Supporting Information

Chemoenzymatic cascades towards methylated tetrahydroprotoberberine and protoberberine alkaloids

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Contents

1. Enzyme sequences, expression and purification

Plasmid details

Plasmid details for M97V, L76V, M97F and wild-type Δ29*Tf*NCS have been previously reported by Lichman *et al*.[1] Plasmid details for the methyltransferase enzymes, *Rn*COMT, *Mx*SafC, *Ec*MAT and *Ec*MTAN have been previously reported by Siegrist *et al*.[2] UniProt codes are: *Tf*NCS (Q67A25), *Rn*COMT (P22734), *Mx*SafC (Q50859), *Ec*MAT (D1LDF1) and *Ec*MTAN (A7ZHQ1). Protein sequences and full plasmid details are given in the Supplementary Methods of these papers.

Enzyme expression

All plasmids were transformed into BL21(DE3) *E. coli*. 100 mL LB medium was inoculated with a single colony and grown at 37 °C for 18 h. 500 mL LB media was inoculated with 1% v/v of overnight culture and grown at 37 °C for 2 h shaking in flasks. Isopropylthiogalactoside (for all NCSs, 0.5 mM final concentration was used and for *Rn*COMT, *Mx*SafC, *Ec*MAT and *Ec*MTAN 0.2 mM final concentration was used) was added and the culture was incubated for 18 h at 25 °C (for all gene expressions except *Mx*SafC, where incubation was performed at 16 °C for 36 h). Cell pellets were isolated by centrifugation and stored at -20 °C until further purification or lysis for enzymatic reactions.

Lysate preparation

To prepare *Rn*COMT lysate for enzymatic reactions, the cell pellet was resuspended in buffer (50 mM HEPES, pH 7.5, 4% v/v of final culture volume) and lysed by sonication (10 s ON, 10 s OFF, 10 x). The solution was centrifuged (10 min, 6000 x g, 4 °C) and the supernatant removed and used directly for enzymatic reactions or stored at -20 °C.[3]

Protein purification

For purification of wild-type-Δ33*Tf*NCS, M97F/V-Δ29*Tf*NCS, L76V-Δ29*Tf*NCS, *Mx*SafC and *Ec*MAT, the cell pellet was resuspended in lysis buffer (100 mM HEPES, 20 mM imidazole, 100 mM NaCl, pH 7.5, 15% v/v culture volume) with a small amount of DNAse1. The cells were lysed by sonication (3 x 3 min ON, 3 min OFF) and centrifuged (15,000 x g, 45 min, 4 °C). The resulting supernatant was removed

and filtered (0.45 μm). A 5 mL His-trap HP column was equilibrated with lysis buffer and the lysate loaded onto the column at 1 mL min^{-1}. The column was washed with lysis buffer to remove any unbound protein (6 column volumes), followed by washings with a stepwise gradient of increasing imidazole concentrations by combining lysis buffer and elution buffer (100 mM HEPES, 500 mM imidazole, 100 mM NaCl, pH 7.5). The column was washed with 8% elution buffer for 6 column volumes, 16% elution buffer for 6 column volumes followed by 100% elution buffer. All washings were performed at a flow rate of 1 mL min^{-1}. Fractions were analysed by SDS-PAGE and fractions containing pure protein were combined and dialysed into buffer (20 mM TRIS, 50 mM NaCl, pH 7.5). The protein sample was centrifuged and concentrated to approximately 10 mg mL^{-1}. Aliquots of protein were stored at -80 ^{o}C until use for enzymatic reactions.[3]

2. SDS-PAGE analysis

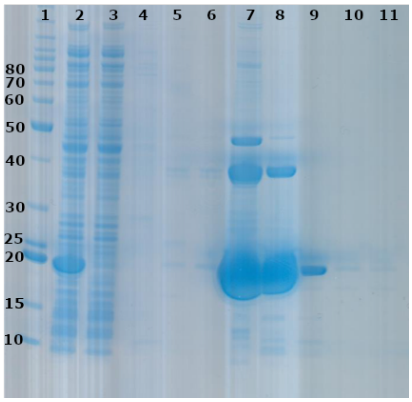
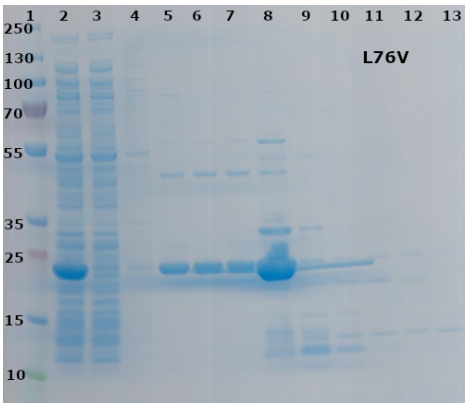
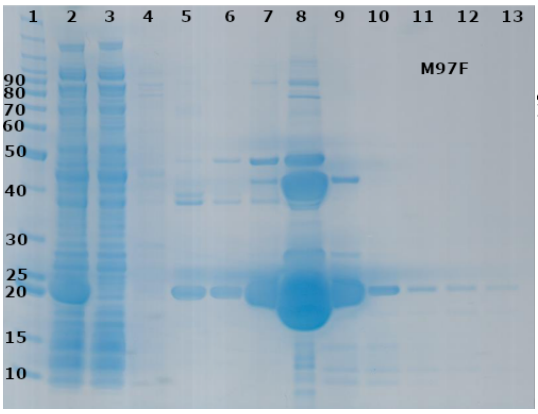


Figure S1: His-trap purification of wild-type∆33*Tf*NCS. Lanes: 1, Benchmark™ Protein Ladder masses given in kDa. 2, clarified cell lysate. 3, flow through with lysis buffer. 4, wash with 6 CV 20 mM imidazole buffer. 5, wash with 6 CV 40 mM imidazole buffer. 6-11, wash with 500 mM imidazole buffer and collected 4 mL fractions.





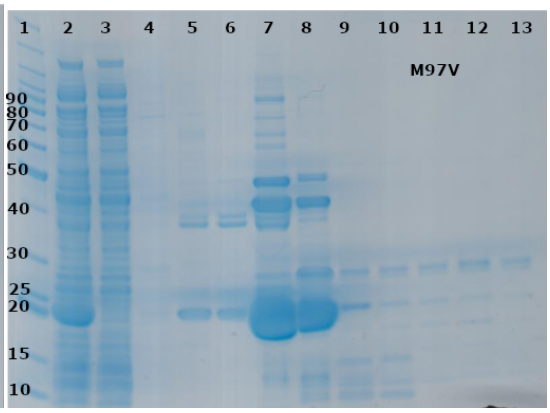


Figure S2: His-trap purification of ∆29*Tf*NCS-M97F, ∆29*Tf*NCS-M97V and ∆29*Tf*NCS-L76V mutants respectively: Lanes: 1, Benchmark™ Protein Ladder (or Prestained PageRuler Plus™ for ∆29*Tf*NCS-L76V), masses given in kDa. 2, clarified cell lysate loaded onto column. 3, flow through with lysis buffer. 4, wash with 6 CV 20 mM imidazole buffer. 5, wash with 6 CV 40 mM imidazole buffer. 6-13, wash with 500 mM imidazole buffer and collected 3 mL fractions.

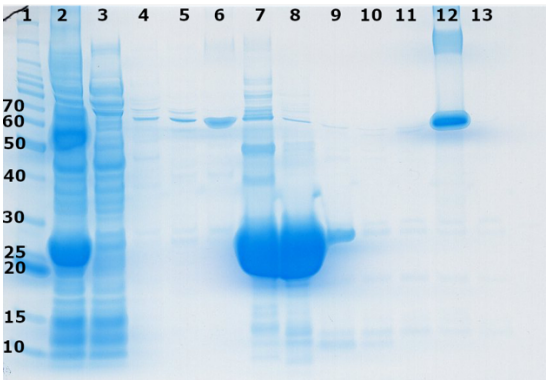


Figure S3: SDS-PAGE analysis of His-trap purification of *Mx*SafC. CV = column volume. Lanes: 1, Benchmark™ Protein Ladder (masses given in kDa) 2, clarified cell lysate loaded onto column. 3, flow through with lysis buffer. 4, wash with 6 CV 20 mM imidazole buffer. 5, wash with 6 CV 40 mM imidazole buffer. 6-13, wash with 500 mM imidazole buffer and collected 3 mL fractions.

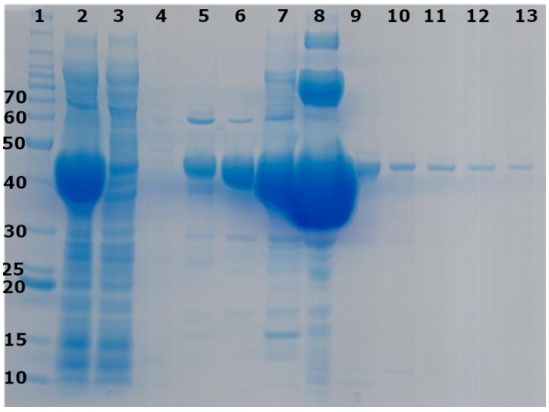


Figure S4: SDS-PAGE analysis of His-trap purification of *Ec*MAT. CV = column volume. Lanes: 1, Benchmark™ Protein Ladder (masses given in kDa) 2, clarified cell lysate loaded onto column. 3, flow through with lysis buffer. 4, wash with 6 CV 20 mM imidazole buffer. 5, wash with 6 CV 40 mM imidazole buffer. 6-13, wash with 500 mM imidazole buffer and collected 3 mL fractions.

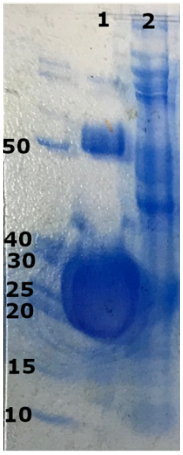


Figure S5: SDS-PAGE analysis. Lane 1: *Ec*MTAN purification, Lane 2: *Rn*COMT expression.

3. HPLC methods

Analytical HPLC

Analytical analysis of samples was performed using an Agilent 1260 Infinity liquid chromatography system with the following units: G1329B autosampler, G1311C quaternary pump, 1260 G1316A column oven and 1260 G1314F variable wavelength detector. A HiChrom ACE C18 column (250 mm x 4.6 mm) was used. In all cases, a flow rate of 1 mL min^{-1} was used. The initial reaction volume was diluted to 25% reaction concentration and 10 μL of sample was injected. The mobile phase used was mixtures of acetonitrile (MeCN) in water (0.1% v/v TFA). UV absorbance was measured at 280 nm.

Achiral HPLC method 1: The gradient used was: 10% MeCN for 1 min, a linear gradient to 70% MeCN over 5 min, 100% MeCN for 30 s, followed by 10% MeCN for 3.5 min.

Achiral HPLC method 2: The gradient used was: 10% MeCN for 1 min, a linear gradient to 70% MeCN over 15 min, 100% MeCN for 30 s, followed by 10% MeCN for 3.5 min.

Preparative HPLC

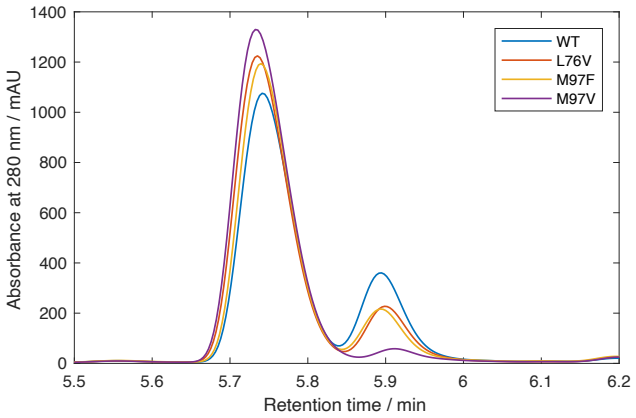
Isolation of compounds by preparative HPLC was performed using Agilent 1200 Infinity series liquid chromatography system equipped with G1361A prep pump, G2260A autosampler, G1364A fraction collector and G7165 multiple wavelength detector. A Supelco Discovery®BIO Wide Pore C18-10 column (25 cm x 21.2 mm, 10 μM) was used with MeCN (0.1% v/v TFA) and water (0.1% v/v TFA) as the mobile phase. UV absorbance was measured at 280 nm**.**

Preparative HPLC Method 1: 20-minute gradient 25-95% MeCN (0.1% v/v TFA) in water (0.1% v/v TFA).

Preparative HPLC Method 2: 30-minute gradient 25-30% MeCN (0.1% v/v TFA) in water (0.1% v/v TFA).

Preparative HPLC Method 3: 30-minute gradient 5-95% MeCN (0.1% v/v TFA) in water (0.1% v/v TFA).

4. Enzymatic assay with *Tf*NCS variants



*Tf*NCS variant used	Diastereomeric ratio of **3a** (1*S*,1'*R*):(1*S*,1'*S*)
Wild type	77:23
L76V	87:13
M97F	82:18
M97V	96:4

Figure S6: Analytical HPLC analysis of product **3a** formed by NCS-mediated Pictet-Spengler reactions. Analytical HPLC analysis was performed using method 1. Based on NMR analysis of purified products and previous work where the stereochemistries were determined, the peak at 5.7 min corresponds to (1*S*,1'*R*)-**3a** and the peak at 5.9 min corresponds to (1*S*,1'*S*)-**3a**. Reaction conditions are as described for all NCS-mediated reactions, using 0.2 mg mL^{-1} Δ29*Tf*NCS (wild-type and variants), 10 mM dopamine HCl (**1a**) and 20 mM aldehyde (**2**). Diastereomeric ratios of the product **3a** formed are given and were determined by taking the analytical HPLC peak area (method 1) of each isomer formed.

5. General Procedures and Synthetic Methodologies

General chemical methods

Geduran® Si 60 silica (43-60 μM) was used for silica column chromatography. Thin layer chromatography analysis was performed using plates with a silica gel matrix on an aluminium support and phosphomolydic acid stain was used for visualisation. All reagents were obtained from commercial sources (Sigma Aldrich, Fischer, ChemCruz, Alfa Aesar, Fluorochem) unless otherwise specified. Key starting materials were purchased from the following sources: **1a** was purchased from Sigma Aldrich and methyl 2-(3,4-dimethoxyphenyl)propanoate (starting material for the synthesis of **2**) was purchased from Fluorochem.

A Bruker Advance III 700 MHz spectrometer was used to obtain ^{1}H and ^{13}C NMR. Chemical shifts reported are relative to trimethylsilane (which is set at 0 ppm) and referenced to the residual, protonated NMR solvent. Coupling constants (*J*) in ^{1}H-NMR spectra are given in Hertz (Hz) and described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m). Data processing was performed using the Bruker Topspin 4.0.8 software package.

Mass spectrometry data was obtained using a Waters Aquity UPLC-MS system (LRMS [ES+]) or a Waters LCT Premier XE ESI Q-TOF mass spectrometer (HRMS). Infrared (IR) data was obtained using a Bruker Alpha Platinum-ATR machine. Optical rotations (α_D) were determined using a Bellingham and Stanley ADP 430 polarimeter.

NCS reactions

Enzymatic reactions were performed on a 10 mL or 50 mL scale. A solution of amine (10 mM), sodium ascorbate (10 mM) and aldehyde (10 or 20 mM) in HEPES buffer (50 mM, pH 7.5), with 10% v/v MeCN was prepared. *Tf*NCS-M97V (at 10 mg mL^{-1} in 20 mM TRIS, 50 mM NaCl, pH 7.5) was added to give a final concentration of 0.2 mg mL^{-1}. The reaction mixture was stirred at 37 ^{o}C for 24 h. After this time, the reaction was quenched by addition of MeCN (1 reaction volume), centrifuged (20 min, 4000 x g, 4 ^{o}C), the supernatant removed and concentrated under reduced pressure. To isolate the products for

characterization purposes, the resulting residue was then purified by preparative HPLC, as described in the characterization of both THIQs generated.

Methyltransferase reaction with *Rn*COMT or *Mx*SafC

To prepare the product, **3a** for methyltransferase reaction, the resulting residue from the NCS reaction was lyophilized and resuspended in 20% v/v NCS reaction volume of buffer (4 mL, 250 mM HEPES, 200 mM $MgCl_2$, 2 M KCl) and 80% NCS reaction volume of dH_2O (16 mL). ATP and L-methionine were prepared as 100 mM stocks in dH_2O and added to give a final concentration of 10 mM. *Ec*MAT (final concentration 0.4 mg mL^{-1}), *Ec*MTAN (final concentration 0.025 mg mL^{-1}) and methyltransferase (*Rn*COMT lysate at 10% v/v or *Mx*SafC at 0.6 mg mL^{-1} final concentration) were added. The reaction volume was adjusted by addition of buffer (50 mM HEPES, pH 7.5) to give a final substrate concentration of 5 mM and a reaction volume of 20 mL. The reaction mixture was stirred at 37 ^{o}C for 24 h then quenched by addition of MeCN (1 reaction volume). The reaction mixture was centrifuged (20 min, 4000 x g, 4 ^{o}C) and the supernatant removed.

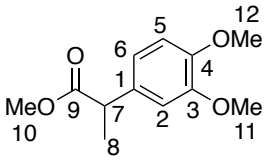
To isolate the product for characterization purposes, the supernatant was concentrated under reduced pressure and purified by preparative HPLC.

To prepare the product for chemical Pictet-Spengler reactions, the supernatant was saturated by addition of $NaHCO_3$ and the product extracted into ethyl acetate (3 x 20 mL). The organic phases were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure.

6. Small molecule characterization

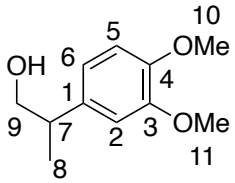
The synthesis and characterisation of 4-methoxytyramine (**1b**) has been reported.[3]

Methyl 2-(3,4-dimethoxyphenyl)propanoate[4]



Under anhydrous conditions, a solution of methyl 2-(3,4-dimethoxyphenyl)acetate (2.00 g, 9.52 mmol) in THF (10 mL) was prepared and cooled to -78 °C. A solution of LDA (7.2 mL, 2.0 M in THF, 14.3 mmol) was added dropwise and the reaction mixture stirred (*ca.* 30 min). Iodomethane (1.2 mL, 19 mmol) was added and the reaction stirred for 1 h, then quenched by the addition of 2 M HCl. The resulting solution was extracted with ethyl acetate (3 x 20 mL), washed with brine (2 x 20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (10% ethyl acetate in petroleum ether 40-60) to give the product as a colourless oil (1.35 g, 63%); ^{1}H-NMR (700 MHz; $CDCl_3$): δ 6.86 - 6.80 (3H, m, 2-H, 5-H and 6-H), 3.88 (3H, s, 12-OC*H*$_3$), 3.86 (3H, s, 11- OC*H*$_3$), 3.66 (1H, q, *J* = 7.3 Hz, 7-H), 3.66 (3H, s, 10-H), 1.48 (3H, d, *J* = 7.3 Hz, 8-H); ^{13}C-NMR (176 MHz; $CDCl_3$): δ 175.3, 149.1, 148.3, 133.2, 119.7, 111.3, 110.8 , 56.0, 56.0, 52.1, 45.1, 18.8; *m/z* (GC-MS): 224 [M+].

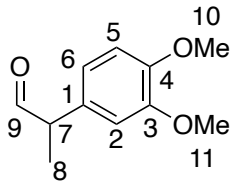
2-(3,4-Dimethoxyphenyl)propan-1-ol[4]



Under anhydrous conditions, a solution of 2-(3,4-dimethoxyphenyl)acetate (858 mg, 3.83 mmol) in THF (15 mL) was prepared, cooled to -78 °C and DIBAL-H (1.0 M in THF, 7.65 mmol, 7.65 mL) was added dropwise over 10 min. The reaction mixture was stirred for 2 h, then quenched by addition of Rochelle's solution (10 mL). The reaction was warmed to 0 °C and stirred for 1 h and then poured into water (10 mL). The reaction mixture was extracted with ethyl acetate (3 x 15 mL), dried with anhydrous

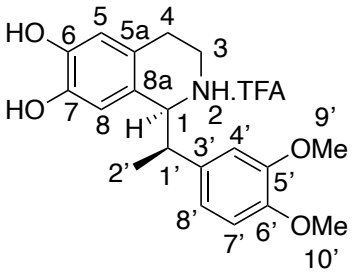
magnesium sulfate and concentrated under reduced pressure to give the product as a yellow oil (710 mg, 95%); ^{1}H-NMR (600 MHz; $CDCl_3$): δ 6.85 – 6.74 (3H, m, 2-H, 5-H and 6-H), 3.88 (3H, s, 10- OC*H*$_3$), 3.86 (3H, s, 11- OC*H*$_3$), 3.70-3.63 (2H, m, 9-H), 2.85 – 2.92 (1H, m, *J* = 6.8 Hz, 7-H), 1.25 (3H, d, *J* = 6.8 Hz, 8-H); ^{13}C-NMR (151 MHz; $CDCl_3$): δ 149.2, 147.9, 136.3, 119.4, 111.5, 110.9, 68.9, 56.1, 56.0, 42.2, 17.9; *m/z* [HRMS ES+] found $[M+Na]^+$ 219.0093; $C_{11}H_{16}O_3Na$ requires 219.0092.[4]

2-(3,4-Dimethoxyphenyl)propanal[4] (2)



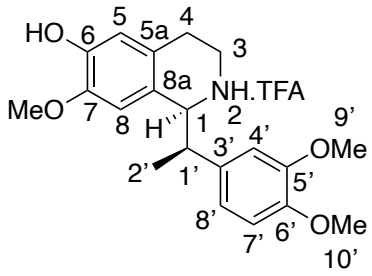
Under anhydrous conditions, a solution of oxalyl chloride (2.0 M in CH_2Cl_2; 1.9 mL, 3.8 mmol) in dichloromethane (10 mL) was prepared at -78 °C. DMSO (400 μL, 7.6 mmol) was added dropwise and the reaction mixture stirred for 30 min. 2-(3,4-Dimethoxyphenyl)propan-1-ol (500 mg, 2.6 mmol) in dichloromethane (*ca.* 5 mL) was then added and the reaction mixture stirred for 30 min, followed by the addition of triethylamine (2.1 mL, 15 mmol) and stirring for 30 min at -30 °C, then 30 min at 0 °C. The mixture was then diluted with pentane (15 mL), washed with sat. aq. $NaHCO_3$ (2 x 20 mL) and water (2 x 20 mL), dried with anhydrous sodium sulfate and concentrated under reduced pressure to give the product as an orange oil (360 mg, 47%). ^{1}H-NMR (700 MHz; $CDCl_3$): δ 9.66 (1H, d, *J* = 1.5 Hz, 9-H), 6.90 – 6.86 (1H, m, Ar*H*), 6.79 – 6.75 (1H, m, Ar*H*), 6.67 – 6.71 (1H, m, Ar*H*), 3.88 (6H, m, 10- OC*H*$_3$ and 11- OC*H*$_3$), 3.91 – 3.87 (1H, m, 7-H), 1.43 (3H, t, *J* = 6.9 Hz, 8-H); ^{13}C-NMR (176 MHz; $CDCl_3$): δ 201.2, 149.6, 148.7, 130.1, 120.6, 111.8, 111.5, 56.1, 56.1, 52.7, 14.7; *m/z* [MS ES+] 195 ($[M+H]^+$, 100%). The aldehyde was taken through to the next step without purification to avoid issues due to its oxidative sensitivity. The NMR data was consistent with that of the previously reported synthesis.[4] The estimated purity by ^{1}H NMR spectroscopy was 85%.

(1*S*,1'*R*)-(5',6'-Dimethoxyphenyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol.TFA (3a)



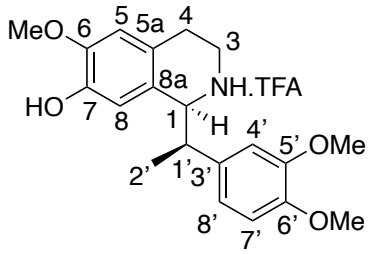
A solution of dopamine hydrochloride (19 mg, 0.10 mmol) and sodium ascorbate (20 mg, 0.10 mmol) in HEPES buffer (9 mL, 50 mM, pH 7.5) was prepared. A solution of 2-(3,4-dimethoxyphenyl)propanal (39 mg, 0.20 mmol) in acetonitrile (1 mL) was prepared and the two solutions mixed. Δ29*Tf*NCS-M97V (2 mg added from a 10 mg mL^{-1} stock in 20 mM Tris, 50 mM NaCl, pH 7.5) was added and the reaction mixture stirred at 37 °C on a heating mantle for 18 h. The reaction was monitored by HPLC and after completion, was quenched with MeCN (10 mL). The solution was then centrifuged (20 min, 5000 x g, rt) and the supernatant lyophilised. The resulting residue was purified by preparative-HPLC (method 1) to isolate the pure product as white solid (7.8 mg, 18% as the TFA salt, d.r. = 96:4 (1*S*,1'*R*):(1*S*,1'*S*)). $[\alpha]_D^{25}$ +15.2 (c = 0.71, MeOH); υ_{max}/ cm^{-1} (thin film): 2971, 2840, 1671, 1609, 1517; ^{1}H-NMR (700 MHz, CD_3OD) δ 6.94 (1H, d, *J* = 8.4 Hz, 7'-H), 6.82 (1H, dd, *J* = 8.4 Hz, 1.9 Hz, 8'-H), 6.78 – 6.75 (1H, m, 4'-H), 6.60 (1H, s, 5-H), 6.46 (1H, s, 8-H), 4.58 (1H, d, *J* = 6.1 Hz, 1-H), 3.82 (3H, s, 9'- OCH_3 or 10'-OCH_3), 3.77 (3H, s, 9'- OCH_3 or 10'-OCH_3), 3.53 – 3.48 (1H, m, 1'-H), 3.42 – 3.37 (1H, m, 3-*H*H), 3.21 – 3.14 (1H, m, 3-H*H*), 2.92 – 2.82 (2H, m, 4-H), 1.31 (3H, d, *J* = 7.3 Hz, 2'-H); ^{13}C-NMR (176 MHz, CD_3OD) δ 150.7, 150.0, 146.8, 145.5, 134.5, 124.2, 122.5, 121.4, 116.0, 114.6, 113.1, 113.0, 61.9, 56.3, 56.3, 43.1, 41.0, 25.4, 15.5, *m/z* [MS ES^+] 330 ($[M+H]^+$, 100%); m/z [HRMS ES+] found [M+H]+ 330.1700; $C_{19}H_{24}NO_4$ requires 330.1700; HPLC (C18, MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 1 min then a linear gradient MeCN (0.1% TFA)/H_2O (0.1% TFA) 70/30 over 5 min then 100% MeCN (0.1% TFA) for 0.5 min then MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 3.5 min, flow rate = 0.1 mL/min, I = 280 nm) t_R = 7.9 min.

((1*S*,1'*R*)-(5',6'-Dimethoxyphenyl)ethyl)-7-methoxy-1,2,3,4-tetrahydroisoquinolin-7-ol.TFA (3b)



An NCS-mediated reaction between 4-methoxytyramine and 2-(3,4-dimethoxyphenyl)propanal was performed as described in the standard NCS reaction conditions or for the reaction to give **3a**, followed by lyophilisation, and **3b** was isolated by preparative HPLC (method 1) as a white solid (35.5 mg, 81% as the TFA salt) with a diastereomeric ratio of 92:8 of (1*S*,1'*R*:1*S*:1'*S*)**.** $[\alpha]_D^{25}$ +1.5 (c = 2.2, MeOH); ν_{max}/ cm^{-1} (thin film): 2919, 1674, 1608, 1516; ^{1}H-NMR (600 MHz; CD_3OD) δ 6.96 (1H, d, *J* = 8.3 Hz, 7'-H), 6.82 (1H, dd, *J* = 8.3, 2.0 Hz, 8'-H), 6.79 (1H, d, *J* = 2.0 Hz, 4'-H), 6.63 (1H, s, 5-H), 6.20 (1H, s, 8-H), 4.85 (1H, d, *J* = 7.7 Hz, 1-H), 3.82 (3H, s, 9'- H or 10'-H), 3.77 (3H, s, 9'-H or 10'-H), 3.55 (3H, s, 7-O*CH*$_3$), 3.54 – 3.42 (2H, m, 1'-H and 3-*H*H), 3.30 – 3.25 (1H, m, 3-H*H*), 3.02 – 2.91 (2H, m, 4-H), 1.38 (3H, d, *J* = 7.1 Hz, 2'-H); ^{13}C-NMR (151 MHz; CD_3OD) δ 150.7, 150.0, 147.8,147.7, 135.1, 125.4, 122.8, 121.6, 116.0, 113.5, 113.2, 111.7, 61.9, 56.5, 56.5, 56.2, 43.5, 40.1, 25.4, 16.3; *m/z* [MS ES$^+$] 344 ([M+H]$^+$, 100%); m/z [HRMS ES+] found [M+H]+ 344.1862; $C_{20}H_{26}NO_4$ requires 344.1856.

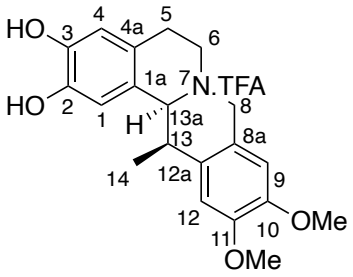
(1*S*,1'*R*)-(5',6'-Dimethoxyphenyl)ethyl)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-7-ol.TFA (3c)



A NCS-mediated reaction between dopamine and 2-(3,4-dimethoxyphenyl)propanal was performed as described in the standard NCS reaction conditions or for the reaction to give **3a**, followed by lyophilisation. The residue was resuspended in buffer (4 mL, 250 mM HEPES, 200 mM $MgCl_2$, 2 M KCl, pH 7.5). ATP (2 mL, 100 mM in dH_2O), L-methionine (2 mL, 100 mM in dH_2O), *Ec*MAT (888 μL, at 9 mg mL^{-1} purified in 50 mM HEPES, pH 7.5, 0.4 mg mL^{-1} final concentration), *Ec*MTAN (200 μL, 2.5 mg mL^{-1} purified, 0.025 mg mL^{-1} final concentration) and *Rn*COMT (10% v/v lysate) were added. The reaction

volume was adjusted to 20 mL by addition of HEPES buffer (50 mM, pH 7.5). Reactions were performed at 37 °C on a heating mantle for 24 h. The resulting residue was lyophilised for a further reaction step or purified by preparative HPLC (method 1) to give the product as a white solid (5.3 mg, 12% isolated yield as the TFA salt over two steps). $[\alpha]_D^{25}$ +4.0 (*c* 0.45, MeOH); ν_{max}/ cm^{-1} (thin film): 2921, 2851, 1710, 1688, 1513; ^{1}H-NMR (700 MHz; CD_3OD) δ 6.95 (1H, d, *J* = 8.2 Hz, 7'-H), 6.83 (1H, dd, *J* = 8.2, 2.1 Hz, 8'-H), 6.80 (1H, d, *J* = 1.8 Hz, 4'-H), 6.76 (1H, s, 5-H), 6.50 (1H, s, 8-H), 4.62 (1H, d, *J* = 6.0 Hz, 1-H), 3.84 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 3.78 (3H, s, OC*H*$_3$), 3.56 – 3.51 (1H, m, 1'-H), 3.46 – 3.41 (1H, m, 3-*H*H), 3.23 – 3.17 (1H, m, 3-H*H*), 3.01 – 2.90 (2H, m, 4-H), 1.30 (2H, d, *J* = 7.2 Hz, 2'-H); ^{13}C-NMR (176 MHz; CD_3OD) δ 150.7, 150.0, 149.1, 146.6, 134.4, 124.2, 123.9, 121.4, 114.5, 113.1, 113.1, 112.4, 61.8, 56.4, 56.4, 56.3, 43.0, 41.1, 25.6, 15.2; *m/z* [MS ES+] 344 ([M+H]$^+$, 100%); *m/z* [HRMS ES+] found [M+H]$^+$ 344.1853; $C_{20}H_{26}NO_4$ requires 344.1862; HPLC (C18, MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 1 min then a linear gradient MeCN (0.1% TFA)/H_2O (0.1% TFA) 70/30 over 5 min then 100% MeCN (0.1% TFA) for 0.5 min then MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 3.5 min, flow rate = 0.1 mL/min, I = 280 nm) t_R = 8.9 min.

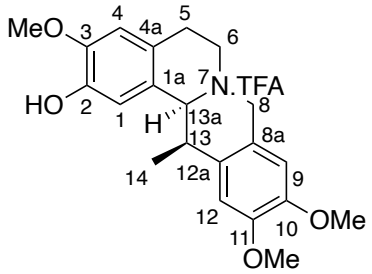
(13*R*,13a*S*)-9,10-Dimethoxy-13-methyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinoline-2,3-diol.TFA[5] (4a)



A NCS-mediated reaction between dopamine and 2-(3,4-dimethoxyphenyl)propanal was performed on a 5 mL, 10 mM scale (as detailed for the synthesis of **3a**) then quenched by addition of 5 mL MeCN. The suspension was centrifuged (20 min, 5000 x g, rt) then the supernatant lyophilised. The residue was resuspended in formic acid (11.6 mL) and formaldehyde (37% in water, 8.4 mL). The reaction mixture was stirred at 50 °C on a heating mantle for 2 h. Solvents were removed under reduced pressure and the residue purified by preparative HPLC (method 3) to give the product as a yellow solid (5.3 mg, 24% as the TFA salt, over two steps). $[\alpha]_D^2$ -35.3 (*c* 0.83, MeOH); ν_{max}/ cm^{-1} (thin film): 2980, 1672, 1614, 1521; ^{1}H-NMR (700 MHz; CD_3OD) δ 6.94 (1H, s, 12-H), 6.79 (1H, s, 9-H), 6.76 (1H, s, 1-H), 6.65

(1H, s, 4-H), 4.91 (1H, d, *J* = 4.0 Hz, 13a-H), 4.54 – 4.51 (2H, m, 8-H), 3.85 (3H, s, OC*H*$_3$), 3.83 (3H, s, OC*H*$_3$), 3.79 – 3.74 (2H, m, 13-H and 6-*H*H), 3.51 (1H, dt, *J* = 3.8 Hz, 6-H*H*), 3.19 – 3.12 (1H, m, 5-*H*H), 2.90 (1H, dd, *J* = 17.2, 2.7 Hz, 5-H*H*), 1.10 (3H, d, *J* = 7.3 Hz, 14-H); ^{13}C-NMR (176 MHz, CD_3OD) δ 150.9, 150.1, 146.7, 146.7, 131.1, 124.2, 121.7, 119.6, 115.9, 113.0, 112.8, 109.7, 65.6, 57.3, 56.4, 56.4, 52.8, 36.7, 26.4, 17.3; *m/z* [MS ES+] 342 ([M+H]$^+$, 100%); *m/z* [HRMS ES$^+$] found [M+H]$^+$ 342.1701; $C_{20}H_{25}NO_4$ requires 342.1700; HPLC (C18, MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 1 min then a linear gradient MeCN (0.1% TFA)/H_2O (0.1% TFA) 70/30 over 5 min then 100% MeCN (0.1%TFA) for 0.5 min then MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 3.5 min, flow rate = 0.1 mL/min, I = 280 nm) t_R = 8.9 min.

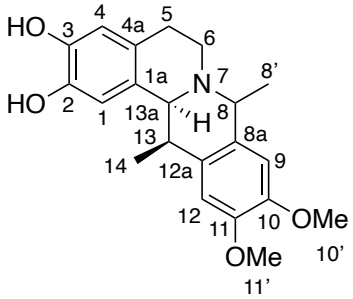
(13*R*,13a*S*)-3,10,11-Trimethoxy-13-methyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinolin-2-ol.TFA[5] (4b)



A crude, lyophilised sample of (**3c**, 0.05 mmol scale reaction) was resuspended in formic acid (11.6 mL) and formaldehyde (37% in water, 8.4 mL). The reaction mixture was stirred at 50 °C on a heating mantle for 2 h.[6] Solvents were removed under reduced pressure and the residue purified by preparative HPLC (method 3) to give the product as a pale yellow solid (5.1 mg, 23% as the TFA salt over 3 steps). $[\alpha]_D^{20}$ +0.92 (*c* 0.72, MeOH)**;** ν_{max}/ cm^{-1} (thin film): 3293, 2967, 2842, 1757, 1668, 1613; ^{1}H-NMR (700 MHz; CD_3OD) δ 6.95 (1H, s, 1-H), 6.83 – 6.81 (2H, m, 4-H and 12-H), 6.80 (1H, s, 9-H), 4.96 (1H, d, *J* = 4.1 Hz, 13a-H), 4.54 (2H, s, 8-H), 3.88 (3H, s, OC*H*$_3$), 3.87 (3H, s, OC*H*$_3$), 3.83 (3H, s, OC*H*$_3$), 3.82 – 3.77 (2H, m, 6-*H*H and 13-H), 3.57 – 3.51 (1H, s, 6-H*H*), 3.25 – 3.17 (1H, m, 5-*H*H), 3.03 – 2.97 (1H, m, 5-H*H*), 1.09 (3H, d, *J* = 7.4 Hz, 14-H); ^{13}C-NMR (176 MHz; CD_3OD) δ 150.9, 150.1, 149.1, 147.7, 131.1, 124.2, 123.0, 119.6, 113.0, 112.8, 112.3, 109.7, 65.5, 57.3, 56.4, 56.4, 56.3, 52.7, 36.5, 26.5, 17.2; *m/z* [MS ES$^+$] 356 ([M+H]$^+$, 100%); *m/z* [HRMS ESI$^+$] found [M+H]$^+$ 356.1854, $C_{21}H_{26}NO_4$ requires 352.1856; HPLC (C18, MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 1 min then a linear gradient MeCN (0.1% TFA)/H_2O (0.1% TFA) 70/30 over 5 min then 100% MeCN (0.1%TFA) for 0.5 min then

MeCN (0.1% TFA)/H_2O (0.1% TFA) = 10/90 for 3.5 min, flow rate = 0.1 mL/min, I = 280 nm) t_R = 9.2 min.

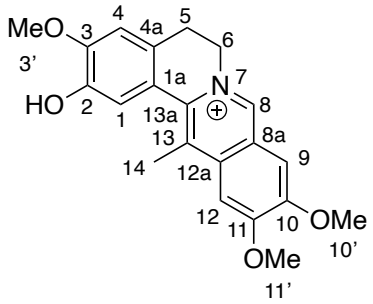
(8*SR*,13*R*,13a*S*)-10,11-Dimethoxy-8,13-dimethyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinoline-2,3-diol.TFA[5] (4c)



A solution of crude, lyophilised **3a** (16.5 mg, 0.05 mmol) in formic acid (2.1 mL), water (1.3 mL) and acetaldehyde (780 μL, 14.0 mmol) was prepared under anhydrous conditions and stirred at 50 °C on a heating mantle for 24 h. Solvents were removed under reduced pressure and the resulting residue was resuspended in a minimum volume of methanol and purified by preparative HPLC (method 2) to give the product as brown solid (4.7 mg, 21% as the TFA salt over two steps) as a mixture of two epimers (8*S*,13*R*,13a*S*):(8*R*,13*R*,13a*S*) in a ratio of 3:1. Stereochemistry at C-13 and C-13a was assigned based upon precedent of NCS reactions involving α-methyl substituted aldehydes and stereochemistry at C-8 was assigned based upon the presence or absence of NOE between 8-H and 13a-H. $[\alpha]_D^{20}$ -24.6 (*c* 0.45, MeOH); ν_{max}/ cm^{-1} (thin film): 3449, 3011, 1673, 1650, 1516; ^{1}H-NMR (700 MHz; CD_3OD) δ 6.92 (0.3H, s, 12-H$_{min}$), 6.88 (1H, s, 12-H$_{maj}$), 6.85 (0.3H, s, 9-H$_{min}$), 6.82 – 6.79 (1.3H, m, 9-H$_{maj}$ and C-1$_{min}$), 6.77 (1H, s, 1-H$_{maj}$), 6.67 (0.3H, s, 4-H$_{min}$), 6.65 (1H, s, 4-H$_{maj}$), 5.12 (1H, d, *J* = 4.1 Hz, 13a-H$_{maj}$), 4.18 (0.3 H, dd, *J* = 12.1, 5.0 Hz, 6-*H*H$_{min}$), 3.87 (0.9H, s, 10'-OC*H*$_3$ or 11'- OC*H*$_3$), 3.86 – 3.83 (7H, m, 10'- OC*H*$_3$ and 11'- OC*H*$_3$), 3.79 – 3.77 (0.3H, m, 6-H*H*$_{min}$), 3.75 – 3.71 (0.3 H, m, 13-H$_{min}$), 3.69 – 3.64 (1H, m, 13-H$_{maj}$), 3.60 – 3.55 (1H, m, 6-*H*H$_{maj}$), 3.52 (1H, td, *J* = 12.3, 2,8 Hz, 6-H*H*$_{maj}$), 3.44 – 3.37 (0.3H, m, 5-*H*H$_{min}$), 3.15 – 3.08 (1H, m, 5-*H*H$_{maj}$), 2.94 (0.3H, dd, *J* = 15.1, 3.3 Hz, 5-H*H*$_{min}$), 2.90 (1H, d, *J* = 17.1 Hz, 5-H*H*$_{maj}$), 1.85 (1H, d, *J* = 6.8 Hz, 8'-H$_{min}$), 1.69 (3H, d, *J* = 6.8 Hz, 8'-H$_{maj}$), 1.17 (1H, d, *J* = 7.3 Hz, 14-H$_{min}$), 1.04 (3H, d, *J* = 7.3 Hz, 14-H$_{maj}$). ^{13}C-NMR (176 MHz; CD_3OD) δ 150.8$_{(maj)}$, 150.5$_{(min)}$, 150.1$_{(maj)}$, 150.1$_{(min)}$, 146.7, 146.5, 131.2$_{(min)}$, 130.1$_{(maj)}$, 126.2$_{(maj)}$, 124.6, 124.3, 122.3$_{(maj)}$, 122.0$_{(min)}$, 115.9$_{(min)}$, 115.8$_{(maj)}$, 113.3$_{(maj)}$, 113.0$_{(min)}$, 112.8$_{(maj)}$, 112.4$_{(min)}$, 110.3$_{(maj)}$, 110.2$_{(min)}$, 62.0, 58.2$_{(maj)}$, 56.6, 56.5, 56.4, 52.0$_{(min)}$, 50.1$_{(maj)}$, 49.4$_{(min)}$, 26.6, 26.6, 19.5$_{(min)}$, 17.3$_{(maj)}$, 17.0$_{(min)}$, 16.9$_{(maj)}$.

m/z [MS ES+] 356 ([M+H]$^+$, 100%); *m/z* [HRMS ES+] found [M+H]$^+$ 356.1855; $C_{21}H_{26}NO_4$ requires 356.1856; HPLC (C18, MeCN (0.1%TFA)/H_2O (0.1%TFA) = 10/90 for 1 min then a linear gradient MeCN (0.1%TFA)/H_2O (0.1%TFA) 70/30 over 5 min then 100% MeCN (0.1%TFA) for 0.5 min then MeCN (0.1%TFA)/H_2O (0.1%TFA) = 10/90 for 3.5 min, flow rate = 0.1 mL/min, I = 280 nm) t_R = 8.6 min.

2-Hydroxy-3,10,11-Trimethoxy-13-methyl-5,6-dihydroisoquinolino[3,2-*a*]isoquinolin-7-ium (5)



Under anhydrous conditions, a crude, lyophilised sample of **3c** (0.10 mmol) was resuspended in DMF (10 mL). Paraformaldehyde (6.0 mg, 0.20 mmol) was added. The reaction mixture was stirred at 120 ^oC on a heating mantle for 18 h. Solvents were removed under reduced pressure and the resulting residue was purified by preparative HPLC (method 3) to give the product as a brown oil (1.7 mg, 4.8% as the TFA salt over three steps).

To prepare from **4b**, a crude, lyophilised sample of **4b** (0.10 mmol) was resuspended in DMF (10 mL) and stirred at 120 ^oC for 18 h. Solvents were removed under reduced pressure and the resulting residue was purified by preparative HPLC (method 3) to give the product as a brown oil (7.4 mg, 21% isolated, 86% conversion, as the TFA salt over three steps). ν_{max}/ cm^{-1} (thin film): 3523, 3321, 3012, 1674, 1605, 1574, 1504; ^{1}H-NMR (700 MHz; CD_3OD) δ 9.32 (1H, s, 8-H), 7.62 (1H, s, 9-H), 7.55 (1H, s, 12-H), 7.33 (1H, s, 1-H), 7.08 (1H, s, 4-H), 4.68 (2H, t, *J* = 5.9 Hz, 6-H), 4.16 (3H, s, 11'-H), 4.06 (3H, s, 10'-H), 3.98 (3H, s, 3'-H), 3.14 (2H, t, *J* = 5.9 Hz, 5-H), 2.97 (3H, s, 14-H); ^{13}C-NMR (176 MHz; CD_3OD) δ 159.6, 154.1, 151.2, 146.5, 144.8, 138.9, 138.7, 131.4, 129.3, 123.1, 121.0, 118.5, 111.7, 107.9, 104.2, 57.9, 57.3, 56.9, 56.5, 28.7, 18.1; *m/z* [MS ES$^+$] 352 ([M]$^+$, 100%); *m/z* [HRMS ESI$^+$] found [M]$^+$ 352.1543, $C_{21}H_{22}NO_4$ requires 352.1534.

7. Calibration curves

The desired compound was purified by preparative HPLC. Methods for each compound are given in synthetic methods when isolated for characterisation purposes. A stock of the purified compound at 2.5 mM in 50% MeCN in dH_2O was prepared. A serial dilution was performed to give varying concentrations of the compound which were analysed by analytical HPLC method 1. Absorbance in mAU was measured at 280 nm. An injection volume of 10 μL was used.

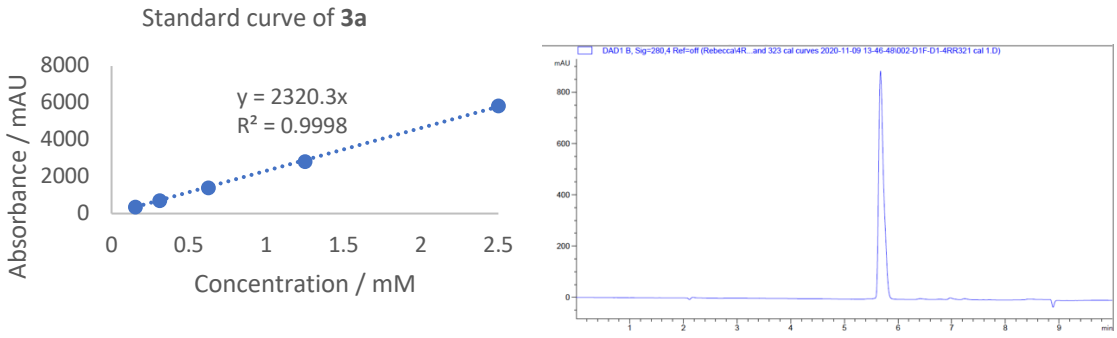


Figure S7: Calibration curve and HPLC spectrum of **3a.**

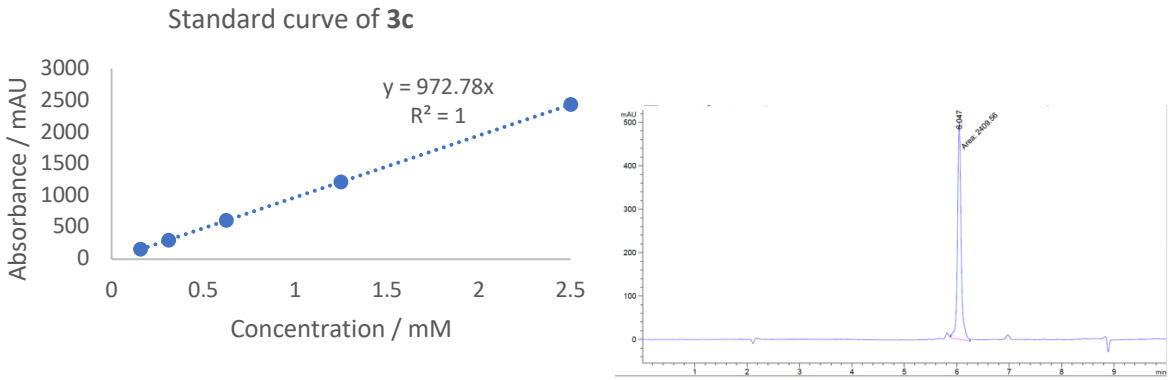


Figure S8: Calibration curve and HPLC spectrum of **3c.**

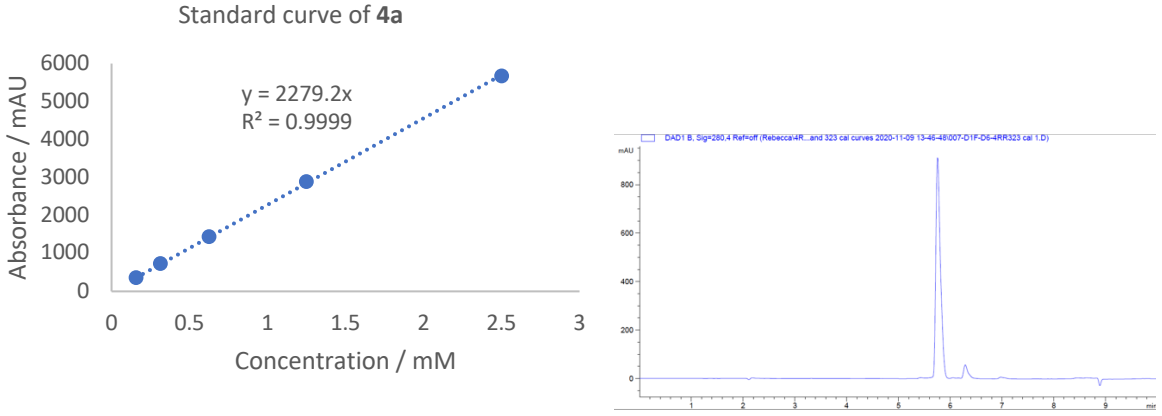


Figure S9: Calibration curve and HPLC spectrum of **4a**.

8. 3D modelling of 4c

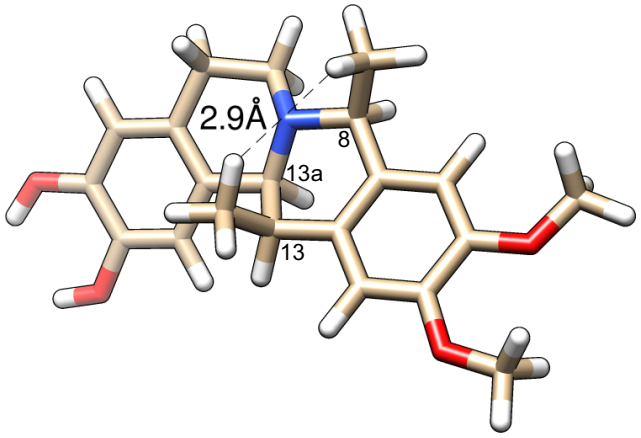
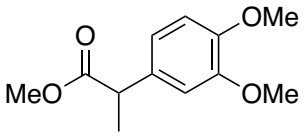
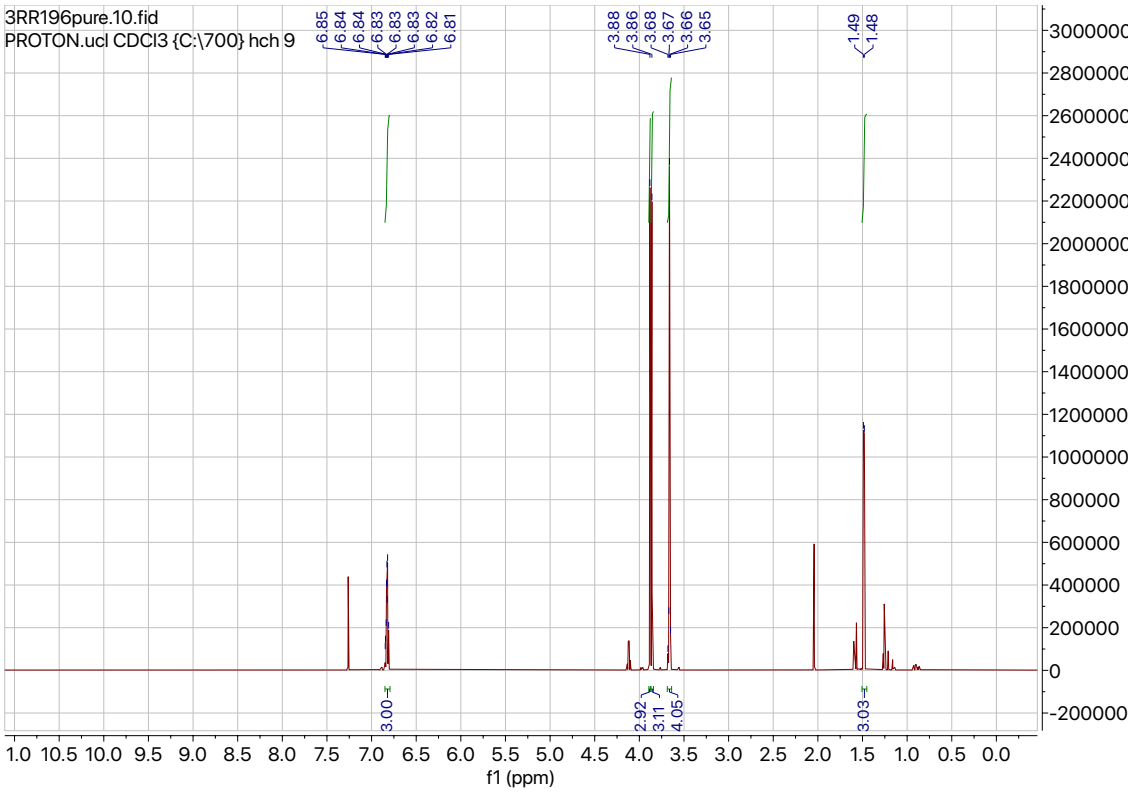


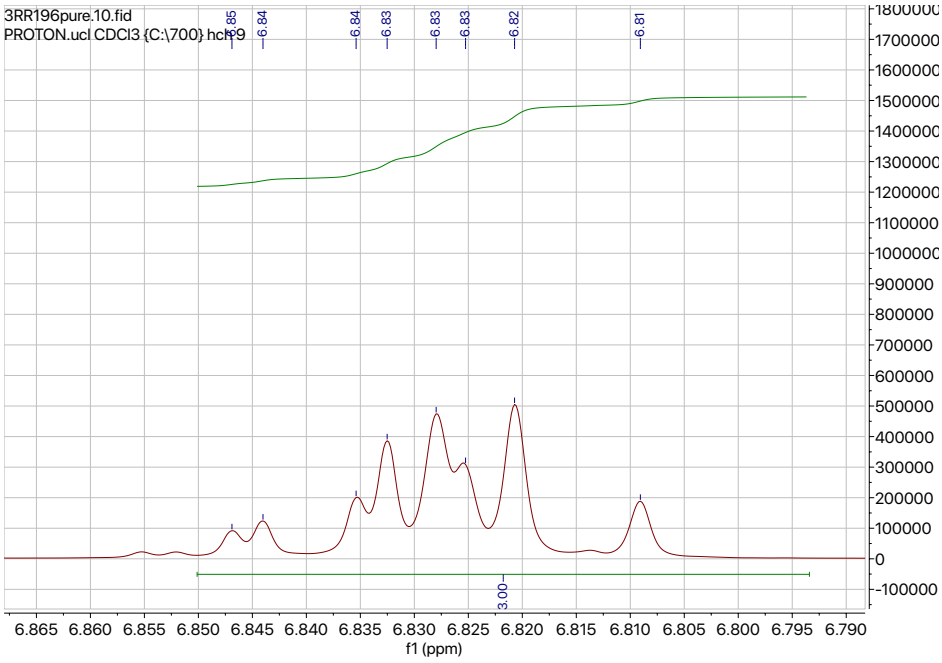
Figure S10: 3D modelling of the minor epimer of 4c generated, (8*R*,13*R*,13a*S*)-4c. The two methyl groups at C-8 and C-13 are in a *cis* relationship, in a pseudo-axial orientation, which is an unfavorable steric interaction.

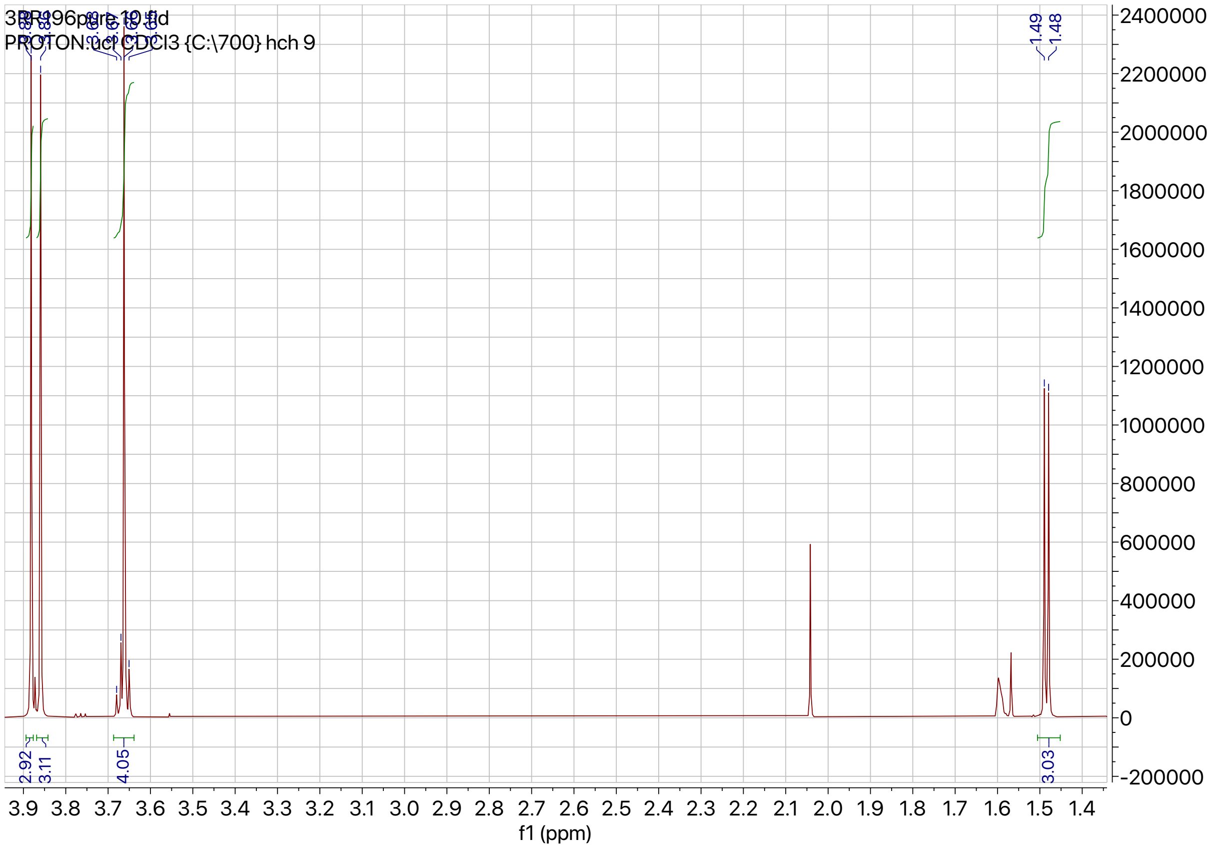
9. NMR spectra

Methyl 2-(3,4-dimethoxyphenyl)propanoate (700 MHz)

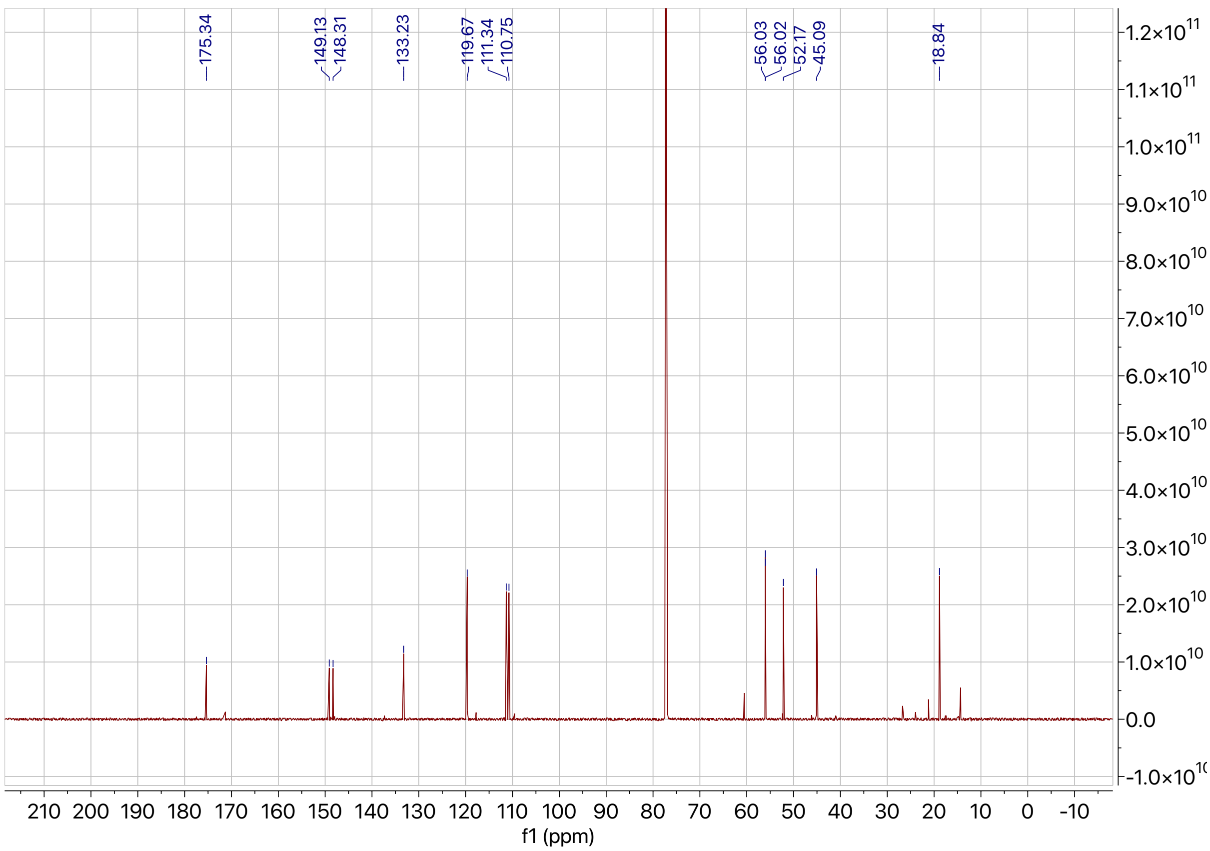






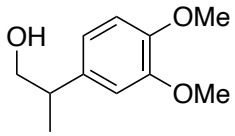


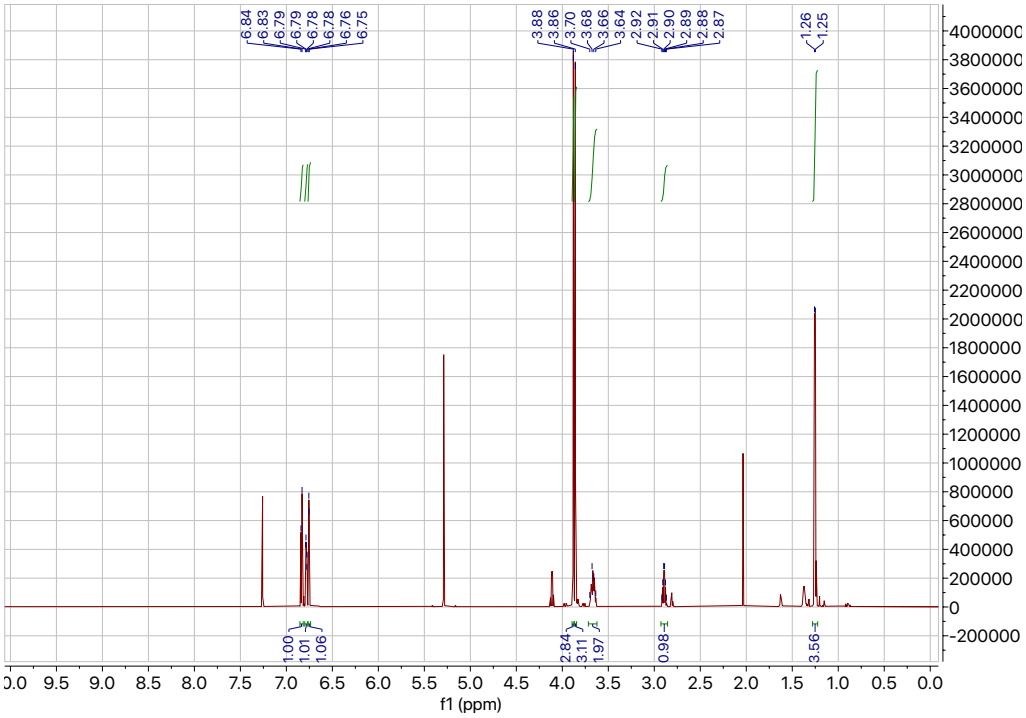
PROTON.ucl CDCl3 {C:\700} hch 9
1.49
1.48
2.92
3.11
4.05
3.03
f1 (ppm)

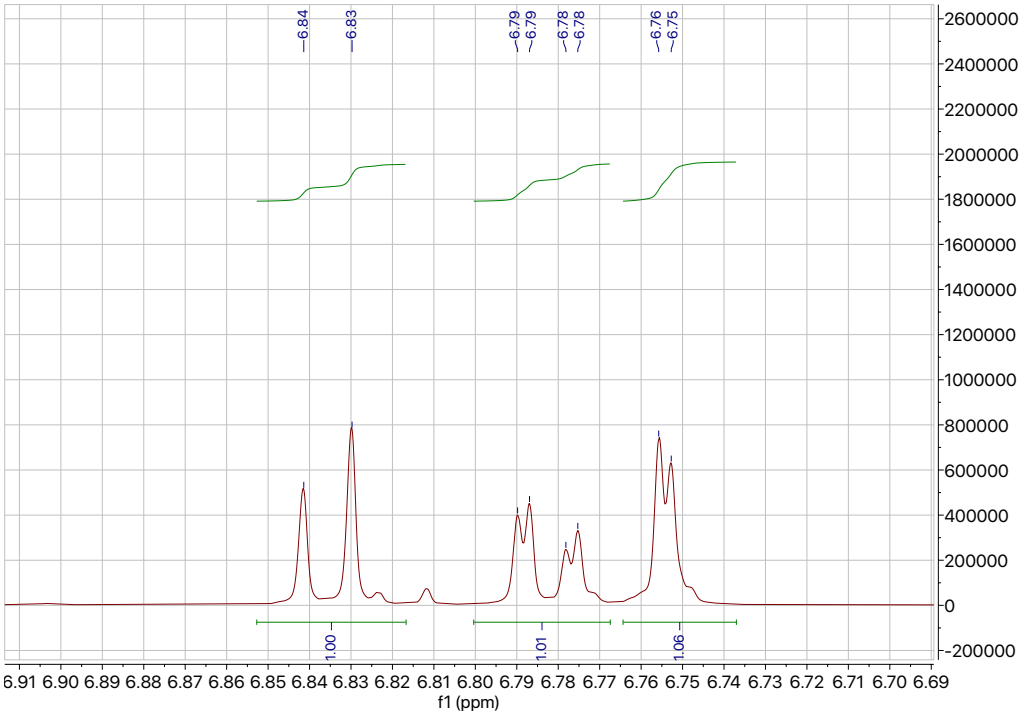


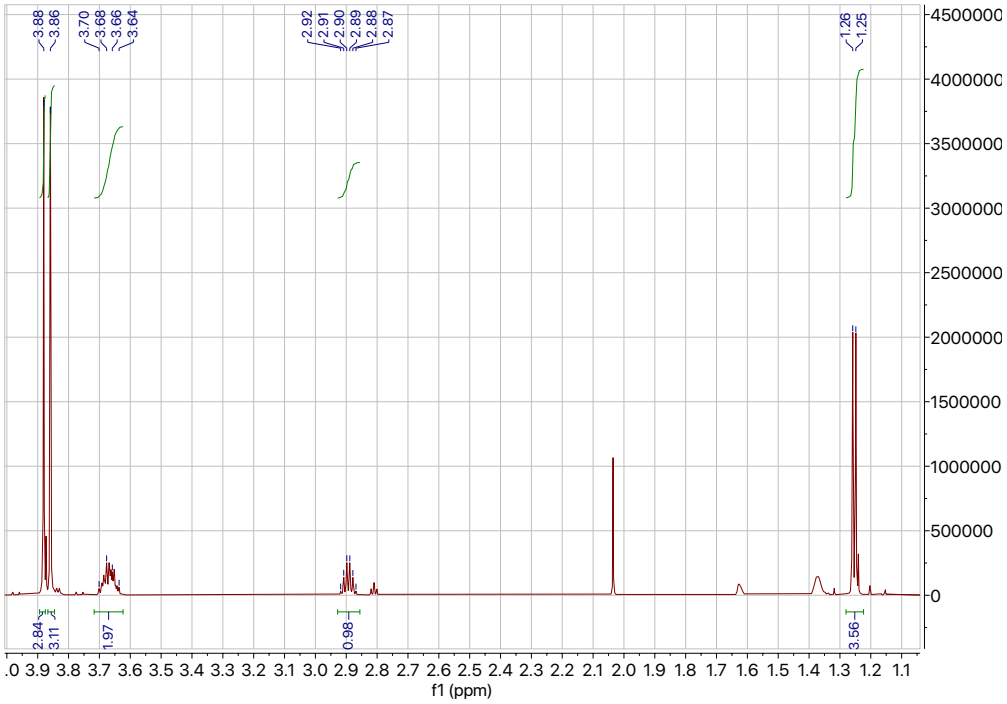
175.34
149.13
148.31
133.23
119.67
111.34
110.75
56.03
56.02
52.17
45.09
18.84
f1 (ppm)

2-(3,4-Dimethoxyphenyl)propan-1-ol (700 MHz)








3.88
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3.64
2.92
2.91
2.90
2.89
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1.25
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1.97
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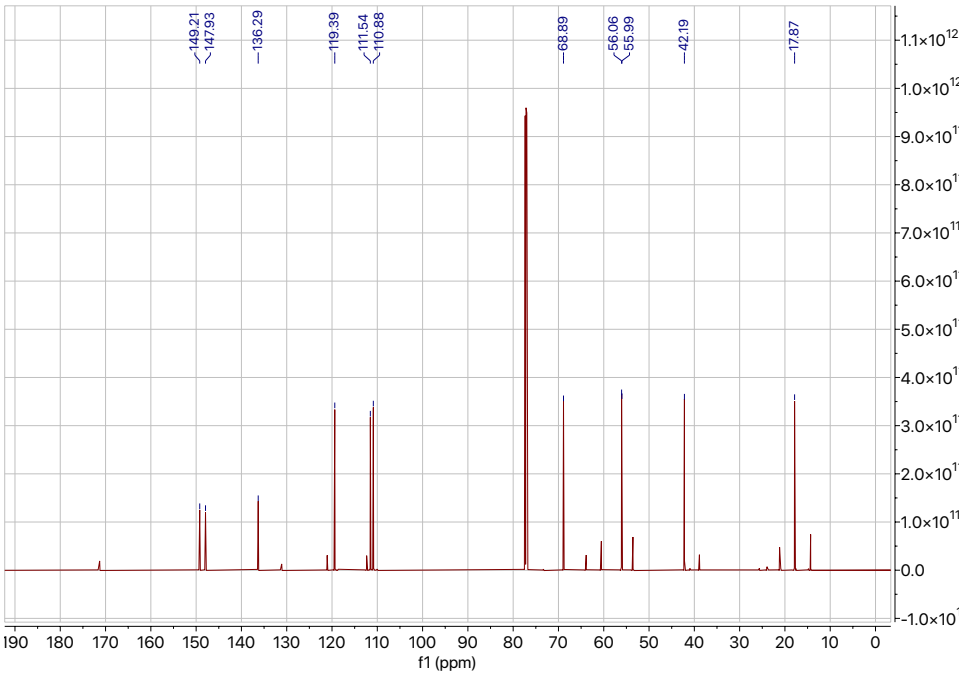
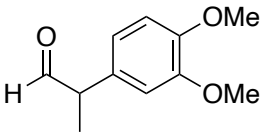
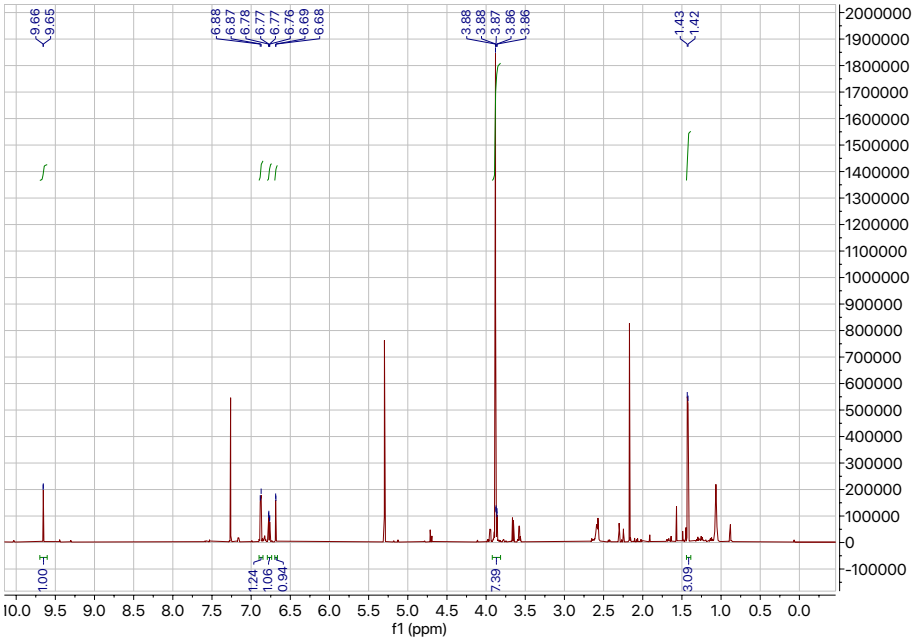
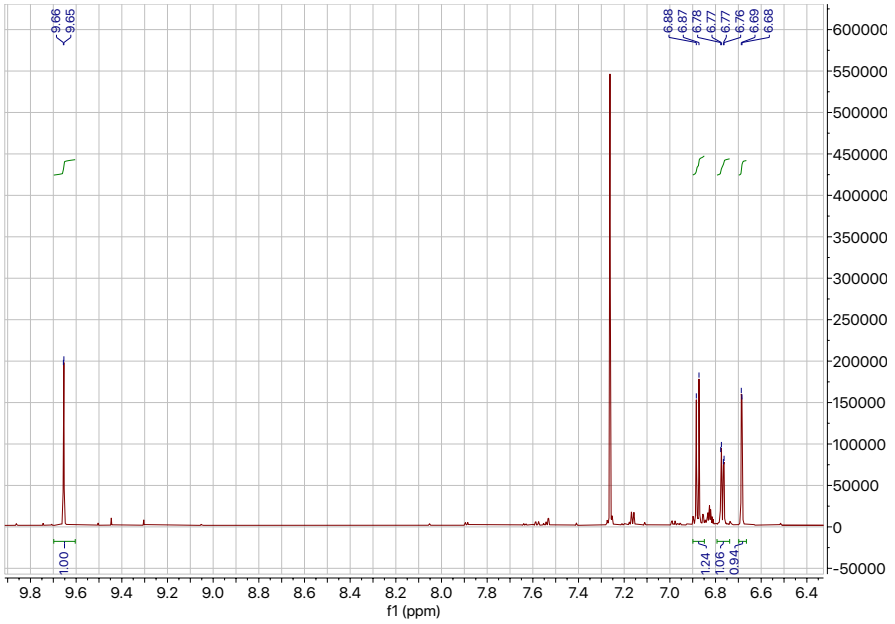
3.56
f1 (ppm)

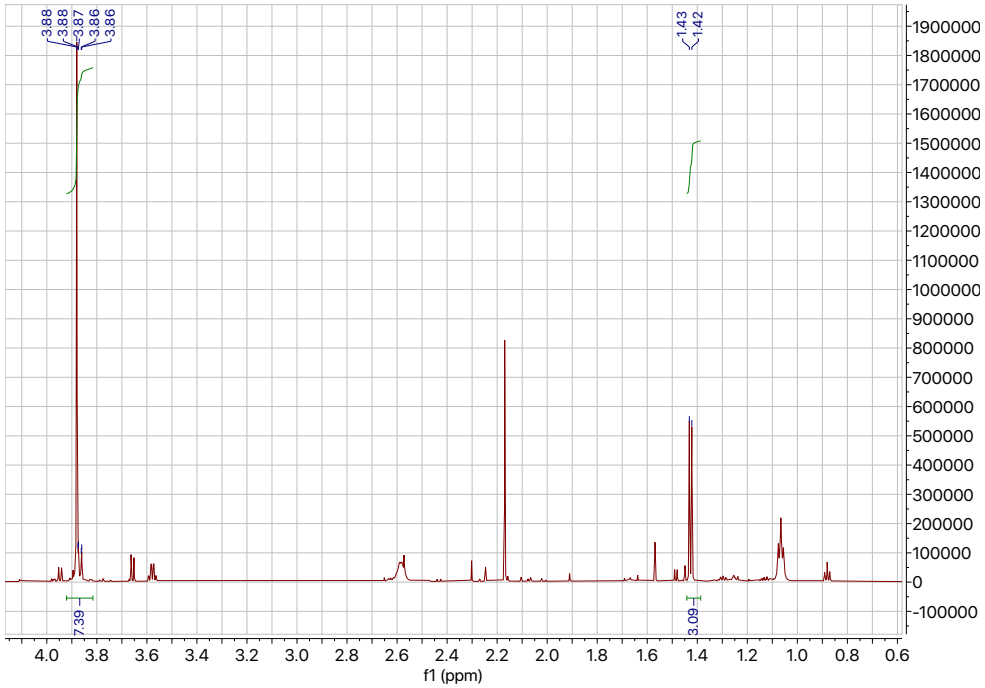

149.21
147.93
136.29
119.39
111.54
110.88
68.89
56.06
55.99
42.19
17.87
f1 (ppm)

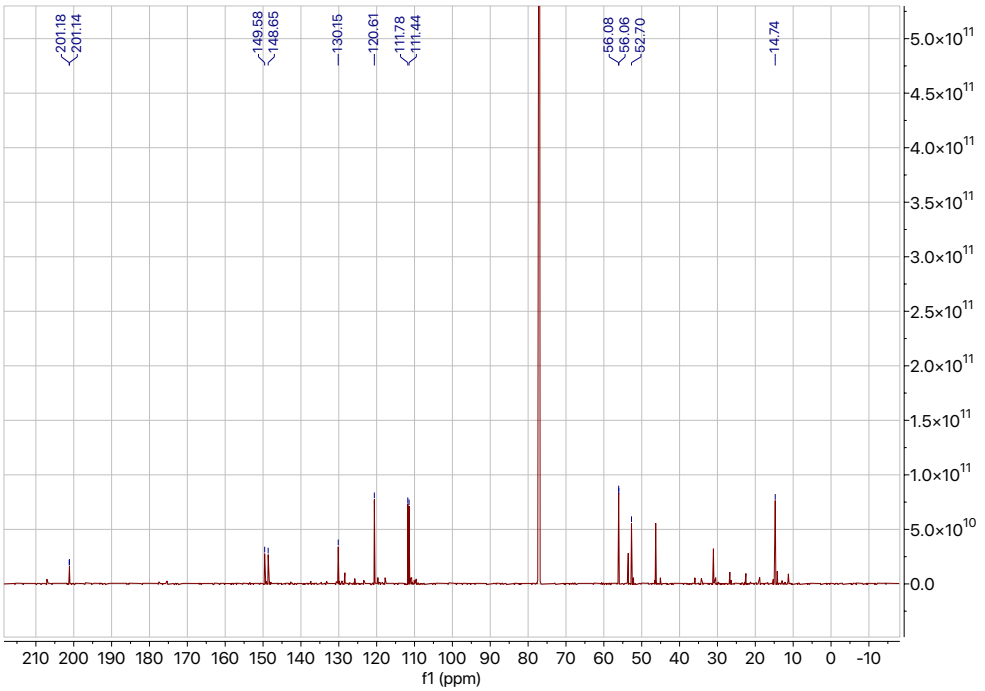
2-(3,4-Dimethoxyphenyl)propanal (2) (700 MHz)



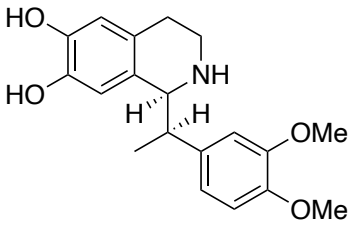


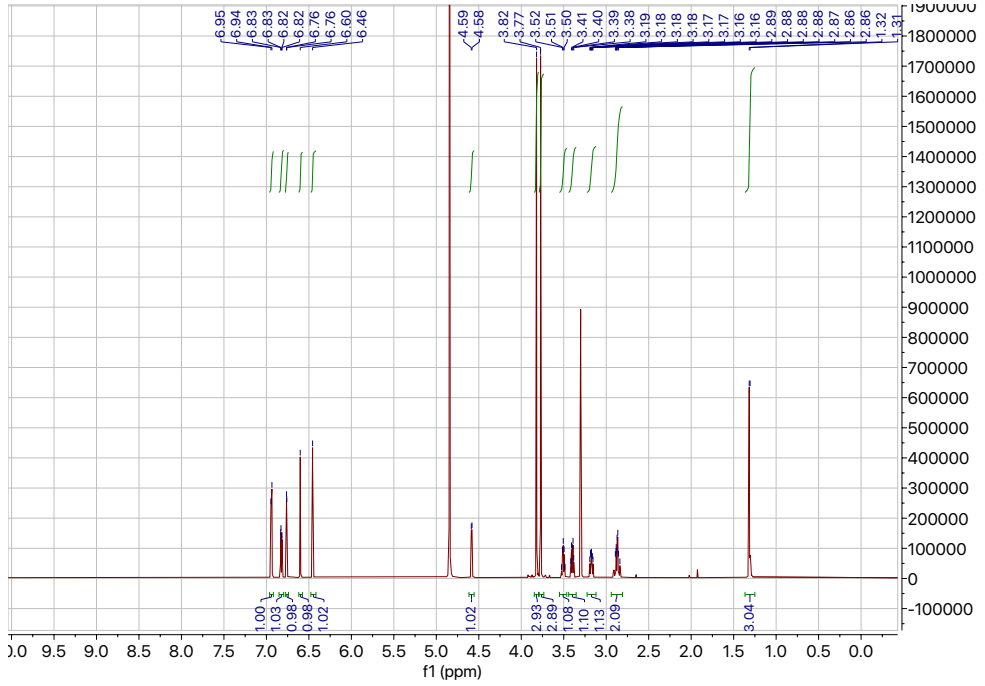


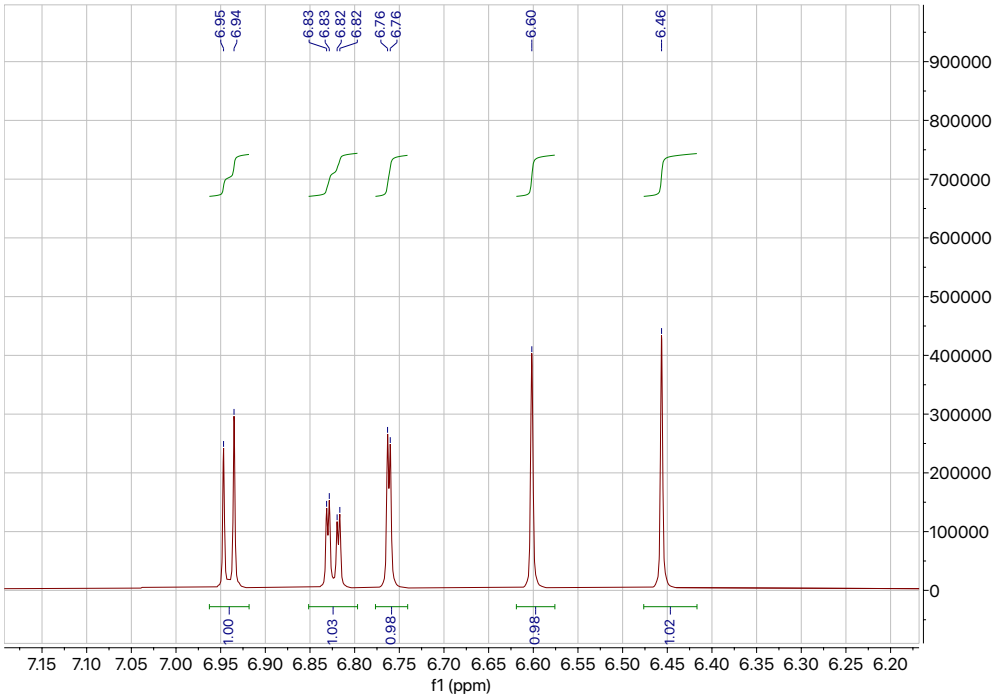

3.88
3.88
3.87
3.86
3.86
1.43
1.42
7.39
3.09
f1 (ppm)

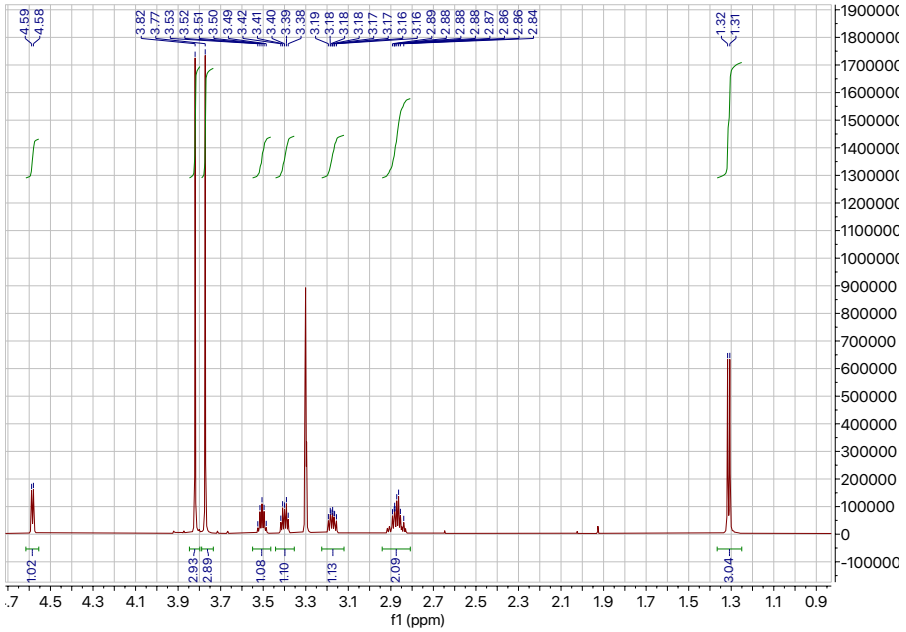

201.18
201.14
149.58
148.65
130.15
120.61
111.78
111.44
56.08
56.06
52.70
14.74
f1 (ppm)

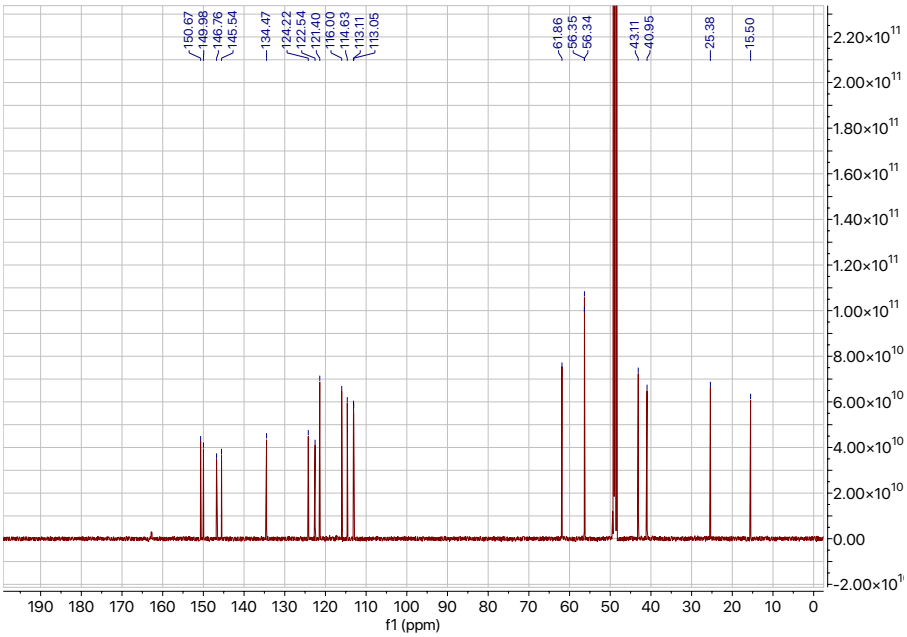
(*S*)-1-((*R*)-1-(3,4-Dimethoxyphenyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol.TFA (3a) (700 MHz)



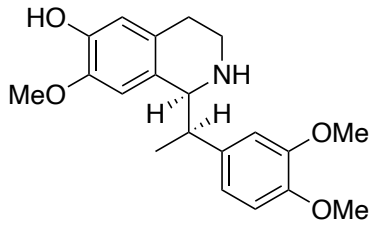


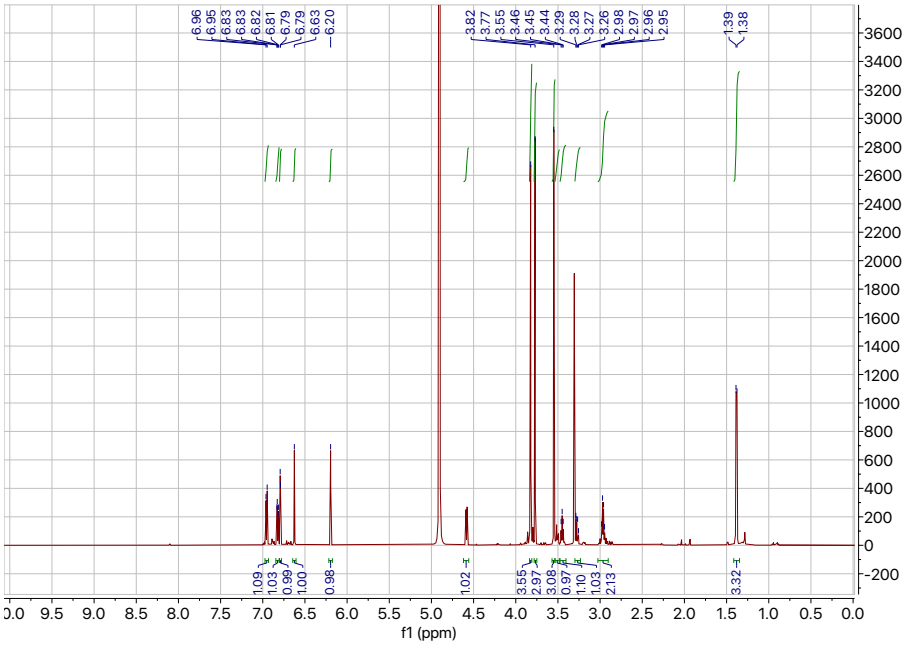


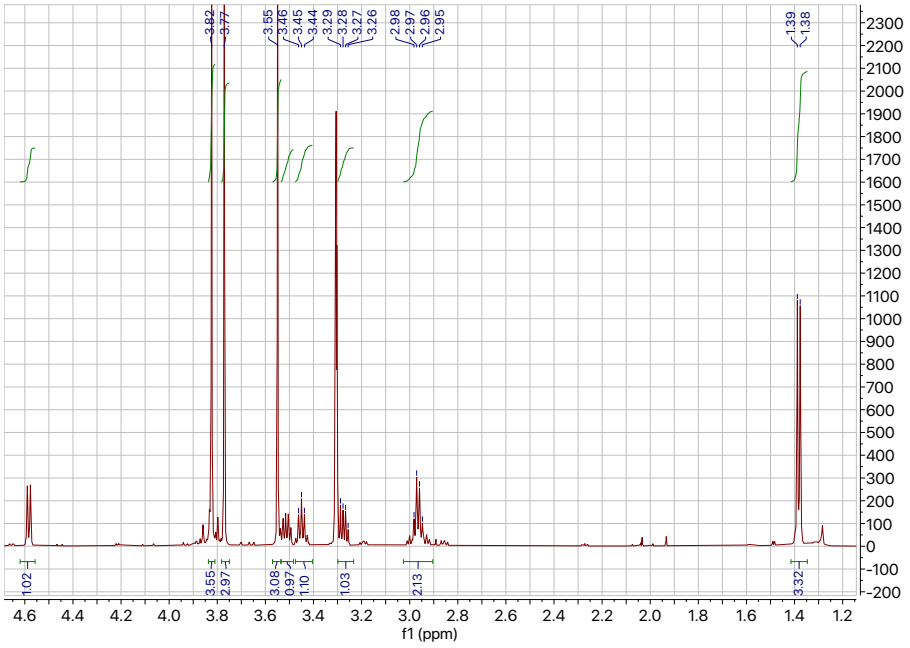

4.59
4.58
3.82
3.77
3.53
3.52
3.51
3.50
3.49
3.42
3.41
3.40
3.39
3.38
3.19
3.18
3.18
3.18
3.17
3.17
3.16
2.89
2.88
2.88
2.88
2.87
2.86
2.86
2.84
1.32
1.31
1.02
2.93
2.89
1.08
1.10
1.13
2.09
3.04
f1 (ppm)

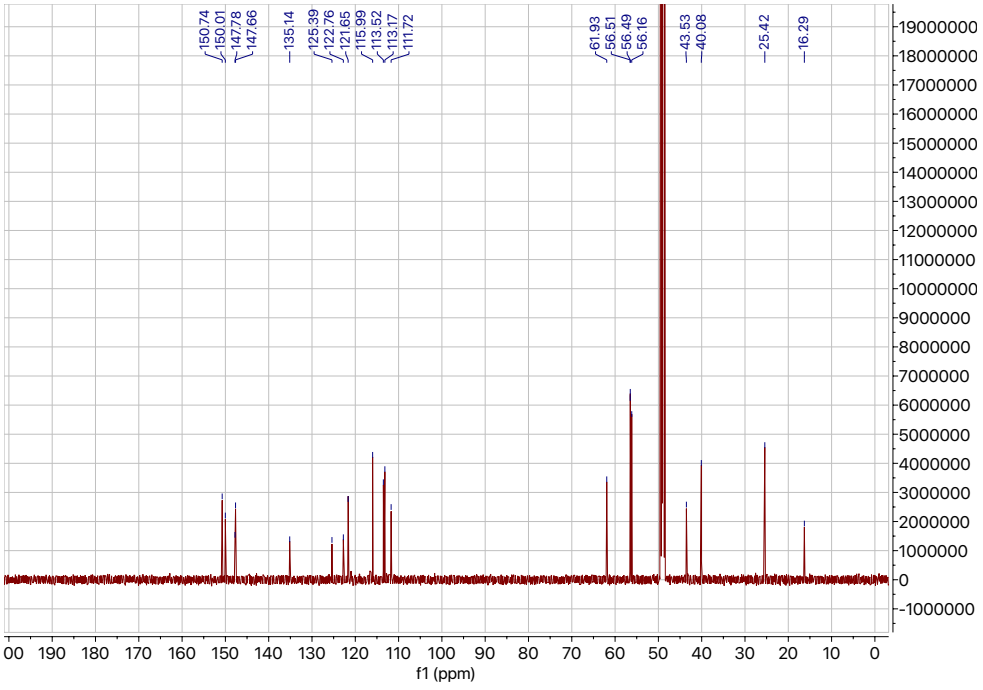

150.67
149.98
146.76
145.54
134.47
124.22
122.54
121.40
116.00
114.63
113.11
113.05
61.86
56.35
56.34
43.11
40.95
25.38
15.50
f1 (ppm)

1-((1*S*)-1-((*R*)-1-(3,4-dimethoxyphenyl)ethyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydro-2λ^4-isoquinolin-2-yl)-2,2,2-trifluoroethan-1-one.TFA (3b) (600 MHz)



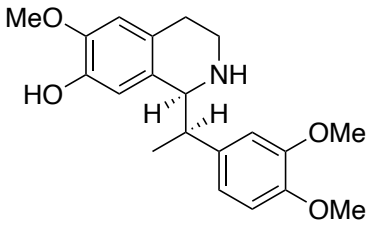


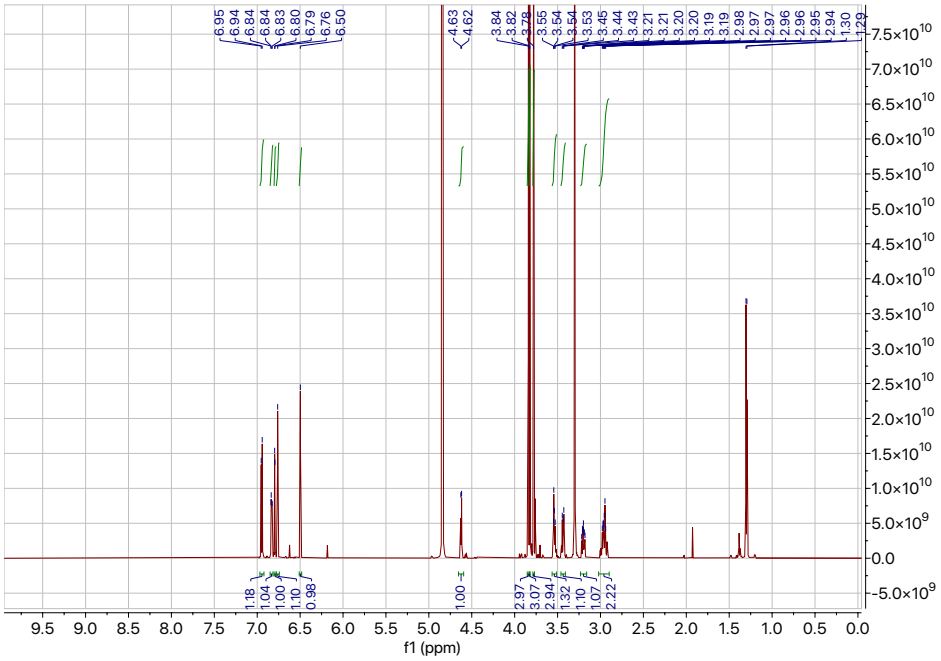

3.82
3.77
3.55
3.46
3.45
3.44
3.29
3.28
3.27
3.26
2.98
2.97
2.96
2.95
1.39
1.38
1.02
3.55
2.97
3.08
0.97
1.10
1.03
2.13
3.32
f1 (ppm)

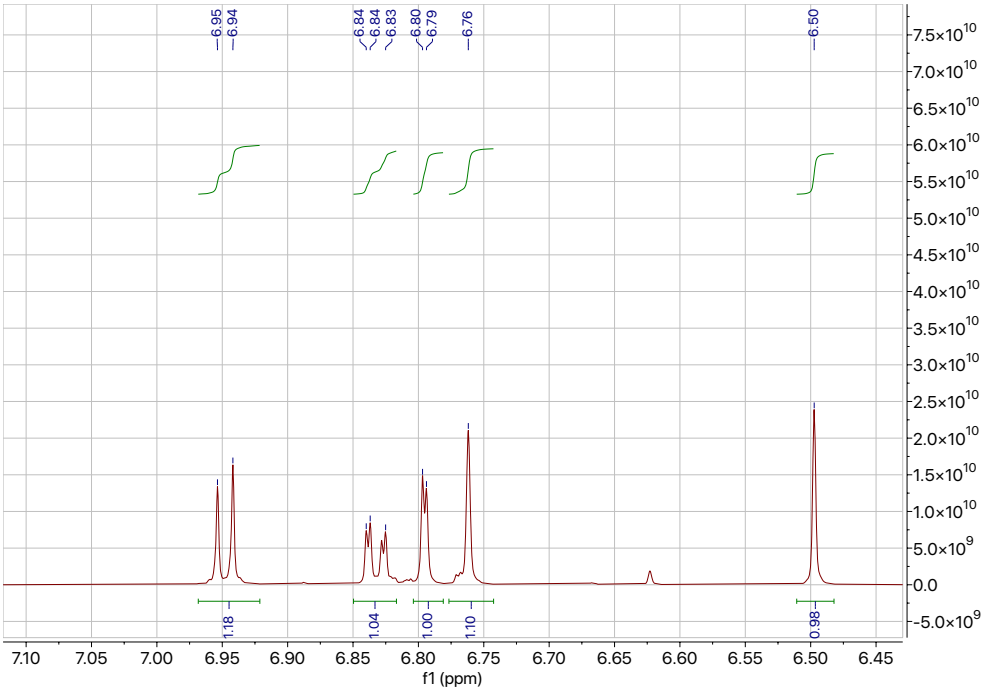

150.74
150.01
147.78
147.66
135.14
125.39
122.76
121.65
115.99
113.52
113.17
111.72
61.93
56.51
56.49
56.16
43.53
40.08
25.42
16.29
f1 (ppm)

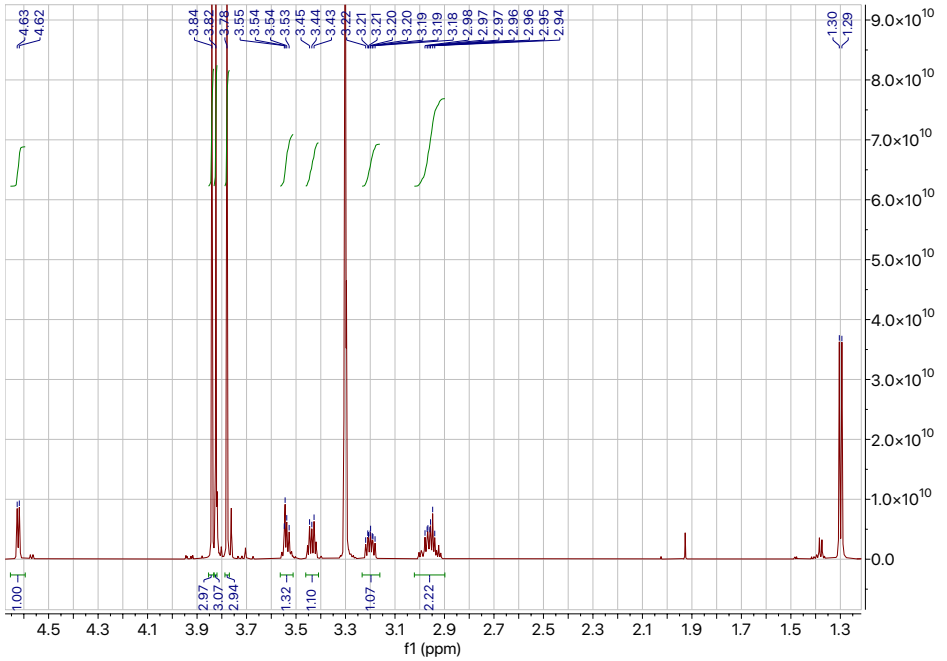
(*S*)-1-((*R*)-1-(3,4-Dimethoxyphenyl)ethyl)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-7-ol.TFA (3c)

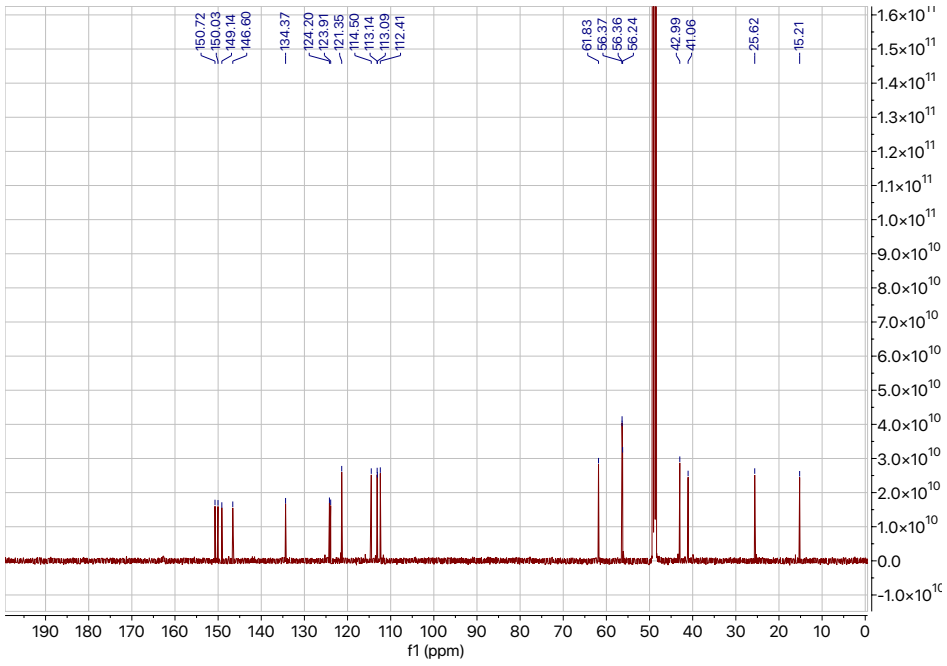
(700 MHz)



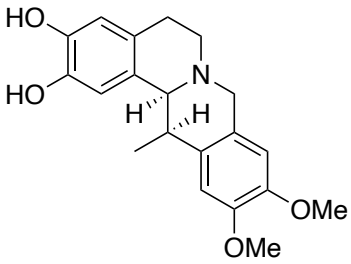


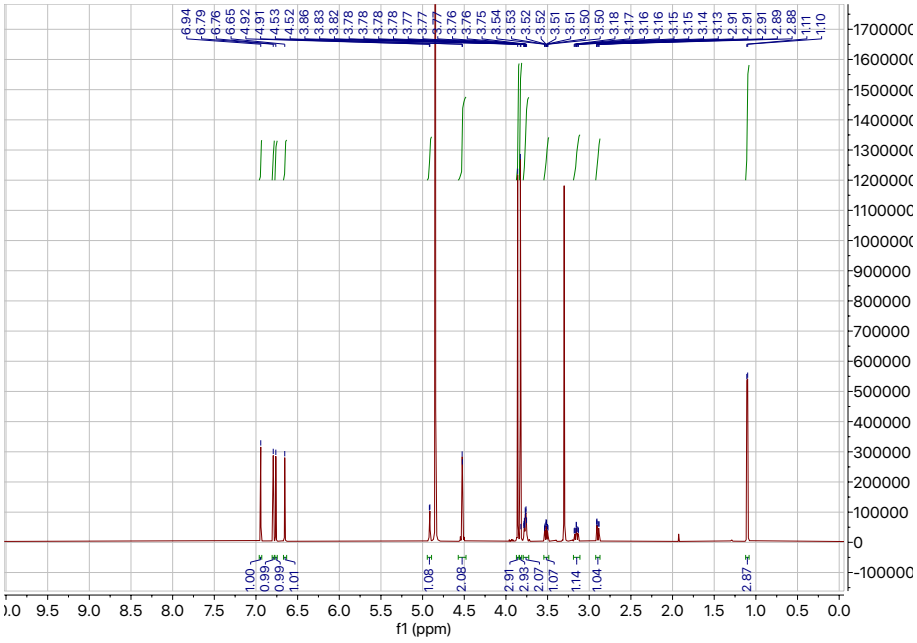


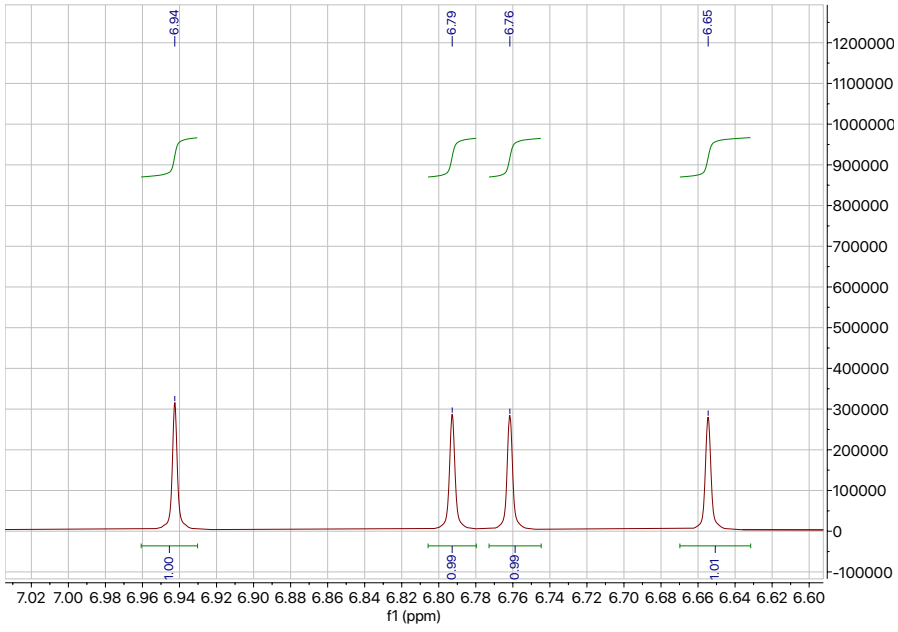

4.63
4.62
3.84
3.82
3.78
3.55
3.54
3.54
3.53
3.45
3.44
3.43
3.22
3.21
3.21
3.20
3.20
3.19
3.19
3.18
2.98
2.97
2.97
2.96
2.96
2.95
2.94
1.30
1.29
1.00
2.97
3.07
2.94
1.32
1.10
1.07
2.22
f1 (ppm)

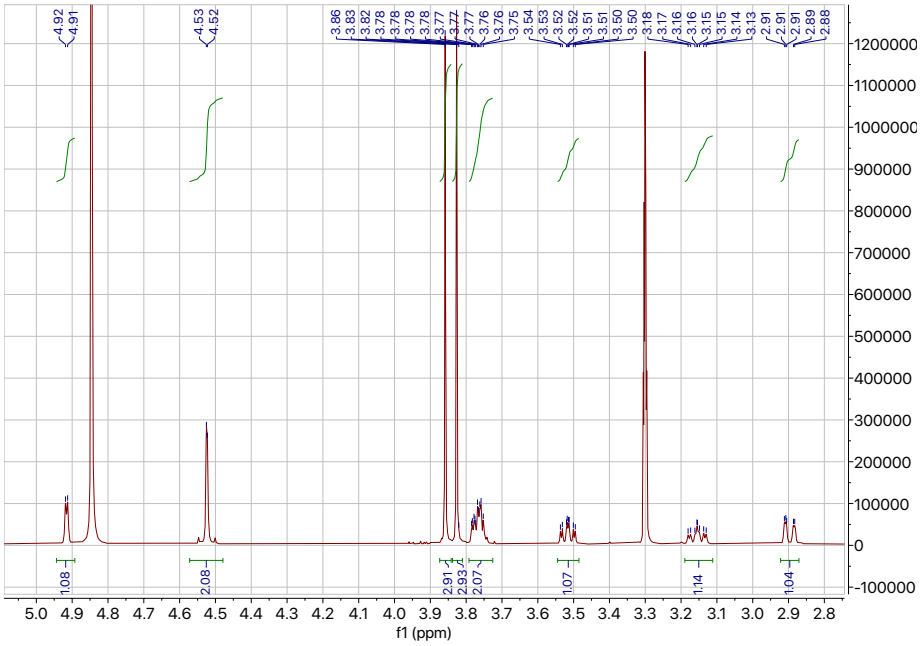

150.72
150.03
149.14
146.60
134.37
124.20
123.91
121.35
114.50
113.14
113.09
112.41
61.83
56.37
56.36
56.24
42.99
41.06
25.62
15.21
f1 (ppm)

(13*R*,13a*S*)-10,11-Dimethoxy-13-methyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinoline-2,3-diol.TFA (4a) (700 MHz)








4.92
4.91
4.53
4.52
3.86
3.83
3.82
3.78
3.78
3.78
3.78
3.77
3.77
3.77
3.76
3.76
3.75
3.54
3.53
3.52
3.52
3.51
3.51
3.50
3.50
3.18
3.17
3.16
3.16
3.15
3.15
3.14
3.13
2.91
2.91
2.91
2.89
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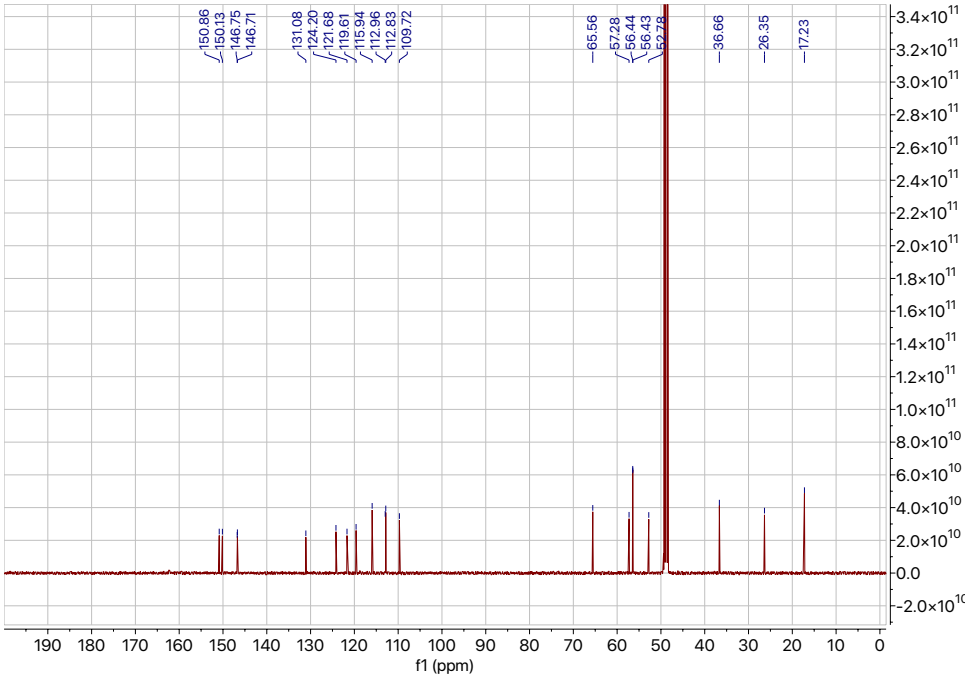
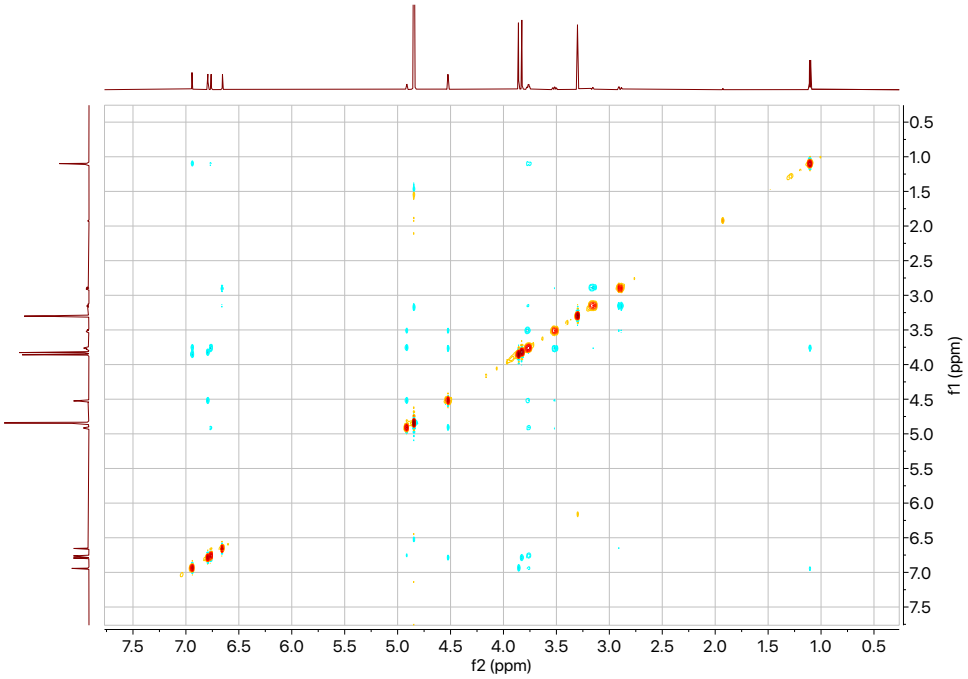
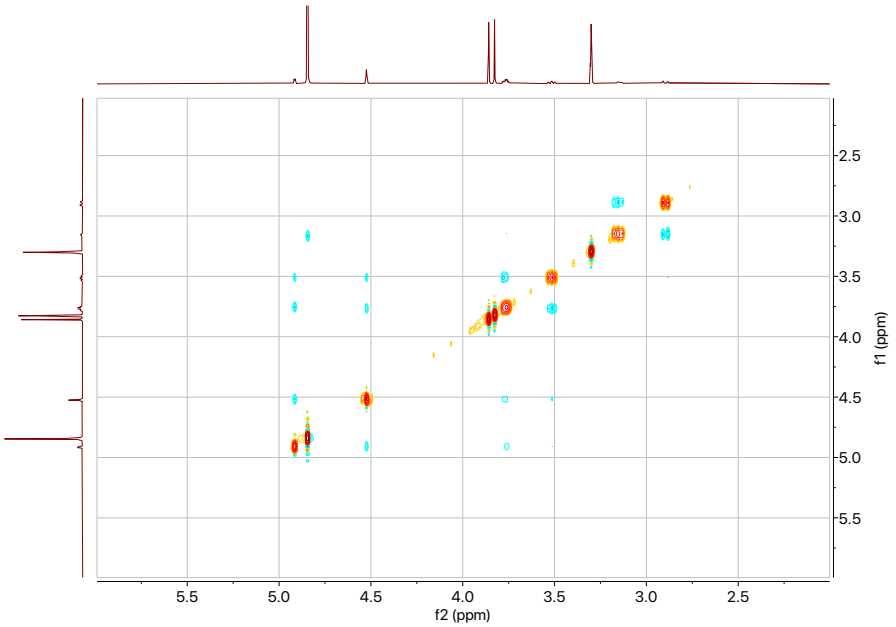
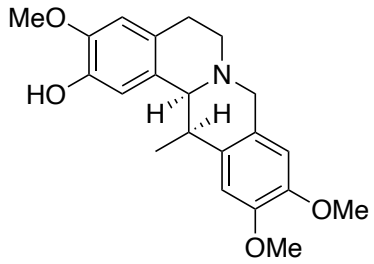
1.08
2.08
2.91
2.93
2.07
1.07
1.14
1.04
f1 (ppm)

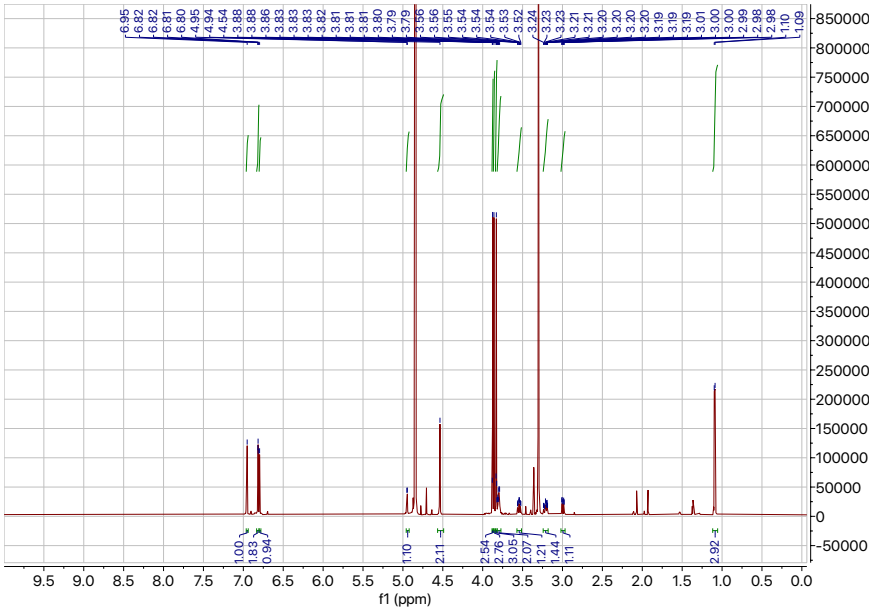

150.86
150.13
146.75
146.71
131.08
124.20
121.68
119.61
115.94
112.96
112.83
109.72
65.56
57.28
56.44
56.43
52.78
36.66
26.35
17.23
f1 (ppm)

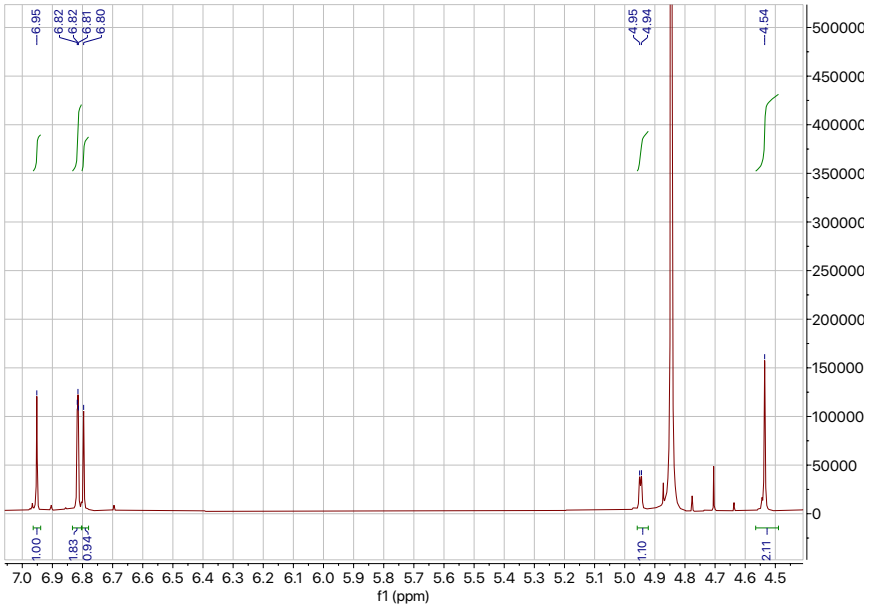

f1 (ppm)
f2 (ppm)

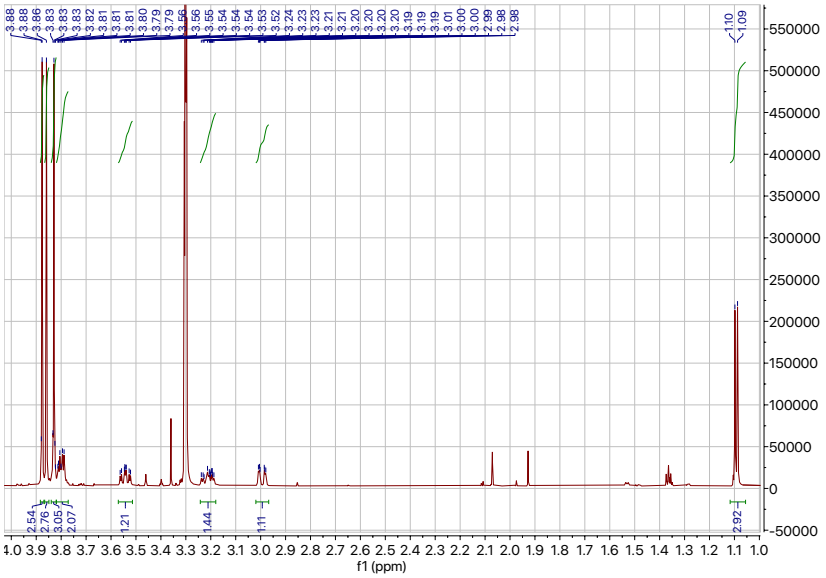

f1 (ppm)
f2 (ppm)

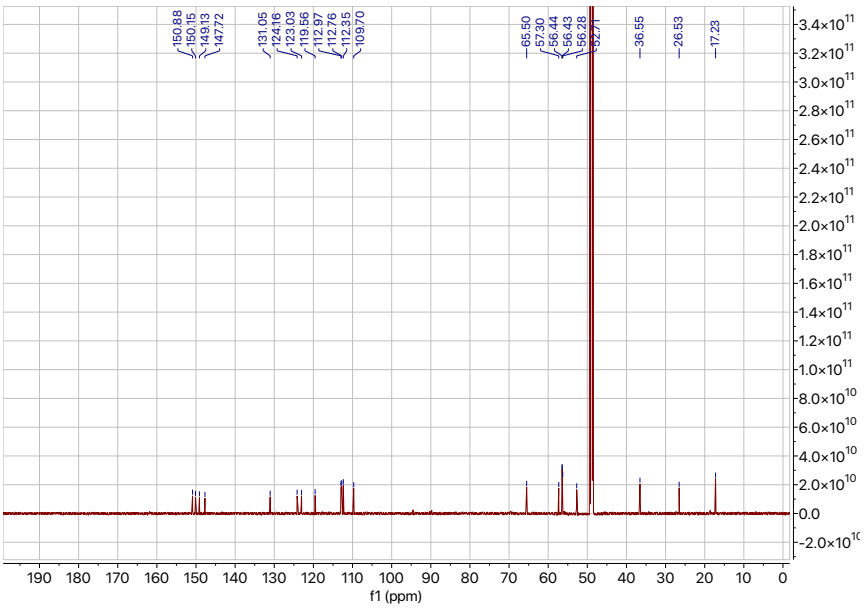
(13*R*,13a*S*)-3,10,11-Trimethoxy-13-methyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinolin-2-ol.TFA (4b) (700 MHz)



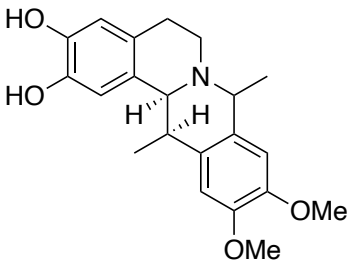


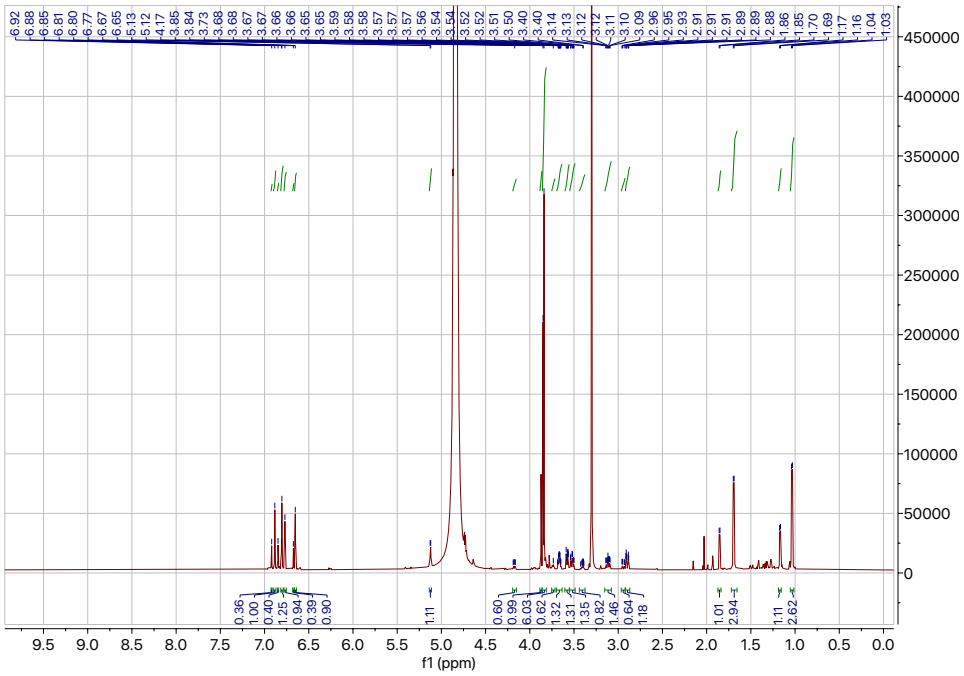


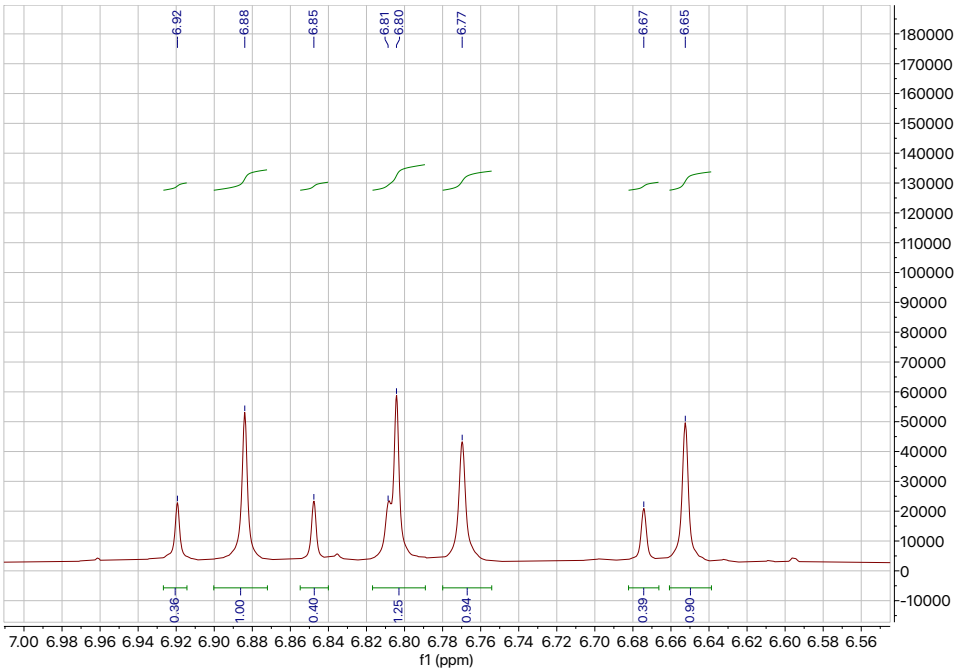

f1 (ppm)

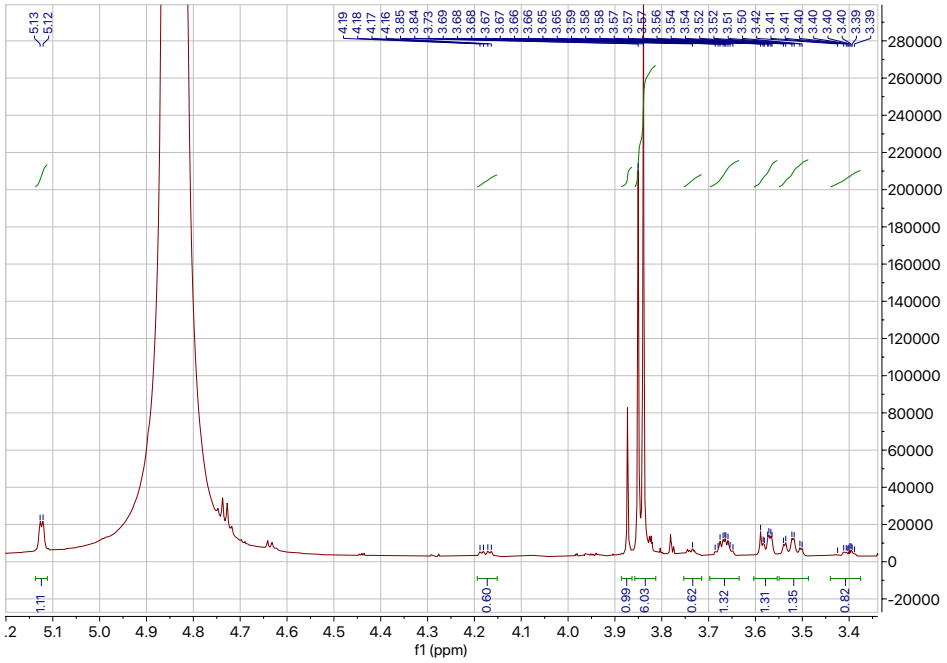

f1 (ppm)

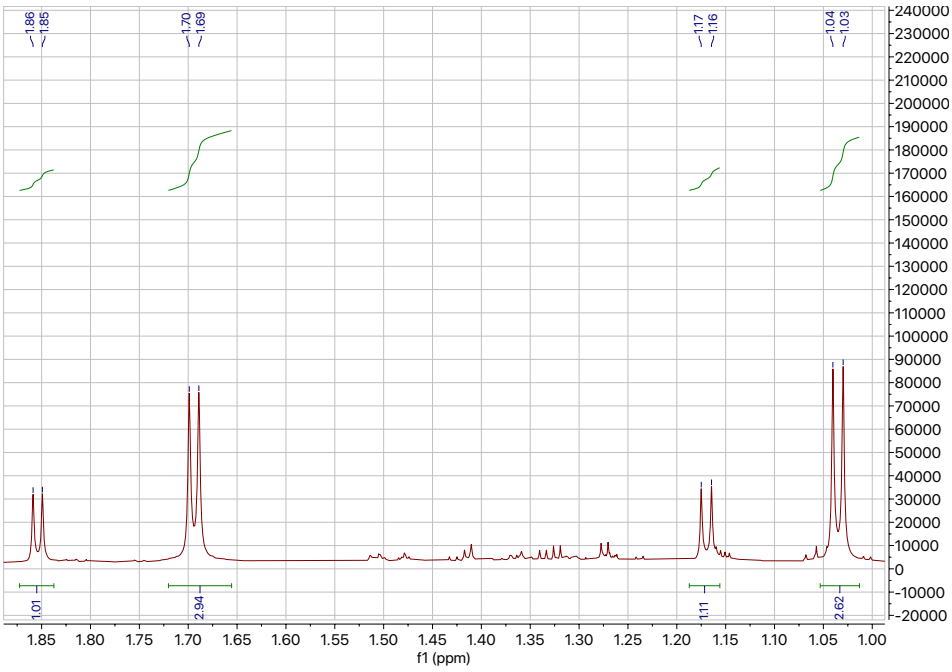
(8*SR*,13*R*,13a*S*)-10,11-Dimethoxy-8,13-dimethyl-5,8,13,13a-tetrahydro-6*H*-isoquinolino[3,2-*a*]isoquinoline-2,3-diol.TFA (4c) (700 MHz)

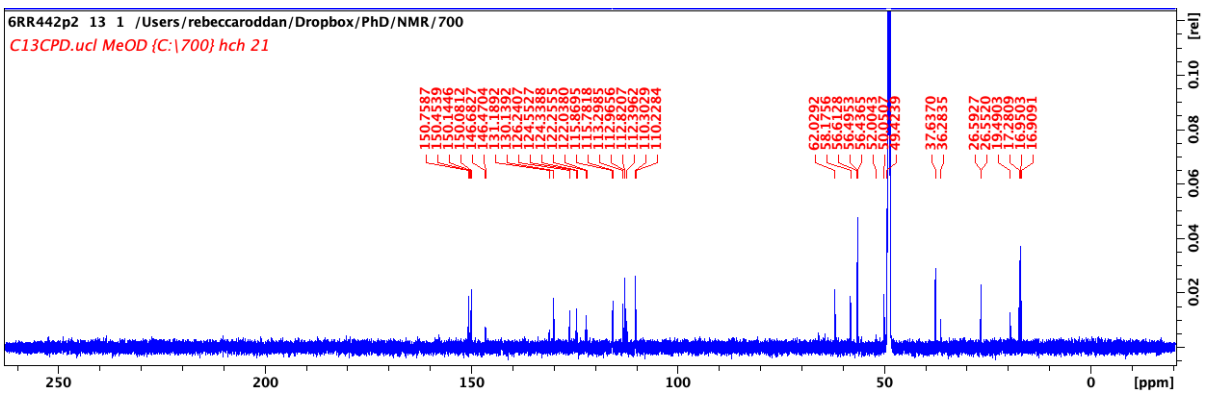


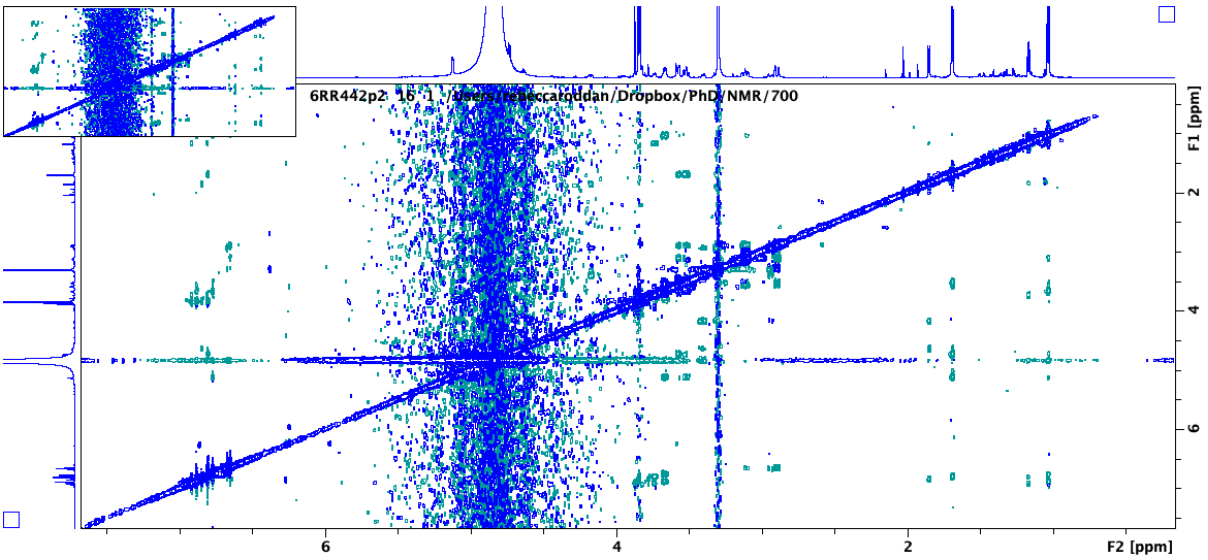


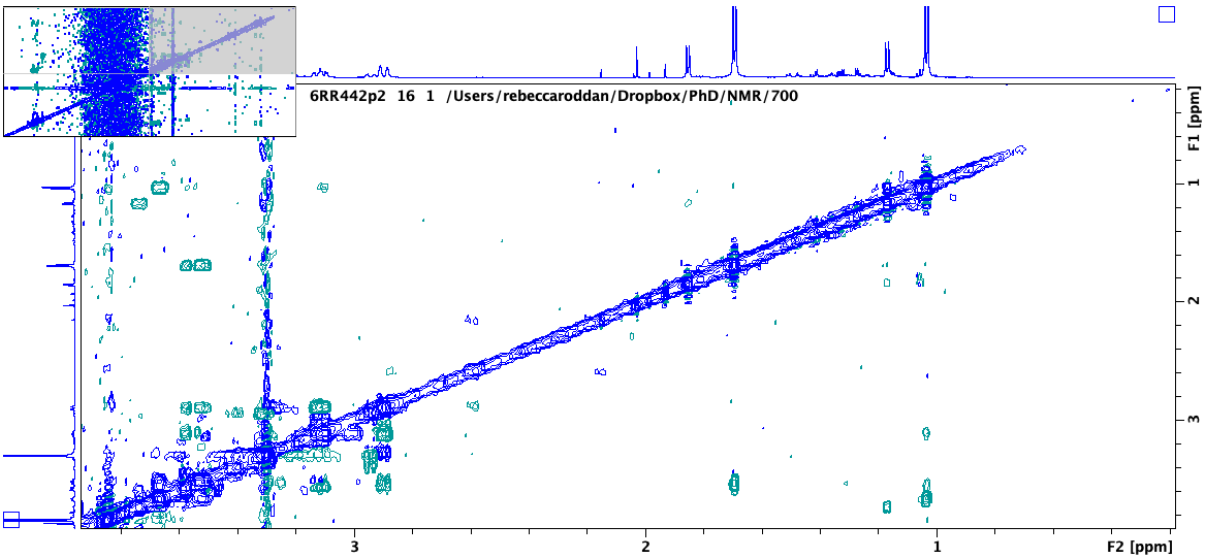



f1 (ppm)


f1 (ppm)

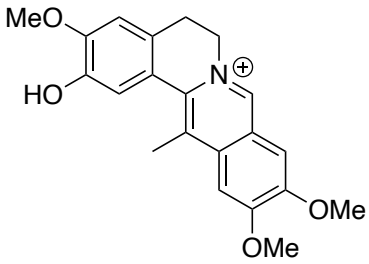

6RR442p2 13 1 /Users/rebeccaroddan/Dropbox/PhD/NMR/700
C13CPD.ucl MeOD {C:\700} hch 21
[ppm]
[rel]

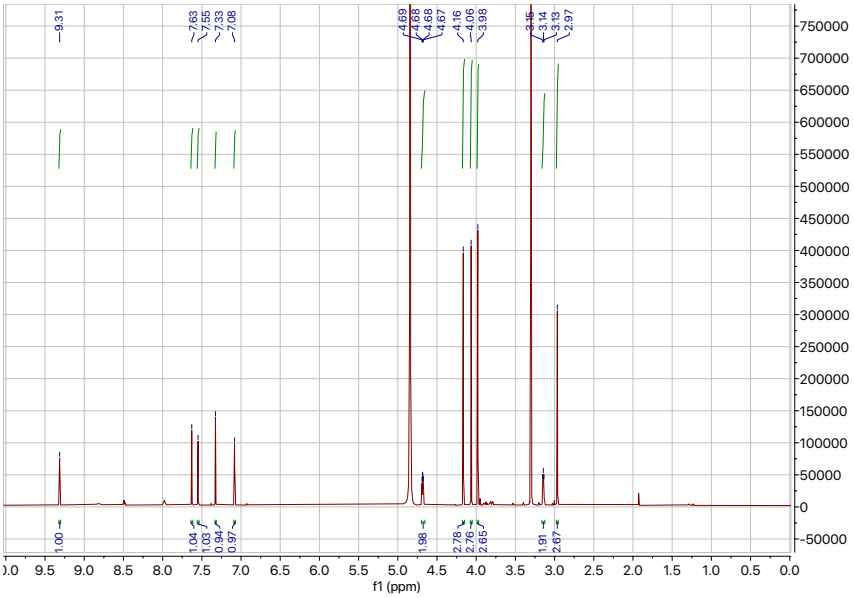

6RR442p2 16 1 /Users/rebeccaroddan/Dropbox/PhD/NMR/700
F1 [ppm]
F2 [ppm]
6
4
2

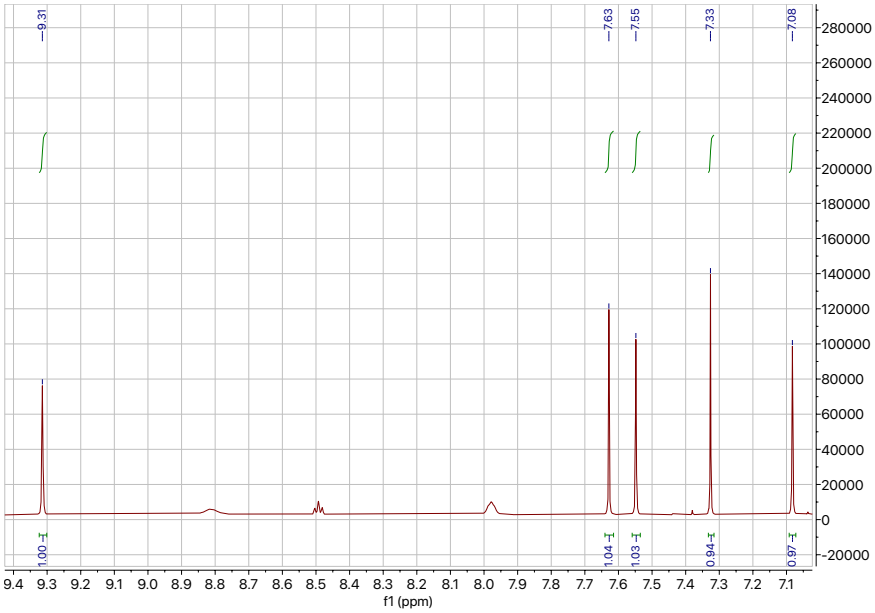

6RR442p2 16 1 /Users/rebeccaroddan/Dropbox/PhD/NMR/700
F1 [ppm]
F2 [ppm]
3
2
1

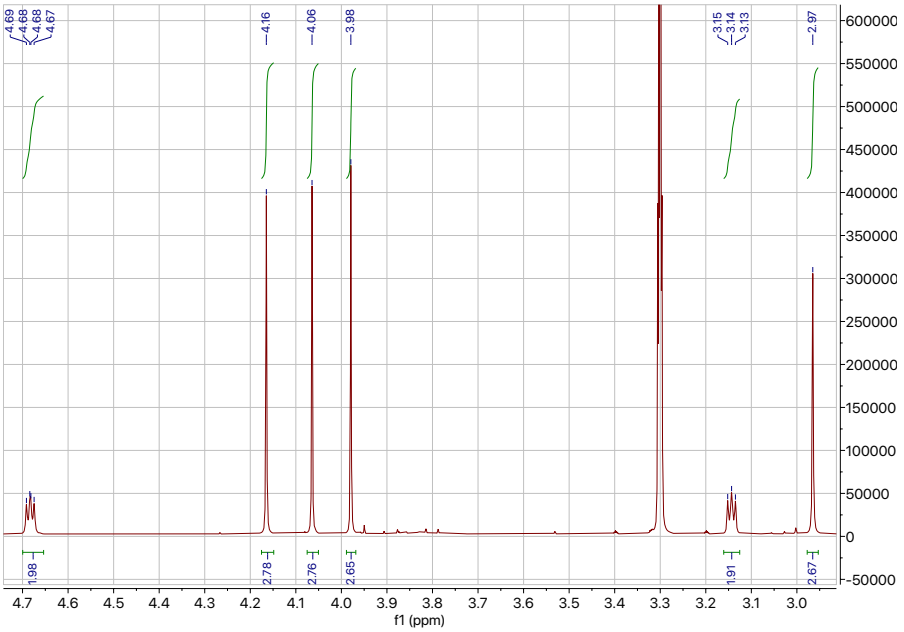
2-Hydroxy-3,10,11-Trimethoxy-13-methyl-5,6-dihydroisoquinolino[3,2-*a*]isoquinolin-7-ium (5)

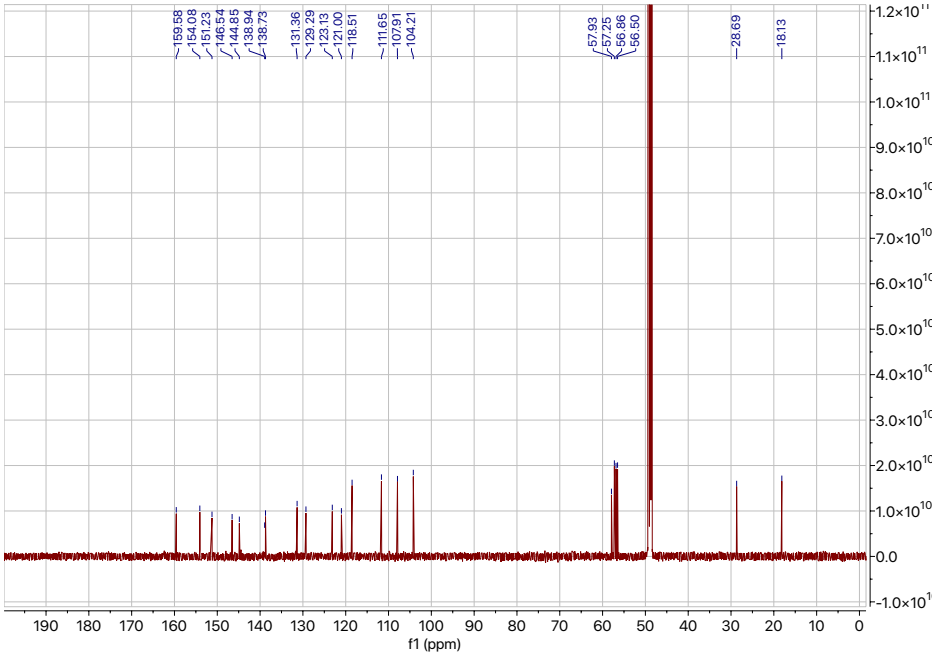
(700 MHz)








4.69
4.68
4.68
4.67
4.16
4.06
3.98
3.15
3.14
3.13
2.97
1.98
2.78
2.76
2.65
1.91
2.67
f1 (ppm)


159.58
154.08
151.23
146.54
144.85
138.94
138.73
131.36
129.29
123.13
121.00
118.51
111.65
107.91
104.21
57.93
57.25
56.86
56.50
28.69
18.13
f1 (ppm)

10. References

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