DESIGN, SYNTHESIS AND REACTIVITY OF CYCLOPROPENONE-CONTAINING NINE MEMBERED CYCLIC ENEDIYNE AND ENYNE-ALLENE PRECURSORS AS PROTOTYPE PHOTOSWITCHABLE ANTITUMOR AGENTS

by

DINESH R. PANDITHAVIDANA

(Under the Direction of Vladimir V. Popik)

ABSTRACT

The extreme cytotoxicity of natural enediyne antibiotics is attributed to the ability of the (*Z*)-3-hexene-1,5-diyne (enediyne) and (*Z*)-1,2,4-heptatrien-6-yne (enyne–allene) fragments incorporated into a 10- or 9-membered ring cyclic system to undergo cycloaromatization producing dDNA-damaging 1,4-diradicals. The rate of cyclization of enediynes to *p*-benzynes strongly depends on the distance between acetylenic termini, which in turn can be controlled by the ring size. Thus, 11-membered ring enediynes are stable, 10-membered ring analogs undergo slow cycloaromatization under ambient conditions or mild heating, 9-membered ring enediynes are virtually unknown and believed to undergo very fast spontaneous cyclization. Cyclic enyne-allenes have not been synthesized so far due to very facile Myers-Saito cyclization.

We have developed thermally stable photo-precursors of 9-membered enediynes (**2.22**, **2.26**) and enyne-allene (**2.44**), in which one of the triple bonds

is replaced by the cyclopropenone group. UV irradiation of **2.14** results in the efficient decarbonylation (Φ_{254}= 0.34) and the formation of reactive enediyne **2.22**. The latter undergoes clean cycloaromatization to 2,3-dihydro-1H-cyclopenta[b]naphthalen-1-ol (**2.24**) with a life-time of ca. 2 h in 2-propanol at 25 °C. The rate of this reaction depends linearly on the concentration of hydrogen donor and shows a primary kinetic isotope effect in 2-propanol-d_8. These observations, along with high negative entropy of activation, indicate that enediyne **2.22** exists in a rapid equilibrium with *p*-benzyne diradical (**3.1**). The rate-limiting step of the cycloaromatization reaction is hydrogen abstraction by the diradical **3.1**.

In order to improve the DNA affinity of the enediyne **2.22**, we designed and synthesized naphthalene-fused enediyne (**2.26**). This structural modification also allowed us to shift the absorbance of the cyclopropenone precursor **2.25**, to 300-350 nm. UV irradiation of the **2.25** results in an efficient decarbonylation (Φ_{300}= 0.50) reaction generating enediyne, **2.26**, which undergoes Bergman cyclization at an elevated temperature.

9-Membered ring enyne-allene (**2.44**), generated by irradiation of the of cyclopropenone-containing precursor **2.43**, undergoes almost instant (life time < 1 μs) cycloaromatization to form cytotoxic 1,4-diradical.

INDEX WORDS: Nine membered enediynes, Enyne-allene, UV irradiation, Cyclopropenones, Decarbonylation, Bergman cyclization, Myers-Saito cyclization

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December 2009

DEDICATION

This dissertation is dedicated to all of my teachers, my parents and family members for their unconditional support and encouragement.

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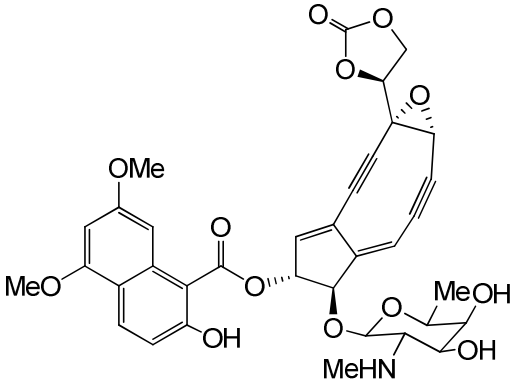
INTRODUCTION

1.1. Naturally occurring enediyne antibiotics

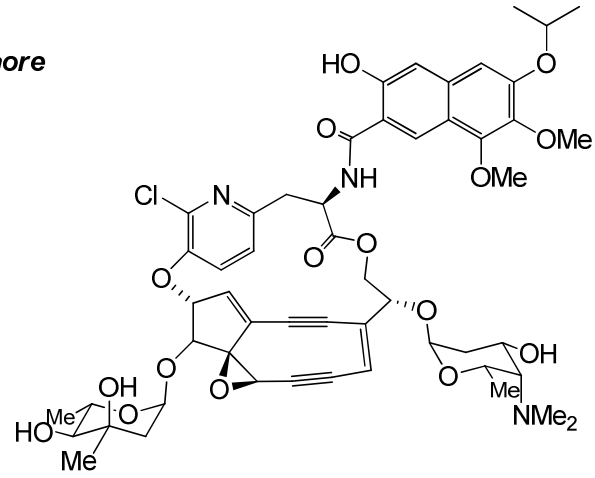
The cyclic enediynes, a relatively new class of antitumor antibiotics, are strong DNA cleavage agents and exhibit remarkable antitumor activities both in vitro and in vivo. Naturally occurring enediyne antitumor antibiotics are divided into two groups according to the 9- or 10-membered ring core structure. The first enediyne antibiotic, neocarzinostatin (NCS), consisting of 1:1 mixture of an apoprotein and a chromophoric molecule (shown in Figure 1.1), was isolated from *Streptomyces carzinostaticus* in 1965.[1,2] However, the structure of the NCS chromophore[3] was determined twenty years later. Following the disclosure of the unprecedented molecular structure of neocarzinostatin, two families of 10-membered ring enediynes were discovered in 1987, as represented by calicheamicin from *Micromonospora echinospora*[4] and esperamicin-A_1 from *Actinomadura verrucospora*.[5]

Another unique subtype of antibiotics, featuring a hybrid of an anthroquinone moiety and a 10-membered ring enediyne core, was isolated in 1989 from the culture of *Micromonospora chersina* as illustrated by the structure of dynemicin-A (Figure 1.2).[6] This shows a high potency against various tumor cell lines that significantly prolongs the life span of mice inoculated with P 388 leukemia and B 16 melanoma cells.[1] In addition, dynemicin-A is unique among

these cyclic enediynes as it contains both a cyclic enediyne ring and an anthraquinone chromophore. Because of its structural novelty, complexity and highly potent activity, the mechanistic and synthetic studies of dynemicin-A have been explored by many researchers including Schreiber,[7] Myers[8] and Danishefsky.[9]



Neocarzinostatin Chromophore

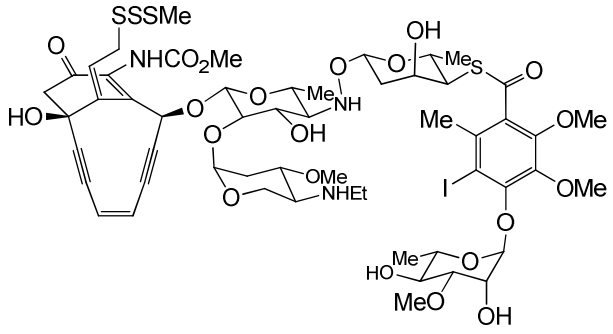


Kedarcidin Chromophore

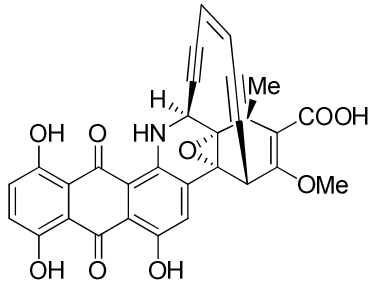
Figure 1.1. Naturally occurring 9-membered ring enediyne anticancer antibiotics.

Natural enediyne antibiotics can be divided into three families: (1) the calicheamicin/ esperamicin familily,[4] possessing a strained bicyclo[7.3.1]tridec-9-ene-2,6-diyne system, polysugar fragment and a methyl trisulfide moiety; (2) the dynemicin family,[6] containing a distinctive structural feature of 1,5-diyn-3-ene unit embedded within a 10-membered ring in association with anthraquinone system;

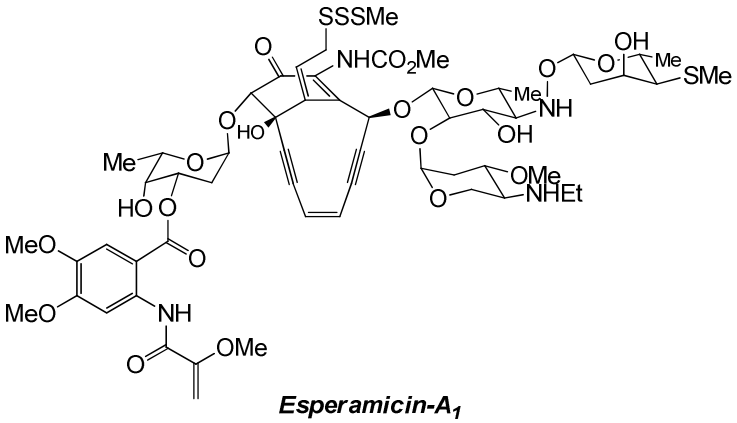
(3) the chromoprotein family,[10,11] consisting of a nonpetide chromophore such as neocarzinostatin, kedarcidin or C-1027 in complex with an apoprotein that acts as a carrier. The related structural features found of this family are a severely strained bicyclo[7.3.0]dodecadienediyne core and the annulated epoxide ring. All members of the first two families have the enediyne unit incorporated into a 10-membered ring and do not need any additional stabilization factors. In contrast, all members of the third family possess a 9-membered ring enediyne core structure that requires a specific associated protein for chromophore stabilization.



Calicheamicin



Dynemicin-A



Esperamicin-A$_1$

Figure 1.2. Naturally occurring 10-membered ring enediyne anticancer antibiotics.

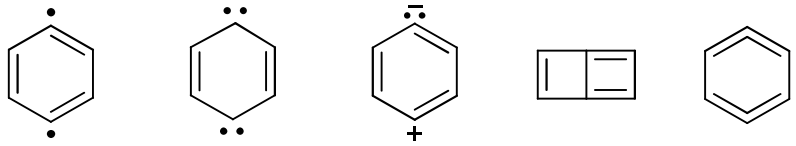
1.2. Bergman cycloaromatization

Nature continually serves to provide scientists with new ideas, often provoking us to reexamine previous studies and leading us in new directions. An example of such instance, which has captured imagination of many scientists, came in 1987 with the unveiling of a new class of natural products, the so-called "enediyne anticancer antibiotics".[1,2] The first studies began some 15 years earlier, in the laboratories of Robert Bergman where an intriguing chemical discovery occurred, providing the key to understand the remarkable activity of these antibiotics.[12] In these experiments, he showed that [1,6-D_2]-(Z)-3-hexene-1,5-diyne (**1.1**) underwent a rapid deuterium scrambling reaction at 200 ^{0}C in gas phase. The ratio of **1.1** to **1.3** isomers was found to be 50:50, and no isomer containing one deuterium atom was detected in the reaction mixture.

Scheme 1.1

1.1 **1.3**

The results of the labeling experiments suggested that the thermal isomerizations proceed through a species with a C_2 axis of symmetry, i.e., a 1,4-dehydrobenzene. A number of possible structures for this species were proposed (in Figure 1.3) during this study. However, no evidence was found in favor of any intermediate by the gas phase experimental work.[13]



1.2a **1.2b** **1.2c** **1.2d** **1.2e**

Figure 1.3. Proposed structures for the intermediate of 1,4-dehydrobenzene

The structure of the intermediate was elucidated by the pyrolysis studies of (Z)-3-hexene-1,5-diyne in the solution. Thermolysis of **1.1** in the medium molecular weight alkane resulted in the disappearance of the starting material along with the quantitative formation of the benzene.[12] Free radicals are virtually the only intermediates that are able to abstract hydrogen atoms from hydrocarbons. Further evidence supporting the formation of a *p*-phenylene-diradical intermediate was obtained by pyrolysis in carbon tetrachloride, where the only volatile isolated product was *p*-dichlorobenzene.[12]

The activation energy for the rearrangement of (Z)-3-hexene-1,5-diyne into *p*-benzyne was estimated to be around 32 kcal/mol. As illustrated in the reaction coordinate diagram (Figure 1.4), the 1,4-benzenoid diradical lies in a very substantial energy (18 kcal/mol) well. In other words, the barrier for the reverse ring opening reaction is 18 kcal/mol.[12]

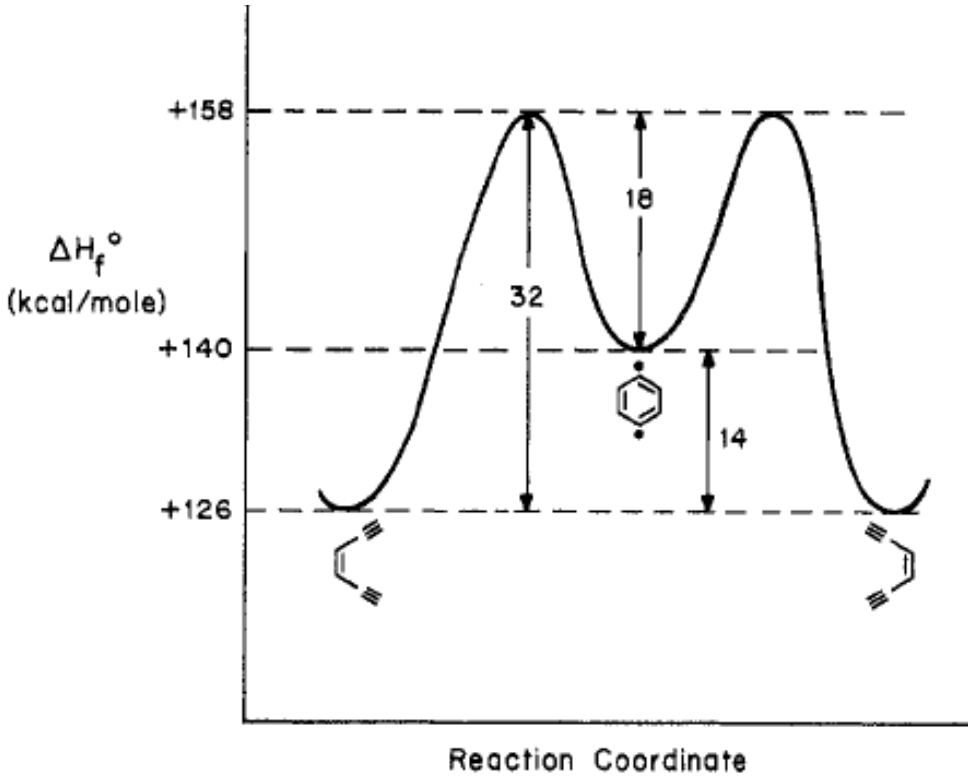
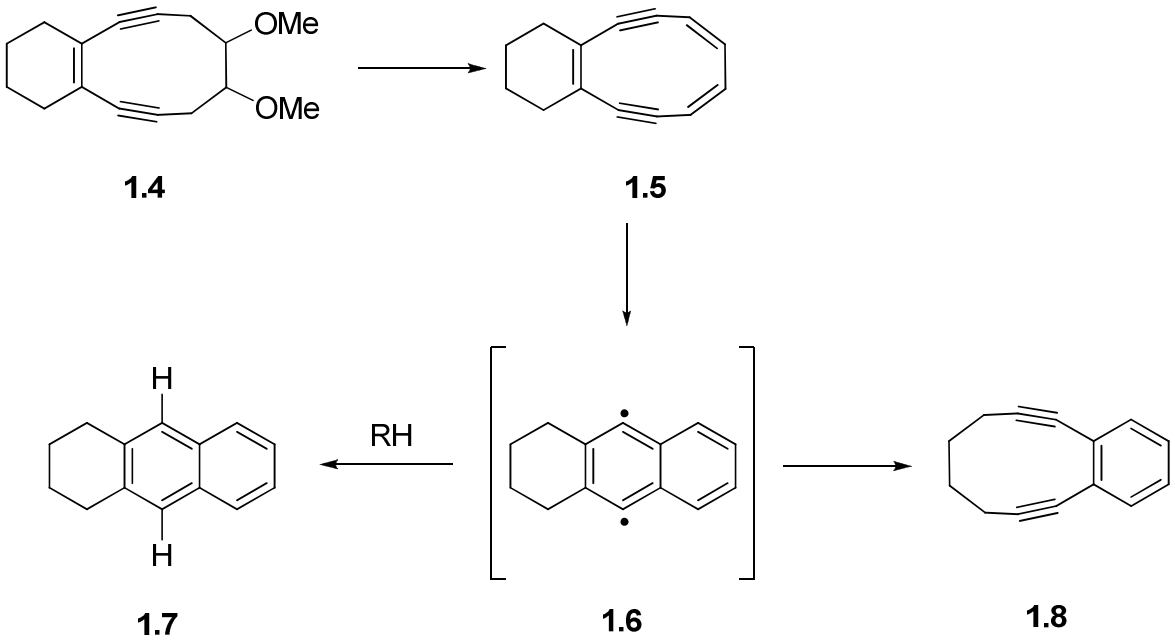


Figure 1.4. Energy profile for the inversion of **1.1**, estimated from group equivalent techniques by Bergman et al.[12]

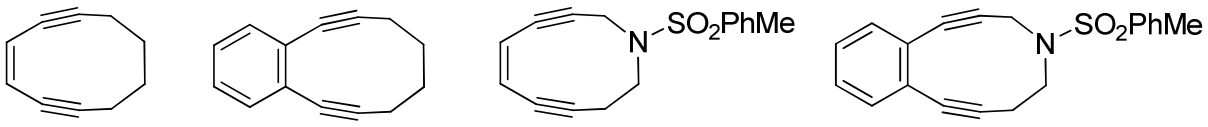
Masamune et al.[14] investigated a treatment of dimesylate **1.4** (Scheme 1.2) with sodium methoxide gaving both tetrahydroanthracene **1.7** and substituted diethynyl benzene **1.8**. This experiment was one of the direct analogs to support Bergman's mechanism by forming both products (**1.7** and **1.8**) of substituted dehydronaphthalene **1.6**. The authors also reported that dimesylate **1.4** invariably provided dehydronaphthalene under various elimination conditions. Furthermore the two new hydrogen atoms had been added at the 9th and 10th positions of intermediate **1.6** to form compound **1.7**.

Scheme 1.2



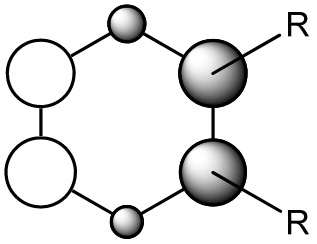
1.4 **1.5** **1.7** **1.6** **1.8**

In contrast to acyclic enediynes, benzannelation of cyclic systems substantially decrease at the rate of Bergman cyclization. The rearrangement of enediyne **1.9** ($\tau_{1/2}$ = 18 h, T= 37 ^{0}C)[15] is much faster than a rearrangement of **1.10** ($\tau_{1/2}$ = 24 h, T= 84 ^{0}C).[16] Interesting enough, a similar effect was reported for aza-enediynes, **1.11** ($\tau_{1/2}$ = 72 h, T= 23 ^{0}C) and **1.12** ($\tau_{1/2}$ = 52 h, T= 65 ^{0}C).[17]

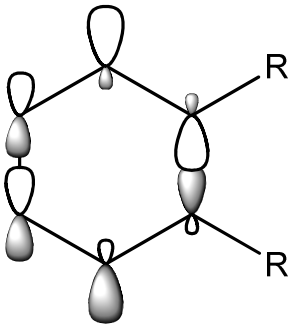


1.9 **1.10** **1.11** **1.12**

A thorough study of the effect of benzannelation revealed the kinetics of the cyclization is not solely determined by the rate of Bergman cycloaromatization, but is greatly affected by the rate of retro-Bergman reaction and hydrogen abstraction.[18] In the case when hydrogen abstraction is the rate-determining step, the observed reaction rate depends on the concentration of hydrogen donor.[16]



1.13



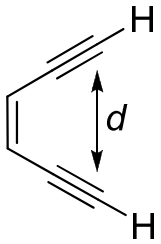
1.14

Figure 1.5. Schematic representation of HOMO (π-bonding, **1.13**) and HOMO-1 (π-antibonding, **1.14**) for the transition state of the Bergman cyclization reaction of substituted enediynes.[19]

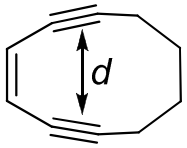
The effect of substituents on the rate of Bergman cyclization was also investigated theoretically[19,20] as well as experimentally.[21,22] Substituents at the propargylic carbon generally affect the kinetics of Bergman cyclization more than at the vinylic positions.[23] The effect of the substitution at the propargylic carbon was rationalized on the basis of MO analysis.[21] Depending on the substituent, the HOMO of the transition state of the cyclization can be σ- or π- type (Figure 1.5). π-Donating substituents increase the occupation of the bonding orbital, and

accordingly decrease the cyclization barrier; σ-acceptors accelerate the Bergman rearrangement by decreasing the occupation of the antibonding σ-orbital.[19] For instance, substitution of hydrogen for fluorine at the propargylic position substantially increases the rate of cyclization.[22] The substituent effect on the energies of the cycloaromatization in the vinylic position is relatively limited. Electron withdrawing substituents generally increase the activation barrier, while electron donating groups slightly increase the activation energy.

The effect of distance between acetylene termini on the rate of Bergman cycloaromatization has also been the subject of theoretical investigations.[24] This transannular distance was found to correlate well with the ground state strain energy, and therefore might be used as a good indicator of the reactivity.[25] Nicolaous's "critical distance rule",[25,26] suggests that the cyclizations occur spontaneously at room temperature, when the distance between two acetylene termini is in the range of 3.2 ± 0.1 Å. Strictly speaking, the reactivity of a given enediyne toward Bergman rearrangement is determined by the free energy of activation and there is no direct correlation between interatomic distance, ***d*** and the energy of activation.[16,25,26]



1.1a



1.9

Nevertheless, the difference between the ground and transition states, the ring strain apparently is an important factor influencing the energetics of Bergman cycloaromatization of monocyclic enediyne.[27] It is understood that only enediyne **1.1a** undergoes cycloaromatization at about 200 ^{0}C (*d* = 4.12 Å, $\tau_{1/2}$ = 30 s),[12] while cyclization of 3-cyclodece-1,5-diyne, **1.9**, occurs at 37 ^{0}C (*d* = 3.25 Å, $\tau_{1/2}$ = 18 h).[16]

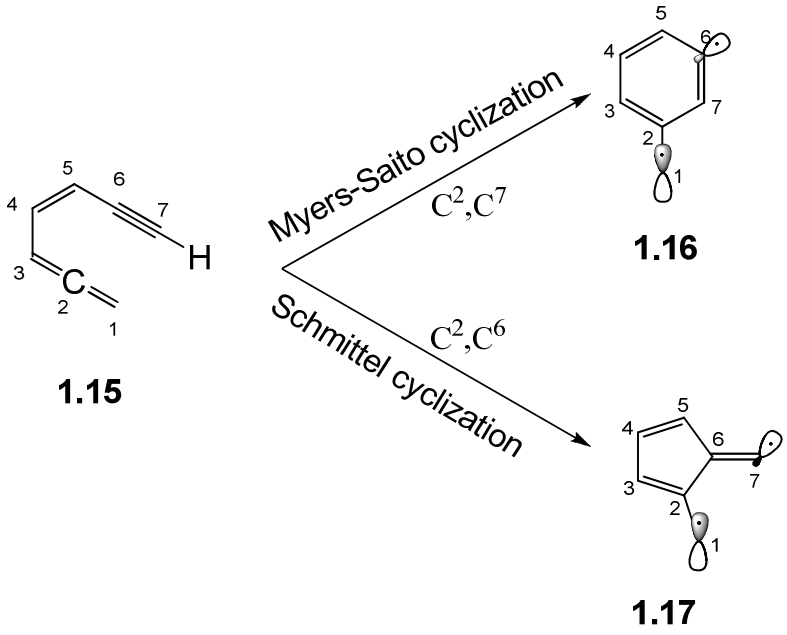
1.3. Cycloaromatization analogs to Bergman rearrangement

Apart from the well known Bergman cyclization, similar rearrangements leading to the following diradicals were proposed as novel types of cyclization mechanisms, called Myers-Saito[28,29] and Schmittel [22,30] reactions (Scheme 1.3). Both reactions in this Scheme start from cis-hepta-1,2,4-triene-6-yne (**1.15**), and lead to a ring closure into a 6-membered ring, **1.16**, (Myers-Saito) or a 5-membered ring, **1.17**, (Schmittel). Both product species are biradicals, but in contrast to the Bergman cyclization, σ,π-biradicals are obtained rather than σ,σ-biradicals. In addition, the cyclization products (**1.16, 1.17**) are partially conjugated σ,π-biradicals, which are less reactive than the *σ,σ*-biradicals obtained from Bergman-type cyclizations.[29,31]

Although the Myers-Saito reaction is thermodynamically much more favorable, Schmittel *et al*. were able to show that the methyl fulvenes **1.17** can be obtained as the main products when the alkyne terminal hydrogen is replaced by phenyl[32] or bulky groups such as *tert*butyl or trimethylsilyl.[33] These substituents raise the cyclization barrier of the Myers-Saito reaction due to steric hindrance. In

the case of phenyl, the barrier of the Schmittel cyclization is lowered by radical stabilization through conjugation.[30]

Scheme 1.3

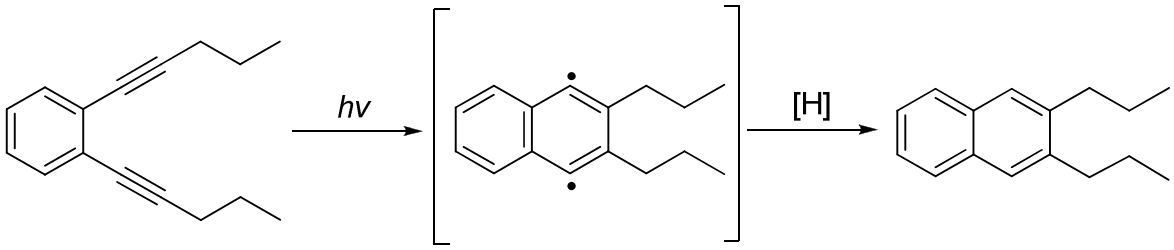


1.4. The photochemical Bergman reaction

The mechanism of the photochemical Bergman reaction is not completely understood, depite the fact that this reaction was discovered in 1968.[34] Substantial attention was attracted to the photochemical cyclization of enediynes due to the observation that in the presence of suitable sensitizers, acyclic enediynes display DNA cleaving activity in addition to *cis-trans* isomerization.[35] The photochemical Bergman rearrangement of the unsubstituted enediyne **1.1**, has also not yet been realized experimentally.[12,36] However several examples of the photochemical cyclization have been reported since 1994. Irradiation of 1,2-di(pent-1-ynyl)benzene, **1.18** gave rise to 1,2-dipropylnaphthalene, **1.20** (Scheme

1.4) in hexanes in the presence of a photosensitizer, which suggest that *p*-benzyne, **1.19** is the intermediate in this reaction.[37]

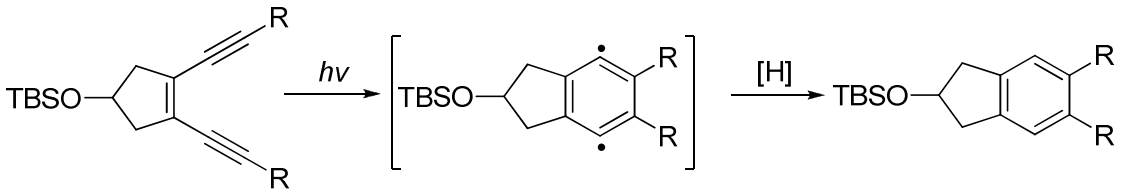
Scheme 1.4



1.18 **1.19** **1.20**

The first example of the photochemical cyclization reaction of a non-benzannulated enediyne was reported by Hirama *et al.* in 1999 (Scheme 1.5).[18,38] Irradiation of enediyne **1.21** (R = Me) with 254 nm light resulted in the formation of indane derivative **1.23**, in 71 % yield. None of the attempts were successful to cyclize **1.21** with a longer wavelength.

Scheme 1.5



1.21 **1.22** **1.23**

1.5. Mechanisms of action of naturally occurring enediynes

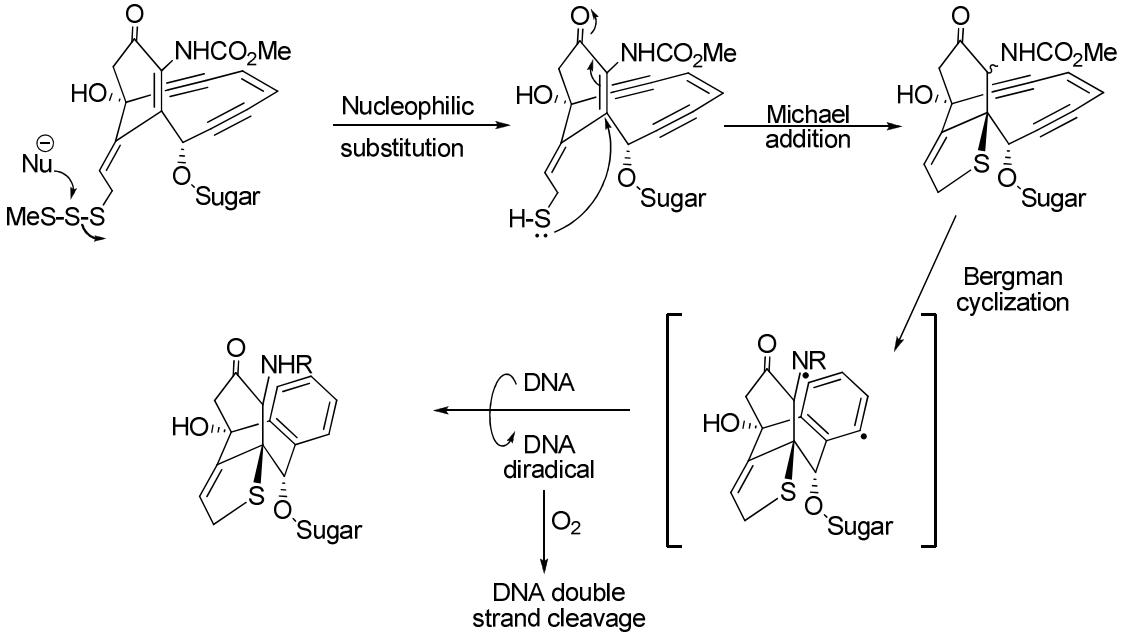
Natural enediyne antibiotics are arguably the most potent antineoplastic agents ever discovered.[39,40] These molecules contain a (Z)-3-hexene-1,5-diyne

unit embedded within their complex architectural frameworks. It was proposed that these natural enediyne molecules bind the enediyne portion within the minor groove of DNA double helix.[41] Reductive or nucleophilic activation of the natural enediynes removes the stereochemical blocking device and the (*Z*)-3-hexene-1,5-diyne fragment undergoes Bergman[12] cyclization to produce a *p*-benzyne diradical,[42,43] which might exist in equilibrium with the enediyne precursor.[44] By its size, *p*-benzyne (ca. 3.8 Å) is perfectly suitable to perform a double hydrogen abstraction from carbohydrate backbones of opposite DNA strands (1′H to 1′H distance is about 5.78 Å).Theoretical studies and experimental data suggest that this diradical has relatively low reactivity.[45,46] Initial hydrogen abstraction from one strand, however, converts *p*-benzyne into a 10-100 times more reactive phenyl radical,[45,47] which immediately abstracts the second hydrogen from the opposite DNA strand. This process leads to an oxidative double-strand dDNA scission.[1]

The structures of many naturally occurring enediynes have three well-defined structural domains, the physical and chemical properties of which play important roles in DNA binding and cleavage. For example, in the case of calicheamicin (in Figure 1.2), the first is the carbohydrate subunit which serves as a DNA-binding agent. The aryltetrasaccharide helps to deliver the drug to its target, binding tightly in the minor groove of double helical DNA and displaying high specificity for sequences such as 5’-TCCT-3’ and 5’-TTTT-3’ through significant hydrophobic interactions and other forces.[48,49] This binding is thought to be facilitated by substantial preorganization of the oligosaccharide into a rigid,

extended conformation[50] with the unusual hydroxylamine linker providing distinctive torsion angles in the central region of the molecule necessary to complement the minor groove.[51] Molecular modeling calculations by Schreiber *et al*.[52] suggest that a significant portion of the sequence selectivity for 5'-TCCT-3' arises from a favorable interaction between the large and polarizable iodo substituent of the hexasubstituted aromatic ring and the exocyclic amino substituents of the two guanines in the 3'-AGGA-5' tract.

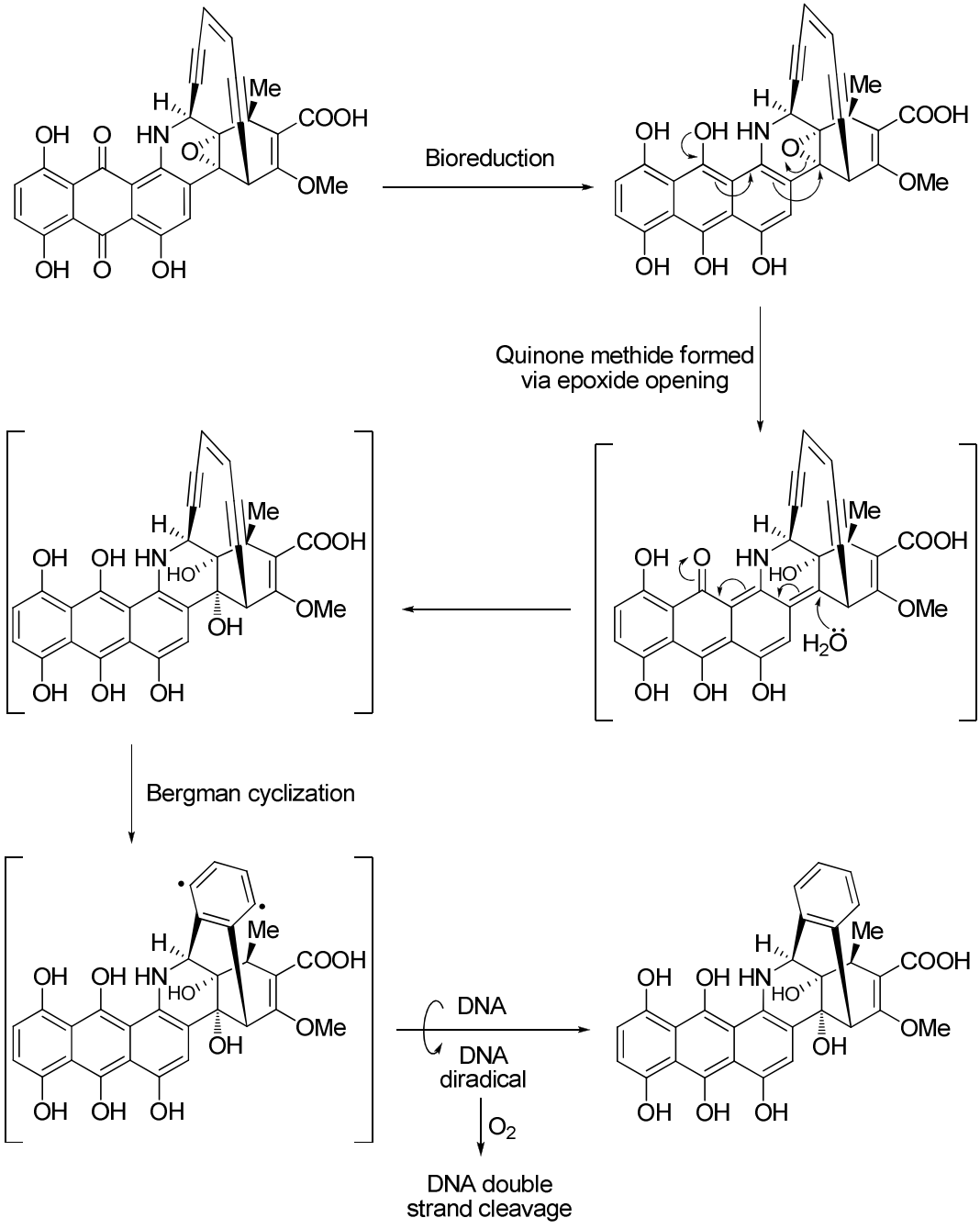
Scheme 1.6



The second is the allylic methyltrisulfide, which plays the part of a control or triggering device. Upon nucleophilic attack on the allylic trisulfide (Scheme 1.6), the allylic thiolate anion is formed, which then adds to the α,β-unsaturated ketone system. This removes the structural rigidity associated with the bridgehead double bond in the DNA-reactive third domain, making it able to undergo

Bergman cycloaromatization reaction.[12,13] This transient *p*-phenylene diradical intermediate that is formed during the cycloaromatization process initiates an oxidative strand scission by abstraction of hydrogen atoms from the deoxyribose sugar backbone. This leads to cleavage of the DNA at both strands.

Scheme 1.7

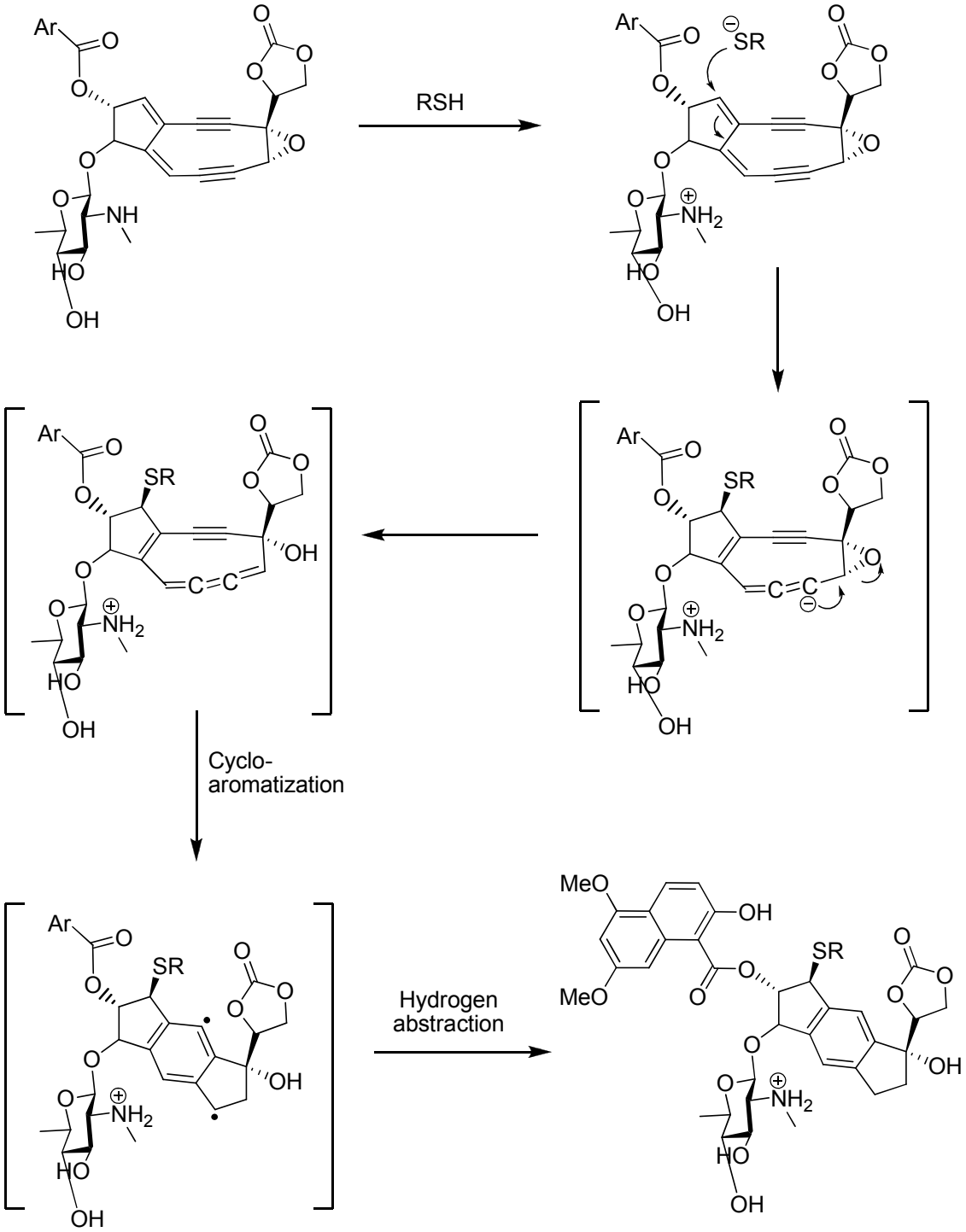


A mechanism for the antitumor activity of dynemicin- A has been proposed[5] which combines mode of actions of the calicheamicin/ esperamicin classe of antibiotics and which is supported by the observation that DNA strand cleavage by dynemicin-A is enhanced by the presence of thiols. In this mechanism[53] (Scheme 1.7), the anthraquinone nucleus intercalates into the DNA and undergoes bioreduction, facilitating the opening of the epoxide. The reaction causes a significant conformational change in the molecule and introduces considerable strain into the enediyne system. This is relieved by the new system undergoing the Bergman reaction, generating the DNA-damaging diradical species.[53]

In most cases the transient *p*-phenylene diradical intermediate, which is formed via Bergman cycloaromatization reaction, causes DNA cleavage. It was shown that the mechanism of DNA cleavage of neocarzinostatin might be slightly different from the mechanisms of the biological activity of the majority of naturally occurring enediynes. The first step in the activation of neocarzinostatin begins with an equilibrium deprotonation through its amino sugar moiety to provide a thiolate anion (Scheme 1.8). The nucleophilic attack by the thiol anion at the endocyclic double bond generates an anion with extensively delocalized negative charge. In the allenyl anion, the negative charge induces a ring opening of the epoxide to generate an enyne[3]cumulenolate intermediate that cyclizes to form an indacene diradicals.[41] This proceeds to attack DNA by hydrogen atom abstraction. It is generally accepted that the NCS chromophore intercalates into

DNA *via* its naphthoate side chain, which positions the rest of the molecule within the minor groove.[54]

Scheme 1.8



1.6. Controlled Bergman cyclization of designed enediynes

Since the discovery of the extremely high antitumor activity of naturally occurring enediynes and elucidation of their structures a number of enediyne analogs equipped with various triggers were synthesized. To control the reactivity of synthetic enediyne model compounds, several strategies were employed: release of a conformational constraint that prohibits spontaneous cyclization,[55] isomerization of enediynes to the more reactive cumulene-enediynes,[56] generation of the enediyne system by retro-Diels-Alder reaction,[57] alkyne deprotection,[58] the induction of the cyclization by catalytic antibodies,[59] metal cations,[60] oxidation[23] or light.[61]

Nicolaou *et al*. were first to identify and study the features of the natural product essential for its activation through the design and synthesis of model systems.[62,63] They reported that the dynemicin model **1.24** (see Figure 1.6) was thermally stable toward cycloaromatization. It was, however, readily triggered by acid-catalyzed epoxide opening, which resulted spontaneous cycloaromatization by analogy with dynemicin-A (Scheme 1.7). By contrast, the deprotected amine **1.25** proved to be very labile and rapidly underwent epoxide opening/ cycloaromatization at ambient temperatures in the absence of cofactors. The reactivity of **1.25** and its ability to cause double-stranded DNA breaks supported the electron donation from the free nitrogen. The epoxide opening results in the formation of an *o*-iminoquinone methide species. Similarly, the phenolic system **1.26** readily cycloaromatized and cleaved DNA as a result of electron push from the phenol opening the epoxide. These findings allowed them to design the 2-

(phenylsulfonyl)ethyl carbamate **1.27**. [64] This compound had an IC_{50} of $2.0x10^{-14}$ M against the MOLT-4 leukemia cell line, making it one of the most potent *in vitro* cytotoxic agents that has been reported to date.[63,64]

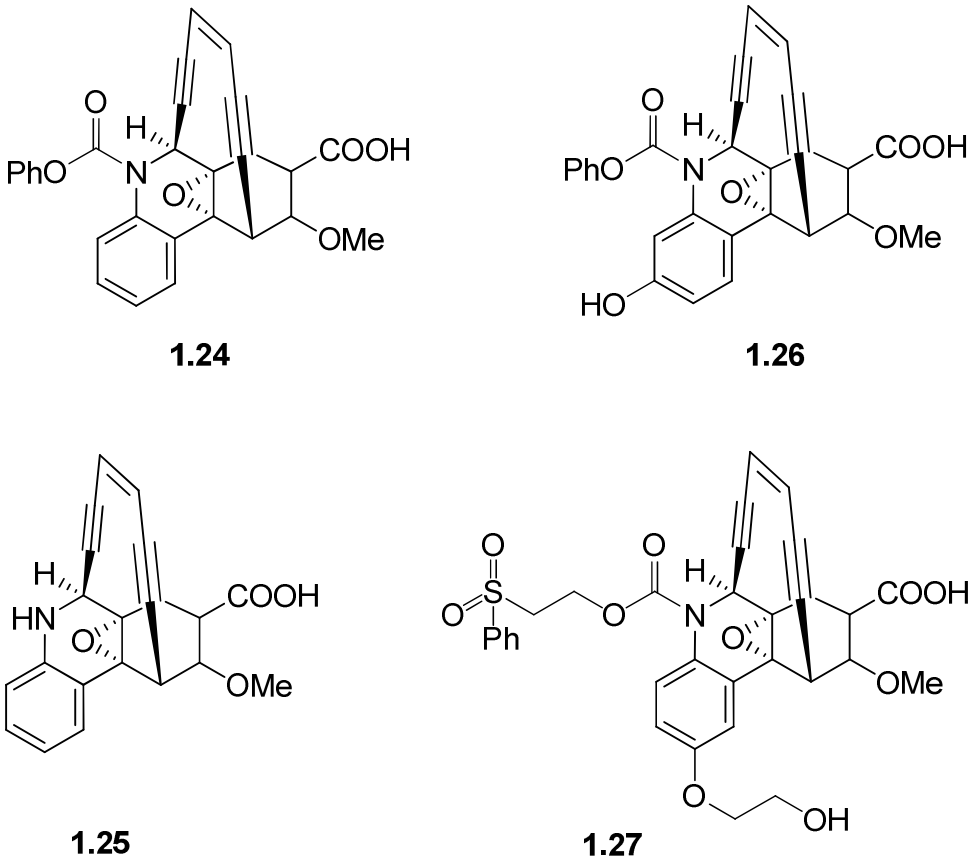
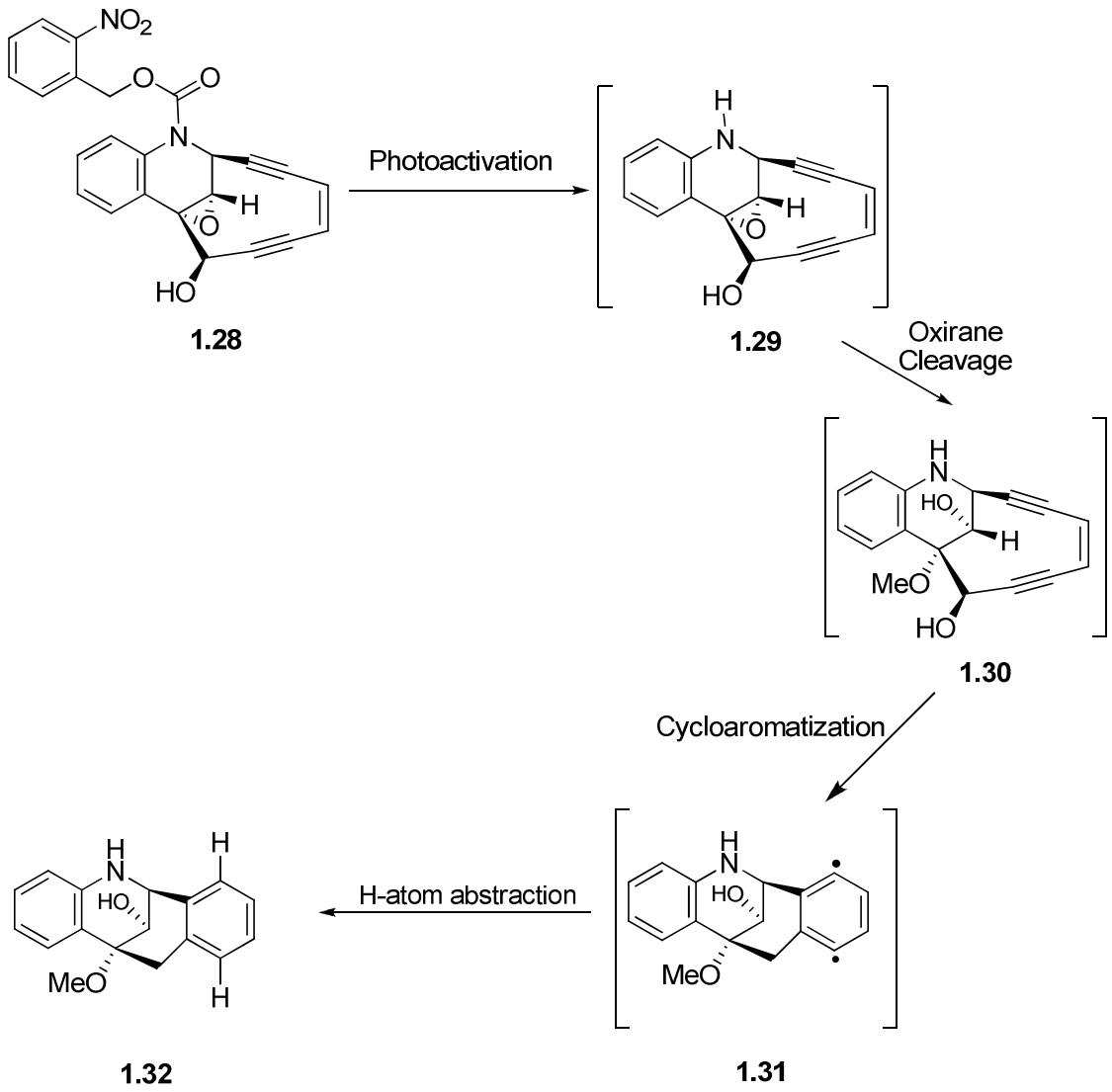


Figure 1.6. Designed enediynes related to dynemicin A (Nicolaou *et al.*).

Wender et al.[65] reported the synthesis of dynemicin analog, **1.28** that can be activated by photo-induced cleavage of the carbamate moiety to the free amine, **1.29** (Scheme 1.9). This photo-deprotection serves to increase electron density proximate to the oxirane bond, thereby facilitating its hydrolytic opening as required for subsequent cycloaromatization of the enediyne, **1.30**. This analog

was induced to undergo Bergman cyclization under neutral conditions in the absence of chemical activators through the use of a photochemically activatable switch.[65]

Scheme 1.9

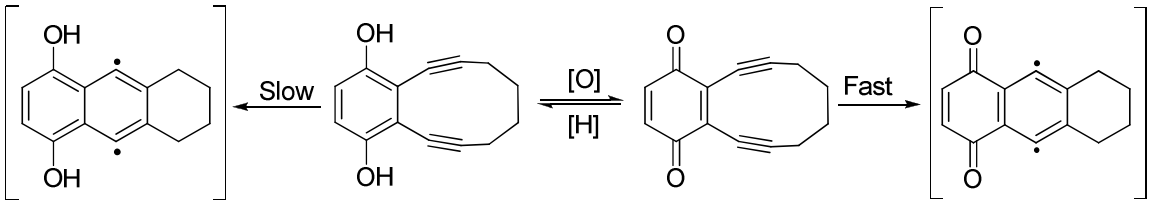


1.28 **1.29** **1.30** **1.31** **1.32**

The synthesis and evaluation of a series of cyclic enediynes was reported by Nicolaou *et al.*[26] in which the Bergman cycloaromatization is controlled by the redox reactions between hydroquinone↔quinone systems. The authors took

advantage of the ease by which the process can be driven in either direction. The hydroquinone system, **1.33** is quite stable toward cycloaromatization to form *p*-benzenoid diradical **1.36** (Scheme 1.10), whereas oxidation of **1.33** to corresponding quinone **1.34**, resulted in a substantial decrease in the activation energy of the Bergman rearrangement.[26]

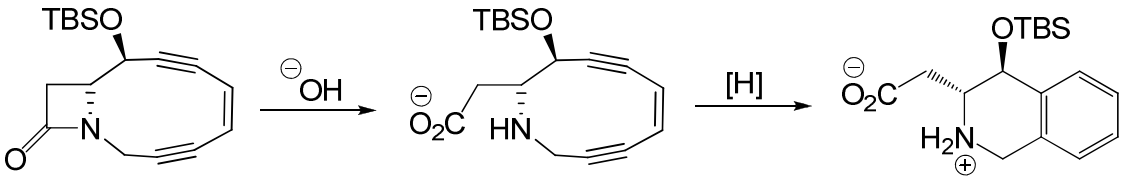
Scheme 1.10



1.36 **1.33** **1.34** **1.35**

The possibility of the activation of enediynes under specific conditions (i.e. base induced), in order to direct the cytotoxicity toward target cell lines was investigated theoretically[66] as well as experimentally.[67] The fusion of a 10-membered enediyne with a β-lactam resulted in a substantial increase of activation energy towards the Bergman rearrangement. The enediyne **1.37** (Scheme 1.11) could be easily hydrolyzed to a highly reactive derivative, **1.38**, by opening of the lactam. The latter undergoes a fast cycloaromatization reaction.

Scheme 1.11



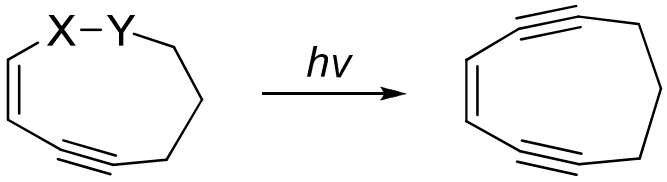
1.37 **1.38** **1.39**

1.7. Goals of the project

Basic principles of organic chemistry were followed to design target enediyne and enyne-allene precursors that have the following characteristics. First, they should be chemically stable under neutral conditions but can undergo the Bergman cycloaromatization reaction upon photoactivation. Second, they should be structurally simple when compared to the natural enediynes thus being easily synthesized. Third, they should be easily tethered to suitable delivery systems and other desirable moieties.

The goal of this project is to explore a concept that combines the reactivity of natural enediyne antibiotics with the selectivity of photoactivation. Ideally, a photoswitchable analogue of natural enediyne antibiotics should be stable in the dark but undergo rapid and reversible cyclization to *p*-benzyne after irradiation. Therefore we plan to modify the core enediyne fragment, so as to make the molecule stable in the dark but convertible into an active form by *in situ* photochemical generation of a triple bond (Scheme 1.12) to complete the enediyne π-system.

Scheme 1.12



The inactive form does not contain the complete enediyne fragment and therefore, it cannot undergo cycloaromatization until irradiation. This concept

envisions a dramatic enhancement of selectivity by allowing the spatial and temporal control of the Bergman cyclization.

To enhance the rate of Bergman cyclization of photoswitchable enediynes, we have designed nine membered ring cyclopropenone-containing enediyne and enyne-allene precursors. Highly strained nine membered cyclic enediynes and enyne-allenes are predicted to undergo very facile cycloaromatization under ambient conditions.[23,68]

These new photo-reactive enediynes were tested and selected for further development on the basis of their stability under ambient conditions, as well as their reactivity with various hydrogen-donors.

1.8. References

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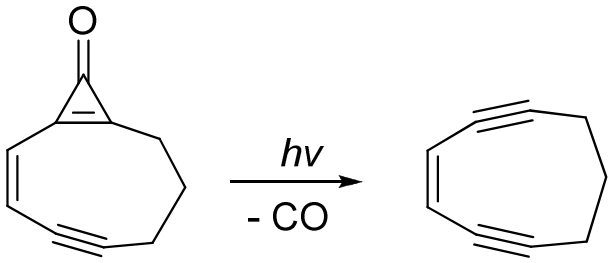
CHAPTER 2

DESIGN AND SYNTHESIS OF NINE MEMBERED RING CYCLIC ENEDIYNES AND ENYNE-ALLENE

2.1. Methods for synthesis of cyclopropenone containing 9-membered cyclic enediynes

The extreme cytotoxicity of natural enediyne antibiotics[1] is attributed to the ability of the (Z)-3-hexene-1,5-diyne fragment incorporated into a 10- or 9-membered ring cyclic system to undergo Bergman cyclization[2] and producing dDNA-damaging *p*-benzyne diradical.[3,4] The rate of this reaction strongly depends on the ring size.[5] While the 11-membered ring enediynes are stable, the 10-membered ring analogs undergo slow cycloaromatization under ambient conditions or mild heating[6-8]. Very little is known about the reactivity of 9-membered ring enediynes due to their instability. Our goal was to develop a thermally stable photo-precursor of a 9-membered enediyne (Scheme 2.1) in which one of the triple bonds is replaced by the cyclopropenone group.

Scheme 2.1

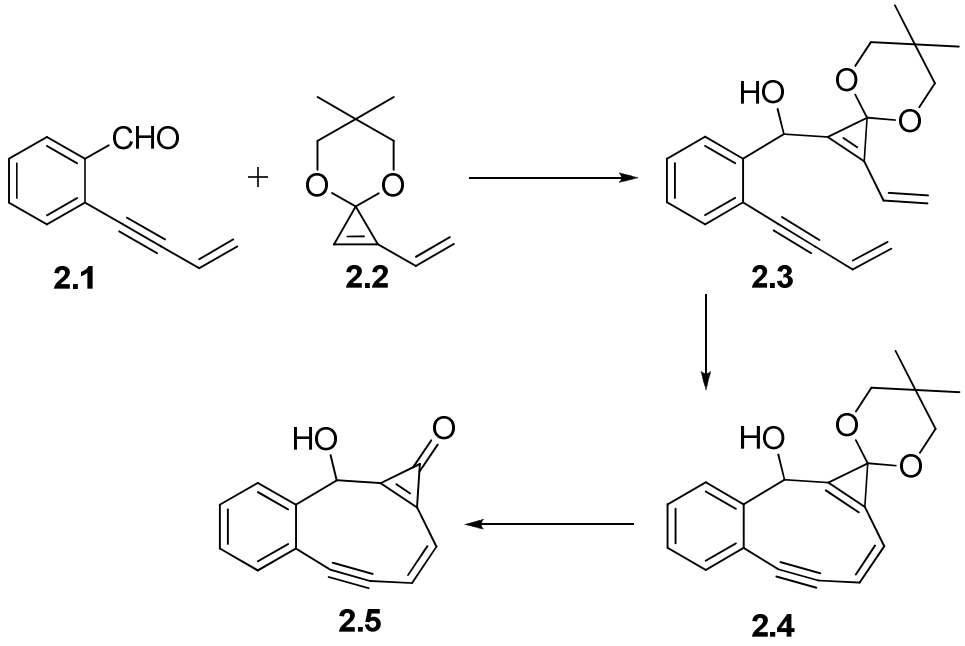


One of the most challenging steps of cyclic enediyne synthesis was the ring closure reaction. We explored two types of cyclization approaches: 1) olefin metathesis reaction; 2) Nozaki-Hiyama-Kishi coupling reaction.

2.2. Attempted synthesis of cyclopropenone containing 9-membered cyclic enediyne precursor via olefin metathesis reaction

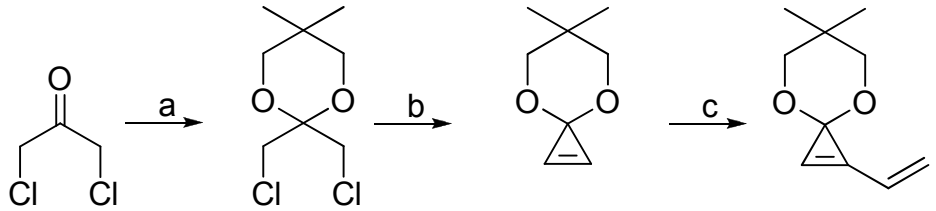
Testing the feasibility of an olefin metathesis route for preparation of enediyne precursors was an attempted synthesis of cyclopropenone, **2.5**. The key steps of the synthesis involve nucleophilic addition of a lithiated intermediate of **2.2** (Scheme 2.2) on compound **2.1** and ring closure under the Grubbs metathesis conditions.[9]

Scheme 2.2



It was found that cyclopropenone ketal **2.2** (6,6-dimethyl-1-vinyl-4,8-dioxaspiro [2.5]oct-1-ene) prepared from 1,3-dichloroacetone and neopentyl glycol, was synthetically useful because it is a good starting material for a number of intriguing reactions.[10] The cyclopropenone ketal **2.2** (Scheme 2.3) was prepared in three steps. 1,3-Dichloroacetone (**2.6**) was protected with neopentyl glycol to obtain compound **2.7**, which was subjected to cyclization with 3.2 equivalents of $NaNH_2$ in liquid NH_3 to afford the ketal **2.8**.[10] Deprotonation of cyclopropenone ketal, **2.8** with n-BuLi produced a lithium salt of **2.8** that reacted with vinyl iodide to give **2.2** in 62% yield.

Scheme 2.3[a]

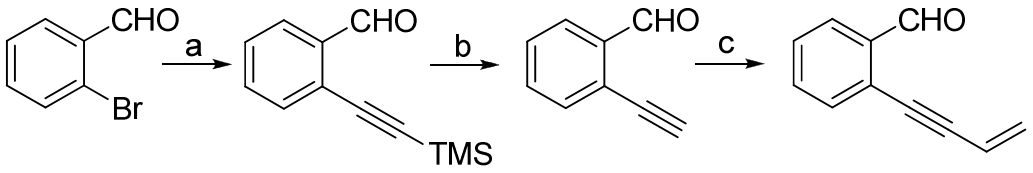


2.6 **2.7** **2.8** **2.2**

[a] Reagents and conditions: (a) neopentyl glycol, p-TsOH, benzene, reflux 19 h, 85%; (b) 3 eq.$NaNH_2$, liq.NH_3, dry ether, NH_4Cl, 48%; (c) n-BuLi, $ZnCl_2$, $Pd(PPh_3)_4$, vinyl iodide, HMPA, THF, 62%.

The reaction of 2-bromobenzaldehyde (**2.9**) with ethynyltrimethylsilane under standard Sonogashira conditions[11] gave product **2.10** in a good yield. Removal of the TMS protecting group was accomplished by using K_2CO_3 in methanol at room temperature. Another Sonogashira cross coupling reaction[11] was used in the next step (in the presence of vinyl bromide) to produce compound **2.1**.

Scheme 2.4[a]

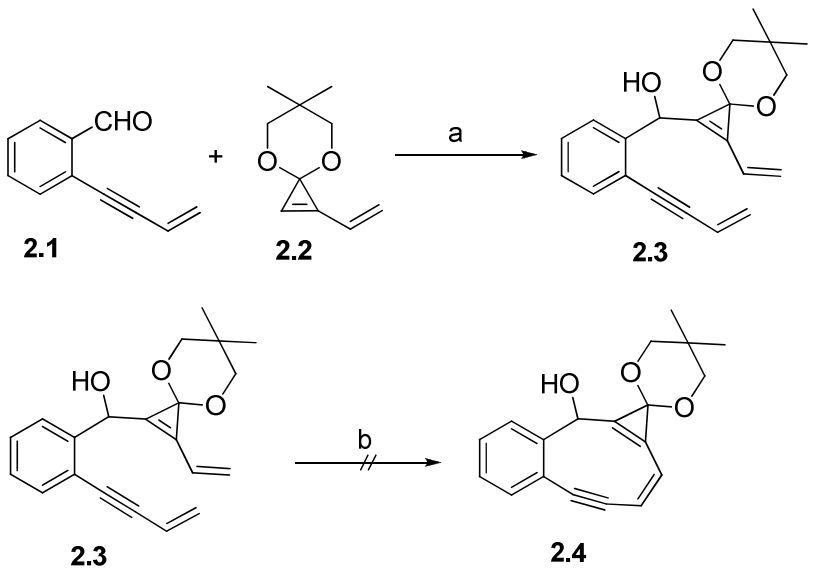


2.9 **2.10** **2.11** **2.1**

[a] Reagents and conditions: (a) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 16 h, 82%; (b) K_2CO_3, MeOH, 2 h, 76%; (c) vinyl bromide, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 8 h, 69%.

Nucleophilic addition of lithiated compund **2.2** in TMEDA to compound **2.1** formed **2.3** in 78 % yield.[12] Grubbs metathesis reaction[9] was selected for the ring closure. Although various types of Grubbs 1st and 2nd generation catalysts[13,14] were tried in metathesis, we were not able to prepare the strained 9-membered ring compound **2.4**.

Scheme 2.5[a]



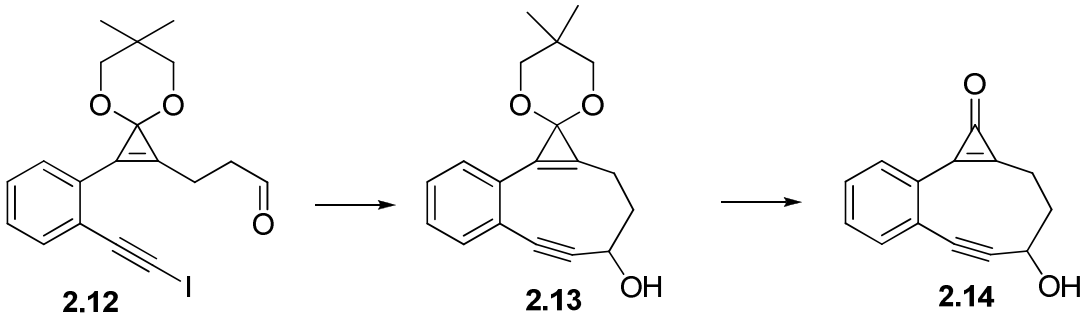
2.1 **2.2** **2.3** **2.3** **2.4**

[a] Reagents and conditions: (a) n-BuLi, TMEDA, THF, -78 ^{0}C, 40 min, 78 %; (b) Grubbs catalyst, CH_2Cl_2, 24 h.

2.3. Synthesis of cyclopropenone containing 9-membered cyclic enediyne precursor via Nozaki-Hiyama-Kishi coupling reaction

Since olefin metathesis didn't work to develop the 9-membered cyclic enediyne, we turned our attention to well established methodology for the preparation of strained macrocycles, i.e. the Nozaki-Hiyama-Kishi coupling.[15] The first stage of the successful synthetic route to the cyclopropenone containing nine membered enediyne precursor involved preparing the key intermediate **2.12** (Scheme 2.6)**,** which was then subjected for crucial ring closure using Cu(II)/ Ni(II) catalytic system,[16] leading to the target macrocycle **2.13**.

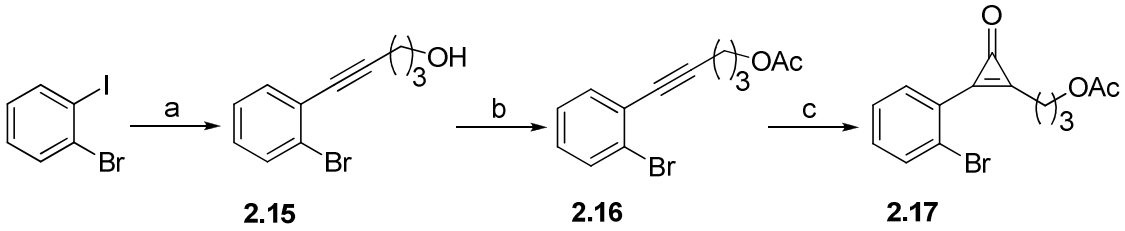
Scheme 2.6



Preparation of cyclopropenone **2.14** began with the Sonogashira coupling of bromo-2-iodobenzene with 4-pentynol which produced 93% of compound **2.15** (Scheme 2.7). Protection with acetic anhydride[12] led to the corresponding acetate, **2.16** (88-96 %). A number of synthetic methods are available for the preparation of the cyclopropenone group, which is the key component of the photoactivatable enediyne precursor **2.14**.[7,17] Few of them, however, are compatible with a highly functionalized macrocycle **2.14**, which forced us to introduce cyclopropenone functionality in early stages of the synthesis. The

addition of difluorocarbene, which was generated by the thermolysis of trimethylsilylfluorosulfonyl difluoroacetate ($FO_2SCF_2CO_2SiMe_3$, TFDA),[18,19] to the acetylene **2.16**, produced 1,1-difluorocyclopropene. The latter was hydrolyzed without isolation on wet silica gel to give cyclopropenone **2.17**.

Scheme 2.7[a]

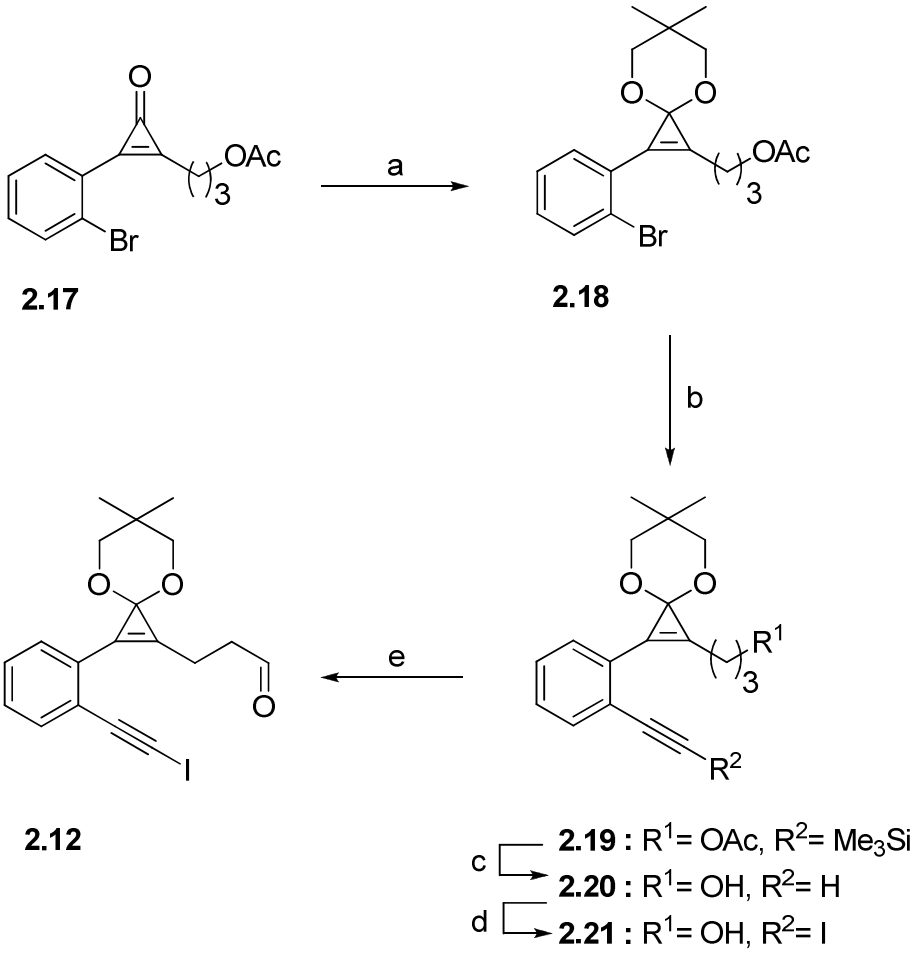


2.15 **2.16** **2.17**

[a] Reagents and conditions: (a) pent-4-ynol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 60 ^{0}C, 24 h, 93%; (b) acetic anhydride, Et_3N, DMAP, CH_2Cl_2, 0 ^{0}C, 2 h, 88-96%; (c) NaF, TFDA, Diglyme, 120 ^{0}C, 2 h, then hydrolyzed with wet silica in hexanes, 65-70%.

It's a difficult task to carry an unprotected cyclopropenone moiety through a multistep synthesis because it is very susceptible to a nucleophilic attack, which usually results in ring opening.[20] Complexation with Lewis acids, on the other hand, produces a relatively unreactive 2π-aromatic oxycyclopropenium cation. Masking the cyclopropenone moiety as a 2,2-dimethyl-1,3-propanediyl acetal **2.18** allows us to avoid these complications and broaden the range of reagents and reaction conditions that can be employed for the preparation of the target compound (Scheme 2.8). The second acetylenic substituent was introduced using conventional Stille coupling conditions to form compound **2.19** in 79% yield. Simultaneous saponification of the acetate and removal of the TMS group followed by iodination[21] and Dess-Martin oxidation[22] gave rise to the iodoaldehyde **2.12** in 84% yield.

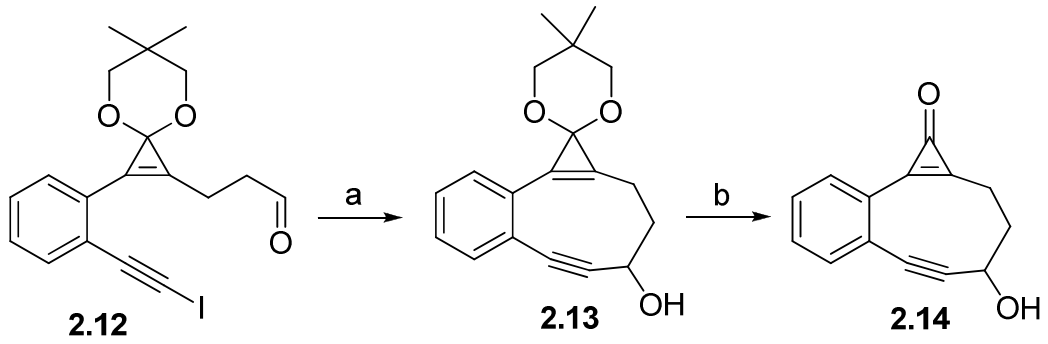
Scheme 2.8[a]



[a] Reagents and conditions: (a) neopentylglycol, Et_3N, CH_2Cl_2, r.t., 12 h, 55-65%; (b) trimethyl[(tributylstannyl) ethynyl]silane, $Pd(PPh_3)_4$, toluene, 90 ^{0}C, 2 h, 79%; (c) K_2CO_3, MeOH, 2 h, 88%; (d) I_2, morpholine, benzene, 78%; (e) Dess-Martin periodinane, CH_2Cl_2, r.t., 3 h, 84%.

The most challenging step, intramolecular Nozaki-Hiyama-Kishi coupling[16,23] was achieved in the presence of the Cr(II)/Ni(II) catalytic system, to afford the desired target macrocycle **2.13,** followed by mild removal of an acetal protection, to produce the target nine-membered ring cyclopropenone **2.14** in decent yield (Scheme 2.9).[12]

Scheme 2.9[a]

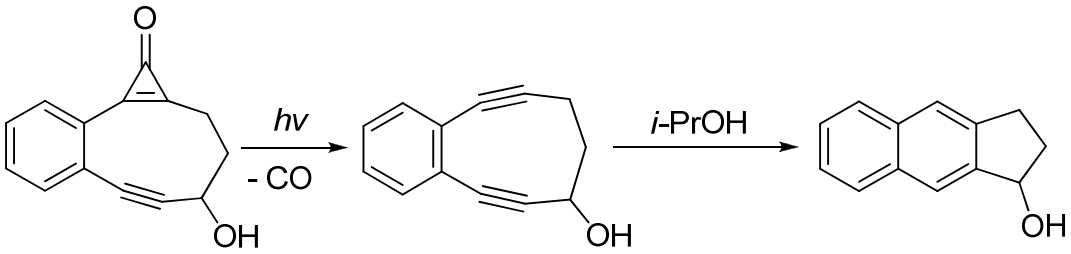


2.12 **2.13** **2.14**

[a] Reagents and conditions: (a) $CrCl_2$, $NiCl_2$, THF, 0 ^{0}C, 3 h, 36%; (b) Amberlyst®, acetone, r.t., 1 h, 77%.

The direct synthesis of benz[*f*]indan-1-ol (**2.24**)[24,25] followed by HPLC analysis supported the structural assignment of the product which was isolated from the photolysate of **2.14** (Scheme 2.10) in 2-propanol.

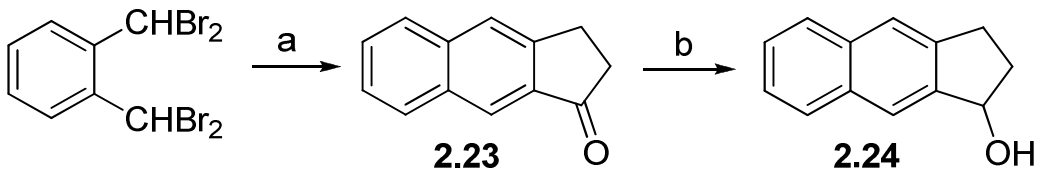
Scheme 2.10



2.14 **2.22** **2.24**

The reaction of α,α,ά,ά-tetrabromo-o-xylene with 2-cyclopentenone in the presence of NaI formed benz[f]indan-1-one (**2.23**) in 69 % yield (Scheme 2.11). $LiAlH_4$ was used to reduce the ketone **2.23** to the corresponding alcohol **2.24** at room temperature.

Scheme 2.11[a]

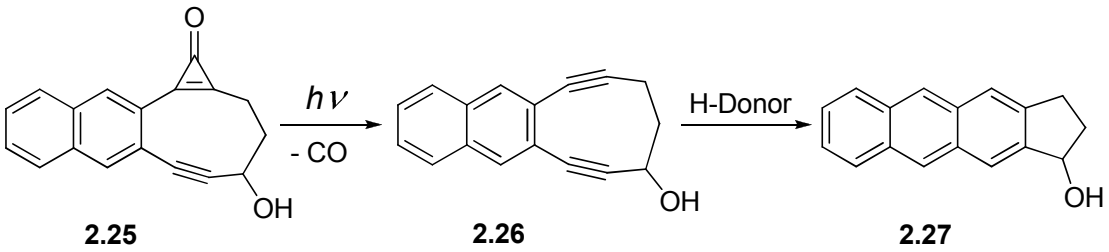


[a] Reagents and conditions: (a) 2-Cyclopentenone, NaI, DMF, 80 ^{0}C, 18 h, 69%; (b) $LiAlH_4$, THF, r.t., 2 h, 74 %.

2.4. Synthesis of naphthalene-fused cyclopropenone containing nine membered enediyne precursor

In order to improve the DNA binding affinity of the enediyne **2.22**, we decided to design and synthesize an analog (**2.26**, Scheme 2.12) of enediyne **2.22,** containing dDNA minor-groove binder or dDNA-intercalating moiety. It was essential to find structural modification that would allow shifting the absorbance of a cyclopropenone to 350 – 400 nm. Numerous studies confirmed that naphthalenes, anthracenes and anthraquinones are good DNA intercalaters.[26-28]

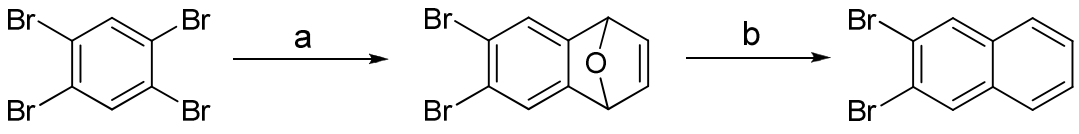
Scheme 2.12



It was found that 2,3-dibromonaphthalene was a versatile starting material for a number of interesting reactions.[29,30] Treatment of 1,2,4,5-tetrabromobenzene (Scheme 2.13) with one equivalent of butyllithium and furan

gave 6,7-dibromo-1,4-epoxide-1,4-dihydronaphthalene[29] (**2.28**) in 76% yield. Iodotrimethyl silane mediated deoxygenation[31] of epoxide **2.28** produced the synthon **2.29** in 87% yield.

Scheme 2.13[a]



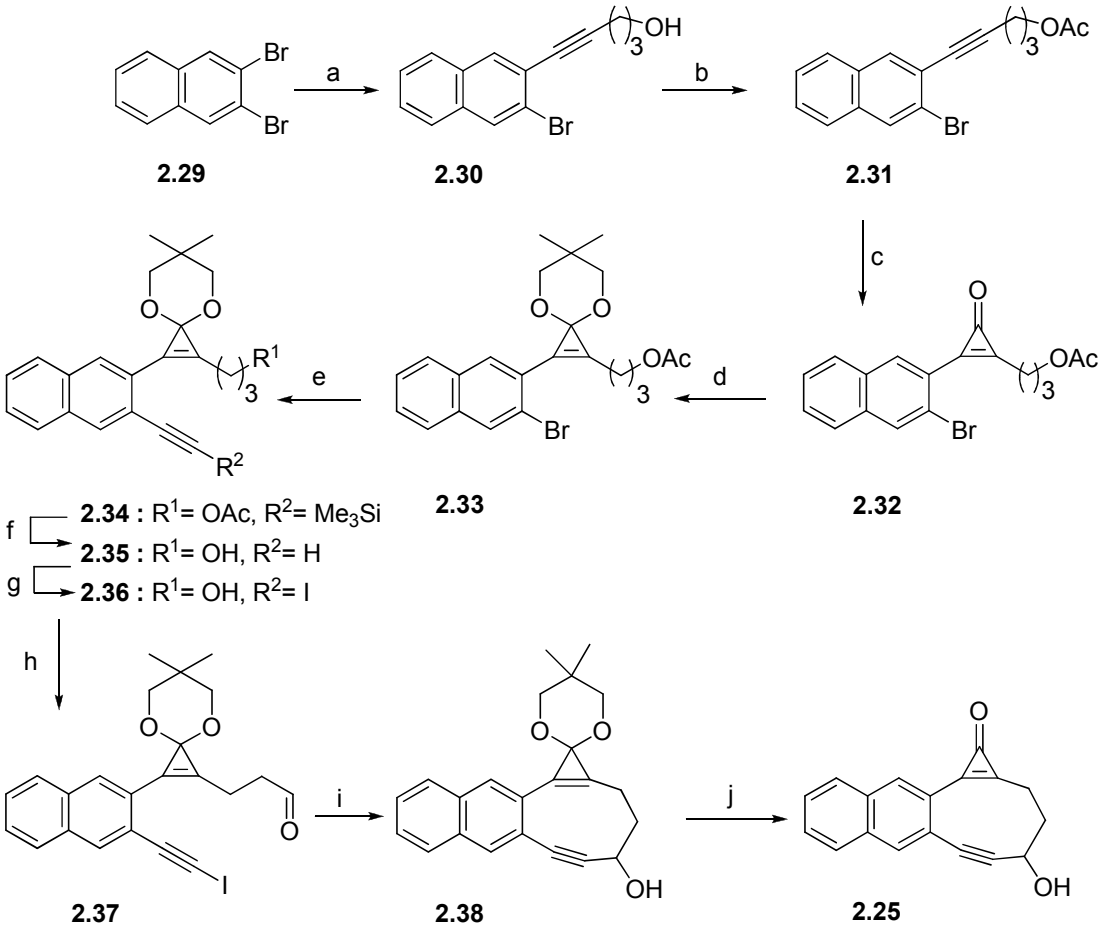
2.28 **2.29**

[a] Reagents and conditions: (a) BuLi, furan, hexane, toluene, 76%; (b) Me_3SiCl, NaI, MeCN, 87 %.

The synthetic route which was established to prepare benzene annulated cyclopropenone **2.14** was used in the preparation of the new target compound, i.e. naphthalene-fused cyclopropenone **2.25**. Sonogashira coupling of 2,3-dibromonaphthalene with 4-pentynol gave 78% of compound **2.30**. Protection with acetic anhydride[12] led to the corresponding acetate, **2.31** (79%). Addition of difluorocarbene to the acetylene **2.31**, produced 1,1-difluorocyclopropene which was hydrolyzed without isolation on wet silica gel to give corresponding cyclopropenone **2.32** (Scheme 2.14). Acetylation of **2.32** in the presence of Et_3OBF_4 and neopentylglycol, produced cyclopropenone-acetal **2.33** in 52% yield. Stille coupling conditions were used to form compound **2.34** in 79% yield. Simultaneous saponification of the acetate and removal of the TMS group followed by iodination[21] and Dess-Martin oxidation[22] gave rise to the iodoaldehyde **2.37**. The intramolecular Nozaki-Hiyama-Kishi coupling[16,23]

conditions were used to afford the desired target macrocycle **2.38** followed by mild removal of an acetal protection, produced the target nine membered ring cyclopropenone **2.25**.

Scheme 2.14[a]

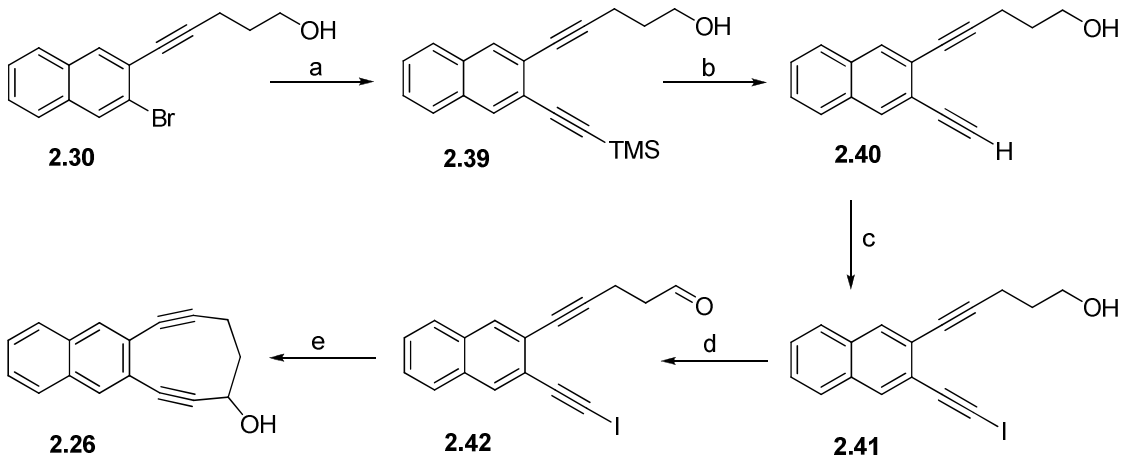


[a] Reagents and conditions: (a) pent-4-ynol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 60 ^{0}C, 24 h, 78%; (b) acetic anhydride, Et_3N, DMAP, CH_2Cl_2, 0 ^{0}C, 2 h, 79%; (c) NaF, TFDA, Diglyme, 120 ^{0}C, 2 h, then hydrolyzed with wet silica in hexanes; (d) neopentylglycol, Et_3N, CH_2Cl_2, r.t., 12 h, 52 %; (e) trimethyl[(tributylstannyl) ethynyl] silane, $Pd(PPh_3)_4$, toluene, 90 ^{0}C, 2 h, 79%; (f) K_2CO_3, MeOH, 2 h, 81%; (g) I_2, morpholine, benzene, 83%; (h) Dess-Martin periodinane, CH_2Cl_2, r.t., 3 h, 74%; (i) $CrCl_2$, $NiCl_2$, THF, 0 ^{0}C, 3 h, 60%; (j) Amberlyst®, acetone, r.t., 1 h, 74%.

Since photochemically generated enediyne **2.26** (Scheme 2.12) was thermally stable at room temperature due to the presence of extended

conjugation effect, we decided to synthesize it independently (Scheme 2.15). Under the conventional Stille coupling conditions, previously synthesized precursor **2.30** was converted to compound **2.39** in 82% yield. The TMS group of compound **2.39** was removed by saponification, followed by iodination and Dess-Martin oxidation to produce 79% of the iodoaldehyde **2.42.** The naphthalene containing 9-membered ring enediyne, **2.26,** was obtained from intramolecular cylcization of the compound **2.42** under the Nozaki-Hiyama-Kishi coupling[16,23] conditions in 54% yield.

Scheme 2.15[a]



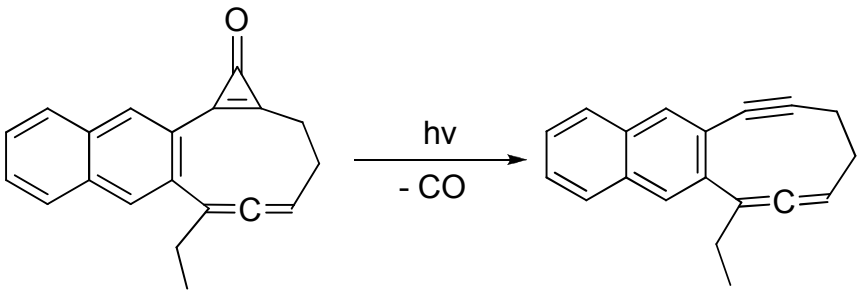
[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, trimethyl[(tributylstannyl)ethynyl]-silane, toluene, 90 ^{0}C, 2 h, 82%; (b) K_2CO_3, MeOH, 2 h, 85%; (c) I_2, morpholine, benzene, 80%; (d) Dess-Martin periodinane, CH_2Cl_2, r.t., 3 h, 79%; (e) $CrCl_2$, $NiCl_2$, THF, 0 ^{0}C, 3 h, 54%.

2.5. Synthesis of naphthalene-fused cyclopropenone containing nine membered enyne-allene precursor

The slow rate of cyclization of naphthalene-fused cyclic enediyne **2.26** (τ = 6 h at 70 ^{0}C) makes it of little use in biological applications. The low reactivity of this naphthalene-fused enediyne was believed to be attributed to the increased

stability of the substrate due to the incorporation of extended conjugation into the benzannulated system **2.22**. The Myers-Saito cyclization reactions of the enediyne moieties, as well as the other natural antitumor antibiotics containing enyne-allene moieties, lead to highly reactive biradicals which are held responsible for the DNA cleavage abilities of potent pharmacophors.[32,33] Cyclopropenone containing enyne-allene precursor **2.43** (Scheme 2.16) was designed and synthesized to achieve three major goals. The first objective was to shift decarbonylation wavelength (activation wavelength) of the system to 350 – 400 nm. The second was to enhance the rate of formation of cytotoxic diradicals. Third, the goal was expected to show some efficient DNA-intercalation properties.

Scheme 2.16

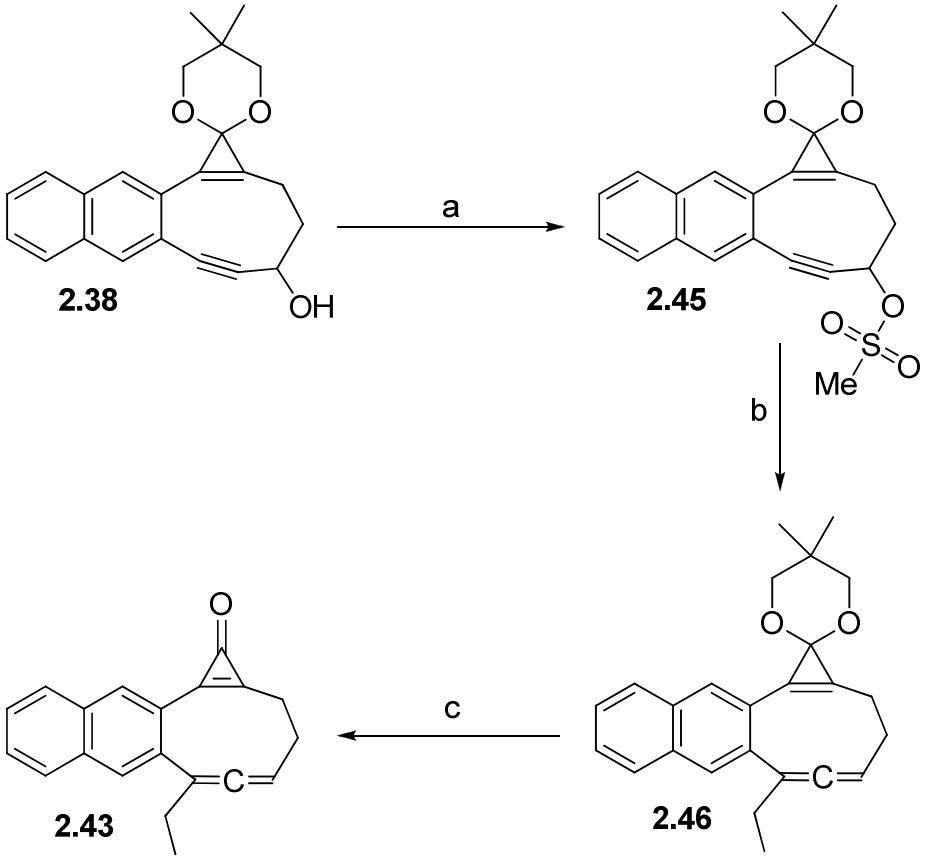


2.43 **2.44**

The key step in the synthesis of enyne–allene precursor **2.43** was the acetylene–allene rearrangement. Several enyne–allenes were prepared by isomerization of propargyl alcohol employing Mitsunobu reaction with 2-nitro-benzenesulfonyl hydrazide.[34,35] Acetal **2.38**, however, is not stable under Mitsunobu conditions and an alternative procedure[36] was adopted. The synthesis

was then commenced with mesylation of cyclopropenone acetal **2.38** in the presence of methanesulfonyl chloride (Scheme 2.17). The reaction of propargylic mesylate **2.38** with EtMgBr in the presence of an excess of CuCN and LiCl led to the exclusive formation of the desired allene **2.46**. Acetal deprotection was achieved using Amberlyst®-15 in acetone to produce targeted enyne-allene precursor **2.43**.

Scheme 2.17[a]



[a] Reagents and conditions: (a) Methane-sulfonyl cholride, Et_3N, CH_2Cl_2, 0 ^{0}C, 1h, 81%; (b) EtMgBr, CuCN, LiCl, THF, -78 ^{0}C, 3 h, 64%; (c) Amberlyst®, acetone, r.t., 1 h, 76%.

2.6. Conclusions

A successful synthetic route was established to synthesize benzene annulated cyclopropenone containing enediyne precursor **2.14**. In order to improve the DNA binding affinity of the enediyne **2.22**, we designed and synthesized naphthalene-fused enediyne **2.26**. This structural modification also allowed us to shift the absorbance of the cyclopropenone precursor **2.25**, to 300-350 nm. This was the first thermally stable, 9-membered cyclic enediyne (**2.26**) ever reported. Synthesis of cyclopropenone containing enyne-allene precursor **2.43** re-established the reactivity of the cycloaromatization reaction. An efficient DNA intercalater is an additional benefit provided by the fused-naphthalene moiety of **2.43**. This could be exploited to develop dDNA cleaving agents, with the spatial and temporal control.

2.7. Experimental

2.7.1 Materials

Moisture and oxygen-sensitive reactions were carried out in flame-dried glassware under an argon atmosphere. Tetrahydrofuran was distilled from sodium, while dichloromethane was distilled from phosphorus pentoxide under argon immediately before use. Hexanes used in column chromatography was distilled from sodium, ethyl acetate and acetone were distilled from anhydrous calcium chloride. Other reagents were obtained from Aldrich or VWR and used as received unless otherwise noted. Transition metal compounds were

purchased from Strem Chemicals. Purification of products by column chromatography was performed using 40-63 μm silica gel.

2.7.2 Instruments

Melting points were determined using Fisher-Johns melting point apparatus and are not corrected. Nuclear magnetic resonance (NMR) spectra were recorded with Varian Mercury 400 MHz and 500 MHz. ^{1}H-NMR and ^{13}C-NMR were monitored using tetramethylsilane (TMS) or residual solvent peak as an internal standard. The chemical shifts are reported in parts per million (ppm) relative to TMS. UV-visible spectra were obtained on Varian Cary 300 UV-Visible spectrometer. IR spectra were recorded on Shimadzu IRPrestige-21 FTIR instrument. GC-MS data were collected using Shimadzu GC-2010 equipped with SHR5XLB column. High resolution mass spectra analyses were performed by the Mass Spectrometry Laboratory, University of Georgia at Athens, GA. The consumption of starting material and formation of product was followed by TLC or HPLC (Shimadzu SCL-10A VP, equipped with Hibar® RT250-4, RP-18).

2.7.3 Experimental Procedures

2,2-bis(chloromethyl)-5,5-dimethyl-1,3-dioxane (2.7).[10] A mixture of 1,3-dichloroacetone (121.5 g, 956 mmol), neopentyl glycol (104 g, 1000 mmol), p-toluenesulfonic acid (3.6 g, 19 mmol) and benzene (80 ml) was heated to reflux for 19 h in a 500 mL, round bottom flask equipped with a Dean-Stark trap and a condenser with azeotropic removal of water. After separation of water ceased, the reaction mixture was partitioned between hexane (500 ml) and saturated sodium bicarbonate (200 ml). The organic phase was washed with water (100

ml) and saturated sodium chloride (100 ml), dried over $MgSO_4$, and concentrated on a rotary evaporator. Distillation of the residue yielded 173 g (85 %) of the acetal, 2,2-bis(chloromethyl)-5,5-dimethyl-1,3-dioxane as a colorless oil (bp 99-100 ^{0}C, 3.5 mm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 6H), 3.56 (s, 4H), 3.79 (s, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 22.39, 29.82, 41.92, 71.02, 97.24.

6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-ene (2.8).[10] An oven-dried, 1 L, three necked round-bottom flask was equipped with a dry ice/acetone condenser protected with a drying tube. The flask was flushed with nitrogen gas and the sodium amide (37.0 g, 950 mmol) was added. The dry ice/acetone bath was placed under the flask and gaseous ammonia was added to the flask condensing ca. 400 ml, with gentle stirring. Then the gas inlet was replaced with a pressure equalizing addition funnel containing a solution of 2,2-bis(chloromethyl)-5,5-dimethyl-1,3-dioxane (64.0 g, 300 mmol) in 150 ml of dry ether. This solution was added drop wise to the slurry of sodium amide and liquid ammonia over 1 h. The cooling bath was removed and the mixture was stirred for 3 h. Then the mixture was cooled with dry ice/ acetone bath again and solid ammonium chloride (20.24 g, 378 mmol) was added in several portions over 5 min. The dry ice condenser was removed and the ammonia was allowed to evaporate. The cooling bath was replaced with a water bath (ca. 30 ^{0}C) and a 1:1 mixture of dry ethyl ether and pentane (400 ml) was added through addition funnel over 10 min with vigorous stirring. After evaporation of most of ammonia (ca. 2 h), the ethereal solution was filtered by suction to remove the inorganic salts. The filter cake was washed with three times with 80 ml of ethyl ether. The combined filtrate and washes were

concentrated under reduced pressure and the residue was distilled to yield 20 g (48%) of ketal of cylopropenone as colorless oil (bp 58-61 ^{0}C, 7 mm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (s, 6H), 3.62 (s, 4H), 7.85 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 22.23, 30.34, 76.57, 80.99, 125.48.

6,6-dimethyl-1-vinyl-4,8-dioxaspiro[2.5]oct-1-ene (2.2). n-BuLi (4 mL of 2.5 M in hexane, 10 mmol) was added to ketal of cyclopropenone (1.4 g, 10 mmol) in HMPA (7.2 mL, 40 mmol) and THF (15 mL) at -78 ^{0}C over 20 min.. The resultant solution was stirred for 30 min at -78 ^{0}C, followed by addition of a solution of $ZnCl_2$ (1.1 g, 8 mmol) in THF (4 mL) and dry ice bath was removed. Vinyl Iodide (0.74 mL, 10 mmol) and Pd $(PPh_3)_4$ (0.35 g, 0.3 mmol) were added to the reaction mixture and then it was stirred for 4 h. The reaction was diluted with 25 % ethyl acetate in hexane, quenched by the addition of saturated phosphate buffer (5 mL, pH= 7.2) The organic layer was separated, passed through a short silica gel column (eluting with 10 % ethyl acetate in hexane, containing 1.5 % of Et_3N) and dried over anhydrous Na_2SO_4 and solvent was removed in vacuum. Chromatography on silica gel (10 % ethyl acetate in hexane, containing 1.5 % of Et_3N) gave 1.0 g (62 %) of the compound **2.2** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (d, 6H, *J*= 6.1 Hz), 3.70 (q, 4H, *J*= 6.1 Hz), 5.75 (d, 1H, J_{cis}= 12.4 Hz), 5.86 (d, 1H, J_{trans}= 20.5 Hz), 6.50 (dd, 1H, *J*= 12.4, 20.5 Hz), 7.48 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 22.24, 22.38, 30.36, 77.86, 82.32, 116.15, 121.75, 126.28, 133.91.

2-((trimethylsilyl)ethynyl)benzaldehyde (2.10). A thoroughly degassed solution of 2-bromobenzaldehyde (8.5 g, 46 mmol), ethynyltrimethylsilane (4.5 g, 46 mmol), Et_3N (15 mL), $Pd(PPh_3)_2Cl_2$ (1.6 g, 2.3 mmol), triphenyl phosphine (0.6 g, 2.3 mmol), CuI (0.5 g, 2.3 mmol) in 300 mL of THF was stirred at 35 ^{0}C for 16 h. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by silica gel chromatography (10 % ethyl acetate in hexane) gave 7.5 g (82 %) of **2.10** as a thick yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.38 (s, 9H), 7.46 (t, 1H, *J*=7.7 Hz), 7.57 (t, 1H, *J*=6.5 Hz), 7.66 (d, 1H, *J*=6.5 Hz), 7.93 (d, 1H, *J*=7.70 Hz), 10.58 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 0.01, 100.29, 102.65, 127.02, 127.09, 129.05, 133.72, 133.88, 136.41, 192.07.

2-ethynylbenzaldehyde (2.11). K_2CO_3 (10.5 g, 76 mmol) was added to a solution of **2.10** (7.7 g, 38 mmol) in MeOH : CH_2Cl_2 mixture (300 mL, 2:1 v/v) and stirred vigorously for 2 h at r.t. 25 mL of water were added to the reaction mixture, organic layer was separated, and aqueous layer was extracted with ether (3 x 200 mL). Combined organic layers were washed with sodium bicarbonate solution, dried over $MgSO_4$, and the solvents were removed in vacuum. Chromatographic purification of the residue (5 % ethyl acetate in hexane) gave 3.8 g (76 %) of **2.11** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46 (s, 1H), 7.48 (t, 1H, *J*=7.8 Hz), 7.56 (t, 1H, *J*=6.5 Hz), 7.60 (d, 1H, *J*=6.5 Hz), 7.94 (d, 1H, *J*=7.8 Hz), 10.54 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 79.42, 84.47, 125.71, 127.48, 129.45, 133.93, 134.16, 136.79, 191.63.

2-(but-3-en-1-ynyl)benzaldehyde (2.1). A thoroughly degassed solution of compound **2.11** (2.5 g, 19.2 mmol), vinyl bromide (4.1 g, 38 mmol), Et_3N (5 mL), $Pd(PPh_3)_2Cl_2$ (0.67 g, 0.96 mmol), triphenyl phosphine (0.25 g, 0.96 mmol), CuI (0.36 g, 1.92 mmol) in 100 mL of THF was stirred at 35 ^{0}C for 8 h. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by silica gel chromatography (5 % ethyl acetate in hexane) gave 2.1 g (69 %) of **2.1** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (d, 1H, J_{cis}=11.26 Hz), 5.86 (d, 1H, J_{trans}=15.76 Hz), 6.09 (dd, 1H, *J*=11.26, 15.76 Hz), 7.46 (t, 1H, *J*=6.76 Hz), 7.57 (t, 1H, *J*=6.42 Hz), 7.58 (d, 1H, *J*=6.42 Hz), 7.94 (d, 1H, *J*=6.76 Hz), 10.56 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 85.59, 95.09, 116.80, 126.95, 127.41, 128.74, 128.89, 133.45, 133.96, 136.07, 191.89.

(2-(but-3-en-1-ynyl)phenyl)(6,6-dimethyl-2-vinyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)methanol (2.3). n-BuLi (0.60 mmol, 0.21 mL of 2.8 M solution in hexane) was added to a solution of 6,6-dimethyl-1-vinyl-4,8-dioxaspiro[2,5]oct-1-ene, **2.2** (0.6 mmol, 0.1 g) in a solution of TMEDA (0.35 mL) and THF (10 mL) at -78 ^{0}C. After 30 min, a solution of aldehyde **2.1** (0.45 mmol, 0.07 g) in THF (2 mL) was added drop wise at -78 ^{0}C. After 10 min, the reaction was quenched by phosphate buffer and promptly warmed to the room temperature. Then the reaction mixture was extracted with EtOAc (2x30 mL) and the combined organic layers were washed with water, brine and dried over sodium sulfate and concentrated in vacuum. Purification of the residue by silica gel chromatography (20 % ethyl acetate in hexane, containing 1.5 % of Et_3N) gave 0.12 g (78 %) of

2.3 as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) ḍ 0.83 (s, 3H), 1.12 (s, 3H), 2.9 (s, broad,1H), 3.34 (d, 1H, *J*=9.6 Hz), 3.48 (d, 1H, *J*=9.6 Hz), 3.58 (d, 1H, *J*=13.7 Hz), 3.69 (d, 1H, *J*=13.7 Hz), 5.58 (m, 2H, *J*=10.96, 18.18 Hz), 5.68 (m, 2H, *J*=12.72, 16.36 Hz), 5.99 (dd, 1H, *J*=10.96, 18.18 Hz), 6.23 (s, 1H), 6.36(dd, 1H, *J*=12.72, 16.36 Hz), 7.28 (t, 1H, *J*=6.76 Hz), 7.35 (t, 1H, *J*=6.42 Hz), 7.46 (d, 1H, *J*=6.42 Hz), 7.53 (d, 1H, *J*=6.76 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 22.19, 22.82, 30.43, 46.39, 68.54, 78.28, 78.31, 84.46, 87.45, 93.71, 117.04, 120.91, 121.28, 125.83, 127.11, 127.88, 128.35, 129.35, 131.82, 132.62, 142.71; HRMS calcd. for $C_{21}H_{22}O_3$ [M+H$^+$] 323.1647, found 323.1633.

5-(2-bromophenyl)pent-4-yn-1-ol (2.15). A thoroughly degassed solution of 1-bromo-2-iodobenzene (20.0 g, 71 mmol), pent-4-yn-1-ol (7.2 g, 85 mmol), Et_3N (15 mL), $Pd(PPh_3)_2Cl_2$ (2.5 g, 3.5 mmol), triphenyl phosphine (1.9 g, 7.1 mmol), CuI (1.4 g, 7.1 mmol) in 300 mL of THF was stirred at 60 ^{0}C for 2 days. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by silica gel chromatography (hexanes:ethyl acetate 4:1 mixture) gave 15.8 g (93 %) of **2.15** as a thick yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, 1H, J=7.77 Hz), 7.45 (d, 1H, J=8.91 Hz), 7.25 (t, 1H, J=8.91 Hz), 7.15 (t, 1H, J=7.77 Hz), 3.90 (t, 2H, J=6.89 Hz), 2.63 (t, 2H, J=6.89 Hz), 1.92 (quintet, 2H, J=6.89 Hz), 1.59 (s, br, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.53, 132.51, 129.09, 127.16, 125.37, 125.67, 94.64, 80.17, 61.97, 31.35, 16.39; HRMS calcd for $C_{11}H_{11}BrO$ [M$^+$] 237.9993, found 237.9992.

5-(2-bromophenyl)pent-4-ynyl acetate (**2.16**). Acetic anhydride (11.4 g, 112 mmol) was added to a stirred solution of 5-(2-bromophenyl)pent-4-yn-1-ol (6.7 g, 28.0 mmol), Et_3N (50 mL), and DMAP (0.7 g, 5.6 mmol) in CH_2Cl_2 (100 mL) and the resulting solution was stirred for 2 h at 0 °C. Solvents were removed in vacuum and the residue was purified by chromatography (hexanes : ethyl acetate 4:1) to give 7.1 g (96 %) of **2.16** as a slightly yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, 1H, *J*=8.85 Hz), 7.42 (d, 1H, *J*=7.08 Hz), 7.22 (t, 1H, *J*=7.08 Hz), 7.12 (t, 1H, *J*=8.85 Hz), 4.27 (t, 2H, *J*=6.28 Hz), 2.57 (t, 2H, *J*=6.28 Hz), 2.05 (s, 3H), 1.95 (quintet, 2H, *J*=6.28 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.33, 133.53, 132.52, 129.12, 127.15, 125.90, 125.70, 93.87, 80.28, 63.39, 27.86, 21.20, 16.58; HRMS calcd. for $C_{13}H_{13}BrO_2$ [M^+] 280.0099, Found 280.0094.

1-(3-Acetoxypropyl)-2-(*o*-bromophenyl)-6,6-dimethyl-4,8-dioxaspiro-[2.5]oct-1-ene (**2.18**). Trimethyl-2,2-difluoro-2-(fluorosufonyl) acetate (10.7 g, 42.7 mmol) was added dropwise over a period of 1 h to a solution of **2.16** (4.0 g, 14.2 mmol) and sodium fluoride (0.06 g, 1.4 mmol) in 5 mL of diglyme at 120 ^{0}C, and stirred for 30 min. The reaction mixture was allowed to cool to r.t., passed through a wet silica gel column (hexanes, containing 3 % water), and left overnight in the column. The crude cyclopropenone **2.17** was eluted with hexanes : ethyl acetate 1:1 mixture and used in the next step without further purification.

A solution of cyclopropenone **2.17** (2.0 g, 6.5 mmol) and triethyloxonium fluoroborate (2.5 g, 13.0 mmol) in dry CH_2Cl_2 (20 mL) were stirred at r.t. for 1 h. A

solution of neopentylglycol (1.4 g, 13.0 mmol) and triethylamine (1.3 g, 13.0 mmol) in CH_2Cl_2 (10 mL) was added to the reaction mixture and left stirring for 12 h. The precipitated salts were removed by filtration, solvent removed in vacuum, and the residue was purified by chromatography (hexanes : ethyl acetate 9:1, containing 1.5 % Et_3N) to give 2.9 g (51 % over two steps) of **2.18** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, 2H, *J*=7.44 Hz), 7.35 (t, 1H, *J*=7.44 Hz), 7.27 (t, 1H, *J*=7.44 Hz), 4.19 (t, 2H, *J*=5.32 Hz), 3.72 (m, 4H), 2.96 (t, 2H, *J*=5.32 Hz), 2.06 (quintet, 2H, *J*=5.32 Hz), 2.04 (s, 3H), 1.13 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.33, 134.41, 133.00, 132.22, 129.21, 126.67, 123.88, 72.05, 63.58, 36.74, 26.04, 25.78, 21.53, 21.09. Compound **2.18** is very unstable in the pure state and undergoes rapid decomposition; it should be put in the next step immediately.

1-(3-Acetoxypropyl)-2-(*o*-((trimethylsilyl)ethynyl)phenyl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-ene (**2.19**). $Pd(PPh_3)_4$ (0.9 g, 0.76 mmol) was added to a solution of **2.18** (2.9 g, 7.3 mmol) and 1-(tributylstannyl)-2-(trimethylsilyl)acetylene (5.9 g, 15.2 mmol) in 150 mL of dry toluene and stirred at for 2 h at 90 ^{0}C. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 9:1, containing 1.5% Et_3N) gave 2.4 g (79 %) of **2.19** as a thick yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, 2H, *J*=7.76 Hz), 7.37 (t, 1H, *J*=7.76 Hz), 7.29 (t, 1H, *J*=7.76 Hz), 4.22 (t, 2H, *J*=5.82 Hz), 3.75 (m, 4H), 2.98 (t, 2H, *J*=5.82 Hz), 2.09 (quintet, 2H, *J*=5.82 Hz), 2.07 (s, 3H), 1.16 (s, 3H),

1.08 (s, 3H), 0.28 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.27, 134.51, 130.72, 130.03, 129.63, 128.98, 128.91, 125.19, 123.04, 105.24, 98.55, 83.79, 78.38, 64.07, 30.56, 27.53, 23.41, 22.54, 21.19, 0.17; HRMS calcd. for $C_{24}H_{33}O_4Si$ [M+H$^+$] 413.2148; found 413.2142.

2-(*o*-Ethynylphenyl)-1-(3-hydroxypropyl)-6,6-dimethyl-4,8-dioxaspiro [2.5]oct-1-ene (**2.20**). K_2CO_3 (1.8 g, 12.7 mmol) was added to a solution of **2.19** (3.5 g, 8.5 mmol) in MeOH : CH_2Cl_2 mixture (120 mL, 2:1 v/v) and stirred vigorously for 1 h at r.t. 25 mL of water were added to the reaction mixture, organic layer was separated, and aqueous layer was extracted with ether (3 x 100 mL). Combined organic layers were washed with sodium bicarbonate solution, dried over $MgSO_4$, and the solvents were removed in vacuum. Chromatographic purification of the residue (hexanes : ethyl acetate 1:1, containing 1.5% Et_3N) gave 2.2 g (7.5 mmol, 88 %) of **2.20** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59-7.61 (d, 2H, *J*=7.76 Hz), 7.41 (t, 1H, *J*=7.76 Hz), 7.32 (t, 1H, *J*=7.76 Hz), 3.81 (t, 2H, *J*=5.82 Hz), 3.76 (m, 4H), 3.39 (s, 1H), 2.98 (t, 2H, *J*=5.82 Hz), 2.01 (quintet, 2H, *J*=5.82 Hz), 1.16 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.03, 130.89, 130.58, 129.21, 129.04, 124.91, 121.99, 83.61, 81.67, 78.38, 62.35, 31.32, 30.59, 22.60, 22.51; HRMS calcd. for $C_{19}H_{22}O_3$ [M+H$^+$] 289.1647; found 299.1642.

1-(3-Hydroxypropyl)-2-(*o*-(iodoethynyl)phenyl)-6,6-dimethyl-4,8-dioxa spiro[2.5]oct-1-ene (**2.21**). A solution of iodine (3.4 g, 13.4 mmol) and morpholine (1.75 mL, 20.1 mmol) in dry benzene (50 mL) was stirred for 45 min at 45°C. A solution of **2.20** (2.0 g, 6.7 mmol) in dry benzene (10 mL) was added

to the reaction mixture, and stirred for 30 min at 45°C. The reaction mixture was allowed to cooled to r.t., diluted with benzene (50 mL), washed with saturated solution of $Na_2S_2O_3$ (10 mL), water (15 mL), and brine (10 mL), dried (MgSO4), and solvents were removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 1:1, containing 1.5% of Et_3N) to give 2.22 g (78 %) of **2.21** as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, 1H, *J*=7.91 Hz), 7.55 (d, 1H, *J*=7.82 Hz), 7.39 (t, 1H, *J*=7.91 Hz), 7.32 (t, 1H, *J*=7.82 Hz), 3.83 (t, 2H, *J*=5.82 Hz), 3.78 (m, 4H,), 2.94 (t, 2H, *J*=5.82 Hz), 2.03 (quintet, 2H, *J*=5.82 Hz), 1.16 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.49, 131.12, 130.63, 130.23, 129.20, 128.95, 124.80, 123.29, 93.68, 83.69, 78.39, 62.58, 23.16, 22.61, 22.55; HRMS calcd. for $C_{19}H_{21}IO_3$ [M+H$^+$] 425.0613, found 425.0621.

2-(*o*-(Iodoethynyl)phenyl)-6,6-dimethyl1-(3-oxopropyl)-4,8-dioxaspiro [2.5]oct-1-ene (**2.12**). A solution of **2.21** (2.0 g, 4.7 mmol) in CH_2Cl_2 (20 mL) was added to a suspension of Dess-Martin periodinane (4.0 g, 9.4 mmol) in CH_2Cl_2 (100 mL). The resulting suspension was stirred for 4 h, diluted with ether (100 mL), solids were removed by filtration, and solvent was removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 1.5 : 1, containing 1.5% of Et_3N) to give 1.67 g (84 %) of the aldehyde **2.12** as a slightly orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.56 (d, 1H, *J*=9.14 Hz), 7.51 (d, 1H, *J*=7.32 Hz), 7.36 (t, 1H, *J*=7.32 Hz), 7.31 (t, 1H, *J*=9.14 Hz), 3.72 (m, 4H), 3.15 (t, 2H, *J*=7.14 Hz), 2.87 (t, 2H, *J*=7.14 Hz), 1.15 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 200.94, 134.43, 130.66, 130.02, 129.99, 129.25,

129.15, 125.29, 123.31, 93.64, 83.52, 42.47, 22.70, 22.46, 19.46; HRMS calcd. for $C_{19}H_{19}IO_3$ [M+H$^+$] 423.0457, found 423.0467.

Neopentylglycol acetal of 6,7-didehydro-2,3,4,5-tetrahydro-5-hydroxy-1*H*-benzo[a]cyclopropa[c]cyclononen-1-one (**2.13**). A solution of aldehyde **2.12** (1.5 g, 3.55 mmol) in THF (50 mL) was added via a syringe pump over a period of 15 min to a degassed suspension of $CrCl_2$ (1.3 g, 10.66 mmol) and $NiCl_2$ (0.23 g, 1.77 mmol) in THF (800 mL) under argon at 0 ^{0}C. After 3 h stirring at room temperature, the reaction mixture was concentrated and solids were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 1:1, containing 1.5% of Et_3N) gave 0.38 g (36 %) of **2.13** as a thick yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J*=6.06 Hz), 7.41-7.30 (m, 3H, *J*=6.06, 8.17 Hz), 4.75 (m, 1H), 3.77 (s, 4H), 3.26 (m, 1H, *J*=9.09, 12.12 Hz), 2.71 (m, 1H, *J*=9.09, 12.12 Hz), 2.36 (m, 1H, *J*=9.09, 12.12 Hz), 2.11 (m, 1H, *J*=9.09, 12.12 Hz), 1.12 (s, 3H), 1.08 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.96, 132.87, 129.64, 129.33, 127.61, 123.22, 99.46, 85.89, 72.05, 62.71, 46.12, 36.76, 21.52, 21.16; HRMS calcd. for $C_{19}H_{20}O_3$ [M+H$^+$] 297.1490, found 297.1492.

6,7-didehydro-2,3,4,5-tetrahydro-5-hydroxy-1*H*-benzo[a]cyclopropa[c] cyclo-nonen-1-one (**2.14**). Amberlyst® resin (0.5 g) was added to a stirred solution of cyclopropenone acetal **2.13** (0.3 g, 1.01 mmol) in distilled acetone (15 mL). The resulting solution was stirred for 1 h at room temperature and the resin was removed by filtration. Solvents were removed in vacuum and the residue

was purified by chromatography (CH_2Cl_2 : MeOH 5:1) to give 0.164 g (0.78 mmol, 77%) of **2.14** as a yellow powder. Decomposed upon heating above 100^oC without melting. ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 1H, *J*=7.01 Hz), 7.49 (m, 2H *J*=7.01 Hz), 7.42 (d, 1H, *J*=7.01 Hz), 4.79 (m, 1H), 3.33 (m, 1H, *J*=11.94 Hz), 2.73 (m, 1H, *J*= 11.94 Hz), 2.37 (m, 1H, *J*=4.18, 11.94 Hz), 2.08 (m, 1H, *J*=4.18, 11.94 Hz), 1.79 (s, br, 1H, OH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.95, 132.84, 129.65, 129.33, 99.35, 62.76, 36.75, 21.15; FTIR (cm^{-1}) 3211 [O-H], 1830 [C=O], 1604, 1583, 1556 [C=C]; HRMS calcd. for $C_{14}H_{10}O_2$ [$M+H^+$] 211.0759, found 211.0760.

Benz[f]indan-1-one[25] (2.23). A solution of α,α,ά,ά-Tetrabromo-o-xylene (5.1 g, 12.2 mmol), NaI (11.9 g, 79.8 mmol), and cyclopent-2-enone (1.0 g, 12.2 mmol) in DMF was heated for 18 h at 80 ^0C, cooled, diluted with CH_2Cl_2, and shaken with 20% aqueous solution of sodium metabisulphite. The aqueous phase was extracted with CH_2Cl_2 and combined organic layers were washed with brine, dried over $MgSO_4$, and solvent removed in vacuum. The residue was purified by chromatography (benzene : ether 19:1) to give 1.53 g (8.42 mmol, 69 %) of **2.23** as a slightly yellow solid. M.p. 143-145^oC; ^1H NMR: 8.33 (s, 1H), 7.99 (d, 1H, *J*=7.18 Hz), 7.86 (d, 1H, *J*=7.18 Hz), 7.59 (t, 1H, *J*=8.38 Hz), 7.50 (t, 1H, *J*=8.38 Hz), 7.26 (s, 1H), 3.33 (t, 2H, *J*=6.83 Hz), 2.81 (t, 2H, *J*=6.83 Hz); ^{13}C NMR: 207.63, 148.12, 137.41, 132.55, 130.64, 128.78, 127.98, 126.34, 125.08, 124.63, 37.19, 25.57; HRMS calcd. for $C_{13}H_{10}O$ [$M+H^+$] 183.0810, found 183.0805.

Benz[f]indan-1-ol[24] (2.24). A solution of **2.23** (0.2 g, 1.1 mmol) in THF (10 mL) was slowly added to a solution of $LiAlH_4$ (0.08g, 2.2 mmol) in dry THF (15 mL) and stirred for 2 h at r.t. The reaction mixture was cooled to 0 ^{0}C on an ice bath and ice water (10 mL) was added dropwise, followed by 10 % aqueous H_2SO_4 (10 mL). The reaction mixture was extracted with ether (150 mL x 3), combined organic layers were washed with brine, dried over $MgSO_4$, and solvent removed in vacuum. The residue was purified by chromatography (benzene : ether 4:1) to give 0.15 g (0.8 mmol, 74 %) of **2.24** as a white solid. M.p. 138-139^{o}C; ^{1}H NMR: 7.87 (s, 1H), 7.84 (d, 1H, J=7.56 Hz), 7.79 (d, 1H, J=7.56 Hz), 7.69 (s, 1H), 7.44 (m, 1H, J=7.56 Hz), 7.37 (s, 1H), 5.38 (t, 1H , J=6.41 Hz), 3.19 (m, 1H), 2.97 (m, 1H), 2.55 (m, 1H), 2.04 (m, 1H), 1.7 (br, 1H); ^{13}C NMR: 141.76, 134.18, 133.14, 128.37, 127.81, 126.03, 125.42, 123.15, 122.87, 76.15, 36.89, 29.55; HRMS calcd. for $C_{13}H_{12}O$ [M+Na$^+$] 207.0786, found 207.0780.

6,7-dibromo-1,4-epoxide-1,4-dihydronaphthalene[29] (2.28). nBuLi (33.0 mmol in 200 mL of dry hexanes) was added dropwise over 2 h to a stirred solution of 1,2,4,5-tetrabromobenzene (10.0 g, 25.4 mmol) and furan (9.1 mL, 127.0 mmol) in dry toluene (200 mL) at -75 ^{0}C, under argon. Then reaction mixture was allowed to warm to room temperature slowly. After stirring the reaction mixture at room temperature for another 1 h, it was quenched with MeOH (10 mL). The reaction mixture was washed with water, dried and concentrated to give a solid. Purification of the residue by chromatography (polarity of mobile phase was increased from 50% dichloromethane in hexanes to 30% ethylacetate in hexanes) gave 5.8 g (76 %) of **2.28** as a white solid. M.p.

116-117 ^{0}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 2H), 7.01 (s, 2H), 5.67 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 150.44, 142.94, 125.73, 120.91, 82.05; HRMS calcd. for $C_{10}H_6Br_2O$ [M+H$^+$] 302.8844, found 302.8837.

2,3-dibromonaphthalene[31] (2.29). Chlorotrimethylsilane (8.2 mL, 64.6 mmol) was added at once to slurry of 6,7-dibromo-1,4-epoxide-1,4-dihydronaphthalene, **2.28** (6.5 g, 21.5 mmol) and NaI (9.7 g, 64.6 mmol) in MeCN (100 mL). In the course of 2 min, the temperature spontaneously rose to 50 ^{0}C. After 25 min, the reaction mixture was poured into a saturated solution of $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with saturated solution of $Na_2S_2O_3$ and brine, dried and concentrated. The residue was purified by chromatography (50 % CH_2Cl_2 in hexanes) to give 5.35 g (87 %) of the compound **2.29** as a white solid. M.p. 137-139 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ8.15 (s, 2H), 7.73 (m, 2H, *J* = 3.67, 5.51 Hz), 7.51 (m, 2H, *J* = 3.67, 5.51 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.27, 132.47, 127.42, 127.09, 122.18; HRMS calcd. for $C_{10}H_6Br_2$ [M$^+$] 285.8816, found 285.8825.

5-(3-bromonaphthalen-2-yl)pent-4-yn-1-ol (2.30). A thoroughly degassed solution of 2,3-dibromonaphthalene (5.0 g, 17.5 mmol), pent-4-yn-1-ol (1.9 g, 22.8 mmol), $Pd(PPh_3)_2Cl_2$ (1.22 g, 1.75 mmol), triphenyl phosphine (0.46 g, 1.75 mmol), CuI (0.33 g, 1.75 mmol) in 200 mL of Et_2NH was stirred at 70 ^{0}C for 20 h. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by silica gel chromatography (hexanes:ethyl acetate 1:1 mixture) gave 3.94 g (78 %) of **2.30** as a yellow oil. ^{1}H NMR (500

MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.95 (s, 1H), 7.74 - 6.85 (m, 2H), 7.49 – 7.46 (m, 2H), 3.91 (t, 2H, *J*= 5.82 Hz), 2.65 (t, 2H, *J*= 5.82 Hz), 1.93 (quintet , 2H, *J*= 5.82 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 133.58, 133.09, 131.99, 131.05, 127.65, 127.49, 127.01, 123.23, 122.35, 94.26, 80.38, 62.02, 31.94, 16.47; HRMS calcd. for $C_{15}H_{13}BrO$ [M^+] 288.0150, found 288.0150.

5-(3-bromonaphthalen-2-yl)pent-4-ynyl acetate (**2.31**). Acetic anhydride (8.6 g, 84 mmol) was added to a stirred solution of alcohol **2.30** (6.0 g, 21.0 mmol), Et_3N (40 mL), and DMAP (0.5 g, 4.2 mmol) in CH_2Cl_2 (80 mL) and the resulting solution was stirred for 2 h at 0 °C. Solvents were removed in vacuum and the residue was purified by chromatography (hexanes : ethyl acetate 4:1) to give 7.1 g (79 %) of **2.31** as a slightly yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.94 (s, 1H), 7.74 – 7.69 (m, 2H), 7.49 – 7.46 (m, 2H), 4.31 (t, 2H, *J*= 6.41 Hz), 2.63 (t, 2H, *J*= 6.41 Hz), 2.08 (s, 3H), 2.01 (quintet , 2H, *J*= 6.41 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.35, 133.60, 133.10, 131.97, 131.07, 127.66, 127.51, 127.01, 123.17, 122.36, 93.51, 80.49, 63.44, 27.91, 21.23, 16.67; HRMS calcd. for $C_{17}H_{15}BrO_2$ [M^+] 330.0255, Found 330.0255.

3-(2-(3-bromonaphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propyl acetate (**2.33**). Trimethyl-2,2-difluoro-2-(fluorosufonyl) acetate (6.75 g, 27.0 mmol) was added dropwise over a period of 1 h to a solution of **2.31** (3.0 g, 9.0 mmol) and sodium fluoride (0.04 g, 0.9 mmol) in 4 mL of diglyme at 120 ^{0}C, and stirred for 30 min. The reaction mixture was allowed to cool to r.t., passed through a wet silica gel column (hexanes, containing 3 % water), and left overnight in the column. The crude cyclopropenone **2.32** was eluted with

hexanes: ethyl acetate 1:1 mixture and used in the next step without further purification.

A solution of cyclopropenone **2.32** (3.0 g, 6.5 mmol) and triethyloxonium fluoroborate (4.8 g, 25.0 mmol) in dry CH_2Cl_2 (25 mL) were stirred at r.t. for 1 h. A solution of neopentylglycol (1.7 g, 16.6 mmol) and triethylamine (2.5 g, 25.0 mmol) in CH_2Cl_2 (10 mL) was added to the reaction mixture and left stirring for 12 h. The precipitated salts were removed by filtration, solvent removed in vacuum, and the residue was purified by chromatography (hexanes: ethyl acetate 4:1, containing 1.5 % Et_3N) to give 2.1 g (52 % over two steps) of **2.33** as a thick yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.092 (s, 1H), 7.85 (m, 1H, *J*= 7.01, 10.5 Hz), 7.73 (m, 1H, *J*= 7.01, 10.5 Hz) 7.52 – 7.49 (m, 2H, *J*= 7.01, 10.5 Hz), 4.21 (t, 2H, *J*= 5.95 Hz), 3.83-3.74 (m, 4H) 3.01 (t, 2H, *J*= 5.95 Hz), 2.11 (quintet , 2H, *J*= 5.95 Hz), 2.06 (s, 3H), 1.29 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.29, 134.58, 132.13, 132.07, 131.39, 128.55, 128.03, 127.07, 127.00, 125.59, 124.74, 119.65, 83.42, 78.44, 63.99, 30.62, 27.79, 23.31, 22.84, 22.41, 21.22; HRMS calcd. for $C_{23}H_{25}BrO_4$ [M^+] 444.0936, Found 444.0929.

3-(6,6-dimethyl-2-(3-((trimethylsilyl)ethynyl)naphthalen-2-yl)-4,8-dioxa spiro[2.5]oct-1-en-1-yl)propyl acetate (**2.34**). $Pd(PPh_3)_4$ (0.5 g, 0.45 mmol) was added to a solution of **2.33** (2.0 g, 4.5 mmol) and 1-(tributylstannyl)-2-(trimethylsilyl)acetylene (3.5 g, 9.0 mmol) in 100 mL of dry toluene and stirred at for 2 h at 90 ^{0}C. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent

was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 4:1, containing 1.5% Et_3N) gave 1.64 g (79 %) of **2.34** as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.02 (s, 1H), 7.83 (m, 1H, *J*= 7.01, 10.5 Hz), 7.75 (m, 1H, *J*= 7.01, 10.5 Hz) 7.50 – 7.47 (m, 2H, *J*= 7.01, 10.5 Hz), 4.21 (t, 2H, *J*= 5.95 Hz), 3.83-3.74 (m, 4H) 3.01 (t, 2H, *J*= 5.95 Hz), 2.09 (quintet , 2H, *J*= 5.95 Hz), 2.04 (s, 3H), 1.23 (s, 3H), 1.04 (s, 3H), 0.28 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.15, 135.06, 133.13, 132.88, 130.63, 130.30, 128.43, 127.65, 127.61, 127.53, 126.24, 124.92, 120.14, 105.58, 97.63, 83.94, 78.51, 64.07, 30.59, 27.68, 23.58, 22.81, 22.54, 21.12, 0.22; HRMS calcd. for $C_{28}H_{34}O_4Si$ [M^+] 462.2226; found 462.2221.

3-(2-(3-ethynylnaphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propan-1-ol (**2.35**). K_2CO_3 (1.4 g, 9.75 mmol) was added to a solution of **2.34** (3.0 g, 6.5 mmol) in MeOH : CH_2Cl_2 mixture (120 mL, 2:1 v/v) and stirred vigorously for 1 h at r.t. 25 mL of water were added to the reaction mixture, organic layer was separated, and aqueous layer was extracted with ether (3 x 100 mL). Combined organic layers were washed with sodium bicarbonate solution, dried over $MgSO_4$, and the solvents were removed in vacuum. Chromatographic purification of the residue (hexanes : ethyl acetate 1:1, containing 1.5% Et_3N) gave 1.83 g (81 %) of **2.35** as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.05 (s, 1H), 7.84 (m, 1H, *J*= 7.01, 10.5 Hz), 7.77 (m, 1H, *J*= 7.01, 10.5 Hz) 7.52 – 7.50 (m, 2H, *J*= 7.01, 10.5 Hz), 3.93-3.77 (t, 2H, *J*= 5.88 Hz and m, 4H), 3.37 (s, 1H), 3.01 (t, 2H, *J*= 5.88 Hz), 2.03 (quintet , 2H, *J*= 5.88 Hz), 1.23 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.62,

133.03, 130.98, 130.59, 128.44, 127.70, 127.68, 126.92, 124.70, 119.11, 83.86, 80.77, 78.55, 62.43, 31.42, 30.63, 23.36, 22.75, 22.51; HRMS calcd. for $C_{23}H_{24}O_3$ [M^+] 348.1725; found 348.1718.

3-(2-(3-(iodoethynyl)naphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5] oct-1-en-1-yl)propan-1-ol (**2.36**). A solution of iodine (1.9 g, 7.4 mmol) and morpholine (1.0 mL, 11.1 mmol) in dry benzene (40 mL) was stirred for 45 min at 45°C. A solution of **2.35** (1.3 g, 3.7 mmol) in dry benzene (40 mL) was added to the reaction mixture, and stirred for 30 min at 45°C. The reaction mixture was allowed to cooled to r.t., diluted with benzene (50 mL), washed with saturated solution of $Na_2S_2O_3$ (10 mL), water (15 mL), and brine (10 mL), dried (MgSO4), and solvents were removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 1:1, containing 1.5% of Et_3N) to give 1.46 g (83%) of **2.36** as a slightly yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 8.03 (s, 1H), 7.83 (m, 1H, *J*= 7.21, 9.32 Hz), 7.77 (m, 1H, *J*= 7.21, 9.32 Hz) 7.51 – 7.49 (m, 2H, *J*= 7.21, 9.32 Hz), 3.86-3.78 (t, 2H, *J*= 5.64 Hz and m, 4H), 2.97 (t, 2H, *J*= 5.64 Hz), 2.04 (quintet , 2H, *J*= 5.64 Hz), 1.23 (s, 3H), 1.06 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 135.26, 132.97, 131.26, 130.60, 128.46, 127.83, 127.77, 126.41, 124.49, 120.37, 93.93, 78.61, 62.67, 31.57, 30.67, 23.33, 22.84, 22.55; HRMS calcd. for $C_{23}H_{21}IO_3$ [M^+] 474.0692, found 474.0681.

3-(2-(3-(iodoethynyl)naphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5] oct-1-en-1-yl)propanal (**2.37**). A solution of **2.36** (1.3 g, 2.74 mmol) in CH_2Cl_2 (50 mL) was added to a suspension of Dess-Martin periodinane (2.39 g, 5.48 mmol) in CH_2Cl_2 (50 mL). The resulting suspension was stirred for 3 h, diluted

with ether (20 mL), solids were removed by filtration, and solvent was removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 3:1, containing 1.5% of Et_3N) to give 0.95 g (74 %) of the aldehyde **2.37** as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 9.93 (S, 1H), 8.05 (s, 1H), 8.03 (s, 1H), 7.83 (m, 1H, *J*= 7.25, 9.41 Hz), 7.75 (m, 1H, *J*= 7.25, 9.41 Hz) 7.52 – 7.49 (m, 2H, *J*= 7.25, 9.41 Hz), 3.84-3.75 (m, 4H), 3.19 (t, 2H, *J*= 6.54 Hz), 2.91 (t, 2H, *J*= 6.54 Hz), 1.25 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 201.00, 135.24, 132.99, 132.95, 130.73, 130.13, 128.51, 127.89, 127.78, 126.08, 124.95, 120.30, 93.90, 83.62, 78.52, 42.60, 30.66, 22.86, 22.45, 19.67; HRMS calcd. for $C_{23}H_{21}IO_3$ [M^+] 472.0535, found 472.0522.

Naphthalene-fused 9-memebred ring cyclopropenone acetal (**2.38**). A solution of aldehyde **2.37** (0.9 g, 1.9 mmol) in THF (30 mL) was added via a syringe pump over a period of 15 min to a degassed suspension of $CrCl_2$ (0.7 g, 5.7 mmol) and $NiCl_2$ (0.12 g, 0.95 mmol) in THF (750 mL) under argon at 0 ^0C. After 3 h stirring at room temperature, the reaction mixture was concentrated and solids were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 1:1, containing 1.5% of Et_3N) gave 0.40 g (60 %) of **2.38** as a thick yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.87 (m, 1H, *J*= 4.41, 7.25 Hz), 7.83 (s, 1H),7.78 (m, 1H, *J*= 4.41, 7.25 Hz), 7.54 – 7.52 (m, 2H, *J*= 4.41, 7.25 Hz), 4.08 (m, 1H), 3.86 (s, 4H), 3.31 (m, 1H, *J*= 8.42, 12.21 Hz), 2.84 (m, 1H, *J*= 8.42, 12.21 Hz), 2.38 (m, 1H, *J*= 8.42, 12.21 Hz), 2.17 (m, 1H, *J*= 8.42, 12.21 Hz), 1.27 (s, 3H), 1.08 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.21,

133.11, 131.03, 130.36, 130.38, 128.87, 128.68, 128.08, 127.76, 127.48, 126.98, 124.99, 120.16, 98.68, 87.00, 82.79, 78.70, 63.15, 38.11, 30.78, 22.78, 22.78, 22.54, 21.01; HRMS calcd. for $C_{23}H_{22}O_3$ [M^+] 346.1569, found 346.1562.

Naphthalene-fused cyclopropenone containing 9-memebred cyclic enediyne precursor (2.25). Amberlyst® resin (0.5 g) was added to a stirred solution of cyclopropenone acetal **2.38** (0.25 g, 0.72 mmol) in distilled acetone (15 mL). The resulting solution was stirred for 1 h at room temperature and the resin was removed by filtration. Solvents were removed in vacuum and the residue was purified by chromatography (CH_2Cl_2 : MeOH 4:1) to give 0.14 g (74 %) of **2.25** as a yellow powdered solid, (Decomposed upon heating above 100^{o}C without melting). ^{1}H NMR (400 MHz, DMSO-D6) δ 8.52 (s, 1H), 8.17 (d, 1H, *J*= 7.22 Hz), 8.11 (s, 1H), 8.01 (d, 1H, *J*= 8.25 Hz), 7.72 – 7.63 (m, 2H, *J*= 7.22, 8.25 Hz), 4.69 (m, 1H), 3.21 (m, 1H, *J*= 8.57, 14.28 Hz), 2.74 (m, 1H, *J*= 8.57, 11.41 Hz), 2.21 (m, 1H, *J*= 6.34, 14.28 Hz), 1.92 (m, 1H, *J*= 6.34, 11.41 Hz); ^{13}C-NMR (400 MHz, DMSO-D6) δ 202.20, 158.44, 135.55, 134.65, 132.71, 130.20, 129.97, 129.51, 128.72, 128.66, 123.49, 119.15, 101.95, 84.26, 61.73, 36.75, 21.54; FTIR (cm^{-1}) 3186 [O-H], 1834 [C=O], 1599, 1573, 1556 [C=C]; HRMS calcd. for $C_{18}H_{12}O_2$ [M^+] 260.0837, found 260.0831.

5-(3-((trimethylsilyl)ethynyl)naphthalen-2-yl)pent-4-yn-1-ol(**2.39**).

$Pd(PPh_3)_4$ (1.6 g, 1.38 mmol) was added to a solution of **2.30** (4.0 g, 13.8 mmol) and 1-(tributylstannyl)-2-(trimethylsilyl)acetylene (8.0 g, 20.7 mmol) in 150 mL of dry toluene and stirred at for 3 h at 90 ^{0}C. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica

gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 3:1) gave 3.5 g (82 %) of **2.39** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.89 (s, 1H), 7.73-7.70 (m, 2H, *J*= 7.21, 9.85 Hz) 7.46 – 7.44 (m, 2H, *J*= 7.21, 9.85 Hz), 3.88 (t, 2H, *J*= 5.72 Hz), 2.64 (t, 2H, *J*= 5.72 Hz), 1.92 (quintet , 2H, *J*= 5.72 Hz), 0.28 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ132.73, 132.65, 132.19, 131.73, 127.75, 127.59, 127.39, 127.08, 123.066, 122.54, 104.19, 97.79, 93.28, 80.17, 62.11, 31.59, 16.51, 0.25; HRMS calcd. for $C_{20}H_{22}OSi$ [M^+] 306.1440; found 306.1433.

5-(3-ethynylnaphthalen-2-yl)pent-4-yn-1-ol (**2.40**). K_2CO_3 (0.6 g, 4.41 mmol) was added to a solution of **2.39** (0.9 g, 2.94 mmol) in MeOH : CH_2Cl_2 mixture (60 mL, 2:1 v/v) and stirred vigorously for 1 h at r.t. 25 mL of water were added to the reaction mixture, organic layer was separated, and aqueous layer was extracted with ether (3 x 15 mL). Combined organic layers were washed with sodium bicarbonate solution, dried over $MgSO_4$, and the solvents were removed in vacuum. Chromatographic purification of the residue (hexanes : ethyl acetate 3:1,) gave 0.59 g (85 %) of **2.40** as a orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.91 (s, 1H), 7.75 – 7.72 (m, 2H, *J*= 7.21, 10.20 Hz), 7.48- 7.46 (m, 2H, *J*= 7.21, 10.20 Hz), 3.91 (t, 2H, *J*= 6.11 Hz), 3.31 (s, 1H), 2.65 (t, 2H, *J*= 6.11 Hz), 1.92 (quintet , 2H, *J*= 6.11 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.09, 132.88, 132.12, 131.82, 127.76, 127.61, 127.60, 127.21, 123.11, 121.50, 93.46, 82.86, 80.31, 80.30, 80.07, 62.08, 31.48, 16.51; HRMS calcd. for $C_{17}H_{14}O$ [M^+] 234.1045; found 234.1042.

5-(3-(iodoethynyl)naphthalen-2-yl)pent-4-yn-1-ol (**2.41**). A solution of iodine (1.3 g, 5.13 mmol) and morpholine (0.67 mL, 7.7 mmol) in dry benzene (30 mL) was stirred for 45 min at 45°C. A solution of **2.40** (0.6 g, 2.56 mmol) in dry benzene (20 mL) was added to the reaction mixture, and stirred for 30 min at 45°C. The reaction mixture was allowed to cooled to r.t., diluted with benzene (20 mL), washed with saturated solution of $Na_2S_2O_3$ (10 mL), water (15 mL), and brine (10 mL), dried (MgSO4), and solvents were removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 3:1) to give 0.74 g (80 %) of **2.41** as a slightly yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.89 (s, 1H), 7.74 – 7.72 (m, 2H, *J*= 7.21, 10.20 Hz), 7.48- 7.46 (m, 2H, *J*= 7.21, 10.20 Hz), 3.94 (t, 2H, *J*= 6.11 Hz), 2.66 (t, 2H, *J*= 6.11 Hz), 1.94 (quintet , 2H, *J*= 6.11 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.22, 132.81, 132.01, 131.56, 127.81, 127.67, 127.63, 127.23, 123.48, 122.81, 93.66, 93.53, 79.88, 62.05, 31.52, 16.42; HRMS calcd. for $C_{17}H_{13}IO$ [$M+H^+$] 361.0089, found 361.0074.

5-(3-(iodoethynyl)naphthalen-2-yl)pent-4-ynal (**2.42**). A solution of **2.41** (0.5 g, 1.4 mmol) in CH_2Cl_2 (10 mL) was added to a suspension of Dess-Martin periodinane (1.2 g, 2.8 mmol) in CH_2Cl_2 (30 mL). The resulting suspension was stirred for 2 h, diluted with ether (20 mL), solids were removed by filtration, and solvent was removed in vacuum. The residue was purified by chromatography (hexanes : ethyl acetate 3 : 1) to give 0.4 g (79 %) of the aldehyde **2.42** as a thick yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.93 (s, 1H), 7.87 (s, 1H), 7.73 – 7.71 (m, 2H, *J*= 7.21, 9.89 Hz), 7.47- 7.45 (m, 2H, *J*= 7.21, 9.89 Hz), 2.84

(s, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 200.76, 133.16, 132.75, 132.09, 131.64, 127.83, 127.72, 127.67, 127.34, 123.14, 122.81, 93.38, 92.08, 80.22, 42.97, 13.24; HRMS calcd. for $C_{17}H_{11}IO$ [M^+] 357.9855, found 357.9846.

Naphthalene-fused 9-membered enediyne (2.26). A solution of aldehyde **2.42** (0.15 g, 0.42 mmol) in THF (10 mL) was added via a syringe pump over a period of 15 min to a degassed suspension of $CrCl_2$ (0.15 g, 1.25 mmol) and $NiCl_2$ (0.03 g, 0.21 mmol) in THF (400 mL) under argon at r.t.. After 3 h stirring at room temperature, the reaction mixture was concentrated and solids were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 2: 1) gave 0.052 g (54 %) of **2.26** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.78 (s, 1H), 7.77 – 7.75 (m, 2H, *J*= 6.24, 9.69 Hz), 7.49- 7.45 (m, 2H, *J*= 6.24, 9.69 Hz), 4.88 (m, 1H), 3.01- 2.94 (m, 1H, *J*= 5.51, 12.9 Hz), 2.66- 2.59 (m, 1H, *J*= 5.51, 14.2 Hz), 2.39- 2.33 (m, 2H, *J*= 5.51, 12.9, 14.2 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 133.95, 132.84, 129.65, 129.33, 99.35, 62.76, 36.75, 21.15; HRMS calcd. for $C_{17}H_{12}O$ [$M+H^+$] 233.0966, found 233.0962.

Naphthalene-fused 9-membered enyne-allene of cyclopropenone acetal (2.46). Methane-sulfonyl cholride (27 µL, 0.35 mmol) was added to a solution of alcohol **2.38** (0.1 g, 0.29 mmol) and triethylamine (49 µL, 0.84 mmol) in CH_2Cl_2 (5 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and quenched with biphosphate buffer (1 mL, pH = 8). Organic layer was washed with biphosphate buffer (2x3 mL, pH = 8), separated, and dried over sodium

sulfate. Solvent was evaporated to give 0.099 g (81 %) of crude mesylate **2.45** as yellowish oil.

A solution of EtMgBr (0.87 mL, 1M in THF) was added an ice-cold suspension of CuCN (0.052 g, 0.58 mmol) and LiCl (0.049 g, 1.16 mmol) in dry THF (4 mL). Reaction mixture was stirred for 25 min, cooled down to -78 °C, and a solution of the crude mesylate **2.45** in dry THF (3 mL) was added dropwise. After 3 hours at - 78 °C, the reaction was quenched by addition of biphosphate buffer (3 mL, pH = 8). The reaction mixture was extracted with ether (3x15 mL), washed with brine (1x20 mL), dried over sodium sulfate. The solvent was removed in a vacuum. Purification of the residue by chromatography (hexanes : ethyl acetate 4 : 1) gave 0.066 g (64 %) of acetal of enyne-allene **2.46** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.83 – 7.775 (m, 2H, *J*= 6.314, 13.25 Hz) 7.75 (s, 1H), , 7.47- 7.43 (m, 2H, *J*= 6.314, 13.25 Hz), 5.50-5.49 (m, 1H, allenic hydrogen), 3.85- 3.79 (m, 4H), 3.07- 2.99 (m, 2H), 2.71- 2.66 (m, 1H), 2.41- 2.34 (m, 2H), 2.18- 2.08 (m, 1H), 1.28 (s, 3H), 1.13 (t, 3H, *J*= 7.24 Hz)1.28 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 204.99, 135.59, 133.95, 132.24, 132.03, 128.43, 128.22, 127.75, 127.32, 126.83, 126.37, 126.15, 123.65, 107.22, 91.58, 78.56, 78.40, 30.85, 26.15, 22.94, 22.88, 22.72, 22.53, 20.89, 12.73; HRMS calcd. for $C_{25}H_{26}O_2$ [M^+] 358.1933, found 358.1924.

Naphthalene-fused cyclopropenone containing 9-memebred cyclic enyne-allene precursor (2.43). Amberlyst® resin (0.05 g) was added to a stirred solution of acetal **2.46** (0.025 g, 0.07 mmol) in distilled acetone (10 mL). The resulting solution was stirred for 30 min at room temperature and the resin was

removed by filtration. Solvents were removed in vacuum and the residue was purified by chromatography (polarity of the mobile phase was increased from 50% ethyl acetate in hexanes to 100% ethyl acetate) to give 0.015 g (76 %) of **2.43** as a thick yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.90 (d, 1H, *J*= 7.79 Hz) 7.82 (s, 1H), 7.81 (d, 1H, *J*= 6.49 Hz), 7.59 (t, 1H, *J*= 6.49, 7.79 Hz), 7.52 (t, 1H, *J*= 6.49, 7.79 Hz), 5.84 (m, 1H, allenic hydrogen), 3.24 (m, 1H), 2.85 (m, 1H), 2.74 (m, 1H), 2.51- 2.39 (m, 2H), 2.11 (m, 1H), 1.17 (t, 3H, *J*= 7.34 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 206.03, 158.01, 152.09, 138.29, 135.37, 134.27, 131.95, 129.36, 129.14, 127.94, 127.20, 126.50, 120.48, 107.52, 91.41, 25.82, 22.76, 18.62, 12.86; HRMS calcd. for $C_{20}H_{16}O$ [$M+H^+$] 273.1279; found 234.1270.

2.7. References

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CHAPTER 3

PHOTOCHEMISTRY AND KINETICS OF NINE MEMBERED RING CYCLIC ENEDIYNES AND ENYNE-ALLENE

3.1. Photochemistry and cycloaromatization of cyclopropenone containing enediynes and enyne-allenes

The photochemical triggering of enediyne cycloaromatization is a very attractive idea as it allows for the spatial and temporal control of the Bergman cyclization.[1] The direct irradiation of acyclic[2] and cyclic[3] enediynes, as well as the natural enediyne antibiotic Dynemicin-A,[4] demonstrate that the Bergman cyclization[5] can be triggered photochemically. The efficiency of the photochemical Bergman cyclization can be substantially improved by adjusting the electronic properties of substituents[6,7] and/or using different modes of excitation energy transfer, for example MLCT.[8,9] In addition, several caged enediynes have been prepared, which undergo conventional chemical activation after the photochemical uncaging step.[10]

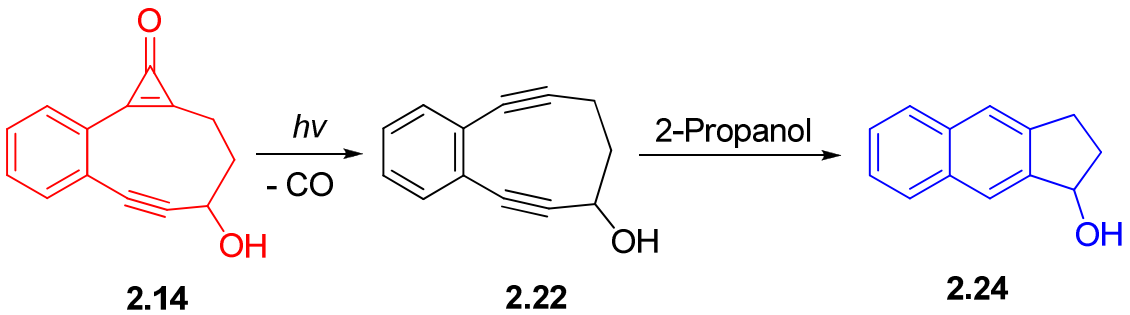
To enhance the rate of Bergman cyclization[5] (for photoswitchable enediynes) and Myers-Saito cyclization[11] (for enyne-allenes), we decided to design the nine membered ring, cyclopropenone-containing enediyne and enyne-allene precursors. Highly strained nine membered enediynes and enyne-allenes are predicted to undergo very facile cycloaromatization under ambient conditions.[12,13] The π-system of the cyclopropenone moiety in the enediyne/

enyne-allene precursors is orthogonal to the plane of the ring. Since these cyclopropenone compounds (**2.14**, **2.25** and **2.43**) lack the crucial in-plane overlap of π-orbitals that is required for cycloaromatization, they cannot undergo cycloaromatization. Thus, irradiation of cyclopropenone **2.14**, **2.25** and **2.43** results in the loss of carbon monoxide and regeneration of the triple bond, makes the substrate susceptible to the Bergman or Myers–Saito cyclizations.

3.2. Photochemical generation of benzannulated nine membered ring enediyne

The UV spectrum of cyclopropenone **2.14** showed two close lying absorbance bands at 231 nm (log ε = 4.5) and 238 nm (log ε = 4.6), red line (in Figure 3.1). Photolysis of **2.14** in 2-propanol with 254 nm light resulted in efficient decarbonylation (Φ_{254nm} = 0.34 ± 0.03) and the formation of the 4,5-benzocyclonona-2,6-diynol (**2.22**, Scheme 3.1). The latter underwent spontaneous Bergman cyclization which was surprisingly clean for a radical reaction, producing quantitative yields of the ultimate product, benz[f]indan-1-ol (**2.24**).

Scheme 3.1



2.14 **2.22** **2.24**

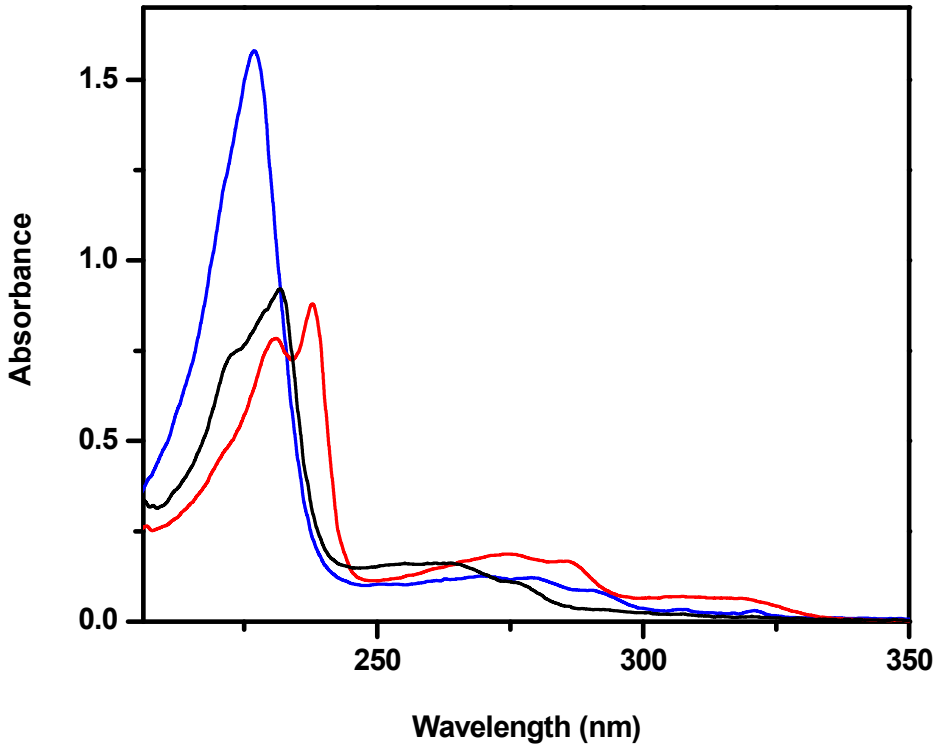


Figure 3.1. UV-spectra of ca. 2 × 10^{-5} M solutions of the cyclopropenone **2.14** (red) and enediyne **2.22** (black) and Bergman cyclized product **2.24** (blue) in 2-propanol.

3.3. Kinetics of the Bergman cyclization of enediyne 2.22

3.3.1 The study of temperature dependent kinetics of the Bergman cyclization of 4,5-Benzocyclonona-2,6-diynol (enediyne 2.22)

The accurate rate measurements of cycloaromatization of the enediyne **2.22** were conducted by UV spectroscopy following the growth of the characteristic 227 nm band of benz[f]indan-1-ol (**2.24**, Figure 3.2). The graph represented in Figure 3.2 fits to a single exponential equation (the reaction followed pseudo first-order equation well). The data evaluated from this

exponential function revealed that the rate of the cycloaromatization at 25 ± 0.1 °C was k = (0.009 ± 0.0001) min^{-1}. Thus the enediyne **2.22** underwent spontaneous but relatively slow Bergman cyclization reaction with a lifetime of ca. 2 h at 25 °C in 2-propanol.

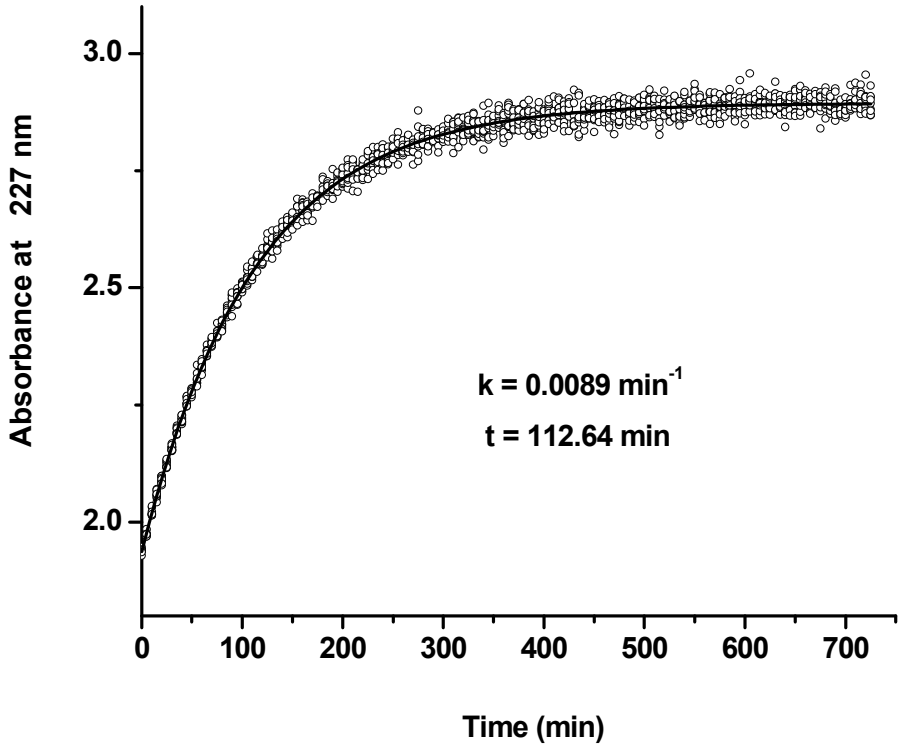


Figure 3.2. Kinetic traces observed at 227 nm, following growth of benz[f]indan-1-ol (**2.24**) at 25.0 ^{0}C in 2-propanol (ca. 10^{-5} M).

The rate of Bergman cyclization of photochemically generated enediyne **2.22** (4,5- Benzocyclonona-2,6-diynol) was measured in degassed solutions of 2-propanol, in the temperature range from 15 to 55 °C with 0.1 °C accuracy. The data obtained is displayed as the temperature rate profile in Figure **3.3**. The temperature dependence of the cyclization rate was surprisingly weak: every 10

K of temperature rise produced only about 2-fold rate increase (Figure 3.3). This observation suggests that the energy barrier ($\Delta G^{\ddagger}$) for the cyclization reaction grows with the temperature. In fact, least-squares fitting of the data to the Eyring equation gave the following activation parameters: $\Delta H^{\ddagger}$ =13.63 ± 0.22 kcal M^{-1} and $\Delta S^{\ddagger}$ = -30.66 ± 0.61 cal M^{-1} K^{-1}.

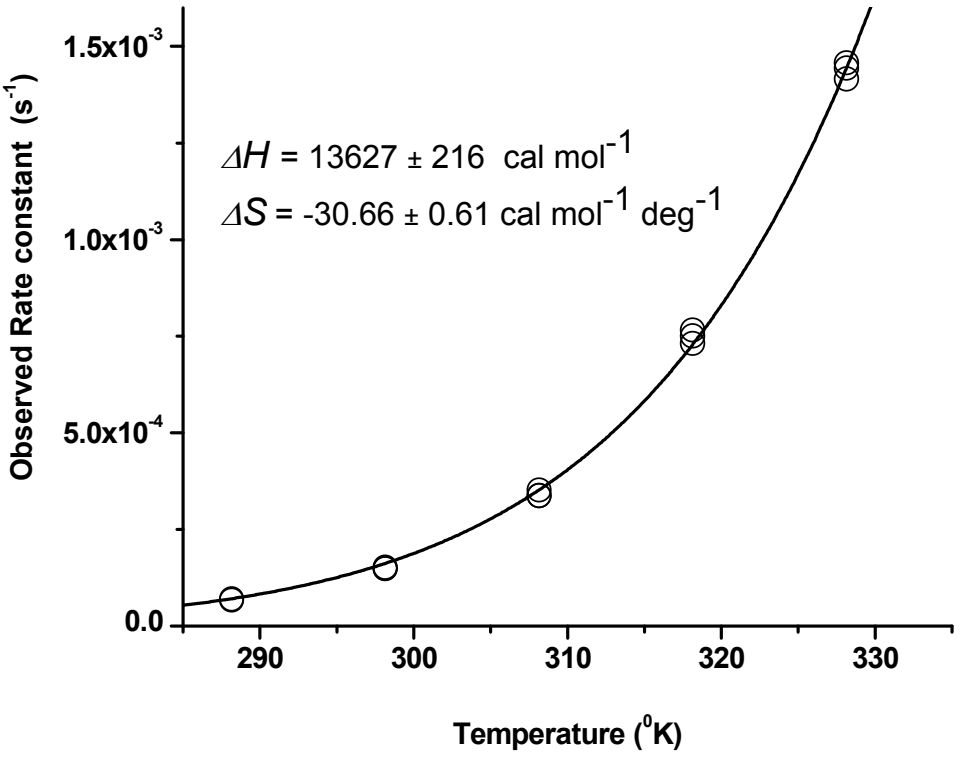
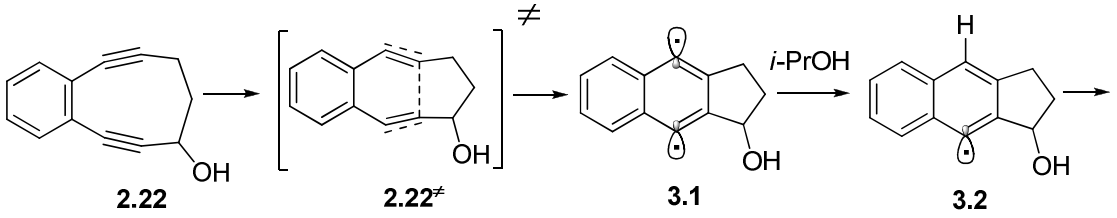


Figure 3.3. Temperature rate profile for the Bergman cyclization of the enediyne **2.22** generated in photolysis of **2.14** in 2-propanol. The line shown was drawn using parameters obtained by least-squares fitting of Eyring equation.

The substantial negative entropy of activation indicates that degrees of freedom in the system are significantly reduced in the transition state for the rate-determining step. The activation energy for the cyclization of the enediyne **2.22** to diradical **3.1** (Scheme 3.2) should have a low entropy component and is not

consistent with the experimental value of $\Delta S^{\ddagger}$. The next step in the process, i.e., hydrogen abstraction, is a bimolecular reaction requiring proper orientation of the diradical **3.1** and a hydrogen donor. Such reactions are usually characterized by pronounced negative entropy of activation. In other words, the temperature dependence on the rate of Bergman cyclization of enediyne **2.22** suggests that hydrogen abstraction rather than formation of **3.1** is the rate limiting step of the reaction.

Scheme 3.2



3.3.2 The study of kinetic isotope effect on the rate of Bergman cyclization of 4,5-benzocyclonona-2,6-diynol

In order to support for the rate-limiting hydrogen abstraction step, the solvent isotope effect on the rate of cyclization was investigated. When enediyne **2.22** was generated in 2-propanol-d_8 at 25 °C, the observed rate of its conversion to **2.24** was substantially lower than in 2-propanol (k_D = (2.669 ± 0.013) × 10^{-5} s^{-1} vs. k_H = (1.497 ± 0.019) × 10^{-4} s^{-1}). The pronounced primary kinetic isotope effect, k_H/k_D = 5.61, clearly indicates that the formation of a carbon-hydrogen bond happens during the rate-limiting step. This value, however, agrees well with both rate-limiting hydrogen transfer, as shown in Scheme 3.2, and rate-limiting proton transfer. This has been shown to induce cyclization of some enediyne

compounds.[14,15] The use of 2-propanol-d_1 (*i*-PrOD) as a hydrogen donor provides a good method for a discrimination of these two pathways. Carbon centered radicals are expected to abstract protium from 2-propanol-d_1 by cleaving the weakest C-H bond of a methine group, while the proton (deuteron) transfer should involve a more acidic OH (OD) group. The rate of the cycloaromatization of **2.22** in 2-propanol-d_1, k_{OBS} = (1.383 ± 0.022) × 10^{-4} s^{-1}, was essentially the same as in an all-protium solvent. This observation clearly showed that the hydrogen, but not proton, transfer was involved in the rate-limiting step of the Bergman cyclization of enediyne **2.22**.

3.3.3 The study of acid catalysis on the rate of Bergman cyclization of 4,5-benzocyclonona-2,6-diynol

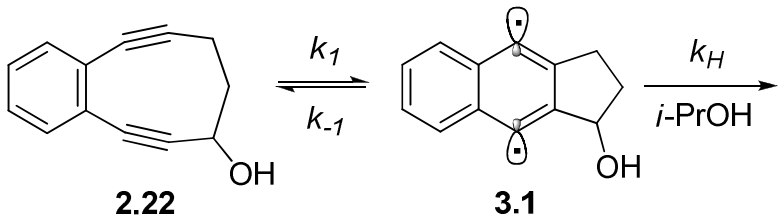
Proton transfer-initiated reactions are also expected to be catalyzed by acid, while conventional Bergman cyclization shows no acid catalysis.[16] Enediyne **2.22** (4,5-Benzocyclonona-2,6-diynol) generated in the presence of 0.025 M hydrochloric acid, or in absence of the acid in 2-propanol, water mixture (4:1) at 25 °C undergoes conversion to **2.24** with the same rate (k_{OBS} = (1.03 ± 0.024) × 10^{-4} vs (9.87 ±0.82) × 10^{-5} s^{-1}, respectively). This experiment clearly evidenced that photochemically generated enediyne **2.22** undergoes Bergman cyclo-aromatization under mild conditions (i.e. at 25 ^{0}C with absence of any acid catalysis).

3.3.4 The study of dependence of Bergman cyclization rate on hydrogen donor concentration

The rate of the Bergman cyclization of enediyne compounds often depends on the concentration and the nature of hydrogen donor.[17,18] This effect is especially prominent in case of benzannulated enediynes.[19,20] We have measured the rate of the cyclization of 4,5-benzocyclonona-2,6-diynol (**2.22**) in 2-propanol-water mixtures of various compositions.

2-Propanol is a good hydrogen donor with an R-C-H bond dissociation energy of 89 kcal mol^{-1}.[21] This value is similar to the C-H bond strength in tetrahydrofuran (92 kcal mol^{-1}),[22] a model compound for DNA deoxyribose. Water, on the other hand, is found to be a poor hydrogen donor ($D_{0(H\text{-}OH)}$ = 118 kcal mol^{-1}).[23] The rate of the cyclization of the enediyne **2.22** to benz[f]indan-1-ol (**2.24**) in 2-propanol-water mixtures was linearly proportional to the concentration of alcohol up to neat 2-propanol solutions (Figure 3.4). This observation indicates that equilibration between enediyne **2.22** and *p*-benzyne **3.1** (Scheme 3.3) is much faster than hydrogen abstraction even at the highest concentration of 2-propanol.

Scheme 3.3



2.22 **3.1**

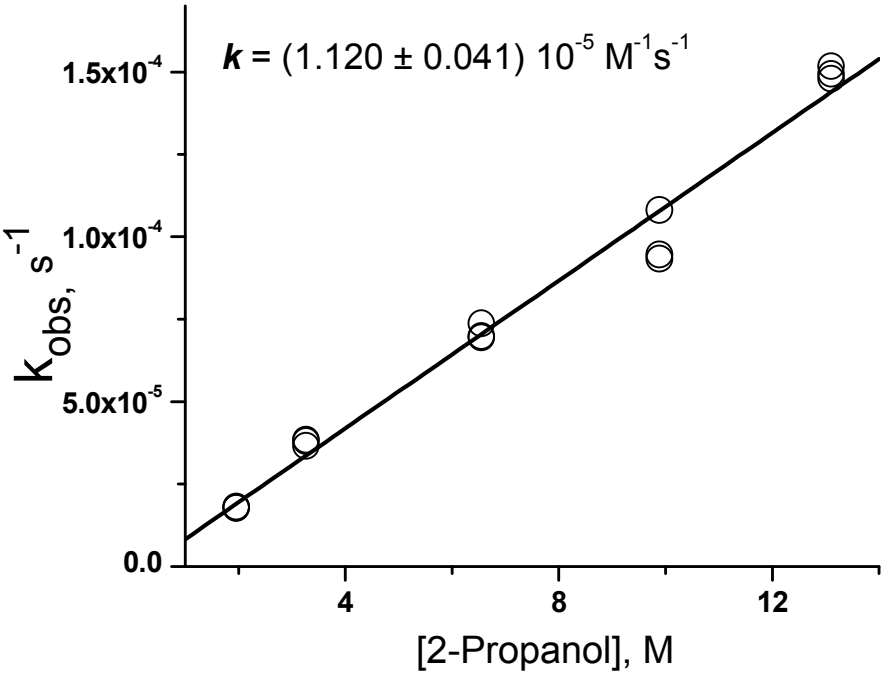


Figure 3.4. Observed rates of the Bergman cyclization of the enediyne **2.22** at various concentrations of 2-propanol in water at 25.0 ± 0.1^{0}C.

The kinetics of the Bergman cyclization can be described by the equation (**1**), where k_1 is the rate constant for the cycloaromatization of **2.22** to **3.1**, k_{-1} representing the rate of cycloreversion, k_H is a second order rate constant for hydrogen abstraction, and [*H*] is a concentration of a hydrogen donor.

$$k_{OBS} = \frac{k_1 \times k_H \times [H]}{k_{-1} + k_H \times [H]} \qquad (\mathbf{1})$$

$$k_{OBS} = K_{eq} \times k_H \times [H] \qquad (\mathbf{2})$$

When the rate of cycloreversion is much faster than the rate of hydrogen abstraction (i.e., $k_{-1} >> k_H \; x \; [H]$), the observed rate is linearly proportional to the hydrogen donor concentration, as shown in equation (**2**), where $K_{eq} = k_1 / k_{-1}$.

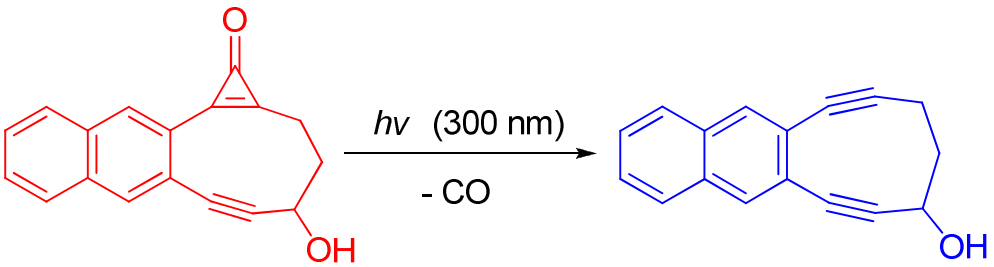
The rate of hydrogen abstraction from methanol by 1,4-dehydronaphthaline is 4×10^5 M^{-1} s^{-1}.[24] Since 2-propanol is ~ 2.7 times more reactive than methanol as a hydrogen donor in radical reactions,[25] we can estimate the rate of hydrogen abstraction by the diradical **3.1** from 2-propanol as 1.1×10^6 M^{-1} s^{-1}. Using this value and equation (**2**) we can evaluate the enediyne **2.22** $\leftrightarrow$ *p*-benzyne **3.1** equilibrium constant at $K_{eq} \approx 10^{-11}$. The free energy difference between **2.22** and **3.1** should be $\Delta G^o \approx 15$ kcal mol^{-1}. At high hydrogen donor concentration the hydrogen abstraction step often becomes faster than the cycloreversion (i.e., $k_H \times [H] >> k\text{-}1$) and observed rate levels off at $k_{OBS} = k_1$. Such rate saturation has been reported for the Bergman cyclization of acyclic[19,24] and cyclic[26] benzannulated enediynes in the presence of 1,4-cyclohexadiene or methanol. The rate of the cycloaromatization of **2.22** showed no signs of leveling off even in neat 2-propanol (Figure 3.4). This observation allowed us to conclude that cycloreversion reaction (k_{-1}, Scheme 3.3) was at least an order of magnitude faster than the reaction of **3.1** in neat 2-propanol. The lower limit for the cycloreversion reaction can be therefore estimated at 1.4×10^8 s^{-1} and for the cycloaromatization reaction (k_1, Scheme 3.3) at 1.4×10^{-3} s^{-1}. The upper limit for the activation barrier for the Bergman cyclization of **2.22** at 25 °C is 21-22 kcal mol^{-1}.

It is also interesting to note that no additional products were observed in the reaction of enediyne **2.22** at low 2-propanol concentration. In completely aqueous solutions UV spectrum of **2.22** remained virtually unchanged for four days at ambient temperatures. In other words, the equilibrated system of enediyne **2.22** and diradical **3.1** had a relatively long lifetime in the absence of good hydrogen donors.

3.4. Photochemical generation of naphthalene-fused nine-membered ring enediyne 2.26

The UV spectrum of cyclopropenone **2.25** has strong absorbance band at 264 nm (log ε = 5.2) and relatively weak absorbance at 300-350 nm. As result of introducing naphthalene chromophore, the photo-activated wavelength could be shifted to 300-350 nm as we expected. The UV photolysis of naphthalene-fused nine membered ring cyclopropenone (**2.25**) resulted in a rapid decarbonylation of the substrate quantitatively producing enediyne analog **2.26** (with λ_{max} = 258 nm, Figure 3.5). This photochemical reaction was also found to be quite efficient with a quantum yield at 300 nm of Φ_{300nm} = 0.50 ± 0.02 (in 2-propanol).

Scheme 3.4



2.25 **2.26**

The naphthalene-fused enediyne **2.26** was a surprisingly stable compound at room temperature, thus allowed us to isolate the photochemically produced enediyne **2.26** by HPLC. To prove the structure of enediyne **2.26**, we have synthesized it independently (in Scheme 2.15). This compound was found to be identical to the sample isolated from reaction mixtures of photolysis of **2.25**.

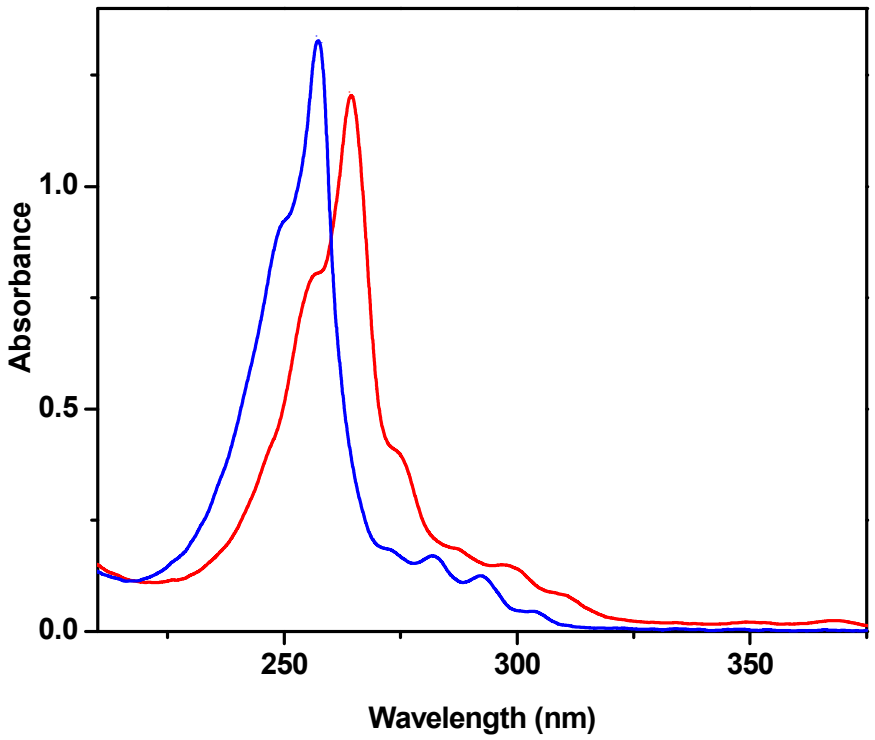


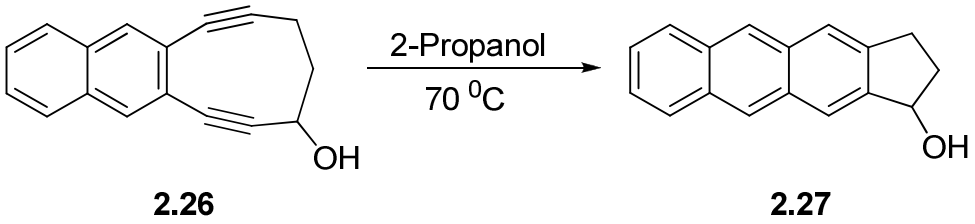
Figure 3.5. UV-spectra of 300 nm photolysis of ca. 2 × 10^{-5} M 2-propanol solutions of the cyclopropenone **2.25** (red) and enediyne **2.26** (blue).

3.5. Kinetics of the Bergman cyclization of naphthalene-fused nine membered ring enediyne 2.26

The enediyne precursor **2.25** was a thermally stable compound. No apparent changes were observed in the UV spectra of the cyclopropenone **2.25**

even after heating for 7 days at 70 ^{0}C and the substrate was quantitatively recovered. The corresponding naphthalene-fused enediyne **2.26**, on the other hand, underwent Bergman cyclization with a life time of ca. 6 h at this temperature. The progress of the reaction was followed by the increase in absorbance at 256 nm, which corresponded to cycloaromatized product **2.27**.

Scheme 3.5



2.26 **2.27**

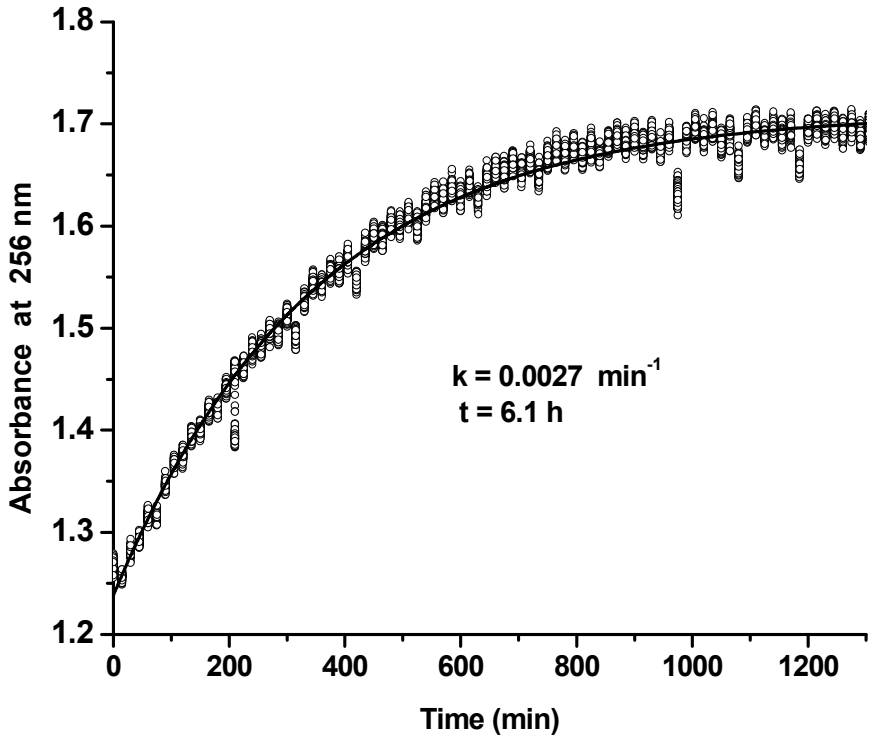


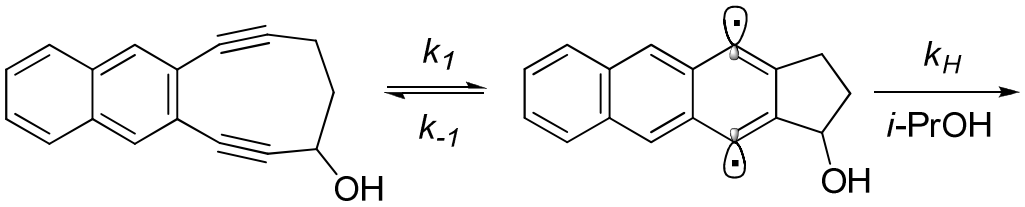
Figure 3.6. Kinetic traces obsereved at 256 nm, following growth of Bergman cyclized product **2.27** at 70.0 ^{0}C in 2-propanol (ca. 10^{-5} M).

As shown in Figure 3.6, the reaction showed pseudo first order kinetics and data fit into the single exponential function. The observed rate of the Bergman cyclization of **2.26** at 70 ^{0}C was k = (0.0027 ± 0.0002) min^{-1}.

3.5.1 Dependence of Bergman cyclization rate of naphthalene-fused enediyne 2.26 on hydrogen donor concentration

The rate of the cycloaromatization of the enediyne **2.26** to Bergman cyclized product **2.27** in 2-propanol-water mixtures was found to be linearly proportional (Figure 3.7) to the concentration of alcohol (hydrogen donor) as we observed in benzannulated enediyne **2.22** system. This observation again confirmed that equilibration between enediyne **2.26** and the corresponding *p*-benzyne **3.3** (Scheme 3.6) was much faster than its hydrogen abstraction step which should be the rate limiting step.

Scheme 3.6



2.26 **3.3**

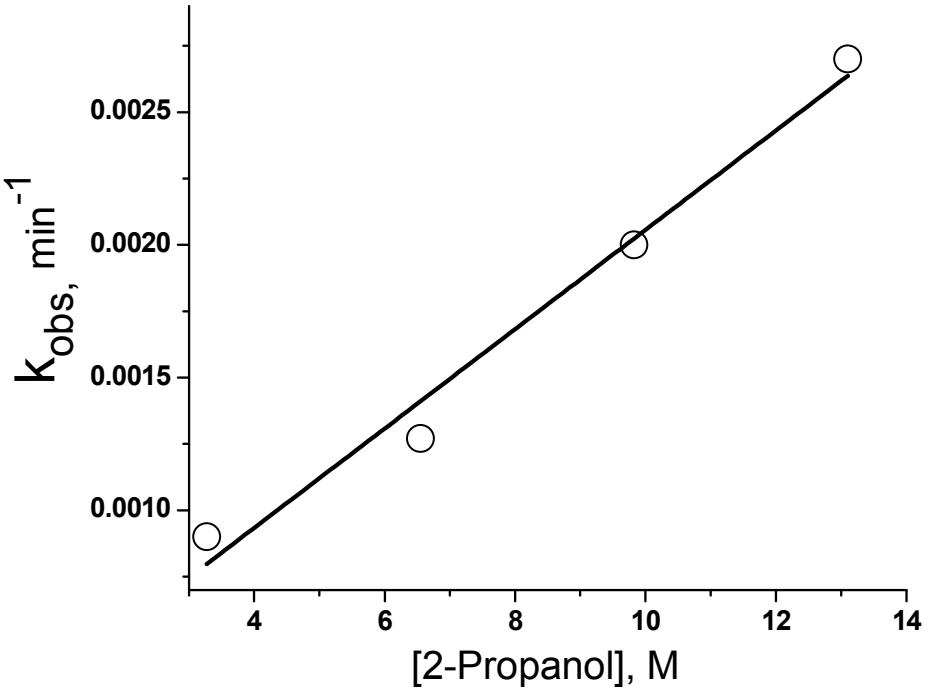


Figure 3.7. Observed rates of the Bergman cyclization of the naphthalene-fused enediyne **2.26** at various concentrations of 2-propanol in water at 70.0 ± 0.1^{0}C.

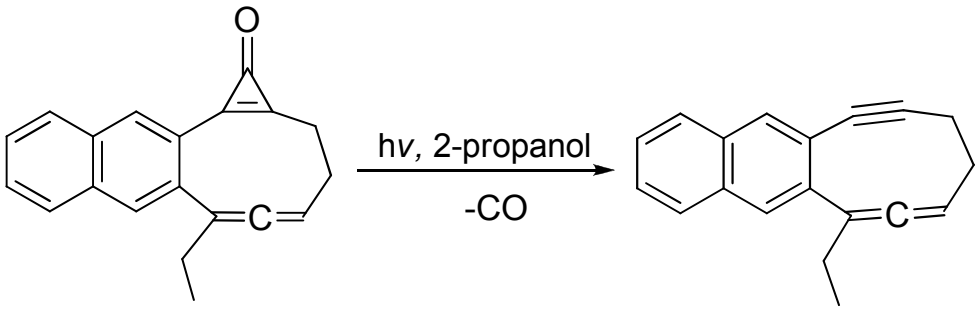
According to our experimental evidences, the naphthalene-fused enediyne **2.26** was relatively stable at room temperature, thus allowing us to separate photochemical enediyne generation and thermal Bergman cyclization steps of the processes shown on Schemes, 3.4 and 3.6. We assume that *p*-benzenoid diradical intermediate **3.3** has been relatively instabilized with compared to the intermediate **3.1** of benzannulated system. As a result, the activation energy barrier for the cycloreversion (k_{-1}) step is lower with compared to cycloaromatization step, k_1 (Scheme 3.6). The net effect is to decrease the spontaneous Bergman cyclization at room temperature. However, it undergoes

efficient cycloaromatization to give a *p*-benzene diradical at elevated temperature.

3.6. Photochemistry of cyclopropenone containing nine membered ring enyne-allene 2.43

The UV spectrum of cyclopropenone containing enyne-allene precursor (**2.43**) has strong absorbance at 266 nm (log ε = 5.2) and weaker band at 258 nm (log ε = 4.9) on the shoulder of the main band. Irradiation of cyclopropenone **2.43** with 300 or 350 nm light resulted in the efficient decarbonylation ($\Phi_{300\ nm}$ = 0.54 ± 0.03) and formation of the target 9-membered ring enyne–allene **2.44** (Scheme 3.7). The latter was quite reactive and expected to undergo facile spontaneous Myers–Saito cyclization or Schmittel cyclization.[27,28] Distinguishable isosbestic points (at 243, 276 and 310 nm, in Figure 3.8) indicated the photochemically clean, decarbonylation reaction of enyne-allene precursor **2.43**.

Scheme 3.7



2.43 **2.44**

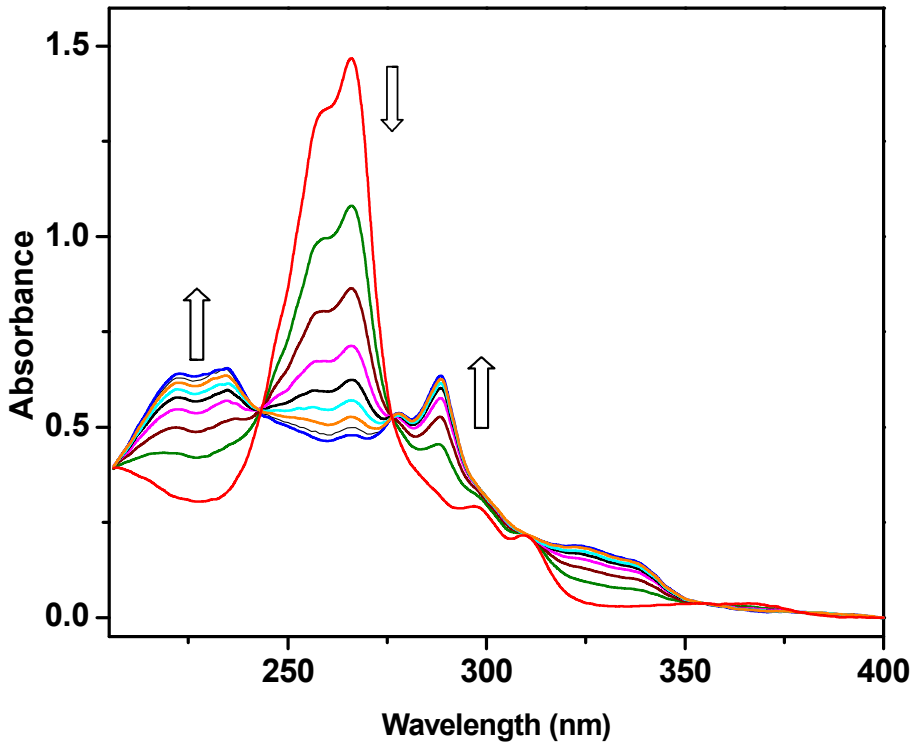


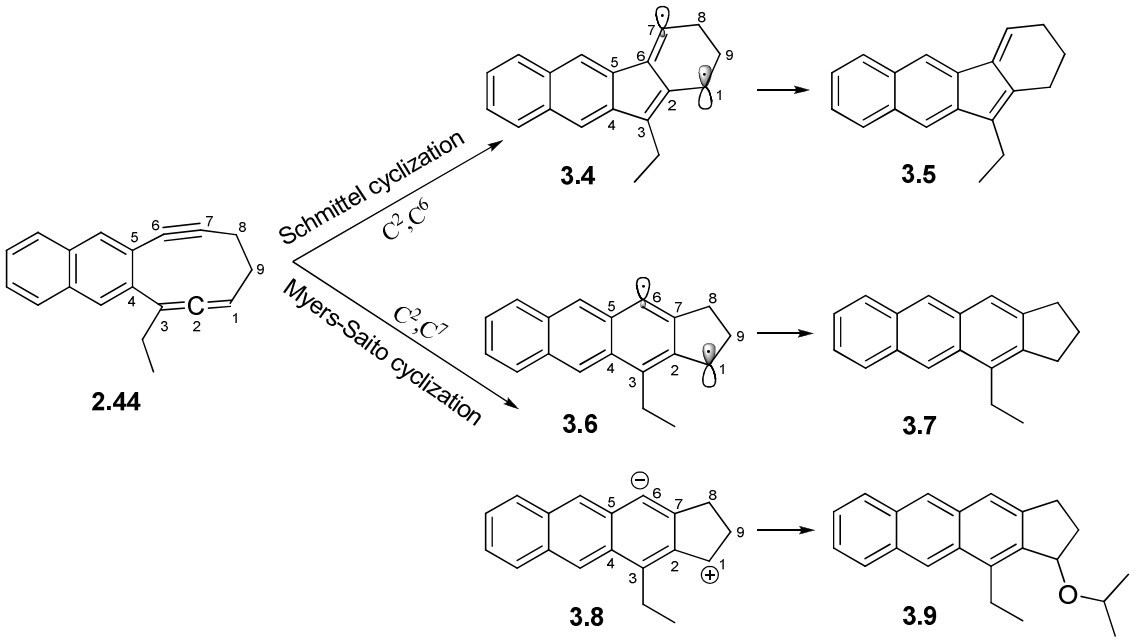
Figure 3.8. UV-spectra of 300 nm photolysis of ca. 5 × 10^{-5} M iso-propanol solutions of cyclopropenone containing enyne-allene precursor **2.43**. Spectra were recorded every 15 second intervals of irradiation.

3.7. Myers-Saito or Schimittel cyclization of naphthalene-fused enyne-allene 2.44

Acyclic enyne–allenes usually undergo spontaneous cyclization under ambient conditions,[29] while cyclic enyne–allenes are virtually unknown apparently due to their ability to undergo very rapid cycloaromatization.[14,30] It is interesting to note that the lifetime of enyne–allenes show little sensitivity to the reaction media.[31] The outcome of the cyclization reaction, on the other hand, strongly depends on the solvent[27,31] and steric effect of transition state formation step.[32,33]

Two major types of cyclization mechanisms (Scheme 3.8) have been proposed for these type enyne-allene systems; i.e. Myers–Saito cyclization (C^2-C^7) or Schimittel cyclization (C^2-C^6)[30].

Scheme 3.8



This type of a 9-membered cyclic enyne-allene **2.44** was expected to undergo the Myers–Saito cyclization to form product **3.7** *via* double hydrogen abstraction by the diradical **3.6** (Scheme 3.8). In 2-propanol, however, insertion of the solvent into the O–H bond, might produce compound **3.9.** The formation of ether **3.9** would be inconsistent with the conventional diradical mechanism of the Myers–Saito cyclization. In 2-propanol, the σ,π-diradical **3.6** was expected to

abstract hydrogen from the secondary carbon of the alcohol, since this C–H bond is much weaker than the O–H bond.[11]

Engels and Schmittel and co-workers[34] have reported that the C^2-C^7 transition state is sensitive to steric influnces around C^7 and ring strain of the system. In this case, the competition of C^2-C^6 cyclization (Schimittel cyclization) with Myers–Saito cyclization might result to form product **3.5**.

3.8. Kinetics of naphthalene-fused enyne-allene 2.44

The laser flash photolysis of the degassed solution of cyclopropenone containing enyne-allene precursor **2.43** in the presence of 2-propanol at 25°C resulted in a formation of a very reactive intermediate which rapidly underwent either Myers-Saito or Schmittel or else cyclization reaction with life time of ca. 1 μs at 25^0C in 2-propanol. The rate of the cyclization of enyne-allene **2.44** was measured in a 2-propanol solution at 25 °C. The progress of the reaction was followed by the decrease in absorbance of the intermediate **2.44** at 300 nm (Figure 3.9). Observed data fit into the first order exponential decay function. The observed rate for the cycloaromatization of cyclic enyne-allene **2.44** was, $k_{25\ ^\circ C}$ = (1.25 ± 0.006) x 10^5 s^{-1}.

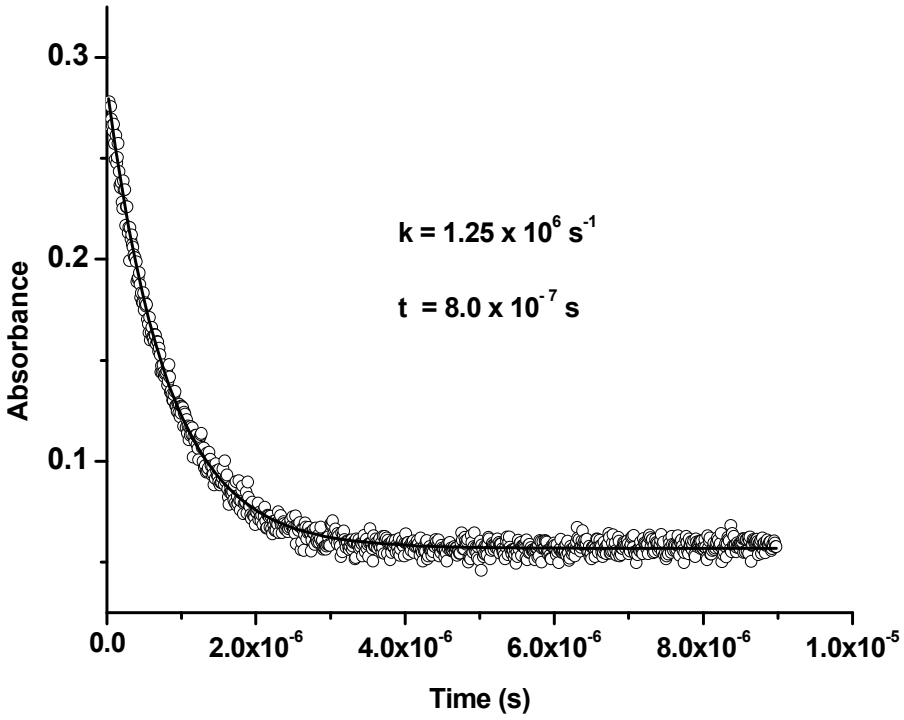


Figure 3.9. Kinetic traces observed at 300 nm, following decay of intermediate which formed by laser-flash photolysis of **2.43** at 25 ^{0}C in 2-propanol (ca. 10^{-5} M). Curve represents the calculated fit to a single exponential equation.

UV spectrum of the cyclized product generated by the laser flash photolysis (at 266 nm) experiment was superimposable with the UV spectrum of the product resulted by conventional UV-Vis experiment (Figure 3.10). This experiment proved that the product formed in laser-flash photolysis (with micro-second life time) was photochemically stable enough to avoid secondary photo-reactions during the conventional UV-Vis photolysis experiment which had relatively long irradiation period.

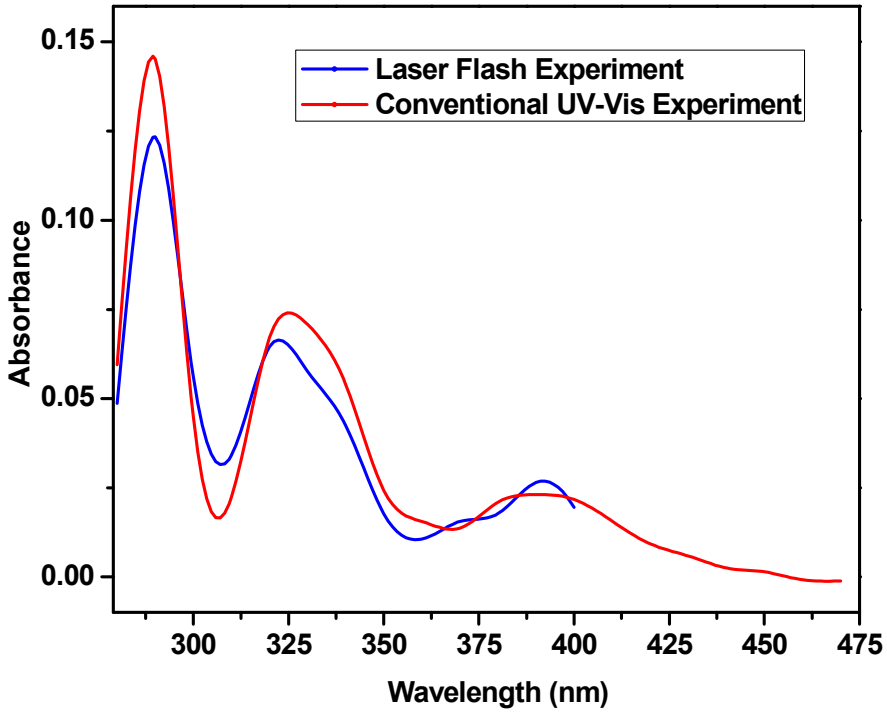


Figure 3.10. UV-spectra of the cyclized product generated from laser-flash photolysis (at 266 nm) and convention irradiation (at 300 nm) of ca. 5 × 10^{-5} M iso-propanol solutions of cyclopropenone containing enyne allene precursor **2.43.**

3.9. Conclusions

Irradiation of the thermally stable cyclopropenone precursor **2.14** produced the first known example of a benzannulated nine membered ring enediyne, 4,5-benzocyclonona-2,6-diynol (**2.22**). The enediyne **2.22** existed in a rapid equilibrium with a corresponding *p*-benzyne analogue, 4,9-didehydro-benz[f]indanol (**3.1**), even at 25 °C (K_{eq} ≈ 10^{-11}). In the presence of hydrogen donors, **3.1** underwent double hydrogen abstraction quantitatively producing benz[f]indanol (**2.24**). Kinetic data indicates that the latter process was the rate-limiting step for the Bergman cyclization of the enediyne **2.22**. While **2.22** was

rapidly consumed in a neat 2-propanol (τ = 111 min), the lifetime in aqueous solution was much longer, τ_{water} > 96 h.

In order to improve the DNA affinity of the enediyne **2.22**, we designed and synthesized naphthalene-fused enediyne, **2.26**. This structural modification also allowed us to shift the absorbance of the cyclopropenone precursor **2.25**, to 300-350 nm. UV irradiation of the thermally stable enediyne precursor **2.25** resulted in an efficient and clean photo-decarbonylation reaction, generation **2.26**, which underwent the Bergman cyclization only at an elevated temperature.

In third project, we have demonstrated that reactive cyclic enyne–allenes could be photochemically generated from thermally stable precursors with good quantum and chemical yields. The naphthalene-fused cyclopropenone containing enyne-allene precursor **2.43** showed no tendency for the formation of **2.44** in the dark. The UV irradiation of **2.43**, on the other hand, resulted in the efficient generation of the extremely reactive nine membered ring enyne-allene **2.44.** Latter underwent cyclization reaction at 25 °C with a life time of 1 μs in 2-propanol.

3.10. Experimental

3.10.1. Photolytic experiments

Analytical photolyses were performed by the irradiation of ca. 10^{-4} M solutions of cyclopropenones in a 1 cm quartz cell using a RMR-600 Rayonet™ photochemical reactor equipped with carousel and 254, 300 or 350 nm florescent

lamps. Determination of a quantum yield was performed using a ferrioxalate chemical actiniometer.[35]

3.10.2. Kinetic experiments

Rate measurements were performed using Varian Carry 300 UV-Vis spectrometer and Varian 700 laser-flash spectrometer equipped with a thermostattable cell holder. The temperature was controlled with 0.1°C accuracy. Observed first-order rate constants were calculated by least-squares fitting of the data to a single exponential function. The transmission coefficient in the Eyring equation was set to unity. The nonlinear least square fitting calculations were conducted using Origin 7.5 by OriginLab. The correlation coefficient for this fit was R^2 = 0.999. The consumption of the starting material and formation of product was followed by TLC or HPLC (Shimadzu SCL-10A VP, equipped with Hibar® RT250-4, RP-18).

3.11. References

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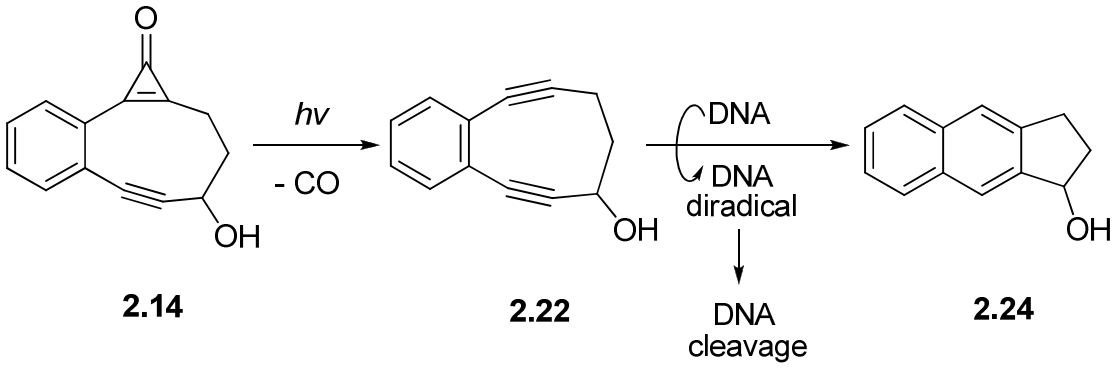
CHAPTER 4

DNA CLEAVAGE EXPERIMENTS

4.1. Introduction

The design and investigation of artificial nucleases,[1] small molecules capable of cleaving duplex or single-stranded DNA, is an exciting area of research that has numerous important biochemical and biomedical applications. While most of these agents are activated thermally, there is an increasing emphasis on photo-activated cleaving agents[2-4] since this methodology processes practical advantages. In particular, nucleases can be triggered by exposure to light. Light is an attractive cofactor since it is easy to manipulate.

Scheme 4.1



2.14 **2.22** **2.24**

In order to test the capability of synthesized model, cyclopropenone **2.14** (Scheme 4.1) to inflict damage to the DNA backbone, we performed a number of DNA cleavage assay experiments, employing different protocols.[5-7] To assess DNA scission ability of small sequence non-specific cleaving agents, their

reactivity towards covalently closed supercoiled DNA, such as φX174 RF- I (5386 base pairs in length, isolated from E. coli HF 4704) relaxes upon the event of a single strand cleavage, forming a relaxed circular structure RF- II (schematically shown on Figure 4.1). Simultaneous scission of both strands of the double-stranded DNA leads to linear DNA with drastically different topology (form RF- III).[8,9]

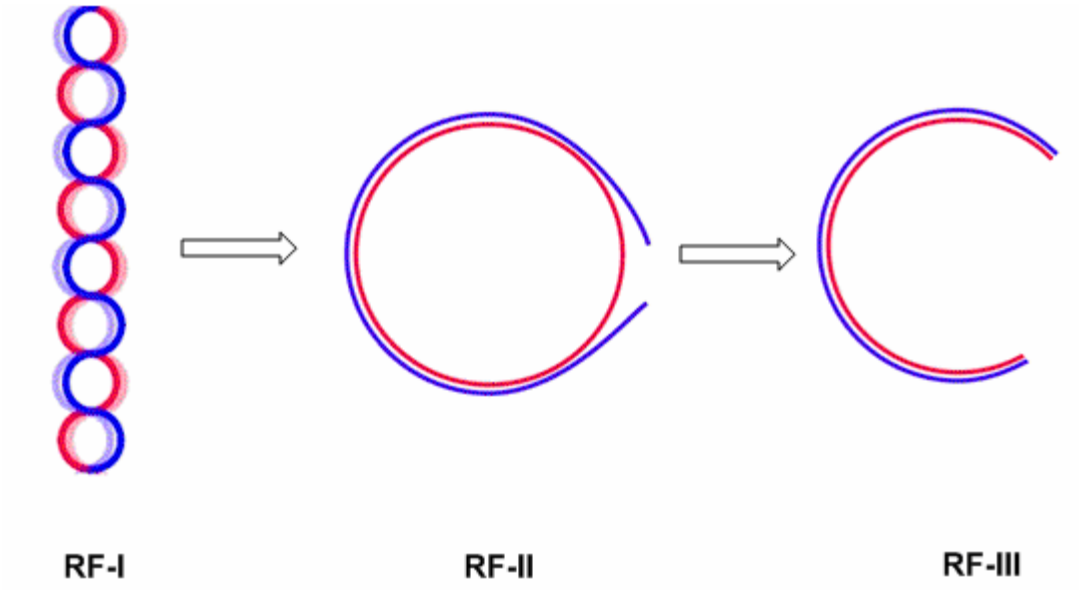


Figure 4.1. Relaxation and linearization of supercoiled (RF- I) DNA

The mixture of the products was then analyzed by agarose gel electrophoresis and the ratio of intensities of reaction products was compared to initial ratio of various DNA forms in the original DNA sample. Electrophoretic mobility of large DNA molecules depends mainly on their net charge (molecular weight) and secondary structure (cross section). Since fragmentation of the DNA chain is typically not observed, all reaction products have the same molecular

weight and their separation is achieved depending purely on topology. Although cleavage of the DNA backbone at random sites produces a multitude of products, they appear as a single band on the gel due to equal molecular weights and very similar topologies. The method also allows us to easily discriminate events of simultaneous double-stranded scission from multiple single strand cleavage events.

4.2. DNA cleavage assay

An evaluation of the photochemically generated nine-membered ring enediyne **2.22** nuclease activity was carried out using supercoiled plasmid DNA cleavage assays. Three forms of this DNA: native (RF I), circular relaxed (RFII, produced by single-strand cleavage), and linear (RF III, formed by scission of both strand in close proximity) are readily separated by the agarose gel electrophoresis. To produce reactive enediyne **2.22**, 1 and 5 mM aqueous solutions of cyclopropenone **2.14** were irradiated with low-pressure mercury lamp until ca. 90% conversion. A solution of *φ*X174 supercoiled circular DNA (10 ng/*μ*L) in TE buffer was added to photolysate and incubated for 16 h at 25 °C.

Incubation of the DNA with cyclopropenone precursor **2.14** (lanes 2 and 4, Figure 4.2) did not induce any detectable DNA cleavage. The photogenerated enediyne **2.22**, on the other hand, induced substantial single strand cleavage of *φ*X174 DNA (RF II), while linearized form (RF III) became prominent only at higher (5 mM) concentration of the cleaving agent (lanes 3 and 5, Figure 5). Integration of fluorescence of bands on the gel shown in Figure 4.2, allowed us to

evaluate the relative abundance of the native, circular, and linearized forms of φX174 DNA. Thus, incubation of the latter with the 1 mM of enediyne **2.22** produced 45% of single strand cleavage (RF II) and less than 5% of the double strand cleavage (RF III, lane 3, Figure 4.2). At 5 mM concentration of **2.22,** DNA-cleavage efficiency grew to 67% and 10%, respectively (lane 5, Figure 4.2). The relatively high concentrations of **2.22**, which are required to achieve doublestrand DNA photoscission indicate that **2.22** has low affinity to a dDNA molecule.

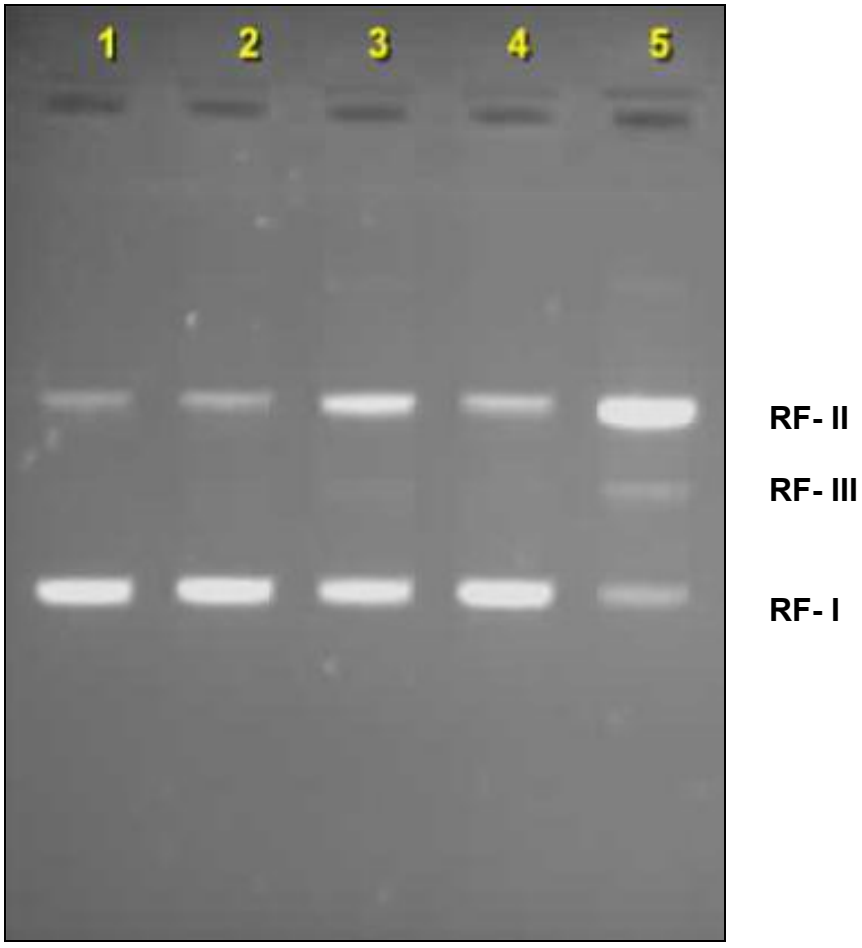


Figure 4.2. Cleavage of φX174 plasmid DNA by the photogenerated enediyne **2.22**. Lane 1: DNA alone incubated for 16 h at 25 °C; lane 2 and 4: DNA incubated in the dark with cyclopropenone precursors **2.14**, 1 and 5 mM; lane 3 and 5: DNA is incubated with the irradiated solution of **2.14**, 1 and 5 mM.

4.3. Conclusions

Enediyne **2.22** induced single and double strand cleavage of dDNA molecules, albeit with moderate efficiency. Cyclopropenone precursor **2.14**, on the other hand, did not produce DNA damage. Therefore, cyclopropenone **2.14** equipped with a DNA-binding moiety is a promising candidate for the development of an efficient in vivo photonuclease.

4.4. Experimental

4.4.1 Materials

*φ*X174 supercoiled DNA (RF- I) and standard DNA ladder marker (1- 8 kb) were purchased from New England BioLabs. Standard buffer solutions, agarose and other consumables were purchased from Fisher Scientifics. Visualizing dye, ethidium bromide was purchased from BioRad.

4.4.2 Instruments

Samples were irradiated using Rayonet photoreactor (RMR – 600) equipped with two lamps, emitting at 254 nm (5 W output power). After the irradiation, percentage conversion of **2.14** was measure by HPLC (Shimadzu SCL-10A VP, equipped with Hibar® RT250-4, RP-18). Gel electrophoresis separations were performed using BioRad Sub-Cell GT. Stained gel were visualized and photographed using Photodyne 60-2100 imaging system with UV (310 nm) transilluminator. The relative intensities of fluorescent bands on the developed gel were calculated using Alpha Ease FC software package by Alpha Innotech, Inc.

4.4.3 Experimental Procedure

In a typical experiment aqueous solutions of cyclopropenone **2.14** (10 μL) were irradiated in a Rayonet photoreactor equipped with 254 nm lamps. After the ~90% conversion was achieved (by HPLC), a solution of plasmid DNA (10 ng/ μL) in TE buffer (pH 8.0, 27 μL) was added and incubated in the dark for 16 h at 25 ^{0}C. Control samples were incubated with cyclopropenone solution in the dark. Incubated samples and the standard marker solution (1.0-8.0 kb) were mixed with a glycerol based loading buffer (7 μL) containing xylene cyanol loading dye and loaded onto a 1% agarose gel containing 0.5 μg/mL of ethidium bromide. Gel was developed at 80 V (400 mA) for 2 h and photographed on the UV transilluminator. Then relative intensities of fluorescent bands on the developed gel were calculated.

4.5. References

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APPENDICES

^{1}H and ^{13}C NMR Spectra of compounds

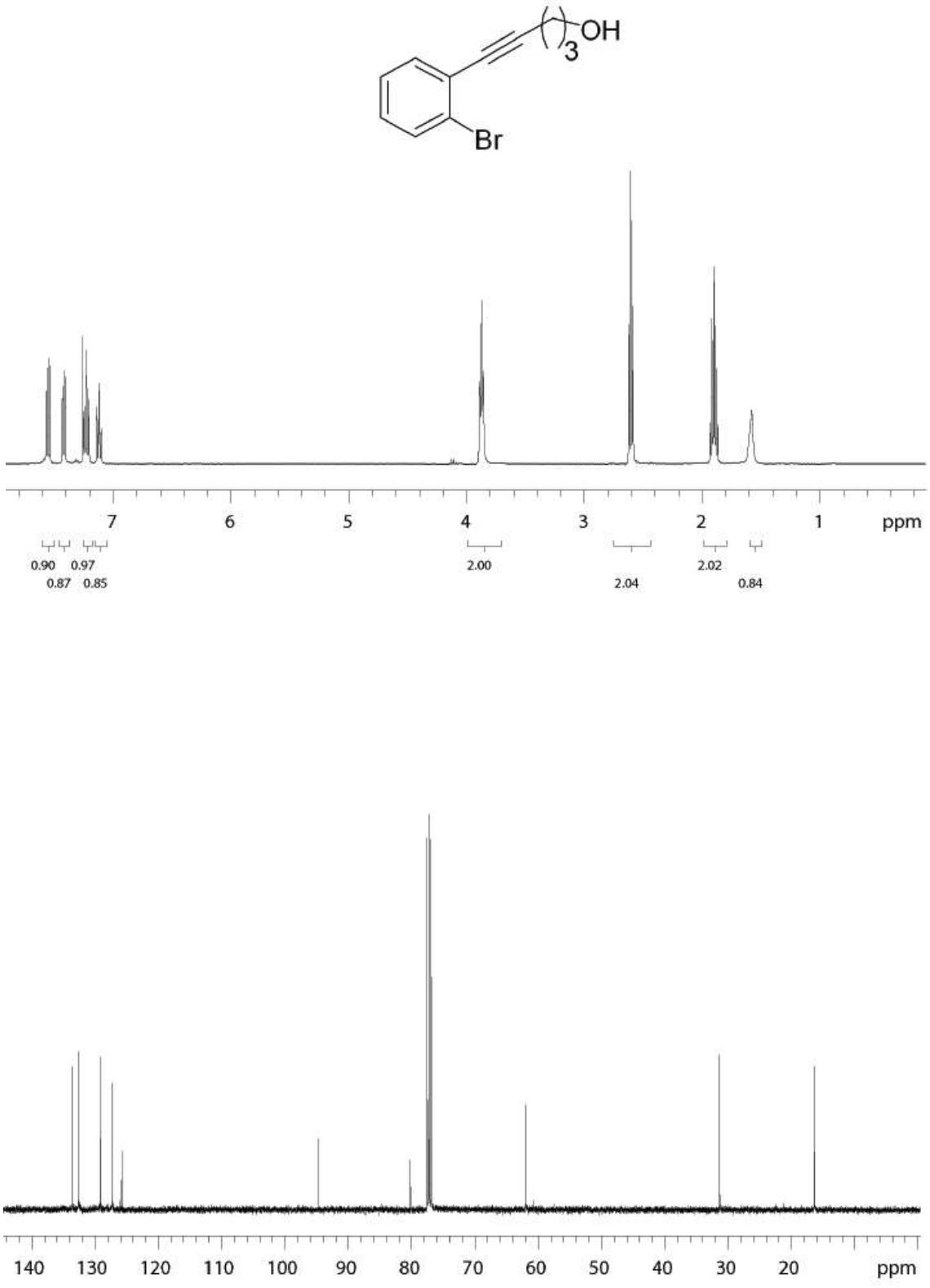

Figure A.1. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.15**

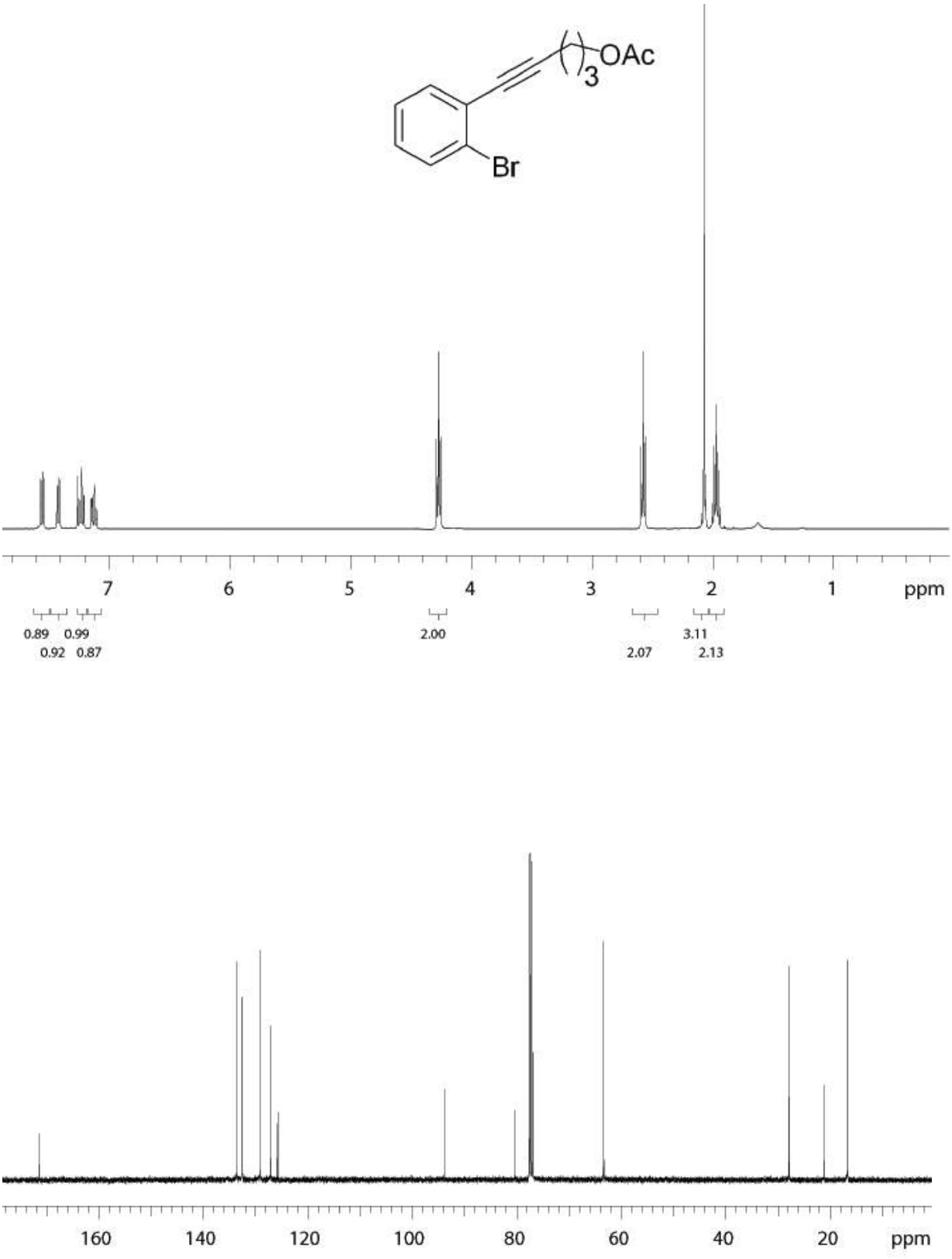

Figure A.2. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.16**

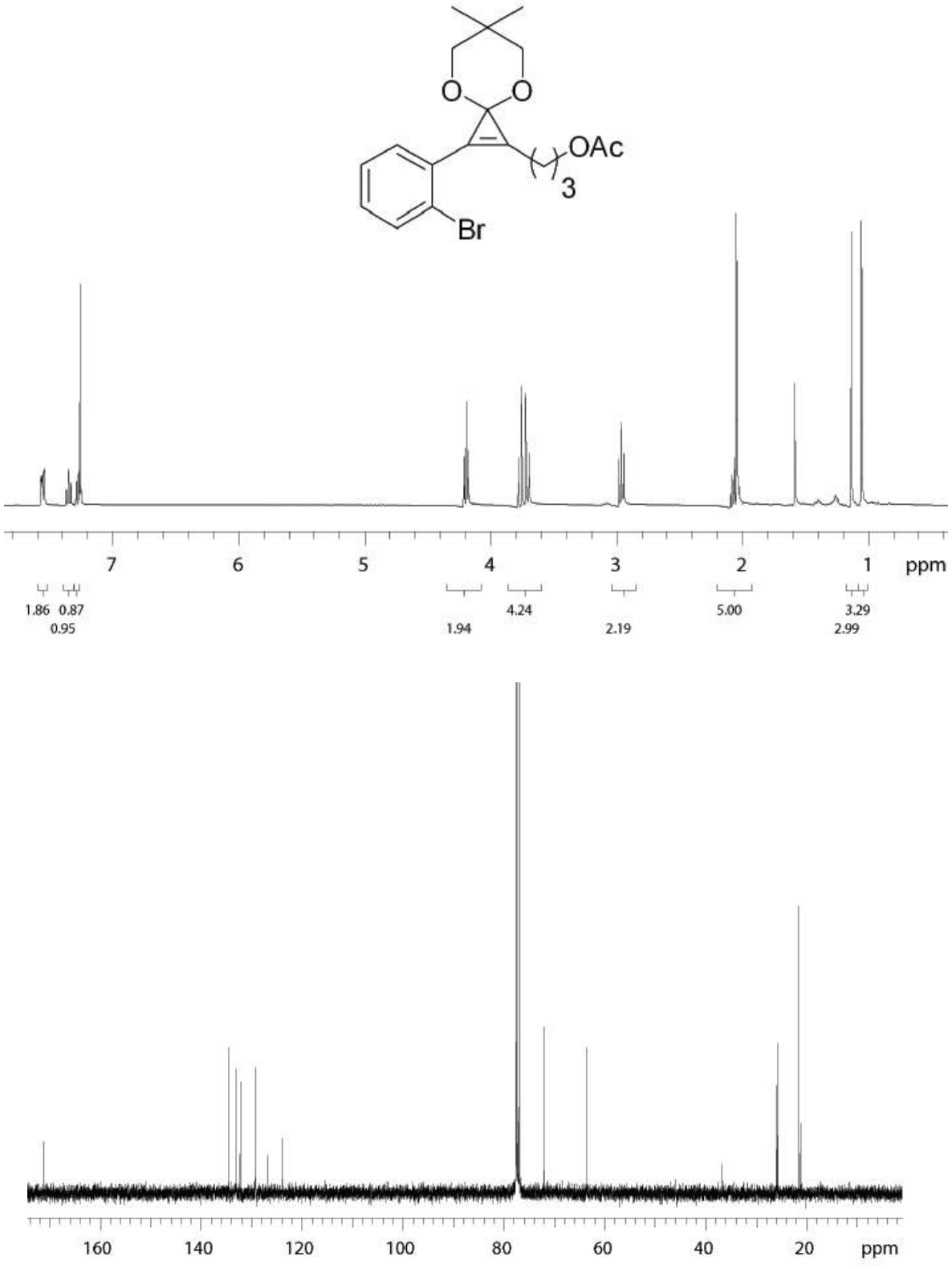

Figure A.3. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.18**

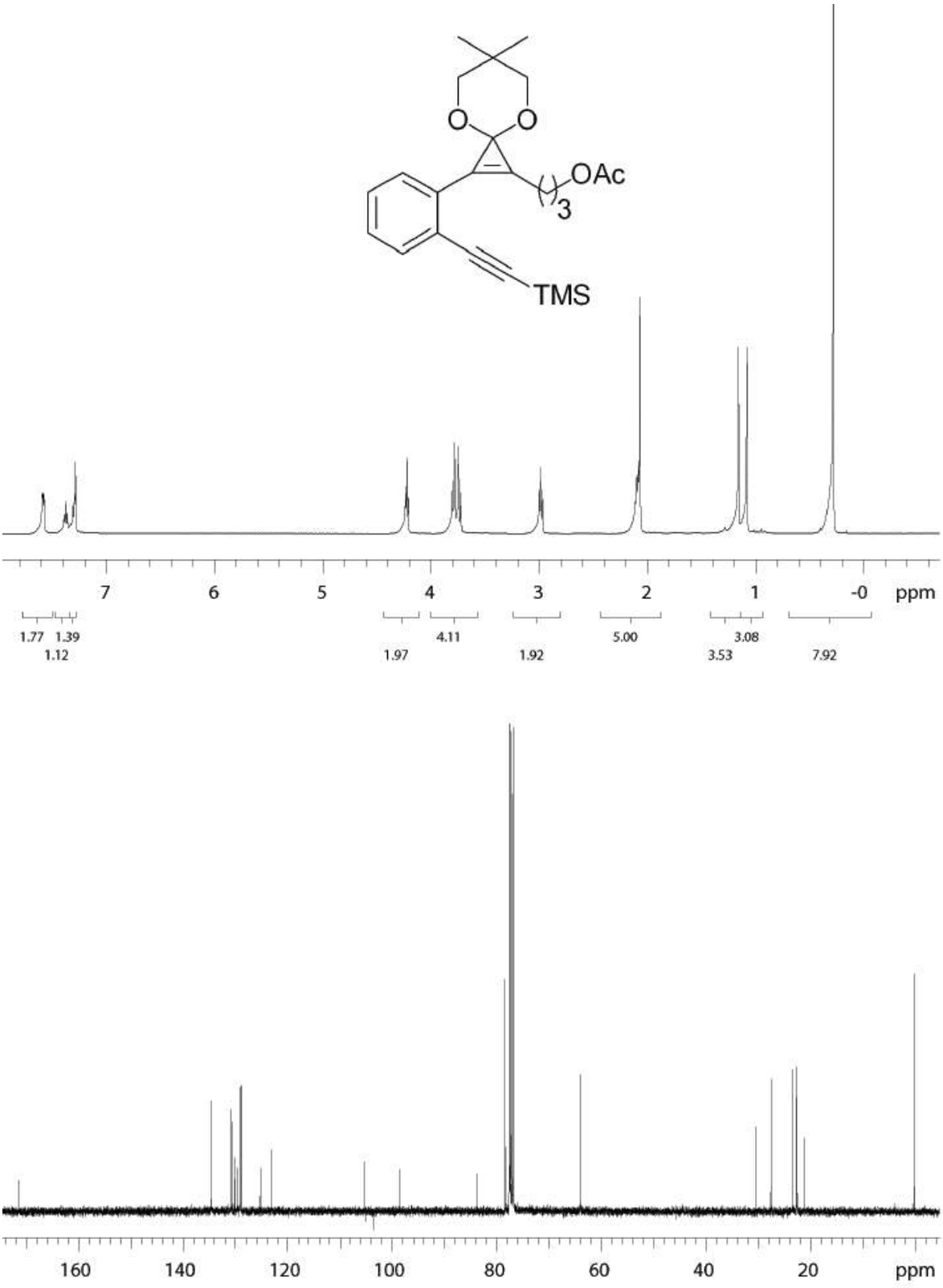

Figure A.4. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.19**

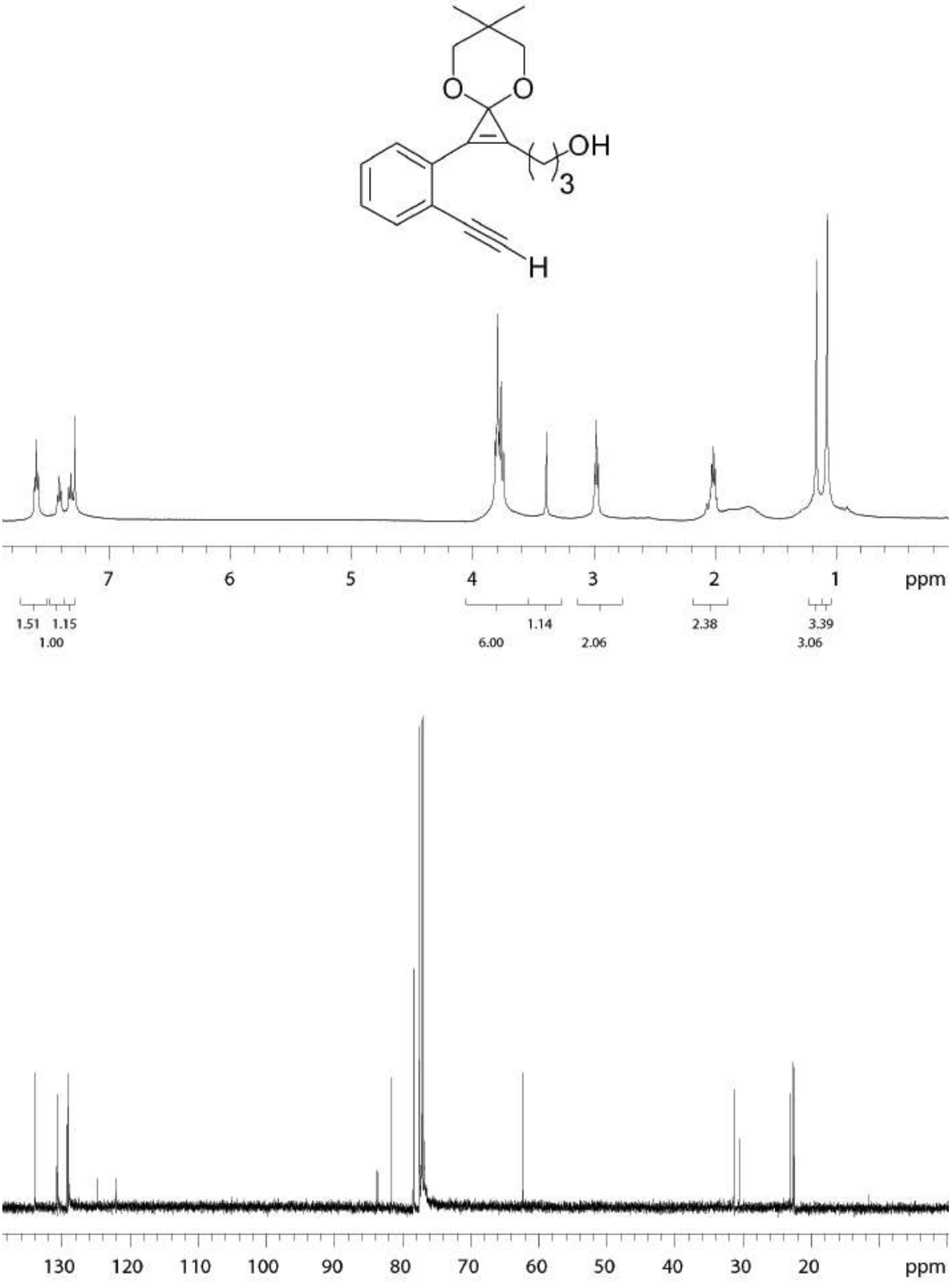

Figure A.5. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.20**

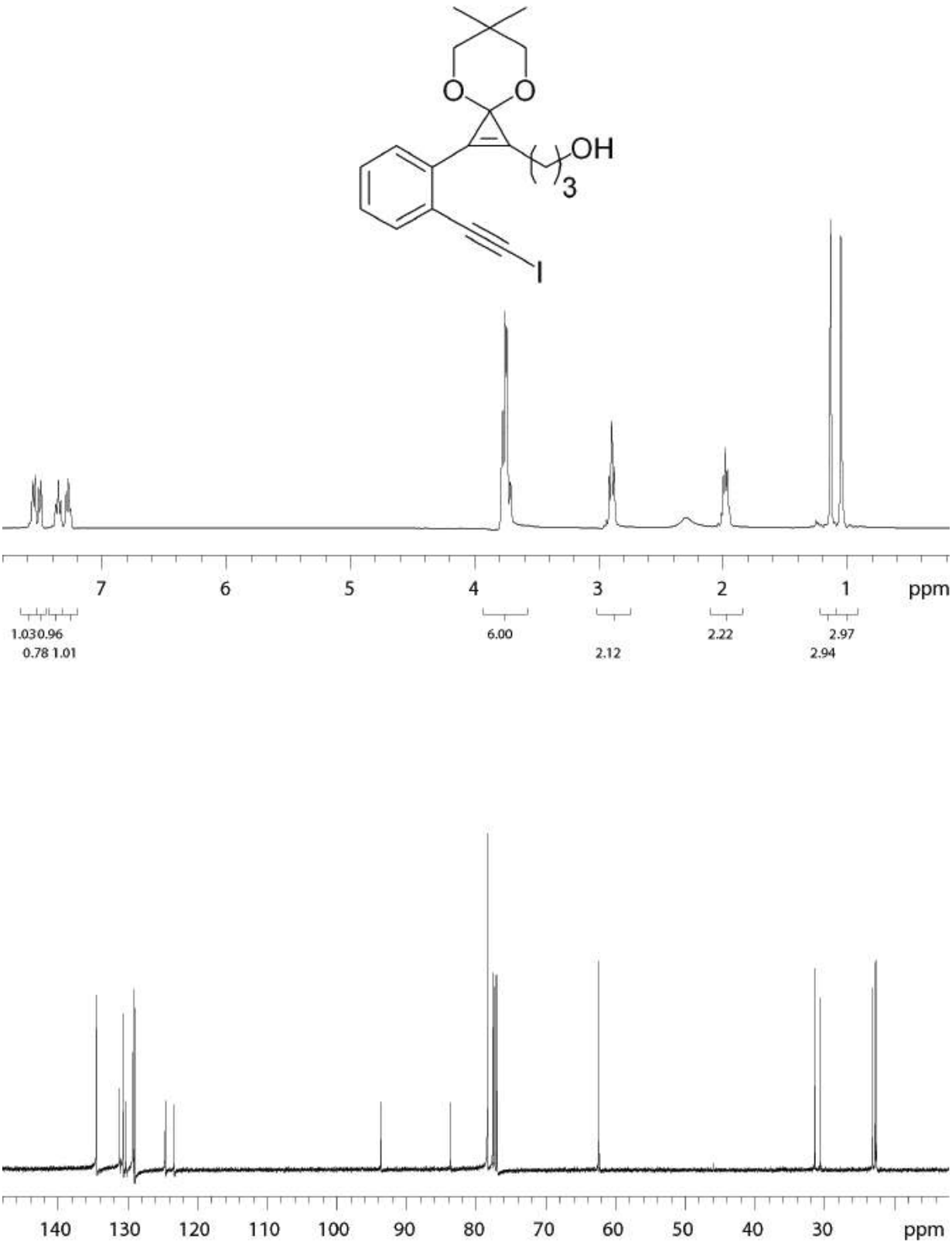

Figure A.6. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.21**

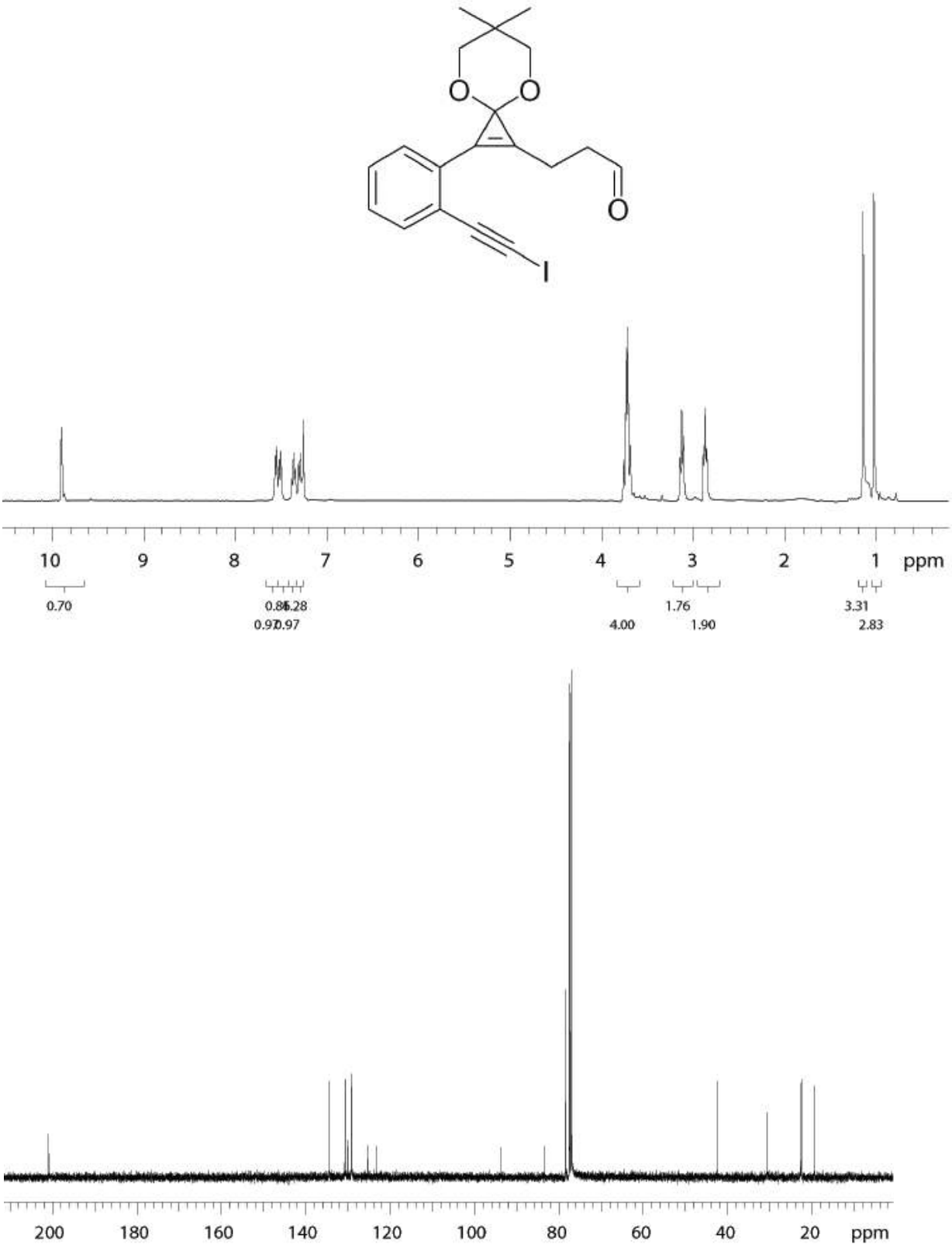

Figure A.7. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.12**

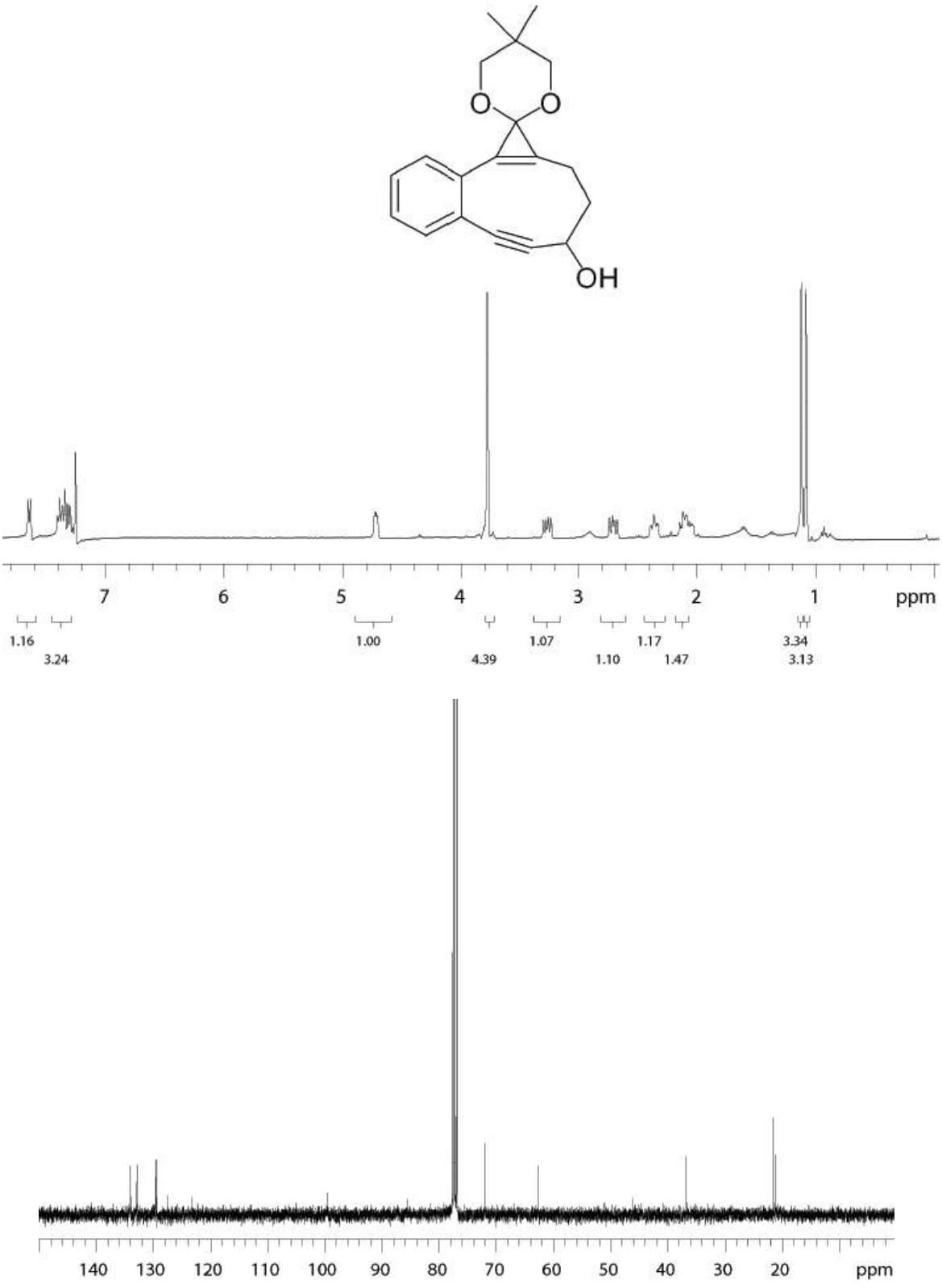

Figure A.8. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.13**

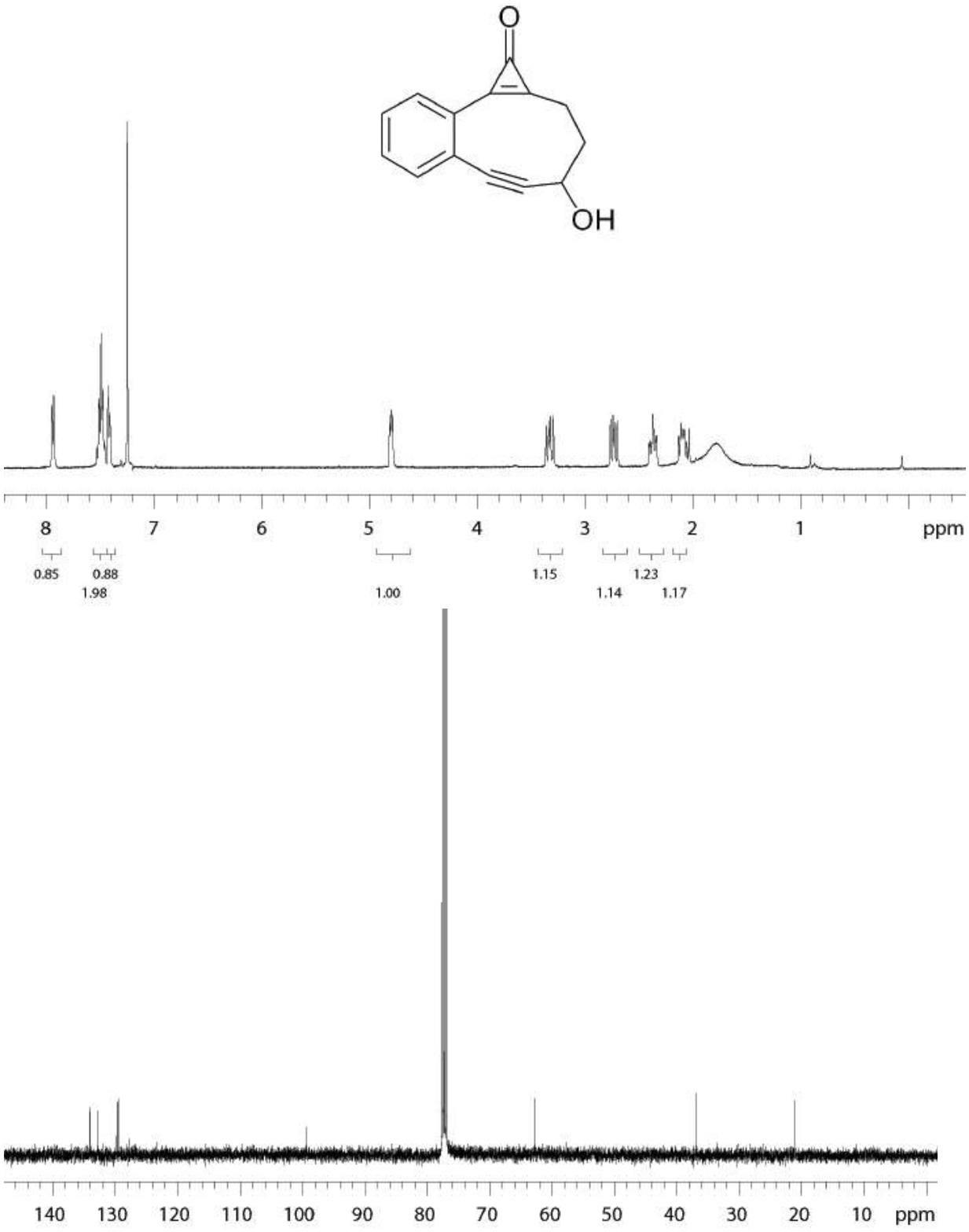

Figure A.9. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.14**

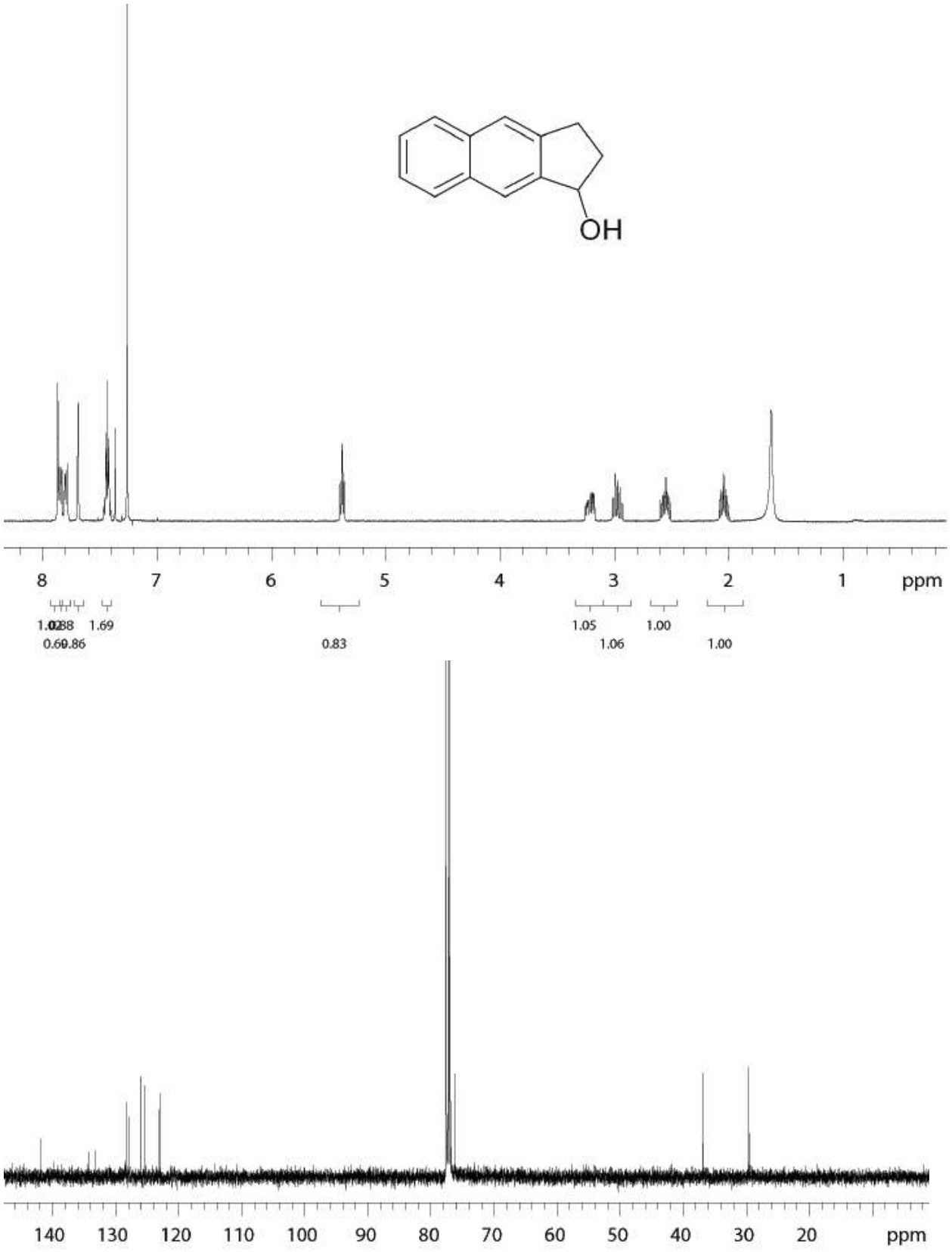

Figure A.10. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.24**

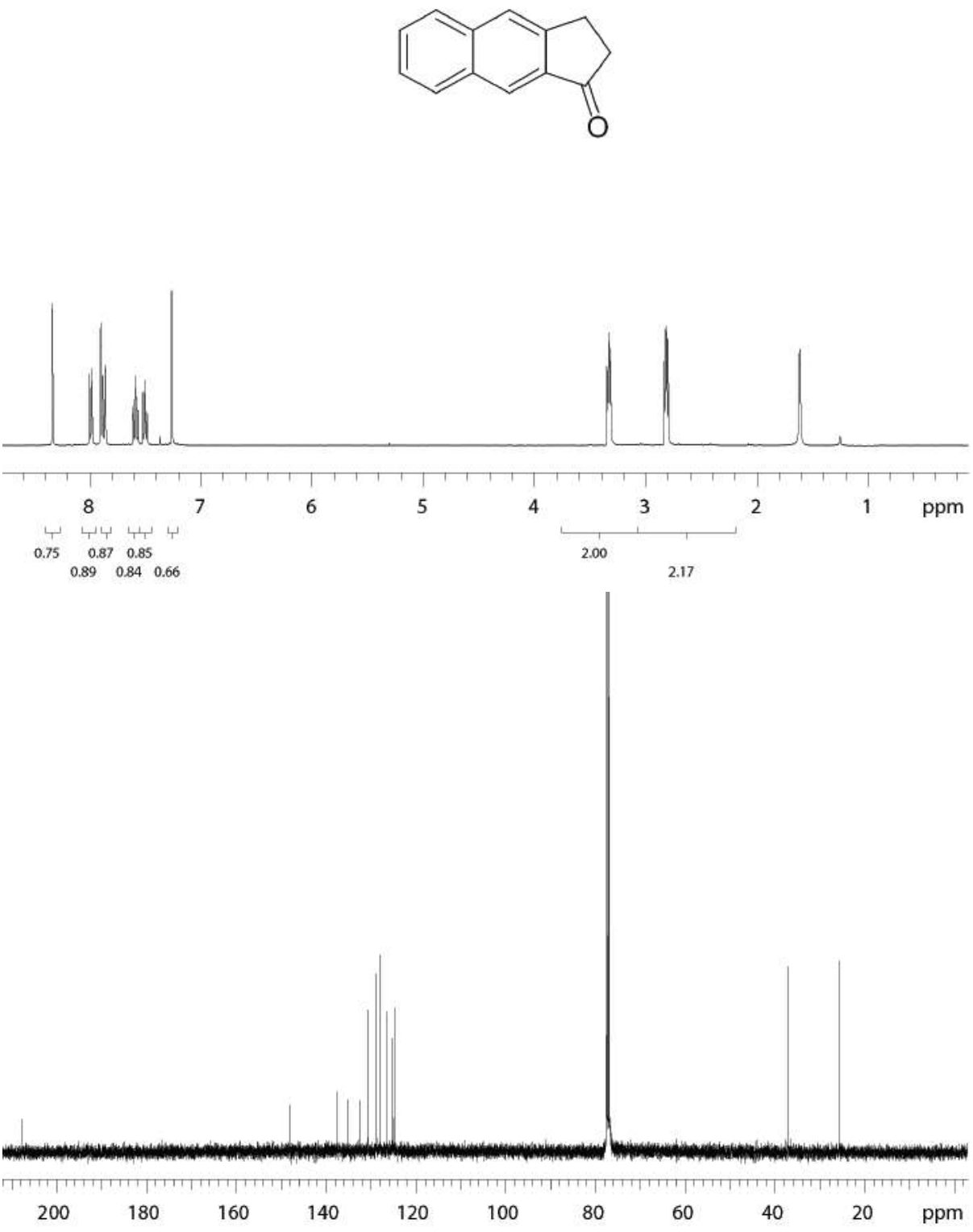

Figure A.11. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.23**

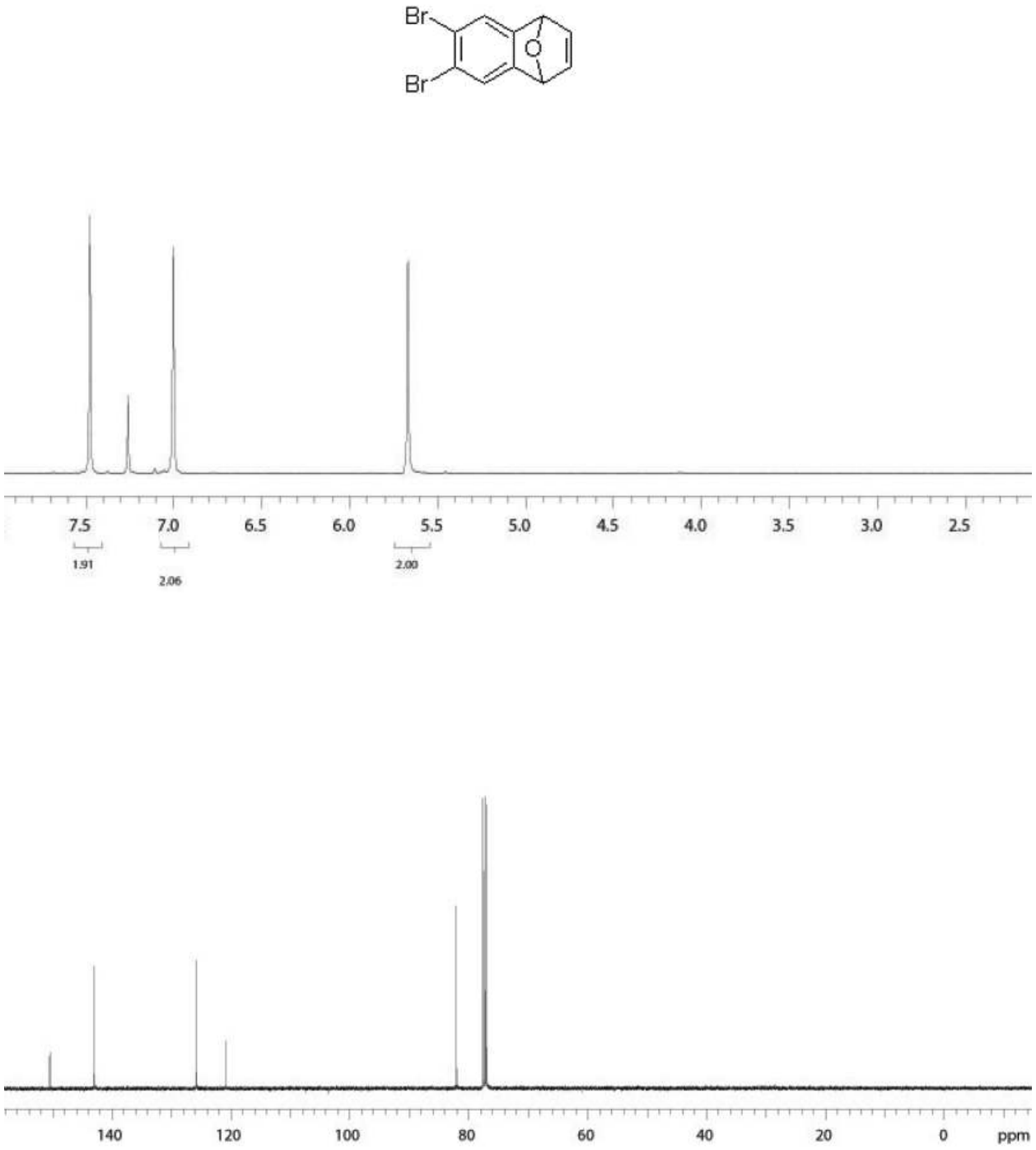

Figure A.12. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.28**

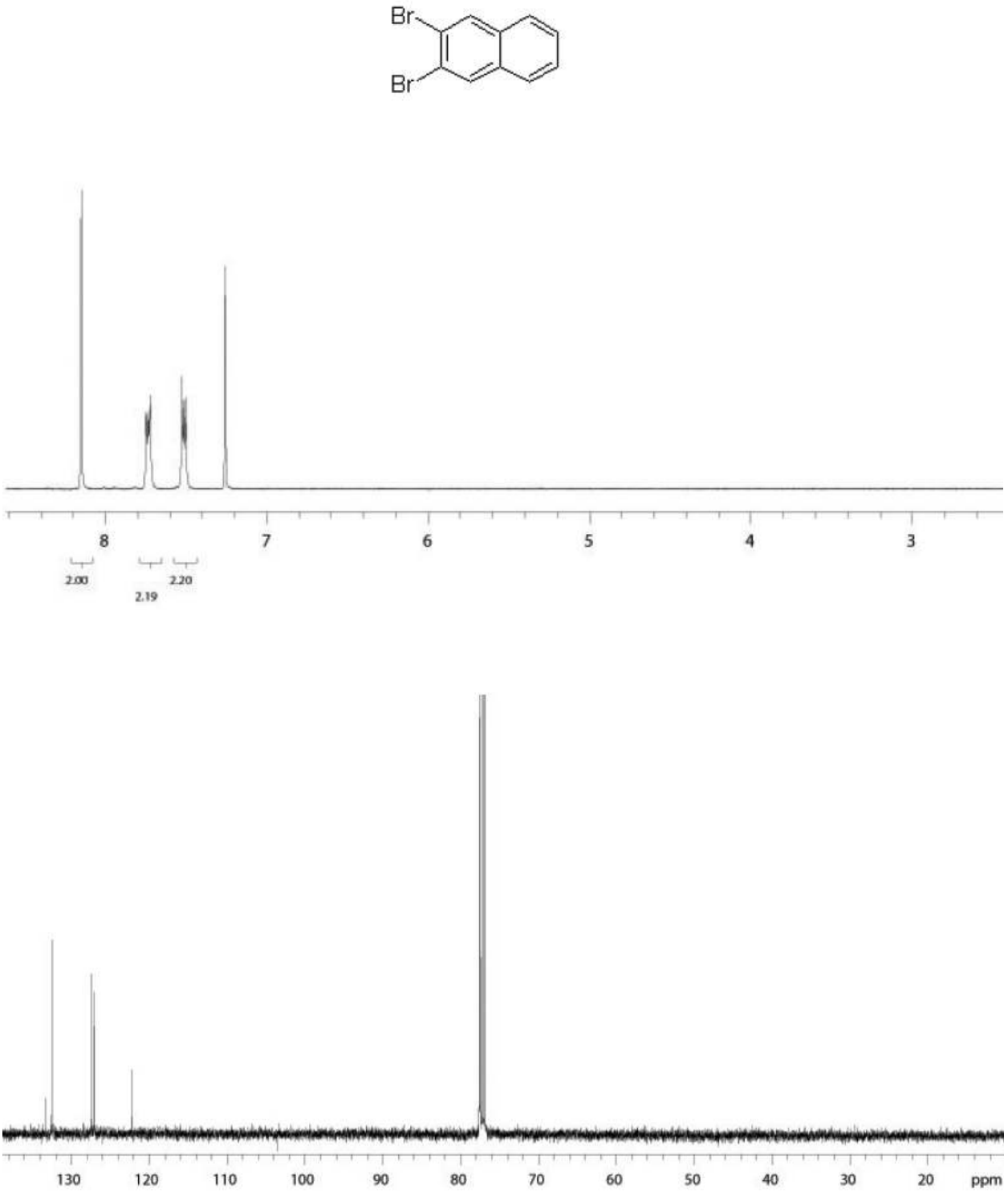

Figure A.13. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.29**

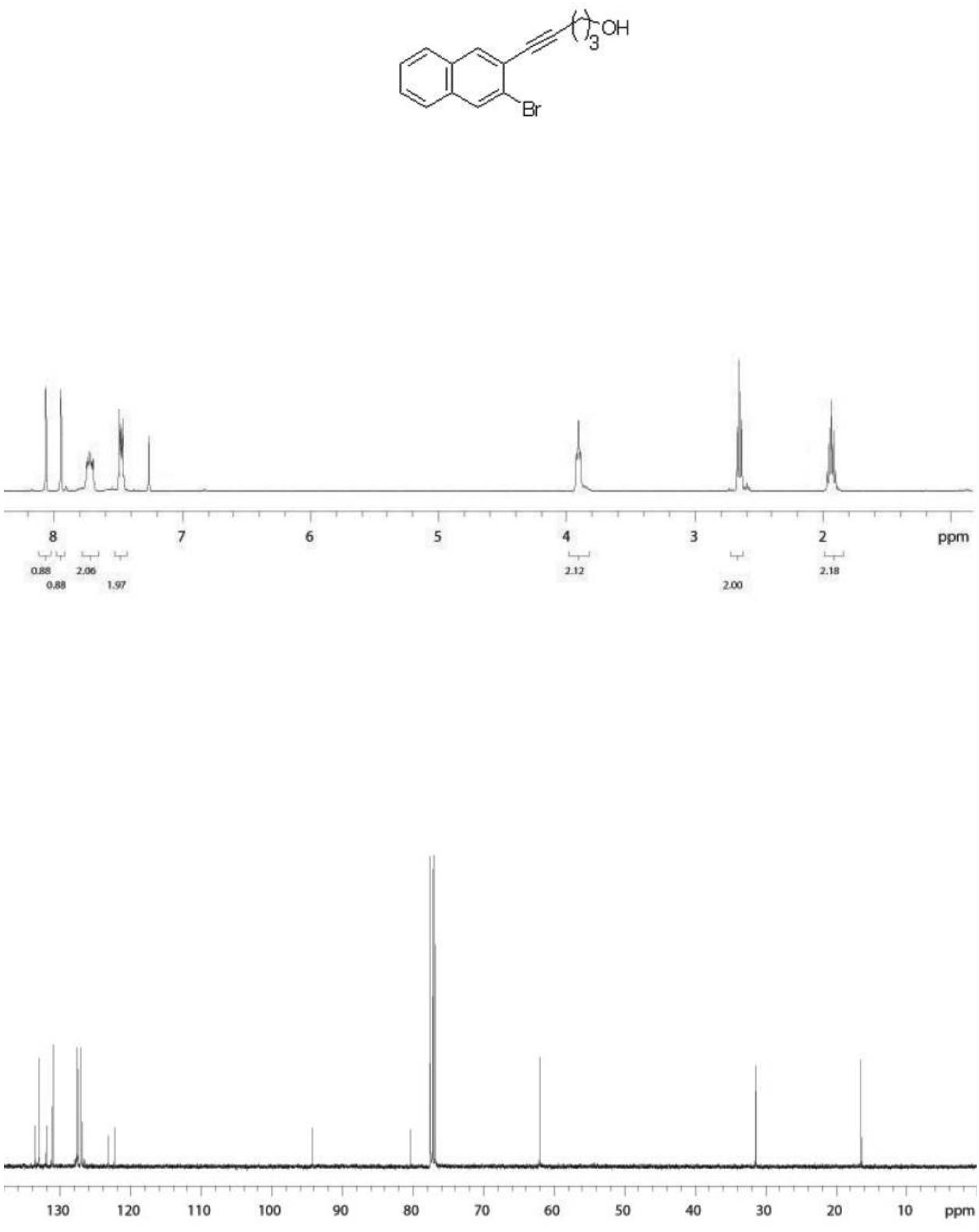

Figure A.14. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.30**

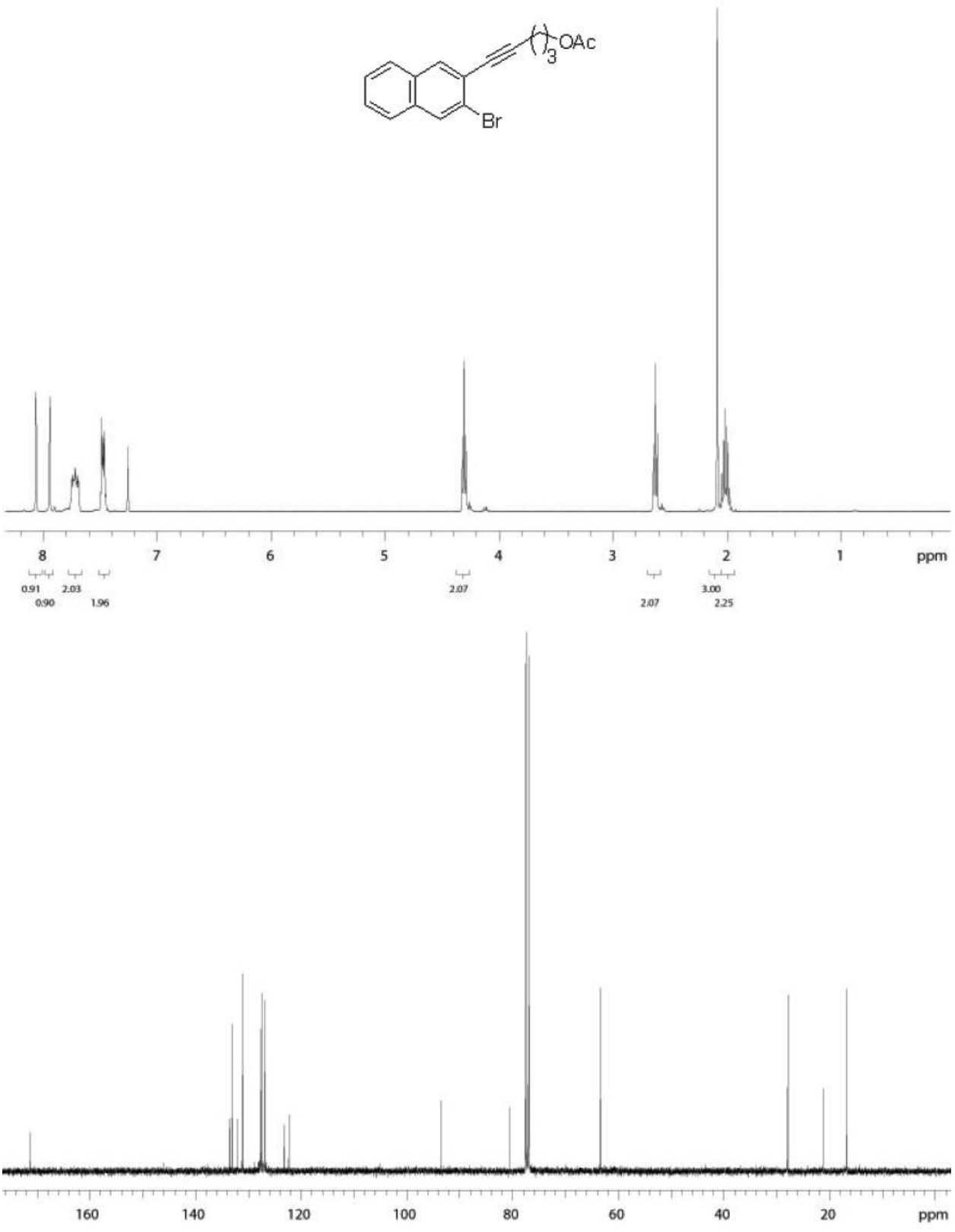

Figure A.15. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.31**

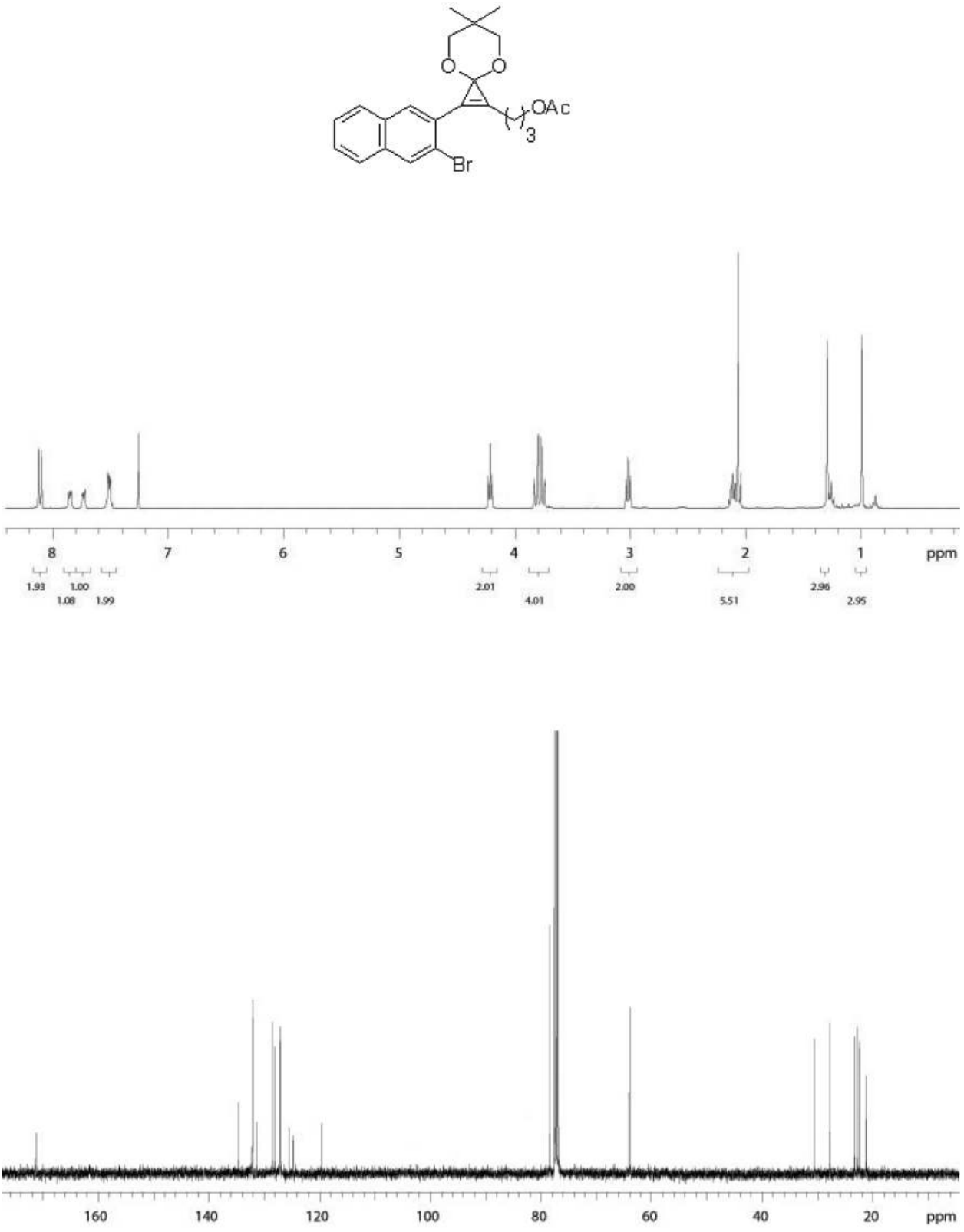

Figure A.16. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.33**

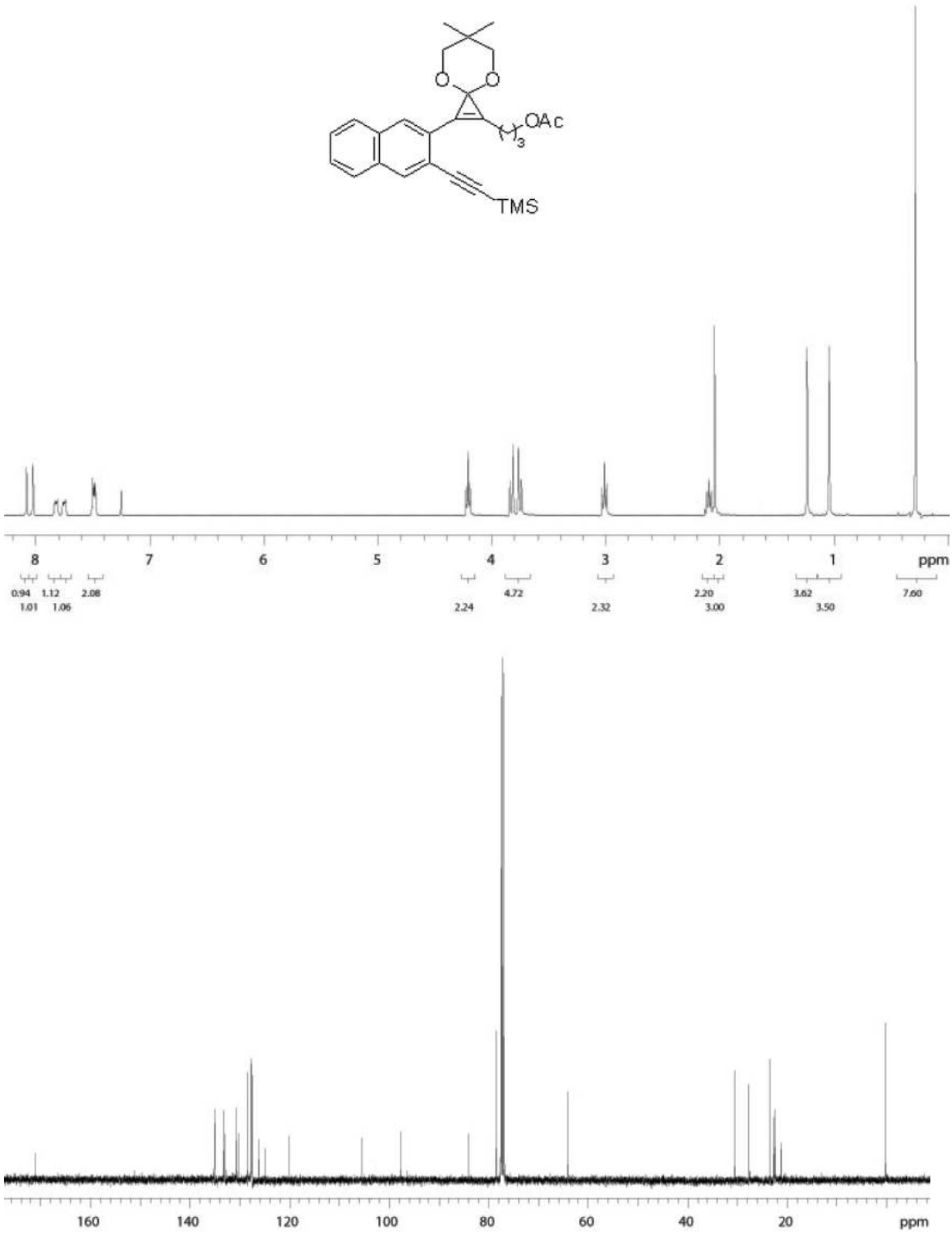

Figure A.17. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.34**

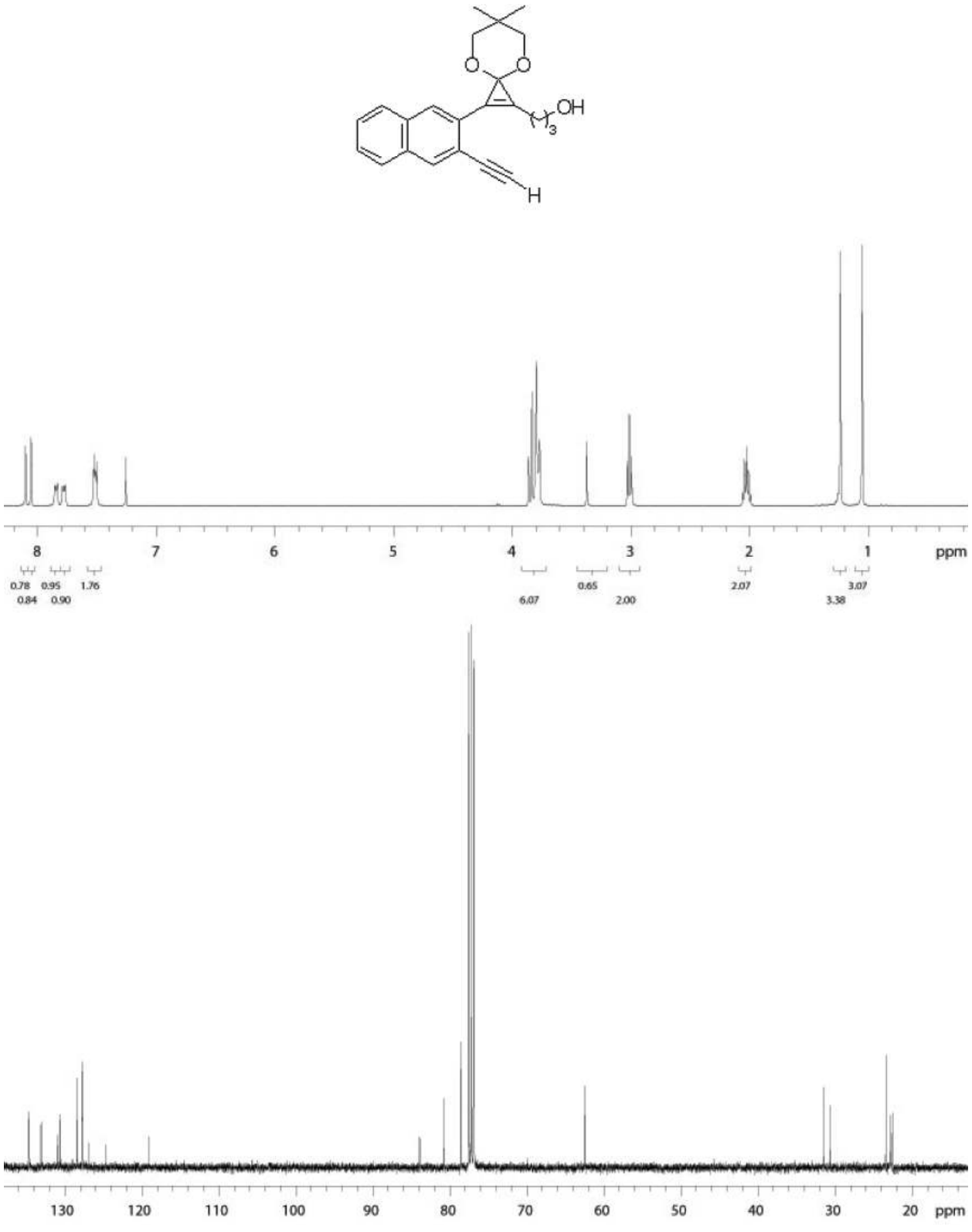

Figure A.18. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.35**

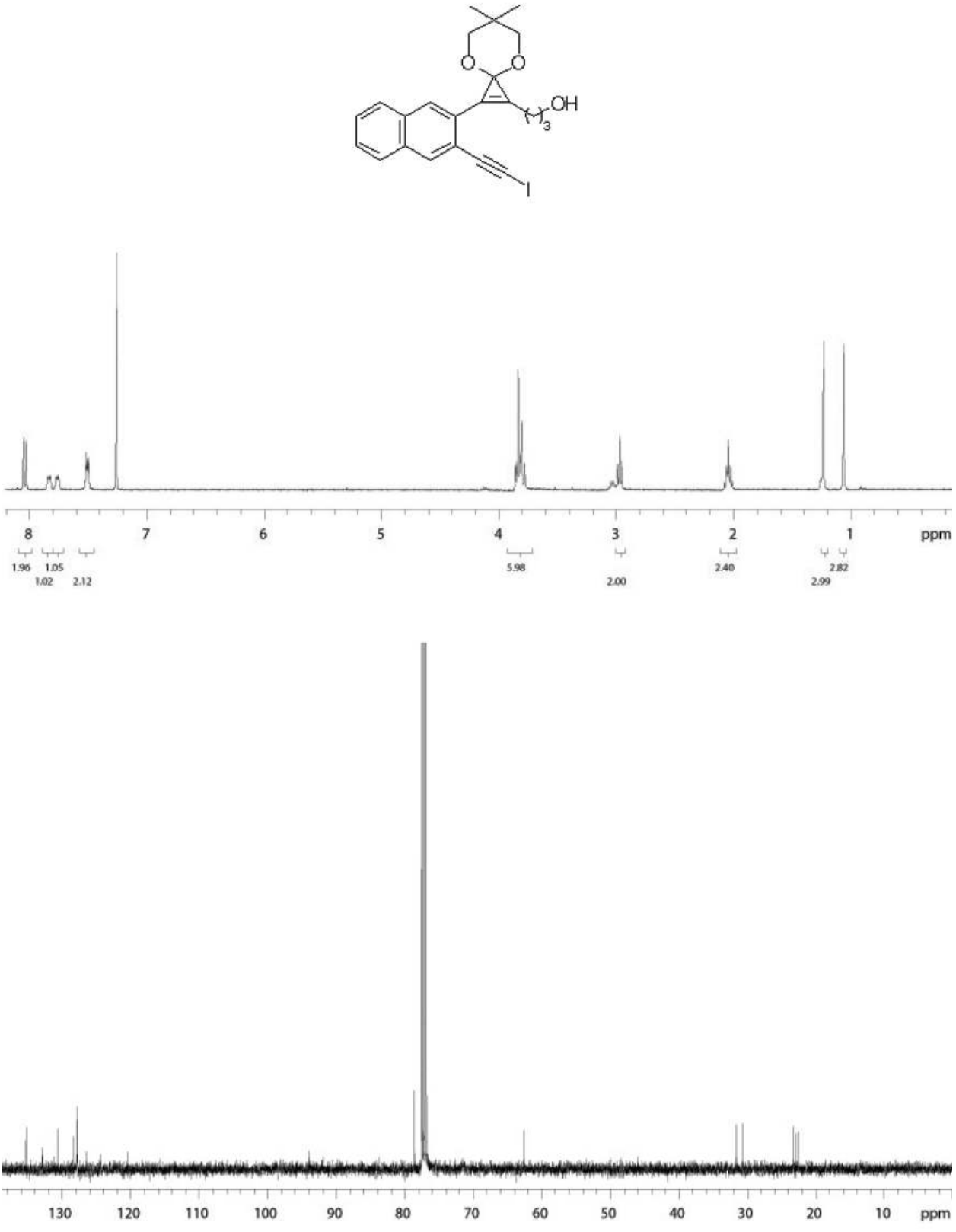

Figure A.19. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.36**

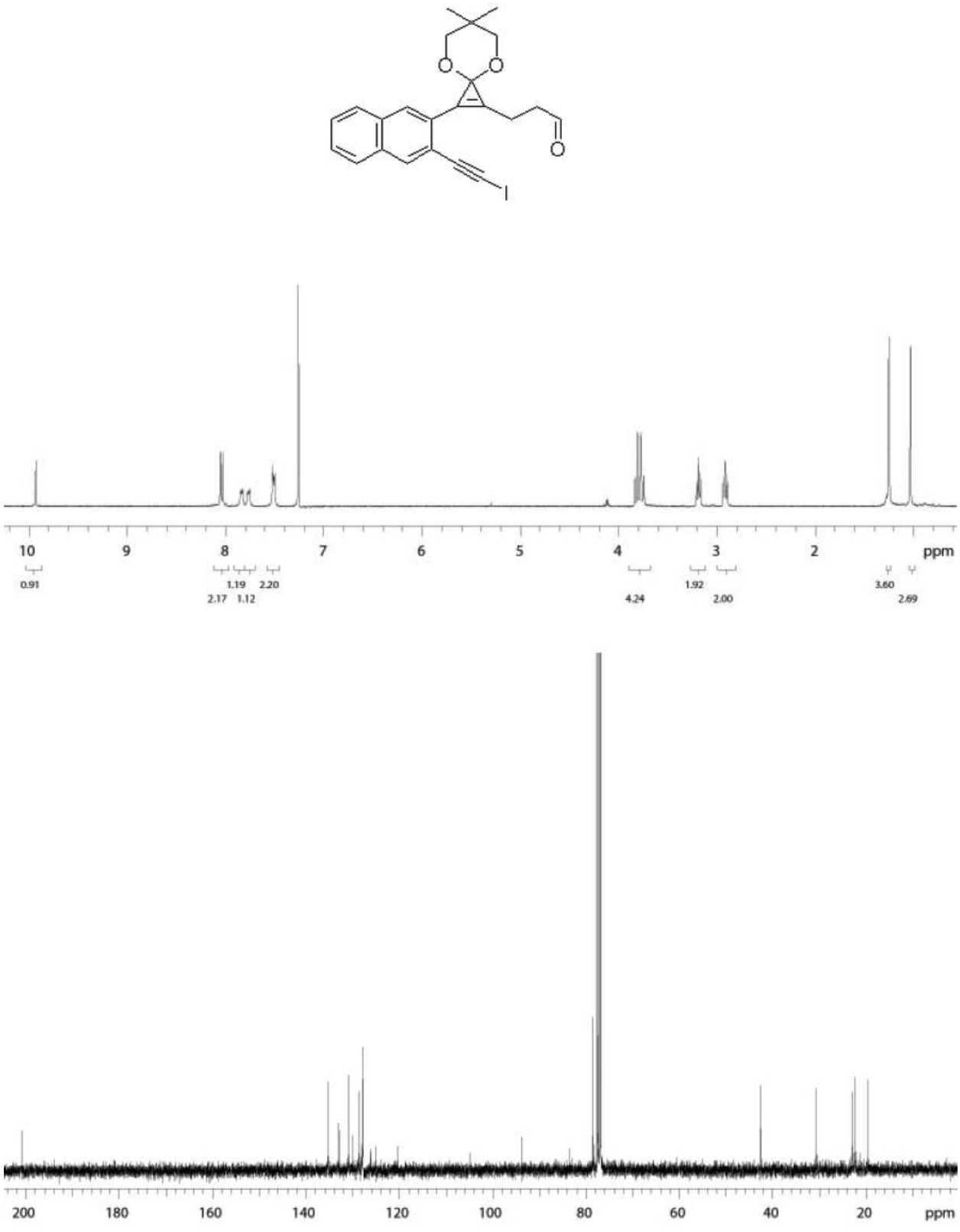

Figure A.20. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.37**

Figure A.21. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.38**

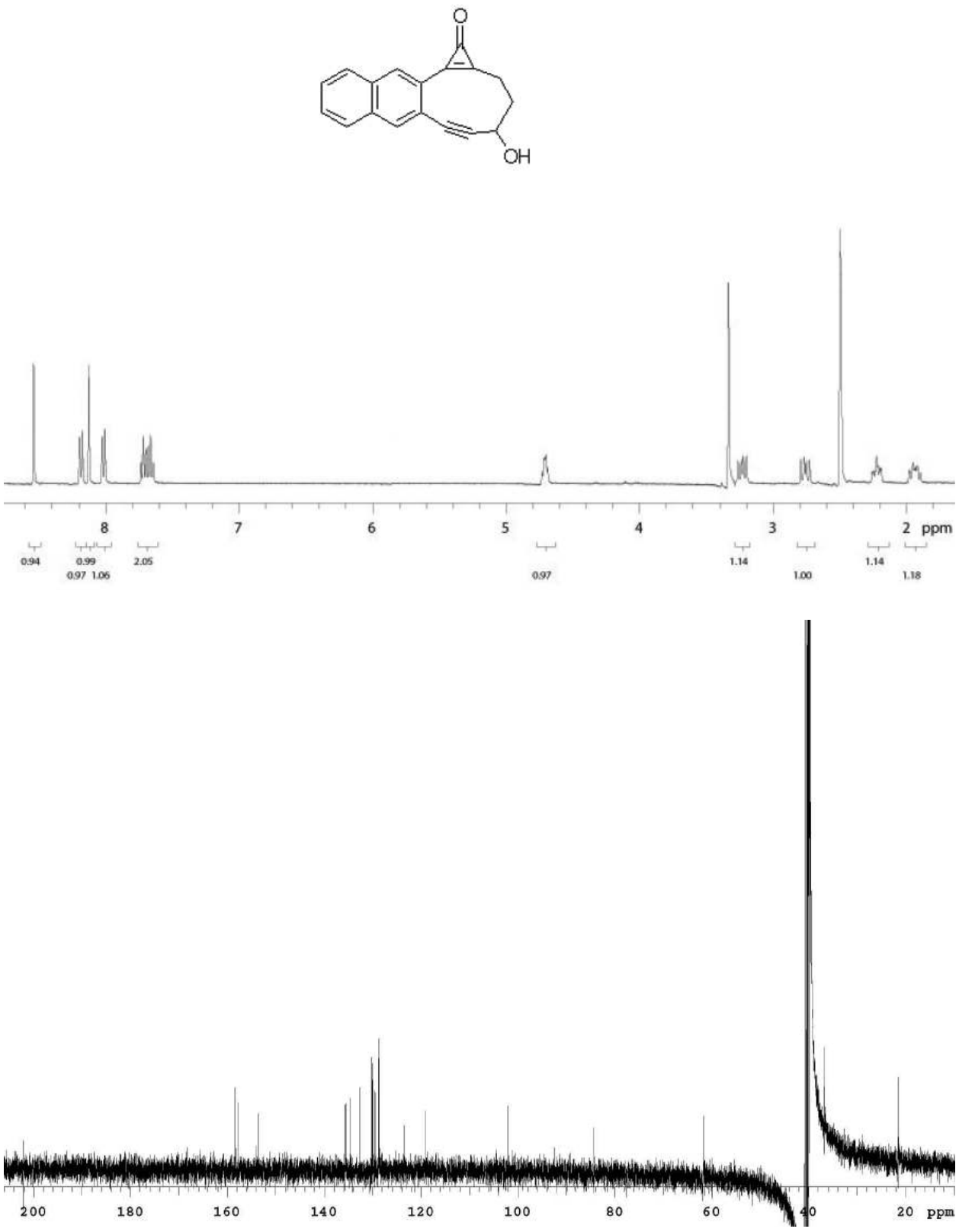

Figure A.22. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.25**

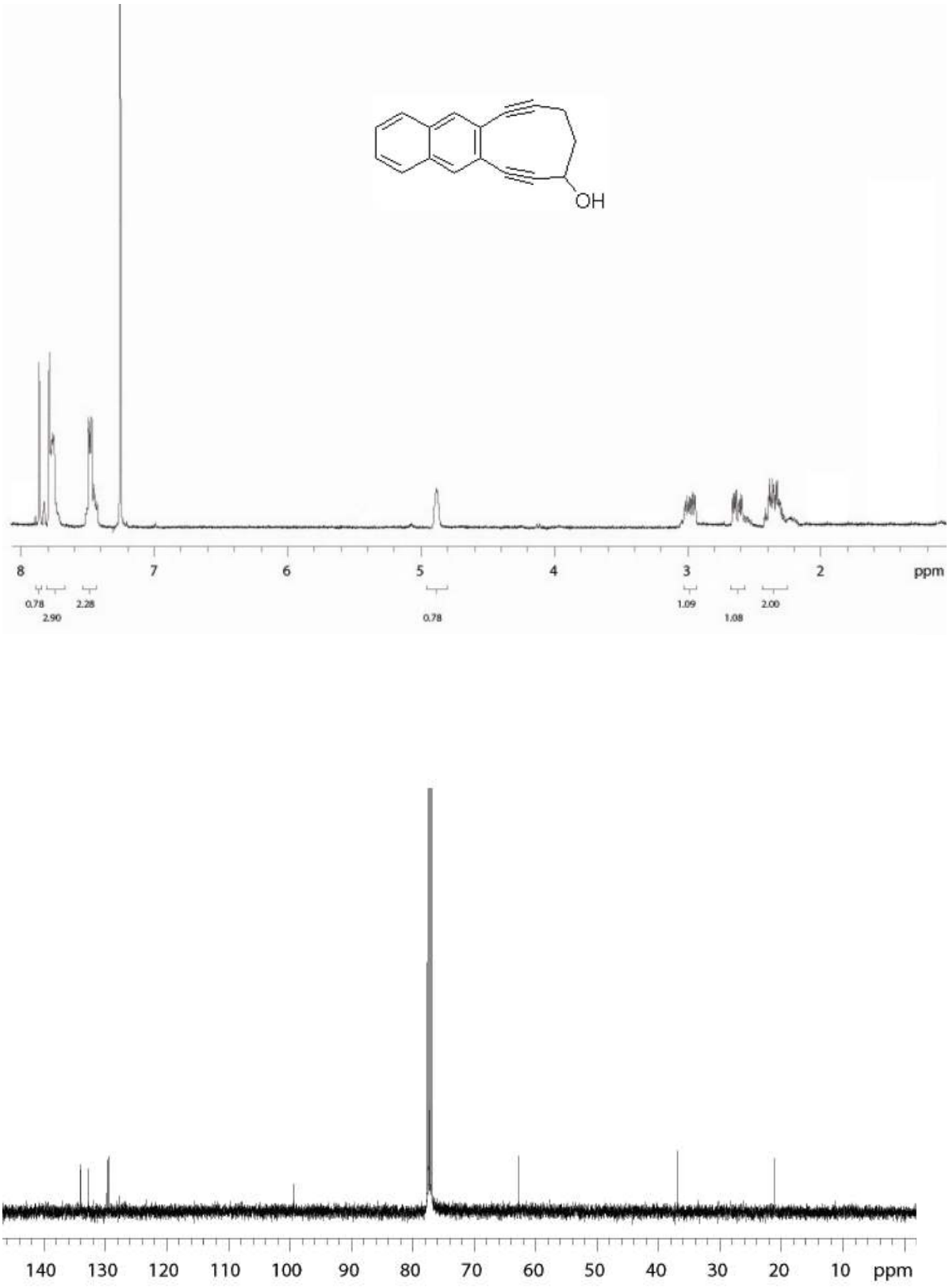

Figure A.23. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.26**

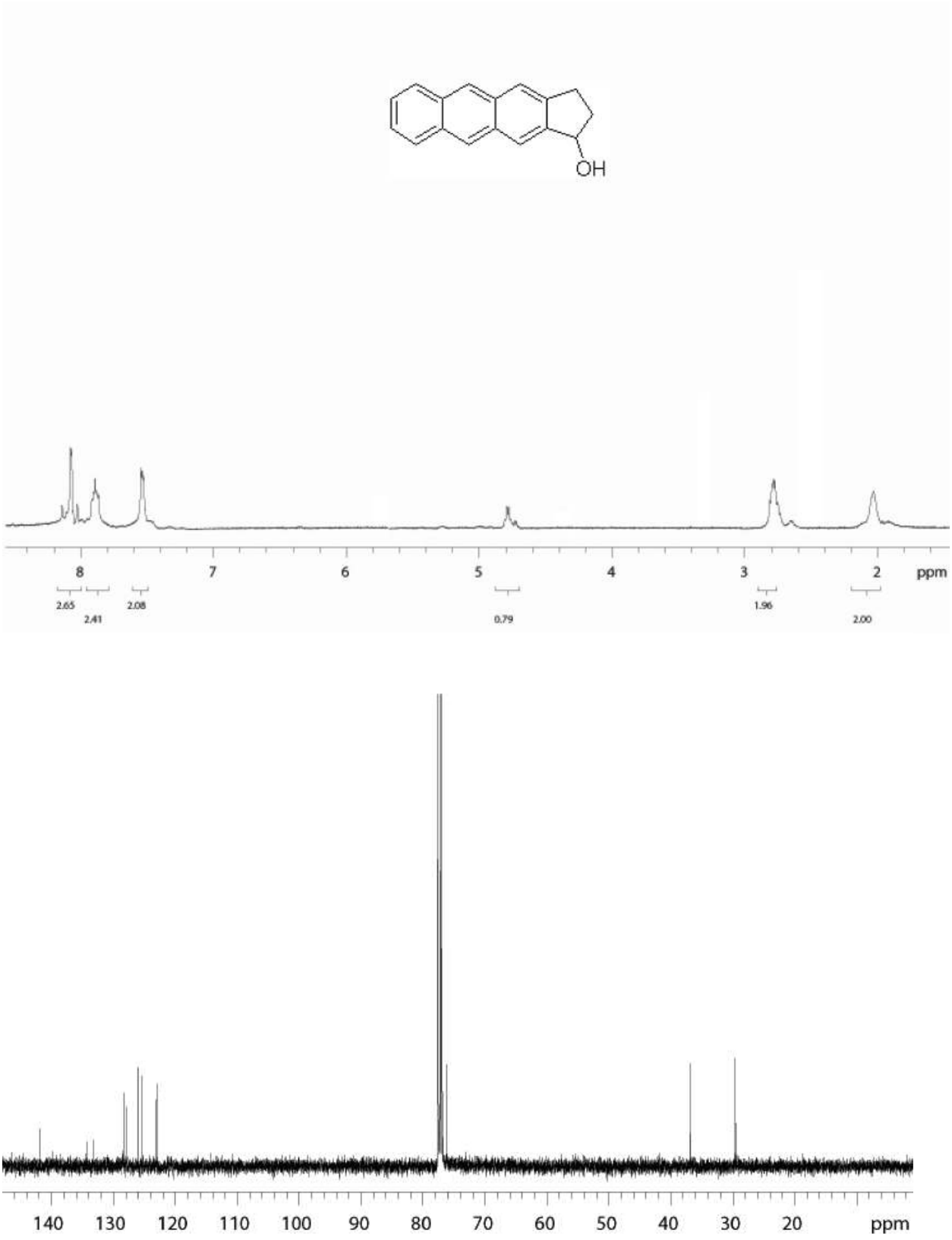

Figure A.24. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.27**

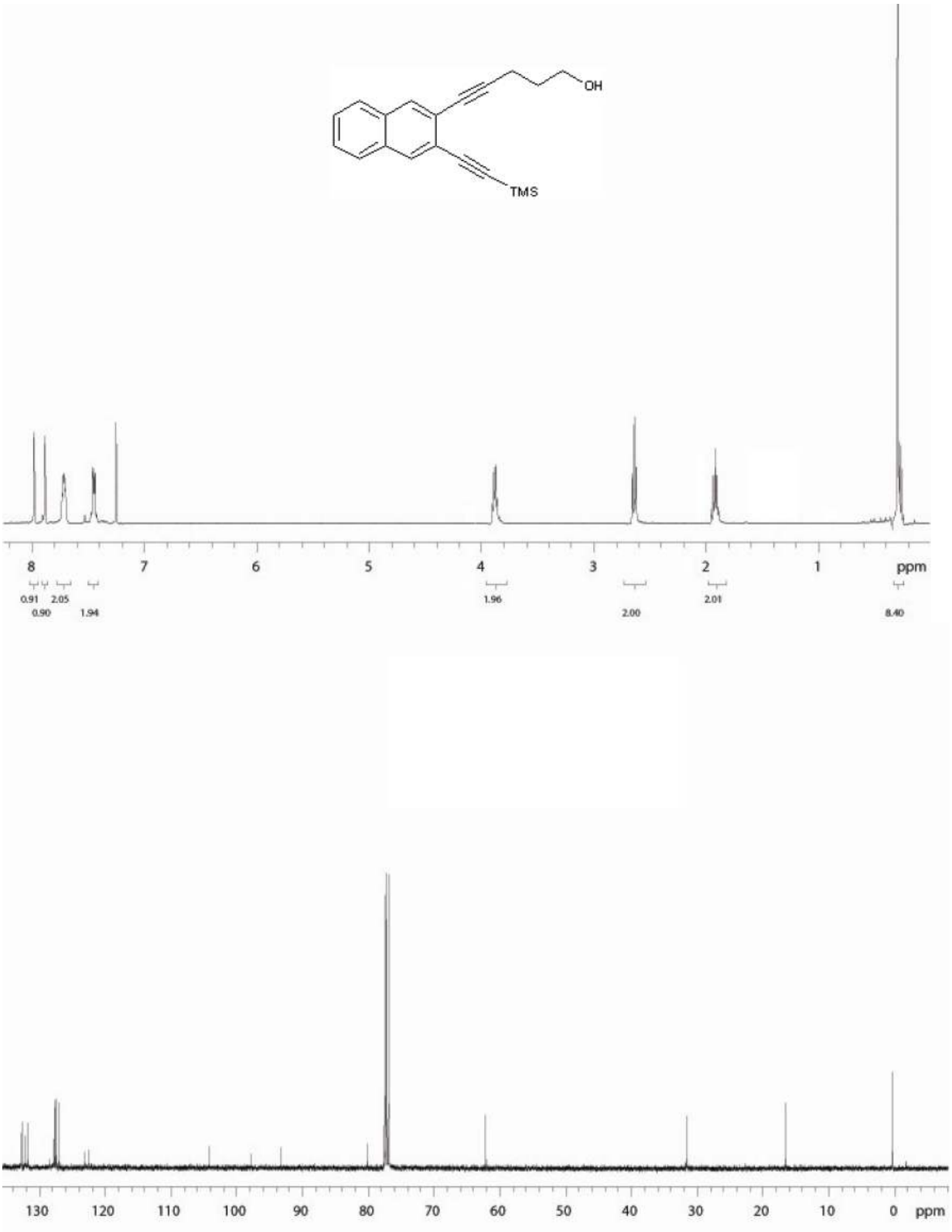

Figure A.25. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.39**

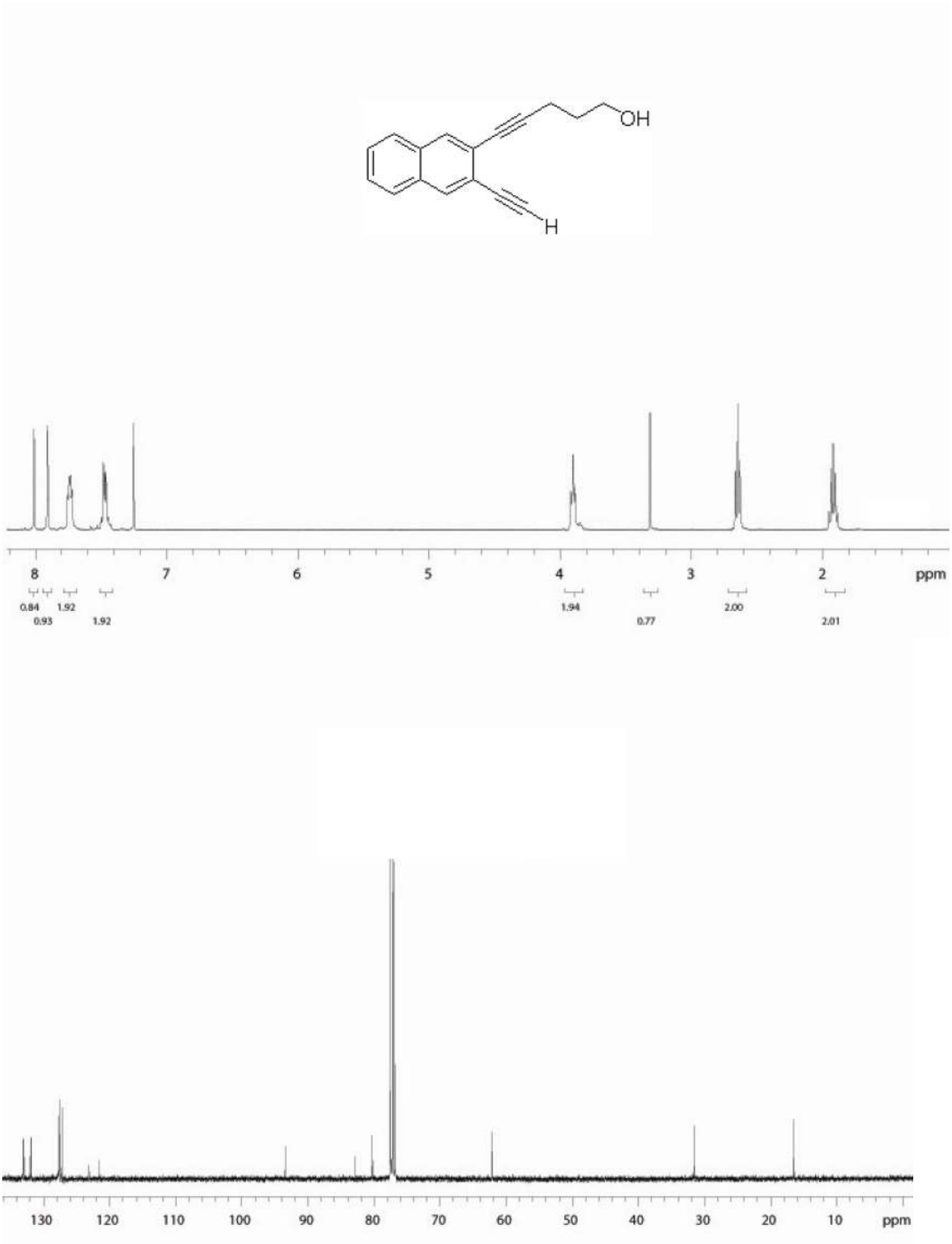

Figure A.26. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.40**

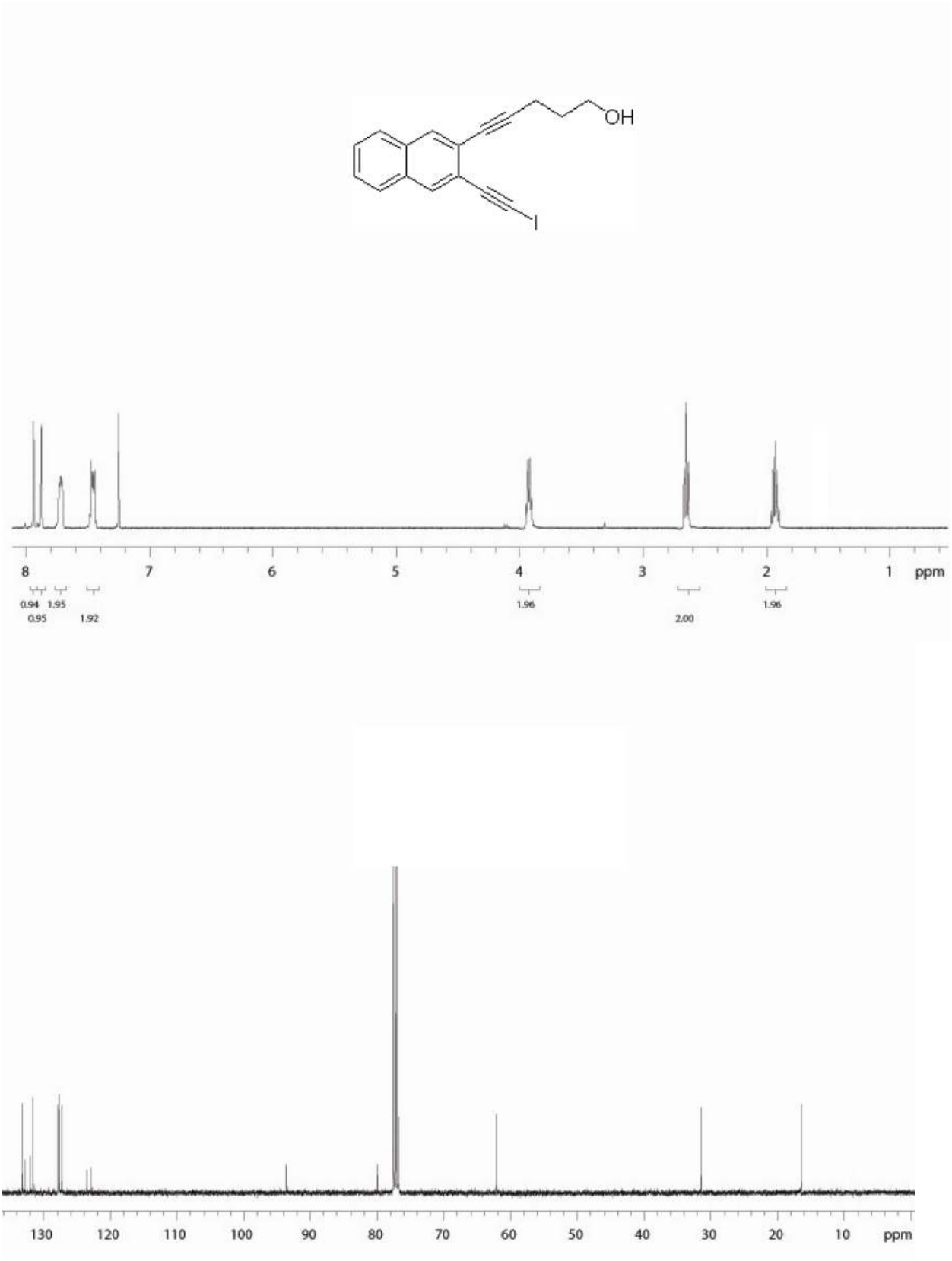

Figure A.27. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.41**

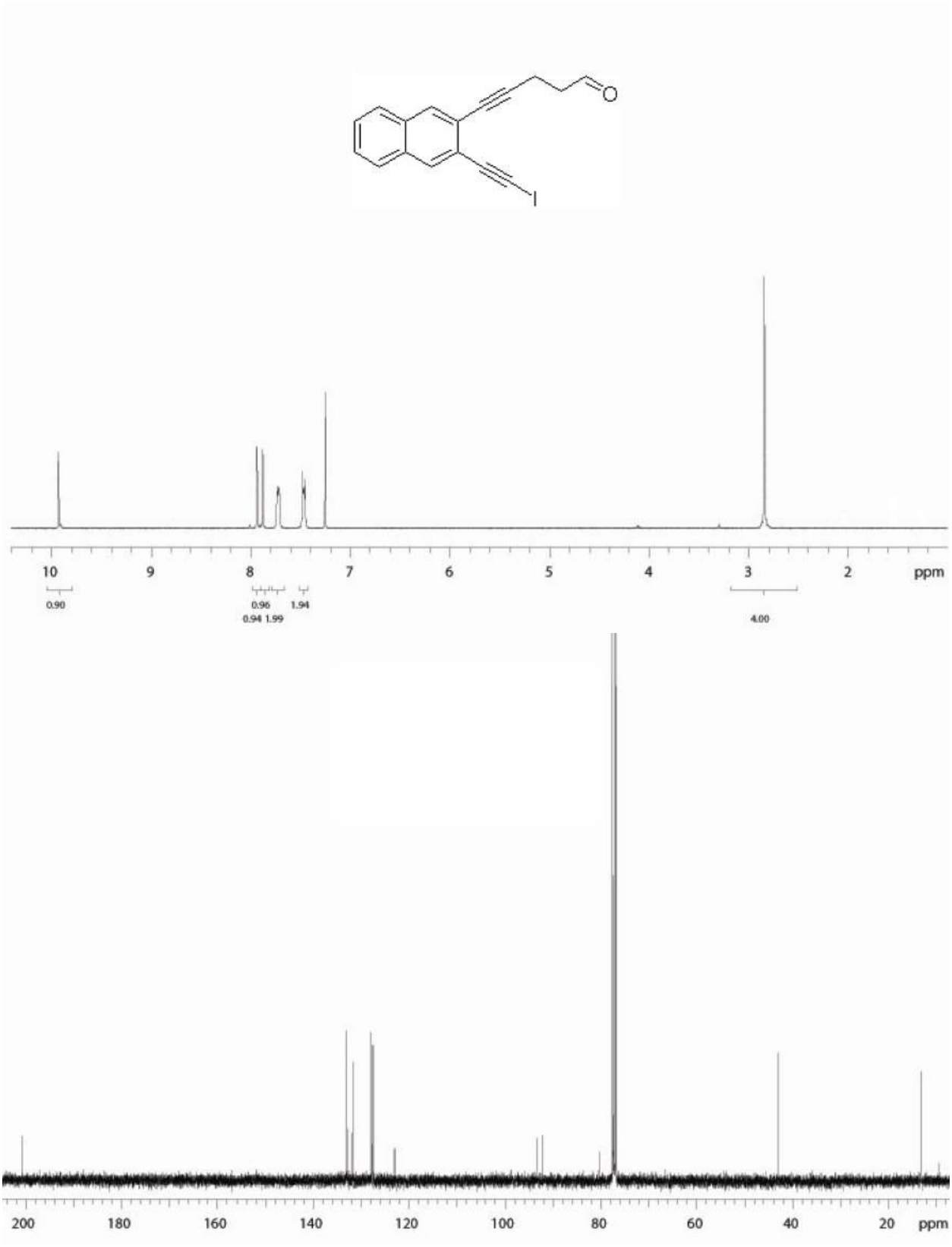

Figure A.28. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.42**

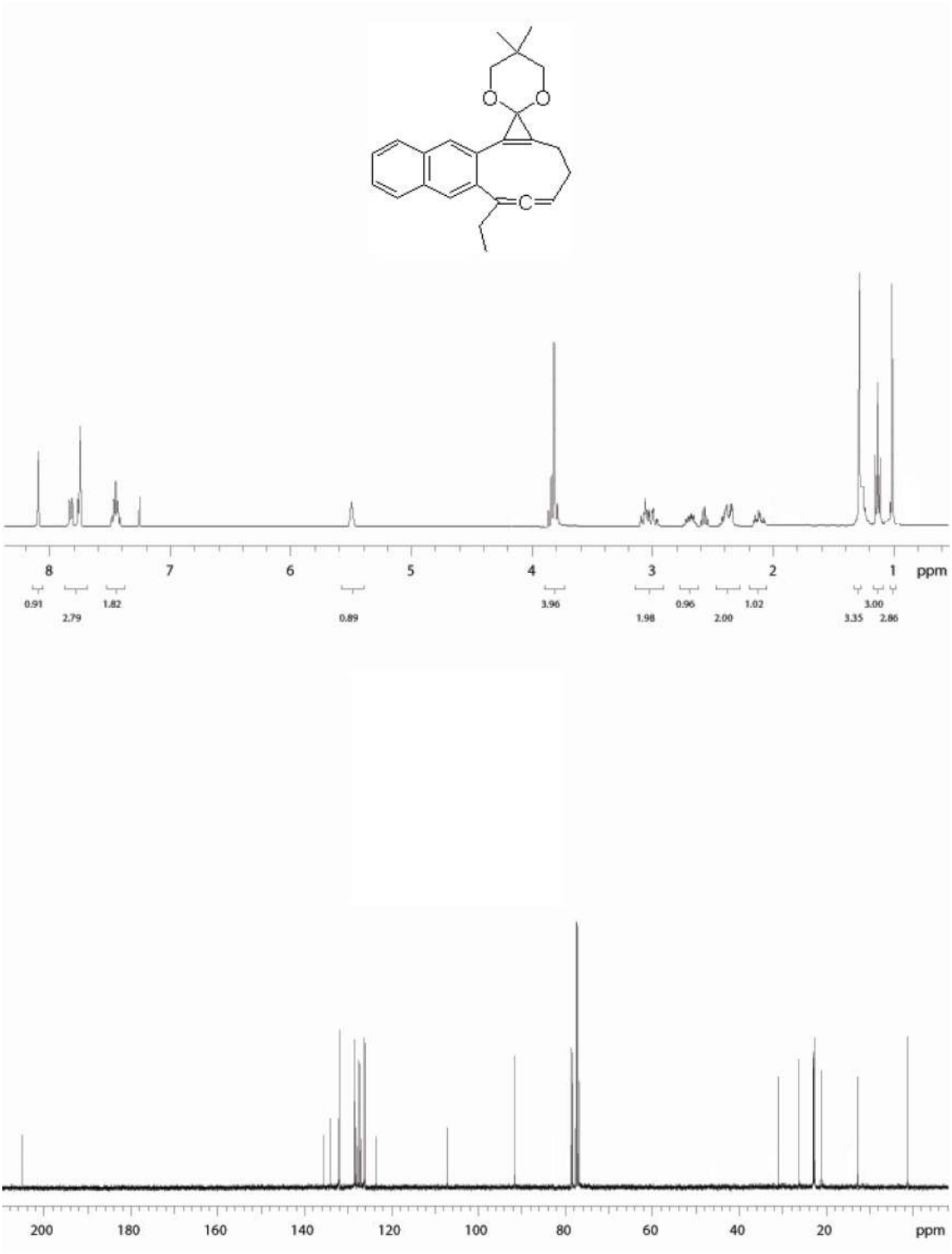

Figure A.29. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.46**

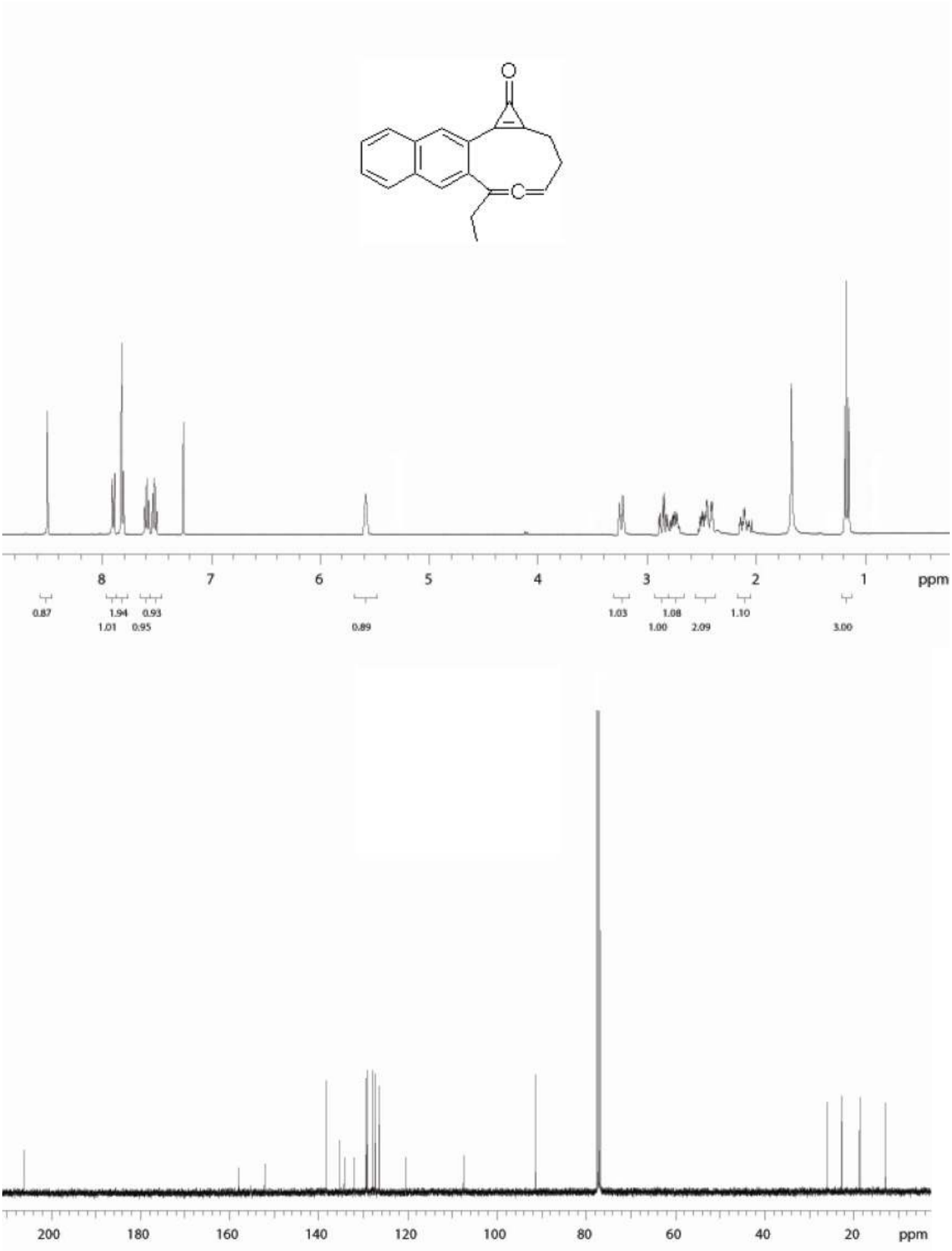

Figure A.30. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **2.43**