

AN ABSTRACT OF THE CAPSTONE REPORT OF

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Title: Synthesis and photochemical studies of two p-hydroxyphenacyl derived photocages

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Date of Project: April 15, 2019-April 29, 2020

Abstract approved:

David M. Chenoweth, Principle Investigator

Abstract:

The synthesis of new p-hydroxyphenylacyl (pHP) derived photocages containing either coumarin or benzothiazole fluorophore cores is described. The newly synthesized photocages exhibit absorption spectra extending beyond 400 nm and good photolysis efficiency. The pHP inspired design, with the insertion of a carbonyl group, provides a 50-60 nm bathochromic shift and efficient photocleavage. Caged chemical probes for controlled protein dimerization were prepared containing the newly developed pHP photocages. In vitro experiments verified efficient photochemical uncaging of the newly developed pHP chemical probes. Cell imaging experiments utilizing the new pHP caged probes are currently in progress.

Synthesis and photochemical studies of two
p-hydroxyphenacyl derived photocages

By

Changfeng Deng

A CAPSTONE REPORT

submitted to the

University of Pennsylvania

in partial fulfilling of
the requirements for
the degree of

Master of Chemical Sciences

Presented April 24, 2020
Commencement May 18, 2020

Master of Chemical Sciences Capstone Report of Changfeng Deng presented on April 24, 2020.

APPROVED:



David M. Chenoweth, Organic Chemistry

I understand that my Capstone Report will become part of the permanent collection of the University of Pennsylvania Master of Chemical Sciences Program. My signature below authorizes release of my final report to any reader upon request.



Changfeng Deng, Author

Acknowledgements

Along the way to my Masters degree, many people have offered their help to advance me to the stage where I am now. First and foremost, I would like to thank my parents and my elder brother for financial and emotional support.

I would like to express my deep and sincere gratitude to my research advisor, Dr. David M. Chenoweth. He is not only offering professional guidance to the research project, but also helping with problems outside the experiments. He is open-minded and inspiring in research ideas. Every time after I talk with him about problems, I would regain my motivation and energy to move forward. I would also express sincere thanks to every group member in the Chenoweth lab. They are not only colleagues, but also friends and family members. When I have problems with synthesis, talking to them always helps me a lot. They are always more than willing to help whenever I am in need.

I am extremely grateful to MCS program director, Dr. Ana-Rita Mayol-Cabassa and her two assistants. Ana-Rita is helpful and patient with any problems students might have. I am also very thankful to all my friends in or outside the program. Without their accompany, I could not have such a meaningful and colorful life at Penn.

Finally, I would like to thank my secondary reader, Prof. E. James Petersson and all the professors who give me lectures. The knowledge they endow me grants me the ability to deal with challenges coming out in the research field.

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Introduction

Protein-protein interactions (PPIs) play a significant role in biological processes, such as signal transduction, cellular metabolism, transport and regulation.¹ One of the common PPI mechanisms exploited by the cell is protein localization. Functional regulation of proteins often relies on specific subcellular context or multi-component assembly.² Appropriate protein localization is crucial to the maintenance of cellular activity, while aberrant localization can result in many metabolic, cardiovascular and neurodegenerative diseases.³

Many methods have been utilized to elucidate PPI *in vivo* and *in vitro*, including fluorescence imaging, protein microarrays, X-ray crystallography and NMR spectroscopy to name a few.^{4,5} One of the most popular techniques for controlling PPIs is chemically-induced dimerization (CID) of proteins, in which two proteins bind together in the presence of small molecules. As shown in **Figure 1A**, a chemical dimerizer is often bifunctional with a recognition site at each end that can be recognized by two different dimerization domains (DDs). When the two DDs recognize and bind to the protein, they are considered dimerized. In **Figure 1B**, any proteins of interest can be attached to a pair of chemically dimerizable protein domains (with DDs) through standard protein engineering technique. Once a chemical dimerizer induces the recruitment of DDs, POI 1 and POI 2 are able to undergo proximity-induced interactions, which can be used to modulate downstream biological effect.

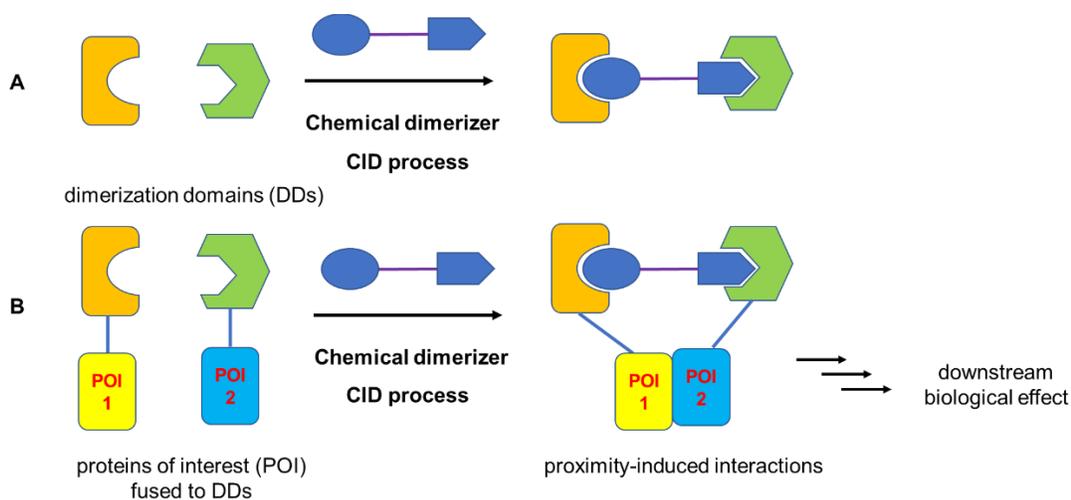


Figure 1. Schematic diagrams illustrating the basic principles of chemically-inducible dimerization(CID). (A) Dimerization of two proteins. (B) CID can be exploited in inducing protein-protein interactions by proximity effects.

As a potent tool to realize spatial and temporal control of protein translocation in cells, tremendous progress has been made in the CID system.⁴ The first reported chemical dimerizer was FK506, developed by the Crabtree and Schreiber groups in 1996, inducing FKBP and calcineurin A (CnA) dimerization.⁶

A major limitation of typical CID systems is the lack of reversibility and precise “switch-on” control. A promising strategy to realize “switch-on” control is to create a photoactivated CID system by introducing photocages to block key molecular recognition interactions that are critical for protein dimerization.

Photocages, often called photoremovable or photocleavable groups, are widely used in biochemistry, neurobiology, biomedicine, polymerization, and fluorescence activation.⁷ When exposed to certain wavelengths of light, the photocage can be released and turn the caged molecule from the inactive state to active state. Common photocages can be categorized into arylcarbonylmethyl groups, nitroaryl groups, (coumarin-4-yl)methyl groups, and arylmethyl groups.⁷ As shown in **Figure 2**, the Chenoweth group has already developed o-nitrobenzyl)-based **1** (NTH) and (coumarin-4-yl)methyl-based **2** (CTH) photocage probes for bioimaging, with 405 nm and 444 nm photocleavage wavelengths, respectively.⁸ In **Figure 2**, the photocage is shown in red, the trimethoprim (TMP), the ligand for *Escherichia coli* dihydrofolate reductase (eDHFR)⁹ is shown in blue, and chlorohexane, the ligand for the HaloTag protein, is shown in green. The purple part serves as a linker to provide space between the two recognition sites. The entire probe molecule consists of three components, photocage(red), carbamate linker(black) and dimerizers (blue, purple, and green). In the photolysis of NTH and CTH in the cell, the probe molecule first only recognizes and binds to the HaloTag protein. The photocage provides a steric block, preventing TMP from binding to eDHFR. Upon irradiation, photocleavage removes the photocage and frees TMP for binding, which binds to eDHFR afterwards. As a result, the eDHFR and HaloTag proteins heterodimerize. In this way, light controlled “switch-on” chemically-induced dimerization is realized.

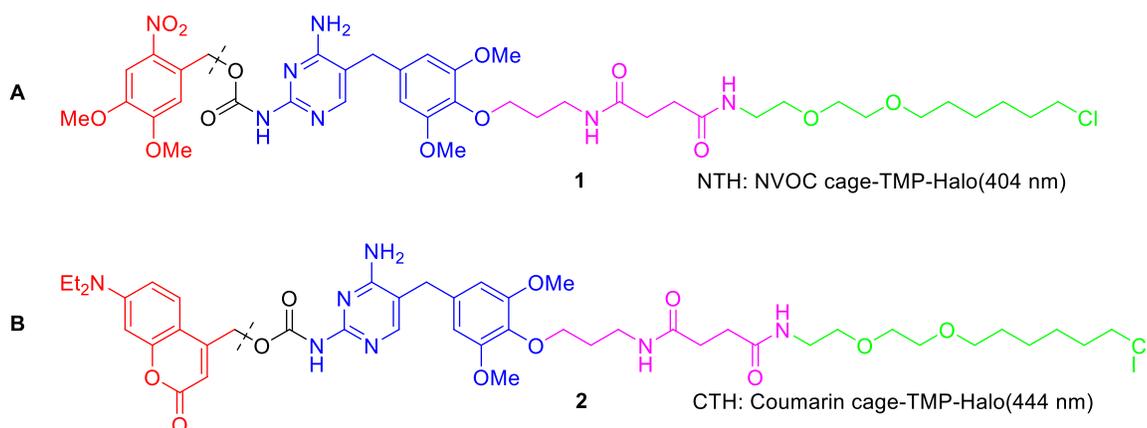


Figure 2. O-Nitrobenzyl group caged and coumarin caged optogenetic probes

Despite the efficiency of NTH and CTH as optogenetic chemical probes, there are still many improvements needed to expand their utility. The NVOC photocage has an absorption maxima around 350 nm and the coumarin cage has an absorption maxima around 375 nm.⁷ Their irradiation wavelength still only barely reaches the visible light region and their quantum efficiency of photocleavage is still relatively low. Therefore, the Chenoweth lab is developing an expanded set of new photocages with higher photocleavage efficiency that can be cleaved by

longer wavelength light such as 400 nm to 600 nm. In the biological imaging field, long wavelength light is easier to penetrate deep inside tissue and causes less damage to the cell.⁷

In this report, several p-hydroxyphenacyl (pHP) chromophores are synthesized, and then their capacity to cage the TMP-Halo dimerizer are explored. The inspiration for focusing on the pHP chromophore originates from their usefulness in caging nicotinamide, part of cofactor NAD(P)/NAD(P)H. Previous studies explored various photolabile groups to cage the nicotinamide, which can be used in the study of enzyme behavior and correlated biocatalytic mechanisms. For example, o-nitrobenzyl caged nicotinamide reported by Patron had short wavelength absorption (<300 nm), as well as low photorelease rate and yield.¹⁰ Wiest and Helquist also reported that coumarin caged nicotinamide shared similar drawbacks even if the absorption wavelength could go above 300 nm. However, the adoption of pHP improved the quantum yield from 1-2% to 11%.¹¹ Hence, if the pHP chromophore is successful in caging nicotinamide, it is believed to maintain superior quantum yield and cleavage yield when applied to the NTH and CTH photocage probe systems developed by the Chenoweth group.

Even if the pHP photocage has high quantum yield, the prototypical pHP chromophore(4-Hydroxyacetophenone) is reported to have an absorption maximum at 273 nm under neutral conditions and 330 nm under basic conditions,¹² which is highly unfavorable according to general criteria for photocages (>300 nm).¹³ Therefore a good modification is to substitute the phenyl core with more electron rich heterocyclic rings. This project provides preliminary assessment of photophysical properties of pHP photocages with altered aromatic cores and their potential to be utilized in the Chenoweth group probe system.

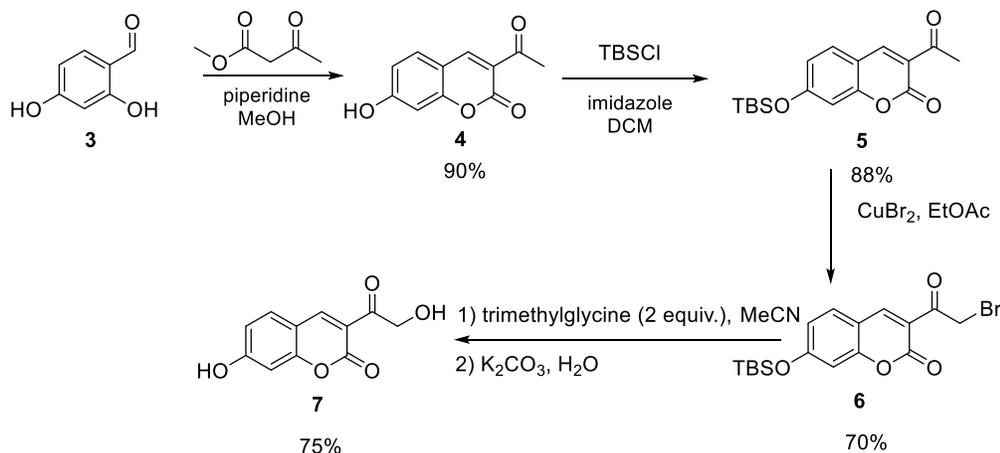
The goal of the project is to develop new photocages with longer absorption wavelength over 400 nm and a quantum yield higher than 5%. More specifically, pHP photocages with coumarin and benothiazole cores will be synthesized, characterized and applied to photolysis experiments. Accordingly, the experiment is divided into three parts, synthesis of target photocages, characterization of their photophysical properties and photolysis experiments *in vitro* and *in vivo*.

Results and Discussion

Scheme 1 shows the synthesis of two photocages used as precursors of the target molecule. In **Scheme 1A**, 2,4-dihydroxybenzaldehyde **3** reacts with methyl acetoacetate via Knoevenagel condensation and successive cyclization to afford 3-acetyl-7-hydroxycoumarin **4**. The subsequent protection of the hydroxy group produces compound **5**, which gets brominated with copper (II) bromide to afford 3-(bromoacetyl)coumarin **6**. The alcohol **7** is obtained with trimethylglycine hydrolysis condition and basic workup. Alcohol **7** is prepared through four steps in 42% overall yield. In **Scheme 1B**, when starting with 4-(N,N-diethylamino)salicylaldehyde **8**, 3-acetyl-7-(diethylamino)coumarin **9** is obtained through the same mechanism. Bromination with Br₂ produces compound **10**, which undergoes hydrolysis to obtain alcohol **11**. The alcohol **11** is prepared through three steps in 44% yield. The optimization and discussion of bromination and hydrolysis step is shown below.

Scheme 1. Synthesis routes of alcohol **7** and alcohol **11**.

A Synthesis of hydroxy coumarin alcohol



B Synthesis of diethylamino coumarin alcohol

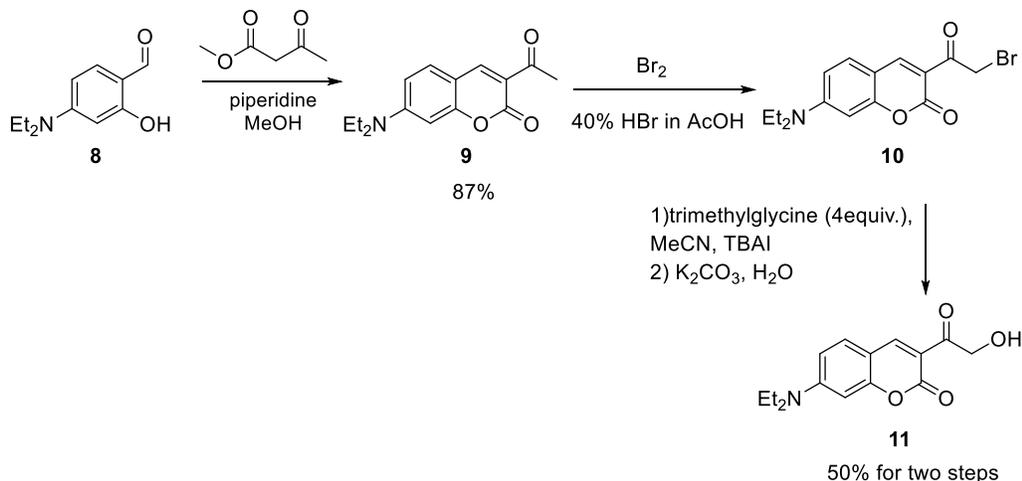


Table 1. Optimization of transformation from ketone to α -bromo ketone

entry	reagent	solvent	yield
1	NBS, p-MePhSO ₃ H	DCM	23% ^a
2	NBS, TFA	DCM	21% ^a
3	NBS, TFA	MeCN	14% ^a
4	TMABr ₃	MeOH/DCM=1:1	59% ^a
5	CuBr ₂	EtOAc/DCM=1:1	100% ^b
6	DDB	Et ₂ O/dioxane=1:1	100% ^b

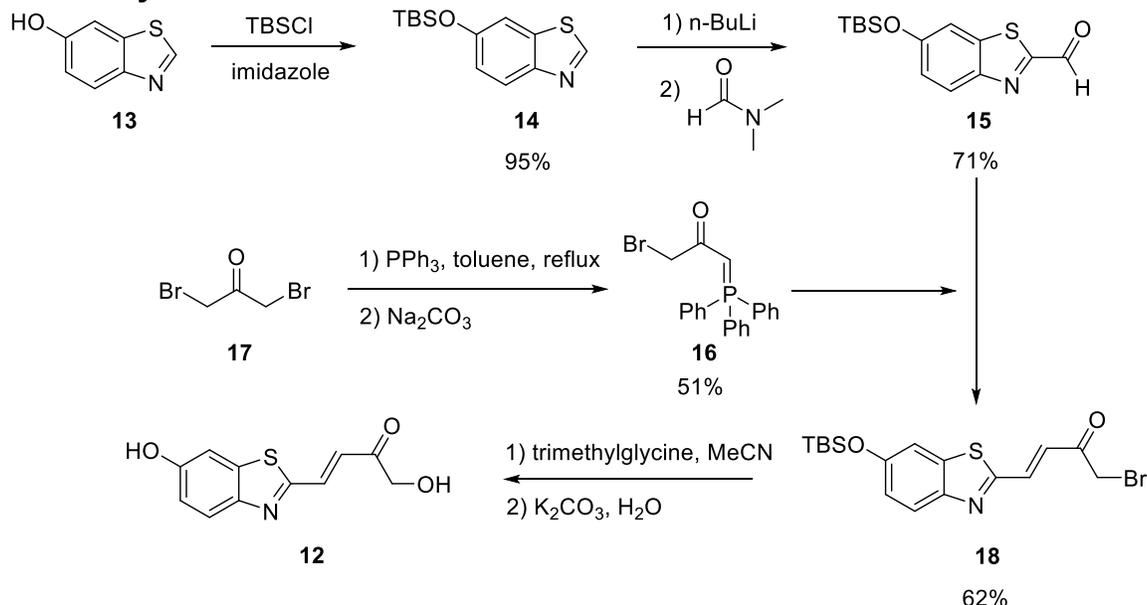
^a NMR yield calculated from starting material and product ratio. ^b LCMS conversion of starting material.

In order to determine a general procedure to prepare α -bromo ketone species, the investigation into bromination conditions of compound **5** is shown in **Table 1**. From the result, a radical pathway (entry 1-3) with NBS results in low yield. Entry 5 with CuBr₂ and entry 6 with DDB (solidified Br₂) stand out as efficient bromination reagents because of the high yield, but CuBr₂ predominates as a greener option without bromine involved. The Br₂/HOAc method is exploited specifically in the preparation of compound **6** instead, because the basicity and coordination ability of -NET₂ group are likely to reduce the efficiency of CuBr₂. For the hydrolytic conversion of **6** to **7**, highly basic conditions such as NaOH in H₂O/THF result in direct cleavage of C-Br bond. Refluxing with HCOONa in MeOH gives alcohol product but also generates many other side products at the same time. After screening many hydrolysis conditions, trimethylglycine is the most promising hydrolysis reagent, which creates an acidic environment for bromide to ester transformation and only requires mildly basic conditions in the workup process. To obtain alcohol **7** more efficiently, tetrabutyl ammonium iodide is used to mediate C-Br bond cleavage through halogen exchange.

The preparation of compound **12** is shown in **Scheme 2**. The synthetic route starts from 2-hydroxybenzothiazole **13**, silylation with TBSCl results in compound **14**. Subsequent formylation with n-BuLi and DMF obtains aldehyde **15**. The Wittig reagent **16** is prepared from 1,3-dibromopropan-2-one **17** in refluxing toluene. The aldehyde reacts with the Wittig reagent to give compound **18**. Standard hydrolysis conditions result in alcohol **12**. Compound **18** can be prepared in three steps in 41% yield. The hydrolysis yield has not been determined yet.

After the synthesis of the photocages, their photophysical properties were measured to determine which wavelength can be used to cleave the photocages. The absorption spectrum of the probe molecule can be considered as the superposition of the absorption of three individual parts. The absorption of the carbamate linker and dimerizer are below 300 nm, and therefore the absorption of the photocages can represent the whole probe molecule in the region above 300 nm.

Scheme 2. Synthesis route of alcohol 12.

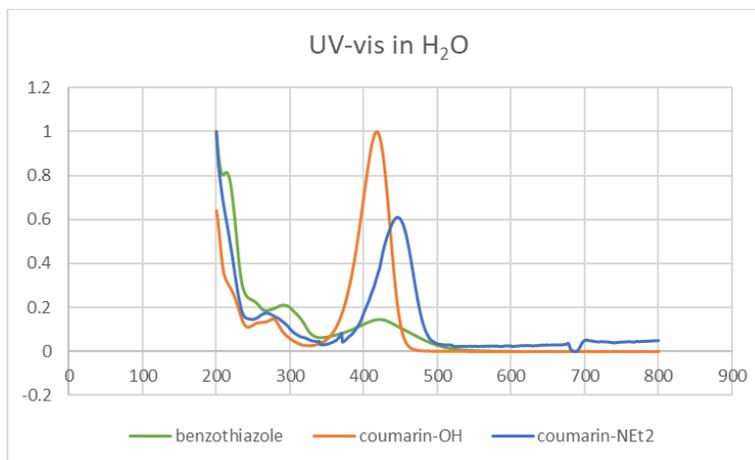


The normalized absorption spectra of alcohols **7**, **11** and **12** are shown in **Figure 3A** (H₂O) and **Figure 3B** (MeOH). The orange line corresponds to alcohol **7**, with two absorption maxima at 363 nm and 426 nm in methanol. A single red-shifted absorption maximum located at 418 nm was observed for alcohol **7** in water. Alcohol **11**, which is shown in blue line, has an absorption maximum at 437 nm in methanol and 446 nm in water. These results confirmed that -NEt₂ is a superior electron donating substituent, resulting in a 10-25 nm red shift in the absorption maximum. Alcohol **12**, which corresponds to green line, has two absorption maxima at 270 nm and 362 nm in methanol, whereas a significant red shift in aqueous environment results in a 290 nm and 421 nm absorption maximum. To explain the trend, literature research into luciferin and oxyluciferin species indicates that the strength of the O-H bond has a large impact on the excitation and emission spectrum.¹⁴

The carbamate linker is used to append the synthesized alcohol onto the TMP-Halo dimerizer, and the carbamate synthesis was investigated. Common carbonyl reagents such as 1,1'-carbonyldiimidazole (CDI), phosgene, triphosgene and miscellaneous carbonates all resulted in low levels of product. A carbon dioxide method to synthesize carbamates represents a green and safe alternative compared to classical CO or phosgene methods.¹⁵ A key step in this reaction relies on the amine reacting with carbon dioxide to generate a carbamic acid (RNHCOOH) or an ammonium carbamate (RNH₃⁺ RNHCOO⁻). Subsequent reactions can either result in direct attack of the bromide species via an S_N2 pathway or by treatment with a dehydrating reagent lead to production of an isocyanate followed by alcohol addition. However, owing to the low nucleophilicity of the amino group in TMP, instability of the carbamate ion and harsh pressure requirements, the CO₂ conditions were found to be recalcitrant. The more reactive carbonyl reagent 1,1'-

Carbonyl-di-(1,2,4-triazole) (CDT) improved the carbamate synthesis and is now used in the standard procedure. The standard method to prepare the complete probe molecule is shown in **Scheme 3**. Alcohol **7**, **11** and **12**, which all have the α -hydroxy ketone as a reactive moiety, reacts with dimerizer **19** to form carbamate probe **20**, **21** and **22**, respectively. Dimerizer **19**, which is currently used in the Chenoweth lab, is the modified version of the previous one shown in **Figure 2**.

A UV-vis of three alcohols in H₂O



B UV-vis of three alcohols in MeOH

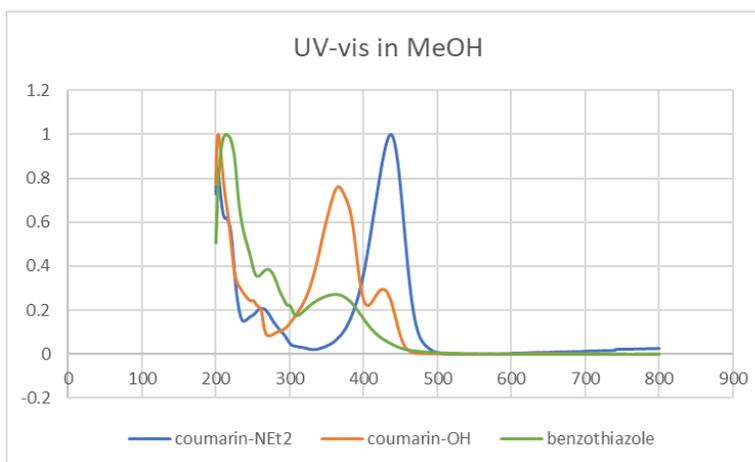
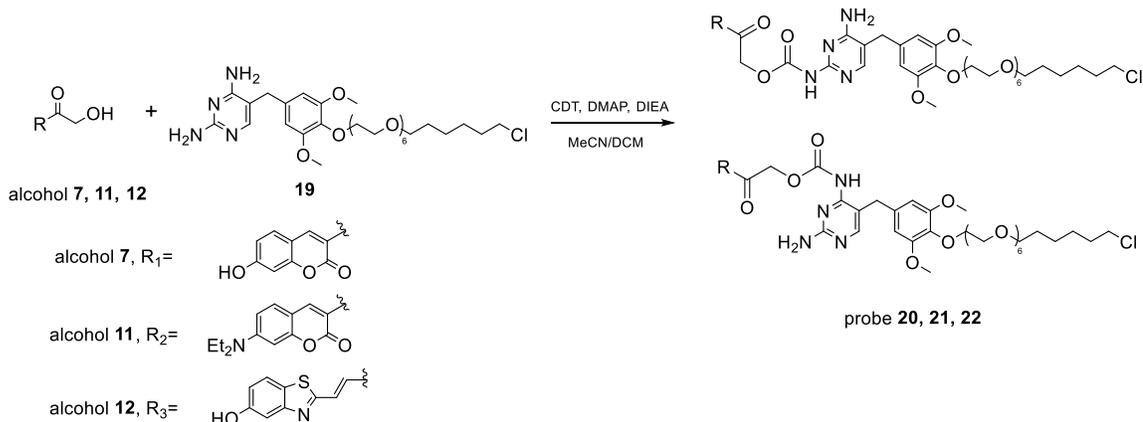


Figure 3. Normalized UV-vis spectrum alcohols **7** (orange line), **11** (blue line) and **12** (green line) in water and methanol.

Scheme 3. Carbamate synthesis

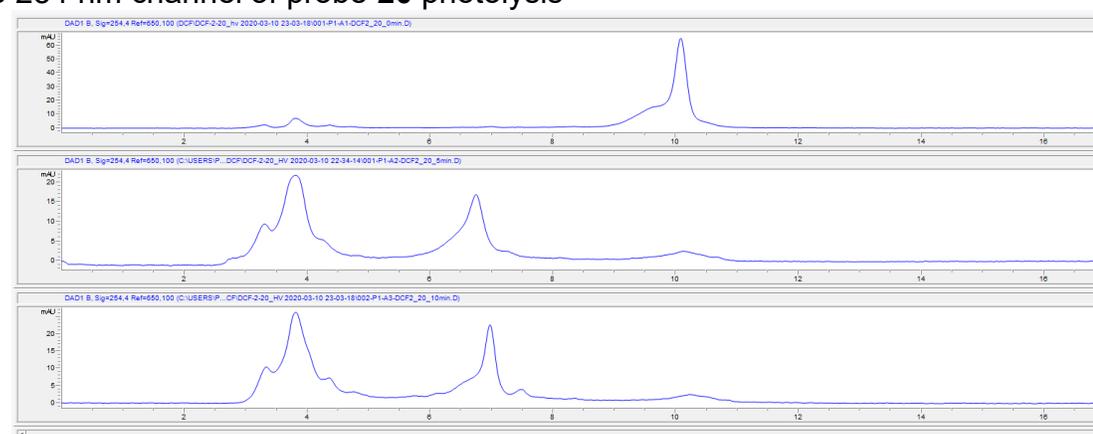


After the synthesis of the probe molecules, they are tested for *in vitro* photolysis efficiency in a photoreactor and the results were monitored by analytical HPLC. Three parallel chromatograms in each subfigure correspond to 0 min, 5 min and 10 min irradiation. The broad peak with a retention time around 4 min is the DMSO solvent front, which has a very strong 214 nm signal and therefore influences the intensity scale of 214 nm channel dramatically. The hydrolysis result of probe **20** is shown in **Figure 4**. From HPLC chromatogram, probe **20** with a retention time around 10 min has an apparent 254 nm and 365 nm absorption. After 5 min irradiation, almost all probe molecule gets consumed and a new peak appears around 7 min without 365 nm signal, which corresponds to the uncaged compound **19**. The Ten-minute trace is the same as the five-minute one, which indicates reaching a balance or completion of photolysis in 5 min. The absorption of probe **21** is 437 nm, which is located between 420 nm and 450 nm. Therefore, two photolysis wavelengths were tested as shown in **Figure 5** (420 nm) and **Figure 6** (450 nm). The data demonstrates that the amount of probe **21** is reduced to one tenth for every five minutes in both 420 nm and 450 nm. The estimated photocleavage half time is 1.5 min. Overall, these results indicate that the two photocages are efficiently cleaved during *in vitro* photolysis experiment. Considering the laser light used for cell imaging experiment is far more efficient than the light source in the photoreactor, the two probes should be excellent candidates for further cell imaging experiments and detailed evaluation.

A 214 nm channel of probe 20 photolysis



B 254 nm channel of probe 20 photolysis



C 365 nm channel of probe 20 photolysis

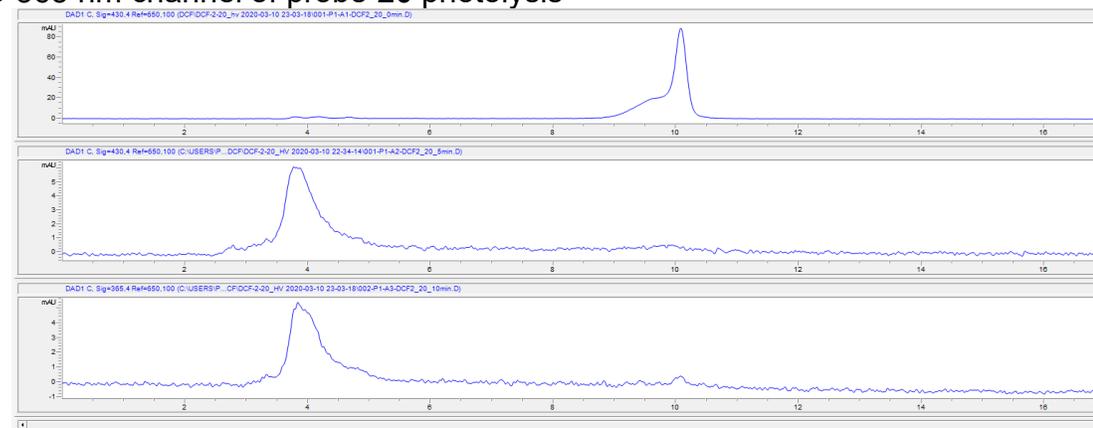
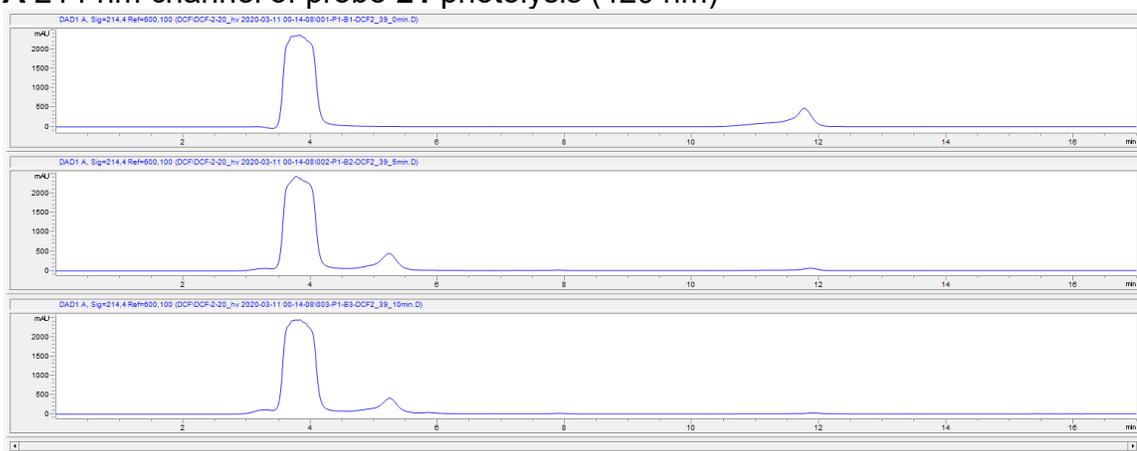
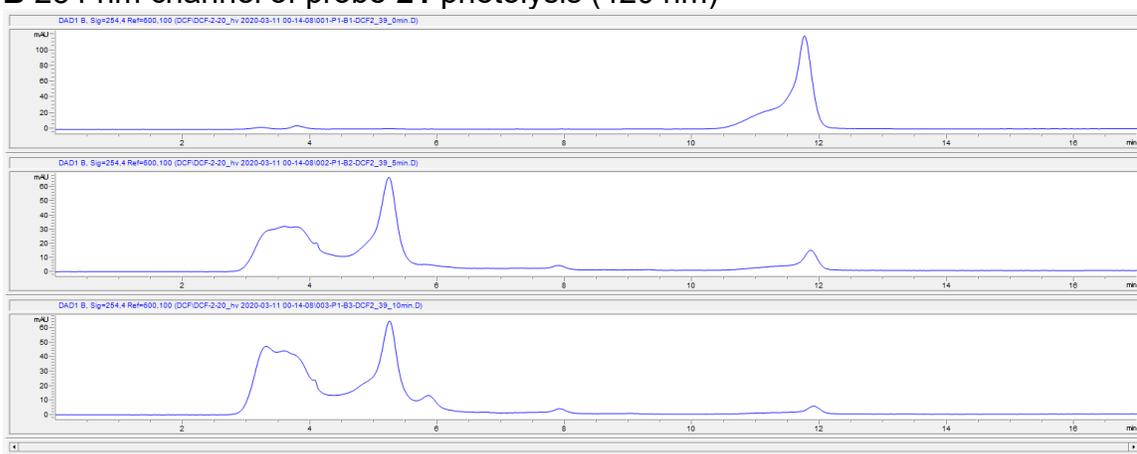


Figure 4. Photolysis of probe 20 under 420 nm (A, B and C correspond to 214 nm, 254nm, 365 nm signal respectively in HPLC)

A 214 nm channel of probe 21 photolysis (420 nm)



B 254 nm channel of probe 21 photolysis (420 nm)



C 430 nm channel of probe 21 photolysis (420 nm)

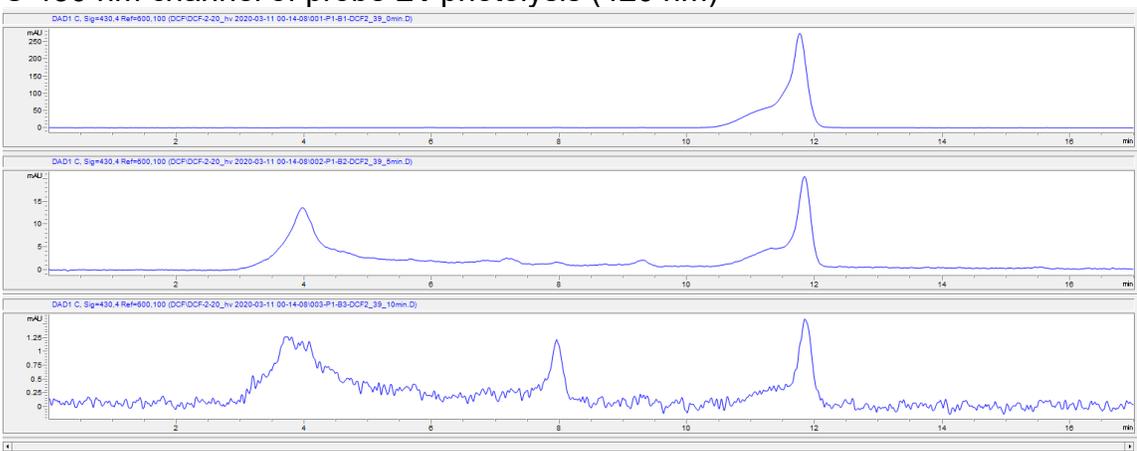
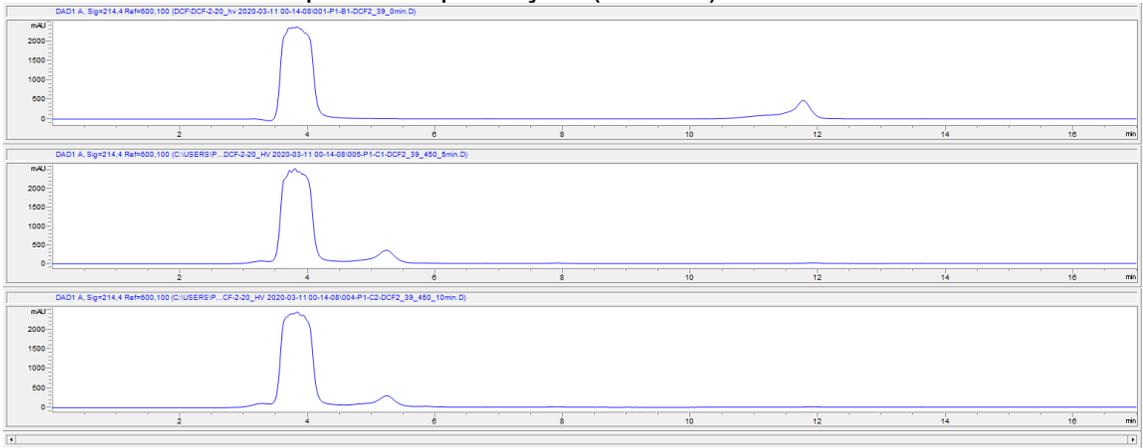
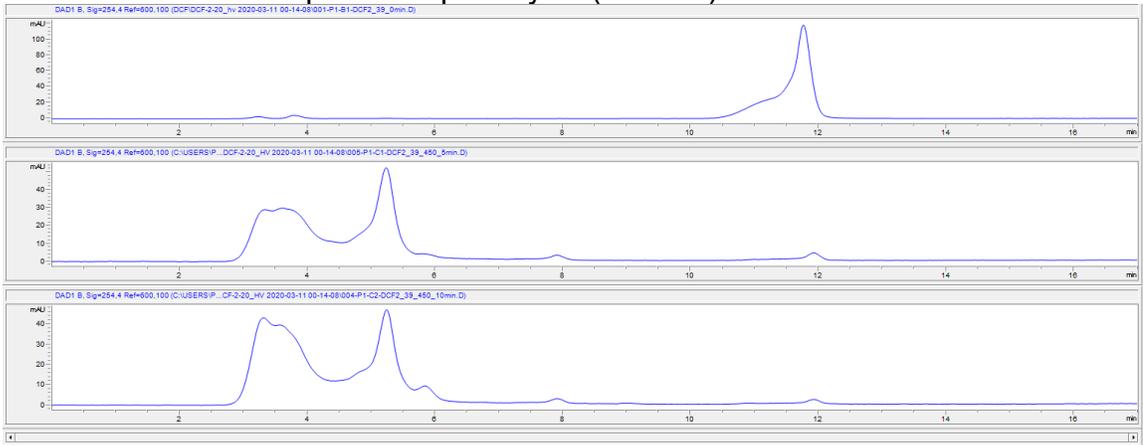


Figure 5. Photolysis of probe 21 under 420 nm (A, B and C correspond to 214 nm, 254 nm, 430 nm signal respectively in HPLC)

A 214 nm channel of probe 21 photolysis (450 nm)



B 254 nm channel of probe 21 photolysis (450 nm)



C 430 nm channel of probe 21 photolysis (450 nm)

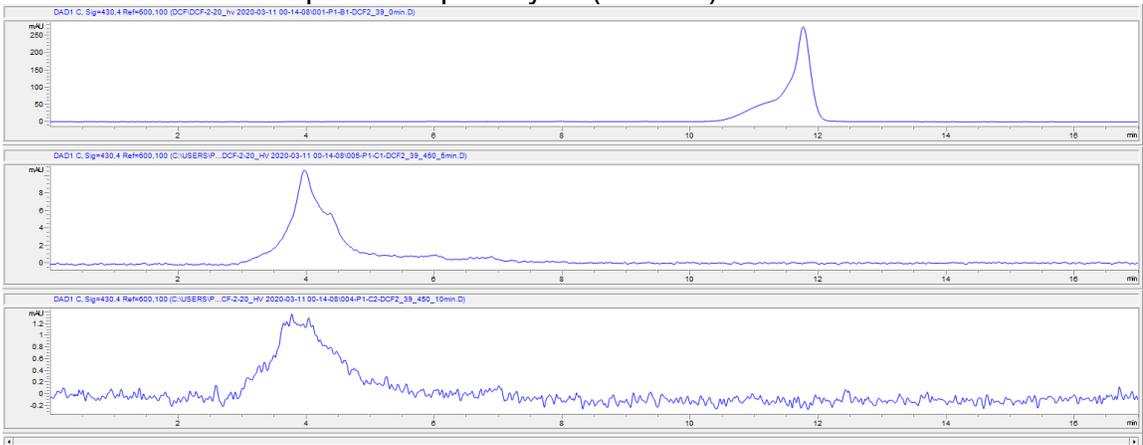


Figure 6. Photolysis of probe 21 under 450 nm (A, B and C correspond to 214 nm, 254 nm, 430 nm signal respectively in HPLC)

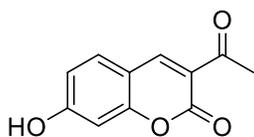
Materials and Methods

All commercially available reagents and solvents were used as received. NaH (60% in mineral oil), and 48% aqueous HBr solution were purchased from Acros Organics. 6-chloro-1-bromohexane, Trimethoprim was purchased from Astatech, Inc. and the rest of the chemicals were purchased from Sigma Aldrich. All chemicals were used without further purification.

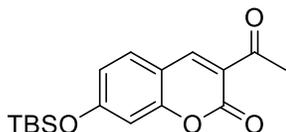
Thin-layer chromatography (TLC) was performed on Sorbent Technologies silica plates (250 μm thickness). Flash column chromatography was performed using Silicycle silica gel (55–65 \AA pore diameter). Automated flash column chromatography was performed using RediSep Rf silica gel on CombiFlash Rf+ system with internal UV detector. The instrument is available from Teledyne Isco, Inc., NE., USA. Ultraviolet-Visible (UV-Vis) absorption spectrophotometry was performed on a JASCO V-650 spectrophotometer with a PAC-743R multichannel Peltier using quartz cells with a 1 cm cell path length.

Proton nuclear magnetic resonance spectroscopy (^1H NMR) and Carbon nuclear magnetic resonance spectroscopy (^{13}C NMR) spectra were recorded on a Bruker UNI 400 MHz, AVII 500 MHz and Biodrx 600 MHz NMR and processed by MestReNova or Topspin software. ^1H NMR data are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet), and the peak integration. NMR solvents are CDCl_3 (7.26, singlet) and DMSO-d_6 (2.50, septet).

Low-resolution mass spectra were obtained using Liquid-Chromatography-Mass-Spectrometry (LCMS) on Waters instrument, electrospray ionization in either positive or negative mode. High-resolution mass spectra (HRMS) were obtained at the University of Pennsylvania's Mass Spectrometry Service Center on Waters LC-TOF mass spectrometer (model LCT-XE Primer) using electrospray ionization in positive or negative mode, depending on the analytes. HRMS data analysis was performed using the automated Waters software.

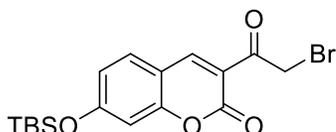


3-acetyl-7-hydroxy-2H-chromen-2-one 4. 2,4-dihydroxybenzaldehyde (2.7 g, 20 mmol) and methyl acetoacetate (2.6 mL, 24 mmol) were added into a round bottom flask and dissolved with 34 mL methanol. Afterwards 1 mL piperidine was added and the reaction was stirred overnight at room temperature. The solid was filtered and recrystallized in MeOH. The yellow solid **4** was obtained in 90% yield. The solid was used directly in the next step.

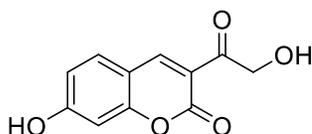


3-acetyl-7-((tert-butyldimethylsilyloxy))-2H-chromen-2-one 5. Compound **4** (0.5288 g, 2.59 mmol), TBSCl (0.5949 g, 3.88 mmol), imidazole (0.3573 g, 5.18

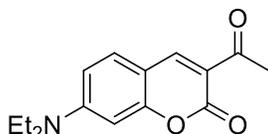
mmol) were added into a round bottom flask with 20 mL DCM. The reaction was stirred at room temperature overnight followed by the removal of solvent in vacuo. The crude mixture was purified by column chromatography with silica gel (25% ethyl acetate:hexanes) to yield product **5** as a light yellow solid (88%). $R_f=0.59$ (25% ethyl acetate:hexanes). $^1\text{H NMR}$ (500 MHz, Chloroform- d) δ 8.49 (s, 1H), 7.52 (d, $J = 8.6$ Hz, 1H), 6.91 – 6.69 (m, 2H), 2.71 (s, 2H), 1.00 (s, 9H), 0.28 (s, 6H). $^{13}\text{C NMR}$ (500 MHz, CDCl_3) δ 195.57, 162.13, 159.75, 157.40, 147.75, 131.58, 120.94, 118.47, 112.63, 107.18, 30.61, 25.51, 18.30, -4.34. HRMS (ESI, m/z): Calcd. for $\text{C}_{17}\text{H}_{22}\text{O}_4\text{SiNa}$ $[\text{M}+\text{Na}]^+$: 341.1185. Found: 341.1198.



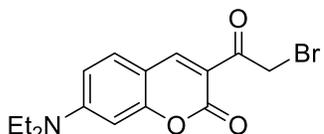
3-(2-bromoacetyl)-7-((tert-butyldimethylsilyl)oxy)-2H-chromen-2-one **6**. Compound **5** (0.2907 g, 0.91 mmol) and CuBr_2 (0.4006 g, 1.82 mmol) were put into a round bottom flask charged with 4 mL ethyl acetate. The reaction was refluxed for 3 hours and the reaction mixture was purified by column chromatography with silica gel (5%-10% ethyl acetate:hexanes) to yield product **5** as a yellow solid (70%). $^1\text{H NMR}$ (500 MHz, Chloroform- d) δ 8.61 (s, 1H), 7.56 (d, $J = 8.5$ Hz, 1H), 6.85 (d, $J = 8.3$ Hz, 1H), 6.81 (s, 1H), 4.75 (s, 2H), 1.00 (s, 12H), 0.29 (s, 5H). $^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 188.77, 162.88, 159.39, 157.61, 149.71, 131.87, 118.82, 118.37, 112.58, 107.29, 36.01, 25.50, 18.31, -4.32. HRMS (ESI, m/z): Calcd. for $\text{C}_{17}\text{H}_{21}\text{BrO}_4\text{Si}$ M^+ : 396.0392. Found: 396.0392.



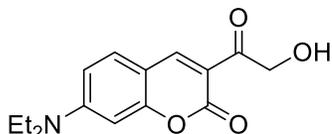
7-hydroxy-3-(2-hydroxyacetyl)-2H-chromen-2-one **7**. Compound **6** (0.1480 mg, 0.37 mmol) was dissolved in 2 mL MeCN and then trimethylglycine (CAS107-43-7) (0.0876 g, 0.74 mmol) was added. The reaction was stirred for 3 hours (LCMS confirms no starting material was left and the remaining is trimethylglycine adduct) and 1 mL water was added to dissolve the solid followed by the addition of K_2CO_3 (0.1557 g, 1.12 mmol). Once phase separation was observed (bottom layer was dark red), the reaction was quenching immediately with 4 N HCl to adjust pH to 1. The crude mixture was extracted with ethyl acetate three times. The organic layer was combined and dried with anhydrous MgSO_4 . The crude mixture was purified by column chromatography with silica gel (50% ethyl acetate:hexanes to 10-20% dichloromethane:methanol) to yield product **3** as a yellow solid (75%). $^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$) δ 8.69 (s, 1H), 7.83 (d, $J = 8.6$ Hz, 1H), 6.86 (d, $J = 8.6$ Hz, 1H), 6.76 (s, 1H), 4.66 (d, $J = 5.2$ Hz, 2H). $^{13}\text{C NMR}$ (400 MHz, DMSO) δ 196.96, 164.99, 159.35, 157.68, 148.64, 133.28, 117.79, 114.85, 111.27, 102.28, 68.52. HRMS (ESI, m/z): Calcd. for $\text{C}_{11}\text{H}_8\text{O}_5$ $[\text{M}-\text{H}]^-$: 219.0293. Found: 219.0299



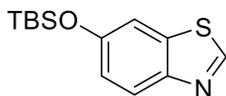
3-acetyl-7-(diethylamino)-2H-chromen-2-one 9. The preparation of **9** is similar to **4**. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.44 (s, 1H), 7.41 (d, $J = 8.9$ Hz, 1H), 6.65 (dd, $J = 8.9, 2.5$ Hz, 1H), 6.50 (d, $J = 2.5$ Hz, 1H), 3.45 (t, $J = 7.1$ Hz, 4H), 2.68 (s, 3H), 1.25 (t, $J = 7.1$ Hz, 6H). $^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 195.75, 160.88, 158.76, 152.97, 147.87, 131.91, 116.21, 109.91, 108.24, 96.67, 45.21, 30.62, 12.45.



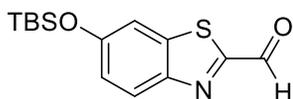
3-(2-bromoacetyl)-7-(diethylamino)-2H-chromen-2-one 10. Compound **9** (0.9540 g, 3.68 mmol) was dissolved in 12 mL 48% HBr/HOAc and cooled to 0 ° C. After stirring for 5 minutes, 0.92 mL (1 equiv.) 4 mol/L Br_2 in 48% HBr/HOAc was slowly added into the above solution. Then the reaction was slowly warmed to room temperature. After stirring for 3 h, the reaction mixture was dumped into 200 mL of water and adjusted pH to basic with sodium bicarbonate. The resultant mixture was extracted with ethyl acetate for three times. The organic phase was combined and dried with anhydrous MgSO_4 . The solvent was removed in vacuo to obtain a yellow solid which was directly used in the next step.



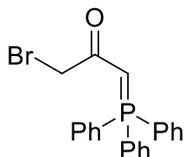
7-(diethylamino)-3-(2-hydroxyacetyl)-2H-chromen-2-one 11. The above solid was dissolved in 700 mL of MeCN followed by addition of 4 equivalents of trimethylglycine (1.7244 g, 14.72 mmol) and 0.1 equiv. TBAI (0.1359 g, 0.368 mmol). After stirring for 4 hours, the solvent was removed in vacuo and the remaining solid was re-dissolved with enough ethyl acetate and 200 mL pH=8 $\text{NaHCO}_3/\text{H}_2\text{O}$. The bilayer was stirred for 30 minutes. The organic phase was collected and evaporated. The reaction mixture was purified by column chromatography with silica gel (50% ethyl acetate:hexanes to 10-20% dichloromethane:methanol) to yield product **7** as a yellow solid (50% in two steps). $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.70 (d, $J = 9.0$ Hz, 1H), 7.01 – 6.34 (m, 2H), 4.70 (dd, $J = 59.0, 5.8$ Hz, 3H), 3.50 (q, $J = 7.0$ Hz, 5H), 1.15 (t, $J = 7.0$ Hz, 8H). $^{13}\text{C NMR}$ (400 MHz, DMSO) δ 196.45, 160.09, 158.70, 153.65, 148.36, 133.06, 113.27, 110.76, 108.09, 96.32, 68.48, 44.93, 12.81. Calcd. for $\text{C}_{15}\text{H}_{17}\text{NO}_4[\text{M}+\text{H}]^+$: 276.1236. Found: 276.1234.



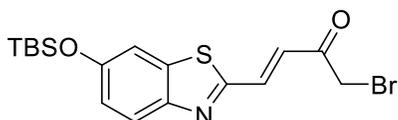
6-((tert-butyldimethylsilyl)oxy)benzo[d]thiazole 14. 6-Hydroxybenzothiazole (2.6104 g, 17.3 mmol), TBSCl (3.1246 g, 20.8 mmol), imidazole (2.355 g, 34.6 mmol) were added into a round bottom flask with 50 mL DCM. The reaction was stirred at room temperature overnight followed by the removal of solvent in vacuo. The crude mixture was purified by column chromatography with silica gel (10% ethyl acetate:hexanes) to yield product **14** as a light yellow liquid (95%). ¹H NMR (400 MHz, CDCl₃) δ 8.83 (d, J = 1.2 Hz, 1H), 7.97 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.04 (dd, J = 8.8, 2.3 Hz, 1H), 1.01 (s, 9H), 0.23 (s, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 154.03, 151.89, 151.82, 148.11, 134.82, 123.82, 123.76, 120.39, 120.28, 111.72, 25.67, 18.24, -4.40.



6-((tert-butyldimethylsilyl)oxy)benzo[d]thiazole-2-carbaldehyde 15. To a flask charged with argon, compound **14** (1.0644 g, 4.02 mmol) was dissolved in 15 mL THF and the solution was cooled down to -78 °C. nBuLi in THF (1.6 mL, 2.5 M) was slowly added into the flask. After stirring for 30 minutes, 0.5 mL DMF in 5 mL THF was added into the flask in one portion and the reaction was further stirred for 2 hours. The reaction was quenched with 10 mL saturated NH₄Cl solution and extracted with ethyl acetate three times. The organic phase was collected and dried with anhydrous MgSO₄. The crude mixture was purified by column chromatography with silica gel (10% ethyl acetate:hexanes) to yield product **15** as an orange-red solid (71%). ¹H NMR (400 MHz, CDCl₃) δ 10.11 (s, 1H), 8.09 (d, J = 9.0 Hz, 1H), 7.37 (d, J = 2.3 Hz, 1H), 7.13 (dd, J = 9.0, 2.4 Hz, 1H), 1.02 (s, 9H), 0.27 (s, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 185.20, 163.37, 156.64, 148.69, 138.27, 126.51, 122.12, 111.82, 25.60, 18.26, -4.35.

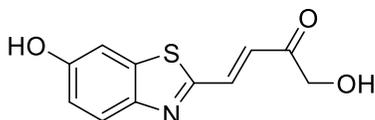


1-bromo-3-(triphenylphosphanylidene)propan-2-one 16. 1,3-dibromopropan-2-one was reflux with PPh₃ in toluene for 6 hours and then 2 equivalent Na₂CO₃ was added. The precipitation was filtered and recrystallized in MeOH/H₂O (v:v=1:1) to obtain a grey solid.



(E)-1-bromo-4-(6-((tert-butyldimethylsilyl)oxy)benzo[d]thiazol-2-yl)but-3-en-2-one 18. Compound **15** (0.3133 g, 1.06 mmol) and compound **16** (0.4653 g, 1.06

mmol) were added into a flask with 5 mL toluene. The reaction was stirred for 4 h at room temperature and the solvent was evaporated. The crude mixture was purified by column chromatography with silica gel (10% ethyl acetate:hexanes) to yield product **18** as a yellow solid (62%). ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, J = 8.9 Hz, 1H), 7.84 (d, J = 15.9 Hz, 1H), 7.31 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 16.0 Hz, 1H), 7.06 (dd, J = 8.9, 2.4 Hz, 1H), 4.12 (s, 2H), 1.01 (s, 9H), 0.25 (s, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 190.22, 160.77, 155.32, 149.05, 136.99, 136.93, 127.80, 124.75, 121.32, 111.53, 32.75, 25.63, 18.26, -4.36.



(E)-1-hydroxy-4-(6-hydroxybenzo[d]thiazol-2-yl)but-3-en-2-one **12.**

Compound **18** (100 mg, 0.43 mmol) was dissolved in 2 mL MeCN and then trimethylglycine (99.7 mg, 0.85 mmol) was added. The reaction was stirred for 3 hours (LCMS confirms no starting material was left and the remaining is trimethylglycine adduct) and 1 mL water was added to dissolve the solid followed by the addition of K₂CO₃ (177.0 mg, 1.28 mmol). Once phase separation was observed (bottom layer was dark red), the reaction was quenched immediately with 4 N HCl to adjust pH to 1. The crude mixture was extracted with ethyl acetate three times. The organic layer was combined and dried with anhydrous MgSO₄. The crude mixture was purified by column chromatography with silica gel (50% ethyl acetate:hexanes to 10-20% dichloromethane:methanol) to yield product **12**. The product **12** was verified with 236.0024 M+1 peak in the LCMS.

Standard procedure for carbamate formation

Compound **19** (100 mg), 2.5 equivalents of 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT), 1 equivalent of DMAP and 2 equivalents of DIEA were stirred in a flask charged with 2 mL DCM at room temperature for 30 minutes. At the same time, 1.1 equivalents of alcohol **7** with 2.2 equivalents DIEA were stirred in a flask charged with 1 mL MeCN for 20 minutes. The two components were combined and continuously stirred for 3 hours. Next, 1 mL saturated NH₄Cl was added to quench the reaction and the mixture was extracted with ethyl acetate (3 x 5 mL). The crude mixture was purified by HPLC.

Procedure for photolysis

The probe was dissolved in DMSO to obtain a 10 mmol/L solution. The final solution for the hydrolysis experiment was prepared with 40 μL probe solution and 960 μL deionized water. The resultant solution was placed into the photoreactor with 100 rpm (revolutions per minute) stirring speed and 420 nm/450 nm light generator. Every five minutes, a 50 μL aliquot was extracted using a pipet and 25 μL was injected into analytical HPLC (Column Luna Omega). Probe **20** used 35%-50% H₂O/MeCN (1% per minutes H₂O/MeCN) method with 214 nm, 254 nm, 354 nm detection signal. Probe **21** used 40%-55% H₂O/MeCN (1% per minute H₂O/MeCN) method with 214 nm, 254 nm, 420 nm detection signal.

Conclusion and Future Development

In conclusion, the synthesis of two pHP derived photocages are presented in this report, with both exhibiting an absorption wavelength extending above 400 nm and simple preparation protocols. Two new caged chemical dimerizer probes derived from these alcohols have been prepared and tested for their photolytic performance in water. Because of their red-shifted absorption wavelength compared to the currently widely used photocages such as NVOc and coumarin, as well as their good photolysis efficiencies, these two new probes show promise as powerful tools for spatial and temporal control of protein localization events and for further applications in biological imaging.

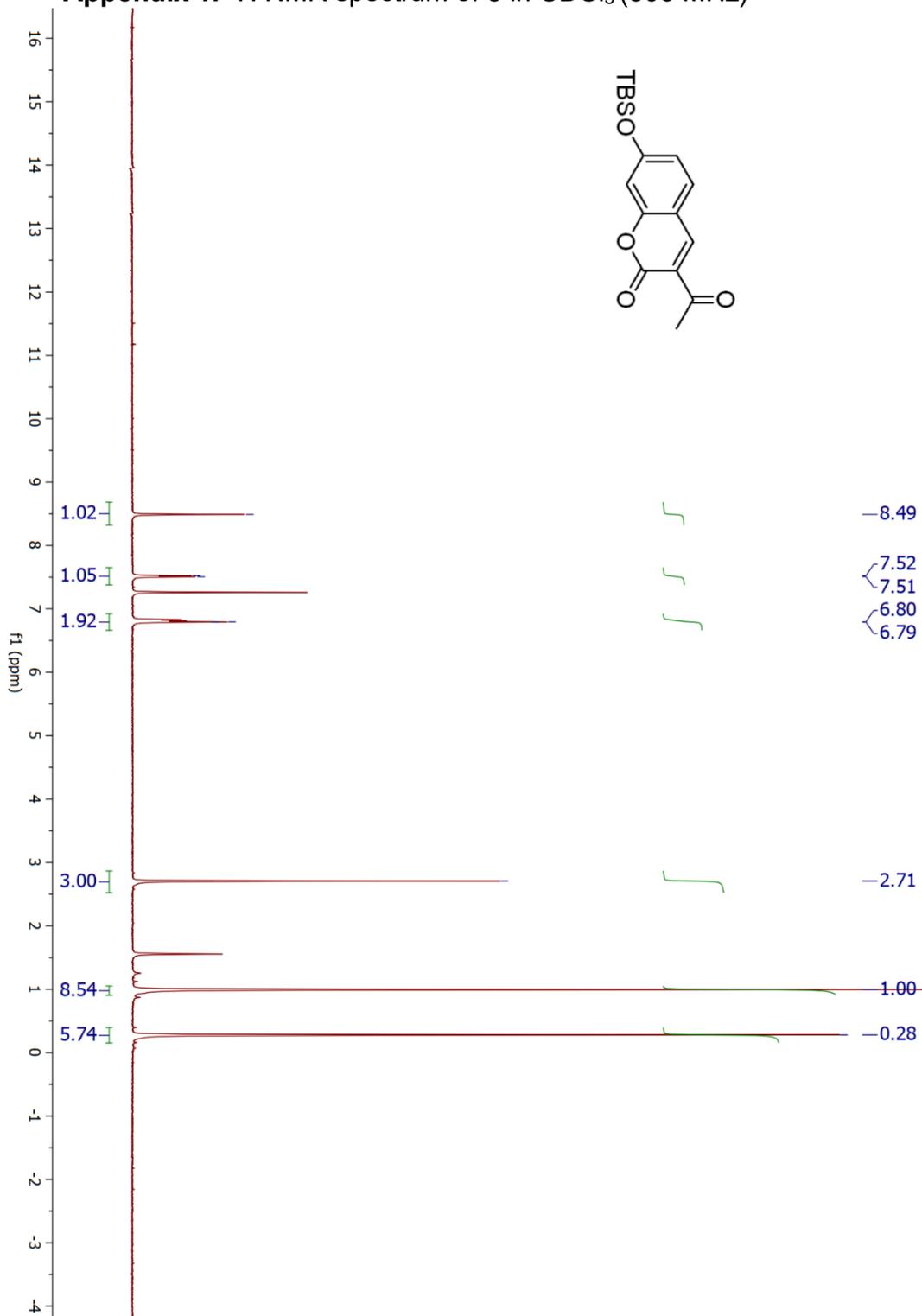
Future developments will focus on two areas. One area is to confirm that the synthesized probes will cause light-induced dimerization in engineered Hela cells and to extend their application in optogenetic control experiments. After verifying their use for these applications, the second area is to diversify the family of pHP derived photocages, using Friedel–Crafts reaction methods, to investigate how to introduce the 2-hydroxyacetyl group in a wide range of aromatic and heteroaromatic rings. The goal is to broaden the available toolbox of organic chromophores for photocaging CIDs by addition of the pHP group. The quantum yield of pHP photocages will also be investigated and compared with commercially available photocages.

References

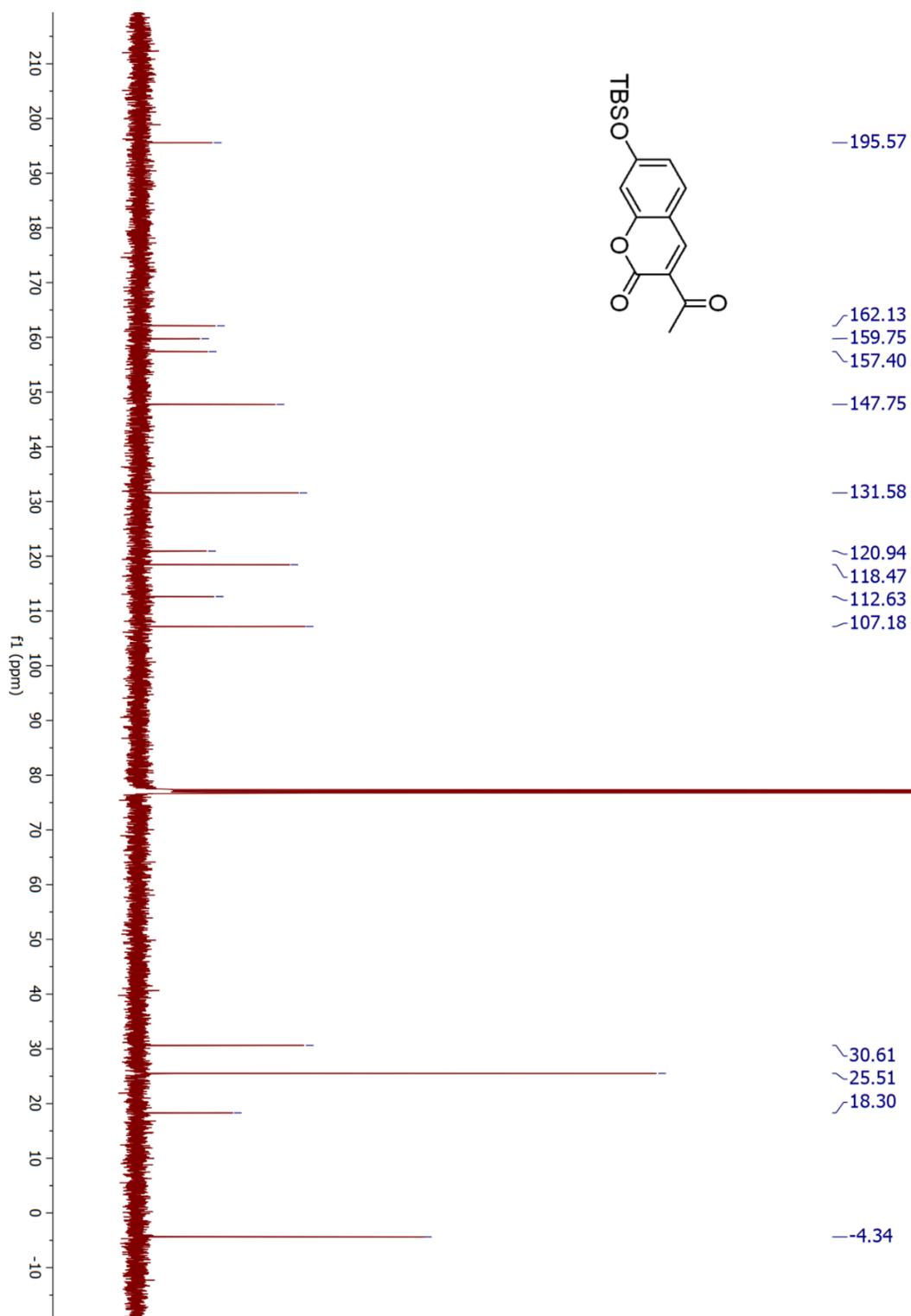
1. Braun, P.; Gingras, A.-C. History of Protein-Protein Interactions: From Egg-White to Complex Networks. *Proteomics* **2012**, *12* (10), 1478–1498.
2. Bauer, N. C.; Doetsch, P. W.; Corbett, A. H. Mechanisms Regulating Protein Localization. *Traffic* **2015**, *16* (10), 1039–1061.
3. Hung, M.-C.; Link, W. Protein Localization in Disease and Therapy. *J. Cell Sci.* **2011**, *124* (20), 3381–3392.
4. Rao, V. S., Srinivas, K., Sujini, G. N. & Kumar, G. N. Protein–protein interaction detection: methods and analysis. *Int. J. Proteom.* 2014, 147648
5. Lippincott-Schwartz, J.; Snapp, E.; Kenworthy, A. Studying Protein Dynamics in Living Cells. *Nat. Rev. Mol. Cell Biol.* **2001**, *2* (6), 444–456.
6. Ho, S. N.; Biggar, S. R.; Spencer, D. M.; Schreiber, S. L.; Crabtree, G. R. Dimeric Ligands Define a Role for Transcriptional Activation Domains in Reinitiation. *Nature* **1996**, *382* (6594), 822–826.
7. Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy *Chem. Rev.* **2012**, *113* (1), 119–191.
8. Zhang, H.; Aonbangkhen, C.; Tarasovetc, E. V.; Ballister, E. R.; Chenoweth, D. M.; Lampson, M. A. Optogenetic Tools for Controlling Kinetochore Function in Live Cells *Nat. Chem. Biol.* **2017**, *13* (10), 1096–1101.
9. Aonbangkhen, C.; Zhang, H.; Wu, D. Z.; Lampson, M. A.; Chenoweth, D. M. Reversible Control of Protein Localization in Living Cells Using a Photocaged-Photocleavable Chemical Dimerizer *J. Am. Chem. Soc.* **2018**, *140* (38), 11926–11930.
10. Salerno, C. P.; Magde, D.; Patron, A. P. Enzymatic Synthesis of Caged NADP Cofactors: Aqueous NADP Photorelease and Optical Properties. *J. Org. Chem.* **2000**, *65* (13), 3971–3981.
11. Salahi, F.; Purohit, V.; Ferraudi, G.; Stauffacher, C.; Wiest, O.; Helquist, P. pHP-Tethered N-Acyl Carbamate: A Photocage for Nicotinamide *Org. Lett.* **2018**, *20* (9), 2547–2550.
12. Altalbawy, F.; Abdelkader, M.; Darwish, E.; Elnagdi, M. Synthesis, Electronic Absorption, Fluorescence and Live Time Spectroscopic Study of Some New 3,7-Disubstituted Coumarin Derivatives as New Fluorescent Probes *Asian J. Chem.* **2016**, *28* (10), 2303–2310.
13. Ma, C.; Kwok, W. M.; Chan, W. S.; Zuo, P.; Kan, J. T.W.; Toy, P. H.; Phillips, D. L. Ultrafast Time-Resolved Study of Photophysical Processes Involved in the Photodeprotection of p-Hydroxyphenacyl Caged Phototrigger Compounds. *J. Am. Chem. Soc.* **2005**, *127*, 1463–1472.
14. Hirano, T.; Hasumi, Y.; Ohtsuka, K.; Maki, S.; Niwa, H.; Yamaji, M.; Hashizume, D. Spectroscopic Studies of the Light-Color Modulation Mechanism of Firefly (Beetle) Bioluminescence. *J. Am. Chem. Soc.* **2009**, *131* (6), 2385–2396.
15. Chaturvedi, D.; Chaturvedi, A. K.; Mishra, V. Carbon Dioxide: Versatile, Cheap and Safe Alternative in the Syntheses of Organic Carbamates. *Curr. Org. Chem.* **2012**, *16*, 1609–1635.

Appendices

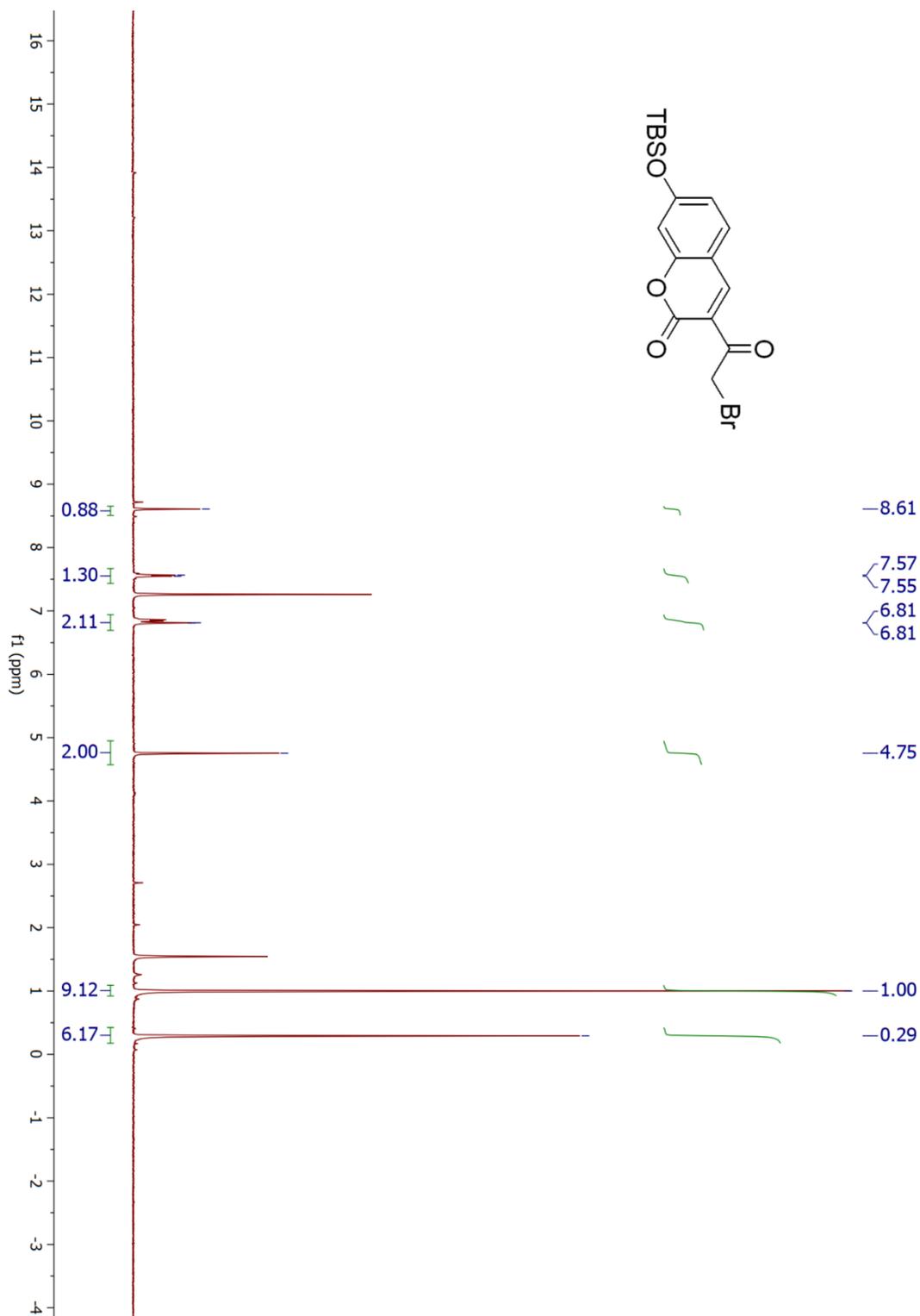
Appendix 1. ^1H NMR spectrum of **5** in CDCl_3 (500 MHz)



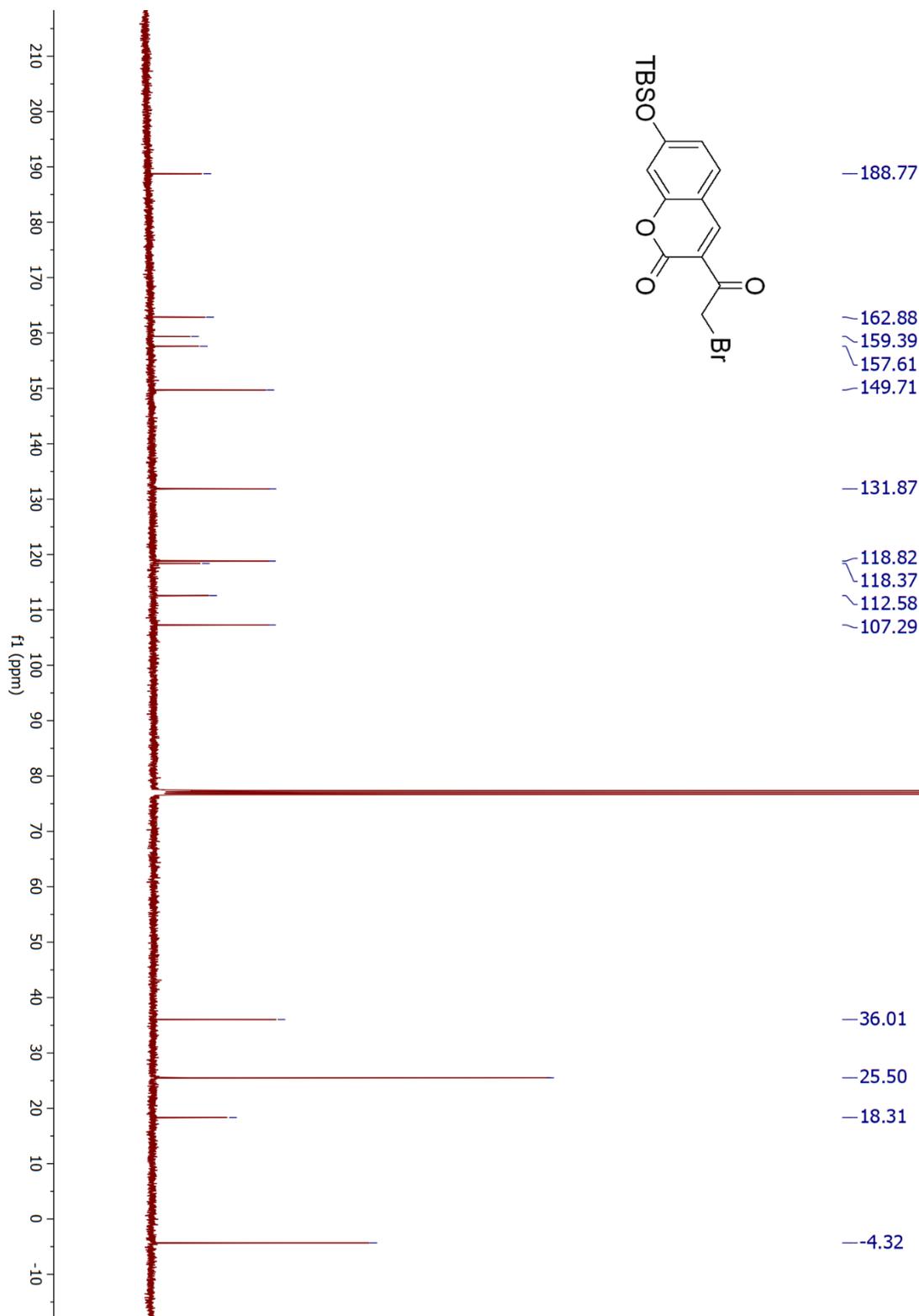
Appendix 2. ^{13}C NMR spectrum of **5** in CDCl_3 (500 MHz)



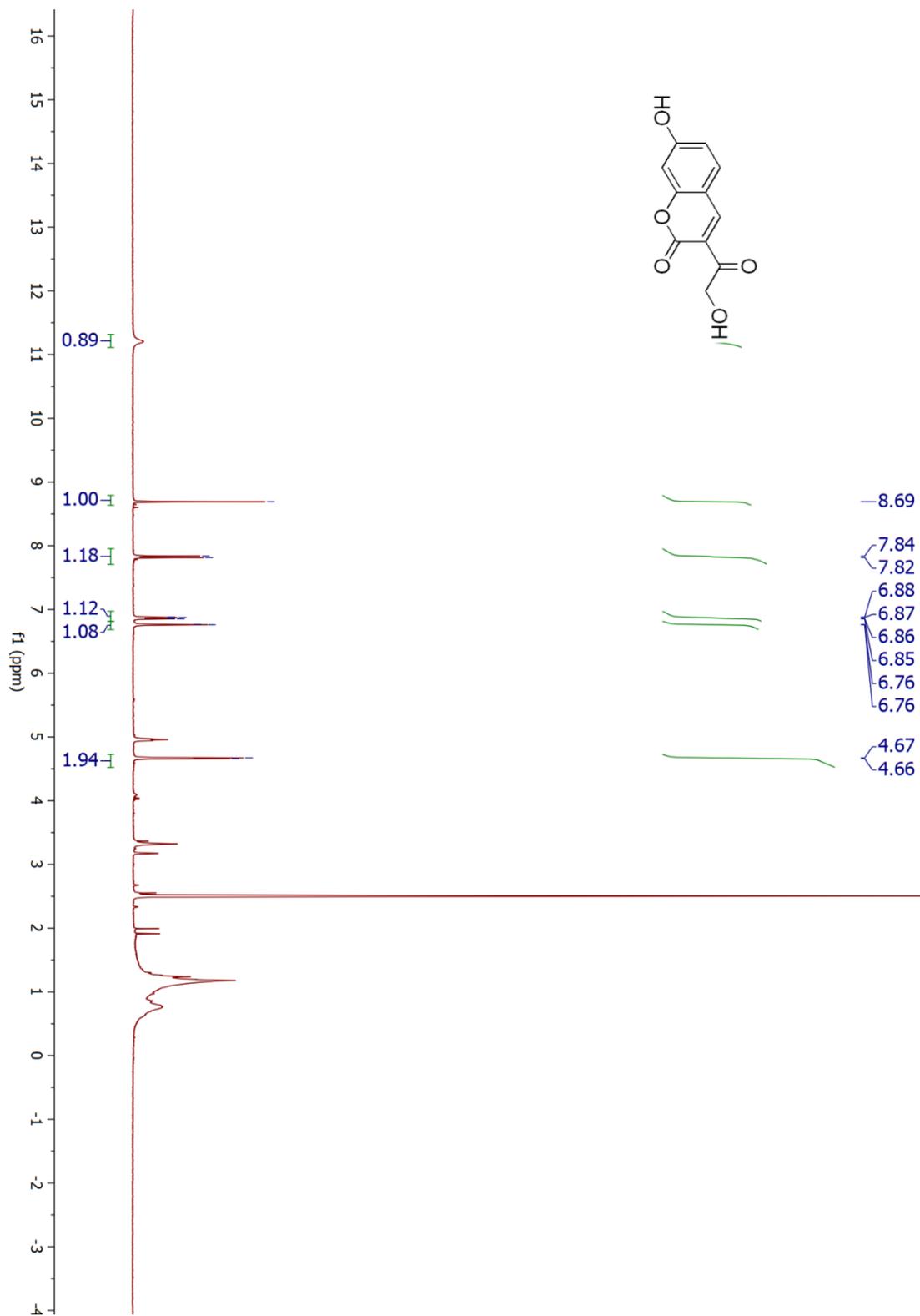
Appendix 3. ^1H NMR spectrum of **6** in CDCl_3 (500 MHz)



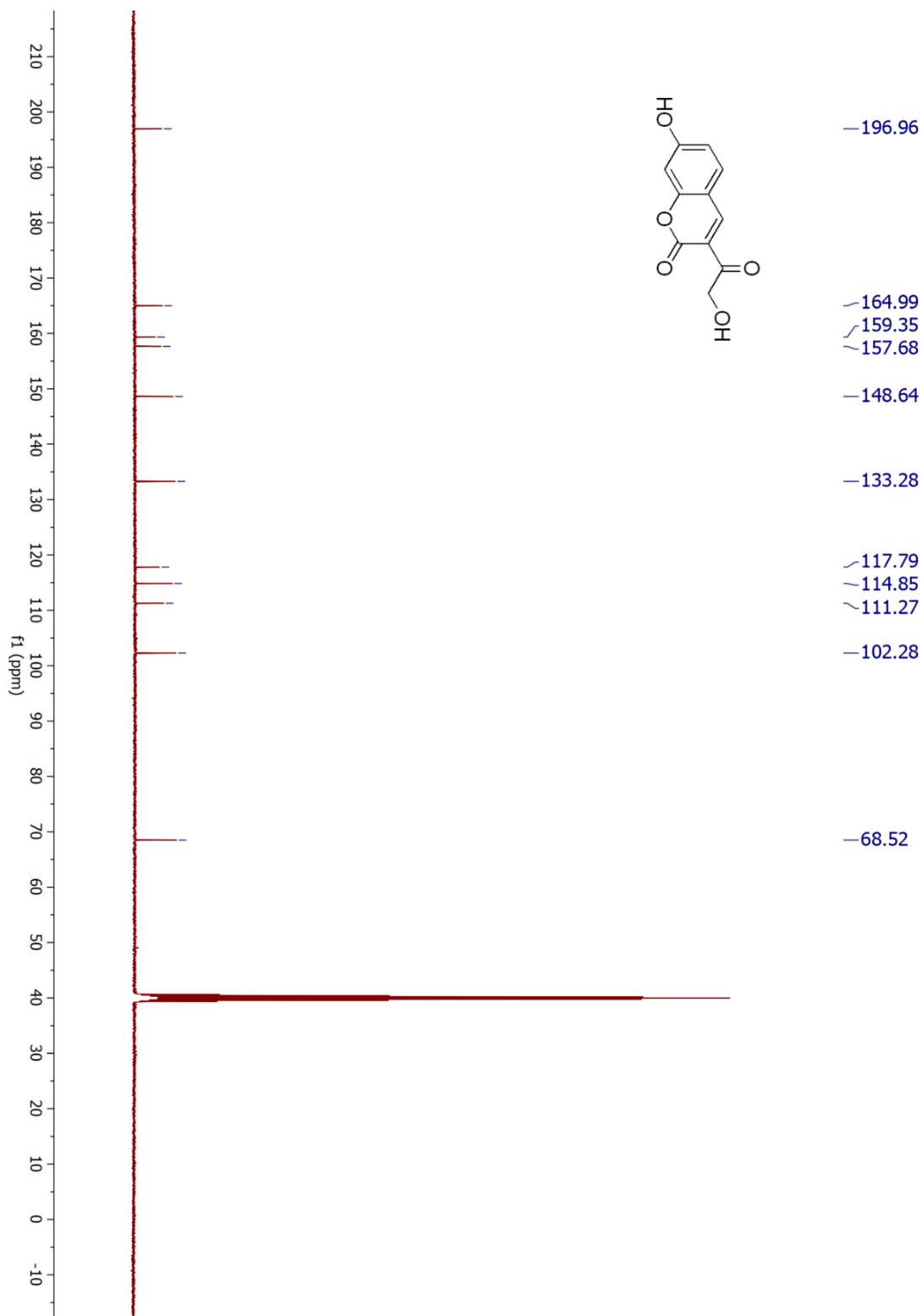
Appendix 4. ^{13}C NMR spectrum of **6** in CDCl_3 (500 MHz)



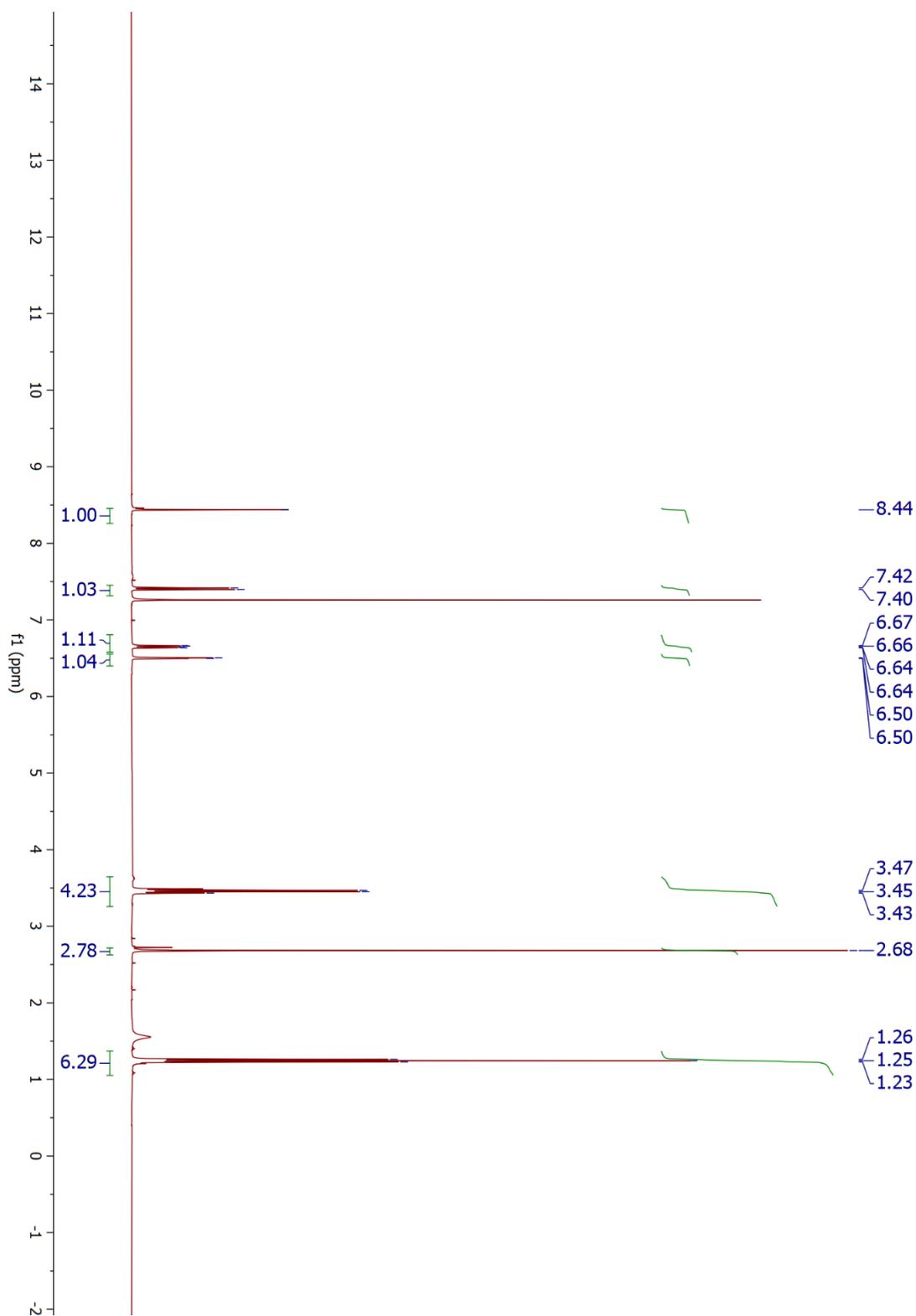
Appendix 5. ^1H NMR spectrum of 7 in DMSO-d_6 (400 MHz)



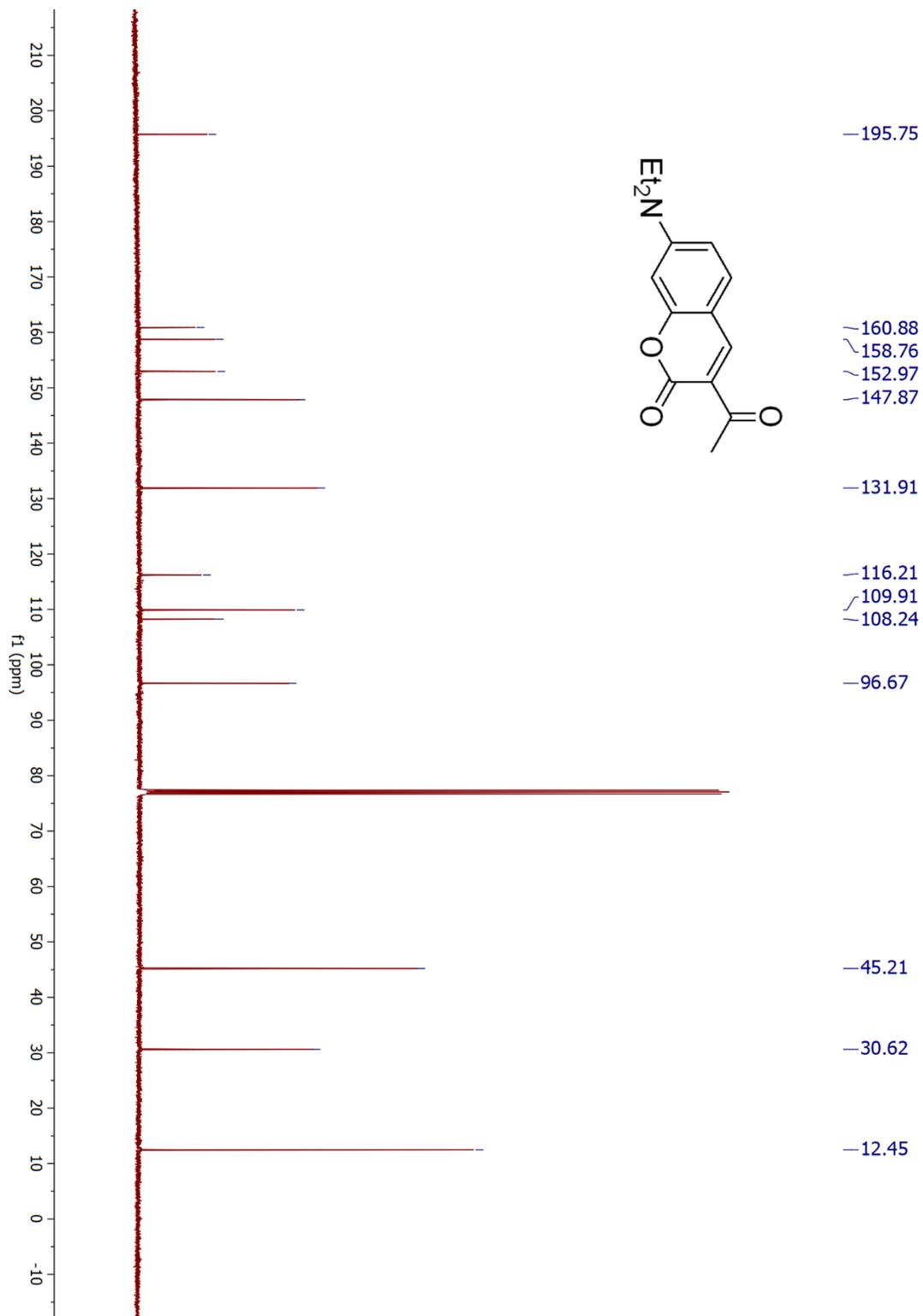
Appendix 6. ^{13}C NMR spectrum of **7** in DMSO-d_6 (400 MHz)



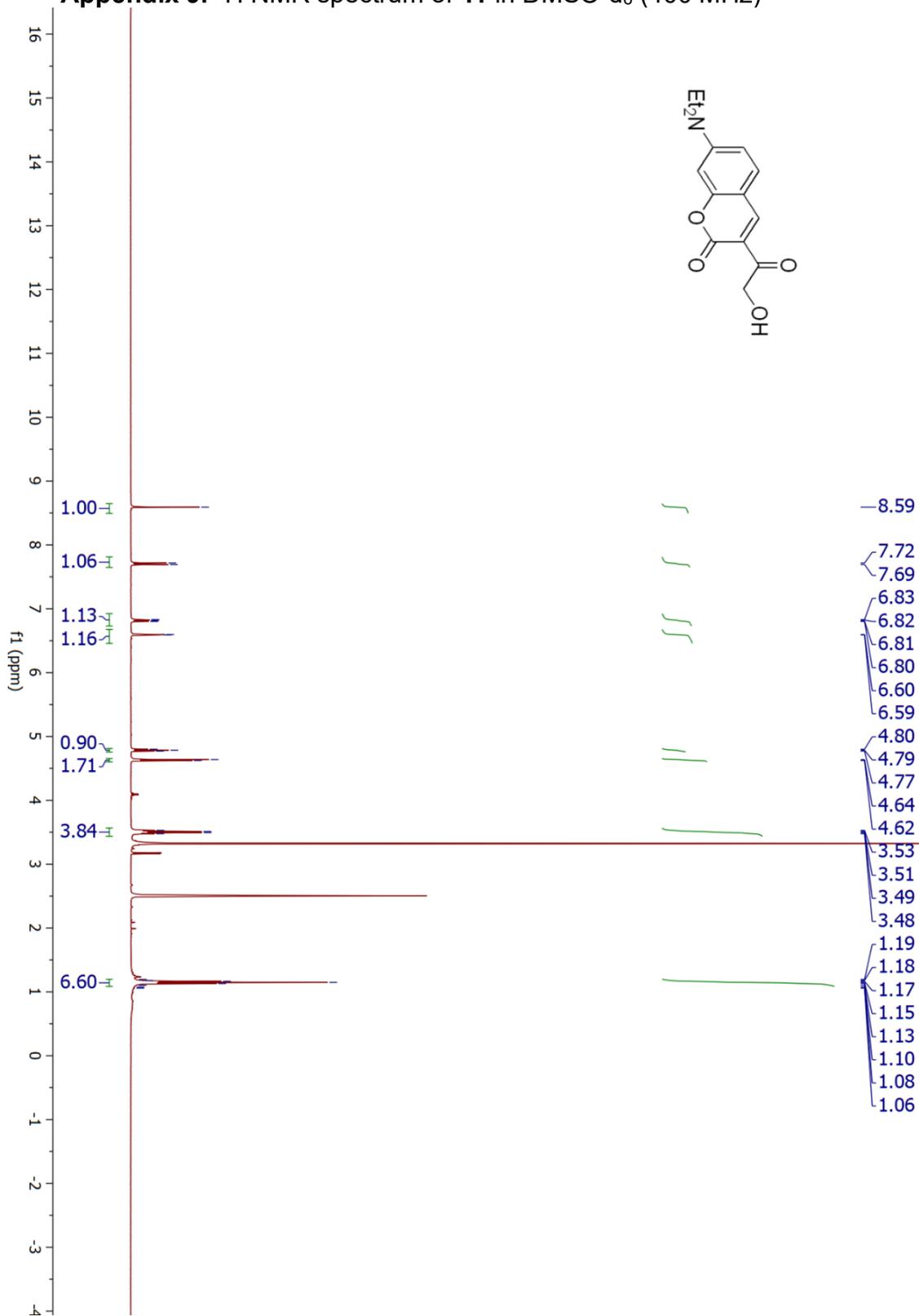
Appendix 7. ^1H NMR spectrum of **9** in CDCl_3 (400 MHz)



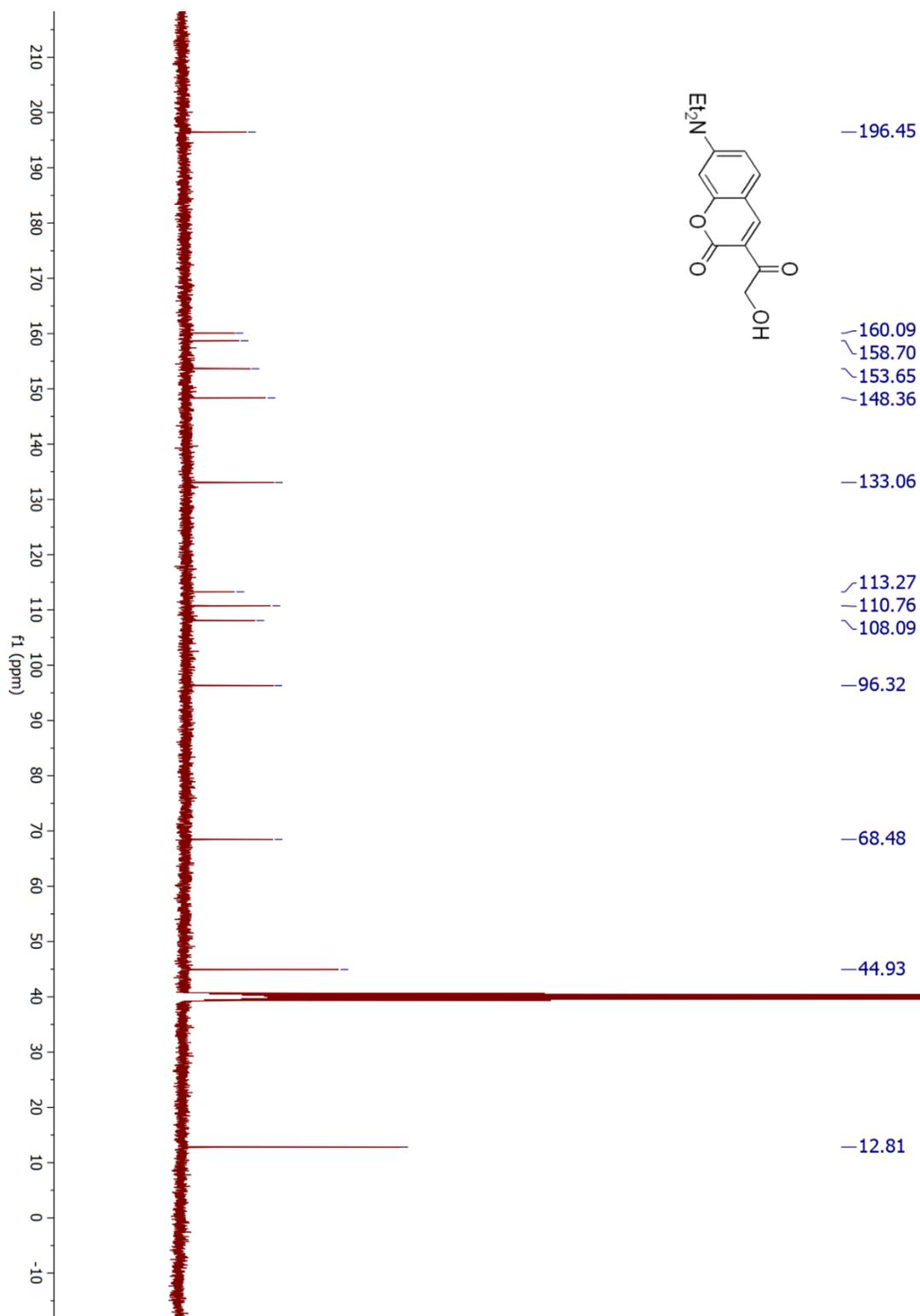
Appendix 8. ^{13}C NMR spectrum of **9** in CDCl_3 (400 MHz)



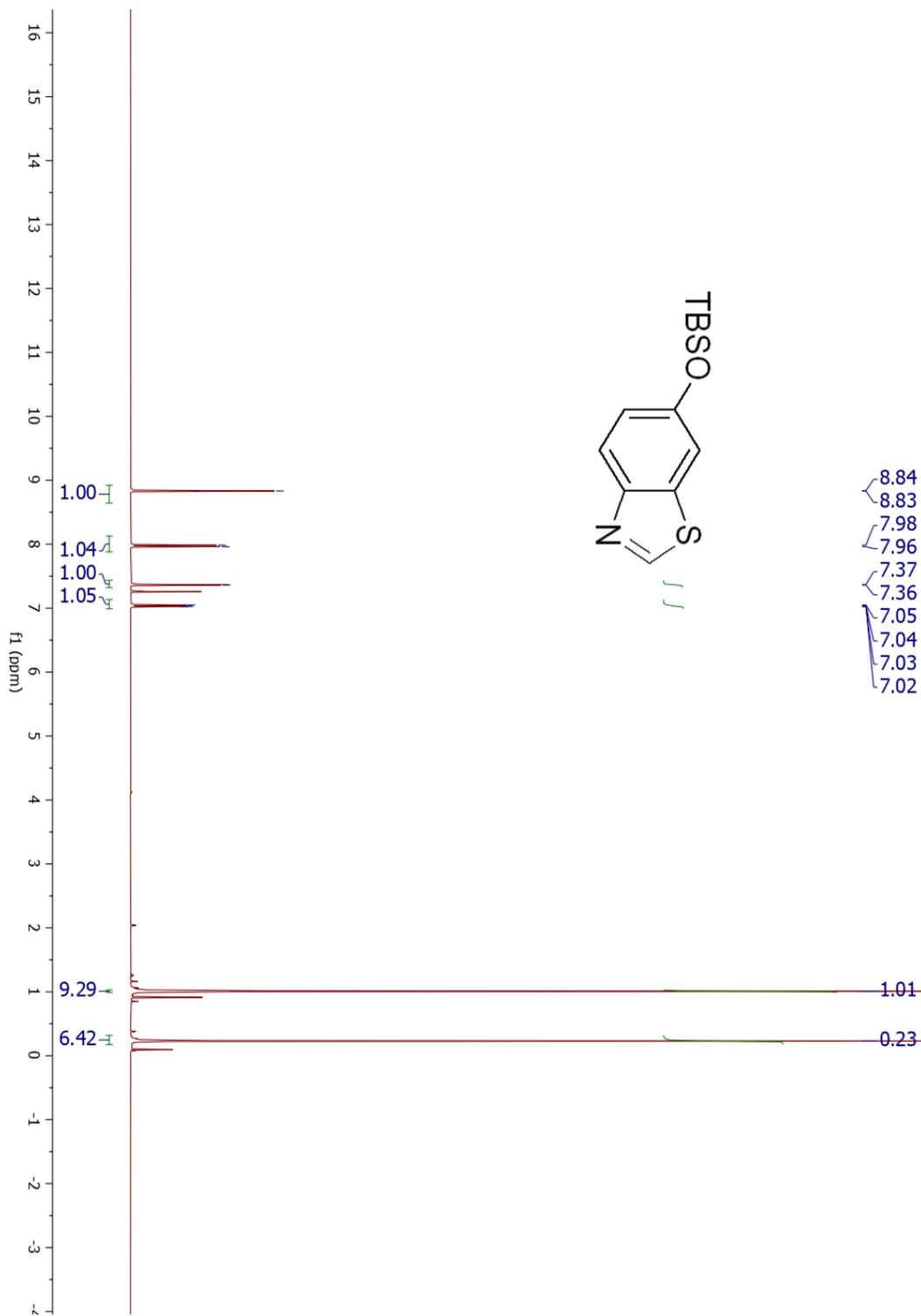
Appendix 9. ¹H NMR spectrum of **11** in DMSO-d₆ (400 MHz)



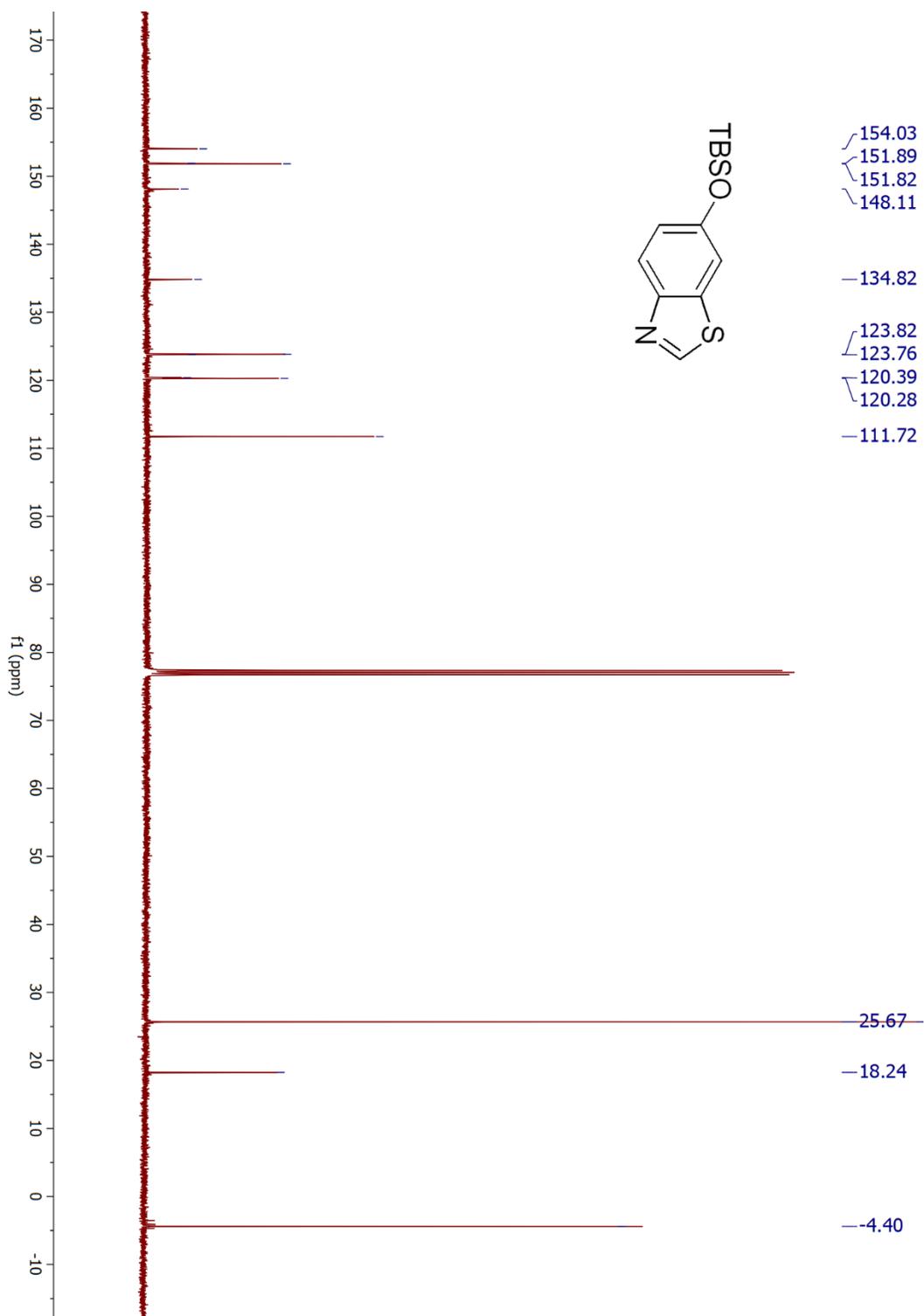
Appendix 10. ^{13}C NMR spectrum of **11** in DMSO- d_6 (400 MHz)



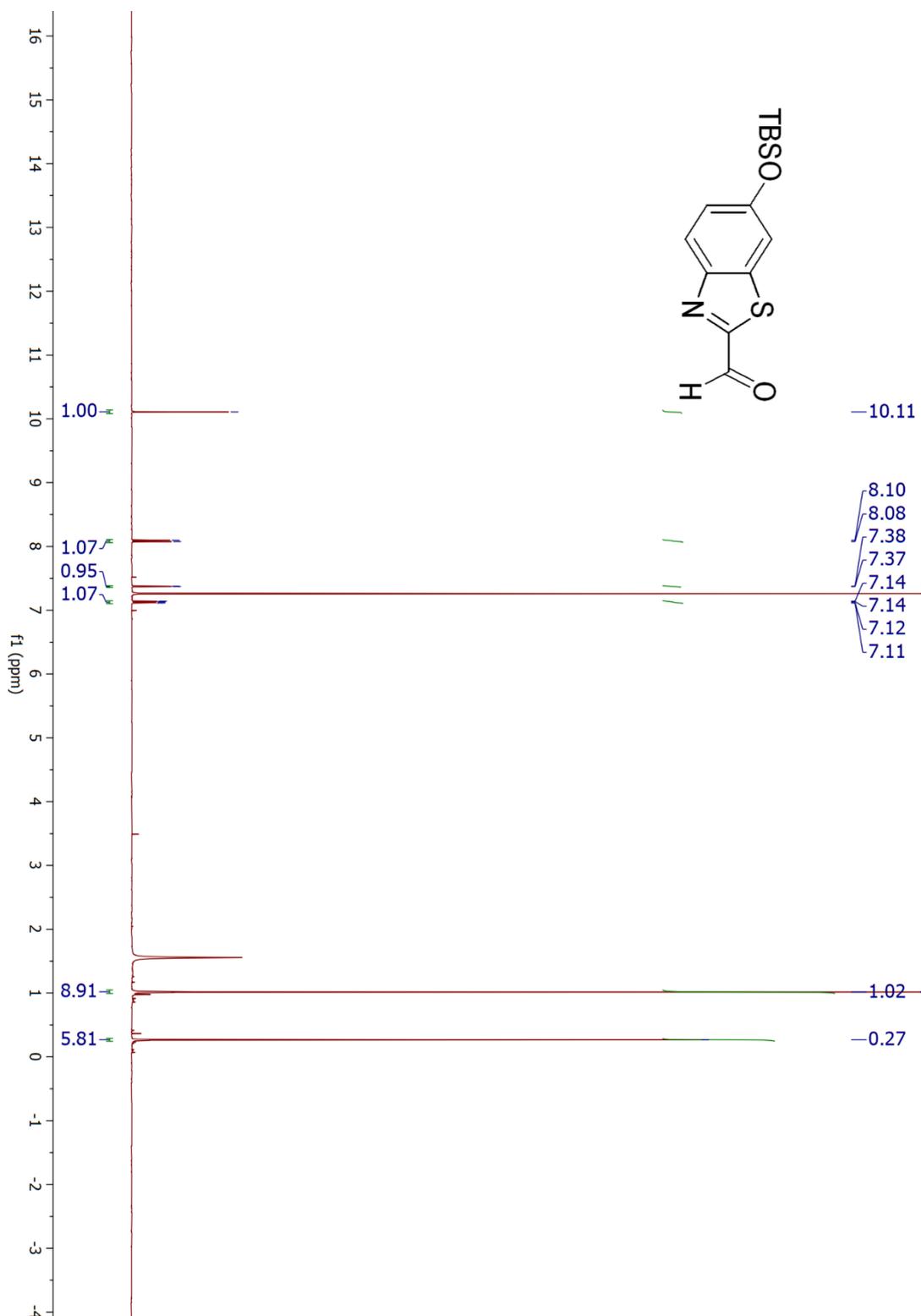
Appendix 11. ^1H NMR spectrum of **14** in CDCl_3 (400 MHz)



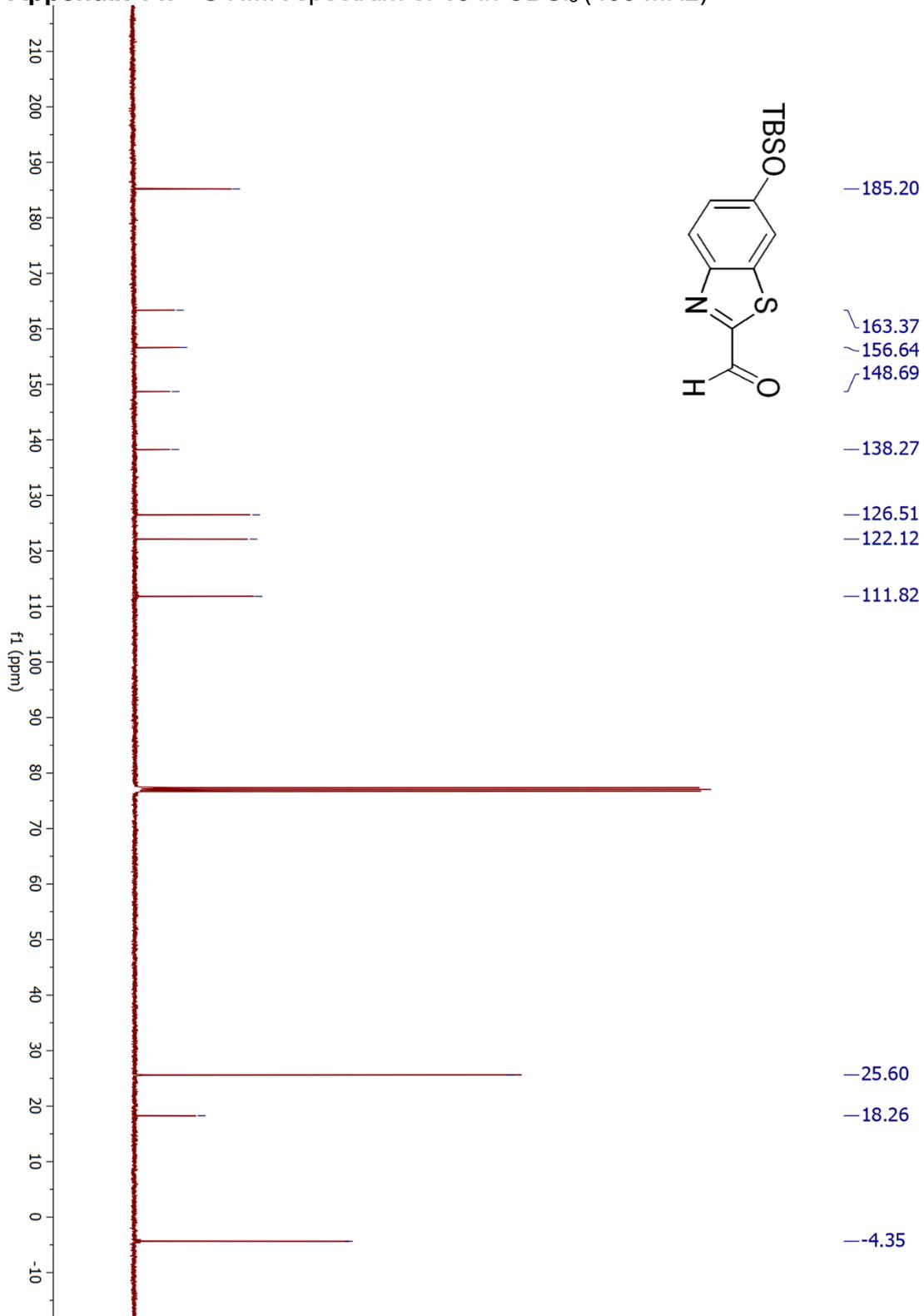
Appendix 12. ^{13}C NMR spectrum of **14** in CDCl_3 (400 MHz)



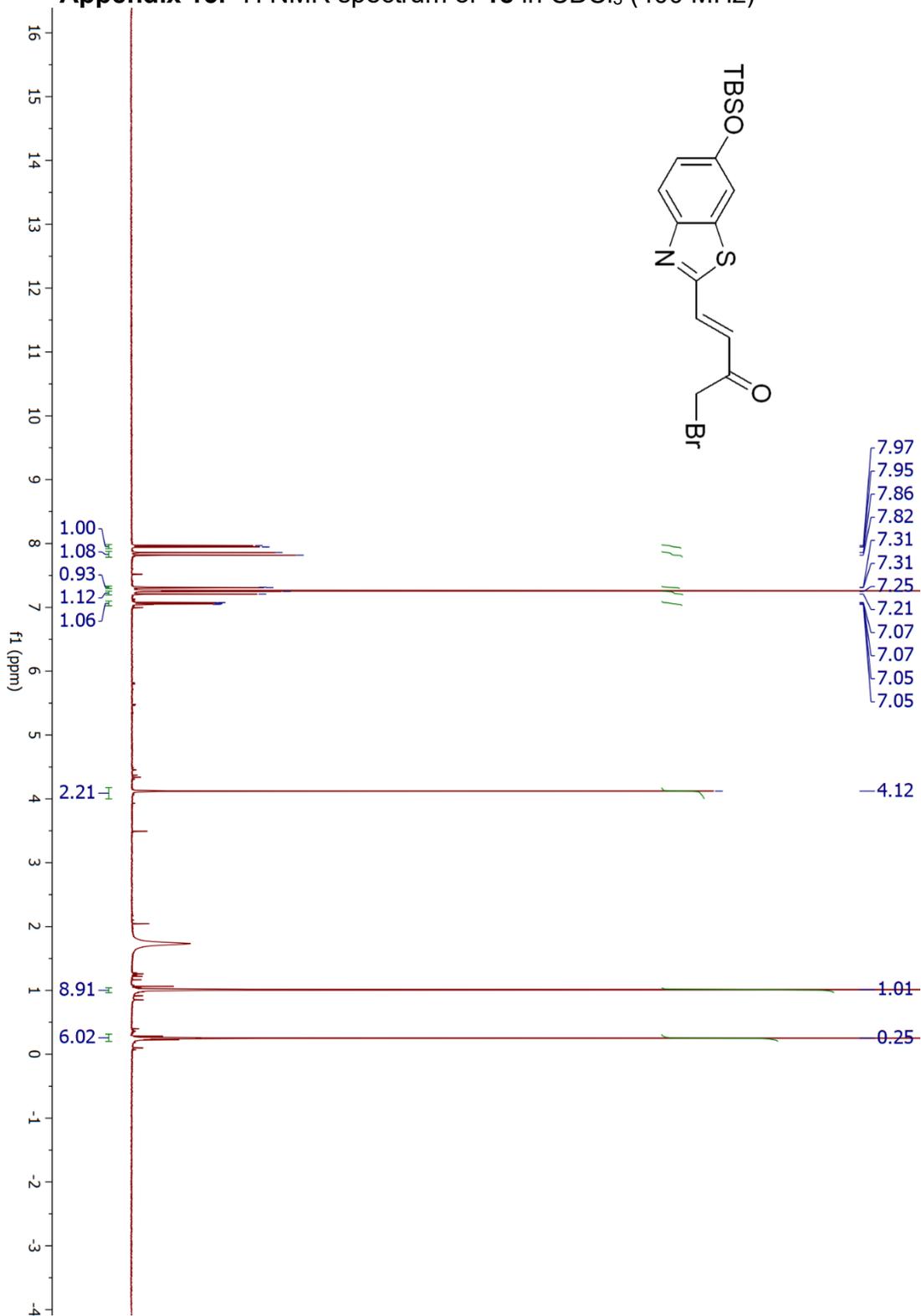
Appendix 13. ^1H NMR spectrum of **15** in CDCl_3 (400 MHz)



Appendix 14. ^{13}C NMR spectrum of **15** in CDCl_3 (400 MHz)



Appendix 15. ¹H NMR spectrum of **18** in CDCl₃ (400 MHz)



Appendix 16. ^{13}C NMR spectrum of **18** in CDCl_3 (400 MHz)

