

Supporting Information

Structure-based screening and optimization of cytosine derivatives as inhibitors of the menin-MLL interaction

Hai-Jing Zhong^a, Bo Ra Lee^c, Joshua William Boyle^c, Wanhe Wang^b, Dik-Lung Ma^{*b}, Philip Wai Hong Chan^{*cd}, Chung-Hang Leung^{*a}

^aState Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China. E-mail: duncanleung@umac.mo

^bDepartment of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China. E-mail: edmondma@hkbu.edu.hk

^cSchool of Chemistry, Monash University, Clayton, Victoria 3800, Australia. E-mail: phil.chan@monash.edu

^dDepartment of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom

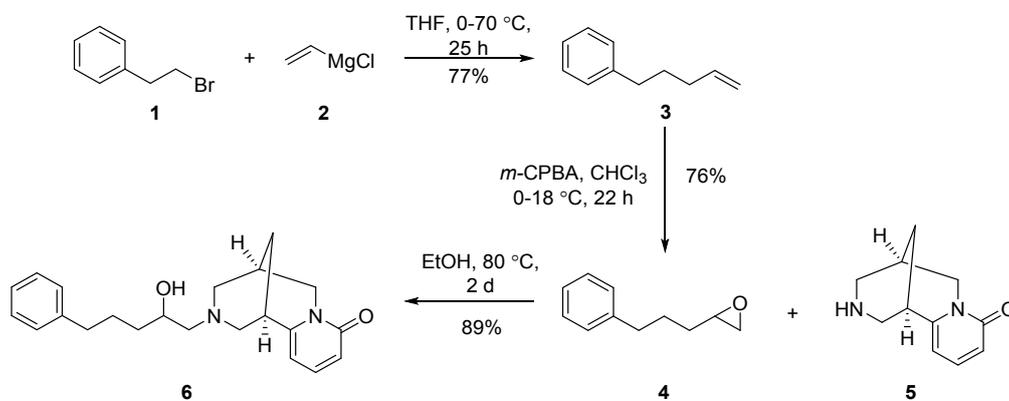
Experimental section

General Remarks

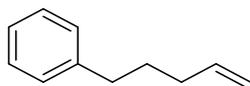
All reactions were performed under a nitrogen atmosphere. Unless specified, all reagents and starting materials were purchased from commercial sources and used as received. Solvents were purified following standard literature procedures. Analytical thin layer chromatography (TLC) was performed using pre-coated silica gel plate. Visualization was achieved by UV light (254 nm). Flash column chromatography was performed using silica gel. ¹H and ¹³C NMR spectra were measured on a 300 and 400 MHz spectrometer with CDCl₃ as solvent and TMS as the internal standard unless specified. Multiplicities are given as: s (singlet), bs (broad singlet), d (doublet), t (triplet), dd (doublet of doublets) or m (multiplet). The number of protons (*n*) for a given resonance is indicated by *n*H and coupling constants are reported as a *J* value in Hz. Infrared spectra were recorded on a FTIR spectrometer. Solid samples were examined as a thin film between NaCl salt plates. High-resolution mass spectra

(HRMS) were obtained on a LC/HRMS mass spectrometer.

General Procedure for the Synthesis of Compound 6



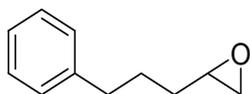
pent-4-en-1-ylbenzene (**3**)¹



To a solution of bromoethylbenzene (**1**) (1.00 g, 5.40 mmol) in THF (13 mL) was added dropwise vinylmagnesium chloride solution (**2**) (1.6 M in THF, 5.06 mL, 8.10 mmol) at room temperature. The resulting mixture was stirred at reflux for 25 h. After completion of the reaction by TLC analysis, the reaction mixture was cooled to room temperature and quenched with saturated NH₄Cl solution (40 mL) and extracted with ethyl acetate (40 mL × 3). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (100% *n*-hexane) gave the title compound. Spectral data for pent-4-en-1-ylbenzene (**3**) was consistent with data reported in the literature.²

Yield 77%; colorless oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.30–7.27 (m, 2H), 7.20–7.17 (m, 3H), 5.89–5.78 (m, 1H), 5.06–4.97 (m, 2H), 2.63 (d, *J* = 7.8 Hz, 2H), 2.12 (dd, *J* = 14, 6.8 Hz, 2H), 1.77–1.69 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ = 142.6, 138.8, 128.6, 128.4, 125.8, 114.8, 35.5, 33.4, 30.8; LRMS (EI): *m/z* (%): 146.2 (35, [M⁺]), 131.0 (16), 104.2 (100), 91.0 (44).

2-(3-phenylpropyl)oxirane (**4**)³

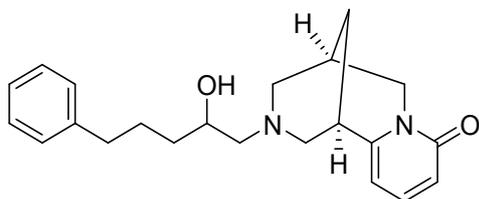


To a solution of pent-4-en-1-ylbenzene (**3**) (93.1 mg, 6.40 mmol) in chloroform was added 50-60% *m*-CPBA (220 mg, 1.27 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was slowly allowed to warm to room temperature and stirred for 22 h. The reaction mixture was quenched with dichloromethane (20 mL) and washed with 2.5 M NaOH solution (25 mL × 4). The organic layer was dried over Na₂SO₄ and

concentrated under reduced pressure. Purification by flash column chromatography on silica gel (100% *n*-hexane) gave the title compound. Spectral data for 2-(3-phenylpropyl)oxirane (**4**) was consistent with data reported in the literature.³

Yield 76%; colorless oil; ¹H NMR (400 MHz, CDCl₃): 7.30–7.26 (m, 2H), 7.21–7.17 (m, 3H), 2.95–2.91 (m, 1H), 2.76–2.74 (m, 1H), 2.68 (t, *J* = 3.6 Hz, 2H), 2.47 (dd, *J* = 4.8, 2.4 Hz, 1H), 1.89–1.72 (m, 2H), 1.66–1.51 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ = 142.1, 128.5, 128.5, 126.0, 52.3, 47.2, 35.7, 32.1, 27.9; LRMS (EI): *m/z* (%): 162.0 (6, [M⁺]), 131.0 (29), 104 (100).

(1S,5R)-3-(2-hydroxy-5-phenylpentyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (6)

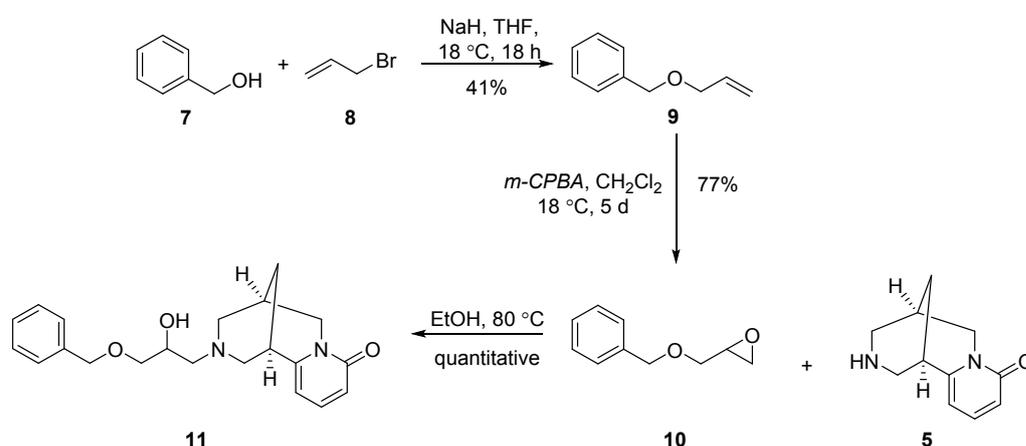


To a solution of 2-(3-phenylpropyl)oxirane (**4**) (187 mg, 1.15 mmol) in ethanol (5 mL), (–)-cytisine (**5**) (241 mg, 1.26 mmol) was added at room temperature under nitrogen atmosphere. The resulting mixture was stirred at reflux for 2 d. The reaction mixture was allowed to cool to room temperature and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (100% methanol) gave the title compound.

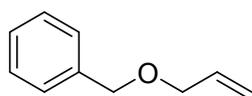
Yield 89%; yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.42–7.34 (m, 3H), 7.28–7.24 (m, 3H), 6.52 (td, *J* = 9.2, 1.2 Hz, 1H), 6.09 (td, *J* = 6.8, 1.2 Hz, 1H), 4.18 (t, *J* = 16.0 Hz, 1H), 3.99 (dd, *J* = 16.0, 7.1 Hz, 1H), 3.70–3.57 (m, 1 H), 3.15–3.08 (m, 2H), 2.98–2.90 (m, 1H) 2.76–2.62 (m, 4H) 2.54 (s, 1H), 2.38–2.19 (m, 3 H), 2.00 (d, *J* =

12.4 Hz, 1 H) 1.92–1.79 (m, 2 H), 1.74–1.64 (m, 1H), 1.49–1.36 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ = 163.4, 163.4, 150.9, 150.6, 142.4, 142.4, 138.9, 138.8, 128.5, 128.4, 128.3, 128.3, 125.7, 117.0, 117.0, 104.7, 104.6, 66.6, 65.9, 64.0, 63.5, 62.7, 62.0, 50.1, 50.0, 36.0, 35.9, 35.8, 35.2, 34.3, 34.1, 28.3, 27.9, 27.4, 27.2, 26.0, 25.9 ; IR (NaCl, neat) ν : 3373, 2934, 3794, 1646, 1645, 1139 cm^{-1} ; LRMS (ESI): m/z = 375.1 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_2\text{Na}$ $[\text{M} + \text{Na}]^+$: 375.2048, found 375.2044.

General Procedure for the Synthesis of Compound 11



((allyloxy)methyl)benzene (9)⁴

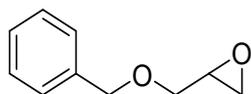


To a suspension of NaH (146 mg, 6.10 mmol) in dry THF (5 mL) was added a solution of benzylalcohol **7** (600 mg, 5.50 mmol) in THF (4 mL) dropwise at 0 °C under a nitrogen atmosphere. The reaction was allowed to stir for 30 mins before allyl bromide (**8**) (789 mg, 6.1 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 18 h. On completion, the reaction mixture was quenched with H_2O (10 mL) and extracted with ethyl acetate (10 mL \times 3). The combined organic layers were washed with saturated NaHCO_3 solution (10 mL) and brine (10 mL) and dried over anhydrous Na_2SO_4 and the solvent removed under

reduced pressure. Purification by flash column chromatography on silica gel (9:1 *n*-hexane/ethyl acetate) gave the title compound. Spectral data for ((allyloxy)methyl)benzene (**9**) was consistent with data reported in the literature.⁴

Yield 41%; colorless oil; ¹H NMR (400 MHz, CDCl₃): δ 7.36 (d, *J* = 4.4 Hz, 4H), 7.34–7.27 (m, 1H), 6.02–5.92 (m, 1H), 5.33 (ddt, *J* = 17.2, 3.2, 1.6 Hz, 1H), 5.22 (ddt, *J* = 10.4, 2.8, 1.2 Hz, 1H), 4.53 (s, 2H), 4.04 (dt, *J* = 5.6, 1.2 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃): δ = 138.4, 136.5, 128.5, 127.9, 127.7, 117.3, 72.6, 71.3; LRMS (EI): *m/z* (%): 148.2 (8, [M⁺]), 107.0 (50), 91.0 (100).

2-((benzyloxy)methyl)oxirane (**10**)⁵

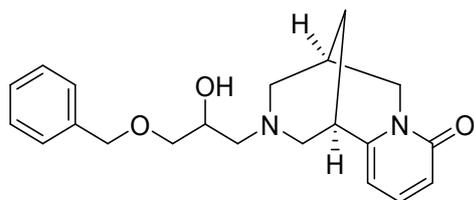


To a suspension of ((allyloxy)methyl)benzene **9** (184 mg, 1.10 mmol) in dry dichloromethane (10 mL) was added 50-60% *m*-CPBA (387 mg, 2.20 mmol) at room temperature under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 5 d. On completion, the reaction mixture was diluted with dichloromethane (5 mL) and washed with 3 M NaOH solution (10 mL × 3) then dried over anhydrous Na₂SO₄ before removing the solvent under reduced pressure. Purification by flash column chromatography on silica gel (9:1 *n*-hexane/ethyl acetate) gave the title compound. Spectral data for 2-((benzyloxy)methyl)oxirane (**10**) was consistent with data reported in the literature.⁶

Yield 77%; colourless oil; ¹H NMR (400 MHz, CDCl₃): δ 7.35 (d, *J* = 4.4 Hz, 4H), 7.33–7.27 (m, 1H), 4.59 (dd, *J* = 23.2, 12.0 Hz, 2H), 3.77 (dd, *J* = 11.6, 3.2 Hz, 1H), 3.45 (dd, *J* = 11.6, 6.0 Hz, 1H), 3.21–3.17 (m, 1H), 2.80 (dd, *J* = 5.2, 4.4 Hz, 1H), 2.62 (dd, *J* = 4.8, 2.8 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 138.0, 128.6, 127.9,

73.5, 71.0, 51.0, 44.5; LRMS (EI): m/z (%): 164.1 (32, [M⁺]), 107.0 (100), 105.0 (51), 91.0 (72).

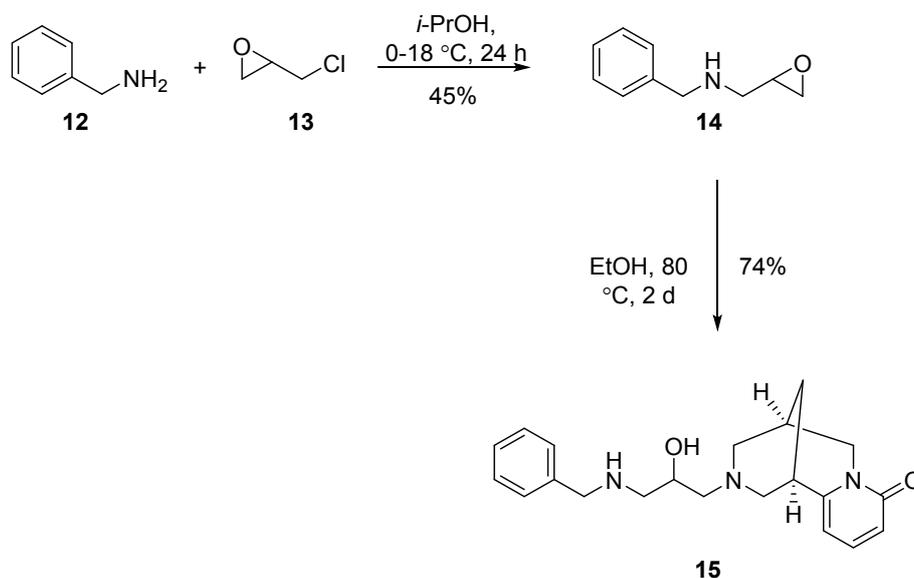
(1S,5R)-3-(3-(benzyloxy)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (11)



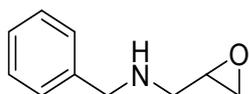
To a solution of 2-((benzyloxy)methyl)oxirane (**10**) (85.4 mg, 0.52 mmol) in ethanol (3 mL), (-)-cytisine (**5**) (94.2 mmol, 0.50 mmol) was added at room temperature under nitrogen atmosphere. The resulting mixture was stirred at reflux for 2 d. The reaction mixture was then allowed to cool to room temperature and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (100% methanol) gave the title compound.

Yield >99%; Yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.35–7.28 (m, 4H), 7.25–7.22 (m, 1H), 6.42 (dt, *J* = 9.2, 1.6 Hz, 1H), 5.97–5.94 (m, 1H), 4.49–4.41 (m, 2H), 4.07 (t, *J* = 1.6 Hz, 1H), 3.92–3.86 (m, 1H), 3.78–3.69 (m, 1H), 3.37–3.25 (m, 2H), 3.00–2.89 (m, 3H), 2.59–2.53 (m, 1H), 2.44–2.40 (m, 2H), 2.39–2.34 (m, 3H), 1.94–1.93 (m, 1H), 1.82–1.78 (m, 1H), 1.68 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 163.5, 150.9, 138.9, 138.8, 138.2, 128.5, 128.5, 127.9, 127.8, 117.1, 117.0, 104.7, 104.6, 73.6, 72.4, 72.3, 67.1, 66.7, 62.0, 61.6, 60.3, 60.0, 59.9, 50.2, 50.1, 35.8, 35.5, 28.4, 28.1, 26.0, 25.9; IR (NaCl, neat) ν: 3345, 2930, 2789, 1644, 1543, cm⁻¹; LRMS (ESI): m/z = 377 [M+Na]⁺ HRMS (ESI) calcd for C₂₁H₂₇N₂O₃ [M + H]⁺: 355.2022, found 355.2023.

General Procedure for the Synthesis of Compound 15



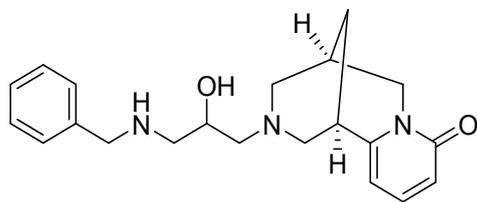
N-benzyl-1-(oxiran-2-yl)methanamine (14)⁷



To a solution of benzylamine (**12**) (1.00 g, 9.33 mmol) in isopropylalcohol (17 mL) was added dropwise (\pm)-epichlorohydrin (**13**) (0.86 g, 9.33 mmol) at room temperature under a nitrogen atmosphere. After stirring for 24 h, the solvent was removed under reduced pressure. Purification by flash column chromatography on silica gel (9:1 *n*-hexane/ethyl acetate) gave the title compound. Spectral data for N-benzyl-1-(oxiran-2-yl)methanamine (**14**) was consistent with data reported in the literature.⁸

Yield 45%; White solid, ¹H NMR (400 MHz, CDCl₃): δ 7.31–7.19 (m, 5H), 3.84–3.80 (m, 1H), 3.76 (d, *J* = 3.6 Hz, 2H), 3.51 (s, 1H), 3.50 (d, *J* = 0.8 Hz, 1H), 2.78 (dd, *J* = 12.4, 4.0 Hz, 1H), 2.67 (d, *J* = 12.4, 8.0 Hz, 1H), 2.35 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 139.8, 128.7, 128.2, 127.4, 69.7, 53.9, 51.7, 47.6; LRMS (EI): *m/z* (%): 163.2 (6, [M⁺]), 120.0 (100), 91.1 (44), 65.0 (14).

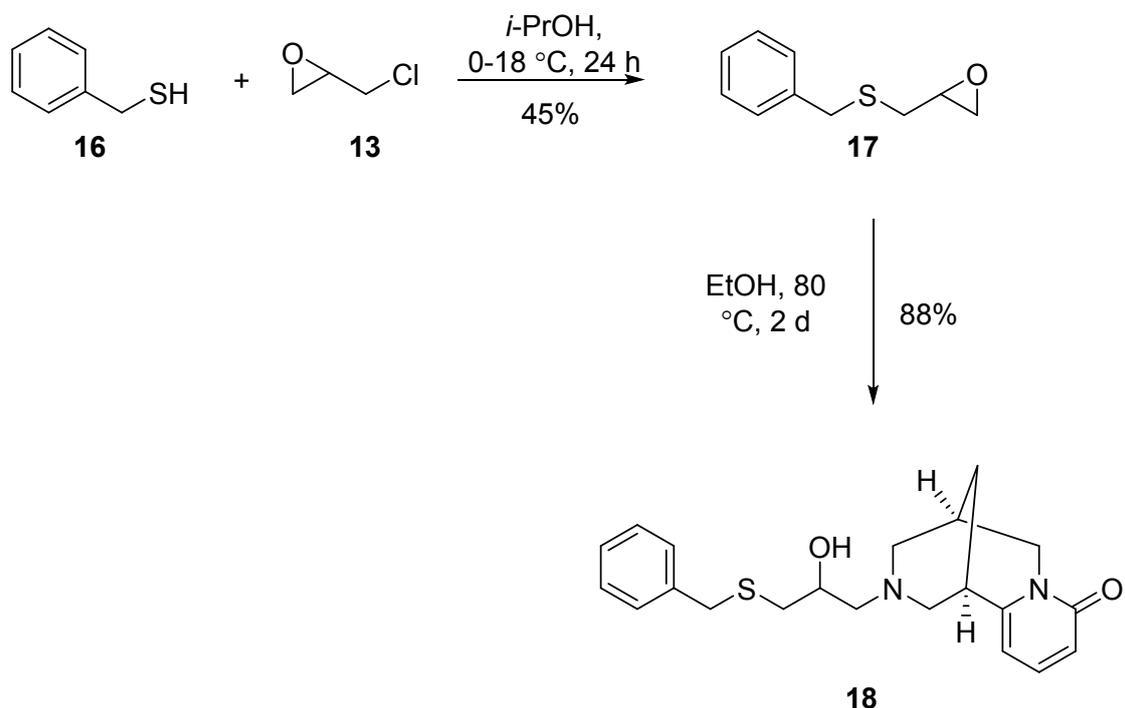
(1S,5R)-3-(3-(benzylamino)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (15)



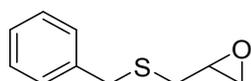
To a solution of *N*-benzyl-1-(oxiran-2-yl)methanamine (**14**) (111 mg, 0.64 mmol) in ethanol (3 mL), (-)-cytisine (**5**) (123 mg, 0.64 mmol) was added at room temperature under nitrogen atmosphere. The resulting mixture was stirred at reflux for 2 d. The reaction mixture was allowed to cool to room temperature and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (100% methanol) gave the title compound.

Yield >74%; Yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.31-7.19 (m, 6H), 6.40 (dd, *J* = 9.2, 1.6 Hz, 1H), 5.96 (dd, *J* = 6.7, 1.6 Hz, 1H), 4.06 (t, *J* = 16.0 Hz, 1H), 3.86 (dd, *J* = 15.6, 6.7 Hz, 1H), 3.74–3.61 (m, 3H), 3.00–2.87 (m, 3H), 2.65 (br s, 2H), 2.53–2.49 (m, 2H), 2.43–2.23 (m, 5H) 1.89 (d, *J* = 12.9 Hz, 1H), 1.78 (dd, *J* = 12.9, 2.8 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 163.4, 151.2, 151.0, 140.0, 139.9, 138.8, 138.8, 137.9, 129.0, 128.6, 128.4, 128.4, 128.2, 128.1, 127.1, 127.1, 127.0, 127.0 116.8, 116.7, 104.7, 104.7, 66.4, 66.2, 64.2, 63.7, 61.5, 61.1, 59.9, 59.8, 53.9, 53.8, 52.7, 52.6, 50.1, 50.0, 35.7, 35.4, 28.2, 28.0, 26.0 26.0; IR (NaCl, neat) ν: 3367, 3058, 2935, 2803, 1646, 1545, 1453, 1354, 1143, 1060, 801 cm⁻¹; LRMS (ESI): *m/z* = 354 [M+H]⁺ HRMS (ESI) calcd for C₂₁H₂₇N₃O₂Na [M + Na]⁺: 376.2001, found 376.1997.

General Procedure for the Synthesis of Compounds 18-21



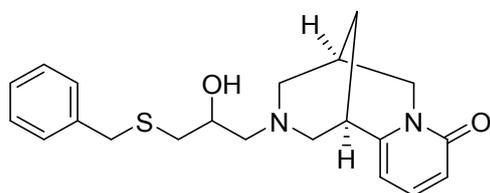
2-((benzylthio)methyl)oxirane (**17**)⁹



To a mixture of KOH (1.68 g, 30 mmol) and (±)-epichlorohydrin (**13**) (2.34 mL, 30 mmol) in water:1,4-dioxane (10 mL of 1:1 v/v mixture) was added benzyl mercaptan (**16**) (1.17 mL, 10 mmol) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 12 h. On completion, the reaction mixture was extracted with dichloromethane (10 mL × 3) and the combined organic layers were washed with water (10 mL × 2) then dried over anhydrous Na₂SO₄ before the solvent was removed under reduced pressure. Purification by flash column chromatography on silica gel (9:1 *n*-hexane/ethyl acetate) gave the title compound. Spectral data for 2-((benzylthio)methyl)oxirane (**17**) was consistent with data reported in the literature.⁹

Yield 99%; colorless oil; ^1H NMR (400 MHz, CDCl_3): δ 7.36–7.23 (m, 5H), 3.81 (d, $J = 2.4$ Hz, 2H), 3.11–3.06 (m, 1H), 2.76 (t, $J = 4.8$ Hz, 1H), 2.63 (dd, $J = 14.4, 5.6$ Hz, 1H), 2.56–2.51 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 138.1, 129.0, 128.6, 127.1, 51.8, 46.8, 36.5, 33.2$; LRMS (EI): m/z (%): 180.2 (46, $[\text{M}^+]$), 122.0 (100), 91.1 (86).

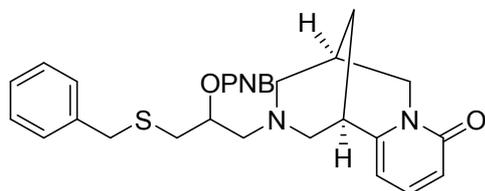
(1S,5R)-3-(3-(benzylthio)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (18)



To a solution of 2-((benzylthio)methyl)oxirane (**17**) (400 mg, 2.20 mmol) in ethanol (7 mL), (-)-cytisine (**5**) (402 mg, 2.10 mmol) was added at room temperature under nitrogen atmosphere. The resulting mixture was stirred at reflux for 2 days before the reaction mixture was allowed to cool to room temperature and the solvent was removed under reduced pressure. Purification by flash column chromatography on silica gel (100% methanol) gave the title compound.

Yield 88%; Yellow oil; ^1H NMR (400 MHz, CDCl_3): $\delta = 7.31\text{--}7.21$ (m, 5H), 6.40 (dt, $J = 9.2, 1.6$ Hz, 1H), 4.06 (t, $J = 16$ Hz, 1H), 3.85 (dd, $J = 15.6, 6.4$ Hz, 1H), 3.73–3.61 (m, 3H), 3.00–2.84 (m, 3H), 2.65 (br s, 2H), 2.53–2.49 (m, 2H), 2.43–2.23 (m, 5H), 1.90–1.76 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 163.5, 163.5, 151.0, 150.7, 138.9, 138.8, 138.5, 129.1, 129.1, 128.6, 128.6, 127.2, 127.1, 117.1, 117.1, 104.8, 104.7, 66.8, 66.5, 62.6, 62.3, 62.2, 61.7, 59.7, 59.6, 50.1, 50.0, 37.3, 37.2, 36.2, 36.0, 35.8, 35.4, 28.4, 28.0, 26.0, 25.9$; IR (NaCl, neat) ν : 3359, 2937, 2792, 2359, 1647, 1545, 1423, 1353, 1141, 800 cm^{-1} ; LRMS (ESI): $m/z = 393$ $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_2\text{SNa}$ $[\text{M} + \text{Na}]^+$: 393.1613, found 393.1604.

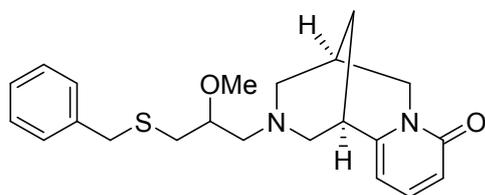
1-(benzylthio)-3-((1R,5R)-8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a][1,5]diazocin-3(4H)-yl)propan-2-yl 4-nitrobenzoate (19)



To a solution of (1S,5R)-3-(3-(benzylthio)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (**18**) (110 mg, 0.30 mmol) in dichloromethane (5 mL), 4-nitrobenzyl chloride (82.6 mg, 0.45 mmol), TEA (0.17 mL, 1.19 mmol) and DMAP (7.3 mg, 0.059 mmol) were added sequentially at room temperature under a nitrogen atmosphere. The reaction was then heated to 40 °C and left to stir for six hours. Upon completion, the reaction mixture was allowed to cool to room temperature and quenched with NH₄Cl (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous Na₂SO₄ and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (100% methanol) gave the title compound.

Yield 88%; Yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 8.03 (dd, *J* = 18.8, 8.8 Hz, 2H), 7.29–7.19 (m, 5H), 7.55 (ddd, *J* = 62.0, 8.8, 2.8 Hz, 1H), 6.14 (ddd, *J* = 50, 8.8, 1.2 Hz, 1H), 5.87 (ddd, *J* = 40.0, 6.8, 1.2 Hz, 1H), 5.27–5.16 (m, 1H), 3.97 (dd, *J* = 27.6, 15.6 Hz, 1H), 3.84–3.78 (m, 1H), 3.64 (d, *J* = 3.6 Hz, 2H), 3.07–2.83 (m, 3H), 2.66–2.47 (m, 5H), 2.40–2.33 (m, 2H), 1.87–1.75 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ = 163.9, 163.9, 163.3, 163.2, 151.2, 151.0, 150.7, 150.6, 138.5, 138.2, 138.0, 137.6, 135.3, 135.2, 131.0, 128.4, 127.3, 127.2, 123.8, 123.6, 123.5, 116.6, 116.5, 104.7, 104.3, 71.1, 71.0, 61.9, 61.4, 60.5, 60.1, 60.0, 59.5, 50.0, 49.7, 37.1, 37.0, 35.5, 33.5, 33.4, 28.2, 28.1, 25.8; IR (NaCl, neat) ν: 3028, 2937, 2788, 2621, 1720, 1648, 1545, 1523, 1343, 1269, 716 cm⁻¹; HRMS (ESI) calcd for C₂₈H₂₉N₃O₅SNa [M + Na]⁺: 542.1726, found 542.1727.

1-(benzylthio)-3-((1R,5R)-8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a][1,5]diazocin-3(4H)-yl)propan-2-yl-methyl ether (20)

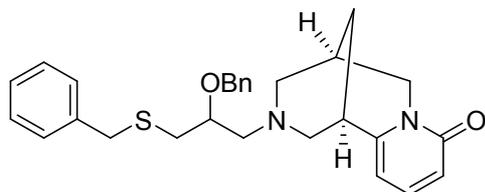


A solution of (1S,5R)-3-(3-(benzylthio)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (**18**) (31 mg, 0.08 mmol) in THF (1 mL) was added dropwise to a suspension of sodium hydride (7.7 mg, 0.32 mmol) in dry THF (0.5 mL) at 0 °C under nitrogen atmosphere. Methyl iodide (34 mg, 0.24 mmol) was then added dropwise and the solution allowed to warm to room temperature. The reaction mixture was allowed to stir for four hours before being quenched with NH₄Cl (4 mL) and extracted with dichloromethane (10 mL × 3). The combined organic layers were dried over anhydrous MgSO₄ and the solvent removed under reduced pressure to give the crude target material as a yellow oil. Purification by flash column chromatography on silica gel (100% ethyl acetate) gave the title compound.

Yield 79%; Yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.34–7.03 (m, 6H), 6.41 (dd, *J* = 9.0, 1.0 Hz, 1H), 5.95 (dt, *J* = 6.9, 1.7 Hz, 1H), 4.06 (d, *J* = 15.3 Hz, 1H), 3.86 (dd, *J* = 15.3, 6.5 Hz, 1H), 3.59 (d, *J* = 4.0 Hz, 2H), 3.18–3.08 (m, 1H), 3.13 (s, 3H), 2.99–2.83 (m, 3H), 2.48–2.22 (m, 7H), 1.87 (d, *J* = 12.8 Hz, 1H), 1.75 (d, 12.8 Hz 1 H); ¹³C NMR (100 MHz, CDCl₃): δ = ¹³C NMR (101 MHz, CDCl₃) δ 163.6, 151.6, 138.7, 129.1, 129.0, 128.5, 128.5, 127.0, 116.7, 104.6, 104.5, 79.3, 78.9, 61.5, 61.4, 61.2, 60.2, 60.0, 57.7, 57.5, 50.2, 37.5, 37.4, 35.8, 35.8, 33.9, 33.8, 28.4, 25.9; IR (NaCl, neat) ν: 2940, 2884, 1952, 1551, 914 cm⁻¹; LRMS (ESI): *m/z* = 407 [M+Na]⁺; HRMS (ESI) calcd for C₂₂H₂₈N₂O₂SNa [M + Na]⁺: 407.1769, found 407.1764.

1-(benzylthio)-3-((1R,5R)-8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-

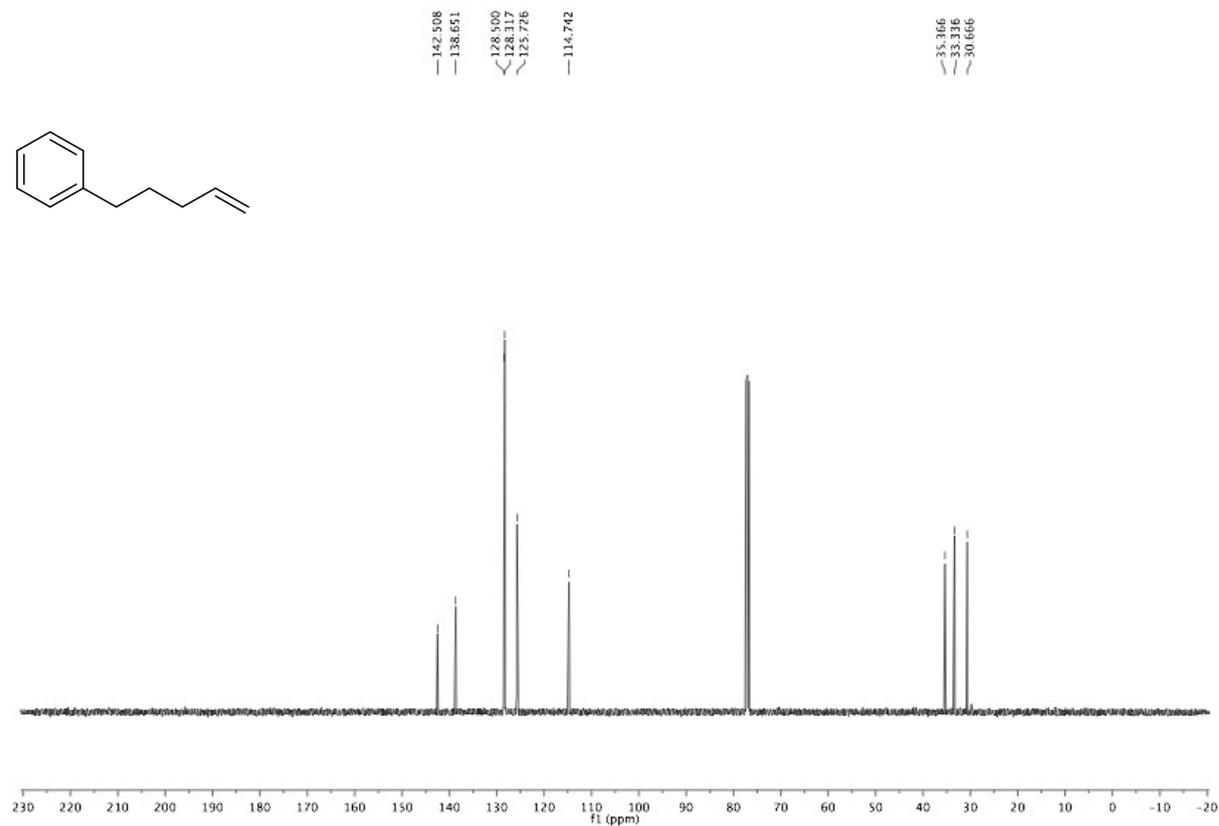
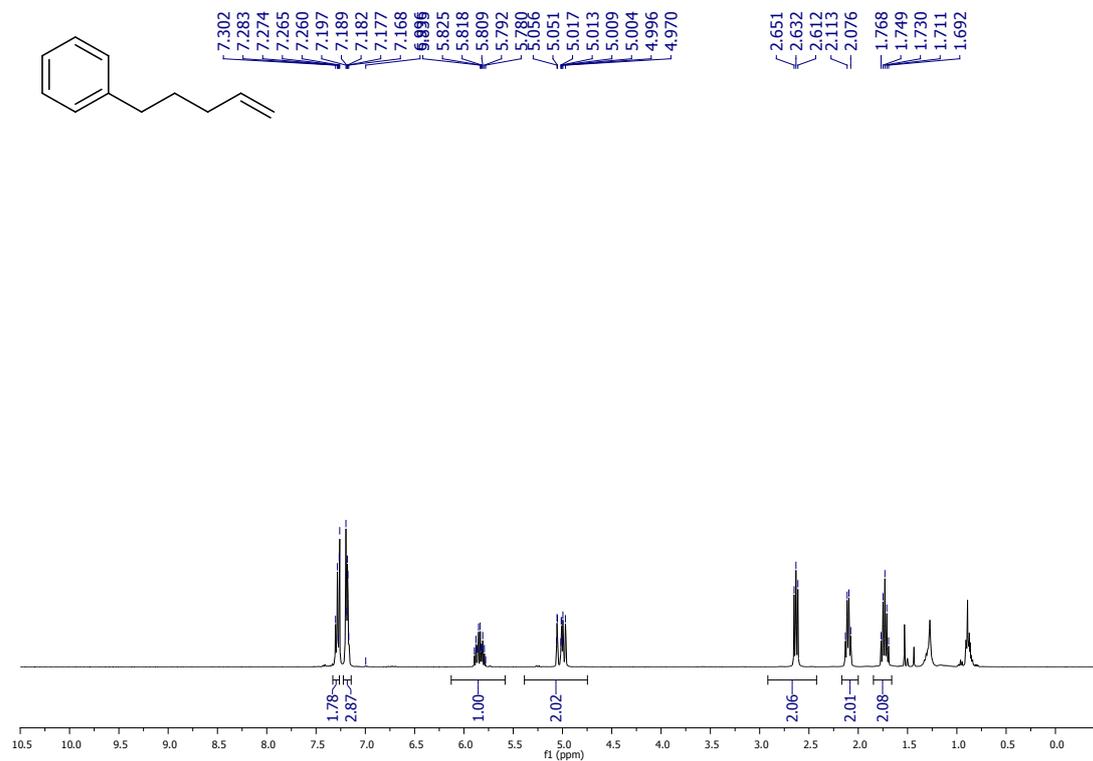
a][1,5]diazocin-3(4H)-yl)propan-2-yl-benzyl ether (21)

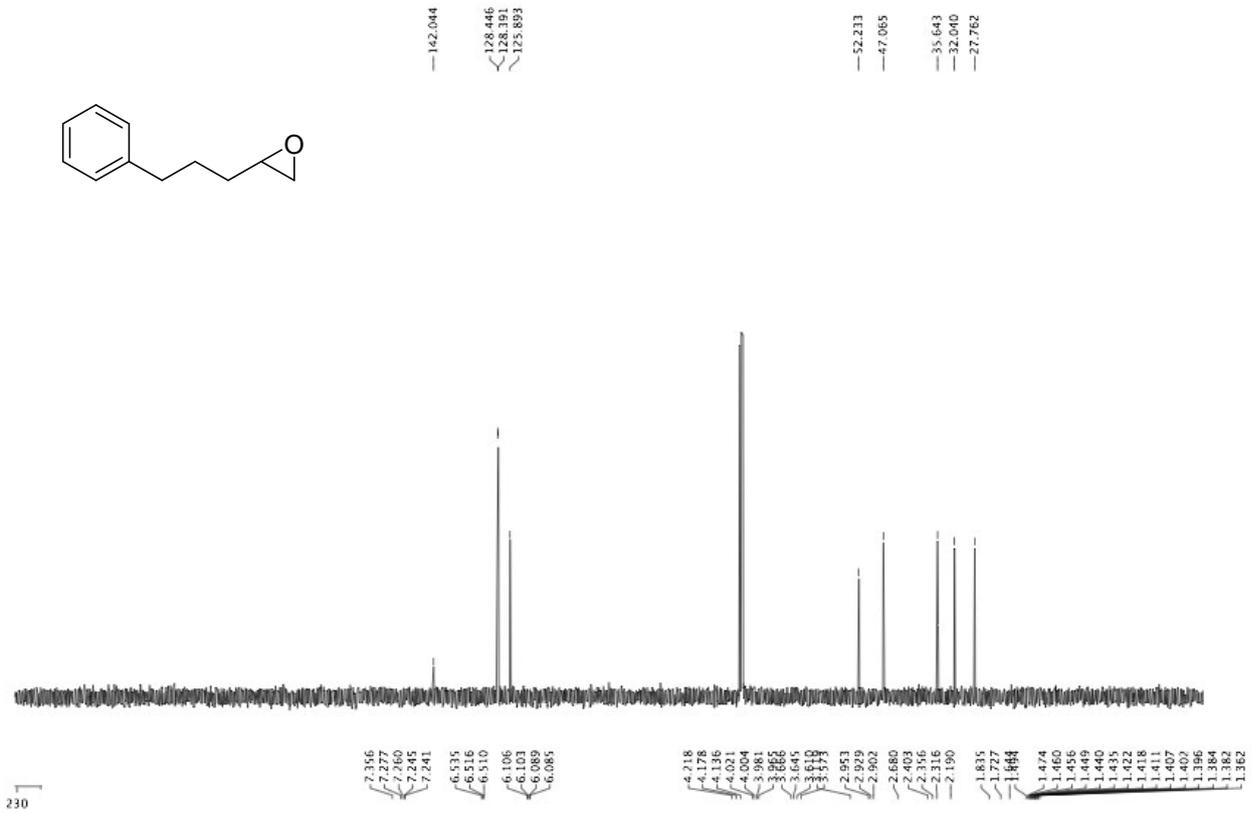
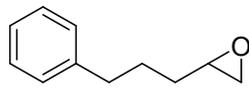
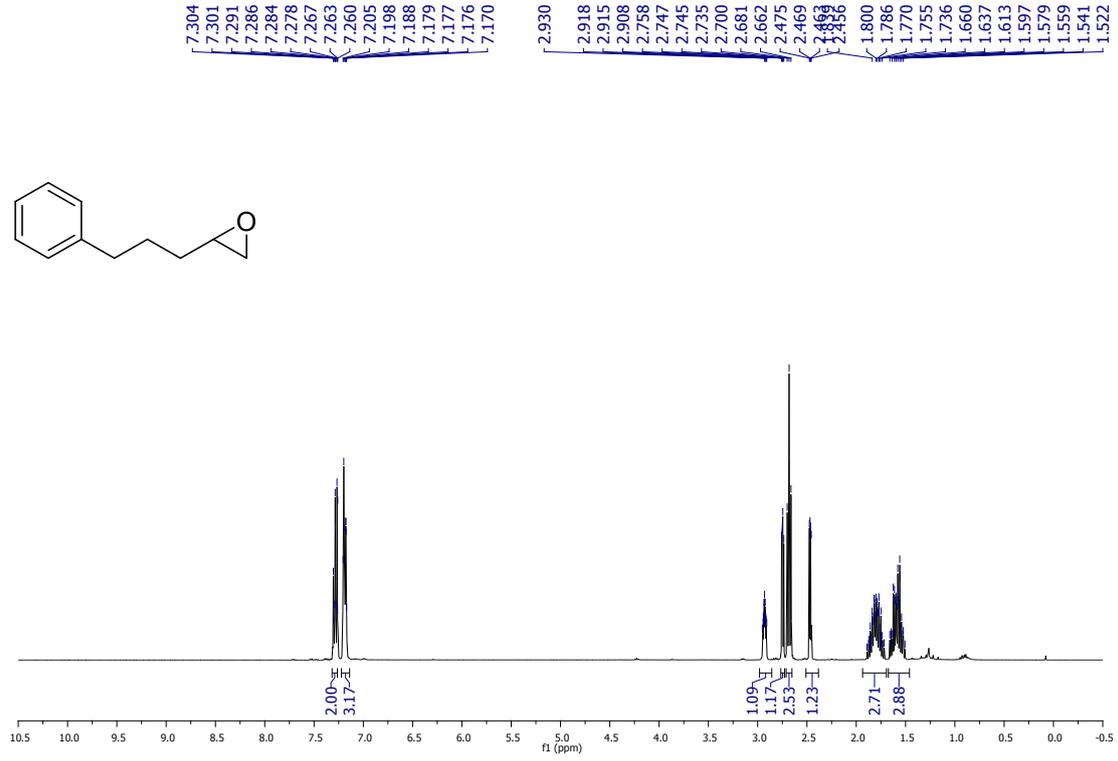
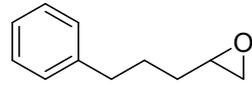


A solution of (1*S*,5*R*)-3-(3-(benzylthio)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8-one (**18**) (31 mg, 0.08 mmol) in THF (1 mL) was added to a suspension of sodium hydride (7.7 mg, 0.32 mmol) in dry THF (0.5 mL) at 0 °C under nitrogen atmosphere. Benzyl bromide (41 mg, 0.24 mmol) was then added dropwise and the solution allowed to warm to room temperature. The reaction mixture was allowed to stir for four hours before being quenched with NH₄Cl (4 mL) and extracted with dichloromethane (10 mL × 3). The combined organic layers were dried over anhydrous MgSO₄ and the solvent removed under reduced pressure to give the crude target material as a yellow oil. Purification by flash column chromatography on silica gel (100% ethyl acetate) gave the title compound.

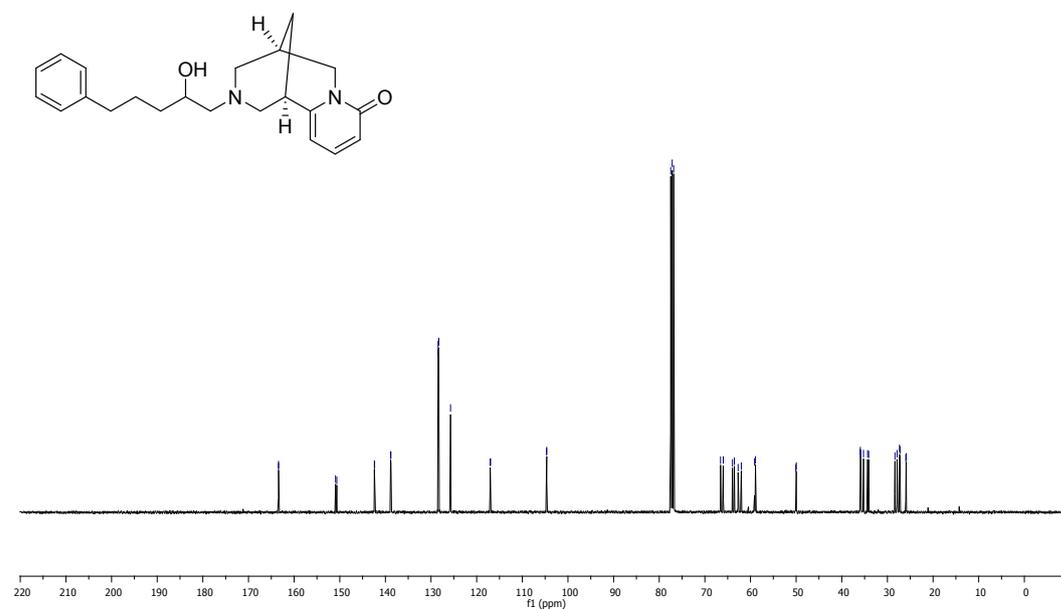
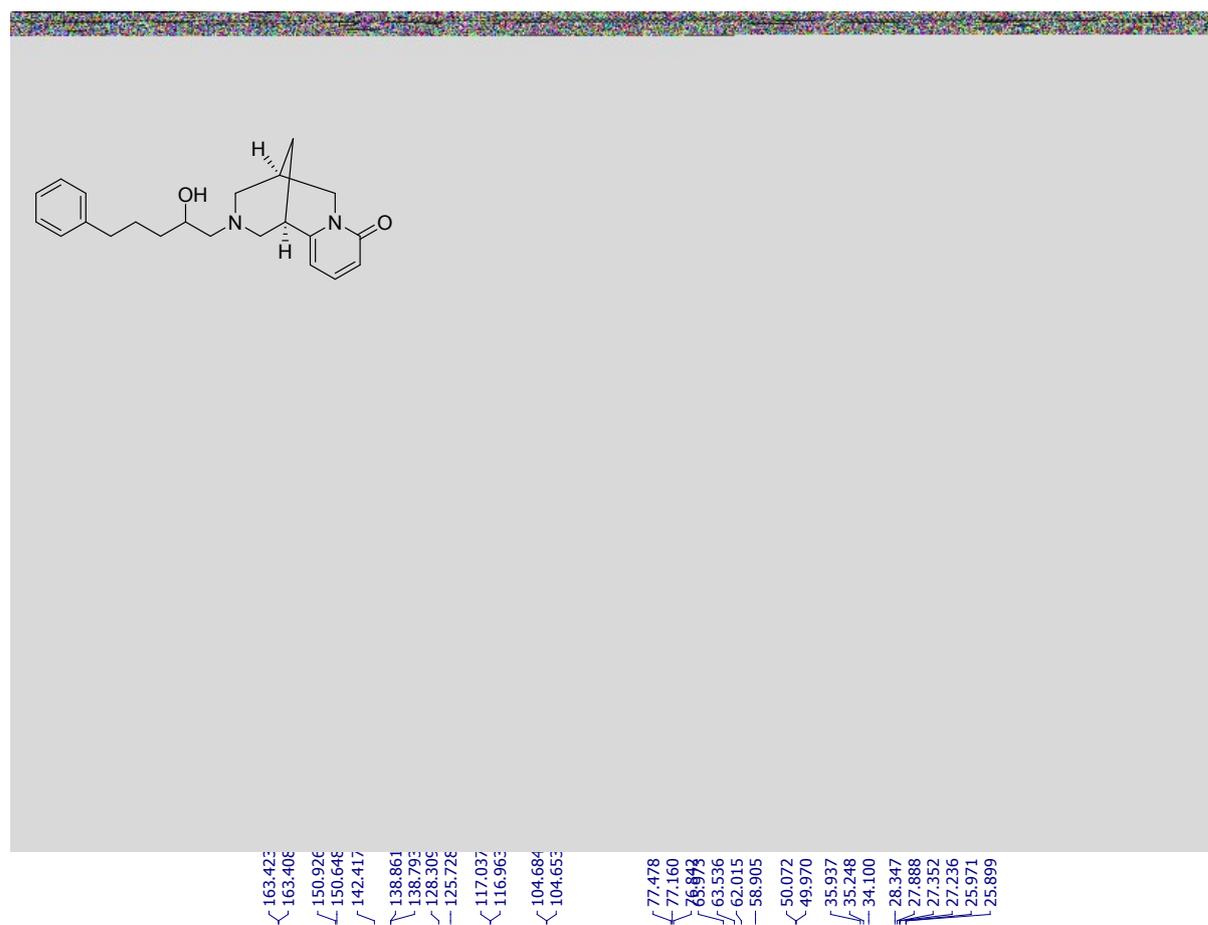
Yield 78%; Yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.35–7.12 (m, 10H), 6.39 (ddd, *J* = 9.0, 3.2, 1.4 Hz, 1H), 5.94 (ddd, *J* = 18.4, 6.9, 1.4 Hz, 1H), 4.37–4.21 (m, 2H), 4.07 (dd, *J* = 15.2, 7.6 Hz, 1H), 3.89 (dd, *J* = 15.2, 7.6 Hz, 1H), 3.58 (s, 2H), 3.47–3.33 (m, 1H), 2.98 (t, *J* = 10.2 Hz, 1H) 2.94–2.85 (m, 2H), 2.52–2.30 (m, 7H), 1.87 (d, *J* = 12.9 Hz, 1H), 1.75 (d, *J* = 12.9 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): ¹³C NMR (101 MHz, CDCl₃) δ = 163.4, 151.4, 151.4, 138.6, 138.5, 138.4, 128.9, 128.4, 128.4, 128.2, 128.2, 127.6, 127.5, 127.5, 127.4, 126.9, 126.8, 116.6, 116.6, 104.4, 104.4, 71.9, 71.8, 61.5, 61.3, 61.1, 61.0, 60.8, 60.5, 50.0, 50.0, 37.4, 37.3, 35.7, 35.6, 34.2, 28.2, 25.8; IR (NaCl, neat) ν: 2929, 1655, 1551, 1145, 1100, 1033, 1071 cm⁻¹; LRMS (ESI): *m/z* = 483 [M+Na]⁺; HRMS (ESI) calcd for C₂₈H₃₂N₂O₂SNa [M + Na]⁺: 483.2082, found 483.2048.

pent-4-en-1-ylbenzene (3)

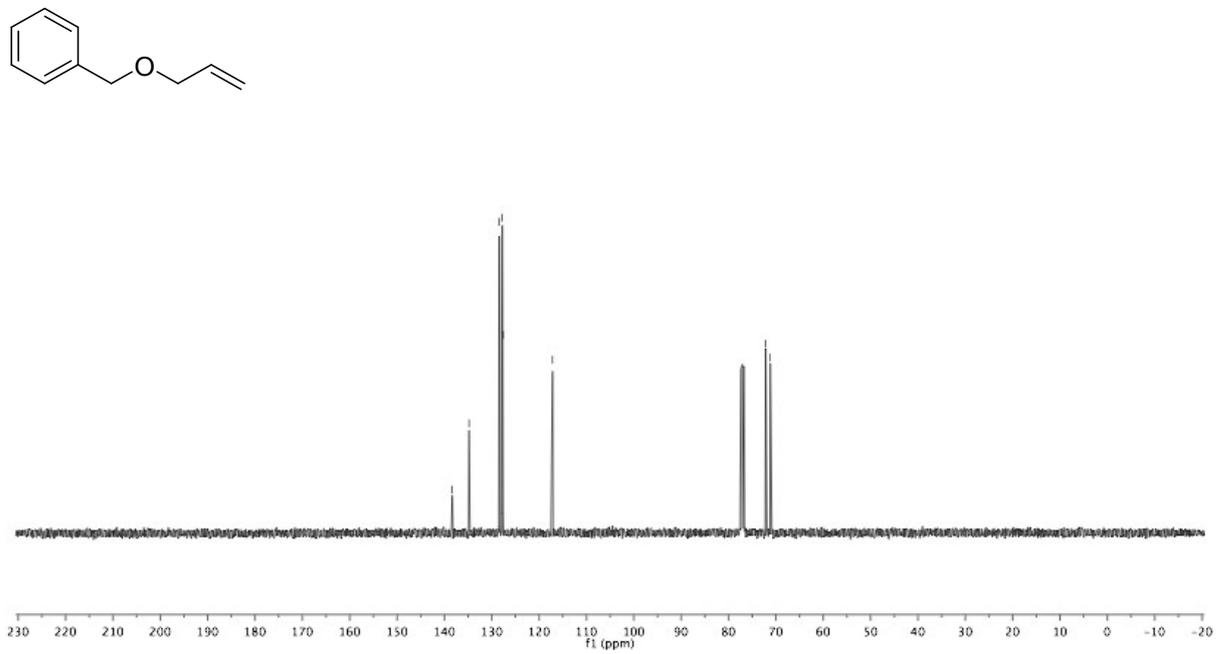
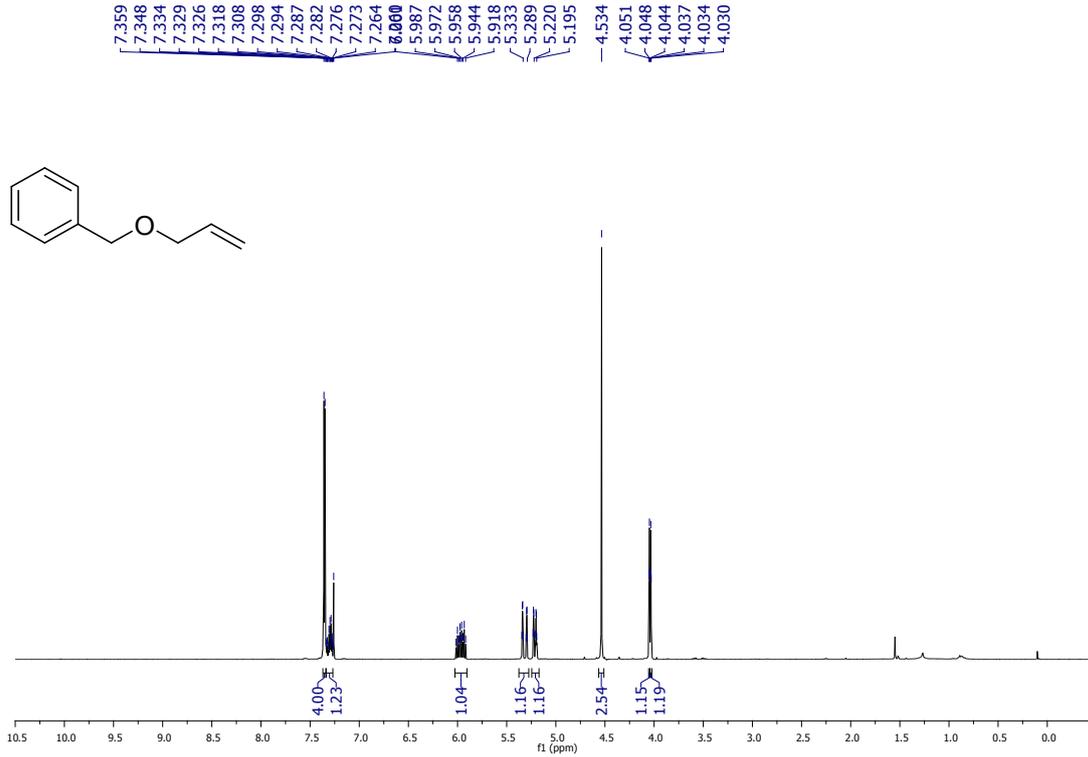


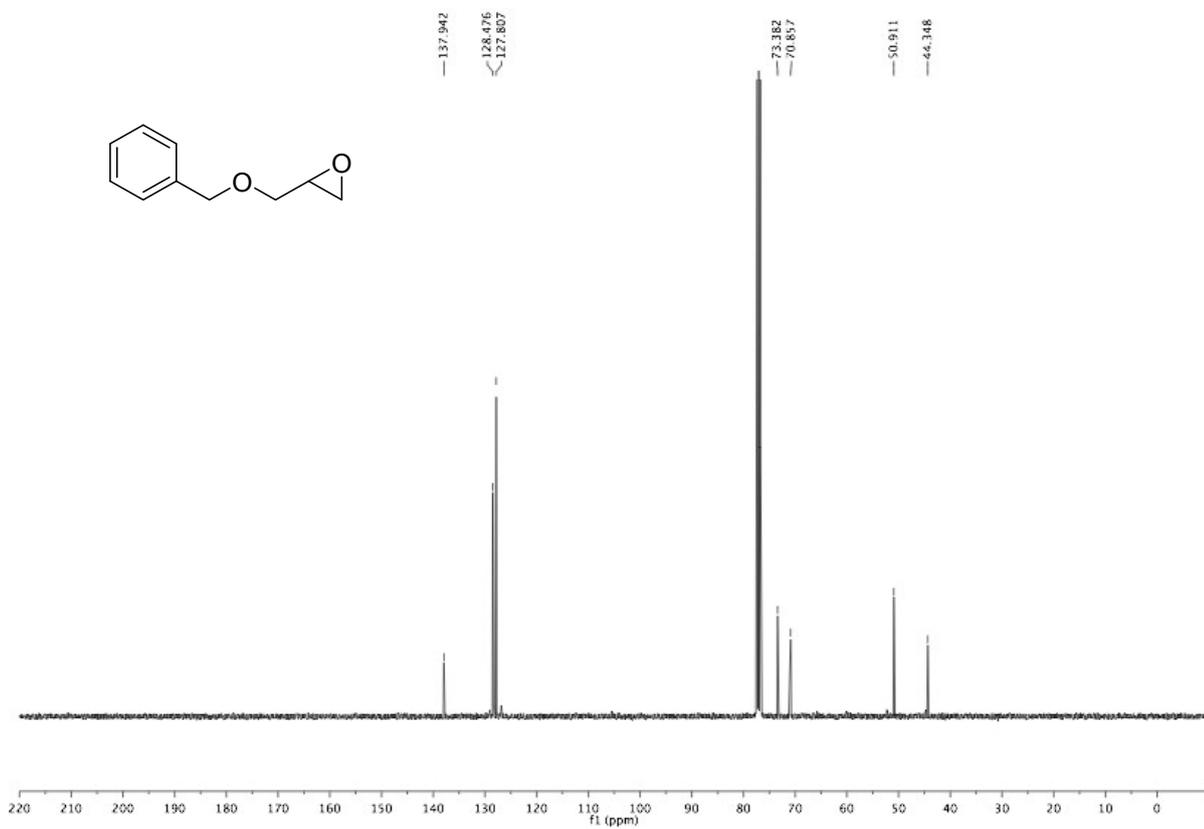
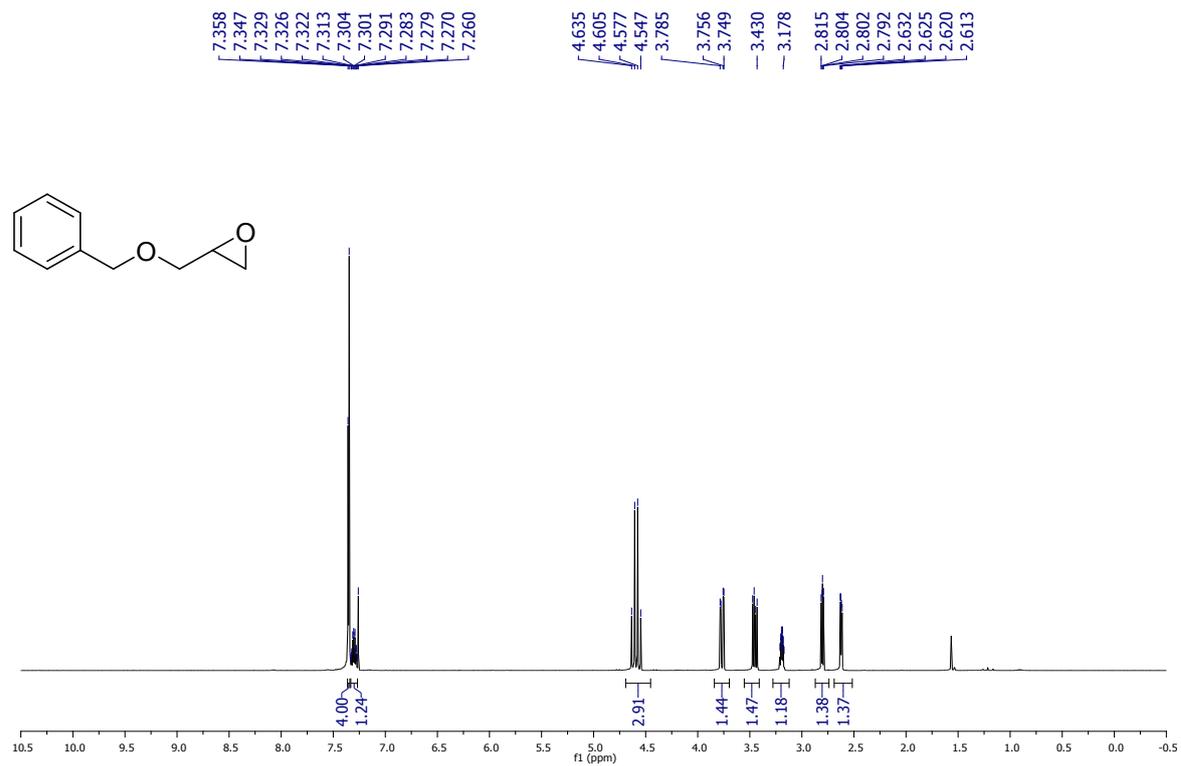


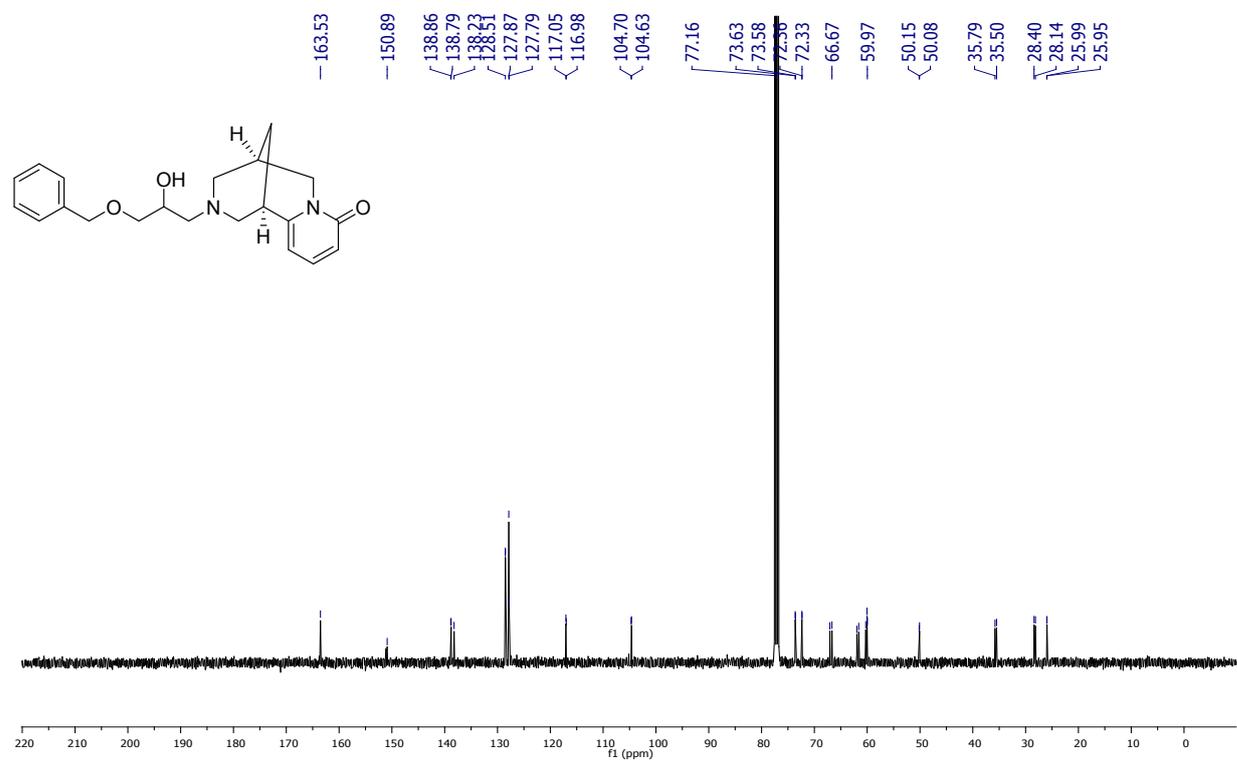
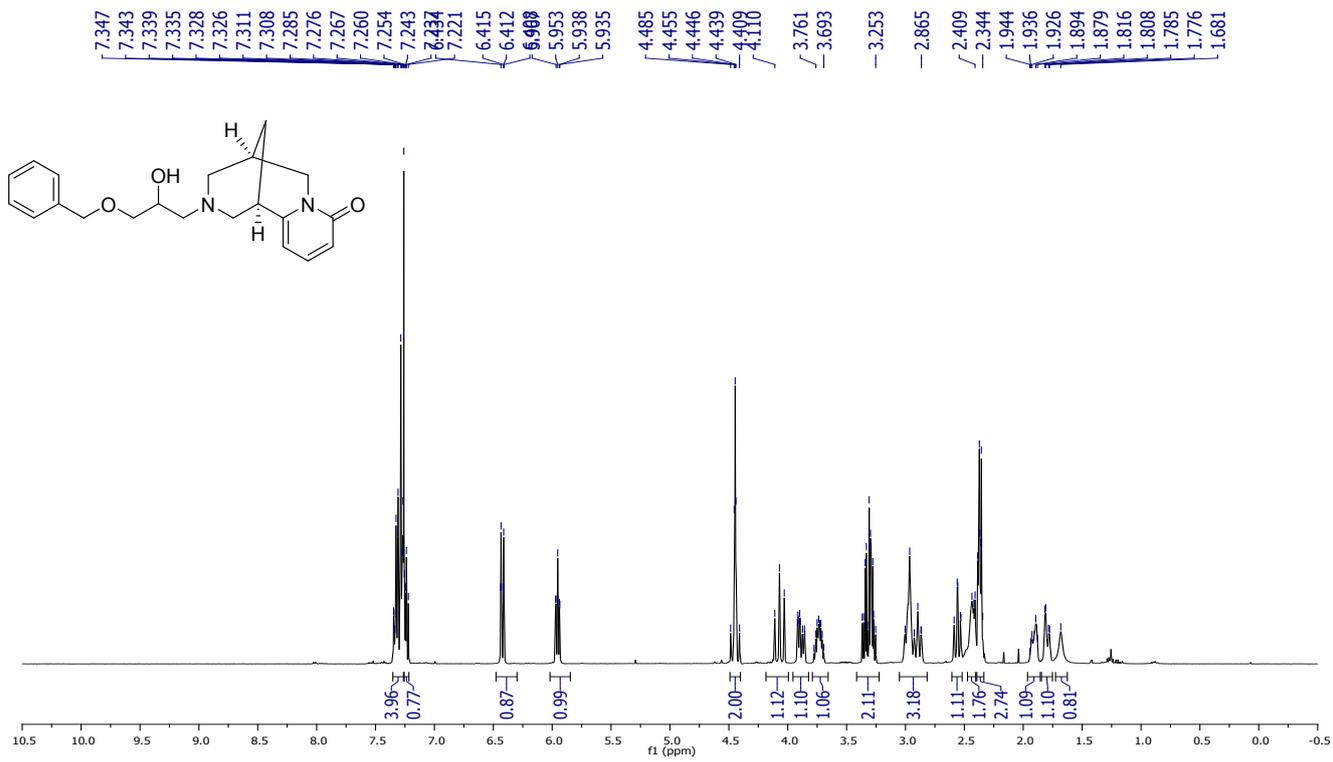
(1S,5R)-3-(2-hydroxy-5-phenylpentyl)-1,2,3,4,5,6-hexahydro-8H-1,5-



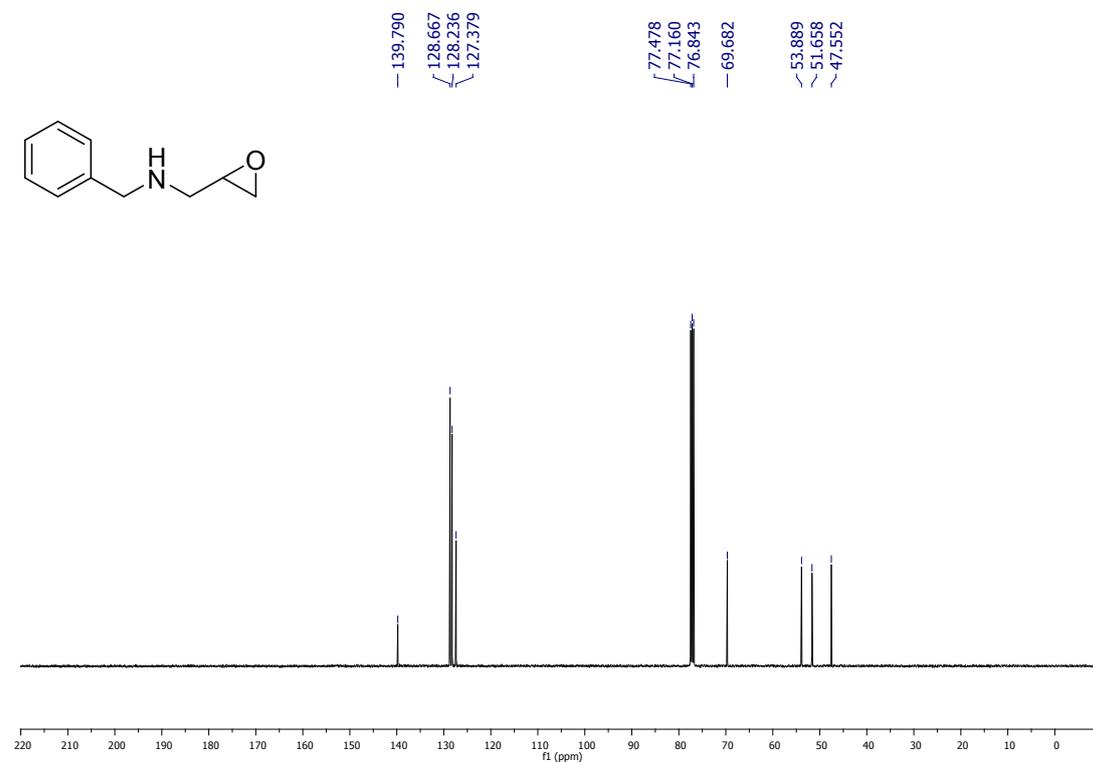
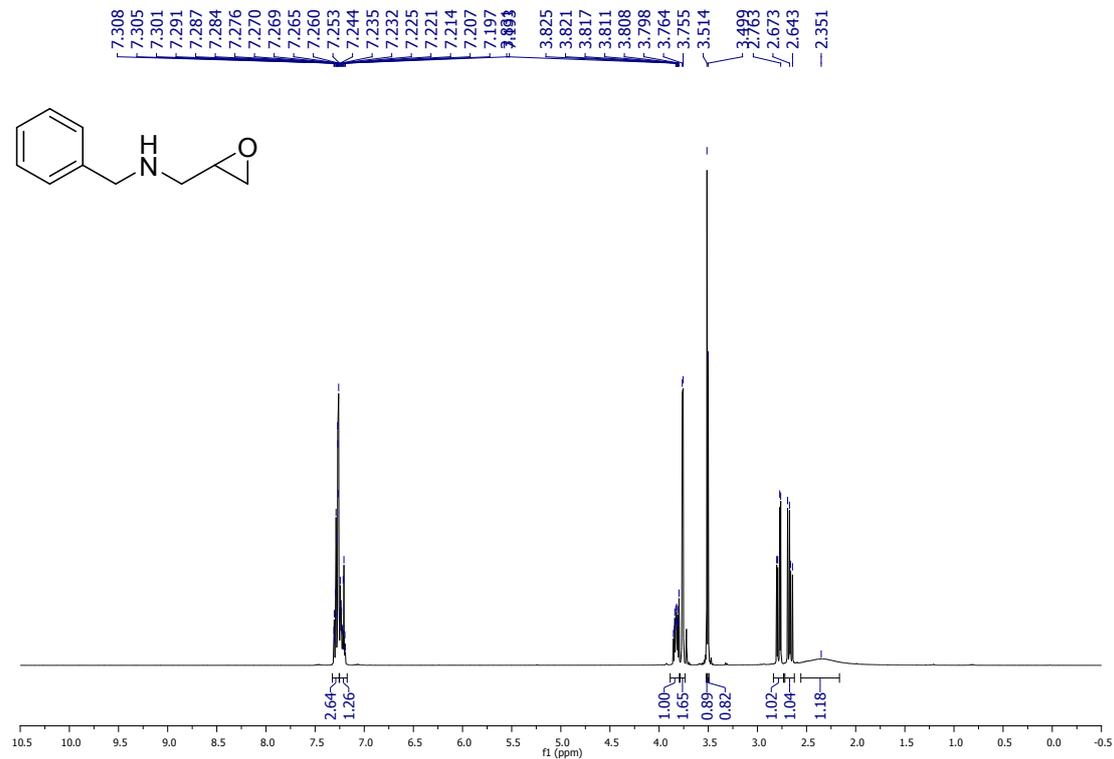
((allyloxy)methyl)benzene (9)



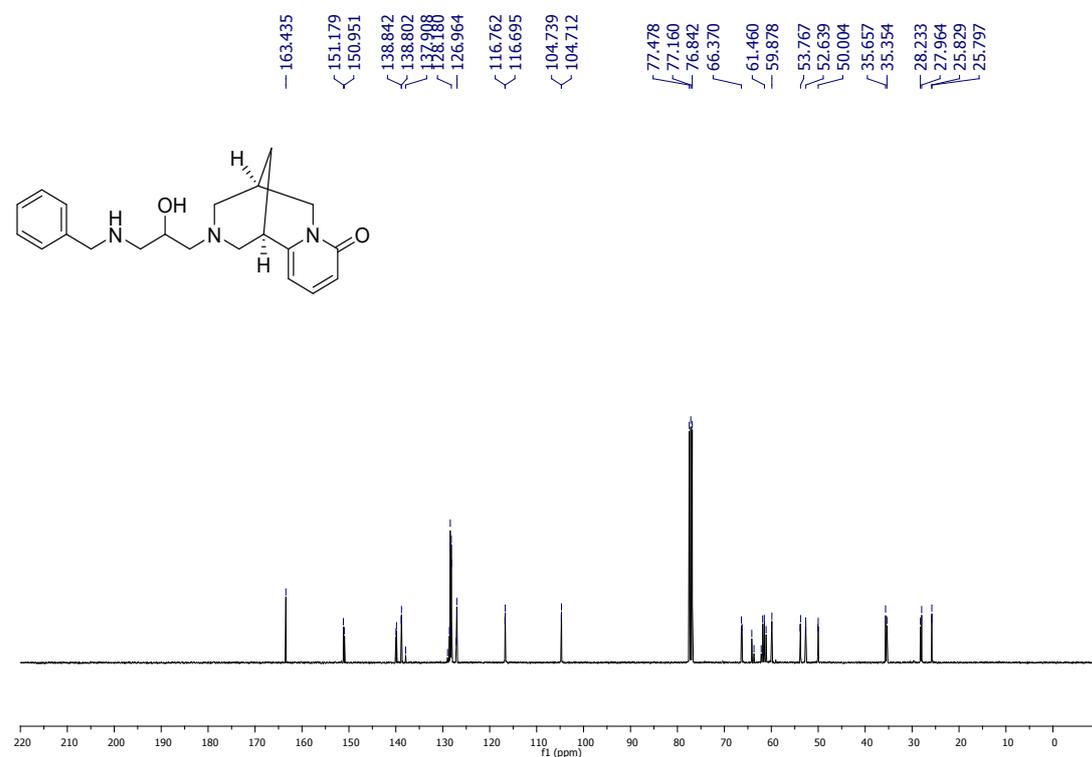
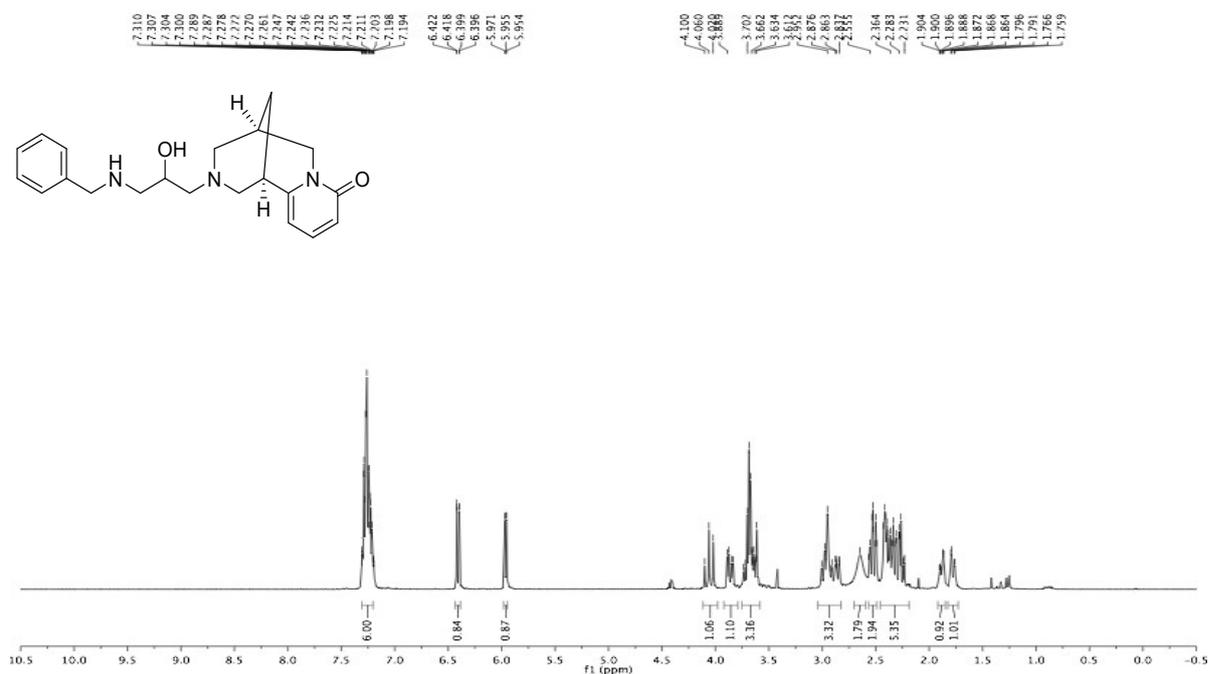




N-benzyl-1-(oxiran-2-yl)methanamine (14)

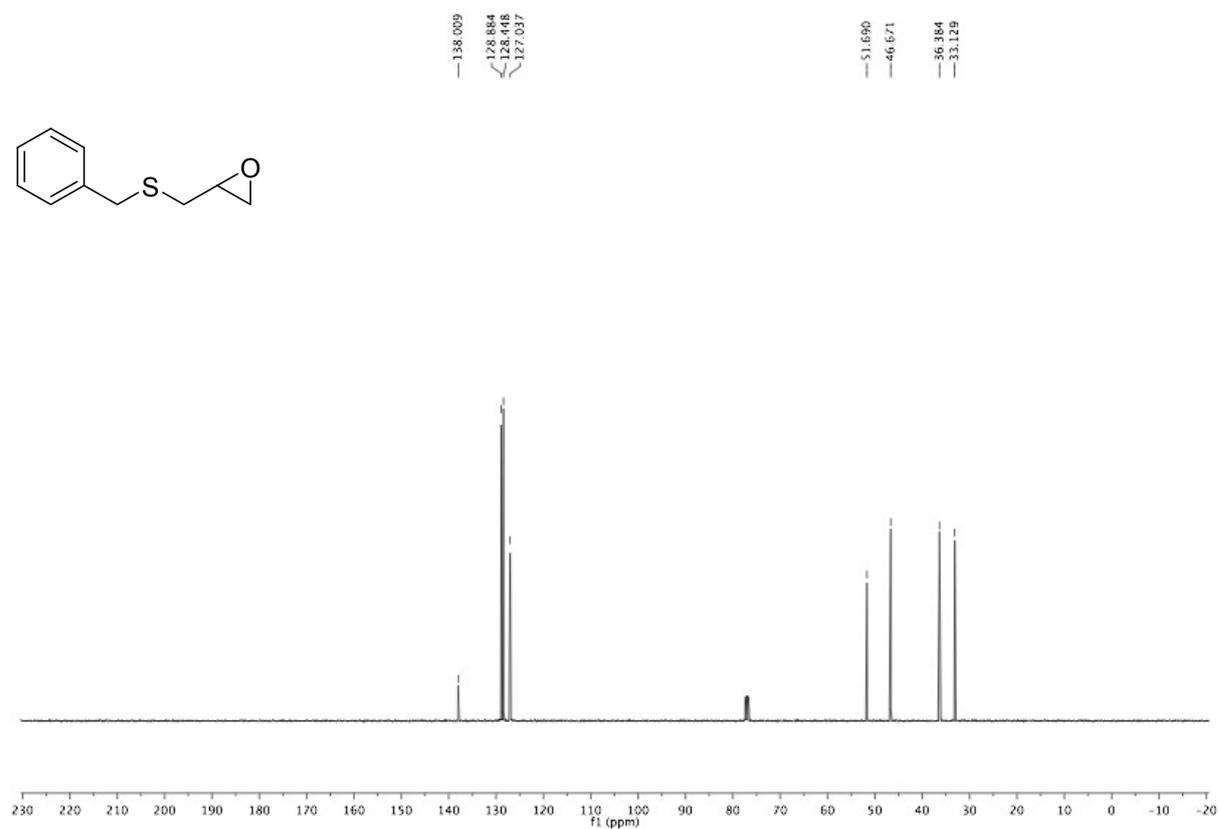
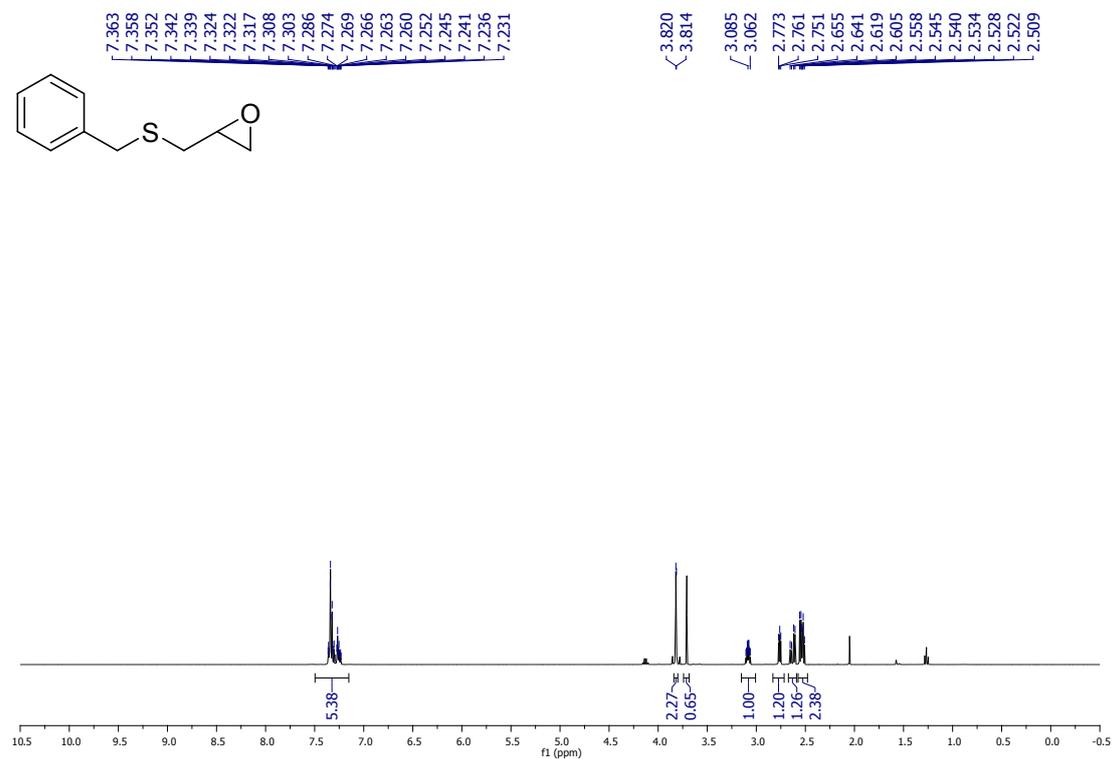


(1S,5R)-3-(3-(benzylamino)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (15)

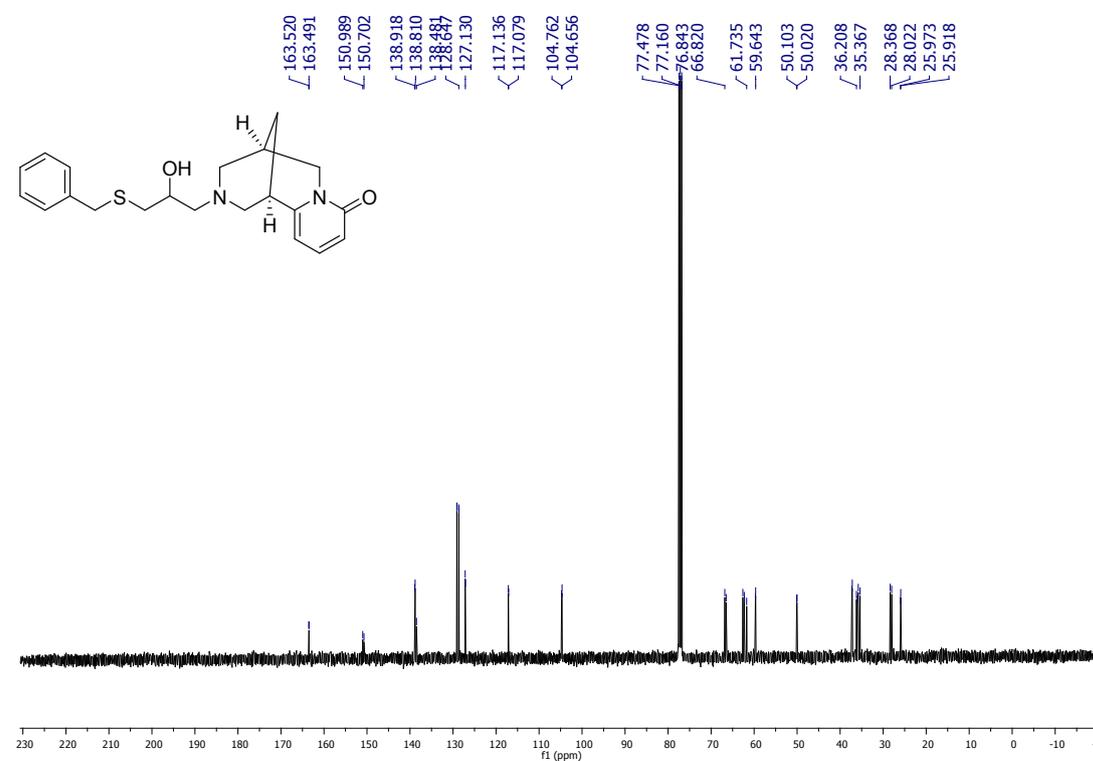
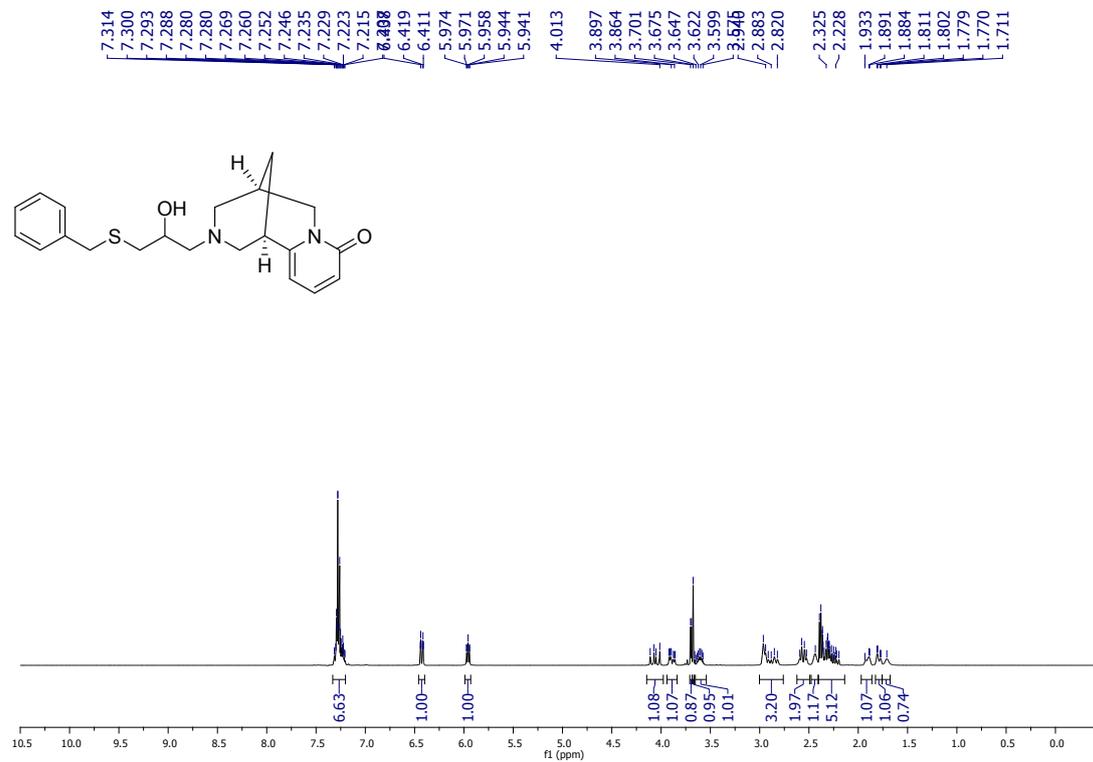


2-((benzylthio)methyl)oxirane

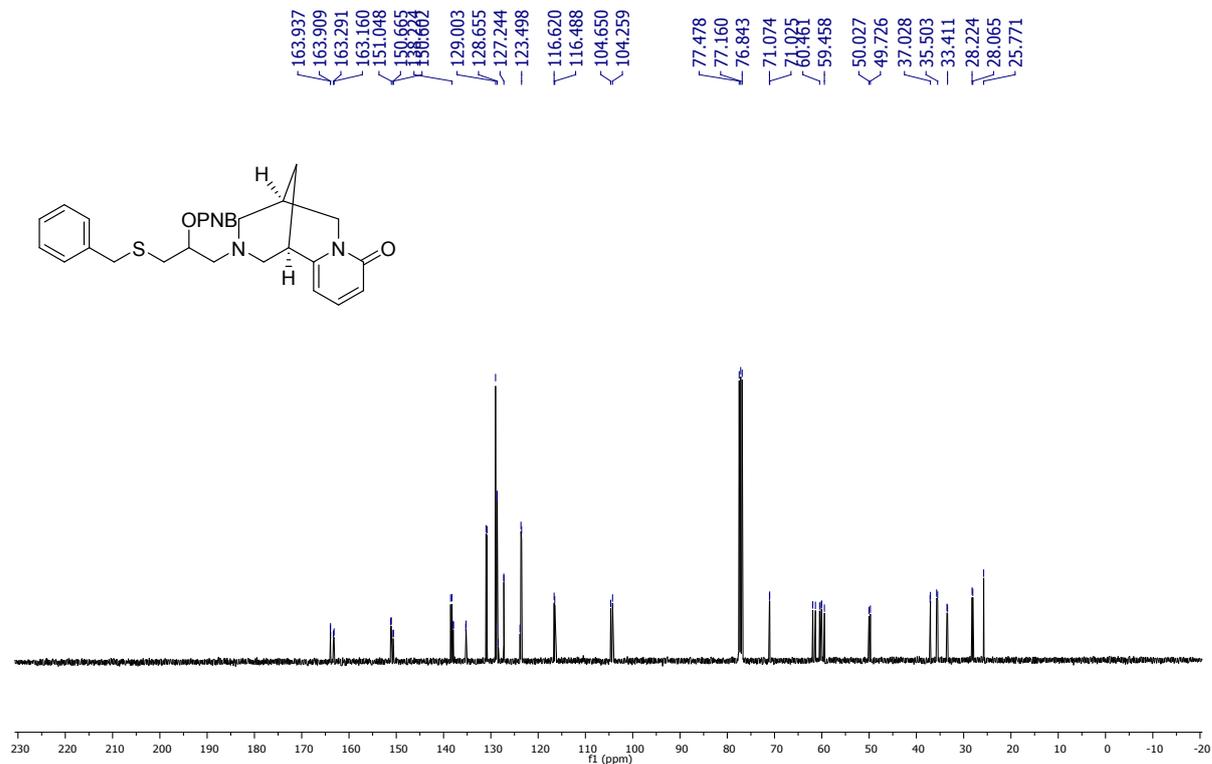
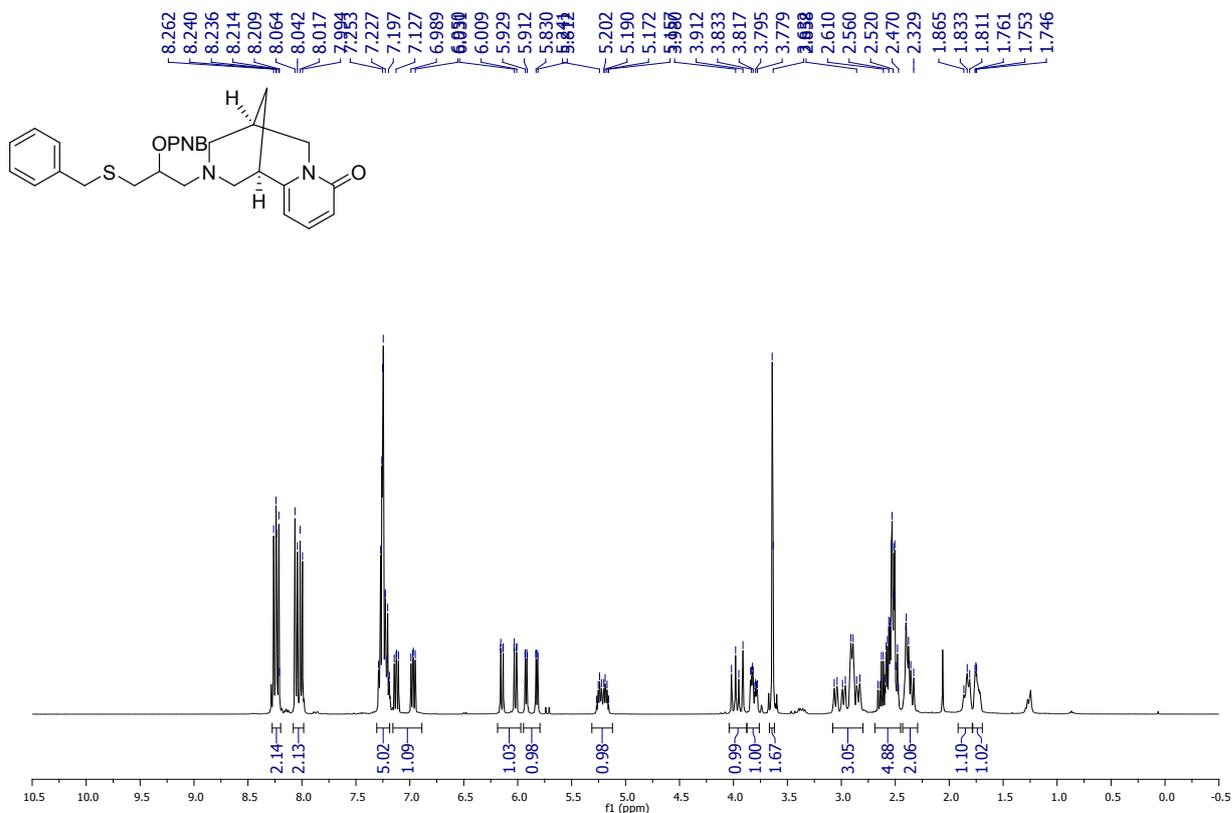
(17)



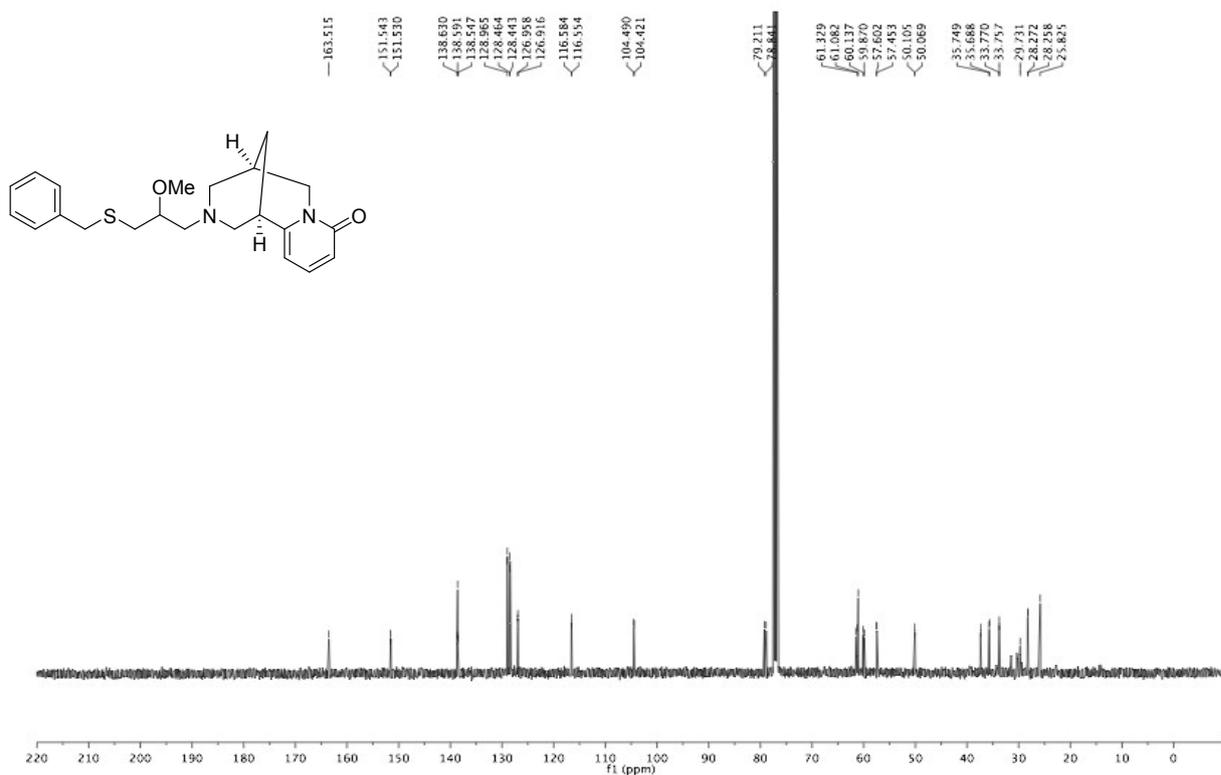
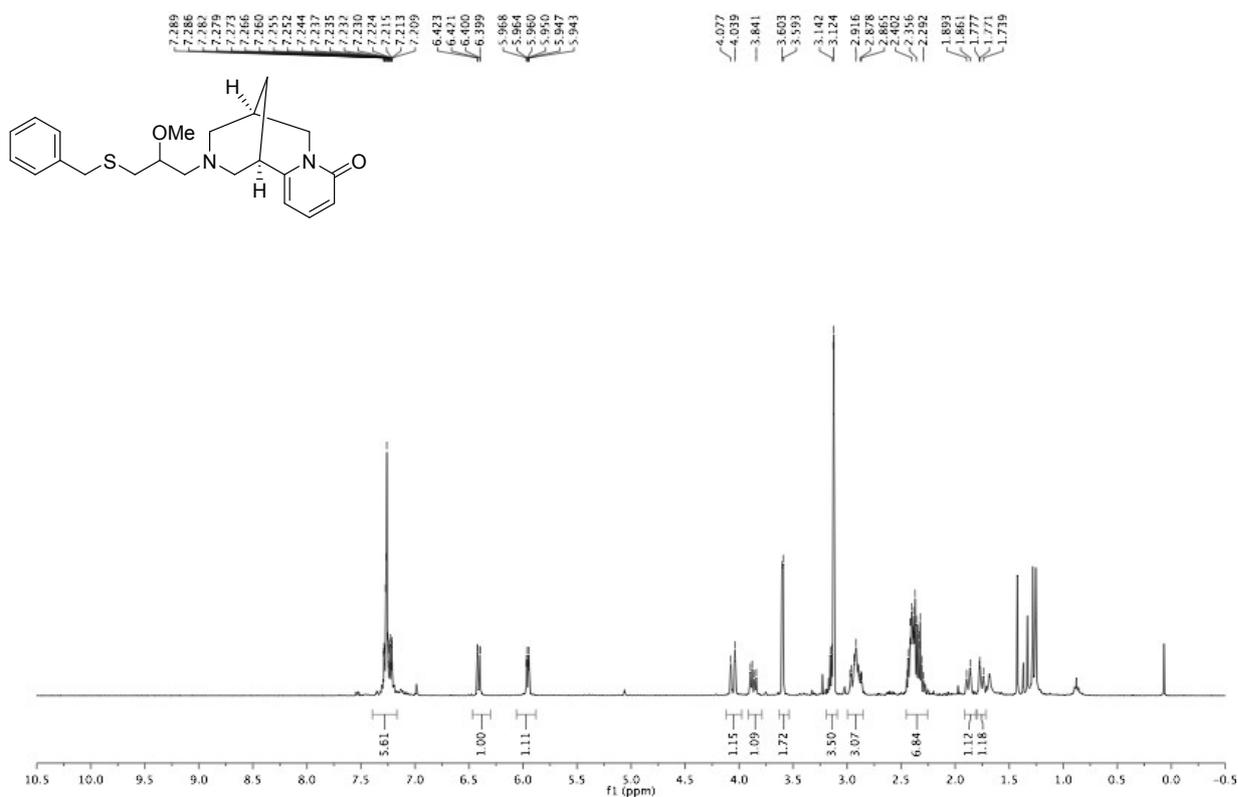
(1S,5R)-3-(3-(benzylthio)-2-hydroxypropyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (18)



(1S,5R)-3-(2-(benzyloxy)-3-(benzylthio)propyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (19)



1-(benzylthio)-3-((1R,5R)-8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a][1,5]diazocin-3(4H)-yl)propan-2-yl-methyl ether (20)



Material and cell lines. All the chemicals, unless specified, were purchased from Sigma-Aldrich and were used as received. Menin and MLL rabbit polyclonal antibodies were obtained from Boston BioChem (Cambridge, MA, USA). HepG2 cells were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Minimum Essential Media containing 10% fetal bovine serum and were incubated at 37 °C/5% CO₂. Deuterated solvents for NMR purposes were obtained from Armar and used as received

Plasmid construction

The Venus and Dronpa cDNA constructs were provided by A. Miyawaki (Riken, Wako, Japan).¹⁰ The plasmids encoding the His6-tagged BiFC probes, MLL-VC210 (the C-terminal fragment 210–238 of Venus fused to the C-terminus of MLL) and VN210-menin (the N-terminal fragment 1-210 of Venus fused to the N-terminus of menin) were constructed as reported previously.¹¹

BiFC assay

Screening of inhibitors of the menin-MLL interaction was performed using the *in vitro* BiFC assay with MLL-VC210 and VN210-menin probes. MLL-VC210 and VN210-menin were co-transfected into HepG2 cells, using TurboFect Transfection Reagent (Thermo Fisher Scientific) for 24 h. The cells were incubated with compounds at the indicated concentrations 16 h at 37 °C in 96-well microtiter plates. Nuclei were then stained with DAPI 1h. YFP signals and DAPI fluorescence were captured using IN Cell Analyzer 2000. The experiments were done using triplicate samples.

MLL binding experiments

Fluorescein-labeled MLL-derived peptides, FITC-MLL⁴⁻⁴³ at 1 nM, FITC-MBM1 at 15 nM, and FITC-MBM2 at 0.2 μM, were titrated with a range of menin concentrations in the FP buffer (50 mM Tris, pH 7.5, 50 mM NaCl, 1 mM DTT) used for binding experiments (K_d measurements). In the competition experiments, fluorescein-labeled MLL peptides, menin, and varying concentrations of the

unlabeled MLL peptides in FP buffer were used for IC₅₀ determination (the concentrations of fluorescein-labeled peptides and menin used for IC₅₀ determination). After a 1-h incubation of the protein-peptide complexes, changes in fluorescence polarization and anisotropy were monitored at 525 nm after excitations at 495 nm using SpectraMax M5 Multi-Mode Microplate Reader. Results were used to assess binding or inhibition for MLL-derived peptides with the Graphpad Prism 6.0.

Co-immunoprecipitation experiments

HepG2 cells were transfected with Flag-MLL plasmid using Fugene 6 (Roche Indianapolis, IN). 48h after transfection cells were treated with compounds (0.25% final DMSO concentration) or DMSO for 12h. Whole cell lysates were immunoprecipitated with ANTIFLAG M-2 Magnetic beads (Sigma-Aldrich, St. Louis, MO) and were analyzed by SDS-PAGE electrophoresis and Western blotting.

Viability assays

HepG2 cells were cultured in DMEM medium with 10% FBS, 1% penicillin/streptomycin and NEAA. 3- (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) viability assay was carried out. For growth curves, 5-6 x 10³ cells were plated in 96 well-plate and treated with compounds or 0.25% DMSO. At 72 hours, cell culture samples were mixed with MTT (Sigma) and recorded.

Colony formation assay

The HepG2 were plated in 12-well plates at the concentration of 5×10³ cells/ml with 1 ml in DMEM medium with 10% FBS, 1% penicillin/streptomycin and 0.25% DMSO or compounds. 6 days later colonies were stained with 100 µl iodinitrotetrazolium chloride (Sigma-Aldrich) at final concentration of 1mg/ml, incubated at 37 °C for 30 min and counted.

Immunoblotting

Cells were harvested to obtain whole-cell extracts by the addition of one volume of 250 mM Tris-HCl (pH 6.8), 20% glycerol, 2% sodium dodecyl sulfate (SDS), 5% 2-

mercaptoethanol, and 0.2% bromophenol blue to cells in one volume of PBS followed by boiling for 5 min. Samples were resolved on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) gels and transferred to polyvinylidene fluoride (PVDF) membranes. Blots were probed with antibodies to H3K4me3, p27, GAPDH and beta-actin (cell signaling). After incubation with secondary antibodies (Santa Cruz Biotechnology), blots were developed with ECL reagent (ThermoFisher).

Molecular docking and virtual screening. *Model construction.* The initial model of Menin-MLL was derived from the X-ray crystal structure (PDB: 4GQ3)¹² using the molecular conversion procedure implemented in the ICM-pro 3.6-1d program (Molsoft).¹³ The molecular conversion procedure implemented in ICM-pro 3.6-1d program can read, build, convert, refine, analyze and superimpose molecules and provide target evaluation to generate three dimensional models.

High throughput molecular docking. A chemical library containing over 90,000 natural product or natural product-like compounds (ZINC natural product database) was docked to the molecular model *in silico*. Molecular docking was performed using the virtual library screening (VLS) module in the ICM-Pro 3.6-1d program (Molsoft). In the ICM fast docking and VLS procedure, the receptor all-atom model was converted into energy potential maps calculated on a fine 3D grid (0.5 Å cell). The grid potential maps account for van der Waals, hydrogen-bonding, hydrophobic, and electrostatic interactions between ligand and receptor. Each compound in the library was assigned the MMFF¹⁴ force field atom types and charges and was then subjected to Cartesian minimization. During the docking analysis, the ligand was represented by an all-atom model and considered fully flexible in the potential field of the receptor, and the binding pose and internal torsions were sampled by the BPMC minimization procedure, which involved local energy minimization after each random move. Each

compound was docked to the protein complex binding pocket, and a score from the docking was assigned to each compound according to the weighed component of the ICM scoring function (see below). Each compound was docked three times to ensure the convergence of the Monte Carlo optimization, and the minimum score of each ligand from the three independent docking experiments was retained and used for ranking. The docking procedure takes about 30 s of time per compound on a Intel Xeon 2.8 GHz CPU using a 100 processor Linux cluster.

ICM full-atom ligand-receptor complex refinement and scoring. Once the ligand-receptor complexes are generated by molecular docking, they have to be subjected to complex refinement and scoring. According to the ICM method,¹⁵ the molecular system was described using internal coordinates as variables. Energy calculations were based on the ECEPP/3 force field with a distance-dependent dielectric constant. The biased probability Monte Carlo (BPMC) minimization procedure was used for global energy optimization. This procedure consisted of four iterative steps. The BPMC global-energy-optimization method consists of 1) a random conformation change of the free variables according to a predefined continuous probability distribution; 2) local-energy minimization of analytical differentiable terms; 3) calculation of the complete energy including non-differentiable terms such as entropy and solvation energy; 4) acceptance or rejection of the total energy based on the Metropolis criterion and return to step (1). The binding between the small molecules and receptor were evaluated with a full-atom ICM ligand binding score¹⁶ from a multi-receptor screening benchmark as a compromise between approximated Gibbs free energy of binding and numerical errors. The scoring function should give a good approximation of the binding free energy between a ligand and a receptor and is usually a function of different energy terms based on a force-field. The ICM scoring function is weighted according to the following parameters (i) internal force-field energy of the ligand, (ii) entropy loss of the ligand between bound and unbound states, (iii) ligand-receptor hydrogen bond interactions, (iv) polar and non-polar solvation energy differences between bound and unbound states, (v) electrostatic energy, (vi)

hydrophobic energy, and (vii) hydrogen bond donor or acceptor desolvation. The lower the ICM score, the higher the chance the ligand is a binder. The score was calculated by:

$$S_{\text{bind}} = E_{\text{int}} + T\Delta S_{\text{Tor}} + E_{\text{vw}} + \alpha_1 E_{\text{el}} + \alpha_2 E_{\text{hb}} + \alpha_3 E_{\text{hp}} + \alpha_4 E_{\text{sf}}$$

where E_{vw} , E_{el} , E_{hb} , E_{hp} , and E_{sf} are van der Waals, electrostatic, hydrogen bonding, and nonpolar and polar atom solvation energy differences between bound and unbound states, respectively. E_{int} is the ligand internal strain, ΔS_{Tor} is its conformational entropy loss upon binding, and $T = 300$ K, and α_i are ligand- and receptor independent constants.¹⁷

References

1. S. W. Youn, S. J. Pastine and D. Sames, *Org. Lett.*, 2004, **6**, 581-584.
2. O. Vechorkin, V. Proust and X. Hu, *J. Am. Chem. Soc.*, 2009, **131**, 9756-9766.
3. J. M. Mitchell and N. S. Finney, *J. Am. Chem. Soc.*, 2001, **123**, 862-869.
4. E. Benedetto, M. Tredwell, C. Hollingworth, T. Khotavivattana, J. M. Brown and V. Gouverneur, *Chem. Sci.*, 2013, **4**, 89-96.
5. E. A. Ilardi, C. E. Stivala and A. Zakarian, *Org. Lett.*, 2008, **10**, 1727-1730.
6. M. Muehlbacher and C. D. Poulter, *JOC*, 1988, **53**, 1026-1030.
7. C. Ho Oh, C. Yun Rhim, C. Ho You and J. Rai Cho, *Synth. Commun.*, 2003, **33**, 4297-4302.
8. V. Pace, P. Hoyos, J. V. Sinisterra, A. R. Alcántara and W. Holzer, *Synlett*, 2011, **2011**, 1831-1834.
9. D. B. G. Williams and A. Cullen, *JOC*, 2009, **74**, 9509-9512.
10. R. Ando, H. Mizuno and A. Miyawaki, *Science*, 2004, **306**, 1370-1373.
11. K. Ohashi, T. Kiuchi, K. Shoji, K. Sampei and K. Mizuno, *Biotechniques*, 2012, **52**, 45-50.
12. T. Cierpicki and J. Grembecka, *Future Med. Chem.*, 2014, **6**, 447-462.
13. Abagyan R, Orry A, Rausch E, Budagyan L and Totrov M, 2009.
14. T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490-519.
15. M. Totrov and R. Abagyan, *Proteins*, 1997, **Suppl 1**, 215-220.
16. M. Schapira, M. Totrov and R. Abagyan, *J. Mol. Recognit.*, 1999, **12**, 177-190.
17. M. Totrov and R. Abagyan, New York: Lyon, France, 1999.

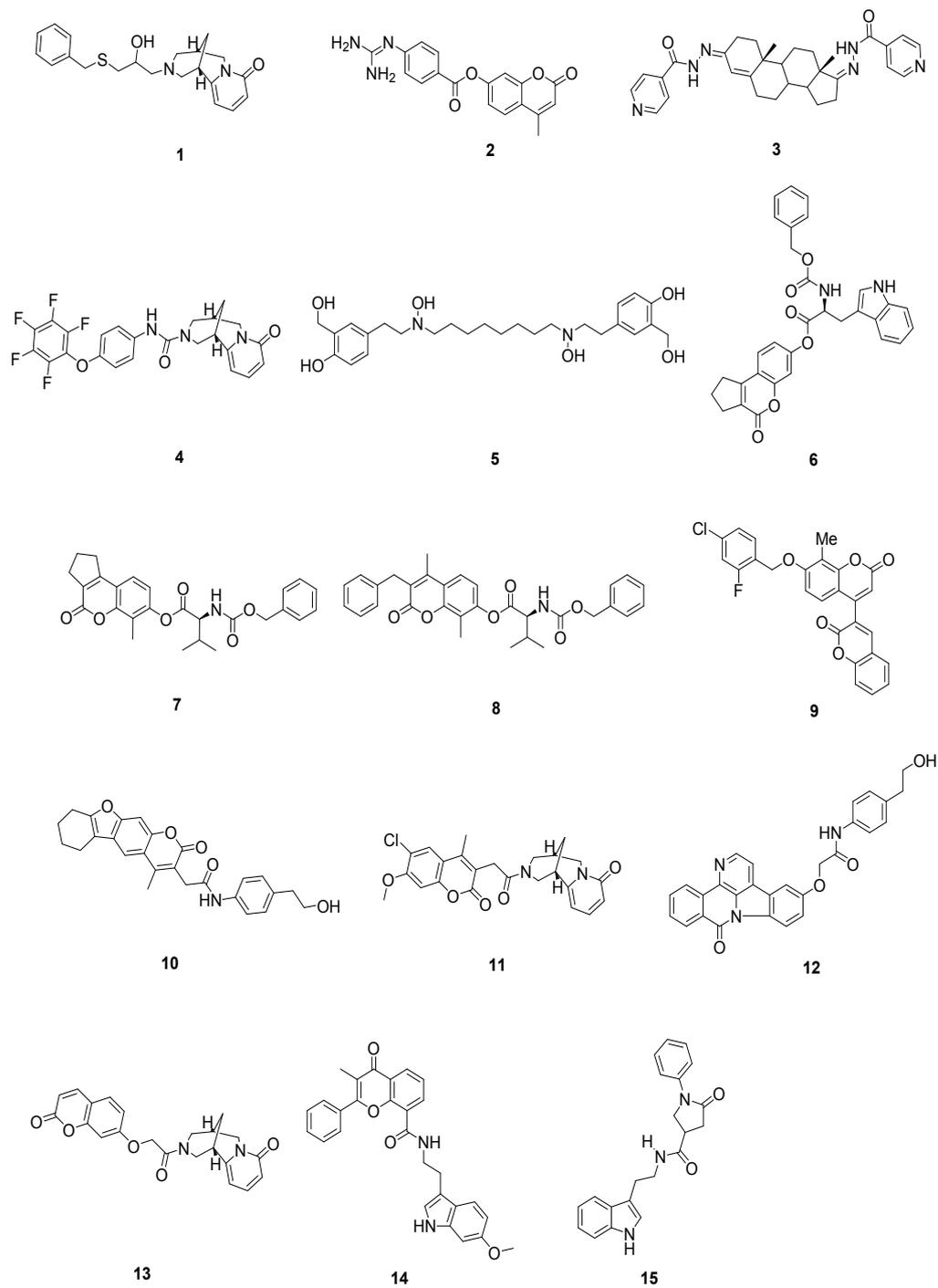


Fig. S1. Chemical structures of natural product-like compounds 1–15 evaluated in this study.

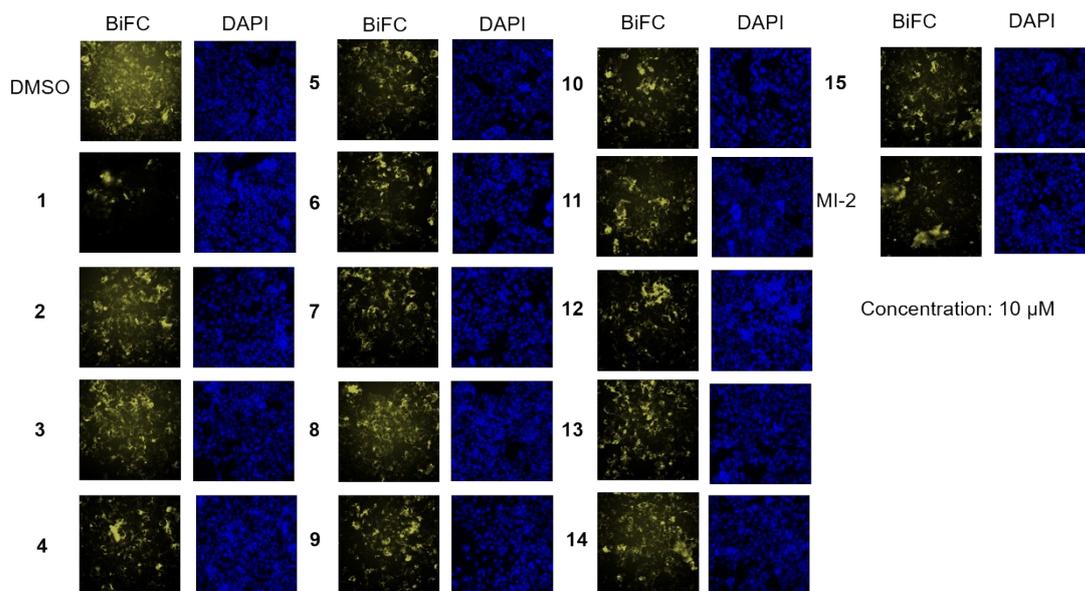


Fig. S2. BiFC assay comparing activities of compound **1–15** and MI-2 (10 μM) for disruption of the menin-MBM1 interaction in HepG2 cells. HepG2 cells were transiently transfected with MLL-VC210 and VN210-menin plasmids. Comparative nuclei staining with DAPI is shown.

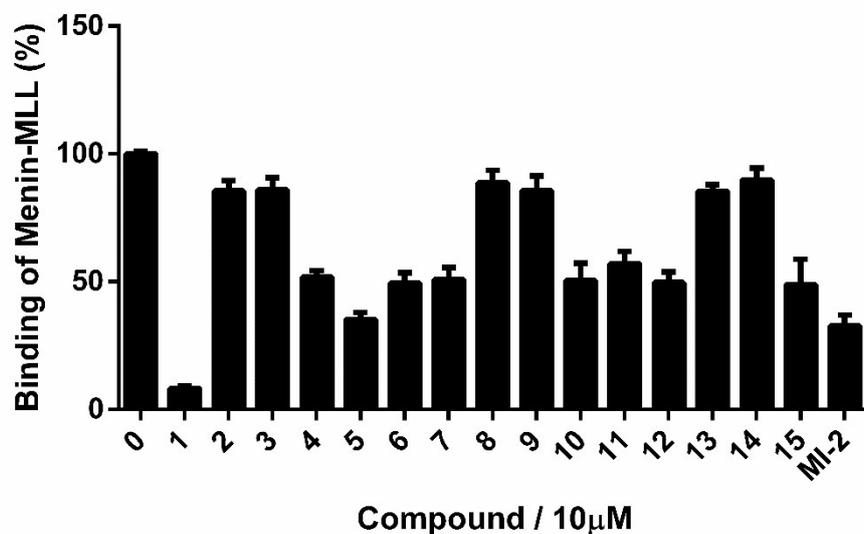


Fig. S3. BiFC assay comparing the activities of compound 1–15 and MI-2 (10 μ M) for disruption of the menin-MBM1 interaction. Graph shows quantitative analysis of distribution of BiFC signals in cells from three independent experiments.

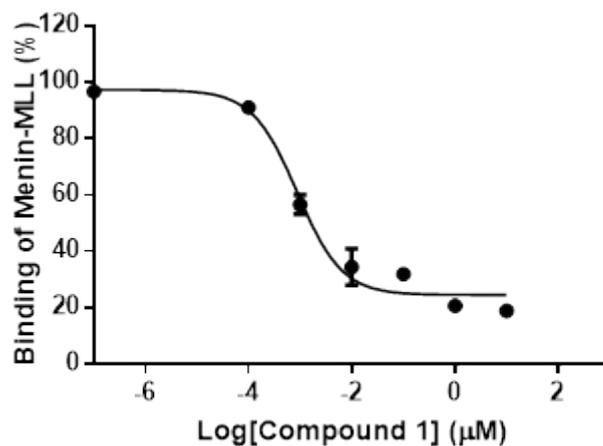


Fig. S4. Inhibition of the menin-MLL interaction by compound 1 measured by fluorescence polarization assay. Menin was treated with various concentrations of compound 1 at room temperature for 1 h, and then incubated with the fluorescent phosphotyrosine peptide MBM1 peptide for 30 min. IC_{50} : 0.04 μ M.

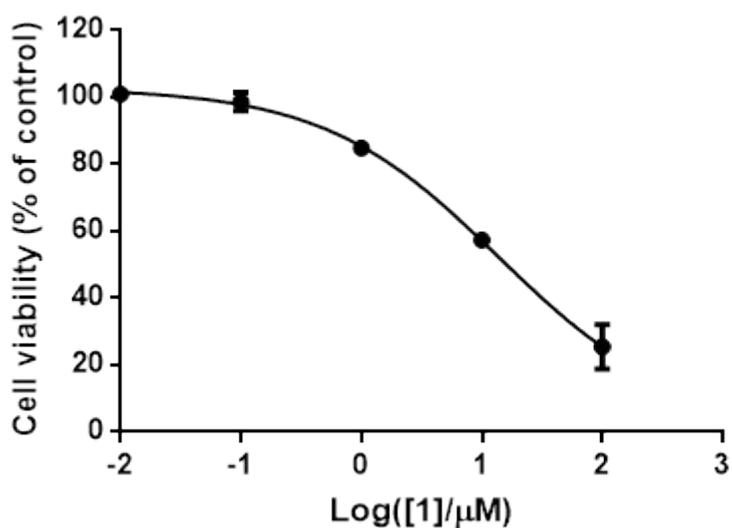


Fig. S5. Compound **1** inhibited HepG-2 cell growth in a dose-dependent manner after 72 h treatment. IC₅₀: 10 μM

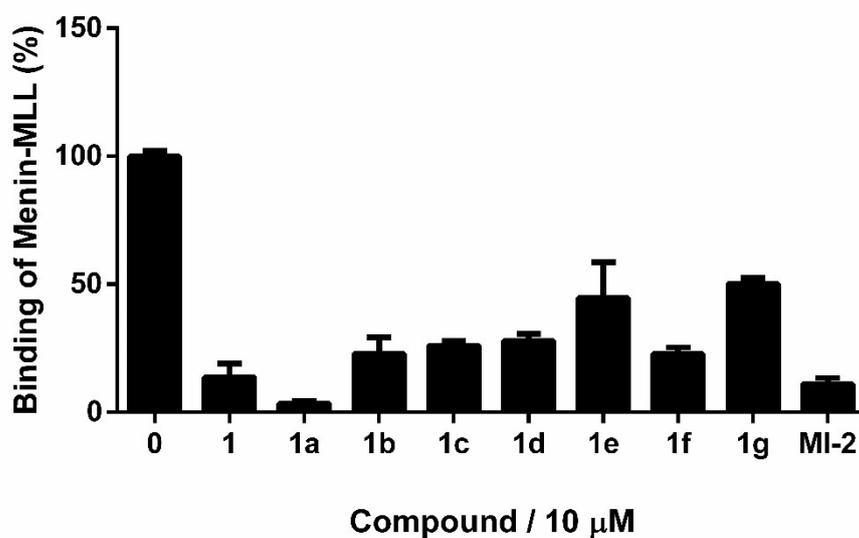


Fig. S6. BiFC assay comparing the activities of compound **1**, **1a–1g** and MI-2 (10 μM) for disruption of the menin-MBM1 interaction. Graph shows quantitative analysis of distribution of BiFC signals in cells from three independent experiments

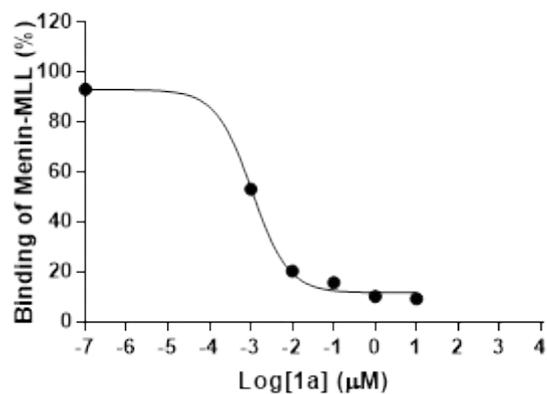


Fig. S7. Inhibition of the menin-MLL interaction by compound 1a measured by fluorescence polarization assay. Menin was treated with various concentrations of compound 1a at room temperature for 1 h, and then incubated with the fluorescent phosphotyrosine peptide MBM1 peptide for 30 min. IC_{50} : 0.001 μ M.

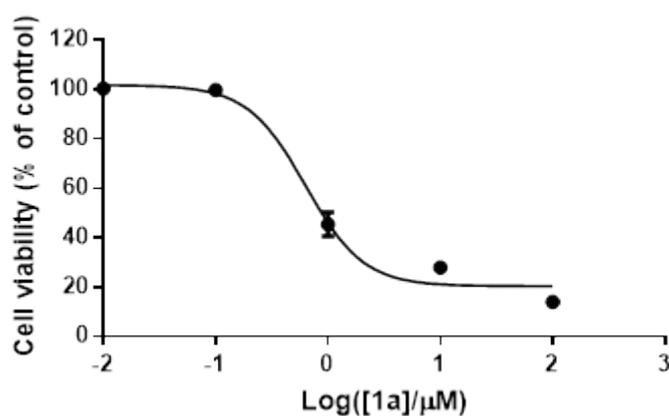


Fig. S8. Compound 1a inhibited HepG-2 cells growth in a dose dependent manner after the treatment of 72 hrs. IC_{50} : 0.63 μ M

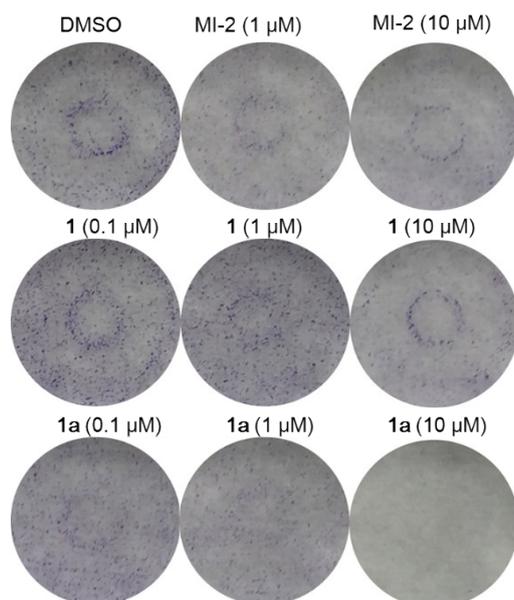


Fig. S9. The inhibitory effects of **1 and **1a** colony formation in HepG2 cells.** Colony formation of HepG2 cells treated with compounds **1** and **1a** are representative of three independent experiments with duplicate plates.