Supplementary Information

Synthesis and characterization of new fluorescent boro-β-carboline dyes

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Experimental

General

All melting points were determined on a Jasco SRS OptiMelt apparatus (Stanford, CA, USA) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solution at room temperature, on a Varian Unity Inova 500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C/APT NMR spectra, respectively), and on a Varian Unity Inova 300 spectrometer (300, 75 MHz and 282 MHz for 1H, APT NMR and ^{19}F NMR spectra, respectively) and on Bruker Avance III 400 (400 and 100 MHz for ^{1}H and ^{13}C NMR spectra, respectively), with the deuterium signal of the solvent as the lock and TMS as the internal standard. Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. High resolution mass spectra were recorded on a Waters Q-TOF Premier mass spectrometer in positive ESI ionization mode. The reactions were followed by analytical thin layer chromatography on silica gel 60 F254 and HPLC–MS chromatography with a Shimadzu LCMS-2020 device using a Reprospher 100 C18 (5 μm; 100 × 3 mm) column and positive-negative double ion source (DUIS) with a quadrupole MS analysator in a range of 50–1000 m/z. All reagents were purchased from commercial sources [e.g., Sigma Aldrich (St. Louis, MO, USA), Fluorochem (Hadfield, Derbyshire, UK), Alfa (Haverhill, MA, USA), Combi-Blocks (San Diego, CA, USA)]. The DMEM, high glucose, pyruvate (Cat. no. 41966052) and Fetal Bovine Serum, qualified, One Shot™ format, Brazil (Cat. no. A3160802) were purchased from Gibco. Analytical samples of new compounds were obtained by trituration or recrystallization from the solvents or solvent mixtures given below in parentheses.

Photophysical measurements

The fluorescence and absorbance measurements were carried out on a Jasco FP8300 spectrofluorometer, in a standard cell quartz cuvette with 1 cm light path length. The widths of the excitation slit and the emission slit were both set to 2.5 nm with the scanning speed at 1000 nm/min. Pure solvents were used as blank correction. We used acetonitrile as solvent if not otherwise mentioned, and the concentration was 1 μM. We measured the absorbance spectra from 250 nm to 500 nm. The excitement spectra were recorded between 250 nm and 500 nm. For the emission spectra, we excited the sample at the excitation maximum, and recorded it from excitation maximum plus 10 nm to 800 nm.

The quantum yields were determined by recording the fluorescence spectra of a series of different concentrations. The gradient of the integrated fluorescence intensities plotted against absorbance at the excitation wavelength was used for the calculation of the quantum yields:

$$\Phi_x = \Phi_{St} \cdot \left(\frac{Grad_x}{Grad_{St}}\right) \cdot \left(\frac{\eta_x^2}{\eta_{St}^2}\right)$$

The **12a-d** compounds were excited at 380 nm, Coumarin-153 was used as reference (Φ_F^{EtOH}=0.55). The **11a,b,e,f** compounds were excited at 339 nm, quinine sulfate was used as reference ($\Phi_{F(0.5\ M\ H_2SO_4)}$=0.54) During the computations we used refraction coefficients from literature[1].

The solvent screen measurement was carried out with 5 μM solutions of the **11f** and **12d** boroisoquinoline in acetonitrile, 1,4-dioxane, water, toluene, dichloromethane, tetrahydrofuran, ethanol, hexane and ethyl acetate.

The photostability measurement was carried out with 5 μM solutions of compound **11f** and **12d** in acetonitrile. We used 8 W, 366 nm emitting UV lamp, and measured the emission spectrum in every hour for 4 hours. During the photophysical measurements the solutions were excited at 380 nm.

The UV/Vis absorbance measurements of trastuzumab conjugate were carried out on Thermo Scientific NanoDrop ™ 1000 Spectrophotometer. Before the measurements buffer exchange was performed six times with Sartorius Vivaspin 500 10000 MWCO at 15000 g for 10 minutes for each time. Sample buffer was used as blank for baseline correction with extinction coefficients; ε_{280}= 218134 $M^{-1}cm^{-1}$ and ε_{407}= 0 $M^{-1}cm^{-1}$ for trastuzumab (in the equation T); ε_{280}= 9923 $M^{-1}cm^{-1}$for ε_{407}= 4173 $M^{-1}cm^{-1}$ for diazaborininocarboline (in the equation βC) scaffold measured in water. FAR values were calculated from Lambert-Beer equation for absorbance at 280 and 403 nm.

$$A_{280\ nm} = \varepsilon_{\beta C,280\ nm} \cdot l \cdot c_{\beta C} + \varepsilon_{T,280\ nm} \cdot l \cdot c_T$$

$$A_{403\ nm} = \varepsilon_{\beta C,403\ nm} \cdot l \cdot c_{\beta C} + \varepsilon_{T,403\ nm} \cdot l \cdot c_T$$

From the concentration values the average FAR value was determined ($c_{\beta C}/c_T$).

SDS-PAGE and densitometry

Non-reducing glycine-SDS-PAGE at 10% acrylamide running were performed following standard lab procedures. A 4% stacking gel was used and a broad-range MW marker (4.6–300 kDa, ProSieve QuadColor Protein Marker, Lonza) was co-run to estimate protein weights. Samples (10 μL at 5 μM) were mixed with loading buffer (3 μL, composition for 6×SDS: 1 g

SDS, 3 mL glycerol, 6 mL 0.5 M Tris buffer pH = 6.8, 2 mg Coomassie-blue R250 in 10 mL), heated at 65 °C for 5minutes. Samples were subsequently loaded into the wells in a volume of 13 µL. All gels were run at constant 200 mA for 45 minutes. Gels were stained using a Coomasie stain (0,12 g Coomasie-blue G-250, 0,10 g Coomasie-blue R-250, 500 mL MeOH, 400 mL distilled water, 100 mL acetic acid), after wash it was rested at room temperature for 16 h in water-ethanol mixture. Than the gels were imaged using a HP DeskjetJ1350? scanner at 600 dpi. Images were saved under default brightness, contrast, and gamma settings. Densitometry was performed using ImageJ. Background subtraction was achieved using the built-in plugin with a rolling ball radius of 30, sliding paraboloid, and smoothing. Brightness and contrast settings were auto-adjusted within the software.

Animals

Six- to 8-week-old female SCID mice from our colony were maintained in our specific pathogen-free units under isothermal conditions. All animal experiments will be conducted following standards and procedures approved by the Animal Care and Use Committee of the National Institute of Oncology, Budapest (license number: PEI/001/2574-6/2015). 4×10^6 SKOV-3 cells, 2×10^6 OVCAR-8 cells and 2×10^6 MDA-MB-231 cells suspended in 0.2 ml serum-free medium were implanted subcutaneously into the back of the mice (5 in each group). Tumour growth was measured, and tumour volume was calculated.

Microscopy imaging

Three types of tumour cell lines were used to test **18** antibody conjugate: A) MDA-MB-231, an epithelial human breast cancer cell line negative for HER2 (human epidermal growth factor receptor 2), B) OVCAR-8, an ovarian carcinoma cell line slightly positive for HER2 and 3) SKOV-3, an ovarian cell line expressing high amounts of HER2. Tumour cells were subcutaneously injected into mice. After 6-8 weeks, the animals were anaesthetised with isoflurane and the tumours were resected. Two SKOV-3 tumours were immersed in HEPES solution, and 200 µm thick slices were cut with a vibratome (Leica VT1200S) at room temperature. The slices were kept in DMEM cell media at 35 °C and incubated with the BK-trastuzumab antibody (**18**) for 1 hour. Control slices were incubated with DMEM cell media for 1 hour. Then slices were washed with DMEM and examined with a two-photon laser scanning system (Femto2D-uncage, Femtonics, Budapest, Hungary). The imaging laser (Chameleon, Coherent, Santa Clara, CA, USA) was set to 850 nm and to 5-10% intensity. The excitation was delivered to the slices and the fluorescent signal was collected using an XLUMPlanFI 20x lens (Olympus, Tokyo, Japan).

Three MDA-MB-2031, three OVCAR-8 and three SKOV-3 tumours were removed from the animals and frozen in isopentane until further use. The tumours were thawed, and immersion fixed overnight in a fixative containing 4% paraformaldehyde and 15% picric acid in 0.1M phosphate buffer (PB). Section of 100 µm thickness were cut with a vibratome and washed in 0.1M PB. The sections were washed in a blocking solution containing 2% normal horse serum, 2% normal goat serum and 0.05% Triton-X for one hour, incubated with the BK-trastuzumab antibody (1:2000) for 24 hours, washed thoroughly with PB, then mounted on slides and covered with Vectashield (Thermo Fischer Scientific, Waltham, MA, USA). The sections were examined with a Zeiss LSM 710 Confocal microscope (Zeiss, Jena, Germany). The laser was set to 405 nm, and photos were taken with a 20x objective.

CYTOTOX assay 24 h and Anti-Proliferative assay 72 h on MRC-5 normal cell lines

Materials and methods

For cytotoxicology and anti-proliferative experiments, compound **12d** was dissolved in DMSO (Sigma Aldrich, St. Louis, MO, USA) to make 20 mM stock.

Cell lines and culture conditions

MRC-5, human lung fibroblast cell line was cultured in DMEM (Dulbecco's Modified Eagle's Medium; Biosera, Nuaille, France), supplemented with 10% heat-inactivated FBS (Fetal Bovine Serum; Biosera), and with 1% Penicillin/Streptomycin (Biosera). Cells were cultured in sterile T75 flasks (Sarstedt, Nümbrecht, Germany) with ventilation cap at 37 °C in a humidified atmosphere with 5% CO_2 in Sanyo Incubator model MCO-17AIC (Sanyo Electric Biomedical Co Ltd., Osaka, Japan). Manipulations with the cells were performed in biosafety cabinet (laminar) ESCO Sentinel Gold class II model AC2-4E8 (ESCO, Friedberg, Germany).

In vitro anti-proliferative activity of the conjugates and free drug

For the evaluation of the in vitro cytotoxic and anti-proliferative activity of the compound **12d**, the cell viability was determined by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; Duchefa Biochemie, Haarlem, The Netherlands). MTT assay is based on mitochondrial enzymes dependent reduction, by MTT, to the colored formazan crystals. After standard harvesting of the cells by trypsin-EDTA (Biosera), 8×10^3 and 15×10^3 cells per well for anti-proliferative and cytotoxicity effect study, respectively, were seeded in serum containing growth medium to 96-well plates with flat bottom (Sarstedt), in a 100 µL final volume per well, and incubated at 37 °C. After 24 h, cells were treated with various concentrations of **12d** (100 µM, 33.3 µM, 11.1 µM, 3.7 µM, 1.23 µM, 411 nM, 137 nM, 45 nM

and 15 nM; dilution factor 3) in volume 100 µL (200 µL final), dissolved in serum free medium and incubated for 24 h and 72 h for cytotoxicity and anti-proliferative effect study, respectively under standard conditions. The control wells were treated with serum free medium. Afterward, the MTT assay was performed, in order to determine cell viability, by adding 20 µL of MTT solution (5 mg/mL in PBS, 0.5 mg/mL final) to each well and after 4 h of incubation at 37 °C, the supernatant was removed. The precipitated purple formazan crystals were dissolved in 100 µL of a 1:1 solution of dimethylsulfoxide (DMSO; Sigma Aldrich) : 96% Ethanol (Molar Chemicals Kft., Halásztelek, Hungary) and the absorbance was measured after 15 min. at λ = 570 nm by using microplate reader CLARIOstar Plus (BMG Labtech, Ortenberg, Germany). Average background absorbance (DMSO-Ethanol) was subtracted from absorbance values of control and treated wells, and cell viability was determined relative to untreated (control) wells where cell viability was arbitrarily set to 100%. Absorbance values of treated samples were normalized versus untreated control samples and interpolated by nonlinear regression analysis with GraphPad Prism 6 software (GraphPad, La Jolla, San Diego, CA, USA) to generate sigmoidal dose-response curves from which the 50% inhibitory concentration (IC_{50}) values of the compound HI63, and presented as micromolar (µM) units. The experiments were done in triplicate and each experiment was repeated twice.

Synthetic Procedures

Oxazaborolo-carboline compounds

The synthesis until the *Kumujian C* was done by following literature process.[2]

9H-carbazole-1-carbaldehyde

Yield: 85%, yellow crystal, m. p. 194–197 °C (MeCN) (lit.[3] 197–199 °C). IR (KBr, cm^{-1}): 3381, 1685, 1202, 1123, 741, 724, 656. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.35 (s, 1H), 10.09 (br s, 1H), 8.64 (d, J = 4.9 Hz, 1H), 8.17–8.16 (m, 2H), 7.64–7.62 (m, 1H), 7.60–7.59 (m, 1H), 7.38–7.35 (m, 1H), 7.26 (s, 1H) ppm.

Grignard reaction

A three-necked round bottom flask was equipped with dropping funnel, gas inlet and magnetic stirrer bar and heated it to get an absolute water free system. Then 10 mL absolute ether and Mg shavings was filled in under nitrogen atmosphere. In another 10 mL absolute ether 10 mmol of the appropriate halogenid {**14a**,**e**: methyl iodide (0.6 mL, 1.4 g, 5 equiv.), **14b**,**f**: ethyl iodide

(0.8 mL, 1.5 g, 5 equiv.), **14c**: benzyl chloride(1.1 mL, 1.3 g, 5 equiv.), **14d**: 4-methoxybenzyl chloride(1.4 mL, 1.6 g, 5 equiv.)} was dissolved and dropped in through the funnel. If the reaction was not started a crystal of iodine was added. From the beginning of reflux the reaction mixture was boiled for 1 h.

An other three-necked round bottom flask was equipped with thermometer, dropping funnel, gas inlet and magnetic stirrer bar and heated it to get an absolute water free system. The Grignard-reagent was filled in the funnel and the 2 mmol of appropriate **13** aldehyde (0.4 g 13a for 14a,b,c,d and 0.42 g 13b for 14e,f) was dissolved in absolute THF. Then under nitrogen atmosphere the system was cooled to -20 °C, and the Grignard reagent was dropped in cautiously. The temperature was not gone over -20 °C. After the complexion of dropping the mixture was let to warm room temperature. After it, it was stirred for another 2 h. The reaction was quenched with NH_4Cl solution and extracted with EtOAc. The organic phase was washed with sodium thiosulphate. Then the organic phase was dried over $MgSO_4$, filtered and evaporated in vacou. The crue residue was purified by flash chromatography on silica gel using hexane/ethyl acetate. After it for NMR purposes the product was recrystallized from EtOAc.

*1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14a**)*

Yield: 0.33 g (77%), yellow crystals, m. p. 168-169 °C (EtOAc) (lit. 168-170 °C[4]); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.20 (s, 1H, NH), 8.21 (d, J =5.1 Hz, 1H, ArH), 8.18 (d, J = 8.4 Hz, ArH), 7.99 (d, J =5.1 Hz, 1H, ArH), 7.65 (d, J =8.1 Hz, 1H, ArH), 7.47 (t, J =8.1 Hz, 1H, ArH), 7.15 (t, J=7.5 Hz,1H, ArH), 5.62 (d, J=4.5 Hz, 1H, OH), 5.31-5.28 (m, 1H, HC), 1.48 (d, J =6.5 Hz, 3H, CH_3) ppm.

*1-(9H-pyrido[3,4-b]indol-1-yl)propan-1-ol (**14b**)*

Yield: 0.3 g (66%), yellow crystals, m. p. 163-164 °C (EtOAc); ^{1}H NMR (300 MHz, MeCN-d_3) δ 9.91 (s, 1H, NH), 8.35 (d, J = 5.2 Hz, 1H, ArH), 8.24 (d, J = 8.0 Hz, 1H, ArH), 8.00 (d, J = 5.2 Hz, 1H, ArH), 7.69 (d, J = 8.2 Hz, 1H, ArH), 7.61 (t, J = 7.8 Hz, 1H, ArH), 7.33 (t, J = 7.5 Hz, 1H, ArH), 5.12 (q, J = 5.3, 4.6 Hz, 1H, OH), 4.37 (s, 1H, HC), 1.96 – 1.86 (m, 2H, CH_2), 1.06 – 1.02 (m, 3H, CH_3); ^{13}C (125 MHz, $CDCl_3$) δ 146.3 (C=), 140.2 (C=), 137.6 (=CH), 132.9 (C=), 129.7 (C=), 128.4 (=CH), 121.6 (=CH), 121.3 (C=), 113.7 (=CH), 111.6 (=CH), 76.2 (CH), 30.2 (CH_2), 9.7 (CH_3); $[M+H]^+$measured = 227.1188, calcd. for $C_{14}H_{15}N_2O$: 227.1178.

*2-phenyl-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14c**)*

Yield: 0.42 g (73%), yellow crystals, m. p. 119-120 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H, NH), 8.14 – 7.96 (m, 2H, ArH), 7.75 (d, J = 5.1 Hz, 1H, ArH), 7.51 (t, J = 7.7 Hz, 1H, ArH), 7.39 (d, J = 8.2 Hz, 1H, ArH), 7.32 – 7.21 (m, 1H, ArH), 7.20 – 7.05 (m, 5H, ArH), 5.46 – 5.26 (m, 1H, CH), 3.35 – 3.02 (m, 2H, CH_2) ppm; ^{13}C (75 MHz, $CDCl_3$) δ 149.0 (C=), 140.6 (C=), 138.1 (C=), 137.6 (=CH), 133.5 (C=), 130.0 (C=), 129.9 (=CH), 128.8 (=CH), 128.7 (=CH), 126.9 (=CH), 121.8 (=CH), 121.3 (C=), 120.1 (=CH), 114.0 (=CH), 111.8 (=CH), 76.3 (CHOH), 44.4 (CH_2) ppm; $[M+H]^+$measured = 289.1337, calcd. for $C_{19}H_{17}N_2O$: 289.1335.

*2-(4-methoxyphenyl)-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14d**)*

Yield: 0.22 g (35%), yellow crystals, m. p. 140-141 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (s, 1H, NH), 8.72 (s, 1H, OH), 8.13 – 7.89 (m, 2H, ArH), 7.80 – 7.70 (m, 1H, ArH), 7.56 – 7.35 (m, 2H, ArH), 7.30 – 7.18 (m, 1H, ArH), 7.06 – 6.79 (m, 2H, ArH), 6.73 – 6.50 (m, 2H, ArH), 5.36 – 5.21 (m, 1H, HC), 3.65 (s, 3H, OCH_3), 3.19 – 2.94 (m, 2H, CH_2) ppm; ^{13}C (75 MHz, $CDCl_3$) δ 158.6 (C=), 146.3 (C=), 141.7 (C=), 140.7 (=CH), 137.4 (C=), 133.5 (C=), 130.8 (=CH), 130.1 (=CH), 130.0 (C=), 129.9 (C=), 128.7 (=CH), 121.8 (=CH), 121.3 (C=), 120.0 (C=), 114.1 (=CH), 113.9 (C=), 111.8 (=CH), 76.3 (CHOH), 55.5 (OCH_3) 44.4 (CH_2) ppm; $[M+H]^+$measured = 319.1447, calcd. for $C_{20}H_{19}N_2O_2$: 319.1441.

*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14e**)*

Yield: 0.33 g (74%), yellow crystals, m. p. 70-72 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 5.2 Hz, 1H), 8.15 – 8.05 (m, 2H), 7.87 (d, J = 5.2 Hz, 1H), 7.64 – 7.56 (m, 1H), 7.47 – 7.40 (m, 1H), 7.33 – 7.24 (m, 1H), 5.64 (q, J = 6.4 Hz, 1H), 4.03 (s, 3H), 1.61 (d, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.0 (C=), 142.6 (C=), 137.0 (=CH), 133.4 (C=), 130.2 (C=), 128.8 (=CH), 121.7 (=CH), 121.4 (C=), 114.2 (=CH), 109.8 (=CH), 66.1 (OCH), 32.6 (CH_3), 28.1 (CH_3) ppm; $[M+H]^+$measured = 227.1180, calcd. for $C_{14}H_{15}N_2O$: 227.1178.

*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)propan-1-ol (**14f**)*

Yield: 0.35 g (72%), yellow crystals, m. p. 70-71 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 5.2 Hz, 1H, ArH), 8.10 (dt, J = 7.8, 1.0 Hz, 1H, ArH), 7.87 (d, J = 5.2 Hz, 1H, ArH), 7.60 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H, ArH), 7.44 (d, J = 8.4 Hz, 1H, ArH), 7.29 (t, J = 7.4 Hz, 1H, ArH), 5.44 (dd, J = 7.9, 3.2 Hz, 1H, CH), 4.03 (s, 3H, NCH_3), 2.02 (dtt, J = 14.8, 7.4, 3.2 Hz, 1H, CH_2), 1.68 (dp, J = 14.7, 7.4 Hz, 1H, CH_2), 1.07 (t, J = 7.4 Hz, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.2 (C=), 142.7 (C=), 136.9 (=CH), 133.6 (=CH), 130.1 (C=), 128.7 (=CH), 121.7 (=CH), 120.2 (=CH), 114.1 (=CH), 109.8 (=CH), 105.0 (C=), 71.0 (CH),

32.9 (NCH_3), 32.6 (CH_2), 10.0 (CH_3) ppm; $[M+H]^+$measured = 241.1336, calcd. for $C_{15}H_{17}N_2O$: 241.1335.

Oxidation

Method A.

In a round bottom flask 1 mmol **14a**,**b** alcohol (**14a**: 0.21 g, **14b**: 0.22 g) was dissolved in 20 mL dioxane. After it 0.9 g MnO_2 (10 mmol, 10 equiv.) was added and refluxed over 2 hours. The reaction was filtered and the filter was washed with warm dioxane. Then it was evaporated in *vacou* and purified by recrystalliztion from ethyl acetate.

1-(9*H*-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15a**)

Yield: 0.15 g (77%), yellow crystals, m. p. 207-208 °C (ethyl acetate) (lit 207-209 °C[4]*)*

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.28 (s, 1H, NH), 9.08 (s, 1H, ArH), 8.40 (d, J = 7.9 Hz, 1H, ArH), 7.79 (t,J = 8.4 Hz,1H, ArH), 7.63 (t, J = 10.3 Hz, ArH), 7.58 – 7.49 (m, 1H ArH), 7.35 – 7.27 (m, 1H, ArH), 2.80 (s, 3H, CH_3) ppm.

*1-(9H-pyrido[3,4-b]indol-1-yl)propan-1-one (**15b**)*

Yield: 0.13 g (57%), yellow crystals, m. p. 120-121 °C (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.34 (s, 1H, NH), 8.55 (d, J = 5.0 Hz, 1H, ArH), 8.18 – 8.13 (m, 2H, ArH), 7.66 – 7.56 (m, 2H, ArH), 7.34 (ddd, J = 8.0, 6.6, 1.4 Hz, 1H, ArH), 3.45 (q, J = 7.3 Hz, 2H, CH_2), 1.33 (t, J = 7.3 Hz, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.8 (C=O), 114.1 (C=), 138.1 (=CH), 135.7 (C=), 135.4 (C=), 131.5 (C=), 129.2 (=CH), 124.3 (C=), 121.8 (=CH), 120.6 (=CH), 118.9 (=CH), 111.9 (=CH), 31.0 (CH_2), 8.1 (CH_3) ppm; $[M+H]^+$measured = 225.1023, calcd. for $C_{14}H_{13}N_2O$: 225.1022.

Method B.

In a round bottom flask 1 mmol **14c-f** alcohol (**14c**: 0.28 g, **14d**: 0.32 g, **14e**: 0.23 g, **14f**: 0.24 g) was dissolved in 20 mL dichloromethane. After it 0.85 g Dess-Martin peridodinane (2 mmol, 2 equiv.) was added and stirred over 2 hours at room temperature. Then it was evaporated in vacou and purified by flash column chromatography on silica gel using hexane/ethyl acetate.

*2-phenyl-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15c**)*

Yield: 0.13 g (46%), yellow crystals, m. p. 155 °C (EtOAc);^{1}H NMR (300 MHz, $CDCl_3$) δ 10.28 (s, 1H, NH), 8.61 (d, J = 4.9 Hz, 1H, ArH), 8.12 (d, J = 6.1 Hz, 2H, ArH), 7.59 (t, J = 7.7 Hz, 1H, ArH), 7.49 (d, J = 7.3 Hz, 3H, ArH), 7.46 – 7.26 (m, 3H, ArH), 4.79 (s, 2H, CH_2) ppm.

*2-(4-methoxyphenyl)-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15d**)*

Yield: 0.16 g (52%), yellow crystals, m. p. 38-40 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.31 (s, 1H, NH), 8.63 (d, J = 4.9 Hz, 1H, ArH), 8.19 (t, J = 6.1 Hz, 2H, ArH), 7.71 – 7.49 (m, 2H, ArH), 7.40 – 7.33 (m, 3H, ArH), 6.93 (d, J = 8.2 Hz, 2H, ArH), 4.72 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.7 (C=O), 158.8 (C=), 141.4 (C=), 138,3 (=CH), 136.2 (C=), 135.5 (C=), 132.0 (C=), 131.3 (=CH), 129.6 (=CH), 127.2 (C=), 122.1 (=CH), 121.1 (=CH), 120.8 (C=), 119.4 (=CH), 114.3 (=CH), 112.2 (=CH), 55.5 (OCH_3), 43.3 (CH_2) ppm; $[M+H]^+$measured = 317.1286, calcd. for $C_{20}H_{17}N_2O_2$: 317.1284.

*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15e**)*

Yield: 0.10 g (46%), yellow crystals, m. p. 100-101 °C (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, J = 4.9 Hz, 1H, ArH), 8.17 – 8.15 (m, 1H, ArH), 8.14 (d, J = 4.9 Hz, 1H, ArH), 7.69 – 7.62 (m, 1H, ArH), 7.53 (d, J = 8.3 Hz, 1H, ArH), 7.37 – 7.32 (m, 1H, ArH), 3.95 (s, 3H, NCH_3), 2.94 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.5 (C=O), 143.5 (C=), 140.0 (C=), 137.1 (=CH), 135.2 (C=), 132.1 (C=), 129.1 (=CH), 121.3 (=CH), 120.7 (C=), 120.3 (=CH), 117.6 (=CH), 110.1 (=CH), 34.0 (NCH_3), 28.3 (CH_3) ppm; $[M+H]^+$measured = 225.1025, calcd. for $C_{14}H_{13}N_2O$: 225.1022.

*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)propan-1-one (**15f**)*

Yield: 0.12 g (46%), yellow crystals, m. p. 53-54 °C (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, J = 4.9 Hz, 1H, ArH), 8.16 – 8.12 (m, 1H, ArH), 8.10 (d, J = 5.0 Hz, 1H. ArH), 7.67 – 7.61 (m, 1H, ArH), 7.54 – 7.48 (m, 1H, ArH), 7.35 – 7.31 (m, 1H, ArH), 3.89 (s, 3H, NCH_3), 3.44 (q, J = 7.3 Hz, 2H, CH_2), 1.33 (t, J = 7.3 Hz, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.6 (C=O), 143.3 (C=), 140.1 (C=), 137.1 (=CH), 135.1 (C=), 131.9 (C=), 129.0 (=CH), 121.3 (=CH), 120.7 (C=), 120.2 (=CH), 117.3 (=CH), 110.0 (=CH), 33.7 (NCH_3), 33.4 (CH_2), 8.5 (CH_3) ppm; $[M+H]^+$measured = 239.1186, calcd. for $C_{15}H_{15}N_2O$: 239.1178.

Oxazaborolo-carbolines (**11a**,**b**,**e**,**f**)

In a round bottom flask 0.5 mmol **15a**,**b**,**e**,**f** ketone (**15a**: 0.1 g, **15b**: 0.11 g, **15e**: 0.11 g, **15f**: 0.12 g) was dissolved in 20 mL dichloromethane. After it 0.62 mL trifluorboranil diethyletherate (0.71g, 2.5 mmol, 5 equiv.) was dropped at 0 °C and stirred over 2 hours at room

temperature. Then it was evaporated in *vacou* and purified by flash column chromatography on silica gel using hexane/ethyl acetate.

*3,3-difluoro-1-methylene-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole(**11a**)*

Yield: 0.11 g (82%), yellow crystals, m. p. 179-181 °C (hexane-EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.47 (s, 1H, NH), 8.66 (d, J = 5.9 Hz, 1H, ArH), 8.55 – 8.42 (m, 2H, ArH), 7.86 – 7.70 (m, 2H, ArH), 7.43 (t, J = 7.4 Hz, 1H, ArH), 5.63 (d, J = 3.0 Hz, 1H, CH_2), 5.14 (d, J = 2.9 Hz, 1H, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO) δ 151.7 (C-O), 143.9 (C=), 134.1 (C=), 131.5 (=CH), 129.2 (C=), 128.8 (=CH), 123.5 (=CH), 122.0 (=CH), 120.3 (=CH), 117.9 (=CH), 113.4 (=CH), 93.1 (=CH_2) ppm; $[M+H]^+$measured = 259.0856, calcd. for $C_{13}H_{10}BN_2OF_2$: 259.0848.

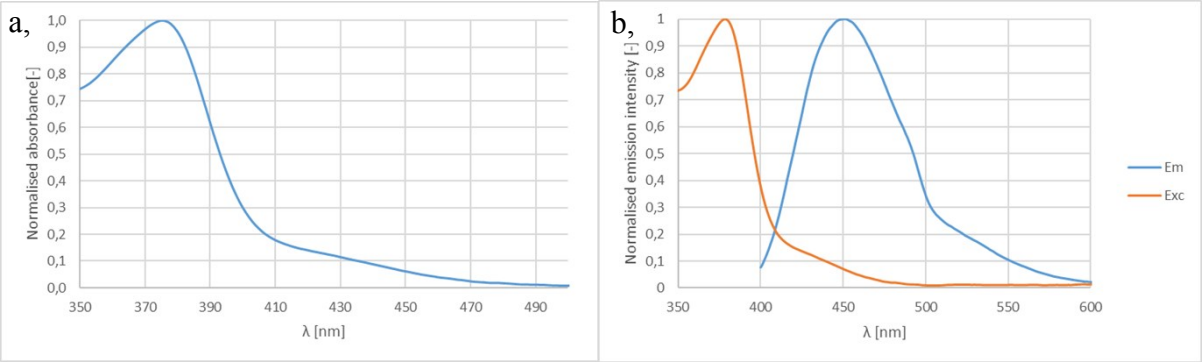

Figure S1: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **11a**

*(Z)-1-ethylidene-3,3-difluoro-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11b**)*

Yield: 0.07 g (55%), yellow crystals, m. p. 178-180 °C (hexane-EtOAc); ^{1}H NMR (300 MHz, Acetone-d_6) δ 11.48 (s, 1H, NH), 8.62 – 8.51 (m, 1H, ArH), 8.52 – 8.43 (m, 1H, ArH), 8.43 – 8.33 (m, 1H, ArH), 7.90 – 7.69 (m, 2H, ArH), 7.57 – 7.42 (m, 1H, ArH), 6.05 – 5.90 (m, 1H, HC=), 1.32 (s, 3H, CH_3) ppm; ^{1}H NMR (300 MHz, CD_3OD) δ 153.9 (C-O), 148.0 (C=), 145.2 (C=), 133.2 (C=), 130.3 (=CH), 128.6 (=CH), 126.4 (C=), 125.8 (C=), 121.4 (=CH), 118.9 (=CH), 117.8 (=CH), 105.0 (=CH), 86.3 (=CH), 36.1 (CH_3) ppm; $[M+H]^+$measured = 273.1012, calcd. for $C_{14}H_{12}BN_2OF_2$: 273.1005.

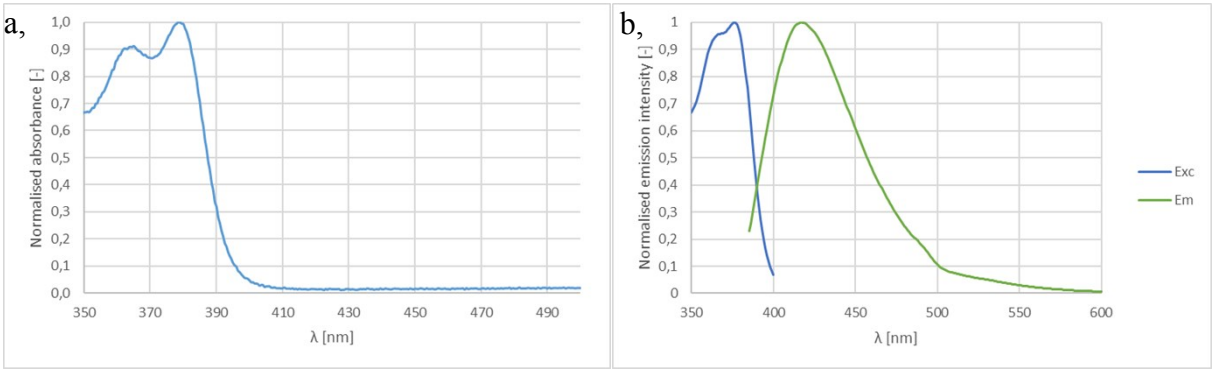

Figure S2: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **11b**

*3,3-difluoro-11-methyl-1-methylene-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11e**)*

Yield: 0.11 g (79%), yellow crystals, m. p. 118-120 °C (hexane-EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (d, J = 5.8 Hz, 1H, ArH), 8.51 (t, J = 6.5 Hz, 2H, ArH), 7.89 – 7.82 (m, 2H, ArH), 7.49 (ddd, J = 8.0, 6.7, 1.2 Hz, 1H, ArH), 5.37 (d, J = 2.8 Hz, 1H, CH_2), 5.21 (d, J = 2.8 Hz, 1H, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO) δ 146.1 (C-O); 137.4 (C=), 135.3 (C=), 131.9 (=CH), 129.5 (d, J = 32.6 Hz, C=), 123.4 (=CH), 122.4 (=CH), 122.1 (C=), 119.37 (d, J = 354.7 Hz, C=), 119.36 (d, J = 238.7 Hz, C=), 112.2 (=CH), 111.1 (=CH), 35.7 (=CH), 28.6 (CH_2) ppm; $[M+H]^+$measured = 273.1011, calcd. for $C_{14}H_{12}BN_2OF_2$: 273.1005.

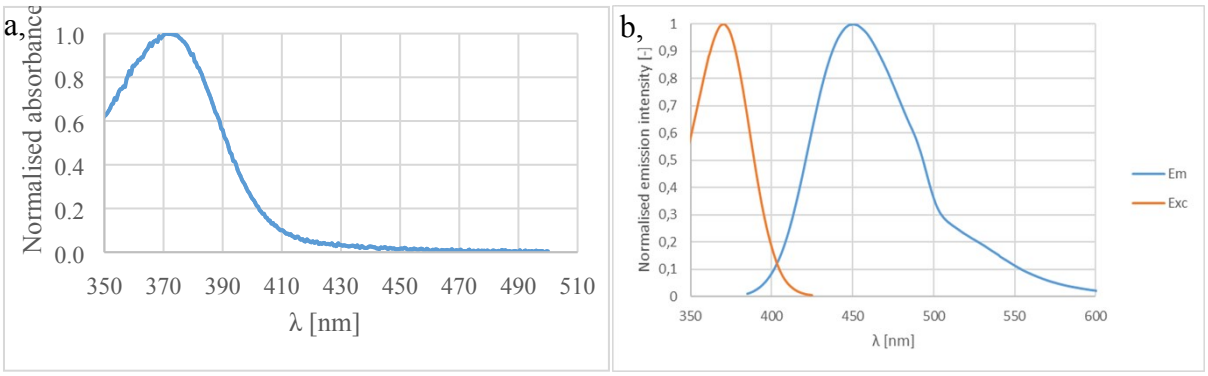

Figure S3: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **11e**

*(Z)-1-ethylidene-3,3-difluoro-11-methyl-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11f**)*

Yield: 0.13 g (92%), yellow crystals, m. p. 75-77 °C (hexane-EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (dd, J = 5.9, 2.5 Hz, 1H), 8.47 – 8.36 (m, 2H), 7.79 (d, J = 7.7 Hz, 2H), 7.44

(t, J = 7.3 Hz, 1H), 5.91 – 5.83 (m, 1H), 4.11 (d, J = 2.3 Hz, 3H), 2.00 – 1.93 (m, 3H) ppm; ^{13}C NMR (125 MHz, DMSO) δ 146.7 (C-O), 145.9 (C=), 134.6 (C=), 131.9 (C=), 131.5 (=CH), 129.1 (=CH), 123.2 (=CH), 122.2 (=CH), 120.4 (C=), 116.8 (=CH), 112.1 (=CH), 109.2 (C=), 60.2 (CH=), 35.6 (NCH_3), 12.3 (CH_3) ppm; $[M+H]^+$measured = 287.1169, calcd. for $C_{15}H_{14}BN_2OF_2$: 287.1161.

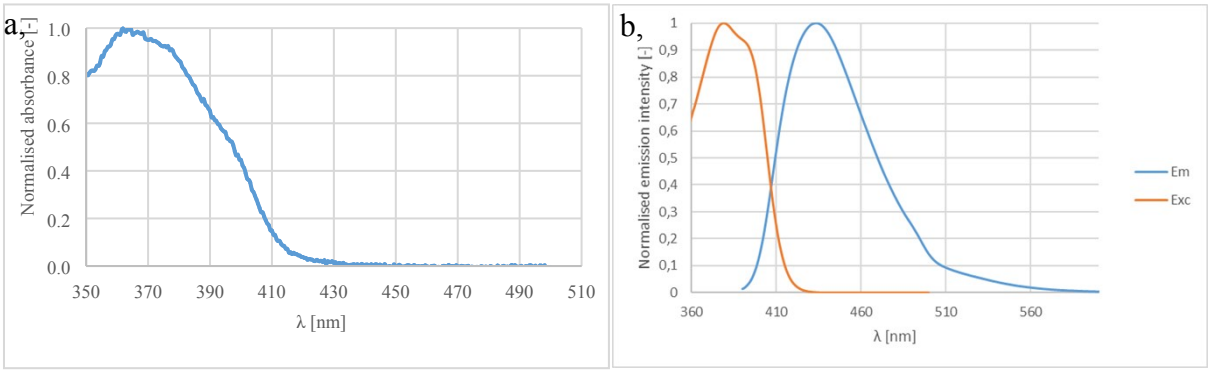

Figure S4: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **11f**

Diazaborinino carbolines

Pictet-Spengler reaction and aromatization

Method A.

In a round bottom flask 1.0 g tryptamine (6.2 mmol) and 6.2 mmol aldehyde [**16a**: 2-formylimidazole (0.6 g, 1 equiv.), **16b:** 1H-benzimidazole-2-carboxaldehyde (0.62, 1 equiv.)] were dissolved in 40 mL dichloroethane. To the reaction mixture was added 954 µl trifluoroacetic acid (1.42 g, 12,48 mmol, 2 equiv.). The reaction mixture was stirred at 100 °C for 2 hours. The solvent was evaporated under reduced pressure and the leftover was dissolved in 40 mL 1,4-dioxane. To this solution was added 920 mg Li_2CO_3 (12.48 mmol, 2 equiv.) and 1.5 g Pd/C catalyst (1.5 weight equiv.). This mixture was stirred at 110 °C for 72 hours. The reaction mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The crude material was dissolved in ethyl acetate, and washed with brine. The combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in vacuo.

*1-(1H-imidazole-2-yl)-9H-pyrido[3,4-b]indole (**16a**)*

Yield: 0.71 g (49 %), brown powder, 269-270 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H, NH), 8.40 (d, J = 5.2 Hz, 1H, ArH), 8.25 (d, J = 7.9 Hz, 1H, ArH), 8.13 (d, J = 5.2 Hz, 1H, ArH), 7.91 (d, J = 8.2 Hz, 1H, ArH), 7.55 (t, J = 7.6 Hz, 1H, ArH), 7.32 (s, 2H, ArH), 7.26 (t, J = 7.4 Hz, 1H, ArH) ppm.

*1-(1H-benzo[d]imidazole-2-yl)-9H-pyrido[3,4-b]indole (**16b**)*

Yield: 0.42 g (24 %), brown powder, m. p. 244-245 °C (water/MeCN); ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.30 (s, 1H, NH), 11.67 (s, 1H, NH), 8.54 (d, J = 5.1 Hz, 1H, ArH), 8.31 (t, 2H, ArH), 8.00 (d, J = 8.2 Hz, 1H, ArH), 7.89 (d, J = 8.4 Hz, 1H, ArH), 7.64 – 7.58 (m, 2H, ArH), 7.34 – 7.25 (m, 3H, ArH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 151.6 C=), 144.5 (C=), 141.7 (=CH), 138.2 (=CH), 134.8 (C=), 133.7 (=CH), 132.0 (C=), 130.1 (=CH), 129.0 (=CH), 123.7 (C=), 122.4 (C=), 122.2 (=CH), 120.9 (=CH), 120.3 (=CH), 119.7 (C=), 116.4 (=CH), 113.8 (=CH), 112.5 (C=) ppm; $[M+H]^+$measured = 288.1141, calcd. for $C_{18}H_{13}N_4$: 285.1134

Method B.

In a three-necked round bottom flask 1 g tryptophan methyl ester (4,6 mmol) was dissolved in 21 mL N-methyl-2-pyrrolidone. To this solution 5.5 mmol of the appropriate aldehyde [**16c:** 1*H*-benzimidazole-2-carboxaldehyde (0.81 g,1.2 equiv.); **16d**: 2-pyridinecarboxaldehyde (0.66 g, 1.2 equiv.)] was added and the mixture was stirred at 140 °C for 24 hours using ambient air as oxidant. The NMP solvent was blown down by nitrogen. The crude material was dissolved in ethyl acetate, and washed with brine. The aqueous layer was extracted with ethyl acetate. The combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in *vacuo*.

*Methyl 1-(1H-benzo[d]imidazol-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**16c**)*

Yield: 1.1 g (67 %), brown powder, m. p. 219-220 °C (NMP), ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.10 (s, 1H, NH), 12.09 (s, 1H, NH), 9.06 (d, J = 4.6 Hz, 1H, ArH), 8.47 (d, J = 8.0 Hz, 1H, ArH), 8.06 (d, J = 8.2 Hz, 1H, ArH), 7.91 (d, J = 7.8 Hz, 1H, ArH), 7.69 (dd, J = 25.2, 7.7 Hz, 2H, ArH), 7.43 – 7.27 (m, 3H, ArH), 4.01 (s, 3H,CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (C=O), 150.6 (=CH), 144.3 (C=), 142.2 (=CH), 136.8 (=CH), 135.1 (C=), 135.0 (C=), 131.9 (=CH), 130.2 (C=), 129.5 (=CH), 124.0 (C=), 122.6 (=CH), 121.3 (C=), 121.2 (=CH), 119.8 (=C), 118.8 (=CH), 114.2 (=CH), 113.0 (C=), 52.7 (OCH_3) ppm; $[M+H]^+$measured = 343.1195, calcd. for $C_{20}H_{15}N_4O_2$: 343.1189.

*Methyl 1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**16d**)*

Yield: 0.21 g (15 %), brown powder, m. p. 180-181 °C (MeOH) (lit. 181-182 °C [5]); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.28 (s, 1H, NH), 9.01 (s, 1H, ArH), 8.91 (dd, J = 5.0, 1.6 Hz, 1H, ArH), 8.67 (d, J = 8.0 Hz, 1H, ArH), 8.45 (d, J = 7.8 Hz, 1H, ArH), 8.09 (td, J = 7.7, 1.8 Hz, 1H, ArH), 7.94 (d, J = 8.2 Hz, 1H, ArH), 7.64 (t, J = 7.7 Hz, 1H, ArH), 7.57 (dd, J = 7.4, 4.9 Hz, 1H, ArH), 7.35 (t, J = 7.5 Hz, 1H, ArH), 3.98 (s, 3H, CH_3) ppm.

Diazaborinino-carbolines (**12a-d**)

In a Schlenk tube 1 mmol of **16** carboline (**16a**: 0.23 g, **16b**: 0.28 g, **16c**: 0.34 g, **16d**: 0.3 g; **16e**: 0.29 g) was dissolved in 20 mL absolute tetrahydrofuran. To this solution 1.6 mL boron trifluoride diethyl etherate (1.9 g, 20 mmol, 20 equiv.) and 0.17 g Na_2CO_3 base (2.5 mmol, 2.5 equiv.) was added. Then the mixture was stirred at 85 °C for 22 hours under argon atmosphere. The reaction mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The product was precipitated by adding diethyl ether to the residue.

*9-(difluoroboranyl)-1-(1H-imidazole-2-yl)-9H-pyrido[3,4-b]indole (**12a**)*

Yield: 0.076 g (27 %), brown powder, m. p. 247-248 °C (Et_2O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51 (d, J = 5.2 Hz, 1H, ArH), 8.33 – 8.27 (m, 2H, ArH), 7.84 – 7.79 (m, 2H, ArH), 7.75 (d, J = 8.1 Hz, 1H, ArH), 7.63 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H, ArH), 7.32 (t, J = 7.5 Hz, 1H, ArH) ppm; ^{13}C NMR (125 MHz, DMSO) δ 144.1 (C=), 142.2 (C=), 138.7 (=CH), 130.5 (C=), 129.5 (=CH), 126.1 (C=), 123.3 (=CH), 122.9 (C=), 122.5 (C=), 121.8 (C=), 120.9 (=CH), 117.9 (=CH), 114.3 (=CH), 113.1 (C=) ppm; $[M+H]^+$measured = 283.0968, calcd. for $C_{14}H_{10}BN_4F_2$: 283.0961.

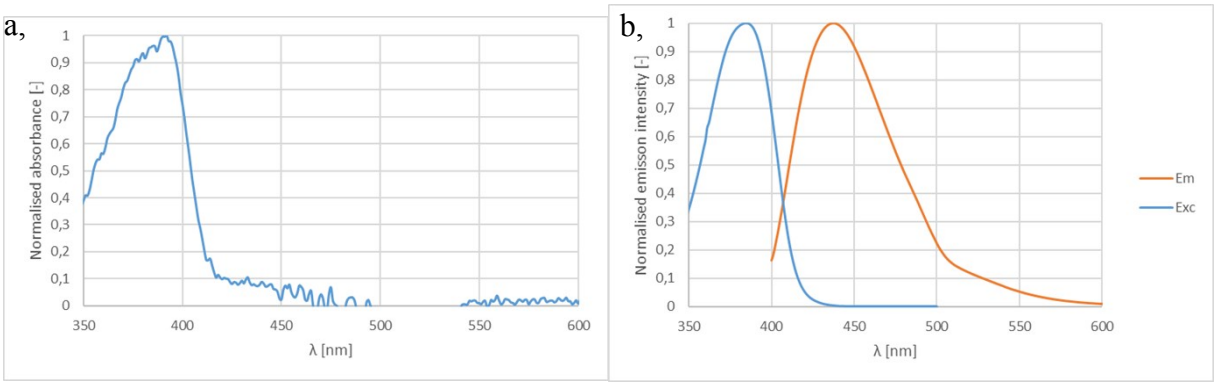

Figure S5: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **12a**

*1-(1H-benzo[d]imidazole-2-yl)-9-(difluoroboranyl)-9H-pyrido[3,4-b]indole (**12b**)*

Yield: 0.32 g (95 %); brown powder, m. p. 261-262 °C (Et_2O);^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (d, J = 5.1 Hz, 1H, ArH), 8.43 (d, J = 5.1 Hz, 1H, ArH), 8.38 (d, J = 7.8 Hz, 1H, ArH), 8.03 (d, J = 7.5 Hz, 1H, ArH), 7.86 – 7.79 (m, 2H, ArH), 7.70 (d, J = 7.7 Hz, 1H, ArH), 7.59 (td, J = 6.9, 6.0, 3.8 Hz, 2H, ArH), 7.38 (t, J = 7.5 Hz, 1H, ArH) ppm; ^{13}C NMR (125 MHz, DMSO) δ 147.3 (C=), 144.3 (C=), 139.4 (=CH), 134.1 (C=), 133.5 (C=), 130.0 (C=), 129.9 (=CH), 126.4 (=CH), 125.9 (=CH), 125.1 (C=), 123.5 (=CH), 122.8 (C=), 121.1 (=CH), 119.3 (=CH), 116.1 (=CH), 114.5 (=CH), 114.3 (=CH) ppm; $[M+H]^+$measured = 333.1125, calcd. for $C_{18}H_{12}BN_4F_2$: 333.1117.

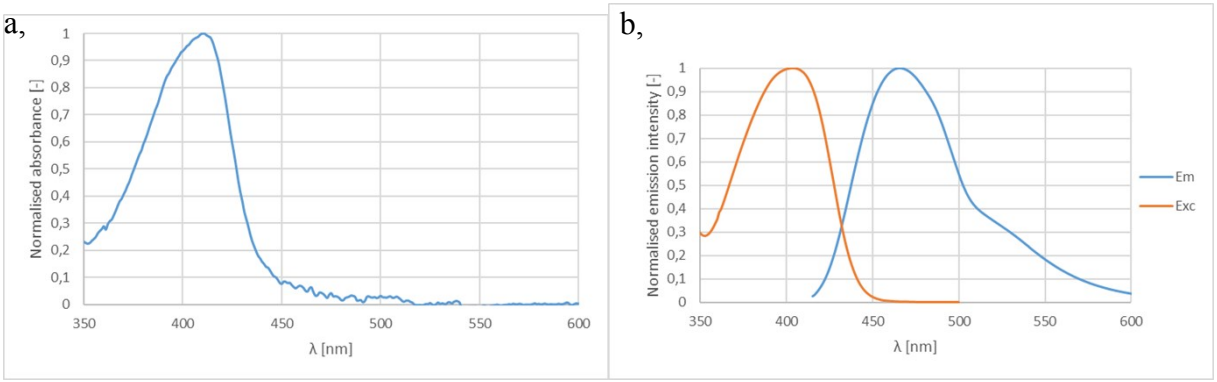

Figure S6: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **12b**

*Methyl 1-(1H-benzo[d]imidazol-2-yl)-9-(difluoroboranyl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12c**)*

Yield: 0.25 g (63 %), brown powder, m. p. 136-137 °C (Et_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (s, 1H, ArH), 8.24 (d, *J* = 7.7 Hz, 2H, ArH), 8.10 (d, *J* = 8.4 Hz, 1H, ArH), 7.78 (t, *J* = 7.8 Hz, 2H, ArH), 7.62 (td, *J* = 5.3, 2.4 Hz, 2H, ArH), 7.45 (t, *J* = 7.6 Hz, 1H, ArH), 4.08 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO) *δ* 165.5 (C=O), 151.0 (C=), 145.4 (C=), 138.9 (C=), 134.2 (C=), 131.9 (C=), 130.9 (C=), 130.3 (=CH), 129.7 (C=), 126.5 (=CH), 126.0 (=CH), 122.5 (=CH), 121.7 (=CH), 121.4 (C=), 120.2 (=CH), 116.9 (=CH), 115.2 (=CH), 113.3 (=CH), 52.8 (OCH_3) ppm; $[M+H]^+$measured = 391.1174, calcd. for $C_{20}H_{14}BN_4O_2F_2$: 391.1172.

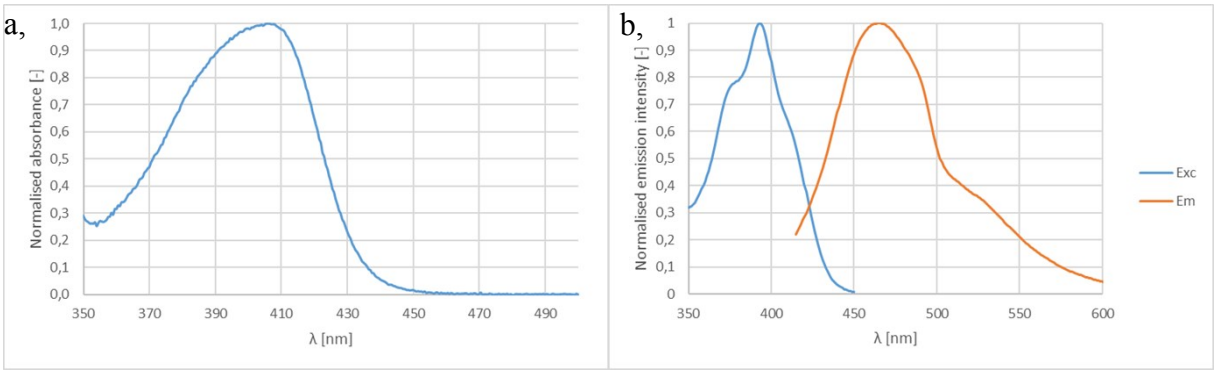

Figure S7: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **12c**

*Methyl 9-(difluoroboranyl)-1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12d**)*

The residue was purified by flash chromatography on silica gel using dichloromethane. For NMR purpose the crude product purified with reverse phase column chromatography using acetonitrile/water.

Yield: 0.26 g (74 %), yellow crystals, m. p. 231-232 °C (water-MeCN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (d, *J* = 8.2 Hz, 1H, ArH), 9.02 (d, *J* = 7.4 Hz, 2H, ArH), 8.37 (t, *J* = 7.8 Hz, 1H,

ArH), 8.24 (d, J = 7.9 Hz, 1H, ArH), 7.99 (d, J = 8.3 Hz, 1H, ArH), 7.82 (t, J = 6.8 Hz, 1H, ArH), 7.70 (t, J = 7.7 Hz, 1H, ArH), 7.42 (t, J = 7.6 Hz, 1H, ArH), 4.10 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, CDCl3) δ 166.3 (C=O), 150.0 (C=), 144.7 (C=), 143.2 (=CH), 142.6 (=CH), 137.9 (C=), 131.2 (C=), 129.9 (=CH), 129.4 (C=), 124.9 (=CH), 123.0 (C=), 122.43 (=CH), 122.35 (=CH), 121.8 (=CH), 120.7 (=CH), 114.8 (=CH), 52.8 (OCH_3) ppm; $[M+H]^+$measured = 391.1174, calcd. for $C_{20}H_{14}BN_4O_2F_2$: 391.1172.

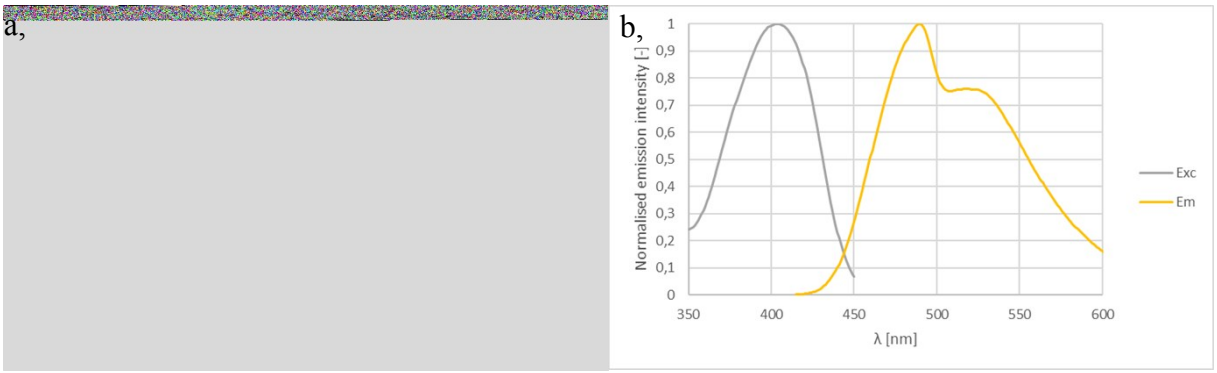

Figure S8: a, Normalised absorbance spectrum and b, normalised excitation and emission spectra of **12d**

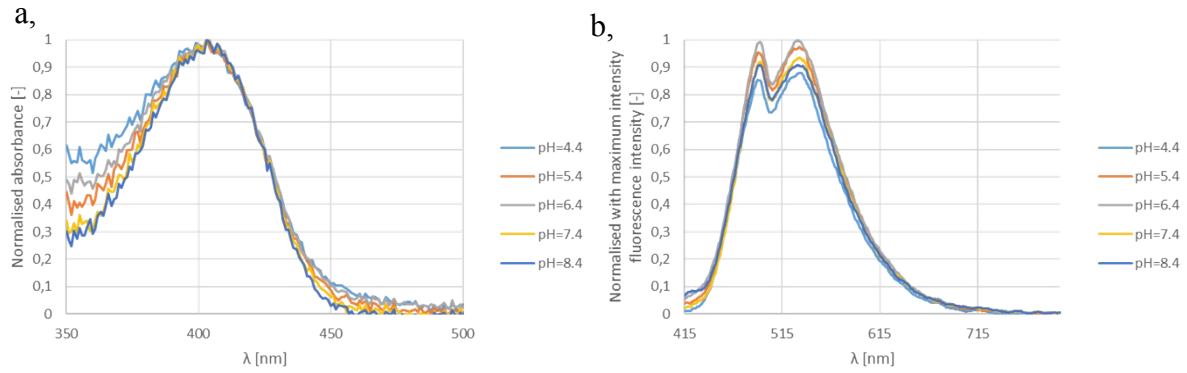

Figure S9: a, Normalised absorbance spectrum and b, normalised emission spectra of **12d** in 10 mM HEPES buffer at different pHs

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a, b,

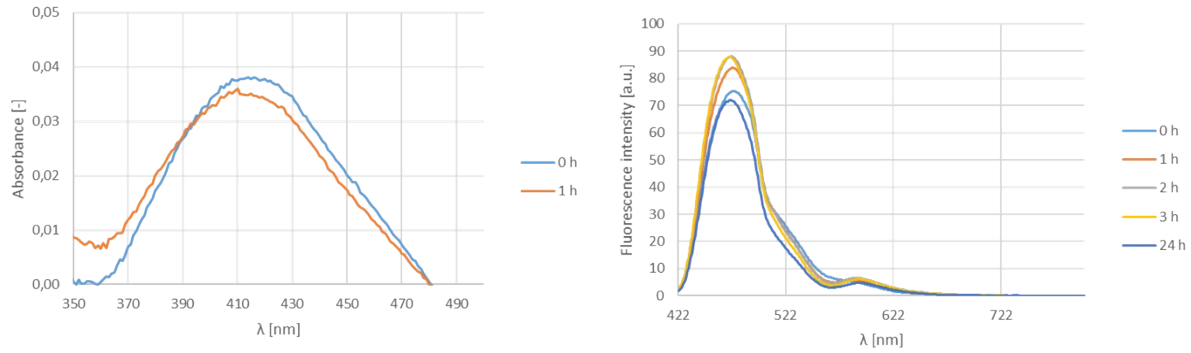


Figure S10: a, Absorbance spectra and b, emission spectra of 5 µM **12d** in 10% fetal bovine serum containing DMEM

Cytotoxic assay 24 h

The treatment with **12d** for 24 h did not show cytotoxicity after 24 h on MRC-5 cells, where IC_{50} was over 100 µM.

Table S1. IC_{50} values in cytotox assay (24h).

Compound	**TOX 24 h IC_{50} [uM]**			
	MRC-5			
	1st	**2nd**	**AVG**	**SD**
HI63	>100	>100	>100	

AVG: Average from 2 experiments. SD: Standard deviation.

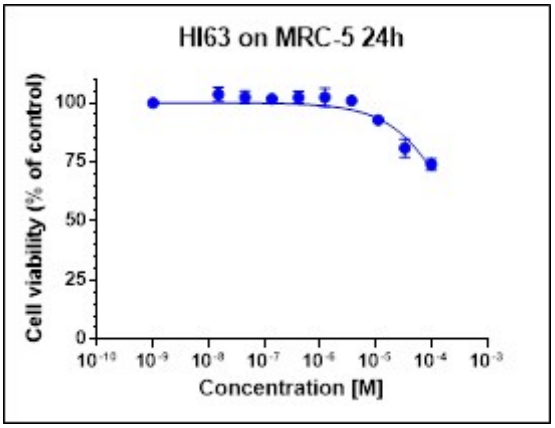


Figure S11. Cell viability curves of MRC-5 under 12d treatment in cytotox assay (24h).

Anti-proliferative assay 72 h

The treatment with **12d** for 24 h did not show anti-proliferative effect after 72 h on MRC-5 cells, where IC_{50} was over 100 μM.

Table S2. IC_{50} values in anti-proliferative assay (72h).

Compound	**Anti-Prolif 72 h IC_{50} [uM]**			
	MRC-5			
	1st	**2nd**	**AVG**	**SD**
HI63	>100	>100	>100	

AVG: Average from 2 experiments. SD: Standard deviation.

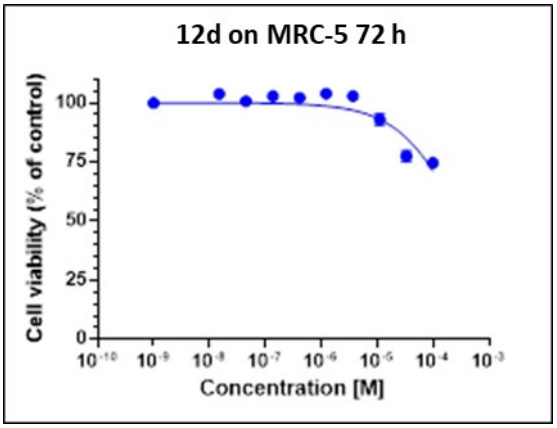


Figure S12. Cell viability curves of MRC-5 under 12d treatment in anti-proliferative assay (72h).

*9-(Difluoroboraneyl)-1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylic acid (**12e**)*

The product was not isolated for spectroscopy.

Yield: 0.11 g (34 %), yellow crystals, m. p. 201-202 °C (DCM-MeOH); $[M+H]^+$measured = 338.0910, calcd. for $C_{17}H_{11}BN_3O_2F_2$: 338.0912.

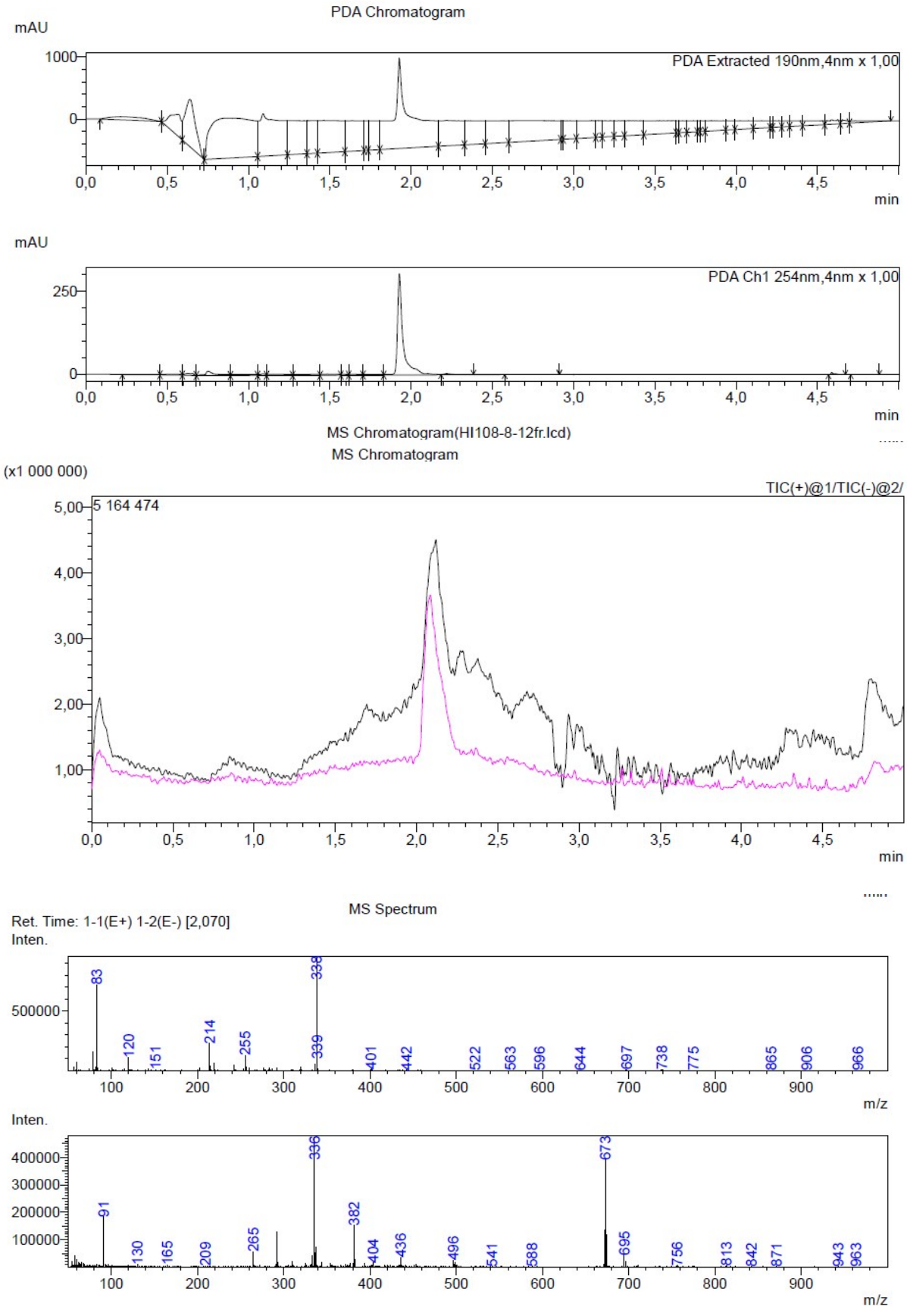


Figure S13:HPLC-MS spectra of **12e**

Hydrolysis of ester-carboline

A two-necked round bottom flask was equipped with thermometer and reflux condenser. 0.5 g of methyl 1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**16d**) (1.6 mmol) was dissolved in 34 ml distilled water and 17 ml THF, and 0.6 g sodium hydroxide (16.5 mmol, 10 equiv.) was added. The reaction mixture was heated at 100 °C for two hours. After the reaction reached the full conversion, the mixture was cooled to room temperature and the THF was evaporated. Then it was acified with 10 % aqueous HCl until reached the pH=3. The precipitated crystals were filtered, washed with distilled water and air dried.

*1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylic acid (**16e**)*

Yield: 0.46 g (97%), orange powder, m. p. 204-205 °C (water), ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.31 (s, 1H, NH), 9.02 (s, 1H, ArH), 8.89 (dd, J = 15.2, 6.4 Hz, 2H, ArH), 8.45 (d, J = 7.9 Hz, 1H, ArH), 8.11 (t, J = 7.8 Hz, 1H, ArH), 7.94 (d, J = 8.3 Hz, 1H, ArH), 7.70 – 7.52 (m, 2H, ArH), 7.35 (t, J = 7.4 Hz, 1H, ArH) ppm; ^{13}C NMR (75 MHz, DMSO) δ 167.0 (COOH), 154.8 (C=), 149.1 (=CH), 142.0 (=CH), 138.1 (C=), 136.9 (=CH), 135.4 (C=), 130.9 (=CH), 129.4 (=CH), 124.5 (=CH), 122.5 (C=), 122.3 (=CH), 121.2 (=CH), 121.0 (C=), 118.2 (C=), 113.8 (C=), 110.0 (=CH) ppm.

*2,5-dioxopyrrolidin-1-yl 9-(difluoroboraneyl)-1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12f**)*

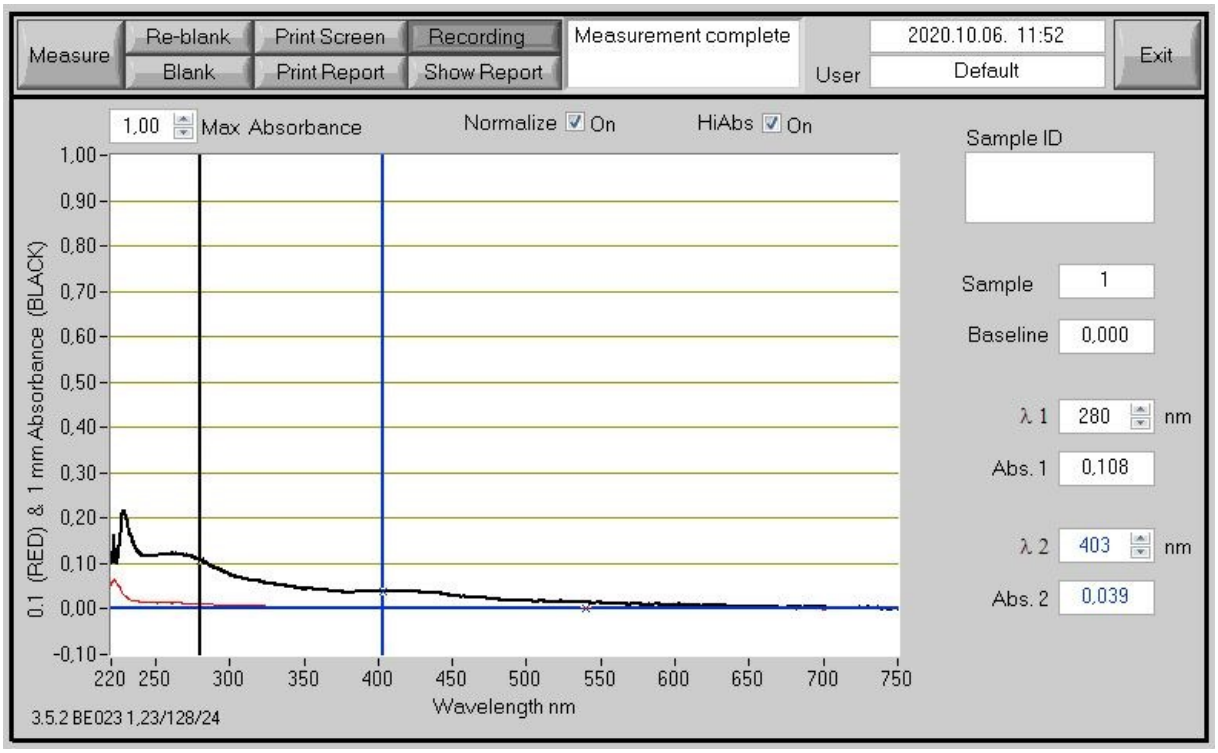
Into a round bottom flask 0. 02 g 9-(difluoroboraneyl)-1-(pyridin-2-yl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**12e**) (0.06 mmol) was solved in 5 ml THF. Then 0.007 g N-hydroxysuccinimide (0.06 mmol, 1 equiv.) and 0.013 g DCC (0.06 mmol, 1 equiv.) was added. The mixture was stirred at room temperature over six hours. After it, the solvent was evaporated and the crude residue was purified by preparative HPLC with water-MeCN (0.1 % HCOOH). The collected fractions were lyophilized.

Yield: 0.0046 g (18%), yellow crystals, m. p. 255-256 °C (water-MeCN); ^{1}H NMR (500 MHz, Acetonitrile-d_3) δ 9.22 (s, 1H, ArH), 9.14 – 9.10 (m, 2H, ArH), 8.60 (td, J = 7.9, 1.5 Hz, 1H, ArH), 8.47 – 8.43 (m, 1H, ArH), 8.05 (ddd, J = 7.4, 6.1, 1.4 Hz, 1H, ArH), 7.99 (d, J = 8.3 Hz, 1H, ArH), 7.80 (ddd, J = 8.4, 7.1, 1.2 Hz, 1H, ArH), 7.56 – 7.51 (m, 1H, ArH), 2.96 (s, 4H, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO) δ 170.4 (C=O), 161.5 (C=O), 152.1 (C=), 148.9 (=CH), 144.8 (C=), 144.4 (=CH), 144.3 (C=), 143.6 (=CH), 133.1 (C=), 131.0 (C=), 130.6 (C=), 130.1 (=CH), 126.5 (=CH), 123.1 (=CH), 122.8 (C=), 122.2 (=CH), 122.1 (=CH), 116.4 (=CH),

114.6 (=CH), 114.4 (=CH) 25.6 (CH_2) ppm;$[M+H]^+$measured = 435.1076, calcd. for $C_{21}H_{14}BN_4O_4F_2$: 435.1076.

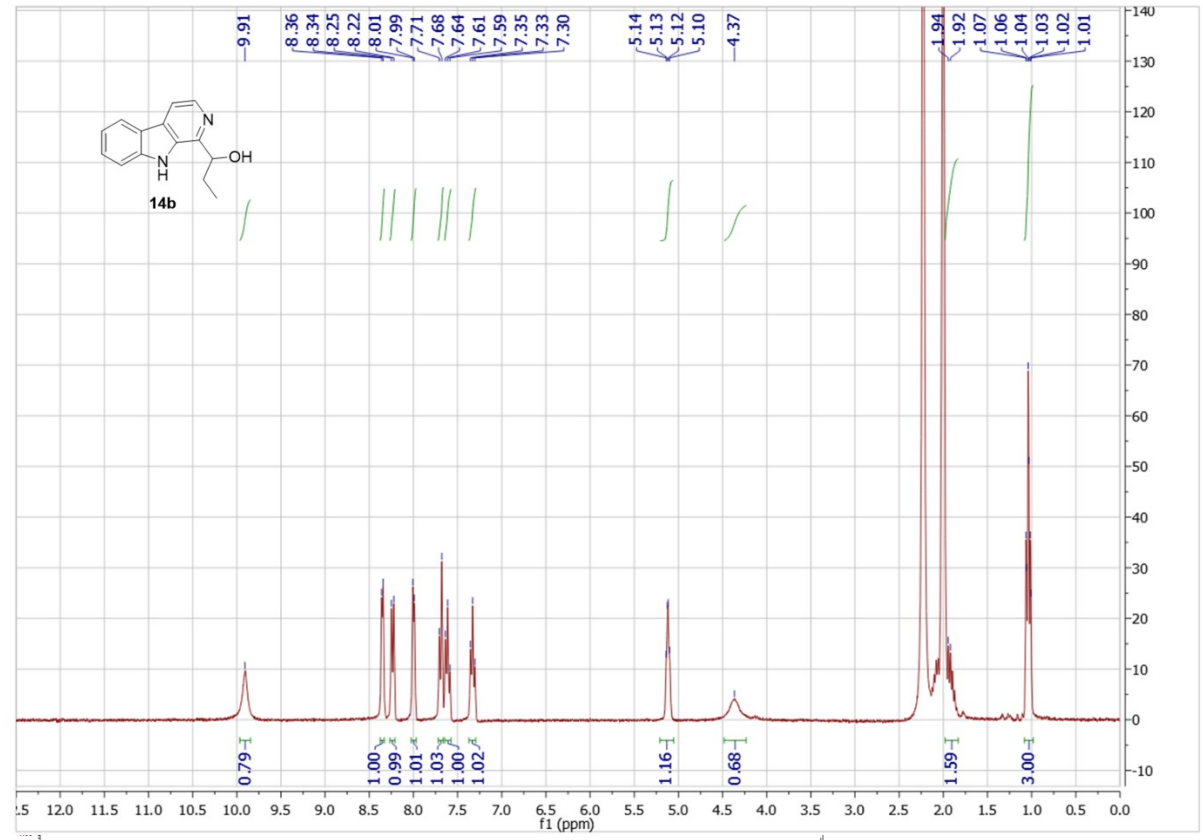
Synthesis of **18** trastuzumab-boro-β-carboline conjugate

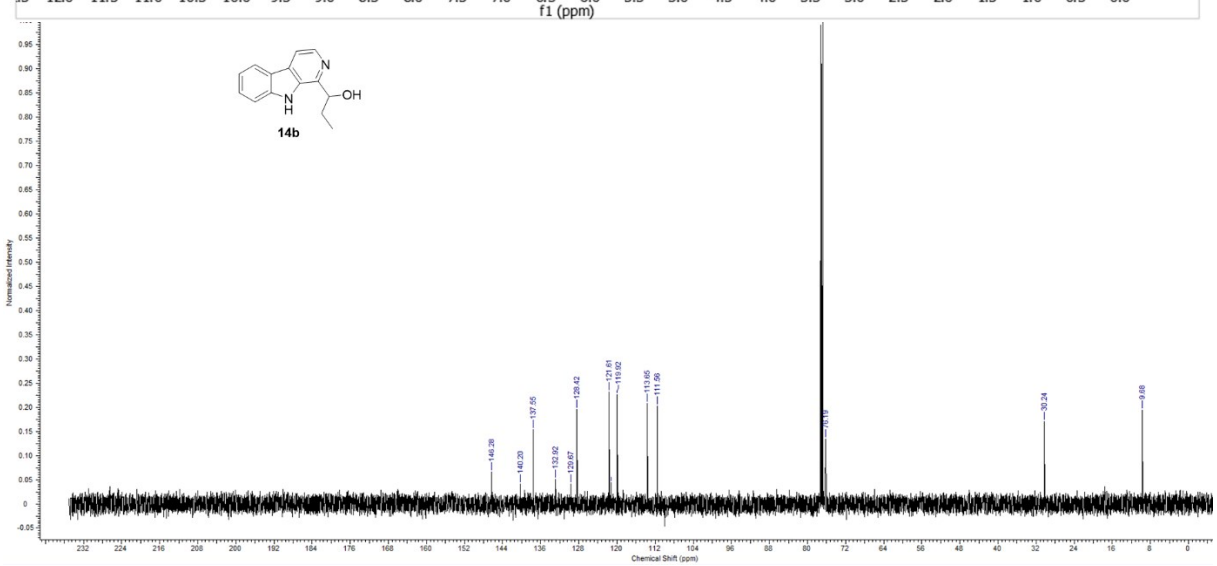
In a microcentrifuge tube 100 µL 10 µM trastuzumab solution in PBS-Na_2CO_3 buffer pH=8.3 was treated with 10 µL 10 mg/mL concentration **12f** carboline solution in DMSO. The mixture was rinsed over 1 hour at room temperature. Then the buffer was changed six times with Sartorius VivaSpin 500 centrifugal filter. The conjugate was investigated with UV/Vis absorbance measurement and non-reducing SDS-PAGE (see at Scheme 3b,). The FAR was turned 18 from the absorbance spectra.



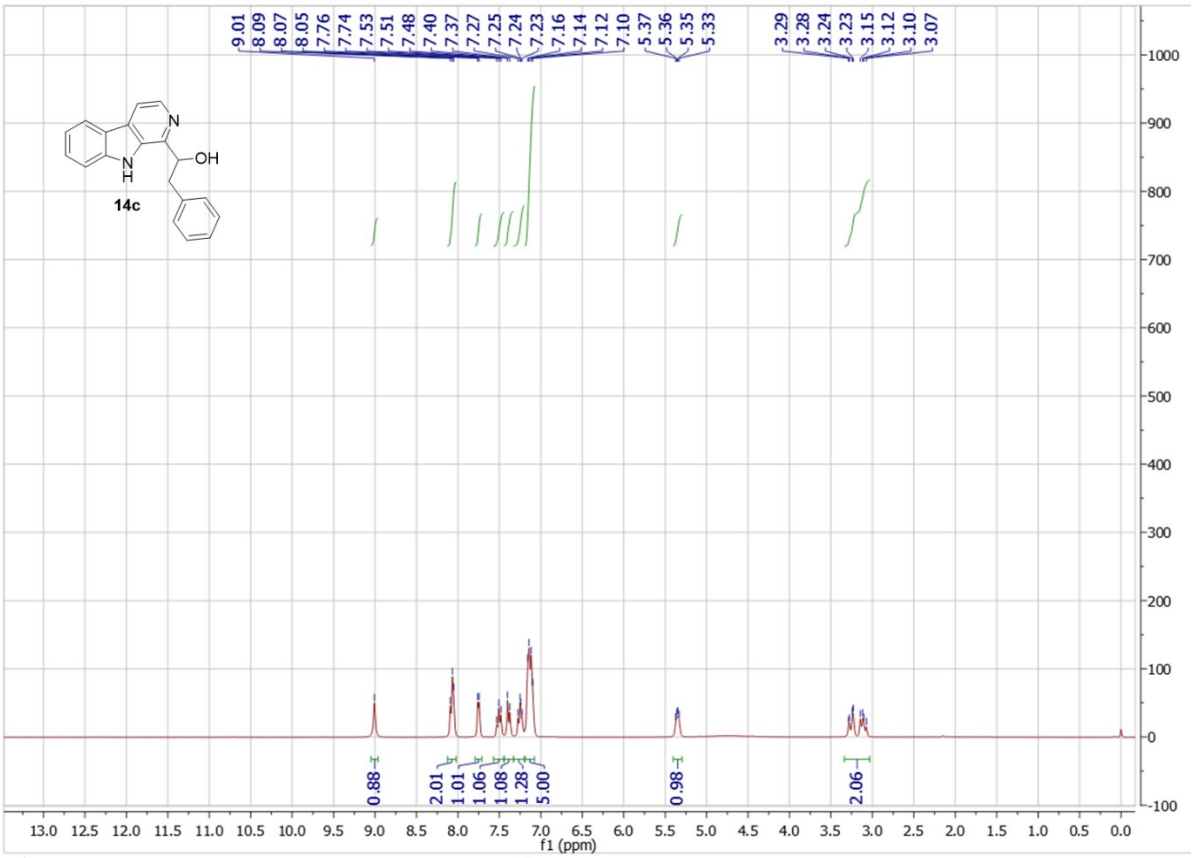
^{1}H and ^{13}C spectra

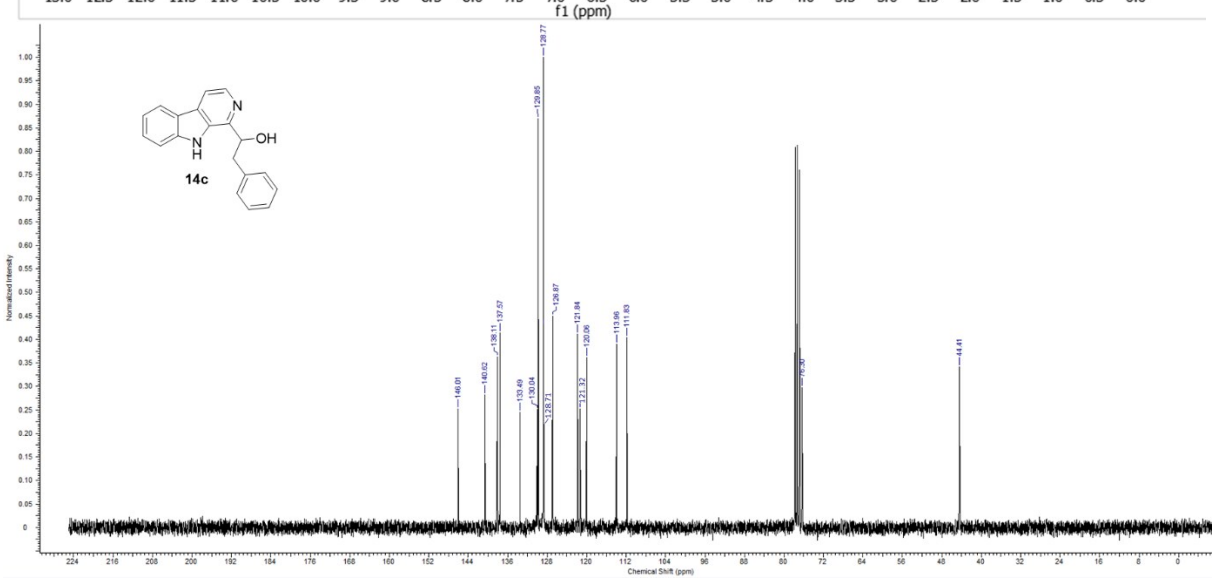
1-(9H-pyrido[3,4-b]indol-1-yl)propan-1-ol ***(14b)***



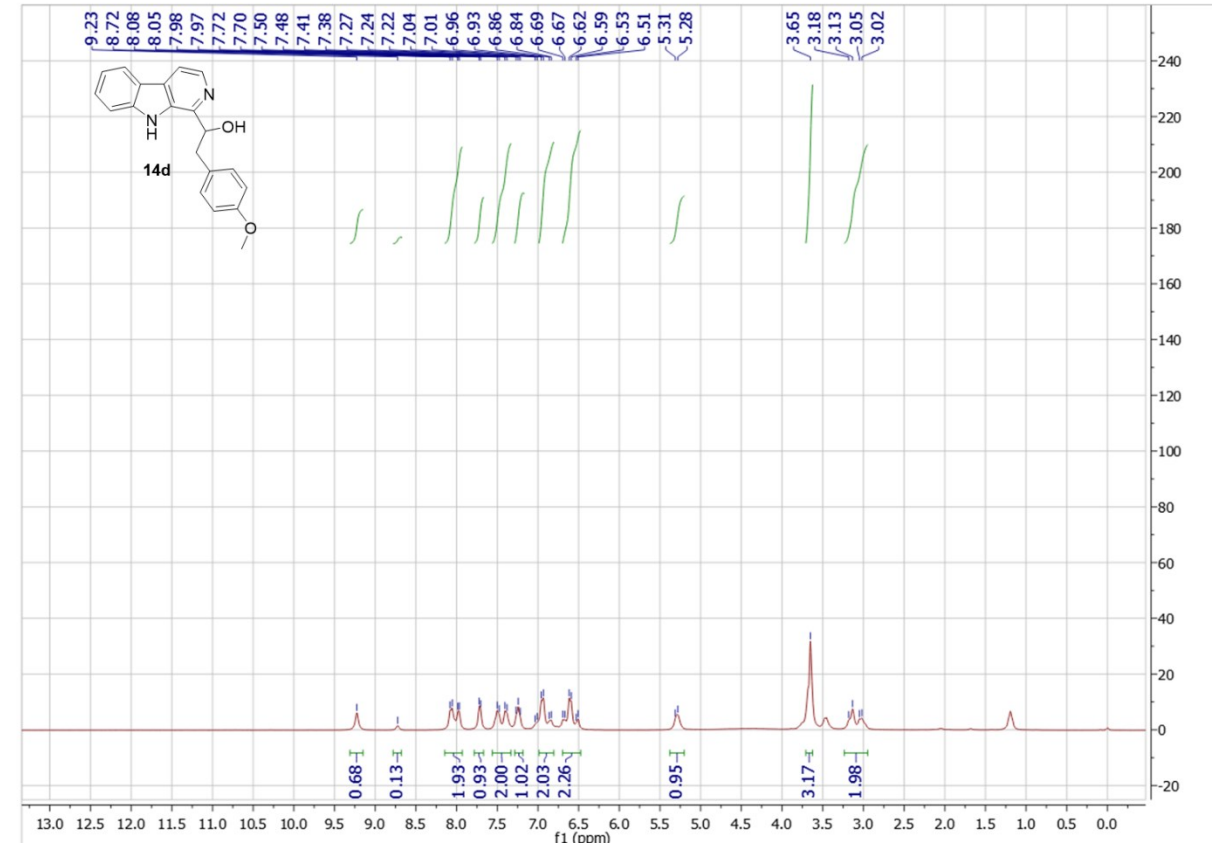


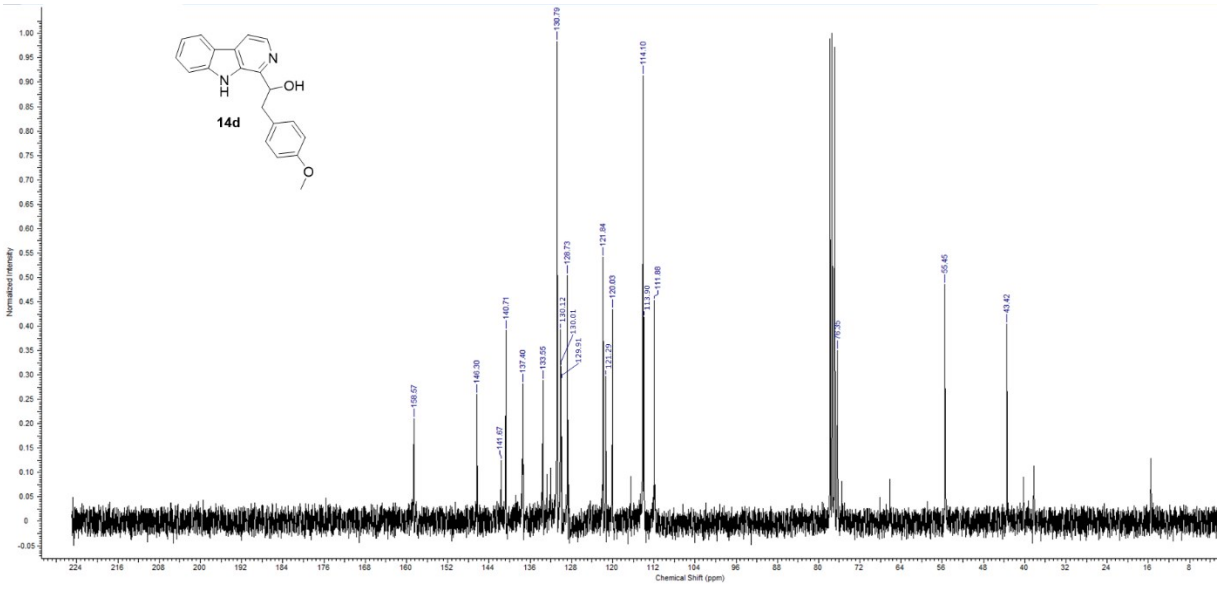
*2-phenyl-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14c**)*



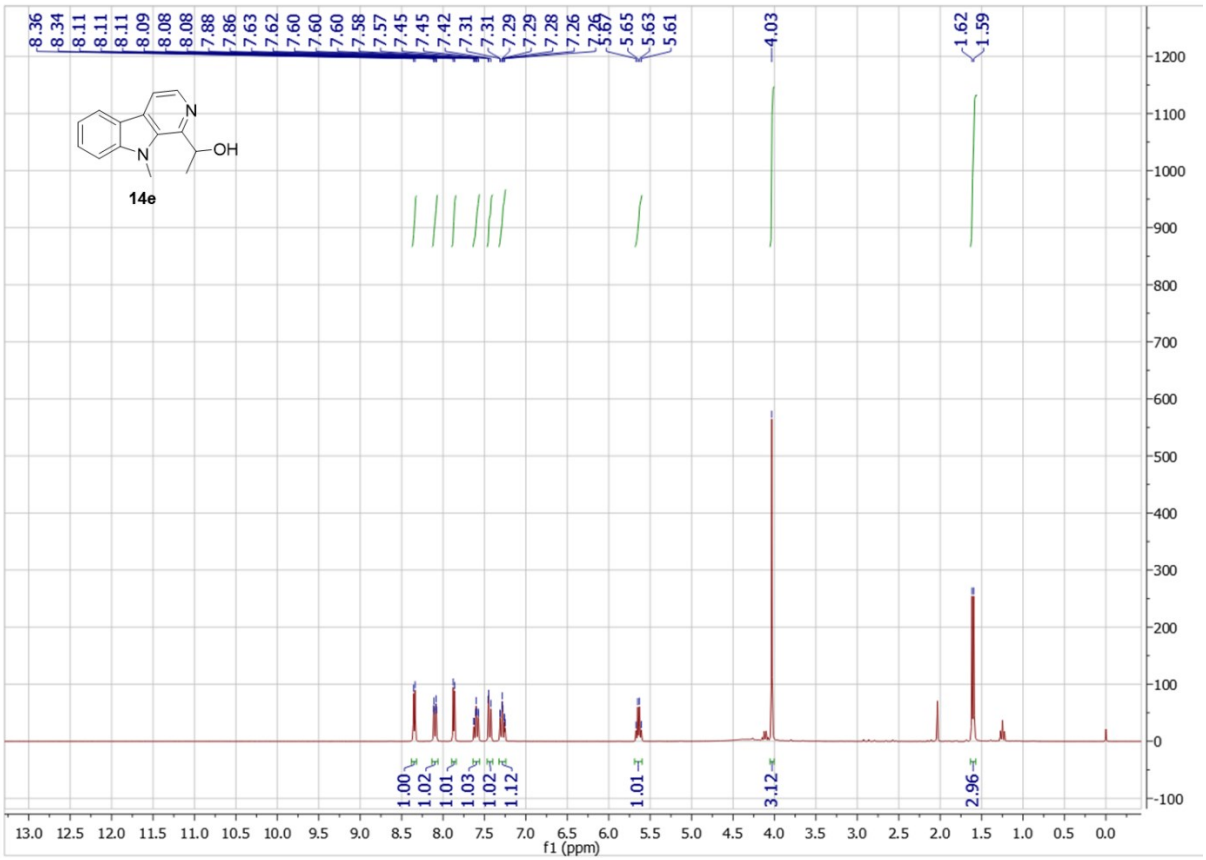


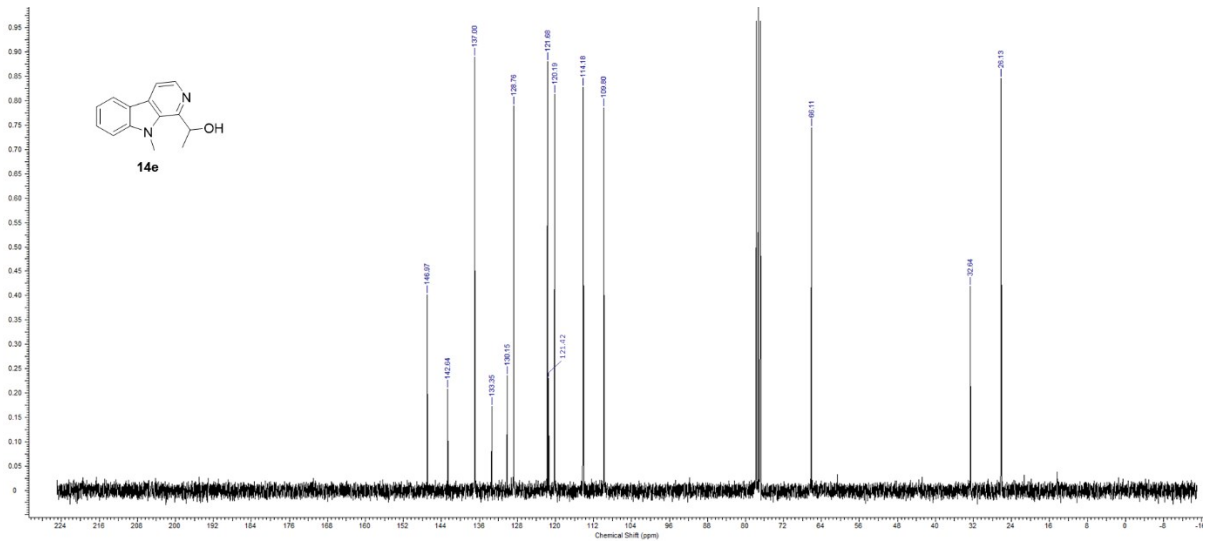
*2-(4-methoxyphenyl)-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14d**)*



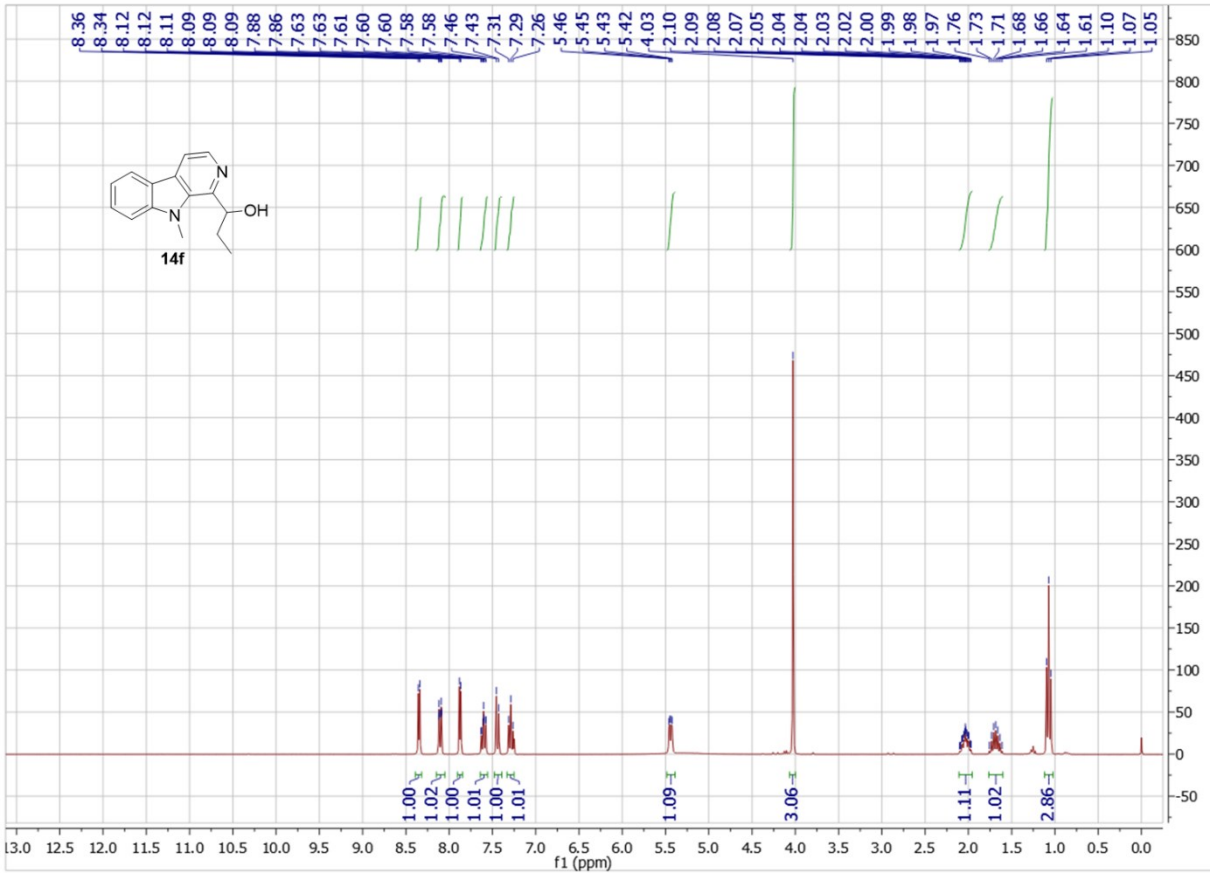


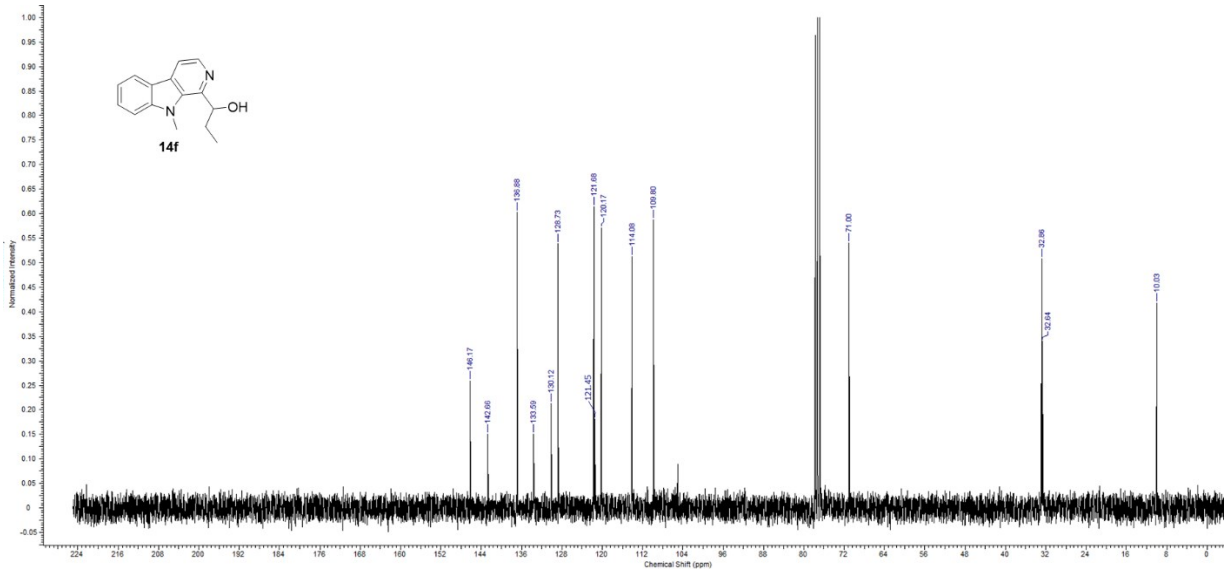
*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)ethan-1-ol (**14e**)*



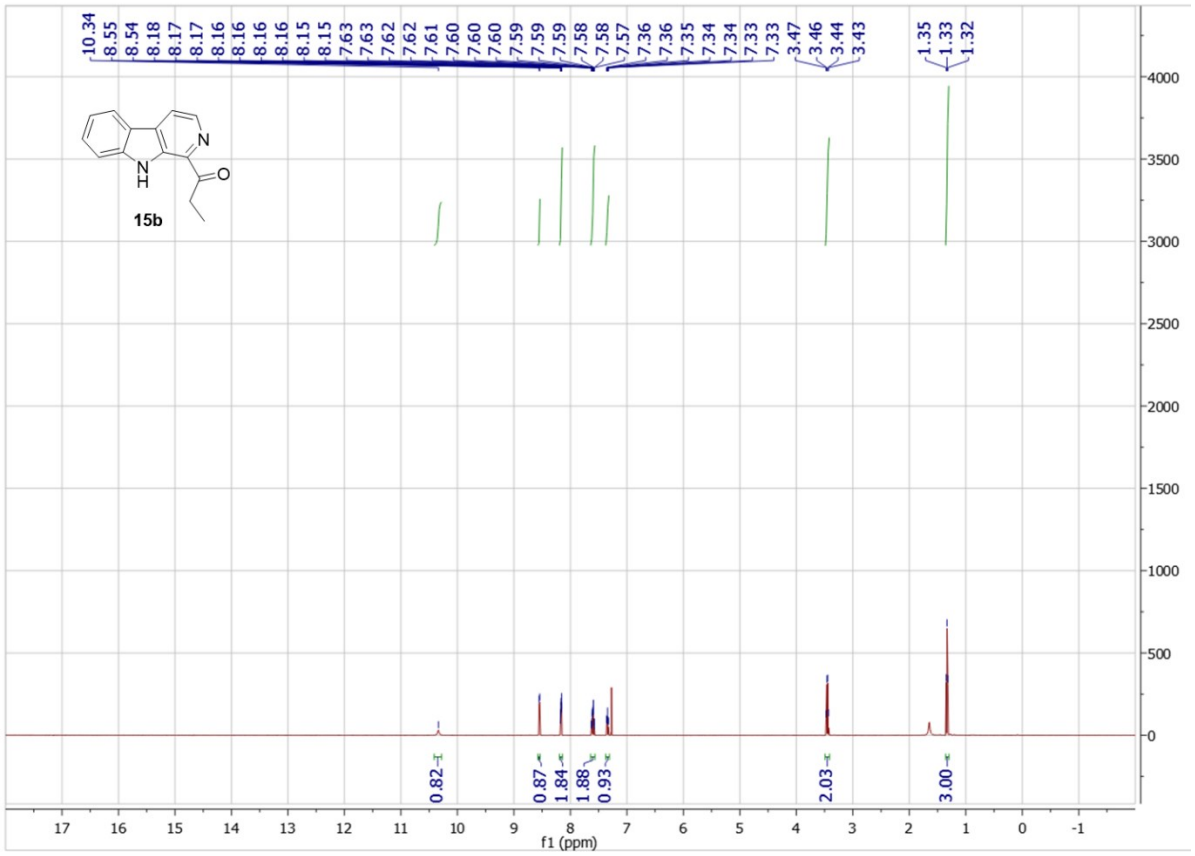


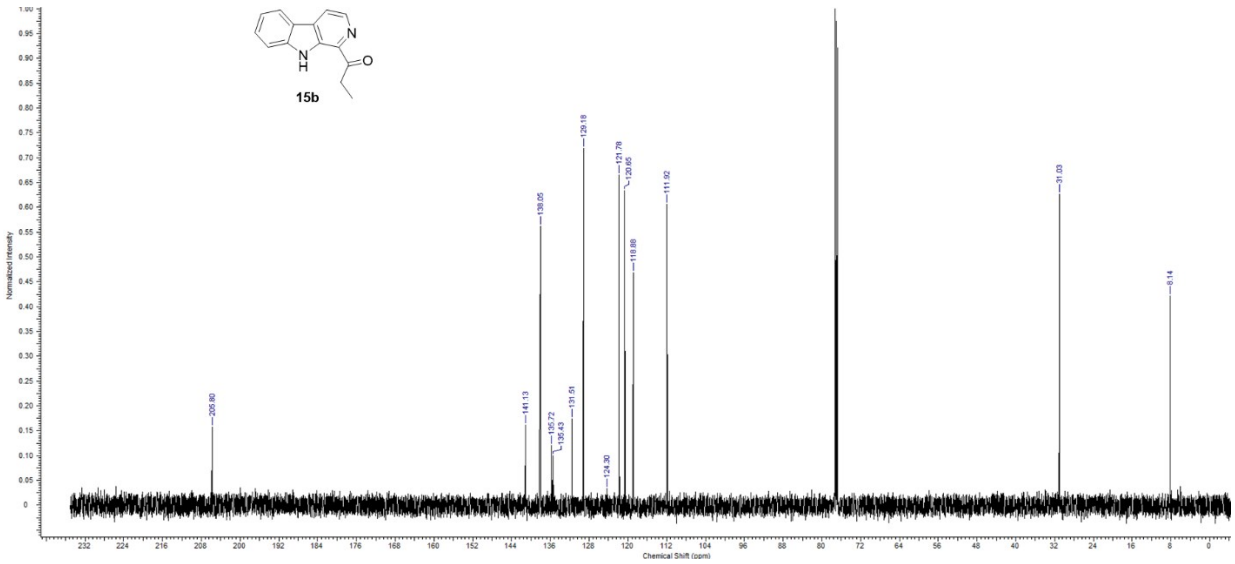
*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)propan-1-ol (**14f**)*



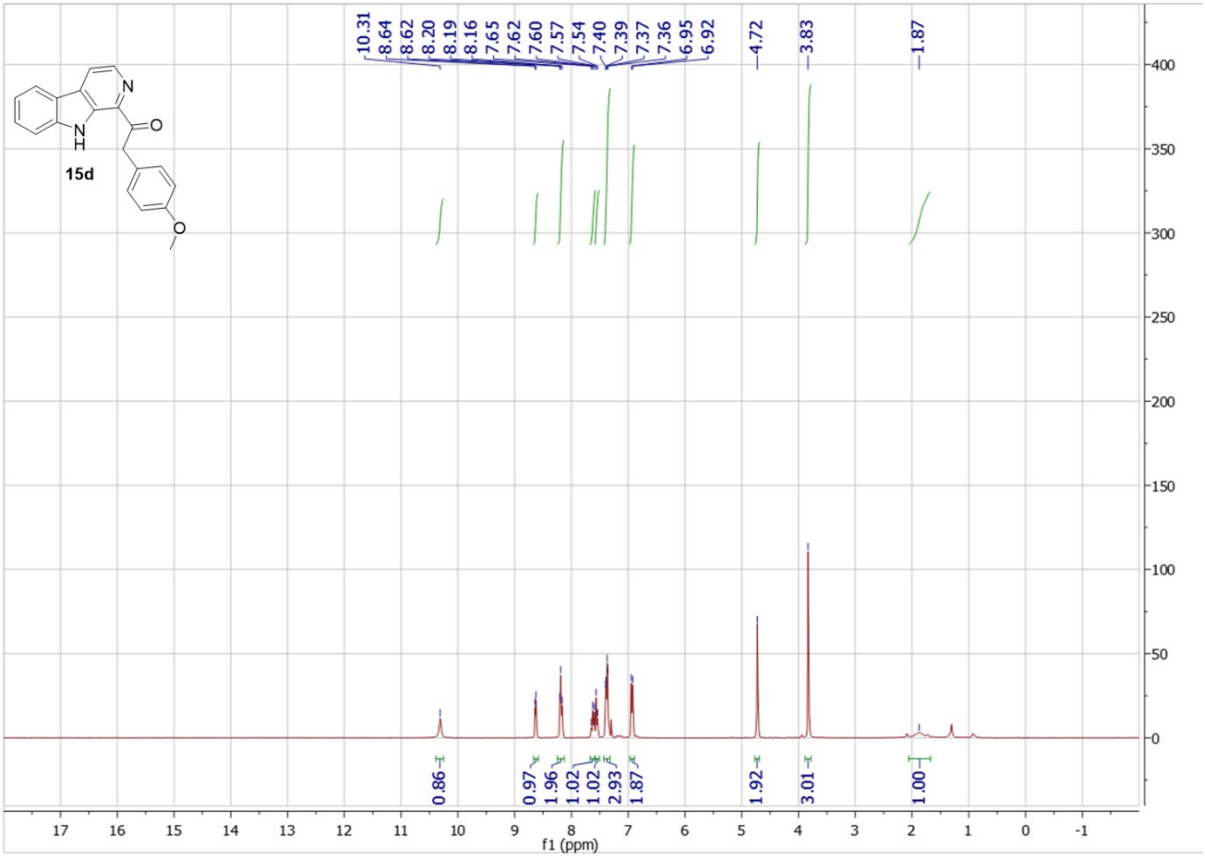


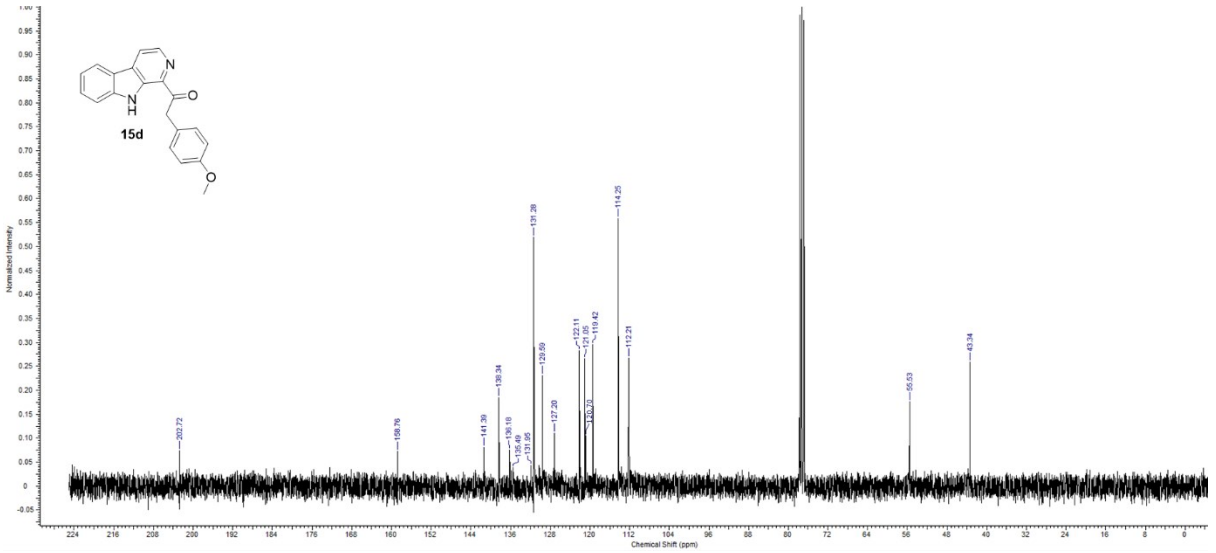
*1-(9H-pyrido[3,4-b]indol-1-yl)propan-1-one (**15b**)*



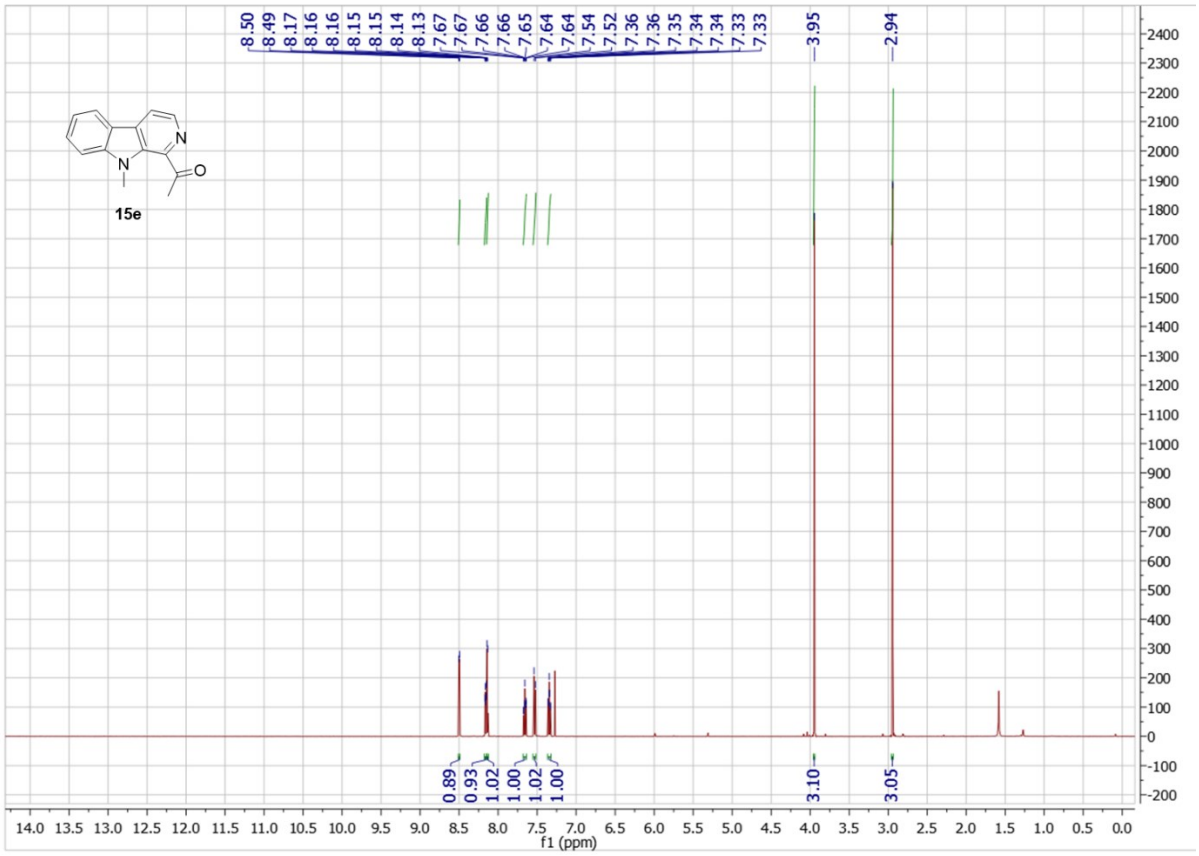


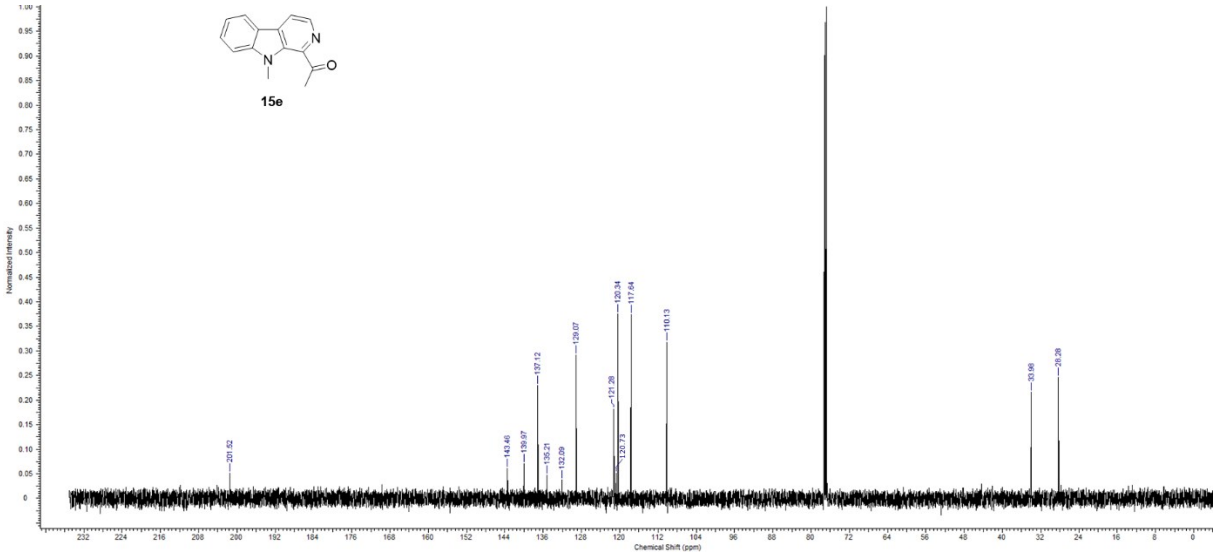
*2-(4-methoxyphenyl)-1-(9H-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15d**)*



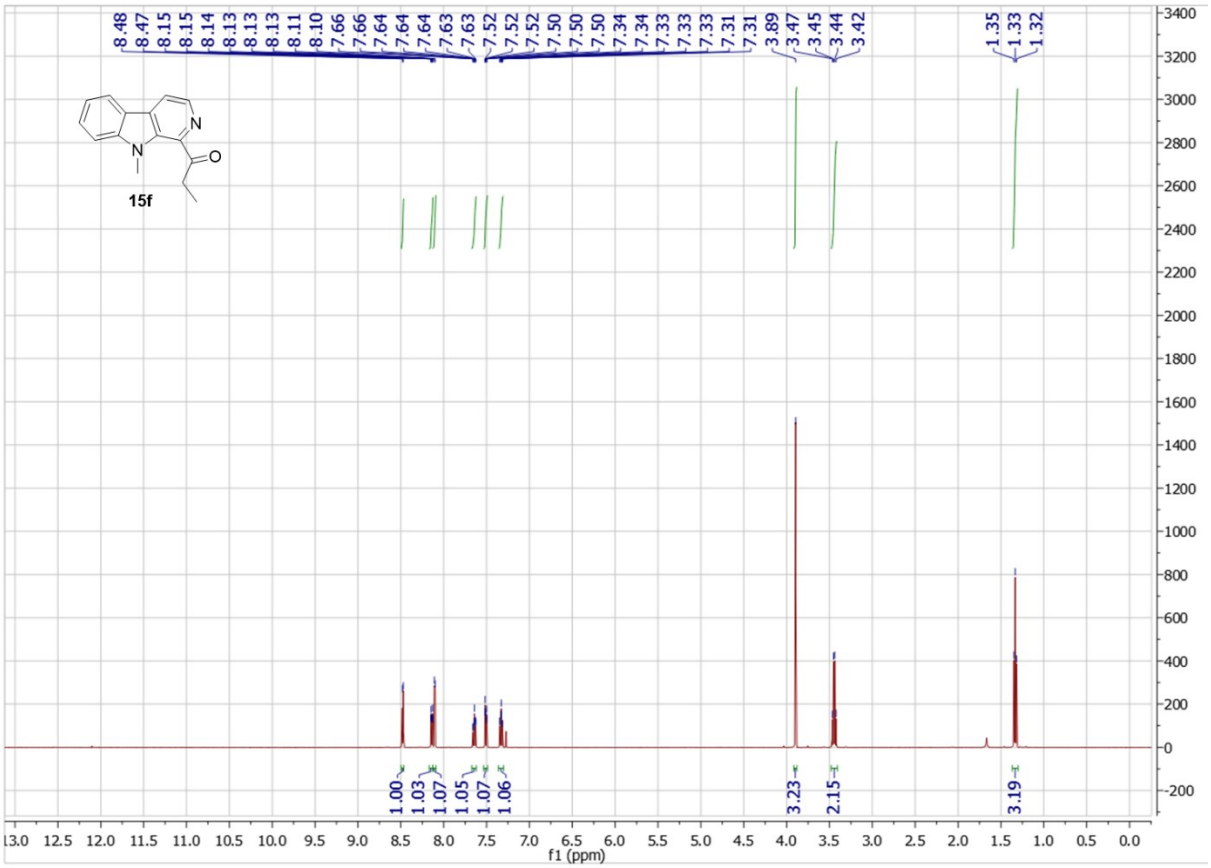


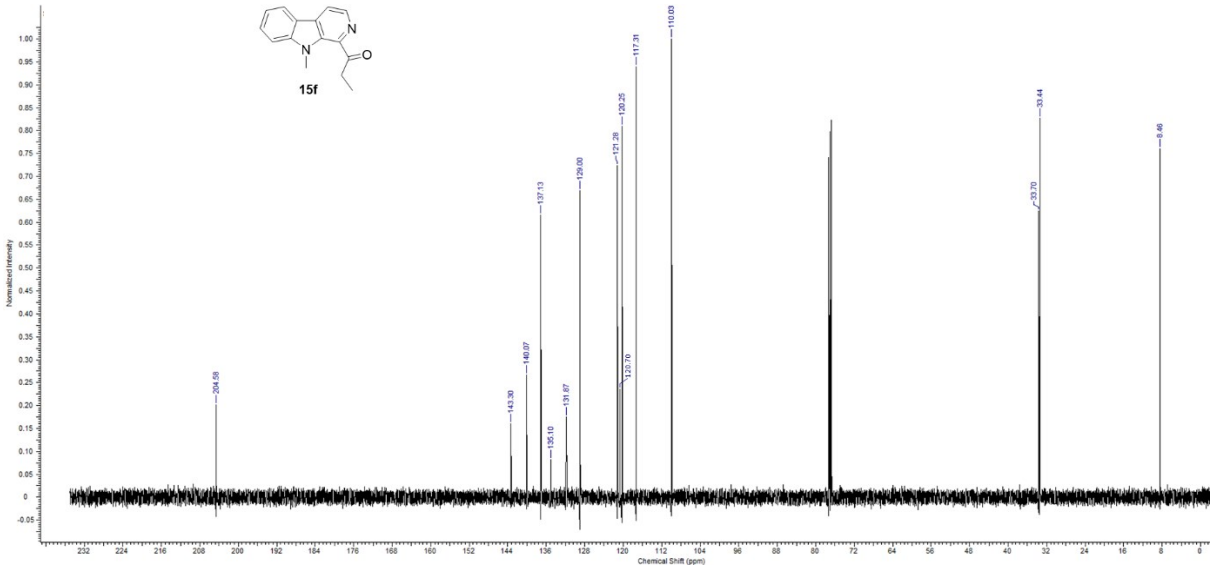
*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)ethan-1-one (**15e**)*





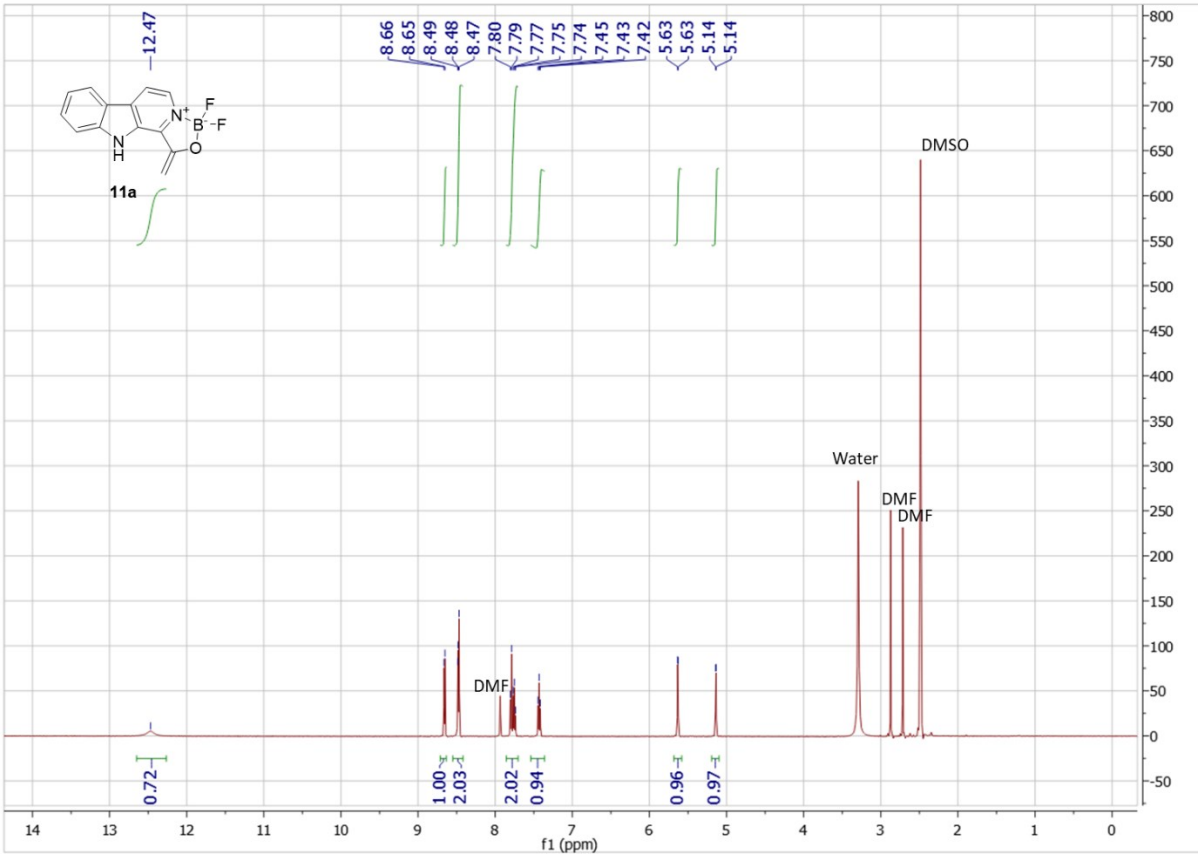
*1-(9-methyl-9H-pyrido[3,4-b]indol-1-yl)propan-1-one (**15f**)*

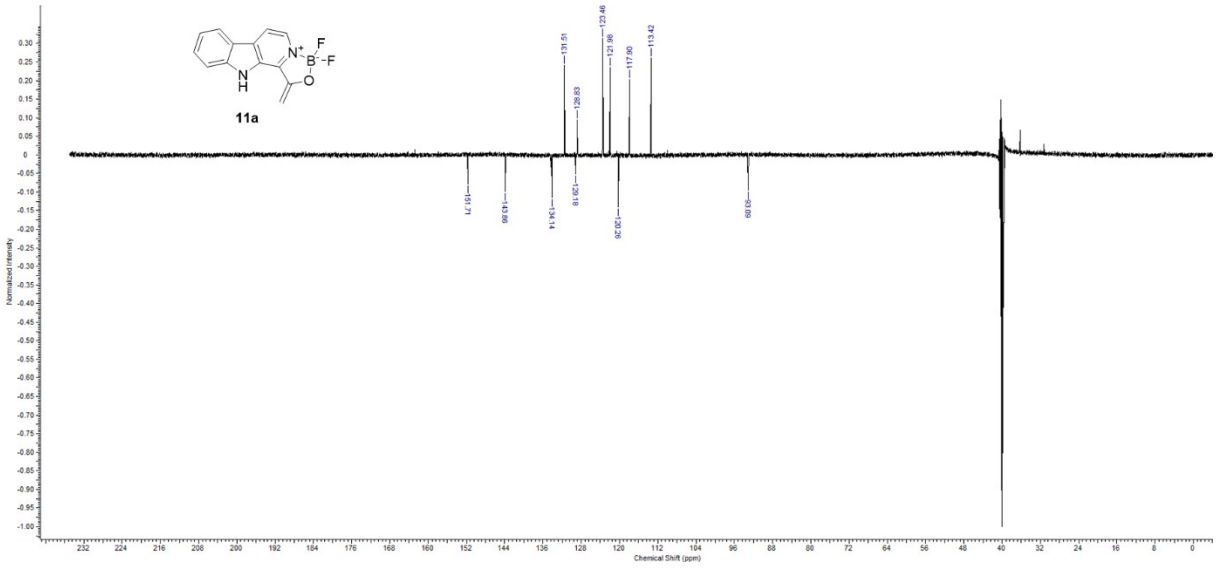




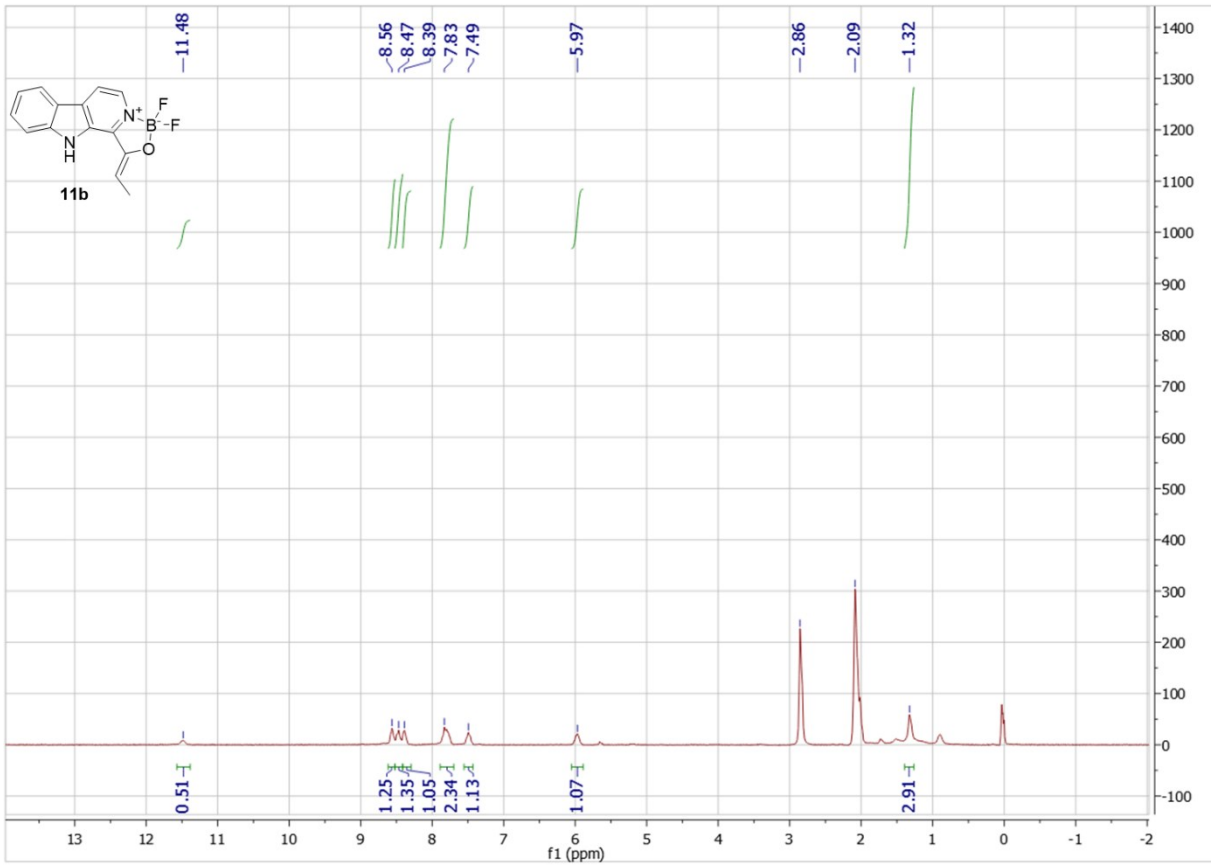
*3,3-difluoro-1-methylene-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole(**11a**)*

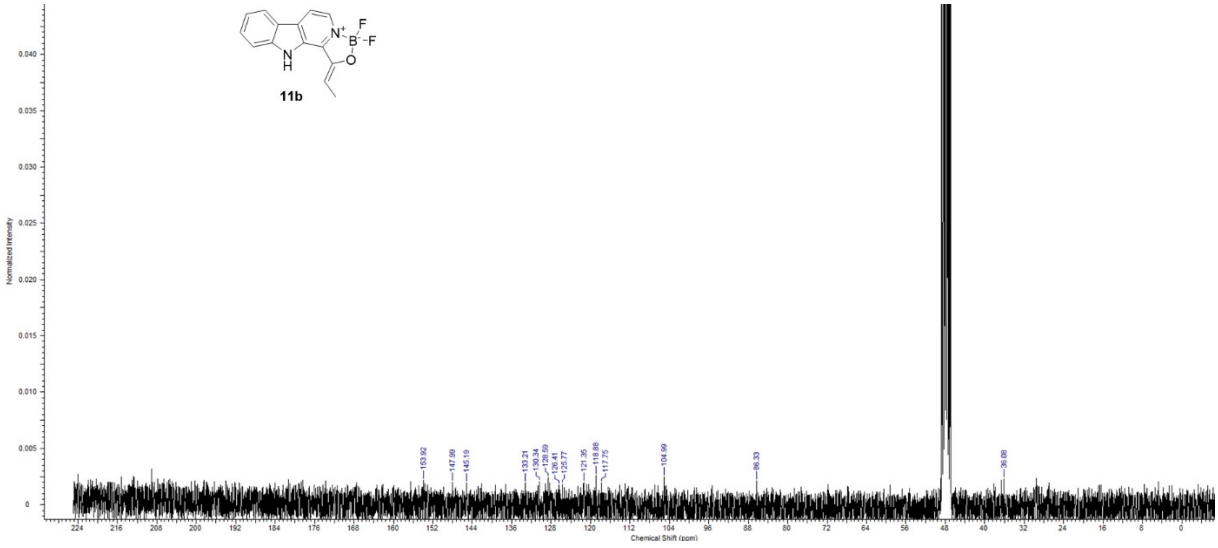
The NMR tube was contaminated with DMF used for tube washing.



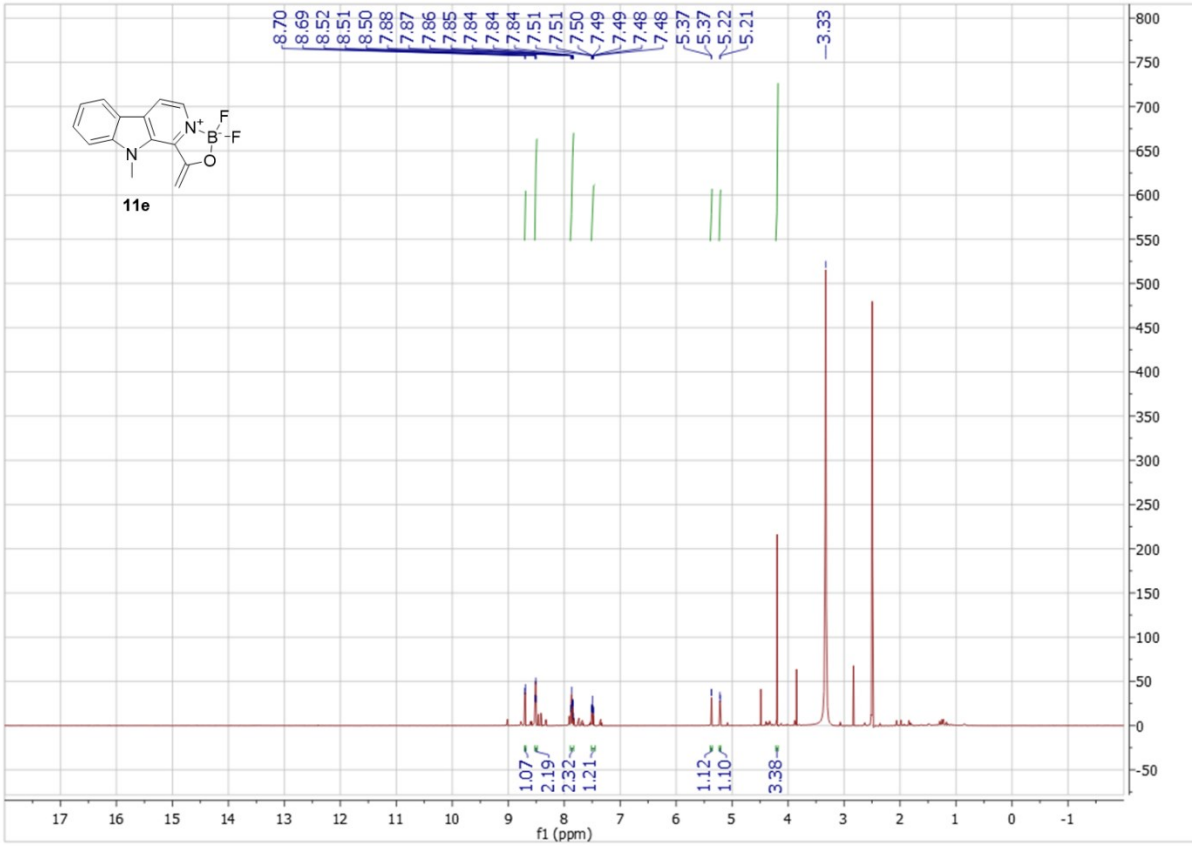


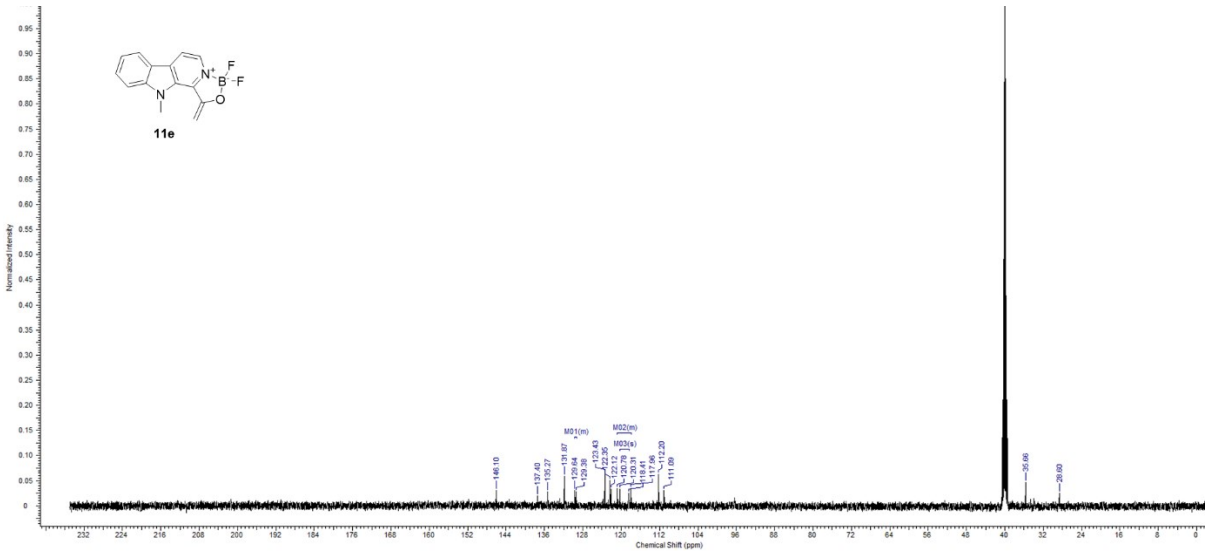
*(Z)-1-ethylidene-3,3-difluoro-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11b**)*



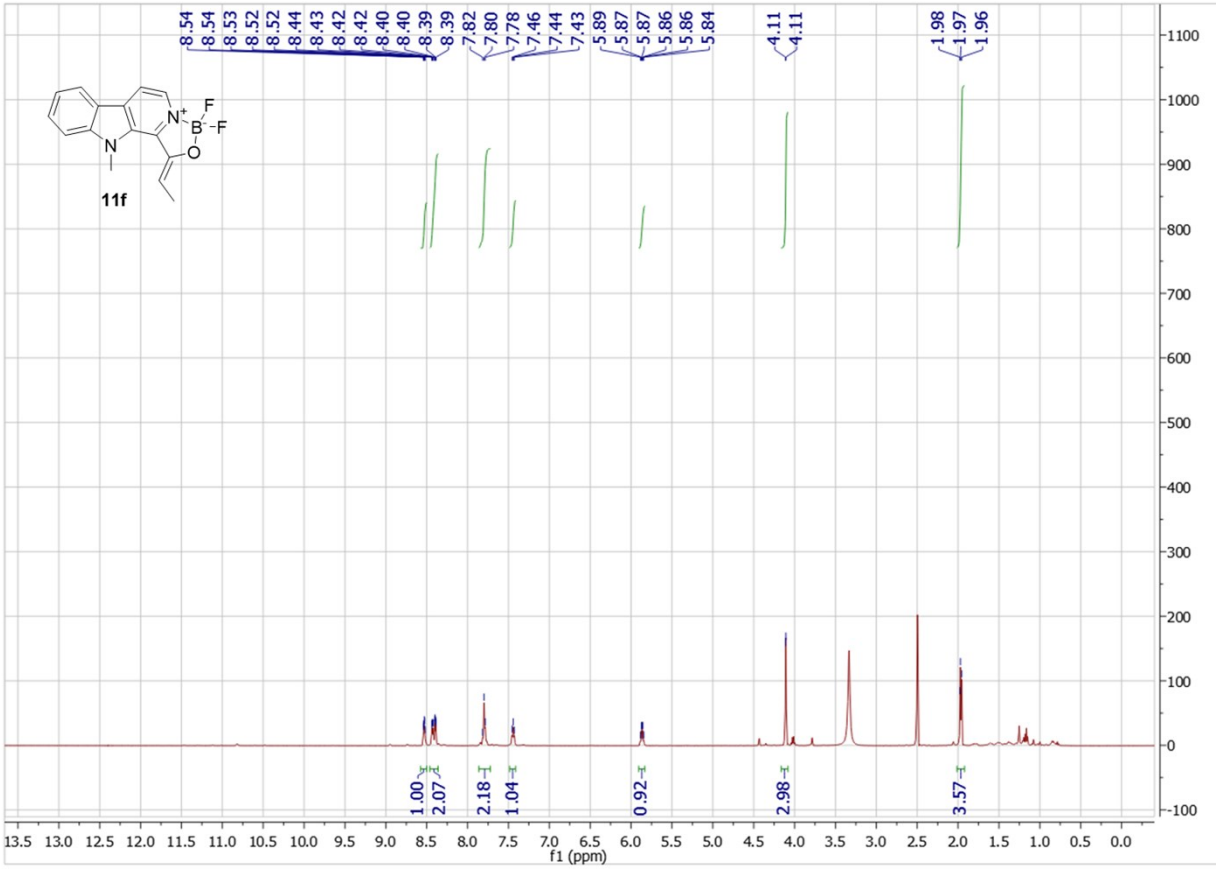


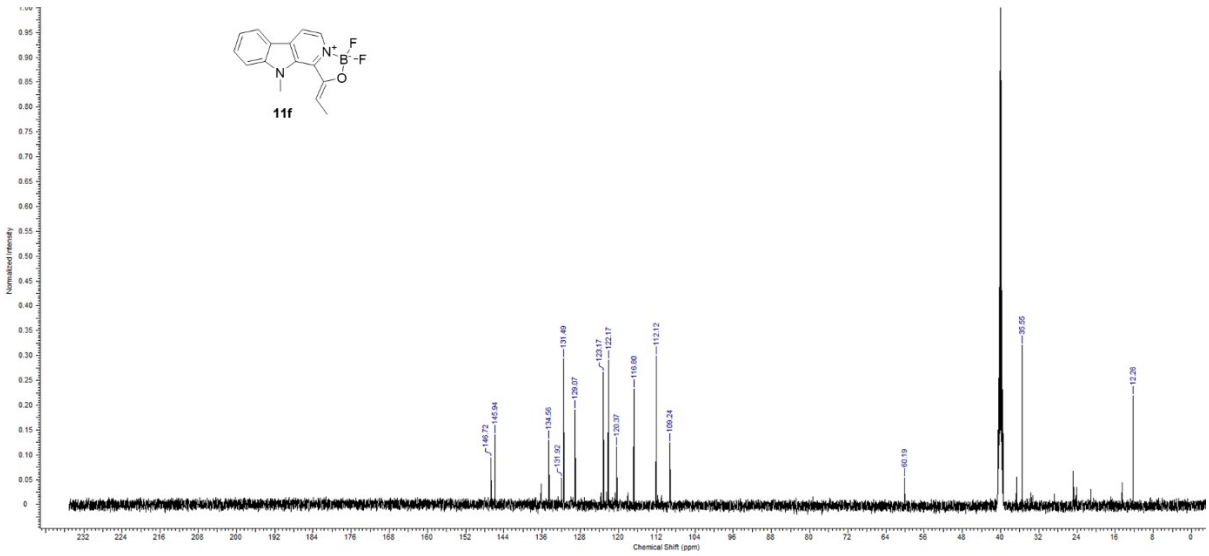
*3,3-difluoro-11-methyl-1-methylene-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11e**)*



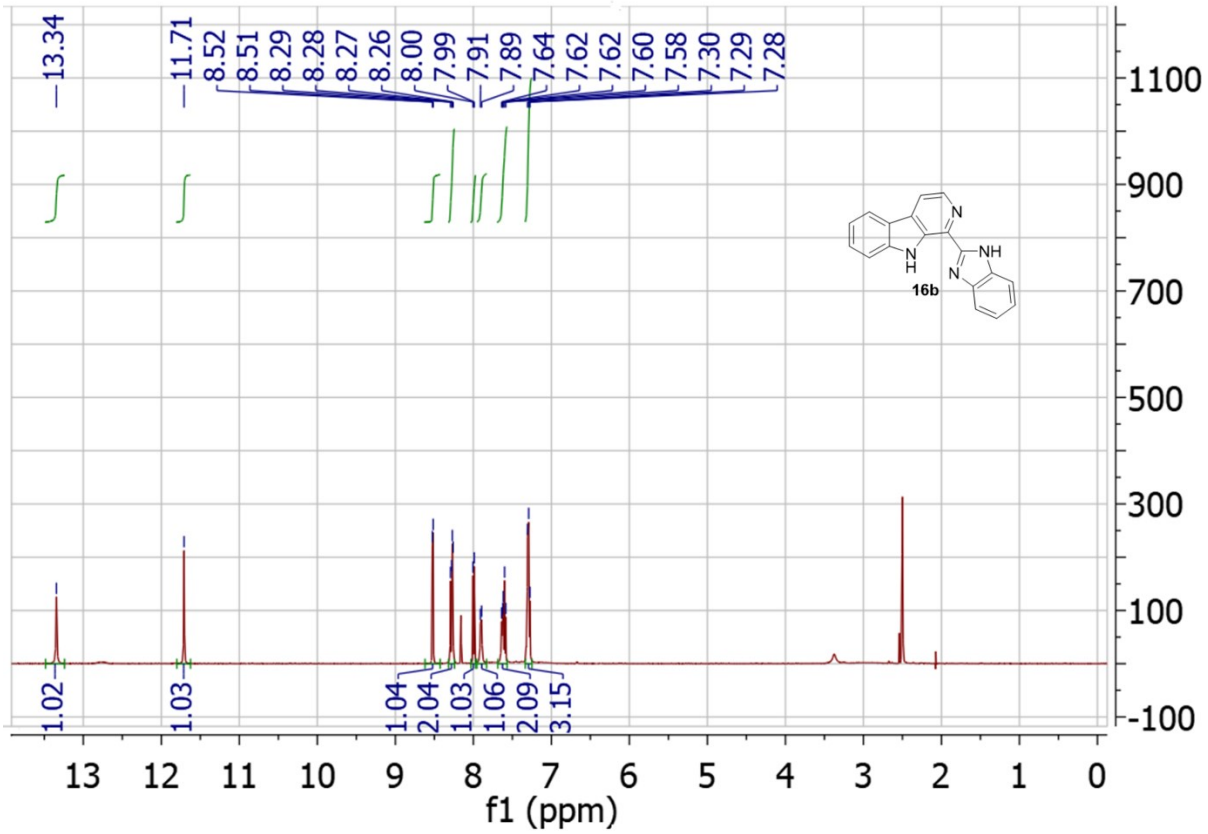


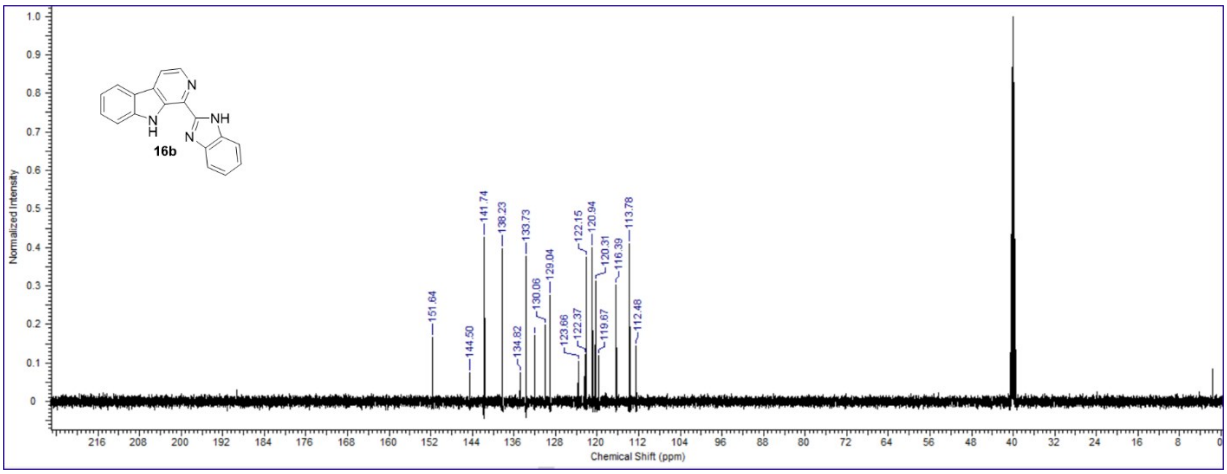
*(Z)-1-ethylidene-3,3-difluoro-11-methyl-3,11-dihydro-1H-3λ⁴,4λ⁴-[1,3,2]oxazaborolo[3',4':1,2]pyrido[3,4-b]indole (**11f**)*



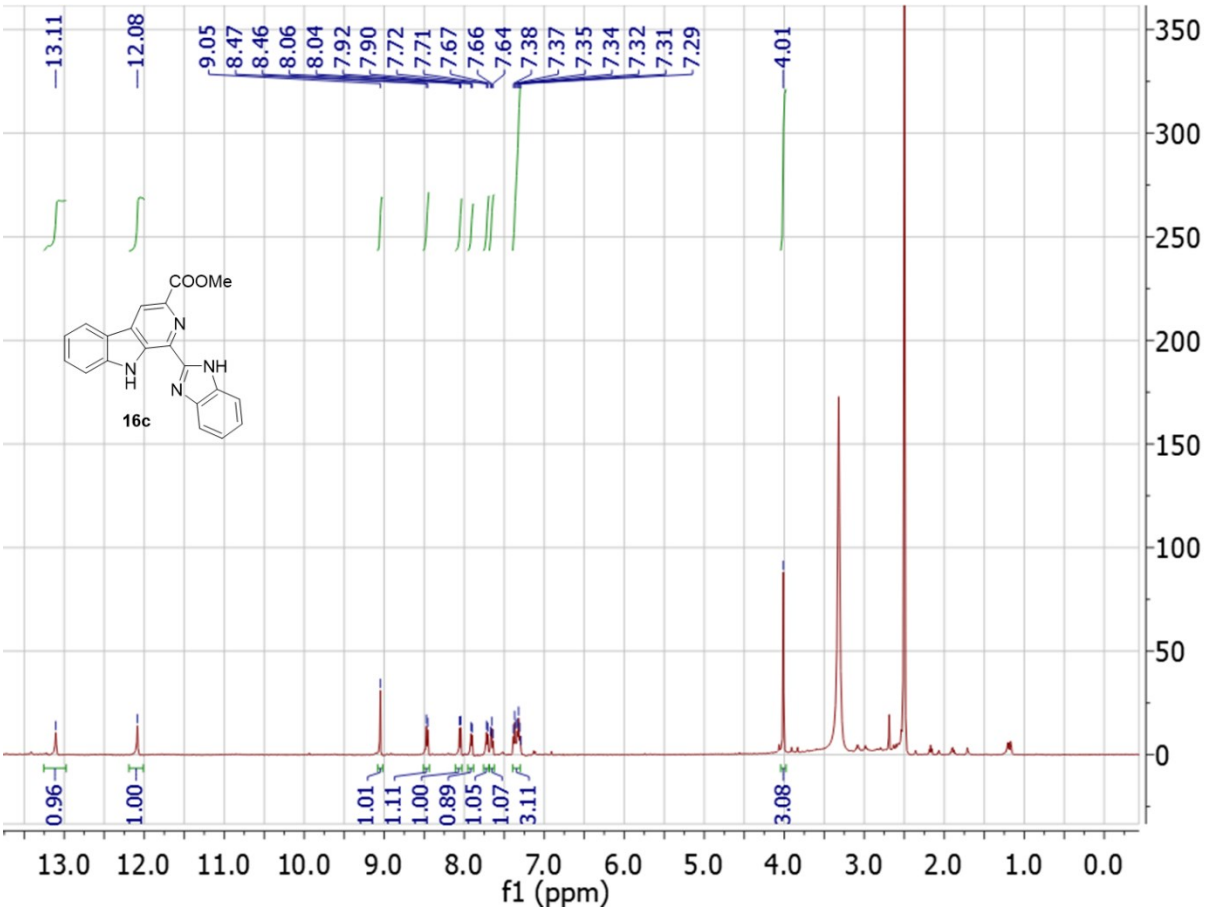


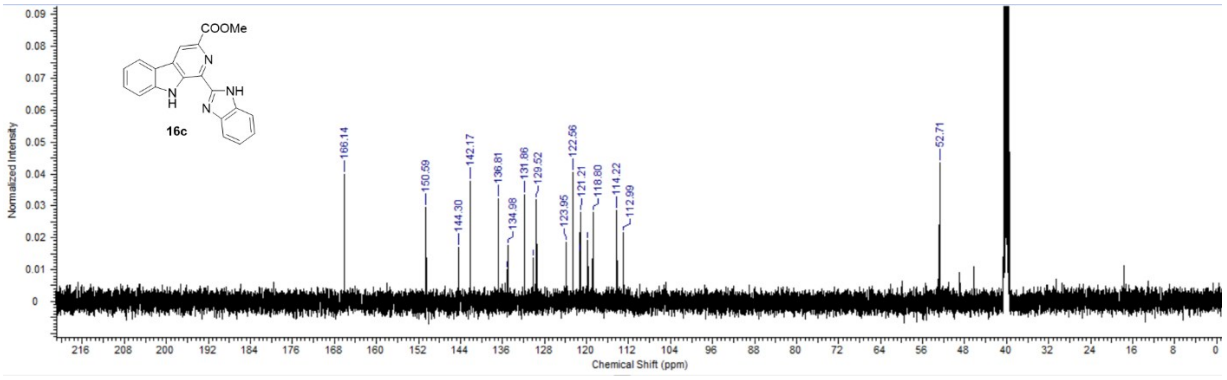
*1-(1H-benzo[d]imidazole-2-yl)-9H-pyrido[3,4-b]indole (**16b**)*



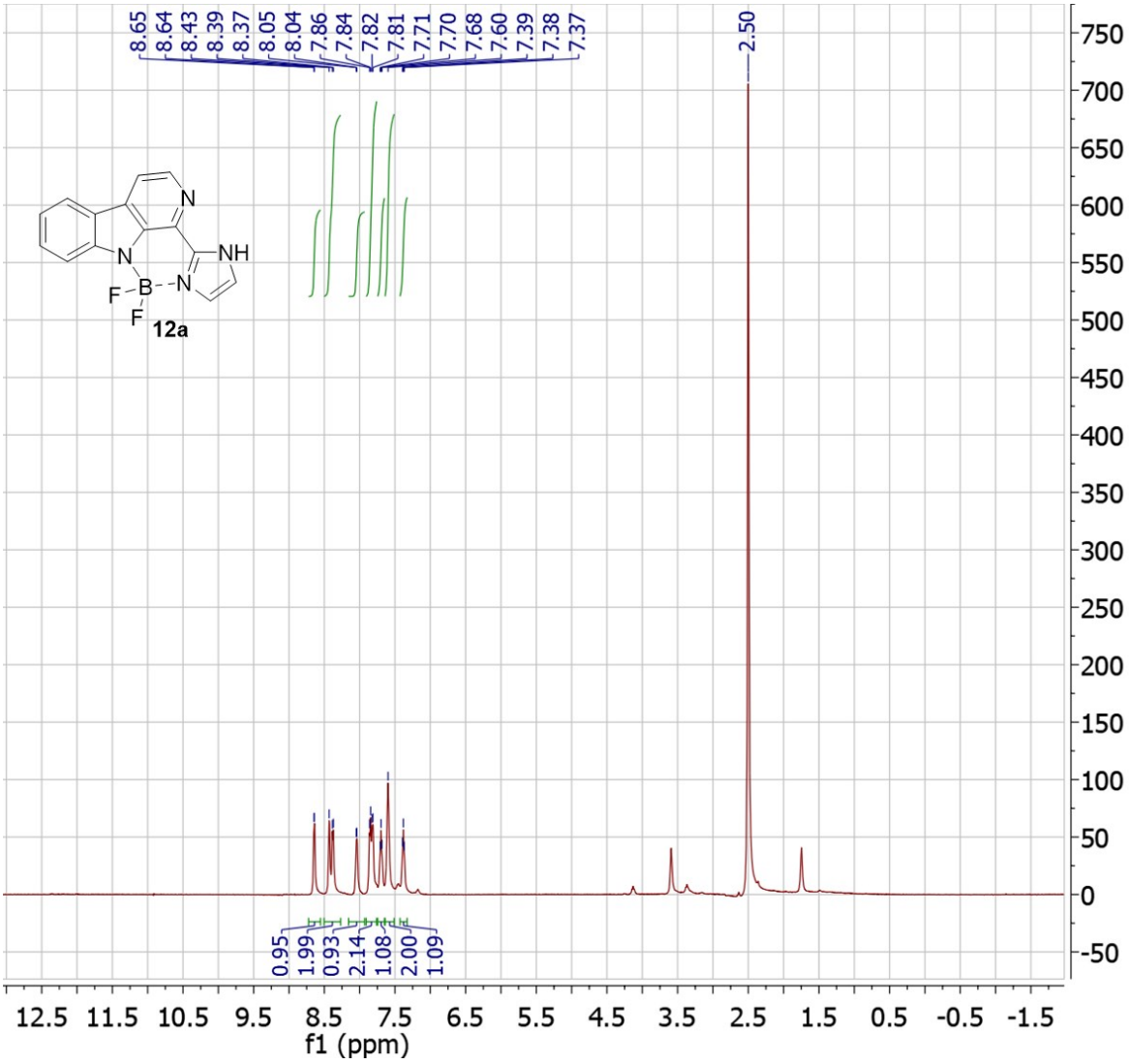


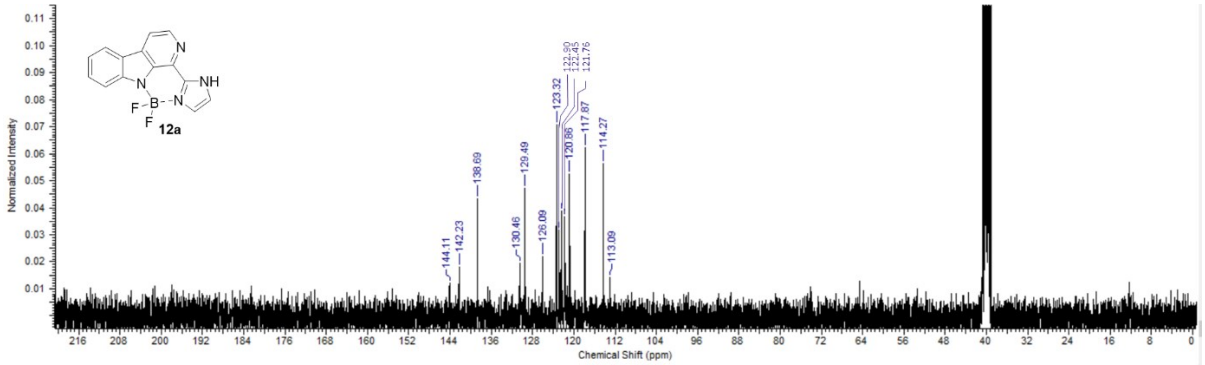
*Methyl 1-(1H-benzo[d]imidazol-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**16c**)*



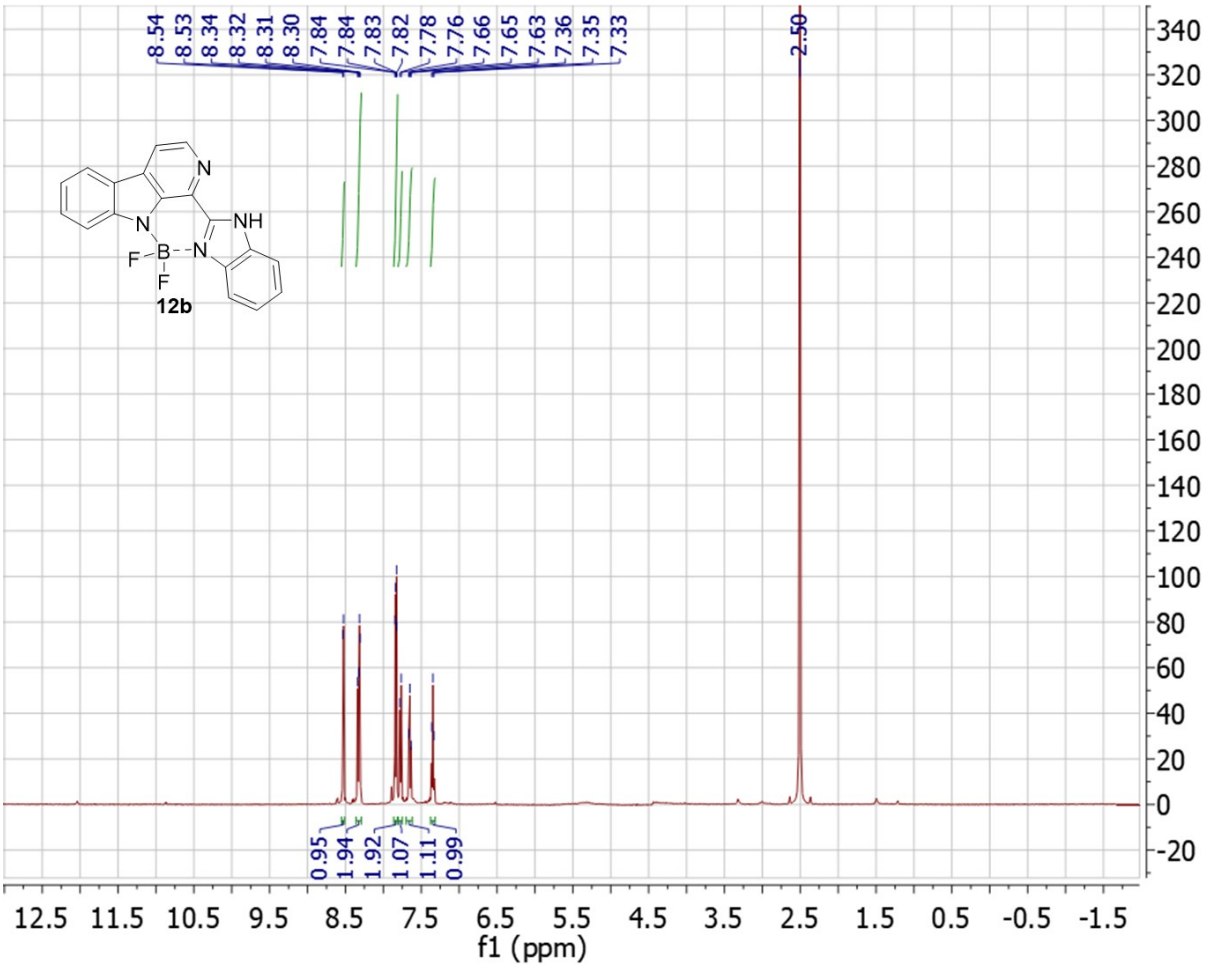


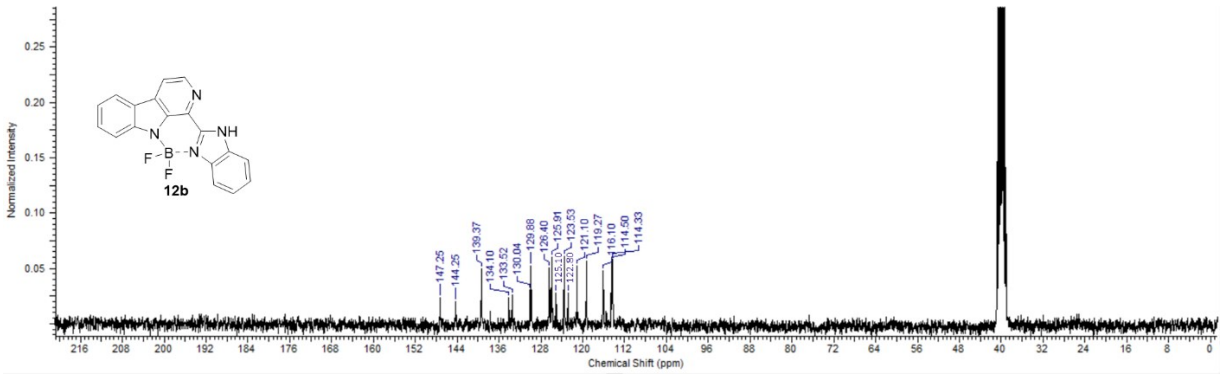
*9-(difluoroboranyl)-1-(1H-imidazole-2-yl)-9H-pyrido[3,4-b]indole (**12a**)*



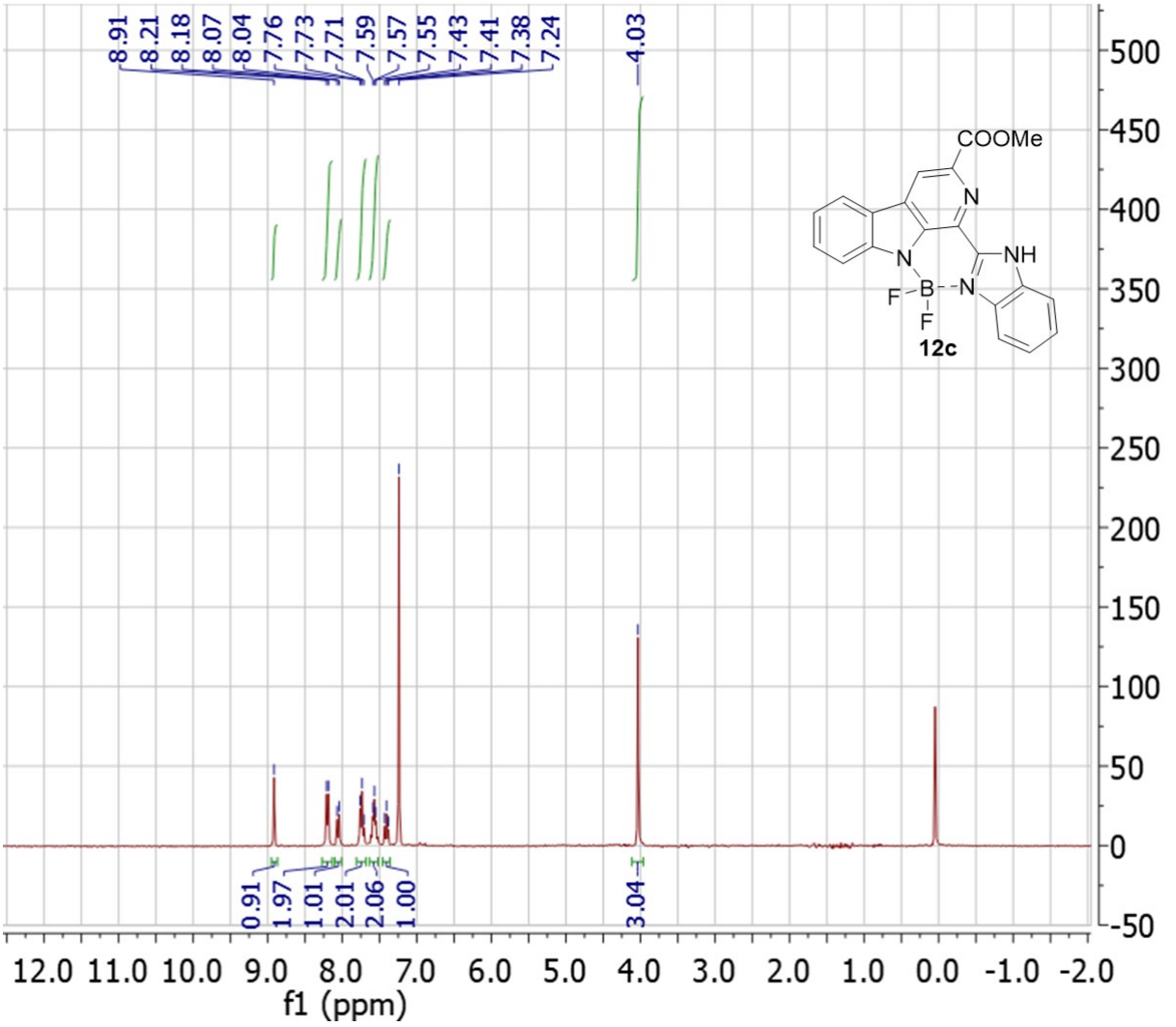


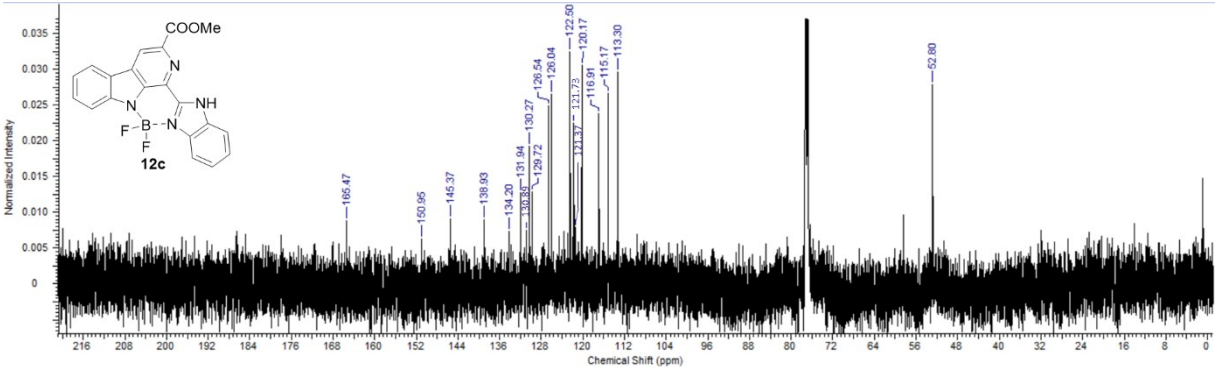
*1-(1H-benzo[d]imidazole-2-yl)-9-(difluoroboranyl)-9H-pyrido[3,4-b]indole (**12b**)*



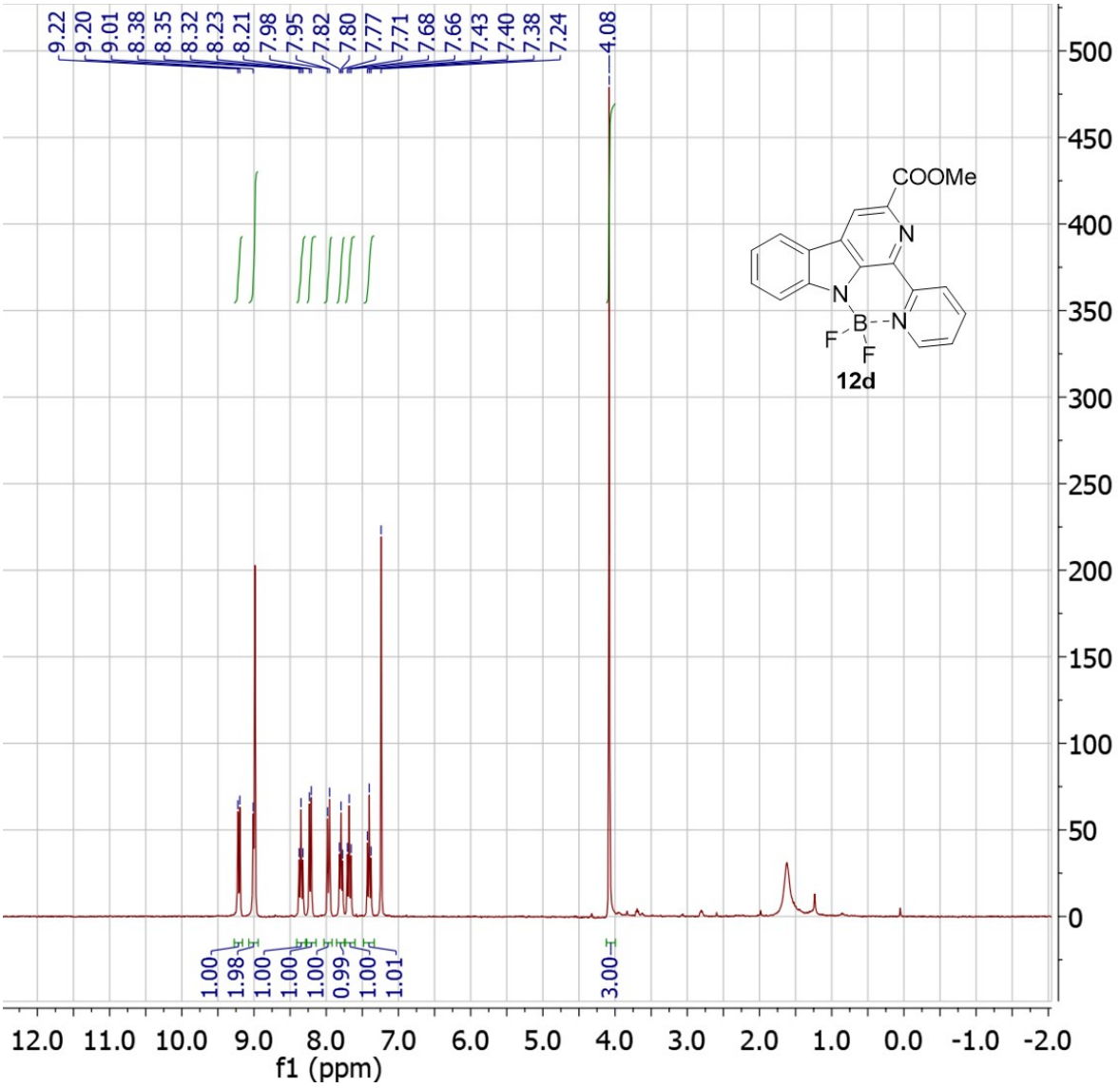


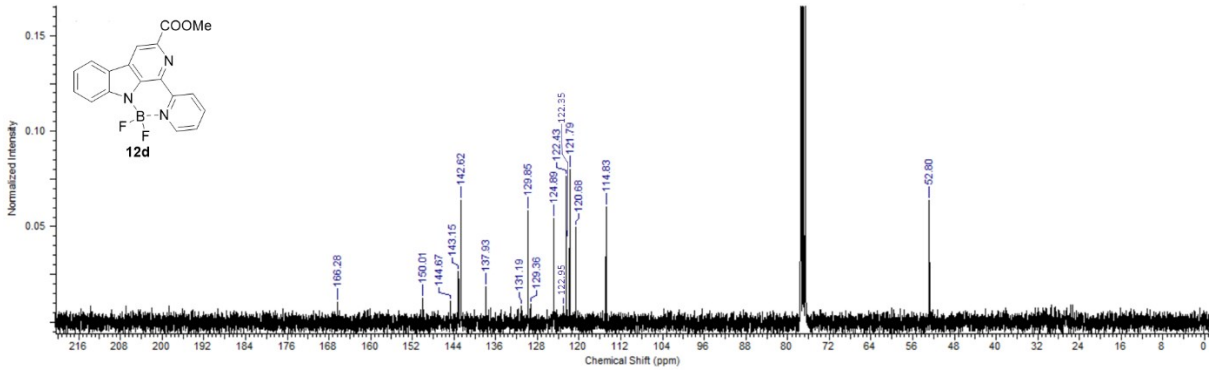
*Methyl 1-(1H-benzo[d]imidazol-2-yl)-9-(difluoroboranyl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12c**)*



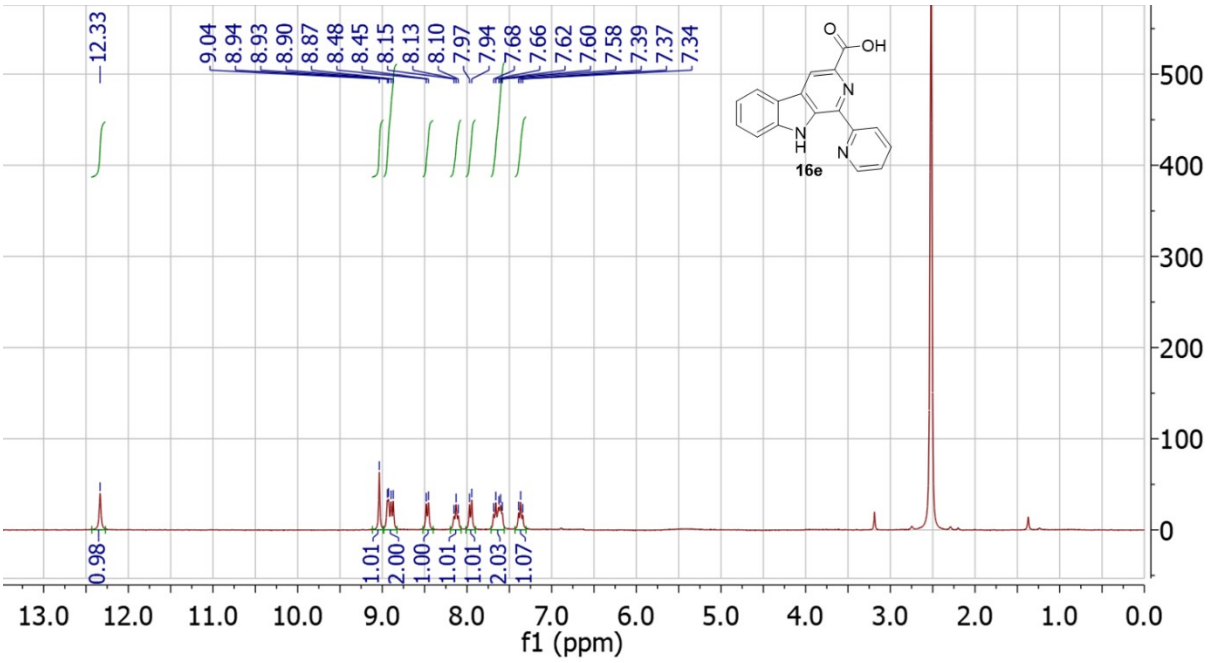


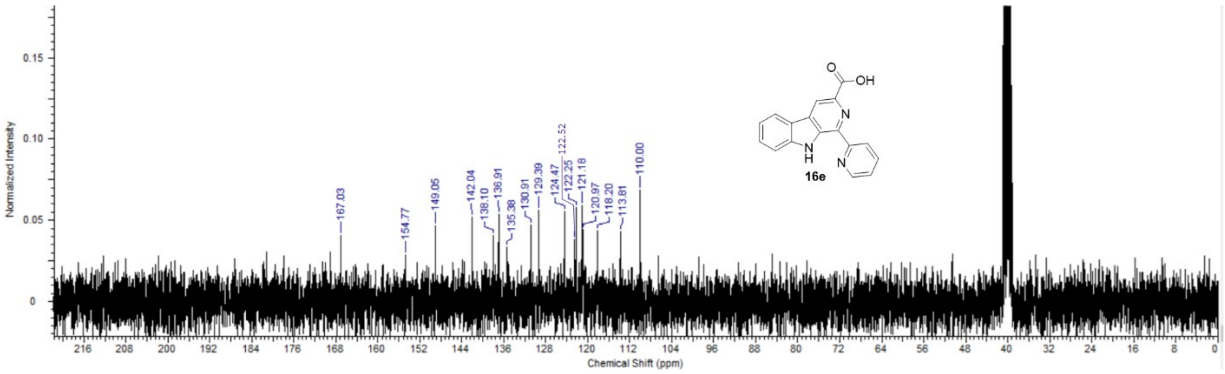
*Methyl 9-(difluoroboranyl)-1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12d**)*



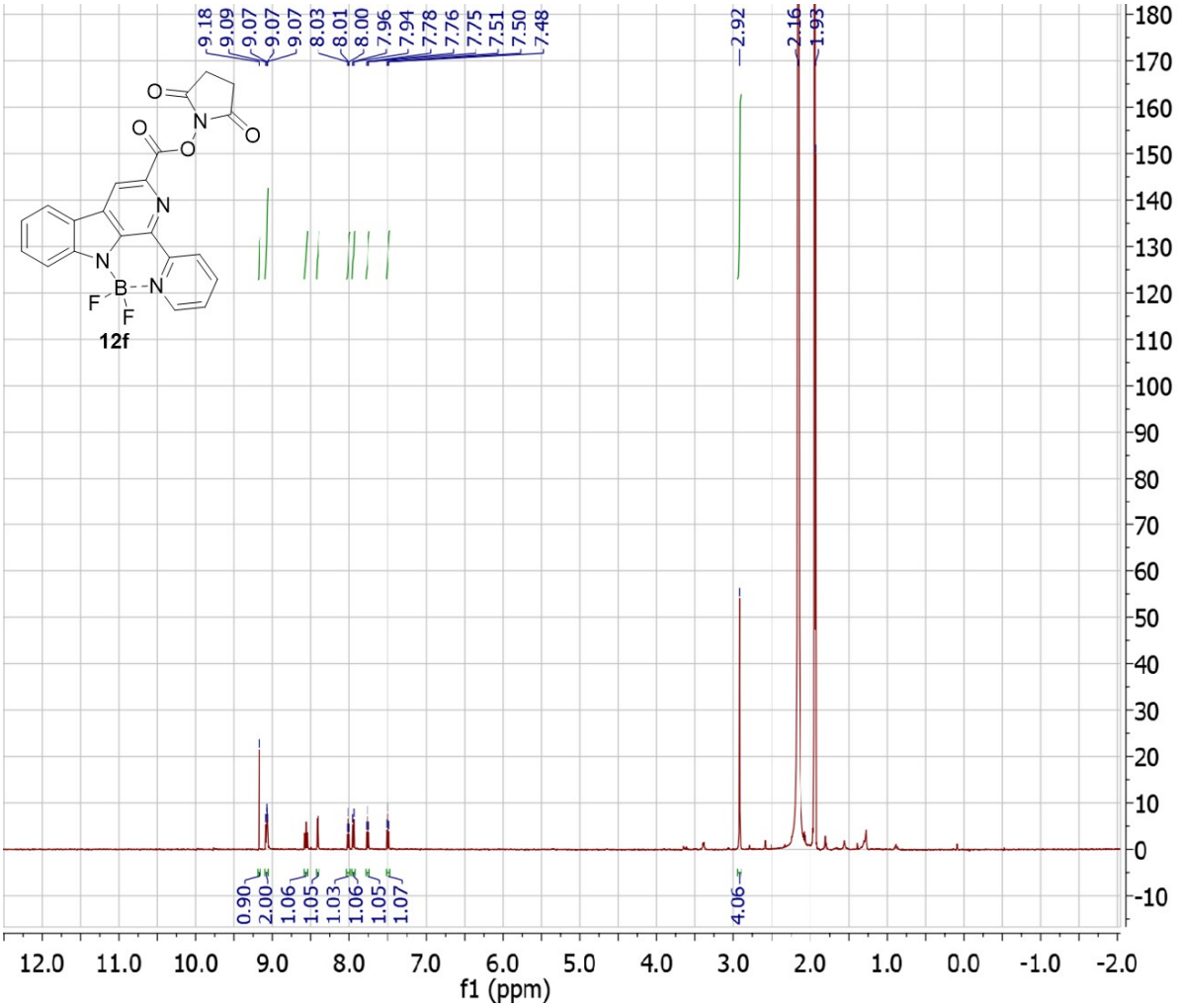


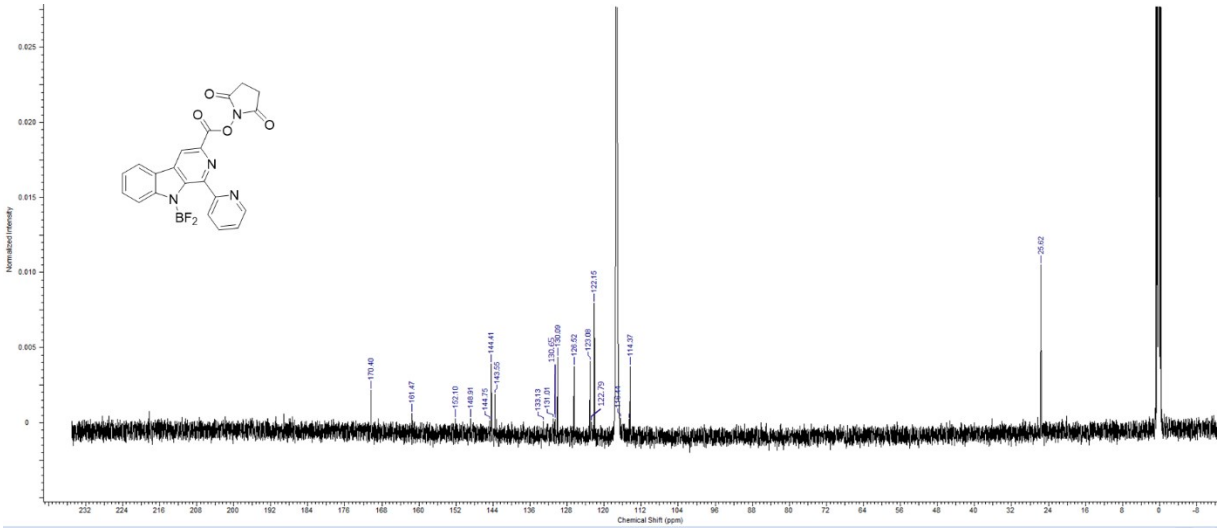
*1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylic acid (**16e**)*





*2,5-dioxopyrrolidin-1-yl 9-(difluoroboraneyl)-1-(pyridin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**12f**)*





Notes and references

1 G. Grabner, K. Rechthaler, B. Mayer, G. Köhler and K. Rotkiewicz, *J. Phys. Chem. A*, 2000, **104**, 1365–1376.

2 T. Szabó, V. Hazai, B. Volk, G. Simig and M. Milen, *Tetrahedron Lett.*, 2019, **60**, 1471–1475.

3 W. Chen, G. Zhang, L. Guo, W. Fan, Q. Ma, X. Zhang, R. Du and R. Cao, *Eur. J. Med. Chem.*, 2016, **124**, 249–261.

4 M.-L. Yang, P.-C. Kuo, T.-L. Hwang, W.-F. Chiou, K. Qian, C.-Y. Lai, K.-H. Lee and T.-S. Wu, *Bioorg. Med. Chem.*, 2011, **19**, 1674–1682.

5 M. G. Abramyants, D. A. Lomov and T. I. Zavyazkina, *Russ. J. Org. Chem.*, 2016, **52**, 1610–1615.