Supplementary Information.

Chain-release of quinoline-based drugs from N-alkoxyquinoline prodrugs upon radiolytic one-electron reduction.

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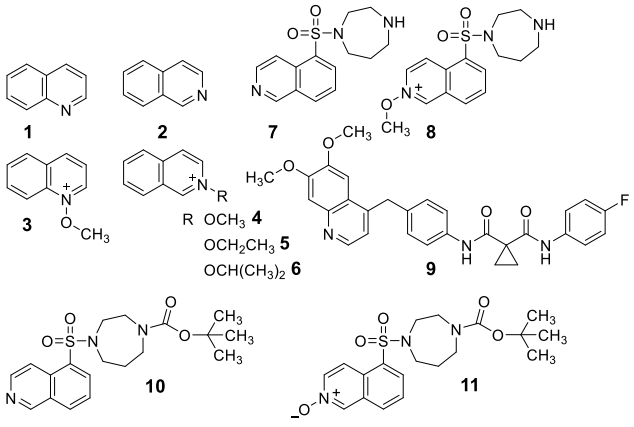
List of Compounds



A. Synthesis of compounds

Materials. Quinoline *N*-oxide, Isoquinoline-*N*-oxide and Fasudil hydrochloride were obtained from Sigma-Aldrich and used as supplied.

General Synthetic Procedures. The *N*-methoxy, *N* -ethoxy- and *N-iso*propoxy- heterocycyles were prepared by alkylation of the corresponding heterocyclic *N*-oxides. Column chromatography was performed on silica gel (Merck 230–400 mesh). Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}), with visualization of components by UV light (254 nm). Compounds were characterized by NMR using a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ^{1}H NMR spectra were referenced to tetramethylsilane at 0 ppm (internal standard) or to residual solvent peak $CHD_2(SO)CD_3$ 2.50 ppm. ^{13}C NMR spectra were referenced to tetramethylsilane at 0 ppm (internal standard) or to the deuterated solvent peak $(CD_3)_2SO$ 39.5 ppm. High resolution electrospray ionization (ESI) mass spectra was undertaken on a micrOTOF-Q mass spectrometer.

Steady-state gamma irradiations were performed using either a ^{60}Co source or a ^{137}Cs Gammacell 1000 irradiator providing radiation dose rates between 0.1 - 20 Gy (J kg^{-1}) min^{-1} (Fricke dosimetry). Time-resolved pulse radiolysis experiments were performed using the University of Auckland 4 MV Dynaray 4 linear accelerator and optical radical detection equipment, as previously described (Ref. 19). UV-visible spectra were recorded on an Ocean Optics HR4000 spectrophotometer.

Preparation of *N*-Methoxyquinolinium triflate (3) (Known compound 188530-00-9)

To quinoline *N*-oxide (0.500 g, 3.42 mmol) in dichloromethane (4.0 mL), cooled on an ice bath was added methyl triflate (3.75 mL, 34.2 mmol) under N_2. The solution was raised to RT with stirring for 4 h. The solution was diluted with further dichloromethane and the product precipitated with di-isopropylether (twice) and filtered, transferred to a vial and pumped dry to give **3**. 738 mg (68.9%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.01 (dd, J = 6.3, 1.2 Hz, 1H), 9.30 (d, J = 8.4 Hz,1H), 8.60-8.54 (m, 2H), 8.38 (ddd, J = 8.6, 7.1, 1.3 Hz, 1H), 8.28 (dd, J = 8.3, 6.3, Hz, 1H), 8.14 (ddd, J = 8.2, 7.1, 1.0 Hz, 1H), 4.54 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 69.7, 116.3, 122.4, 130.2, 130.7, 130.8, 135.7, 136.6,144.5, 146.3. Purity, 99.8%

Preparation of *N*-Methoxyisoquinolinium perchlorate (4) (Known compound 24206-36-8)

Isoquinoline *N*-oxide (1.00 g, 6.9 mmol) was heated in dimethysulfate (1.30 mL, 13.8 mmol) at 80 °C for 2 h. The reaction was cooled to rt, and the brown residue was triturated with diethyl ether twice. The residue was taken up in MeOH (0.5 mL) and 5 drops of perchloric acid added. This was dilute with MeOH, stirred with activated charcoal, filtered through celite and concentrated to an oil. The residue was crystalized from MeOH/diethyl ether three times to give **4** (130 mg, 7.3%) as crystalline white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.50 (d, J = 2.1 Hz, 1H), 9.16 (dd, J = 7.2, 2.2 Hz, 1H), 8.72 (d, J = 7.2 Hz, 1H), 8.51 (d, J = 8.2 Hz, 1H), 8.41 (d, J = 8.1 Hz, 1H), 8.29 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 8.13 (ddd, J = 8.2, 7.1, 1.0 Hz, 1H), 4.52 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ, 145.5, 138.3, 138.1, 133.0, 132.3, 131.2, 129.0, 128.7, 118.5, 70.3. HRMS (ESI^+) Calcd for $C_{10}H_{10}NO$: 160.0757; found (M^+) 160.0753. Purity 97.5%.

Preparation of *N*-Ethoxyisoquinolinium perchlorate (5)

Similar reaction of Isoquinoline *N*-oxide (1.00 g, 6.9 mmol) and diethylsulfate (1.80 mL, 13.7 mmol) for 4 h, and purification as above gave **5** (800 mg, 42.4%) as a crystalline white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.48 (d, J = 2.1 Hz, 1H), 9.14 (dd, J = 7.2, 2.1 Hz, 1H), 8.71 (d, J = 7.2 Hz, 1H), 8.50 (dd, J = 8.3, 0.7 Hz, 1H), 8.41 (dd, J = 8.3, 0.5 Hz, 1H), 8.30 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 8.13 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 4.79 (q, J = 7.0 Hz, 2H), 1.45 (t, J = 7.0 Hz, 3H). ^{13}C NMR (400 MHz, DMSO) δ 145.8, 136.9, 136.5, 132.3, 131.6, 130.3, 127.55, 127.50, 127.3, 78.8, 13.0. HRMS (ESI^{+}) Calcd for $C_{11}H_{12}NO$: 174.0913; found (M^{+}) 174.0907. Purity 93.7%.

Preparation of *N*-Isopropoxyisoquinolinium perchlorate (6)

Similar reaction of Isoquinoline *N*-oxide (1.00 g, 6.9 mmol) and isopropyl methanesulfonate (4.75, 34 mmol) for 12 h, and purification as above gave **6** (250 mg, 12.6%) as a waxy solid. ^{1}H NMR (400 MHz, DMSO) δ 1H NMR (400 MHz, Solvent) d ppm 10.44 (d, J = 2.0 Hz, 1H), 9.09 (dd, J = 7.1, 2.1 Hz, 1H), 8.71 (d, J = 7.2 Hz, 1H), 8.50 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 7.8 Hz, 1H), 8.30 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 8.13 (ddd, J = 7.0, 5.3, 1.1 Hz, 1H), 5.11 (sept., J = 6.1, 6.1, 6.1, 6.1, 6.1, 6.1 Hz, 1H), 1.42 (d, J = 6.1 Hz, 6H). ^{13}C NMR (400 MHz, DMSO) δ 146.4, 136.9, 136.6, 133.1, 131.6, 130.4, 127.57, 127.54, 127.3, 86.7, 20.0. HRMS (ESI^{+}) Calcd for $C_{12}H_{14}NO$: 188.1070; found (M^{+}) 188.1068. Purity 96.0%, 0.27% isoquinoline.

Preparation of Fasudil-Boc (10). (Known compound 612483-90-6)

di-tert-butyldicarbonate (500 mg, 2.29 mmol) was added to a stirred solution of fasudil hydrochloride (500 mg, 1.5 mmol) and NaOH (122 mg, 3.05 mmol) in THF/water (50 mL/20 mL) at room temperature. After 2h, the reaction was extracted with EtOAc, and the organic layer was washed with brine and dried (Na_2SO_4). Purification by flash column chromatography (neat dichloromethane) gave **10** (500 mg, 80%) as a white solid? ^{1}H NMR (400 MHz, DMSO) δ 9.49 (s, 1H), 8.70 (d, J = 6.1 Hz, 1H), 8.46 (d, J = 8.2 Hz, 1H), 8.37 – 8.28 (m, 2H), 7.84 (td, J = 7.9, 3.2 Hz, 1H), 3.51 – 3.41 (m, 4H), 3.41 – 3.32 (m, 4H), 1.80 – 1.71 (m, 2H), 1.37 (s, 9H).

Preparation of *N*-oxide-Fasudil-Boc (11). (Known compound 802902-39-2)

To **10** (500 mg, 1.20 mmol) dissolved in dichloromethane (20 mL) was added Na_2HPO_4 (340 mg, 2.39 mmol) and *meta*-chloroperoxybenzoic acid (300 mg, 1.44 mmol) and stirred for 16 h at RT. The reaction was diluted with dichloromethane (30 mL) and stirred with K_2CO_3 (331 mg, 2.4 mmol) for 1h, filtered, and the white ppt was washed well with dichloromethane. The supernatant was concentrated and purified by purified by flash column chromatography (silica gel, dichloromethane to EtOAc) to give **11** (440 mg, 90%) as a white semi-solid. ^{1}H NMR (400 MHz, DMSO) δ 9.11 (s, 1H), 8.38 (dd, J = 7.6, 2.7 Hz, 1H), 8.32 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 8.3 Hz, 1H), 8.07 (dd, J = 7.5, 1.1 Hz, 1H), 7.78 (td, J = 7.9, 3.1 Hz, 1H), 3.50 – 3.31 (m, 8H), 1.81 – 1.68 (m, 2H), 1.37 (s, 9H).

Preparation of *N*-Methoxy-Fasudil perchlorate (8).

The *N*-oxide **11** (50 mg, 0.122 mmol) was heated in dimethyl sulfate (0.115 mL, 1.22 mmol) at 85 °C for 1.5h. The reaction was then cooled, and diethyl ether was added (5.0 mL) and the supernatant was removed. This was repeated twice to give an oily residue. The oil was dissolved in MeOH (0.20 mL) and a few drops of perchloric acid was added, followed by diethyl ether. The supernatant was

removed, and the white solid was dried under high vacuum to give **8** (35 mg, 68%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.72 (d, J = 2.3 Hz, 1H), 9.26 (dd, J = 7.6, 2.3 Hz, 1H), 8.99 (d, J = 7.6 Hz, 1H), 8.78 (d, J = 1.0 Hz, 1H), 8.73 (dd, J = 7.6, 1.2 Hz, 1H), 8.71 (bs s, 1H), 8.26 (t, J = 7.5 Hz, 1H), 4.56 (s, 3H), 3.73 – 3.66 (m, 2H), 3.53 (t, J = 6.1 Hz, 2H), 3.33 – 3.20 (m, 4H), 2.04 (p, J = 5.9 Hz, 2H). (The resonance at 8.71 exchanges with D_2O). Two unknown singlets at 3.36 and 3.16 ppm. ^{13}C (400 Hz, DMSO) 145.8., 137.2, 135.8, 134.6, 133.5, 132.0, 131.1, 129.4, 124.1, 46.9, 46.4, 44.5, 44.1, 25.3. HRMS (ESI$^+$) Calcd for $C_{15}H_{20}N_3O_3S$: 322.1220; found (M^+) 322.1212. Purity 93.5%.

Stability of *N*-Methoxy-fasudil (8) in water and media.

HPLC Analysis

The samples were analyzed using an Agilent 1260 Infinity HPLC with a diode array absorbance detector and autosampler at 4 °C (Agilent Technologies). Samples prepared in H_2O and αMEM containing 1% DMSO, were injected directly (typically 20 mL). The calibration standards for the prodrug and fasudil were prepared freshly in water or αMEM (typically 0.3 – 100 μM for each analysis. All calibration curves were linear (R2 > 0.999). The column was an Agilent Zorbax Eclipse (XBD-C8, 5u, 2.1 x150 mm, Agilent Technologies) with a flow rate of 0.5 mL/min. The mobile phase was 0.1% TFA/H_2O, pH 2 (A) and 0.1% TFA/ACN (B), eluting with linear gradients of 5%-100% B over 18 min, 100% - 5% over 2 min and 5% - post run over 3 min. The analytes were quantified using 334 nm (with ref 550 nm) with retention times of 7.5 min for fasudil, 8.0 min for prodrug for samples in αMEM, and 7.6 min for prodrug in H_2O.

A. Analysis of Compound **8** at t = 0, and Stability in H_2O at 37 °C over 24 h.
B. Analysis of Compound **8** at t = 0 and Stability in αMEM at 37 °C over 24 h.

A. Data at t = 0 (H_2O)

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Last changed    : 4/12/2014 1:41:52 p.m. by Sisira Kumara
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                  05% - 100% B over 18 min.
                  100% - 05%  B over 2 min.
                  05% B - Post run over 3 min.

Sample Info     : Solubility & stability in water with 1% DMSO
                  At ambient temp. at t=0 hrs.
                  Method - A, A0(H2O + 1% DMSO)
                  Target conc. - 1 mM
                  no apperant ppt + clear colourless solution
                  Test soln. - solution (spun down twice) diluted 2 fold with water
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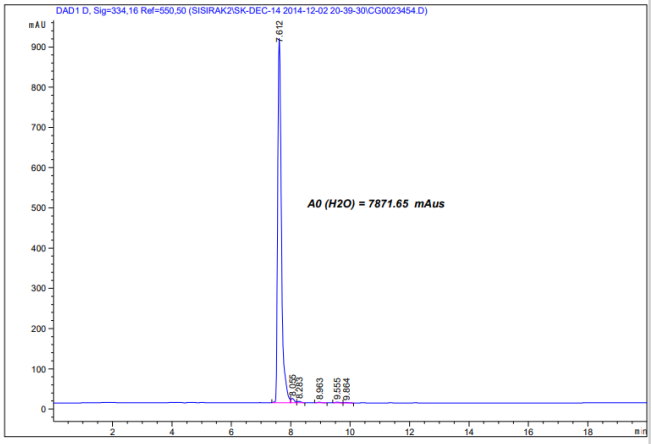


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Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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   2   8.055 VV    0.1151   85.52671   10.94934   1.0657
   3   8.283 VB    0.1185   27.41184    3.53237   0.3416
   4   8.963 BB    0.1362   13.35423    1.49551   0.1664
   5   9.555 BV    0.1409   17.46041    1.80453   0.2176
   6   9.864 VB    0.1313    9.87939    1.11529   0.1231

Totals :                  8025.27809  929.14558

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A. Data at t = 24 h (H_2O) 99.4% stable

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Last changed    : 2/12/2014 8:25:37 p.m. by Sisira Kumara
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Last changed    : 4/12/2014 7:29:20 p.m. by Sisira Kumara
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                  Gradient-   A= 0.1% TFA/H2O &  B= 0.1% TFA/ACN
                  05% - 100% B over 18 min.
                  100% - 05%  B over 2 min.
                  05% B - Post run over 3 min.

Sample Info     : Stability in water with 1% DMSO
                  At 37.5C temp. at t=24 hrs.
                  Method - A, A1(H2O + 1% DMSO)
                  Test soln. - supernatant (spun down twice) diluted 2 fold with water
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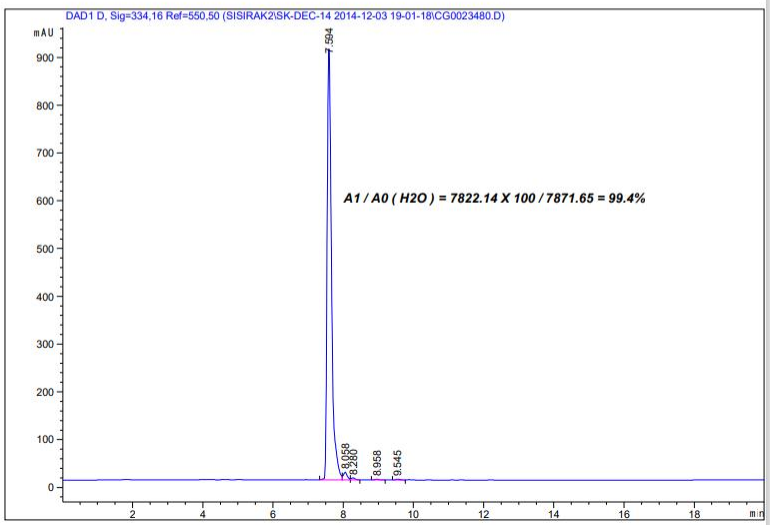


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   3   8.280 VB    0.1145   32.18347    4.14879   0.4018
   4   8.958 BB    0.1234   12.02106    1.46949   0.1501
   5   9.545 BV    0.1436   18.34241    1.85141   0.2290

Totals :                  8009.26245  925.42824


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B. Data at t = 0 (αMEM)

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                  Flow rate-  0.5 ml/min.
                  Gradient-   A= 0.1% TFA/H2O &  B= 0.1% TFA/ACN
                  05% - 100% B over 18 min.
                  100% - 05%  B over 2 min.
                  05% B - Post run over 3 min.

Sample Info     : Solubility & stability in aMEM with 1% DMSO
                  At ambient temp. at t=0 hrs.
                  Method - A, A0 (aMEM + 1% DMSO)
                  Target conc. - 1 mM
                  no apperant ppt + clear colourless solution
                  Test soln. - solution (spun down twice) diluted 2 fold with water

Additional Info : Peak(s) manually integrated
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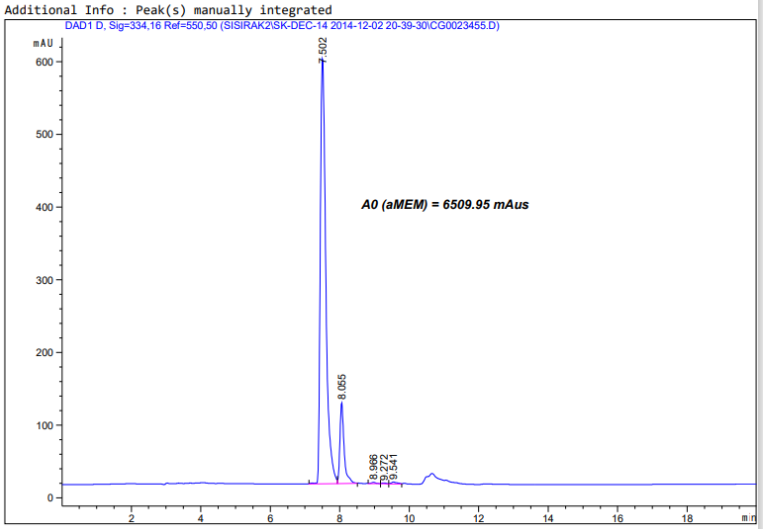


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Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 D, Sig=334,16 Ref=550,50

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
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   2   8.055 VB    0.1304  940.52704  111.47801  12.5310
   3   8.966 BB    0.1310   15.11527    1.71283   0.2014
   4   9.272 BV    0.1266    9.56016    1.13137   0.1274
   5   9.541 VV    0.1692   30.46482    2.51653   0.4059

Totals :                  7505.61261  702.77306


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C. Data at t= 24 h (αMEM) 72.8% stable

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Analysis Method : C:\CHEM32\1\METHODS\ZORBAX-C8-ECLIPSE METHODS\ZE0515TFA0.5V40.M
Last changed    : 4/12/2014 7:29:20 p.m. by Sisira Kumara
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                  Flow rate-  0.5 ml/min.
                  Gradient-   A= 0.1% TFA/H2O &  B= 0.1% TFA/ACN
                  05% - 100% B over 18 min.
                  100% - 05%  B over 2 min.
                  05% B - Post run over 3 min.

Sample Info     : Stability in aMEM with 1% DMSO
                  At 37.5C temp. at t=24 hrs.
                  Method - A, A1(aMEM + 1% DMSO)
                  Test soln. - supernatant (spun down twice) diluted 2 fold with aMEM

Additional Info : Peak(s) manually integrated
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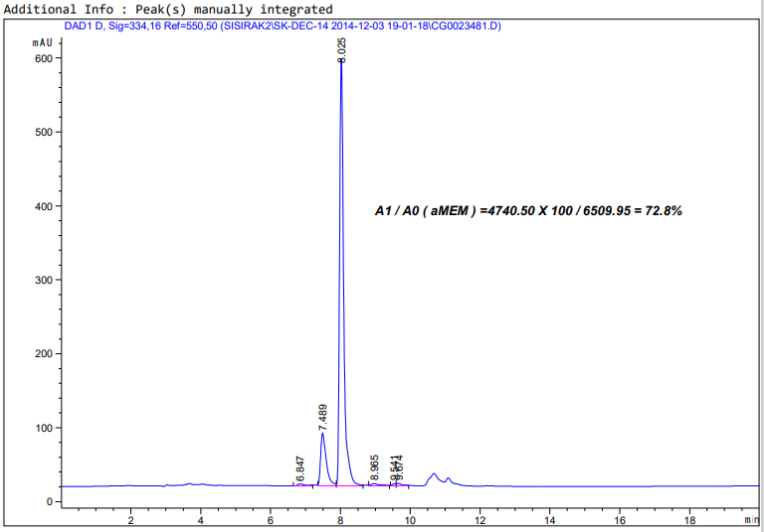


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Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 D, Sig=334,16 Ref=550,50

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
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   3   8.025 VB    0.1233 4740.49707  580.25916  83.3828
   4   8.965 BB    0.2322   52.80037    3.07717   0.9287
   5   9.541 BV    0.1058   19.02917    2.71418   0.3347
   6   9.674 VB    0.1537   35.68443    3.21046   0.6277

Totals :                  5685.22249  663.31856

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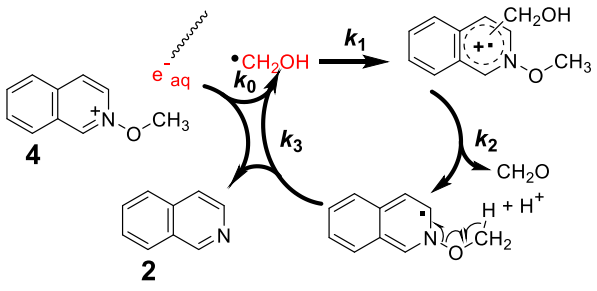
B. Radiation Chemistry Data

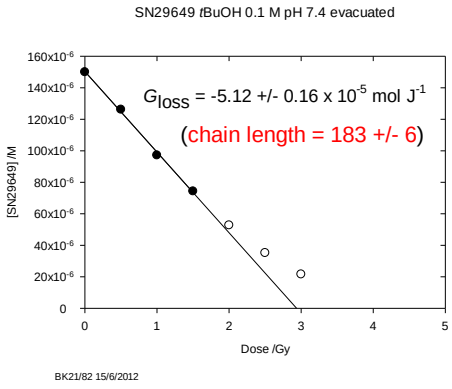
Pulse Radioysis Spectra

Time-resolved spectra are displayed as the change in extinction coefficient, ε with wavelength, λ. Values are calculated using the radiolytic yields (G-values) of the e^-_{aq} in N_2-saturated solution as 0.27 µM Gy^{-1} (µmol J^{-1}) and the hydroxymethyl radical in methanol (0.2 M) in N_2O-saturated solution as 0.54 µM Gy^{-1}. (ε / M^{-1} cm^{-1} = absorbance/Gy divided by G-value of reducing species in a 1 cm pathlength cell).

N-Methoxyisoquinolinium tetrafluoroborate (4)

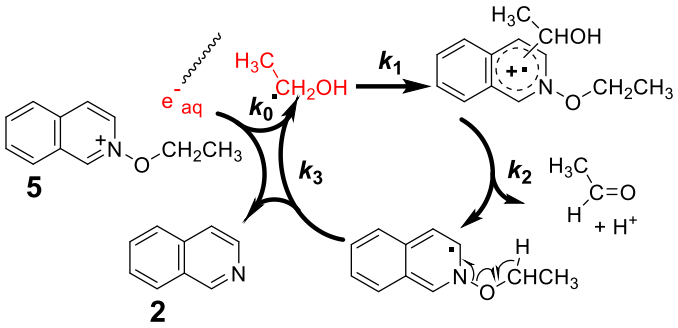
Figure S1. Scheme and loss of compound **4** (G value and chain length) with radiation dose in evacuated solution containing 2-methylpropan-2-ol (0.2 M) at pH 7.

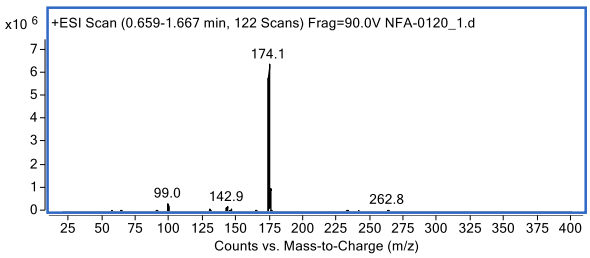


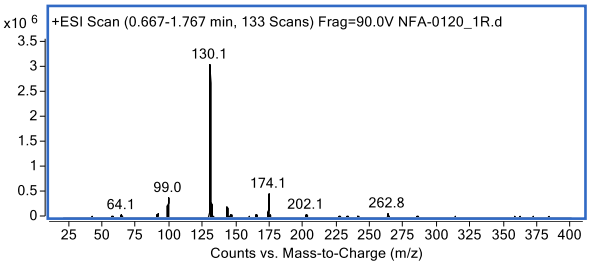


***N*-Ethoxyisoquinolinium tetrafluoroborate (5)**

Figure S2. Scheme and loss of compound **5** (G value and chain length) with radiation dose in evacuated solution containing 2-methylpropan-2-ol (0.2 M) at pH 7. Mass spectra before and after irradiation showing loss of sidechain. UV-visible spectra of the transition of **5** to **2** with increasing radiation dose.







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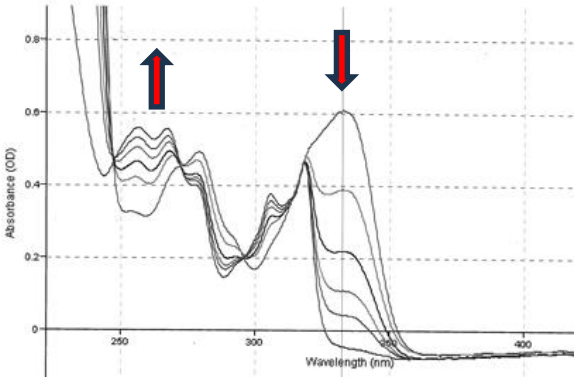

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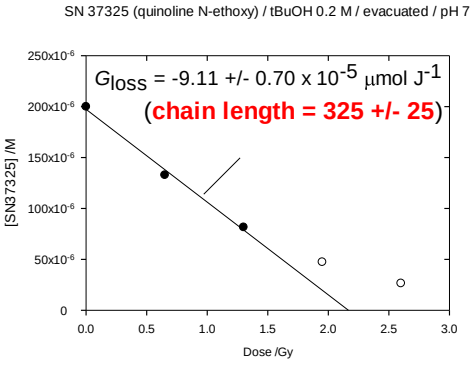
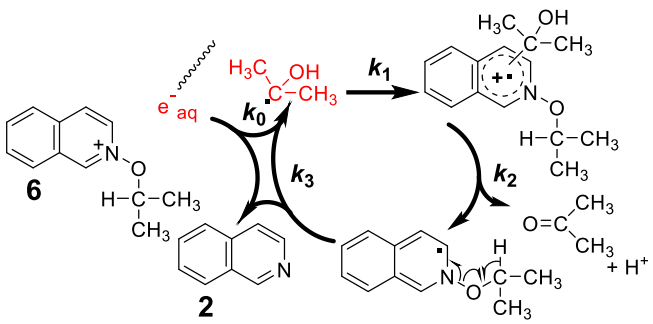
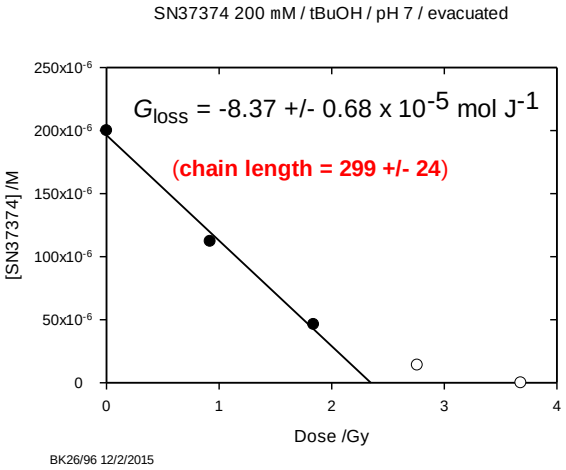


A. Changes in absorption of **5** (200 µM) in evacuated aqueous solution containing 2-methylpropan-2-ol (0.2 M) at pH 7 (2 mM phosphate) with accumulated radiation dose. **B.** Loss in the concentration of **5** with accumulated radiation dose.

N-Isopropoxyisoquinolinium tetrafluoroborate (6)

Figure S3. Scheme and loss of compound (G value and chain length) with radiation dose in evacuated solution containing 2-methylpropan-2-ol (0.2 M) at pH 7.

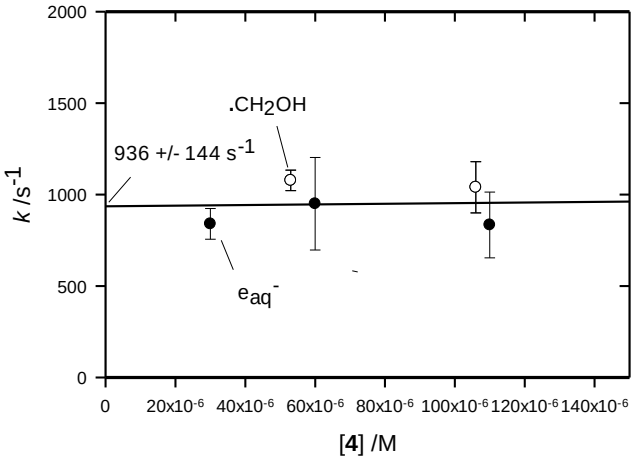




Loss in the concentration of **6** with accumulated radiation dose.

Independence of k_2 on the concentration of prodrug (4)

Figure S4. Rate constants, k_2, for the decay of the one-electron reduced intermediate at 370 nm following irradiation of increasing concentrations of **4** in (i) N_2-saturated solution containing 2-methylpropan-2-ol (0.2 M), ○ , (ii) N_2O-saturated solutions containing methanol (0.2 M), ●



Determination of formaldehyde following irradiation of compound (3).

The method of Hantzch was used (Nash, T. *Nature*, December 6th 976, 1952: Cinti, D.L. & Thal, S.E. *Anal. Biochem.*83, 91, 1977) where two molecules of acetylacetone and one of formaldehyde-ammonium combine to specifically form the chromogen 3,5-diacetyl-1,4-dihydrolutidine. It has a broad band centered around 412 nm with extinction coefficient, ε, of 8,000 M^{-1} cm^{-1}.

A 151 μM duplicate samples of **3** in water containing 2-methylpropan-2-ol (0.2 M) were purged with N_2 and evacuated x3 and irradiated on the Gammacell at a dose rate of 2 Gy min^{-1}. Reduction of **3** was followed to completion by uv-visible spectrophotometry in the closed system. To 8 mL irradiation solution and non-irradiated control, 1 mL each of 50 mM acetylacetone and 33.3 mM glacial acetic acid, both in 1 M aqueous ammonium acetate, were added and the mixtures left for 2 hours for colour to develop. A standard solution of 150 μM formaldehyde was prepared and treated with the reagents in the same way. UV-visible spectra of the mixtures and standard exhibited the same broad spectrum and absorptions at 412 nm were recorded (spectra displayed below, Figure S5). The formaldehyde standard abs 1.232 compared to the irradiation solutions of 0.053 ± 0.001 indicated that only 3.7% of the possible maximum concentration of formaldehyde is in solution. This is understood by the known reaction of formaldehyde with carbon-centred radicals to form a range of diverse molecules. Such a reactive radical is formed on *tert*-butanol in the test system, which is added to scavenge the radiolytically-formed .OH radical and H-atom.

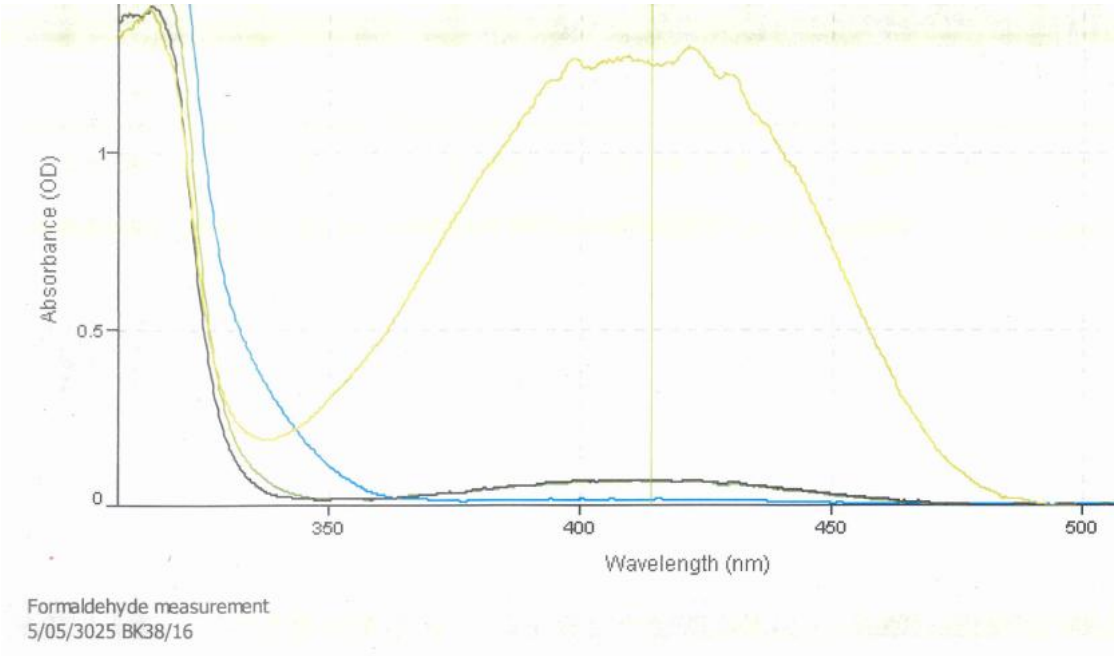
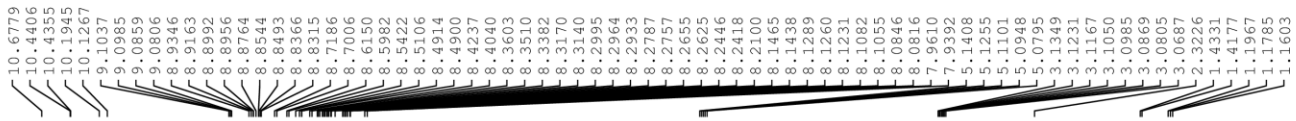
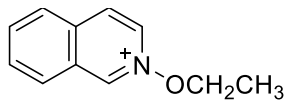


Figure S5. Spectra formed on reaction of formaldehyde standard and irradiated solutions with reagents described above after 2 h incubation time. Yellow spectrum, 151 μM formaldehyde, Black and Green, spectra from duplicate experiments of irradiated solutions of compound **3** and Blue spectrum, unirradiated solution containing compound **3** treated in the same way with reagents.

HSS SN 37374



1H NMR of **5** (d_6-DMSO)

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FIDRES                  0.250144 Hz
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DE                        13.93 usec
TE                        298.0 K
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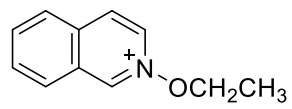
HSS SN 37374

APT NMR of **5** (d_6-DMSO)

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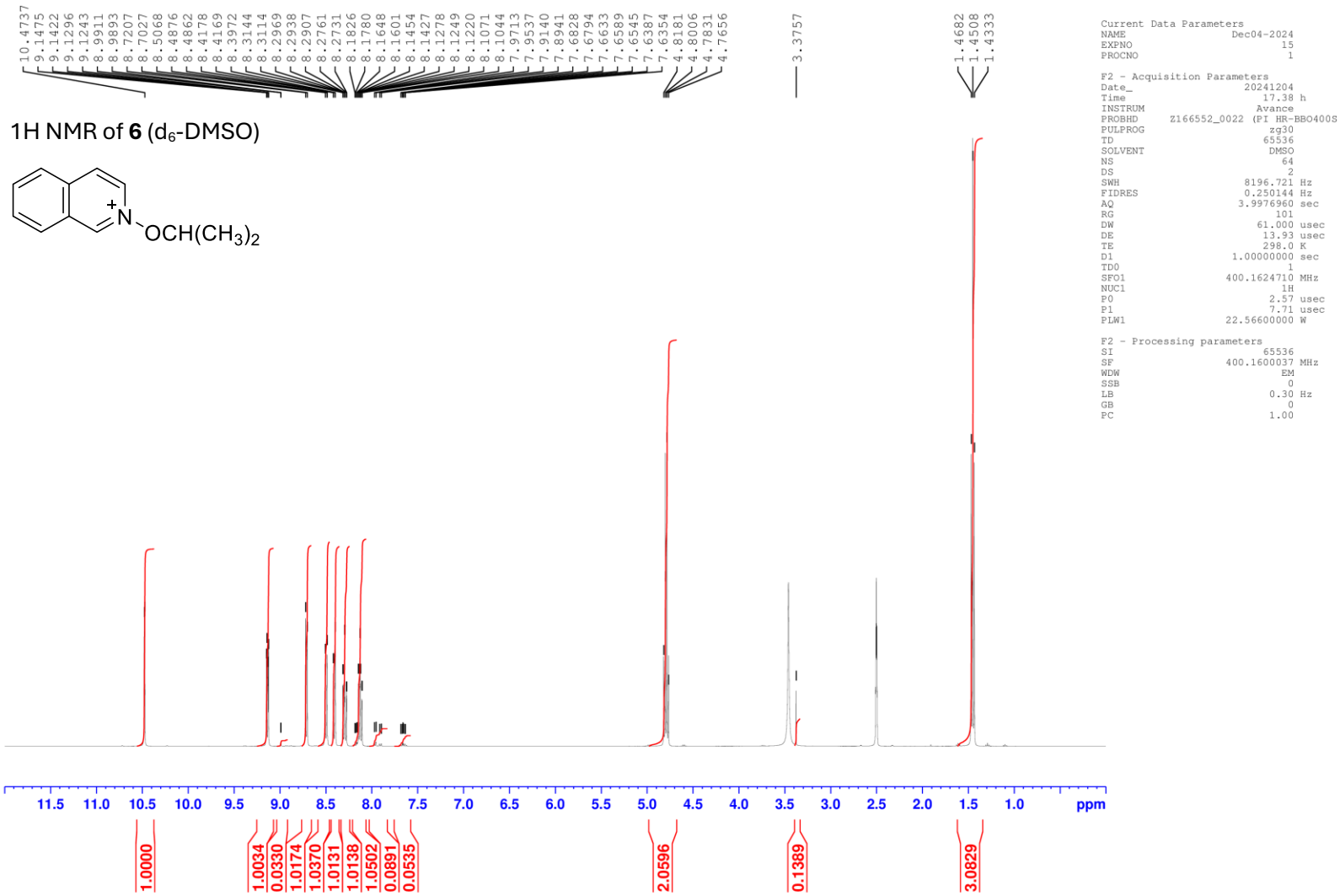
HSS SN 37325



1H NMR of **6** (d_6-DMSO)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FIDRES                 0.250144 Hz
AQ                     3.9976960 sec
RG                          101
DW                       61.000 usec
DE                       13.93 usec
TE                       298.0 K
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NUC1                         1H
P0                        2.57 usec
P1                        7.71 usec
PLW1                 22.56600000 W

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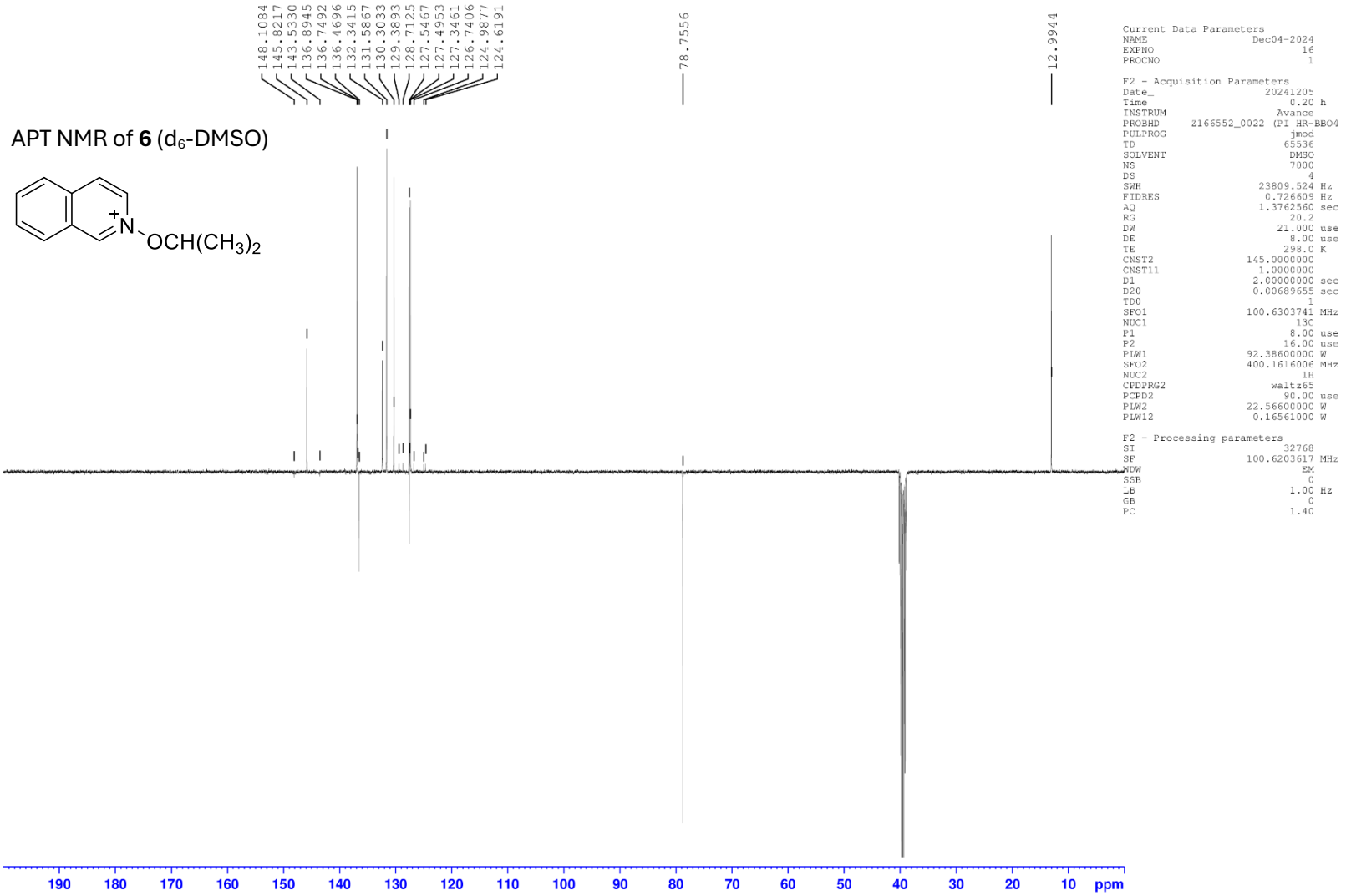
HSS SN 37325

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TD                         65536
SOLVENT                     DMSO
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FIDRES                 0.726609 Hz
AQ                     1.3762560 sec
RG                          20.2
DW                        21.000 use
DE                         8.00 use
TE                        298.0 K
CNST2                145.0000000
CNST11                 1.0000000
D1                     2.00000000 sec
D20                    0.00689655 sec
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NUC1                         13C
P1                         8.00 use
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CPDPRG2                 waltz65
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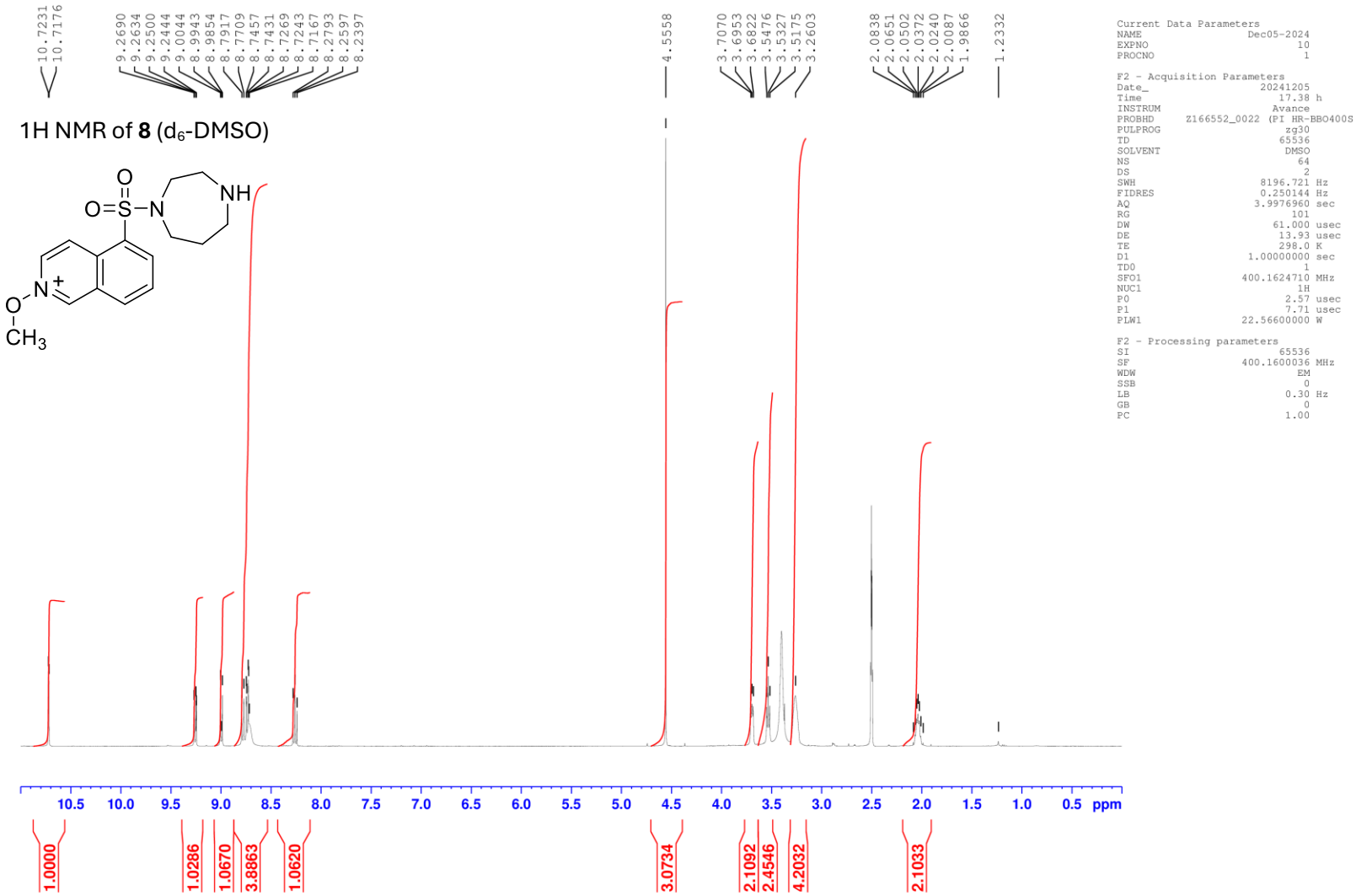
HSS SN 37146



1H NMR of **8** (d_6-DMSO)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PROCNO                         1

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TD                        65536
SOLVENT                    DMSO
NS                            64
DS                             2
SWH                     8196.721 Hz
FIDRES                  0.250144 Hz
AQ                      3.9976960 sec
RG                           101
DW                        61.000 usec
DE                        13.93 usec
TE                        298.0 K
D1                      1.00000000 sec
TD0                            1
SFO1                  400.1624710 MHz
NUC1                          1H
P0                         2.57 usec
P1                         7.71 usec
PLW1                   22.56600000 W

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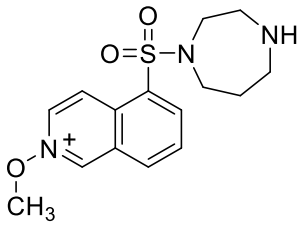
HSS SN 37146

APT NMR of **8** (d_6-DMSO)

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SOLVENT                     DMSO
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TE                        298.0 K
CNST2                145.0000000
CNST11                 1.0000000
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D20                    0.00689655 sec
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NUC1                         13C
P1                         8.00 use
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