Electronic Supplementary Information

A near-infrared fluorescent long-chain fatty acid toward optical imaging of cardiac metabolism in living mice

Mahadeva M. M. Swamy,[a.b] Mohamad Zarif Mohd Zubir,[b] Mutmainah,[b] Setsuko Tsuboi,[a] Yuta Murai,[a,b] Kenji Monde,[a,b] Ken-ichi Hirano[c] and Takashi Jin[a*]

[a] *Center for Biosystems Dynamics Research, RIKEN, Furuedai 6-2-3, Suita, Osaka 565-0874, Japan.*

[b] *Graduate School of Life Science, Hokkaido University, Kita 21 Nishi 11, Sapporo, Hokkaido 001-0021, Japan.*

[c] *Laboratory of Cardiovascular Disease, Novel, Non-Invasive, and Nutritional Therapeutics (CNT), Graduate School of Medicine, Osaka University, 6-2-4, Furuedai, Suita, Osaka 565-0874, Japan.*

Table of Contents

1. Synthesis of BMIPP

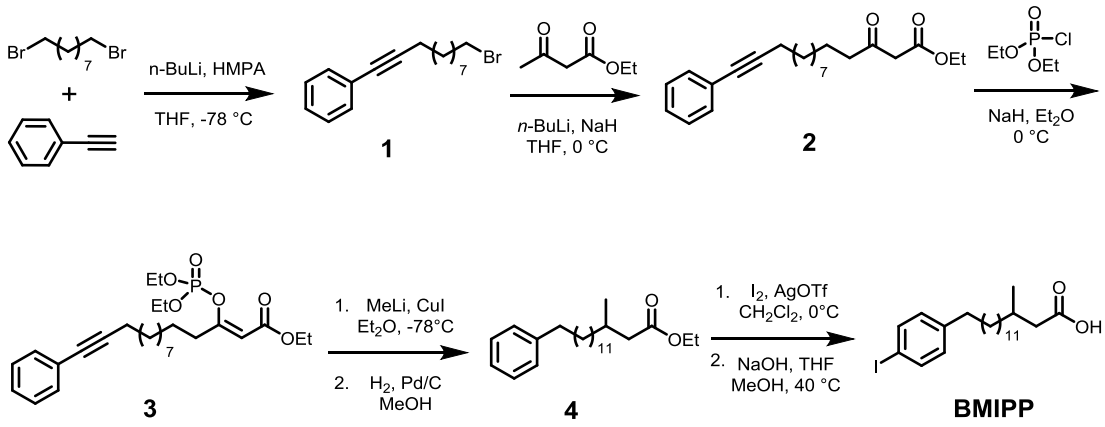


Fig. S1 Synthetic scheme for the preparation of BMIPP.

(11-bromoundec-1-yn-1-yl) benzene (1)[1]

A solution of n-butyllithium (8.3 mL, 2.6 M in n-hexane, 21.69 mmol) was added dropwise to a stirred solution of ethynylbenzene (2.2 mL, 19.72 mmol) in dry THF (100 mL) under N_2 atmosphere at −78°C and stirred 30 min. Hexamethylphosphoramide (4.0 mL, 23.07 mmol) was then added into the mixture and was stirred for another 30 min. Then 1,9-dibromononane (4.0 mL, 19.72 mmol) was added and stirred at room temperature (rt) for 2 hours. Water was added into the reaction mixture, extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (n - hexane) to give (11-bromoundec-1-yn-1-yl) benzene, **1** (3.57 g, 59%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.37 - 7.40 (2H, m), 7.25 - 7.30 (3H, m), 3.40 (2H, t, J=6.8 Hz), 2.42 (2H, t, J=7.1 Hz), 1.82 - 1.88 (2H, m), 1.56 - 1.63 (2H, m), 1.40 - 1.49 (4H, m) 1.30 - 1.36 (6H, m).

Ethyl 3-oxo-15-phenylpentadec-14-ynoate (2)

Sodium hydride (0.90 g, 60% in mineral oil, 22.42 mmol) was suspended in dry THF (70 mL) at 0°C under N_2 atmosphere. Ethyl acetoacetate (2.65 g, 20.39 mmol) was added dropwise over

15 min and continued stirring for an additional 15 min. A solution of n-butyllithium (8.2 mL, 2.6 M in n-hexane, 21.40 mmol) was added dropwise to the stirred solution and the mixture was stirred for 15 min before compound **1** (3.12 g, 10.19 mmol) was added. The stirring was continued for one hour at 0°C and brought up to room temperature for 1 hour. Upon completion of the reaction, 2 M HCl was added and extracted with diethyl ether, washed with water, saturated $NaHCO_3$ solution, brine, dried over MgSO4 and evaporated to dryness. The crude product was purified using column chromatography (n-hexane) to give ethyl 3-oxo-15-phenylpentadec-14-ynoate, **2** (3.21 g, 88%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.37 - 7.41 (2H, m), 7.24 - 7.30 (3H, m), 4.17 - 4.22 (2H, m), 3.42 (2H, s), 2.52 (2H, t, J=7.45 Hz), 2.39 (2H, t, J=7.08 Hz), 1.56 - 1.63 (4H, m), 1.40 - 1.47 (2H, m), 1.25 - 1.34 (14H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm, 204.48, 167.24, 131.49, 128.13, 127.40, 124.06, 90.41, 80.52, 61.29, 49.28, 43.01, 33.99, 32.78, 29.41 - 28.71 (5C), 23.42, 19.37, 14.08. HRMS (m/z): $[M + H]^+$ calculated for $C_{23}H_{33}O_3$: 357.2424; found 357.2396.

Ethyl -3-((diethoxyphosphoryl)oxy)-15-phenylpentadec-2-en-14-ynoate (3)

A solution of **2** (2.60 g, 7.30 mmol) in dry diethyl ether (5 mL) was added to a suspension of sodium hydride (0.34 g, 60% in mineral oil, 8.39 mmol) in dry diethyl ether (50 mL) at 0°C under nitrogen atmosphere and continued stirring for 20 min at rt. Then the mixture was cooled to 0°C and diethyl phosphorochloridate (1.6 ml, 10.95 mmol) was added and stirring was continued for two hours at rt. Then the reaction mixture was quenched with NH_4Cl solution. The ether layer was extracted and washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified using column chromatography (ethyl acetate/n-hexane = 1:3) to yield ethyl -3-((diethoxyphosphoryl)oxy)-15-phenylpentadec-2-en-14-ynoate, **3** (2.95 g, 82%) as yellow oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.37 -7.40 (2H, m), 7.24 - 7.30 (3H, m), 5.34 (1H, s), 4.22 - 4.29 (4H, m), 4.15 (2H, q, J=7.3 Hz), 2.38 - 2.44 (4H, m), 1.53 - 1.63 (4H, m), 1.40 - 1.48 (2H, m), 1.25 - 1.38 (19H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm, 206.22, 163.70, 131.41, 128.05, 127.33, 123.99, 90.31, 80.46, 64.66, 64.60, 59.74, 35.05, 33.87, 32.70, 29.39 - 28.59 (5C), 26.27, 19,30, 16.01, 15.95, 14.15. HRMS (m/z): $[M + H]^+$ calculated for $C_{27}H_{41}O_6P$: 493.2713; found 493.2719.

Ethyl 3-methyl-15-phenylpentadecanoate (4)

A solution of methyllithium (30.0 mL, 1.1 M in diethyl ether, 33.00 mmol) was added dropwise to a suspension of cuprous iodide (2.10 g, 11.00 mmol) in dry diethyl ether (40 mL) at 0°C under nitrogen atmosphere. Then the reaction was stirred for 30 min and cooled to −78°C followed by a dropwise addition of **3** (2.79 g, 5.50 mmol) in dry diethyl ether (10 mL) for 20 min. The reaction mixture was left to stirred below −46°C for 4 h. The mixture was poured into the ice-cold mixture (4:1) of saturated NH_4Cl solution and concentrated NH_4OH solution and stirred for 1 hour. The aqueous layer was separated and extracted with diethyl ether. The combined organic extract was washed with saturated 10% NH_4OH hydroxide solution, water, brine, dried over $MgSO_4$, filtered, concentrated by rotary evaporation and vacuum drying to give the crude product. Then it was purified using column chromatography (ethyl acetate/n-hexane = 1:20) to yield ethyl-3-methyl-15-phenylpentadec-2-en-14-ynoate **4a** (1.65 g, 87%) as clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.37 - 7.41 (2H, m), 7.24 - 7.30 (3H, m), 5.65 (1H, d, J=1.0 Hz) 4.14 (2H, q, J=6.8 Hz), 2.40 (2H, t, J=7.1 Hz), 2.15 (3H, d, J=1.0 Hz), 2.12 (2H, t, J=7.6 Hz), 1.56 - 1.64 (2H, m), 1.40-1.49 (4H, m) 1.25 - 1.35 (13H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm, 166.79, 160.22, 131.43, 128.07, 127.34, 124.03, 90.32, 80.50, 59.32, 40.87, 33.87, 32.75, 29.42 – 28.68 (5C), 28.08, 27.31, 19.33, 18.62, 14.27. HRMS (m/z): [M +

H]$^+$ calculated for $C_{24}H_{34}O_2$: 355.2631; found 355.2602.

Subsequently, **4a** (1.40 g, 3.95 mmol) was added into a flask with methanol (20 mL) and a catalytic amount of Pd/C catalyst fixed with hydrogen. It was left to stir at room temperature for 24 hours. Upon completion the mixture was filtered, the solvent was removed and dried completely to yield pure ethyl 3-methyl-15-phenylpentadecanoate, **4** (1.39 g, 97%) as a clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.24 - 7.28 (2H, m), 7.15 - 7.19 (3H, m), 4.12 (2H, q, J=7.2 Hz), 2.57 - 2.62 (2H, m), 2.26 - 2.31 (1H, m), 2.06 - 2.12 (1H, m), 1.90 - 1.98 (1H, m), 1.57 - 1.65 (2H, m), 1.22 - 1.36 (23H, m), 0.93 (3H, d, J=6.4 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm, 204.49, 142.94, 128.37, 128.18, 125.51, 60.04, 41.95, 36.73, 35.98, 31.52, 30.37, 29.77 - 29.49 (7C), 29.34, 26.90, 19.72, 14.28. HRMS (m/z): [M + H]$^+$ calculated for $C_{24}H_{40}O_2$: 361.3101; found 361.3072.

BMIPP (β-methyl iodophenyl-pentanedecanoic acid)[2]

Iodine (0.11 g, 0.42 mmol) was added to a mixture of **4** (0.15 g, 0.42 mmol) and silver trifluoromethanesulfonate (0.15 g, 0.58 mmol) in dry dichloromethane (10 ml) at 0°C. Then it was allowed to warm slowly to room temperature and was stirred for 1 hour. The solution was filtered, and the filtrates were washed with 5% Na_2SO_3 solution, water, brine, dried over $MgSO_4$, filtered, and evaporated to dryness to give the crude product which was used in the next step without any further isolation. The crude mixture was dissolved in THF (10 mL) and MeOH (10 mL) and 1 M NaOH (2.5 mL) was added to the reaction mixture and the reaction was stirred for 4 h at 40 °C. The solvent was reduced to remove the MeOH and extracted water and EtOAc mixture. The water phase was acidified by 2 M HCl and extracted with EtOAc. The organic phase was washed with brine and dry with $MgSO_4$ and evaporated to obtain BMIPP (14.0 mg, 88%) without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm, 7.57 (2H, d, J=8.3 Hz),

6.92 (2H, d, J=8.3 Hz), 2.51-2.56 (2H, m), 2.35 (1H, dd, J= 14.7, 5.9 Hz), 2.14 (1H, dd, J=14.7, 8.3), 1.19 - 2.00 (1H, m), 1.53 - 1.61 (2H, m), 1.16 - 1.43 (21H, m), 0.96 (3H, d, J=6.8 Hz).

2. Synthesis of Amino-BMPP

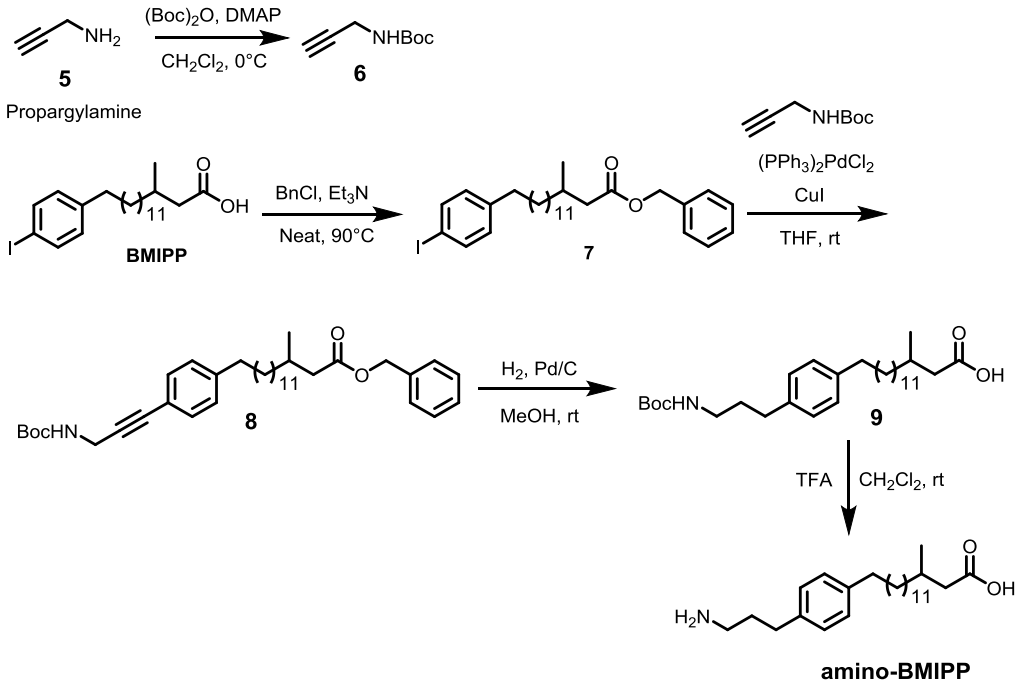


Fig. S2 Synthetic scheme for the preparation of Amino-BMPP.

Tert-butyl prop-2-yn-1-ylcarbamate (6)

Tert-butoxycarbonyl (Boc) anhydride (4 g, 18.2 mmol) in CH_2Cl_2 was added to a stirred solution of propargylamine (1 g, 18.2 mmol) and DMAP (44mg, 0.36 mmol) in CH_2Cl_2 (25 mL) at 0°C. The temperature was slowly increased to room temperature (rt) and stirred for an additional 2 h. After completion of the reaction, CH_2Cl_2 was evaporated *in vacuo*, and the residue was extracted using EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue obtained was purified by silica gel column chromatography using

hexane/EtOAc (8:2) to obtain the desired Boc-protected propargylamine (**6**) (2.4 g, yield 95%), in the form of a pale-yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.71 (1H, s), 3.92 (2H, s), 2.22 (1H, t, J=2.5 Hz), 1.45 (9H, s).

Benzyl 15-(4-iodophenyl)-3-methylpentadecanoate (7)

Benzyl chloride (275 mg, 2.2 mmol) was added to a stirred solution of **BMIPP** (200 mg, 0.44 mmol) in triethylamine (approx. 0.3 mL, 1:1 v/v) at 90°C. The reaction mixture was stirred at 90°C for 2 h. After completion of the reaction, the reaction mixture was cooled to rt and the residue was extracted using EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (9:1) to obtain the desired compound **7** (215 mg, yield 90%), in the form of a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.60 (2H, d, J=8.3 Hz), 7.36 (5H, m), 6.94 (2H, d, J=8.1 Hz), 5.12 (2H, s), 2.54 (2H, t, J=7.8 Hz), 2.36 (1H, dd, J=14.9, 6.1 Hz), 2.17 (1H, dd, J=14.6, 8.0 Hz), 1.56-1.59 (2H, m), 1.24-1.29 (20H, m), 0.93 (3H, d, J=6.6 Hz). ESI-MS (m/z): $[M + Na]^+$ calculated for $C_{29}H_{41}INaO_2$: 571.53 Found: 571.95.

Benzyl 15-(4-(3-((tert-butoxycarbonyl)amino)prop-1-yn-1-yl)phenyl)-3-methylpentadecanoate (8)

Triethylamine (110 mg, 1.1 mmol) and bis(triphenylphosphine)palladium(II) dichloride (51 mg, 0.073 mmol) were added to a stirred solution of **7** (200 mg, 0.37 mmol) in THF. The reaction mixture was stirred at room temperature for a few minutes. Boc-protected propargylamine **6** (170 mg, 1.1 mmol) in THF was added slowly to the reaction mixture and stirred for 5 min. Next, copper iodide (7 mg, 0.037 mmol) was added, and the reaction mixture was stirred overnight at rt. After completion of the reaction, THF was removed *in vacuo*. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (8:2) to obtain

the desired compound **8** (115 mg, yield 55%), in the form of a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.31-7.38 (7H, m), 7.12 (2H, d, J=8.3 Hz), 5.12 (2H, s), 4.75 (1H, s) 4.15 (2H, s), 2.58 (2H, t, J=7.8 Hz), 2.36 (1H, dd, J=14.6, 6.1 Hz), 2.17 (1H, dd, J=14.6, 8.0 Hz), 1.57-1.62 (2H, m), 1.47 (9H, s), 1.24-1.30 (20H, m), 0.93 (3H, d, J=6.8). ESI-MS (m/z): $[M + Na]^+$ calculated for $C_{37}H_{53}NNaO_4$: 598.82 Found: 599.18.

15-(4-(3-((tert-butoxycarbonyl)amino)propyl)phenyl)-3-methylpentadecanoic acid (9)

10% Pd/C (120 mg, 0.12 mmol) was added to a stirred solution of (**8**) (100 mg, 0.174 mmol) in MeOH (10 mL). The reaction mixture was stirred overnight at rt under an H_2 atmosphere. The solid was filtered off and the filtrate was concentrated under vacuum to obtain a residue that was purified by silica gel column chromatography using $CHCl_3$/MeOH (9.5:0.5) to obtain the desired compound (**9**) (75 mg, 92%), in the form of a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.09 (4H, m), 4.53 (1H, s), 3.15 (1H, s), 2.61 (2H, t, J=7.7 Hz), 2.57 (2H, t, J=7.3), 2.33-2.37 (1H, dd, J=15.9, 6.1 Hz), 2.16 (1H, dd, J=14.9, 8.0 Hz), 1.93-1.98 (1H, m), 1.77-1.82 (2H, m), 1.56-1.60 (2H, m), 1.45 (9H, s), 1.24-1.30 (20H, m), 0.97 (3H, d, J=6.6 Hz). ESI-MS (m/z): $[M + Na]^+$ calculated for $C_{30}H_{51}NNaO_4$: 512.73 Found: 513.04.

15-(4-(3-aminopropyl)phenyl)-3-methylpentadecanoic acid (Amino-BMPP)

TFA (160 mg, 1.43 mmol) was added to a stirred solution of (**4**) (70 mg, 0.143 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was stirred at rt for 1.5 hours. After completion of the reaction, the solvent was evaporated *in vacuo*, and the residue was extracted using EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to obtain the desired amino-BMPP (52 mg, yield 93%), in the form of a white solid. ^{1}H NMR (CD_3OD, 500 MHz): δ = 7.11 (4H, s), 2.91 (2H, t, J=7.5 Hz), 2.68 (2H, t, J=7.5 Hz), 2.57 (2H, t, J=8.5), 2.28 (1H, dd, J=14.4,

5.7 Hz), 2.07 (1H, dd, J=14.4, 7.1 Hz), 1.91-1.95 (2H, m), 1.57-1.60 (2H, m), 1.20-1.31 (20H, m), 0.95 (3H, d, J=6.6 Hz). ESI-MS (m/z): $[M+H]^+$ calculated for $C_{25}H_{44}NO_2$: 390.63 Found: 390.77.

3. References:

1. L-M. Yang, L-F. Huang, T-Y. Luh, *Org. Lett.*, 2004, **6**, 1461-1463.
2. M. M. Goodman, G. Kirsch, F. F. Knapp Jr, *J. Med. Chem.*, 1984, **27**, 390-397.
3. V. G. Bandi, M. P. Luciano, M. Saccomano, N. L. Patel, T. S. Bischof, J. G. P. Lingg, P. T. Tsrunchev, M. N. Nix, B. Ruehle, C. Sanders, L. Riffle, C. M. Robinson, S. Difilippantonio, J. D. Kalen, U. Resch-Genger, J. Ivanic, O. T. Bruns, M. J. Schnermann, *Nat. Methods*, 2022, **19**, 353-358.
4. M. Oshima, S. Higashi, Y. Kikuchi, T. Shirai, T. Yokokawa, T. Kaminaga, H. Yasukochi and S. Furui, *Ann. Nucl. Med.*, 1998, **1**2, 133-137.

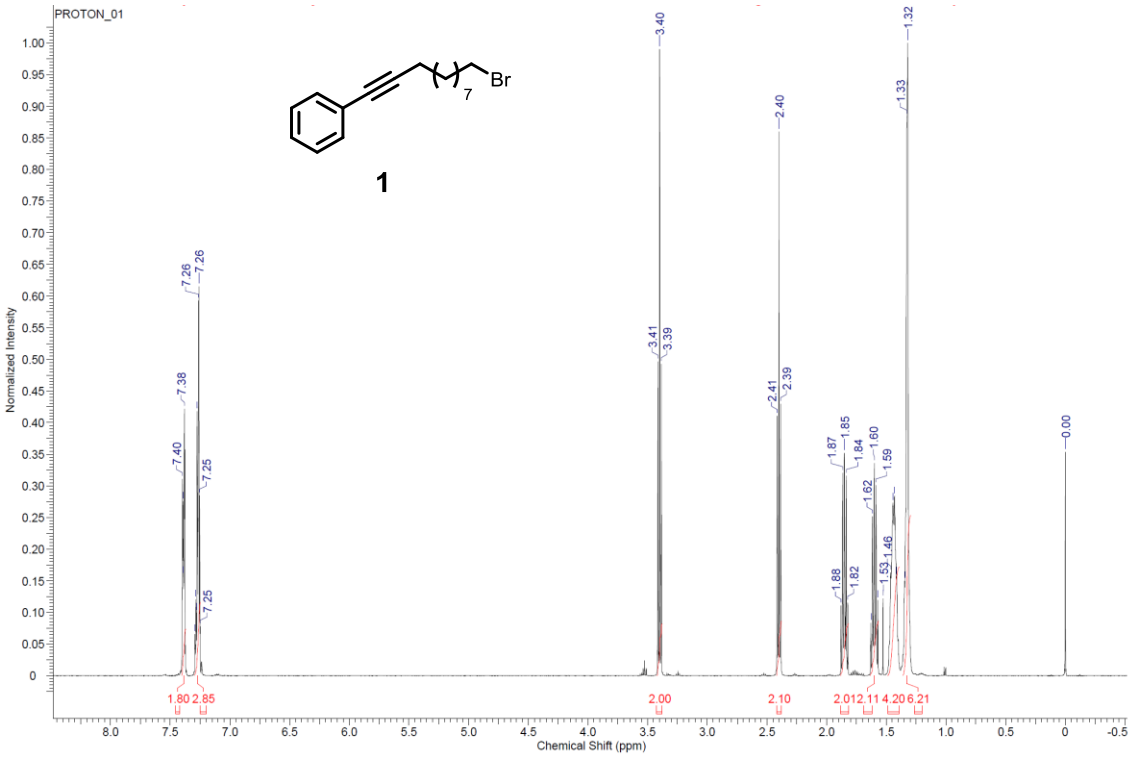
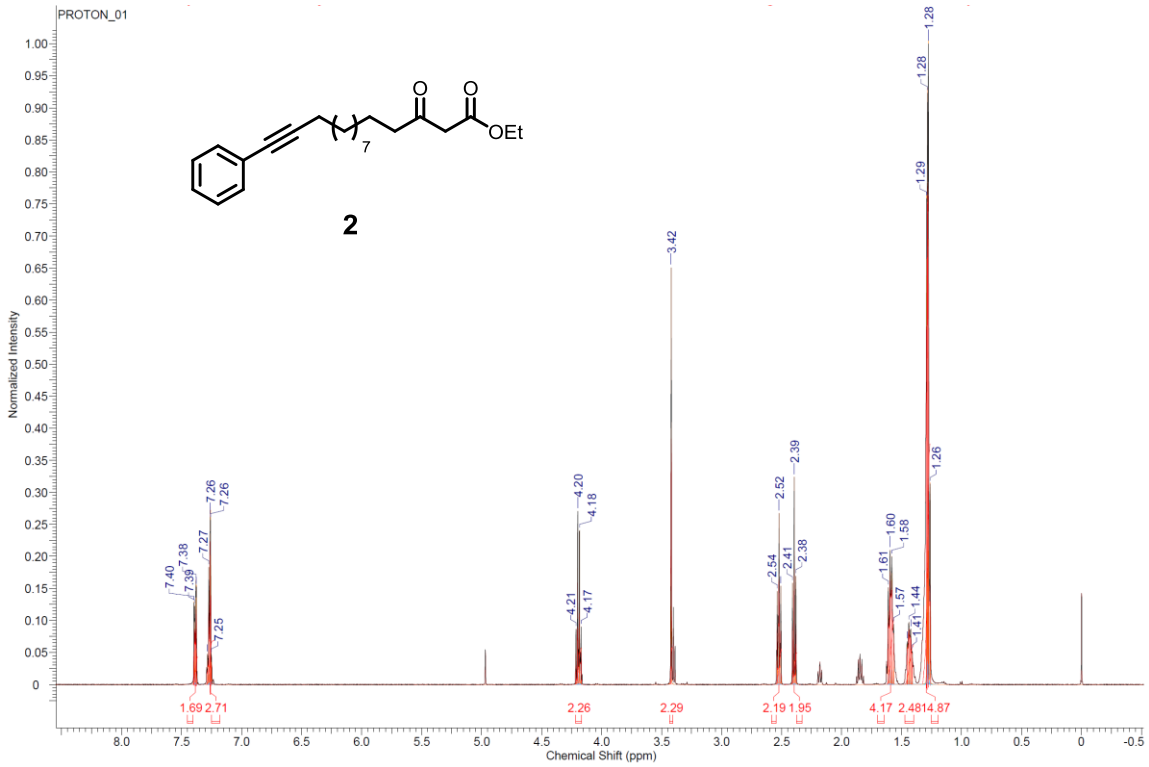


Fig. S3 ^{1}H and ^{13}C NMR spectra


PROTON_01
Normalized Intensity
Chemical Shift (ppm)
OEt
2
7.40
7.39
7.38
7.27
7.26
7.26
7.25
4.21
4.20
4.18
4.17
3.42
2.54
2.52
2.41
2.39
2.38
1.61
1.60
1.58
1.57
1.44
1.41
1.29
1.28
1.28
1.26
1.69
2.71
2.26
2.29
2.19
1.95
4.17
2.48
14.87

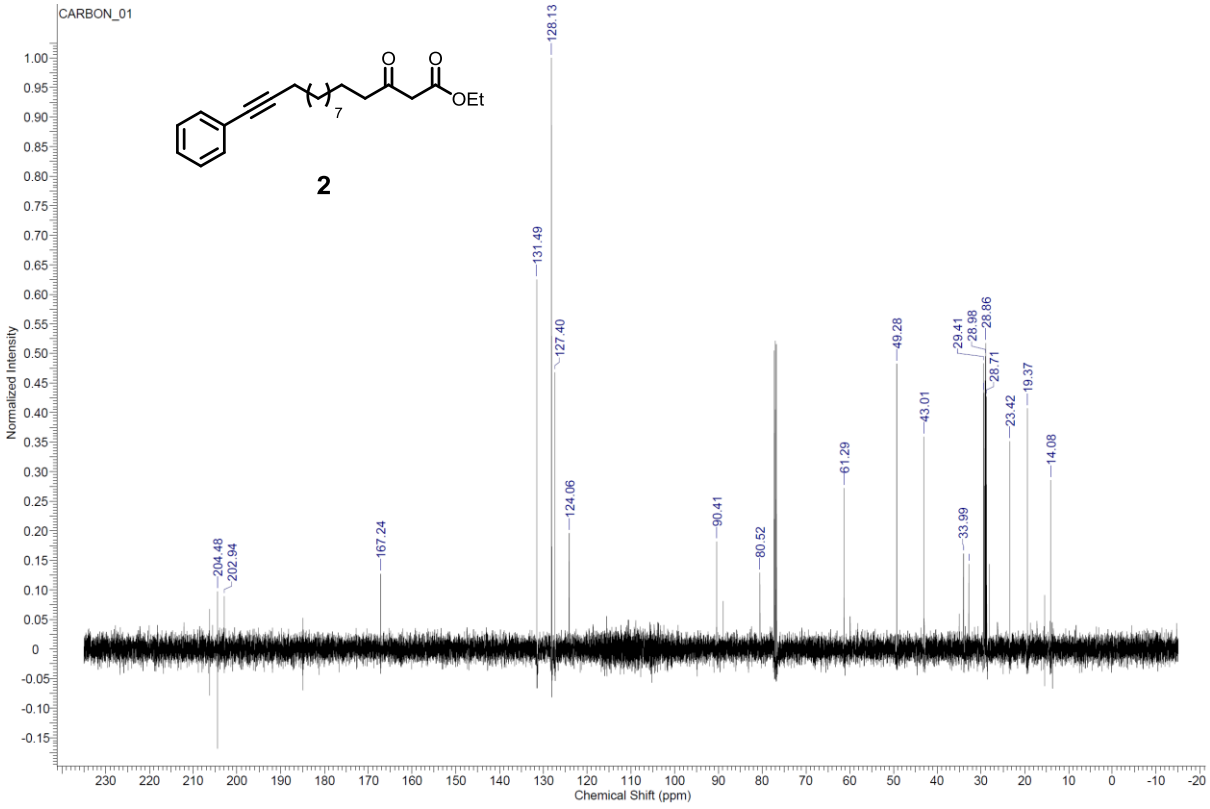

CARBON_01
Normalized Intensity
Chemical Shift (ppm)
OEt
2
204.48
202.94
167.24
131.49
128.13
127.40
124.06
90.41
80.52
61.29
49.28
43.01
33.99
29.41
28.98
28.86
28.71
23.42
19.37
14.08

Fig. S3 Cont.

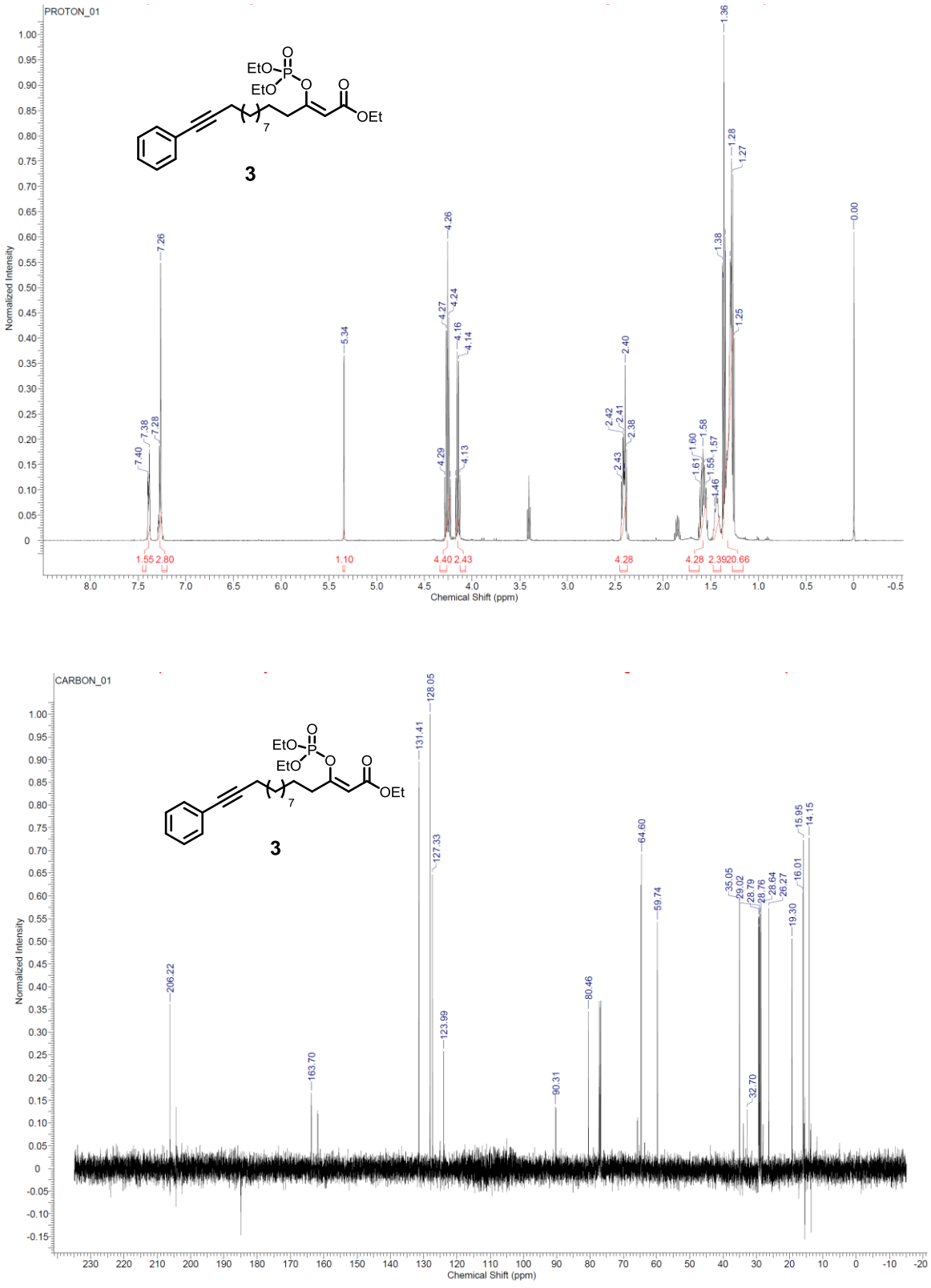

PROTON_01
CARBON_01
Normalized Intensity
Chemical Shift (ppm)
3
EtO
EtO
OEt
7
1.55 2.80 1.10 4.40 2.43 4.28 4.28 2.39 0.66

Fig. S3 Cont.

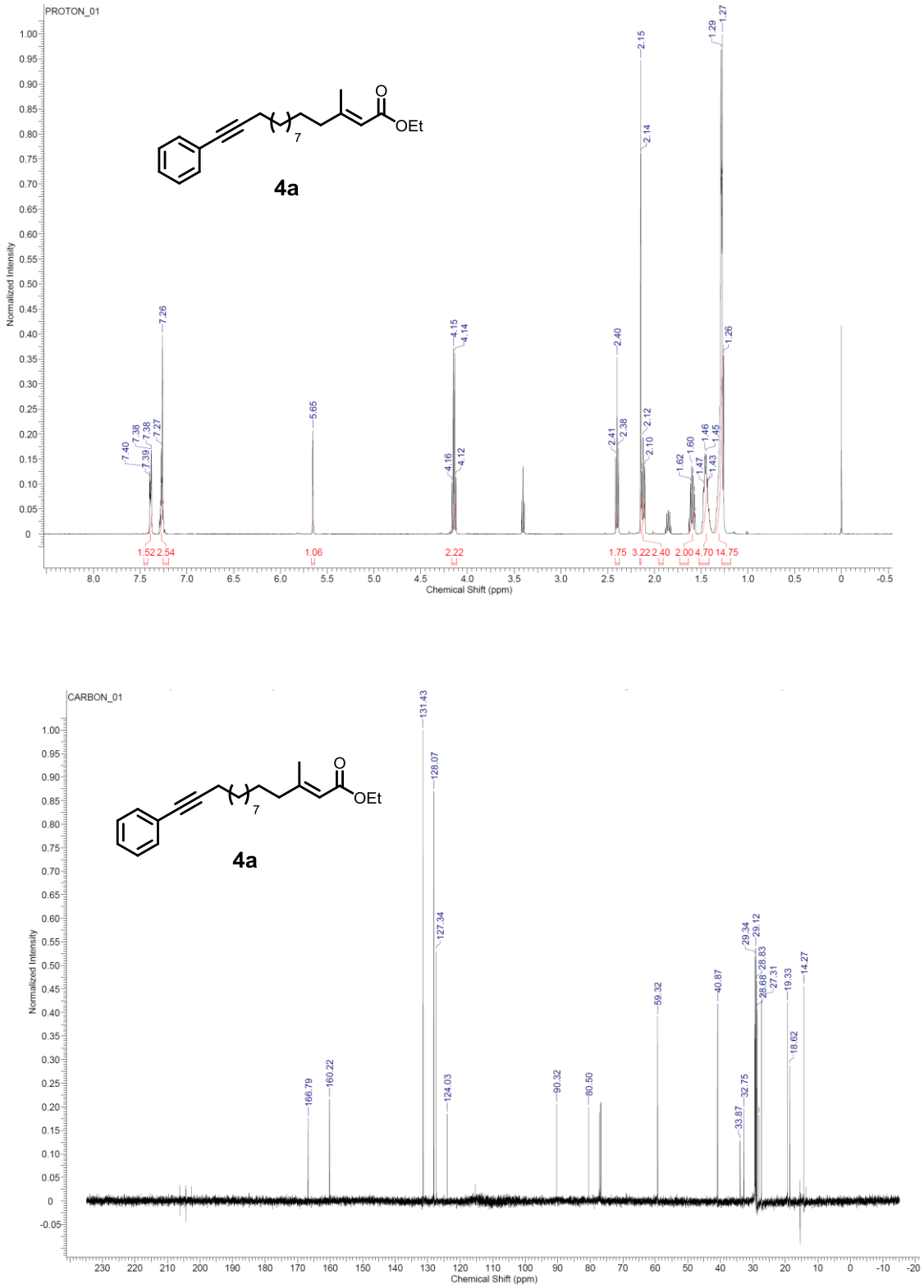

PROTON_01
4a
Normalized Intensity
Chemical Shift (ppm)
CARBON_01
4a
Normalized Intensity
Chemical Shift (ppm)

Fig. S3 Cont.

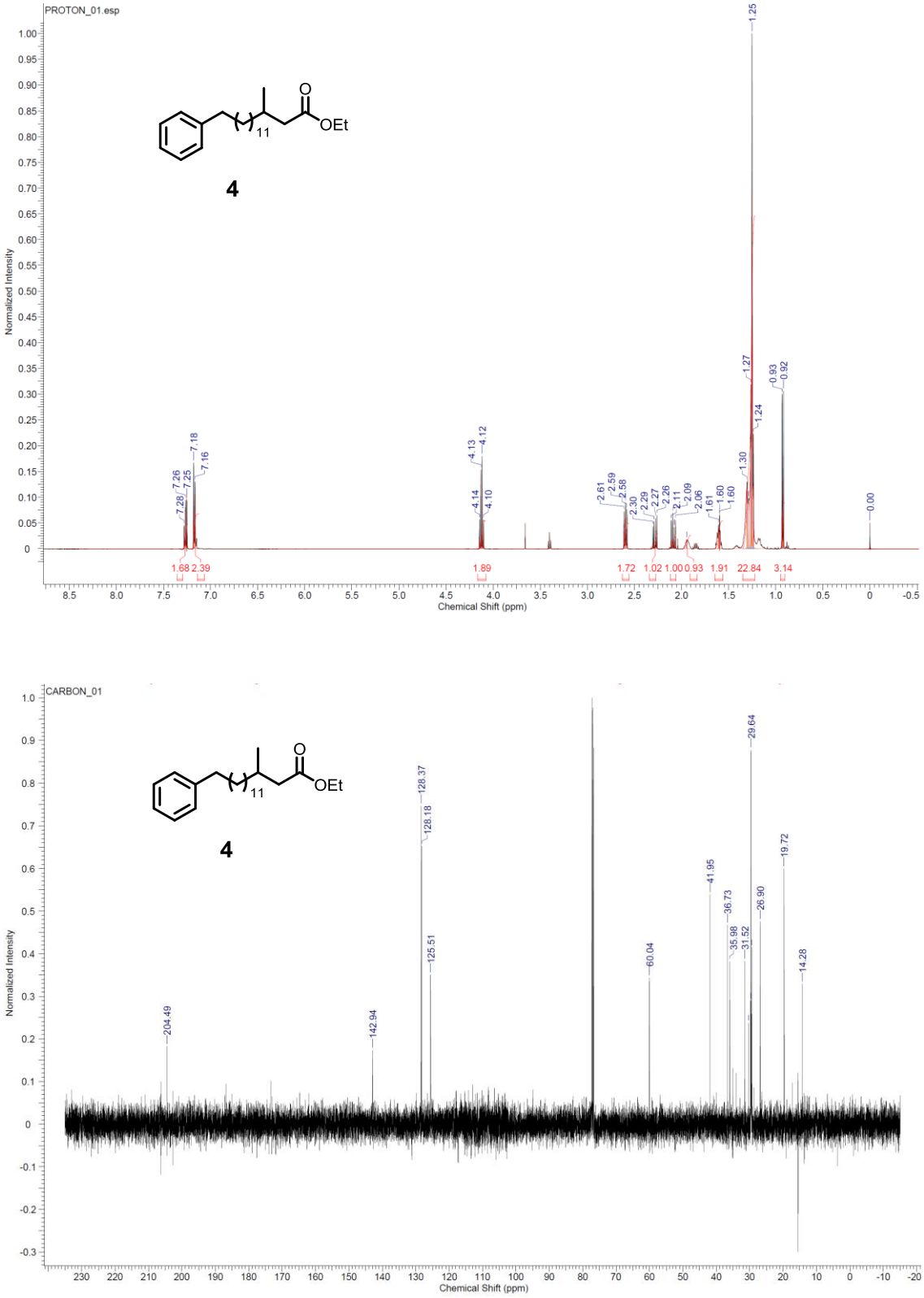
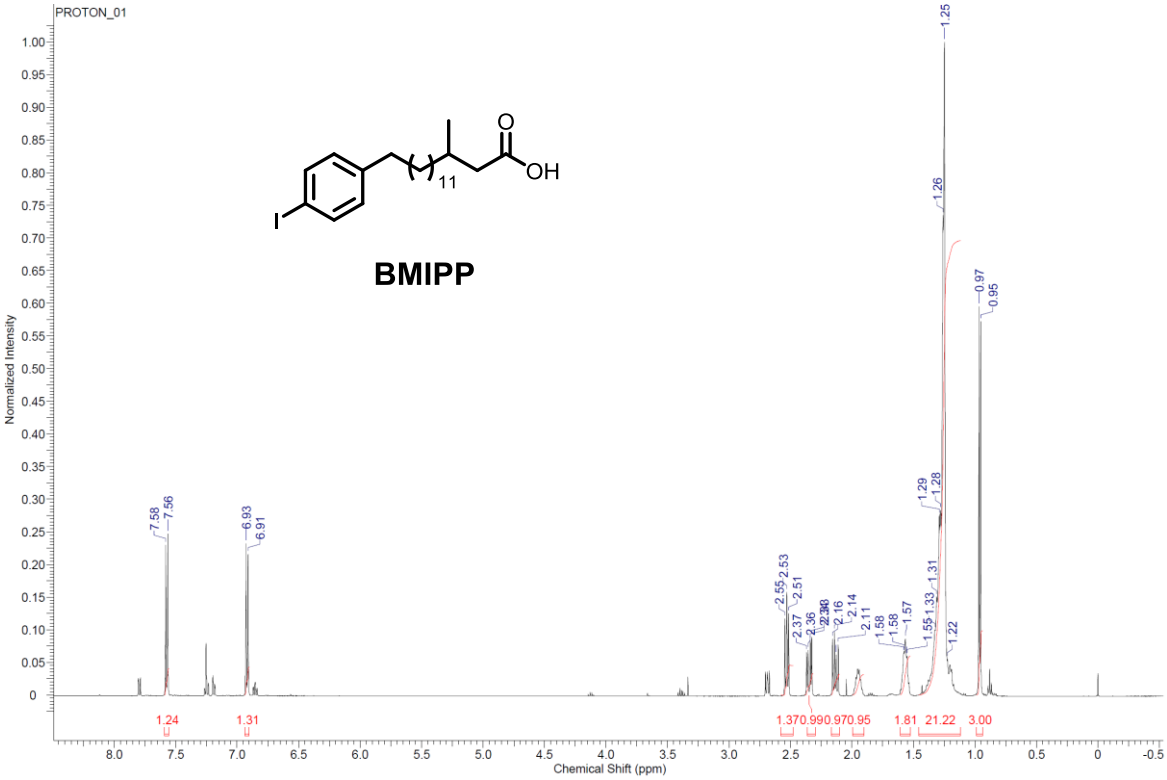

PROTON_01.esp
Normalized Intensity
Chemical Shift (ppm)
4
CARBON_01
Normalized Intensity
Chemical Shift (ppm)
4

Fig. S3 Cont.


PROTON_01
BMIPP
Normalized Intensity
Chemical Shift (ppm)

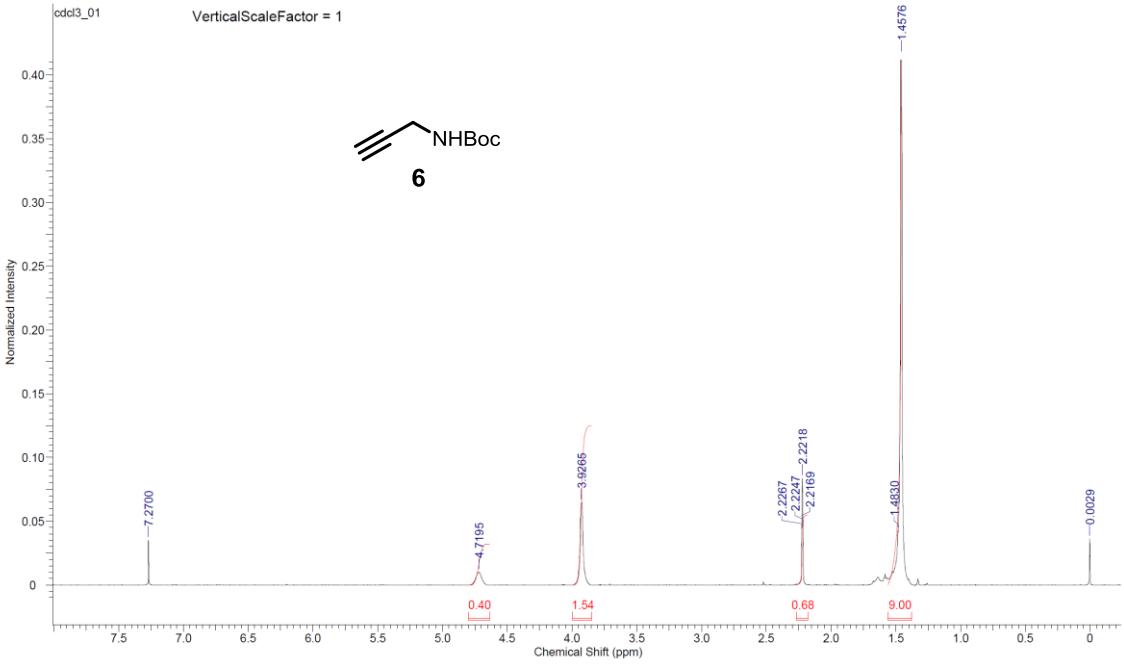

cdcl3_01
VerticalScaleFactor = 1
NHBoc
6
Normalized Intensity
Chemical Shift (ppm)

Fig. S3 Cont.

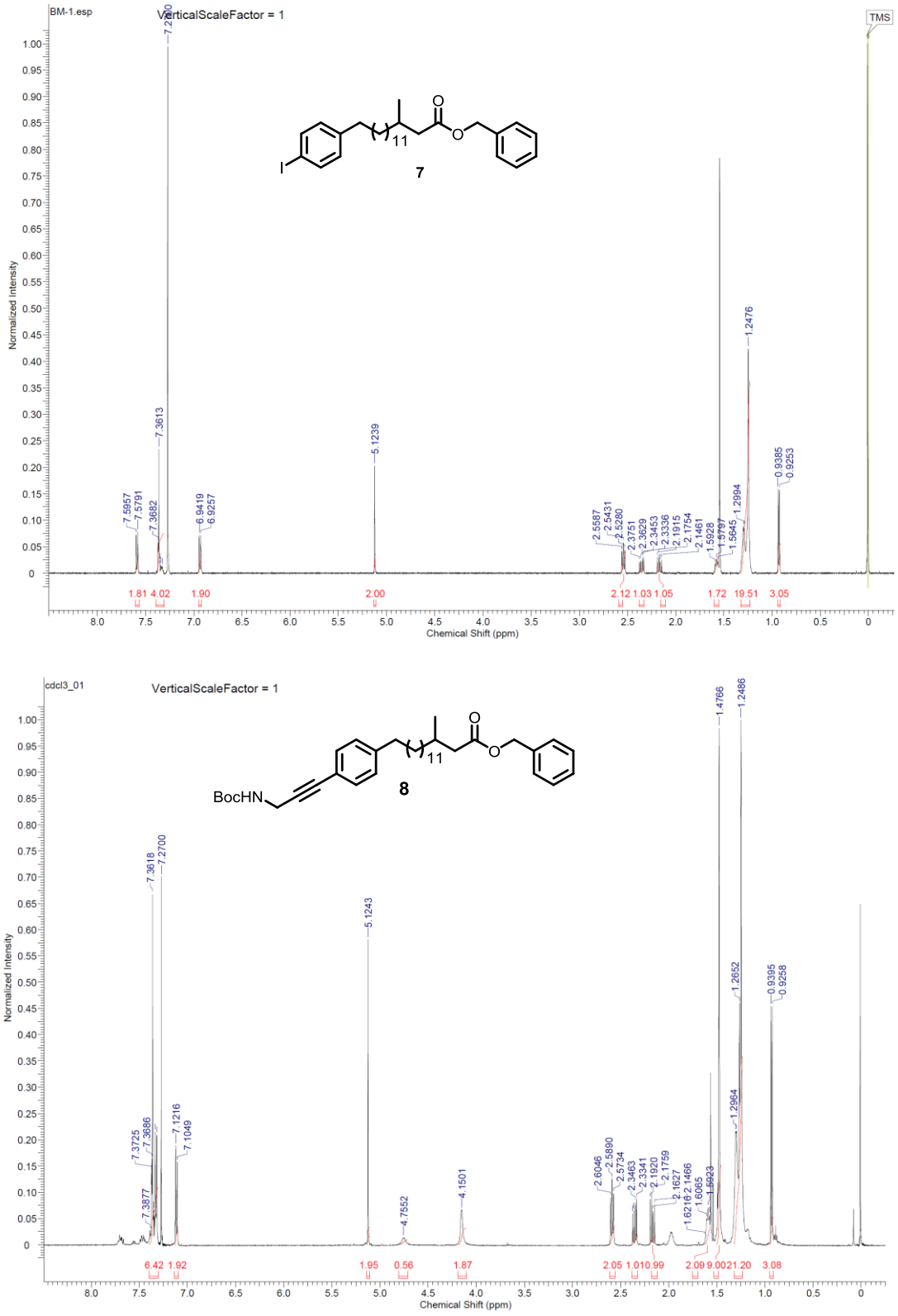

BM-1.esp
VerticalScaleFactor = 1
TMS
7
Normalized Intensity
Chemical Shift (ppm)
cdcl3_01
VerticalScaleFactor = 1
BocHN
8
Normalized Intensity
Chemical Shift (ppm)

Fig. S3 Cont.

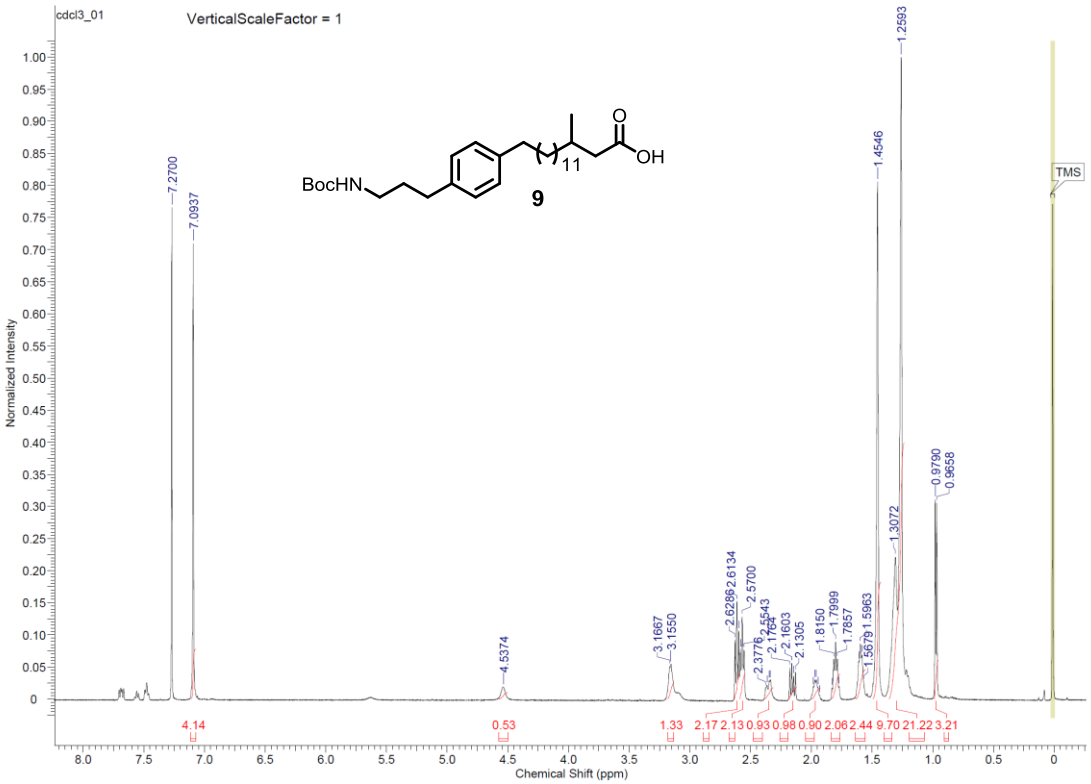
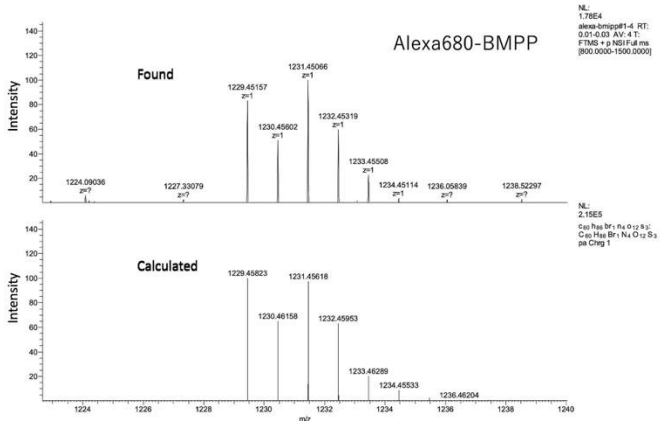
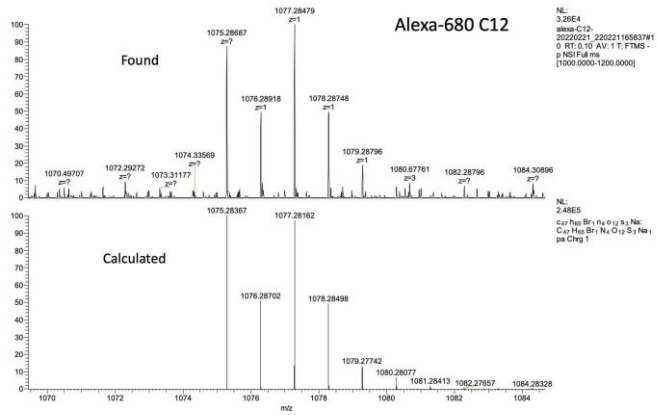

cdcl3_01
VerticalScaleFactor = 1
BocHN
O
OH
11
9
Normalized Intensity
Chemical Shift (ppm)
TMS
7.2700
7.0937
4.5374
3.1667
3.1550
2.6286
2.6134
2.5700
2.3776
2.1603
2.1305
1.8150
1.7999
1.7857
1.5679
1.5963
1.4546
1.3072
1.2593
0.9790
0.9658
4.14
0.53
1.33
2.17
2.13
0.93
0.98
0.90
2.06
2.44
9.70
2.21
2.22
3.21

Fig. S3 Cont.

Alexa680-BMPP



Alexa680-C12



Alexa680-C6

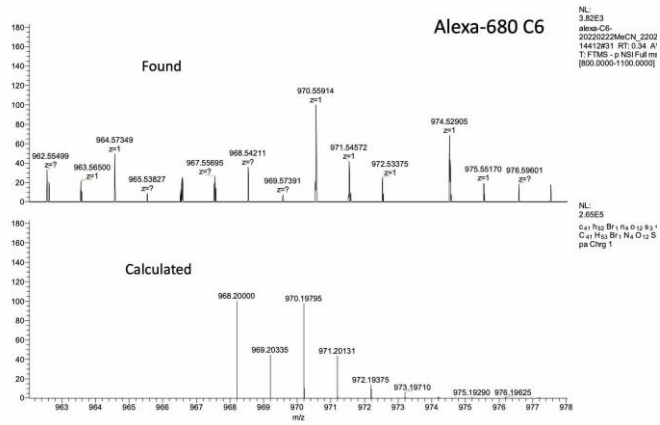


Fig. S4 High resolution mass spectra[3] of Alexa680-BMPP, Alexa680-C12, and Alexa680-C6.

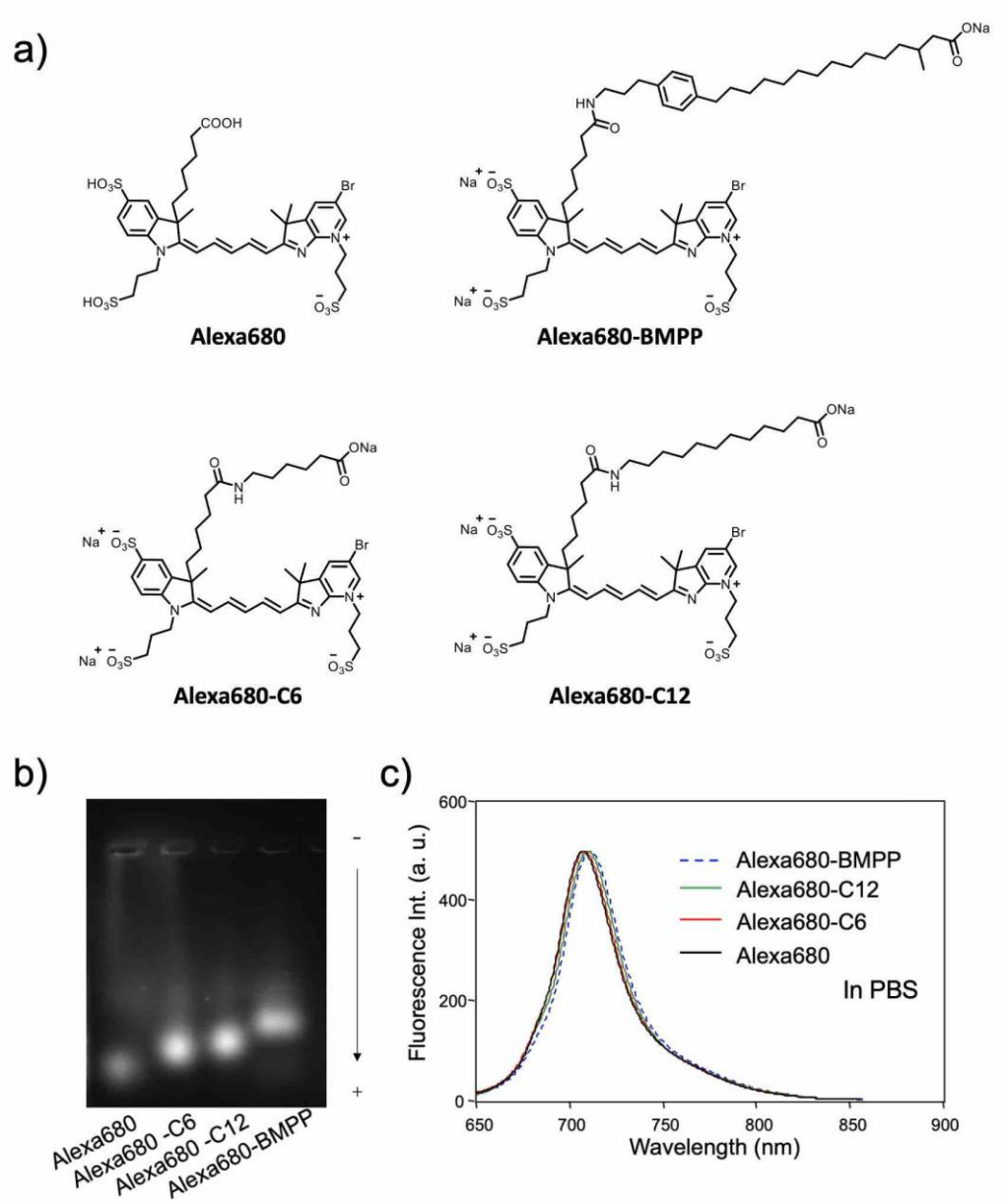


Fig. S5 Characteristics of compounds: (a) Chemical structures, (b)agarose gel electrophoresis, and (c) fluorescence spectra of Alexa680-BMPP, Alexa680-C12, Alexa680-C6, and Alexa680.

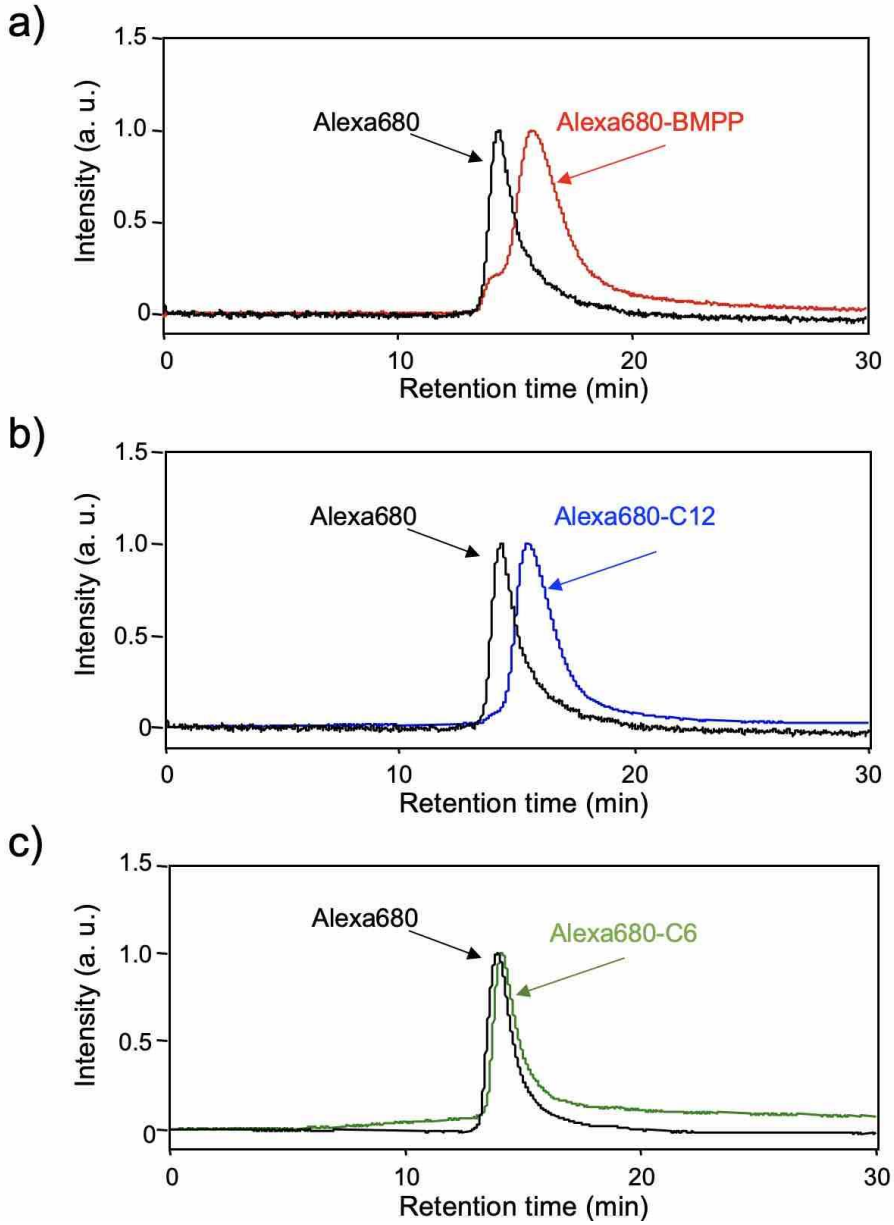


Fig. S6 HPLC chromatogram of Alexa680-BMPP, Alexa680-C12, Alexa680-C6, and Alexa680. The compounds were monitored using the fluorescence at 700 nm with excitation at 650 nm. HPLC column: TSKgel G4000SW$_{XL}$(Tosoh bioscience), Mobile phase: PBS (pH=7.4), Flow rate: 1 mL/min. Temp: 25 ^{o}C.

Table S1. Optical property of Alexa680 and Alexa680 labeled fatty acids

	λ_{max} (nm)	Em_{max} (nm)	Quantum yield
Alexa680	676	707	0.36
Alexa680-C6	677	708	0.36
Alexa680-C12	680	710	0.38
Alexa680-BMPP	680	711	0.39

Sample concentrations were 2 μM in phosphate buffered saline (pH =7.4)

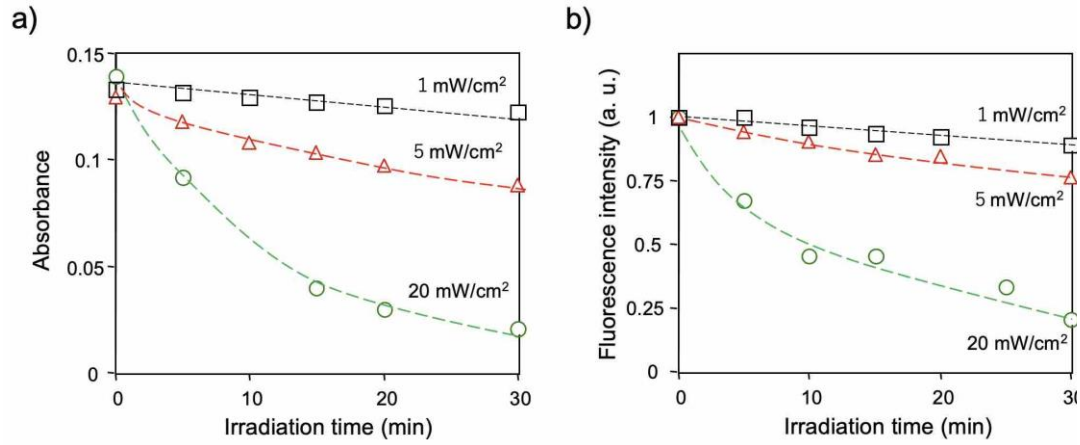


Fig. S7 Photostability of Alexa680-BMPP in PBS. The changes in the absorption at 680 nm (a) and fluorescence at 711 nm (b) of aqueous solutions (3. 8 μM) of Alexa680-BMPP by irradiation of a 670 nm laser light. Laser power: 1mW/cm^2, 5mW/cm^2 and 20 mW/cm^2.

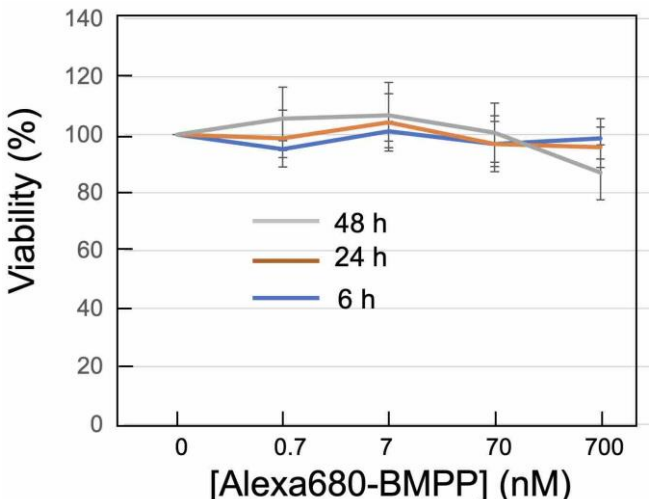


Fig. S8 Viability of HeLa cells in the presence of Alexa680-BMPP.

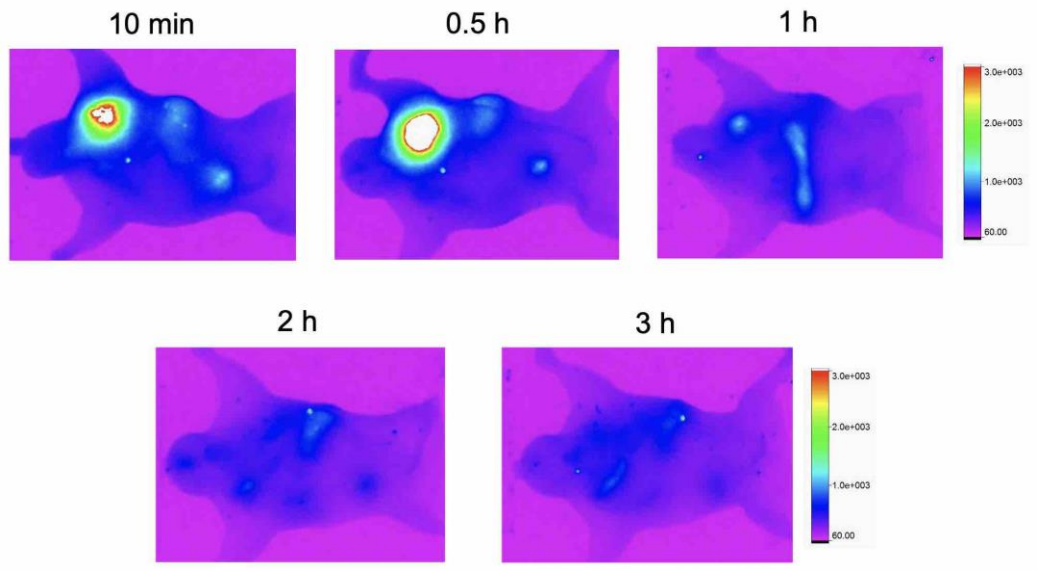


Fig. S9 In vivo NIR fluorescence images of a whole-body of a hairless mouse. The images were taken after the intravenous injection of Alexa680 (200 μL of 2 μM aqueous solution).

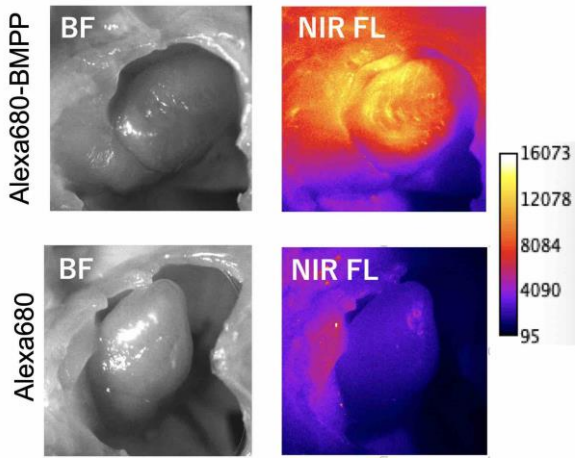


Fig. S10 NIR fluorescence images of heart tissues with skin and ribs removed. The images were taken after the intravenous injection of Alexa680-BMPP and Alexa680 (200 μL of 2 μM aqueous solution).

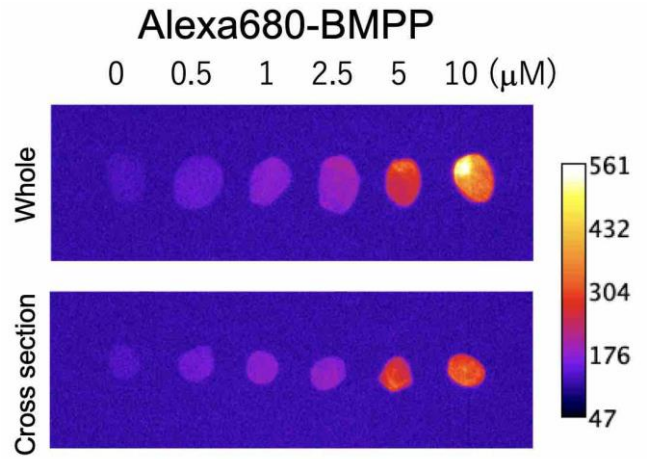


Fig. S11 Ex vivo NIR fluorescence images of heart tissues. The images were taken after the intravenous injection of 200 μL of Alexa680-BMPP (0-10 μM aqueous solution).

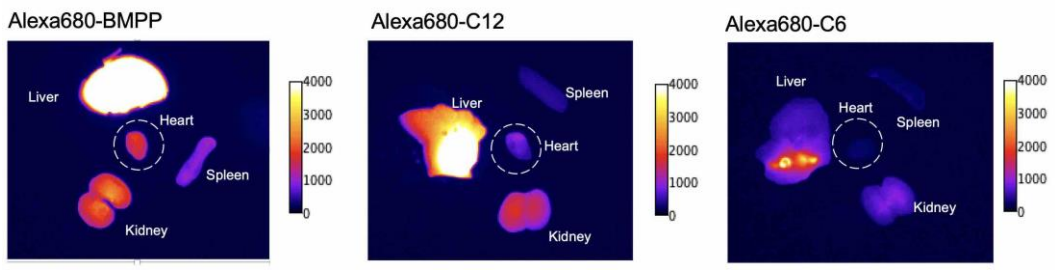


Fig. S12 Biodistribution of Alexa680-BMPP, Alexa680-C12, and Alexa680-C6. Ex vivo NIR fluorescence images of heart tissues and organs were taken 30 min after the intravenous injection of 200 μL of the probes (2 μM solution).

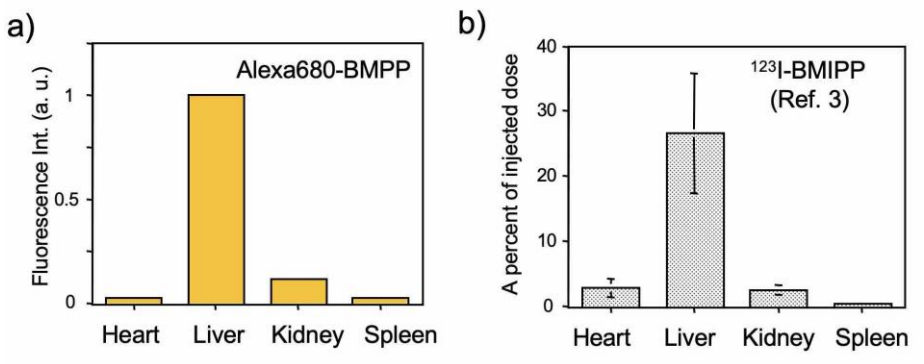


Fig. S13 Comparison of biodistribution of Alexa680-BMPP and ^{123}I-BMIPP in mice. a) A hairless mouse injected with 200 μL of Alexa680-BMPP (2 μM, PBS). b) C57BL/6J mice injected with 370 kBq of ^{123}I-BMIPP in 200 μL saline (data from ref 4: Oshima et al., *Ann. Nucl. Med.*, 1998, **12**, 133-137).