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The Chemistry of Aza-enediynes, Aza-enyne Allenes and Related Aza-Bergman and Aza-Myers-Saito Rearrangements

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The Chemistry of Aza-enediynes, Aza-enyne Allenes, and Related Aza-Bergman and Aza-Myers-Saito Rearrangements

by

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Dedication

To my husband, Shiju Wang, for his endless encouragement and patience

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The Chemistry of Aza-enediynes, Aza-enyne Allenes and Related Aza-Bergman and Aza-Myers-Saito Rearrangements

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Naturally occurring enediynes are a class of potent antibiotic antitumor agents that cleave DNA by generating reactive diradicals via the Bergman cyclization or Myer-Saito type cyclization. However, the lack of tumor specificity of these compounds has limited their application in cancer chemotherapy. We have undertaken an approach to achieve cancer cell specific targeting by enediynes involving the re-design of the enediyne core. Classes of *C*,*N*-dialkynyl imines (3-ene-3-aza-1,5-diynes, aza-enediynes) and 3-ene-4-aza-1,6-diynes (skipped aza-enediynes) have been designed and synthesized. These aza-enediynes undergo a facile aza-Bergman cyclization to generate 2,5-didehydropyridine diradicals that undergo a rapid retro-aza-Bergman rearrangement to (*Z*)-β-alkynyl acrylonitriles. Certain aza-enediynes can undergo reaction under acidic conditions to afford carbene intermediates which can be trapped efficiently. On the other hand, aza-enediynes that undergo aza-Bergman rearrangement slowly can convert to enediynes through a dimerization mechanism. The skipped aza-enediynes can isomerize to the corresponding *C*-alkynyl-*N*-allenyl imines (aza-enyne allenes) under basic

conditions. The resulting aza-enyne allenes undergo a facile aza-Myers-Saito cyclization to generate previously un-reported α,5-didehydro-3-picoline diradicals that can be efficiently trapped. Both 2,5-didehydropyridine diradicals and α,5-didehydro-3-picoline diradicals have the potential to cleave DNA. DNA cleavage abilities of these aza-enediynes and skipped aza-enediynes are under investigation.

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Chapter 1 Enediynes

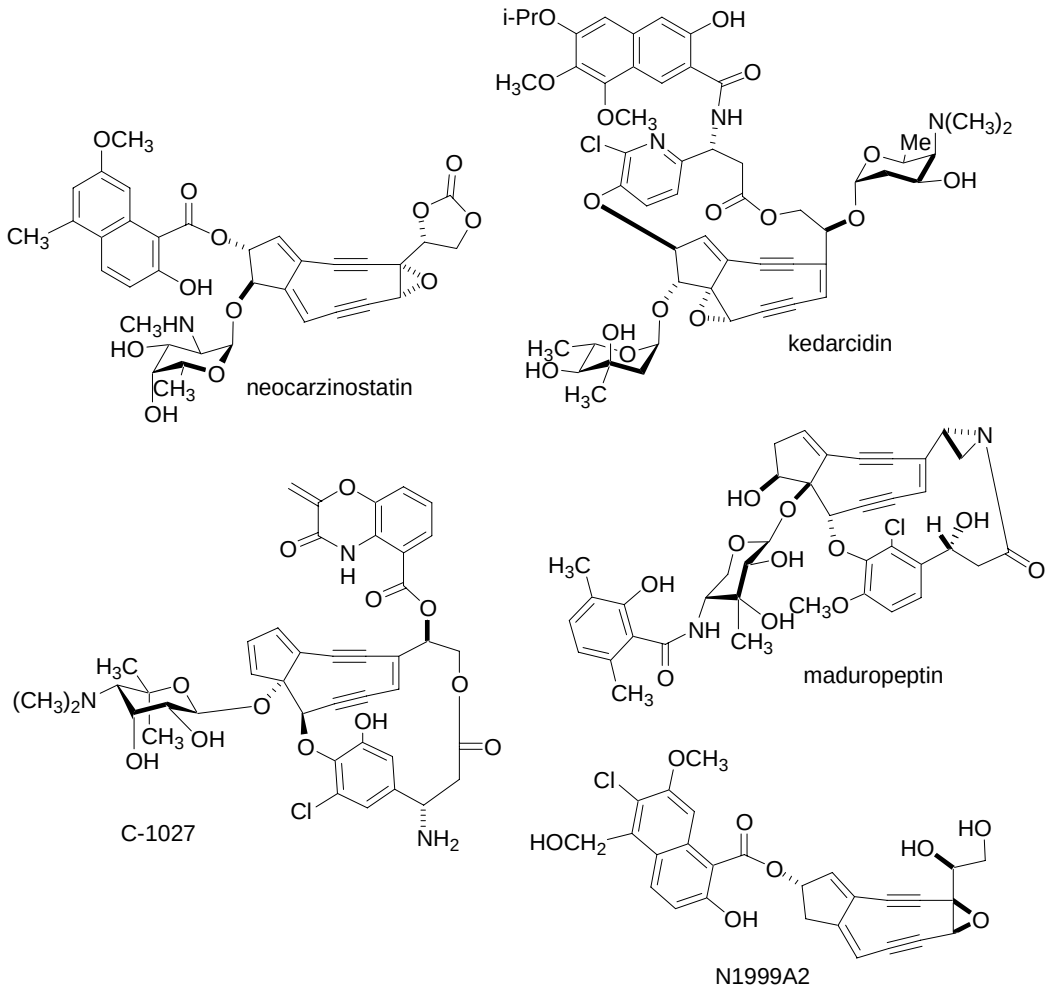
1.1 NATURALLY OCCURRING ENEDIYNES

Naturally occurring enediynes belong to a class of very potent antitumor antibiotics.[1,2] Except shishijimicins and namenamicins, which are produced by marine didemnum,[3] the rest of naturally occurring enediynes discovered so far are produced by the soil bacteria.[2] The structures of naturally occurring enediynes are highly conserved; they all have a conserved unsaturated core structure with two acetylenic groups conjugated to a double bond or incipient double bond, the so called enediyne core structure. These naturally occurring enediynes can be classified into two subfamilies based on the ring size of the enediyne core structure: those possessing a nine-membered ring chromophore core and those possessing a ten-membered ring chromophore core.

1.1.1 Nine-membered ring naturally occurring enediynes

All of nine-membered ring enediynes with an exception of N1999A2 are not stable and cannot exist without the related apo-protein which tightly, but non-covalently binds and stabilizes the enediyne chromophore.[4] To date, the nine-membered ring enediyne family consists nine compounds: neocarzinostatin (NCS), kedarcidin, C-1027, maduropeptin, N1999A2, actinoxanthin, largomycin, auromomycin, and sporamycin.[5] These all contain a common bicycle[7.3.0]dodecdadiynene chromophore.[5] Among these nine-membered ring enediynes, there are only five whose structures have been fully established (Scheme 1-1). They are NCS,[6] kedarcidin,[7] C-1027,[8a] maduropeptin,[9a] N1999A2.[10] These enediynes, except C-1027[8b] and maduropeptin,[9b] require a diradical initiation step which normally is thiol activation to trigger the electronic rearrangement

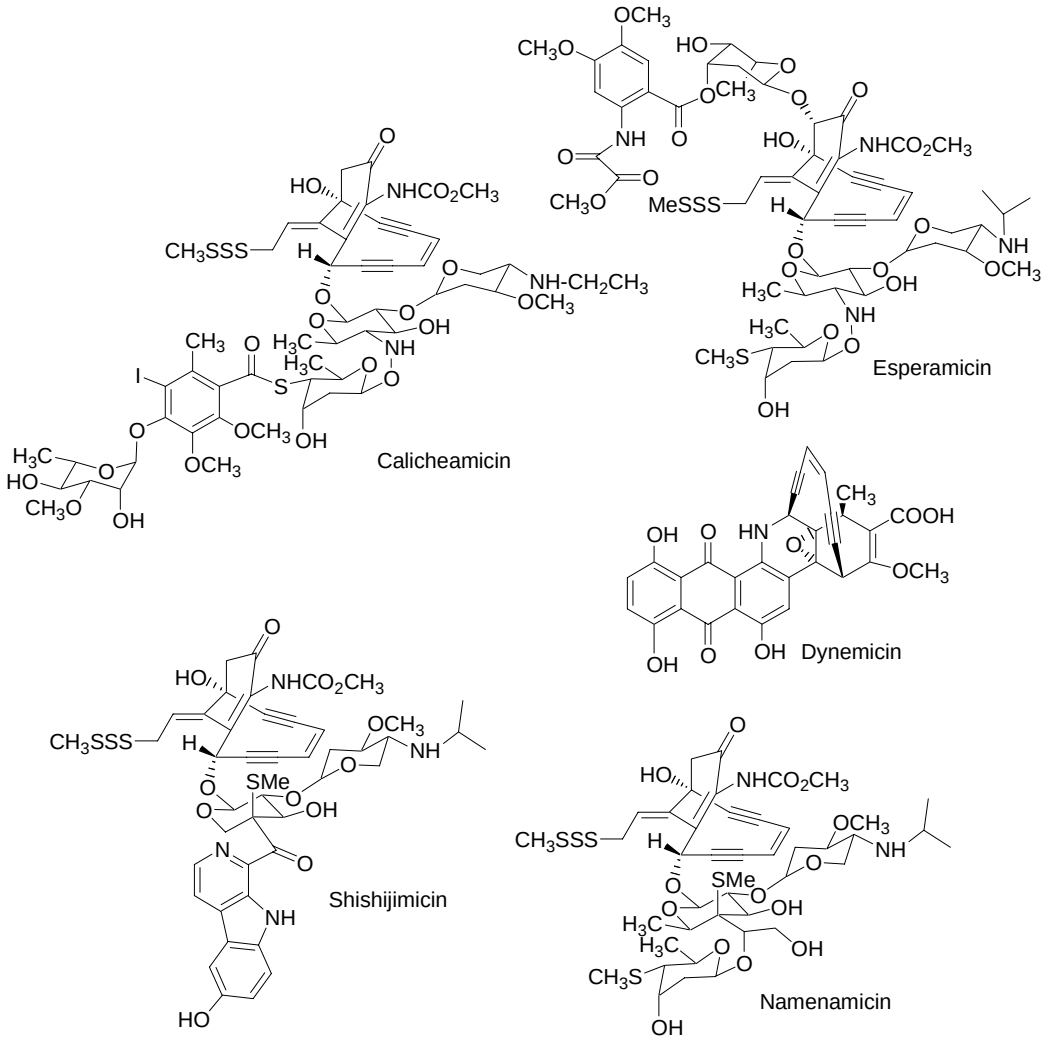
and diradical formation.[2] Shen[11] and co-workers cloned and characterized the complete polyketide synthase (PKS) gene cluster responsible for C-1027 biosynthesis from *S. globisporus*. These findings allowed Shen and co-workers to prepare a new enediyne which is analogous to C-1027 by manipulating genes governing C-1027 biosynthesis.[11a]



Scheme 1-1 The structures of nine-membered ring naturally occurring enediynes

1.1.2 Ten-membered ring naturally occurring enediynes

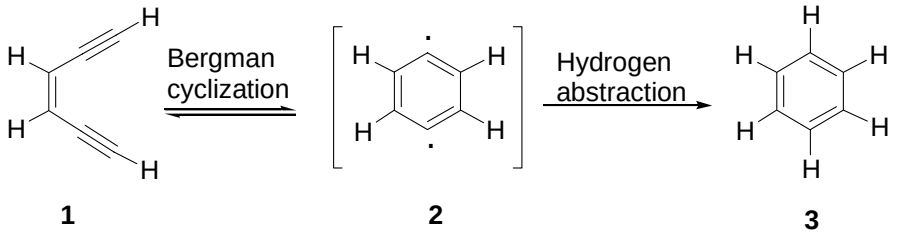
Similar to nine-membered ring enediynes, ten-membered ring enediynes also contain the enediyne moiety and require a diradical initiation step (usually thiol activation).[5] In contrast to nine-membered enediynes, ten-membered enediynes do not require sequestration by an apo-protein.[2] To date, this subfamily includes calicheamicin,[12] esperamicin,[13] namenamicin,[3] shishijimicin,[3] and dynemicin[14] (Scheme 1-2). Thorson and Farnet and co-workers[15] discovered the calicheamicin gene cluster from *Micromomospora ethinospora ssp. Calichensis* consisting a polyketide synthase (PKS), which is a homolog of the PKS gene cluster for C-1027 biosynthesis. These findings together with the findings from Shen[11] and co-workers suggest that PKS is critical for enediyne formation and all enediynes may share a common biosynthetic origin.



Scheme 1-2 The structures of ten-membered ring naturally occurring enediynes

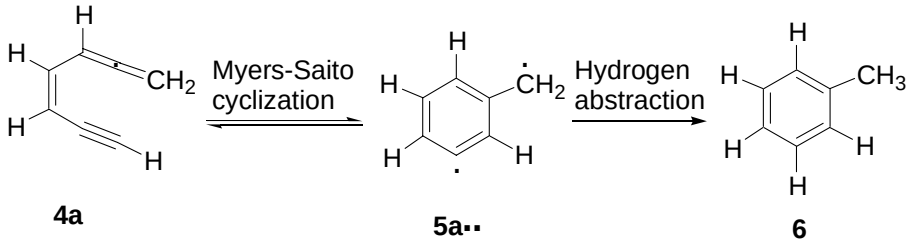
1.2 PROPOSED MECHANISMS OF THE ACTION OF NATURALLY OCCURRING ENEDIYNES

In 1972, Robert Bergman[16] and co-workers reported a simple enediyne **1** undergoes a cyclization (so-called Bergman cyclization) to produce 1,4-didehydrobenzene (1,4-ddb) diradical **2** which then abstracts two hydrogen atoms from solvent to form benzene **3** (Scheme 1-3).



Scheme 1-3 Bergman cyclization

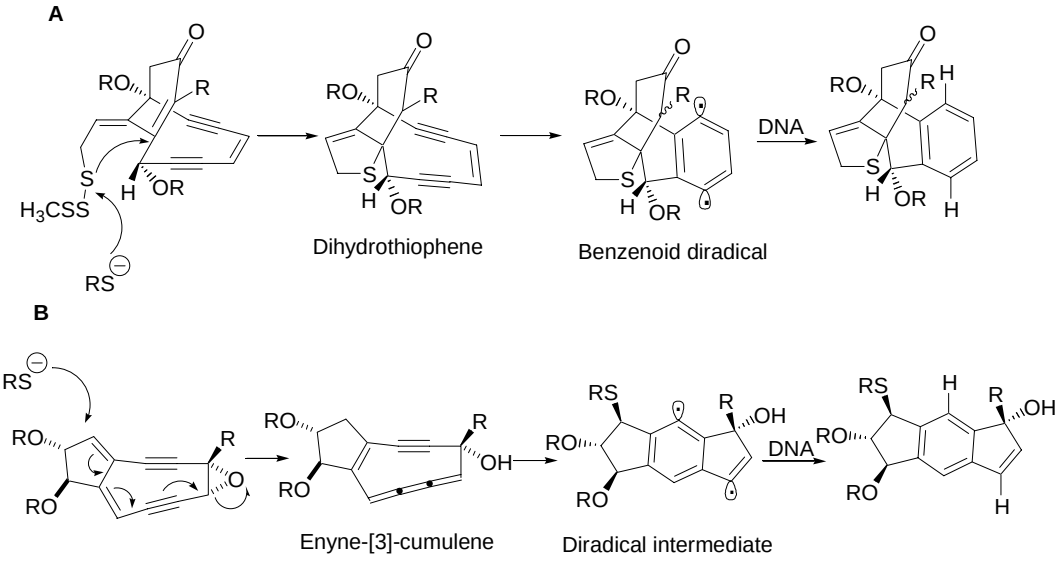
Later, Myers[17] and Saito[18] and their co-workers independently reported that enyne allene **4a** can undergo a cyclization to produce α,3-didehydrotoluene diradical **5a••** (Scheme 1-4), which can also abstract hydrogen atoms from hydrogen donor reagents such as 1,4-cyclohexadiene (1,4-CHD).



Scheme 1-4 Myers-Saito cyclization

Studies have shown that naturally occurring enediynes (Scheme 1-1 and Scheme 1-2) non-specifically bind to DNA minor groove, followed by tracking along the groove for a target site for specific binding.[2] It is not clear whether enediynes bind to DNA before or after their thiol activation step. Once bound to DNA and activated, enediynes then either undergo reductively-induced Bergman cyclization (Scheme 1-5, path **A**) or a Myers-Saito type cyclization of the enediyne via enyne-cumulene (Scheme 1-5, path **B**),

depending on their structures,[2] to produce reactive diradical intermediates, which then abstract a hydrogen atom from the DNA sugar phosphate backbone, forming a aryl radical.[19] This newly formed aryl radical is more reactive than the previous diradical and rapidly abstracts another hydrogen atom from DNA.[19,20] The molecular oxygen can react with the resulting DNA radicals leading to site-specific DNA breaks and ultimately cell death.[19,21,22]



Scheme 1-5 Proposed mechanisms of the action of naturally occurring enediynes (taken from Ref. 2b); **A**: Bergman cyclization type; **B**: Myers-Saito cyclization type (in the case of neocarzinostatin).

1.3 LIMITATIONS OF NATURALLY OCCURRING ENEDIYNES IN CANCER CHEMOTHERAPY AND STRATEGIES TO OVERCOME THESE LIMITATIONS

Although these naturally occurring enediynes are among the most potent anticancer antibiotics ever discovered, they cannot distinguish normal cells and cancer cells. The lack of tumor specificity causes the problem of general toxicity and subsequent systemic side effects.[23] Many approaches have been employed to achieve improved

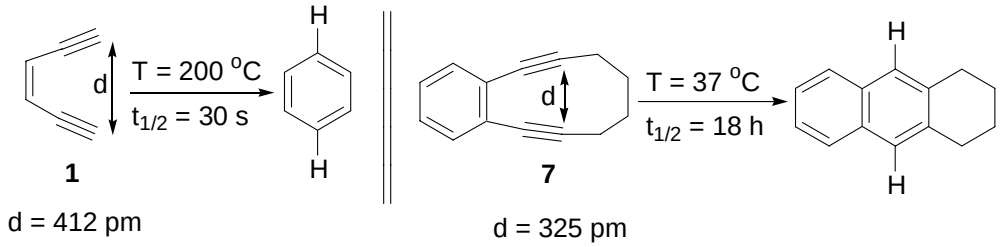
cancer-cell specific targeting by enediynes. One approach involves the creation of monoclonal antibody (mAB)-enediyne conjugates. The most successful example is anti-CD33 mAB-calicheamicin, also known as gemtuzumab ozogamicin (GO), which is currently approved by the FDA under the trade name of Mylotarg to treat acute myeloid leukemia (AML).[24a,b] GO is formed through a covalent linkage between a humanized anti-CD33 mAB and a semisynthetic derivative of calicheamicin.[24a,b] The anti-CD33 mAB directs the mAB-calichemicin to the CD33 cell surface glycoprotein antigen expressed by blast cells in 90% of patients with AML.[24c] Several mAB-C-1027 conjugates have been prepared and currently are being evaluated for clinical application as anticancer drugs.[25] An alternative approach relies on a linkage of an enediyne-specific activation enzyme to tumor cells via a tumor specific mAB. Upon administration, the enediyne will be activated only at the tumor site by the tumor associated mAB-enzyme.[26] The more general approach employs re-design of the enediyne core to achieve more selective tumor cell targeting in combination with minimum toxicity towards normal cells.[27]

1.4 BERGMAN CYCLIZATION

1.4.1 The factors affecting Bergman cyclization

To design new enediynes which target tumor cells with minimum toxicity towards normal cells, it is necessary to understand the factors which influence the Bergman cyclization. The Bergman cyclization is an endothermic process. The experimentally determined activation energy for cyclization of the parent enediyne (Z)-hex-3-ene-1,5-diyne **1** is 28.5 kcal/mol.[28] Enediyne **1** cyclizes only at rather high temperature, for example, at 200 °C with a half-life 30 s. Therefore, overcoming this activation barrier is

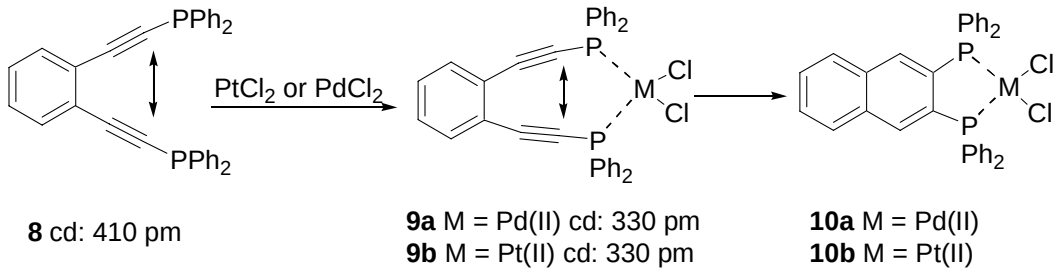
important for re-designing enediynes. All naturally occurring enediynes mentioned in Section 1.1 are embedded into either a nine-membered or ten-membered ring system. The distance between the terminal acetylenic carbon atoms (so called c-d distance[29a]) among these enediynes decreases dramatically due to the ring strain compared to the parent (*Z*)-enediyne **1**. After the thiol activation step, these enediynes cyclize spontaneously at physiological temperature. Scheme 1-6 shows the comparison of c-d distances and half-lives between parent enediyne (Z)-hex-3-ene-1,5-diyne **1** and a simple 10-membered cyclic enediyne **7**.[16,30] Nicolaou[29] and co-workers suggested that the c-d distance should be smaller than 331 pm for enediynes to cyclize at room temperature. In contrast to c-d distance, Magnus[31] and Snyder[32] and their co-workers suggested that the difference in ring strain between ground and transition state indeed accounts for the change in the energetics of the Bergman cyclization.



Scheme 1-6 Comparison of c-d distances and half-lives between acyclic enediyne **1** and cyclic enediyne **7**

Enediyne-metal chelation can also alter the geometry of enediynes and lower the Bergman cyclization barriers (in some cases, electronic effects cannot be ruled out). Buchwald[33] and co-workers prepared the acyclic enediyne **8**, which undergoes a facile Bergman cyclization upon chelation with $PdCl_2$ or $PtCl_2$ (Scheme 1-7). Enediyne **8** cyclizes at 243 °C; in contrast, enediynes **9a** and **9b** formed upon chelation of **8** with Pd

and Pt, respectively, cyclize at relatively low temperatures: 61 °C and 81 °C, respectively.



8 cd: 410 pm

9a M = Pd(II) cd: 330 pm
9b M = Pt(II) cd: 330 pm

10a M = Pd(II)
10b M = Pt(II)

Scheme 1-7 Enediyne-metal chelation facilities Bergman cyclization

Basak[34] and co-workers reported a novel diazoenediyne **11** and its macrocyclic analogue **12** (Figure 1-1) and demonstrated that Cu (II) complexation of these enediynes could promote the Bergman cyclization at reduced temperature. Zaleski[35] and co-workers showed that enediyne ligands can be activated by non-hazardous Mg^{2+} salts.

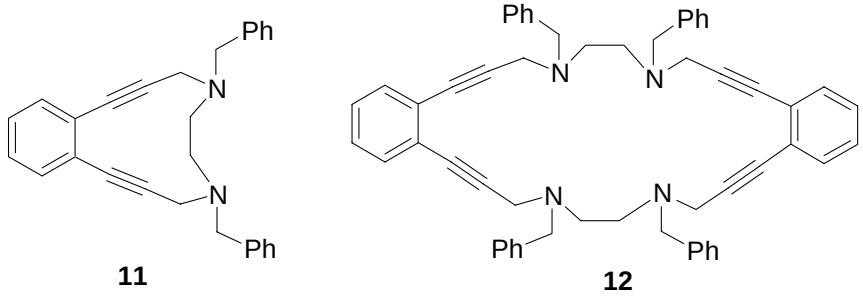


Figure 1-1 Cyclization temperatures of enediynes **11** and **12** reduced upon chelation with Cu (II)

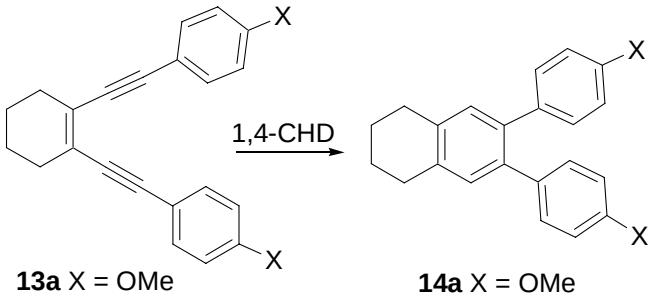
Geometric effects are not the only factor to influence the Bergman cyclization. Electronic effects, including the effects of substituents in the vinylic and terminal alkyne

position, and the influence of benzo-fusion, also, have important impacts on the Bergman cyclization of enediynes as discussed below.[36,37,38,39,40]

Substitution of the terminal alkyne position

Schreiner[36] and co-workers theoretically predict that strong σ-acceptors and/or π-donors such as -F, -OH, $-OH_2^+$ groups lower the Bergman cyclization barrier, while π-withdrawing groups such as $-BH_2$, $-AlH_2$ substituents raise the barrier of the Bergman cyclization due to the interaction with σ-antibonding and π-bonding orbitals in the transition state.

Schmittel and Kiau[37] confirmed this prediction by proving that replacing of the electron-donating methoxy group at alkyne position of enediyne **13** by electron-withdrawing nitro group (Scheme 1-8) leads to a decrease in the activation enthalpy of the Bergman cyclization.



13a X = OMe
13b X = H
13c X = NO_2

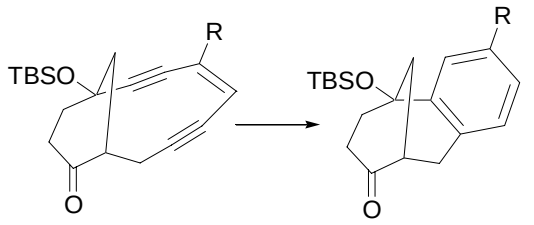
14a X = OMe
14b X = H
14c X = NO_2

	T (^oC) for $t_{1/2}$ = 71 min	$\triangle G^{\ddagger}$ kJ/mol
13a	285.9	180.0
13b	280.2	178.5
13c	249.6	170.6

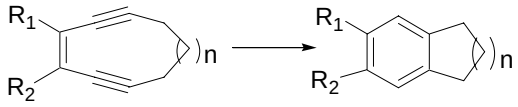
Scheme 1-8 Nitro group facilities the Bergman cyclization of enediyne **13**

Substitution of the vinyl position

Enediyne **15a** cyclizes spontaneously at room temperature (Scheme 1-9); enediyne **15b**, having 4-methoxyphenyl substituent in the vinyl position, does not cyclize at temperature below 80 °C. Maier[38] and Greiner suggested that the 4-methoxyphenyl substituent stabilizes the ground state more than the reaction intermediate, disfavoring the Bergman cyclization. In addition, the electron-donating aryl group may increase the repulsion in the transition state, also leading to retardation of the Bergman cyclization. Jones and Warner[39] prepared a series of cyclic enediynes **16a-e** with chlorine in the vinyl position (Scheme 1-9). The nine-membered enediyne **16a** is extremely labile, but the chlorinated analogue **16b** has a half-life of 8 h at 0 °C. The same retardation trend by addition of chlorine in the vinyl position is also found for the ten-membered enediynes **16c-e**. Significantly, the addition of the second chlorine in **16e** retards the Bergman cyclization rate further.[39]



15a R = H
15b R = 4-(MeO)Ph



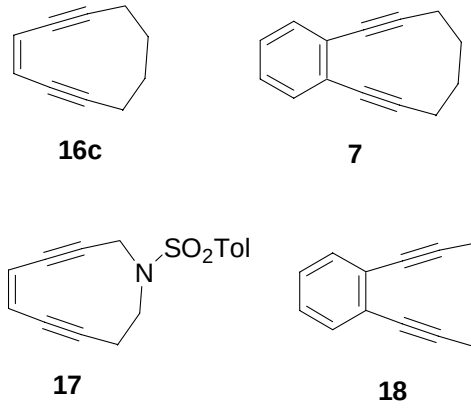
16a $R_1 = R_2 = H$, n = 1
16b R_1 = Cl, R_2 = H, n = 1
16c $R_1 = R_2 = H$, n = 2
16d R_1 = Cl, R_2 = H, n= 2
16e $R_1 = R_2$ =Cl, n = 2

Compound	Cyclization T	$t_{1/2}$ (T)
15a	spontaneously at r. t.	
15b	80 °C	
16a	extremely labile	
16b		8 h (0 °C)
16c		18 h (37 °C)
16d		18 h (50 °C)
16e		24 h (170 °C)

Scheme 1-9

Benzo-fusion

Benzo-fusion in the ene position of the enediynes increases the cyclization barrier compared with non-fused system.[34c,40] Benzo-fused enediyne **7** is stable over several weeks at room temperature, in contrast, enediyne **16c** has a half-life time of 18 h at 37 °C (Scheme 1-10). Similar trend was found in enediyne **17** and its benzo-fused analogue **18**.[34c]



Compound	$t_{1/2}$
16c	18 h at 37 °C
7	Stable at r.t.
17	72 h at 23 °C
18	52 h at 65 °C

Scheme 1-10 Comparison of reactivity of benzo-fused and non-benzo-fused enediynes

1.4.2 Reactivity of 1,4-didehydrobenzene diradical intermediates (1,4-ddb)

In the presence of hydrogen atom source, the 1,4-ddb diradical generated from the Bergman cyclization can either abstract two hydrogen atoms or undergo retro-Bergman cyclization (Fig 1-2). In general, the overall Bergman cyclization is first order reaction with the formation of 1,4-ddb as the rate-limiting step. In the case of the benzannulated enediynes, hydrogen atom abstraction is the rate-determining step.[30]

Chen[41a] and co-workers have emphasized that the reactivity of 1,4-ddb intermediate is largely determined by the small separation between its singlet - triplet (S-

T) gap (3.8 kcal/mol for the enediyne **1** determined experimently[42a]). The stronger the interaction between radical centers, the larger the S-T gap is and the less reactive the diradical is towards hydrogen atoms abstraction.[41a]

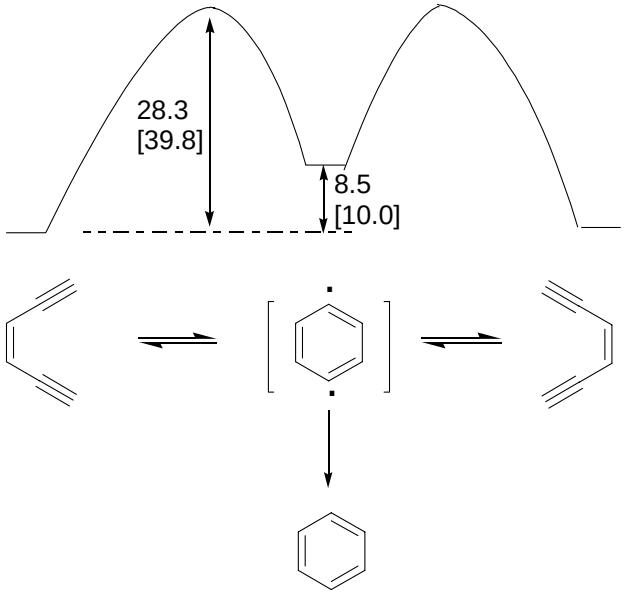
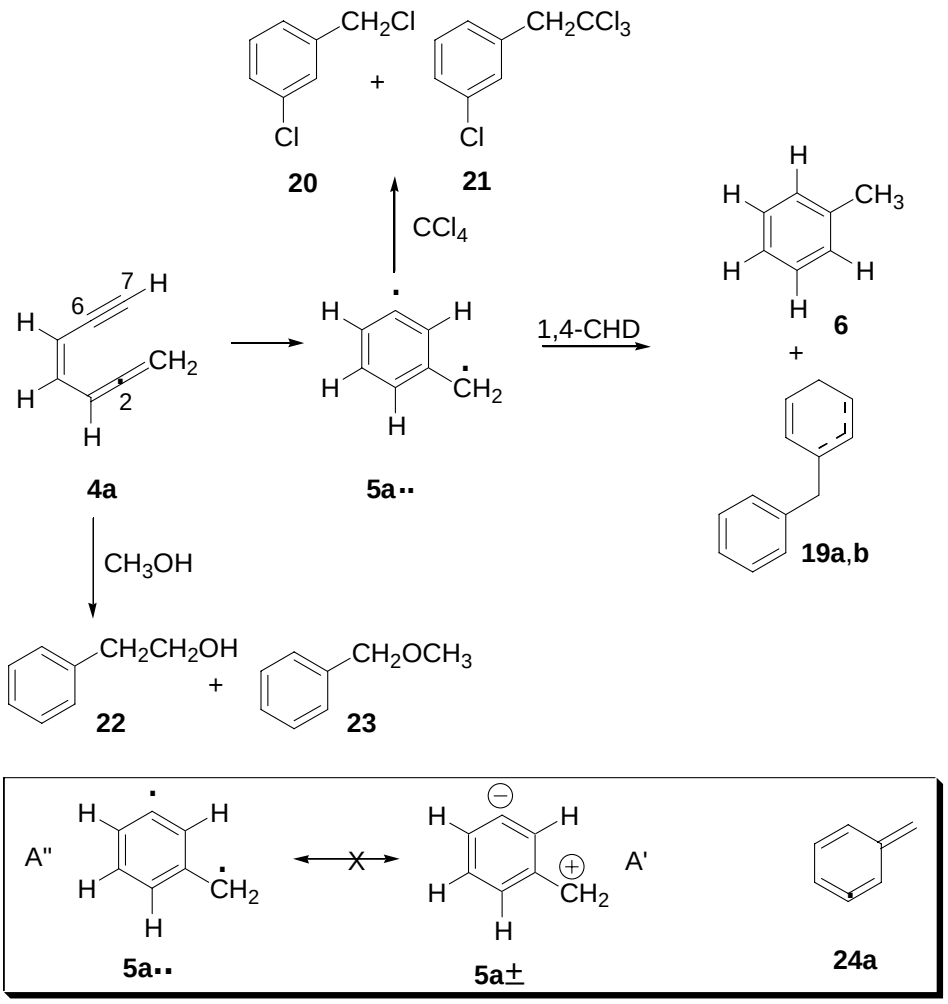


Figure 1-2 One-dimensional reaction coordinate for the parent Bergman cyclization. Energy differences in kcal/mol derived from experimental data (ref. 42b) and from CASSCF level (numbers in brackets, ref. 41b).

1.5 MYERS-SAITO CYCLIZATION AND SCHMITTEL CYCLIZATION

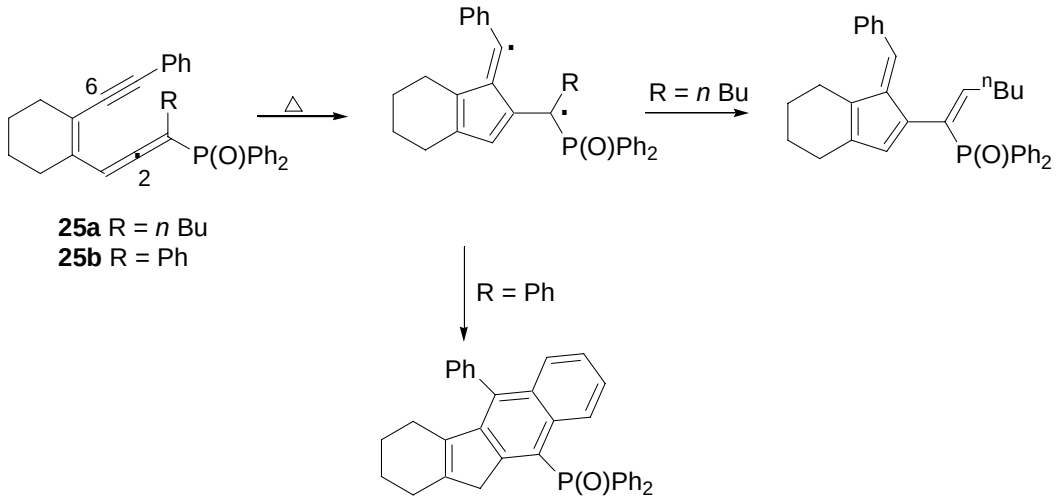
Myers[17] and Saito[18] and their co-workers reported the cyclization of (*Z*)-1,2,4-heptatrien-6-ynes (enyne-allenes, such as compound **4a**) (Scheme 1-11). Enyne allene **4a** undergoes C^2-C^7 cyclization to produce α,3-didehydrotoluene diradical which abstracts the hydrogen atoms as shown in Scheme 1-11. This pathway is similar to that found in naturally occurring enediyne neocarzinostatin, except that the α,3-didehydrotoluene

diradical is a σ, π–diradical instead of one having σ, σ-character in neocarzinostatin. The σ, π–diradical is less reactive than the σ, σ–diradical.[17] Unlike Bergman cyclization, the Myers-Saito cyclization is exothermic by 15 kcal/mol and was found to have a half-life of 20.5 h at 39 °C for the parent enyne allene **4a**, corresponding to an activation barrier of 22 kcal/mol.[17a] Pyrolysis of compound **4a** in methane led to the products corresponding to the both polar reaction (methyl benzyl ether, Scheme 1-11, **23**) and free diradical trapping reaction[17] (2-phenylethanol, Scheme 1-11, **22**). Myers[17] and co-workers proposed that methyl benzyl ether **23** was formed via zwitterionic intermediate **5a±** instead of diradical **5a••** and suggested that intermediates **5a±** and **5a••** share a common rate-limiting cyclization step and may be a pair of rapidly equilibrating species. Later, Carpenter[43] and Cramer[44] and their co-workers pointed out that diradical **5a••** and zwitterions **5a±** cannot co-exist as a pair of equilibrating species. Diradical **5a••** has A'' symmetry and zwitterions **5a±** has A' symmetry; mixing of these electronic configurations is therefore symmetry forbidden. Computational studies[43] showed that ground state closed shell structure for α,3-didehydrotoluene was in fact not planar, but a puckered cyclic allene **24a**, which may account for the formation of product **23**. Recently, Carpenter[45] and co-workers ruled out three mechanisms mentioned above, and proposed that enyne-allene **4a** must directly form the zwitterion **5a±** and exhibit a post-rate-determining bifurcation to explain the formation of both **23** and trapping products **6**, **19a**,**b**. Apparently, more research needs to be done to clarify the mechanism of the Myers-Saito cyclization.



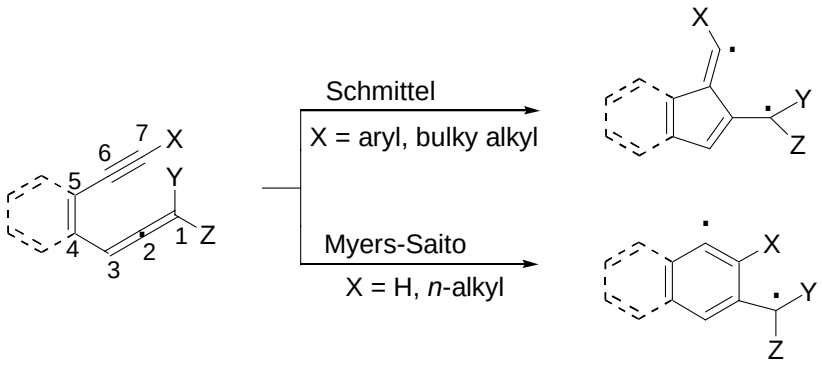
Scheme 1-11 Myers-Saito cyclization (C^2-C^7) of enyne-allene **4a**

The initial reports on the thermolysis of enyne allenes stimulated a number of studies of the thermal cyclization of related enyne allenes to aromatic diradicals. Among these studies, Schmittel[46] and co-workers and others[47,48] discovered an alternate cyclization of enyne allenes – Schmittel cyclization (Scheme 1-12). Schmittel cyclization joins positions C^2 and C^6 to generate a fulvene-like diradical, which also has σ, π-character.



Scheme 1-12 Schmittel cyclization (C^2-C^6) of enyne-allene **25**

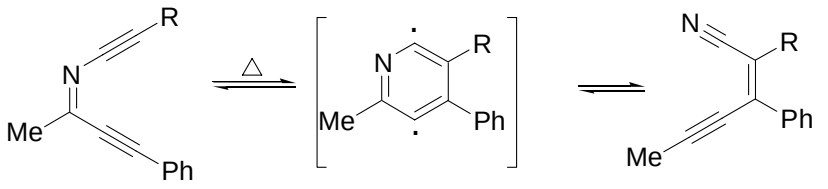
Computational studies demonstrated that Schmittel cyclization has much higher activation barriers than Myers-Saito cyclization does (25 kcal/mol for Myers-Saito cyclization, 35 kcal/mol for Schmittel cyclization).[49] Under normal circumstances, enyne allenes favor Myers-Saito cyclization. Schmittel[50] and co-workers discovered that enyne allenes may favor Schmittel cyclization when radical stabilizating substituents such as aryl or bulky alkyl groups are introduced at C^7 position (Scheme 1-13). Other factors such as steric effect[51a] and ring strain effect[51b] also play important roles on the relative barriers of the Myers-Saito cyclization and Schmittel cyclization.



Scheme 1-13 A switch of Myers-Saito and Schmittel cyclization by changing substituent at C^7 position.

1.6 AZA-ENEDIYNE AND AZA-BERGMAN CYCLIZATION

Kerwin and David[52] reported the first synthesis of a new class of enediynes, the 3-aza-enediynes, or *C,N*-dialkynyl imines **26e,f** and their Bergman cyclization in 1997 (Scheme 1-14). The replacement of a sp^2 carbon with a sp^2 nitrogen would have a profound impact on facility of the Bergman cyclization due to the following reasons: the presence of nitrogen in the aza-enediyne would disturb π-delocalization compared with the enediyne, which destabilizes aza-enediyne; in addition, nitrogen also decreases repulsion of the in plane π-orbital in transition state.[52] Aza-enediynes **26e,f** undergo a cyclization analogous to the Bergman cyclization (aza-Bergman cyclization) to produce 2,5-didehydropyridine (2,5-ddp) diradical intermediates[52] **27e,f** (Scheme 1-14).



26e R = Ph
26f R = CH_2CH_2CH=CHPh

27e R = Ph
27f R = CH_2CH_2CH=CHPh

28e R = Ph
28f R = CH_2CH_2CH=CHPh

Scheme 1-14 Thermolysis of aza-enediynes **26e,f**

Although these aza-enediynes undergo an aza-Bergman cyclization to produce 2,5-didehydropyridine diradical intermediates, all attempts to trap these intermediates only result in the formation of the retro-aza-Bergman rearrangement products (*Z*)-*β*-alkynyl acrylonitriles **28e,f**[52] (Scheme 1-14). The only experimental support for the existence of the 2,5-didehydropyridine diradical intermediate had been reported by Chen[41b] and co-workers, who reported the detection of miniscule amounts of pyridine products by GC/MS in the thermolysis of the aza-enediyne **26e** under acidic conditions or upon prolonged standing in freezer.

The S-T gap of 2,5-ddp is calculated to be 8.2 kcal/mol at CASMPS level compared with 2.1 kcal/mol for 1,4-ddb at the same level.[41b,53] Chen[41b] and co-workers and Cramer[53] proposed that the larger S-T gap of 2,5-ddp diradical compared to 1,4-ddb makes 2,5-ddp less diradical in character and less reactive towards hydrogen atom abstraction reaction. In addition, 2,5-ddp diradical easily undergoes retro-aza-Bergman rearrangement to nitrile. The activation barrier for this retro rearrangement is calculated to be only 7 kcal/mol, and strongly exothermic with the formation of more stable nitrile[41b] (Fig 1-3).

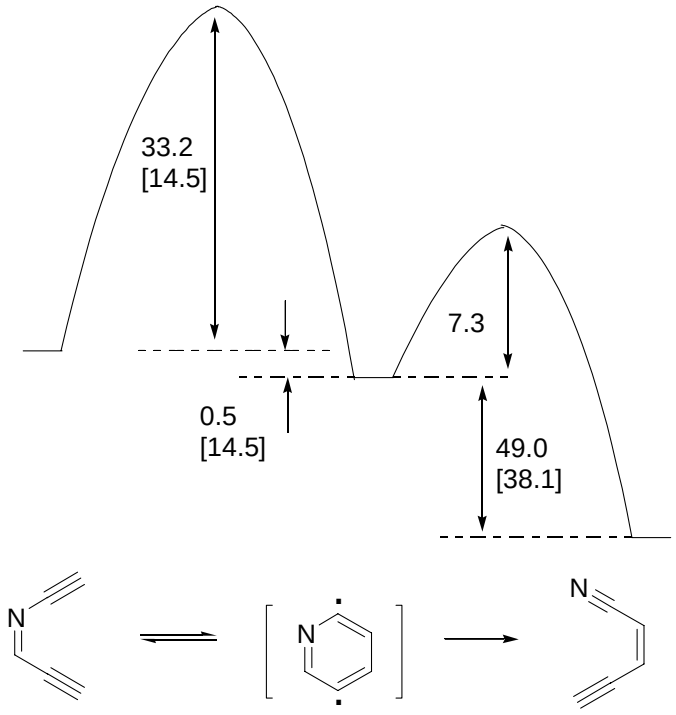


Figure 1-3 One dimensional reaction coordinate for the parent aza-Bergman cyclization. Energy differences in kcal/mol at the CASSCF (ref. 41b) and BPW91 levels (numbers in brackets, ref. 53).

The difficulties encountered in trapping of 2,5-ddp diradicals are in agreement with the theoretical predictions. Protonation of nitrogen atom is predicted to reduce the S-T gap of 2,5-ddp.[41b] In addition, protonation of the nitrogen atom is also predicted to increase the barrier for retro-aza-Bergman cyclization[41b] (Fig 1-4). As mentioned in section 1.3, the application of naturally occurring enediynes in chemotherapy has been limited due to their lack of cancer cell specificity and toxicity. It has been previously reported certain drugs such as amiloride, nigericin, and hydralazine preferentially lower the intracellular pH in tumor cells, with no such effect in normal cells.[54] These drugs can drop the intracellular pH in tumor cells from 7.2 to between 6.2 and 6.6.[54] A switch of the reactivity of 2,5-ddp diradical upon protonation of nitrogen atom may provide the basis

for the design of novel aza-enediynes which undergo aza-Bergman cyclization and abstract hydrogen atoms from DNA only in tumor cells.

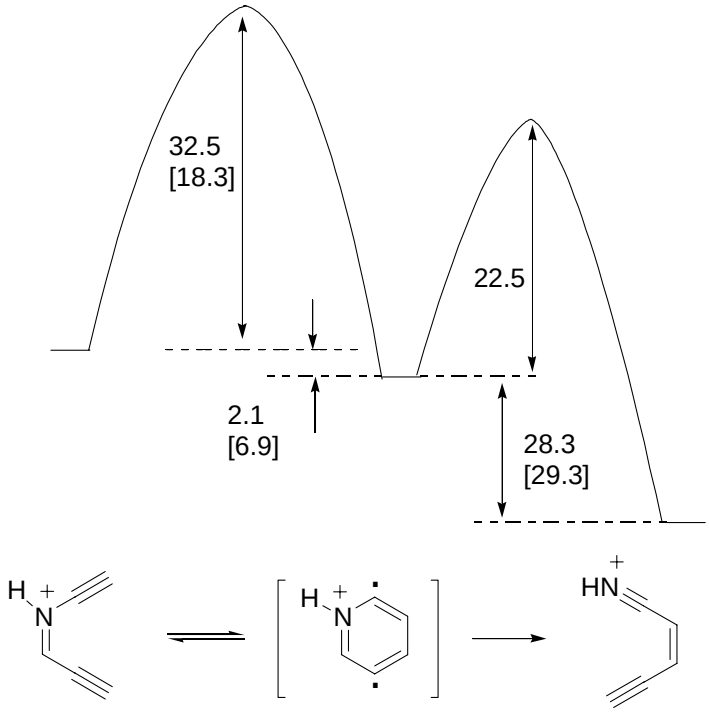
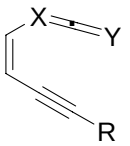


Figure 1-4 One dimensional reaction coordinate for the protonated parent aza-Bergman cyclization. Energy differences in kcal/mol at the CASSCF (ref. 41b) and BPW91 levels (numbers in brackets, ref. 53).

1.7 HETEROATOM-SUBSTITUTED ENYNE ALLENES

All of the heteroatom-substituted enyne allene analogues examined to date involve heteroatom substitution on the allene moiety, including enyne ketenes, enyne ketenimines, enyne carbodiimides, and enyne-isocyanate (Fig. 1-5).

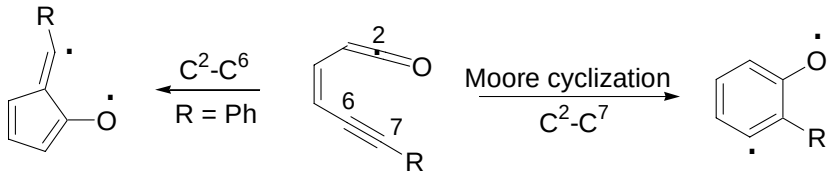


enyne-ketenes: X = CH, Y = O
enyne-ketenimines: X = N, Y = CR'R''
enyne-carbodiimides: X = N, Y = NR'
enyne-isocyanate: X = N, Y = O

Figure 1-5 Heteroatom-substituted enyne allene analogues

1.7.1 Enyne-ketenes and Moore cyclization

Enyne-ketenes having an oxygen atom in the allene termini undergo a cyclization analogous to Myers-Saito cyclization (also known as Moore cyclization after H. W. Moore[55] who first reported the cyclization in 1985) via diradical intermediates (Scheme 1-15). Enyne-ketenes having a phenyl substituent or other radical stabilizing groups at the alkynyl terminius undergo a cyclization analogous to the Schmittel cyclization to form the fulvene-like diradicals.[55]

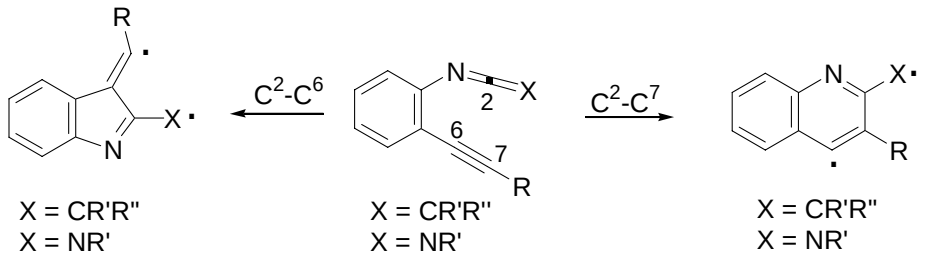


Scheme 1-15 Enyne-ketenes

1.7.2 Enyne ketenimines and enyne carbodiimides

Wang[56] and co-workers reported that benzannulated enyne-ketenimines (Scheme 1-16, X = CR'R'') and benzannulated enyne carbodiimides (Scheme 1-16, X = NR') also

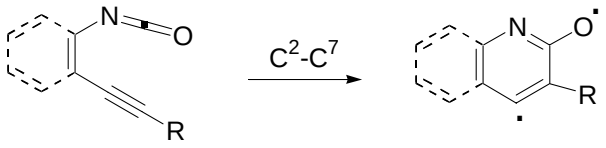
undergo the C^2-C^7 and/or C^2-C^6 cyclization via diradical formation depending on the alkynyl substituents.



Scheme 1-16 Enyne ketenimines and enyne carbodiimides

1.7.3 Enyne-isocyanate

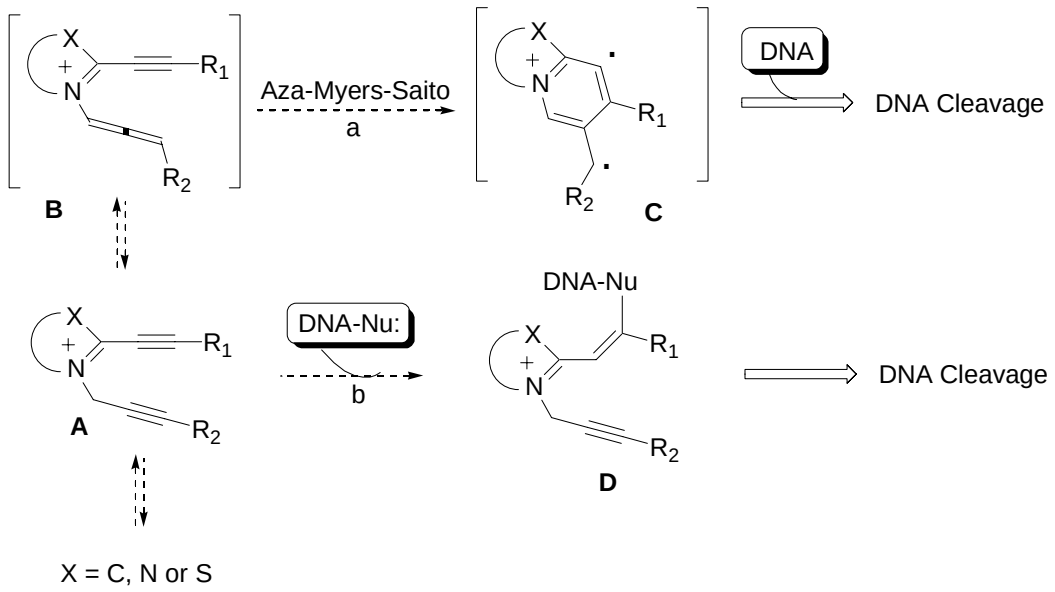
Enyne-isocyanates prepared by Wang[57] and co-workers undergo a cyclization analogous to the Myers-Saito cyclization via diradical formation pathway (Scheme 1-17). In contrast to other hetero-enyne allene systems, such as enyne-allene, enyne-ketene, enyne-ketenimine and enyne-carbodiimide, the cycloaromatization reactions of enyne-isocyanates appear to require higher temperature and are more in favor of the C^2-C^7 cyclization pathway.[57]



Scheme 1-17 Enyne-isocyanates

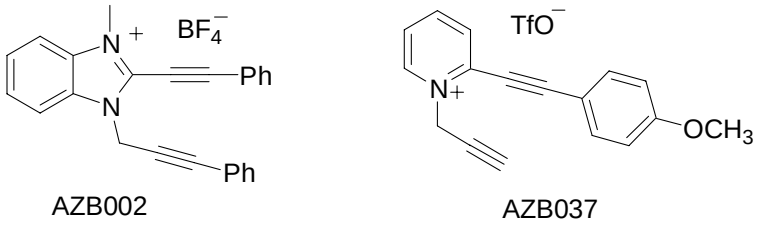
1.8 SKIPPED AZA-ENEDIYNE (3-ENE-4-AZA-1,6-DIYNE): A POTENTIAL ROUTE TO AZA-ENYNE ALLENE

Kerwin[58] and co-workers first designed the 3-ene-4-aza-1,6-diyne (skipped aza-enediyne) with a methylene carbon interrupting the enediyne conjugation system (Scheme 1-18, **A**). These skipped aza-enediynes may isomerize to the corresponding *C*-alkynyl-*N*-allenyl imines (aza-enyne allenes) under basic conditions[58] (Scheme 1-18, **B**). The potential aza-Myers-Saito cyclization or aza-Schmittel cyclization of the resulting aza-enyne allene (Scheme 1-18, path **a**) may afford reactive diradical intermediates such as diradical **C** (Scheme 1-18). These diradical intermediates may cleave DNA by abstracting hydrogen atoms from DNA sugar phosphate backbone. In addition, if the nitrogen atom of these skipped aza-enediyne systems is incorporated into an electron-deficient heterocyclic ring, the alkyne moeity can serve as an electrophile which may cleave DNA as DNA, a nucleophile, attacks this electrophile forming DNA-skipped enediyne adducts **D** (Scheme 1-18).



Scheme 1-18 Potential reactions of skipped aza-enediyne

The heterocyclic skipped aza-enediynes such as AZB002[58,60] and AZB037[59] (Fig. 1-6) have shown the promising pH and temperature dependent DNA cleavage abilities and have the potential to function as effective antitumor agents.[58,59,60] Compound AZB002 was treated with strong base in order to generate the corresponding aza-enyne allene which may undergo an aza-Myers-Saito cyclization to reactive diradical intermediates with the potential to cleave DNA. Although no trapping products of these diradical intermediates were observed, ^{13}C NMR of the crude reaction demonstrated the formation of very unstable aza-enyne allene with resonances at δ 198, 111, and 106 ppm corresponding to resonances of a typical allene system.[58] The mechanism of DNA cleavage affected by AZB002 and AZB037 remains to be elucidated. It is possible that this cleavage is from the combination of DNA-adduct and diradical derived from an aza-Myers-Saito cyclization.[58,59,60]



AZB002 AZB037

Figure 1-6 Heterocyclic aza-skipped enediynes with DNA cleavage abilities

1.9 DISSERTATION OUTLINE

Here, I present our studies on *C*,*N*-dialkynyl imines (aza-enediynes) and 3-ene-4-aza-1,6-diynes (skipped aza-enediynes). Briefly, these aza-enediynes undergo a facile aza-Bergman cyclization to generate 2,5-didehydropyridine diradicals that undergo a rapid retro-aza-Bergman rearrangement to (*Z*)-β-alkynyl acrylonitriles (Chapter 2). Certain aza-enediynes can undergo reaction under acidic conditions to afford carbene intermediates which can be trapped efficiently (Chapter 3). On the other hand, aza-enediynes that undergo aza-Bergman rearrangement slowly can undergo a remarkable transformation to produce enediynes through a dimerization mechanism (Chapter 5). Skipped aza-enediynes can isomerize to the corresponding *C*-alkynyl-*N*-allenyl imines (aza-enyne allenes) under basic conditions. The resulting aza-enyne allenes undergo a facile aza-Myers-Saito cyclization to generate previously un-reported α,5-didehydro-3-picoline diradicals that can be efficiently trapped (Chapter 4).

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Chapter 2 An extremely facile aza-Bergman rearrangement of sterically unencumbered acyclic 3-aza-3-ene-1,5-diynes

2.1 INTRODUCTION

We have investigated a new class of enediynes – *C*,*N*-dialkynyl imines (also known as aza-enediynes, Scheme 1-11, **26e,f**).[1] The replacement of a sp^2 carbon with a sp^2 nitrogen is predicted to have a profound impact on facility of the Bergman cyclization. These aza-enediynes undergo aza-Bergman cyclization to produce 2,5-didehydropyridine diradicals (Scheme 1-11, **27e,f**) which then undergo retro-aza-Bergman rearrangement to (*Z*)-*β*-alkynyl acrylonitriles (Scheme 1-11, **28e,f**).[1] Since the initial report of the aza-enediynes and the corresponding aza-Bergman cyclization reaction, these compounds have been the subject of a number of theoretical studies.[2,12,13,15,17] However, the factors that affect the kinetics of the aza-Bergman cyclization of aza-enediynes have not been elucidated. To date, there are only two reports of kinetic studies of this process,[1,2] and both of these were carried out on similar aza-enediynes. In this chapter, I present our kinetic studies on a series of acyclic 3-aza-enediynes.[5]

2.2 EXPERIMENT DESIGN

The goals of this part of my dissertation research are to design and synthesize a series of aza-enediynes with different R substitution groups as shown in Figure 2-1 and to study the thermal rearrangements of these aza-enediynes. These aza-enediynes will be synthesized according to our previously reported procedure[1] with some modification if necessary. The substituent effects on the kinetics of aza-Bergman rearrangement will be

studied. UV/Vis spectroscopy and/or ^{1}H NMR will be used to follow the reaction kinetics.

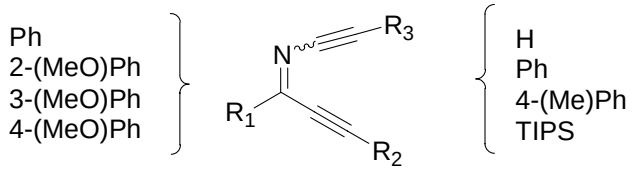
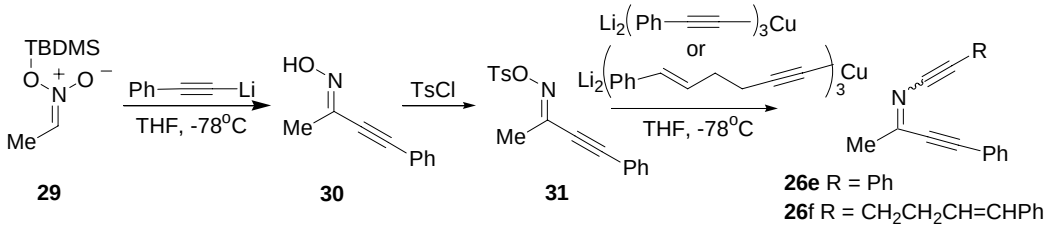


Figure 2-1 Designed aza-enediynes with different R substitution groups

2.3 SYNTHESIS OF A SERIES OF ACYCLIC AZA-ENEDIYNES

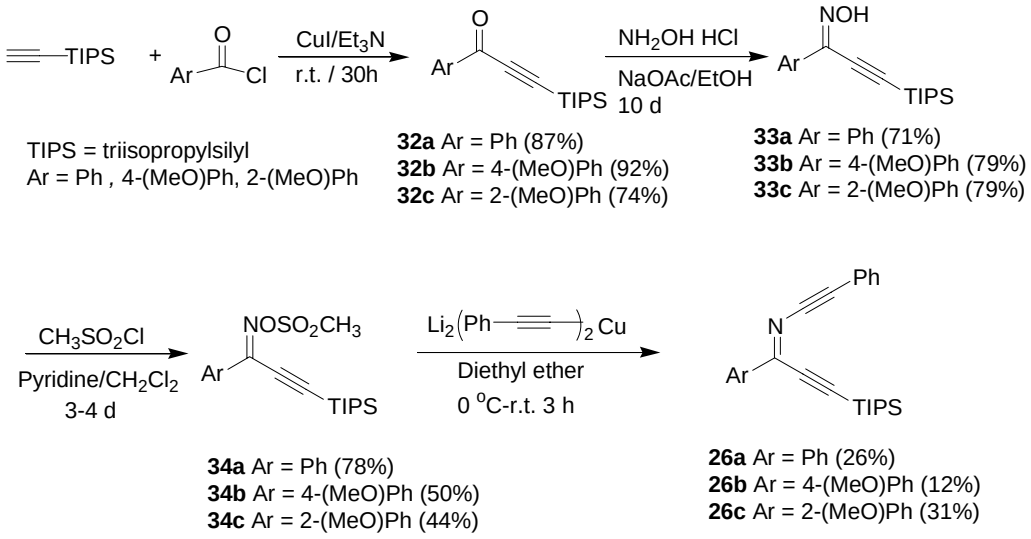
Scheme 2-1 shows our previous procedure[1] to synthesize aza-enediynes **26e,f**. (*E*)-oxime **30** was obtained from the addition of lithium phenylacetylide to the *tert*-butyldimethylsilyl nitronate ester of nitroethane **29**. The oxime **30** was then activated to its sulfonate ester **31**. Addition of the cuprate reagent[3] derived from phenylacetylene or 6-phenyl-5-ene-hexyne to the sulfonate ester **31** afforded the *C*,*N*-dialkynyl imines **26e,f** in modest yields (Scheme 2-1).



Scheme 2-1 Previously reported procedure for the synthesis of aza-enediynes **26e,f**

Employing the same route to access other aza-enediynes was met with difficulty.[1b] Addition of lithium phenylacetylide to the silyl nitronate derived from nitromethyl benzene led to intractable mixtures from which the desired oxime could not be isolated. Condensation of 1,3-diphenylpropynone with hydroxylamine only afforded 3,5-diphenylisoxazole.[1b] To avoid the problem of isoxazole formation, we chose to prepare propynone oximes bearing a large substituent on the terminal acetylenic carbon. Thus, a series of aza-enediynes with bulky triisopropylsilyl group at C^6 terminal acetylenic carbon were synthesized according to the route shown in Scheme 2-2. The first step is the cuprous iodide-catalyzed addition[4] of triisopropylsilyl acetylene to benzoyl chloride, *p*-anisoyl chloride, or *o*-anisoyl chloride to afford the corresponding propynones **32a-c** in good yield. The conversion of these acetylenic ketones to the corresponding oximes **33a-c** proceeded in good yield, although the reactions required 10 days for completion. The oximes **33a-c** were isolated as predominately single isomers of undetermined stereochemistry, as judged from ^1H and ^{13}C NMR spectra. The oximes were then activated to their methylsulfonate esters **34a-c** by the treatment with methylsulfonyl chloride. The corresponding mesylates **34a-c** were also isolated as single isomers of undetermined stereochemistry. Addition of the cuprate reagent[3] derived from phenylacetylene to the mesylates **34a-c** afforded the *C*,*N*-dialkynyl imines **26a-c** in modest yields (12% -31%) along with variable amounts of reduction products 1-aryl-3-(triisopropylsilyl)-prop-2-ynylidenamines **35a-c**.[6] The mechanism for the formation of reduction products **35a-c** is not clear. It may involve water induced cleavage of the imidocopper intermediate complex.[7,8] The cuprate coupling reaction is very sensitive to moisture. All glassware used for this reaction has been dried in a 160 °C oven for a period of time before use, and then transferred directly into a desiccator to cool. The aza-enediynes **26a-c** can be purified by flash chromatography on silica gel and isolated as

relatively stable, yellow oils that can be stored at 0 °C for several months without any signs of decomposition. The aza-enediynes **26a-c** were isolated as predominantly one isomer, as judged by ^{1}H and ^{13}C NMR.

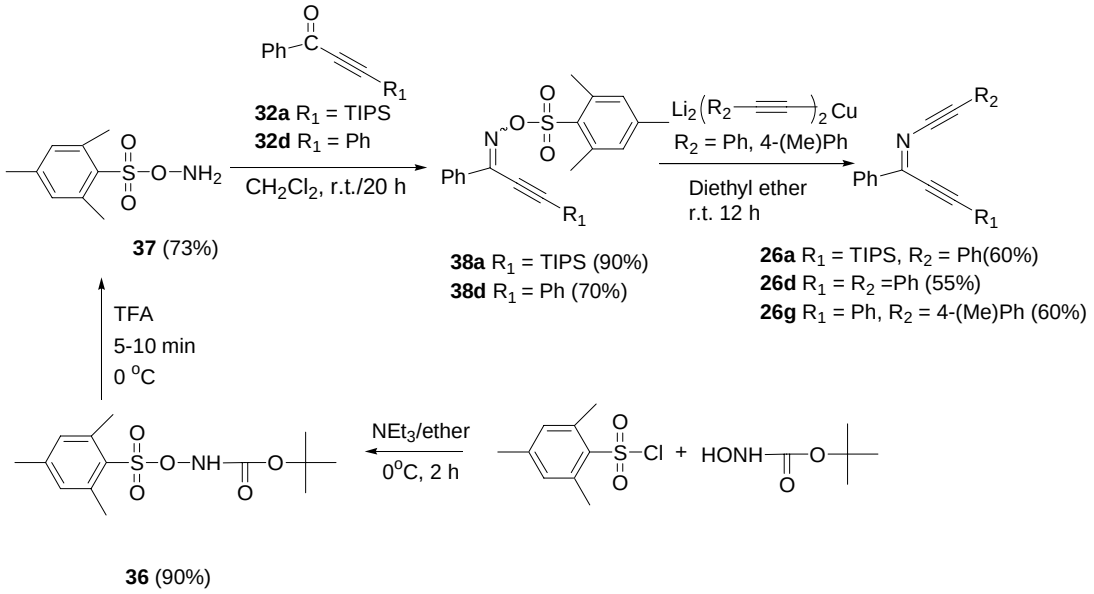


Scheme 2-2 Synthesis of a series of acyclic aza-enediynes **26a-b**

The yields for cuprate addition were relatively poor (12-31%). An alternative synthetic route was developed as shown in Scheme 2-3. Instead of the methylsulfonate esters **34**, the propynones reacted with mesitylenesulfonyl hydroxylamine **37** to afford the mesitylenesulfonate esters **38** directly.[9] There are several advantages to this approach. First of all, mesitylenesulfonyl group is a better leaving group than methylsulfonyl group; it may facilitate the cuprate addition reaction and therefore increasing the yield for this reaction. Secondly, the oxime formation step which took 10 days for completion was avoided. In addition, some propynones such as 1,3-diphenylpropynone reacted with hydroxylamine only to afford 3,5-diphenylisoxazole.[1b] By switching to

mesitylenesulfonate ester, propynones were converted to mysitylenesulfonate ester directly. Mesitylenesulfonyl hydroxylamine **37** was synthesized according to the previously reported procedure.[9a] Briefly, condensation of 2-mesitylenesulfonyl chloride with *t*-butyl *N*-hydroxycarbamate afforded *t*-butyl *N*-mesitylenesulfonyl oxycarbamate **36** in near quantitative yield, which then underwent hydrolysis by the treatment with trifluoroacetic acid (TFA) to yield mesitylenesulfonyl hydroxylamine **37** in good yield. Compound **37** was not stable; it decomposed even when stored in a 4 °C fridge. In addition, compound **37** is extremely explosive;[9b] therefore, it is used immediately to react with propynones.

Mesitylate **38a**,**d** were obtained from the condensation of the propynones **32a,d** with mesitylenesulfonyl hydroxylamine **37** in CH_2Cl_2 in good yield. The resulting mesitylates **38a,d** were treated with cuprate reagent derived from the phenylactylene or 4-methylphenylactylene to afford the corresponding aza-enediynes **26a,d,g** (Scheme 2-3) in good yields (> 50%). The yields for the cuprate addition were improved from 12-31% to 55%. Aza-enediynes **26a**,**d,g** were isolated as one isomer of undetermined stereochemistry, as judged by ^{1}H NMR and ^{13}C NMR. Unlike the cuprate addition to mesylates **34a**, no reduction by-product 1-phenyl-3-(triisopropylsilyl)-prop-2-ynylidenamine **35a** was isolated from cuprate reaction employing **38a**. Further studies revealed that cuprate addition reactions carried out on more than 300 mg of mesitylates gave lower yields (less than 50%).



Scheme 2-3 Optimized synthetic route for aza-enediynes **26a,d,g**

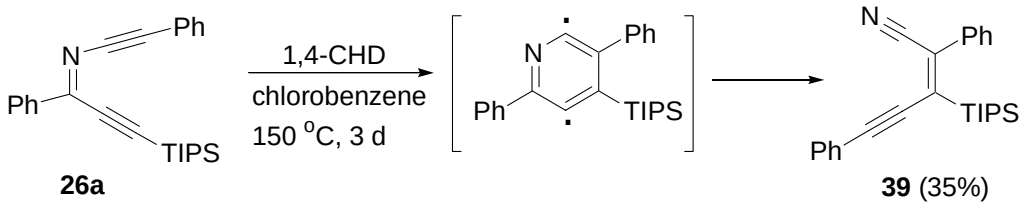
2.4 AZA-BERGMAN REARRANGEMENT

2.4.1 Aza-Bergman kinetics

David and Kerwin[1] reported that aza-enediynes **26e,f** undergo aza-Bergman-retro-aza-Bergman rearrangement to the corresponding nitriles under relatively mild condition. The thermolysis of aza-enediyne **26e** follows first order kinetics and has a half-life of 47 min at 110 °C in CH_3CN. In contrast, the half-life for the Bergman cyclization of the corresponding (*Z*)-1,6-diphenylhex-3-ene-1,5-diyne is reported to be 71 min at 280 °C.[10]

Thermolysis of **26a** was performed by heating compound **26a** in chlorobenzene in the presence of 20 equiv of 1,4-cyclohexadiene (1,4-CHD) as proton donor in a sealed tube at 150 °C. The disappearance of starting material was monitored by TLC, which demonstrated that the reaction required 3 days to reach completion. We were unable to identify the product derived from trapping of the intermediate 2,5-didehydropyridine

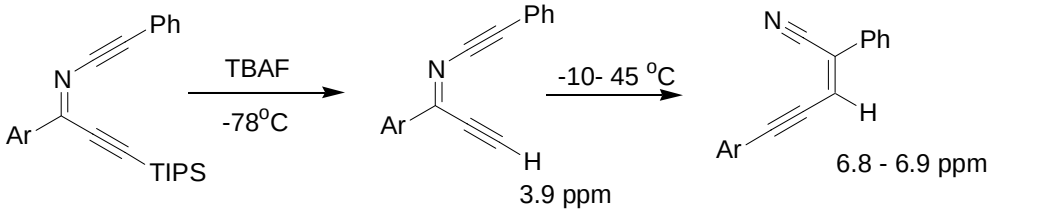
diradical from the reaction mixture; however, the retro-Bergman cyclization product, 2,5-diphenyl-3-triisopropylsilyl-pent-2-en-4-ynenitrile **39** (Scheme 2-4), was isolated in 35% yield.



26a **39** (35%)

Scheme 2-4 Thermolysis of aza-enediyne **26a**

Initial attempts to deprotect aza-enediyne **26a** by treatment with TBAF in THF afforded only the rearranged nitrile **28a** as the sole product in 89% yield (Scheme 2-5).



26a Ar = Ph
26b Ar = 4-(MeO)Ph
26c Ar = 2-(MeO)Ph

40a Ar = Ph
40b Ar = 4-(MeO)Ph
40c Ar = 2-(MeO)Ph

28a Ar = Ph (89%)
28b Ar = 4-(MeO)Ph (72%)
28c Ar = 2-(MeO)Ph (91%)

Scheme 2-5 Deprotection of aza-enediynes **26a-c**.

Despite the inability to isolate and purify the intermediate deprotected aza-enediyne **40a**, rapid workup of the deprotection reactions at low temperature followed by ^{1}H NMR of the unpurified reaction product in $CDCl_3$ demonstrated the presence of the deprotected aza-enediyne **40a** (Figure 2-2). The terminal alkyne resonance for **40a** at 3.9

ppm diminished with time and was replaced by a resonance for the alkene proton at 6.8 ppm as **40a** underwent conversion to the nitrile **28a** over a period of minutes (Figure 2-2). The relative concentrations of aza-enediyne **40a** was calculated by normalizing the integrals of the alkyne resonance for **40a** at 3.9 ppm obtained at different time intervals against the integral of the alkyne resonance for **40a** at 3.9 ppm obtained at reaction time equals zero minute. Based on the first-order kinetic equation (eq 2-2), the first-order kinetics for the conversion of **40a** to **28a** was obtained by plotting the relative concentration of **28a** vs reaction time (Figure 2-4, **A**). The conversion of **40a** to **28a** determined by ^{1}H NMR follows first-order kinetics for both the disappearance of **40a** and the appearance of **28a**. At 20 °C, the ^{1}H NMR-determined half-life for the conversion of **40a** to **28a** is 79 ± 4 min as an average of two runs (Figure 2-4, **A**).

$$-d[\text{reactant}] / dt = d[\text{product}] / dt = k[\text{reactant}] \quad \text{(eq 2-1)}$$

[reactant], [product]: concentrations of reactant and product, respectively;

k: first-order kinetic rate constant; t: reaction time.

Integrating eq 2-1 to yield eq 2-2:

$$\ln[\text{reactant}] = -kt + \ln[\text{reactant}]_o \quad \text{(eq 2-2)}$$

$[\text{reactant}]_o$: the initial reactant concentration

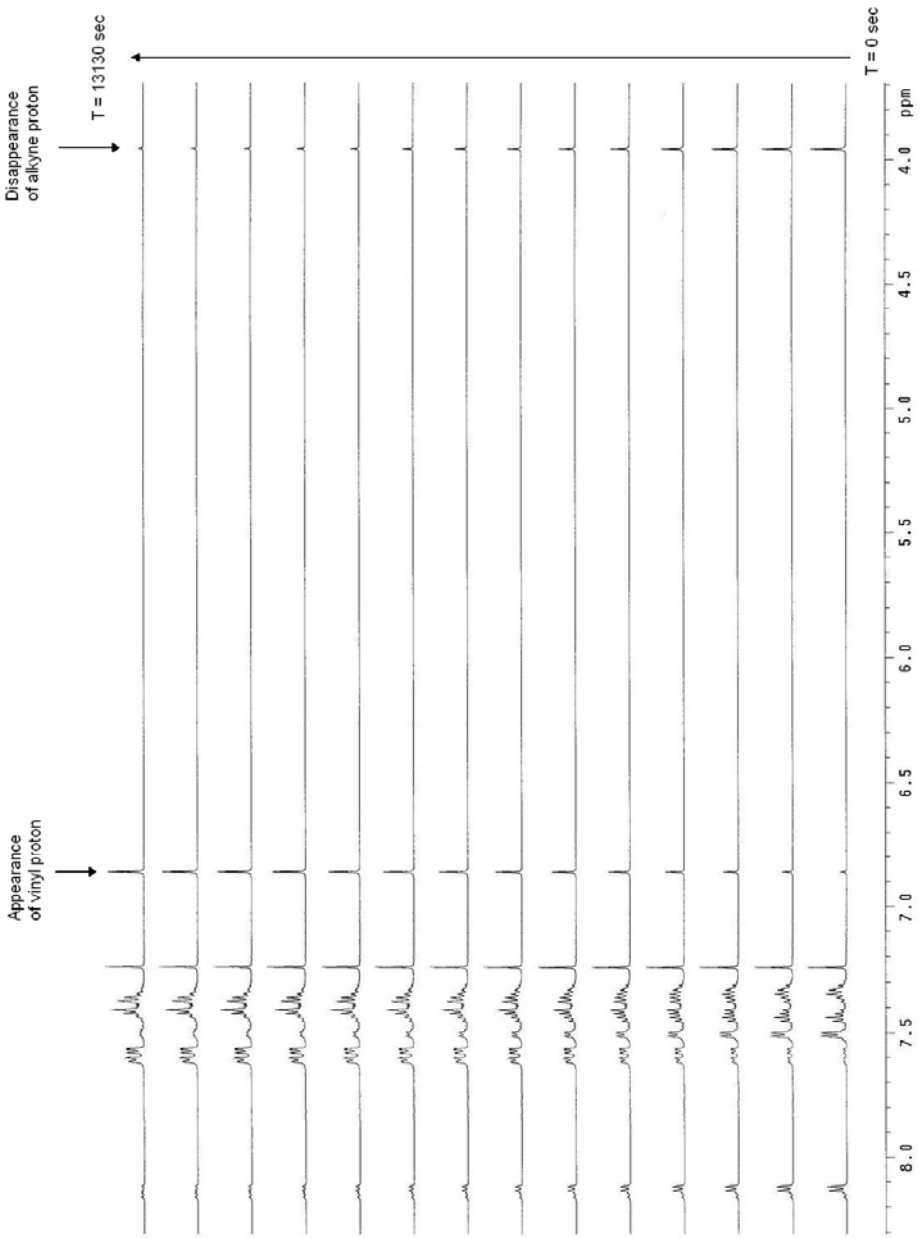


Figure 2-2 The conversion of aza-enediyne **40a** (alkyne resonance at 3.9 pm) to nitrile **28a** (alkene resonance at 6.8 ppm) followed by ^{1}H NMR (500 MHz) in $CDCl_3$ at 20 °C. Spectra were acquired every 1010 sec, starting with the bottom spectrum.

The conversion of the aza-enediyne **40a** to nitrile **28a** could also be followed by UV spectroscopy. Nitrile **28a** has a UV absorbance peak at 345 nm and the aza-enediyne **40a** has an absorbance peak at 390 nm. Dilute (μM) solutions of the product mixture from the deprotection of **26a** in $CHCl_3$ were quickly prepared and placed in temperature-controlled cuvettes to monitor the UV absorbance change with time. Figure 2-3 shows the conversion of aza-enediyne **40a** to nitrile **28a** followed by UV-Vis spectroscopy in $CHCl_3$ at 45 °C; the spectra were taken every 1.8 min for 20 min. The arrows indicate the decrease in absorbance due to the aza-enediyne **40a** at 390 nm and the increase in absorbance due to the nitrile **28a** at 345 nm with time. The absorbance spectra demonstrate isobestic behavior and the expected first-order kinetics.

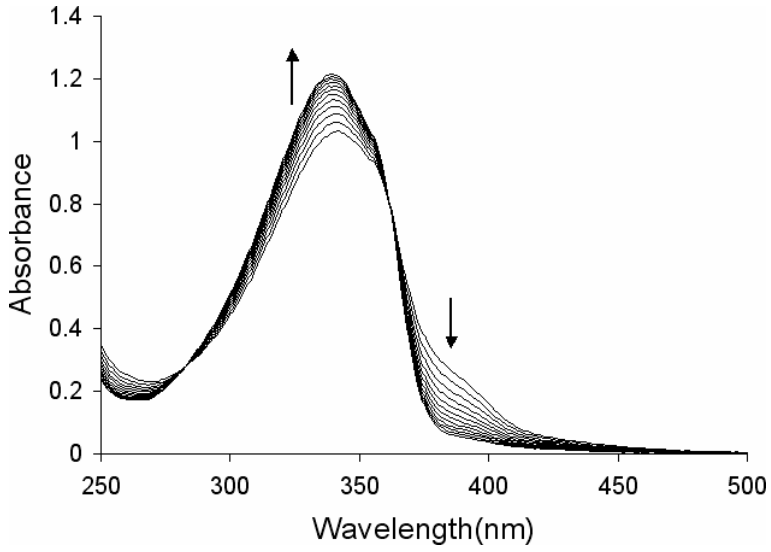
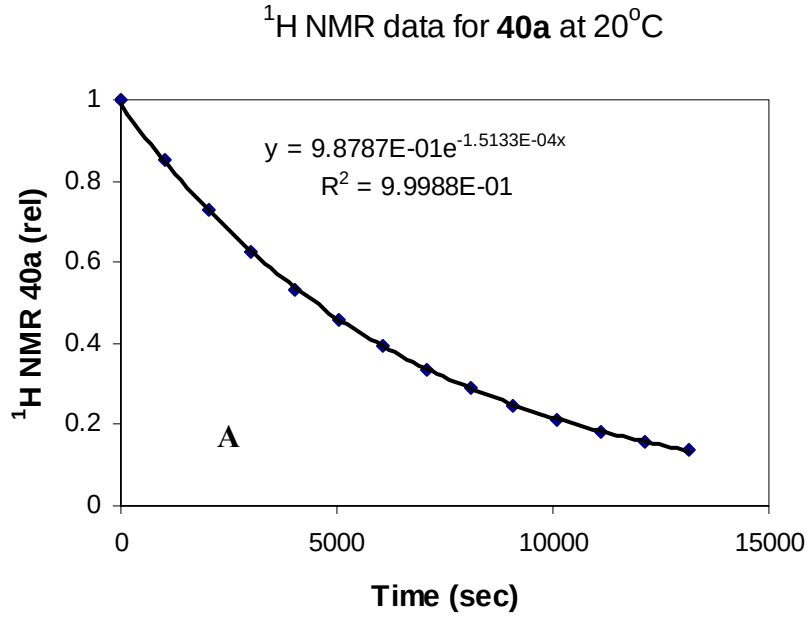


Figure 2-3 The conversion of aza-enediyne **40a** to nitrile **28a** followed by UV-absorption spectroscopy in $CHCl_3$ at 45 °C (The arrows indicate the decrease in absorbance due to the aza-enediyne **40a** at 390 nm and the increase in absorbance due to the nitrile **29a** at 345 nm with time).

At 20 °C, the half-life for the conversion of **40a** to **28a** determined spectrophotometrically is 71 ± 1 min as an average of two runs (Figure 2-4, **B**), which is in agreement with that determined by ^{1}H NMR (79 ± 4 min, Figure 2-4, **A**). Our results indicate that both ^{1}H NMR and UV spectroscopy are good tools to follow the conversion of aza-enediyne **40a** to nitrile **28a**.



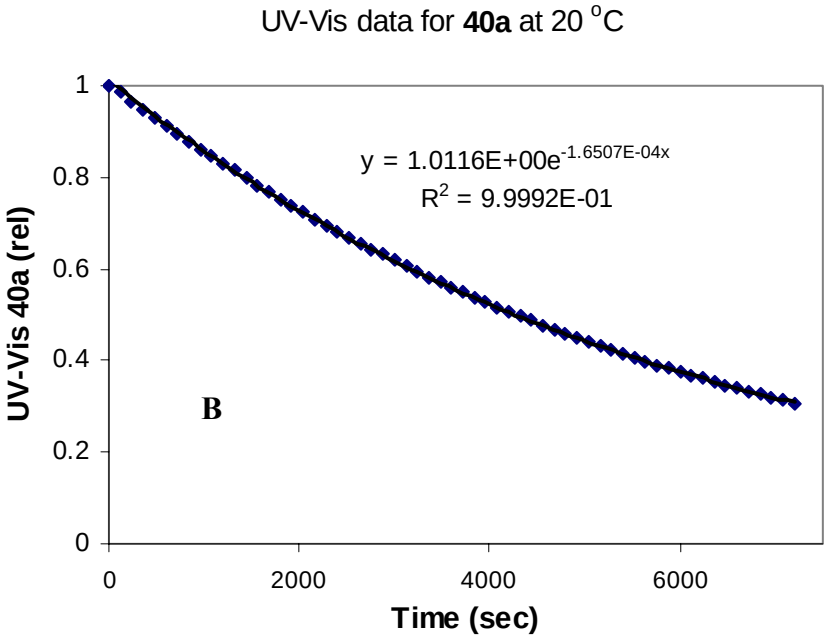


Figure 2-4 The first-order kinetics of the conversion of **40a** to **28a** followed by ^{1}H NMR in $CDCl_3$ (**A**) and by UV-absorption spectroscopy in $CHCl_3$ (**B**) at 20 °C.

2.4.2 Substituent effects

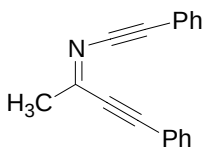
The substituent effects that influence the kinetics of the aza-Bergman cyclization of aza-enediynes have not been elucidated. Aza-enediyne **26a** is relatively stable and undergoes aza-Bergman cyclization at rather forcing conditions (150 °C for 3 d); however, removal of the bulky TIPS group from **26a** to give **40a** results in a facile aza-Bergman cyclization spontaneously even at -10 °C. Our kinetic data shows that **40a** is a good model for kinetic study since the conversion of aza-enediyne **40a** to nitrile **28a** can be easily followed by ^{1}H NMR and UV spectroscopy. Therefore, *p*-(methoxy)phenyl-substituted aza-enediyne **40b** and *o*-(methoxy)phenyl-substituted aza-enediyne **40c** were prepared to study the substituent effects on the kinetics of the aza-Bergman cyclization.

A combination of ^{1}H NMR and UV-Vis were used to follow the conversion of **40b** to **28b** at 20 °C. The time-dependent ^{1}H NMR spectra of crude **40b** in $CDCl_3$ demonstrates the same features noted above for **40a**, namely, the first-order disappearance of a peak at 3.9 ppm corresponding to the terminal alkyne proton in **40b** and the appearance of a new peak at 6.9 ppm, corresponding to the alkene proton in **28b**. In addition, in the case of **40b**, the methoxy protons' resonance for the aza-enediyne (3.86 ppm) is well resolved from the methoxy protons' resonance for the nitrile **28b** (3.82 ppm), and these signals also undergo a first-order decay and increase, respectively, with a kinetic yield of > 99%. The time-dependent UV absorbance spectra of dilute solutions of **40b** in $CHCl_3$ are characterized by a decrease in a peak at 415 nm, corresponding to the aza-enediyne, and an increase in a peak at 360 nm, corresponding to the nitrile **28b**.

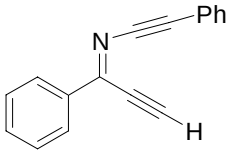
The conversion of the *o*-(methoxy)phenyl-substituted aza-enediyne **40c** to nitrile **28c** can also be followed by ^{1}H NMR spectroscopy in $CDCl_3$. The overlap of the ^{1}H NMR signals due to the terminal alkyne proton and methoxy group of **40c** and the methoxy group of **28c** preclude the use of these signals to accurately follow the course of

the reaction. Instead, a distinctive aromatic ^{1}H NMR resonance for **40c** at 7.9 ppm can be used to determine a first-order half-life of 35 min at 20 °C (one run). The UV absorbance changes accompanying the conversion of **40c** to **28c** in $CHCl_3$ are similar to those described above for the para-isomer **40b**, and give a first-order half-life of 36 ± 1 min at 20 °C (for two runs). Table 2-1 summarizes the half-lives for the aza-Bergman cyclization of aza-enediynes **40a**-**c** and **26e**.

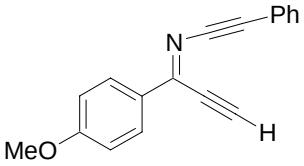
Table 2-1 Half-lives ($t_{1/2}$) for the aza-Bergman cyclization of aza-enediynes **26e** and **40a**,**b**,**c**



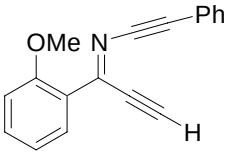
26e



40a



40b



40c

Aza-enediyne	Temperature (°C)	$t_{1/2}$ (min)
40a	0	667[a]
40a	10	276 ± 13[b]
40a	20	75 ± 6[c]
40a	25	40.6 ± 0.1[d]
40a	30	21.5 ± 0.1[d]
40a	37	10.4 ± 0.1[d]
40a	45	4.3 ± 0.1[d]
40b	20	77 ± 3[e]
40c	20	35.6 ± 0.5[e]
26e	110	47[f]

[a]Based on one ^{1}H NMR run in $CDCl_3$. [b]Average value for two ^{1}H NMR runs in $CDCl_3$. [c]Based on two ^{1}H NMR runs in $CDCl_3$ and two spectrophotometric runs in $CHCl_3$. [d]Average of two spectrophotometric runs in $CHCl_3$. [e]Based on one ^{1}H NMR run in $CDCl_3$ and two spectrophotometic runs in $CHCl_3$. [f]Data from reference 1 in CH_3CN.

As shown in Table 2-1, at 20 °C, the first-order half-life for the conversion of **40b** to **28b** is 77 ± 3 min; the first-order half-life for the conversion of **40a** to **28a** is 75 ± 6 min. Thus, there is no apparent electronic substituent effect on the rate of the aza-Bergman cyclization when comparing **40a** and **40b**. This result is in contrast to a number of reports of substituent effects in the Bergman cyclization. Maier and Greiner[11] have reported a pronounced retardation of the Bergman cyclization of the bicyclo[7.3.1]enediyne **15b** (Scheme 1-9) when the double bond is substituted with an electron-donating group (*p*-C_6H_4OMe). Jones and Plourde have reported the retardation of the Bergman cyclization of a series of cyclic enediynes **16a-e** (Scheme 1-9) when the double bond is substituted with chlorine. On the other hand, calculations of a variety of aza-enediynes in which the imine double bond is replaced with an amide, amidine, or amidinium group predict that the barrier to aza-Bergman cyclization is relatively insensitive to these changes.[12,13] The lack of an observable difference in the rate of aza-Bergman reaction of **40a** and **40b** is not surprising in light of these theoretical studies.

The first-order half-life for the conversion of **40c** to **28c** is 35.6 ± 0.5 min at 20 °C. Thus, the aza-Bergman reaction of **40c** at 20 °C occurs approximately two-times faster than that of either **40a** or **40b** (Table 2-1). The origin of this modest substituent effect is not known; however, it is likely that the steric interaction between the *o*-methoxy group and the adjacent alkyne moiety in **40c** plays a role.

The relative insensitivity of the rate of aza-Bergman cyclization to the nature of the imine double bond substituent indicates that this region of the aza-enediynes provides a great deal of flexibility in the design of potential warheads for targeting DNA and other receptors. This is significant because the imine double bond of aza-enediynes is a site of hydrolytic instability. Chen[2] and co-workers note that thermolysis reactions of **26e** under acidic conditions afford primarily products of imine hydrolysis. This hydrolytic

instability could be remedied by replacing the imine double bond with the amidine group as proposed by Kraka and Cremer,[12,13] or by incorporating the imine double bond into a heterocyclic ring.[14]

In contrast to the modest substituent effects observed when comparing the aza-Bergman cyclization rate of **40a-c**, the nature of the alkyne substituents appears to play an important role in the rate of aza-Bergman cyclization. The aza-enediyne **26a**, which bears a bulky triisopropylsilyl substituent on the C-alkynyl group, undergoes aza-Bergman cyclization only under rather forcing conditions (150 °C, 3 d for completion). Removal of the triisopropylsilyl group from **26a** to give **40a** results in a facile aza-Bergman cyclization. The phenyl-substituted aza-enediyne **26e** undergoes aza-Bergman cyclization at a rate that is intermediate between that for **26a** and **40a** (Table 2-1). Schreiner[15] and co-workers have performed calculations on substituent effects on the Bergman cyclization of (*Z*)-1,5-hexadiyne-3-enes. According to these calculations, 1,6-diphenyl-3-hexene-1,5-diyne, a diphenyl-substituted enediyne, has a Bergman cyclization transition state that is 10 kcal/mol higher than that for 6-phenyl-3-hexene-1,5-diyne, mono-phenyl-substituted enediyne. The authors explained that this effect is due to a combination of additional stabilization of the starting enediyne and destabilization of the transition state due to steric repulsion between the phenyl groups in the diphenyl-substituted case. In the parent enediyne case, the effect of changing the size of the terminal substituents on the facility of Bergman cyclization cannot be employed as a triggering mechanism at physiological temperatures due to the large barrier to Bergman cyclization of the unsubstitued enediyne; however, in the case of the aza-enediynes studied here, the terminal alkyne substituent effect can be viewed as a potential triggering device due to the facility with which sterically unencumbered aza-enediynes such as **40a-c** undergo aza-Bergman cyclization.

2.4.3 Activation energy for the aza-Bergman cyclization

As shown in the Table 2-1, the half-lives of the conversion of **40a** to **28a** were measured at different temperatures (0, 10, 20, 25, 30, 37, 45 °C). At 10 °C, the ^{1}H NMR-determined half-life is 4.6 h, and at 0 °C the half-life is 11 h (Table 1). The half-life for the aza-Bergman reaction of **40a** at 37 °C is only 10.4 min, as determined spectrophotometrically (Table 2-1). A plot of the logarithmic rate of disappearance of **40a** versus temperature yields an Arrhenius plot for the aza-Bergman reaction of **40a** to nitrile **28a**, shown in Figure 2-5. Arrhenius activation parameters were extracted from the linear fit in Figure 2-5 giving E_a = 19.9 ± 0.3 kcal/mol and log A = 11.1 ± 0.2. Chen[2] and co-workers determined the activation energy for 1,6-bis(4-*tert*-butylphenyl)-3-aza-4-methylhex-3-ene-1,5-diyne to be E_a = 23.1 ± 1.5 kcal/mol. The lower activation energy for the aza-Bergman reaction of **40a** relative to the 1,6-diaryl aza-enediyne studied by Chen and co-workers is most likely due to the substituent effects discussed above in the comparison of the facility of cyclization of **40a** versus **26e**. The activation energy for the aza-Bergman cyclization of **40a** is lower than the barrier for aza-Bergman cyclization of the parent aza-enediyne system calculated at the CASMP2 level of theory (27.7 kcal/mol),[2] but close to the value determined by B3LYP calculations (21.8 kcal/mol),[12,13] and that calculated by Cramer using a composite energy term (19.1 kcal/mol).[16,17]

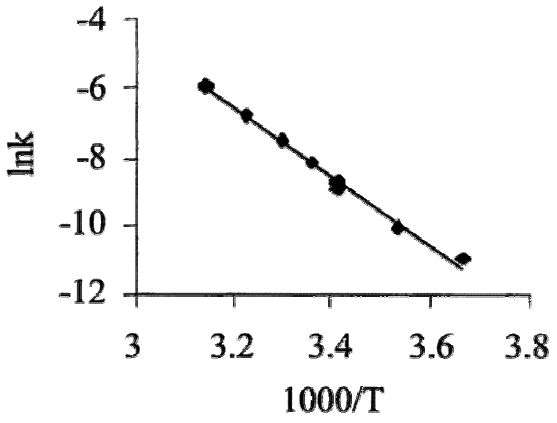
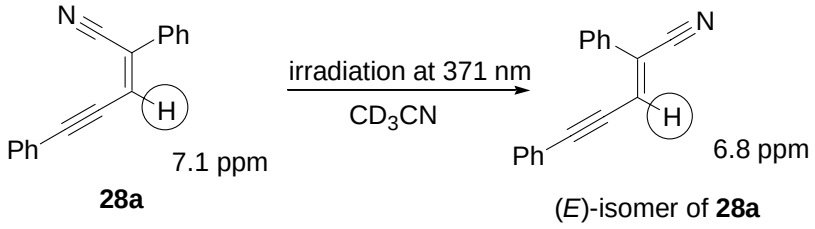


Figure 2-5 Arrhenius plot of the aza-Bergman cyclization of aza-enediyne **40a**. Fifteen points at seven temperatures (0, 10, 20, 25, 30, 37, 45 °C) yield E_a = 19.9 ± 0.3 kcal/mol and log A = 11.1 ± 0.2 (R^2 = 0.993).

2.4.4 Stereochemical aspects of aza-Bergman cyclization

The aza-Bergman cyclization of aza-enediynes can only occur through the (*Z*)-imine isomer. Previous studies of the aza-Bergman cyclization have employed aza-enediynes **26e,f** that were mixtures of stereochemical isomers about the imine double bond.[1,2] As a result, the observed kinetics for the conversion of these aza-enediynes to products of aza-Bergman cyclization may have been affected by the rate of (*E*)- to (*Z*)-imine isomerization. Aza-enediyne **26a-c** and the deprotected aza-enediynes **40a-c** appear to be single isomers (> 9:1) by ^{1}H NMR. Density functional theory calculations[18] at B3LYP/6-31G** level[19] predict that the (*Z*)-isomer of **40a** is more stable than the (*E*)-isomer by 4.4 kcal/mol. Assuming that these aza-enediynes undergo thermodynamic equilibration during their preparation and isolation, the predominant isomers observed for **40a-c** are the more thermodynamically stable (*Z*)-isomer. The nitriles produced by the

aza-Bergman-retro-aza-Bergman rearrangement can only be (*Z*)-stereoisomer. The spontaneous rearrangement of **40a-c** to the nitriles **28a-c** occurs stereoselectively to afford the nitrile products as single isomers as judged by ^{1}H NMR and ^{13}C NMR. Assignment of the (*Z*)-stereochemistry of these nitriles is based upon their isomerization to a mixture of (*E*)- and (*Z*)-isomers (Scheme 2-6).



Scheme 2-6 Isomerization of nitrile **28a** upon irradiation at 371 nm

The ^{1}H NMR spectrum of **28a** in CD_3CN contains a singlet resonance at 7.1 ppm, corresponding to the alkene hydrogen (Figure 2-6, **A**). When this solution is subjected to irradiation at 371 nm, using the excitation beam of a spectroflourimeter,[20] the ^{1}H NMR spectrum shows a new single peak at 6.8 ppm, which is due to the alkene proton of the isomer of **28a** (Figure 2-6, **B**, after 1.5 h irradiation). Because the resonance of the alkene proton of (*E*)-**28a** is predicted to lie 0.34 ppm upfield of that for the (*Z*)-isomer by the ChemDraw Ultra program, we assign the 7.1-ppm peak to the (*Z*)-isomer and the 6.8-ppm peak to the (*E*)-isomer. After 4.5 h of irradiation, 34% (*E*)-**28a** was formed based on the ratio of the peaks at 6.8 and 7.1 ppm (Figure 2-6). For conversion of aza-enediyne **40b** to nitrile **28b**, the ^{1}H NMR ($CDCl_3$) of the product mixture prior to purification showed one singlet peak at 6.8 ppm, due to the (*Z*)-isomer of **28b**. Photochemical isomerization of

28b results in the formation of an isomer with a new ^{1}H NMR resonance at 6.6 ppm, which is associated with the (*E*)-nitrile (20%).

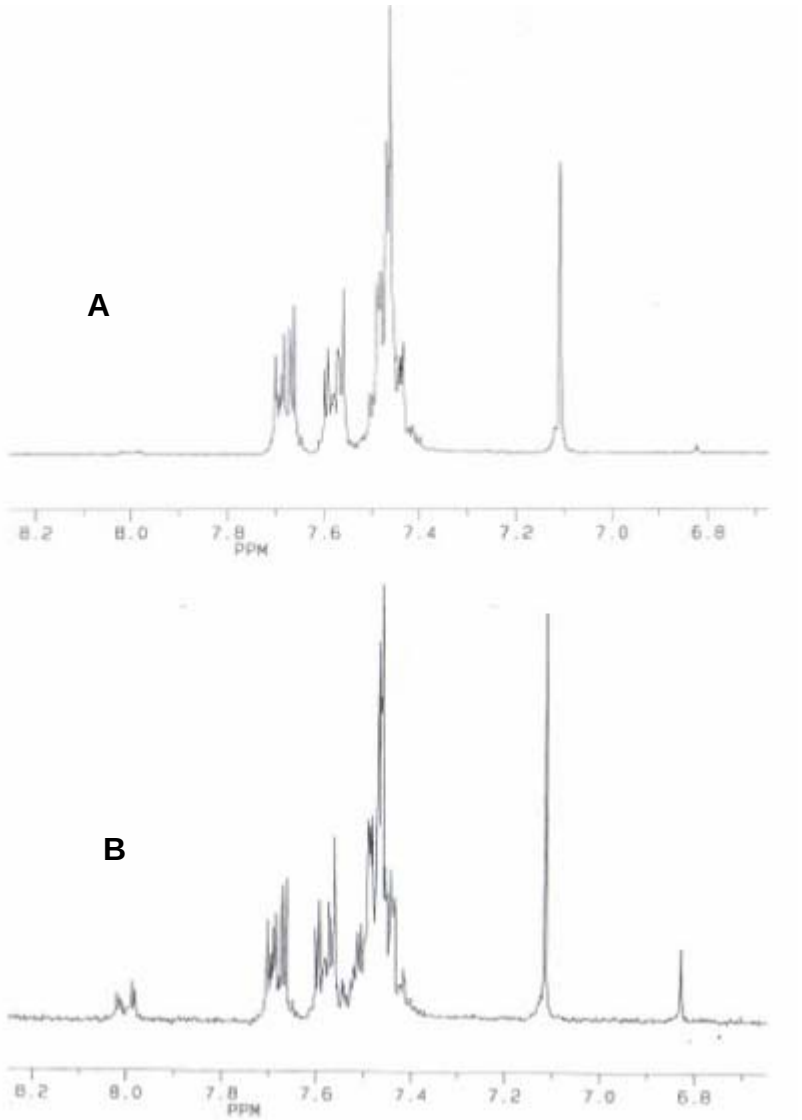


Figure 2-6 Isomerization of (*Z*)-nitrile **28a** to its (*E*) isomer upon irradiation at 371 nm. ^{1}H NMR of the nitrile **28a** in CD_3CN before irradiation (**A**); ^{1}H NMR of the nitrile **28a** in CD_3CN after irradiation at 371 nm for 1.5 h (**B**).

2.4.5 Solvent effects on the aza-Bergman cyclization

We have performed kinetic studies of **40a** in various solvents to determine solvent effects on the aza-Bergman cyclization. The conversion of **40a** to **28a** at 45 °C in various solvents was followed by UV-vis spectroscopy. Kinetic data in all solvents were first-order and analysis of the reaction mixtures by TLC and mass spectroscopy confirmed the presence of **28a** as the sole reaction product. The aza-Bergman cyclization rates were found to be solvent dependent, with slightly faster rates in nonpolar solvents (Table 2-2). The effect is not large; the half-lives varied from 3.6 min in hexanes to 8.2 min in acetonitrile. There are examples of enediynes that undergo solvent-dependent Bergman cycloaromatization rates,[21,22] but these cases are complicated by the solvent effects on the rate of hydrogen atom abstraction by the diradical intermediate, which impacts the observed rate of disappearance of enediyne. In the case of the conversion of **40a** to **28a**, it is clear that the rate determining step is the initial aza-Bergman cyclization. We believe that the solvent-dependent rate of aza-Bergman reaction of **40a** shown in Table 2-2 is due to preferential solvation of **40a** by more polar solvents, resulting in ground-state stabilization and concomitant decreased rate. Although the solvent dependence of the rate of aza-Bergman cyclization of **40a** is small, the increased facility of aza-Bergman cyclization in nonpolar solvents implies that aza-enediynes such as **40a** would be somewhat more stable to aza-Bergman cyclization in aqueous solution as compared to when bound to a target receptor, where the microenvironment is less polar.

Table 2-2 First-order half-lives ($t_{1/2}$) for the aza-Bergman cyclization of aza-enediyne **40a** in various solvents at 45 °C

Solvent[a]	Half-life[b] ($t_{1/2}$, min)	Dielectric constant (D)
Hexanes	3.61±0.03	1.89
Chlorobenzene	4.2±0.1	2.71
Chloroform	4.3±0.1	4.81
THF	6.4±0.2	7.60
Isopropyl alcohol	7.91±0.04	18.30
Acetonitrile	8.14±0.03	37.50

[a]All solvents were HPLC or ACS grade and used directly without further purification. [b]Average value ± standard deviation for two spectrophotometric runs.

2.5 CONCLUSIONS

In this chapter, I report the synthesis and kinetic studies of the aza-Bergman cyclization of a series of 6-triisopropylsilyl and 6-unsubstituted 4-aryl-3-aza-3-ene-1,4-diynes. There are two types of substituent effects in this system: The aza-Bergman reaction rate is not greatly affected by changing the 4-aryl substituent on the aza-enediyne imine double bond, but is very sensitive to the nature of the 6-substitutent at the alkyne terminus. The aza-Bergman cyclization of sterically unencumbered, 6-unsubstituted aza-enediynes occurs spontaneously at temperatures as low as -10 °C. We hope that these observations provide the basis for the design of a novel aza-Bergman triggering device involving the conversion of a sterically blocked aza-enediyne to a sterically unencumbered one mediated by a cancer cell-specific enzymatic activity. One issue that remains to be addressed is the reactivity of the 2,5-didehydropyridine intermediates involved in the aza-Bergman cyclization of these aza-enediynes. In this study, there is no evidence of any products corresponding to trapping of these 2,5-didehydropyridine diradical intermediates. These results are in accord with many

theoretical predictions of an extremely low barrier to retro-aza-Bergman cyclization of these intermediates to afford the observed (*Z*)-*β*-alkynyl acrylonitrile products.[1,12,15,23]

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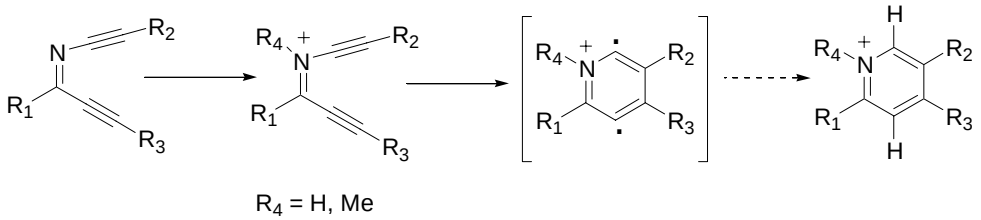
Chapter 3 Attempts to trap the 2,5-didehydropyridine diradical intermediates

3.1 INTRODUCTION

We have shown that aza-enediynes undergo a rapid aza-Bergman cyclization through a 2,5-didehydropyridine diradical intermediate to afford the isomeric (*Z*)-*β*-alkynyl acrylonitriles.[1,2a] Unlike the Bergman cyclization of enediynes, the aza-Bergman cyclization of aza-enediynes does not afford any products corresponding to trapping of the intermediate diradicals.[1,2a] A number of computational studies of the aza-Bergman cyclization and the resulting 2,5-didehydropyridine intermediate have been carried out.[3,4,5] These theoretical studies predicted that the parent 2,5-didehydropyridine diradical can undergo a rapid retro-aza-Bergman reaction to the thermodynamically stable nitrile (Figure 1-3). In addition to the small activation barrier for the nitrile formation, the 2,5-ddp diradical has a large S-T gap due to the interaction between the nitrogen and the adjacent radical center. The large S-T gap and small activation barrier for the nitrile formation make the 2,5-ddp diradical intermediate an unreactive, very short-lived species. In contrast, the protonated 2,5-ddp diradical (Figure 1-4) is predicted to be more resistance to retro-aza-Bergman rearrangement and have a smaller S-T gap, indicating that this intermediate would more readily undergo trapping reactions.[3,4] These predictions have led to the proposal that these aza-enediynes might serve as pH-dependent DNA cleavage agents. Significantly, the only experimental support for the existence of the elusive 2,5-didehydropyridine intermediate has come from studies of the aza-Bergman cyclization reaction. Chen[3] and co-workers reported the detection of miniscule amounts of pyridine product by GC/MS in the thermolysis of the aza-enediyne **26e** under acidic conditions. We propose that alkylation of the nitrogen atom would have the same impact on the reactivity of 2,5-ddp intermediates. In this chapter, I report our attempts to

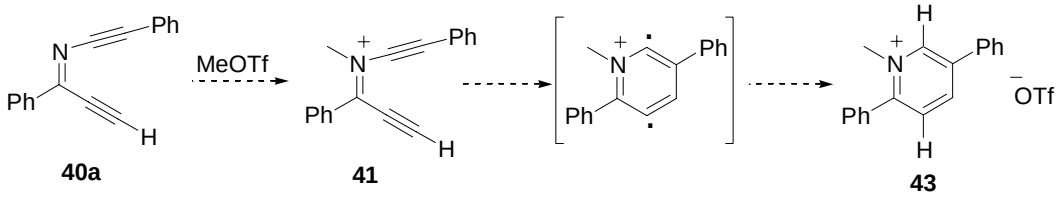
protonate and methylate the nitrogen atom of aza-enediynes and our studies on the effect of added acid on the aza-Bergman cyclization and the fates of the reactive diradical intermediates from aza-Bergman cyclization under acidic conditions (Scheme 3-1). Under acidic conditions, no pyridine products were detected; rather, a cyclopropane derivative of 1,4-cyclohexadiene derived from a 5-oxazolylcarbene was isolated.[2b]



Scheme 3-1 Protonation and methylation of aza-enediynes

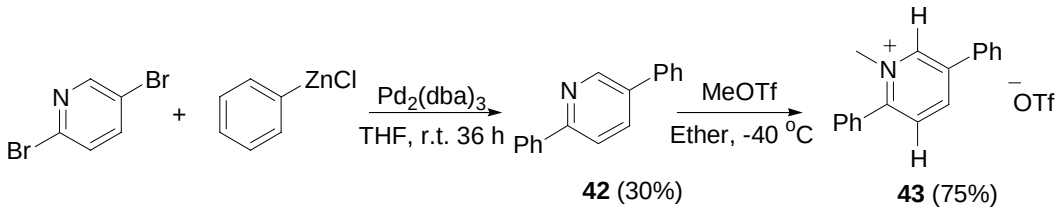
3.2 ATTEMPTS TO METHYLATE AZA-ENEDIYNE 40A

In chapter 2, we[2a] report that aza-enediyne **40a** undergoes a facile aza-Bergman cyclization to 2,5-ddp diradical intermediate spontaneously even at -10 °C. We propose that methylated aza-enediyne **40a** undergoes aza-Bergman cyclization to diradical intermediate which may abstract hydrogen atoms to form 1-methyl-2,5-diphenylpyridinium triflate salt **43** (Scheme 3-2).



Scheme 3-2 Proposed formation of pyridinium salt **43** via diradical pathway

Pyridinium triflate salt **43** would be the trapping product of methylated 2,5-ddp diradical if it undergoes hydrogen atom abstraction reaction. Therefore, compound **43** was prepared independently as the authentic sample (Scheme 3-3). Palladium (0) catalyzed coupling of 2,5-dibromopyridine with freshly prepared phenyl zinc chloride afforded 2,5-diphenylpyridine **42** in 30% yield along with some amount of 2-phenyl-5-bromo-pyridine and biphenyl.[6,7] Methyl triflate is a powerful *N*-methylating reagent.[8] Diphenylpyridine **42** was treated with methyl triflate at -40 ºC to give pyridinium triflate salt **43** in 75% yield.



Scheme 3-3 Synthesis of the authentic pyridinium triflate salt **43**

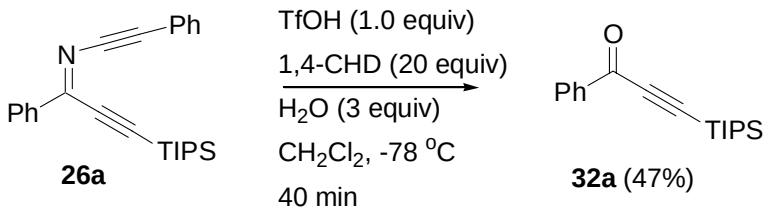
Aza-enediyne **26a** underwent desilylation reaction to afford **40a** under the treatment with TBAF in THF at -78 ˚C followed by rapid aqueous work-up. The organic extracts were dried over Na_2SO_4, and passed through a plug of silica gel to remove the

tetrabutylammonium species. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2. To this solution was added 8.0 equiv of methyl triflate and 20 equiv of 1,4-cyclohexdiene as hydrogen atom donor. The reaction mixture was warmed up to 0 °C and kept at this temperature for 24 h. TLC of the reaction mixture and the authentic pyridinium salt **43** demonstrated that there was no product corresponding to the trapping of methylated 2,5-ddp diradical intermediate. ^{1}H NMR of the reaction mixture indicated that majority was unidentified polymer. We conducted this reaction again, this time 1.2 equiv of methyl triflate was used instead of 8.0 equiv. The MS and TLC analysis of the crude reaction mixture did not show any peak corresponding to the methylated aza-enediyne **40a** or the trapping of methylated 2,5-ddp intermediate; instead, nitrile **28a** was isolated in 35% along with other unidentified polar species, presumably polymer.

3.3 ISOLATION OF A CYCLOPROPANE-CONTAINING PRODUCT 44 FROM THE REARRANGEMENT OF AZA-ENEDIYNE 40A UNDER ACID CATALYSIS

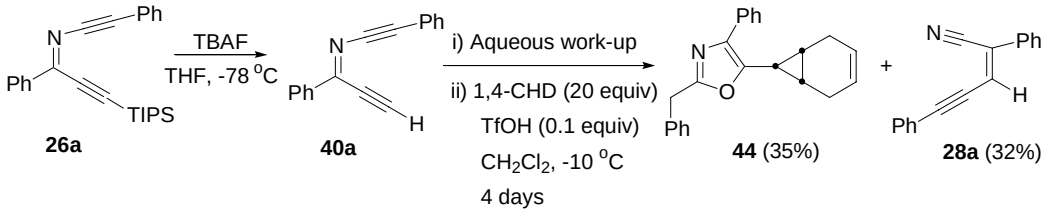
Chen[3] and co-workers reported the detection of miniscule amounts of pyridine product by GC/MS in the thermolysis of the aza-enediyne **26e** under acidic conditions. They also found that imine double bond hydrolytic product ketone (4-phenylbut-3-yn-2-one) and aza-Bergman-retro-aza-Bergman rearrangement product nitrile (phenylacetonitrile) were formed in high yield.[3] Imine nitrogen is very acidic and it needs a strong acid to protonate it. We conducted the trapping experiment on aza-enediyne **26a** and **40a**, respectively, under the treatment with trifluoromethane sulfonic acid (TfOH). 1.0 equiv of TfOH and 20 equiv of 1,4-CHD were added to the solution of aza-enediyne **26a** in CH_2Cl_2 at -78 °C. The reaction mixture was kept at -60 °C for 40 min and afforded complex mixtures from which 1-phenyl-3-triisopropylsilanyl-propynone **32a** was isolated in 47% along with other unidentified polar products (Scheme 3-4). No pyridine products

corresponding to trapping of 2,5-ddp diradical were detected. It is not surprising since the imine double bond is the site of hydrolytic instability.



Scheme 3-4 Hydrolysis of imine double bond of aza-enediyne **26a** under acidic condition

Desilylation of aza-enediyne **26a** was carried out as described before. After aqueous work-up, the organic extracts were cooled in a dry-ice bath, and dried over Na_2SO_4. Upon removal of the solvent, the residue was dissolved in CH_2Cl_2 and treated with catalytic TfOH (0.1 equiv) in the presence of 20 equiv of 1,4 CHD at -10 °C for 4 days. The reaction was monitored by TLC, which demonstrated the disappearance of aza-enediyne **40a** and appearance of two new products, one corresponds to retro-aza-Bergman rearrangement product nitrile **28a** and the other is a more polar unknown product **44** (Scheme 3-5).



Scheme 3-5 Formation of the cyclopropyloxazole **44** from the aza-enediyne **40a** under acidic conditions

After column chromatography, compound **44** was re-crystallized in hexanes and afforded colorless lathes and needles. The unexpected structure of compound **44** has been determined by NMR and single-crystal X-ray diffraction analysis as shown in Figure 3-1.

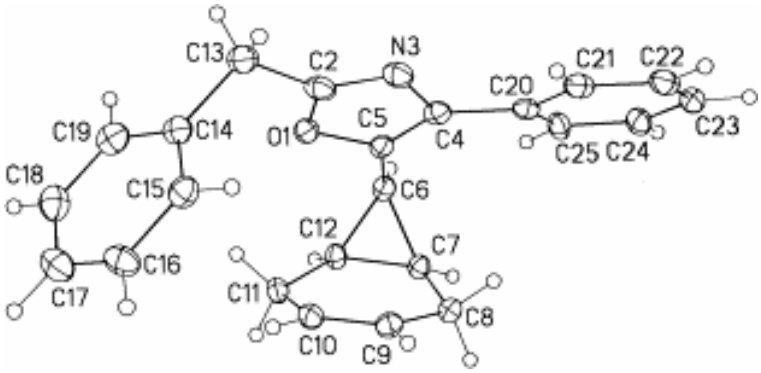
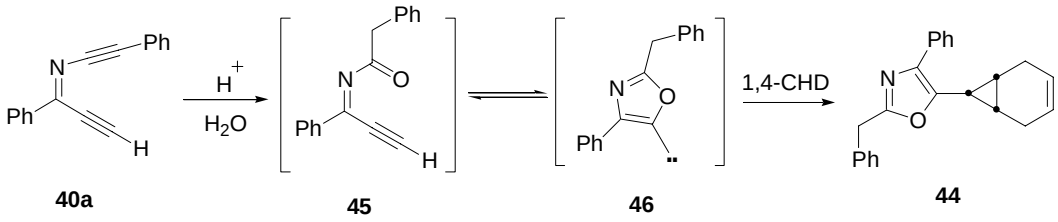


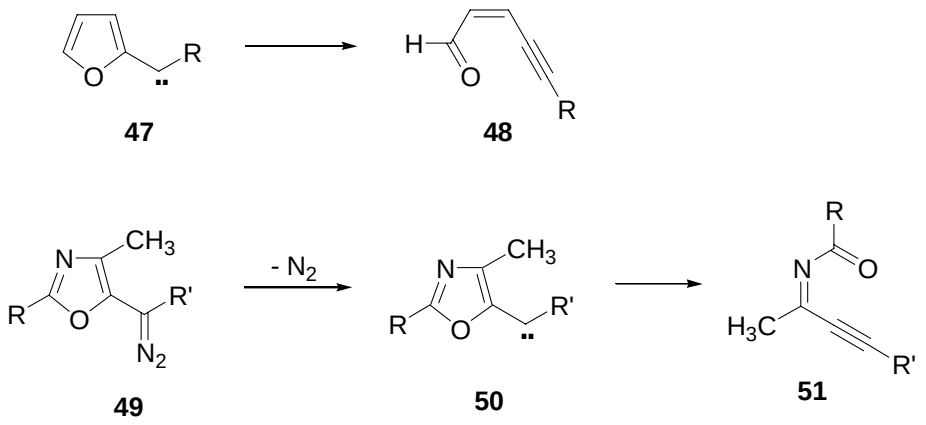
Figure 3-1 X-Ray structure of **44** showing the atom labeling scheme. Displacement ellipsoids are scaled to the 30% probability level. The hydrogen atoms are drawn to an arbitrary size.

In order to understand the mechanism of this cyclopropyloxazole **44** formation, a set of control experiments were designed and conducted. When the reaction was carried out in the presence of stoichiometric TfOH, the aza-enediyne **40a** was consumed within 30 min at -78 °C, and the compound **44** was isolated in 30% yield along with a 15% yield of the nitrile **28a**. When the reaction was conducted in the absence of TfOH, only nitrile **28a** was isolated in 89% yield. When the nitrile **28a** was treated with TfOH, only nitrile itself was recovered from the reaction; nitrile **28a** can not be converted to cyclopropyloxazole **44** under these acidic conditions. Based on the results obtained from these reactions, we proposed that the cyclopropyloxazole **44** is the trapping product of an intermediate carbene **46** (Scheme 3-6).



Scheme 3-6 Proposed mechanism for the formation of compound **44**

Although the origin of the carbene **46** is uncertain, it is possible that water molecule adds to the ynamine triple bond of the aza-enediyne **40a** under the acid catalysis to afford an intermediate *N*-acyl-*C*-alkynyl imine **45**. Feustel and Himbert[9] observed the addition of water to ynamine triple bond to generate *N*-acyl compound under acid catalysis. The resulting imine **45** can be in rapid equilibrium with the thermodynamically less stable carbene species **46** which then trap 1,4-CHD by addition to its double bond to afford cyclopropyloxazole **44**. There are several experimental results supporting the carbene formation hypothesis. It is well known that 2-furylcarbenes **47** undergo ring-opening rearrangement to aldehyde acetylene **48** (Scheme 3-7).[10,11,12] Moreover, Schecter[13] and co-workers reported that certain 5-oxazolylcarbenes **50**, generated from the thermolysis or photolysis of the corresponding diazo compounds, undergo C-H insertion and cyclopropanation reactions as well as ring-opening to *N*-acyl-*C*-alkynyl imines **51** (Scheme 3-7). In addition, it has been reported that several heteroatom-substituted dieneyne system undergoes thermal cyclization to a carbene intermediate.[14,15] Therefore, we propose that the formation of the carbene **46** from the *N*-acyl-*C*-alkynyl imine **45** followed by addition of carbene **46** to the double bond of 1,4-CHD leads to consumption of the imine **45** and the formation of cyclopropyloxazole **44**.



Scheme 3-7

Carefully examining our trapping experiment conditions, it is not hard to find out that there may be adventitious water present in the crude desilylated aza-enediyne **40a**. The role of water in the formation of compound **44** was established by carrying out the following reactions. The isolation of crude aza-enediyne **40a** was conducted under strictly anhydrous conditions; organic extracts were distilled off through a short path distillation apparatus in the presence of 4 Å molecular sieves. The aza-enediyne **40a** thus obtained was subjected to TfOH treatment as previously described. Under these conditions, no cyclopropane **44** was observed; instead, only nitrile **28a** was isolated in nearly quantitative yield. In a separate experiment, 20 equiv of $H_2{}^{18}O$ along with 0.1 equiv of TfOH and 20 equiv of 1,4-CHD was added into the solution of anhydrous **40a** in CH_2Cl_2. Under these conditions, cyclopropyloxazole **44** was isolated in 21% yield along with 30% of nitrile **28a**. The MS analysis of the compound **44** demonstrated the incorporation of ^{18}O atom into its oxazole ring. These experiments clearly establish that the oxygen atom in oxazole ring is from adventitious water presented in the crude desilylation reaction.

3.4 CONCLUSIONS

The isolation of the cyclopropyloxazole **44** from the aza-enediyne **40a** under acidic conditions is not in agreement with the results of Chen[3] and co-workers, who obtained the trapping of 2,5-ddp intermediate product and the products corresponding to the hydrolysis of imine double bond under acidic conditions. In contrast, aza-enediyne **26a** bearing a bulky TIPS group hydrolyzes to afford the hydrolysis product propynone **32a** under acidic conditions. No pyridine product corresponding to trapping of 2,5-ddp diradical was detected by either ^{1}H NMR or TLC. Aza-enediyne **40a** obtained from the removal of TIPS from **26a** was treated under acidic conditions; no pyridine products corresponding to trapping of 2,5-ddp intermediate was detected by either ^{1}H NMR or TLC; rather, a cyclopropane derivative of 1,4-cyclohexadiene **44** derived from a 5-oxazolylcarbene **46** was isolated. We understand that our analysis would not be able to detect the extremely low yields (~0.05%) of pyridine product reported by Chen.[3] Here we demonstrated that certain aza-enediynes can undergo reaction under acidic conditions to afford carbene intermediates that can be efficiently trapped.

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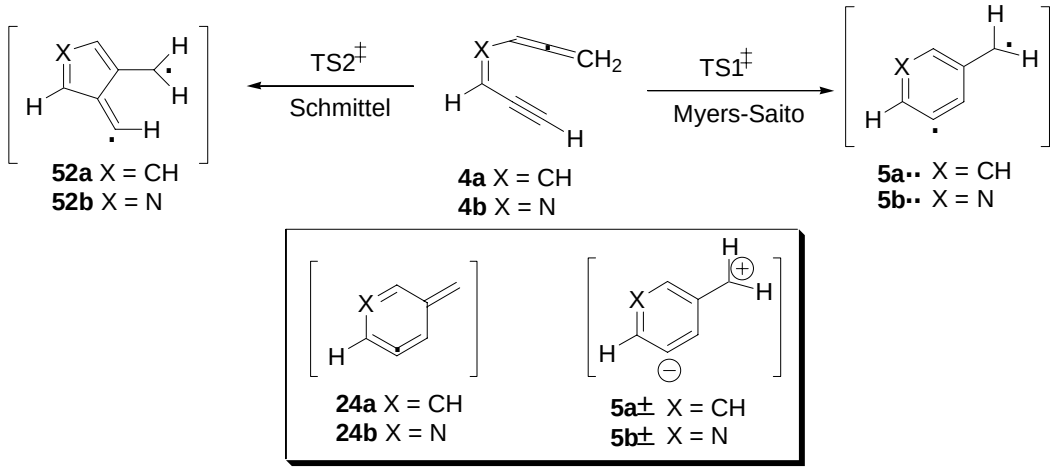
Chapter 4 α,5-Didehydro-3-picoline diradicals from skipped aza-enediynes: computational and trapping studies of an aza-Myers-Saito cyclization

4.1 INTRODUCTION

The naturally occurring antitumor antibiotics neocarzinostatin (Scheme 1-2) undergoes the Myers-Saito type cyclization to produce a reactive diradical (Scheme 1-5, pathway **B**).[1,2] This highly reactive diradical intermediate abstracts hydrogen atoms from the sugar phosphate backbone of DNA, leading to oxidative DNA strand scission and ultimately cell death.[3,4,5] When the enyne allene functionality is not incorporated into a ring, two different cyclization pathways, Myers-Saito cyclization and Schmittel cyclization, have been demonstrated as shown in Scheme 4-1 (X = CH).



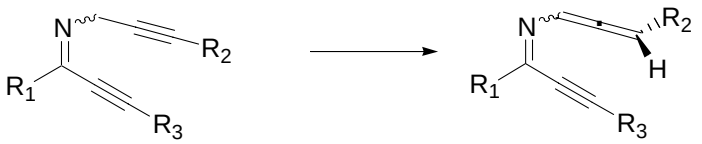
Scheme 4-1 Cyclization paths for enyne allenes and aza-enyne allenes

The Myers-Saito pathway which generates an α,3-didehydrotoluene diradical is similar to that found in neocarzinostatin. The Schmittel pathway generates a fulvene-like

diradical. Both α,3-didehydrotoluene diradical and fulvene-like diradical are σ,π-diradicals unlike the σ,σ-diradicals generated from neocarzinostatin.[6,7] The Myers-Saito and Schmittel cyclizations have drawn lot of attention due to the involvement of these cyclizations in the DNA cleavage mechanism of natural and designed compounds and the potential synthetic usefulness of these cyclizations, particularly when coupled to free radical cyclization cascades.

A number of heteroatom-substituted enyne allenes have been reported.[8-11] As mentioned in section 1.7, all of the heteroatom-substituted enyne allene analogues examined to date involve heteroatom substitution on the allene moiety, including enyne ketenes,[8] enyne ketenimines,[9] enyne carbodiimides,[10] and enyne-isocyanate[11] (Figure 1-5). These heteroatom-substituted enyne allene analogues undergo cyclizations analogous to the Myers-Saito and/or Schmittel cyclization of enyne allenes to generate diradical or zwitterion intermediates. We propose that the replacement of a sp^2 carbon with a sp^2 nitrogen to afford such previously unreported aza-enyne allenes, or *C*-alkynyl-*N*-allenyl imines (Scheme 4-1, **4b**) will have a profound impact on the facility of these rearrangements. These aza-enyne allenes may have the potential to undergo cyclizations analogous to the Myers-Saito and Schmittel cyclization to produce the previously un-reported diradical or zwitterions intermediates.

The skipped aza-enediynes (Scheme 4-2, **53**) in which a single methylene group interrupts the conjugation of the aza-enediyne system have the potential to isomerize to the corresponding aza-enyne allenes (Scheme 4-2, **4**) under basic conditions.[18] Here I report our computational and experimental studies of the thermal rearrangements of aza-enyne allenes derived from isomerization of skipped aza-enediynes (Scheme 4-2).[21]



53b $R_1 = R_2 = R_3 = H$
53c $R_1 = R_3 = H$, $R_2 = Me$
53d $R_1 = Ph$, $R_2 = R_3 = H$
53e $R_1 = R_2 = H$, $R_3 = Ph$

4b $R_1 = R_2 = R_3 = H$
4c $R_1 = R_3 = H$, $R_2 = Me$
4d $R_1 = Ph$, $R_2 = R_3 = H$
4e $R_1 = R_2 = H$, $R_3 = Ph$

Scheme 4-2 Proposed isomerization of the skipped aza-enediynes **53b-e** to aza-enyne allenes **4b-e**

4.2 A FACILE ISOMERIZATION OF THE SKIPPED AZA-ENEDIYNES TO AZA-ENYNE ALLENES BASED ON COMPUTATIONAL STUDIES AT B3LYP/6-31G* LEVEL

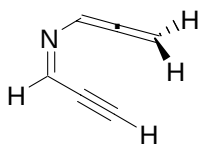
We conducted the computational calculations at B3LYP/6-31G* level[12] on the isomerization of the skipped aza-enediynes **53b-d** to aza-enyne allenes **4b-d** (Scheme 4-2). Each skipped aza-enediynes **53b-d** and aza-enyne allenes **4b-d** can have two isomers (*Z* and *E* isomer) about the imine double bond. Each isomer of **4b-d** can have two conformers (*s-trans* and *s-cis*) about *N-C* single bond. The *s-cis* conformers of (*Z*)-**4b**,**c** or (*E*)-**4d** (Table 4-1) have the right geometry for the Myers-Saito/Schmittel cyclization.

Table 4-1 summarizes the calculated electronic energies at B3LYP/6-31G* level for the skipped aza-enediynes **53b-d** and the corresponding aza-enyne allenes **4b-d**. In each series, the relative energies are calculated relative to the more stable *s-trans* conformers of (*Z*)-**53b**,**c** or *s-trans* comformer of (*E*)-**53d**. Our computational data show that (*Z*)-aza-enediynes **53b**,**c** are only less stable than the corresponding (*E*)-aza-enediyne **53b**,**c** by just 1.9 and 0.2 kcal/mol, respectively. Thus, the (*Z*)-isomers of **53b**,**c** required for aza-Myers-Saito/Schmittel cyclization should be thermodynamically accessible. Our computational studies also demonstrate that the aza-enyne allenes **4b**,**c** are more stable than the corresponding skipped aza-enediynes **53b**,**c** by 12.0 and 7.3 kcal/mol,

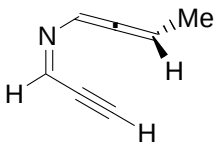
respectively (Table 4-1), supporting the proposed isomerization. There is only a small predicted energy difference between the less stable (*Z*)-aza-enyne allenes **4b**,**c** and the corresponding (*E*)-isomers (0.1 kcal/mol). The *s-cis* conformers of (*Z*)-**4b**,**c** which are needed for the cyclization are only 3.5 kcal/mol less stable than the corresponding *s-trans* conformers of (*Z*)-**4b**,**c**. Therefore, the *s-cis* conformers of (*Z*)-aza-enyne allenes **4b**,**c** required for the Myers-Saito/Schmittel cyclization should be thermodynamically accessible. In the case of ketimine **4d**, the reactive (*E*)-imine isomer (corresponding to (*Z*)-**4b**,**c**) should be predominate.

Table 4-1 B3LYP/6-31G* Energies for the skipped aza-enediynes **53b-d** and corresponding aza-enyne allenes **4b-d**

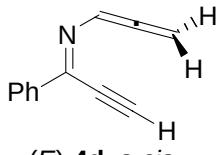
The geometries required for the aza-Myers-Saito/Schmittel cyclization:



(*Z*)-**4b**, *s-cis*



(*Z*)-**4c**, *s-cis*



(*E*)-**4d**, *s-cis*

Compound	Electronic energy[a] (Hartree)	ZPE[b] (kcal/mol)	Relative energy (kcal/mol)
(*E*)-Skipped aza-enediyne **53b**	-286.22186	54.7	10.1[c]
(*Z*)-Skipped aza-enediyne **53b**	-286.21899	54.9	12.0[c]
(*E*)-Aza-enyne allene **4b** (*s-trans*)	-286.23766	54.5	-0.1[c]
(*E*)-Aza-enyne allene **4b** (*s-cis*)	-286.23454	54.7	-2.0[c]
(*Z*)-Aza-enyne allene **4b** (*s-trans*)	-286.23795	54.8	0[c]
(*Z*)-Aza-enyne allene **4b** (*s-cis*)	-286.23233	54.7	3.5[c]
(*E*)-Skipped aza-enediyne **53c**	-325.54553	72.8	7.1[d]
(*Z*)-Skipped aza-enediyne **53c**	-325.54547	73.0	7.3[d]
(*E*)-Aza-enyne allene **4c** (*s-trans*)	-325.55668	72.5	-0.1[d]
(*E*)-Aza-enyne allene **4c** (*s-cis*)	-325.55353	72.6	2.0[d]
(*Z*)-Aza-enyne allene **4c** (*s-trans*)	-325.55682	72.8	0[d]
(*Z*)-Aza-enyne allene **4c** (*s-cis*)	-325.55124	72.7	3.5[d]
(*E*)-Aza-enyne allene **4d** (*s-trans*)	-517.30170		0[e]
(*E*)-Aza-enyne allene **4d** (*s-cis*)	-517.29516		4.1[e]
(*Z*)-Aza-enyne allene **4d** (*s-trans*)	-517.29068		6.9[e]
(*Z*)-Aza-enyne allene **4d** (*s-cis*)	-517.28537		10.2[e]

[a]Absolute energies in Hartree; [b]Zero-point energy correction in kcal/mol; [c]ZPE-corrected energy relative to (*Z*)-aza-enyne allene **4b** (*s-trans*) in kcal/mol; [d]ZPE-corrected energy relative to (*E*)-aza-enyne allene **4c** (*s-trans*) in kcal/mol; [e]Energy relative to (*E*)-aza-enyne allene **4d** (*s-trans*) in kcal/mol.

4.3 A FACILE AZA-MYERS-SAITO/AZA-SCHMITTEL CYCLIZATION OF AZA-ENYNE ALLENES BASED ON COMPUTIONAL STRUDIES AT (U)B3LYP/6-31* LEVEL

The Myers-Saito/Schmittel cyclization and the related diradical intermediates have been the subjects of a number of computational studies. Studies have shown that the diradicals derived from either Myers-Saito or Schmittel cyclization have a small singlet-triplet energy gap; therefore, they have high degrees of diradical character.[13,14] As a consequence, single-determinant such as Hartree-Fock, SCF approaches are not accurate for this situation. Schmittel[15] and co-workers carried out comparative calculations on enyne-allene cyclization at semiempirical AM1/CI level of theory. However, semiempirical theory such as AM1 does not handle singlet-triplet splitting well.[16] Density functional theory (DFT) methods are quite suitable to describe the Myers-Saito and Schmittel cyclization barriers and the corresponding diradicals and are nearly as accurate as much time-consuming high-level methods such as coupled cluster [CCSD(T)] and Brueckner doubles [BCCD(T)].[17] Schreiner and Prall[17] suggested that hybrid DFT such B3LYP approach towards singlet α,3-didehydrotoluene diradical species suffers from artifactual symmetry breaking; Lipton and Wenthold[19] discovered that symmetry breaking for the diradical under B3LYP level can be avoided by using the GUESS = ALTER command in the input. The wave function obtained from this procedure is a mixture of singlet and triplet states (spin operator is larger than one). Since singlet-triplet gap of α,3-didehydrotoluene is relatively small, the energy difference between this mixtured wave function and pure open-shell singlet wave function is rather small.[19] In the present study, the geometries and energies for the diradicals and related species and transition states of the aza-Myers-Saito/Schmittel cyclizations are computated at hybrid density functional theory (U)B3LYP level with a basis set 6-31G*. In the case of singlet diradicals, the geometries and energies are computed by using an unrestricted B3LYP

wave function and the command GUESS = ALTER in the input. In comparison, we also compute the geometries and energies for the diradicals and related species and transition states of the Myers-Saito/Schmittel cyclizations at the same level.

Table 4-2 summarizes the relative energies of transition states, zwitterions and diradicals relative to their respective (*Z*)-reactants **4a,b**. The results demonstrate that aza-Myers cyclization is more exothermic than Myers cyclization by about -4 kcal/mol and it has a slightly lower barrier than that of Myers cyclization. There is a closed-shell singlet planar zwitterion **5±** which is much higher in energy than that of the corresponding open-shell singlet diradical **5••**, in both the Myers-Saito and aza-Myers-Saito cases. A non-planar closed-shell singlet cyclic-allene **24b** that has lower energy than aza-zwitterion **5±** was found for aza-Myers cyclization, as has been reported for Myers-Saito case.[14,20] The calculation results predict a facile aza-Myers cyclization of aza-enyne allene **4b** via either diradical pathway or ionic pathway. (U)B3LYP calculations also indicates that both transition states for aza-Myers-Saito (**TSb1**) and aza-Schmittel (**TSb2**) do not have diradical character, which is also the case for the Myers-Saito[17,20,22] and Schmittel cyclization.[17,22] This indicates that a crossing to the open-shell surface from the closed shell surface occurs after these transition states. Although aza-enyne allenes favor aza-Myers-Saito cyclization over aza-Schmittel cyclization, as shown in Table 4-2, the difference of the barriers between these cyclizations is less than 5 kcal/mol, which indicates there might be a competition between aza-Myers cyclization and aza-Schmittel cyclization.

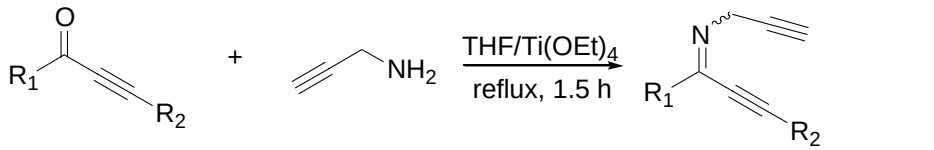
Table 4-2 Calculated energies[a] for the Myers, Schmittel, aza-Myers and aza-Schmittel cyclizations of **4a**,**b** at (U)B3LYP/6-31G* level

	Myers Cyclization					Schmittel Cyclization		
	TS1‡	**s5••**[b]	**t5••**[c]	**5±**[d]	**24**	**TS2**‡	**S52••**[b]	**t52••**[c]
4a	26.3[e]	-11.6	-10.2	20.9	0.1	34.5[e]	13.1	10.5
Exp	23.3±0.5[f]	-13.3±4[g]						
	Aza-Myers Cyclization					Aza-Schmittel Cyclization		
4b	23.2[e]	-15.4	-14.0	16.5	-2.6[e]	27.7[e]	7.3	5.2

[a]calculated energies with zero-point correction relative to respective (*Z*)-*trans* **4** isomer in kcal/mol; [b]the singlet diradical; [c]the triplet diradical; [d]closed-shell singlet with C_s restriction enforced; [f]data from ref 6a; [g]data from ref 14.

4.4 SYNTHESIS OF ACYCLIC SKIPPED AZA-ENEDIYNE 53E,F

The skipped aza-enediynes **53e,f** were synthesized from the condensation of 1-phenyl-3-triisopropylsilyl-propynone **32a** or phenyl propynal **32g** with excess amount of propargyl amine in the presence of titanium (IV) ethoxide in modest yield (Scheme 4-3). The skipped aza-enediyne **53f** can be purified by flash chromatography on silica gel and isolated as relatively stable, red-yellow oil. The skipped aza-enediyne **53e** can be purified by re-crystallization from hexanes as relatively stable colorless cubic crystal. Compound **53f** was obtained as predominant single isomer of undetermined stereochemistry, based on ^{1}H NMR and ^{13}C NMR. Compound **53e** was isolated as two isomers (1:3) about imine double bond as judged by ^{1}H NMR and ^{13}C NMR.



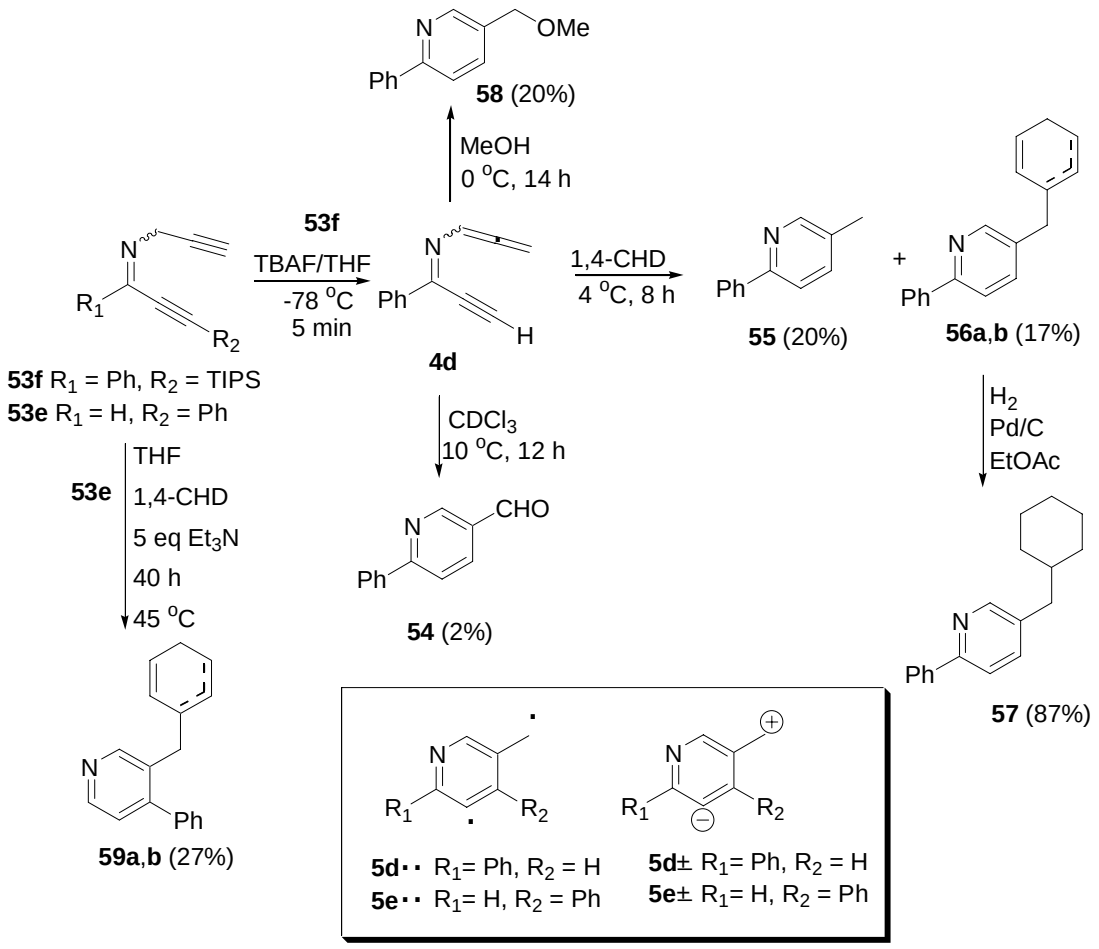
32a R_1 = Ph, R_2 = TIPS
32g R_1 = H, R_2 = Ph

53f R_1 = Ph, R_2 = TIPS (52%)
53e R_1 = H, R_2 = Ph (60%)

Scheme 4-3 Synthesis of acyclic skipped aza-enediyne **53e,f**

4.5 ISOMERIZATION OF SKIPPED AZA-ENEDIYNE 53E,F TO AZA-ENYNE ALLENE 4D,E UNDER BASIC CONDITIONS AND TRAPPING OF A,5-DIDEHYDRO-3-PICOLINE DIRADICALS

Attempts to desilylate compound **53f** by treatment with TBAF at -78 °C only afforded isomerization product **4d** as the sole product. We did not detect any desilylated skipped aza-enediyne (Scheme 4-4).



Scheme 4-4 Isomerization of **53e,f** under basic conditions and trapping of α,5-didehydro-picoline diradicals

Although the instability of **4d** prevented its chromatographic purification, its structure was confirmed by NMR. MS analysis of the reaction mixture after aqueous workup further confirms the loss of the triisopropylsilyl group. The ^{1}H NMR of **4d** contains distinctive resonance for the allene moiety; it has a methylene doublet at 5.36 ppm and a methine triple at 7.60 ppm. Decoupled ^{1}H NMR demonstrates that methylene doublet at 5.36 ppm couples with the methine triplet at 7.60 ppm with a coupling constant

6.0 Hz. We proposed that the downfield shift of the methine triplet (7.60 ppm) is due to the favorable (Z)-s-*trans* conformation of **4d** (Figure 4-1). In this conformation, the allene methine is proximal to and in the deshielding region of the alkyne triple bond, and the resulting deshielding shifts methine downfield. The ^{13}C NMR of reaction mixture contains expected resonance for the allene *sp* carbon at 212 ppm.

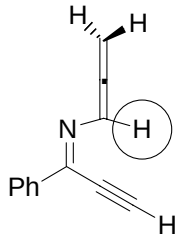


Figure 4-1 (*Z*)-*s*-*trans* conformation of aza-enyne allene **4d**

Solutions of aza-enyne allene **4d** in $CDCl_3$ are unstable, even when refrigerated, and afford complex mixtures from which aldehyde **54** could be isolated (Scheme 4-4). Solutions of **4d** in 1,4-CHD were allowed to stand at 4 °C. TLC demonstrated that all aza-enyne allene **4d** was consumed after 8 hours at 4 °C. The reaction afforded a complex mixture of products that included 6-phenyl-3-picoline **55** isolated in 20% yield, and a mixture of cyclohexadiene adducts **56a,b** in 17% yield (~ 1:1 ratio as judged by ^1H NMR, Scheme 4-4). We were unable to separate **56a** and **56b** by TLC under different solvent systems. Therefore, compounds **56a**,**b** were subjected to hydrogenation to afford the reduced product **57**, which was fully characterized.

The formation of **54**, **55**, and **56a,b** are all commensurate with the intermediacy of the previously unreported α,5-didehydro-3-picoline diradical **5d••**. Rapid cyclization of **4d** to diradical **5d••** (compare Myers cyclization of **4a** with $t_{1/2}$ of 20.5 h at 39 °C),

followed by hydrogen atom abstraction from 1,4-CHD affords a radical pair that can recombine to give **56a,b** or undergo subsequent hydrogen atom abstraction to afford **55**. Aldehyde **54** presumably arises from interception of adventitious oxygen by diradical **5d••**. When the solution of **4d** in d_8-THF was stored at 4 °C for 10 hour, the pyridine derivative **55** was isolated in 2% yield. ^{1}H NMR and MS analysis demonstrated that ~ 33% of **55** have two deuteriums, which further support for the intermediacy of **5d••**. THF apparently is a poor hydrogen atom donor towards diradical **5d••**. No trapping product which corresponds to the trapping of the aza-Schmittel cyclization diradical **52d••** was isolated from any of the conditions mentioned above.

The solution of the skipped aza-enediyne **53e** in THF was maintained at 50 ºC for 1.5 h in the presence of 5 equiv of Et_3N. The NMR analysis of crude reaction mixture demonstrated that 10% of **53e** was converted to aza-enyne allene **4e**. All attempts to purify aza-enyne allene **4e** failed due to its instability. In the presence of large excess of 1,4-CHD, the solution of **53e** in THF in the presence of 5 equiv of Et_3N afforded a complex mixture of products including the cyclohexadiene adducts **59a**,**b** in 27% (~ 1:1 ratio, as judged by ^{1}H NMR) (Scheme 4-4). We were unable to isolate the trapping product containing two hydrogen atoms derived from 1,4-CHD from the reaction mixture. Similar to aza-enyne allene **4d**, aza-enyne allene **4e** undergoes a cyclization analogous to Myers-Saito cyclization to produce diradical **5e••**, which abstracts hydrogen atom from 1,4-CHD to afford a radical pair that can recombine to give adducts **59a**,**b**.

When solutions of **4d** in methanol were stored at 0 °C, 5-methoxymethyl-2-phenylpyridine **58** was isolated in 20% yield. Myers[6a,b] and co-workers reported a similar methanol addition product from the thermolyses of enyne allene **4a** in methanol, and proposed that this is due to a partitioning between diradical and ionic reaction pathways. Recently, Carpenter[23] and co-workers examined this reaction in detail by the combination

of experimental and computational means, and proposed that enyne-allene **4a** must directly form the zwitterion **5a±** and exhibit a post-rate-determining bifurcation to explain the formation of both **23** and trapping products **6**, **19a,b** (Scheme 1-11). Alternatively, the methoxymethyl pyridine **58** may arise from a different mechanism involving methanol addition to the aminoallene functionality of **4d**, followed by cyclization of the resulting azadienyne.[21]

Despite the overall similarity in trapping products from the thermal rearrangements of **4a** and **4d**, there are differences between these two systems. In general, the isolated yields of trapped products from **4d** are lower than those reported for **4a**. It may be that a competing aza-Schmittel pathway in the case of **4d** leads to more complex reaction mixtures and lower isolated yields.

4.6 CONCLUSIONS

In conclusion, the formation of α,5-didehydro-3-picoline diradical intermediates from skipped aza-enediynes via isomerization to aza-enyne allenes and aza-Myers-Saito cyclization has been predicted on the basis of DFT calculations. Skipped aza-enediynes are easily prepared, and their facile aza-Myers cyclization provides a route to the previously unreported picolinyl diradicals. In addition, the isomerization of skipped aza-enediynes and their subsequent rapid aza-Myers-Saito cyclization may have relevance to the recently reported DNA cleavage ability of cationic heterocyclic skipped aza-enediynes such as AZB002[24] and AZB037[25] (Figure 1-6).

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Chapter 5 Aza-enediynes undergo both aza-Bergman rearrangement to nitriles and dimerization to enediynes simultaneously

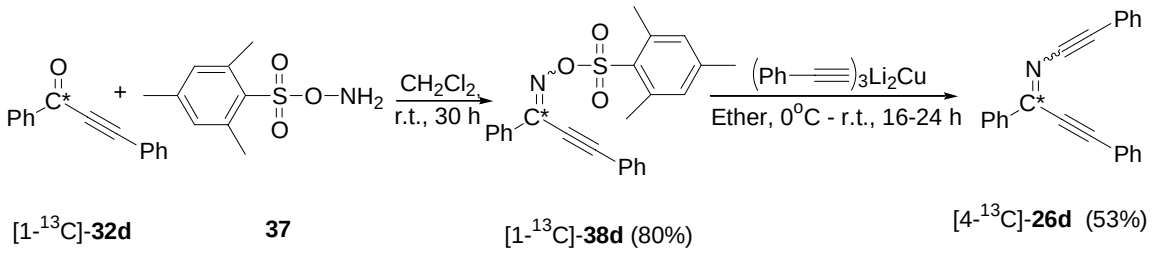
5.1 INTRODUCTION

Naturally occurring enediynes (Scheme 1-1, Scheme 1-2) are a class of potent anticancer agents that undergo Bergman cyclization or Myers-Saito-type cyclization to reactive diradical intermediates (Scheme 1-5),[1,2] which abstract hydrogen atoms from the sugar phosphate backbone of DNA, leading to oxidative DNA strand scission and ultimately cell death.[1b,c] The applications of these enediynes in cancer chemotherapy are limited due to their lack of tumor specificity and toxicity.[3,4] In 1997, David and Kerwin[5] first reported a new class of enediynes, the aza-enediynes, or *C,N*-dialkynyl imines (Scheme 1-14, **26e,f**) and reported that these aza-enediynes undergo an aza-Bergman cyclization to produce 2,5-didehydropyridine intermediate diradicals that may have the potential to cleave DNA (Scheme 1-14, **27e,f**), since then, these compounds have been the subjects of a number of theoretical[6,7,8,9,10] and experimental studies.[10,11,12] In contrast to 1,4-ddb, 2,5-ddp diradicals are very short-lived un-reactive species and can undergo a rapid retro-aza-Bergman reaction to nitriles[5,10,12] (Scheme 1-14, **28e,f**). Chen[10] and co-workers reported the trapped pyridine product of 2,5-ddp from the thermolysis of aza-enediyne **26e** (Scheme 1-14) carried out under acidic conditions, and the isolation of this trapped pyridine product from samples of **26e** stored at -4 ºC for months. We have been unable to confirm this finding. Instead, we isolated (*E*)-, and (*Z*)-enediynes[13] **60e** and bisnitrile **61** (Scheme 5-2) from samples of **26e** stored at -10 ºC for 24 months. The transformation of aza-enediyne to enediynes has also been observed in the case of triphenyl aza-enediyne **26d,g** (Scheme 2-3). The unusual behavior of compounds **26d,e,g** trigged our interests to further study this remarkable transformation reaction. Here I

report our kinetic and mechanistic studies of aza-enediyne **26d,e,g** including ^{13}C labeled [4-^{13}C]-**26d**.[26]

5.2 SYNTHESIS OF [4-^{13}C]-26D

Aza-enediyne [4-^{13}C]-**26d** was prepared according to the procedure analogous to that for the preparation of **26d** (Scheme 2-3) by using ^{13}C labeled 1,3-diphenyl-propynone [1-^{13}C]-**32d** (Scheme 5-1). Briefly, the addition of the cuprate reagent derived from the phenyl acetylene to the mesitylate[16] [1-^{13}C]-**38d**, that was obtained from the condensation of the [1-^{13}C]-**32d** with *O*-mesitylenesulfonyl hydroxylamine **37**, afforded [4-^{13}C]-**26d** in 53% yield (Scheme 5-1).



[1-^{13}C]-**32d** **37** [1-^{13}C]-**38d** (80%) [4-^{13}C]-**26d** (53%)

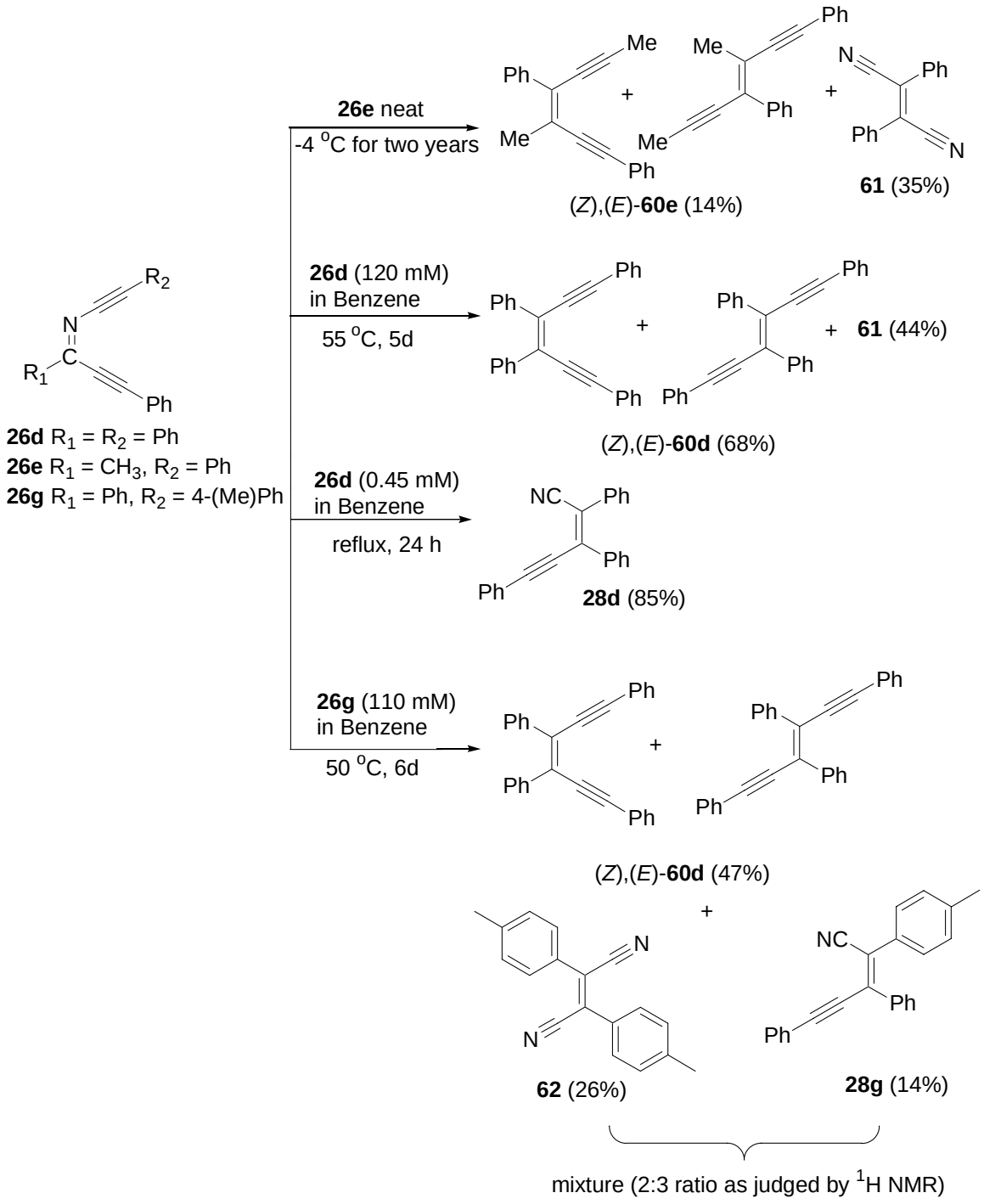
Scheme 5-1 Synthesis of [4-^{13}C]-**26d**

Mesitylate [1-^{13}C]-**38d** was isolated as predominant single isomer of undetermined stereochemistry as judged from ^{1}H NMR and ^{13}C NMR. The aza-enediyne [4-^{13}C]-**26d** can be purified by chromatography and isolated as single isomer based on ^{1}H NMR and ^{13}C NMR. Our previous calculations[12] at B3LYP level demonstrated that the (*Z*)-isomer of aza-enediyne **40a** (Scheme 2-5) is more stable than the (*E*)-isomer by 4.4 kcal/mol. We predict that the (*Z*)-isomer of **26d** is more stable than the (*E*)-isomer.

Therefore, the single isomer observed for **26d** is the more thermodynamically stable (*Z*)-isomer.

5.3 KINETIC AND MECHANISTIC STUDIES OF THE TRANSFORMATION OF AZA-ENEDIYNES 26D,E,G TO ENEDIYNES 60D,E

The neat aza-enediyne **26d** is not stable even stored in a -10 °C freezer. After being stored in a freezer for weeks, TLC of the samples of **26d** showed that the small amounts of **26d** converted to new unidentified compounds. A solution of 90 mg of aza-enediyne **26d** (120 mM) in benzene was stirred at 55 °C for 5 days until aza-enediyne **26d** disappeared upon TLC analysis. The reaction generated a complex mixture from which (*E*)- and (*Z*)-tetraphenyl enediynes **60d** (68%, 1:1 ratio after chromatograph) and (*E*)-dicyano compound **61** (44%) (Scheme 5-2) were isolated along with other unidentified more polar materials, presumably polymers. The structures of compounds **60d** and **61** were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS analysis.[17,18]

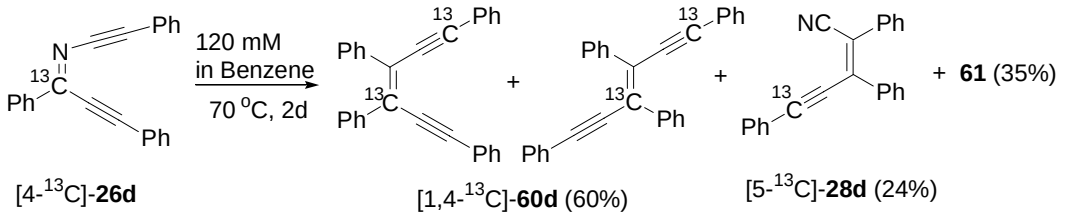


Scheme 5-2 Thermolyses of aza-enediynes **26d**,**e,g**

Thermolysis of aza-enediyne **26g** with a 4-(Me)Ph substituent (110 mM in benzene) at 50 ºC afforded enediynes (*Z*)-, (*E*)-**60d** (1:1 after flash chromatography, 47%), nitrile **28g** with one 4-(Me)Ph substituent (14%) and bisnitrile **62** with two 4-(Me)Ph substituents (26%) (Scheme 5-2). Compounds **28g** and **62** were isolated as the mixtures (2:3 as judged by ^{1}H NMR). We propose that nitrile **28g** was generated from the aza-Bergman-retro-aza-Bergman rearrangement of aza-enediyne **26g**. The small amount of pure bisnitrile **62** was obtained by re-crystallization of the mixtures of **28g** and **62** from hexanes and ethyl acetate as white needle crystal. The structure of **62** was confirmed by ^{1}H NMR and CI-MS.

When the solution of aza-enediyne **26d** (0.45 mM) in benzene was refluxed at 90 ˚C for 24 hours, the retro-aza-Bergman rearranged nitrile **28d** was isolated as the sole product in 85% yield (Scheme 5-2). To gain further insight of this transformation of aza-enediyne to enediynes, ^{13}C labeled aza-enediyne [4-^{13}C]-**26d** was prepared. Thermolysis of aza-enediyne [4-^{13}C]-**26d** (120 mM in benzene) at 70 ºC afforded di-^{13}C labeled enediynes (*Z*)-, (*E*)-**60d** (1:3 after flash chromatography, 60%), mono-^{13}C labeled retro-aza-Bergman rearranged nitrile **28d** (24%), and bisnitrile **61** (35%) (Scheme 5-3). No label was detected in the bisnitrile **61**. ^{13}C-labeled nitrile **28d** was generated from the aza-Bergman-retro-aza-Bergman rearrangement. NMR analysis of enediynes **60d** demonstrated that both (*Z*)-**60d** and (*E*)-**60d** have two labels, one is alkynyl carbon ((*E*)-**60d**: δ 98.56; (*Z*)-**60d**: δ 96.84), and the other one is olefinic carbon ((*E*)-**60d**: δ 128.63; (*Z*)-**60d**: δ 129.23); the alkynyl carbon is coupled with the olefinic carbon to give the coupling constant 4.6 Hz for (*E*)-**60d** and 4.1 Hz for (*Z*)-**60d**, respectively. After comparing our coupling constants with the ones obtained from reported similar structures such as hept-1-yne[19,20] ($^{3}J_{1,4}$ = 3.1 Hz), $^{2}J_{1,3}$ = 12.5 Hz) and pent-4-en-2-yn-1-ol[19,21] ($^{3}J_{1,4}$

= 2.0 Hz, $^2J_{1,3}$ = 12.4 Hz and $^2J_{2,4}$ = 11.9 Hz), we assign that two labels are at 1- and 4-positions.



[4-^{13}C]-**26d** [1,4-^{13}C]-**60d** (60%) [5-^{13}C]-**28d** (24%)

Scheme 5-3 Thermolysis of [4-^{13}C]-**26d** in benzene

The transformation of aza-enediyne **26d** to enediynes **60d** and the conversion of **26d** to nitrile **28d** can be followed by ^{1}H NMR. Briefly, a NMR tube containing degassed aza-enediyne **26d** solution in d_6-benzene (120 mM) and 2,5-dimethylfuran (1.5 equiv, internal standard) was sealed under flame. ^{1}H NMR of the sample was taken as initial spectrum (reaction time equals zero second). After ^{1}H NMR, the tube was kept at 70 °C. The conversion of aza-enediyne to enediyne and nitrile was followed spectroscopically by taking ^{1}H NMR spectra 15 times over a period of time. Intervals varied from 35 minutes to 5 hours. Every time, the ^{1}H NMR tube was taken out of a 70 °C oil bath, and immediately inserted into a beaker containing ice-water to stop the reaction. Reaction time was measured as the time during which the tube maintained at 70 °C. Aza-enediyne **26d** has a doublet resonance at 7.45 ppm which is well resolved from other peaks. The disappearance of the aza-enediyne **26d** was monitored as the disappearance of the resonance at 7.45 ppm. The integrals of the resonance for **26d** at 7.45 ppm were normalized against the resonance for the internal standard 2,5-dimethylfuran at 5.76 ppm. The relative concentrations of aza-enediyne **26d** were calculated by normalizing the integrals of the resonance for **26d** at 7.45 ppm obtained at different intervals against the

integral of the resonance for **26d** at 7.45 ppm obtained at reaction time equals zero second. The kinetic data was obtained by fitting plots of the concentrations of aza-enediyne **26d** versus time.[23] ^{1}H NMR kinetic data demonstrated that the disappearance of **26d** does not follow either first-order kinetics or second-order kinetics, but follows independent first-order and second-order kinetics simultaneously (Table 5-1).[23] We have demonstrated that the conversion of aza-enediynes to nitriles follow first-order kinetics for both the disappearance of aza-enediynes and the appearance of nitriles.[12] Here we discover that the conversion of aza-enediyne **26d** to nitrile **28d** in benzene follows first-order kinetics with a half-life of 4.3 hours; the transformation of aza-enediyne **26d** to enediyne **60d** follows second-order kinetics (Table 5-1).

The conversion of aza-enediyne [4-^{13}C]-**26d** to enediyne [1,4-$^{13}C_2$]-**60d** and nitrile [5-^{13}C]-**28d** can be followed by ^{13}C NMR by a procedure analogous to that described for ^{1}H NMR kinetics. The resonance for [4-^{13}C]-**26d** at 159 ppm (^{13}C) diminished over time and was replaced by the resonance for [1,4-$^{13}C_2$]-**60d** at 97 ppm and 129 ppm (^{13}C) and the resonance for [5-^{13}C]-**28d** at100 ppm (^{13}C). The integrals of the resonance for [4-^{13}C]-**26d** at 159 ppm were normalized against the resonance for the solvent (benzene, THF or DMSO). The relative concentrations of aza-enediyne [4-^{13}C]-**26d** were calculated by normalizing the integrals of the resonance for [4-^{13}C]-**26d** at 159 ppm obtained at different intervals against the integral of the resonance for [4-^{13}C]-**26d** at 159 ppm obtained at reaction time equals zero second. The kinetic data was obtained by fitting plots of the concentrations of aza-enediyne [4-^{13}C]-**26d** versus time.[23] Kinetic data obtained from ^{13}C NMR studies were also independent first-order and second-order (Table 5-1). At 70 ºC, the second-order transformation reaction is faster than the first-order aza-Bergman cyclization in either benzene, THF or DMSO. We have performed kinetic studies of **26d** in different solvents to determine the solvent effect (Table 5-1). We

have reported that the conversion of aza-enediyne to nitrile is solvent dependent; proceeding more rapidly in less polar solvents.[12] Here we report that the conversion of aza-enediyne **26d** to nitrile **28d** follows first-order kinetics and proceeds more rapidly in benzene than in more polar solvents such as THF and DMSO (Table 5-1). Importantly, ^{13}C NMR kinetic studies demonstrate that more than 90% of enediyne generated from the transformation is (*Z*)-**60d** which slowly isomerizes to thermodynamic more stable (*E*)-**60d** isomer during extended heating or chromatography on silica gel to mixtures of (*E*)-, and (*Z*)-**60d**.

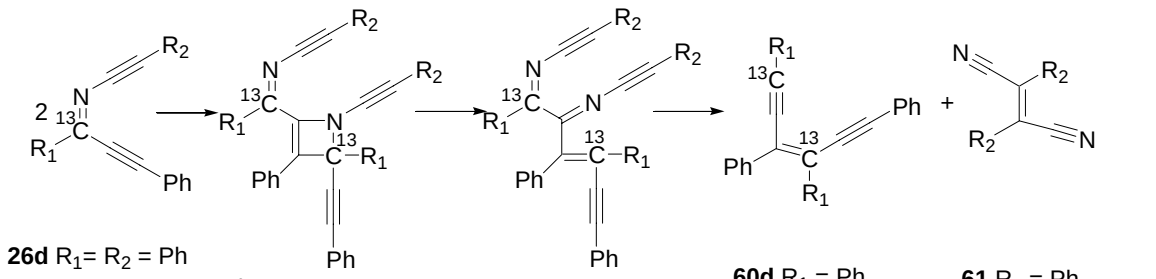
Table 5-1 Rate constants for the first-order aza-Bergman rearrangement of **26d** and the second-order transformation of **26d** to **60d** at 70 ± 1 ºC

Aza-enediyne[a]	First-order reaction		Second-order reaction
	First-order rate constant k_1 (s^{-1})	Half-life $t_{1/2}$ (h)	Second-order rate constant k_2 (L $mol^{-1}s^{-1}$)
d_6-Benzene[b]	$4.4 \pm 0.3 \times 10^{-5}$	4.3 ± 0.2	$4.1 \pm 0.2 \times 10^{-4}$
d_8-THF[c]	$1.8 \pm 1.3 \times 10^{-5}$	10.7 ±6.2	$2.0 \pm 0.8 \times 10^{-4}$
d_6-DMSO[c]	$7.2 \pm 4.5 \times 10^{-6}$	26.7 ±16.4	$1.6 \pm 0.1 \times 10^{-3}$

[a]Initial **26d** or [4-^{13}C]-**26d** concentration of 120 mM. [b]avarage values of one ^{1}H NMR run and one ^{13}C NMR run; [c]values from one ^{13}C NMR run.

Kamigata[17] and co-workers reported the synthesis of (*Z*)-, (*E*)-**60d** from selenium-substituted allenes and proposed the formation of diphenyl propargyl carbene via the homolytic cleavage of the carbon-selenium bond of allene followed by the attack of the seleno radical to the selenium atom. Compound **60d** generated from aza-enediyne **26d** would also be formed through the diphenyl propargyl carbene. However, thermolyses of aza-enediyne **26d** in benzene at 50 ºC in the presence of cyclohexene or 1,4-CHD (both are good carbene trapping reagents) only gave enediynes **60d** and bisnitrile **61**, and did

not afford any carbene trapping products. Based on the kinetic and mechanistic studies of aza-enediynes **26d,e,g**, we proposed that the transformation of aza-enediynes to enediynes occurs through a "head-to-tail" coupling of the propargyl vinylidene units and a "tail-to-tail" coupling of the ynamine units. One possible mechanism is shown in Scheme 5-4. "Head-to-tail" [2+2] cyclization affords an aza-cyclobutene which opens to give an α-diimine. Diimine then leads to enediyne **60d,e** and bisnitrile **61**/**62**.



Scheme 5-4 Proposed mechanism of the formation of enediyne **60d,e** from aza-enediyne **26d,e,g**

It has been reported that certain transition metals such as osmium[24(a)] and ruthenium[24(b)] can catalyze olefin cross metathesis. The transformation of aza-enediyne **26d** to enediyne **60d** in the presence of 1.0 equiv of copper (I) bromide dimethyl sulfide complex in THF (120 mM) at 50 ºC proceeded as slowly as the reaction in the absence of copper (I) salt. Therefore, trance amounts of Cu(I), if introduced from cuprate addition reaction, do not affect the transformation reaction.

5.4 CONCLUSIONS

We[5,12] have demonstrated that aza-enediynes undergo aza-Bergman cyclization to 2,5-ddp diradical intermediates, which then undergo retro-aza-Bergman rearrangement to nitriles (Scheme 2-5). On the other hand, certain aza-enediynes[25] can undergo reaction under acidic conditions to afford carbene intermediates which can be trapped efficiently (Scheme 3-5). Here we[26] present the evidence for a remarkable transformation of aza-enediynes **26d,e,g** to enediynes **60d,e**. Aza-enediynes (**40a-c**) that undergo rapid aza-Bergman cyclization do not undergo the transformation to enediynes. Aza-enediynes (**26a**) having bulky terminal substituents such as TIPS group do not undergo the transformation reaction. Aza-enediynes such as **26d**,**e,g** that undergo aza-Bergman rearrangement slowly can convert to enediynes through a dimerization mechanism (Scheme 5-4). In this case, at high concentration of aza-enediynes, the transformation to enediynes can predominate.

5.5 SUMMARY AND FUTURE DIRECTIONS

Classes of *C*,*N*-dialkynyl imines (3-ene-3-aza-1,5-diynes, aza-enediynes) and 3-ene-4-aza-1,6-diynes (skipped aza-enediynes) have been designed and synthesized in the present study. The substitution of nitrogen for carbon in the enediyne system can have a profound impact on the facility of the Bergman rearrangement and on the nature of the intermediates that are involved. These aza-enediynes undergo a facile aza-Bergman cyclization to generate 2,5-didehydropyridine diradicals that undergo a rapid retro-aza-Bergman rearrangement to (*Z*)-β-alkynyl acrylonitriles. The convertion of aza-enediynes to the nitriles follows first-order kinetics. The rate of aza-Bergman cyclization is solvent dependent, proceeding more rapidly in less polar solvents. The kinetic studies of the aza-Bergman cyclization of a series of 6-triisopropylsilyl and 6-unsubstituted 4-aryl-3-aza-3-

ene-1,4-diynes demonstrate that there are two types of substituent effects in this system; the aza-Bergman reaction rate is not greatly affected by changing the 4-aryl substituent on the aza-enediyne imine double bond, but is very sensitive to the nature of the 6-substitutent at the alkyne terminus.

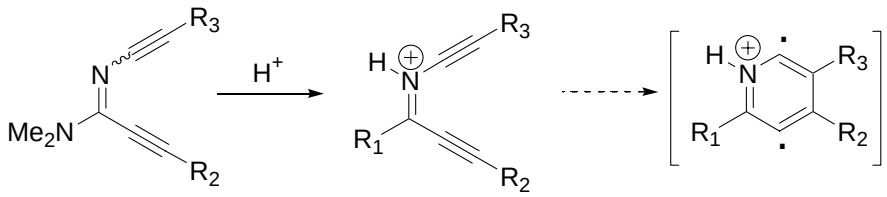
Certain aza-enediynes (**40a**) can undergo reaction under acidic conditions to afford carbene intermediates which can be trapped efficiently. No pyridine products corresponding to trapping of 2,5-ddp intermediates from the thermolysis of aza-enediynes **26a**/**40a** under acidic conditions are detected, which is in contrast with Chen[10] and co-workers' report of a pyridine product from the thermolysis of aza-enediyne **26e** under acidic conditions or from the samples of **26e** stored for months at -4 ºC. We were unable to isolate the pyridine product from the samples of **26e** stored for two years at -10 ºC. On the other hand, aza-enediynes such as **26d,e,g** that undergo aza-Bergman rearrangement slowly can undergo a remarkable transformation to produce enediynes through a dimerization mechanism. We also demonstrate that aza-enediynes (**26a**) having bulky terminal substituents such as TIPS group do not undergo the transformation to enediynes. The transformation of aza-enediynes to enediynes follows second-order kinetics.

The skipped aza-enediynes can isomerize to the corresponding *C*-alkynyl-*N*-allenyl imines (aza-enyne allenes) under basic conditions. The resulting aza-enyne allenes undergo a facile aza-Myers-Saito cyclization to generate previously un-reported α,5-didehydro-3-picoline diradicals that can be efficiently trapped. The isomerization of skipped aza-enediynes and their subsequent rapid aza-Myers-Saito cyclization may have relevance to the recently reported DNA cleavage ability of cationic heterocyclic skipped aza-enediynes such as AZB002 and AZB037 (Figure 1-6).

The 1,4-didehydrobenzenoid diradicals produced by the Bergman cyclization of enediynes are responsible for the DNA cleavage ability of these enediynes.[1] However,

unlike enediynes, aza-enediynes studied cannot cleave DNA, since 2,5-didehydropyridine diradicals produced by the aza-Bergman cyclization of aza-enediynes are a very short lived and unreactive species, and these intermediates undergo a rapid retro-aza-Bergman rearrangement to (*Z*)-β-alkynyl acrylonitriles.[5,12]

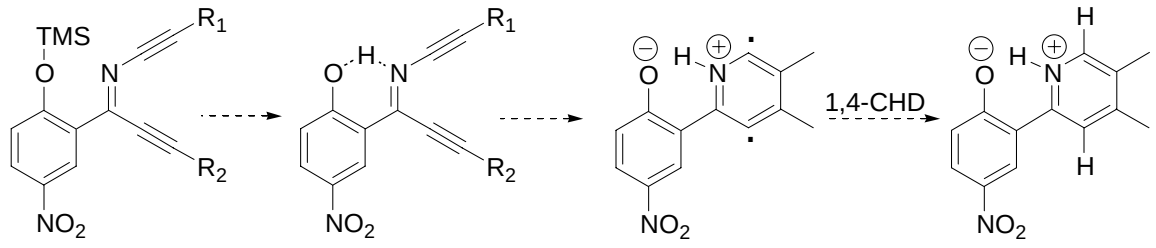
Protonation of the nitrogen of the aza-enediyne is predicted to increase the diradical reactivity towards hydrogen atom abstraction, which would lead to the proposal of a pH-selective aza-enediyne.[10] However, attempts to trap these 2,5-ddp diradicals under acidic conditions afforded cyclopropane derivatives derived from carbene intermediates (Scheme 3-6).[25] It is possible that under acidic conditions the ynamine carbon of aza-enediyne can be protonated followed by addition of water affording carbene intermediate responsible for the formation of cyclopropane derivative. Increasing the pK_a value of imine nitrogen would help protonate the nitrogen under acidic conditions. Based on our studies, the aza-Bergman cyclization rate is not greatly affected by the nature of the imine double bond substituent.[12] We propose replacing the imine double bond of the aza-enediyne system with the amidine group to afford aza-enediynes such as **63** shown in Scheme 5-5 would facilitate nitrogen protonation due to the increased pK_a value of nitrogen. In addition, incorporating amidine goup into aza-enediyne system would prevent the hydrolysis of imine double bond. Therefore, it would be interesting to prepare aza-enediynes **63** (Scheme 5-5) and to study thermolyses of these aza-enediynes under acidic conditions.



63 **64** **65**

Scheme 5-5

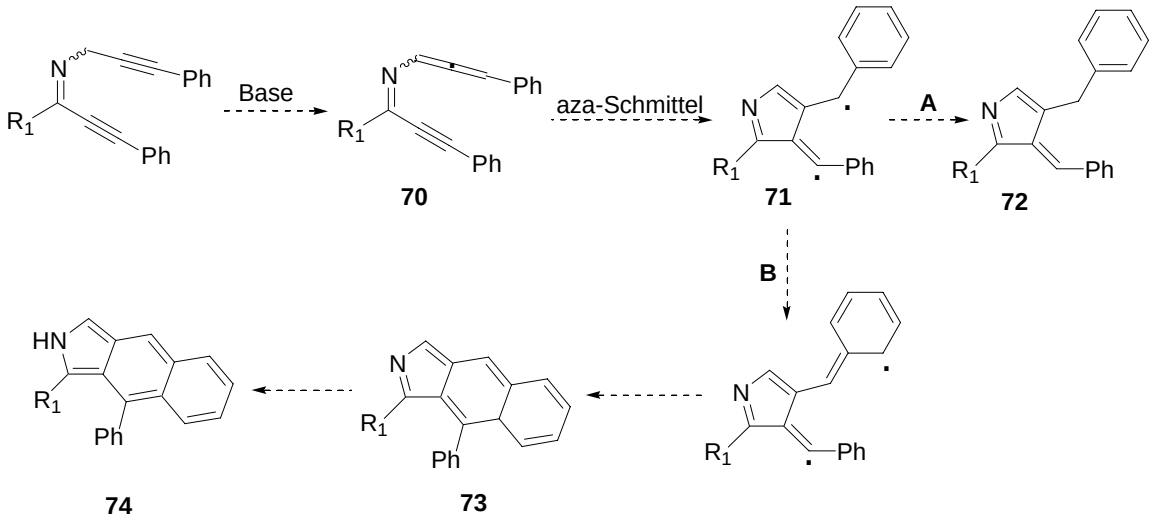
Since 2,5-ddp diradical intermediates are a very short lived and unreactive species, these intermediates undergo a rapid retro-aza-Bergman rearrangement to produce thermodynamically more stable nitrile. We hypothesize that protonation of the nitrogen in aza-enediyne system through an intra-molecular proton transfer pathway may proceed much more rapidly than that through an inter-molecular proton transfer.[27] Hence, under this conditions, 2,5-ddp diradical intermediate may have potential to be protonated before it undergoes retro-aza-Bergman rearrangement to produce nitrile. Based on our hypothesis, we propose that aza-enediyne **67** undergoes aza-Bergman cyclization to produce diradical **68**, whose nitrogen atom may be protonated by the adjacent acidic phenol group (Scheme 5-6). The protonated diradical **68** can abstract two hydrogen atoms from 1,4-CHD.



66 **67** **68** **69**

Scheme 5-6 Protonation of the nitrogen atom of 2,5-ddp diradical **68** via an intramolecular proton transfer from the adjacent acidic phenol group

As mentioned in Chapter 4, aza-enyne allenes **4d**,**f** undergo a facile aza-Myers-Saito cyclization to produce α,5-didehydro-3-picoline diradicals which can be trapped efficiently (Scheme 4-4). We were unable to detect any products corresponding to the trapping of diradicals produced by Schmittel cyclization of aza-enyne allenes **4d**,**f**. It has been reported that the enyne allenes with radical stabilizing substituents such as aryl or with bulky groups at the C^7 position (alkyne terminus) favor the Schmittel cyclization (Scheme 1-12).[28] We propose that an aza-enyne allene with a phenyl substituent at the alkyne terminus such as **70** may undergo aza-Schmittel cyclization to produce a pyrrole-like diradical **71**, which may have potential to abstract two hydrogen atoms from hydrogen donor such as 1,4-CHD to afford a pyrrole-like product **72** (Scheme 5-7, path **A**) or undergo intramolecular radical recombination followed by rearrangement of **73** to afford product **74** (Scheme 5-7, path **B**).



Scheme 5-7 Proposed aza-Schmittel cyclization of aza-enyne allene **70**

The initial goal of this study was to design novel enediyne core structures that target cancer cell with minimum toxicity towards normal cells. However, all of the aza-enediynes studied here are unsuitable for DNA cleavage. Our studies suggest that aza-enyne allenes may have the potential to cleave DNA via diradical imtermediates produced by aza-Myers-Saito cyclization of aza-enyne allenes. We hope that the findings presented in this study lay a foundation for the design of a novel enediyne core structure that can target cancer cells.

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13. The assignment of the structures of (*E*)-, (*Z*)-3,6-dimethyl-1,4-diphenyl-3-hexene-1,5-diynes **60e** is based on the comparison of ^{1}H NMR of **60e** with ^{1}H NMR of reported 1,6-dimethyl-3,4-diphenyl-3-hexene-1,5-diyne (ref. 15) and 3,4-dimethyl-1,6-diphenyl-3-hexene-1,5-diyne (ref. 14,15); **60e**: ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 2.30 (s, 3H), 7.23-7.36 (m, 16H), 7.49-7.51 (m, 2H), 7.77 (d, 2H, J = 7.6 Hz); HRMS m/z 257.1323 (calcd 257.1330, $C_{20}H_{17}$).

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23. TableCurve 2D® from SYSTAT Software Inc. was used to analyze the kinetic data. Kinetic data fits independent first-order and second-order kinetics with an equation: $\frac{d[A]}{dt} = -k_1[A] - k_2[A]^2$, [A]: concentration of aza-enediyne **26d**; k_1: first-order constant; k_2: second-order constant.

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Chapter 6 Experimental section

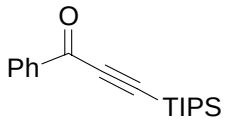
6.1 GENERAL

All commercial chemicals were used without further purification unless otherwise noted. Tetrahydrofuran (THF) was re-distilled from Na/benzophenone, methylene chloride, diethyl ether, and pyridine were re-distilled from calcium hydride prior to use. All reactions were performed under argon. Unless otherwise noted, organic extracts were dried with Na_2SO_4, filtered through a fritted glass funnel, and concentrated with a rotary evaporator (20-30 mmHg). R_f values are reported for thin-layer chromatography (TLC) performed on silica gel TLC plates, using the indicated mobile phase. Flash chromatography was preformed with EM silica gel. ^{1}H NMR spectra were recorded at 500, 300, and 250 MHz and ^{13}C NMR spectra were recorded at 100, 75 and 62 MHz. Spectroscopic studies were performed on a spectrophotometer with a Peltier thermostated cuvette holder. All mass spectra were obtained by chemical ionization with methane as the ionizing gas. Melting points are uncorrected. The yields for compounds **60d**,**e** and **61/62** were calculated based on 2:1 stoichiometry between **26d**,**e,g** and **60d**,**e**, **61/62**.

All calculations were carried out by using (U)B3LYP/6-31G* with Gaussian 98 program.[35] Closed-shell singlet states were computed using B3LYP/6-31G*. Open-shell singlet diradicals and triplet diradicals were computed using unrestricted UB3LYP/6-31G*, and the open-shell singlet states were specified by using the command GUESS = ALTER in the input and switching the HOMO and LUMO for the β electron. All stationary structures were identified as minima (number of imaginary frequencies = 0) or transition states (number of imaginary frequencies = 1) by computing vibrational frequencies. The energies reported here were corrected to zero-point vibrational energies obtained by computing vibration frequencies.

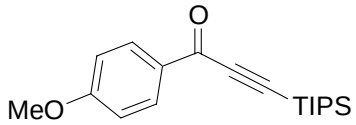
6.2 SYNTHESES

6.2.1 1-Phenyl-3-(triisopropylsilyl)-propynone 32a



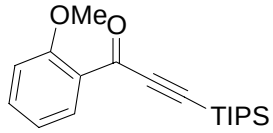
To a mixture of triisopropylsilyl acetylene (0.5 g, 2.74 mmol) and cuprous iodide (26.6 mg, 0.14 mmol) in triethylamine (9 mL) was added benzoyl chloride (0.5 g, 3.56 mmol). The reaction mixture was stirred at room temperature under argon for 30 h. After the removal of the solvent, methanol (3 mL) was added and the mixture was stirred for an additional 5 min, during which time the mixture became clear. The methanol was distilled off under vacuum, and the residue was poured into water and extracted with dichloromethane (2 × 20 mL) and dried over Na_2SO_4. The solvent was distilled off and the product was purified by Kugelrohr distillation (110 °C, 1 mmHg) to afford 0.69 g (87%) of propynone **32a** as a clear oil: ^{1}H NMR ($CDCl_3$) δ1.01-1.22 (m, 21H), 7.46-7.55 (m, 3H), 8.16-8.23 (m, 2H); ^{13}C NMR ($CDCl_3$) δ11.07, 18.53, 97.91, 103.01, 128.53, 129.49, 134.00, 136.70, 177.43; HRMS *m*/*z* 287.1826 (calcd 287.1831, $C_{18}H_{26}OSi$).

6.2.2 1-(4-Methoxyphenyl)-3-(triisopropylsilyl)-propynone 32b



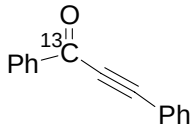
To a mixture of triisopropylsilyl acetylene (3.0 g, 16.45 mmol) and cuprous iodide (156.4 mg, 0.82 mmol) in triethylamine (35 mL) was added *p*-Anisoyl chloride (3.5 g, 20.57 mmol). The reaction mixture was stirred at room temperature under argon for 30 h. After the removal of the solvent, methanol (10 mL) was added and the mixture was stirred for an additional 5 min, during which time the mixture became clear. The methanol was distilled off under vacuum, and the residue was poured into water and extracted with dichloromethane (2 × 20 mL) and dried over Na_2SO_4. The solvent was distilled off and the product was purified by flash chromatography (0-30% CH_2Cl_2 in hexanes) to afford 4.8 g (92%) of propynone **32b** as a transparent oil: R_f 0.85 (CH_2Cl_2); ^{1}H NMR (CD_2Cl_2) δ1.11-1.30 (m, 21H), 3.86 (s, 3H), 6.95 (d, J = 8.0 Hz, 2H), 8.13 (d, J = 8.0 Hz, 2H); ^{13}C NMR (CD_2Cl_2) δ11.54, 18.74, 55.99, 97.21, 103.87, 114.24, 130.80, 132.12, 165.04, 176.23; HRMS *m*/*z* 317.1930 (calcd 317.1936, $C_{19}H_{28}O_2Si$).

6.2.3 1-(2-Methoxyphenyl)-3-(triisopropylsilyl)propynone 32c



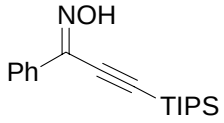
To a mixture of triisopropylsilyl acetylene (0.5 g, 2.74 mmol) and cuprous iodide (26.6 mg, 0.14 mmol) in triethylamine (9 mL) was added o-anisoyl chloride (0.5 g, 3.56 mmol). The reaction mixture was stirred at room temperature under argon for 30 h. After the removal of the solvent, methanol (3 mL) was added and the mixture was stirred for an additional 5 min, during which time the mixture became clear. The methanol was distilled off under vacuum, and the residue was poured into water and extracted with dichloromethane (2 × 20 mL) and dried over Na_2SO_4. The solvent was distilled off and the product was purified by flash chromatography (10% EtOAc in hexanes) to afford 4.62 g (74%) of **32c** as a transparent oil: R_f 0.85 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.95-1.20 (m, 21H), 3.85 (s, 3H), 6.90-7.01 (m, 2H), 7.45 (ddd, J = 8.2, 6.0, 2.0 Hz, 1H), 7.99 (dd, J = 8.2, 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ10.94, 18.34, 55.51, 95.50, 105.11, 111.84, 119.96, 126.25, 132.56, 134.72, 159.96, 176.11; HRMS *m*/*z* 317.1944 (calcd 317.1936, $C_{19}H_{28}O_2Si$).

6.2.4 1,3-Diphenyl-1-^{13}C-propynone 32d



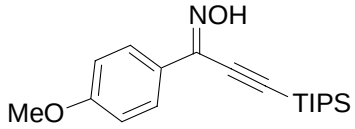
To a mixture of phenyl acetylene (0.22 g, 2.2 mmol) and cuprous iodide (17.0 mg, 0.08 mmol) in triethylamine (5.0 mL) was added benzoyl chloride (0.20 g, 1.4 mmol). The reaction mixture was stirred at room temperature under argon for 48 h. After filtering off the salt, the solvent was distilled off and the product was purified by flash chromatography (hexanes/EtOAc, 20:1) to afford 0.35 g (60%) of 1,3-diphenyl-1-^{13}C-propynone **32d** as a yellow oil: R_f 0.42 (EtOAc/hexanes, 1:9).[3] ^{1}H NMR ($CDCl_3$) δ 7.31-7.53 (m, 5H), 7.56-7.70 (m, 3H), 8.15-8.23 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 86.78 (d, J = 91.2 Hz), 93.01 (d, J = 13.7 Hz), 119.98 (d, J = 1.7 Hz), 128.54 (d, J = 5.0 Hz), 128.60, 129.46 (d, J = 3.3 Hz), 130.73, 132.96, 134.05, 136.74 (d, J = 61.1 Hz), 177.89 (^{13}C); HRMS *m/z* 208.0842 (calc'd 208.0843, $^{12}C_{14}{}^{13}CH_{11}O$).

6.2.5 1-Phenyl-3-(triisopropylsilyl)-propynone oxime 33a



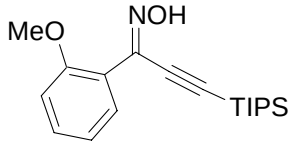
To a solution of **32a** (0.64 g, 2.23 mmol) in 8 mL of EtOH was added hydroxylamine hydrochloride (186 mg, 2.68 mmol) and sodium acetate (220 mg, 2.68 mmol). The mixture was stirred at room temperature under argon for 10 days. The reaction mixture was poured into water (20 mL), and the water layer was extracted by CH_2Cl_2 (3 × 15 mL). The residue upon drying and concentration of the organic layer was purified by flash chromatography (0-20% CH_2Cl_2 in hexanes) to afford 0.51 g (77%) of oxime **33a** as a transparent oil: R_f 0.54 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.04-1.30 (m, 21H), 7.38-7.42 (m, 3H), 7.80-7.89 (m, 2H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.13, 18.61, 94.61, 107.42, 126.46, 128.46, 129.82, 133.12, 141.97; HRMS *m*/*z* 302.1946 (calcd 302.1940, $C_{18}H_{27}NOSi$).

6.2.6 1-(4-Methoxyphenyl)-3-(triisopropylsilyl)propynone oxime 33b



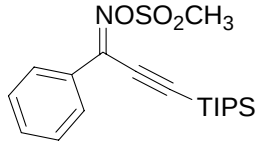
To a solution of **32b** (2.46 g, 7.77 mmol) in 15 mL of EtOH was added hydroxylamine hydrochloride (647 mg, 9.32 mmol) and sodium acetate (764 mg, 9.32 mmol). The mixture was stirred at room temperature under argon for 10 days. The reaction mixture was poured into water (40 mL), and the water layer was extracted by CH_2Cl_2 (3 × 20 mL). The residue upon drying and concentration of the organic layer was purified by flash chromatography on silica gel (0-20% CH_2Cl_2 in hexanes) to afford 2.01 g (79%) of oxime **33b** as a light yellow oil: R_f 0.50 (CH_2Cl_2); ^{1}H NMR (CD_2Cl_2) δ 0.96-1.35 (m, 21H), 3.85 (s, 3H), 6.93 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 8.80 (s, 1H); ^{13}C NMR (CD_2Cl_2) δ 11.58, 18.80, 55.74, 97.90, 107.23, 114.26, 126.14, 128.26, 142.00, 161.52; HRMS m/z 332.2036 (calcd 332.2045, $C_{19}H_{29}NO_2Si$).

6.2.7 1-(2-Methoxyphenyl)-3-(triisopropylsilyl)propynone oxime 33c



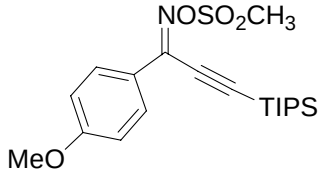
To a solution of **32c** (0.64 g, 2.23 mmol) in 8 mL of EtOH was added hydroxylamine hydrochloride (186 mg, 2.68 mmol) and sodium acetate (220 mg, 2.68 mmol). The mixture was stirred at room temperature under argon for 10 days. The reaction mixture was poured into water (20 mL), and the water layer was extracted by CH_2Cl_2 (3 × 15 mL). The residue upon drying and concentration of the organic layer was purified by flash chromatography (0-20% CH_2Cl_2 in hexanes) to afford 1.24 g (79%) of oxime **33c** as a white solid: R_f 0.40 (CH_2Cl_2); mp 120.2-121.0 °C; ^{1}H NMR (CD_2Cl_2) δ 1.10-1.22 (m, 21H), 3.89 (s, 3H), 6.97-7.08 (m, 2H), 7.39 (ddd, J = 8.0, 7.0, 2.0 Hz, 1H), 7.80 (dd, J = 8.0, 2.0 Hz, 1H); ^{13}C NMR (CD_2Cl_2) δ 11.57, 18.78, 55.71, 96.46, 105.56, 111.84, 120.77, 122.20, 131.01, 131.39, 140.42, 157.68; HRMS *m*/*z* 332.2058 (calcd 332.2045, $C_{19}H_{29}NO_2Si$).

6.2.8 1-Phenyl-3-(triisopropylsilyl)-propynone mesylate 34a



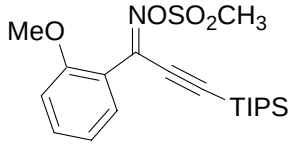
To a solution of oxime **33a** (2.7 g, 8.96 mmol) in 60 mL of dry CH_2Cl_2 was added dry pyridine (3.6 mL, 44.8 mmol). The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of methanesulfonyl chloride (1.28 g, 11.2 mmol) in 5 mL of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 3 days. The solvent volume was reduced by rotary evaporation, and the residue was poured into ice-water. The aqueous layer was extracted by CH_2Cl_2 three times. The residue upon drying and concentration of the organic layer was purified by flash chromatography (0-20% CH_2Cl_2 in hexanes) to afford 2.6 g (78%) of mesylate **34a** as a yellow oil: R_f 0.76 in CH_2Cl_2; ^{1}H NMR ($CDCl_3$) δ 1.04-1.28 (m, 21H), 3.25 (s, 3H), 7.39-7.52 (m, 3H), 7.94 (dd, J = 8.0, 1.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.01, 18.50, 36.71, 93.37, 111.53, 127.52, 128.68, 130.89, 131.79, 148.58; HRMS m/z 380.1718 (calcd 380.1715, $C_{19}H_{29}NOSSi$).

6.2.9 1-(4-Methoxyphenyl)-3-(triisopropylsilyl)-propynone mesylate 34b



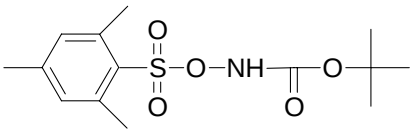
To a solution of oxime **33b** (0.77 g, 2.33 mmol) in 30 mL of dry CH_2Cl_2 was added dry pyridine (0.94 mL, 11.6 mmol). The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of methanesulfonyl chloride (0.33 g, 2.92 mmol) in 5 mL of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 3 days. The solvent volume was reduced by rotary evaporation, and the residue was poured into ice-water. The aqueous layer was extracted by CH_2Cl_2 three times. The residue upon drying and concentration of the organic layer was purified by flash chromatography on silica gel (0-30% CH_2Cl_2 in hexanes) to afford 450 mg (50%) of mesylate **34b** as a yellow solid: mp 85.8-86.9 °C; R_f 0.73 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.05-1.20 (m, 21H), 3.20 (s, 3H), 3.81 (s, 3H), 6.91 (d, J = 8.7 Hz, 2H), 7.87 (d, J = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 10.98, 18.46, 36.59, 55.35, 93.53, 110.61, 114.05, 123.27, 129.19, 148.20, 162.53; HRMS m/z 410.1828 (calcd 410.1821, $C_{20}H_{31}NO_4SSi$).

6.2.10 1-(2-Methoxyphenyl)-3-(triisopropylsilyl)-propynone mesylate 34c



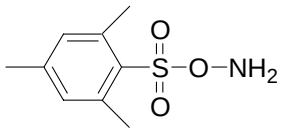
To a solution of oxime **33c** (2.7 g, 8.96 mmol) in 60 mL of dry CH_2Cl_2 was added dry pyridine (3.6 mL, 44.8 mmol). The reaction mixture was cooled to 0 °C, followed by dropwise addition of a solution of methanesulfonyl chloride (1.28 g, 11.2 mmol) in 5 mL of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 3 days. The solvent volume was reduced by rotary evaporation, and the residue was poured into ice-water. The aqueous layer was extracted by CH_2Cl_2 three times. The residue upon drying and concentration of the organic layer was purified by flash chromatography (0-15% CH_2Cl_2 in hexanes) to afford 90 mg of a yellow oil consisting of an inseparable mixture of mesylate **34c** along with Beckman rearrangement product 3-(triisopropylsilyl)-propynoic acid (2-methoxyphenyl)amide in 6 to 1 ratio, as judged by ^{1}H NMR. A pure sample of 3-(triisopropylsilyl)-propynoic acid (2-methoxyphenyl)amide was obtained as a yellow solid in 72% yield after chromatography from a reaction allowed to proceed for 6 days. Mesylate **34c**: R_f 0.65 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.01-1.19 (m, 21H), 3.18 (s, 3H), 3.83 (s, 3H), 6.93-7.05 (m, 2H), 7.43 (ddd, J = 9.3, 7.5, 1.8 Hz, 1H), 7.55 (dd, J = 7.5, 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 10.93, 18.38, 36.57, 55.55, 94.39, 110.12, 111.55, 120.45, 120.59, 130.64, 132.43, 147.87, 157.91; HRMS *m*/*z* 410.1825 (calcd 410.1821, $C_{20}H_{31}NO_4SSi$). 3-(Triisopropylsilyl)-propynoic acid (2-methoxyphenyl)amide: R_f 0.67 (CH_2Cl_2); mp 32.0-32.9 °C; ^{1}H NMR ($CDCl_3$) δ 1.00-1.19 (m, 21H), 3.88 (s, 3H), 6.82-6.98 (m, 2H), 7.00-7.08 (m, 1H), 8.05 (s, 1H), 8.29 (dd, J = 8.7, 2.0, 1H); ^{13}C NMR ($CDCl_3$) δ 10.98, 18.45, 55.76, 89.10, 100.31, 109.86, 120.21, 121.08, 124.32, 127.15, 147.43, 149.81; HRMS *m*/*z* 332.2042 (calcd 332.2045, $C_{19}H_{29}NO_2Si$).

6.2.11 *t*-Butyl N-mesitylenesulfonyl oxycarbamate 36



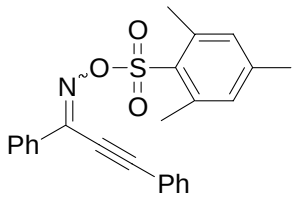
TEA (1.9 mL, 13.7 mmol) was added dropwise to a mixture of 2-mesitylenesulfonyl chloride (3.0 g, 13.7 mmol) and *t*-butyl *N*-hydroxycarbamate (1.8 g, 13.7 mmol) in 40 mL dry Et_2O at 0 °C. The resulting mixture was stirred at the same temperature for 55 min. Upon filtering off TEA hydrochloride salt, the organic layer was washed by water (20 mL x 2). The residue upon drying and concentration of the organic layer was purified by flash chromatography (10% EtOAc in hexanes) to afford 3.9 g (90%) of oxycarbamate **36** as a white solid: R_f 0.50 (hexanes/EtOAc, 1:9); m.p 100.2-102.1 °C, lit.[4] 102-104 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 9H), 2.29 (s, 3H), 2.64 (s, 6H), 6.96 (s, 2H), 7.80 (br, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 21.05, 23.07, 27.66, 83.74, 128.43, 131.59, 1414.88, 144.37, 154.27; HRMS *m/z* 316.1226 (calc'd 316.1218, $C_{14}H_{21}NO_5S$).

6.2.12 Mesitylenesulfonyl hydroxylamine 37



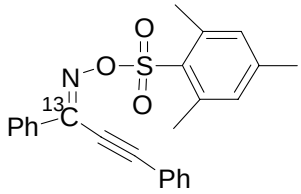
Trifluoroacetic acid (8 mL) was added to *t*-Butyl *N*-mesitylenesulfonyloxycarbamate (2.0 g) at 5 °C. The mixture was stirred at 5 °C for 5 - 10 min, and then poured into ice-water (80 mL). After filtration, the white solid was collected and dissolved in a minimum amount of Et_2O (2 mL). The white crystal was precipitated by the addition of petroleum ether (30-60 °C, 10 mL). The crystal was filtered to afford 0.99 g (73%) of compound **37** as white needles:[5,6] ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.61 (s, 6H), 5.53 (br, 2H. NH_2), 6.97 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 21.05, 22.86, 128.99, 131.67, 140.93, 143.79; HRMS *m/z* 216.0689 (calc'd 216.0694, $C_9H_{14}NO_3S$).

6.2.13 1,3-Diphenylpropynone mesitylate 38d



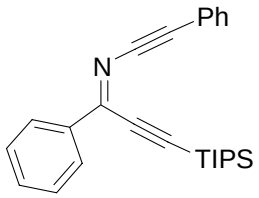
To a solution of compound **32d** (0.25 g, 1.21 mmol) in 6 mL dry CH_2Cl_2 was added compound **37** (0.48 g, 2.32 mmol). The reaction mixture was stirred at room temperature for 30 h, and poured into ice-water. The aqueous layer was extracted by CH_2Cl_2 (15 mL x 3). The residue upon drying and concentration of the organic layer was purified by flash chromatography (5% EtOAc in hexanes) to afford 0.34 g (70%) of mesitylate **38d** as a white solid: R_f 0.34 (10% EtOAc in hexanes); m.p 111.0-113.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.79 (s, 6H), 7.01 (s, 2H), 7.39-7.51 (m, 6H), 7.68 (dd, 2H, J = 7.8, 1.5 Hz), 7.87 (dd, 2H, J = 7.8, 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 20.99, 22.77, 77.79, 104.29, 120.57, 127.29, 128.51, 128.59, 130.32, 130.47, 131.29, 131.40, 131.61, 132.44, 140.70, 143.69, 147.59; HRMS *m/z* 404.1304 (calc'd 404.1320, $C_{24}H_{22}NO_3S$).

6.2.14 1,3-diphenyl-1-^{13}C-propynone mesitylate 38d



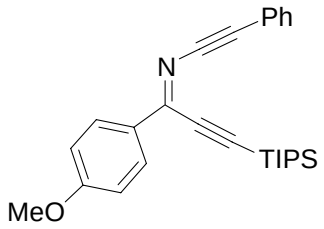
To a solution of 1,3-diphenyl-1-^{13}C-propynone **32d** (0.11 g, 0.53 mmol) in 4 mL dry CH_2Cl_2 was added mesitylenesulfonyl hydroxyamine **37** (0.30 g, 1.39 mmol). The reaction mixture was stirred at room temperature for 48 h, and poured into ice-water. The aqueous layer was extracted by CH_2Cl_2 (15 mL x 3). The residue upon drying and concentration of the organic layer was purified by flash chromatography (5% EtOAc in hexanes) to afford 0.15 g (70%) of mesitylate **38d** as a white solid: R_f 0.34 (10% EtOAc in hexanes); m.p 112.0-114.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H), 2.77 (s, 6H), 6.97 (s, 2H), 7.37-7.49 (m, 6H), 7.64 (dd, 2H, *J* = 7.8, 1.5 Hz), 7.82-7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.99, 22.77, 77.78 (d, *J* = 95.1 Hz), 104.28 (d, *J* = 11.5 Hz), 120.56 (d, *J* = 2.2 Hz), 127.29 (d, *J* = 2.2 Hz), 128.51 (d, *J* = 5.0 Hz), 128.59, 130.32, 130.47, 131.27 (d, *J* = 63.8 Hz) , 131.40, 131.61, 132.44, 140.70, 143.69, 147.59 (^{13}C); HRMS *m/z* 405.1354 (calc'd 405.1354, $^{12}C_{23}{}^{13}CH_{22}NO_3S$).

6.2.15 1,4-Diphenyl-6-(triisopropylsilyl)-3-aza-3-ene-1,5-diyne 26a



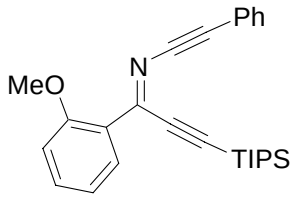
To a solution of phenylacetylene (410 mg, 4.02 mmol) in 5 mL of dry ether at -20 °C was added dropwise a solution of *n*-butyllithium (2.89 mL, 1.6 M in hexanes, 4.02 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (251 mg, 1.32 mmol) in 5 mL of dry ether was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesylate **38a** (500 mg, 1.32 mmol) in 5 mL of dry ether at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 2 h. The solvent was evaporated, and the product was purified by flash chromatography on silica gel (hexanes) to afford 110 mg (26%) of aza-enediyne **26a** as a yellow oil: R_f 0.56 (40% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.10-1.25 (m, 21H), 7.28-7.39 (m, 3H), 7.39-7.55 (m, 5H), 8.14 (dd, J = 7.6, 1.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.55, 18.79, 93.75, 100.27, 100.60, 106.79, 124.90, 128.26, 128.52, 128.66, 128.92, 131.77, 132.49, 136.22, 158.96; HRMS *m*/*z* 386.2310 (calcd 386.2304, $C_{26}H_{31}NSi$).

6.2.16 1-Phenyl-4-(4-methoxyphenyl)-6-(triisopropylsilyl)-3-aza-3-ene-1,5-diyne 26b



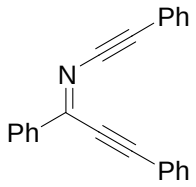
To a solution of phenylacetylene (78 mg, 0.76 mmol) in 2 mL of dry ether at -20 °C was added dropwise a solution of *n*-butyllithium (0.64 mL, 1.6 M in hexanes, 0.76 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (48 mg, 0.25 mmol) in 2 mL of dry ether was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesylate **38b** (100 mg, 0.24 mmol) in 2 mL of dry ether at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 1 h. The solvent was evaporated, and the product was purified by preparative TLC on silica gel (hexanes) to afford 12 mg (15%) of aza-enediyne **26b** as a yellow oil: R_f 0.35 (30% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.08-1.25 (m, 21H), 3.85 (s, 3H), 6.92 (d, J = 9.0 Hz, 2H), 7.25-7.34 (m, 3H), 7.44 (dd, J = 8.4, 1.5 Hz, 2H), 8.08 (d, J = 9.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.16, 18.65, 55.47, 93.60, 97.86, 100.00, 105.35, 113.94, 124.90, 127.82, 128.14, 128.99, 129.86, 131.38, 131.92, 163.00; HRMS *m*/*z* 416.2407 (calcd 416.2409, $C_{27}H_{33}NOSi$).

6.2.17 1-Phenyl-4-(2-methoxyphenyl)-6-(triisopropylsilyl)-3-aza-3-ene-1,5-diyne 26c



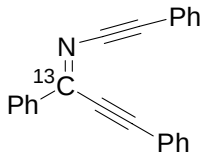
To a solution of phenylacetylene (410 mg, 4.02 mmol) in 5 mL of dry ether at -20 °C was added dropwise a solution of *n*-butyllithium (2.89 mL, 1.6 M in hexanes, 4.02 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (251 mg, 1.32 mmol) in 5 mL of dry ether was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesylate **38c** (500 mg, 1.32 mmol) in 5 mL of dry ether at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 2 h. The solvent was evaporated, and the product was purified by flash chromatography (0-20% CH_2Cl_2 in hexanes) to afford 22 mg (31%) of aza-enediyne **26c** as a yellow oil: R_f 0.45 (40% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.05-1.19 (m, 21H), 3.88 (s, 3H), 6.95-7.02 (m, 2H), 7.26-7.34 (m, 3H), 7.36-7.48 (m, 3H), 7.86 (d, J = 7.6, 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.19, 18.60, 55.70, 93.21, 100.66, 101.82, 105.05, 111.77, 120.44, 124.86, 125.99, 127.77, 128.11, 130.81, 131.27, 132.64, 157.66, 158.60; HRMS m/z 416.2404 (calcd 416.2409, $C_{27}H_{33}NOSi$).

6.2.18 1,4,6-triphenyl-3-aza-3-ene-1,5-diyne 26d



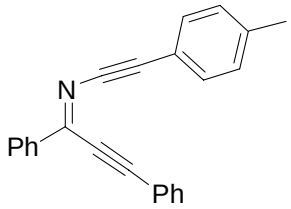
To a solution of phenyl acetylene (262 mg, 2.57 mmol) in 3 mL of dry Et_2O at -20 °C was added dropwise a solution of *n*-butyllithium (2.57 mL, 1.0 M in hexanes, 2.67 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (161 mg, 0.85 mmol) in 3 mL of dry Et_2O was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesitylate **38d** (280 mg, 0.51 mmol) in 3 mL of dry Et_2O at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 24 h. The solvent was evaporated, and the product was purified by flash chromatography on silica gel (hexanes) to afford 76 mg (50%) of aza-enediyne **26d** as a yellow oil: R_f 0.68 (50% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 7.31-7.58 (m, 11H), 7.70 (dd, 2H, J = 7.5, 1.5 Hz), 8.22 (dd, 2H, J = 7.5, 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 84.36, 93.94, 99.83, 102.07, 121.14, 124.70, 127.97, 128.24, 128.40, 128.55, 128.68, 130.33, 131.60, 132.09, 132.61, 135.82, 159.03; HRMS *m*/*z* 306.1272 (calcd 306.1282, $C_{23}H_{16}N$).

6.2.19 1,4, 6-triphenyl-4-^{13}C-3-aza-3-ene-1,5-diyne 26d



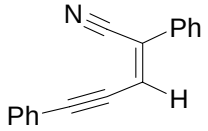
To a solution of phenyl acetylene (124 mg, 1.22 mmol) in 2 mL of dry Et_2O at -20 °C was added dropwise a solution of *n*-butyllithium (0.87 mL, 1.6 M in hexanes, 1.22 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (70 mg, 0.37 mmol) in 2 mL of dry Et_2O was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesitylate [1-^{13}C]-**38d** (140 mg, 0.34 mmol) in 2 mL of dry Et_2O at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 20 h. The solvent was evaporated, and the product was purified by flash chromatography on silica gel (hexanes) to afford 55 mg (53%) of aza-enediyne [4-^{13}C]-**26d** as a red-yellow oil: R_f 0.70 (50% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 7.29-7.57 (m, 11H), 7.69 (dd, 2H, J = 8.1, 1.8 Hz), 8.18-8.23 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 84.34 (d, J = 91.2 Hz), 93.94 (d, J = 6.6 Hz), 99.83 (d, J = 7.0 Hz), 102.06 (d, J = 10.4 Hz), 121.13 (d, J = 2.2 Hz), 124.70 (d, J = 2.2 Hz), 127.96 (d, J = 2.8 Hz), 128.23, 128.40, 128.54 (d, J = 5.0 Hz), 128.68, 130.32, 131.60 (d, J = 1.7 Hz), 132.07, 132.61, 135.79 (d, J = 67.6 Hz), 159.03 (^{13}C); HRMS *m*/*z* 307.1323 (calcd 307.1316, $^{12}C_{22}{}^{13}CH_{16}N$).

6.2.20 1-(Methylphenyl)-4,6-diphenyl-3-ene-3-aza-1,5-diyne 26g



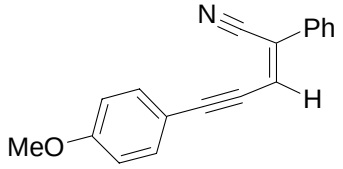
To a solution of 4-methylphenyl acetylene (152 mg, 1.30 mmol) in 3 mL of dry Et_2O at -20 °C was added dropwise a solution of *n*-butyllithium (1.04 mL, 1.6 M in hexanes, 1.30 mmol) such that the temperature was maintained at -20 °C. The reaction mixture was stirred for 20 min at the same temperature. In a separate flask, a suspension of cuperous iodide (82 mg, 0.43 mmol) in 3 mL of dry Et_2O was cooled to -40 °C. The phenylacetylide solution was added slowly via cannula to this suspension. After the addition was complete, the mixture was stirred at room temperature for 40 min. The resulting light yellow suspension was added to a solution of mesitylate **38d** (165 mg, 0.41 mmol) in 3 mL of dry Et_2O at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 20 h. The solvent was evaporated, and the product was purified by flash chromatography on silica gel (hexanes) to afford 67 mg (55%) of aza-enediyne **26g** as a yellow oil: R_f 0.70 (50% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 7.17 (d, 2H, J = 8.1 Hz), 7.42-7.4 (m, 8H), 7.69 (dd, 2H, J = 8.1, 1.5), 8.20 (dd, 2H, J = 8.1, 1.5); ^{13}C NMR ($CDCl_3$) δ 21.53, 84.40, 93.69, 100.42, 101.86, 121.22, 121.65, 127.88, 128.52, 128.66, 129.20, 130.25, 131.60, 131.94, 132.59, 135.89, 138.54, 158.44; HRMS *m*/*z* 320.1445 (calcd 320.1439, $C_{24}H_{18}N$).

6.2.21 (*Z*)-2,5-Diphenyl-pent-2-en-4-ynenitrile 28a



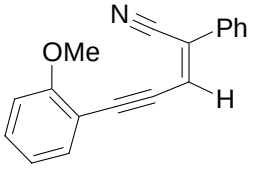
A solution of **26a** (68 mg, 0.176 mmol) in dry THF (6 mL) was cooled to -78 °C and then a 1 M solution of TBAF (0.194 mmol) was added slowly. After being stirred at -78 °C for 5-10 min the mixture was poured into ice-water (10 mL) and extracted by CH_2Cl_2 (2 × 15 mL). The dry organic solution was plugged through a small column before evaporation. The conversion of aza-enediyne **40a** to (*Z*)-nitrile **28a** was followed by ^{1}H NMR and/or UV-vis spectroscopy. After the conversion was complete, the (*Z*)-nitrile **28a** was purified by flash chromatography on silica gel (0-10% CH_2Cl_2 in hexanes) to afford 36 mg (89%) of nitrile **28a** as a yellow solid: R_f 0.41 (50% CH_2Cl_2 in hexanes); mp 79.3-80.7 °C; ^{1}H NMR (CD_2Cl_2) δ 6.92 (s, 1H), 7.38-7.50 (m, 6H), 7.55-7.67 (m, 4H); ^{1}H NMR (CD_3CN) δ 7.1 (s, 1H), 7.41-7.50 (m, 6H), 7.54-7.61 (m, 2H), 7.65-7.71 (m, 2H); ^{13}C NMR (CD_2Cl_2) δ 86.45, 102.23, 116.90, 121.87, 122.43, 124.09, 126.03, 128.99, 129.54, 130.15, 130.52, 132.48, 133.04; HRMS *m*/*z* 230.0972 (calcd 230.0969, $C_{17}H_{11}N$).

6.2.22 (*Z*)-5-(4-Methoxyphenyl)-2-phenyl-pent-2-en-4-ynenitrile 28b



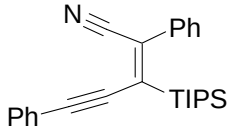
A solution of **26b** (9 mg, 0.022 mmol) in dry THF (2 mL) was cooled to -78 °C and then a 1 M solution of TBAF (0.024 mmol) was added slowly. After being stirred at -78 °C for 5-10 min the mixture was poured into ice-water (10 mL) and extracted by CH_2Cl_2 (2 × 10 mL). The dry organic solution was plugged through a small column before evaporation. The conversion of imine **40b** to nitrile **28b** was followed by ^{1}H NMR and/or UV-vis. The nitrile **28b** was purified by flash chromatography on silica gel (0-5% CH_2Cl_2 in hexanes) to afford 4 mg (72%) of (*Z*)-nitrile **28b** as a light yellow solid: R_f 0.35 (40% CH_2Cl_2 in hexanes); mp 85.5-86.6 °C; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 6.84 (s, 1H), 6.88 (d, J = 8.9 Hz, 2H), 7.36-7.44 (m, 3H), 7.52 (d, J = 8.9 Hz, 2H), 7.57-7.62 (dd, J = 7.7, 1.5 Hz, 2H); HRMS m/z 260.1070 (calcd 260.1075, $C_{18}H_{13}NO$).

6.2.23 (Z)-5-(2-Methoxyphenyl)-2-phenyl-pent-2-en-4-ynenitrile 28c



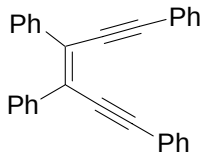
A solution of **26c** (68 mg, 0.176 mmol) in dry THF (6 mL) was cooled to -78 °C and then a 1 M solution of TBAF (0.194 mmol) was added slowly. After being stirred at -78 °C for 5-10 min the mixture was poured into ice-water (10 mL) and extracted by CH_2Cl_2 (2 × 15 mL). The dry organic solution was plugged through a small column before evaporation. The conversion of imine **40c** to nitrile **28c** was followed by ^{1}H NMR and/or UV-vis. The nitrile **28c** was purified by flash chromatography (0-10% CH_2Cl_2 in hexanes) to afford 10 mg (91%) of (*Z*)-nitrile **28c** as a light yellow solid: R_f 0.30 (40% CH_2Cl_2 in hexanes); mp 57.7-58.6 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 6.88-6.98 (m, 3H), 7.33-7.48 (m, 4H), 7.50-7.57 (dd, J = 7.8, 1.8 Hz, 1H), 7.58-7.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 55.87, 90.44, 99.41, 110.74, 111.21, 120.65, 120.82, 121.56, 122.95, 125.61, 129.14, 129.94, 131.41, 132.50, 134.27, 160.54; HRMS *m*/*z* 260.1077 (calcd 260.1075, $C_{18}H_{13}NO$).

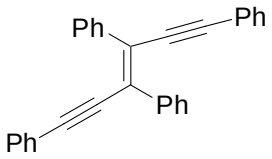
6.2.24 (*E*)-2,5-Diphenyl-3-(triisopropylsilyl)-pent-2-ene-4-ynenitrile 39

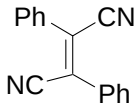


To a solution of the aza-enediyne **26a** (20 mg, 0.0518 mmol) in chlorobenzene (1.5 mL) was added 1,4-cyclohexadiene (83.19 mg, 1.036 mmol, 20 equiv). The mixture was heated at 150 °C for 3 days. The solvent was evaporated and the residue purified by chromatography to afford nitrile **39** (7 mg, 35%) as a yellow oil: R_f 0.51 (40% CH_2Cl_2 in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.10-1.22 (d, J = 7.5 Hz, 18H), 1.68-1.85 (p, J = 7.5 Hz, 3H), 7.24-7.34 (m, 5H), 7.37-7.48 (m, 3H), 7.84 (dd, J = 7.7, 1.9, 2H); ^{13}C NMR ($CDCl_3$) δ 12.27, 18.78, 91.31, 108.25, 120.38, 122.98, 128.12, 128.51, 128.70, 129.23, 129.42, 131.44, 134.10, 136.07, 139.69; HRMS *m*/*z* 386.2317 (calcd 386.2304, $C_{26}H_{31}NSi$).

6.2.25 (*E*) and (*Z*)-1,3,4,6-tetraphenyl-3-hexene-1,5-diynes 60d and (*E*)-1,2-dicyano-1,2-diphenylethylene 61



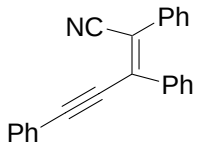




A benzene solution (2.5 mL) of compound **26d** (90 mg, 0.29 mmol) was stirred at 55°C for 5 days under argon. The residue upon concentration of the organic layer was purified by flash chromatography to afford 38 mg (70%) of (*Z*)-**60d** and (*E*)-**60d** mixtures (1:1 ratio judged by ^{1}H NMR) as a yellow solid and 15 mg (45%) of (*E*)-dicyano compound **61** as a white solid. Re-crystallization of the mixtures of (*Z*) and (*E*) tetraphenyl enediynes **60d** from hexanes/EtOAc afforded pure (*E*)-isomer (purity > 95% as judged by ^{1}H NMR) and (*Z*)-isomer rich fraction (containing 20% of (*E*)-isomer). (*E*)-**60d**: yellow prisms from hexanes/EtOAc; R_f 0.65 (10% EtOAc in hexanes); m.p. 155.0-157.0 °C, lit.[7] 156.2-157.2 °C; ^{1}H NMR ($CDCl_3$) δ 7.23-7.25 (m, 10H), 7.39 (t, 2H, J = 8.0 Hz), 7.45 (t, 4H, J = 8.0 Hz), 7.97 (dd, 4H, J = 8.0, 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ 90.90, 98.53, 123.21, 127.83, 128.28, 128.45, 128.60, 129.23, 131.40, 139.01; (*Z*)-**60d**: yellow needles from hexanes/EtOAc, after 3 times of re-crystallization, there is still at least 20% of (*E*)-**60d** in the sample; R_f 0.65 (10% EtOAc in hexanes); m.p. 96.0-99.0 ºC; ^{1}H NMR ($CDCl_3$) δ 7.18-7.21 (m, 6H), 7.26-7.32 (m, 10H), 7.52-7.55 (m, 4H); Mixture of (*E*)-**60d** and (*Z*)-**60d** (1:1): yellow solid; R_f 0.65 (10% EtOAc in hexanes); m.p. 95.0-99.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.19-7.23 (m, 6H), 7.26-7.40 (m, 20H), 7.39 (t, 2H, J = 8.0 Hz), 7.45 (t, 4H, J = 8.0 Hz), 7.53- 7.57 (m, 4H), 7.97 (dd, 4H, J = 8.0, 1.2 Hz); ^{13}C NMR δ 90.90, 91.77, 96.84, 98.53, 123.21, 123.37, 127.83, 128.04, 128.28, 128.36, 128.45, 128.52, 128.60, 129.23, 129.72, 131.40, 131.65, 137.45, 139.01; HRMS *m*/*z* (**60d**) 381.1636 (calcd 381.1643, $C_{30}H_{21}$); (*E*)-**61**: white needle from hexanes; R_f 0.40 (10% EtOAc in hexanes);

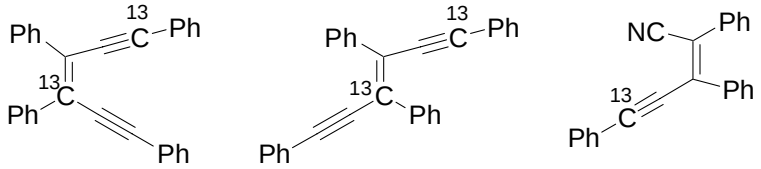
m.p. 161.5-162.5 °C, lit.[8] 161-162 °C; ^{1}H NMR ($CDCl_3$) δ 7.50-7.55 (m, 6H), 7.80-7.84 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 116.6, 125.57, 128.67, 129.27, 131.70, 131.95; HRMS *m*/*z* 231.0924 (calcd 231.0922, $C_{16}H_{11}N_2$).

6.2.26 2,3,5-Triphenyl-pent-2-en-4-ynenitrile 28d



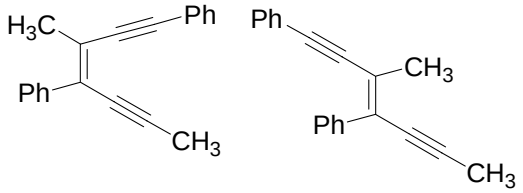
The solution of aza-enediyne **26d** (22 mg) in benzene (0.45 mM), kept in dark, was stirred at 90 °C for 24 hours. The solvent was evaporated, and the product was purified by flash chromatography on silica gel (1% EtOAc in hexanes) to afford 18.6 mg (85%) of nitrile **28d** as a light-yellow solid: R_f 0.50 (10% EtOAc in hexanes); mp: 132-134 ºC; ^{1}H NMR ($CDCl_3$) δ 7.22-7.40 (m, 13H), 7.62-7.66 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 89.47, 100.48, 118.24, 119.24, 121.91, 128.49, 128.60, 129.05, 129.42, 129.48, 129.65, 132.23, 132.87, 135.32, 137.34, 138.26; FT-IR: 2185 cm^{-1}; HRMS *m*/*z* 306.1284 (calcd 306.1283, $C_{23}H_{16}N$).

6.2.27 [1,4-$^{13}C_2$]-60d, and [5-^{13}C]-28d



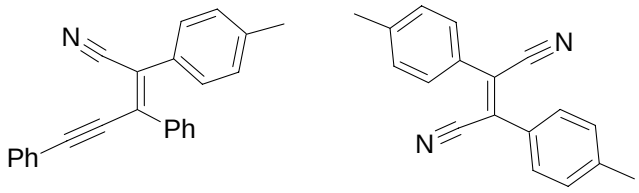
A benzene solution (1.1 mL) of compound **26d** (42 mg, 0.14 mmol) was stirred at 70 °C for 2 days under argon. The residue upon concentration of the organic layer was purified by flash chromatography to afford 16 mg (60%) of (*Z*), (*E*)-[1,4-$^{13}C_2$]-**60d** mixtures (1:3 ratio judged by ^{1}H NMR) as a yellow solid, 6 mg (35%) of (*E*)-dicyano compound **61** as a white solid and 10 mg (24%) of nitrile [5-^{13}C]-**28d** as yellow solid. (*E*)-[1,4-$^{13}C_2$]-**60d**: ^{13}C NMR ($CDCl_3$) δ 96.84 (^{13}C), 129.23 (^{13}C); (*Z*)-[1,4-$^{13}C_2$]-**60d**: ^{13}C NMR ($CDCl_3$) δ 98.56 (^{13}C), 128.63 (^{13}C); HRMS *m*/*z* 383.1708 (calcd 383.1710); [5-^{13}C]-**28d**: ^{13}C NMR ($CDCl_3$) δ 100.48 (^{13}C); HRMS *m*/*z* 307.1314 (calcd 307.1316, $^{12}C_{22}{}^{13}CH_{16}N$).

6.2.28 (*E*), (*Z*)-3,6-Dimethyl-1,4-diphenyl-3-hexene-1,5-diynes 60e



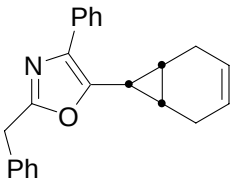
Flash chromatography of the samples of aza-enediyne **26e** (20 mg) stored in a -10 ºC freezer for 24 months affords 1.2 mg (14%) of (Z), (*E*)-**60e** (1:1 ratio as judged by ^{1}H NMR) as a yellow solid, 2.8 mg (35%) of (*E*)-1,2-dicyano-1,2-diphenylethylene **61** as a white solid. (*Z*), (*E*)-**60e**: R_f 0.60 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 2.30 (s, 3H), 7.23-7.36 (m, 16H), 7.49-7.51 (m, 2H), 7.77 (d, 2H, J = 7.6 Hz); HRMS *m*/*z* 257.1323 (calcd 257.1330, $C_{20}H_{17}$).

6.2.29 2-(Methylphenyl)-3,5-diphenyl-pent-2-en-4-ynenitrile 28g (*E*)-1,2-dicyano-1,2-di-(methylphenyl)ethylene 62



A benzene solution (1.4 mL) of compound **26g** (48 mg, 0.14 mmol) was stirred at 50 °C for 6 days under argon. The residue upon concentration of the organic layer was purified by flash chromatography to afford 12.5 mg (47%) of (*Z*), (*E*)-**60d** mixtures (1:1 ratio judged by ^{1}H NMR) as a yellow solid, 16.5 mg of the mixtures of (*E*)-dicyano compound **62** (26%, based on ^{1}H NMR) and nitrile **28d** (14%, based on ^{1}H NMR) as light yellow solid. The small amount of pure **62** was obtained by re-crystallization of the mixtures from hexanes and ethyl acetate. **62**: ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 7.31 (d, J = 7.5 Hz, 4H), 7.71 (d, J = 8.1 Hz, 4H); CIMS m/z 259 (MH^+).

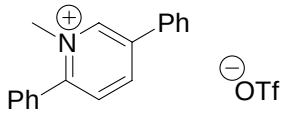
6.2.30 2-Benzyl-5-bicyclo[4.1.0]hept-3-en-7-yl-4-phenyl-oxazole 44



To a solution of 1,4-diphenyl-6-(triisopropylsilyl)-3-aza-3-ene-1,5-diyne **26a** (60 mg, 0.156 mmol) in 5 ml dry THF cooled to -78 ºC was added slowly 1M solution of TBAF (0.172 mmol). After stirred at -78 ºC for 5-10 min, the mixture was poured into ice-water (10 ml) and extracted by CH_2Cl_2 (2 x 15 ml). The dry organic solution was plugged through a small column before evaporation. The residue was further dried under vacuum for 10-15 min, and then re-dissolved in 4 ml dry CH_2Cl_2. To this red solution cooled to -78˚C was added 20 equiv freshly distilled 1,4-CHD, followed by 0.1 equiv trifluoromethane sulfonic acid. The mixture was stirred at -78˚C for 30 min, and then at -10 °C for 4 days. The oxazole derivative **44** and nitrile **28a** were purified by flash chromatography on silica gel (0-30% CH_2Cl_2 in hexanes) to afforded 15 mg oxazole **44** as white solid: R_f = 0.25 (50% CH_2Cl_2 in hexanes) and 12 mg nitrile **28a** as yellow solid. The product **44** was re-crystallized in hexanes and afforded colorless lathes and needles crystal: mp = 85.0-86.4 °C. Oxazole **44**: ^{1}H NMR ($CDCl_3$) δ 7.88 (dd, J = 8.5, 1.4 Hz, 2H), 7.37 (t, J = 8.1 Hz, 2H), 7.29 (dd, J = 7.1, 1.0 Hz, 2H), 7.28-7.15 (m, 4H), 4.89 (s(br), 2H), 4.04 (s, 2H), 2.22 (d(br), J = 19.8 Hz 2H), 2.18-2.00 (m, 3H), 1.60 (dd, J = 7.7, 4.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 13.77, 15.79, 20.78, 34.85, 123.15, 126.00, 126.83, 126.84, 128.39, 128.45, 128.86, 132.77, 135.67, 136.01, 145.72, 160.45; HRMS m/z 328.1711 (Calc. 328.1702, $C_{23}H_{21}NO$). The data crystal was cut from a long lathe and had approximate dimension: 0.27×0.17×0.04 mm. The data were collected on a Nonius Kappa CCD diffractimeter using a graphite monochromator with MoKα radiation (λ =

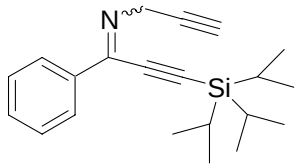
0.71073 Å). A total of 400 frames of data were collected using ω-scans with a scan range of 1 º and a counting time of 136 seconds per frame. The data were collected at 153 K using an Oxford Cryostream low temperature device. Crystallographic data for compound **44** has been deposited with the Cambridge Crystallographic Data Center as CCDC 204592 and can be obtained free of charge from CCDC at deposit@ccdc.cam.ac.uk.

6.2.31 2,5-Diphenylpyridinium triflate salt 43



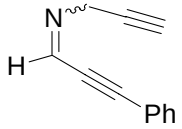
To a solution of 2,5-diphenylpyridine **42** (8 mg, 0.04 mmol) in 1 mL CH_2Cl_2 at -40 º was added methyl triflate (6.5 mg, 0.05 mmol) in 1 mL ether solution via cannula. The reaction mixture was stirred at -20 ºC for 1 h, and then at 0 ºC for 12 h. The triflate salt **43** (10 mg, 75%), precipitated from ether as light yellow solid, was collected by filtering off solvent. ^{1}H NMR ($CDCl_3$) δ 4.25 (s, 3H), 7.40-7.85 (m, 11H), 8.51 (d, J = 1.8 Hz, 1H), 9.17 (s, 1H).

6.2.32 1-Triisopropylsilyl-3-phenyl-3-ene-4-aza-1,6-diyne 53e



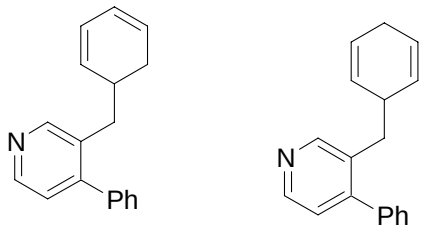
Propargylamine (200.0 mg, 3.49 mmol) was added dropwise to a mixture of 1-phenyl-3-(triisopropylsilyl)-propynone (500.0 mg, 1.75 mmol) and titanium (IV) ethoxide (600.0 mg, 2.62 mmol) in 10 mL dry THF. The resulting yellow mixture was heated to reflux under argon for 1.5 h and then cooled to room temperature. The mixture was poured into water (30 mL), and the water layer was extracted by CH_2Cl_2 (2 x 20 mL). The residue upon drying and concentration of the organic layer was purified by flash chromatography (0-2% ethyl acetate in hexanes) to afford 290.0 mg (52%) of compound **53e** as a yellow oil: R_f 0.50 (ethyl acetate : hexanes 1:9); ^{1}H NMR ($CDCl_3$) δ 1.12-1.21 (m, 21H), 2.28 (t, 1H, J = 2.8 Hz), 4.58 (d, 2H, J = 2.8 Hz), 7.36-7.44 (m, 3H), 8.07 (dd, 2H, J = 8.0, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 11.09, 18.61, 45.07, 70.87, 81.26, 97.15, 105.18, 127.69, 128.24, 130.92, 136.59, 153.72; HRMS *m/z* 324.2153 (calcd 324.2147, $C_{21}H_{29}NSi$).

6.2.33 3-Phenyl-prop-2-ynylidene)-prop-2-ynyl-amine 53f



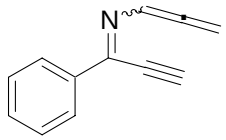
Propargylamine (169.0 mg, 3.08 mmol) was added dropwise to a mixture of phenyl propynal (200.0 mg, 1.54 mmol) and titanium (IV) ethoxide (600.0 mg, 2.62 mmol) in 3 mL dry THF. The resulting yellow mixture was stirred at room temperature for 30 min. The mixture was poured into water (30 mL), and the water layer was extracted by CH_2Cl_2 (2 x 20 mL). The residue upon drying and concentration of the organic layer was purified by re-crystallization (in hexanes) to afford 155.0 mg (60%) of compound **53f** as a white cube crystal (two isomers, 1:3 ratio as judged by ^{1}H NMR): R_f 0.40 (ethyl acetate : hexanes 1:9); ^{1}H NMR (CD_2Cl_2) δ 2.34 (t, J = 2.4 Hz, 0.3H), 2.63 (t, J = 2.4 Hz, 0.7H), 4.45-4.51 (m, 2H), 7.36-7.44 (m, 3H), 7.52-7.58 (m, 2H), 7.76 (t, J = 3.0 Hz, 0.3H), 8.08 (t, J = 1.8 Hz, 0.7H); ^{13}C NMR (CD_2Cl_2) δ 44.95, 48.04, 71.22, 76.84, 78.15, 81.21, 81.50, 86.78, 92.88, 99.53, 121.11, 121.71, 128.86, 128.96, 130.01, 130.49, 132.52, 132.54, 145.67, 146.87; HRMS *m/z* 168.0807 (calcd 168.0807, $C_{12}H_{10}N$).

6.2.34 3-Cyclohexa-2,4-dienylmethyl-4-phenylpyridine 59a and 3-Cyclohexa-2,5-dienylmethyl-4-phenylpyridine 59b



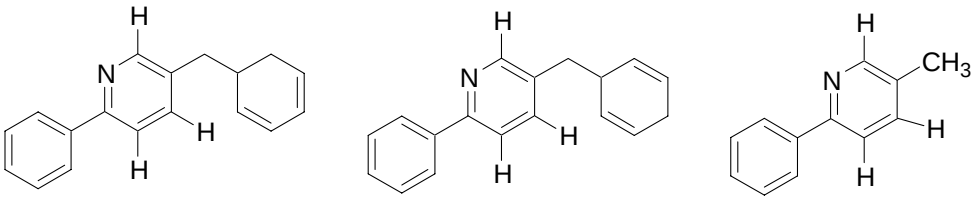
To a solution of **53f** (190 mg, 1.14 mmol) in 2 mL THF and 2 mL 1,4-CHD was added Et_3N (580 mg, 5.69 mmol) at room temperature. The reaction mixture was then stirred at 45 ºC for 40 h. The compounds **59a,b** were purified by flash chromatography (0-10% CH_2Cl_2 in hexanes) to afford 47 mg (27%) of **59a,b** as a light yellow wax product: R_f 0.25 (25% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$) (**59a/59b**) δ 1.95-2.08 and 2.09-2.22 (two m, 1H), 2.35-2.40 (m, 0.5H), 2.58-2.65 (m, 1H), 3.05-3.18 (m, 0.5H), 3.55-3.68 (m, 0.5H) 3.80-3.96 (m, 1H), 5.45-5.55 (m, 2H), 5.68-5.5.78 (m, 2H), 5.88-5.95 (m, 0.5H), 7.12-7.7.32 (m, 5H), 7.52-7.60 (m, 1H), 8.42 (t, 1H, J = 4.8 Hz), 8.55 (brs, 1H) HRMS m/z 248.1437 (calcd 248.1439, $C_{18}H_{18}N$).

6.2.35 (1-Phenyl-prop-2-ynylidene)-propa-1,2-dienyl-amine 4d



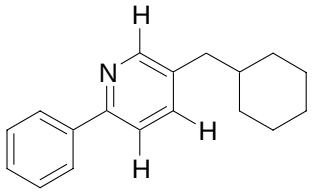
A 1 M solution of TBAF (1.46 mmol) was slowly added to a solution of the compound **53e** (130.0 mg, 0.40 mmol) in dry THF (3 mL) cooled to -78 °C. After being stirred at -78 °C for 5 min, the mixture was poured into ice-water (10 mL) and extracted by CH_2Cl_2 (2 x 15 mL). The organic layer upon drying, concentration, and removal of trace of solvent under vacuum (0.05-0.1 mmHg/5min) afforded aza-enyne allene **4d** as a yellow oil. R_f 0.45 (ethyl acetate : hexanes 1:9); ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 1H), 5.36 (d, 2H, J = 6.0 Hz), 7.38-7.41 (m, 3H), 7.60 (t, 1H, J = 6.0 Hz), 8.07 (dd, 2H, J = 7.6, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 68.17, 81.06, 89.72, 91.43, 110.11, 127.99, 128.54, 131.12, 136.72, 147.10, 213.72. CIMS m/z 168 (MH^+).

6.2.36 5-Cyclohexa-2,4-dienylmethyl-2-phenylpyridine 56a, 5-cyclohexa-2,5-dienylmythyl-2-phenylpyridine 56b, and 6-phenyl-3-picoline 55



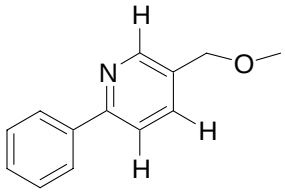
Compound **4d** (0.40 mmol) prepared as above was placed in a round bottle flask that was then cooled to 0 °C. To this was added freshly distilled 1,4-chd (2 mL). The reaction mixture was then kept at 4 °C for 8 h. The residue upon concentration was purified by flash chromatography (0-2% ethyl acetate in hexanes) to afford 16.5 mg (17%) of 1:1 ratio mixture of **56a** and **56b** as a yellow oil and 13.0 mg (20%) of compound **55** as a light yellow solid: R_f (**56a/56b**) 0.40 (ethyl acetate : hexanes 1:9); ^{1}H NMR ($CDCl_3$) (**56a/56b**) δ 1.98-2.08 and 2.20-2.31 (two m, 1H), 2.50-2.60 (m, 0.5H), 2.60-2.71 (m, 1H), 2.72 (d, 2H, J = 6.9 Hz), 2.97-3.10 (m, 0.5H), 5.58-5.78 (m, 3H), 5.88-5.94 (m, 1H), 7.37-7.48 (m, 3H), 7.52-7.58 (m, 1H), 7.63 (dd, 1H, J = 8.4, 1.8 Hz), 7.96 (d, 2H, J = 7.2 Hz), 8.49 (brs, 1H); CI-MS *m/z* (**56a/56b**) 248; R_f (**55**) 0.36 (ethyl acetate : hexanes, 1:9); mp (**55**) 52-54 °C, Lit:[1] mp 56 °C; ^{1}H NMR ($CDCl_3$) (**55**) δ 2.35 (s, 3H), 7.35-7.40 (m, 1H), 7.41-7.47 (m, 2H), 7.54 (dd, 1H, J = 7.6, 2.0 Hz), 7.60 (d, 1H, J = 7.6 Hz), 7.93 (dd, 2H, J = 8.4, 1.6 Hz), 8.50 (brs, 1H); ^{13}C NMR ($CDCl_3$) (6b) δ 17.67, 120.09, 126.68, 128.58, 128.68, 131.61, 137.34, 139.36, 150.02, 154.80; CI-MS *m/z* (**55**) 170.

6.2.37 5-Cyclohexylmethyl-2-phenylpyridine 57



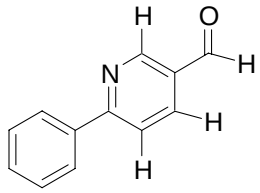
A 5 mL empty round bottle flask was charged with 10% palladium on activated carbon (10.0 mg, 0.0063 mmol); the flask was purged and then filled with hydrogen gas (this process was repeated three times). To this flask was added **56a/56b** (15.0 mg, 0.063 mmol) in degassed ethyl acetate (1 mL). The reaction mixture was stirred under an atmosphere of H_2 at room temperature for 2 h. The reaction mixture was filtered, concentrated, and purified by flash chromatography (0.5% ethyl acetate in hexanes) to afford 13.0 mg (87%) of compound **57** as a white solid: mp 60-62 °C; R_f 0.47 (ethyl acetate : hexanes, 1:9); ^{1}H NMR ($CDCl_3$) δ 0.90-1.02 (m, 2H), 1.14-1.1.24 (m, 4H), 1.48-1.58 (m, 1H), 1.60-1.74 (m, 4H), 2.50 (d, 2H, J = 7.2 Hz), 7.37 (t, 1H, J = 7.2 Hz), 7.40-7.48 (m, 2H), 7.51 (dd, 1H, J = 8.0, 2.0 Hz), 7.63 (d, 1H, J = 8.0 Hz), 7.96 (dd, 2H, J = 8.8, 1.6 Hz), 8.46 (d, 1H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 26.19, 26.42, 32.96, 39.53, 40.69, 119.98, 126.68, 128.62, 128.69, 134.99, 137.42, 139.29, 150.23, 154.89; HRMS *m/z* 252.1747 (calcd 252.1752, $C_{18}H_{21}N$).

6.2.38 5-Methoxymethyl-2-phenylpyridine 58



Compound **4d** (0.17 mmol) prepared as above was placed in a round bottle flask that was then cooled to 0 °C. To this was added freshly distilled methanol (2 mL). The reaction mixture was then kept at 0 °C for 14 h. The residue upon concentration was purified by flash chromatography (0-10% ethyl acetate in hexanes) to afford 6.7 mg (20%) of compound **58** as a yellow oil: R_f 0.20 (ethyl acetate : hexanes, 1:9); ^{1}H NMR ($CDCl_3$) δ 3.41 (s, 3H), 4.50 (s, 2H), 7.38-7.49 (m, 3H), 7.71-7.74 (m, 2H), 7.94-7.99 (m, 2H), 8.62 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 58.32, 71.97, 120.32, 126.91, 128.75, 128.98, 131.94, 136.41, 139.09, 149.05, 156.97; HRMS *m/z* 200.1074 (calcd 200.1075, $C_{13}H_{13}NO$).

6.2.39 6-phenylpyridine-3-carbaldehyde 54



Compound **4d** (0.31 mmol) prepared above was dissolved in 1 mL of $CDCl_3$. The solution was stored at 10 °C in a fridge for 12 h. The residue upon concentration was purified by flash chromatography (10% ethyl acetate in hexanes) to afford 1.0 mg (1.8%) of compound **54** as a yellow wax product:[2] R_f 0.12 (ethyl acetate : hexanes, 1:9); ^{1}H NMR ($CDCl_3$) δ 7.45-7.52 (m, 3H), 7.90 (d, 1H, J = 8.4 Hz), 8.03-8,08 (m, 2H), 8.22 (dd, 1H, J = 8.4, 2.0 Hz), 9.11 (dd, 1H, J = 2.0, 0.4 Hz), 10.13 (s, 1H); HRMS *m/z* 184.0765 (calcd 184.0762, $C_{12}H_9NO$).

6.3 SPECTROSCOPIC (UV-VIS) KINETICS (40A-C)

To a solution of aza-enediyne **26a-c** (0.0052 mmol) in dry THF (2 mL) cooled to -78 ºC was added slowly a 1 M solution of tetrabutylammonium floride in dry THF (0.0059 mmol). The reaction mixture was allowed to stir at -78 ºC for 5-10 min, and the mixture was poured into ice-water (10 mL) and extracted by CH_2Cl_2 (2 × 15 mL). The dry organic solution was chromatographed through a small plug of silica gel to remove tetrabutylammonium species before evaporation. The residue was stored at -80 ºC and quickly redissolved in $CHCl_3$ or other solvents (THF, *i*PrOH, chlorobenzene, hexanes, CH_3CN) and the resulting solution transferred to a thermostated cuvette at 20, 25, 30, 37, or 45 ºC. The conversion of aza-enediyne **40a-c** to (*Z*)-nitrile **28a-c** was followed spectrophotometrically by periodically acquiring UV absorbance spectra (250-500 nm) over a period of at least 2 half-lives. The first-order rate constants were obtained by fitting plots of the normalized absorbance at 390 nm (**40a**) or 415 nm (**40b/40c**) versus time to an exponential decay.

6.4 SPECTROSCOPIC (^{1}H NMR AND ^{13}C NMR) KINETICS (26D)

A NMR tube containing the degassed (freeze-pump-thaw) solution of aza-enediyne **26d** (120 mM) and 2,5-dimethylfuran (1.5 equiv, internal standard) in d_6-benzene was sealed under flame. ^{1}H NMR of the sample was taken as initial spectrum (reaction time equals zero second). After ^{1}H NMR, the tube was kept at 70 °C. The conversion of aza-enediyne to enediyne and nitrile was followed spectroscopically by taking ^{1}H NMR spectra 15 times over a period of time. Intervals varied from 35 minutes to 5 hours. Every time, the ^{1}H NMR tube was taken out of a 70 °C oil bath, and immediately inserted into a baker containing ice-water to stop the reaction. Reaction time was measured as the time during which the tube maintained at 70 °C. Aza-enediyne **26d** has a doublet resonance at 7.45 ppm which is well resolved from other peaks. The disappearance of the aza-enediyne **26d** was monitored as the disappearance of the resonance for **26d** at 7.45 ppm. The integrals of the resonance for **26d** at 7.45 ppm were normalized against the resonance for the internal standard 2,5-dimethylfuran at 5.76 ppm. The relative concentrations of aza-enediyne **26d** were calculated by normalizing the integrals of the resonance for **26d** at 7.45 ppm obtained at different intervals against the integral of the resonance for **26d** at 7.45 ppm obtained at reaction time equals zero second. The kinetic data was obtained by fitting plots of the relative concentrations of aza-enediyne **26d** versus time.TableCurve 2D® from SYSTAT Software Inc. was used to analyze the kinetic data. Kinetic data fits independent first-order and second-order kinetics with an equation: $\frac{d[A]}{dt} = -k_1[A] - k_2[A]^2$, [A]: concentration of aza-enediyne **26d**; k_1: first-order constant; k_2: second-order constant.

The conversion of aza-enediyne [4-^{13}C]-**26d** to enediyne [1,4-$^{13}C_2$]-**60d** and nitrile [5-^{13}C]-**28d** can be followed by ^{13}C NMR by a procedure analogous to that described for ^{1}H NMR kinetics. The resonance for [4-^{13}C]-**26d** at 159 ppm (^{13}C)

diminished over time and was replaced by the resonance for [1,4-$^{13}C_2$]-**60d** at 97 ppm (^{13}C) and the resonance for [5-^{13}C]-**28d** at100 ppm (^{13}C). The integrals of the resonance for [4-^{13}C]-**26d** at 159 ppm were normalized against the resonance for solvent (benzene, THF or DMSO). The relative concentrations of aza-enediyne [4-^{13}C]-**26d** were calculated by normalizing the integrals of the resonance for [4-^{13}C]-**26d** at 159 ppm obtained at different intervals against the integral of the resonance for [4-^{13}C]-**26d** at 159 ppm obtained at reaction time equals zero second. The kinetic data was obtained by fitting plots of the relative concentration versus time. TableCurve 2D® from SYSTAT Software Inc. was used to analyze the kinetic data. Kinetic data fits independent first-order and second-order kinetics with an equation: $\frac{d[A]}{dt} = -k_1[A] - k_2[A]^2$, [A]: concentration of aza-enediyne **26d**; k_1: first-order constant; k_2: second-order constant.

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Appendices

APPENDIX A ENERGIES CALCULATED AT THE (U)B3LYP/6-31* LEVEL OF THEORY

Table 1 Energies calculated on Myers-Saito and Schmittel cyclizations of **4a** at the (U)B3LYP/6-31* level

	Electronic energy[a]	ZPE[b]	Relative energy[c]
(*Z*)-Enyne allene (S-*cis*) **4a**	-270.20169	62.2	4.0
(*Z*)-Enyne allene (S-*trans*) **4a**	-270.20832	62.3	0
(*E*)-Enyne allene (S-*cis*) **4a**	-270.20436	62.1	-2.2
(*E*)-Enyne allene (S-*trans*) **4a**	-270.20816	62.1	-0.1
α,3-Didehydrotoluene triplet diradical **5a**	-270.22722	64.0	-10.2
α,3-Didehydrotoluene singlet diradical **5a**	-270.22906	63.7	-11.6
Zwitterion[d] $\mathbf{5a}^{\pm}$	-270.17919	65.0	20.9
Cyclic allene **24a**	-270.21139	64.4	0.1
Myers-Saito transition state[e] **TS1a**	-270.16603	62.1	26.3
Schmittel triplet diradical **52a**, anti-addition	-270.19203	62.6	10.5
Schmittel singlet diradical **52a**, anti-addition	-270.18754	62.4	13.1
Schmittel transition state[e] **TS2a**, anti-addition	-270.1521095	61.5	34.5

[a]Absolute energies in Hartrees; [b]ZPE in kcal/mol; [c]Energies relative to (*Z*)-Enyne allene (S-*trans*) **4a** in kcal/mol; [d]1 imaginary frequency at the HF/6-31G* level; [e]1 imaginary frequency at the B3LYP/6-31G* level.

Table 2 Energies calculated on aza-Myers-Saito and aza-Schmittel cyclizations of **4b** at the (U)B3LYP/6-31G* level

	Electronic energy[a]	ZPE[b]	Relative energy[c]
(*Z*)-Aza-enyne allene (S-*cis*) **4b**	-286.23233	54.7	3.5
(*Z*)-Aza-enyne allene (S-*trans*) **4b**	-286.23795	54.8	0
(*E*)-Aza-enyne allene (S-*cis*) **4b**	-286.23454	54.7	-2.0
(*E*)-Aza-enyne allene (S-*trans*) **4b**	-286.23766	54.5	-0.1
α,5-Didehydro-3-picoline triplet diradical **5b**	-286.26314	56.6	-14.0
α,5-Didehydro-3-picoline singlet diradical **5b**	-286.26503	56.4	-15.4
Aza-zwitterion[d] **5b**$^{\pm}$	-286.21523	57.0	16.5
Aza-cyclic allene **24b**	-286.24552	56.9	-2.6
(*Z*)-Aza-skipped enediyne **53b**	-286.21899	54.9	12.0
(*E*)-Aza-skipped enediyne **53b**	-286.22186	54.7	10.1
Aza-Myers transition state[d] **TS1b**	-286.20081	54.6	23.2
Aza-Schmittel singlet diradical **52b**, syn-addition	-286.22656	54.8	7.2
Aza-Schmittel triplet diradical **52b**, syn-addition	-286.23075	55.0	4.8
Aza-Schmittel transition state[d] **TS2b**, syn-addition,	-286.18804	53.8	30.4
Aza-Schmittel singlet diradical **52b**, anti-addition	-286.22630	54.8	7.3
Aza-Schmittel triplet diradical **52b**, anti-addition	-286.23001	54.9	5.2
Aza-Schmittel transition state[d] **TS2b**, anti-addition	-286.19269	54.1	27.7

[a]Absolute energies in Hartrees; [b]ZPE in kcal/mol; [c]Energies relative to (*Z*)-Aza-enyne allene (S-*trans*) **4b** in kcal/mol; [d]1 imaginary frequency at the B3LYP/6-31G* level.

APPENDIX B SPECTROSCOPIC KINETICS FOR AZA-ENEDIYNE 26D

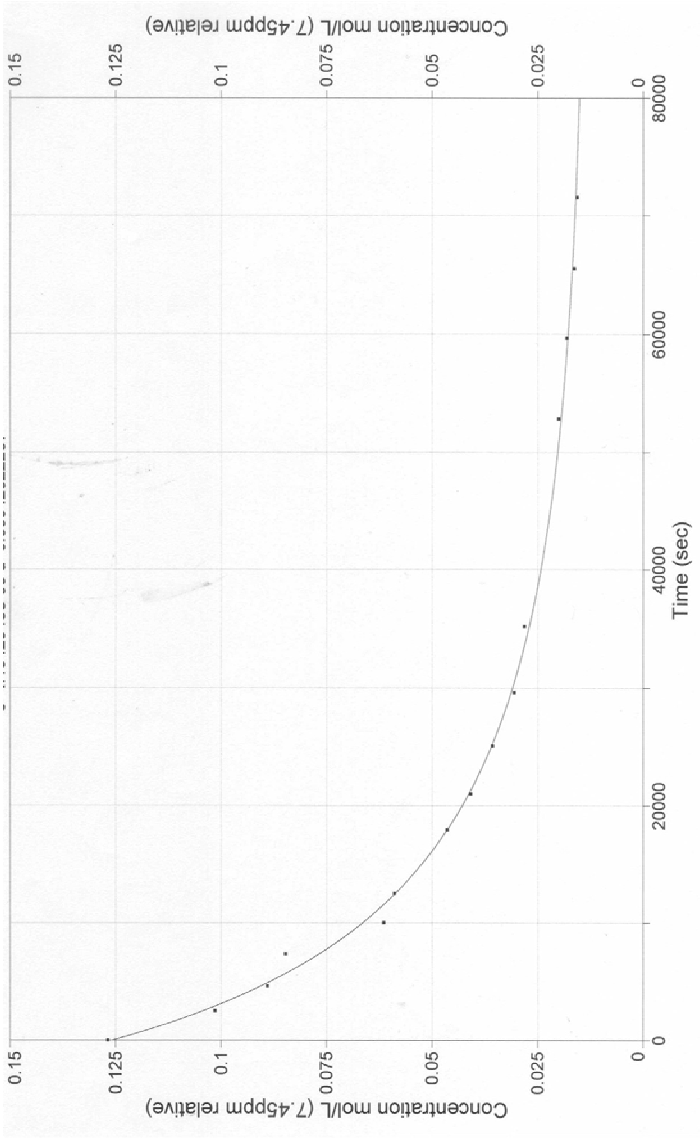


Figure 1 Kinetic plot of the transformation of aza-enediyne **26d** to enediyne **60d** and the conversion of **26d** to nitrile **61** followed by ^{1}H NMR in d_6-benzene. The data fit independent first-order and second-order kinetics simultaneously by using TableCurve 2D® from SYSTAT Software Inc. The x-axis represents reaction time in seconds; the y-axis represents the concentrations of **26d** in mol/L calculated from the ratio of the integals of resonance at 7.45 ppm from **26d** to the one at 5.76 ppm from internal standard 2,5-dimethyl furan (see section 6.4 for details). The first-order rate constant $k_1 = 4.15 \times 10^{-5}$ s^{-1}, the second-order rate constant $k_2 = 4.29 \times 10^{-4}$ $s^{-1}mol^{-1}L$.

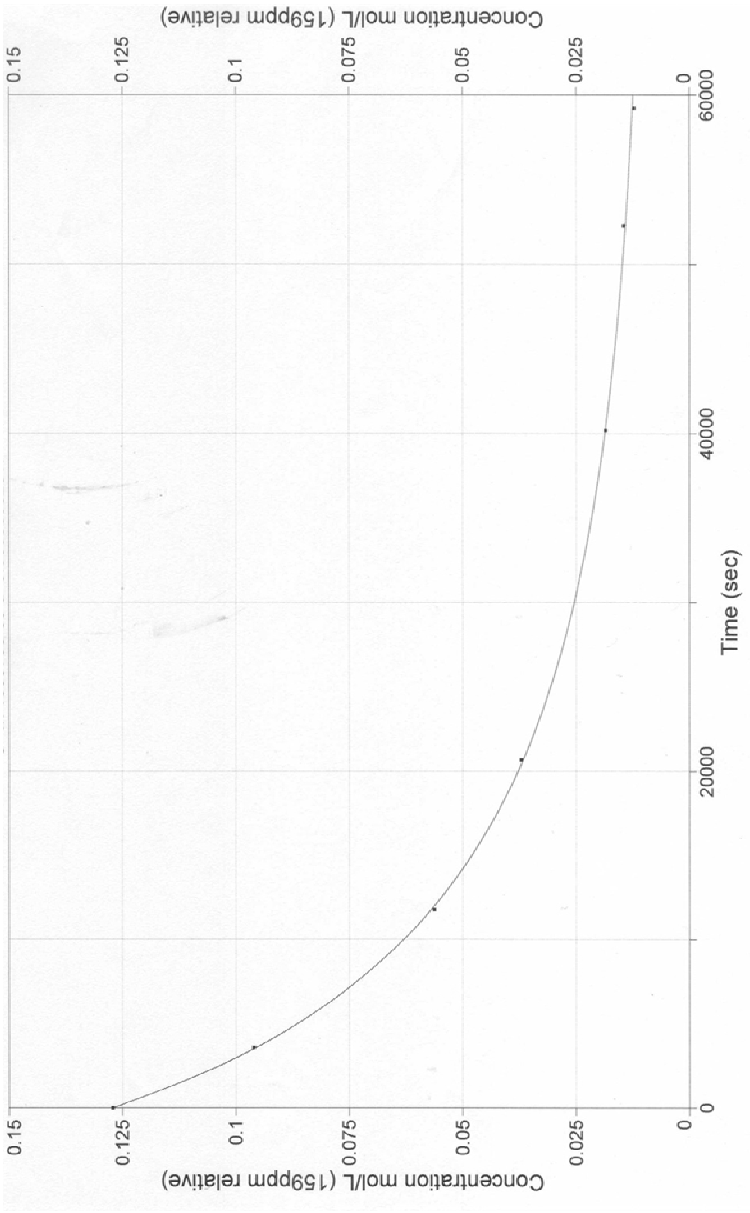


Figure 2 Kinetic plot of the transformation of aza-enediyne [4-^{13}C]-**26d** to enediyne [1,4-$^{13}C_2$]-**60d** and the conversion of [4-^{13}C]-**26d** to nitrile **61** followed by ^{13}H NMR in d_6-benzene. The data fit independent first-order and second-order kinetics simultaneously by using TableCurve 2D® from SYSTAT Software Inc. The x-axis represents reaction time in seconds; the y-axis represents the concentrations of [4-^{13}C]-**26d** in mol/L calculated from the ratio of the integals of resonance at 159 ppm from [4-^{13}C]-**26d** to the one from d_6-benzene (see section 6.4 for details). The first-order rate constant $k_1 = 4.71 \times 10^{-5}$ s^{-1}, the second-order rate constant $k_2 = 3.83 \times 10^{-4}$ $s^{-1}mol^{-1}L$.

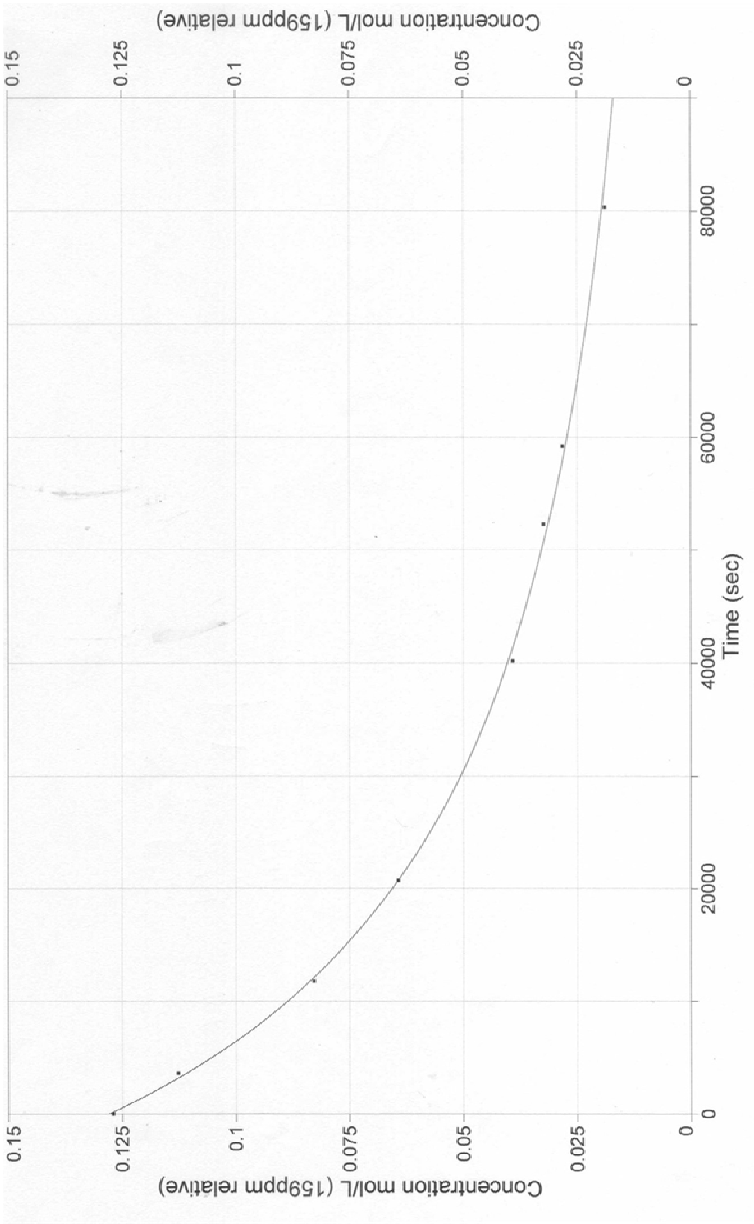


Figure 3 Kinetic plot of the transformation of aza-enediyne [4-^{13}C]-**26d** to enediyne [1,4-$^{13}C_2$]-**60d** and the conversion of [4-^{13}C]-**26d** to nitrile **61** followed by ^{13}H NMR in d_8-THF. The data fit independent first-order and second-order kinetics simultaneously by using TableCurve 2D® from SYSTAT Software Inc. The x-axis represents reaction time in seconds; the y-axis represents the concentrations of [4-^{13}C]-**26d** in mol/L calculated from the ratio of the integals of resonance at 159 ppm from [4-^{13}C]-**26d** to the one from d_8-THF (see section 6.4 for details). The first-order rate constant $k_1 = 1.79 \times 10^{-5}$ s^{-1}, the second-order rate constant $k_2 = 2.04 \times 10^{-4}$ $s^{-1}mol^{-1}L$.

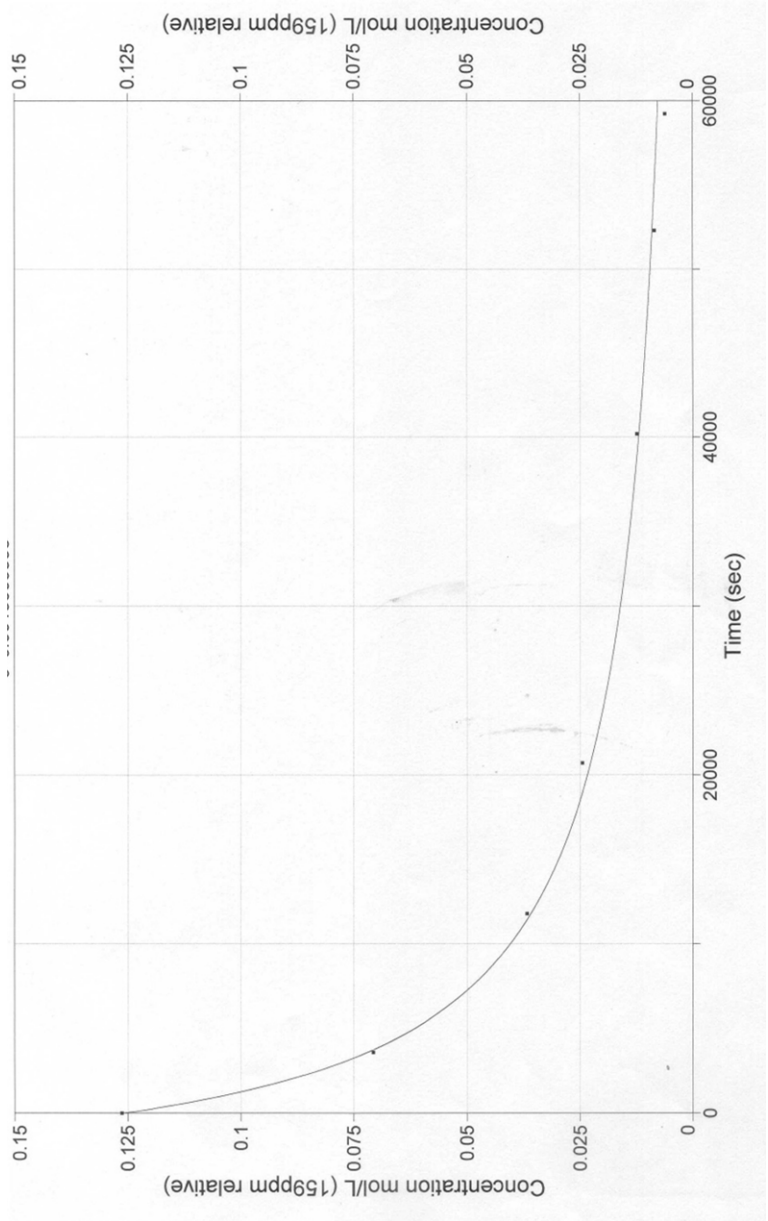
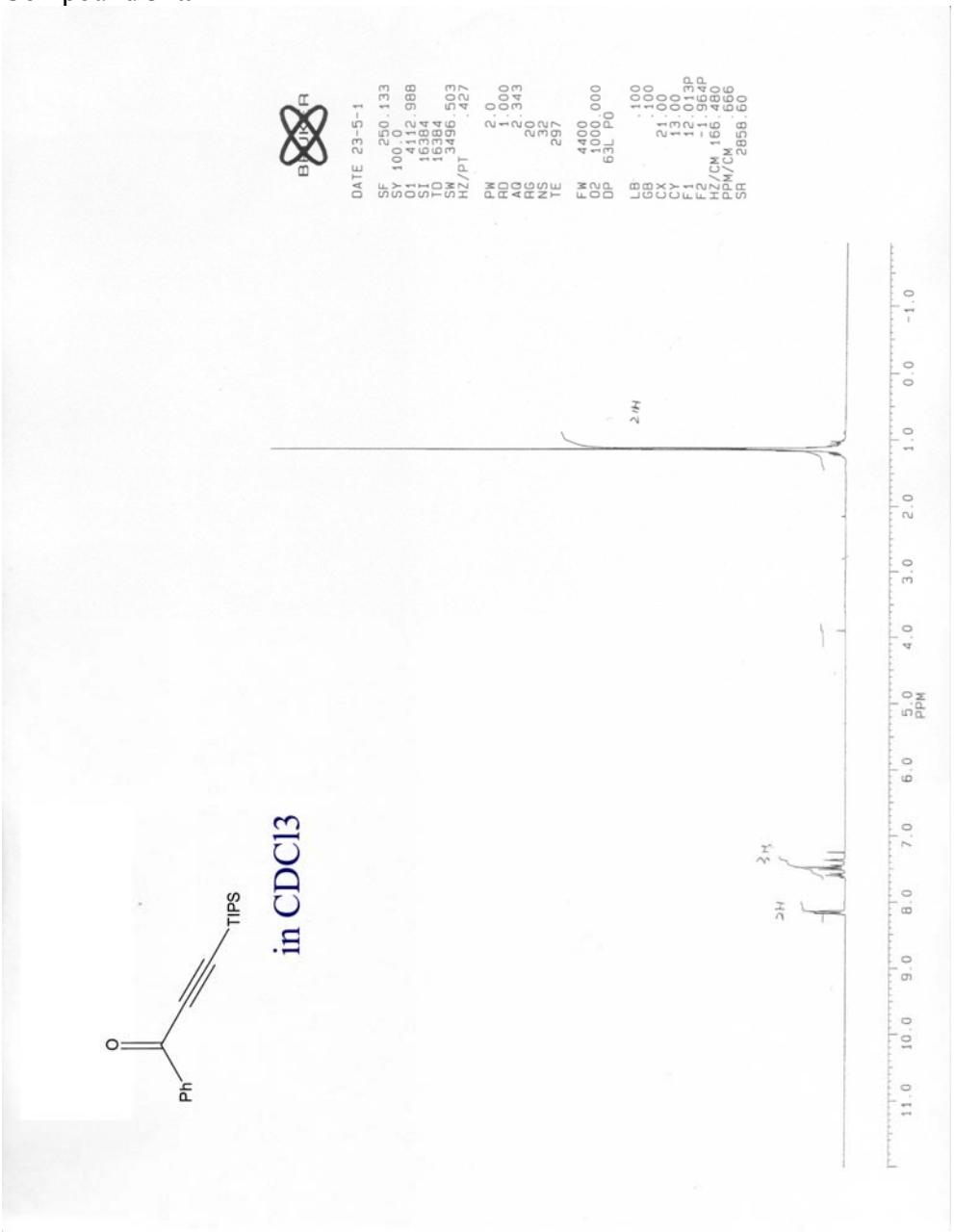


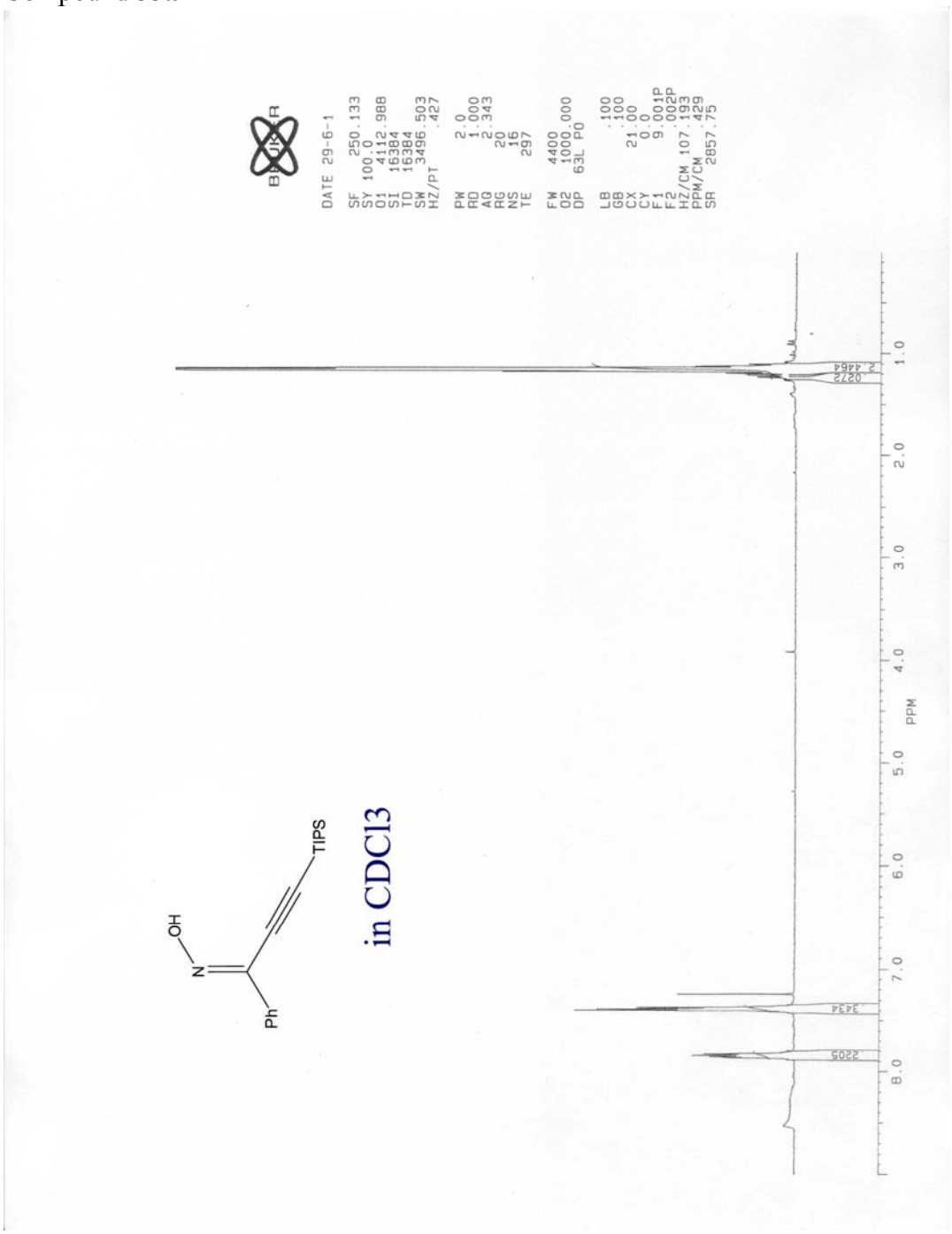
Figure 4 Kinetic plot of the transformation of aza-enediyne [4-^{13}C]-**26d** to enediyne [1,4-$^{13}C_2$]-**60d** and the conversion of [4-^{13}C]-**26d** to nitrile **61** followed by ^{13}H NMR in d_6-DMSO. The data fit independent first-order and second-order kinetics simultaneously by using TableCurve 2D® from SYSTAT Software Inc. The x-axis represents reaction time in seconds; the y-axis represents the concentrations of [4-^{13}C]-**26d** in mol/L calculated from the ratio of the integals of resonance at 159 ppm from [4-^{13}C]-**26d** to the one from d_6-DMSO (see section 6.4 for details). The first-order rate constant $k_1 = 7.18 \times 10^{-6}$ s^{-1}, the second-order rate constant $k_2 = 1.56 \times 10^{-3}$ $s^{-1}mol^{-1}L$.

APPENDIX C SELECTED ^{1}H NMRS FOR COMPOUNDS PREPARED IN EXPERIMENTAL SECTION

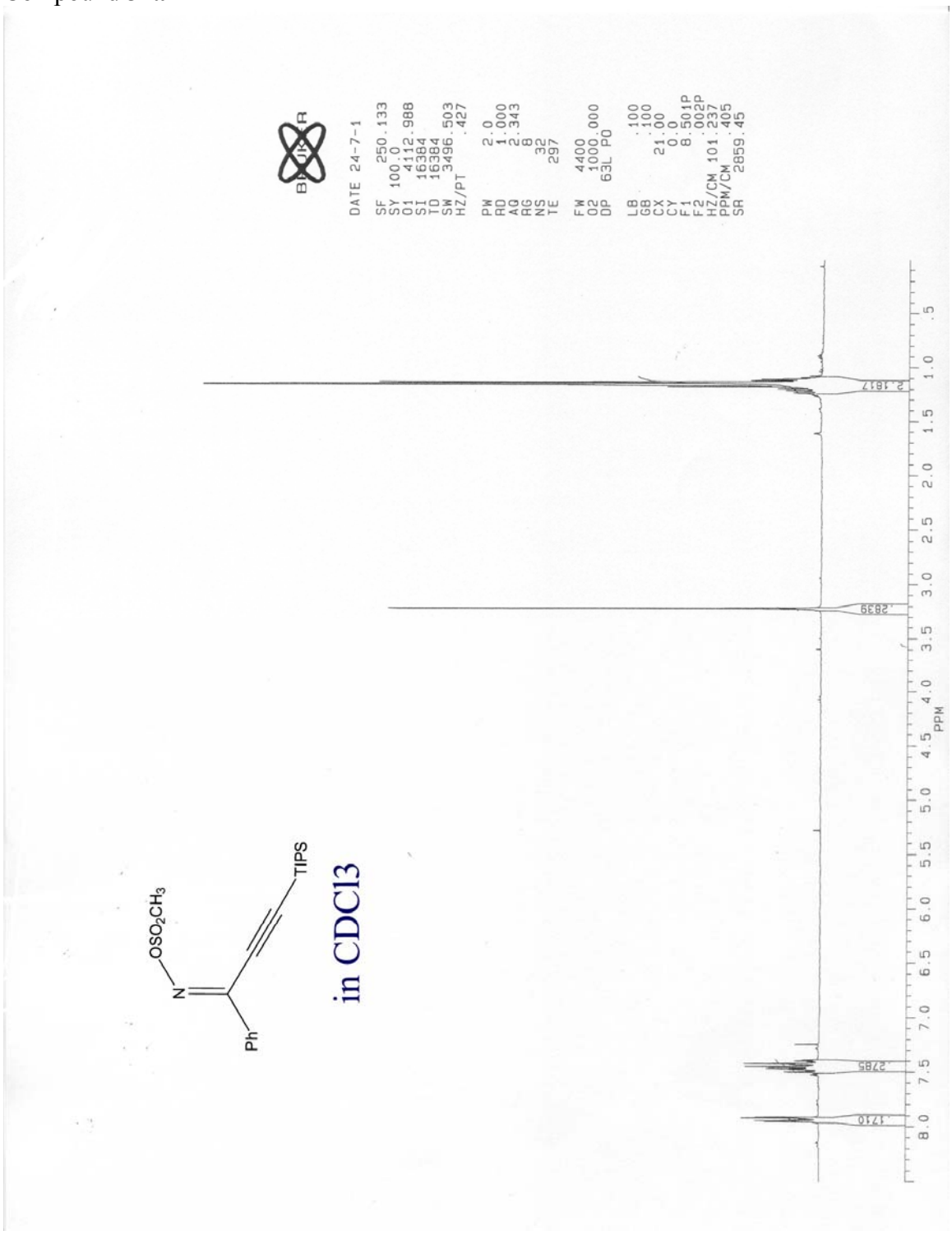
Compound **32a**



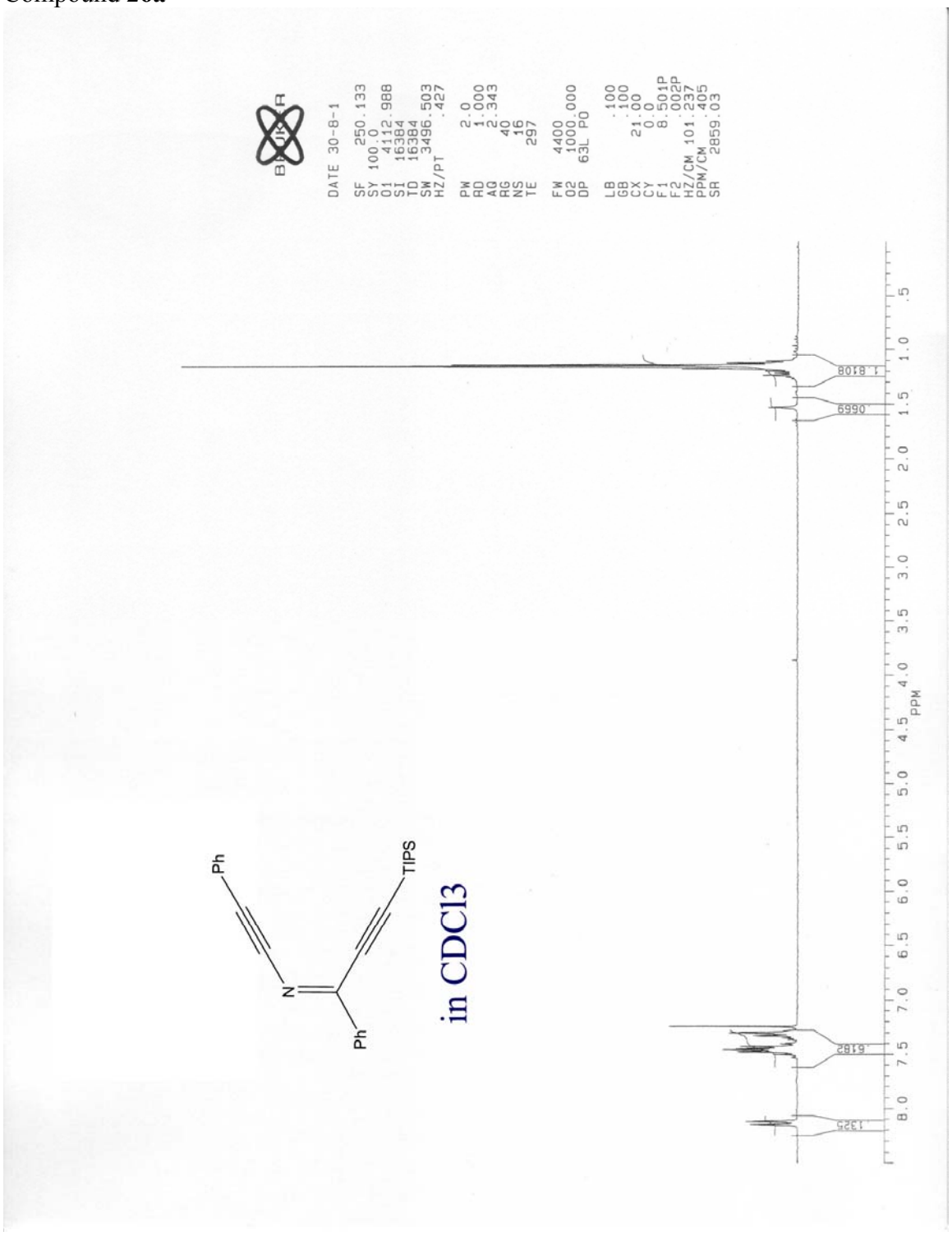
Compound **33a**



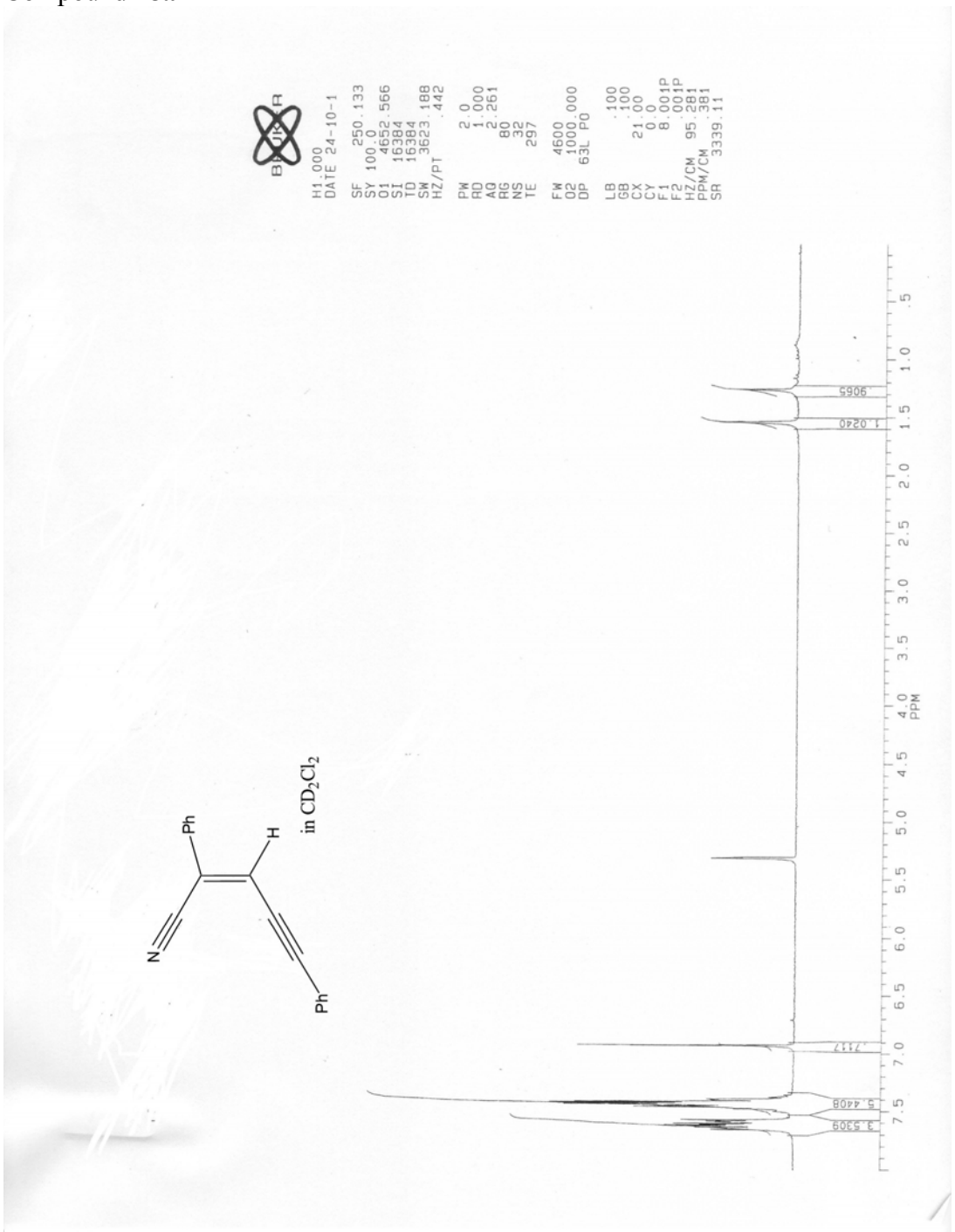
Compound **34a**



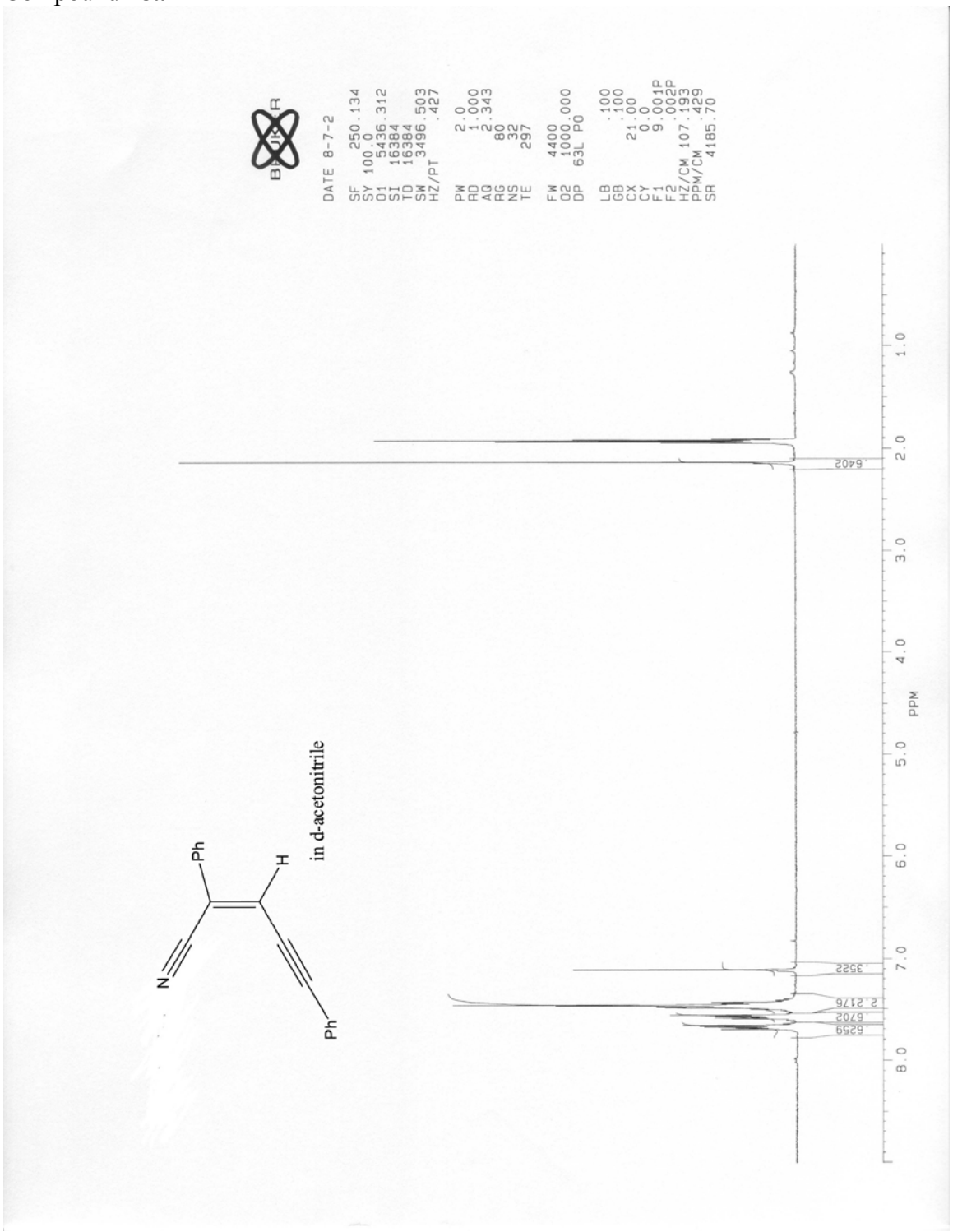
Compound **26a**



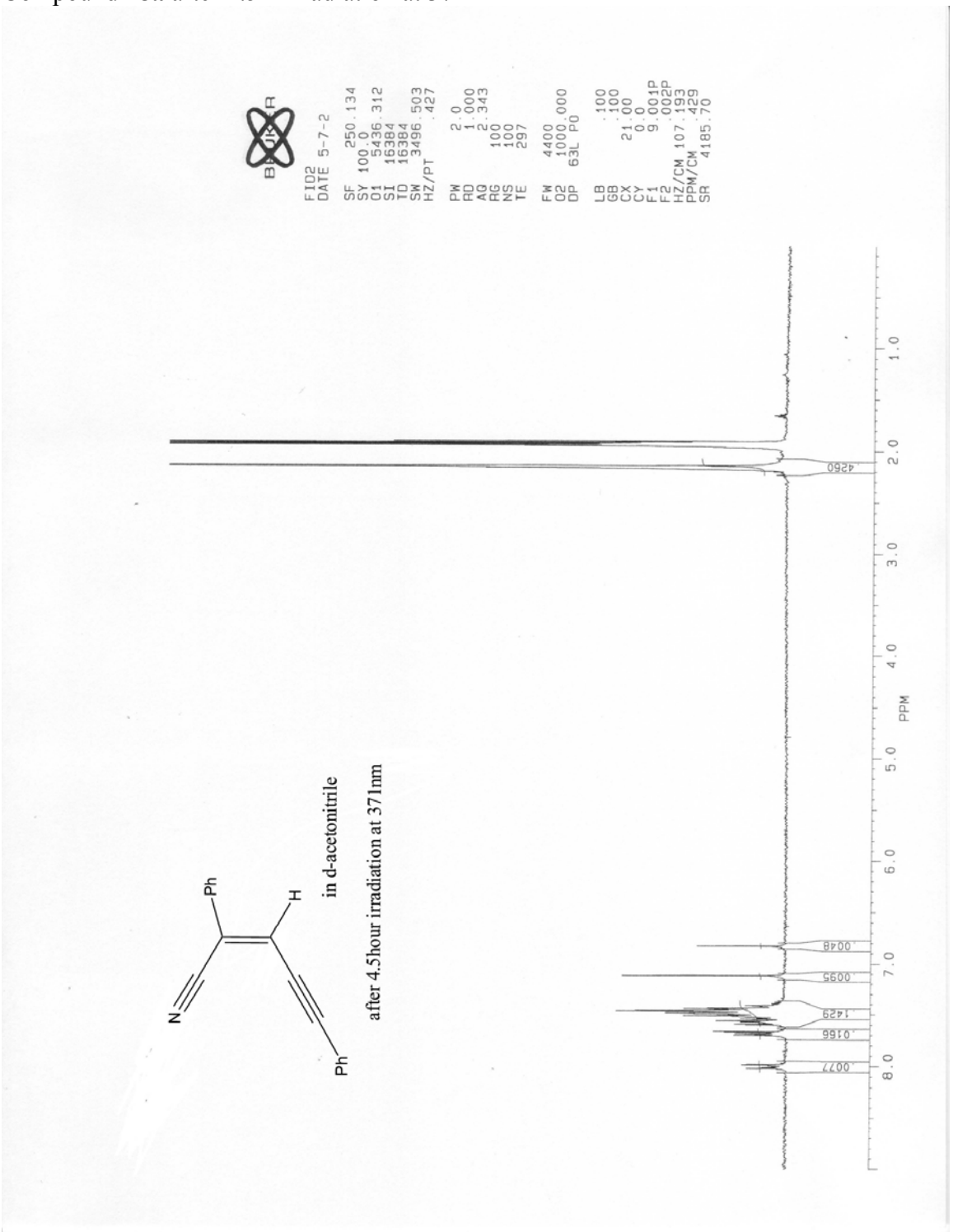
Compound **28a**



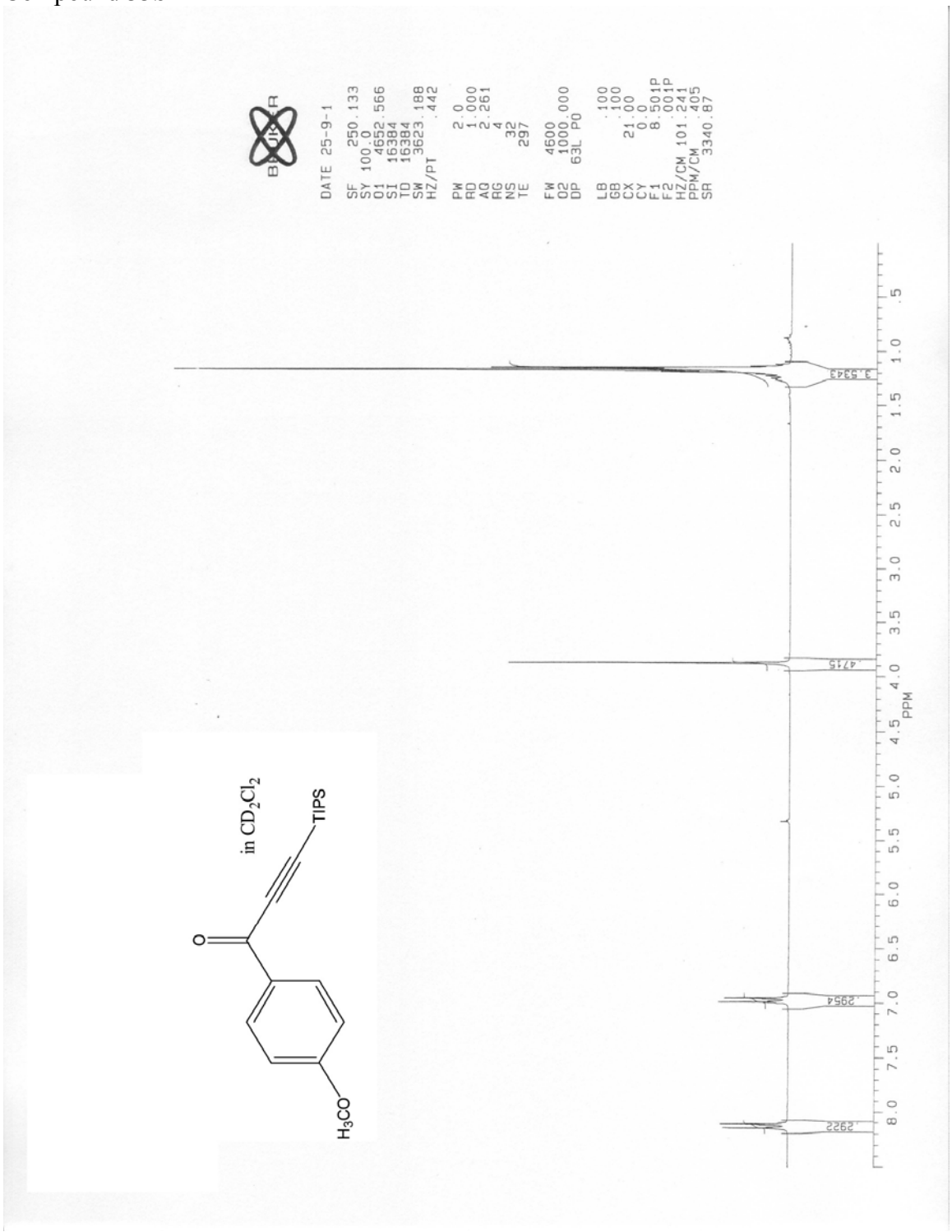
Compound **28a**



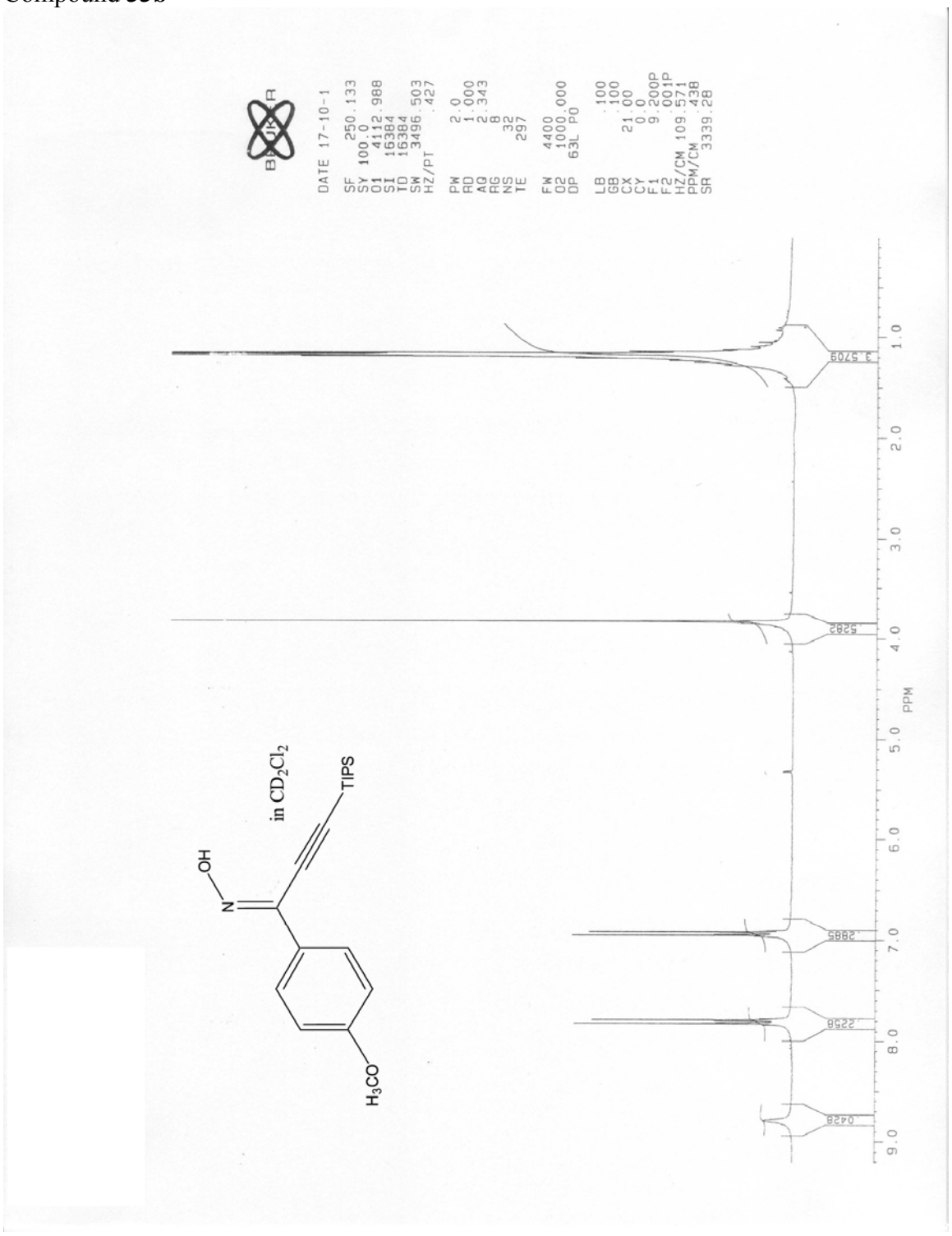
Compound **28a** after 4.5 h irradiation at 371 nm



Compound **33b**

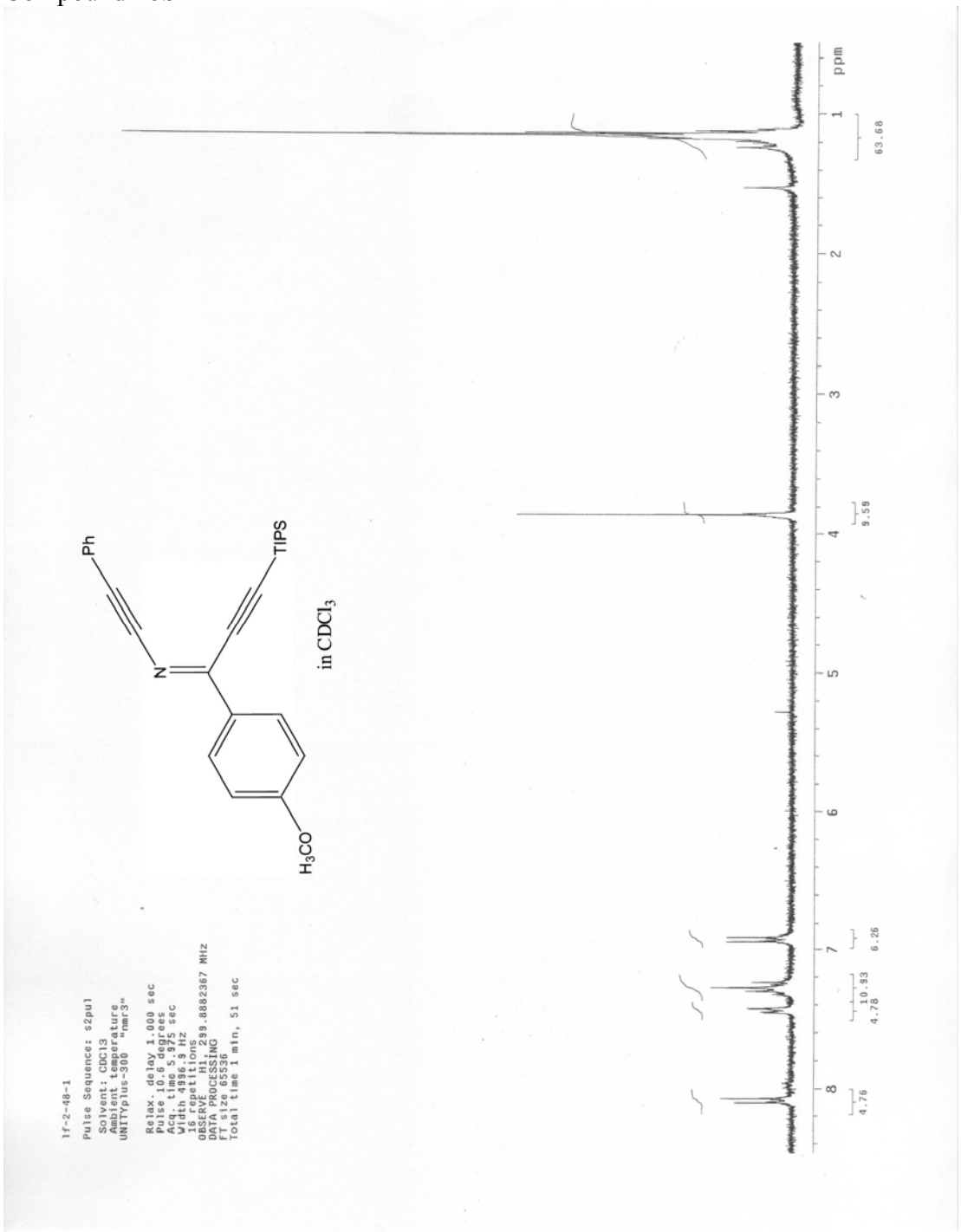


Compound **33b**

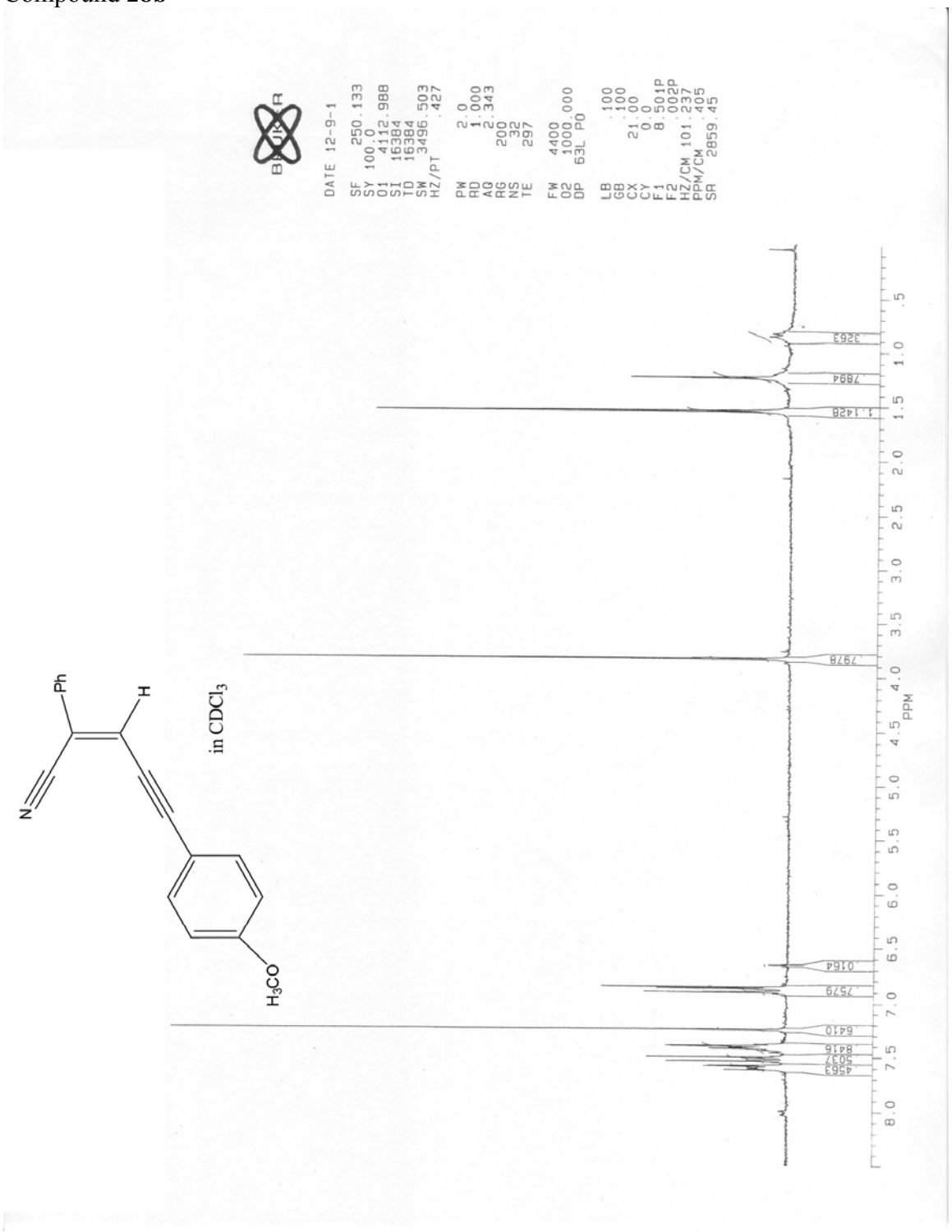


Compound **34b**

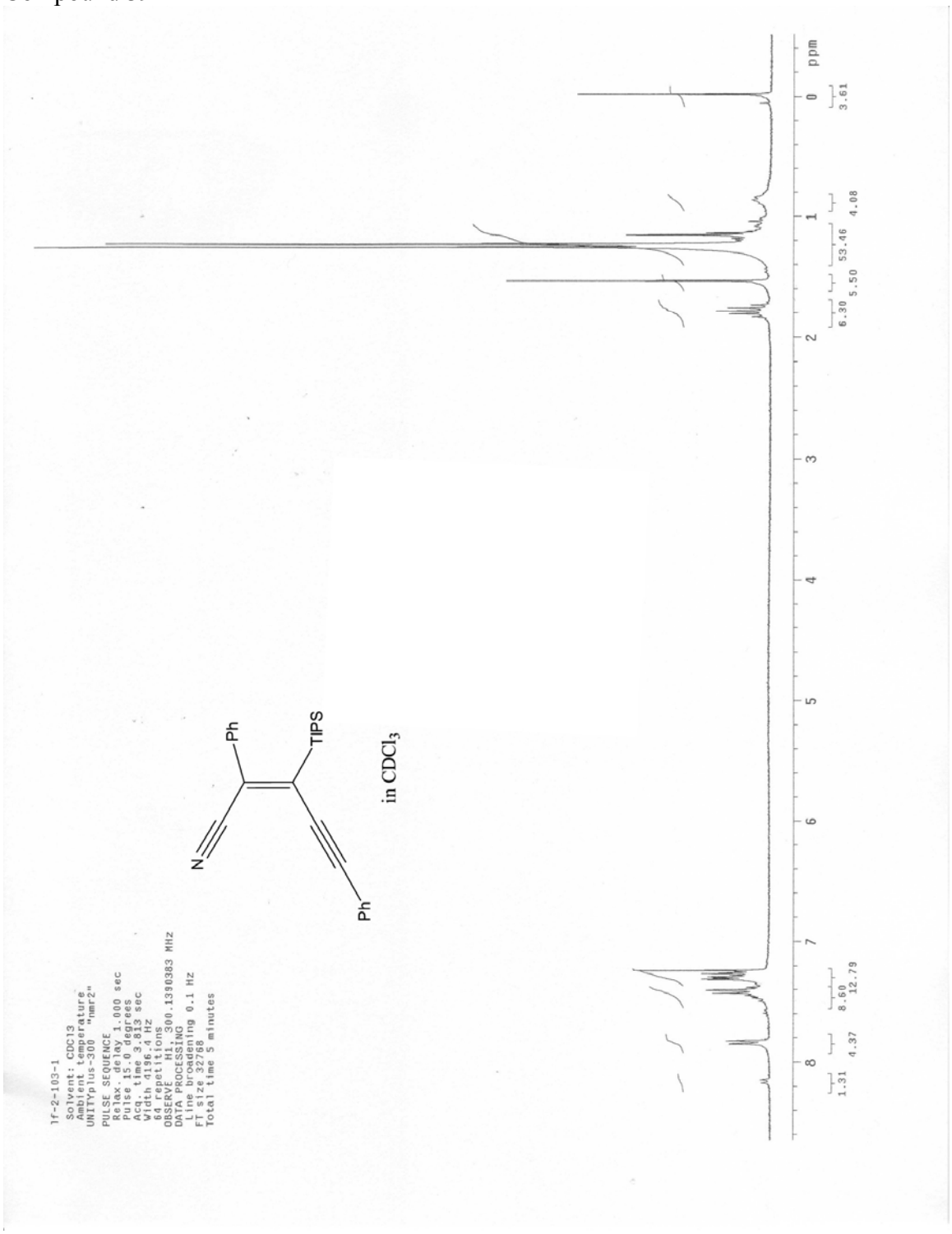
Compound **26b**



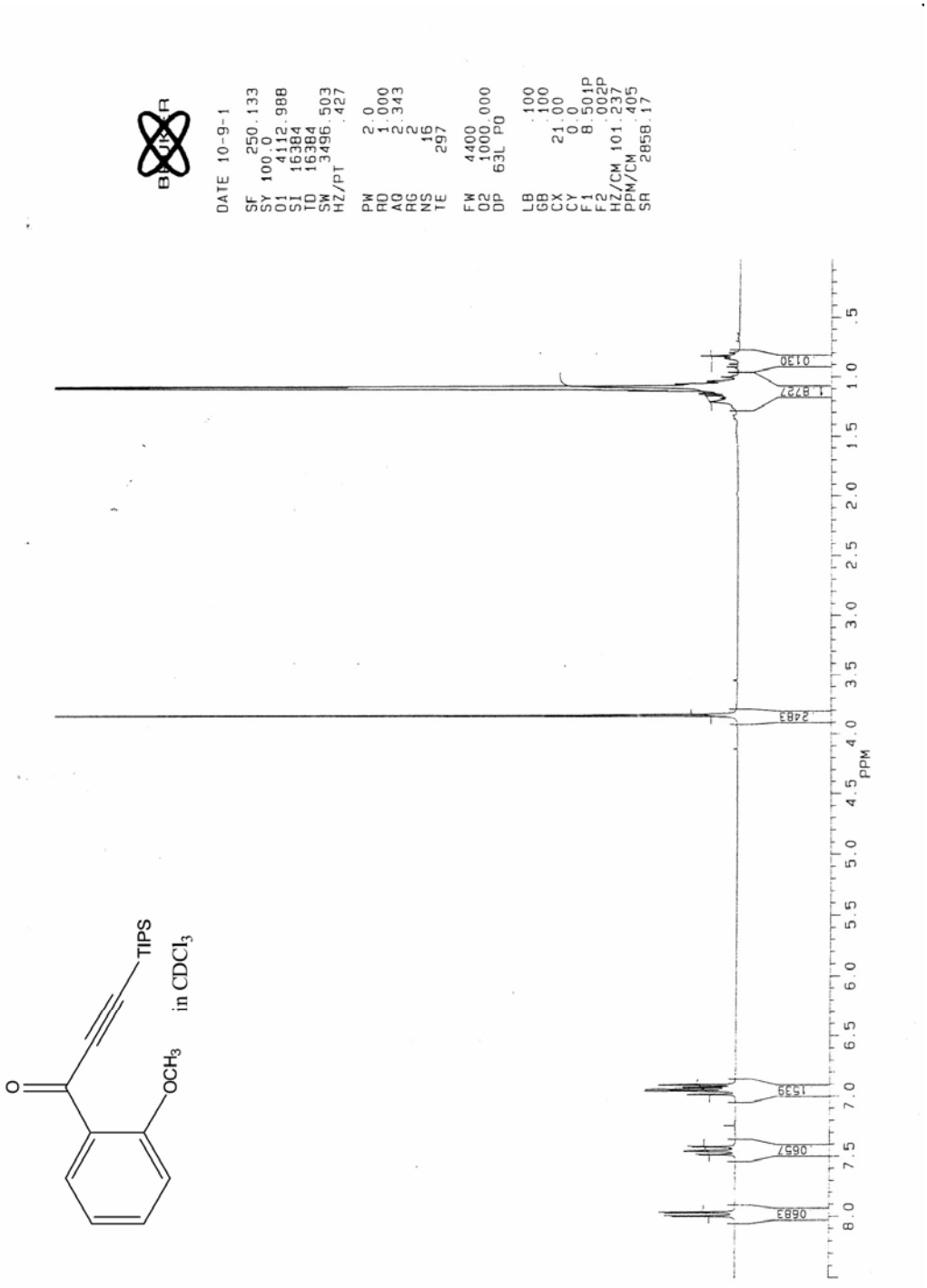
Compound **28b**



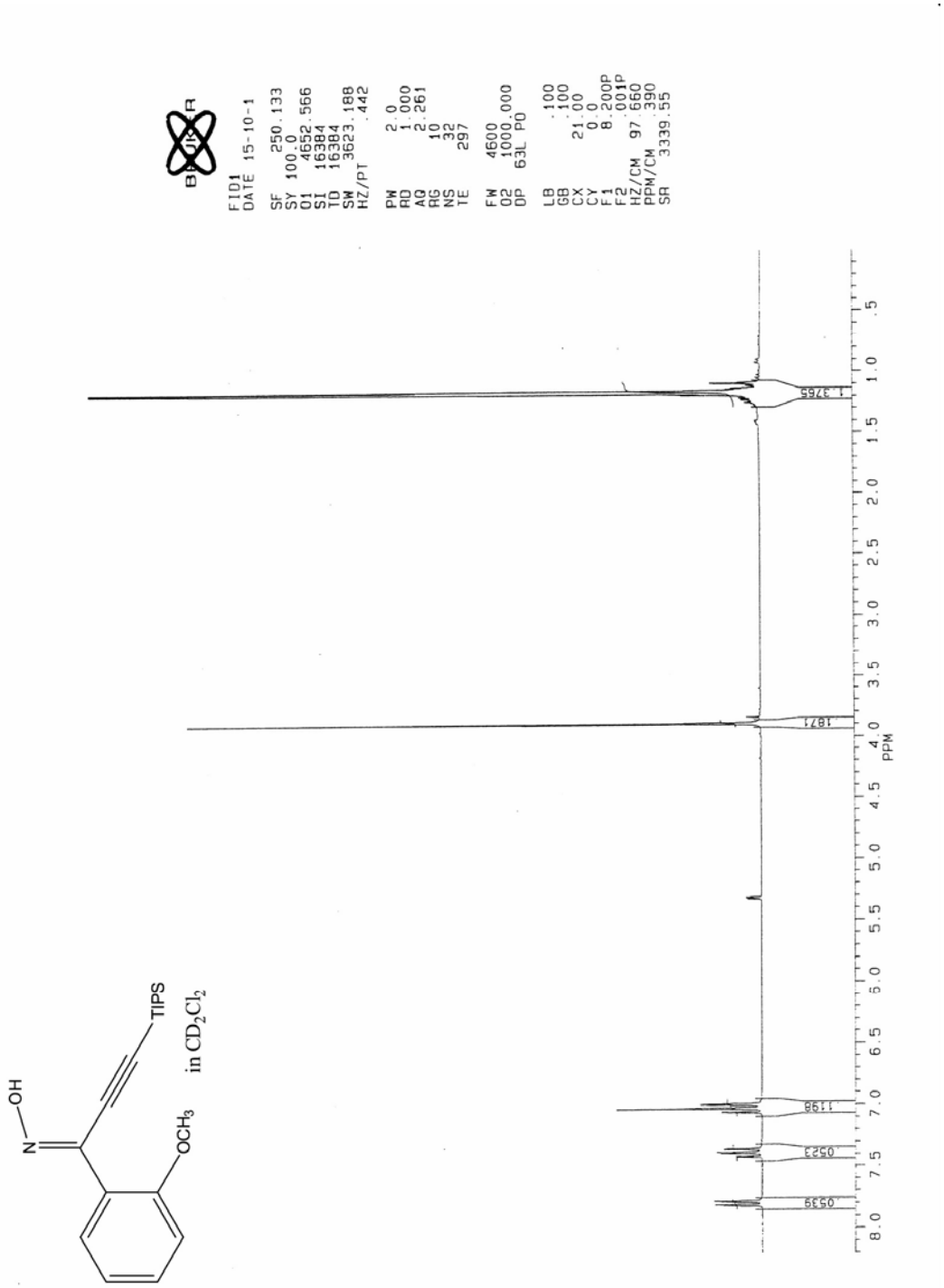
Compound **39**

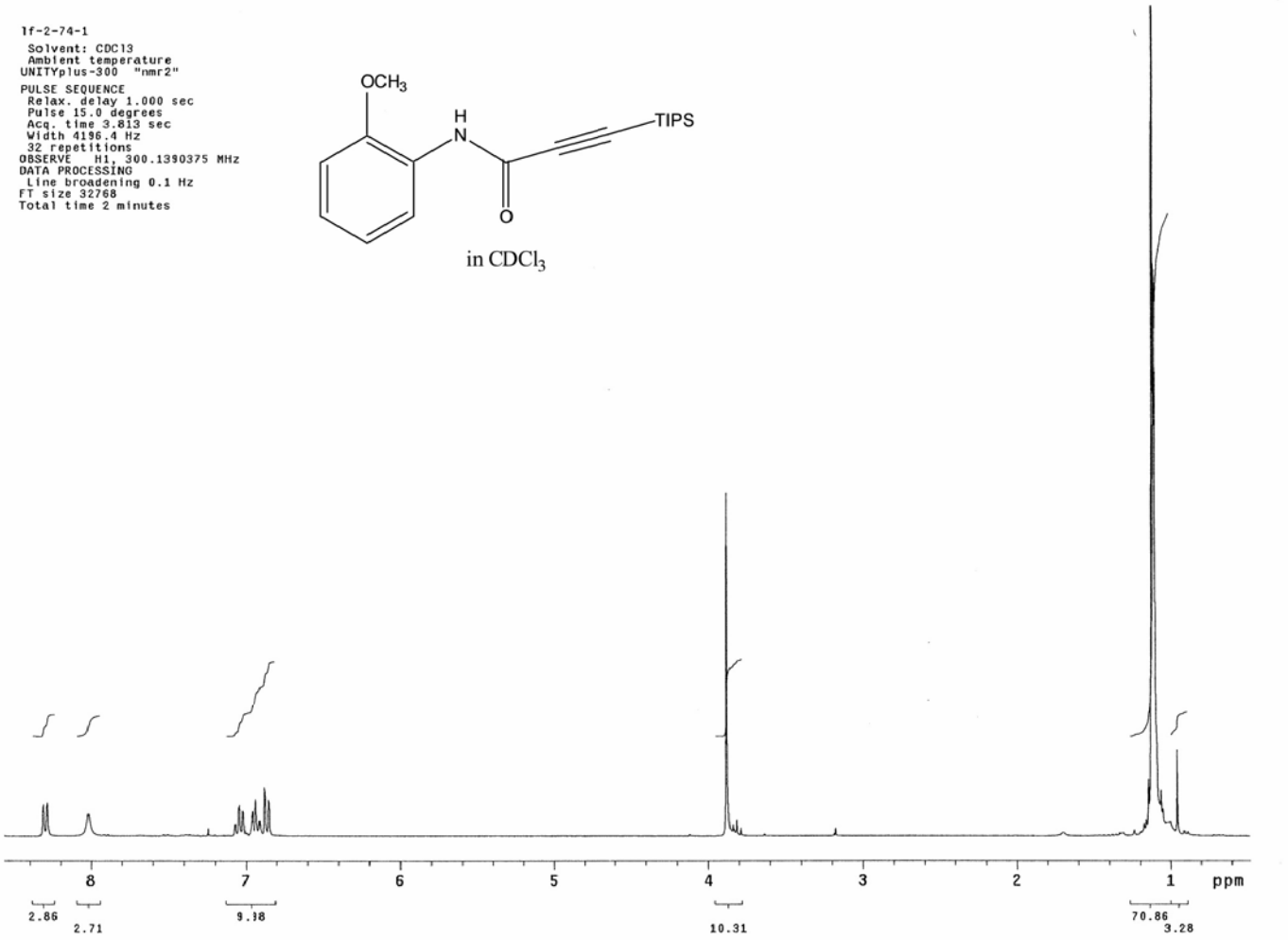

1f-2-103-1
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
64 repetitions
OBSERVE H1, 300.1390383 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 5 minutes
Ph
TIPS
N
Ph
in CDCl3
ppm
8.60
12.79
1.31
4.37
6.30
5.50
53.46
4.08
3.61

Compound **32c**

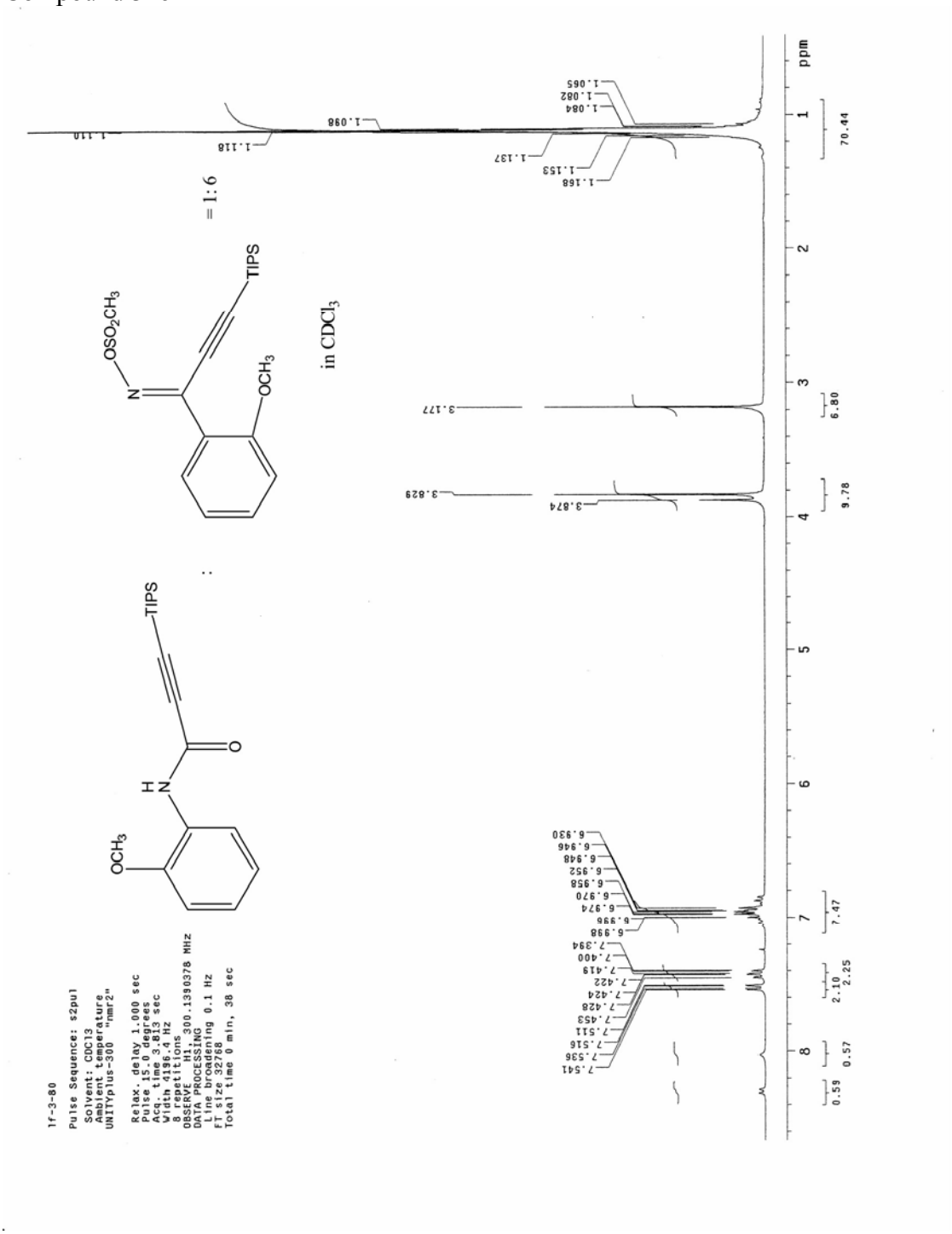


Compound **33c**

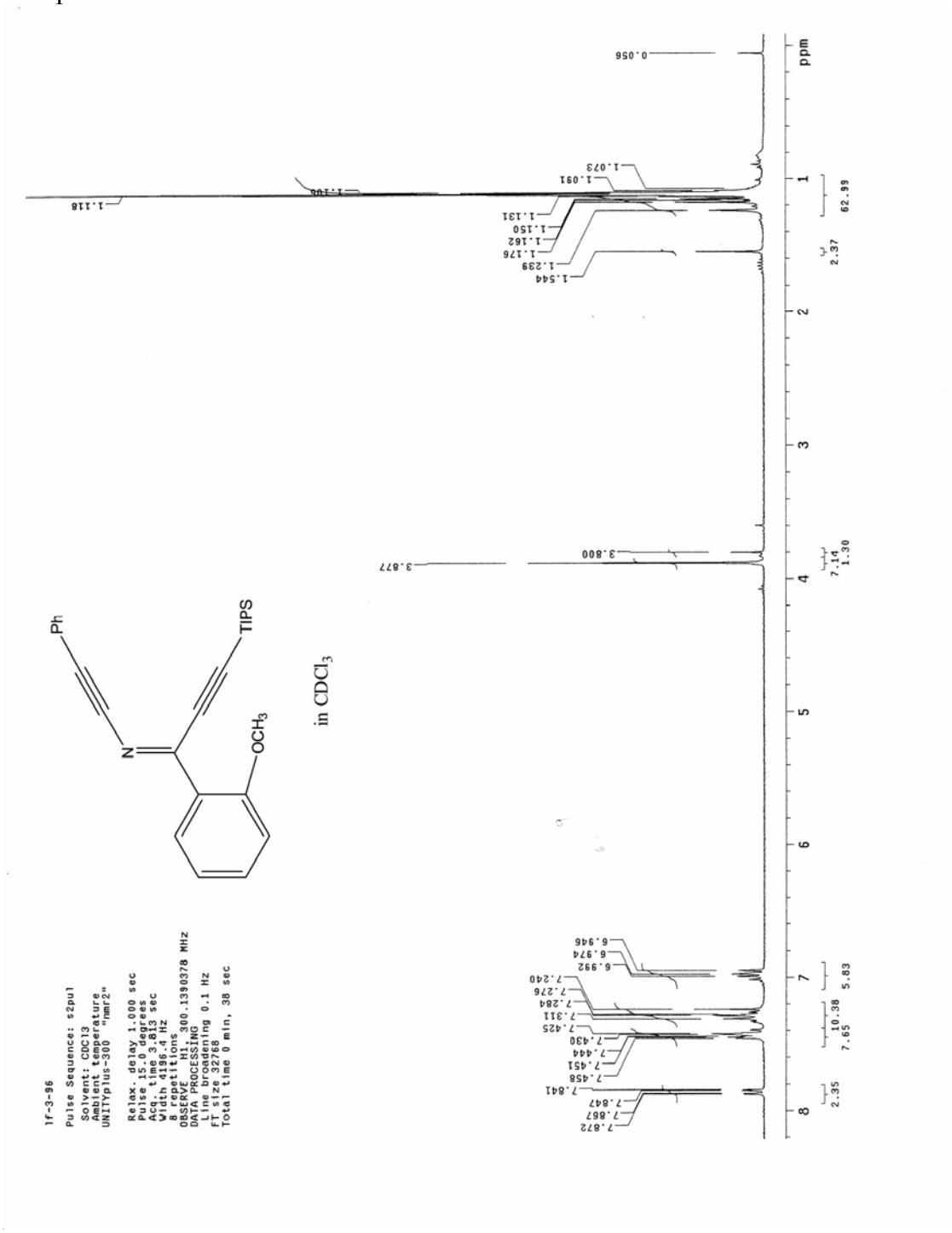



lf-2-74-1
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
32 repetitions
OBSERVE H1, 300.1390375 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 2 minutes
OCH3
H
N
O
TIPS
in CDCl3
8
7
6
5
4
3
2
1
ppm
2.86
2.71
9.18
10.31
70.86
3.28

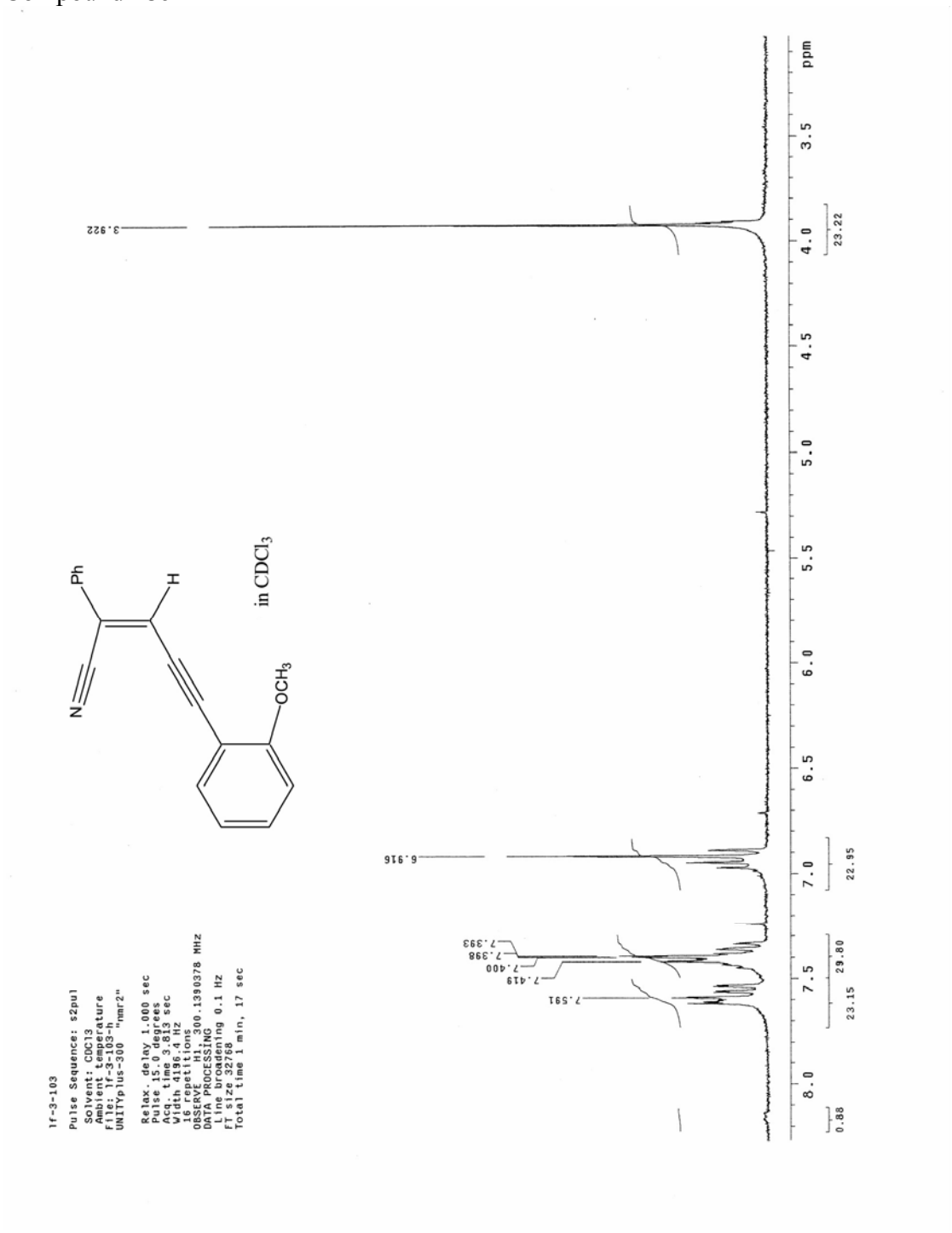
Compound **34c**



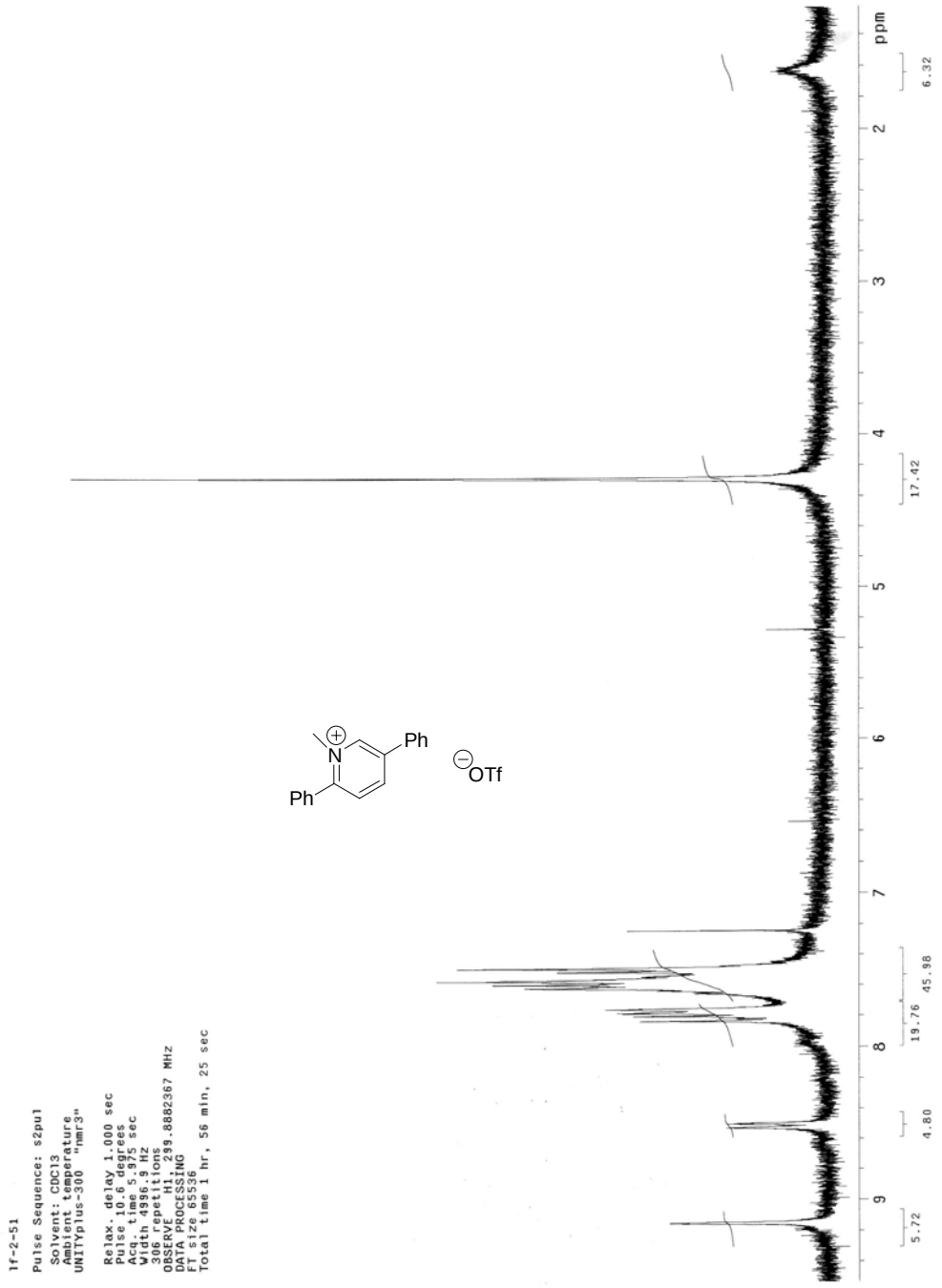
Compound **26c**



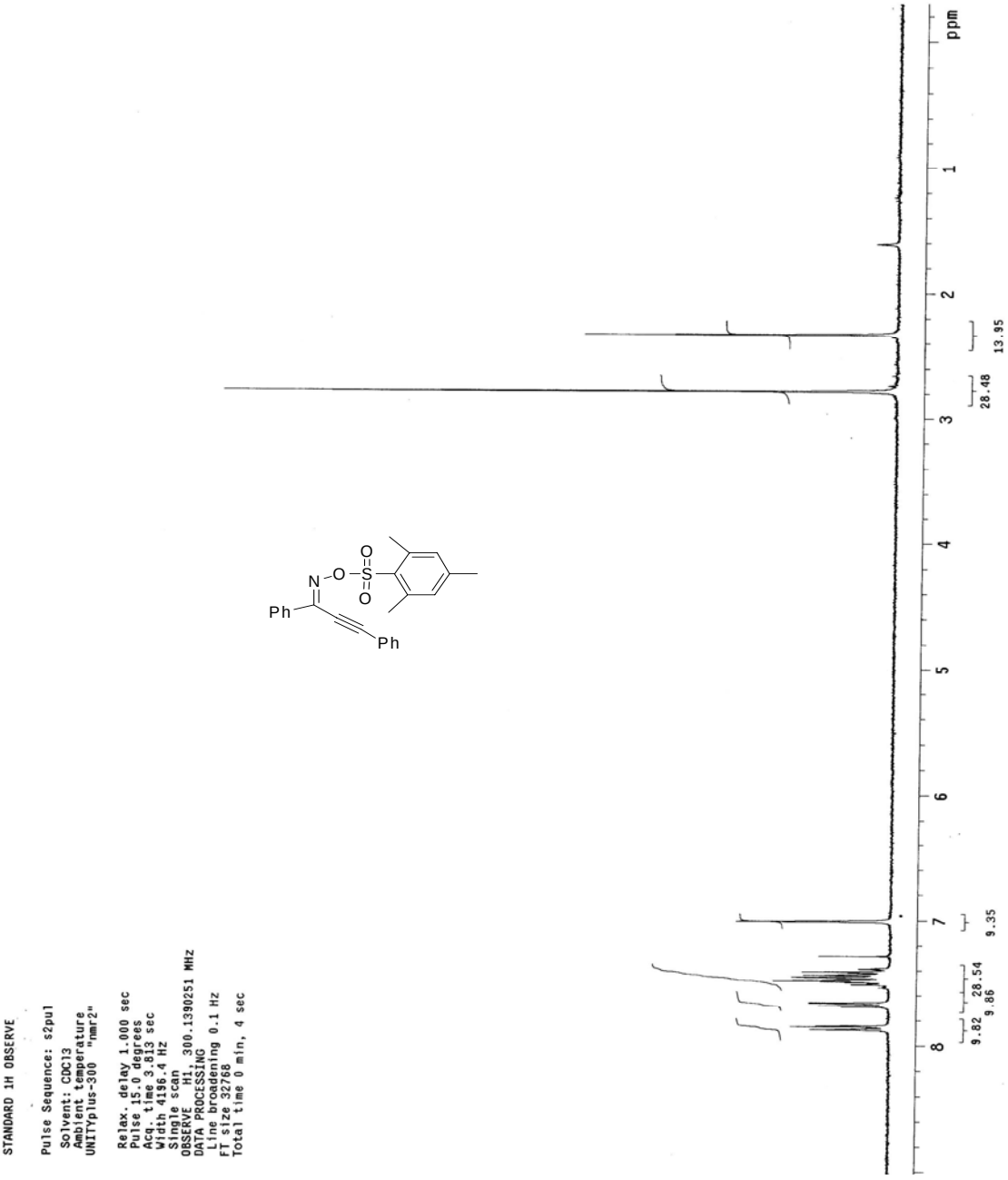
Compound **28c**



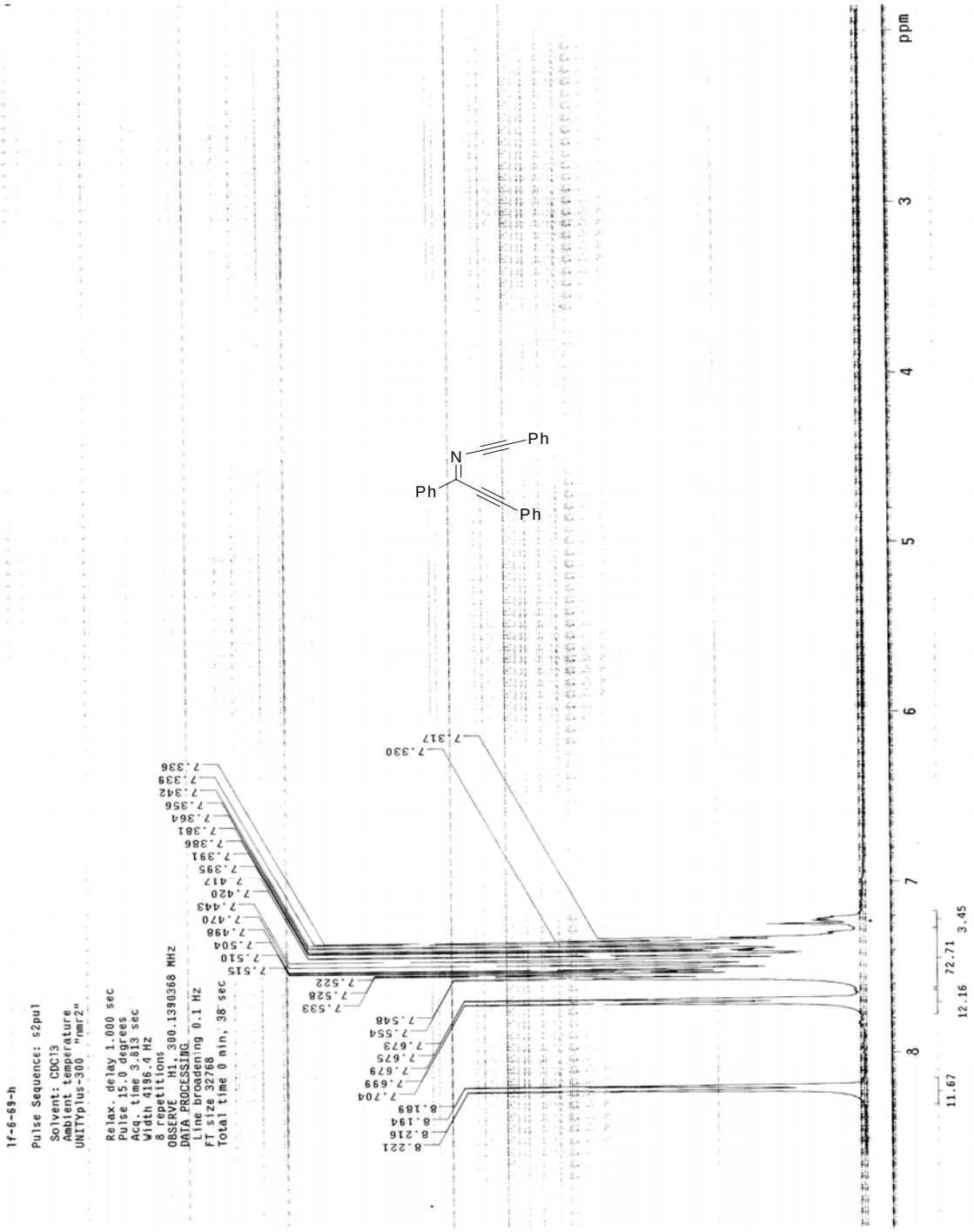
Compound **43**


1f-2-51
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "mar3"
Relax. delay 1.000 sec
Pulse 10.6 degrees
Acq. time 5.375 sec
Width 4996.9 Hz
306 repetitions
OBSERVE H1, 299.8882367 MHz
DATA PROCESSING
FT size 65536
Total time 1 hr, 56 min, 25 sec
Ph
Ph
OTf
ppm
17.42
6.32
19.76
45.98
5.72
4.80

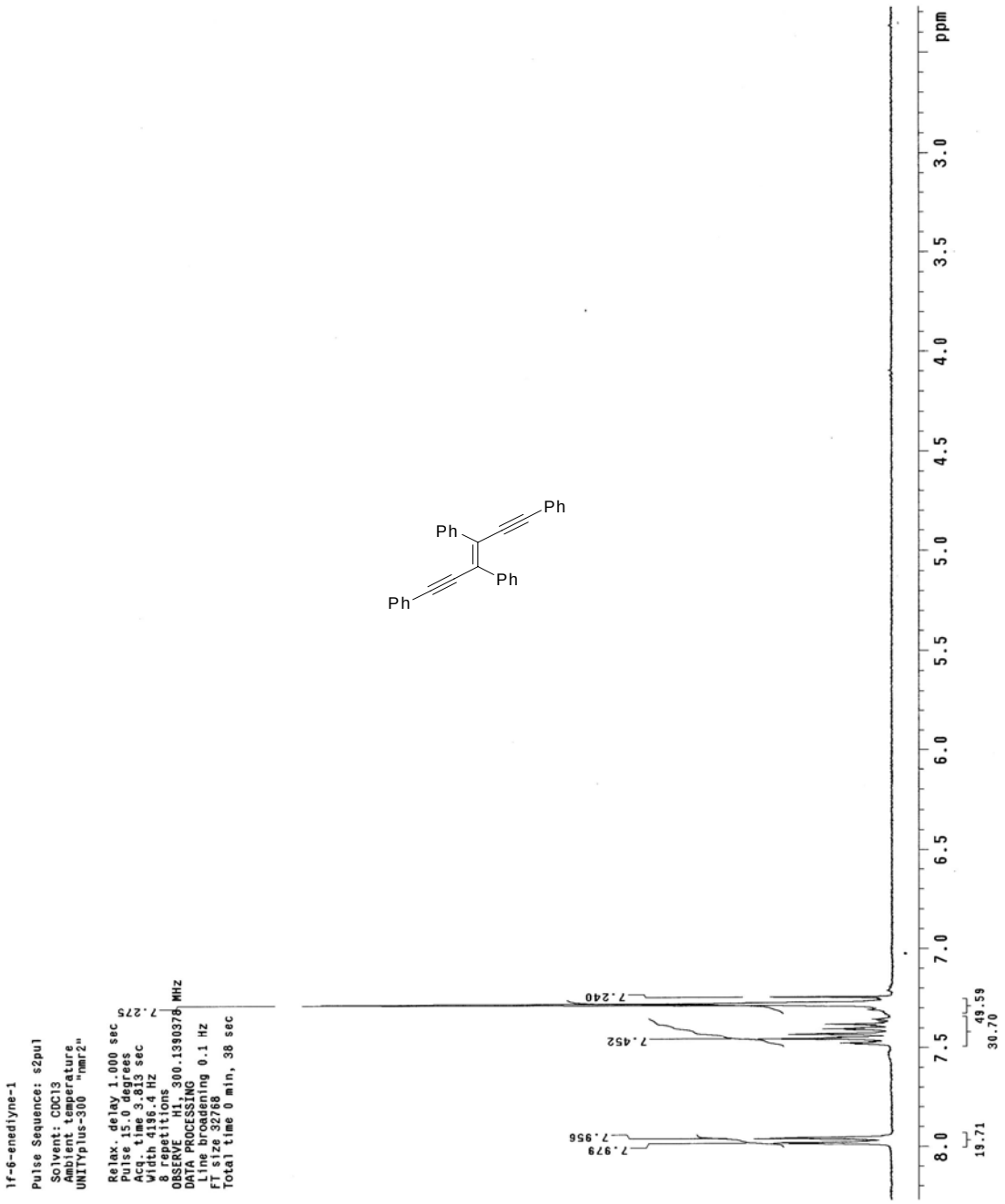
Compound **38d**



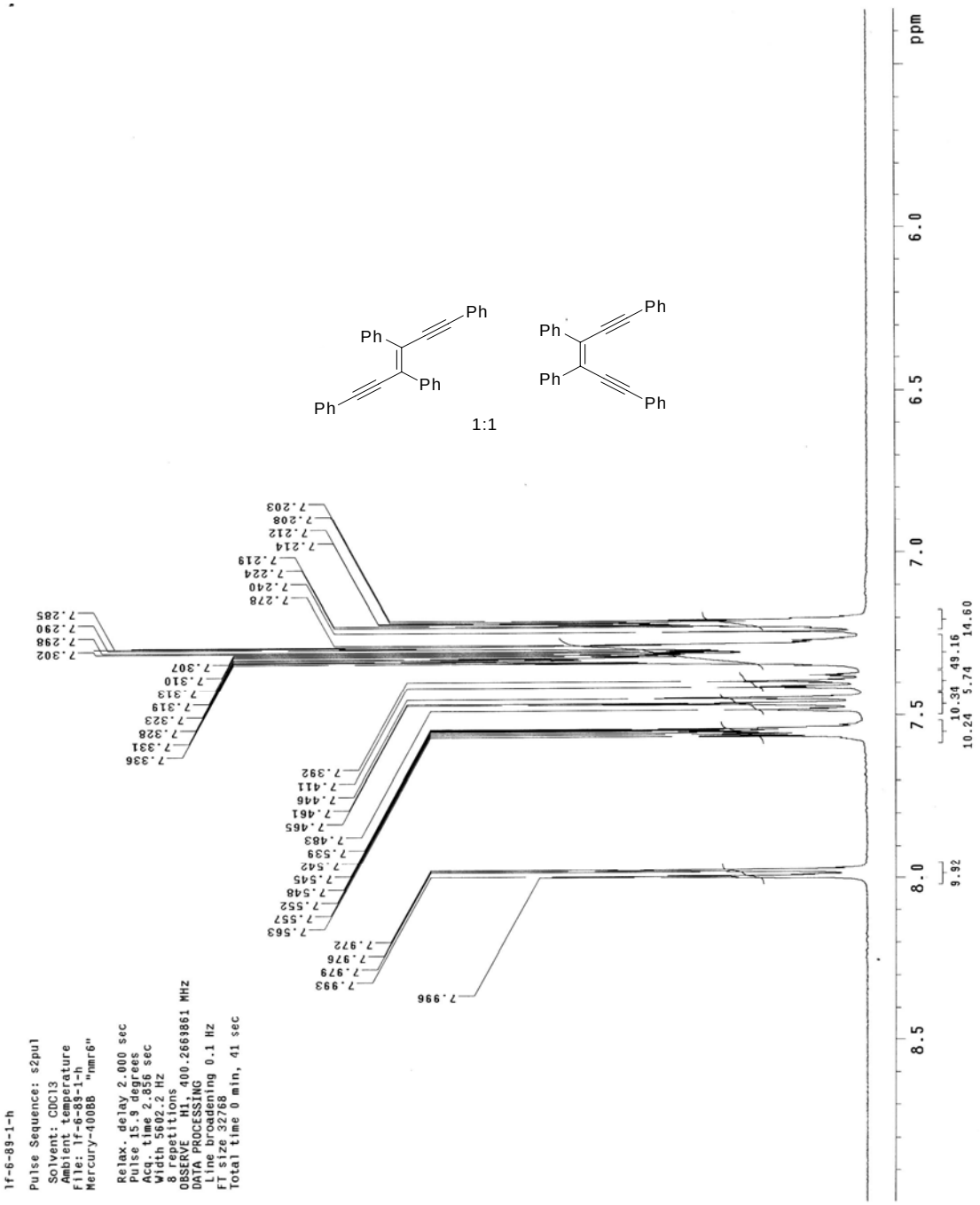
Compound **26d**



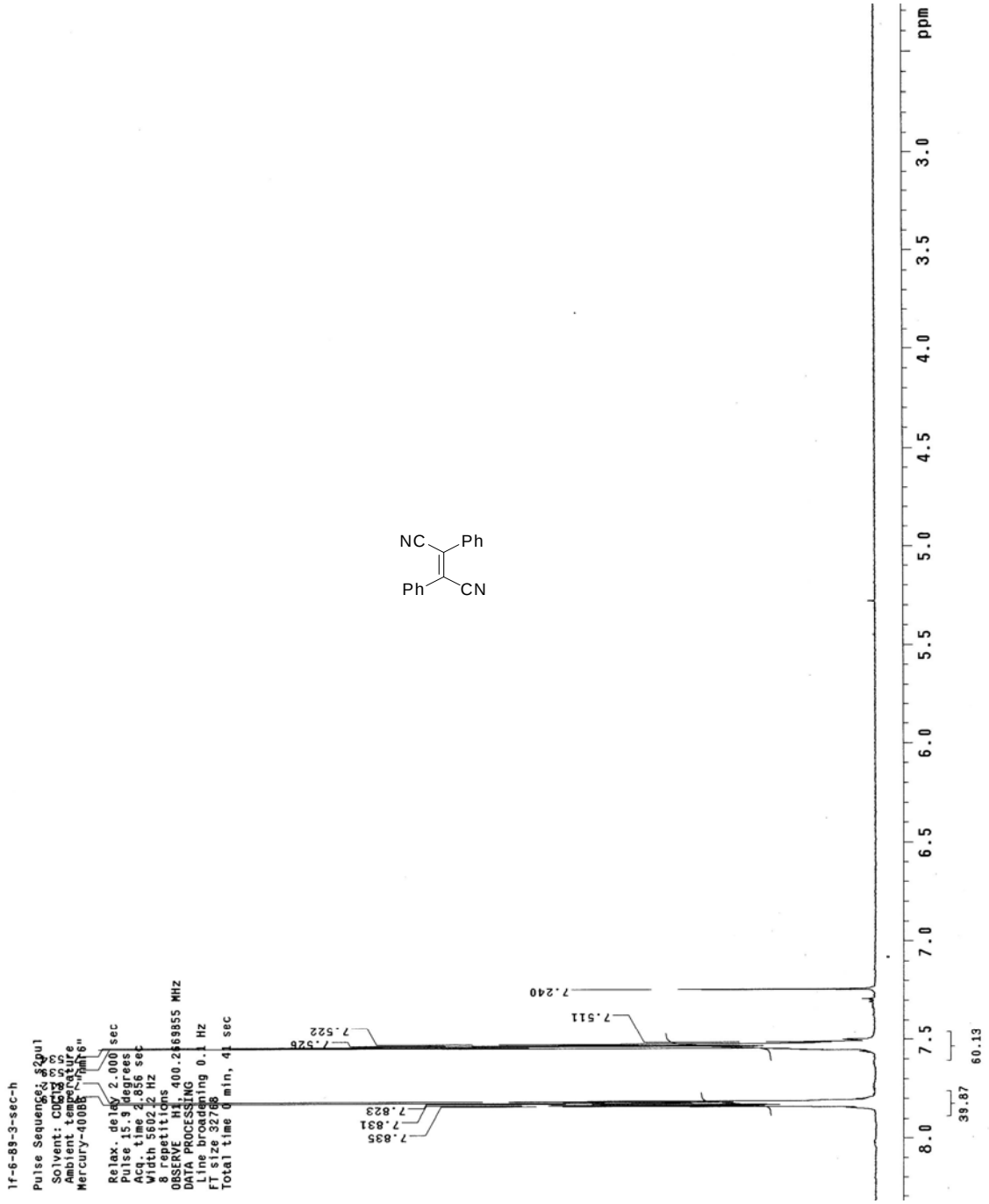
Compound **(*E*)-60d**



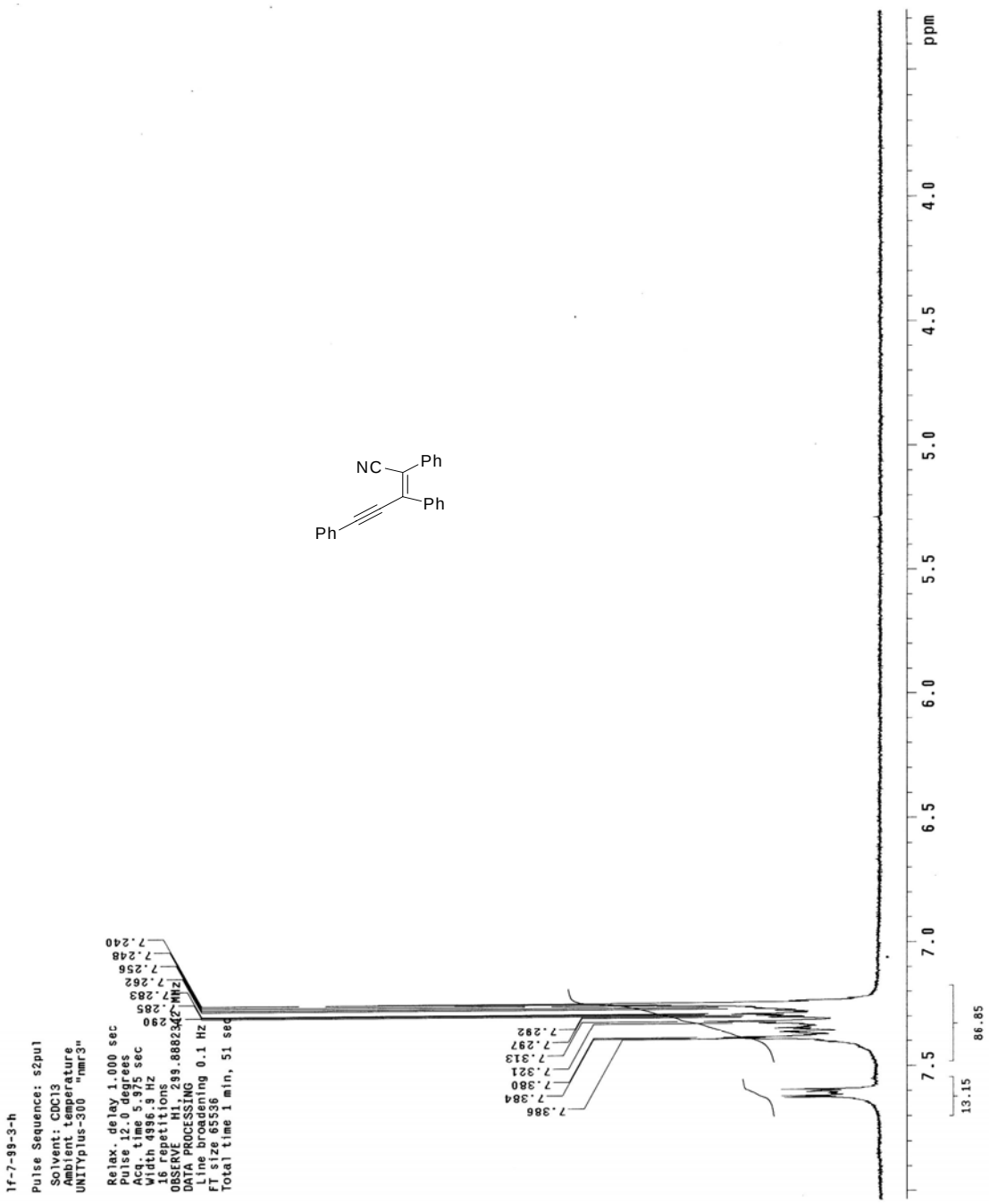
Compound **60d**



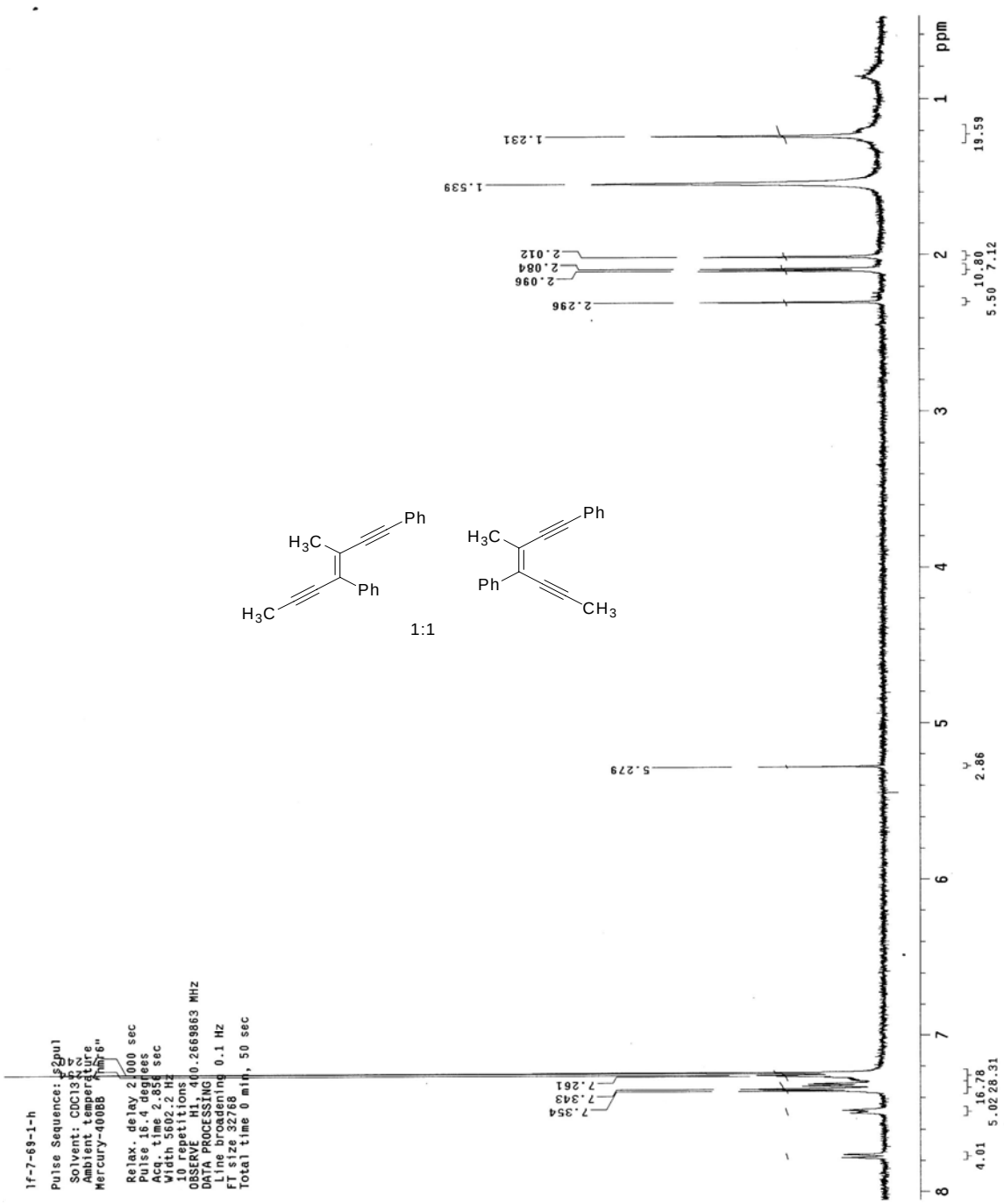
Compound **(*E*)-61**



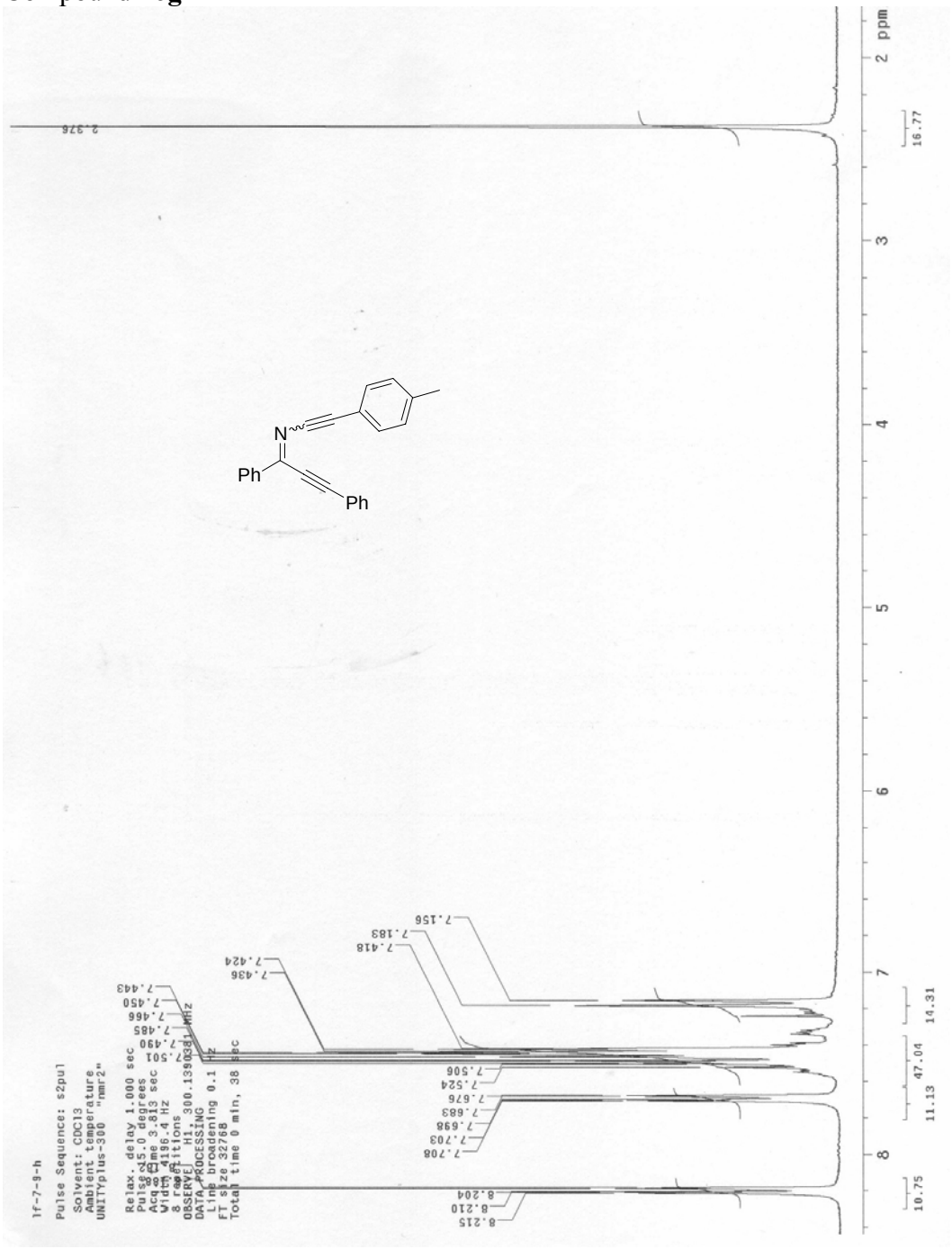
Compound **28d**



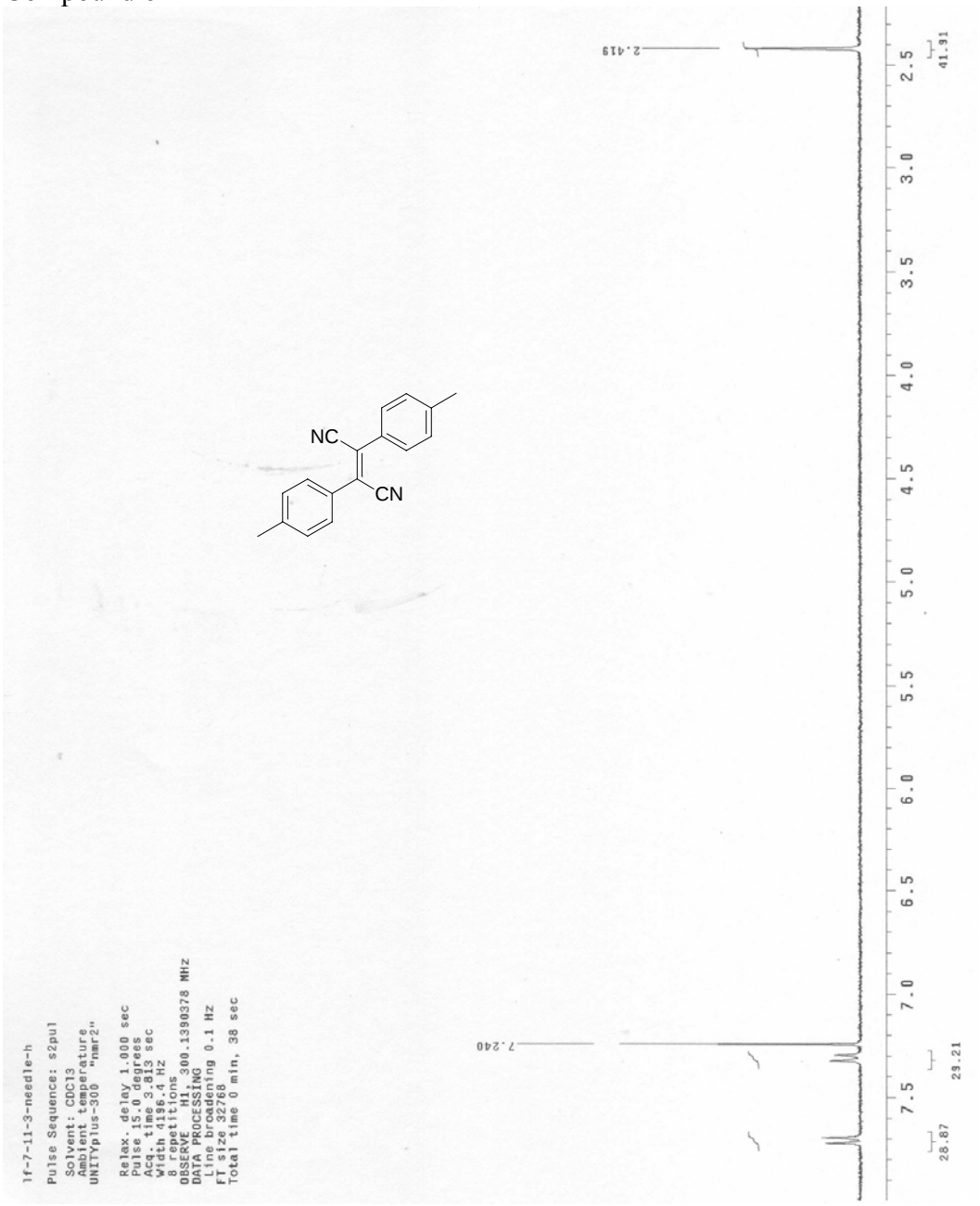
Compound **60e**



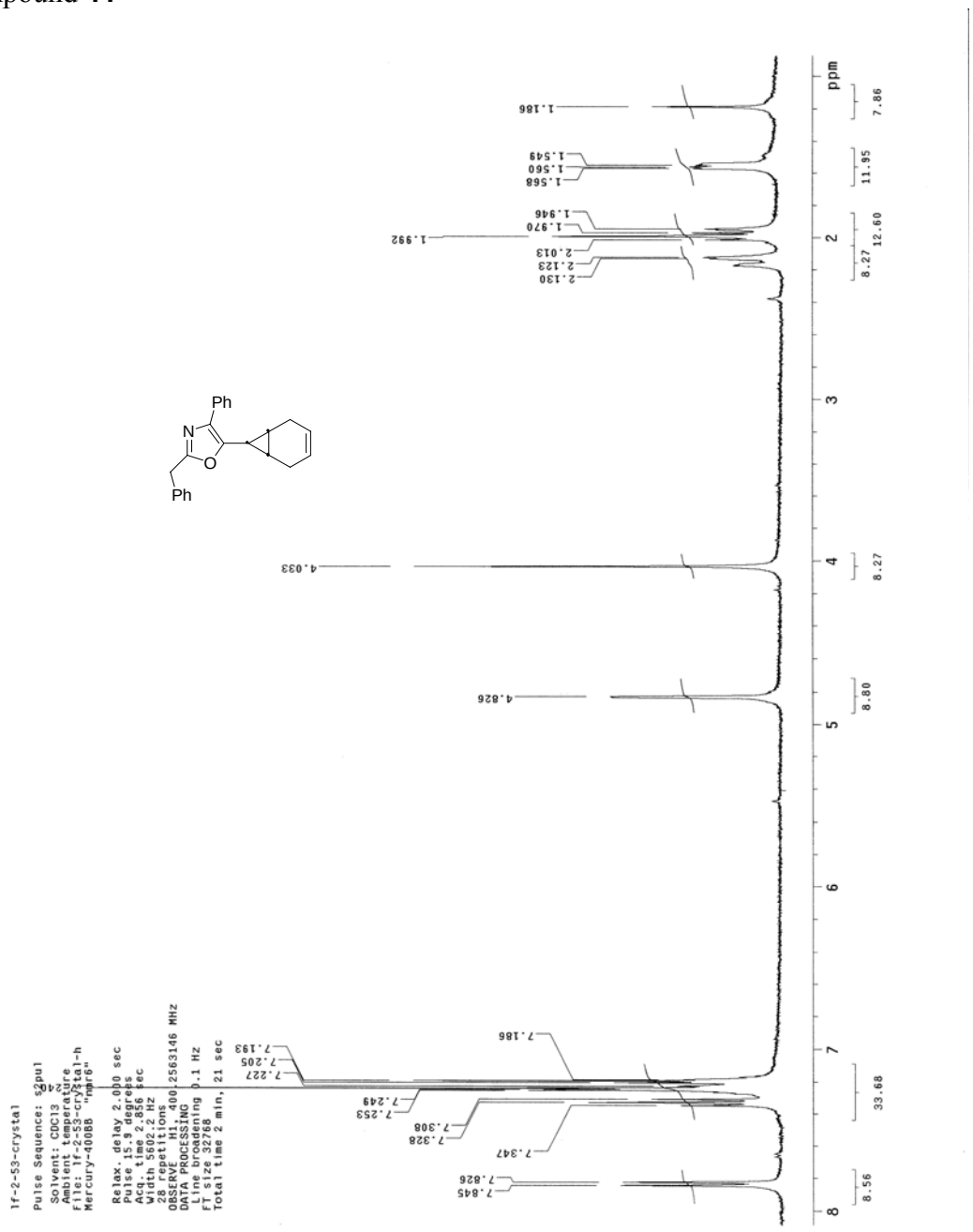
Compound **26g**



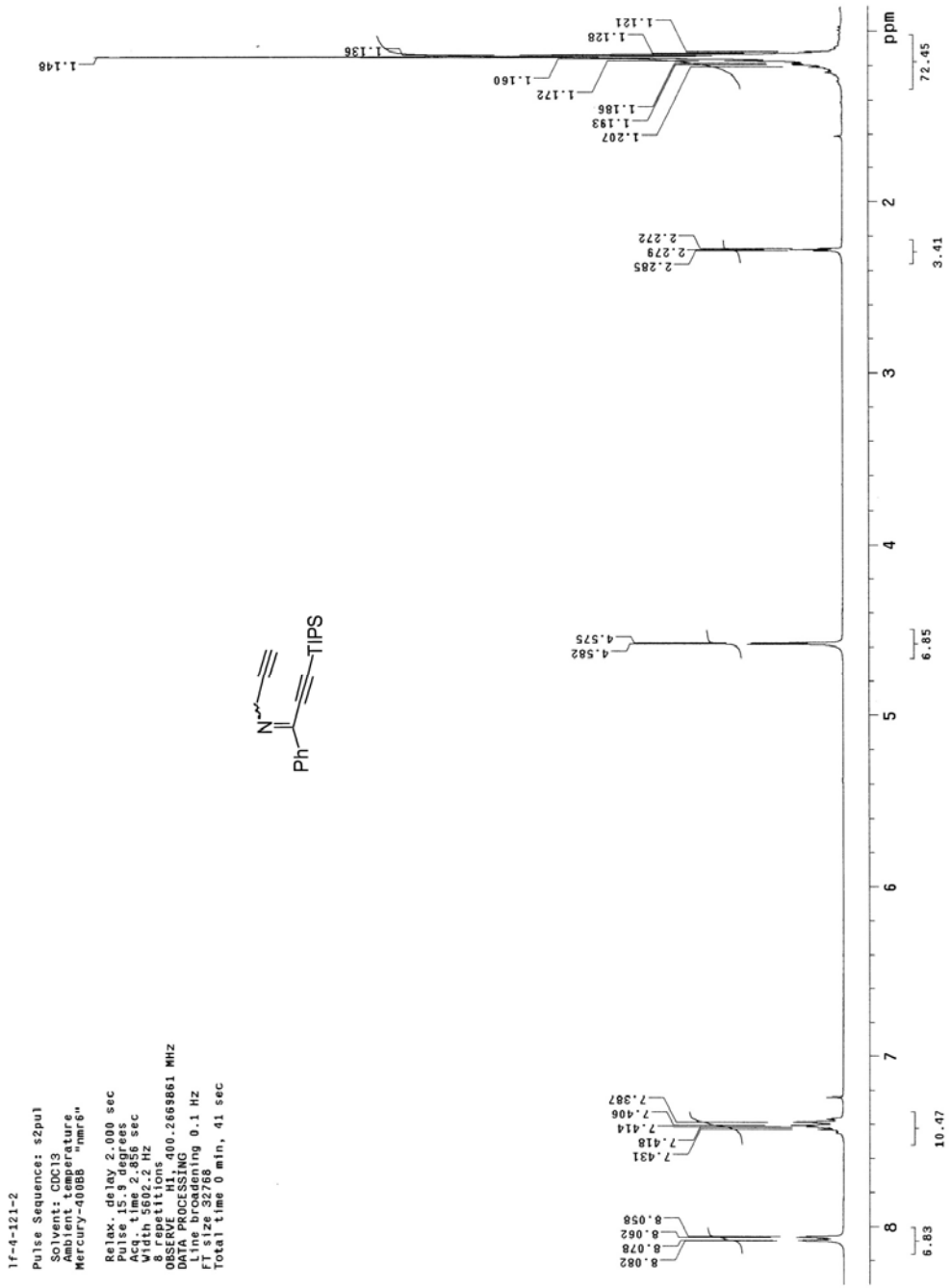
Compound **62**


1f-7-11-3-needle-h
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
8 repetitions
OBSERVE H1 300.1390378 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 38 sec
NC
CN
7.240
2.419
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
28.87
23.21
41.91

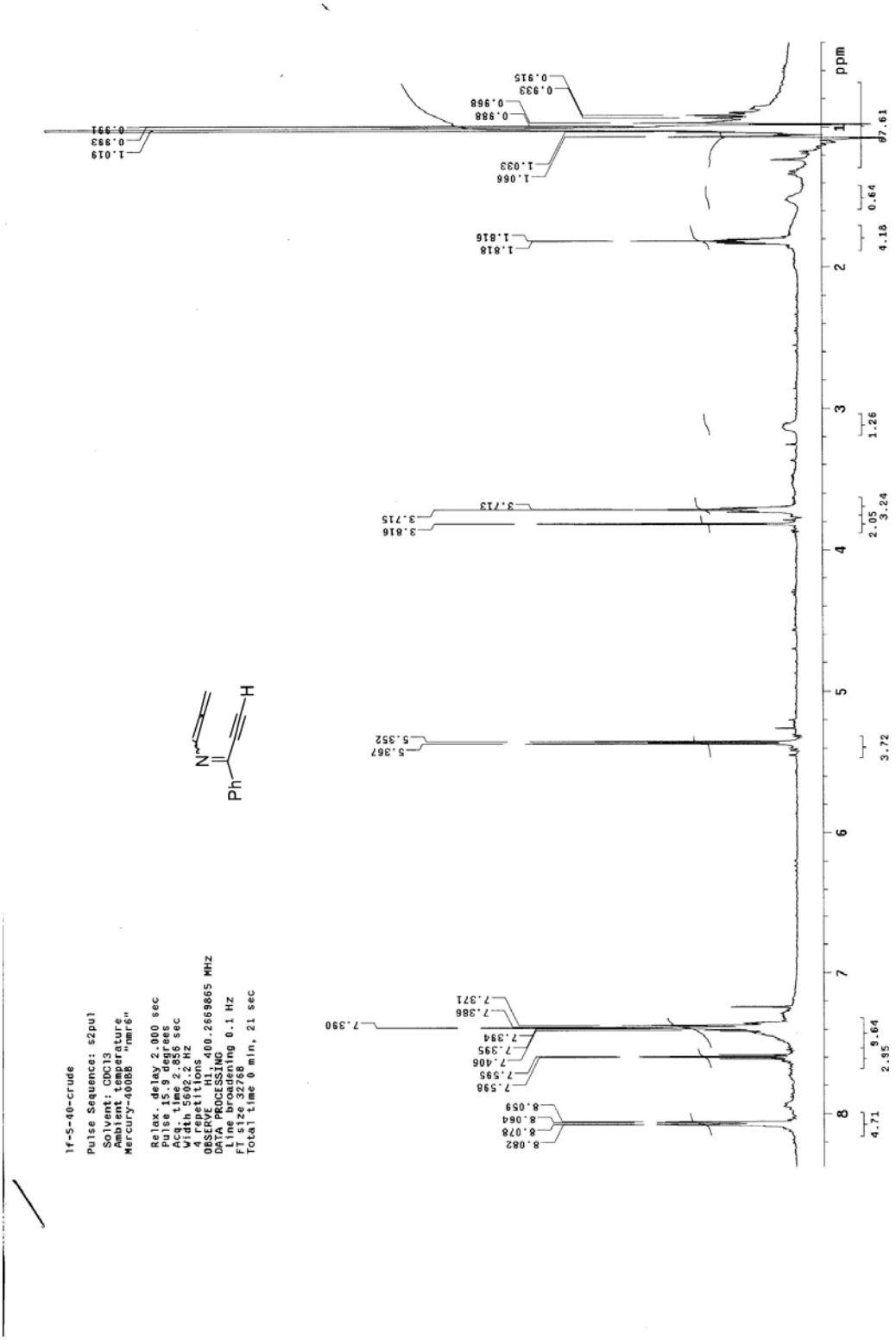
Compound **44**


1f-2-53-crystal
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: 1f-2-53-crystal-h
Mercury-400BB "mpr6"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
28 repetitions
OBSERVE H1, 400.2563146 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 2 min, 21 sec
Ph
N
O
Ph
ppm

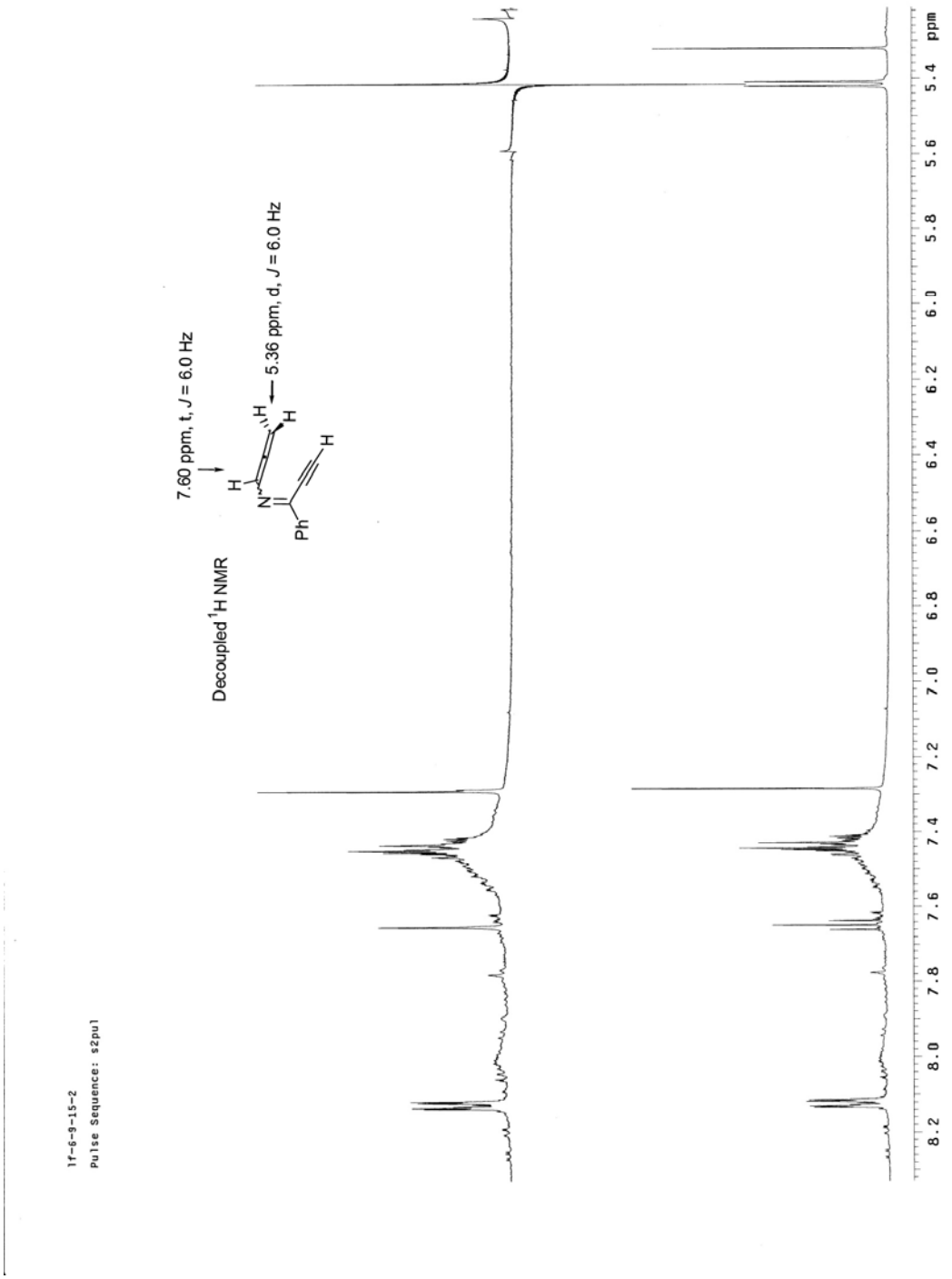
Compound **53e**



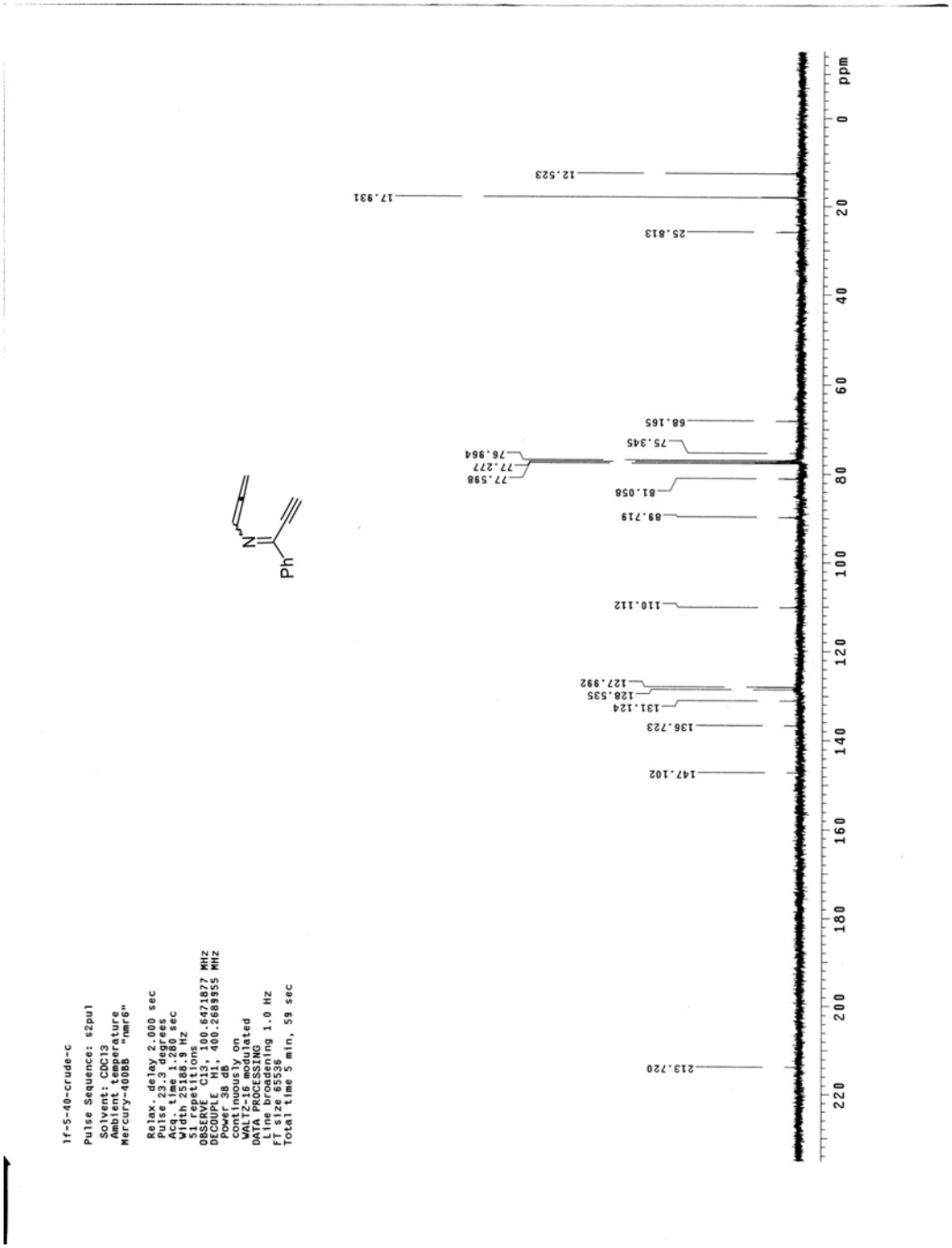
Compound **4d**


1f-5-40-crude
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "nmrs"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
4 repetitions
OBSERVE H1, 400.2669865 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 21 sec
N
Ph
H
ppm

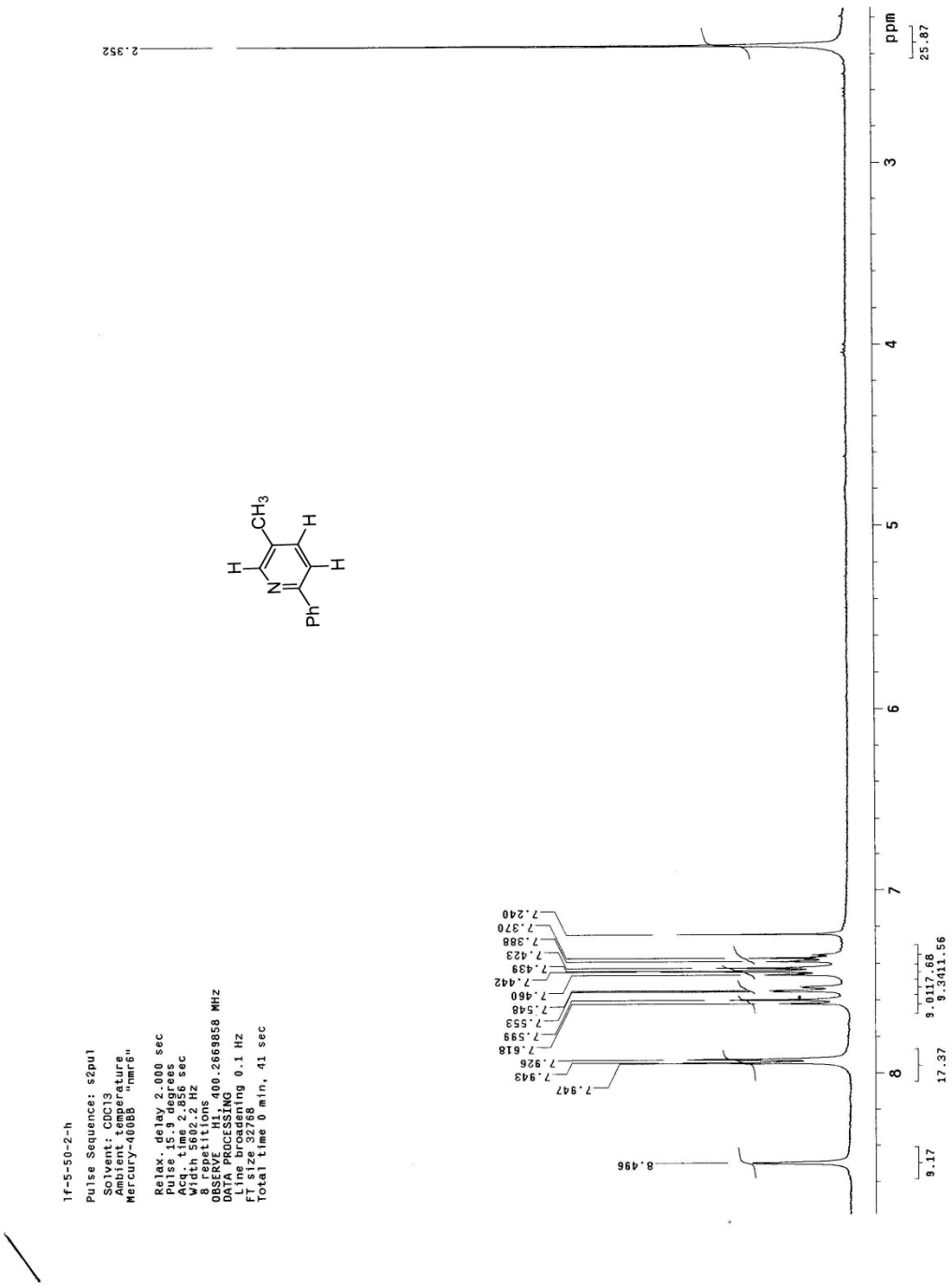
Compound **4d** (decoupled ^{1}H NMR)



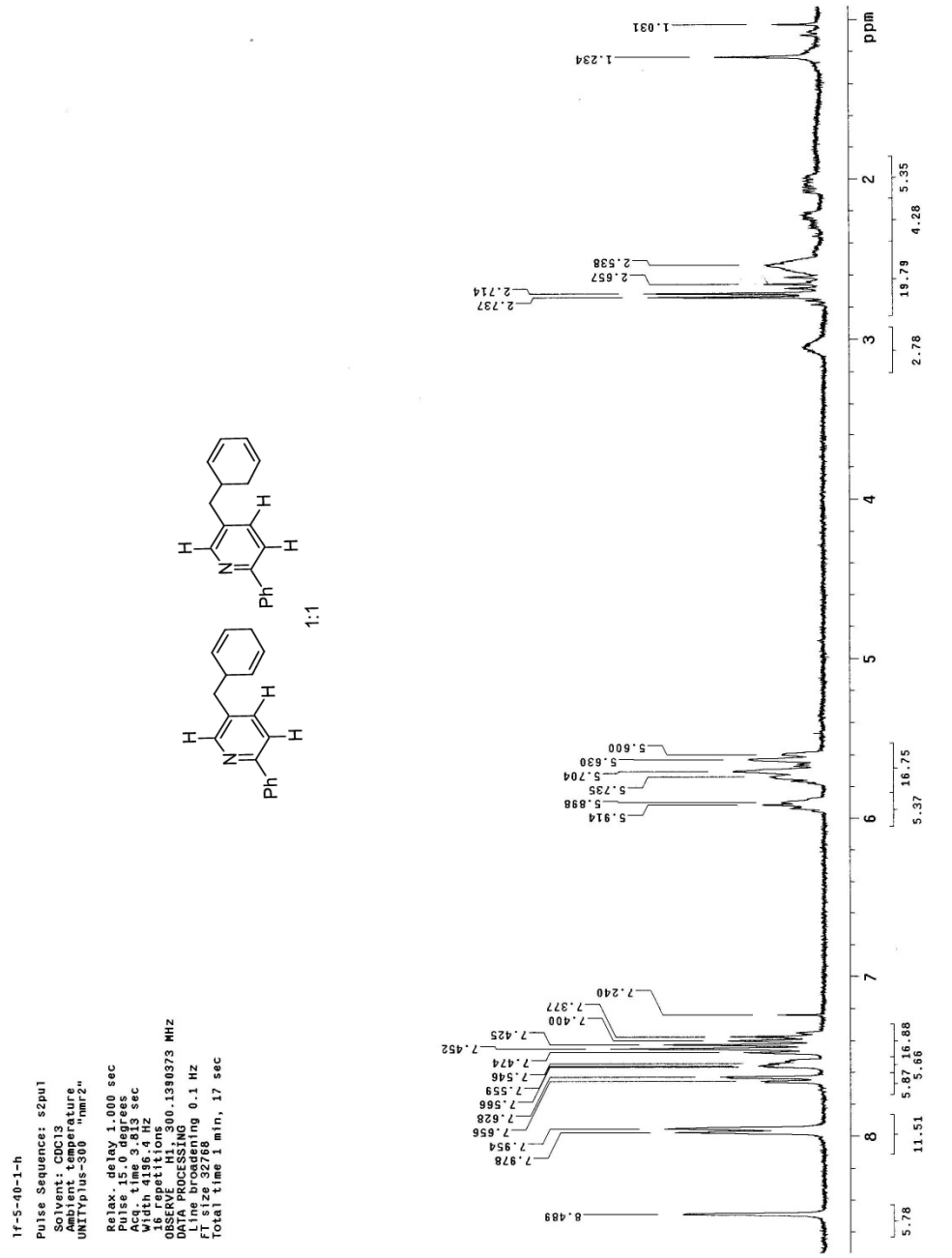
Compound **4d** (^{13}C NMR)



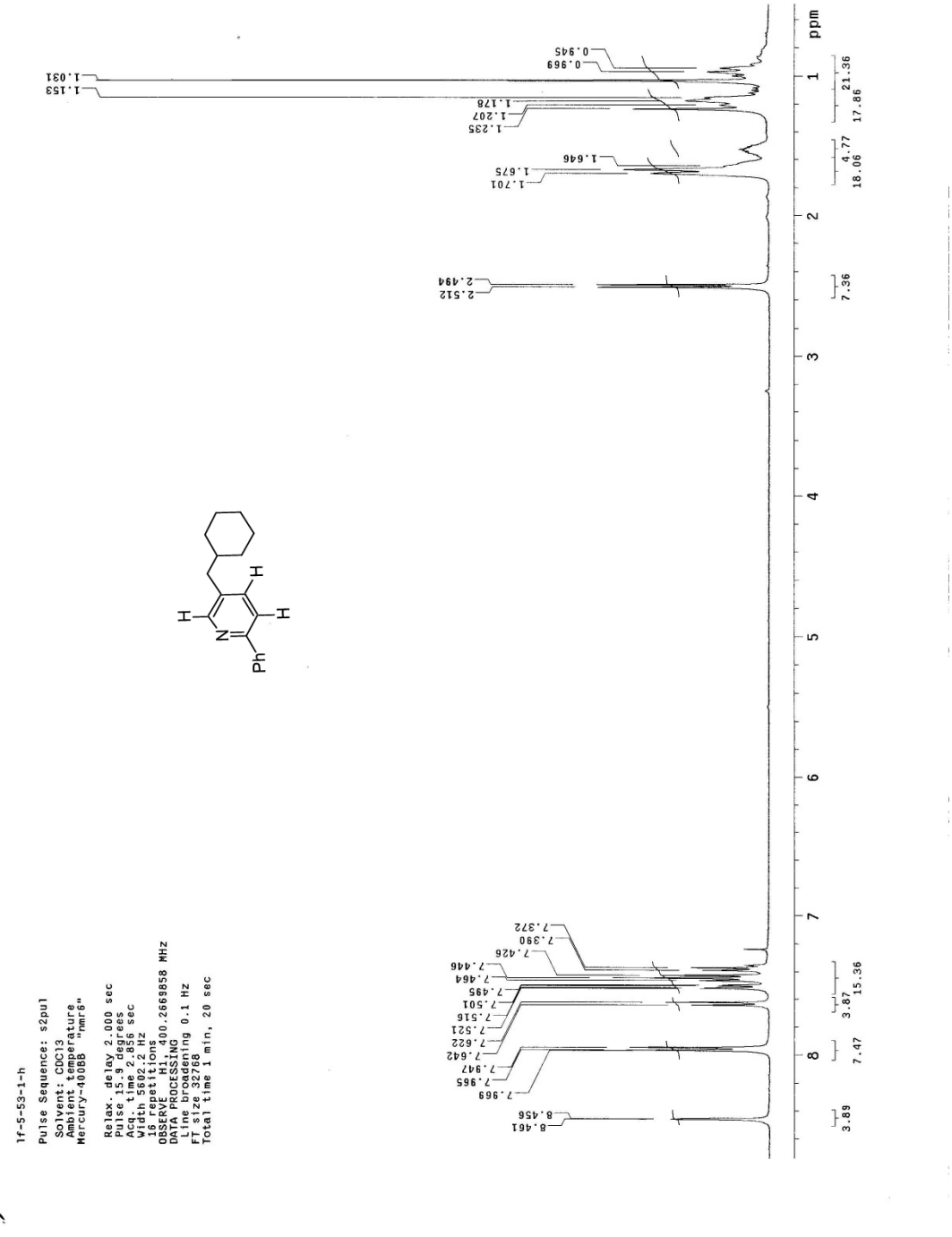
Compound **55**


1f-5-50-2-h
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "nmr6"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
8 repetitions
OBSERVE H1, 400.2669858 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 41 sec
CH3
H
H
H
N
Ph
2.352
8.496
7.947
7.943
7.926
7.618
7.599
7.553
7.548
7.460
7.442
7.439
7.423
7.388
7.370
7.240
ppm
25.87
9.17
17.37
9.0117.68
9.3411.56

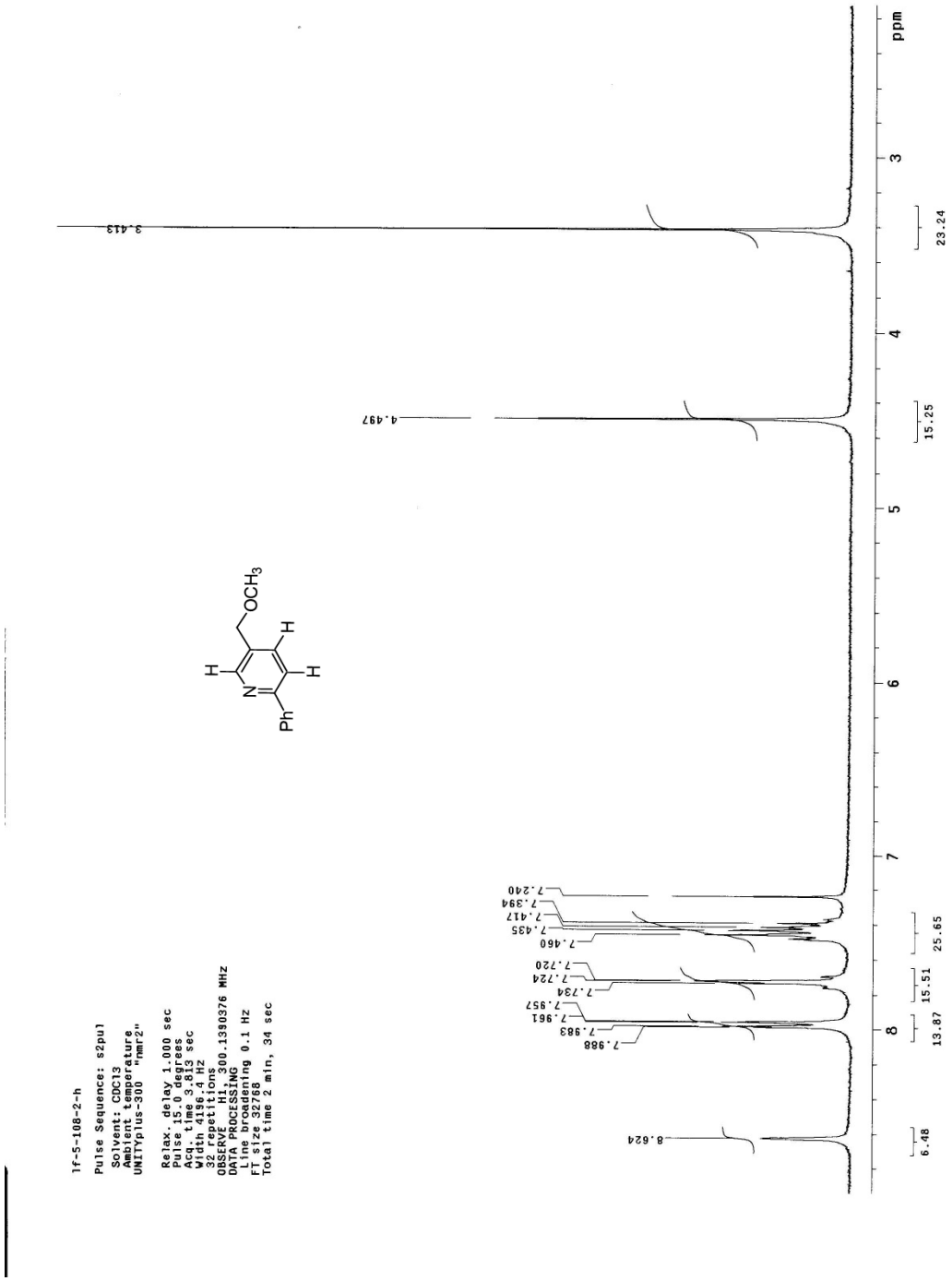
Compound **56a,b**



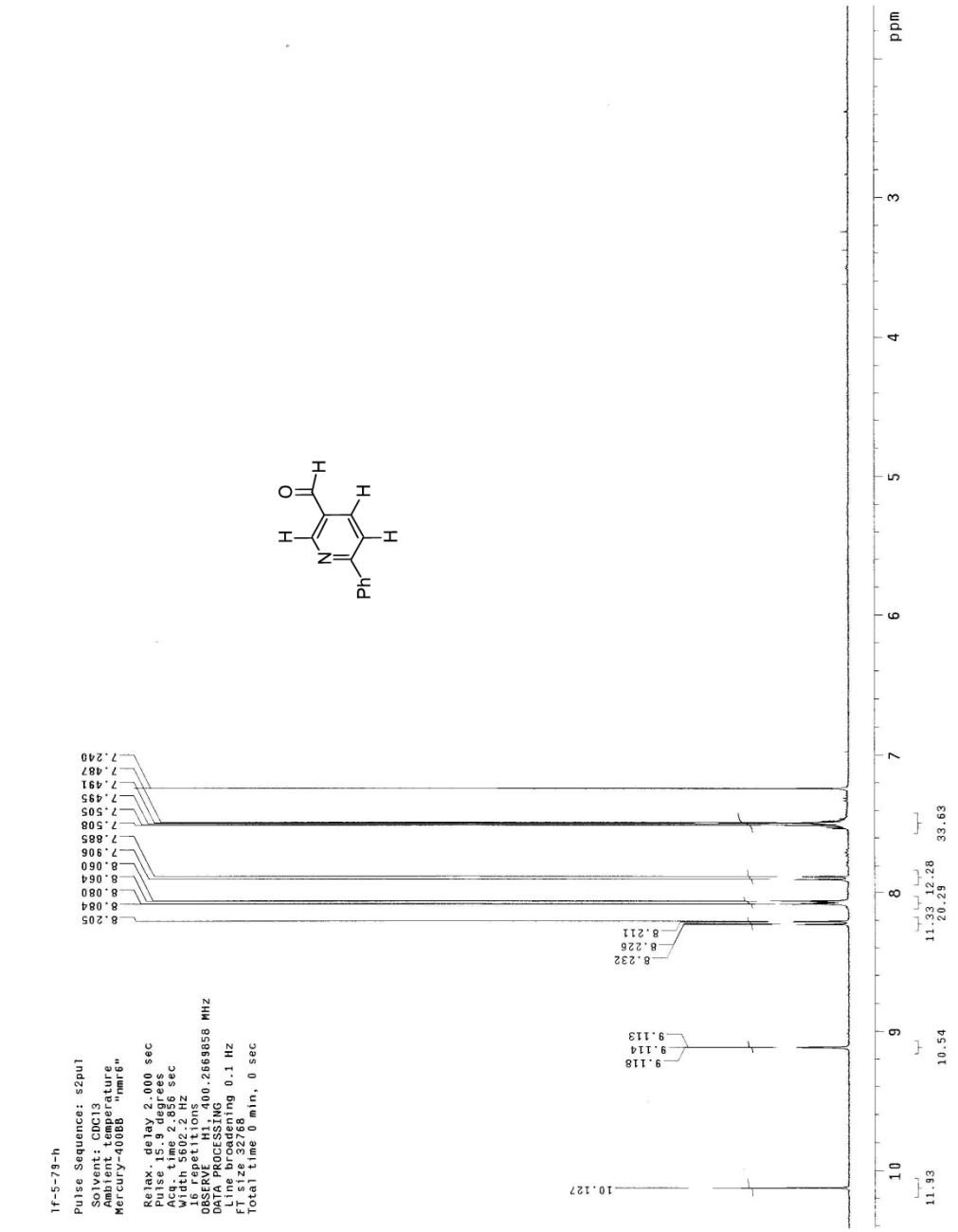
Compound **57**


1f-5-53-1-h
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "nmr6"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
16 repetitions
OBSERVE H1, 400.2669858 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 min, 20 sec
ppm
1.031
1.153
0.969
0.945
1.178
1.207
1.235
1.646
1.675
1.701
2.494
2.512
7.372
7.390
7.426
7.446
7.464
7.495
7.501
7.516
7.521
7.622
7.642
7.947
7.965
7.969
8.456
8.461
Ph

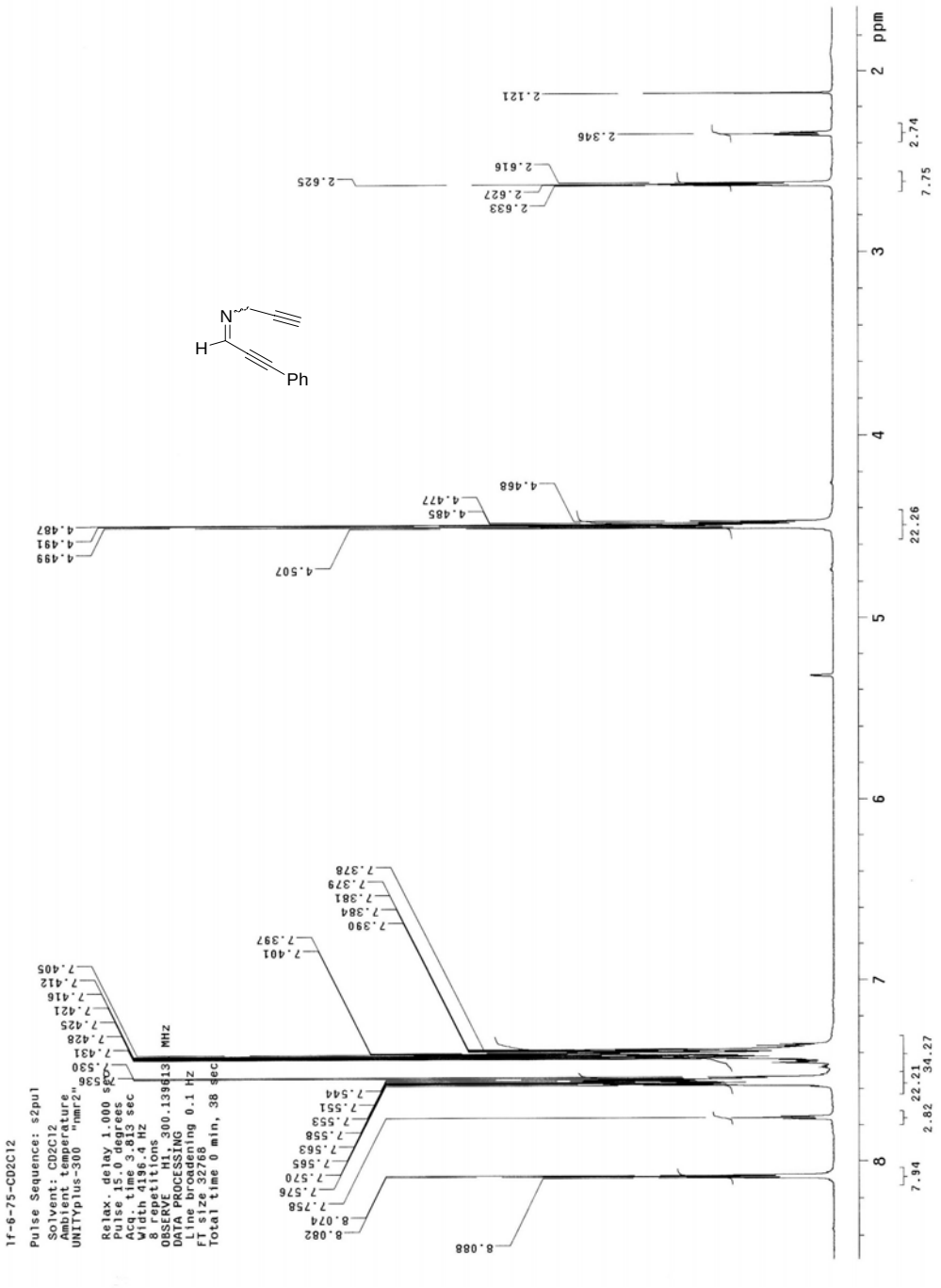
Compound **58**


1f-5-108-2-h
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "rmr2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
32 repetitions
OBSERVE H1, 300.1390376 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 2 min, 34 sec
OCH3
H
H
H
N
Ph
3.413
4.497
7.240
7.394
7.417
7.435
7.460
7.720
7.724
7.734
7.957
7.961
7.983
7.988
8.624
ppm
23.24
15.25
25.65
15.51
13.87
6.48

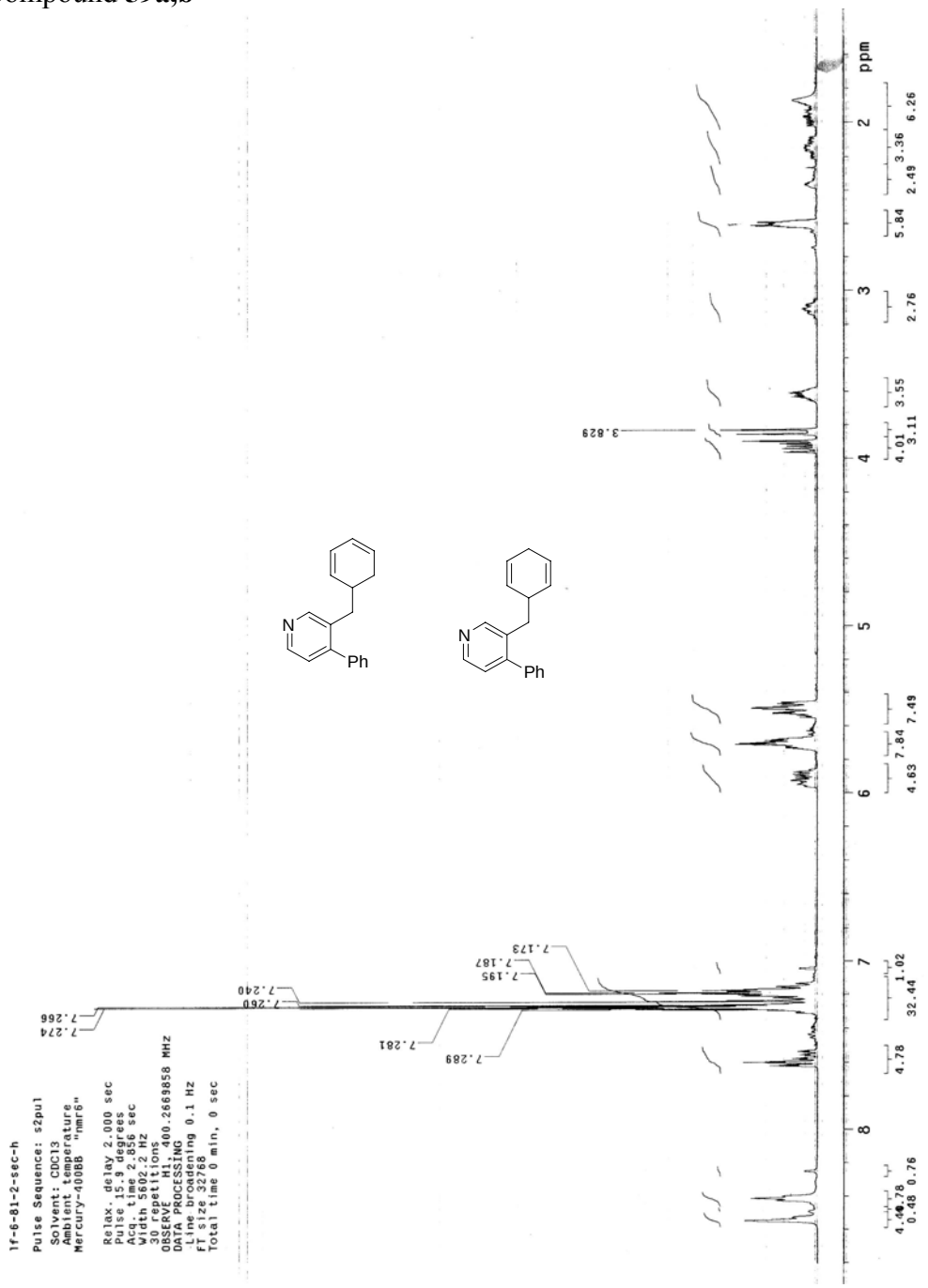
Compound **54**


1f-5-79-h
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "mmr6"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
16 repetitions
OBSERVE H1, 400.2669858 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 0 sec
O
H
H
H
H
N
Ph
8.205
8.084
8.080
8.064
8.060
7.906
7.885
7.508
7.505
7.495
7.491
7.487
7.240
8.232
8.226
8.211
9.118
9.114
9.113
10.127
11.93
10.54
11.33
12.28
20.29
33.63
ppm

Compound **53f**



Compound **59a,b**



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