Supporting Information

Discovery of 1,4-Didydroxy-2-naphthoate Prenyltransferase Inhibitors: New Drug Leads for Multidrug-Resistant Gram-positive Pathogens

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General Procedures and Methods: All glassware was oven dried, assembled hot and cooled under a stream of nitrogen before use. Reactions with air sensitive materials were carried out by standard syringe techniques. Commercially available reagents were used as received without further purification. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with potassium permanganate solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230-400 mesh, Merck). IR absorptions on NaCl plates were run on a Perkin Elmer FT-IR 1600. ^{1}H NMR spectral data were obtained using Varian 300, 400 MHz instruments. The residual solvent signal was utilized as an internal reference. ^{13}C NMR spectral data were obtained using a Varian 100 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS, using the middle resonance of $CDCl_3$ (77.0 ppm) as an internal standard. For all NMR spectra, δ values are given in ppm and *J* values in Hz. Mass spectra were obtained at Colorado State University's Central Instrument Facility.

Reagents and solvents are commercial grade and were used as supplied. Reaction vessels were flame-dried or oven-dried and cooled under an inert atmosphere when necessary.

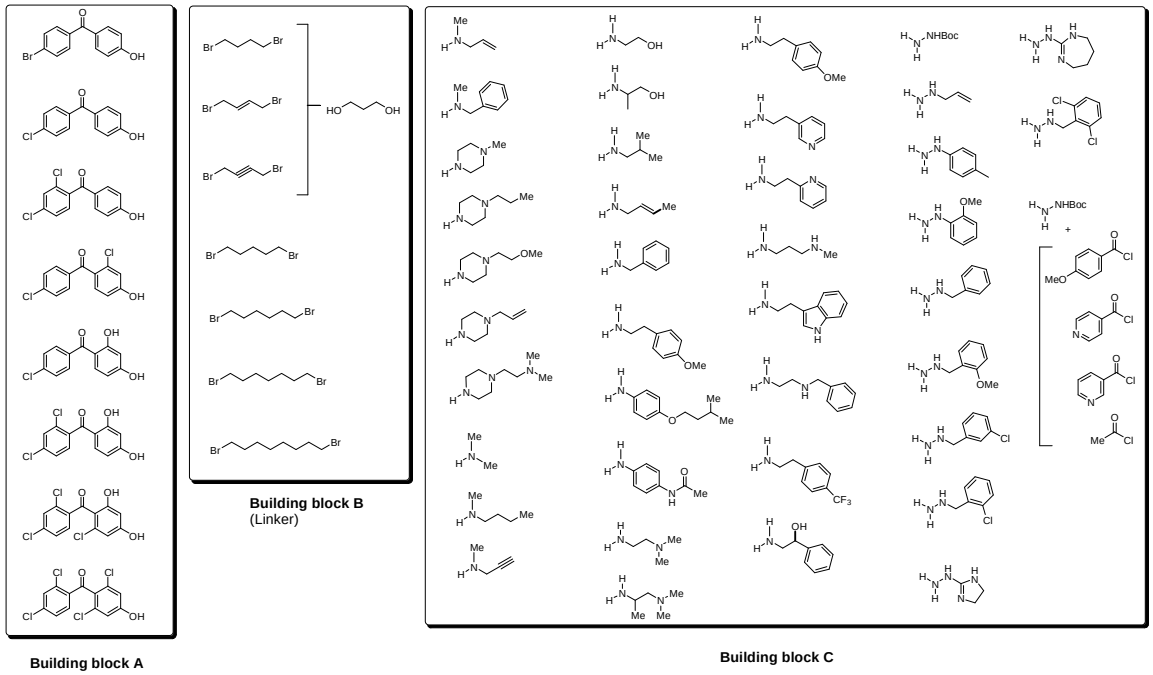
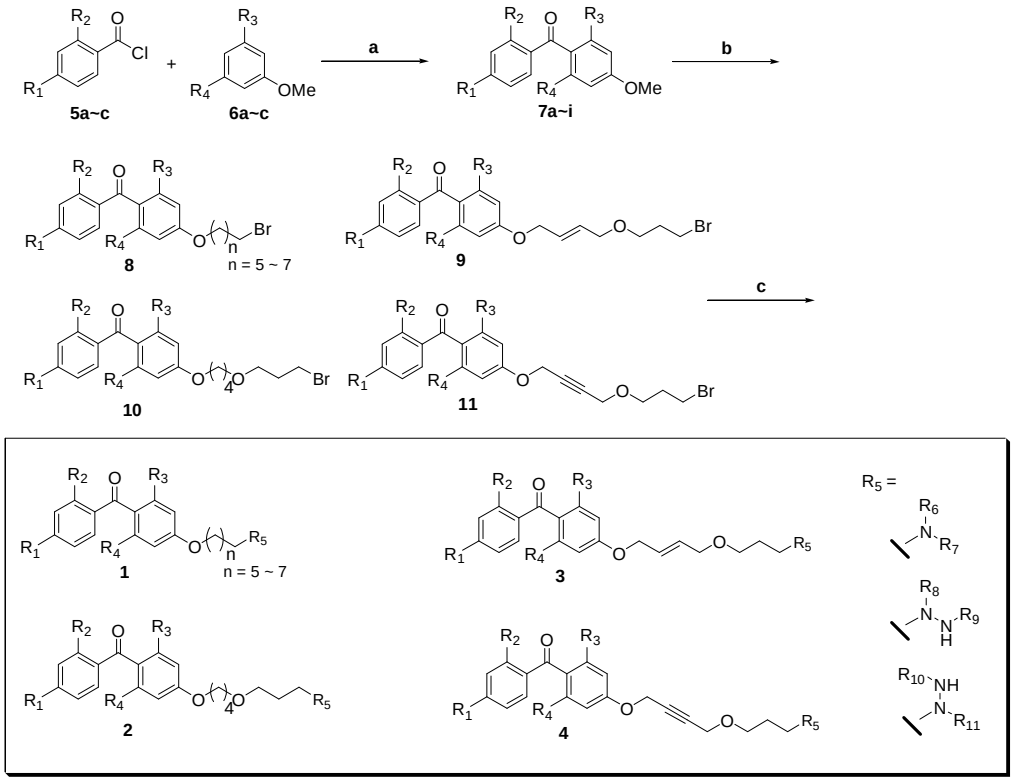


Figure 1. Building blocks utilized in the library production.

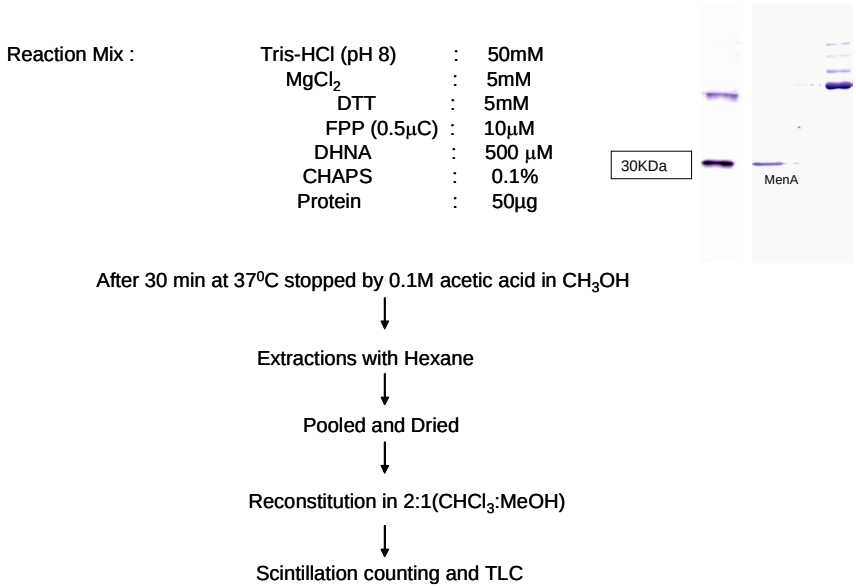
Scheme 1. Synthetic scheme for the generation of a library of molecules in solution.[1]



[a] Reagents and conditions: (a) $AlCl_3$, $PhNO_2$ (75~90%); (b) (i) 48% HBr, AcOH-water (90%); (ii) 1,5-dibromopentane or 1,6-dibromohexane or 1,7-dibromoheptane or 1,8-dibromooctane, K_2CO_3, DMF (for **1**) (80~95%); 1,4-dibromobutane, K_2CO_3, DMF; 1,3-propanediol, NaH, DMF; CBr_4, PPh_3, CH_2Cl_2 (for **2**) (65%); 1,4-dibromobutene, K_2CO_3, DMF; 1,3-propanediol, NaH, DMF; CBr_4, PPh_3, CH_2Cl_2 (for **3**) (65%); 1,4-dibromobutyne, K_2CO_3, DMF; 1,3-propanediol, NaH, DMF; CBr_4, PPh_3, CH_2Cl_2 (for **4**) (65%); (iii) R_5, $NaHCO_3$, DMF (50~98%); (iv) acylchloride, pyridine (v) TFA, CH_2Cl_2 (for Boc-protected R_5) (100%).

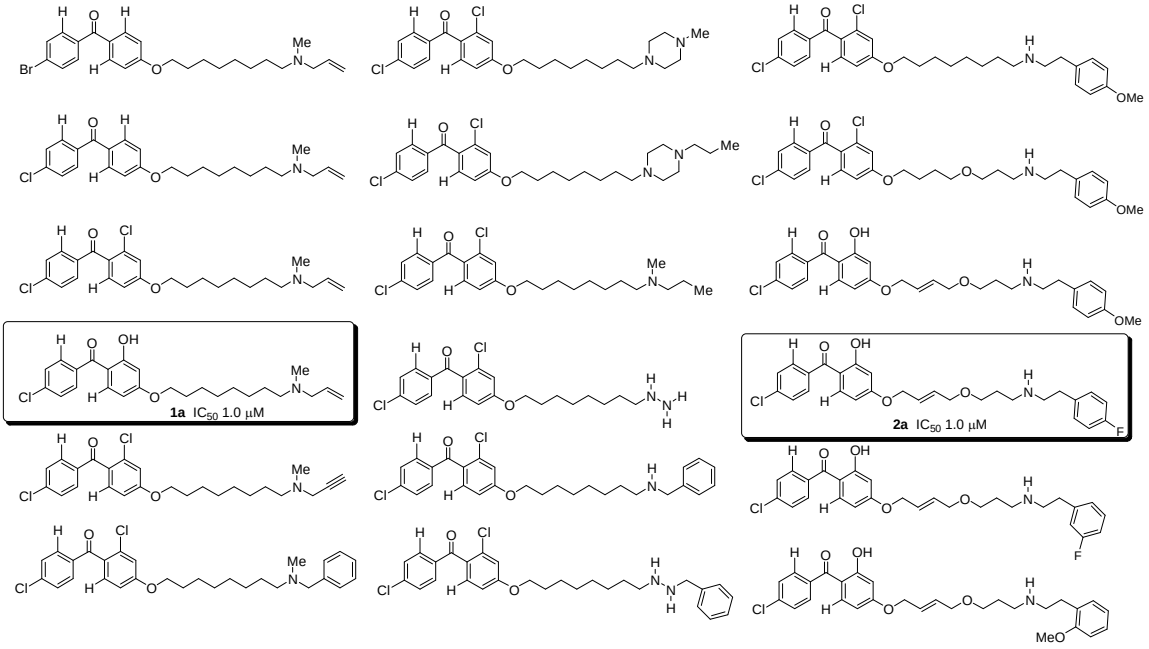
Figure 2. Enzymatic assay against MenA.

MenA inhibitory Assay:



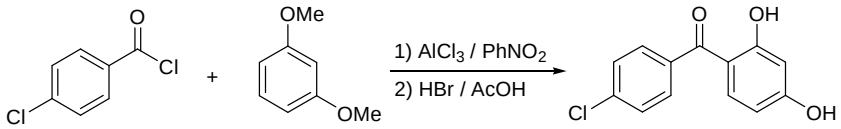
The enzymatic activity was characterized using membrane fractions prepared from *M. tuberculosis* essentially as previously described.[2] *M. tuberculosis* (H37Rv) was grown to mid-log phase in glycerol-alanine-salts medium, washed with saline and harvested by centrifugation.[3] The resulting pellet was irradiated for 18 h at 2,315 Rads/min using a JL Shepard instrument with a 137Cs source. This exposure was calculated to kill 100% of the bacteria but retain 90% of enzyme activity. The washed cell pellet was resuspended in homogenization buffer containing 50 mM MOPS (pH 7.9), 0.25 M sucrose, 10 mM $MgCl_2$ and 5 mM 2-mercaptoethanol and disrupted by probe sonication on ice with a Sanyo Soniprep 150 (10 cycles of 60 sec on and 90 sec off). The resulting suspension was centrifuged at 27,000 X g for 15 min. The pellet was discarded and the supernatant was centrifuged at 100,000 X g for 1 h in a Beckman Ti70.1 rotor. The pellet (membranes) was resuspended in homogenization buffer, divided into aliquots and frozen at -70 ºC. The protein concentration was estimated using a BCA protein assay kit (Pierce, Rockford, IL). MenA assays were conducted essentially as previously described.[4] Assay mixtures contained 500 µM DHNA, 10 µM [^{3}H]-farnesyl diphosphate (American Radiolabeled Chemicals, St. Louis, MO), 5 mM $MgCl_2$[5] and 0.1% CHAPS in 100 mM MOPS (pH 8) and an appropriate amount of membrane protein. Reactions were stopped by the addition of 1 ml of 0.1 M AcOH in MeOH. The resulting mixture was extracted twice with 3 ml of hexanes and the combined extracts were evaporated to dryness under N_2 and redissolved in $CHCl_3$: MeOH (2:1). An aliquot was taken for liquid scintillation counting and the remaining material was subjected to TLC on silica gel plates, which were developed in hexanes : Et_2O (95:5). Radioactive spots on the thin layer plated were located and quantitated using a Bioscan System 200 Imaging Scanner. IC_{50} values were calculated for the inhibitors and compared to the MIC values, in all cases tested there was a good correlation between IC_{50} and MIC.

Figure 2. Representative structures which exhibited MenA inhibitory activity at IC_{50} of lower than 20 μM concentrations.



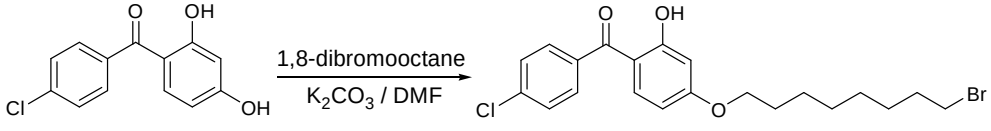
Experimental Procedures and Compound Data:

(4-Chlorophenyl)(2,4-dihydroxyphenyl)methanone



Anhydrous $AlCl_3$ (4.5 g, 33.9 mmol) was taken in a round bottom flask and $PhNO_2$ (50 mL) was added. At – 40 ^oC, 1,3-dimethoxybenzene (3.9 g, 28.2 mmol) and 4-chlorobenzoyl chloride (4.7 mL, 33.9 mmol) were added. The reaction was warmed up to r.t. and stirred at 50 ^oC for 12 h. The reaction mixture was quenched with 2N NaOH at 0^oC and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vaccuo* ($PhNO_2$ was removed at 90 ^oC under high vaccum). The crude mixture in AcOH (50 mL) was added 48% HBr (25 mL) and heated at 120 ^oC for 36 h. All volatiles were evaporated. Purification by silica gel chromatography (5:1, hexanes:EtOAc) to provide (4-chlorophenyl)(2,4-dihydroxyphenyl)methanone (7.0 g, 26.3 mmol, 93%) as a white powder. Data for (2-chloro-4-hydroxyphenyl)(4-chlorophenyl)methanone: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.52 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.34 (m, 1H), 6.37 (s, 1H), 6.30 (d, *J* = 8.7 Hz, 1H), 3.99 (bs, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 198.9, 166.1, 165.6, 137.9, 136.9, 135.9, 130.5(2c), 128.8(2c), 112.5, 108.6, 103.4; IR (neat, cm^{-1}): 3288, 2923, 1629, 1599, 1540; HRMS (FAB) calcd. for $C_{13}H_{10}O_3Cl$ ($M+H^+$) 249.0313, and observed 249.0305.

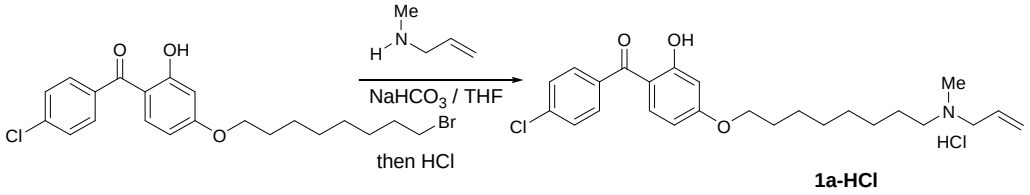
(4-(8-Bromooctyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone



To a stirred solution of (4-chlorophenyl)(2,4-dihydroxyphenyl)methanone (1.86 g, 7.51 mmol) in DMF (20 mL) were added K_2CO_3 (5.2 g, 37.6 mmol) and 1,8-dibromooctane (5.1 g,18.8 mmol). After 12 h, the

reaction mixture was quenched with water and extracted with hexanes. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vaccuo*. Purification by silica gel chromatography (30:1, hexanes:EtOAc) to provide (4-(8-bromooctyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (2.8 g, 6.38 mmol, 85%) as a liquid. Data for (4-(8-bromooctyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 12.6 (s, 1H), 7.59 (m, 2H), 7.48 (m, 3H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.41 (dd, *J* = 2.7, 9.0 Hz, 1H), 4.03 (t, *J* = 6.3 Hz, 2H), 3.43 (t, *J* = 6.9 Hz, 2H), 1.86 (m, 4H), 1.43 (m, 8H); ^{13}C-NMR ($CDCl_3$, 75 MHz): 198.5, 166.3, 165.9, 137.7, 136.2, 134.8, 130.3(2c), 128.6(2c), 112.7, 107.9, 101.5, 68.4, 33.9, 32.7, 29.1, 28.8, 28.6, 28.0, 25.8; HRMS (FAB) calcd. for $C_{21}H_{25}O_3ClBr$ ($M+H^+$) 439.0597, and observed 439.0595.

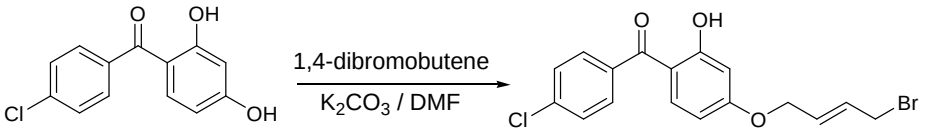
(4-(8-(Allyl(methyl)amino)octyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (allylaminomethanone-A(1a))



1a-HCl

To a stirred solution of (4-(8-bromooctyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (20 mg, 0.046 mmol) in THF (1 mL) were added $NaHCO_3$ (36.2 mg, 0.46 mmol) and *N*-methylprop-2-en-1-amine (32.6 mg, 0.46 mmol). After 3 days at r.t., the reaction mixture was evaporated. The residue was dissolved in $CHCl_3$ and filtered. Purification by PTLC (10:1, $CHCl_3$:MeOH) to provide **1a** (18.9 mg,0.044 mmol, 95 %). This was transformed to its HCl salt with sat. HCl in Et_2O. Data for **1a•HCl**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.20 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.40 (m, 3H), 6.97 (d, *J* = 5.4 Hz, 1H), 6.90 (s,1H), 6.16 (m, 1H), 5.57 (d, *J* = 10.2 Hz, 2H), 3.82 (t, *J* = 6.0 Hz, 2H), 3.61 (m, 2H), 3.04 (m, 1H), 2.89 (m, 1H), 2.73 (s, 3H), 1.79 (m, 2H), 1.23 (m, 10H).; ^{13}C-NMR ($CDCl_3$, 100 MHz): 194.8, 158.9, 139.1, 138.3, 137.9, 130.7(3c), 128.1(2c), 126.1, 125.9(2c), 120.8, 113.8, 68.0, 58.2, 55.2, 39.3, 28.9, 28.8, 28.7, 26.5, 25.4, 23.6.; HRMS (FAB) calcd. for $C_{25}H_{33}O_3ClN$ ($M+H^+$) 430.2071, and observed 430.2065.

(*E*)-(4-(4-Bromobut-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone

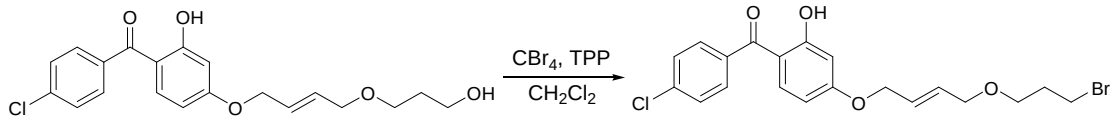


To a stirred solution of (4-chlorophenyl)(2,4-dihydroxyphenyl)methanone (1.0 g, 4.03 mmol) in DMF (20 mL) were added K_2CO_3 (1.1 g, 8.1 mmol) and 1,4-dibromobutene (2.1 g,10.1 mmol). After 3 h at 5 ^{o}C, the reaction mixture was quenched with water and extracted with hexanes. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vaccuo*. Purification by silica gel chromatography (15:1, hexanes:EtOAc) to provide (*E*)-(4-(4-bromobut-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (1.37 g, 3.62 mmol, 90 %) as a liquid. Data for (*E*)-(4-(4-bromobut-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 12.50 (s, 1H), 7.58 (m, 2H), 7.48 (m, 3H), 6.51 (d, *J* = 2.7 Hz, 1H), 6.43 (dd, *J* = 2.4, 9.0 Hz, 1H), 6.08 (m, 1H), 6.00 (m, 1H), 4.62 (dd, *J* = 0.6, 5.1 Hz, 2H), 4.01 (d, *J* = 7.5 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 198.6, 166.2, 164.9, 137.8, 136.4, 134.9, 131.0, 130.3(2c), 129.9, 128.6(2c), 113.1, 107.8, 101.8, 67.4, 31.2; IR (neat, cm^{-1}): 2925, 1630, 1598, 1570, 1469; HRMS (FAB) calcd. for $C_{17}H_{15}O_3ClBr$ ($M+H^+$) 380.9815, and observed 380.9813.

(*E*)-(4-Chlorophenyl)(2-hydroxy-4-(4-(3-hydroxypropoxy)but-2-enyloxy)phenyl)methanone

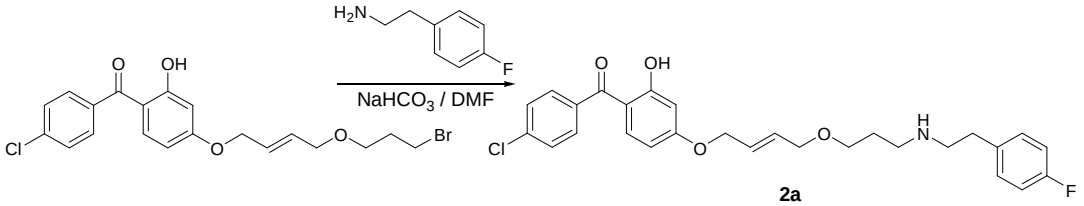
To a stirred solution of 1,3-propanediol (275 mg, 18.1 mmol) in DMF (10 mL) at 0 ^{o}C was added NaH (60%, 2.1 g, ~54.3 mmol). After 10 min, (*E*)-(4-(4-bromobut-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (1.37 g, 3.62 mmol) in DMF (4 mL) was added. The reaction mixture was stirred for 2h and quenched with 0.5 N HCl, and extracted with hexanes. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vaccuo*. Purification by silica gel chromatography (20:1, hexanes:EtOAc) to provide (*E*)-(4-chlorophenyl)(2-hydroxy-4-(4-(3-hydroxypropoxy)but-2-enyloxy)phenyl)methanone (1.36 g, 3.44 mmol, 95%) as a liquid. Data for (*E*)-(4-chlorophenyl)(2-hydroxy-4-(4-(3-hydroxypropoxy)but-2-enyloxy)phenyl)methanone: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 12.54 (s, 1H), 7.58 (m, 2H), 7.47 (m, 3H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.44 (dd, *J* = 2.4 & 9.0 Hz, 1H), 5.95 (m, 2H), 4.61 (m, 2H), 4.06 (m, 2H), 3.80 (t, *J* = 5.7 Hz, 2H), 3.66 (t, *J* = 5.7 Hz, 2H), 1.88 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 198.6, 166.2, 165.2, 137.8, 136.5, 134.9, 130.8, 130.3(2c), 128.6(2c), 126.3, 112.9, 107.9, 101.9, 70.7, 69.6, 68.1, 61.8, 32.0; IR (neat, cm^{-1}): 3368, 3202, 2930, 1639, 1593, 1520; HRMS (FAB) calcd. for $C_{20}H_{22}O_5Cl$ ($M+H^+$) 377.1150 and observed 377.1151.

(*E*)-(4-(4-(3-Bromopropoxy)but-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone



To a stirred solution of (*E*)-(4-chlorophenyl)(2-hydroxy-4-(4-(3-hydroxypropoxy)but-2-enyloxy)phenyl)methanone (1.36 g, 3.44 mmol) in CH_2Cl_2 (10 mL) were added TPP (4.51 g, 17. 2 mmol) and CBr_4 (5.6 g, 17.2 mmol). After 1h at r.t., the reaction mixture was evaporated in vaccuo. The crude mixture was purified by silica gel chromatography (20:1, hexanes:EtOAc) to provide (*E*)-(4-(4-(3-bromopropoxy)but-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (1.51 g, 3.44 mmol, 100%) as a liquid. Data for (*E*)-(4-(4-(3-bromopropoxy)but-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 12.53 (s, 1H), 7.60 (m, 2H), 7.47 (m, 3H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.44 (dd, *J* = 2.7, 9.0 Hz, 1H), 5.96 (m, 2H), 4.61 (m, 2H), 4.06 (m, 2H), 3.59 (t, *J* = 6.0 Hz, 2H), 3.54 (t, *J* = 6.6 Hz, 2H), 2.14 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 198.6, 166.2, 165.2, 137.8, 136.5, 134.9, 131.0. 130.3(2c), 128.6(2c), 126.2, 112.9, 107.9, 101.9, 70.5, 68.1, 67.8, 32.8, 30.6. IR (neat, cm^{-1}): 3208, 2930, 1745, 1635,1590; HRMS (FAB) calcd. for $C_{20}H_{21}O_4ClBr$ ($M+H^+$) 439.0306 and observed 439.0289.

(*E*)-(4-Chlorophenyl)(4-(4-(3-(4-fluorophenethylamino)propoxy)but-2-enyloxy)-2-hydroxyphenyl)methanone (Phenethylaminomethanone-A (2a))



2a

To a stirred solution of (*E*)-(4-(4-(3-bromopropoxy)but-2-enyloxy)-2-hydroxyphenyl)(4-chlorophenyl)methanone (10 mg, 0.023 mmol) in THF (1 mL) were added $NaHCO_3$ (20.5 mg, 0.23 mmol) and 2-(4-fluorophenyl)ethanamine (16.7 mg, 0.12 mmol). After 24 h at r.t., the reaction mixture was evaporated. The residue was dissolved in $CHCl_3$ and filtered. Purification by PTLC (10:1, $CHCl_3$: MeOH) to provide **2a** (10.9 mg, 0.022 mmol, 96%) as a white powder. Data for **2a**: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ

7.41 (m, 2H), 7.06 (m, 2H), 6.96 (m, 2H), 6.85 (m, 2H), 6.54 (d, *J* = 9.0 Hz, 1H), 6.43 (d, *J* = 2.7 Hz, 1H), 6.16 (dd, *J* = 2.4, 9.0 Hz, 1H), 5.94 (m, 2H), 4.55 (m, 2H), 4.03 (m, 2H), 3.58 (t, *J* = 6.0 Hz, 2H), 3.53 (m, 4H), 2.93 (t, *J* = 6.6 Hz, 2H), 2.13 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 198.5, 168.6, 162.8, 160.2, 137.8, 132.6(2c), 130.6, 130.4, 130.3(2c), 130.1, 130.0, 128.9, 128.7, 128.6(2c), 126.8, 115.3, 106.2, 102.5, 70.6, 69.0, 67.7, 47.2, 36.3, 35.4, 29.9; IR (neat, cm^{-1}): 3052, 2917, 1601, 1509, 1487; HRMS (FAB) calcd. for $C_{28}H_{30}O_4ClFN$ ($M+H^+$) is 498.1769 and observed 498.1763.

MIC determinations against Gram-positive bacteria:

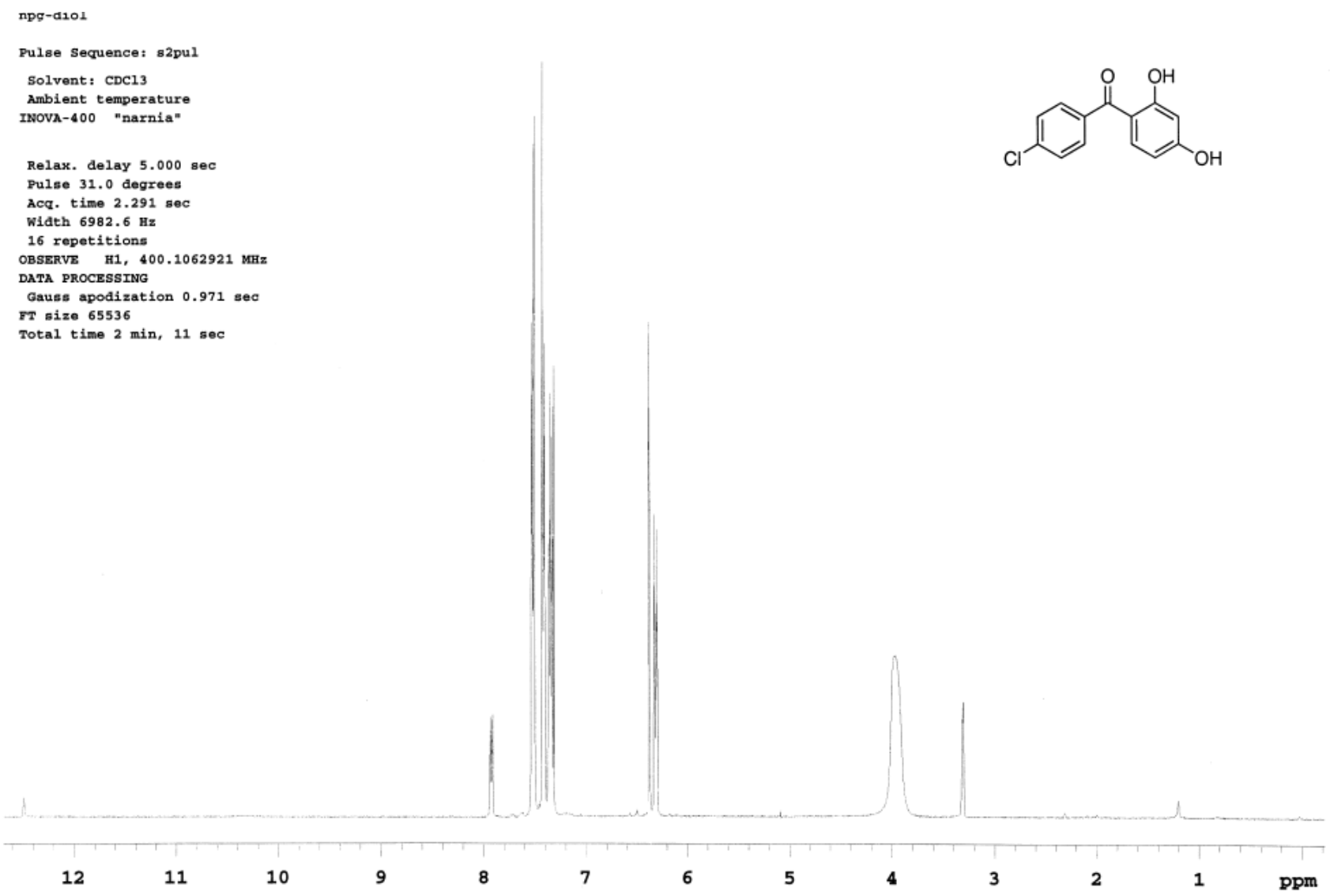
Following the standard method recommended by the National Committee for Clinical Laboratory Standards, we used an agar dilution method to determine MICs of MenA inhibitors and known compounds as references. All of the *S. aureus* strains and the others were tested at a final inoculum concentration of 104 c.f.u. per spot using a multipoint inoculator and incubated at 35 ^{o}C for 18h.[6]

MIC determinations against *M. tuberculosis* H37Rv:

We also applied colorimetric microtiter plate based method with Alamar blue/visual inspection for the MIC determination with *M. tuberculosis*.

The MIC of all compounds synthesized will be determined first against H37Rv by the colorimetric microtiter plate based method with Alamar blue/visual inspection.[7] Compounds demonstrating good activity against H37Rv will be tested against MDR-TB strains. The MDR strains are all resistant to isoniazid (INH), streptomycin, rifampicin (RIF), or ethambutol (EMB), and some are resistant to pyrazinamide (PZA) and/or additional second line drugs.[8] Drug susceptibility test methods can vary depending on the size of the inoculum (i.e. density of the cells). We will standardize the size of the inoculum using the frozen stocks with known CFU/mL (approx. 1 x104).

Compounds were tested at 20 μM initially; each microtitre plate has both positive and negative controls and the outside wells are filled with water to help prevent dehydration. If inhibitory activity occurs at 20 μM (this is usually 10 μg/ml of a compound with approx. 500 MW) in the primary screen, serial dilutions of each compound were prepared, and MICs were determined.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FT size 65536
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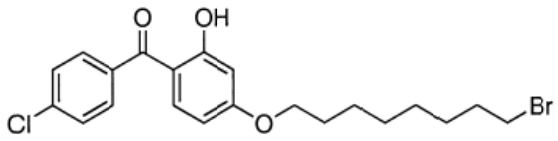

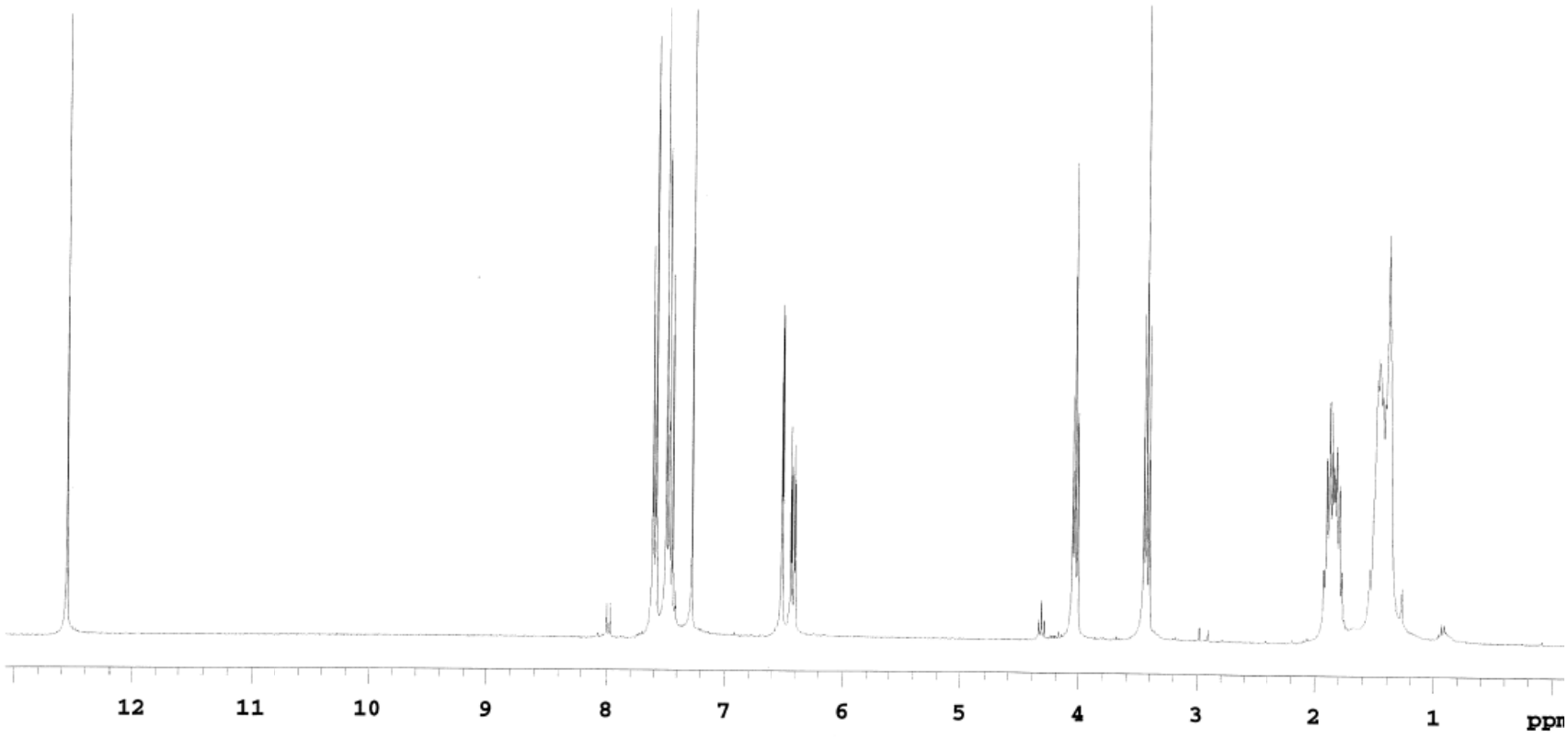

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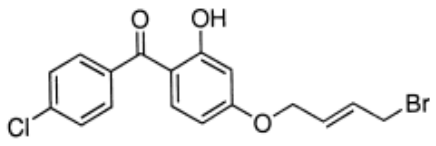
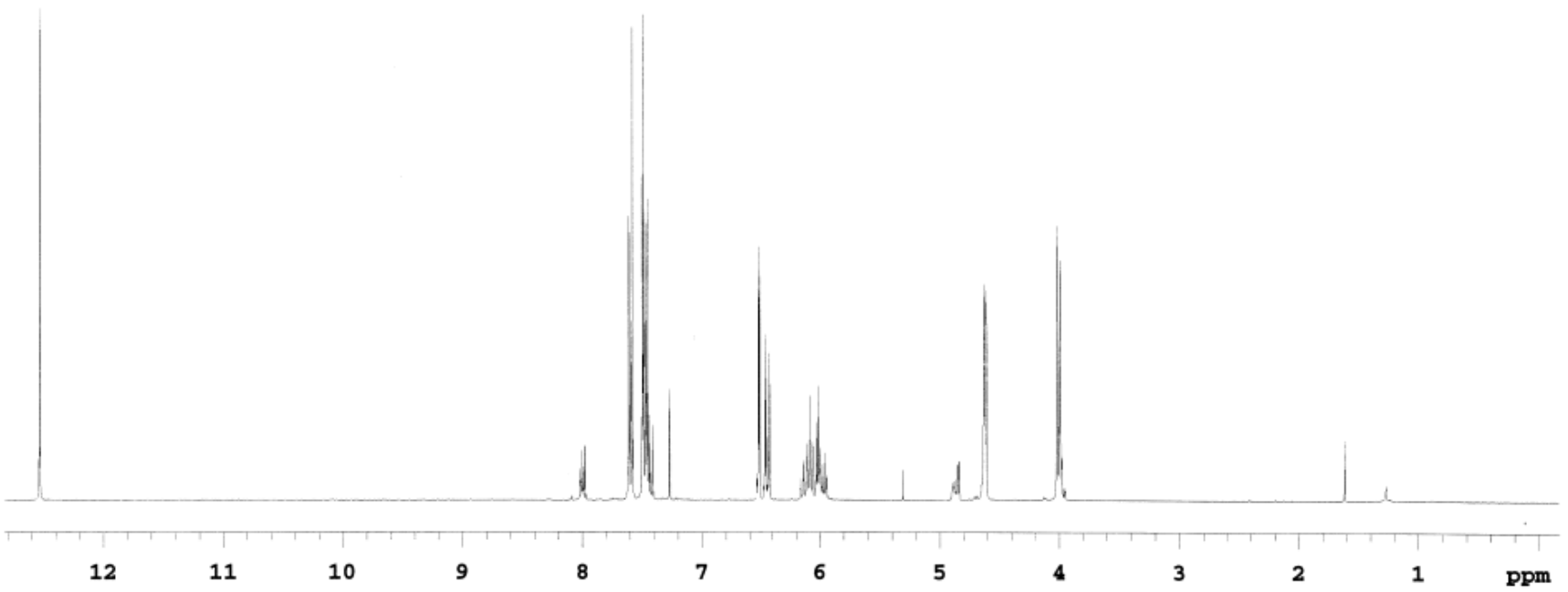
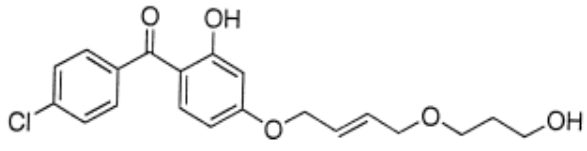
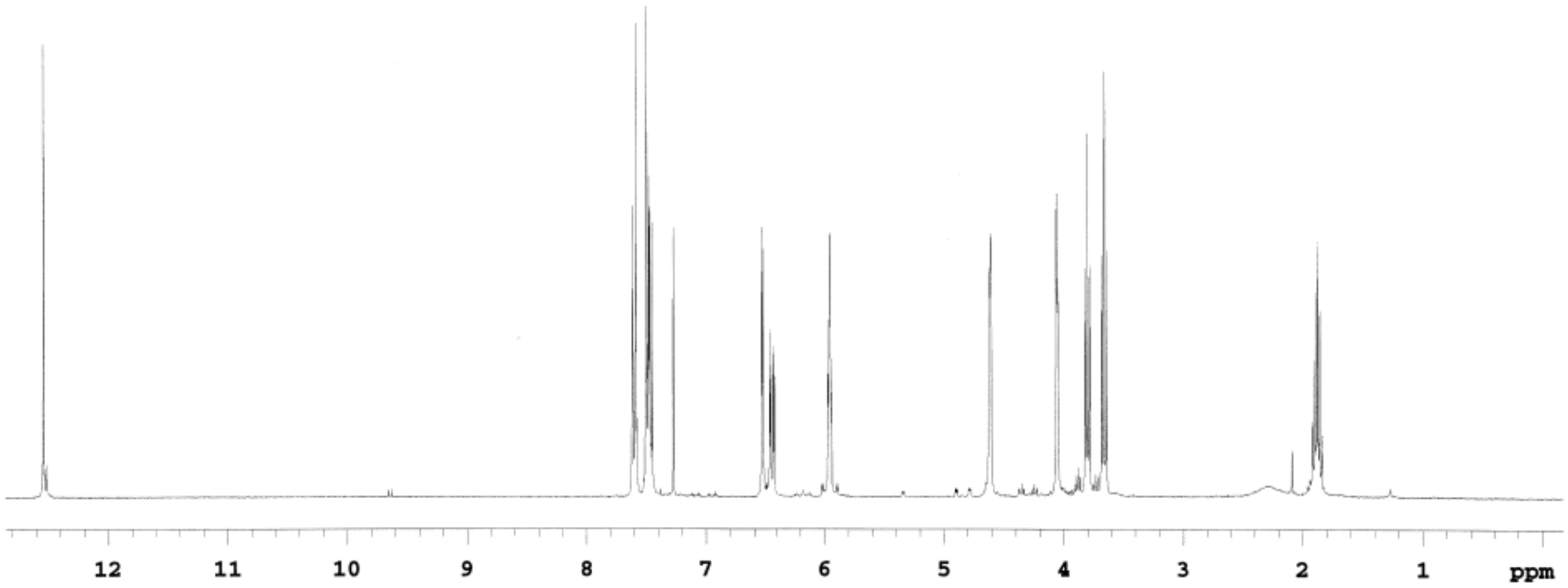




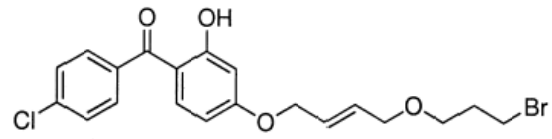
npg2-oh١٢٤

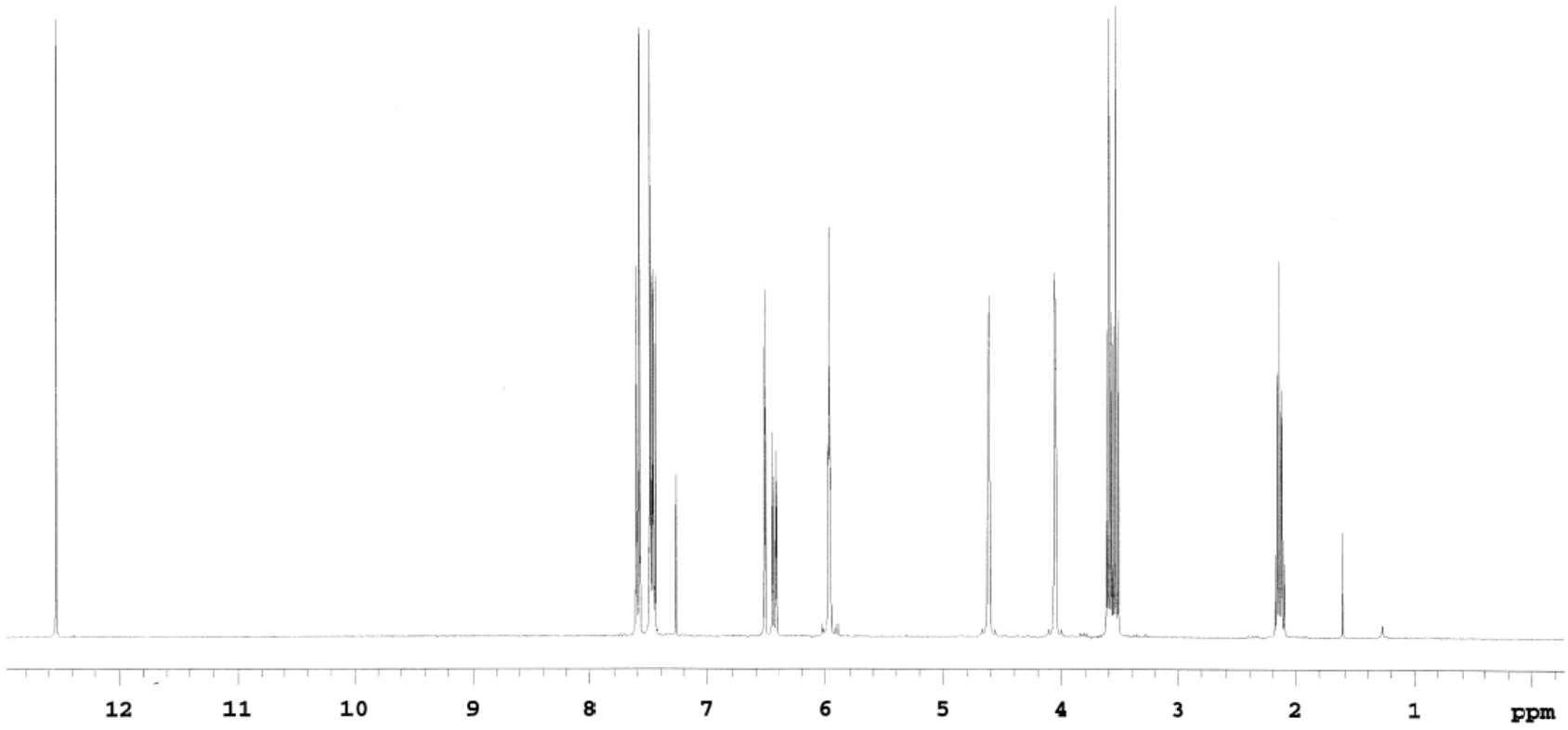
Pulse Sequence: s2pul

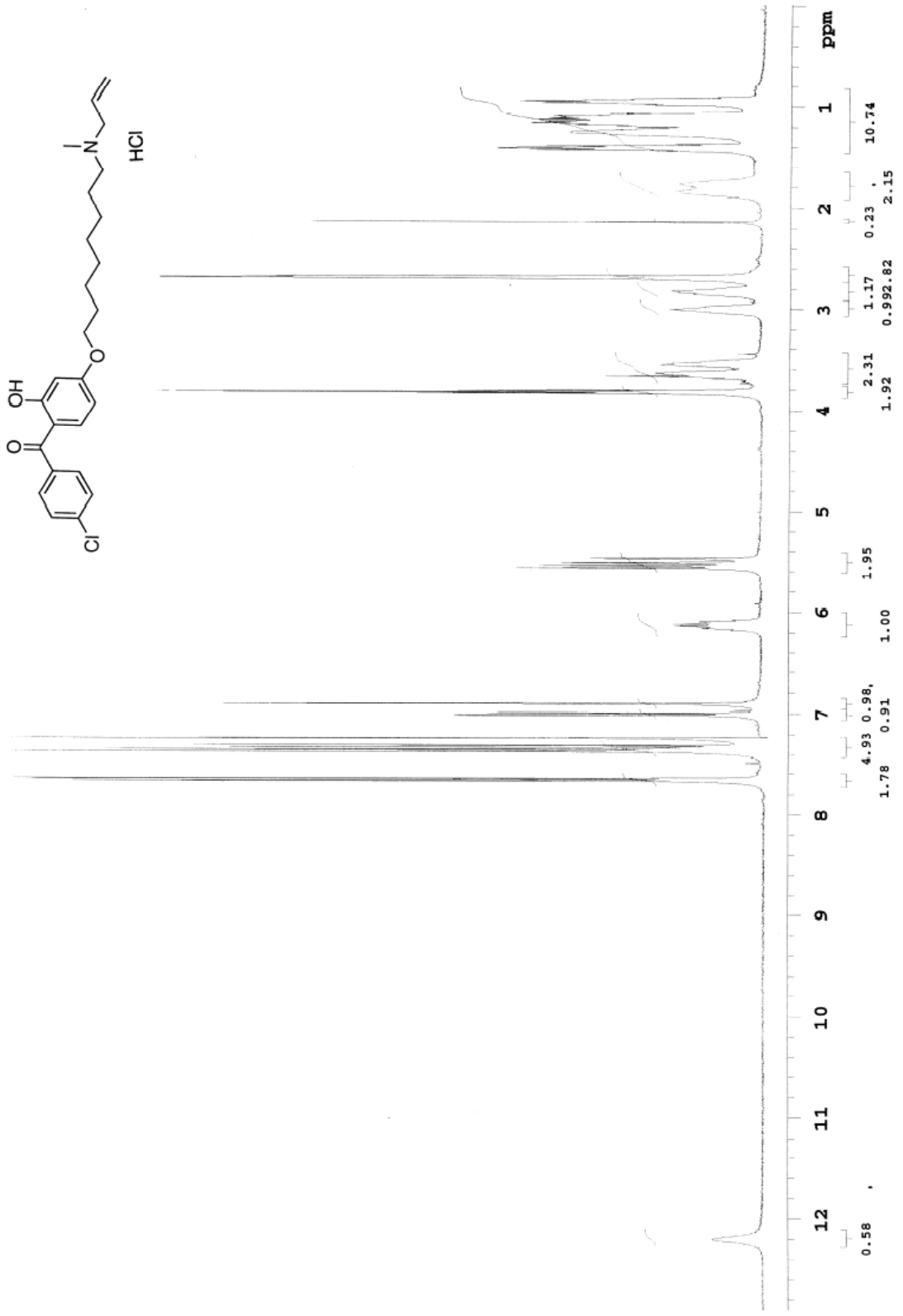


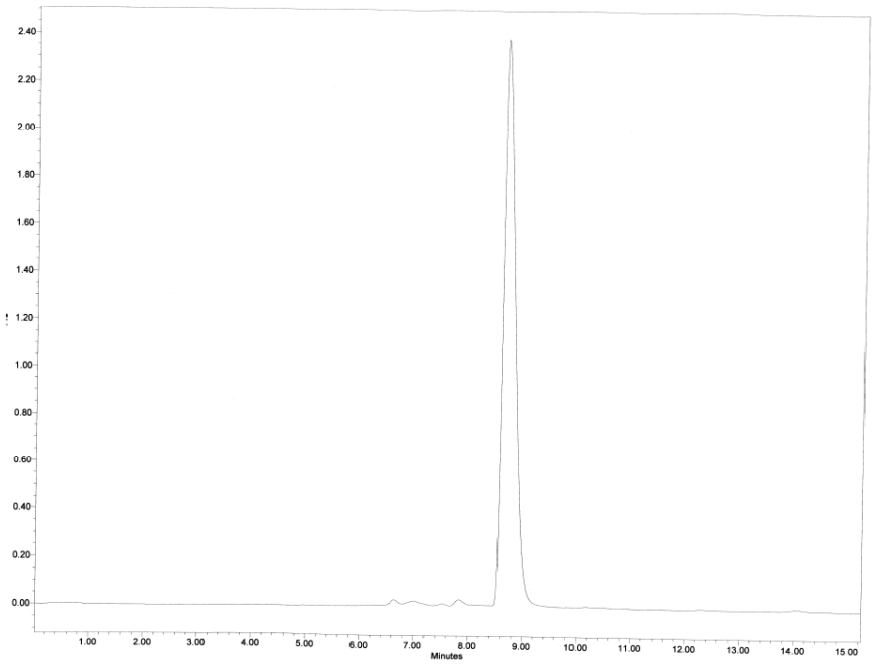


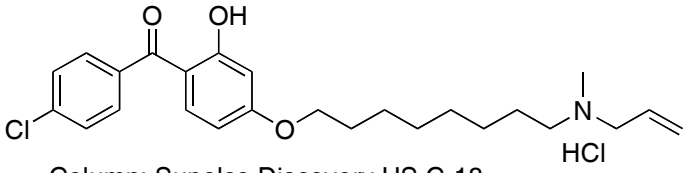
Pulse Sequence: s2pul



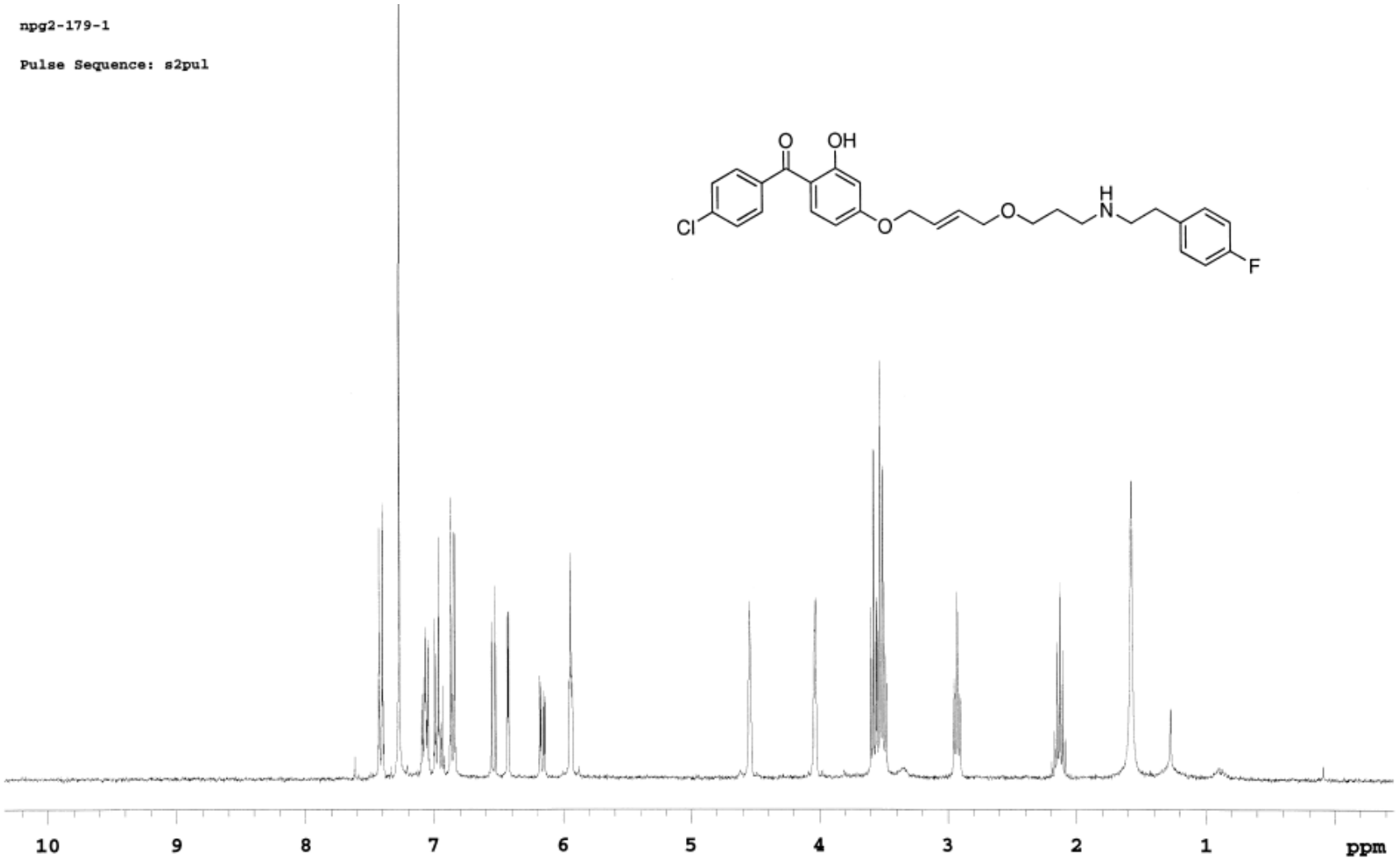


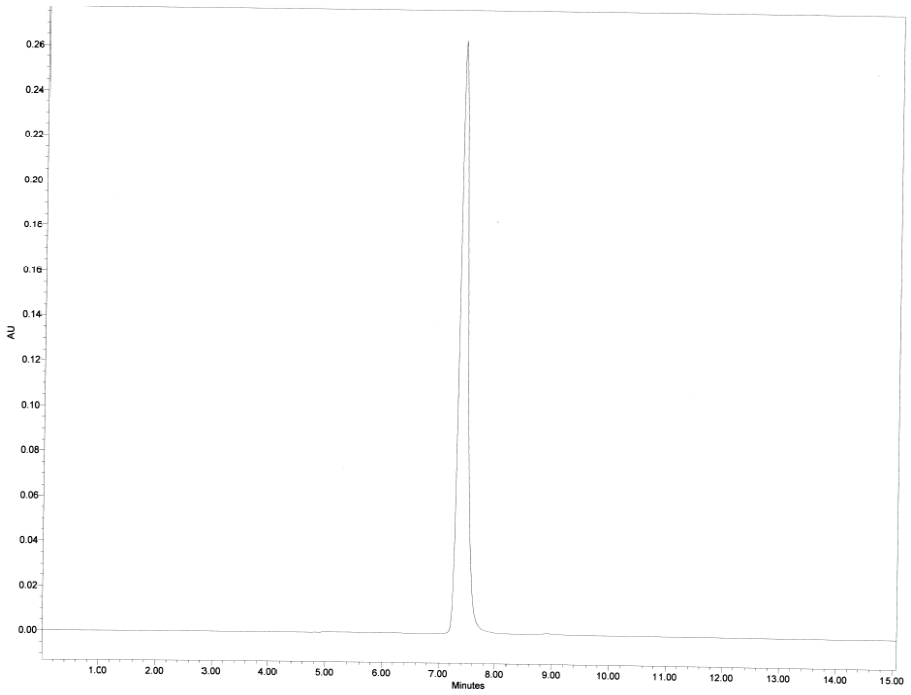

OH
O
O
N
HCl
Cl
ppm
12
11
10
9
8
7
6
5
4
3
2
1
0.58
4.93 0.98
1.78 0.91
1.00
1.95
2.31
1.92
1.17
0.992.82
0.23
2.15
10.74

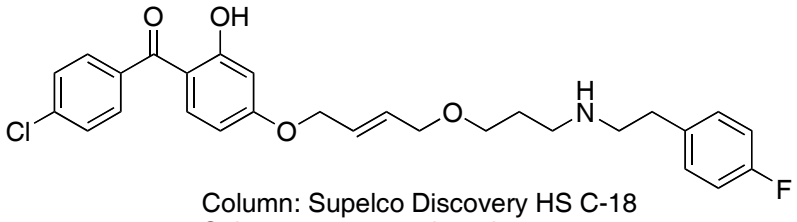




Column: Supelco Discovery HS C-18
Solvent : 100% methanol
flow rate: 0.5 mL / min
retention time: 8.8 min


npg2-179-1
Pulse Sequence: s2pul
O
OH
Cl
O
O
H
N
F
10
9
8
7
6
5
4
3
2
1
ppm





Column: Supelco Discovery HS C-18
Solvent : 100% methanol
flow rate: 0.5 mL / min
retention time: 7.4 min

References and Note

[1] We have synthesized only 100 analogues out of a 3,240-membered library which will be delivered using the building blocks A, B, and C summarized in Figure 1. All compounds were purified by chromatography on silica gel.
[2] Crick, D. C., Schulbach, M. C.; Zink, E. E.; Macchia, M.; Barontini, S.; Besra, G. S.; Brennan, P. J. *J. Bacteriol*. **2000**, *182*, 5771.
[3] We cloned Rv0534c, which is responsible for encoding the *menA* gene, and expressed in the pET28a vector and successfully purified Men enzyme.
[4] Shineberg, B.; Young, I. G. *Biochemistry* **1976**, *15*, 2754.
[5] MenA activity is absolutely dependent on the presence of the divalent cations as the treatment with EDTA or Biorex-70 (a cation exchange resin) completely abolished the activity.
[6] *National Committee for Clinical laboratory standards* **2003a**.
[7] Yajko, D. M., Sanders, C. A.; Madej, J. J.; Cawthon, V. L.; Hadley, W. K. *Antimicrob. Agents Chemother*. **1996**, *40*, 743.
[8] Lenaerts, A. J., Gruppo, V.; Marietta, K. S.; Johnson, C. M.; Driscoll, D. K.; Tompkins, N. M.; Rose, J. D.; Reynolds, R. C.; Orme, I. M. *Antimicrob. Agents Chemother*. **2005**, *49*, 2294.