SYNTHETIC APPROACHES TO FLUORINATED TEN-MEMBERED ENEDIYNE

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ABSTRACT

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Enediyynes are the most potent antitumor agents ever discovered. This antibiotic family is represented by Calicheamicin, Esperamicin A₁, Neocarzinostatin, and Dynemicin A. With the exception of Neocarzinostatin, all of these molecules contain a 1, 5-diyne-3-ene unit within a strained ten-membered ring. Apart from their diversity, enediyne compounds share some structural and functional similarities.

Their unique mode of biological action is to destroy double-helical DNA by double strand scission, caused by H-atom abstraction from the sugar phosphate backbone of both duplex DNA strands by a 1, 4-benzenoid diradical formed as a reactive intermediate in the Bergman cyclization of the enediyne. Unfortunately the DNA cleavage observed is very non-selective so modified enediynes with more controlled Bergman reactivity must be developed. In this thesis, we describe synthetic schemes designed to create uniquely substituted enediyne structures in hopes of developing a more thorough understanding of the Bergman reaction.
To My Dad

Binali Obali
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1. INTRODUCTION

1.1 Enediynes

For the last twenty five years, the chemistry of (Z)-1,5-diyne-3-enes, the so called “enediynes,” has been drawing the attention of organic chemists and biochemists alike due to remarkable chemical and biochemical reactivity of some natural enediynes. The conjugated enediynes of primary interest are included in a nine or ten-membered ring and contain the two triple bonds separated by a double bond (1).

![Diagram of (Z)-hexa-3-en-1, 5-diyne (1)]

Enediyne antibiotics are a small and relatively new family of natural compounds. Although one of them (neocarzinostatin) was isolated by Ishida, et al. in 1965, the story of the enediyne antibiotics began to develop only in 1986.\(^1\) The molecular architecture of these compounds was very surprising since they were so different from other natural products. Subsequently, these natural enediynes were found to be highly cytotoxic and characterized by a potent antitumor activity. Their remarkable reactivity generated active interest in the study of several synthetic enediyne model compounds, some of which possessed unique biological properties.\(^2\)
The natural enediynes discovered thus far have been classified into four different families represented by Calicheamicin (2), Dynemicin A (3), Esperamicin A1 (4), and Neocarzinostatin (5) (Figure 1).

Figure 1. Natural enediynes
With the exception of Neocarzinostatin (5), which is converted to a nine-membered cyclic enediyne, all of these molecules contain a 1,5-diyne-3-ene unit within a strained ten-membered ring. The ten-membered cyclic enediyynes are more stable than nine-membered cyclic enediyynes.

Enediyne antibiotics are presently the most intriguing agents in anti-cancer therapy due to their unique structures and sophisticated mechanism by which they cleave DNA (Figure 2). These natural enediyne compounds have four similar reaction groups: Delivery system; the fragment of the structure that is responsible for transporting and forming a strong and specific complexation with DNA. In Calicheamicin (2), this is the oligosaccharide part. In Dynemicin A (3), it is the planar anthraquinone which intercalates into the DNA backbone. Warhead; the reactive enediyne unit which undergoes Bergman cyclization and produces a 1,4-benzenoid diradical compound capable of double hydrogen abstraction from the sugar phosphate backbone of DNA, thereby initiating double-stranded DNA cleavage leading to cell death. All natural enediyynes are extremely cytotoxic and cause cleavage of both single and double stranded DNA. Obviously double strand cleavage is more fatal than single strand cleavage. Safety catch; a “restraining” unit which prevents the enediyne Bergman reaction until triggered. With this unit, the enediyne does not “explode” until a particular chemical event takes place. In Calicheamicin (2), the safety catch is represented by the enamine double bond. In Dynemicin A (3), it is the epoxide. Chemical trigger; a reaction that causes the removal of the safety catch and therefore initiates the cyclization of the enediyne. In Calicheamicin (2), this unit is represented by the trisulfide group. In Dynemicin A (3), it is the quinone.
Figure 2. Mechanism of DNA cleavage by the Calicheamicin family of enediyne antibiotics.²

Most of these natural enediyynes are available by means of difficult total synthesis but certainly more commonly by fermentation of cultured bacterial species. These compounds remain highly expensive. The natural antitumor antibiotics have found little use in medicinal practice due to their high inherent toxicity and lack of selectivity which causes damage and death of healthy cells in addition to cancer cells. During the 1990s, organic chemists made a considerable effort to mimic the extraordinary efficiency of natural enediyne antibiotics by synthesizing simplified model systems. Organic chemists continue to synthesize non-natural enediyynes, designed to have greater selectivity towards cancer cells over healthy cells.
1.2. Bergman Cyclization

Since cyclization of natural enediynes into a highly reactive diradical is their main mechanism of action, a thorough understanding of the Bergman cyclization is necessary if better synthetic enediynes are to be developed as medicinal agents. A discussion of known facts about this reaction follows.

The thermal rearrangement of enediynes to 1,4-benzenoid diradicals (1,4-dehydrobenzene or p-benzyne) was discovered in the early 1970s by Masamune\(^5\) and fully studied by Bergman\(^6\) in 1972, and is now commonly called the Bergman cycloaromatization. In the classical Bergman experiment, \([1,6-\text{D}_2]-\text{(Z)-3-hexene-1,5-diyne (10)}\) underwent a rapid deuterium scrambling reaction at 200 \(^\circ\)C in the gas phase. The reaction to form the diradical is endergonic, and therefore occurs only at high temperatures.\(^7\) The ratio of isomers \([1,6-\text{D}_2]-\text{(Z)-3-hexene-1,5-diyne (10)}\) and, \([3,4-\text{D}_2]-\text{(Z)-3-hexene-1,5-diyne (12)}\) was found to be 50: 50, and no product containing only one vinyl deuterium (13) or (14) was detected in the reaction mixture (Figure 3).\(^6\) This proves that deuterium atom migration from carbon to carbon does not occur.
Figure 3. Bergman cyclization of [1, 6-D2]-Z-1-hexene-1, 5-diyne.²

Heating a 0.01M solution of 1,5-diyne-3-ene (1) in 2,6,10,14-tetramethylpentadecane at 200 °C, resulted in the disappearance of the starting material and the quantitative production of benzene. Because free radicals are virtually the only intermediates capable of removing hydrogens from a hydrocarbon, this immediately suggested that the structure of the intermediate was 1,4-dehydrobenzene (1,4-benzyne)diradical (16).² Several additional trapping experiments where performed which supported this hypothesis (Figure 3).
Since Bergman cyclization is the heart of the chemistry of enediynes as well as the origin of their biological activity, understanding factors controlling the kinetics of the Bergman cyclization is crucial for the design of any new and effective enediynes. The thermal cyclization of enediynes to benzene 1, 4-diradicals (Bergman cyclization) is affected by geometrical and electronic conditions. Combined experimental and computational methods were used to identify the parameters that affect reactivity of Z-enediynes in the Bergman reaction and they can be classified as follows; a) The \( c-d \) distance between the two terminal acetylenic carbons; b) difference in strain energy between the enediyne and the transition state, c) concentration of trapping agents, and d) electronic substituent effects.

Since most of natural enediynes contain the 1,5-diyne-3-ene unit (1) within a larger (nine- or ten-membered) ring system, much attention has been paid to the influence of the distance \( (d) \)
between the terminal acetylenic carbon atoms. In 1992, Nicolaou\textsuperscript{3} et al. analyzed the mechanism of Calicheamicin’s action. They highlighted the change in the distance between the acetylene termini on the Calicheamicin itself ($d = 3.35$ Å) and in the triggered enediyne ($d = 3.16$ Å) (Figure 2). This modification was used in simple monocyclic enediynes to examine the validity of their hypothesis (Table 1). The stability data for the monocyclic compound showed that compounds with ($d$) distance less than 3.2 Å rapidly cyclize at 25 °C, while those with ($d$) distance of greater than about 3.35 Å are thermally stable at 25 °C. Those compounds which fall between this range displays limited stability at ambient temperatures.\textsuperscript{3} Not surprisingly, the crucial ring size was found to be ten as in the case of most natural enediynes. The so-called Nicolaou’s “critical distance” ($c-d$) rule, however, does not apply to strained enediynes such as those in bicyclic and more complex systems. It appears that the rate of Bergman cyclization is, in the absence of other factors, directly proportional to the $c-d$ distance.

![Diagram of monocyclic enediyne with labels $c-d$ and $n$ values](image-url)
Table 1. Calculated c-d distances and stabilities of cyclic enediynes.

<table>
<thead>
<tr>
<th>compound</th>
<th>ring size</th>
<th>c-d dist.(Å)</th>
<th>stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>9</td>
<td>2.84</td>
<td>unknown</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>3.25</td>
<td>$t_{1/2}=18$ h, 25 °C</td>
</tr>
<tr>
<td>22</td>
<td>11</td>
<td>3.66</td>
<td>stable at 25 °C</td>
</tr>
<tr>
<td>23</td>
<td>12</td>
<td>3.90</td>
<td>stable at 25 °C</td>
</tr>
<tr>
<td>24</td>
<td>13</td>
<td>4.14</td>
<td>stable at 25 °C</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>4.15</td>
<td>stable at 25 °C</td>
</tr>
<tr>
<td>26</td>
<td>15</td>
<td>4.33</td>
<td>stable at 25 °C</td>
</tr>
<tr>
<td>27</td>
<td>16</td>
<td>4.20</td>
<td>stable at 25 °C</td>
</tr>
</tbody>
</table>

Apart from ring strain, the Bergman reaction is quite sensitive to substituent effects. The effect of functional group substituents on the rate of Bergman cyclization was investigated theoretically as well as experimentally. The effect on the energetics of the cycloaromatization of a substituent in the vinylic position is relatively limited. Jones and co-workers reported that chlorine substituents on the vinyl group significantly reduced rate of cyclization. The nine-membered enediyne (Z)-cyclonona-3-en-1,5-diyne (20) is extremely reactive so its half-life time cannot be measured whereas chlorinated enediyne (28) can be isolated and its life-time measured as 8h at 0 °C.
Similar results were found for ten-membered cyclic enediynes and bicyclic enediynes. The rate of cyclization is slower for enediyne (E)-3-chlorocyclodeca-3-en-1, 5-diyne (29) compared with the unsubstituted enediyne (Z)-cyclodeca-3-en-1,5-diyne (21). The addition of a second chlorine as in 30 further reduced the rate of cyclization significantly. There are three possible factors that could be responsible: 1) the cyclization barriers are higher for chloro substituted enediynes; 2) the \( p \)-benzyne ring-opening barriers are lower for the chloro enediynes; 3) the chloro substituted \( p \)-benzyynes are relatively more stable to H atom abstraction, which increase their half-lives.

The bicyclic enediyne 31 is highly reactive and cyclizes spontaneously at room temperature. By introducing a 4-methoxyphenyl substituent in the vinyl position, the enediyne 32 is stabilized. Upon heating to 80 °C this compound cyclizes. Morokumo\textsuperscript{13} et al. suggests that the 4-methoxyphenyl substituent stabilizes the ground state more than the reaction intermediate, disfavoring reaction.
Another form of “vinyl substitution” is benzo-fusion. Studies show that benzannulation alters the rate limiting step in enediyne cycloaromatization. Bergman and co-workers established that the cyclization step is rate-determining in the cycloaromatization reaction of aliphatic enediynes. A thorough study of the effect of benzannulation disclosed that the kinetics of the cyclization is not solely determined by the rate of Bergman reaction, but is also greatly affected by the rate of retro-Bergman reaction and hydrogen abstraction. Hirama et al. examined 21 and 35 in different concentrations of 1,4-cyclohexadiene and compared the kinetic data. The kinetic data indicate that the decay of 21 is independent of the concentration of trapping agent. Consequently, the cyclization step of 21 should be rate-determining step as expected. The kinetic data for 35 shows that the rate depends on the concentration of trapping agent. The hydrogen abstraction step of 35 is kinetically significant in contrast to that of 21.
Table 2. Effect of 1,4-cyclohexadiene concentration on the disappearance rate of 21 (10mM) in benzene-d6 at 57 °C.  

<table>
<thead>
<tr>
<th>1, 4-cyclohexadiene</th>
<th>k/10⁻⁴ s⁻¹</th>
<th>t₅₀/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1.19</td>
<td>1.61</td>
</tr>
<tr>
<td>0.25</td>
<td>1.13</td>
<td>1.71</td>
</tr>
<tr>
<td>0.50</td>
<td>1.10</td>
<td>1.75</td>
</tr>
<tr>
<td>1.32</td>
<td>1.14</td>
<td>1.69</td>
</tr>
<tr>
<td>2.48</td>
<td>1.14</td>
<td>1.69</td>
</tr>
<tr>
<td>5.29</td>
<td>1.13</td>
<td>1.71</td>
</tr>
<tr>
<td>10.50</td>
<td>1.12</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Table 3. Effect of 1,4-cyclohexadiene concentration on the disappearance rate of 35 (10mM) in benzene-d6 at 89 °C.  

<table>
<thead>
<tr>
<th>1, 4-cyclohexadiene</th>
<th>k/10⁻⁴ s⁻¹</th>
<th>t₅₀/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>3.88</td>
<td>49.6</td>
</tr>
<tr>
<td>0.25</td>
<td>7.65</td>
<td>25.2</td>
</tr>
<tr>
<td>0.50</td>
<td>13.7</td>
<td>14.2</td>
</tr>
<tr>
<td>1.32</td>
<td>28.2</td>
<td>6.83</td>
</tr>
<tr>
<td>2.48</td>
<td>37.6</td>
<td>5.13</td>
</tr>
<tr>
<td>5.29</td>
<td>48.2</td>
<td>4.00</td>
</tr>
<tr>
<td>10.50</td>
<td>46</td>
<td>4.19</td>
</tr>
</tbody>
</table>
This benzannulation effect should be attributable to the change of the relative rate between the retro Bergman cyclization from the diradical intermediate and the corresponding hydrogen abstraction step. An explanation is that the rate from 36 to 35 becomes faster than that from 36 to 37 because only part of the resonance energy of 36 is lost in the reversion process in comparison with the full loss of aromaticity in the corresponding reaction of 33. Another possibility is that the hydrogen abstraction might be retarded in 36 compared to 33. Based on Chen’s rational that \( p \)-benzyne diradical in a low-lying singlet is a poor hydrogen abstraction agent, Hirama et al. assume that benzannulation induces a substantial singlet-triplet splitting. Thus, the aromatic ring annulated diradical 36 would have a lower reactivity than diradical 33 because of the larger singlet-triplet gap.

A further example which is consistent with this rationale explanation is the following; 38 (\( t_{1/2} = 72 \text{ h}, 23 ^\circ \text{C} \)) cyclizes much faster than 39 (\( t_{1/2} = 52 \text{ h}, 65 ^\circ \text{C} \)).

Since a hydrogen abstraction is the rate-determining step, the observed reaction rate depends on the concentration of hydrogen donor. And also recent studies with benzannulated systems showed that the influence of the solvent on the rate of cyclization can be significant. For
example, half-lives ranging from 16 min (THF) to almost 6 h (acetonitrile) were observed for 40 at 168 °C.\textsuperscript{17}

![Chemical structure](image)

**Table 4.** Kinetic data for thermal cyclization of 40 in various solvents at 168 °C.\textsuperscript{17}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>rate constant ($k_{obs}$)</th>
<th>half-life ($t_{1/2}$; min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>$3.3 \times 10^{-5}$</td>
<td>361</td>
<td>7</td>
</tr>
<tr>
<td>Methanol</td>
<td>$3.7 \times 10^{-5}$</td>
<td>312</td>
<td>2</td>
</tr>
<tr>
<td>Benzene</td>
<td>$1.2 \times 10^{-4}$</td>
<td>96</td>
<td>12</td>
</tr>
<tr>
<td>Dioxane</td>
<td>$1.9 \times 10^{-4}$</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>THF</td>
<td>$7.1 \times 10^{-7}$</td>
<td>16</td>
<td>36</td>
</tr>
</tbody>
</table>

Russell\textsuperscript{17} et al. showed that there was no direct relation between half-life and solvent dipole moment. These results can be explained if the ground state of 40 is more polar than its transition state so that polar solvents stabilize the ground state better than the transition state. For compound 40, polar solvents increase the activation energy of Bergman cyclization resulting in larger half-lives with low yield whereas non-polar solvents resulted in shorter half-lives and gave better yields.\textsuperscript{17}
Nicolaou and co-workers employed well-known quinone and hydroquinone redox chemistry in studies of the rate of Bergman cyclization (Figure 5). While the electron-rich hydroquinone moiety (43) highly retarded the cyclization, the quinone (44) accelerated cyclization by simultaneously reducing the electron density on the enediyne fragment and increasing the aromatization energy.\textsuperscript{18}

\[ \text{[O]} \rightarrow \text{[H]} \]

\[ \begin{array}{c}
\text{43} \\
\text{44}
\end{array} \]

\[ \begin{array}{c}
\text{45} \\
\text{46}
\end{array} \]

\textbf{Figure 5.} Redox-controlled Bergman cyclization of quinone and hydroquinone.

More extended aromatic systems in the enediyne fragment as in 48 and 50 provide less influence on the rate of Bergman cyclization than more limited ones as placed in 47 and 49.
Substituents at the propargylic position generally affect the kinetics of Bergman cyclization more than those at the vinylic positions.\textsuperscript{12} Studies showed that $\sigma$-acceptors and/or $\Pi$-donors ($-F$, $-OH$, $-NH_3^+$, $-OH_2^+$) lower, while $\Pi$ – withdrawing groups ($-BH_2$, $-AlH_2$) raise the barrier of the Bergman cyclization due to interactions with $\sigma$-antibonding and $\Pi$- bonding orbitals in the transition state.\textsuperscript{8} Therefore, hydroxy, nitro and especially the fluoro substituents increase the thermal reactivity of enediynes. For benzannulated enediynes, carbonyl groups do not enhance Bergman cyclization rates, in the case of compound 4,4'-(1,2-phenylene)dibut-3-yn-2-one (51) which cyclizes considerably slower than unsubstituted 1,2-diethynylbenzene 53.
Figure 6. Thermal cyclization of 4,4’-(1,2-phenylene)dibut-3-yn-2-one (51) and 1,2-diethynylbenzene (53).

This surprising result was explained as the effect of the predominantly \( \Pi \) – acceptor character of the carbonyl group, whereas \( \sigma \)-acceptors are required for activation.\(^{19}\) For cyclic benzannulated enediynes, electron-withdrawing groups, whether \( \sigma \) or \( \Pi \)-type, accelerate the Bergman cyclization, while electron-donating substituents retard it.\(^{20}\)
Depending on the substituent, the HOMO of the cyclization transition state can be either of σ or Π type. Π-donors decrease of the barrier through the stabilization of the Π-binding orbitals. σ-acceptors favor the cyclization by lowering the occupation of antibonding σ-orbitals.

The barriers for disubstituted products depend on steric hindrance caused by substituents in the transition states. In the case of fluoro substituted enediynes, computational results predicted that the cyclization of unknown difluoro-enediyne 57 has the lowest barrier all of computed enediynes. Calculations predict that difluoro-enediyne 57 is highly reactive and unstable at physiological temperatures and would immediately cyclize exergonically to o-difluoro-p-benzynę 59. Because of the small size of the fluoro substituent, there is little steric repulsion in the transition state leading to cyclization of 57 and both substituents electronically lower the activation energies. ⁸
2. RESULTS AND DISCUSSION

On the basis of previous knowledge described in the Introduction, we propose to synthesize molecules which contain strong $\sigma$-electron withdrawing groups which have no $\Pi$ donation capability and study the thermal Bergman cyclization of these compounds. Our immediate target was 60 which possesses the powerful difluoromethylene groups attached at the propargyl position.

In contrast to the natural enediynes, a stable enediyne would be activated essentially by raising the energy of the ground state of the enediyne by strain modulation. Therefore, in our project we planned to make use of the more reactive ten-membered cyclic enediyne. The ten-membered cyclic enediyne compound 60 containing the strong sigma-electron withdrawing groups directly attached to the propargylic carbons of the enediyne fragment should show accelerated rate of Bergman cyclization (see Introduction). Although benzofusion system alters the rate limiting step of Bergman cyclization and retards the rate of cyclization, computational and experimental studies$^9$ show that substituents at the propargylic position generally affect the rate of Bergman cyclization more than at the vinylic positions, we suggest that the fluoromethylene groups of 60 will accelerate the rate of Bergman cyclization.
Acetylenes fluorinated in propargyl position are rare in the organic literature, so synthesis was expected to be the major difficulty in this. Based on a previous report, we designed our syntheses to ultimately employ a ring closing reaction that occurs between an aldehyde and an organometallic reagent derived from an alkynyl halide. This particular coupling reaction is known as the Nozaki-Hiyama-Kishi reaction, and utilizes Ni(II) and Cr(II) to generate the acetylenyl organometallic reagent. This reaction system has been previously shown to be successful in closing medium and large ring enediynes and example is shown in Figure 7.

![Figure 7. The Nazaki-Hiyama-Kishi coupling reaction of 3-(tert-butyldimethylsilyoxy)-7-(2-(idoethynyl)phenyl)hept-6-ynal.](image)

2.1 Synthetic Scheme One

A literature report describes the synthesis of 1,2-diethynlbenzene (53) in two steps. In the initial reaction, phthalaldialdehyde (63) is converted to 1,2-bis(2’,2’-dibromoethenyl) benzene (64) by the use of triphenylphospine (PPh₃) and tetrabromomethane (CBr₄) in dichloromethane. In a second step, 1,2-bis(2’,2’-dibromoethenyl) benzene (64) is transformed in to 1,2-
diethynlbenzene (53) by the use of n-buthyllithium and diisopropylamine in dry THF. The structure of 1,2-diethynylbenzene (53) was confirmed by NMR and mass spectral data.

Protection of a single acetylene group of 1,2-diethynylbenzene (53) was very important, because 6-(2-ethynlyphenyl)-4,4-difluorohex-5-ynal (69) is required for the cyclization. For the protection of 1,2-diethynylbenzene (53), ethylmagnesiumbromide and tert-butyldimethylsilyl-triflate (TBDMS-OTf) were used. After addition of ethylmagnesiumbromide to 1,2-diethynylbenzene (53) in dry THF, the reaction mixture was checked using D2O by GCMS to confirm Grignard reaction. After the Grignard reaction step, tert-butyldimethylsilyl-triflate (TBDMS-OTf) was added at 0 °C and the mixture was stirred for 3 h at room temperature. The mass spectrum of the product showed a molecular ion peak at m/z 240 and a peak at 183 which corresponded to the cleavage of a t-buty group. Tert-butyl((2-ethynlyphenyl)ethynyl)dimethylsilane (65) could not be isolated in pure form using chromatographic or vacuum distillation techniques.

The fourth step was the synthesis of ((2-(3-bromo-3,3-difluoroprop-1-ynyl)phenyl) ethynyl)tert-butyldimethylsilane (66) from crude tert-butyl((2-ethynlyphenyl)ethynyl)
dimethylsilane (65). Therefore, firstly was prepared the lithium salt of (65), then
dibromodifluromethane was added at -78 °C and the reaction was stirred for 1 day. The mass
spectrum of the reaction mixture showed peaks at m/z 311/313 which would be result of
cleavage of 57 mass units (t-butyl) from M⁺ 368 of 66. However, ((2-(3-bromo-3,3-difluoroprop-
1-ynyl)phenyl)ethynyl)tert-butylimethyldimethylsilane (66) could not be isolated from the crude
reaction mixture. It was apparently easily decomposed at room temperature. Therefore, progress
on proposed synthetic scheme one was stopped at molecule 66.
1. n-BuLi, THF, -78°C
2. CF₂Br₂, -78°C

1. Co III, Zn
2. O₂Et
2.2 Synthetic Scheme Two

Since we were unsuccessful in our attempt to isolate \((2-(3\text{-bromo-3,3-difluoroprop-1-ynyl})\text{phenyl)ethynyl})\ tert-butyl dimethylsilane (66), we decided to change our synthetic
approach by avoiding early introduction of the group TBDMS. This second synthetic approach differs from the first by first converting 1,2-diethynylbenzene (53) to the mono bromodifluoromethyl derivative 73. 1,2-Diethynylbenzene (53) was dissolved in dry THF, cooled and treated with 1.1 mol equiv. of n-butyllithium. The reaction mixture was stirred for 1 hour at -78 °C and then dibromodifluoro was added portion wise to the yellow-brown mixture and stirred at room temperature for 24 hours under argon. Before purification of the product, the crude was checked by GC/MS and the mass spectrum showed peaks at m/z 254/256 for the molecular ion and a peak at m/z 175. This suggested the cleavage of bromo group from 1-(3-bromo-3,3-difluoroprop-1-ynyl)-2-ethynylbenzene (73). However, 1-(3-bromo-3,3-difluoroprop-1-ynyl)-2-ethynylbenzene (73) could not be isolated in pure form from crude reaction mixture [which include 1,2-bis (3-bromo-3,3-difluoroprop-1-ynyl) benzene (76) an (75)] by column chromatography or vacuum distillation techniques.

The next step was to be the synthesis of ethyl 6-(2-ethynylphenyl)-4,4-difluorohex-5-ynoate (74) from the impure sample of 1-(3-bromo-3,3-difluoroprop-1-ynyl)-2-ethynylbenzene (73). Cobaloxime (III) / Zn was used to promote the conjugate addition of ethyl acrylate to give ethyl 6-(2-ethynylphenyl)-4,4-difluorohex-5-ynoate (74), following a literature report. Since bromo (pyridine) cobaloxime is not commercially available, it was prepared from cobalt (II)
nitrate hexahydrate and dimethylglyoxime\textsuperscript{24}. A mixture of bromo (pyridine) cobaloxime (III) and zinc powder was stirred for 1 hour at room temperature; then 1-(3-bromo-3,3-difluoroprop-1-ynyl)-2-ethynylbenzene (73) and ethyl acrylate were added. After the mixture was stirred for 24 hours at room temperature, the reaction mixture was investigated using $^{19}$F-NMR spectroscopy. The $^{19}$F-NMR and the mass spectrum showed unchanged 73 but no additional fluorine containing transformation products. The procedure was modified by increasing the reaction time to 48 hours, but again there was no evidence for addition to form 74.

\[
\begin{align*}
&\text{CF}_2\text{Br} \\
&\text{1. Co III, Zn} \\
&\text{2. OEt} \\
\text{73} \quad \rightarrow \quad \text{74}
\end{align*}
\]
2.3 Synthetic Scheme Three
Since we were unsuccessful in our attempt to produce ethyl 6-(2-ethynylphenyl)-4,4-difluorohex-5-ynoate (74), we decided to modify our synthetic approach by avoiding initial introduction of the bromodifluoromethyl group. Instead of using 1,2 diethynylbenzene a costly starting material, we decided to use simple commercially available phenylacetylene to study appropriate model reactions (Synthetic Scheme Four). According to the new projected synthesis Scheme Four, 6-hydroxy-1-phenylhex-1-yn-3-one (82) was to be synthesized in two steps. In the initial reaction, phenylacetylene was deprotonated using \( n \)-butyllithium in dry THF at -78 °C. Then the yellow reaction mixture was added to a solution of \( \alpha \)-butyrolactone at -78 °C. The mass spectrum of the product showed a molecular ion at \( m/z \) 170 which amounts to the loss of water from the expected molecular ion. A peak at 129 corresponded to fragmentation adjacent to the carbonyl group. However, 6-hydroxy-1-phenylhex-1-yn-3-one (82) could not be isolated from the reaction mixture.

The second step was the protection of alcohol group using acetic anhydride. Since we plan to convert the carbonyl group to -CF\(_2\) group, this protection step is highly important to prevent conversion of alcohol to fluoro. Therefore, firstly acetic anhydride and 4-dimethylaminopyridine (DMAP) was added to solution of crude 6-hydroxy-1-phenylhex-1-yn-3-one (82) in pyridine at room temperature. The crude was checked by GC/MS and the GC showed two peaks at the different retention times but their mass spectra were exactly the same. This could be the result of efficient fragmentation of ketene from the acetate protected molecule.

Since we could not separate the two chemical compounds, we decided to try the fluorination reaction with the crude reaction mixture. A literature report\(^25\) describes the conversion molecules containing an alkyne and carbonyl group to a –CF\(_2\) group. A mixture of
diethylaminosulfur-trifluoride (DAST) and 4-oxo-6-phenylhex-5-ynyl acetate (83) was heated to 55 °C. After the mixture was stirred for 6 hours at 55 °C, the reaction was investigated using by 19F –NMR spectroscopy. Unfortunately, 19F –NMR and mass spectrum showed that the reaction did not work. Therefore, the procedure was modified by increasing the reaction time to 24 hour. This change did not give a successful result either.

Since the conversion reaction did not work with 4-oxo-6-phenylhex-5-ynyl acetate (83), we decided to try the same reaction with 6-hydroxy-1-phenylhex-1-yn-3-one (82). After the crude compound 82 and DAST mixture was stirred 6 hours at 55 °C, the reaction mixture was checked by 19F –NMR and mass spectrum. Spectral results showed that the 6-hydroxy-1-phenylhex-1-yn-3-one (82) was successfully fluorinated by DAST and converted to the (3,3,6-trifluorohex-1-ynyl)benzene (85).
2.4 Synthetic Scheme Four

\[
\begin{align*}
\text{81} 
\xrightarrow{\text{LDA, } -78^\circ C} 
\text{82} 
\xrightarrow{\text{DAST, } 55^\circ C} 
\text{85} \\
\text{82} 
\xrightarrow{\text{DMAP, pyridine, } Ac_2O} 
\text{83} 
\xrightarrow{\text{DAST, } 55^\circ C} 
\text{84}
\end{align*}
\]
3. EXPERIMENTAL

3.1 Materials

All moisture and oxygen sensitive reactions were carried out in oven-dried glassware under argon atmosphere. All solvents were purified and dried by distillation immediately before use. All other materials were purchased from Aldrich or Alfa Aesar. GC/MS analyses were performed using Shimadzu QP5050A GC mass spectrometers equipped with ZB5MS 30 m x 0.25 mm x 0.25 µm columns. Nuclear magnetic resonance (NMR) spectra were recorded in deuterated solvent using a 300 MHz or 500 MHz Varian Unityplus NMR spectrometers. ¹H-NMR chemical shifts (δ) are reported in ppm versus TMS reference. Column chromatography and preparative TLC were carried out with Merck Silica Gel (60 - 200 Mesh size).

3.2 Experimental Procedure

3.2.1 Synthesis of 1,2-Bis(2',2'-dibromoethenyl)benzene (64).

Triphenylphosphine (27.52g, 104.9 mmol) was added portionwise to a cold (0 °C) solution of tetrabromomethane (17.39 g, 52.4 mmol) in 250 mL of dichloromethane. The resulting orange-red solution was stirred at room temperature for 20 min. After cooling to 0 °C a solution of 3.07 g (22.8 mmol) of o-phthalaldehyde in 100 mL of dichloromethane was added slowly and the mixture was stirred in the dark at room temperature for 7 h. The reaction mixture was extracted with distilled water (2 x 100 mL) and the water layers washed with dichloromethane (2 x 50 mL). The combined organic layers were dried (MgSO₄) and concentrated. Pentane (4 x 150 mL) was added and the resulting suspension was decanted after stirring. After addition of another 150
mL of pentane to the suspension it was filtered and all pentane fractions were combined, concentrated, and purified by column chromatography (SiO₂, 1% Et₂O in pentane, \( R_f = 0.5 \)) yielding a yellow oil (9.85 g, 22.1 mmol, 40%). \(^1\)H NMR (CDCl₃): \( \delta \) 7.47-7.56 (m, 2H, \( o\)-ArH), 7.42 (s, 2H, CH=), 7.35-7.39 (m, 2H, \( m\)-ArH). MS m/z (%): 446 (5) [M⁺], 367 (20) [M⁺ - Br], 286 (100) [M⁺ - 81Br - 79Br], 126 (38) [M⁺ - Br₄].

3.2.2 Synthesis of 1,2-Diethynylbenzene (53).

\( n\)-BuLi (48.8 mL, 78.1 mmol, 1.6 M in hexanes) was added to a cold (-78 °C) solution of 10.3 mL (78.1 mmol) of diisopropylamine in 250 mL of THF. After warming to room temperature the solution was added carefully to a cold (-78 °C), well stirred solution of 5.82 g (13.0 mmol) of 1, 2-Bis (2′,2′-dibromoethenyl) benzene (64) in 50 mL of THF. After 20 min. of stirring at -78 °C, the reaction mixture was stirred 24 h at room temperature, then quenched with 130 mL of saturated aq. (NH₄)₂SO₄ and stirred for 2 h at room temperature. This mixture was poured into 200 mL of pentane, the organic layer was separated and washed with water, dried (MgSO₄), concentrated, and purified using column chromatography (SiO₂, 1% Et₂O in pentane, \( R_f = 0.42 \)). A colorless liquid 53 (1.54 g, 12.2 mmol, 70%) was obtained. \(^1\)H NMR (CDCl₃): \( \delta \) 7.48-7.54 (m, 2H, \( o\)-ArH), 7.27-7.34 (m, 2H, \( m\)-ArH), 3.33 (s, 2H, CH).

3.2.3 Synthesis of tert-butyl((2-ethynylphenyl)ethynyl)dimethylsilane (65).

Ethylmagnesium bromide in THF (3.3 mL, 5.25 mmol) was added dropwise with stirring, at 3 °C, to 1,2-diethynylbenzene (53) (0.55 g, 4.37 mmol) in tetrahydrofuran (4 mL) and the color turned to brownish-yellow. The cooling bath was then removed and the stirred solution was
allowed to reach 20 °C and kept 1.5 h at this temperature. Tert-butylidemethylsilyl-triflate (TBDMS-OTf, 1mL, 6.55 mmol) was added at 0 °C. The mixture was stirred for 3 h at room temperature, then quenched with water (10 mL) at 0 °C and extracted with ether (10 mL). The organic layer was washed with ammonium chloride solution (2 x 5 mL) and dried over MgSO4. The mass spectrum showed a molecular ion at m/z 240 and a peak at m/z 183 corresponded to the cleavage of a t-butyl group. However, the product could not be purified using column chromatography or vacuum distillation techniques.

3.2.4 Synthesis of 2-(3-bromo-3,3-difluoroprop-1-ynyl)phenyl)ethynyl)(tert-butyl)dimethylsilane (66).
To a solution of tert-butyl((2-ethynylphenyl)ethynyl)dimethylsilane (65) (3.5 g, 14.6 mmol) in anhydride THF (30 mL), a solution of n-BuLi (2.5 M in hexane; 6.1 mL, 15.3 mmol) was added dropwise at -78 °C under an argon atmosphere. After the reaction mixture had been stirred for 30 min at -78 °C, cold (-78 °C) CF2Br2 (4.60 g, 21.9 mmol) was added to the reaction mixture at -78 °C. After stirring for 20 h at room temperature, the THF solution was washed with saturated aq NH4Cl solution (10 mL). The aqueous layer was extracted with hexane (2 x 20 mL) and the combined organic layers were dried over MgSO4. After evaporation of the solvent, the crude was checked by GC/MS. The mass spectrum showed peaks at m/z 311/313, and the peak at 165 indicated the loss of bromo, difluoro, and dimethyl groups. Since the product was not stable, it could not be purified.

3.2.5 Synthesis of 1-(3-bromo-3,3-difluoroprop-1-ynyl)-2-ethynylbenzene (73).
To a solution of 1,2-diethynlbenzene (53) (1.84 g, 14.6 mmol) in dry THF (30 mL), a solution of n-BuLi (2.5 M in hexane; 6.1 mL, 15.3 mmol) was added dropwise at -78 °C under an argon atmosphere. After the reaction mixture had been stirred for 30 min. at -78 °C, cold (-78 °C)
CF₂Br₂ (4.60 g, 21.9 mmol) was added to the reaction mixture at -100 °C. After stirring for 16 h at room temperature, the THF solution was washed with saturated aq NH₄Cl solution (10 mL). The aqueous layer was extracted with hexane (2 x 20 mL) and the combined organic layer was dried over MgSO₄. After evaporation of the solvent, column chromatography, preparative TLC, and vacuum distillation techniques were applied to isolate the desired compound (73) from the crude reaction mixture, but these efforts gave unsuccessful results.

3.2.6 Synthesis of ethyl 6-(2-ethynylphenyl)-4,4-difluorohex-5-ynoate (74).
A mixture of bromo(pyridine)cobaloxime (0.1 mmol), Zinc powder (0.65 g, 10 mmol) and ethanol (20 mL) was vigorously stirred at room temperature under argon, for ca. 30 min during which time, the color of the suspension changed from brown to light green; further starting material (73) (crude) (10 mmol) and ethylacrylate (12 mmol) was then added to it. After the mixture had been stirred at room temperature for ca. 1 day, the reaction was checked by ¹⁹F-NMR and GC/MS spectroscopy. However, these spectrometric results showed that reaction did not occur.

3.2.7 Synthesis of 6-hydroxy-1-phenylhex-1-yn-3-one (82).
To a stirred solution of phenyl acetylene (1.84 g, 20 mmol) in dry THF (50 mL), n-BuLi (13 mL, 1.6 M) was added. The reaction mixture was stirred at -78 °C for 3 h. The resulting yellow solution was added to a solution of γ-butyrolactone (2.6 g, 30 mmol) in 20 mL of dry THF at -78 °C through a syringe. The solution was stirred at -78 °C for 1 h quenched with 10 ml of methanol, and warmed to 0 °C. Water was added, and the product was isolated with ether. Combined organic phases were dried with MgSO₄ and evaporated under reduced pressure. At the end of this period GC/MS of the sample of the reaction mixture showed two major products.
Crude product was purified by preparative TLC. 6-hydroxy-1-phenylhex-1-yn-3-one (82) was found to quickly decompose upon exposure to daylight. It was also found to have limited storage life in the dark at room temperature or in the freezer. The mass spectrum showed a peak at m/z 188, and a larger peak at 170 indicated the loss of H₂O.

3.2.8 Synthesis of 4-oxo-6-phenylhex-5-ynyl acetate (83).
A mixture of 6-hydroxy-1-phenylhex-1-yn-3-one (82) (0.1 mmol), acetic anhydride (36 mg, 0.3 mmol), and dimethylaminopyridine (5 mg) in 2 mL of pyridine was stirred at room temperature for 3 hours. The mixture was diluted with 30 mL of hexane-ethylacetate (7:3) solution. The reaction mixture was washed with saturated CuSO₄, the product was isolated with ether. Combined organic phases were dried (MgSO₄) and evaporated under reduced pressure. At the end of this period GC/MS of the sample of the reaction mixture showed two major products which had the same mass spectrums. Crude product was purified by preparative TLC.

3.2.9 Synthesis of 4,4-difluoro-6-phenylhex-5-ynyl acetate (84).
To a stirred oily product (83) (0.55 g, 3 mmol) was added dropwise diethylaminosulfur trifluoride (DAST, 2.4 g, 15 mmol) and a mixture heated at 55 °C under Argon. The resulting mixture was stirred for 1 day at 55 °C under Argon. The reaction mixture was poured into ice-water (50 mL) and extracted with ether (3x 50 mL). The crude product was checked by F-NMR and GC/MS but the spectral results showed that conversion of carbonyl to difluoromethylene did not occur.
3.2.10 Synthesis of (3,3,6-trifluorohex-1-ynyl)benzene (85).

To a stirred oily product 6-hydroxy-1-phenylhex-1-yn-3-one (82) (0.55g, 3 mmol) was heated at 55 °C, was added dropwise diethylaminosulfur-trifluoride (DAST, 2.4g, 15 mmol) under argon. The resulting dark brown mixture was stirred for 6 h at 55 °C. The dark color reaction mixture was poured into ice-water (50 mL) and extracted with ether (3 x 50). This mixture was purified by preparative TLC. The mass spectrum of the isolated material showed a correct molecular ion at m/z 212 and a peak at m/z 193 indicating the loss of fluorine.
4. CONCLUSION

In summary, during this project we investigated several different approaches for preparation of fluorinated ten-membered cyclic edeniynes. In many cases, we encountered product reaction mixtures which had low stability at room temperature and even at lower temperatures. This instability and complex reaction product mixture did not allow us complete our synthetic approaches. However, it is important to mention that fluorination of carbonyl group adjacent to an alkyne was successful. To the best of our knowledge, this is the first successful case of fluorination of a compound which contains a conjugated phenylalkyne group.

We suggest that further research on this topic follow a revised synthetic sequence. Instead of preparing 6-(2-ethynylphenyl)-4,4-difluorohex-5-ynal (69), we suggest first synthesizing B from A using commercial iodoaniline using well-known reactions and reactions described in this thesis. Introduction of the second acetylene side chain using Sonigashira-Hagihara coupling would produce C which would be utilized for ring closure and final conversion to the target molecule as described in Synthetic Scheme One of this thesis.
APPENDIX
REFERENCES


