Synthesis of the fungal metabolite YWA1 and related constructs as tools to study MelLec-mediated immune response to Aspergillus infec-tions

Journal:	*The Journal of Organic Chemistry*
Manuscript ID	jo-2020-023242.R1
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Piras, Monica; University of Aberdeen Institute of Medical Sciences, Patruno, Ilaria; University of Aberdeen College of Life Sciences and Medicine Nikolakopoulou, Christina; University of Exeter Willment, Janet ; University of Exeter Sloan, Nikki; Loughborough University, Chemistry Department Zanato, Chiara ; Universite de Cergy-Pontoise, CNRS, BioCIS Brown, Gordon; University of Exeter Zanda, Matteo; Loughborough University,

Synthesis of the fungal metabolite YWA1 and related constructs as tools to study MelLec-mediated immune response to Aspergillus infections†

Monica Piras,[a,*] Ilaria Patruno,[a] Christina Nikolakopoulou,[b,c] Janet A. Willment,[b,c] Nikki Sloan,[d] Chiara Zanato,[e] Gordon D. Brown,[b,c,*] Matteo Zanda[a,d,f,*]

[a]Kosterlitz Centre for Therapeutics, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK

[b]Aberdeen Fungal Group, MRC Centre for Medical Mycology, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK

[c]Medical Research Council Centre for Medical Mycology at the University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, EX4 4QD, UK

[d]Chemistry Department, Loughborough University, LE113TU Loughborough, UK

[e]CY Cergy Paris Université, CNRS, BioCIS, 95000, Cergy Pontoise (France)

[f]C.N.R.-SCITEC, via Mancinelli 7, 20131 Milan (Italy)

ABSTRACT: We describe the chemical synthesis of the fungal naphthopyrones YWA1 and fonsecin B, as well as their functionalization with an amine-spacer arm and the conjugation of the resulting molecules to three different functional tags (i.e. biotin, Oregon green, PyMPO). The naphthopyrone-biotin and -PyMPO constructs maintained the ability to bind the C-type lectin receptor MelLec, whose interaction with immunologically active fungal metabolites (i.e. DHN-melanin and YWA1) is a key step in host recognition and induction of protective immune responses against A. fumigatus. The fluorescent Fonsecin B-PyMPO construct **21** was used to selectively visualize MelLec-expressing cells, thus validating the potential of this strategy for studying the role and functions of MelLec in immunity.

INTRODUCTION

Naphthopyrones are important aromatic polyketides displaying a characteristic poly-hydroxy-naphthalene system fused to a pyran-4-one ring. These fungal metabolites have attracted a great deal of interest due to their broad range of biological activities, encompassing immunomodulatory,[1] antiproliferative,[2] cytotoxic[3] and antibacterial[4] properties (**Figure 1a**). The hemiketal naphthopyrone YWA1, whose structure was unambiguously determined by spectroscopic methods two decades ago,[5] was isolated from the human opportunistic pathogen *Aspergillus fumigatus* and other fungal species expressing specific types of nonreducing polyketide synthases (NR-PKS). These multidomain enzymes assemble acyl and malonyl units in a polyketide chain and successively catalyze regiospecific cyclization/aromatization reactions to convert the polyketide chain into the YWA1 aromatic scaffold.[6,7] YWA1 is the first intermediate in the biosynthetic pathway leading to 1,8-dihydroxynaphthalene-(DHN)-melanin (**Figure 1b**), a highly insoluble conidial pigment formed by polymerization of 1,8-DHN monomers that confers fungal cell resistance against the host immune response, oxidative stress and ultraviolet light.[8] Although melanin represents an important protective factor contributing to fungal virulence and pathogenesis, we recently demonstrated that recognition of DHN-melanin components by the host immune system has a crucial role in the control of *A. fumigatus* infections in both mice and humans.[9] Fungal DHN-melanin and its metabolic precursors (YWA1, THN and DHN) are sensed by the host immune system through the C-type lectin receptor (MelLec).[9] Our findings indicate that the interaction of MelLec with immunologically active components of DHN-melanin is a key step in host recognition and induction of protective immune responses against *A. fumigatus*. To investigate the interaction of the melanin-sensing receptor MelLec with YWA1 and evaluate how structural modifications of the naphthopyrone scaffold could affect the ligand-receptor binding properties, we have now developed the first synthesis of YWA1. Until now, YWA1 - whose structure offers very limited options for chemical modification - could only be obtained in tiny amounts by extraction from *Aspergillus* conidia. Despite the biological importance of polyketide naphthopyrones, very few methods have been described for their preparation[10–13] and none of these turned out to be suitable for our aims, which were: (i) to have ready access to sufficient quantities of YWA1 for biological studies and (ii) to generate YWA1-

based functional chemical probes for studying the activity of MelLec in cells, with the view of enabling the study of MelLec-mediated immune responses *in vivo* and the biosynthesis of DHN-melanin in fungi.

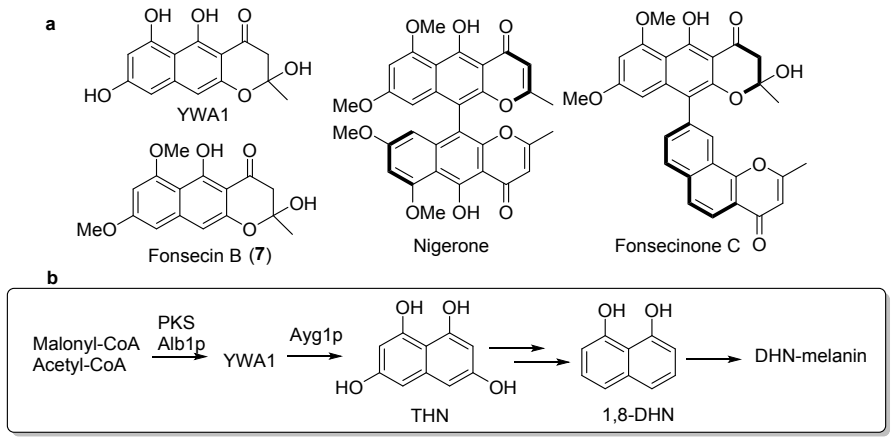


Figure 1. (**a**) Structure of YWA1 and other biologically active fungal metabolites incorporating a naphthopyrone system: fonsecin B (stabilising ligand of c-myc G-quadruplex DNA),[2] nigerone (cytotoxic agent)[3] and fonsecinone C (antibiotic).[4] (**b**) biosynthetic pathway to DHN-melanin.

Herein, we report (1) a concise synthesis of YWA1 and some of its structural analogues, which are recognized by MelLec protein,[9] (2) the functionalization of the YWA1 scaffold with an amine-spacer arm and its conjugation with Oregon green (**19**), biotin (**20**) and PyMPO (**21**), without significantly altering its ability to bind MelLec; (3) the capacity of the fluorescent conjugate **21** to specifically visualize MelLec in cell cultures.

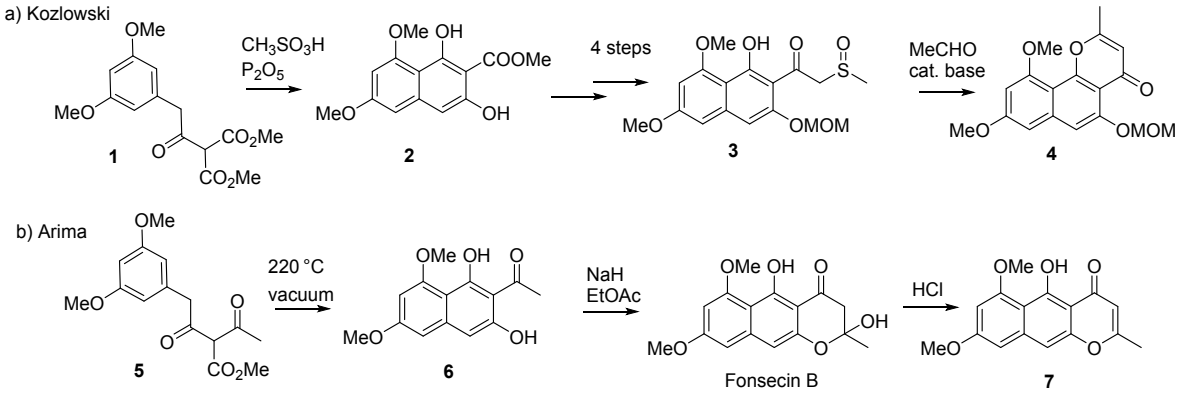
RESULTS AND DISCUSSION

Synthesis of YWA1 and Fonsecin B

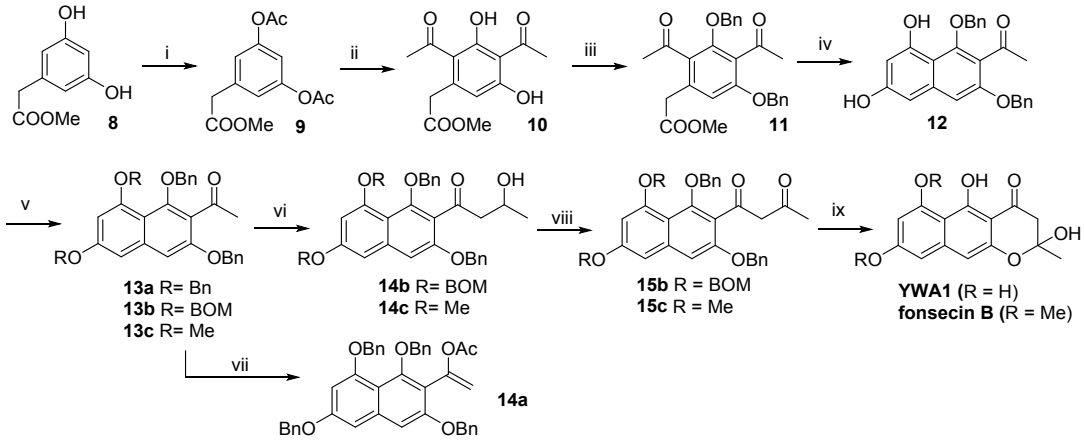
Existing synthetic procedures for the preparation of tricyclic oxygen-heterocycles, similar to YWA1, generally start with the formation of a naphthalene unit followed by condensation of a pyran-4-one ring.[10–12] In their work on the total synthesis of nigerone, Kozlowski et al. used a 2-naphthoate precursor (**2**) to generate the naphthopyrone heterocycle **4** (**Scheme 1a**).[10] According to the authors, the formation of the pyrone ring was the most challenging part of the synthesis, where the obvious disconnection involving addition of acetone to the methyl ester of **2** failed to supply the requisite pyrone ring and an alternative approach - based on addition of dimesyl anion and condensation of acetaldehyde - was instead required. Our attempts to adapt this strategy to prepare YWA1 from the naphthol intermediate **2** were unsuccessful, mainly because the pyrone ring could not be obtained in its hemiketal form, characteristic of YWA1. Additionally, this route seemed to lack the synthetic flexibility we required to develop YWA1 analogues suitable for bioconjugation to chemical handles and functional probes. In earlier studies based on the preparation of naphthopyrones, Arima and co-workers used a carboxy-diketone precursor (**5**) to access the 2-acetyl naphthol **6**, which was then readily converted into Fonsecin B by Claisen condensation with EtOAc (**Scheme 1b**).[11] Although this latter strategy seemed particularly well-suited for the synthesis of our target molecule, numerous attempts to convert **5** into the desired **6** turned out to be unsuccessful in our hands. In a more recent publication,[6] a concise approach to the 2-acetyl naphthol unit **12** was described (**Scheme 2**), comprising (i) Fries rearrangement of phenyl diacetate **9**, (ii) benzylation of the key intermediate **10** and (iii) Dieckmann cyclization of the non-symmetrical penta-substituted phenyl ester **11**. Following this procedure and optimizing the reaction conditions for the Fries rearrangement, we successfully obtained **12** in satisfactory yields. When the Fries rearrangement was conducted using $AlCl_3$ in refluxing DCE for 72 hours, as previously reported, we could only obtain small quantities of the desired product **10** (15% yield). A considerable amount of unreacted starting material was recovered along with a complex mixture of by-products resulting from the hydrolysis of the *O*-acetyl/methyl ester groups. Significant improvements were made by treating neat **9** with $AlCl_3$ at 150 °C for 1 hour and then performing a Fisher esterification to repristinate the methyl ester partially hydrolyzed during the acylation step. *O*-Benzylation of **10** followed by intramolecular Dieckmann condensation of **11**, afforded the desired naphthol **12**. During the *O*-benzylation step, the acidic character of the methylene protons of **10**, led to the formation of the over-alkylated side-product **11a** (**Scheme 3**). The nature of the solvent was found to have a remarkable effect on the chemoselectivity of the process, with *O*-benzylation being favored in acetone, while *C*-alkylation being predominant in DMF. Interestingly, the over-alkylated by-product **11a** could also undergo intramolecular Dieckmann cyclization giving the 5-benzyl substituted naphthalene analogue **12a** (**Scheme 3**). The newly formed hydroxyl groups of **12** were then fully protected before testing the formation of the hemiketal pyrone ring. While *O*-methylation of **12** to **13c** was achieved smoothly, *O*-benzylation proved to be particularly laborious. Treatment of **12** with BnBr under different reaction conditions did not afford the desired fully protected intermediate **13a**, while Mitsunobu alkylation gave low yields (17%). In both cases, *C*-alkylation of the

naphthol ring in position 5 was the main side reaction observed.

Scheme 1. Examples of syntheses of polyketide naphthopyrones

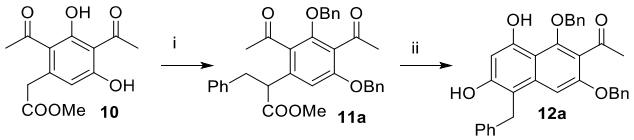


Scheme 2. Synthesis of YWA1 and Fonsecin B



Reagents and conditions: i) Ac_2O, pyridine, r.t., overnight (70%); ii) $AlCl_3$, 150 °C, 1.5 h, then MeOH, H_2SO_4, r.t., 4 h (60%); iii) BnBr, K_2CO_3, acetone, 60 °C, overnight (70%); iv) LiHMDS, THF, 0 °C, 2 h (90%); v) a) BnOH, PPh_3, DIAD, Et_2O, r.t., 4 h for compound **13a** (17%); b) BOMCl, DIPEA, CH_2Cl_2, r.t., overnight for compound **13b** (50%); c) Me_2SO_4, K_2CO_3, acetone, 60 °C, overnight for compound **13c** (70%); vi) LiHMDS, THF, –40 °C, acetaldehyde, 15 min (90%); vii) LiHMDS, THF, 0 °C, Ac_2O, 15 min (50%); viii) DMP, $CHCl_3$, 40 °C ; ix) H_2, $Pd(OH)_2$ 20% wt. on C, AcOH, MeOH, r.t., 3–12h (90%).

Scheme 3. Synthesis of 12a

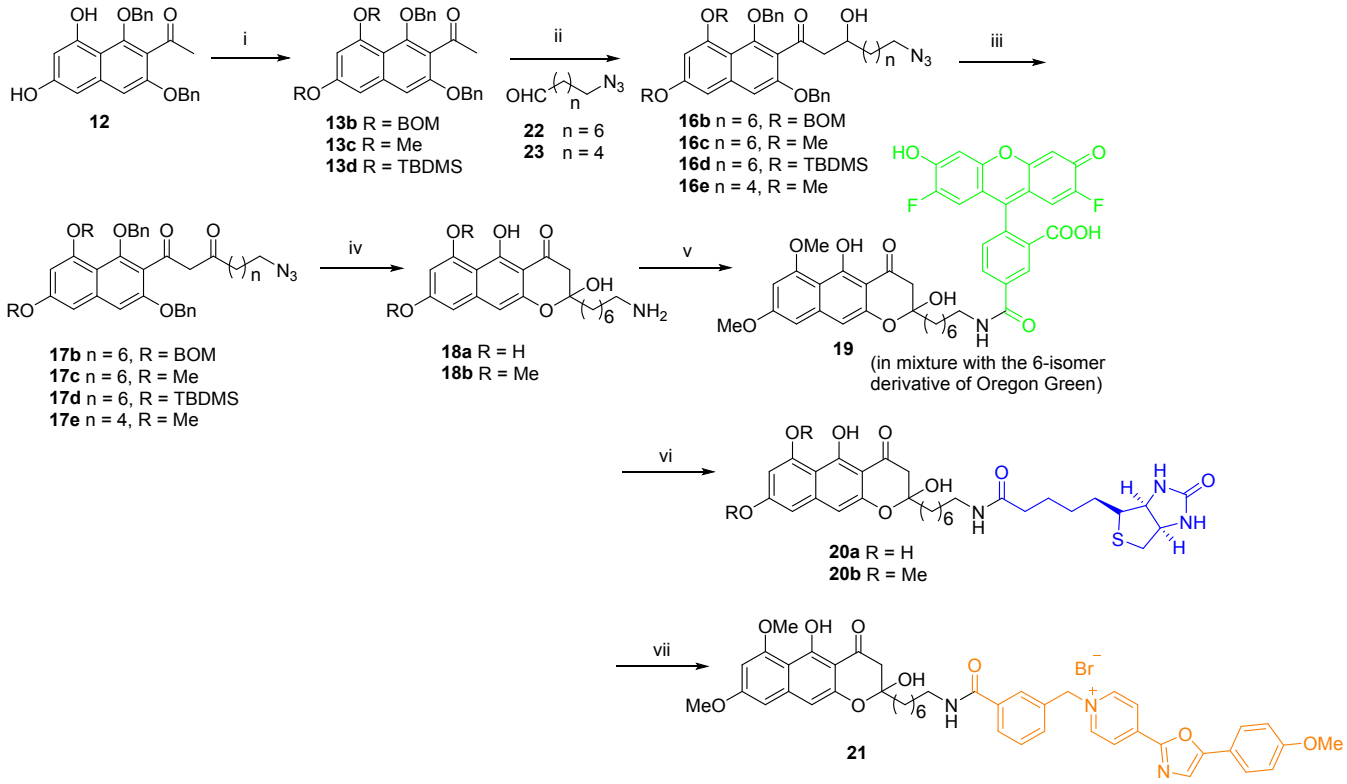


Reagents and conditions: i) BnBr, K_2CO_3, DMF, r.t., 4 h (95%); ii) LiHMDS, THF, -40 °C, acetaldehyde, 2h (90%).

Better results were obtained using the benzyloxymethyl (BOM) group as an alternative to benzyl protection (**13b**). With the protected 2-acetyl naphthols **13b-c** in our hands, we then tried to access the final product YWA1 and its methylated analogue fonsecin B by Claisen condensation with EtOAc and subsequent hydrogenolysis of the protecting groups. Surprisingly, numerous attempts to install a diketone moiety via Claisen condensation were unsuccessful. A variety of solvents, bases and temperatures were screened to condense **13b–c** to EtOAc but only starting material was recovered, while an enol acetate was formed (**14a**, see Supporting Information) when Ac_2O and LiHMDS were used. Applying a different strategy based on (i) aldol condensation of **13b–c** with acetaldehyde, (ii) oxidation of β-hydroxyketones **14b–c** and (iii) final hydrogenolysis of the resulting 1,3-diketones **15b–c**, we finally accomplished the synthesis of YWA1 (10.7% overall yield) and its *O*-dimethyl analogue fonsecin B (15.0% overall yield), both in 8 steps. Various procedures were tested for oxidizing the β-hydroxy ketone group in **14b–c**

(Swern, Parikh-Doering, PCC, IBX) but only DMP led to the conversion of the secondary alcohol into the desired diketone moiety.

Scheme 4. Synthesis of bioconjugated derivatives



Reagents and conditions: i) a) BOMCl, DIPEA, CH_2Cl_2, r.t., overnight for compound **13b** (50%); b) Me_2SO_4, K_2CO_3, acetone, 60 °C, overnight, for compound **13c** (70%); c) TBDMSCl, DIPEA, DMF, r.t., overnight for compound **13d** (99%); ii) LDA, THF, -78 °C, 45 min, then **22**, -78 °C, 15 min (99%); iii) DMP, $CHCl_3$, 40 °C or IBX, EtOAc, 80 °C overnight (for **16c**); iv) a) TBAF, THF, 0 °C, 3 h, then H_2, $Pd(OH)_2$ on carbon 20%, AcOH, MeOH, r.t., 3h for compound **18a**; b) H_2, $Pd(OH)_2$ on carbon 20%, AcOH, MeOH, r.t., 3h for compound **18b**; v) Oregon Green-NHS, DIPEA, DMF, r.t., 0.5 h (50%); vi) Biotin-NHS, DIPEA, DMF, r.t., 0.5 h (50%); vii) PyMPO-NHS, DIPEA, DMF, r.t., 0.5 h (60%).

Design and synthesis of bioconjugated derivatives

This synthetic strategy was used to functionalize YWA1 with an amine-spacer arm and perform a chemoselective ligation of the naphthopyrone scaffold to different tags (**Scheme 4**). Aldol condensation of the fully protected intermediate **13b–c** with 8-azido octanal **22** and subsequent oxidation of the β-hydroxyketones **16b-c** with DMP (or IBX for **16c**), afforded the desired diketones **17b-c.** Hydrogenolysis of the protecting groups of **17c**, concomitantly with azide reduction, quantitatively produced intermediate **18b**, while cleavage of the BOM protecting groups from **17b** was impaired by the presence of the newly formed amino group.

Attempts to force the removal of the BOM groups by adding more catalyst or acetic acid, led to the formation of a complex mixture of by-products. To overcome this problem, we decided to protect the hydroxyl groups of **12** as TBDMS ethers. The protected intermediate **13d** was successfully condensed with 8-azido octanal to afford **16d** which was then oxidized to give the diketone **17d.** Treatment of **17d** with TBAF followed by hydrogenolysis of the Bn groups afforded **18a**. The final intermediates (**18a–b**) equipped with an amine-spacer arm were successfully conjugated to the NHS active esters of Oregon green (commercial mixture of 5- and 6-isomers), biotin and PyMPO affording the constructs **19** (9.2% overall yield)**, 20a** (13.0% overall yield), **b** (9.2% overall yield) and **21** (11.0% overall yield) respectively, each in 9 steps. It should be noted that fonsecin B and derivatives are more stable than YWA1 counterparts, and therefore more reliable tools for performing biological studies, as OH-groups methylation prevents oxidation to the corresponding quinones.

It is also worth noting that the spacer's arm length is important for the synthetic accessibility of these constructs: hydrogenolysis of an azido diketone analogue **17e** synthesized using ω-azido hexanal **23** - instead of ω-azido octanal **22** - produced a complex mixture of unidentified products.

Biology

We have previously shown that MelLec is able to recognize 1,8-DHN-melanin and other structural isomers in the DHN-melanin biosynthetic pathway due to the presence of the

naphthalene-diol unit.[9] To test our compounds, we used the Fc-MelLec probe (the extracellular domain of MelLec, fused to the Fc-portion of human Ig)[9] which was pre-treated with or without YWA1, 1,8-DHN, fonsecin B, 2,6-DHAP, **21** and **20b**, and then incubated with *A. fumigatus* ΔrodA conidia (**Figure 2**). Fc-MelLec bound to the conidia was detected with APC-conjugated donkey anti-human antibody. Binding of Fc-MelLec to these compounds was determined by flow cytometry where lack of a signal on the *A. fumigatus* conidia indicates inhibition of binding through competitive inhibition. As we had described previously,[9] YWA1 inhibited binding of Fc-MelLec to ΔrodA conidia, whereas the structurally unrelated 2,6-DHAP had no effect on binding. Notably, fonsecin B and conjugates, **20b** and **21**, also inhibited the recognition of A. fumigatus ΔrodA conidia by Fc-MelLec. This shows that addition of the amine-spacer arm and subsequent conjugation do not impair ability to bind the receptor. Immunofluorescence experiments showed that, compared to the untreated cells, RAW 264.7 cells expressing hMelLec can bind to the fluorescent conjugate **21** (**Figure 3**), and the binding is concentration-dependent (data not shown). The binding of **21** was shown to be specific by treating RAW 264.7 cells expressing hMelLec in the presence of competing unlabelled YWA1 (**Figure 3**), which resulted in a strong decrease of fluorescence at the highest concentrations, such as 25 μM and 50 μM (for further competition experiments and data see Figure S1, Supporting Information).

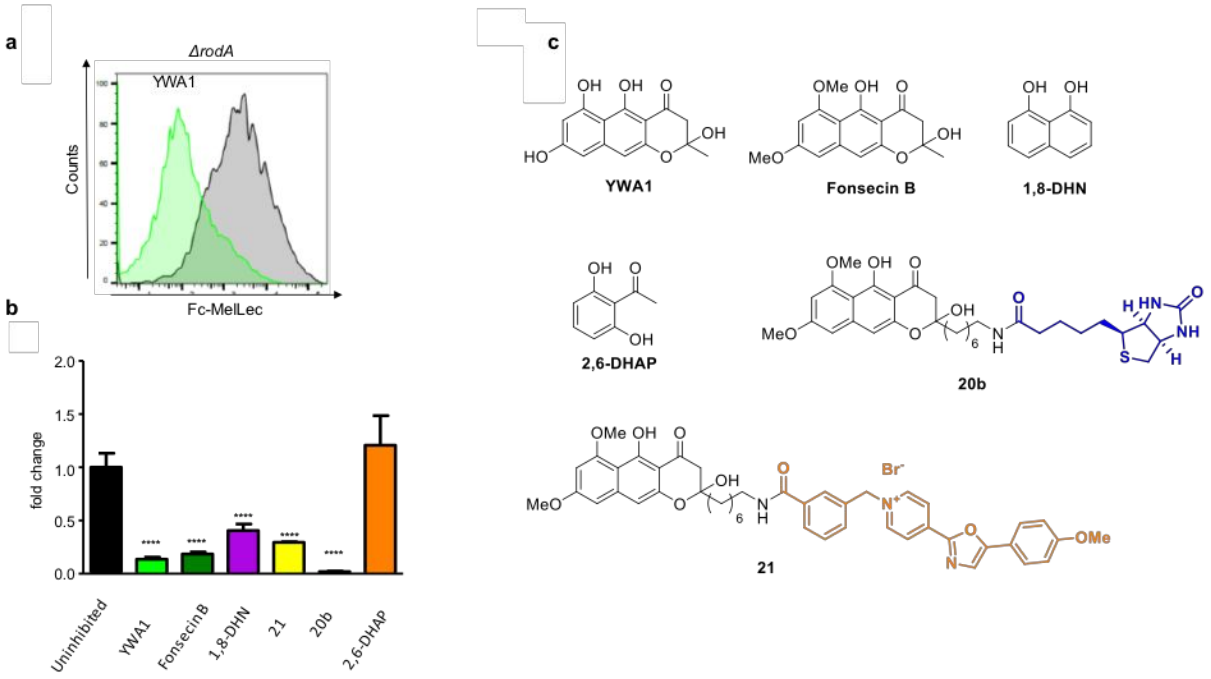


Figure 2. Fc-MelLec binds to 1,8-DHN-melanin precursors and other structurally related bio-conjugates. **a**, Flow cytometry histogram showing that YWA1 (1,8-DHN melanin precursor) inhibits recognition of *A. fumigatus ΔrodA* conidia by Fc-MelLec (green) compared to the uninhibited Fc-MelLec control (grey). **b**, Inhibition of Fc-MelLec binding to *ΔrodA* conidia of *A. fumigatus* by 1,8-DHN-melanin precursors (YWA1, 1, 8-DHN), Fonsecin B and the bio-conjugates **21** and **20b** compared to uninhibited Fc-MelLec control. 2,6-DHAP is included as a negative control, as it lacks the naphthol unit. The experiment was repeated independently twice, with similar results. Two technical replicates were used for each sample in each experiment. Data shown here is pooled from the two independent experiments. Fold change was calculated by dividing the mean value of pre-treated Fc-MelLec with each compound by the mean value of the uninhibited Fc-MelLec. Values show mean ± SEM, ****p<0.05 (One-way ANOVA and Bonferroni Post-hoc test comparing inhibition of Fc-MelLec binding to *ΔrodA* conidia by the different compounds versus uninhibited Fc-MelLec). **c**, Structures of the compounds tested.

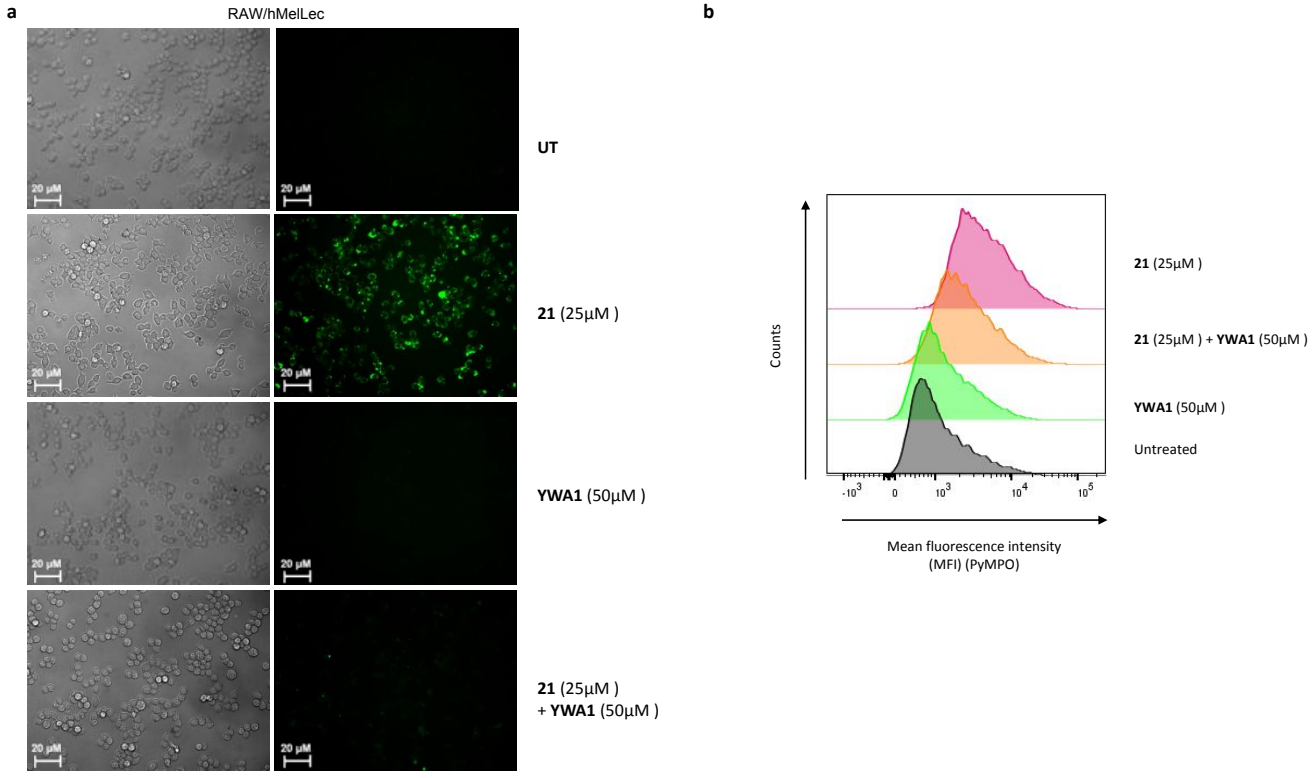


Figure 3. Specificity control of the binding of the fluorescent conjugate **21** to hMelLec-expressing cells with or without competition by YWA1. **a:** Representative light microscopy images and immunofluorescence micrographs of hMelLec expressing RAW 264.7 cells untreated or treated with 25μM concentration of **21** (green) for 3h at 37 °C 5% CO_2 with or without YWA1 (50 μM), or with YWA1 (50 μM) alone. **b:** histograms showing the binding of MelLec expressing RAW 264.7 cells to **21** compared to untreated cells, or treated with YWA1 alone, or treated with both **21** and YWA1, as determined by flow cytometry.

CONCLUSIONS

We described an efficient synthesis of YWA1 and other structurally related fungal naphthopyrones. The flexibility of the synthetic approach allowed us to functionalize the YWA1 scaffold with an amine-spacer arm and conjugate the resulting molecules to three different functional tags (i.e. biotin, Oregon green, PyMPO) without impairing the ability to bind to MelLec. The ability of MelLec to recognize YWA1 and its structural analogues was determined by flow cytometry experiments, using a Fc-MelLec probe[9]. The capacity to bind to MelLec was determined as the ability of naphthopyrone ligands to inhibit the interaction of Fc-MelLec with melanin components localized on the surface of *A. fumigatus* conidia. The fluorescent probe **21** was then used to selectively visualize MelLec-expressing cells using fluorescence microscopy, thus validating the potential of this strategy for studying *Aspergillus* infections. These compounds therefore offer exciting new tools to study the cellular and immunological functions of MelLec *in vitro* and *in vivo.*

EXPERIMENTAL SECTION

General information

Dry solvents were obtained from commercial sources and used without further purification. The reactions performed under nitrogen atmosphere were carried out in dry solvents. Reactions requiring heating were performed using an oil bath. Reactions were monitored by thin-layer chromatography (TLC), unless otherwise noted. TLCs were performed on Merck silica gel glass plates (60 F254). Visualisation was accomplished by UV light (254 nm) or staining with ceric ammonium molybdate or $KMnO_4$ solution. Flash chromatography was performed manually using Silica gel (60 Å, particle size 40–63 μm) purchased from Merck or automatically using Interchim PuriFlash instrument and normal phase Silica columns. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer and calibrated using residual undeuterated solvent as internal reference. ^{1}H δ = 7.26 ($CDCl_3$), ^{13}C δ = 77.16 ($CDCl_3$), ^{1}H δ = 3.31 (CD_3OD), ^{13}C δ = 49.15 (CD_3OD), ^{1}H δ = 2.05 ($(CD_3)_2CO$), ^{13}C δ = 29.84 ($(CD_3)_2CO$), ^{1}H δ = 2.50 ($(CD_3)_2SO$), ^{13}C δ = 39.52 ($(CD_3)_2SO$). ^{19}F NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer and were referenced to $CFCl_3$. ^{13}C NMR spectra were recorded with complete proton decoupling. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz), multiplicity is described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, or combinations thereof. Mass analyses were performed using Agilent 1200 HPLC system coupled to Agilent G6120 single quadrupole detector equipped with an electrospray ionization (ESI) source in direct infusion modality. ESI-MS spectra were recorded in positive mode. RP (reverse phase)-HPLC-MS analyses were performed with an Agilent 1200 HPLC system equipped with a DAD

and an ESI-MS detector. High resolution mass spectra were recorded using a Thermofisher Q-Exactive orbitrap ion trap mass spectrometer. HPLC conditions for analytical analyses: Phenomenex Luna C18 column, 5 µm, 100 Å, 250 × 4.6 mm (L × ID) injected volume 10 µL, flow rate 1 mL/min, unless otherwise specified. HPLC preparative purification were performed using an Agilent 1260 HPLC system equipped with DAD detector, HPLC conditions for preparative purification: Phenomenex Luna C18 column, 5 µm, 100 Å, 250 × 21.2 mm (L × ID), flow rate 20 mL/min.

5-(2-methoxy-2-oxoethyl)-1,3-phenylene diacetate (9)

Commercially available methyl 3,5-dihydroxyphenylacetate (12.0 g, 65.9 mmol, 1.0 equiv) was dissolved in Ac_2O (31.2 mL, 0.33 mol, 5.0 equiv) then pyridine (26.5 mL, 0.33 mol, 5.0 equiv) was added dropwise at 0 °C. The reaction was stirred at room temperature overnight, quenched with 10 mL of an ice-cold aqueous solution of HCl (2 M) and extracted with Et_2O (3 x 100 mL). The combined organic phases were washed with 50 mL of brine, 20 mL of an aqueous solution of HCl (1 M), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **9** as a pale-yellow oil (12.0 g, 70% yield). R_f= 0.6 *n*-hexane/EtOAc, 1:1. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

Methyl 2-(2,4-diacetyl-3,5-dihydroxyphenyl)acetate (10)

A mixture of $AlCl_3$ (5.20 g, 39.4 mmol, 3.5 equiv) and **9** (3.0 g, 11.2 mmol, 1.0 equiv) was stirred at 150 °C for 1.5 h. After cooling, the reaction was poured into a solution of ice and aqueous HCl (2 M) and the whole suspension was stirred for 10 min. The mixture was extracted with EtOAc (3 x 100mL), the combined organic phases were dried over Na_2SO_4 and concentrated under vacuum. In order to repristinate the partially hydrolysed ester, the crude mixture was submitted to Fisher esterification using H_2SO_4 (0.6 mL, 11.2 mmol, 1.0 equiv) in MeOH (20 mL) and stirring at room temperature for 4 h. The product was then extracted with EtOAc (3 x 100 mL), washed with brine (50 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by automated flash chromatography (100% DCM) to give product **10** as a white solid (1.9 g, 60% yield). R_f = 0.5 *n*-hexane/EtOAc, 1:1. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 15.33 (s, 1H), 13.94 (br, 1H), 6.35 (s, 1H), 3.92 (s, 2H), 3.76 (s, 3H), 2.77 (s, 3H), 2.64 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 205.6, 203.6, 170.4, 169.5, 168.6, 143.3, 116.4, 114.1, 109.6, 52.6, 42.1, 33.6, 31.9. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{13}H_{15}O_6$ 267.08; found 267.1. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

Methyl 2-(2,4-diacetyl-3,5-bis(benzyloxy)phenyl)acetate (11)

To a suspension of **10** (1.10 g, 4.20 mmol, 1.0 equiv) and K_2CO_3 (1.20 g, 8.40 mmol, 2.0 equiv) at room temperature in acetone (25 mL), BnBr (1.4 g, 8.4 mmol, 2.0 equiv) was added. The suspension was heated to 60 °C and stirred overnight, quenched with 10 mL of an ice-cold aqueous solution of HCl (1 M) and extracted with Et_2O (3 x 100 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **11** as a white solid (1.3 g, 70% yield). R_f = 0.3 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 – 7.34 (m, 10H), 6.70 (s, 1H), 5.14 (s, 2H), 4.85 (s, 2H), 3.74 (s, 2H), 3.71 (s, 3H), 2.57 (s, 3H), 2.54 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 204.0, 201.3, 171.2, 156.5, 154.3, 136.0, 135.7, 135.1, 129.6, 128.7, 128.6, 128.5, 128.3, 127.3, 125.8, 111.2, 79.5, 70.7, 52.2, 38.6, 32.5, 32.2. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{27}O_6$ 447.17; found 447.2. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

Methyl 2-(2,4-diacetyl-3,5-bis(benzyloxy)phenyl)-3-phenylpropanoate (11a)

To a suspension of **10** (1.10 g, 4.20 mmol, 1.0 equiv) and K_2CO_3 (1.70 g, 12.6 mmol, 3.0 equiv) in DMF (25 mL), BnBr (2.5 g, 14.7 mmol, 3.5 equiv) was added. This suspension was stirred at room temperature for 4 h, quenched with 10 mL of an ice-cold aqueous solution of HCl (1 M) and extracted with Et_2O (3 x 30 mL). The organic phase was washed with brine (10 mL), dried over Na_2SO_4 and concentrated under vacuum. The mixture was then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **11a** as a white solid (2.0 g, 95% yield). R_f= 0.4 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 – 7.03 (m, 13H), 6.98 – 6.93 (m, 2H), 6.85 (s, 1H), 5.06 (s, 2H), 4.72 (d, *J* = 10.4, 1H), 4.68(d, *J* = 10.4, 1H), 3.92 (dd, *J* = 8.2, 6.8 Hz, 1H), 3.48 (s, 3H), 3.19 (dd, *J* = 13.5, 8.2 Hz, 1H), 2.87 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.38 (s, 3H), 2.16 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 204.4, 201.2, 173.3, 156.3, 153.4, 138.7, 138.4, 136.0, 135.9, 129.1, 128.8, 128.61, 128.56, 128.52, 128.50, 128.3, 127.4, 126.7, 125.7, 107.8, 79.3, 70.7, 52.3, 48.8, 40.1, 32.6, 32.5. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{34}H_{33}O_6$ 537.22; found 537.2.

General procedure for the Dieckman condensation (procedure A):

To a solution of **11** or **11a** (1.0 equiv, 0.28 M in THF), LiHMDS (1.0 M in THF, 3.0 equiv) was added dropwise at 0 °C. The resulting orange suspension was stirred at 0 °C under nitrogen atmosphere for 2 h, then quenched with an aqueous solution of HCl (1 M), extracted with Et_2O (50 mL x 3), washed with brine (20 mL), dried over Na_2SO_4. and concentrated under vacuum to afford the title compound **12** or **12a**.

1-(1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)ethan-1-one (12)

From compound **11** (1.23 g, 2.76 mmol) according to general procedure A. Product **12** was obtained as a white solid (1.0 g, 90%). R_f = 0.2 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 9.19 (s, 1H), 8.71 (s, 1H), 7.55 – 7.32 (m, 10 H), 7.12 (s, 1H), 6.72 (d, *J* = 2.3 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 5.27 (s, 2H), 5.15 (s, 2H), 2.58 (s, 3H). $^{13}C\{^1H\}$ (101 MHz, $(CD_3)_2CO$) δ: 200.8, 158.0, 155.9, 153.8, 152.1, 138.8, 136.8, 135.8, 128.9, 128.8, 128.7, 128.5, 128.0, 127.6, 122.2, 107.7, 103.2, 101.1, 100.8, 80.0, 70.2, 32.2. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{23}O_5$ 415.15 found 415.2. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

1-5(benzyl-1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)ethan-1-one (12a)

From compound **11a** (1.00 g, 1.86 mmol) according to general procedure A. Product **12a** was obtained as a yellow solid (850 mg, 90% yield). R_f = 0.3 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 9.12 (s, 1H), 8.71 (s, 1H), 7.41 – 6.94 (m, 16H), 6.47 (s, 1H), 5.01 (s, 2H), 4.98 (s, 2H), 4.16 (s, 2H), 2.41 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $(CD_3)_2CO$) δ: 200.8, 155.3, 154.4, 153.6, 152.5, 141.7, 137.1, 136.7, 135.6, 129.0, 128.9, 128.7, 128.6, 128.3, 128.2, 128.0, 127.6, 125.5, 121.9, 110.3, 108.2, 101.2, 100.6, 80.2, 70.2, 32.3, 30.3. HRMS (ESI), *m/z*: $[M + Na]^+$ Calcd for $C_{33}H_{28}O_5Na$ 527.1829; found 527.1828.

1-(1,3,6,8-tetrakis(benzyloxy)naphthalen-2-yl)ethan-1-one (13a)

To a solution of **12** (230 mg, 0.56 mmol, 1.0 equiv), PPh_3 (308 mg, 1.2 mmol, 2.1 equiv), and BnOH (122 µL, 1.17 mmol, 2.1 equiv) in Et_2O (10 mL) at 0 °C, DIAD (232 µL, 1.17 mmol, 2.1 equiv) was added dropwise. The reaction was stirred for 4 h at room temperature and then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 30% EtOAc) to afford compound **13a** as a white solid (50.0 mg, 17% yield). R_f = 0.3 *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 – 7.14 (m, 20H), 6.95 (s, 1H), 6.79 (d, *J* = 2.2 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 5.21 (s, 2H), 5.19 (s, 2H), 5.15 (s, 2H), 5.02 (s, 2H), 2.51 (s, 3H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$) δ: 203.0, 158.2, 156.9, 153.7, 152.9, 138.8, 137.4, 136.6, 136.5, 136.3, 128.7, 128.63, 128.59, 128.2, 128.0, 127.8, 127.7, 127.5, 127.1, 125.5, 111.8, 103.8, 100.2, 99.6, 78.7, 71.3, 70.2, 70.1, 32.8. HRMS (ESI), *m/z*: $[M + Na]^+$ Calcd for $C_{40}H_{34}O_5Na$ 617.2298; found 617.2298.

1-(1,3-bis(benzyloxy)-6,8-bis((benzyloxy)methoxy) naphtalen-2-yl) ethan-1-one (13b)

To a solution of **12** (930 mg, 2.24 mmol, 1.0 equiv) in CH_2Cl_2 (16 mL), DIPEA (3.8 mL, 22.4 mmol, 10.0 equiv) and BOMCl (1.2 mL, 8.96 mmol, 4.0 equiv) were added at 0 °C. The reaction was stirred at room temperature overnight under nitrogen atmosphere. The mixture was quenched with 10 mL of a saturated aqueous solution of NH_4Cl, extracted with Et_2O (3 x 50 mL), washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 30% EtOAc) to afford compound **13b** as a yellow oil (730 mg, 50% yield). R_f = 0.6 *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 – 7.11 (m, 20H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.88 – 6.83 (m, 2H), 5.29 (s, 2H), 5.21 (s, 2H), 5.10 (s, 2H), 4.98 (s, 2H), 4.68 (s, 2H), 4.54 (s, 2H), 2.46 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 202.7, 156.5, 155.2, 153.6, 152.2, 138.6, 137.8, 137.2, 137.1, 136.4, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.1, 125.9, 112.1, 104.0, 103.9, 102.9, 93.6, 92.2, 78.5, 70.3, 70.2, 70.2, 32.8. HRMS (ESI), *m/z*: $[M + Na]^+$ Calcd for $C_{42}H_{38}O_7Na$ 677.2510; found 677.2510.

1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphtalen-2-yl)ethan-1-one (13c)

Me_2SO_4 (0.8 mL, 8.90 mmol, 10.0 equiv) and K_2CO_3 (616 mg, 4.46 mmol, 5.0 equiv) were added to a solution of **12** (370 mg, 0.89 mmol, 1.0 equiv) in acetone (15 mL) and the reaction was stirred overnight at 60 °C. The mixture was quenched with 10 mL of an aqueous solution of NaOH (1 M) and extracted with Et_2O (3 x 50 mL). The solvent was removed under vacuum and the residue was stirred with 10 mL of an aqueous solution of NH_4OH (1 M) for 2 h. The aqueous phase was extracted with Et_2O (3 x 50 mL) and the combined organic phases were washed with H_2O (2 x 30 mL), brine (1 x 30 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/ *n*-hexane gradient from 10% EtOAc to 20 % EtOAc) to afford product **13c** as a yellow oil (275 mg, 70% yield). R_f = 0.6, *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.32 (m, 10H), 6.96 (s, 1H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 5.21 (s, 2H), 5.02 (s, 2H), 3.92 (s, 3H), 3.88 (s, 3H), 2.58 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 202.9, 159.2, 157.6, 153.7, 152.5, 138.8, 137.6, 136.46, 128.6, 128.4, 128.2, 128.0, 127.9, 127.1, 125.2, 111.2, 103.7, 98.6, 97.4, 78.9, 70.2, 55.8, 55.3, 32.8. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{28}H_{26}O_5Na$ 465.1672; found 465.1672.

1-(1,3-bis(benzyloxy)-6,8-bis((tert-butyldimethyl-silyl)oxy)naphtalen-2-yl)ethan-1-one (13d)

TBDMSCl (85.1 mg, 0.56 mmol, 2.5 equiv) and DIPEA (0.1 mL, 0.68 mmol, 3.0 equiv) were added to a solution of **12** (100 mg, 0.23mmol, 1.0 equiv) in DMF (1 mL) and the reaction was stirred at room temperature overnight under nitrogen atmosphere. The mixture was extracted with Et_2O (3 x 10 mL), washed with 10 mL of brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 20% EtOAc) to afford compound **13d** as a yellow oil (140 mg, 99% yield). R_f= 0.6 *n*-hexane/EtOAc, 9:1. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45-7.37 (m, 10 H), 6.90 (s, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 5.19 (s, 2H), 5.08 (s, 2H), 2.50 (s, 3H), 1.07 (s, 9H), 0.94 (s, 9H), 0.31 (s, 6H), 0.11 (s, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 202.4, 154.9, 153.4, 153.3, 153.0, 138.6, 137.7, 136.5, 128.6, 128.1, 128.0, 127.5, 127.5, 127.1, 125.0, 113.8, 110.8, 109.0, 103.1, 78.0, 70.1, 32.9, 26.21, 25.7, 18.9, 18.3, -4.0,-4.2. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{38}H_{50}O_5Si_2Na$ 665.3089; found 665.3088.

1-(1,3,6,8-tetrakis(benzyloxy)naphtalen-2-yl)vinyl acetate (14a)

LiHMDS (33.0 µL, 0.17 mmol, 2.0 equiv) was added to a solution of **13a** (50.0 mg, 0.08 mmol, 1.0 equiv) in THF (2 mL) at 0 °C and the reaction was stirred for 15 min. Then acetic anhydride (9.5 mg, 0.09 mmol, 1.1 equiv) was added dropwise and the reaction was monitored by LC-MS confirming the complete conversion of the starting material into the desired product after 2 h. The reaction was quenched with H_2O (5 mL) and the mixture was extracted with EtOAc (3 x 20 mL), washed with 10 mL of brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was then purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford **14a** as a white solid (25.0 mg, 50% yield). R_f= 0.4 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 – 7.26 (m, 14H), 7.23 – 7.13 (m, 6H), 6.90 (s, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 6.54 (d, *J* = 2.2 Hz, 1H), 5.45 (d, *J* = 1.3 Hz, 1H), 5.31 (d, *J* = 1.3 Hz, 1H), 5.20 (s, 2H), 5.12 (s, 2H), 5.11 (s, 2H) 5.05 (s, 2H), 1.94 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 168.5, 158.1, 157.0, 155.5, 155.3, 146.3, 138.5, 138.2, 136.9, 136.7, 136.4, 128.7, 128.5, 128.4, 128.1, 128.0, 127.8, 127.71, 127.67, 127.4, 127.3, 127.1, 118.1, 112.1, 108.3, 103.6, 100.0, 99.6, 76.4, 71.2, 70.2, 70.0, 21.3. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{42}H_{37}O_6$ 637.25; found 637.2.

General procedure for the aldol condensation with acetaldehyde (procedure B1)

To a solution of **13b** or **13c** (1.0 equiv, 0.11 M in THF), LiHMDS (1 M in THF, 3.0 equiv) was added dropwise at -40 °C under nitrogen atmosphere. The solution was stirred for 10 min and then acetaldehyde (5 M in THF, 4.0 equiv) was added. The reaction was stirred for 15 min keeping the temperature at -40 °C, then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with Et_2O, washed with brine dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by Flash chromatography eluting with the indicated solvent.

1-(1,3-bis(benzyloxy)-6,8-bis((benzyloxy) methxy)naphtalen-2-yl)-3-hydroxybutan-1-one (14b)

Compound **13b** (750 mg, 1.14 mmol) was reacted according to the general procedure B1. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **14b** as a yellow oil (700 mg, 90% yield). R_f = 0.3 *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 – 7.25 (m, 18H), 7.24 – 7.18 (m, 2H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.96 (s, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 5.38 (s, 2H), 5.29 (s, 2H), 5.17 (s, 2H), 5.08 (d, *J* = 10.5 Hz, 1H), 5.04 (d, *J* = 10.5 Hz, 1H), 4.76 (s, 2H), 4.62 (s, 2H), 4.30 – 4.21 (m, 1H), 3.13 (br, 1H), 3.02 (dd, *J* = 17.1, 2.7 Hz, 1H), 2.86 (dd, *J* = 17.1, 9.0 Hz, 1H), 1.12 (d, *J* = 6.4 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 206.0, 156.7, 155.2, 153.4, 152.3, 138.8, 137.4, 137.14, 137.07, 136.09, 128.7, 128.52, 128.48, 128.40, 128.18, 128.16, 128.0, 127.9, 127.8, 127.7, 127.2, 125.1, 112.0, 104.1, 103.9, 103.0, 93.6, 92.2, 78.78, 70.4, 70.36, 70.27, 64.4, 53.7, 22.3. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{44}H_{43}O_8$ 699.29; found 699.3.

1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphtalen-2-yl)-3-hydroxybuta-1-one (14c)

Compound **13c** (275 mg, 0.62 mmol) was reacted according to the general procedure B1. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **14c** as a yellow oil (272 mg, 90% yield). R_f = 0.5 *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 – 7.21 (m, 10H), 6.86 (s, 1H), 6.57 (d, *J* = 2.2 Hz, 1H), 6.35 (d, *J* = 2.2 Hz, 1H), 5.09 (s, 2H), 4.93 (d, *J* = 10.5 Hz, 1H), 4.88 (d, *J* = 10.5 Hz, 1H) 4.19 (m, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 2.96 (dd, *J* = 17.2, 2.7 Hz, 1H), 2.78 (dd, *J* = 17.2, 9.0 Hz, 1H), 1.04 (d, *J* = 6.3 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 206.2, 159.4, 157.63, 153.60, 152.7, 139.0, 137.3, 136.2, 128.7, 128.5, 128.2, 128.1, 128.0, 127.1, 124.4, 111.1, 103.9, 98.6, 97.6, 79.1, 70.3, 64.3, 55.8, 55.4, 53.6, 22.3. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{30}H_{31}O_6$ 487.20; found 487.2

General procedure for the synthesis of 8-azido octanal (22) or 6-azido hexanal (23)

Commercially available 8-bromo-1-octanol (100 mg, 0.48 mmol, 1.0 equiv) or 6-bromo-1-hexanol (87.0 mg, 0.48 mmol, 1.0 equiv) was dissolved in DMF (4mL), followed by the addition of NaN_3 (31.2 mg, 0.96 mmol, 2.0 equiv). The reaction was stirred at room temperature for 12 h. The mixture was extracted with Et_2O (3 x 50 mL), washed with brine (10 mL), dried over Na_2SO_4 and evaporated. The crude product was directly submitted to the next step without further purification. DMP (140 mg, 0.72 mmol, 1.5 equiv) was added to the solution of the azide in $CHCl_3$ (7 mL). The reaction was stirred at room temperature for 30 min and the crude product was purified by flash chromatography (*n*-hexane/CH_2Cl_2 4:6) to afford product **22** or **23** as a pale oil (99% yield for both **22** and **23**). **22** R_f = 0.5 *n*-hexane/CH_2Cl_2, 3:7. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.79 (t, *J* = 1.8 Hz, 1H), 3.28 (t, *J* = 6.9 Hz, 2H), 2.45 (td, *J* = 7.3, 1.8 Hz, 2H), 1.71 – 1.58 (m, 4H), 1.44 – 1.34 (m, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 202.60, 51.36, 43.77, 28.94, 28.85, 28.72, 26.47, 21.88. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_8H_{15}N_3ONa$ 192.1107; found 192.1107. For **23** analytical and spectroscopic data were consistent with those reported in the literature.[15]

General procedure for the aldol condensation with 22 or 23 (procedure B2)

LDA (1 M in THF) was freshly prepared according to the following procedure: 280 µL of 1,2-diisopropylamine were added to 920 µL of THF in dry conditions under inert atmosphere and cooled to -78°C, then 800 µL of *n*-BuLi (2.5 M in *n*-hexane) were added and the mixture was stirred at 0 °C for 40 min. LDA (1 M in THF, 1.5 equiv) was added dropwise to a solution of **13b** or**13c** or **13d** (1.0 equiv, 0.04 M in THF) at -78 °C and the reaction was stirred for 45 min under nitrogen keeping the temperature at -78 °C. Then **22** or **23** (2.0 equiv) – freshly prepared and stored under inert atmosphere until needed - was added dropwise to the solution and the reaction was stirred for 15 min at -78 °C. The reaction was quenched with a saturated aqueous solution of NH_4Cl, extracted with Et_2O (3 x 30 mL), washed with brine (5 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography or directly submitted to the following step without further purification.

10-azido-1-(1,3-bis(benzyloxy)-6,8-bis((benzyloxy)methoxy)naphthalen-2-yl)-3-hydroxydecan-1-one (16b)

Compound **13b** (30.0 mg, 0.04 mmol) was reacted according to general procedure B2. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 20% EtOAc) to afford compound **16b** as a yellow oil (38.0 mg, 99%). R_f = 0.4 *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 – 7.09 (m, 20H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.88 (s, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 5.30 (s, 2H), 5.21 (s, 2H), 5.09 (s, 2H), 5.02 (d, *J* = 10.5 Hz, 1H), 4.96 (d, *J* = 10.5 Hz, 1H), 4.68 (s, 2H), 4.54 (s, 2H), 3.98 (br, 1H), 3.15 (t, *J* = 7.0 Hz, 2H), 3.02 (d, *J* = 3.1 Hz, 1H), 2.97 (dd, *J* = 17.1, 2.5 Hz, 1H), 2.77 (dd, *J* = 17.1, 9.1 Hz, 1H), 1.53 – 1.14 (m, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 206.2, 156.7, 155.2, 153.4, 152.3, 138.8, 137.5, 137.1, 137.07, 136.1, 128.7, 128.52, 128.46, 128.40, 128.2, 128.1, 128.0, 127.88, 127.85, 127.81, 127.6, 127.2, 125.1, 112.1, 104.1, 103.9, 103.0, 93.6, 92.2, 78.7 , 70.4, 70.3, 70.3, 68.1, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.6, 25.3. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{50}H_{54}N_3O_8$ 824.38; found 824.4.

10-azido-1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxydecan-1-one (16c)

Compound **13c** (450 mg, 1.0 mmol) was reacted according to general procedure B2. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **16c** as a yellow oil (588 mg, 99%). R_f = 0.4 *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 -7.31 (m, 10H), 6.95 (s, 1H), 6.66 (d, *J* = 2.2 Hz, 1H), 6.44 (d, *J* = 2.2

Hz, 1H), 5.17 (s, 2H), 5.03 (d, *J* = 10.5 Hz, 1H), 4.97 (d, *J* = 10.5 Hz, 1H) 4.09 (br, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.24 (t, *J* = 7.0 Hz, 2H), 3.08 (dd, *J* = 17.1, 2.5 Hz, 1H), 2.86 (dd, *J* = 17.1, 9.1 Hz, 1H), 1.62 – 1.11 (m, 12H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) *δ*: 206.4, 159.4, 157.6, 153.6, 152.7, 139.0, 137.4, 136.2, 128.7, 128.5, 128.2, 128.1, 128.0, 127.1, 124.5, 111.1, 103.9, 98.6, 97.6, 79.1, 70.3, 68.0, 55.8, 55.4, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.6, 25.3. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{36}H_{42}N_3O_6$ 612.30; found 612.3.

10-azido-1-(1,3-bis(benzyloxy)-8-((*tert*-butyldimethylsilyl)oxy)-6-((isopropyldimethylsilyl)oxy)naphthalen-2-yl)-3-hydroxydecan-1-one (16d)

Compound **13d** (130 mg, 0.2 mmol) was reacted according to general procedure B2. The crude product was purified by Flash chromatography (*n*-hexane/EtOAc 9:1) to afford compound **16d** as a yellow oil (162 mg, 99%). R_f = 0.3 *n*-hexane/EtOAc, 9:1. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 7.35 – 7.17 (m, 10H), 6.78 (s, 1H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.35 (d, *J* = 2.2 Hz, 1H), 5.05 (s, 2H), 4.98 (d, *J* = 10.5 Hz, 1H), 4.93 (d, *J* = 10.5 Hz, 1H), 3.91 (br, 1H), 3.15 (t, *J* = 7.0 Hz, 2H), 2.98 (br, 1H), 2.90 (dd, *J* = 17.1, 2.4 Hz, 1H), 2.64 (dd, *J* = 17.1, 9.1 Hz, 1H), 1.54 – 1.44 (m, 2H), 1.30 – 1.13 (m, 10H), 0.94 (s, 9H), 0.82 (s, 9H), 0.18 (s, 6H), -0.00 (d, *J* = 2.4 Hz, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) *δ*: 205.8, 155.1, 153.5, 153.25, 153.16, 138.7, 137.3, 136.3, 128.6, 128.2, 128.1, 127.6, 127.2, 124.1, 113.7, 111.0, 109.1, 103.2, 78.3, 70.2, 67.9, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.7, 26.20, 26.16, 25.7, 25.4, 18.9, 18.3, -3.89, -3.93, -4.19. MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{46}H_{66}N_3O_6Si_2$ 812.44; found 812.4.

8-azido-1-(1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)-3-hydroxyoctan-1-one (16e)

Compound **13c** (450 mg, 1.0 mmol) was reacted following procedure B2. The crude product was directly submitted to the next step without further purification. R_f = 0.4 *n*-hexane/EtOAc, 7:3. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 7.47 – 7.32 (m, 10H), 6.94 (s, 1H), 6.66 (d, *J* = 2.2 Hz, 1H), 6.44 (d, *J* = 2.2 Hz, 1H), 5.17 (s, 2H), 5.02 (d, *J* = 10.5 Hz, 1H), 4.96 (d, *J* = 10.5 Hz, 1H), 4.05 (br, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.20 (t, *J* = 7.0 Hz, 2H), 3.14 (br, 1H) ,3.05 (dd, *J* = 17.1, 2.5 Hz, 1H), 2.84 (dd, , *J* = 17.1, 9.1 Hz, 1H), 1.57 – 1.11 (m, 8H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{34}H_{38}N_3O_6$ 584.27; found 584.3.

General procedure for the Dess-Martin Oxidation (procedure C)

To a solution of β-hydroxyketone **14b-c** or **16b-e** (1.0 equiv, 0.04 M in $CHCl_3$) DMP (1.5 equiv) was added in small portions. The suspension was stirred at 40 °C or at room temperature until complete consumption of the starting material (reaction monitored by TLC). The crude product was filtered on silica pad to remove the excess of DMP and directly submitted to the next step without further purification.

(*Z*)-1-(1,3-bis(benzyloxy)-6,8-bis((benzyloxy)methoxy)naphthalen-2-yl)-3-hydroxybut-2-en-1-one (15b)

Compound **14b** (700 mg, 1.0 mmol) was reacted according to general procedure C. The crude product was filtered on a silica pad to give **15b** which was directly submitted to the next step without further purification. R_f = 0.4 *n*-hexane/EtOAc, 8:2. Ketone **15b** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labelled with an asterisk when clearly distinguishable from the enol. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 15.49 (s, 1H), 7.41 – 7.10 (m, 26H), 6.95 (d, *J* = 2.2 Hz, 1.3H), 6.87 – 6.83 (m, 2.6H), 5.73 (s, 1H), 5.28 (s, 2.6H), 5.20 (s, 2.6H), 5.10 (s, 2H), 5.06* (s, 0.6H), 4.98 (s, 2.6H), 4.66 (s, 2.6H), 4.55 (s, 2H), 4.51* (s, 0.6H), 3.81* (s, 0.6H), 1.96 (s, 3H) 1.95*(s, 0.9H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{44}H_{41}O_8$ 697.27; found 697.3.

(*Z*)-1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxybut-2-en-1-one (15c)

Compound **14c** (395 mg, 0.81 mmol) was reacted according to general procedure C. The crude product was filtered on silica pad to afford **15c** which was directly submitted to the next step without further purification. R_f *n*-hexane/EtOAc, 7:3 = 0.4. Ketone **15c** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labelled with an asterisk when clearly distinguishable from the enol. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 15.50 (s, 1H), 7.42 – 7.20 (m, 14H), 6.85 (s, 1.4H), 6.56 (d, *J* = 2.2 Hz, 1.4H), 6.34 (d, *J* = 2.2 Hz, 1.4H), 5.77 (s, 1H), 5.13 (s, 2H), 5.09* (s, 0.8 H), 4.93* (s, 0.8 H), 4.90 (s, 2H), 3.82 (s, 4.2H), 3.79 (s, 4.2H), 1.98 (s, 4.2H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{30}H_{29}O_6$ 485.19; found 485.2.

(*Z*)-10-azido-1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxydec-2-en-1-one (17c)

Compound **16c** (269 mg, 0.44 mmol) was reacted according to general procedure C. The crude product was filtered on silica pad to afford **17c** which was directly submitted to the next step without further purification. R_f = 0.5 *n*-hexane/EtOAc, 7:3. Ketone **17c** is in equilibrium with its enolic form. According the NMR, the enol is predominant. The signals of the ketone are labelled with an asterisk when clearly distinguishable from the enol. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 15.61 (s, 1H), 7.48 – 7.30 (m, 14H), 6.93 (s, 1.4H), 6.64 (d, *J* =2.2 Hz, 1.4H), 6.42 (d, *J* = 2.2 Hz, 1.4), 5.83 (s, 1H), 5.20 (s, 2H), 5.17* (s, 0.8H), 4.99* (s, 0.8H), 4.98 (s, 2H), 3.90 (s, 4.2H), 3.86 (s, 4.2H), 3.23 (t, *J* = 7.0, 2.8H), 2.35* (t, *J* = 7.4 Hz, 0.8H), 2.27 (t, *J* = 8.0 Hz, 2H), 1.55-1.23 (m, 14H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{36}H_{40}N_3O_6$ 610.28; found 610.3.

Alternative procedure. Stabilised 2-iodoxybenzoic acid (275 mg, 0.98 mmol, 3 equiv) was added to compound **16c** (200 mg, 0.33 mmol) in EtOAc (10 mL) at room temperature. The reaction mixture was heated to 80 °C and stirred overnight. After cooling to room temperature, the reaction was filtered on silica pad, washing with EtOAc. The filtrate was washed with saturated $NaHCO_3$ (3 x 10 mL), dried ($MgSO_4$), filtered and concentrated under vacuum to give **17c** as a yellow oil.

(*Z*)-10-azido-1-(1,3-bis(benzyloxy)-8-((*tert*-butyldimethylsilyl)oxy)-6-((isopropyldimethylsilyl)oxy)naphthalen-2-yl)-3-hydroxydec-2-en-1-one (17d)

Compound **16d** (140 mg, 0.17 mmol) was reacted according to general procedure C (room temperature). The crude product was filtered on silica pad to afford **17d** which was directly submitted to the next step without further purification. R_f = 0.4 *n*-hexane/EtOAc, 9:1. Ketone **17d** is in equilibrium with its enolic form. According the NMR, the enol is predominant. The signals of the ketone are labelled

with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.52 (br, 1H), 7.46 – 7.27 (m, 14H), 6.88 (s, 1.4H), 6.77 (d, J = 2.2 Hz, 1.4H), 6.45 (d, J = 2.2 Hz, 1.4H), 5.73 (s, 1H), 5.19 (s, 2H), 5.15* (s, 0.8H), 5.07 (s, 2.8H), 3.84* (s, 0.8H), 3.25 (t, J = 6.9 Hz, 2.8H), 2.36* (t, J = 8 Hz, 0.8H), 2.26 (t, J = 8 Hz, 2H), 1.62 – 1.54 (m, 5.6H), 1.42 – 1.25 (m, 8.4H), 1.05 (s, 12.6H), 0.92 (s, 12.6H), 0.29 (s, 8.4H), 0.08 (s, 8.4H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{46}H_{64}N_3O_6Si_2$ 810.43; found 810.4.

(*Z*)-8-azido-1-(1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)-3-hydroxyoct-2-en-1-one (17e)

Compound **16e** (255 mg, 0.44 mmol) was reacted according to general procedure C. The crude product was filtered on silica pad to afford **17e** which was directly submitted to the next step without further purification. R_f = 0.5 *n*-hexane/EtOAc, 7:3. Ketone **17e** is in equilibrium with its enolic form. According the NMR, the enol is predominant. The signals of the ketone are labelled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.61 (br, 1H), 7.55 – 7.29 (m, 14H), 6.94 (s, 1.4H), 6.65 (d, J = 2.2 Hz, 1.4H), 6.43 (d, J = 2.2 Hz, 1.4H), 5.85 (s, 1H), 5.20 (s, 2H), 5.16* (s, 0.8H), 5.00 (s, 2.8H), 3.92* (s, 0.8H), 3.89 (s, 4.2H), 3.86 (s, 4.2 H) 3.21-3.15 (m, 2.8H), 2.36* (t, J = 8 Hz, 0.8H), 2.29 (t, J = 8 Hz, 2H), 1.64 – 1.38 (m, 8.4H). MS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{34}H_{36}N_3O_6$ 582.25; found 582.2.

General procedure for the hydrogenolysis (procedure D)

Glacial AcOH (1.0 equiv) and a catalytic amount of $Pd(OH)_2$ on carbon 20% were added to a suspension of 1,3-diketone **15b-c** or **17b-d** (1.0 equiv, 0.014 M in MeOH) under nitrogen. The reaction was stirred under hydrogen atmosphere and monitored via LC-MS using a gradient from 30% of ACN (containing 0.1 % of TFA) to 100% of ACN (containing 0.1% of TFA) in water over 10 min (METHOD 1) confirming the complete conversion of the starting material into the desired product. $Pd(OH)_2$ was removed by filtration on a cotton pad and the solvent was slowly evaporated at low temperature under vacuum. The crude product was purified by preparative RP-HPLC using a gradient from 30% of ACN (containing 0.05% of TFA) to 100% of ACN (containing 0.05% of TFA) in water over 10 min (METHOD 2) to obtain the final products YWA1 and fonsecin B. Intermediates **18a** and **18b** instead, due to the reactivity of the amine group, proved to be unstable in solution and therefore were used immediately in the next conjugation step without further purification.

YWA1

Compound **15b** (100 mg, 0.14 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the complete conversion of the starting material to the desired product in 12 h. The product was submitted to preparative RP-HPLC (METHOD 2) and isolated as a yellow solid (36.0 mg, 90% yield), rt = 7.8 min. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 15.37 (s, 1H), 9.45 (s, 1H), 9.20 (s, 1H), 6.54 (d, J = 2.2 Hz, 1H), 6.47 (s, 1H), 6.30 (d, J = 2.2 Hz, 1H), 6.05 (s, 1H), 3.19 (d, J = 17.1 Hz, 1H), 2.88 (d, J = 17.1 Hz, 1H), 1.74 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $(CD_3)_2CO$) δ: 198.8, 165.0, 162.8, 160.8, 154.1, 143.6, 105.0, 102.9, 102.8, 102.4, 101.3, 100.8, 47.8, 28.4. MS (ESI) m/z: $[M + H]^+$ Calc. for $C_{14}H_{13}O_6$ 277.06; found 277.1. Analytical and spectroscopic data were consistent with those reported in the literature.[5]

Fonsecin B

Compound **15c** (100 mg, 0.21 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the complete conversion of the starting material into the desired product in 3 h. The product was submitted to preparative RP-HPLC (METHOD 2) and isolated as a yellow solid (56.0 mg, 90% yield), rt = 8.5 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 14.21 (s, 1H), 6.45 (s, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.24 (d, J = 2.2 Hz, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 2.95 (d, J = 17.0 Hz, 1H), 2.85 (d, J = 17.0 Hz, 1H), 1.68 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 196.0, 164.9, 162.5, 161.4, 153.1, 143.3, 107.4, 103.1, 102.6, 100.0, 98.6, 96.7, 56.1, 55.4, 47.0, 28.7. HRMS (ESI), *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{16}O_6Na$ 327.0839; found 327.0840.

General procedure for the coupling reaction (procedure E)

Intermediate **18a** or **18b** (1.0 equiv) was dissolved in DMF and then the desired fluorescent tag (1.5 equiv) was added in one portion. After the addition of DIPEA (2.0 equiv), the solution was stirred for 0.5 h under nitrogen atmosphere. The reaction was monitored by LC-MS using a gradient from 30% of ACN (containing 0.1% of TFA) to 100% of ACN (containing 0.1% of TFA) in water over 10 min (METHOD 1) confirming the total conversion of the starting material into the desired product. The compound was then purified by preparative RP-HPLC with a gradient from 30% of ACN (containing 0.05% of TFA) to 100% of ACN (containing 0.05% of TFA) in water over 10 min (METHOD 2).

Oregon Green derivative 19

Compound **17c** (25.0 mg, 0.04 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the complete conversion of the starting material into the desired product **18b** in 3 h, rt = 5.7 min. MS (ESI, m/z): $C_{22}H_{30}NO_6$ $[M+H]^+$ calc. 404.20 found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with Oregon green-NHS (30.0 mg, 0.06 mmol, 1.5 equiv) – previously prepared as reported by Sun *et al.*[15] (see Supporting Information) – and DIPEA (15.0 µL, 0.08 mmol, 2.0 equiv) in 1 mL of DMF following general procedure E. The reaction was monitored by LC-MS (METHOD 1), observing the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (METHOD 2) to give **19** as a yellow solid (15.0 mg, 50% yield over two steps), rt = 10.1 min. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 14.13 (s, 1H), 8.22 (d, J = 8.0, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.96 (t, J = 4Hz, 1H), 7.76 (s, 1H), 6.96 (d, J = 7.5 Hz, 2H), 6.64 – 6.57 (m, 3H), 6.51 (d, J = 3.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.36 – 3.32 (m, 2H), 3.08 (d, J = 16.9 Hz, 1H), 2.75 (d, J = 16.9 Hz, 1H), 1.58 – 1.51 (m, 4H) 1.40 – 1.23 (m, 8H). HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{43}H_{37}F_2NO_{12}Na$ 820.2176; found 820.2172.

Biotin derivative 20a

TBAF (1 M in THF, 0.1 mL, 0.13 mmol, 2.5 equiv) was added to a solution of **17d** (45.0 mg, 0.05 mmol, 1.0 equiv) in THF (4 mL) at 0 °C. The reaction was stirred for 3 h under nitrogen at 0 °C and quenched with 5 mL of a saturated

aqueous solution of NH_4Cl, extracted with Et_2O (3 x 5 mL) washed with brine (5 mL), dried over Na_2SO_4 and the solvent was evaporated under vacuum. The crude product was filtered on silica pad and directly reacted with glacial AcOH (4 µL, 0.05 mmol, 1.0 equiv) and a catalytic amount of $Pd(OH)_2$ on carbon 20% in 3 mL of MeOH under hydrogen according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the conversion of the starting material into **18a** in 3 h, rt = 5.0 min. MS (ESI, m/z): $C_{20}H_{26}NO_6$ $[M+H]^+$ calc. 376.17 found 376.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with Biotin-NHS (28.0 mg, 0.08 mmol, 1.5 equiv) – previously prepared as reported by Susumu *et al.*[16] – and DIPEA (10.0 µL, 0.10 mmol, 2.0 equiv) in 1mL of DMF according to general procedure E. The reaction was monitored by LC MS (METHOD 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (METHOD 2) to give 20a as a white solid (15.0 mg, 50% yield over three steps), rt = 7.5 min. ^1H NMR (400 MHz, CD_3OD) δ: 6.33 (s, 1H), 6.30 (d, J = 2.1 Hz, 1H), 6.11 (d, J = 2.1 Hz, 1H), 4.37 (dd, J = 7.8, 4.7 Hz, 1H), 4.18 (dd, J = 7.8, 4.7 Hz, 1H), 3.10 – 3.06 (m, 3H), 2.99 – 2.88 (m, 1H), 2.81 (ddd, J = 12.7, 4.7, 1.3 Hz, 1H), 2.67 – 2.62 (m, 1H), 2.60 (d, J = 12.7 Hz, 1H), 2.10 (t, J = 7.3 Hz, 2H), 1.85 – 1.74 (m, 2H), 1.63 – 1.29 (m, 16H). HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{30}H_{39}N_3O_8SNa$ 624.2350; found 624.2350.

Biotin derivative 20b

Compound **17c** (25.0 mg, 0.04 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the complete conversion of the starting material into the desired product **18b** in 3 h, rt = 5.7 min. MS (ESI, m/z): $C_{22}H_{30}NO_6$ $[M+H]^+$ calc. 404.20 found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with Biotin-NHS (21.0 mg, 0.06 mmol, 1.5 equiv) – previously prepared as reported by Susumu *et al.*[16] – and DIPEA (8.0 µL, 0.08 mmol, 2.0 equiv) in 1 mL of DMF according to general procedure E. The reaction was monitored by LC MS (METHOD 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (METHOD 2) to give 20b as a white solid (13.0 mg, 50% yield over two steps), rt = 9.3 min. ^1H NMR (400 MHz, $(CD_3)_2CO$) δ: 14.02 (s, 1H), 6.92 (br, 1H), 6.52 (d, J = 2.2 Hz, 1H), 6.40 (s, 1H), 6.22 (d, J = 2.2 Hz, 1H), 5.70 (br, 1H), 5.52 (br, 1H), 4.37 (dd, J = 7.4, 4.8 Hz, 1H), 4.20 (dd, J = 7.4, 4.8 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.10 – 3.03 (m, 3H), 2.97 (d, J =16.8 Hz, 1H), 2.84 – 2.76 (m, 2H), 2.65 (d, J = 16.8 Hz, 1H), 2.57 (d, J = 12.8 Hz, 1H), 2.04 (t, J = 7.2 Hz, 2H), 1.88 – 1.80 (m, 2H), 1.70 – 1.57 (m, 2H), 1.56 – 1.18 (m, 14H). HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{43}N_3O_8SNa$ 652.2663; found 652.2661.

PyMPO derivative 21

17c (7.0 mg, 0.01 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (METHOD 1) confirming the complete conversion of the starting material into **18b** in 3 h, rt = 5.7 min. MS (ESI, m/z): $C_{22}H_{30}NO_6$ $[M+H]^+$ calc. 404.20 found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with the commercially available PyMPO succinimidyl ester (5.0 mg, 0.01 mmol, 1.5 equiv) and DIPEA (5.0 µL, 0.02 mmol, 2.0 equiv) in 0.5 mL of DMF following procedure E. The reaction was monitored by LC-MS (METHOD 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (METHOD 2) to give 21 as an orange solid (5.0 mg, 60% over two steps), rt = 8.0 min. ^1H NMR (400 MHz, CD_3OD) δ: 9.02 (d, J = 6.4 Hz, 2H), 8.44 (d, J = 6.4 Hz, 2H), 8.01 (s, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.76 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.06 (d, J = 8.9 Hz, 2H), 6.45 (d, J = 2.2 Hz, 1H), 6.43 (s, 1H), 6.24 (d, J = 2.2 Hz, 1H), 5.87 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.44 (t, J = 6.6 Hz, 2H), 2.95 (d, J = 16.9 Hz, 1H), 2.64 (d, J = 16.9 Hz, 1H), 1.97 – 1.81 (m, 2H), 1.73 – 1.63 (m, 2H), 1.55 – 1.40 (m, 8H). $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ: 197.7, 167.7, 163.58, 163.56, 162.5, 161.5, 160.9, 156.0, 154.8, 153.8, 145.0, 143.4, 140.9, 135.9, 133.6, 131.8, 129.6, 128.2, 128.1, 126.6, 124.8, 122.8, 118.9, 114.4, 106.4, 103.1, 102.3, 101.6, 98.2, 96.0, 63.4, 54.8, 54.6, 54.5, 45.2, 40.3, 39.3, 28.7, 28.66, 28.1, 25.7, 22.6. HRMS (ESI) *m/z*: M^+ Calcd for $C_{45}H_{46}N_3O_9^+$ 772.3229; found 772.3228.

Flow cytometry

A. fumigatus ΔrodA conidia[16] were incubated in flow cytometry buffer (1.5% (w/v) BSA and 5 mM EDTA in PBS) on ice for 30 min. Fc-MelLec (5 µg/ml) was pre-treated with or without YWA1, Fonsecin B, 2, 6-DHAP, 1, 8-DHN, **20b** and **21** (all dissolved in DMSO and used at 70 µM resulting in a 1% DMSO solution in PBS) for 30 min at room temperature and then incubated with *ΔrodA* conidia on ice for 40 min. Following incubation, fungal particles were washed in flow cytometry buffer and Fc-MelLec bound to the conidia was detected with allophycocyanin (APC)-conjugated donkey anti-human IgG antibody (Jackson ImmunoResearch), and then fixed in 1% (v/v) formaldehyde and analysed by flow cytometry. One-way ANOVA and Bonferroni Post-hoc tests were performed to compare inhibition of Fc-MelLec binding to *ΔrodA* conidia by the different compounds versus uninhibited Fc-MelLec control. Fold change was calculated by dividing the mean value of the pre-treated Fc-MelLec with each compound by the mean value of the uninhibited Fc-MelLec.

Cell culture and growth conditions

RAW 264.7 cells expressing human MelLec (hMelLec)[9] were maintained at 37°C and 5% CO_2 in DMEM medium supplemented with 10% heat-inactivated foetal calf serum (FCS), 100 units per mL penicillin, 0.1 mg/mL streptomycin, 2 mM L-glutamine and 400 µg/ml geneticin (G418) (Life Technologies, Inc.).

Immunofluorescence microscopy

RAW 264.7 cells expressing human MelLec (hMelLec) ($1.25x10^5$ cells/ well) were seeded in 48-well plate at 37°C and 5% CO_2. Next day, the cells were washed with PBS (1X) and then incubated with 10, 20, 50 or 100 µM of 21 at 37°C and 5% CO_2 for 3h. The cells were washed with PBS (1X) and then were visualised under an inverted immunofluorescent microscope (Zeiss). Cells without 21 were included as a negative control (untreated).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXXX.

- Experimental procedure for the synthesis of Oregon Green NHS, precursor of compound **19**.
- Immunofluorescence microscopy and image analysis experiments on binding of **21** to human MelLec-expressing RAW264.7 cells with competition by YWA1 at different concentrations (1, 5, 25 and 50 μM).
- Copies of ^{1}H and ^{13}C NMR spectra

AUTHOR INFORMATION

Corresponding Authors

* matteo.zanda@scitec.cnr.it; gordon.brown@exeter.ac.uk

Author Contributions

M.P., I.P. and C.Z. designed and carried out the synthesis of all the compounds. N.S. repeated the synthesis of all compounds. C.N. and J.A.W. performed the biological studies. G.D.B. and M.Z. designed, supervised and coordinated the study. M.P., G.D.B. and M.Z. wrote the manuscript. I.P., M.P., N.S., C.Z., C.N. and J.A.W. wrote the Supporting Information.

Funding Sources

Funding was provided by the Wellcome Trust (102705, 097377), the Medical Research Council Centre for Medical Mycology and the University of Aberdeen (MR/N006364/1).

ACKNOWLEDGMENT

We thank Ian Fraser Flow Cytometry and Microscopy and Histology facilities, University of Aberdeen.

REFERENCES

†This article is dedicated to the memory of Dr Leonardo Manzoni.

(1) Xu, G.-B.; Yang, T.; Bao, J.-K.; Fang, D.-M.; Li, G.-Y. Isochaetomium A2, a New Bis(Naphthodihydropyran-4-One) with Antimicrobial and Immunological Activities from Fungus Chaetomium Microcephalum. *Arch. Pharm. Res.* **2014**, *37* (5), 575–579.

(2) Lee, H.-M.; Chan, D. S.-H.; Yang, F.; Lam, H.-Y.; Yan, S.-C.; Che, C.-M.; Ma, D.-L.; Leung, C.-H. Identification of Natural Product Fonsecin B as a Stabilizing Ligand of C-Myc G-Quadruplex DNA by High-Throughput Virtual Screening. *Chem. Commun.* **2010**, *46* (26), 4680.

(3) Koyama, K.; Ominato, K.; Natori, S.; Tashiro, T., Tsuruo, T. Cytotoxicity and Antitumor Activities of Fungal Bis (Naphtho-γ-Pyrone) Derivatives. *Journal of pharmacobio-dynamics* **1988**, *11* (9), 630–635.

(4) He, Y.; Tian, J.; Chen, X.; Sun, W.; Zhu, H.; Li, Q.; Lei, L.; Yao, G.; Xue, Y.; Wang, J.; et al. Fungal Naphtho-γ-Pyrones: Potent Antibiotics for Drug-Resistant Microbial Pathogens. *Sci Rep* **2016**, *6* (1), 24291.

(5) Watanabe, A.; Timberlake, W. E.; Ebizuka, Y. Re-Identification Of Aspergillus nidulans wA Gene to Code for a Polyketide Synthase of Naphthopyrone. *Tetrahedron Letters* **1999,** *40*, 91-94.

(6) Vagstad, A. L.; Hill, E. A.; Labonte, J. W.; Townsend, C. A. Characterization of a Fungal Thioesterase Having Claisen Cyclase and Deacetylase Activities in Melanin Biosynthesis. *Chemistry & Biology* **2012**, *19* (12), 1525–1534.

(7) Newman, A. G.; Vagstad, A. L.; Storm, P. A.; Townsend, C. A. Systematic Domain Swaps of Iterative, Nonreducing Polyketide Synthases Provide a Mechanistic Understanding and Rationale for Catalytic Reprogramming. *J. Am. Chem. Soc.* **2014**, *136* (20), 7348–7362.

(8) Tsai, H.-F.; Fujii, I.; Watanabe, A.; Wheeler, M. H.; Chang, Y. C.; Yasuoka, Y.; Ebizuka, Y.; Kwon-Chung, K. J. Pentaketide Melanin Biosynthesis in *Aspergillus Fumigatus* Requires Chain-Length Shortening of a Heptaketide Precursor. *J. Biol. Chem.* **2001**, *276* (31), 29292–29298.

(9) Stappers, M. H. T.; Clark, A. E.; Aimanianda, V.; Bidula, S.; Reid, D. M.; Asamaphan, P.; Hardison, S. E.; Dambuza, I. M.; Valsecchi, I.; Kerscher, B.; et al. Recognition of DHN-Melanin by a C-Type Lectin Receptor Is Required for Immunity to Aspergillus. *Nature* **2018**, *555* (7696), 382–386.

(10) DiVirgilio, E. S.; Dugan, E. C.; Mulrooney, C. A.; Kozlowski, M. C. Asymmetric Total Synthesis of Nigerone. *Org. Lett.* **2007**, *9* (3), 385–388.

(11) Arima, Y., S., S. ,. Metabolic Products of Fungi. XXV. Synthesis of Ribofusarin and Its Derivatives.Pdf. *Chem. Pharm. Bull.* **1967**, *15* (11), 1757–1764.

(12) Bycroft, B. W.; Roberts, J. C. 929. Studies in Mycological Chemistry. Part XIV. Synthesis of Flavasperone. *J. Chem. Soc.* **1963**, *929*, 4868-4872.

(13) Qi, C.; Wang, W.; Reichl, K. D.; McNeely, J.; Porco, J. A. Total Synthesis of Aurofusarin: Studies on the Atropisomeric Stability of Bis-Naphthoquinones. *Angew. Chem. Int. Ed.* **2018**, *57* (8), 2101–2104.

(14) Vagstad, A. L.; Hill, E. A.; Labonte, J. W.; Townsend, C. A. Characterization of a Fungal Thioesterase Having Claisen Cyclase and Deacetylase Activities in Melanin Biosynthesis. *Chem. Biol.* **2012**, *19* (12), 1525–1534.

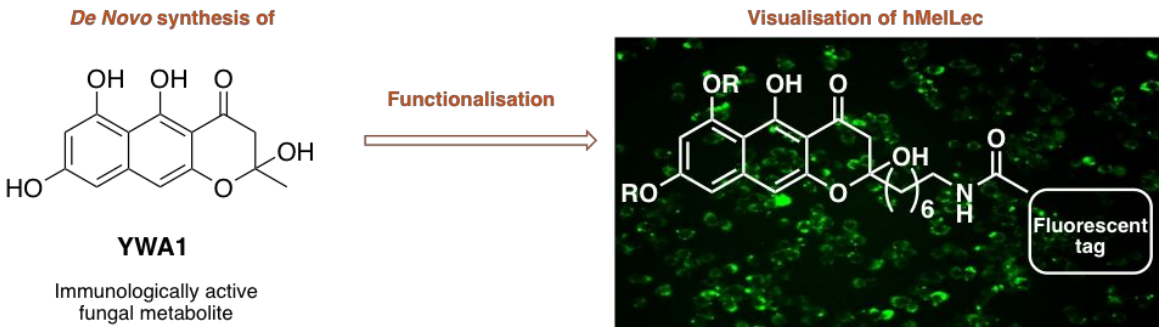
(15) Sun, W.-C.; Gee, K. R.; Klaubert, D. H.; Haugland, R. P. Synthesis of Fluorinated Fluoresceins. *J. Org. Chem.* **1997**, 62, 6469-6475.

(16) Susumu, K.; Uyeda, H. T.; Medintz, I. L.; Pons, T.; Delehanty, J. B.; Mattoussi, H. Enhancing the Stability and Biological Functionalities of Quantum Dots via Compact Multifunctional Ligands. *J. Am. Chem. Soc.* **2007**, *129* (45), 13987–13996.

(17) Rakers, L.; Grill, D.; Matos, A. L. L.; Wulff, S.; Wang, D.; Börgel, J.; Körsgen, M.; Arlinghaus, H. F.; Galla, H.-J.; Gerke, V.; et al. Addressable Cholesterol Analogs for Live Imaging of Cellular Membranes. *Cell Chem. Biol.* **2018**, *25* (8), 952-961.

(18) Thau. N, Monod M., Crestani B., Rolland C., Tronchin G., Latgè JP, Paris S., Rodletless Mutants of Aspergillus Fumigatus. *Infect. Immun.* **1994**, *62* (12), 4380–4388.

Table of Contents



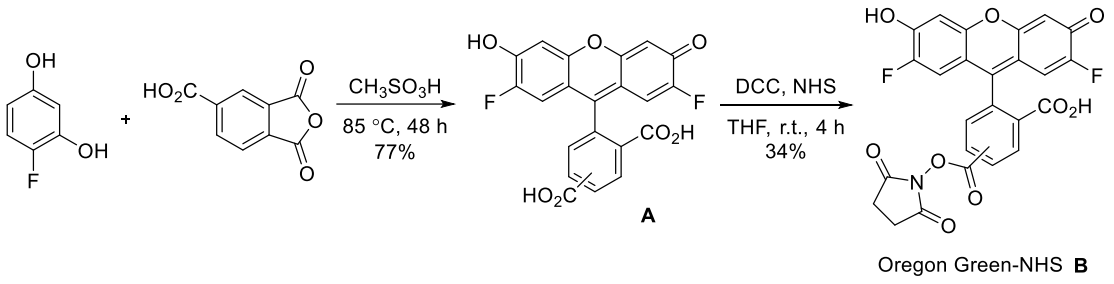
Supporting information

Synthesis of the fungal metabolite YWA1 and related constructs as tool to study MelLec receptor-mediated immune response to *Aspergillus* infections

Monica Piras,* Ilaria Patruno, Christina Nikolakopoulou, Janet A. Willment, Nikki Sloan, Chiara Zanato, Gordon D. Brown,* Matteo Zanda*

Table of Contents

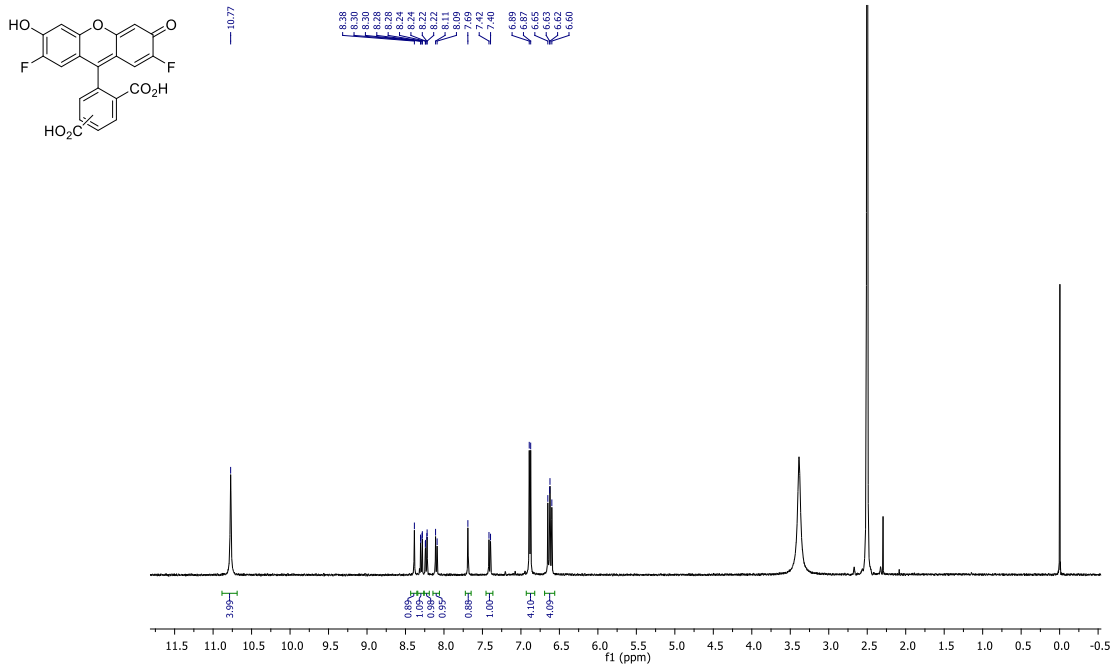
1. Synthesis of Oregon Green NHS Ester (Precursor of compound 19)



Oregon green 488 carboxylic acid (A)

4-Fluororesorcinol (150 mg, 1.17 mmol, 2 equiv.) was dissolved in methanesulfonic acid (2 mL), before addition of 1,2,4-benzenetricarboxylic anhydride (112 mg, 0.58 mmol, 1 equiv.) and heating to 85 °C for 48 h. The reaction mixture was cooled to room temperature and diluted in ice water before filtration and washings with cold water. The filtrate was dissolved in a minimum amount of methanol and precipitated in cold water before refiltration. The solid was dried under vacuum to constant weight to afford Oregon green 488 carboxylic acid **A** (mixture of 5- and 6-isomer) as an orange solid (186 mg, 77% yield). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ: 10.77 (s, 4H), 8.38 (s, 1H), 8.29 (dd, J = 8.0, 1.4 Hz, 1H), 8.23 (dd, J = 8.0, 1.4 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.41 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 7.5 Hz, 4H), 6.62 (dd, J = 11.2, 9.6, 4H). HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{21}H_{11}F_2O_7$ 413.0467; found 413.0467. Analytical and spectroscopic data were consistent with those reported in the literature for the separated isomers.[1]

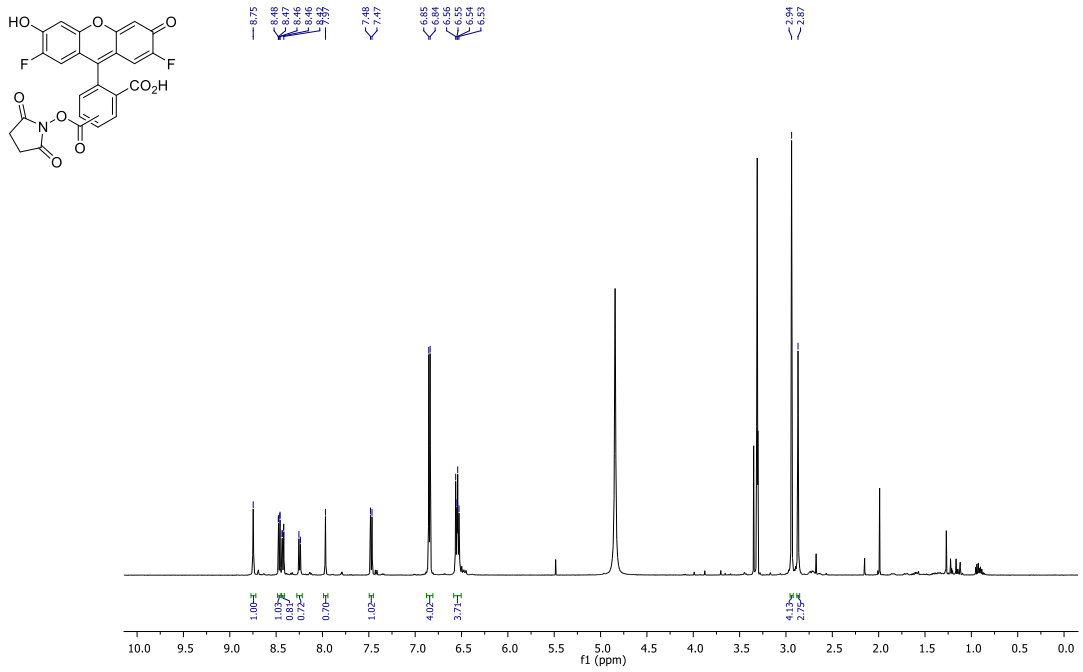
^{1}H NMR (400 MHz, $(CD_3)_2SO$)



Oregon Green 488 Carboxylic acid, succinimidyl ester (B)

Oregon Green 488 carboxylic acid **A** (50 mg, 0.12 mmol, 1 equiv.), *N,N'*-dicyclohexylcarbodiimide (30 mg, 0.15 mmol, 1.2 equiv.) and *N*-hydroxysuccinimide (20 mg, 0.18 mmol, 1.5 equiv.) were dissolved in THF and stirred at room temperature under nitrogen atmosphere for 4 h. The reaction mixture was filtered, washed with THF and concentrated under vacuum. The crude product was filtered on silica pad to afford Oregon green 488 carboxylic acid, NHS ester **B** (mixture of 5- and 6-isomer) as an orange solid, which was used in the next step without further purification. ^{1}H-NMR (500 MHz, CD_3OD) δ: 8.75 (s, 1H), 8.47 (dd, J = 8.0, 1.4 Hz, 1H), 8.43 (dd, J = 8.0, 1.4 Hz, 0.7H), 8.25 (d, J = 8.0 Hz, 0.7H), 7.97 (s, 0.7H), 7.47 (d, J = 8.0 Hz, 1H), 6.85 (d, J = 7.4 Hz, 4H), 6.55 (dd, J = 11.0, 7.0, 4H), 2.94 (s, 4H), 2.87 (s, 2.8H). HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{14}F_2N_1O_9$ 510.0631; found 510.0632.

^{1}H NMR (400 MHz, CD_3OD)



REFERENCES

(1) Sun, W.-C.; Gee, K. R.; Klaubert, D. H.; Haugland, R. P. Synthesis of Fluorinated Fluoresceins. *J. Org. Chem.* **1997**, 62, 6469-6475.

2. Immunofluorescence microscopy and image analysis experiments on binding of 21 to human MelLec-expressing RAW264.7 cells with competition by YWA1 at different concentrations (1, 5, 25 and 50 μM).

Methodology

Immunofluorescence microscopy and image analysis

RAW264.7 cells expressing human MelLec (hMelLec) were seeded in an 8-well ibidiTreat dish (Ibidi) ($1.25x10^5$ cells/ well) and incubated at 37°C and 5% CO_2 overnight. Next day, the cells were incubated with or without 1, 5, 25 or 50 μM of YWA1 at 37°C and 5% CO_2 for 1h. After that, the cells were incubated with or without 25 μM of **21** at 37°C and 5% CO_2 for further 3h. The cells were washed with PBS (1X) and then visualised under an inverted fluorescent microscope (Delta Vision, Applied Precision) with a sCMOS_4.2 camera (Photometrics), running softWoRx 6.x using UPLSAPO 20X/0.85 NA oil immersion objective and a standard FITC filter. Cells without YWA1 or **21** were included as a positive or untreated control, respectively.

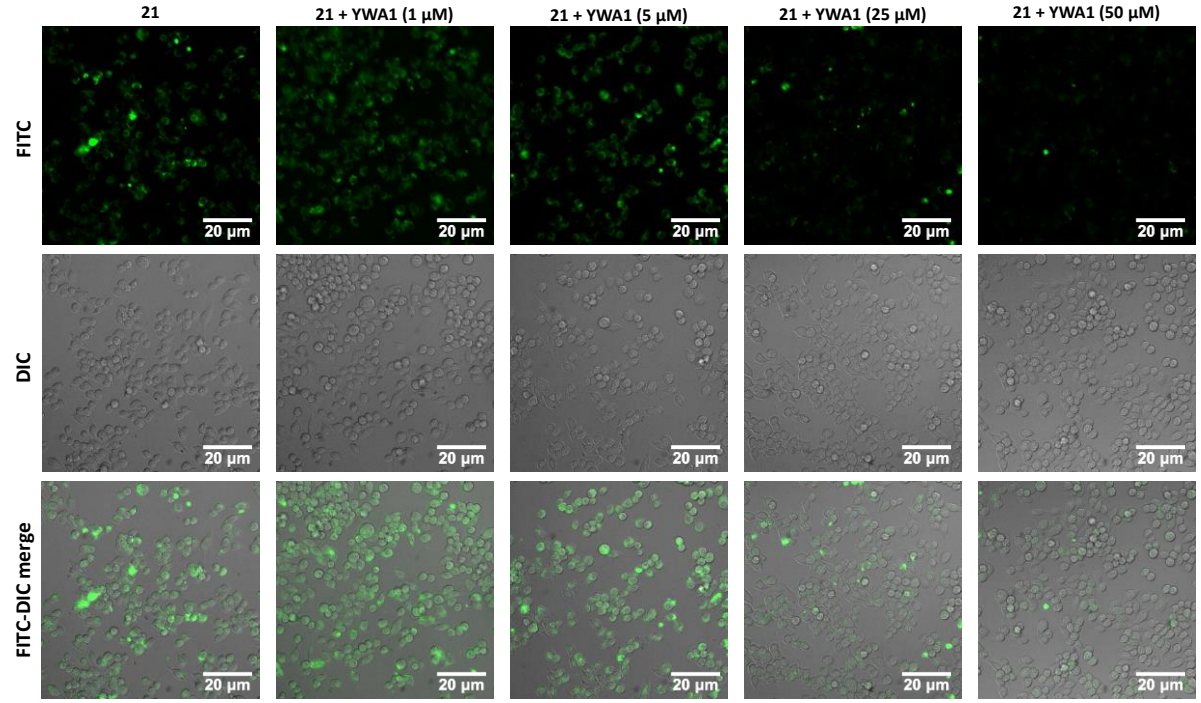
For image processing, the background of each image was subtracted in ImageJ V2.1.0 (Rolling ball radius: 20 pixels with sliding paraboloid) and the brightness/contrast was adjusted equally across all images for visual presentation. For fluorescence intensity measurements, the background of each image was subtracted as described above without adjusting brightness and contrast. The mean fluorescence intensity (MFI) was measured for 40 cells per image for each condition (1 image was processed for each condition).

Statistical analyses

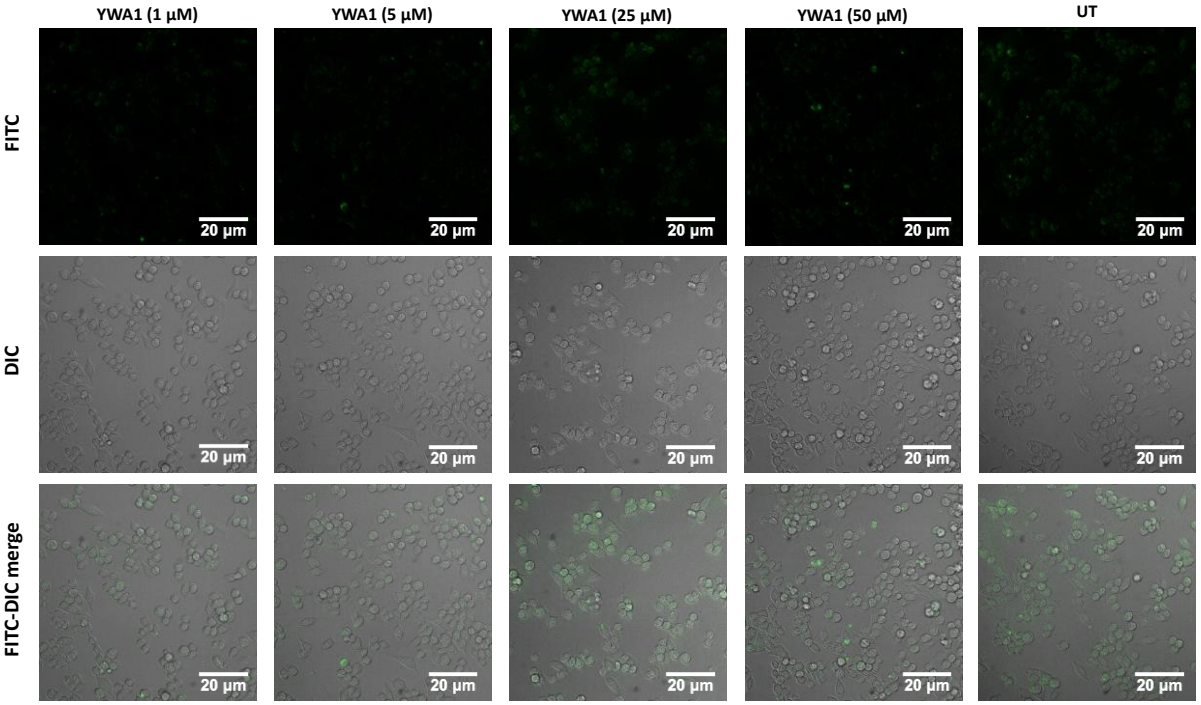
Data generated in this study were imported into GraphPad Prism V9 for Windows (GraphPad Software). The statistical tests used, and level of significance are described in figure legends.

Figure S1.

(a)


21
21 + YWA1 (1 µM)
21 + YWA1 (5 µM)
21 + YWA1 (25 µM)
21 + YWA1 (50 µM)
FITC
DIC
FITC-DIC merge
20 µm

(b)


YWA1 (1 µM)
YWA1 (5 µM)
YWA1 (25 µM)
YWA1 (50 µM)
UT
FITC
DIC
FITC-DIC merge
20 µm

(c)

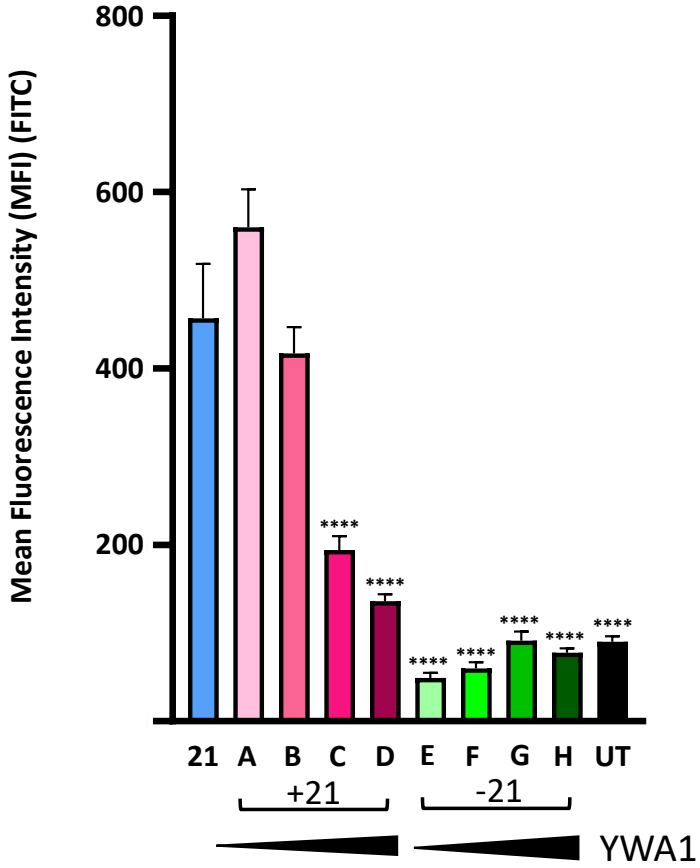
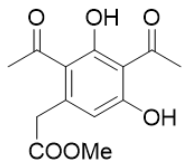


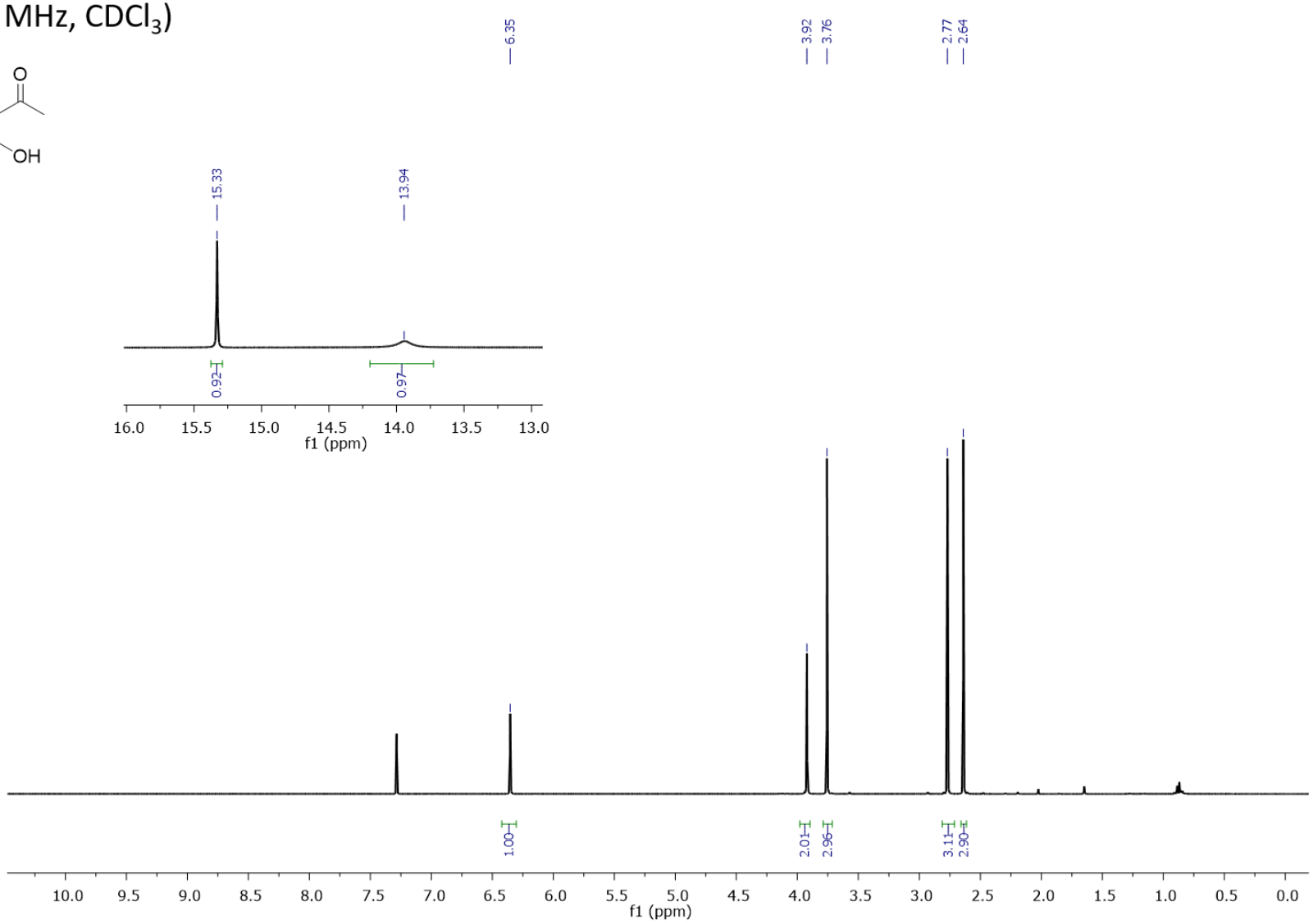
Figure S1. Specificity control of the binding of the fluorescent conjugate **21** to hMelLec-expressing cells with or without competition by **YWA1.** (a): Representative differential interference contrast (DIC) images and immunofluorescence micrographs of hMelLec expressing RAW264.7 cells treated with **21** (25 μM), 5% CO_2 for 3h at 37°C, with or without **YWA1** (1 μM, 5 μM, 25 μM or 50 μM); (b) Representative differential interference contrast (DIC) images and immunofluorescence micrographs of hMelLec expressing RAW264.7 cells either treated with 5% CO_2 for 3h at 37°C and **YWA1** (1 μM, 5 μM, 25 μM or 50 μM) or untreated (**UT**); (c): Bar graphs showing the binding of hMelLec-expressing RAW264.7 cells to **21** (blue bar) compared to cells treated with both **21** and YWA1 (bars **A-D**, respectively 1, 5, 25 and 50 μM of YWA1), or treated with YWA1 alone (bars **E-H**, respectively 1, 5, 25 and 50 μM), or untreated (**UT**), as determined by image quantification. Data were analysed using One-way ANOVA with Dunnett's *post hoc* test, values show mean ± SEM, **P* ≤ 0.05, *****P* ≤ 0.0001; *n* = 1 (40 cells/ condition).

3. NMR SPECTRA

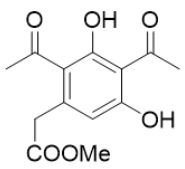
^{1}H NMR (400 MHz, $CDCl_3$)



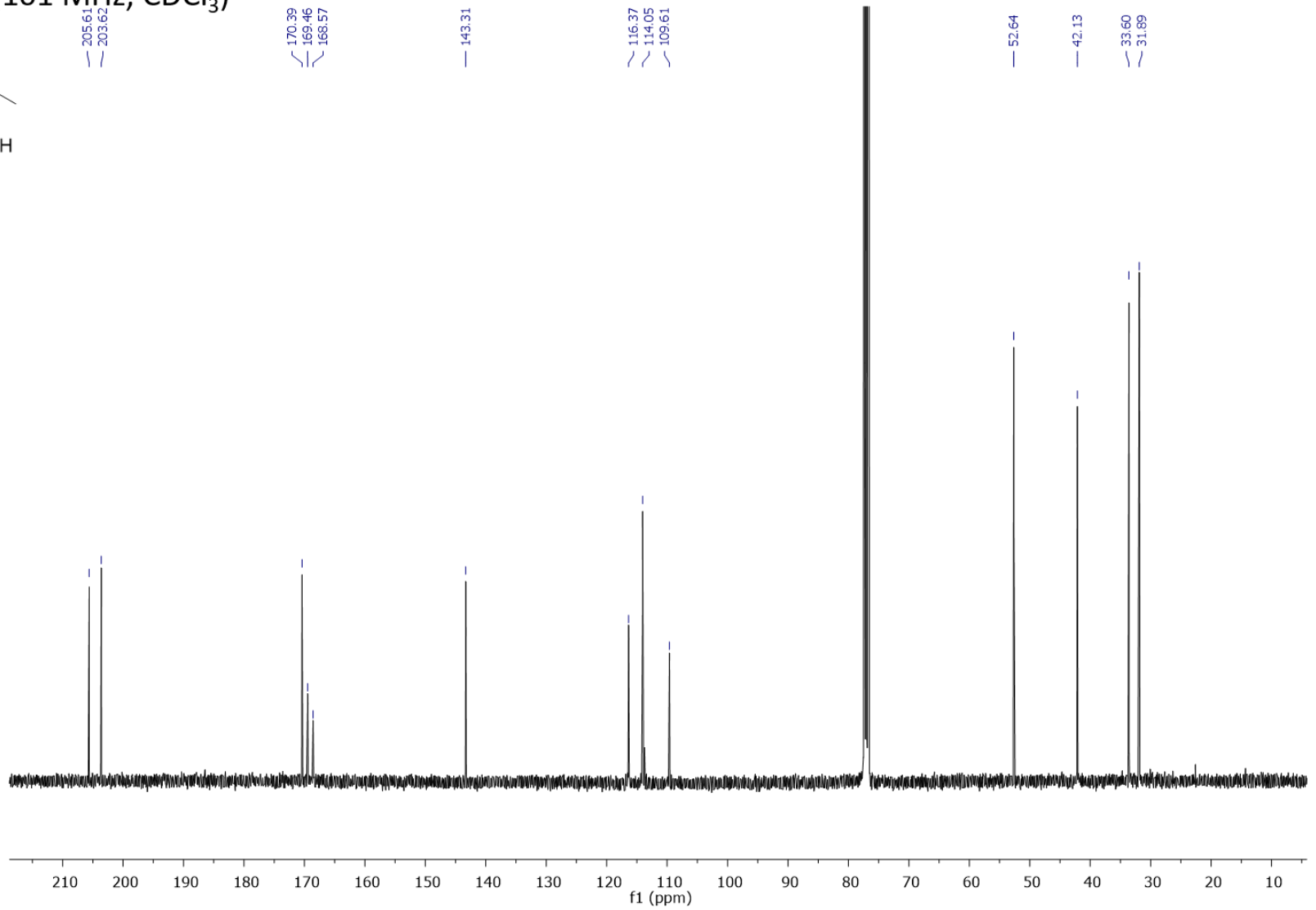
10



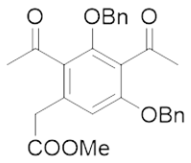
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



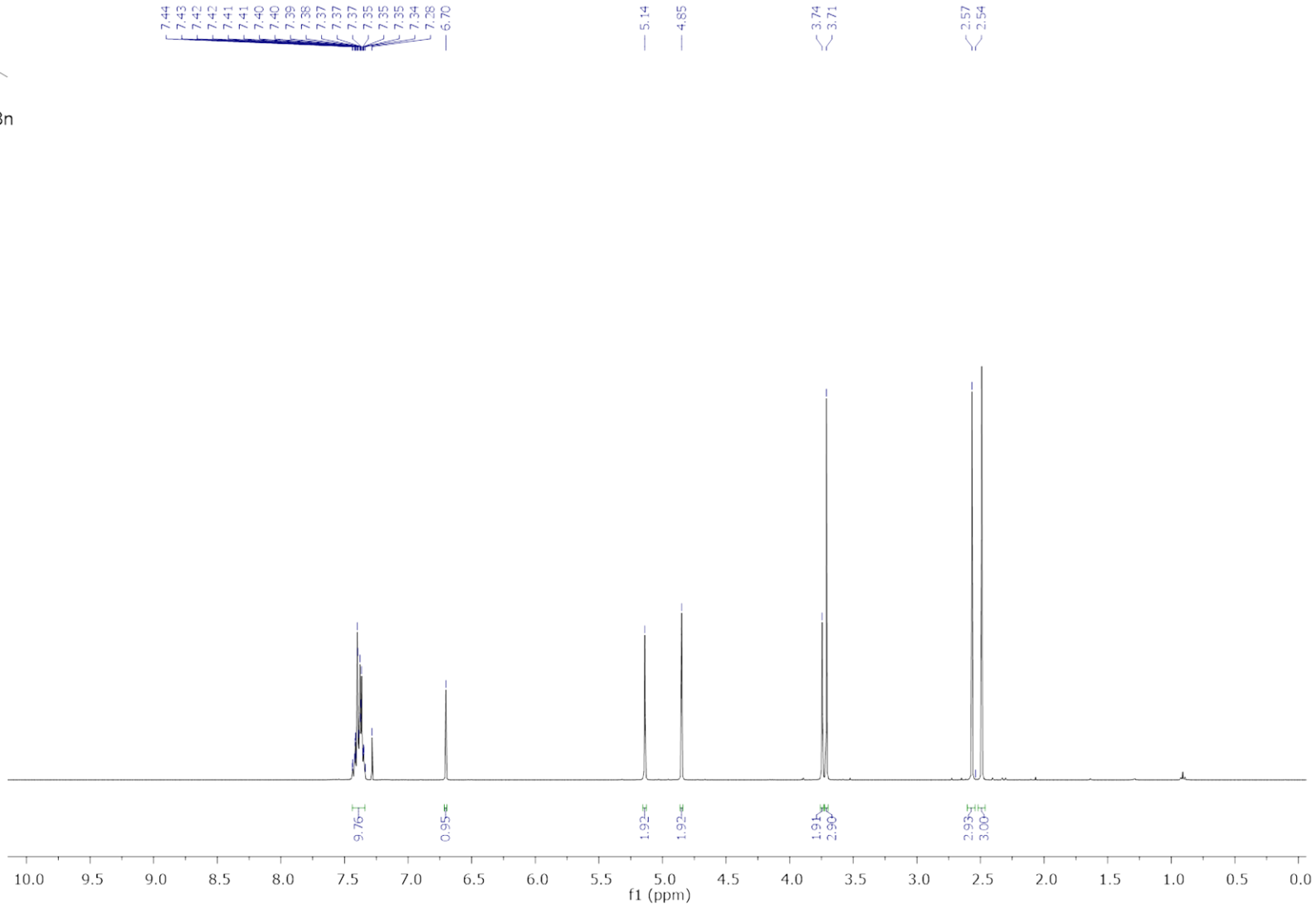
10



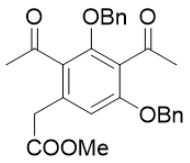
^{1}H NMR (400 MHz, $CDCl_3$)



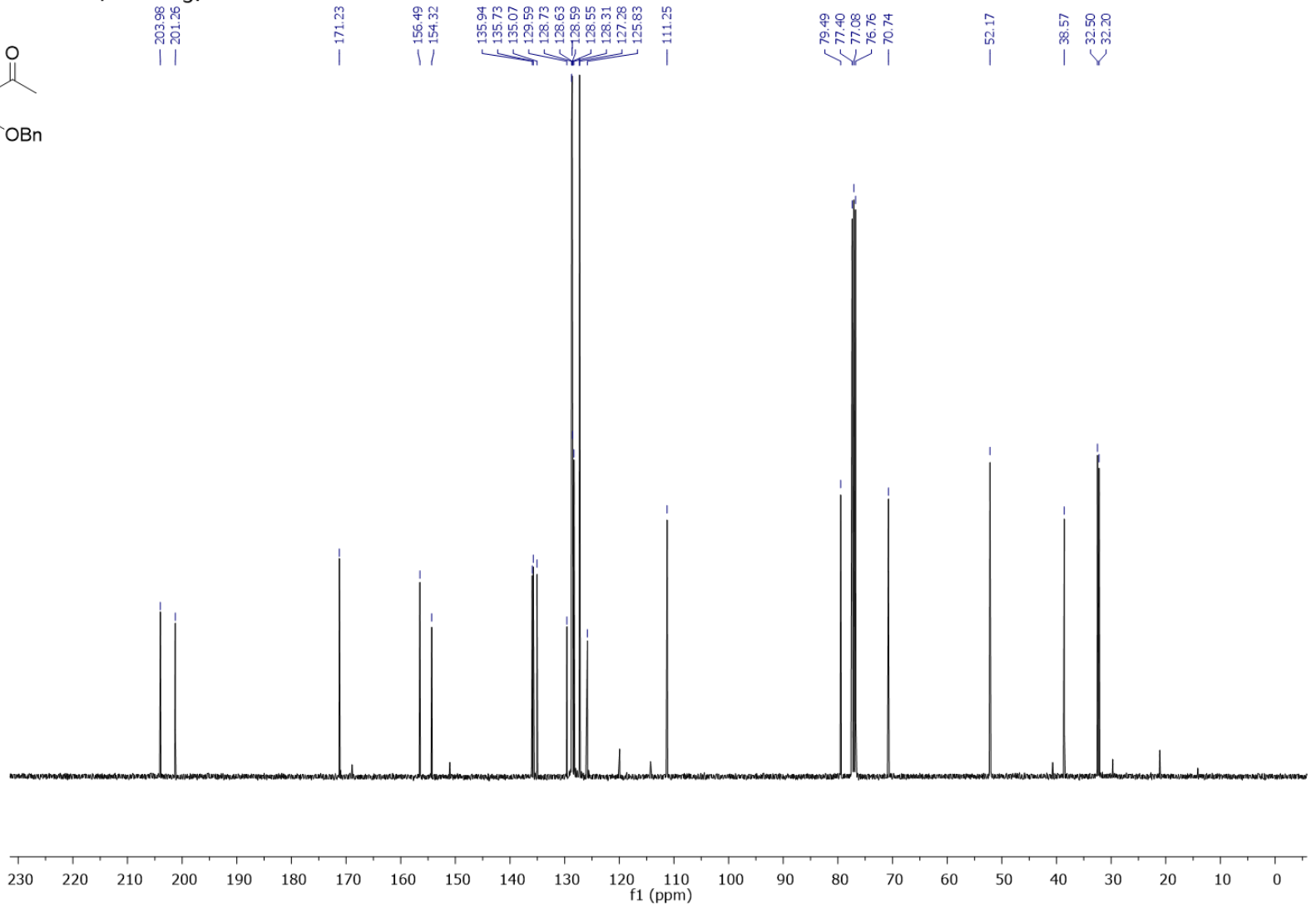
11



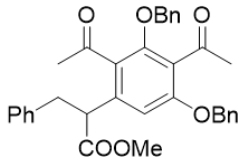
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



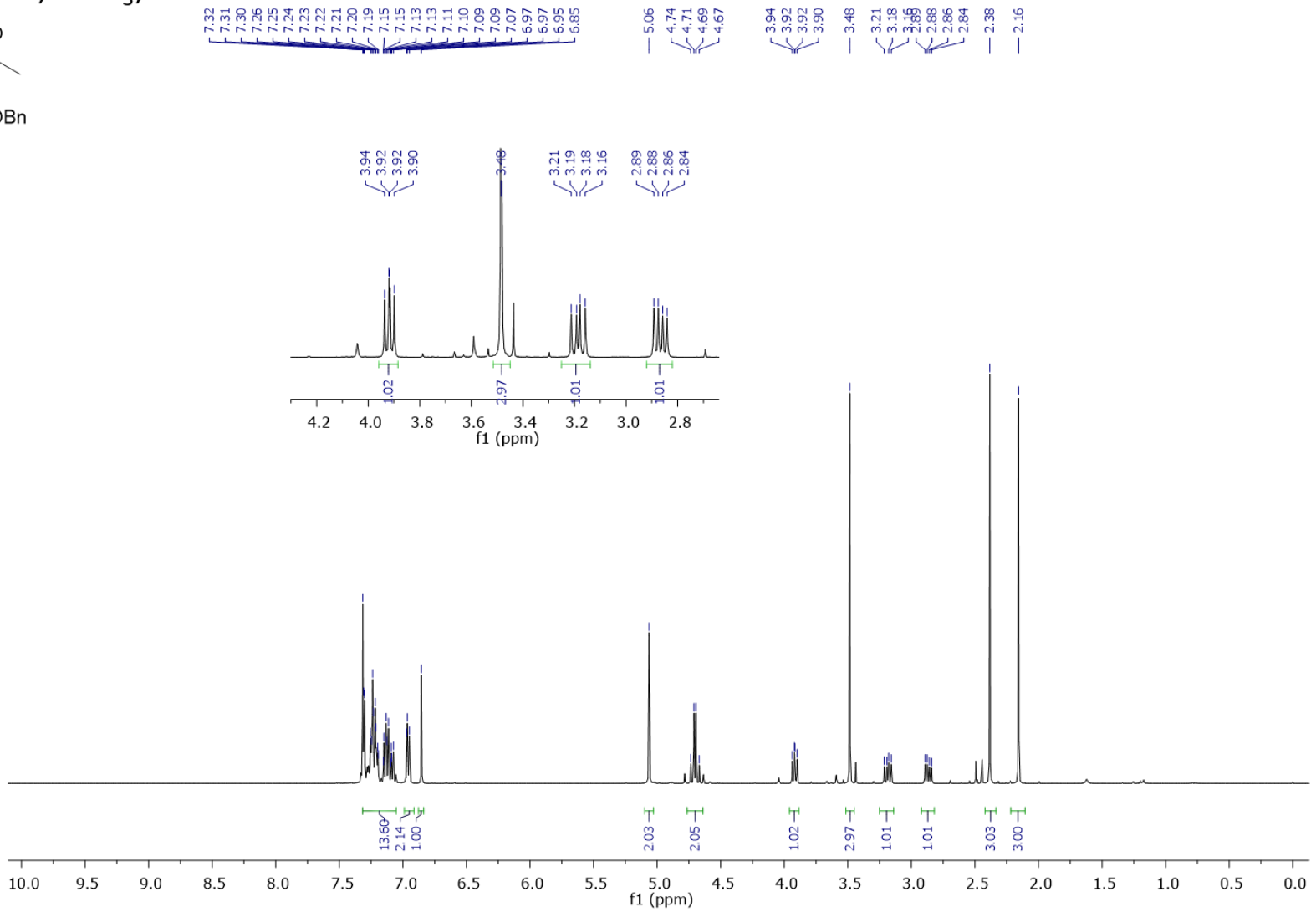
11



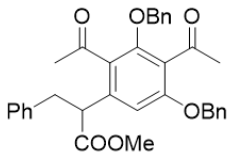
^{1}H NMR (400 MHz, $CDCl_3$)



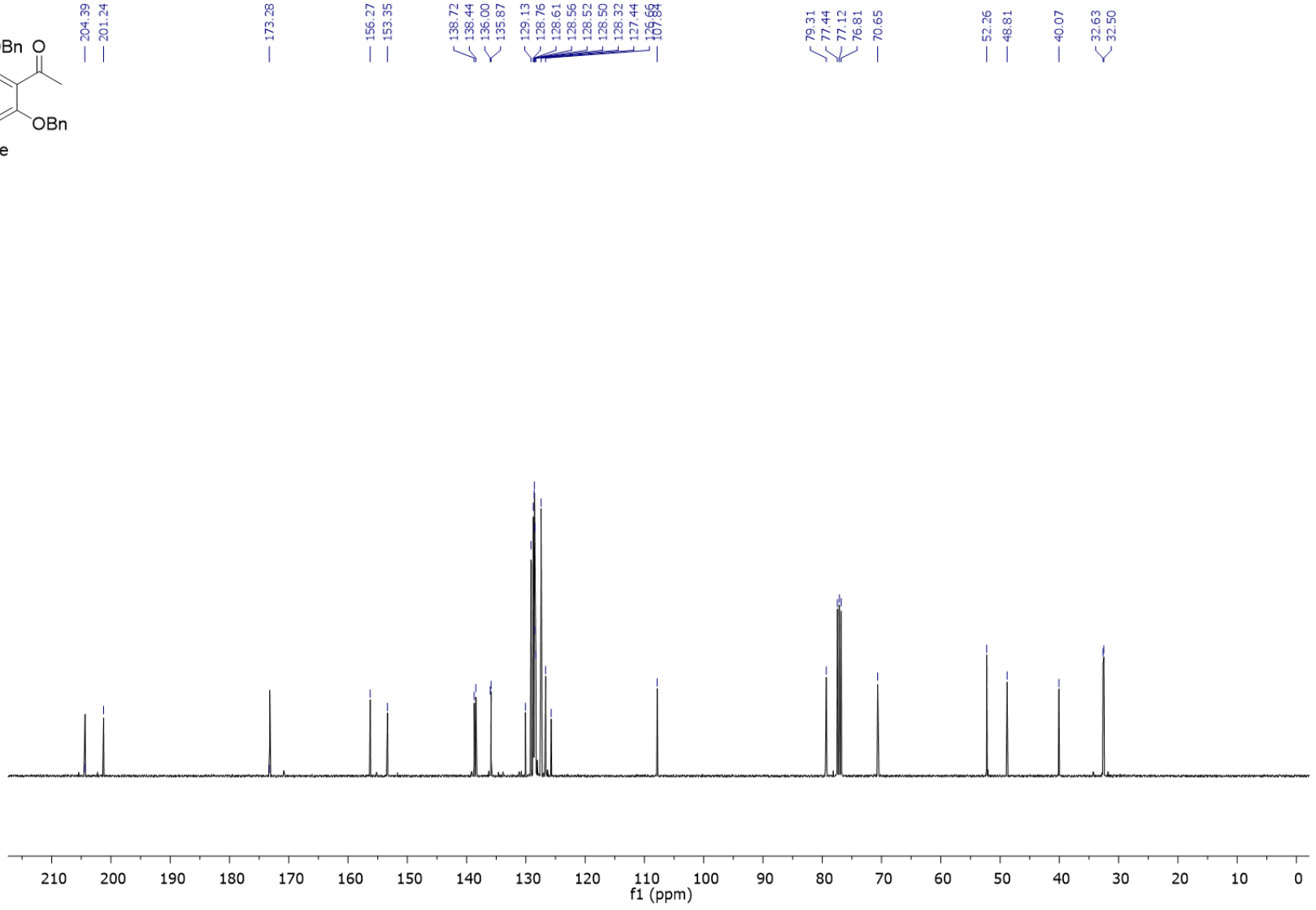
11a



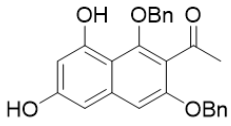
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



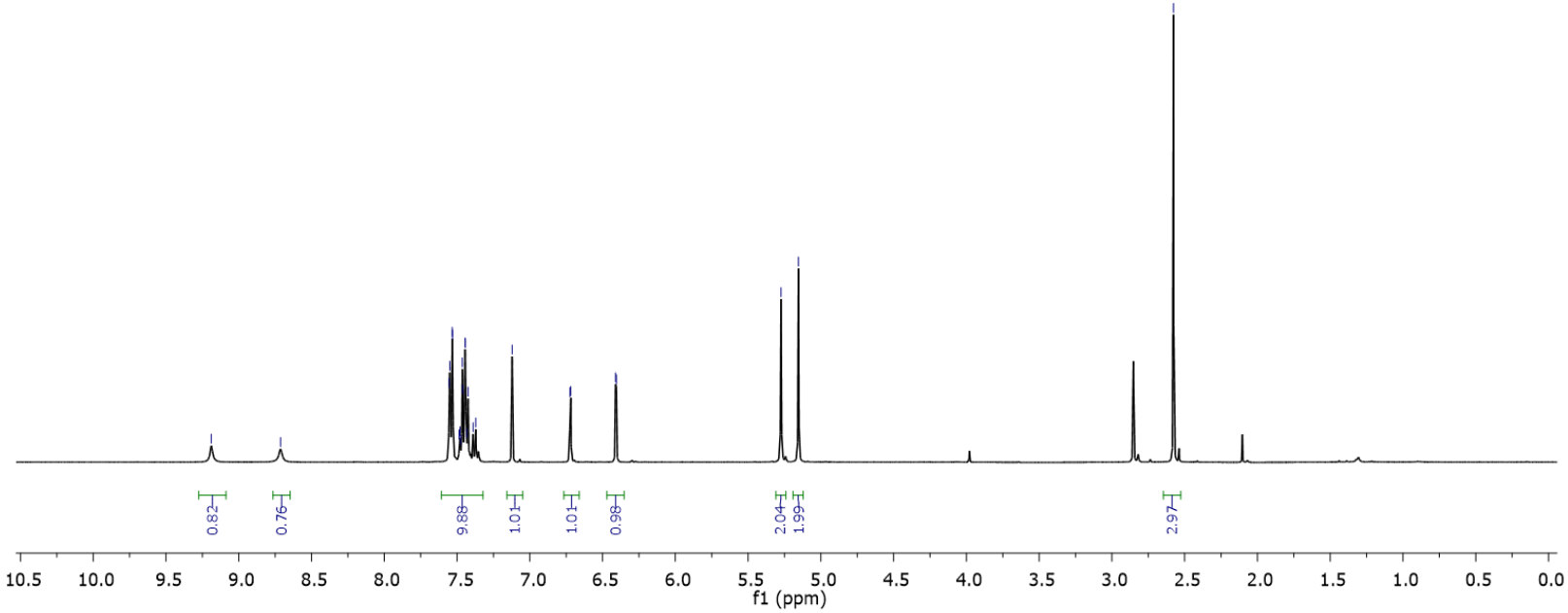
11a



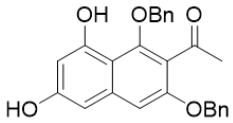
^{1}H-NMR (400 MHz, $(CD_3)_2CO$)



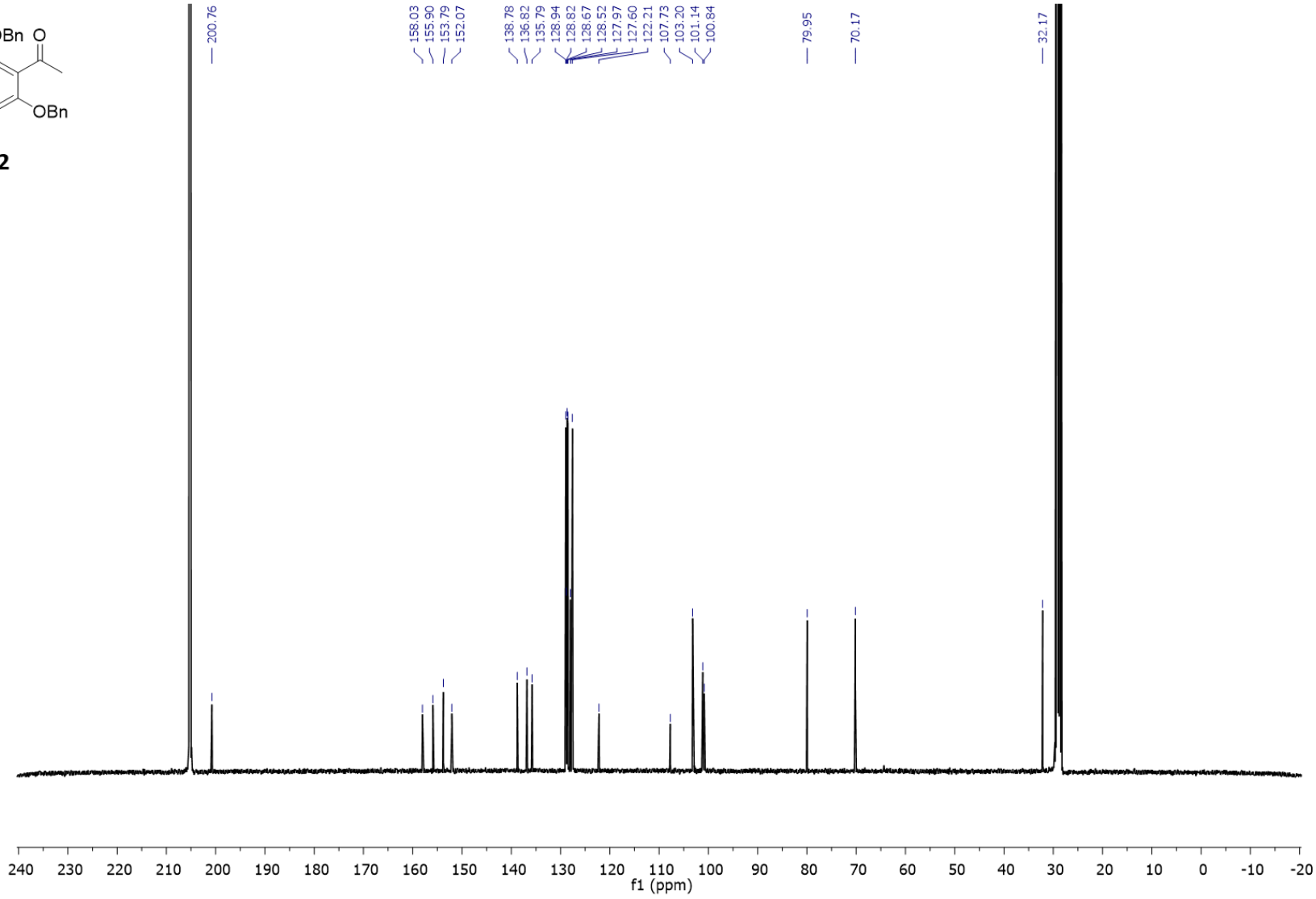
12



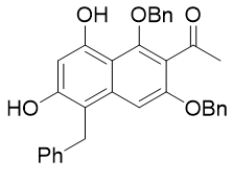
$^{13}C\{^{1}H\}$ NMR (101 MHz, $(CD_3)_2CO$)



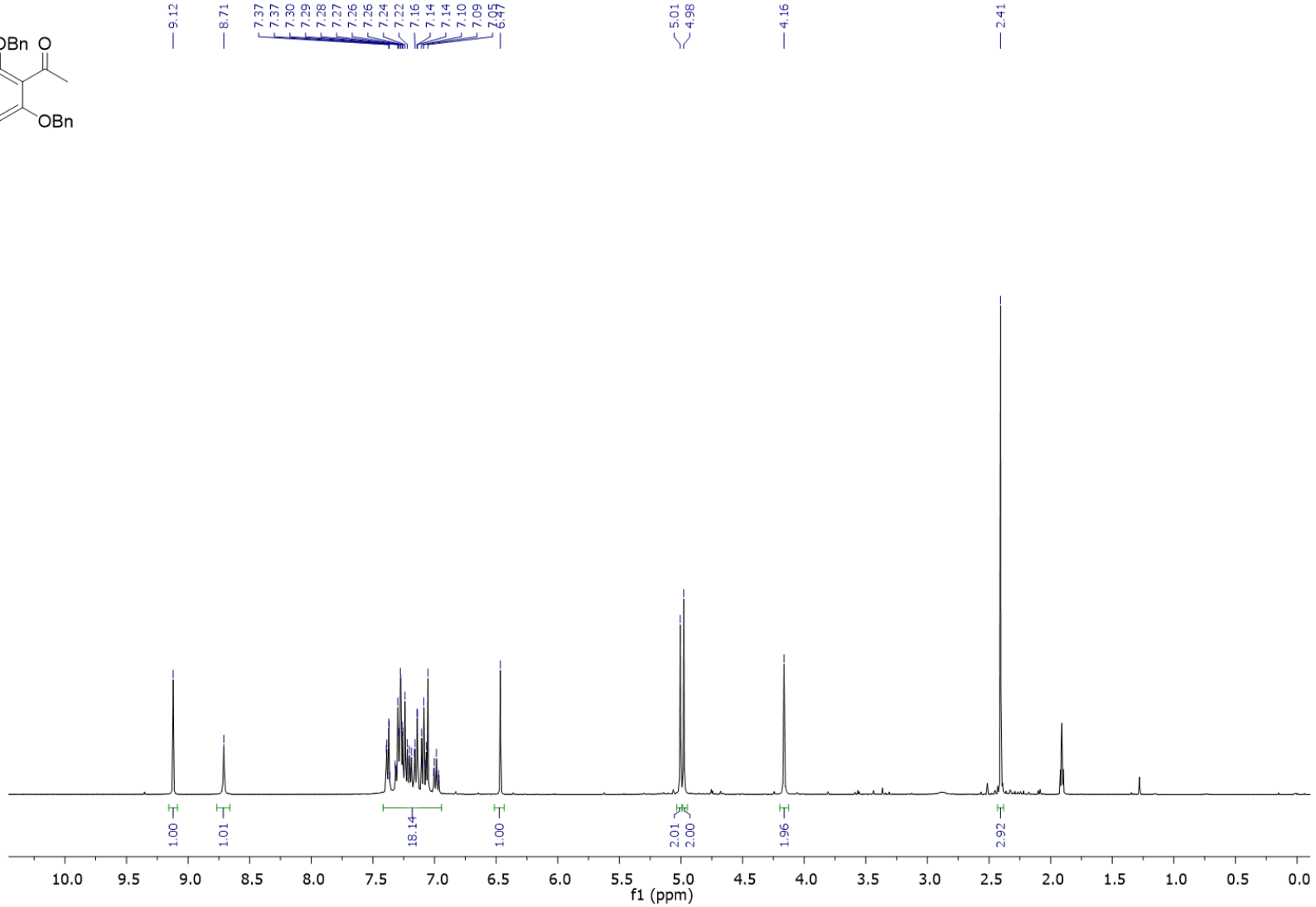
12



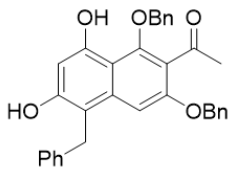
^{1}H-NMR (400 MHz, $(CD_3)_2CO$)



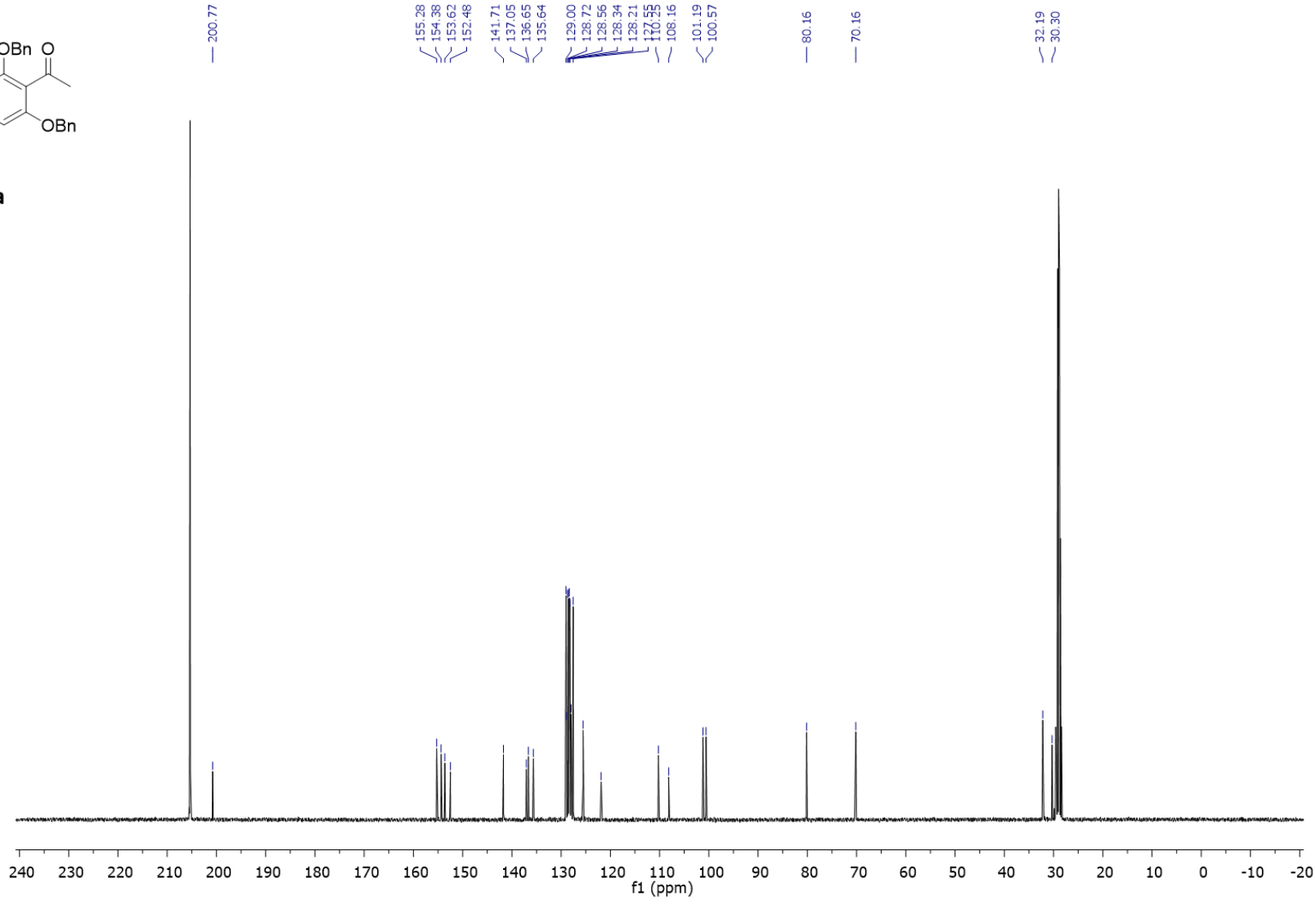
12a



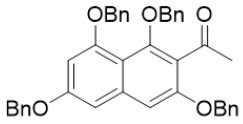
$^{13}C\{^{1}H\}$ NMR (101 MHz, $(CD_3)_2CO$)



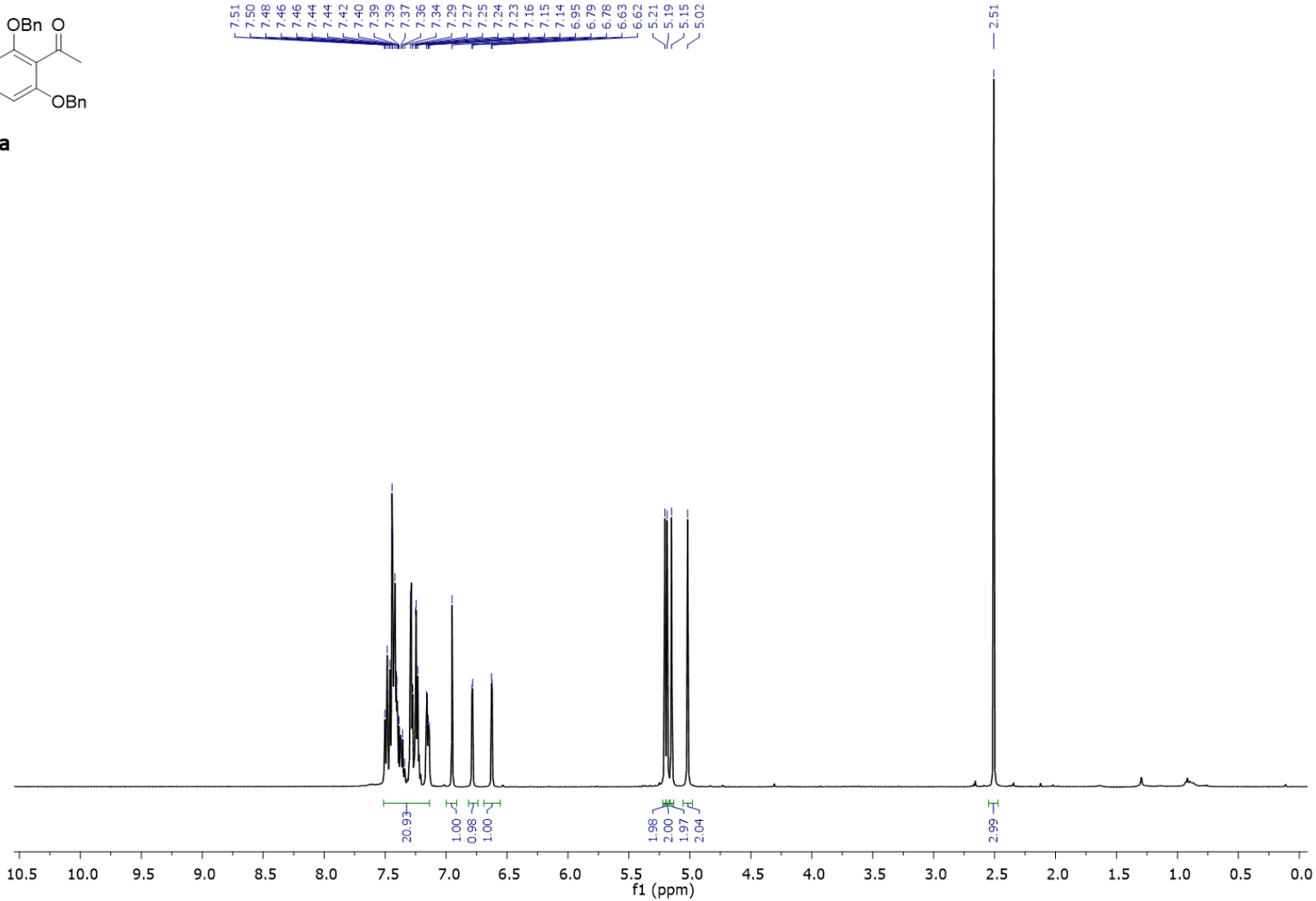
12a



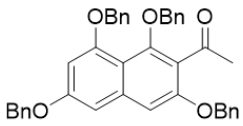
^{1}H NMR (400 MHz, $CDCl_3$)



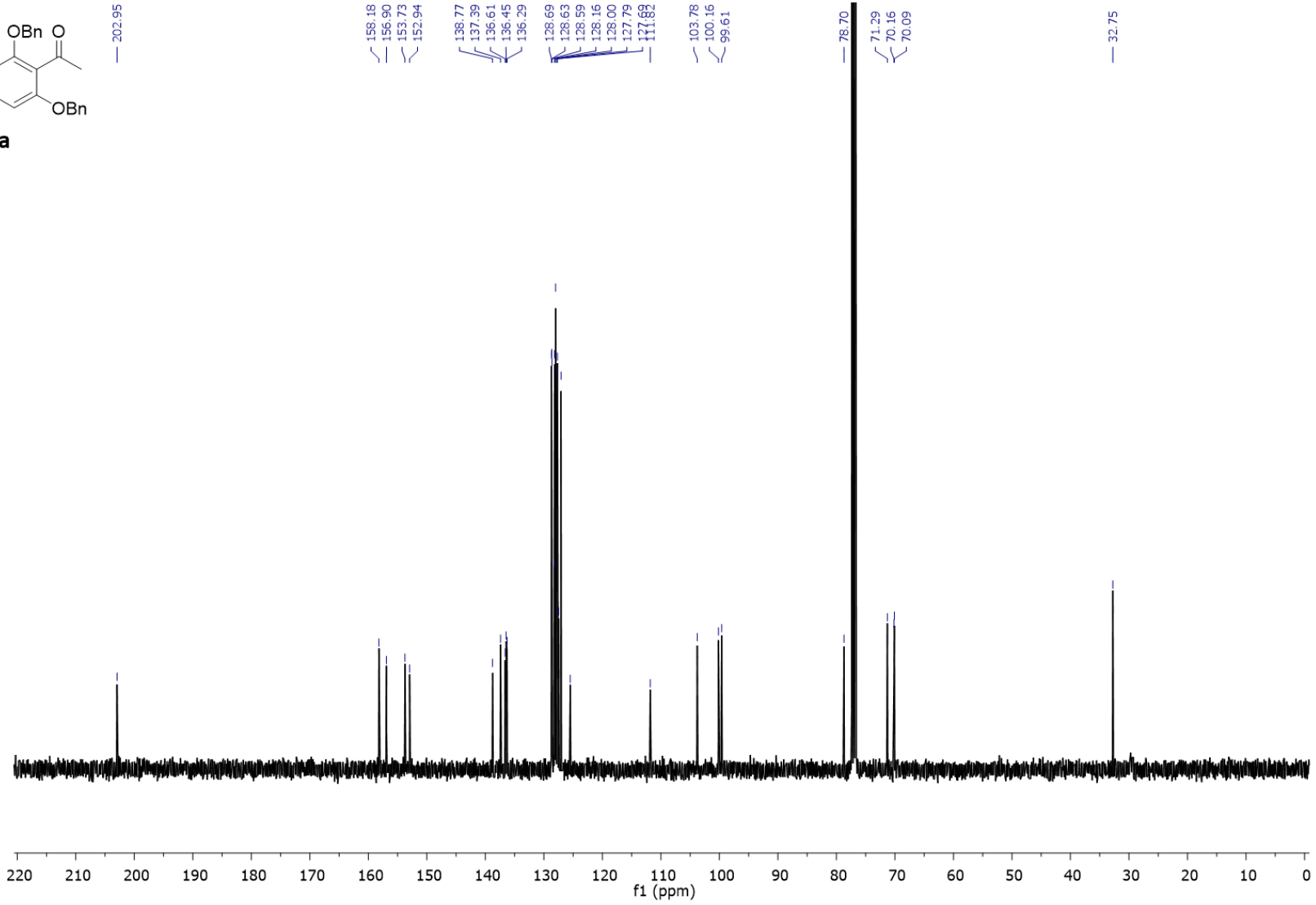
13a



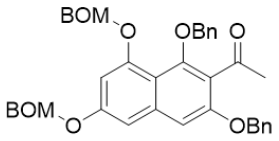
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



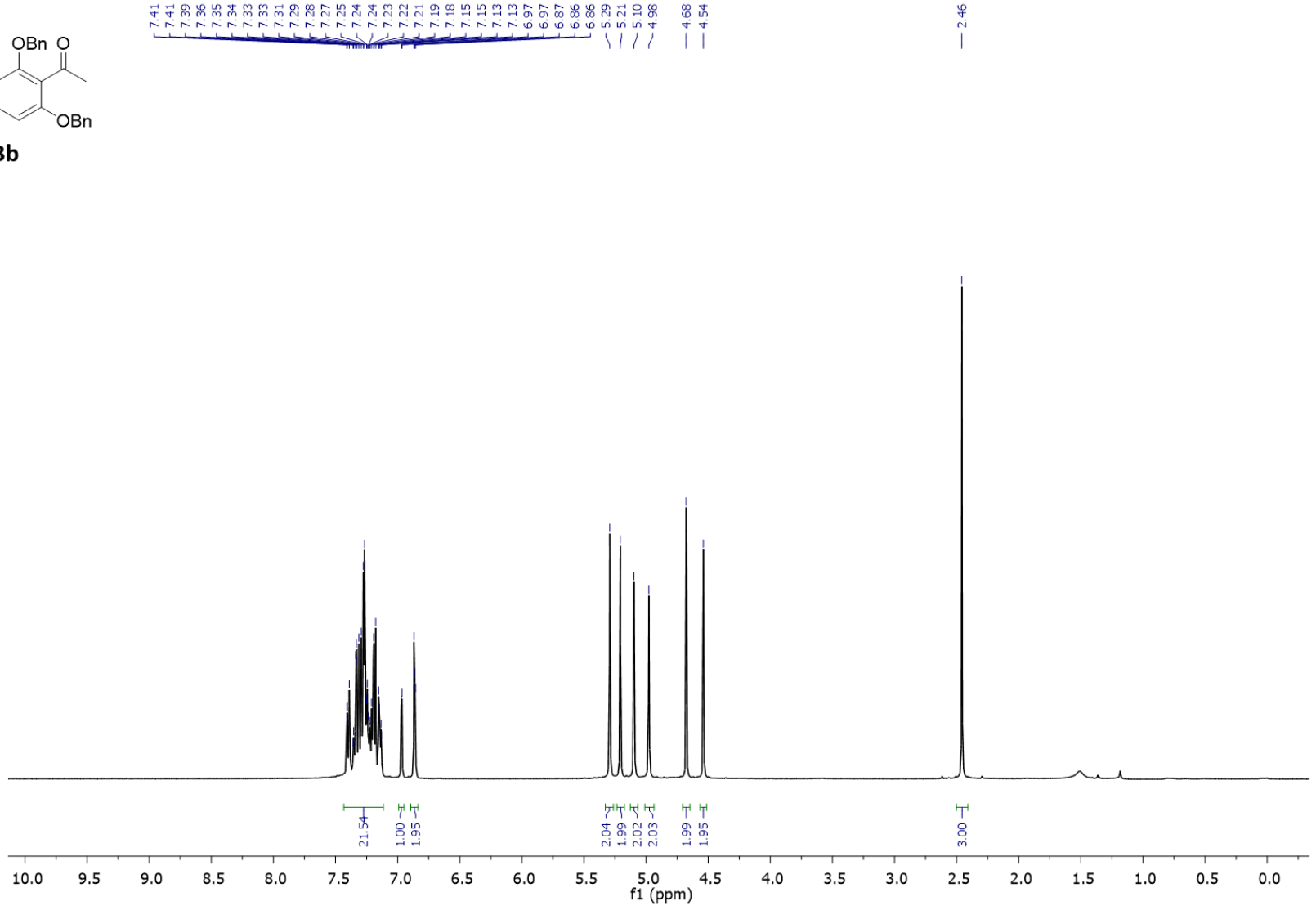
13a



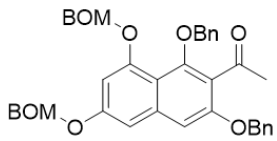
^{1}H NMR (400 MHz, $CDCl_3$)



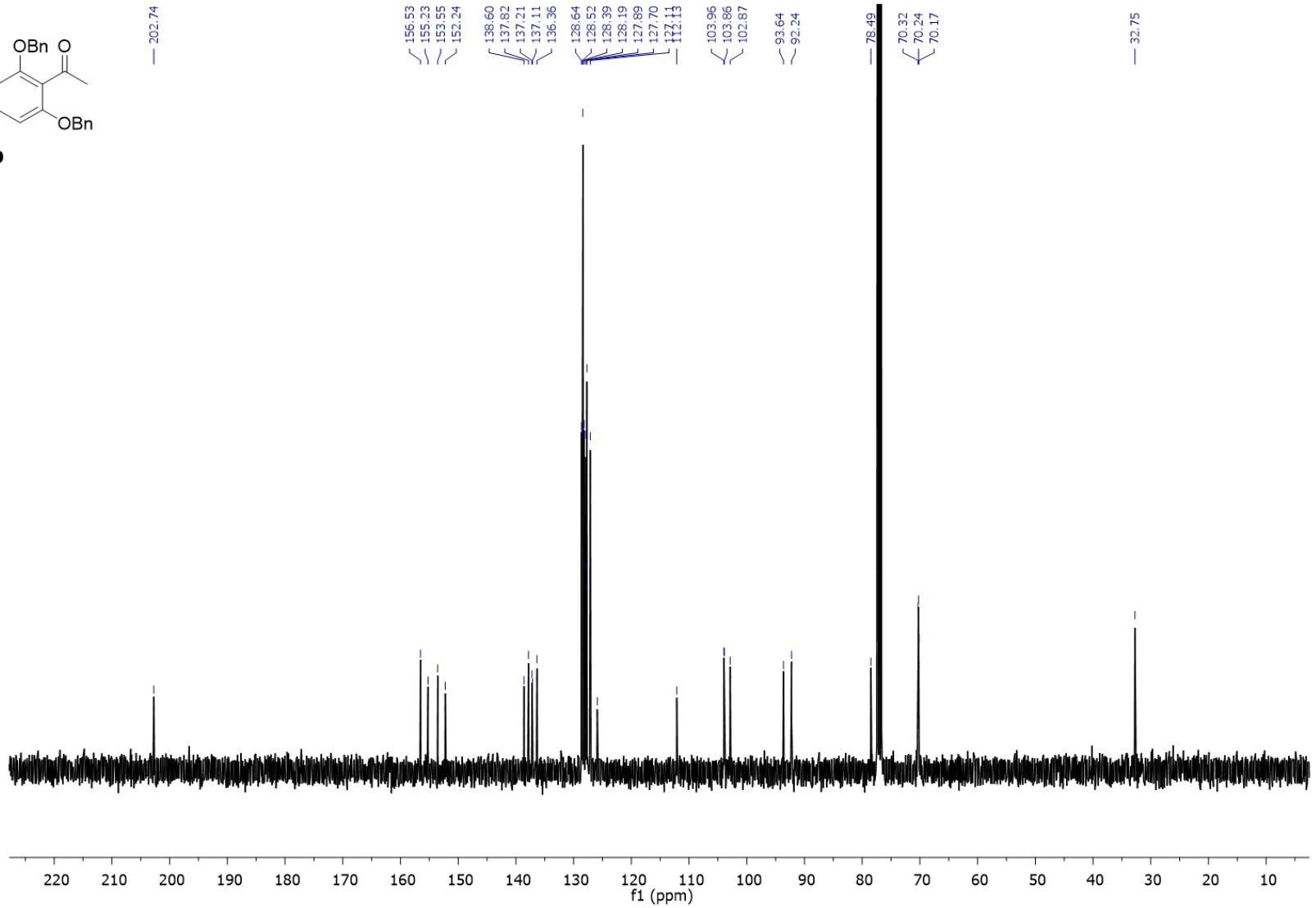
13b



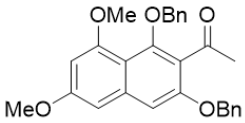
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



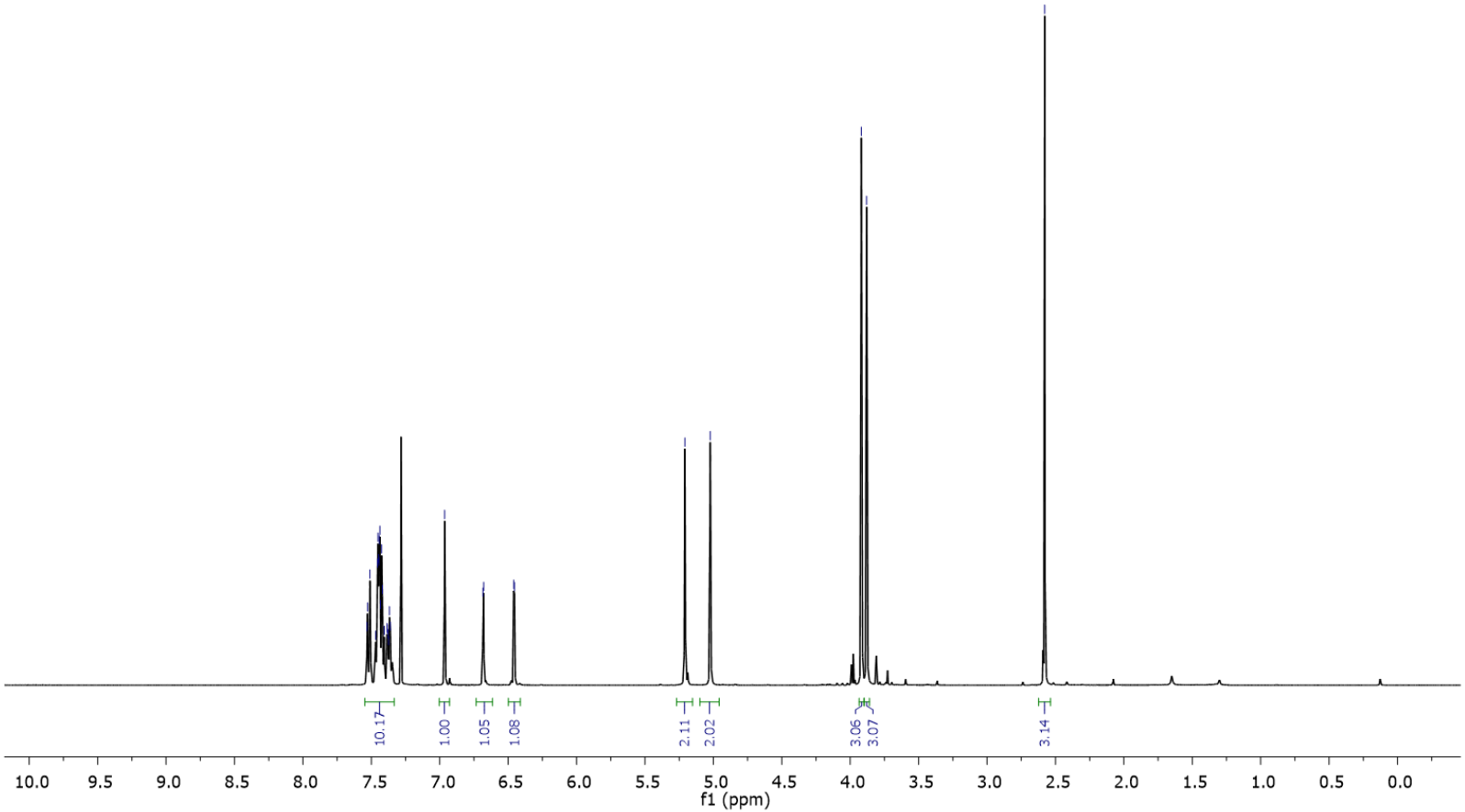
13b



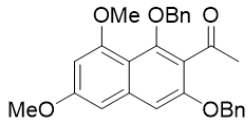
^{1}H NMR (400 MHz, $CDCl_3$)



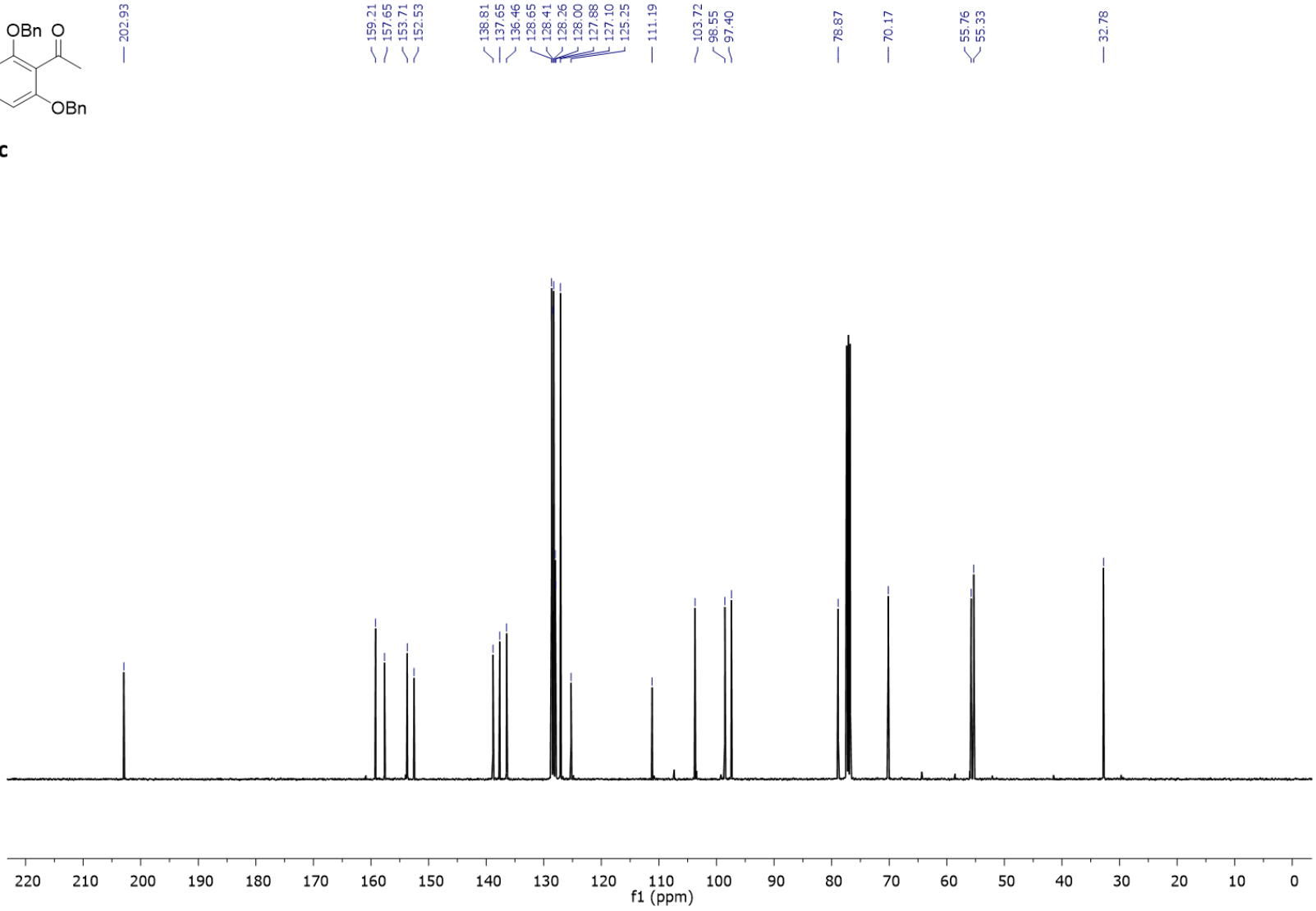
13c



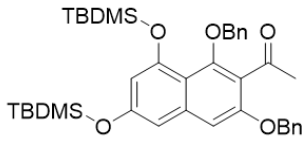
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



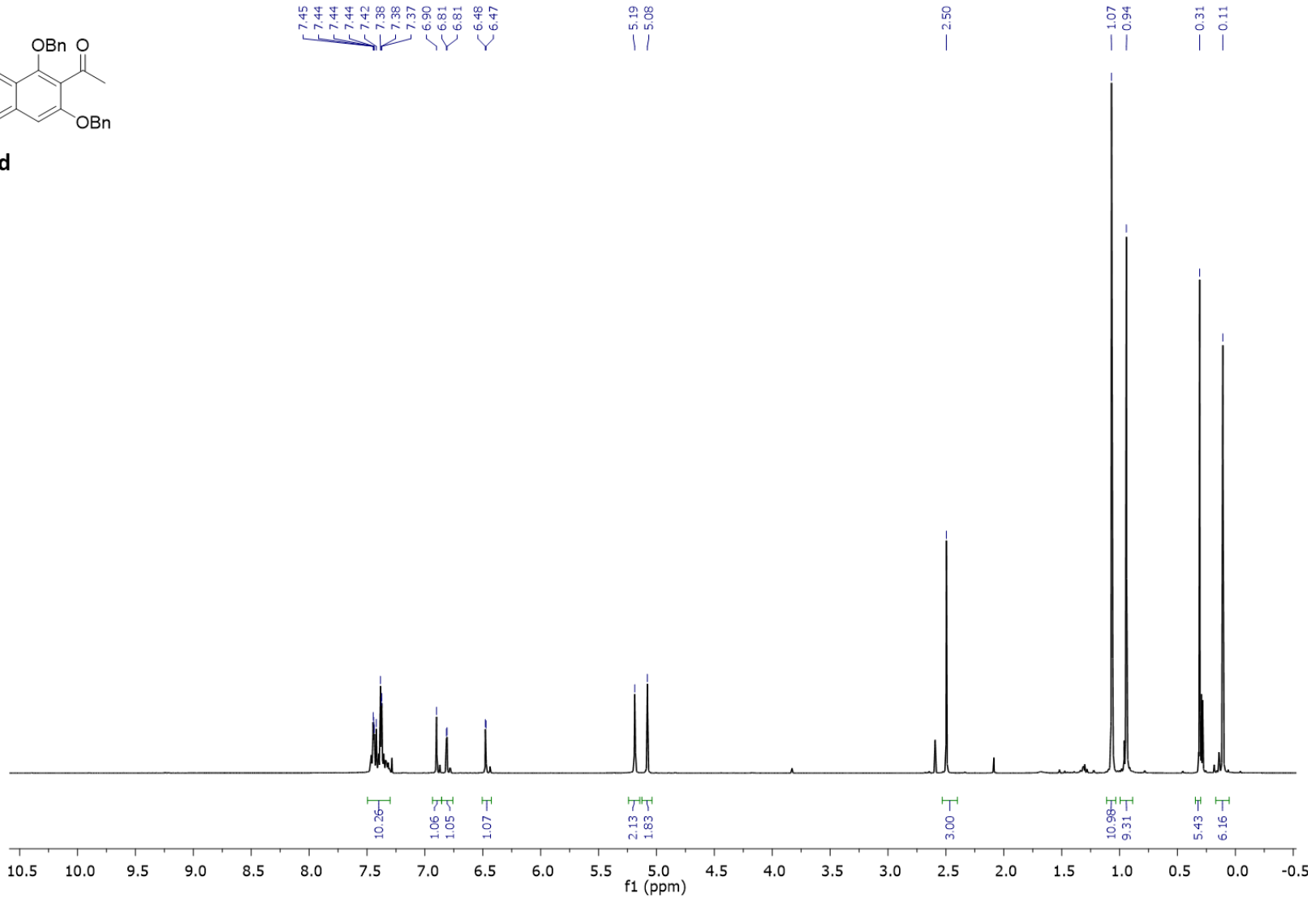
13c



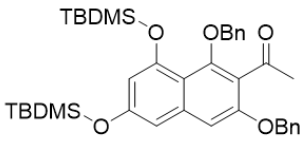
^{1}H NMR (400 MHz, $CDCl_3$)



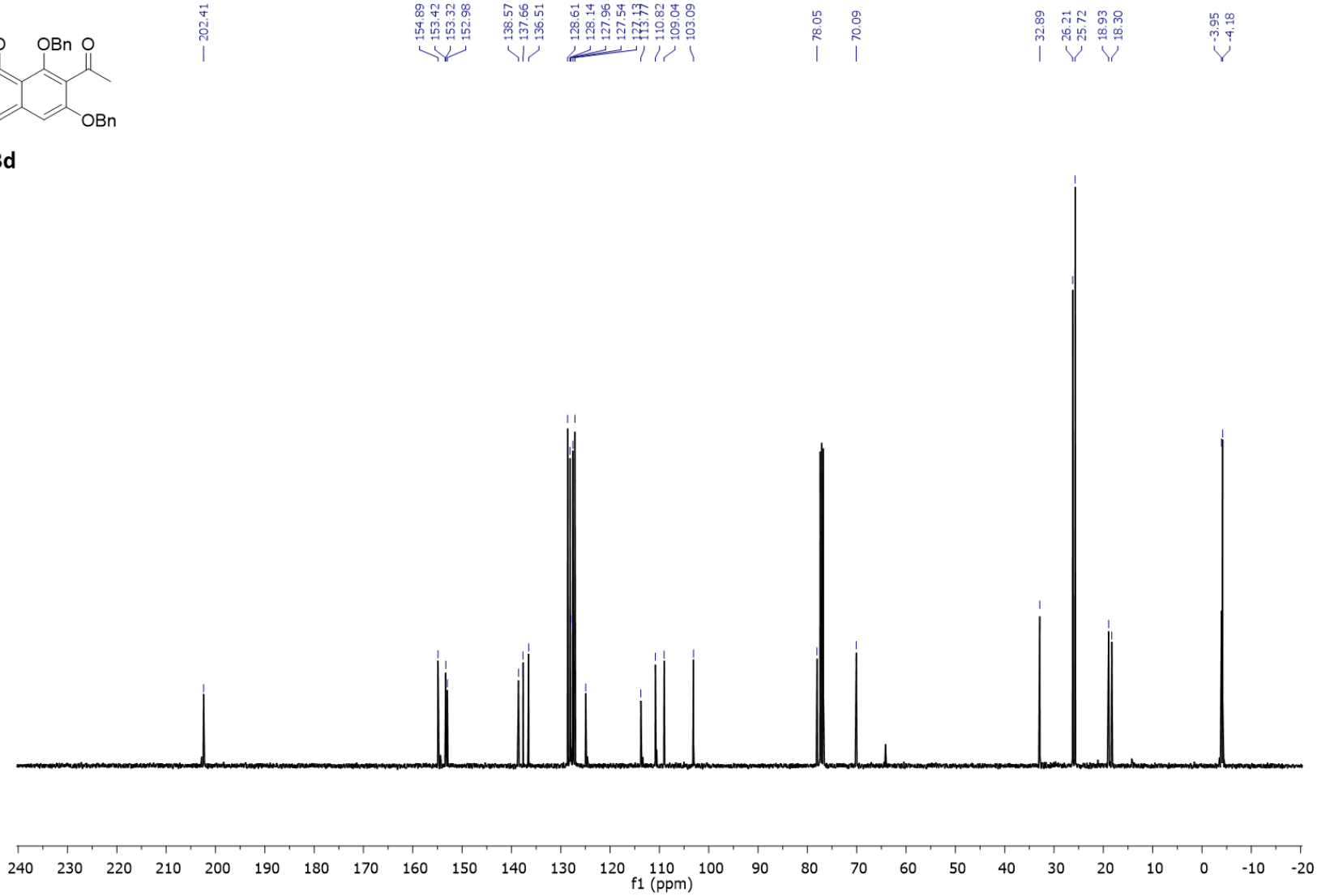
13d



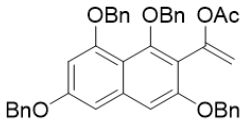
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



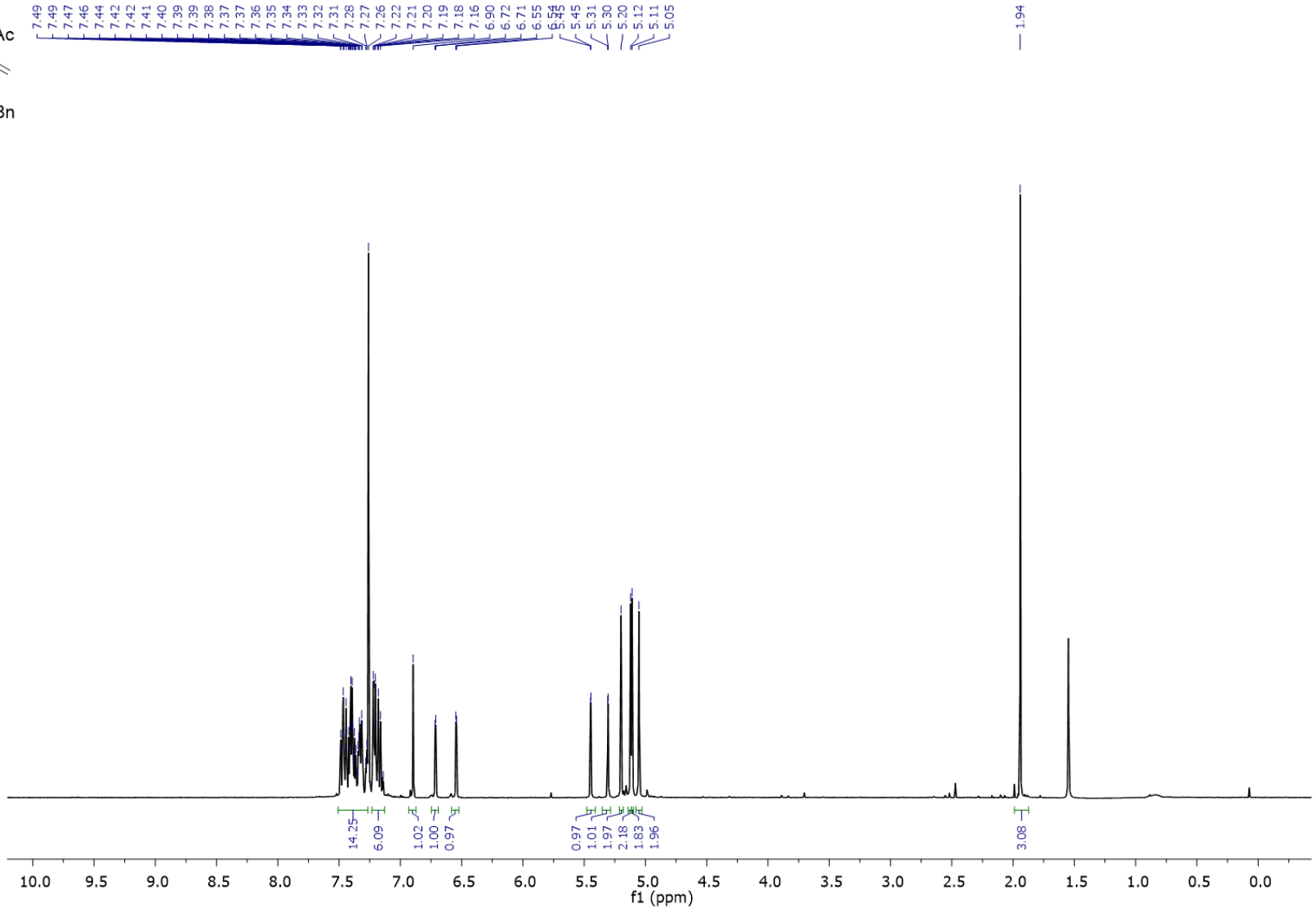
13d



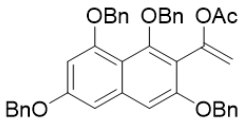
^{1}H NMR (400 MHz, $CDCl_3$)



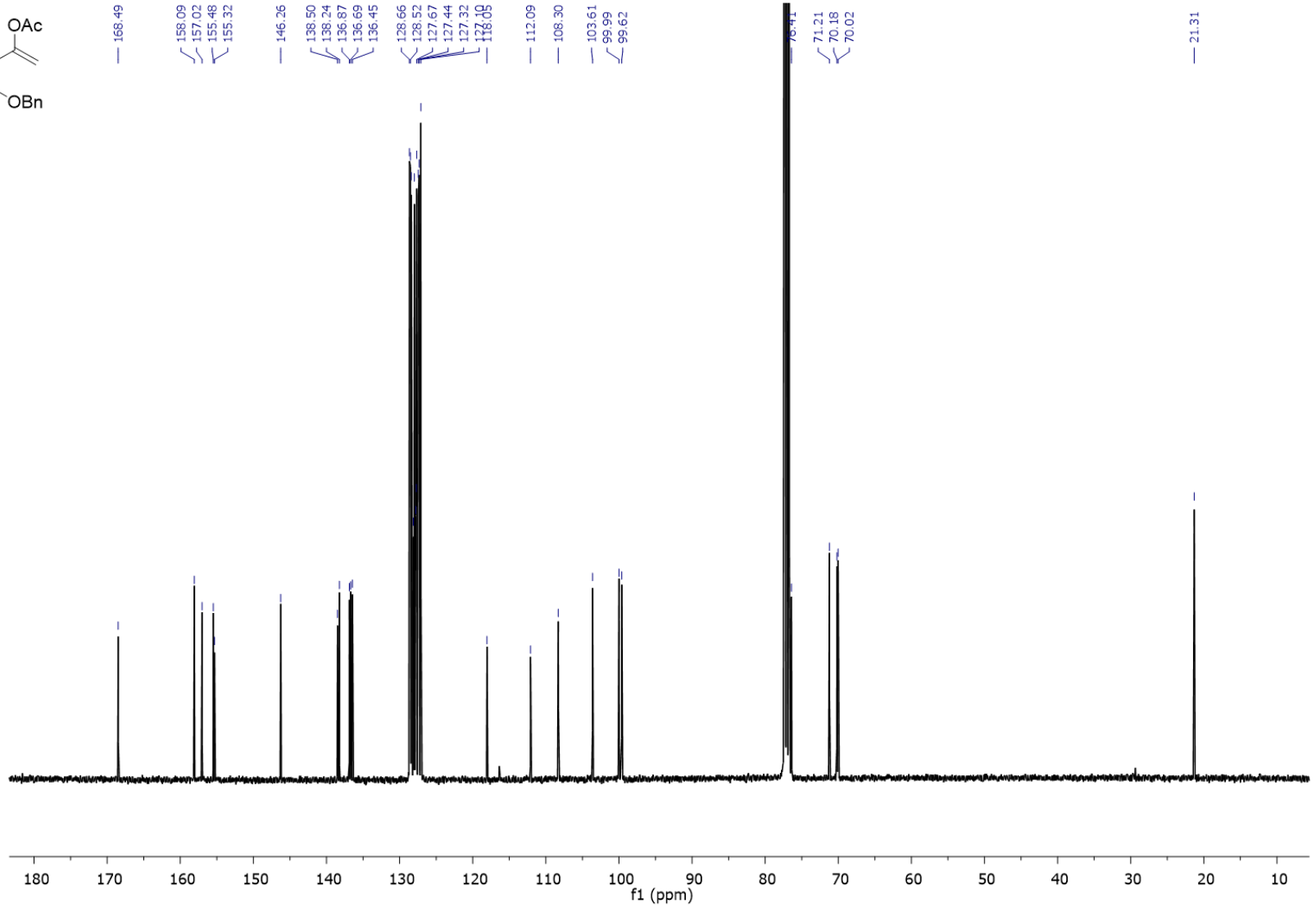
14a



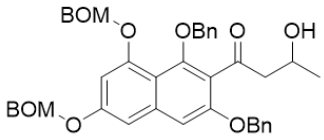
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



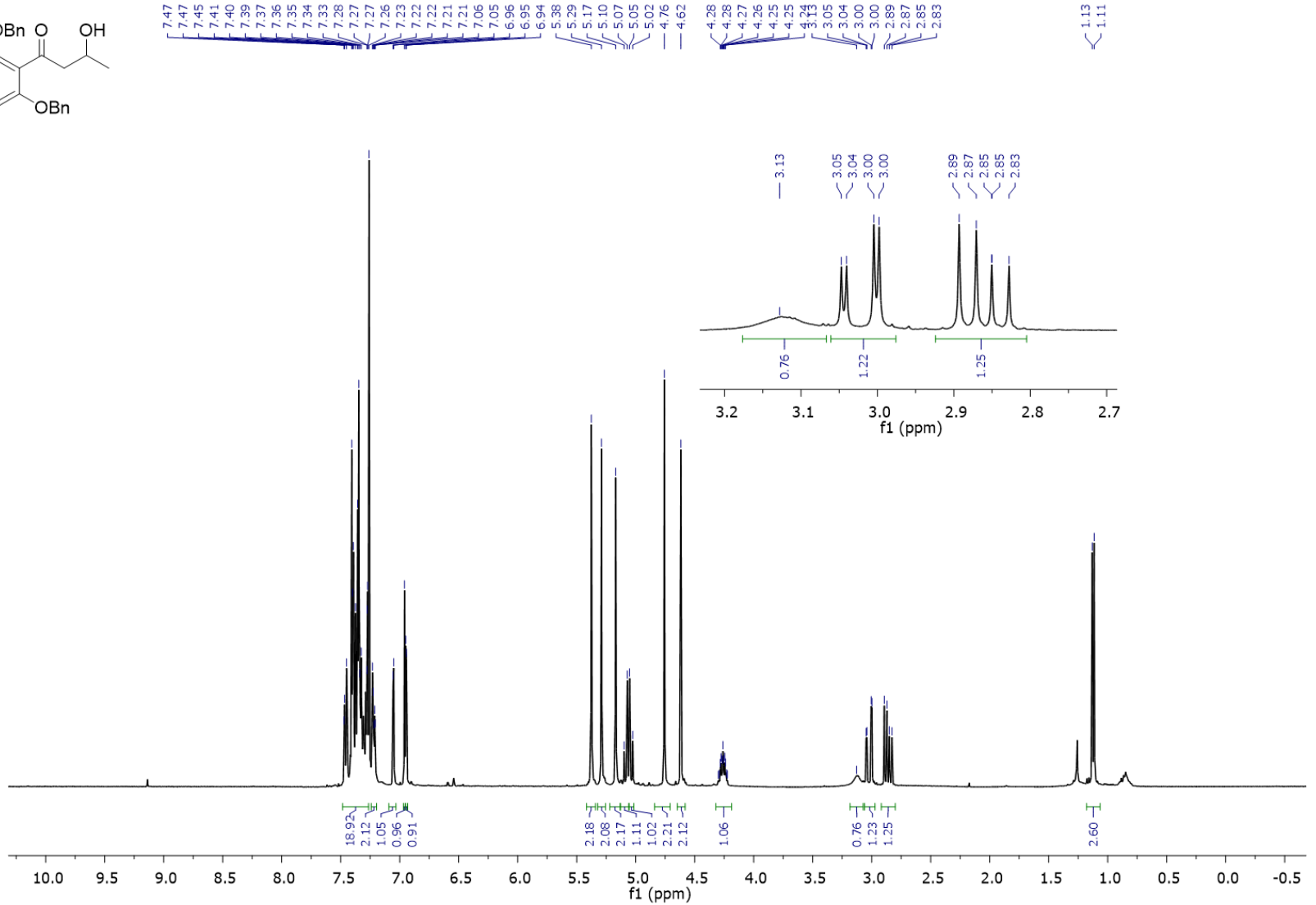
14a



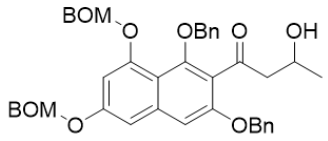
^{1}H NMR (400 MHz, $CDCl_3$)



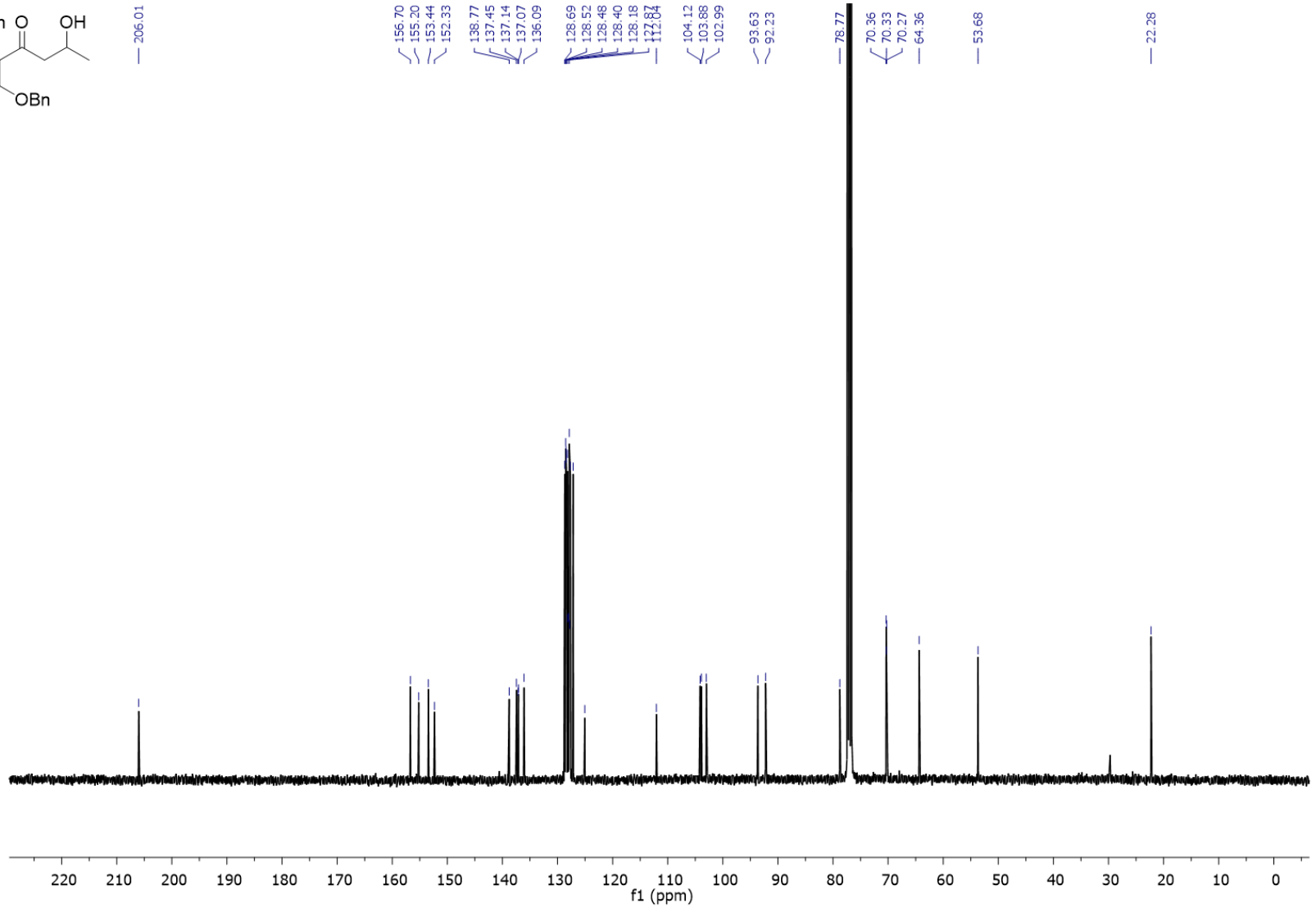
14b



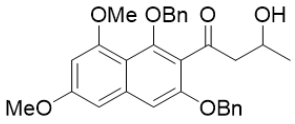
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



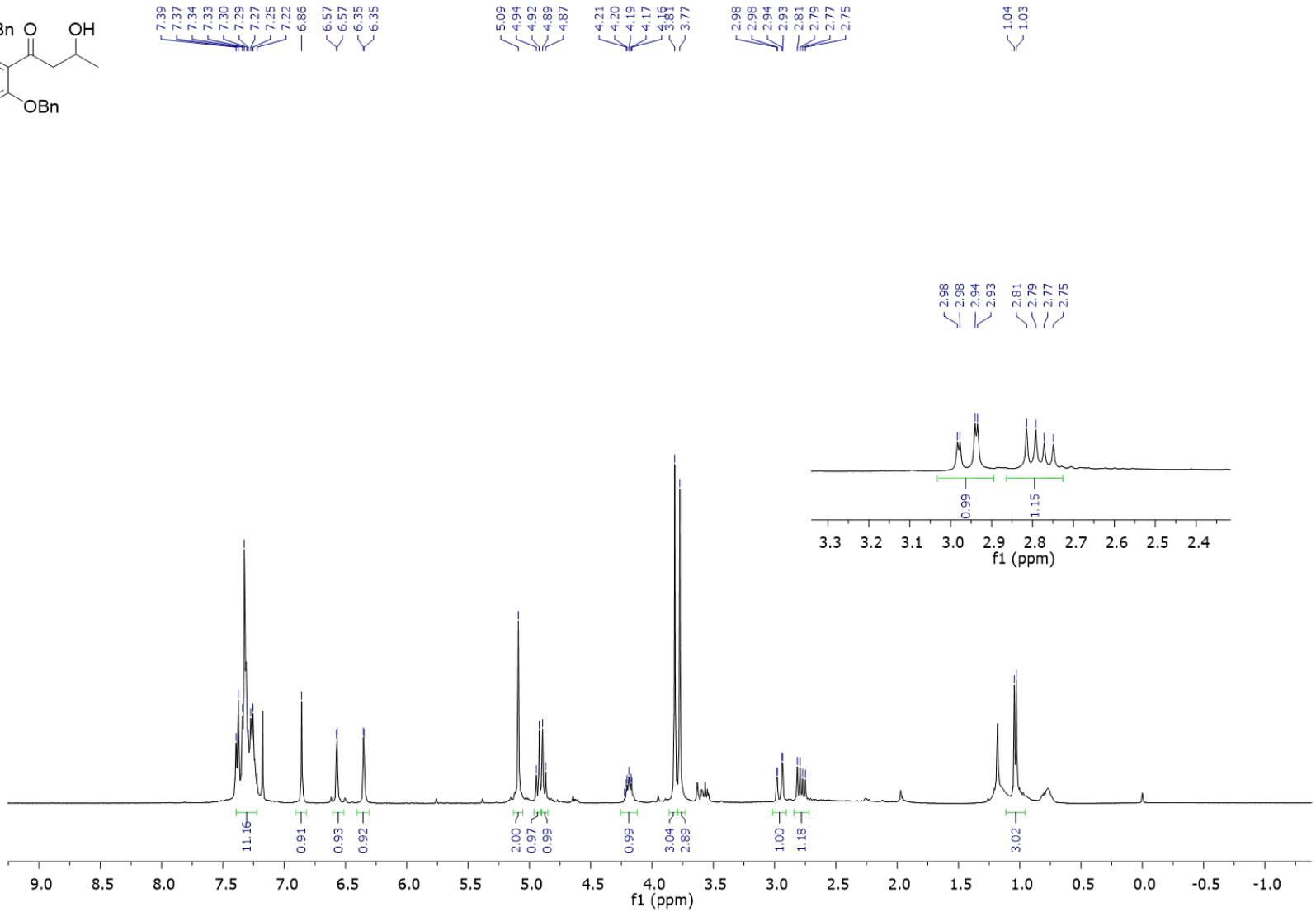
14b



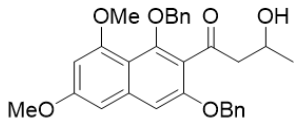
^{1}H NMR (400 MHz, $CDCl_3$)



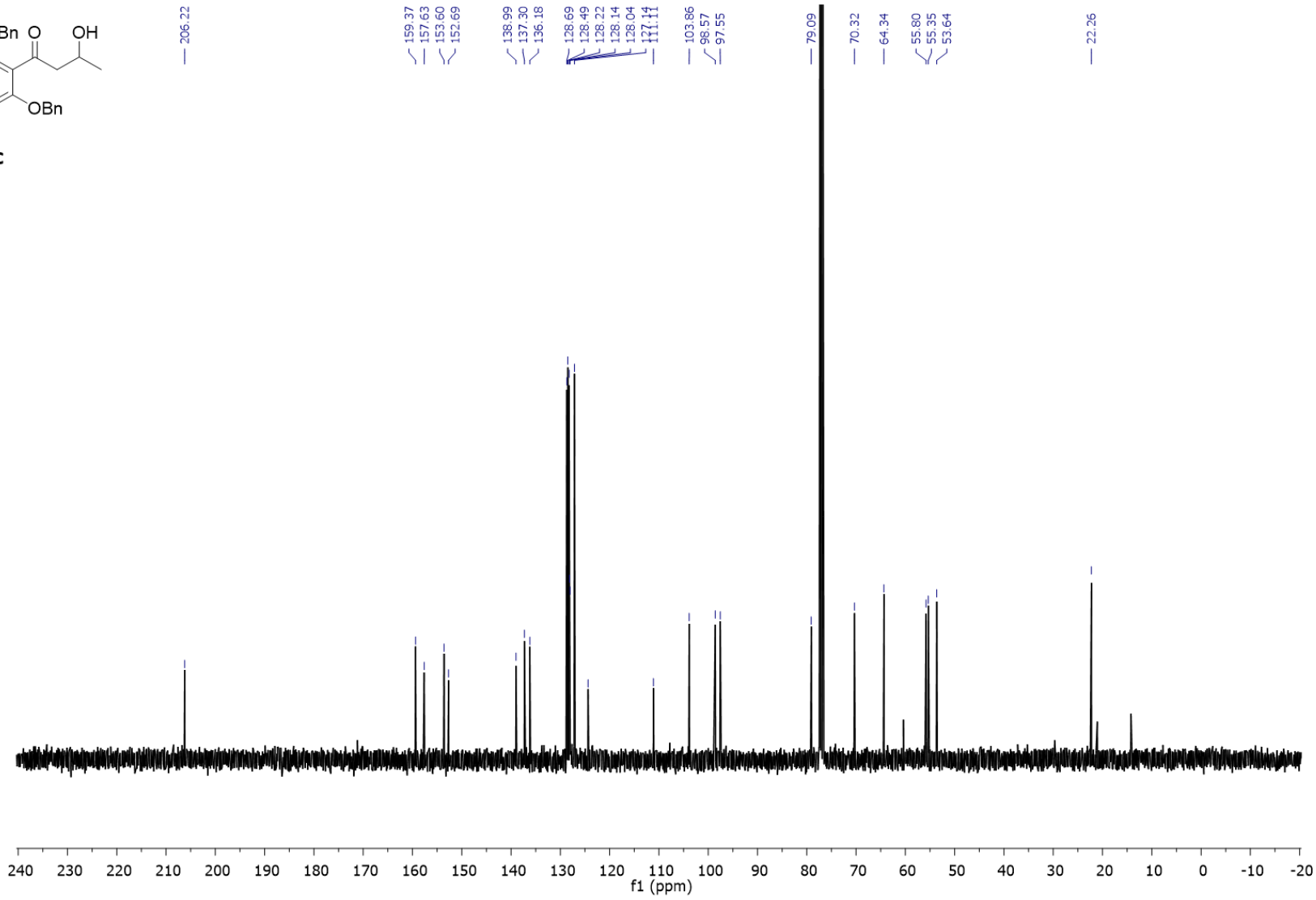
14c



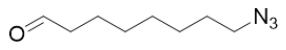
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



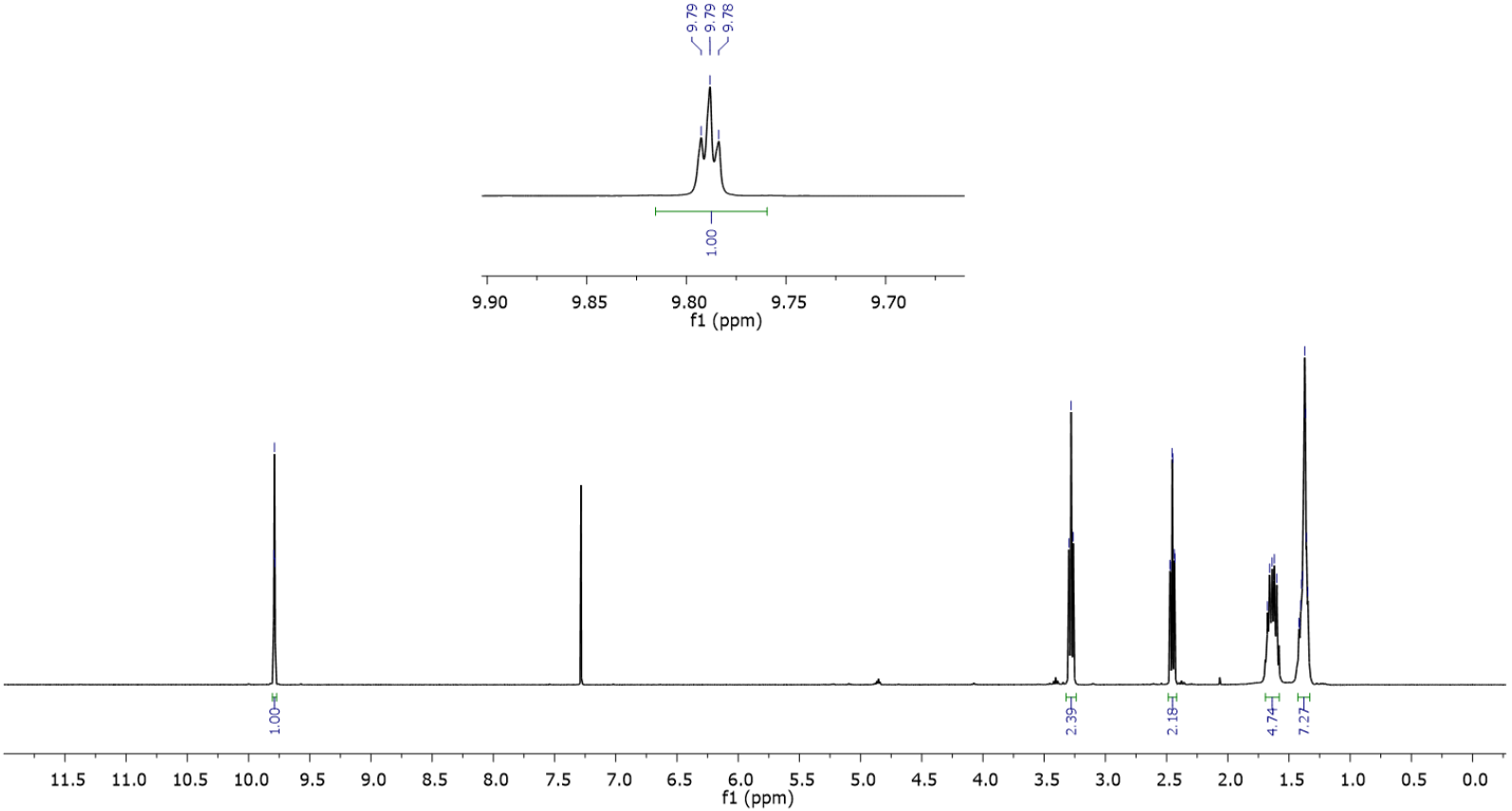
14c



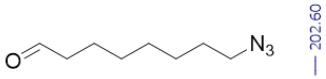
^{1}H NMR (400 MHz, $CDCl_3$)



22

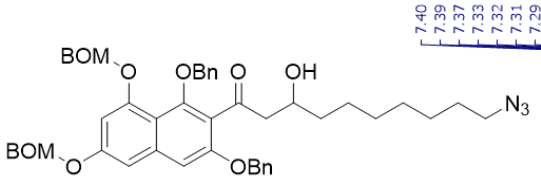


$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



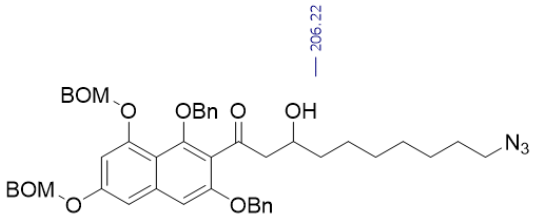
22

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



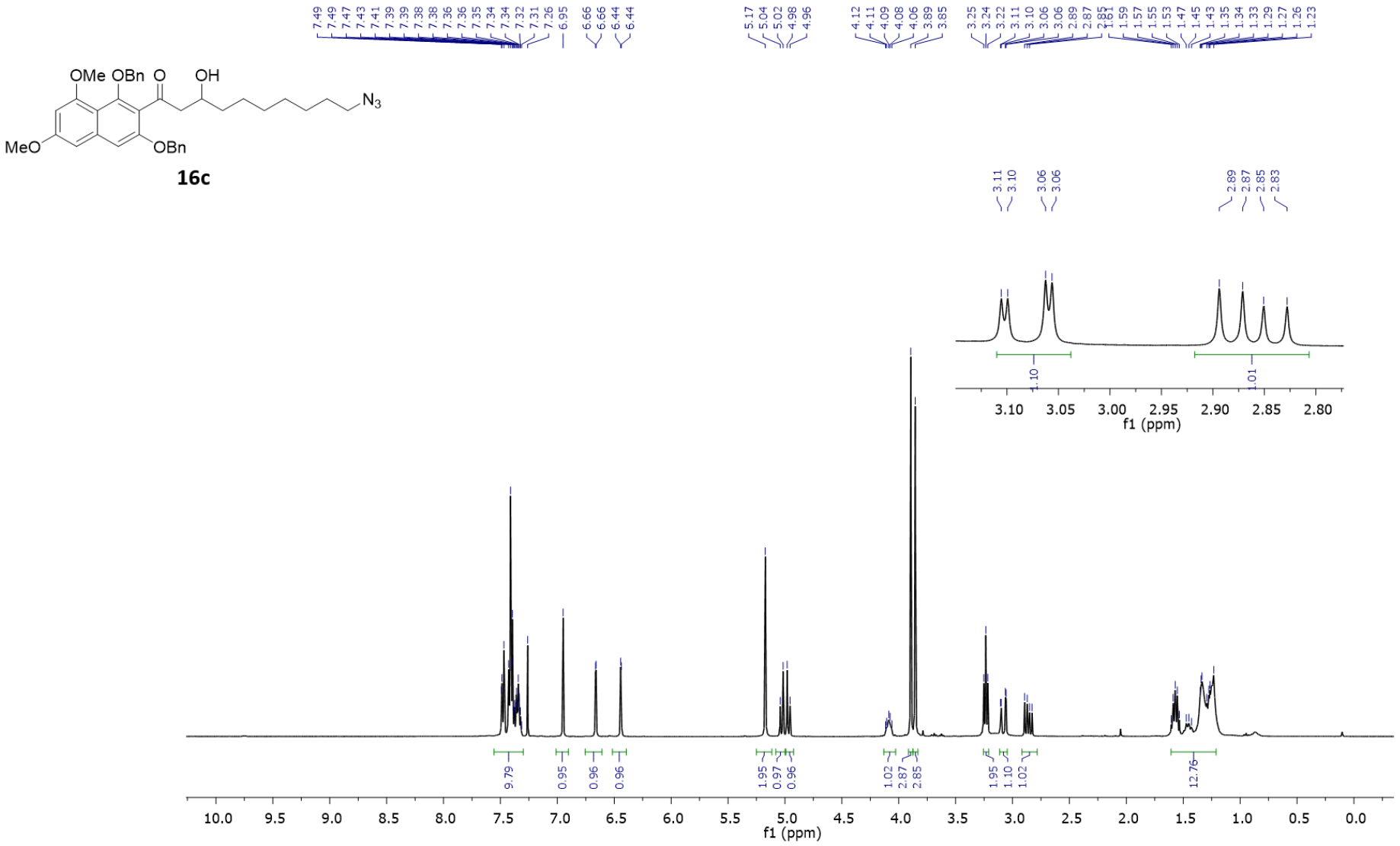
16b

$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)

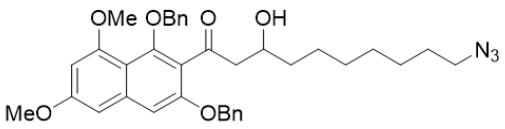


16b

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

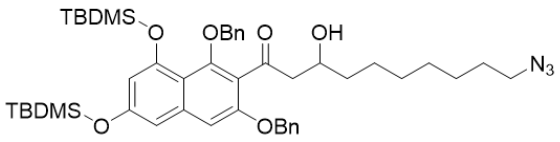


$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



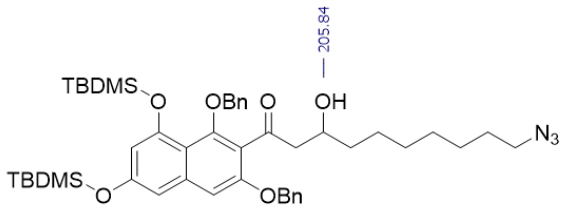
16c

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



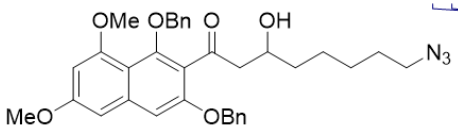
16d

$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



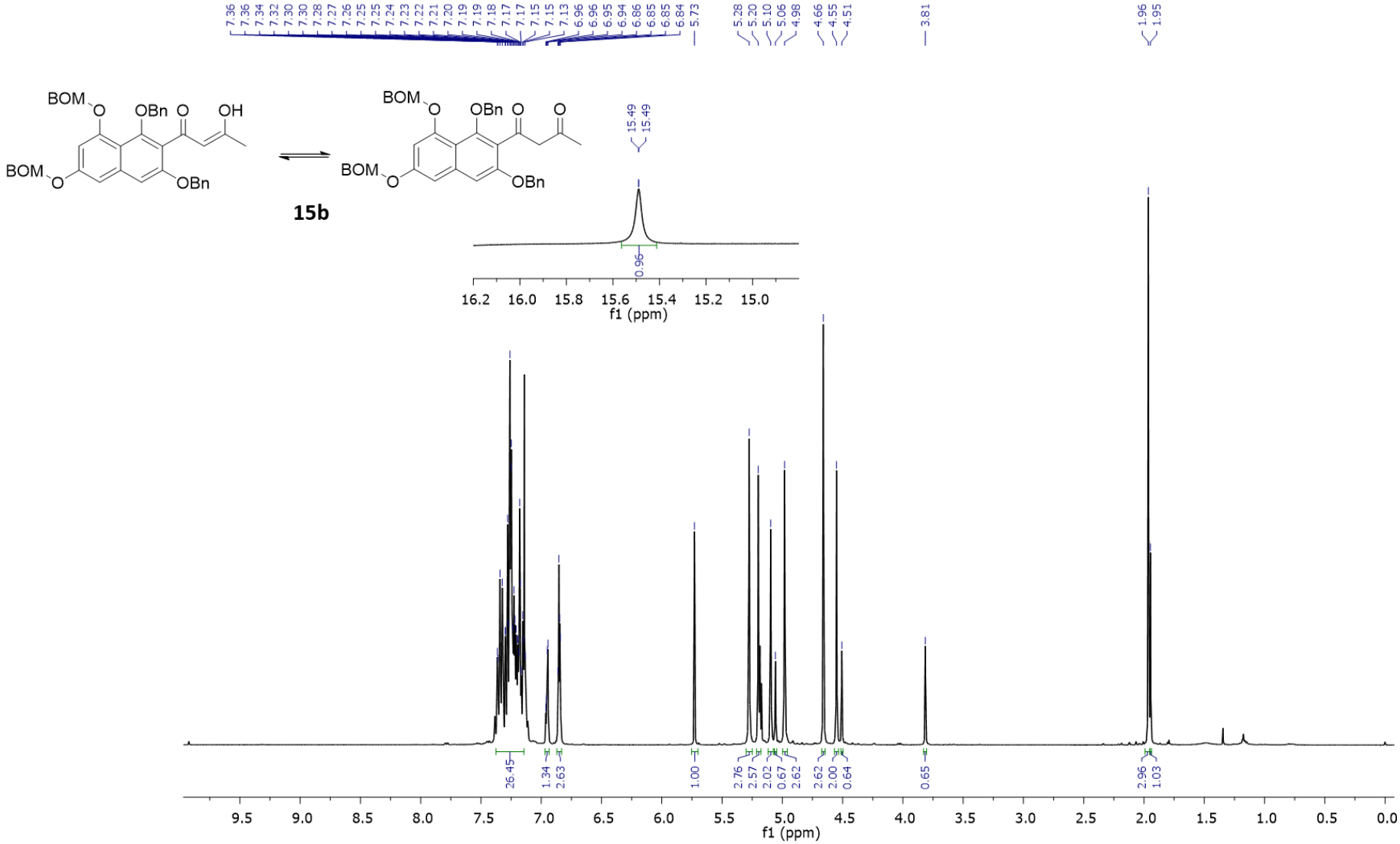
16d

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

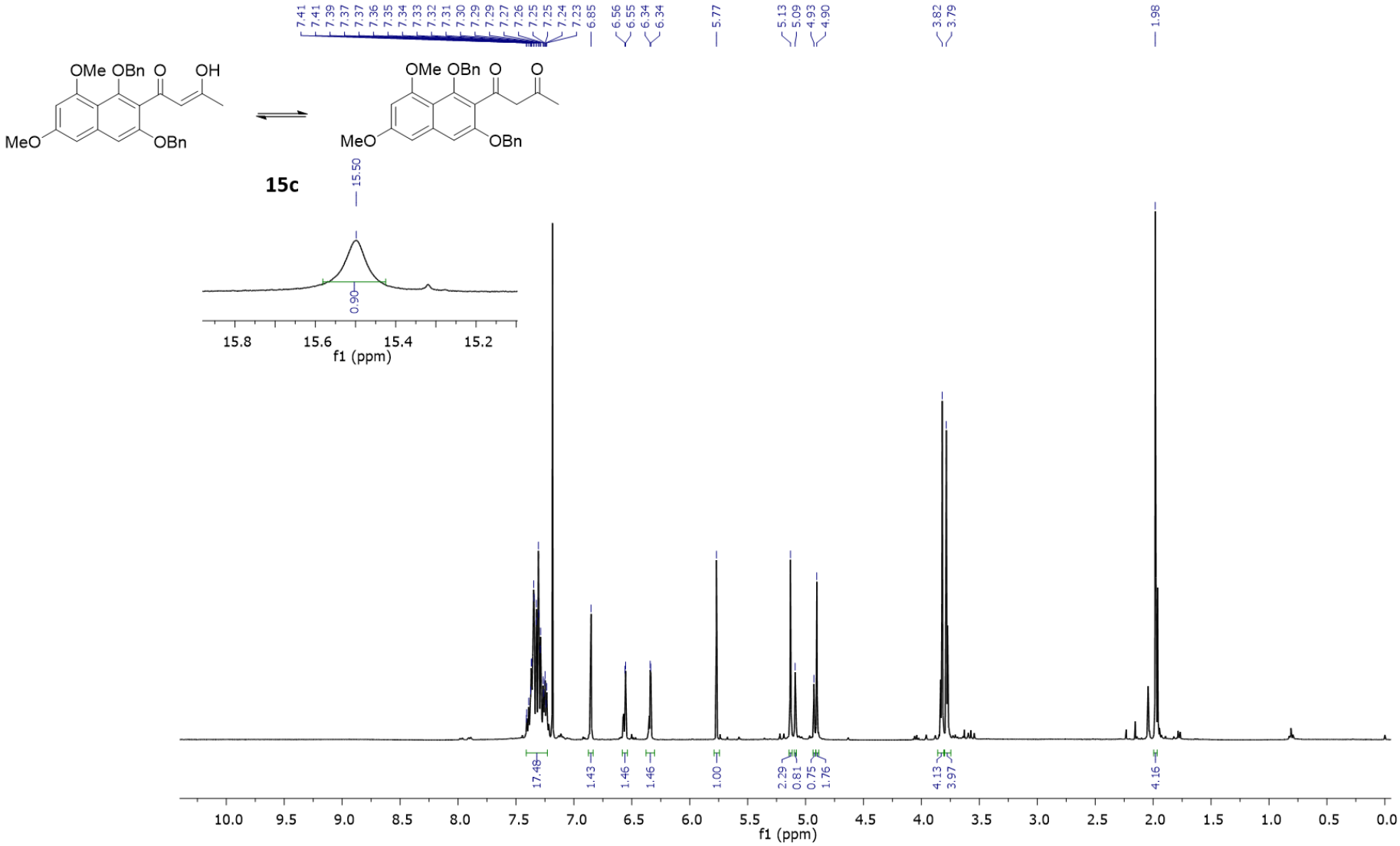


16e

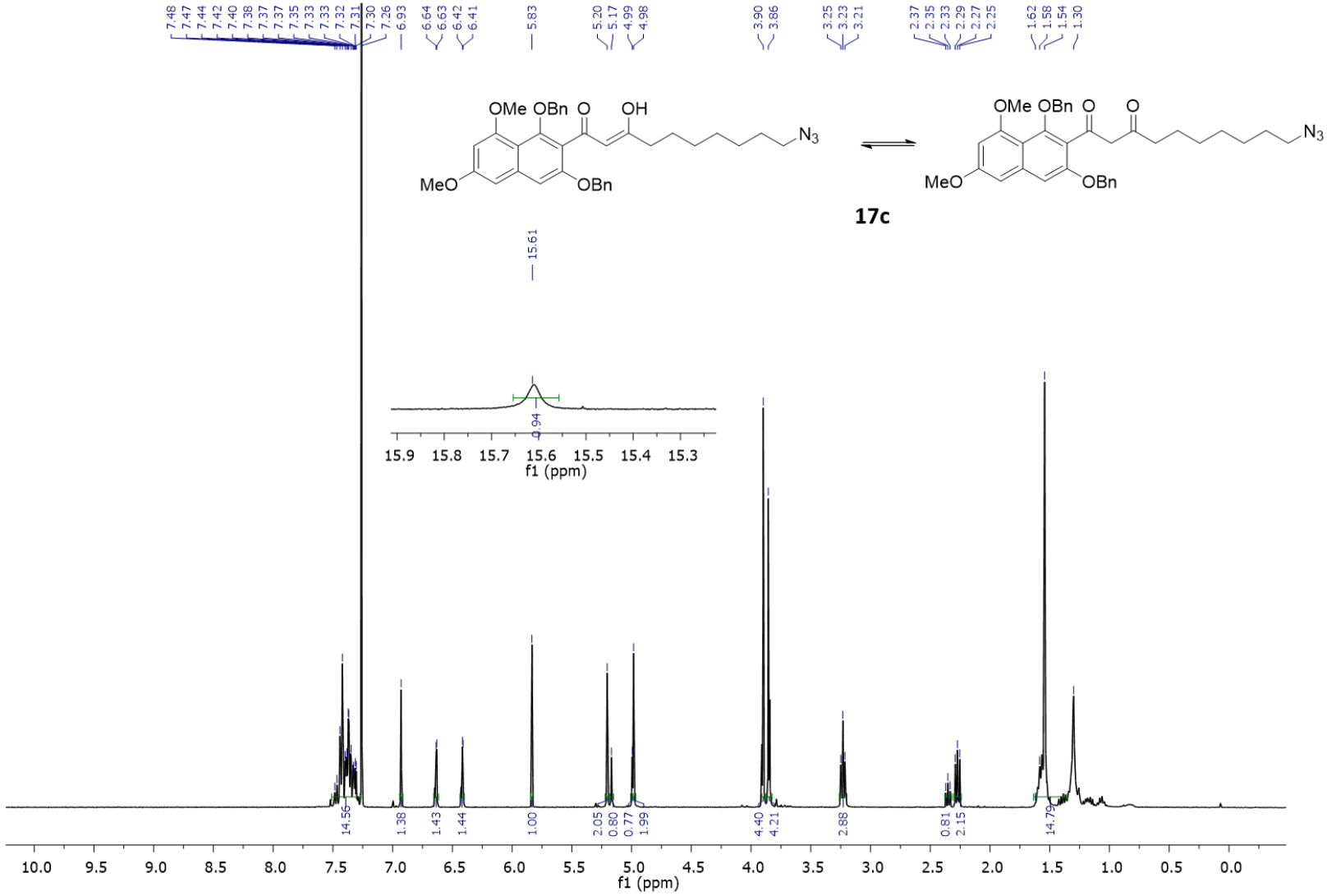
^{1}H NMR (400 MHz, $CDCl_3$)



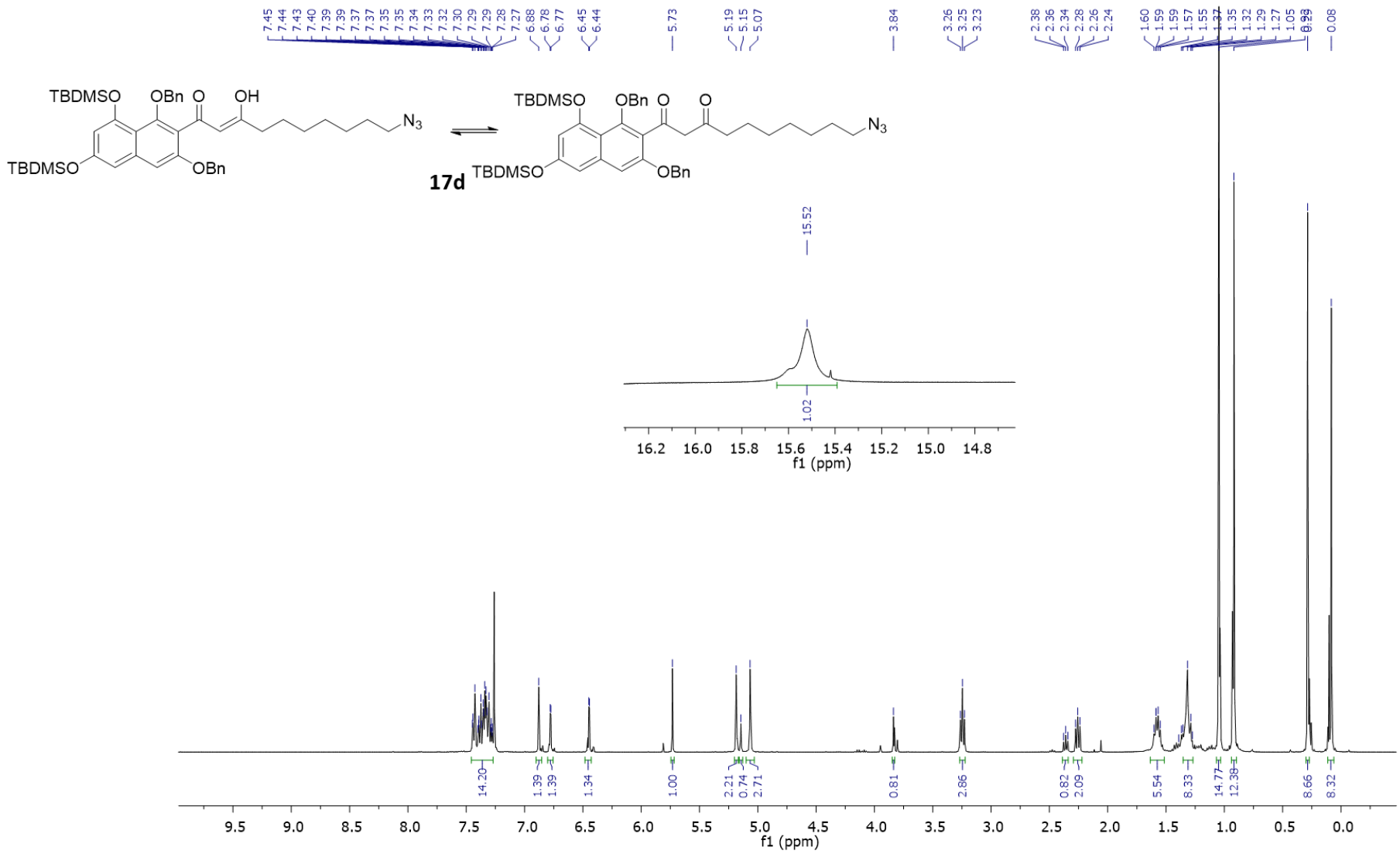
^{1}H NMR (400 MHz, $CDCl_3$)



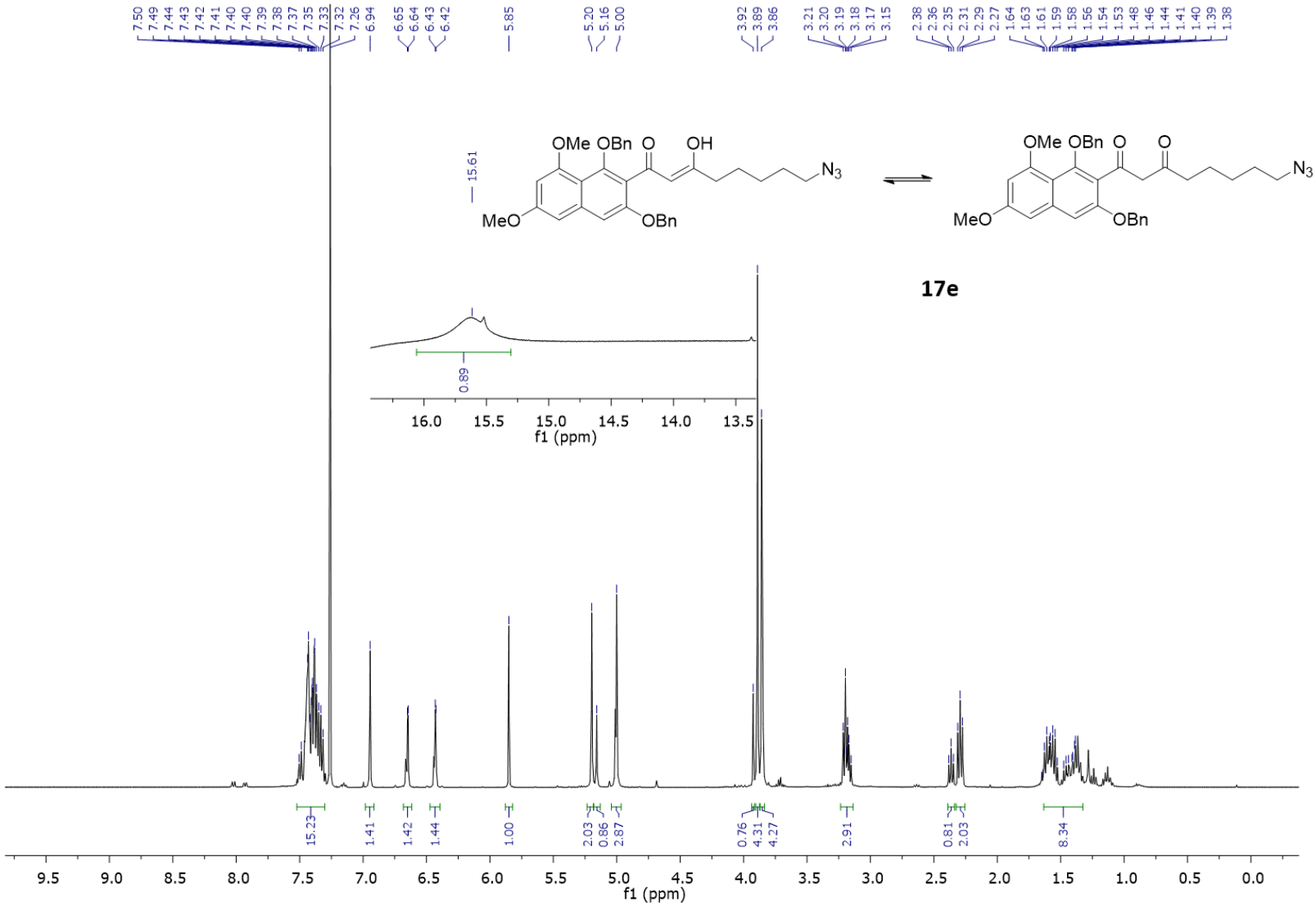
^{1}H NMR (400 MHz, $CDCl_3$)



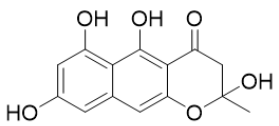
^{1}H NMR (400 MHz, $CDCl_3$)



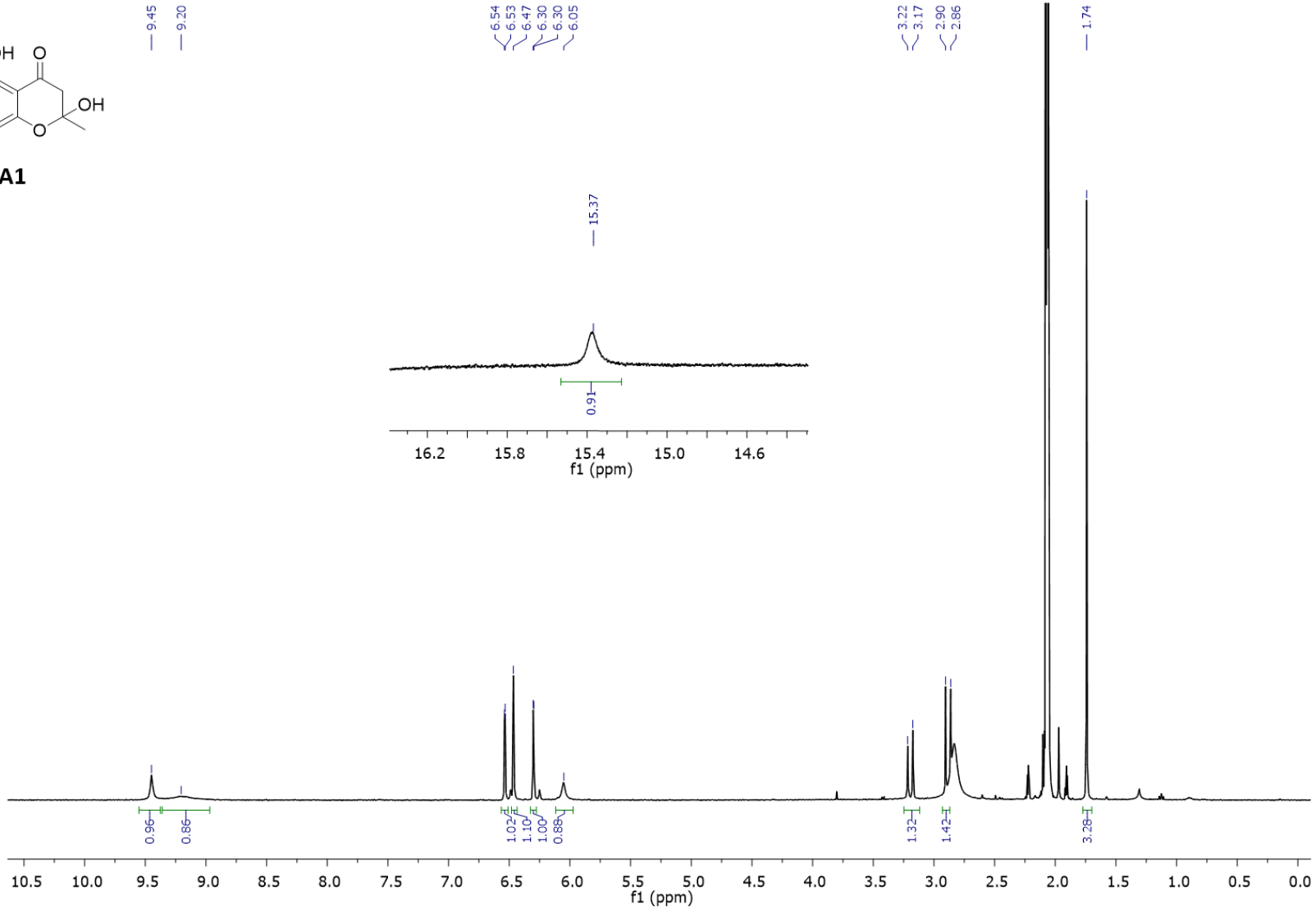
^{1}H NMR (400 MHz, $CDCl_3$)



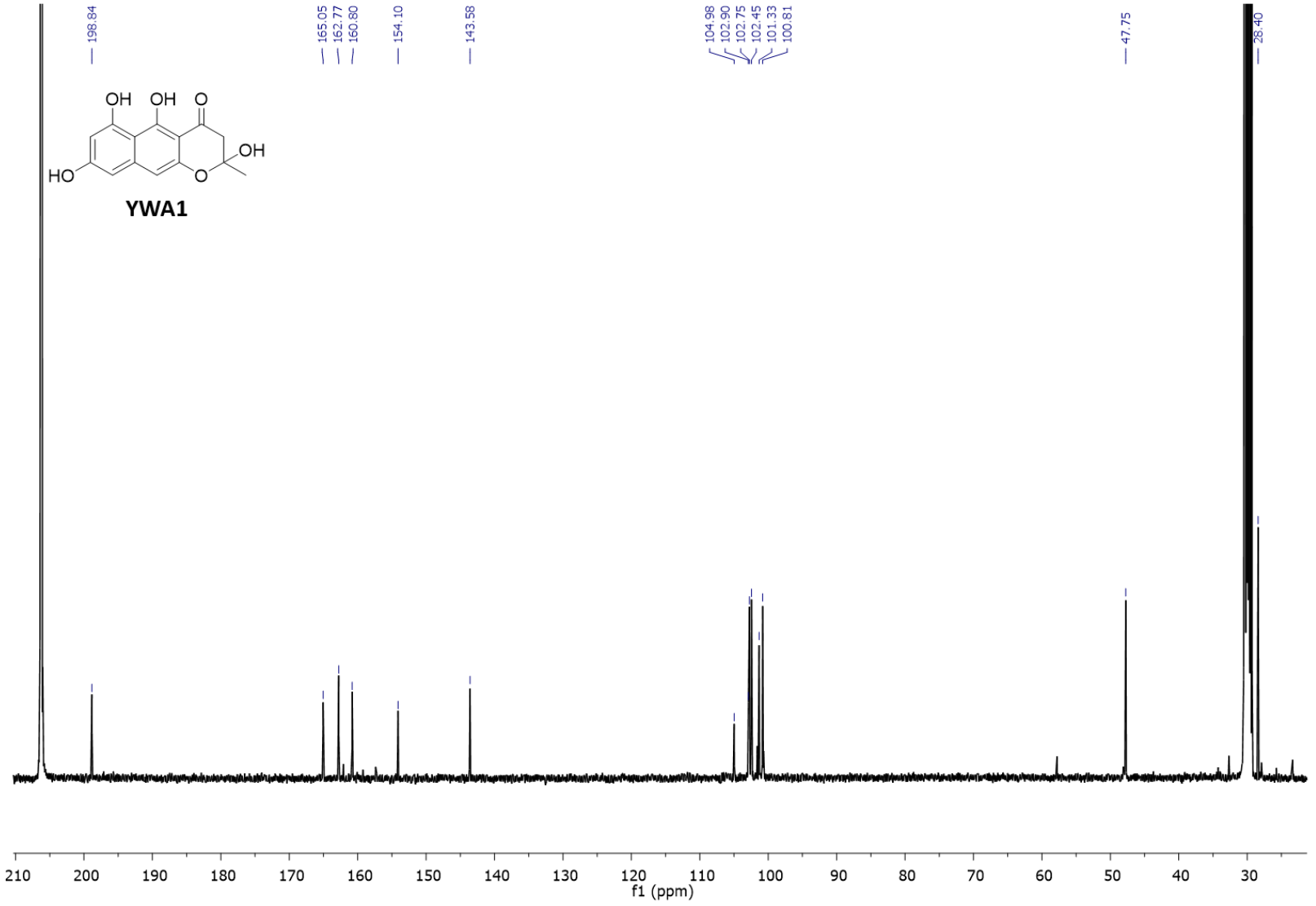
^{1}H NMR (400 MHz, $(CD_3)_2CO$)



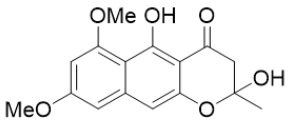
YWA1



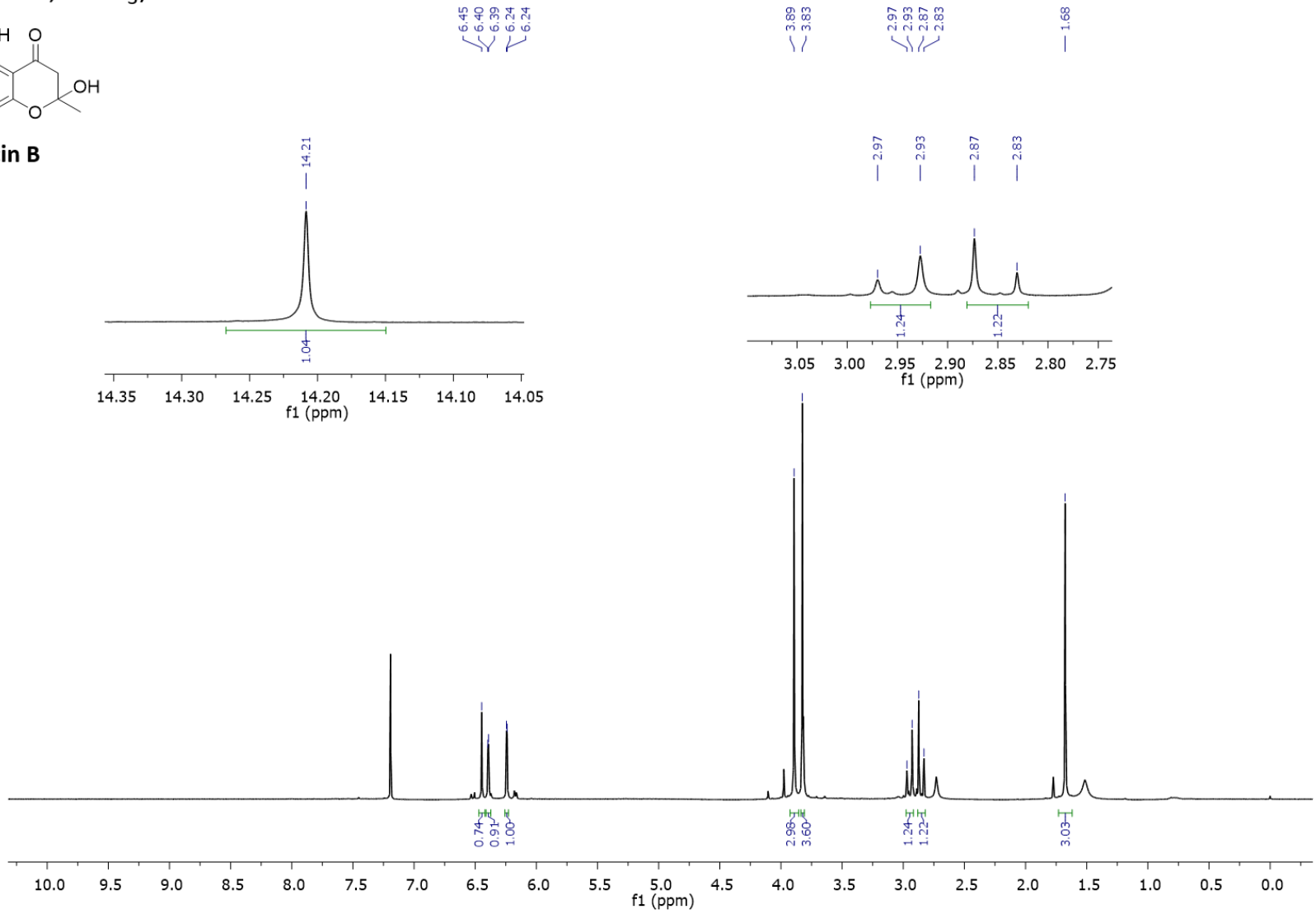
$^{13}C\{^{1}H\}$ NMR (101 MHz, $(CD_3)_2CO$)



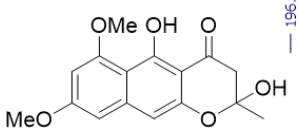
^{1}H NMR (400 MHz, $CDCl_3$)



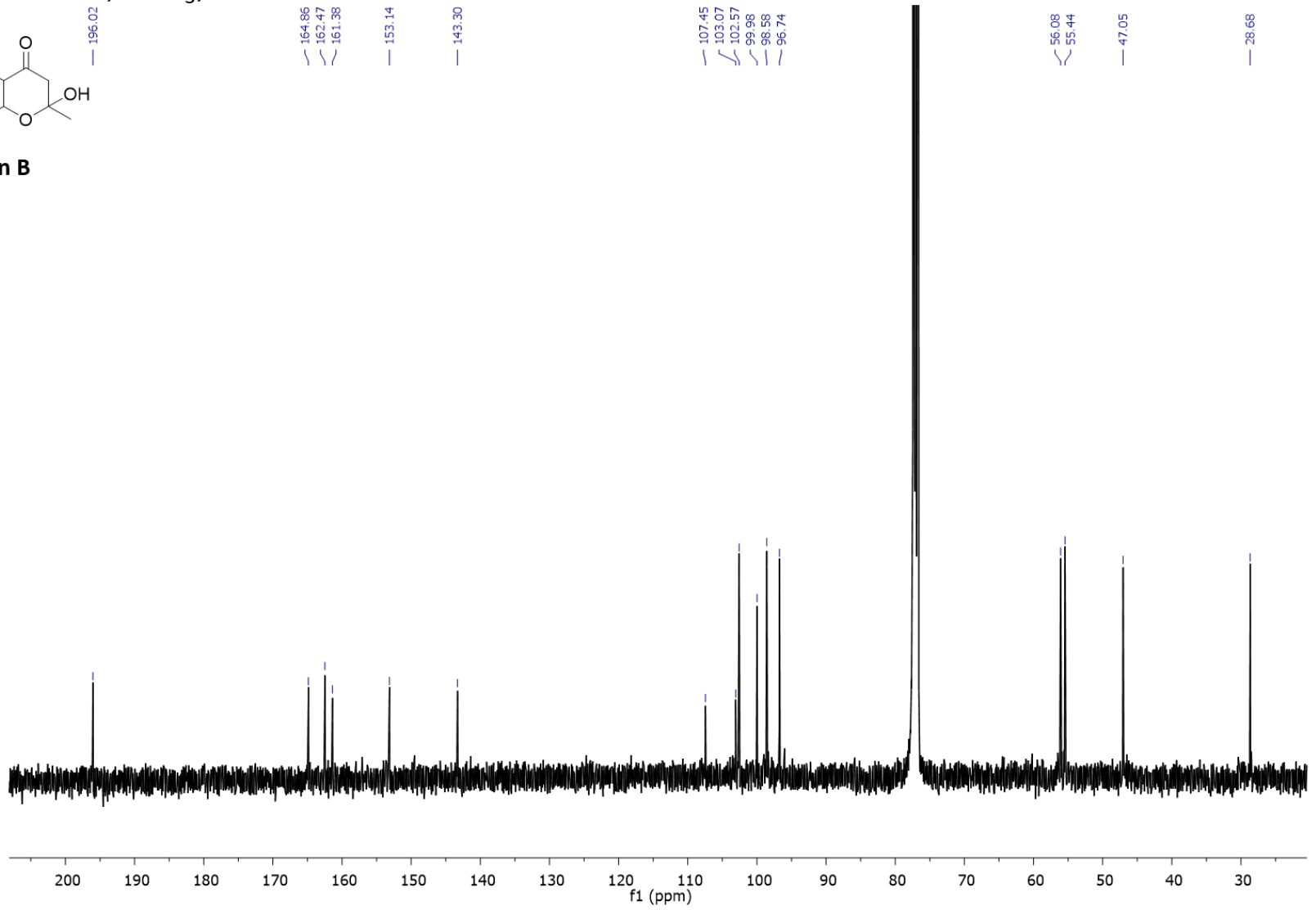
Fonsecin B



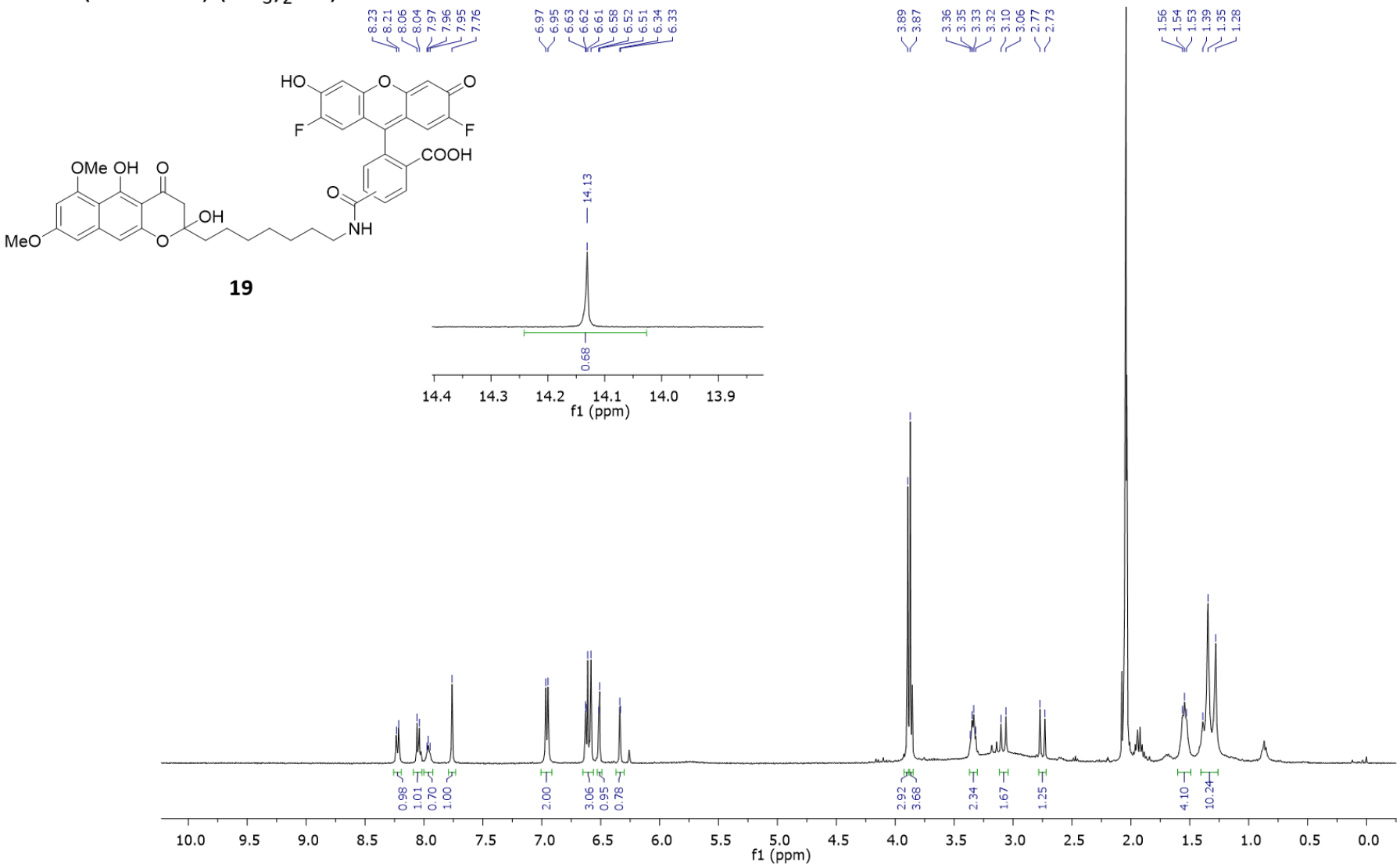
$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$)



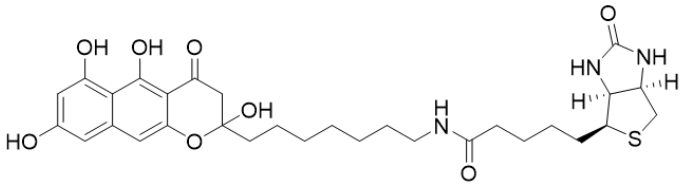
Fonsecin B



^{1}H NMR (400 MHz, $(CD_3)_2CO$)

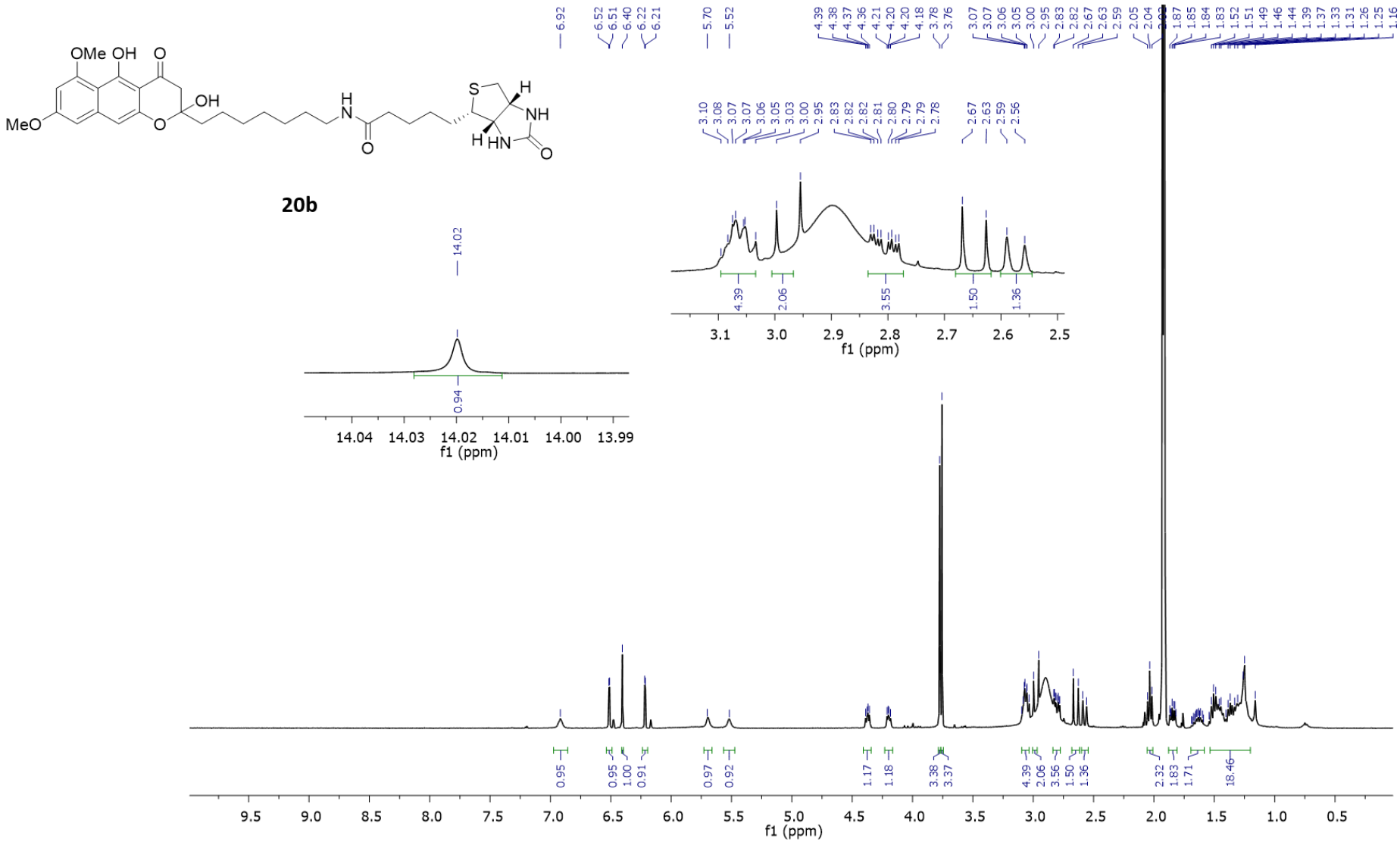


^{1}H NMR (400 MHz, (CD_3OD)

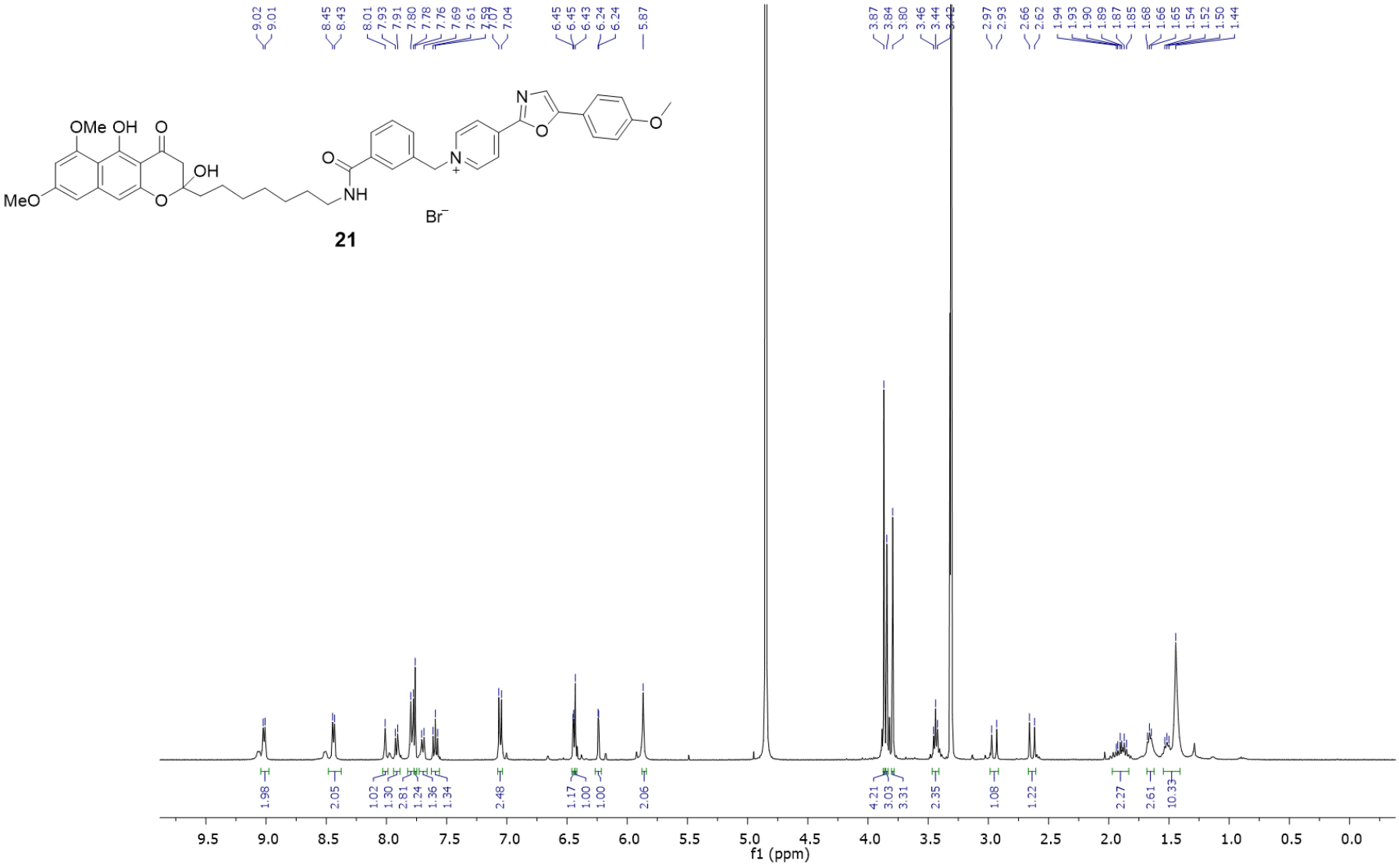


20a

^{1}H NMR (400 MHz, $(CD_3)_2CO$)



^{1}H NMR (400 MHz, (CD_3OD)



$^{13}C\{^{1}H\}$ NMR (101 MHz, (CD_3OD)