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SYNTHESIS OF AN ENEDIYNE TOXINE MODEL SYSTEM

Abstract

In an attempt to find compounds with large margins of therapeutic usefulness, models resembling the calicheamicin/esperamicin framework are studied. In this paper, a presentation of the enediynes is first proposed, before explaining the Bergman cyclization which is the main reaction involved in the cytotoxicity of these antitumor antibiotics. Then, the action of enediynes on DNA and the way of DNA cleavage is described. Finally, the synthesis of a two-atom 1,4-bridged enediyne is reported, with explanations of the reasons for the choice of this molecule and the synthesis strategy.

Keywords

Enediyne, calicheamicin, antitumor antibiotics, Bergman cyclization, DNA cleavage

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Introduction

Since the discovery of penicillin by Fleming in 1929, chemists tried to synthesize drugs to fight against illnesses. But new illnesses imply new drugs with new way of action. One of the most important illness of our time may be the cancer and today some cancers remain incurable.

In the middle of the 80's, several members of a new family of molecules, the enediynes, were discovered. The enediynes were found to be very active against tumor cells, and since this discovery, research on enediynes has been in the forefront of chemistry, biology and medicine because these compounds could be developed into very useful drugs.
Enediynes are active because they cyclize to a diradical which can cleave DNA (in a single or double-stranded fashion). Therefore, chemists have been trying to imitate nature and to synthesize new enediynes which could cleave DNA (with preference with double-stranded cleavage), but which would be much simpler to make than natural enediynes and which would be selective for tumor cells: the natural products are generally not selective for tumor cells but analogues might be able to achieve this selectivity. If such a useful DNA cleaving agent could be made, it would be a significant advance in the fight against cancers. In 2002, clinical development of neocarzinostation analogues were continuing in Japan and Europe, US trials of a monoclonal antibody conjugate of calicheamicin (Mylotarg) were entering phase III and esperamicins were entering phase II trials [1].

Several enediynes have already been synthesized, some with biological activity and others without activity. In this paper, the synthesis of a two-atom 1,4-bridged enediyne is reported.

Chapter 1

Background

1.1 The enediynes

In 1965, neocarzinostatin was discovered and in 1985 the structure of the neocarzinostatin chromophore was disclosed by Edo *et al.* [2], a discovery which received little attention. Then in 1987, Lee *et al.* [3] reported the discovery of a new family of molecules, the calichemicins (also know as the LL-E33288 antibiotics) (produced by *Micromonospora echinospora ssp. Calichensis*), whereas Golik *et al.* [4] reported the discovery of the esperamicins (produced by *Actinomadura verrucosospora*). A fourth structure was reported in 1989 by Konishi *et al.* [5]: dynemicin. (Figure 1.1)

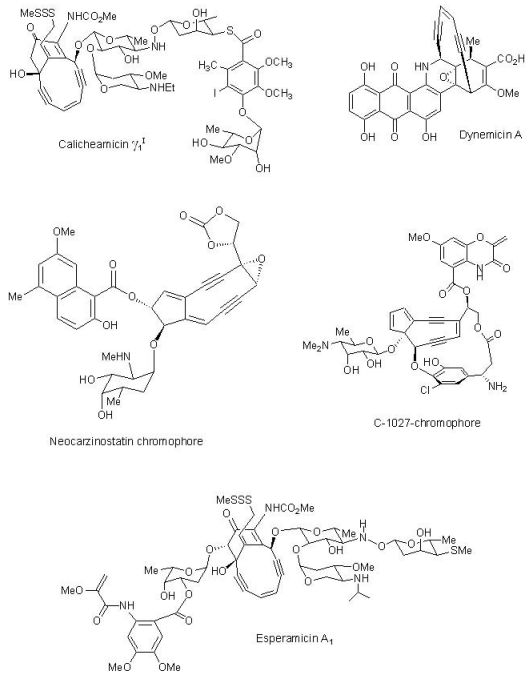


Figure 1.1: Main enediynes

These discoveries were the beginnings of the chemistry on a new kind of molecules, the enediynes. The enediyne moiety is the (Z)-3-hexene-1,5-diyne. Over 20 natural enediynes have been discovered to date [1] (Appendix A). These natural molecules, which contain the enediyne functional group, are not biologically active prior to a triggering event, after

which the enediyne moiety cyclizes to a diradical which can cleave DNA by hydrogen atom abstraction. The enediynes are said to be "one of the most potent antitumor agents (both in vitro and in vivo) ever evaluated" [1]. Although the exact mechanisms of their biological activity are not perfectly understood, it was shown that they are very good DNA cleaving agents, and mechanism for this cleaving was suggested (§ 1.3).
There are two distinct subcategories of enediynes: those containing a ten-membered 3-hexene-1,5-diyne subunit (the calicheamicins, esperamicins, dynemicins) and those containing a nine-membered enediyne, which are typically isolated as complexes with an associated apoprotein (the neocarzinostatins) [1].
The enediynes have three functional domains: a "warhead" which provides the fragment which can damage DNA after activation (i.e. the enediyne system); a "delivery system" which delivers the warhead to its target (DNA) and binds to it (ex. the oligosaccharide fragment for the calicheamicin $\gamma_1{}^I$); a triggering domain which, after activation, initiates the reactions leading to DNA cleavage (ex. the trisulfide moiety in calicheamicin $\gamma_1{}^I$) [6] (Figure 1.2).

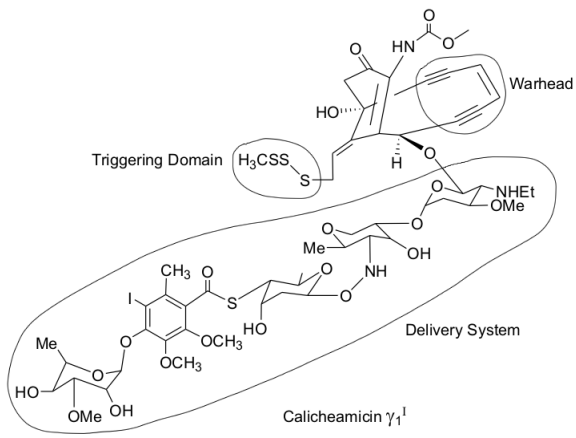


Figure 1.2: The functional domains of calicheamicin $\gamma_1{}^I$

Interest in enediynes, especially in calicheamicins and esperamicins, was heightened by the development of monoclonal antibody conjugates of these enediynes. These conjugates induced remission in up to 40% of patients with aculate myeloid leukemia (AML) who had previously relapsed following other chemotherapies [1].

1.2 The Bergman cycloaromatization

The biological activity of enediynes is due to the possibility for these compounds to become a diradical after a cycloaromatization. In 1972, Robert G. Bergman studied the cyclization of (Z)-3-hexene-1,5-diyne into 1,4-dehydrobenzene [7] (Figures 1.3 and 1.4).

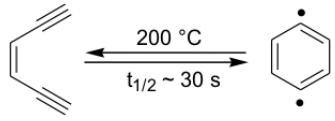


Figure 1.3: The Bergman cyclization

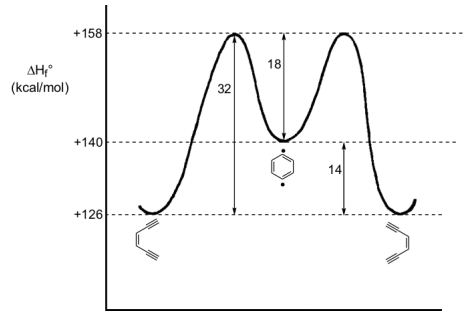


Figure 1.4: Equilibrium between (Z)-3-hexene-1,5-diyne and 1,4-dehydrobenzene

He noticed that the stability of dehydroaromatic compounds is increased if there is an interaction between the two single electrons; he gave the example of the naphthalene which can be seen as a 10-membered ring and whose stability is due to the strength of the 1,6 σ bond (Figure 1.5).

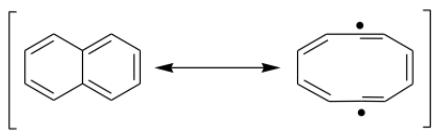


Figure 1.5: Naphtalene seen as a 10-membered ring

1,4-Dehydrobenzene has no interaction between the two electrons and is therefore not very stable. In presence of cyclohexa-1,4-diene, 1,4-dehydrobenzene can lead to benzene by hydrogen atom abstraction: cyclohexa-1,4-diene is a very good donor of hydrogen atom because the resulting radical is stabilized by a resonance structure (Figure 1.6).

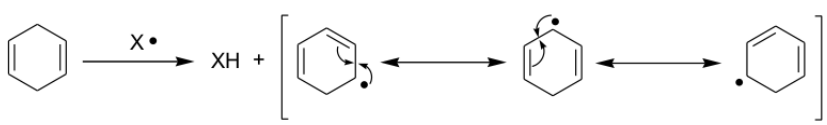
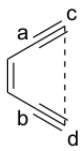


Figure 1.6: Resonance form of 3-dehydro-cyclohexa-1,4-diene

Nicolaou showed that for simple enediynes the stability can be predicted by looking at the cd distance [8]. The ab distance is the distance between the two carbons of the enediyne closer to the alkene, while the cd distance is the distance between the two carbons of the enediyne further from the alkene (Figure 1.7). If the cd distance is greater than 3.31 Å the enediyne is stable at room temperature and if it is lower than 3.20 Å, it is unstable[1]. For example, (Z)-3-Hexene-1,5-diyne has a cd distance of 4.12 Å (MM2 calculations) and is stable at room temperature.
An interesting molecule is cyclodec-3-ene-1,5-diyne (Figure 1.8) which has a cd distance of 3.25 Å and cyclizes at 37°C with a half-life ($t_{1/2}$) of 18h, a rate constant (k_r) of 6.4×10^{-4}/min and a energy of activation (E_a) of 23.8 kcal/mol; this 10-membered ring is the framework of calicheamicins, esperamicins and dynemicin A [8].

[1]It must be emphasized that this hypothesis is based on first approximations and is meant for simple systems, and that exceptions, particularly in bicyclic systems with complex frameworks and centers, do exist.



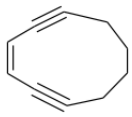


Figure 1.7: ab and cd distances Figure 1.8: cyclodec-3-ene-1,5-diyne

The typical half-lives of most unstrained 10-membered enediynes (for Bergman cyclization) are approximately 3 to 18h [1].

<u>*NotaBene*</u>: in some case (such as for neocarzinostatin), the molecule undergo a Myers cycloaromatization instead of a Bergman cycloaromatization (Figure 1.9).

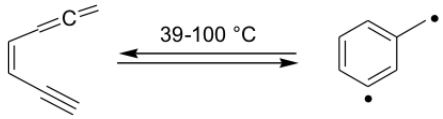


Figure 1.9: Myers cycloaromatization

1.3 Action of enediynes on DNA

Enediynes are studied because they are a family of antitumor antibiotics which can cleave DNA at low concentration. The activity of enediynes can be divided into three reactions: a chemical triggering event which modifies the structure of the molecule; the Bergman cycloaromatization; and then the DNA cleavage by the diradical [9] (Figure 1.10).

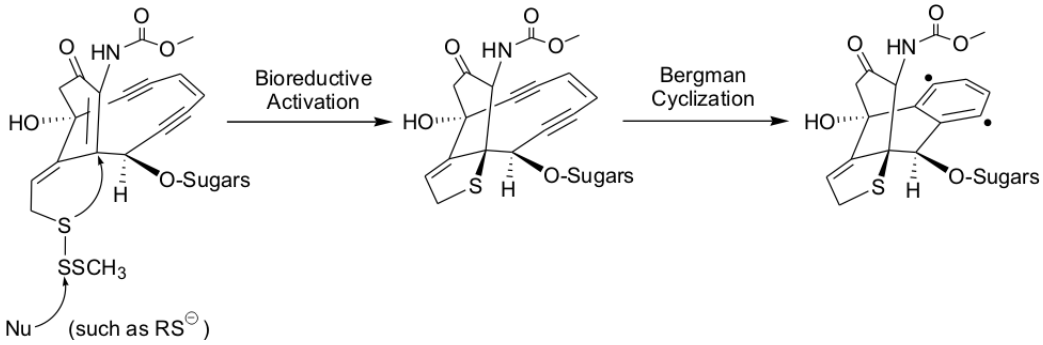


Figure 1.10: Activation of enediyne and cleavage of DNA

One of the most studied enediynes is calicheamicin $\gamma_1{}^I$ (which is the most prominent member of the calicheamicin family): it is 1000-fold more active than adriamycin against murine tumors and is optimal at 0.5 to 1.5 μg per kilogram of body weight. This drug can also cause chromosome aberrations in human and mutagenesis in *Escherichia Coli* [10]; it exhibits extraordinary potency against murine tumors such as P338 and L1210 leukemias and solid neoplasms such as colon 26 and B-16 melanoma.
If an enediyne could cleave the DNA of a tumor cell but not DNA of the organism, it would

be a very active drug. The goal, therefore, is to find a way to activate the enediyne in the tumor cell selectively.
Calicheamicin $\gamma_1{}^I$ has a labile methyltrisulfide grouping that upon reduction with thiols (probably assisted by the basic nitrogen of the carbohydrate moiety of the agent [6]) initiates aromatization of the enediyne moiety via the diradical species (Figure 1.11)[2]. It was demonstrated that other kinds of triggering can happen, such as photo-activation, redox activation, or acid/base activation.

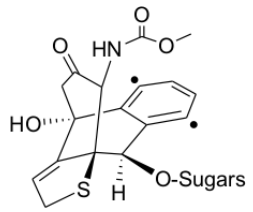


Figure 1.11: Structure responsible for the DNA damaging

The geometrical change which follows the triggering is crucial: the saturation of the former double bond after the reduction is thought to result in the shortening of the cd distance which goes from 3.35 Å to 3.16 Å (MM2 calculations) [8]; the structure presented Figure 1.11 has a half-life of 4sec at 37°C after the triggering. The conversion of the bridgehead sp^2 carbon atom into an sp^3 carbon implies a conformational change in the architecture which lead to a more favourable orientation for the cycloaromatization event, leading to the benzenoid diradical.

If the Bergman cycloaromatization of the drug occurs in the presence of DNA, the diradical compound abstracts a hydrogen atom from deoxyriboses from each strand of the DNA backbone, initiating strand scission: a 5'-hydrogen atom is abstracted from the second nucleotide (Figure 1.12) within the recognition sequence while a 4'-hydrogen atom is abstracted from the nucleotide three base pairs in the 3'-direction on the flanking strand (Figure 1.13) [11][3].

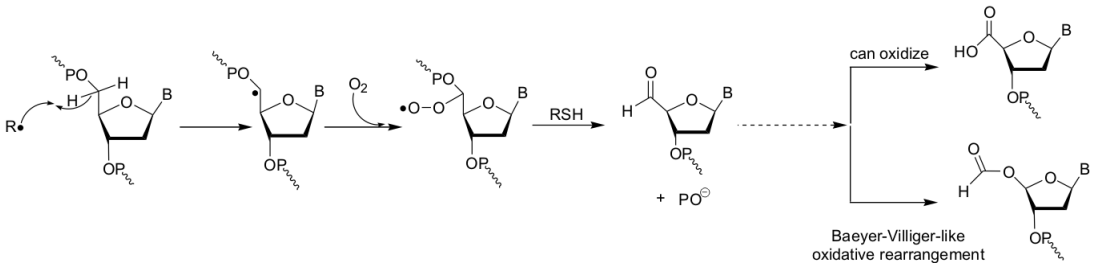


Figure 1.12: Cleavage mechanism at 5'C

All the natural enediynes can make both single and double-stranded cleavages to DNA (with different mechanisms) and add to DNA site specifically. Double-stranded cleavages are more effective because single-stranded cuts are easily repaired by cell. Therefore, the most powerful, toxic and effective natural enediynes are those which make more double-stranded cuts than single-stranded cuts, such as calicheamicin $\gamma_1{}^I$. In the other hand, many

[2]It was shown that calicheamicin $\gamma_1{}^I$ is stable in the presence of DNA if no thiols are present [11].
[3]In some case, the cleavage can result from 4'-hydrogen atom and 1'-hydrogen atom abstraction.

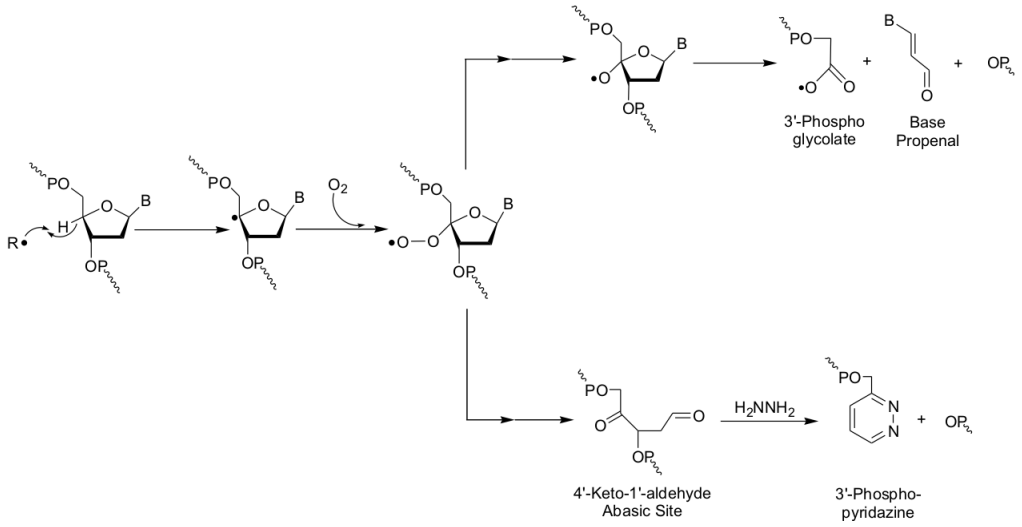


Figure 1.13: Cleavage mechanism at 4'C

synthetic models can cause single-stranded cleavages to DNA, but not double-stranded cleavage.

Calicheamicin $\gamma_1{}^I$ cleaves DNA at oligopyrimidine/oligopurine runs such as TCCT and CTCT mainly, and also at runs such as AGGA, TCCA, ACCT, TCCC and TTTT and their complementary sequences (by orienting its oligosaccharide tail towards the 3'-ends of DNA fragments [6] and without distortion of the enediyne) [11].

Chapter 2

Project

2.1 Explanations and interests of my project

The goal of a part of the research about enediynes is the identification of compounds with greater margins of therapeutic usefulness, as well as the development of a better understanding of the natural products. In an attempt to achieve these ends, a model resembling calicheamicin/esperamicin framework which can exist either in boat or chair conformation were studied and experimental half-lives of 10 min at 21°C were observed. DFT calculations showed that the chair form would cyclize more quickly than the boat form (Figure 2.1) (the difference in transition state energy is of 4.1 kcal/mol) [9].

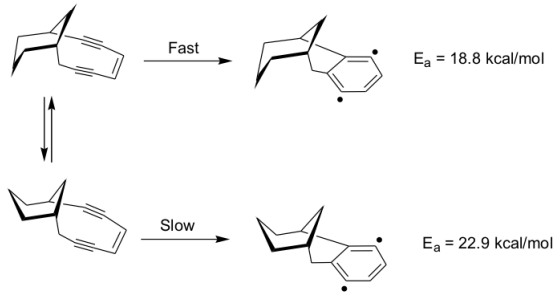


Figure 2.1: Cycloaromatization of boat and chair conformation

Because cyclohexane has a preference for the chair conformer, the conversion between the boat and the chair conformation could serve as a triggering event. In order to allow selective triggering, it is necessary to hold the conformation in the boat form. In his thesis, Lingwyun Wu tried to synthesize the structure as shown in Figure 2.2, which contains a 1,3-bridge to restrict the cyclohexane to the boat confromer, but without a lot of success and with low yields.

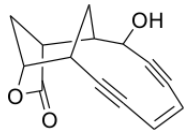


Figure 2.2: Lingwyun Wu's work

Another idea is to synthesize the structure with a 1,4-bridge, as shown in Figure 2.3.

DFT calculations suggest that if the 1,4-bridge consists of two atoms, the molecules will not be stable at room temperature, but if the bridge is larger are more, it could be stable.

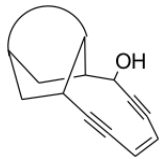


Figure 2.3: Current work

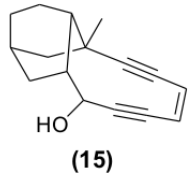
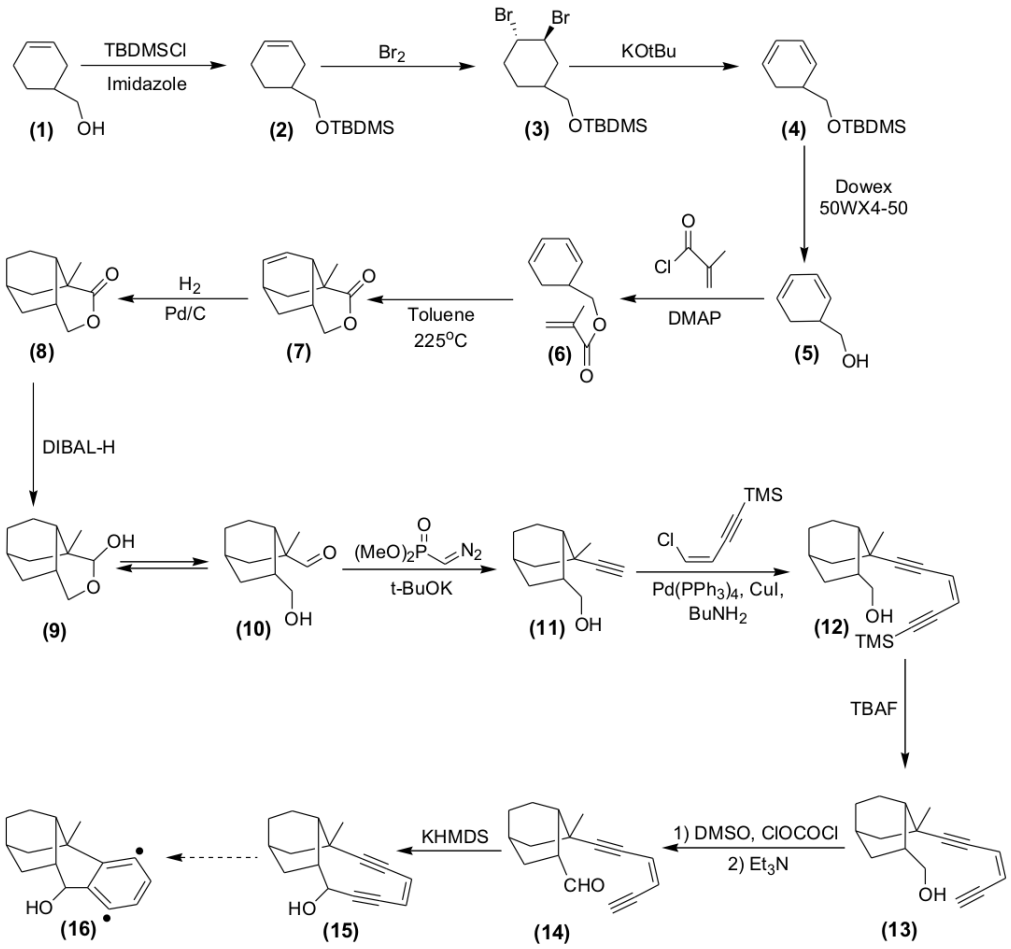


Figure 2.4: Goal of my project

My project was to synthesise the molecule **(15)** (Figure 2.4) and see if it is stable at room temperature or if it will directly lead to the diradical compound. Moreover, the study of the stability of this molecule can help to assess accuracy of calculations for this type of framework.

2.2 Synthesis

In order to synthesise the product **(15)** and to study its stability at room temperature, the following steps were proposed.



2.3 Experimental part

(The full protocol is given in Appendix C.)

In order to synthesize the product **(15)**, I started the synthesis with 5.6 g of 1,2,3,6-tetrahydrobenzylalcohol **(1)** (which had been distilled before); I first protected the alcohol. Then I oxidized the product with bromine and then reduced it by elimination with tert-butoxide. I had some troubles with the distillation under reduce pressure for the purification of **(4)** because of the "bumping". In this synthesis, the first purification were made only after 3 steps[1] so I had a lot of impurities: during the distillation a part of the product were "jumping" into the flask where was already the distilled product, so I had to do the distillation very carefully. I then unprotected the alcohol and made the 5-hydroxymethylcyclohexa-1,3-diene **(5)** but I had only 0.466 g of this product which represents a yield of 8.7% for the three steps, so I tried to make some more product.

I began the synthesis again with some modifications of the procedure (for example I had noticed that for the third step I needed more potassium tert-butoxide than what was written in the procedure I had and the fourth step required more of the ion-exchange resin). The methacryloyl chloride was distilled under reduce pressure (102mmHg, 54°C) and then was kept in the freezer in dry ice and wrapped in aluminium foil to avoid polymerization. The cyclohexa-2,4-dienylmethyl methacrylate **(6)** can also polymerize (Appendix D) so the purification was done just after the reaction (which was performed in aluminium foil). After the purification, the risks of polymerization decrease a lot, and the product can be kept at room temperature for 2-3 days and at 0°C for several months. The hydroquinone was added to avoid polymerization; it was added twice because the crystal was dissolved in the saturated $NaHCO_3$ solution.

The reaction **(6)** to **(7)** is a Diels-Alder reaction. We had to find the optimal time and temperature for this reaction. Lara had already performed this reaction and she had found that the optimal temperature was 225°C (at 215°C after 24 hours there was only starting material and at 250°C all the product decomposed). I first attempted this reaction in a NMR tube with deuterated toluene in order to find the optimal time. The first time I tried the Diels-Alder reaction, I heated at 235°C (because of a mistake) and all my product decomposed. I attempted it again at 225°C and I took NMR spectra after 26, 51, 63, 70, 82 and 92 hours. The goal was to find the optimal time between a high yield and the decomposition of too much of the product. We decided that between 70 and 82 hours was a good time.
For this step, the tube had to be sealed, and I had to wait several days for glassblower to seal it, so I did two things: first Lara gave me 50 mg of the Diels-Alder product **(7)** and I attempted the two next step; then I started the synthesis a third time[2].

I repeated the five first steps while the Diels-Alder reaction was occuring in the NMR tube. Then I attempted the Diels-Alder reaction with all my product. After that I hydrogenated the product and then reduced it with DIBAL-H to make **(9)**. To accomplish the following step I had to synthesize the dimethyl (diazomethyl)phosphonate which is known

[1] The lack of purification for the first two steps explains the very high yields, close to 100%, given in the procedures in Appendix C and the yield of 45% for the third step.

[2] The procedure which is given in Appendix C is the one which was used the third time I performed the synthesis.

as the Seyferth/Gilbert Reagent and I had a lot of trouble making this reagent (another possibility would have been to use dimethyl-1-diazo-2-oxopropylphosphonate).

When I first tried to make the reagent, I used 4-acetamidobenzenesulfonyl azide (p-ABSA) which had been previously synthesized in the lab but I didn't manage to make the product; I tried a second time, and I still couldn't synthesize the reagent. I took NMR spectra of all the compounds I had used, and I realised that the p-ABSA wasn't pure (it may have degraded). So we had to order N-acetylsulfanilyl chloride and then make p-ABSA which took me some time.

I didn't finish the synthesis and I stopped it at the eighth step (product **(9)**); I purified this compound once but it wasn't clean enough, which is why no yield is given in the procedure.
The next steps would have been first a Sonogashira coupling [12], then the deprotection of the terminal alkyne, followed by a Swern oxidation and the cyclization of the enediyne moiety which can be performed in different ways: Kende [13] and Danishefsky [14] described the cyclization with different procedures (the first one with $LiN[Si(Me)_3]_2$ and the other one with $KN[Si(Me)_3]_2$).

Conclusion

Today, the enediyne antitumor antibiotics are appreciated for several reasons, such as their remarkable biological activities, their fascinating mode of action and their new molecular architecture [15]. Enediynes and their relatives are in the forefront of chemical, biological and medical research [6]. Jones *et al.* [1] studied the action of designing enediynes towards specific proteins, which could increase the interest in enediynes.

Current researches are focused on developing new frameworks which could increase margins of therapeutic usefulness. In this project, I tried to synthesize one of these framework but without success because of a lack of time. This work was linked with Lara's one, who is currently trying to synthesize a three-atom 1,4-bridged enediyne (with an ester moiety in the bridge). Other project exists such as a four-atom 1,4-bridge made with C=N–N=C which could easily be opened.

But some questions about enediynes still need some answers such as for example how does binding to DNA initiate activation, how does nature make enediynes (this question is not precisely determined) [15], and how does the producing organism protect itself from such a dangerous molecule [6] ?

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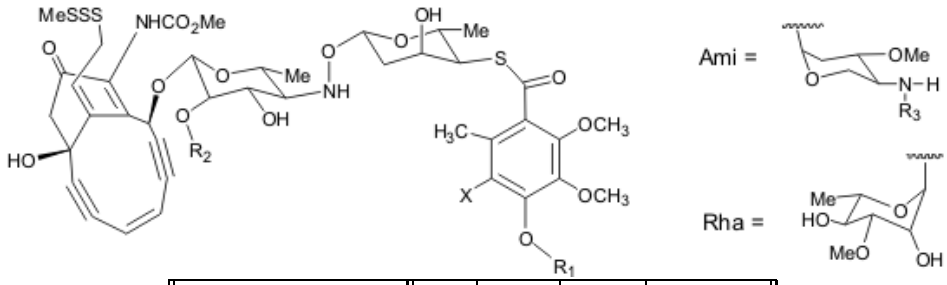
Appendix A

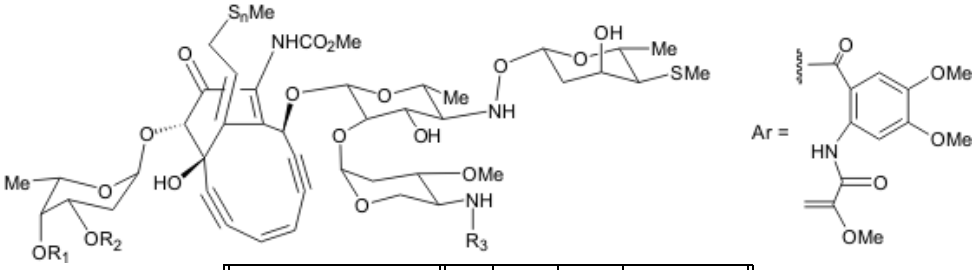
Some natural enediynes

Calicheamicins



Calicheamicin	X	R_1	R_2	R_3
β_1^{Br}	Br	Rha	Ami	$CHMe_2$
γ_1^{Br}	Br	Rha	Ami	Et
α_2^{I}	I	H	Ami	Et
α_3^{I}	I	Rha	H	–
β_1^{I}	I	Rha	Ami	$CHMe_2$
γ_1^{I}	I	Rha	Ami	Et
δ_1^{I}	I	Rha	Ami	Me

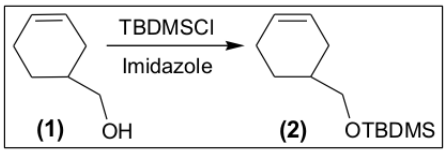
Esperamicins

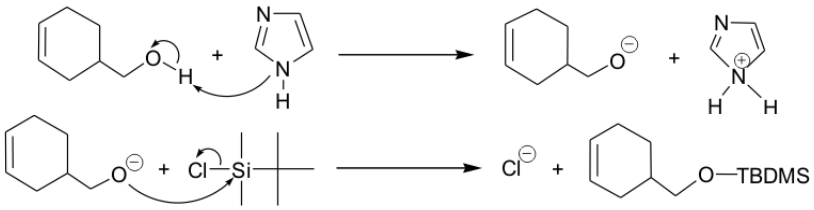


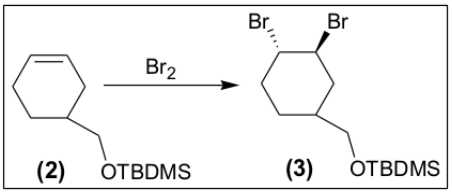
Esperamicin	n	R_1	R_2	R_3
A_1	3	H	Ar	$CHMe_2$
A_{1b}	3	H	Ar	Et
A_{1c}	3	H	Ar	Me
P	4	H	Ar	$CHMe_2$
A_2	3	Ar	H	$CHMe_2$
A_{2b}	3	Ar	H	Et
A_{2c}	3	Ar	H	Me

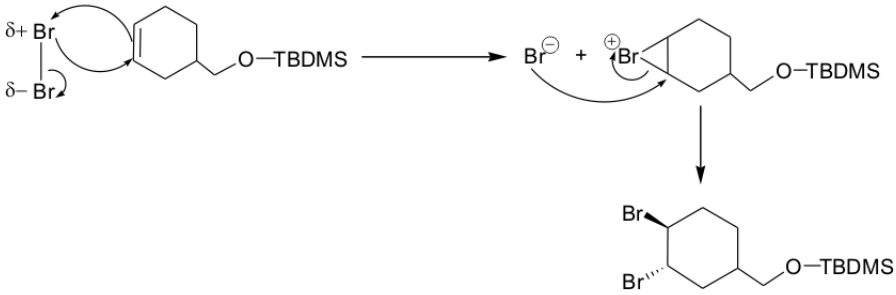
Appendix B

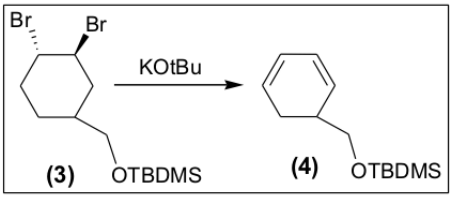
Mechanisms

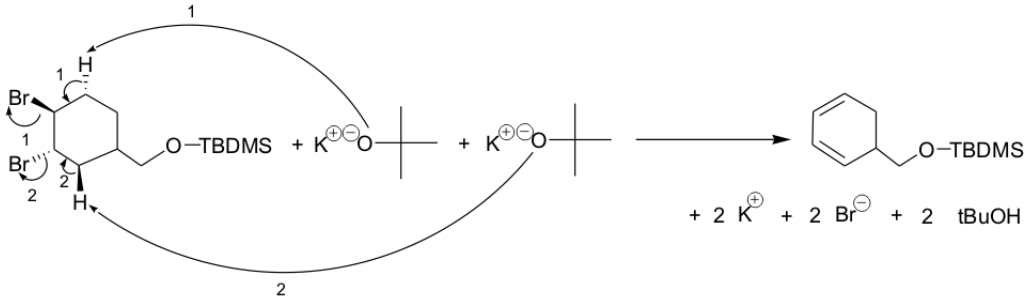


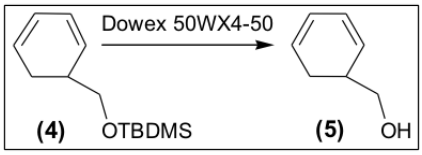


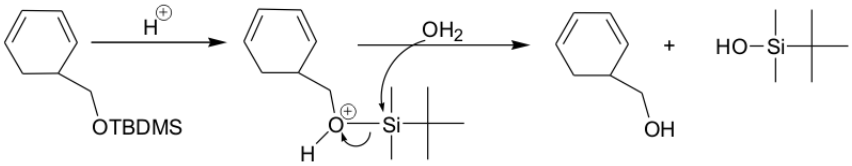


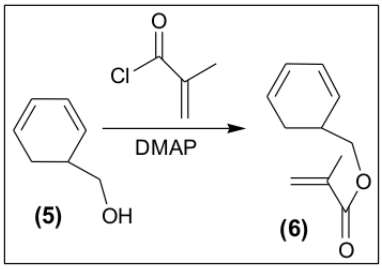


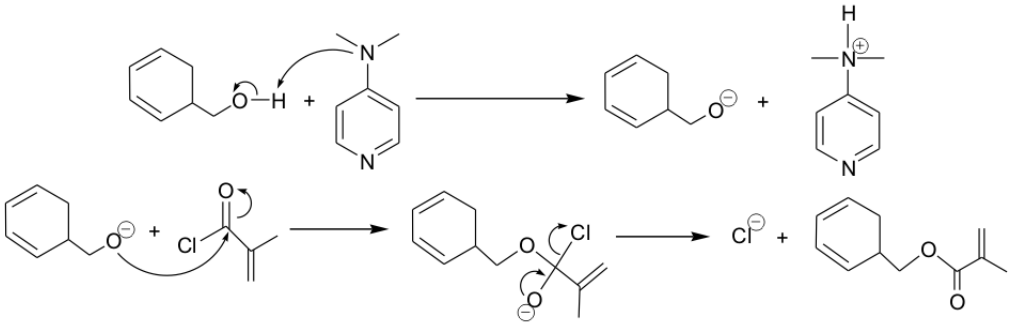


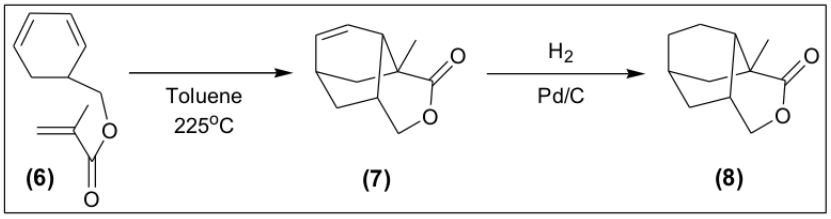

Br
Br
H
H
O–TBDMS
+ 2 K⊕ + 2 Br⊖ + 2 tBuOH

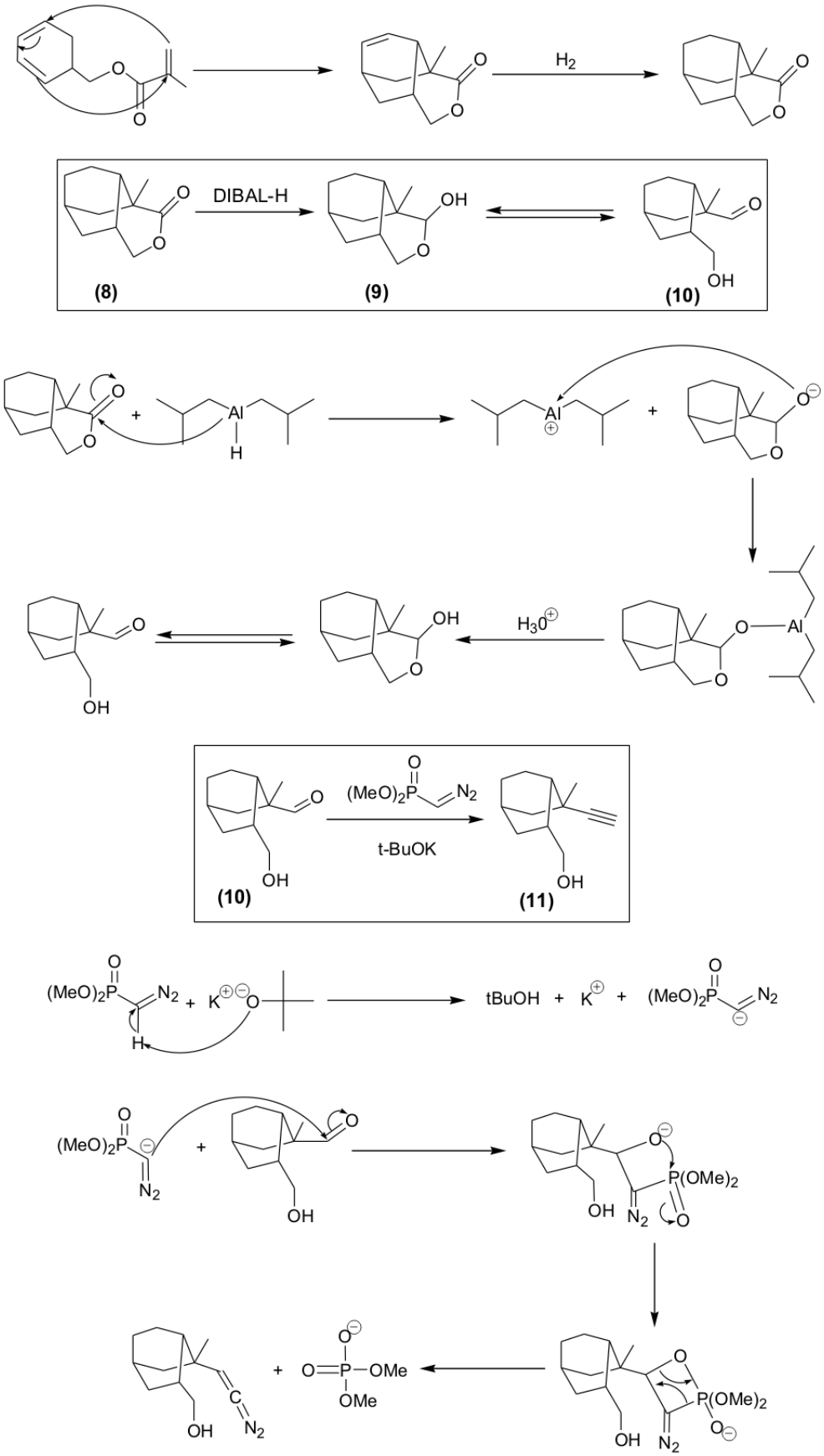

Dowex 50WX4-50
(4)
OTBDMS
(5)
OH

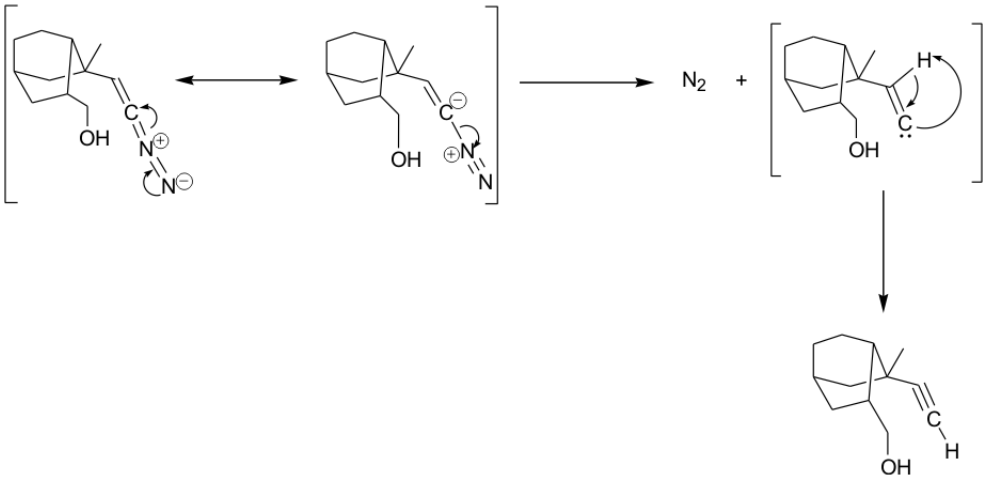

H⊕
OTBDMS
OH2
Si
H
OH
HO–Si

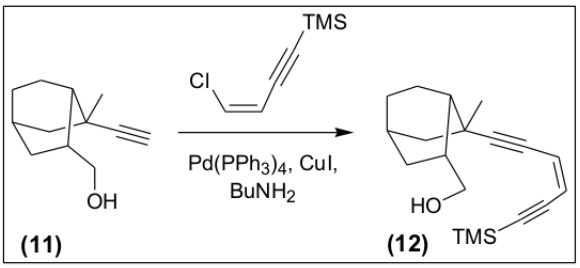

Cl
DMAP
(5)
OH
(6)


O–H
N
H
Cl
Cl⊖

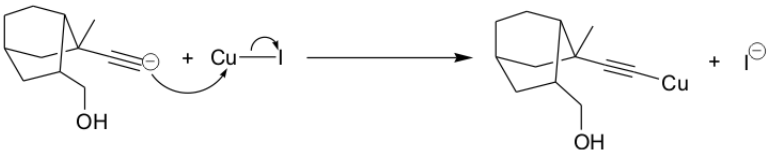

Toluene
225°C
H2
Pd/C
(6)
(7)
(8)

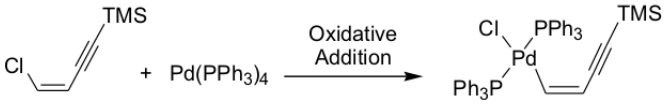

H2
DIBAL-H
(8)
(9)
(10)
Al
H
H30⊕
t-BuOK
(10)
(11)
(MeO)2P
N2
tBuOH
K⊕
P(OMe)2
OMe
OH

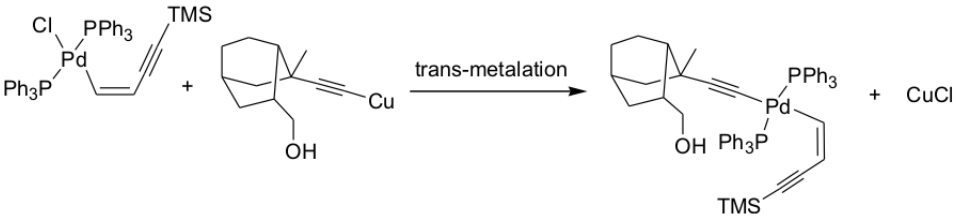

OH
C
N
N
OH
C
N
N
N2
+
H
C
OH
C
H
OH

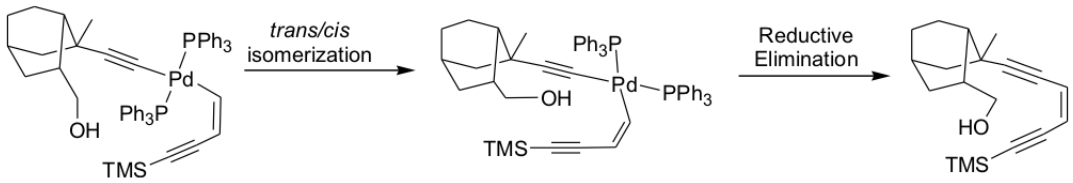

TMS
Cl
Pd(PPh3)4, CuI,
BuNH2
OH
(11)
HO
TMS
(12)

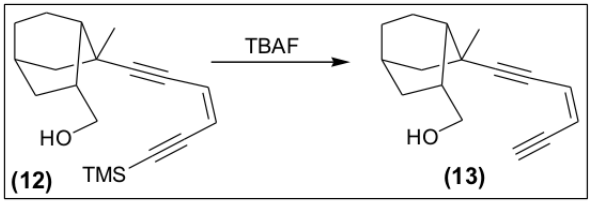
H
+ BuNH2
BuNH3
+
OH
OH

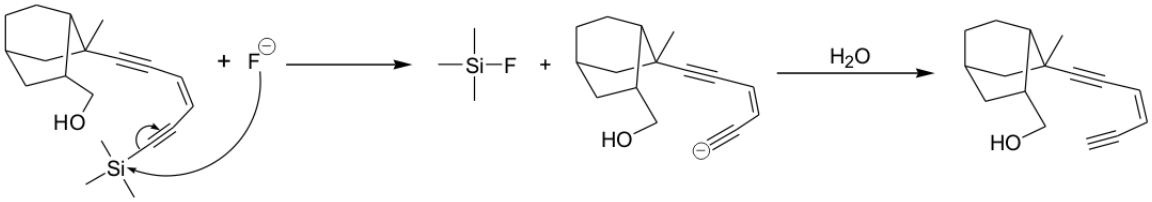

+ Cu
I
OH
Cu
+ I
OH

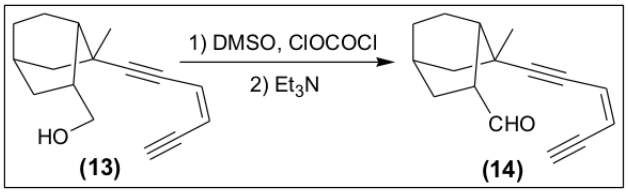

TMS
Cl
+ Pd(PPh3)4
Oxidative
Addition
Cl
PPh3
TMS
Pd
Ph3P

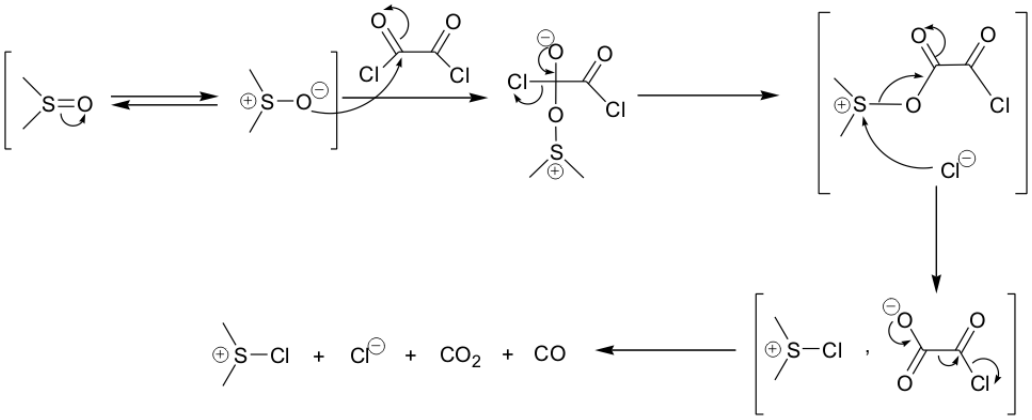

Cl
PPh3
TMS
Pd
Ph3P
+
Cu
OH
trans-metalation
PPh3
Pd
OH
Ph3P
TMS
+ CuCl

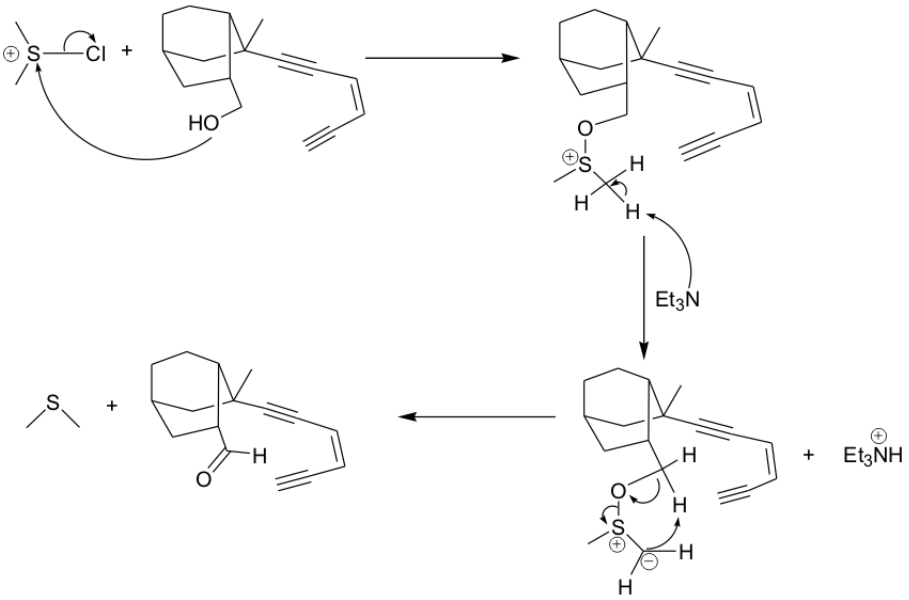

PPh3
Pd
Ph3P
OH
TMS
trans/cis
isomerization
Ph3P
Pd
PPh3
OH
TMS
Reductive
Elimination
HO
TMS

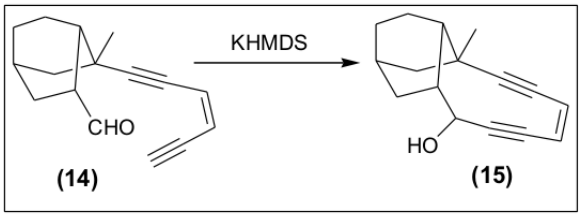

TBAF
HO
TMS
(12)
HO
(13)

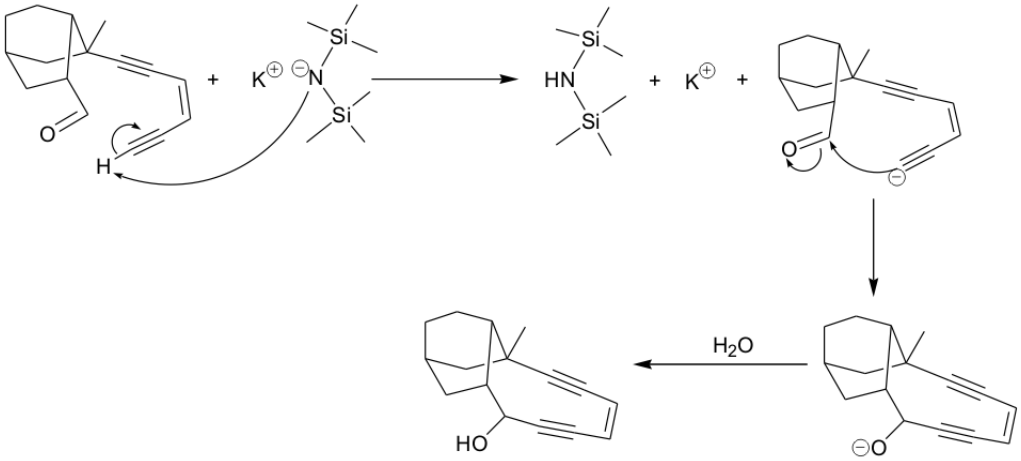

+ F
HO
Si
Si—F
+
HO
H2O
HO


1) DMSO, ClOCOCl
2) Et3N
HO
(13)
CHO
(14)


S=O
S—O
Cl
Cl
O
O
Cl
Cl
O
O
S
Cl
O
O
S
O
Cl
Cl
S—Cl
Cl
O
O
O
Cl
S—Cl
+
Cl
+
CO2
+
CO


S
Cl
HO
O
S
H
H
H
Et3N
S
O
H
H
O
S
H
H
H
+ Et3NH

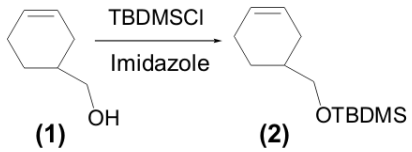

KHMDS
CHO
HO
(14)
(15)


O
H
K
N
Si
Si
HN
Si
K
O
H2O
HO
O

Appendix C

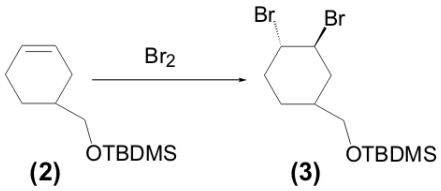
Procedure

4-(tert-Butyldimethylsilyloxymethyl)-cyclohex-1-ene (2)



To a stirred solution of 7.78 g (69.36 mmol) of 1,2,3,6-tetrahydrobenzylalcohol **(1)** and 9.5 g (139.54 mmol) of imidazole in 70 mL of DMF was added 12.59 g (83.53 mmol) of TBDMSCl. The solution was stirred 24 hours, then the mixture was treated with 500 mL of brine, and extracted 4 times with 250 mL of hexane; the combined organic layers were washed with 500 mL of brine, dried over $MgSO_4$ and evaporated in vacuo to obtain 15.31 g (67.61 mmol) of **(2)** (yield: 97.5 %; NMR spectrum **1**[1]). [22]

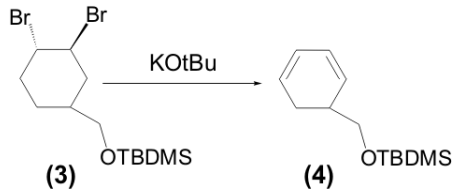
trans-1,2-Dibromo-4-(tert-butyldimethylsilyloxymethyl)-cyclohexane (3)



To a stirred solution of 15.31 g (67.61 mmol) of **(2)** in 110 mL of CCl_4, was added dropwise a solution of 3.5 mL (68.1 mmol) of bromine (d=3.11) in 9 mL of CCl_4. The solution was stirred 1 hour 30 min. Then the mixture was diluted in 370 mL of CH_2Cl_2, washed with 370 mL of 10% $Na_2S_2O_3$ solution and then with 370 mL of water. The organic layer was dried over $MgSO_4$, and evaporated in vacuo to obtain 26.09 g (67.55 mmol) of **(3)** (yield: 99.9 %; NMR spectrum **2**). [22]

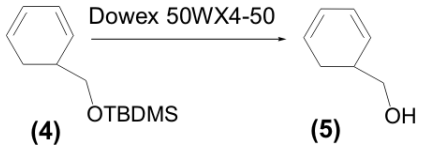
5-(tert-Butyldimethylsilyloxymethyl)-cyclohexa-1,3-diene (4)

[1] All the NMR spectras were made on a Mercury 300 MHz



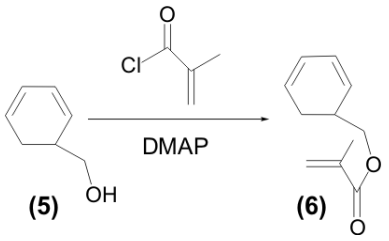
To a stirred 0°C solution of 26.09 g (67.55 mmol) of dibromide **(3)** and 0.65 g (1.61 mmol, 0.024 equiv) of Aliquat 336 in 150 mL of dry THF under argon was added 24.2 g (216 mmol, 3.2 equiv) of KOtBu. A yellow precipitate appeared. After 5 min, the cooling bath was removed, and the mixture was stirred 2 hours 30 min. The slurry was evaporated in vacuo, diluted with 100 mL of hexane, and washed with 100 mL of a saturated NH_4Cl solution. The aqueous layer was extracted with 70 mL of hexane, and then the organic layers were combined, washed with 100 mL of water, dried over $MgSO_4$ and evaporated in vacuo to give **(4)**. The product was distilled under reduce pressure (2 mmHg, 60-70°C) to get 6.961 g (31.02 mmol) of **(4)** (yield: 45.9 %; NMR spectrum **3**). [22]

5-Hydroxymethylcyclohexa-1,3-diene (5)



6.834 g (30.45 mmol) of **(4)** were dissolved in 70 mL of methanol, and treated with 10.5 g of Dowex 50WX4-50[2]. The mixture was stirred strongly 3 hours. The resin was filtered and washed with methanol; the solution was evaporated in vacuo. The crude product was distilled under reduce pressure (2 mmHg, 65°C) to obtain 2.402 g (21.8 mmol) of **(5)** (yield: 71.6 %; NMR spectrum **4**). [22]

Cyclohexa-2,4-dienylmethyl methacrylate (6)

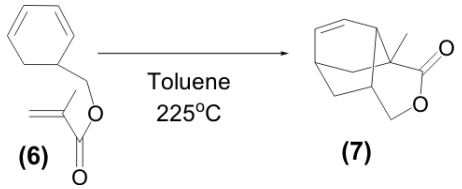


A solution of 2.499 g (22.69 mmol) of 5-Hydroxymethylcyclohexa-1,3-diene **(5)** in 200 mL of CH_2Cl_2 was wrapped in aluminium foil and cooled to 0°C. Under argon, 5.694 g (46.61 mmol, 2.05 equiv) of DMAP in 50 mL of CH_2Cl_2 was added. To the stirred solution was added 2.64 mL (27.28 mmol, 1.2 equiv) of methacryloyl chloride (d=1.08) dropwise. The solution was stirred at 0°C for 1 hour, 1 crystal of hydroquinone was added, the mixture was diluted with 600 mL of ether and then washed with 300 mL of saturated $NaHCO_3$ solution and with 300 mL of brine. A crystal of hydroquinone was added again, then the solution was evaporated in vacuo. The product was purified by column chromatography

[2] Dowex 50WX4-50 is a strong ion-exchange resin made on polystyrene with sulfonic acid

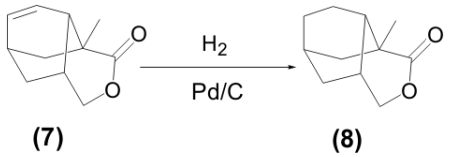
(silica gel; 30:1 hexane:EtOAc; visualized with anisaldehyde) to get 1.097 g (6.15 mmol) of **(6)** (yield: 27.13 %; NMR spectrum **5**). [23]

3-Methyl-5-oxa-tricyclo[5.3.1.03,8]undec-9-en-4-one (7)



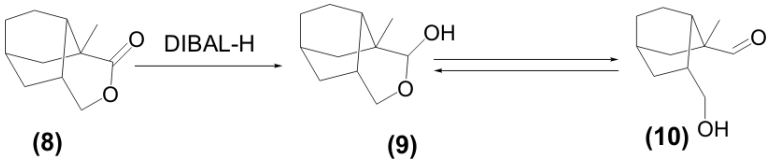
In a sealed glass tube, 1.00 g of cyclohexa-2,4-dienylmethyl methacrylate **(6)** were heated to 225°C in 10 mL of toluene for 79 hours. The heating apparatus was a graduated cylinder wrapped with heating tape and filled with sand which was placed in a beaker filled with sand for insulation. The product was purified by column chromatography (silica gel; 12:1 hexane:EtOAc; visualized with $KMnO_4$) to obtain 0.266 g (1.49 mmol) of **(7)** (yield: 26.6 %; NMR spectrum **6**). [23]

3-Methyl-5-oxa-tricyclo[5.3.1.03,8]undecan-4-one (8)



0.300 g of 5% Pd/C was placed under an H_2 atmosphere. To the Pd was added 0.266 g (1.49 mmol) of the Diels-Alder product **(7)** in 15 mL of methanol. The mixture was stirred 1 hour, filtered, and the palladium was washed with methanol. After evaporation in vacuo, the product was purified by column chromatography (silica gel; 1,5:1 ether:hexane; visualized with $KMnO_4$) to obtain 150 mg (0.83 mmol) of **(8)** (yield: 56.4 %; NMR spectrum **7**). [23]

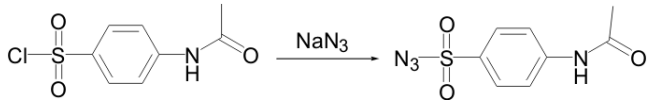
3-Methyl-5-oxa-tricyclo[5.3.1.03,8]undecan-4-ol (9)



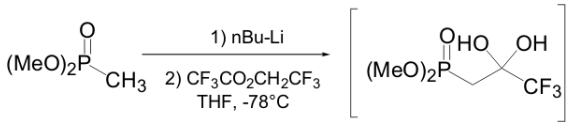
To a solution of lactone **(8)** (150 mg, 0.83 mmol) in toluene (4 mL) at -78°C was added DIBAL-H (0.22 mL, 1.1 mmol, d=0.701). After 20 min the reaction mixture was quenched with 20 mL of acetone, warmed to room temperature, diluted with 15 mL of a saturated aqueous potassium sodium tartrate solution, stirred for 2 hours, and diluted with 15 mL of water and then with 15 mL of brine. The aqueous phase was extracted three times with ether (3x35 mL) and the combined organic layers were washed with 60 mL of brine and dried over $MgSO_4$. The solution was then evaporated in vacuo to obtain **(9)** (which is in equilibrium with **(10)**) which was purified by column chromatography (silica gel; 1,25:1

ether:hexane; visualized with $KMnO_4$) (NMR spectrum **8**). *[24][3]

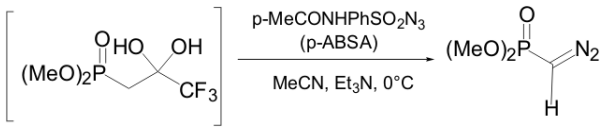
Dimetyl (diazomethyl)phosphonate



Under argon, 2.85 g (43.8 mmol) of sodium azide was dissolved in 500 mL of acetone. The mixture was cooled to 0°C and 10.0 g (42.75 mmol) of N-acetylsulfanilyl chloride was added slowly (ca. 5 min) in small portions. The reaction was allowed to warm to room temperature and stir for 48h. The slurry was filtered and concentrated by rotary evaporation to give p-acetamidobenzenesulfonyl azide (p-ABSA) which was used without further purification in the next step. [25]



In a flame-dried flask under argon was put 20 mL of dry THF. Dimethyl methylphosphonate (1 mL, 9.3 mmol, d=1.145) was added, and the mixture was cooled to -78°C. n-Butyllithium (3.8 mL of a 2.50 M solution in hexanes, 9.5 mmol) was added over 5 min, and the solution was allowed to stir at -78°C for 30 min. 2,2,2-Trifluoroethyl trifluoroacetate (1.9 mL, 14.1 mmol, d=1.46) was added rapidly (1-2 s), and the resulting mixture was stirred at -78°C for 15 min. The solution was then warmed to room temperature. The crude reaction mixture was partitioned between diethyl ether (125 mL) and 3 % HCl (5 mL). The ether layer was washed with saturated $NaHCO_3$ (5 mL) and saturated NaCl (5 mL), dried with $MgSO_4$, and concentrated by rotary evaporation. [25]

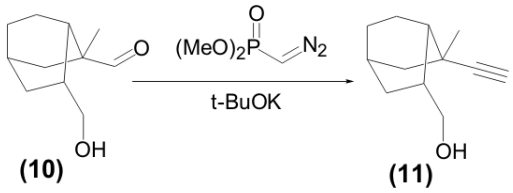


The crude sample was immediately dissolved in dry CH_3CN (20 mL). 4-Acetamidobenzenesulfonyl azide (p-ABSA) (2 g, 8.35 mmol) was added, and the solution was cooled to 0°C. Triethylamine (1.16 mL, 8.35 mmol, d=0.726) was slowly added (ca. 5 min). The mixture was allowed to warm to room temperature and was stirred overnight. Solvent was removed by rotary evaporation from the resulting slurry, which contained precipitated 4-acetamidobenzenesulfonamide. The residual orange oily solid was suspended in $CHCl_3$ and filtered through a coarse glass frit to remove 4-acetamidobenzenesulfonamide, which was washed with a small portion of additional $CHCl_3$. Column chromatography of the concentrated filtrate (silica gel; EtOAc; visualized with I_2) gave dimethyl(diazomethyl)phosphonate. [25]

[3]The * means that the procedure was modified for my substrate; when the * does not appear it means that I used the exact same substrate as in the procedure.

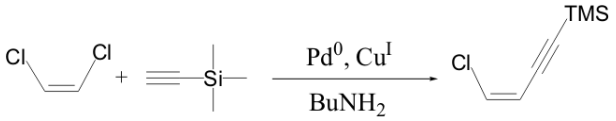
(The following steps have not been attempted and the procedures are just proposed.)

(6-Ethynyl-6-methyl-bicyclo[2.2.2]oct-2-yl)-methanol (11)



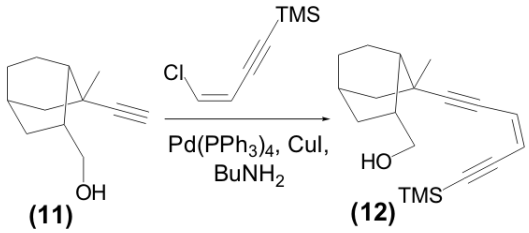
To a THF (3 mL) solution of KOtBu (112 mg, 1 mmol, 1.2 equiv) at -78°C was added $N_2CHP(O)(OMe)_2$ (150.1 mg, 1 mmol, 1.2 equiv) dropwise over 5 min under argon. The solution was stirred for 30 min at -78°C, and then aldehyde **(10)** (151.3 mg, 0.83 mmol, 1 equiv) in THF (3 mL) was added dropwise over 10 min. After being stirred at -78°C for 9 hours, the mixture was warmed to 23°C and stirred an additional 1 hour. It was then quenched by addition of saturated aqueous ammonium chloride solution (6 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3x6 mL). The combined organic solution was washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by flash column chromatography (silica gel; hexane:ether) to obtain **(11)**. *[26]

(Z)-1-chloro-4-trimethylsilyl-1-buten-3-yne



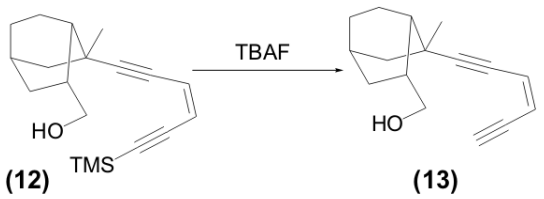
To a solution of (Z)-1,2-dichloroethene (5.8 g, 60 mmol) in benzene (40 mL), $Pd(PPh_3)_4$ (1.73 g, 1.5 mmol), n-butylamine (4.4 g, 60 mmol) and terminal alkyne (30 mmol) was rapidly added. After 10 min, cuprous iodide (0.57 g, 3 mmol) was introduced into the stirred solution; the reaction is exothermic and the flask was cooled in a water bath. After 5 hours at room temperature, the mixture was hydrolyzed with a saturated aqueous solution of ammonium chloride and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated by rotary evaporation. Most of triphenylphosphine was precipitated with a minimum volume of ether/pentane (50/50). The crude product was purified by flash column chromatography to obtain (Z)-1-chloro-4-trimethylsilyl-1-buten-3-yne. [27]

[6-Methyl-6-(6-trimethylsilanyl-hex-3-ene-1,5-diynyl)-bicyclo-[2.2.2]oct-2-yl]-methanol (12)



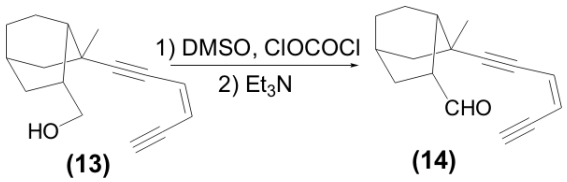
Compound **(11)** (148 mg, 0.83 mmol) and CuI (47.1 mg, 0.166 mmol, 20 mol-%) were placed in an oven-dried flask. The system was purged with argon and 2 mL of benzene was added. The mixture was cooled to -5/-10°C and $BuNH_2$ (0.367 mL, 2.51 mmol, 3 equiv) was added, followed by (Z)-1-chloro-4-trimethylsilyl-1-buten-3-yne (196.9 mg, 1.24 mmol, 1.5 equiv) and $Pd(PPh_3)_4$ (71.4 mg, 0.042 mmol, 5 mol-%) in sequence. The mixture was warmed to 23°C and allowed to stir for 24 hours. The reaction was quenched by adding saturated aqueous ammonium chloride solution (8 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3x8 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by column chromatography (silica gel; ether:hexane) to provide **(12)**. *[26]

(6-Hex-3-ene-1,5-diynyl-6-methyl-bicyclo[2.2.2]oct-2-yl)-methanol (13)



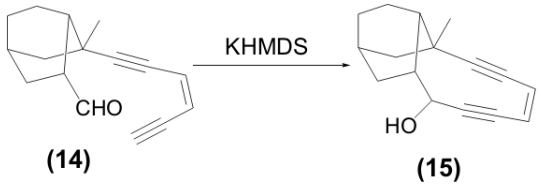
To a solution of **(12)** (249.4 mg, 0.83 mmol) in THF (180 mL) was added TBAF (1.63 mL, 1.63 mmol) at -78°C. The reaction mixture was stirred for 3 hours at -78°C and quenched by adding saturated aqueous ammonium chloride solution at -78°C. After being warmed to 23°C, the mixture was extracted with CH_2Cl_2 (3x350 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation to obtain **(13)**. *[26]

6-Hex-3-ene-1,5-diynyl-6-methyl-bicyclo[2.2.2]octane-2-carbaldehyde (14)



To a solution of $(COCl)_2$ (0.46 mL, 2.0 M, 0.91 mmol, 1.1 equiv) in CH_2Cl_2 (2.5 mL) at -78°C under argon was added DMSO (0.13 mL, 1.82 mmol, 2.2 equiv) dropwise over 5 min. The mixture was stirred at -78°C for 15 min, then a solution of the alcohol **(13)** (189.5 mg, 0.83 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 5 min. After the mixture was stirred for 15 min at -78°C, triethylamine (0.3 mL, 2.48 mmol, 3 equiv) was added and warmed to 23°C over 30 min. The reaction mixture was quenched by adding saturated aqueous ammonium chloride solution (3.5 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3x3.5 mL). The combined organic layers was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (silica gel; ether:EtOAc) to provide **(14)**. *[26]

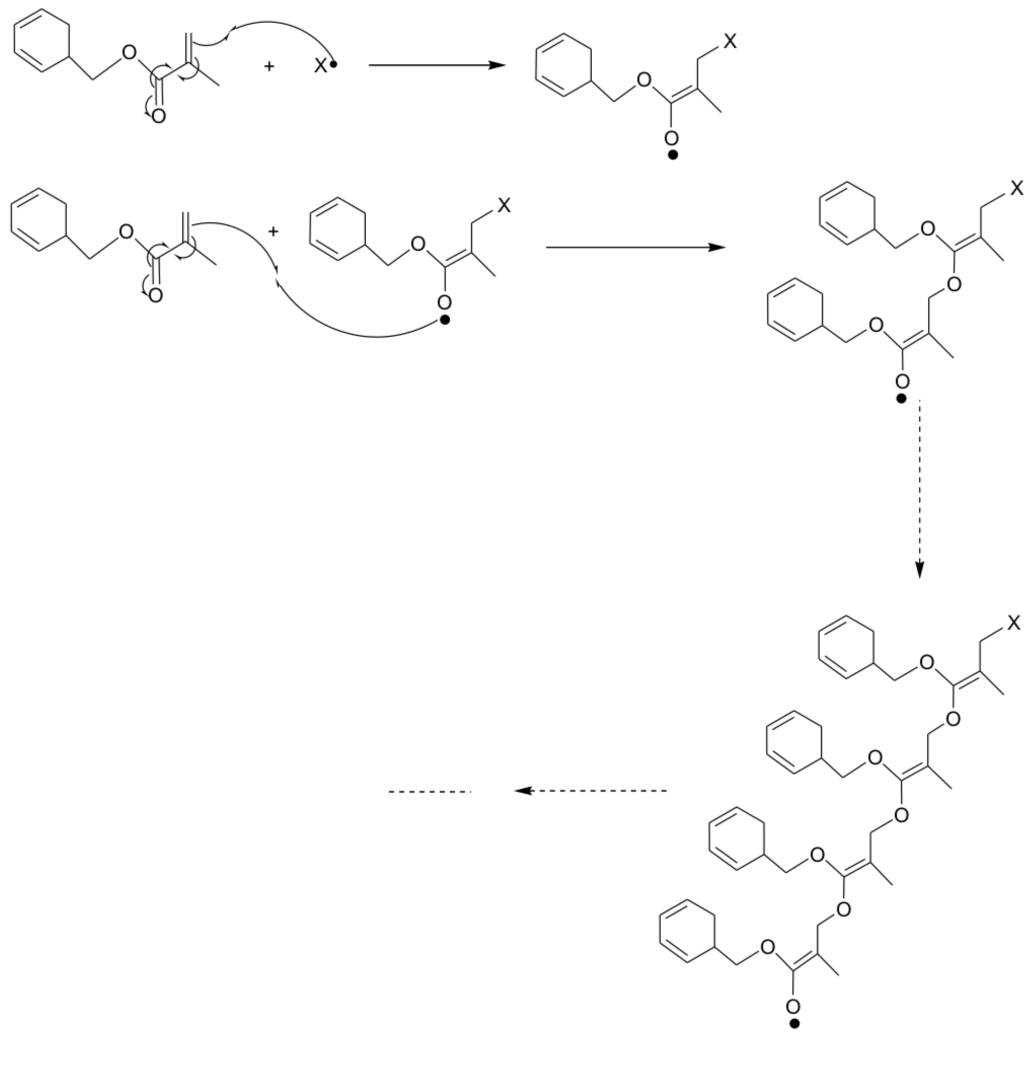
11-Methyl-tricyclo[9.3.1.03,12]pentadec-7-ene-5,9-diyn-4-ol (15)



The enediyne was inversely added to a dilute solution of KHMDS in THF and the solution was stirred 6 hours. After washing the solution with a saturated ammonium chloride solution, the product was purified by column chromotagrphy (silica gel). *[13]

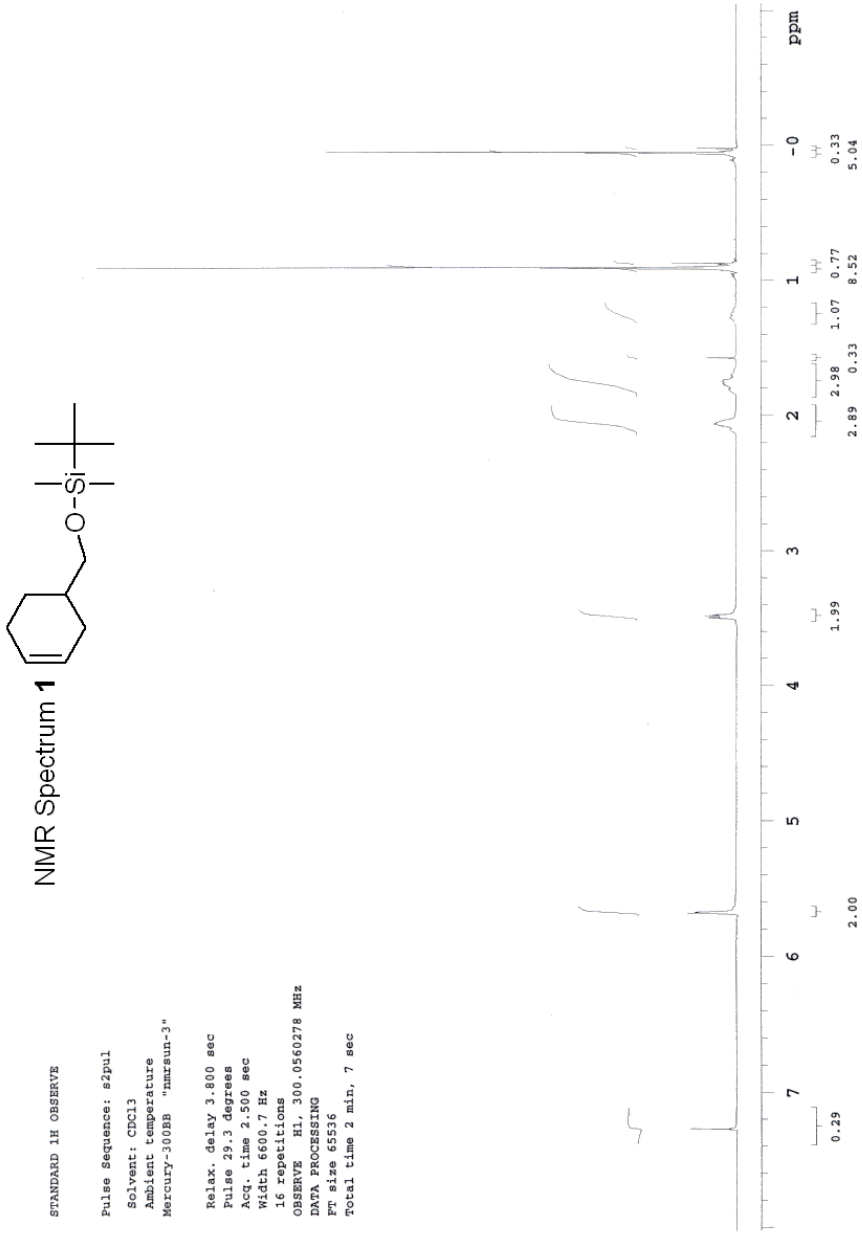
Appendix D

Polymerization of (6)

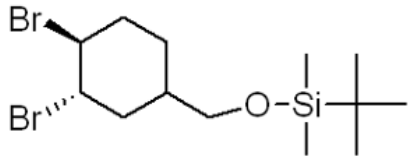


Appendix E

NMR spectra



NMR Spectrum **2**



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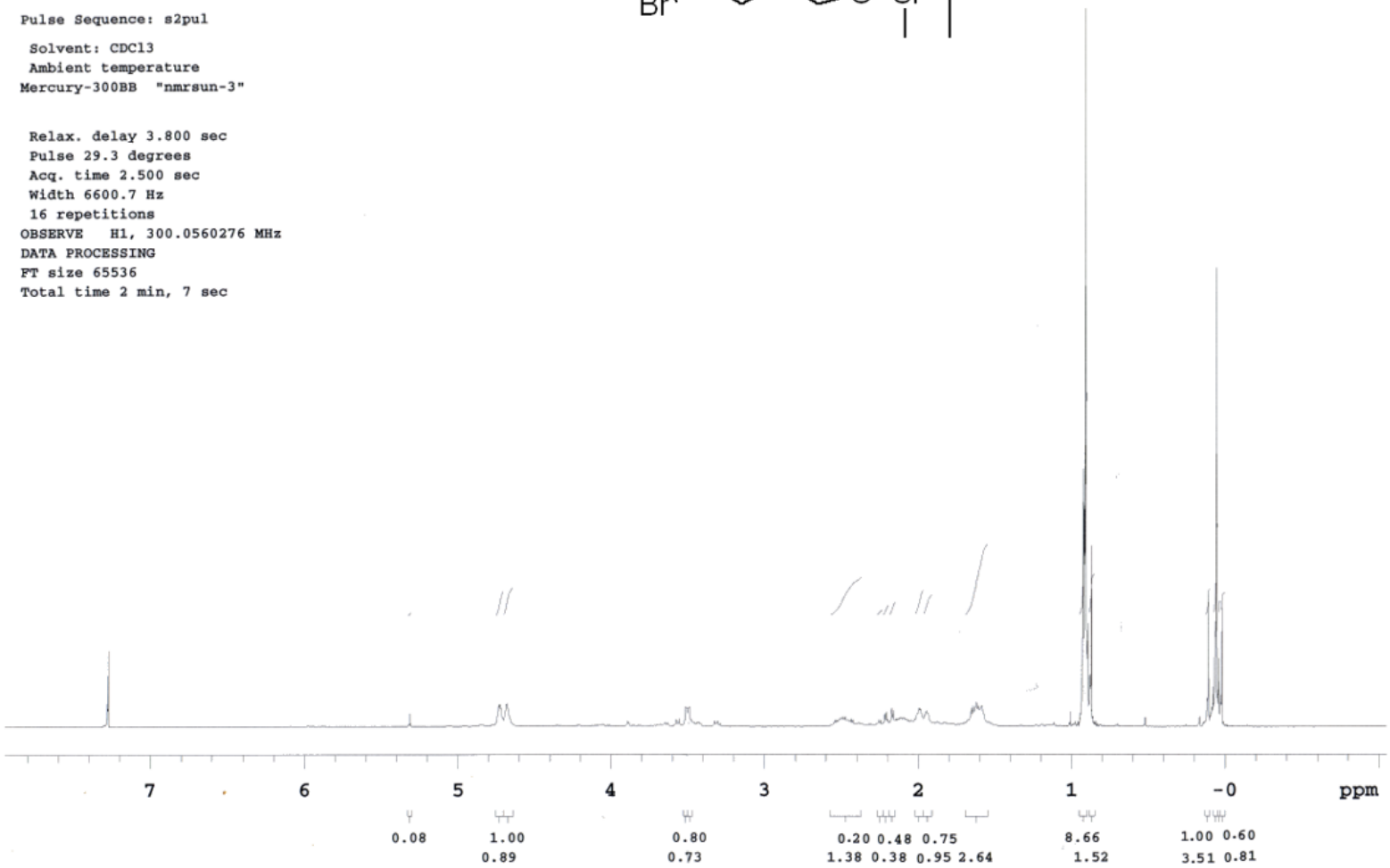
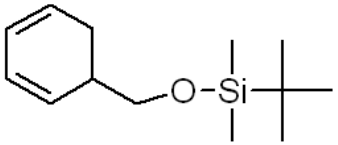


NMR Spectrum **3**



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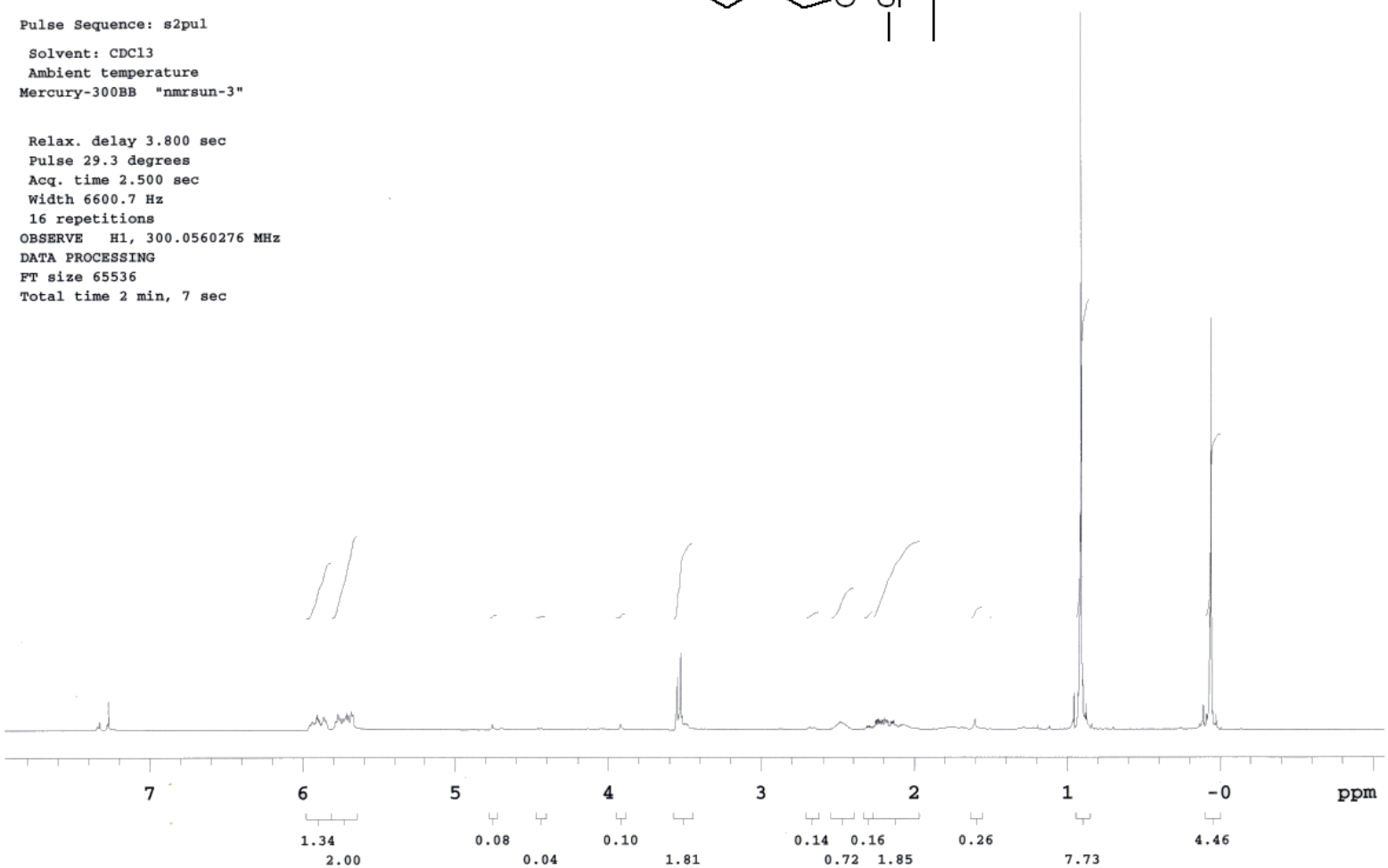
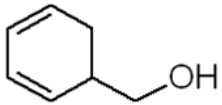


NMR Spectrum **4**



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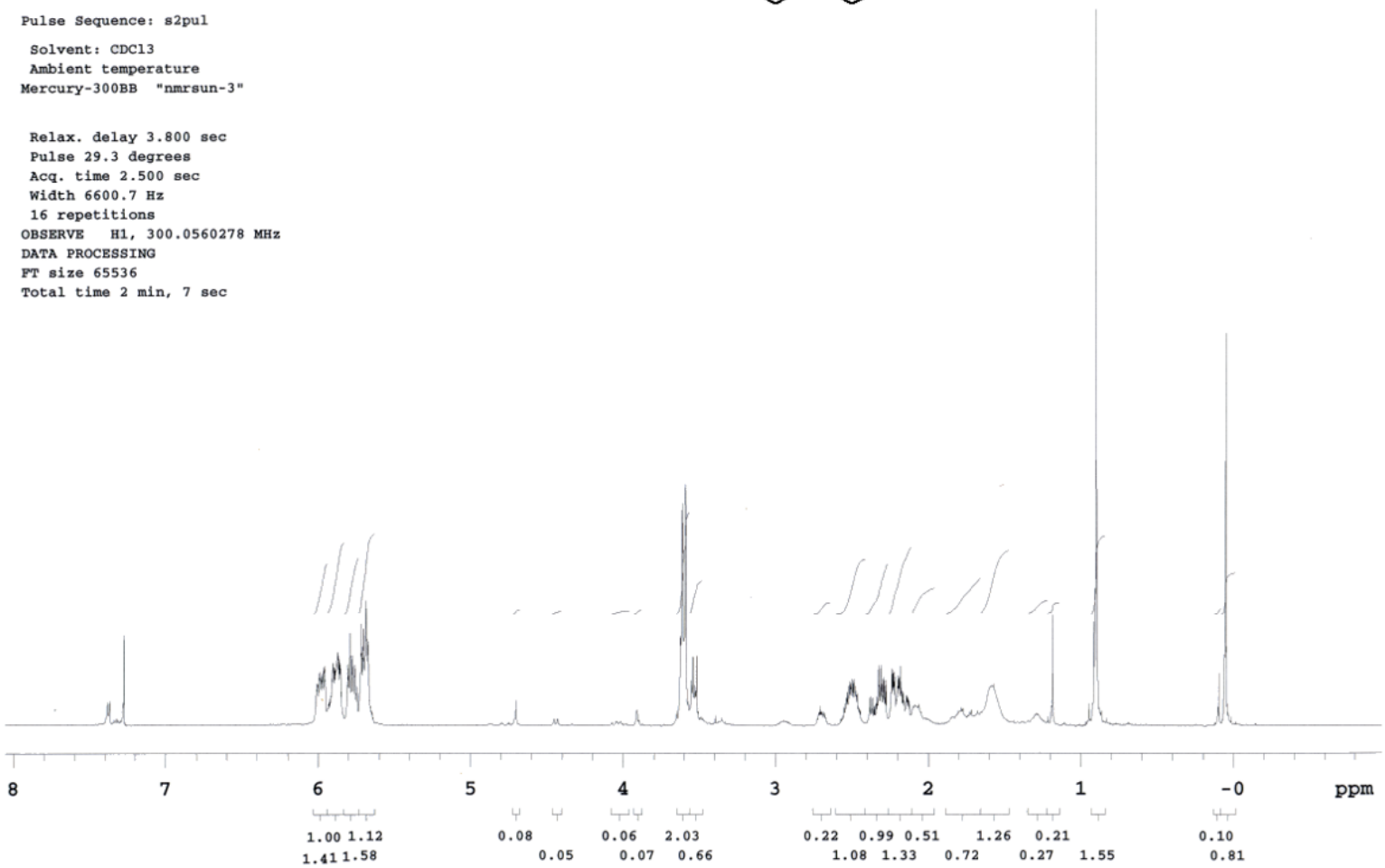
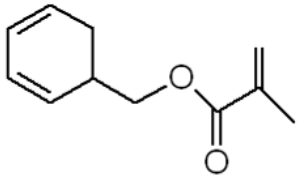


NMR Spectrum **5**



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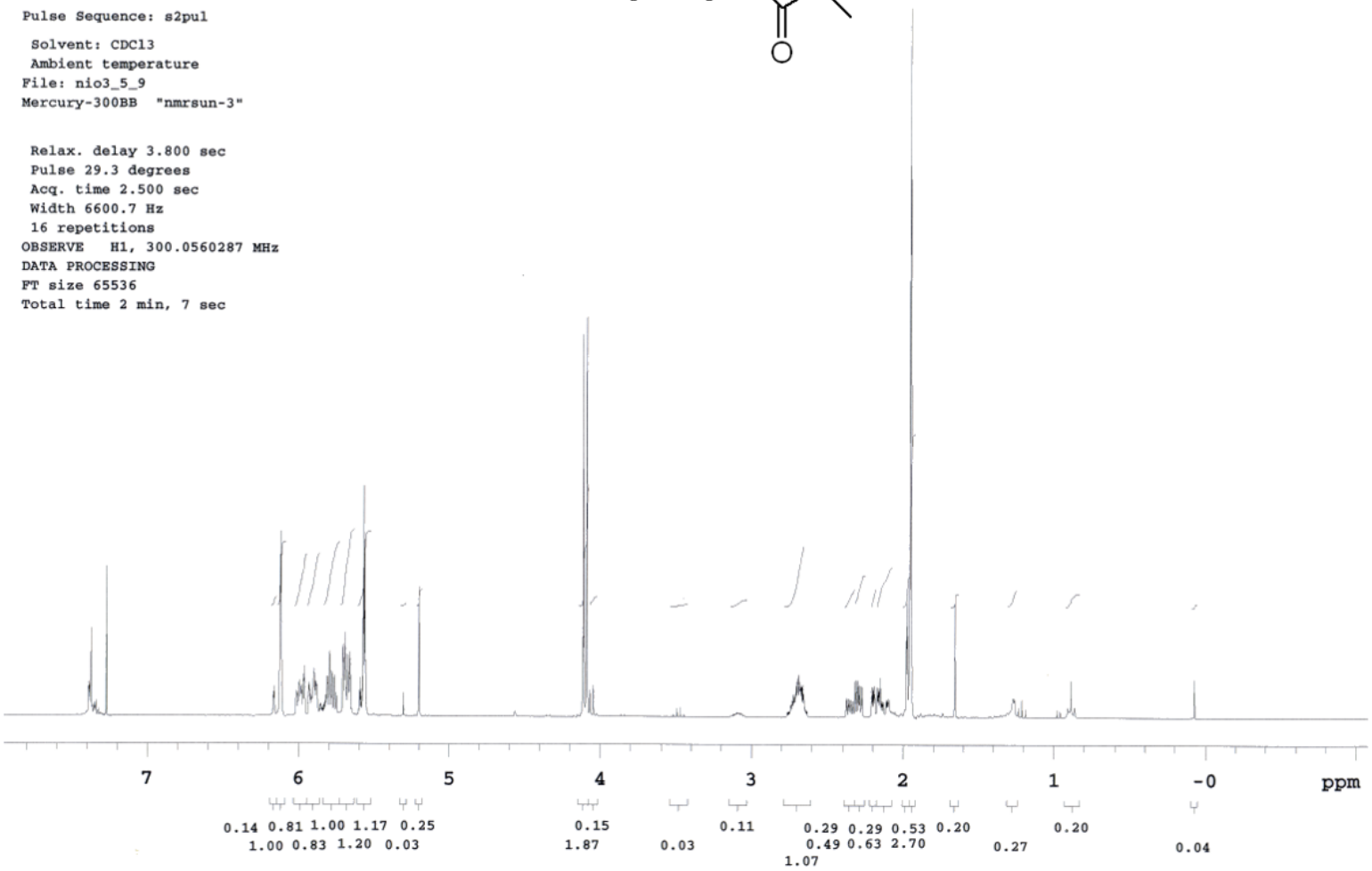
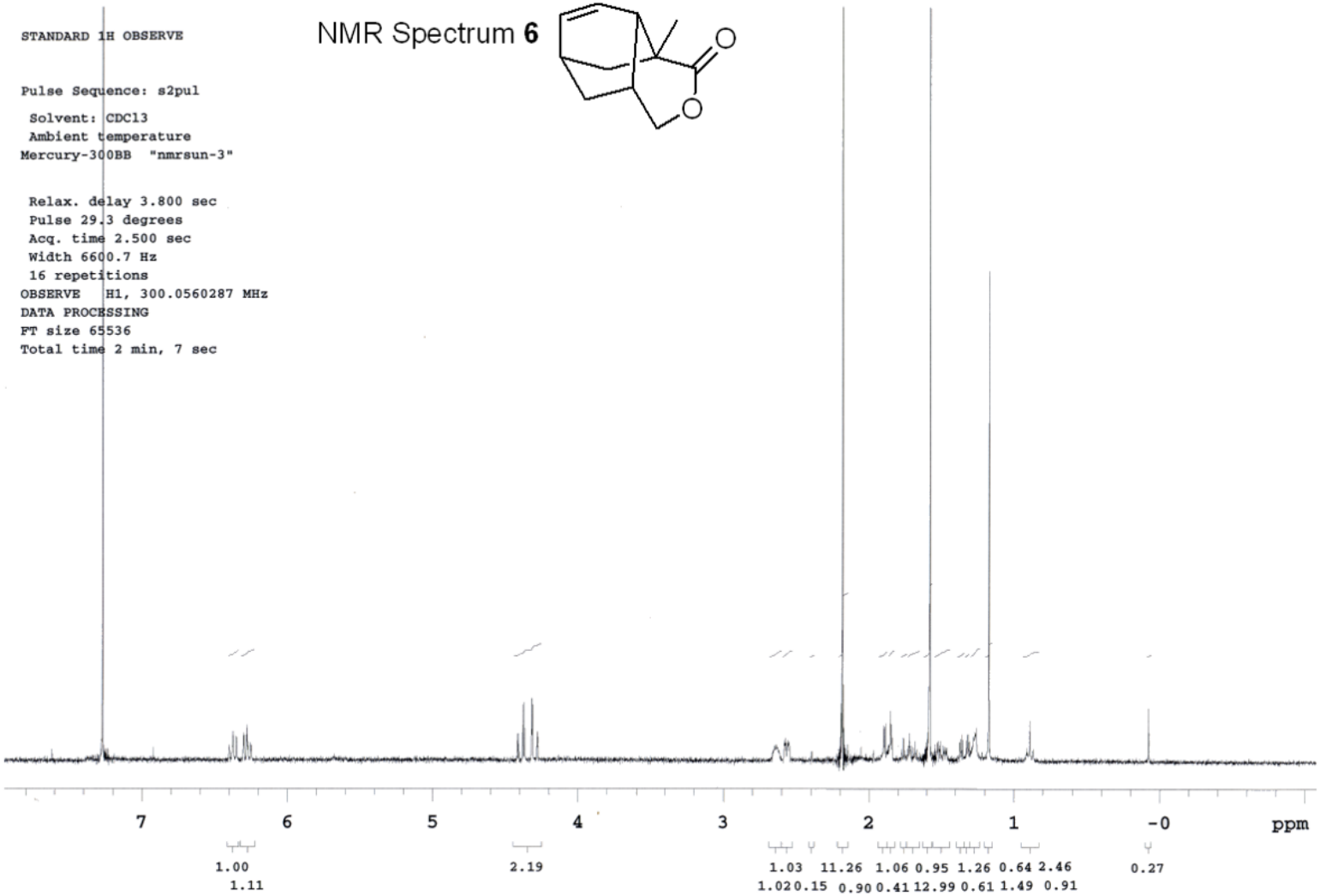
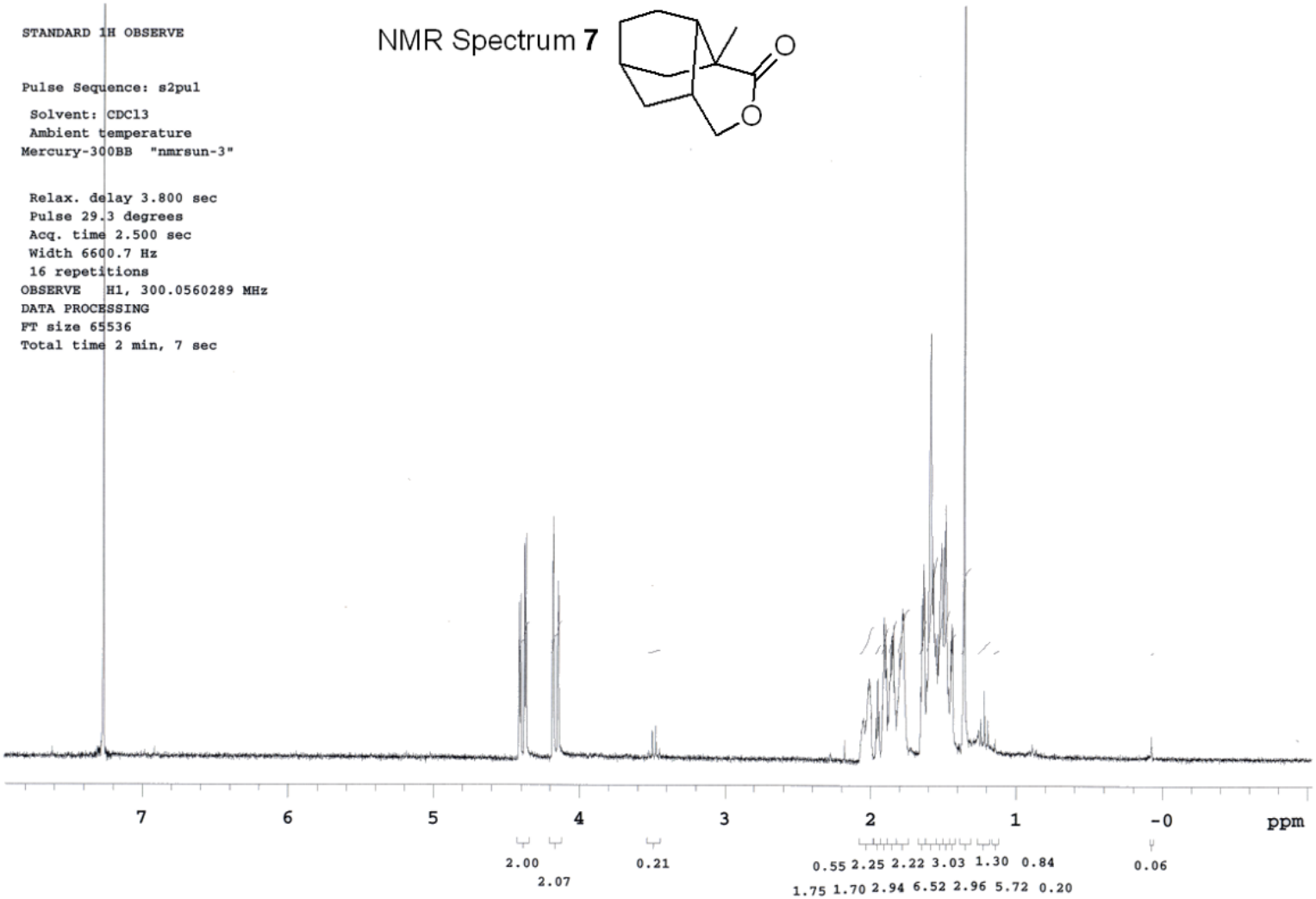


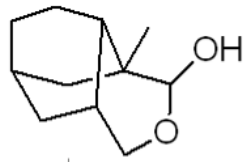
NMR Spectrum **6**



NMR Spectrum **7**



NMR Spectrum **8**



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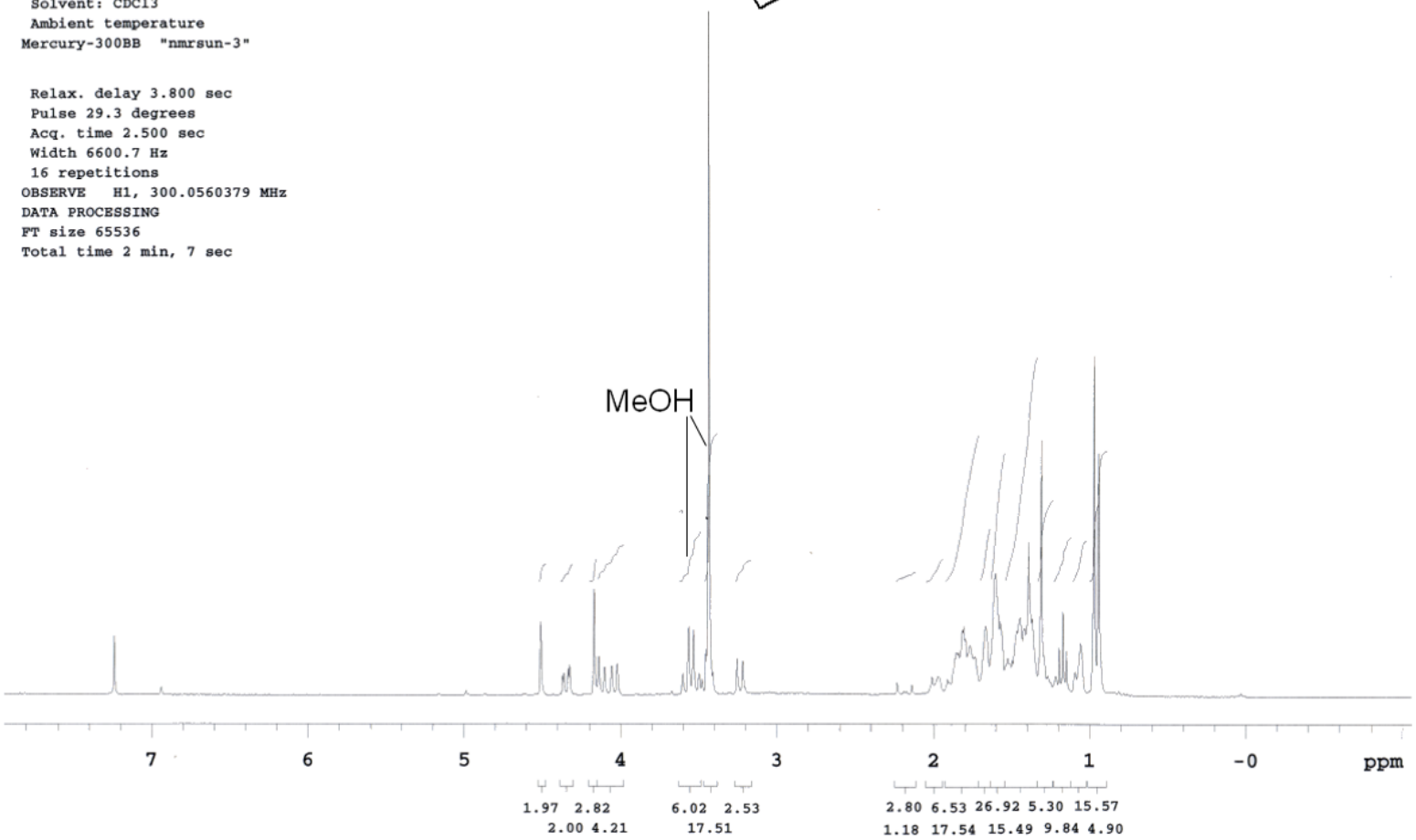
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