Supporting information for

The first fluorogenic sensor for sphingosine-1-phosphate lyase activity in intact cells

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Table of contents

General synthetic procedures

Unless otherwise stated, reactions were carried out under argon atmosphere. Dry solvents were obtained by passing through an activated alumina column on a Solvent Purification System (SPS). Methanol and ethanol were dried over CaH_2 and distilled prior to use. Commercially available reagents and solvents were used with no further purification. All reactions were monitored by TLC analysis using ALUGRAM® SIL G/UV_{254} precoated aluminum sheets (Machery–Nagel). UV light was used as the visualizing agent and a 5% (w/v) ethanolic solution of phosphomolybdic acid as the developing agent. Flash column chromatography was carried out with the indicated solvents using flash–grade silica gel (37–70 μm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. . High Resolution Mass Spectrometry analyses were carried out on an Acquity UPLC system coupled to a LCT Premier orthogonal accelerated time–of–flight mass spectrometer (Waters) using electrospray ionization (ESI) technique. Optical rotations were measured at room temperature on a Perkin Elmer 341 polarimeter.

General procedure 1: Ru–catalyzed olefin cross metathesis reactions

To a stirred solution of the starting olefins (1.5 and 6 mmol, respectively) in degassed CH_2Cl_2 (20 mL), Grubbs catalyst 2nd generation (0.03 equiv/mol) was added portionwise at rt. The resulting mixture was refluxed in the dark for 2 h, cooled down to rt and concentrated *in vacuo* to afford a crude, which was purified as indicated for each compound.

General procedure 2: Regioselective phosphorylation of 1,3–diols

A solution of the starting diol (0.5 mmol) in CH_2Cl_2 (15 mL) at 0 ºC was treated successively with *N*–methylimidazole (1.5 equiv/mol) and dimethyl chlorophosphate (1.2 equiv/mol). The reaction mixture was stirred at rt for 1 h, cooled down to 0 ºC and quenched by the dropwise addition of saturated aqueous NH_4Cl (10 mL). The resulting mixture was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent afforded a crude mixture, which was purified as indicated for each compound.

General procedure 3: TMSBr–mediated deprotection of dimethyl phosphates and *N*–Boc amino groups.

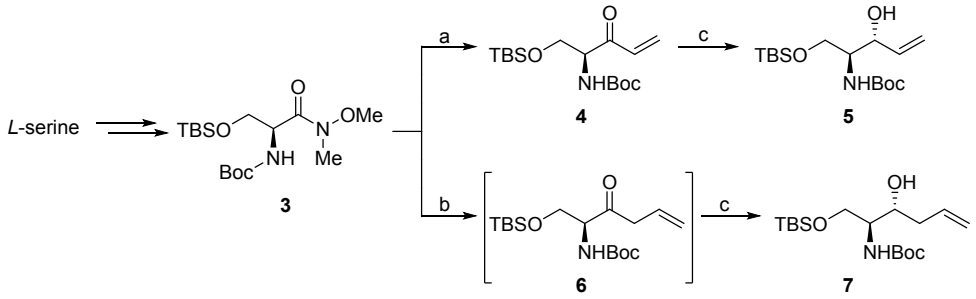
To an ice cooled solution of the starting dimethyl phosphate (0.2 mmol) in dry CH_3CN (8 mL), was added dropwise TMSBr (5 equiv/mol). After stirring for 3 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was then redissolved in MeOH/H_2O (95:5, same reaction volume) and stirred for an additional hour at rt. Evaporation of the solvent afforded a crude mixture, which was purified as indicated for each compound.

General procedure 4: fluoride–mediated deprotection of TBS groups

To a solution of the corresponding TBS–protected alcohol (1 mmol) in THF (10 mL) was added dropwise TBAF (1 M in THF, 2 equiv/mol) at 0 ºC. After stirring at the same temperature for 30 min, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) and the resulting mixture was extracted with Et_2O (3 x 15 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to give the crude products. Purification by flash chromatography on silica gel (from 0 to 3 % MeOH in CH_2Cl_2) afforded the required alcohols.

Synthesis of probes RBM77 and RBM148

Synthesis of building blocks 5 and 7



Scheme S1. Reagents and conditions: (a) vinylmagnesium bromide, THF, 0 ºC to rt (71 %). (b) allylmagnesium chloride, THF, -20 ºC to rt. (c) $LiAlH(O\textit{t}\text{-}Bu)_3$, EtOH, -78 ºC (for **5**: 90 %, dr = 98:2 *anti*/*syn*; for **7**: 78 % over 2 steps, dr = 99:1 *anti*/*syn*)

(*S*)–*tert*–butyl (1–((*tert*–butyldimethylsilyl)oxy)–3–oxopent–4–en–2–yl)carbamate (4)

Vinylmagnesium bromide (82.8 mL, 57.9 mmol, 0.70 M in THF) was added dropwise to a solution of **3** [1] (6.0 g, 16.6 mmol) in THF (50 mL) at 0 ºC. After stirring at rt for 1 h,

the reaction mixture was added dropwise *via* canula to a 1 M aq. solution of HCl (50 mL) at 0 ºC and the resulting mixture was extracted with EtOAc (3 x 50 mL) The organic layers were combined, washed with brine (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. Flash chromatography (from 0 to 5 % EtOAc in hexane) gave **4** (3.85 g, 71 %) as a colourless oil.

$[\alpha]^{20}_D$ = +62.2 (*c* 1.0, $CHCl_3$) [lit.[1] $[\alpha]^{24}_D$ = +63.3 (*c* 0.62, $CHCl_3$)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (dd, *J* = 17.5, 10.6 Hz, 1H), 6.35 (dd, *J* = 17.4, 1.3 Hz, 1H), 5.83 (d, *J* = 10.6 Hz, 1H), 5.52 (br d, *J* = 7.0 Hz, 1H), 4.60 (dt, *J* = 7.6, 3.9 Hz, 1H), 4.01 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.85 (dd, *J* = 10.3, 4.4 Hz, 1H), 1.45 (s, 9H), 0.84 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.0, 155.4, 133.3, 129.5, 79.9, 63.6, 59.7, 28.5, 25.9, 18.3, –5.5, –5.5. HRMS calcd. for $C_{16}H_{31}NO_4SiNa$ ($[M + Na]^+$): 352.1920, found: 352.1937.

tert–butyl ((2*S*,3*R*)–1–((_tert_–butyldimethylsilyl)oxy)–3–hydroxypent–4–en–2–yl) carbamate (5)

A solution of **4** (1.01 g, 3.08 mmol) in ethanol (12 mL) was added dropwise to a suspension of lithium tri–*tert*–butoxyaluminum hydride (1.72 g, 6.78 mmol) in ethanol (30 mL) at –78 ºC. After stirring at the same temperature for 30 min, the reaction mixture was allowed to warm to 0 ºC and was quenched with 10 % (w/v) aqueous citric acid (20 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to give a 98:2 *anti*/*syn* crude mixture of diastereomers. Flash chromatography of the residue (from 0 to 11 % EtOAc in hexane) gave pure *anti*–**5** (922 mg, 90 %) as a colourless oil.

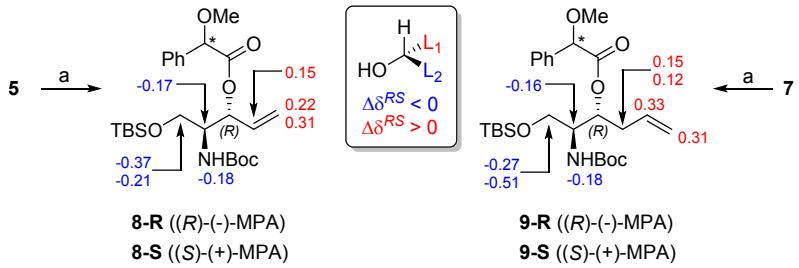
$[\alpha]^{20}_D$ = +28.1 (*c* 1.0, $CHCl_3$) [lit.[1] $[\alpha]^{23}_D$ = +24.8 (*c* 1.45, $CHCl_3$)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (ddd, *J* = 17.2, 10.6, 4.9 Hz, 1H), 5.39 (dt, *J* = 17.2, 1.7 Hz, 1H), 5.32 – 5.19 (m, 2H), 4.27 (s, 1H), 3.93 (dd, *J* = 10.4, 2.9 Hz, 1H), 3.76 (dd, *J* = 10.3, 2.6 Hz, 1H), 3.63 (dd, *J* = 7.3, 3.5 Hz, 2H), 3.44 (br s, 1H), 1.45 (s, 9H), 0.90 (s, 9H), 0.07 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 138.0, 116.0, 79.7, 75.0, 63.6, 54.2, 28.5, 25.9, 18.3, –5.5, –5.5. HRMS calcd. for $C_{16}H_{34}NO_4Si$ ($[M + H]^+$): 332.2257, found: 332.2267.

tert–butyl ((2_S_,3_R_)–1–((_tert_–butyldimethylsilyl)oxy)–3–hydroxyhex–5–en–2–yl) carbamate (7)

Allylmagnesium chloride (6.9 mL, 13.8 mmol, 2.0 M in THF) was added dropwise to a solution of **3** (2.50 g, 6.90 mmol) in THF (30 mL) at –20 ºC. The reaction mixture was allowed to warm to 0 ºC over 5 h with stirring and was then quenched with saturated aqueous NH_4Cl (25 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue, containing crude **6**, was dissolved in ethanol (15 mL) and was added dropwise to a suspension of lithium tri–_tert_–butoxyaluminum hydride (3.86 g, 15.2 mmol) in ethanol (60 mL) at –78 ºC. After stirring at the same temperature for 30 min, the reaction mixture was allowed to warm to 0 ºC and was quenched with 10 % (w/v) aqueous citric acid (40 mL). The resulting mixture was carefully concentrated under reduced pressure and then extracted with EtOAc (3 x 50 mL). The organic layers were combined and were washed with brine (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to give a 99:1 *anti*/*syn* crude mixture of diastereomers. Flash chromatography of the residue (from 0 to 9 % EtOAc in hexane) gave pure *anti*-**7** (1.85 g, 78 %) as a colourless oil.

$[\alpha]^{20}_D$ = +32.6 (*c* 1.0, $CHCl_3$) [lit.[2] $[\alpha]_D$ = +32.2 (*c* 1.32, $CHCl_3$)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H), 5.23 (br d, *J* = 7.9 Hz, 1H), 5.19 – 5.06 (m, 2H), 3.98 (dd, *J* = 10.6, 2.9 Hz, 1H), 3.84 – 3.76 (m, 1H), 3.76 – 3.67 (m, 1H), 3.60 – 3.48 (m, 1H), 3.02 (br s, 1H), 2.42 – 2.27 (m, 2H), 1.45 (s, 9H), 0.90 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.8, 134.7, 117.9, 79.6, 73.0, 63.4, 53.8, 39.5, 28.5, 26.0, 18.3, –5.5, –5.5. HRMS calcd. for $C_{17}H_{35}NO_4NaSi$ ($[M + Na]^+$): 368.2233, found: 368.2235.

Configurational assignment of 5 and 7



8-R ((*R*)-(-)-MPA)
8-S ((*S*)-(+)-MPA)

9-R ((*R*)-(-)-MPA)
9-S ((*S*)-(+)-MPA)

Scheme S2. Reagents and conditions: (a) (*R*)-(-)- or (*S*)-(+)-MPA, EDC, DMAP, CH_2Cl_2, 0 ºC to rt.(64 % for **8-R**; 82 % for **8-S**; 36 % for **9-R**; 68 % for **9-S**).

Synthesis of MPA esters.

DMAP (1.1 equiv/mol) was added portionwise at 0 ºC to a solution of the starting alcohol (0.2 mmol) in CH_2Cl_2 (2 mL) containing (*R*)–(–)– or (*S*)–(+)–MPA (1.6 equiv/mol) and EDC (1.5 equiv/mol). After stirring for 6 h at rt, the reaction mixture was washed with saturated aq. $NaHCO_3$ (2 x 2 mL) and the organic phase was separated, dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The resulting residue was flash chromatographed on silica gel (from 0 to 8 % EtOAc in hexane) to give the corresponding MPA esters

(*R*)–(–)–MPA ester of alcohol 5 (8–R)

Compound **8–R** (colorless oil, 52 mg, 64 %) was obtained from **5** (56 mg, 0.17 mmol), (*R*)–(–)–MPA (45 mg, 0.27 mmol), EDC (49 mg, 0.25 mmol) and DMAP (23 mg, 0.19 mmol), according to the above procedure.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.40 (m, 2H), 7.40 – 7.29 (m, 3H), 5.82 (ddd, *J* = 17.3, 10.5, 6.8 Hz, 1H), 5.37 – 5.21 (m, 3H), 4.75 (s, 1H), 4.49 (br d, *J* = 9.4 Hz, 1H), 3.78 – 3.69 (m, 1H), 3.43 – 3.36 (m, 4H), 3.19 (dd, *J* = 10.3, 3.5 Hz, 1H), 1.40 (s, 9H), 0.81 (s, 9H), –0.10 (s, 3H), –0.13 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 155.4, 136.4, 133.3, 128.9, 128.8, 127.3, 119.3, 82.6, 79.6, 74.2, 61.2, 57.5, 53.9, 28.5, 25.9, 18.3, –5.5, –5.6. HRMS calcd. for $C_{25}H_{42}NO_6Si$ ($[M + H]^+$): 480.2781, found: 480.2782.

(*S*)–(+)–MPA ester of alcohol 5 (8–S)

Compound **8–S** (colorless oil, 72 mg, 82 %) was obtained from **5** (61 mg, 0.184 mmol), (*S*)–(+)–MPA (49 mg, 0.29 mmol), EDC (53 mg, 0.28 mmol) and DMAP (25 mg, 0.20 mmol), according to the above procedure.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 2H), 7.38 – 7.29 (m, 3H), 5.68 (ddd, *J* = 17.1, 10.7, 6.3 Hz, 1H), 5.39 (t, *J* = 6.5 Hz, 1H), 5.07 (d, *J* = 10.7 Hz, 1H), 4.95 (d, *J* = 17.2 Hz, 1H), 4.77 (s, 1H), 4.67 (br d, *J* = 9.4 Hz, 1H), 3.95 – 3.84 (m, 1H), 3.62 (dd, *J* = 10.3, 3.5 Hz, 1H), 3.55 (dd, *J* = 10.3, 4.6 Hz, 1H), 3.43 (s, 3H), 1.43 (s, 9H), 0.87 (s, 9H), 0.00 (s, 3H), –0.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 155.5, 136.3, 132.5, 128.8, 128.7, 127.3, 118.9, 82.7, 79.6, 74.1, 61.8, 57.6, 53.7, 28.5, 25.9, 18.3, –5.4, –5.5. HRMS calcd. for $C_{25}H_{42}NO_6Si$ ($[M + H]^+$): 480.2781, found: 480.2762.

(*R*)–(–)–MPA ester of alcohol 7 (9–R)

Compound **9–R** (yellow oil, 38 mg, 36 %) was obtained from **7** (75 mg, 0.22 mmol), (*R*)–(–)–MPA (58 mg, 0.35 mmol), EDC (62 mg, 0.33 mmol) and DMAP (29 mg, 0.24 mmol), according to the above procedure.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.38 (m, 2H), 7.38 – 7.28 (m, 3H), 5.83 – 5.69 (m, 1H), 5.09 – 4.98 (m, 3H), 4.70 (s, 1H), 4.53 (br d, *J* = 9.5 Hz, 1H), 3.73 – 3.63 (m, 1H), 3.40 (s, 3H), 3.31 (dd, *J* = 10.3, 3.7 Hz, 1H), 3.06 (dd, *J* = 10.4, 3.5 Hz, 1H), 2.55 – 2.45 (m, 1H), 2.38 – 2.28 (m, 1H), 1.41 (s, 9H), 0.81 (s, 9H), –0.12 (s, 3H), –0.15 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 155.5, 136.5, 133.6, 128.9, 128.8, 127.3, 118.1, 82.6, 79.6, 72.5, 61.3, 57.5, 53.5, 36.0, 28.5, 25.9, 18.3, –5.5, –5.6. HRMS calcd. for $C_{26}H_{44}NO_6Si$ ($[M + H]^+$): 494.2938, found: 494.2926.

(*S*)–(+)–MPA ester of alcohol 7 (9–S)

Compound **9–S** (yellow oil, 73 mg, 68 %) was obtained from **7** (75 mg, 0.22 mmol), (*S*)–(+)–MPA (58 mg, 0.35 mmol), EDC (62 mg, 0.33 mmol) and DMAP (29 mg, 0.24 mmol), according to the above procedure.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.37 (m, 2H), 7.37 – 7.28 (m, 3H), 5.43 (ddt, *J* = 17.0, 10.2, 7.2 Hz, 1H), 5.01 (td, *J* = 7.5, 4.0 Hz, 1H), 4.81 – 4.65 (m, 4H), 3.89 – 3.79 (m, 1H), 3.57 (d, *J* = 3.6 Hz, 2H), 3.42 (s, 3H), 2.40 – 2.31 (m, 1H), 2.26 – 2.16 (m, 1H),

1.44 (s, 9H), 0.87 (s, 9H), 0.01 (s, 3H), –0.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 155.5, 136.4, 132.9, 128.8, 128.6, 127.4, 118.0, 82.8, 79.7, 73.1, 61.8, 57.6, 53.2, 35.4, 28.5, 26.0, 18.3, –5.4, –5.5. HRMS calcd. for $C_{26}H_{44}NO_6Si$ ($[M + H]^+$): 494.2938, found: 494.2933.

Synthesis of precursors 12 and 14

***tert*–butyl ((2*S*,3*R*,*E*)–1–((*tert*–butyldimethylsilyl)oxy)–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–4–en–2–yl)carbamate (12)**

Compound **12** (inseparable 94:6 *E*/*Z* mixture, colourless oil, 860 mg, 63 %) was obtained from **5** (875 mg, 2.64 mmol), **10**[3] (2.28 g, 10.6 mmol) and Grubbs catalyst 2nd generation (67 mg, 0.08 mmol), according to general procedure 1. The title compound was purified by flash chromatography on silica gel (from 0 to 1 % CH_3OH in CH_2Cl_2). Early–eluting fractions were independently collected to give a sample of pure *E*–alkene, from which the following data were acquired.

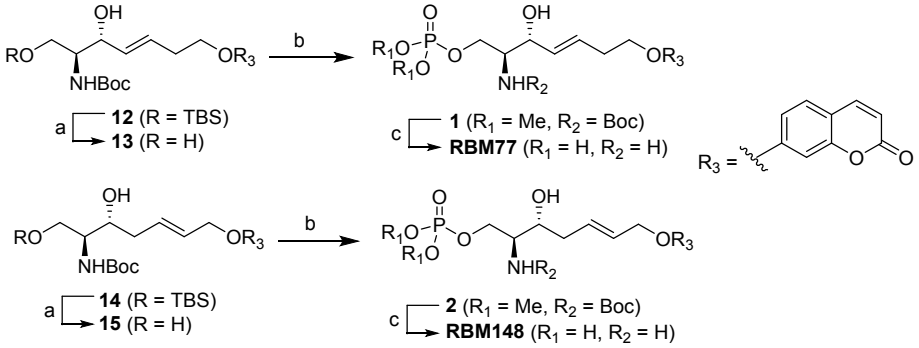
$[\alpha]^{20}_D$ = +11.1 (*c* 1.0, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 6.85 – 6.76 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 5.92 – 5.81 (m, 1H), 5.71 (dd, *J* = 15.4, 5.4 Hz, 1H), 5.24 (br d, *J* = 8.2 Hz, 1H), 4.29 – 4.22 (m, 1H), 4.06 (t, *J* = 6.5 Hz, 2H), 3.94 (dd, *J* = 10.4, 2.9 Hz, 1H), 3.80 – 3.70 (m, 1H), 3.65 – 3.55 (m, 1H), 2.60 (app q, *J* = 6.6 Hz, 2H), 1.45 (s, 9H), 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 161.3, 156.0, 155.9, 143.5, 132.9, 128.9, 127.5, 113.2, 113.0, 112.7, 101.5, 79.7, 74.5, 67.8, 63.5, 54.5, 32.1, 28.5, 25.9, 18.2, –5.5, –5.5. HRMS calcd. for $C_{27}H_{41}NO_7NaSi$ ($[M + Na]^+$): 542.2550, found: 542.2557.

***tert*–butyl ((2*S*,3*R*,*E*)–1–((*tert*–butyldimethylsilyl)oxy)–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–5–en–2–yl)carbamate (14)**

Compound **14** (93:7 *E*/*Z* mixture, colourless oil, 865 mg, 66 %) was obtained from **135** (868 mg, 2.51 mmol), **11**[4] (2.03 g, 10.1 mmol) and Grubbs catalyst 2nd generation (64 mg, 0.08 mmol), according to general procedure 1. The title compound was purified by flash chromatography on silica gel (from 0 to 27 % EtOAc in hexane). Pure *E*–alkene was obtained after purification of the diastereomeric mixture by flash chromatography using the same gradient.

$[\alpha]^{20}_D$ = +16.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.87 – 6.78 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 6.00 – 5.90 (m, 1H), 5.80 (dt, *J* = 15.5, 5.8 Hz, 1H), 5.22 (br d, *J* = 8.3 Hz, 1H), 4.55 (d, *J* = 5.8 Hz, 2H), 3.97 (dd, *J* = 10.6, 2.9 Hz, 1H), 3.85 – 3.69 (m, 2H), 3.60 – 3.49 (m, 2H), 3.14 (br s, 1H), 2.46 – 2.32 (m, 2H), 1.45 (s, 9H), 0.90 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 161.3, 155.9, 155.8, 143.5, 132.1, 128.9, 126.9, 113.2, 113.1, 112.7, 101.8, 79.7, 73.1, 69.1, 63.4, 53.9, 37.9, 28.5, 25.9, 18.3, –5.5, –5.5. HRMS calcd. for $C_{27}H_{41}NO_7NaSi$ ($[M + Na]^+$): 542.2550, found: 542.2556.

Conversion of 12 and 14 into probes RBM148 and RBM77



Scheme S3. Reagents and conditions: (a) TBAF, THF, 0 ºC; (b) $(MeO)_2POCl$, 1-methylimidazole, CH_2Cl_2, 0 ºC to rt; (c) (i) TMSBr, CH_3CN, 0 ºC to rt. (ii) CH_3OH/H_2O (95% v/v), rt;

***tert*–butyl ((2*S*,3*R*,*E*)–1,3–dihydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–4–en–2–yl) carbamate (13)**

Diol **13** (colorless oil, 345 mg, 95 %) was obtained from **12** (465 mg, 0.90 mmol) and TBAF (1.79 mL, 1.79 mmol), according to general procedure 4.

$[\alpha]^{20}_D$ = –3.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.86 – 6.76 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 5.94 – 5.83 (m, 1H), 5.74 (dd, *J* = 15.5, 6.0 Hz, 1H), 5.33 (br d, *J* = 7.2 Hz, 1H), 4.40 – 4.35 (m, 1H), 4.07 (t, *J* = 6.4 Hz, 2H), 3.94 (dd, *J* = 11.4, 3.5 Hz, 1H), 3.73 (dd, *J* = 11.4, 3.7 Hz, 1H), 3.65 (br s, 1H), 2.65 – 2.55 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 161.5, 156.4, 155.9, 143.6, 132.6, 128.9, 128.3, 113.1, 113.1, 112.7, 101.5, 80.0, 74.4, 67.8,

62.5, 55.4, 32.1, 28.5. HRMS calcd. for $C_{21}H_{27}NO_7Na$ ([M + Na]$^+$): 428.1685, found: 428.1691.

***tert*–butyl ((2*S*,3*R*,*E*)–1–((dimethoxyphosphoryl)oxy)–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–4–en–2–yl)carbamate (1)**

Compound **1** (colorless oil, 105 mg, 69 %) was obtained from alcohol **13** (120 mg, 0.30 mmol), *N*–methylimidazole (35 µL, 0.44 mmol) and dimethyl chlorophosphate (38 µL, 0.36 mmol), according to general procedure 2. The title compound was purified by flash chromatography on silica gel (from 0 to100 % EtOAc in hexane).

$[\alpha]^{20}_D$ = +6.4 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 6.87 – 6.77 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.91 – 5.81 (m, 1H), 5.70 (dd, *J* = 15.5, 6.4 Hz, 1H), 5.09 (br d, *J* = 7.8 Hz, 1H), 4.35 (ddd, *J* = 10.8, 8.2, 4.7 Hz, 1H), 4.20 (t, *J* = 6.3 Hz, 1H), 4.16 – 4.08 (m, 1H), 4.05 (t, *J* = 6.5 Hz, 2H), 3.88 – 3.73 (m, 7H), 2.62 – 2.53 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 161.33, 156.0, 155.8, 143.5, 132.0, 128.9, 113.2, 113.0, 112.7, 101.5, 80.0, 72.2, 67.8, 66.7 (d, $J_{C–P}$ = 5.7 Hz), 55.0 (br d, $J_{C–P}$ = 4.4 Hz), 54.7 (d, $J_{C–P}$ = 5.9 Hz), 32.1, 28.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 2.65. HRMS calcd. for $C_{23}H_{32}NO_{10}NaP$ ([M + Na]$^+$): 536.1662, found: 536.1670.

(2*S*,3*R*,*E*)–2–ammonio–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–4–en–1–yl hydrogen phosphate (RBM77)

Compound **RBM77** (white solid, 49 mg, 85 %) was obtained from dimethyl phosphate **1** (77 mg, 0.15 mmol) and TMSBr (99 µL, 0.75 mmol), according to general procedure 3. The crude reaction mixture was dissolved in methanol and loaded on an Amberlite ® XAD4 column (10 g), which had been washed thoroughly with acetone and then equilibrated with water. Elution with a linear gradient from 0 to 50 % CH_3CN in H_2O provided pure **RBM77**.

$[\alpha]^{20}_D$ = +14.7 (*c* 0.7, DMSO). ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (d, *J* = 9.5 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 6.99 – 6.89 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 6.06 – 5.93 (m, 1H), 5.68 (dd, *J* = 15.4, 6.6 Hz, 1H), 4.34 (t, *J* = 5.6 Hz, 1H), 4.20 – 4.07 (m, 3H), 4.00 (dt, *J* = 11.8, 7.9 Hz, 1H), 3.43 – 3.36 (m, 1H), 2.67 – 2.58 (m, 2H). ^{13}C NMR (101 MHz, DMSO–d_6) δ 161.6, 160.3, 155.4, 144.3, 131.0, 129.5, 128.4, 112.7, 112.4, 112.3, 101.1, 69.0, 67.5, 61.5 (br d, $J_{C–P}$ = 2.7 Hz), 56.2 (br d, $J_{C–P}$ = 2.7 Hz), 31.3. ^{31}P NMR (162

MHz, DMSO–d_6) δ 1.84. HRMS calcd. for $C_{16}H_{21}NO_8P$ ([M + H]$^+$): 386.1005, found: 386.0997.

***tert*–butyl ((2*S*,3*R*,*E*)–1,3–dihydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–5–en–2–yl) carbamate (15)**

Diol **15** (colorless oil, 427 mg, 88 %) was obtained from **14** (620 mg, 1.19 mmol) and TBAF (2.39 mL, 2.39 mmol), according to general procedure 4.

$[\alpha]^{20}_D$ = +4.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 6.87 – 6.78 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 5.99 – 5.88 (m, 1H), 5.83 (dt, *J* = 15.5, 5.5 Hz, 1H), 5.31 (br d, J = 10.4 Hz, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 4.01 (dd, *J* = 11.4, 3.4 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.77 (dd, *J* = 11.4, 3.4 Hz, 1H), 3.61 – 3.50 (m, 1H), 2.46 – 2.33 (m, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 161.5, 156.2, 155.8, 143.7, 131.7, 128.9, 127.4, 113.3, 113.1, 112.7, 101.8, 80.0, 72.9, 69.0, 62.5, 54.8, 37.5, 28.5. HRMS calcd. for $C_{21}H_{27}NO_7Na$ ([M + Na]$^+$): 428.1685, found: 428.1682.

***tert*–butyl ((2*S*,3*R*,*E*)–1–((dimethoxyphosphoryl)oxy)–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–5–en–2–yl)carbamate (2)**

Compound **2** (colorless oil, 232 mg, 78 %) was obtained from alcohol **15** (236 mg, 0.58 mmol), *N*–methylimidazole (70 µL, 0.87 mmol) and dimethyl chlorophosphate (75 µL, 0.70 mmol), according to general procedure 2. The title compound was purified by flash chromatography on silica gel (from 0 to100 % EtOAc in hexane).

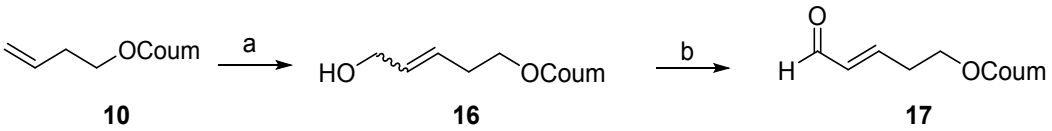
$[\alpha]^{20}_D$ = +14.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.87 – 6.78 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 6.02 – 5.91 (m, 1H), 5.82 (dt, *J* = 15.5, 5.8 Hz, 1H), 5.05 (br d, *J* = 8.1 Hz, 1H), 4.56 (d, *J* = 5.7 Hz, 2H), 4.50 – 4.41 (m, 1H), 4.15 – 4.05 (m, 1H), 3.81 (d, *J* = 3.4 Hz, 3H), 3.78 (d, *J* = 3.4 Hz, 3H), 3.71 (br s, 2H), 3.34 (br s, 1H), 2.53 – 2.40 (m, 1H), 2.31 (dt, *J* = 14.1, 6.9 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 161.3, 155.9, 155.6, 143.6, 131.9, 128.9, 127.3, 113.2, 113.2, 112.7, 101.8, 80.1, 70.1, 69.1, 67.0 (d, $J_{C–P}$ = 5.7 Hz), 54.8 (d, $J_{C–P}$ = 6.0 Hz), 54.8 (d, $J_{C–P}$ = 6.0 Hz), 54.7 (br d, $J_{C–P}$ = 5.4 Hz), 36.8, 28.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 2.96. HRMS calcd. for $C_{23}H_{32}NO_{10}NaP$ ([M + Na]$^+$): 536.1662, found: 536.1660.

(2*S*,3*R*,*E*)–2–ammonio–3–hydroxy–7–((2–oxo–2*H*–chromen–7–yl)oxy)hept–5–en–1–yl hydrogen phosphate (RBM148)

Compound **RBM148** (white solid, 45 mg, 82 %) was obtained from dimethyl phosphate **2** (73 mg, 0.14 mmol) and TMSBr (94 µL, 0.71 mmol), according to general procedure 3. The title compound was purified as described above for **RBM77**.

$[\alpha]^{20}_{D}$ = +4.2 (*c* 1.0, DMSO). ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (d, *J* = 9.5 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 6.98 – 6.90 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 6.01 – 5.84 (m, 2H), 4.65 (d, *J* = 4.9 Hz, 2H), 4.18 (ddd, *J* = 10.8, 6.9, 3.7 Hz, 1H), 4.08 (dt, *J* = 11.5, 7.9 Hz, 1H), 3.88 (dt, *J* = 9.4, 4.8 Hz, 1H), 3.39 (dt, *J* = 8.2, 3.9 Hz, 1H), 2.45 – 2.29 (m, 2H). ^{13}C NMR (101 MHz, DMSO–d_6) δ 161.4, 160.3, 155.3, 144.3, 131.7, 129.5, 126.6, 112.8, 112.4, 112.3, 101.4, 68.8, 68.1, 61.4 (d, $J_{C–P}$ = 5.7 Hz), 55.8 (d, $J_{C–P}$ = 3.4 Hz), 35.6. ^{31}P NMR (162 MHz, DMSO–d_6) δ 2.08. HRMS calcd. for $C_{16}H_{21}NO_8P$ ($[M + H]^+$): 386.1005, found: 386.0994.

Synthesis of aldehyde 17



Scheme S4. Reagents and conditions: (a) allyl alcohol, Grubbs cat. 2nd gen., CH_2Cl_2, reflux (33 %, dr = 85:15 *E*/*Z*). (b) DMP, CH_2Cl_2, 0 ºC to rt (64 %).

(*E*)–7–((5–hydroxypent–3–en–1–yl)oxy)–2*H*–chromen–2–one (16)

Compound **16** (inseparable 85:15 *E*/*Z* mixture, brownish oil, 114 mg, 33 %) was obtained from **10**[4] (300 mg, 1.39 mmol), allyl alcohol (377 µL, 5.55 mmol) and Grubbs catalyst 2nd generation (35 mg, 42.0 µmol), according to general procedure 1. The title compound was obtained in 33% yield after flash chromatography (from 0 to 1.5 % MeOH in CH_2Cl_2).

^{1}H NMR (400 MHz, $CDCl_3$, major isomer) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 6.86 – 6.76 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.87 – 5.74 (m, 2H), 4.15 (br s, 2H), 4.06 (t, *J* = 6.6 Hz, 2H), 2.66 – 2.52 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, major isomer) δ 162.2, 161.4, 156.0, 143.6, 132.2, 128.9, 127.6, 113.2, 113.1, 112.7, 101.5, 68.0, 63.5, 32.0. HRMS calcd. for $C_{14}H_{15}O_4$ ($[M + H]^+$): 247.0970, found: 247.0961.

(*E*)–5–((2–oxo–2*H*–chromen–7–yl)oxy)pent–2–enal (17)

Dess–Martin periodinane (530 mg, 1.25 mmol) was added to a solution of allylic alcohol **16** (205 mg, 0.83 mmol) in CH_2Cl_2 (4 mL) at 0°C. After stirring for 2 h at rt, the reaction mixture was filtered through a plug of Celite and the filtrate was evaporated to dryness. The residue was purified by flash chromatography (from 0 to 50 % EtOAc in hexane) to afford aldehyde **17** (130 mg, 64 %) as a white solid. Late–eluting fractions were independently collected to give a 95:5 *E*:*Z* mixture of **17**, from which the following data were acquired.

^{1}H NMR (400 MHz, $CDCl_3$, major isomer) δ 9.57 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 9.5 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 6.93 (dt, *J* = 15.7, 6.7 Hz, 1H), 6.88 – 6.78 (m, 2H), 6.31 – 6.21 (m, 2H), 4.20 (t, *J* = 6.1 Hz, 2H), 2.91 – 2.83 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, major isomer) δ 193.7, 161.7, 161.2, 156.0, 153.0, 143.4, 134.9, 129.0, 113.6, 113.0, 101.5, 66.2, 32.3. HRMS calcd. for $C_{14}H_{13}O_4$ ($[M + H]^+$): 245.0814, found: 245.0810.

Fluorometric characterization of probes RBM77 and RBM148

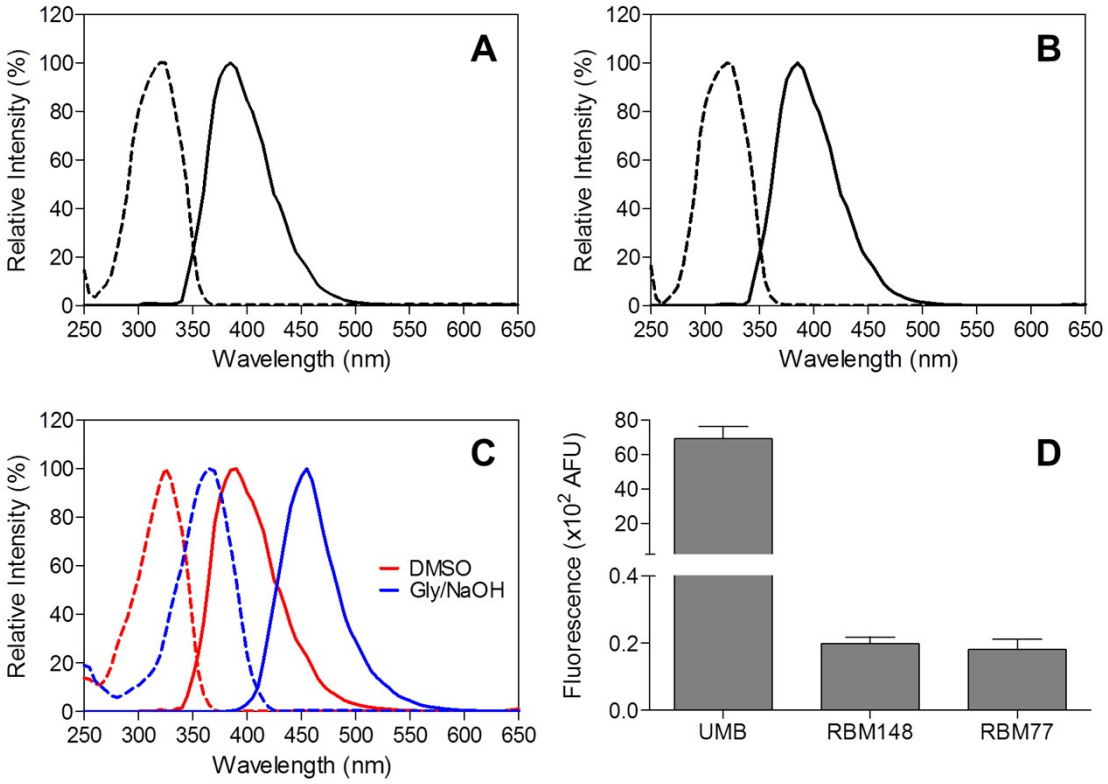


Figure S1**.** Excitation (dashed line) and emission (bold line) spectra of compounds **RBM148** (A) and **RBM77** (B) in DMSO. $\lambda_{ex}/\lambda_{em}$ were 325/385 nm for both compounds. (C) Excitation (dashed line) and emission (bold line) spectra of Umbelliferone in DMSO (red) or in Gly/NaOH buffer pH 10.6 (blue). $\lambda_{ex}/\lambda_{em}$ were 325/385 (DMSO) and 365/455 nm (Gly/NaOH buffer pH 10.6), respectively. (D) Fluorescence intensity ($\lambda_{ex}/\lambda_{em}$ = 365/455 nm) of 10 µM solutions of umbelliferone, **RBM148** or **RBM77** in Gly/NaOH buffer pH 10.6.

Table S1. Spectral characterization of the probes

	DMSO		Gly/NaOH buffer pH 10.6	
Compound	$\lambda_{ex}/\lambda_{em}$ (nm)	ε ($M^{-1}\cdot cm^{-1}$)	$\lambda_{ex}/\lambda_{em}$ (nm)	ε ($M^{-1}\cdot cm^{-1}$)
Umbelliferone	325/385	17996	365/455	25976
RBM148	325/385	13456	325/390	14122
RBM77	325/385	11154	325/390	11540

Release of umbelliferone from 17

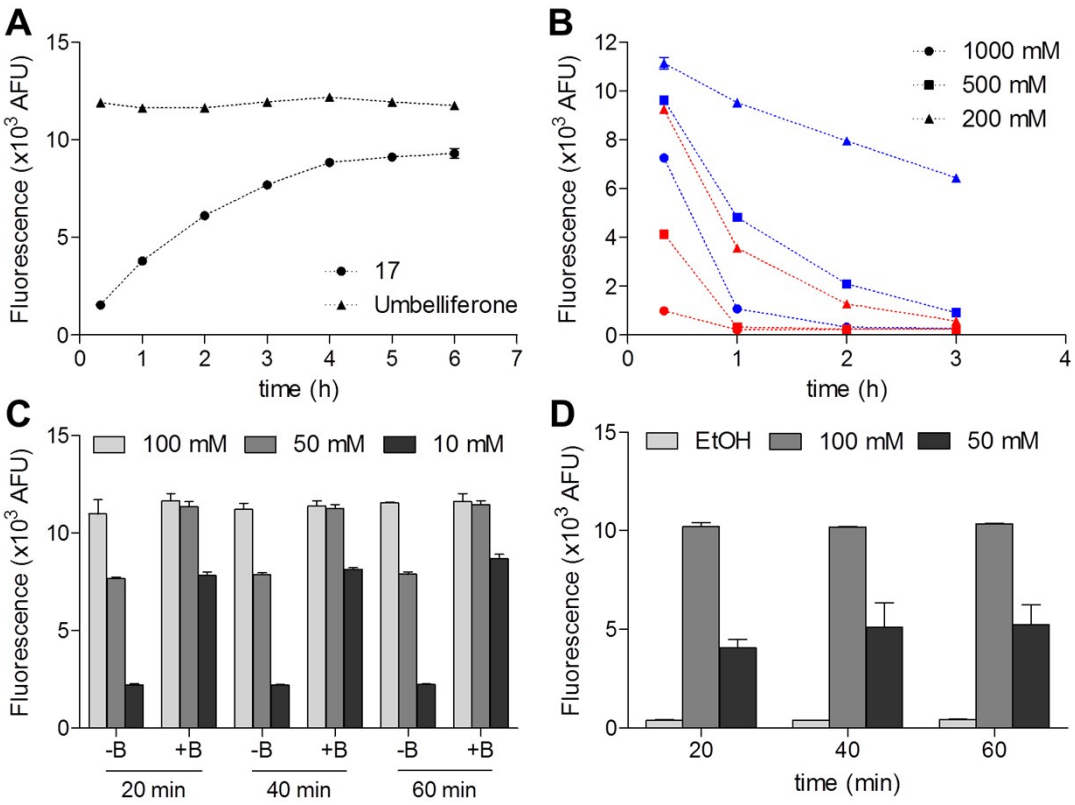
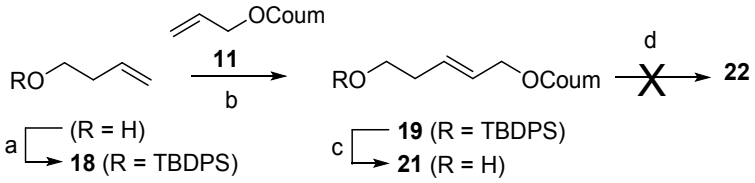


Figure S2**.** Optimization of the conditions for the base–mediated release of umbelliferone. A 5 µM solution (100 µL) of either **17** or umbelliferone in a 100 mM HEPES buffer pH 7.4, containing 0.1 mM EDTA, 0.05 % Triton X–100, 0.01 % Pluronic F127 (Biotium), and 100 µM pyridoxal 5′–phosphate were successively treated with the corresponding basic solution (at the indicated concentrations) and/or solvent and incubated at 37 ºC for the indicated times before fluorescence reading ($\lambda_{ex/em}$ = 355/460 nm). In all cases, umbelliferone and **17** were added from 100 µM stock solutions in DMSO (5 µL). (A) Umbelliferone and **17** were treated with 50 µL of MeOH and 100 µL of a 200 mM glycine/NaOH buffer solution (pH 10.6). (B) Umbelliferone was treated with 50 µL of MeOH and 100 µL of KOH in EtOH (blue) or aq. NaOH (red) at the indicated concentrations. (C) Umbelliferone was treated with 50 µL of MeOH and 100 µL of KOH in EtOH, at the indicated concentrations, and incubated at 37 ºC for the indicated times without further treatment (–B) or treated with 100 µL of KOH in EtOH, at the indicated concentrations, incubated at 37 ºC for the indicated times and further treated with 50 µL of a 200 mM glycine/NaOH buffer solution immediately before reading (+B). (D) Aldehyde **17** was treated with 100 µL of KOH in EtOH at the indicated concentrations,

incubated at 37 ºC for the indicated times and further treated with 50 µL of a 200 mM glycine/NaOH buffer solution immediately before reading. Data are means ± SD of one representative experiment with triplicates.

Attempts to synthesize aldehyde 22



Scheme S5. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, rt (quant. yield). (b) **11**, Grubbs cat. 2nd gen., CH_2Cl_2, reflux (58 %, dr = 91:9 *E*/*Z*). (c) TBAF, THF, 0 ºC to rt (96 %). (d) DMP, CH_2Cl_2, 0 ºC to rt.

(But–3–en–1–yloxy)(*tert*–butyl)diphenylsilane (18)

A solution of 3–buten–1–ol (500 mg, 6.93 mmol) in DMF (10 mL) was treated successively with imidazole (1.04 g, 15.3 mmol) and TBDPSCl (1.98 mL, 7.63 mmol). The resulting mixture was stirred overnight at rt, diluted with Et_2O (10 mL) and poured into 1 M aqueous HCl (10 mL). The mixture was extracted with Et_2O (3 x 10 mL) and the combined organic layers were washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Flash chromatography of the residue (from 0 to 1 % EtOAc in hexane) gave **18** (2.17 g, quant. yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.64 (m, 4H), 7.46 – 7.33 (m, 6H), 5.83 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H), 5.09 – 4.99 (m, 2H), 3.71 (t, *J* = 6.7 Hz, 2H), 2.32 (qt, *J* = 6.8, 1.3 Hz, 2H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 135.6, 134.1, 129.7, 127.7, 116.5, 63.7, 37.4, 27.0, 19.4. HRMS calcd. for $C_{20}H_{27}OSi$ ($[M + H]^+$): 311.1831, found: 311.1834.

(*E*)–7–((5–((*tert*–butyldiphenylsilyl)oxy)pent–2–en–1–yl)oxy)–2*H*–chromen–2–one (19)

Compound **19** (inseparable 91:9 *E*/*Z* mixture, colourless oil, 347 mg, 58 %) was obtained from **11** (250 mg, 1.24 mmol), **18** (1.54 g, 4.95 mmol and Grubbs catalyst 2nd generation (32 mg, 0.04 mmol), according to general procedure 1 (see main text). The title compound was purified by flash chromatography on silica gel (from 0 to 16 % EtOAc in hexane).

Late–eluting fractions were independently collected to give a 94:6 *E*:*Z* mixture of **19**, from which the following data were acquired.

^{1}H NMR (400 MHz, $CDCl_3$, major isomer) δ 7.69 – 7.60 (m, 4H), 7.46 – 7.32 (m, 6H), 6.86 – 6.76 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 5.93 – 5.82 (m, 1H), 5.73 (dt, *J* = 15.5, 5.9 Hz, 1H), 4.51 (d, *J* = 5.8 Hz, 2H), 3.74 (t, *J* = 6.5 Hz, 2H), 2.42 – 2.31 (m, 2H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, major isomer) δ 162.0, 161.4, 156.0, 143.5, 135.7, 134.0, 133.1, 129.8, 128.9, 127.8, 125.8, 113.2, 112.7, 101.8, 69.3, 63.3, 35.8, 27.0, 19.4. HRMS calcd. for $C_{30}H_{32}O_4NaSi$ ($[M + Na]^+$): 507.1968, found: 507.1972.

(*E*)–7–((5–hydroxypent–2–en–1–yl)oxy)–2*H*–chromen–2–one (21)

To an ice cooled solution of **19** (175 mg, 0.36 mmol) in THF (3.5 mL) was added dropwise TBAF (1 M in THF, 722 µL, 0.72 mmol). After completion of addition the ice bath was removed and the mixture was stirred for 2 h at rt. The reaction was then quenched with saturated aqueous NH_4Cl (5 mL) and the resulting mixture was extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to give the crude product. Purification by flash chromatography on silica gel (from 0 to 70 % EtOAc in hexane) afforded homoallylic alcohol **21** (85 mg, 96 %) as a colourless oil. Late–eluting fractions were independently collected to give a 98:2 *E*:*Z* mixture of **21**, from which the following data were acquired.

^{1}H NMR (400 MHz, $CDCl_3$, major isomer) δ 7.63 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.88 – 6.78 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.95 – 5.86 (m, 1H), 5.81 (dt, *J* = 15.5, 5.6 Hz, 1H), 4.55 (dd, *J* = 5.5, 0.7 Hz, 2H), 3.72 (t, *J* = 6.3 Hz, 2H), 2.46 – 2.35 (m, 2H), 1.61 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$, major isomer) δ 161.9, 161.4, 155.8, 143.6, 132.4, 128.9, 126.5, 113.2, 113.0, 112.6, 101.7, 69.1, 61.7, 35.7. HRMS calcd. for $C_{14}H_{14}O_4Na$ ($[M + Na]^+$): 269.0790, found: 269.0785.

S1PL activity with recombinant hS1PL

a) Fluorogenic assay with RBM77 as substrate

hS1PL (50 µL from stock solutions in a 100 mM HEPES buffer pH 7.4, containing 0.1 mM EDTA, 0.05 % Triton X–100, 0.01 % Pluronic F127 (Biotium), and 100 µM pyridoxal 5′–phosphate., final concentration: 0.8 µg/mL) was added to a mixture of

RBM77 (final concentration: 125 μM) and putative inhibitors (at the indicated concentrations) in the same buffer solution (final volume: 100 μL). The mixture was incubated at 37 ºC for 1 h and the enzymatic reaction was stopped by the addition of 100 μL of KOH/EtOH (100 mM). After incubation at 37 ºC for 20 min, the resulting mixture was treated with 50 μL of a 200 mM glycine–NaOH buffer, pH 10.6, and the amount of umbelliferone formed was determined on either a SpectraMax M5 (Molecular Devices) or Synergy 2 (BioTek) microplate readers ($\lambda_{ex/em}$ = 355/460 nm), using a calibration curve. In all cases, the fluorescence of a blank solution, without enzyme, was subtracted from the total fluorescence.

b) Fluorogenic assay with RBM148 as substrate

hS1PL (50 μL from stock solutions in a 100 mM HEPES buffer pH 7.4, containing 0.1 mM EDTA, 0.05 % Triton X–100, 0.01 % Pluronic F127 (Biotium), and 100 μM pyridoxal 5′–phosphate., final concentration: 0.8 μg/mL) was added to a mixture of **RBM148** (final concentration: 125 μM) and putative inhibitors (at the indicated concentrations) in the same buffer solution (final volume: 100 μL). The mixture was incubated at 37 ºC for 1 h and the enzymatic reaction was stopped by the addition of 50 μL of MeOH. Finally, 100 μL of a 200 mM glycine–NaOH buffer, pH 10.6, were added to the resulting solution and the mixture was incubated for 20 additional min at 37 ºC in order to complete the β–elimination reaction. The amount of umbelliferone formed was determined as described for **RBM77** (see above).

Kinetic parameters of the enzyme reaction were obtained by fitting the data to the Michaelis–Menten equation in Prism 5 (GraphPad Software, La Jolla). IC_{50} values were determined by plotting percent activity versus log [I] and fitting the data to the log(inhibitor) vs. response equation in Prism 5 (GraphPad Software, La Jolla). In both cases, settings for curve adjustments were kept with their default values.

Validation of the probes as hS1PL substrates

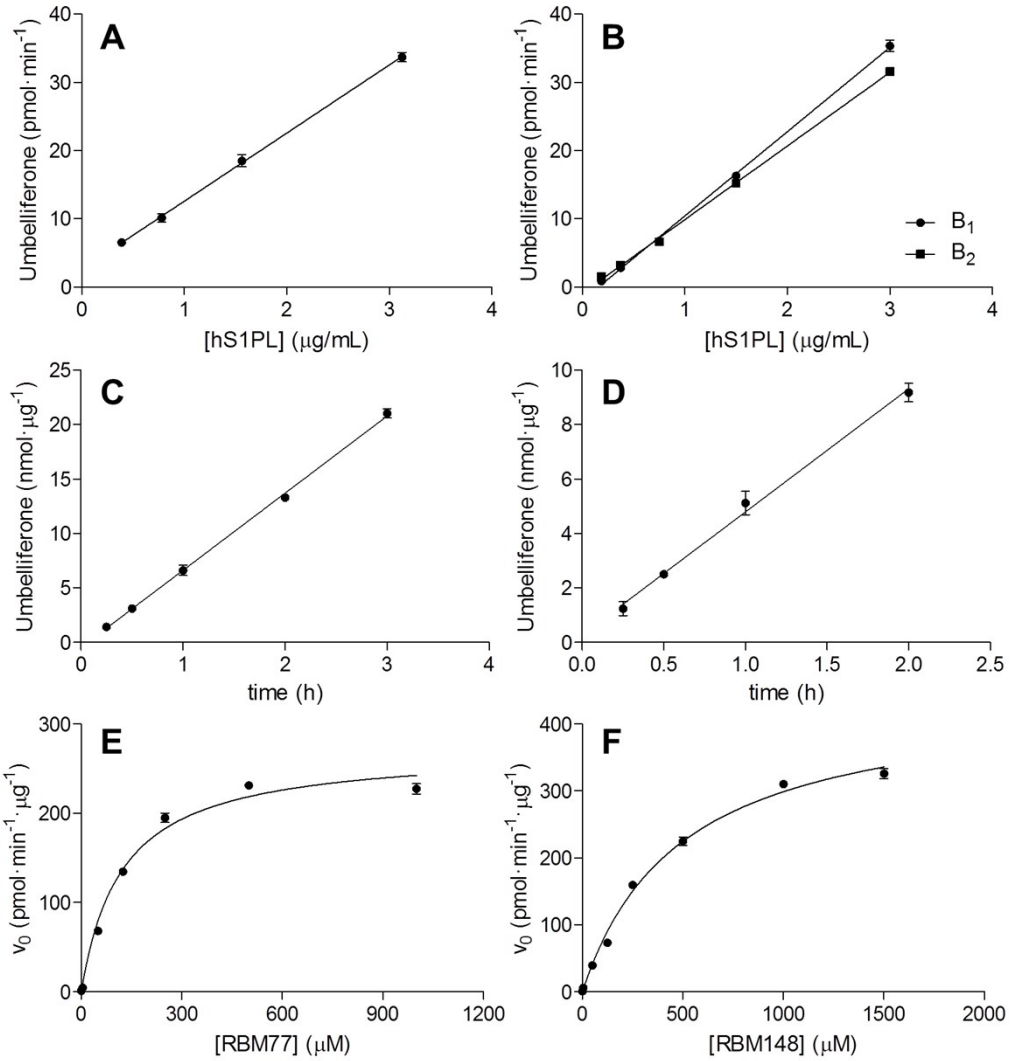


Figure S3. Enzyme concentration (A,B) and time (C,D) dependence of the hS1PL catalyzed reaction using **RBM77** (A,C) and **RBM148** (B,D) as substrates at a final concentration of 125 μM. A and B: Incubation time was 60 min. C and D: hS1PL concentration was 0.8 μg/mL. In B, the resulting enzymatic reaction was stopped with MeOH (50 μL) and treated with 100 μL of 200 mM glycine/NaOH buffer (pH 10.6) (B_1) or alternatively, treated with 100 mM KOH/EtOH (100 μL), incubated for 20 min and the pH was further adjusted with the addition of 50 μL of a 200 mM glycine/NaOH buffer solution (pH 10.6) immediately before reading (B_2). Data are means ± SD of one representative experiment with triplicates. In E and F, the substrate concentration dependence of the initial velocity (V_O, expressed as pmol of umbelliferone formed per minute and per μg of enzyme) of the hS1PL catalyzed reaction is represented. In both cases, hS1PL (0.8 μg/mL) was incubated for 60 min with **RBM77** (E) or **RBM148** (F) at

graded concentrations. Data correspond to the mean ± SD of one representative experiment out of three performed with triplicates.

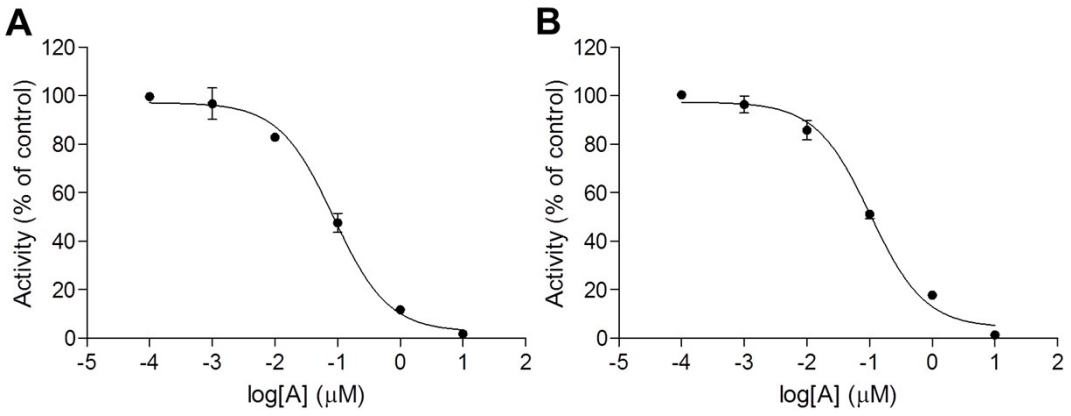


Figure S4. Activity (% of control) of hS1PL (final concentration: 0.8 μg/mL) at graded concentrations of **A**[5] using **RBM77** (A) or **RBM148** (B) as substrate (final concentration: 125 μM). Data correspond to the mean ± SD of one representative experiment out of three performed with triplicates

Computational Methods

General data

All the molecular modelling was carried out with the package Schrödinger Suite 2016,[6] through its graphical interface Maestro.[7] The program Macromodel[8] with the OPLS3 force-field,[9] a modified version of the OPLS-AA force-field,[10,11] and GB/SA water solvation conditions[12] were used for energy minimization. Molecular dynamics simulations were performed with the program Desmond [13–16] using the OPLS3 force-field.

Coordinates of hS1PL (PDB code 4Q6R)[5] were obtained from the Protein Data Bank[17] at Brookhaven National Laboratory. The protein X-ray structure was prepared using the Protein Preparation Wizard[18,19] included in Maestro to remove solvent molecules, ligands and ions, adding hydrogens, setting protonation states[20] and minimizing the energies. To model the aldimines, the imine bond between the essential Lys353 and the PLP cofactor was cleaved and structures **20a–c** were manually built on the resulting PLP moiety. The structures were then minimized considering the 3-hydroxypyridine and imino groups of the aldimine-bound PLP prosthetic group in their ionized state. From these, simulation systems for molecular dynamics were built using the System Builder of the Maestro-

Desmond interface,[21] which automatically assigns parameters to all atom. Each protein-aldimine complex was immersed in a 120 x 120 x 120 Å cubic box of TIP3P water with enough Cl^- anions to achieve neutrality (~52000 water molecules, ~170000 atoms in total). Systems were relaxed by minimization (initial steepest descent followed by LBFGS minimization), first with the solute restrained and then without restrains, until a gradient threshold of 0.1 kcal $mol^{-1}Å^{-1}$ was reached. Then, they were heated stepwise up to 300 K with short MD runs under periodic boundary conditions (PBC) (25 ps at 0.1, 10, 100 and 300 K), and equilibrated for 2 ns at the same temperature and 1.0 bar, in the NPT ensemble. Production MD simulations (50 ns, 2 fs timestep) were performed under the same conditions (PBC, NPT ensemble, 300 K and 1.0 bar) using the Nose-Hoover thermostat method[22,23] with a relaxation time of 1.0 ps and the Martyna-Tobias-Klein barostat method[24] with isotropic coupling and a relaxation time of 2 ps. Integration was carried out with the RESPA integrator[23] using time steps of 2.0, 2.0, and 6.0 fs for the bonded, short range and long range interactions, respectively. A cut-off of 9.0 Å was applied to van der Waals and short-range electrostatic interactions, while long-range electrostatic interactions were computed using the smooth particle mesh Ewald method with an Ewald tolerance of 10^{-9}.[25,26] Bond lengths to hydrogen atoms were constrained using the Shake algorithm.[27] Coordinates were saved every 50 ps, hence 1000 snapshots were obtained from each MD run. The Simulation Event Analysis application included in the Desmond-Maestro interface was used to analyze the trajectories.

Docking studies

Substrates **RBM13**, **RBM77** and **RBM148** were modelled in the form of external aldimines **20a–c** (Figure 1, paper) bound to the hS1PL (PDB 4Q6R) active site. In all cases, the reactive C3–OH group can establish hydrogen bond interactions with the phosphate group of the PLP cofactor and with the ε-NH of the imidazole side chain of H242. Comparison of the spatial arrangement of aldimines **20b** and **20c** revealed that the position of the double bond does not have a significant influence in the disposition of both the linker and the coumarin group.

Molecular dynamics

To further asses the plausibility of such structures, the modelled bound aldimines were used as starting point to run molecular dynamics simulations in explicit water (50 ns, 300

K, periodic boundary conditions, NPT ensemble; see above for complete details). Figure S6 illustrates the results obtained for aldimine **20c**. The simulations showed that the structures were mostly stable after an initial slight rearrangement of the whole protein, with an RMSD around 1.4 Å relative to the starting structure. Analysis of the hydrogen–bond interactions established by aldimine **20c** showed that the hydrogen bond between the C3–OH and the PLP–phosphate was kept for more than 95 % of the simulation time whilst that between the C3–OH and H242 was established only intermittently (Figure S6B). Similar results were obtained with the complexes from aldimines **20b** and **20a** (Figures S5 and S7, respectively), although in those cases an additional intermittent hydrogen bond interaction with T387 was also observed. Thus, these simulations would suggest that the PLP-phosphate group could be the base responsible for the C3–OH deprotonation that is previous to the C–C bond cleavage in the enzymatic cycle. The only other candidate base that apparently is close enough to the reactive C3–OH would be H242. However, this seems less likely, since the crystal structure of hS1PL shows that the δ–N of its imidazole ring accepts a hydrogen bond from the backbone amide of A244, thus forcing the ε–N-atom to be protonated and becoming unable to act as acceptor of another proton. Instead, as observed in our simulations, the ε–NH of the imidazole is suitable to act as a hydrogen–bond donor if an acceptor group is placed appropriately, such as the C3–OH present in aldimines **20a-c**. Therefore, it seems reasonable to propose that the hydrogen–bond interaction between H242 and the C3–OH of the substrate, as well as that formed with Y387, could serve to increase the acidity of this reactive OH, thus promoting the cleavage of the C–C bond and the concerted release of the corresponding aldehyde, both essential steps of the catalytic cycle of S1PL.

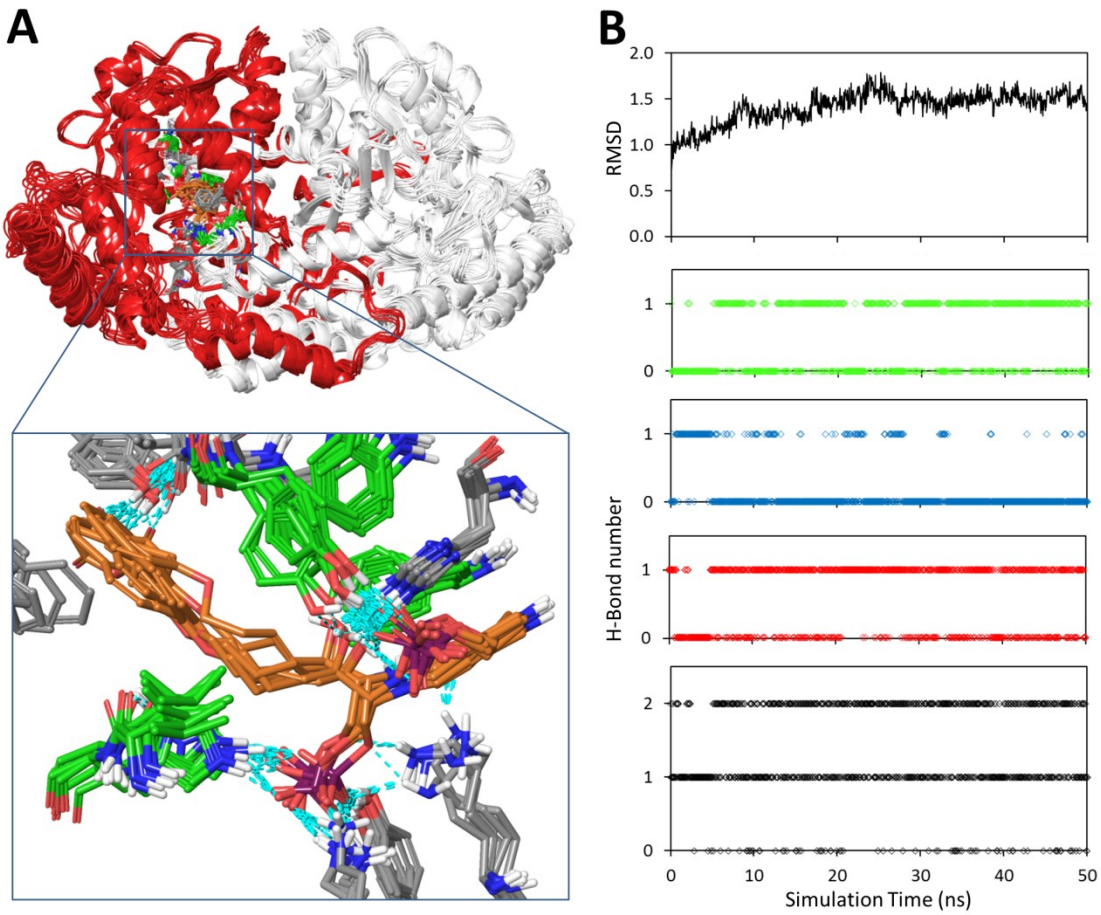


Figure S5: (A) Snapshots from the 50 ns MD simulation of the complex between aldimine **20b** (orange) and hS1PL (gray, residues forming a hydrophobic patch close to the PLP-binding site are highlighted in green). (B) RMSD plot and dependence of the number of hydrogen bond interactions established between the C3-OH of aldimine **20b** and the rest of the protein (black), the PLP phosphate group (red), His242 (blue) or Tyr387 (green), with simulation time.

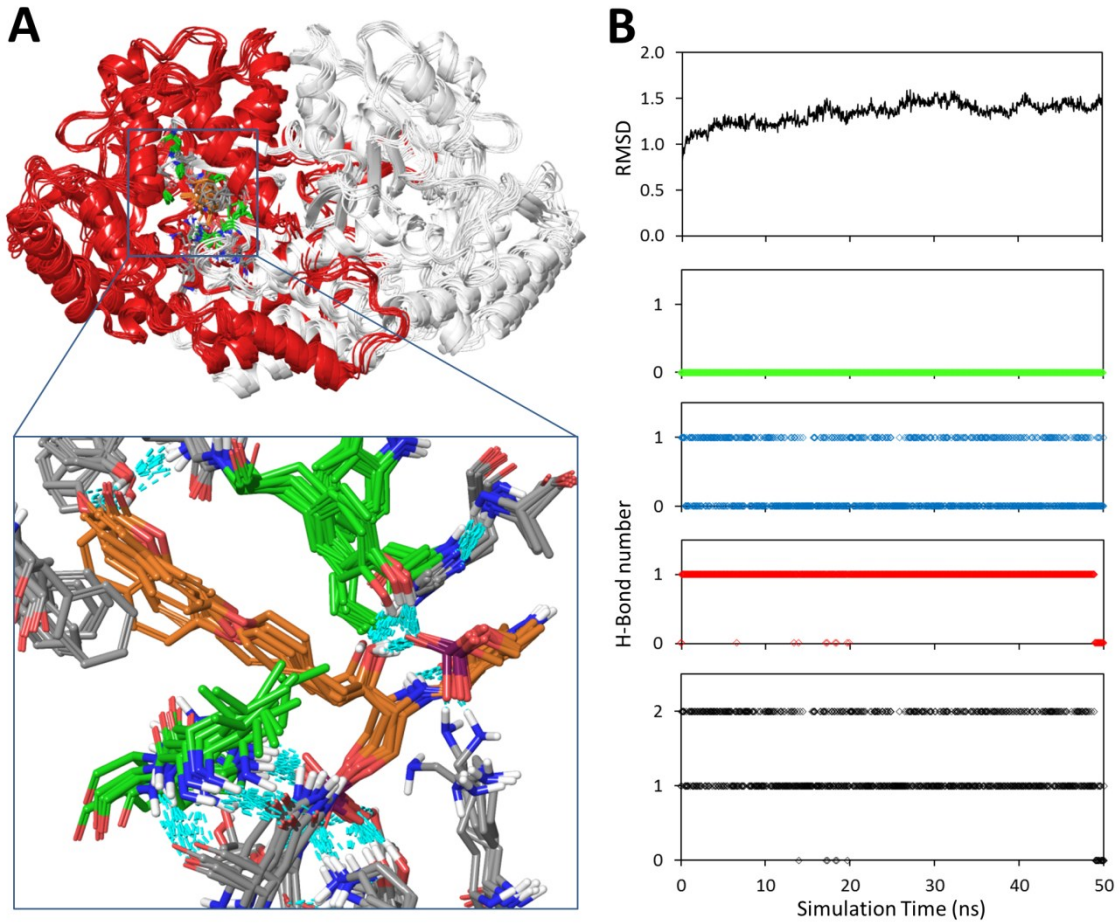


Figure S6. (A) Snapshots from the 50 ns MD simulation of the complex between aldimine **20c** (orange) and hS1PL (gray, residues forming a hydrophobic patch close to the PLP-binding site are highlighted in green). (B) RMSD plot and time dependence of the number of hydrogen bond interactions between the C3-OH of aldimine **20c** and the rest of the protein (black), the PLP phosphate group (red), H242 (blue) or Y387 (green)..

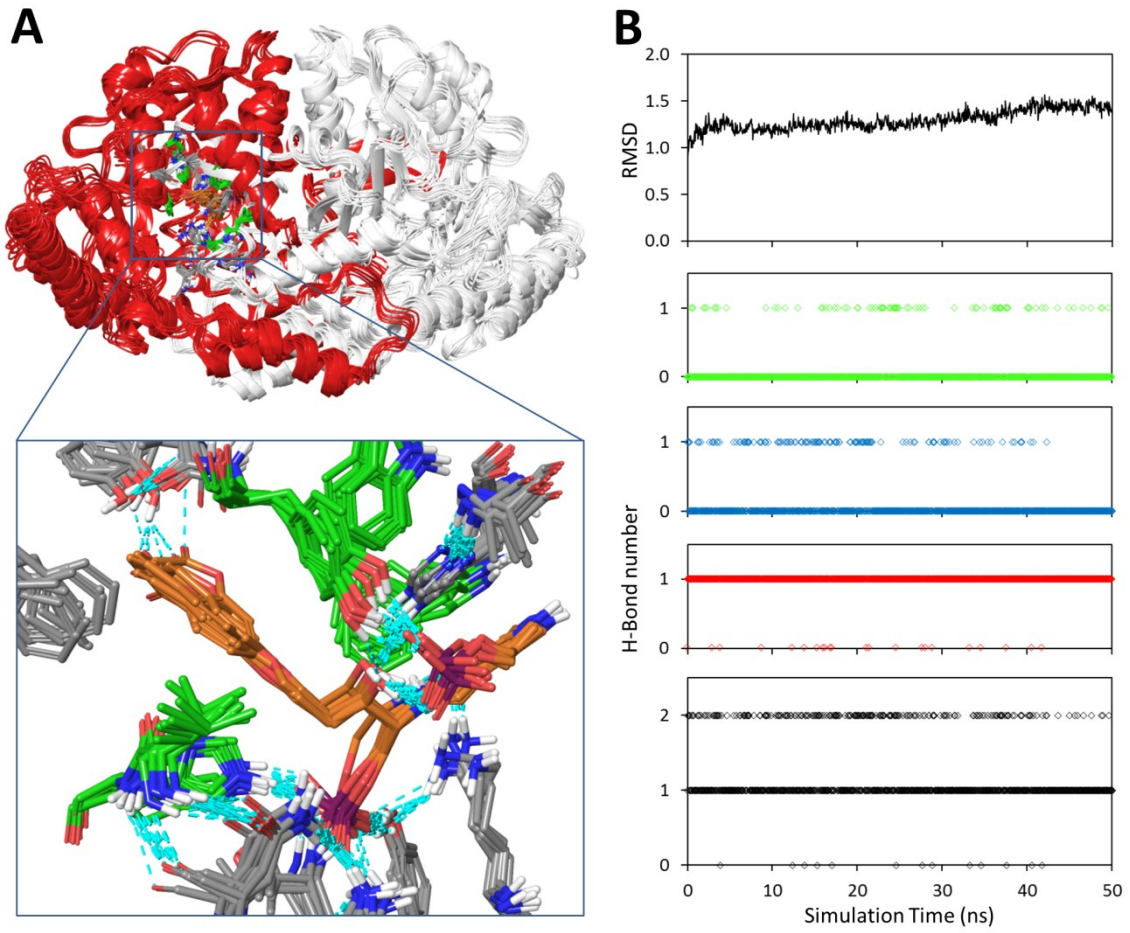


Figure S7: (A) Snapshots from the 50 ns MD simulation of the complex between aldimine **20a** (orange) and hS1PL (gray, residues forming a hydrophobic patch close to the PLP-binding site are highlighted in green). (B) RMSD plot and dependence of the number of hydrogen bond interactions established between the C3-OH of aldimine **20a** and the rest of the protein (black), the PLP phosphate group (red), His242 (blue) or Tyr387 (green), with simulation time.

Cell assays

Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin/streptomycin solution, Hank's balanced salt solution were from Sigma. Opti-MEM and Lipofectamine 2000 were from Invitrogen. *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methyl sulfate (DOTAP) and Lipoid S-100, whose main component (>94%) is soybean phosphatidylcholine, were from Lipoid GmbH. pCMV6 vector harboring the mouse *Sgpl1* gene was from OriGene.

Human embryonic kidney cells HEK293T and mouse embryo fibroblasts MEF were cultured at 37ºC in 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 ng/mL each of penicillin and streptomycin.

Overexpression of *Sgpl1*

HEK29T cells, plated in 12-well plates ($3x10^5$ cells per well) for 24 h, were transfected with 1 μg/well of pCMV6 vector harbouring the mouse *Sgpl1* gene using opti-MEM/Lipofectamine 2000 (L2000), following the manufacturer's instructions. Control cells were treated only with the transfection reagent L2000. Test compounds were added 48 h after transfection.

Cell lysates

Cell pellets were resuspended in the appropriate volume of a 25 μM Na_3VO_4 solution in 0.2 M phosphate buffer (pH 7.4). The suspension was submitted to three cycles of a 5 s sonication (probe) at 10 watts/5 s resting on ice. The cell lysate was centrifuged at 600 g for 5 min. The supernatant was collected and protein concentration was determined as specified below.

Determination of protein concentration

Protein concentrations were determined with BSA as a standard using a BCA protein determination kit (Thermo Scientific) according to the manufacturer's instructions.

S1PL activity in cell lysates

S1PL activity determination in cell lysates was done in 96-well plates at a final volume of 100 μL/well. The reaction mixture contained per well 100 μg of cell lysate, 125 μM of substrate (5 μL/well from 2.5 mM stock solution), 250 μM of pyridoxal 5'-phosphate (5 μL/well from 5 mM stock solution) and up to 100 μL of phosphate buffer. The reaction

mixture was incubated at 37ºC for 1 h. The enzymatic reaction was stopped with 50 µL/well of methanol and then 100 µL of 200 mM glycine-NaOH buffer (pH 10.6) were added. After incubation at 37ºC for 20 min in the dark, fluorescence was measured spectrophotometrically at excitation and emission wavelengths of 355 and 460 nm, respectively. The same reaction mixture without cell lysate was used as blank. Using **RBM77** as substrate, the reaction was stopped with 100 µL/well of 100 mM KOH-MeOH solution and the plate was incubated at 37ºC for 30 min in the dark. Then 50 µL of 200 mM glycine-NaOH buffer (pH 10.6) were added and the fluorescence was measured at the same wavelengths.

S1PL activity in intact cells

Cells (2-3x10^5 per well) were seeded in a 12-well plate. MEF cells were incubated for 24 h at 37ºC and 5% CO_2. HEK293T cells were transfected with the *Sgpl1* encoded plasmid as mentioned above. Medium was replaced by 300 µL of HBSS to which the required volume of a 3.5 mM stock solution of the probes liposomes had been added to obtain the desired probe concentration. The plate was incubated for 3 h at 37ºC and 5% CO_2. The reaction was stopped with 250 µL/well of methanol and then 500 µL of 200 mM glycine-NaOH buffer (pH 10.6) were added. After incubation at 37ºC for 30 min in the dark, fluorescence was measured spectrophotometrically at excitation and emission wavelengths of 355 and 460 nm, respectively. The same reaction mixture without cells was used as blank.

Liposome preparations

Cationic liposomes were formulated as a 1:1 mixture of phosphatydilcholine and DOTAP at 3.5 mM total lipid concentration. The lipids were dissolved and mixed in chloroform. Once the lipids were mixed in the organic solvent, the solvent was removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film was thoroughly dried to remove residual organic solvent by placing the flask on a vacuum pump overnight. The film was hydrated with 1 or 2 mM HBSS solutions of the probes. Three dilutions of liposomes were made in each case to give final total lipid concentrations of 350 µM, 700 µM and 1000 µM, containing probe concentrations of 100 µM, 200 µM and 290 µM, respectively (from the initial 1 mM probe solution), and 200 µM, 400 µM and 580 µM, respectively (from the initial 2 mM probe solution).

The lipid suspension was then placed on a rotatory evaporation system without vacuum and kept turning in warm water with gentle agitation. The product of hydration is a

dispersion of large vesicles but with cycles of 5 min sonication smaller liposomes were obtained.

Measurement of liposomes size distribution

The average size and size distribution of liposomes were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments). The measurements by DLS were performed at room temperature (see Figure S8).

A B

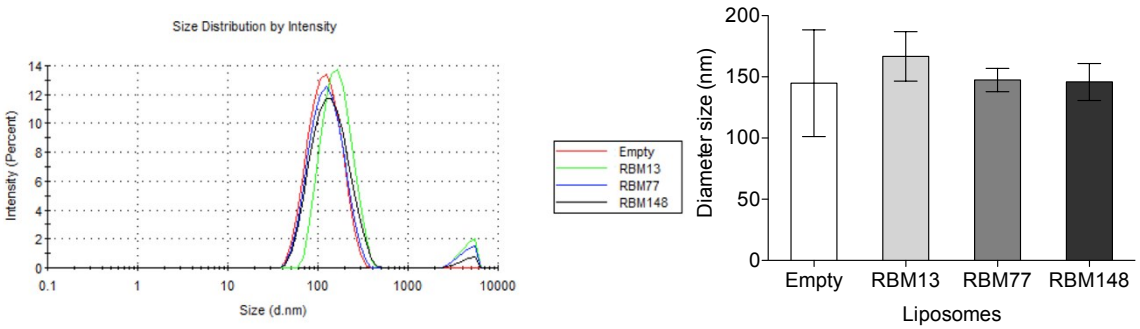


Figure S8. Particle size distribution curves of liposome suspension analysed by intensity. **A**. Size distribution curves of different liposome formulation (empty and encapsulating **RBM13**, **RBM77** and **RBM148** probes) were measured using a Zetasizer Nano ZS instrument. Curves correspond to one representative measurement. **B**. Average diameters of liposomes. Data are the mean ± SD of three experiments with triplicates. There is no statistical difference in diameters between liposome preparations: empty (145±40 nm), **RBM13** (167±20 nm), **RBM77** (147±9 nm) and **RBM148** (146±15 nm).

Determination of encapsulation efficiency

The encapsulation efficiency (EE) of liposomes was determined referring to the following equation EE (%) = (T-F) x 100/T, where T is the total probe and F is the free probe amount. Separation of liposome-incorporated and free probe was accomplished by centrifugal ultrafiltration. Liposomal suspension containing encapsulated probes as well as free probes were loaded into Amicon 3K (Millipore) centrifuge ultrafiltration units and centrifuged at 21ºC for 30 min at 4500 g. An aliquot from total preparation before centrifugation and from the filtrate (after centrifugation) were collected and analyzed by UPLC-MS and the amount of RBM148 and its dephosphorylated derivative was quantified (see Figure S9).

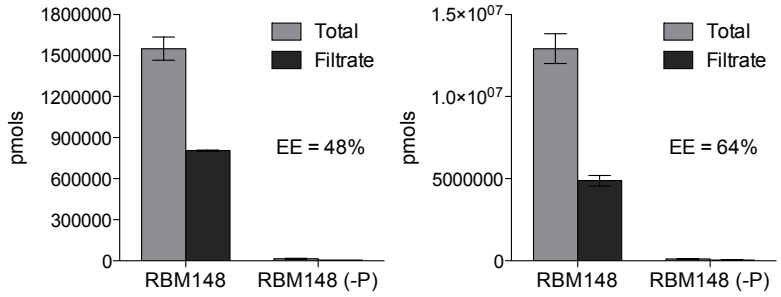


Figure S9**.** Entrapment efficiency analysis of **RBM148** encapsulated liposomes. Encapsulation efficiency of two different **RBM148** liposomes composition was calculated. 3.5 mM (**A**) or 7 mM (**B**) of lipid both containing 2 mM of **RBM148**. Each preparation was analyzed by UPLC-MS. Data are the mean ± SD of three experiments with duplicates.

Effect of liposomes and encapsulated RBM148 on cell viability

a) Empty liposomes: Cell viability was determined by cell counting. MEF SGPL1$^{+/+}$cells were seeded in 12--well plates at a density of 2x10^5 cells per well and HEK293T cells were used after 48 h of *Sgpl1* transfection. Cells were exposed to different empty liposome dilutions for 3h. At the end of the treatment, cells were washed with 200 µL PBS and harvested with 200 µL Trypsin-EDTA and 300 µL of medium. Cells were counted using a Neubauer chamber (see Figure S10)

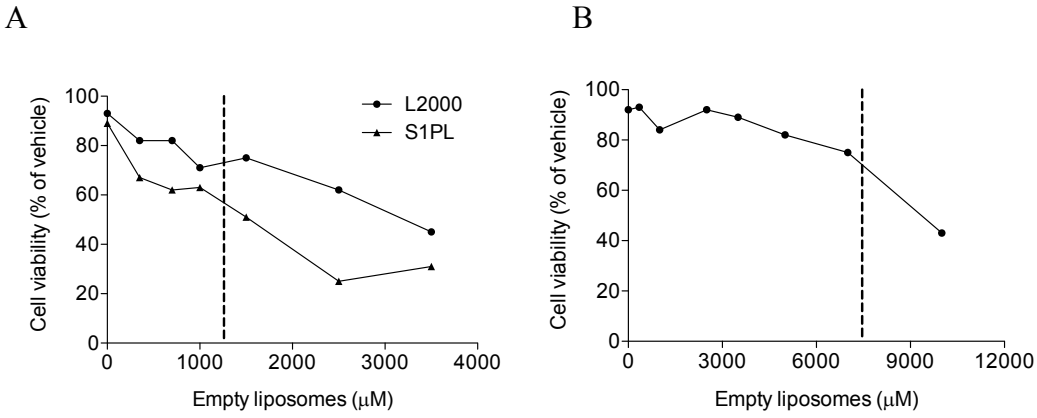


Figure S10. Effect of liposomes on cell viability. HEK293T cells overexpressing S1PL (**A**) and MEF SGPL1$^{+/+}$ cells (**B**) were incubated with empty liposomes for 3 h and cell viability was determined by cell counting. Dotted line showed the maximum allowable

liposome concentration (based on toxicity assay) for each cell line; L2000: Lipofectamine 2000.

Due to the transfection agent (lipofectamine 2000), HEK293T cells were more sensitive to liposomes, reaching higher cytotoxicity levels. On the other hand, on MEF SGPL1$^{+/+}$ cells, cell viability was not affected up to a liposome concentration of 7 mM. This result was extrapolated to other MEF cells used in this work (SGPL1$^{+/-}$ and SGPL1$^{-/-}$).

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b) Encapsulated RBM148 and comparison of cell viability with empty liposomes. No significant effect on cell viability is observed between empty and loaded liposomes.

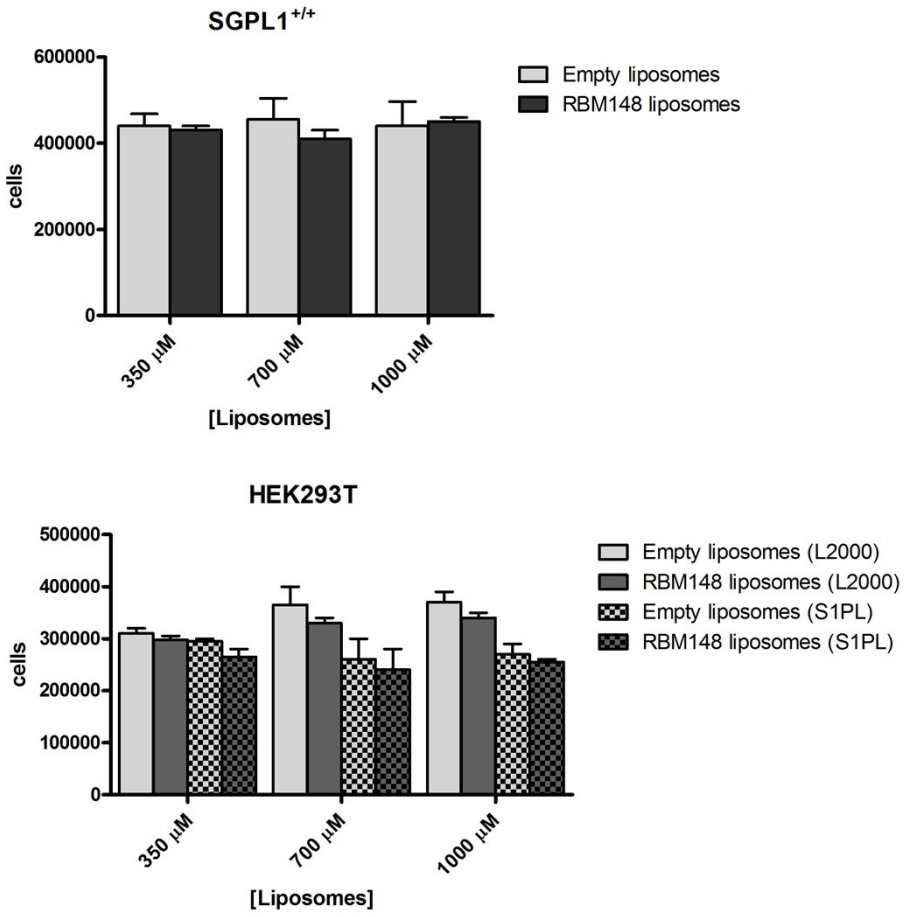

Figure S11. Effect of empty and RBM148 encapsulated liposomes on cell viability. HEK293T overexpressing S1PL and S1PL$^{+/+}$ MEF cells were incubated with 350 µM, 700 µM and 1000 µM of empty liposomes or liposomes containing RBM148 200 µM, 400 µM and 580 µM, respectively, for 3 h. Cell viability was determined by cell counting. Data are the average ± SD of two experiments.

S1PL activity in intact cells with encapsulated RBM77

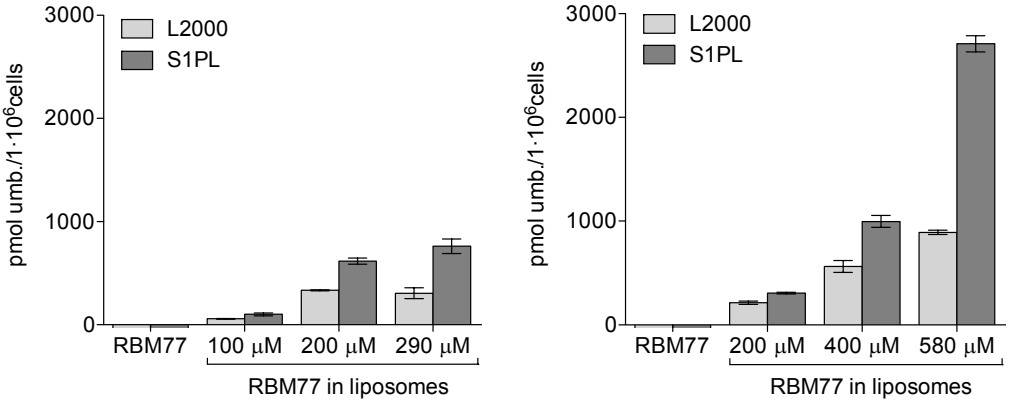

Figure S12. S1PL activity determination in intact cells using **RBM77** encapsulated liposomes. HEK293T overexpressing S1PL were incubated 48 h after transfection with different dilution of encapsulated liposomes obtained from 1 mM (**left**) or 2 mM (**right**) **RBM77** solution for 3 h. Non-encapsulated **RBM77** at 200 μM was also tested. Data are the average ± SD of three experiments with duplicates.

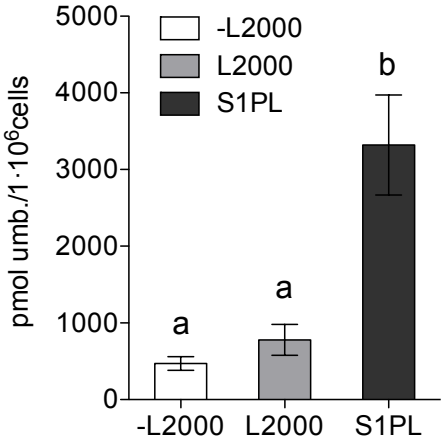

Figure S13. Amounts of umbelliferone released in non-transfected (L2000) and S1PL overexpressing (SPL) HEK293T cells incubated with 200 μM **RBM148** encapsulated in liposomes for 3 h. –L2000, cells used as a control without lipofectamine 2000®. Data are the average ± SD of two experiments with duplicates. Data were analyzed by one way ANOVA following by Bonferroni's multiple comparison post-test if ANOVA $P<0.05$. Different letters atop each denote statistical significance ($P<0.05$).

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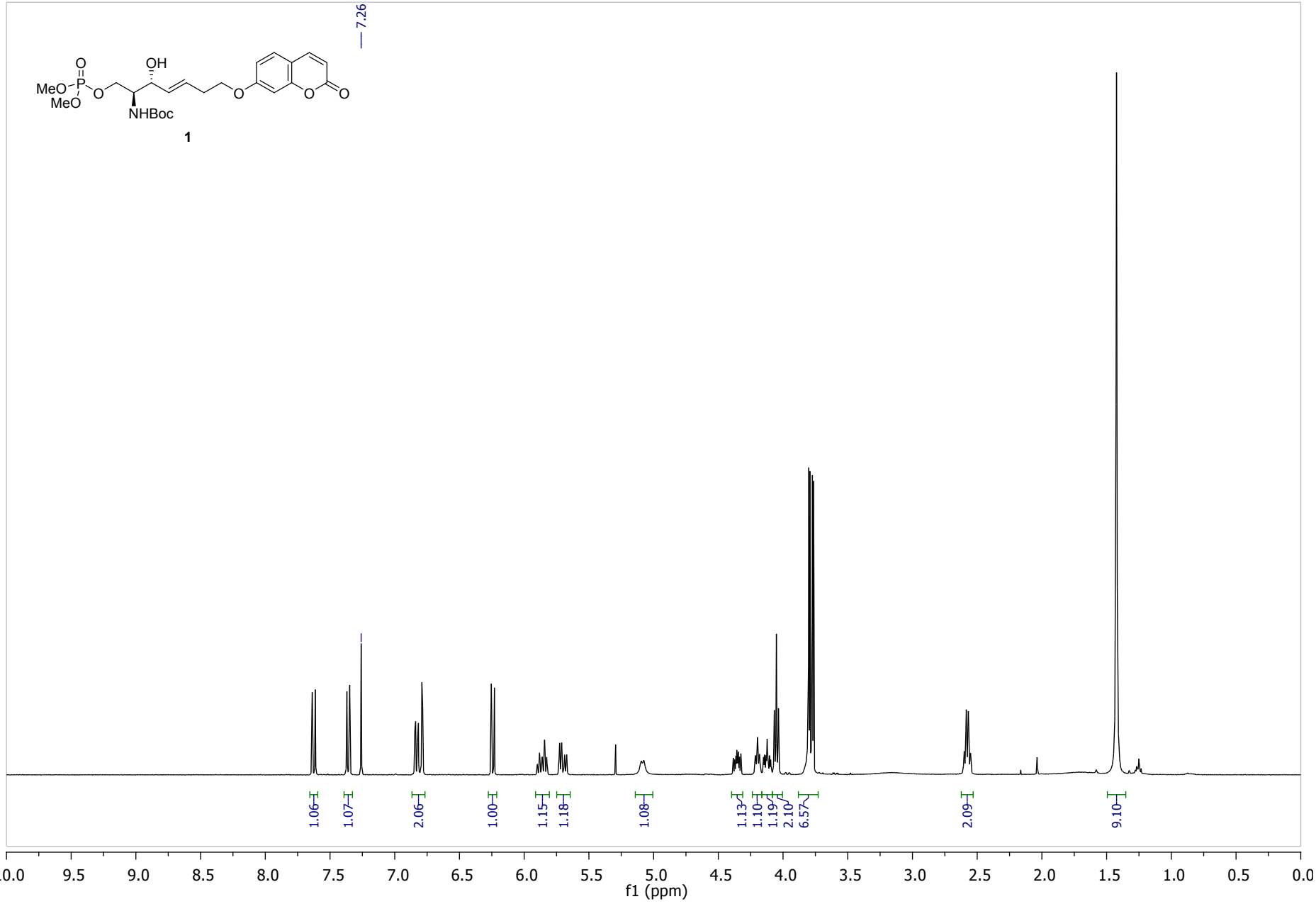
NMR SPECTRA

NMR spectra were recorded at room temperature on a Varian Mercury 400 instrument. The chemical shifts (δ) are reported in ppm relative to the solvent signal, and coupling constants (*J*) are reported in Hertz (Hz). ^{31}P chemical shifts are relative to a 85 % H_3PO_4 external reference (0 ppm). The following abbreviations are used to define the multiplicities in ^{1}H NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad signal and app = apparent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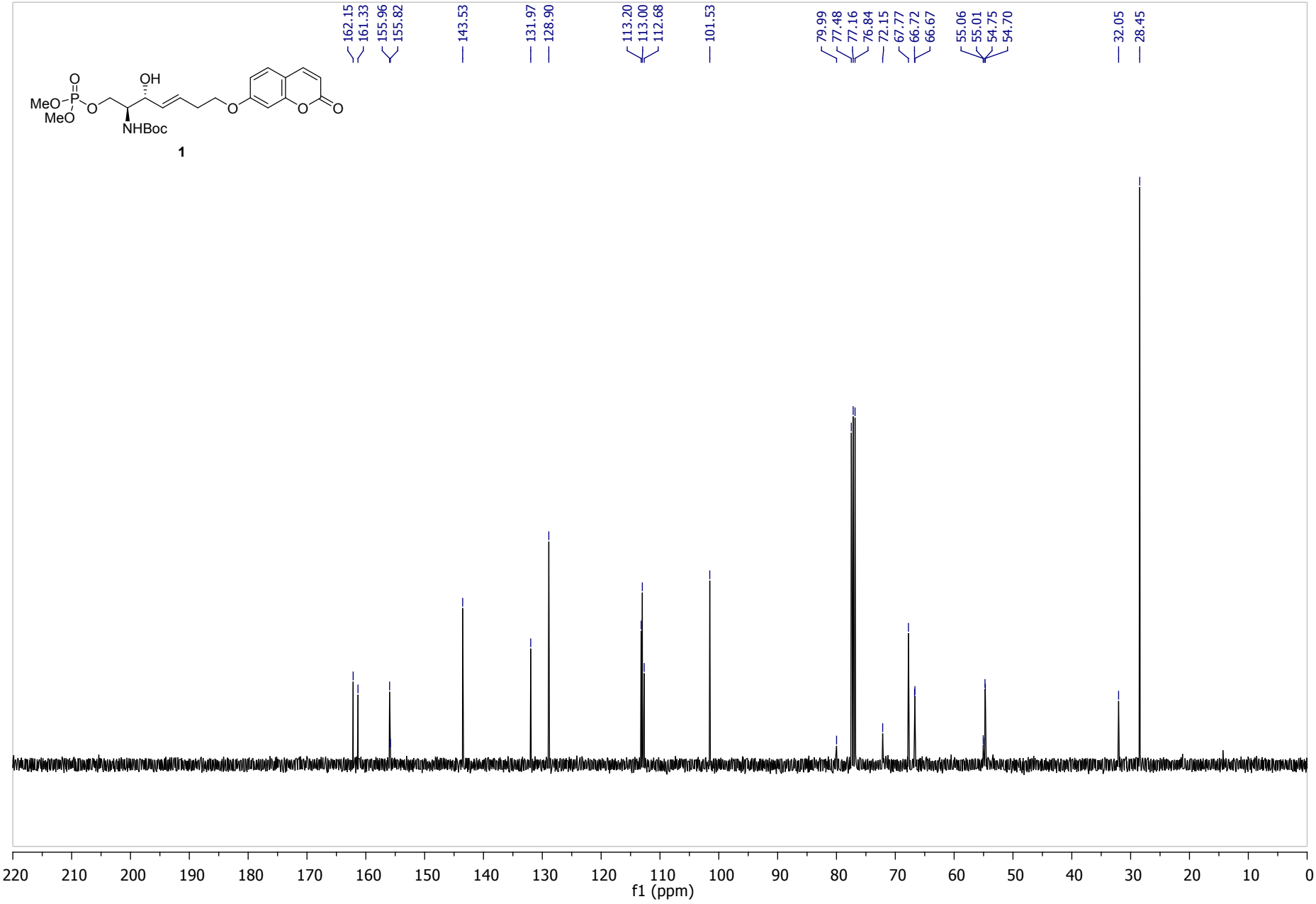
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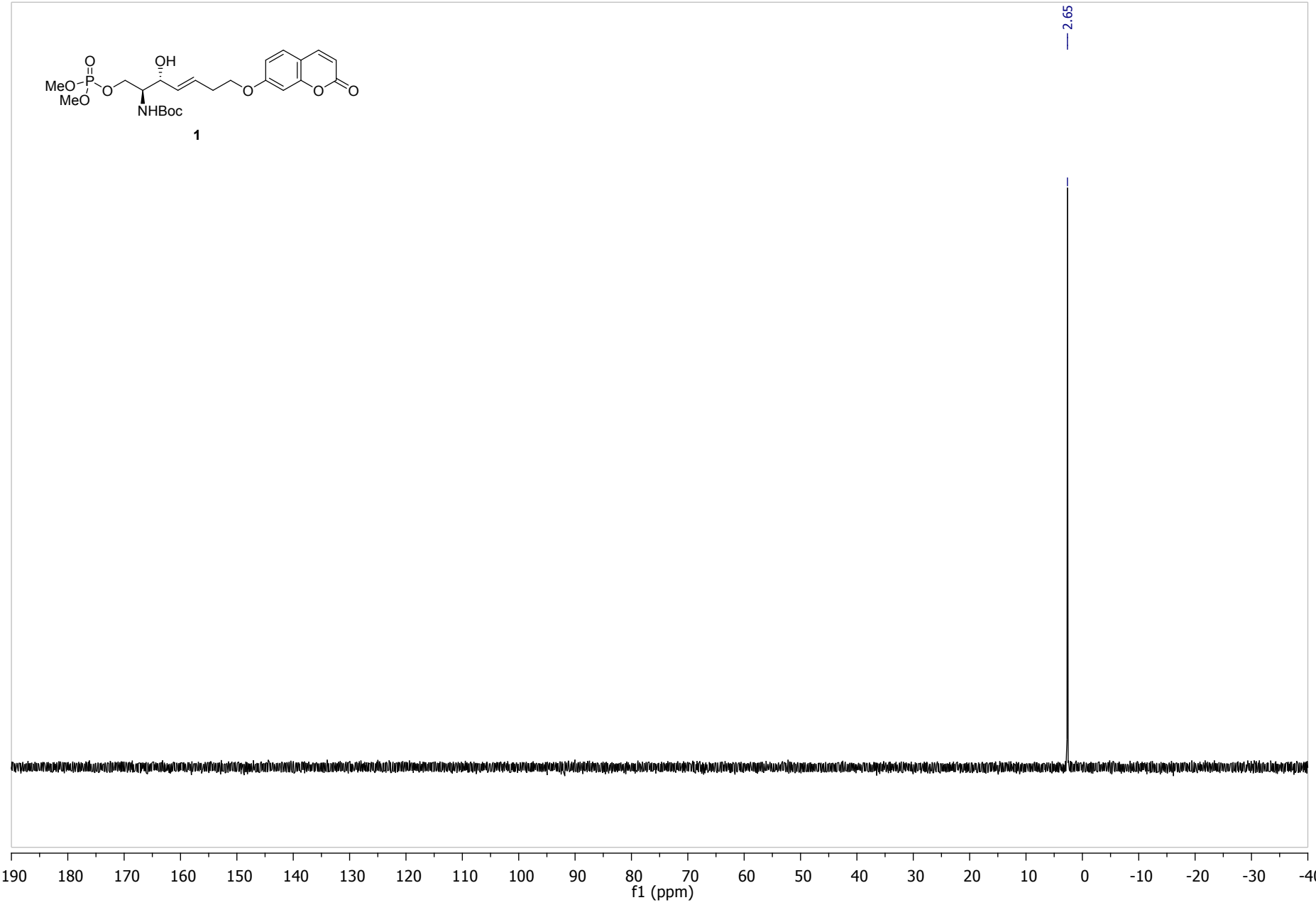
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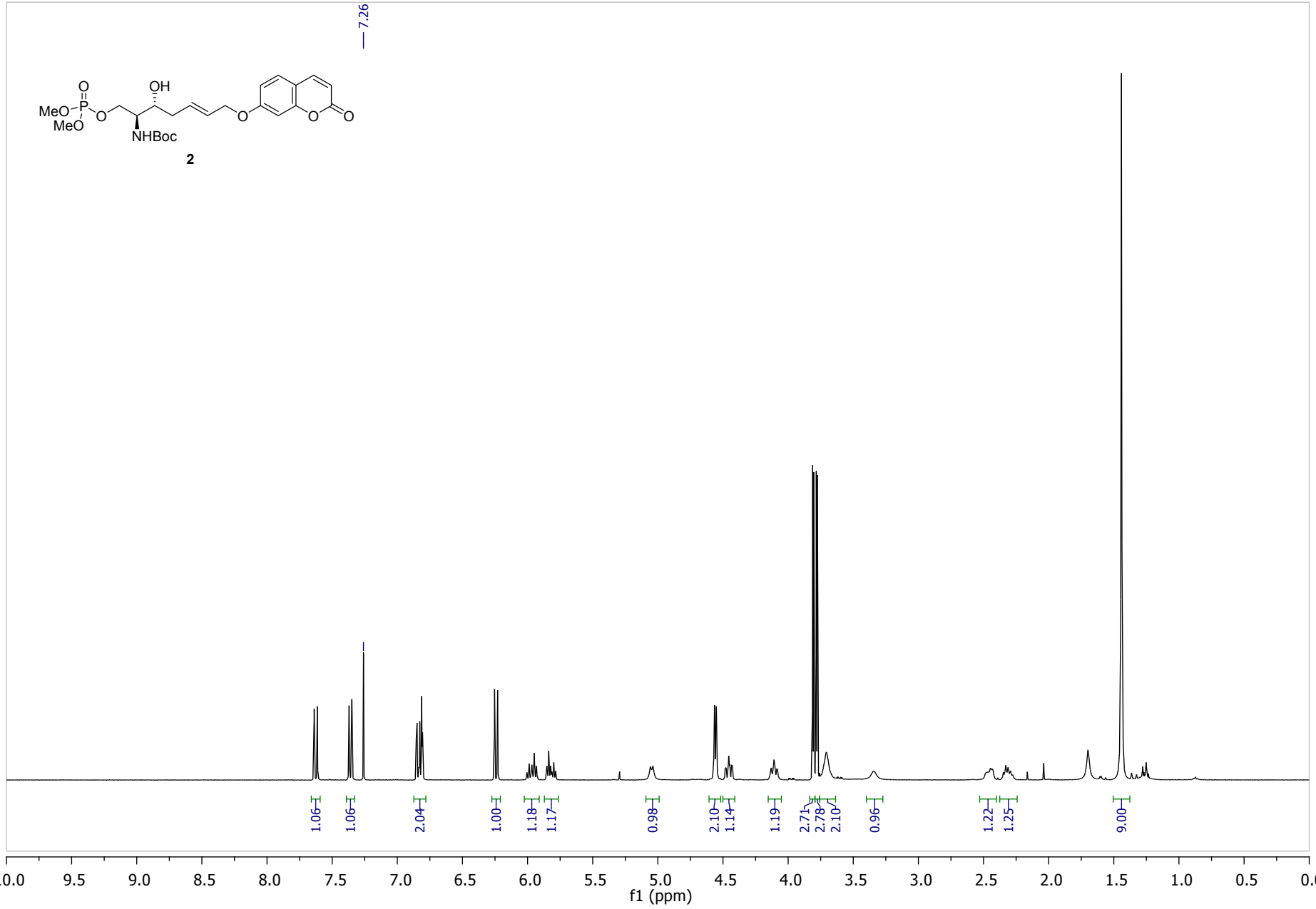
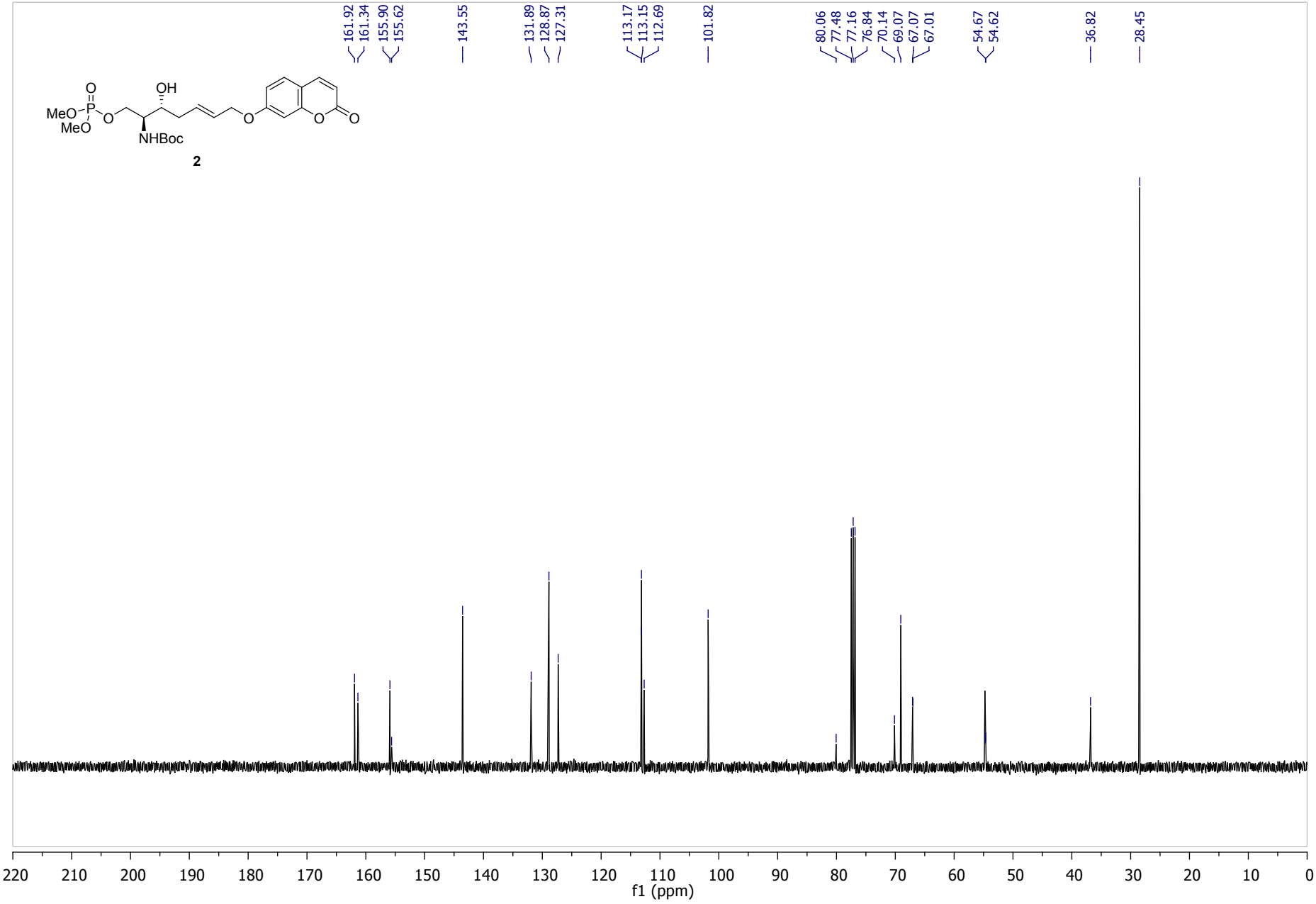
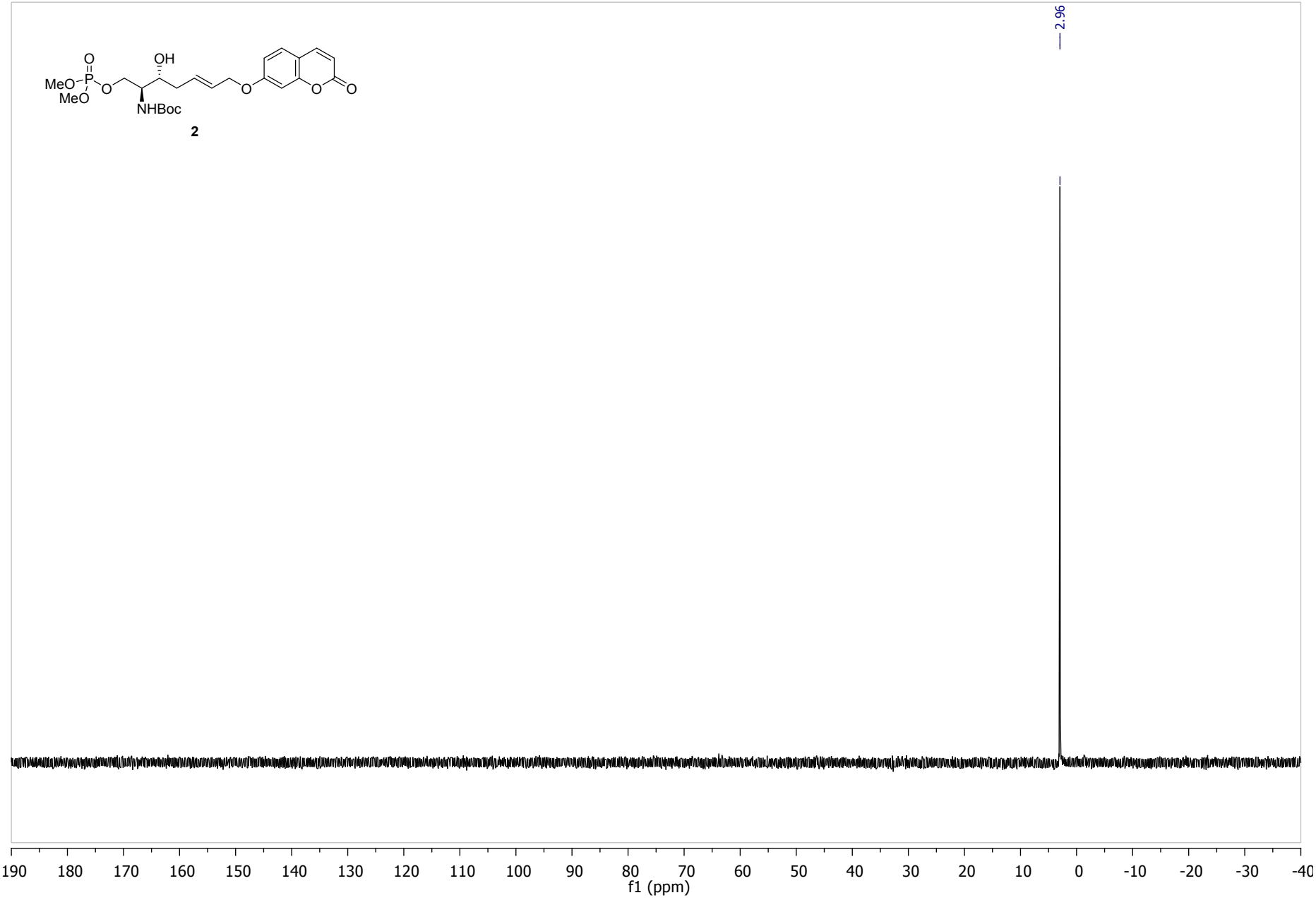
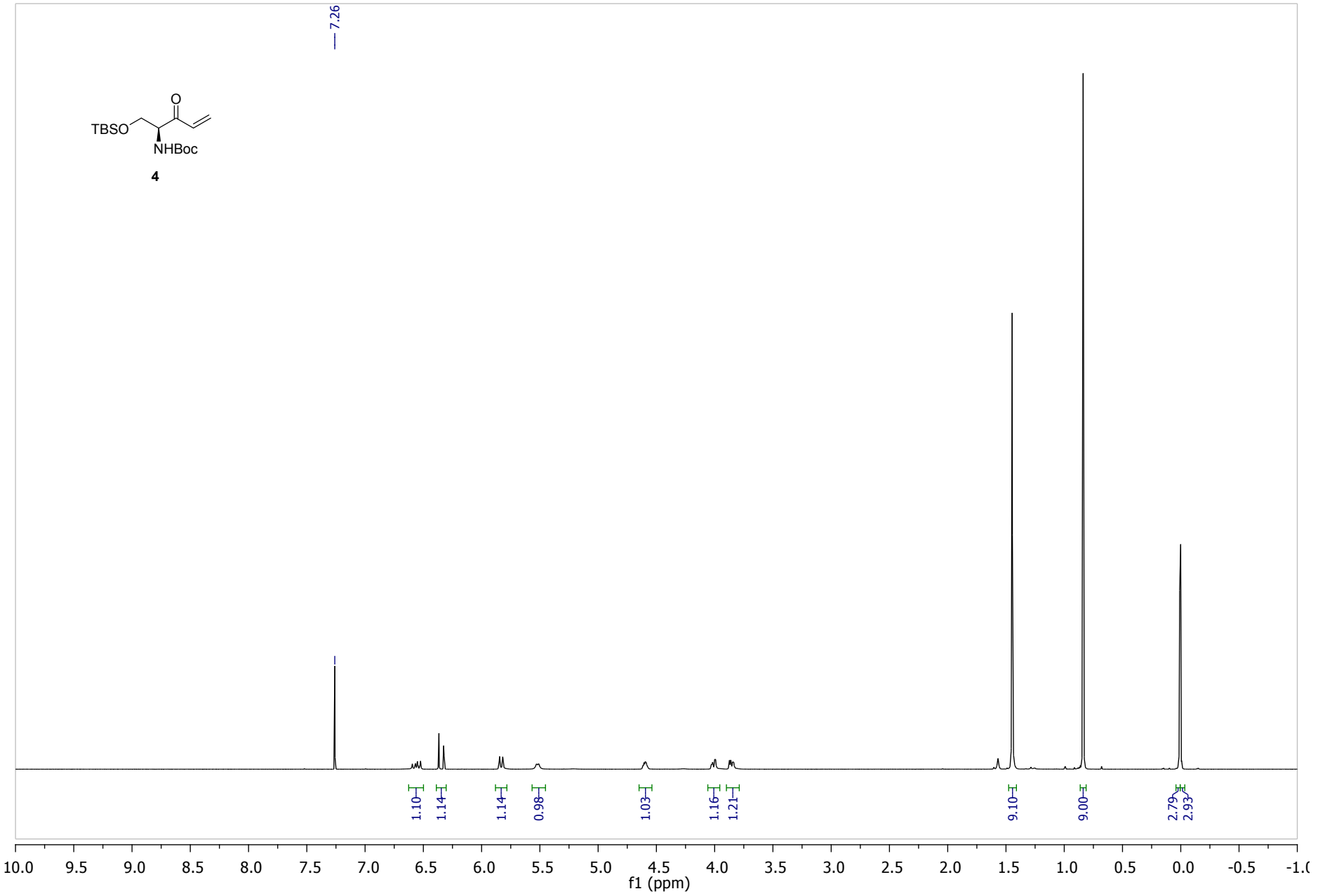
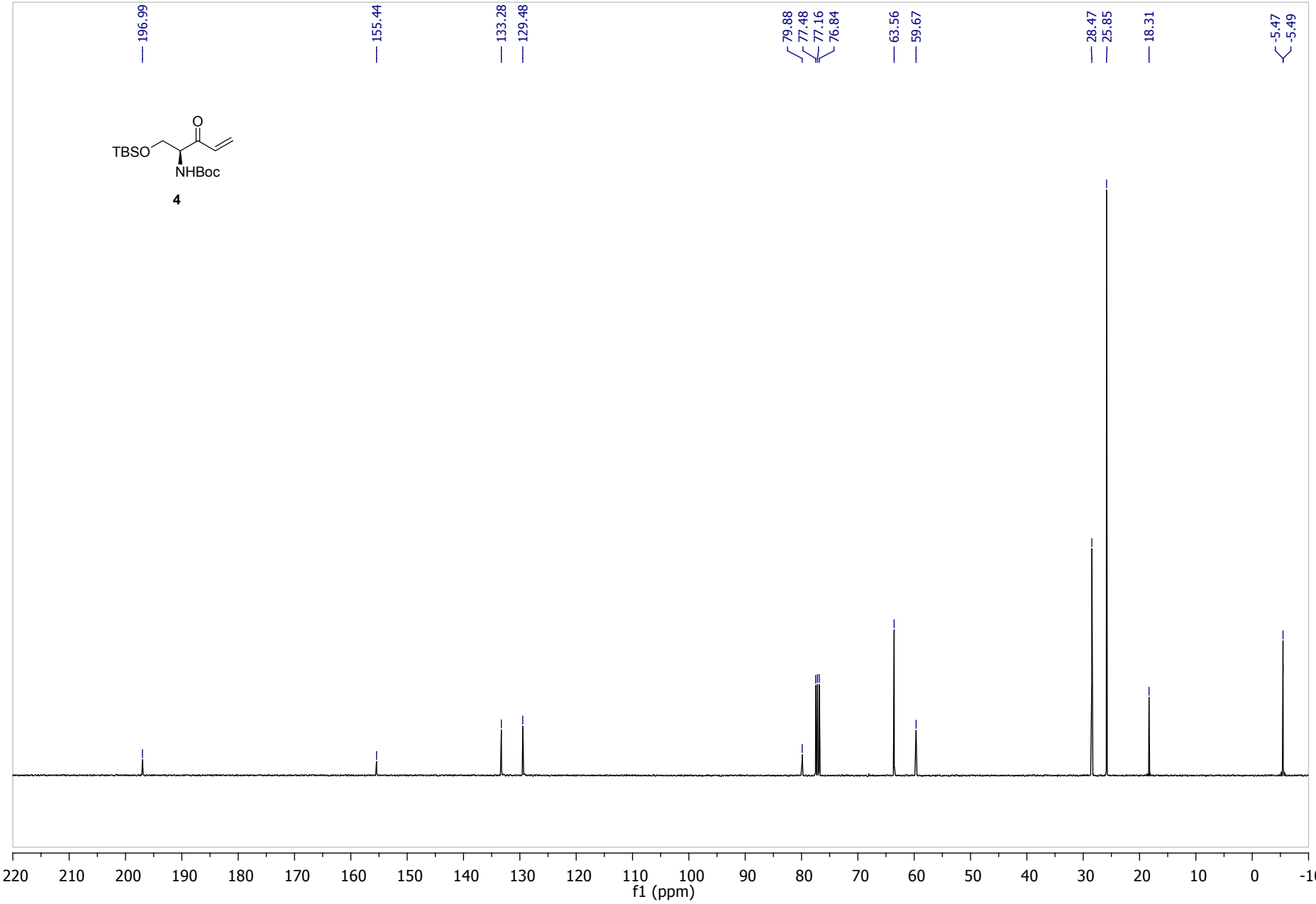
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)

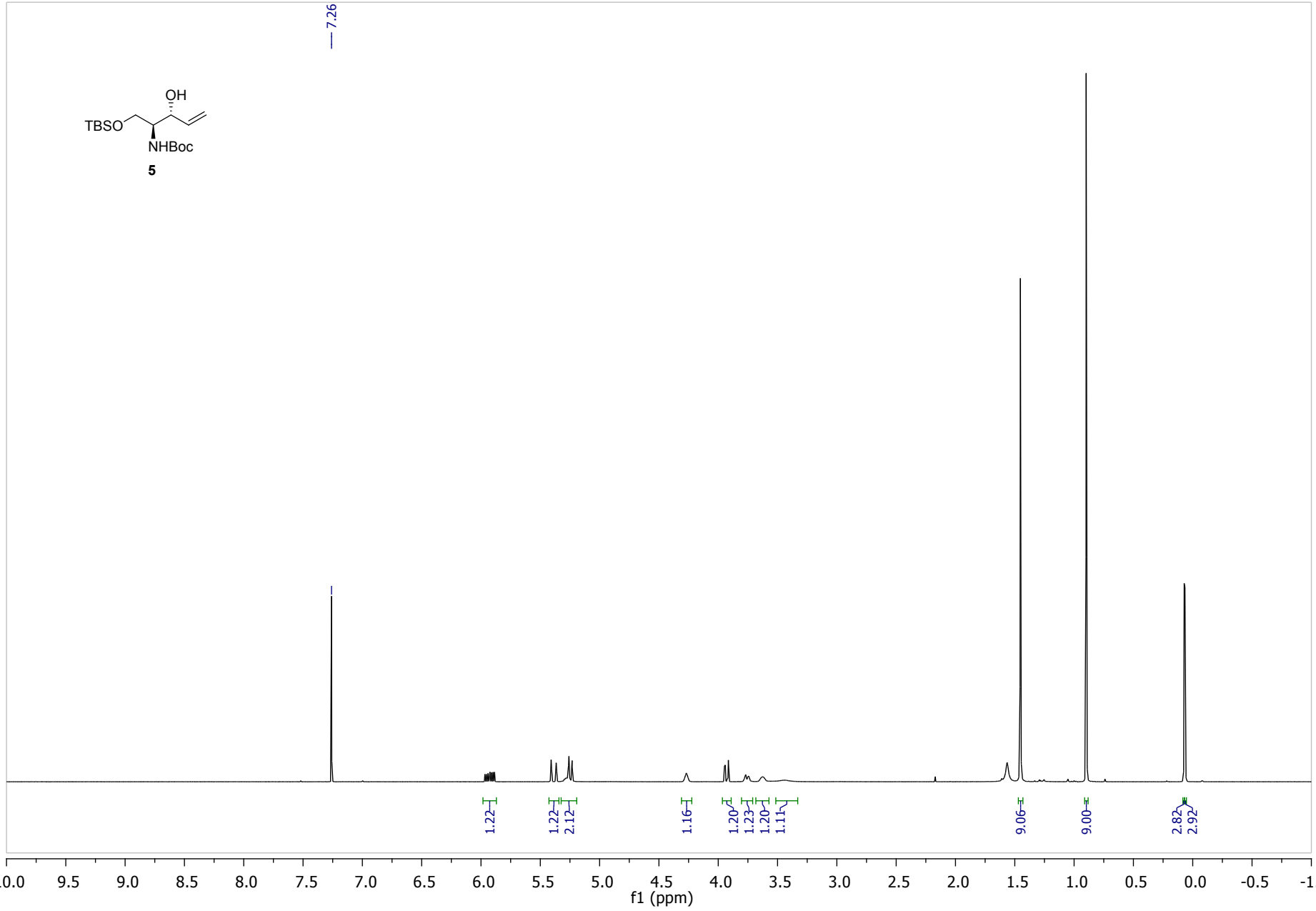

7.26
MeO
MeO
O
P
O
O
OH
NHBoc
O
O
O
2
1.06
1.06
2.04
1.00
1.18
1.17
0.98
2.10
1.14
1.19
2.71
2.78
2.10
0.96
1.22
1.25
9.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

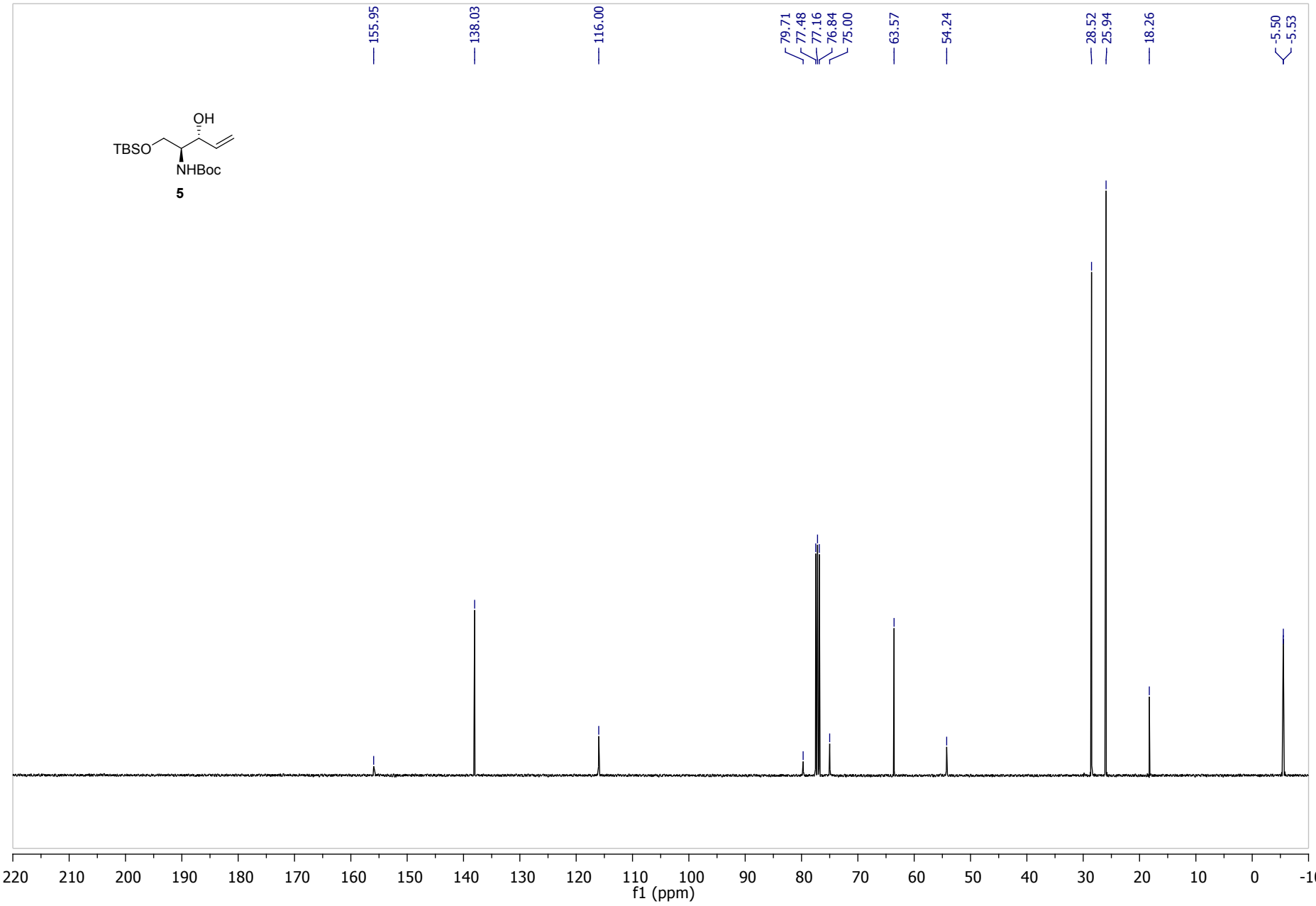

161.92
161.34
155.90
155.62
143.55
131.89
128.87
127.31
113.17
113.15
112.69
101.82
80.06
77.48
77.16
76.84
70.14
69.07
67.07
67.01
54.67
54.62
36.82
28.45
MeO
MeO
O
P
O
O
OH
NHBoc
O
O
O
2
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

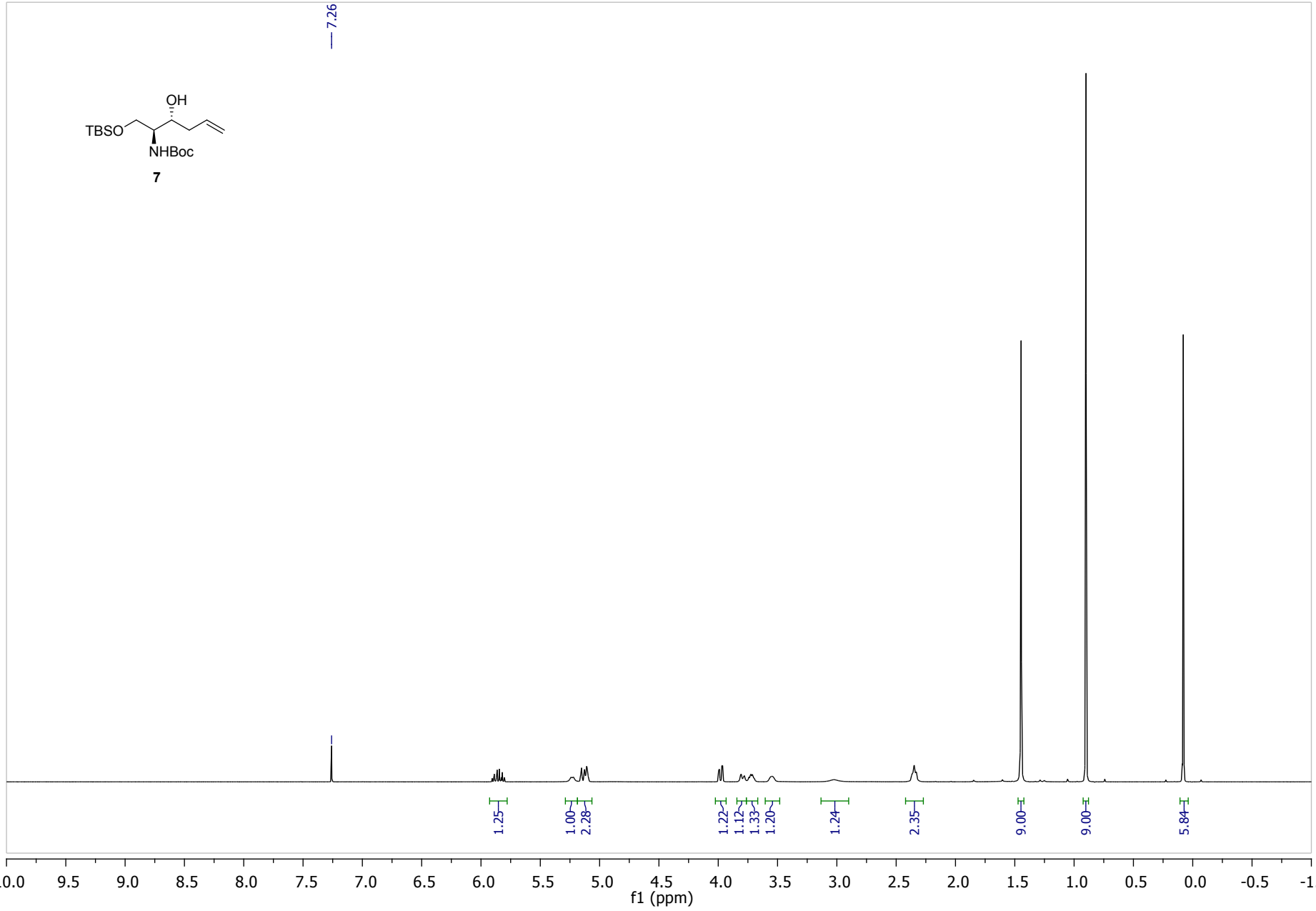

2.96
MeO
MeO
O
P
O
OH
NHBoc
O
O
O
2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)

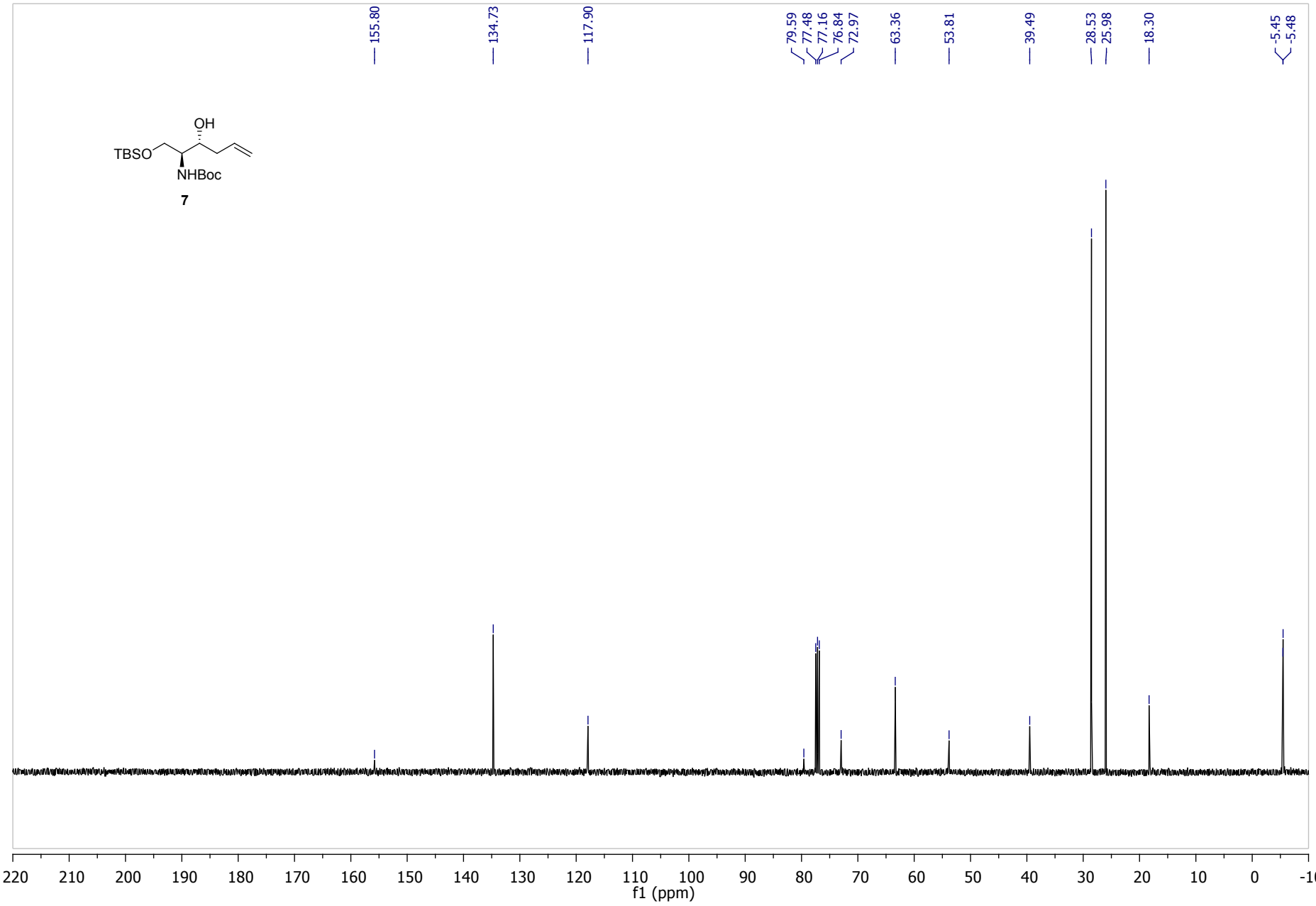

7.26
TBSO
O
NHBoc
4
1.10
1.14
1.14
0.98
1.03
1.16
1.21
9.10
9.00
2.79
2.93
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

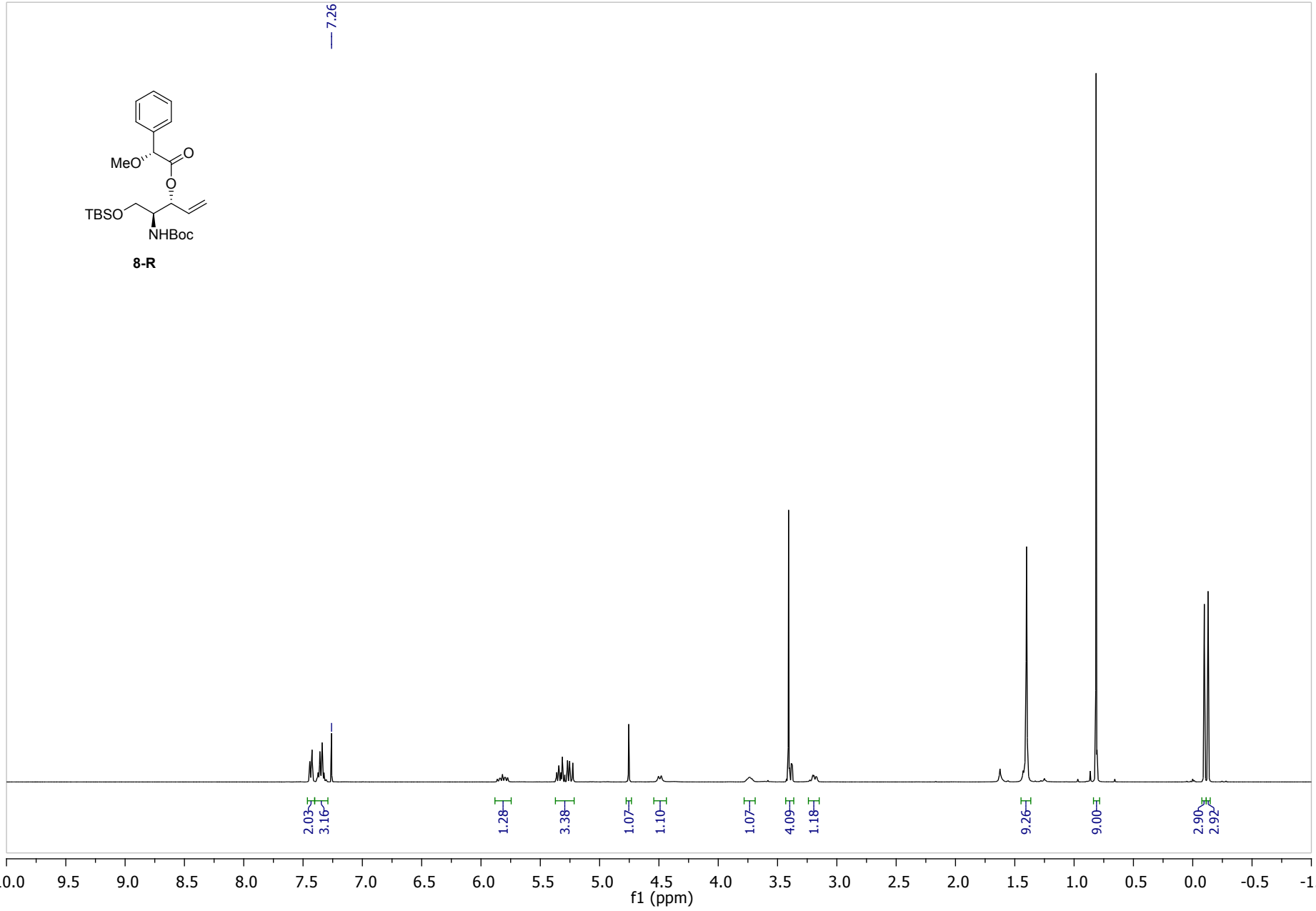

196.99
155.44
133.28
129.48
79.88
77.48
77.16
76.84
63.56
59.67
28.47
25.85
18.31
-5.47
-5.49
TBSO
O
NHBoc
4
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

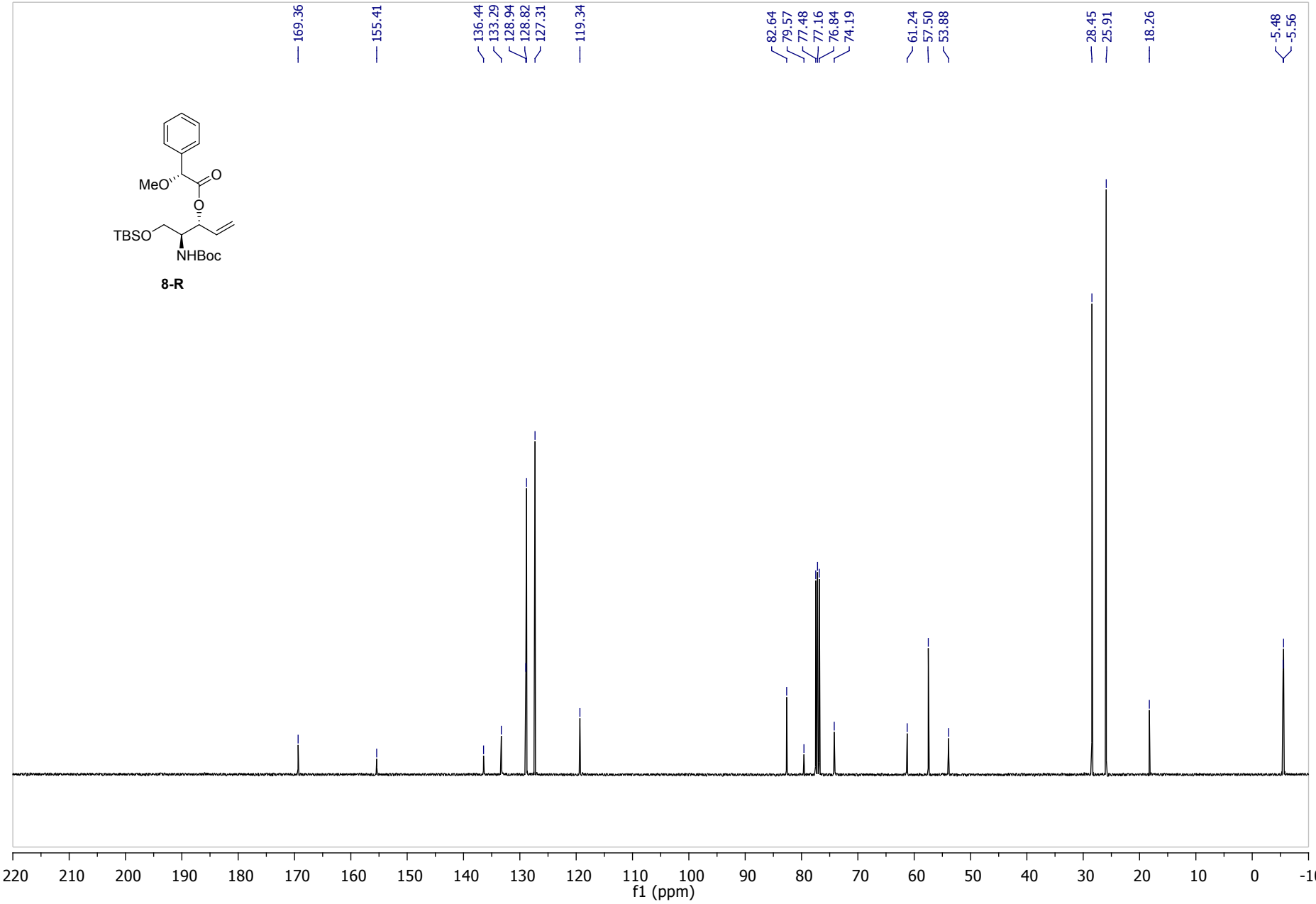

7.26
OH
TBSO
NHBoc
5
1.22
1.22
2.12
1.16
1.20
1.23
1.20
1.11
9.06
9.00
2.82
2.92
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

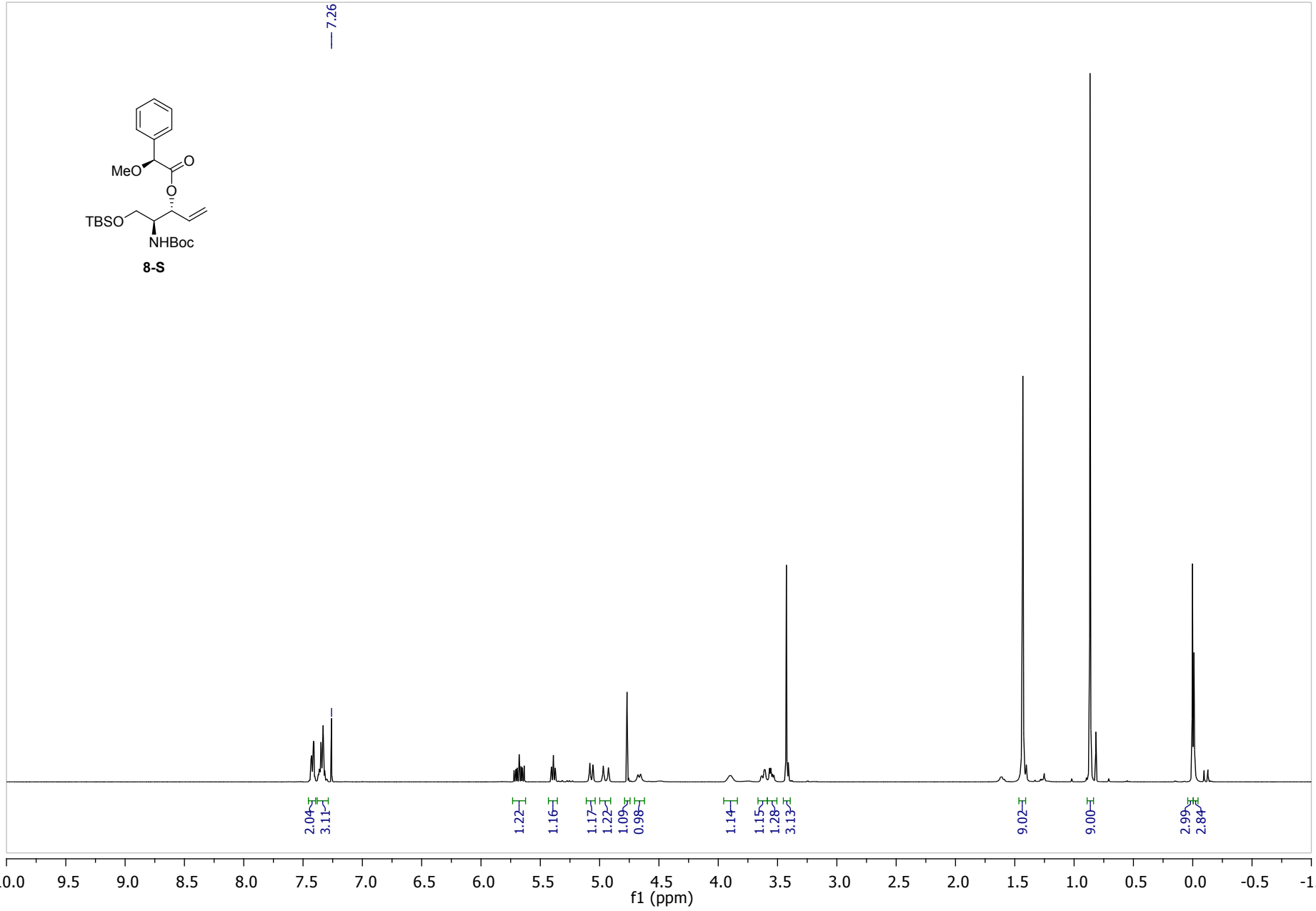

155.95
138.03
116.00
79.71
77.48
77.16
76.84
75.00
63.57
54.24
28.52
25.94
18.26
-5.50
-5.53
OH
TBSO
NHBoc
5
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

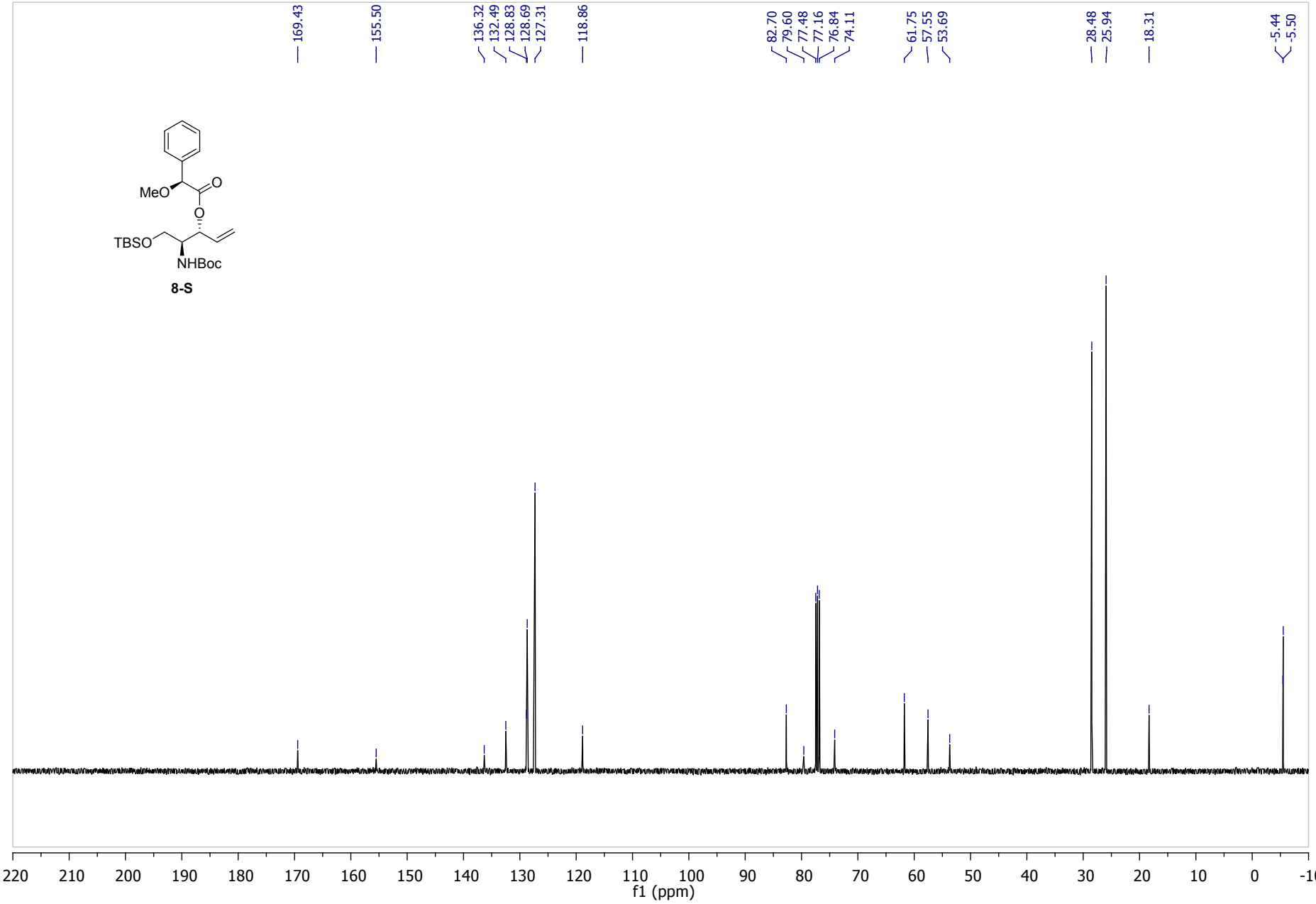

7.26
OH
TBSO
NHBoc
7
1.25
1.00
2.28
1.22
1.12
1.33
1.20
1.24
2.35
9.00
9.00
5.84
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

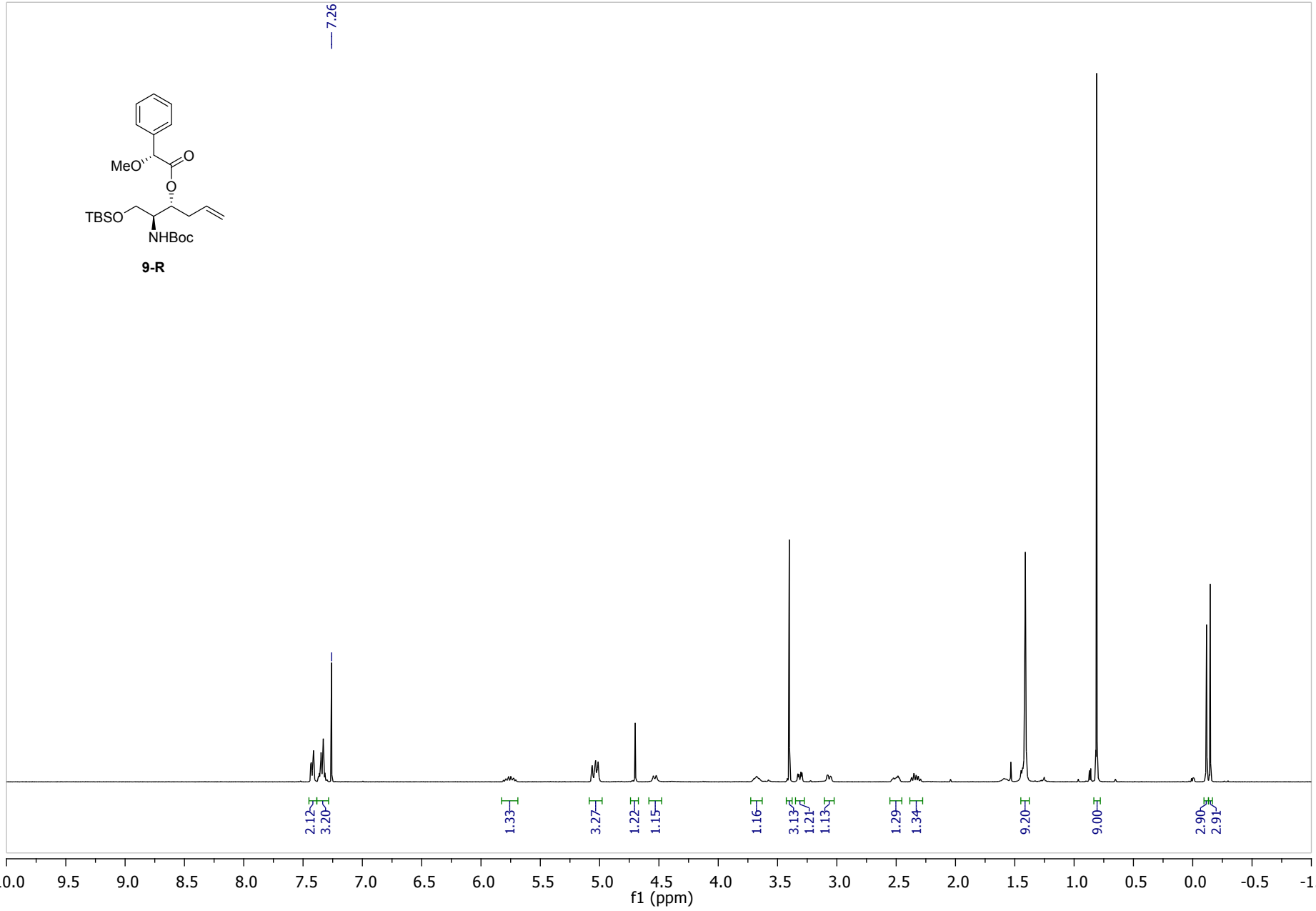

155.80
134.73
117.90
79.59
77.48
77.16
76.84
72.97
63.36
53.81
39.49
28.53
25.98
18.30
-5.45
-5.48
OH
TBSO
NHBoc
7
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

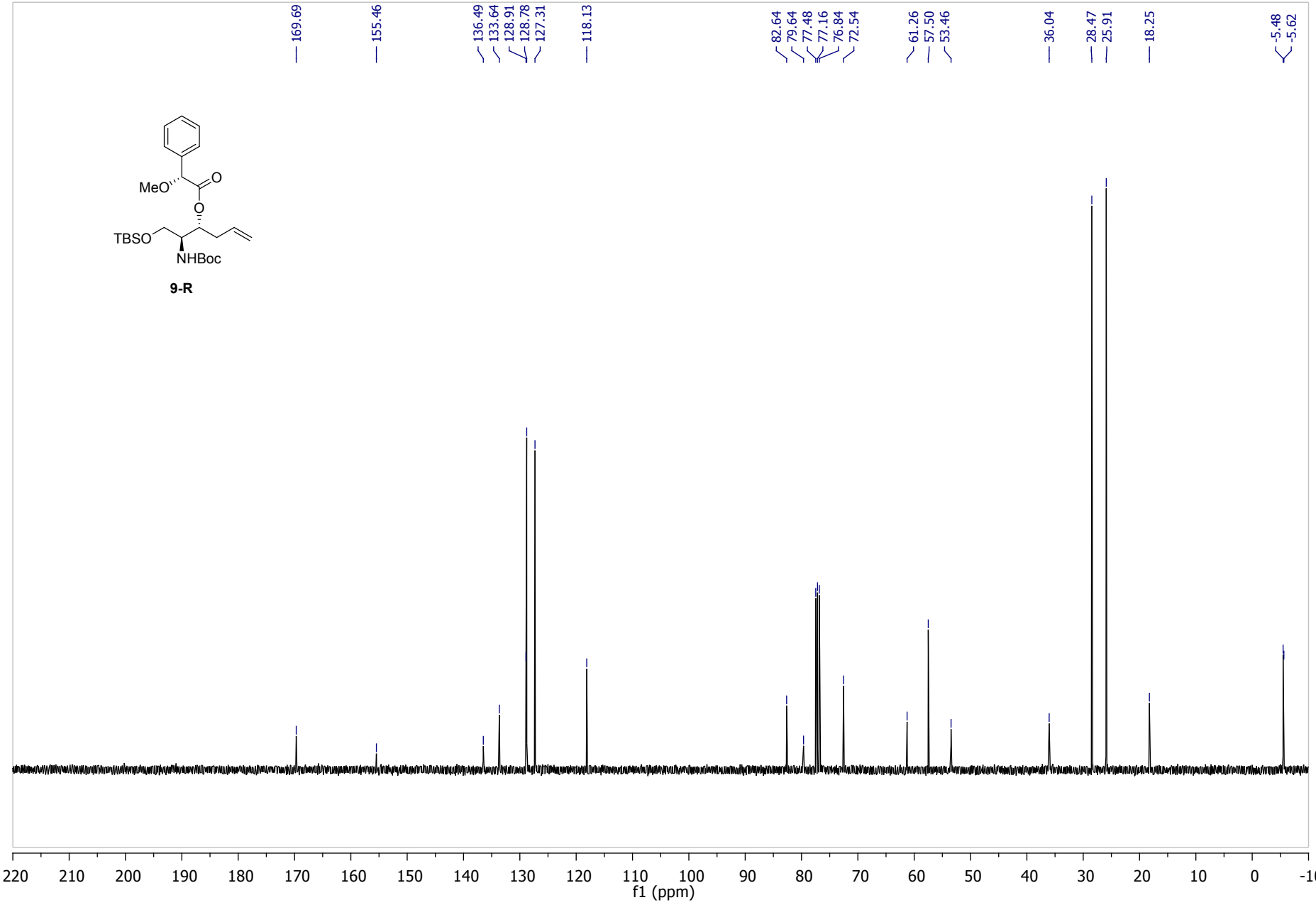

7.26
MeO
O
O
TBSO
NHBoc
8-R
2.03
3.16
1.28
3.38
1.07
1.10
1.07
4.09
1.18
9.26
9.00
2.90
2.92
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

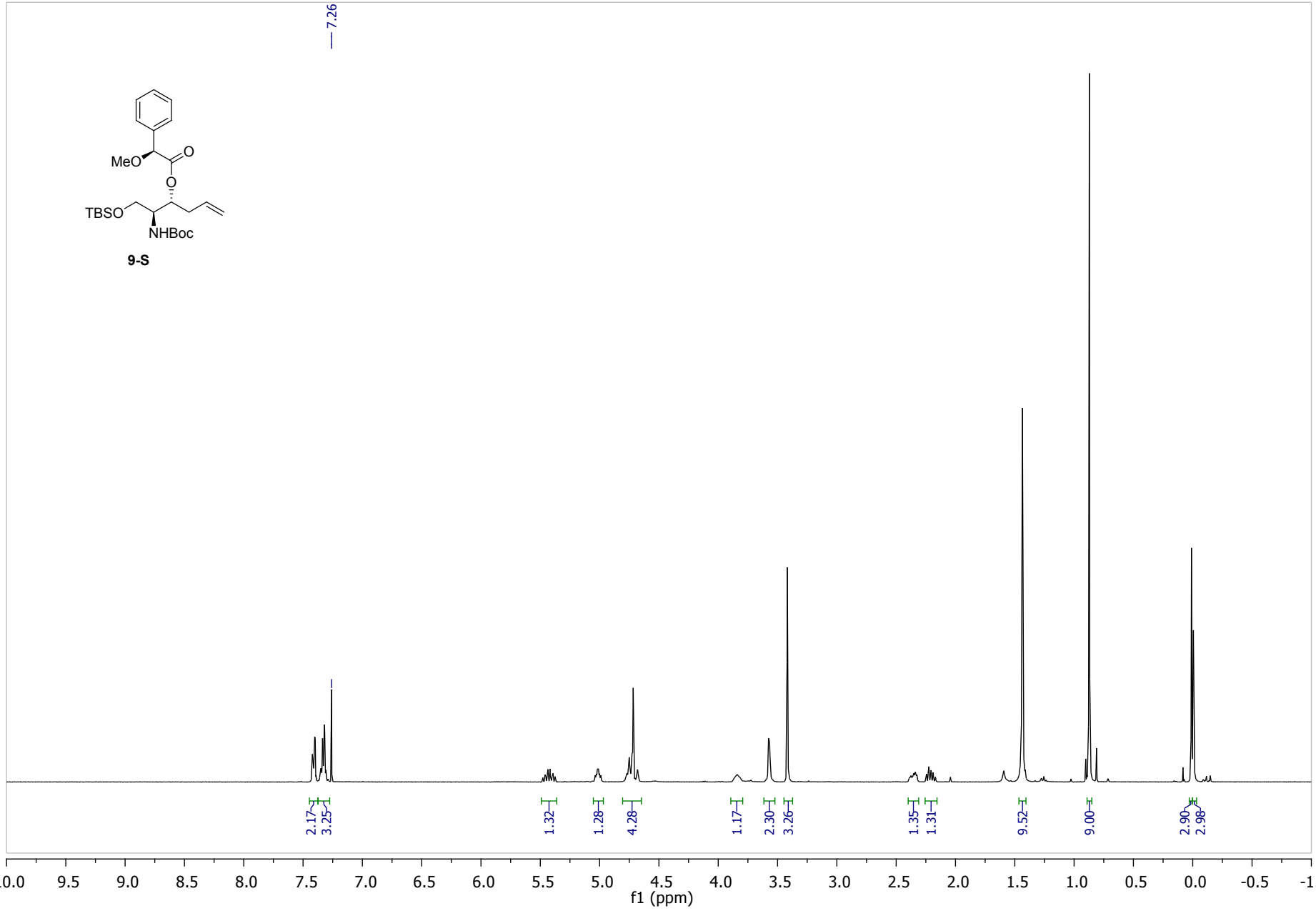

169.36
155.41
136.44
133.29
128.94
128.82
127.31
119.34
82.64
79.57
77.48
77.16
76.84
74.19
61.24
57.50
53.88
28.45
25.91
18.26
-5.48
-5.56
MeO
O
O
TBSO
NHBoc
8-R
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

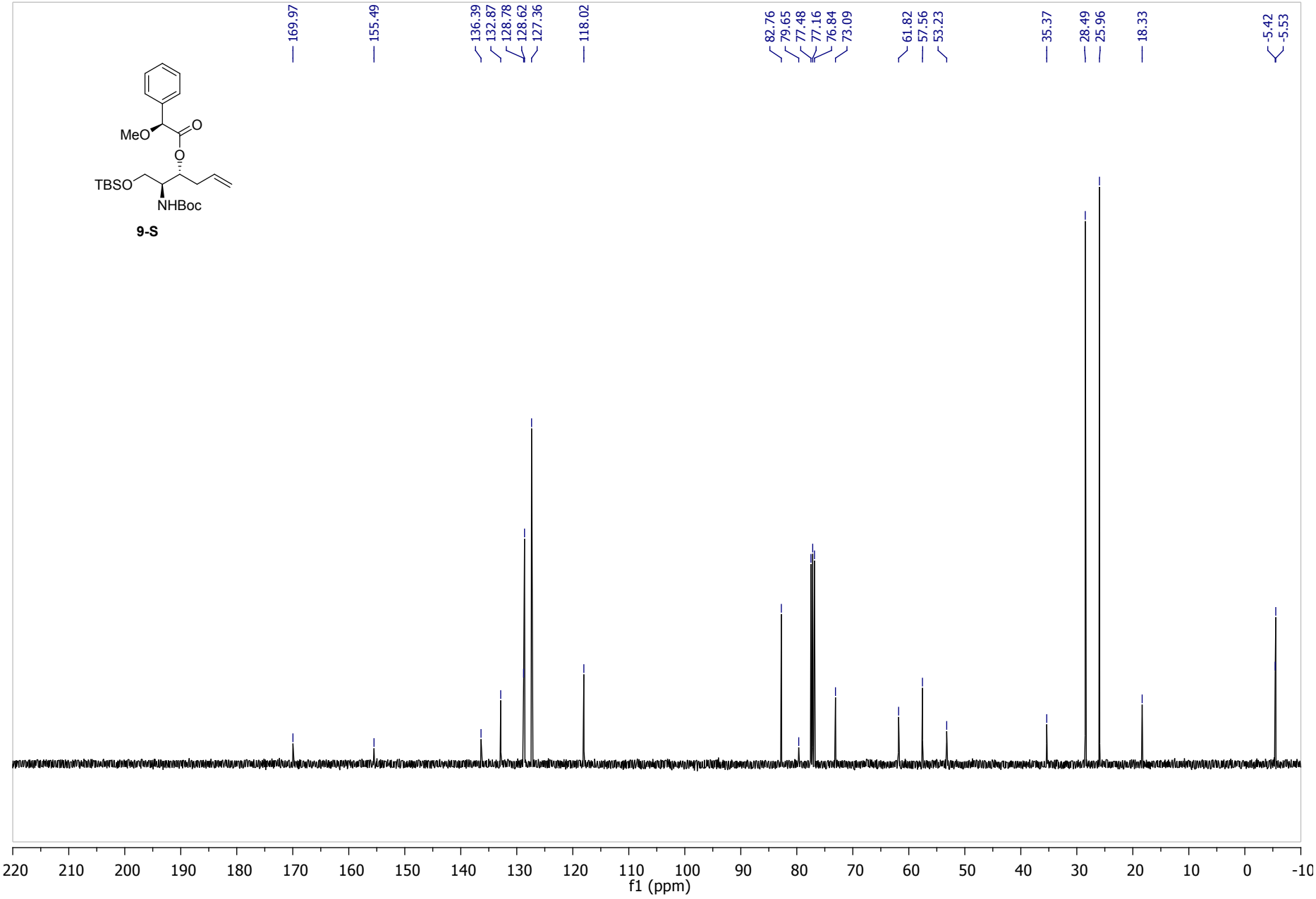

7.26
MeO
O
O
TBSO
NHBoc
8-S
2.04
3.11
1.22
1.16
1.17
1.22
1.09
0.98
1.14
1.15
1.28
3.13
9.02
9.00
2.99
2.84
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

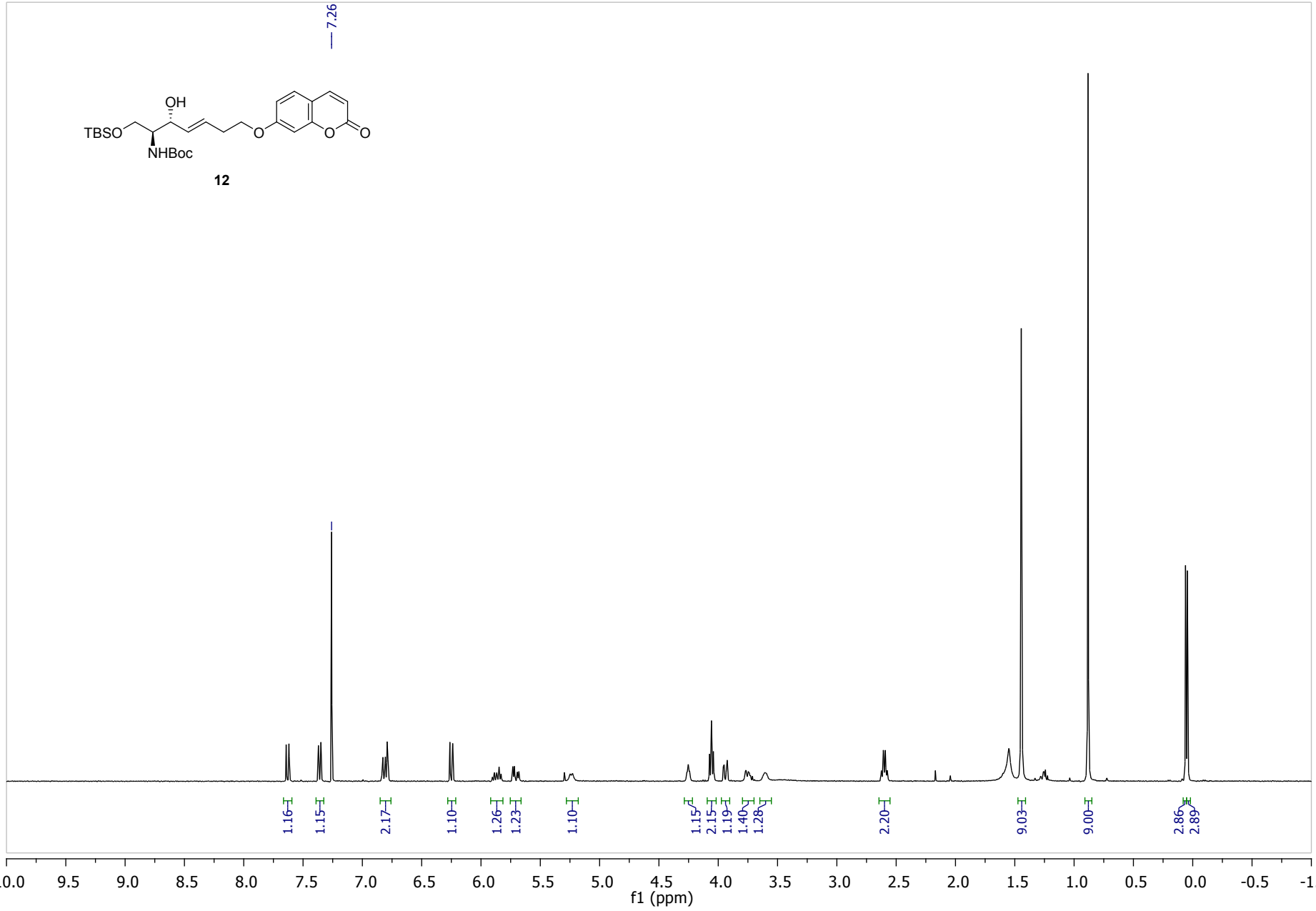

169.43
155.50
136.32
132.49
128.83
128.69
127.31
118.86
82.70
79.60
77.48
77.16
76.84
74.11
61.75
57.55
53.69
28.48
25.94
18.31
-5.44
-5.50
MeO
O
O
TBSO
NHBoc
8-S
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

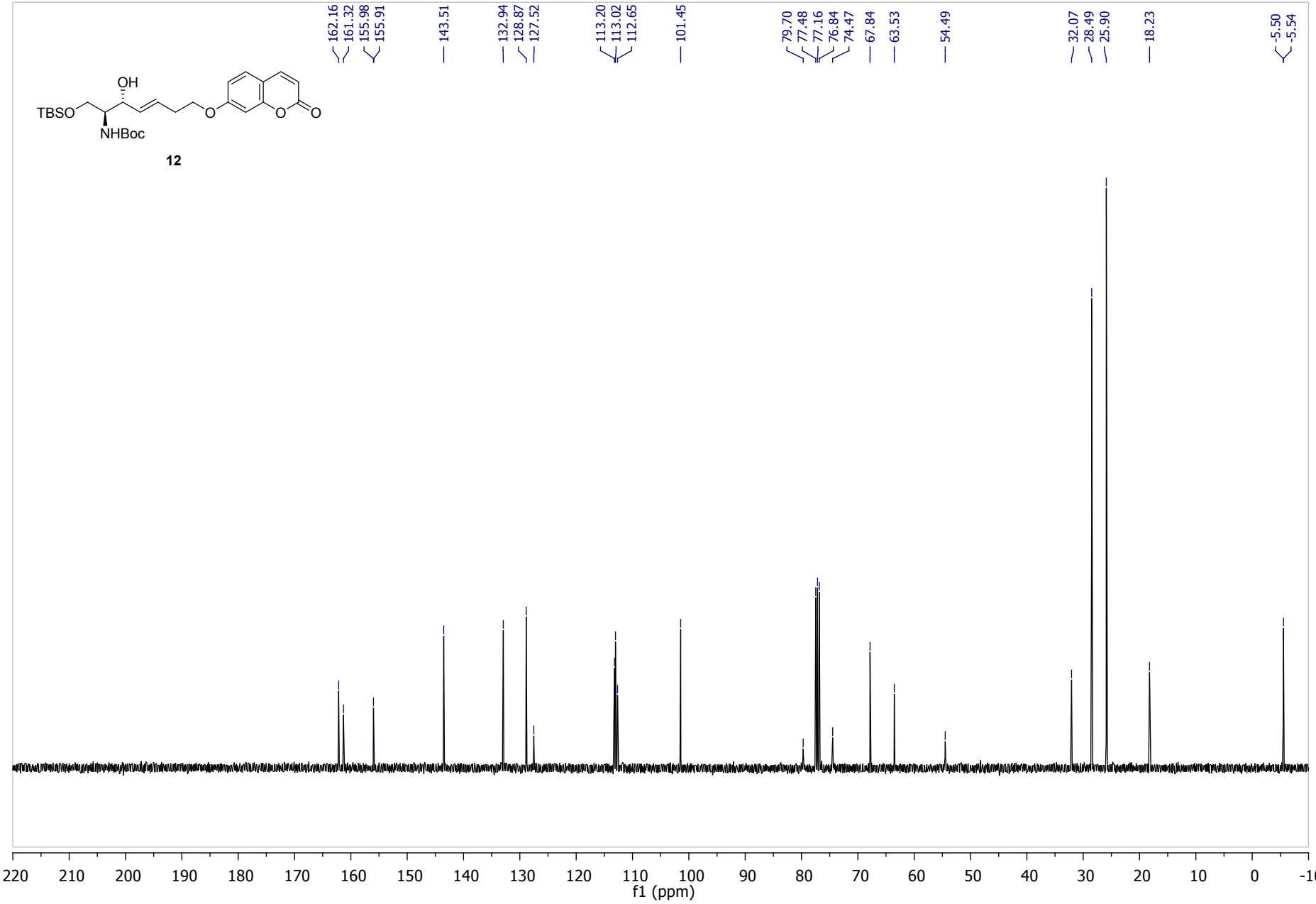

7.26
MeO
O
O
TBSO
NHBoc
9-R
2.12
3.20
1.33
3.27
1.22
1.15
1.16
3.13
1.21
1.13
1.29
1.34
9.20
9.00
2.90
2.91
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

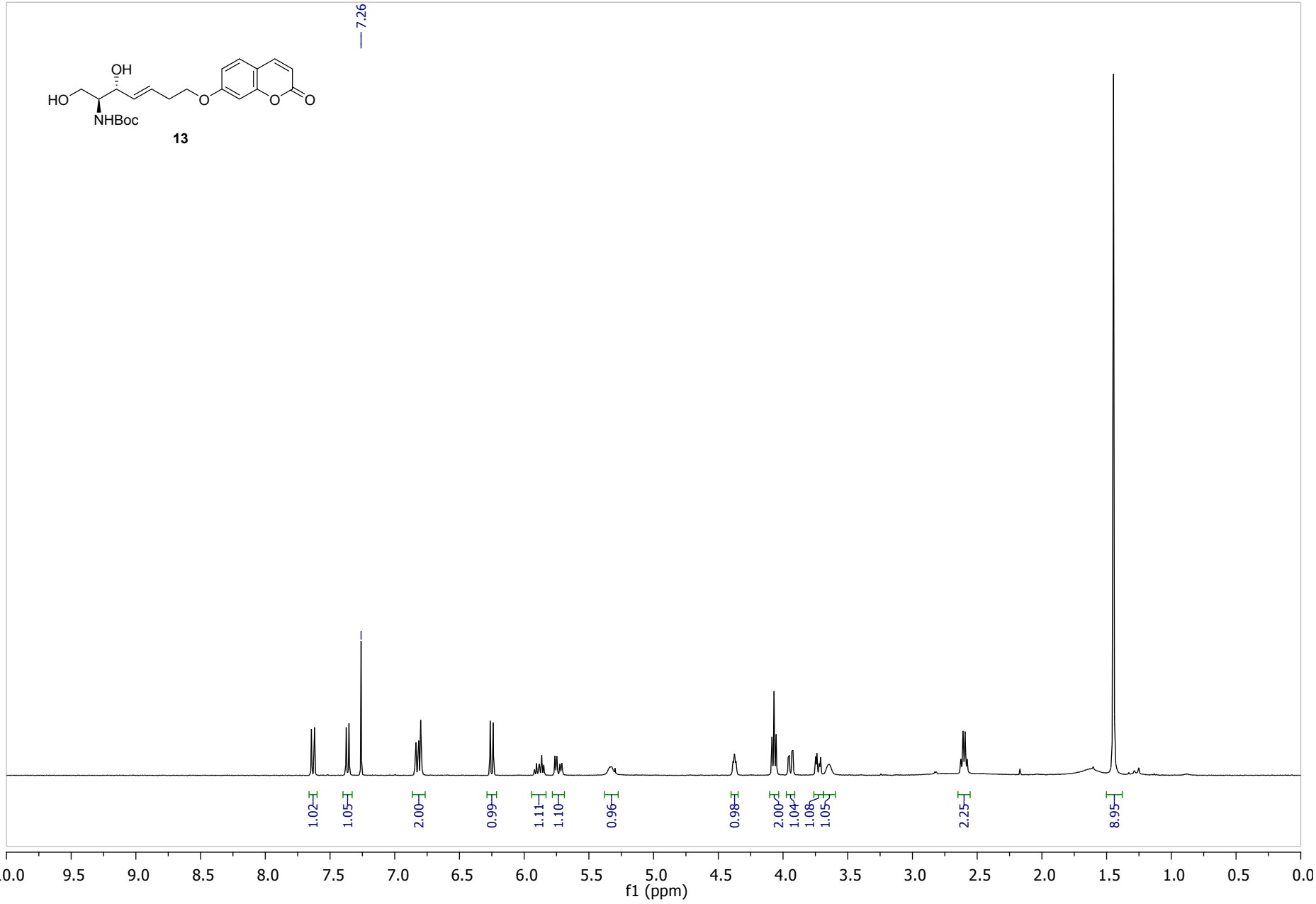

169.69
155.46
136.49
133.64
128.91
128.78
127.31
118.13
82.64
79.64
77.48
77.16
76.84
72.54
61.26
57.50
53.46
36.04
28.47
25.91
18.25
-5.48
-5.62
MeO
O
O
TBSO
NHBoc
9-R
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

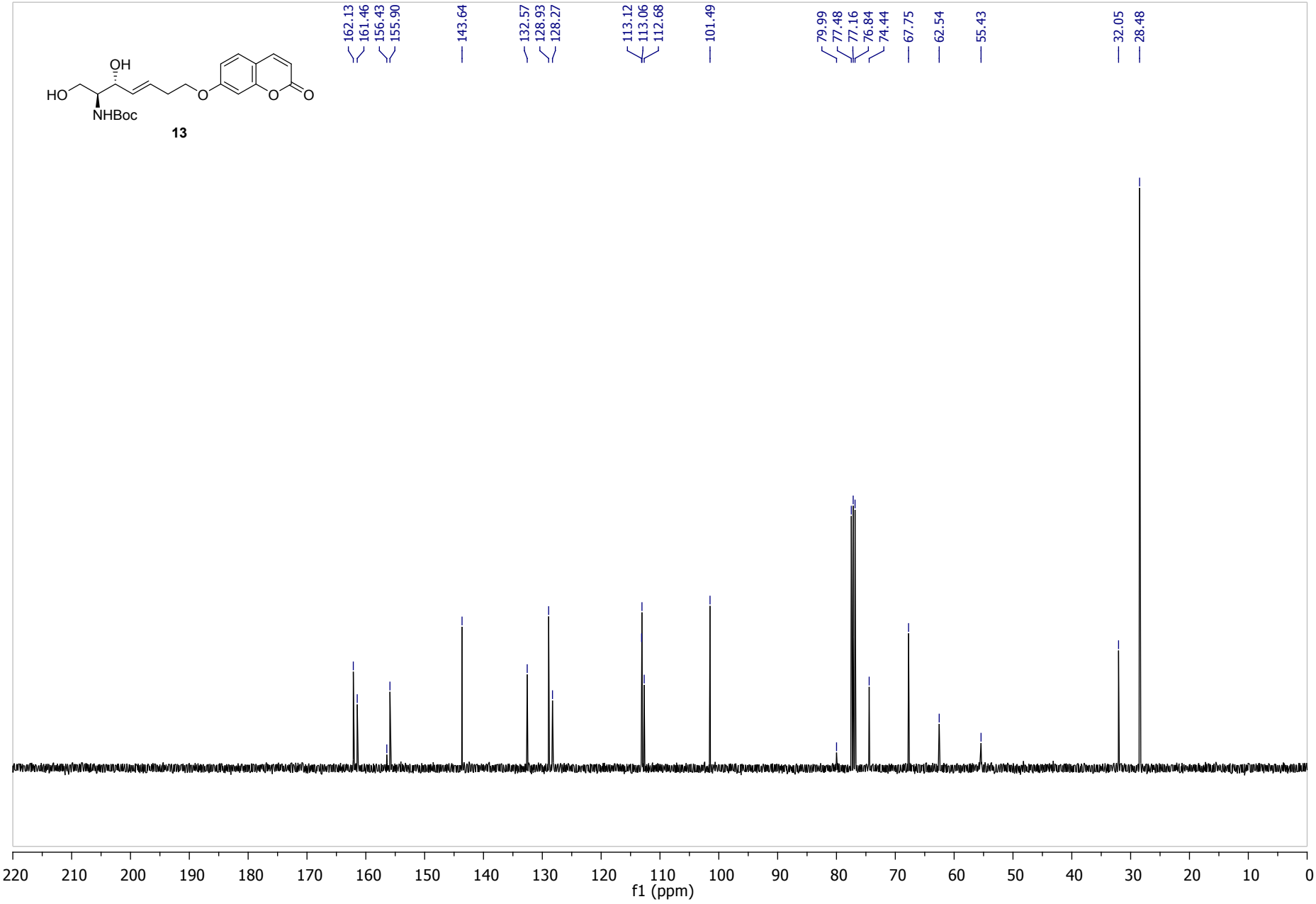

7.26
MeO
O
O
TBSO
NHBoc
9-S
2.17
3.25
1.32
1.28
4.28
1.17
2.30
3.26
1.35
1.31
9.52
9.00
2.90
2.98
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

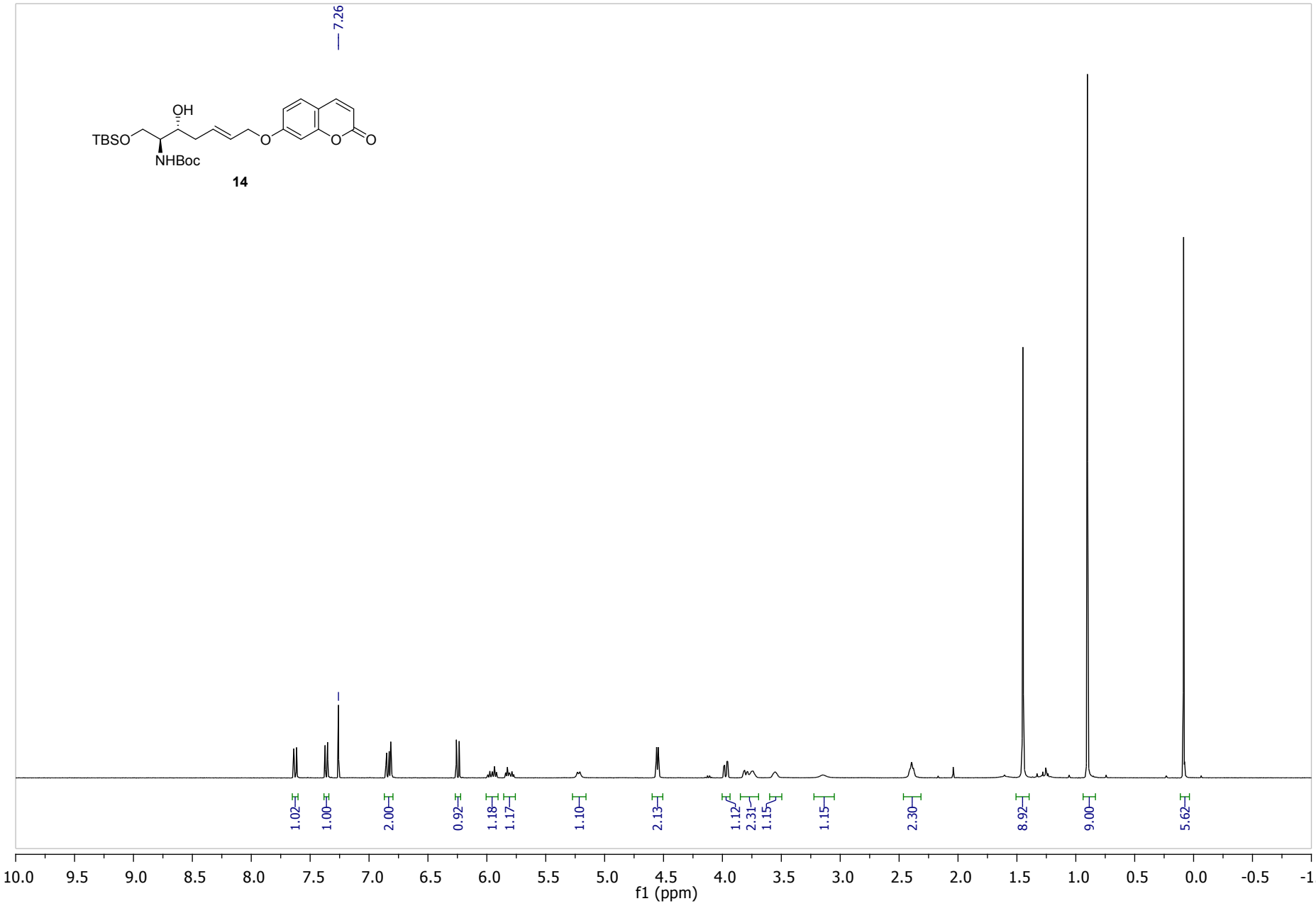

169.97
155.49
136.39
132.87
128.78
128.62
127.36
118.02
82.76
79.65
77.48
77.16
76.84
73.09
61.82
57.56
53.23
35.37
28.49
25.96
18.33
-5.42
-5.53
MeO
O
O
TBSO
NHBoc
9-S
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

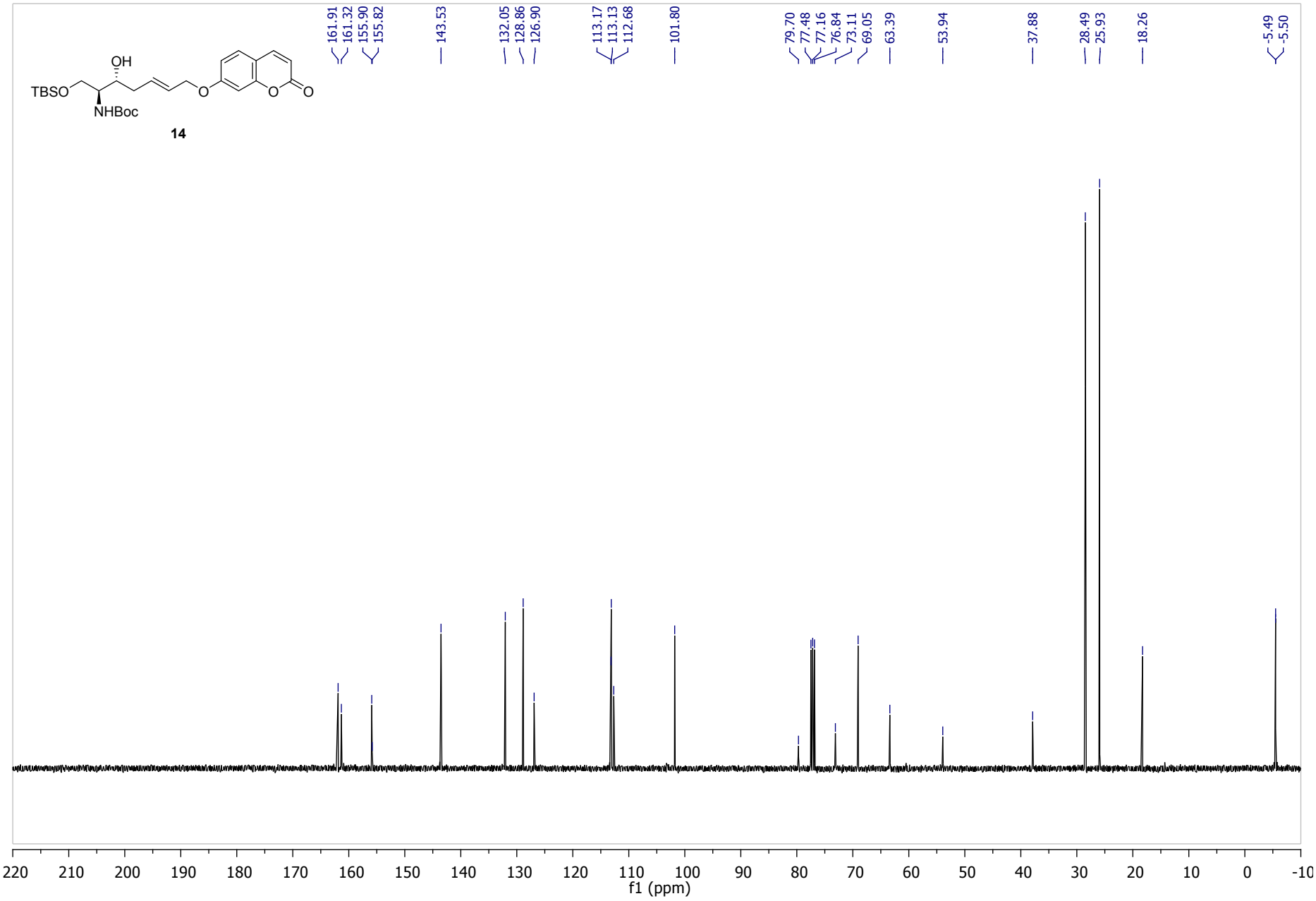

7.26
OH
TBSO
NHBoc
O
O
O
12
1.16
1.15
2.17
1.10
1.26
1.23
1.10
1.15
2.15
1.19
1.40
1.28
2.20
9.03
9.00
2.86
2.89
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

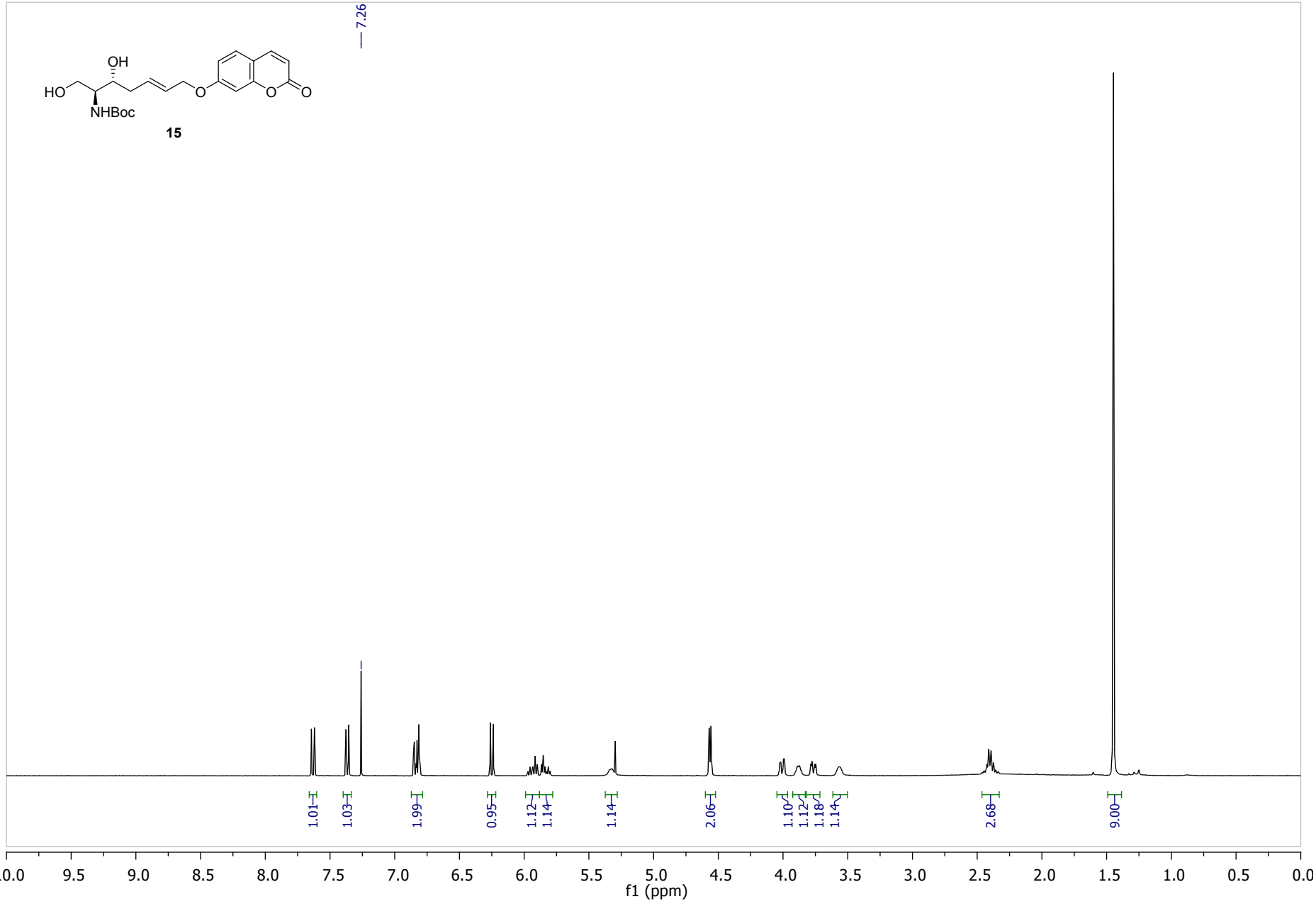

162.16
161.32
155.98
155.91
143.51
132.94
128.87
127.52
113.20
113.02
112.65
101.45
79.70
77.48
77.16
76.84
74.47
67.84
63.53
54.49
32.07
28.49
25.90
18.23
-5.50
-5.54
OH
TBSO
NHBoc
O
O
O
12
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

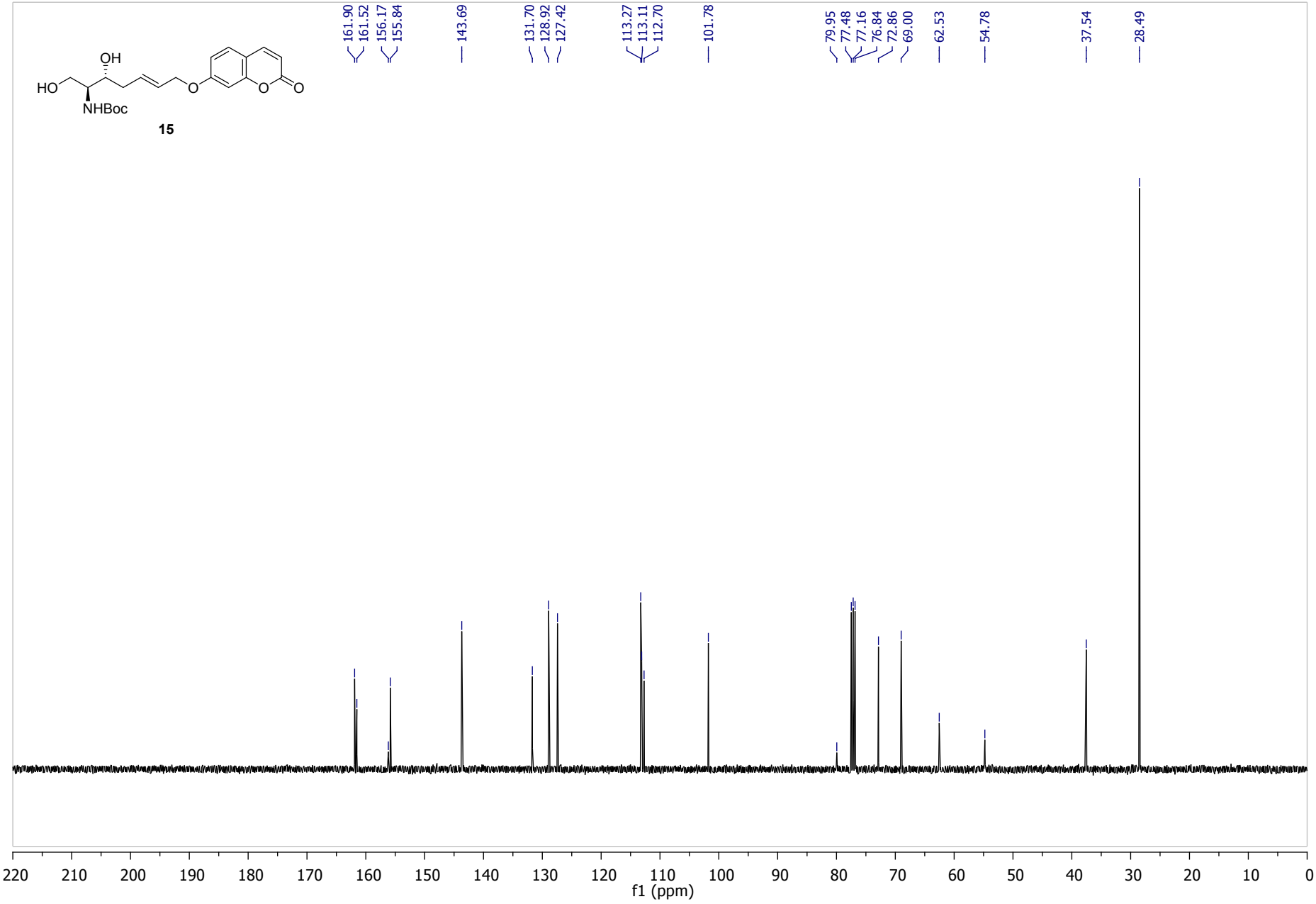

7.26
OH
HO
NHBoc
O
O
O
13
1.02
1.05
2.00
0.99
1.11
1.10
0.96
0.98
2.00
1.04
1.08
1.05
2.25
8.95
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

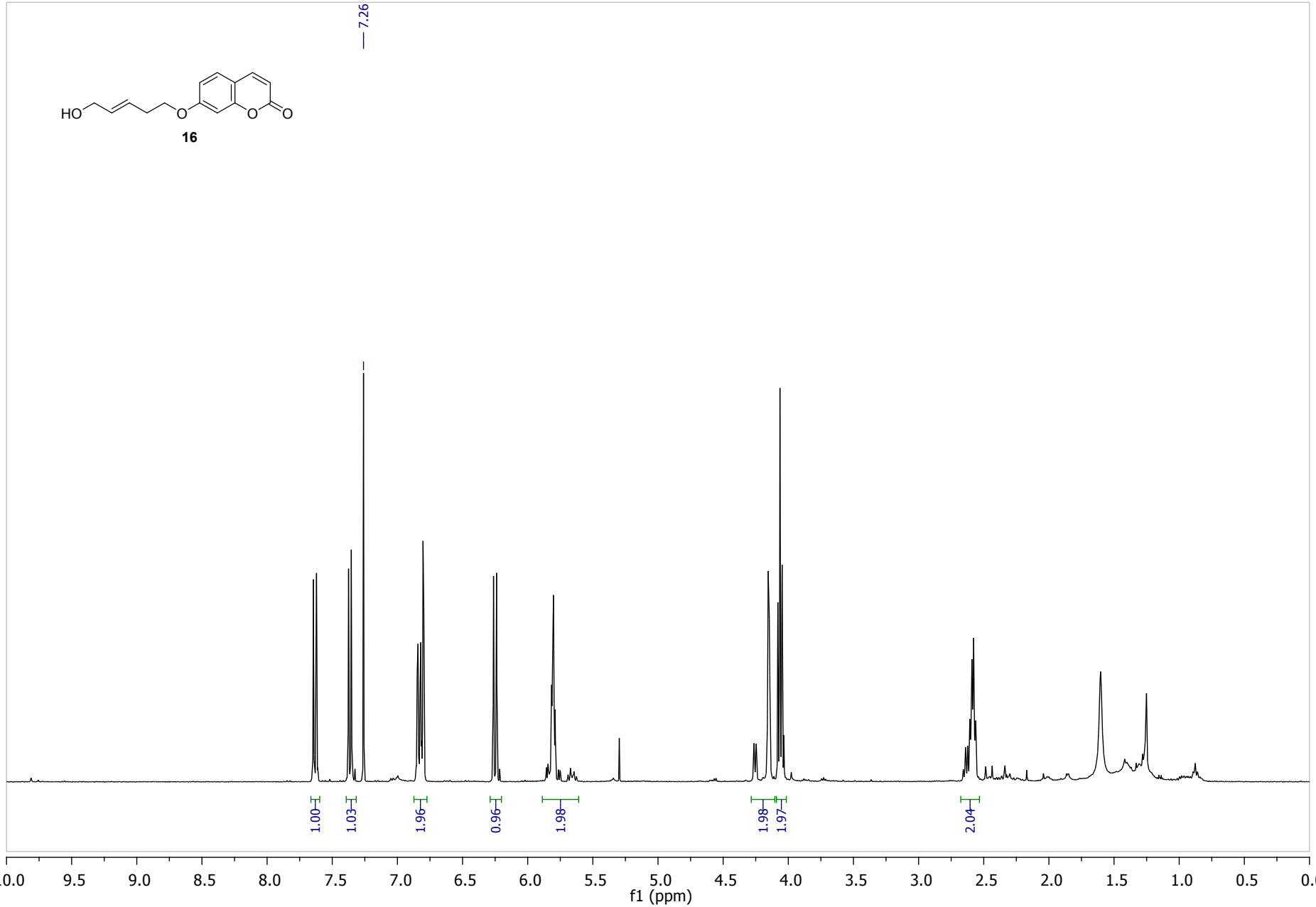

OH
HO
NHBoc
O
O
O
13
162.13
161.46
156.43
155.90
143.64
132.57
128.93
128.27
113.12
113.06
112.68
101.49
79.99
77.48
77.16
76.84
74.44
67.75
62.54
55.43
32.05
28.48
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

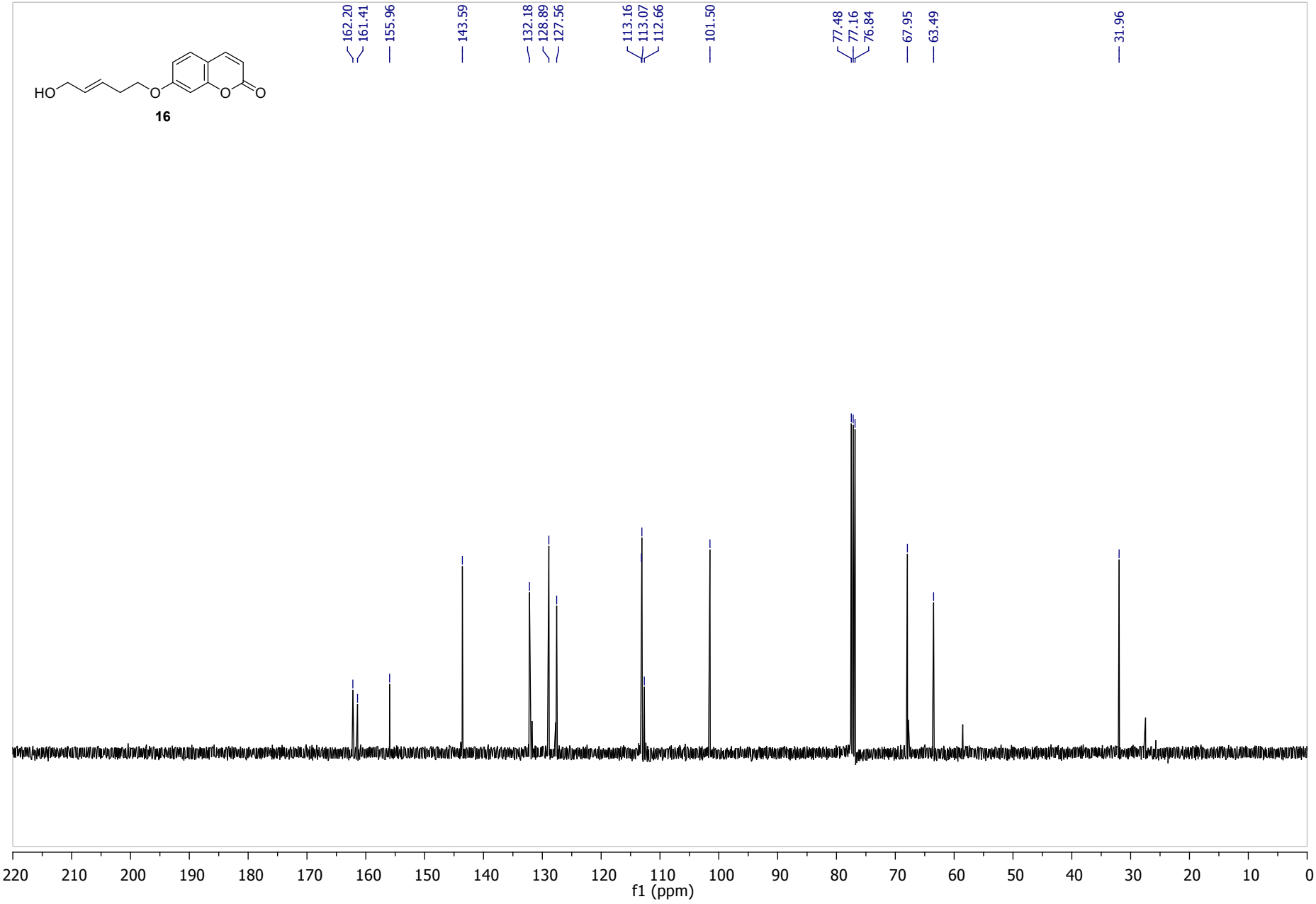

7.26
OH
TBSO
NHBoc
O
O
O
14
1.02
1.00
2.00
0.92
1.18
1.17
1.10
2.13
1.12
2.31
1.15
1.15
2.30
8.92
9.00
5.62
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

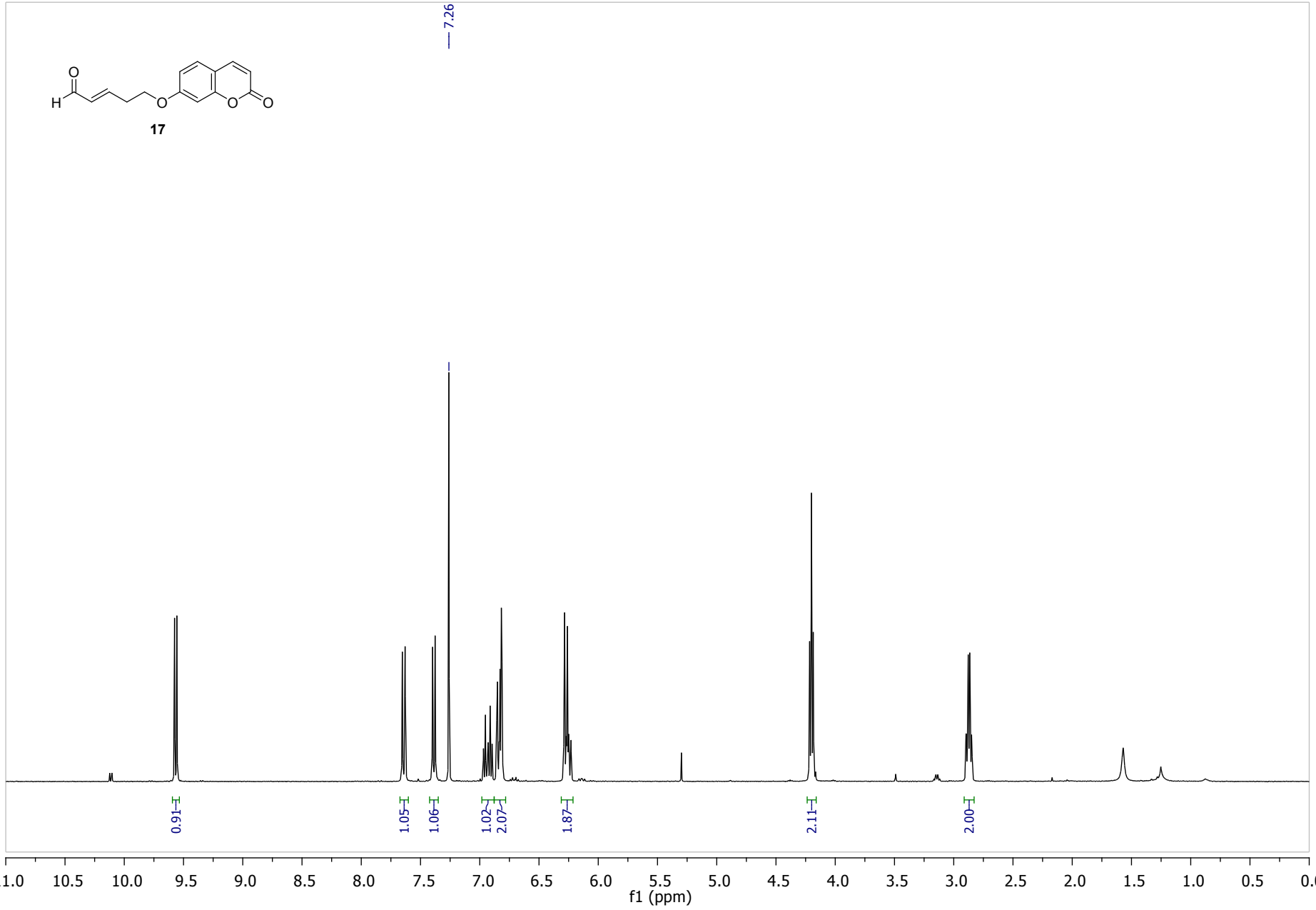

OH
TBSO
NHBoc
O
O
O
14
161.91
161.32
155.90
155.82
143.53
132.05
128.86
126.90
113.17
113.13
112.68
101.80
79.70
77.48
77.16
76.84
73.11
69.05
63.39
53.94
37.88
28.49
25.93
18.26
-5.49
-5.50
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

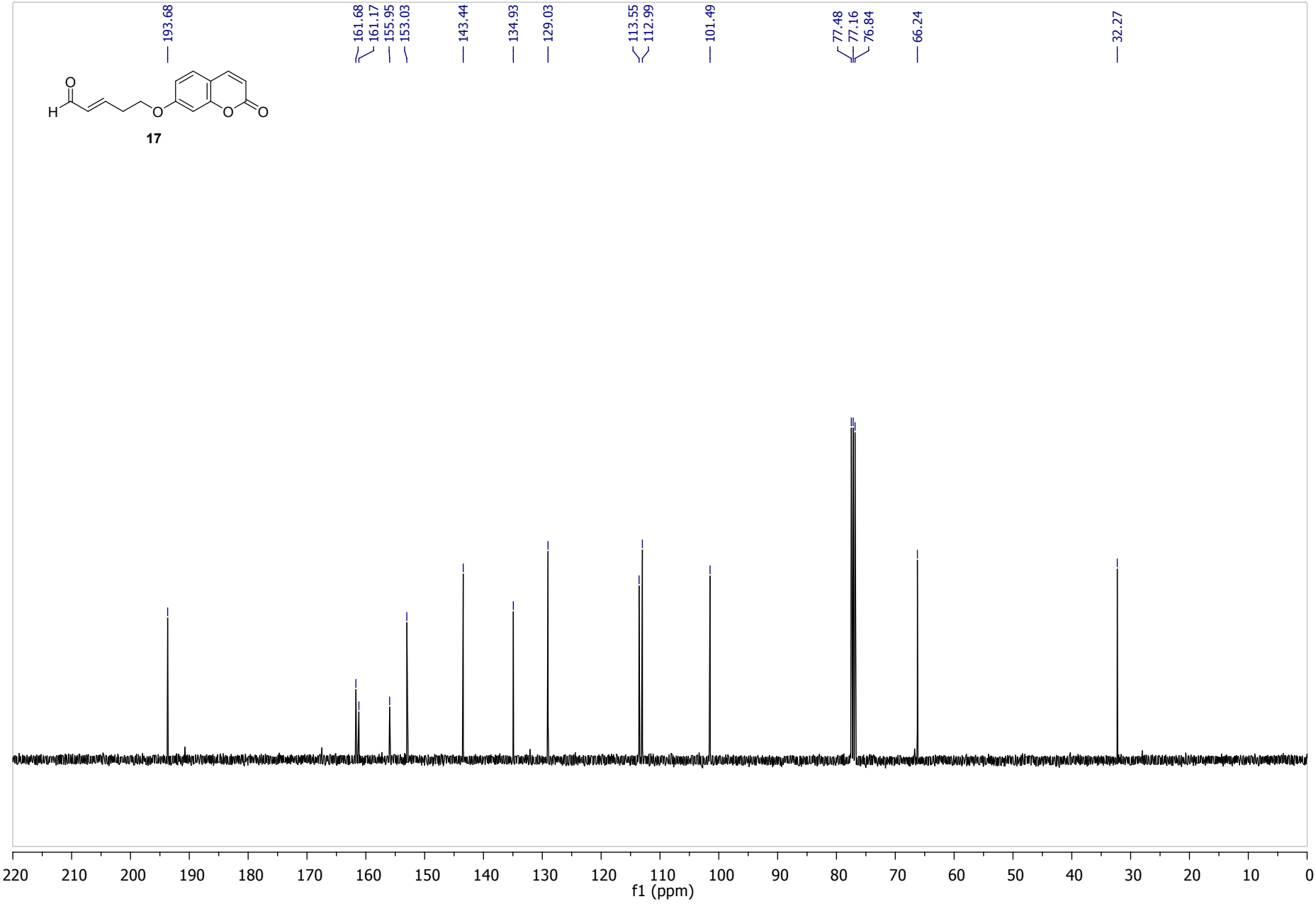

OH
HO
NHBoc
O
O
O
15
7.26
1.01
1.03
1.99
0.95
1.12
1.14
1.14
2.06
1.10
1.12
1.18
1.14
2.68
9.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

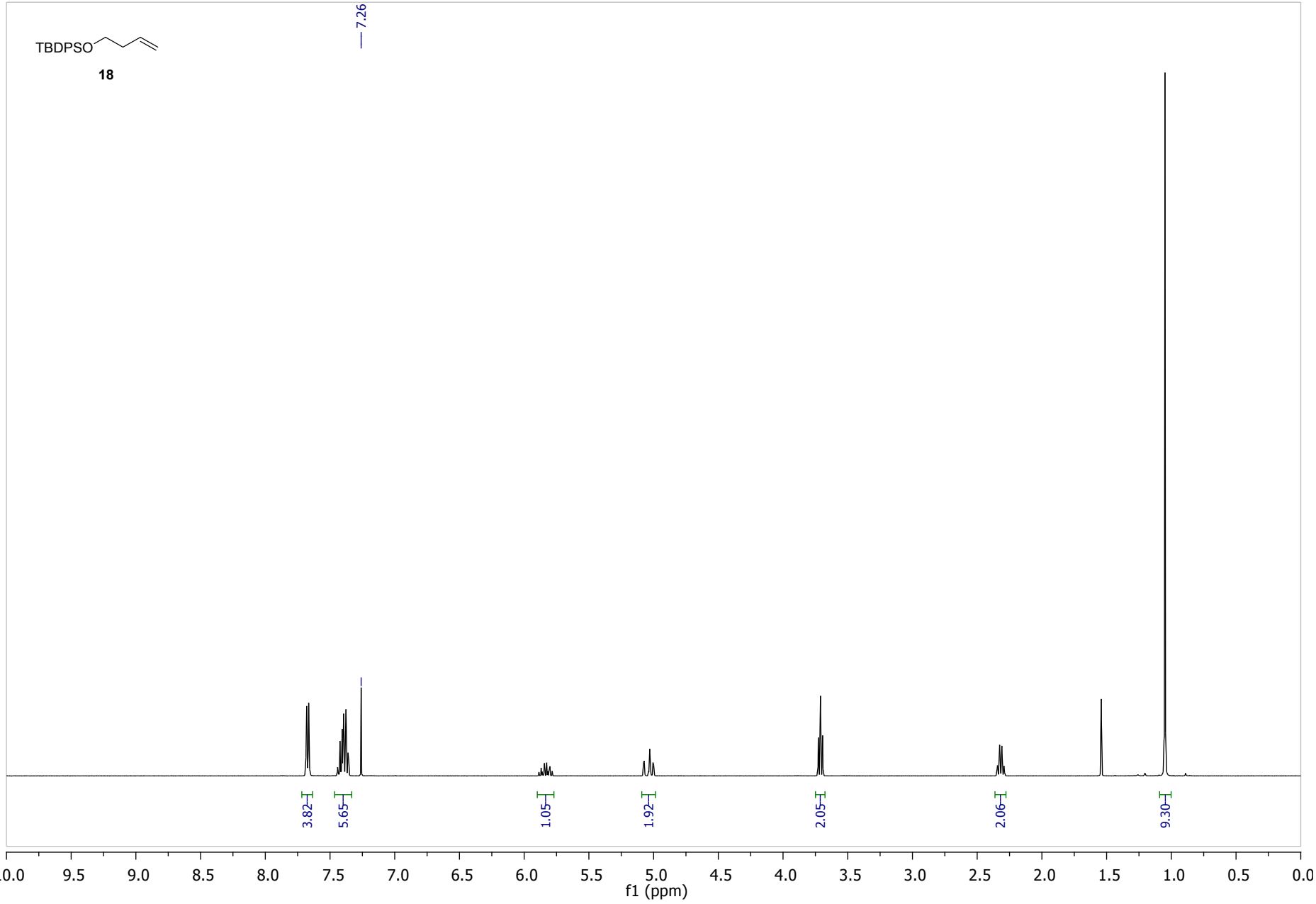

OH
HO
NHBoc
O
O
O
15
161.90
161.52
156.17
155.84
143.69
131.70
128.92
127.42
113.27
113.11
112.70
101.78
79.95
77.48
77.16
76.84
72.86
69.00
62.53
54.78
37.54
28.49
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

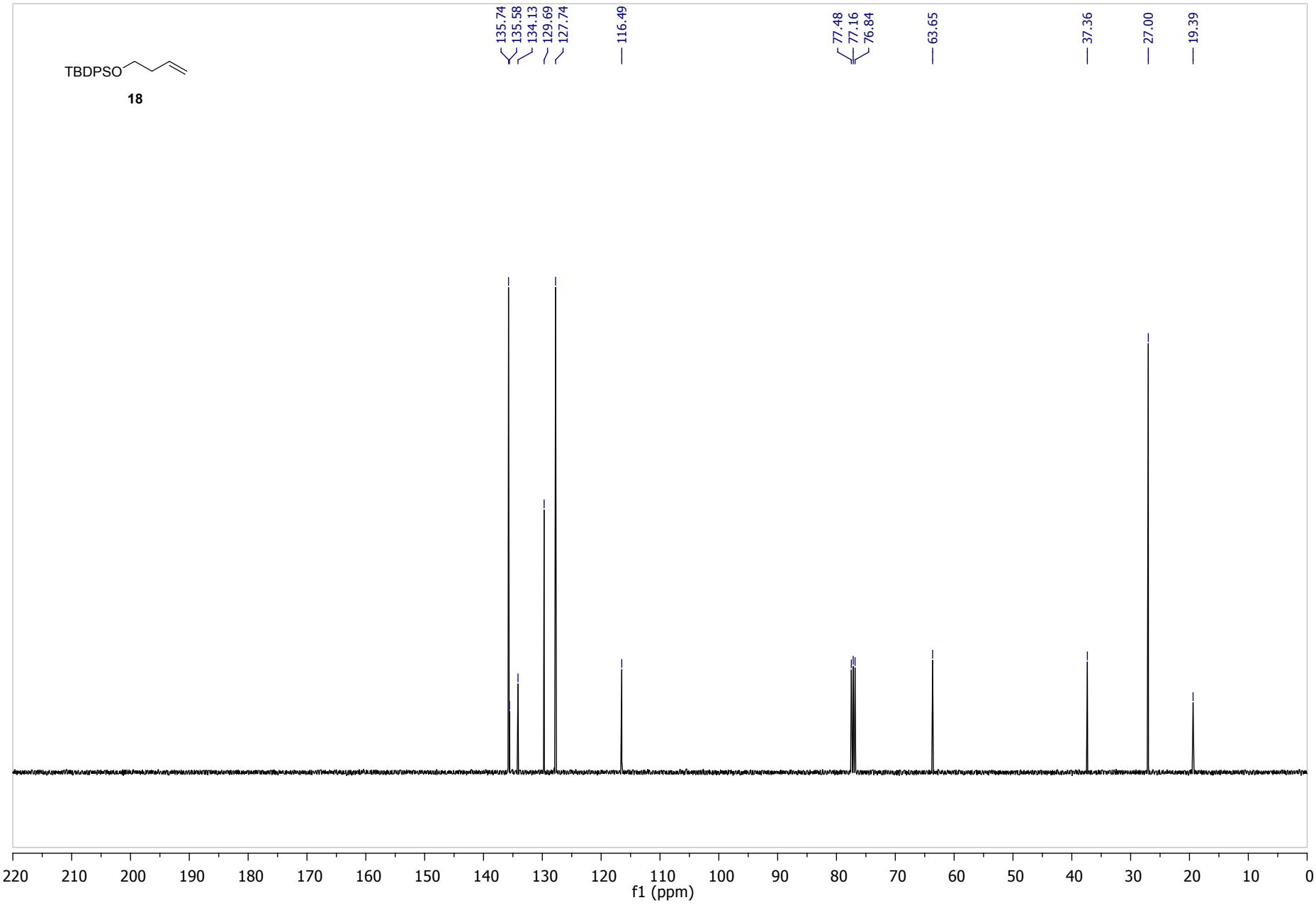

7.26
HO
O
O
O
16
1.00
1.03
1.96
0.96
1.98
1.98
1.97
2.04
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

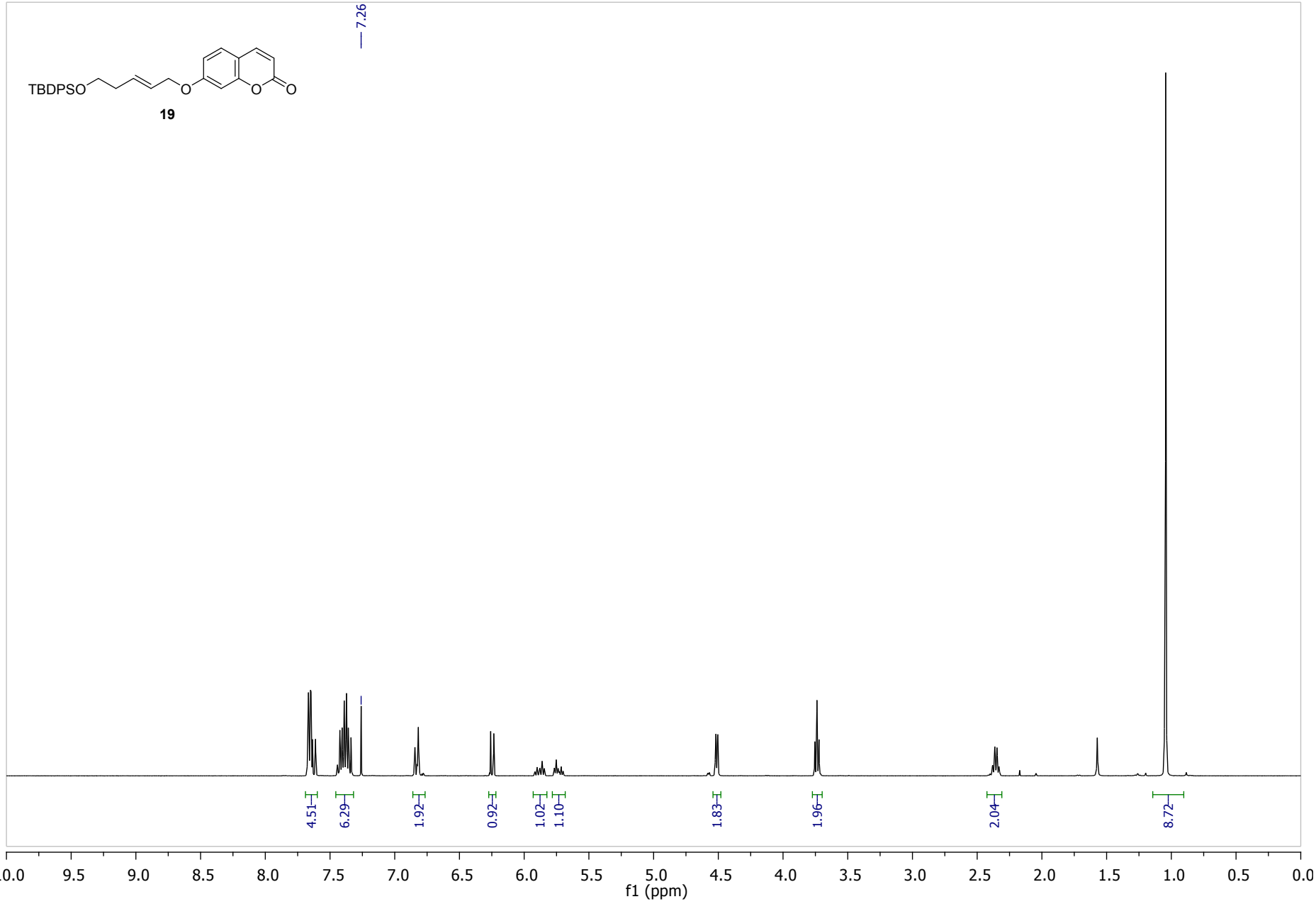

HO
O
O
O
16
162.20
161.41
155.96
143.59
132.18
128.89
127.56
113.16
113.07
112.66
101.50
77.48
77.16
76.84
67.95
63.49
31.96
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

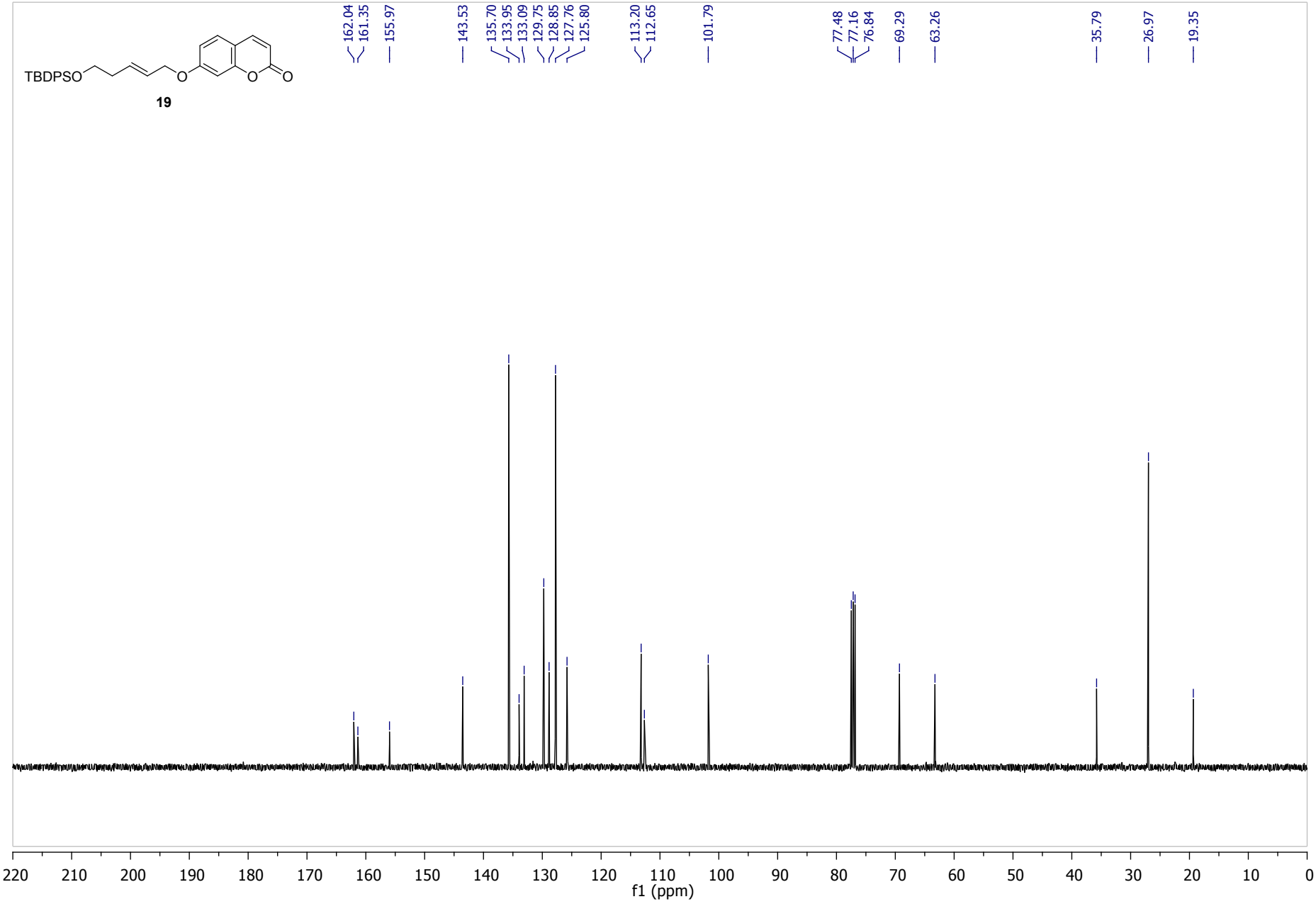

7.26
O
H
O
O
O
17
0.91
1.05
1.06
1.02
2.07
1.87
2.11
2.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

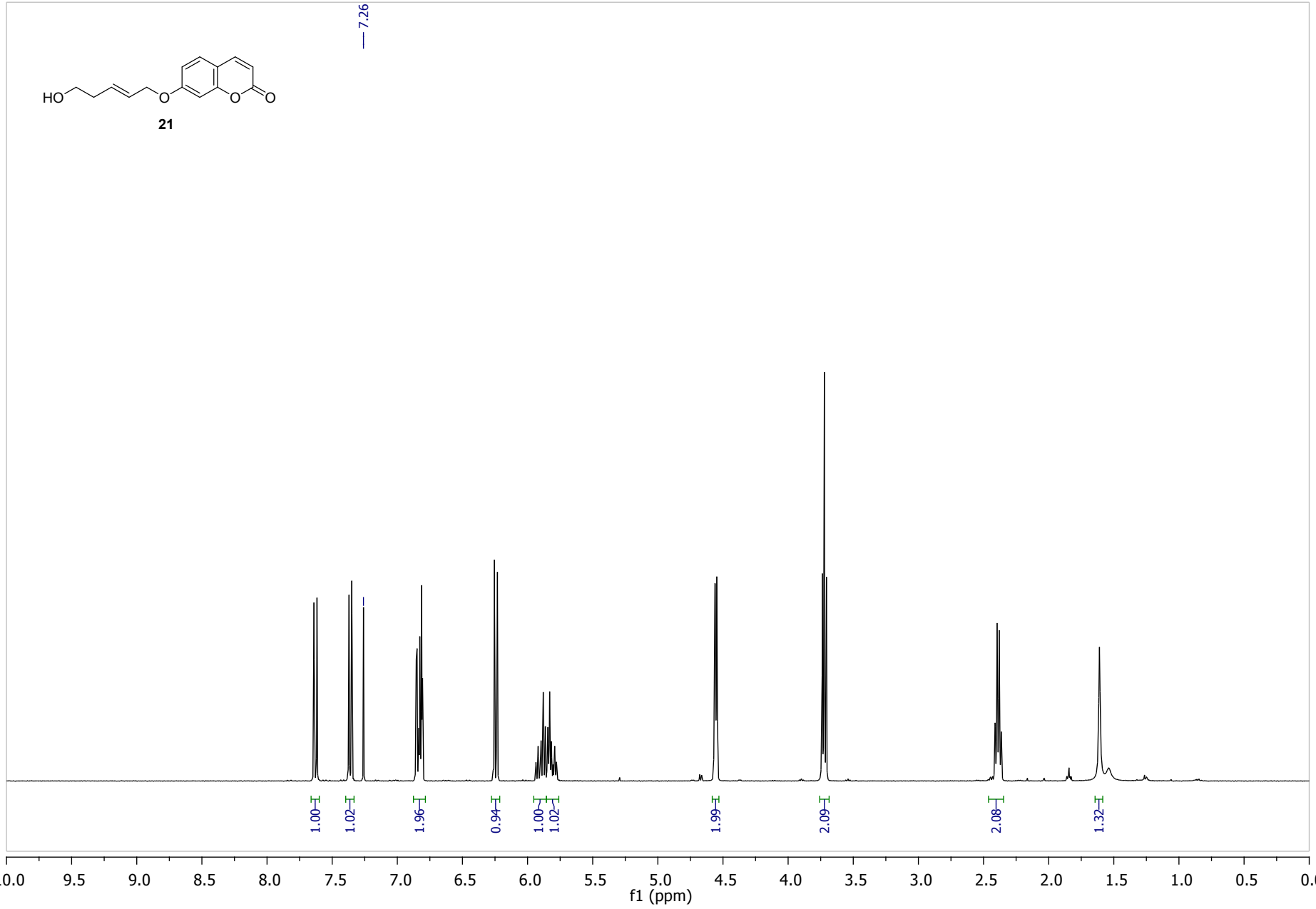

193.68
161.68
161.17
155.95
153.03
143.44
134.93
129.03
113.55
112.99
101.49
77.48
77.16
76.84
66.24
32.27
O
H
O
O
O
17
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

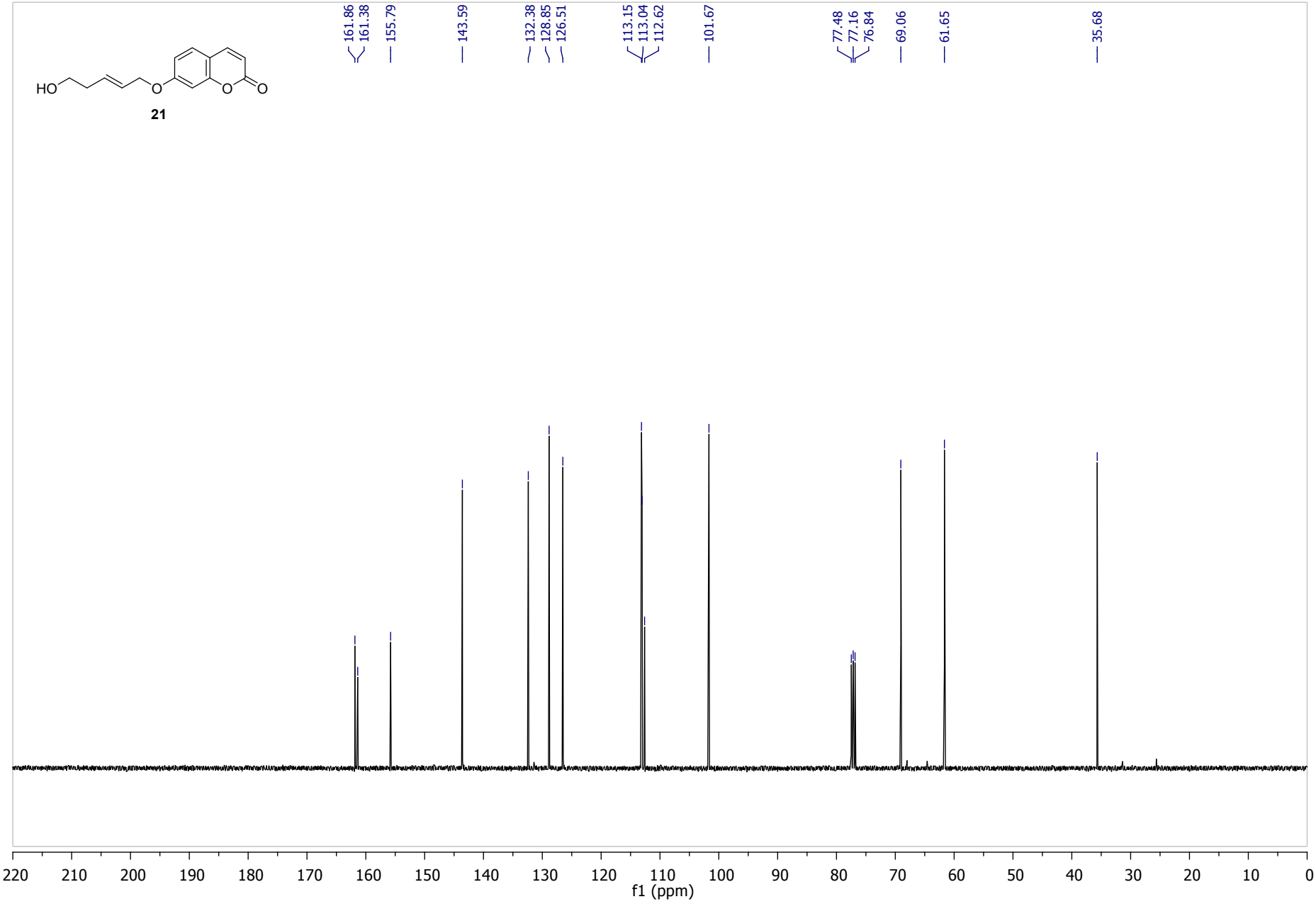

TBDPSO
18
7.26
3.82
5.65
1.05
1.92
2.05
2.06
9.30
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

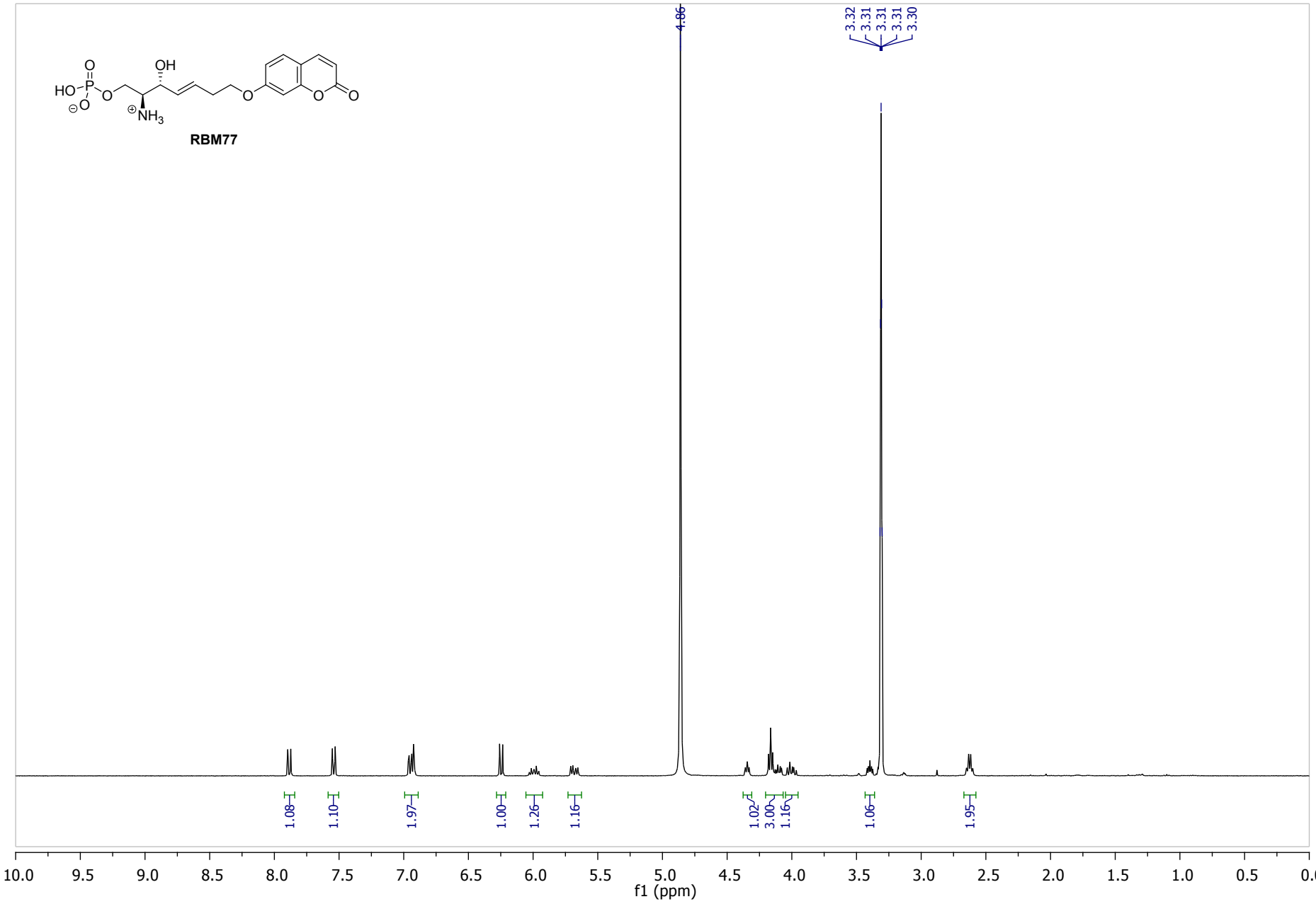

TBDPSO
18
135.74
135.58
134.13
129.69
127.74
116.49
77.48
77.16
76.84
63.65
37.36
27.00
19.39
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

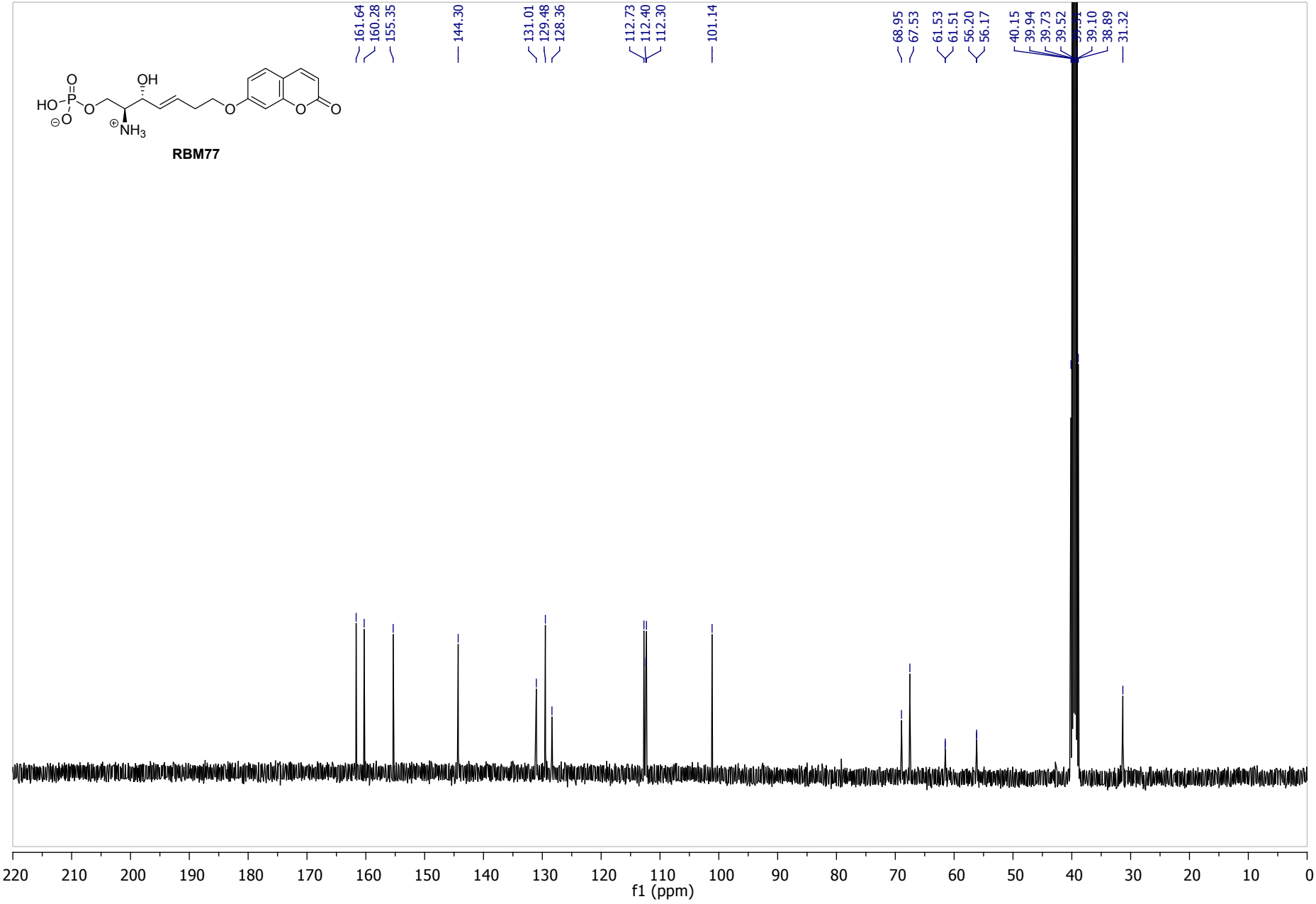

TBDPSO
O
O
O
19
7.26
4.51
6.29
1.92
0.92
1.02
1.10
1.83
1.96
2.04
8.72
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

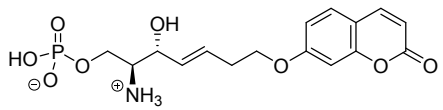

TBDPSO
O
O
O
19
162.04
161.35
155.97
143.53
135.70
133.95
133.09
129.75
128.85
127.76
125.80
113.20
112.65
101.79
77.48
77.16
76.84
69.29
63.26
35.79
26.97
19.35
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.26
HO
O
O
O
21
1.00
1.02
1.96
0.94
1.00
1.02
1.99
2.09
2.08
1.32
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


HO
O
O
O
21
161.86
161.38
155.79
143.59
132.38
128.85
126.51
113.15
113.04
112.62
101.67
77.48
77.16
76.84
69.06
61.65
35.68
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

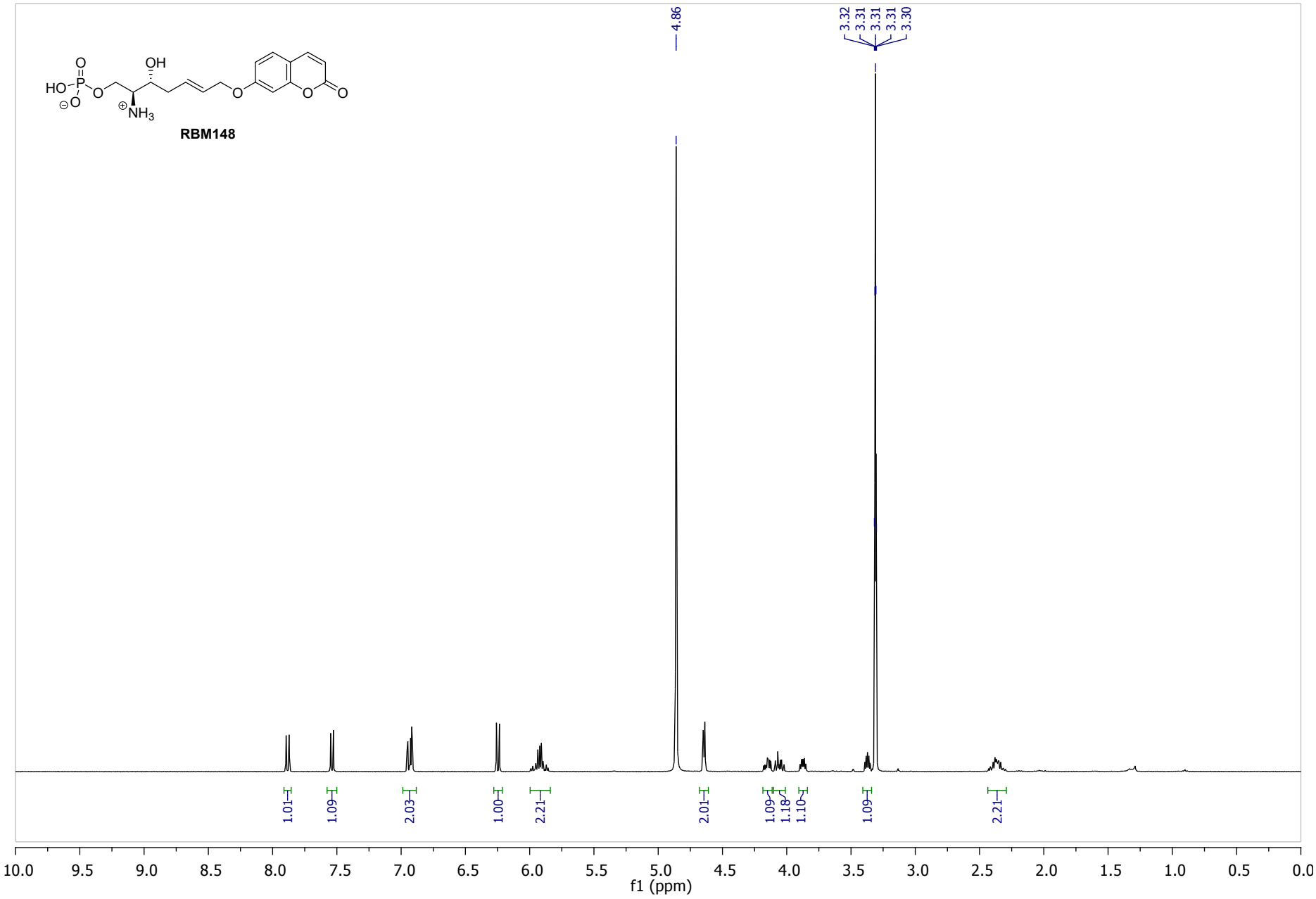

RBM77
4.86
3.32
3.31
3.31
3.31
3.30
1.08
1.10
1.97
1.00
1.26
1.16
1.02
3.00
1.16
1.06
1.95
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

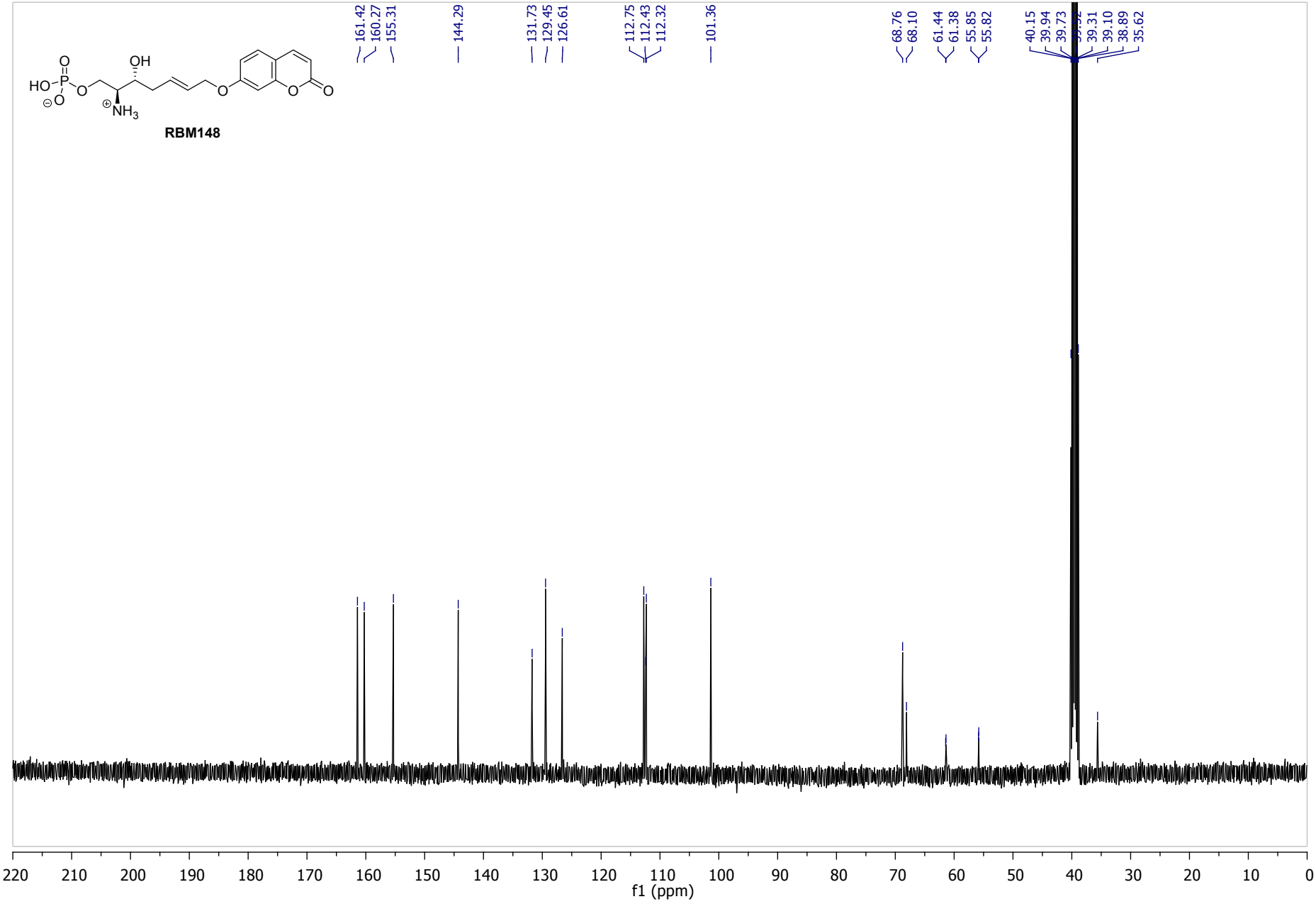

161.64
160.28
155.35
144.30
131.01
129.48
128.36
112.73
112.40
112.30
101.14
68.95
67.53
61.53
61.51
56.20
56.17
40.15
39.94
39.73
39.52
39.10
38.89
31.32
RBM77
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

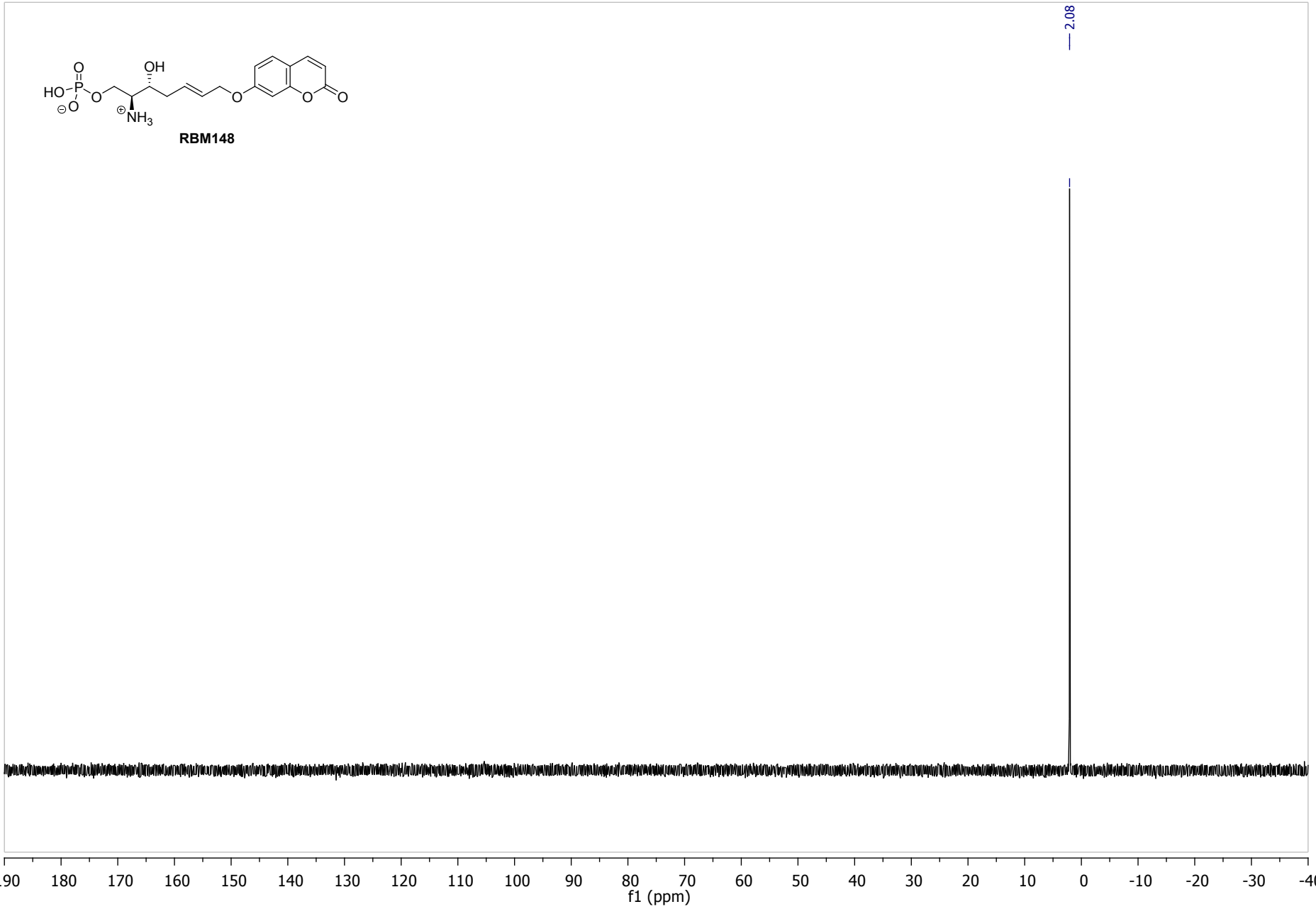

HO
P
O
O
O
OH
NH3
O
O
O

RBM77

1.84
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)


RBM148
4.86
3.32
3.31
3.31
3.31
3.30
1.01
1.09
2.03
1.00
2.21
2.01
1.09
1.18
1.10
1.09
2.21
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


RBM148
161.42
160.27
155.31
144.29
131.73
129.45
126.61
112.75
112.43
112.32
101.36
68.76
68.10
61.44
61.38
55.85
55.82
40.15
39.94
39.73
39.52
39.31
39.10
38.89
35.62
f1 (ppm)


RBM148
2.08
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)