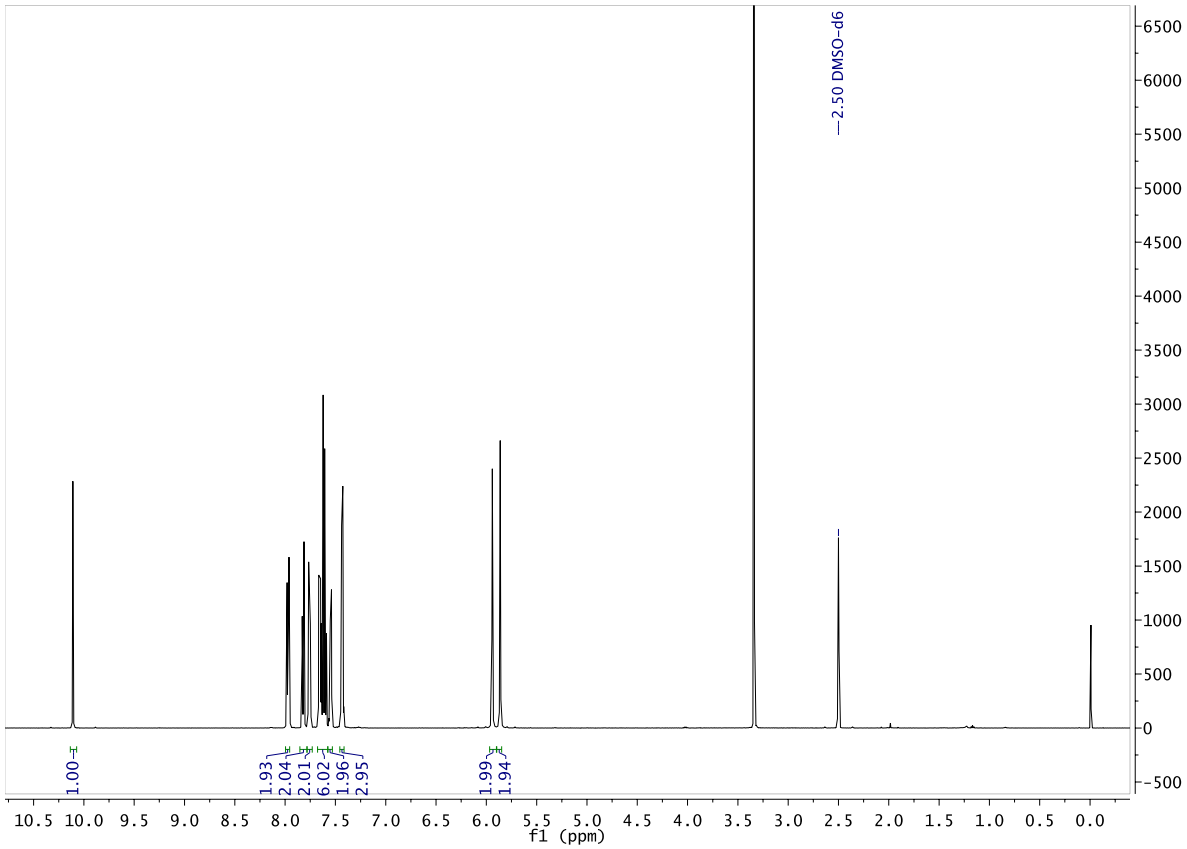
3-(4-bromobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (11).



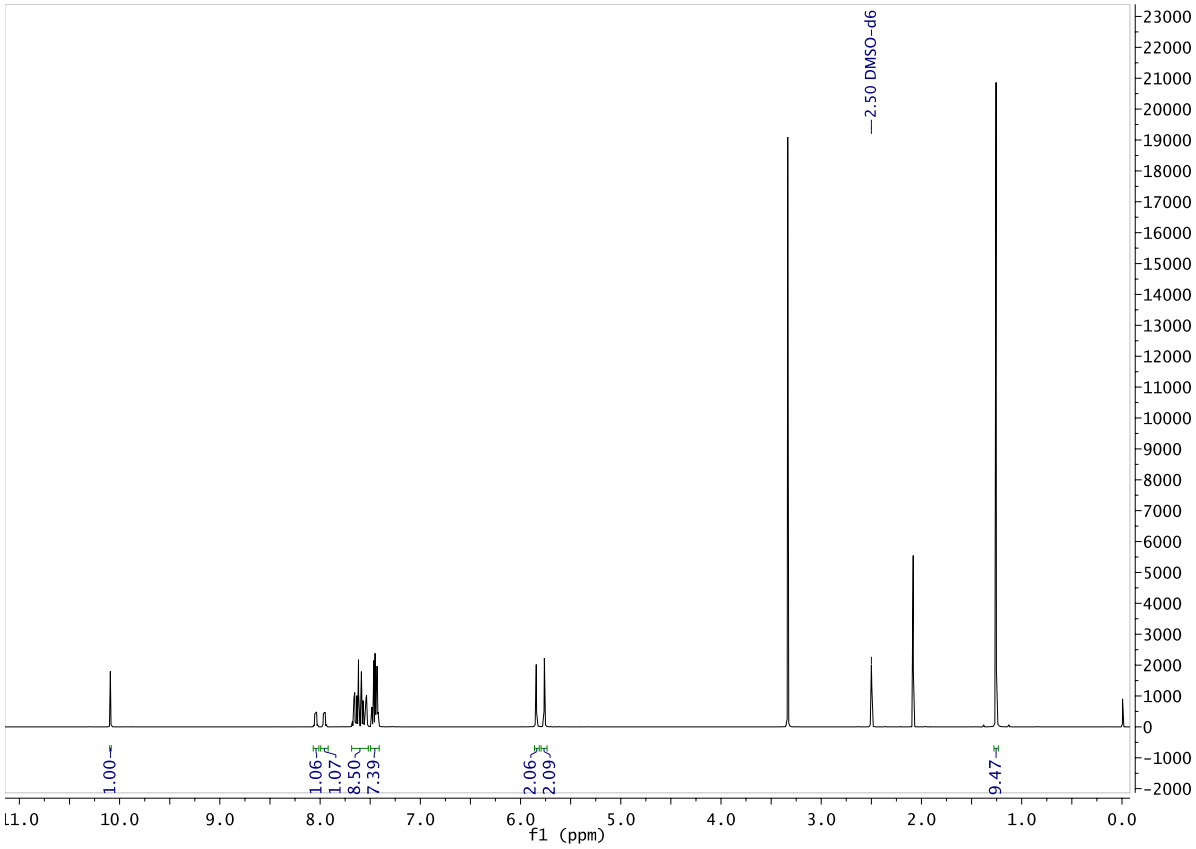
3-(4-iodobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (12).



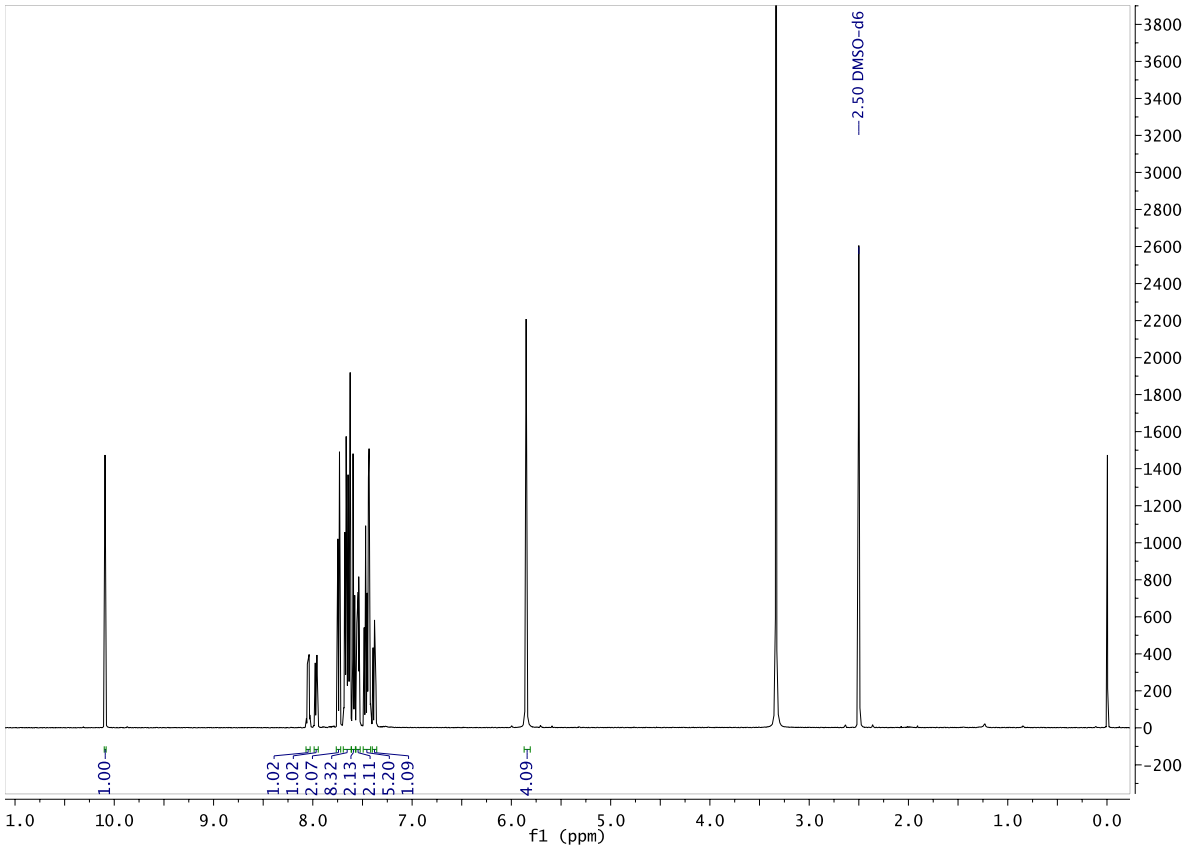
1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (13).



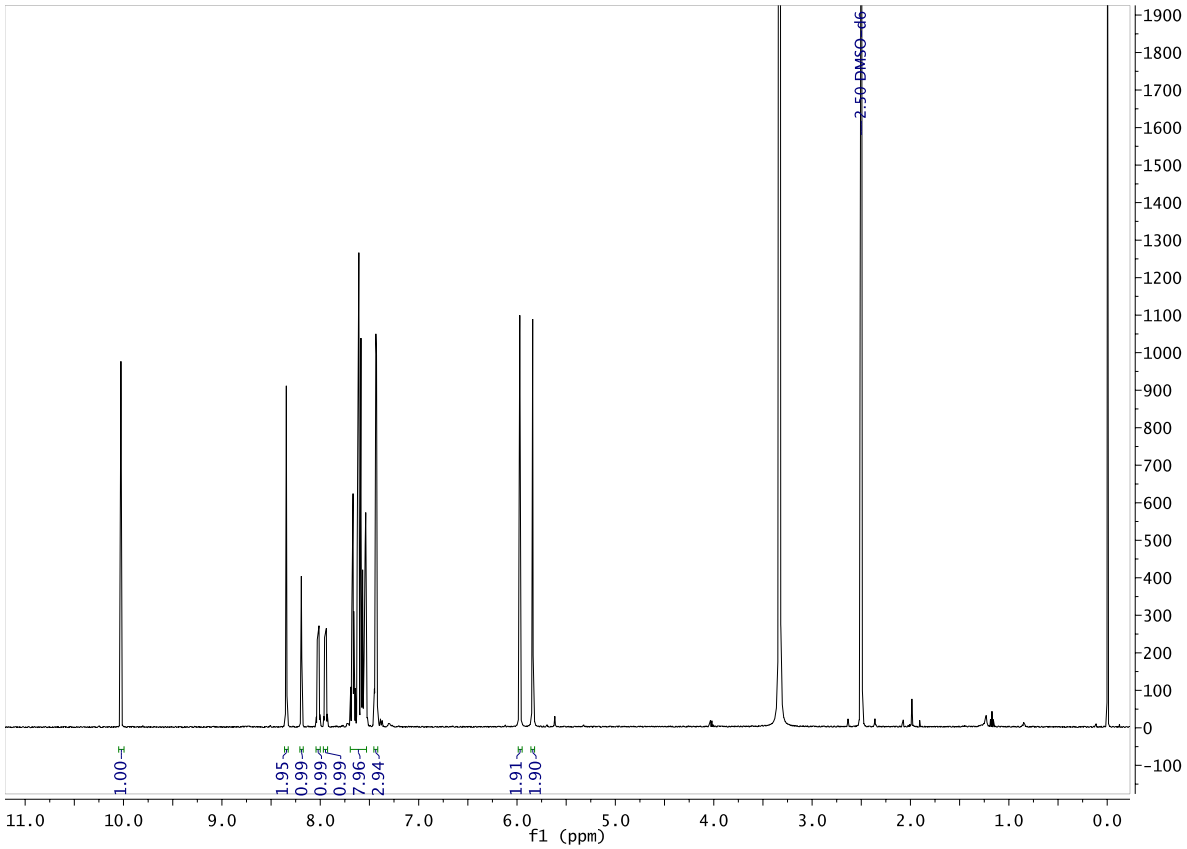
3-(4-(*tert*-butyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (14).



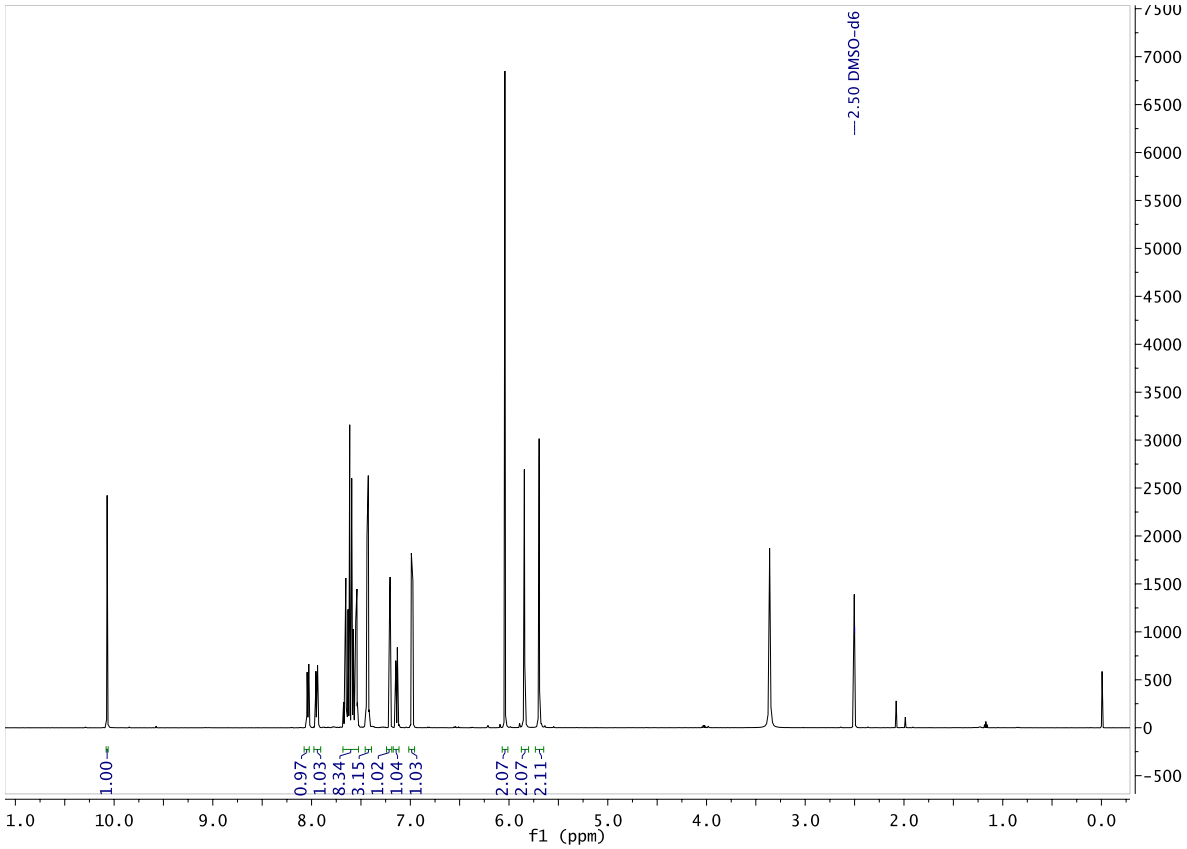
3-([1,1'-biphenyl]-4-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (15).



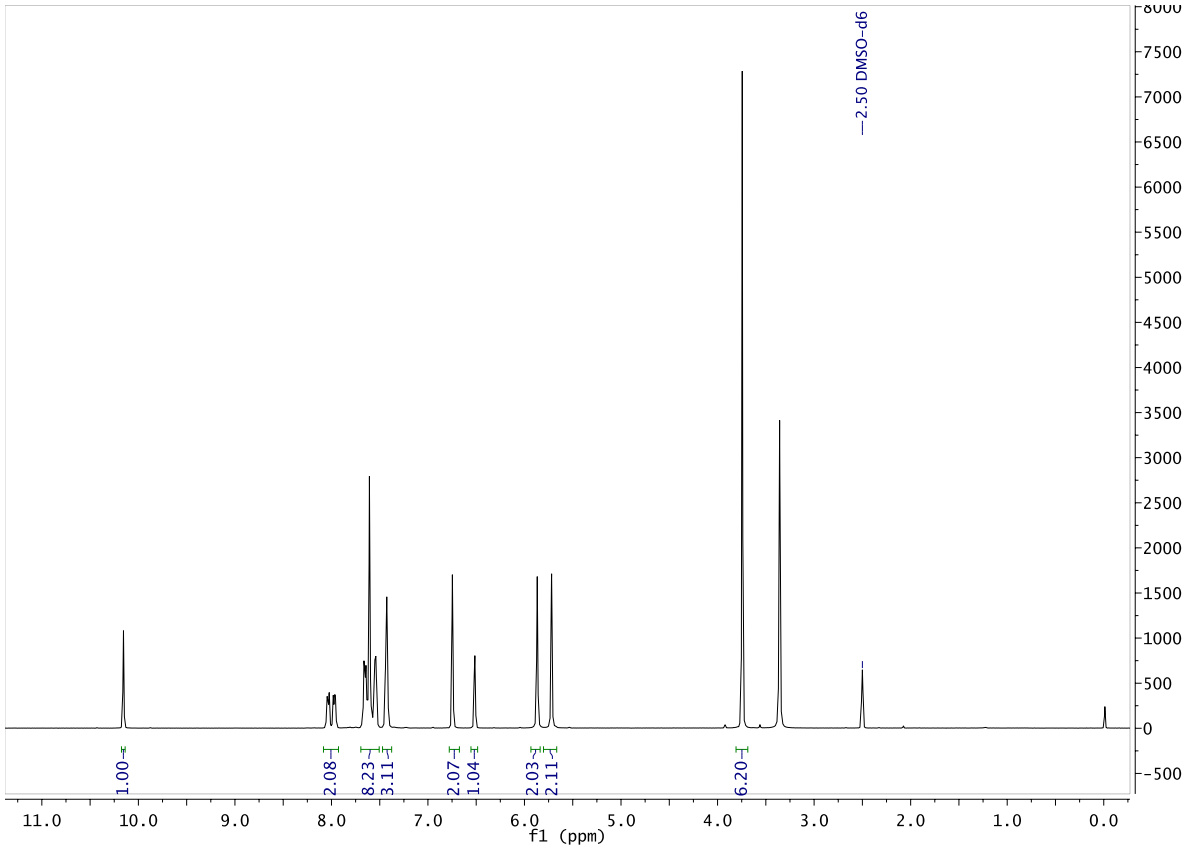
3-(3,5-bis(trifluoromethyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (16).



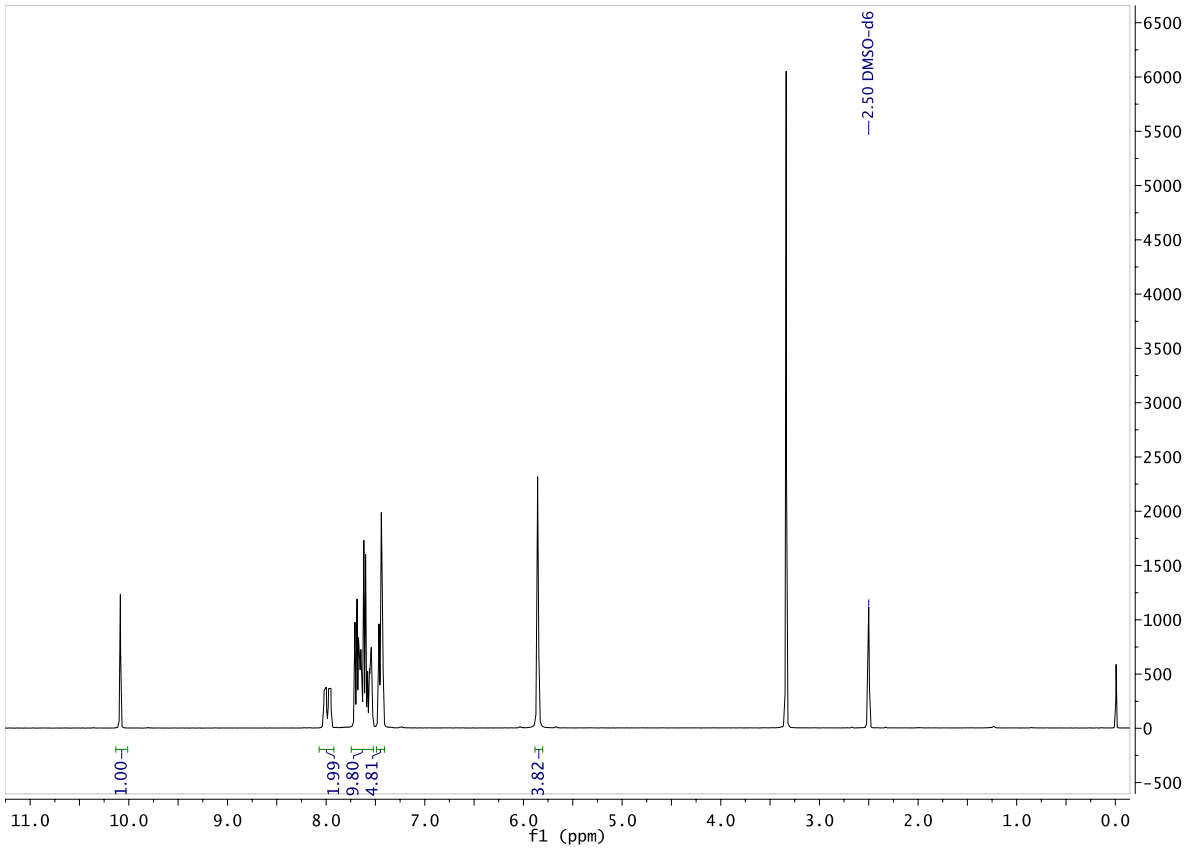
3-(benzo[*d*][1,3]dioxol-5-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (17).



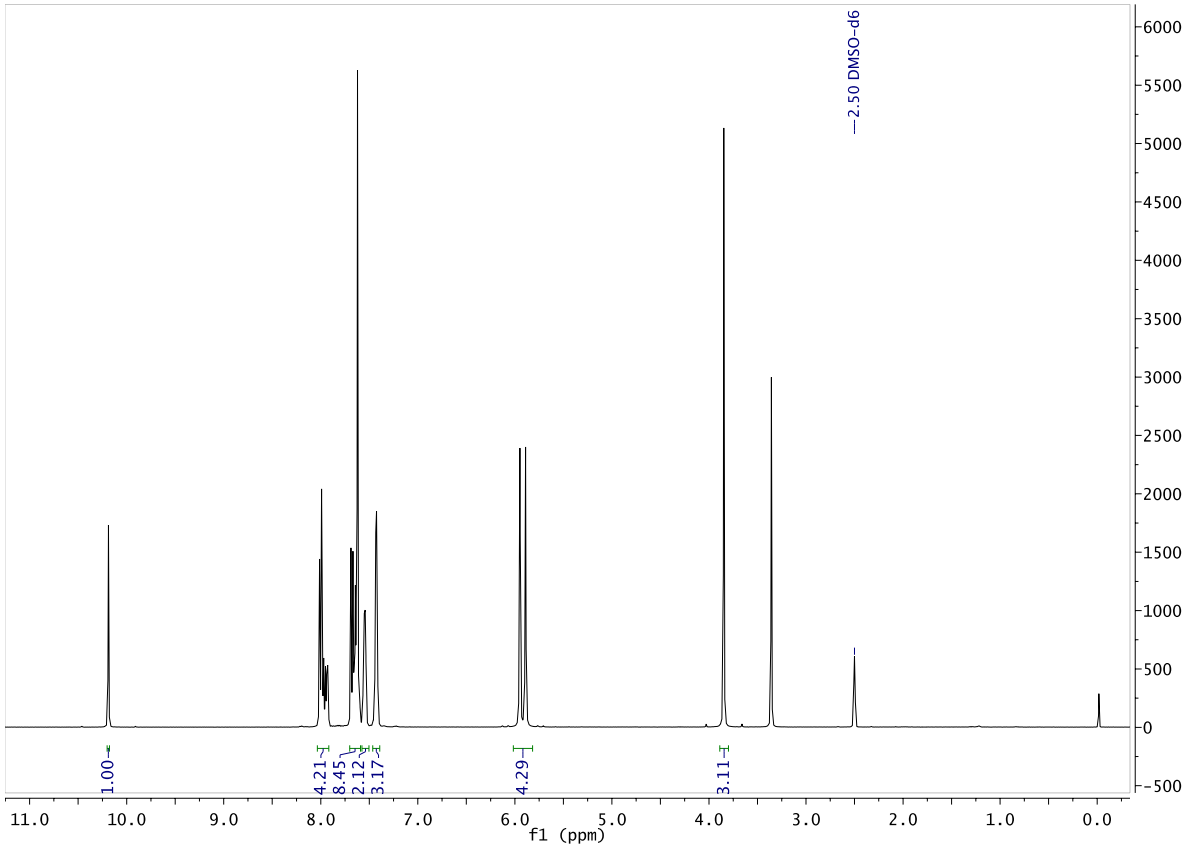
3-(3,5-dimethoxybenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (18).



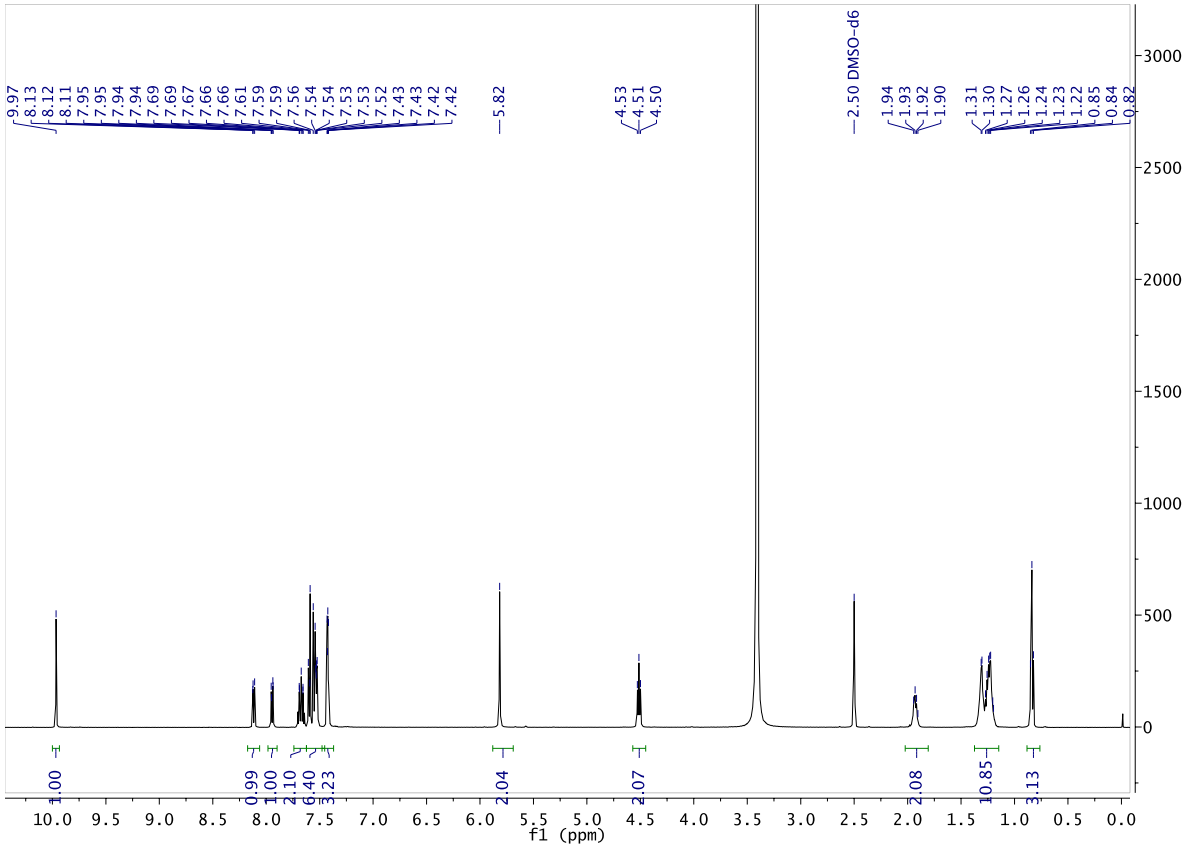
1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethoxy)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (19).



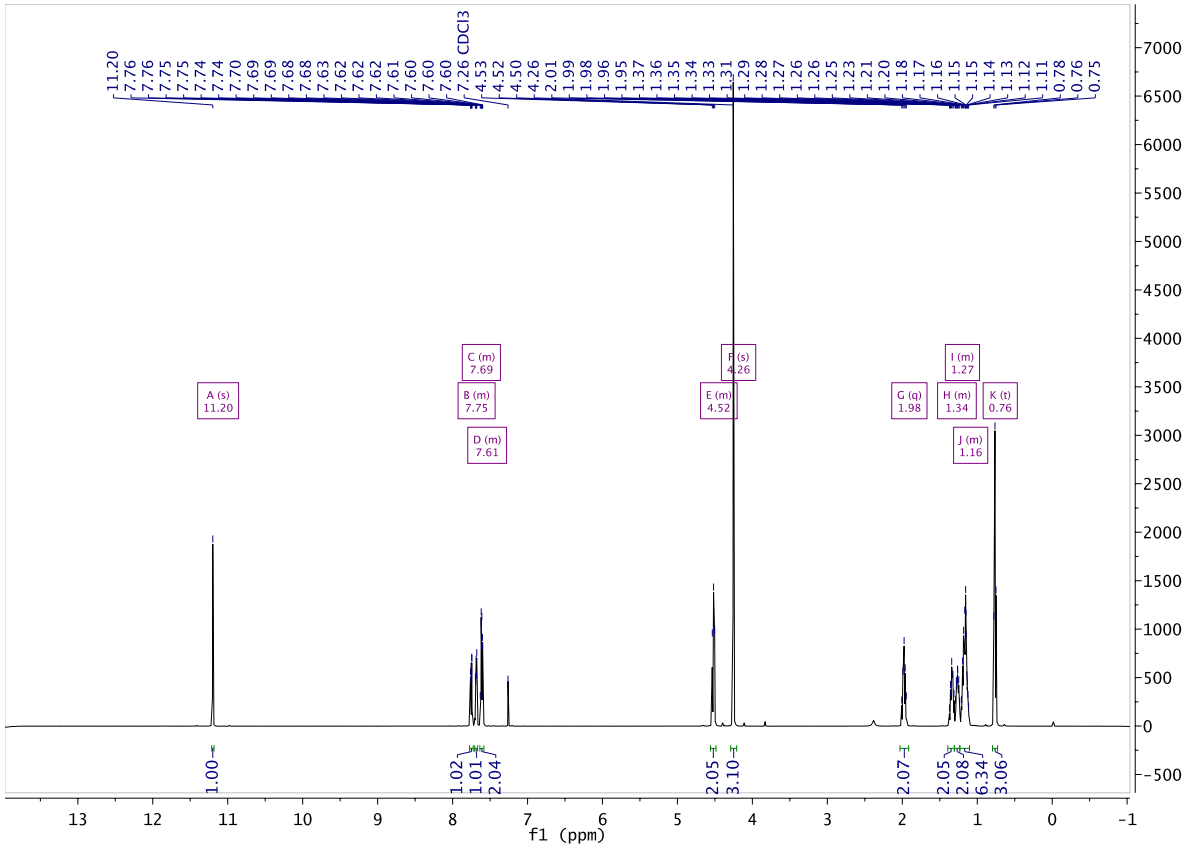
3-(4-(methoxycarbonyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (20).



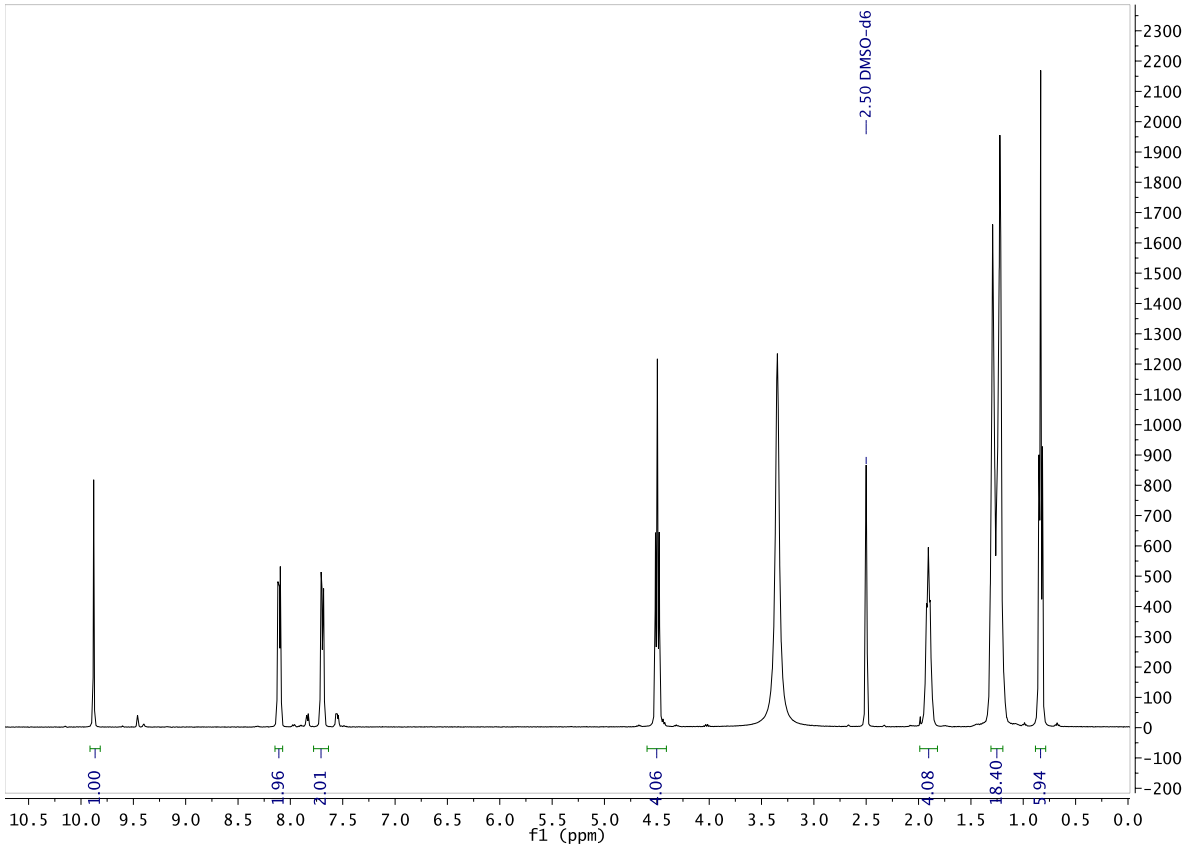
3-octyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (21).



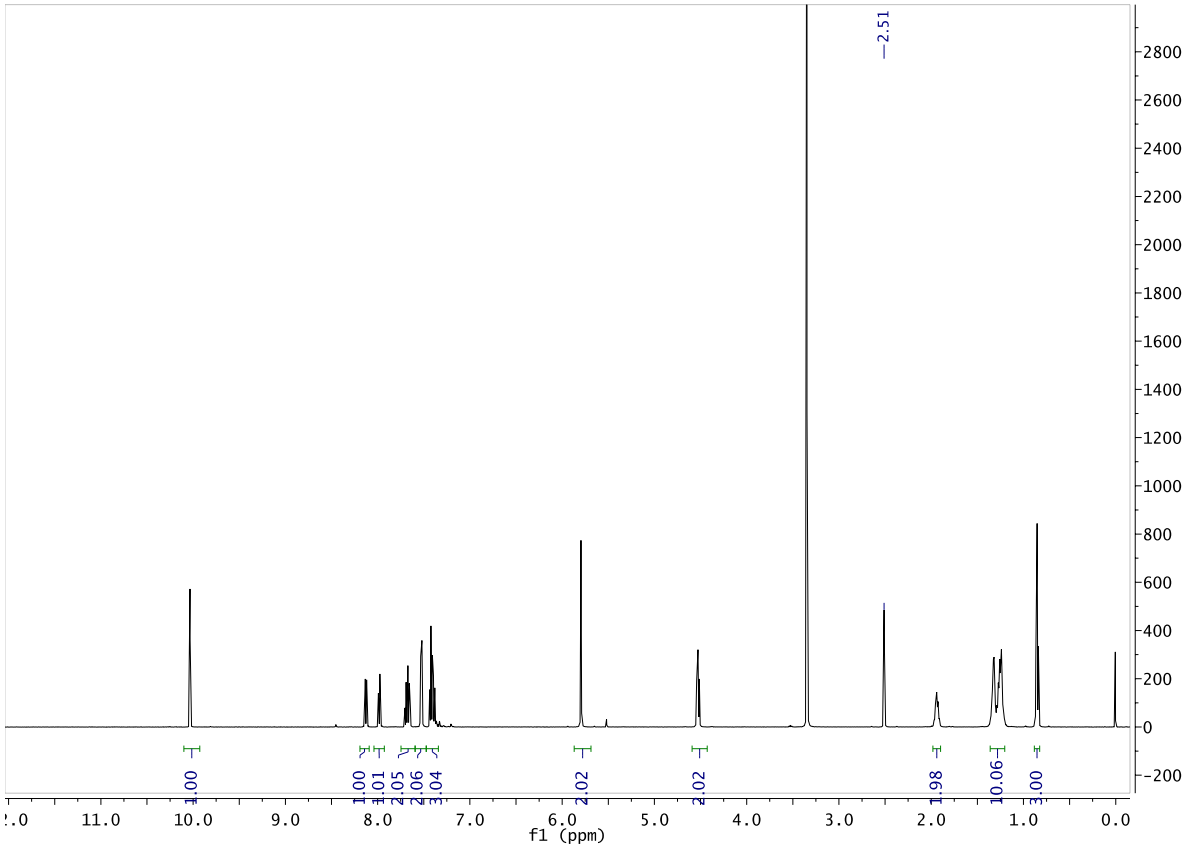
1-methyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (33).



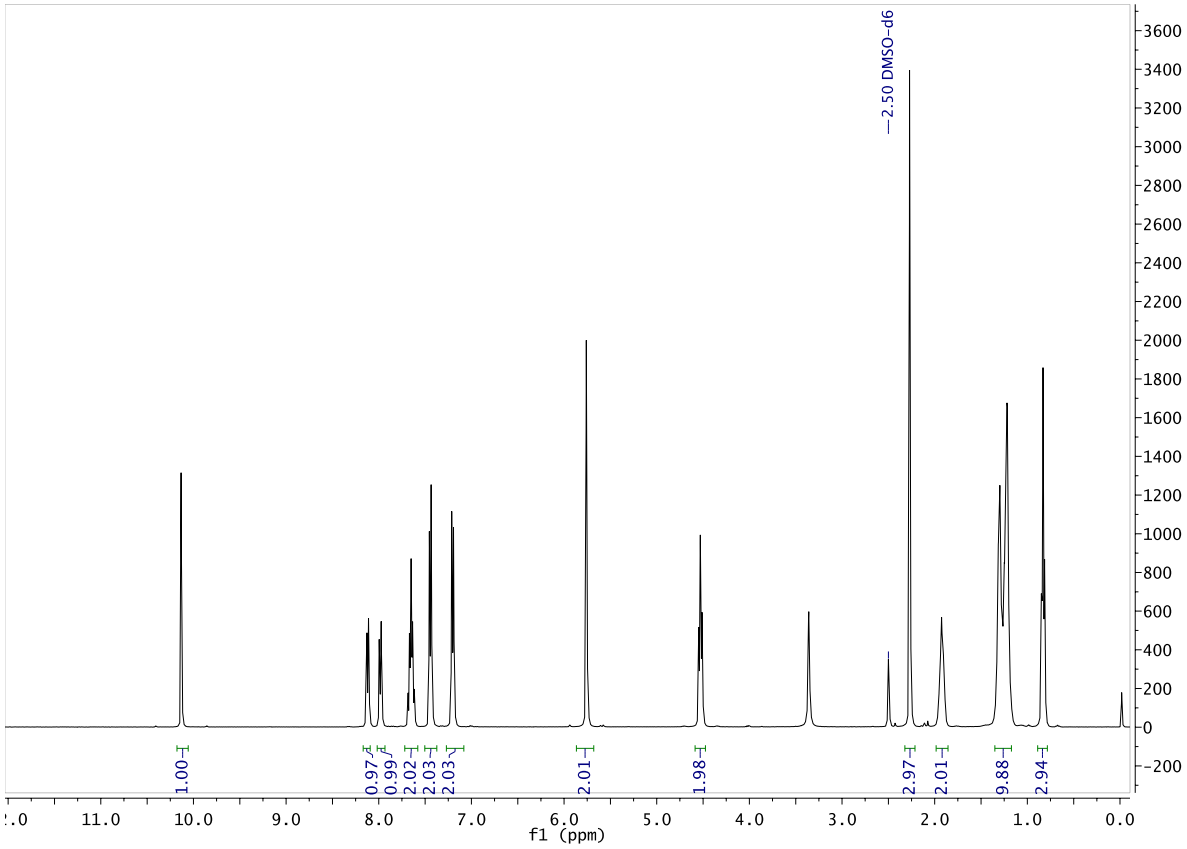
1,3-dioctyl-1*H*-benzo[*d*]imidazol-3-ium bromide (34).



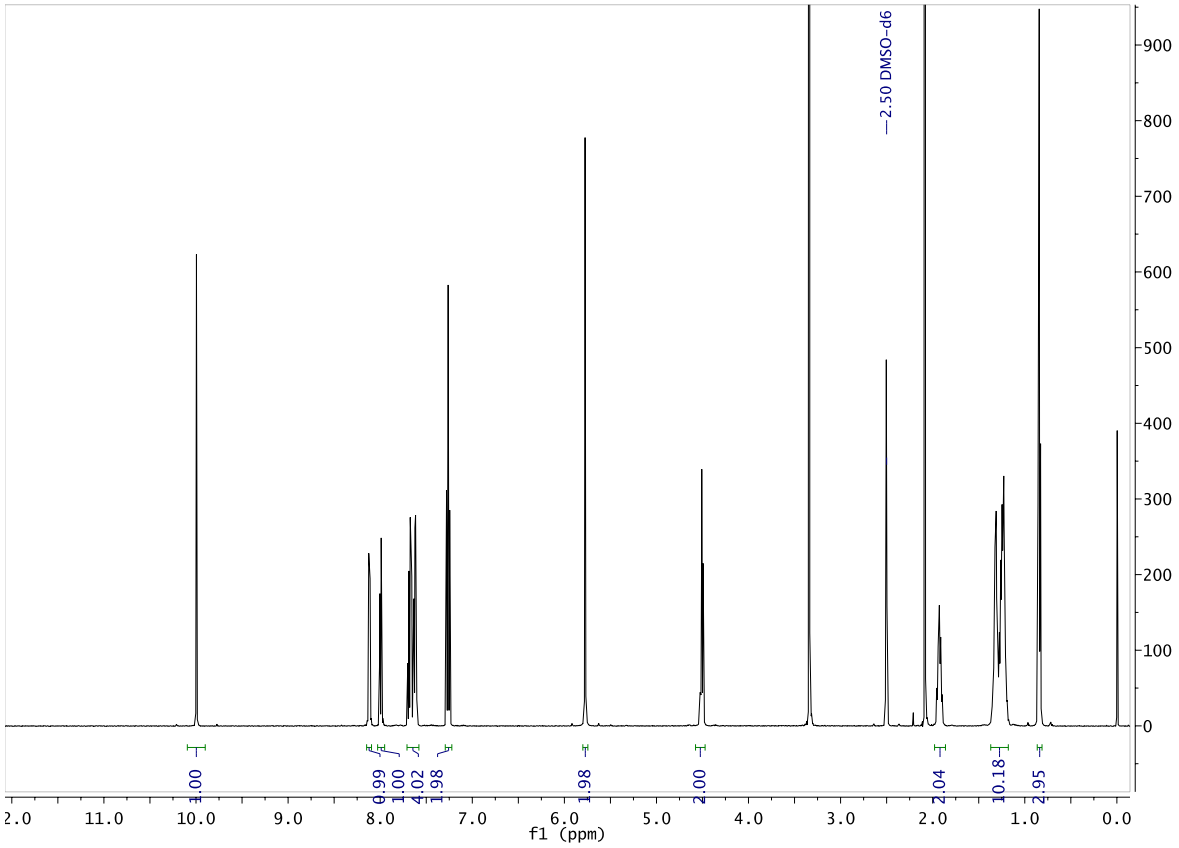
1-benzyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (35).



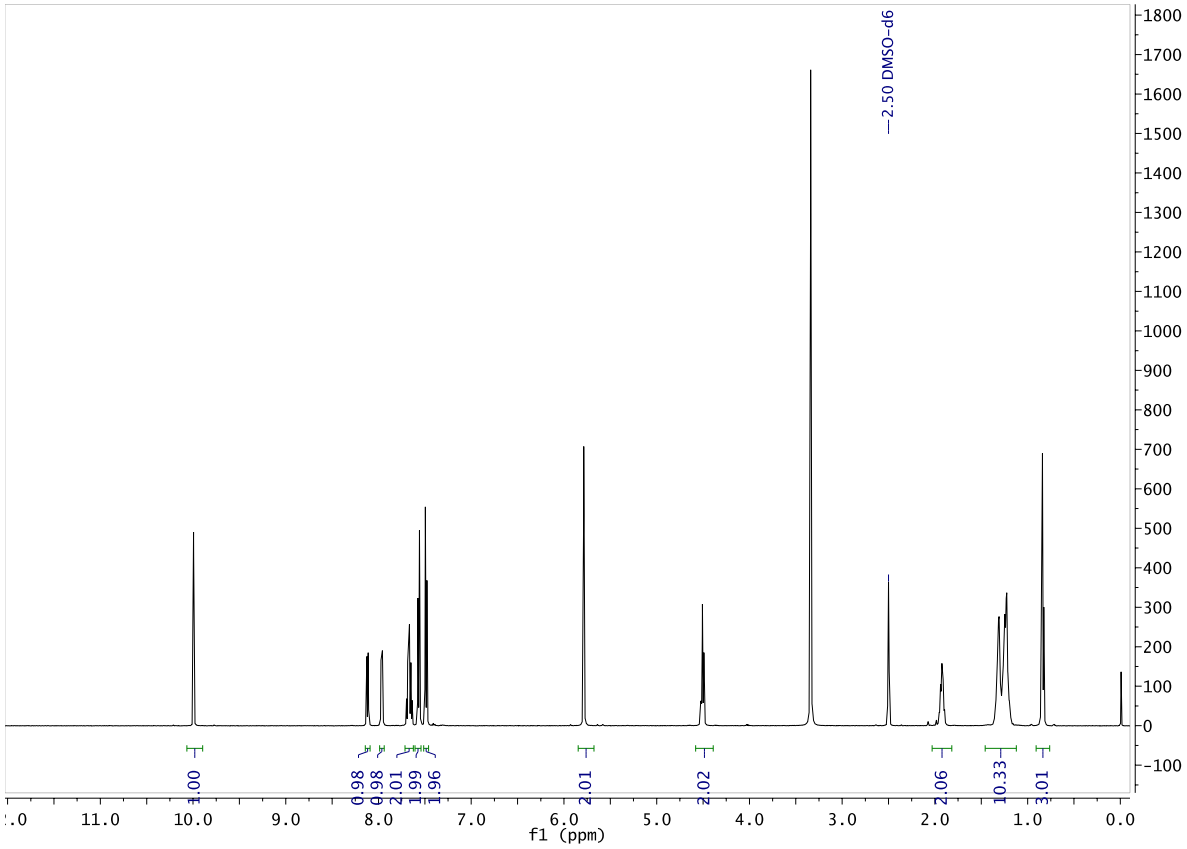
1-(4-methylbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (36).



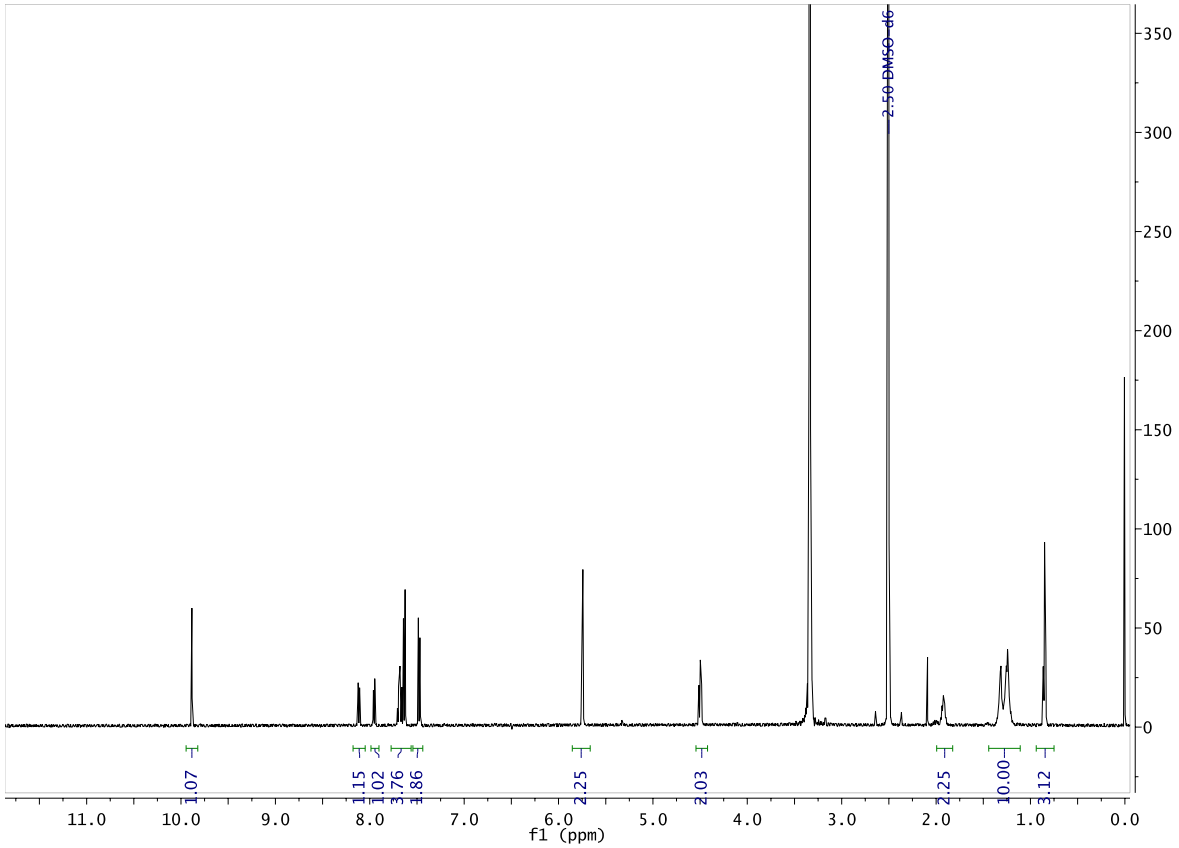
1-(4-fluorolbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (37).



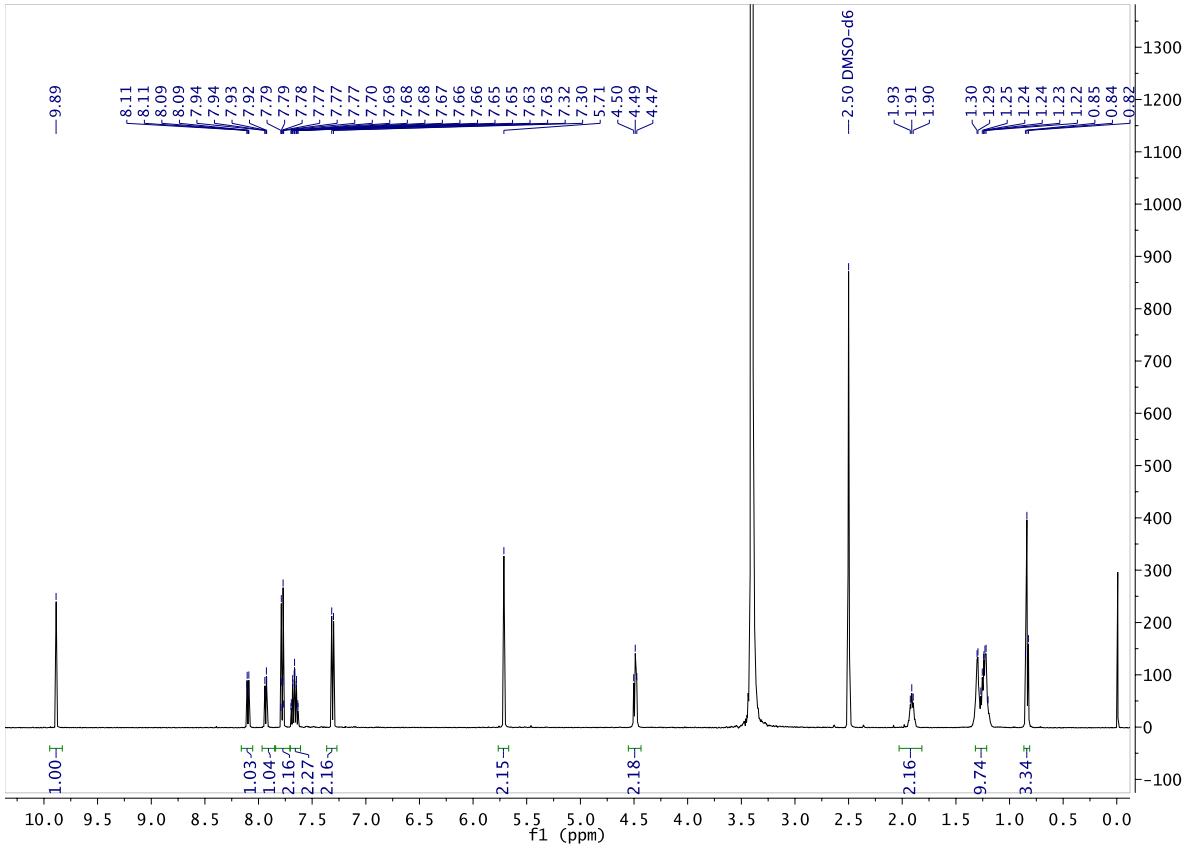
1-(4-chlorobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (38).



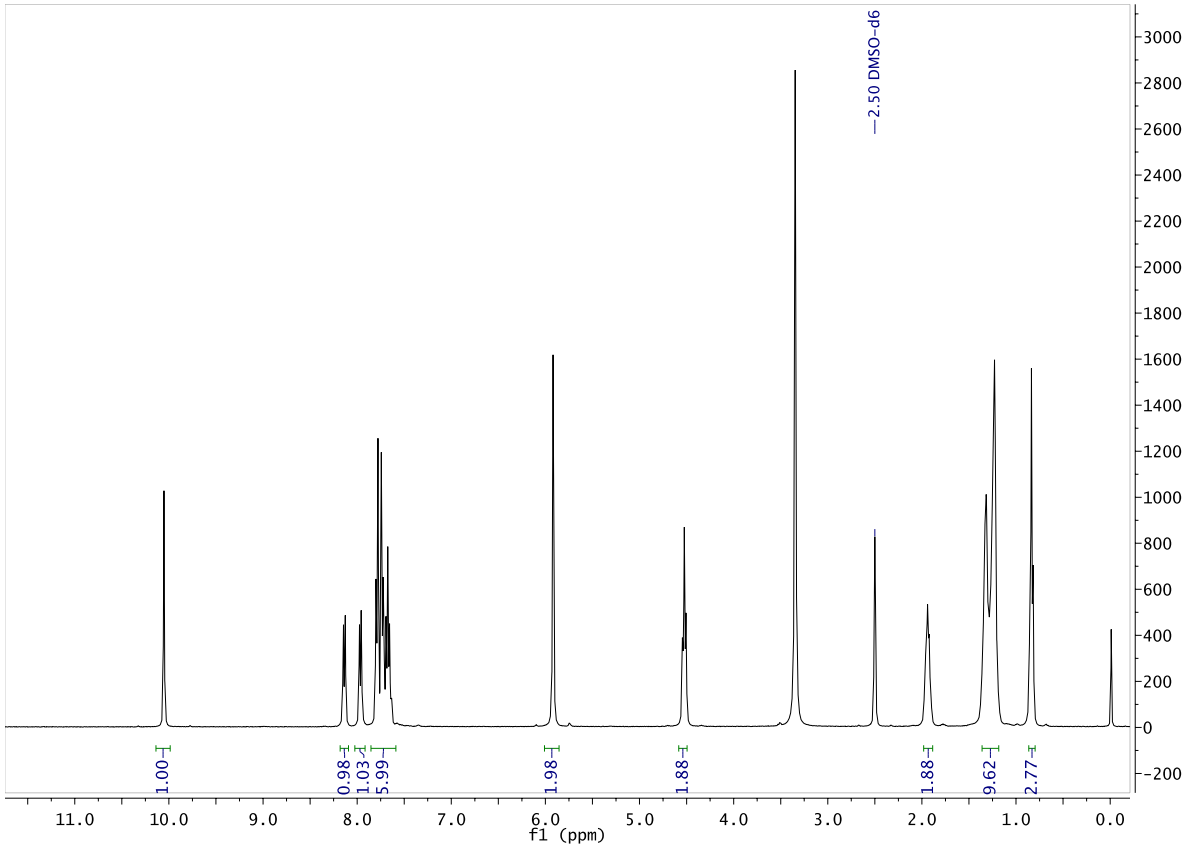
1-(4-bromobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (39).



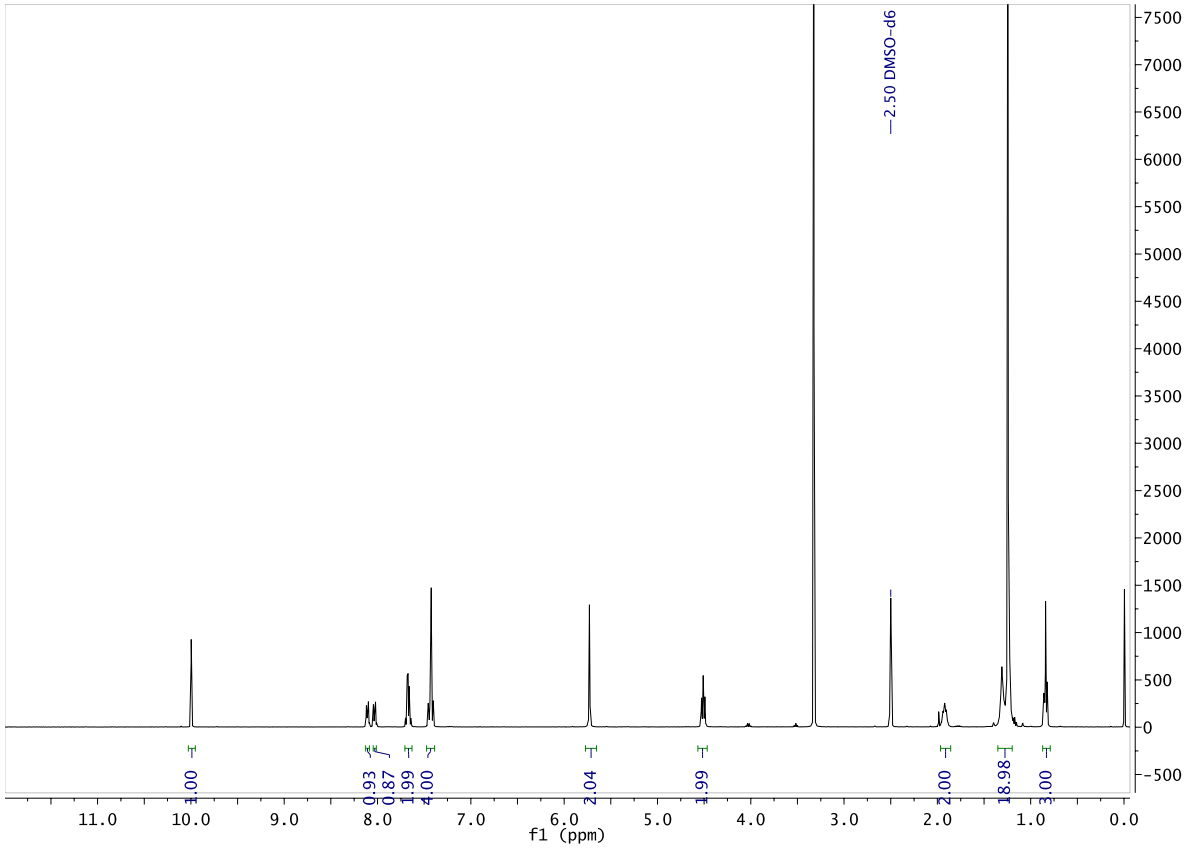
1-(4-iodobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (40).



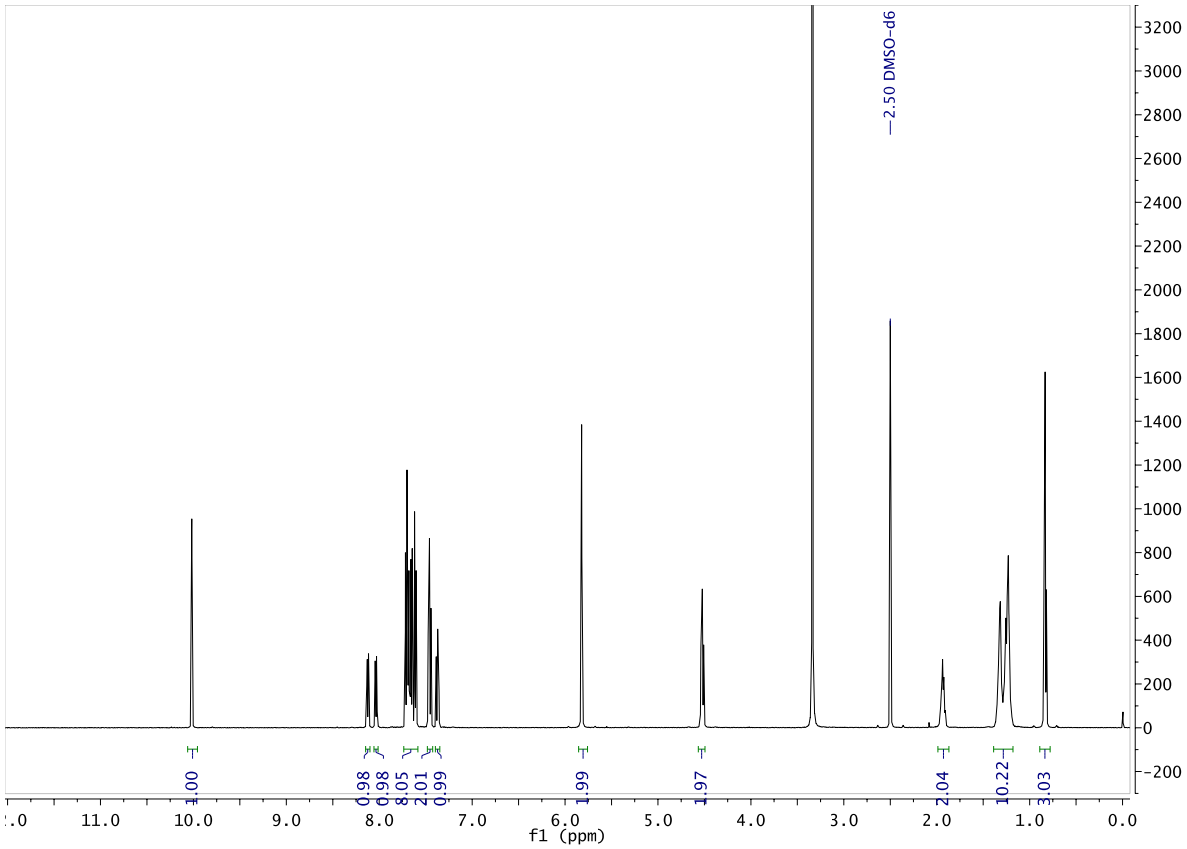
3-octyl-1-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (41).



1-(4-(*tert*-butyl)benzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (42).

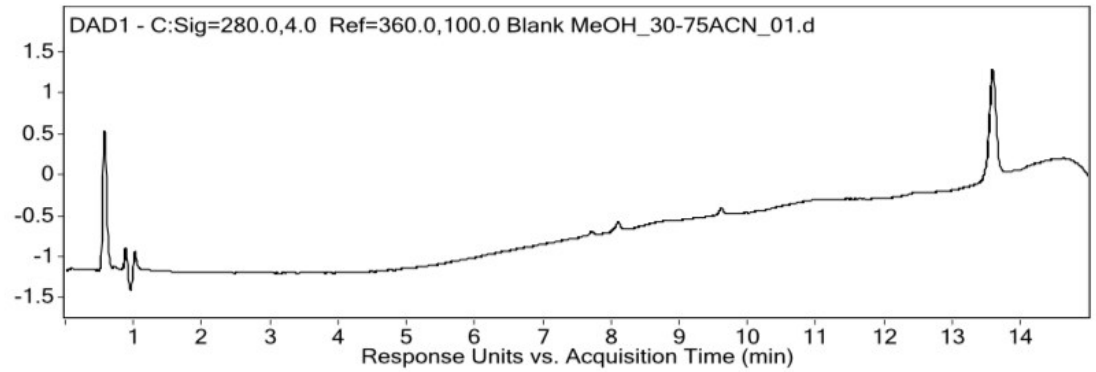


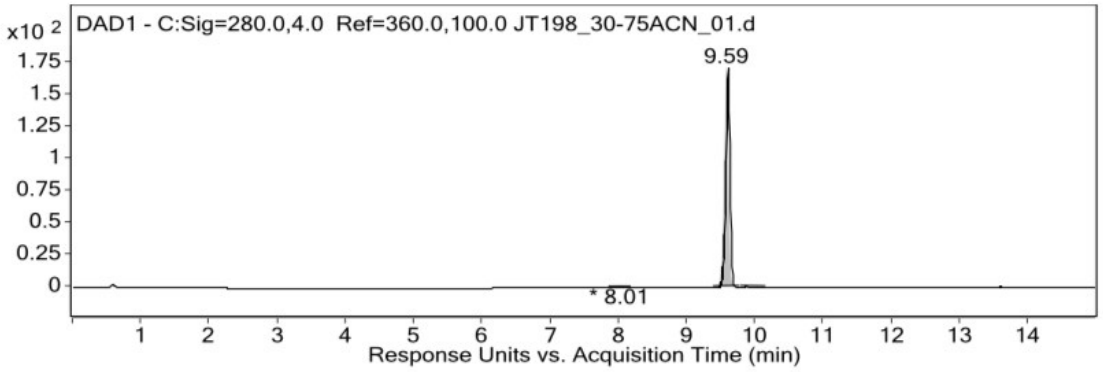
1-([1,1'-biphenyl]-4-ylmethyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (43).



HPLC analysis of analogues (6-21) and (33-43).

1,3-bis(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (6).

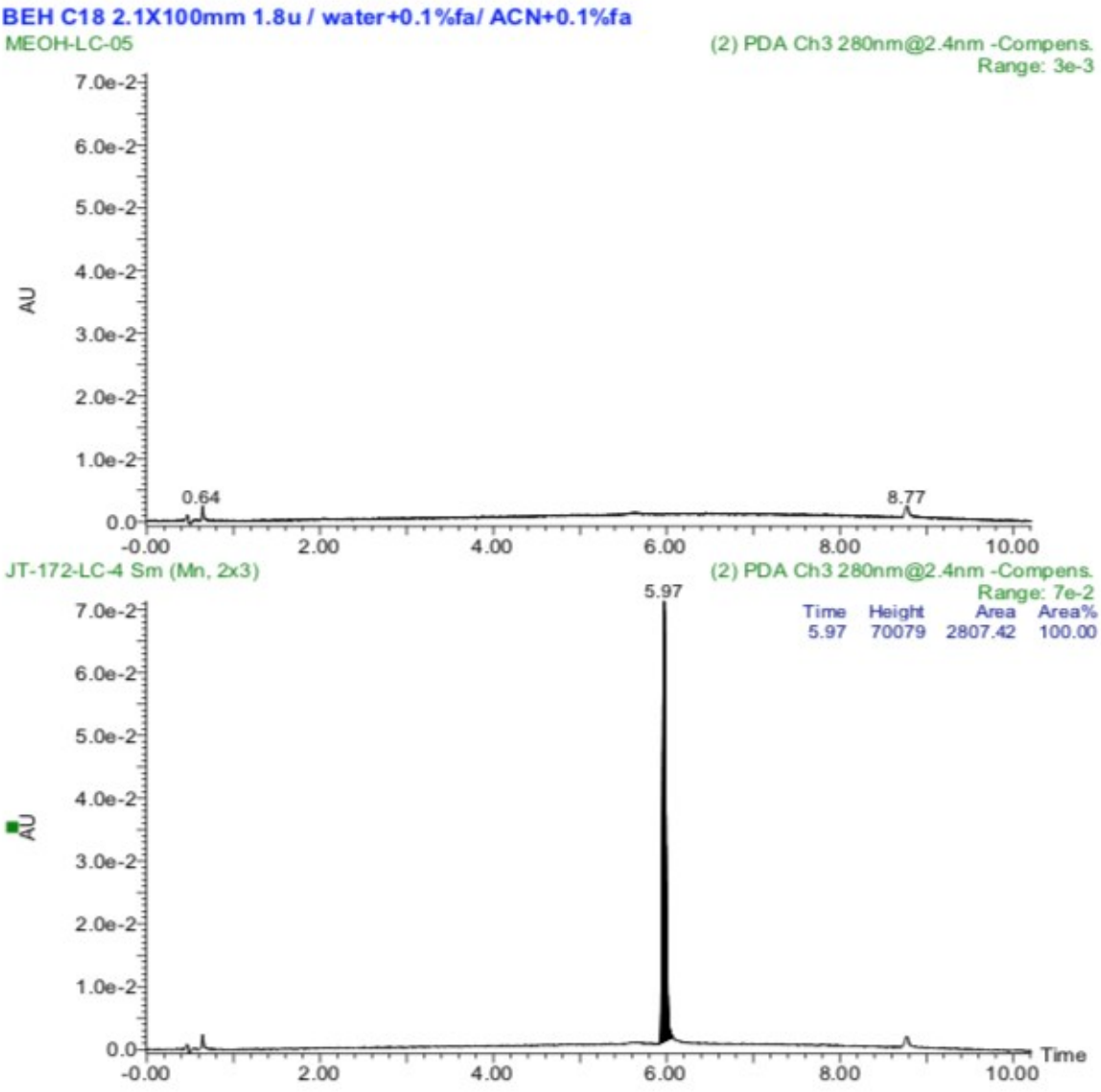




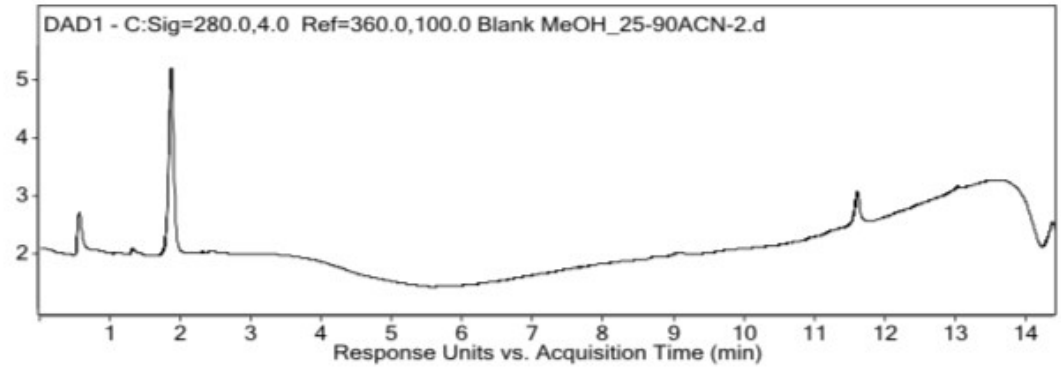
Integration Peak List

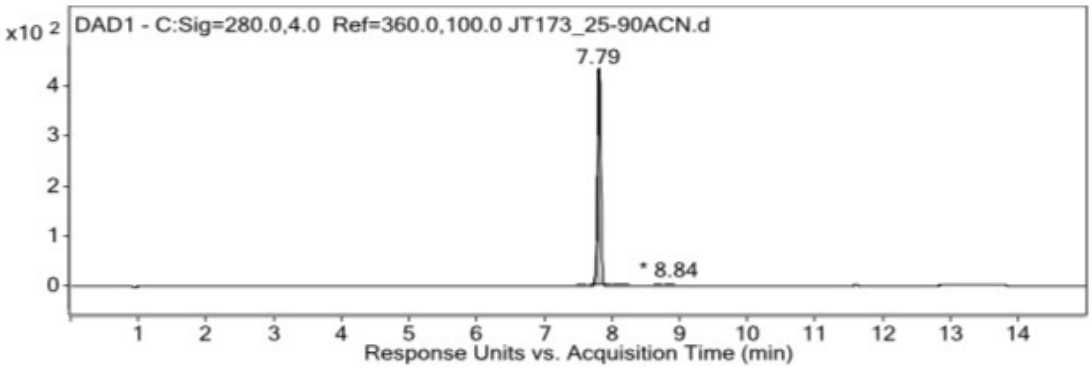
Peak	RT	Height	Area	Area (%)
1	8.01	0.56	3.81	0.52
2	9.59	171.23	727.99	98.99
3	9.86	0.49	3.65	0.5

3-benzyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (7).



3-(4-methylbenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (8).

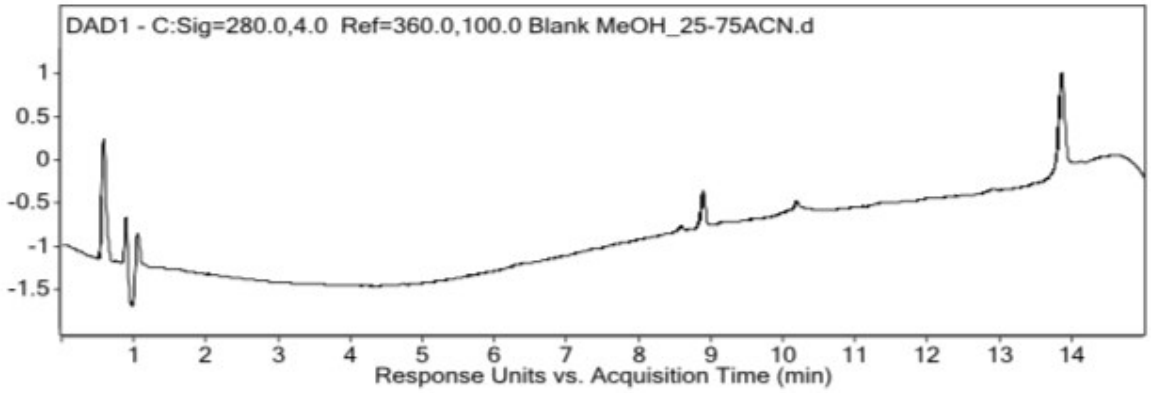


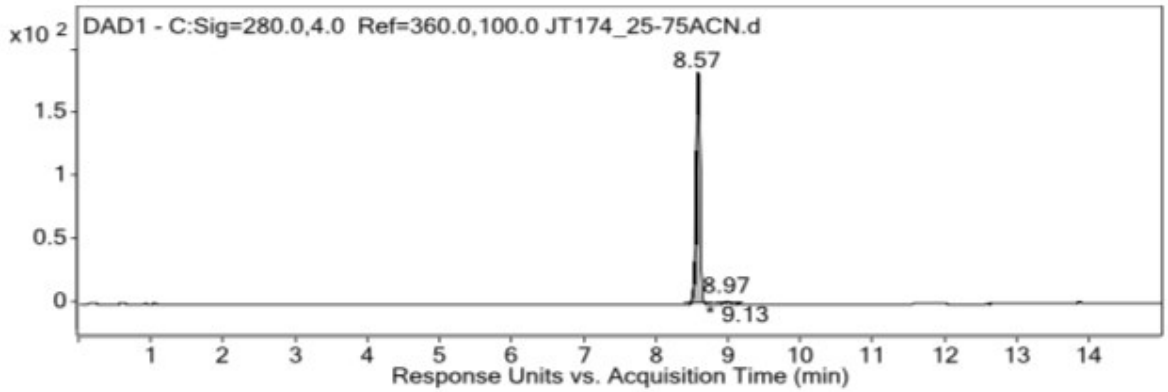


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	7.53	0.18	0.53	0.04
2	7.79	435.63	1413.48	99.48
3	8.16	0.77	3.87	0.27
4	8.7	0.17	0.45	0.03
5	8.84	0.87	2.6	0.18

3-(4-fluorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (9).

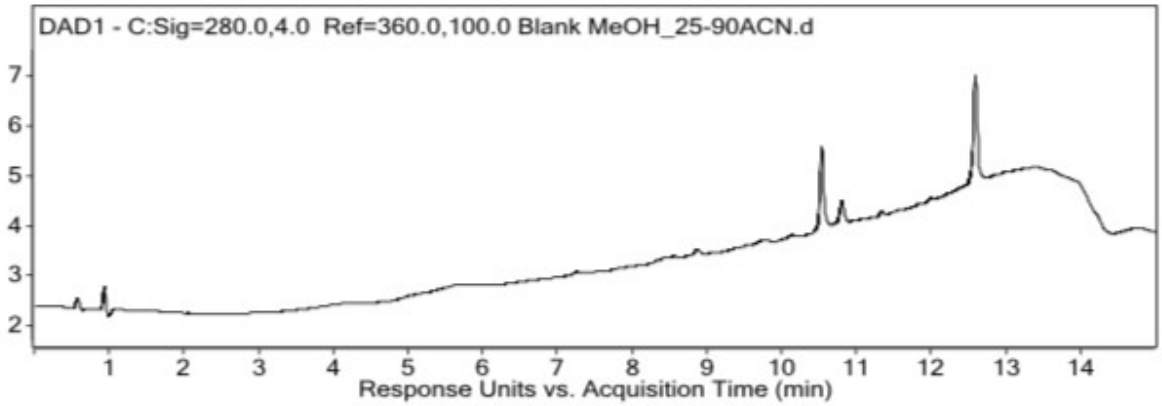


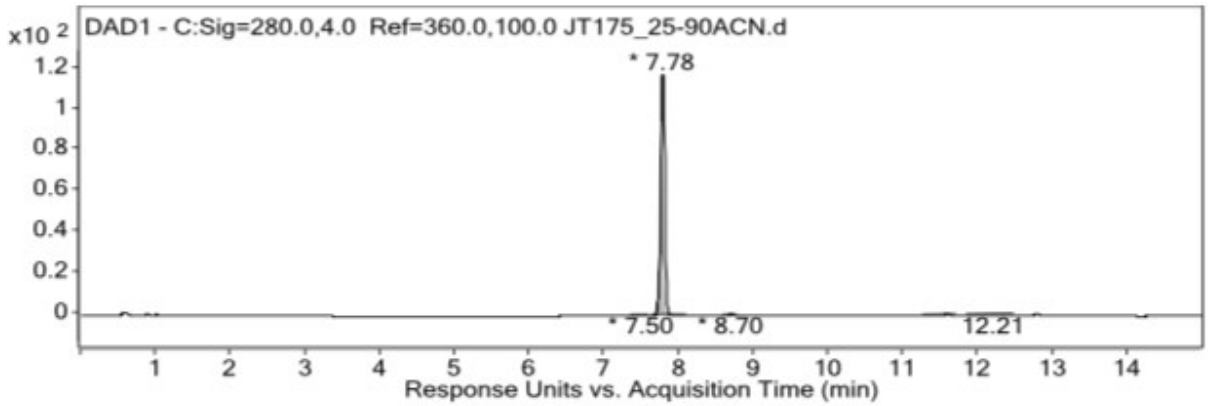


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	8.57	183	682.99	98.65
2	8.97	1.8	8.07	1.17
3	9.13	0.32	1.27	0.18

3-(4-chlorobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (10).

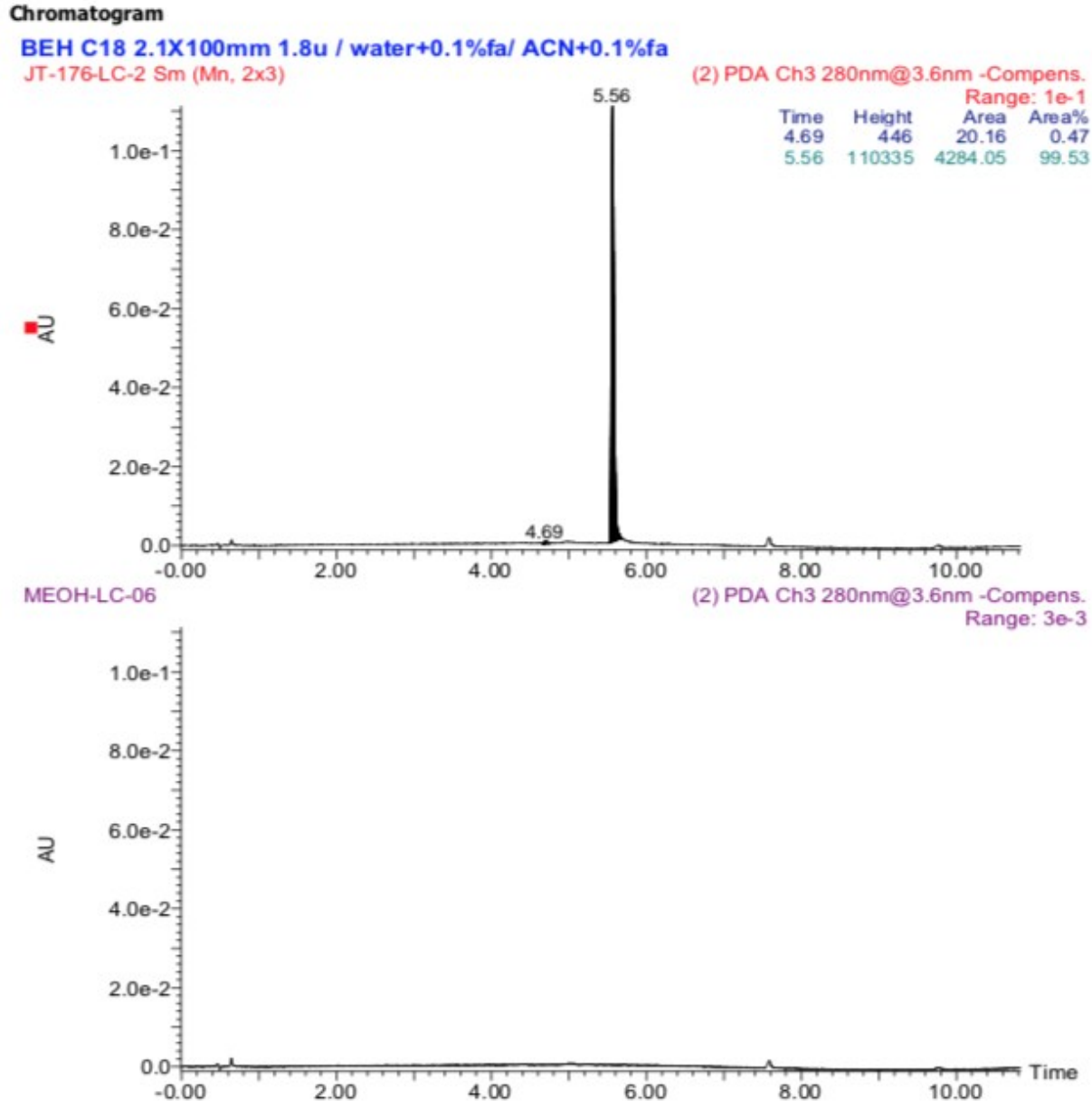




Integration Peak List

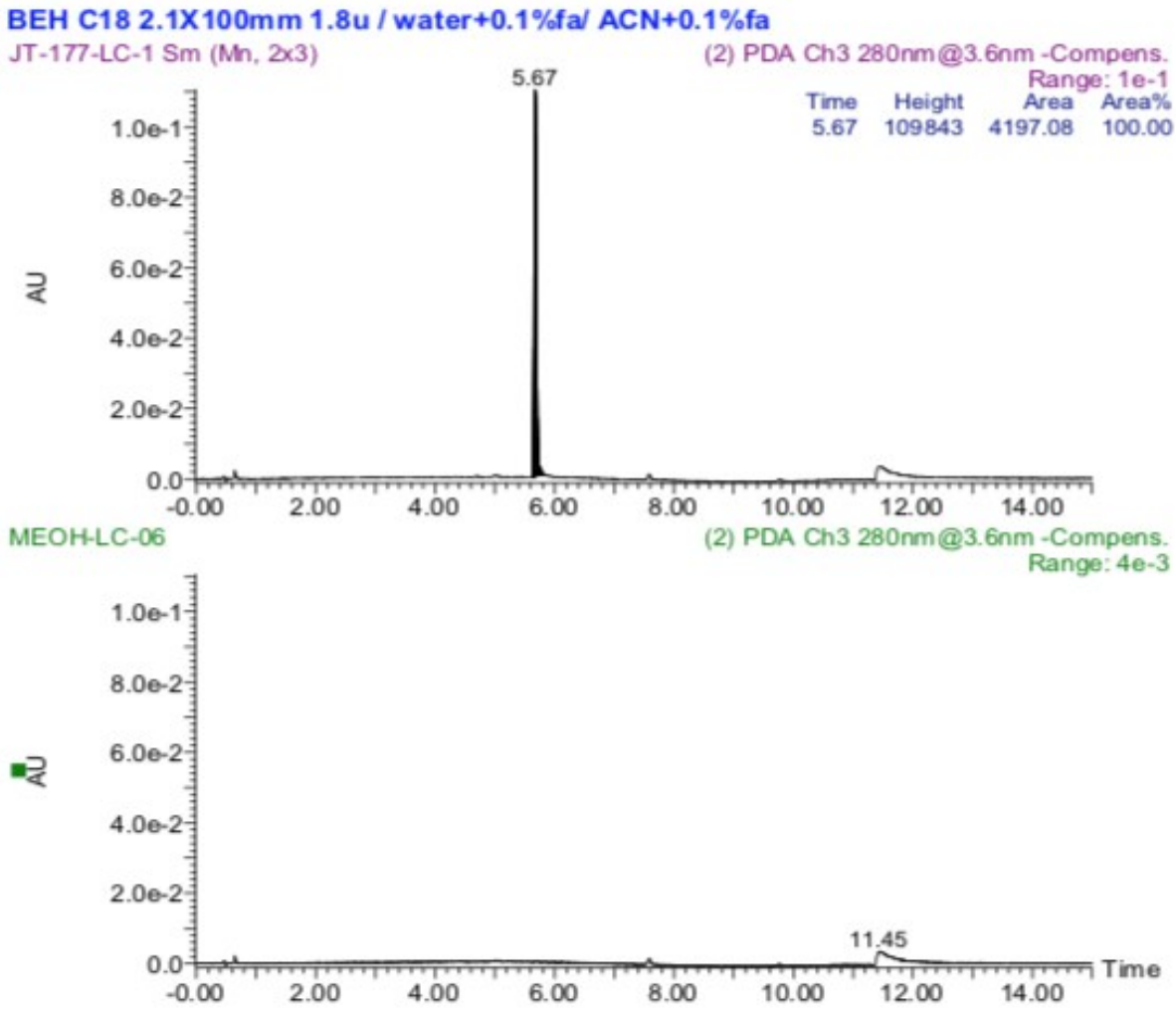
Peak	RT	Height	Area	Area (%)
1	7.5	0.08	0.32	0.07
2	7.78	117.67	463.08	96.89
3	7.89	0.23	0.84	0.17
4	8.7	1.04	3.7	0.77
5	11.58	0.94	5.18	1.08
6	12.21	0.27	4.82	1.01

3-(4-bromobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (11).

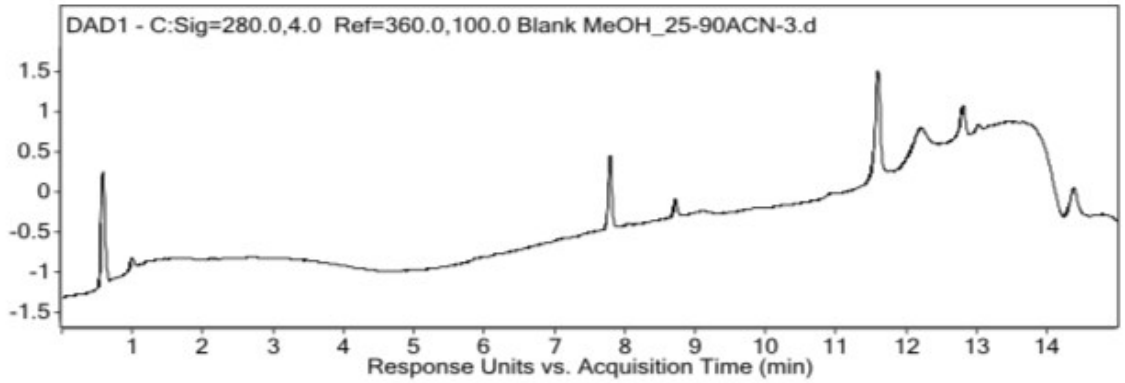


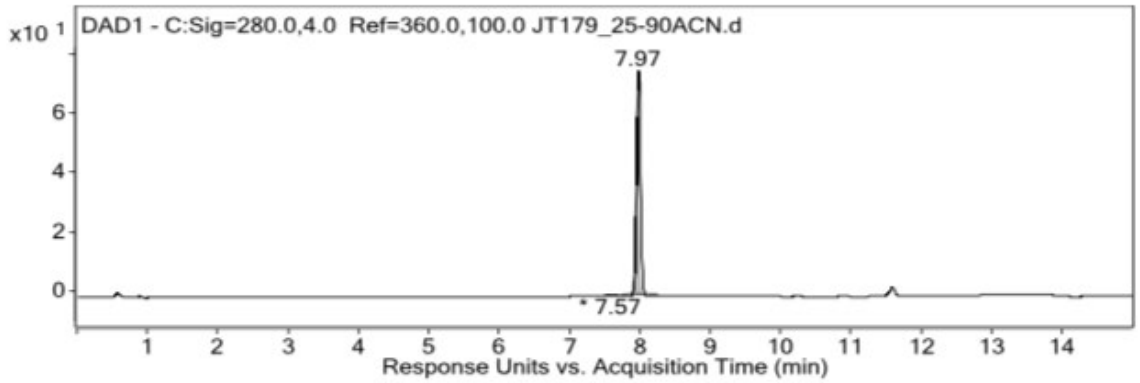
3-(4-iodobenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (12).

Chromatogram



1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (13).

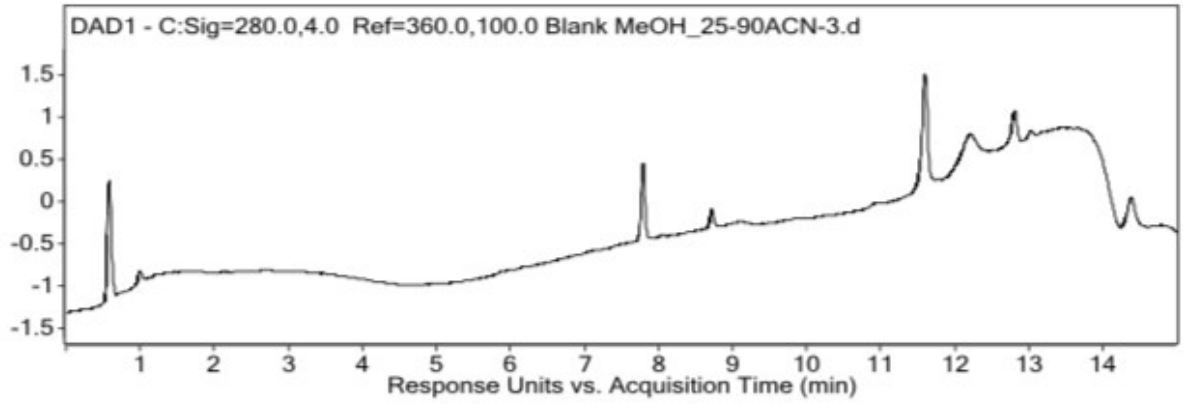


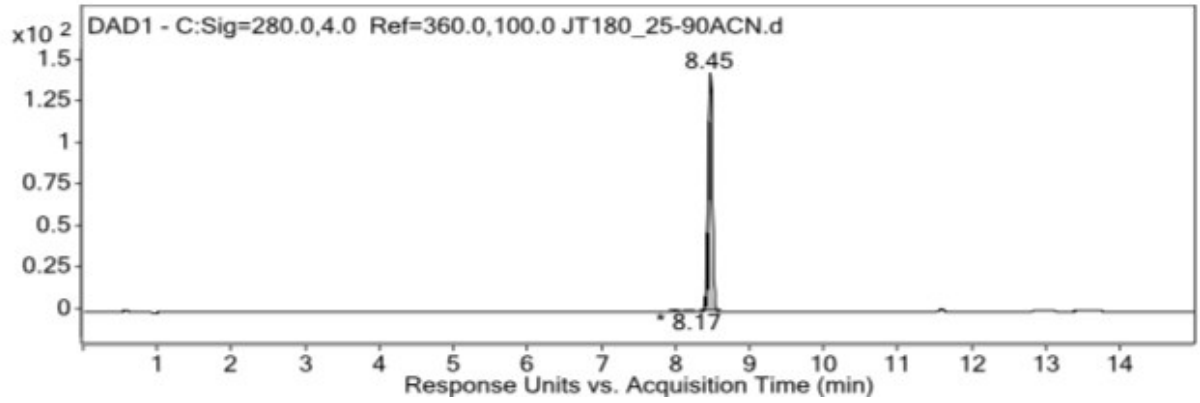


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	7.57	0.13	0.5	0.17
2	7.81	0.16	0.59	0.19
3	7.97	75.81	301.27	99.57
4	8.16	0.06	0.22	0.07

3-**(4-(*tert*-butyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (14).**

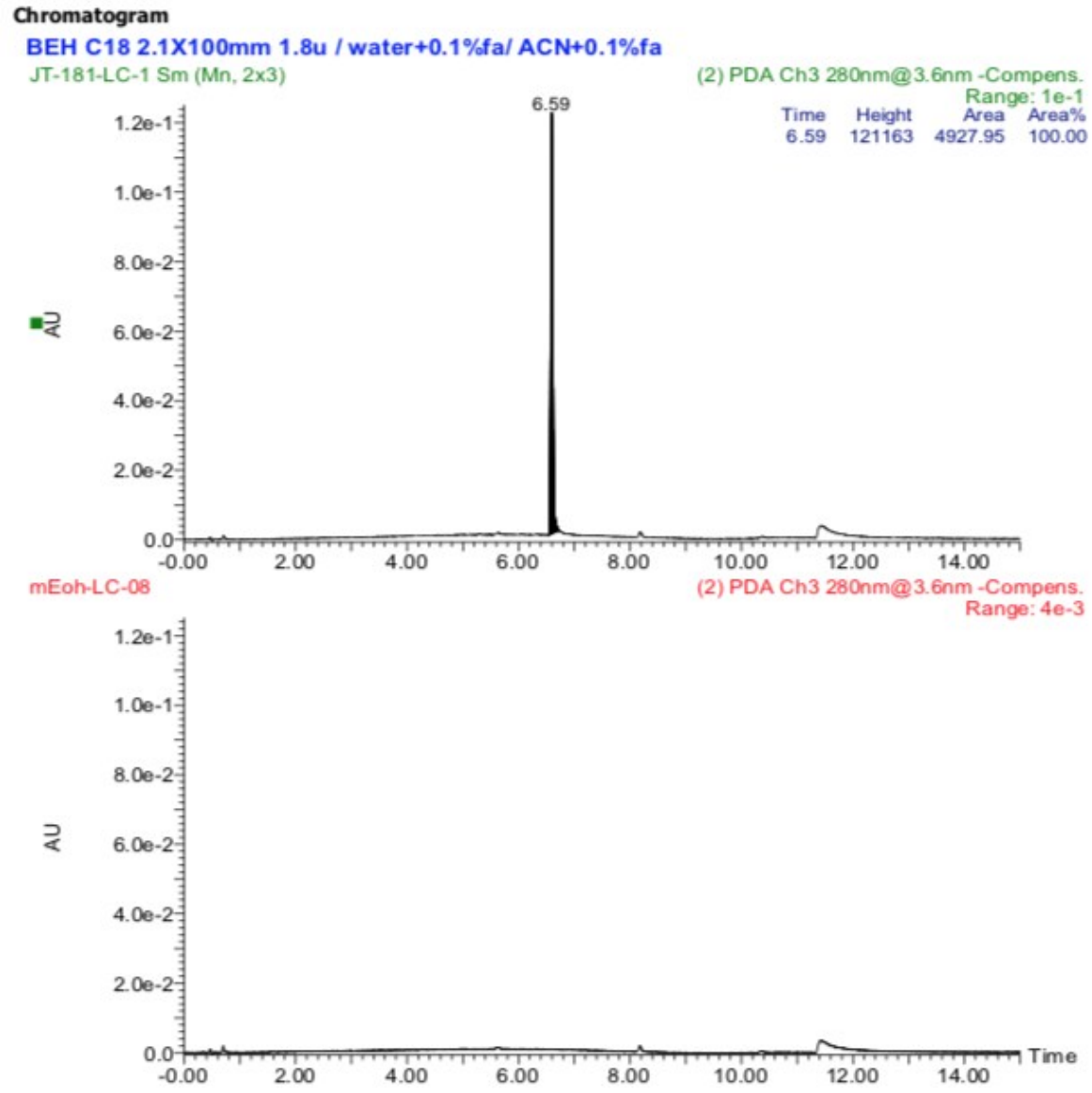




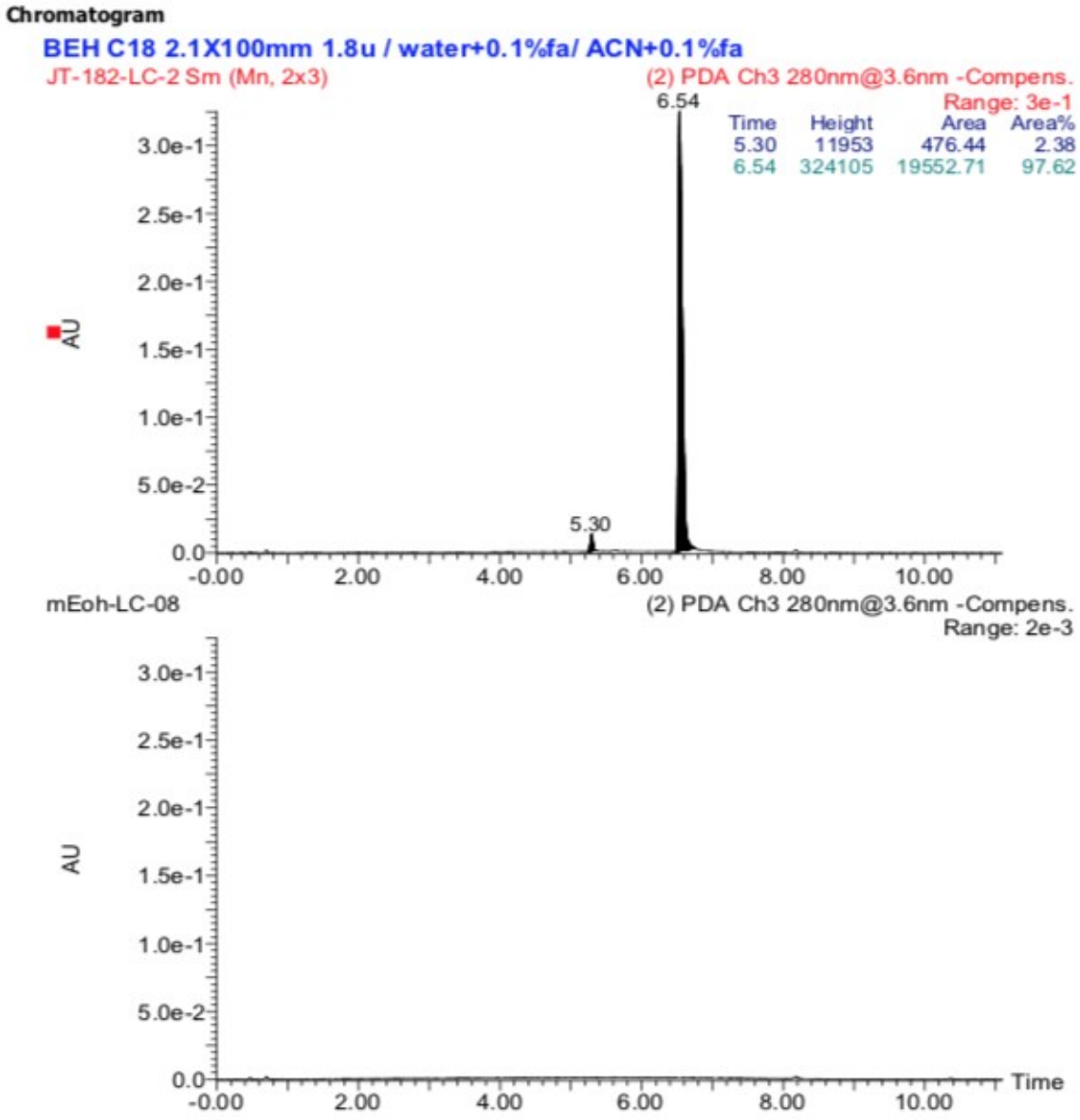
Integration Peak List

Peak	RT	Height	Area	Area (%)
1	7.97	0.64	2.25	0.41
2	8.17	0.09	0.4	0.07
3	8.45	143.22	544.89	99.52

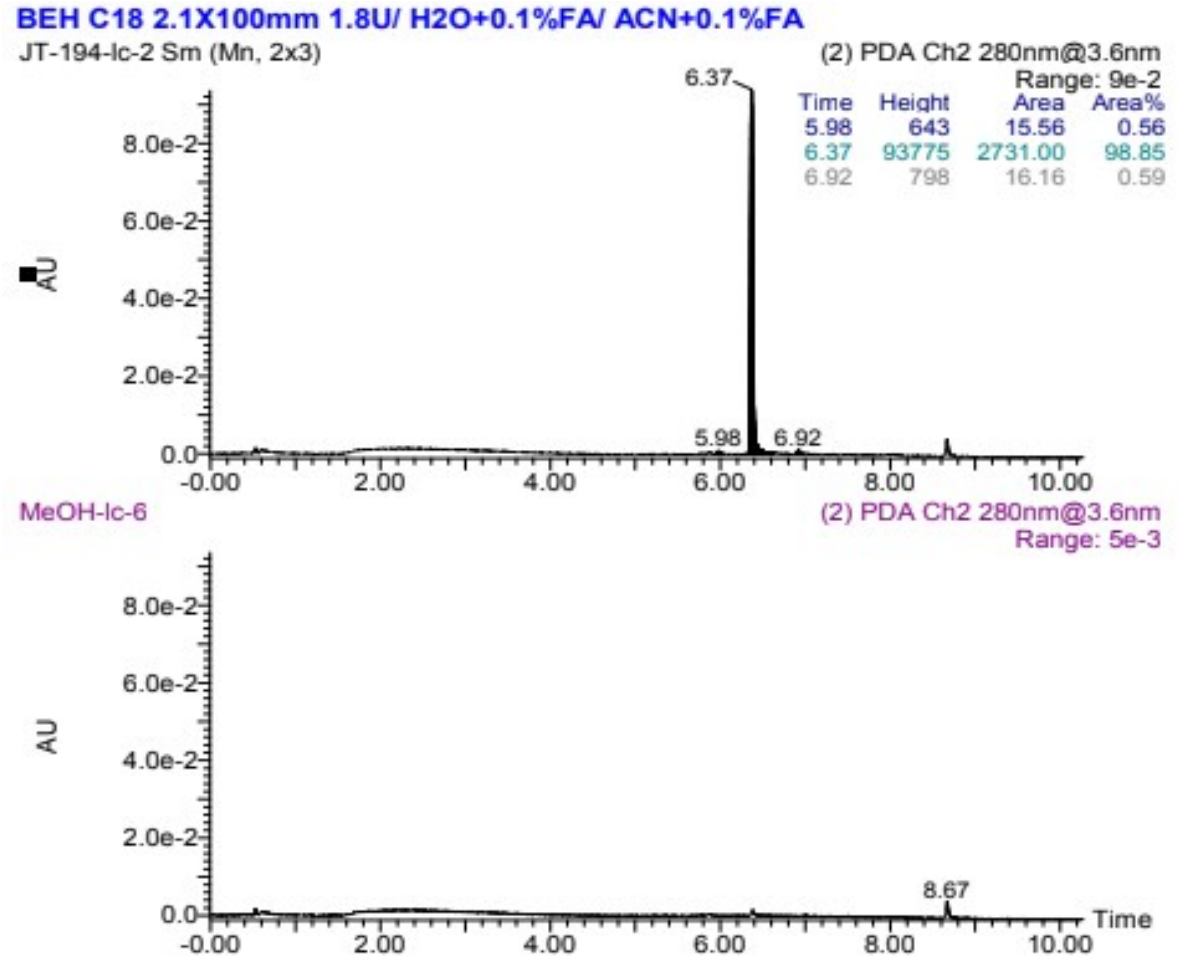
3-([1,1'-biphenyl]-4-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (15).



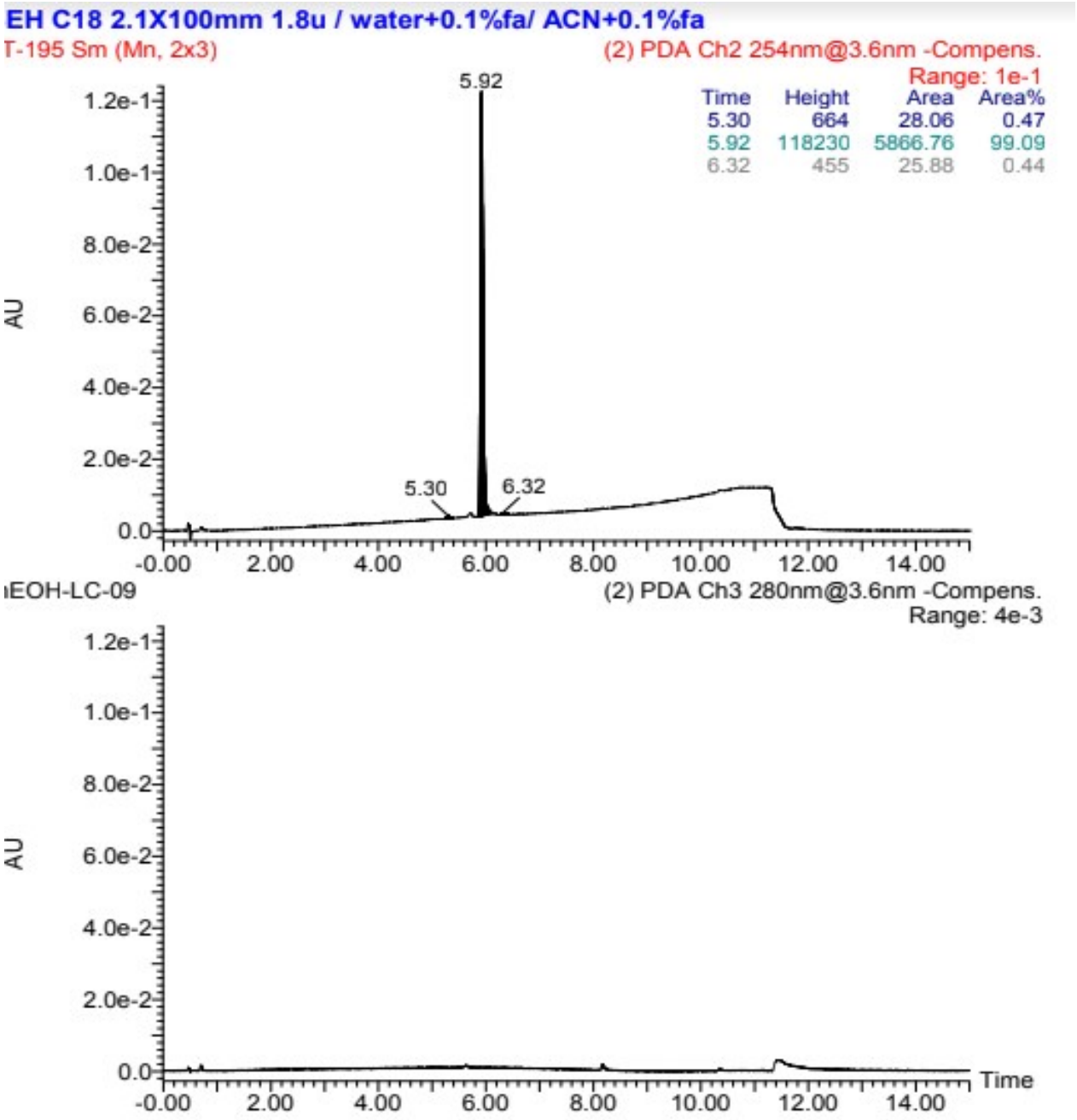
3-(3,5-bis(trifluoromethyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (16).



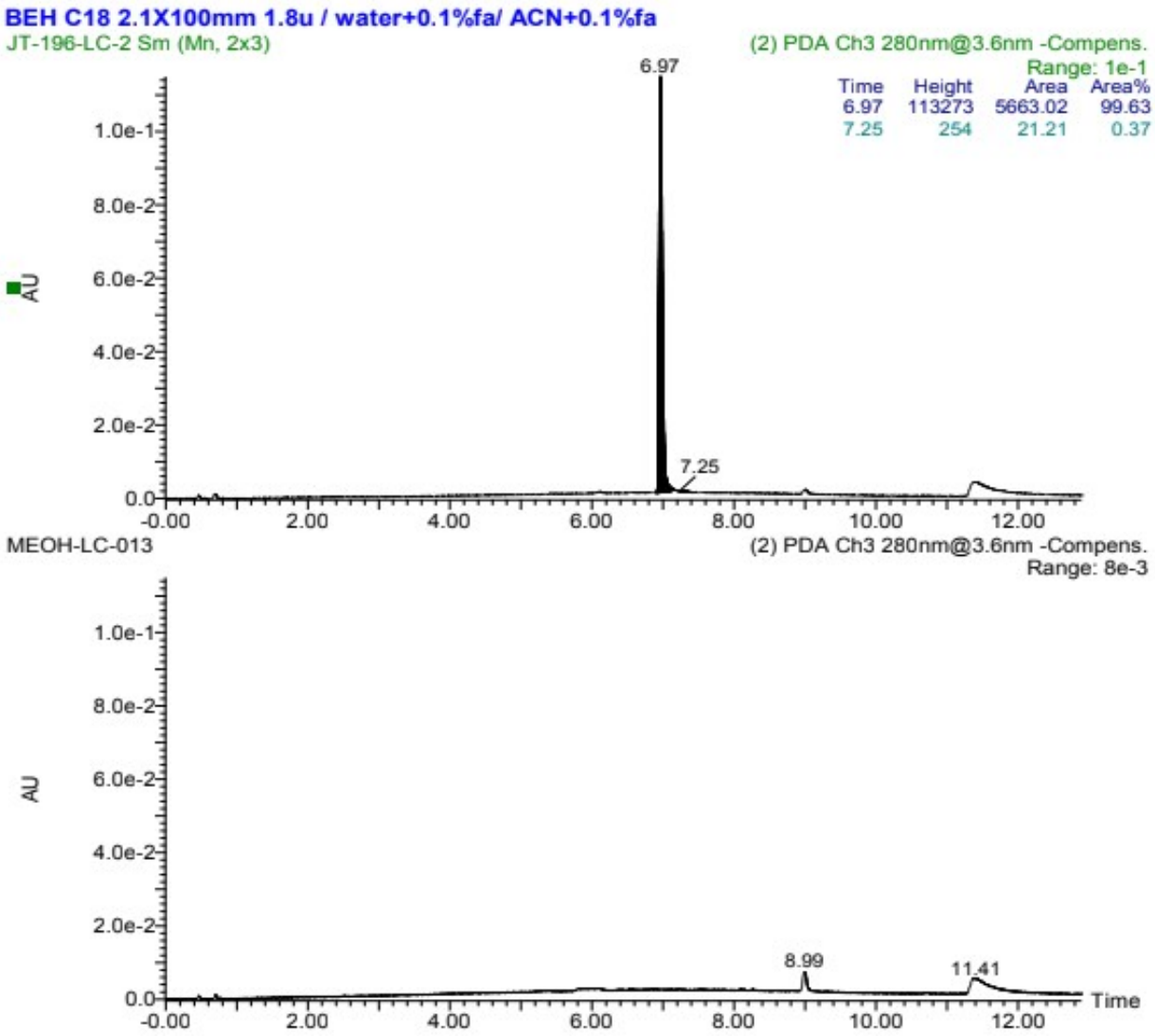
3-(benzo[*d*][1,3]dioxol-5-ylmethyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (17).



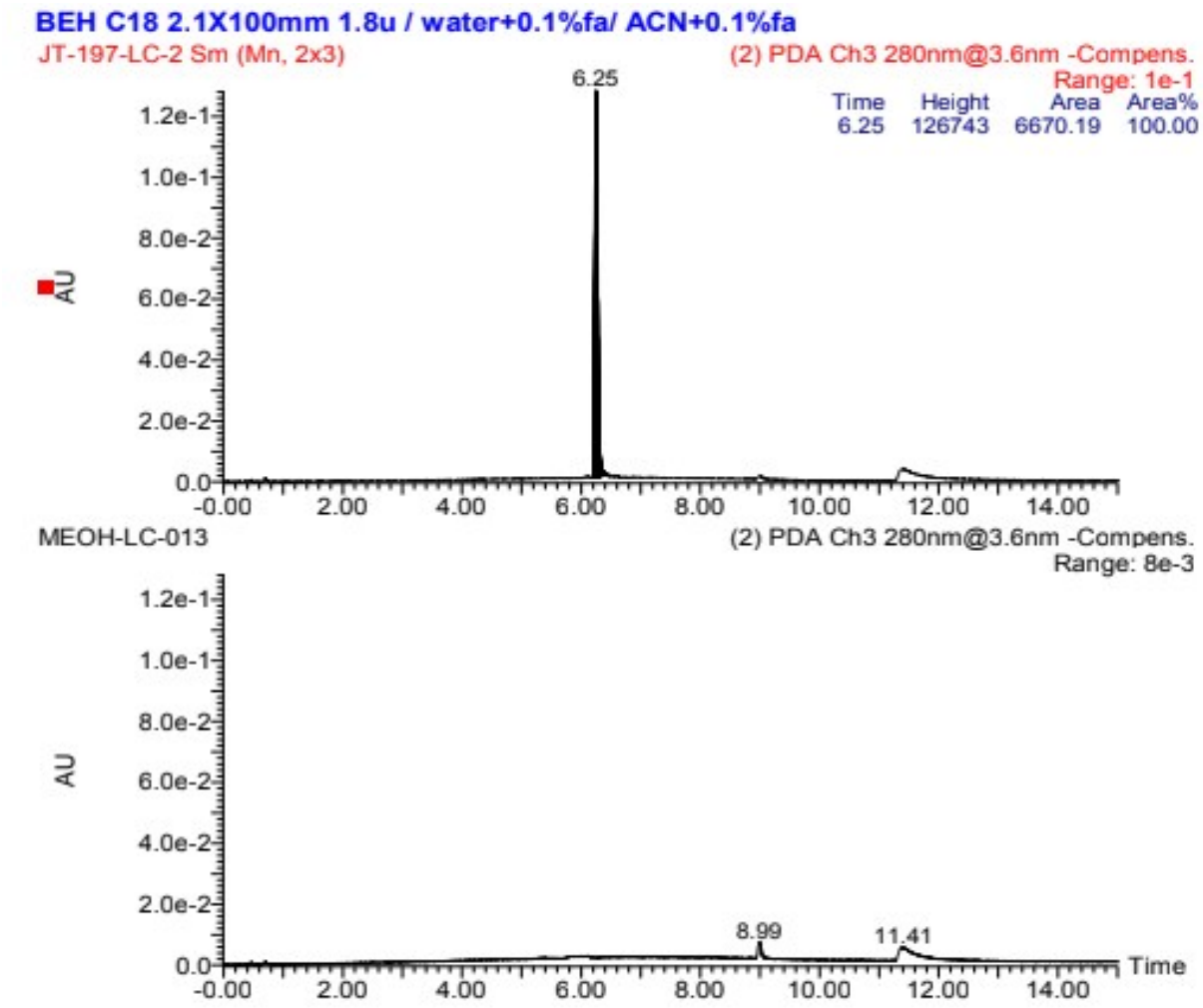
3-(3,5-dimethoxybenzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (18).



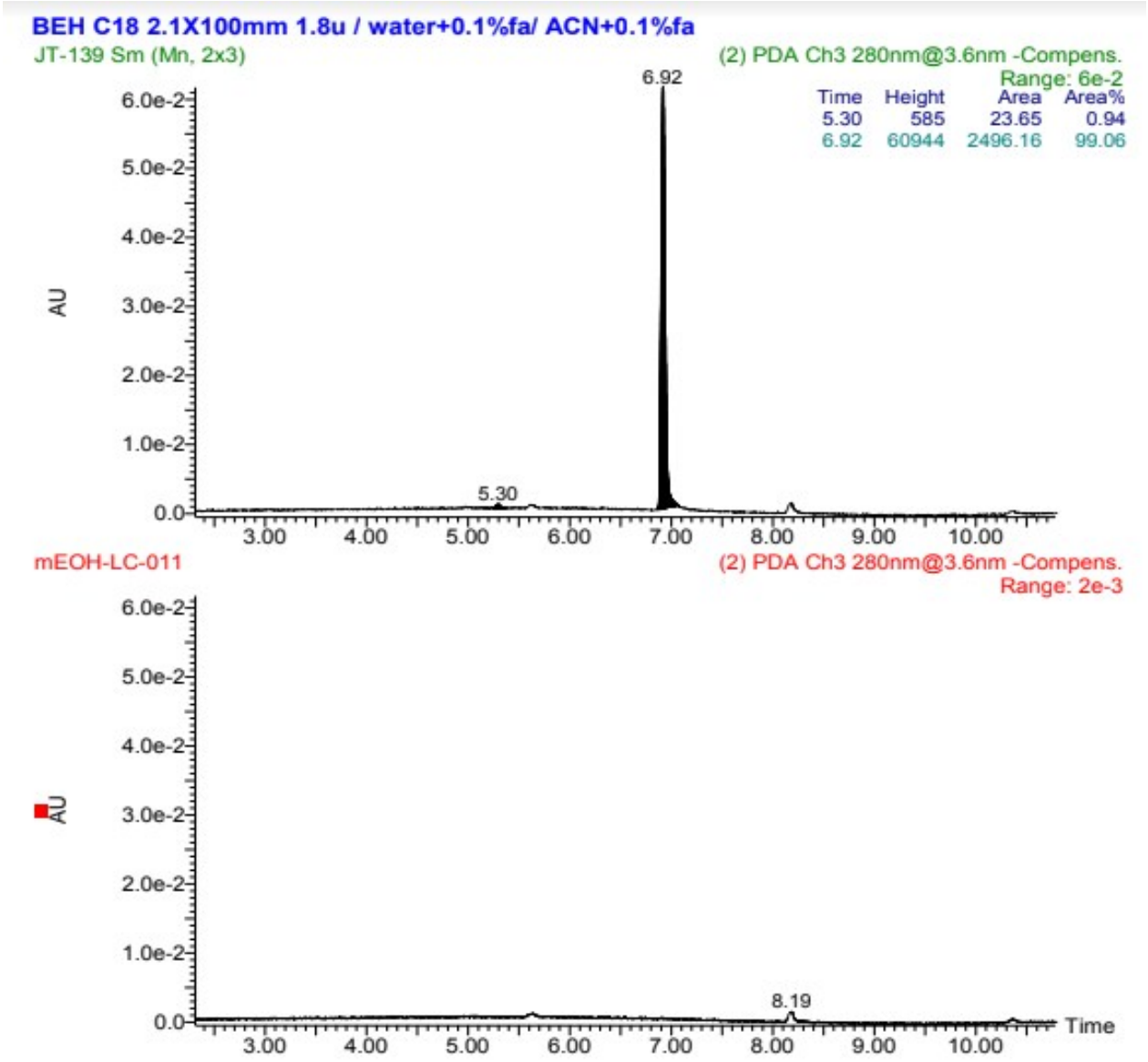
1-(4-(phenylethynyl)benzyl)-3-(4-(trifluoromethoxy)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (19).



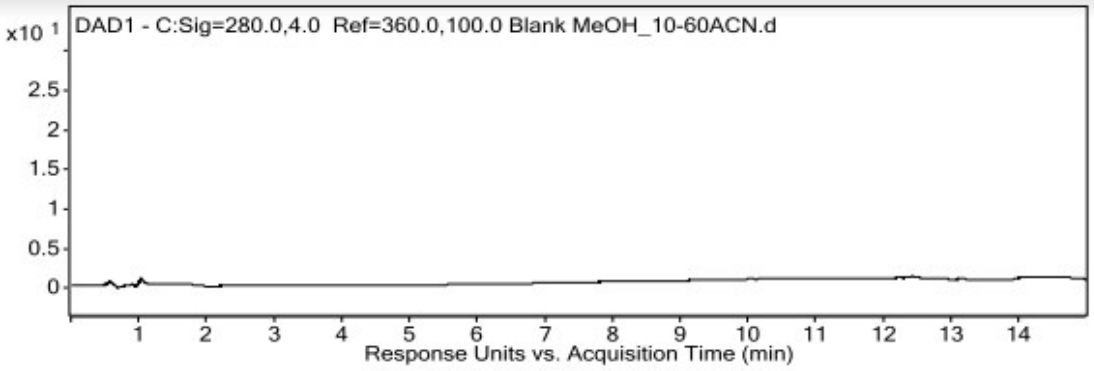
3-(4-(methoxycarbonyl)benzyl)-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (20).

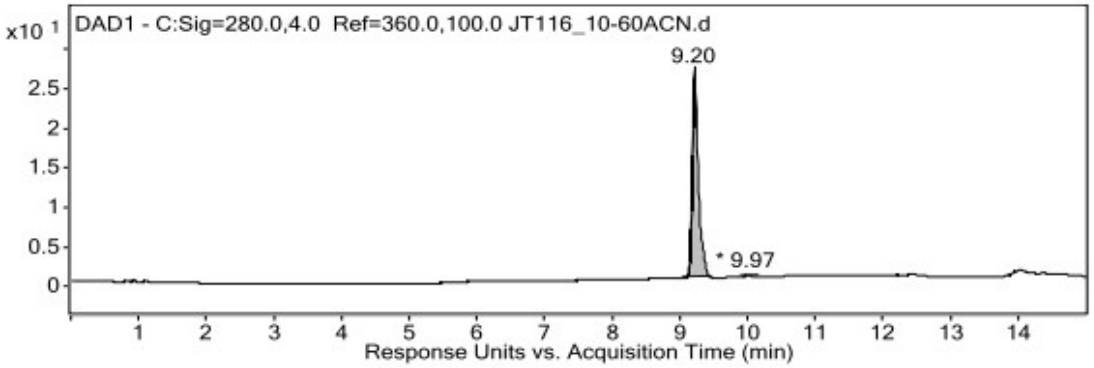


3-octyl-1-(4-(phenylethynyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (21).



1-methyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (33).

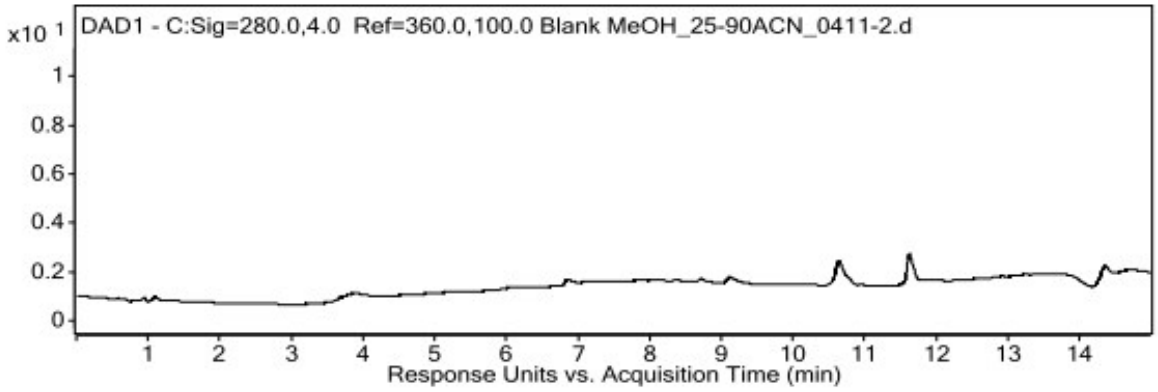


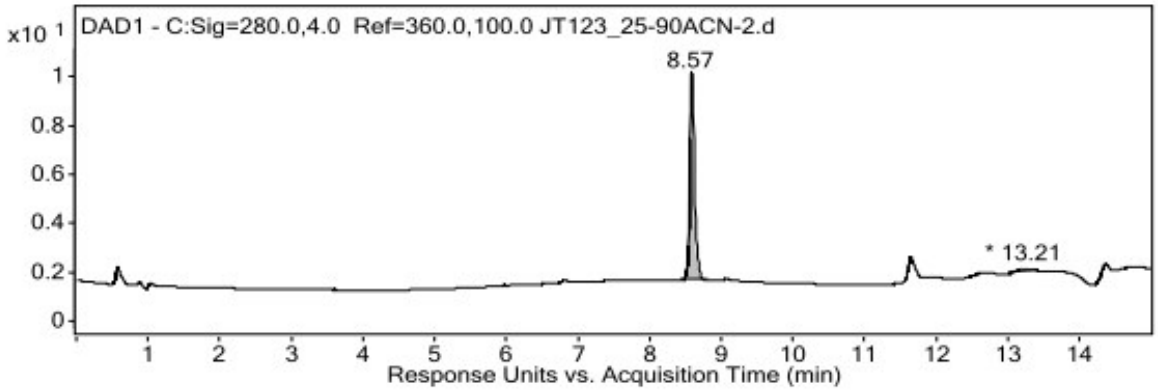


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	9.2	26.56	158.31	99.36
2	9.97	0.14	1.01	0.64

1,3-dioctyl-1*H*-benzo[*d*]imidazol-3-ium bromide (34).

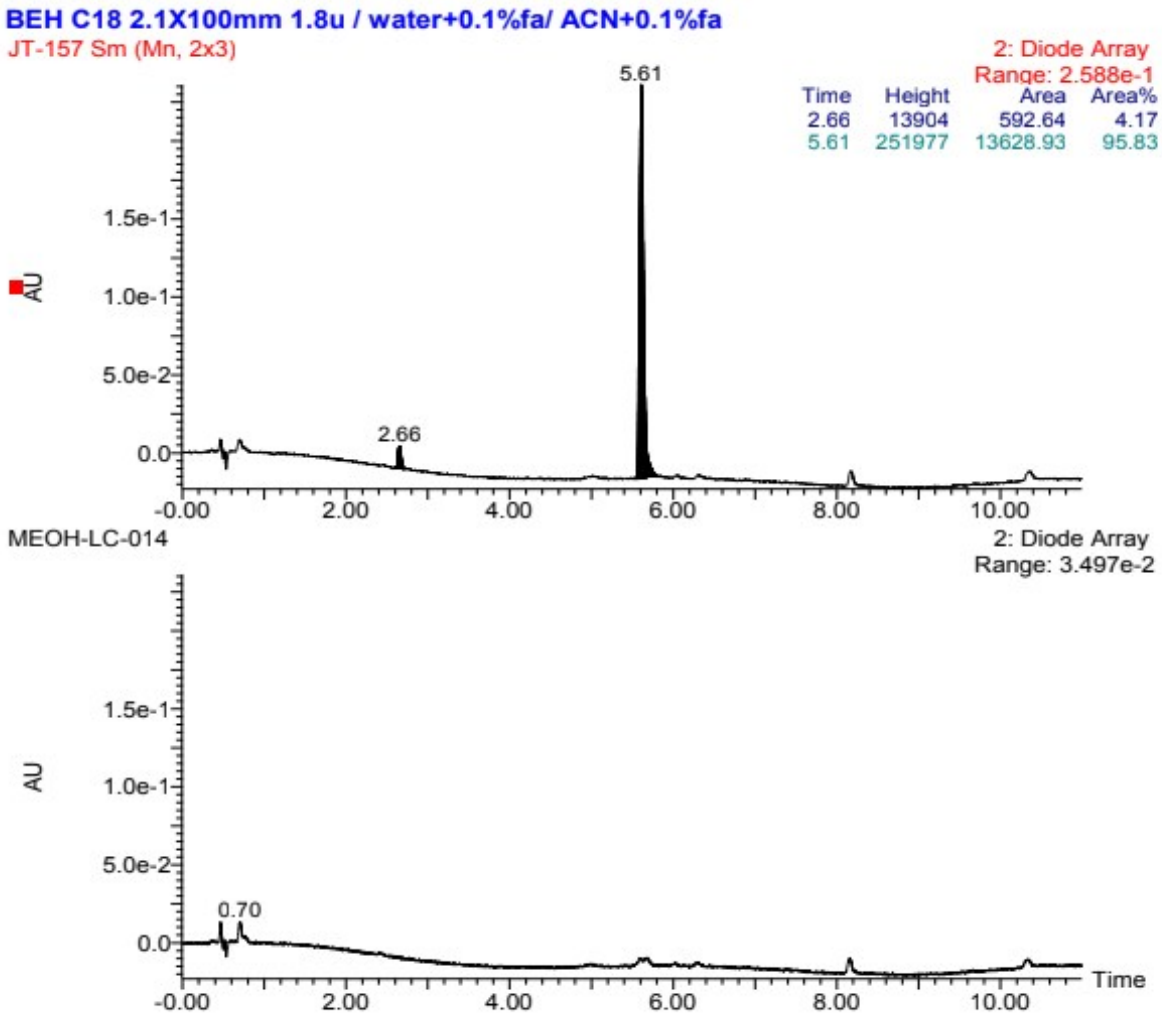




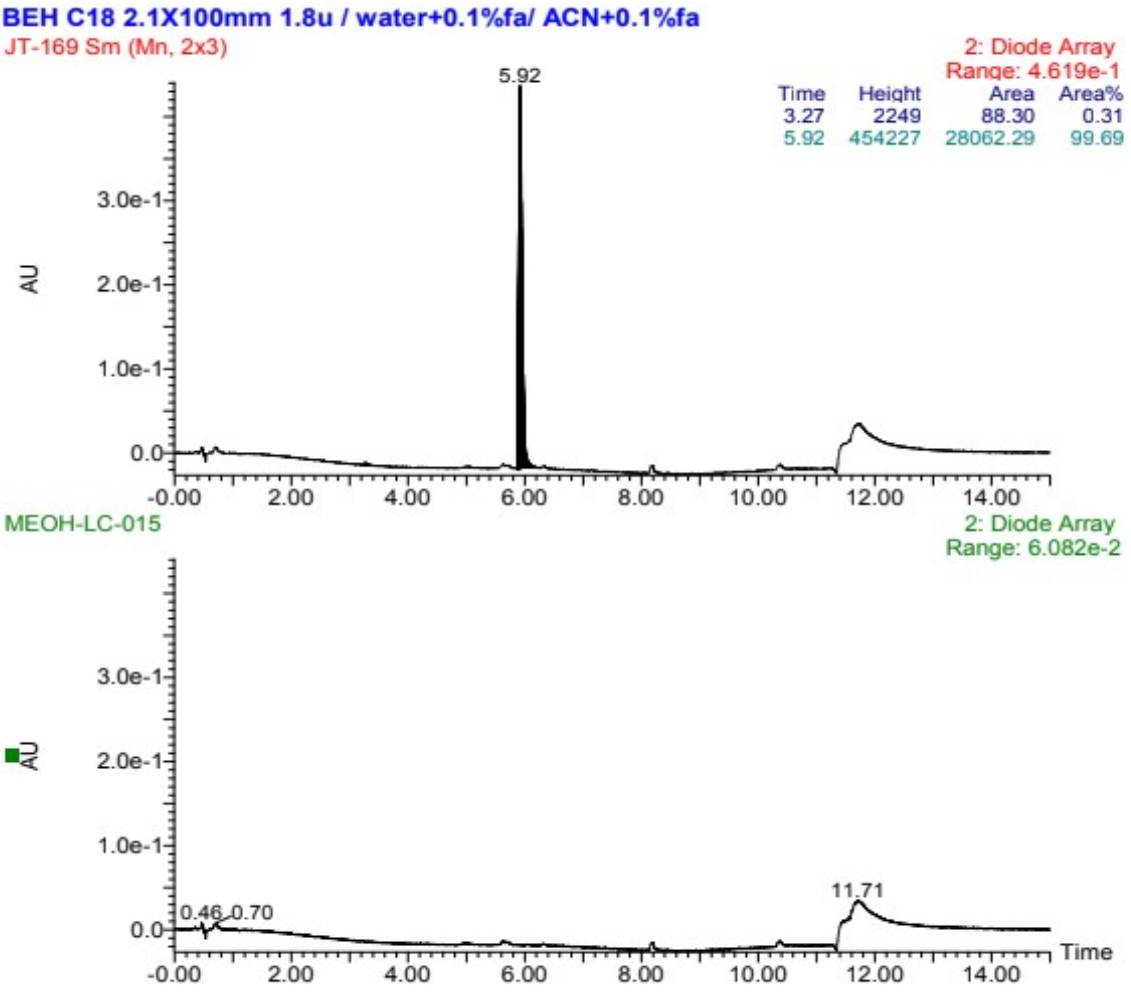
Integration Peak List

Peak	RT	Height	Area	Area (%)
1	8.57	8.47	38.42	95.46
2	13.21	0.13	1.83	4.54

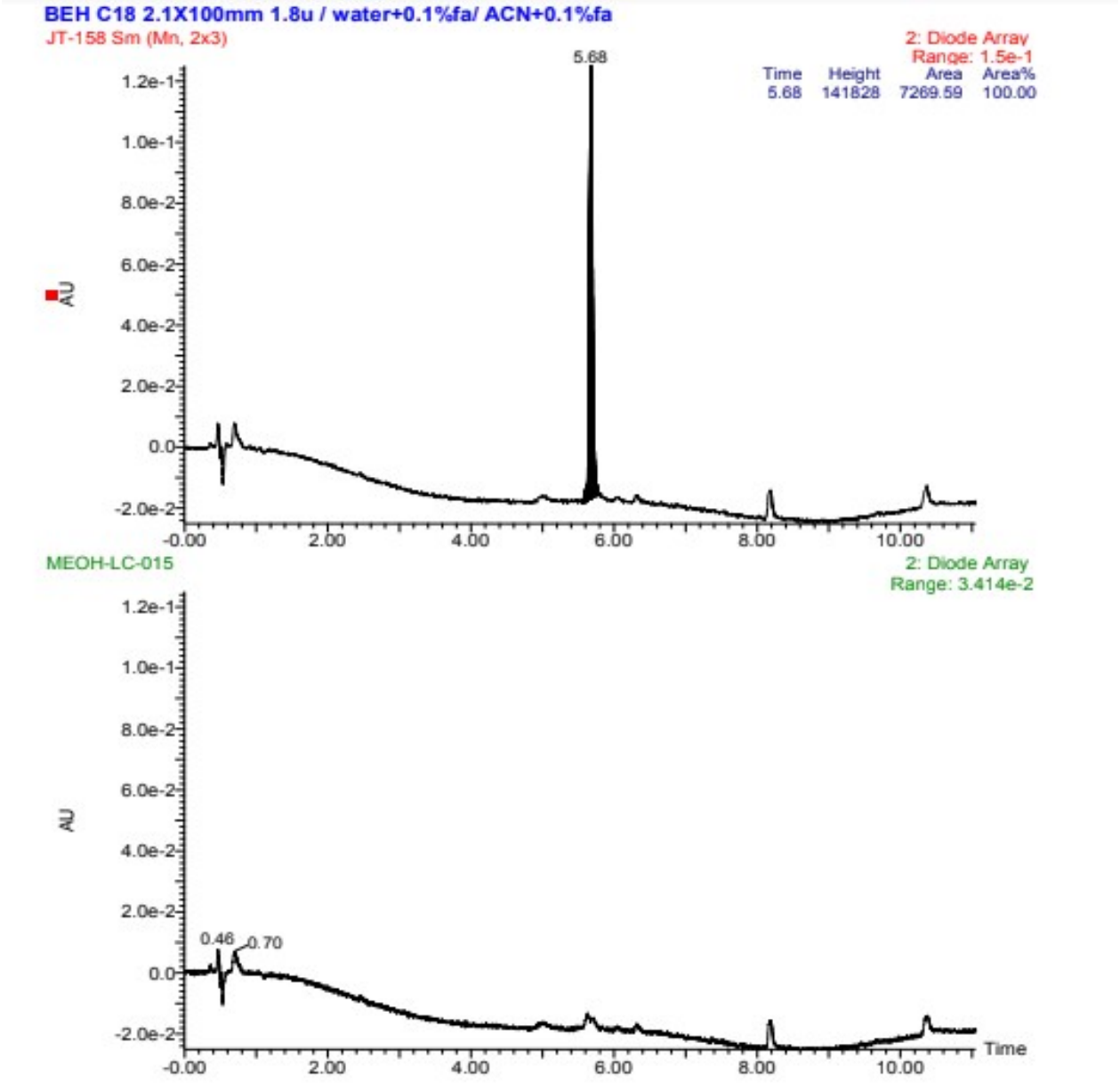
1-benzyl-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (35).



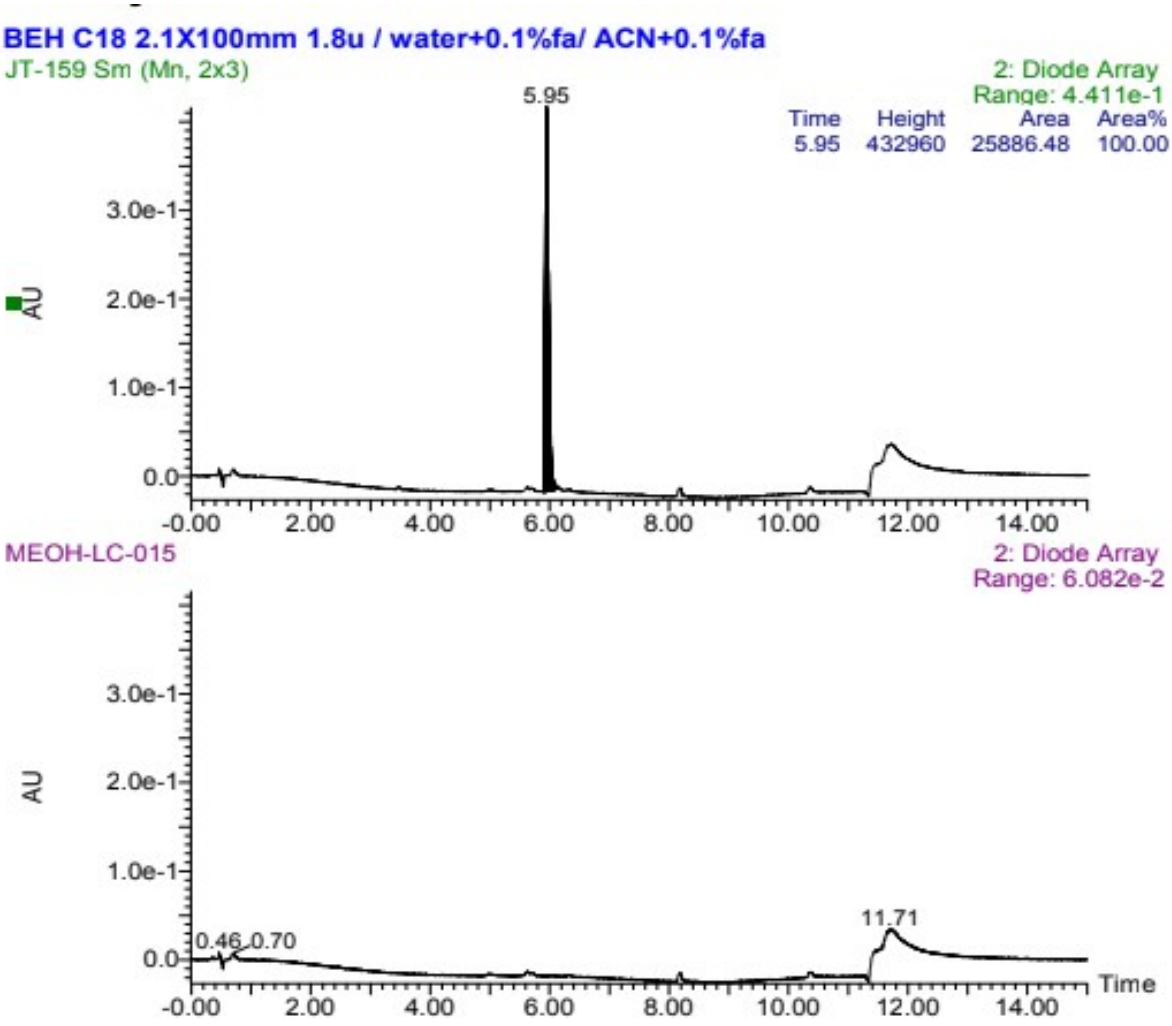
1-(4-methylbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (36).



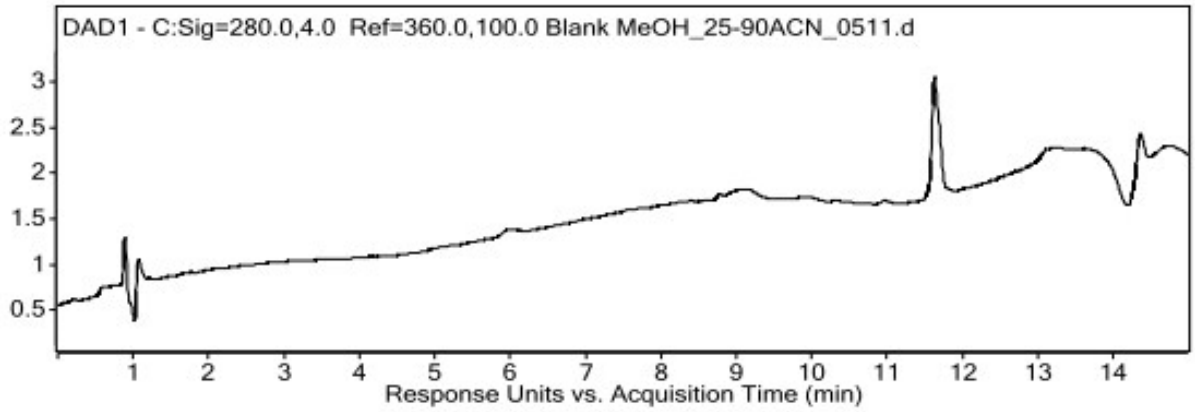
1-(4-fluorolbenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (37).

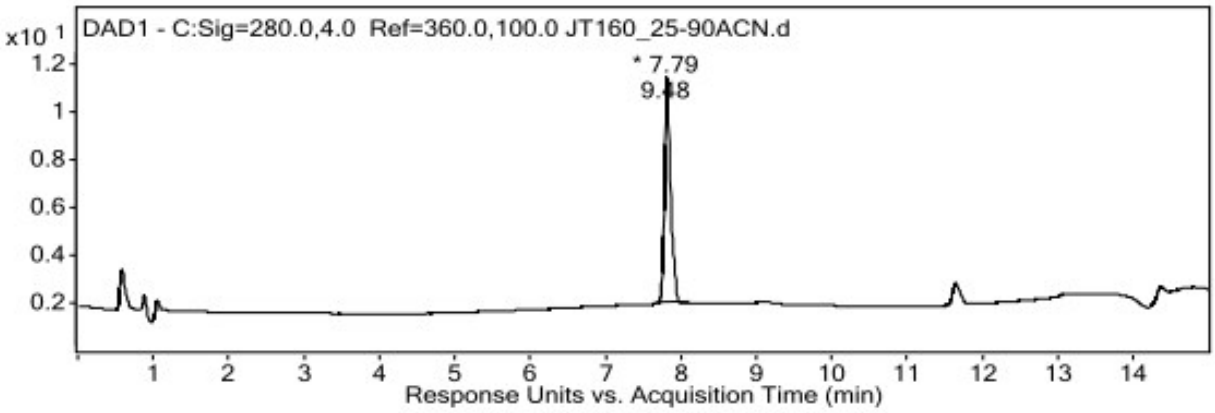


1-(4-chlorobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (38).



1-(4-bromobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (39).

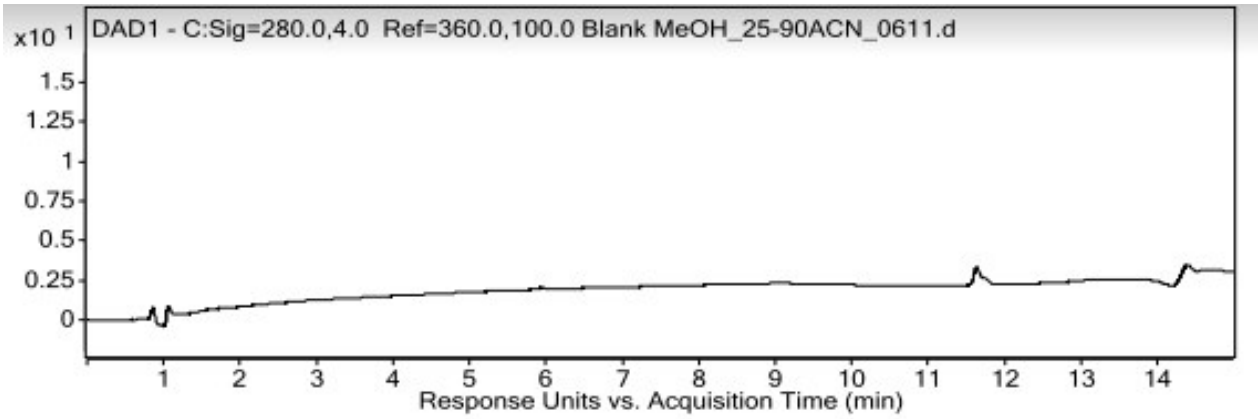


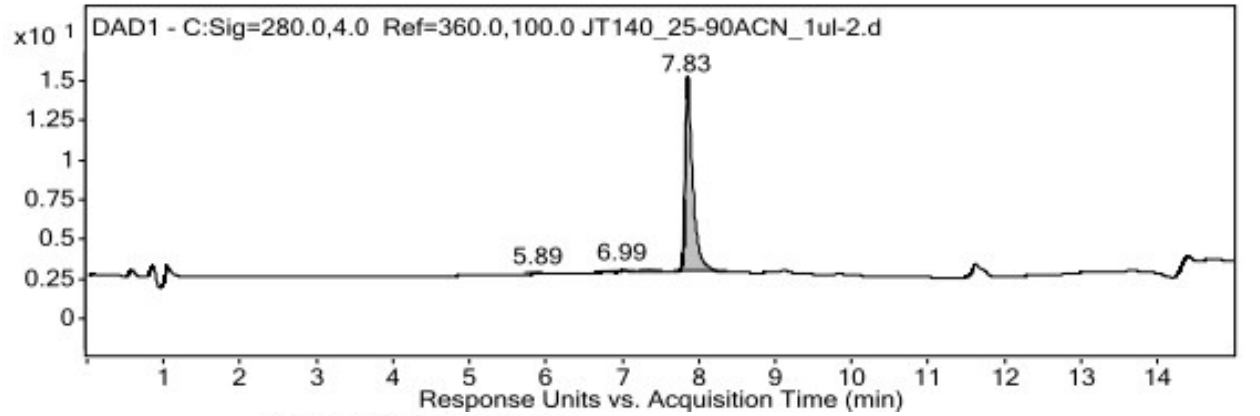


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	7.79	9.48	53.16	100

1-(4-iodobenzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (40).

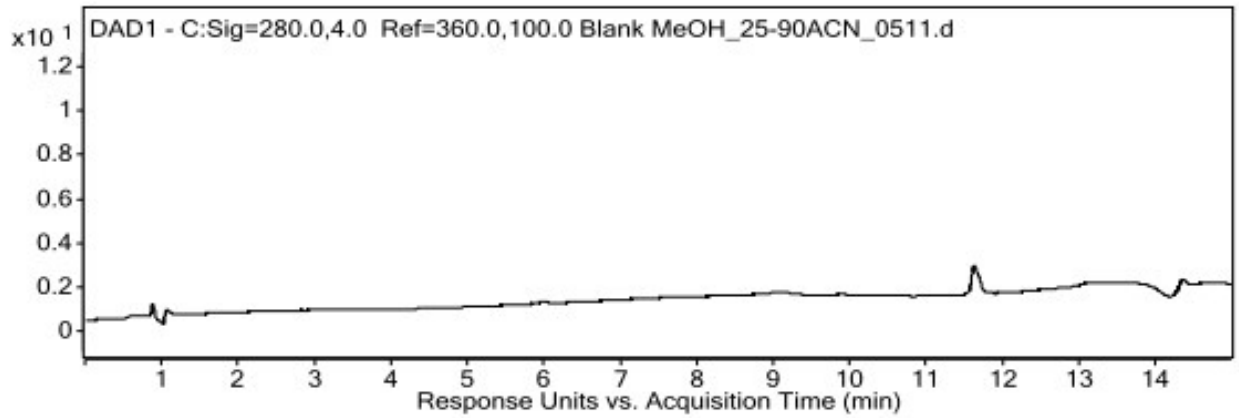


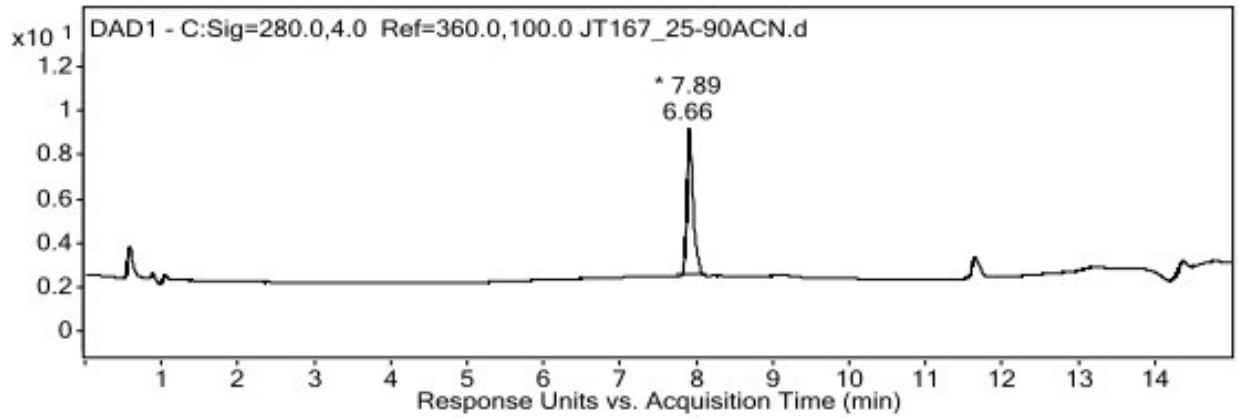


Integration Peak List

Peak	RT	Height	Area	Area (%)
1	5.89	0.02	0.18	0.21
2	6.75	0.08	0.52	0.61
3	6.99	0.18	0.98	1.15
4	7.27	0.04	0.14	0.17
5	7.36	0.03	0.11	0.13
6	7.83	12.22	83.36	97.73

3-octyl-1-(4-(trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazol-3-ium bromide (41).

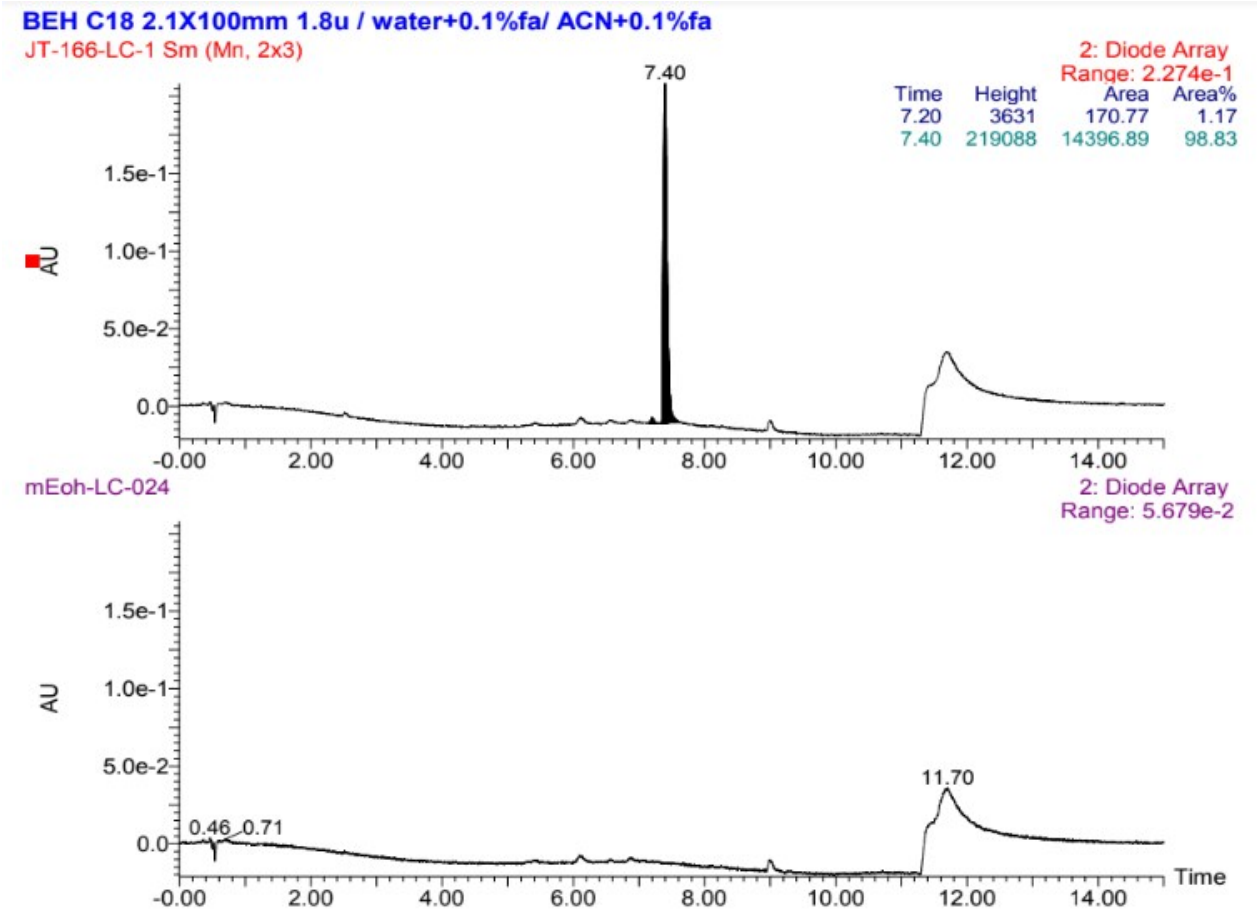




Integration Peak List

Peak	RT	Height	Area	Area (%)
1	7.89	6.66	37.39	100

1-(4-(*tert*-butyl)benzyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (42).



1-([1,1'-biphenyl]-4-ylmethyl)-3-octyl-1*H*-benzo[*d*]imidazol-3-ium bromide (43).