Electronic Supplementary Material (ESI) for Chemical Communications

Strain-promoted cycloadditions of cyclic nitrones with cyclooctynes for labeling human cancer cells

Supporting Information

Craig S. McKay, [a,b] Jessie A. Blake, [a,b] Jenny Cheng, [a] Dana C. Danielson, [a,c] and John Paul Pezacki*[a,b,c]

[a]Steacie Institute for Molecular Sciences, National Research Council Canada, 100 Sussex Dr., Ottawa, ON, K1A 0R6, Canada
[b]Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, ON, K1N 6N5, Canada
[c]Department of Chemistry Biochemistry, Microbiology and Immunology, University of Ottawa 451 Smyth Rd., Ottawa, ON, K1H 8M5, Canada

John.Pezacki@nrc-cnrc.gc.ca

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Materials and Methods

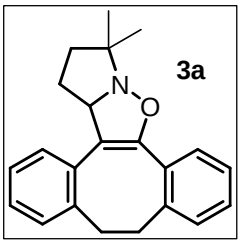
All chemical reagents, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, **1a**) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich and were used without further purification. Human recombinant epidermal growth factor (EGF), tetramethyl rhodamine cadaverine, Alexa Fluor® 488 cadaverine sodium salt and FITC-streptavidin were purchased from Invitrogen. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Cell line and culture media were purchased from ATCC. Thin layer chromatography (TLC) was carried out on Analtech Uniplate® silica gel plates (60 Å F254, layer thickness 250μm) using UV light to visualize the course of the reaction. Flash column chromatography was performed using silica gel (60 Å, particle size 40–63 μm). ^{1}H NMR and ^{13}C NMR spectra were obtained using a 400 MHz Bruker NMR spectrometer. Chemical shifts are reported as δ referenced to solvent and coupling constants (*J*) are reported in Hz. All other cyclic nitrones: 5-ethoxycarbonyl-5-methyl-1-pyrroline-N-oxide (EMPO, **1b**)[1] prolinol nitrones **1c** and **1f** and 3-N-Boc pyrrolidine N-Oxide **1d**,[2] piperazinone N-oxide **1e**,[3] ketonitrone **1g**,[4] acyclic nitrone[5] and dibenzocyclooctynes, **2a**,[6] **2b** and **2b**-Biotin[7] were each prepared by standard literature procedures. The cycloadduct **3d** has been characterized previously.[6]

General Procedure for SPANC in model systems:

The appropriate cyclic nitrone **1a-1e** (0.015mmol) and **2a** or **2b** (0.015mmol) were pre-dissolved in either C_6D_6 (0.3 mL) or CD_3CN (0.3 mL) and mixed at equimolar concentrations of ~25 mM (0.6 mL). Percent conversion was monitored both by disappearance of starting materials and by appearance of product as determined by integration at multiple chemical shifts in the ^{1}H NMR spectrum. No other products were detected by ^{1}H NMR and all reactions were performed in triplicate. Upon completion, the triplicate reactions were combined and the solvent was removed by rotary evaporation. The crude triplicate reactions were purified by flash column chromatography.

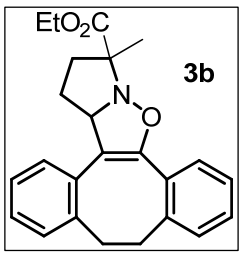
Characterization Data for New Compounds:

Compound **3a**:



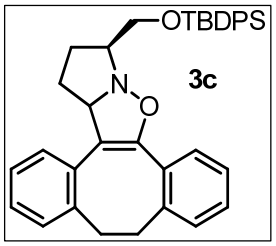
The crude reaction mixture was concentrated under reduced pressure and **3a** was purified by flash column chromatography (9:1/Hx:EtOAc, R_f = 0.20). **3a** was obtained as a white solid (13.0 mg, 0.04 mmoles, 91 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50-7.48 (1H, m), 7.29-7.08 (7H, m), 5.18 (1H, dd, J = 7.7, 2.8 Hz), 3.56 – 3.49 (1H, M), 3.46-3.39 (1H, m), 2.14-2.04 (1H, m), 2.01-1.91 (H, m), 1.88-1.82 (1H, m), 1.78-1.73 (1H, m), 1.47 (3H, s), 1.20 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 147.5, 140.7, 138.3, 132.7, 130.8, 129.9, 129.7, 128.3, 128.3, 128.1, 126.9, 125.9, 125.5, 110.1, 74.5, 69.5, 36.8, 35.1, 33.3, 31.5, 26.7, 23.7. HRMS (ESI+): m/z calculated for $C_{22}H_{24}NO_2$ = 318.1858, found: 318.1832.

Compound **3b**:



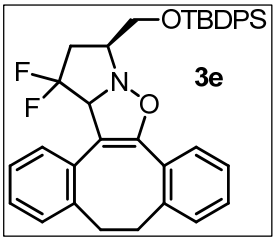
The crude reaction mixture was concentrated under reduced pressure and **3b** and **3b'** were separated by flash column chromatography (8:2/Hx:EtOAc, R_f = 0.32 for **3b** and 0.25 for **3b'**). A 72:28 mixture of **3b** and **3b'** were obtained as off white solids (16.2 mg, 0.043 mmoles, 96 %). **3b** major**:** ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53-7.52 (1H, m), 7.24-7.22 (2H, m), 7.14-7.09 (5H, m), 5.32 (1H, dd, J=7.1, 2.9 Hz), 4.26 (2H, q), 3.56-3.49 (1H, m), 3.44-3.37 (1H, m), 3.18-3.10 (1H, m), 3.00-2.94 (1H, m), 2.43-2.36 (1H, m), 2.00-1.85 (1H, m), 1.69 (3H, s), 1.32 (3H, t). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.8, 148.0, 140.7, 138.2, 132.2, 130.8, 129.8, 129.7, 127.0, 126.0, 125.6, 109.8, 76.5, 75.2, 61.4, 36.9, 33.6, 33.3, 30.6, 19.8, 14.2. **3b'** minor**:** ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42 (1H, d, J=7.6 Hz), 7.26-7.04 (m, 7H), 3.85 (1H, dd, J=8.1, 1.9 Hz), 4.31-4.21 (2H. m), 3.56-3.40 (2H, m), 3.15-3.00 (1H, m), 2.99-2.94 (1H, m), 2.68-2.62 (1H, m), 2.13-2.05 (1H, m), 1.93 (2H, m), 1.45 (3H, s), 1.28 (3H, t). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.6, 147.3, 141.0, 138.4, 132.1, 130.8, 129.8, 129.8, 128.4, 138.3, 127.5, 127.2, 126.0, 125.4, 109.8, 74.5, 61.1, 36.8, 33.2, 30.6, 30.4, 22.6, 14.1. HRMS (ESI+): m/z calculated for $C_{24}H_{26}NO_3$ = 376.1913, found: 376.1915.

Compound **3c**:



The reaction mixture was concentrated under reduced pressure and **3c** was purified by flash column chromatography (9:1/Hx:EtOAc, R_f = 0.50 for **3c** and 0.39 for **3c'**). An 87:13 ratio **3c** and **3c'** was obtained as a white solid (22.1 mg, 0.040 mmoles, 88 %). **3c** major: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70-7.67 (4H, m), 7.42-7.32 (7H, m), 7.26-7.23 (1H, m), 7.17-7.08 (6H, m), 5.18 (1H, dd, J=6.5, 4.5Hz), 4.04 (1H, dd, J= 9.6, 4.2 Hz), 3.75-3.64 (2H, m), 3.55-3.48 (1H, m), 3.44-3.37 (1H, m), 3.19-3.11 (1H, m), 3.02-2.95 (1H, m), 2.12-2.04 (2H, m), 1.86-1.82 (1H, m), 1.74-1.69 (1H, m), 1.08 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 147.7, 140.8, 138.1, 135.7, 135.6, 133.7, 133.7, 132.5, 130.8, 129.7, 129.6, 129.4, 128.3, 128.3, 127.6, 126.9, 126.0, 125.5, 109.9, 73.8, 71.4, 66.0, 36.9, 33.3, 29.1, 26.9, 25.5, 19.3. **3c'** minor: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70-7.68 (3H, m), 7.44-7.08 (15H, m), 5.20-5.19 (1H, m), 4.33-4.29 (1H, m), 3.93-3.89 (1H, m), 3.51-3.33 (3H, m), 3.12-3.06 (1H, m), 2.95-2.88 (1H, m), 2.08-2.04 (1H, m), 1.88-1.81 (2H, m), 1.07 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.2, 140.8, 135.6, 133.8, 130.8, 129.9, 129.7, 129.6, 128.4, 128.3, 127.6, 126.0, 125.4, 75.9, 72.8, 50.3, 36.9, 33.2, 32.0, 26.8, 26.6, 19.2. HRMS (ESI+): m/z calculated for $C_{37}H_{40}NO_2Si$ = 558.2828, found: 558.2775.

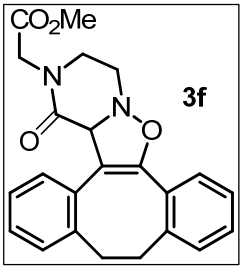
Compound **3e**:



The reaction mixture was concentrated under reduced pressure and **3e** was purified by flash column chromatography (85:15/Hx:EtOAc, R_f = 0.50). **3e** was obtained as a white-solid (22.5 mg, 0.038 mmoles 84 %). %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70-7.66 (4H, m), 7.44-7.34 (7H, m), 7.24-7.17 (4H, m), 7.15-7.19 (3H,m), 5.31 (1H, dd, J = 11.7, 9.5 Hz), 4.80 (1H, dd, J = 10.5, 4.1 Hz), 3.87 (1H, dd, J = 10.5, 6.5 Hz), 3.78-3.71 (1H, m), 3.56-3.47 (2H, m), 3.13-2.94 (2H, m), 2.51-2.42 (1H, m), 2.32-2.17 (1H, m), 1.08 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.93, 139.8, 139.2, 135.7, 135.6, 133.3, 133.2, 131.4, 130.6, 130.3, 129.8, 129.2, 129.1, 127.7, 127.4, 127.3, 127.1, 125.9, 125.6, 103.8, 103.7, 79.3, 79.1, 79.0, 78.8, 69.5, 69.4,

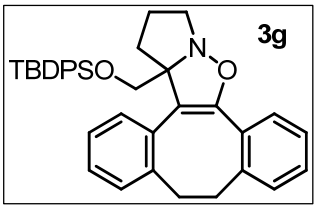
64.5, 36.0 (t), 33.7, 26.9, 19.3. HRMS (ESI+): m/z calculated for $C_{37}H_{38}F_2NO_2Si$ = 594.2640, found: 594.2665.

Compound **3f**:



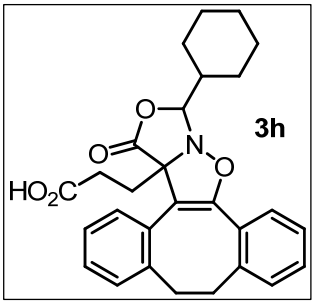
The reaction mixture was concentrated under reduced pressure and **3f** was purified by flash column chromatography (8:2/Hx:EtOAc, R_f = 0.52). **3f** was obtained as an off-white solid (15.2 mg, 0.039 mmoles, 87 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43 (1H, m), 7.32-7.29 (1H, m), 7.20-7.11 (6H, m), 5.46 (1H, s), 4.35 (1H, d, J = 17.4 Hz), 4.06-4.00 (m, 1H), 3.99 (1H, d, J = 17.4 Hz), 3.80-3.70 (2H, m), 3.73 (3H, s), 3.64-3.57 (1H, m), 3.54-3.47 (1H, m), 3.41-3.31 (2H, m), 3.23-3.15 (1H, m), 3.02-2.96 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.0, 165.7, 149.9, 140.3, 139.2, 131.1, 131.0, 129.6, 129.4, 129.3, 129.1, 127.5, 127.2, 125.6, 125.6, 107.8, 71.9, 52.3, 50.5, 48.3, 13.6, 36.7, 33.0. HRMS (ESI+): m/z calculated for $C_{23}H_{23}N_2O_4$ = 391.1658, found 391.1611.

Compound **3g**:



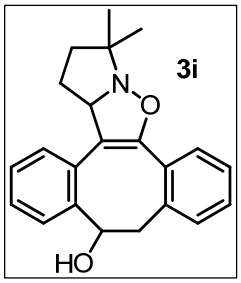
The reaction mixture was concentrated under reduced pressure and **3g** was purified by flash column chromatography (95:5/Hx:EtOAc, R_f = 0.25). **3g** was obtained as an off-white solid (22.2 mg, 0.040 mmoles, 89 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.78-7.72 (4H, m), 7.45-7.36 (7H, m), 7.28-7.26 (2H, m), 7.18-7.03 (5H, m), 3.93 (1H, d, J=10.6 Hz), 3.73 (1H, d, J=10.6 Hz), 3.62-3.56 (1H, m), 3.45-3.32 (2H, m), 3.29-3.20 (2H, m), 3.18-3.08 (1H, m), 2.12-2.02 (1H, m), 1.93-1.79 (2H, m), 1.70-1.63 (1H, m), 1.07 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.2, 142.3, 137.6, 136.0, 135.8, 133.8, 133.4, 132.9, 131.3, 130.3, 129.5, 128.9, 128.6, 128.4, 128.2, 127.6, 127.5, 127.2, 125.9, 125.2, 108.3, 85.3, 68.0, 59.4, 37.9, 32.7, 32.1, 26.9, 23.5, 19.4. HRMS (ESI+): m/z calculated for $C_{37}H_{40}NO_2Si$ = 558.2828, found 558.2817.

Compound **3h**:



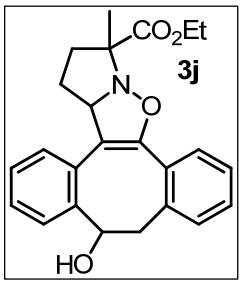
The reaction mixture was concentrated under reduced pressure and **3h** was purified by flash column chromatography (7:3/Hx:EtOAc, R_f = 0.34). **3h** was obtained as a white solid (20.0 mg, 0.044 mmoles, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29-7.10 (8H, m), 4.98 (1H, d, J = 6.3Hz), 3.44-3.23 (3H, m), 3.04-2.99 (1H, m), 2.67-2.59 (1H, m), 2.55-2.47 (1H, m), 2.42-2.35 (1H, m), 2.32-2.24 (2H, m), 2.01-1.98 (2H, m), 2.86-1.73 (4H, m) 1.39-1.22 (1H, 6m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 177.6, 170.6, 151.7, 142.1, 138.5, 131.7, 130.2, 129.4, 129.2, 128.8, 128.6, 126.3, 125.9, 125.6, 106.2, 101.4, 78.9, 42.1, 37.6, 32.0, 29.0, 28.7, 27.3, 27.0, 26.2, 25.5, 25.4. HRMS (ESI+): m/z calculated for $C_{28}H_{30}NO_5$ = 460.2124, found 460.2112.

Compounds **3i**:



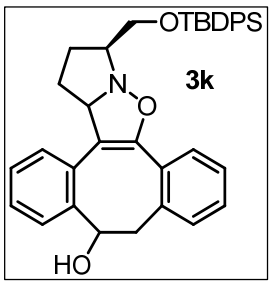
The reaction mixture was concentrated under reduced pressure and **3i** was purified by flash column chromatography (7:3/Hx:EtOAc, R_f = 0.30). **3j** was obtained as a mixture of regio- and diastereomers as a off-white solid (14.7 mg, 0.044 mmoles, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.69-7.63 (2.0H, m), 7.52-7.50 (1.3H, m), 7.39-7.35 (2.0H, m), 7.29-7.09 (11H, m), 5.65-5.61 (1.0H, m), 5.24-5.20 (1.8H, m), 5.08-5.03 (0.9H, m), 3.82-3.77 (1H, dd, J = 16.0, 5.2 Hz), 3.44-3.38 (0.9H, dd, J = 12.6, 11.2 Hz), 3.32-3.27 (0.9H, dd, 12.7, 6.1 Hz), 3.22-3.15 (1.0H, dd, J = 16.0, 10.5 Hz), 2.12-1.88 (6.0H, m), 1.79-1.74 (4.2H, m), 1.49, 1.46, 1.21, 1.20 (4x3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.6, 147.5, 146.8, 143.6, 139.3, 136.7, 135.3, 132.8, 132.6, 131.3, 130.5, 130.4, 130.0, 129.6, 129.0, 128.6, 127.8, 127.7, 127.6, 127.5, 127.2, 127.0, 126.6, 125.9, 125.2, 110.5, 109.1, 74.3, 74.2, 69.6, 69.1, 45.7, 40.5, 35.1, 35.0, 31.1, 30.9, 26.6, 26.5, 23.7. HRMS (ESI+): m/z calculated for $C_{22}H_{24}NO_2$ = 334.1807, found 334.1775.

Compounds **3j**:



The reaction mixture was concentrated under reduced pressure and **3j** was purified by flash column chromatography (65:35/Hx:EtOAc, R_f = 0.28). **3j** was obtained as a mixture of regio- and diastereomers as a white solid (16.9 mg, 0.043 mmoles, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.69-7.66 (1.4H, m), 7.55-7.50 (0.9 H, m), 7.46-7.43 (0.4H, m), 7.37-7.34 (1.6H, m), 7.30-7.06 (9H, m), 5.67-5.83 (0.8H, m), 5.37-5.35 (1H, m), 5.29-5.25 (0.6H, m), 5.08-5.04 (0.4H, m), 5.01-4.96 (0.2H, m), 4.31-4.09 (3.8H, m), 3.82-3.75 (0.8H, m), 3.54-3.13 (2.1H, m), 2.71-2.59 (0.4H, m), 2.43-2.33 (1.2H, m), 2.20-1.77 (8.0H, m), 1.71, 1.69, 1.48, 1.46 (4x3H, s), 1.3-1.23 (4x3H, t). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 171.7, 171.4, 171.2, 150.0, 149.3, 148.0, 143.8, 143.6, 139.6, 139.2, 137.1, 136.6, 135.4, 135.2, 132.9, 132.6, 132.4, 132.3, 131.3, 130.2, 130.1, 129.9, 129.9, 129.8, 129.6, 129.6, 129.1, 129.0, 128.7, 128.7, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.4, 127.2, 127.1, 127.0, 126.6, 126.5, 126.2, 125.9, 125.8, 125.7, 125.3, 125.1, 110.3, 110.1, 108.8, 108.8, 74.9, 74, 9, 74.4, 74.1, 61.5, 61.4, 61.2, 60.4, 45.8, 45.8, 40.8, 40.5, 33.7, 33.6, 31.3, 30.5, 30.4, 30.2, 30.1, 29.7, 29.5, 23.1, 22.6, 21.0, 19.8, 19.8, 14.2, 14.1, 14.0. HRMS (ESI+): m/z calculated for $C_{24}H_{26}NO_4$ = 392.1862, found 392.1799.

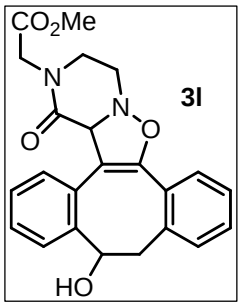
Compounds **3k**:



The reaction mixture was concentrated under reduced pressure and **3j** was purified by flash column chromatography (8:2/Hx:EtOAc, R_f = 0.25). **3j** was obtained as a mixture of regio- and diastereomers as a white solid (24.0 mg, 0.041 mmoles, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.71-7.65 (33.2H, m), 7.63-7.51 (6.2H, m), 7.43-7.14 (93.5H, m), 5.65-5.60 (3.5H, m), 5.53-5.49 (1.4H, m), 5.39-5.35 (1.0H, m), 5.30-5.16 (5.5H, m), 5.09-4.99 (3.1H, m), 2.13-2.05 (16.8H, m), 1.96-1.85 (7.2H, m), 1.80-1.68 (8.6H, m), 1.08, 1.07, 1.07, 1.06 (4x3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 143.6, 139.1, 136.7, 135.7, 135.7, 135.7, 135.5, 133.7, 133.6, 132.7, 131.6, 131.3, 131.2, 130.4, 130.1, 130.1, 129.8, 129.7, 129.5, 128.9, 128.6, 128.1, 127.6, 127.0, 126.8, 126.6, 125.9, 125.3, 110.3, 109.1, 107.1, 103.5, 73.7, 72.3,

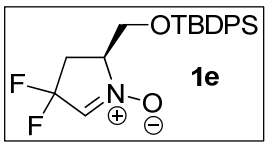
71.5, 69.0, 65.7, 60.4, 45.8, 45.8, 42.1, 40.6, 30.2, 29.6, 29.0, 26.9, 26.8, 26.8, 25.5, 25.4, 21.1, 19.3, 19.2, 14.2. HRMS (ESI+): m/z calculated for $C_{37}H_{40}NO_3Si$ = 574.2777, found 574.2763.

Compounds **3l**:



The reaction mixture was concentrated under reduced pressure and **3l** was purified by flash column chromatography (6:4/Hx:EtOAc, R_f = 0.35). **3l** was obtained as a mixture of regio- and diastereomers as a white solid (17.4 mg, 0.043 mmoles, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67-7.64 (2.1H, m), 7.58-7.55 (1.1H, m), 7.48-7.10 (29H, m), 5.81-5.77 (1.0H, dd, J = 11.6, 5.4 Hz), 5.55-5.23 (2.1H, m), 5.46-5.43 (3.1H, m), 5.09-5.04 (1.0H, dd, J = 10.5, 7.3 Hz), 4.99-4.94 (1.2H, dd, J = 10.1, 8.1 Hz), 4.72-4.47 (3.6H, m), 4.38-4.25 (2.3H, m), 4.17-4.05 (4.7H, m), 3.95-3.78 (7.8H, m), 3.75, 3.74, 3.73, 3.69 (4x3H, s), 3.68-3.43 (8.9H, m), 3.34-3.10 (8.9H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.4, 169.2, 169.1, 169.0, 167.3, 165.8, 165.5, 165.2, 152.2, 149.9, 149.8, 148.7, 144.5, 142.8, 140.4, 140.3, 138.2, 136.5, 136.6, 136.4, 133.8, 132.7, 132.4, 131.7, 131.5, 130.7, 130.4, 130.2, 130.1, 130.0, 130.0, 129.8, 129.7, 129.5, 129.3, 129.2, 129.0, 129.0, 128.8, 128.7, 128.7, 128.6, 128.2, 128.0, 128.0, 127.7, 127.6, 127.5, 127.3, 127.0, 126.8, 126.6, 126.5, 126.4, 126.3, 126.2, 125.9, 125.7, 125.6, 125.4, 125.2, 124.8, 124.5, 110.2, 109.3, 108.5, 105.8, 76.1, 74.2, 72.0, 71.9, 71.8, 71.5, 71.3, 68.1, 65.5, 64.3, 60.4, 57.1, 52.6, 52.5, 52.4, 52.3, 52.0, 51.9, 50.5, 50.2, 50.1, 49.2, 48.8, 48.5, 48.4, 48.3, 45.6, 43.7, 43.6, 43.6, 43.5, 43.1, 42.4, 40.6, 35.1, 30.9, 30.6, 21.0, 19.1, 14.2, 13.7. HRMS (ESI+): m/z calculated for $C_{23}H_{23}N_2O_5$ = 407.1617, found 407.1634.

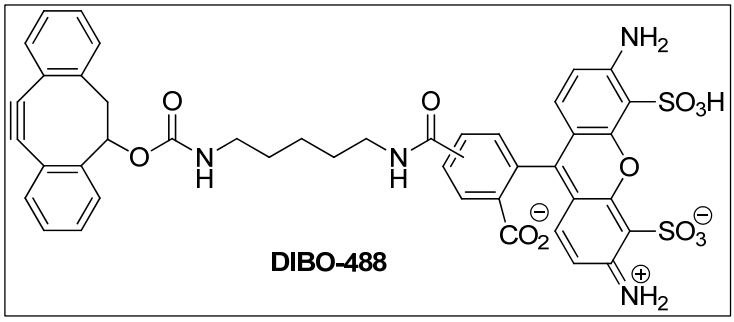
Compound **1e**:



N-Boc-4,4-difluoro-*L*-proline (500 mg, 1.99 mmoles) was dissolved in THF (3.85 mL) and BH_3·THF (3.85mL, 3.85 mmoles from a 1.0 M solution in THF) was added at 0 ˚C. The resultant solution was stirred at 0 ˚C for 2 hrs. Upon completion, ice water (7 mL) was added and the solution was extracted with EtOAc (14 mL x3), the combined organic phases were washed with brine (7 mL), sat'd $NaHCO_3$ (7 mL) and H_2O (7mLx2). The organic phase

was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography (86:6:6/Hx:EtOAc:EtOH, R_f = 0.1). N-Boc-4,4-difluoro-*L*-prolinol was obtained as a colorless oil (467 mg, 1.97 mmoles, 99 %). N-Boc-4,4-difluoro-*L*-prolinol (104 mg, 0.44 mmoles) was dissolved in dry CH_2Cl_2 (4.4 mL) at 0 ˚C. TBDPS-Cl (236 µL, 0.92 mmoles) and Imidazole (66 mg, 0.97 mmoles) were successively added and the reaction was stirred at 0 ˚C for 15 minutes, then was warmed to r.t. and stirred overnight. Upon completion, the reaction mixture was filtered through a plug of celite, diluted with a sat'd NH_4Cl (10 mL) and extracted with CH_2Cl_2 (10mLx3). The combined organic phases where dried over anhydrous Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography (95:5/Hx:EtOAc, R_f = 0.35). This colourless oil (159 mg, 0.33 mmoles) was dissolved in CH_2Cl_2 (4 mL) and TFA (250 µL, 3.3 mmoles), stirred for 2 hrs at r.t. and the solvent was removed under reduced pressure. The crude OTBDPS-*L*-prolinol (~0.3mmoles) was dissolved in MeCN:THF/4:1 (1mL) at 5 ˚C. The EDTA (0.5mL from a 0.01 M solution) and $NaHCO_3$ (252 mg, 3 mmoles) were added. Subsequently over a 2 hr. period, oxone (203 mg, 0.33 mmoles) was added portion wise at 5 ˚C. The reaction mixture was allowed to stir for an additional 20 minutes at 5 ˚C. Upon completion, the mixture was extracted into EtOAc (3x5 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated. Crude **1e** was separated from the keto-regioisomer by reverse phase preparative HPLC. Eluting 70-80 % MeCN:H_2O during 15 minutes (column: Sunfire C_{18} 19 x 100 mm, flow rate = 17.0 mL/min). ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.70-7.65 (4H, m), 7.46-7.39 (6H, m), 7.09 (1H, m), 4.44 (1H, dd, J = 11.3, 1.5 Hz), 4.20 (1H,m), 3.63 (1H, dd, J = 11.3, 1.9 Hz), 3.07-2.95 (1H, m), 2.85-2.73 (1H, m), 1.05 (9H, s). ^{13}C NMR ($CDCl_3$, 100MHz): δ 135.6, 135.5, 132.8, 132.1, 130.0, 127.8, 127.8, 73.3, 59.9, 55.6 (t), 26.5, 19.3. MS (ESI+): m/z calculated for $C_{21}H_{26}F_2NO_2Si$ = 390.17, found 390.2.

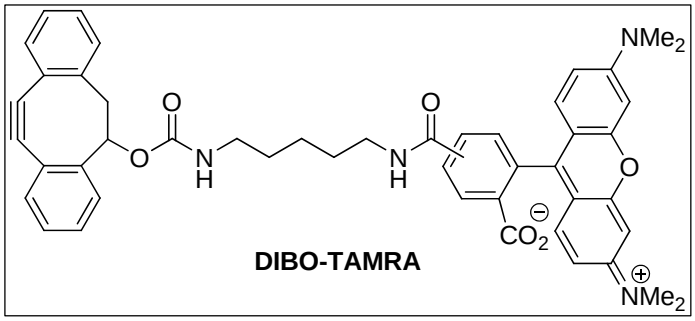
Compound **DIBO-488**:



DIBO-488

Alexa Fluor® 488 cadaverine, sodium salt (1mg, 0.00156 mmoles) was dissolved in a dry DMF (0.2 mL) solution containing N,N-diisopropyl amine (1.4 µL, 0.0078 mmoles). Then 4-nitrophenyl chloroformate dibenzocyclooctyne[7] (0.8mg, 0.00312 mmoles) was added in at r.t. and the reaction was stirred overnight. The solvent was removed by rotary evaporation and **DIBO-488** was purified by reverse-phase preparative HPLC. Eluting 10-95% MeCN+0.1%TFA:H_2O+0.1%TFA gradient in 10 minutes. Column: Sunfire C_{18}, 19x100mm, flow rate: 17.0 mL/min. **DIBO-488** was obtained as a dark orange solid (0.8 mg, 0.0009 mmoles, 58 %). MS (ESI+): m/z calculated for $C_{43}H_{37}N_4O_{12}S_2$ = 865.18, found 865.2.

Compound **DIBO-TAMRA**:



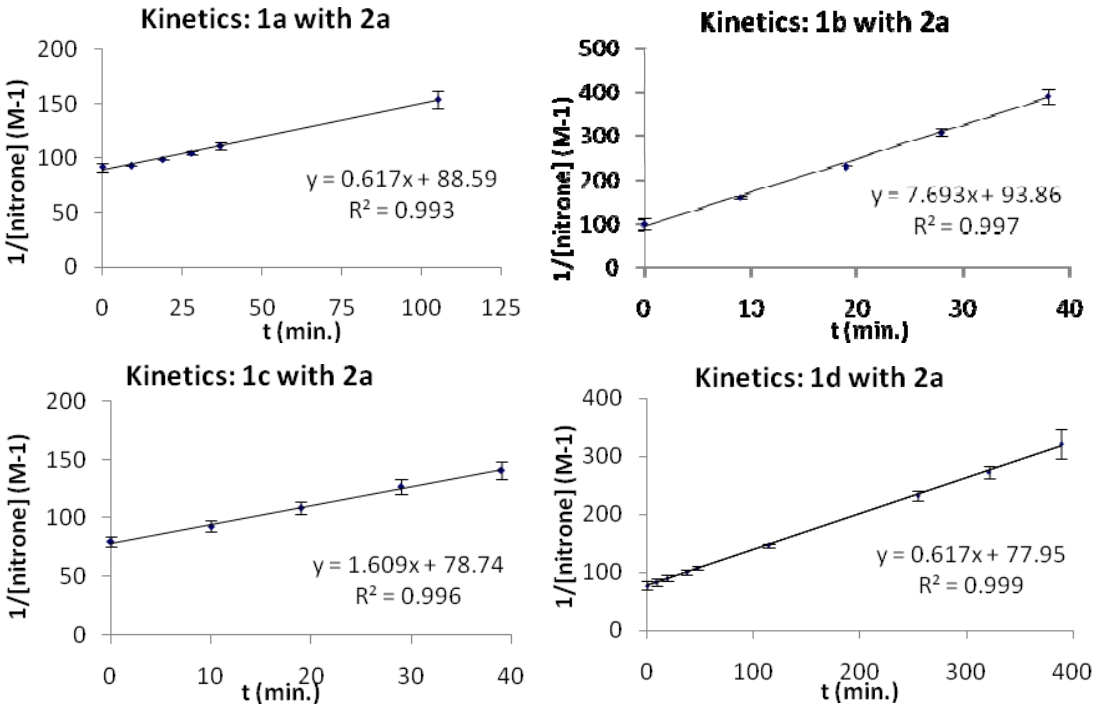
DIBO-TAMRA

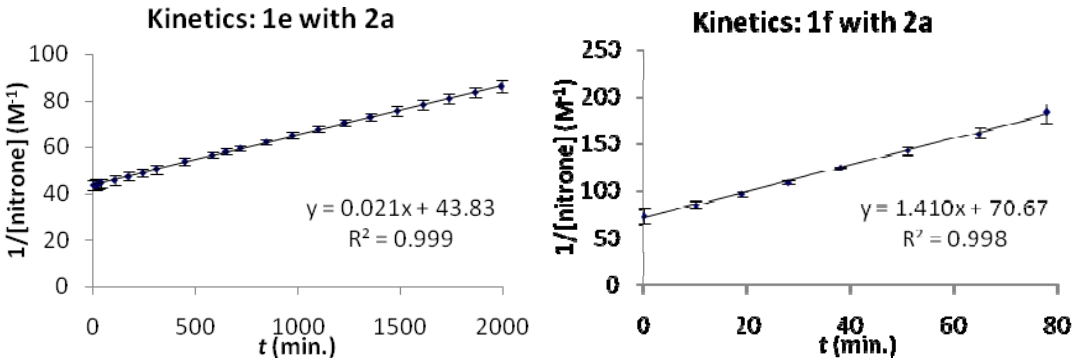
5-(and-6)-((*N*-(5-aminopentyl)-amino)carbonyl)tetramethylrhodamine (10 mg, 0.019 mmoles) was dissolved in DMF (0.5 mL). Then 4-nitrophenyl chloroformate dibenzocyclooctyne[7] (11.2 mg, 0.029 mmoles) and Et_3N (8 µL, 0.057 mmoles) were added. The reaction was allowed to stir at r.t. in the absence of light for 4 hrs. After the allotted time, the solvent was removed under reduced pressure. **DIBO-TAMRA** was purified by reverse-phase preparative HPLC. Eluting 40-65% MeCN+0.1%TFA/H_2O+0.1%TFA gradient in 20 minutes. Column: Sunfire C_{18} 19x100mm, flow rate: 17.0 mL/min. **DIBO-TAMRA** was obtained as a dark purple solid (11.9 mg, 0.015 mmoles). MS (ESI+): m/z calculated for $C_{47}H_{45}N_4O_6$ = 761.33, found 761.3.

Kinetics measurements of SPANC by ^{1}H NMR:

The appropriate cyclic nitrone (**1a-f**) and dibenzocyclooctyne (**2a** or **2b**) were pre-dissolved in either C_6D_6 or CD_3CN and mixed at equimolar concentrations of ~25mM. Percent conversion was monitored both by disappearance of starting materials and by appearance of product as determined by integration at multiple chemical shifts in the ^{1}H NMR spectrum. No other products were detected by ^{1}H NMR and all reactions were performed in triplicate. Second order rate constants in units of $M^{-1}s^{-1}$ were determined by plotting 1/[nitrone] versus time and subsequently using analysis by linear regression. The second order rate constant k_2 ($M^{-1}s^{-1}$) corresponds to the determined slope.

(a) Reactions of cyclic nitrones (**1a-1e**) with Dibenzocyclooctyne (**2a**) in C_6D_6 (25mM).





(b) Reactions of cyclic nitrones **(1a, 1b, 1c** and **1e)** with DIBO (**2b**) in CD_3CN (25mM).

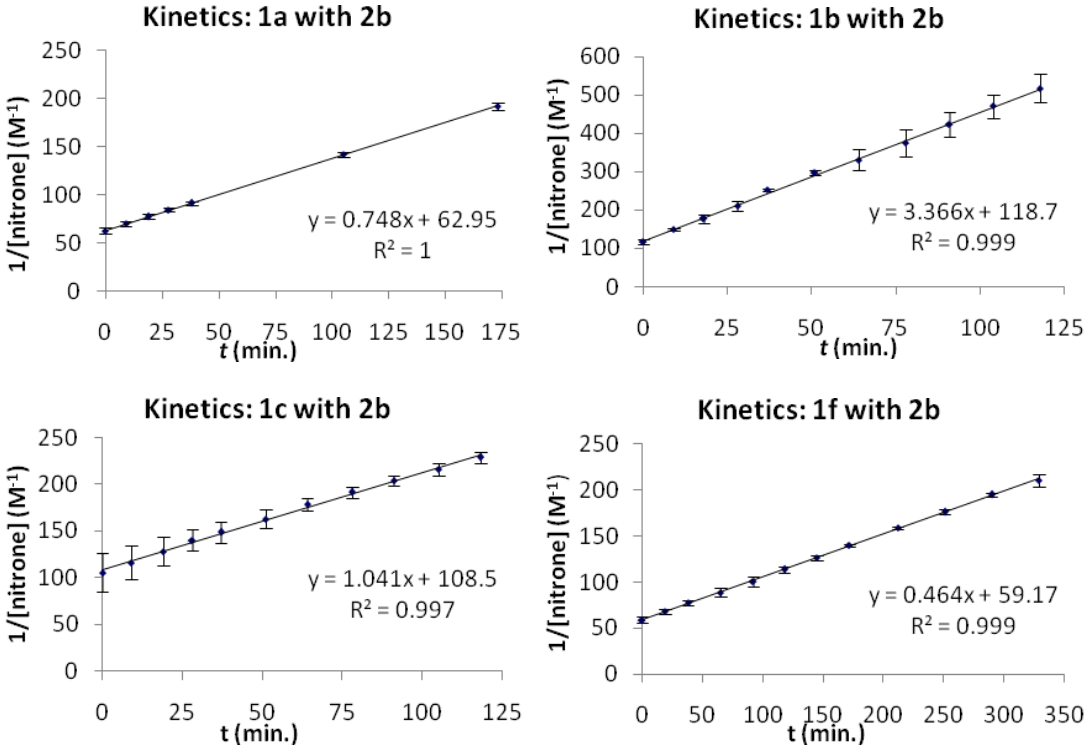
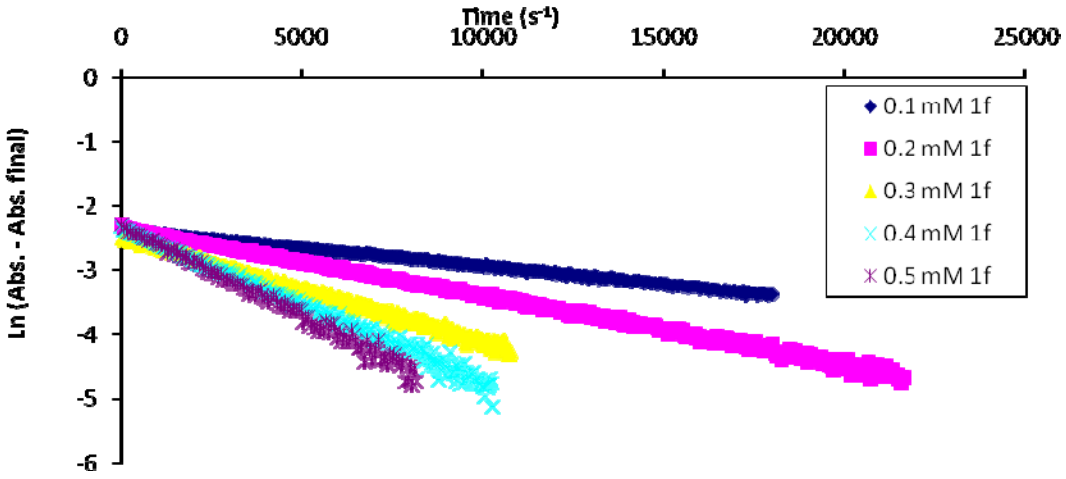


Figure S1. Kinetic studies of Doubly-Strained SPANC by ^{1}H NMR. (a) Reactions of cyclic nitrones (**1a-1e**) with Dibenzocyclooctyne (**2a**) in C_6D_6 (25mM). (b) Reactions of cyclic nitrones **(1a, 1b, 1c** and **1e)** with DIBO (**2b**) in CD_3CN (25mM).

Kinetics measurements of SPANC by absorption spectroscopy:

Cyclic nitrone **1f** and dibenzocyclooctyne (**2a** or **2b**) were pre-dissolved in either toluene or acetonitrile and mixed at 25°C. This was repeated for multiple excess concentrations of **1f**. For each sample the absorbance at 305 nm (Abs.) was monitored until reaction was complete as determined by no further change in absorbance. Pseudo first order rate constants (k_{obs}) in units of s^{-1} were determined as the slope obtained by plotting Ln(Abs. – Abs. final) versus time (Figure S2 a). The second order rate constant k_2 ($M^{-1}s^{-1}$) was determined by plotting k_{obs} against the concentration of **1f** (Figure S2 b); the slope of this line corresponds to k_2.

(a)



(b)

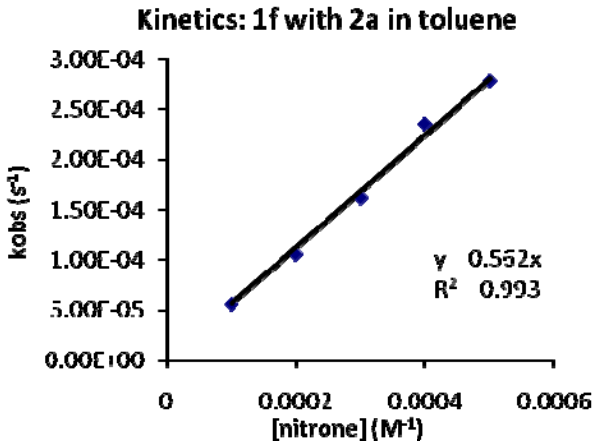
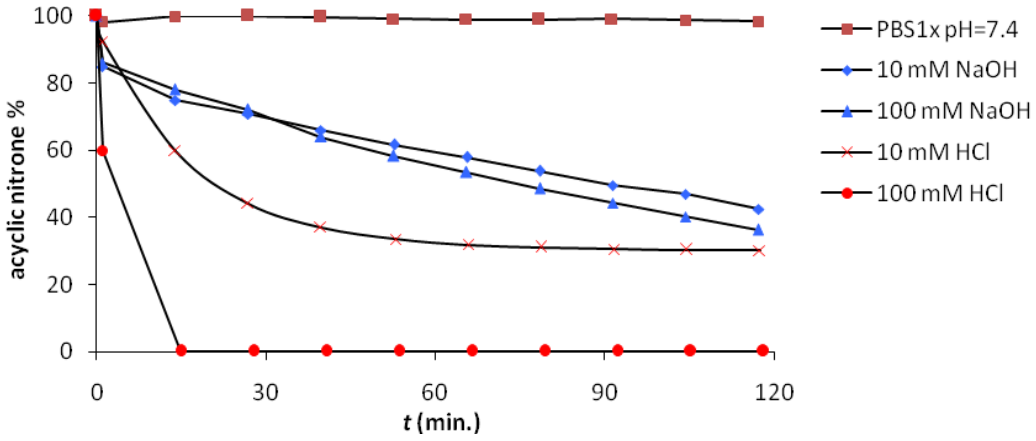


Figure S2. Kinetic studies of SPANC by absorption spectroscopy. (a) Reaction of cyclic nitrones (**1f**) (0.1, 0.2, 0.3, 0.4, 0.5 mM) with DIBCO (**2a**, 5 μM) in acetonitrile. (b) Determination of second order rate constant k_2 ($M^{-1}s^{-1}$) from multiple pseudo-first order rate constants ($k = k_{obs}$[**1f**]).

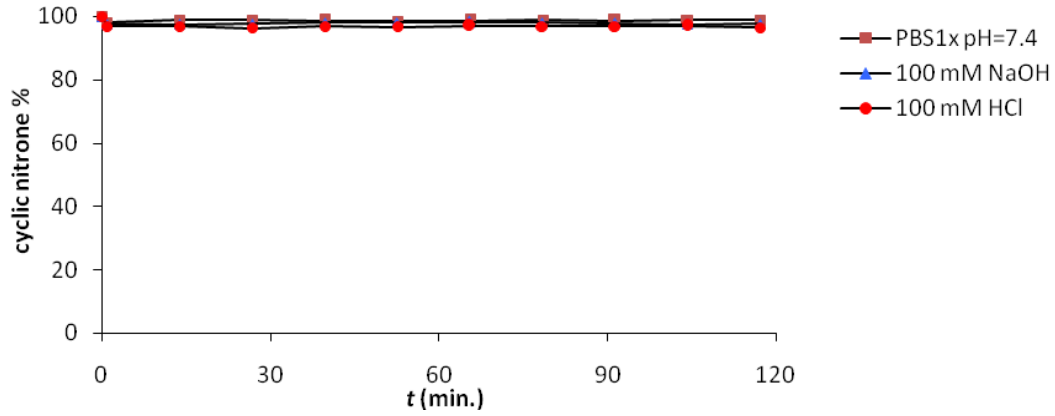
Hydrolysis studies of acyclic and cyclic nitrones at variable pH of solution:

The acyclic nitrone[7] (20 µL, 0.01 mmoles from a 500 mM stock in DMSO) or cyclic nitrone (20 µL, 0.01 mmoles from a 500 mM stock in DMSO) was added to either PBS 1x (980 µL), 10 mM NaOH (980 µL), 100 mM NaOH (980 µL), 10 mM HCl (980 µL) or 100 mM HCl (980 µL). Hydrolysis was monitored by disappearance of the acyclic nitrone as determined by reverse phase analytical HPLC. The concentration of nitrone remaining (nitrone %) was determined by substituting the peak area from the HPLC chromatogram into the equations of nitrone calibration curves.

(a) Hydrolysis of Acyclic nitrone (10mM) in the presence of PBS 1x, 10mM NaOH, 100 mM NaOH, 10 mM HCl and 100 mM HCl.



(b) Hydrolysis study of Cyclic nitrone **2a** (10mM) in the presence of PBS 1x, 100 mM NaOH or 100 mM HCl.



(c) Calibration curves for acyclic and cyclic nitrones.

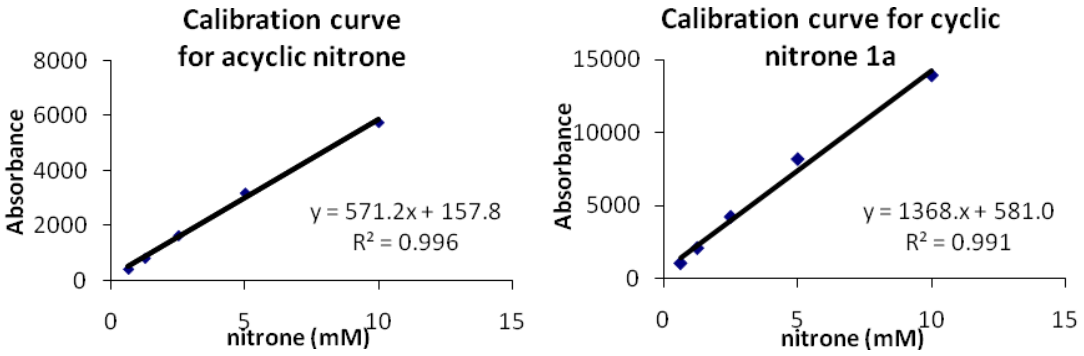
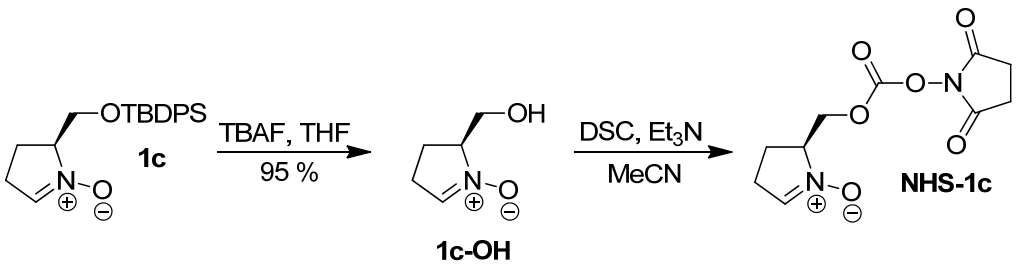


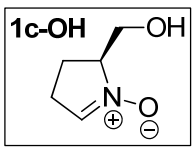
Figure S3. Hydrolytic stabilities of acyclic and cyclic nitrones at variable pH. (a) Reaction of acyclic nitrone[5] (10mM) in the presence of PBS1x, 10mM NaOH, 100 mM NaOH, 10 mM HCl and 100 mM HCl for 2 hrs. (b) Cyclic nitrone **2a** (10mM) in the presence of PBS 1x, 100 mM NaOH or 100 mM HCl. (c) Plot of absorption versus time for acyclic and cyclic nitrones at a range of concentration (0.625 mM, 1.25 mM, 2.5 mM, 5.0 mM and 10.0 mM).

General procedure for *in vitro* SPANC labeling of BSA and EGF:

A. Preparation of NHS-1c:



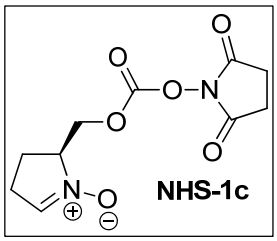
Compound **1c-OH**:



To a stirring solution of **1c** (39.2 mg, 0.11 mmoles) in dry THF (1.5 mL) was added TBAF (132 µL, 0.13 mmoles from a 1.0 M solution in THF). The resultant solution was stirred at r.t. for 3hrs. Upon completion, the solvent was removed by rotary evaporation and the crude orange oil was purified by flash column chromatography (85:15/CH_2Cl_2:CH_3OH, R_f = 0.3). **1c-OH** was isolated as an off white solid (12.0 mg, 0.10 mmoles, 95 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.93 (1H, m),

4.15 (1H, m), 4.02 (1H, dd, J = 12.1, 2.8 Hz), 3.80 (1H, dd, J = 12.1, 6.9 Hz), 2.70-2.66 (2H, m), 2.36-2.30 (1H, m), 2.02-1.97 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.9, 72.2, 63.8, 27.1, 21.4. MS (ESI+): m/z calculated for $C_5H_{10}NO_2$ = 116.07, found 116.1.

Compound **NHS-1c**:



To a stirring solution of 1c-OH (3.3 mg, 0.029 mmoles) in dry CH_3CN (0.29 mL), was added DSC (8.7 mg, 0.034 mmoles) and Et_3N (5.2 μL, 0.037 mmoles). The reaction was stirred overnight under an atmosphere of argon. Upon completion, **NHS-1c** was used directly for coupling with BSA or EGF.

B. Cyclic nitrone modification of BSA or EGF *via* NHS coupling:

The NHS-activated nitrone, **NHS-1c** (2 μL, 200 nmoles from a 100 mM freshly prepared stock in CH_3CN) was added to a solution of BSA (10 nmoles) or EGF (8 nmoles) in phosphate buffered saline pH =7.4 (100 μL, PBS: 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4). The reaction was incubated on a shaking platform over night at r.t. Upon completion, in the first case, **BSA-1c** was first diluted with PBS (15 mL) and then concentrated in an Amicon Ultra-15 centrifugal filter device (Millipore) with a 10-kDa molecular weight cut off (MWCO) by centrifugation at 4000 g. In the second case, **EGF-1c** was diluted with PBS (15 mL) in an Amicon Ultra-15 filter device (Millipore) with a 3-kDa MWCO and was concentrated by centrifugation at 4000 g. In both cases, the process was repeated twice more to remove excess **NHS-1c**, and coupling reagents and byproducts from the NHS activation of **1c-OH**. The samples were concentrated to final volume of 200-500 μL. The final concentration of **BSA-1c** was determined by a Bio-Rad Bradford Protein Assay (BIO-RAD), against BSA standards. The final concentration of **EGF-1c** was calculated based on the volume remaining in device. MALDI-MS of **BSA-1c** and unmodified BSA samples indicate that there are on average four cyclic nitrones incorporated (**Figure S4.** (a) and (b) respectively). The MALDI-MS of **EGF-1c** and EGF suggests that there were one and two nitrone groups incorporated (**Figure S5.** (a) and (b) respectively).

C. *In vitro* SPANC labeling of BSA-1c with DIBO-488:

The crude **BSA-1c** (7.36 µL, 0.25 nmoles from a 34 µM stock in PBS) was diluted with PBS (993 µL) and was reacted with **DIBO-488** (1.25 µL, 1.25 µmoles from a 100 mM stock solution in PBS). The reaction was incubated on a shaking platform at r.t. in the absence of light for 0-60 minutes. During this time course experiment, a negative control (-) sample contained BSA (0.25 nmoles) solution in PBS (1 mL) in the presence of **DIBO-488** (1.25µmoles). This was used to confirm specific time dependent labeling of BSA via SPANC. At each time point, a 40 µL aliquot from the SPANC reaction and negative control was quenched with cyclic nitrone **1b** (2 µL, 200µmoles from a 100 mM stock solution in DMSO). Each quenched BSA sample (20 µL) and negative control (20 µL) was diluted with 4 µL of 6X sodium dodecyl sulphate polyacrylamide gel electrophoresis sample buffer (SDS-PAGE, 300 mM Tris pH 6.8, 12% w/v SDS, 60% glycerol, 600 mM dithiothreitol) and was heated at 95 °C for 10 minutes. The samples and negative controls were separated by 10% Tris-glycine SDS-PAGE. After electrophoresis, the gel was imaged using the Fluorescent Method Bio Image Analyzer FMBIO III (Hitachi, Tokyo, Japan). The band intensities corresponding to fluorescently labeled BSA (**Figure S4.** (c) upper gel) were quantified with ImageJ software (National Institutes of Health, Bethesda, MD). After the fluorescence measurement, the same gel was silver stained for protein content (**Figure S4.** (c) lower gel).

D. *In vitro* SPANC labeling of EGF-1c with DIBO-TAMRA:

The crude **EGF-1c** (64.8 µL, 1.25 nmoles from a 19.3 µM stock in PBS) was diluted with PBS (435.2 µL) and was reacted with **DIBO-TAMRA** (62.5 µL, 6.25 µmoles from a 100 µM stock solution in DMSO). The reaction was incubated on a shaking platform at r.t. in the absence of light for 0-60 minutes. During this time course experiment, a negative control (-) sample contained EGF (1.25 nmoles) solution in PBS 1x (0.5 mL) in the presence of **DIBO-TAMRA** (6.25 µmoles). At each time point, a 40 µL aliquot from the SPANC reaction and negative control was quenched with cyclic nitrone **1b** (2 µL, 200µmoles from a 100 mM stock solution in DMSO). Each quenched EGF sample (20

μL) and negative control (20 μL) was diluted with 4 μL of 6X sodium dodecyl sulphate polyacrylamide gel electrophoresis sample buffer (SDS-PAGE, 300 mM Tris pH 6.8, 12% w/v SDS, 60% glycerol, 600 mM dithiothreitol) and was heated at 95 °C for 10 min. The samples and negative controls were separated by a 16% tricine SDS-PAGE gel. After electrophoresis, the gel was imaged using the Fluorescent Method Bio Image Analyzer FMBIO III (Hitachi, Tokyo, Japan). The band intensities corresponding to fluorescently labeled EGF (**Figure S5.** (c) upper gel) were quantified with ImageJ software (National Institutes of Health, Bethesda, MD). After the fluorescence measurement, the same gel was silver stained for protein content (**Figure S5.** (c) lower gel).

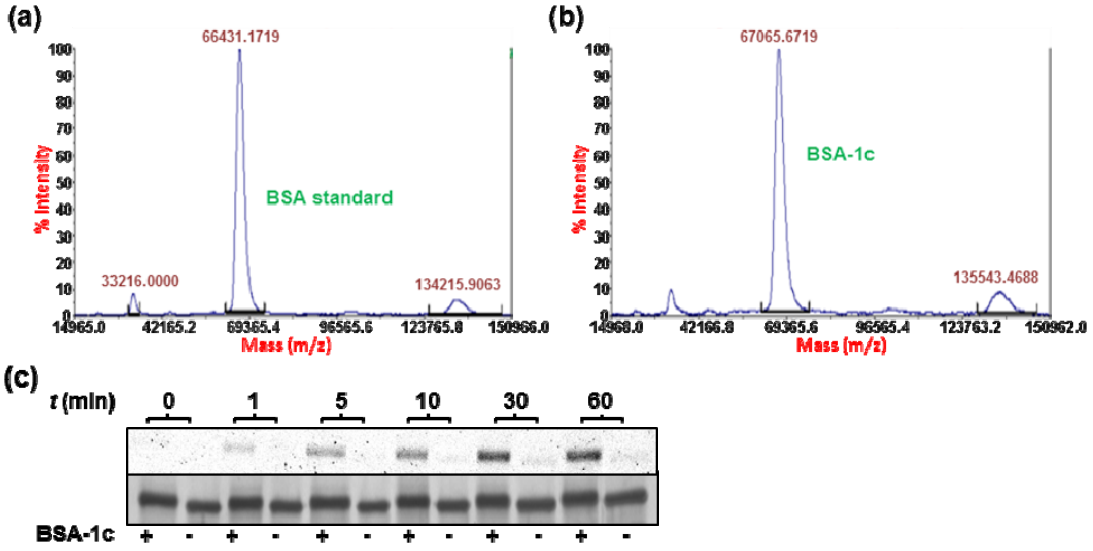


Figure S4. Modification of BSA with **NHS-1c** and subsequent SPANC labeling with **DIBO-488**. (a) MALDI MS of BSA. (b) MALDI MS post incubation with **NHS-1c**. (c) Time course labeling of BSA by SPANC was detected by SDS-PAGE with fluorescence scan (upper gel) and silver stain as the loading control (lower gel).

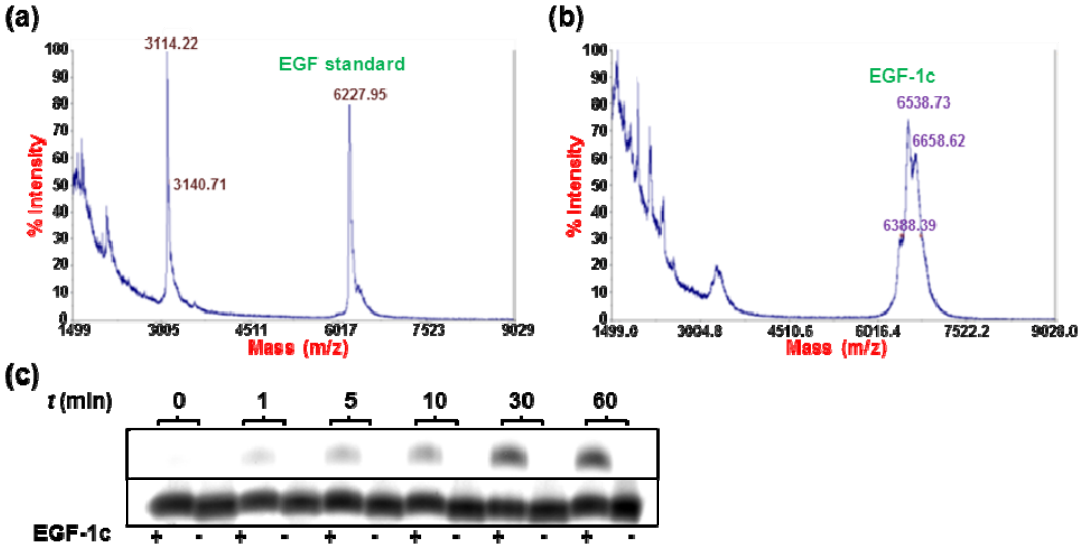


Figure S5. Modification of EGF with **NHS-1c** and subsequent SPANC labeling with **DIBO-TAMRA**. (a) MALDI MS of EGF. (b) MALDI MS post incubation with **NHS-1c**. (c) Time course labeling of EGF by SPANC was detected by SDS-PAGE with fluorescence scan (upper gel) and silver stain as the loading control (lower gel).

***In situ* imaging of cancer cells via SPANC:**

Cell culture:

The MDA-MB-468 (breast cancer) cell line was maintained in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS; CANSERA), 50 U/mL penicillin and 50 µg/mL streptomycin in a humidified incubator at 37°C with 100% atmospheric air.

Fixed cell labeling of EGFRs by SPANC:

MDA-MB-468 cells were seeded at 20 x 10^4 cells/well in 12 well tissue culture plates in 1 mL of Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS; CANSERA), 50 U/mL penicillin and 50 µg/mL streptomycin. After 24 hours, appropriate samples were treated with BSA (1% in serum free medium) for 30 minutes at r.t. to block non-specific binding of EGF. The samples were subsequently, treated with

cyclic nitrone **1c**-modified EGF (1 μM) and incubated for 5 minutes at 4˚C. In parallel, the negative control samples were treated with unmodified EGF (1 μM). The cells were washed with media (3X) and PBS (3X) and treated with DIBO-biotin (10 μM) for 30 minutes at r.t. in serum-free media. The cells were washed with media (3X) and PBS (3X), blocked with 1% BSA (in serum free media) for 30 minutes at room temperature, and then stained with FITC-streptavidin (5 μg/mL in PBS) for 30 minutes at room temperature. Cells were then washed with media (2X) and PBS (2X), and then fixed by applying fixing solution (4% paraformaldehyde, 4% sucrose in dH_2O) for 15 minutes at 4˚C. Once the coverslips were mounted on slides, the cells were imaged with an Olympus 1X81 spinning-disk confocal microscope equipped with a FITC filter (Semrock, Excitation: 465-499nm, Emission: 516-556 nm) and a Photometrics (Coolsnap ES) camera using 100x magnification. Images were taken of samples and controls using both bright-field and the FITC channel (1 second exposure). Image processing was done using ImageJ software, applying pseudocolour to FITC channel images. The same pixel-intensity ranges were applied and displayed for all images taken.

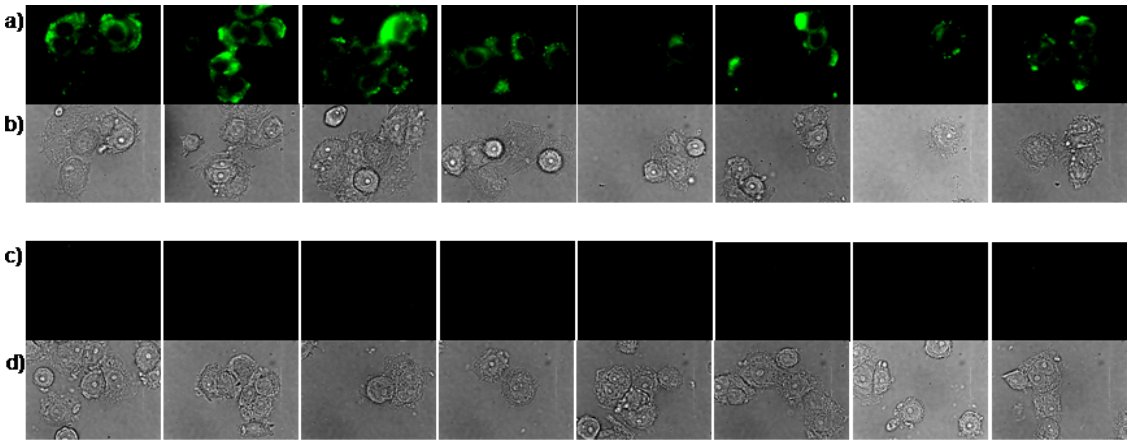
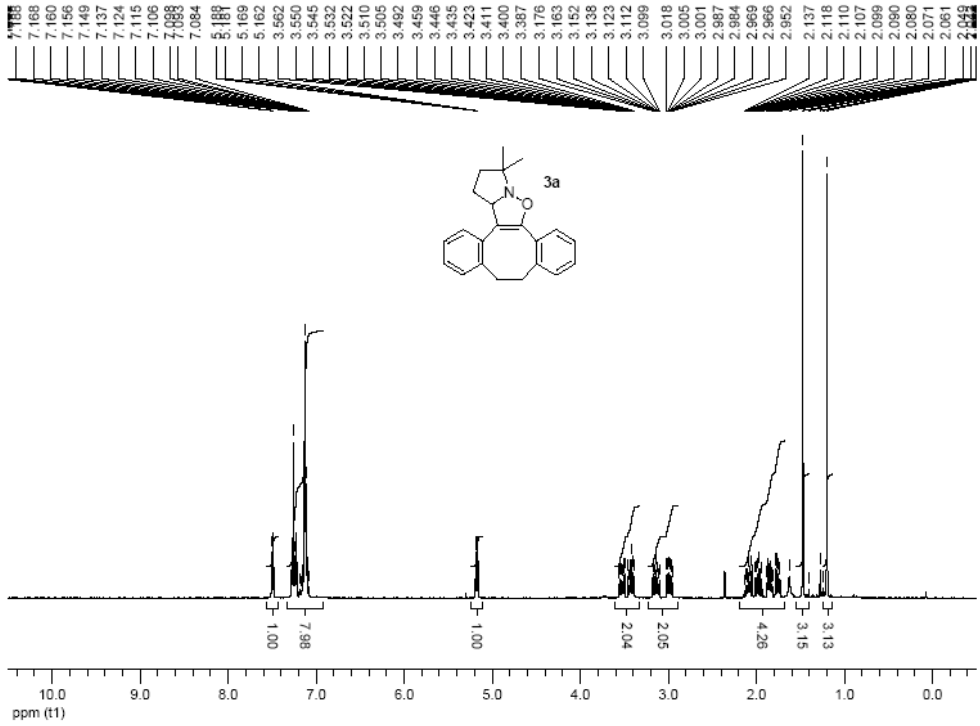
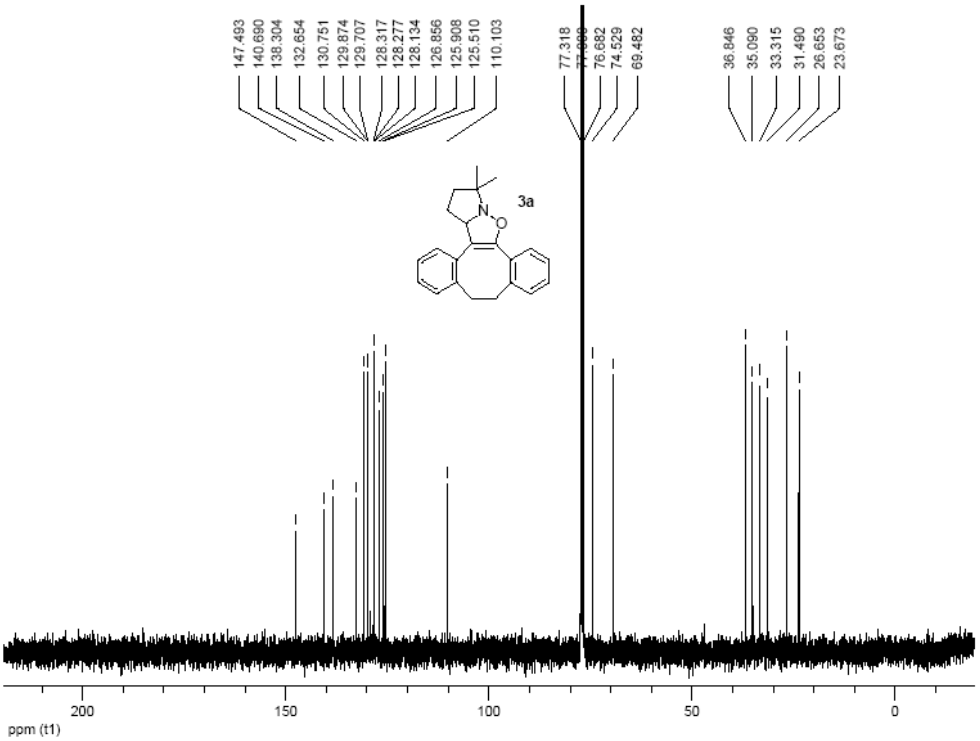
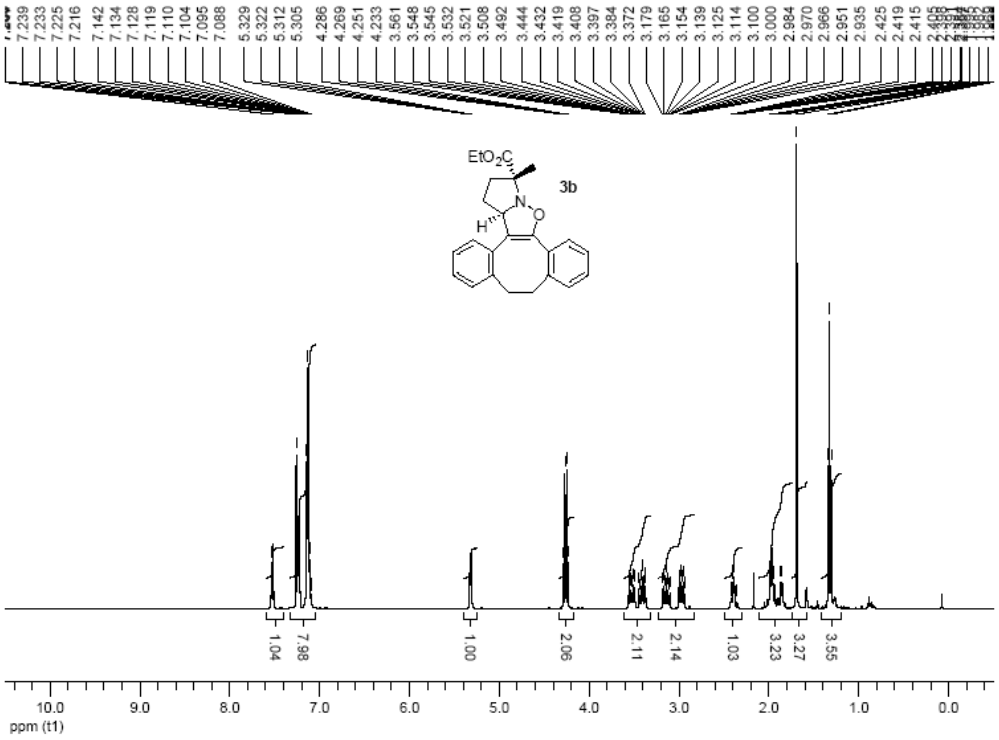


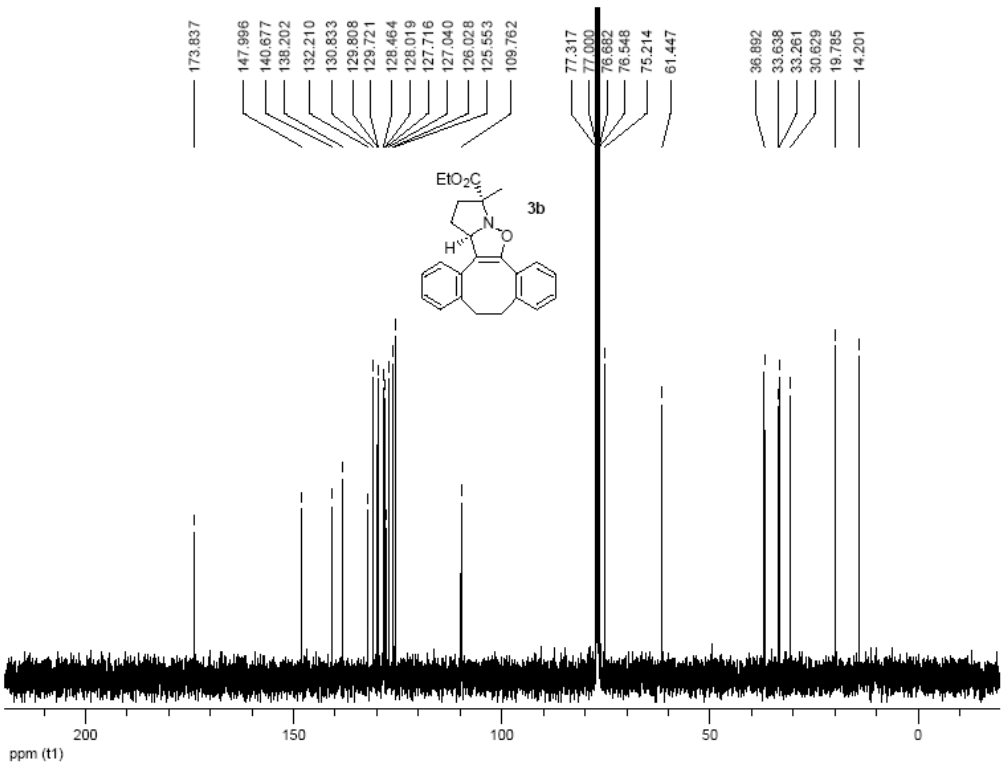
Figure S6. Fixed cell imaging of EGFRs via SPANC. Highly specific fluorescent labeling for 8 fields of view (a and b, n = 41) relative to the negative controls (c and d, n = 47). For each image, fluorescence mode is on top and bright field is on the bottom (b and d). The labeling experiment was repeated once with consistent results.

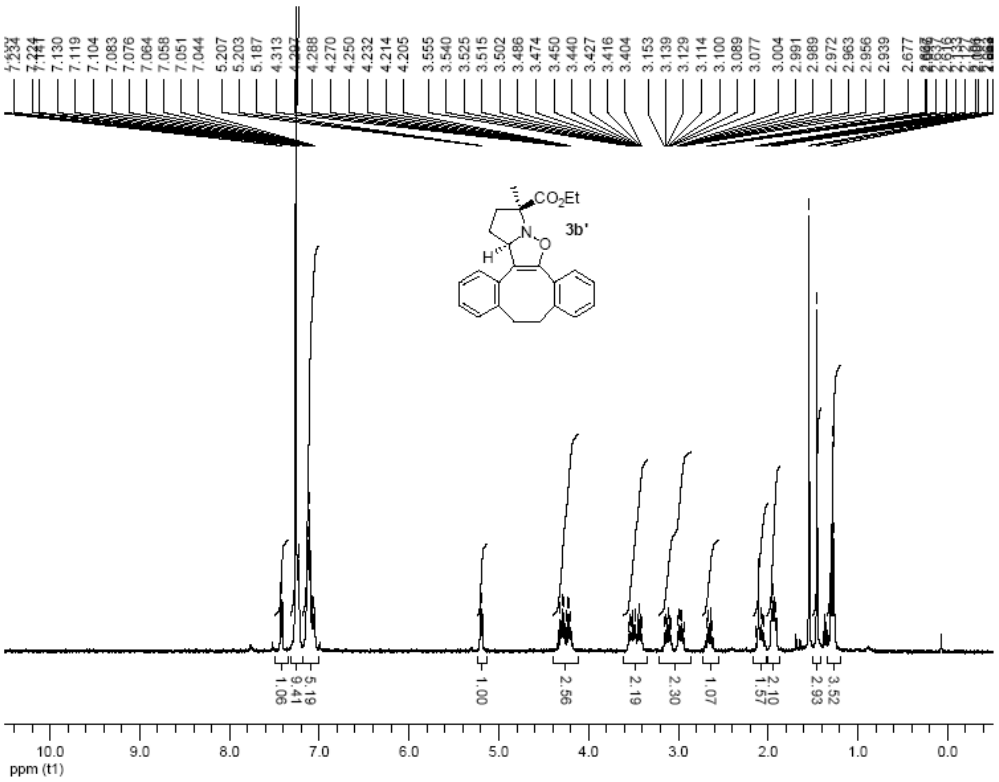
^{1}H and ^{13}C NMR Spectra:

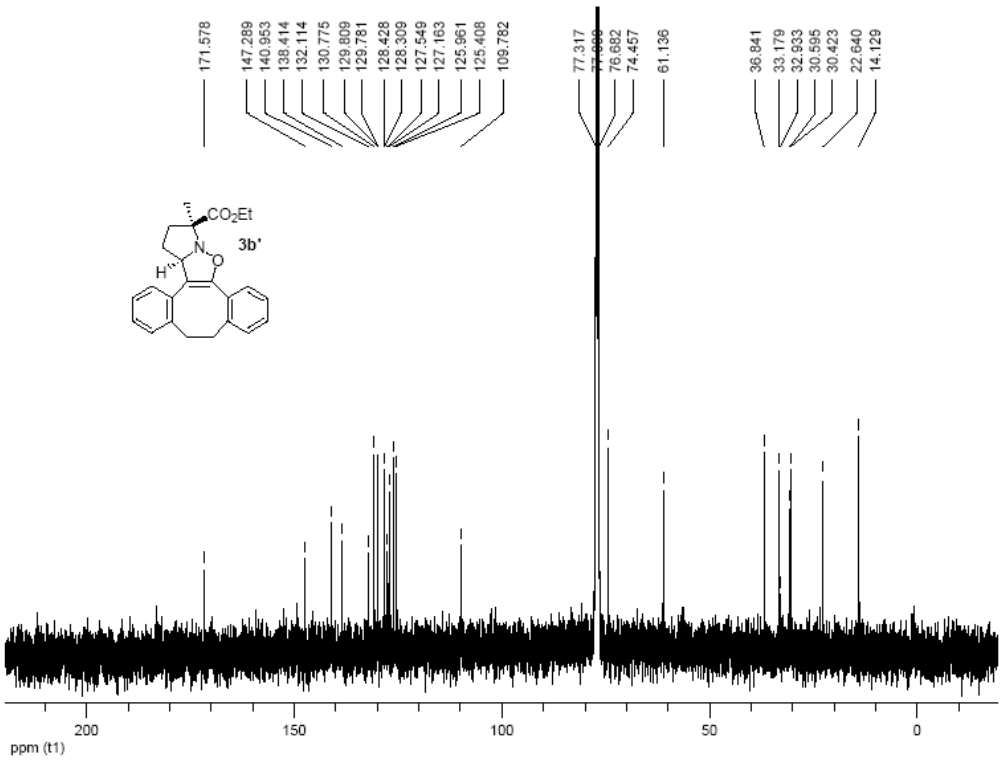


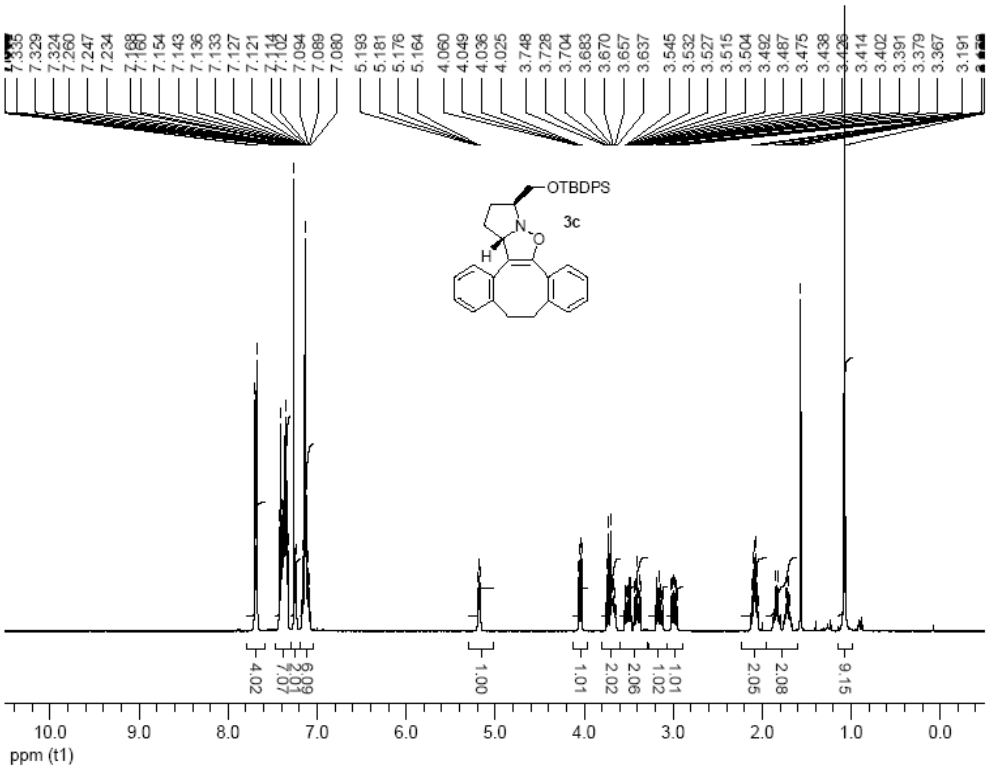


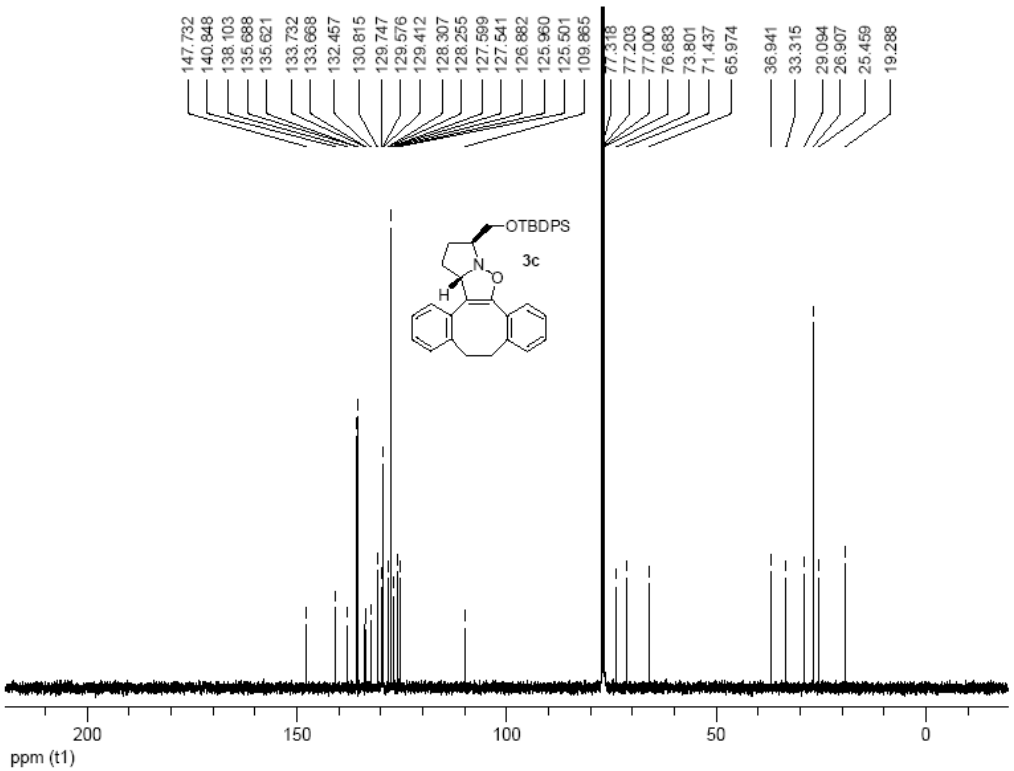

7.239
7.233
7.225
7.216
7.142
7.134
7.128
7.119
7.110
7.104
7.095
7.088
5.329
5.322
5.312
5.305
4.286
4.269
4.251
4.233
3.561
3.548
3.545
3.532
3.521
3.508
3.492
3.444
3.432
3.419
3.408
3.397
3.384
3.372
3.179
3.165
3.154
3.139
3.125
3.114
3.100
3.000
2.984
2.970
2.966
2.951
2.935
2.425
2.419
2.415
EtO2C
3b
N
O
H
1.04
7.98
1.00
2.06
2.11
2.14
1.03
3.23
3.27
3.55
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

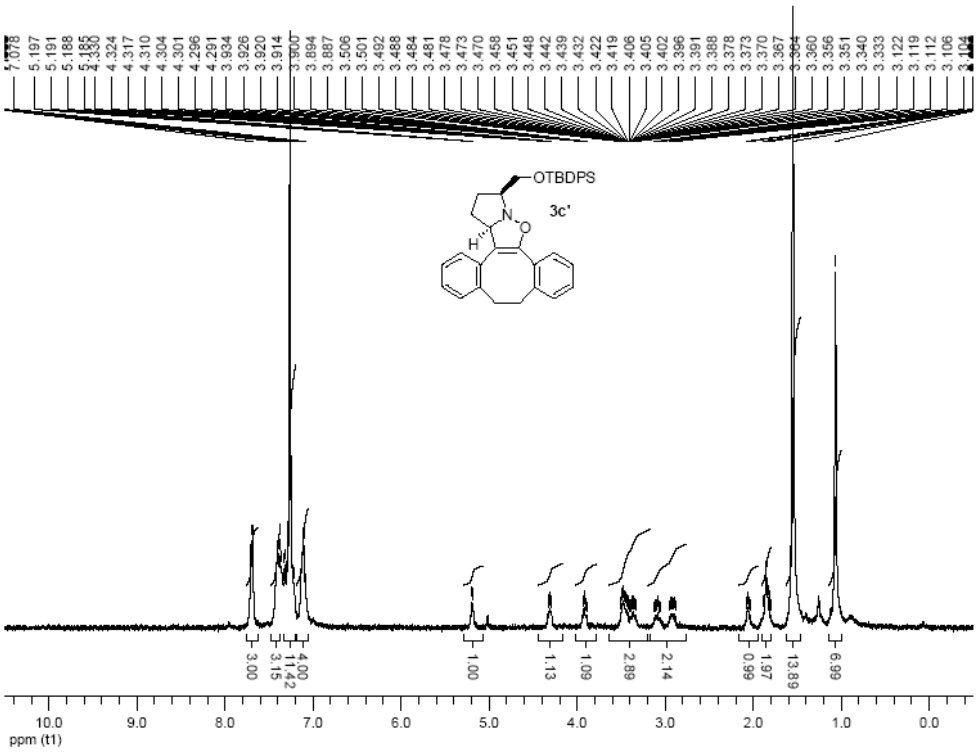

173.837
147.996
140.677
138.202
132.210
130.833
129.808
129.721
128.464
128.019
127.716
127.040
126.028
125.553
109.762
77.317
77.000
76.682
76.548
75.214
61.447
36.892
33.638
33.261
30.629
19.785
14.201
EtO2C
3b
N
O
H
200
150
100
50
0
ppm (t1)

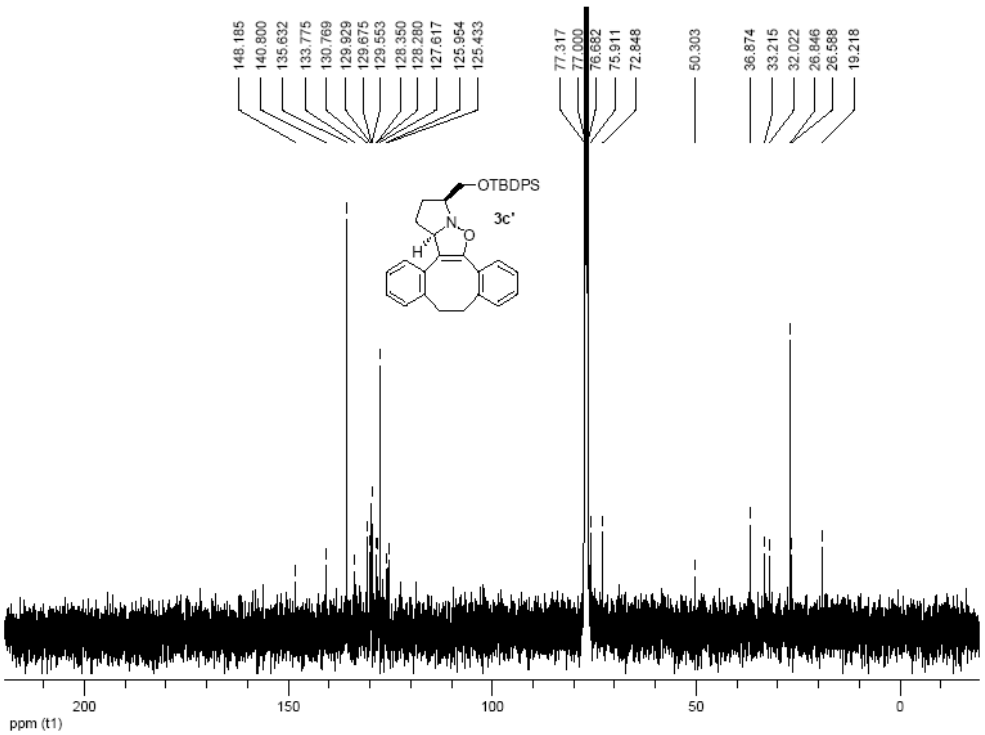

7.234
7.130
7.119
7.104
7.083
7.076
7.064
7.058
7.051
7.044
5.207
5.203
5.187
4.313
4.288
4.270
4.250
4.232
4.214
4.205
3.555
3.540
3.525
3.515
3.502
3.486
3.474
3.450
3.440
3.427
3.416
3.404
3.153
3.139
3.129
3.114
3.100
3.089
3.077
3.004
2.991
2.989
2.972
2.963
2.956
2.939
2.677
CO2Et
3b'
H
N
O
1.06
1.41
1.19
1.00
2.66
2.19
2.30
1.07
1.57
2.10
2.93
3.52
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

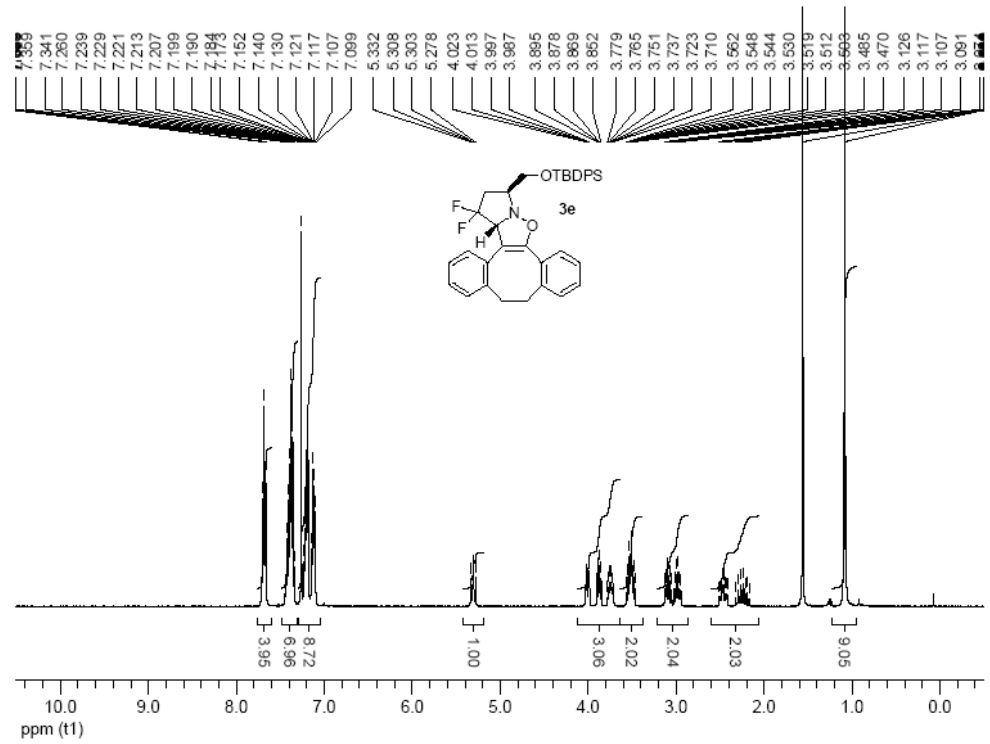

171.578
147.289
140.953
138.414
132.114
130.775
129.809
129.781
128.428
128.309
127.549
127.163
125.961
125.408
109.782
77.317
76.682
74.457
61.136
36.841
33.179
32.933
30.595
30.423
22.640
14.129
CO2Et
3b'
H
N
O
200
150
100
50
0
ppm (t1)

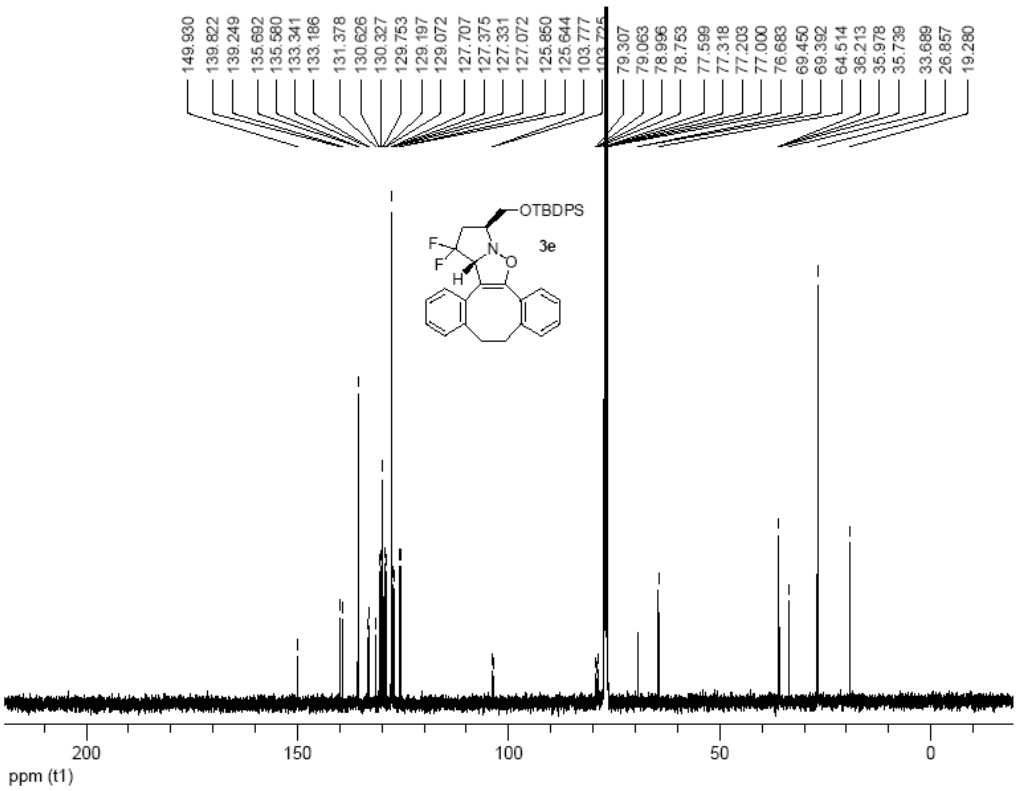

OTBDPS
3c
ppm (t1)

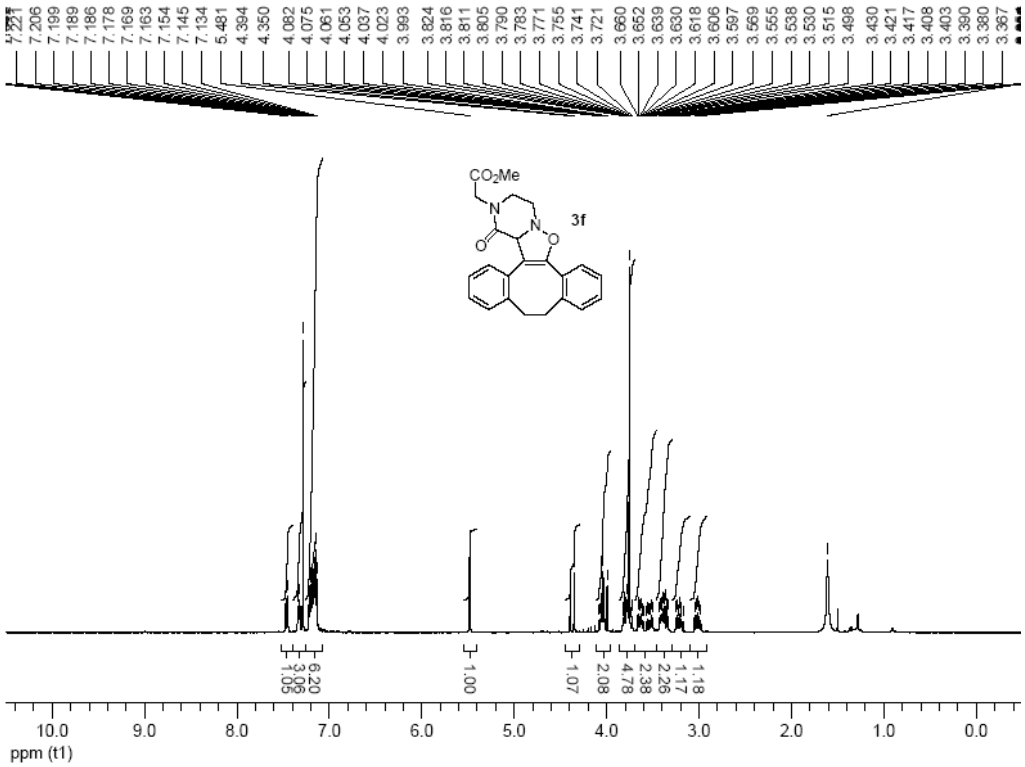

OTBDPS
3c
ppm (t1)

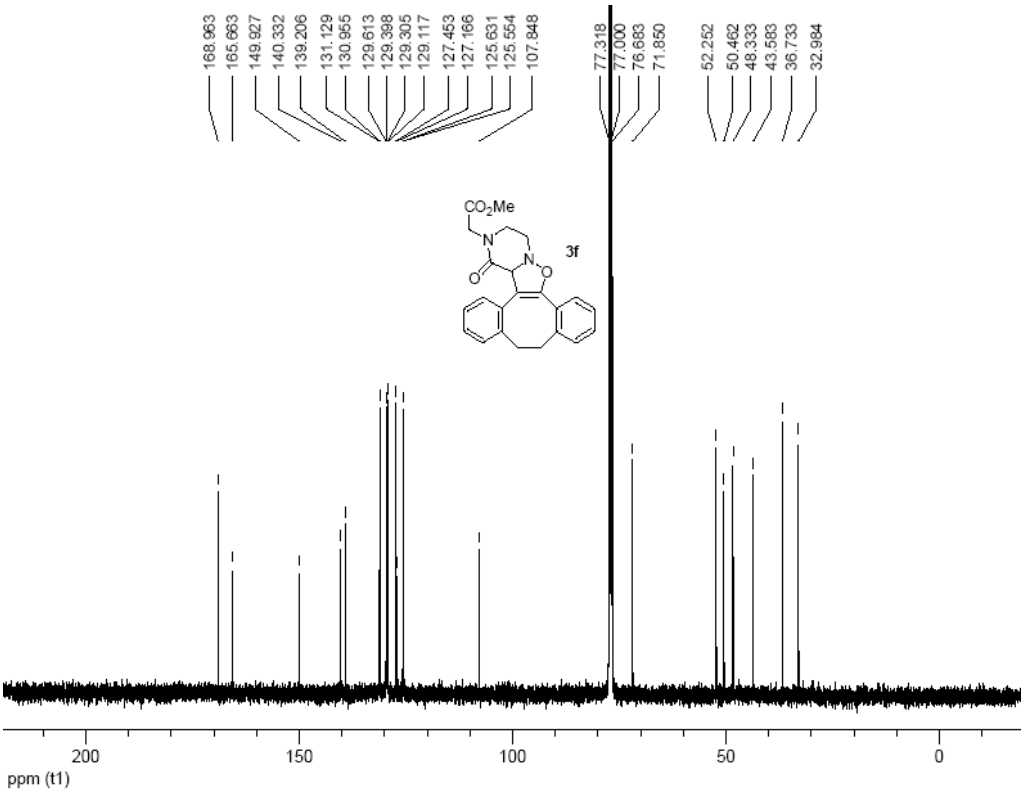

OTBDPS
3c'
3.00
3.15
1.12
4.00
1.00
1.13
1.09
2.89
2.14
0.99
1.97
13.89
6.99
ppm (t1)

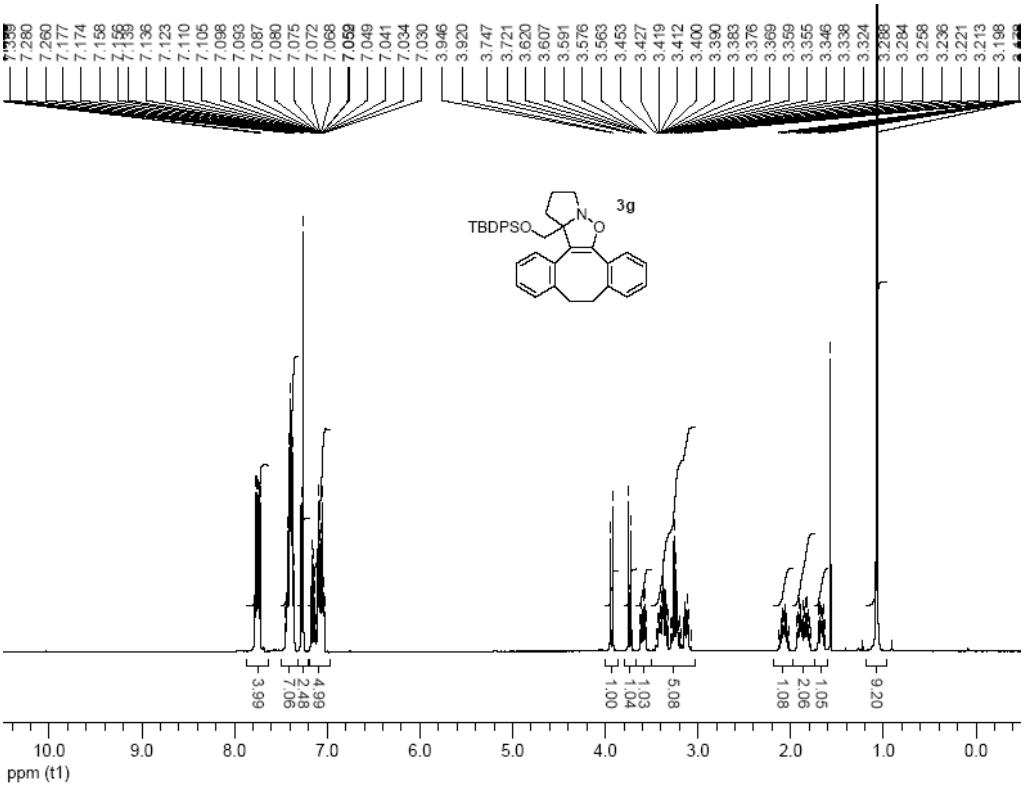

148.185
140.800
135.632
133.775
130.769
129.929
129.675
129.553
128.350
128.280
127.617
125.954
125.433
77.317
77.000
76.682
75.911
72.848
50.303
36.874
33.215
32.022
26.846
26.588
19.218
OTBDPS
3c'
ppm (t1)

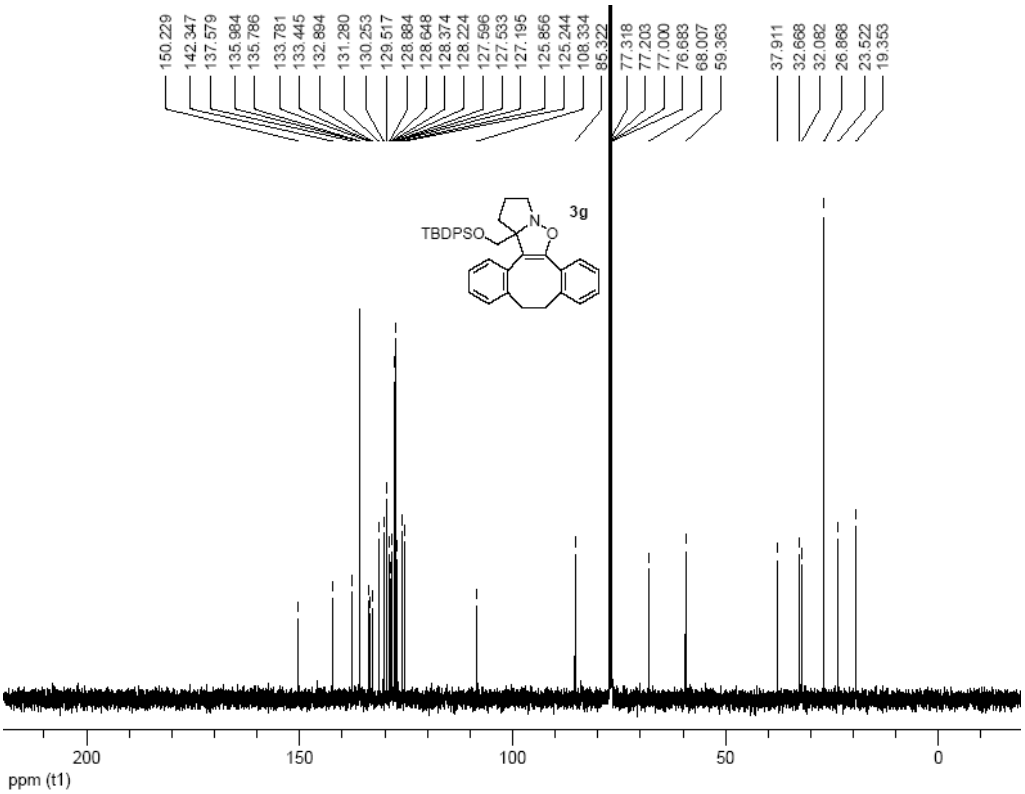

OTBDPS
3e
F
F
H
N
O
3.96
6.96
8.72
1.00
3.06
2.02
2.04
2.03
9.05
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

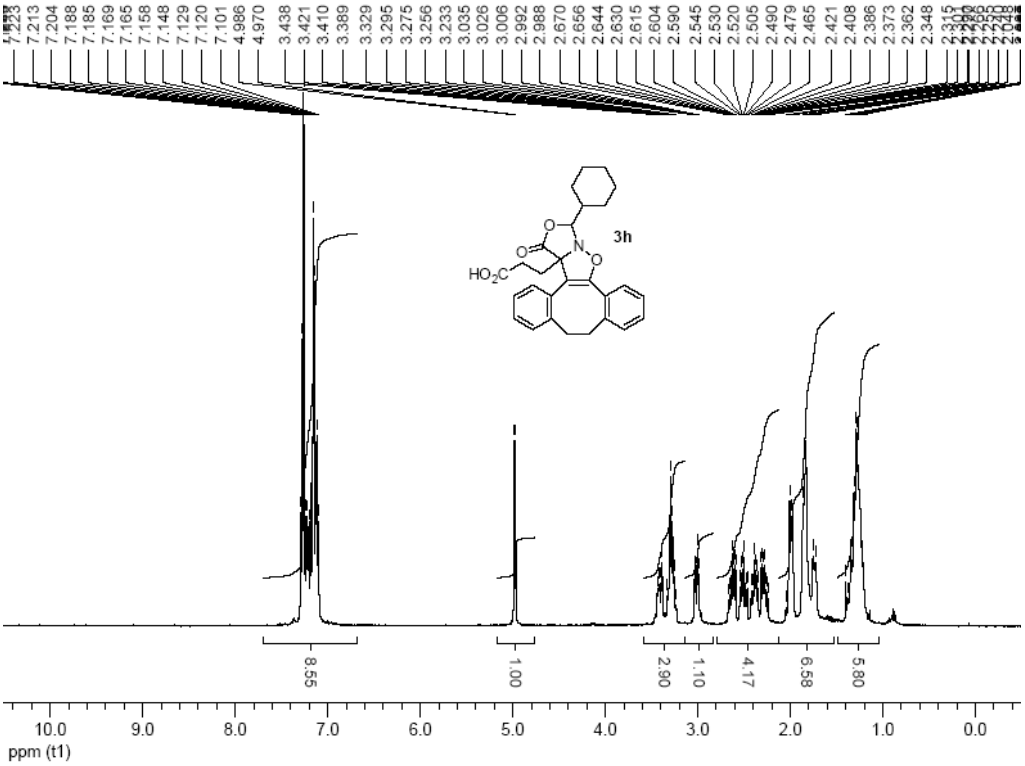

OTBDPS
3e
F
F
H
N
O
200
150
100
50
0
ppm (t1)

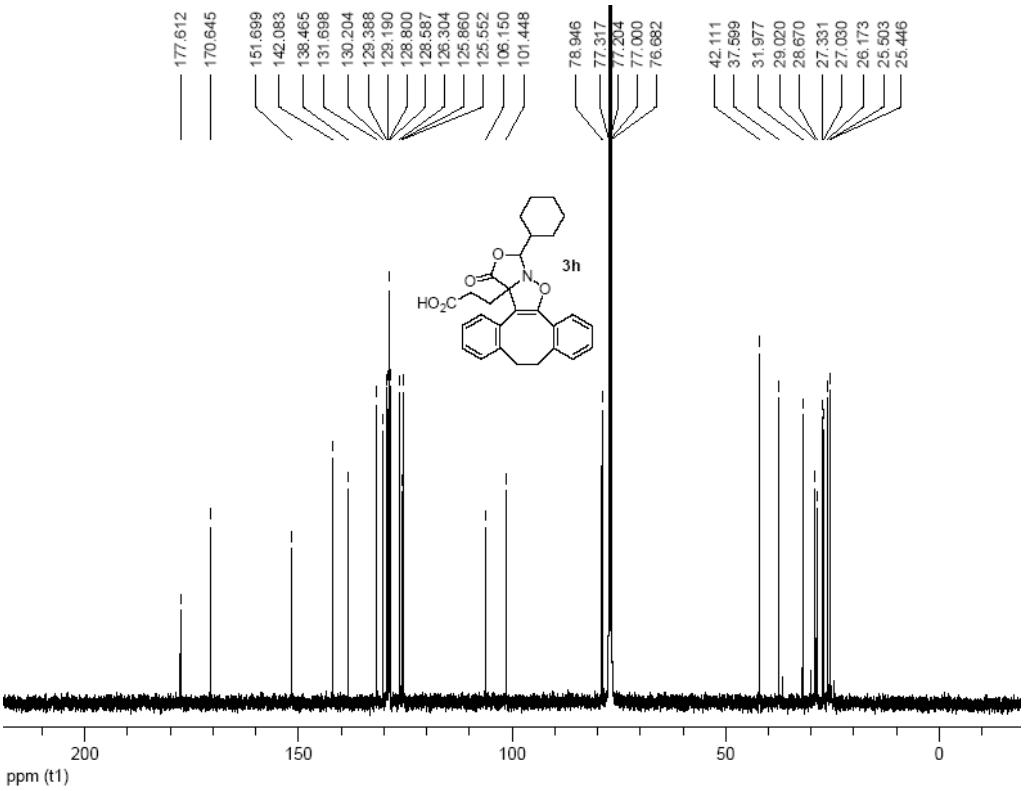

7.206
7.199
7.189
7.186
7.178
7.169
7.163
7.154
7.145
7.134
5.481
4.394
4.350
4.082
4.075
4.061
4.053
4.037
4.023
3.993
3.824
3.816
3.811
3.805
3.790
3.783
3.771
3.755
3.741
3.721
3.660
3.652
3.639
3.630
3.618
3.606
3.597
3.569
3.555
3.538
3.530
3.515
3.498
3.430
3.421
3.417
3.408
3.403
3.390
3.380
3.367
CO2Me
3f
1.00
6.20
1.00
1.07
2.08
4.78
2.38
2.26
1.17
1.18
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

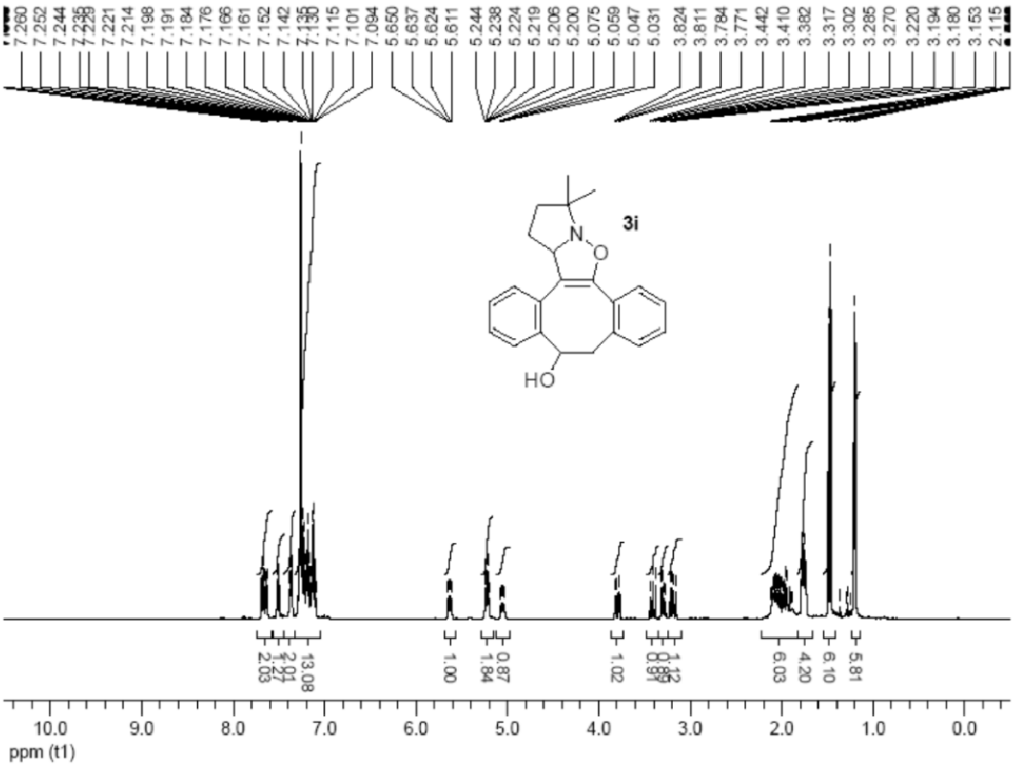

168.963
165.663
149.927
140.332
139.206
131.129
130.955
129.613
129.398
129.305
129.117
127.453
127.166
125.631
125.554
107.848
77.318
77.000
76.683
71.850
52.252
50.462
48.333
43.583
36.733
32.984
CO2Me
3f
200
150
100
50
0
ppm (t1)

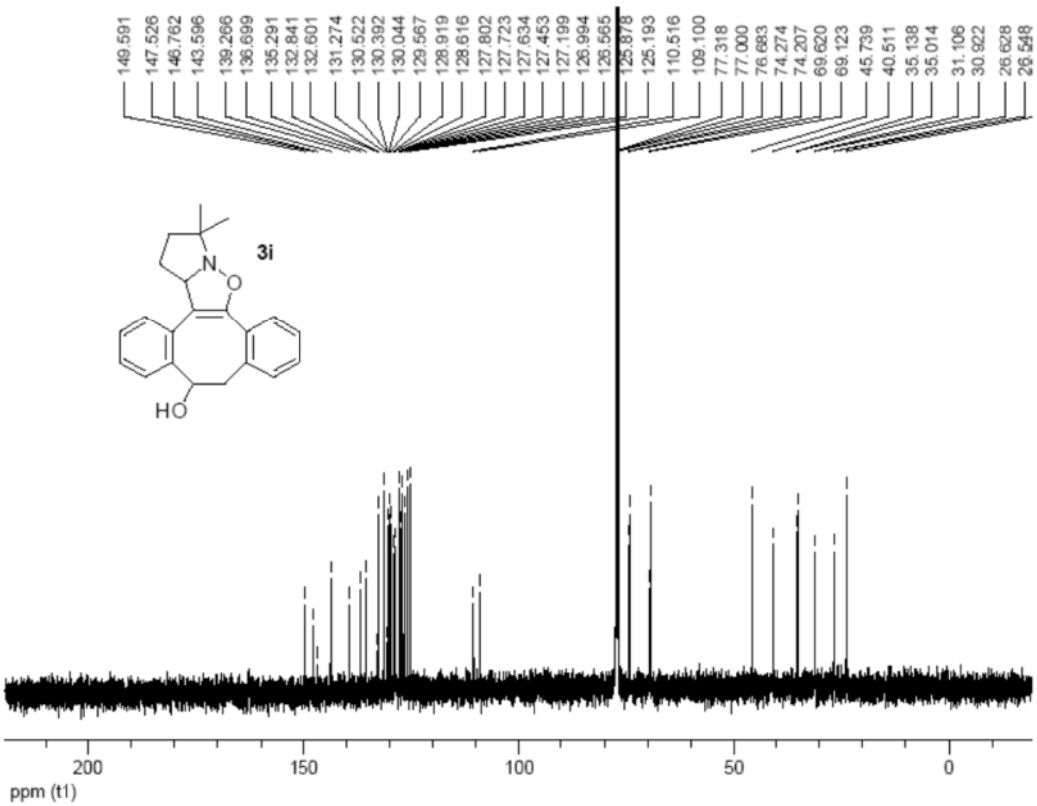

3g
TBDPSO
3.99
7.06
2.48
4.99
1.00
1.04
1.03
5.08
1.08
2.06
1.05
9.20
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

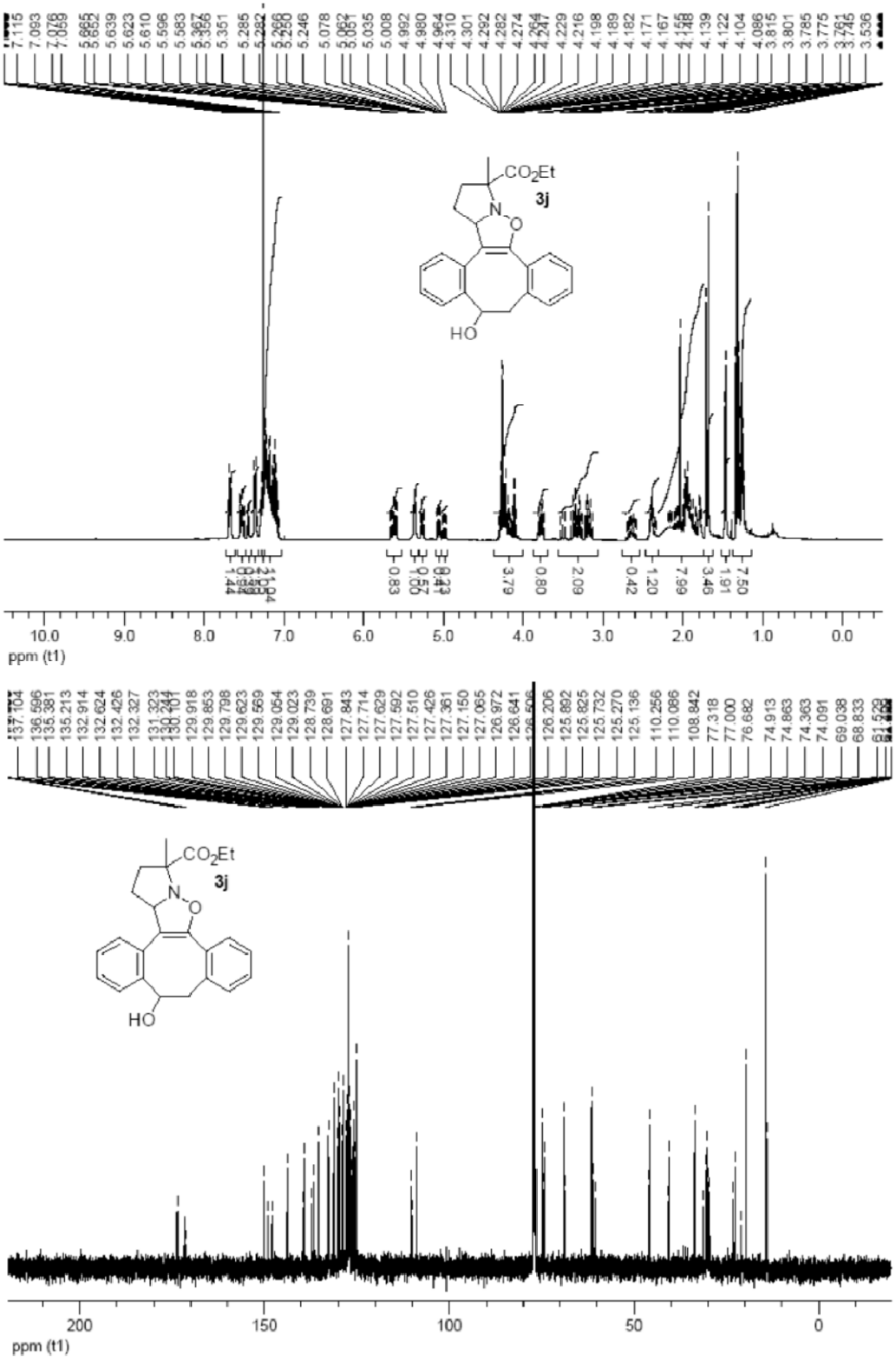

3g
TBDPSO
150.229
142.347
137.579
135.984
135.786
133.781
133.445
132.894
131.280
130.253
129.517
128.884
128.648
128.374
128.224
127.596
127.533
127.195
125.856
125.244
108.334
85.322
77.318
77.203
77.000
76.683
68.007
59.363
37.911
32.668
32.082
26.868
23.522
19.353
200
150
100
50
0
ppm (t1)

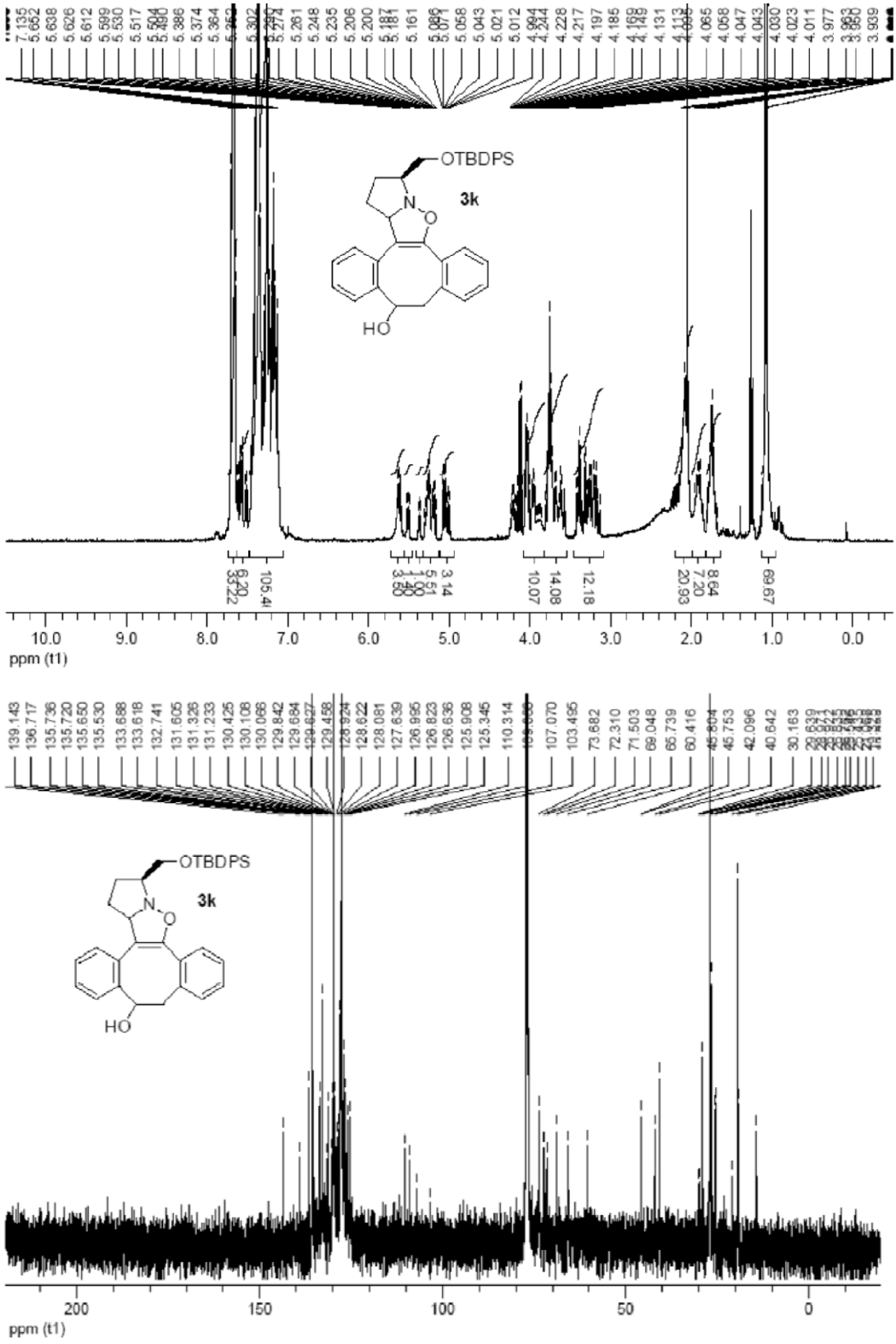

3h
HO2C
8.56
1.00
2.90
1.10
4.17
6.58
5.80
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

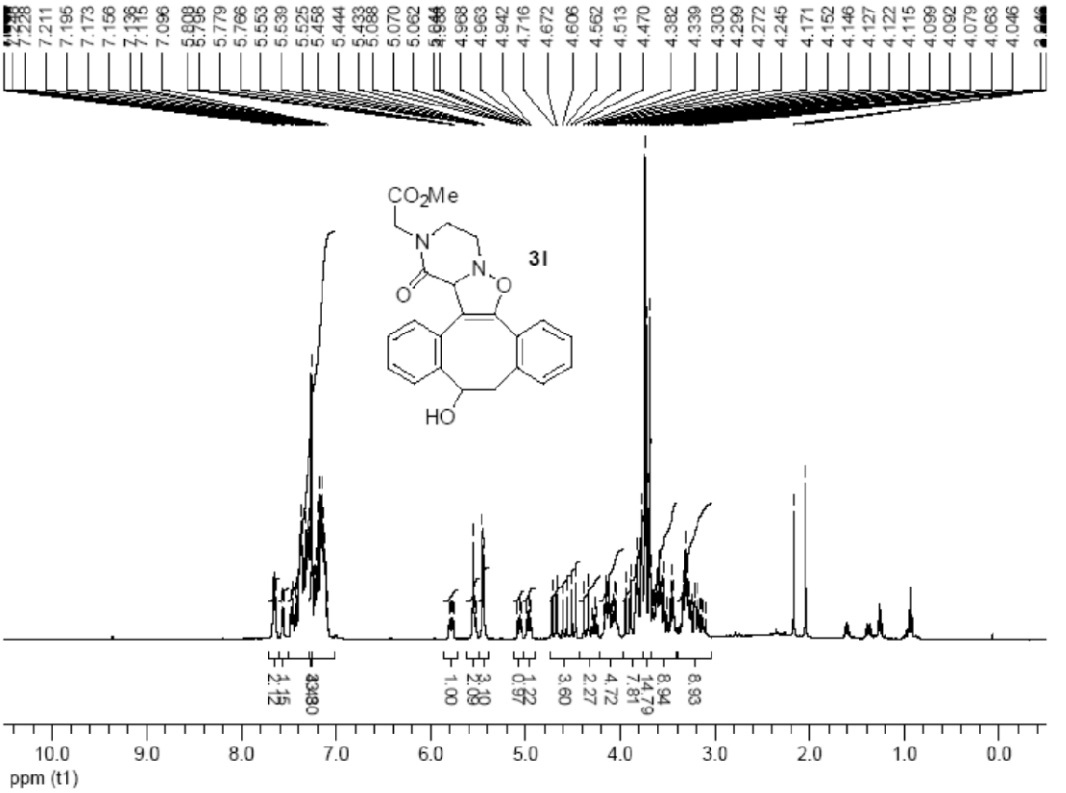

177.612
170.645
151.699
142.083
138.465
131.698
130.204
129.388
129.190
128.800
128.587
126.304
125.860
125.552
106.150
101.448
78.946
77.317
77.204
77.000
76.682
42.111
37.599
31.977
29.020
28.670
27.331
27.030
26.173
25.503
25.446
3h
HO2C
200
150
100
50
0
ppm (t1)

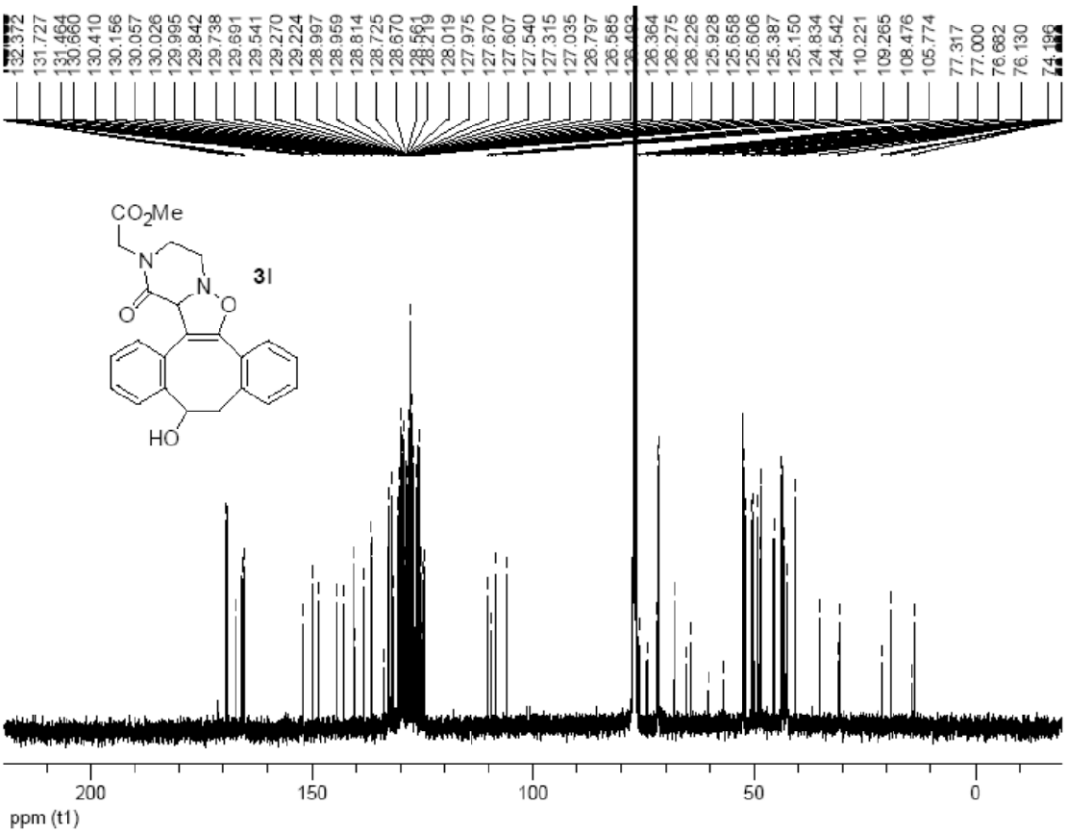

7.260
7.252
7.244
7.221
7.214
7.198
7.191
7.184
7.176
7.166
7.161
7.152
7.142
7.115
7.101
7.094
5.650
5.637
5.624
5.611
5.244
5.238
5.224
5.219
5.206
5.200
5.075
5.059
5.047
5.031
3.824
3.811
3.784
3.771
3.442
3.410
3.382
3.317
3.302
3.285
3.270
3.220
3.194
3.180
3.153
2.115
3i
HO
2.03
2.27
2.01
2.27
13.08
1.00
1.84
0.87
1.02
0.89
0.87
2.13
6.03
4.20
6.10
5.81
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

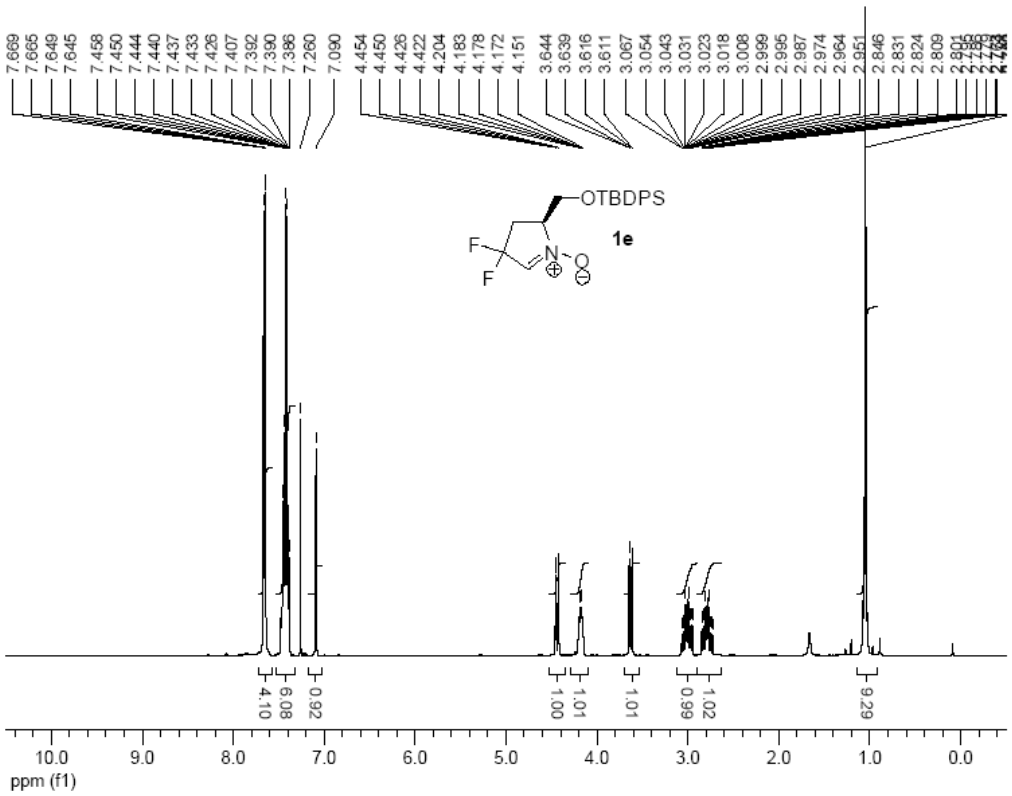

149.591
147.526
146.762
143.596
139.266
136.699
135.291
132.841
132.601
131.274
130.522
130.392
130.044
129.567
128.919
128.616
127.802
127.723
127.634
127.453
127.199
126.994
126.565
125.878
125.193
110.516
109.100
77.318
77.000
76.683
74.274
74.207
69.620
69.123
45.739
40.511
35.138
35.014
31.106
30.922
26.628
26.548
3i
HO
200
150
100
50
0
ppm (t1)

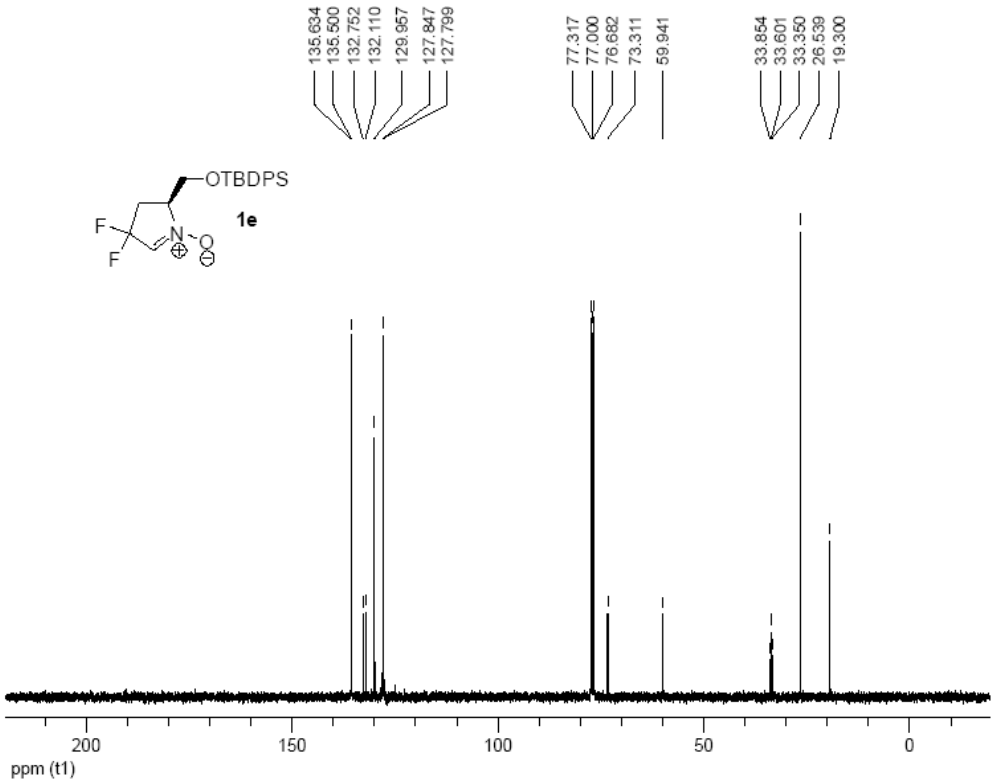

CO2Et
3j
N
O
HO
ppm (t1)
CO2Et
3j
N
O
HO
ppm (t1)

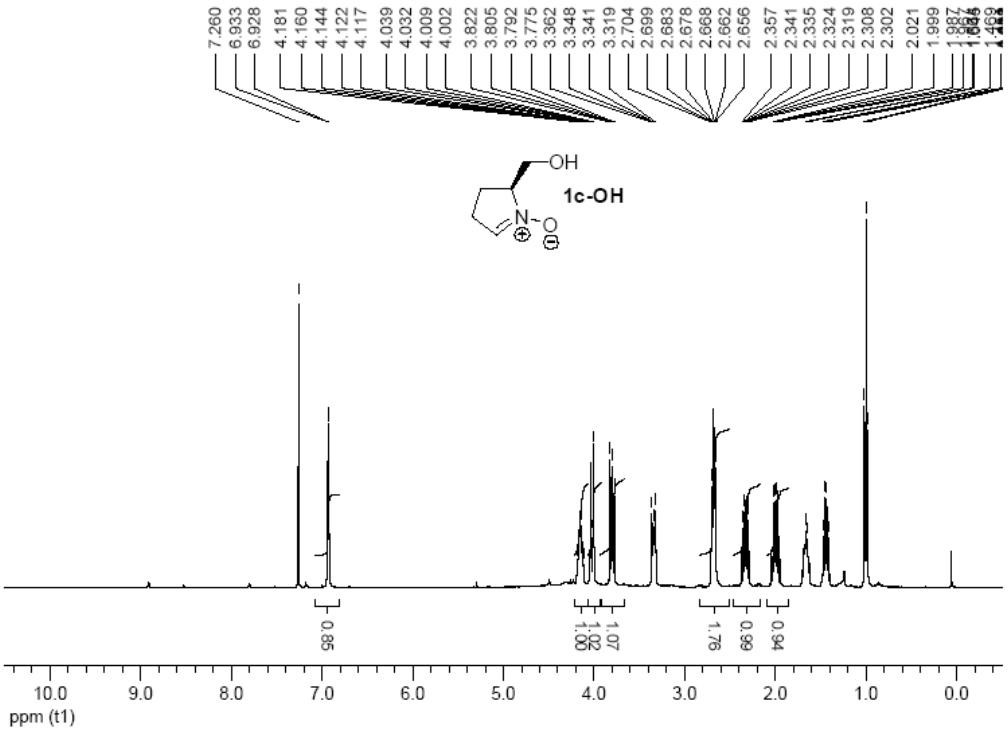

OTBDPS
3k
HO
ppm (t1)
OTBDPS
3k
HO
ppm (t1)

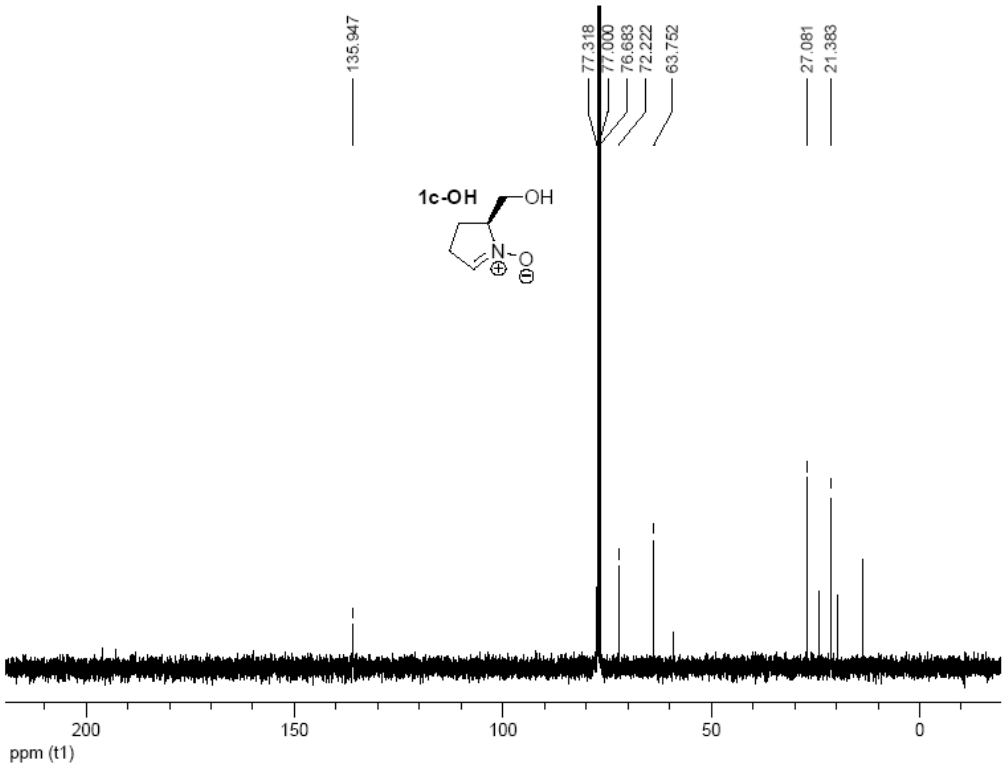

CO2Me
N
N
O
O
HO
3l
ppm (t1)


CO2Me
N
N
O
O
HO
3l
ppm (t1)


7.669
7.665
7.649
7.645
7.458
7.450
7.444
7.440
7.437
7.433
7.426
7.407
7.392
7.390
7.386
7.260
7.090
4.454
4.450
4.426
4.422
4.204
4.183
4.178
4.172
4.151
3.644
3.639
3.616
3.611
3.067
3.054
3.043
3.031
3.023
3.018
3.008
2.999
2.995
2.987
2.974
2.964
2.951
2.846
2.831
2.824
2.809
OTBDPS
1e
F
F
N
O
4.10
6.08
0.92
1.00
1.01
1.01
0.99
1.02
9.29
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


135.634
135.500
132.752
132.110
129.957
127.847
127.799
77.317
77.000
76.682
73.311
59.941
33.854
33.601
33.350
26.539
19.300
OTBDPS
1e
F
F
N
O
200
150
100
50
0
ppm (t1)


7.260
6.933
6.928
4.181
4.160
4.144
4.122
4.117
4.039
4.032
4.009
4.002
3.822
3.805
3.792
3.775
3.362
3.348
3.341
3.319
2.704
2.699
2.683
2.678
2.668
2.662
2.656
2.357
2.341
2.335
2.324
2.319
2.308
2.302
2.021
1.999
OH
1c-OH
0.86
1.00
1.02
1.07
1.76
0.99
0.94
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


135.947
77.318
77.000
76.683
72.222
63.752
27.081
21.383
1c-OH
OH
200
150
100
50
0
ppm (t1)

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