Design, Synthesis and Evaluation of Scaffolds for Thiol-Mediated Tunable Drug Release

A thesis submitted towards partial fulfillment of
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CERTIFICATE

This is to certify that this dissertation entitled "***Design, Synthesis and Evaluation of Scaffolds for Thiol-Mediated Tunable Drug Release***" towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by Rohan Kumbhare at IISER Pune under the supervision of **Dr. Harinath Chakrapani**, Assistant Professor, Department of Chemistry, IISER Pune during the academic year 2012-2013.

Date:

Place:

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Assistant Professor,
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DECLARATION

I hereby declare that the matter embodied in the report entitled "***Design, Synthesis and Evaluation of Scaffolds for Thiol-Mediated Tunable Drug Release***" are the results of the investigations carried out by me at the Department of Chemistry, IISER Pune, under the supervision of **Dr. Harinath Chakrapani** and the same has not been submitted elsewhere for any other degree.

Date: **Rohan Kumbhare**

Place:

ACKNOWLEDGEMENTS

Working on this project has not only added to my knowledge and experience, but has also boosted my experimental skills and confidence in tackling research problems. Thus, I would like to express my sincere gratitude to my mentor Dr. Harinath Chakrapani for giving me this wonderful opportunity to work on an interesting project.

I would like to thank my labmates Dharma, Abhijeet, Satish, Kundan, Kavita and Vinayak for their useful help in the lab. I cannot forget the invigorating late night discussions with Dharma.

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ABSTRACT

Spatiotemporal control over drug delivery is highly desirable, but challenging. In particular, limited examples of small molecule-based scaffolds for tunable drug release are known. A routinely used metabolic trigger for drug release is cysteine-containing proteins and peptides. Here, we present results of our design, synthesis and evaluation of a new thiol-activated scaffold for drug release. We designed cinnamate-based benzoate esters as prototype scaffolds for tunable drug release. These compounds were prepared using a Baylis-Hillman reaction as the first step followed by a Mitsunobu reaction to install a self-immolable 2-nitroaryl linker. This 2-nitroaryl linker was attached to benzoic acid, a model for a carboxylic acid containing drug. We provide evidence for high stability of this prototype in physiological pH in the absence of a thiol trigger but in presence of a physiological thiol entity such as glutathione, release of a carboxylic acid drug mimic was initiated. We found the prototype to have a half-life of about 8h in the presence of 1 mM glutathione. A potential advantage of this scaffold is the availability of structural handles to control rate of reaction with glutathione. The synthesis and evaluation of such modified derivatives are currently underway.

INTRODUCTION

Development of new drug delivery technologies for controlled and localised delivery of drugs has been the centre of interest among medicinal chemists. By definition, drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans and animals.[1] Drug delivery methods can control the drug release profile, absorption, distribution of active drug molecules as well as elimination of drug molecules and by-products from the system. Recent efforts in these technologies are concentrated in sustained drug delivery which shows promise in development of new drug delivery candidates. Sustained (or slow) drug delivery deals with prolonging the time of release of active drug in the system, thus, posing a better alternative than conventional (or immediate) drug delivery methods.

Conventional drug delivery methods have been classically defined by the 'Hill and Valley' phenomenon (Figure 1).[2] When a drug is administered, plasma drug concentration increases and reaches a maximum (usually in the toxic range). Then, it decreases reaching a minimum (in the sub-therapeutic range), till a next dose is administered. A drug is most effective in the concentration corresponding to the therapeutic window. Thus, in this case, the effective drug concentration is achieved intermittently. The drug needs to be taken at frequent short intervals, especially for drugs having short half life. On the other hand, chances of patient missing the dose increases. Other drawbacks include under-medication or over-medication due to fluctuations in the drug concentration, difficulty in maintaining steady-state condition. The fluctuations may also lead to precipitation of drug resulting in adverse effects, specifically in case of over-medication of drug having low therapeutic index **TI**, which is the ratio of lethal or toxic dose to the therapeutic dose. The higher the TI, the higher the drug concentration is tolerable.[3] Most importantly, immediate drug release increases the chances of off-site, undesirable and potentially harmful reactions.

A clinical study in this regard has been conducted by Lammer and colleagues.[4] They reported a sustained drug-eluting bead which produced better results than conventional transcatheter arterial chemoembolization (TACE) technique for doxorubicin delivery in hepatocellular carcinoma patients. The drug-eluting bead not

only increased the drug's effectiveness, but also the toxicity related to immediate release of doxorubicin significantly decreased.

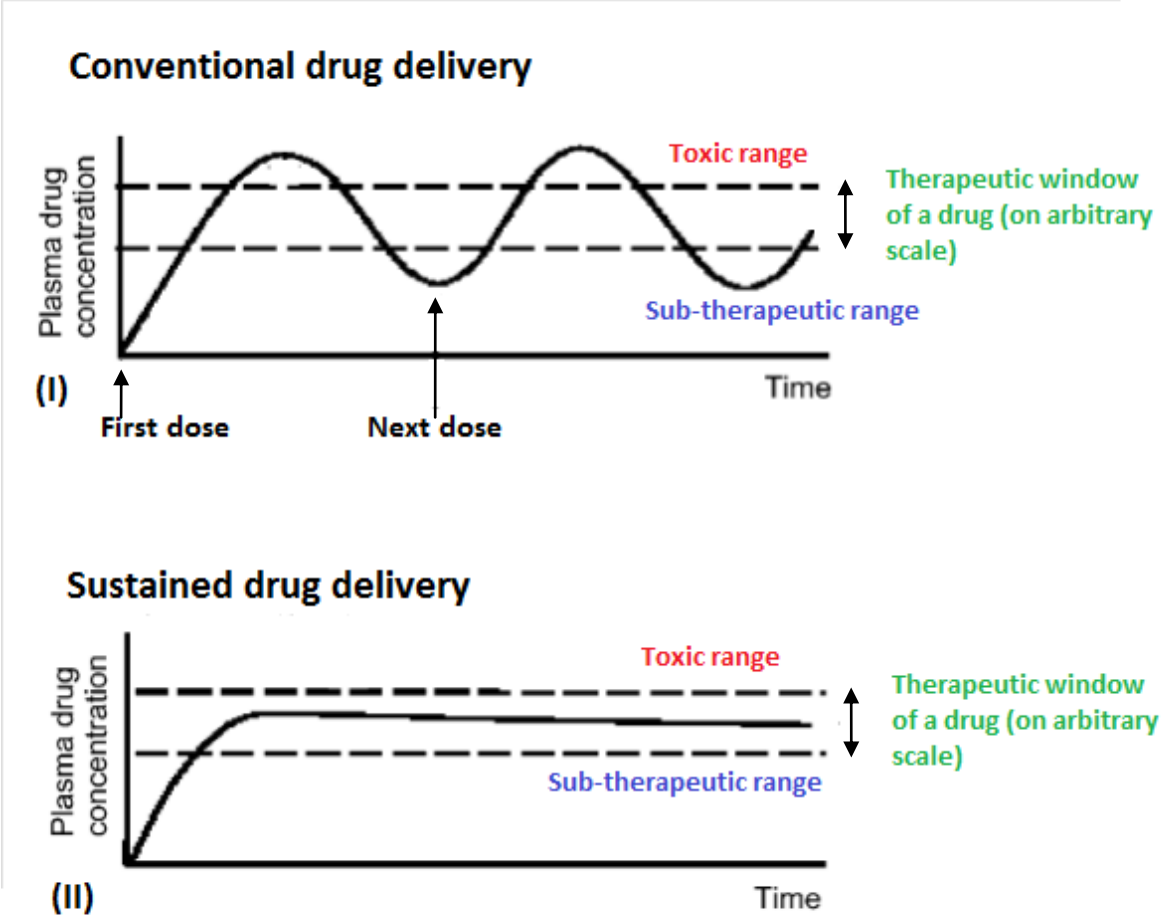


Figure 1: Comparison of profiles of plasma drug concentration in conventional drug delivery and controlled drug delivery over time. Adapted from Teo, A. L.; et al. *Mater. Sci. Eng. B* **2006**, *132*, 151–154.

With the help of sustained drug delivery systems, active drug concentration can be maintained in the therapeutic window for a prolonged duration, thus increasing the efficacy of the drug and minimising the above mentioned side effects and risks. A number of such systems have been reported till now. Examples of delivery methods include diffusion controlled release, pH-dependent release, dissolution controlled systems, methods using osmotic pressure and ion exchange.[5] A number of dendrimer-based systems[6] and some cyclodextrin-based carriers[7] are reported that encapsulate drug molecules and release them slowly. Drug delivery systems using liposomes and hydrophobic polymerosomes are also known for slow drug release.[8]

Nanoporous inorganic membranes have also been prepared showing sustainable drug release characteristics.[9] But, most of these slow drug release candidates lack a target-specific trigger and are activated even non-physiological milieu, resulting in non-specific delivery and off-target effects. Hence, it is necessary to increase the drug payload of these systems to attain the drug concentration in the therapeutic window at the active site.

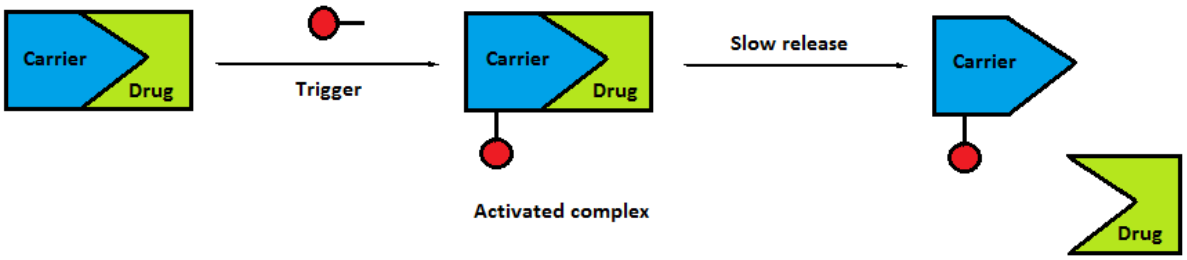


Figure 2: Schematic picture of slow drug release from a carrier activated by a trigger (red).

Prodrugs are small molecule-based systems that are useful for directed delivery of drug molecules. These molecules are designed such that they undergo cleavage only in the presence of a metabolic trigger to release the active drug molecule. An example of a metabolic trigger for such prodrug-based system is to use reactive cysteine residues. Reactive cysteine residues, although very low in abundance compared to other amino acids in a proteome, are present in many functionally active sites in a protein scaffold. The active thiol group is known to mediate crucial and very specific reactions in protein function, including nucleophilic catalysis, 1,4-nucleophilic addition on Michael acceptors, disulfide bond formation and metal-ligand interactions.[10] Molecules targeting such cysteine residues can potentially be used for site-specific detection, inhibition or drug release purposes.

A number of examples are reported with glutathione as prodrug activation trigger.[11,12,13] Glutathione is the most abundant tripeptide with a reactive thiol present in cellular milieu. Intracellular levels of reduced glutathione are much more compared to extracellular levels, making it a successful trigger. But, a number of these prodrug strategies that depend on such activation use the property of glutathione in making and breaking disulfide bonds (Figure 3). Although, disulfide bonds are specific for thiol entities, chemistry of disulfide bonds does not offer a good handle at controlling

these reactions in terms of selectivity and rate and thus, a lot of thiol species (even disulfide reductase enzymes) can activate such prodrugs. Serum albumin, the most abundant protein found in plasma, can also potentially compete for activation of these prodrugs before even these molecules can reach the cells.

Figure 3: Glutathione activated prodrug: Glutathione reacts with disulfide bonds to give an active thiol group that subsequently eliminates the drug molecule. Adapted from Chen, S.; et al. *Bioconjug. Chem.* **2010**, *21*, *5*, 979–987.

Recently, M.G. Finn and co-workers reported a strategy for thiol activated molecules with tunable drug release characteristics[14] (Figure 4). The norbornadiene-based molecules, after thiol addition, undergo retro-Diels-Alder (rDA) reaction. These thiol adducts have half-lives in the timescales of hours to several days, in which these adducts degrade to release the drug molecule. Thus, these molecules can even be injected intravenously and after a quick activation by serum albumin, these scaffolds can retain the drug molecule and release them slowly, over a prolonged period of time. Here too, the major drawback of this work is the non-specificity towards activation by thiols.

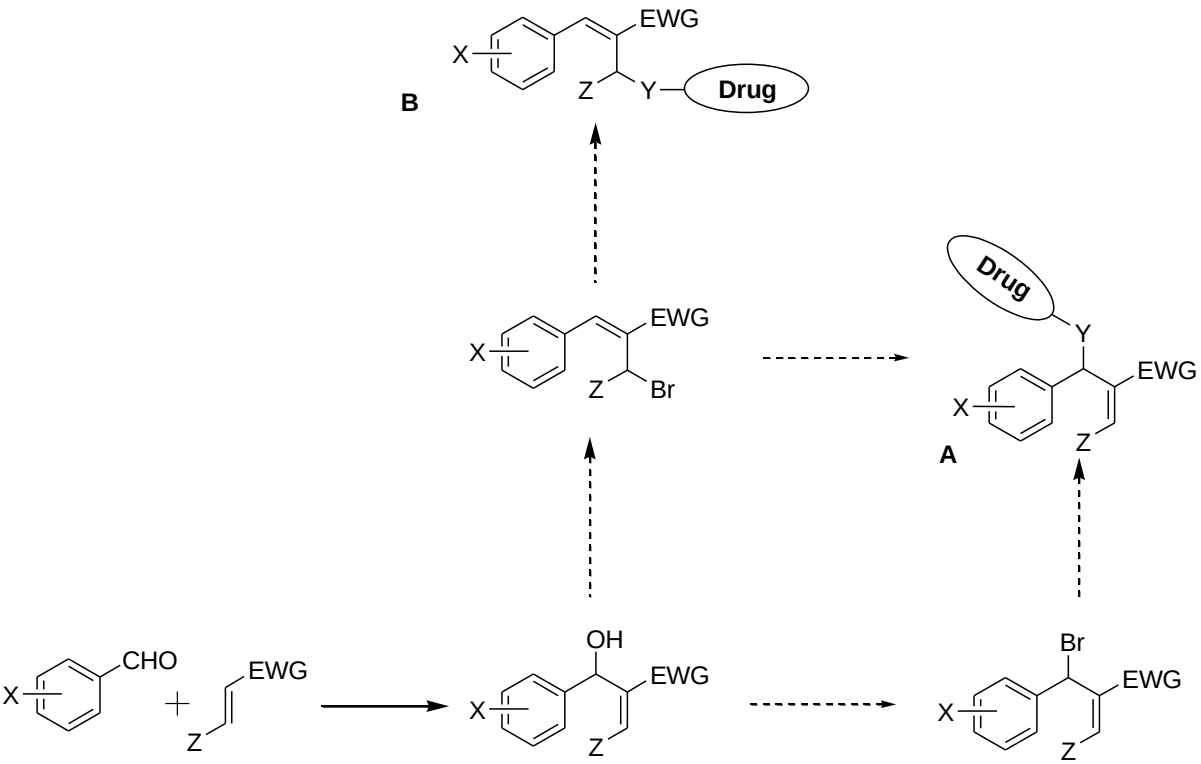
Figure 4: Nucleophile reacts with norbornadiene-based molecules which give corresponding adducts. These adducts undergo slow retro-Diels-Alder (rDA) reaction to release the drug. Adapted from Kislukhin, A. A.; et al. *J. Am. Chem. Soc.* **2012**, *134*, 6491−6497.

In this work, we proposed a drug release scaffold that can be specifically activated by thiols. We focussed on the ability of thiols to undergo 1,4-nucleophilic addition to be the activation trigger. A generic scaffold with design principles (Figure 5) shows the various parameters considered for tuning the rate of drug release.

Figure 5: Generic scaffold with the design principles of compounds of type **A** (I) and **B** (II) along with mechanism of reaction with thiol. (III) Drug with carboxylic acid

functionality can be attached as an ester, while amine functionality can be attached by carbamate linkage.

Compounds of type **A** and **B** (Figure 5) can be synthesized from the same scaffold. The position of attachment of drug molecule through linker Y differs in these scaffolds. The drug molecule having a carboxylic acid (for e.g., methotrexate) or amine functionality (for eg. doxorubicin, dactinomycin) can be attached through introduction of linker Y. Perturbation of moieties X, Z and EWG can be anticipated to have an electronic and/or steric control as shown in the Figure 5. The similarity in reaction mechanism can be seen from Figure 5. We propose that all these factors can have an influence on reaction kinetics of compounds of type **A** and **B** with thiols. An electron withdrawing group at X is likely to increase the rate of thiol addition, while electron donating group may decrease the rate. Z group may have a steric or electronic control as it may affect the thiol addition or the leaving group kinetics.



Scheme 1: Proposed synthetic scheme – Starting with aldehydes and acrylic compounds, type **A** and type **B** compounds are proposed to be synthesized according to known literature procedures.

RESULTS AND DISCUSSION

Having described the design principles of the prodrug, we started with the Morita-Baylis-Hillman (MBH) reaction to prepare compounds **1a-f** and **3a-j** from a number of benzaldehyde derivatives and methyl acrylate or acrylonitrile. Following a literature procedure,[15] we obtained the MBH adducts (MBHA) in excellent to nearly quantitative yields (Table 1).

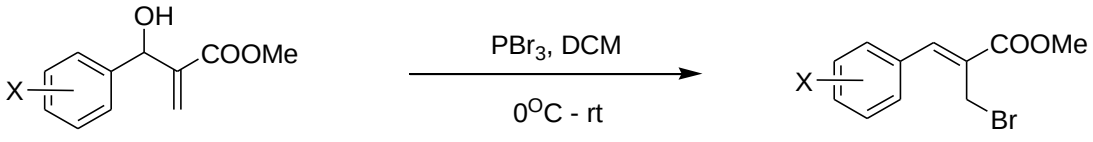
Table 1: Synthesis of compounds **1a**-**1f** and **3a**-**3j** using DABCO**.**

1a-f; E = CO_2Me
3a-3j; E = CN

Entry	EWG	X	Product	Yield (%)
1	CO_2Me	H	**1a**	87
2	CO_2Me	4-NO_2	**1b**	89
3	CO_2Me	2-NO_2	**1c**	99
4	CO_2Me	3-NO_2	**1d**	92
5	CO_2Me	4-Cl	**1e**	88
6	CO_2Me	4-Me	**1f**	99
7	CN	H	**3a**	99
8	CN	4-NO_2	**3b**	99
9	CN	2-NO_2	**3c**	99
10	CN	3-NO_2	**3d**	99
11	CN	4-Cl	**3e**	81
12	CN	4-Br	**3g**	97
13	CN	4-F	**3h**	99
14	CN	2-Br	**3i**	96
15	CN	4-CN	**3j**	99

Further, bromination on some of these compounds was tried by using phosphorus tribromide (PBr_3) which gave good yields with compounds **1a-c** (Table 2), but very low yields with compounds **3a-d**. However, compounds **4a-d** were synthesized in moderate to good yields by using Appel reagents (CBr_4 and PPh_3)[16] (Table 3).

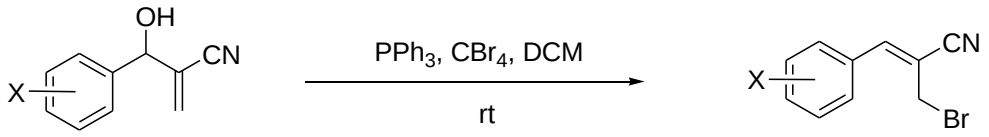
Table 2: Synthesis of compounds **2a-c** using PBr_3.



1a-c **2a-c**

Compound	X	Yield (%)
2a	H	79
2b	4-NO_2	89
2c	2-NO_2	79

Table 3: Synthesis of compounds **4a-d** using Appel reagents.



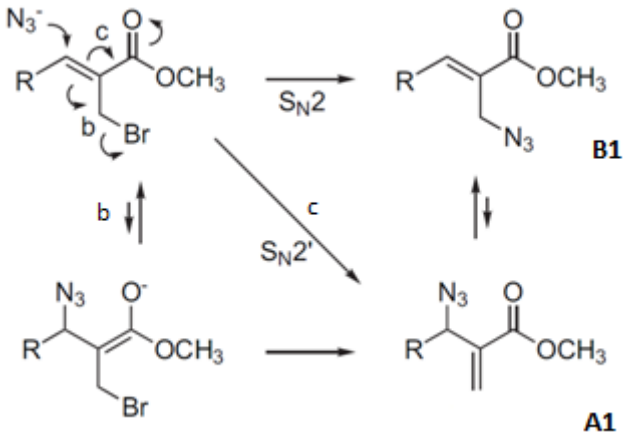
3a-d **4a-d**

Compound	X	Yield (%)
4a	H	49
4b	4-NO_2	58
4c	2-NO_2	80
4d	3-NO_2	88

Then, **2a-c** were subjected to thiol reactions (β-mercaptoethanol, reduced L-glutathione and L-cysteine) under physiological conditions, to study its reactivity. The reaction of **2a-c** with stoichiometric quantities of glutathione showed very fast

reaction kinetics ($t_{1/2}$ < 1h). Even β-mercaptoethanol and L-cysteine showed similar reactivity pattern.

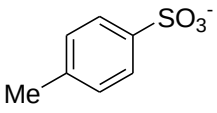
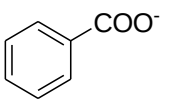
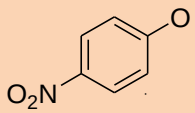
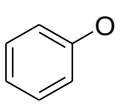
A mechanistic study on these substrates was conducted by Sá and colleagues.[17] They reported that, on azide reaction, molecules similar to compounds **2** can proceed via S_N2 or S_N2' reaction (Scheme 2). But, the products **A1** and **B1** formed by both the routes can potentially be converted into one another by nucleophilic substitution. Formation of both the products is seen in case of R as an alkyl group, while for R as an aryl group, only compounds of type **B1** (Figure 5) are obtained. Hence, a complete understanding of the mechanism is still unavailable. But it appears that the intermediates are short-lived and the rate-determining step is attack by a nucleophile.



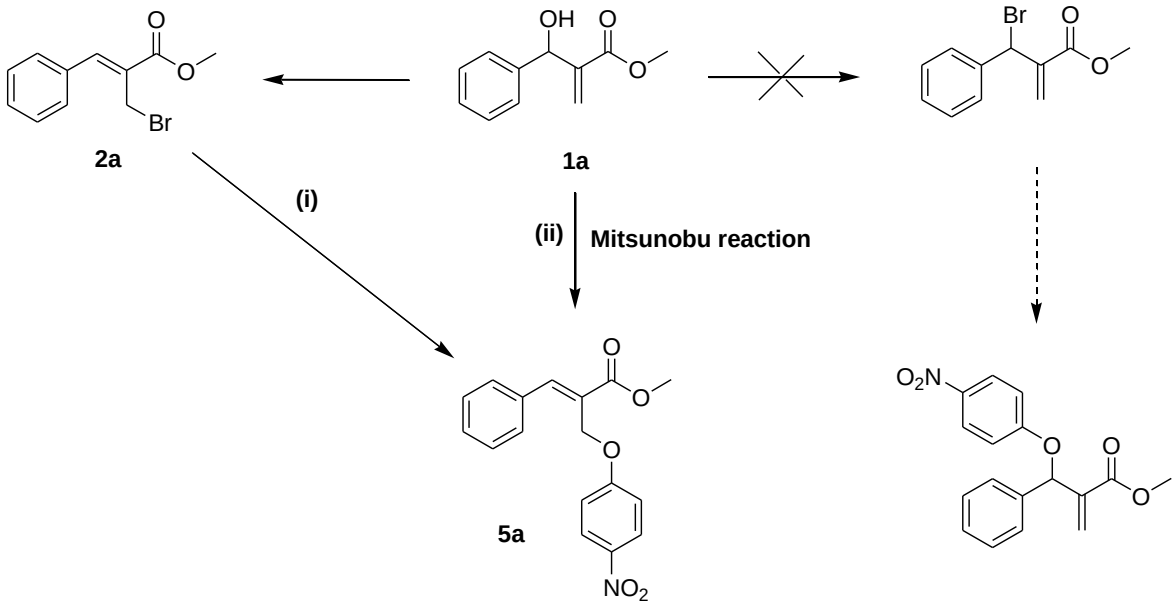
Scheme 2: Mechanistic view into formation of compounds of type **A1** and **B1**. Compounds of type **A1** and **B1** can be converted into one another by nucleophilic substitution. Adapted from Sá, M. M.; et al. *Tetrahedron* **2006**, *62*, 11652–11656.

Assessing the high reactivity of **2a-c**, it was contemplated that a weak leaving group than bromide might help increase the half-lives of the compounds. Here, leaving group ability was directly compared to pK_a of corresponding conjugate acids. Among different leaving groups considered, p-nitrophenol was chosen (Table 4). pK_a values of conjugate acids of bromide and p-nitrophenolate anion shows considerable increase (about 10^{16} fold) hinting to the lower reactivity with thiols (or lower reaction rates).

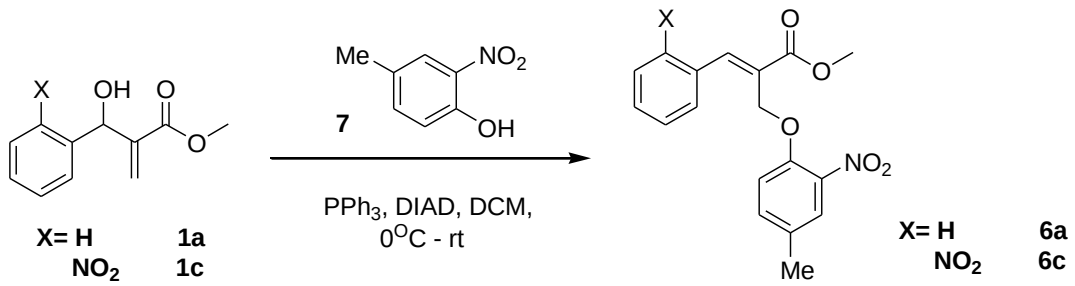
Table 4: Selection of appropriate leaving group by analysis of pK_a of their corresponding conjugate acids.

Leaving group	Br^-	Cl^-				
pK_a of conjugate acid	-8.7	-6.3	-2.8	4.21	7.16	9.9

Now, having determined the leaving group, efforts were concentrated to prepare compounds of type **A**. Mitsunobu reaction procedure was anticipated to give series of type **A** compounds from compounds **1** with different nucleophiles. But these reactions only yielded type **B** compounds, probably via indirect substitution reaction S_N2'. Even bromination reaction on compounds **1** gave rearranged brominated products (compounds **2**). Another strategy to prepare type **A** compounds was to prepare DABCO salt of the brominated products **2** and then on subjecting it to different nucleophiles would yield corresponding type **A** compounds.[18] Even attempts on this strategy failed giving compounds of type **B**.



Scheme 3: Bromination of **1a** only leads to the rearranged product **2a**. Further, reactions on compound **2a** with p-nitrophenol gives compound **5a**. Compound **5a** can also be obtained by Mitsunobu reaction on compound **1a**. (i) NaH, 4-nitrophenol, THF : DMF (9 : 1). (ii) 4-nitrophenol, PPh_3, DIAD, DCM, 0^OC – rt.



Scheme 4: Synthesis of **6a**, **6c** by Mitsunobu reaction on **1a**, **1c** with **7.**

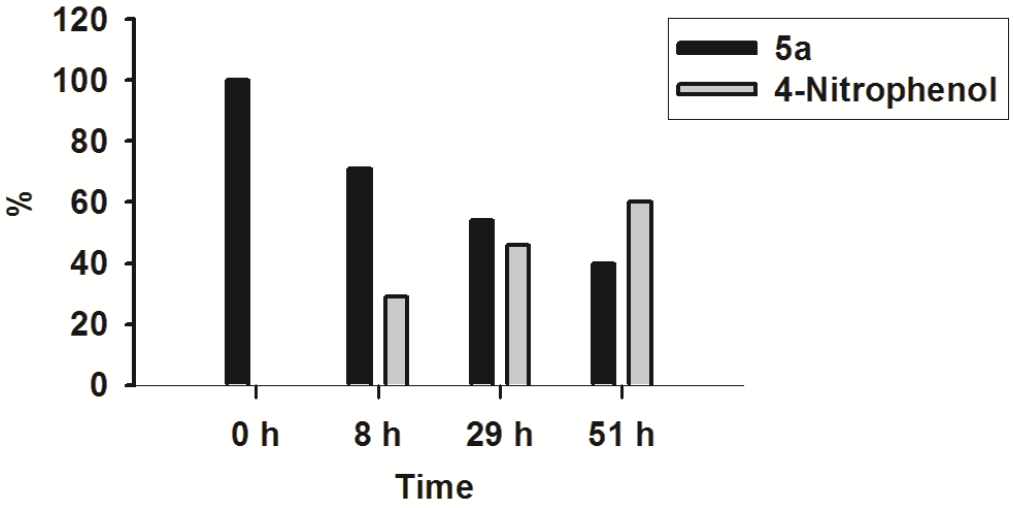


Figure 6: HPLC analysis on glutathione reaction with **5a** showing the decrease in concentration of **5a**, while an increase in concentration of 4-nitrophenol.

After several failed attempts to make compounds of type **A**, we focussed our attention on synthesizing type **B** compounds. Compound **1a** was selected as a precursor for further development of the delivery system. The procedure developed for Mitsunobu reaction was employed to synthesize **5a**. Surprisingly, **5a** on reaction with excess (10 equiv.) of glutathione under physiological conditions showed a significant decrease in reactivity ($t_{1/2}$ = ca. 29h) (Figure 6). Even **6a** was synthesized and subjected to glutathione reaction, which showed similar reactivity as compound **5a** ($t_{1/2}$ = ca. 29h) (Figure 7).

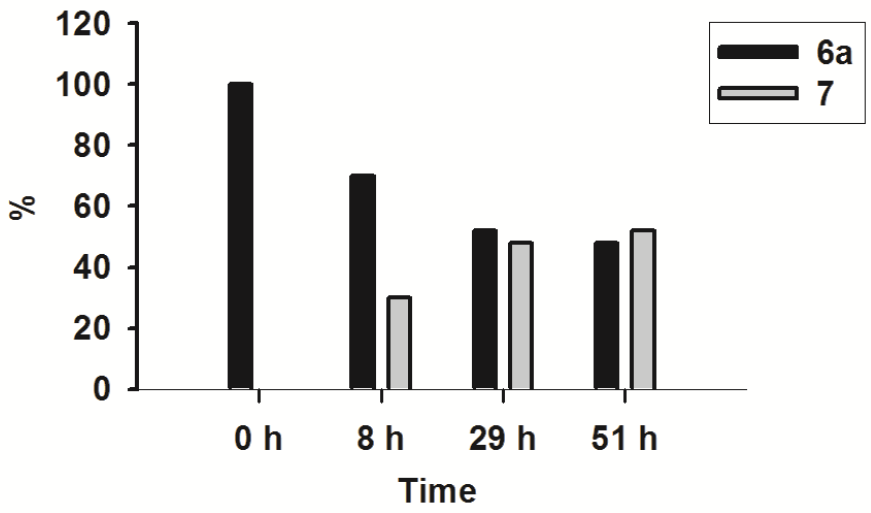


Figure 7: HPLC analysis on reaction rates of **6a** with glutathione showing an increase in concentration of **7** while a decrease in concentration of **6a**.

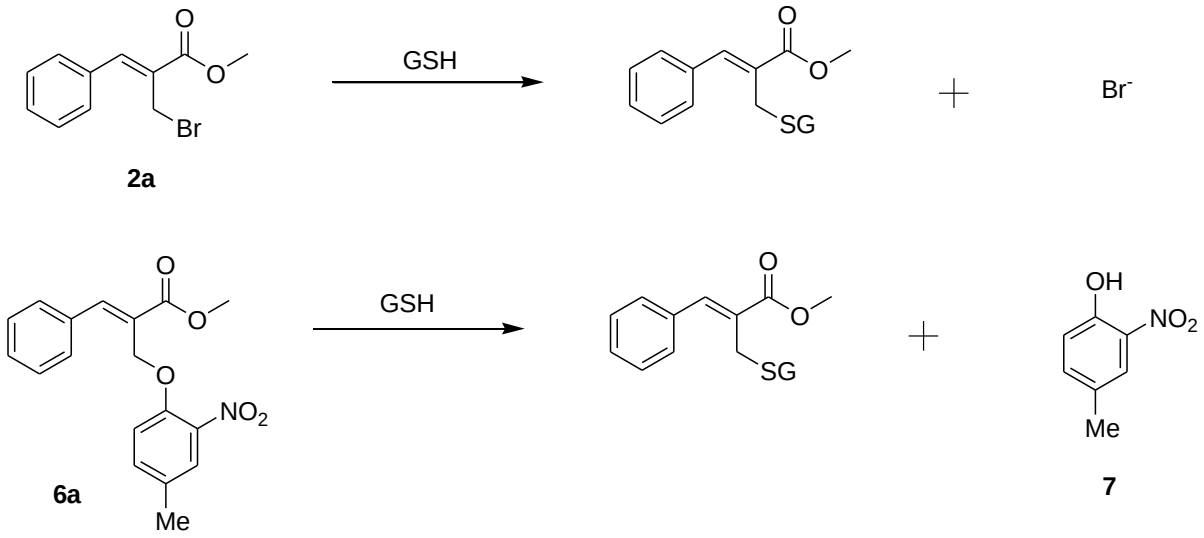


Figure 8: Thiol reaction on **2a** and **6a** giving the thiol adduct and the leaving group. (GSH = Glutathione, with reactive thiol moiety).

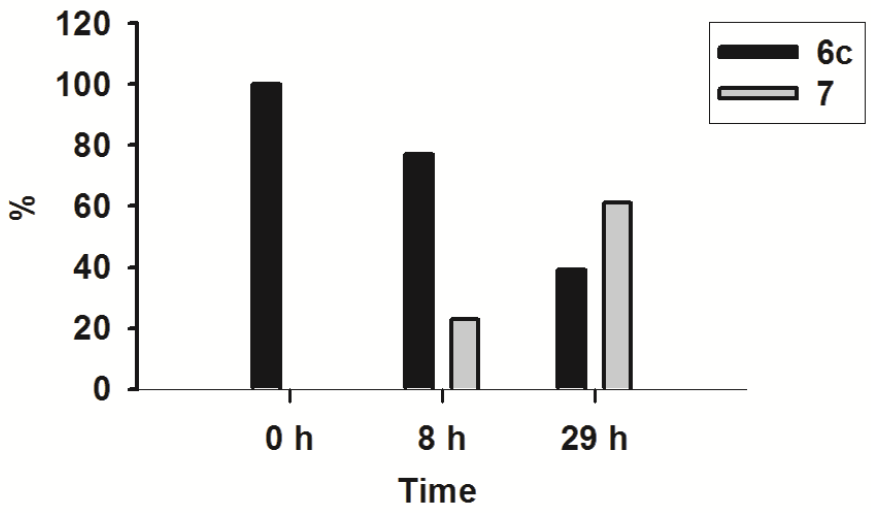


Figure 9: HPLC analysis of glutathione reaction with **6c**. Concentration of leaving group **7** increases as concentration of **6c** decreases.

On having these preliminary results, compound **7** (Figure 8) was used as a linker molecule to which the molecule of interest can be attached, especially, a drug molecule. Self-immolative behaviour of compounds similar to **7** is well-documented[19,20] (Figure 10(I)). So, on releasing such linkers under physiological condition, these linkers undergo 1,4- or 1,6-elimination reaction, without any external influence, releasing the entity attached to the linker. This system can be perceived as an efficient drug delivery medium.

Further, to check the reactivity of the proposed linker with glutathione, compounds **8** and **9** (Figure 10(II)) were prepared. These compounds act as mimics of the linker and with the phenolic hydroxyl group protected by methyl group. On reaction with glutathione, **8** and **9** showed no reactivity, even when incubated for 51 hours. These results indicate that esters and carbamates remain unreacted in the presence of glutathione and hence, compatible with drug molecules containing amines and carboxylic acid groups. Also, only self-immolative behaviour of the linker would be active, once glutathione reacts with the core of the prodrug.

A general description of the prodrug entity (Figure 10(III)) shows the prodrug can be differentiated into three parts – core, linker and drug molecule. Glutathione reaction is restricted to the core part where rate of the glutathione reaction can be tuned, by changing the X moiety on the aryl part and EWG. The linker part remains constant and is used for slow as well as efficient drug release. Glutathione does not affect this

part and hence, the rate of drug release can be only be determined by glutathione reaction rate with the core part. Then, the drug part is where different drug molecules can be attached, for e.g., gemcitabine, an anticancer drug[21] or even DNA alkylating agent, nitrogen mustard.[22]

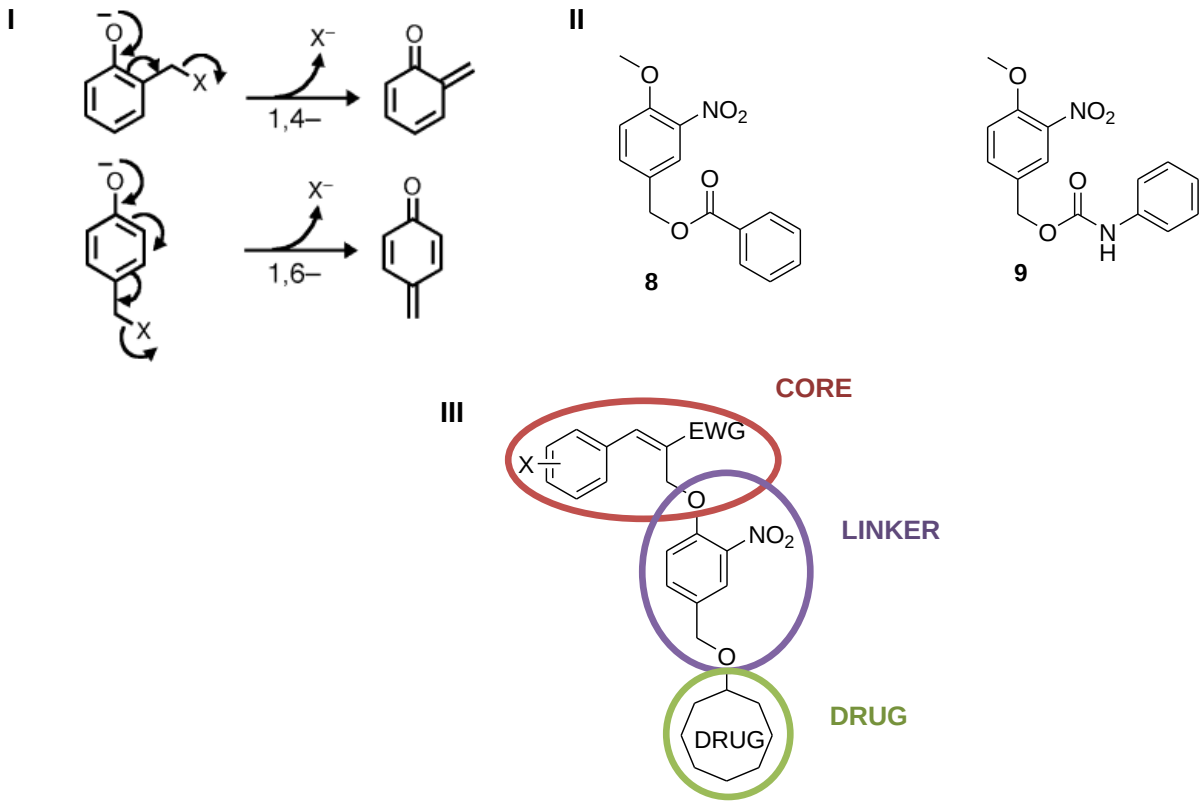
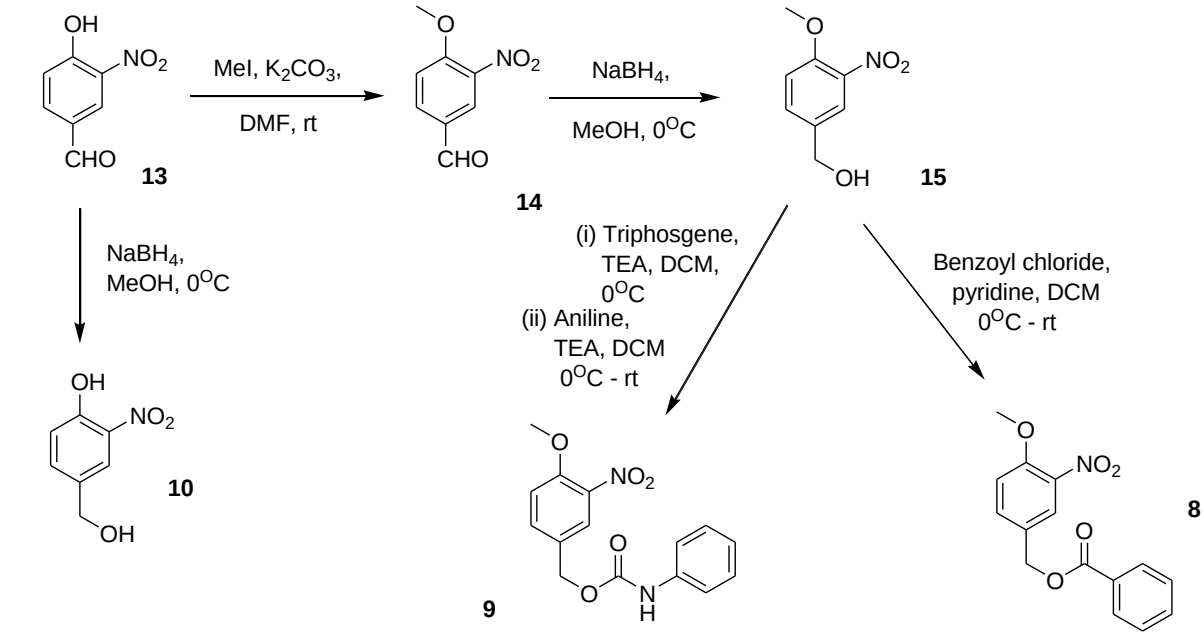
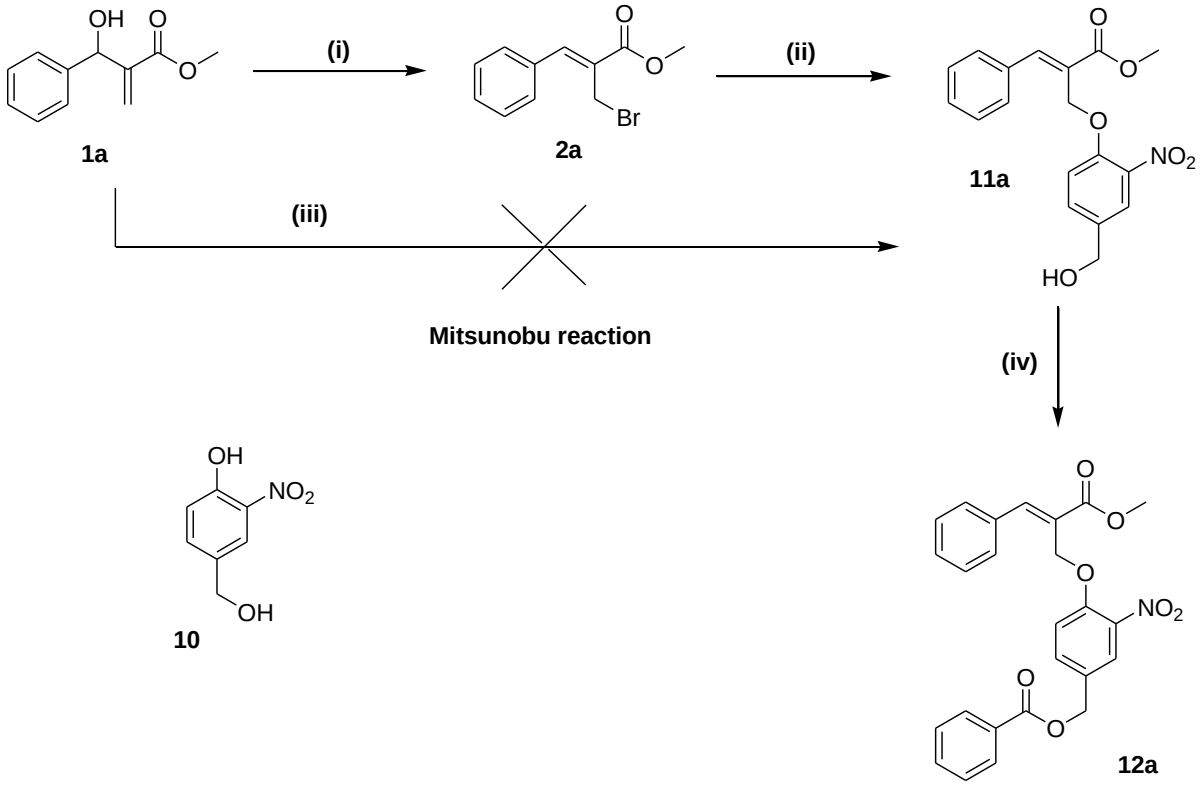


Figure 10: (I) 1,4- and 1,6- elimination reaction of self-immolative linkers. Adapted from Lee, H. Y.; et al. *Org. Lett.* **2009**, *11*, *10*, 2065-2068. (II) Linker mimics **8** and **9** prepared to study linker behaviour on thiol reaction. (III) Generic design of developed prodrug showing three parts - core, linker and drug.

On having all the results necessary for construction of the prodrug, a prodrug mimic **12a** was successfully synthesized (Scheme 5). Conversion of **2a** to **11a** was achieved by reaction with compound **10**, in presence of cesium carbonate as base. Mitsunobu reaction on **1a** with **10** failed, not giving the desired product **11a**. Compound **12a** was synthesized by benzoyl chloride reaction in presence of pyridine as base. Benzoic acid was used as a drug mimic for preliminary analysis of these compounds. Compound **11c** was also, synthesized following the same synthetic scheme (Scheme 5).



Scheme 5: Synthesis of **8**, **9** and **10**.



Scheme 6: Reaction scheme for synthesis of prodrug mimic **12a**. Attempts at synthesizing **11a** directly from **1a** failed. (i) PBr_3, DCM, 0^OC; (ii) **10**, Cs_2CO_3, CH_3CN, rt; (iii) **10**, PPh_3, DIAD, DCM, 0^OC - rt; (iv) benzoyl chloride, pyridine, DCM, 0^OC - rt.

A proposed mechanism for glutathione reaction on prodrug mimic **12a** (Figure 8, reaction details included) shows a two-step process of drug release. After the glutathione reaction on the core of the molecule, linker with the drug attached (compound **13**) is eliminated. Under physiological conditions, **13** undergoes self-immolative reaction (see arrow pushing, Figure 12) to give quinone methide **14** and release the drug mimic, benzoic acid. Compound **14**, then, reacts with water to yield compound **10**.

Further, compound **12a** was subjected to glutathione reaction. However, HPLC traces showed that benzoic acid and compound **10** elutes at almost the same retention time. Thus, these preliminary results have been determined by just monitoring the compound **12a** concentration in the reaction mixture. Compound **12a** shows faster reaction kinetics compared to compounds **5a** and **6a** (Figure 11). This result may be due to difference in solubility of **12a** or interference of compound **10**, formed during the reaction, by competing in reactivity with glutathione.

Also, compound **6c** showed similar reaction kinetics with glutathione compared to **6a** (Figure 9). This result supports the possibility of a tunable prodrug structure, with aforementioned design principles. However, more such experiments are needed to be conducted and analysed before this prodrug strategy can be established.

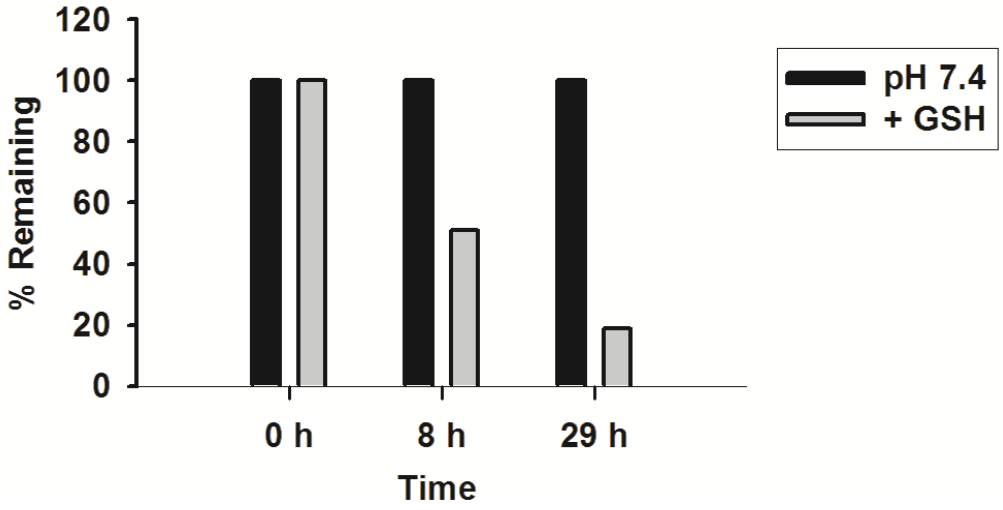


Figure 11: HPLC analysis of reaction of **12a** with glutathione with control experiment (absence of glutathione).

This strategy has an advantage over the work M.G. Finn and co-workers have published.[14] In their case, although the drug is released slowly into the system after a rate-limiting retro-Diels-Alder reaction, the drug released is attached to a furan moiety. This might affect the drug activity. Whereas, in this case, the drug released is in its native form (as should be administered to obtain the necessary effect), without any linker attached to it.

On delving into the possible applications of this technology, targeted drug delivery can be one of them. Compound **12a** reacts with glutathione to release the drug mimic (benzoic acid). Cancer cells are known to have high metabolism rates and thus, have elevated levels of glutathione compared to normal cells. These prodrugs can be used for preferential targeting of cancer cells in comparison to normal cells. Only a few glutathione-activated prodrugs are known. These compounds may have the advantage of tunable drug release characteristics, which the other strategies do not fulfill.

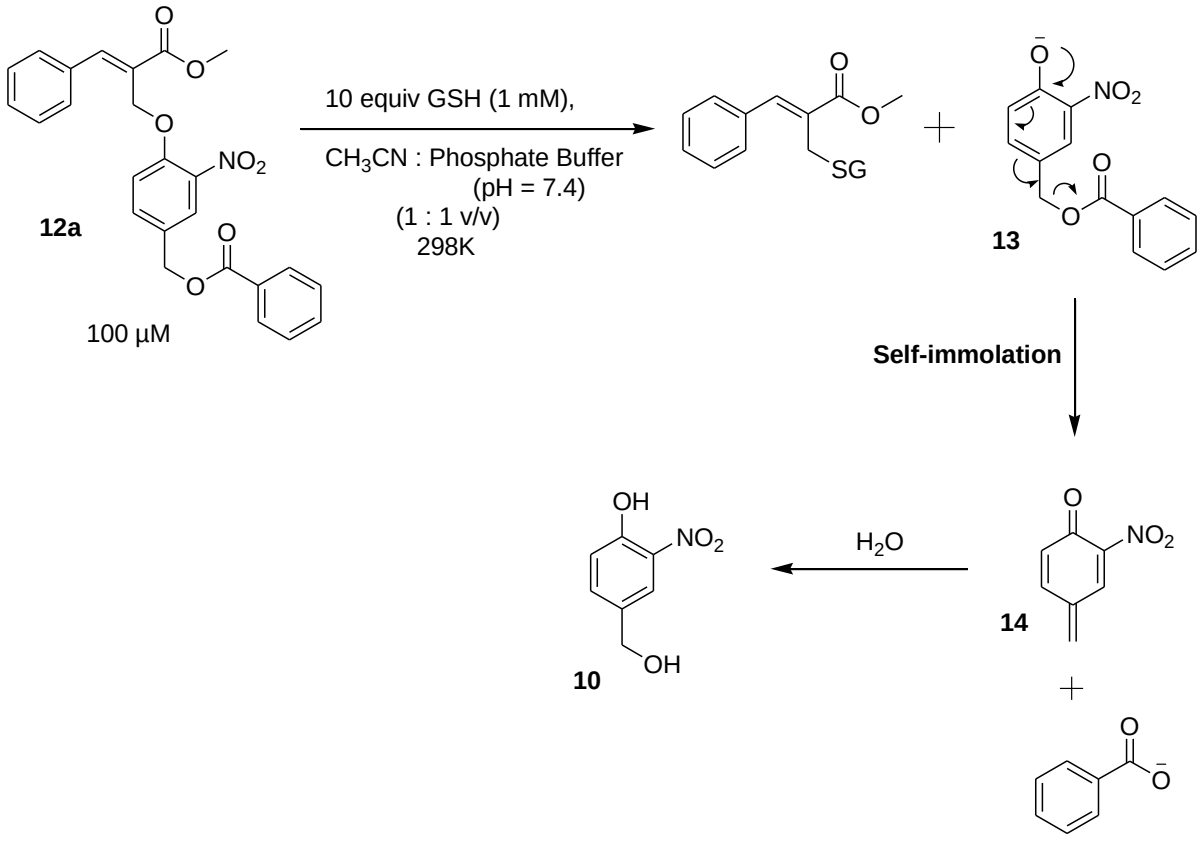


Figure 12: A proposed reaction mechanism of the prodrug mimic **12a**, with reaction details. After glutathione reaction, self-immolation of linker-drug conjugate **13** giving drug mimic benzoic acid.

This strategy can be anticipated to have applications outside the field of drug delivery. Differentiating individual proteins from a complex proteome is a major challenge for studying proteins implicated in diseased condition. Recently, Weerpana and co-workers reported a strategy using triazine-based small molecular probes which can differentially react with cysteine residues of proteins in a complex proteome and can be used for identification.[23] A particular emphasis is given to reactive cysteine moieties that show some specific thiol targeted reactions with appropriate electrophiles. Using this strategy, the reactivity of cysteine functionalities can be exploited and possibly differentiated. Thus, these compounds can act as small molecule mediators that specifically label proteins. With necessary changes made, visual cues can be attached to these molecules, like, an alkyne group to facilitate click-chemistry with a fluorescent tag or to attach biotin moiety for binding of avidin-fluorescent complex (Figure 13(I)).

An emergence of the theranostic ("therapy + diagnostic") approach will be crucial in the development of personalised medicine. This approach deals with simultaneous detection and diagnosis of a particular diseased condition. Recently, a glutathione-activated anticancer prodrug having drug-fluorescent tag complex have been shown to have theranostic properties.[21] With foresight, this approach can also be extended to prepare such prodrugs. Such fluorescent tag-drug complex can be synthesized with the use of an appropriate linker that can accommodate both of these entities[24] (Figure 13(II)).

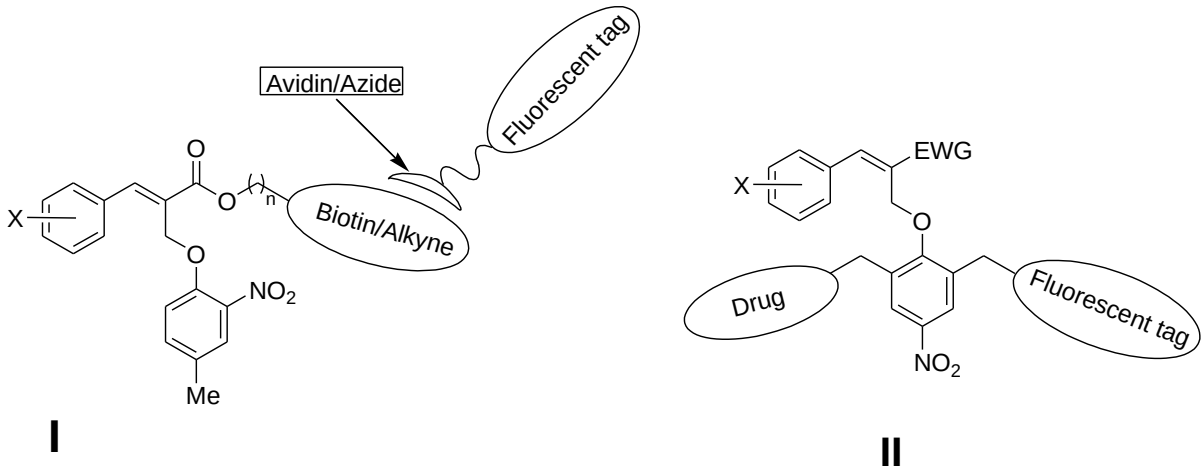


Figure 13: (I) Labelling of proteins with reactive cysteine residues can be achieved by biotin/avidin complex or click chemistry on the core part of the molecule with a

fluorescent tag attached. (II) Prodrug having a linker with drug and fluorescent tag for theranostic purposes.

CONCLUSION

In this project, we have presented a novel scaffold for potential use as thiol-activated tunable drug release candidates. A number of 2-hydroxyphenylmethylacrylates and 2-hydroxyphenylacrylonitriles were prepared using a Baylis-Hillman reaction. Selected number of these compounds was converted to the desired cinnamates with appropriate leaving groups. Our initial assessment of rate of reaction with thiols in pH 7.4 revealed that a leaving group such as bromide was highly reactive with thiols with a half-life of <1 h. Hence, based on these observations, the design was modified to incorporate poorer leaving groups such as 2-nitro and 4-nitrophenols. These phenols were substantially diminished in their reaction rate with thiols in pH 7.4 in comparison with the bromides suggesting that modulating leaving group ability could provide a handle for controlling thiol-mediated release rate. Also, we provide evidence for high stability of this scaffold in the absence of a thiol trigger. Further, a prototype was prepared which showed slow drug release properties with excess of physiological thiols like glutathione. Finally, the potential applications for this strategy are discussed, which go beyond the concept of drug delivery and can be implemented to selective detection of proteins as well.

EXPERIMENTAL SECTION

Abbreviations

DABCO – 1,4-diazabicyclo[2.2.2]octane

DCM – Dichloromethane

DIAD – Diisopropyl azodicarboxylate

DMF – Dimethylformamide

TEA – Triethylamine

THF – Tetrahydrofuran

Materials and Methods

All reagents were bought from commercial suppliers and used without further purification unless otherwise mentioned. Analytical thin-layer chromatography (TLC) was performed on Merck TLC Plate Silica Gel 60 F254 thin-layer chromatographic plates and visualised under a UV lamp. Proton and carbon NMR were recorded on a JEOL 400 MHz (100 MHz for ^{13}C) using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded from MALDI-TOF/TOF mass spectrometer (Applied Biosystems). Silica gel was used as a solid support for column chromatography was standard silica gel (60-120 mesh, Merck silica gel).

Synthetic protocols and compound characterization

Compounds **1a-e**,[15] **1f**,[25] **2a-b**,[16] **2c,**[26] **3a-h**,[15] **3i**,[27] **4a**,[16] **4c**,[28] **4d**,[16] **10**,[29] **13**,[30] **14**,[31] **15**[32] were previously reported and spectral data that we obtained was consistent with the literature reported values.

4-(2-cyano-1-hydroxyallyl)benzonitrile **(3j)**

4-cyanobenzaldehyde (1.00 g, 7.63 mmol) and acrylonitrile (3.11 mL, 47.3 mmol) were mixed and cooled to $0^{O}C$. DABCO (1.71 g) was added to this mixture at $0^{O}C$. Reaction mixture was stirred at $0^{O}C$ for 2 days. After disappearance of starting material as confirmed by TLC, this mixture was filtered through silica gel bed with 50% ethyl acetate in petroleum ether. The solvent was evaporated to yield

compound **3j** as a white solid (1.39 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J* = 8.26 Hz, 2H), 7.55 (d, *J* = 8.11 Hz, 2H), 6.17 (d, *J* = 1.03, 1H), 6.09 (s, 1H), 5.39 (broad, OH). ^{13}C (100 MHz, $CDCl_3$): δ 144.4, 132.8, 130.9, 127.3, 125.6, 118.5, 116.5, 112.6, 73.5. MALDI TOF/TOF: $C_{11}H_8N_2O$ $[M+Na]^+$: 207.0529. Found $[M+Na]^+$: 206.9584.

(Z)-2-(bromomethyl)-3-(4-nitrophenyl)acrylonitrile (4b)

To a stirred solution of **3b** (100 mg, 0.49 mmol) and PPh_3 (256.8 mg, 0.98 mmol) in anhydrous DCM (3.0 mL), CBr_4 (325.0 mg, 0.98 mmol) was added under nitrogen atmosphere. The mixture was stirred at room temperature and the reaction was monitored by TLC. After completion as seen by disappearance of starting material, the reaction mixture was diluted with cold water (10 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were dried over sodium sulphate (Na_2SO_4) and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate in petroleum ether) as eluent to yield **4b** as a white solid (76 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, *J* = 8.63 Hz, 2H), 7.94 (d, *J* = 8.57 Hz, 2H), 7.31 (s, 1H), 4.24 (s, 2H).

***Procedure A: (E)-methyl 2-((4-nitrophenoxy)methyl)-3-phenylacrylate* (5a)**

To a stirred solution of 4-nitrophenol (81.8 mg, 0.59 mmol) in anhydrous THF : DMF (900 µL : 100 µL) at 0^{O}C, NaH (25.2 mg, 60% suspension in oil, 0.63 mmol) was added and the resulting mixture was stirred for 10 min. Compound **2a** was added to this mixture at 0^{O}C. Reaction mixture was then warmed to room temperature and stirred for additional 3h. Reaction mixture was again cooled to 0^{O}C and slowly quenched with ice-cold water (10 mL). Aqueous solution was washed with DCM (3 × 10 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (5 × 5 mL), brine (10 mL), dried over sodium sulphate (Na_2SO_4) and concentrated. The filtrate obtained is purified by silica gel column chromatography (13% ethyl acetate in petroleum ether as eluent) to yield compound **5a** as a white solid (73 mg, 60%).

***Procedure B: (E)-methyl 2-((4-nitrophenoxy)methyl)-3-phenylacrylate* (5a)**

To a stirred solution of **1a** (100 mg, 0.52 mmol) in anhydrous DCM (5.0 mL), 4-nitrophenol (72.4 mg, 0.52 mmol) and PPh_3 (272.8 mg, 1.04 mmol) were added under nitrogen atmosphere. This solution was cooled to 0^{O}C. DIAD (204.8 µL, 1.04

mmol) was slowly added to this solution and the solution was gradually warmed to room temperature. The solution was stirred for additional 2h. After disappearance of starting material as confirmed by TLC, reaction mixture was diluted by adding water (10 mL), followed by extraction with DCM (4 × 5 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (5 × 5 mL), brine (10 mL), dried over sodium sulphate (Na_2SO_4) and concentrated to yield crude product. Product was purified by silica gel column chromatography (13% ethyl acetate in petroleum ether as eluent) to yield a white solid (31%, 50 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (dd, *J* = 2.07, 7.20 Hz, 2H), 8.10 (s, 1H), 7.40 (m, 5H), 7.02 (dd, *J* = 2.15, 9.23 Hz, 2H), 4.94 (s, 2H), 3.87 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 167.3, 163.5, 146.5, 141.9, 134.2, 130.0, 129.6, 128.9, 126.3, 126.0, 115.0, 63.7, 52.6. MALDI TOF/TOF: $C_{17}H_{15}NO_5$ $[M+Na]^+$: 336.0842. Found $[M+Na]^+$: 336.0499.

General procedure for synthesis of compounds ***6***

To a stirred solution of compound **1a** (1.0 equiv) in anhydrous DCM (5.0 mL) was added 4-methyl-2-nitrophenol (1.0 equiv) and PPh_3 (2.0 equiv) under nitrogen atmosphere. This solution was cooled to 0^OC. DIAD (2.0 equiv) was slowly added to this solution and the resulting mixture was gradually warmed to room temperature. The solution was stirred for additional 2h. After disappearance of starting material as confirmed by TLC, reaction mixture was diluted by adding water (10 mL), followed by extraction with DCM (4 × 5 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (5 × 5 mL), brine (10 mL), dried over sodium sulphate (Na_2SO_4) and concentrated to yield crude product. Product was purified by silica gel column chromatography (13-15% ethyl acetate in petroleum ether as eluent) to yield the desired product.

(E)-methyl 2-((4-methyl-2-nitrophenoxy)methyl)-3-phenylacrylate **(6a)**

Starting from **1a** (50 mg, 0.26 mmol), **6a** was obtained as light yellow viscous liquid (25 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.64 (d, *J* = 1.64 Hz, 1H), 7.53 (m, 2H), 7.40 (m, 3H), 7.31 (dd, *J* = 2.20, 8.96 Hz, 1H), 7.11 (d, *J* = 8.52 Hz, 1H), 4.95 (s, 2H), 3.85 (s, 3H), 2.35 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 167.5, 149.9, 146.6, 140.4, 134.6, 134.3, 131.1, 129.8, 128.8, 127.3, 126.5, 125.7, 116.3, 65.2, 52.5, 20.3. MALDI TOF/TOF: $C_{18}H_{17}NO_5$ $[M+Na]^+$: 366.0738. Found $[M+Na]^+$: 366.0457.

***(E)-methyl 2-((4-methyl-2-nitrophenoxy)methyl)-3-(2-nitrophenyl)acrylate* (6c)**

Starting from **1c** (50 mg, 0.21 mmol), **6c** was obtained as yellow viscous liquid (28 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.19 (d, *J* = 8.22 Hz, 1H), 7.76 (d, *J* = 7.39 Hz, 1H), 7.69 (dd, *J* = 7.57, 7.35 Hz, 2H), 7.61 (d, *J* = 1.69 Hz, 1H), 7.57 (dd, *J* = 7.17, 8.37 Hz, 1H), 7.29 (dd, *J* = 2.03, 8.68 Hz, 1H), 7.08 (d, *J* = 8.52 Hz, 1H), 4.79 (s, 2H), 3.89 (s, 3H), 2.34 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 166.5, 149.7, 147.3, 143.2, 134.8, 134.2, 132.1, 131.5, 130.4, 130.2, 127.8, 125.6, 125.0, 116.5, 65.4, 52.7, 20.3. MALDI TOF/TOF: $C_{18}H_{16}N_2O_7$ $[M+Na]^+$: 395.0855. Found $[M+Na]^+$: 395.0601.

***4-methoxy-3-nitrobenzyl benzoate* (8)**

In anhydrous DCM (2.0 mL), **15** (50 mg, 0.27 mmol) and pyridine (33.1 µL, 0.41 mmol) were added under nitrogen atmosphere. This solution was cooled to 0^{O}C. Benzoyl chloride (47.8 µL, 0.410 mmol) was slowly added to this solution and the resulting solution was gradually warmed to room temperature. The solution was stirred for additional 2 hours. After completion of reaction as confirmed by TLC, reaction mixture was diluted with water (5.0 mL) and extracted with DCM (3 × 10 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (3 × 5 mL), brine (10 mL), dried over sodium sulphate (Na_2SO_4) and concentrated. The filtrate was purified by silica gel column chromatography (20% ethyl acetate in petroleum ether as eluent) to yield a white solid (76 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (dd, *J* = 8.4, 1.2 Hz, 2H), 7.94 (d, *J* = 2.2 Hz, 1H), 7.63 (dd, *J* = 2.2, 8.6 Hz, 1H), 7.55 (tt, *J* = 1.2, 6.9 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.08 (d, *J* = 8.7 Hz, 1H), 5.31 (s, 2H), 3.95 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 166.3, 153.0, 139.6, 134.4, 133.4, 129.8, 128.6, 125.9, 113.8, 65.2, 56.7. MALDI TOF/TOF: $C_{15}H_{13}NO_5$ $[M+Na]^+$: 310.0686. Found $[M+Na]^+$: 309.9955.

***4-methoxy-3-nitrobenzyl phenylcarbamate* (9)**

Under nitrogen atmosphere, **15** (50 mg, 0.273 mmol) and TEA (38 µL, 0.273 mmol) were dissolved in anhydrous DCM (5.0 mL). This solution was cooled to 0^{O}C. Triphosgene (81.0 mg, 0.273 mmol) was added to this solution and the resulting solution was allowed to stir at 0^{O}C for 30 min. Solvent was evaporated from this solution and the crude product was dried by putting it to high vacuum for 30 min.

This crude was dissolved in anhydrous DCM (3.0 mL) and cooled to 0^{O}C. A solution of aniline (25.0 µL, 0.273 mmol) and TEA (38 µL, 0.273 mmol) in anhydrous DCM (2.0 mL) was prepared separately and slowly added to the other solution at 0^{O}C. The reaction mixture was warmed to room temperature and stirred overnight. Reaction was monitored by TLC. After reaction completion, reaction mixture was diluted by water (10 mL) and extracted by DCM (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried over sodium sulphate (Na_2SO_4) and concentrated. The filtrate was purified by silica gel column chromatography (20% ethyl acetate in petroleum ether as eluent) to yield a reddish yellow solid (38 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, *J* = 2.2 Hz, 1H), 7.53 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.16 (dd, *J* = 7.4, 8.5 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.72 (t, *J* = 7.3 Hz, 1H), 6.59 (dd, *J* = 0.8, 7.7 Hz, 2H), 4.31 (s, 2H), 3.93 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 152.2, 147.6, 139.7, 133.0, 132.2, 129.4, 124.5, 118.2, 113.9, 113.0, 56.7, 47.0.

*General procedure for synthesis of compounds **11***

To stirred solution of **2** (1.0 equiv) in acetonitrile (5.0 mL), **10** (1.0 equiv) and Cs_2CO_3 (1.0 equiv) was added at room temperature. This mixture was stirred at room temperature for 24h. Reaction was monitored by TLC. After completion, acetonitrile was evaporated from reaction mixture and water (10 mL) was added to it. Aqueous layer was washed with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (5 × 5 mL), brine (15 mL), dried over sodium sulphate (Na_2SO_4) and concentrated. The filtrate was purified by silica gel column chromatography (43-45% ethyl acetate in petroleum ether as eluent).

***(E)-methyl 2-((4-(hydroxymethyl)-2-nitrophenoxy)methyl)-3-phenylacrylate* (11a)**

Starting from **2a** (50 mg, 0.20 mmol), **11a** was obtained as a white solid (63 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.84 (d, *J* = 2.23 Hz, 1H), 7.51 (m, 3H), 7.40 (dd, *J* = 1.98, 5.05 Hz, 3H), 7.19 (d, *J* = 8.62 Hz, 1H), 4.98 (s, 2H), 4.67 (s, 2H), 3.85 (s, 3H), 2.07 (broad, OH). ^{13}C (100 MHz, $CDCl_3$): δ 167.5, 151.3, 146.9, 140.4, 134.2, 134.2, 132.5, 129.9, 129.8, 128.9, 126.2, 124.0, 116.1, 65.1, 63.7, 52.5. MALDI TOF/TOF: $C_{18}H_{17}NO_6$ $[M+Na]^+$: 366.0948. Found $[M+Na]^+$: 366.1022.

***(E)-methyl 2-((4-(hydroxymethyl)-2-nitrophenoxy)methyl)-3-(2-nitrophenyl)acrylate* (11c)**

Starting from **2a** (50 mg, 0.20 mmol), **11a** was obtained as light yellow viscous liquid (60 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.20 (d, *J* = 8.22 Hz, 1H), 7.81 (s, 1H), 7.72 (dd, *J* = 7.58, 8.00 Hz, 2H), 7.57 (t, *J* = 7.79 Hz, 1H), 7.49 (d, *J* = 8.63 Hz, 1H), 7.14 (d, *J* = 8.54 Hz, 1H), 4.82 (s, 2H), 4.67 (s, 2H), 3.88 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 166.5, 150.9, 147.2, 143.5, 140.3, 134.6, 134.3, 132.6, 131.9, 130.4, 130.3, 127.5, 125.0, 123.9, 116.3, 65.2, 63.6, 52.7. MALDI TOF/TOF: $C_{18}H_{16}N2O_8$ $[M+Na]^+$: 411.0804. Found $[M+Na]^+$: 411.1011.

***(E)-4-(2-(methoxycarbonyl)-3-phenylallyloxy)-3-nitrobenzyl benzoate* (12a)**

Under a nitrogen atmosphere, **11a** (50 mg, 0.146 mmol) and pyridine (17.7 µL, 0.219 mmol) were dissolved in anhydrous DCM (3.0 mL). This solution was cooled to 0^{O}C. Benzoyl chloride (25.4 µL, 0.219 mmol) was slowly added to this solution while stirring. The resulting solution was warmed to room temperature and stirred for additional 2 hours. Reaction mixture was diluted by water (5 mL) and extracted with DCM (3 × 5 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ (3 × 5 mL), brine (10 mL), dried over sodium sulphate (Na_2SO_4), filtered and concentrated. The filtrate was purified by silica gel column chromatography (20% ethyl acetate in petroleum ether as eluent) to yield **12a** as a white solid (60 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 8.07 (d, *J* = 1.12 Hz, 1H), 8.05 (d, *J* = 1.40 Hz, 1H), 7.95 (d, *J* = 2.21 Hz, 1H), 7.61 (dd, *J* = 2.24, 8.63 Hz, 1H), 7.57 (dt, *J* = 1.54, 6.94 Hz, 1H), 7.52 (dd, *J* = 2.10, 7.31 Hz, 2H), 7.46 (m, 2H), 7.42 (m, 2H), 7.40 (d, *J* = 1.80 Hz, 1H), 7.23 (d, *J* = 8.65 Hz, 1H), 5.33 (s, 2H), 5.00 (s, 2H), 3.85 (s, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 166.1, 165.0, 150.6, 145.6, 139.1, 132.9, 132.8, 132.1, 128.6, 128.5, 127.9, 127.6, 127.3, 124.8, 124.4, 114.8, 75.8, 75.5, 63.9, 63.7, 51.2. MALDI TOF/TOF: $C_{25}H_{21}NO_7$ $[M+K]^+$: 486.0950. Found $[M+Na]^+$: 486.0843.

High performance liquid chromatography (HPLC) method

Analytical reverse- phase high performance liquid chromatography experiments were carried out in an Agilent 1100 series HPLC system with a C18 column (phenomenex, 30 cm × 2.1 mm). A multistep gradient system for 30 min with a solvent flow rate of 1

mL min^{-1} (50 to 75% of acetonitrile in water over 0 to 30 min). The compound elution was monitored using ultraviolet profiles of multiple wavelengths (254, 280 and 400 nm).

HPLC Experiment

10 mM stock solutions of compounds were prepared in acetonitrile, 100 mM stock solution of reduced L-glutathione was prepared in pH = 7.4 (25 mM) phosphate buffer. In a typical experiment, 30 µL (10 mM) solution of compounds were dissolved into 1470 µL acetonitrile and 1470 µL pH = 7.4 (25 mM) phosphate buffer. 30 µL glutathione solution (100 mM) was added to this solution and the reaction mixture was allowed to stir at room temperature. The time was calculated from the moment of addition of glutathione. Control experiment contained 30 µL (10 mM) solution of compounds were dissolved into 1485 µL acetonitrile and 1485 µL pH = 7.4 (25 mM) phosphate buffer. At specific time points, an aliquot of the reaction mixture was injected into HPLC instrument. All experiments were conducted in duplicate.

^{1}H and ^{13}C spectral data

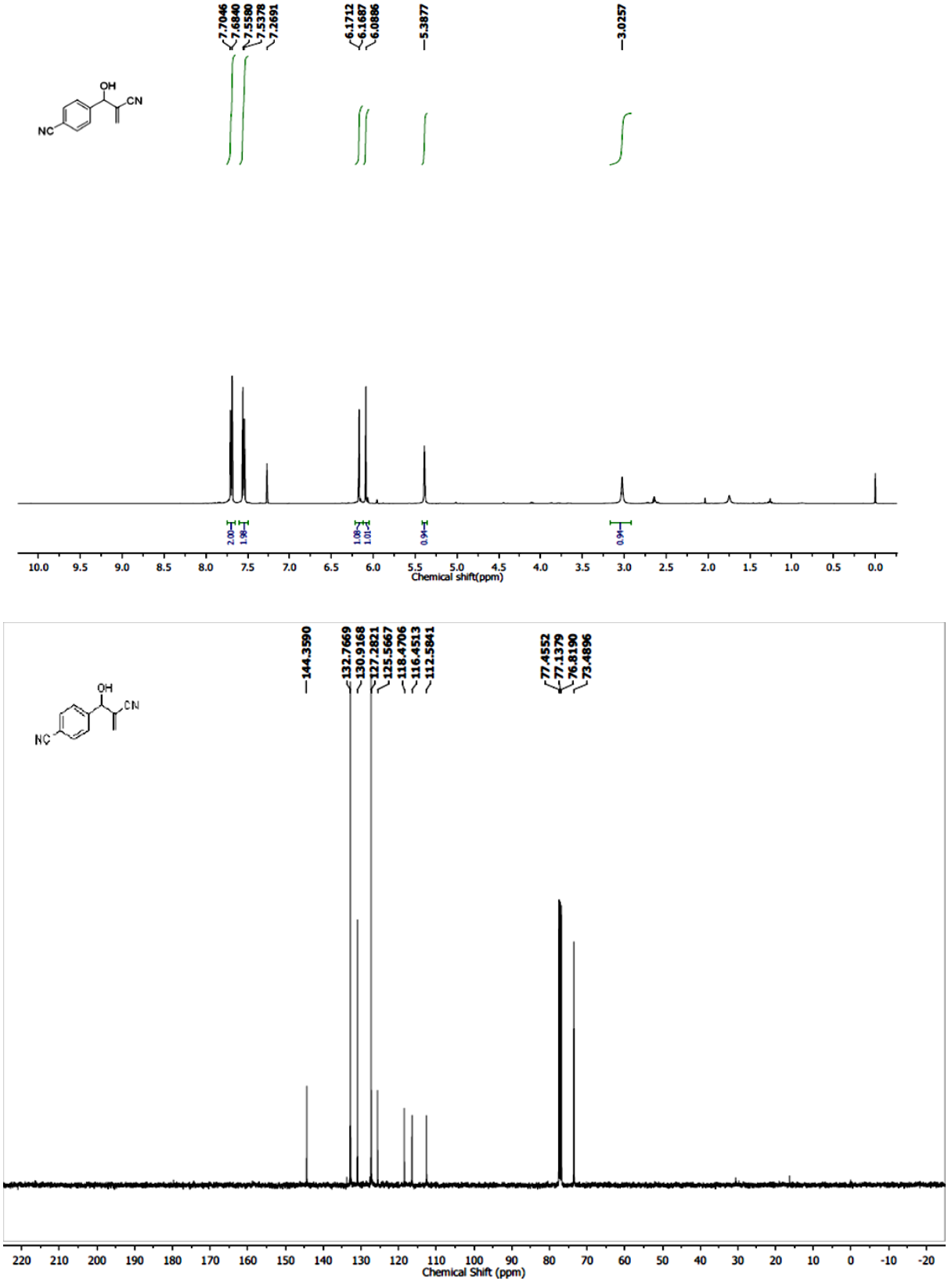


Figure 14: ^{1}H and ^{13}C spectrum of **3j**.

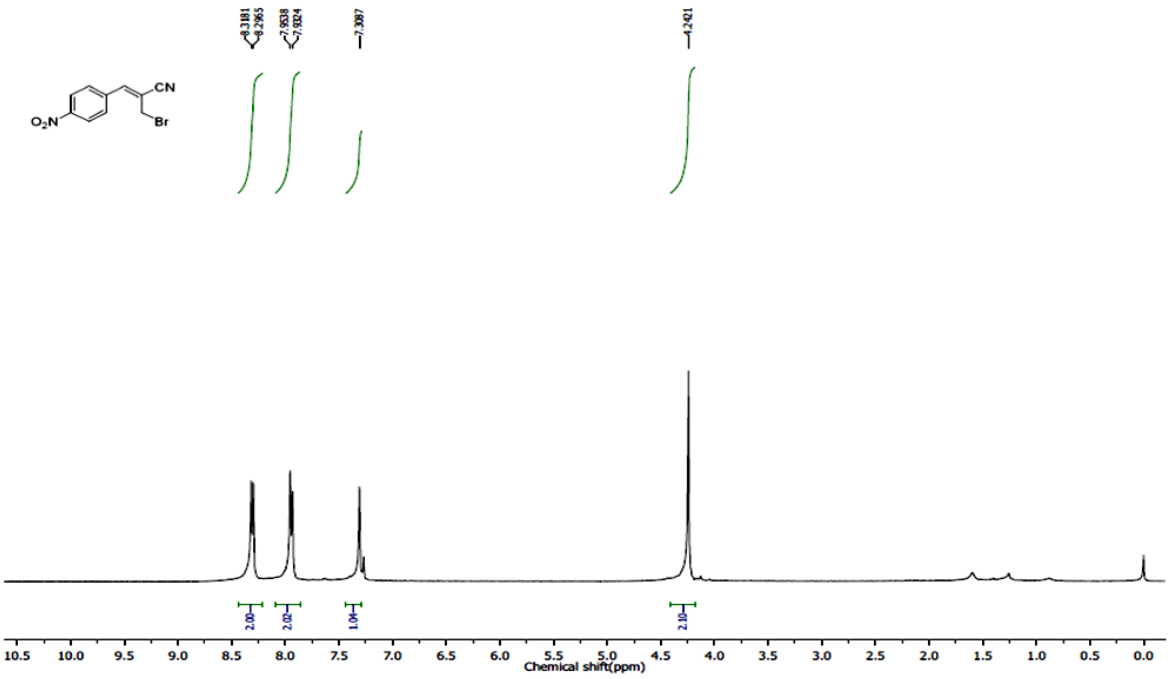


Figure 15: ^{1}H spectrum of **4b**.

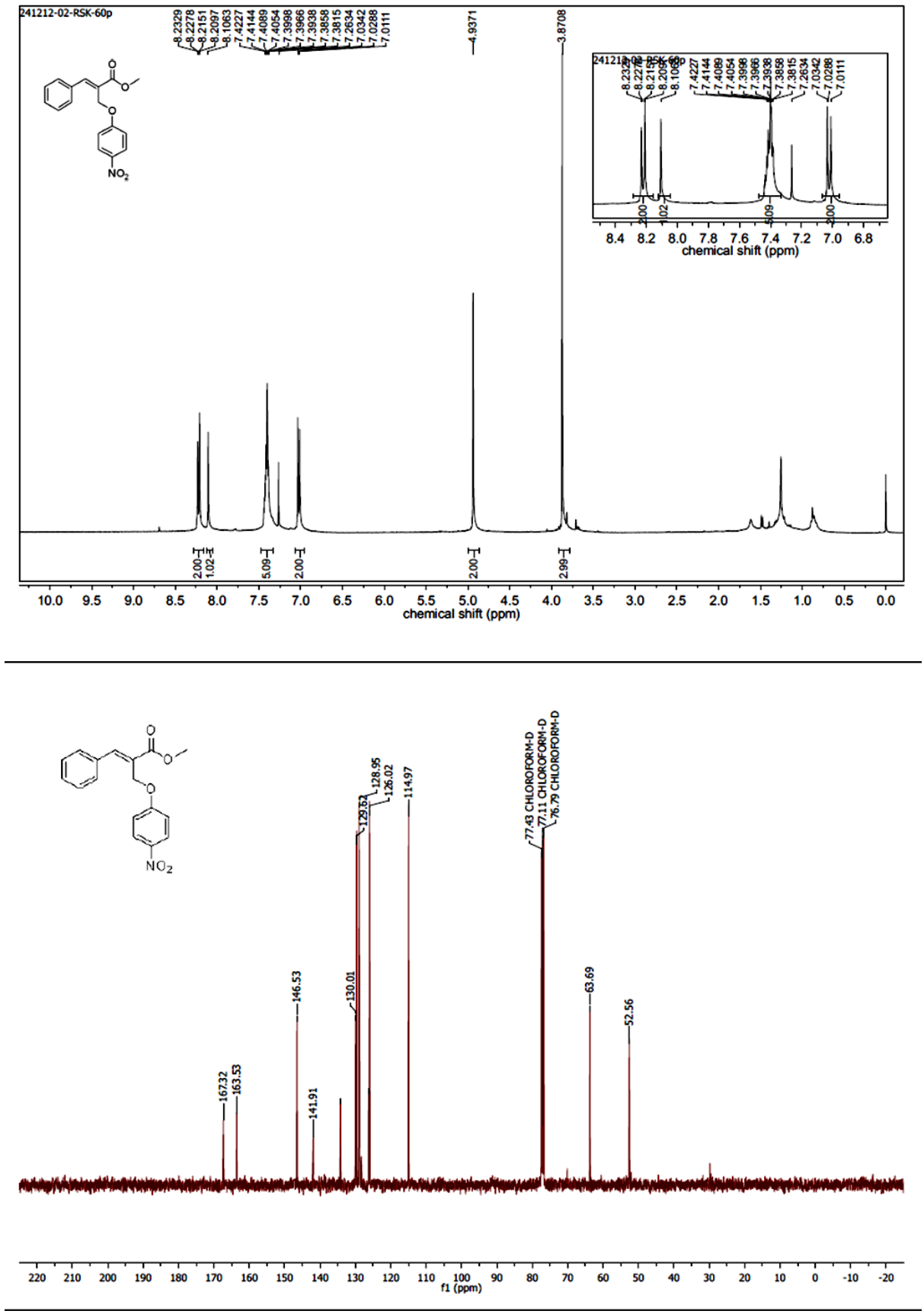


Figure 16: ^{1}H and ^{13}C spectra of **5a**.

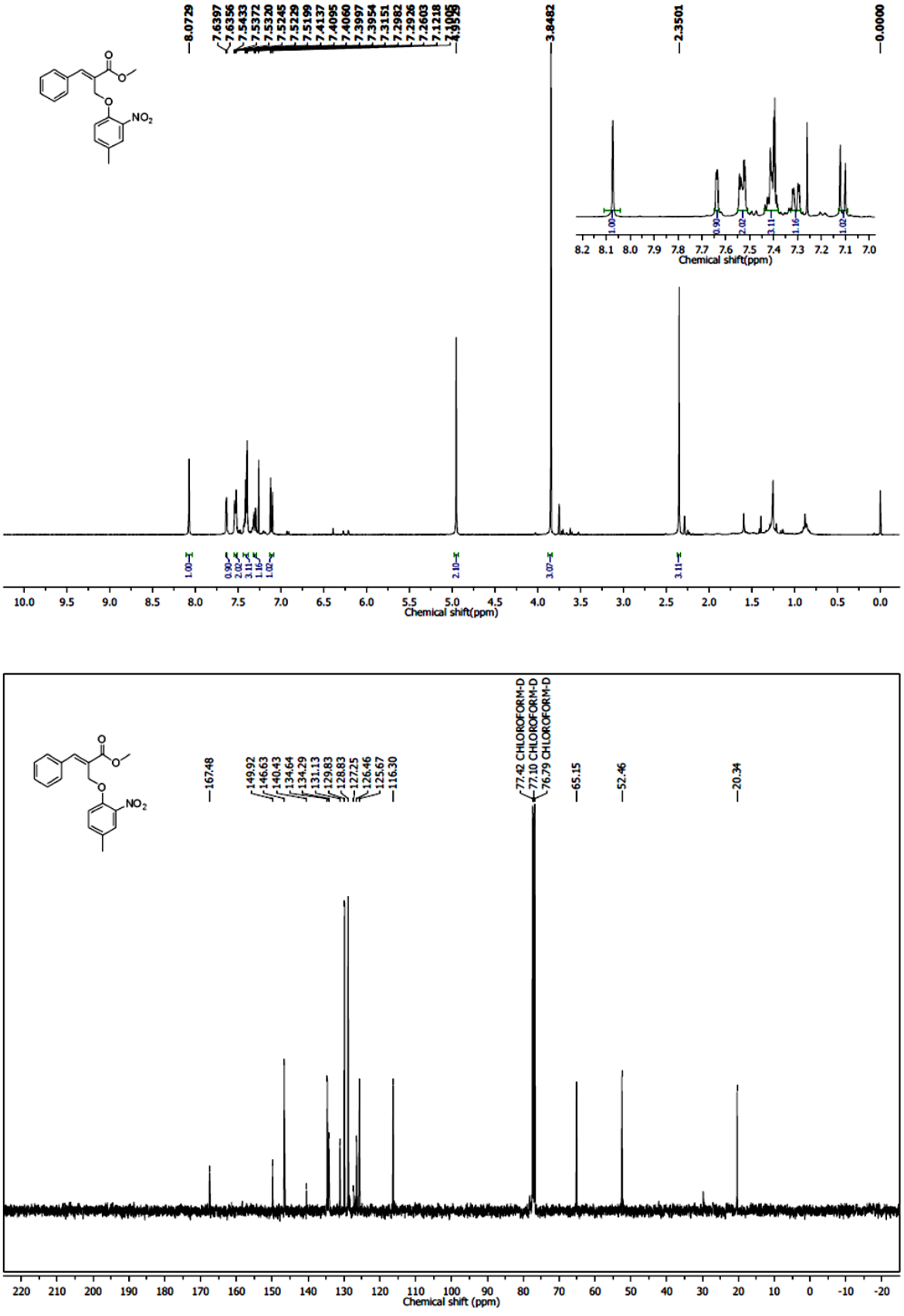


Figure 17: ^{1}H and ^{13}C spectra of **6a**.

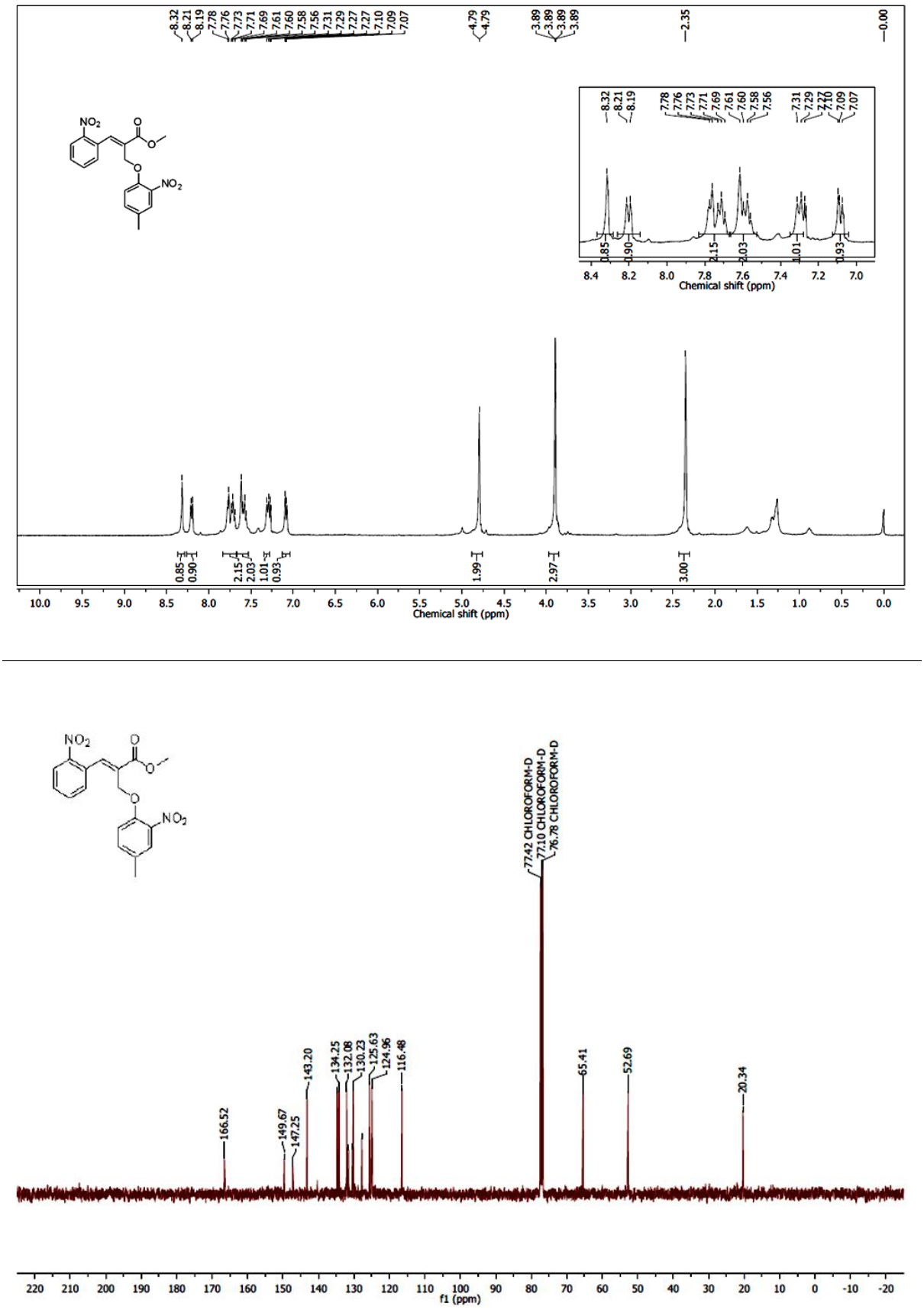


Figure 18: ^{1}H and ^{13}C spectra of **6c**.

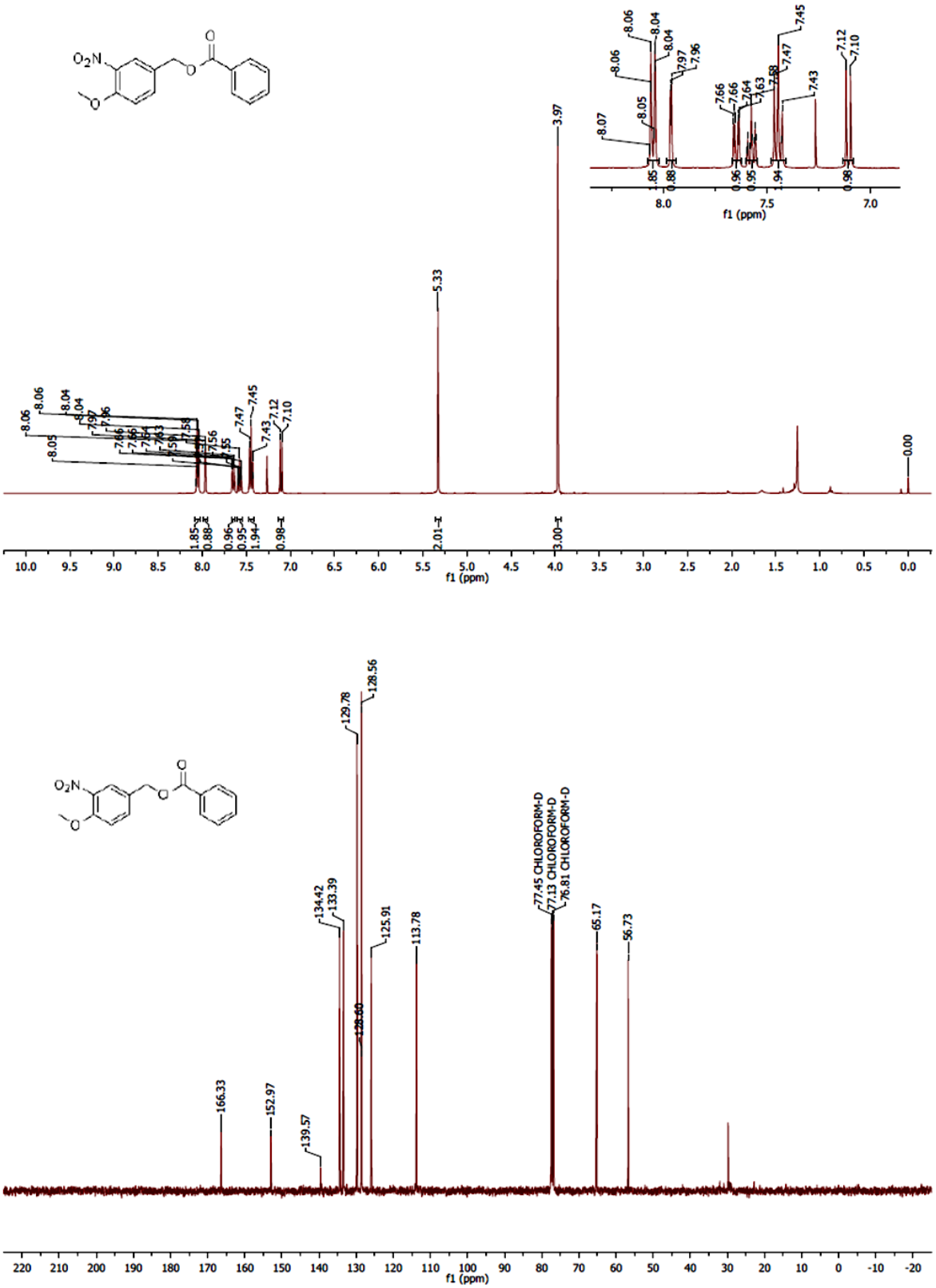


Figure 19: ^{1}H and ^{13}C spectra of **8**.

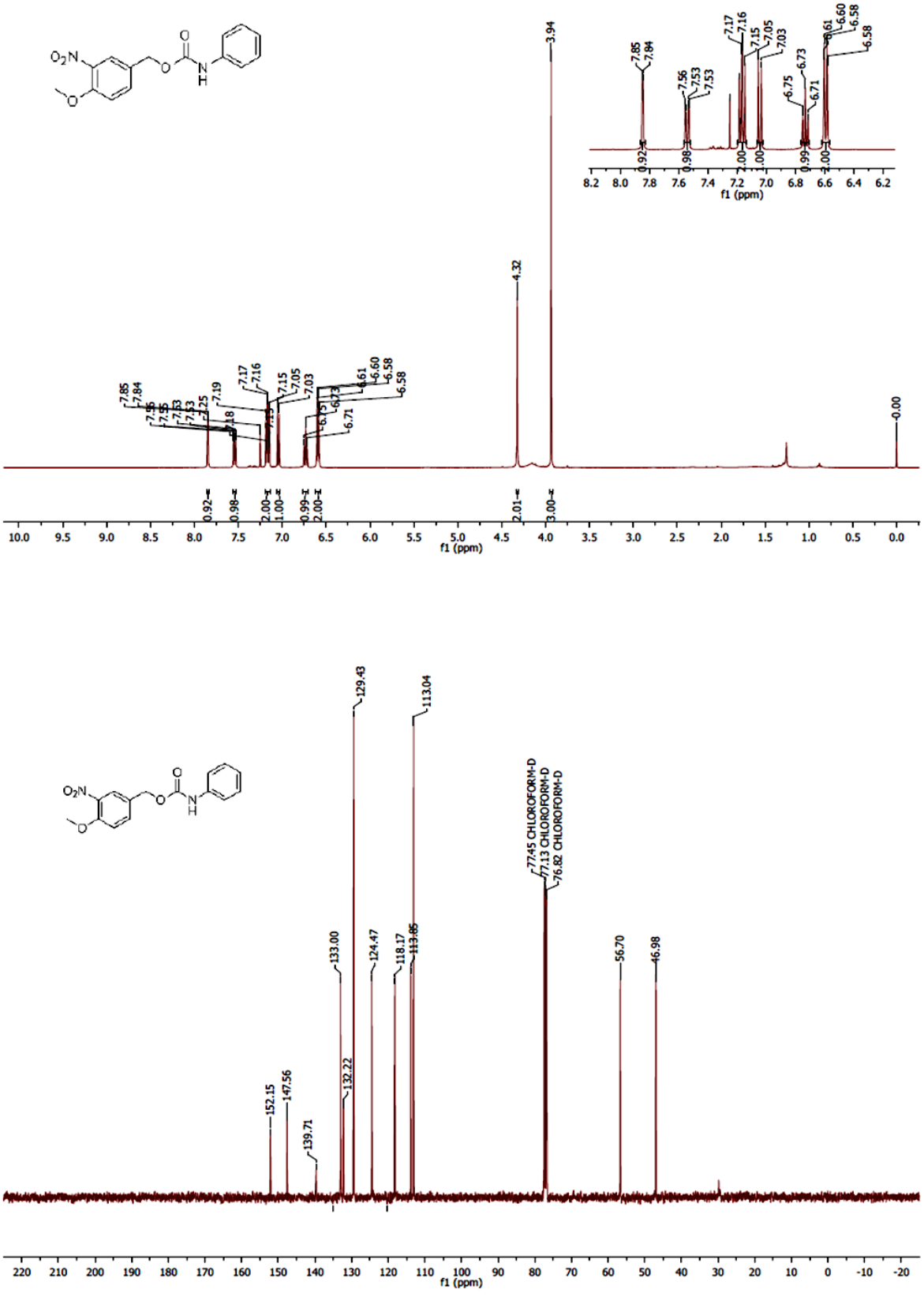


Figure 20: ^{1}H and ^{13}C spectra of **9**.

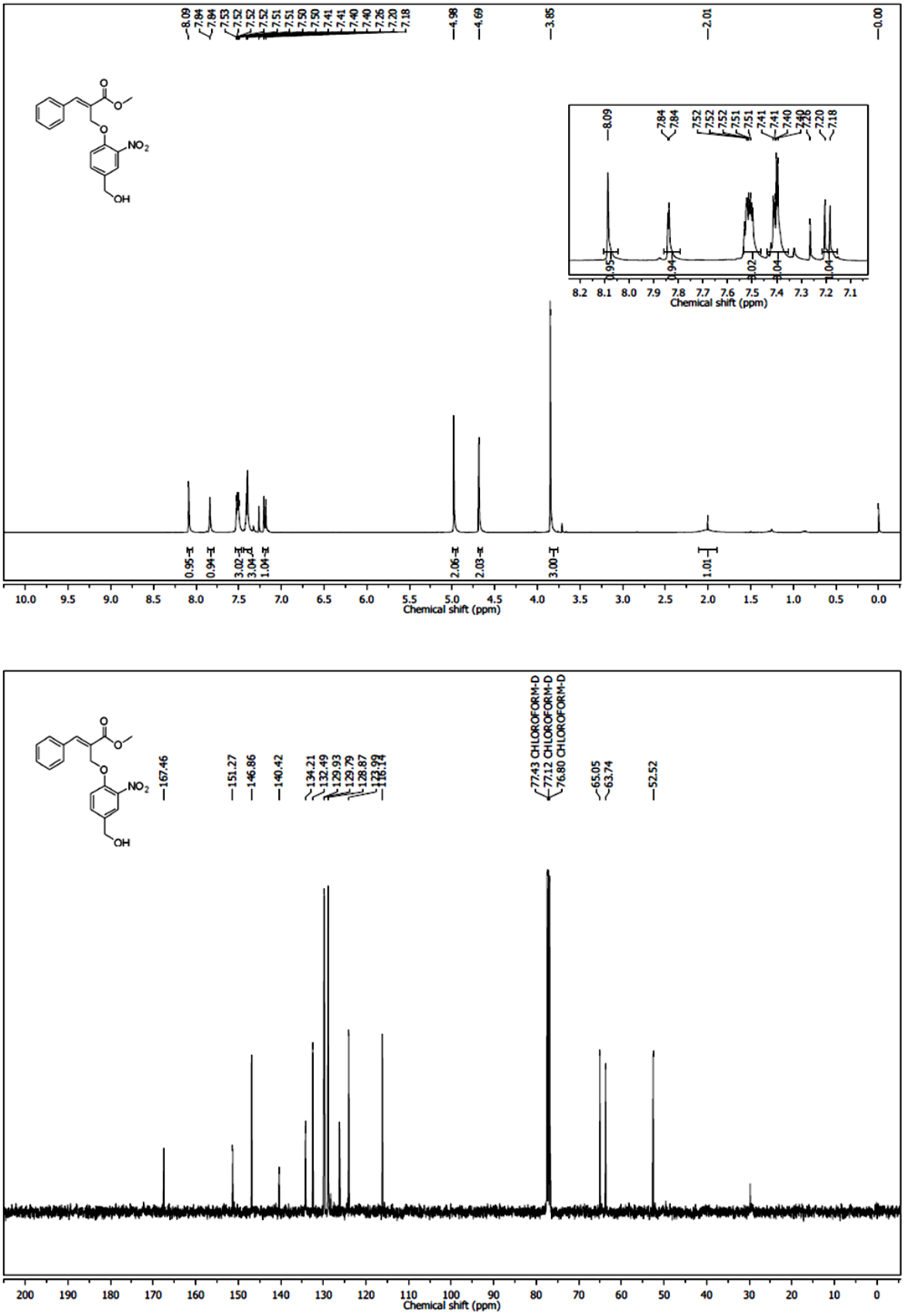


Figure 21: ^{1}H and ^{13}C spectra of **11a**.

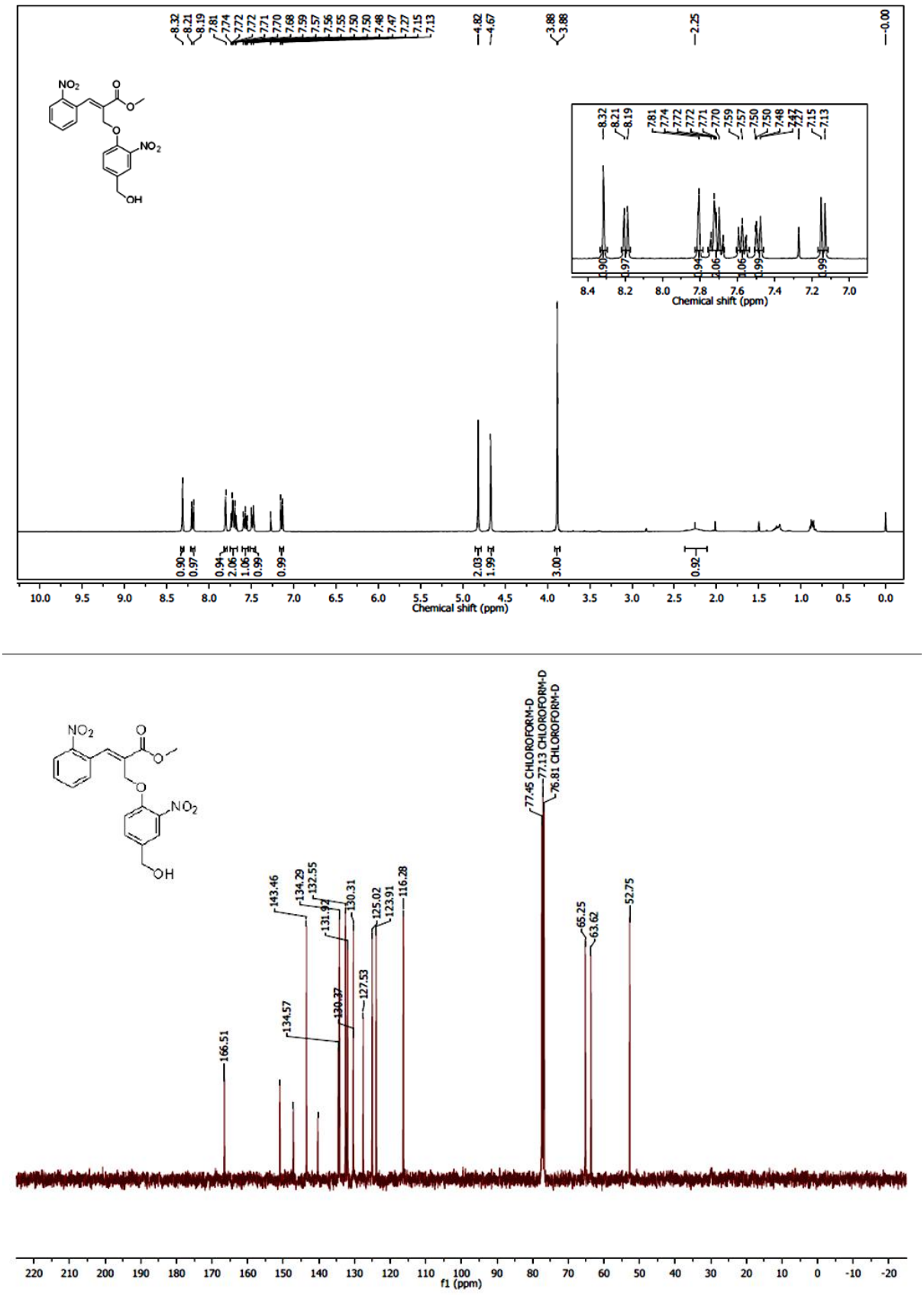


Figure 22: ^{1}H and ^{13}C spectra of **11c**.

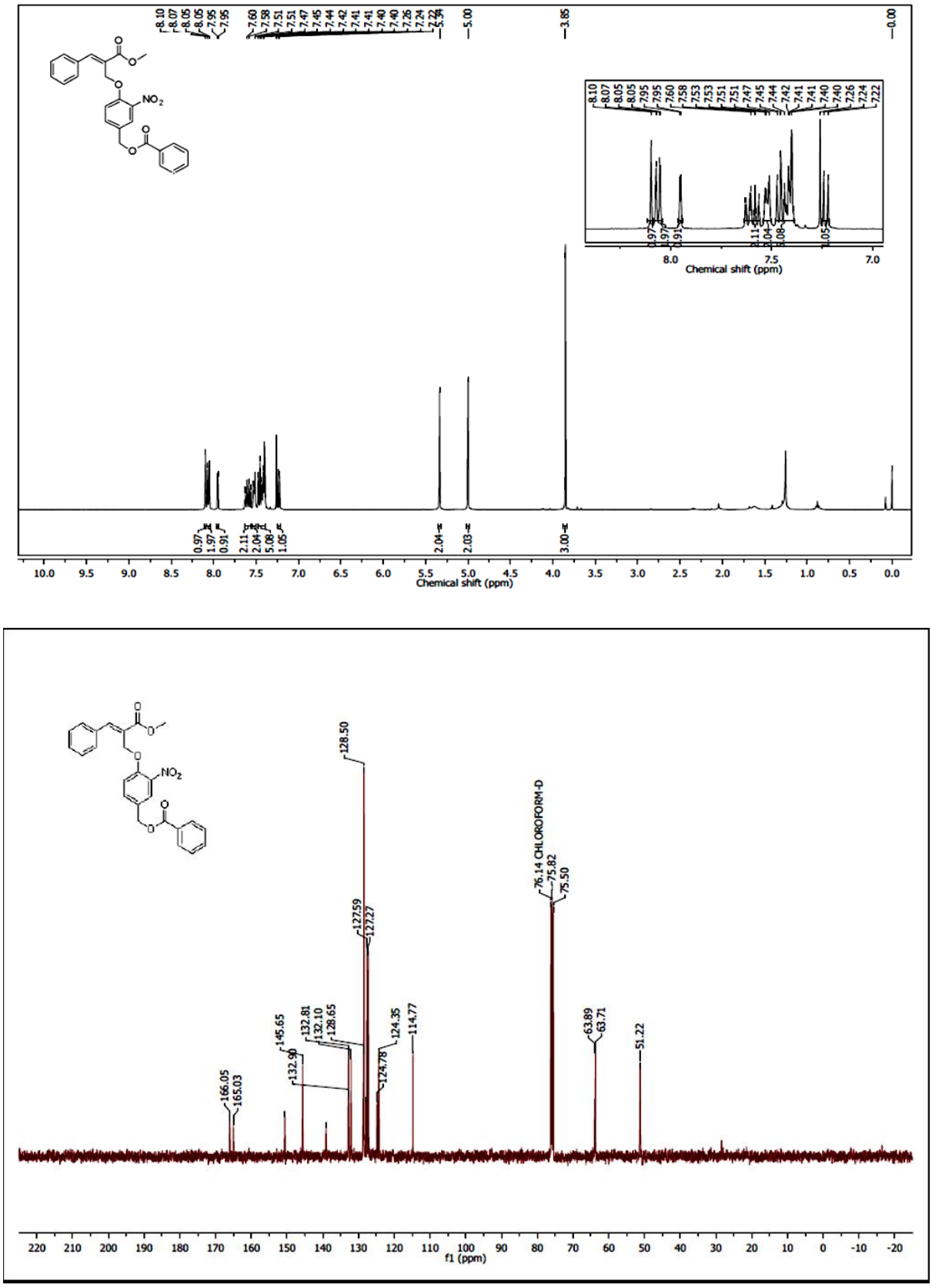


Figure 23: ^{1}H and ^{13}C spectra of **12a**.

HPLC Purity Traces

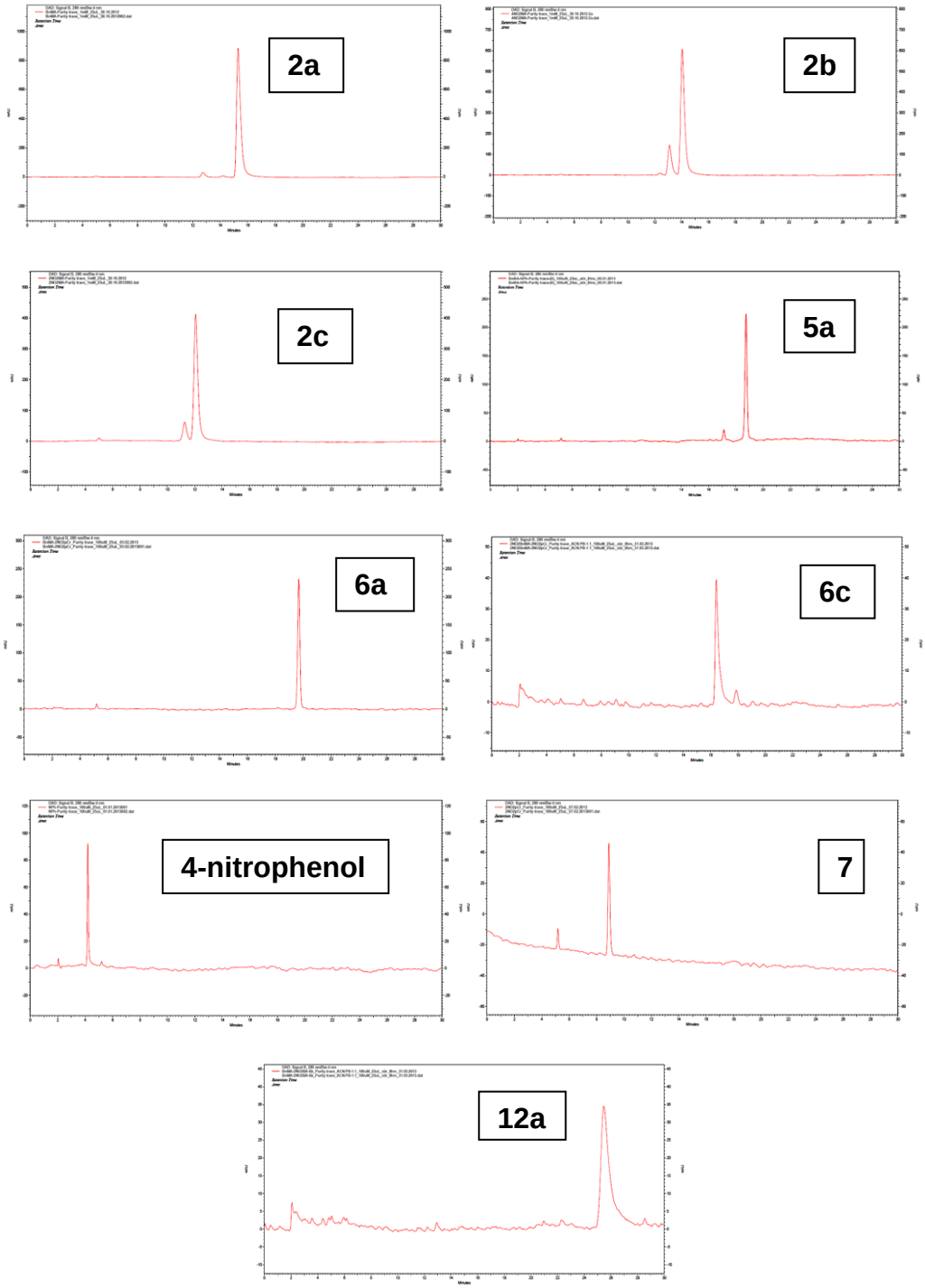


Figure 24: HPLC purity traces of **2a**, **2b**, **2c**, **5a**, **6a**, **6c**, **7**, **12a** and 4-nitrophenol. HPLC purity traces of **2a**, **2b** and **2c** shows presence of E, Z isomers.

Calibration Plots

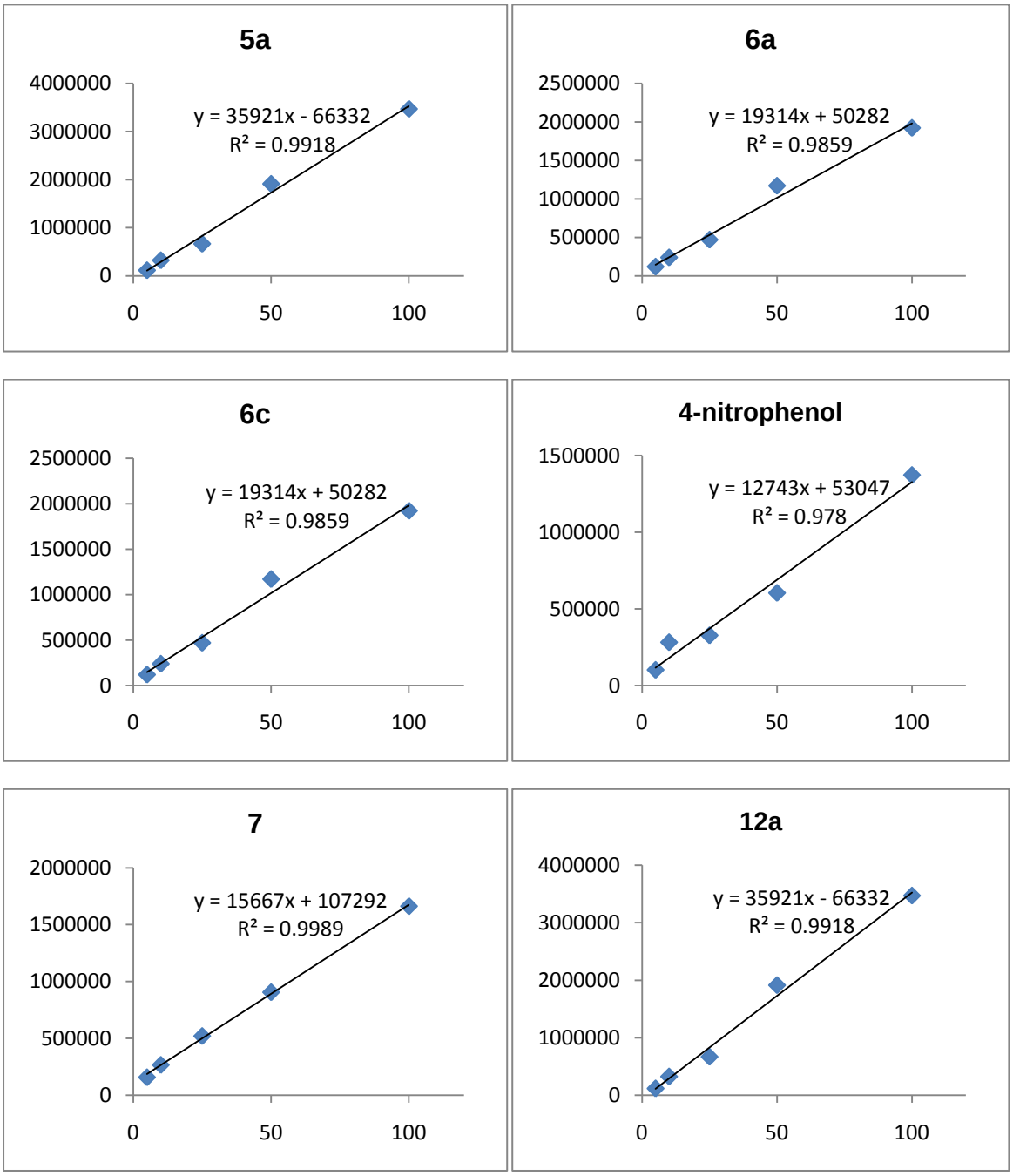


Figure 25: Calibration curves of **5a**, **6a**, **6c**, **7**, **12a** and 4-nitrophenol. X-axis denotes concentration in µM and Y-axis denotes absorbance in AU.

REFERENCES

1. M. N. V. Ravi Kumar. Handbook of Particulate Drug Delivery (2-Volume Set), *American Scientific Publishers*, **2008.**
2. Teo, A. L.; et al. Transdermal microneedles for drug delivery applications. *Mater. Sci. Eng. B* **2006**, *132*, 151-154**.**
3. Modi, S. A.; Gaikwad, P. D.; Bankar, V. H.; Pawar S. P. Sustained release drug delivery system: A review. *IJPRD* **2011**, *2*, *12*, 147-160.
4. Lammer, J.; et al. Prospective Randomized Study of Doxorubicin-Eluting-Bead Embolization in the Treatment of Hepatocellular Carcinoma: Results of the PRECISION V Study. *Cardiovasc. Intervent. Radiol.* **2010**, *33*, 41–52.
5. Chugh I.; Seth N.; Rana A.C., Gupta S. Oral sustained release drug delivery system: An overview. *IRJP* **2012**, *3*, *5*, 57-62.
6. Mignani, S., et al. Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview. *Adv. Drug Deliv. Rev.* **2013**, http://dx.doi.org/10.1016/j.addr.2013.01.001
7. Hirayama, F.; Uekama, K. Cyclodextrin-based controlled drug release system. *Adv. Drug Deliv. Rev.* **1999**, *36*, 125-141.
8. Hearnden, V. et al. New developments and opportunities in oral mucosal drug delivery for local and systemic disease. *Adv. Drug Deliv. Rev.* **2012**, *64*, 16-28.
9. Gultepe, E.; Nagesha, D.; Sridhar, S.; Amiji, M. Nanoporous inorganic membranes or coatings for sustained drug delivery in implantable devices. Adv. Drug Deliv. Rev. **2010**, *62*, 305–315.
10. Pace, N.J.; Weerapana, E. Diverse functional roles of reactive cysteines. *ACS Chem. Bio.* **2013**, *8*, 283-296.
11. Hemenway, J. N.; Nti-Addae, K.; Guarino, V.R.; Stella, V. J. Preparation, characterization and in vivo conversion of new water-soluble sulfenamide prodrugs of carbamazepine. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6629–6632.
12. Chen, S.; et al. Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated Endocytosis and Drug Release. *Bioconjug. Chem.* **2010**, *21*, *5*, 979–987.
13. Townsend, D. M.; et al. NOV-002, a Glutathione Disulfide Mimetic, as a Modulator of Cellular Redox Balance. *Cancer Res.* **2008**, *68*, *8*, 2870-2877.

14. Kislukhin, A. A.; Higginson, C. J.; Hong V. P.; Finn, M. G. Degradable Conjugates from Oxanorbornadiene Reagents. *J. Am. Chem. Soc.* **2012**, *134*, 6491−6497.
15. Vasconcellos M. L. A. A., et al. Microwave Irradiation or Low Temperature Improved Synthesis of Antiparasitic Morita-Baylis-Hillman Adducts. *J. Braz. Chem. Soc.* **2011**, *22*, *11*, 2220-2224.
16. Das, B.; Damodar, K.; Bhunia, N.; Shashikanth, B. Mild and practical stereoselective synthesis of (Z)- and (E)-allyl bromides from Baylis–Hillman adducts using Appel agents (PPh_3/CBr_4): a facile synthesis of semiplenamides C and E. *Tet. Lett.* **2009**, *50*, 2072–2074.
17. Sá, M. M.; Ramos, M. D.; Fernandes, L. Fast and efficient preparation of Baylis–Hillman-derived (E)-allylic azides and related compounds in aqueous medium. *Tetrahedron* **2006**, *62*, 11652–11656.
18. Zulykama, Y.; Uma, U.; Devi, P. C.; Perumal, P. T. Chemo- and region-selective functionalization of Morita-Baylis-Hillman bromides with anthranilic acid. *Can. J. Chem.*, **2009**, *87*, 1682-1691.
19. Blencowe, C. A.; et al. Self-immolative linkers in polymeric delivery systems. *Polym. Chem.* **2011**, *2*, 773–790.
20. Lee, H.Y.; Jiang, X.; Lee, D. Kinetics of Self-Immolation: Faster Signal Relay over a Longer Linear Distance? *Org. Lett.,* **2009**, *11*, *10*, 2065-2068.
21. Maiti, S.; et al. Gemcitabine−Coumarin−Biotin Conjugates: A Target Specific Theranostic Anticancer Prodrug. *J. Am. Chem. Soc.* **2013**, *135*, 4567-4572.
22. Kuang, Y.; Balakrishnan,K.; Gandhi, V.; Peng, X. Hydrogen Peroxide Inducible DNA Cross-Linking Agents: Targeted Anticancer Prodrugs. *J. Am. Chem. Soc.* **2011**, *133*, *48*, 19278–19281.
23. Banerjee, R., Pace, N.J., Brown, D.R., Weerapana, E. 1,3,5-Triazine as a Modular Scaffold for Covalent Inhibitors with StreamLined Target Identification. *J. Am. Chem. Soc.*, **2013**, *135*, 2497-2500.
24. Tanabe, K.; et al. Emission under Hypoxia: One-Electron Reduction and Fluorescence Characteristics of an Indolequinone–Coumarin Conjugate. *ChemBioChem* **2008**, *9*, 426-432.
25. Cai, J.; Zhou, Z.; Zhao, G.; Tang, C. Dramatic Rate Acceleration of the Baylis-Hillman Reaction in Homogeneous Medium in the Presence of Water. *Org. Lett.* **2002**, *4*, *26*, 4723-4725.

26. Kim, Y.; Lim, H. N.; Lee, K. A new route to allyl thiols and allyl thiocarbamates from Baylis-Hillman adducts. *Jour. Heter. Chem.* **2009**, *46*, *1*, 23-27.
27. Shadakshari, U.; Nayak, S. Reductive dehydroxylation of Baylis-Hillman adducts with low-valent titanium reagent: syntheses of stereoselective trisubstituted alkenes. *Tetrahedron* **2001**, *57*, 4599-4602.
28. Singh, V.; Hutait, S.; Batra, S. Reductive-Cyclization-Mediated Synthesis of Fused Polycyclic Quinolines from Baylis–Hillman Adducts of Acrylonitrile: Scope and Limitations. *Eur. J. Org. Chem.* **2009**, 3454-3466.
29. Bouvier, E.; Thirot, S.; Schmidt, F.; Monneret, C. First enzymatically activated Taxotere prodrugs designed for ADEPT and PMT. *Bioorg. Med. Chem.* **2004**, *12*, 969-977.
30. Kitano, Y.; et al. A Convenient Method for the Preparation of Benzyl Isocyanides. *Synthesis* **2006**, *3*, 405-410.
31. Aridoss, G.; Laali, K.K. Ethylammonium Nitrate (EAN)/Tf_2O and EAN/TFAA: Ionic Liquid Based Systems for Aromatic Nitration. *J. Org. Chem.* **2011**, *76*, *19*, 8088-8094.
32. Okada, I.; Kitano, Y. One-Pot Synthesis of Isocyanides from Alcohols. *Synthesis* **2011**, *24*, 3997-4002.