Supporting Information

Lipid-Soluble 3-Pyridinol Antioxidants Spare α-Tocopherol and Do Not Efficiently Mediate Peroxidation of Cholesterol Esters in Human Low-Density Lipoprotein

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I. Supplemental Oxidation Data

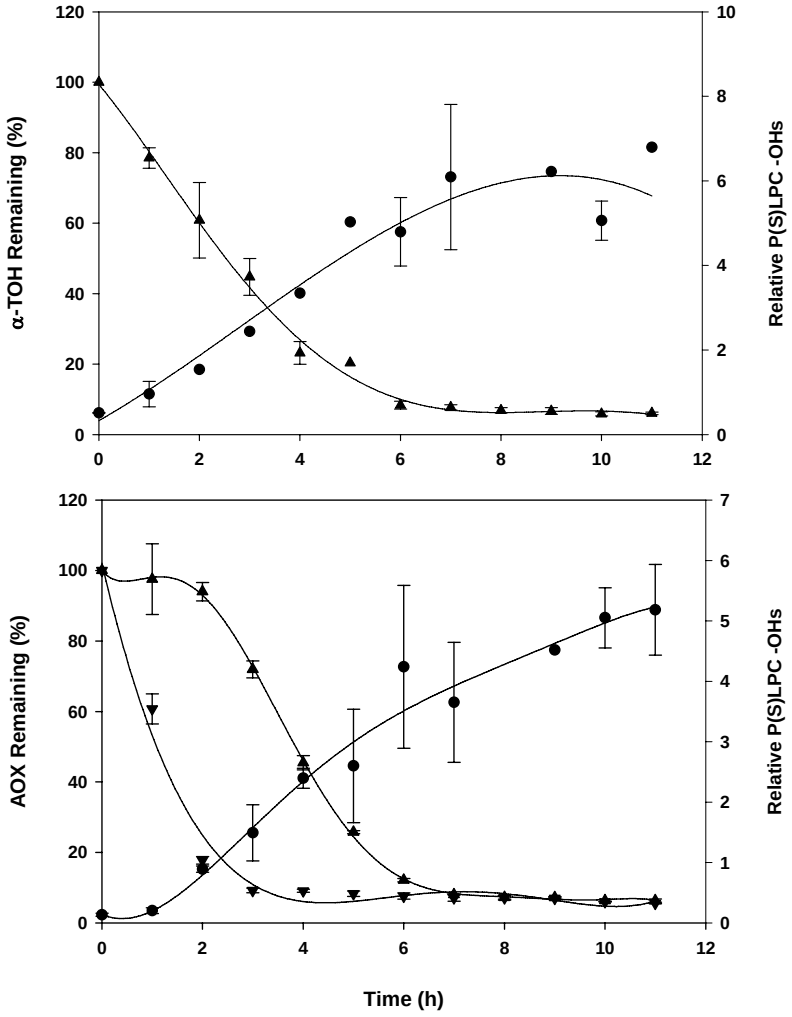


Figure S1. Oxidation of human LDL (0.75 mg/ml) in PBS initiated by decomposition of the azo-initiator C-0 (0.5 mM) at 37ºC. Relative total 1-palmitoyl-2-linoleyl-*sn*-glycero-3-phosphatidylcholine (PLPC) and 1-steroyl-2-linoleyl-*sn*-glycero-3-phosphatidylcholine (SLPC) alcohols (●, the hydroperoxides were reduced with PPh_3 prior to analysis as the pentafluorobenzyl esters by APCI-LC-MS/MS) are given on the right axis and the number of antioxidant molecules remaining per LDL (**6,** ▼; α-TOH, ▲) are given on the left axis.

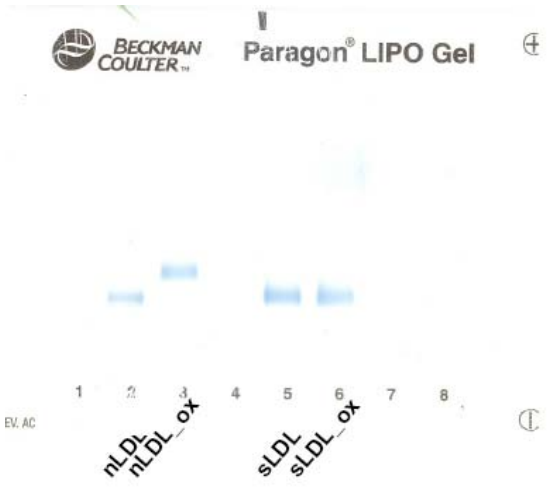
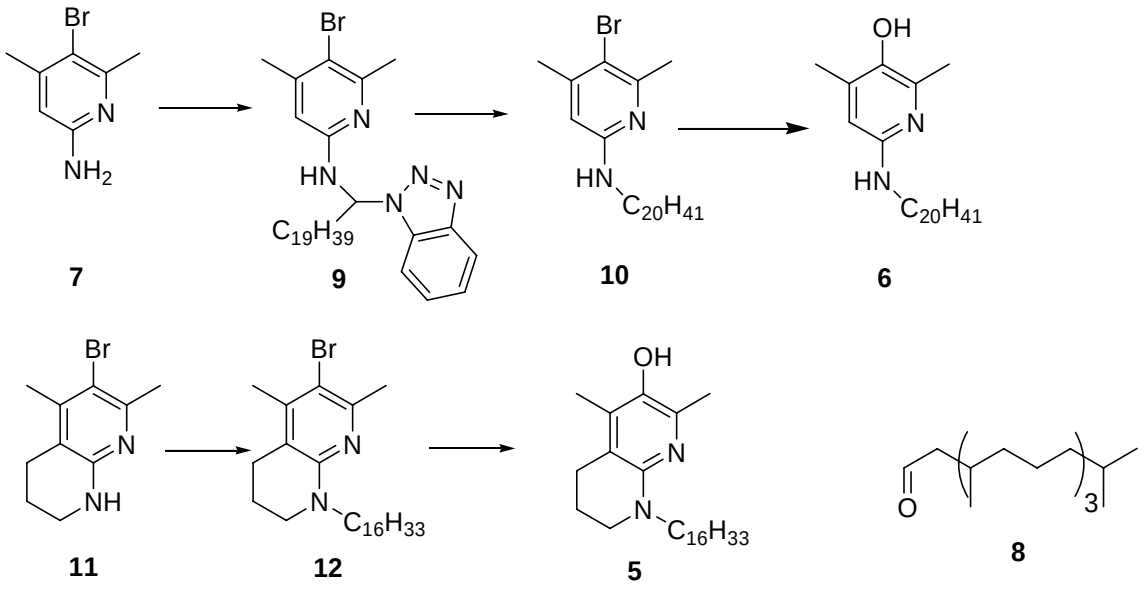


Figure S2. Agarose gel electrophoresis ran @100V for 30 min. Both native and supplemented LDL were oxidized @ 37 °C for 21h initiated by C-0 (final concentration 0.5 mM). Bis-Tris gel supported extreme degradation of protein after extensive oxidation of LDL (data not shown).

II. Preparation of compounds 5 and 6



2-Amino-5-bromo-4,6-dimethyl-pyridine (7). The synthesis and spectral data of this compound have been reported by us.[1]

3,7,11,15-Tetramethyl-hexadecanal (8). 3,7,11,15-Tetramethyl-hexadecanol[2] (2.1 g, 7.1 mmol) and TEMPO[3] (0.010 g, 0.06 mmol) were dissolved in CH_2Cl_2 (50 mL). Water (1 mL) and KBr (0.08 g, 0.67 mmol) were added and the solution was cooled in an icebath. Commercial Chlorox bleach (5.25 %, 0.81 M, 12.3 mL, 9.9 mmol) was mixed with $NaHCO_3$ (1.4 g) and this mixture was added to the reaction mixture at once. A red color developed, which disappeared after 2 – 5 min indicating completion of the oxidation. If necessary, more bleach was added to complete the reaction (TLC analysis). The layers were allowed to settle. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated to afford 1.9 g of crude aldehyde. The product was purified by column chromatography (5 : 1 hexanes : EtOAc) to afford a yellowish oil (1.67 g, 79 %). Spectral data were in agreement with the literature.[4]

2-[(1-Benzotriazol-1-yl-3,7,11,15-tetramethyl-hexadecyl)-amino]-5-bromo-4,6-dimethyl-pyridine (9). A synthetic protocol reported by Katritzky was used.[5] Bromide **7** (1.62 g, 8.1 mmol), benzotriazole (0.955 g, 8.1 mmol) and aldehyde **8** (2.62 g, 8.8 mmol) were dissolved in EtOH (25 mL). The reaction mixture was heated at reflux for 30 min and then stirred at ambient temperature for 16 h. During this time, the product precipitated. The solvent was evaporated and the resulting solid dried to give a white product (4.8 g) of sufficient purity for the next step. An analytical sample was reprecipitated and filtered from EtOH. m.p. 82 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, 1H, HBt, J=8.1 Hz), 7.89 (d, 1H, HBt, J=8.4 Hz), 7.45 (t, 1H, HBt, J=7.2 Hz), 7.32 (t, 1H, HBt, J=7.8 Hz), 6.9 (m, 1H, NCHN), 6.18 (s, 1H, ArH), 5.73 (m, 1H, NH), 2.44 (s, 3H, 6-Me), 2.13 (s, 3H, 4-Me), 1.28 – 0.9 (24H, alkyl), 0.85 (t, 15H, alkyl-CH_3, J=6.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.5, 154.4, 149.2, 127.5, 126.7, 124.3, 120.1, 118.7, 113.8, 111.3, 108.1, 65.2, 39.77, 37.79, 37.69, 37.57, 37.47, 33.18, 33.06, 29.91, 29.64, 28.38, 25.52, 25.49, 25.22, 24.86, 24.54, 23.88, 23.76, 23.15, 23.05, 20.17, 20.09, 19.96, 19.69; HRMS for $C_{33}H_{52}BrN_5B$ [M + H] 598.3479, found 479.2938 (complete cleavage of C-Bt bond: - 119.048 amu, found: - 119.054 amu).

5-Bromo-4,6-dimethyl-2-[(3,7,11,15-tetramethyl-hexadecyl)-amino]-pyridine (10). The benzotriazole adduct **9** (4.7 g, ca. 7.83 mmol) was dissolved in THF (35 mL) and the solution was cooled in an ice bath. Solid $LiAlH_4$ (0.3 g, 7.9 mmol) was slowly added and the reaction mixture was warmed to room temperature and stirred for 30 min. Sequentially were then added 300 μL of water, 300 μL of 15 % NaOH and 600 μL of water. The mixture was filtered over Celite and the cake was washed with ether. The filtrate was concentrated and the residue was purified by column chromatography (9 : 1 hexanes : EtOAc) to give the product as a yellow oil (3.3 g, 85 % starting from **7**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.13 (s, 1H, ArH), 4.35 (t, 1H, NH, J=5.4 Hz), 3.14 (m, 2H, NCH_2), 2.46 (s, 3H, 6-Me), 2.19 (s, 3H, 4-Me), 1.48 – 0.9 (24H, alkyl), 0.77 (t, 15H, alkyl-CH_3, J=6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.2, 155.4, 148.3, 111.0, 105.2, 51.4, 40.86, 39.70, 37.80, 37.75, 37.64, 37.58, 37.04, 36.95, 33.0, 30.9, 28.3, 25.65, 25.17, 24.88, 24.85, 24.82, 24.79, 24.74, 24.0, 23.13, 23.04, 20.12, 20.05, 20.00, 20.93; IR (film) ν (cm^{-1}) 2900, 2450, 1580, 1510, 1100, 730; HRMS for $C_{27}H_{49}BrN_2$ [M + H] 481.3152, found 481.3155.

2,4-Dimethyl-3-hydroxy-6-(3,7,11,15-tetramethyl-hexadecylamino)-pyridine (6). This compound was obtained using a lithiation/oxidation sequence recently reported by us.[1] Bromide **10** (790 mg, 1.7 mmol) was dissolved in THF (20 mL) and the solution was cooled to –78 °C. Then, s-BuLi (1.3 M in pentane, 4.0 mL, 5.5 mmol) was added and the yellow mixture was stirred at – 78 °C for 15 – 30 min (use of 2 eq of *s*-BuLi or 3 eq of *n*-BuLi led to incomplete exchange which is a result of concomitant deprotonation of the –NH group). Subsequently, dry 2-nitro-*m*-xylene (1.1 mL, 8.1 mmol) was added, resulting in a brown-green mixture. After stirring for 2 h at –78 °C, the reaction mixture was warmed up and quenched with satd. aq. NH_4Cl-soln. The THF layer was separated and the aqueous layer extracted once with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (2 : 1 hexanes : EtOAc) to afford a yellow oil (200 mg, 28 %). λ_{max} (MeOH) = 323 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.06 (s, 1H, ArH), 4.10 (br s, 1H, NH and OH), 3.08 (m, 2H, NCH_2), 2.28 (s, 3H, 6-Me), 2.19 (s, 3H, 4-Me), 1.48 – 0.9 (24H, alkyl), 0.83 (t, 15H, alkyl-CH_3, J=6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.6, 143.5, 141.7, 137.5, 105.3, 41.7, 39.8, 37.90, 37.85, 37.80, 37.69, 37.28, 37.19, 33.2, 31.1, 28.4, 25.2, 24.87, 24.79, 23.11, 23.01, 20.15, 20.08, 20.05, 19.99, 18.98, 16.89; IR (film) ν (cm^{-1}) 3400, 2930, 1610, 1480, 1420, 1220, 1050, 730; HRMS (FAB) for $C_{27}H_{50}N_2O$ [M] 418.3931, found 418.3928; HPLC purity: >95 area % (detection at 330 nm), >96 area % (electrochemical detection at +0.5 V).

6-Bromo-5,7-dimethyl-1,2,3,4-tetrahydro-[1,8]naphthyridine (11). The synthesis and spectral data of this compound have been reported by us.

6-Bromo-5,7-dimethyl-1-hexadecyl-1,2,3,4-tetrahydro-[1,8]naphthyridine (12). A mixture of hexadecanal (2.19 g, 9.1 mmol), bromide **11** (2.0 g, 8.3 mmol) and AcOH (0.47 mL, 8.3 mmol) was stirred in dichloroethane (40 mL). To this was added $NaB(OAc)_3H$ (2.64 g, 12.5 mmol) and the mixture was stirred at room temperature for 1 h. More aldehyde (0.8 g, 3.32 mmol) was added and stirring was continued for an additional hour. Aq. 1.0 M NaOH was added and the mixture was extracted with EtOAc (3x). The combined organic layers were concentrated and redissolved in MeOH (20 mL). Excess $NaBH_4$ (ca. 1 g, 29 mmol) was added and the mixture was stirred overnight at room temperature (this served to completely reduce remaining aldehyde since it co-eluted with the product). The mixture was quenched with 40 mL of 1.0 M HCl (gas evolution!!!), the MeOH was evaporated and the residue was extracted with aq. 1.0 M NaOH and CH_2Cl_2 (3x). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The product was purified by column chromatography (10 : 1 hexanes : EtOAc) to afford the product as a colorless oil (3.16 g, 82 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.6 (t, 2H, NCH_2-ring, J=6.8 Hz), 3.3 (t, 2H, NCH_2-chain, J=5.6), 2.6 (t, 2H, Ar-CH_2, J=6.3 Hz), 2.4 (s, 3H, 6-Me), 2.2 (s, 3H, 4-Me), 1.9 (m, 2H, CH_2-ring), 1.5 (m, 2H, $NCH_2C\underline{H}_2$-chain), 1.3– 1.2 (m, 26H, alkyl), 0.9 (t, 3H, alkyl-CH_3, J=6.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0, 152.2, 143.6, 114.2, 110.7, 48.9, 47.4, 32.3, 30.1, 29.9, 29.8, 27.4, 27.3, 26.2, 26.0, 23.2, 22.2, 19.5, 14.5. HRMS for $C_{26}H_{45}BrN_2$ [M + H] 465.2766 / 467.2746, found 465.2819 / 467.2812.

2,4-Dimethyl-8-hexadecyl-5,6,7,8-tetrahydro-[1,8]naphthyridin-3-ol (5). This compound was obtained using a lithiation/oxidation sequence recently reported by us.[1]

Bromide **12** (343 mg, 0.74 mmol) was dissolved in THF (10 mL) and the solution was cooled to –78 °C. Then *n*-BuLi (2.5 M in hexanes, 1.19 mL, 1.67 mmol) was added and the yellow reaction mixture was stirred at – 78 °C for 15 – 30 min (the Br/Li exchange was monitored by TLC and was quantitative). Then dry 2-nitro-*m*-xylene (0.5 mL, 3.7 mmol) was added, resulting in a brown mixture. After stirring for 2 h at –78 °C, the reaction mixture was warmed up and quenched with satd. aq. NH_4Cl-soln. The THF layer was separated and the aqueous layer extracted once with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (9 : 1 hexanes : EtOAc) to afford a light brown powder (88 mg, 0.22 mmol, 30 %). λ_{max} (MeOH) =331 nm; ^{1}H NMR (DMSO-d_6 + CD_2Cl_2, 300 MHz) δ 7.32 (s, 1H, Ar-OH), 3.45 (t, 2H, NCH_2-chain, J=7.2), 3.17 (t, 2H, NCH_2-ring, 5.4), 2.53 (t, 2H, Ar-CH_2, J=6.3), 2.18 (s, 3H, 6-Me), 1.98 (s, 3H, 4-Me), 1.83 (m, 2H, CH_2-ring), 1.46 (m, 2H, $NCH_2C\underline{H}_2$-chain), 1.23 (m, 26H, alkyl), 0.85 (t, 3H, alkyl-CH_3). ^{13}C NMR (d^6-DMSO + CD_2Cl_2, 75 MHz) δ149.5, 140.4, 140.2, 133.8, 113.1, 48.3, 47.3, 31.7, 29.5, 29.3, 29.1, 26.9, 26.7, 24.7, 22.5, 22.2, 19.7, 14.1. HRMS for $C_{26}H_{46}N_2O$ [M + H] 403.3683, found 403.3685; HPLC purity: >96 area % (detection at 330 nm), >98 area % (electrochemical detection at +0.5 V).

Spectral data for final compounds.

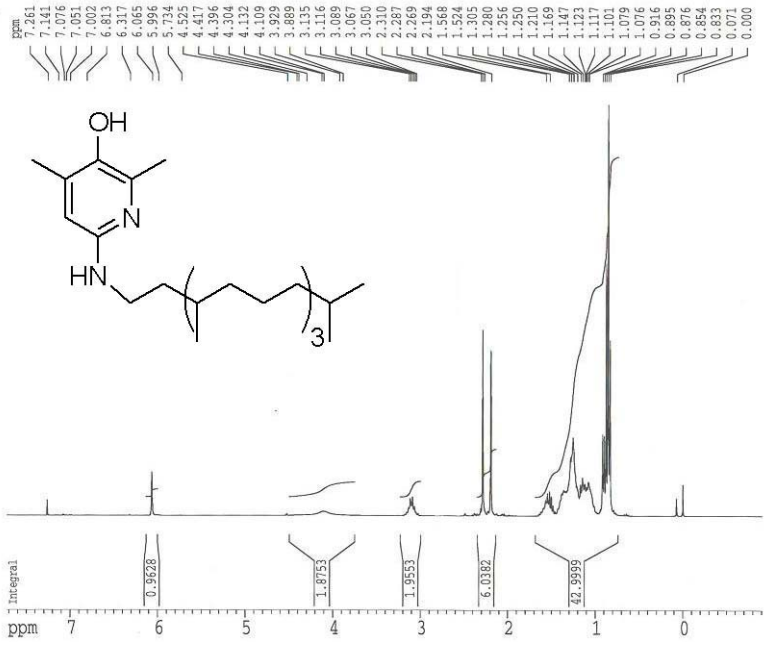


Figure S3. ^{1}H-NMR spectrum of pyridinol **6** in $CDCl_3$.

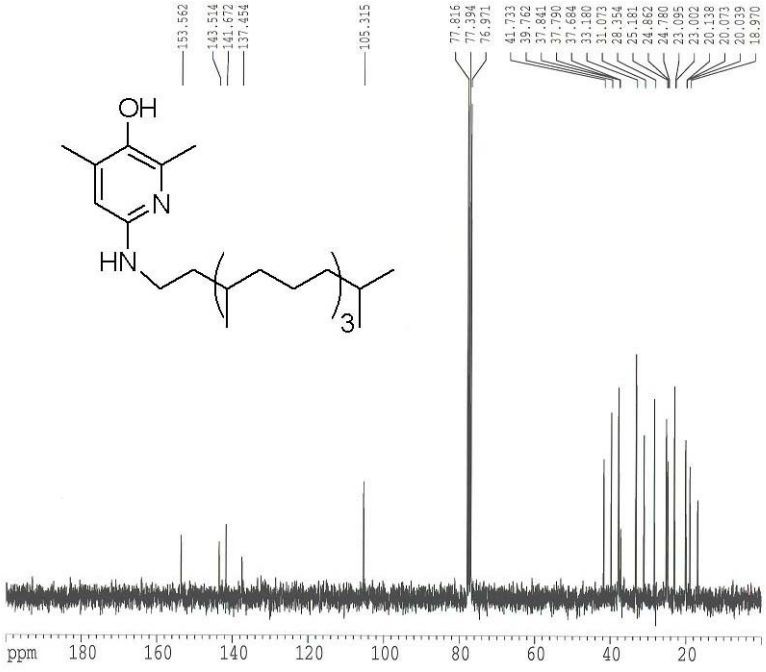


Figure S4. ^{13}C-NMR spectrum of pyridinol **6** in $CDCl_3$.

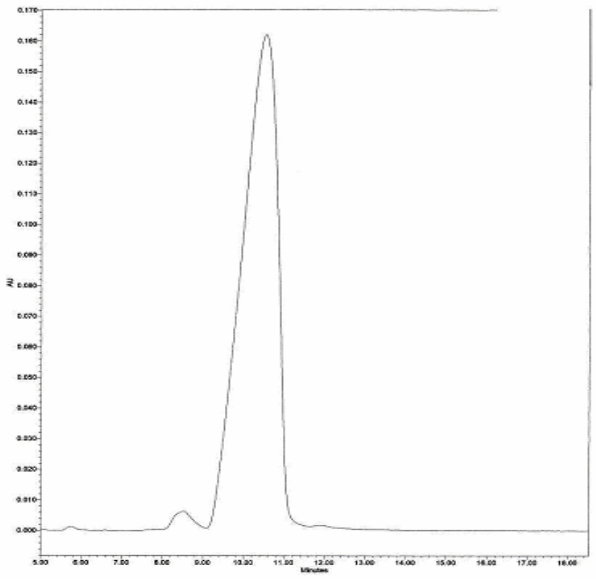


Figure S5. RP-HPLC chromatogram of pyridinol **6** with detection at 330 nm using reverse phase conditions (1.5 mL/min, eluent: 4 L of MeOH mixed with 8.85 g $LiClO_4$, 200 mL water and 8.0 mL of distilled pyridine).

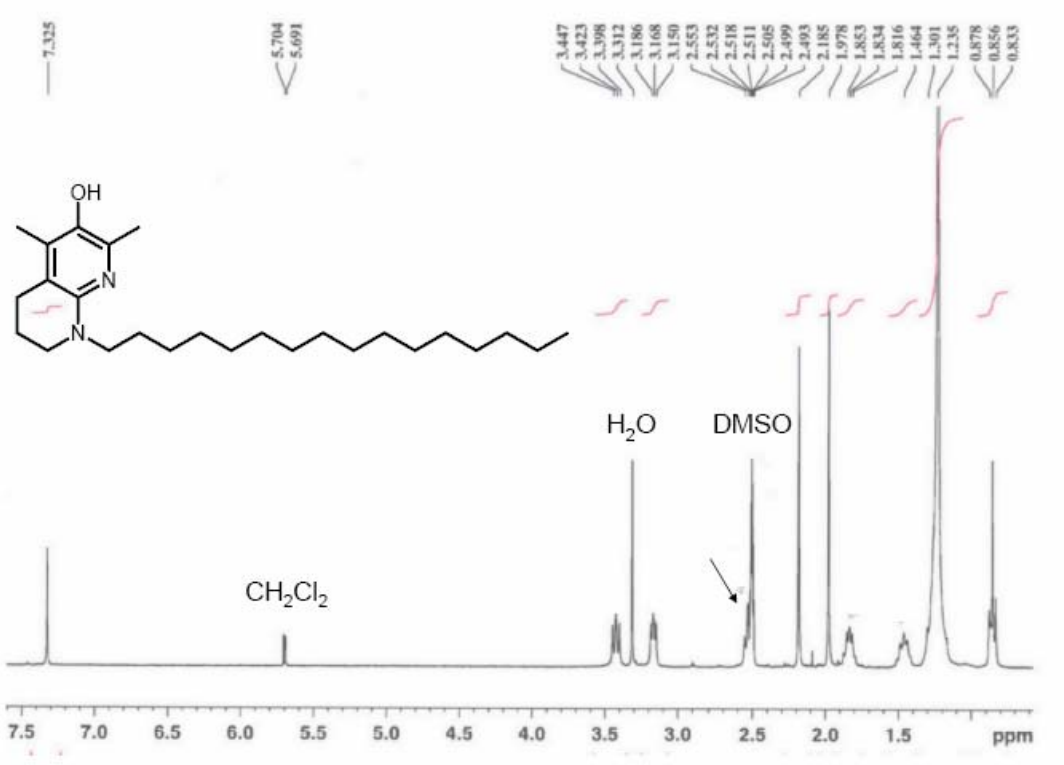


Figure S6. ^{1}H-NMR spectrum of pyridinol **5** in DMSO-d_6/CD_2Cl_2. The arrow indicates methylene protons under the DMSO.

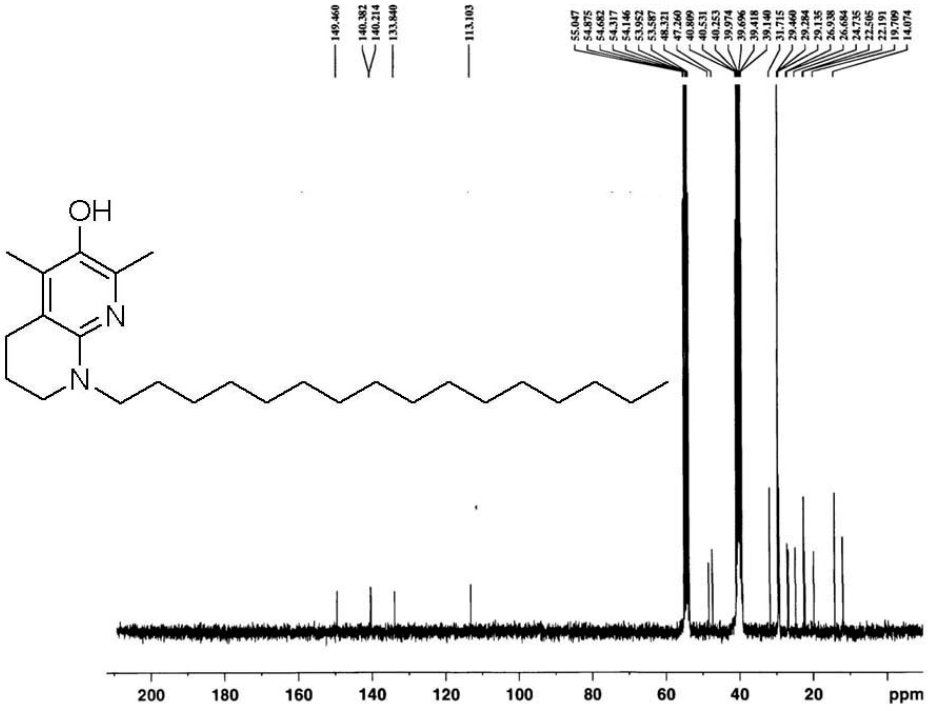


Figure S7. ^{13}C-NMR spectrum of pyridinol **5** in DMSO-d_6/CD_2Cl_2.

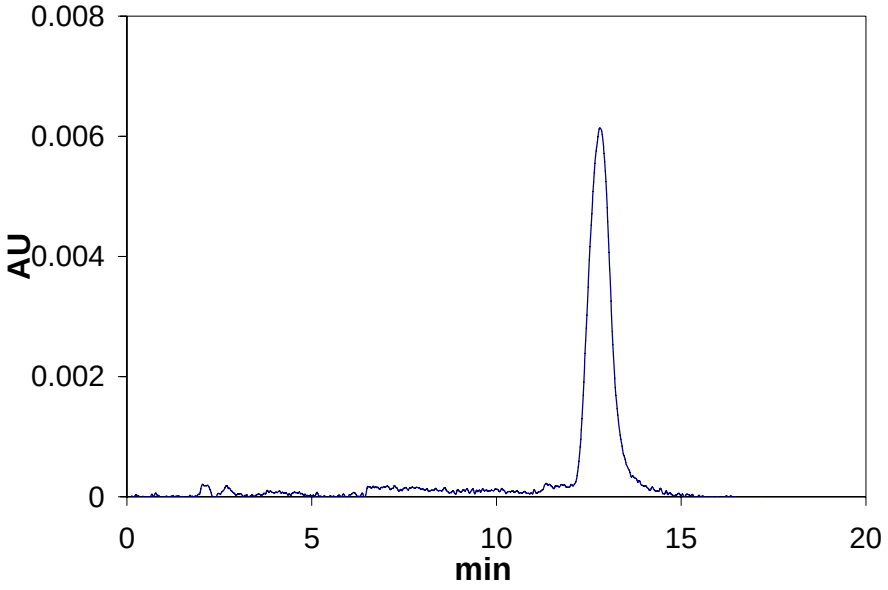


Figure S8. RP-HPLC chromatogram of pyridinol **5** with detection at 330 nm using reverse phase conditions (1.5 mL/min, eluent: 4 L of MeOH mixed with 8.85 g $LiClO_4$, 200 mL water and 8.0 mL of distilled pyridine).

References

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