Supplementary Tables

Supplementary Table 1: Primers for cloning into pMRX-IP-GFP. *Related to Figure 4, Extended Data Figure 4, Extended Data Figure 55.*	
ACSL4 F	CTGTACAAGGGTTCAGGCTCGGGATCCATGGCAAAGAGAATAAAAGCT
ACSL4 R	AATTTACGTAGCGGCCGCTCTCGAGTCATTTGCCCCCATACATTCGTT
FUNDC1 F	TGTACAAGGGTTCAGGCTCGGGATCCATGGCGACCCGGAA
FUNDC1 R	AGCGGCCGCTCTCGAGTCAAGATGCAAGTCCGAGCAAAAA
PLA2G16 cyb5 F1	AGGGTTCAGGCTCGGGATCC
PLA2G16 cyb5 R1	ACGGCCACTGCAGAGATGGCAGGGATCACCCAGTTGGTCCACCAACTGGAACTAGAATCAATAGTAGTGATTTGCTTCTGTTTCTTGTTT
PLA2G16 cyb5 F2	GGGTGATCCCTGCCATCTCTGCAGTGGCCGTCGCCTTGATGTATCGCCTATACATGGCAGAGGACTGACTCGAGAGCGGCCGCTACGT
PLA2G16 cyb5 R2	GCGAGGGTGCGTACG
FUNDC1-cyb5 R1	ACGGCCACTGCAGAGATGGCAGGGATCACCCAGTTGGTCCACCAACTGGAACTAGAATCAATAGTAGTGATAGATGCAAGTCCGAGCAAA
GFP F1	TGCCGGATCTGCCATCGATATA
GFP R1	AGGAATTCCCGTACCACCACACTGGGATCCCGAGCCTGAACCTCACTTGTACAGCTCGTCCAT

GFP F2	CTCGGGATCCCAGTGTGGTGGTACGGGAATTCCTGCAGGC CTCGAGAGCGGCCGCTACGTAAATT
GFP-Cyb5 F	TGTACAAGGGTTCAGGCTCGGGATCCATCACTACTATTGAT TCTA
Nterm-FUNDC1-cyb5 R1	AATCAATAGTAGTGATTTTTTCTACCATAGGTCCCGAA
Nterm-FUNDC1-cyb5 F2	ACCTATGGTAGAAAAAATCACTACTATTGATTCTA

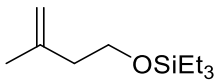
Supplementary Table 2: qPCR primers.	
ACSL1 F	CAATCCTTGCCCAGATGATAC
ACSL1 R	TAGCTCCATGACACAGCATTAC
ACSL3 F	ATCTGTTTCTGCTGTCCTGTT
ACSL3 R	CCACTCTGCCAGTATTGTAGTC
ACSL4 F	CAAGTAGACCAACGCCTTCA
ACSL4 R	GTCCCAGTCCAGGTATTCTTTC
ACSL5 F	GGAGAATACATTGCACCAGAGA
ACSL5 R	AGGAACCACCACTCCTACTAA
ACSL6 F	GCAGGTTAAAGCCATTCACATC
ACSL6 R	CTCTCTCAGCTCAGGTCTCTTA
AGPAT3 F	TGCTGTGAAAGACAGGGATAAA
AGPAT3 R	CCTTGGAGACATCTATGCAGAC
FAM134B F	GTCTTGCAGTGGGTGGTATTA
FAM134B R	GTGCTCCCAACATCCCTATC
RTN3 F	GAGTTCCTTCAGGTTCTCACTC

RTN3 R	CCGTACTTTGGGACCATCAA
SEC62 F	AGGATGGTAGGGTGCCTATT
SEC62 R	GGAAAGCAGGGAAGGAGTTT

Supplementary Table 3: MISSION shRNA plasmids.	
Gene	Plasmid ID
ACSL1	SHCLNG-NM_001995 TRCN0000045518
ACSL3	SHCLNG-NM_004457 TRCN0000045530
ACSL4	SHCLNG-NM_004458 TRCN0000045541
ACSL5	SHCLNG-NM_016234 TRCN0000419516
ACSL6	SHCLNG-NM_015256 TRCN0000417643
AGPAT3	SHCLNG-NM_020132 TRCN0000427286
FAM134B	SHCLNG-NM_019000 TRCN0000421826
RTN3	SHCLNG-NM_006054 TRCN0000203633
SEC62	SHCLNG-NM_003262 TRCN0000306854

Synthetic Methods

Triethylsilyl 3-methylbut-3-ene ether (10) [1]

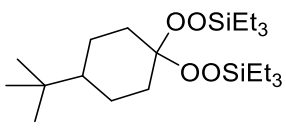


Under a N_2 atmosphere, DMAP (352 mg, 2.89 mmol), NEt_3 (17.0 mL, 122 mmol) and 3-methyl-3-butenol (6.8 mL, 67 mmol) were added to CH_2Cl_2 (48.0 mL). The resulting mixture was cooled to 0 °C followed by addition of Et_3SiCl (10.0 mL, 59.6 mmol) and the reaction mixture was warmed to ambient temperature and stirred for 15 h. The reaction mixture was diluted with sat. aq. $NaHCO_3$ (50 mL), the organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude yellow oil was passed through a silica plug (7 cm) and washed with hexanes to afford the title compound (10.0 g, 84%) as a clear oil:

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (s, 1H), 4.70 (s, 1H), 3.71 (t, *J* = 7.2, 2H), 2.26 (t, *J* = 7.2, 2H), 1.74 (s, 3H), 0.96 (t, *J* = 7.9, 9H), 0.60 (q, *J* = 7.9, 6H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 143.2, 111.6, 62.0, 41.3, 23.1, 6.9, 4.6.

4-*tert*-Butylcyclohexanone bis(triethylsilylperoxy) ketal (11) [1]



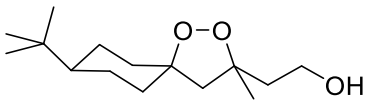
A mixture of 4-*tert*-butylcyclohexanone (2.03 g, 13.2 mmol) and PMA·H_2O (1.15 g, 0.630 mmol) in Et_2O (80 mL) was stirred vigorously followed by the addition of H_2O_2 (50% aq., 22.0 mL, 387 mmol). The reaction mixture was stirred for 15 h and then diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic phases were washed with a sat. aq. NaCl (75 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a white powder. The hydroperoxy ketal intermediate was placed under a N_2 atmosphere followed by addition of DMAP (163 mg, 1.36 mmol) and anhydrous DMF (32.0 mL). The reaction mixture was stirred followed by addition of NEt_3 (4.8 mL, 34 mmol) then Et_3SiOTf (7.4 mL, 33 mmol). The mixture was stirred for an additional 5 h.

The reaction was diluted with H_2O (30 mL) and extracted with pentane (4 × 30 mL). The combined organic phases were washed with sat. aq. NaCl (60 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (hexanes, *rf* = 0.79) afforded the title compound (4.18 g, 73%) as a clear oil:

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31 (d, *J* = 11.8, 2H), 1.64 (dd, *J* = 12.3, 2.1, 2H), 1.34−1.17 (m, 4H), 1.01 (app. q, *J* = 7.8, 19H), 0.85 (s, 9H), 0.70 (app. sex, *J* = 7.8, 12H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 109.2, 47.7, 32.5, 30.6, 27.7, 23.7, 6.95, 6.94, 4.1, 4.0.

(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(2'-hydroxyethyl)-1,2-dioxaspiro[4.5]decane ($FlNO_2$) (1)** [1]

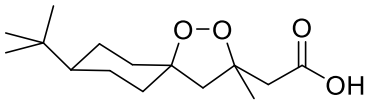


A flame-dried flask containing diperoxyketal **11** (20.8 g, 48.1 mmol) and alkene (28.9 g, 144.2 mmol) was placed under N_2 and dissolved in anhydrous CH_2Cl_2 (300 mL). Once cooled to −20 °C, $SnCl_4$ (25.0 g, 96.0 mmol) was added *via* cannula and the reaction mixture was stirred for a further 2 hours at −20 °C, and then it was warmed to ambient temperature. The mixture was stirred for an additional 1 h before being diluted with sat. aq. Rochelle's salt (100 mL) and stirred for 20 h. The biphasic mixture was extracted with CH_2Cl_2 (4 × 50 mL), and the combined organic phases were washed with sat. aq. NaCl (200 mL), dried over$MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (20:80 EtOAc:hexanes, *rf* =0.25) afforded the title compound (8.0 g, 65%) as a white powder:

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85−3.74 (m, 2H), 2.23 (d, *J* = 12.0, 1H), 2.12−2.08 (m, 3H), 2.05−1.98 (m, 2H), 1.81 (ddd, J = 14.6, 6.4, 5.4, 1H), 1.61 (dt, *J* = 12.3, 2.6, 2H), 1.51−1.38 (m, 2H), 1.37 (s, 3H), 1.33−1.21 (m, 2H), 0.96 (tt, *J* = 11.9, 3.0, 1H), 0.84 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 85.5, 85.0, 59.5, 57.8, 47.2, 41.6, 36.2, 35.8, 32.5, 27.7, 24.6, 24.0, 23.8.

(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-carboxymethyl-1,2-dioxaspiro[4.5]decane (12)** [1,2]

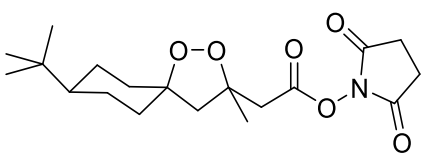


A modified procedure from Zhao et al. was followed. A vigorously stirred mixture of alcohol **FINO₂** (558 mg, 2.18 mmol), TEMPO (28 mg, 0.18 mmol), CH_3CN (11.0 mL), and aq. phosphate buffer (0.67 M, pH = 6.7, 7.5 mL) was heated to 50 °C. Aqueous solutions of NaClO (0.045 M, 2.0 mL, 0.09 mmol) and $NaClO_2$ (1.6 M, 5.6 mL, 9.0 mmol) were added to the mixture simultaneously and heating was continued for 16 h. After cooling to ambient temperature, the reaction mixture was diluted with aq. NaOH (1 M, 15 mL) and washed with CH_2Cl_2 (2 × 15 mL). The aqueous phase was acidified to pH 1 using 5% aq. HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with sat. aq. NaCl (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material is typically pure without purification but can be purified using flash column chromatography (30:70 EtOAc:hexanes, *rf* = 0.20) to afford the title compound (469 mg, 80%) as a white powder:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (br s, 1H), 2.78 (d, *J* = 15.0, 1H), 2.73 (d, *J* = 15.0, 1H) 2.40 (d, *J* = 12.4, 1H), 2.15−2.12 (m, 2H), 1.95 (dd, *J* = 13.9, 2.6, 1H), 1.62−1.50 (m, 3H), 1.47 (s, 3H), 1.41−1.18 (m, 3H), 0.96 (t, *J* = 11.6, 1H), 0.84 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 85.2, 83.3, 56.7, 47.0, 43.5, 36.0, 35.7, 32.4, 27.5, 24.0, 23.6, 23.5.

(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-succinimidylcarboxymethyl-1,2-dioxaspiro[4.5]decane (13)**



Carboxylic acid **12** (399 mg, 1.47 mmol), EDCI (327 mg, 1.71 mmol), and *N*-hydroxysuccinimide (265 mg, 2.30 mmol) were dissolved in CH_2Cl_2 (5.9 mL) and stirred for 17 h. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with sat. aq. $NaHCO_3$ (15 mL), aq. HCl (5%, 15 mL), sat. aq. NaCl (15 mL), and dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude solids were dissolved and filtered through a silica plug (5 cm) and washed

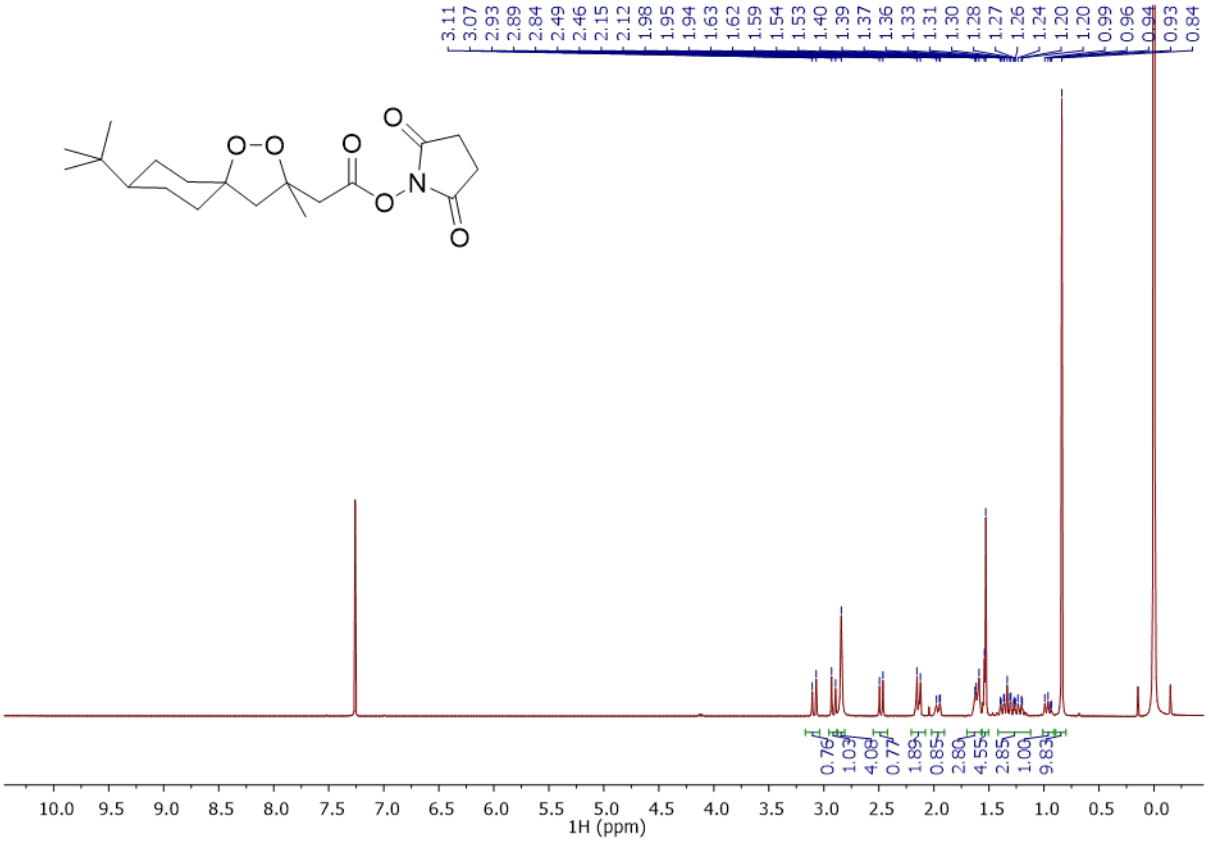
with 40:60 EtOAc:hexanes (100 mL, *rf* = 0.54). The filtrate was concentrated *in vacuo* to afford the title compound (459 mg, 85%) as a white powder:

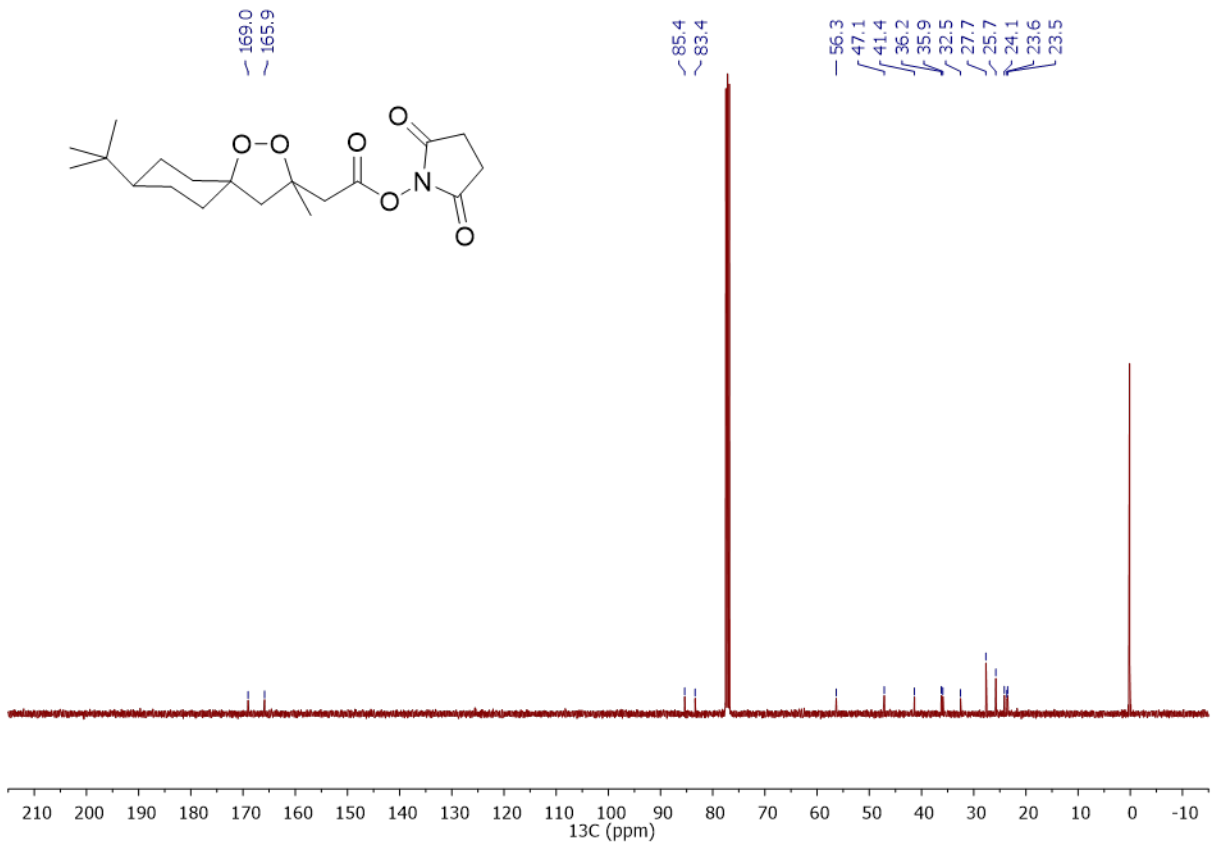
mp = 151–152 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.09 (d, *J* = 14.6, 1H), 2.91 (d, *J* = 14.5, 1H), 2.84 (br s, 4H), 2.48 (d, *J* = 12.6, 1H), 2.17−2.12 (m, 2H), 1.95 (d, *J* = 14.1, 1H), 1.63−1.59 (m, 3H), 1.53 (s, 3H), 1.43−1.16 (m, 3H), 0.96 (t, *J* = 11.8, 1H), 0.84 (s, 9H);

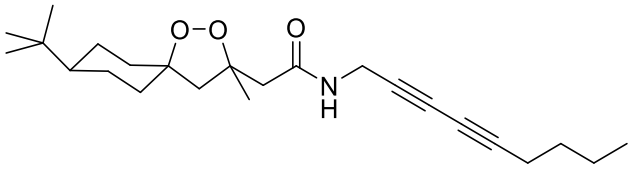
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0 (C), 165.9 (C), 85.4 (C), 83.4 (C), 56.3 (CH_2), 47.1 (CH), 41.4 (CH_2), 36.2 (CH_2), 35.9 (CH_2), 32.5 (C), 27.7 (CH_3), 25.7 (CH_2), 24.1 (CH_2), 23.6 (CH_2), 23.5 (CH_3);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{19}H_{33}N_2O_6$ $[M+NH_4]^+$ 385.2333, found 385.2328.





(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(*N*-nona-4',6'-diynecarbamoylmethyl)-1,2-dioxaspiro[4.5]decane ($FINO_2$-2) (6)**



To a solution of Boc-protected amine **30** (181 mg, 0.467 mmol) in CH_2Cl_2 (7.8 mL) under a N_2 atmosphere was added TFA (1.5 mL, 20 mmol). After 30 min, the reaction mixture was concentrated *in vacuo* and coevaporated with $CHCl_3$ (2 × 3 mL) to afford the ammonium·TFA salt. In a microwave vial, carboxylic acid **12** (170 mg, 0.629 mmol), EDCI (195 mg, 1.02 mmol), and HOBt (124 mg, 0.928 mmol) were added to $CHCl_3$ (1.5 mL). The ammonium·TFA salt was transferred to the microwave vial using $CHCl_3$ (1.7 mL), followed by addition of NEt_3 (150 µL, 1.08 mmol). The microwave vial was sealed and irradiated at 70 °C for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (10 mL), washed with aq. NaOH (1 M, 10 mL), aq. HCl (5%, 10 mL), sat. aq. NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (20:80

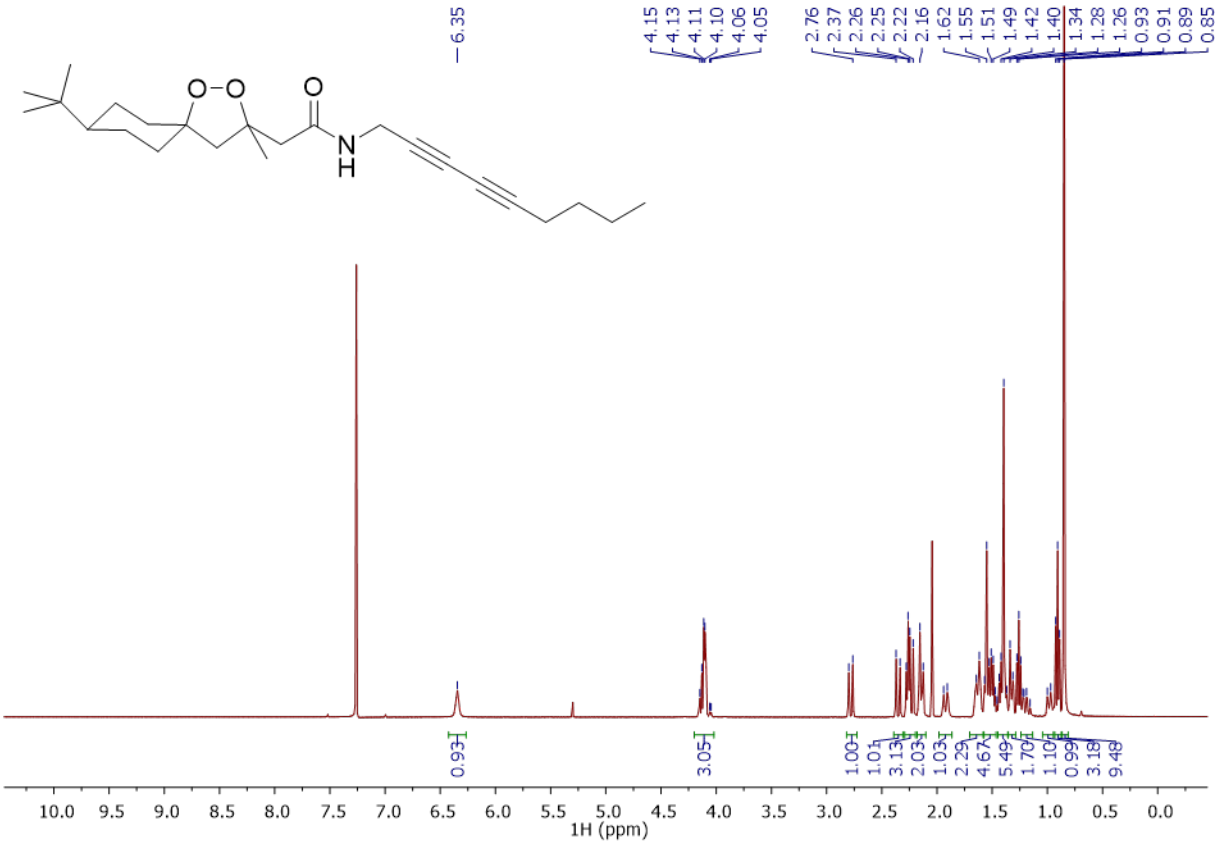
EtOAc:hexanes, *rf* = 0.15) afforded the title compound (136 mg, 56%) as a white solid. The isolated product was stored at −20 °C under static N_2 to prevent decomposition:

mp **=** slow dec. 21 °C;

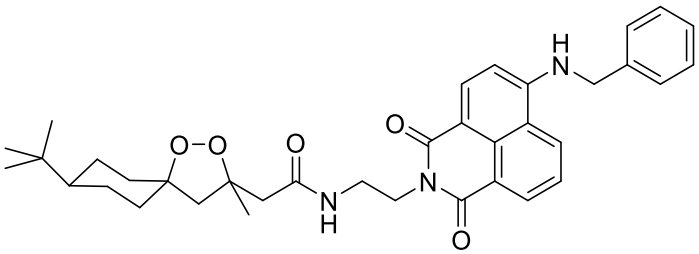
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (s, 1H), 4.15−4.05 (m, 2H) 2.78 (d, *J* = 14.4, 1H), 2.35 (d, *J* = 14.4, 1H), 2.28−2.22 (m, 3H), 2.14 (d, *J* = 12.1, 2H), 1.92 (d, *J* = 13.4, 1H), 1.63 (d, *J* = 10.8, 2H), 1.57−1.47 (m, 3H), 1.46−1.37 (m, 5H), 1.34−1.31 (m, 2H), 1.22-1.16 (m, 1H), 0.97 (t, *J* = 12.2, 1H), 0.91 (t, *J* = 7.2, 3H), 0.85 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4 (C), 85.1 (C), 83.8 (C), 80.3 (C), 71.3(C), 68.4 (C), 64.6 (C), 57.5 (CH_2), 47.0 (CH), 46.0 (CH_2), 36.1 (CH_2), 35.7 (CH_2), 32.4 (C), 30.2 (CH_2), 29.7 (CH_2), 27.5 (CH_3), 24.1 (CH_2), 23.5 (CH_2), 23.3 (CH_3), 21.9 (CH_2), 18.9 (CH_2), 13.5 (CH_3);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{24}H_{38}NO_3$ $[M+H]^+$ 389.2879, found 389.2872.



(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(9'-(benzylamino)-4'-naphthalimido-*N*-ethylcarbamoylmethyl)-1,2-dioxaspiro[4.5]decane (FINO$_2$-1) (2).**



To a solution of Boc-protected amine **24** (72 mg, 0.16 mmol) in CH_2Cl_2 (1.6 mL) was added TFA (330 µL, 4.31 mmol). The resulting mixture was stirred for 1 h, and then concentrated *in vacuo* and coevaporated with $CHCl_3$ (2 × 3 mL) to afford the ammonium·TFA salt. In a microwave vial, carboxylic acid **12** (40 mg, 0.15 mmol), EDCI (43 mg, 0.22 mmol), and HOBt (33 mg, 0.24 mmol) were added to $CHCl_3$ (680 µL). The ammonium·TFA salt was transferred to the microwave vial using $CHCl_3$ (2.0 mL) followed by addition of NEt_3 (150 µL, 1.1 mmol). The microwave vial was sealed and irradiated at 70 °C for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (10 mL), washed with aq. NaOH (1 M, 10 mL), aq. HCl (5%, 10 mL), sat. aq. NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (2:49:49 MeOH:EtOAc:hexanes, *rf* = 0.18) afforded the title compound (52 mg, 60%) as yellow glass-like solid:

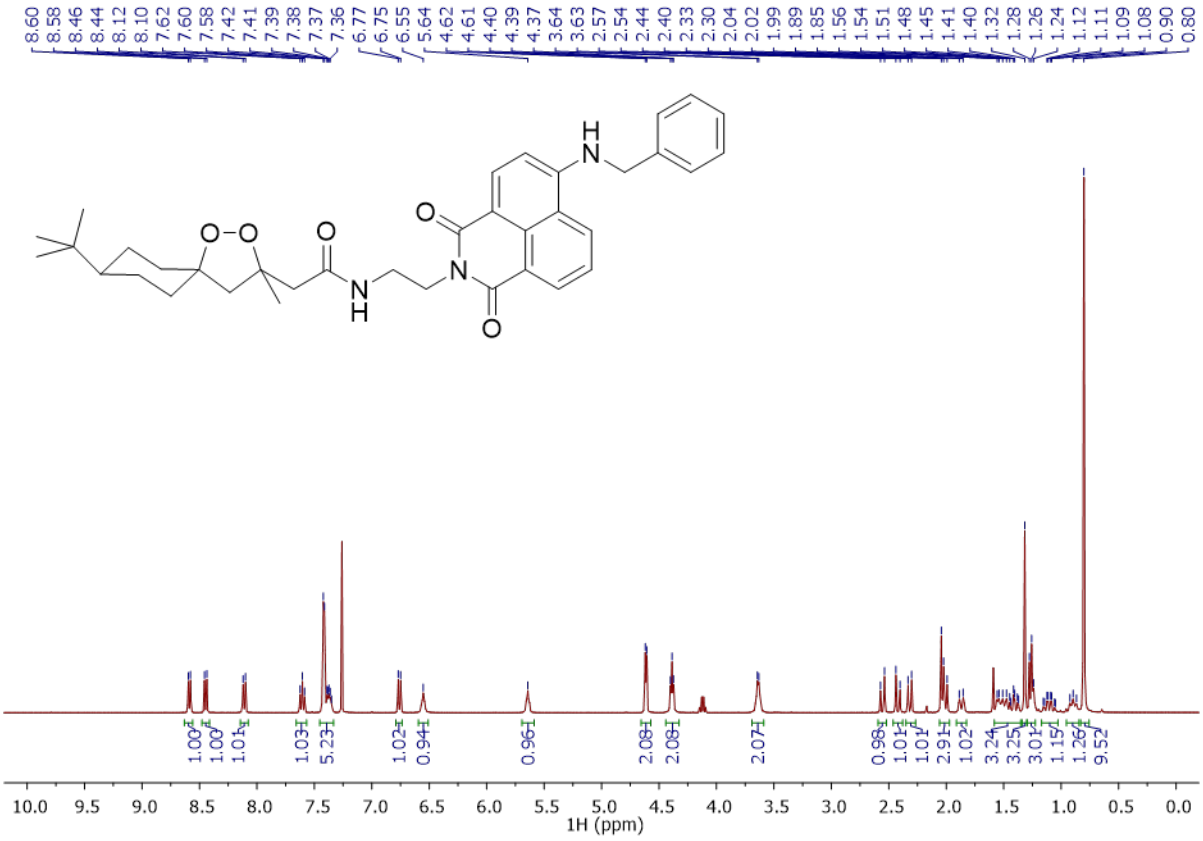
mp = dec. 124 °C;

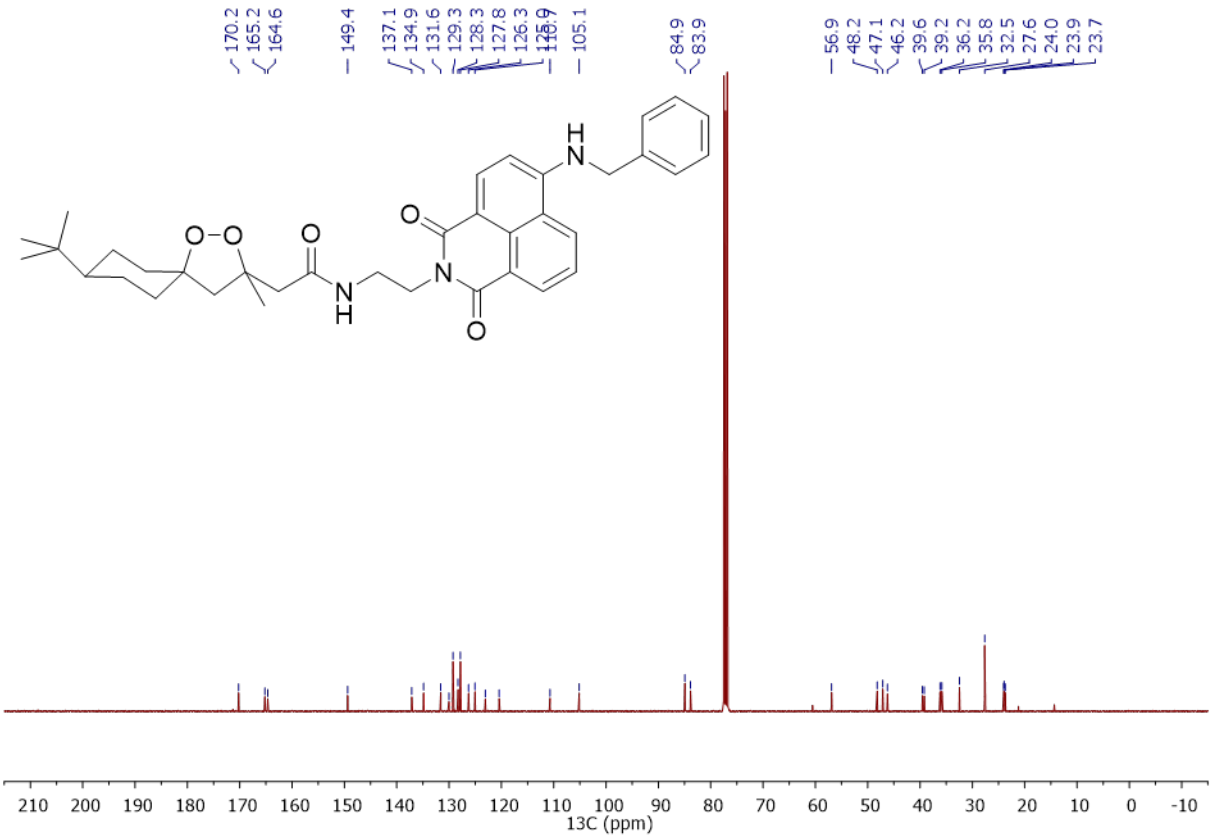
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 7.3, 1H), 8.44 (d, *J* = 8.4, 1H), 8.11 (d, *J* = 8.4, 1H), 7.60 (t, *J* = 7.8, 1H), 7.42−7.35 (m, 5H), 6.77 (d, *J* = 8.4, 1H), 6.55 (br s, 1H), 5.64 (br s, 1H), 4.61 (d, *J* = 5.0, 2H), 4.39 (t, *J* = 5.4, 2H), 3.64 (d, *J* = 5.3, 2H), 2.55 (d, *J* = 14.1, 1H), 2.42 (d, *J* = 14.1, 1H), 2.32 (d, *J* = 12.3, 1H), 2.04−1.99 (m, 2H), 1.87 (d, *J* = 13.4, 1H), 1.56−1.37 (m, 3H), 1.32 (s, 3H), 1.28−1.24 (m, 2H), 1.15−1.06 (m, 1H), 0.90 (t, *J* = 11.4, 1H), 0.90 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2 (C), 165.2 (C), 164.6 (C), 149.4 (C), 137.1 (C), 134.9 (CH), 131.6 (CH), 130.0 (C), 129.3 (CH), 128.3 (CH), 127.8 (CH), 126.3 (CH), 125.0 (CH), 123.1 (C), 110.7 (C), 105.1 (CH), 84.9 (C), 83.9 (C), 56.9 (CH_2), 48.2 (CH_2), 47.1 (CH), 46.2 (CH_2), 39.6

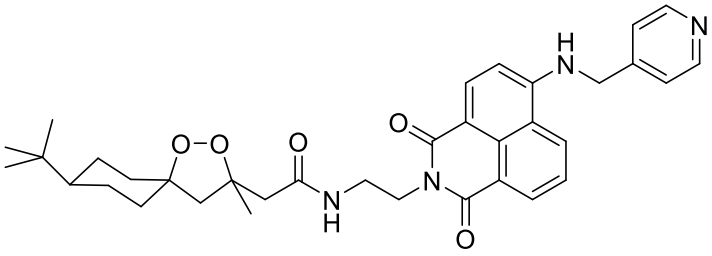
(CH_2), 39.2 (CH_2), 36.2 (CH_2), 35.8 (CH_2), 32.5 (C), 27.6 (CH_3), 24.0 (CH_2), 23.9 (CH_3), 23.7 (CH_2);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{36}H_{44}N_3O_5$ $[M+H]^+$ 598.3236, found 598.3268.





(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(9'-(*p*-picoylamino)-4'-naphthalimido-*N*-ethylcarbamoylmethyl)-1,2-dioxaspiro[4.5]decane (FINO$_2$-7) (9).**



Boc-protected amine **25** (119 mg, 0.270 mmol) was dissolved in CH_2Cl_2 (2.7 mL) followed by addition of TFA (540 µL, 7.1 mmol) and the resulting solution was stirred for 4 h. The reaction mixture was concentrated *in vacuo* and coevaporated with $CHCl_3$ (3 × 2 mL) to afford the ammonium·TFA salt. NHS-ester **13** (118 mg, 0.321 mmol) was added to the ammonium salt, followed by $CHCl_3$ (4.6 mL) and NEt_3 (170 µL, 1.2 mmol), and the resulting slurry was stirred for 15 h. The reaction mixture was diluted with aq. NaOH (1 M, 10 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography

(1:2:97 NEt_3:MeOH:CH_2Cl_2, *rf* = 0.26) afforded the title compound (146 mg, 91%) as a yellow glass-like solid:

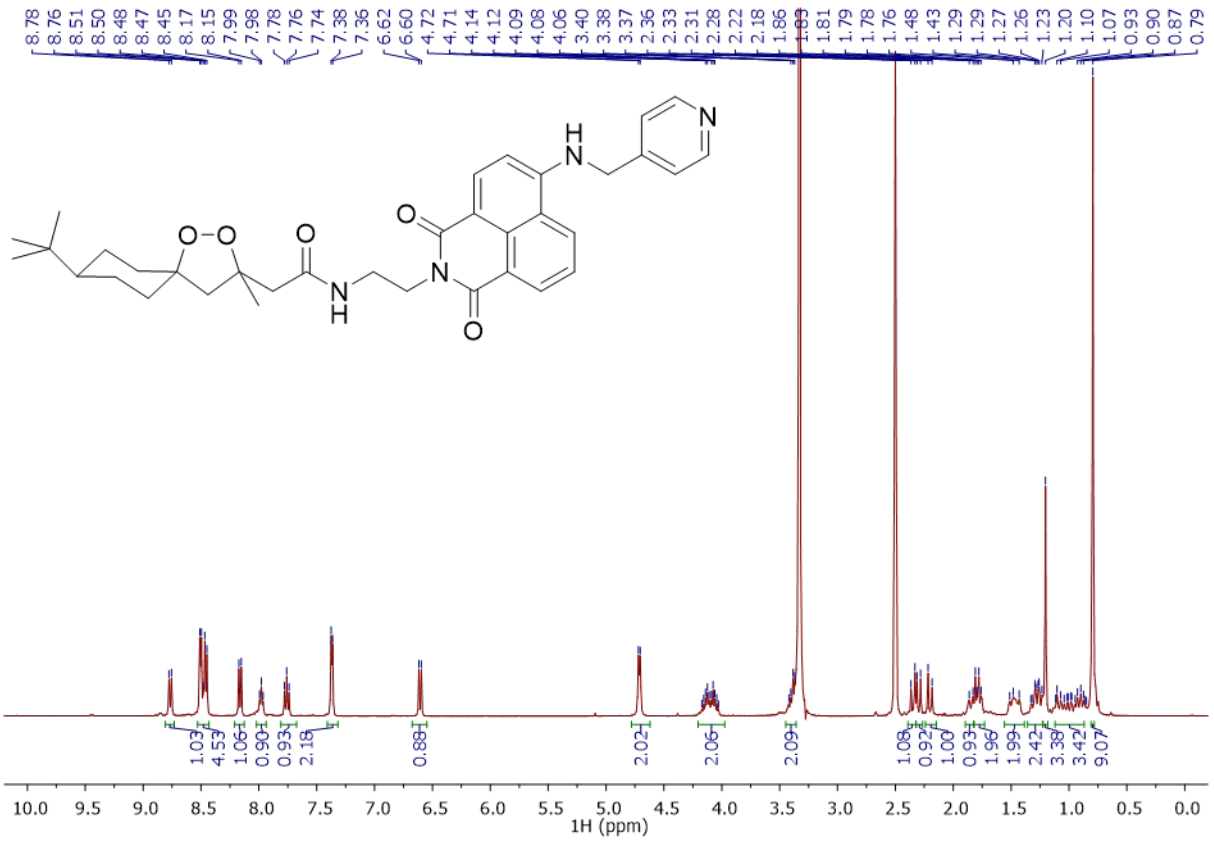
mp = 134–138 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, *J* = 8.4, 1H), 8.51−8.45 (m, 4H), 8.16 (d, *J* = 8.4, 1H), 7.98 (t, *J* = 5.8, 1H), 7.76 (t, *J* = 7.9, 1H), 7.37 (d, *J* = 2H), 6.61 (d, *J* = 8.6, 1H), 4.07 (d, *J* = 5.8, 2H), 4.17−4.03 (m, 2H), 3.42−3.37 (m, 2H), 2.35 (d, *J* = 13.6, 1H), 2.30 (d, *J* = 12.4, 1H), 2.20 (d, *J* = 13.6, 1H), 1.85 (d, *J* = 13.6, 1H), 1.81−1.76 (m, 2H), 1.51−1.43 (m, 2H), 1.29−1.23 (m, 2H), 1.20 (s, 3H), 1.12−0.87 (m, 3H), 0.79 (s, 9H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.1 (C), 163.9 (C), 163.1 (C), 150.0 (C), 149.7 (CH), 147.7 (C), 133.8 (CH), 130.7 (CH), 129.5 (C), 128.3 (CH), 124.7 (CH), 122.3 (C), 122.0 (CH), 120.3 (C), 108.9 (C), 104.5 (CH), 83.8 (C), 83.4 (C), 55.4 (CH_2), 46.2 (CH), 44.9 (CH_2), 44.7 (CH_2), 36.5 (CH_2), 35.2 (CH_2), 34.9 (CH_2), 32.0 (C), 27.3 (CH_3), 24.1 (CH_3), 23.4 (CH_2), 23.3 (CH_2);

HSQC confirmed that there was a $\underline{C}H_2$ resonance under the solvent peak;

HRMS (TOF MS ESI+) *m/z* calc. for $C_{35}H_{43}N_4O_5$ $[M+H]^+$ 599.3228, found 599.3225.


8.78
8.76
8.51
8.50
8.48
8.47
8.45
8.17
8.15
7.99
7.98
7.78
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7.74
7.38
7.36
6.62
6.60
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1.76
1.48
1.43
1.29
1.29
1.27
1.26
1.23
1.20
1.10
1.07
0.93
0.90
0.87
0.79
O
O
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N
H
N
H
N
1.03
4.53
1.06
0.90
0.93
2.18
0.88
2.02
2.06
2.09
1.08
0.92
1.00
0.93
1.96
1.99
2.42
3.38
3.42
9.07
10.0
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8.5
8.0
7.5
7.0
6.5
6.0
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5.0
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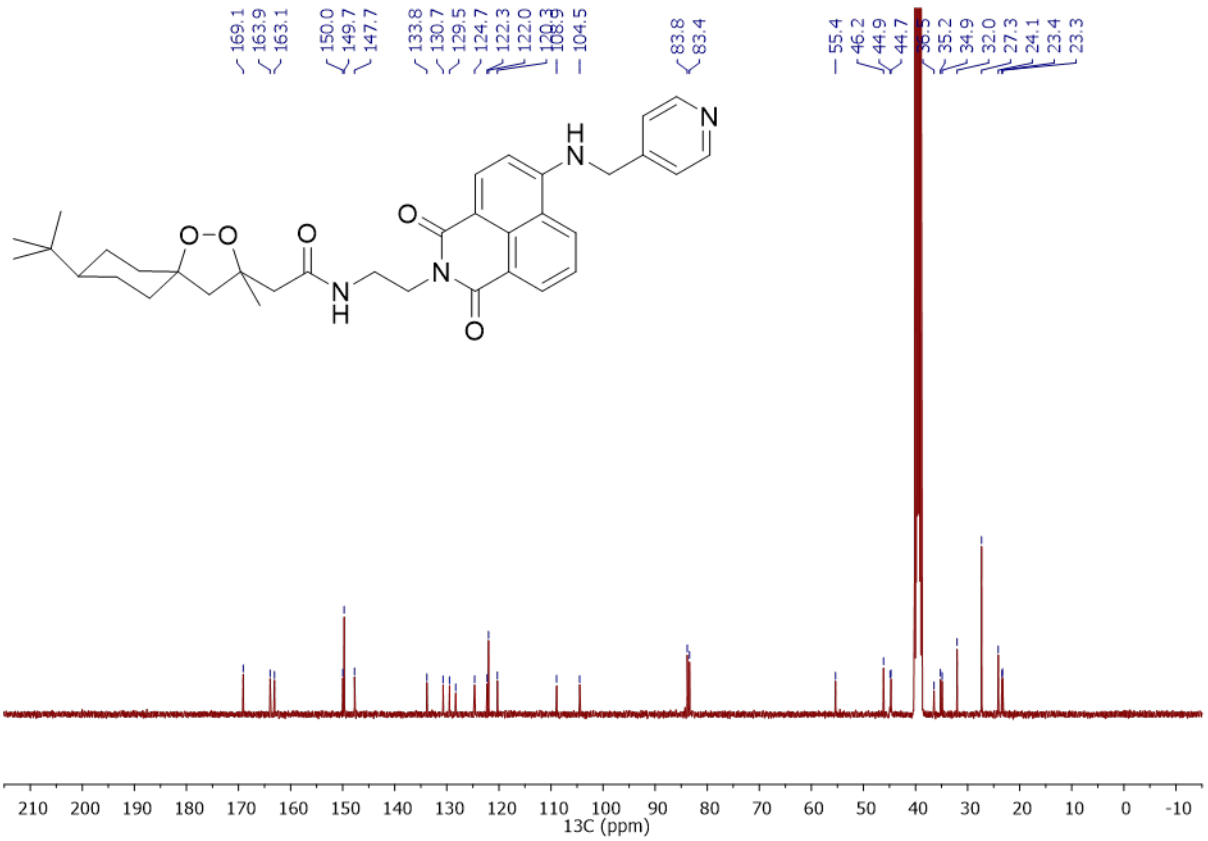
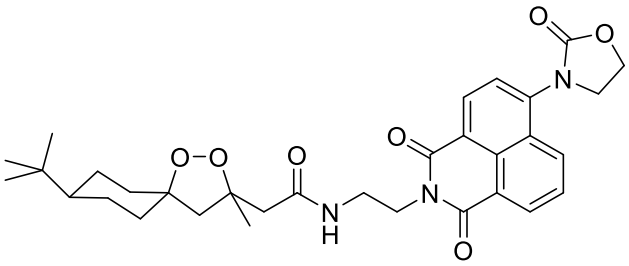
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1H (ppm)



(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(9'-(oxazlidin-16'-onyl)-4'-naphthalimido-*N*-ethylcarbamoylmethyl)-1,2-dioxaspiro[4.5]decane (FINO$_2$-3) (3).**



To a solution of Boc-protected amine **27** (203 mg, 0.439 mmol) in CH_2Cl_2 (4.4 mL) was added TFA (880 µL, 12 mmol), and the mixture was stirred for 6 h. The reaction mixture was concentrated *in vacuo* and coevaporated with $CHCl_3$ (3 × 2 mL) to afford the ammonium·TFA salt. NHS-ester **13** (195 mg, 0.531 mmol) was added to the ammonium salt, followed by $CHCl_3$ (4.4 mL) and NEt_3 (450 µL, 3.2 mmol), and the resulting slurry was stirred for 17 h. The reaction mixture was diluted with aq. NaOH (1 M, 20 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were washed with sat. aq. NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (25:75

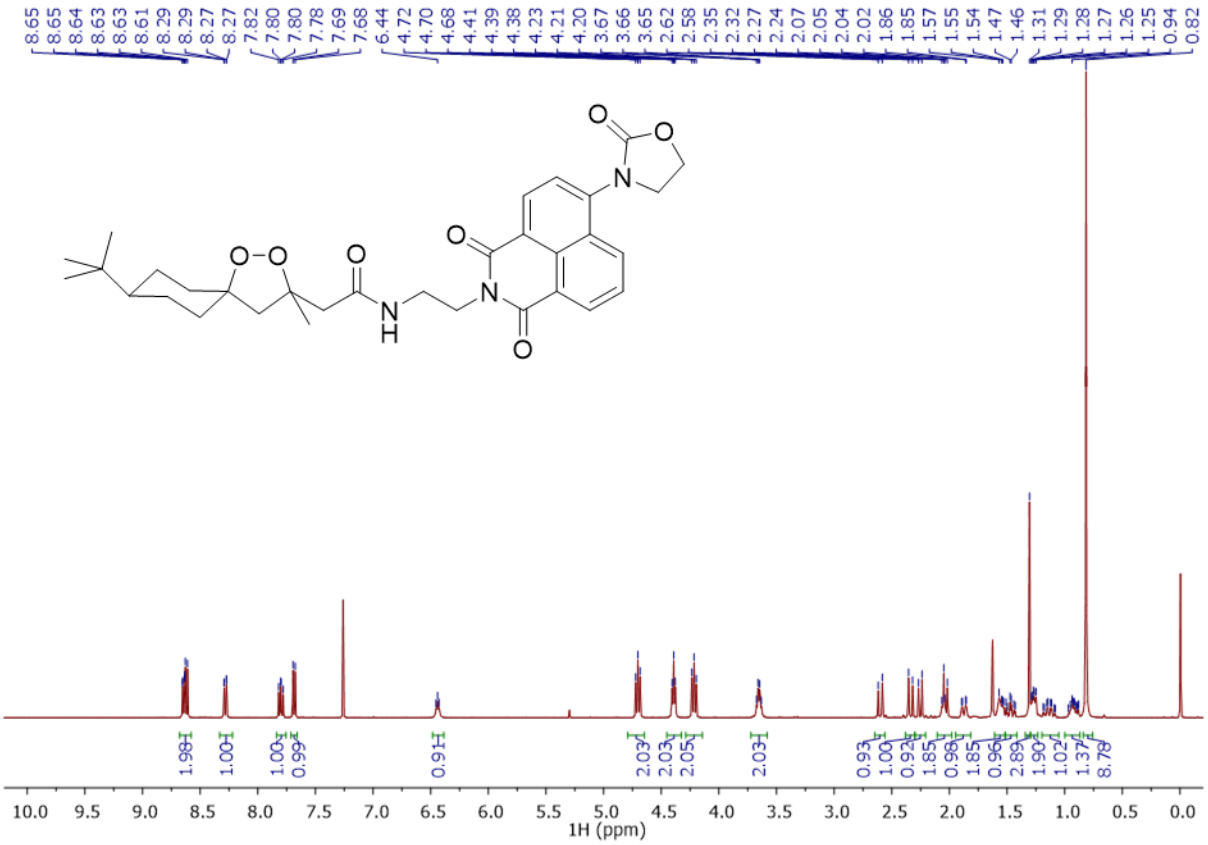
CH_3CN:CH_2Cl_2, *rf* = 0.27) afforded the title compound (259 mg, 96%) as a yellow glass-like solid:

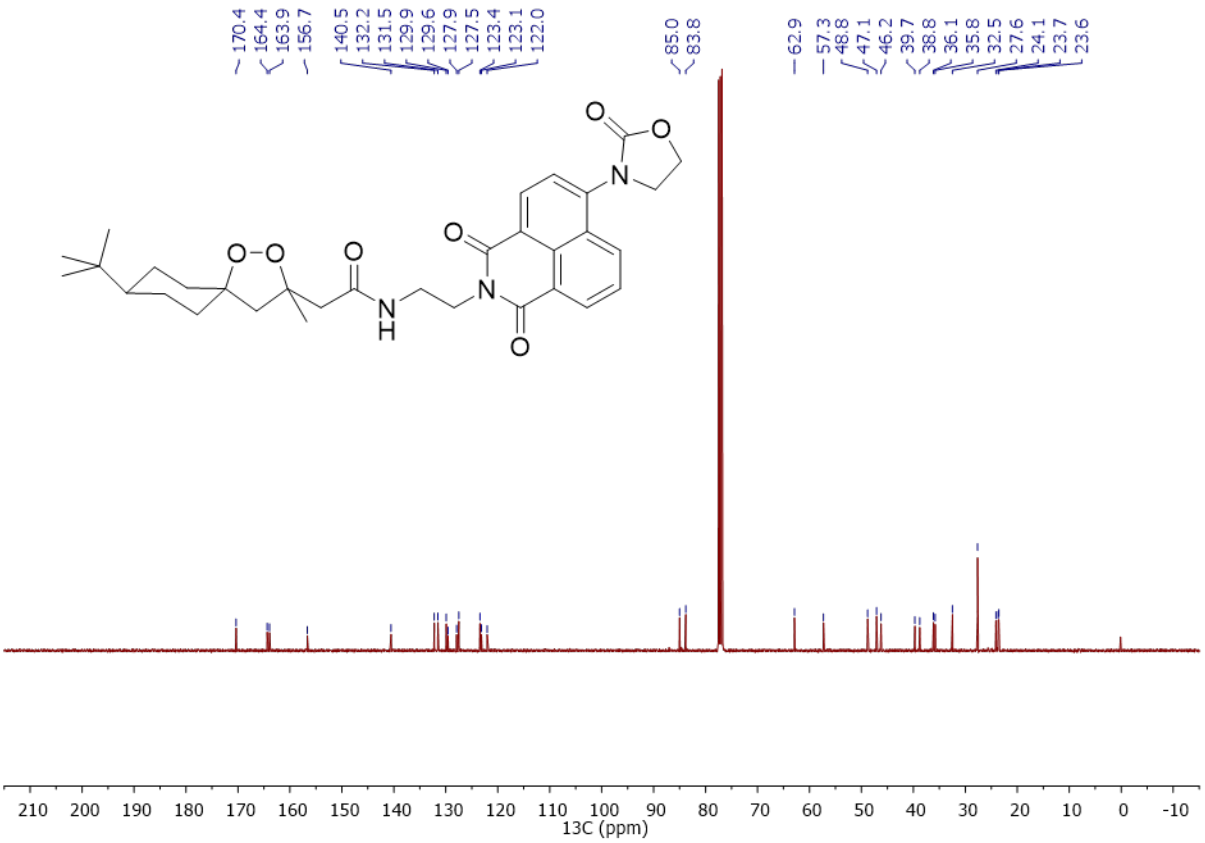
mp = 129–132 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, *J* = 7.3, 0.8, 1H), 8.62 (d, *J* = 7.9, 1H), 8.28 (dd, *J* = 8.5, 0.8, 1H), 7.80 (dd, *J* = 8.4, 7.4, 1H), 7.69 (d, *J* = 7.9, 1H), 6.44 (t, *J* = 5.3, 1H), 4.70 (t, *J* = 7.7, 2H), 4.39 (t, *J* = 5.8, 2H), 4.22 (t, *J* = 7.7, 2H), 3.65 (q, *J* = 5.6, 2H) 2.60 (d, *J* = 14.2, 1H), 2.34 (d, *J* = 14.2, 1H), 2.25 (d, *J* = 12.3, 1H), 2.07−2.02 (m, 2H), 1.87 (dd, *J* = 13.6, 2.6, 1H), 1.57−1.53 (m, 2H), 1.47 (td, *J*; 13.6, 4.1, 1H), 1.31 (s, 3H), 1.29−1.25 (m, 2H), 1.13 (app. qd, *J* = 12.8, 3.4, 1H), 0.97−0.89 (m, 1H), 0.82 (s, 9H);

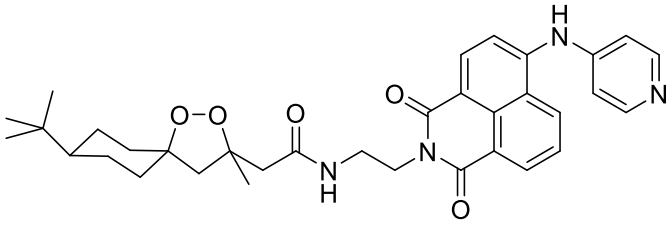
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4 (C), 164.4 (C), 163.9 (C), 156.7 (C), 140.5 (C), 132.2 (CH), 131.5 (CH), 129.9 (CH), 129.6 (C), 127.9 (C), 127.5 (CH), 123.4 (CH), 123.1 (C), 122.0 (C), 85.0 (C), 83.8 (C), 62.9 (CH_2), 57.3 (CH_2), 48.8 (CH_2), 47.1 (CH), 46.2 (CH_2), 39.7 (CH_2), 38.8 (CH_2), 36.1 (CH_2), 35.8 (CH_2), 32.5 (C), 27.6 (CH_3), 24.1 (CH_2), 23.7 (CH_2), 23.6 (CH_3);

HRMS (TOF MS ESI+) *m/z* calc. for $C_{32}H_{40}N_3O_7$ $[M+H]^+$ 578.2861, found 578.2870.


8.65 8.65 8.64 8.63 8.63 8.61 8.29 8.29 8.27 8.27 7.82 7.80 7.80 7.78 7.69 7.68 6.44 4.72 4.70 4.68 4.41 4.39 4.38 4.23 4.21 4.20 3.67 3.66 3.65 2.62 2.58 2.35 2.32 2.27 2.24 2.07 2.05 2.04 2.02 1.86 1.85 1.57 1.55 1.54 1.47 1.46 1.31 1.29 1.28 1.27 1.26 1.25 0.94 0.82
O
O
N
O
O-O
O
N
N
H
O
1.90 1.00 1.00 0.99 0.91 2.03 2.03 2.05 2.03 0.93 1.00 0.92 1.85 0.98 1.85 0.96 2.89 1.90 1.02 1.37 8.78
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)



(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(9'-(*p*-pyridylamino)-4'-naphthalimido-*N*-ethylcarbamoylmethyl)-1,2-dioxaspiro[4.5]decane (FINO$_2$-5) (7).**



Boc-protected amine **26** (141 mg, 0.326 mol) was dissolved in CH_2Cl_2 (3.4 mL) followed by addition of TFA (650 µL, 8.5 mmol) and the resulting solution was stirred for 5 h. The reaction mixture was concentrated *in vacuo* and coevaporated with $CHCl_3$ (3 × 2 mL) to afford the ammonium·TFA salt. NHS-ester **13** (103 mg, 0.280 mmol) was added to the ammonium salt, followed by $CHCl_3$ (5.6 mL) and NEt_3 (240 µL, 1.7 mmol), and the resulting slurry was stirred for 16 h. The reaction mixture was diluted with aq. NaOH (1 M, 25 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with sat. aq. NaCl (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column

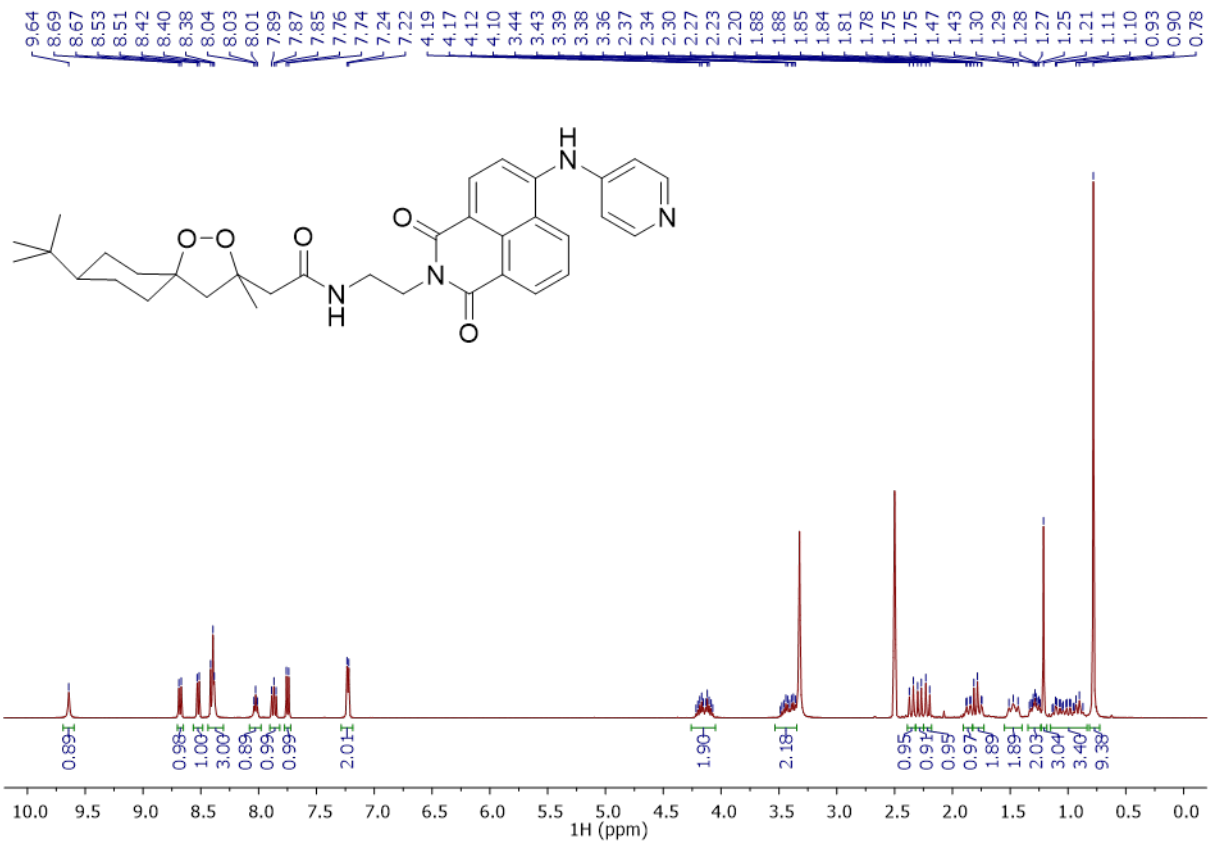
chromatography (5:95 CH_3OH:CH_2Cl_2, *rf* = 0.09) afforded the title compound (110 mg, 58%) as a yellow glass-like solid:

mp: = 144–146 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (br s, 1H), 8.68 (d, *J* = 8.5, 1H), 8.52 (d, *J* = 7.2, 1H), 8.42−8.38 (m, 3H), 8.03 (t, *J* = 6.0, 1H), 7.87 (t, *J* = 7.9, 1H), 7.75 (d, *J* = 8.2, 1H), 7.23 (d, *J* = 6.2, 2H), 4.22−4.07 (m, 2H), 3.49−3.36 (m, 2H), 2.35 (d, *J* = 13.7, 1H), 2.28 (d, *J* = 12.4, 1H), 2.21 (d, *J* = 13.6, 1H), 1.86 (dd, *J* = 13.5, 2.3, 1H), 1.81−1.75 (m, 2H), 1.51−1.43 (m, 2H), 1.29 (tt, *J* = 14.2, 5.0, 2H), 1.21 (s, 3H), 1.14−0.87 (m, 3H), 0.78 (s, 9H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.2 (C), 163.7 (C), 163.1 (C), 150.4 (CH), 149.0 (C), 143.6 (C), 132.3 (CH), 131.1 (CH), 129.3 (C), 129.2 (CH), 126.1 (CH), 124.0 (C), 122.6 (C), 115.5 (C), 114.0 (CH), 112.3 (CH), 83.8 (C), 83.4 (C), 55.4 (CH_2), 46.1 (CH), 44.7 (CH_2), 36.3 (CH_2), 35.2 (CH_2), 34.9 (CH_2), 32.0 (CH), 27.3, (CH_3), 24.1 (CH_3), 23.4 (CH_2), 23.3 (CH_2); HSQC confirmed that there was a $\underline{C}H_2$ resonance under the solvent peak;

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{34}H_{41}N_4O_5$ $[M+H]^+$ 585.3071, found 585.3073.


9.64 8.69 8.67 8.53 8.51 8.42 8.40 8.38 8.04 8.03 8.01 7.89 7.87 7.85 7.76 7.74 7.24 7.22 4.19 4.17 4.12 4.10 3.44 3.43 3.39 3.38 3.36 2.37 2.34 2.30 2.27 2.23 2.20 1.88 1.88 1.85 1.84 1.81 1.78 1.75 1.75 1.47 1.43 1.30 1.29 1.28 1.27 1.25 1.21 1.11 1.10 0.93 0.90 0.78
H
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H
N
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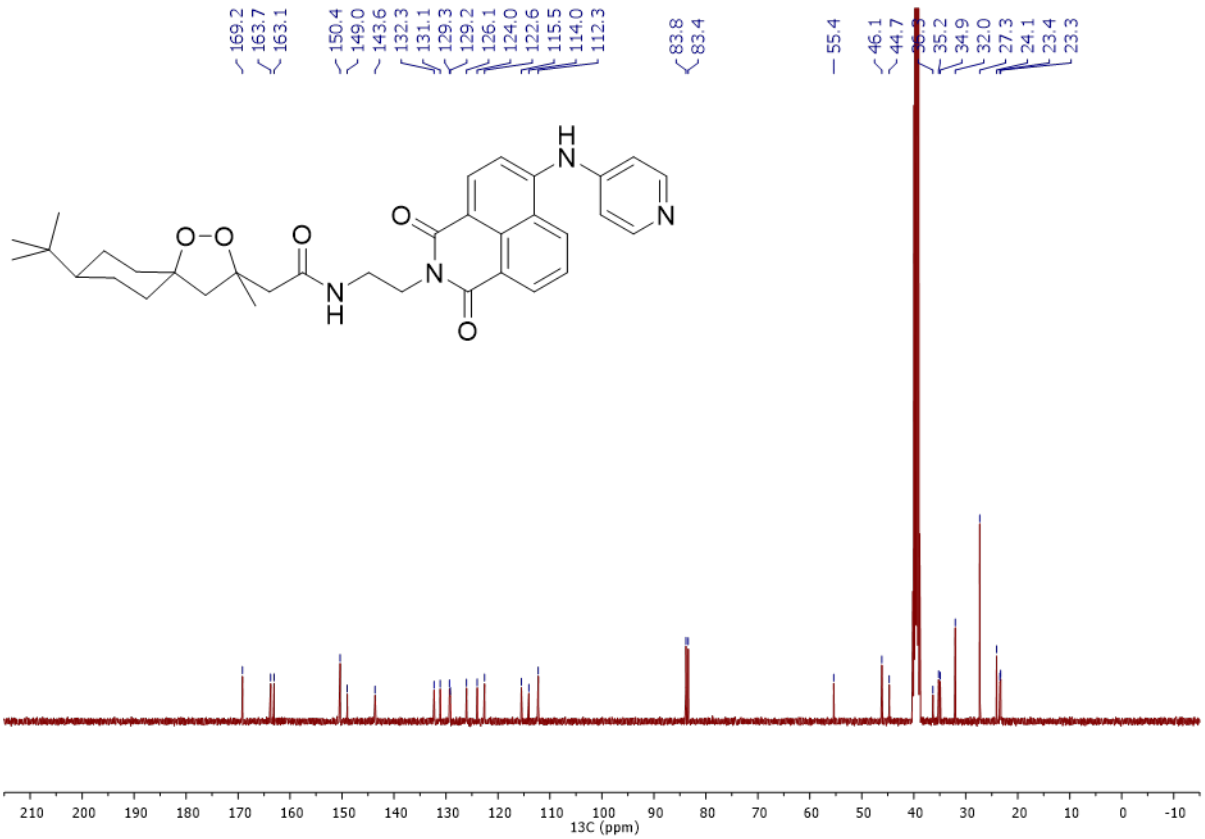
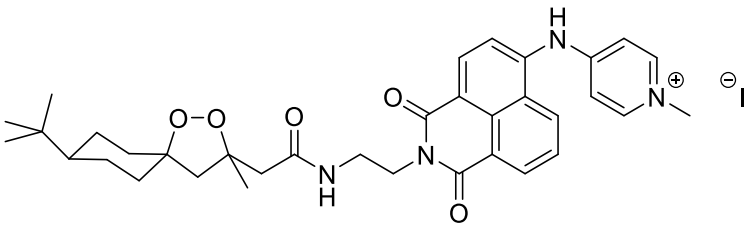
0.89 0.98 1.00 3.00 0.89 0.99 0.99 2.01 1.90 2.18 0.95 0.91 0.99 0.97 1.89 1.89 2.03 3.04 3.40 9.38
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)



(5*R,8*R**)-8-*tert*-Butyl-3-methyl-3-(9'-(*p*-methylpyridiniumamino)-4'-naphthalimido-*N*-ethylcarbamoylmethyl)-1,2-dioxaspiro[4.5]decane iodide (FINO$_2$-6) (8).**



Pyridine **FINO$_2$-5** (22 mg, 0.038 mmol) was dissolved in acetone (370 µL) followed by addition of CH_3I (7.5 µL, 0.12 mmol). The mixture was stirred for 20 h. The solid material was collected by vacuum filtration and washed with acetone (3 × 2 mL) to afford the title compound (16 mg, 58%) as an orange powder:

mp = dec. 205 °C;

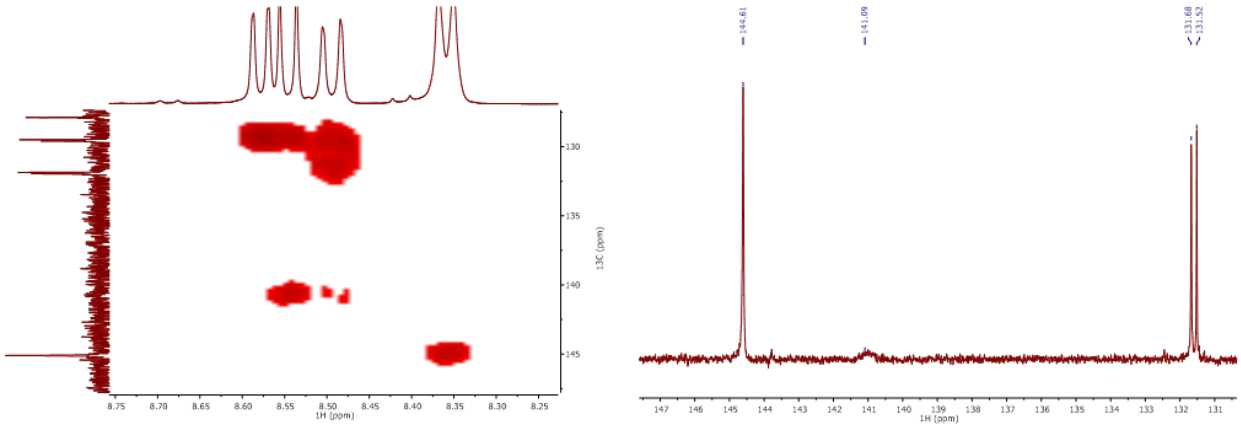
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (br s, 1H), 8.58 (d, J = 7.2, 1H), 8.55 (d, J = 7.9, 1H), 8.49 (d, J = 8.5, 1H), 8.36 (d, J = 7.1, 2H), 8.05 (t, J = 6.1, 1H), 7.94 (t, J = 7.9, 1H), 7.88 (d, J = 7.9, 1H), 7.24 (d, J = 7.1, 2H), 4.22−4.21 (m, 2H), 4.01 (s, 3H), 3.47−3.38 (m, 2H), 2.37 (d, J =

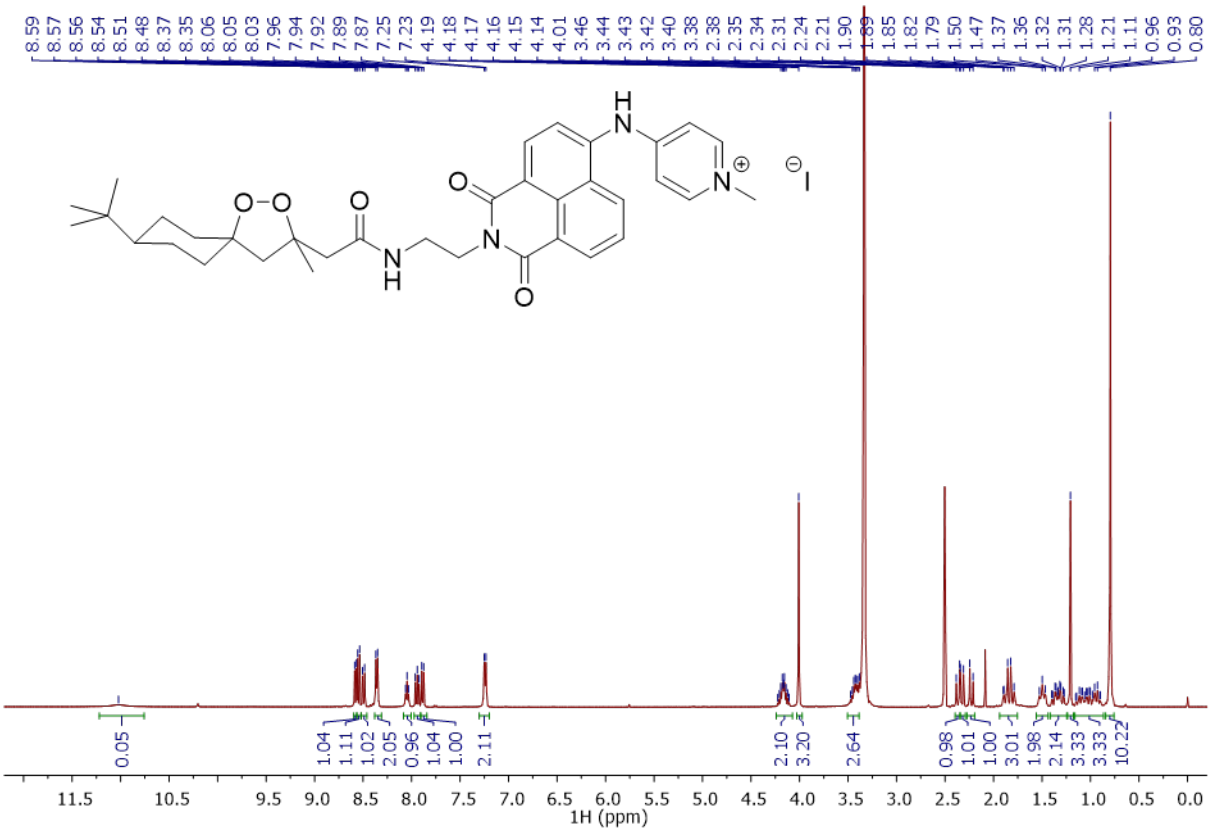
13.8, 1H), 2.33 (d, *J* = 12.4, 1H), 2.23 (d, *J* = 13.8, 1H), 1.90−1.79 (m, 3H), 1.50 (app. t, *J* = 12.4, 2H), 1.40−1.27 (m, 2H), 1.21 (s, 3H), 1.52−0.90 (m, 3H), 0.80 (s, 9H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.2 (C), 163.5 (C), 163.0 (C), 155.6 (C), 144.6 (CH), 131.5 (CH), 131.4 (CH), 129.1 (CH), 129.0 (C), 127.4 (CH), 126.3 (C), 122.9 (C), 121.3 (CH), 120.0 (C), 110.7 (CH), 83.9 (C), 83.5 (C), 55.5 (CH_2), 46.1 (CH), 45.0 (CH_3), 44.8 (CH_2), 36.3 (CH_2), 35.2 (CH_2), 34.9 (CH_2), 32.1 (C), 27.3 (CH_3), 24.0 (CH_3), 23.5 (CH_2), 23.3 (CH_2); HSQC confirmed that there was a $\underline{C}H_2$ resonance under the solvent peak;

HMBC confirmed ^{13}C at ≈ δ 141 ppm assigned as the aniline carbon of the naphthalimide ring based on 2D correlations. The ^{13}C NMR data was collected on a 201 MHz NMR for 20000 scans in an attempt to resolve this peak but only a broad peak was observed;

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{35}H_{43}N_4O_5$ $[M^*]^+$ 600.3260, found 600.3262.




8.59 8.57 8.56 8.54 8.51 8.48 8.37 8.35 8.06 8.05 8.03 7.96 7.94 7.92 7.89 7.87 7.25 7.23 4.19 4.18 4.17 4.16 4.15 4.14 4.01 3.46 3.44 3.43 3.42 3.40 3.38 2.38 2.35 2.34 2.31 2.24 2.21 1.90 1.89 1.85 1.82 1.79 1.50 1.47 1.37 1.36 1.32 1.31 1.28 1.21 1.11 0.96 0.93 0.80
H
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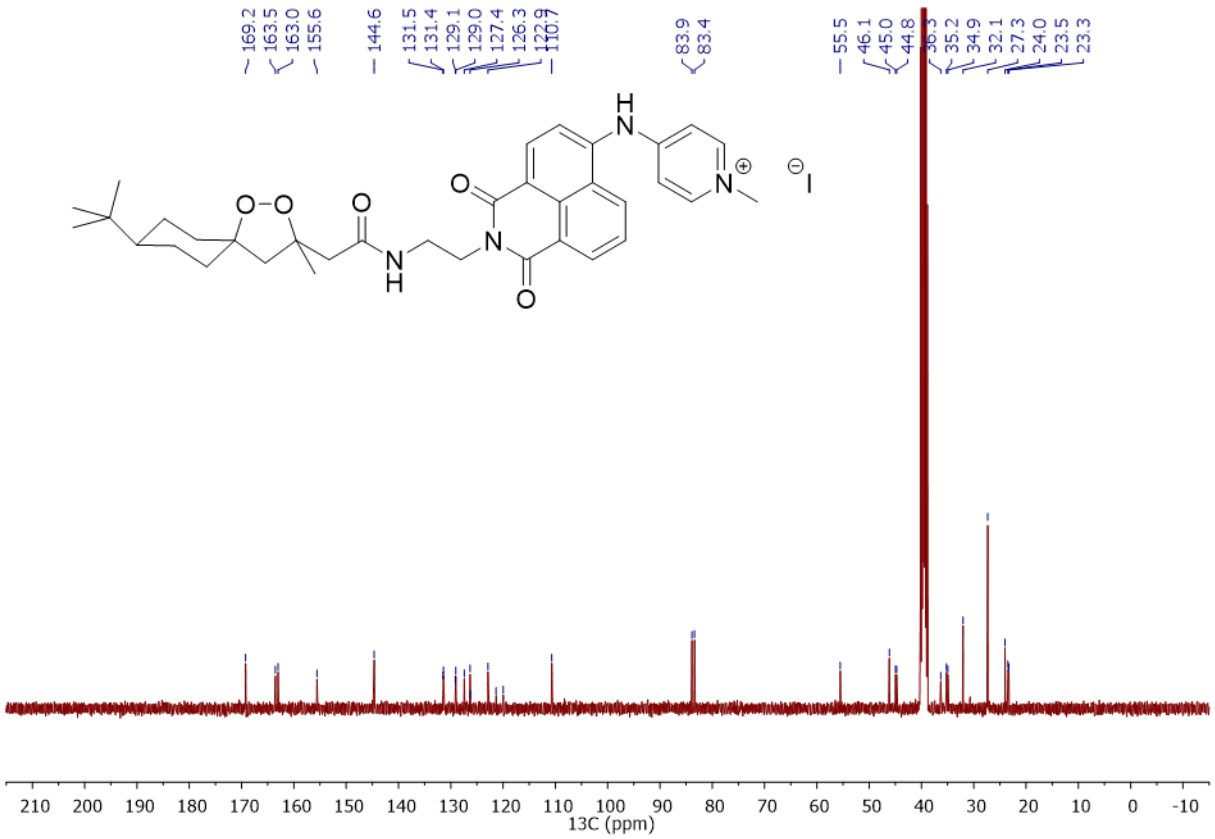
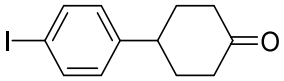
O
O
O
N
H
N
I
0.05 1.04 1.11 1.02 2.05 0.96 1.04 1.00 2.11 2.10 3.20 2.64 0.98 1.01 1.00 3.01 1.98 2.14 3.33 3.33 10.22
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)



4-(*p*-Iodophenyl)cyclohexanone (14) [3]



A modified procedure of Tang et al. was followed. A slurry of 4-phenylcyclohexanone, (2.02 g, 11.5 mmol) and *N*-iodosuccinimide (5.13 g, 22.8 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, 46 mL) was stirred for 64 h at reflux (80 °C). Upon cooling, the solvent was removed *in vacuo* and the residual slurry was resuspended in sat. aq. $NaHCO_3$ (25 mL). The product was extracted into CH_2Cl_2 (4 × 25 mL), and the combined organic phases were washed with sat. aq. $Na_2S_2O_3$ (50 mL) and sat. aq. NaCl (50 mL) and were then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (10:90 –70:30 EtOAc:hexanes, *rf* = 0.28 in 10%) afforded the title compound (1.89 g, 55%) as a white powder:

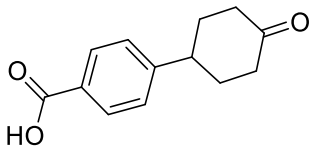
mp **=** 98–100 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.4, 2H), 7.00 (d, *J* = 8.2, 2H), 2.98 (tt, *J* = 12.1, 3.3, 1H), 2.51−2.48 (m, 4H), 2.22−2.17 (m, 2H), 1.96−1.85 (m, 2H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 210.7 (C), 144.4 (C), 137.7 (CH), 128.8 (CH), 91.6 (C), 42.3 (CH), 41.2 (CH_2), 38.8 (CH_2);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{12}H_{14}OI$ $[M+H]^+$ 301.0084, found 301.0079.

4-(*p*-benzoic acid)cyclohexanone (15) [4,5]



A modified procedure Cacchi et al. was followed. Under a N_2 atmosphere HCOONa (688 mg, 10.1 mmol), Ac_2O (640 µL, 6.8 mmol), and Et*i*-Pr_2 (1.12 mL, 6.43 mmol) were added to dry DMF (4.0 mL) and the mixture was stirred for 1 h. In a separate flask under N_2 was combined iodophenylcyclohexanone **14** (1.01 g, 3.37 mmol), $Pd_2(dba)_3$ (80 mg, 0.09 mmol), LiCl (468 mg, 11.0 mmol), and dry DMF (6.0 mL) followed by transfer by cannula into the reaction mixture. The mixture was heated at 80 °C for 19 h before being allowed to cool, at which time it was diluted with aq. NaOH (1 M, 25 mL), washed with EtOAc (2 × 20 mL), acidified to pH 1 using aq. HCl [3.0 M], and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with sat. aq. NaCl (25 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude mixture was typically used directly in the next reaction but could be purified by column chromatography (50%−60% EtOAc/hexanes, *rf* = 0.26 in 50%) to afford the title compound (508 mg, 69%) as a white powder:

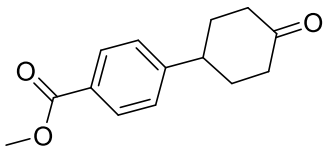
mp >200 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (br s, 1H), 8.06 (d, *J* = 8.2, 2H), 7.36 (d, *J* = 8.2, 2H), 3.11 (tt, *J* = 12.1, 3.2, 1H), 2.55−2.51 (m, 4H), 2.27−2.23 (m, 2H), 2.03−1.92 (m, 2H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 210.6, 171.1, 151.1, 130.6, 127.7, 126.9, 42.9, 41.2, 33.6;

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{13}H_{15}O_3$ $[M+H]^+$ 202.0944, found 202.0948;

Methyl *p*-(4-cyclohexanone)benzoate (16) [6]

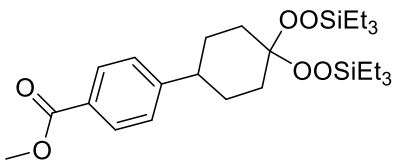


To a solution of carboxylic acid **15** (508 mg, 2.33 mmol) in MeOH (22.5 mL) was added H_2SO_4 (2.3 mL, 42 mmol) dropwise and the mixture was stirred for 16 h. The reaction mixture was diluted with aq. NaOH (1 M, 80 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with sat. aq. NaCl (20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude mixture is sufficiently pure to use in the next reaction but could be purified by column chromatography (10:90−30:70 EtOAc:hexanes *rf* = 0.36 in 30:70 EtOAc:hexanes) to afford the title compound (438 mg, 81%) as a white powder:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4, 2H), 7.32 (d, *J* = 8.2, 2H), 3.91 (s, 3H), 3.09 (tt, *J* = 12.1, 3.2, 1H), 2.54−2.51 (m, 4H), 2.25−2.21 (m, 2H), 2.04−1.91 (m, 2H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 210.7, 167.1, 150.2, 130.1, 128.8, 126.9, 52.2, 43.0, 41.4, 33.8.

Methyl *p*-(4,4-bis(triethylsilylperoxy)cyclohexanone)benzoate (17)

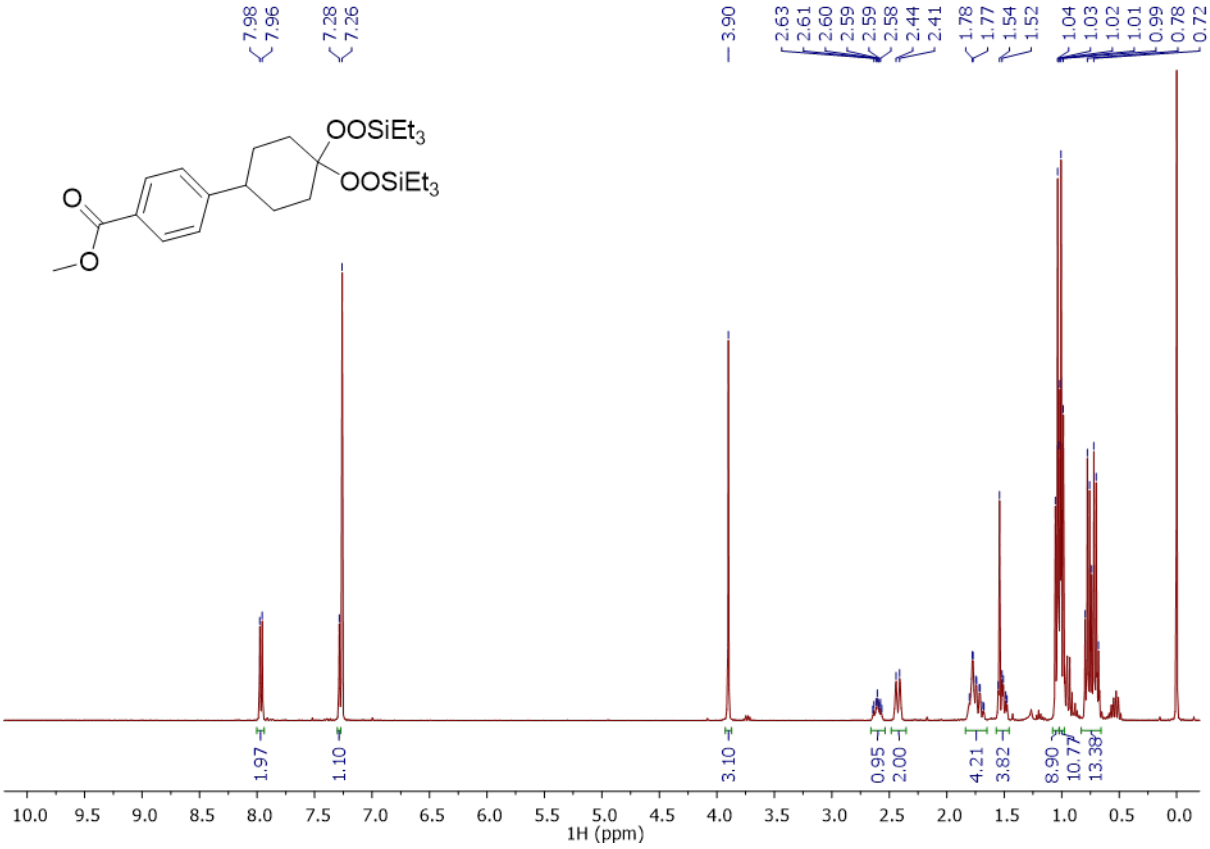


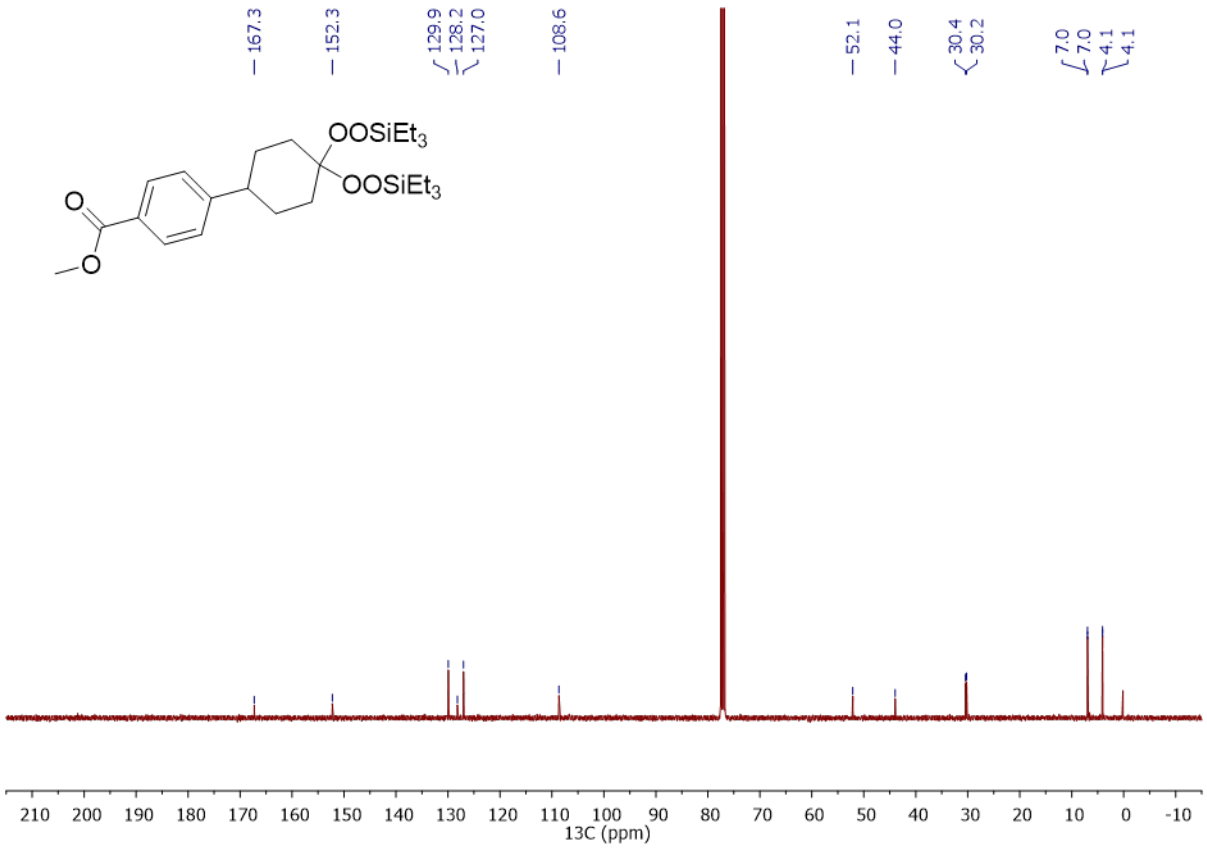
To a vigorously stirred mixture of ketone **16** (919 mg, 3.96 mmol) and PMA·H_2O (728 mg, 0.399 mmol) in Et_2O (25.0 mL) was added aq. H_2O_2 (50% aq., 6.8 mL, 120 mmol). The reaction mixture was stirred for 5 h and then diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (4 × 10 mL). The combined organic phases were washed with sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a white powder. The hydroperoxyketal intermediate was then combined with DMAP (50 mg, 0.41 mmol) and placed under a N_2 atmosphere before being dissolved in DMF (10.0 mL). The reaction was stirred followed by addition of NEt_3 (1.4 mL, 10 mmol) then Et_3SiOTf (2.2 mL, 9.9 mmol). The mixture was stirred for a further 16 h. The mixture was diluted with H_2O (25 mL) and extracted with pentane (4 × 25 mL). The combined organic phases were washed with sat. aq. NaCl (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (2:98 EtOAc:hexanes, *rf* = 0.23) afforded the title compound (1.81 g, 89%) as a colorless oil:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.2, 2H), 7.28 (d, 2H), 3.90 (s, 3H), 2.60 (tt, *J* = 11.6, 4.1, 1H), 2.43 (d, *J* = 12.3, 2H), 1.80−1.68 (m, 4H), 1.55−1.48 (m, 2H), 1.04 (t, *J* = 7.9, 9H), 1.01 (t, *J* = 7.9, 9H), 0.77 (q, *J* = 7.9, 6H), 0.71 (q, *J* = 8.0, 6H); two aromatic protons, visible by HSQC, were obscured by the peak for residual $CHCl_3$;

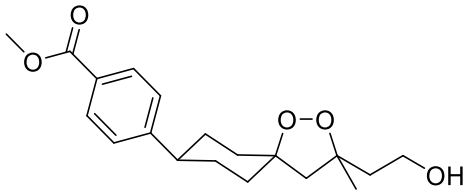
^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 (C), 152.3 (C), 129.9 (CH), 128.2 (C), 127.0 (CH), 108.6 (C), 52.1 (CH_3), 44.0 (CH), 30.4 (CH_2), 30.2 (CH_2), 6.98 (CH_3), 6.95 (CH_3), 4.07 (CH_2), 4.06 (CH_2);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{26}H_{47}O_6Si_2$ $[M+H]^+$ 511.2960, found 511.2901.





(5*R,8*R**)-8-(*p*-Methyl benzoate)-3-methyl-3-(2'-hydroxyethyl)-1,2-dioxaspiro[4.5]decane (18).**



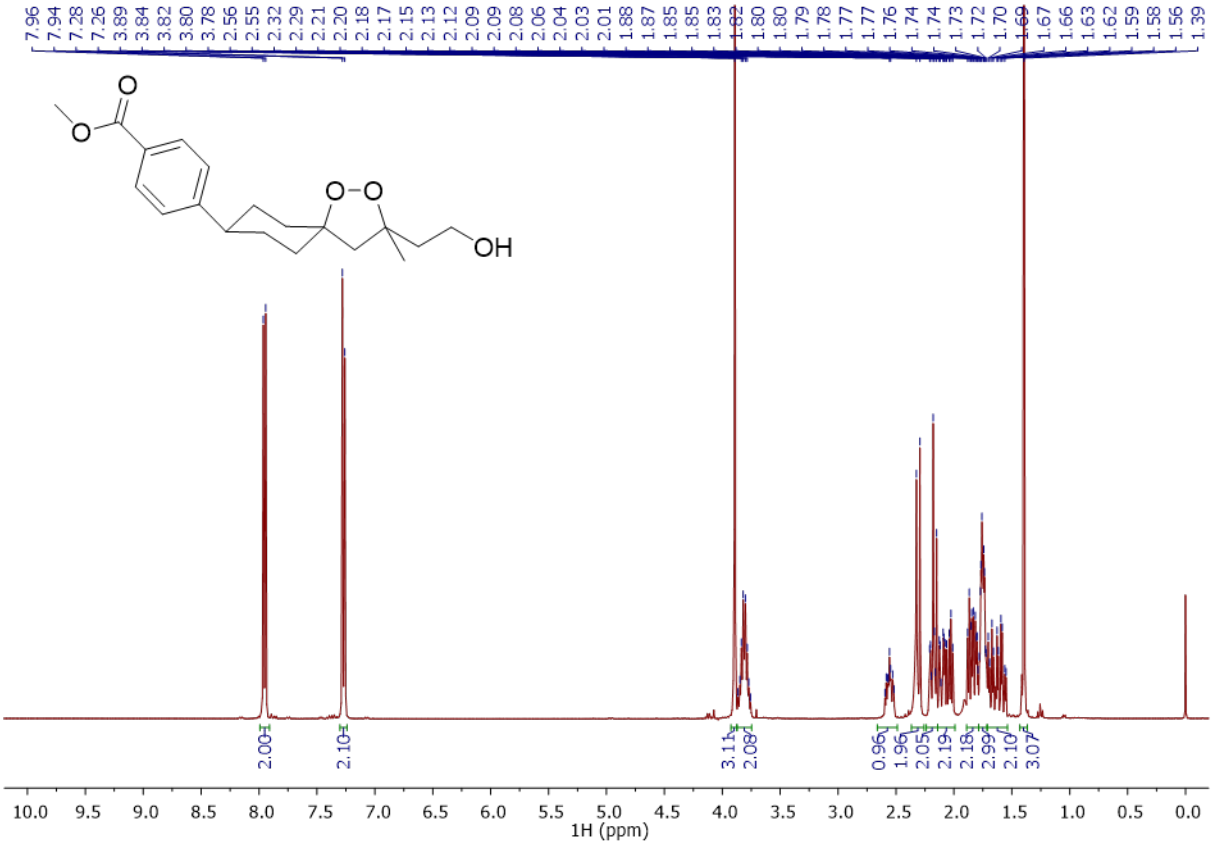
A flask containing diperoxyketal **17** (1.75 g, 3.43 mmol) and alkene **10** (2.13 g, 10.6 mmol) was placed under N_2 and dissolved in CH_2Cl_2 (22.0 mL) prior to stirring at −20 °C. Once the mixture had reached the desired temperature, $SnCl_4$ (830 µL, 7.1 mmol) was added and the mixture was stirred for an additional 2 h at −20 °C before it was warmed to ambient temperature and stirred for an additional 1 h. The reaction mixture was diluted with sat. aq. Rochelle's salt (50 mL) and stirred for 20 h. The biphasic mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (4 × 40 mL). The organic phases were combined with the emulsion and washed with aq. HCl (5%, 50 mL), sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (40:60 EtOAc:hexanes, *rf* = 0.29) afforded the title compound (840.0 g, 72%) as a white powder:

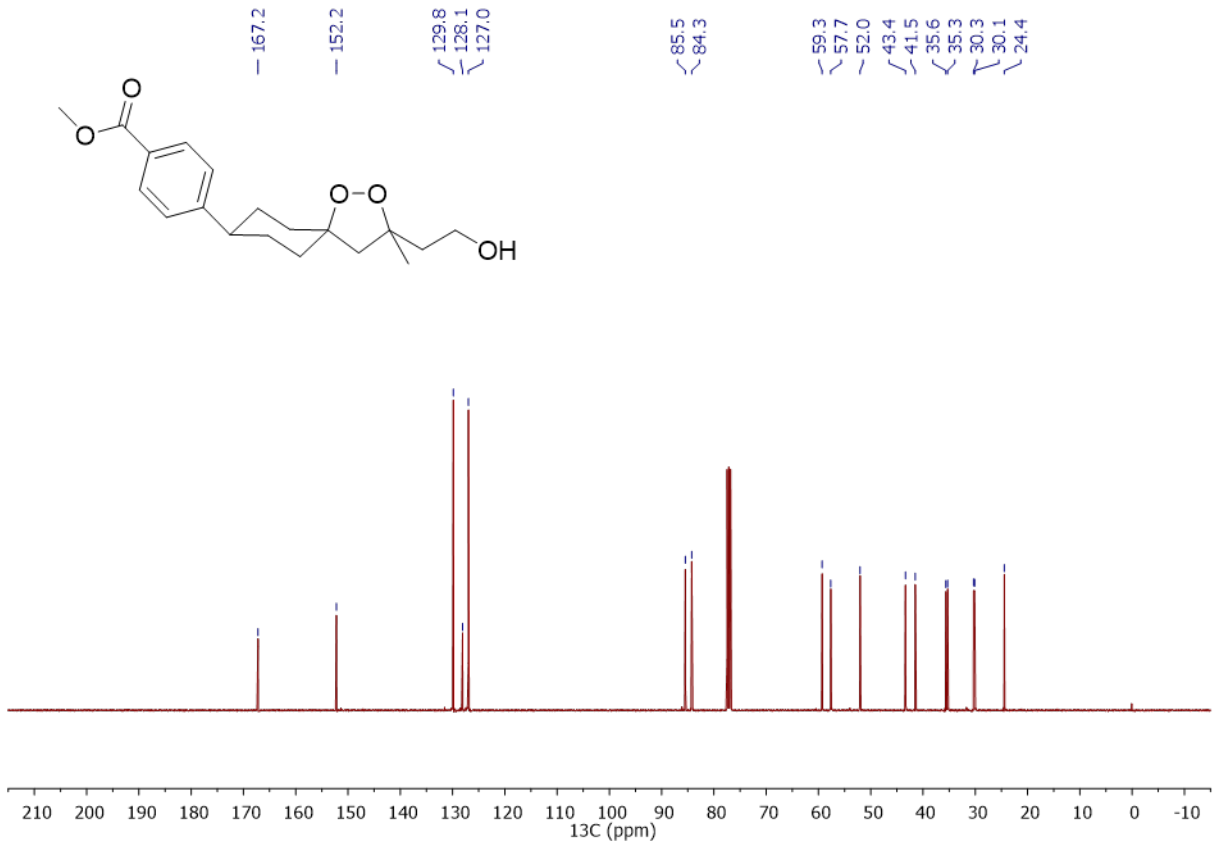
mp **=** 95–97 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3, 2H), 7.27 (d, *J* = 8.4, 2H), 3.89 (s, 3H), 3.86−3.76 (m, 2H), 2.60−2.52 (m, 1H), 2.32 (br s, 1H), 2.31 (d, *J* = 12.1, 1H), 2.21−2.15 (m, 2H), 2.13−2.01 (m, 2H), 1.88−1.79 (m, 2H), 1.78−1.72 (m, 3H), 1.71−1.55 (m, 2H), 1.39 (s, 3H);

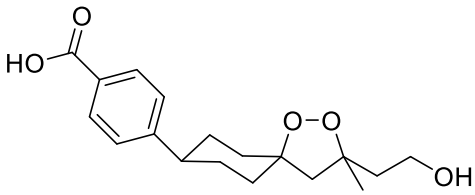
^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2 (C), 152.2 (C), 129.8 (CH), 128.0 (C), 127.0 (CH), 85.5 (C), 84.2 (C), 59.3 (CH_2), 57.7 (CH_2), 52.1 (CH_3), 43.4 (CH), 41.5 (CH_2), 35.6 (CH_2), 35.3 (CH_2), 30.3 (CH_2), 30.1 (CH_2), 24.5 (CH_3);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{19}H_{25}O_4$ $[(M+H)-H_2O]^+$ 317.1747, found 317.1754.





(5*R,8*R**)-8-(*p*-Benzoic acid)-3-methyl-3-(2'-hydroxyethyl)-1,2-dioxaspiro[4.5]decane (19).**



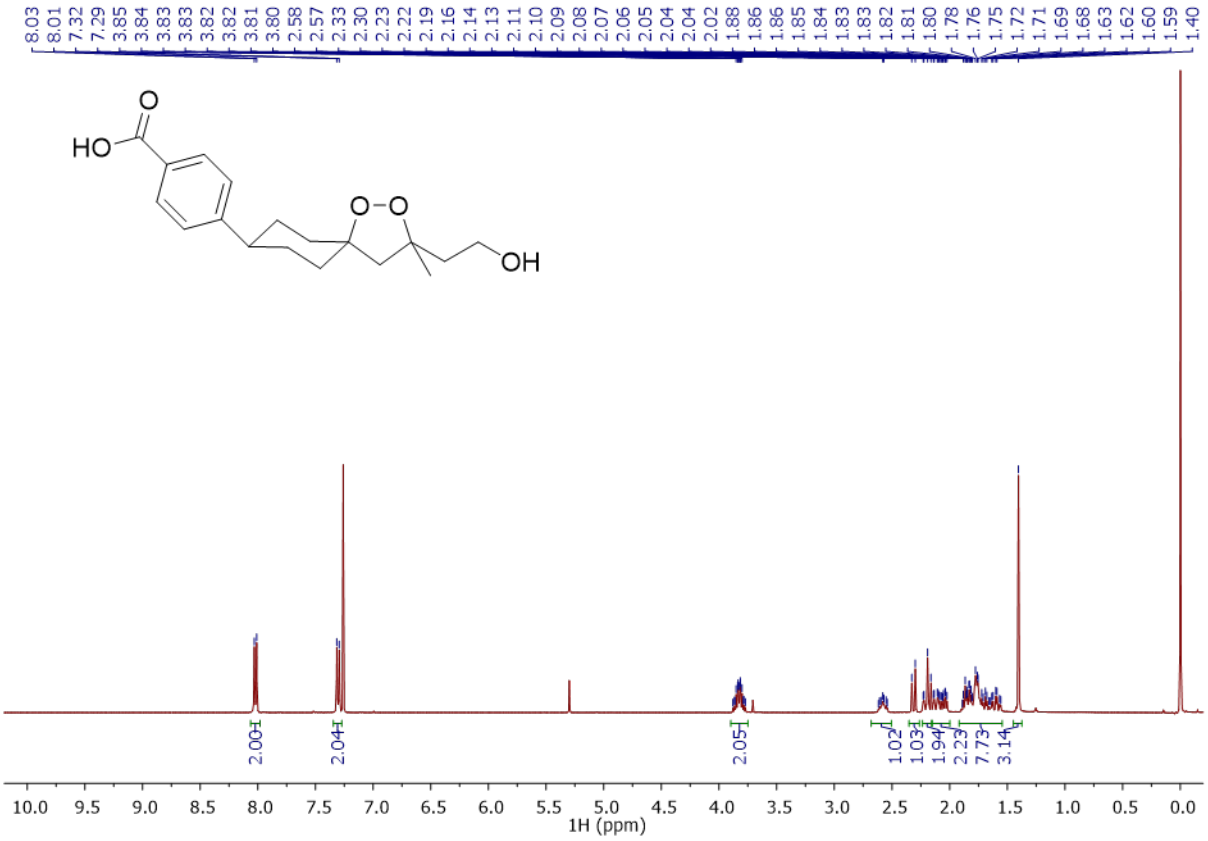
To a stirring solution of ester **18** (626 mg, 1.87 mmol) in dioxane (9.4 mL) was added aq. LiOH (2.0 M, 9.4 mL, 19 mmol). The reaction mixture was stirred for 2 h before being acidified with aq. HCl (5%, 25 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic phases were washed with sat. aq. NaCl (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford the title compound (570 mg, 95%) as a white powder:

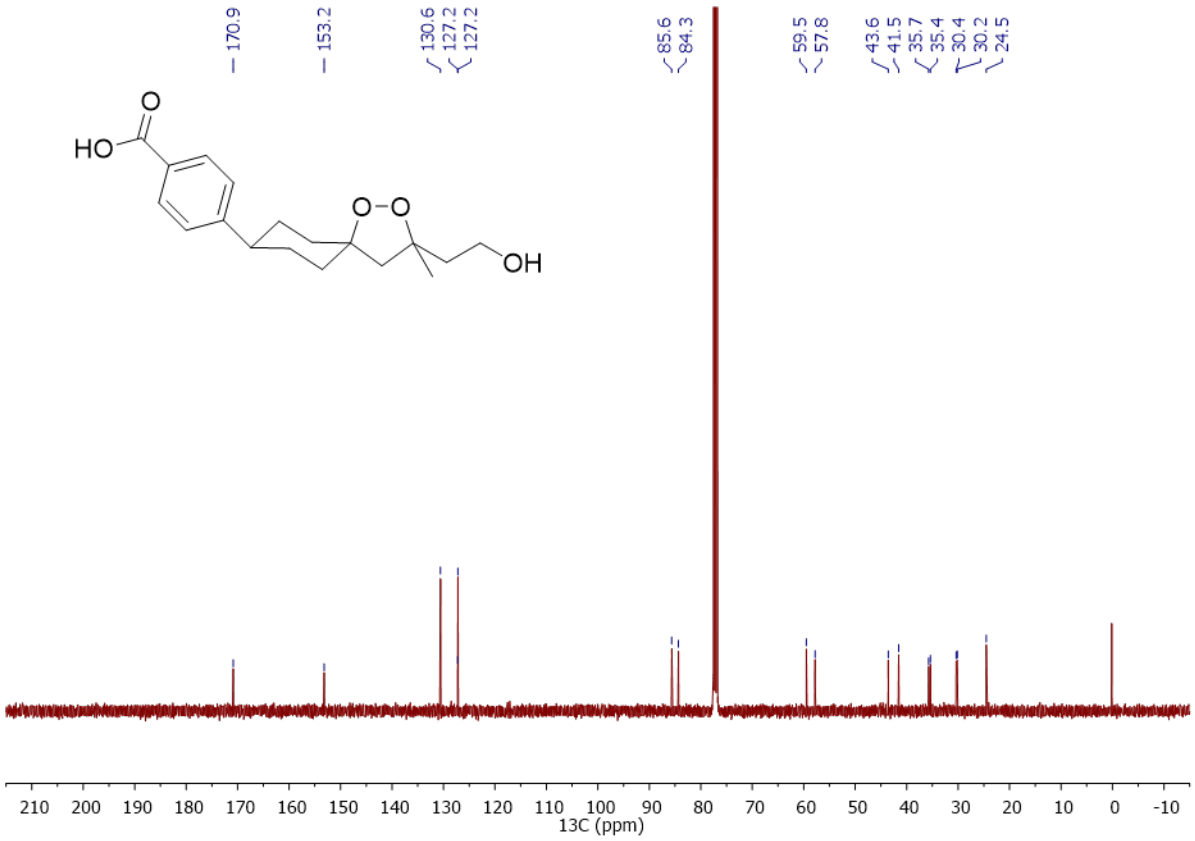
mp = slow dec. 162 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.3, 2H), 7.30 (d *J* = 8.3, 2H), 3.88−3.77 (m, 2H), 2.62−2.54 (m, 1H), 2.31 (d, *J* = 12.0, 1H), 2.23−2.16 (m, 2H), 2.14−2.02 (m, 2H), 1.86−1.56 (m, 8H), 1.40 (s, 3H);

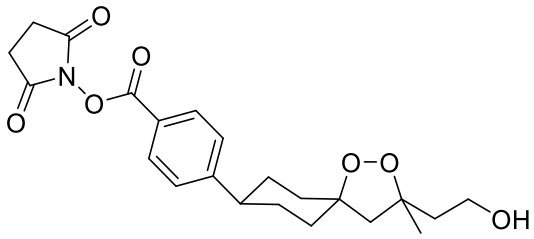
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9 (C), 153.2 (C), 130.6 (CH), 127.22 (C), 127.19 (CH), 85.6 (C), 84.3 (C), 59.5 (CH_2), 57.8 (CH_2), 43.6 (CH), 41.5 (CH_2), 35.7 (CH_2), 35.4 (CH_2), 30.4 (CH_2), 30.2 (CH_2), 24.5 (CH_3);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{18}H_{23}O_4$ $[(M+H)-H_2O]^+$ 303.1591, found 303.1599.





(5*R,8*R**)-8-(succinimidyl-*p*-Benzoate)-3-methyl-3-(2'-hydroxyethyl)-1,2-dioxaspiro[4.5]decane (20).**



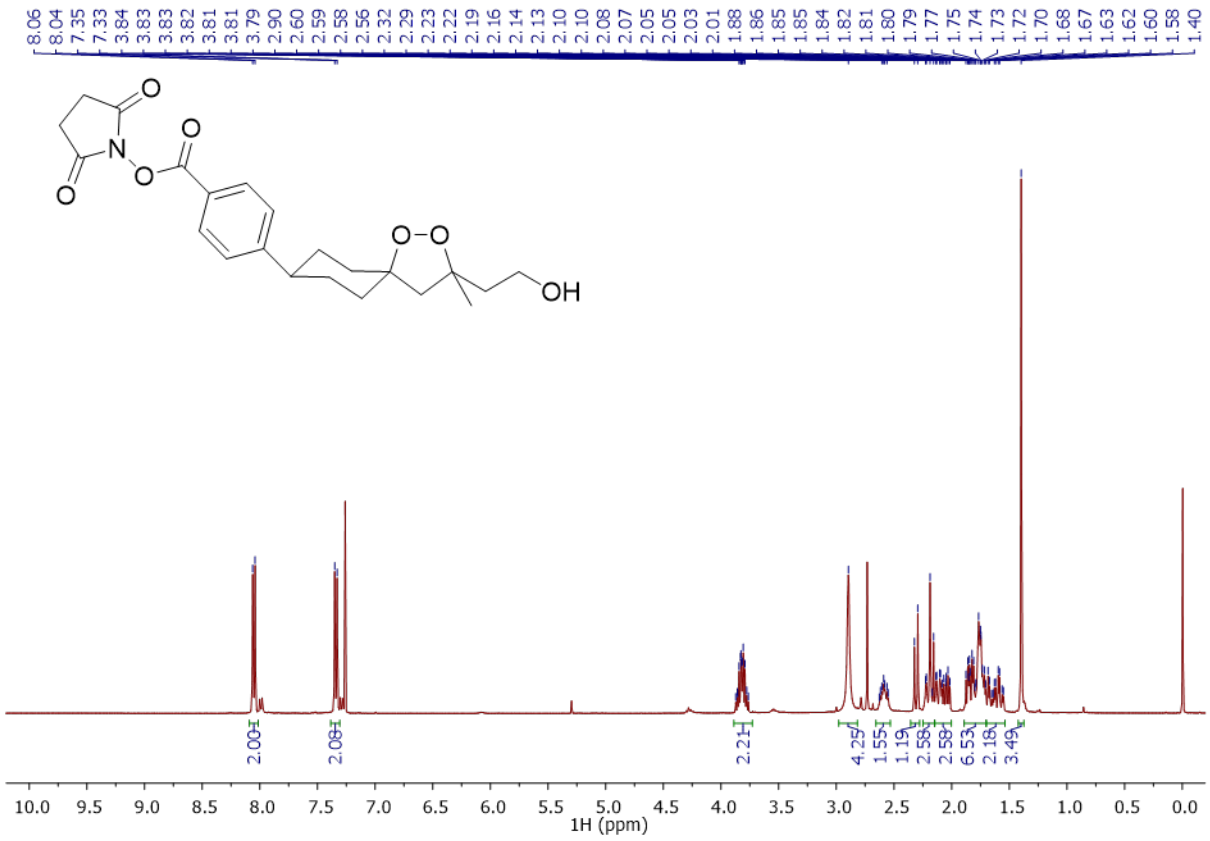
A mixture of carboxylic acid **19** (144 mg, 0.449 mmol), EDCI (137 mg, 1.15 mmol), and NHS (78 mg, 0.68 mmol) were dissolved in CH_2Cl_2 (4.3 mL) and stirred for 13 h. The reaction mixture was diluted with aq. HCl (5%, 10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (60:40 EtOAc:hexanes, *rf* = 0.29) afforded the title compound (118 mg, 66%) as a white powder with minor impurities (≈10% starting carboxylic acid **19**) that were carried through to the next reaction:

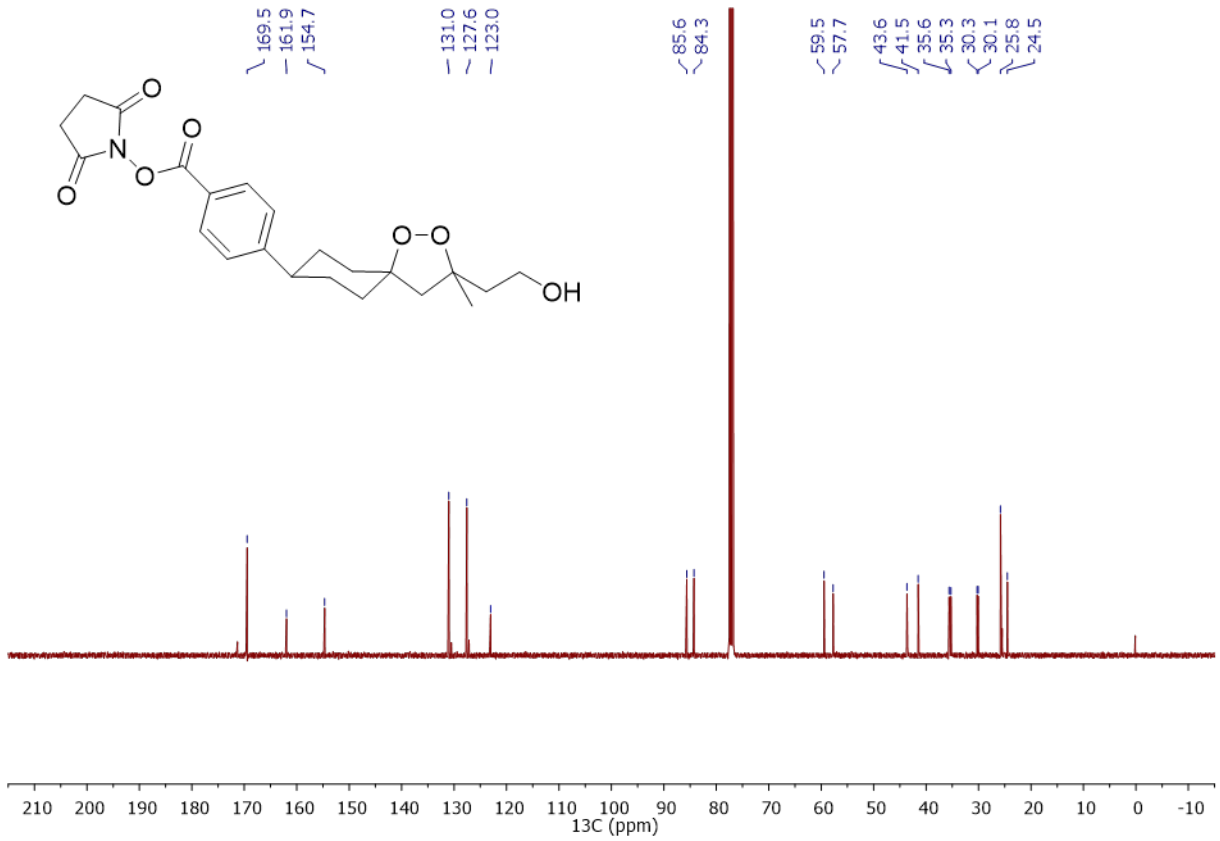
mp = slow dec. 139 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05, (d, *J* = 8.4, 2H), 7.34 (d, *J* = 8.3, 2H), 3.87−3.76 (m, 2H), 2.89 (br s, 4H), 2.61−2.54 (m, 1H), 2.31 (d, *J* = 12.1, 1H), 2.22−2.15 (m, 2H), 2.14−2.01 (m, 2H), 1.88−1.73 (m, 6H), 1.68−1.55 (m, 2H), 1.40 (s, 3H);

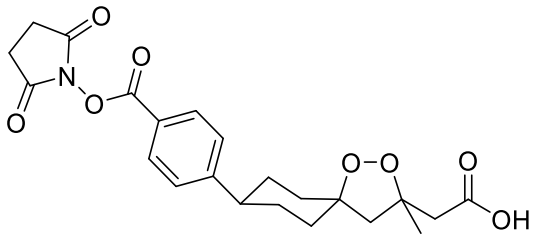
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3 (C), 161.8 (C), 154.5 (C), 130.9 (CH), 127.4 (CH), 122.9 (C), 85.5 (C), 84.1 (C), 59.3 (CH_2), 57.6 (CH_2), 43.5 (CH), 41.4 (CH_2), 35.5 (CH_2), 35.1 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 25.7 (CH_2), 24.4 (CH_3);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{22}H_{26}NO_6$ $[(M+H)-H_2O]^+$ 400.1755, found 400.1763.





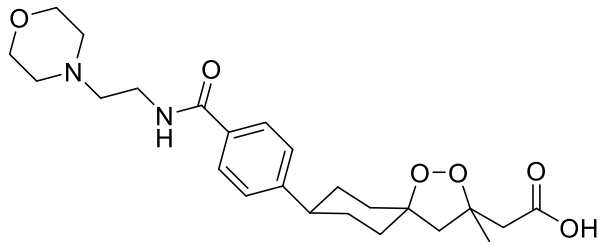
(5*R,8*R**)-8-(succinimidyl-*p*-Benzoate)-3-methyl-3 carboxymethyl-1,2-dioxaspiro[4.5]decane (21).**



To a stirring mixture of alcohol **20** (179 mg, 0.429 mmol) and TEMPO (13 mg, 0.08 mmol) in CH_2Cl_2 (2.8 mL) was added H_2O (1.4 mL), diacetoxyiodobenzene (420 mg, 1.30 mmol), and TBAB (143 mg, 0.444 mmol). The mixture was stirred for 14 h before being diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with sat. aq. NaCl (15 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was used directly in the next step without purification or characterization due to sensitivity to silica gel.

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{22}H_{29}N_2O_8$ $[M+NH_4]^+$ 449.1918, found 449.1925.

(5*R,8*R**)-8-(succinimidyl-*p*-Benzoate)-3-methyl-3 carboxymethyl-1,2-dioxaspiro[4.5]decane (22).**



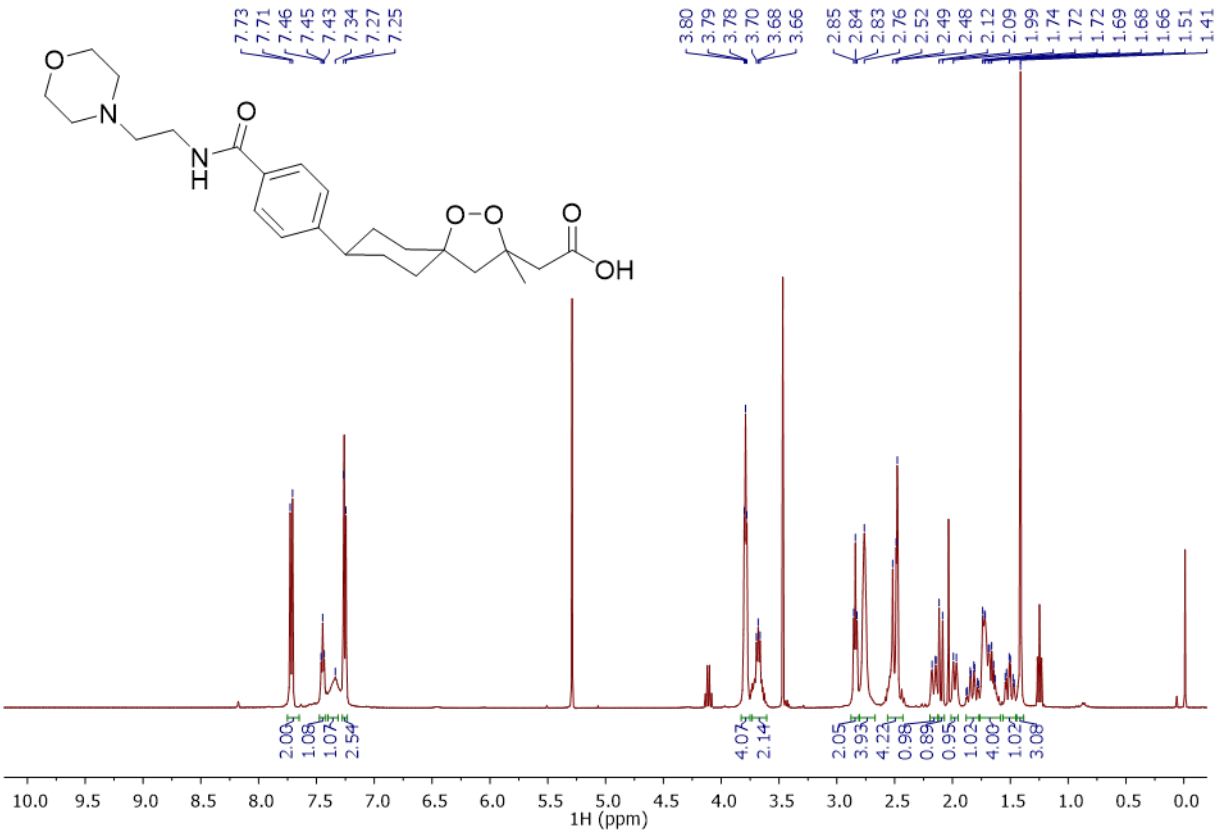
To a stirring solution of NHS-ester **21** (147 mg, 0.341 mmol) in CH_2Cl_2 (6.8 mL) was added aminoethylmorpholine (90 µL, 0.69 mmol). The reaction mixture was stirred for 9 h before being diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (4 × 15 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography (Davisil, 5:95 MeOH:CH_2Cl_2, *rf* = 0.23 in 10%) to afford the title compound (105 mg, 55% from the alcohol **20**) as a white solid:

mp = dec. 129 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.2, 2H), 7.45 (t, *J* = 5.3, 1H), 7.34 (br s, 1H), 7.26 (d, *J* = 8.2, 2H), 3.79 (t, *J* = 4.5, 4H), 3.68 (t, *J* = 6.4, 2H), 2.84 (t, *J* = 5.5, 2H), 2.76 (br s, 4H), 2.52−2.48 (m, 4H), 2.16 (dd, *J* = 13.5, 1.8, 1H), 2.01 (d, *J* = 12.3, 1H), 1.98 (d, *J* = 10.5, 1H), 1.83 (qd, *J* = 12.8, 3.2, 1H), 1.74−1.63 (m, 4H), 1.50 (td, *J* = 13.5, 4.0, 1H), 1.41 (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.5 (C), 167.9 (C), 150.8 (C), 132.1 (C), 127.5 (CH), 127.1 (CH), 84.3 (C), 83.7 (C), 65.5 (CH_2), 57.3 (CH_2), 56.8 (CH_2), 52.8 (CH_2), 45.3 (CH_2), 43.2 (CH), 35.6 (CH_2), 35.2 (CH_2), 30.7 (CH_2), 30.2 (CH_2), 24.0 (CH_3);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{24}H_{35}N_2O_6$ $[M+H]^+$ 442.2490, found 442.2501.


7.73
7.71
7.46
7.45
7.43
7.34
7.27
7.25
3.80
3.79
3.78
3.70
3.68
3.66
2.85
2.84
2.83
2.76
2.52
2.49
2.48
2.12
2.09
1.99
1.74
1.72
1.72
1.69
1.68
1.66
1.51
1.41
O
N
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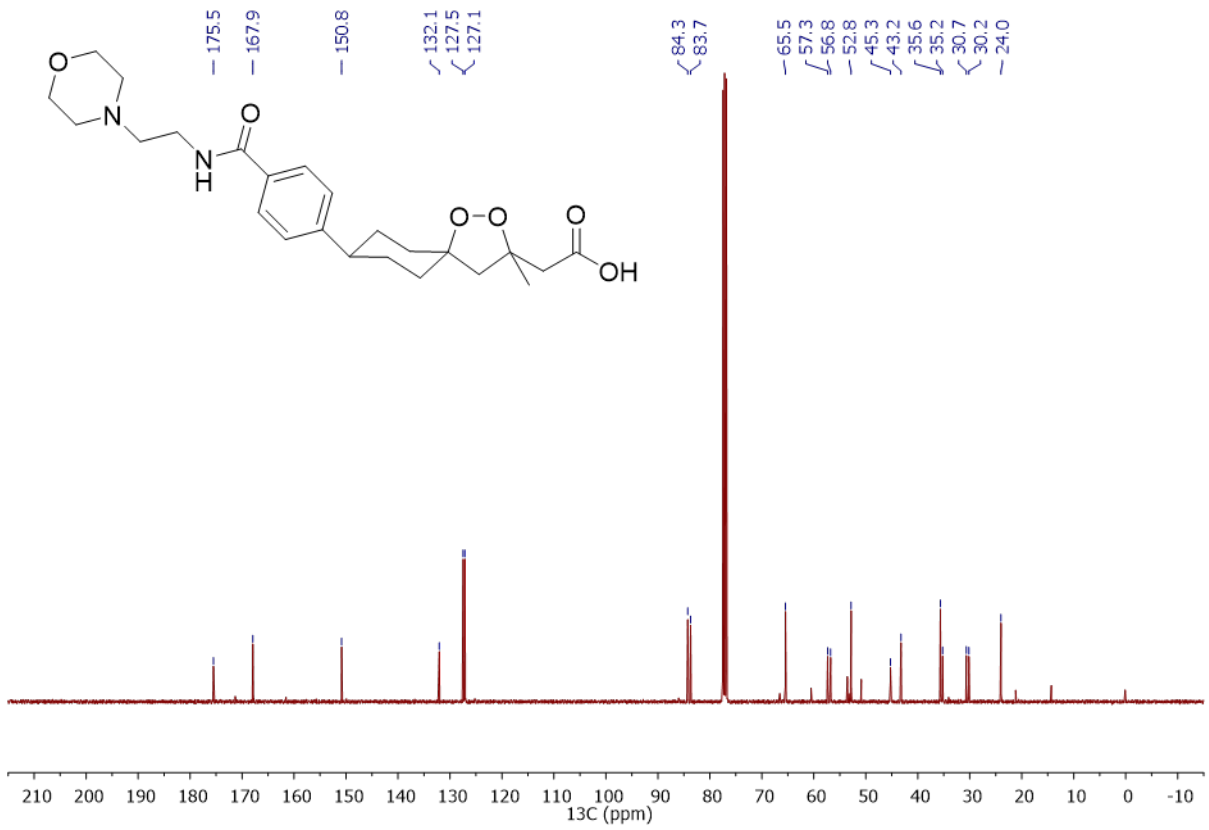
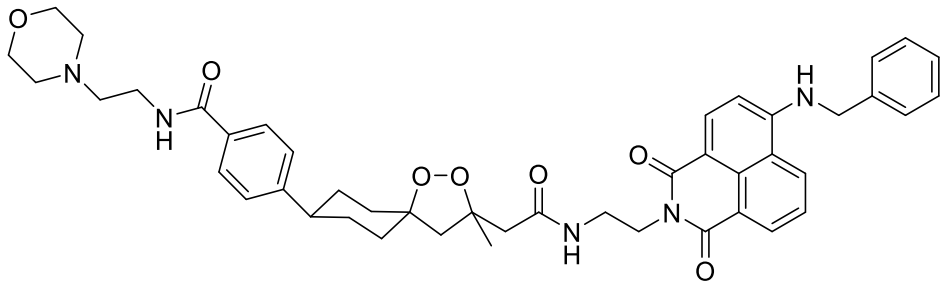
N
H
O-O
O
OH
2.00
1.09
1.07
2.54
4.07
2.14
2.05
3.93
4.22
0.99
0.89
0.95
1.02
4.00
1.02
3.08
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)



4-((5*R,8*S**)-3-(2-((2-(6-(benzylamino)-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl)amino)-2-oxoethyl)-3-methyl-1,2-dioxaspiro[4.5]decan-8-yl)-*N*-(2-morpholinoethyl)benzamide (FINO$_2$-4) (4).**



To a solution of amine **24** (64 mg, 0.14 mmol), prepared according to the general procedure described for the synthesis of **FINO$_2$-1**, was added carboxylic acid **22** (52 mg, 0.12 mmol) in CH_2Cl_2 (2.4 mL) followed by addition of HATU (83 mg, 0.22 mmol) and NEt_3 (82 µL, 0.59 mmol). The reaction mixture was stirred for 13 h before being diluted with CH_2Cl_2 (10 mL) and washed with NaOH (1 M aq., 10 mL), H_2O (10 mL), and sat. aq. NaCl (10 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column

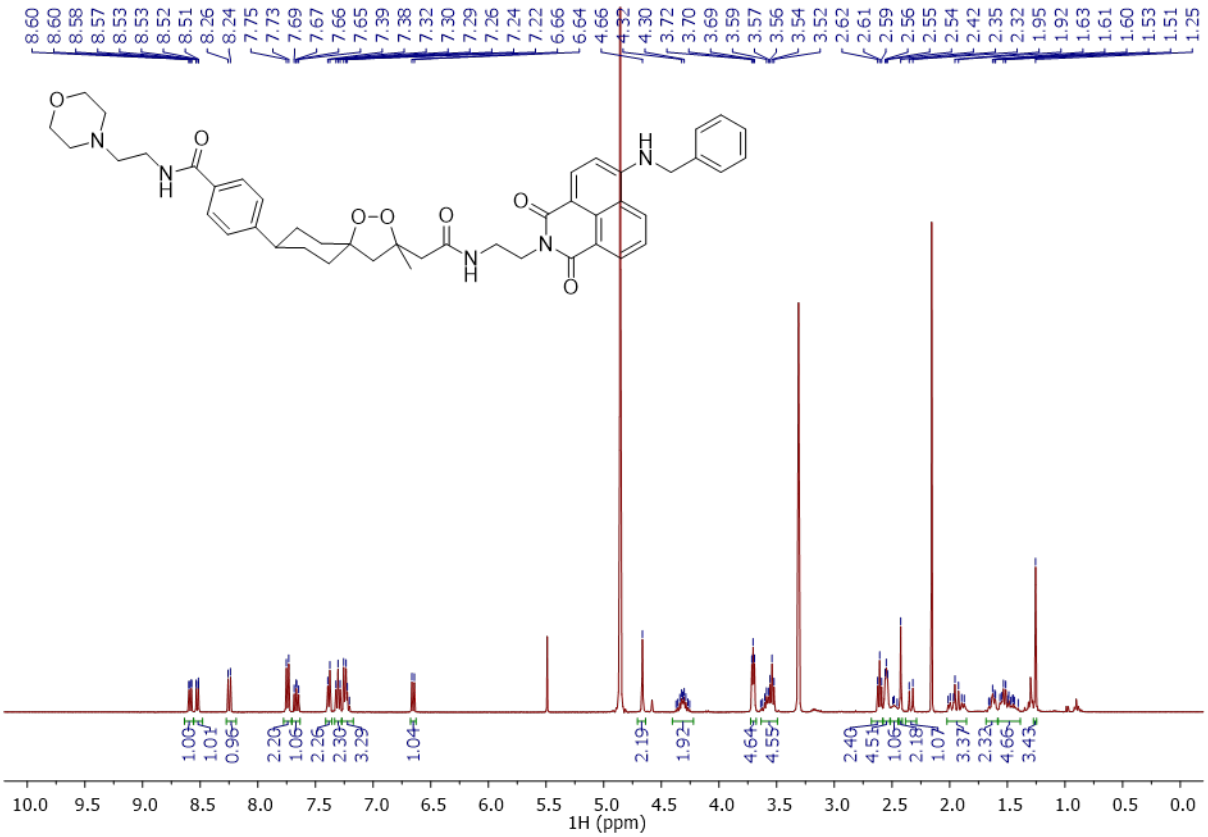
chromatography (Davisil, 2:98–5:95 MeOH:CH_2Cl_2, *rf* = 0.21 in 5:95 MeOH:CH_2Cl_2%) to afford the title compound as a yellow solid:

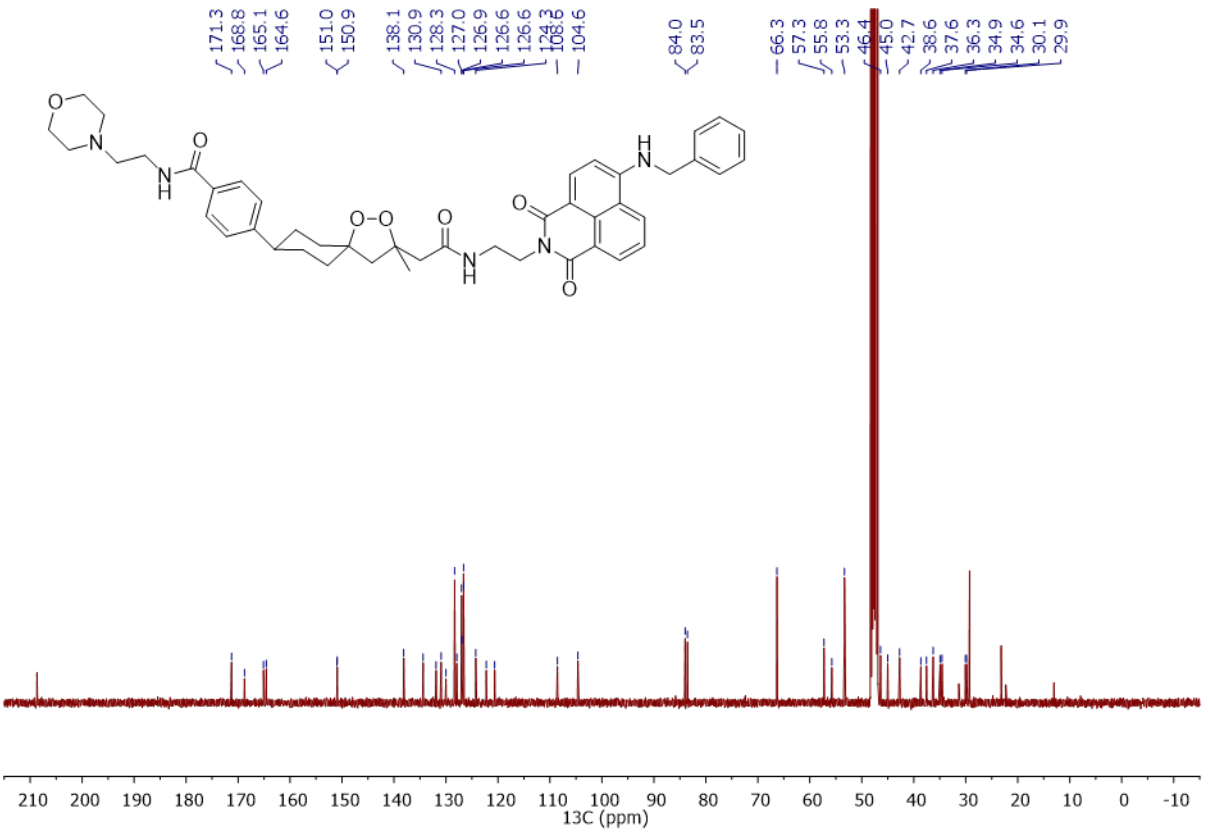
mp = dec. 128 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (dd, *J* = 8.4, 0.9, 1H), 8.52 (dd, *J* = 7.3, 0.8, 1H), 8.25 (d, *J* = 8.6, 1H), 7.74 (d, *J* = 8.3, 2H), 7.67 (dd, *J* = 8.3, 7.5, 1H), 7.38 (d, *J* = 7.3, 2H), 7.30 (t, *J* = 7.5, 2H), 7.26−7.21 (m, 3H), 6.65 (d, *J* = 8.6, 1H), 4.66 (s, 2H), 4.37−4.26 (m, 2H), 3.70 (t, *J* = 4.6, 4H), 3.64−3.52 (m, 4H), 2.61 (t, *J* = 6.7, 2H), 2.55 (t, *J* = 4.2, 4H), 2.49−2.46 (m, 1H), 2.42 (s, 2H), 2.33 (d, *J* = 12.4, 1H), 2.01−1.87 (m, 3H), 1.66−1.60 (m, 2H), 1.56−1.40 (m, 4H), 1.25 (s, 3H);

^{13}C NMR (101 MHz CD_3OD) δ171.3 (C), 168.8 (C), 165.1 (C), 164.6 (C), 151.0 (C), 150.9 (C), 138.1 (C), 134.4 (CH), 131.9 (C), 130.9 (CH) 130.0 (C), 128.3 (CH), 127.9 (CH), 127.0 (CH), 126.9 (CH), 126.62 (CH), 126.58 (CH), 124.3 (CH), 122.3 (C), 120.6 (C), 108.6 (C), 104.6 (CH), 84.0 (C), 83.5 (C), 66.3 (CH_2), 57.3 (CH_2), 55.8 (CH_2), 53.3 (CH_2), 46.4 (CH_2), 45.0 (CH_2), 42.7 (CH), 38.6 (CH_2), 37.6 (CH_2), 36.3 (CH_2), 34.9 (CH_2), 34.6 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.3 (CH_3);

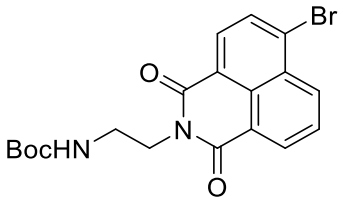
HRMS (TOF MS ESI+) *m / z* calcd. for $C_{45}H_{52}N_5O_7$ $[M+H]^+$ 774.3861, found 774.3868.


8.60 8.60 8.58 8.57 8.53 8.53 8.52 8.51 8.26 8.24 7.75 7.73 7.69 7.67 7.66 7.65 7.39 7.38 7.32 7.30 7.29 7.26 7.24 7.22 6.66 6.64 4.66 4.32 4.30 3.72 3.70 3.69 3.59 3.57 3.56 3.54 3.52 2.62 2.61 2.59 2.56 2.55 2.54 2.42 2.35 2.32 1.95 1.92 1.63 1.61 1.60 1.53 1.51 1.25
1.00 1.01 0.96 2.20 1.06 2.25 2.30 3.29 1.04 2.19 1.92 4.64 4.55 2.40 4.51 1.06 2.18 1.07 3.37 2.32 4.66 3.43
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)


171.3 168.8 165.1 164.6 151.0 150.9 138.1 130.9 128.3 127.0 126.9 126.6 126.6 124.3 108.8 104.6 84.0 83.5 66.3 57.3 55.8 53.3 46.4 45.0 42.7 38.6 37.6 36.3 34.9 34.6 30.1 29.9
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

Synthesis of fluorophores and localization tags

4-Bromo-*N*-(2"-*tert*-butoxycarbonylaminoethyl)-1,8-naphthalimide (23) [7]

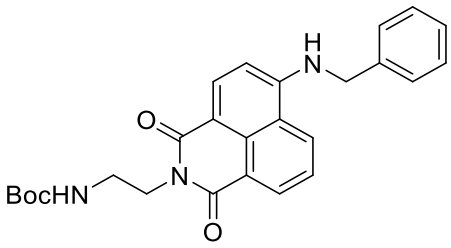


A mixture of 4-bromo-1,8-naphthalic anhydride (516 mg, 1.86 mmol), *N*-Boc-ethylenediamine (300 mg, 1.87 mmol), and EtOH (5.0 mL) was placed in a microwave vial and sealed. The vessel was sonicated briefly to create a slurry and heated using microwave irradiation to 120 °C for 1 h. The resulting tan solid was collected by vacuum filtration, washed repeatedly with H_2O (≈200 mL total), and dried under vacuum to afford the title compound (688 mg, 88%) as a white solid:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, *J* = 6.8, 1H), 8.52 (d, *J* = 8.4, 1H), 8.37 (d, *J* = 7.8, 1H), 8.00 (d, *J* = 7.8, 1H), 7.81 (app. t, *J* = 7.9, 1H), 4.33 (t, *J* = 5.6, 2H), 3.52 (d, *J* = 5.1, 2H), 3.22 (br s, 1H), 1.27 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) 163.93, 163.91, 156.1, 133.3, 132.2, 131.4, 131.1, 130.6, 130.4, 129.0, 128.1, 122.9, 122.0, 79.2, 40.0, 39.5, 28.2.

4-Benzyl-*N*-(2"-*tert*-butoxycarbonylaminoethyl)-1,8-naphthalimide (24)



A pressure vessel was charged with aryl bromide **23** (1.03 g, 2.46 mmol), copper powder (13 mg, 0.20 mmol), H_2O (2.5 mL), NEt_3 (680 µL, 4.9 mmol), and benzylamine (540 µL, 4.9 mmol).The vessel was sealed and heated at 100 °C for 15 h before being diluted with H_2O (20 mL). The solids were collected by vacuum filtration and washed with H_2O (2 × 20 mL). Purification by flash column chromatography (50:50−100:0 EtOAc:hexanes, *rf* = 0.48) afforded the title compound (554 mg, 51%) as a yellow, glass-like solid:

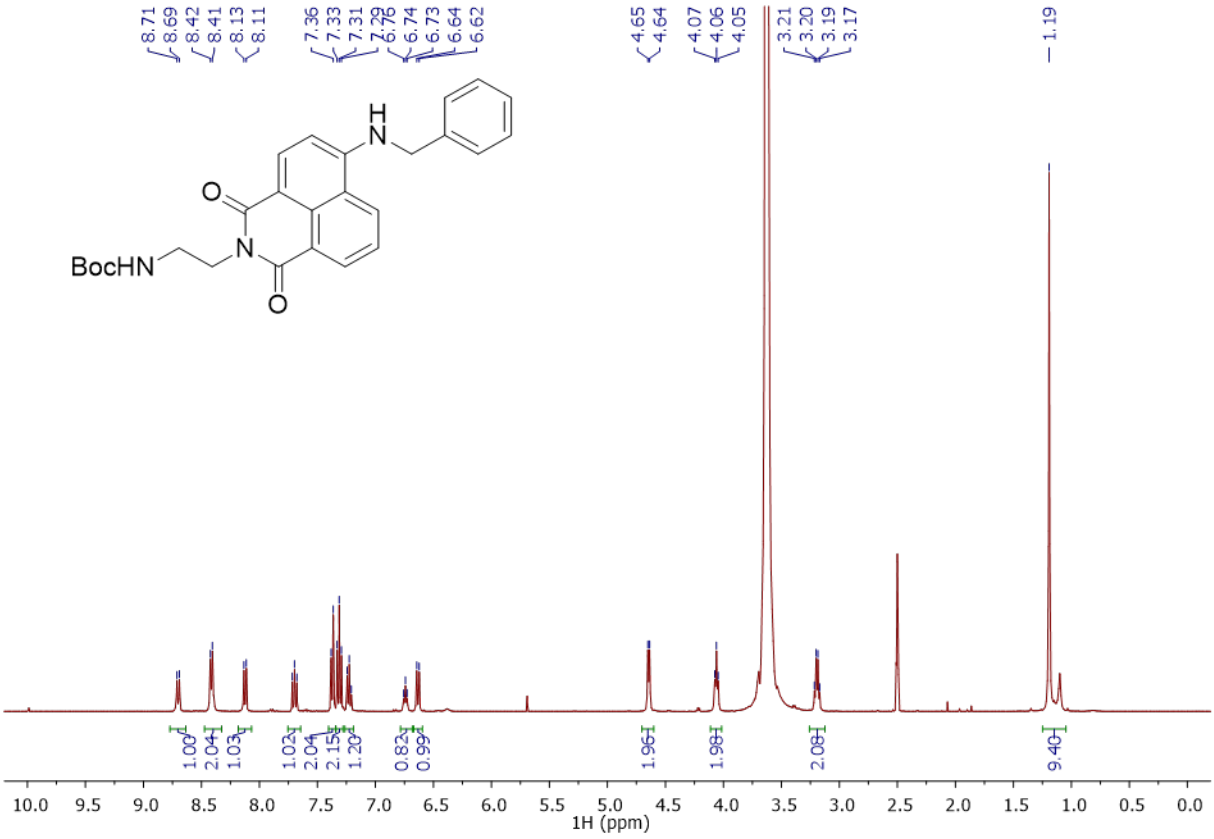
mp = dec. 196 °C;

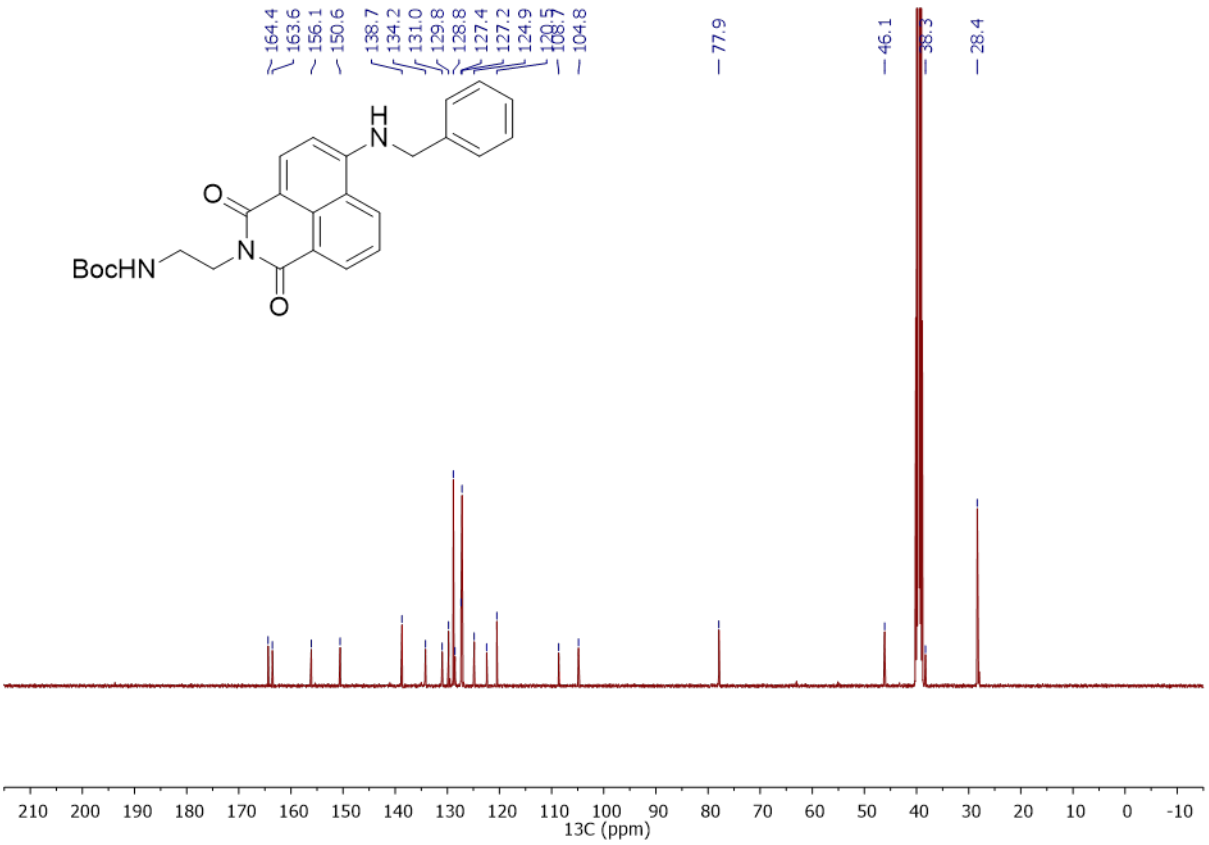
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (d, J = 8.4, 1H), 8.41 (d, J = 6.9, 2H), 8.12 (d, J = 8.5, 1H), 7.70 (t, J = 7.9, 1H), 7.37 (d, J = 7.2, 2H), 7.31 (t, J = 7.9, 2H), 7.23 (t, J = 7.2, 1H), 6.74 (t, J = 6.0, 1H), 6.63 (d, J = 8.6, 1H), 4.64 (d, J = 5.9, 2H), 4.06 (t, J = 5.8, 2H), 3.19 (q, J = 6.0, 2H), 1.19 (s, 9H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 164.4 (C), 163.6 (C), 156.1, (C) 150.6 (C), 138.7 (C), 134.2 (CH), 131.0 (CH), 129.8 (C), 128.8 (CH), 128.5 (CH), 127.4 (CH), 127.2 (CH), 124.9 (CH), 122.4 (C), 120.5 (C), 108.7 (C), 104.8 (CH), 77.9 (C), 46.1 (CH_2), 38.3 (CH_2), 28.4 (CH_3) ;

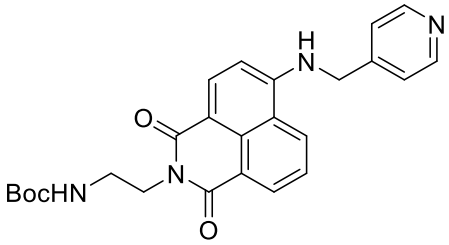
HSQC confirmed that there was a CH_2 resonance under the solvent peak;

HRMS (TOF MS ESI+) m / z calcd. for $C_{26}H_{27}N_3O_4Na$ $[M+Na]^+$ 468.1894, found 468.1893.





***N*-(2"-*tert*-Butoxycarbonylaminoethyl)-4-(5'-picolyl)-1,8-naphthalimide (25)**



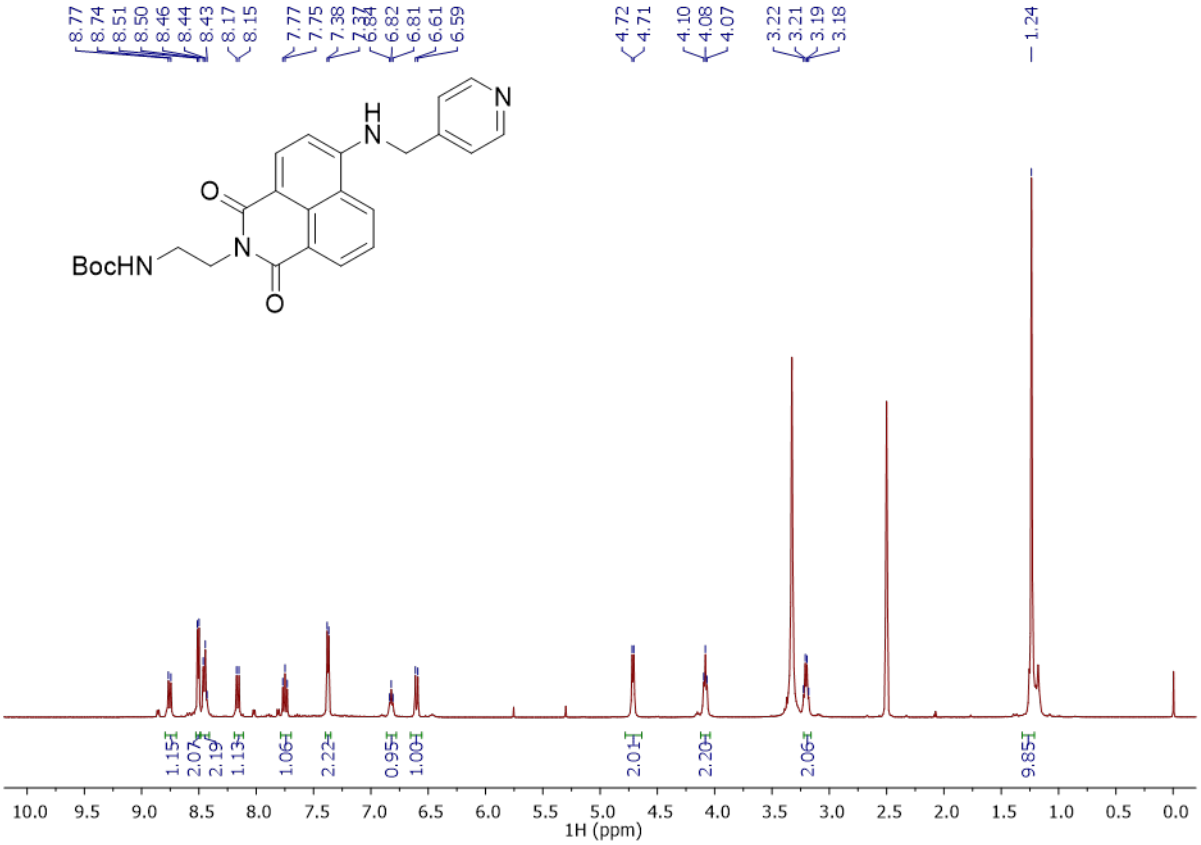
A mixture of aryl bromide **23** (500 mg, 1.19 mmol), XantPhos (69 mg, 0.12 mmol), $Pd_2(dba)_3$ (98 mg, 0.11 mmol), and Cs_2CO_3 (1.20 g, 3.67 mmol) was placed under a N_2 atmosphere. The solids were suspended in toluene (24.0 mL) and picolylamine (760 µL, 7.5 mmol) was added. The resulting slurry was heated at 80 °C for 22 h. After cooling, silica (1.06 g) was added and the slurry was concentrated *in vacuo*. Purification by flash column chromatography (50:50 CH_3CN:CH_2Cl_2, *rf* = 0.17) afforded the title compound (315 mg, 59%) as a yellow powder:

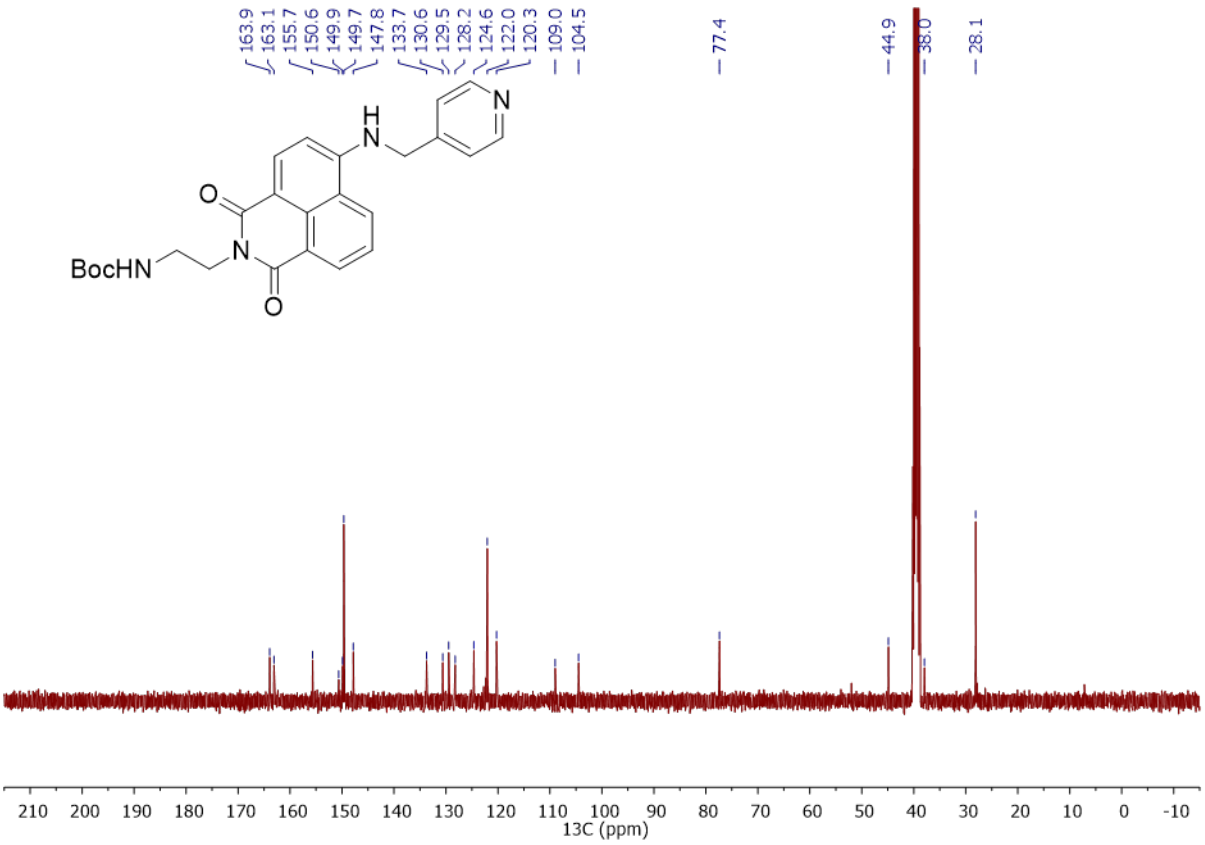
mp = dec. 195 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 8.8, 1H), 8.51 (d, J = 5.9, 2H), 8.46−8.43 (m, 2H), 8.16 (d, J = 8.4, 1H), 7.75 (t, J = 7.9, 1H), 7.37 (d, J = 5.7, 2H), 6.82 (t, J = 5.8, 1H), 6.60 (d, J = 8.5, 1H), 4.71 (d, J = 5.9, 2H), 4.08 (t, J = 5.8, 2H), 3.20 (q, J = 5.9, 2H), 1.24 (s, 9H);

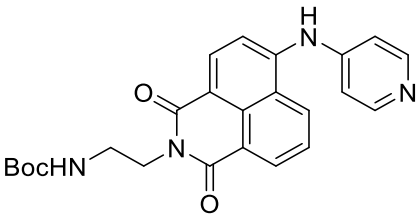
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C), 163.1 (C), 155.7 (C), 150.6 (C), 149.9 (CH), 149.7 (C), 147.8 (C), 133.7 (CH), 130.6 (CH), 129.5 (C), 128.2 (CH), 124.6 (CH), 122.0 (CH), 120.3 (C), 109.0 (C), 104.5 (CH), 77.4 (C), 44.9 (CH_2), 38.0 (CH_2), 28.1 (CH_3); HSQC confirmed that there was a $\underline{C}H_2$ resonance under the solvent peak;

HRMS (TOF MS APCI+) m/z calcd. for $C_{25}H_{27}N_4O_4$ $[M+H]^+$ 447.2027, found 447.2033.





***N*-(2"-*tert*-Butoxycarbonylaminoethyl)-4-(4'-pyridyl)-1,8-naphthalimide (26)**



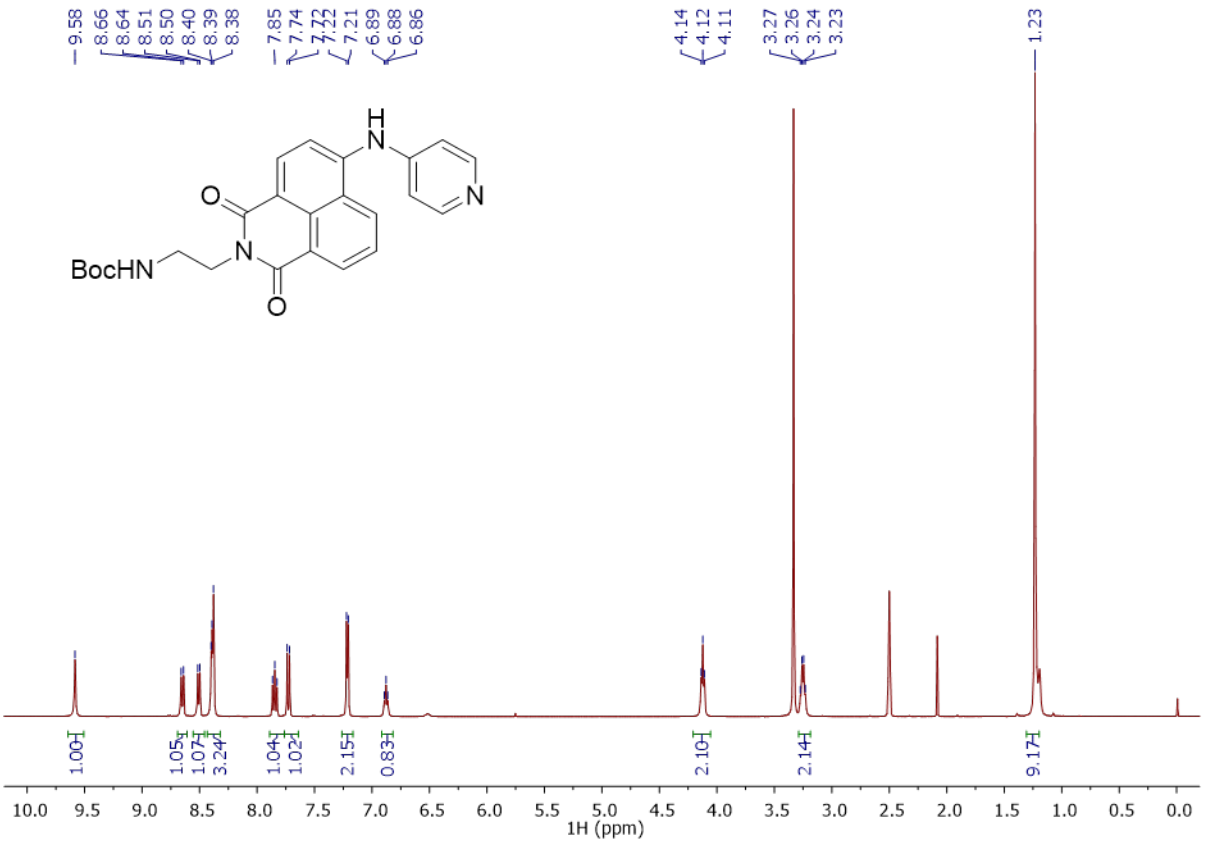
A mixture of aryl bromide **23** (499 mg, 1.19 mmol), XantPhos (27 mg, 0.05 mmol), $Pd_2(dba)_3$ (45 mg, 0.05 mmol), Cs_2CO_3 (1.140 g, 3.499 mmol), and 4-aminopyridine (353 mg, 3.75 mmol) was placed under a N_2 atmosphere. The solids were suspended in toluene (24.0 mL) and the resulting slurry was heated at 80 °C for 43 h. After cooling, silica (492 mg) was added and the slurry was concentrated *in vacuo*. Purification by flash column chromatography (10:90 MeOH:CH_2Cl_2, *rf* = 0.45) afforded the title compound (314 mg, 61%) as a yellow powder:

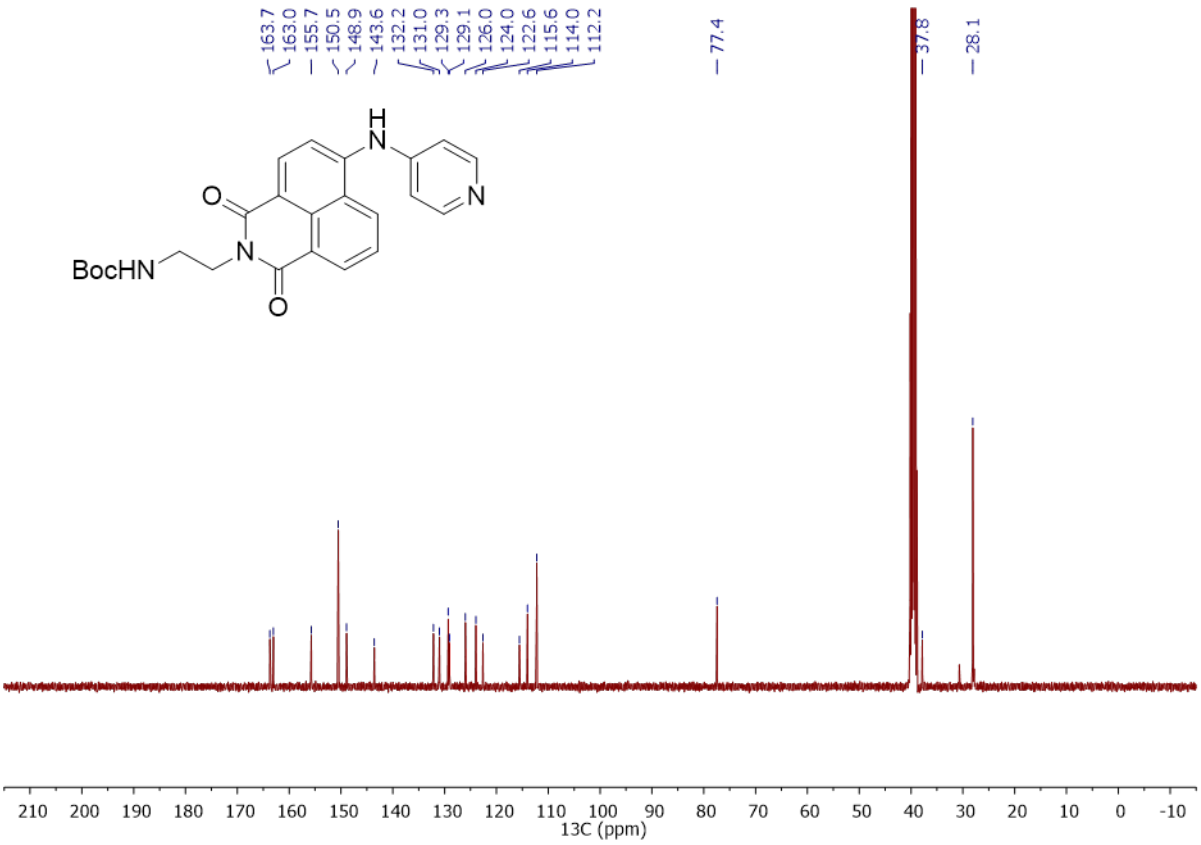
mp = dec. 163 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (br s, 1H), 8.65 (d, *J* = 8.4, 1H), 8.51 (d, *J* = 7.2, 1H), 8.39−8.38 (m, 3H), 7.85 (app. t, *J* = 7.9, 1H), 7.73 (d, *J* = 8.2, 1H), 7.21 (d, *J* = 6.2, 2H), 6.88 (t, *J* = 6.0, 1H), 4.12 (t, *J* = 5.7, 2H), 3.25 (q, *J* = 5.7, 2H), 1.23 (s, 9H);

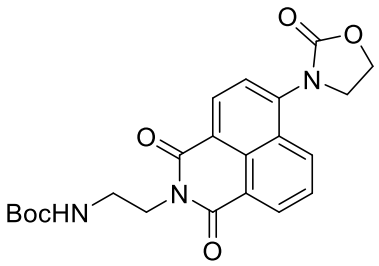
^{13}C NMR (101 MHz, DMSO-d_6) 163.7 (C), 163.0 (C), 155.7 (C), 150.5 (CH), 148.9 (C), 143.6 (C), 132.2 (CH), 131.0 (CH), 129.3 (C), 129.1 (CH), 126.0 (CH), 124.0 (C), 122.6 (C), 115.6 (C), 114.0 (CH), 112.2 (CH), 77.4 (C), 37.8 (CH_2), 28.1 (CH_3); HSQC confirmed that there was a CH_2 resonance under the solvent peak;

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{24}H_{25}N_4O_4$ $[M+H]^+$ 434.1901, found 434.1905;





***N*-2''-*tert*-Butoxycarbonylaminoethyl-4-3'-oxazolidin-2'-onyl-1,8-naphthalimide (27).**



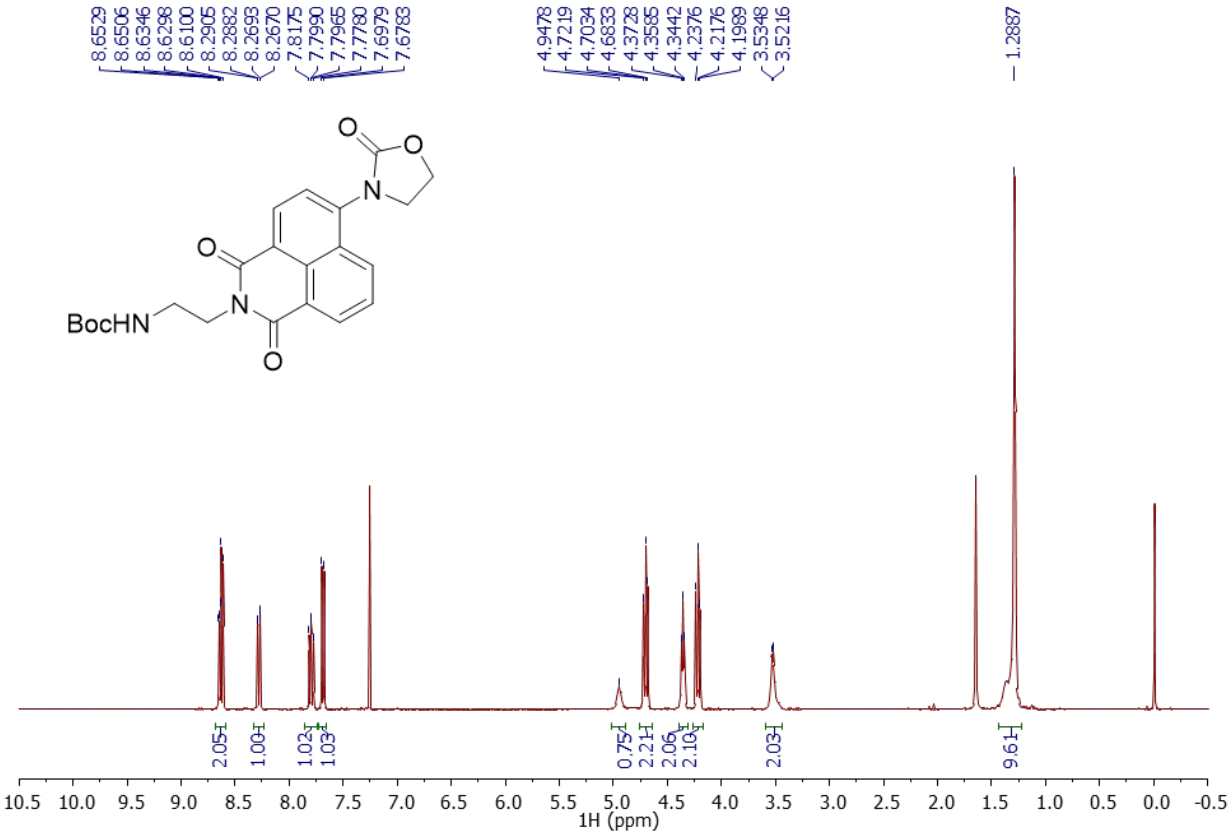
A mixture of aryl bromide **23** (499 mg, 1.19 mmol), XantPhos (69 mg, 0.12 mmol), $Pd_2(dba)_3$ (109 mg, 0.117 mmol), Cs_2CO_3 (2.426 g, 7.446 mmol), and 2-bromoethylamine hydrobromide (758 mg, 3.70 mmol) was placed under a N_2 atmosphere. The solids were suspended in toluene (24.0 mL) and the resulting slurry was heated at 80 °C for 24 h. After cooling, silica (1.03 g) was added and the slurry was concentrated *in vacuo*. Purification by flash column chromatography (50:50 EtOAc:hexanes, *rf* = 0.25) afforded the title compound (212 mg, 39%) as a yellow powder:

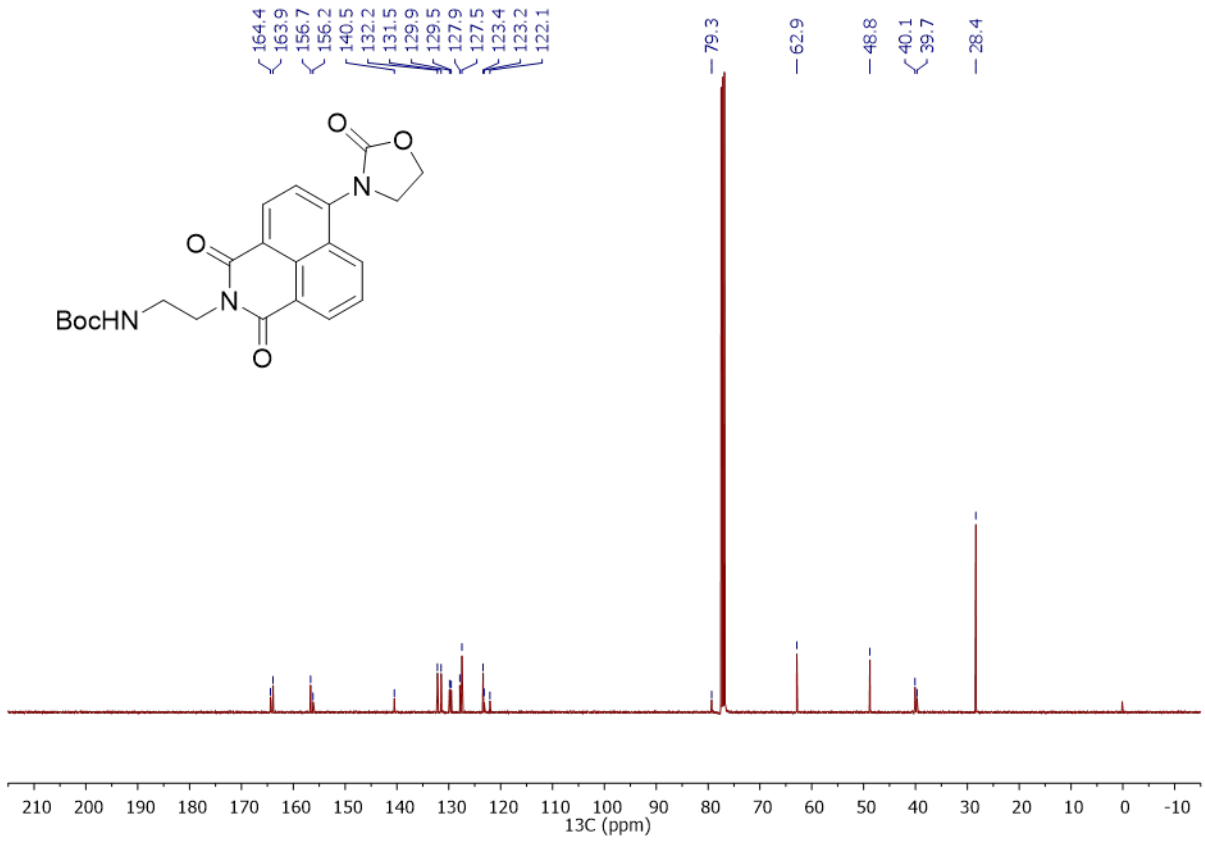
mp = 182−185 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, *J* = 7.3, 0.9, 1H), 8.62 (d, *J* = 7.9, 1H), 8.27 (dd, *J* = 8.5, 0.9, 1H), 7.80 (dd, *J* = 8.4, 7.4, 1H), 7.69 (d, *J* = 7.8, 1H), 4.95 (br s, 1H), 4.70 (t, *J* = 7.7, 2H), 4.36 (t, *J* = 5.6, 2H), 4.22 (t, *J* = 7.7, 2H), 3.53 (app. d, *J* = 5.3, 2H), 1.29 (s, 9H);

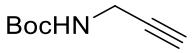
^{13}C NMR (101 MHz, $CDCl_3$) δ 164.4 (C), 163.9 (C), 156.7 (C), 156.2 (C), 140.5 (C), 132.2 (CH), 131.5 (CH), 129.9 (C), 129.5 (CH), 127.9 (C), 127.5 (CH), 123.4 (CH), 123.2 (C), 122.1 (C), 79.3 (C), 62.9 (CH_2), 48.8 (CH_2), 40.1 (CH_2), 39.7 (CH_2), 28.4 (CH_3);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{22}H_{23}N_3O_6Na$ $[M+Na]^+$ 448.1479, found 448.1497.





***N-tert*-Butoxycarbonylprop-2-ynamine (28)** [8]

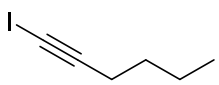


A solution of Boc_2O (2.03 g, 9.32 mmol) in CH_2Cl_2 (17 mL) was added dropwise (10 min) to a stirring solution of propargylamine (500 µL, 7.81 mmol) in CH_2Cl_2 (16 mL). The reaction mixture was stirred for an additional 75 min before being concentrated *in vacuo* to afford the title compound (1.19 g, 98%) as a yellow oil:

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.69 (br s, 1H), 3.92 (s, 2H), 2.22 (s, 1H), 1.45 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.4, 85.3, 80.2, 71.4, 28.5, 27.6.

1-Iodohexyne (29) [9]



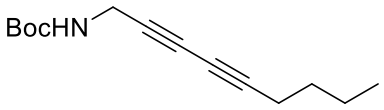
To a solution of hexyne (1.0 mL, 8.7 mmol) and $AgNO_3$ (68 mg, 0.40 mmol) in acetone (22.0 mL) in a foil-covered round bottom flask was added *N*-iodosuccinimide (691 mg, 3.07 mmol). The reaction mixture was stirred for 4 h before being concentrated *in vacuo*, suspended in hexanes (1.0 mL), filtered through a silica plug (2 cm),

washed with hexanes (50 mL), and concentrated *in vacuo* to afford the title compound (482 mg, 41%) as a colorless oil:

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (t, *J* = 6.9, 2H), 1.50 (app. quin, *J* = 7.1, 2H), 1.40 (app. q, *J* = 7.3, 2H), 0.91 (t, *J* = 7.2, 3H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 95.0, 30.7, 22.0, 20.7, 13.7, −7.59.

***N-tert*-Butoxycarbonylnon-2,4-diynamine (30)**

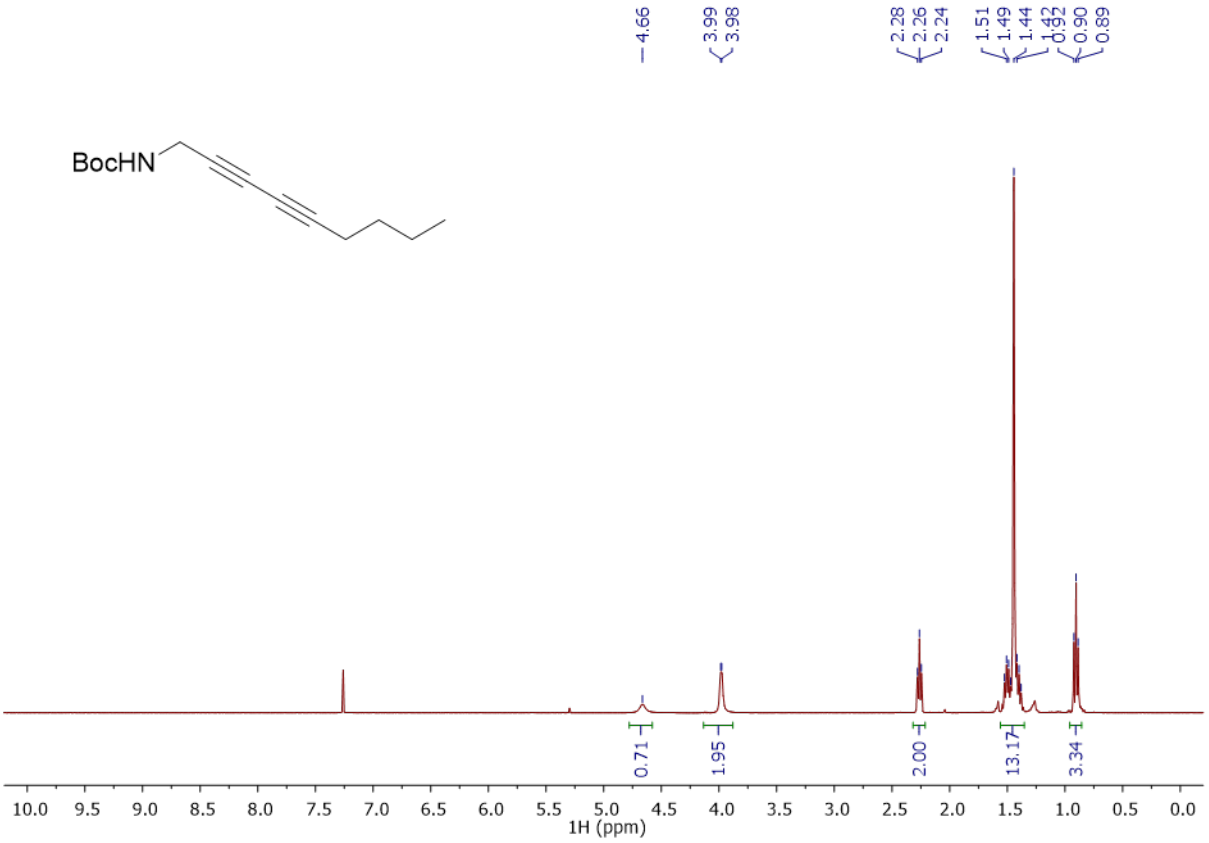


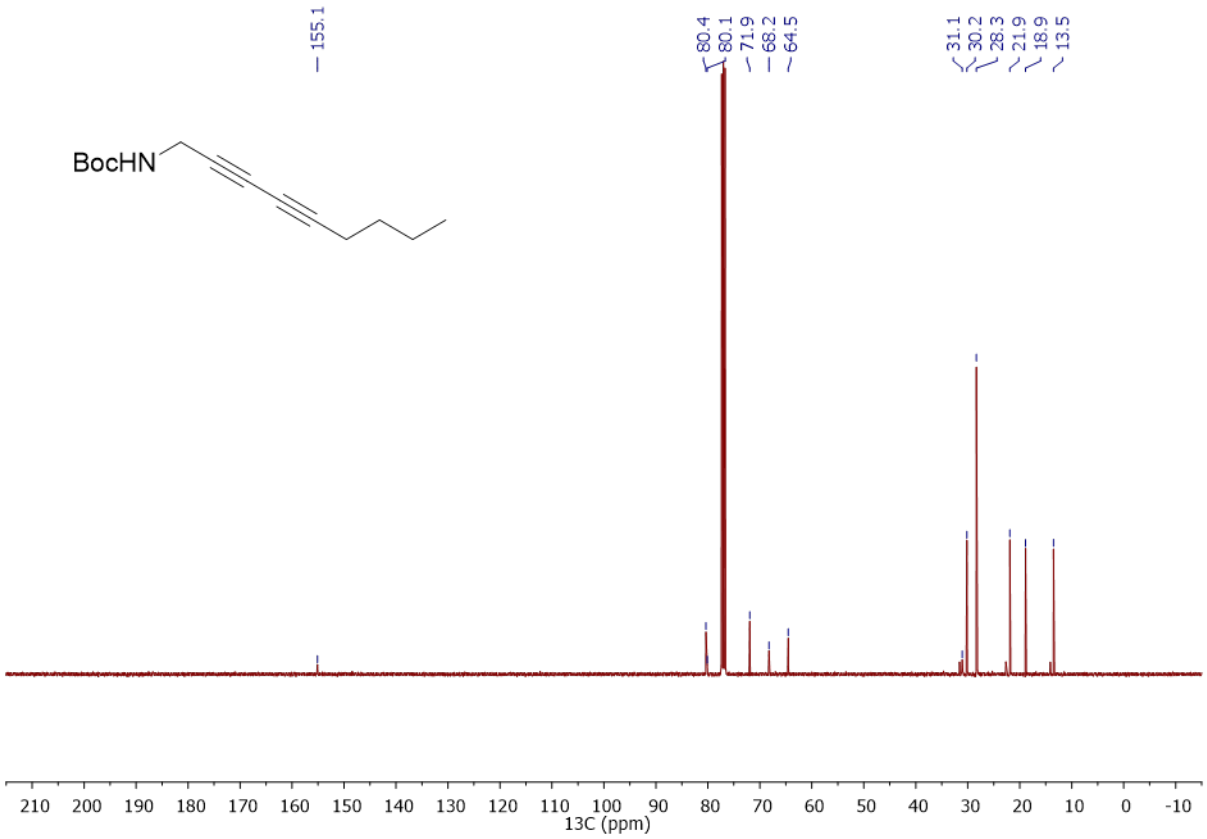
To a stirring mixture of ethylamine (70% aq., 1.9 mL, 22 mmol), H_2O (13.0 mL), and alkyne **28** (430 mg, 2.76 mmol) in THF (43.0 mL) was added $NH_2OH{\cdot}HCl$ (143 mg, 2.05 mmol), CuCl (60 mg, 0.61 mmol). The reaction mixture was stirred for 5 mins. A solution of iodohexyne (**29**, 275 mg, 1.32 mmol) in THF (12.0 mL) was then added and the mixture was stirred for 90 mins during which time the clear colorless solution turned green then dark blue in color. A solution of KCN (5.6 g, 86 mmol) in H_2O (108.0 mL) was added, and the mixture was extracted with Et_2O (4 × 40 mL). The combined organic phases were washed with sat. aq. NaCl (80 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was stored at 2−8 °C prior to purification by flash column chromatography (0:100−2.5:97.5−5:95 EtOAc:hexanes, *rf* = 0.2 in 5:95 EtOAc:hexanes) to afford the title compound (182 mg, 59%) as a clear oil, which was stored in a darkened vial under N_2 at −20 °C:

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (br s, 1H), 3.98 (d, *J* = 3.3, 2H), 2.26 (t, *J* = 6.9, 2H), 1.52−1.38 (m, 13H), 0.90 (t, *J* = 7.2, 3H);

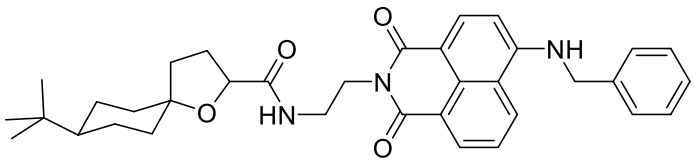
^{13}C NMR (101 MHz, $CDCl_3$) δ 155.1 (C), 80.4 (C), 80.1 (C), 71.9 (C), 68.2 (C), 64.5 (C), 31.1 (CH_2), 30.2 (CH_2), 28.3 (CH_3), 21.9 (CH_2), 18.9 (CH_2), 13.5 (CH_3);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{14}H_{21}NO_2Na$ $[M+Na]^+$ 259.1497, found 259.1496.


4.66
3.99
3.98
2.28
2.26
2.24
1.51
1.49
1.44
1.42
0.92
0.90
0.89
BocHN
0.71
1.95
2.00
13.17
3.34
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)



(5*R,8*R**)-2-(8'-(Benzylamino)-3'-naphthalimido-*N*-ethylcarbamoylmethyl)-8-*tert*-butyl-1-oxaspiro[4.5]decane (FINO$_2$-0) (5)**



To a solution of Boc-protected amine **24** (161 mg, 0.371 mmol) in CH_2Cl_2 (3.7 mL) was added TFA (740 µL, 9.67 mmol) and the mixture was stirred for 16 h. The reaction mixture was concentrated *in vacuo* and the orange glass-like solid was coevaporated with $CHCl_3$ (3 x 2 mL). The solid material was combined with the NHS ester **31** (100 mg, 0.296 mmol) in $CHCl_3$ (5.9 mL), and NEt_3 (150 µL, 1.08 mmol) was added. The reaction mixture was stirred for an additional 3 h before being diluted with aq. NaOH (1 M, 7 mL) and extracted with CH_2Cl_2 (3 x 7 mL). The combined organic phases were washed with sat. aq. NaCl (11 mL), dried over $MgSO_4$, filtered, and concentrated

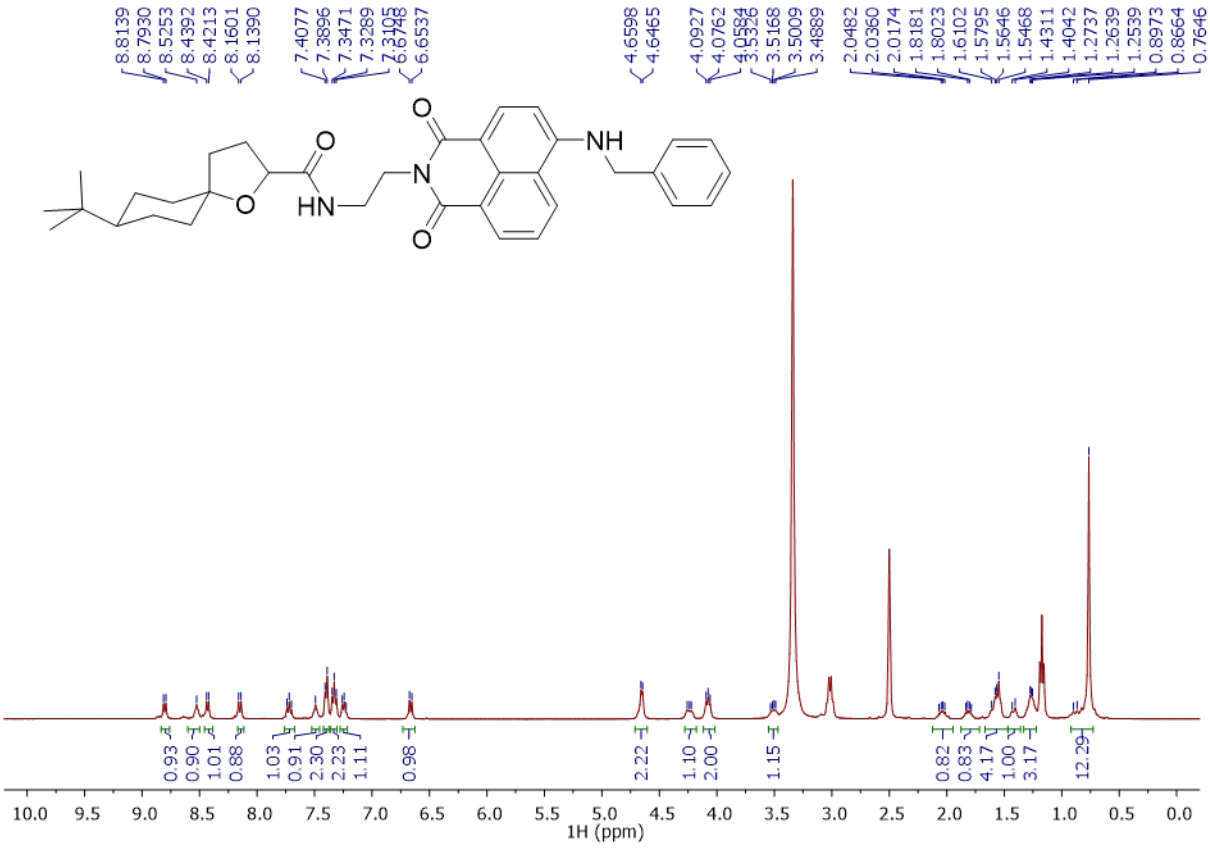
in vacuo. The orange solids were triturated in EtOAc (50 mL), and the solids were collected by vacuum filtration to afford the title compound (72 mg, 43%) as a bright orange powder:

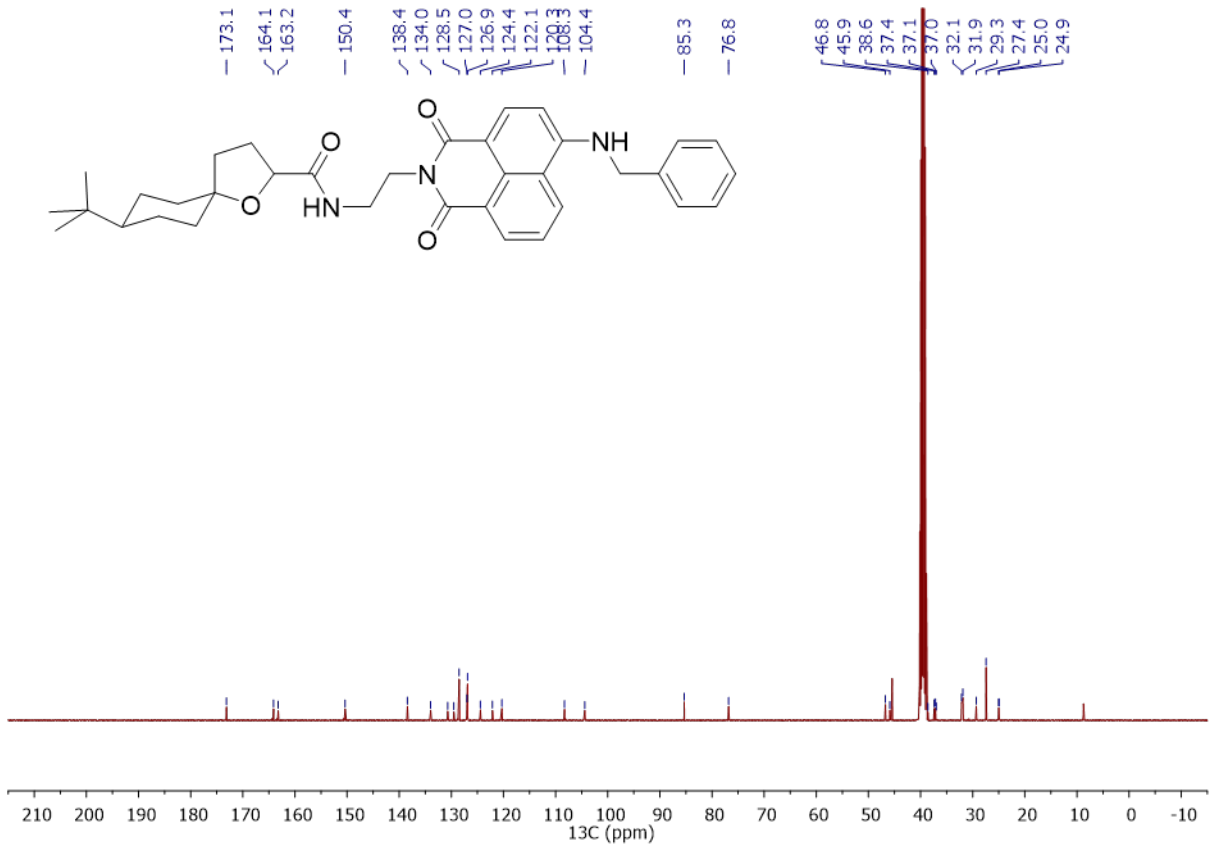
mp = dec. 198 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, J = 8.4, 1H), 8.53 (br s, 1H), 8.43 (d, J = 7.2, 1H), 8.15 (d, J = 8.4), 7.72 (t, J = 7.9, 1H), 7.49 (br s, 1H), 7.40 (d, J = 7.2, 2H), 7.33 (t, J = 7.3, 2H), 7.24 (t, J = 6.9, 1H), 6.66 (d, J = 8.4), 4.65 (d, J = 5.32, 2H), 4.26−4.22 (m, 1H), 4.08 (t, J = 7.1, 2H), 3.53−3.49 (m, 1H), 2.07−2.02 (m, 1H), 1.83−1.79 (m, 1H), 1.61−1.55 (m, 4H), 1.42 (d, J = 10.8), 1.27−1.25 (m, 3H), 0.88−0.76 (m, 12H);

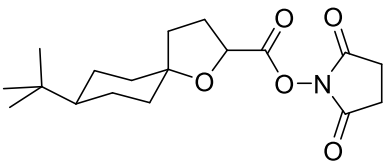
^{13}C NMR (101 MHz, DMSO-d_6) δ 173.1 (C), 164.1 (C), 163.2 (C), 150.4 (C), 138.4 (C), 134.0 (CH), 130.7 (CH), 129.5 (C), 128.5 (CH), 127.0 (CH), 126.9 (CH), 124.4 (CH), 122.1 (C), 120.3 (C), 108.3 (C), 104.4 (CH), 85.3 (C), 76.8 (CH), 46.8 (CH), 45.9 (CH_2), 38.6 (CH_2), 37.4 (CH_2), 37.1 (CH_2), 37.0 (CH_2), 32.1 (CH_2), 31.9 (C), 29.3 (CH_2), 27.4 (CH_3), 25.0 (CH_2), 24.9 (CH_2);

HRMS (TOF MS APCI+) *m / z* calcd. for $C_{35}H_{42}N_3O_4$ $[M+H]^+$ 568.3170, found 568.3164.





Succinimidyl (5*R,8*R**)-8-*tert*-Butyl-1-oxaspiro[4.5]decane-2-carboxylate (31)**

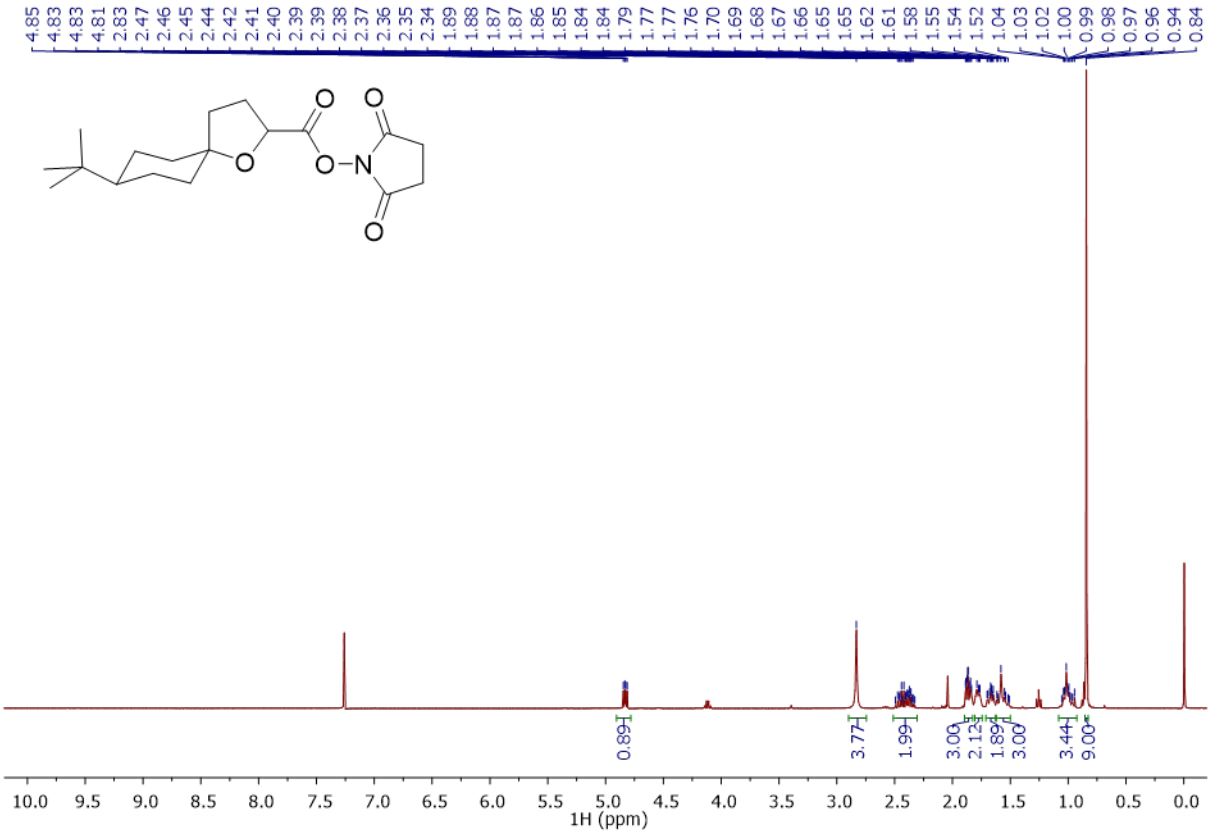


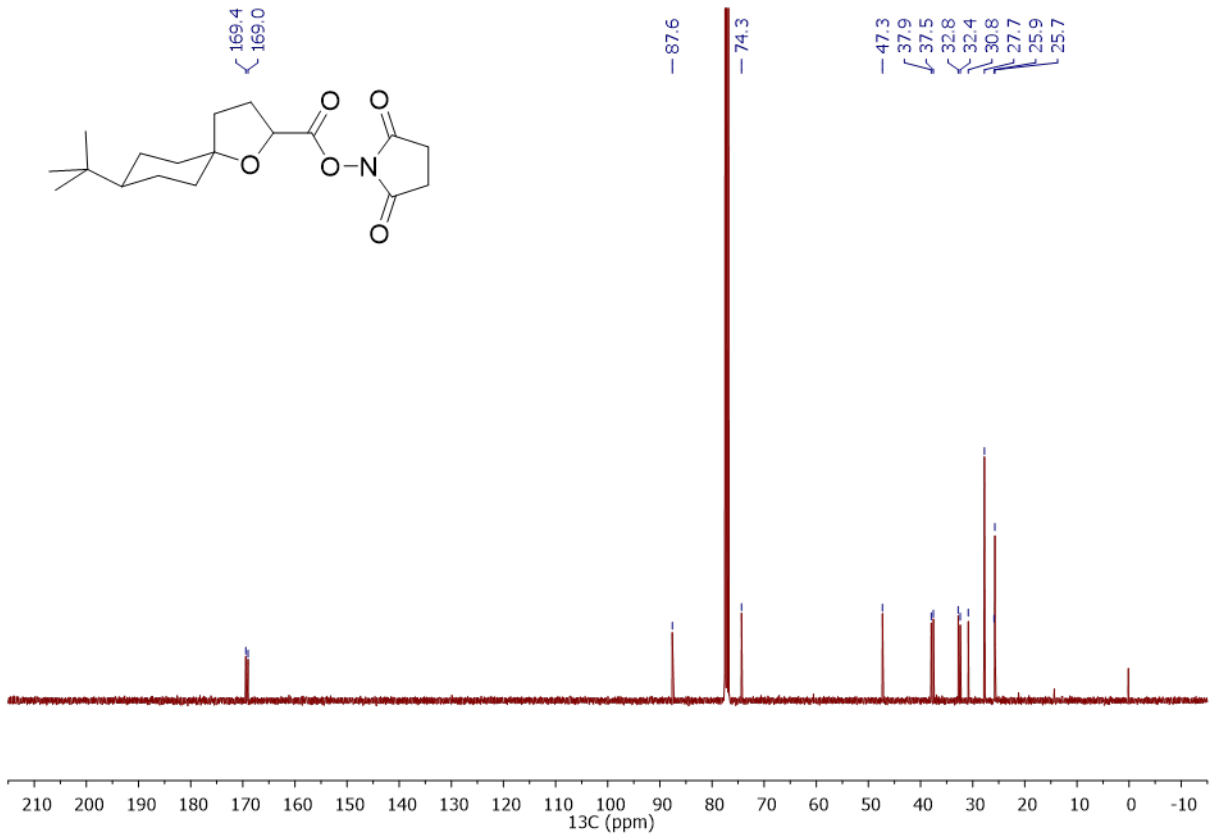
Carboxylic acid **34** (500 mg, 2.07 mmol), EDCI (482 mg, 3.11 mmol), and *N*-hydroxysuccinimide (358 mg, 3.11 mmol) were suspended in CH_2Cl_2 (8.3 mL) and stirred for 18 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with sat. aq. $NaHCO_3$ (20 mL), HCl (5% aq., 20 mL), and sat. aq. NaCl (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was passed through a silica plug and washed with 40:60 EtOAc:hexanes (*rf* = 0.58) to afford the title compound (431 mg, 62%) as a white solid, which was used directly in the next step:

mp = 68−71 °C;

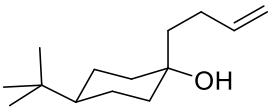
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.83 (dd, *J* = 8.3, 5.1, 1H), 2.83 (s, 4H), 2.49−2.33 (m, 2H), 1.89−1.84 (m, 3H), 1.79−1.76 (m, 2H), 1.70−1.65 (m, 2H), 1.62−1.51 (m, 1H), 1.05−0.94 (m, 3H), 0.84 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4 (C), 169.0 (C), 87.6 (C), 74.3 (CH), 47.3 (CH), 37.9 (CH_2), 37.5 (CH_2), 32.8 (CH_2), 32.4 (C), 30.8 (CH_2), 27.7 (CH_3), 25.9 (CH_2), 25.74 (CH_2), 25.73; HRMS (TOF MS ESI+) *m / z* calcd. for $C_{18}H_{31}N_2O_5$ $[M+NH_4]^+$ 355.2227, found 355.2226.





1-But-3'-ene-4-*tert*-butylcyclohexanol (32) [10]

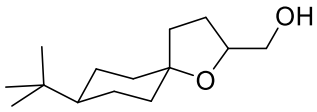


A flask containing Mg (4.278 g, 176.0 mmol) and I_2 (129 mg, 0.508 mmol) under N_2 atmosphere was suspended in Et_2O (100 mL) and cooled to 0 °C. Homoallyl bromide (10.0 mL, 98.0 mmol) was added, and the reaction mixture was stirred for 30 min. A solution of cyclohexanone (5.022 g, 32.58 mmol) in Et_2O (20 mL) was then added, and the mixture was stirred at 0 °C for an additional 3 h. The reaction mixture was diluted with aq. HCl (1 M, 50 mL) and extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with sat. aq. NaCl (150 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column chromatography (5:95 EtOAc:hexanes) afforded the title compound (1.477 g, 22%, *rf* = 0.30) as a white solid:

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93−5.83 (m, 1H), 5.06 (dq, *J* = 17.1, 1.7, 1H), 4.97 (dq, *J* = 10.2, 1.5, 1H), 2.16−2.11 (m, 2H), 1.84−1.80 (m, 2H), 1.70−1.67 (m, 2H), 1.62−1.58 (m, 3H), 1.38−1.32 (m, 2H), 1.09−1.02 (m, 3H), 0.85 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) 139.5, 114.6, 72.4, 47.7, 39.0, 35.7, 32.4, 27.8, 27.5, 24.6.

(5*R,8*R**)-8-*tert*-Butyl-3-hydroxymethyl-1-oxaspiro[4.5]decane (33)** [10]

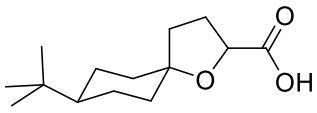


To a stirring solution of alkene **32** (1.00 g, 4.76 mmol) in CH_2Cl_2 (150 mL) was added *m*-CPBA (2.54 g, 11.3 mmol) and the mixture was stirred for 3 h. The reaction mixture was diluted with sat. aq. $Na_2S_2O_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with NaOH (1 M aq., 50 mL), sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column chromatography (40:60 EtOAc:hexanes *rf* = 0.47) afforded the title compound (1.01 g, 93%) as a white solid;

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12−4.06 (m, 1H), 3.70−3.65 (m, 1H), 3.49−3.43 (m, 1H), 2.01 (t, *J* = 6.3, 1H), 1.97−1.87 (m, 1H), 1.80−1.70 (m, 6H), 1.64−1.60 (m, 1H), 1.54−1.42 (m, 2H), 1.05−1.00 (m, 3H), 0.85 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) 84.4, 78.1, 65.3, 47.5, 38.7, 38.1, 34.0, 32.4, 27.8, 27.4, 25.9, 25.4.

(5*R,8*R**)-8-*tert*-Butyl-1-oxaspiro[4.5]decane-2-carboxylic acid (34)**



A stirring solution of alcohol **33** (620 mg, 2.73 mmol), TEMPO (30 mg, 0.19 mmol), aq. phosphate buffer (0.67 M, pH = 6.7, 10.1 mL) in CH_3CN (13.6 mL) was heated to 50 °C. Simultaneously, aq. solutions of NaOCl (0.045 M, 2.4 mL, 0.11 mmol) and NaO_2Cl (2.0 M, 5.5 mL, 11 mmol) were added, and the reaction mixture was stirred for 16 h at 50 °C. Upon cooling, the reaction mixture was diluted with aq. NaOH (1 M, 30 mL) and washed with CH_2Cl_2 (2 × 30 mL) and the organic layer was discarded. The aqueous layer was acidified to pH = 1 using aq. HCl (5%) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with sat. aq. NaCl (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (3:7

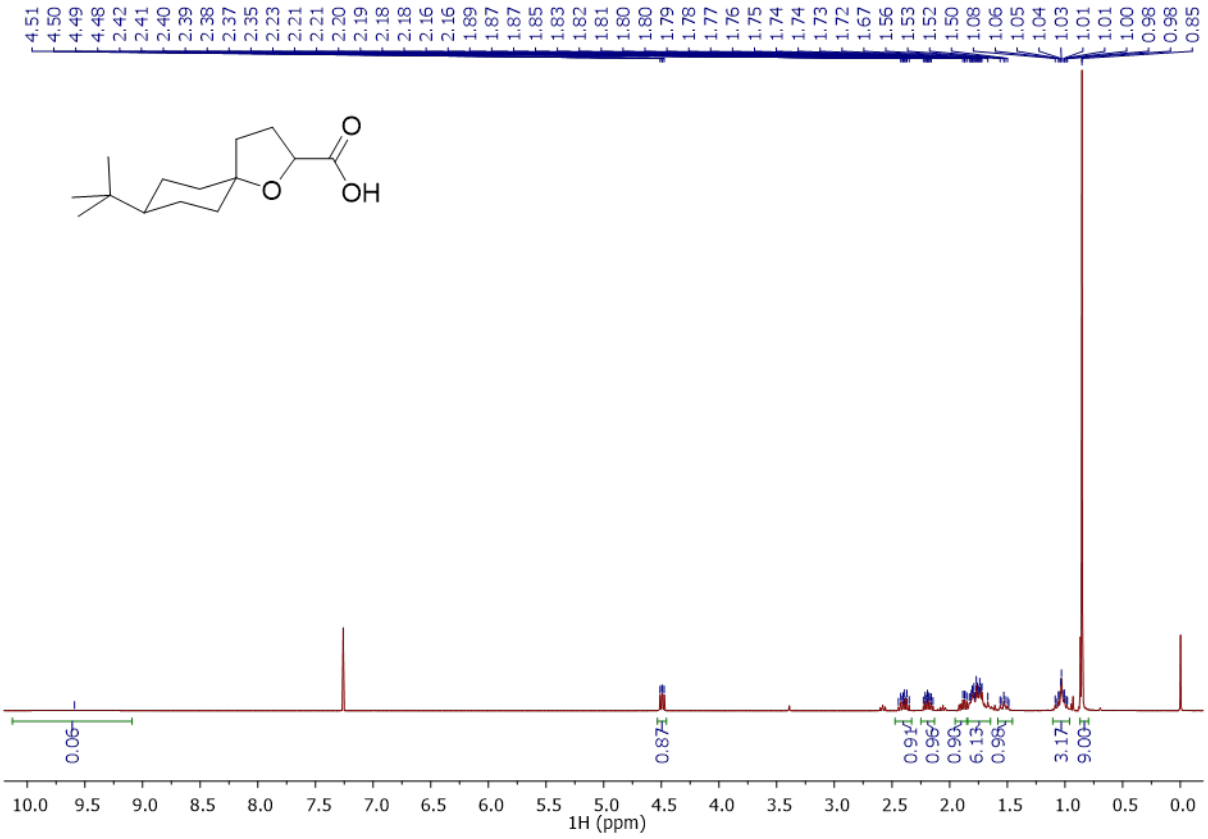
EtOAc:hexanes with 1% AcOH *rf* = 0.27) afforded the title compound (529 mg, 81%) as a white solid:

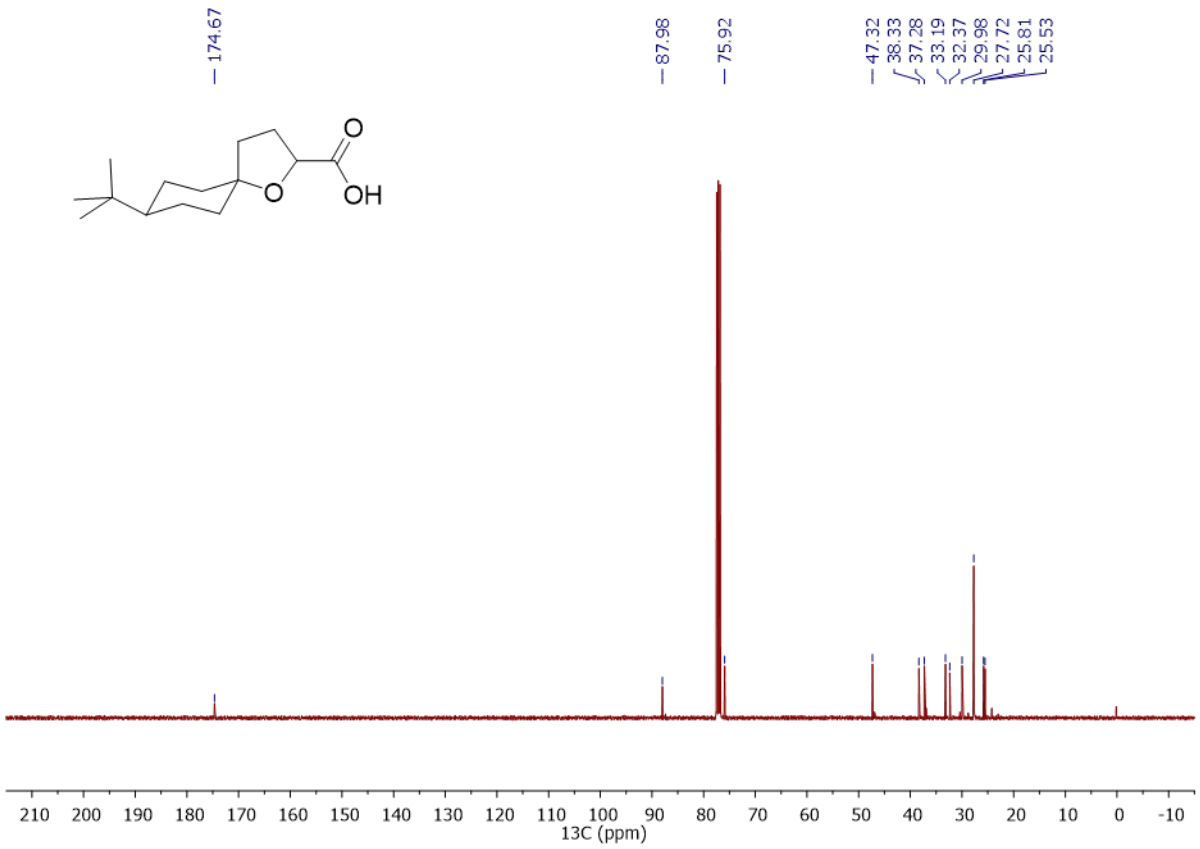
mp = 64−65 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (br s, 1H), 4.49 (dd, *J* = 8.4, 5.9, 1H), 2.44−2.35 (m, 1H), 2.23−2.14 (m, 1H), 1.89−1.85 (m, 1H), 1.82−1.67 (m, 6H), 1.56−1.49 (m, 1H), 1.08−0.98 (m, 3H), 0.85 (s, 9H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7 (C), 88.0 (C), 75.9 (CH), 47.3 (CH), 38.3 (CH_2), 37.3 (CH_2), 33.2 (CH_2), 32.4 (C), 30.0 (CH_2), 27.7 (CH_3), 25.8 (CH_2), 25.5 (CH_2);

HRMS (TOF MS ESI+) *m / z* calcd. for $C_{14}H_{24}O_3Na$ $[M+Na]^+$ 263.1618, found 263.1622.





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