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Supporting Information

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Reuben B. Leveson-Gower, Ruben M. de Boer, and Gerard Roelfes*

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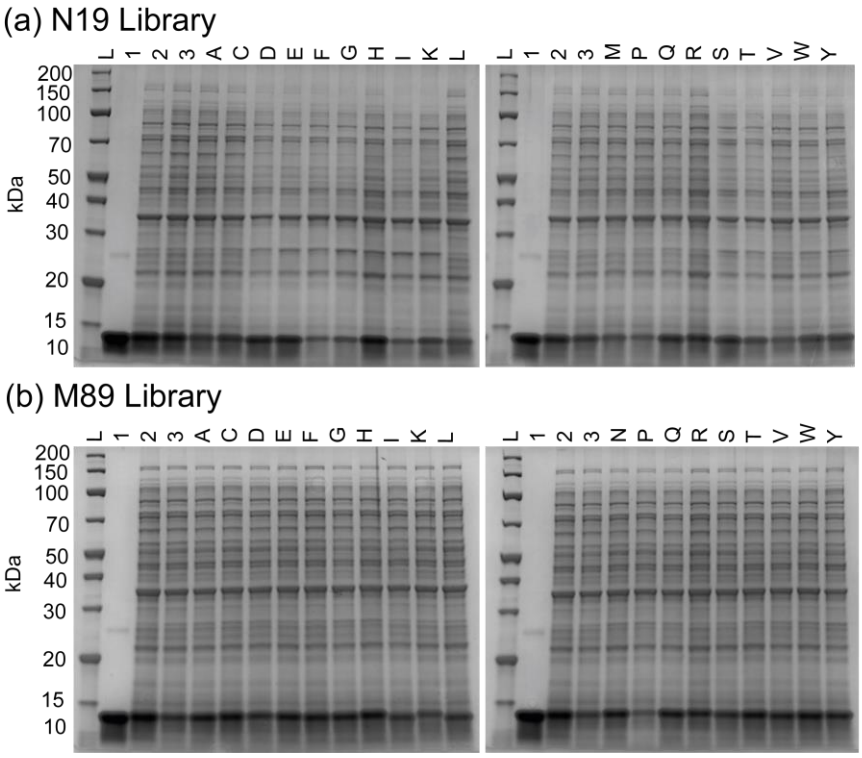
# 1. Supporting Figures




Supporting Figure 1: SDS-PAGE (ExpressPlus™ 12%, GenScript) analysis of soluble fraction of libraries expressing (a) LmrR_pAF_N19X mutants and (b) LmrR_pAF_M89X mutants. L = Thermo-Fisher broad-range unstained protein ladder; 1 = LmrR_pAF (purified) 5 µM; 2 = LmrR; 3 = LmrR_pAF.

# 2. Supporting Tables

**Supporting Table 1.** Optimisation of enzyme loading and substrates concentration for LmrR_pAF catalysed production of **3a**.[a]

| [LmrR_pAF] µM[b] | [1a] mM | [2a] mM | Time (h) | Yield (%)[c] | ee (%)[d] | TON[e] |
|---|---|---|---|---|---|---|
| 10 | 6 | 1 | 6 | 71 ± 3 | 88 ± 0 | 71 ± 3 |
| 5 | 6 | 1 | 16 | 69 ± 4 | 84 ± 0 | 138 ± 8 |
| 2 | 6 | 1 | 40 | 70 ± 14 | 76 ± 3 | 275 ± 70[f] |
| 1 | 6 | 1 | 40 | 28 ± 9 | 40 ± 21 | 130 ± 90[f] |
| 0 | 6 | 1 | 40 | 15 ± 0 | - | - |
| 10 | 1 | 6 | 16 | 97 ± 2 | 82 ± 2 | 97 ± 2 |
| 10 | 2 | 12 | 16 | 87 ± 8 | 78 ± 3 | 171 ± 15 |
| 10 | 3.5 | 21 | 16 | 61 ± 4 | 63 ± 2 | 214 ± 15 |
| 10 | 5 | 30 | 16 | 61 ± 11 | 54 ± 12 | 304 ± 57 |

[a]Reaction conditions: 300 µL volume reaction in phosphate buffer (50 mM , pH 6.5) containing NaCl (150 mM) and DMF (8 vol %). Reactions conducted at 4 °C for specified time. [b]LmrR_pAF concentration refers to that of the dimer. [c]Analytical yield calculated using normal-phase HPLC with a calibration curve. [d]Enantiomeric excess determined with chiral normal-phase HPLC. [e]TON = [**3a**]/[LmrR_pAF]. [f]40 hours background reaction subtracted from these entries. Experiments were conducted with two batches of protein, and in duplicate, of the four values obtained the results presented are the mean, whilst the errors given are the standard deviation.

**Supporting Table 2.** Background reactions for FC and FC-EP reactions[a]

| pH | Yield **3a** (%) | Yield **4** (%) |
|---|---|---|
| 6 | 3 ± 0 | <1 |
| 6.5 | 3 ± 0 | <1 |
| 7 | 3 ± 0 | <1 |

[a]Reaction conditions: 300 µL volume reaction in phosphate buffer (50 mM , pH 6.5) containing NaCl (150 mM) and DMF (8 vol %) [2-methyl-indole] = 1 mM and [methacrolein] = 6 mM or [crotonaldehyde] = 5 mM. Reactions conducted at 4 °C for 6 hours, followed by reduction with $NaBH_4$. Analytical yield calculated using normal-phase HPLC with a calibration curve. Experiments were conducted in triplicate, the results are the mean of the values obtained and the errors are the standard deviation.

# 3. Materials and Equipment

Chemicals were purchased from commercial suppliers (Sigma (UK), Acros (Germany), TCI (Belgium/Japan) and Fluorochem (UK)) and used without further purification unless specified. Flash column chromatography was performed on silica gel (Silica-P flash silica gel from Silicycle, 0.040-0.063 mm, 230-400 mesh). The unnatural amino acid pAzF was purchased as racemic mixture from Bachem (Switzerland) or as the enantiopure hydrochloride salt from Iris-Biotech (Germany). NMR $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian 400 (400 MHz) spectrometer in $CDCl_3$ or $(CD_3)_2SO$. Chemical shifts values (δ) are denoted in ppm using residual solvent peaks as the internal standard ($CHCl_3$: δ 7.26 for $^{1}$H; 77.16 for $^{13}$C. $(CD_3)_2SO$: δ 2.50 for $^{1}$H; 39.52 for $^{13}$C). HPLC analysis was conducted using a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector. Plasmid pEVOL-pAzF was obtained from Addgene (pEvol-pAzF was a gift from Prof. Peter Schultz (The Scripps Research Institute))[1]. Plasmid pEVOL_pAzFRS.2.t1 was obtained from Addgene (pEVOL_pAzFRS.2.t1 was a gift from Prof. Farren Isaacs, Yale University)[2]. *E. coli* strains, NEB5-alpha, NEB10-beta and BL21(DE3) (New England Biolabs) were used for cloning and expression. Primers were synthesized by Eurofins MWG Operon (Germany) and Sigma-Aldrich (UK). Plasmid Purification Kits were obtained from QIAGEN (Germany) and DNA sequencing carried out by GATC-Biotech (Germany). Phusion polymerase and DpnI were purchased from New England Biolabs. Strep-tactin columns (Strep-Tactin® Superflow® high capacity) and Desthiobiotin were purchased from IBA-Lifesciences (Germany). Concentrations of DNA and protein solutions were determined based on the absorption at 260 nm or 280 nm on a Thermo Scientific Nanodrop 2000 UV-Vis spectrophotometer. Molar extinction coefficients were approximated using the ProtParam Expasy web server https://web.expasy.org/protparam/. UPLC/MS analysis was performed on Waters Acquity Ultra Performance LC with Acquity TQD detector. Water (solvent A) and acetonitrile (solvent B) containing 0.1% formic acid by volume, were used as the mobile phase at a flow rate of 0.3 mL/min. Gradient: 90% A for 2 min, linear gradient to 50% A in 2 min, linear gradient to 20% A in 5 min, followed by 2 min at 5% A. Re-equilibration of the column with 2 min at 90% A. SFC analysis was performed using a Water Acquity UPC$^2$ system. High-Resolution Mass Spectrometry (HRMS) measurements were performed using a Thermo LTQ Orbitrap XL. Low-Resolution Mass Spectrometry (LRMS) measurements were performed using a Waters Acquity H-class UPLC with Waters Xevo G2 QTOF with the column set to bypass (direct-injection).

# Methods

## 3.1 Protein Production and Purification

LmrR_pAF variants were produced and purified as previously described[3]. The identity and purity of proteins and the successful reduction of pAzF were determined by mass spectrometry. Protein concentration was determined by correcting the calculated extinction coefficients for LmrR variants for the absorbance of pAF ($\varepsilon_{280}$ = 1333 $M^{-1}$ $cm^{-1}$)

## 3.2 Construction of Mutants

LmrR_V15TAG_L18A, LmrR_V15TAG_N19A, LmrR_V15TAG_K22A, LmrR_V15TAG_M89A, LmrR_V15TAG_F93A, LmrR_V15TAG_W96A, LmrR_V15TAG_D100A and LmrR_V15TAG_RGN were prepared and characterised previously[4,5]. The 18 remaining possible mutants at positions N19 and M89 were constructed by site-directed mutagenesis (QuikChange, Agilent Technologies). Primers are described in supporting information section 5. 25 µL reactions were set-up using Phusion polymerase (New England Biolabs) according to the manufacturer protocol with pET17b+_LmrR_V15TAG as template. The following PCR protocol was used: (1) initial denaturation at 98 °C for 1 min, (2) 20 cycles of denaturation at 98 °C for 30 s, annealing at 56-68 °C for 30 s, and extension at 72 °C for 2 min, (3) a final extension at 72 °C for 10 min. The crude reaction mixture was transformed into chemically competent *E. coli* cells of either NEB5α or NEB10β strains and spread onto LB agar plates containing ampicillin (100 µg/mL). Single colonies were sent for sequencing (GATC Biotech.). The isolated plasmid was then co-transformed with pEVOL_pAzFRS.2.t1 or pEVOL_pAzF into chemically-competent *E. coli* BL21(DE3) cells or transformed into chemically-competent *E. coli* BL21(DE3) cells containing pEVOL_pAzF_RS2.t1 or pEVOL_pAzFRS.2.t1 which were spread onto LB agar plates containing ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL). Single colonies from these plates were grown overnight in 5 mL LB containing ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL) and these culture were used to prepare glycerol stocks which were stored at -70 ºC until further use. pEVOL_pAzF or pEVOL_pAzFRS.2.t1 were used in the preparation of purified LmrR_pAF mutants, whilst only pEVOL_pAzFRS.2.t1 was used for the preparation of cell-free extracts in deep-well format, owing to the higher activity of cell-free extracts prepared using this OTS plasmid. These glycerol stocks were used for protein production.

## 3.3 Preparation of Cell-Free Extracts in Deep Well Format

Conducted in a similar manner to our previous works[4,6]. Glycerol stocks of the relevant mutants (along with LmrR_WT and LmrR_pAF as controls) were used to inoculate 1.5 mL deep well plates containing 500 µL LB media and appropriate antibiotics in triplicate. The resulting deep well plates were incubated overnight at 37 °C while shaking at 950 rpm (Titramax 1000 & Incubator 1000, Heidolph). The next morning, 50 µL of the densely grown overnight cultures were transformed into fresh 96-deep well plates containing 1150 µL LB media and appropriate antibiotics. Glycerol (500 µL, 50 % with miliQ water) was added to the remaining overnight culture, mixed thoroughly and stored at - 70 °C. Bacteria were cultured at 37 °C for 5 - 6 hours while shaking at 950 rpm. Subsequently, protein production was induced by addition of 50 µL LB media, containing IPTG (1.2 µL of a 1 M stock solution), arabinose (1.2 µL of a 20% arabinose stock solution) and p-azidophenylalanine at a concentration of 30 mM (final concentrations: IPTG = 1 mM, arabinose = 0.02%, pAzF = 1.2 mM). To avoid precipitation of the unnatural amino acid, pAzF was dissolved by addition of base (1 M NaOH) prior to addition to the LB media. Plates were then incubated at 30 °C for 16 hours while shaking (950 rpm) and then 50 µL was removed from each triplicate and combined to give 150 µL for each distinct mutant. This was used for SDS-PAGE analysis using the BugBuster® (primary amine-free) Extraction Reagent (Millipore). The remaining culture was harvested by centrifugation (3,500 rpm at 4 °C for 15 minutes). After removing the supernatant, cells were washed by addition of 500 µL of buffer A (50 mM sodium phosphate, 150 mM NaCl, pH = 6), and the supernatant was again discarded after centrifugations (3,500 rpm for 10 minutes). For the preparation of cell-free extracts, bacteria were resuspended in 300 µL buffer A, containing protease inhibitor (Roche cOmplete), lysozyme (1 mg/mL). DNase I (0.1 mg/mL) and $MgSO_4$ (10 mM) to assist in cell lysis and prevent protein degradation. Resuspended cells were incubated for 2 hours at 30 °C at 800 rpm and then stored until further use at -20 °C. The lysates were defrosted and 30 µL of a TCEP stock solution (100 mM in buffer A, adjusted to pH 6 by addition of 6 M NaOH) was added to individual wells. The reduction was initially performed for 2 hours at 30 °C, after which incubation was continued overnight at 4 °C. Subsequently, cell debris was removed by centrifugation (4,000 rpm, 1 hour, 4 °C) and 276 µL of cell-free lysate was transferred into 2 mL microcentrifuge tubes for catalysis, stored at 4 °C and used within 8 hours.

## 3.4 Catalysis with Cell-Free Extracts and Purified Protein

Reactions were conducted in 300 µL total volume in a 2 mL microcentrifuge tube. Stock solutions of protein in PBS buffer (50 mM NaCl, 150 mM $NaH_2PO_4$, pH as specified) to give the specified final concentration and the same buffer was added to make up 276 µL volume. For screening of N19 and M89 mutant libraries, 276 µL of cell-free lysate was used instead. Stock solutions of indole (25 mM in DMF, 12 µL added, final concentration 1 mM) and enal (150 mM or 750 mM when using cell-free lysate, 12 µL added to give final concentrations of 6 mM or 18 mM with cell free lysate) substrates were added. The microcentrifuge tubes were then mixed by continuous inversion in a cold room at 4 °C for the specified reaction time. After the reaction time had elapsed, $NaBH_4$ solution (60 µL, 20 mg/mL in 0.5 w/v % NaOH) and 3-(3-hydroxypropyl)indole internal standard solution (12 µL, 5 mM in DMF) were added. The micro-centrifuge tubes were mixed by continuous inversion for a further 30 minutes.

For HPLC analysis (Products **3a**, **3b** and **3d-i**): the reaction products and internal standard were then extracted by vortex mixing with EtOAc (1 mL) and the organic extract was dried over $Na_2SO_4$, filtered and evaporated to dryness. The residue thus obtained was redissolved by vortex mixing with HPLC grade solvent (heptane:isopropanol 4:1, 90 µL) and analysed by normal phase HPLC to determine yield and enantioselectivity with a 20 µL injection volume (injection volumes were reduced proportionately when indole concentrations above 1 mM were used).

For SFC analysis (cell free extract libraries with product **3a**, purified protein with product **3c** and **3l**) 400 µL n-butanol was added to the reactions and vortexed for one minute. The layers were separated with the aid of centrifugation (14,500 rpm, 5 minutes) and 150 µL of the organic layer was taken for SFC analysis, using a 10 µL injection volume. For product **3k**, the extraction and sample preparation procedure described for HPLC analysis was used, but the samples were analysed by SFC.

## 4. Primer List

| Primer | Sequence |
|---|---|
| M89C_fw | TGAAAACTGTCGCCTGGCGTTCGAAT |
| M89C_rv | CCAGGCGACAGTTTTCATGGCCGATT |
| M89D_fw | TGAAAACGATCGCCTGGCGTTCGAAT |
| M89D_rv | CCAGGCGATCGTTTTCATGGCCGATT |
| M89E_fw | ATCGGCCATGAAAACGAGCGCCTG |
| M89E_rv | TCGAACGCCAGGCGCTCGTTTTCA |
| M89F_fw | TGAAAACTTTCGCCTGGCGTTCGAAT |
| M89F_rv | CCAGGCGAAAGTTTTCATGGCCGATT |
| M89G_fw | TGAAAACGGTCGCCTGGCGTTCGAAT |
| M89G_rv | CCAGGCGACCGTTTTCATGGCCGATT |
| M89H_fw | TGAAAACCACCGCCTGGCGTTCGAAT |
| M89H_rv | CCAGGCGGTGGTTTTCATGGCCGATT |
| M89I_fw | TGAAAACATTCGCCTGGCGTTCGAAT |
| M89I_rv | CCAGGCGAATGTTTTCATGGCCGATT |
| M89K_fw | TGAAAACAAGCGCCTGGCGTTCGAAT |
| M89K_rv | CCAGGCGCTTGTTTTCATGGCCGATT |
| M89L_fw | TGAAAACCTGCGCCTGGCGTTCGAAT |
| M89L_rv | CCAGGCGCAGGTTTTCATGGCCGATT |
| M89N_fw | TGAAAACAATCGCCTGGCGTTCGAAT |
| M89N_rv | CCAGGCGATTGTTTTCATGGCCGATT |
| M89P_fw | TGAAAACCCGCGCCTGGCGTTCGAAT |
| M89P_rv | CCAGGCGCGGGTTTTCATGGCCGATT |
| M89Q_fw | TGAAAACCAGCGCCTGGCGTTCGAAT |
| M89Q_rv | CCAGGCGCTGGTTTTCATGGCCGATT |
| M89R_fw | TGAAAACCGTCGCCTGGCGTTCGAAT |
| M89R_rv | CCAGGCGACGGTTTTCATGGCCGATT |
| M89S_fw | TGAAAACTCTCGCCTGGCGTTCGAAT |
| M89S_rv | CCAGGCGAGAGTTTTCATGGCCGATT |
| M89T_fw | TGAAAACACCCGCCTGGCGTTCGAAT |
| M89T_rv | CCAGGCGGGTGTTTTCATGGCCGATT |
| M89V_fw | TGAAAACGTGCGCCTGGCGTTCGAAT |
| M89V_rv | CCAGGCGCACGTTTTCATGGCCGATT |
| M89W_fw | TGAAAACTGGCGCCTGGCGTTCGAAT |
| M89W_rv | CCAGGCGCCAGTTTTCATGGCCGATT |
| M89Y_fw | TGAAAACTATCGCCTGGCGTTCGAAT |
| M89Y_rv | CCAGGCGATAGTTTTCATGGCCGATT |
| N19C_fw | CTGCTGTGCGTCCTGAAACAAGGC |
| N19C_rv | TCAGGACGCACAGCAGGATCTAATTGGT |
| N19D_fw | CTGCTGGATGTCCTGAAACAAGGC |
| N19D_rv | TCAGGACATCCAGCAGGATCTAATTGGT |
| N19E_fw | CTGCTGGAAGTCCTGAAACAAGGC |
| N19E_rv | TCAGGACTTCCAGCAGGATCTAATTGGT |
| N19F_fw | CTGCTGTTTGTCCTGAAACAAGGC |
| N19F_rv | TCAGGACAAACAGCAGGATCTAATTGGT |
| N19G_fw | CTGCTGGGCGTCCTGAAACAAGGC |
| N19G_rv | TCAGGACGCCCAGCAGGATCTAATTGGT |
| N19H_fw | CTGCTGCATGTCCTGAAACAAGGC |
| N19H_rv | TCAGGACATGCAGCAGGATCTAATTGGT |
| N19I_fw | CTGCTGATTGTCCTGAAACAAGGC |
| N19I_rv | TCAGGACAATCAGCAGGATCTAATTGGT |
| N19K_fw | CTGCTGAAAGTCCTGAAACAAGGC |
| N19K_rv | TCAGGACTTTCAGCAGGATCTAATTGGT |
| N19L_fw | CTGCTGCTGGTCCTGAAACAAGGC |
| N19L_rv | TCAGGACCAGCAGCAGGATCTAATTGGT |
| N19M_fw | CTGCTGATGGTCCTGAAACAAGGC |
| N19M_rv | TCAGGACCATCAGCAGGATCTAATTGGT |
| N19P_fw | CTGCTGCCGGTCCTGAAACAAGGC |
| N19P_rv | TCAGGACCGGCAGCAGGATCTAATTGGT |
| N19Q_fw | CTGCTGCAGGTCCTGAAACAAGGC |

| | |
|---|---|
| N19Q_rv | TCAGGACCTGCAGCAGGATCTAATTGGT |
| N19R_fw | CTGCTGCGCGTCCTGAAACAAGGC |
| N19R_rv | TCAGGACGCGCAGCAGGATCTAATTGGT |
| N19S_fw | CTGCTGAGCGTCCTGAAACAAGGC |
| N19S_rv | TCAGGACGCTCAGCAGGATCTAATTGGT |
| N19T_fw | CTGCTGACCGTCCTGAAACAAGGC |
| N19T_rv | TCAGGACGGTCAGCAGGATCTAATTGGT |
| N19V_fw | CTGCTGGTGGTCCTGAAACAAGGC |
| N19V_rv | TCAGGACCACCAGCAGGATCTAATTGGT |
| N19W_fw | CTGCTGTGGGTCCTGAAACAAGGC |
| N19W_rv | TCAGGACCCACAGCAGGATCTAATTGGT |
| N19Y_fw | CTGCTGTATGTCCTGAAACAAGGC |
| N19Y_rv | TCAGGACATACAGCAGGATCTAATTGGT |

# 5. Preparation and Characterisation of Reference Products

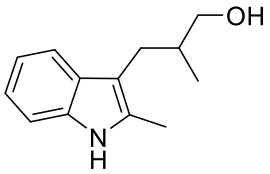
## 5.1 General Procedure for Preparation of Reference Products

The relevant indole (0.5 or 1 mmol) and enal (approximately 3 equivalents) were dissolved in hexafluoroisopropanol (2 or 4 mL) and benzylamine (12 µL, 0.11 mmol or 24 µL, 0.22 mmol) was added. The solution was stirred at ambient temperature until consumption of the indole as evidenced by TLC. Methanol (2 or 4 mL) was added, followed by portionwise additions of $NaBH_4$ (113 mg, 3 mmol or 227 mg, 3 mmol). The reaction was stirred for 30 mins at ambient temperature and then partitioned between EtOAc (~20 mL) and brine:water 1:1 (~10 mL) and shaken. The organic phase was dried over $Na_2SO_4$ and concentrated *in vacuo* and then purified by silica-gel flash chromatography (pentane:EtOAc 4:1). Fractions containing the product were combined and concentrated in vacuo to afford the title compounds.

Note on mass spectrometric analysis: attempts to obtain a HRMS of the molecular ion or its adducts failed despite the use of several ionisation techniques, instead we observed the loss of hydroxide for all reference compounds when using positive electrospray ionisation. This is likely because loss of hydroxide results in a stabilised tertiary carbocation after 1,2-H migration. The molecular proton adduct could be observed without fragmentation when we used a lower resolution technique with a different instrument, for all reference products. The abundance of this ion was very low in all cases, supporting the propensity of these compounds to lose hydroxide under mass spectrometry conditions.

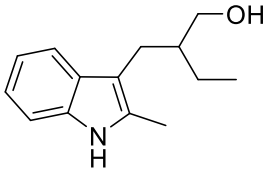
## 5.2 Characterisation of Reference Products

**2-methyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3a)**



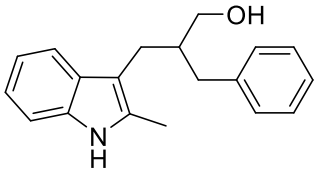

Prepared via the general procedure outlined above from 2-methyl-indole (66 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a yellow oil (36 mg, 35 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.27 (m, 1H), 7.18 – 7.02 (m, 2H), 3.59 (dd, *J* = 10.5, 5.7 Hz, 1H), 3.50 (dd, *J* = 10.5, 5.9 Hz, 1H), 2.79 (dd, *J* = 14.3, 6.9 Hz, 1H), 2.55 (dd, *J* = 14.3, 7.5 Hz, 1H), 2.38 (s, 3H), 2.09 – 1.96 (m, 1H), 1.30 (s, 1H), 0.97 (d, *J* = 6.8 Hz, 3H). The spectral data are in accordance with the literature[7]. LRMS calc'd for $C_{13}H_{18}NO$ ($[M+H]^+$) 204.1; measured 204.2. HRMS: calc'd for $C_{13}H_{16}N$ ($[M\text{-}OH]^+$) 186.1277; measured 186.1274. HPLC analysis: Chiracel OJ-H heptane:isopropanol 80:20 1 mL/min retention times 9.8 min and 10.8 min. SFC analysis: super-critical $CO_2$ (A) and methanol (B) were used for the mobile phase at a flow rate of 1.8 mL/min and a Trefoil AMY1 column was used for chiral separation. Program: 97% A with a linear gradient to 59% A in 3 min, 40% A for 1 min, 97% A for 1 min. Retention times of the enantiomers – 2.37 min and 2.48 min. The configuration of the major product from the enzyme catalysed reaction was tentatively assigned as (S) based on comparison of the order of elution of the enantiomer peaks with that reported in the literature using the same stationary phase. In that report, the configuration was assigned based on analogy to another product whose structure was determined with single-crystal X-ray diffraction[7]. Chiralpak AS-H heptane:isopropanol 90:10 0.5 mL/min retention times 12.9 min (major) and 13.9 min (minor). Literature: Chiralpak AS-H hexane:isopropanol 80:20 0.5 mL/min 13.09 (major – (S)) and 15.41 (minor – (R)).

**2-((2-methyl-1H-indol-3-yl)methyl)butan-1-ol (3b)**



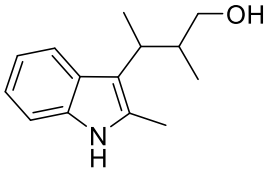

Prepared via the general procedure outlined above from 2-methyl-indole (66 mg, 0.5 mmol) and 2-ethyl-acrolein (150 µL, 1.53 mmol). Product obtained as a yellow oil (41 mg, 38 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.14 – 7.04 (m, 1H), 3.63 – 3.52 (m, 2H), 2.75 – 2.64 (m, 2H), 2.38 (s, 3H), 1.86 – 1.75 (m, 1H), 1.54 – 1.37 (m, 2H), 1.20 (s, 1H), 1.00 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 135.5, 131.6, 129.2, 121.2, 119.4, 118.4, 110.8, 110.3, 65.4, 43.8, 26.1, 24.2, 12.0, 11.8. LRMS calc'd for $C_{14}H_{20}NO$ ($[M+H]^+$) 218.2; measured 218.3. HRMS calc'd for $C_{14}H_{18}N$ ($[M\text{-}OH]^+$) 200.1434; measured 200.1433. HPLC analysis Chiracel AS-H heptane:isopropanol 90:10 0.5 mL/min retention times of the enantiomers 11.8 and 12.7 min.

**2-benzyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3c)**



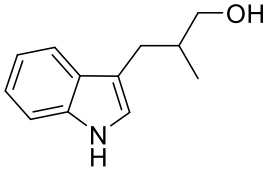

Prepared via the general procedure outlined above from 2-methyl-indole (66 mg, 0.5 mmol) and 2-benzyl-acrolein (220 mg, 1.5 mmol). Product obtained as a yellow oil (20 mg, 14 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.34 – 7.27 (m, 3H), 7.22 (d, *J* = 7.1 Hz, 3H), 7.11 (td, *J* = 7.5, 1.3 Hz, 1H), 7.06 (td, *J* = 7.4, 1.2 Hz, 1H), 3.58 – 3.48 (m, 2H), 2.85 – 2.67 (m, 4H), 2.35 (s, 3H), 2.24 – 2.14 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 141.1, 135.4, 131.7, 129.3, 129.1, 128.5, 126.1, 121.1, 119.3, 118.2, 110.4, 110.3, 64.9, 44.2, 38.3, 26.0, 11.9. LRMS calc'd for $C_{19}H_{22}NO$ ($[M+H]^+$) 280.2; measured 280.3. HRMS calc'd for $C_{19}H_{20}N$ ($[M-OH]^+$) 262.1590; measured 282.1591. SFC analysis: super-critical $CO_2$ (A) and methanol (B) were used for the mobile phase at a flow rate of 1.8 mL/min and a Chiralcel OJ-3 column was used for chiral separation. Program: 99% A with a linear gradient to 90% A in 8.5 min, linear gradient to 50% A in 0.5 min, 50% A for 1 min, 99% A for 1 min. Retention times of the enantiomers – 9.43 min and 9.61 min.

**2-methyl-3-(2-methyl-1H-indol-3-yl)butan-1-ol (3d)**



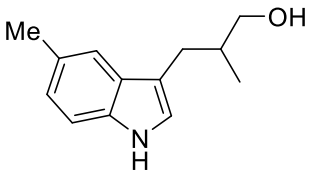

Tiglic aldehyde (568 µL, 5.87 mmol) was added to a solution of 2-Me-indole (250 mg, 1.81 mmol) in 8 mL DCM thereafter catalysts tosylic acid (31 mg) and benzylamine (19 µL) were added. The reaction mixture was stirred at 30 °C for 70 h. The solvent was removed *in vacuo* after which the intermediate was dissolved in 8 mL MeOH. $NaBH_4$ (400 mg, 6 eq) was added and the mixture was stirred at room temperature for 60 minutes. The solvent was removed *in vacuo* and the crude product was dissolved in 20 mL EtOAc thereafter it was washed with brine (3x 10 mL) in a separation funnel. The organic layer was dried over $Na_2SO_4$. The crude was purified by silica column (6:1, pentane:EtOAc). The product was obtained as a yellow oil in a mixture of diastereomers (44.8 mg, 10.7 %). First diastereomer: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.64 – 7.58 (m, 1H), 7.29 – 7.24 (m, 1H), 7.13 – 7.07 (m, 1H), 7.07 – 7.00 (m, 1H), 3.82 (dd, *J* = 10.5, 3.8 Hz, 1H), 3.66 (dd, *J* = 10.5, 6.3 Hz, 1H), 3.00 – 2.87 (m, 1H), 2.38 (s, 3H), 2.22 – 2.09 (m, 1H), 1.45 (d, *J* = 7.2 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H). Second diastereomer: $^{1}$H NMR (400 MHz, $cdcl_3$) δ 7.71 (s, 1H), 7.64 – 7.58 (m, 1H), 7.29 – 7.24 (m, 1H), 7.13 – 7.07 (m, 1H), 7.07 – 7.00 (m, 1H), 3.45 (dd, *J* = 10.5, 4.1 Hz, 1H), 3.33 – 3.22 (m, 1H), 2.86 – 2.74 (m, 1H), 2.38 (s, 3H), 2.22 – 2.09 (m, 1H), 1.42 (d, *J* = 7.1 Hz, 3H), 1.15 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 135.6, 130.7, 121.0, 120.7, 119.8, 119.6, 119.1, 118.9, 115.6, 110.5, 110.4, 67.7, 66.8, 41.3, 40.9, 34.0, 33.3, 18.8, 18.8, 16.0, 15.9, 12.5, 12.4. (Peaks for both diastereomers). LRMS calc'd for $C_{14}H_{20}NO$ ($[M+H]^+$) 218.2; measured 218.3; HRMS calc'd for $C_{14}H_{18}N$ ($[M-OH]^+$) 200.1432; measured 200.1429. HPLC analysis Chiralcel OD-H heptane:isopropanol 90:10 0.5 mL/min retention times 25.9 and 27.2 min (enantiomers of first diastereomer) and 32.6 and 33.9 min (enantiomers of second diastereomer)

**3-(1H-indol-3-yl)-2-methylpropan-1-ol (3e)**



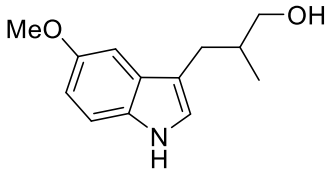

Prepared via the general procedure outlined above from indole (118 mg, 1 mmol) and methacrolein (228 µL, 2.76 mmol). Product obtained as a slightly turbid colourless oil (108 mg, 53 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.20 (t, J = 7.5 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.00 (s, 1H), 3.60 (dd, J = 10.5, 5.9 Hz, 1H), 3.52 (dd, J = 10.5, 6.0 Hz, 1H), 2.87 (dd, J = 14.4, 6.6 Hz, 1H), 2.63 (dd, J = 14.4, 7.4 Hz, 1H), 2.15 – 2.03 (m, 1H), 1.35 (s, 1H), 0.99 (d, J = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 136.5, 128.0, 122.2, 122.1, 119.4, 119.3, 114.9, 111.3, 68.3, 36.9, 29.1, 17.2. LRMS calc'd for $C_{12}H_{16}NO$ ($[M+H]^+$) 190.1; measured 190.2. HRMS calc'd for $C_{12}H_{14}N$ ($[M-OH]^+$) 172.1121; measured 172.1121. HPLC analysis Chiralcel OD-H heptane:isopropanol 85:15 1 mL/min retention times of the enantiomers 14.4 and 15.5 min.

**2-methyl-3-(5-methyl-1H-indol-3-yl)propan-1-ol (3f)**



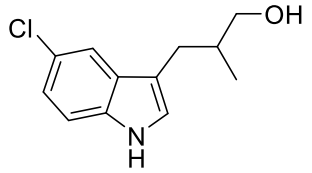

Prepared via the general procedure outlined above from 5-methyl-indole (66 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a colourless oil (31 mg, 31 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.39 (s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 6.96 (s, 1H), 3.60 (dd, J = 10.5, 5.8 Hz, 1H), 3.52 (dd, J = 10.5, 6.0 Hz, 1H), 2.84 (dd, J = 14.3, 6.6 Hz, 1H), 2.60 (dd, J = 14.3, 7.5 Hz, 1H), 2.46 (s, 3H), 2.16 – 2.04 (m, 1H), 0.99 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 134.9, 128.7, 128.2, 123.8, 122.4, 118.9, 114.4, 110.9, 68.4, 36.8, 29.2, 21.7, 17.3. LRMS calc'd for $C_{13}H_{18}NO$ ($[M+H]^+$) 204.1; measured 204.2. HRMS calc'd for $C_{13}H_{16}N$ ($[M\text{-}OH]^+$) 186.1277; measured 186.1278. HPLC analysis Chiralcel OJ-H heptane:isopropanol 80:20 retention time of the enantiomers 8.6 and 10.9 min.

**3-(5-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3g)**



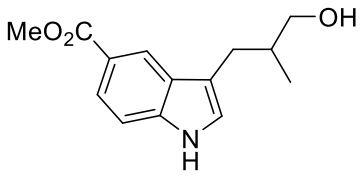

Prepared via the general procedure outlined above from 5-methoxy-indole (74 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a yellow oil (40 mg, 37 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.25 (d, J = 9.3 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.98 (d, J = 2.4 Hz, 1H), 6.86 (dd, J = 8.8, 2.3 Hz, 1H), 3.87 (s, 3H), 3.60 (dd, J = 10.5, 6.1 Hz, 1H), 3.52 (dd, J = 10.5, 6.0 Hz, 1H), 2.83 (dd, J = 14.4, 6.6 Hz, 1H), 2.60 (dd, J = 14.4, 7.4 Hz, 1H), 2.16 – 2.01 (m, 1H), 1.35 (s, 1H), 0.99 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.1, 131.7, 128.5, 123.1, 114.6, 112.2, 112.0, 101.3, 68.3, 56.2, 36.8, 29.2, 17.3. LRMS calc'd for $C_{13}H_{18}NO_2$ ($[M+H]^+$) 220.1; measured 220.2. HRMS calc'd for $C_{13}H_{16}NO$ ($[M\text{-}OH]^+$) 202.1226; measured 202.1225. HPLC analysis Chiracel OJ-H heptane:isopropanol 80:20 1 mL/min retention time of the enantiomers 11.0 and 12.1 min.

**3-(5-chloro-1H-indol-3-yl)-2-methylpropan-1-ol (3h)**




Prepared via the general procedure outlined above from 5-chloro-indole (76 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). 1 mL methanol was added to the reaction mixture to ensure complete dissolution of the indole starting material. Product obtained as a slightly turbid colourless oil (21 mg, 19 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.58 (s, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.13 (dd, J = 8.6, 1.7 Hz, 1H), 7.03 (d, J = 1.7 Hz, 1H), 3.64 – 3.47 (m, 2H), 2.84 (dd, J = 14.4, 6.4 Hz, 1H), 2.57 (dd, J = 14.4, 7.6 Hz, 1H), 2.11 – 1.99 (m, 1H), 1.31 (s, 1H), 0.97 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 134.8, 129.2, 125.3, 123.7, 122.4, 118.8, 114.8, 112.3, 68.1, 36.8, 28.9, 17.1. LRMS calc'd for $C_{12}H_{15}{}^{35}ClNO$ ($[M+H]^+$) 224.1; measured 224.2; calc'd for $C_{12}H_{15}{}^{37}ClNO$ ($[M+H]^+$) 226.1; measured 226.2. HRMS calcd for $C_{12}H_{13}{}^{35}ClN$ ($[M\text{-}OH]^+$) 206.0731; measured 206.0729; calc'd $C_{12}H_{13}{}^{37}ClN$ ($[M\text{-}OH]^+$) 208.0702; measured 208.0702. HPLC analysis Chiralcel OJ-H heptane:isopropanol 76:24 1 mL/min retention time of the enantiomers 6.0 and 6.6 min.

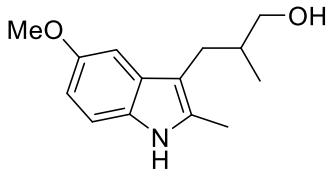
**methyl 3-(3-hydroxy-2-methylpropyl)-1H-indole-5-carboxylate (3i)**




Prepared via the general procedure outlined above from methyl indole-5-carboxylate (88 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a white solid (43 mg, 35 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ

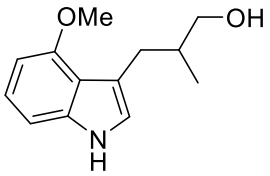
8.38 (s, 1H), 8.16 (s, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 7.07 (s, 1H), 3.94 (s, 3H), 3.63 – 3.50 (m, 2H), 2.91 (dd, *J* = 14.4, 6.5 Hz, 1H), 2.65 (dd, *J* = 14.4, 7.7 Hz, 1H), 2.20 – 2.04 (m, 1H), 1.34 (s, 1H), 0.99 (d, *J* = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.4, 139.1, 127.7, 123.6, 123.5, 122.3, 121.6, 116.5, 110.9, 68.1, 52.1, 36.9, 28.9, 17.1.. LRMS calc'd for $C_{14}H_{18}NO_3$ ($[M+H]^+$) 248.1; measured 248.2. HRMS calc'd for $C_{14}H_{16}NO_2$ ($[M-OH]^+$) 230.1176; measured 230.1176. HPLC analysis Chiralcel AS-H heptane:isopropanol 90:10 0.5 mL/min retention time of the enantiomers 18.9 and 20.2 min.

**3-(5-methoxy-2-methyl-1H-indol-3-yl)-2-methylpropan-1-ol (3j)**



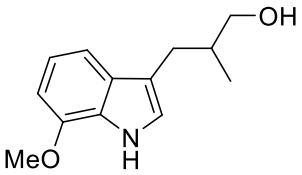

Prepared via the general procedure outlined above from 5-methoxy-2-methly-indole (81 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a yellow oil (48 mg, 41 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.15 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 6.77 (dd, J = 8.7, 2.1 Hz, 1H), 3.86 (s, 3H), 3.59 (dd, J = 10.4, 5.7 Hz, 1H), 3.50 (dd, J = 10.4, 6.0 Hz, 1H), 2.74 (dd, J = 14.3, 6.9 Hz, 1H), 2.52 (dd, J = 14.3, 7.5 Hz, 1H), 2.36 (s, 3H), 2.13 – 1.95 (m, 1H), 0.97 (d, J = 6.7 Hz, 3H) $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.1, 132.7, 130.6, 129.7, 110.9, 110.5, 110.5, 101.2, 68.4, 56.3, 37.4, 28.2, 17.3, 12.1. LRMS calc'd for $C_{14}H_{20}NO_2$ ($[M+H]^+$) 234.1; measured 234.2. HRMS calc'd for $C_{14}H_{18}NO$ ($[M-OH]^+$) 216.1383; measured 216.1382. HPLC analysis Chiralcel OD-H heptane:isopropanol 85:15 1 mL/min retention time of the enantiomers 14.2 and 15.3 min.

**3-(4-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3k)**



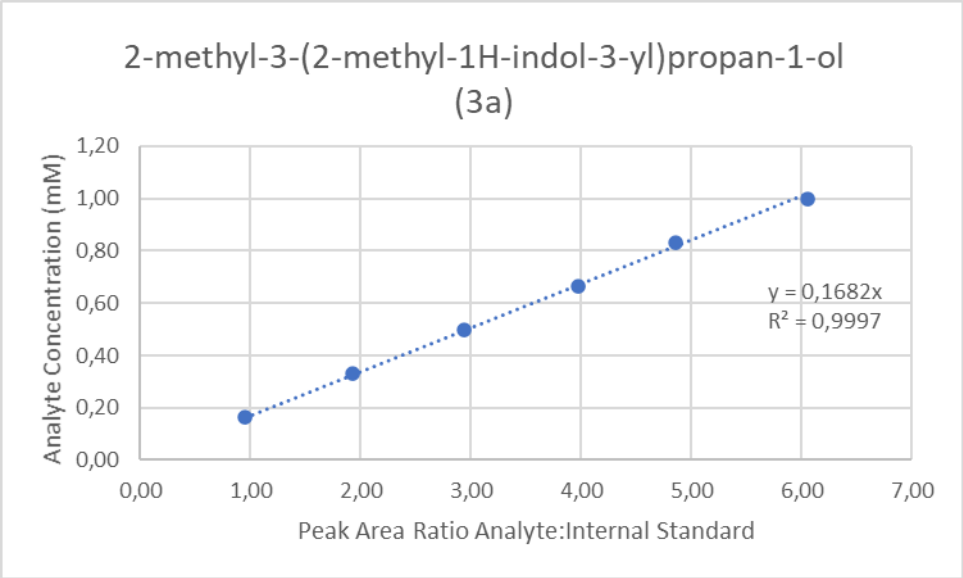

Prepared via the general procedure outlined above from 4-methoxy-indole (74 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a colourless oil (33 mg, 30 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.08 (t, J = 7.9 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 6.86 (d, J = 2.3 Hz, 1H), 6.51 (d, J = 7.7 Hz, 1H), 3.93 (s, 3H), 3.48 (s, 2H), 2.97 (dd, J = 14.1, 6.7 Hz, 1H), 2.77 (dd, J = 14.0, 6.6 Hz, 1H), 2.03 (m, 2H), 0.98 (d, J = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.7, 138.2, 122.8, 121.4, 117.7, 114.9, 104.9, 99.8, 67.5, 55.3, 38.0, 30.0, 16.9. LRMS calc'd for $C_{13}H_{18}NO_2$ ($[M+H]^+$) 220.1; measured 220.1. HRMS calc'd for $C_{13}H_{16}NO$ ($[M-OH]^+$) 202.1226; measured 202.1223. SFC analysis: super-critical $CO_2$ (A) and methanol (B) were used for the mobile phase at a flow rate of 1.8 mL/min and a Trefoil CEL1 column was used for chiral separation. Program: 97% A for 3 min, linear gradient to 50% A in 13 min, 50% A for 1 min, 97% A for 1 min. Retention times of the enantiomers – 5.62 min and 5.78 min.

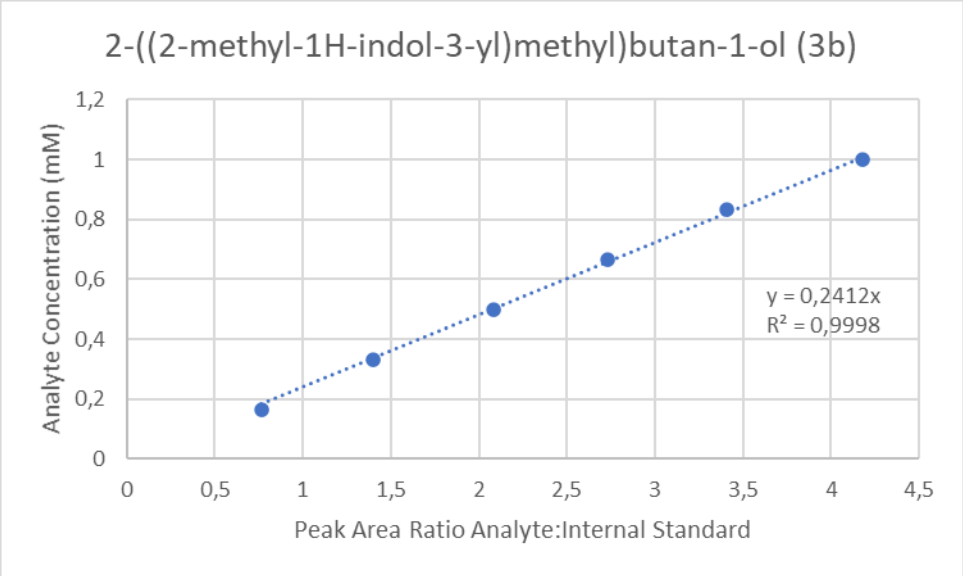
**3-(7-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3l)**

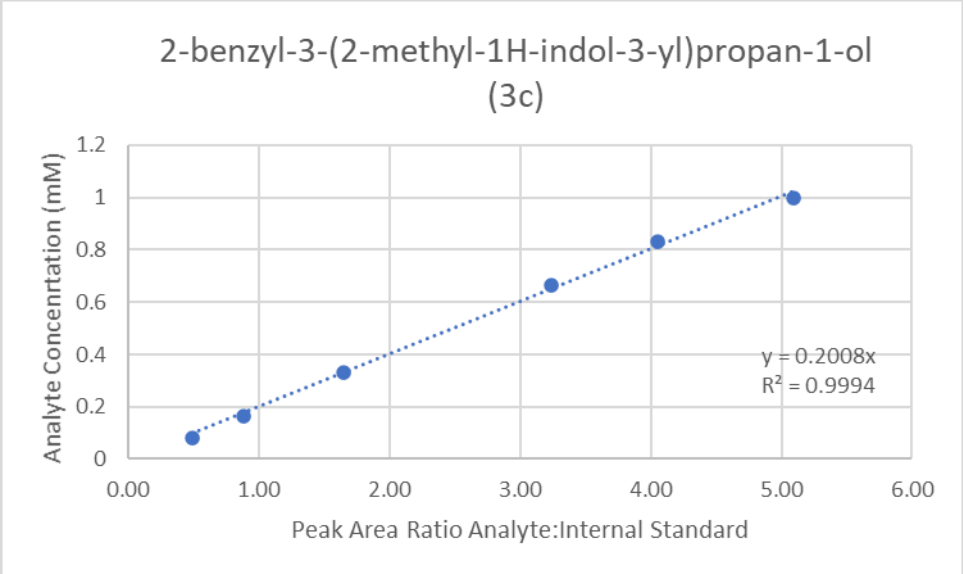


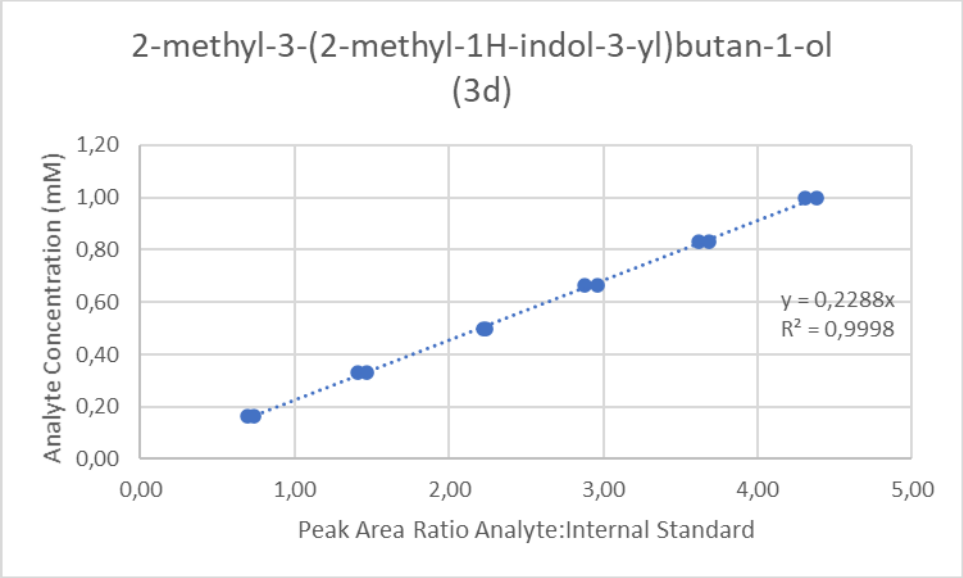

Prepared via the general procedure outlined above from 7-methoxy-indole (74 mg, 0.5 mmol) and methacrolein (114 µL, 1.38 mmol). Product obtained as a colourless oil (66 mg, 60 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.23 (d, J = 8.0 Hz, 1H), 7.03 (t, J = 7.9 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 6.65 (d, J = 7.7 Hz, 1H), 3.96 (s, 3H), 3.59 (dd, J = 10.6, 6.0 Hz, 1H), 3.51 (dd, J = 10.6, 6.0 Hz, 1H), 2.84 (dd, J = 14.4, 6.6 Hz, 1H), 2.61 (dd, J = 14.3, 7.4 Hz, 1H), 2.07 (dq, J = 13.2, 6.6 Hz, 1H), 1.36 (s, 1H), 0.98 (d, J = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 146.3, 129.3, 126.9, 121.8, 119.7, 115.2, 112.0, 101.9, 68.2, 55.4, 36.9, 29.2, 17.1. LRMS calc'd for $C_{13}H_{18}NO_2$ (M+H) 220.1; measured 220.1. HRMS calc'd for $C_{13}H_{16}NO$ ($[M-OH]^+$) 202.1226; measured 202.1222. SFC analysis: super-critical $CO_2$ (A) and methanol (B) were used for the mobile phase at a flow rate of 1.8 mL/min and a Trefoil CEL1 column was used for chiral separation. Program: 97% A with a linear gradient to 50% A in 3 min, 50% A for 1 min, 97% A for 1 min. Retention times of the enantiomers – 2.58 min and 2.69 min.

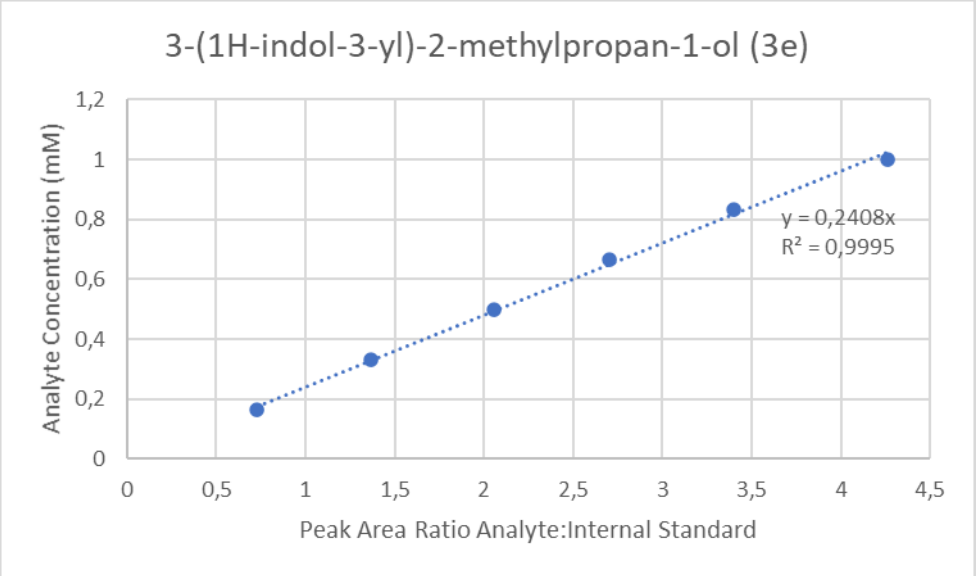
## 6. Calibration Curves

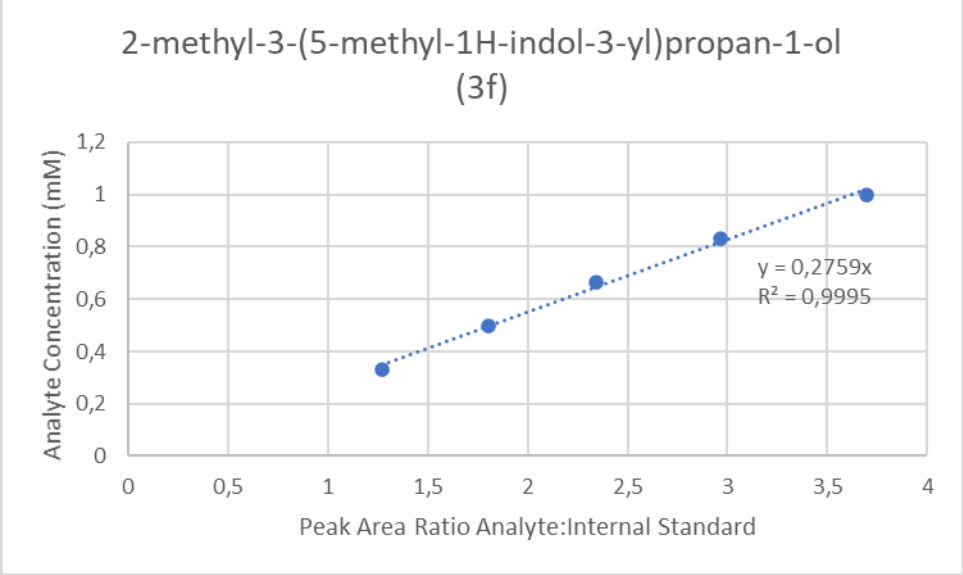


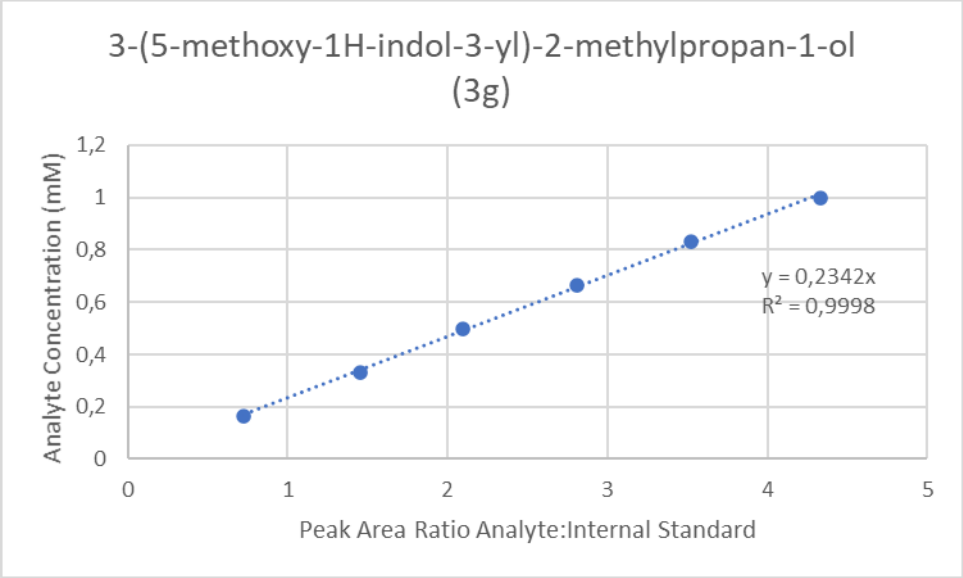



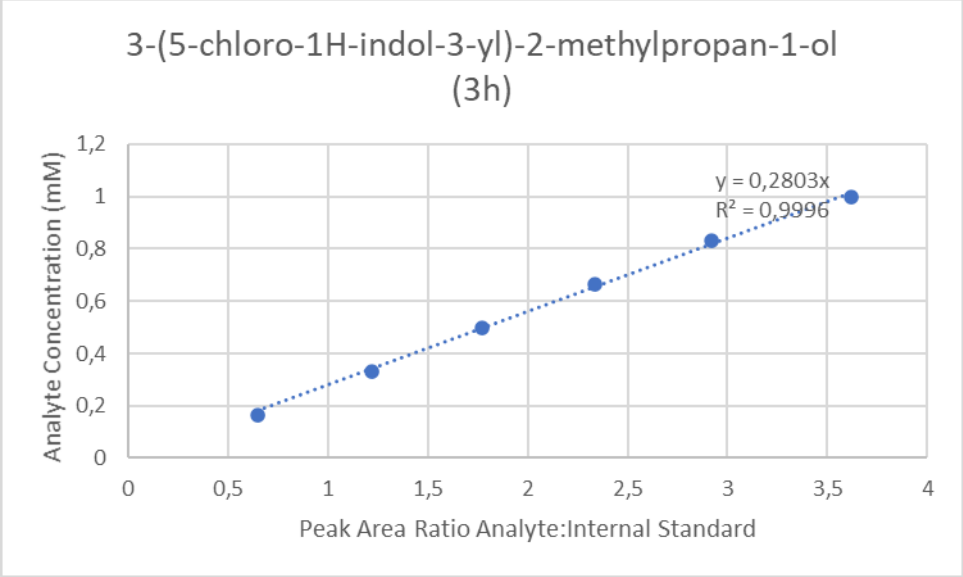



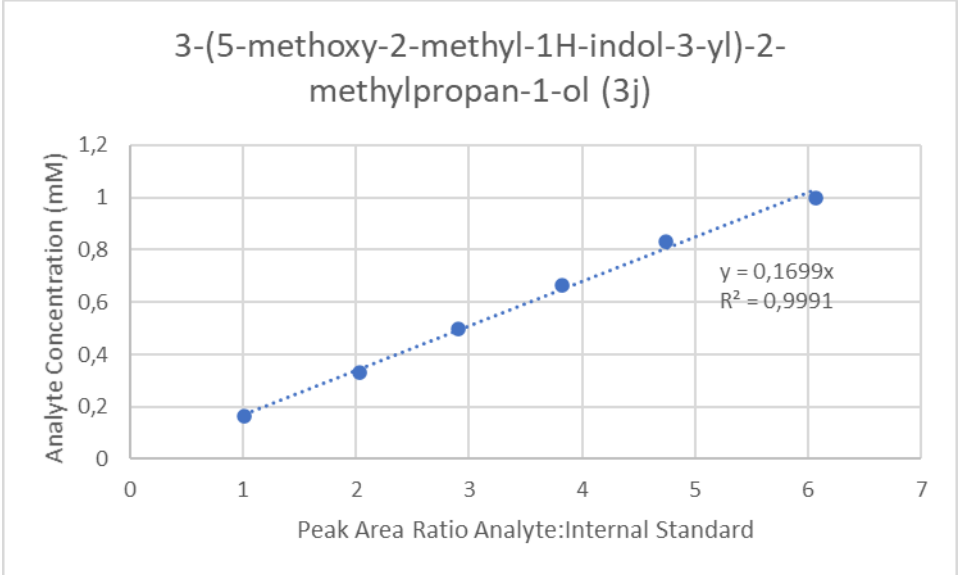

2-methyl-3-(2-methyl-1H-indol-3-yl)butan-1-ol (3d)
Analyte Concentration (mM)
1,20
1,00
0,80
0,60
0,40
0,20
0,00
0,00
1,00
2,00
3,00
4,00
5,00
Peak Area Ratio Analyte:Internal Standard
y = 0,2288x
R² = 0,9998

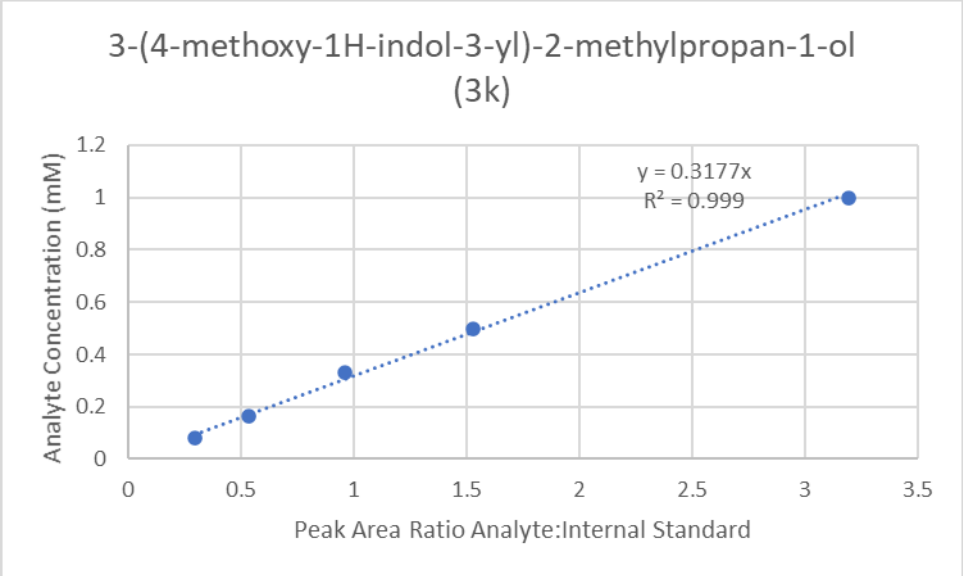

3-(1H-indol-3-yl)-2-methylpropan-1-ol (3e)
Analyte Concentration (mM)
1,2
1
0,8
0,6
0,4
0,2
0
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
Peak Area Ratio Analyte:Internal Standard
y = 0,2408x
R² = 0,9995

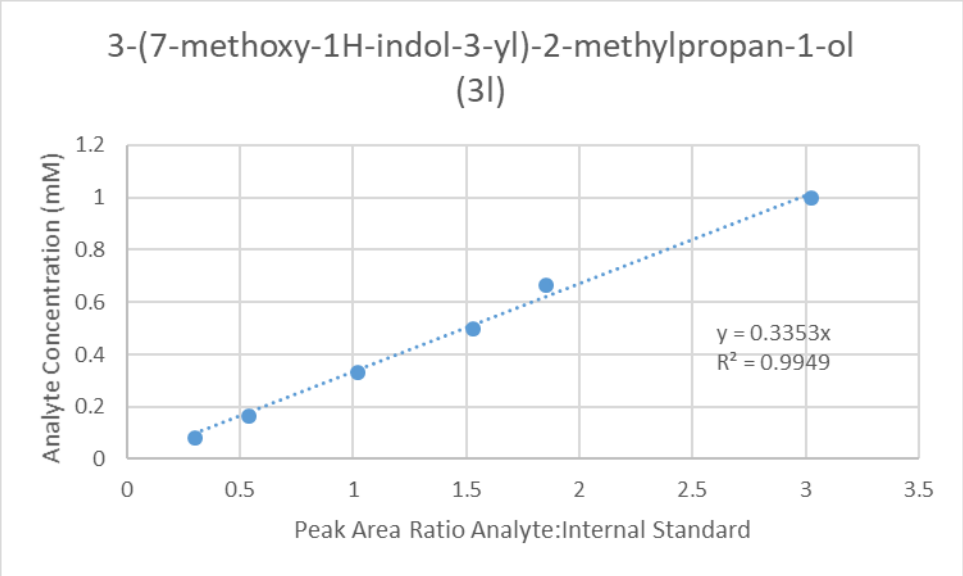

2-methyl-3-(5-methyl-1H-indol-3-yl)propan-1-ol (3f)
Analyte Concentration (mM)
1,2
1
0,8
0,6
0,4
0,2
0
0
0,5
1
1,5
2
2,5
3
3,5
4
Peak Area Ratio Analyte:Internal Standard
y = 0,2759x
R² = 0,9995


3-(5-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3g)
Analyte Concentration (mM)
1,2
1
0,8
0,6
0,4
0,2
0
0
1
2
3
4
5
y = 0,2342x
R² = 0,9998
Peak Area Ratio Analyte:Internal Standard


3-(5-chloro-1H-indol-3-yl)-2-methylpropan-1-ol (3h)
Analyte Concentration (mM)
1,2
1
0,8
0,6
0,4
0,2
0
0
0,5
1
1,5
2
2,5
3
3,5
4
y = 0,2803x
R² = 0,9996
Peak Area Ratio Analyte:Internal Standard


3-(5-methoxy-2-methyl-1H-indol-3-yl)-2-methylpropan-1-ol (3j)
Analyte Concentration (mM)
1,2
1
0,8
0,6
0,4
0,2
0
0
1
2
3
4
5
6
7
y = 0,1699x
R² = 0,9991
Peak Area Ratio Analyte:Internal Standard

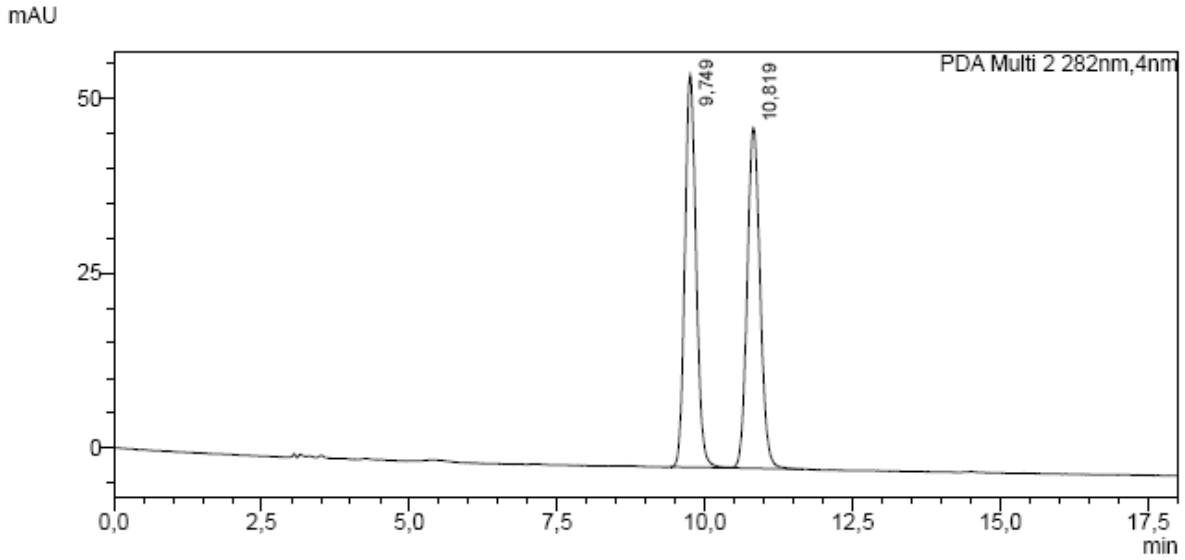

3-(4-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3k)
y = 0.3177x
R² = 0.999
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
0
0.2
0.4
0.6
0.8
1
1.2
0
0.5
1
1.5
2
2.5
3
3.5


3-(7-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3l)
y = 0.3353x
R² = 0.9949
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
0
0.2
0.4
0.6
0.8
1
1.2
0
0.5
1
1.5
2
2.5
3
3.5

# 7. HPLC Chromatograms

**2-methyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3a)**

Reference product:

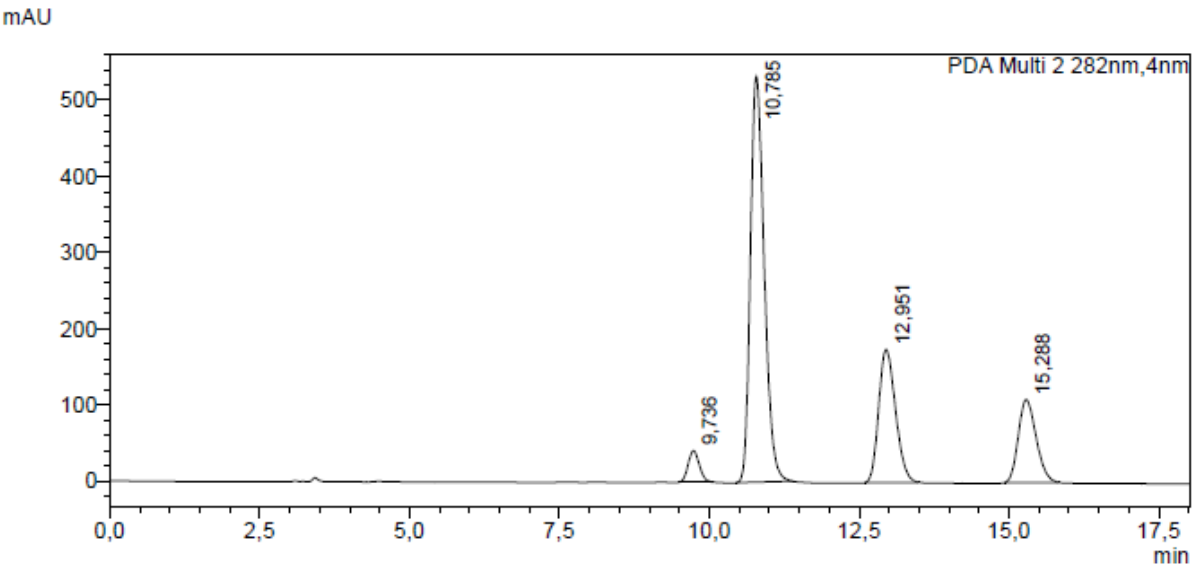



**<Peak Table>**

PDA Ch2 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 9,749 | 739769 | 56018 | 49,970 | | | |
| 2 | 10,819 | 740666 | 48623 | 50,030 | | | |
| Total | | 1480435 | 104641 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 6 hours reaction time at 4°. 13.0 min = 2-methyl-indole. 15.3 min = 3-(3-hydroxypropyl)-indole (internal standard).

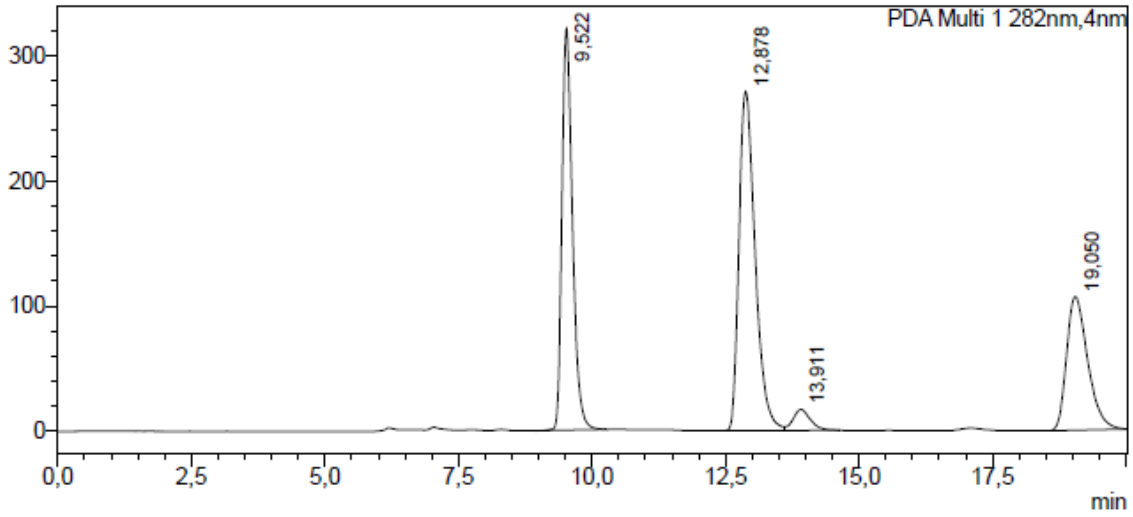



**<Peak Table>**

PDA Ch2 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 9,736 | 536858 | 41397 | 0,000 | | | |
| 2 | 10,785 | 8547512 | 532680 | 0,000 | | | |
| 3 | 12,951 | 3412447 | 174615 | 0,000 | | | |
| 4 | 15,288 | 2250590 | 108690 | 0,000 | | | |
| Total | | 14747407 | 857382 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 2 hours reaction time at 4°. 9.5 min = 2-methyl-indole. 12.9 min and 13.9 product enantiomers. 19.5 min = 3-(3-hydroxypropyl)-indole (internal standard). Using Chiralpak AS-H, heptane:isopropanol 90:10 0.5 mL/min.



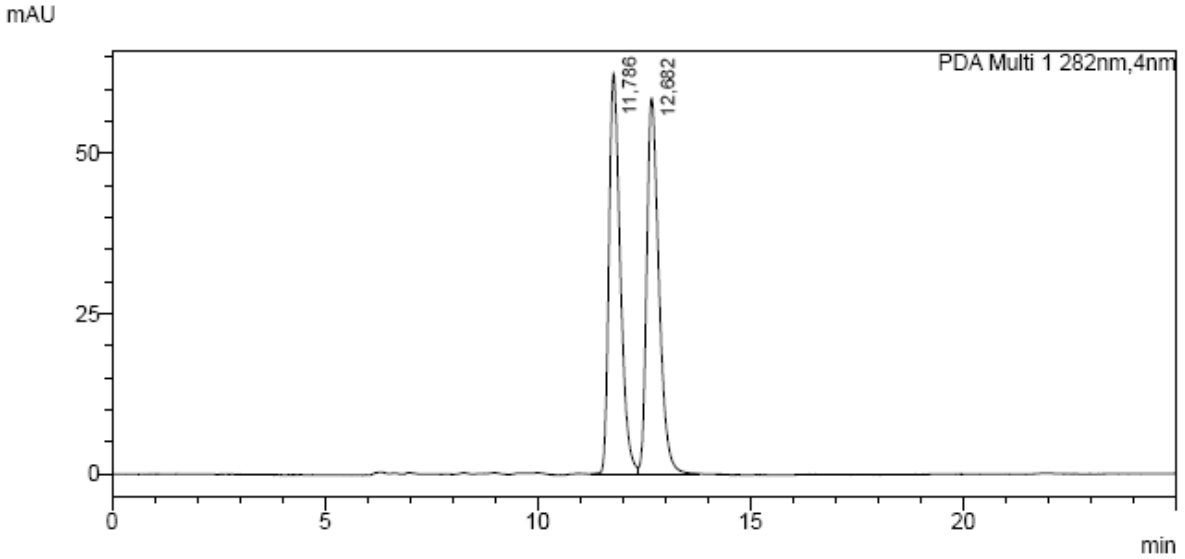

**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 9,522 | 4408506 | 320162 | 32,861 | | | |
| 2 | 12,878 | 5691292 | 270269 | 42,423 | | | |
| 3 | 13,911 | 376102 | 16678 | 2,803 | | V | |
| 4 | 19,050 | 2939663 | 106700 | 21,912 | | | |
| Total | | 13415562 | 713809 | | | | |

**2-((2-methyl-1H-indol-3-yl)methyl)butan-1-ol (3b)**

Reference product:

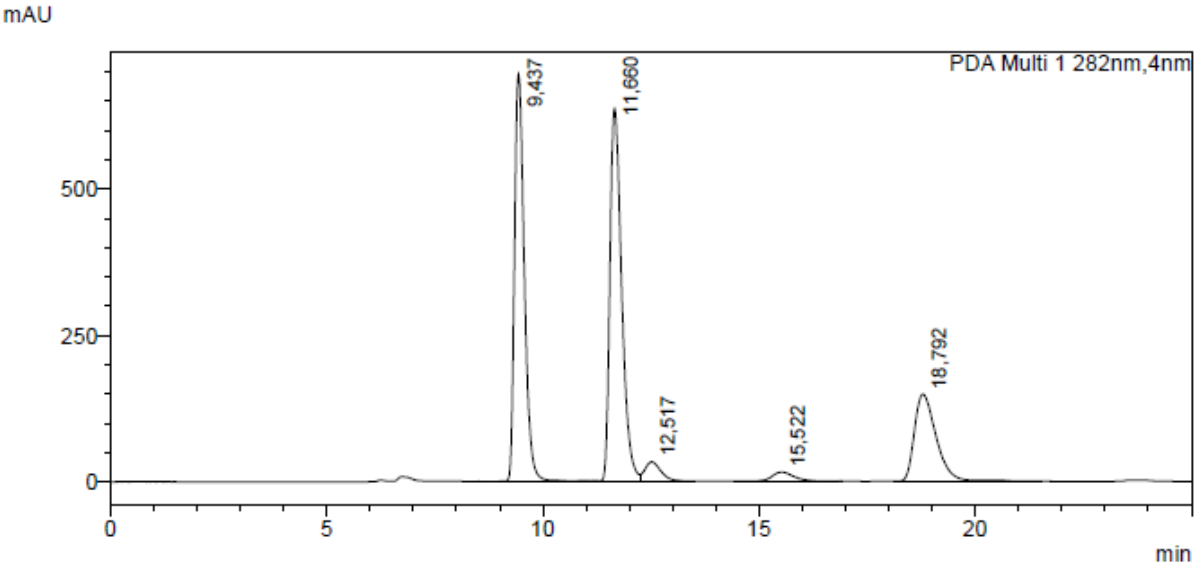



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 11,786 | 1156851 | 62322 | 0,000 | | | |
| 2 | 12,682 | 1163857 | 58529 | 0,000 | | SV | |
| Total | | 2320708 | 120850 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 6 hours reaction time at 4°. 9.4 min = 2-methyl-indole. 18.8 min = 3-(3-hydroxypropyl)-indole (internal standard). 15.5 min = unidentified impurity.



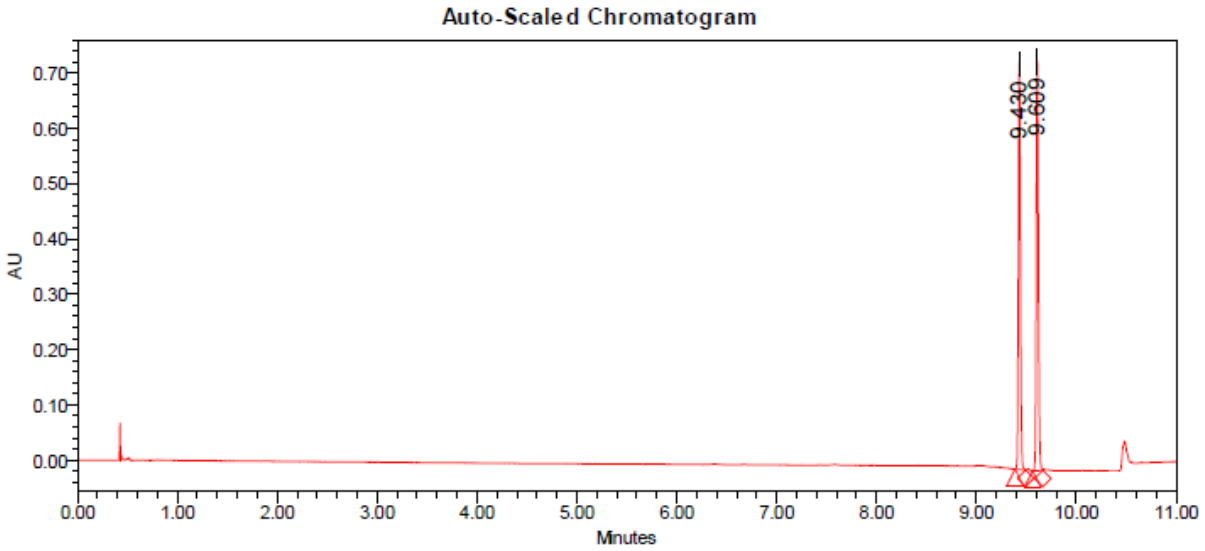

**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 9,437 | 11082272 | 696206 | 37,062 | | SV | |
| 2 | 11,660 | 12047177 | 637707 | 40,289 | | V | |
| 3 | 12,517 | 909755 | 33637 | 3,042 | | V | |
| 4 | 15,522 | 599441 | 16129 | 2,005 | | V | |
| 5 | 18,792 | 5263220 | 149035 | 17,602 | | S | |
| Total | | 29901865 | 1532714 | | | | |

**2-benzyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3c)**

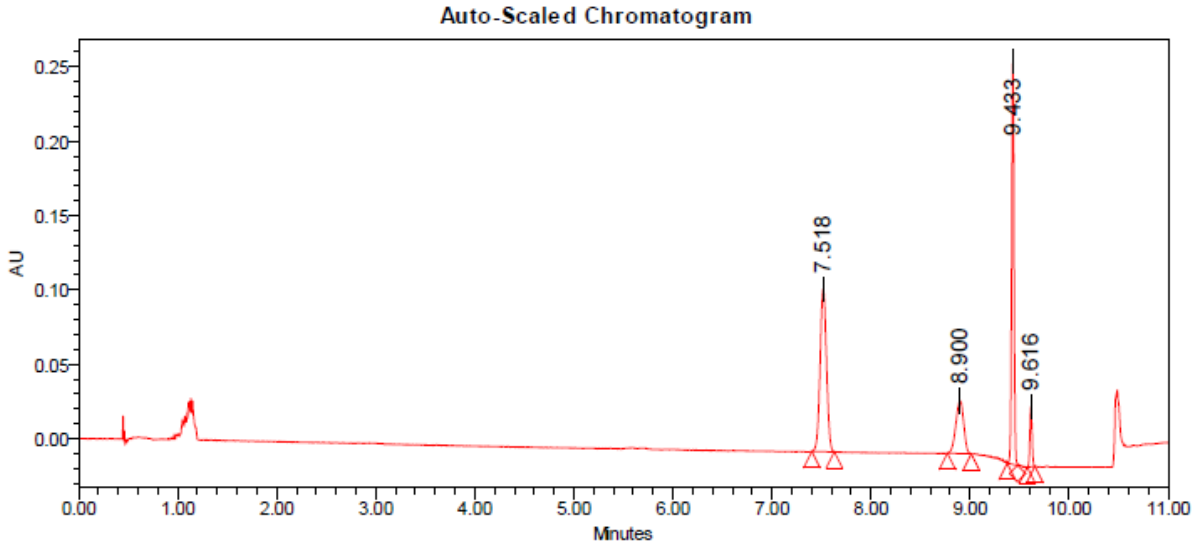
Reference product:




Peak Results

| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 9.430 | 1053118 | 735226 | 49.83 |
| 2 | | 9.609 | 1060366 | 741880 | 50.17 |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 7.5 min = 2-methyl-indole. 8.9 min = 3-(3-hydroxypropyl)-indole (internal standard). Peak around 1 min = unidentified. Apparent peaks around 10.5 min are the result of baseline shift during rapid solvent gradient.

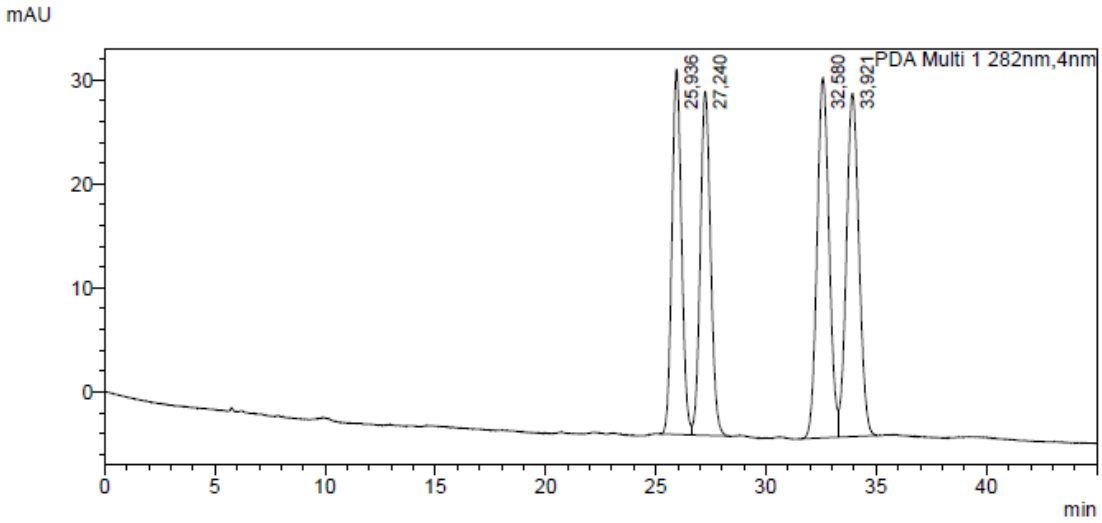



Peak Results

| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 7.518 | 490516 | 109294 | 40.69 |
| 2 | | 8.900 | 196370 | 35396 | 16.29 |
| 3 | | 9.433 | 457094 | 272588 | 37.92 |
| 4 | | 9.616 | 61381 | 40605 | 5.09 |

**2-methyl-3-(2-methyl-1H-indol-3-yl)butan-1-ol (3d)**

Reference product:

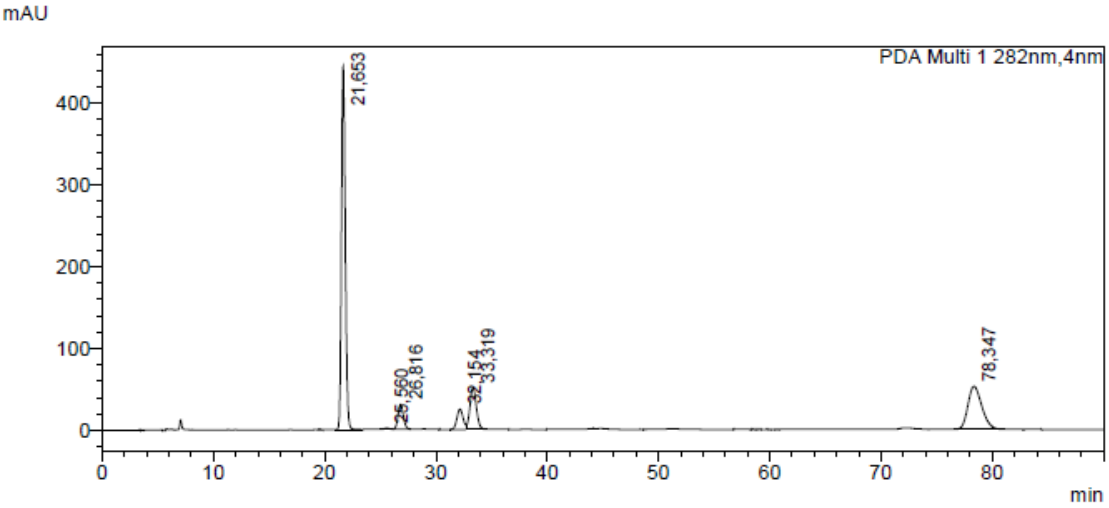



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 25,936 | 1064487 | 35056 | 22,342 | | | |
| 2 | 27,240 | 1078263 | 33040 | 22,631 | | V | |
| 3 | 32,580 | 1312015 | 34614 | 27,538 | | | |
| 4 | 33,921 | 1309699 | 32971 | 27,489 | | V | |
| Total | | 4764463 | 135681 | | | | |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 21.7 min = 2-methyl-indole. 78.3 min = 3-(3-hydroxypropyl)-indole (internal standard).

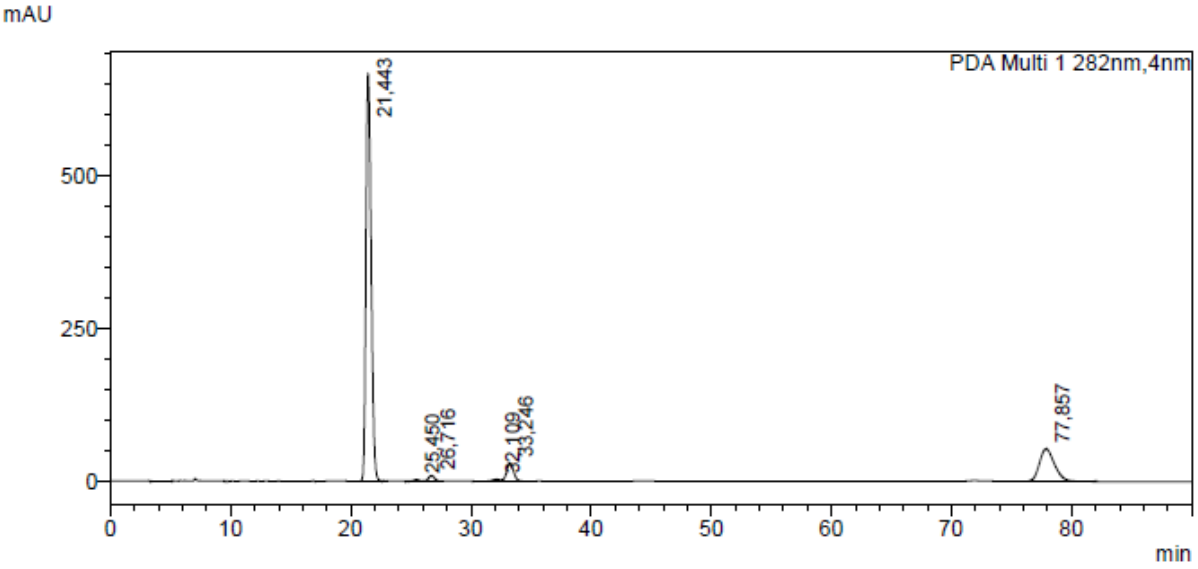



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 21,653 | 10659024 | 443565 | 55,058 | | | |
| 2 | 25,560 | 46761 | 1493 | 0,242 | | | |
| 3 | 26,816 | 1029775 | 30523 | 5,319 | | V | |
| 4 | 32,154 | 958332 | 24775 | 4,950 | | | |
| 5 | 33,319 | 2054049 | 51266 | 10,610 | | V | |
| 6 | 78,347 | 4611513 | 52665 | 23,820 | | | |
| Total | | 19359454 | 604288 | | | | |

Catalysis sample: 25 µM LmrR_pAF_RGN pH 6. 16 hours reaction time at 4°. 21.4 min = 2-methyl-indole. 77.9 min = 3-(3-hydroxypropyl)-indole (internal standard).



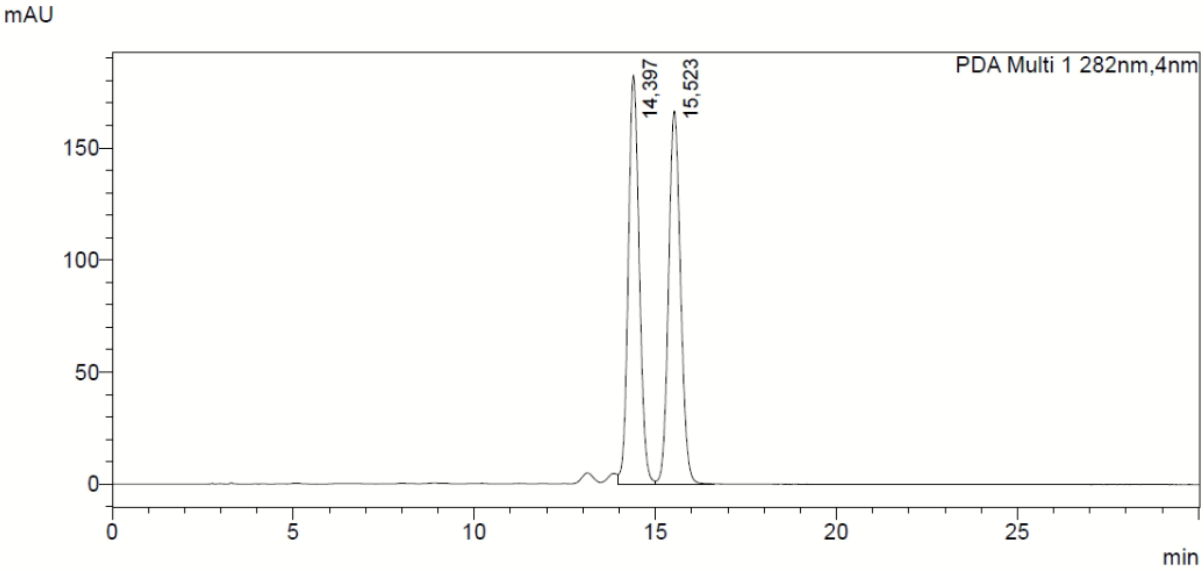

**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 21,443 | 20051618 | 667605 | 75,783 | | | |
| 2 | 25,450 | 84052 | 2597 | 0,318 | | | |
| 3 | 26,716 | 322964 | 9377 | 1,221 | | V | |
| 4 | 32,109 | 135608 | 3536 | 0,513 | | | |
| 5 | 33,246 | 1206446 | 30227 | 4,560 | | V | |
| 6 | 77,857 | 4658434 | 53273 | 17,606 | | S | |
| Total | | 26459123 | 766615 | | | | |

**3-(1H-indol-3-yl)-2-methylpropan-1-ol (3e)**

Reference product:

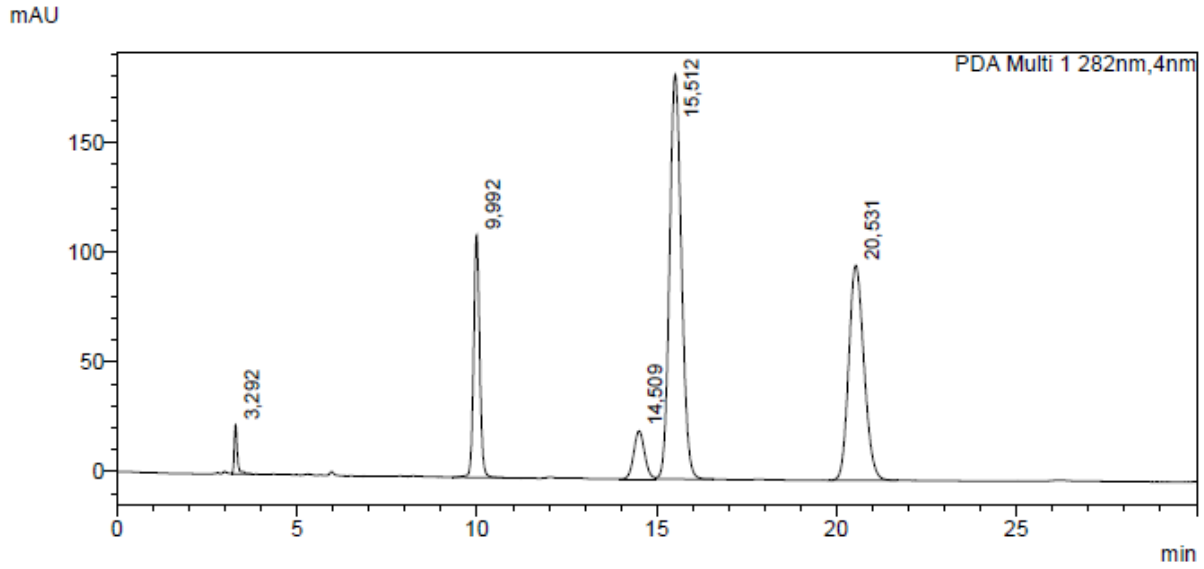



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 14,397 | 3841134 | 182233 | 50,091 | | V | |
| 2 | 15,523 | 3827240 | 166374 | 49,909 | | V | |
| Total | | 7668374 | 348607 | | | | |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 9.9 min = indole. 20.5 min = 3-(3-hydroxypropyl)-indole (internal standard). 3.3 min = unidentified impurity




**<Peak Table>**

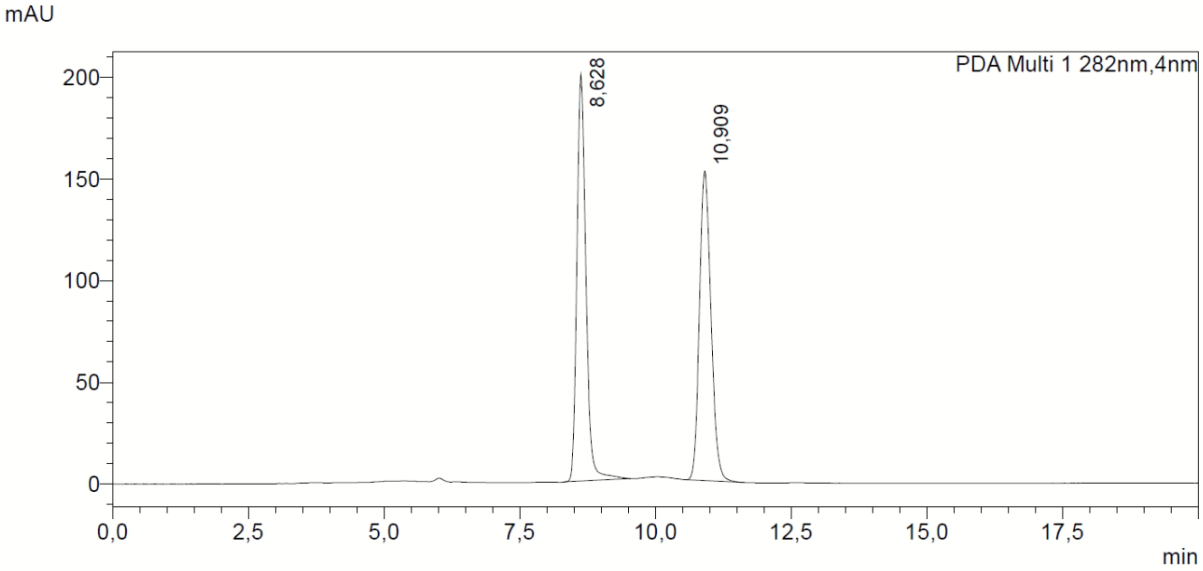
PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 3,292 | 134660 | 22413 | 0,000 | | SV | |
| 2 | 9,992 | 1287082 | 110493 | 0,000 | | | |
| 3 | 14,509 | 457834 | 21866 | 0,000 | | | |
| 4 | 15,512 | 4240937 | 184187 | 0,000 | | V | |
| 5 | 20,531 | 2895440 | 97955 | 0,000 | | | |
| Total | | 9015953 | 436914 | | | | |

**2-methyl-3-(5-methyl-1H-indol-3-yl)propan-1-ol (3f)**

Reference product:

## <Chromatogram>

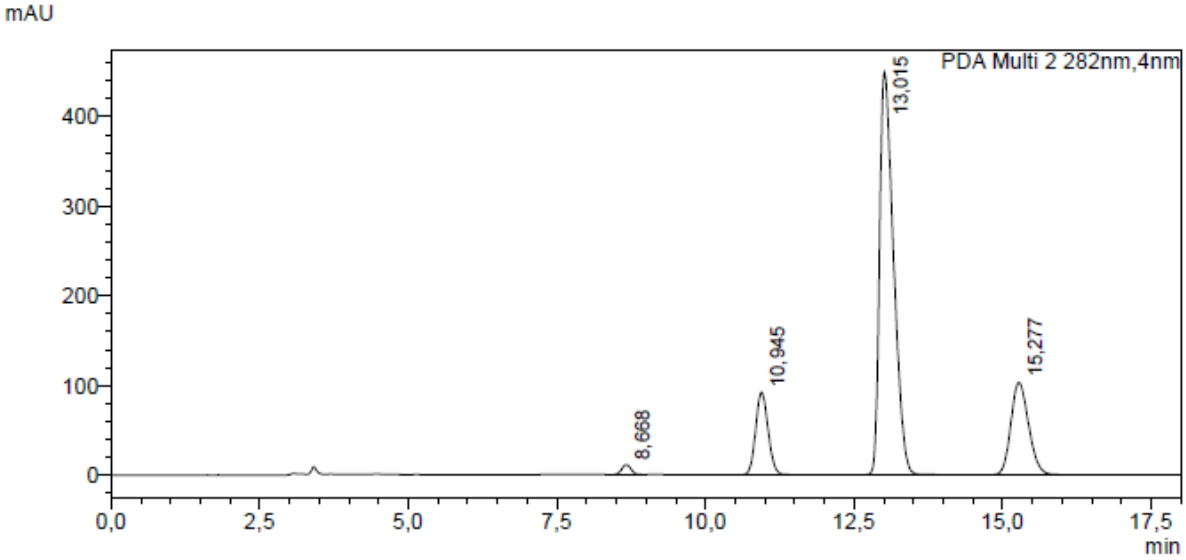



## <Peak Table>

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 8,628 | 2313819 | 199883 | 50,658 | | | |
| 2 | 10,909 | 2253692 | 152113 | 49,342 | | | |
| Total | | 4567511 | 351996 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 6 hours reaction time at 4°. 13.0 min = 5-methyl-indole. 15.3 min = 3-(3-hydroxypropyl)-indole (internal standard).



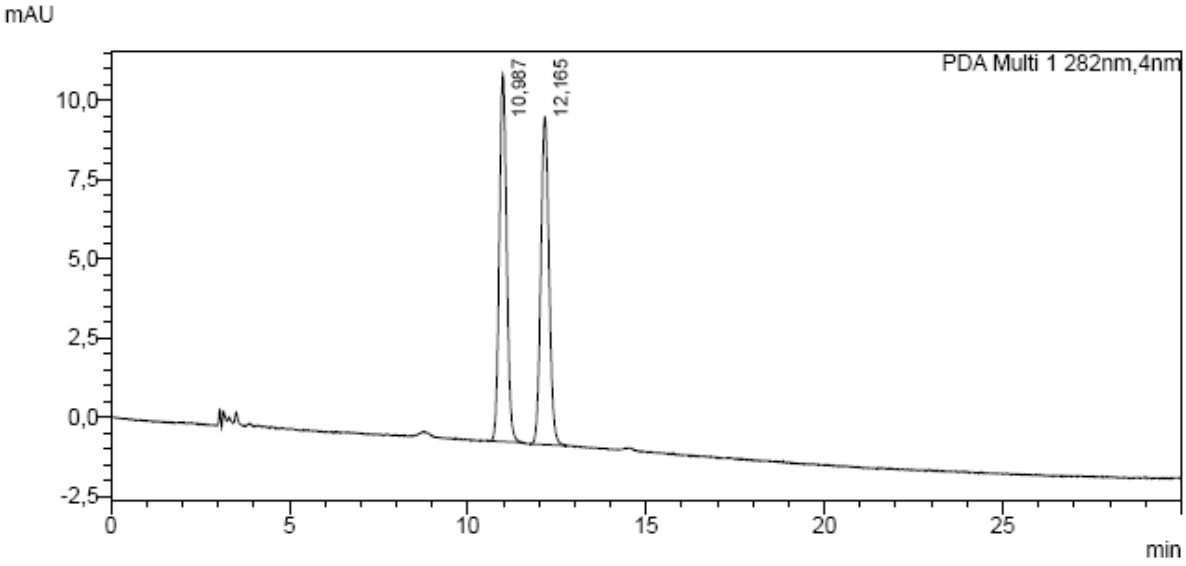

## <Peak Table>

PDA Ch2 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 8,668 | 124316 | 11260 | 0,000 | | | |
| 2 | 10,945 | 1345720 | 92429 | 0,000 | | | |
| 3 | 13,015 | 7346666 | 449543 | 0,000 | | | |
| 4 | 15,277 | 2145366 | 103057 | 0,000 | | | |
| Total | | 10962068 | 656289 | | | | |

**3-(5-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3g)**

Reference product:

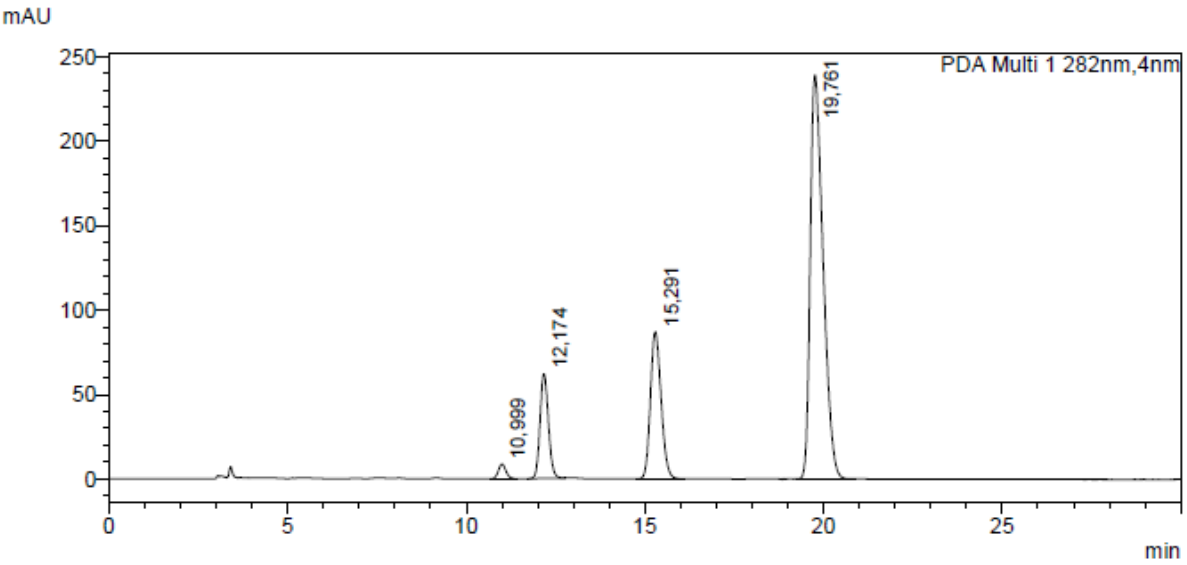



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 10,987 | 165688 | 11581 | 0,000 | | | |
| 2 | 12,165 | 166202 | 10334 | 0,000 | | | |
| Total | | 331889 | 21915 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 6 hours reaction time at 4°. 15.3 min = 3-(3-hydroxypropyl)-indole (internal standard). 19.8 = 5-methoxy-indole.



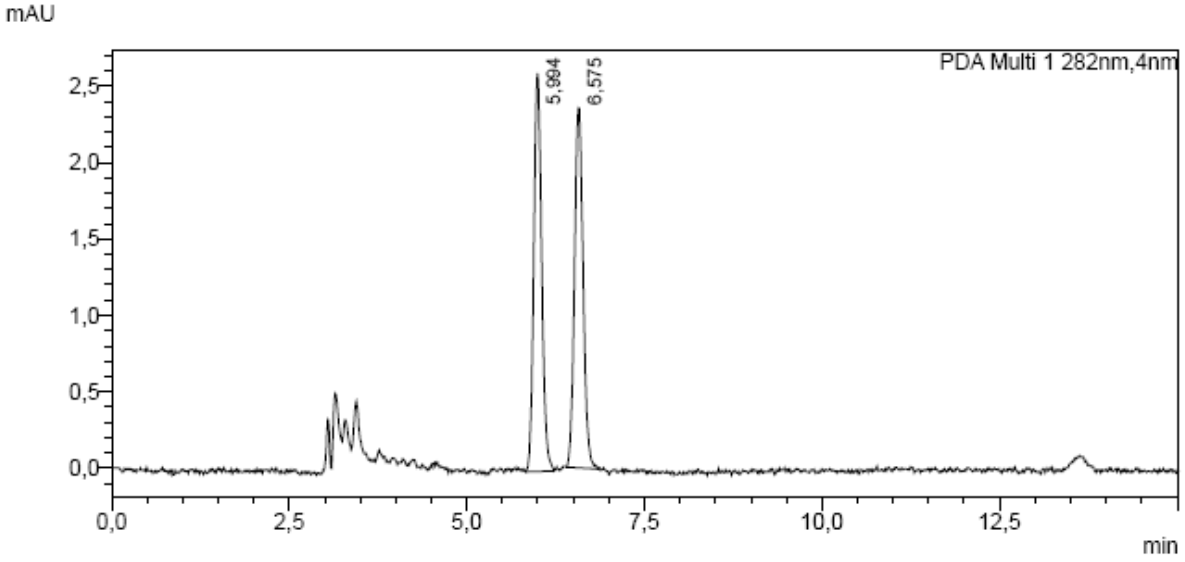

**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 10,999 | 126744 | 8633 | 0,000 | | | |
| 2 | 12,174 | 1007665 | 62018 | 0,000 | | | |
| 3 | 15,291 | 1812097 | 87076 | 0,000 | | | |
| 4 | 19,761 | 5996364 | 239017 | 0,000 | | | |
| Total | | 8942870 | 396745 | | | | |

**3-(5-chloro-1H-indol-3-yl)-2-methylpropan-1-ol (3h)**

Reference product:

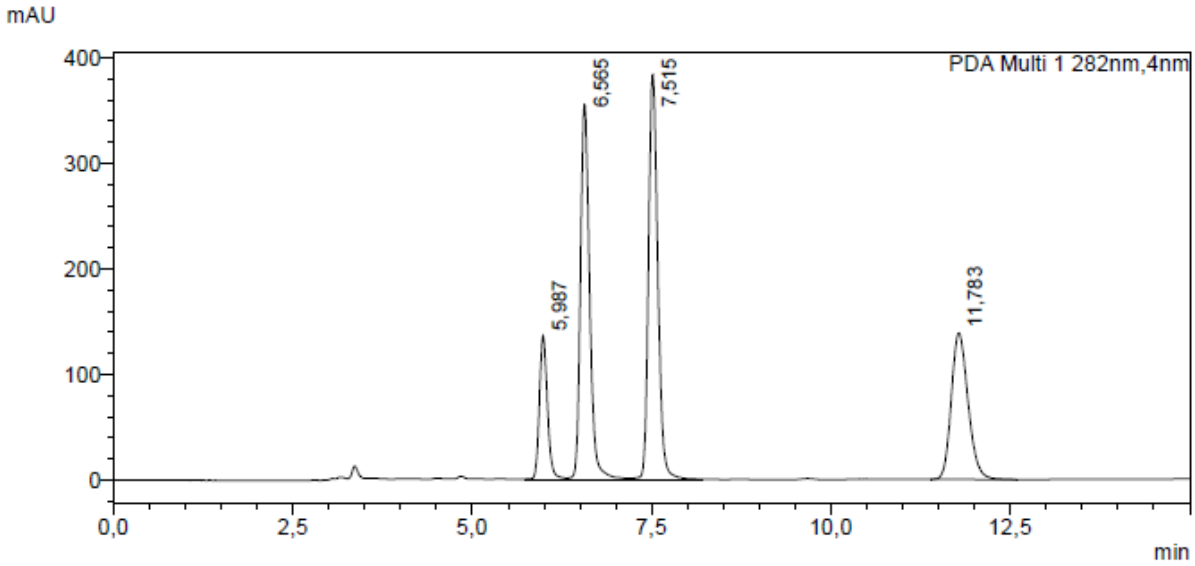



**<Peak Table>**

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 5,994 | 20099 | 2607 | 50,272 | | | |
| 2 | 6,575 | 19881 | 2357 | 49,728 | | | |
| Total | | 39980 | 4964 | | | | |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 7.5 min = 5-chloro-indole. 11.8 min = 3-(3-hydroxypropyl)-indole (internal standard).




**<Peak Table>**

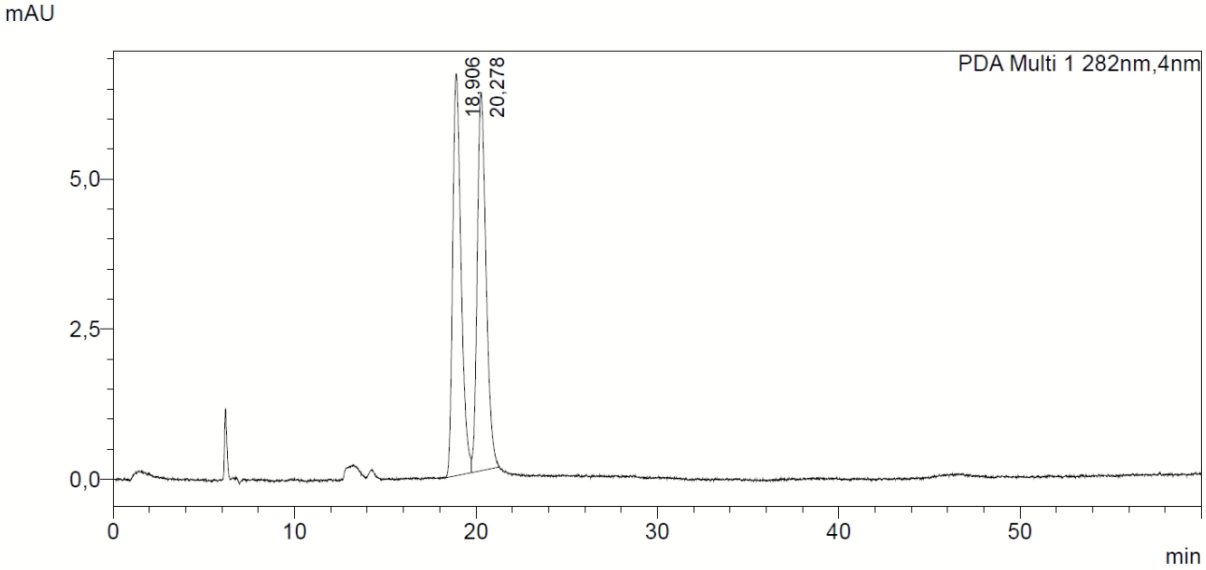
PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 5,987 | 1056534 | 135794 | 10,662 | | | |
| 2 | 6,565 | 3161184 | 355043 | 31,901 | | V | |
| 3 | 7,515 | 3438740 | 383302 | 34,702 | | V | |
| 4 | 11,783 | 2252776 | 138563 | 22,734 | | | |
| Total | | 9909233 | 1012702 | | | | |

**methyl 3-(3-hydroxy-2-methylpropyl)-1H-indole-5-carboxylate (3i)**

Reference product:

<Chromatogram>




<Peak Table>

PDA Ch1 282nm

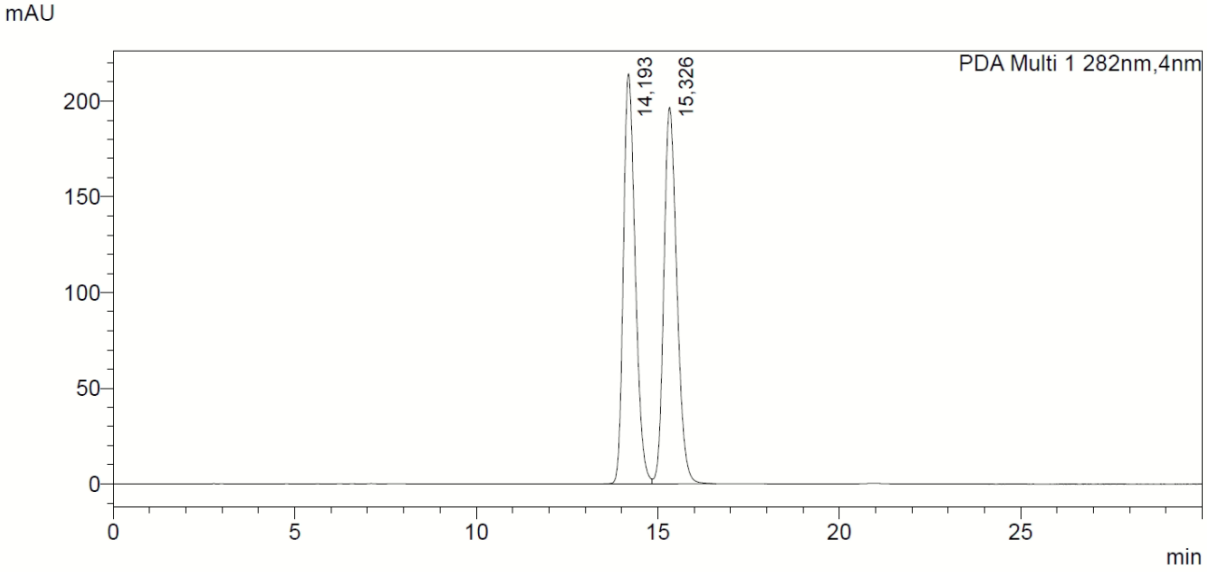
| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 18,906 | 215153 | 6694 | 50,257 | | | |
| 2 | 20,278 | 212956 | 6292 | 49,743 | | V | |
| Total | | 428108 | 12986 | | | | |

No product peaks found in the catalysis sample.

**3-(5-methoxy-2-methyl-1H-indol-3-yl)-2-methylpropan-1-ol (3j)**

Reference product:

<Chromatogram>

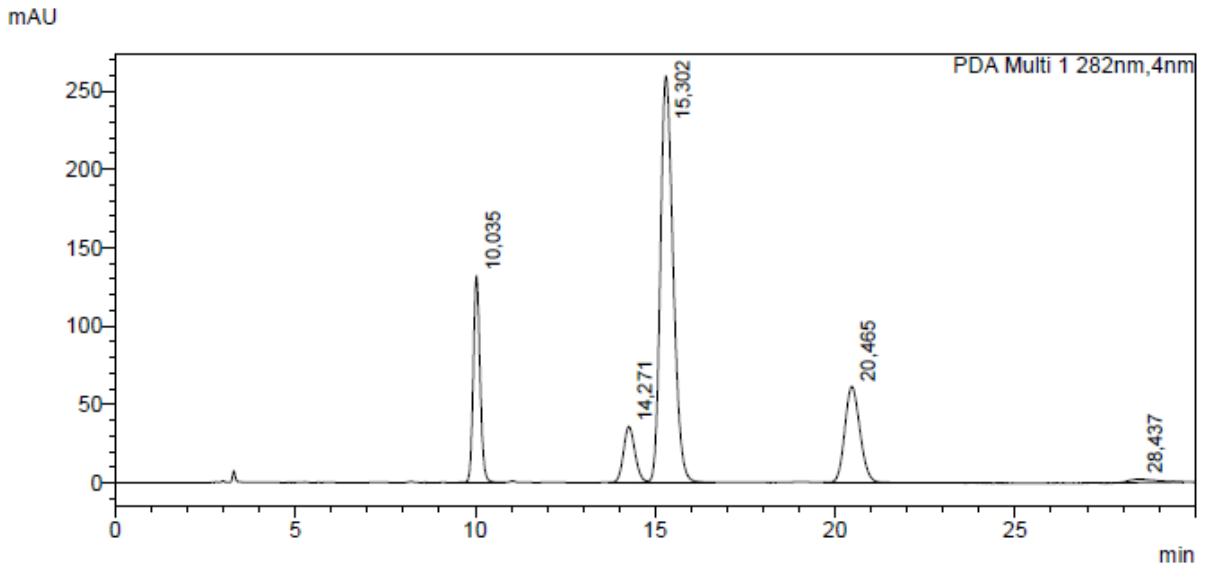



<Peak Table>

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 14,193 | 4865836 | 214184 | 49,894 | | | |
| 2 | 15,326 | 4886432 | 196494 | 50,106 | | V | |
| Total | | 9752267 | 410677 | | | | |

Catalysis sample: 10 µM LmrR_pAF pH 6. 6 hours reaction time at 4°. 10.0 min = 5-methoxy-2-methyl-indole. 20.5 min = 3-(3-hydroxypropyl)-indole (internal standard).



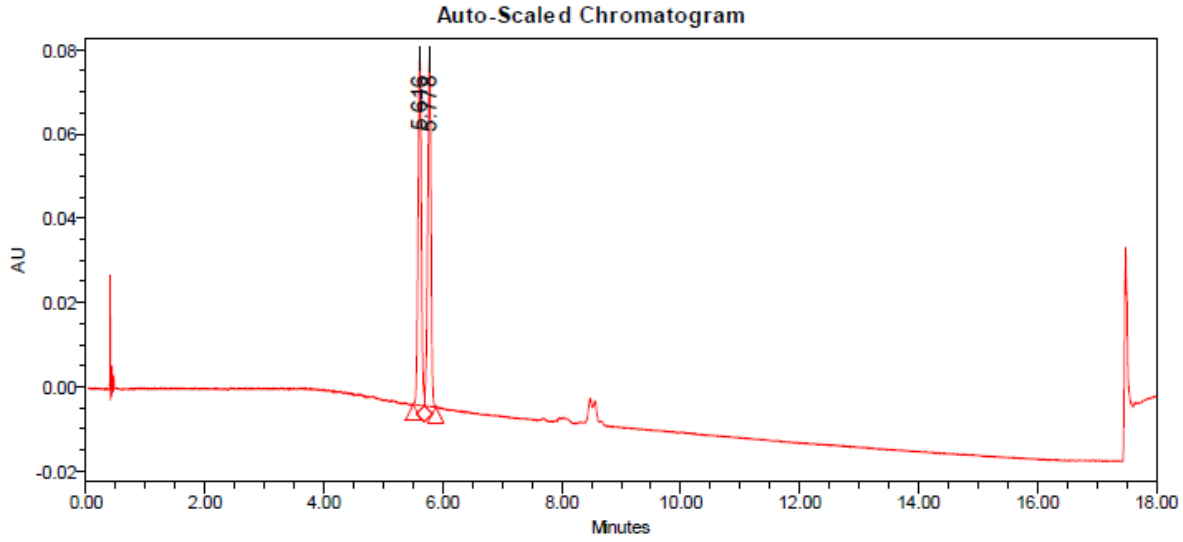

<Peak Table>

PDA Ch1 282nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 10,035 | 1737204 | 131556 | 0,000 | | | |
| 2 | 14,271 | 796343 | 35656 | 0,000 | | | |
| 3 | 15,302 | 6394485 | 259084 | 0,000 | | V | |
| 4 | 20,465 | 1796785 | 61284 | 0,000 | | V | |
| 5 | 28,437 | 114997 | 2208 | 0,000 | | | |
| Total | | 10839813 | 489786 | | | | |

**3-(4-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3k)**

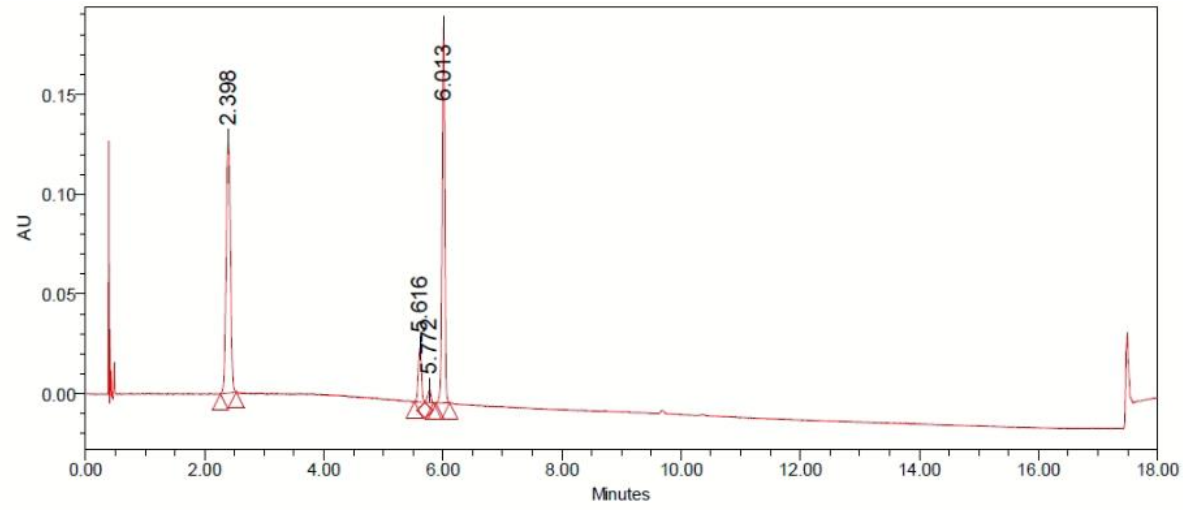
Reference product:




Peak Results

| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 5.616 | 317132 | 82361 | 49.75 |
| 2 | | 5.778 | 320363 | 82609 | 50.25 |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 2.4 min = 4-methoxy-indole. 6.0 min = 3-(3-hydroxypropyl)-indole (internal standard).

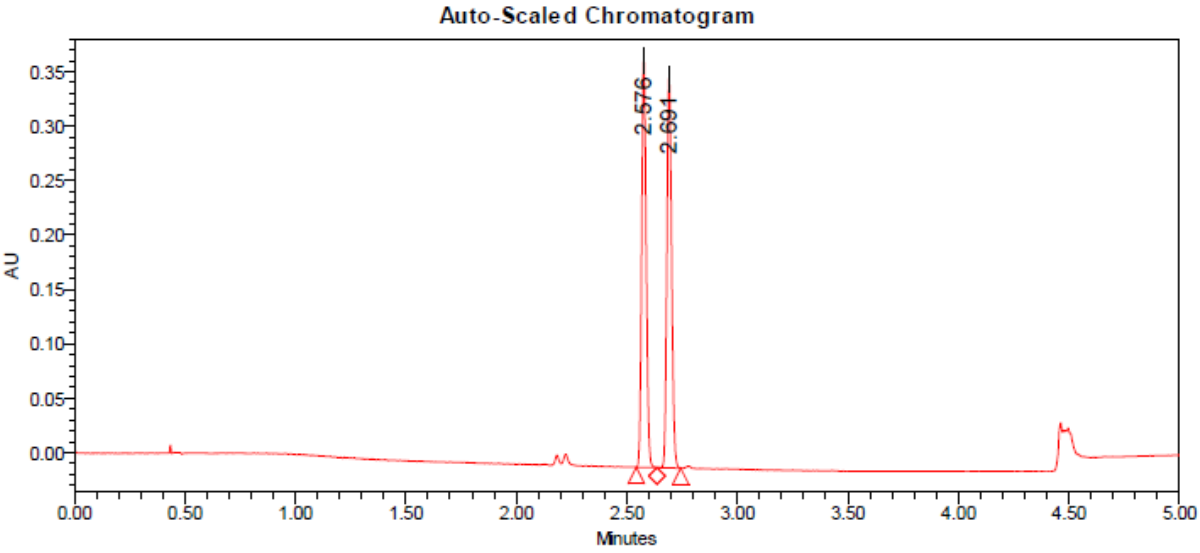



Peak Results

| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 2.398 | 637957 | 125951 | 48.10 |
| 2 | | 5.616 | 96044 | 27070 | 7.24 |
| 3 | | 5.772 | 24347 | 6341 | 1.84 |
| 4 | | 6.013 | 567984 | 188122 | 42.82 |

**3-(7-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3l)**

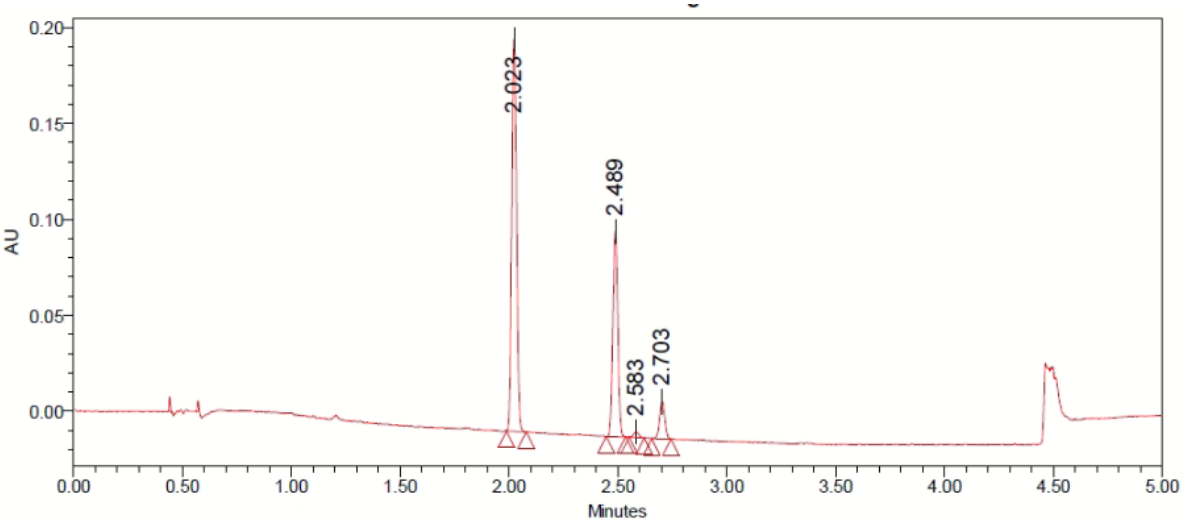
Reference product:




Peak Results

| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 2.576 | 535318 | 374828 | 49.98 |
| 2 | | 2.691 | 535794 | 357830 | 50.02 |

Catalysis sample: 25 µM LmrR_pAF pH 6. 16 hours reaction time at 4°. 2.0 min = 7-methoxy-indole. 2.5 min = 3-(3-hydroxypropyl)-indole (internal standard).

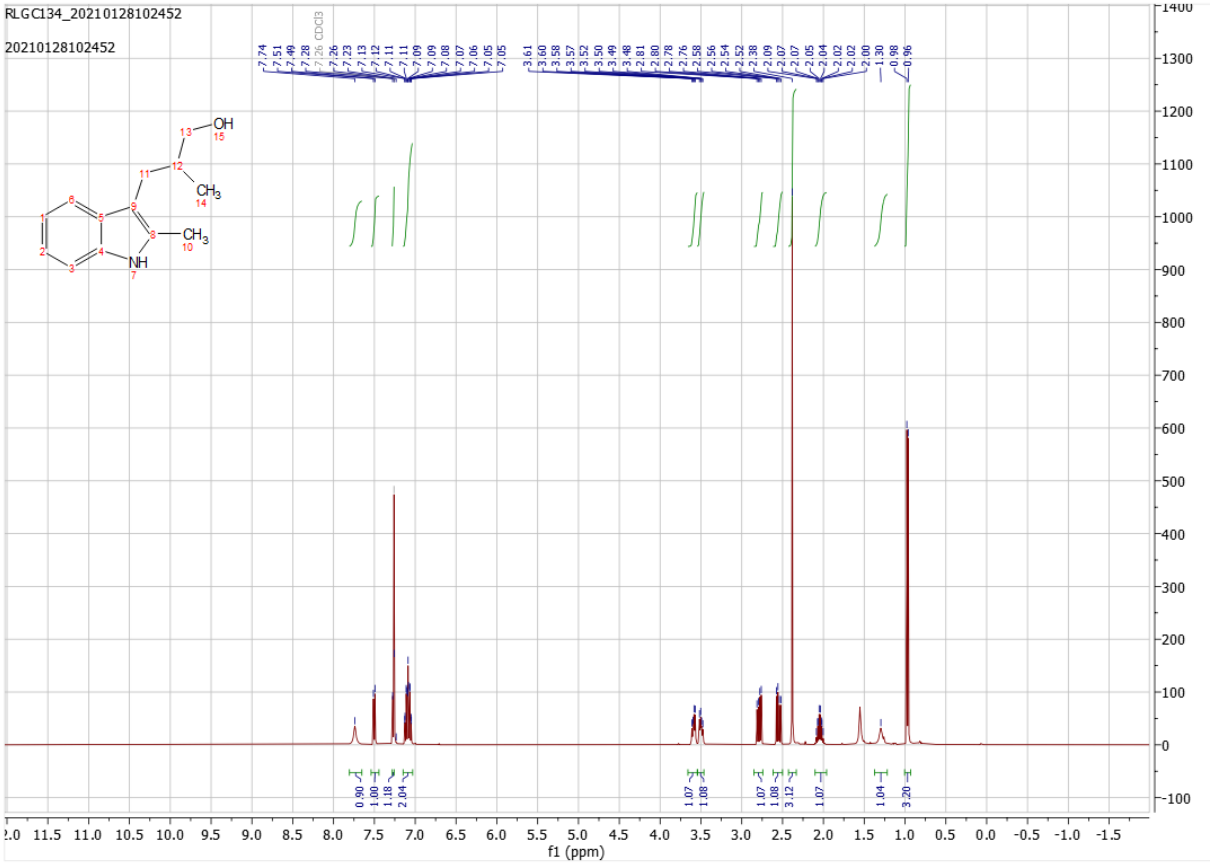



Peak Results

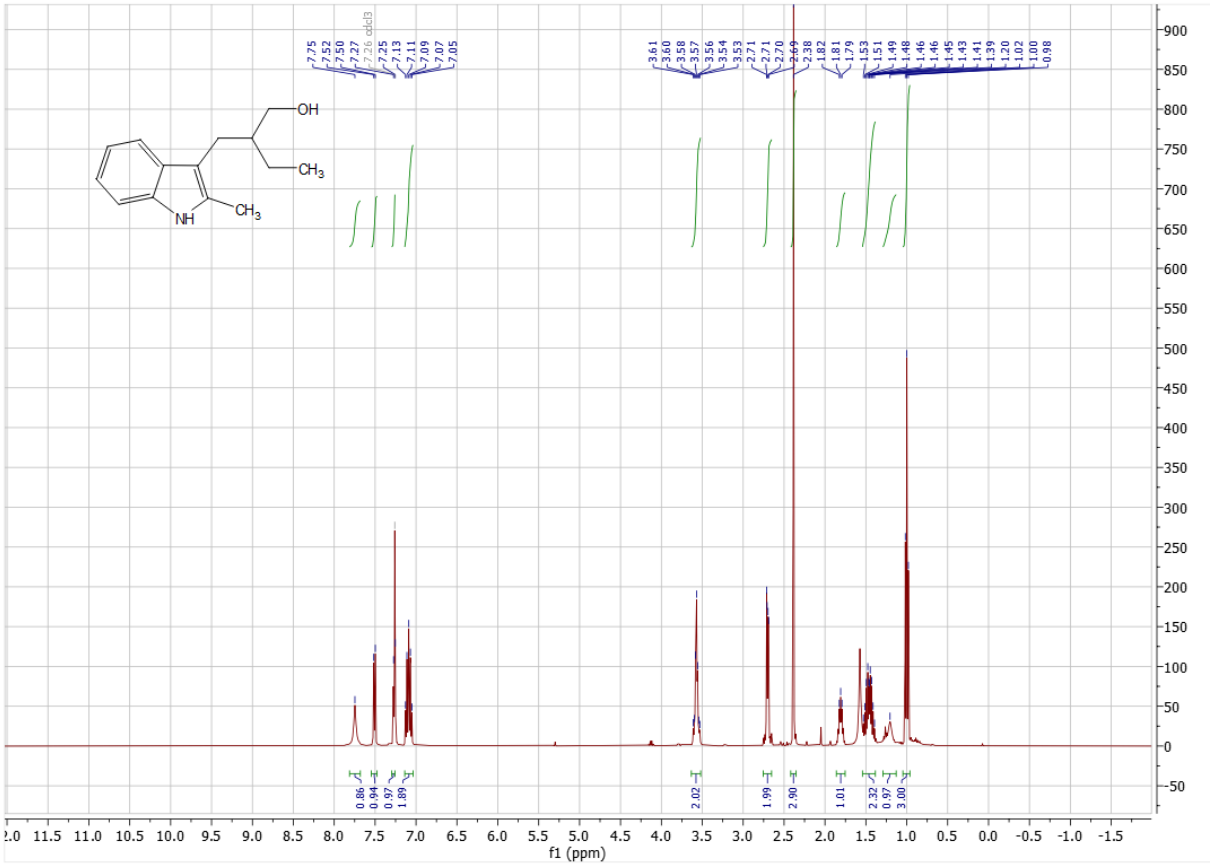
| | Name | RT | Area | Height | % Area |
|---|---|---|---|---|---|
| 1 | | 2.023 | 315719 | 204522 | 60.22 |
| 2 | | 2.489 | 170107 | 107173 | 32.45 |
| 3 | | 2.583 | 5002 | 2982 | 0.95 |
| 4 | | 2.703 | 33462 | 19443 | 6.38 |

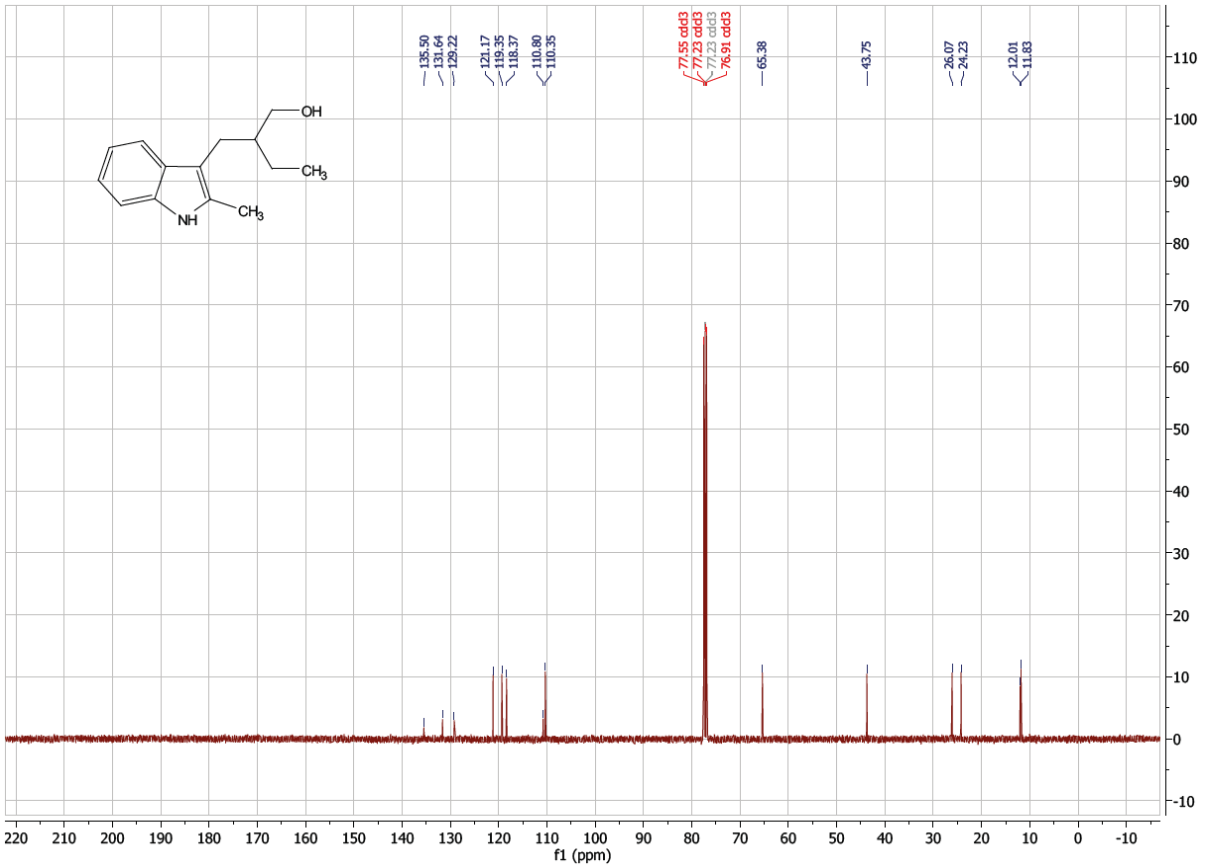
# 8. NMR Spectra

**2-methyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3a)**

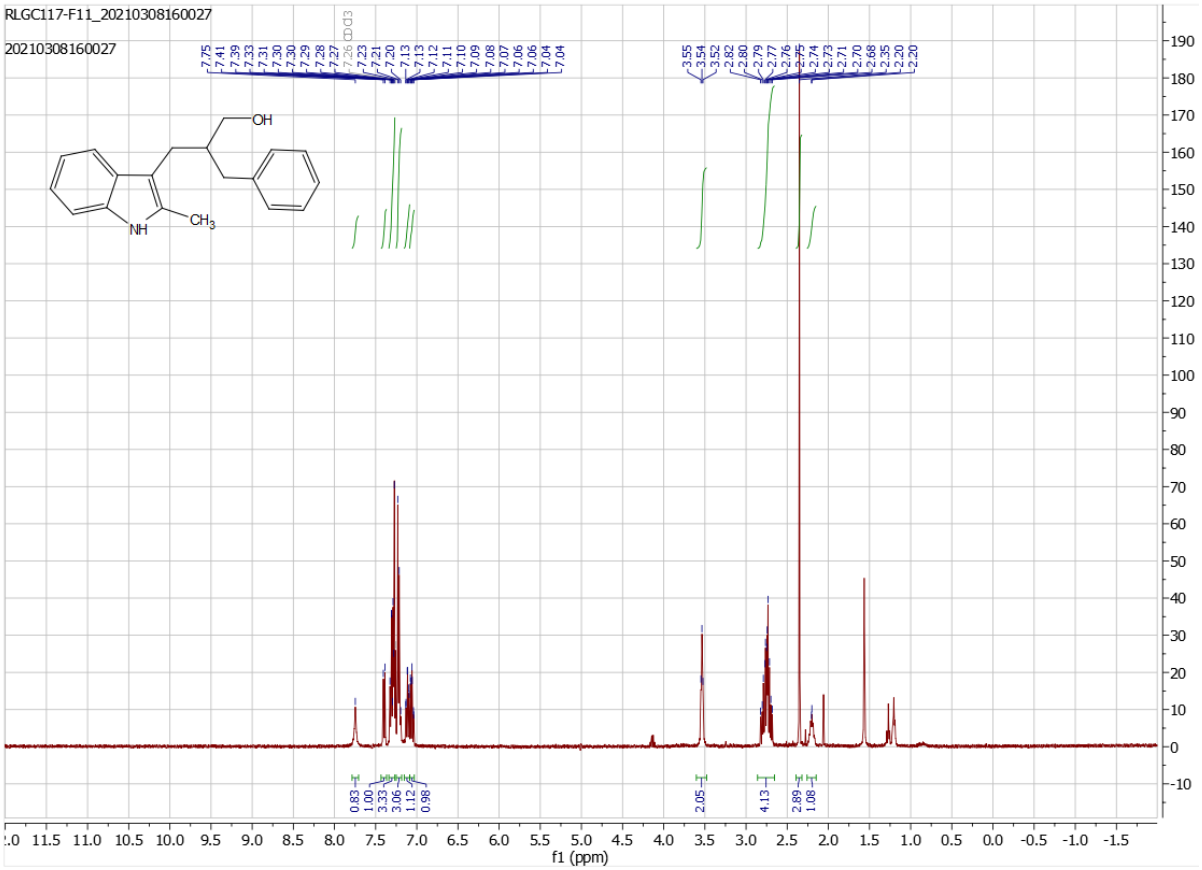


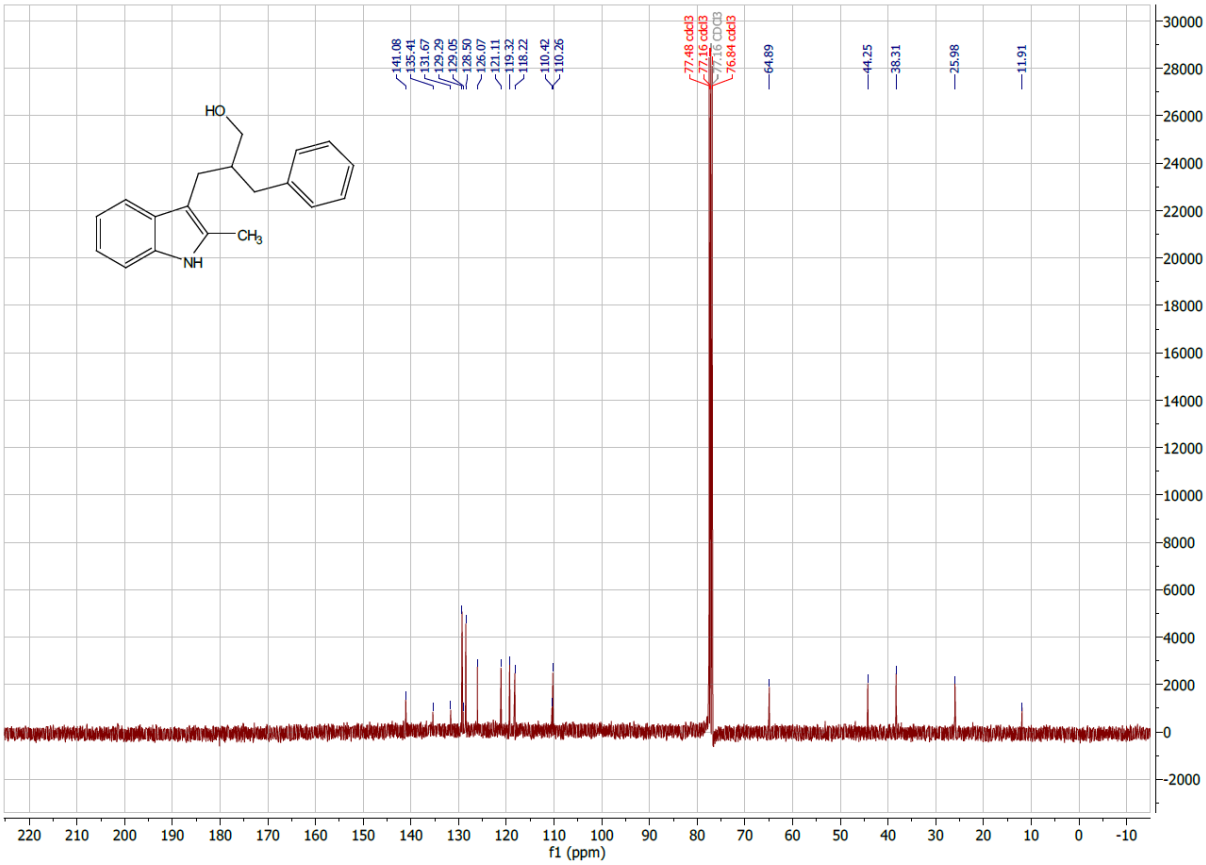
**2-((2-methyl-1H-indol-3-yl)methyl)butan-1-ol (3b)**



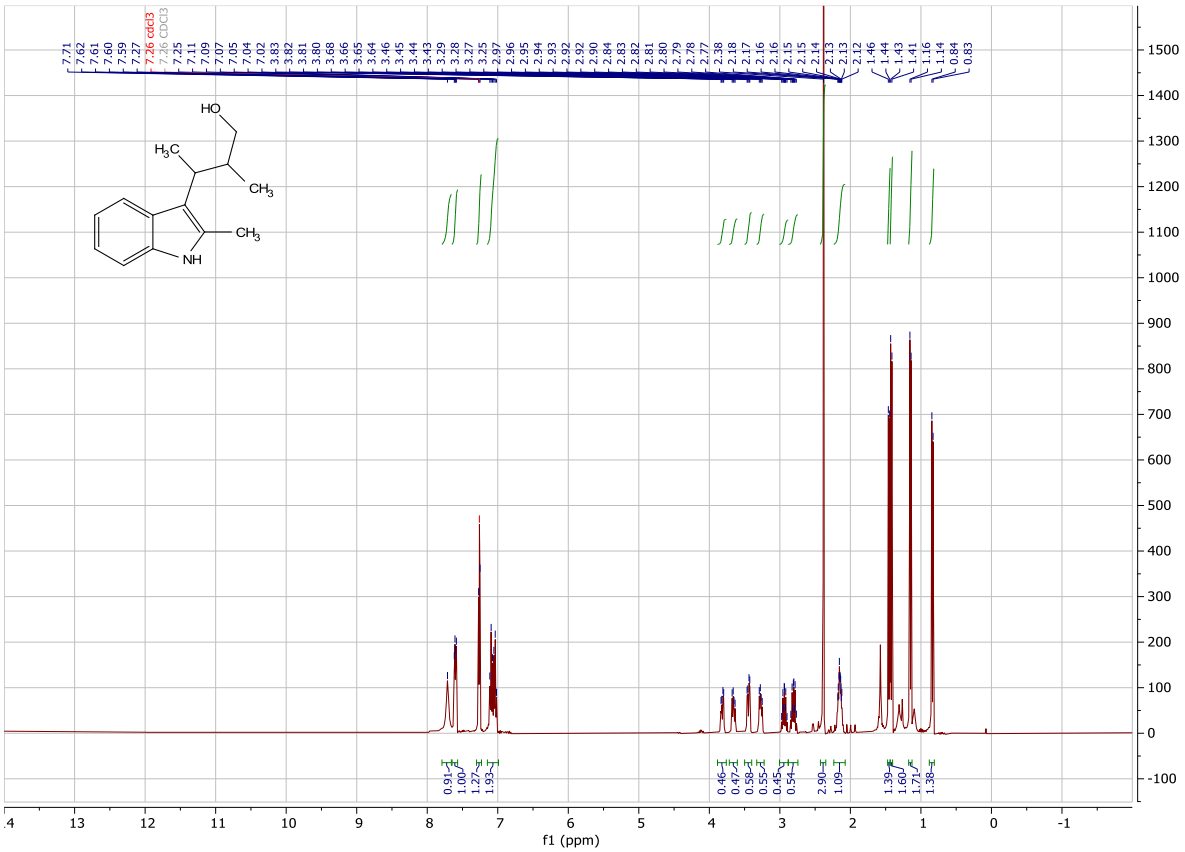



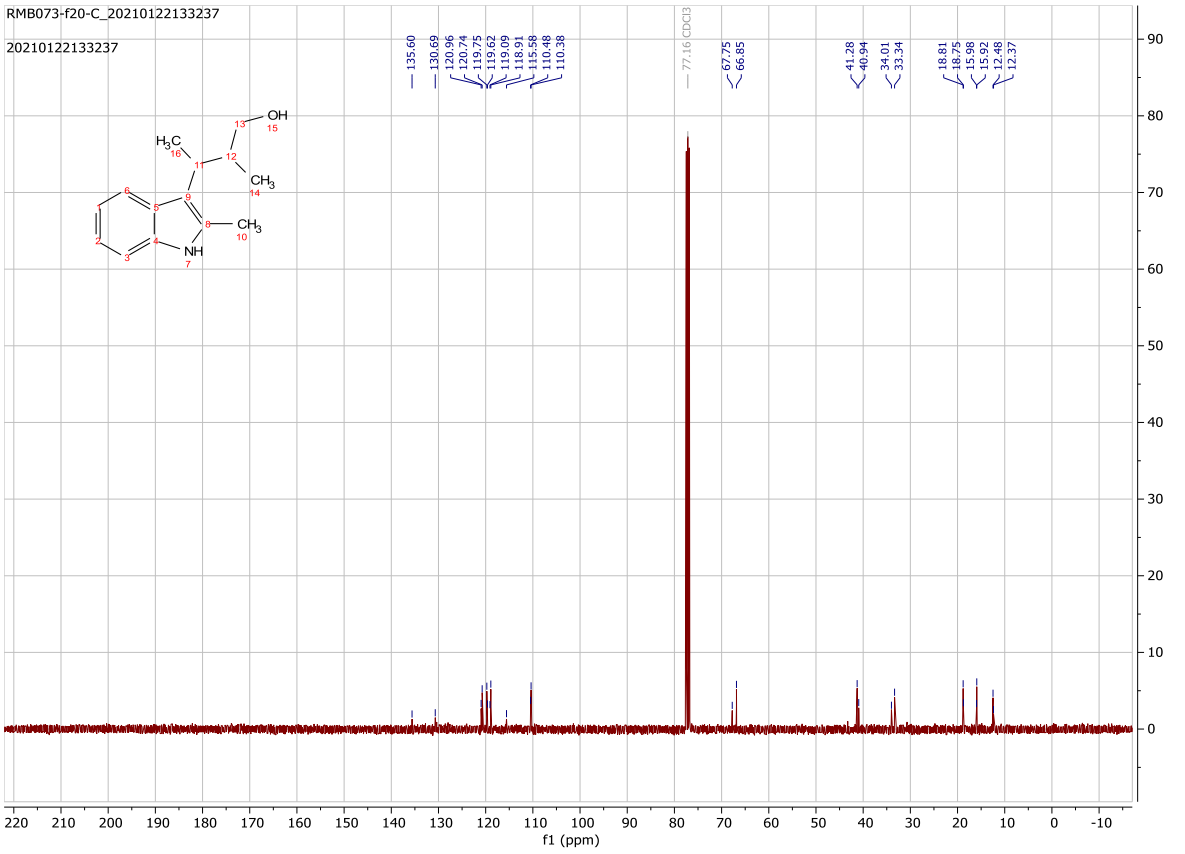
**2-benzyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3c)**



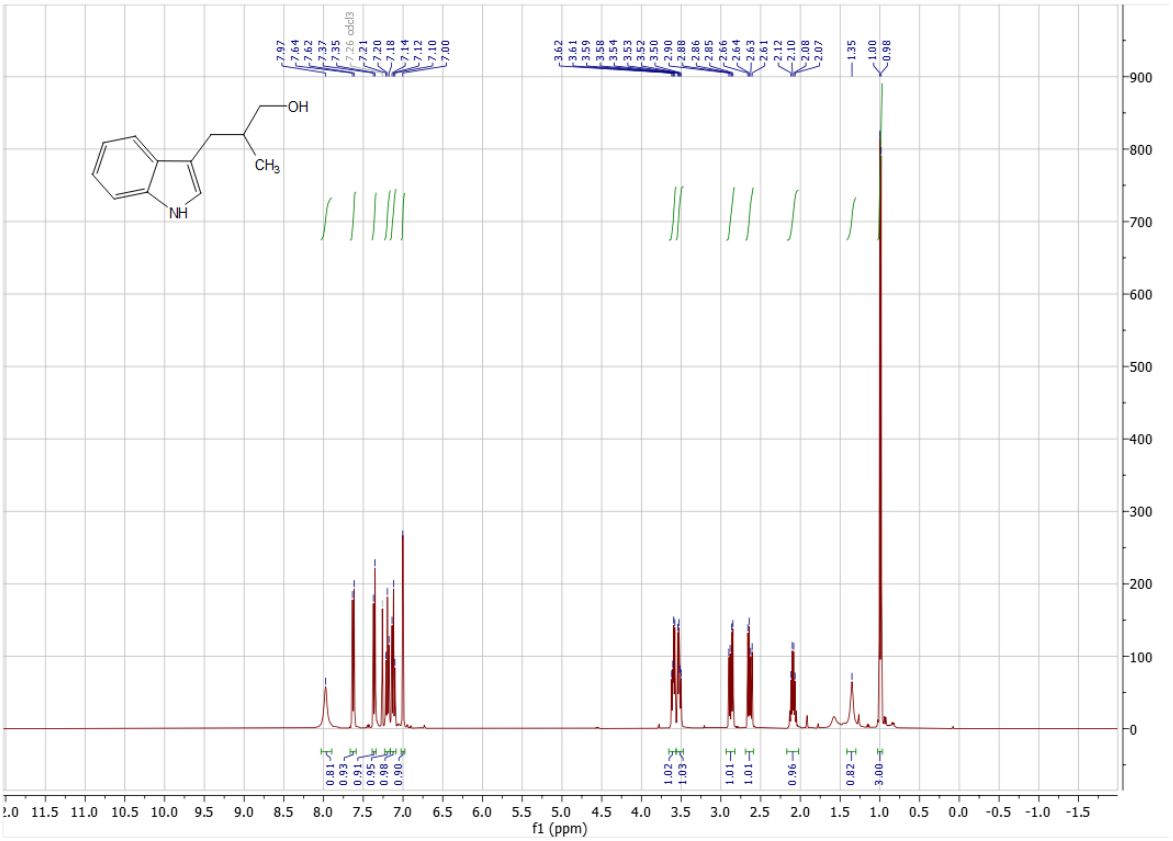



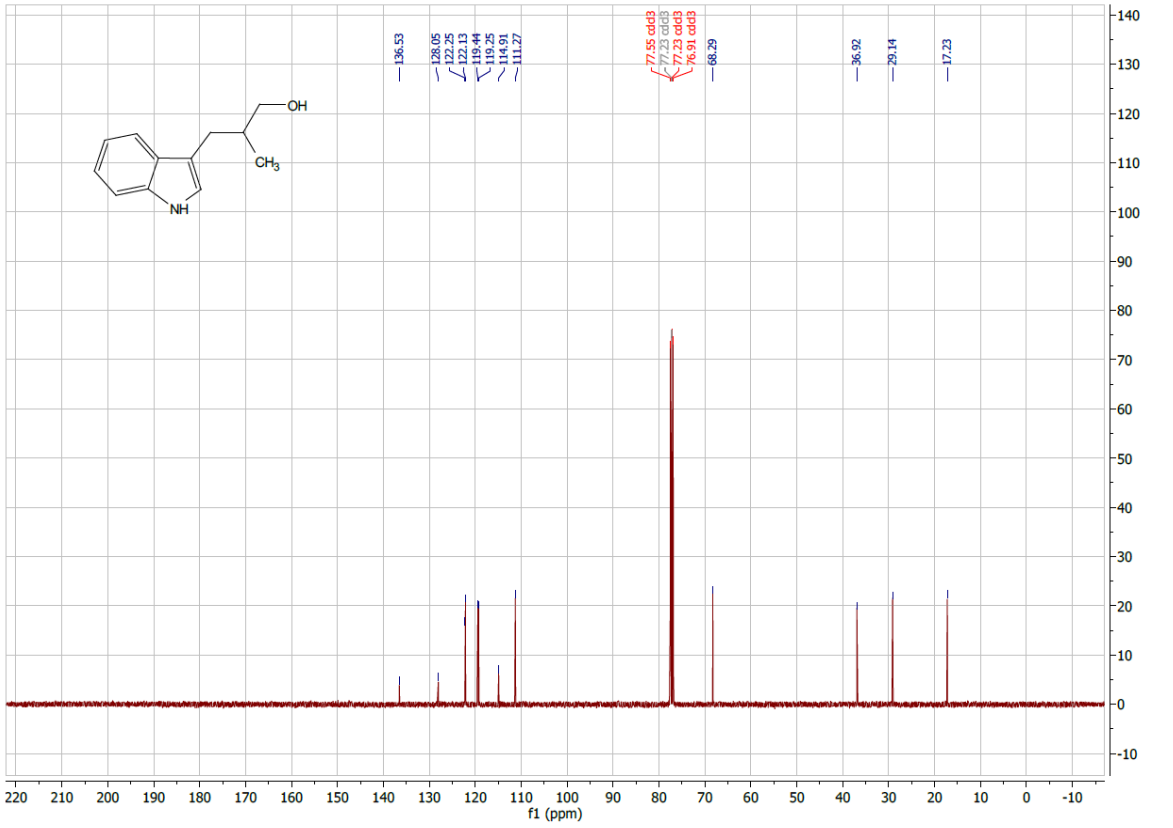
**2-methyl-3-(2-methyl-1H-indol-3-yl)butan-1-ol (3d)**



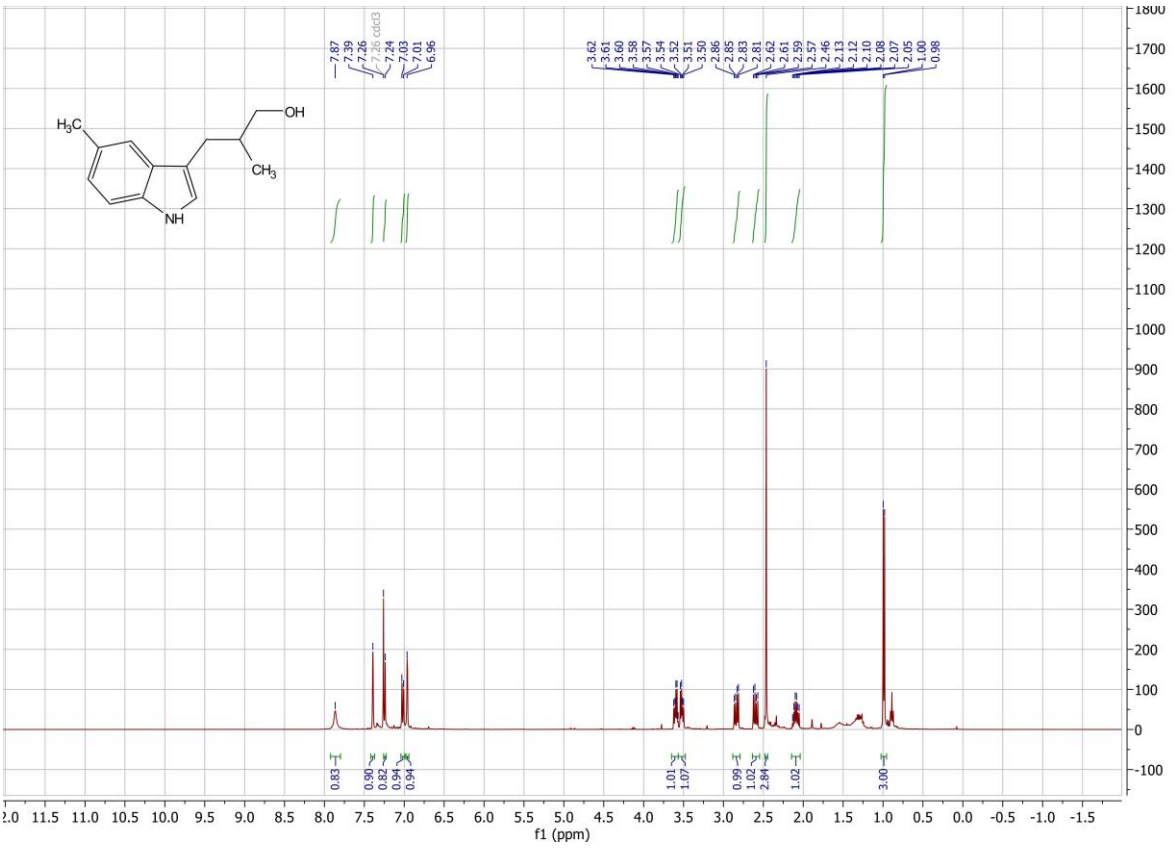



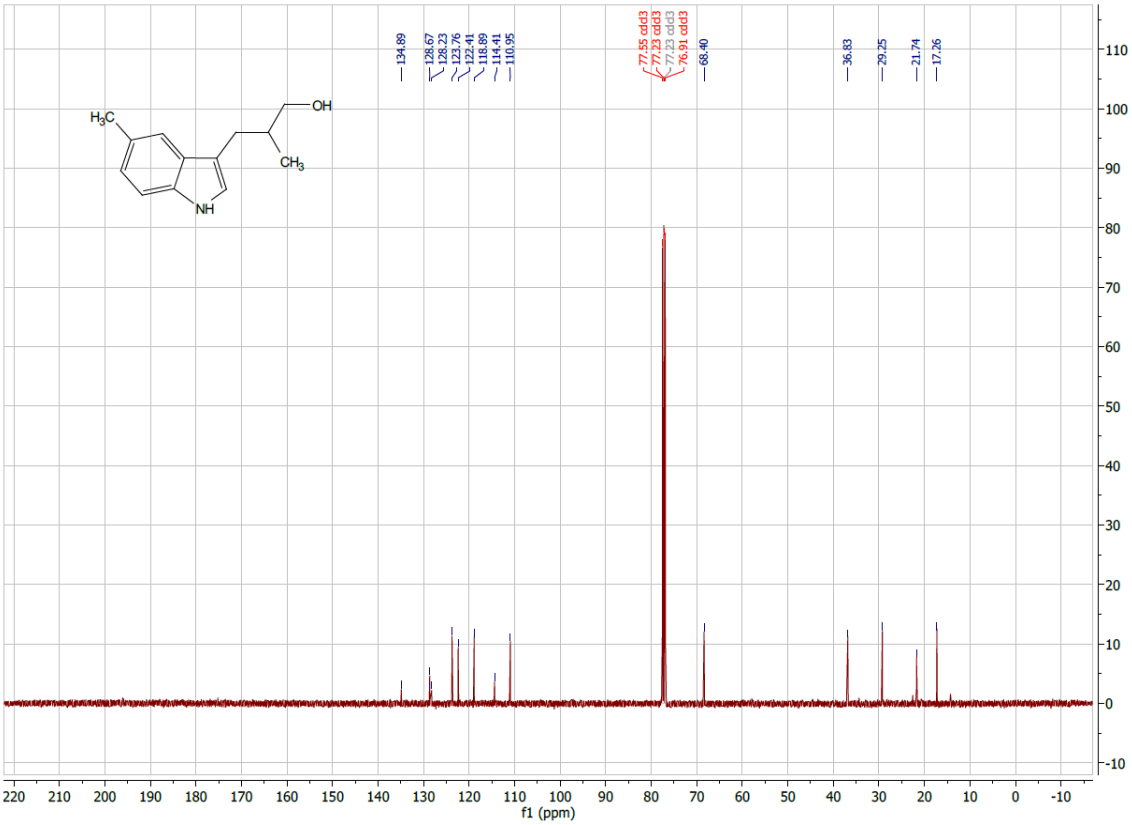
**3-(1H-indol-3-yl)-2-methylpropan-1-ol (3e)**



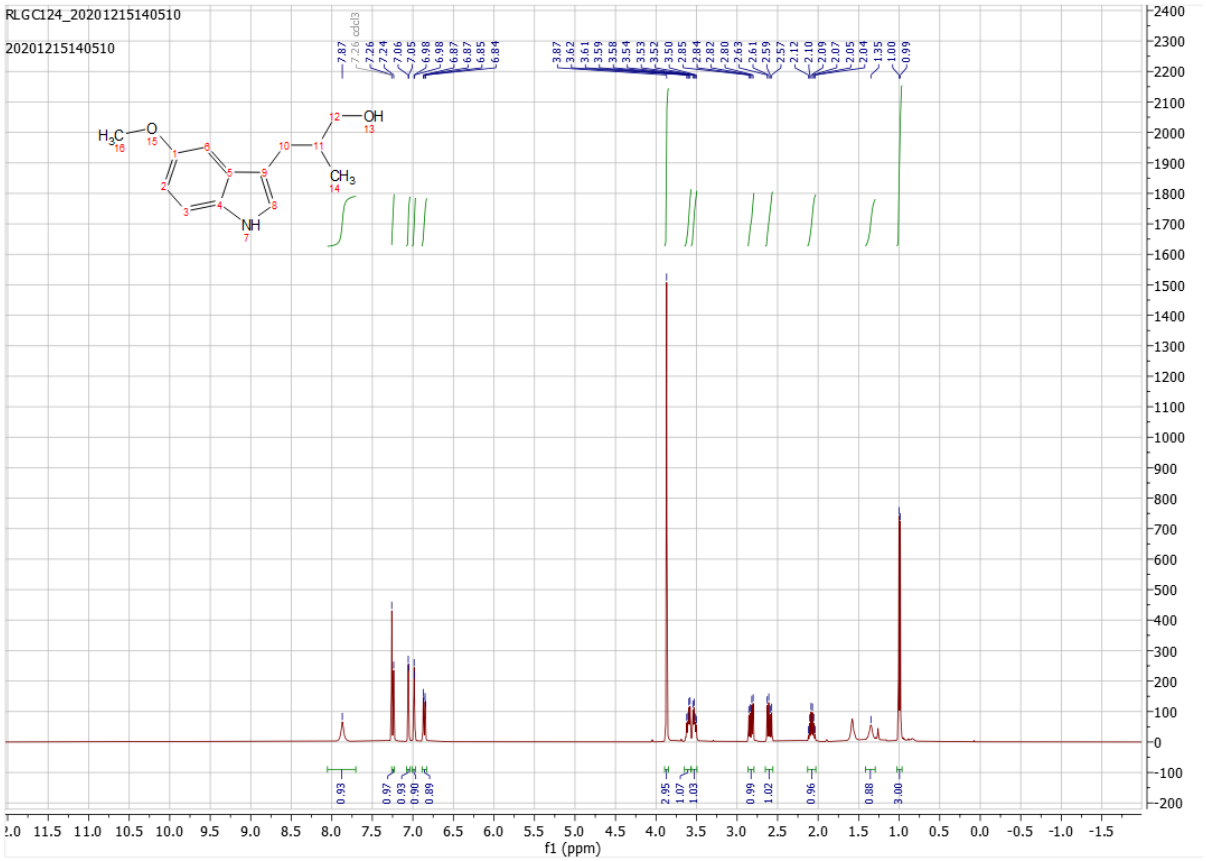



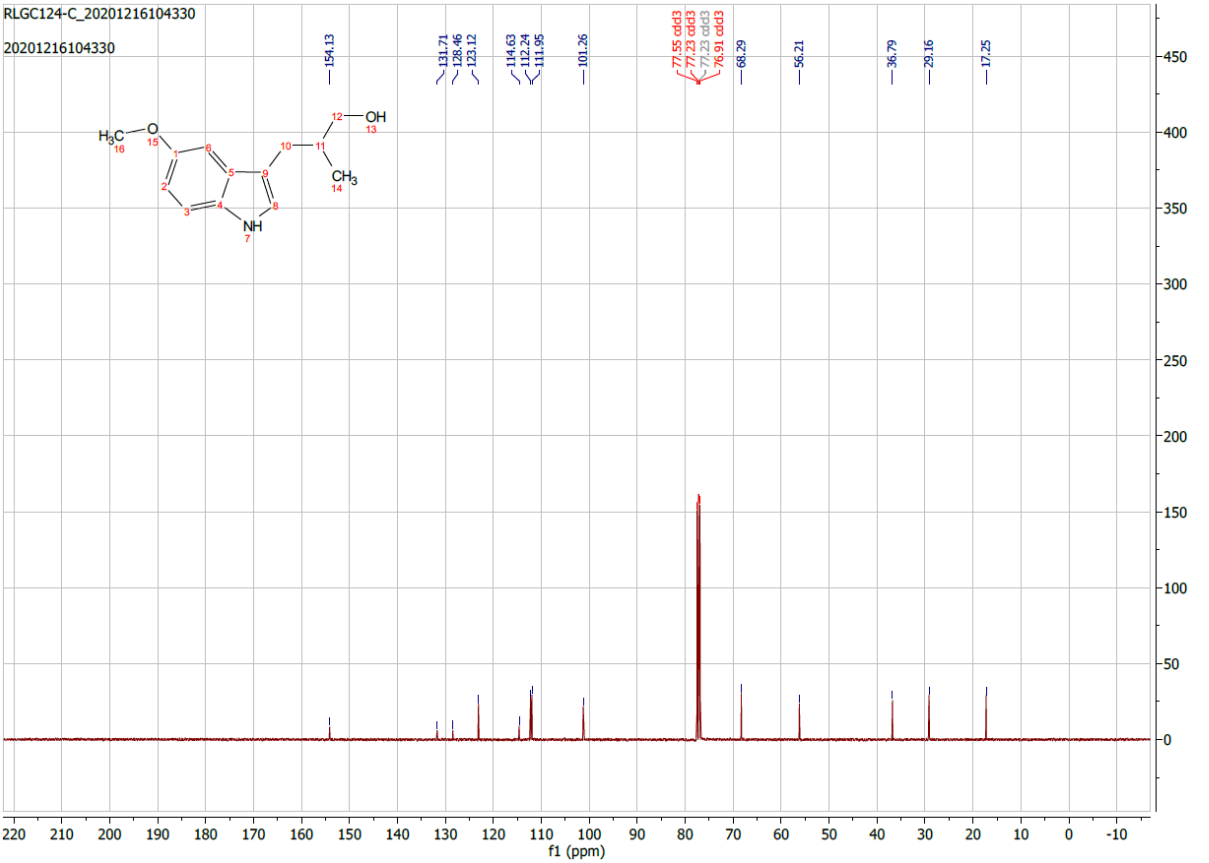
**2-methyl-3-(5-methyl-1H-indol-3-yl)propan-1-ol (3f)**



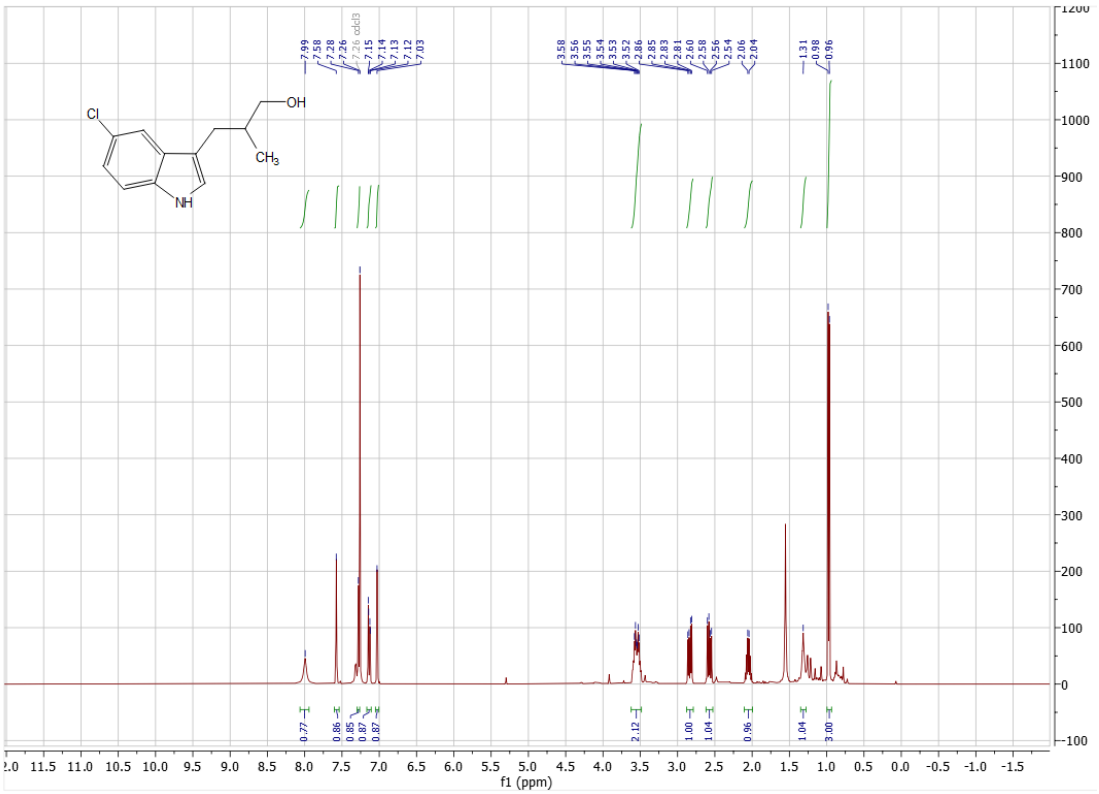



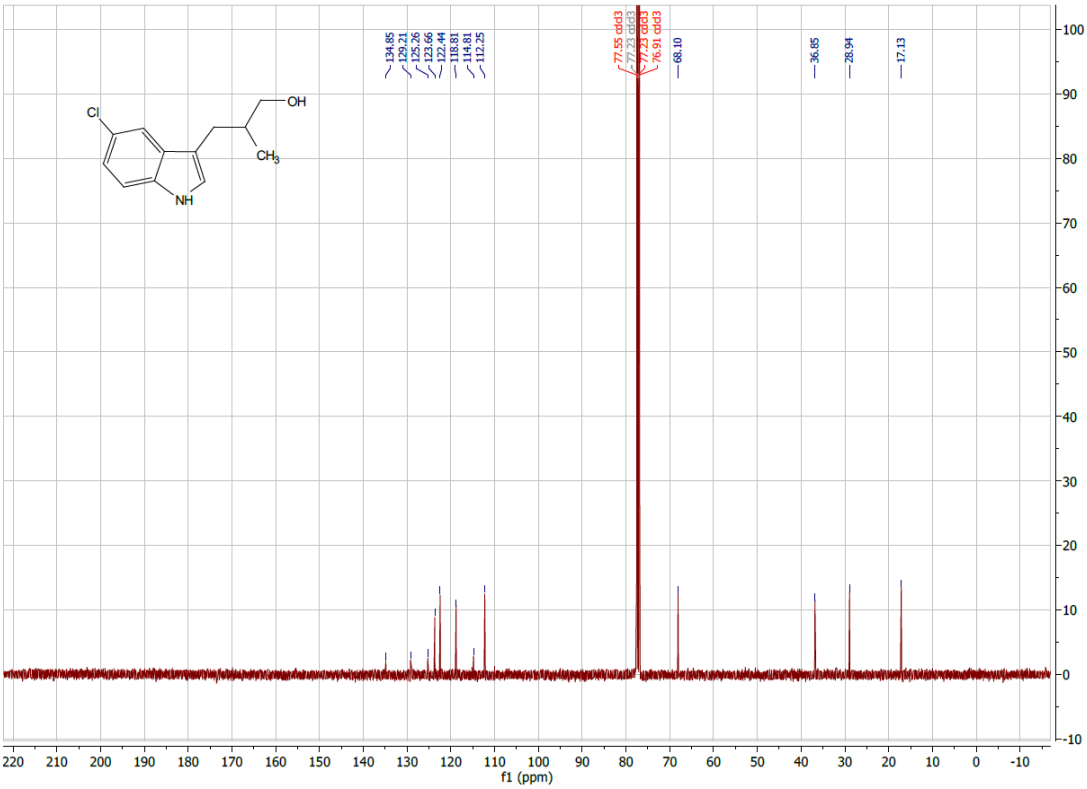
**3-(5-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3g)**



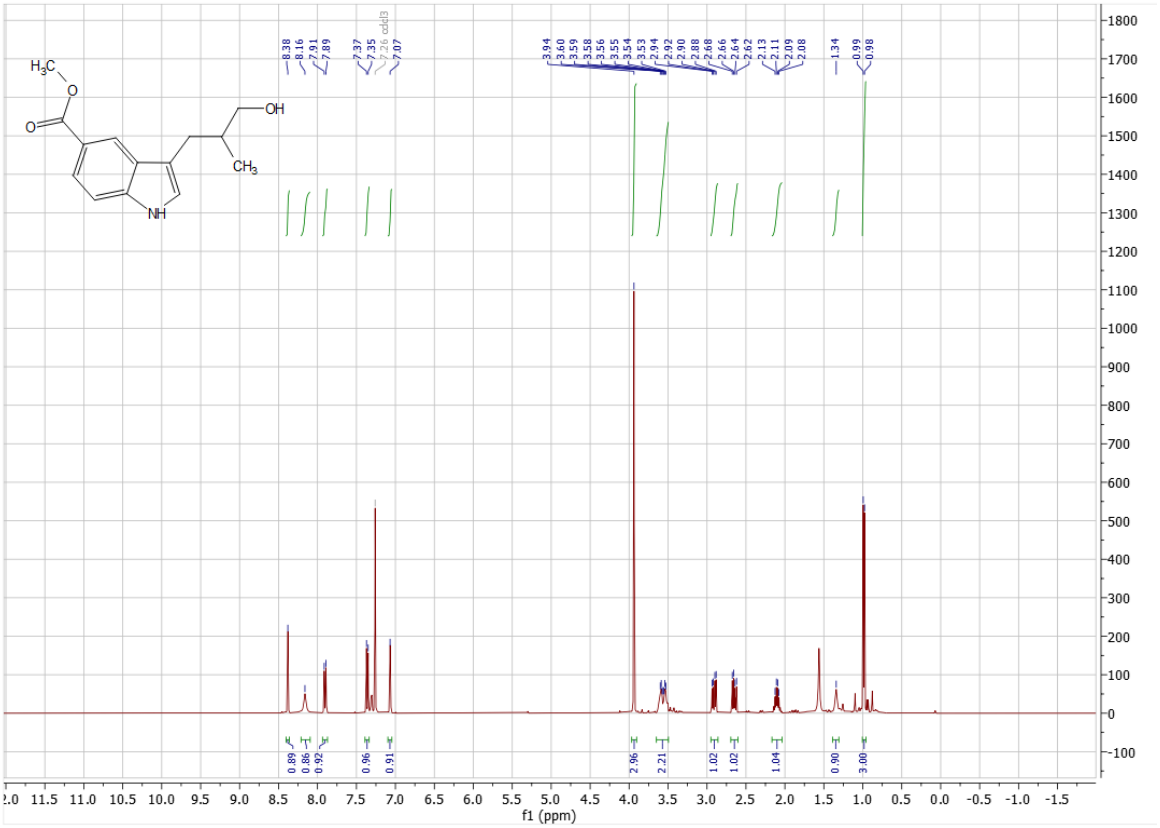



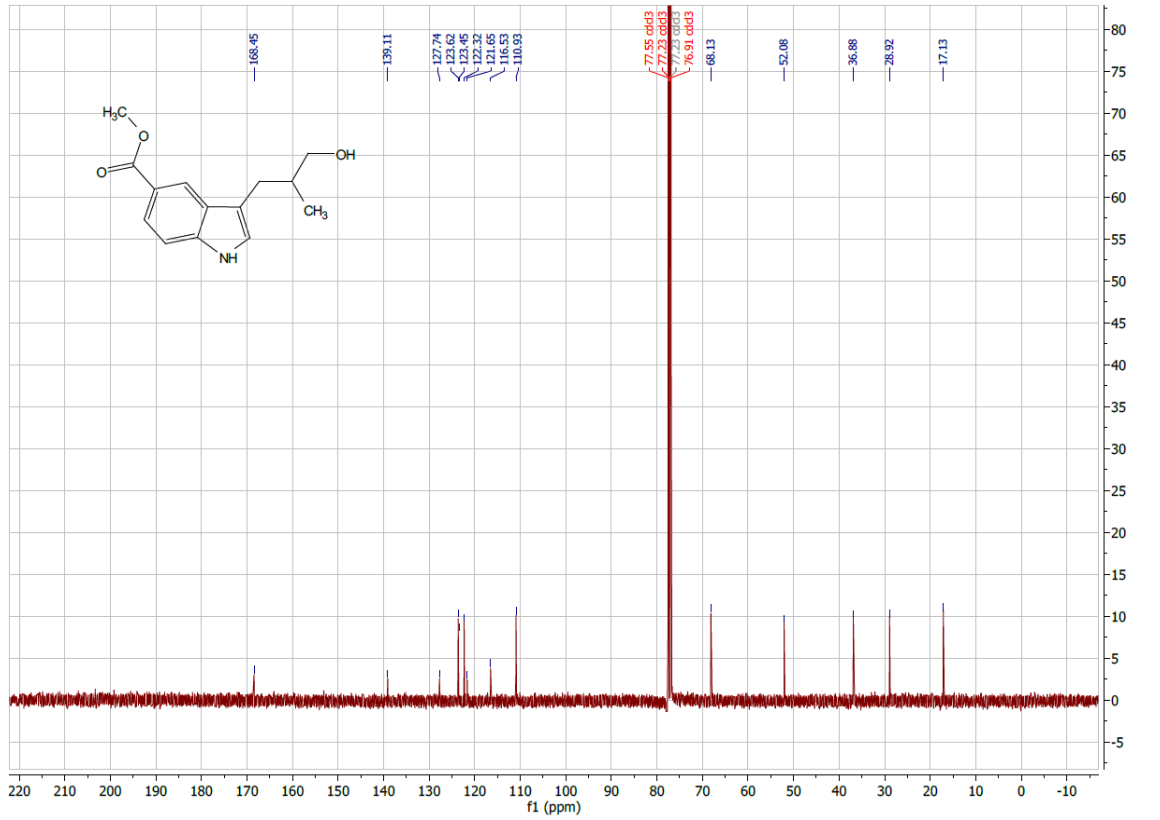
**3-(5-chloro-1H-indol-3-yl)-2-methylpropan-1-ol (3h)**



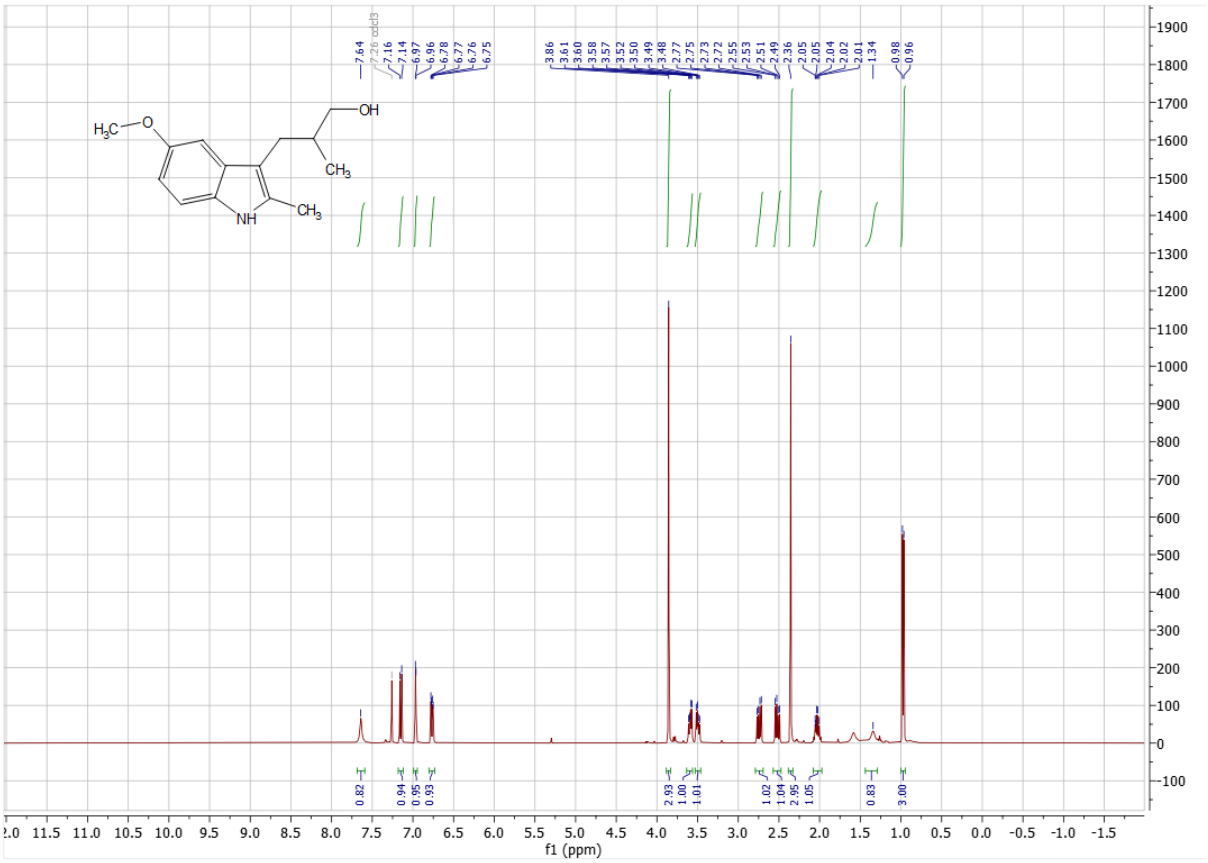



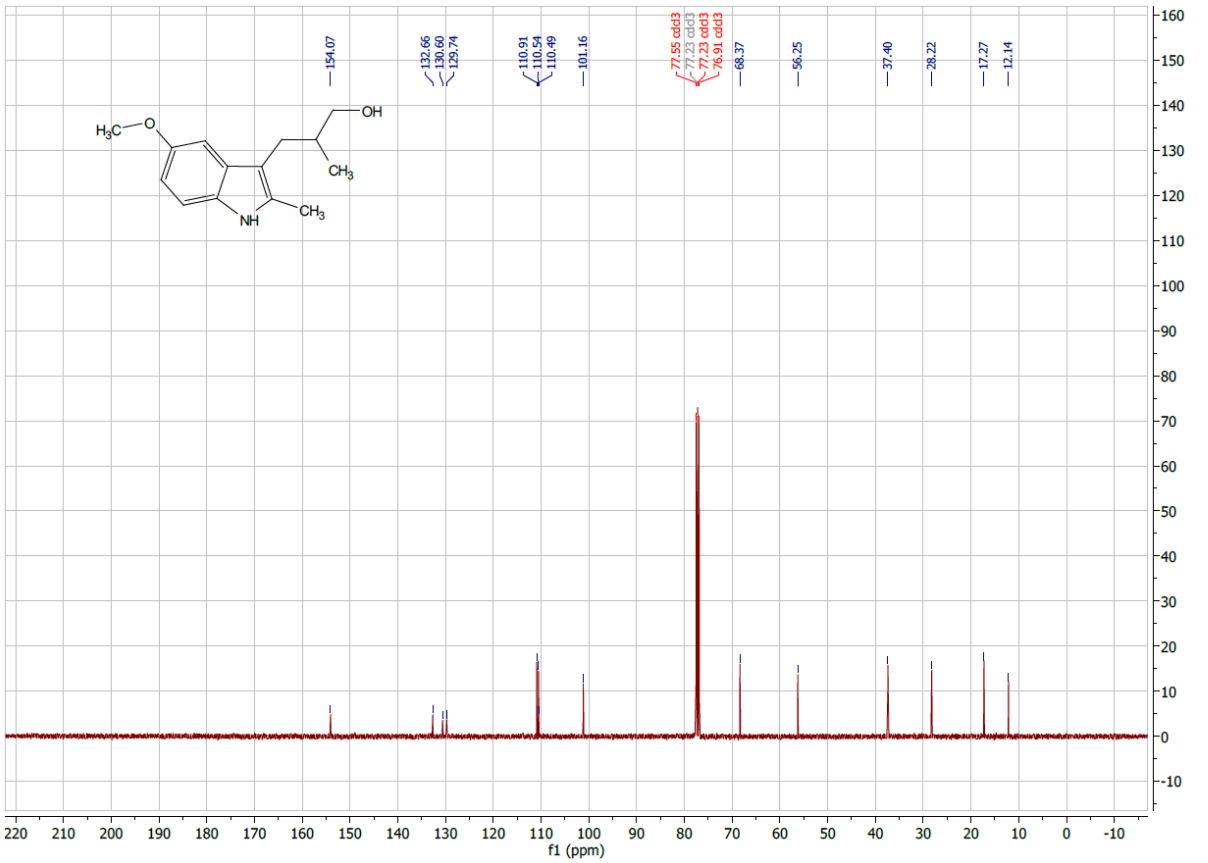
**methyl 3-(3-hydroxy-2-methylpropyl)-1H-indole-5-carboxylate (3i)**



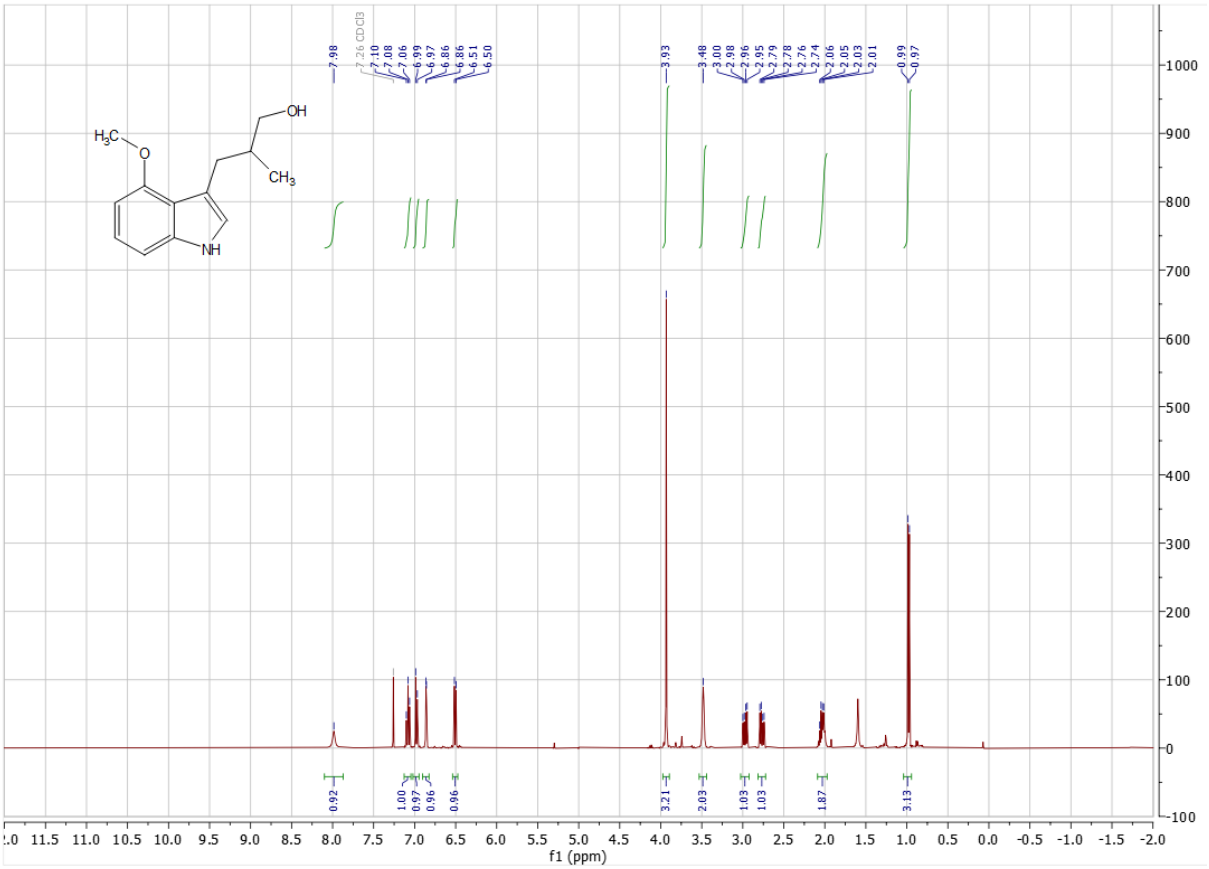



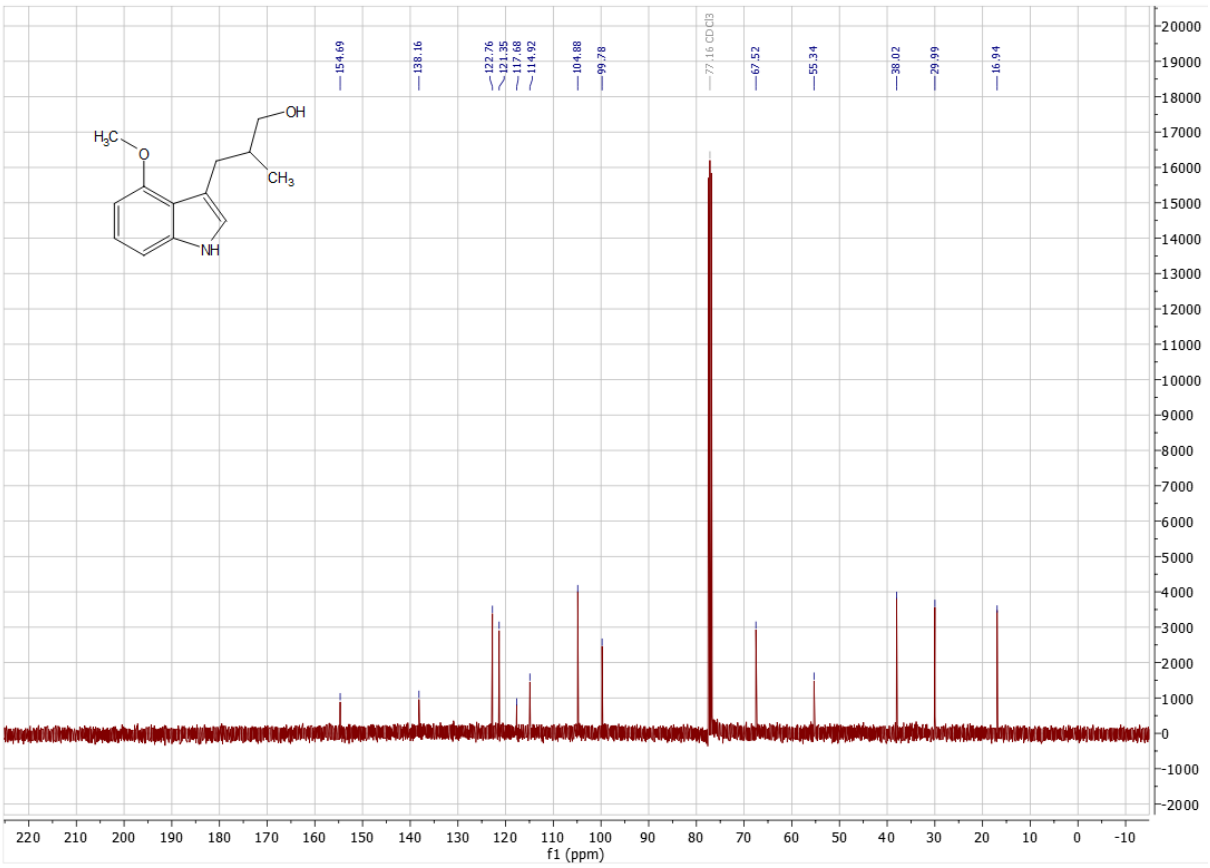
**3-(5-methoxy-2-methyl-1H-indol-3-yl)-2-methylpropan-1-ol (3j)**



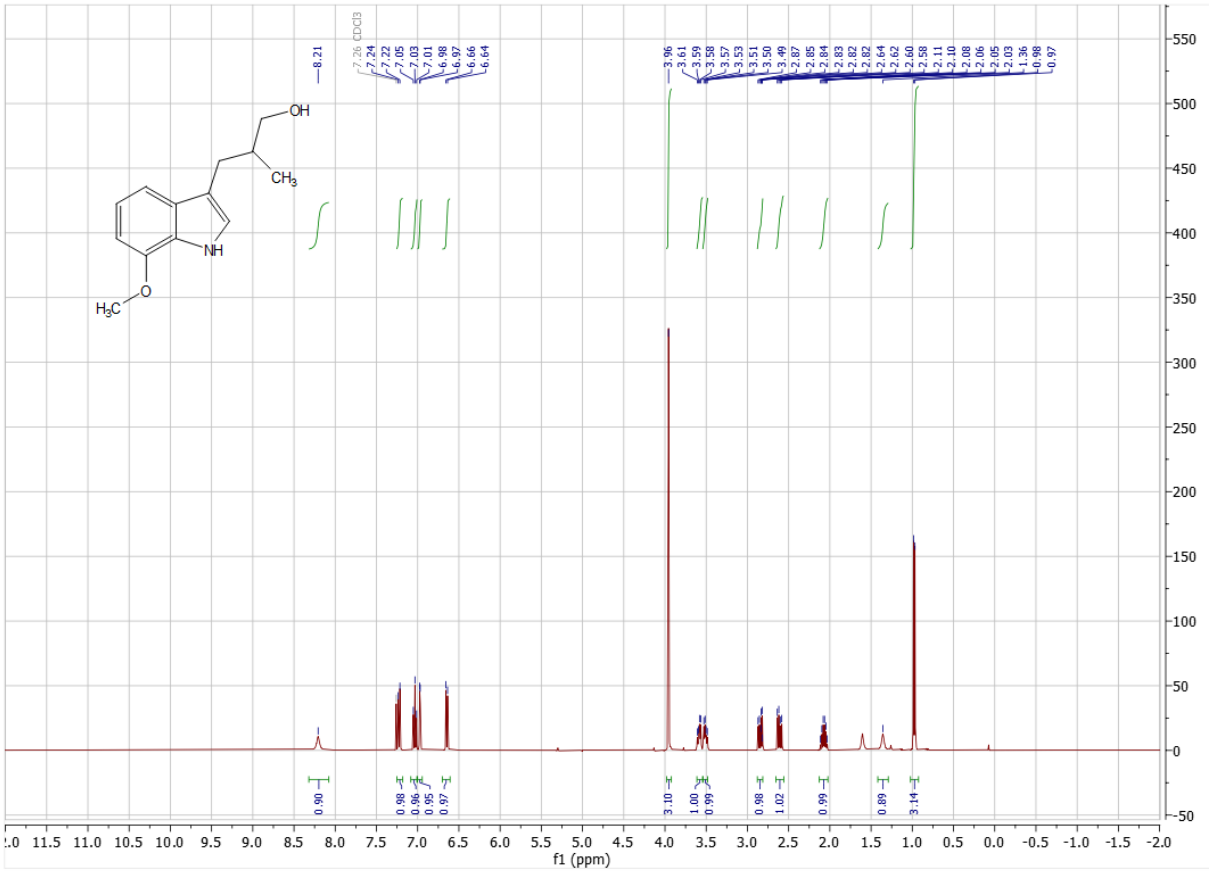



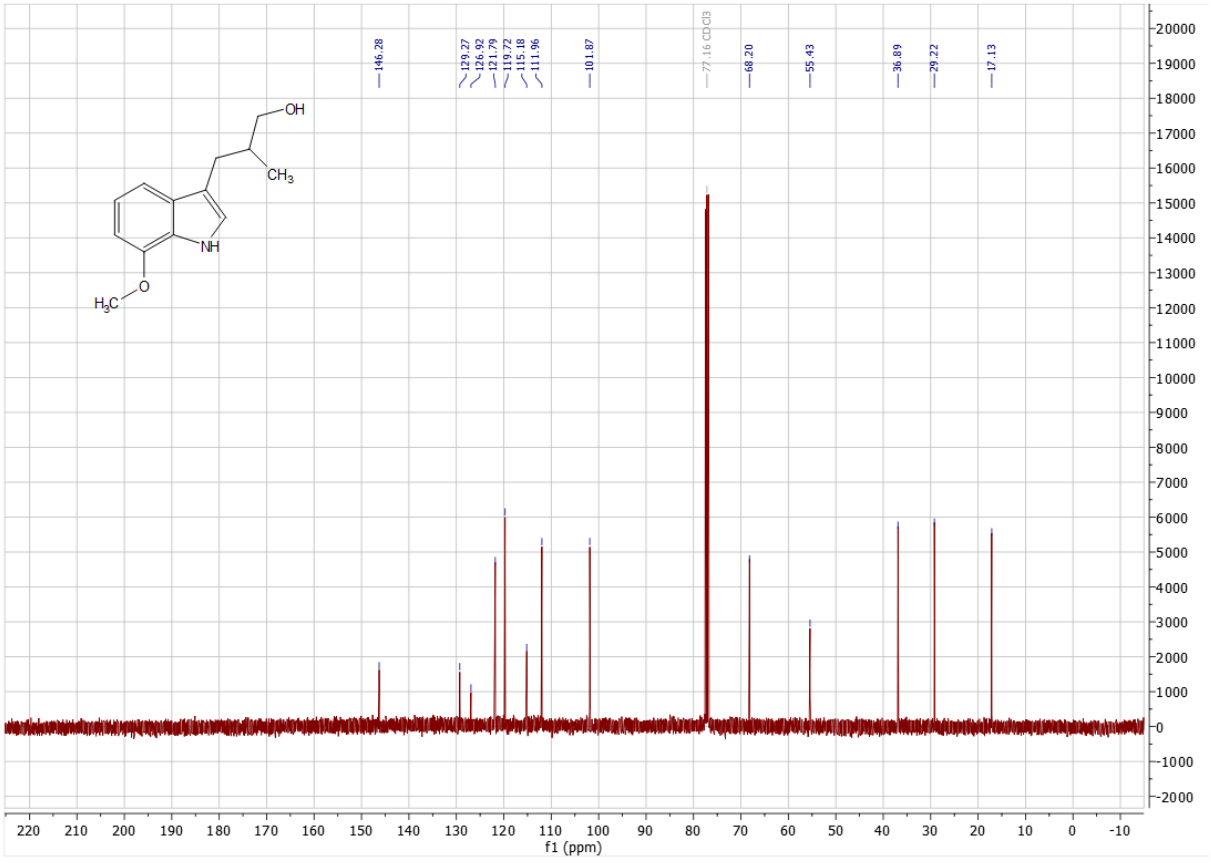
**3-(4-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3k)**



**3-(7-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3l)**

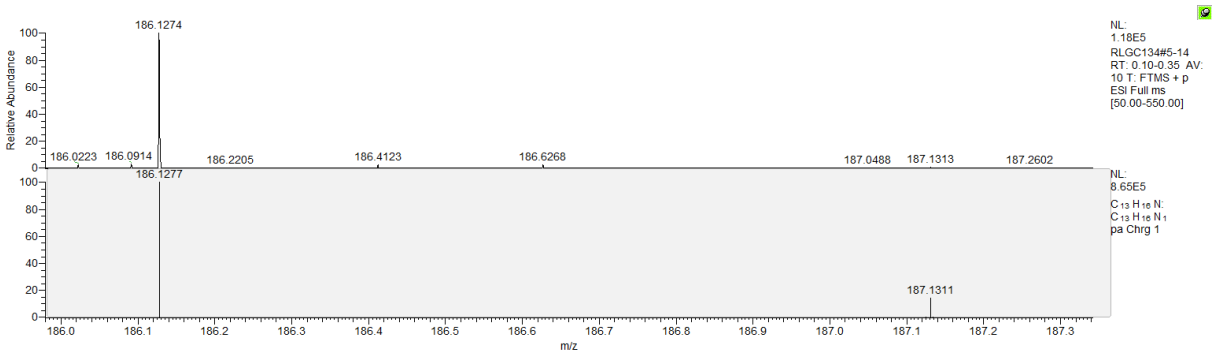


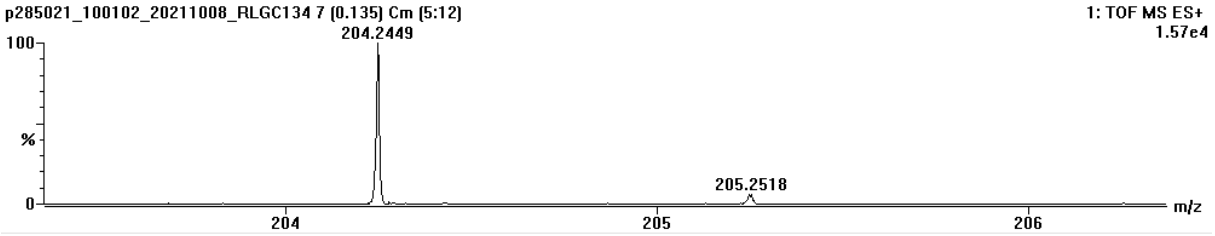
# 9. Mass Spectra of Reference Compounds

**2-methyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3a)**

HRMS ([M-OH]$^+$)

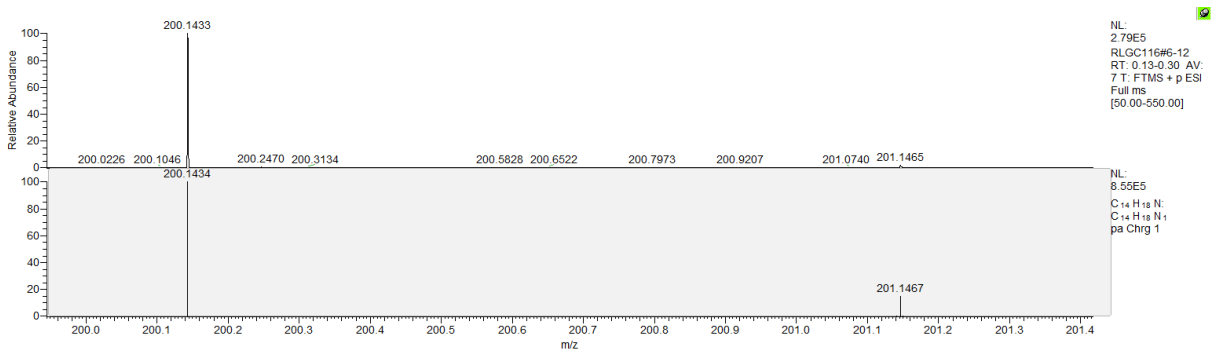



LRMS ([M+H]$^+$)




**2-((2-methyl-1H-indol-3-yl)methyl)butan-1-ol (3b)**

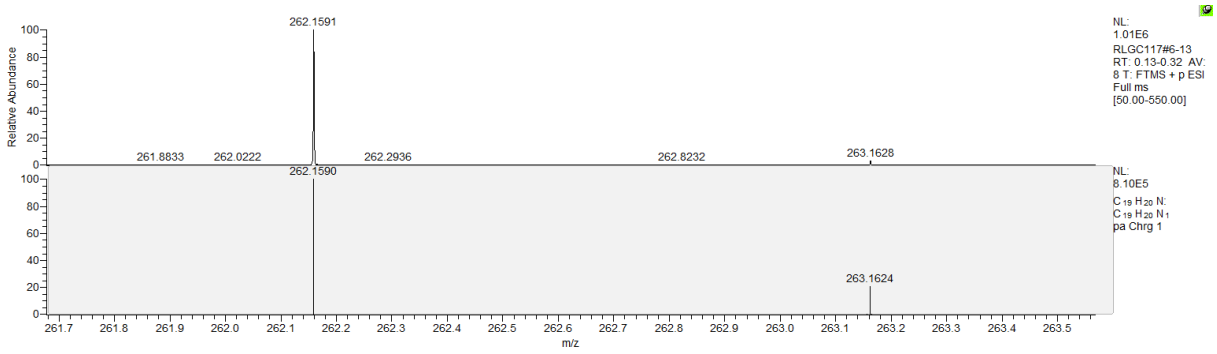
HRMS ([M-OH]$^+$)



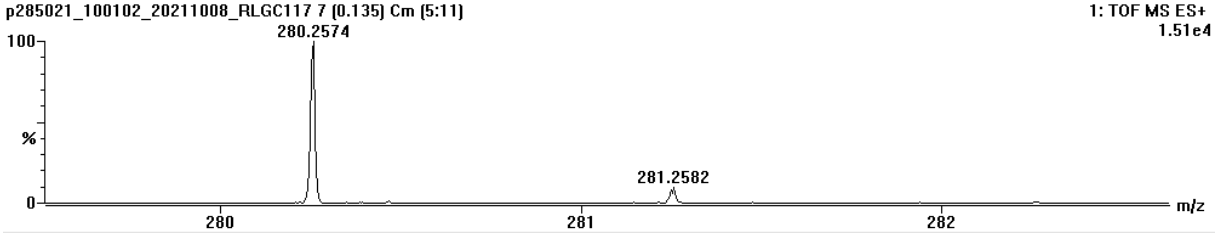

LRMS ([M+H]$^+$)

**2-benzyl-3-(2-methyl-1H-indol-3-yl)propan-1-ol (3c)**

HRMS ([M-OH]$^+$)

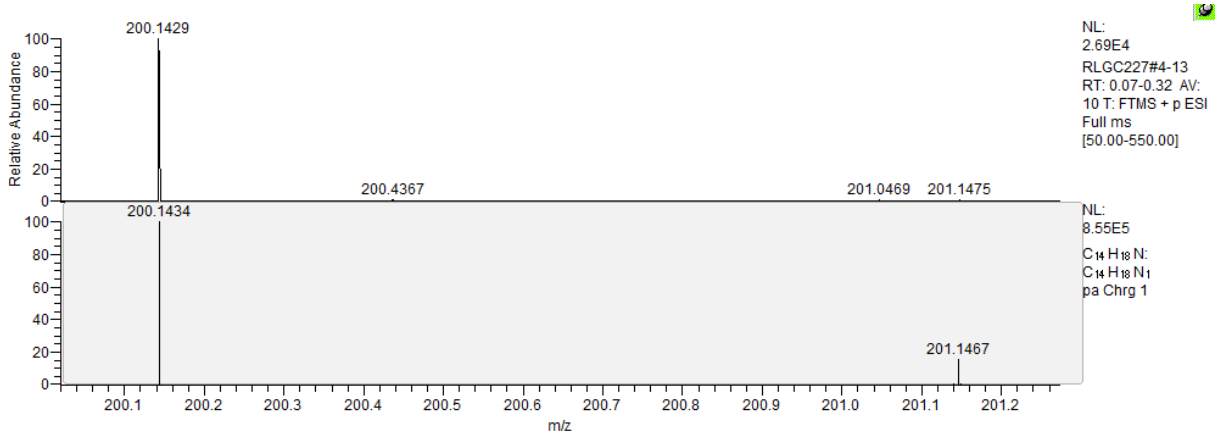



LRMS ([M+H]$^+$)

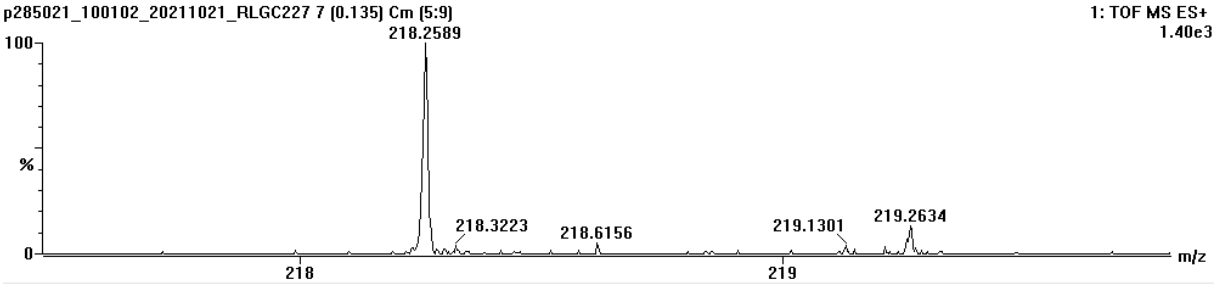



**2-methyl-3-(2-methyl-1H-indol-3-yl)butan-1-ol (3d)**

HMRS ([M-OH]$^+$)

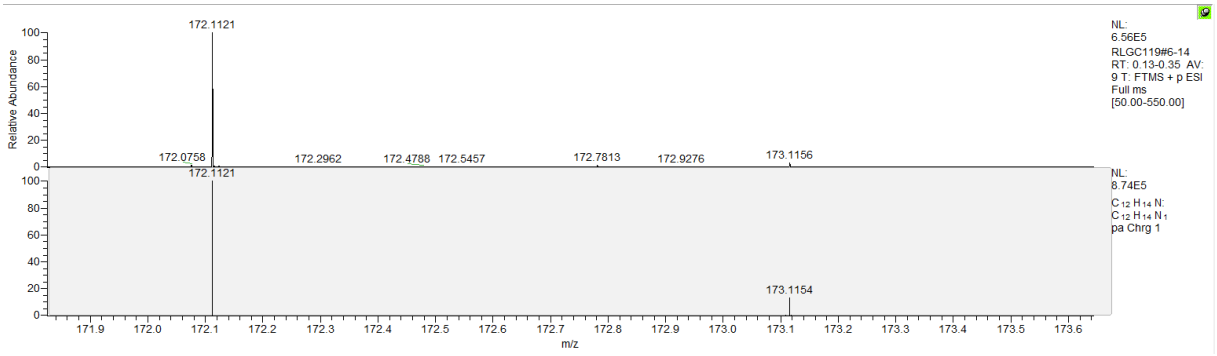



LRMS ([M+H]$^+$)

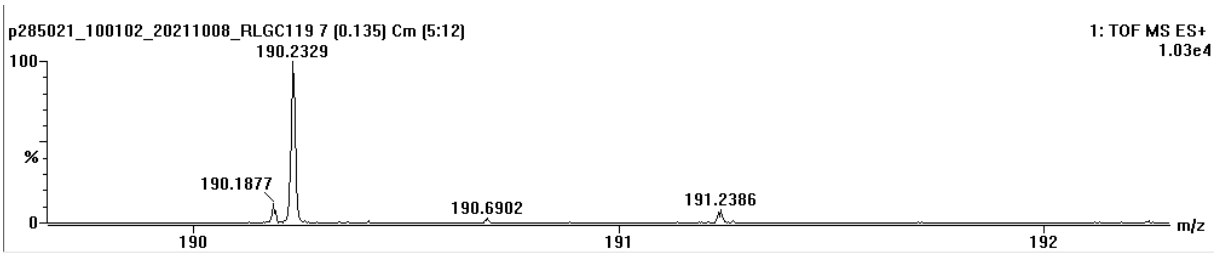


**3-(1H-indol-3-yl)-2-methylpropan-1-ol (3e)**

HRMS ([M-OH]$^+$)

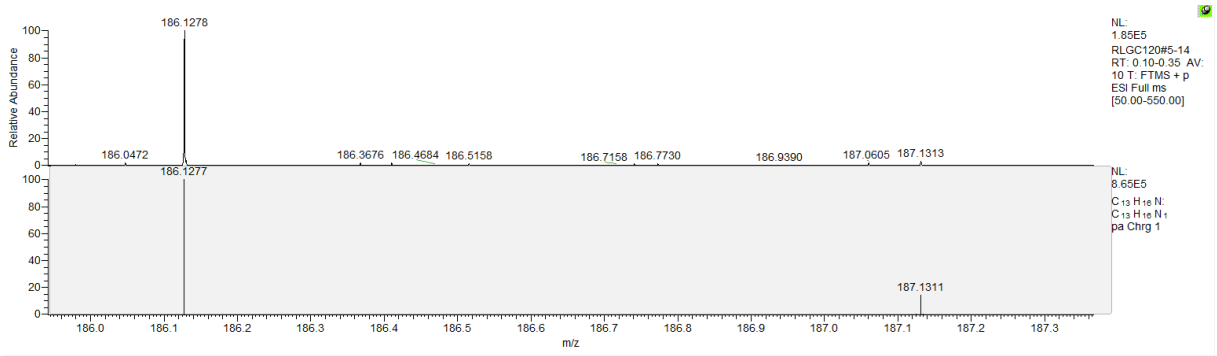



LRMS ([M+H]$^+$)

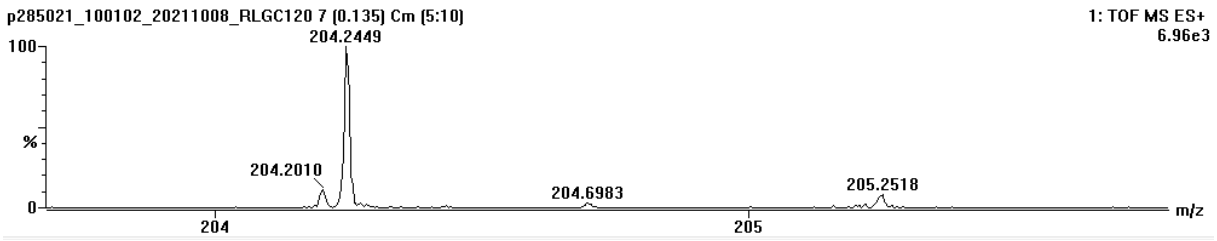



**2-methyl-3-(5-methyl-1H-indol-3-yl)propan-1-ol (3f)**

HRMS ([M-OH]$^+$)

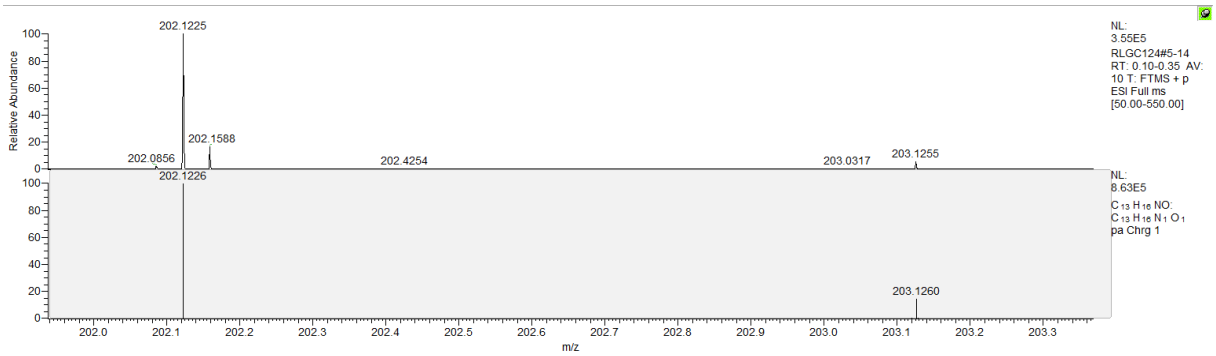



LRMS ([M+H]$^+$)

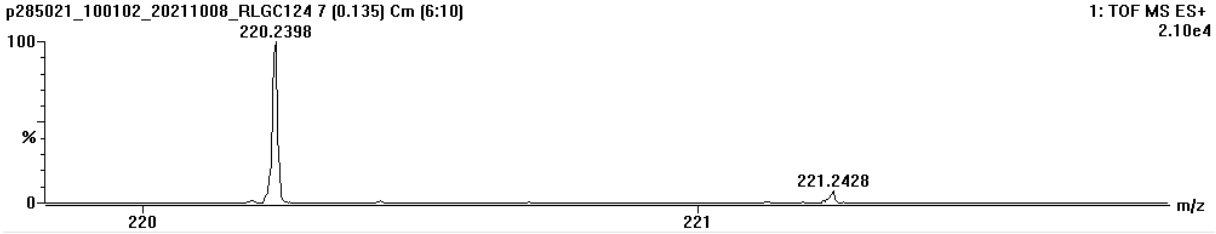


## 3-(5-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3g)

HRMS ([M-OH]$^+$)

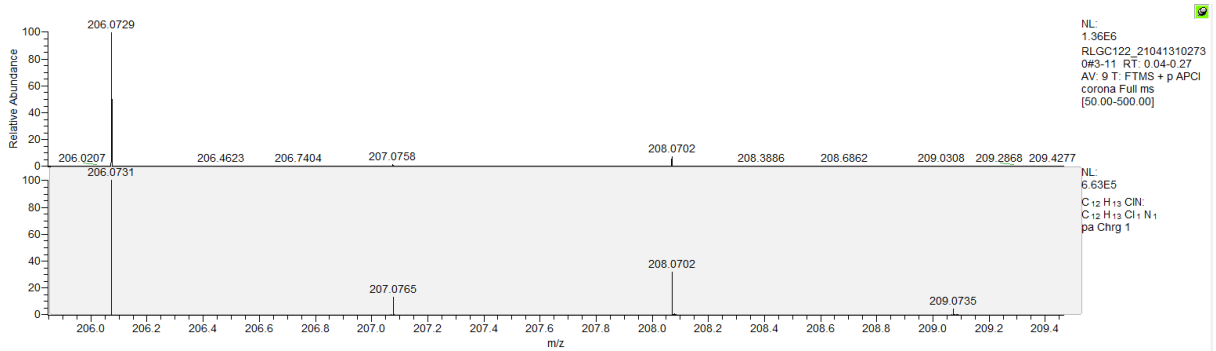



LRMS ([M+H]$^+$)

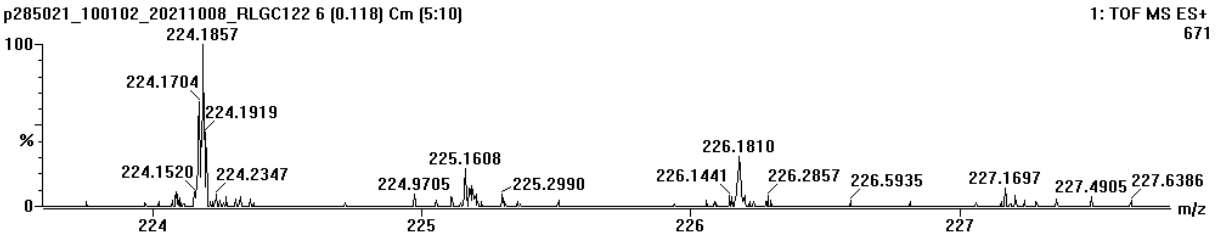



## 3-(5-chloro-1H-indol-3-yl)-2-methylpropan-1-ol (3h)

HRMS ([M-OH]$^+$)

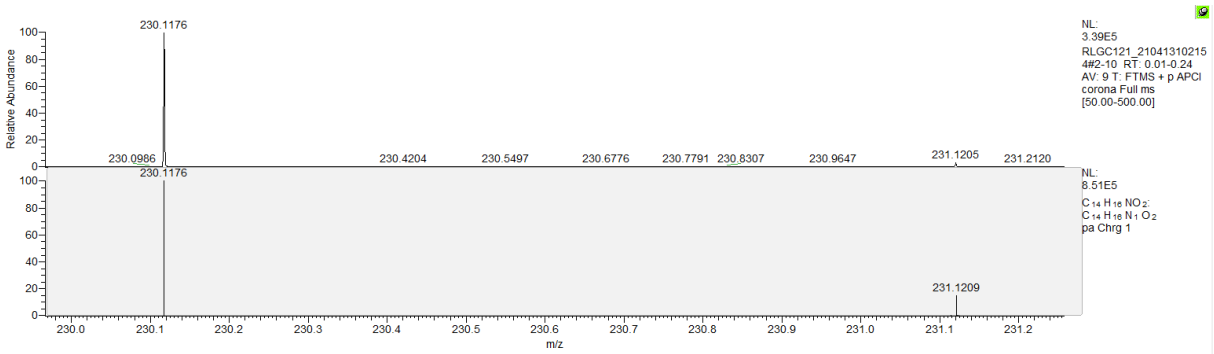



LRMS ([M+H]$^+$)

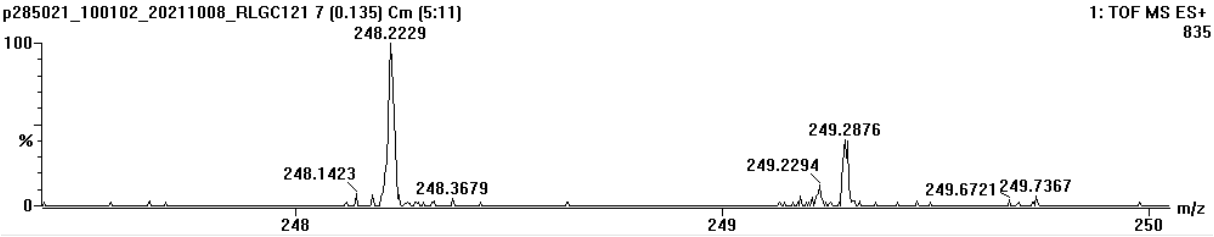


**methyl 3-(3-hydroxy-2-methylpropyl)-1H-indole-5-carboxylate (3i)**

HRMS ([M-OH]$^+$)

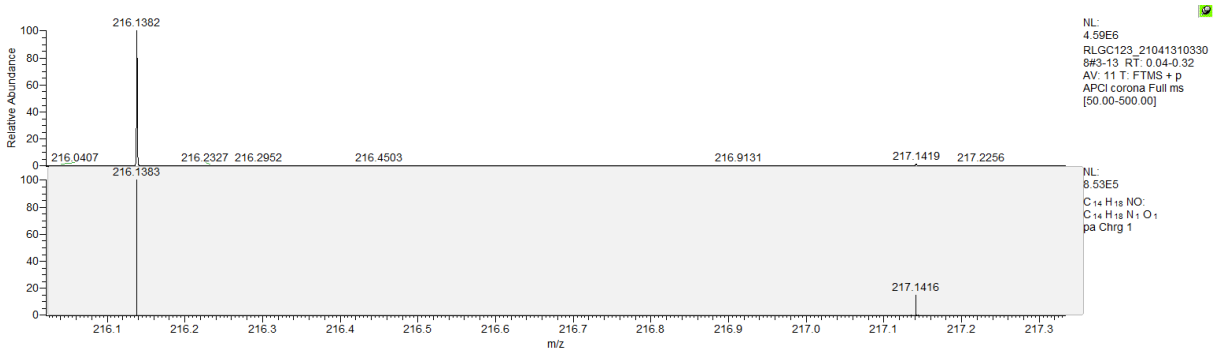



LRMS ([M+H]$^+$)




**3-(5-methoxy-2-methyl-1H-indol-3-yl)-2-methylpropan-1-ol (3j)**

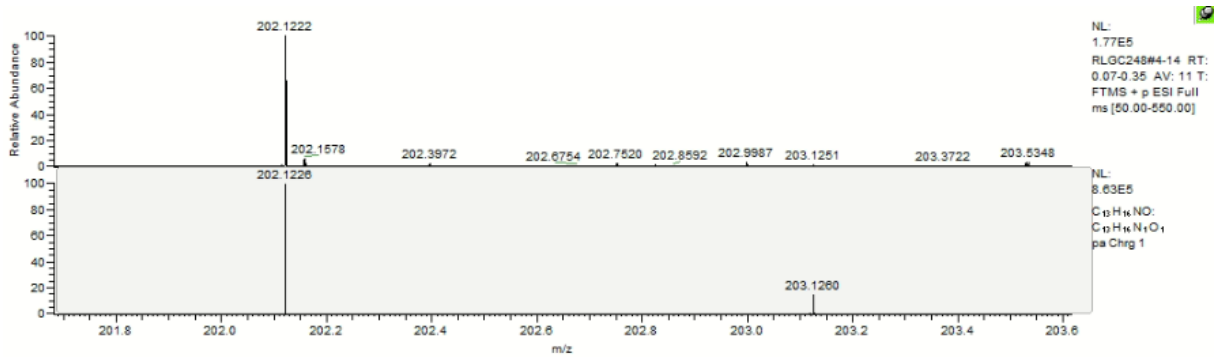
HRMS ([M-OH]$^+$)



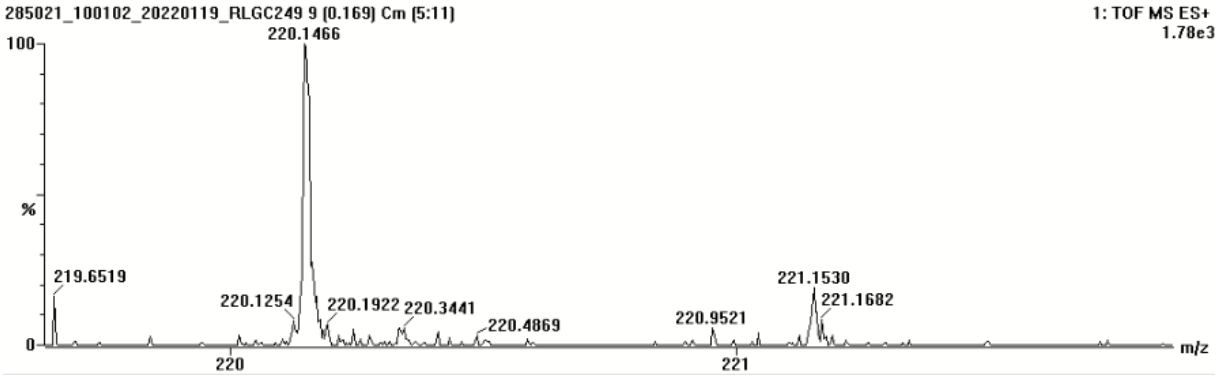

LRMS ([M+H]$^+$)

**3-(4-methoxy-1H-indol-3-yl)-2-methylpropan-1-ol (3k)**

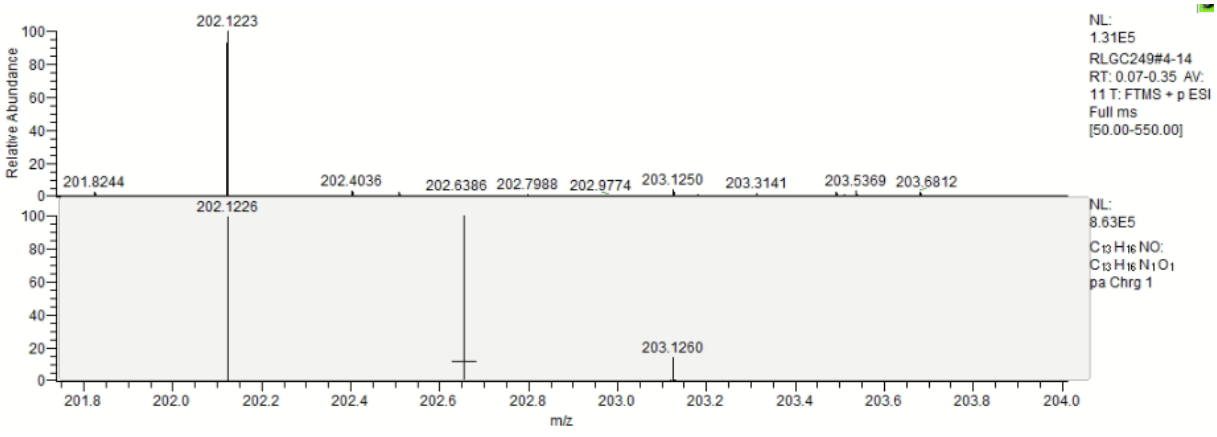
HRMS ([M-OH]$^+$)

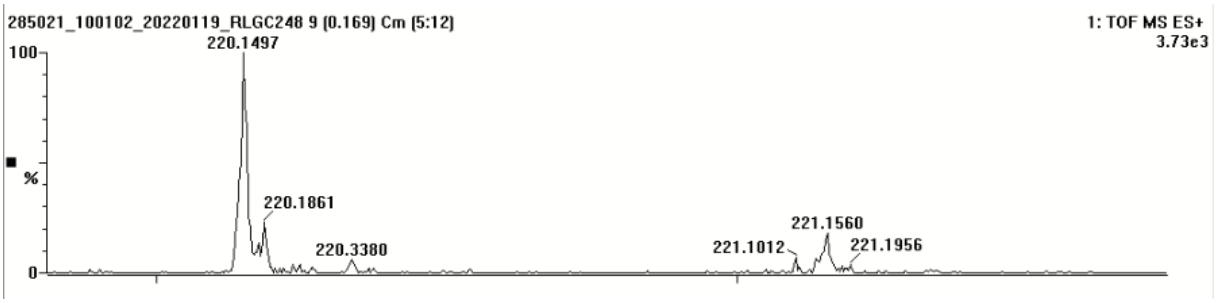



LRMS ([M+H]$^+$)




3l



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