Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Synthesis and bioactivity of psilocybin analogues containing a stable carbon-phosphorus bond

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General considerations

The synthetic procedures made use of commercially available reagents and solvents primarily purchased from Sigma-Aldrich®, Acros Organics®, Apollo scientific® and TCI®. Distilled water was obtained by the demineralization of tap water by an Aquadem ion exchanger from the 22DF type. Biological testing was performed by Eurofins. The melting point of compounds was determined by a Büchi Melting Point M-560.

Anhydrous solvents

Dry solvents such as toluene and THF were dried using the MBraun SPS-800 solvent purification system. Solvents were available in Pure-Pac containers of 17 L contained in a safety closet.

Automated column chromatography

The Grace™ Reveleris™ Flash Chromatography system was used for reversed-phase (C18) purification, whereas the Büchi Reveleris® X2 Flash Chromatography system was used for normal-phase (SiO_2) purification. The particle diameter for C18 and SiO_2 ranges from 20-40 µm and from 40-63 µm respectively. The stationary phase is contained in a cartridge of which several sizes (4-120 g) are available. The size of the cartridge and the elution rate (18-60 mL) depend on the weight of the coated sample. Detection of the compounds was accomplished *via* two UV detectors for the Grace™ Reveleris™ and three UV detectors for the Büchi Reveleris® system. Characteristic UV signals were determined *via* LC-MS.

Liquid Chromatography coupled with Mass Spectrometry (LC-MS)

LC-MS analyses were performed on an Agilent 1200 Series HPLC equipped with a Supelco Ascentis® Express C18 column (3 cm x 4.6 mm, 2.7 µm fused-core particles, 90 Å), a Phenomenex Guard column (SecurityGuard Standard) and a UV-DAD detector. The liquid chromatography system is coupled to an Agilent 1100 Series MS with electrospray ionization (4000 V, 70 eV) and a single quadrupole detector.

High Resolution Mass Spectrometry (HRMS)

High Resolution Mass Spectrometry (HRMS) analyses are conducted on a Thermo Fisher Q-Exactive Orbitrap HRMS with mass resolution of 70000, a loop injection of 10 µL and HESI ionization POS.

Nuclear Magnetic Resonance spectrometry (NMR)

^{1}H-NMR, ^{31}P-NMR and ^{13}C-NMR spectra were obtained from a Bruker Avance III HD Nanobay spectrometer, equipped with a 1H/BB z-gradient probe (BBO, 5 mm). Measurements were taken at 400 MHz, 162 MHz and 100.6 MHz respectively. Chemical shifts were reported as δ-values (ppm) and coupling constants (J) were expressed in Hertz (Hz). Deuterated solvents ($CDCl_3$, MeOD, DMSO, acetone-d_6, D_2O) containing TMS as an internal standard were used to dissolve samples. Additional two-dimensional spectra were obtained as well to assign signals with certainty. Spectra could be consulted and processed *via* TOPSPIN 4.3.0 software.

Infrared spectroscopy (IR)

Infrared spectra were obtained from solid or dissolved samples with a signal-to-noise ratio (S/N) of 30 000:1 using a Shimadzu IRAffinity-1S Fourier Transform Infrared Spectrophotometer with a (FTIR)Quest ATR (Attenuated Total Reflectance) accessory with diamond crystal pucks. The infrared spectra were accessed and processed with LabSolutions IR software.

Thin Layer Chromatography (TLC)

Glass-backed silica plates (Merck Silicagel 60 $F2_{54}$, precoated, thickness 0.25 mm) were used in combination with an appropriate solvent mixture. All compounds could be visualized by UV irradiation (254 or 365 nm).

Safety

Caution! *The synthesised molecules are potentially highly toxic. It is important to comply with personal protective equipment directives and general laboratory regulations. The experimental work takes place in hoods; crude reaction mixtures, or purified substances will be kept in closed recipients.*

Caution! *Sodium hydroxide is considered highly corrosive and can cause severe skin burns and eye damage.*

Caution! *Dimethylamine has a boiling point of only 7 °C and subsequently forms a vapor at room temperature that is extremely flammable. The recipient may contain gas under pressure that can explode if heated. Additionally, the product may cause eye damage, skin irritation and be harmful and irritating if inhaled.*

Caution! *Dimethyl sulfate is a colorless liquid that is toxic if swallowed, causes severe skin burns and eye damage. The reagent is fatal upon inhalation and is suspected of causing genetic effects and cancer.*

Caution! *Activated zinc dust should be added slowly to the reaction mixture.*

Caution! *Hydrochloric acid is considered highly corrosive and can cause severe skin irritation and eye damage.*

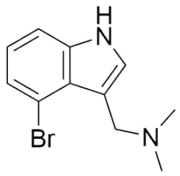
Caution! *Sodium cyanoborohydride emit flammable gases which may ignite spontaneously in contact with water.*

Caution! *$Pd(OAc)_2$ and diethyl phosphite can cause severe eye damage.*

Caution! *Toluene is a colorless, highly flammable liquid and vapor that can cause skin irritation and drowsiness or dizziness. Swallowing or inhalation of the reagent may be fatal.*

Synthesis

1-(4-Bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylmethanamine (2)



A solution of 4-bromoindole (**1**) (2.65 g, 13.50 mmol, 1 eq) in acetonitrile (26.48 mL) and glacial acetic acid (8.83 mL) under nitrogen was cooled to 0 °C. Formaldehyde (1.35 mL of a 37% aqueous solution, 17.96 mmol, 1.33 eq) and dimethylamine (1.98 mL of a 40% solution in water, 15.80 mmol, 1.17 eq) were added to the reaction mixture and stirred at room temperature for approximately 2 h. The reaction mixture was concentrated using a rotary evaporator. A solution of 30% sodium hydroxide was added to the residue and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over $MgSO_4$ and again evaporated using a rotary evaporator. If the product remained a dense oil, the extraction was repeated with basified water. If white solids were obtained after evaporation, a recrystallisation was performed with acetonitrile to yield white crystals of 1-(4-bromo-1*H*-indol-3-yl)-*N,N*-dimethylmethanamine (**2**) (1.39 g, 42%).

^{1}H-NMR (400 MHz, MeOD): δ = 2.31 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 3.93 (2*H*, *s*, C<u>H</u>$_2$-N); 6.97 (1*H*, *t*, J_{H-H} = 7.9 Hz, CH-C<u>H</u>-CH); 7.20 (1H, *d x d*, J_{H-H} = 7.8, 0.7 Hz, Br-C$_q$-C<u>H</u>-CH); 7.28 (1*H*, *s*, C<u>H</u>-NH); 7.35 (1*H*, *d x d*, J_{H-H} = 7.8, 0.7 Hz, C<u>H</u>-C$_q$-NH). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 44.9 (2*C*, 2 x <u>C</u>H$_3$-N); 54.7 (1*C*, <u>C</u>H$_2$-N); 112.1 (1*C*, <u>C</u>H-C$_q$-NH); 112.8 (1*C*, CH$_2$-<u>C</u>$_q$-CH); 114.7 (1*C*, <u>C</u>$_q$-Br); 123.4 (1*C*, CH-<u>C</u>H-CH); 124.9 (1*C*, Br-C$_q$-<u>C</u>H-CH); 126.6 (1*C*, <u>C</u>$_q$-C$_q$-Br); 128.2 (1*C*, <u>C</u>H-NH); 139.6 (1*C*, CH-<u>C</u>$_q$-NH).

Spectral data is in accordance with literature.[1]

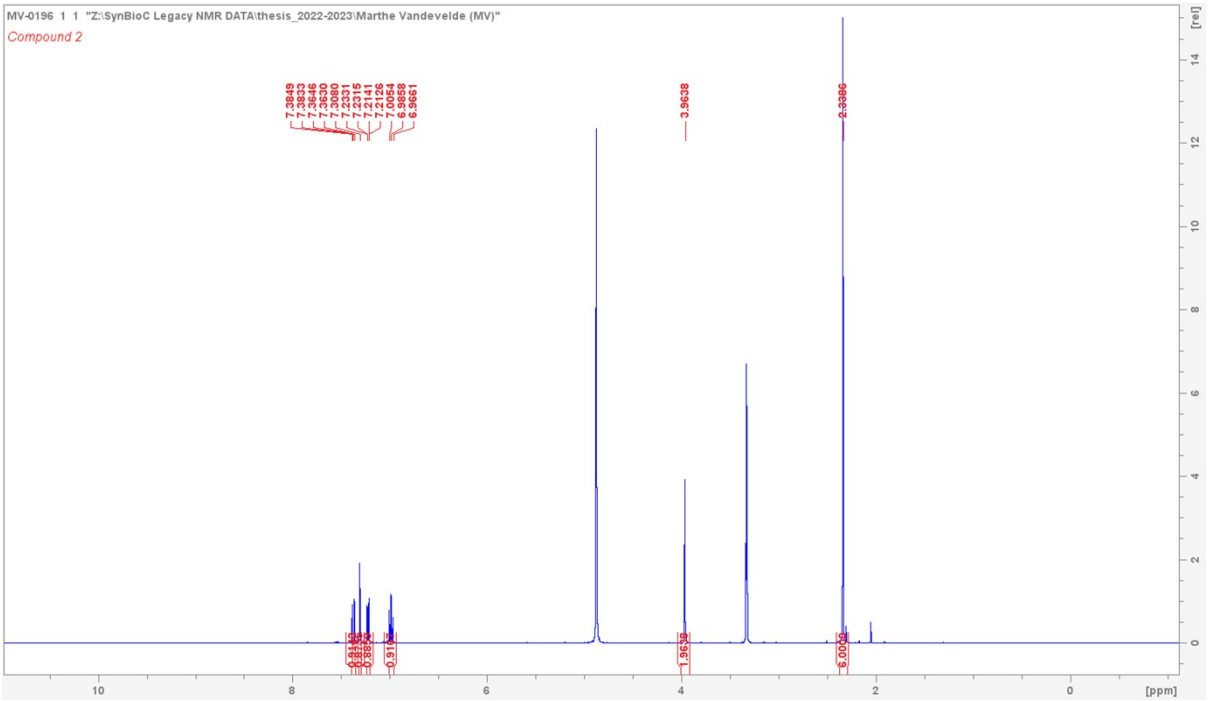
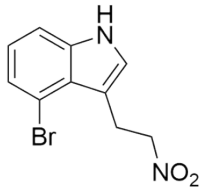


Figure S1. ^{1}H-NMR of compound 2 in MeOD.

4-Bromo-3-(2-nitroethyl)-1*H*-indole (3)



1-(4-Bromo-1*H*-indol-3-yl)-*N,N*-dimethylmethanamine (**2**) (1.39 g, 5.48 mmol, 1 eq) was dissolved in a solution of nitromethane (23.60 mL) and methanol (11.80 mL). Next, sodium methoxide (0.41 g, 7.51 mmol, 1.37 eq) and dimethyl sulfate (1.38 g, 10.96 mmol, 2 eq) were added and the reaction mixture was stirred for *circa* 24 h. The progress of the reaction was followed by LC-MS or ^{1}H-NMR analysis. Upon completion, the reaction mixture was concentrated using a rotary evaporator employing a moderate temperature for the water bath. Next, the residue was dissolved in dichloromethane and was successively extracted with 5% NH_4OH, 1 N HCl and brine. The residual organic phase was dried over $MgSO_4$ and evaporated using a rotary evaporator to yield a dark-pink oil of 4-bromo-3-(2-nitroethyl)1*H*-indole (**3**) (1.17 g, yield 79%).

^{1}H-NMR (400 MHz, MeOD): δ = 3.63 (2*H*, *t*, J_{H-H} = 7.1 Hz, $C\underline{H}_2$-CH_2-NO_2); 4.74 (2*H*, *t*, J_{H-H} = 7.1 Hz, CH_2-$C\underline{H}_2$-NO_2); 6.95 (1*H*, *t*, J_{H-H} = 7.8 Hz, CH-C<u>H</u>-CH); 7.14 (1*H*, s, C<u>H</u>-NH); 7.18 (1*H*, *d x d*, J_{H-H} = 8.1, 0.7 Hz, Br-C_q-C<u>H</u>-CH); 7.33 (1*H*, *d x d*, J_{H-H}= 8.1, 0.7 Hz, C<u>H</u>-C_q-NH); 10.77 (1*H*, *s*, N<u>H</u>). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 25.5 (1*C*, $\underline{C}H_2$-CH_2-NO_2); 78.3 (1*C*, CH_2-$\underline{C}H_2$-NO_2); 110.9 (1*C*, CH_2-$\underline{C}_q$-CH); 112.2 (1*C*, $\underline{C}H$-C_q-NH); 114.7(1*C*, $\underline{C}_q$-Br); 123.5 (1*C*, CH-$\underline{C}H$-CH); 124.4 (1*C*, Br-C_q-$\underline{C}H$-CH); 126.7 (1*C*,$\underline{C}_q$-C_q-Br); 127.3 (1*C*, $\underline{C}H$-NH); 139.5 (1*C*, CH-$\underline{C}_q$-NH).

Spectral data is in accordance with literature.[1-2]

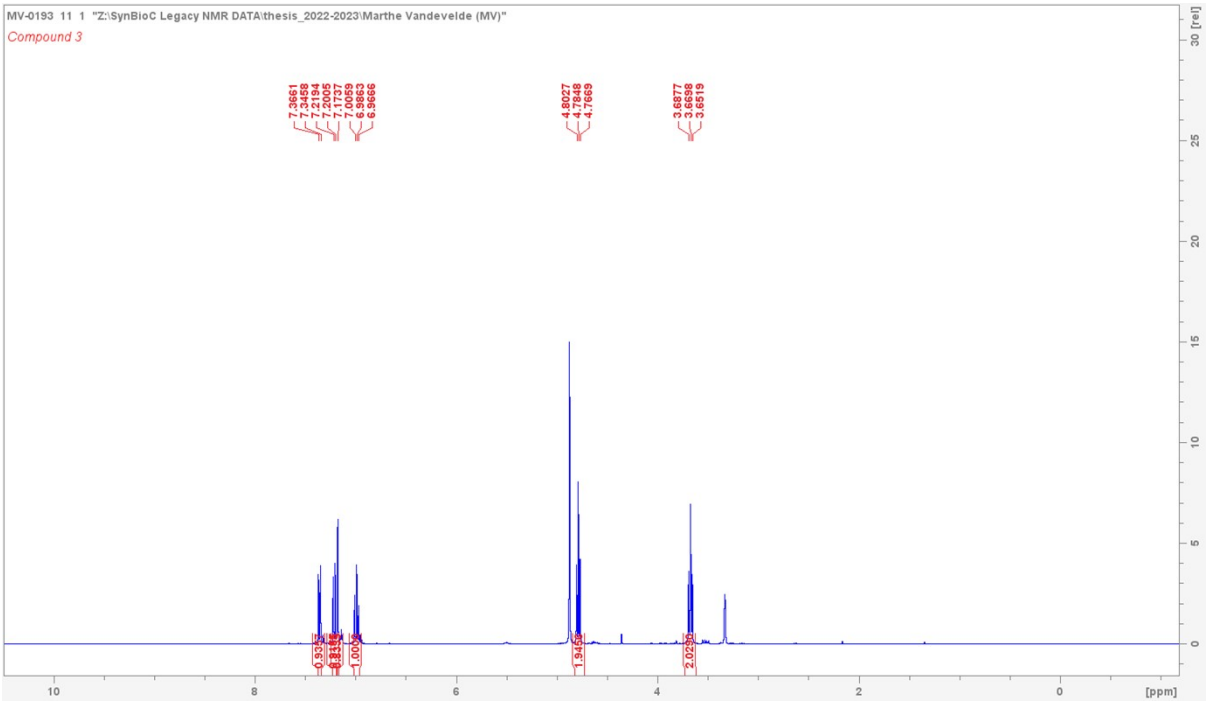
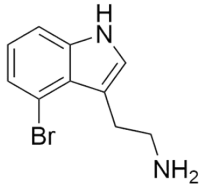


Figure S2. ^{1}H-NMR of compound 3 in MeOD

2-(4-Bromo-1*H*-indol-3-yl)ethan-1-amine (4)



4-Bromo-3-(2-nitroethyl)-1*H*-indole (**3**) (1.00 g, 3.73 mmol) was dissolved in methanol (74.40 mL) and aqueous HCl (2 M, 74.40 mL). Activated zinc dust (4.38 g, 66.96 mmol) was added in portions over 15 min. After the addition was finished, the suspension was stirred and heated at reflux for 3 h. After being cooled down

to room temperature, the reaction mixture was filtered, and the residue was washed with methanol. The organic solution was concentrated under reduced pressure until almost all solvents were removed. Potassium carbonate was added in small portions until the pH of the mixture became alkaline. In case the solubility of potassium carbonate was too low, a very small amount of water was added. The aqueous phase was extracted with ethyl acetate. The organic extracts were combined and dried with anhydrous $MgSO_4$. Removal of the solvent using a rotary evaporator gave 2-(4-bromo-1*H*-indol-3-yl)ethan-1-amine (**4**) (0.65 g, 73%) as a dark pink oil.

^{1}H-NMR (400 MHz, MeOD): δ = 2.96 (2*H*, *t*, J_{H-H} = 7.1 Hz, CH_2-C$\underline{H}_2$-NH_2); 3.12 (2*H*, *t*, J_{H-H} = 7.1 Hz, C$\underline{H}_2$-CH_2-NH_2); 6.93 (1*H*, *t*, J_{H-H} = 7.7 Hz, CH-C$\underline{H}$-CH); 7.13 (1*H*, *s*, C$\underline{H}$-NH); 7.15 (1*H*, *d x d*, J_{H-H} = 7.7, 0.7 Hz, Br-C_q-C$\underline{H}$-CH); 7.32 (1*H*, *d x d*, J_{H-H} = 7.7, 0.7 Hz, C$\underline{H}$-C_q-NH); 10.77 (1*H*, *s*, N$\underline{H}$). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 30.3 (1*C*, $\underline{C}H_2$-CH_2-NH_2); 44.4 (1*C*, CH_2-$\underline{C}H_2$-NH_2); 111.9 (1*C*, $\underline{C}$H-C_q-NH); 114.0 (1*C*, CH_2-$\underline{C}_q$-CH); 114.6 (1*C*, $\underline{C}_q$-Br); 123.2 (1*C*, CH-$\underline{C}$H-CH); 124.1 (1*C*, Br-C_q-$\underline{C}$H-CH); 126.0 (1*C*, $\underline{C}$H-NH); 126.4 (1*C*, $\underline{C}_q$-C_q-Br); 139.7 (1*C*, CH-$\underline{C}_q$-NH).

Spectral data is in accordance with literature.[1-2]

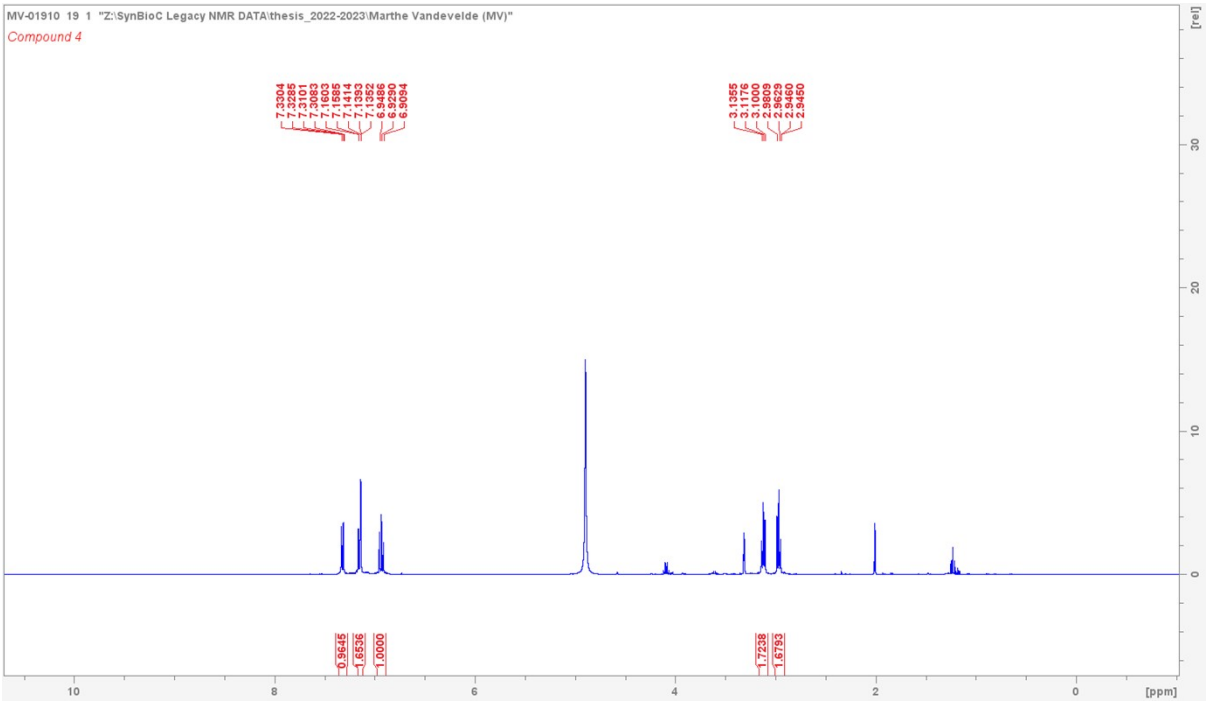
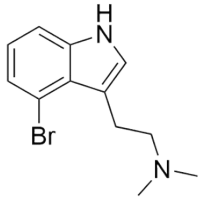


Figure S3. ^{1}H-NMR of compound 4 in MeOD.

2-(4-Bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylethan-1-amine (5a)



To a stirred solution of 2-(4-bromo-1*H*-indol-3-yl)ethan-1-amine (**4**) (0.65 g, 2.72 mmol, 1 eq) in MeOH (56.37 mL) acetic acid (0.63 mL, 11.00 mmol, 4.0 eq) was added followed by sodium cyanoborohydride (0.35 g, 5.55 mmol, 2.0 eq) under nitrogen atmosphere at 0 °C. A solution of formaldehyde (0.49 mL of 37% aqueous solution, 6.58 mmol, 2.4 eq) in MeOH (18.60 mL) was then added dropwise over 20 min, and the resulting solution was stirred at room temperature for 4 h. The reaction was quenched by slowly adding aqueous Na_2CO_3 (2 N) until pH 8–9 was obtained and the solvents were removed *in vacuo*. The residue was partitioned between chloroform and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated at a rotary

evaporator to yield 2-(4-bromo-1*H*indol-3-yl)-*N*,*N*-dimethylethan-1-amine (**5a**) without further purification as a white-pink oil (0.656 g, 90%).

^{1}H-NMR (400 MHz, MeOD): δ = 2.34 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 2.65-2.69 (2*H*, *m*, CH$_2$-C<u>H</u>$_2$-N); 3.14-3.18 (2*H*, *m*, C<u>H</u>$_2$-CH$_2$-N); 6.92 (1*H*, *t*, J_{H-H} = 7.9 Hz, CH-C<u>H</u>-CH); 7.12 (1*H*, *s*, C<u>H</u>-NH); 7.15 (1*H*, *d x d*, J_{H-H} = 7.9, 0.7 Hz, Br-C$_q$-C<u>H</u>-CH); 7.31 (1*H*, *d x d*, J_{H-H} = 7.9, 0.7 Hz, C<u>H</u>-C$_q$-NH). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 24.9 (1*C*, <u>C</u>H$_2$-CH$_2$-N); 45.4 (2*C*, 2 x <u>C</u>H$_3$-N); 63.1(1*C*, CH$_2$-<u>C</u>H$_2$-N); 111.9 (1*C*, <u>C</u>H-C$_q$-NH); 114.4 (1*C*, CH$_2$-<u>C</u>$_q$-CH); 114.5 (1*C*, <u>C</u>$_q$-Br); 123.1 (1*C*, CH-<u>C</u>H-CH); 124.1 (1*C*, Br-C$_q$-<u>C</u>H-CH); 125.7 (1*C*, <u>C</u>H-NH); 126.5 (1*C*, <u>C</u>$_q$-C$_q$-Br); 139.6 (1*C*, CH-<u>C</u>$_q$-NH).

Spectral data is in accordance with literature.[2]

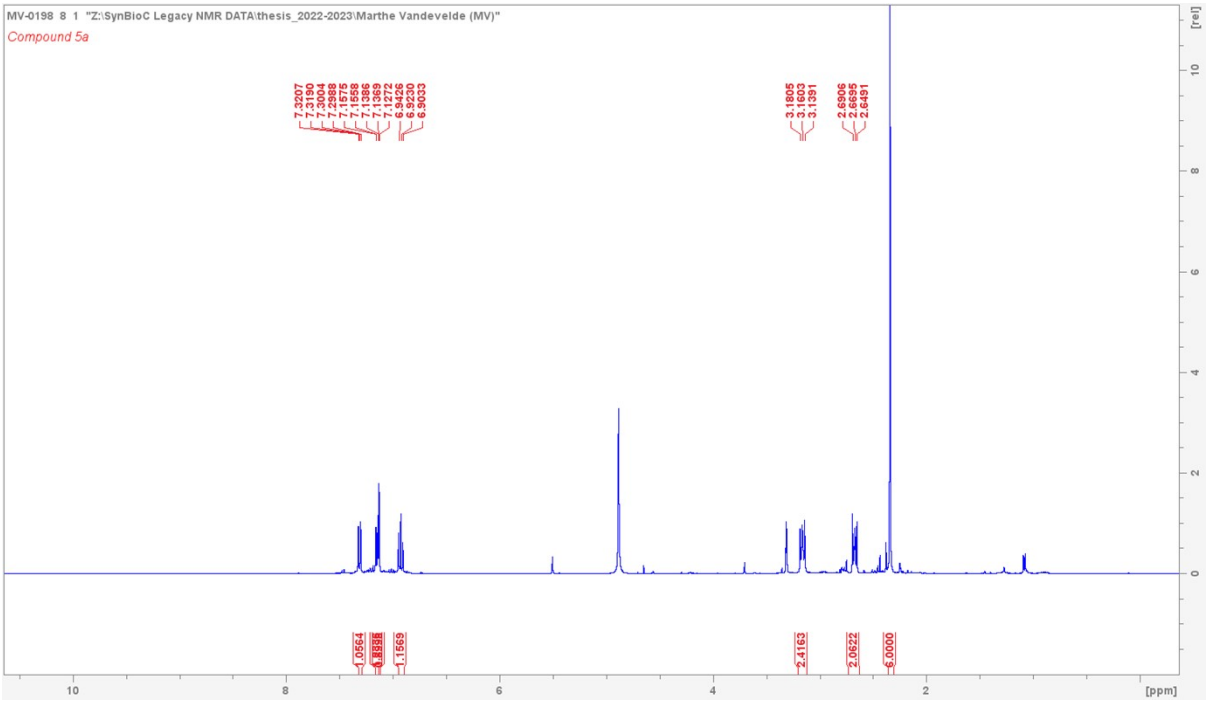
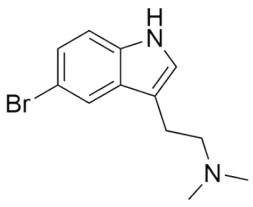


Figure S4. ^{1}H-NMR of compound 5a in MeOD

Method II

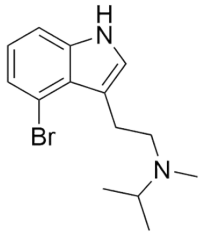
A mixture of 4-bromo indole (**1**) (200 mg, 0.13 mL, 1.02 mmol), Cs_2CO_3 (91 mg, 1.12 mmol), $[Cp^*IrCl_2]_2$ (8 mg, 0.0102 mmol) and 2-dimethylaminoethanol (682 mg, 0.77 mL, 3.06 mmol) was stirred under N_2 atmosphere in a sealed microwave vial at 150 °C for 2 h 30. After cooling to room temperature the reaction mixture was dissolved in EtOAc/MeOH 9:1 and filtered through a silica plug. The filtrate was concentrated *in vacuo* and the residue was purified by and automatic column chromatography (C18, CH_3CN in H_2O, 30% → 100%) to yield 2-(4-Bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylethan-1-amine (**5a**) (66 mg, 24%) as a white oil.

2-(5-Bromo-1H-indol-3-yl)-*N*,*N*-dimethylethan-1-amine



The compound was prepared according to the reported method.[3]

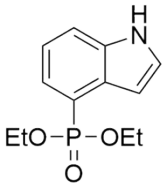
N-(2-(4-Bromo-1*H*-indol-3-yl)ethyl)-*N*-methylpropan-2-amine (5b)



^{1}H-NMR (400 MHz, MeOD): δ = 0.99 (6*H*, *d*, J_{H-H} = 6.6 Hz, 2 x CH-C$\underline{H}_3$); 2.26 (3*H*, *s*, C$\underline{H}_3$-N); 2.64-2.69 (*2H*, *m*, CH_2-C$\underline{H}_2$-N); 2.81 (1*H*, *septet*, J_{H-H} = 6.6 Hz, C$\underline{H}$-CH_3); 3.08-3.12 (2*H*, *m*, C$\underline{H}_2$-CH_2-N); 6.89 (1*H*, *t*, J_{H-H} = 7.8 Hz, CH-C$\underline{H}$-CH); 7.09 (1*H*, *s*, C$\underline{H}$-NH); 7.12 (1*H*, *d x d*, J_{H-H} = 7.8, 0.7 Hz, Br-C_q-C$\underline{H}$-CH); 7.29 (1*H*, *d x d*, J_{H-H}= 7.8, 0.7 Hz, C$\underline{H}$-C_q-NH). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 15.4 (2*C*, 2 x CH-$\underline{C}H_3$); 22.7 (1*C*, $\underline{C}H_2$-CH_2-N); 36.6 (1*C*, $\underline{C}H_3$-N); 55.6 (1*C*, CH_2-$\underline{C}H_2$-N); 58.8 (1*C*, $\underline{C}H$-CH_3); 110.4 (1*C*, CH_2-$\underline{C}_q$-CH); 112.4 (1*C*, $\underline{C}H$-C_q-NH); 114.0 (1*C*, $\underline{C}_q$-Br); 123.6 (1*C*, CH-$\underline{C}H$-CH); 124.3 (1*C*, Br-C_q-$\underline{C}H$-CH); 125.9 (1*C*, $\underline{C}_q$-C_q-Br); 127.2 (1*C*, $\underline{C}H$-NH); 139.5 (1*C*, CH-$\underline{C}_q$-NH). **IR (cm^{-1})**: 3157, 2978, 2646, 1477, 1190, 743. **MS** (70 eV): *m*/Z % 295.1 ([*M*+H]$^+$, 50.0); 297.1 ([*M*+H]$^+$, 50.0). **HRMS** (ESI): *m*/Z calcd for $C_{14}H_{20}BrN_2$+: 295.08044 , 297.07839 [*M*+H]$^+$; found: 295.08063, 297.07818.

To a stirred solution of 2-(4-bromo-1*H*-indol-3-yl)ethan-1-amine (**4**) (0.65 g, 2.72 mmol, 1 eq) in MeOH (56.4 mL), acetic acid (0.63 mL, 11.00 mmol, 4.0 eq) was added followed by sodium cyanoborohydride (0.35 g, 5.55 mmol, 2.0 eq) under nitrogen atmosphere at 0 °C. A solution of formaldehyde (0.49 mL of 37% aqueous solution, 6.58 mmol, 2.4 eq) in MeOH (18.60 mL) was then added dropwise over 20 min, and the resulting solution was stirred at room temperature for 4 h. The reaction was quenched by slowly adding aqueous Na_2CO_3 (2 N) until pH 8–9 was obtained and the solvents were removed *in vacuo*. The residue was partitioned between chloroform and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Next, the product was purified with a normal-phase chromatography with acetone (no gradient) as mobile phase and *N*-(2-(4-bromo-1*H*-indol-3-yl)ethyl)-*N*-methylpropan-2-amine (**5b**) was obtained as a white oil (0.113 g, 17%). Note that this product is considered an unwanted side product that is solely formed in the presence of acetone.

Diethyl (1*H*-indol-4-yl)phosphonate (8)



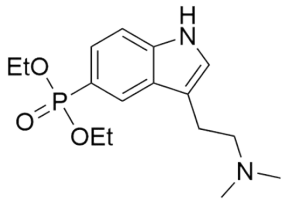
^{1}H-NMR (400MHz, $CDCl_3$): δ = 1.30 (6*H*, *t*, J_{H-H} = 7.1 Hz, 2 x C$\underline{H}_3$-CH_2-O); 4.04−4.20 (4*H*, *m*, 2 x CH_3-C$\underline{H}_2$-O); 6.88 (1*H*, *br. s*, NH-CH=C$\underline{H}$); 7.23 (1*H*, *t x d*, J_{H-H} = 7.7 Hz, J_{H-P} = 3.9 Hz, CH-C$\underline{H}$-CH); 7.32 (1*H*, *t*, J_{H-H} = 2.8 Hz, NH-C$\underline{H}$); 7.60 (1*H*, *d*, J_{H-H} = 8.1 Hz, C$\underline{H}$-C_q-NH); 7.70 (1*H*, *d x d*, J_{H-H} = 7.3 Hz, J_{H-P} = 14.9 Hz, P-C_q-C$\underline{H}$); 9.19 (1*H*, *s*, N$\underline{H}$). **^{13}C-NMR** (100MHz, $CDCl_3$): δ = 16.3 (2*C*, J_{C-P} = 6.6 Hz, 2 x $\underline{C}H_3$-CH_2-O); 62.0 (2*C*, J_{C-P} = 4.4 Hz, 2 x CH_3-$\underline{C}H_2$-O); 102.1 (1*C*, J_{C-P} = 1.9 Hz, NH-CH=$\underline{C}$H); 116.5 (1*C*, J_{C-P} = 3.0 Hz, $\underline{C}$H-C_q-NH); 117.1 (1*C*, J_{C-P} = 187.5 Hz, P-$\underline{C}_q$-CH); 120.5 (1*C*, J_{C-P} = 16.1 Hz, CH-$\underline{C}$H-CH); 125.5 (1*C*, J_{C-P} = 9.5 Hz, P-C_q-$\underline{C}$H); 126.8 (1C, $\underline{C}$H-NH); 128.5 (1*C*, J_{C-P} = 11.7 Hz, P-C_q-$\underline{C}_q$); 136.2 (1*C*, J_{C-P} = 16.9 Hz, $\underline{C}_q$-NH). **^{31}P-NMR** (162MHz, $CDCl_3$): δ = 20.2 (*s*). **IR (cm^{-1})**: 3186, 1227, 972, 766, 546. **MS** (70 eV): *m*/Z % 254.1 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/z calcd for $C_{12}H_{17}NO_3P$+: 254.09406 [*M*+H]$^+$; found: 254.09403.

In a flask, 1.5 equivalents (0.2 ml, 1.5 mmol) diethyl phosphite were added to 4-bromoindole (**1**) (200 mg, 1 mmol), together with 3 equivalents K_2CO_3 (0.414 g, 3 mmol) and 2 mol% Pd[(IPr)(Cin)(Cl)] (13 mg, 0.02 mmol). In advance, diethyl phosphite was bubbled through with nitrogen gas to avoid the presence of oxygen. Next, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas. The mixture was heated to 120 °C for 7 h. After cooling down to room temperature, the mixture was filtered through silica and celite and rinsed with ethyl acetate. Distilled water was added to the mixture and this was washed 3 times with ethyl acetate. The combined organic layers are washed one last time with distilled water. The organic phase is dried over $MgSO_4$ and the solvent was evaporated. The residue is coated on silica gel and flash chromatography (silica, 5 CV: 15%, increased to 50% over 10 CV, 10 CV: 50%, increased to 100% over 10 CV, 10 CV: 100% ethyl acetate in hexane) was performed to give pure diethyl(1*H*-indol-4-yl)phosphonate (**8**) as a white crystal (yield: 38%; Rf (80/20 ethyl acetate/hexane): 0.23, melting point: 108.3 °C).

Method II:

In a flask, 4-bromo indole (**1**) (0.24 mL, 360 mg, 1.84 mmol, 1 eq), DPPF (102 mg, 0.18 mmol, 0.1 eq), $Pd(OAc)_2$ (41 mg, 0.18 mmol, 0.1 eq) and K_2CO_3 (508 mg, 3.67 mmol, 2 eq) were added. Next, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas while adding dry toluene (18.36 mL). Meanwhile, the bottle of diethyl phosphite was flushed with nitrogen and 0.47 mL (507 mg, 3.67 mmol, 2 eq) was added to the sealed flask. The flushing of the flask was continued for another five minutes after which the whole was sealed and allowed to stirr for 14 h 30 at 90 °C. When the reaction was finished, the solvents were removed under reduced pressure using a rotary evaporator. The residue is coated on silica gel and flash chromatography (silica, 5 CV: 15%, increased to 50% over 10 CV, 10 CV: 50%, increased to 100% over 10 CV, 10 CV: 100% ethyl acetate in hexane) was performed to give pure diethyl(1*H*-indol-4-yl)phosphonate (**8**) as a white crystal (407 mg, 68%; Rf (80/20 ethyl acetate/hexane): 0.23, melting point: 108.3 °C)

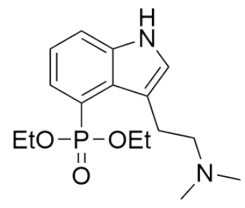
Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)phosphonate (9)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.31 (6*H*, *t*, J_{H-H} = 7.1 Hz, 2 x C<u>H</u>$_3$-CH$_2$-O); 2.32 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 2.63 (2*H*, *t*, J_{H-H} = 7.9 Hz, CH$_2$-C*H*$_2$-N); 2.94 (2*H*, *t*, J_{H-H} = 7.9 Hz, C<u>H</u>$_2$-CH$_2$-N); 4.00-4.18 (4*H*, *m*, 2 x CH$_3$-C<u>H</u>$_2$-O); 7.08 (1*H*, *br. s*, C<u>H</u>-NH); 7.39 (1*H*, *d x d*, J_{H-H} = 8.4 Hz, J_{H-P} = 3.3 Hz, C<u>H</u>-C$_q$-NH); 7.52 (1*H*, *d x d x d*, J_{H-H} = 8.4, 1.2 Hz, J_{H-P} = 12.0 Hz, P-C$_q$-C<u>H</u>-CH); 8.14 (1*H*, *d*, J_{H-P} = 14.5 Hz, P-C$_q$-C<u>H</u>-C$_q$). **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 16.4 (2*C*, J_{C-P} = 6.6 Hz, 2 x <u>C</u>H$_3$-CH$_2$-O); 23.4 (1*C*, <u>C</u>H$_2$-CH$_2$-N); 45.5 (2*C*, 2 x <u>C</u>H$_3$-N); 60.1 (1*C*, CH$_2$-<u>C</u>H$_2$-N); 61.8 (2*C*, J_{C-P} = 5.1 Hz, 2 x CH$_3$-<u>C</u>H$_2$-O); 111.7 (1*C*, J_{C-P} = 16.9 Hz, <u>C</u>H-C$_q$-NH); 115.2 (1*C*, <u>C</u>$_q$-CH$_2$); 117.4 (1*C*, J_{C-P} = 190.7 Hz, P-<u>C</u>$_q$-CH); 123.2 (1*C*, <u>C</u>H-NH); 124.4 (1*C*, J_{C-P} = 8.4 Hz, P-C$_q$-<u>C</u>H-CH); 124.5 (1*C*, J_{C-P} = 8.5 Hz, P-C$_q$-<u>C</u>H-C$_q$); 127.3 (1*C*, J_{C-P} = 17.6 Hz, <u>C</u>$_q$-CH-C$_q$-P); 138.5 (1*C*, J_{C-P} = 2.2 Hz, <u>C</u>$_q$-NH) **^{31}P-NMR** (162 MHz, $CDCl_3$): δ = 23.1 (*s*). **IR (cm^{-1})**: 3174, 2768, 1439, 1355, 1116, 1018, 959, 797. **MS** (70 eV): *m*/Z % 325.2 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{16}H_{26}N_2O_3P$+: 325.16756 [*M*+H]$^+$; found: 325.16731.

In a flask, 2-(5-bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylethan-1-amine (766 mg, 2.87 mmol, 1 eq), DPPF (159 mg, 0.29 mmol, 0.1 eq), $Pd(OAc)_2$ (64 mg, 0.29 mmol, 0.1 eq) and K_2CO_3 (792 mg, 5.73 mmol, 2 eq) were added. After this, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas while adding dry toluene (28.66 mL). Meanwhile, the bottle of diethyl phosphite was flushed with nitrogen and 0.73 mL (792 mg, 5.73 mmol, 2 eq) was added to the sealed flask. The flushing of the flask was continued for another five minutes after which the whole was sealed and allowed to stir for 16 h at 90 °C. When the reaction was finished, the solvents were removed under reduced pressure using a rotary evaporator. Next, the residue was dissolved in ethyl acetate and filtered through a silica and celite plug. Subsequently, methanol was sent over the plug and collected in a different recipient. Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-5yl)phosphonate (**9**) (727 mg, yield: 95%) was obtained as a brown oil.

Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-4-yl)phosphonate (6)

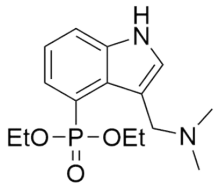


^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.30 (6*H*, *t*, J_{H-H} = 7.1 Hz, 2 x C<u>H</u>$_3$-CH$_2$- O); 2.34 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 2.69 (2*H*, *t*, J_{H-H} = 7.7 Hz, CH$_2$-C<u>H</u>$_2$-N); 3.26 (2*H*, *t*, J_{H-H} = 7.7 Hz, C<u>H</u>$_2$-CH$_2$-N); 4.05-4.20 (4*H*, *m*, 2 x CH$_3$-C<u>H</u>$_2$-O); 7.10 (1*H*, *br. s*, C<u>H</u>-NH); 7.13 (1*H*, *d x d*, J_{H-H} = 7.6 Hz, J_{H-P} = 3.5 Hz, CH-C<u>H</u>-CH); 7.48 (1*H*, ~*d x t*, J_{H-H} = 7.6 Hz, J_{H-P} = 1.3 Hz, C<u>H</u>-C$_q$-NH); 7.76 (1*H*, *d x d x d*, J_{H-H} = 7.6, 0.9 Hz, J_{H-P} = 15.7 Hz, P-C$_q$-C<u>H</u>-CH); 9.82 (1*H*, *s*, NH). **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 16.4 (2*C*, J_{C-P} = 6.6 Hz, 2 x <u>C</u>H$_3$-CH$_2$-O); 23.6 (1*C*, <u>C</u>H$_2$-CH$_2$-N); 45.4 (2*C*, 2 x <u>C</u>H$_3$-N); 60.1 (1*C*, CH$_2$-<u>C</u>H$_2$-N); 62.1 (2*C*, J_{C-P} = 5.9 Hz, 2 x CH$_3$-<u>C</u>H$_2$-O); 114.6 (1*C*, J_{C-P} = 2.2 Hz, CH$_2$-<u>C</u>$_q$-CH); 116.7 (1*C*, J_{C-P} = 2.9 Hz, <u>C</u>H-C$_q$-NH); 117.5 (1*C*,

J_{C-P} = 187.1 Hz, P-$\underline{C}_q$-CH); 120.1 (1*C*, J_{C-P} = 16.1 Hz, CH-$\underline{C}$H-CH); 124.7 (1*C*, $\underline{C}$H-NH); 126.6 (1*C*, J_{C-P} = 12.4 Hz, P-C_q-$\underline{C}_q$); 127.1 (1*C*, J_{C-P} = 8.8 Hz, P-C_q-$\underline{C}$H); 137.1 (1*C*, J_{C-P} = 16.9 Hz, $\underline{C}_q$-NH). **^{31}P-NMR** (162 MHz, $CDCl_3$): δ = 21.2 (*s*). **IR (cm^{-1})**: 3206, 2938, 1227, 1022, 968, 750. **MS** (70 eV): *m*/Z % 325.2 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{16}H_{26}N_2O_3P$+: 325.16756 [*M*+H]$^+$; found: 325.16700.

In a flask, 2-(4-bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylethan-1-amine (**5a**) (408 mg, 1.53 mmol, 1 eq), DPPF (85 mg, 0.15 mmol, 0.1 eq), $Pd(OAc)_2$ (34 mg, 0.15 mmol, 0.1 eq) and K_2CO_3 (422 mg, 3.05 mmol, 2 eq) were added. Next, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas while adding dry toluene (15.27 mL). Meanwhile, the bottle of diethyl phosphite was flushed with nitrogen and 0.39 mL (422 mg, 3.05 mmol, 2 eq) was added to the sealed flask. The flushing of the flask was continued for another five minutes after which the whole was sealed and allowed to stir for 16 h at 90 °C. When the reaction was finished, the solvents were removed under reduced pressure using a rotary evaporator. Subsequently, the residue was dissolved in ethyl acetate and filtered through a silica and celite plug. Hereafter, methanol was sent over the plug and collected in a different recipient. The methanol phase containing the product was subsequently subjected to automatic column chromatography (C18, CH_3CN in H_2O, 20% → 100%). Next, the purity was controlled *via* ^{31}P-NMR. If only one peak was visible, no further purification was needed. If a peak around 4.0-4.5 ppm was still present, the product was dissolved in water, basified with $NaHCO_3$ and extracted with dichloromethane. Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-4-yl)phosphonate (**6**) (129 mg, yield: 26%) was obtained as an oil with a slightly blue shine.

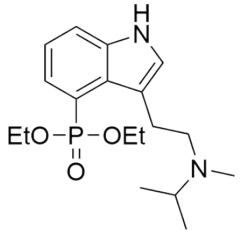
Diethyl (3-((dimethylamino)methyl)-1*H*-indol-4-yl)phosphonate (10)



^{1}H-NMR (400 MHz, MeOD): δ = 1.36 (6*H*, *t*, J_{H-H} = 7.0 Hz, 2 x $C\underline{H}_3$- CH_2-O); 2.85 (6*H*, *s*, 2 x $C\underline{H}_3$-N); 4.09-4.28 (4*H*, *m*, 2 x CH_3-$C\underline{H}_2$-O); 4.57 (2*H*, *s*, $C\underline{H}_2$-N); 7.38 (1*H*, *t x d*, J_{H-H} = 7.8 Hz, J_{H-P} = 3.6 Hz, CH-C$\underline{H}$-CH); 7.64 (1*H*, *d x d x d*, J_{H-H} = 7.8, 0.9 Hz, J_{H-P} = 15.1 Hz, P-C_q-C$\underline{H}$-CH); 7.79 (1*H*, *br. s*, C$\underline{H}$-NH); 7.80-7.82 (1*H*, *m*, C$\underline{H}$-C_q-NH). **^{13}C-NMR** (100.6 MHz, MeOD): δ = 16.5 (2*C*, J_{C-P} = 6.9 Hz, 2 x $\underline{C}H_3$-CH_2-O); 42.2 (2*C*, 2 x $\underline{C}H_3$-N); 55.7 (1*C*, $\underline{C}H_2$-N); 64.6 (2*C*, J_{C-P} = 6.6 Hz, 2 x CH_3-$\underline{C}H_2$-O); 105.1 (1*C*, J_{C-P} = 3.7 Hz, CH_2-$\underline{C}_q$-CH); 117.5 (1*C*, J_{C-P} = 187.6 Hz, P-$\underline{C}_q$-CH); 119.0 (1*C*, J_{C-P} = 3.7 Hz, $\underline{C}$H-C_q-NH); 122.6 (1*C*, J_{C-P} = 15.4 Hz, CH-$\underline{C}$H-CH); 126.7 (1*C*, J_{C-P} = 13.2 Hz, P-C_q-$\underline{C}_q$); 127.4 (1*C*, J_{C-P} = 8.0 Hz, P-C_q-$\underline{C}$H); 133.6 (1*C*, $\underline{C}$H-NH); 139.4 (1*C*, J_{C-P} = 16.9 Hz, $\underline{C}_q$-NH). **^{31}P-NMR** (162 MHz, MeOD): δ = 21.0 (*s*). **IR (cm^{-1})**: 3202, 2862, 1410, 1227, 1022, 968, 787. **MS** (70 eV): *m*/Z % 311.1 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{15}H_{24}N_2O_3P$+: 311.15191 [*M*+H]$^+$; found: 311.15111.

In a flask, 1-(4-bromo-1*H*-indol-3-yl)-*N,N*-dimethylmethanamine (**2**) (1157 mg, 4.57 mmol, 1 eq), DPPF (253 mg, 0.46 mmol, 0.1 eq), $Pd(OAc)_2$ (103 mg, 0.46 mmol, 0.1 eq) and K_2CO_3 (1264 mg, 9.14 mmol, 2 eq) were added. Next, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas while adding dry toluene (45.71 mL). Meanwhile, the bottle of diethyl phosphite was flushed with nitrogen and 1.17 mL (1262 mg, 9.14 mmol, 2 eq) was added to the sealed flask. The flushing of the flask was continued for another five minutes after which the whole was sealed and allowed to stir for 16 h at 90 °C. When the reaction was finished, the solvents were removed under reduced pressure using a rotary evaporator. Subsequently, the residue was dissolved in ethyl acetate and filtered through a silica and celite plug. Hereafter, methanol was sent over the plug and collected in a different recipient. The methanol phase containing the product was subsequently subjected to automatic column chromatography (C18, CH_3CN in H_2O, 20% → 100%). Next, the purity was controlled *via* ^{31}P-NMR. If only one peak was visible, no further purification was needed. If a peak around 4.0-4.5 ppm was still present, the product was dissolved in water, basified with NaOH and extracted with dichloromethane. Diethyl (3((dimethylamino)methyl)-1*H*-indol-4-yl)phosphonate (**10**) (128 mg, yield: 9%) was obtained as an oil with a slightly blue shine.

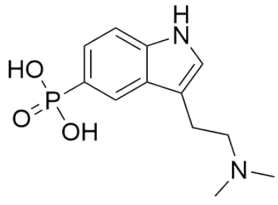
Diethyl (3-(2-(isopropyl(methyl)amino)ethyl)-1*H*-indol-4-yl)phosphonate (11)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.13 (6*H*, *d*, J_{H-H} = 7.1 Hz, 2 x CH-C<u>H</u>$_3$); δ = 1.33 (6*H*, *t*, J_{H-H} = 7.1 Hz, 2 x C<u>H</u>$_3$-CH_2-O); 2.44 (3*H*, *s*, C<u>H</u>$_3$-N); 2.96 (2*H*, *t*, J_{H-H} = 7.7 Hz, CH_2-C<u>H</u>$_2$-N); 3.06-3.17 (1*H*, *m*, C<u>H</u>-CH_3); 3.33 (2*H*, *t*, J_{H-H} = 7.7 Hz, C<u>H</u>$_2$-CH_2-N); 7.18 (1*H*, *t x d*, J_{H-H} = 7.7 Hz, J_{H-P} = 3.6 Hz, CH-C<u>H</u>-CH); 7.24 (1*H*, *s*, C<u>H</u>-NH); 7.59 (1*H*, *d*, J_{H-H} = 7.7 Hz, C<u>H</u>-C_q-NH); 7.74 (1*H*, *d x d*, J_{H-H} = 7.7 Hz, J_{H-P} = 15.7 Hz, P-C_q-C<u>H</u>-CH). **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 16.5 (2*C*, J_{C-P} = 6.6 Hz, 2 x C<u>H</u>$_3$-CH_2-O); 17.8 (2*C*, 2 x CH-C<u>H</u>$_3$); 22.9 (1*C*, C<u>H</u>$_2$-CH2-N); 36.0 (1*C*, C<u>H</u>$_3$-N); 54.2 (1*C*, CH_2-C<u>H</u>$_2$-N); 54.2 (1*C*, C<u>H</u>-CH_3); 62.2 (2*C*, J_{C-P} = 5.6 Hz, 2 x CH_3-C<u>H</u>$_2$-O); 113.8 (1*C*, CH_2-C<u>$_q$</u>-CH); 116.8 (1*C*, J_{C-P} = 2.9 Hz, C<u>H</u>-C_q-NH); 117.4 (1*C*, J_{C-P} = 186.7 Hz, P-C<u>$_q$</u>-CH); 120.2 (1*C*, J_{C-P} = 16.1 Hz, CH-C<u>H</u>-CH); 125.6 (1*C*, C<u>H</u>-NH); 126.6 (1*C*, J_{C-P} = 12.5 Hz, P-C_q-C<u>$_q$</u>); 126.8 (1*C*, J_{C-P} = 9.8 Hz, P-C_q-C<u>H</u>); 137.2 (1*C*, J_{C-P} = 16.9 Hz, C<u>$_q$</u>-NH). **^{31}P-NMR** (162 MHz, $CDCl_3$): δ = 20.7 (*s*). **IR (cm^{-1})**: 3180, 2972, 1572, 1391, 1225, 1024, 970, 786. **MS** (70 eV): *m*/Z % 353.2 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{18}H_{30}N_2O_3P$+: 353.19886 [*M*+H]$^+$; found: 353.19821.

In a flask, a mixture of 2-(4-bromo-1*H*-indol-3-yl)-*N*,*N*-dimethylethan-1-amine (**5b**) and *N*-(2-(4bromo-1*H*-indol-3-yl)ethyl)-*N*-methylpropan-2-amine (682 mg, 2.55 mmol, 1 eq), DPPF (142 mg, 0.26 mmol, 0.1 eq), $Pd(OAc)_2$ (57 mg, 0.26 mmol, 0.1 eq) and K_2CO_3 (706 mg, 5.11 mmol, 2 eq) were added. After this, the flask was sealed with an overfolded septum and parafilm and flushed with nitrogen gas while adding dry toluene (25.53 mL). Meanwhile, the bottle of diethyl phosphite was flushed with nitrogen and 0.65 mL (705 mg, 5.11 mmol, 2 eq) was added to the sealed flask. The flushing of the flask was continued for another five minutes after which the whole was sealed and allowed to stir for 16 h at 90 °C. When the reaction was finished, the solvents were removed under reduced pressure using a rotary evaporator. Next, the residue was dissolved in ethyl acetate and filtered through a silica and celite plug. Subsequently, methanol was sent over the plug and collected in a different recipient. Diethyl (3-(2-(isopropyl(methyl)amino)ethyl)-1*H*-indol-4-yl)phosphonate (**11**) (102 mg, yield: 11%) was obtained after automatic column chromatography on silica with acetone:methanol (9:1) as a brown oil.

(3-(2-(Dimethylamino)ethyl)-1*H*-indol-5-yl)phosphonic acid (12)



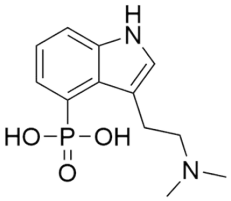
^{1}H-NMR (400 MHz, MeOD): δ = 2.97 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 3.27 (2*H*, *m*, J_{H-H} = 7.9 Hz, C<u>H</u>$_2$-CH_2-N); 3.48 (2*H*, *t*, J_{H-H} = 7.9 Hz, CH_2-C<u>H</u>$_2$-N); 7.35 (1*H*, *br. s*, C<u>H</u>-NH); 7.49 (1*H*, *d x d*, J_{H-H} = 8.3 Hz, J_{H-P} = 3.0 Hz, C<u>H</u>-C_q-NH); 7.59 (1*H*, *d x d*, J_{H-H} = 8.3 Hz, J_{H-P} = 11.7 Hz, P-C_qC<u>H</u>-CH); 8.12 (1*H*, *d*, J_{H-P} = 14.6 Hz, P-C_q-C<u>H</u>-C_q). **^{13}C-NMR** (100.6 MHz, DMSO): δ = 20.1 (1*C*, C<u>H</u>$_2$-CH_2-N); 42.4 (2*C*, 2 x C<u>H</u>$_3$-N); 56.8 (1*C*, CH_2-C<u>H</u>$_2$-N); 109.8 (1*C*, CH_2-C<u>$_q$</u>-CH) ; 111.4 (1*C*, J_{C-P} = 15.6 Hz, C<u>H</u>-C_q-NH); 121.9 (1*C*, J_{C-P} = 3.7 Hz, P-C_q-C<u>H</u>-C_q); 122.9 (1*C*, J_{C-P} = 160.9 Hz, P-C<u>$_q$</u>-CH); 123.9 (1*C*, J_{C-P} = 5.1 Hz, P-C_q-C<u>H</u>-CH); 124.5 (1*C*, C<u>H</u>-NH); 126.9 (1*C*, J_{C-P} = 16.9 Hz, C<u>$_q$</u>-CH-C_q-P); 137.5 (1*C*, J_{C-P} = 2.2 Hz, C<u>$_q$</u>-NH). **^{31}P-NMR** (162 MHz, MeOD): δ = 19.7 (*s*). **IR (cm^{-1})**: 3260, 3189, 2700, 1479, 1118, 980, 744. **MS** (70 eV): *m*/Z % 269.1 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{12}H_{18}N_2O_3P$+: 269.10496 [*M*+H]$^+$; found: 269.10471.

Because of the very low solubility in methanol, a ^{13}C-NMR spectrum was obtained in DMSO with 6144 scans and 2 seconds delay. The ^{1}H-NMR spectrum showed a very low resolution in DMSO and was thus obtained from a very low concentration in MeOD.

Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)phosphonate (**9**) (439 mg, 1.35 mmol, 1 eq) was dissolved in 50 mL dry dichloromethane in a sealed flask. Bromotrimethylsilane (0.45 mL, 518 mg, 3.38 mmol, 2.5 eq) was added *via* a syringe and the mixture was allowed to stir for 20 h. Solvents were

removed under reduced pressure with a rotary evaporator, followed by the immediate addition of methanol. The resulting solution was stirred for one hour. Solvents were removed again under reduced pressure and the residue was dissolved in acidified water (HCl) and extracted with dichloromethane. Water was removed to give (3(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)phosphonic acid (**12**) (296 mg, yield: 82%).

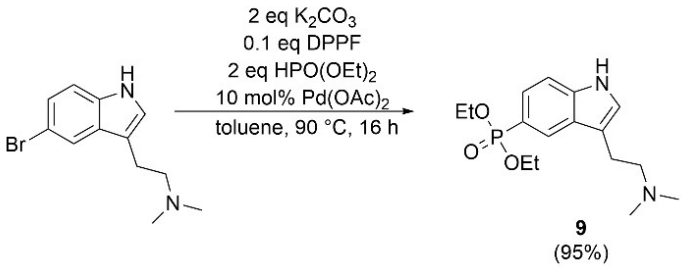
(3-(2-(Dimethylamino)ethyl)-1*H*-indol-4-yl)phosphonic acid (7)



^{1}H-NMR (400 MHz, MeOD): δ = 2.92 (6*H*, *s*, 2 x C<u>H</u>$_3$-N); 3.45 (2*H*, *m*, J_{H-H} = 7.9 Hz, CH_2-C<u>H</u>$_2$-N); 3.57 (2*H*, *t*, J_{H-H} = 7.9 Hz, C<u>H</u>$_2$-CH_2-N); 7.20 (1*H*, *t x d*, J_{H-H} = 7.8 Hz, J_{H-P} = 3.2 Hz, CH-C<u>H</u>-CH); 7.43 (1*H*, *br. s*, C<u>H</u>-NH); 7.63 (1*H*, *br. d*, J_{H-H} = 7.8 Hz, C<u>H</u>-C_q-NH); 7.68 (1*H*, *d x d*, J_{H-H} = 7.8 Hz, J_{H-P} = 15.8 Hz, P-C_q-C<u>H</u>-CH) . **^{13}C-NMR** (100.6 MHz, MeOD): δ = 22.8 (1*C*, <u>C</u>H$_2$-CH_2-N); 43.8 (2*C*, 2 x <u>C</u>H$_3$-N); 60.8 (1*C*, CH_2-<u>C</u>H$_2$-N); 110.9 (1*C*, J_{C-P} = 2.9 Hz, CH_2-<u>C</u>$_q$-CH); 117.3 (1*C*, J_{C-P} = 3.7 Hz, <u>C</u>H-C_q-NH); 122.2 (1*C*, J_{C-P} = 184.8 Hz, P-<u>C</u>$_q$-CH); 121.4 (1*C*, J_{C-P} = 15.4 Hz, CH-<u>C</u>H-CH); 126.9 (1*C*, J_{C-P} = 13.2 Hz, P-C_q-<u>C</u>$_q$); 127.6 (1*C*, <u>C</u>H-NH); 126.1 (1*C*, J_{C-P} = 8.9 Hz, P-C_q-<u>C</u>H); 138.8 (1*C*, J_{C-P} = 16.9 Hz, <u>C</u>$_q$-NH). **^{31}P-NMR** (162 MHz, MeOD): δ = 17.2 (*s*). **IR (cm^{-1})**: 3624, 3356, 2982, 1479, 974. **MS** (70 eV): *m*/Z % 269.1 ([*M*+H]$^+$, 100.0). **HRMS** (ESI): *m*/Z calcd for $C_{12}H_{18}N_2O_3P$+: 269.10496 [*M*+H]$^+$; found: 269.10465.

Diethyl (3-(2-(dimethylamino)ethyl)-1*H*-indol-4-yl)phosphonate (**6**) (86 mg, 0.26 mmol, 1 eq) was dissolved in 6.9 mL dry dichloromethane in a sealed flask. Bromotrimethylsilane (0.09 mL, 101 mg, 0.66 mmol, 2.5 eq) was added *via* a syringe and the mixture was allowed to stir for 20 h. After this, the reaction mixture was heated at reflux and additional bromotrimethylsilane was added until completion (*circa* 30 h, progression followed by LC-MS and ^{31}P-NMR). Solvents were removed under reduced pressure with a rotary evaporator, followed by the immediate addition of methanol. The resulting solution was stirred for one hour. Solvents were removed again and the residue was further purified *via* automatic column chromatography on silica employing an acetone:methanol (9:1, no gradient) eluent to give (3-(2-(dimethylamino)ethyl)-1*H*-indol-4-yl)phosphonic acid (**7**) (16 mg, yield: 22%).

Overview of tested experimental conditions



Entry	Catalyst	Base	Solvent	Conditions	Conversion (%)	Yield compound **9** (%)
1	BF3•Et2O	/	THF	50-55 °C, 6 days	0	/
2	BF3•Et2O	/	[bbim]Br	50-55 °C, 24 h	0	/
3	$NiCl_2$	/	/	160 °C, 54 h	0	/
4	Pd[(IPr)(Cin)(Cl)]	K_2CO_3	DMF	90 °C, 10 days	29	/
5	$Pd(OAc)_2$	K_2CO_3	Toluene	90 °C, 15 h	49	/
6	$Pd(OAc)_2$	K_2CO_3	Toluene	90 °C, 26 h	24	/
7	$Pd(OAc)_2$	Et_3N	THF	Reflux, 8 days	12	/
8	$Pd(OAc)_2$	K_2CO_3	Toluene	90 °C, 16 h	100	95

Table S1. Overview of tested phosphonylation reaction conditions on 5-bromo-N,N-dimethyltryptamine

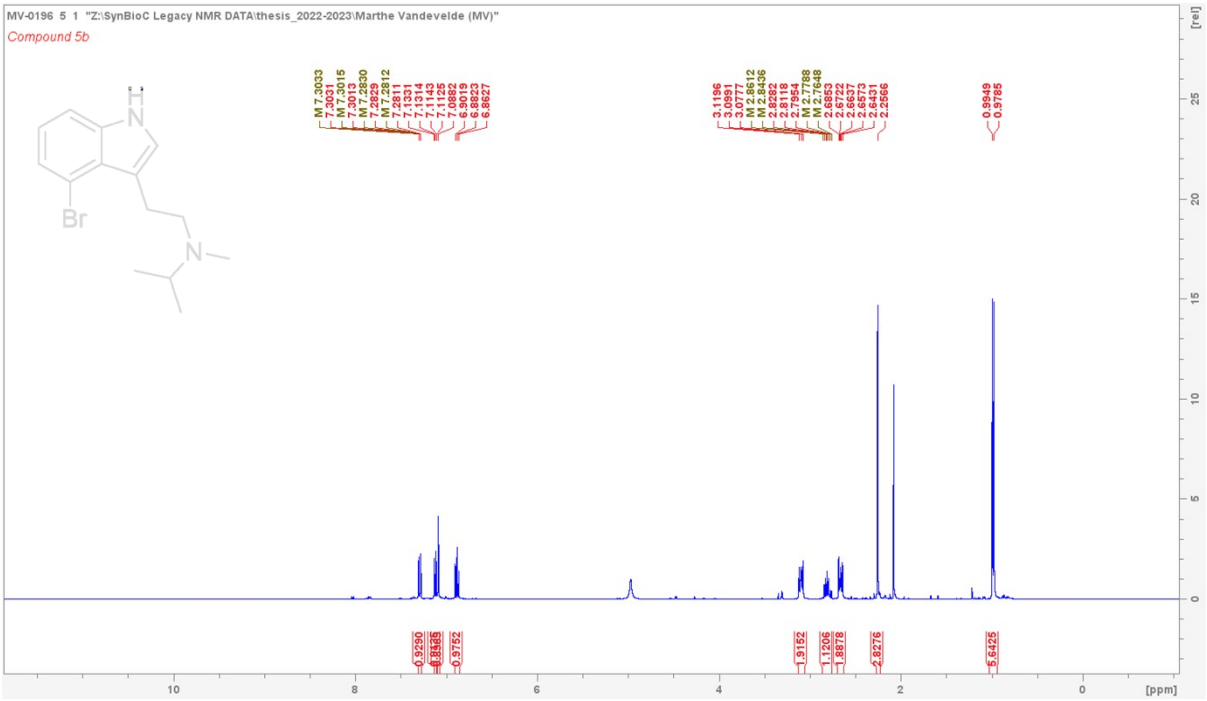
Entry	Catalyst	Base	Solvent	Conditions	Yield compound **8** (%)
1	$Pd(OAc)_2$	K_2CO_3	DMF	100 °C, 8 h	22
2	$Pd(OAc)_2/PPh_3$	K_2CO_3	DMF	100 °C, 8 h	14
3	Pd[(IPr)(Cin)(Cl)]	K_2CO_3	DMF	100 °C, 8 h	32
4	Pd[(IPr)(Cin)(Cl)]	K_2CO_3	DMF	120 °C, 8 h	38
5	$Pd(OAc)_2$	K_2CO_3	Toluene	90 °C, 14 h	68

Table S2. Overview of tested Hirao coupling conditions on 4-bromo indole

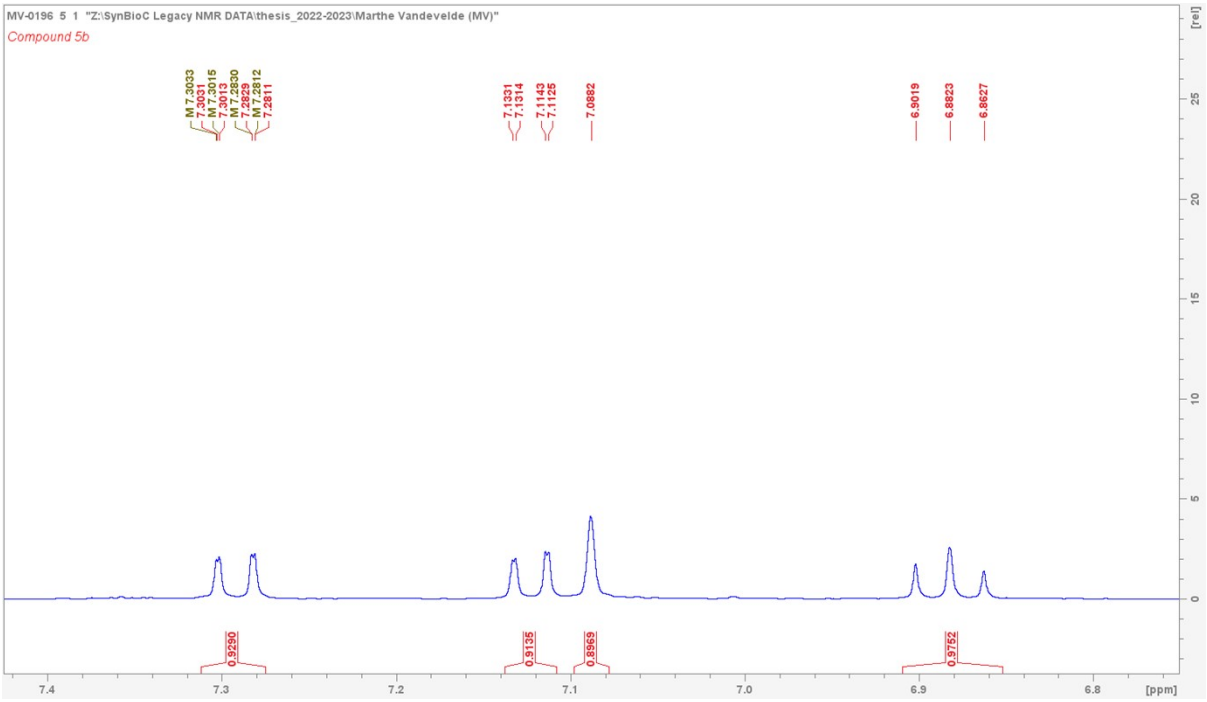
NMR spectra of new compounds

Compound 5b

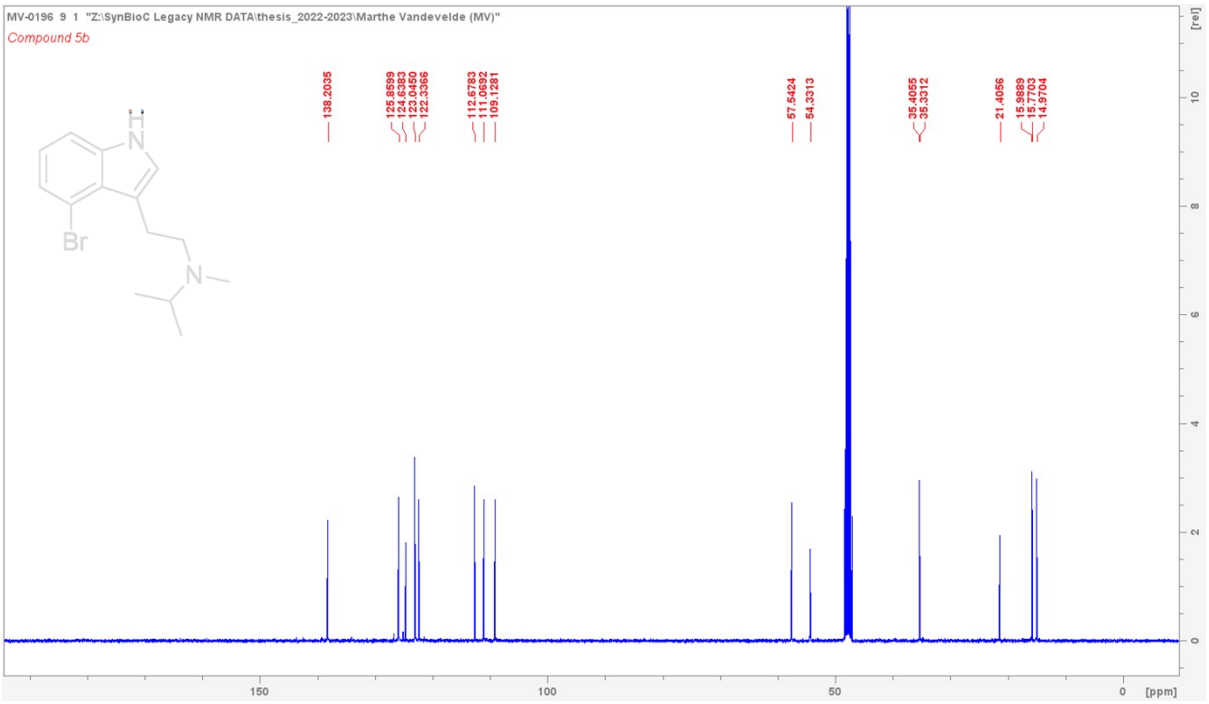
^{1}H-NMR (400 MHz, MeOD)



Zoom of the aromatic region



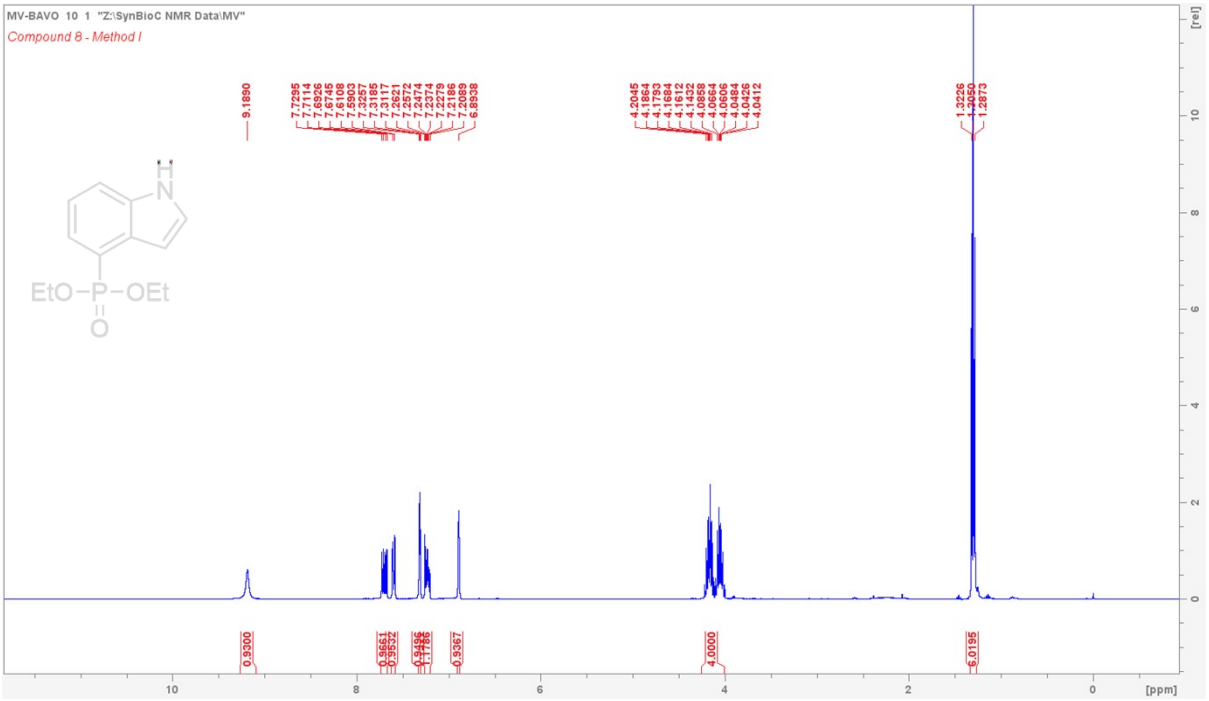
^{13}C-NMR (100.6 MHz, MeOD)



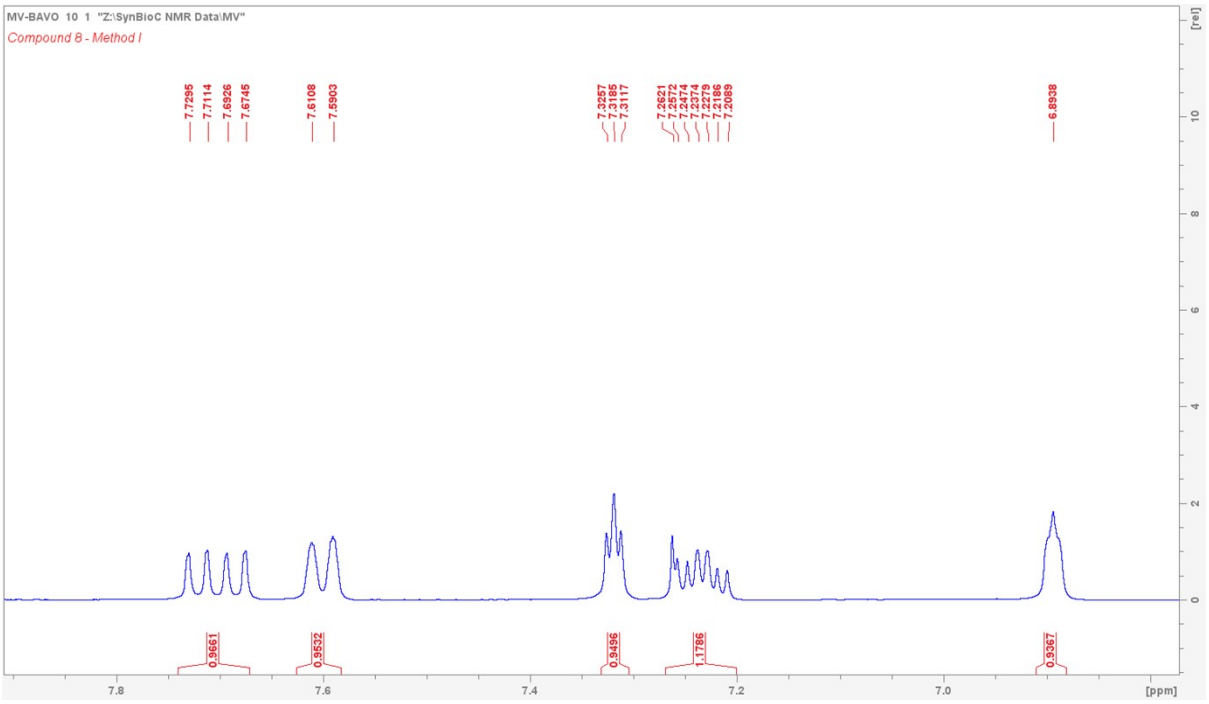
Compound 8

Method I

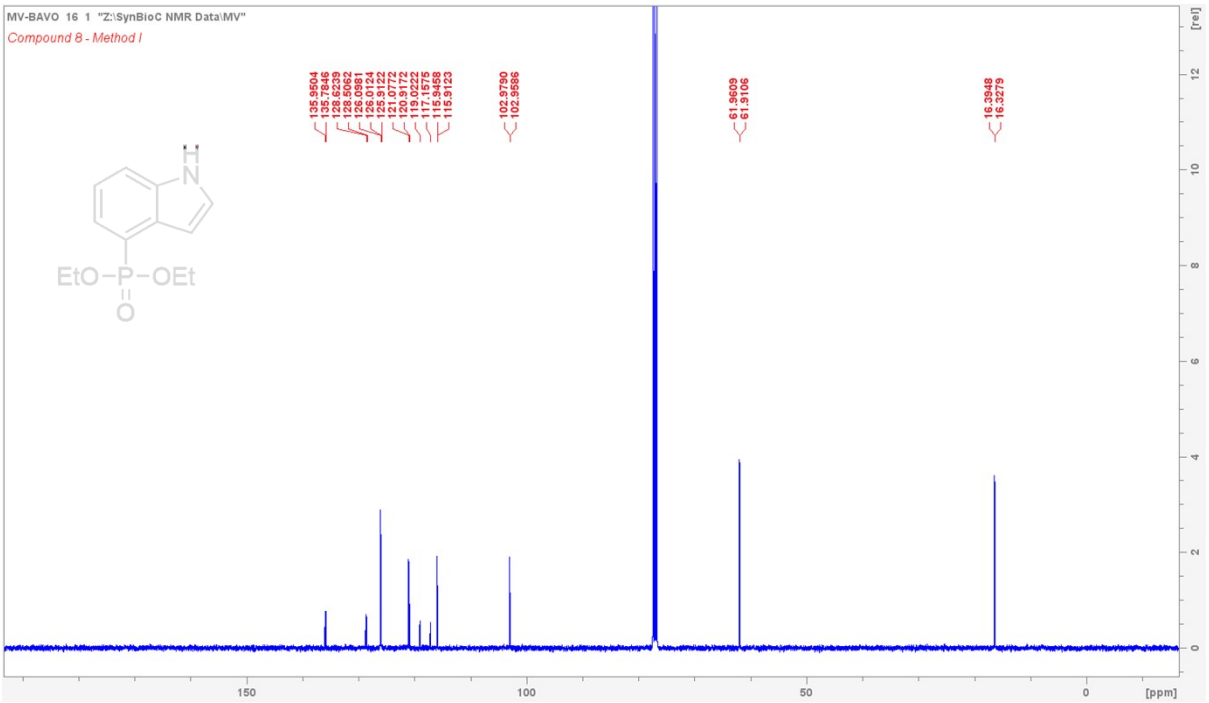
^{1}H-NMR (400 MHz, $CDCl_3$)



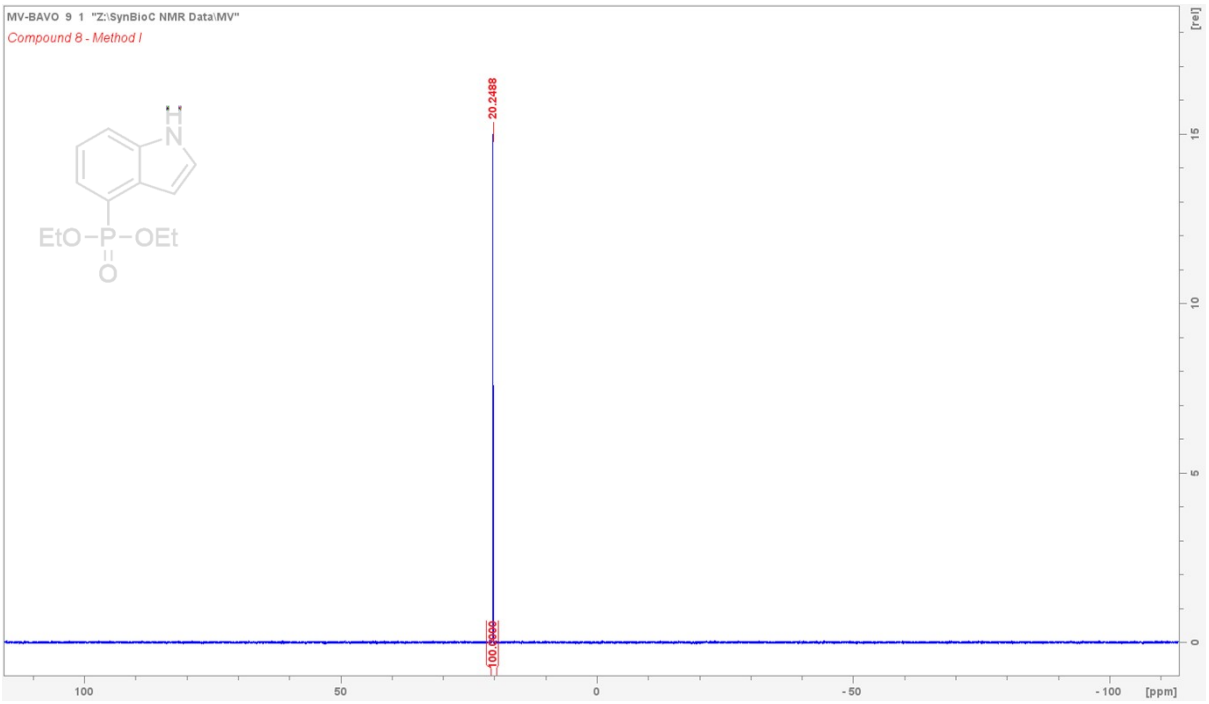
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, $CDCl_3$)

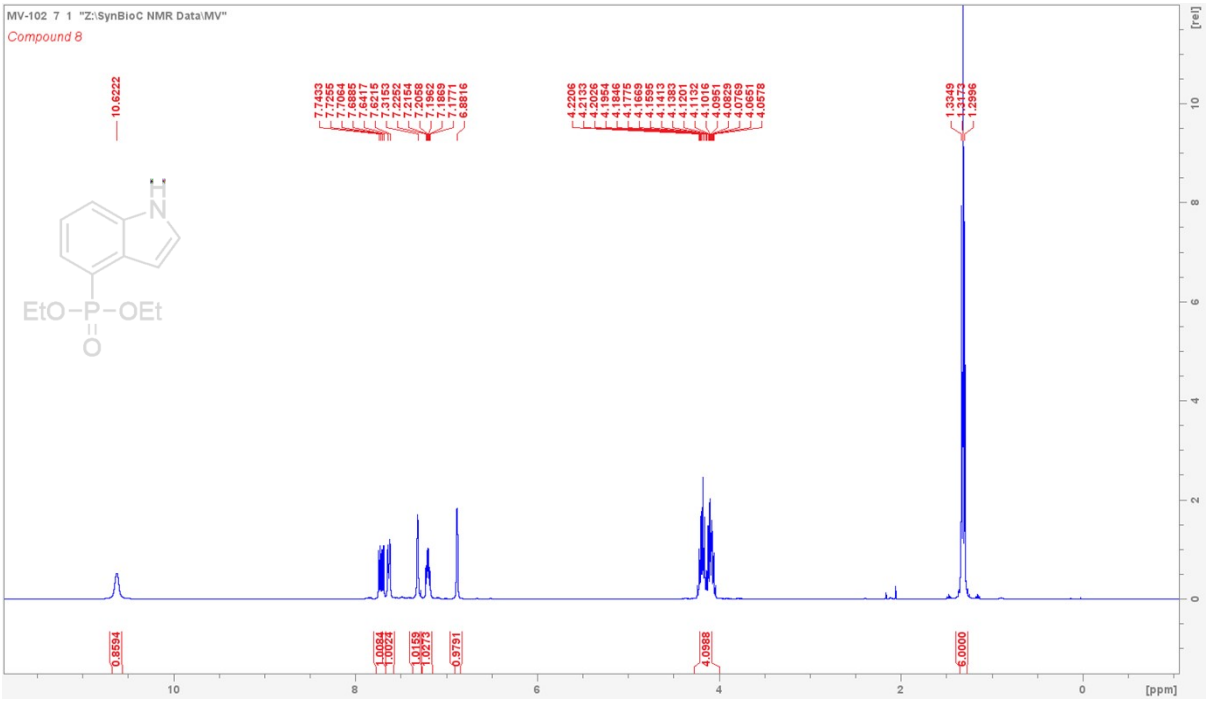


^{31}P-NMR (162MHz,$CDCl_3$)

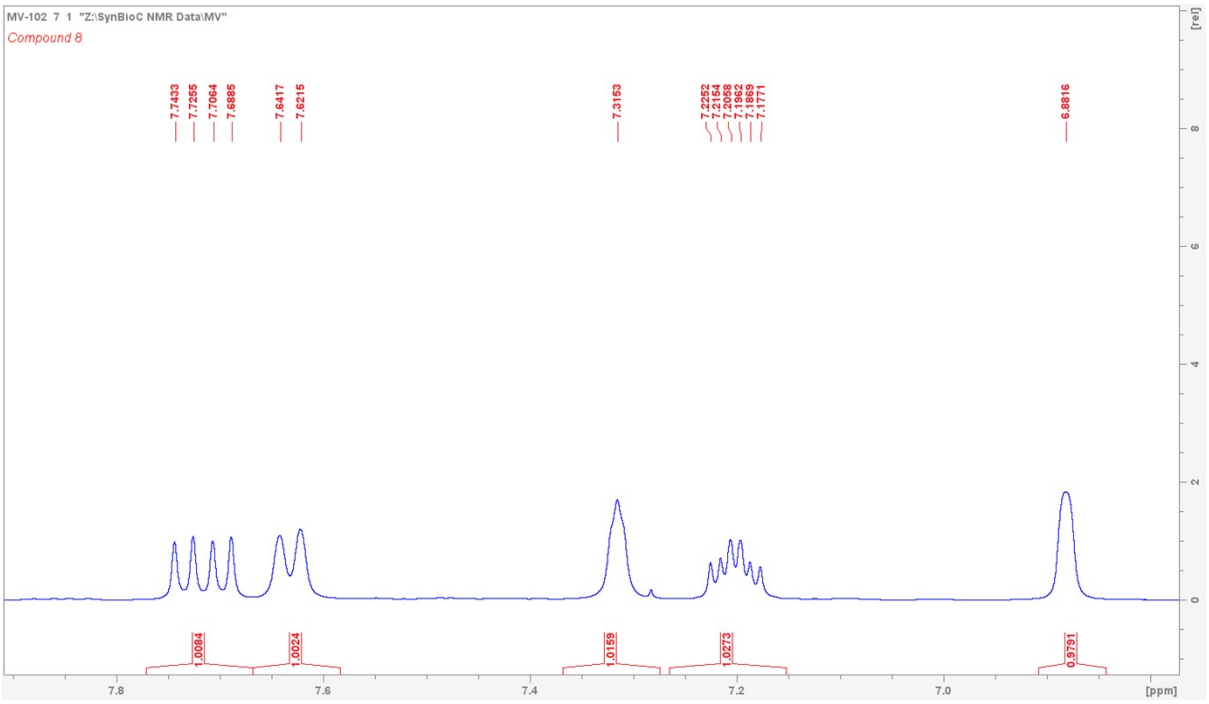


Method II

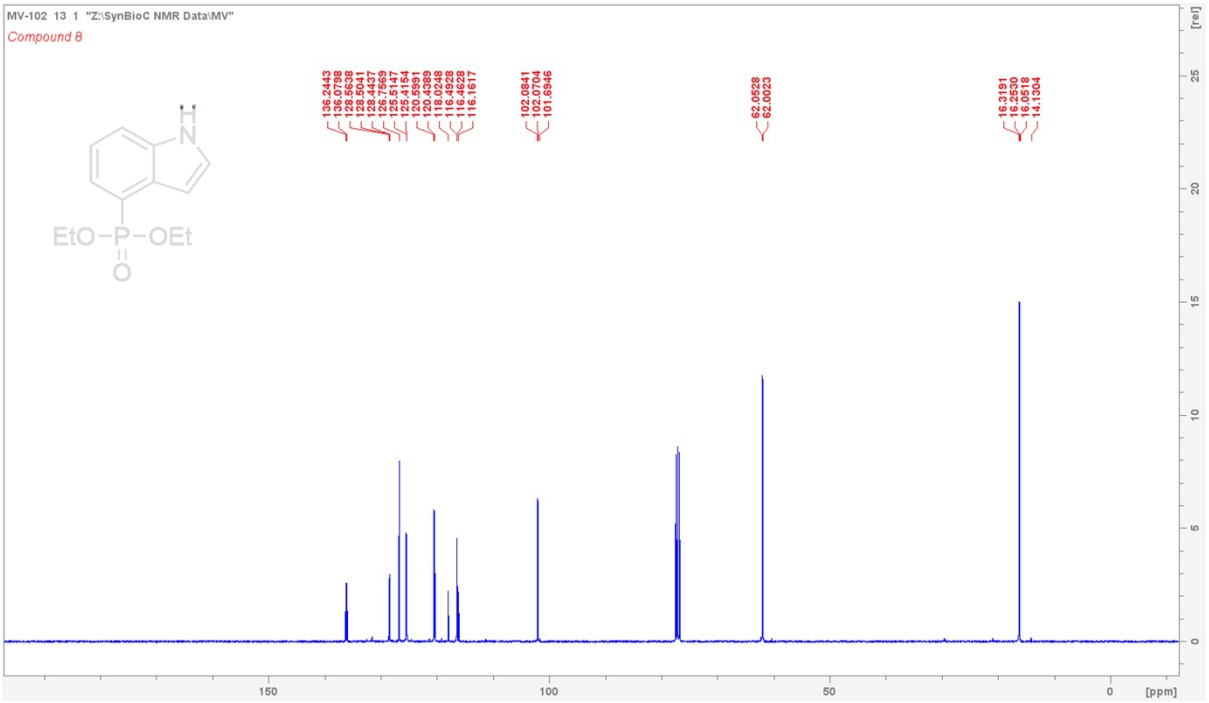
^{1}H-NMR (400 MHz, $CDCl_3$)



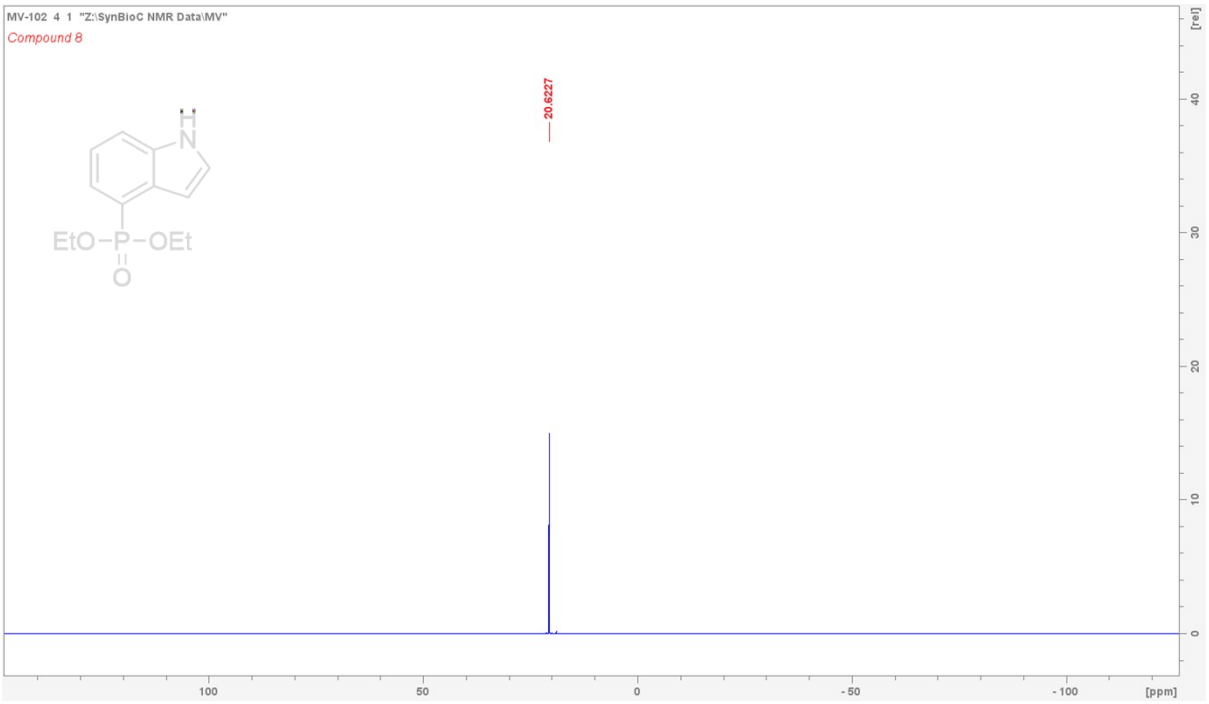
Zoom of aromatic region



^{13}C-NMR (100.6 MHz, $CDCl_3$)

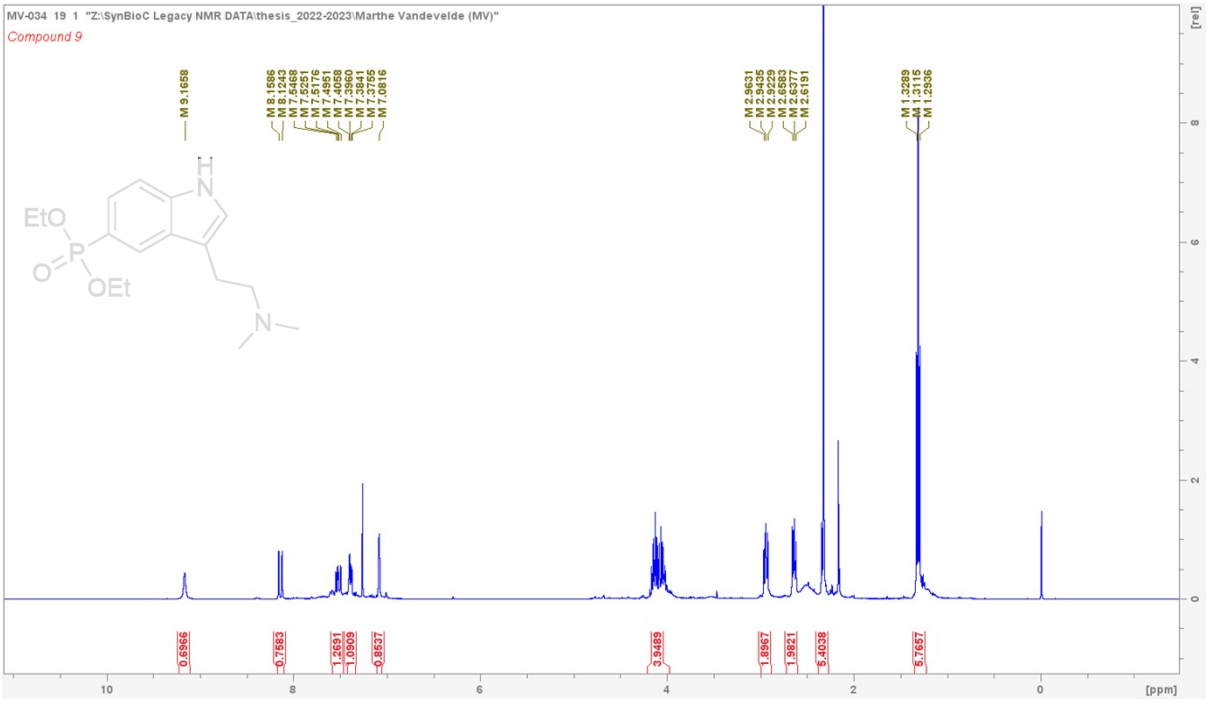


^{31}P-NMR (162MHz,$CDCl_3$)

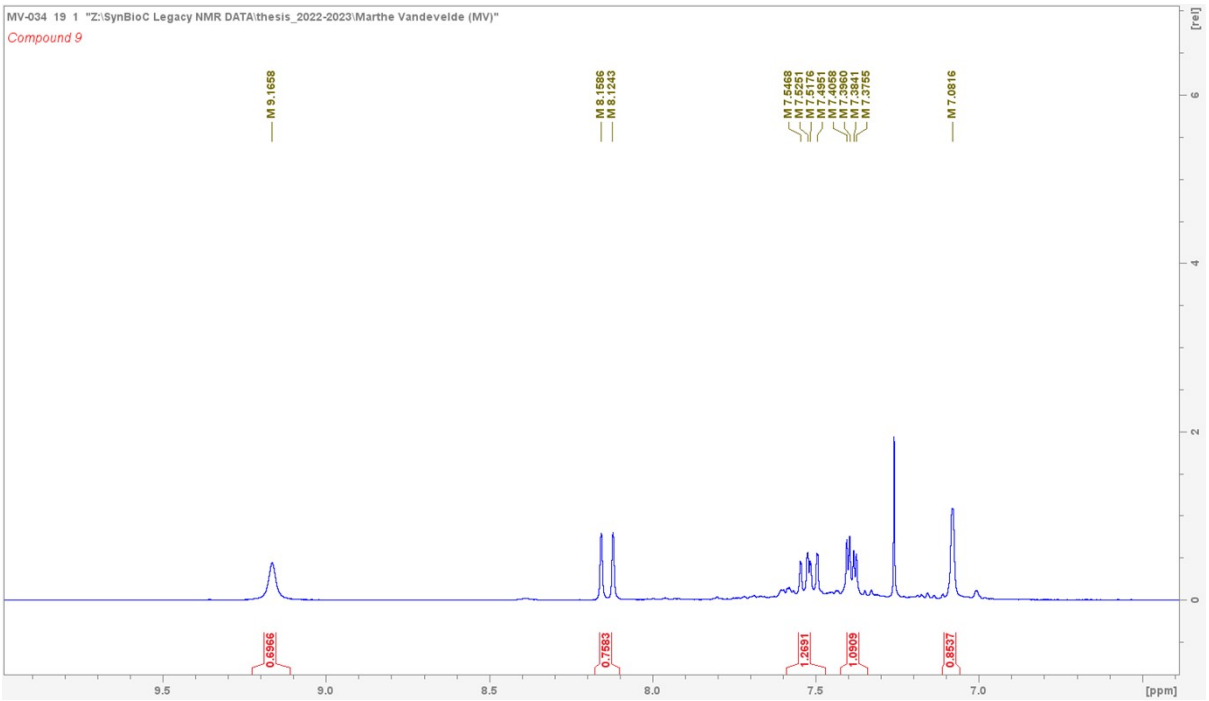


Compound 9

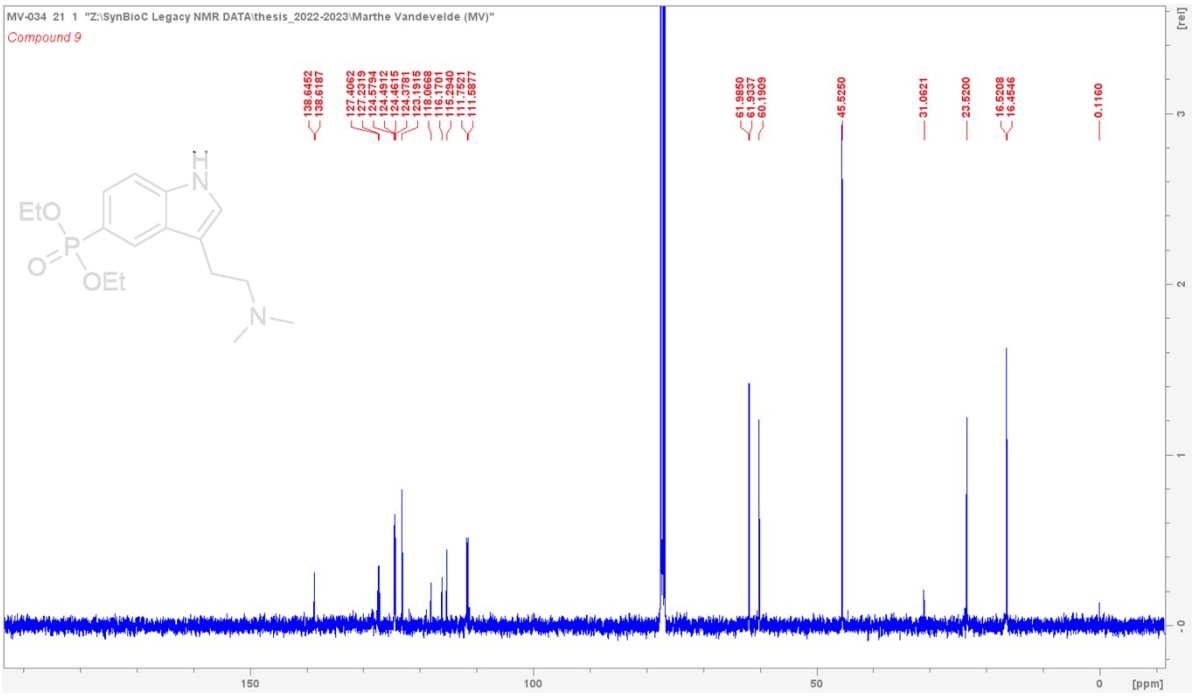
^{1}H-NMR (400 MHz, $CDCl_3$)



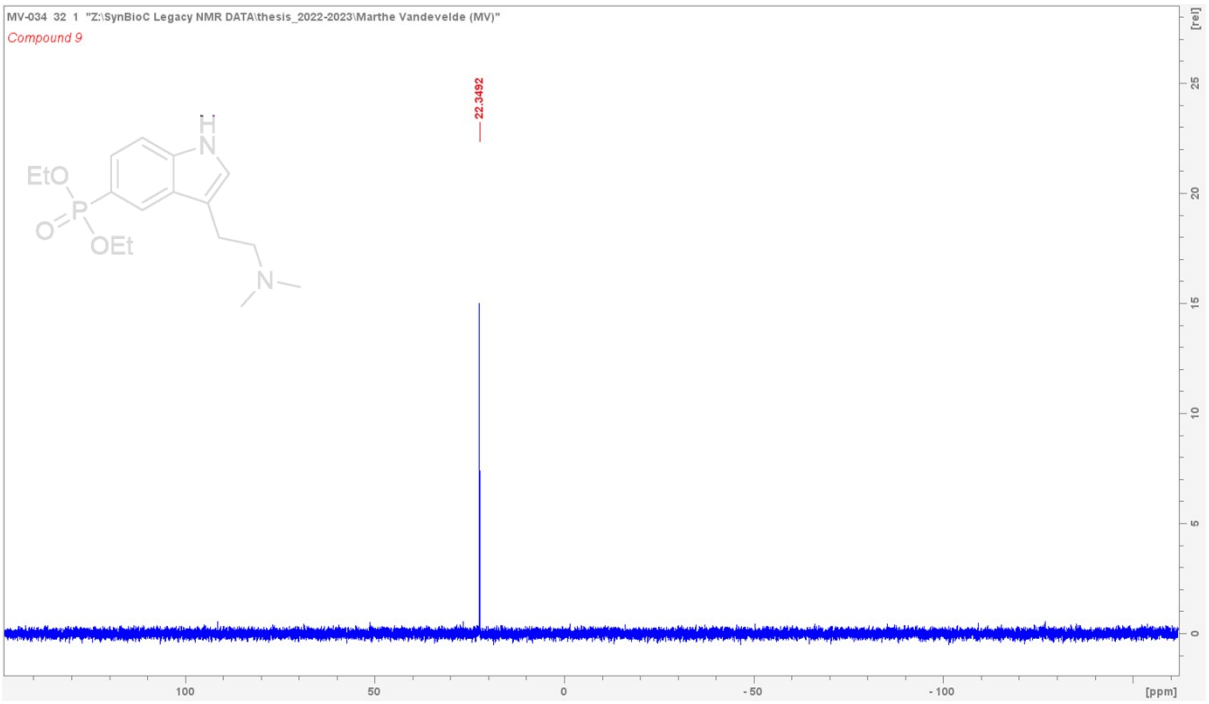
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, $CDCl_3$)

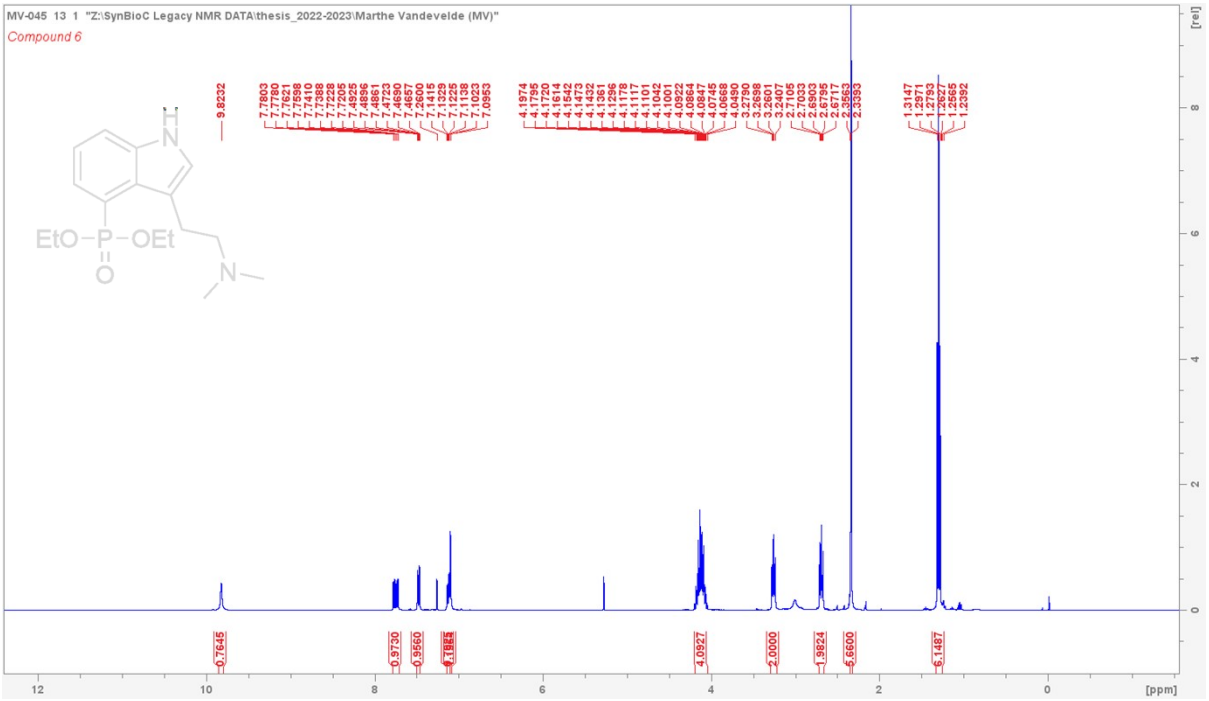


^{31}P-NMR (162MHz,$CDCl_3$)

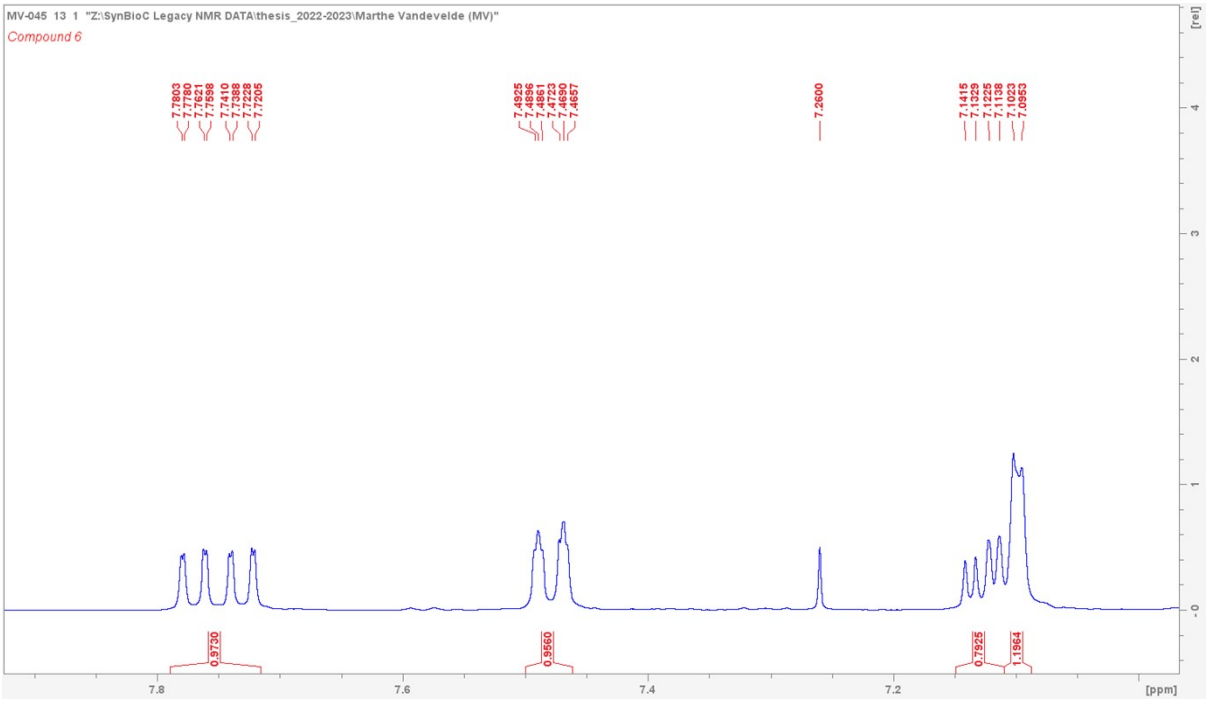

MV-034 32 1 "Z:\SynBioC Legacy NMR DATA\thesis_2022-2023\Marthe Vandevelde (MV)"
Compound 9
22.3492
EtO
O
P
OEt
H
N
N
[rel]
25
20
15
10
5
0
100
50
0
- 50
- 100
[ppm]

Compound 6

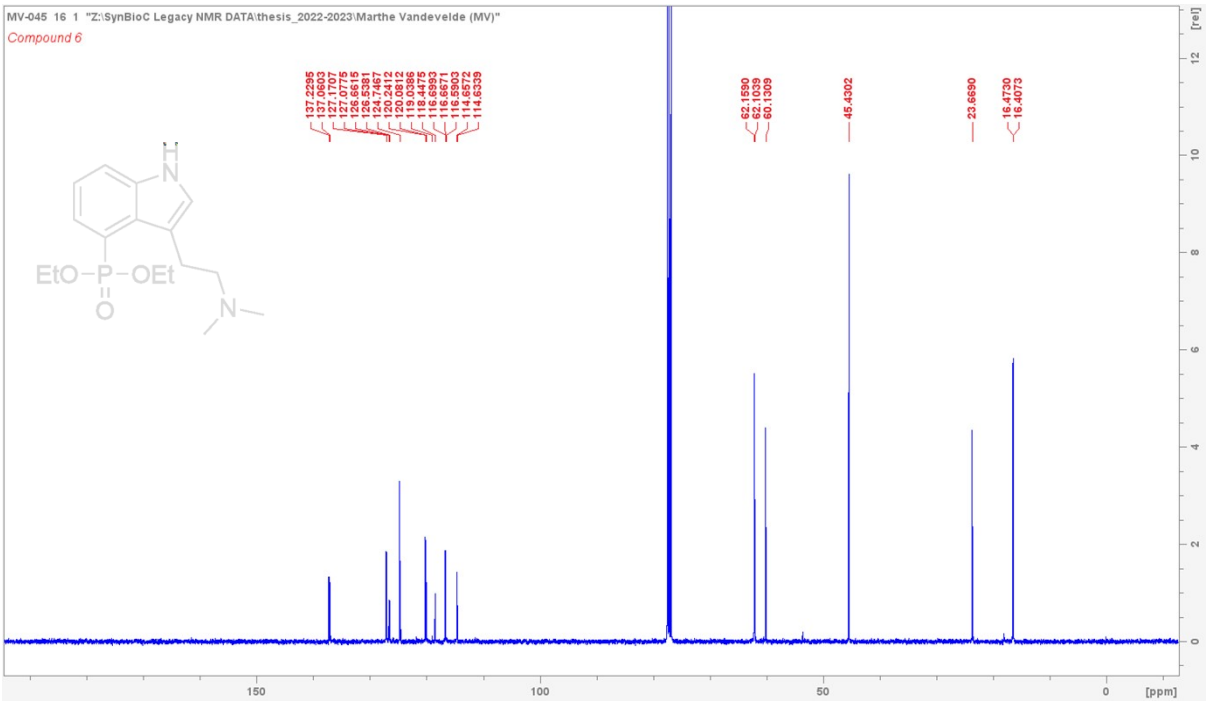
^{1}H-NMR (400 MHz, $CDCl_3$)



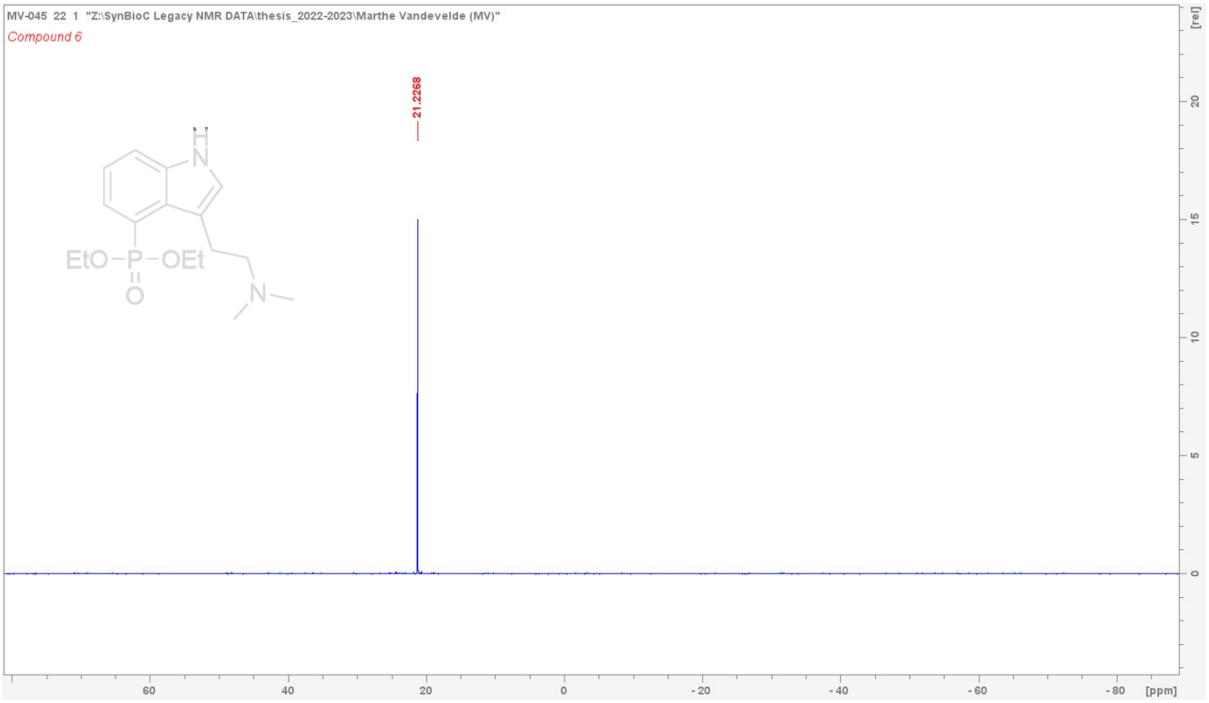
Zoom of aromatic region



^{13}C-NMR (100.6 MHz, $CDCl_3$)

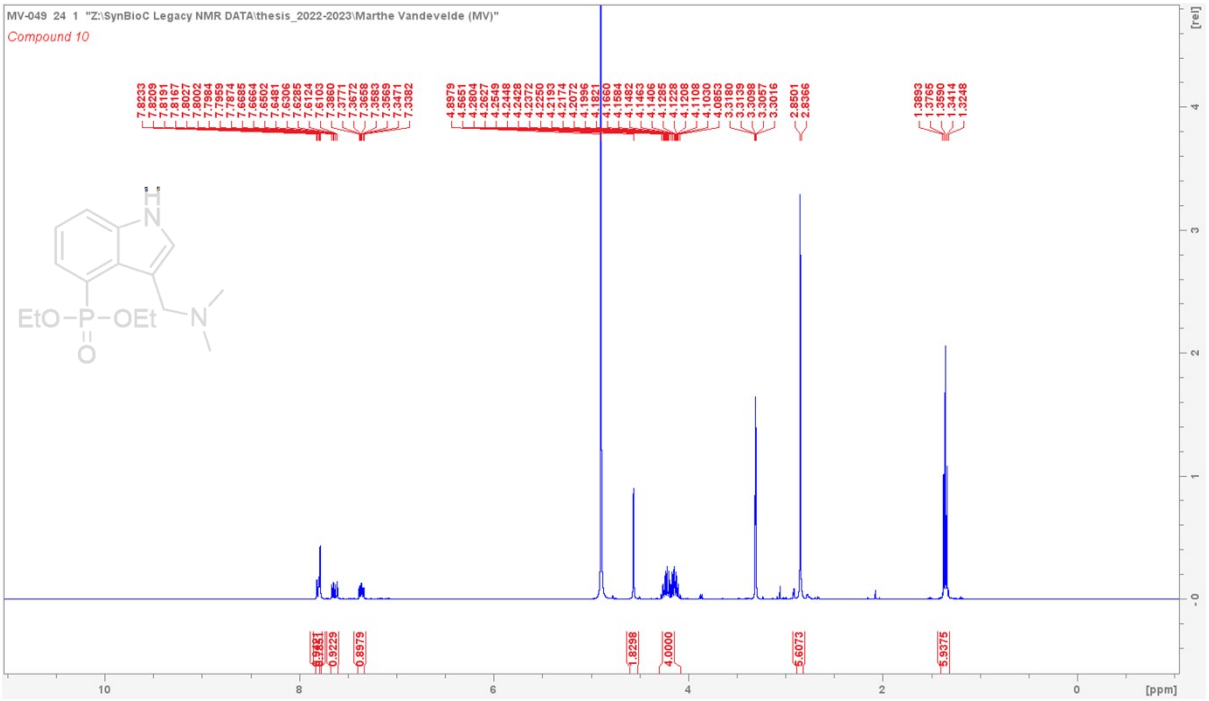


^{31}P-NMR (162MHz,$CDCl_3$)

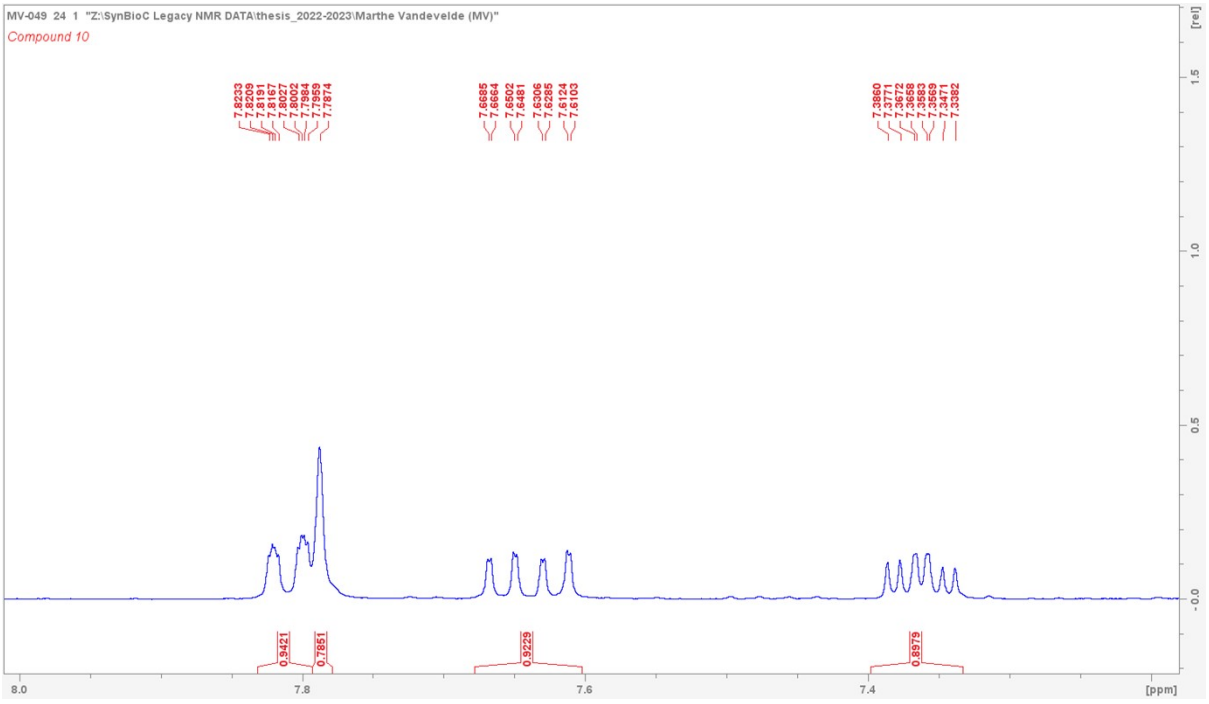


Compound 10

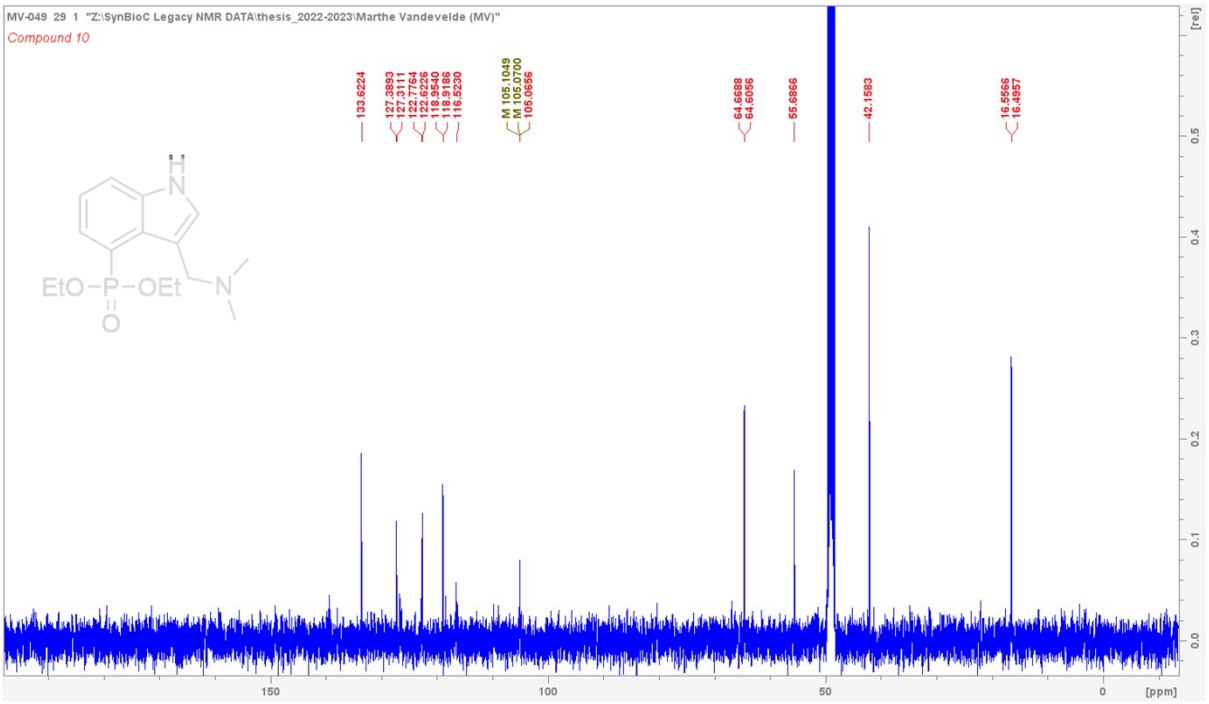
^{1}H-NMR (400 MHz, MeOD)



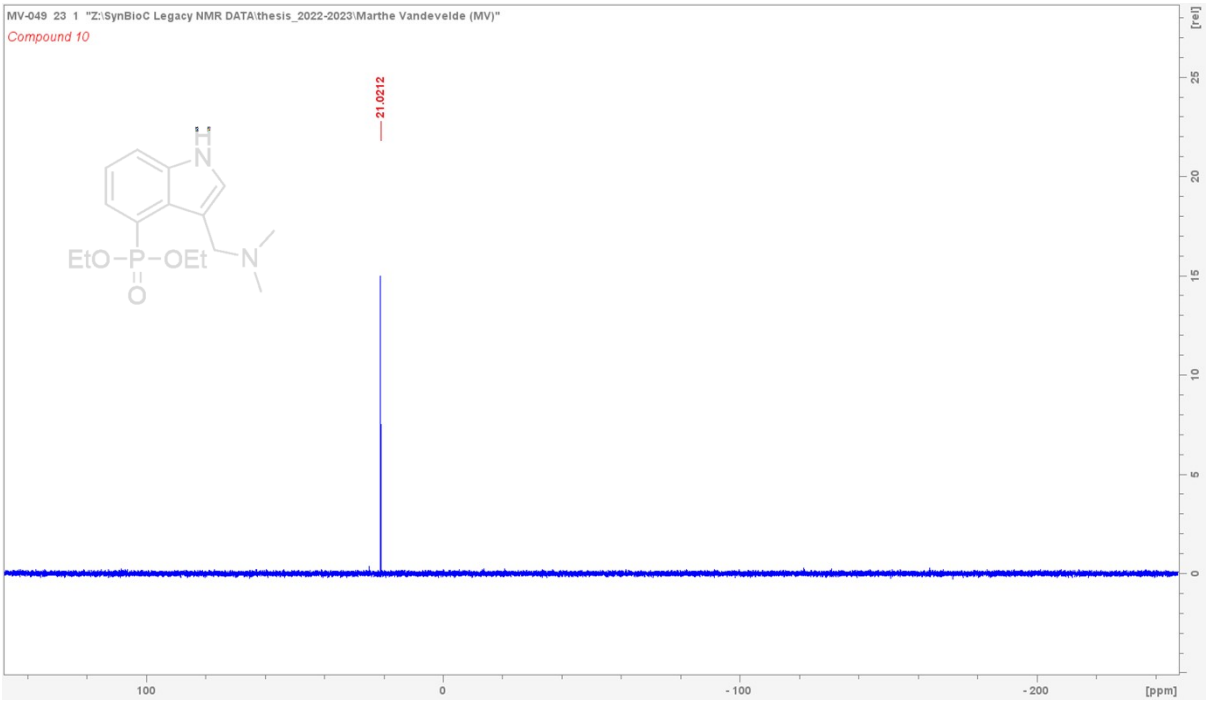
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, MeOD)

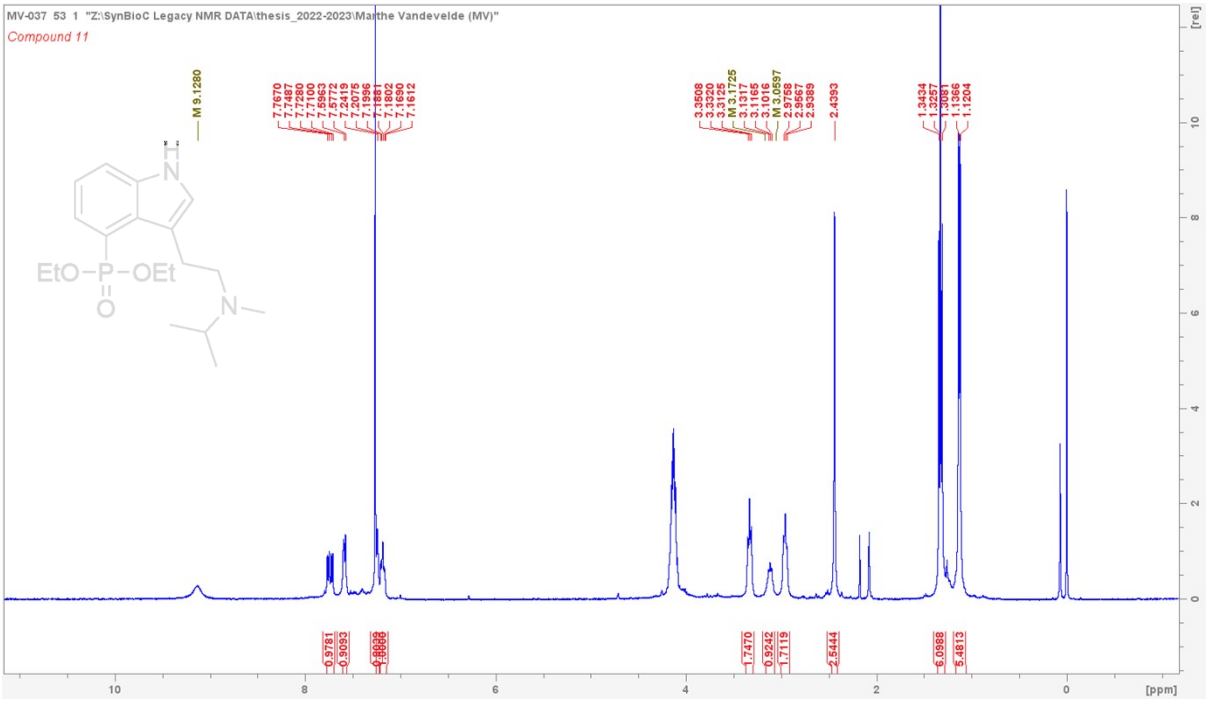


^{31}P-NMR (162MHz,MeOD)

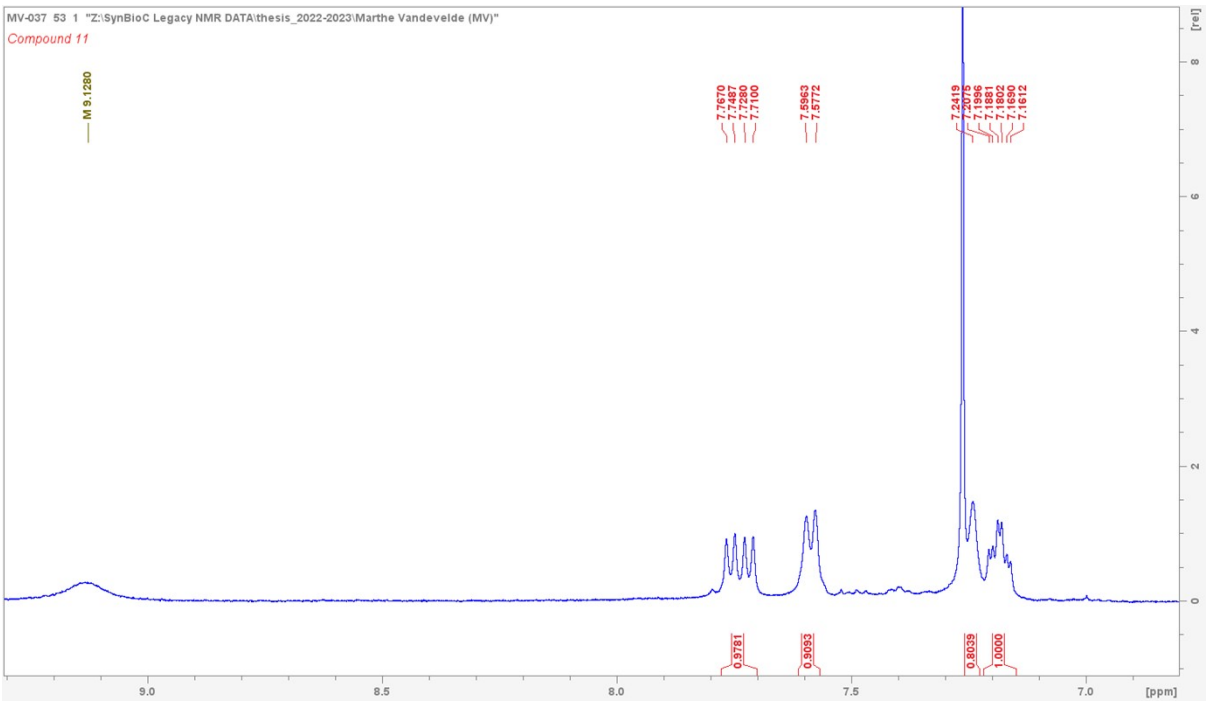


Compound 11

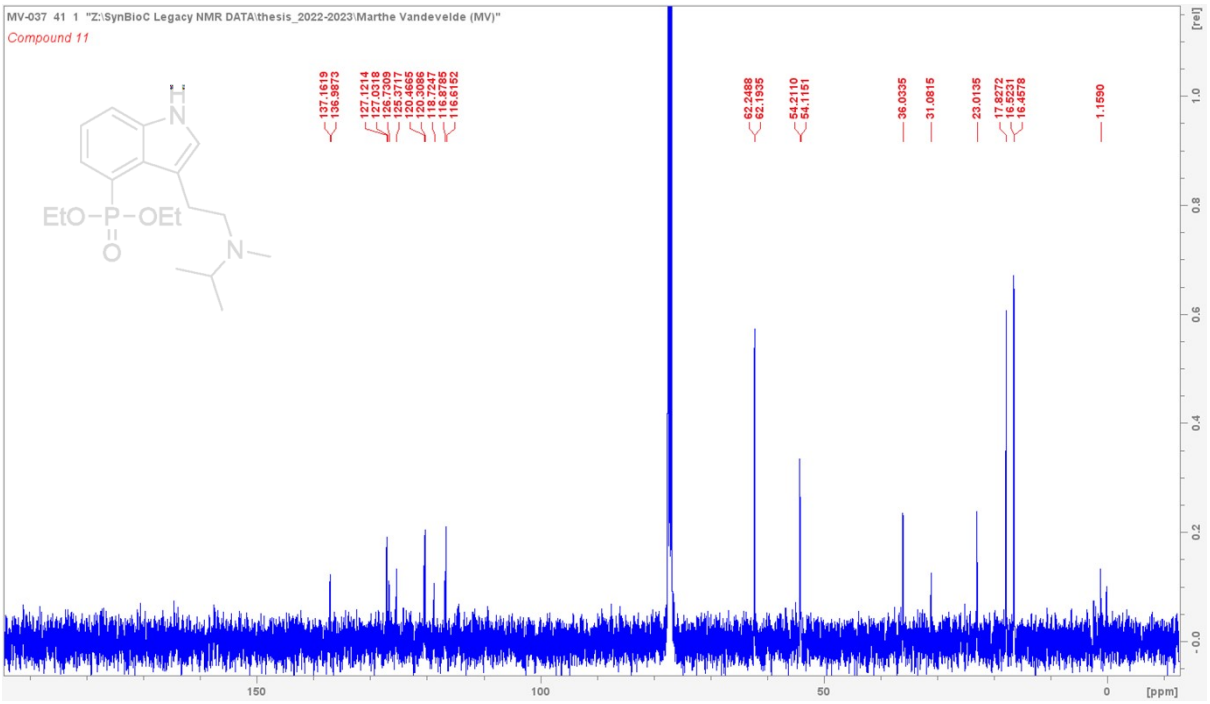
^{1}H-NMR (400 MHz, $CDCl_3$)



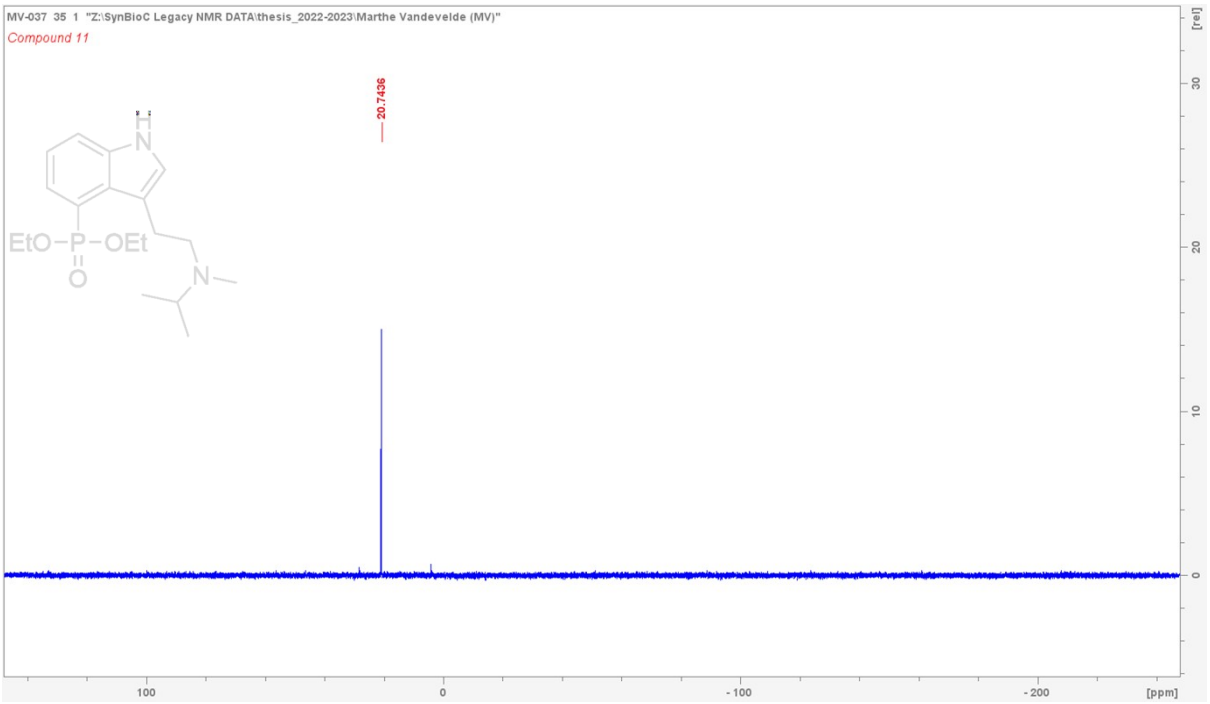
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, $CDCl_3$)

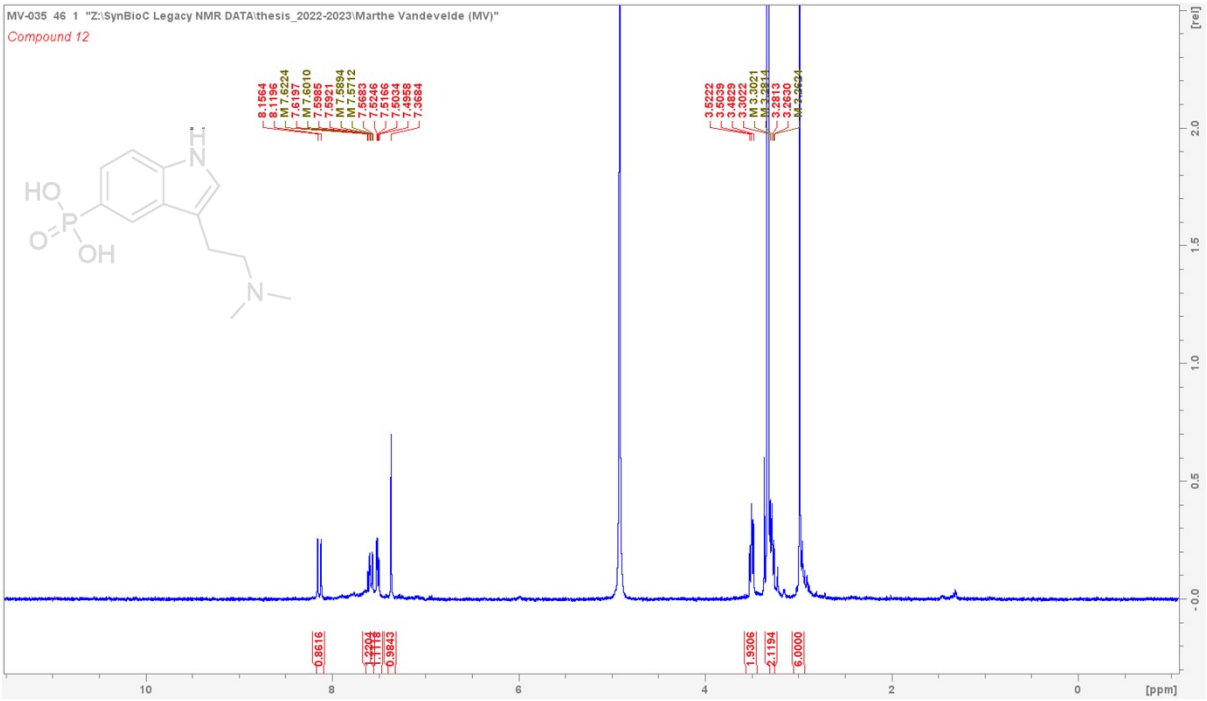


^{31}P-NMR (162MHz,$CDCl_3$)

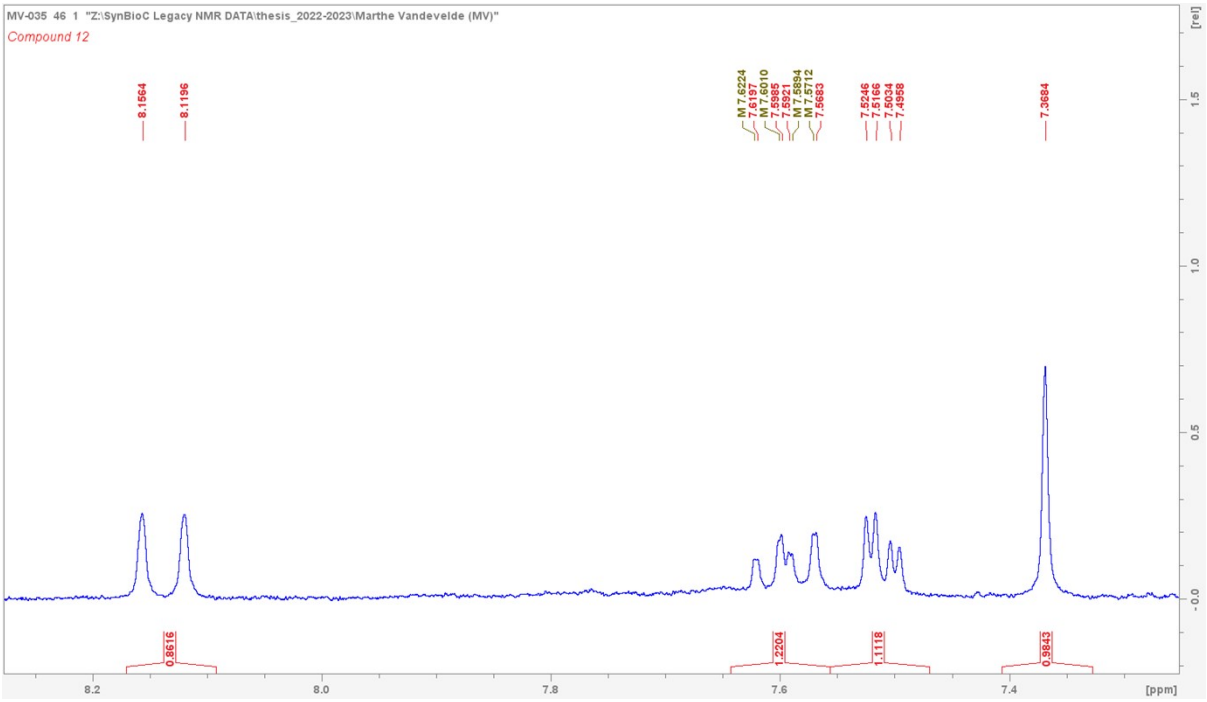


Compound 12

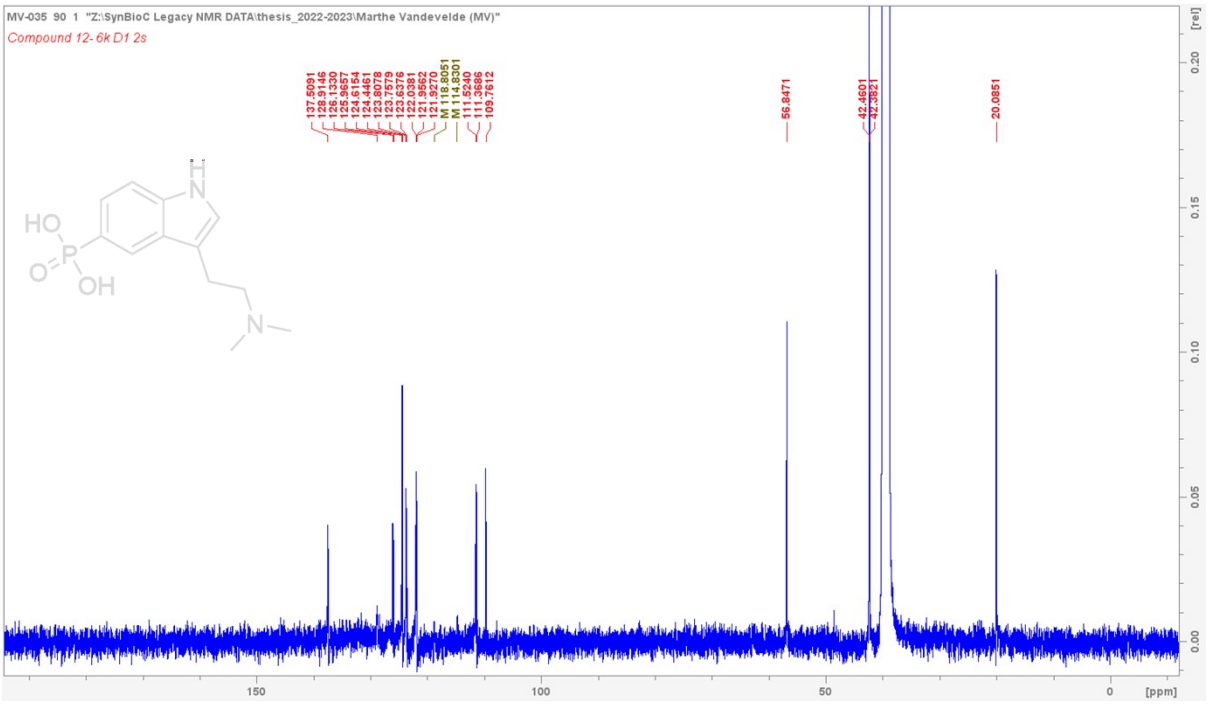
^{1}H-NMR (400 MHz, MeOD)



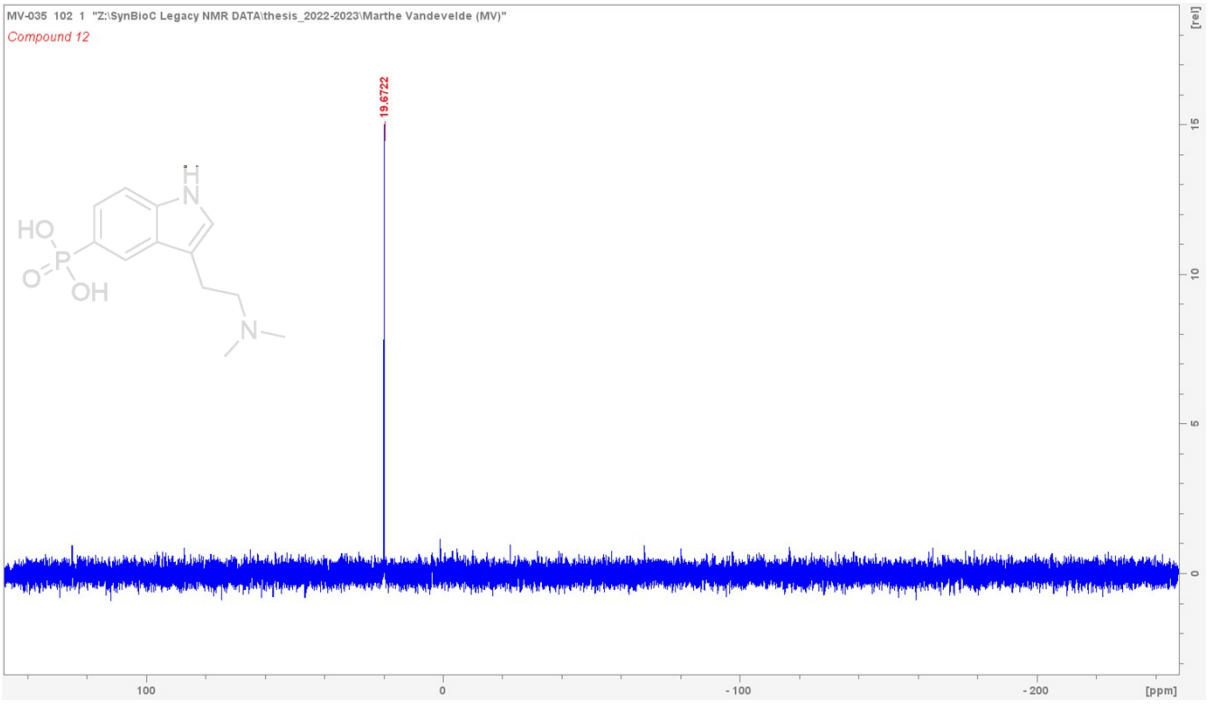
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, DMSO-d_6)

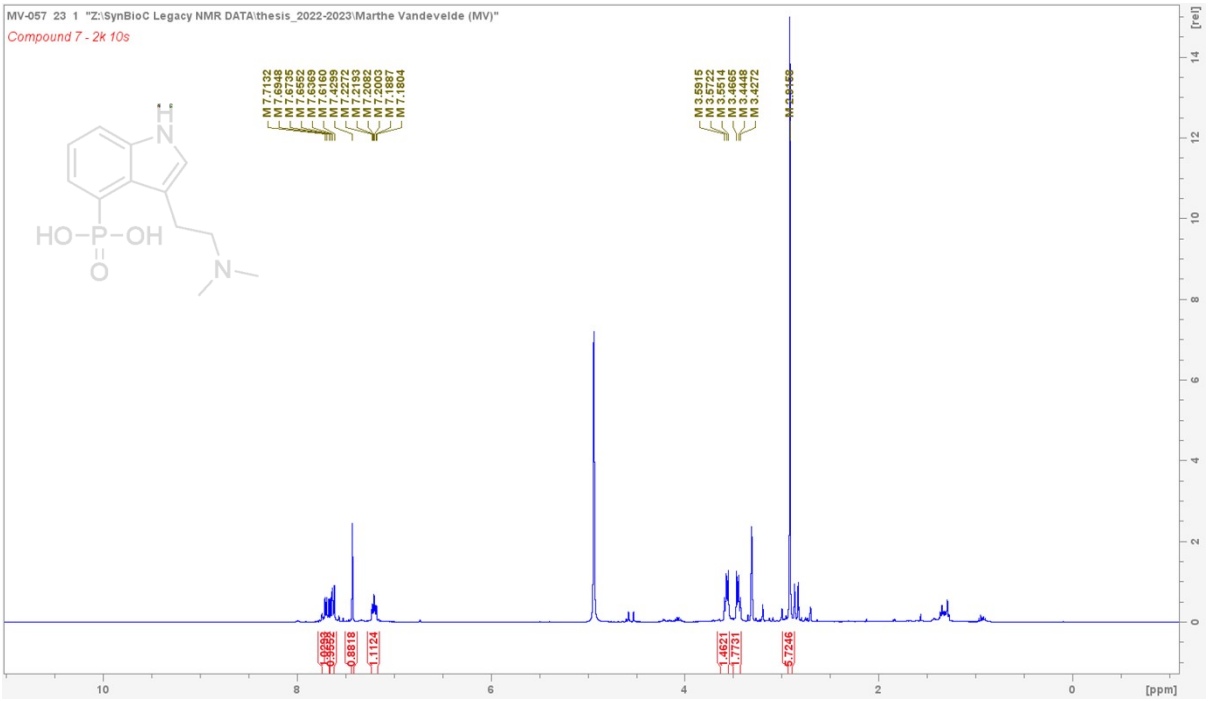


^{31}P-NMR (162MHz,MeOD)

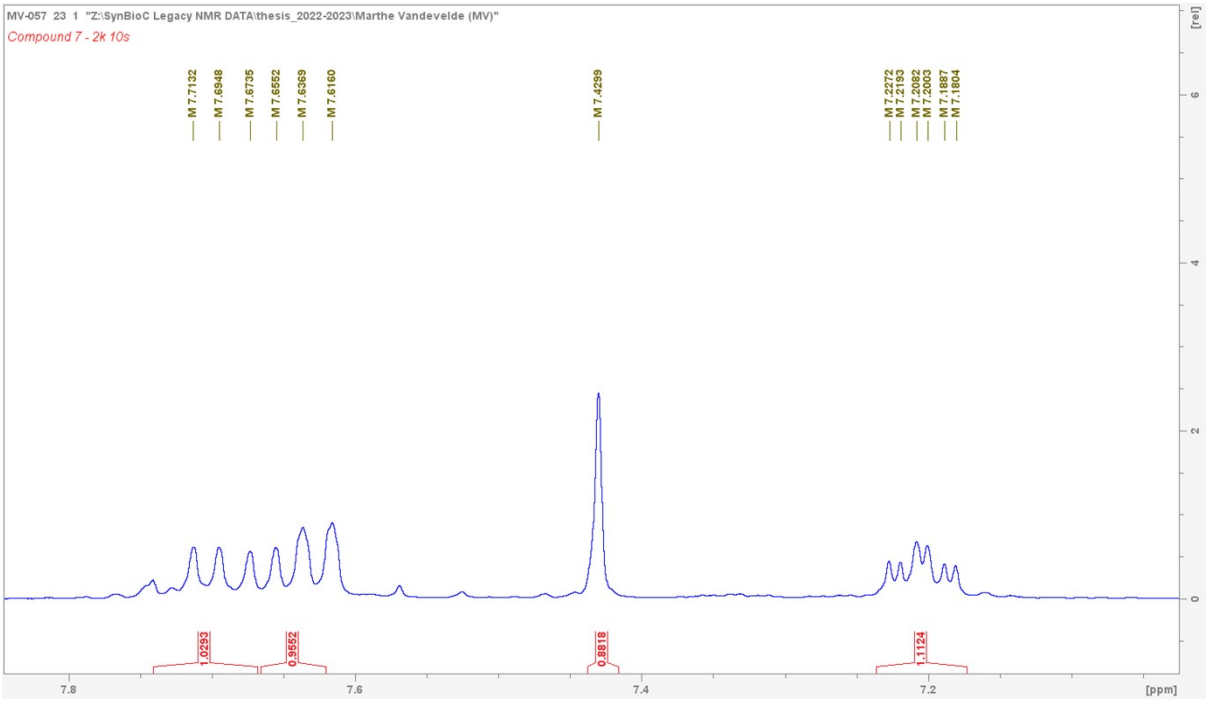


Compound 7

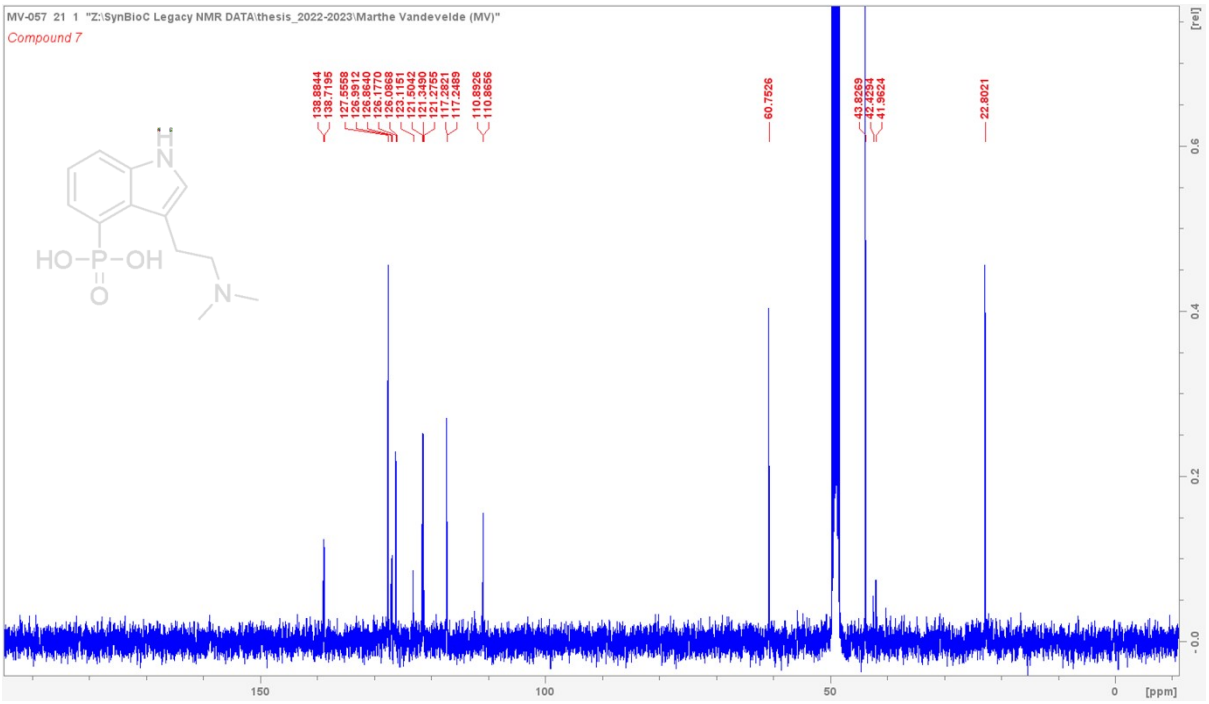
^{1}H-NMR (400 MHz, MeOD)



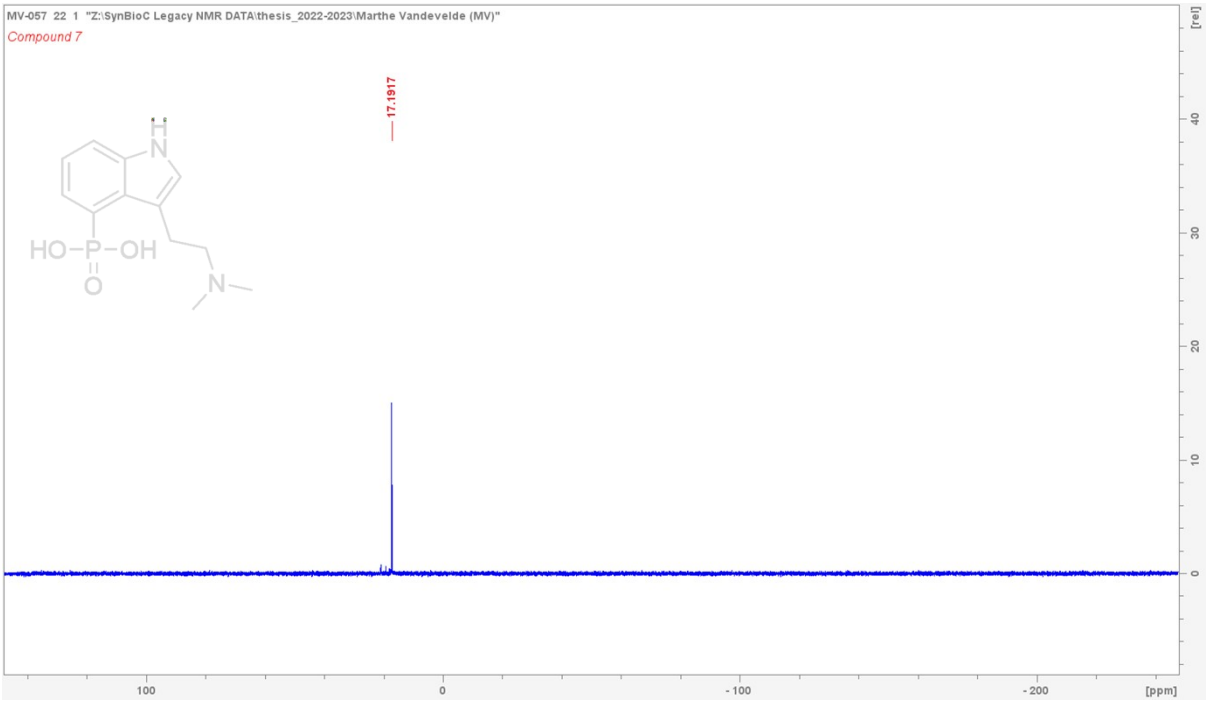
Zoom of the aromatic region



^{13}C-NMR (100.6 MHz, MeOD)

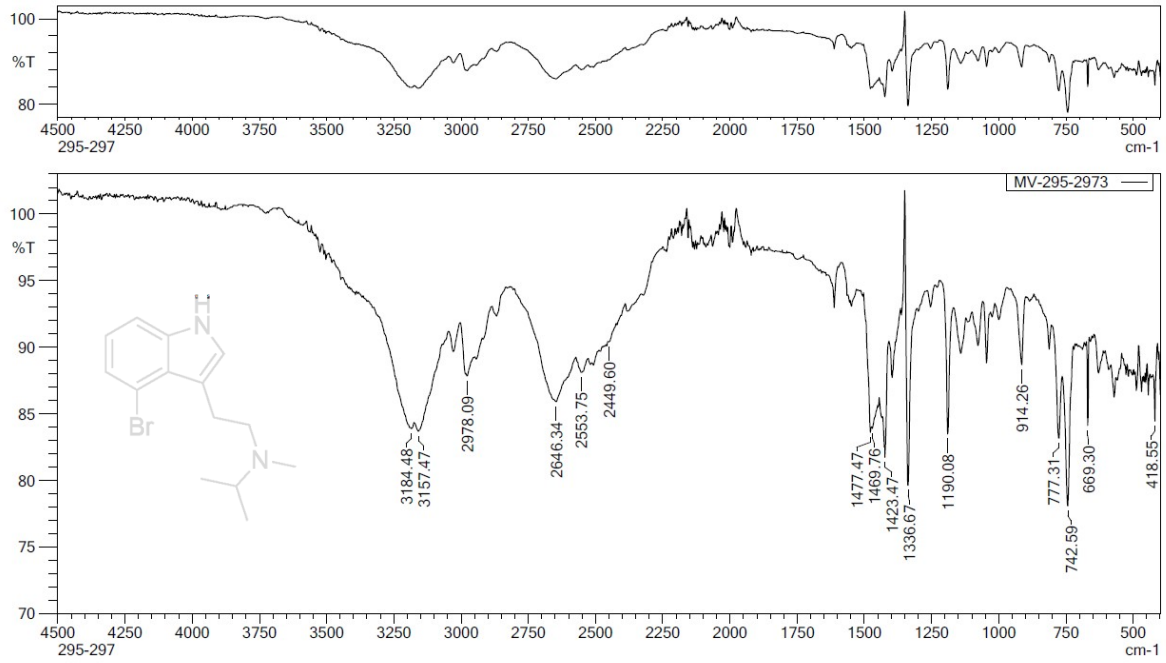


^{31}P-NMR (162MHz, MeOD)

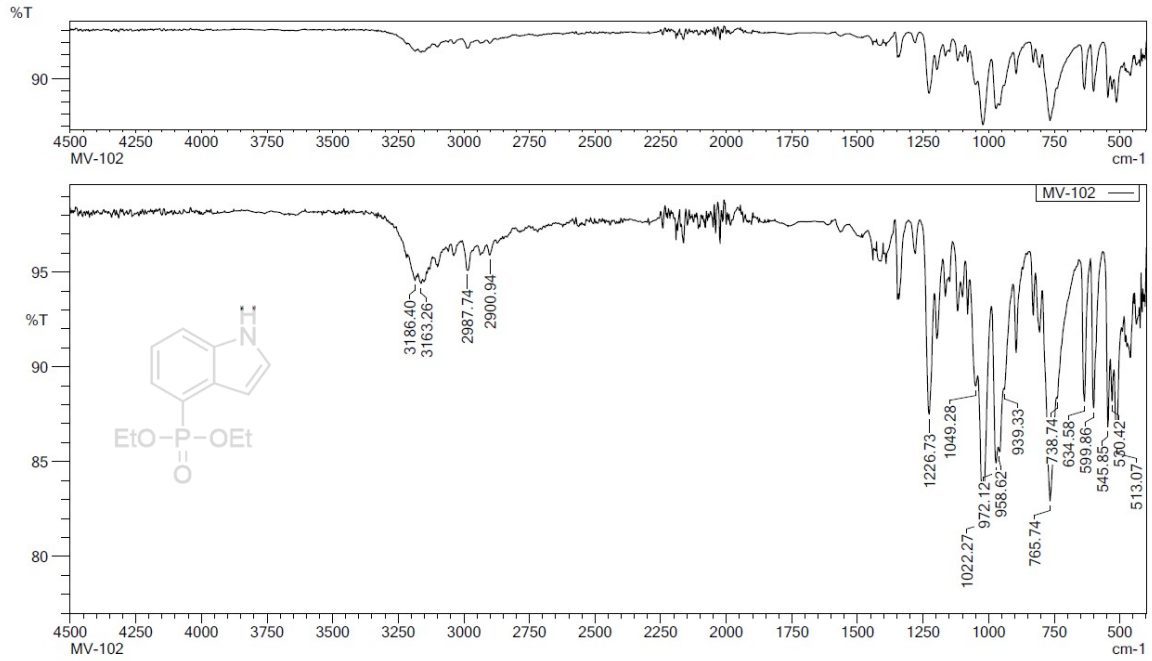


IR spectra of new compounds

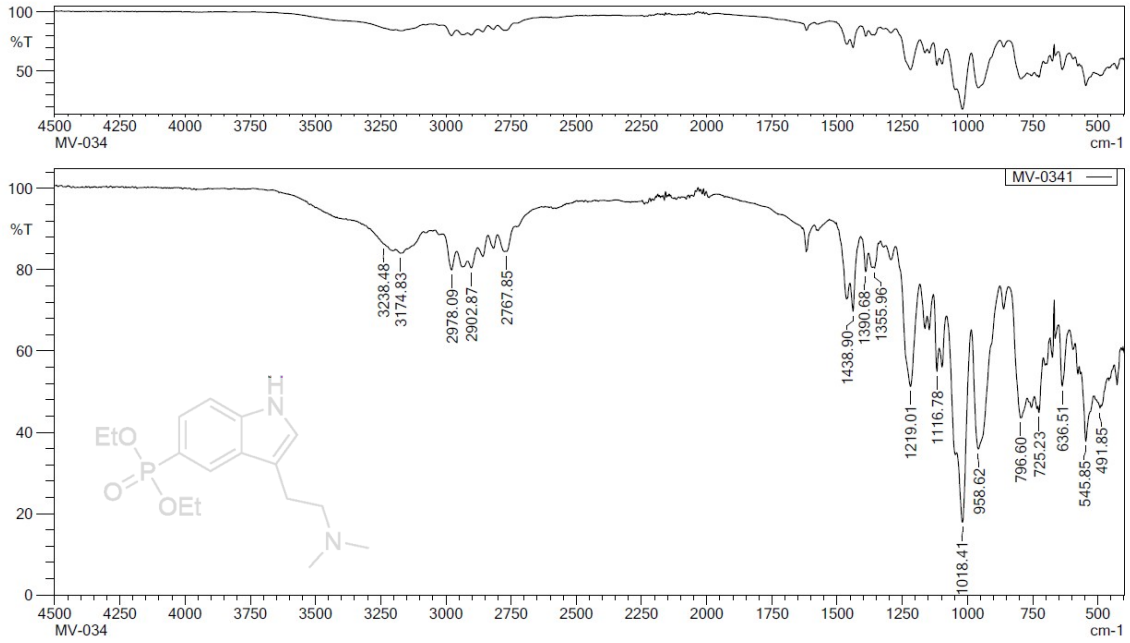
Compound 5b



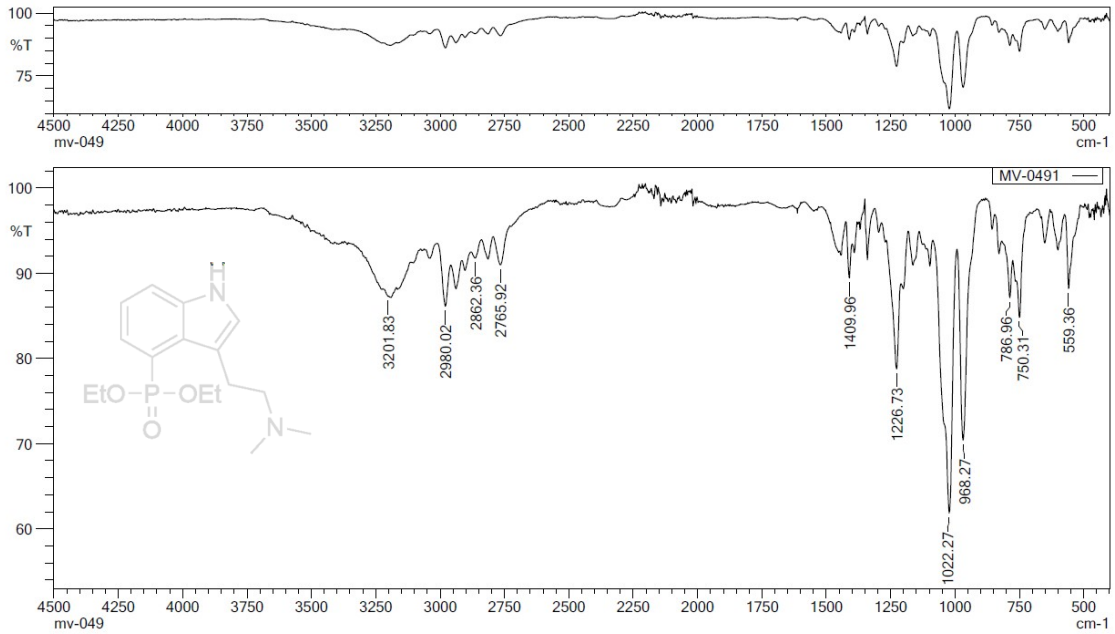
Compound 8



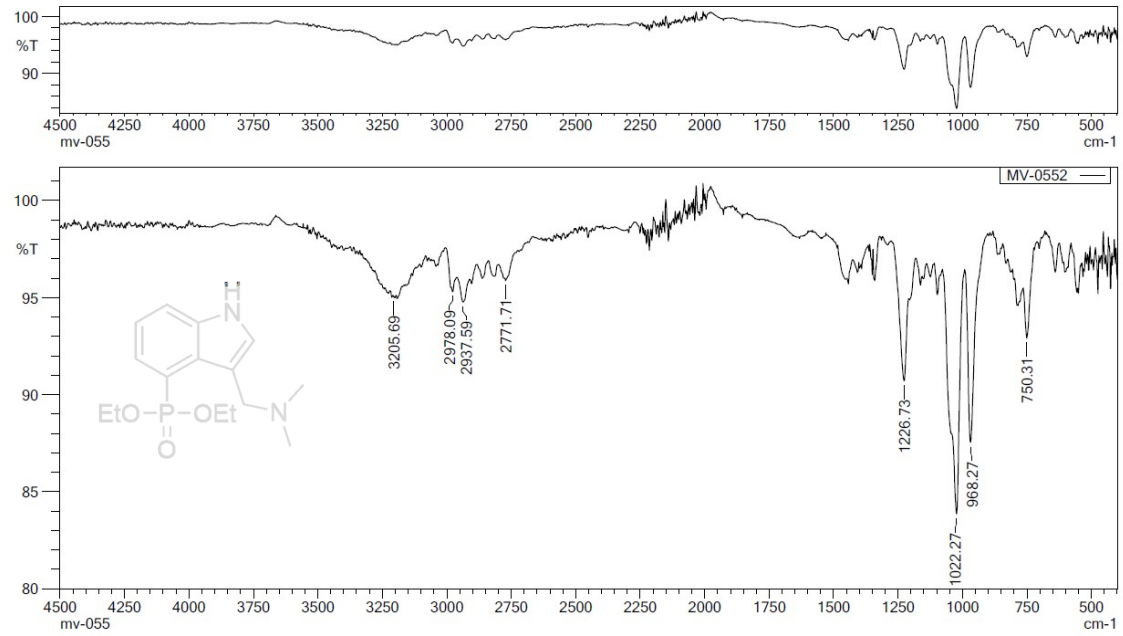
Compound 9



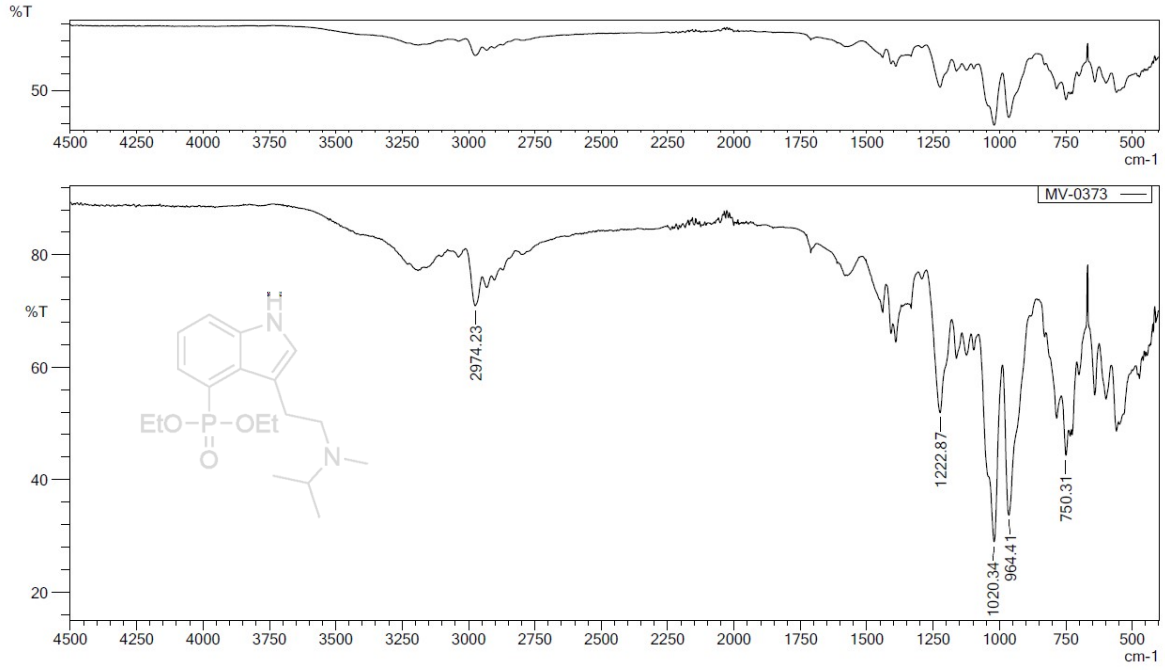
Compound 6



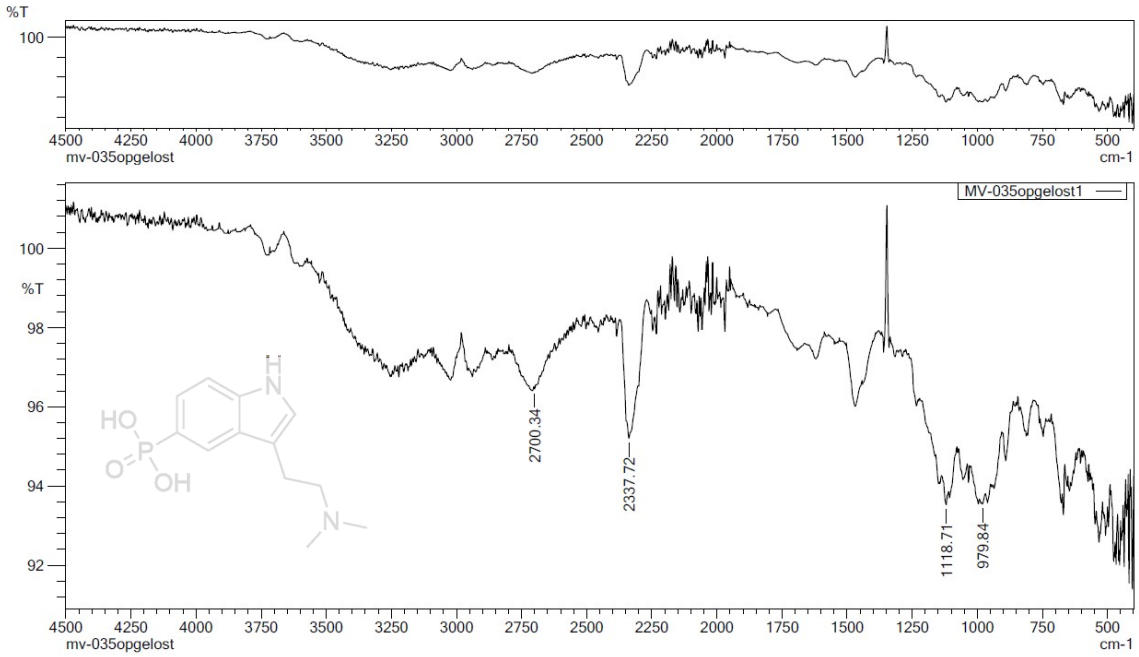
Compound 10



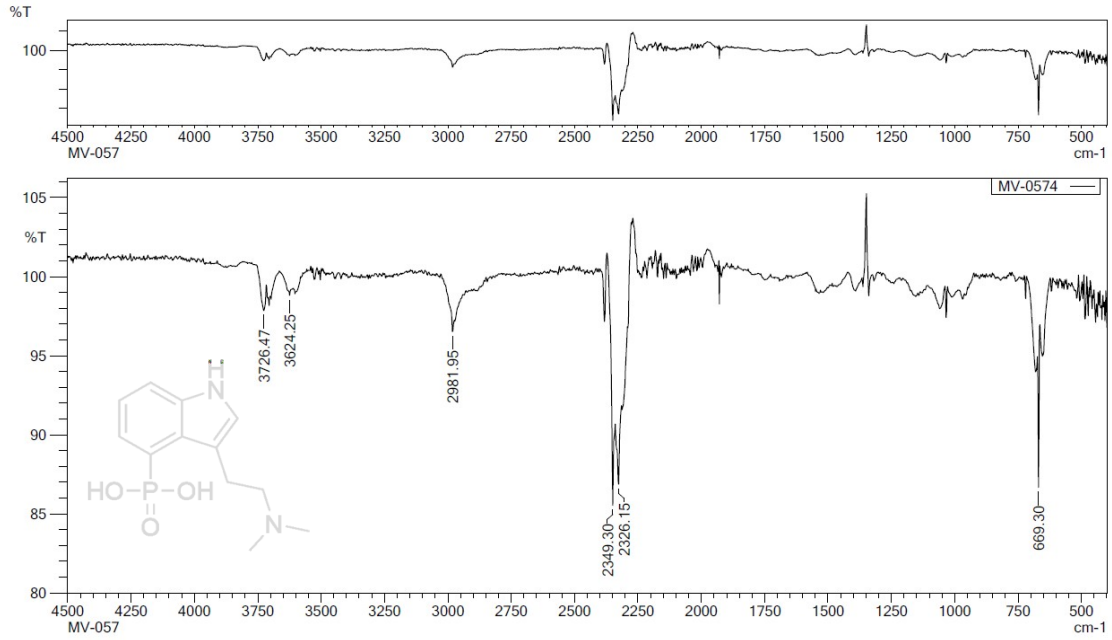
Compound 11



Compound 12



Compound 7



Cellular assay

Cellular assays were performed by Eurofins CEREP SA in Celle-Lévescault, France. The 5-HT_{2A} and 5-HT_{2B} receptors were expressed in HEK-293 or CHO cells respectively.[4] Homogenates of the cell membranes were subsequently incubated for 60 minutes at 22 °C with the radioligand ([^{125}I](±)DOI). Next, 60 µL of a 10 mM stock of the to-be-investigated compound in a buffer was added. The buffer contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbic acid. After incubation, the samples were filtered rapidly under vacuum through glass fibre filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). After drying the filters, radioactivity was measured in a scintillation counter using a scintillation cocktail (Microscint 0, Packard). If the compound has an affinity for the receptor, it will inhibit the binding of [^{125}I](±)DOI.

Results were expressed in a percentage of inhibition of the control radioligand-specific binding. Human recombinant TNAP (Tissue-nonspecific Alkaline Phosphatase) was encoded by the ALPL gene and was expressed in a mouse myeloma cell line. The test compound (10 µM) and/or vehicle (1.0% DMSO) was first preincubated with 1.25 ng/mL enzyme in TNAP at 25 °C for 15 minutes. Next, DiFMUP (5 µM) was added to initiate the reaction after another incubation of 30 minutes at 25 °C. The incubation buffer consisted of 50 mM TrisHCl and 1 mM $MgCl_2$ with a pH of 9.0. Unless TNAP was inhibited by another compound, DiFMUP was hydrolyzed to DiFMU. The amount of DiFMU was subsequently measured by a spectrofluorimeter at 358nm/450 nm.

The results are expressed as a percent of control specific binding (1)

$$\frac{measured\ specific\ binding * 100}{control\ specific\ binding} \quad (1)$$

and as a percent inhibition of control specific binding (2)

$$100 - (\frac{measured\ specific\ binding * 100}{control\ specific\ binding}) \quad (2)$$

obtained in the presence of the investigated compounds.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation (3) curve fitting.

$$Y = D + [\frac{A - D}{1 + \left(\frac{C}{C_{50}}\right)^{nH}}] \quad (3)$$

where Y = specific binding, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, C_{50} =IC_{50}, and nH = slope factor. This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the

commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.).The inhibition constants (K_i) were calculated using the Cheng Prusoff equation (4)

$$K_i = \frac{IC_{50}}{(1 + \frac{L}{K_D})} \quad (4)$$

where L = concentration of ligand in the assay, and KD = affinity of the ligand for the receptor.

In Vitro Pharmacology: binding assays

The results of the radioligand assays for the 5-HT_{2A}R and 5-HT_{2B}R are expressed as the inhibition of the binding of [^{125}I](±)DOI at the receptor caused by the examined compound (10 µM). Results for the third receptor under investigation, the human phosphatase enzyme TNAP, are expressed as the percentage inhibition of the binding of 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP).

Table S3. Results of an initial in vitro screening of the synthesized compounds. The activity of the compounds is expressed as the inhibition percentage of [^{125}I](±)DOI at the tested receptor or as inhibition of DiFMUP at TNAP

Compound	5-HT_{2B}R (%)	5-HT_{2A}R (%)	TNAP
9	96	88	-4
6	94	87	-2
10	73	57	0
11	89	77	0
12	81	80	3
7	85	70	2

Compound I.D.	Client Compound I.D.	Test Concentration	% Inhibition of Control Specific Binding 1st	Mean
5-HT_{2A}*(h)* (agonist radioligand)				
100067644-1	34	1.0E-05 M	88.4	**88.4**
100067644-2	35	1.0E-05 M	80.1	**80.1**
100067644-3	37	1.0E-05 M	76.5	**76.5**
100067644-4	57	1.0E-05 M	69.6	**69.6**
100067644-5	45	1.0E-05 M	87.0	**87.0**
100067644-6	49	1.0E-05 M	56.6	**56.6**

Figure S5. Results of a first screening on 5-HT_{2A}R of compound 9, 12, 11, 7, 6 and 10 (from top to bottom) (Eurofins Report)

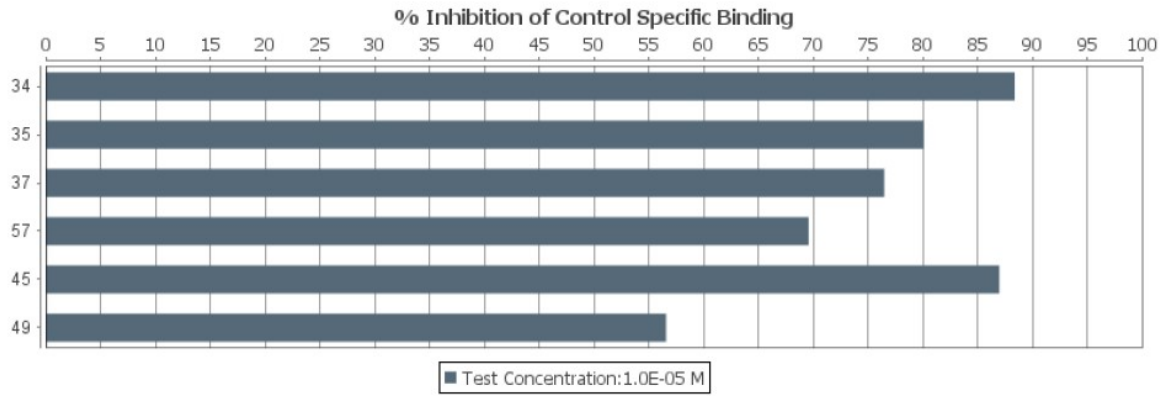


Figure S6. Histogram for 5-HT_{2A}R of compound 9, 12, 11, 7, 6 and 10 (from top to bottom) (Eurofins Report)

Compound I.D.	Client Compound I.D.	Test Concentration	% Inhibition of Control Specific Binding 1st	Mean
5-HT$_{2B}$*(h)* (agonist radioligand)				
100067644-1	34	1.0E-05 M	95.5	**95.5**
100067644-2	35	1.0E-05 M	80.7	**80.7**
100067644-3	37	1.0E-05 M	89.4	**89.4**
100067644-4	57	1.0E-05 M	84.8	**84.8**
100067644-5	45	1.0E-05 M	93.5	**93.5**
100067644-6	49	1.0E-05 M	73.1	**73.1**

Figure S7: Results of a first screening on 5-HT$_{2B}$R of compound 9, 12, 11, 7, 6 and 10 (from top to bottom) (Eurofins Report)

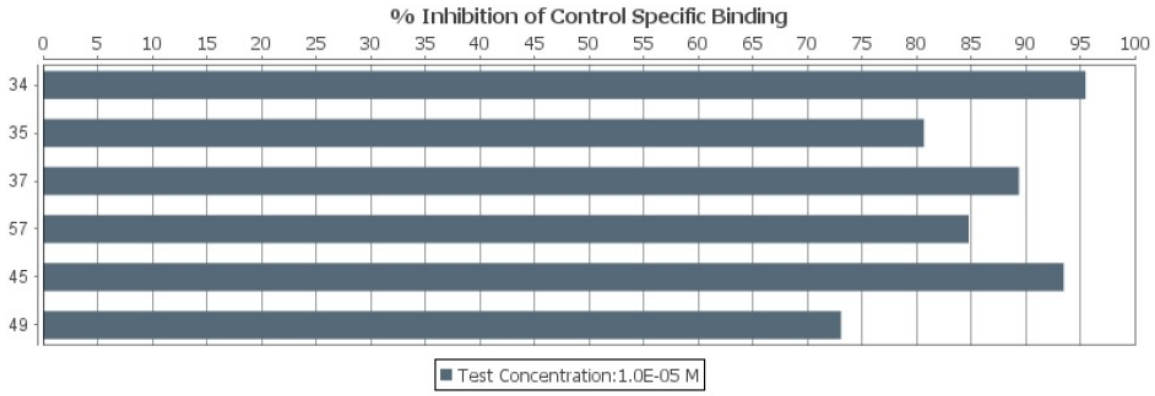


Figure S8. Histogram for 5-HT$_{2B}$R of compound 9, 12, 11, 7, 6 and 10 (from top to bottom) (Eurofins Report)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC$_{50}$*	K$_i$	n$_H$	R
Compound: 34, PT #: 1276245										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	-4				
Compound: 35, PT #: 1276246										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	3				
Compound: 37, PT #: 1276247										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	0				
Compound: 45, PT #: 1276249										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	-2				
Compound: 49, PT #: 1276250										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	0				
Compound: 57, PT #: 1276248										
107070	Alkaline Phosphatase/ALPL (TNAP)	502976	hum	2	10 µM	2				

Figure S9. Results of a first screening on TNAP of compound 9, 12, 11, 6, 10 and 7 (from top to bottom) (Eurofins Report)

IC_{50} Determination: Compound 9

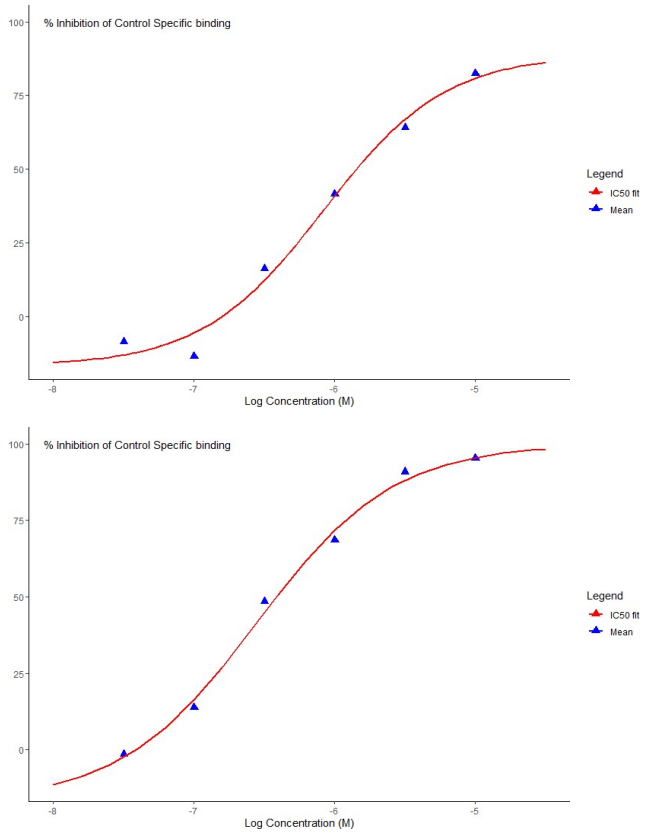


Figure S10. Dose–response competition binding assays for compound 9 on 5-HT_{2A}R (above) and 5-HT_{2B}R (below)

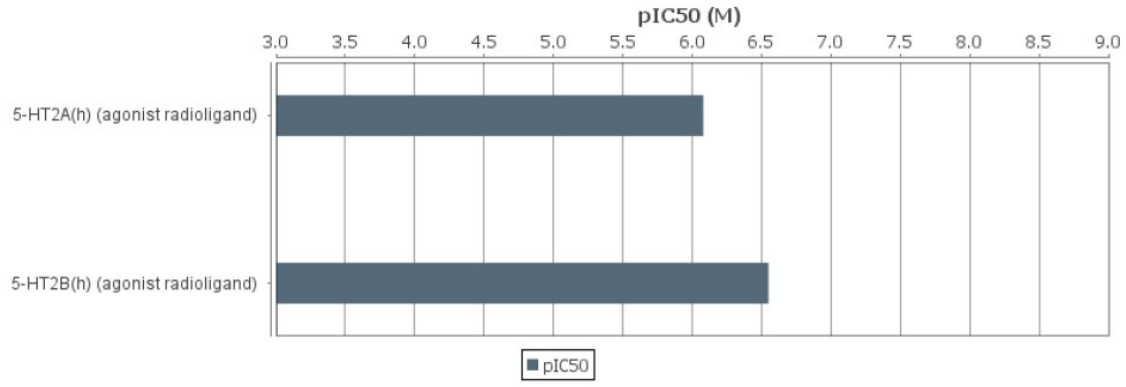


Figure S11. Histogram for compound 9 (Eurofins Report)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	Mean
5-HT_{2A}*(h)* (agonist radioligand)							
100068736-1	MV-034	8.3E-07 M	6.2E-07 M	1.0	3.162E-08 M	-8.6	-8.6
					1.0E-07 M	-13.6	-13.6
					3.1623E-07 M	16.2	16.2
					1.0E-06 M	41.6	41.6
					3.1623E-06 M	64.0	64.0
					1.0E-05 M	82.4	82.4

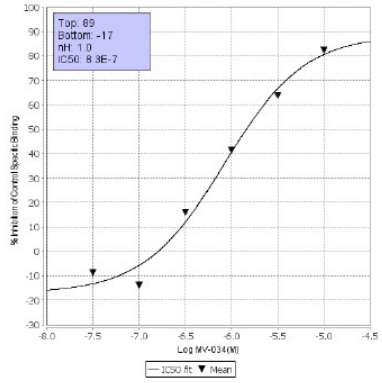


Figure 2. MV-034 on 5-HT_{2A}*(h)* (agonist radioligand)

Figure S12. Results for compound 9 on 5-HT_{2A}R (Eurofins Report)

Compound I.D.	Client Compound I.D.	IC_{50}(M)	K_i(M)	nH	Test Concentration	% Inhibition of Control Specific Binding 1st	Mean
5-HT_{2B}*(h)* (agonist radioligand)							
100068736-1	MV-034	2.8E-07 M	1.4E-07 M	0.9	3.162E-08 M	-1.6	-1.6
					1.0E-07 M	13.7	13.7
					3.1623E-07 M	48.4	48.4
					1.0E-06 M	68.5	68.5
					3.1623E-06 M	90.9	90.9
					1.0E-05 M	95.4	95.4

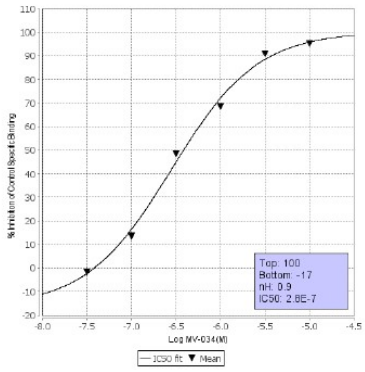


Figure 3. MV-034 on 5-HT_{2B}*(h)* (agonist radioligand)

Figure S13. Results for compound 9 on 5-HT_{2B}R (Eurofins Report)

Compound I.D.	IC_{50}(M)	K_i(M)	nH
5-HT_{2A}*(h)* (agonist radioligand)			
(±)DOI	7.2E-10 M	5.4E-10 M	0.9
5-HT_{2B}*(h)* (agonist radioligand)			
(±)DOI	3.0E-09 M	1.5E-09 M	0.7

Figure S14. Reference compound results (Eurofins Report)

References

(1) Alzweiri, M.; S. Alsegiani, A.; Karaj, E.; A. Almarghalani, D.; Tabaza, Y.; A. Shah, Z.; Tillekeratne, L. M. V. The structural simplification of lysergic acid as a natural lead for synthesizing novel anti-Alzheimer agents. *Bioorganic & Medicinal Chemistry Letters* **2021**, *47*, 128205.
(2) Olsen, E. K.; Hansen, E.; W. K. Moodie, L.; Isaksson, J.; Sepčić, K.; Cergolj, M.; Svenson, J.; Andersen, J. H. Marine AChE inhibitors isolated from Geodia barretti: natural compounds and their synthetic analogs. *Organic & Biomolecular Chemistry* **2016**, *14* (5), 1629-1640.
(3) Chen, C.-y.; Senanayake, C. H.; Bill, T. J.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. Improved Fischer Indole Reaction for the Preparation of *N*,*N*-Dimethyltryptamines: Synthesis of L-695,894, a Potent 5-HT_{1D} Receptor Agonist. *The Journal of Organic Chemistry* **1994**, *59* (13), 3738-3741.
(4) (a) Bryant, H. U.; Nelson, D. L.; Button, D.; Cole, H. W.; Baez, M. B.; Lucaites, V. L.; Wainscott, D. B.; Whitesitt, C.; Reel, J.; Simon, R.; Koppel, G. A. A novel class of 5-HT_{2A} receptor antagonists: Aryl aminoguanidines. *Life Sciences* **1996**, *59* (15), 1259-1268. (b) Choi, D.-S.; Birraux, G.; Launay, J.-M.; Maroteaux, L. The human serotonin 5-HT_{2B} receptor: Pharmacological link between 5-HT_2 and 5-HT_{1D} receptors. *FEBS Letters* **1994**, *352* (3), 393-399.