BIS- AND MONO-CYCLOPROPENONE FUSED DIBENZOCYCLOOCTADIYNES: PHOTOACTIVATABLE LINCHPINS FOR SEQUENTIAL CLICK LIGATIONS

by

DEWEY ANDREW SUTTON

(Under the Direction of Vladimir V. Popik)

ABSTRACT

We have synthesized the first fully conjugated bis-cyclopropenone (photo-DIBOD), a derivative of dibenzo[a,e][8]annulene. 350-420 nm irradiation of these robust compounds results in the efficient formation of dibenzo[a,e]cyclooctadiyne (DIBOD), an unstable, but useful reagent for strain-promoted alkyne azide cycloaddition (SPAAC). Photochemical release of DIBOD allows for full spatial and temporal control of ligations therefrom. We have demonstrated the utility of this platform by derivatizing a model protein, bovine serum abumin (BSA) with a variety of functional moieties.

Additionally, we have synthesized a mono-cyclopropenone caged dibenzocyclooctadiyne (MC-DIBOD). An order of magnitude difference in photo-reactivity between bis- (photo-DIBOD) and mono-cyclopropenone - caged dibenzocyclooctadiynes (MC-DIBOD,) allows for selective mono-decarbonylation of photo-DIBOD. Alternatively, MC-DIBOD is prepared by selective mono-cyclopropanation of dibenzo[*a,e*]cyclooctadiyne. MC-DIBOD permits efficient sequential SPAAC cross-linking of azide-derivatized substrates. Cycloaddition of MC-DIBOD converts an azide moiety into a photo-caged form of triazole-fused dibenzo[*a,e*]cyclooctyne. While azide reactivity MC-DIBOD and dibenzo[*a,e*] cyclooctadiyne (DIBOD) is similar to other cyclooctynes, fusion of a triazole to the

dibenzocyclooctyne system results in three orders of magnitude enhancement in SPAAC rates. In methanol, this triazole reacts with butyl azide at an astonishing rate constant of 34 $M^{-1}s^{-1}$, thus representing the most reactive cyclooctyne analog reported so far. MC-DIBOD was utilized in the preparation of mixed bis-triazoles and derivatization of a protein (BSA) with fluorescent dye and polyethylene glycol.

We have also worked towards the development of a targeted, cleavable photoaffinity label for immunoglobulin G's (IgG). Such a molecule can reversibly bind to the Z domain of Staphylococcal protein A (Z protein), allowing for targeted labelling of IgG's. Subsequent cleavage of Z protein would provide homogeneous antibody-drug conjugates without the immunogenicity associated with Z protein.

INDEX WORDS: click chemistry, SPAAC, dibenzocyclooctadiyne, dibenzocyclooctyne, dipolar cycloaddition, copper-free click reaction, azide, cyclopropenone, photochemistry, photo-labile, Sondheimer diyne

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DEDICATION

I would like to dedicate this work to my parents, Sam and Tina Sutton, and my girlfriend, Mariia Sorokina, for their continuous love and support.

ACKNOWLEDGEMENTS

First of all I would like to thank my research advisor, Dr. Vladimir Popik, for allowing me to join his group and giving me several really nice projects. He is undoubtedly the nicest and most patient P.I. anyone could ever work for.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In his highly acclaimed review, Sharpless coined and described the term click chemistry as reactions which are: "modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods, and [are] stereospecific."[1] One of the reactions mentioned in this work fulfilling these criteria was the 1,3-dipolar (Huisgen) cycloaddition of alkynes and azides to form 1,2,3-triazoles (Figure 1.1a).[2] The triazole products generated from the cycloaddition are extremely robust, stable in biological media, and can be used as convenient isosteres for amide bonds in a variety of applications.[3]

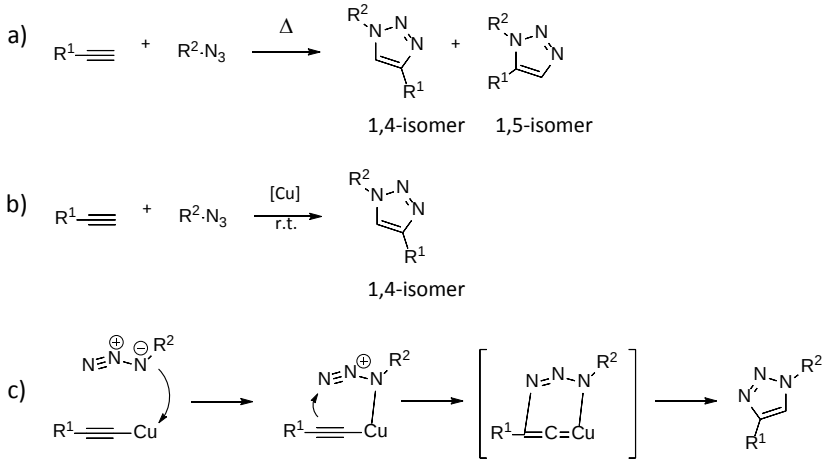


Figure 1.1 Uncatalyzed and Cu-catalyzed azide-alkyne cycloaddition

1.1 Introduction of azides to substrates

The azide moiety is unique in that it is the most stable 1,3-dipole. Azides are stable to oxygen and water, and can be introduced early without interference in subsequent synthetic steps.[4] Importantly, installation of an azide group is straightforward and examples exist in a variety of

disciplines. In organic synthesis, azides can be introduced by both electrophilic and nucleophilic processes.[5] For materials science applications, the azide moiety is easily introduced via nucleophilic substitution.[6] Azides are relatively biologically inert and can be incorporated into biological systems without side reactions or cytotoxicity.[7] Several methods have been developed for the introduction of azides into biological molecules, including pre- and post-synthetic modification of oligonucleotides,[8] metabolic incorporation by feeding cells with azidosugars,[9] and genetic incorporation via an orthogonal synthetase/tRNA pair.[10]

1.2 Copper catalyzed Alkyne-Azide Cycloaddition

The reaction of terminal alkynes with azides is highly exergonic ($\Delta G^{\circ} \approx$-61kcal mol^{-1}), but suffers from a relatively high activation barrier; in fact, reaction of methyl azide and propyne requires activation energy of ca. 25 kcal mol^{-1}.[11] The resulting slow kinetics of reaction limits the applicability of these cycloadditions. Another drawback of the Huisgen cycloaddition of terminal alkynes is its indiscriminate regioselectivity. Because the HOMO-LUMO gaps of alkynes and azides are of similar size, both standard and inverse electron demand pathways are operational, resulting in a mixture of 1,5- and 1,4- adducts.[12] Later, Sharpless[4] and Meldal[13] independently reported a copper-catalyzed variant of this reaction (Figure 1.1b), the copper-catalyzed alkyne-azide cycloaddition, featuring an ultra-fast reaction rate and high regioselectivity. In contrast to the thermal variant, DFT calculations suggest CuAAC proceeds via a stepwise mechanism (Figure 1.1c)[4, 12] which accounts for its selectivity. CuAAC has become the gold standard for the functionalization of a variety of substrates under very mild conditions. In materials science, CuAAC has been utilized in dendrimer synthesis,[14] polymer synthesis,[15] and formation of hydrogels.[16] The reaction is particularly useful in biotechnology for functionalization of nanoparticles.[17] In the field of drug discovery, CuAAC has found use as a valuable tool for high-throughput screening.[18] Chemical biologists have exploited CuAAC to label nucleotides,[19] as well as proteins and carbohydrates.[20] CuAAC has even been used to label the surface of living cells.[21] Unfortunately, the high cytotoxicity of

copper limits the *in vivo* applicability of CuAAC. Copper has been shown to cause oxidative damage to cells by catalyzing formation of hydroxyl radicals.[22] Additionally, Cu^{2+} can induce direct strand scission of DNA.[23] Biological ligations with CuAAC also require the use of protective additives to prevent protein denaturation.[5] Additionally, terminal alkynes have been shown to inhibit intracellular cysteine proteases.[24]

1.3 Strain Promoted Azide-Alkyne Cycloaddition (SPAAC)

To circumvent the use of copper in biological settings, a copper-free variant, strain promoted alkyne-azide cycloaddition (SPAAC) has been developed.[25] In addition to terminal acetylenes, small cyclic alkynes are known to undergo [3+2] cycloaddition with azides. Huisgen first noted that cycloaddition of 1,3-dipoles with strained dipolarophiles such as norbornene proceeds over 100 times faster than with unstrained dipolarophiles, and attributed this to an unknown "factor X."[26] The cycloaddition of cyclooctyne with phenyl azide was first reported by Wittig and Krebs,[27] who found a dramatic rate increase compared to acyclic alkynes. The increased reactivity of cyclooctyne compared to acyclic acetylenes is facilitated by bond angle deformation of the acetylene moiety (163° for unsubstituted cyclooctyne), resulting in a ring strain of ≈18 kcal mol^{-1}.[28] Houk has proposed the increased reactivity of cycloalkynes can be explained by a "distortion/interaction model."[29] This model suggests that the difference in cycloalkyne reactivity is more closely related to the differences of their distortion energies rather than the amount of ring strain released in the product. Presumably, the distortion of the acetylene bonds creates a conformer that more closely resembles the transition state geometry, thereby lowering $\Delta G^{\ddagger}$.[30] In 2004, Bertozzi and coworkers were the first to report the use of a functionalized cyclooctyne (OCT) for labelling of living cells (Figure 1.2).[25] Jurkat cells with metabolically introduced azide moieties on their cell surface displayed dosage and time dependent fluorescence when incubated with a biotinylated cyclooctyne, followed by fluor-avidin stain. The labelled cells also

exhibited no change in viability. This seminal work paved the way for what would become the diverse field of SPAAC conjugation.

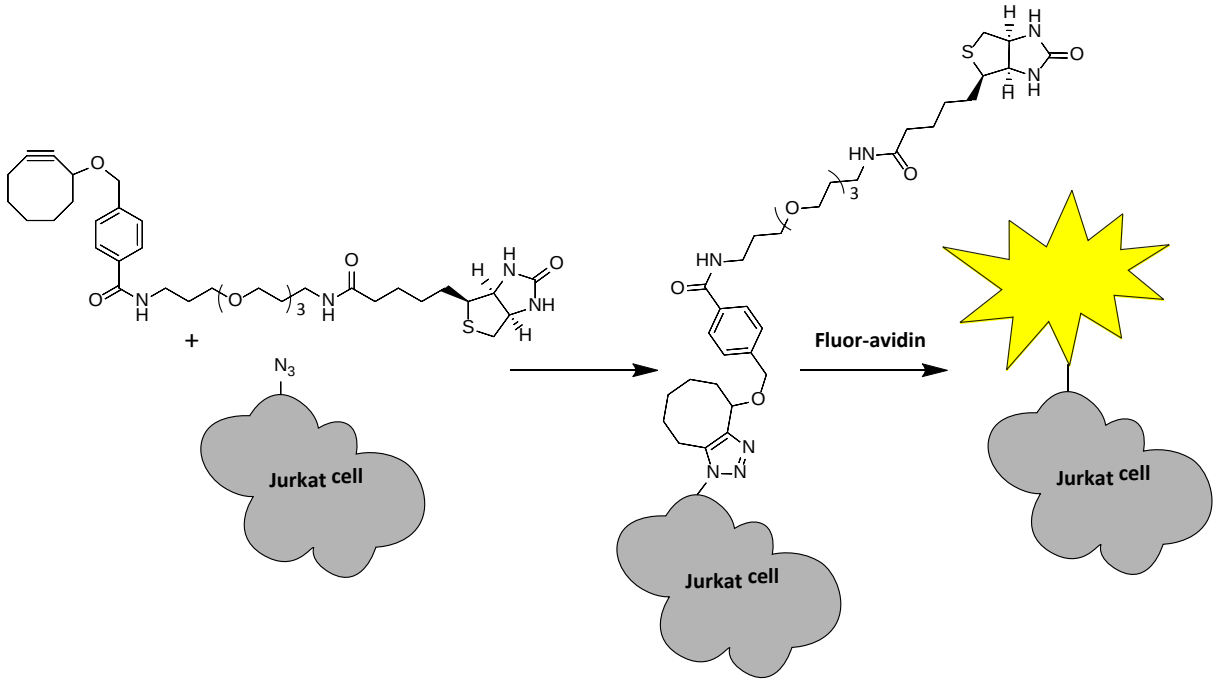


Figure 1.2 Fluorescence labelling of Jurkat cells with OCT

1.4 Diversity and Reactivity of Cyclooctynes for SPAAC

Unfortunately, the parent cyclooctyne OCT **1.2** suffers from rather sluggish reaction kinetics with azides (≈0.001 M^{-1} s^{-1} for benzyl azide). Many recent efforts have been made towards synthesis of more reactive cyclooctynes (Figure 1.3).

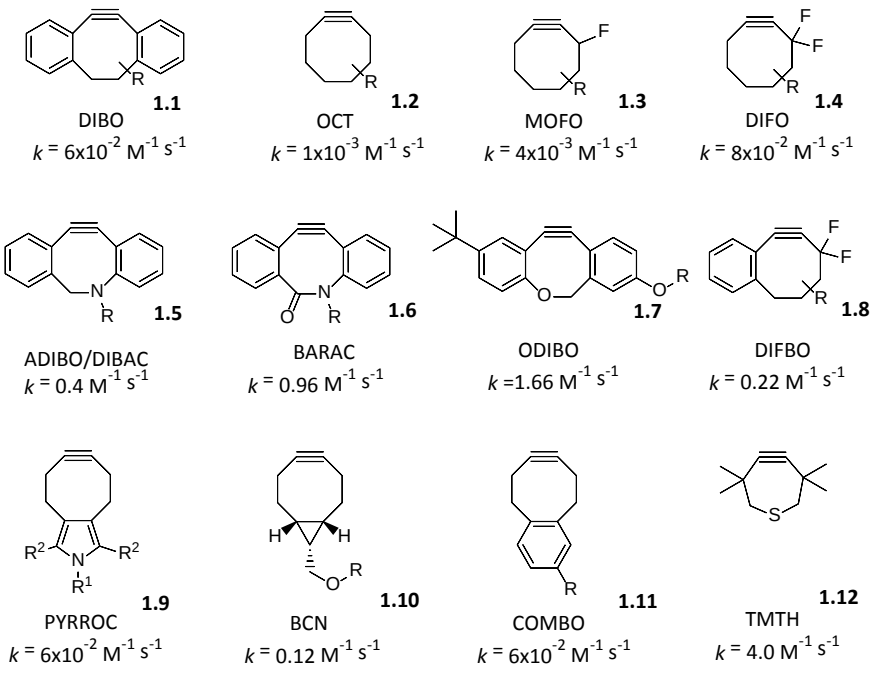


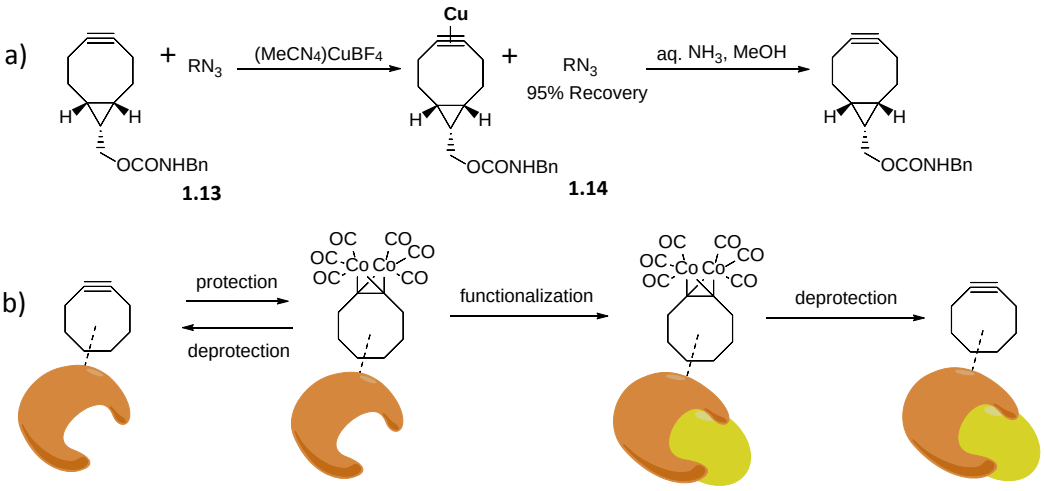
Figure 1.3 Common cyclooctynes and rate constants with benzyl azide

Rate enhancement can be achieved via electronic modification of the strained acetylene. Propargylic substitution with electron withdrawing groups lowers the LUMO of the octyne, increasing its interaction with HOMO of the dipole.[30b] In this case only fluoro- substitution is appropriate, as carbonyl groups encourage Michael addition by intracellular nucleophiles. Bertozzi has synthesized a series of fluorinated cyclooctynes. Mono-fluorinated cyclooctyne **1.3** (MOFO)[31] provides a modest rate enhancement, with second order rate constant ≈0.004 M^{-1} s^{-1} with benzyl azide. Di-fluorinated analogues **1.4** (DIFO)[31-32] feature rate enhancement up to 60-fold (k ≈ 0.08 M^{-1} s^{-1} for benzyl azide). Structural modifications have also been exploited to increase reactivity of cyclooctynes. Fusion of benzene rings to the cyclooctyne core increases ring strain with subsequent rate enhancement. Mono-benzene fused cyclooctyne **1.11** (COMBO)[33] (k ≈ 0.24 M^{-1} s^{-1} with benzyl azide) and di-benzene fused cyclooctyne **1.1** (DIBO)[34] (k ≈ 0.06 M^{-1} s^{-1} with benzyl azide) both display increased reactivity. DIBO in particular receives much attention due to the stabilizing effect of its *ortho* hydrogens, which help to prevent nucleophilic attack on the acetylene. Fusion of a cyclopropane to the cyclooctyne core produces

similar increased reactivity. In 2010, van Delft and co-workers synthesized bicyclo[6.1.0]non-4-yne **1.10** (BCN) ($k \approx 0.14$ M^{-1} s^{-1} with benzyl azide).[35] BCN has enjoyed widespread popularity because of its combination of reduced lipophilicity, relatively fast kinetics, and high stability towards nucleophiles. Interestingly, BCN displays inverse electron demand reactivity, and reacts much faster with electron poor azides. This reactivity has been exploited by van Delft to perform orthogonal ligations with standard electron demand cyclooctynes.[36] Recently, Berg has reported the development of a pyrrole-fused cyclooctyne **1.9** (PYRROC) ($k \approx 0.06$ M^{-1} s^{-1} with benzyl azide),[37] which is unique among SPAAC reagents as it generates isomer-free triazole products. Combination of electron withdrawing groups and benzene fusion produces even further rate enhancement, albeit at the cost of stability. The di-fluorinated, benzene-fused cyclooctyne **1.8** (DIFBO)[38] features high rate of cycloaddition ($k \approx 0.22$ M^{-1} s^{-1} with benzyl azide) but quickly trimerizes in solution. Addition of a heteroatom into the cyclooctyne ring also increases SPAAC reactivity. In 2010, three independent amide-bearing cyclooctynes were reported, each with high reactivity: ADIBO[39]/DIBAC **1.5** ($k \approx 0.30$ M^{-1} s^{-1} with benzyl azide),[40] and BARAC **1.6** ($k \approx 0.96$ M^{-1} s^{-1} with benzyl azide).[41] In 2012, the Popik group published the synthesis of the fastest cyclooctyne described to date, oxa-dibenzocyclooctyne **1.7** (ODIBO) ($k \approx 1.66$ M^{-1} s^{-1} with benzyl azide).[42] Reduction in ring size to cycloheptynes has been explored to increase the kinetics of cycloaddition. The high reactivity of these derivatives comes with the price of extreme instability. Cycloheptyne, for example, has been prepared but only exists as a transient species. The ring strain in cycloheptynes can be partially mitigated by incorporation of a sulfur atom into, due to its greater bond length. Krebs demonstrated this in 1970 by synthesizing a tetramethylthiacycloheptyne **1.12** (TMTH) derivative which was found to be somewhat stable.[43] Bertozzi expounded upon this work in 2012 by exploring the rate of TMTH in SPAAC.[44] The rate was indeed fast ($k \approx 4.0$ M^{-1} s^{-1} with benzyl azide), but TMTH is still too unstable for practical use.

1.5 Protection of Cyclooctynes

While SPAAC has become a paradigm shifting technology, it is not without limitations. One limitation of SPAAC is the low stability of reactive cyclooctynes. Cyclooctyne design always requires a balancing act between reactivity and stability. Increased reactivity of the acetylene often correlates with proclivity towards nucleophilic attack. For example, the less reactive cyclooctyne DIBO has been reported to produce significantly lower azide-independent labelling of cells than the more facile ADIBO and BCN.[45] Cyclooctynes bearing electron withdrawing groups, while displaying much faster kinetics of cycloaddition, are known to display non-specific binding to nucleophilic residues.[46] DIFO, in particular, has been shown to react with glutathione at a rate similar to cycloaddition.[47] Strained cyclooctynes often feature low thermal stability, in fact most commonly used SPAAC reagents undergo rapid hydration in neutral buffer at 60°C.[42] To prevent non-specific labelling with SPAAC reagents, a few strategies have been employed. One can remove all accessible cysteine moieties from the sample by blocking them prior to SPAAC with an electrophile.[45] Alternatively, thiols such as β-mercaptoethanol can be added to the SPAAC mixture to outcompete any accessible thiols.[48] A method to prevent both nucleophilic attack and thermal decomposition is protection of the acetylene moiety with a masking group. In some applications, cyclooctynes can be masked by Cu(I) complexes (Scheme 1.1a).[49]

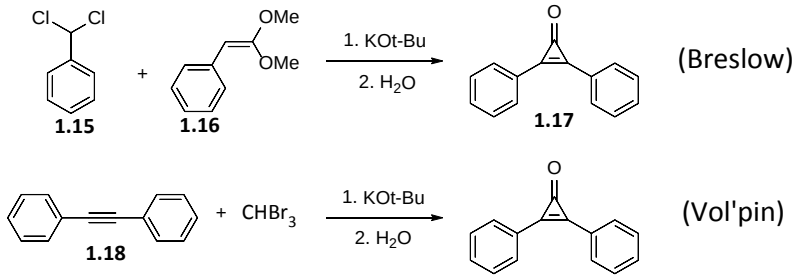


Scheme 1.1 Transient protection of cyclooctynes with metals

Treatment of BCN carbamate **1.13** with 1 equivalent of the cationic Cu(I) salt $(MeCN)_4CuBF_4$ gave rise to a tricoordinate Cu(I) cyclooctyne complex **1.14**. The copper cage can be removed when necessary by treatment with aqueous ammonia. Another protection method has been pioneered by Nicholas,[50] in which an acetylene is masked as a dicobalt hexacarbonyl cluster (Scheme 1.1b).[51] Deprotection of the dicobalt hexacarbonyl cluster can be achieved by oxidative treatment with cerium ammonium nitrate (CAN). These reactions, however, tend to be rather finicky,[49b, 52] and dicobalt octacarbonyl required alkyne protection is highly toxic.[53] A better option that has been developed by Popik is the photo-caging of reactive alkynes as cyclopropenones.

1.6 Cyclopropenones and photo-caging of reactive acetylenes

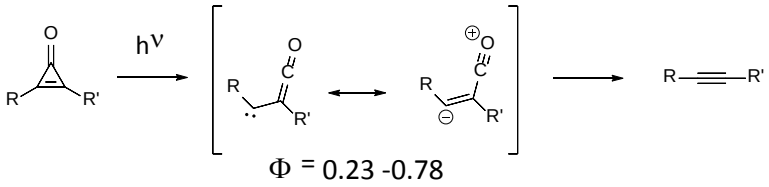
Cyclopropenones have been intensively studied for well over 50 years. The first synthesis of a cyclopropenone was reported independently by Breslow[54] and Vol'pin[55] in 1959 (Scheme 1.2).



Scheme 1.2 Original synthesis of a cyclopropenone

Breslow attained diphenylcyclopropenone **1.17** (DPCP) by addition of phenylchlorocarbene to phenylketene, while Vol'pin was afforded the same product by treatment of diphenylacetylene **1.18** with dibromocarbene. Cyclopropenones possess many unique characteristics which make them intriguing from a fundamental perspective. Cyclopropenones are highly charge delocalized, and represent the smallest Hückel aromatics.[56] Cyclopropenones have been used extensively in organic synthesis (discussion of which is beyond the scope of this work) and summaries can be found in these excellent reviews.[57]

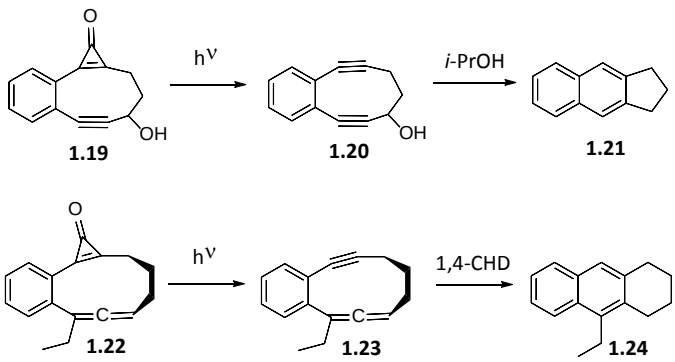
Cyclopropropenones feature high thermal stability due to their high resonance stabilization energy.[58] For example, DPCP displays a reversible melting point of 120°C, with temperatures of 130-140°C necessary to induce thermal decarbonylation, producing carbon monoxide and the corresponding acetylene.[54] Photo-decarbonylation of these species, on the other hand, is remarkably facile. Transient absorption studies reveal complete conversion within several hundred picoseconds of irradiation.[59] In 2003, Popik and coworkers explored the photochemistry of a series of substituted cyclopropenones (Scheme 1.3).[60]



Scheme 1.3 Photo-decarbonylation cyclopropenones

The authors found that photo-decarbonylation proceeded with high efficiency (Φ = 0.23-0.78) and gave the acetylenes in near quantitative yield. Preliminary DFT calculations and transient observation suggested the reaction proceeded via a resonance hybrid intermediate, possessing both carbene and zwitterionic character.[59c, 60] The high stability of the cyclopropenone moiety, combined with the speed and efficiency of photo-decarbonylation, makes it an excellent candidate for use as a photo-cleavable protecting group.[61] Additionally, the use of light as a reagent allows for precise temporal and spatial resolution of the de-protection.

Numerous examples have exploited cyclopropenones to mask unstable or otherwise highly reactive molecules. Popik has used the moiety extensively to photo-cage highly reactive enediynes **1.20** and enyne allenes **1.23** (Scheme 1.4).[60, 62]



Scheme 1.4 Photochemical generation of enediynes and enyne-allenes

These highly strained molecules (**1.19** and **1.22**) undergo thermal Bergman[63] and Myers-Saito[64] cyclization at low temperatures, and the photo-cage allowed for their protection during synthesis, as well as full control of their release. Cyclopropenone masks have been used to protect sensitive cyclooctynes for SPAAC. In 2009, Popik and coworkers synthesized a cyclopropenone-fused 5,6-dihydrodibenzo[*a*,*e*][8]annulene **1.25** (photo-DIBO)[65] and utilized it to label cells with metabolically incorporated azides (Figure 1.4). Bertozzi has used a cyclopropenone photo-cage to release highly unstable dibenzoselenacycloheptynes **1.28** for SPAAC (Scheme 1.5).[66]

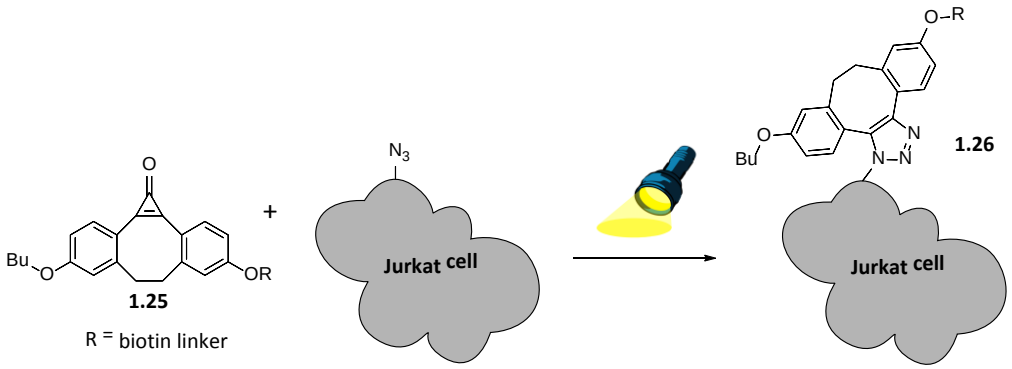
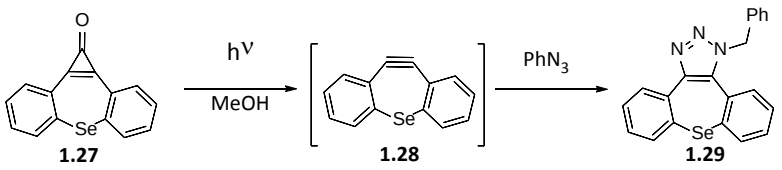


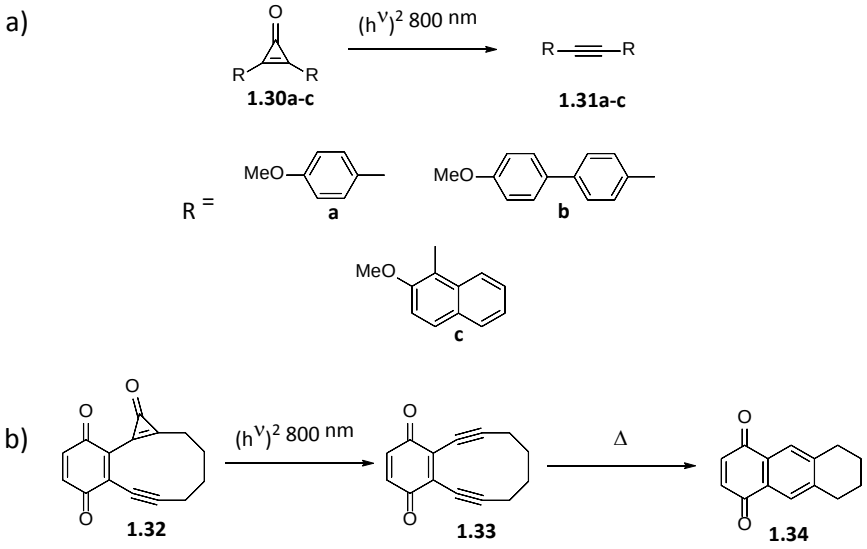
Figure 1.4 Photo-initiated labelling of living cells with photo-DIBO

This methodology can also be applied to polymer synthesis. In 2010, Nuckolls used cyclopropenone caged DIBO derivatives to synthesize reagents for living ring opening alkyne-metathesis polymerization (ROMP).[67]



Scheme 1.5 Photochemical release of dibenzoselenacycloheptynes

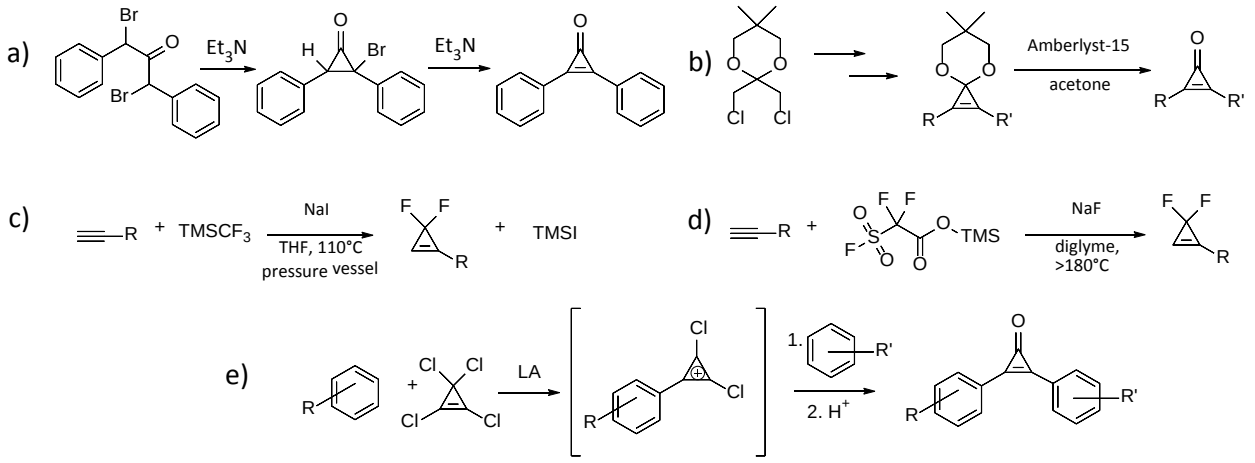
It is important to note that cyclopropenones are adaptable to two-photon excitation, which allows for irradiation in the "photo-therapeutic window"[68] which exists between 650 - 950 nm. Popik has demonstrated two photon excitation for decarbonylation of three substituted cyclopropenones **1.30a-c** with an 800 nm Ti:sapphire laser (Scheme 1.6a).[69] Although the derivatives displayed relatively small two-photon cross sections, the authors attributed this to low extinction of single photon excitation (400 nm). Later work provided proof of concept for two photon release of reactive enediynes **1.33** (Scheme 1.6b).[70]



Scheme 1.6 Two-photon decarbonylation of cyclopropenones

Another feature that makes cyclopropenone protection so appealing is the ease and variety of methods to install them. A number of strategies exist for the synthesis of cyclopropenones (Scheme 1.7). One can envision a *de novo* type strategy in which the cyclopropenone is built from scratch via ring closure, followed by deprotonation and nucleophilic addition to attach the substituents (Scheme 1.7b).

This strategy has been employed by Popik.[60] A strategy for synthesis of aryl cyclopropenones was developed by West in 1974.[71] Friedel-Crafts alkylation of arenes with tetrachlorocyclopropene provides an arylcyclopropenium which is hydrolyzed upon work-up to give aryl cyclopropenones (Scheme 1.7e).

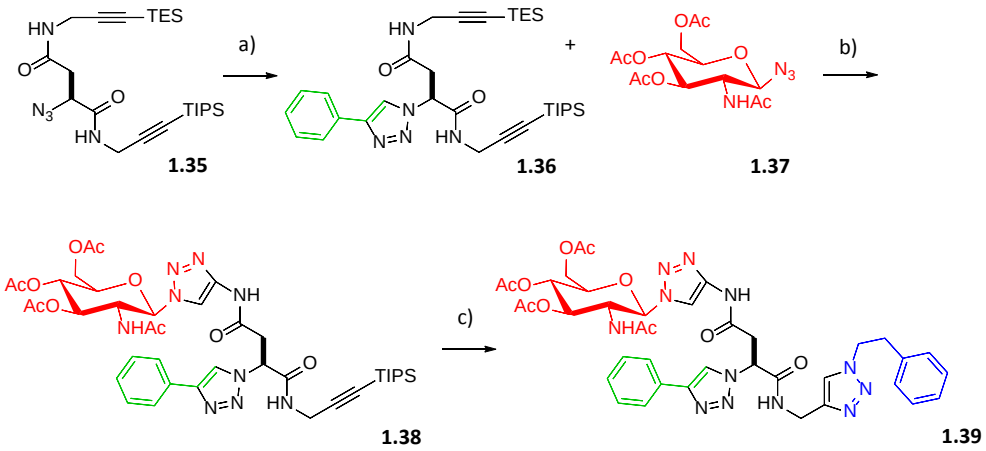


Scheme 1.7 Common strategies for cyclopropenone synthesis

This method has been widely utilized in the literature.[42, 57a, 65-66] Another method for generation of substituted cyclopropenones is Favorskii-type elimination of HBr from α,α'-dibromoketones (Scheme 1.7a).[72] The most common strategy for cyclopropenone synthesis is insertion of dihalocarbenes to alkynes. Difluorocarbene is the most attractive of these, because its greater stability provides the highest yields.[73] The standard method for difluorocarbene insertion is via thermolysis of TFDA (Scheme 1.7d).[73] Unfortunately, this reaction is not well suited for protection of reactive alkynes due to the long exposure to harsh conditions required. Recently, a novel reagent for generation of difluorocarbene was introduced, trifluoromethyltrimethylsilane.[74] The mild conditions offer easy access to a variety of protected cyclooctynes (Scheme 1.7c).

1.7 Sequential Click and Click Crosslinking

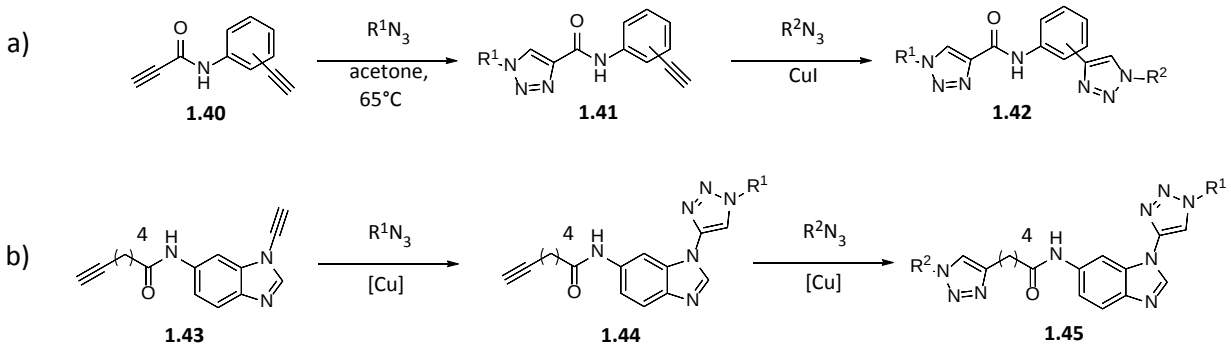
Many applications call for linkage of multiple reporter molecules to a particular substrate. A couple of approaches have been taken towards this end. One strategy is to synthesize a multifunctional linker featuring variably protected alkynes. A good example has been reported by Aucagne (Scheme 1.8).[75]



Reagents and conditions: a)phenylazide, $CuSO_4$' ascorbate; b) 1. $AgNO_3$' MeOH/H_2O, 2. $CuSO_4$' ascorbate; c) 1. TBAF, 2. $PhCH_2CH_2N_3$' $CuSO_4$' ascorbate, 72% overall yield

Scheme 1.8 Aucagne *et Al*' sequential click ligation

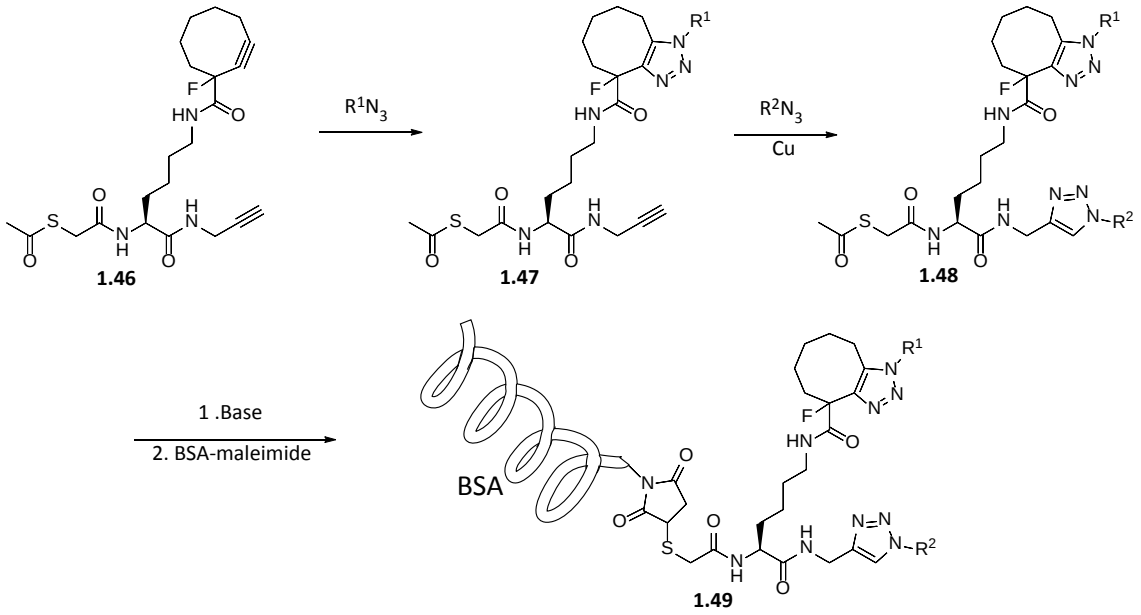
The trifunctional linker **1.35** was first treated with phenylazide under standard CuAAC conditions to give the triazole **1.36**. Selective deprotection of the TES-group with silver nitrate followed by a second CuAAC coupling with glucosamine azide **1.37** provided the bis-triazole **1.38**. Finally, treatment with TBAF followed by a third CuAAC coupling provided the triply functionalized conjugate **1.39**. Selective deprotection of silyl-alkynes has also been used by Carell to conjugate multiple reporters to dNA molecules for FRET imaging.[76] In a similar fashion, Girard has exploited reactivity differences in alkynes **1.40** to prepare multifunctional conjugates **1.42** (Scheme 1.9a).[77]



Scheme 1.9 Dual conjugation via alkyne orthogonality

A thermal Huisgen cycloaddition was used to selectively functionalize conjugate to the electron deficient alkyne of the linker, followed by standard CuAAC with another azide to form triazole. Recently, Burley

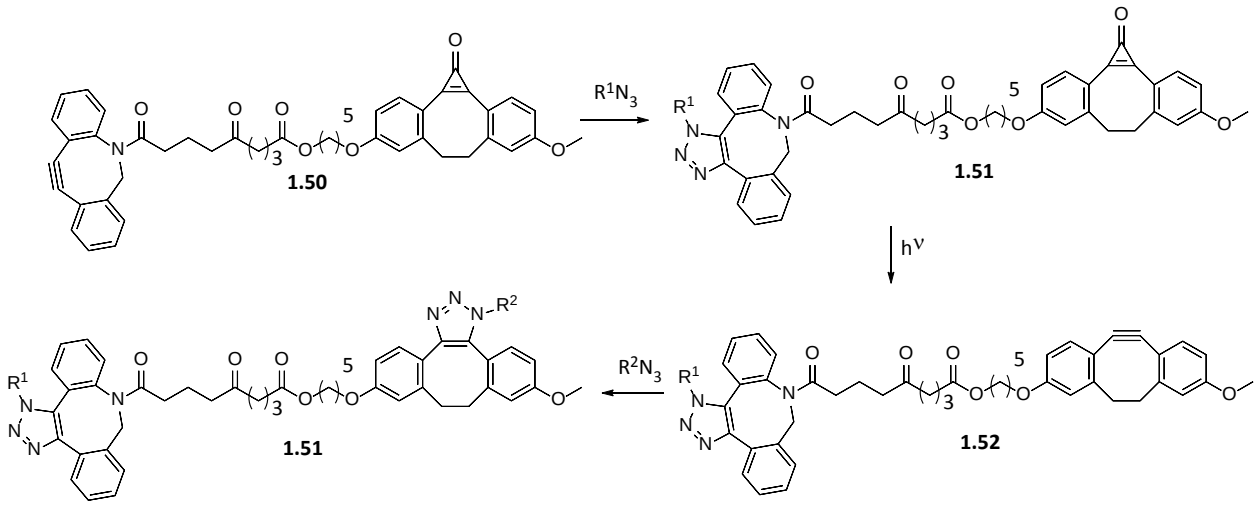
has reported the use of ethynylbenzimidazole derivatives **1.43** for sequential CuAAC ligations (Scheme 1.9b).[78] The authors found that functionalization occurs exclusively on the ynamine moiety when **1.43** is treated with one equivalent of azide and cat. copper. Despite these advancements, this strategy has significant shortcomings. The biggest disadvantage is the need for multiple synthetic steps for installation, deprotection, and purification of silyl-alkynes. Furthermore, the electron deficient alkynes used in Scheme 1.9a are highly susceptible to nucleophilic attack (*vide supra*), and ynamines (Scheme 1.9b) are known to be unstable in water. Another method to prepare multifunctional conjugates is to employ a linker decorated with orthogonal labelling moieties. A typical example of this was published by Beal in 2012 (Scheme 1.10).[79]



Scheme 1.10 Sequential functionalization of BSA

The authors first synthesized the trifunctional linker **1.46**, and the first reporter moiety was attached to the cyclooctyne via SPAAC. A second reporter was added to the terminal alkyne of **1.47** via CuAAC. The linker **1.48** was then treated with base to deprotect the thiol moiety, and incubation with BSA-maleimide provided the desired bis-labelled BSA conjugate **1.49**. This strategy has been widely adopted, and has been applied to the synthesis of peptides,[80] dendrimers,[81] nanoparticles,[82] and functionalized

surfaces.[83] While this strategy requires fewer chemical steps to the linker, it still necessitates extensive pre-functionalization with the appropriate orthogonal groups. Additionally, the highly reactive nature of many cyclooctynes limits the synthetic palette of reactions available for functionalization. A limited number of pre-functionalized cyclooctynes are commercially available, but are rather expensive. Alternatively, two azide-derivatized substrates can be ligated using a bi-functional linker. Functionalization of a wide variety of substrates with azides is well known and relatively straightforward, making this strategy a desirable improvement. The most common method for this is to attach two independent alkyne moieties via a flexible tether. Popik has developed a platform for sequential click conjugation by tethering a cyclooctyne to a cyclopropenone-caged cyclooctyne (Scheme 1.11).[84]



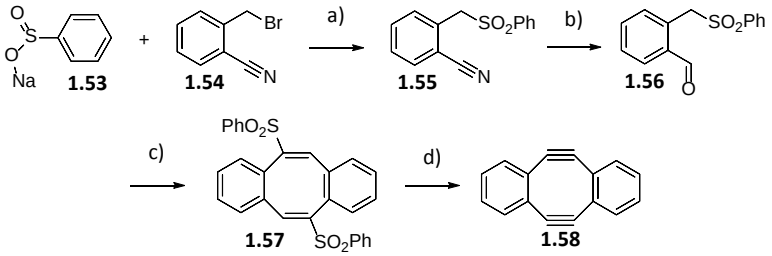
Scheme 1.11 Sequential SPAAC conjugation via tethered cyclooctynes

The linker **1.50** is mono-functionalized via SPAAC to form the monotriazole **1.51**, followed by photochemical decarbonylation of the cyclopropenone. The linker can then undergo further SPAAC reaction to form a bis-triazole **1.52**. The authors demonstrated the utility of this platform by conjugating BSA-azide to an azido fluorophore. A similar strategy employing tethered cycloalkynes has recently employed in the synthesis of hydrogels.[49a] The drawback of this approach is the requirement of lengthy

convergent synthesis of the multifunctional linker. Others have explored the development of a simple, compact platform that can act as a "linchpin" to bind together two functional azides.

1.8 Reactivity and Applications of DIBOD

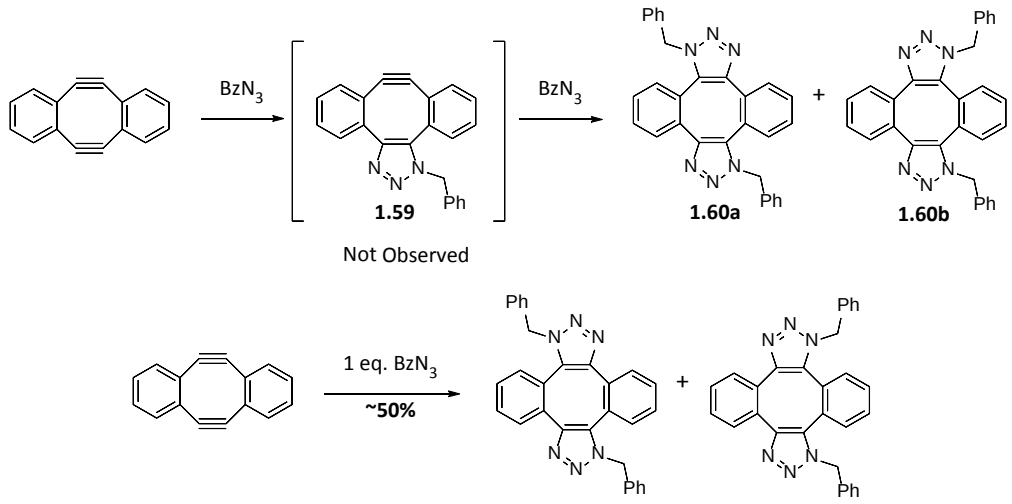
In 2010, Hosoya demonstrated that both simultaneous and sequential click crosslinking are accessible through such a "linchpin" platform, dibenzo[*a*,*e*]cyclooctadiyne **1.58** (DIBOD) aka "Sondheimer diyne."[85] This intriguing molecule was originally synthesized by Sondheimer in 1974,[86] but the very low-yielding preparation limited access to the structure until improved methodologies were developed by Otera and coworkers (Scheme 1.12).[87] The same group has since described the facile synthesis of a host of substituted DIBOD derivatives.[88]



Reagents and conditions: a)DMF, 70°C, 89%; b)DIBAL, -78°C, 78%;
c) $ClP(O)(OEt)_2$' LHMDS, -78°C, 65%; d) LDA, -78°C, 99%

Scheme 1.12 Otera *et Al*. synthesis of DIBOD

Hosoya synthesized DIBOD using the new procedure and studied its reactivity with azides. Treatment of DIBOD with 2 equivalents of azides yielded a mixture of *cis*- **1.60b** and *trans*- **1.60a** bis-triazoles. Kinetic studies revealed a similar rate constant as DIBO ($k \approx 0.06\ M^{-1}\ s^{-1}$) for dual addition of benzyl azide (Scheme 1.13). Interestingly, when DIBOD was treated with 1 equivalent of azide, only the bis-triazole adducts **1.60a-b** could be isolated, implying the second cycloaddition occurred with a much faster rate than the first.



Scheme 1.13 Dual SPAAC reaction of DIBOD aka "Sondheimer diyne"

DFT calculations supported these findings, indicating the activation for the second addition was much lower than the first. The computed activation barrier for the first cycloaddition was 12.4 kcal mol^{-1}, while the activation energy required for the second reaction was 8.8 and 9.5 kcal mol^{-1} (for *trans* and *cis* isomers respectively).

In spite of the increased reactivity of the intermediate mono-triazole **1.59**, sequential functionalization could still be achieved if the first azide substrate was very bulky (i.e. a protein). Hosoya exploited this by functionalizing a resin-bound, azido labelled protein with an azido fluorophore via a DIBOD linchpin (Figure 1.5). Resin bound HaloTag protein **1.61** was incubated in the presence of azido HaloTag ligand **1.62**, DIBOD **1.58**, and TAMRA-azide **1.63** in both sequential and simultaneous procedures. After incubation, the conjugated HaloTag proteins **1.64** were cleaved from the resin and analyzed via SDS-PAGE gel against control without DIBOD. Conjugation yield based on fluorescence intensity measurements were comparable with samples incubated with commercially available TAMRA-HaloTag.

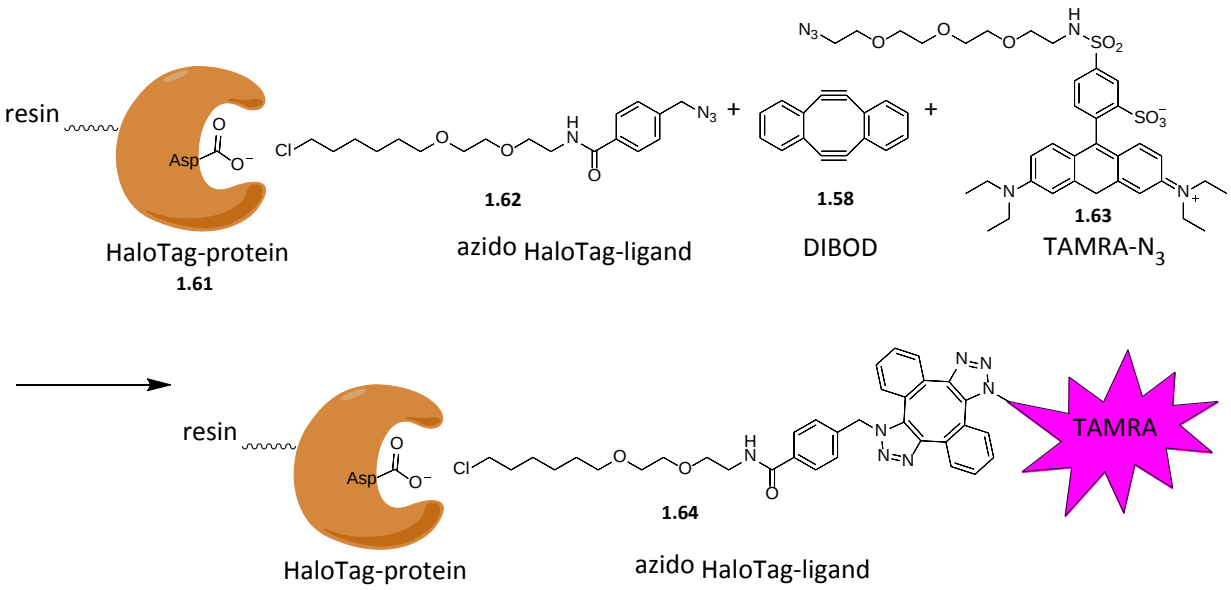
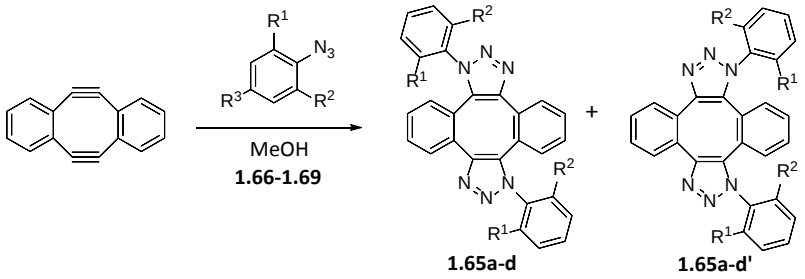


Figure 1.5 Hosoya *et. Al* labelling of HaloTag protein

Later work published by the same authors found that DIBOD reacts much faster with sterically hindered aryl azides (Scheme 1.14).[89] The cycloaddition of di-isopropylphenyl azide **1.69** proceeds ca. 76 fold faster in MeOH than the analogous reaction with phenyl azide **1.66**. Further analysis with other cyclooctynes demonstrated that this trend was not unique to DIBOD, but general to all SPAAC reactions.

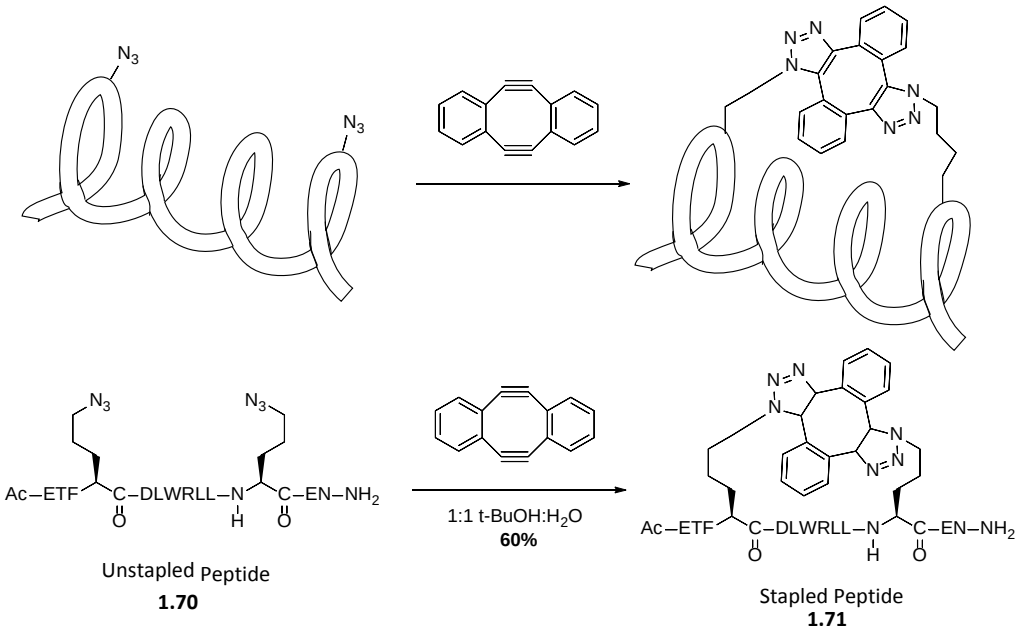


1.66 $R^1 = R^2 = H$ $k = 0.0088\ M^{-1}\ s^{-1}$
1.67 $R^1 = R^2 = Me$ $k = 0.32\ M^{-1}\ s^{-1}$
1.68 $R^1 = R^2 = Et$ $k = 0.38\ M^{-1}\ s^{-1}$
1.69 $R^1 = R^2 = i\text{-Pr}$ $k = 0.67\ M^{-1}\ s^{-1}$

Scheme 1.14 Reactivity of DIBOD with hindered azides

DFT calculations suggested that resonance inhibition of the azide – benzene interaction by the bulky substituent decreased the distortion energy of the azide, in agreement with Houk's model (*vide supra*). The authors proposed that a sequential click strategy could be performed wherein a bis-azide containing one hindered azide could react quantitatively with one equivalent of cyclooctyne, followed by addition of a second cyclooctyne to conjugate the less reactive azide. This method, however, would be subject to all the problems associated with cyclooctyne-bearing substrates.

Peptide stapling is a rapidly growing area of interest in drug discovery. Native peptides mitigate a host of protein-protein interactions, opening up new avenues for possible drugs.[90] An alpha helical region of the peptide typically interacts with an active face of the protein target. Unfortunately, these peptides often change conformations in solution, and are rapidly degraded by proteases.[91] The alpha helical conformers can be stabilized via peptide stapling.[92] To achieve this, different areas of the peptide are functionalized with a linker, and then stapled in place using a macrocyclisation technique.[91] The SPAAC reaction of DIBOD with azides has recently been applied to the field of peptide stapling (Scheme 1.15).[93]



Unstapled Peptide
1.70

Stapled Peptide
1.71

Scheme 1.15 Peptide Stapling with DIBOD

In 2015, Spring and coworkers used DIBOD to staple a library of azide-modified, MDM2 binding peptides **1.70**. The authors noted that peptide stapling with DIBOD proceeded with higher rates than other stapling methods they tried, perhaps due to the rapid kinetics of the second cycloaddition relative to the first.

Despite the recent successes of dual SPAAC ligation with DIBOD, there are still potential pitfalls to its application. The major drawback of the DIBOD platform is stability. Sondheimer reported that some decomposition of DIBOD was noted after 2 days on the benchtop.[86] This thermal instability makes long-term storage impractical. Additionally DIBOD's susceptibility to nucleophilic attack is unknown. Despite the recent interest in the compound, there are no reports of its stability towards nucleophiles, which could limit its applicability. Lastly, DIBOD is a rigid, planar compound unlikely to have favorable aqueous solubility.

1.9 Conclusions and Goals of the Project

CuAAC is the gold standard for decorating a variety of substrates. Unfortunately, the toxicity of copper catalysts limits the applicability of this method. SPAAC has emerged as an alternative technique, but suffers shortcomings as well. Two of the biggest limitations, in particular are: (1) the requirement of lengthy synthesis of coupling partners, and (2) the instability of cyclooctynes needed for rapid functionalization. There is also a growing demand for reagents to use in sequential click ligations. To address these issues, there is a need for a modular SPAAC platform that can act as a linchpin to bind two robust, easily synthesized coupling partners. Azides make excellent candidates for the coupling partners, due to their stability and ease of installation onto a variety of substrates. The platform must possess a simple and scalable synthetic route for its access. The platform should be easily derivatized with a synthetic handle to attach reporter tags or substrates. This allows for conjugation of further components in addition to the two azide substrates. In addition, the broad applicability of this platform must be demonstrated. The platform should be stable under standard biological conditions: aqueous solution,

neutral buffers, intracellular nucleophiles. Photo-caging the platform as a cyclopropenone would provide the necessary stability as well as permit the on-demand release of cyclooctyne. In addition to chemical and biochemical functionalizations, the platform should perform equally as well in materials applications such as polymers, vesicles, hydrogels, surfaces, and nanoparticles.

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CHAPTER 2

CYCLOPROPENONE-CAGED SONDHEIMER DIYNE (DIBENZO[*A*,*E*]CYCLOOCTADIYNE): A PHOTOACTIVATABLE LINCHPIN FOR EFFICIENT CLICK CROSSLINKING[1]

[1] Sutton, D. A., Yu, S.-H., Steet, R., Popik, V. V. *Chem. Commun*., **2016**, *52*, 553-556.

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2.1 Abstract

The first fully conjugated bis-cyclopropenone (photo-DIBOD), a derivative of dibenzo[*a,e*][8]annulene, has been synthesized. 350-420 nm irradiation of this robust compounds results in the efficient formation of dibenzo [*a, e*] cyclooctadiyne, an unstable, but useful SPAAC cross-linking reagent. Since photo-DIBO does not react with organic azides, this method allows for the spatiotemporal control of the ligation of two azide-tagged substrates.

2.2 Introduction

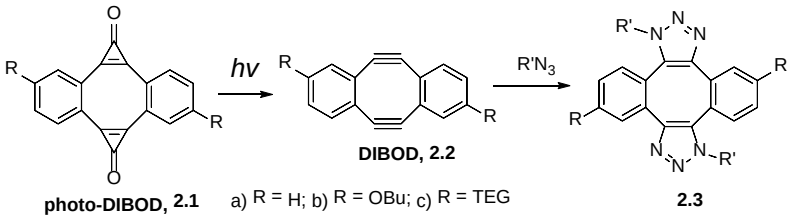
In recent years the copper (I) - catalyzed 1,3-dipolar cycloaddition of azides to terminal acetylenes (CuAAC),[1] as well as the strain-promoted, catalyst – free version of this reaction (SPAAC),[2] have become very popular tools for the functionalization, cross-linking, and immobilization of various substrates.[3] These quintessential "click-chemistries" have found numerous applications in molecular biology,[3b, 3c, 4] drug development,[5] biotechnology,[6] and materials science.[3a, 7] The SPAAC ligation is better suited for applications in living systems as it avoids the use of cytotoxic copper catalysts.[8] In addition, terminal acetylenes were found to inhibit cysteine proteases by forming thioethers with the catalytically active thiol.[9] The utility of the SPAAC technique, on the other hand, is somewhat restricted by the requirement for the derivatization of the coupling partners with two different functionalities. While introduction of an azide group is well developed for a large variety of substrates, the incorporation of cyclooctyne moieties often requires multiple synthetic steps. A limited number of pre-functionalized SPAAC reagents are available commercially, but are rather expensive. Alternatively, two azide-derivatized substrates can be ligated using a bifunctional linker.[10]

Dibenzo[a,e]cyclooctadiyne (DIBOD **2.2a**, a.k.a. Sondheimerdiyne), represents a unique cross-linking platform in this sense,as it contains two azide-reactive strained triple bonds within the same cyclic structure and allows for double-SPAAC click ligation of azide-tagged substrates.[11] Diyne **2.2a** has been employed for the cross-coupling of various azide-functionalized substrates from biomolecules to

metal–organic frameworks.[11-12] The utility of this method, however, is limited by the low stability of the Sondheimer diyne. In the neat form, **2.2a** completely decomposes within two days and exhibits half lifetime of only 10 min in a neutral aqueous solution (1 mM in PBS, pH 7.4, r.t.).[13]

2.3 Synthesis and characterization of photo-DIBOD

To address this deficiency of the DIBOD cross - linker, we envisaged protecting (or "caging") triple bonds in the molecule with a photolabile functionality. Photochemical decarbonylation of cyclopropenones is one of the most efficient methods for the generation of reactive acetylenes, as it is characterized by very fast rate, quantitative chemical and high quantum yields.[14] This reaction can be induced by NIR light under two-photon excitation conditions[15] and proceeds smoothly in the solid state.[16] We have already employed this reaction for the generation of reactive triple bonds in ynols,[17] enediynes,[15, 18] and dibenzocyclooctynes.[19] Irradiation of bis-cyclopropenone **2.1** (photo-DIBOD) results in the formation of the Sondheimer diyne **2.2**, which, in turn reacts with two azide-functionalized substrates to create a stable covalent cross-link (Scheme 2.1). It is important to note that the addition of second equivalent of azide to the diyne **2.2a** proceeds at much higher rate than the first cycloaddition.[11a]

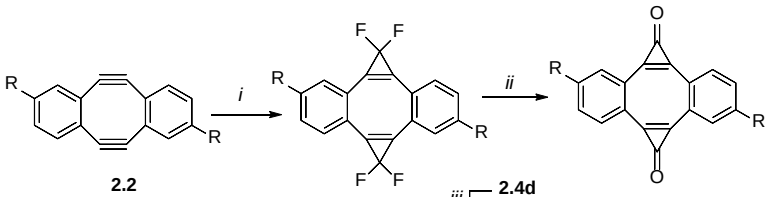


Scheme 2.1 Photochemical generation of DIBOD

Photochemical generation of cross-linker **2.2** from azide-inert precursor **2.1** is not only reagent-free, but also allows for the spatiotemporal control of the ligation process. This reaction expands the family of recently developed "photo-click" ligation methods,[20] including cycloaddition of alkenes to photochemically generated nitrile imines,[21] photo-initiated thiol-ene,[22] and thiol-yne[23] reactions, light-induced Diels-Alder,[24] as well as photo-Michael reaction.[25] Additionally, photo-DIBOD (**2.1**) is an

interesting molecule in its own right, representing the first known fully conjugated bis-cyclopropenone. Despite the presence of the cyclooctatetraene core and the obvious angle strain, this compound is surprisingly stable in the solid state and in solutions (*vide infra*).

We envisioned the preparation of dibenzo[*a,e*]dicyclopropa[*c,g*] [*8*]annulene-1,6-dione (**2.1a**) and its derivatives by the double addition of dihalocarbene across the triple bonds in dibenzocyclooctyne **2.2** followed by the controlled hydrolysis. To address the limited solubility of the parent diyne **2.2a**, we have also synthesized 2,8-dibutoxy- (**2.2b**) and 2,8-bis-[tetra(ethylene glycol)]- (**2.2c**) substituted analogs. Diynes **2.2a-c** were prepared using the modified method of Otera and coworkers.[13, 26] Standard protocols for the generation of difluorocarbene require elevated temperature and harsh conditions[27] that are not compatible with the highly sensitive diynes **2.2a-c**. The milder conditions of the recently developed Hu-Prakash reaction[28] allow for the efficient formation of difluorocarbene without significant decomposition of the substrate. Treatment of diynes **2.2a**,**b**,**d** with $TMSCF_3$ resulted in the formation of the bis-difluorocyclopropenes **2.4a**,**b**,**d**, which were subsequently hydrolyzed on wet silica gel to afford the target bis-cyclopropenones **2.1a-c** (Scheme 2.2). The tetra(ethylene glycol) substituents in **2.2c** were capped with acetyl groups prior to cyclopropenation. The ester protection was later removed by base-promoted hydrolysis (Scheme 2.2).[13]



Reagents and conditions: i) $TMSCF_3$, NaI, 110°C, 2.4a: 60%, 2.4b: 80%, 2.4c: 25%; ii) wet silica gel, 2.1a: 81%, 2.1b: 73%; iii) K_2CO_3, MeOH (2.1c: 53% over 2 steps, ii and iii)

Scheme 2.2 Synthesis of photo-DIBOD

Bis-cyclopropenones **2.1a-c** are bright yellow high-melting crystalline compounds[13] that have a long shelf life if stored in the dark. The spectrum of photo-DIBOD **2.1a** in the UVA region (Fig. 2.1) contains two close-lying bands at 342 (log ε=4.0) and 361 nm (log ε= 4.1), as well as two intense bands at shorter wavelengths (260 and 268 nm, log ε~ 4.8). Two weaker bands in the visible region (409 nm, log ε= 2.9; 438 nm, log ε= 2.6) are responsible for the color of these compounds (Fig. 2.1). Alkoxy-substitution in **2.1b** and **2.1c** results in the 17 nm red shift of the UVA bands, which is accompanied by a moderate hyperchromic effect (360 nm, log ε ~ 4.5, 378 nm, log ε~ 4.4). Photo-DIBODs **2.1a-c** do not react with organic azides under ambient conditions. Irradiation of a methanol solution of photo-DIBO **2.1a** with 350 nm light in the presence of butyl azide results in efficient decomposition of the starting material (Φ=0.05), which could be observed by the bleaching of the 342-361 nm bands. An isomeric mixture of head-to-tail (**2.5**) and head-to-head (**2.6**) bis-triazole adducts was isolated from the photolysate in 77% preparative yield (Fig. 2.1).

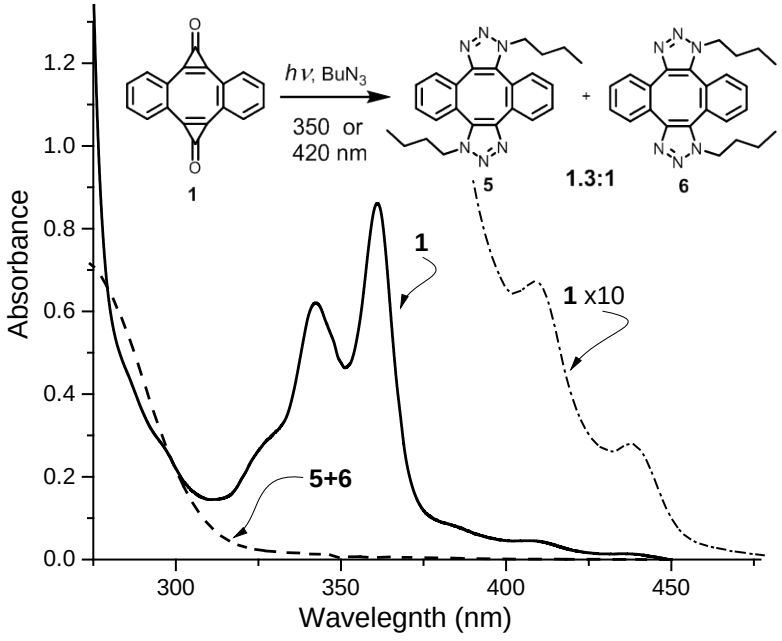


Figure 2.1. UV spectra of ca. 80 μM solutions of *photo*-DIBOD (**2.1a**, solid line) and a mixture of bis-triazoles (**2.5** and **2.6**, dashed line), as well as 0.78 mM solution *photo*-DIBOD (**2.1a**, dash-dotted line) in MeOH-CH_2Cl_2 (9:1).

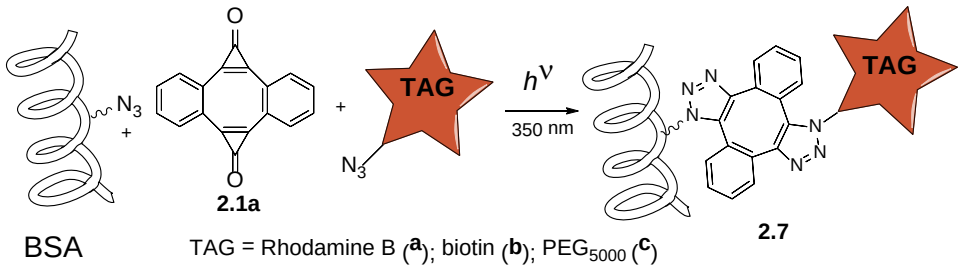
Interestingly, while the absorbance bands at 409 and 438 nm have relatively low intensity, irradiation of photo-DIBOD **2.1a** with 420 nm fluorescent tubes in the presences of butyl azide produced the same mixture of triazoles **2.5** and **2.6**. Complete conversion, however, requires longer irradiation times than with 350 nm lamps. Sensitivity to the violet light makes the photo-DIBOD cross-linking procedure compatible with the majority of modern confocal fluorescent microscopes.

Since photo-DIBOD derivatives **2.1a-c** have very low aqueous solubility, we decided to explore the feasibility of conducting photo-crosslinking experiments employing nanocrystalline suspension of photo-DIBOD. Garcia-Garibay has previously reported that irradiation of the nanocrystalline suspension of diphenylcyclopropenone results in the efficient decarbonylation of the substrate.[29] In fact, the quantum yield of the process in the solid state is about 4 times higher than in solution due to "quantum chain reaction."[29-30] The nanocrystalline suspension of photo-DIBOD **2.1a** was prepared using reprecipitation techniques.[29] A methanol solution of the substrate was slowly added to an aqueous PBS buffer containing 15 mM of SDS under sonication. Dynamic light scattering (DLS) studies performed on a photo-DIBOD suspension revealed a mean distribution of particles at 572±130 nm.[13] The aqueous suspension of photo-DIBOD is chemically stable in the dark. While the intensity of the absorbance bands of **2.1a** slowly decreases with time, this observation can be attributed to the precipitation of the substrate. Even after 48 h, full absorbance value was restored by sonication of the mixture for 15 min. In accordance with previously reported observations,[16, 29-30] the efficiency of photodecarbonylation of **2.1a** shows dramatic enhancement in crystalline state (Φ = 0.41±0.8).

2.4 SPAAC ligations with photo-DIBOD

To demonstrate the utility of the photo-DIBOD crosslinker for biochemical ligations, we have derivatized a model protein, azide-tagged bovine serum albumin (azido-BSA), with a variety of functional moieties. The latter was prepared by treating the commercially available BSA with 1-azido-3-iodopropane in TRIS buffer.[13] Fluorescent labeling of azido-BSA was achieved by irradiation of 0.4 μM

PBS solution of the protein containing 4 μM of the nanocrystalline suspension of **2.1a** and 4 μM of Rhodamine B azide for 8 min using 350 nm fluorescent tubes (Scheme 2.3).



Scheme 2.3 Photo-labelling of azido-BSA

The SDS-PAGE analysis of the photolysate, conducted after overnight incubation in the dark, clearly shows the efficient cross-conjugation of azido-BSA with fluorescent dye (Fig. 2.2). Incubation of the same mixture in the dark overnight, as well as irradiation of azido-BSA – Rhodamine-azide mixture, does not produce labelled BSA **2.7a**. As a positive control experiment, we have incubated azido-BSA – Rhodamine-azide mixture with freshly prepared Sondheimer diyne **2.2a**. As the gel image illustrates, the photo-DIBOD conjugation is at least as efficient as the use of dibenzocyclooctadiyne **2.2a** (Fig. 2.2).

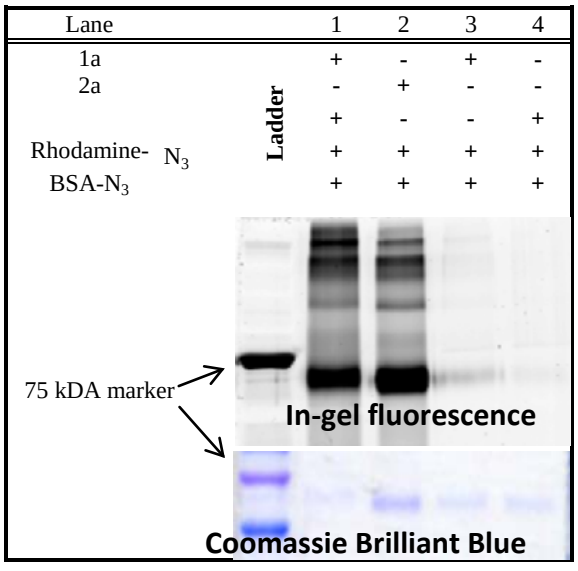


Figure 2.2 SDS-PAGE analysis of BSA - Rhodamine B conjugation. Lane 1: photo-DIBOD 1a, azido-BSA and Rhodamine B - azide irradiated; Lane 2: 2a, azido-BSA, and Rhodamine B - azide incubated overnight;

Lane 3: photo-DIBOD 1a, azido-BSA, and Rhodamine B - azide incubated overnight in the dark. Lane 4: azido-BSA and Rhodamine B - azide incubated overnight**.**

Photo-biotinylation of azido-BSA has been conducted following a similar procedure. A PBS solution of azido-BSA (0.4 μM), biotin-azide (4 μM), and nanocrystalline suspension of **2.1a** (4 μM) was irradiated with 350 nm light for 5 min and incubated overnight. The protein was separated from low-molecular weight compounds by spin-filtration and further purified by size-exclusion chromatography. Western blot - anti-biotin-HRP analysis demonstrates the efficient photo-biotinylation of azido-BSA (Fig. 2.3).[13] As in the previous experiment, azido-BSA – biotin-azide cross-linking using Sondheimer diyne **2.2a** in the dark has been employed as a positive control.

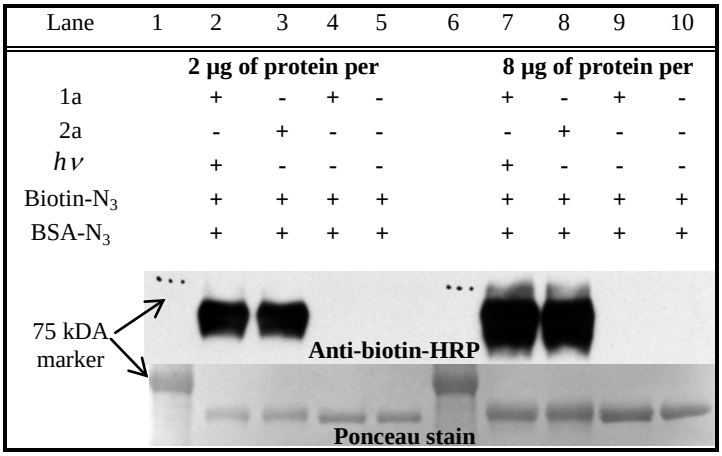
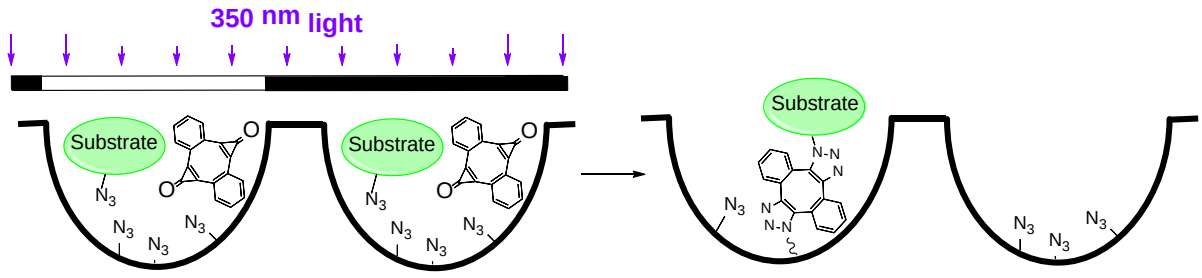


Figure 2.3. Western blot analysis of azido-BSA – Biotin-azide conjugation using different protein loading: lanes 1-5, 2 μg per lane and lanes 6-7, 2 μg per lane. Lanes 2,7: photo-DIBOD **2.1a**, azido-BSA and Biotin-azide irradiated; Lanes 3,8: **2.2a**, azido-BSA, and Biotin-azide incubated overnight; Lanes 4,9: photo-DIBOD **2.1a**, azido-BSA, and Biotin-azide incubated overnight in the dark; Lane 5,10: azido-BSA and Biotin-azide incubated overnight.

The labeling of BSA with Rhodamine B and biotin demonstrated the utility of photo-DIBOD for protein functionalization with relatively small moieties. To test the capability of this platform for the conjugation of larger substrates, we conducted the cross-linking of azido-BSA with PEG_{5000}-azide[31] (Scheme 2.3). A PBS solution of azido-BSA (0.4 μM), PEG_{5000}-azide (4 μM), and nanocrystalline suspension of **2.1a** (4 μM) was irradiated with 350 nm light for 8 min and incubated overnight. The functionalized protein (**2.7c**) was concentrated via spin filtration (MWCO 10,000), purified on a Sephadex column, and lyophilized. Surprisingly, the MALDI-TOF mass spectrum revealed a mixture of BSA derivatives with various degrees of functionalization (Fig. 2.4).[13] To rule out the possibility of non-specific reaction of photo-DIBOD **2.1a** or of photo-generated **2.2a** with the protein, we conducted copper-catalyzed "click" conjugation of azido-BSA with PEG_{5000}-acetylene.[32] Gratifyingly, the MALDI-TOF spectrum of the resulting product shows the same distribution of PEGylated BSA derivatives as in the photo-conjugation experiment (Fig. 2.5).[13] These experiments demonstrate that the photo-derivatization of proteins with **2.1a** occurs only at azide moieties and is as efficient as CuAAC ligations. Incubation of azido-BSA, photo-DIBOD, and PEG_{5000}-azide in the dark does not produce any BSA-PEG_{5000} conjugates.[13]

Finally, photo-DIBOD can be useful for the patterned immobilization of various substrates. To illustrate this capability of the new photo-crosslinking platform, we immobilized fluorescent dye in selected wells of 96-well plates. Amine binding plates were functionalized with azide moieties by incubation with 3-azidopropan-1-amine. Methanol solution of Rhodamine B-azide and photo-DIBOD were added to each well and the loaded plate was irradiated with a 350 nm handheld fluorescent lamp via a shadow mask (Scheme 2.4). The plate was thoroughly washed with methanol and fluorescent intensity of individual wells was recorded using a plate reader (Table 2.1).[13] The intense fluorescence of the irradiated wells demonstrates covalent immobilization of the dye.



Scheme 2.4. Light-directed Immobilization of Rhodamine B on a 96-well plate

2.5 Conclusions and Acknowledgements

In conclusion, we have reported the first synthesis of a conjugated bis-cyclopropenone-fused dibenzo[*a,e*]cyclo-octatetraene (photo-DIBOD **2.1a**) and its butoxy- (**2.1b**) and tetra-ethylene glycol (**2.1c**) derivatives. Irradiation of photo-DIBODs gives the corresponding bis-alkyne with a quantum yield of Φ =0.05, which acts as a linchpin in conjugating two azides. In aqueous solutions, nanocrystalline suspension of photo-DIBOD **2.1a** serves as an efficient photo-crosslinker. The use of the nanocrystalline suspension has an additional benefit by enhancing the quantum yield of the process by almost an order of magnitude. We have demonstrated the general applicability of this platform by functionalizing a model protein, BSA, with a variety of functional fragments. In addition, photo-DIBOD was employed for the light-directed immobilization of azide-derivatized substrates.

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2.6 Supporting Information

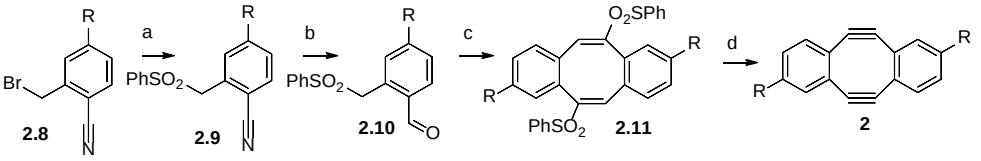
General Methods

Tetrahydrofuran was freshly distilled from sodium/ benzophenone ketyl prior to use. Dichloromethane was freshly distilled from CaH_2 prior to use. Solutions were prepared using HPLC grade water and methanol. Flash chromatography was performed using 40-63 μm silica gel. Electronic spectra

were recorded using Cary 300 Bio UV-Vis spectrometer. Photolyses were conducted using a Rayonet photoreactor equipped with sixteen 4W 350 or 420 nm fluorescent lamps. A handheld lamp equipped with 2 fluorescent UV lamps (4W, 350 nm) was employed for the irradiation of 96-well plates. The quantum yields of photolysis were measured against the 4-nitroveratrole actinometer.[33] All NMR spectra were recorded on a 400M Hz spectrometer in deuterochloroform and referenced to TMS unless otherwise noted. Dynamic light scattering analysis was performed using a Zetasizer Nano S90 size analyzer (Malvern Corp).

Materials

All reagents were purchased from Sigma Aldrich or VWR and used as received unless otherwise noted. Amino-binding 96-well plates were obtained from G-Biosciences. Rhodamnie B – azide,[34] PEG_{5000}-azide,[32] PEG_{5000}-propargyl ether,[35] 4-butoxy-2-methylbenzonitrile,[36] 4-methoxy-2-methylbenzonitrile,[37] 1-azido-3-iodopropane,[38] and N-(2-(2-(2-azidoethoxy)ethoxy)-ethyl)-2-iodoacetamide[39] were prepared following the procedures reported previously.



R= H (a); OBu (b); $O(CH_2CH_2O)_4TBDPS$ (e)

Reagents and conditions: (a) DMF, 70°C, $PhSO_2Na$; (b) DIBAL, THF, -78°C; (c) $ClP(O)O_2Et_2$, LHMDS, -78°C; (d) LDA, -78°C

Scheme 2.5. Syntheses of dibenzo[*a,e*]cyclooctadiynes

2-[(phenylsulfonyl)methyl]benzonitrile[26] (2.9a). Sodium benzenesulfinate dihydrate (4.80 g, 24.0 mmol) was added to a solution of 2-(bromomethyl)benzonitrile (3.92 g, 20.0 mmol), in DMF (30mL). The mixture was stirred at 80°C for 2 h, cooled to r.t., diluted with ethyl acetate, washed with distilled water, and solvents evaporated *in vacuo*. The residue was recrystallized from EtOAc/hexanes to give 4.78g

(93%) as a white solid. ^{1}H NMR: 4.57 (s, 2H,), 7.27 (m, 4H), 7.62 (m, 5H). ^{13}C NMR: 137.66, 134.27, 132.92, 132.82, 132.21, 131.70, 129.58, 129.29, 128.70, 116.56, 114.95, 60.55.

2-[(phenylsulfonyl)methyl]benzaldehyde (**2.10a**).[26] DIBAL (1.0 M in hexane, 102 mL, 102 mmol) was added to a solution of ortho-(phenylsulfonylmethyl)benzonitrile (**2.9a**, 11.42 g, 44.4 mmol) in DCM (444 mL) at 78°C, and stirred for 2 h. A saturated aqueous solution of NH_4Cl was added to the mixture, solution diluted with DCM, washed with 1M HCl, and the solvent was evaporated *in vacuo*. The residue was filtered through a short silica gel plug and recrystallized from DCM/hexane to give 6.50g (58%) as a white solid. ^{1}H NMR: 5.03 (s, 2H,), 7.43 (m, 3H), 7.55 (m, 3H), 7.69 (m, 3H), 9.83 (s, 1H). ^{13}C NMR: 191.89, 138.33, 134.71, 134.30, 133.82, 133.75, 133.48, 129.43, 128.88, 128.81, 128.65, 57.70.

5,11-bis(phenylsulfonyl)-Dibenzo[a,e][8]annulene (**2.11a**).[26] A THF solution of LHMDS (38.4mL, 38.4 mmol) was added dropwise to a mixture of aldehyde **2.10a** (5.00g, 19.21 mmol) and diethyl phosphorochloridate (3.98g, 23.05 mmol) in THF (291mL) at -78^{0}C and stirred for 30 min. The reaction mixture was slowly warmed to r.t., stirred for 1.5 h, quenched with a saturated aqueous solution of NH_4Cl, and diluted with EtOAc. The organic layer was washed with brine, the solvents evaporated *in vacuo*, and the residue purified via flash chromatography (DCM in Hexanes, gradient 1:5 -> 1:1) to yield 2.48g (53%) of **2.11a** as a white solid. ^{1}H NMR: 7.61 (t, J=7.2Hz, 2H), 7.49-7.40 (m, 9H), 7.35 (s, 2H), 7.24 (m, 4H), 6.98-6.95 (d, J=6.6Hz, 2H). ^{13}C NMR: 144.76, 138.89, 138.86, 135.62, 133.83, 130.71, 129.33, 128.96, 128.91, 128.30, 128.08, 126.93.

5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2.2a**).[26] A THF solution of n-BuLi (23.3 mL, 53.7 mmol) was added dropwise to a solution of diisopropylamine (7.67 mL, 54.7 mmol) in THF (200 mL) at -78°C and stirred for 1 h. A solution of the **2.11a** (2.60 g, 5.37 mmol) in THF (15 mL) was added to the reaction

mixture, stirred for 2 h, and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with DCM, washed with distilled water and brine, dried over $MgSO_4$, and the solvents evaporated *in vacuo*. The crude product was triturated with DCM to give 1.01g (94%) of **2.2a** as a bright yellow solid. ^{1}H NMR: 6.92 (m, 4H), 6.74 (m, 4H). ^{13}C NMR: 132.90, 129.02, 126.88, 109.31.

1,1,6,6-Tetrafluorodibenzo[a,e]dicyclopropa[c,g][8]annulene (**2.4a**). A solution of diyne **2.2a** (0.207 g, 1.03 mmol), $TMSCF_3$ (0.607 mL, 4.14 mmol), NaI (0.682 g, 4.55 mmol) in THF (5 mL) was heated in a pressure vessel for 2 h at at 110^{0}C. The reaction was quenched by adding saturated aqueous sodium bicarbonate solution (20 mL), diluted with DCM (30 mL) and EtOAc (100 mL), washed with with deionized water and brine. The organic phase was dried over anhydrous potassium carbonate, partially concentrated, and cooled to -40°C. The resulting crystals were separated and washed with EtOAc to afford 0.186g (59%) of yellow solid, m.p. = 183 - 185^{0}C. ^{1}H NMR: 6.75 (dd, J=8.8, 3.3Hz, 4H), 6.44 (dd, J=8.8, 3.3Hz, 4H. ^{19}F NMR: -116.82. ^{13}C NMR: 132.65, 132.05, 125.37, 125.13 (t, J=12.1Hz), 100.05 (t, J=27.5Hz). **2.4a** used in the next step without further purification.

Dibenzo[*a*,*e*]dicyclopropa[*c*,*g*][8]annulene-1,6-dione (**2.1a**)**.** The crude dicyclo-propene **2.4a** (0.155g, 0.516mmol) was loaded onto a silica gel column (1:1 DCM/hexane + 0.5% H_2O), spread by recycling the eluent, and allowed to react overnight. The remaining starting material was flushed with DCM, and then the bis-cyclopropenone was eluted with 10%MeOH/DCM to give 0.107g (81%) as a bright yellow solid. M.p. (dec)= 259^{0}C. ^{1}H NMR: 7.82 (dd, J = 5.6, 3.3 H-z, 4H), 7.54 (dd, J = 5.6, 3.3 Hz, 4H). ^{13}C NMR: 152.99, 148.86, 135.76, 134.60, 123.25. ESI HRMS cald. ($M+H^+$): $C_{18}H_9O_2$ 257.0297, found: 257.0603; IR (cm^{-1}): 3040, 2960, 1854, 1619, 1430, 1352, 1258, 1017, 801, 776.

1,8-dibutyldibenzo[3,4:7,8]cycloocta[1,2-*d*:5,6-*d*]bis([1,2,3])triazole) (**2.5**) and **1,10-dibutyldibenzo[3,4:7,8]cycloocta[1,2-*d*:5,6-*d*]bis([1,2,3])triazole)** (**2.6**). A solution of dicyclopropenone **2.1a** (120 mg, 0.468 mmol) in DCM/MeOH (1:10, 100 mL) and butyl azide (93 mg, 0.937 mmol) was irradiated at 350 nm. After 15 min irradiation, the bis-cyclopropenone peak at 360 nm had disappeared. The solution was stirred overnight and separated via column chromatography (DCM - MeOH gradient, 1:0 -> 200:1) to give 81 mg (43%) of the head-to-tail (**2.5**) and 64 mg (34%) of the head-to-head adducts, both as yellow solids.

Bis-triazole **2.5**, m.p.= 175 - 179^{0}C; ^{1}H NMR: 7.68 (d, J=7.6Hz, 2H), 7.45 (m, 4H), 7.20 (d, J=7.5Hz, 2H), 4.20 (m, 4H), 1.58 (m, 4H), .98 (m, 4H), .67 (t, J=7.4Hz, 6H). ^{13}C NMR: 144.92, 134.49, 132.74, 131.26, 130.00, 129.39, 128.82, 126.80, 48.32, 31.74, 19.30, 13.14.

Bis-triazole **2.6**, m.p.= 189 - 190^{0}C. ^{1}H NMR: 7.62 (dd, J=9.2, 2.3Hz, 2H), 7.53 (dd, J=9.2, 2.3Hz, 2H), 7.42 (dd, J=9.2, 2.3Hz, 2H), 7.32 (dd, J=9.2, 2.3Hz, 2H), 4.25 (m, 4H), 1.77 (m, 4H), 1.21 (m, 4H), .82 (t, J=7.4Hz, 6H). ^{13}C NMR: 145.96, 133.27, 130.98, 130.66, 130.37, 128.94, 128.46, 48.21, 32.18, 19.75, 13.41. ESI HRMS cald. (M+H$^+$): $C_{24}H_{27}N_6$ 399.2292, found: 399.2288.

2-(Bromomethyl)-4-butoxybenzonitrile (**2.8b**). A mixture of 4-butoxy-2-methylbenzonitrile (20.5 g, 108 mmol), NBS (28.9 g, 163 mmol), and AIBN (1.78 g, 10.8 mmol) in cyclohexane (157 mL) was refluxed for 3 h under inert atmosphere, cooled to r.t., filtered, and washed with water and brine. The reaction mixture was concentrated under vacuum and purified by flash chromatography (Hexanes to 5% EtOAc in hexanes) to give 16.8 g (57%) of **2.8b** as a yellow oil. ^{1}H NMR: 7.53 (d, J=8.6Hz, 1H,), 6.98 (d, J=2.4Hz, 1H), 6.85 (dd, J=8.6, 2.4Hz, 1H) 4.54 (s, 2H), 3.98 (t, J=6.5Hz, 2H), 1.75 (m, 2H), 1.46 (m, 2H), 0.94 (t, J=7.4Hz, 3H). ^{13}C NMR: 162.58, 142.93, 134.77, 116.46, 115.05, 103.53, 68.30, 30.84, 29.60, 19.10, 13.76.

4-Butoxy-2-((phenylsulfonyl)methyl)benzonitrile (**2.9b**). A mixture of 2-(bromomethyl)-4-butoxybenzonitrile (**2.8b**, 4.33 g, 16.15 mmol) and sodium benzenesulfinate (3.18 g, 19.4 mmol) in DMF (25 mL) was stirred for 3 h under inert atmosphere at 80°C. The crude mixture was cooled to r.t., diluted with ether, and washed with water and brine. The mixture was concentrated under vacuum and purified by flash chromatography (25% EtOAc-hexanes) to yield 4.23g (80%) of **2.9b** as a white solid. ^{1}H NMR: 7.72 (m, 2H), 7.64 (t, 1H), 7.48 (m, 2H), 7.42 (d, J=8.7Hz, 1H), 7.06 (d, J=2.4Hz, 1H), 6.90 (dd, J=8.7, 2.4Hz, 1H), 4.49 (s, 2H), 3.99 (t, J=6.5Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), .97 (t, J=7.5Hz, 3H). ^{13}C NMR: 162.29, 137.59, 134.22, 133.51, 129.22, 128.67, 117.75, 117.03, 115.96, 105.51, 68.39, 60.52, 30.89, 19.08, 13.75.

4-Butoxy-2-((phenylsulfonyl)methyl)benzaldehyde (**2.10b**). A DIBAL solution (1.0 M in Hexanes, 25.7 mL, 25.7 mmol) was added dropwise to a solution of 4-butoxy-2-((phenylsulfonyl)methyl)benzonitrile (**2.9b**, 5.65 g, 17.15 mmol) in DCM (170mL) at -78°C under inert atmosphere. The mixture was stirred for 2 h and quickly quenched with a saturated aqueous solution of NH_4Cl, diluted with DCM, and washed with 1M HCl followed by brine. The mixture was concentrated under vacuum and purified by flash chromatography (25% EtOAc in hexanes) to produce 4.36g (76%) of **2.10b** as a white solid, m.p.= 99 - 102^{0}C. ^{1}H NMR: 9.59 (s, 1H), 7.64 (d, J=8.1Hz, 2H,), 7.56 (m, 2H), 7.39 (t, J=7.8Hz, 2H), 6.93 (dd, J=8.5, 2.4Hz, 1H), 6.88 (s, 1H), 4.98 (s, 2H), 3.96 (t, J=6.5Hz, 2H), 1.73 (m, 2H), 1.45 (m, 2H), .94 (t, J=7.4Hz, 3H).^{13}C NMR: 190.61, 162.87, 138.26, 137.19, 133.74, 131.10, 128.75, 128.63, 127.61, 119.76, 114.77, 68.26, 57.55, 30.99, 19.08, 13.78. ESI HRMS cald. (M+H)+: $C_{18}H_{21}O_4S$ 333.1155, found: 333.1161

(5E,11E)-2,8-Dibutoxy-6,12-bis(phenylsulfonyl)dibenzo[*a,e*][8]annulene (**2.11b**). A solution of LHMDS (29.2 mL, 29.2 mmol) was added dropwise to a solution of 4-butoxy-2-((phenylsulfonyl)methyl)benzaldehyde (**2.10b**, 4.86 g, 14.6 mmol) and diethyl chlorophosphate (3.03g,

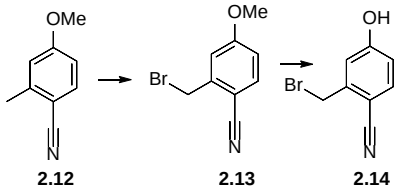
17.5mmol) in THF (443 mL) at -78°C under inert atmosphere. The reaction mixture solution was stirred for 30 min, warmed to r.t., and stirred for 2 h. The mixture was diluted with ethyl acetate and washed with 1 M HCl, followed by water, then brine. The mixture was concentrated under vacuum and purified by flash chromatography (30% EtOAc in Hexanes). The crude product was recrystallized from hexanes – EtOAc to give 1.55 g (34%) of **2.11b** as a white solid, m.p.= 192 - 195^{0}C. ^{1}H NMR: 7.59 (m, 2H,), 7.42 (m, 8H), 7.31 (s, 2H), 6.95-6.75 (m, 6H), 3.84 (t, J=6.5Hz, 4H), 1.68 (m, 4H), 1.40 (m, 4H), .91 (t, J=7.4Hz, 6H). ^{13}C NMR: 162.97, 148.01, 143.43, 143.25, 137.80, 134.57, 133.02, 132.36, 132.15, 131.60, 120.67, 119.82, 71.92, 35.19, 23.24, 17.94. ESI HRMS calcd. (M+H)+: $C_{36}H_{37}O_6S_2$ 629.2026, found: 629.2029.

2,8-Dibutoxy-5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2.2b**). A solution of n-BuLi (12.9 mL, 29.7 mmol) was added dropwise to a solution of diisopropylamine (4.30 mL, 30.2 mmol) in THF (100 mL) at -78°C. The reaction mixture was stirred for 1 h and a solution of **2.11b** (1.86 g, 2.96 mmol) in THF (20 mL) was added. The mixture was stirred for 2 h and quenched with a saturated aqueous solution of NH_4Cl. The reaction mixture was diluted with EtOAc, washed with distilled water and brine, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by flash chromatography (2:3 DCM/hexanes) to yield 530 mg (52%) of **2.2b** as a yellow solid, m.p. (decmp)= 134^{0}C. ^{1}H NMR: 6.63 (d, J=8.4Hz, 2H), 6.37 (dd, J=8.4, 2.6Hz, 2H), 6.32 (d, J=2.6Hz, 2H), 3.84 (t, J=6.5Hz, 4H), 1.70 (m, 4H), 1.42 (m, 4H), 0.94 (t, J=7.4Hz, 6H). ^{13}C NMR: 159.82, 134.82, 127.77, 123.54, 114.92, 112.48, 110.13, 107.29, 67.81, 31.05, 19.11, 13.76. HRMS calcd. ($C_{24}H_{24}O_2$) 344.1776, found: 344.1774.

3,8-Dibutoxydibenzo[*a,e*]dicyclopropa[*c,g*][8]annulene-1,6-dione (**2.1b**). A solution of **2.2b**, (0.512 g, 1.49 mmol), $TMSCF_3$ (0.881 mL, 5.95 mmol), NaI (0.936g, 6.24 mmol), and THF (5 mL) was heated at 110°C for 2 h in a pressure vessel. The reaction was quenched by adding saturated sodium bicarbonate solution (20 mL), diluted with DCM (30 mL) and EtOAc (100 mL), and washed with deionized water and

brine. The organic phase was dried over anhydrous K_2CO_3, partially concentrated, and purified by flash chromatography (DCM-hexanes). The reaction yielded 400 mg (60%) of 3,8-dibutoxy-1,1'6,6'-tetrafluorodibenzo[a,e]dicyclopropa[c,g][8]annulene (**2.4b**) as yellow solid. ^{1}H NMR: 7.20 (d, J=8.4Hz, 2H), 6.81 (d, J=2.5Hz, 2H), 6.69 (dd, J=8.4, 2.5Hz, 2H), 3.97 (t, J=6.4Hz, 4H), 1.76 (m, 4H), 1.47 (m, 4H), .97 (t, J=7.4Hz, 6H). ^{18}F NMR: -116.48. ^{13}C NMR: 162.56, 133.78, 127.48, 125.68 (t, J=11.2Hz), 121.30 (t, J=11.7Hz), 118.70, 117.06, 116.32, 100.33, 68.27, 30.98, 19.09, 13.75.

Crude **2.4b** (661 mg, 1.48 mmol) was loaded onto a silica gel column pre-packed with "wet" hexanes (1% of water), spread throughout the column, and incubated for 72 h. After 72 h, the unreacted starting material and mono-adduct were eluted with 20% EtOAc in hexanes and polarity was increased to 5% MeOH/DCM to elute the target compound producing 433 mg (73%) of **2.1b** as a bright yellow solid, m.p. (decmp) = 201^{0}C. ^{1}H NMR: 7.70 (d, J=8.5Hz, 2H), 7.26 (m, 2H), 6.90 (dd, J=8.5, 2.5Hz, 2H), 4.04 (t, J=6.5Hz, 4H), 1.70 (m, 4H), 1.42 (m, 4H), .98 (t, J=7.4Hz, 6H). ^{13}C NMR 164.12, 152.87, 149.39, 143.73, 137.79, 125.45, 121.49, 118.82, 115.50, 68.79, 30.88, 19.02, 13.69. HRMS cald. (M+H)+: $C_{26}H_{25}O_4$ 401.1747, found: 401.1738. IR (cm^{-1}): 2953, 2871, 1838, 1631, 1585, 1557, 1326, 1233, 1093, 1065, 1020, 973, 884, 682.



2.12 **2.13** **2.14**

Scheme 2.6. Synthesis of intermediate **2.14**

2-(Bromomethyl)-4-methoxybenzontrile (**2.13**). AIBN (1.22 g, 7.41 mmol) was added to a suspension of 4-methoxy-2-methylbenzonitrile (5.45 g, 37 mmol) and NBS (9.89 g, 55.5 mmol) in carbon tetrachloride (185 mL) and the mixture was refluxed fo 3 h. The reaction was quenched with aqueous sodium thiosulfate solution, washed with water, and the solvents evaporated in vacuo. The crude solid was purified by flash chromatography (20% EtOAc in hexanes) to give 5.3 g (63%) **2.13** as a clear oil. ^{1}H NMR:

7.59 (d, J=8.6Hz, 1H), 7.03 (d, J=2.3Hz, 1H), 6.91 (dd, J=8.6, 2.3Hz, 1H), 4.58 (s, 2H), 3.87 (s, 3H). ^{13}C NMR: 162.99, 143.06, 134.88, 117.20, 116.02, 114.71, 103.91, 55.79, 29.49.

2-(Bromomethyl)-4-hydroxybenzonitrile (**2.14**)**.** Boron tribromide (6.05 g, 250 mmol) was added to a solution of the **S5** (5.45 g, 22.1 mmol) in DCM (120 mL) at -10°C. After 30 min the reaction mixture was slowly warmed to r.t., stirred for 4 days, quenched with water, and washed with saturated aqueous sodium bicarbonate solution. The solvents were evaporated and the residue purified through a short silica gel plug to give 4.32 g (82%) of **2.14** as a white solid. ^{1}H NMR: 10.78 (s, 1H), 7.64 (d, J=8.5Hz, 1H), 7.01 (d, J=2.2Hz, 2H), 6.84 (dd, J=8.5, 2.3Hz, 1H), 4.66 (s, 2H). ^{13}C NMR: 162.05, 143.62, 135.77, 118.00, 116.80, 101.88, 31.31. ESI HRMS cald. (M-H^{-}): C_8H_5BrNO 209.9560, found: 209.9565.

2-(Bromomethyl)-4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)benzonitrile (**2.8e**). DIAD (4.12 g, 20.4 mmol) was added to a solution of 2-(bromomethyl)-4-hydroxybenzonitrile (**2.14**, 4.32 g, 20.4 mmol), triphenylphosphine (5.88 g, 22.4 mmol), and 2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (8.81 g, 20.4 mmol) in THF (204 mL) at 0°C. The reaction mixture was warmed to r.t. and stirred for 12 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (20% EtOAc in hexanes) to give 10.3 g (81%) of **2.8e** as a clear oil. ^{1}H NMR: 7.67 (m, 4H), 7.56 (d, J=8.6Hz, 1H), 7.39 (m, 6H), 7.04 (d, J=2.3Hz, 1H), 6.89 (dd, J=8.6, 2.4Hz, 1H), 4.56 (s, 2H), 4.15 (t, J=4.7Hz, 2H), 3.85 (t, J=4.7Hz, 2H), 3.80 (t, J=5.3Hz, 2H), 3.64 (m 10H), 1.04 (s, 9H). ^{13}C NMR: 162.24, 143.02, 135.58, 134.80, 133.65, 129.60, 127.62, 117.15, 116.64, 115.19, 104.05, 72.43, 70.92, 70.76, 70.73, 70.68, 69.33, 67.96, 63.42, 26.81, 19.19. ESI HRMS cald. (M+Na)+: $C_{32}H_{40}BrNNaO_5Si$ 648.1751, found: 648.1754.

4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-2-(2-(phenylsulfonyl)ethyl)benzonitrile (**2.9e**). A solution of 2-(bromomethyl)-4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)benzonitrile (**2.8c**, 10.3g, 16.6 mmol) and sodium benzenesulfinate (3.24 g, 20.0 mmol) in DMF (110 mL) was heated at 80°C and for 2 h. The solution was cooled to r.t., diluted in ether, and washed with water and brine. Solvent was evaporated in vacuum and the residue purified by flash chromatography (30%EtOAc in hexanes) to give 6.04 g (53%) of **2.9e** as a clear oil. ^{1}H NMR: 7.74-7.68 (m, 7H), 7.50 (t, J=7.8Hz, 2H), 7.38 (m, 7H), 7.14 (s, 1H), 6.95 (dd, J=8.6, 2.4Hz, 1H), 4.51 (s, 2H), 4.17 (t, J=4.7Hz, 2H), 3.87 (t, J=4.7Hz, 2H), 3.82 (t, J=5.3Hz, 2H), 3.72-3.62 (m, 10H), 1.05 (s, 9H). ^{13}C NMR: 161.97, 137.52, 135.59, 134.28, 134.21, 133.63, 133..59, 129.62, 129.26, 128.71, 127.63, 117.86, 116.91, 116.13, 105.94, 96.11, 72.44, 70.93, 70.76, 70.73, 70.67, 69.28, 68.06, 63.43, 60.48, 26.82, 19.20. ESI HRMS cald. (M-H)-: $C_{38}H_{44}NO_7SSi$ 686.2613, found: 686.2594.

4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-2-(2-(phenylsulfonyl)ethyl)benzaldehyde (**2.10e**). A DIBAL solution (20.0 mL, 20.0 mmol) was added dropwise to solution of **2.9e** (5.00 g, 7.27 mmol) in DCM (22 mL) at -78°C under inert atmosphere. The mixture was stirred for 2 h and quickly quenched with a saturated aqueous ammonium chloride solution. The crude mixture was diluted with DCM, and washed with 1M HCl and brine. The solvents were removed in vacuum and the residue purified by flash chromatography (25% EtOAc in hexanes) to give 3.71 g (74%) **2.10e** as a yellow oil.^{1}H NMR: 9.64 (s, 1H), 7.74-7.68(m, 6H), 7.59 (t, 2H), 7.49-7.38 (m, 8H), 7.01 (m, 2H), 5.03 (s, 2H), 4.20 (t, J=4.7Hz, 2H), 3.87 (t, J=4.6Hz, 2H), 3.82 (t, J=5.3Hz, 2H), 3.72-3.62 (m, 10H), 1.05 (s, 9H). ^{13}C NMR: 190.63, 162.52, 138.29, 137.24, 135.59, 133.75, 133.65, 131.18, 129.60, 128.77, 128.71, 127.93, 127.62, 119.87, 114.98, 72.43, 70.93, 70.76, 70.67, 69.32, 67.96, 63.42, 57.52, 26.81, 19.19. ESI HRMS cald. (M-H)-: $C_{38}H_{45}O_8SSi$ 689.2610, found: 689.2588

(((5E,11E)-6,12-Bis(phenylsulfonyl)dibenzo[a,e][8]annulene-1,8-diyl)bis(oxy)bis- (2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecane) (**2.11e**). A solution of LiHMDS (1.0M in THF, 10.74 mL, 10.74 mmol) was added dropwise to a solution of **2.10e** (3.71 g, 5.37 mmol) and ClP(O)(OEt)2 (0.93 mL, 6.44 mmol) in THF (160 mL) at -78°C. The reaction mixture was stirred at -78°C for 30 min, then at r.t. for 2 h, and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with ethyl acetate, washed with water and brine, and then evaporated *in vacuo*. The residue was purified by chromatography (AcOEt-hexanes 1:1) to give 1.3g (36%) of **2.11e** as a clear oil. ^{1}H NMR: 7.68 (m, 8H), 7.62 (m, 2H), 7.5-7.33 (m, 20H), 7.31 (s, 2H), 7.00 (m, 2H), 6.84 (m, 4H), 4.02 (m, 4H), 3.80 (m, 8H), 3.63 (m, 22H), 1.04 (s, 18H). ^{13}C NMR: 158.53, 143.99, 139.19, 139.11, 135.58, 133.69, 130.47, 129.52, 128.51, 128.21, 128.06, 127.87, 127.60, 116.74, 115.81, 72.42, 70.82, 70.74, 70.69, 70.63, 69.43, 67.56, 63.42, 26.81, 19.18. . ESI HRMS cald. (M+H)+: $C_{76}H_{88}O_{14}S_2Si_2$ 1345.5232, found: 1345.5237.

2,8-bis((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2.2e**). A solution of n-BuLi (8.88 mL, 20.4 mmol) was dropwise added to a solution diisopropylamine (2.97 mL, 20.8 mmol) in THF (82mL) at -78°C. The reaction mixture was stirred for 1 h and a solution of **2.11e** (2.04g, 1.52 mmol) in THF (10ml) was added. The mixture was stirred for 2 h and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with DCM, washed with distilled water and brine, dried over $MgSO_4$, and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography (1:1 hexanes - EtOAc) to give 1.22 g (56%) of the diyne **2.2e** as a bright yellow solid. ^{1}H NMR: 7.69 (m, 10H), 7.37 (m, 10H), 6.62 (d, J=8.4Hz, 2H), 6.39 (dd, J=8.4, 2.6Hz, 2H), 6.35 (d, J=2.6Hz, 2H) 4.01 (t, J=4.8Hz, 4H), 3.81 (m, 8H), 3.67 (m, 20H), 1.05 (s, 18H). ^{13}C NMR: 159.47, 135.58, 134.84, 133.70, 129.58, 127.74, 127.60, 124.05, 115.07, 112.70, 110.12, 107.37, 72.43, 70.85, 70.75, 70.71, 70.65, 69.45, 67.54, 63.43, 26.82, 19.19. ESI HRMS cald. (M+H)+: $C_{64}H_{77}O_{10}Si_2$ 1061.5055, found: 1061.5057.

(((((((((5,6,11,12-Tetradehydrodibenzo[*a,e*][8]annulene-2,8-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy)) diethanol (**2.2c**)**.** A solution of TBAF (2.87 mL, 2.87 mmol) was dropwise added to a solution of **2.2e** (1.22 g, 1.15 mmol) in THF (12 mL) at r.t. and stirred for 45 min, and quenched with saturated aqueous ammonium chloride solution. The organic layer was washed with brine, dried over $MgSO_4$, and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (DCM - MeOH, gradient from 0.5% to 5%) to give 400 mg (60%) of **2.2c** as a bright yellow solid. ^{1}H NMR: 6.81 (d, J=8.4Hz, 2H), 6.59 (dd, J=8.4, 2.6Hz, 2H), 6.52 (d, J=2.6Hz, 2H), 4.56 (s, 2H), 4.04 (m, 4H), 3.69 (m, 4H), 3.51 (m, 20H), 3.41 (m, 4H). ^{13}C NMR: 159.47, 133.89, 128.18, 122.59, 114.98, 113.33, 110.01, 107.22, 72.31, 69.87, 69.80, 69.75, 69.73, 68.65, 67.48, 60.19. ESI HRMS cald. (M+H)+: $C_{32}H_{41}O_{10}$ 585.2655, found: 585.2696.

(((((((5,6,11,12-Tetradehydro-dibenzo[a,e][8]annulene-2,8-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis (ethane-2,1-diyl) diacetate (**2.2d**)**.** Acetic anhydride (0.52 mL, 5.5 mmol) and DMAP (17 mg, 0.137 mmol) were added to a solution of triethylamine (2.5 mL) and **2.2c** (400 mg, 0.68 mmol) at r.t., stirred for 3 h, diluted with DCM (200 mL), washed with distilled water and brine, the organic layer dried over $NaSO_4$, and the solvents evaporated *in vacuo*. The crude product was purified by flash chromatography (1:1 hexanes-EtOAc) to give 360 mg (80%) of **2.2d** as a bright yellow solid. ^{1}H NMR: 6.67 (d, J=8.4Hz, 2H), 6.42 (dd, J=8.4, 2.6Hz, 2H), 6.37 (d, J=2.6Hz, 2H), 4.23 (t, J=4.8Hz, 4H), 4.04 (t, J=4.7Hz, 4H), 3.80 (m, 4H), 3.69 (m, 20H), 2.08 (s, 3H). ^{13}C NMR: 170.99, 159.47, 134.84, 127.75, 124.06, 115.08, 112.71, 110.11, 107.36, 70.85, 70.64, 70.61, 70.57, 69.48, 69.13, 67.55, 63.57, 20.94. ESI HRMS cald. (M+H)+: $C_{36}H_{45}O_{12}$ 669.2911, found: 669.2902.

(((((((((1,1,6,6-Tetrafluoro-dibenzo[*a,e*]dicyclopropa[c,g][8]annulene-3,8-diyl)bis (oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) diacetate (**2.4d**). A solution of **2.2d** (360 mg, 0.538 mmol), $TMSCF_3$ (0.207 mL, 1.40 mmol), and NaI (0.218 g, 1.45 mmol) in THF (8 mL) were heated at 110°C for 2 h in a pressure vessel. The reaction was quenched by a saturated sodium bicarbonate solution (20 mL), diluted with DCM (200 mL), washed with deionized water and brine. The organic phase was dried over anhydrous K_2CO_3, concentrated, and purified on a silica gel column (50% EtOAc in hexanes with 1% of triethylamine) to give 93 mg (23%) of **2.4d** as a yellow solid. ^{1}H NMR: 7.26 (d, J=8.5Hz, 2H), 6.88 (d, J=2.4Hz, 2H), 6.77 (dd, J=8.4,2.6Hz, 2H), 4.23 (t, J=4.8Hz, 4H), 4.17 (t, J=4.7Hz, 4H), 3.87 (t, J=4.6Hz, 4H), 3.67 (m, 20H), 2.08 (s, 3H).: ^{19}F-NMR: -116.57 ^{13}C-NMR: 171.01, 162.21, 133.78, 127.46, 121.66, 118.91, 117.49, 116.55, 70.93, 70.65, 70.62, 70.51, 69.13, 69.40, 67.98, 63.56, 20.94. ESI HRMS cald. (M+Na)+: $C_{38}H_{44}F_4NaO_{12}$ 791.2666, found: 791.2660.

3,8-bis(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)dibenzo[*a,e*]dicyclopropa [*c,g*][8]annulene-1,6-dione (**2.1c**). A solution of **2.4d** (93 mg, 0.121 mmol) and potassium carbonate (42 mg, 0.302mmol) in 2:1 MeOH:DCM (3ml) was stirred at r.t. for 1 h. The reaction mixture was filtered and evaporated *in vacuo*. The crude mixture was hydrolyzed immediately during chromatography (1%MeOH/DCM) to give 41 mg (53%) of the bis-cyclopropenone **2.1c** as a yellow solid, m.p. (decmp) = 137-139°C. ^{1}H NMR: 7.65 (d, J=8.2Hz, 2H), 7.17 (m, 4H), 4.56 (t, J=5.5Hz, 2H), 4.27 (m, 4H), 3.76 (m, 4H), 3.59-3.39 (m, 24H). ^{13}C NMR: 163.83, 152.95, 149.18, 143.80, 137.86, 125.35, 121.78, 119.15, 115.78, 72.55, 70.68, 70.56, 70.27, 69.27, 68.44, 61.61. ESI HRMS cald. (M+H)+: $C_{34}H_{41}O_{12}$ 641.2598, found: 641.2591. IR (cm^{-1}): 3348, 2876, 1861, 1827, 1604, 1585, 1346, 1258, 1106, 1068, 956, 848.

Azido-BSA.[10e] Derivatization of BSA with 1-azido-3-iodopropane in PBS (pH = 7.4) did not produce significant incorporation of azido groups into protein structure. Increasing the pH resulted in the

incorporation of several azide units. Thus, a solution of 1-azido-3-iodopropane (4.2 mg, 19 μmol) in acetonitrile (1 mL) was added to a solution of BSA (131 mg, 2 μmol) in Tris buffer (6 mL, pH 8.1) and stirred for 12 h. The resulting protein was purified by spin filtration (MWCO=10,000 amu) and lyophilized to give 100 mg (76%) of azido-BSA as a white powder. Conjugation of the azido-BSA produced by this method with PEG_{5000} using both SPAAC and CuAAC techniques (*vide infra*) indicate that it contained a mixture of unmodified BSA (60%) and BSA molecules derivatized with one (24%), two (10%), three (4%), and four (2%) azide groups. Using 100 equivalents of 1-azido-3-iodopropane at pH= 8.1 produces a mixture of BSA derivatives with a higher fraction of multi-labelled molecules (native – 19%, one azide – 26%, two – 23%, three - 17%, four – 10%, five – 5%). BSA can be functionalized at pH=7.4 using stronger electrophile, N-(2-(2-(2-azidoethoxy)ethoxy)-ethyl)-2-iodoacetamide, but this reaction also resulted in incorporation of multiple azide units per protein molecule.

Methods

The nanocrystalline suspensions of photo-DIBOD 1a were prepared employing reprecipitation technique.[29] Photo-DIBOD **1a** (1 mg, 3.9 μmol) was taken up in 0.5 mL DCM and diluted in MeOH (4 mL). The DCM was evaporated by heating in a sonicator at 40°C for 30 min. The nanocrystalline suspension was prepared by by slow addition of the methanol solution into 10 mL of PBS buffer (7 mM, pH 7.4) containing 15 mM of SDS under sonication. Dynamic light scattering analysis of three independently prepared samples produced mean values of 509 nm, 753 nm, 453 nm. Photo-DIBOD **1a** nanocrystalline suspensions for BSA labeling experiments were prepared without the use of SDS by the direct addition of **1a** in 2 mL of methanol to PBS solution of protein under sonication.

Stability of Sondheimer diyne in aqueous buffer. A suspension of the diyne **2a** (0.96 mM) in a 20% MeOH/PBS (pH 7.4) containing 15 mM of SDS was prepared by re-pricipitation technique.

Decomposition was followed by disappearance of absorbance of **2a** at 350 nm. The half lifetime of decomposition was 10 ± 2 minutes.

Photo-conjugation azido-BSA with Rhodamine B – azide. The mixture of the nanocrystalline suspension of **1a** (1 mg, 3.90 μmol), azido-BSA (25.8 mg, 0.390 μmol), and Rhodamine B - azide (2.06 mg, 3.90 μmol) in PBS (10 mL) was irradiated for 8 min at 350 nm and stirred overnight in the dark. The resulting protein was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The BSA-Rhodamine conjugate, as well as controls, were resolved on 12% SDS-PAGE gel and visualized through in-gel fluorescence using a GE Typhoon scanner with excitation wavelength fixed at 532 nm and emission wavelength fixed at 580 nm.

Photo-conjugation azido-BSA with biotin-azide. The mixture of the nanocrystalline suspension of **1a** (1 mg, 3.90 μmol), azido-BSA (25.8 mg, 0.390 μmol), and biotin azide (1.74mg, 3.90 μmol) in PBS (6 mL) was irradiated for 5 min at 350 nm, and incubated overnight. The mixture was spin filtered (MWCO=10,000amu), purified on a PD-10 column, and lyophilized. Experimental and control samples were dissolved in Dulbecco's Phosphate Buffered saline (DPBS) and the concentration of BSA in each sample was determined by bicinchoninic acid assay (BCA). Each 2 μg (or 6 μg) of the samples were loaded and separated on 8% SDS-PAGE gel and then transferred to nitrocellulose membrane. Immunoblotting was done by anti-biotin antibody conjugated with Horseradish peroxidase (HRP) (1:100,000, Jackson Immunology Lab). For the loading control, the nitrocellulose membrane was stained by 0.5% Ponceau S in 1 % acetic acid.

Conjugation of azido-BSA and PEG_{5000}-azide using photo-DIBOD. The mixture of the nanocrystalline suspension of **1a** (2 mg, 7.8 μmol), azido-BSA (25.8 mg, 0.390 μmol), and PEG_{5000} azide (19.51 mg, 3.90

μmol) in PBS (10 mL) was irradiated for 8 min at 350 nm, then stirred overnight. The resulting protein solution was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The MALDI-TOF analysis of the BSA-PEG_{5000} conjugate indicated that it contained a mixture of unmodified BSA (60%) and BSA molecules derivatized with one (24%), two (10%), three (4%), and four (2%) PEG_{5000} fragments (Figure 2.4). Stirring the mixture containing the nanocrystalline suspension of **1a**, azido-BSA, and PEG_{5000}-azide in PBS overnight, as well as incubation of azido-BSA with PEG_{5000}-azide, didn't produce any BSA-PEG_{5000} conjugate (Fig. 2.5).

Copper-catalyzed click (CuAAC) conjugation of azido-BSA and propargyl-PEG_{5000}. A solution azido-BSA (13.2 mg, 0.20 μmol), propargyl-PEG_{5000} (10 mg, 2.0 μmol), ascorbic acid (7.0 μg, 0.040 μmol) and $CuSO_4$ (3.2 μg, 0.020 μmol) in PBS (7 mM, 6 mL) was stirred for 12 h, concentrated to 1.5 mL by spin filtration (MWCO 10,000), purified over a PD-10 column, and lyophilized. The MALDI-TOF analysis of the protein shows that it contains a mixture of unmodified BSA and BSA molecules derivatized with one, two, three, and four PEG_{5000} fragments (Fig. 2.6). The ratio of these proteins (60 : 23 : 11 : 4 : 2) was almost identical to photo-DIBOD pegylation experiment.

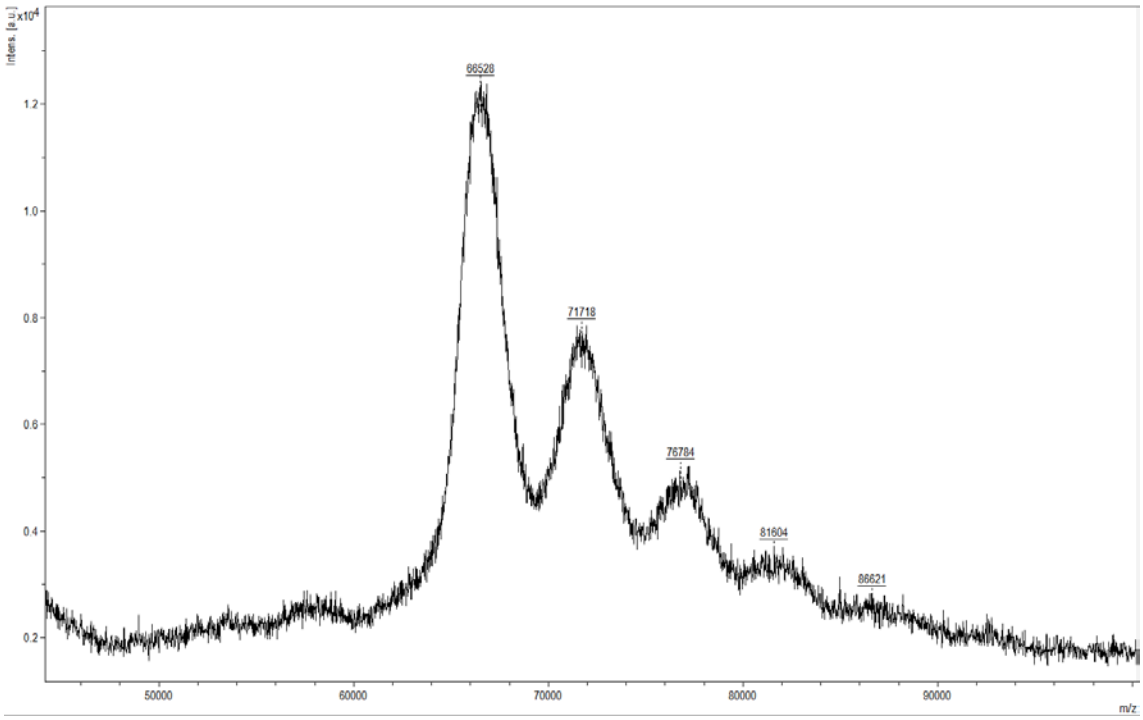


Figure 2.4. Photo-derivatization of azido-BSA-azide with PEG_{5000}-azide using **1a**.

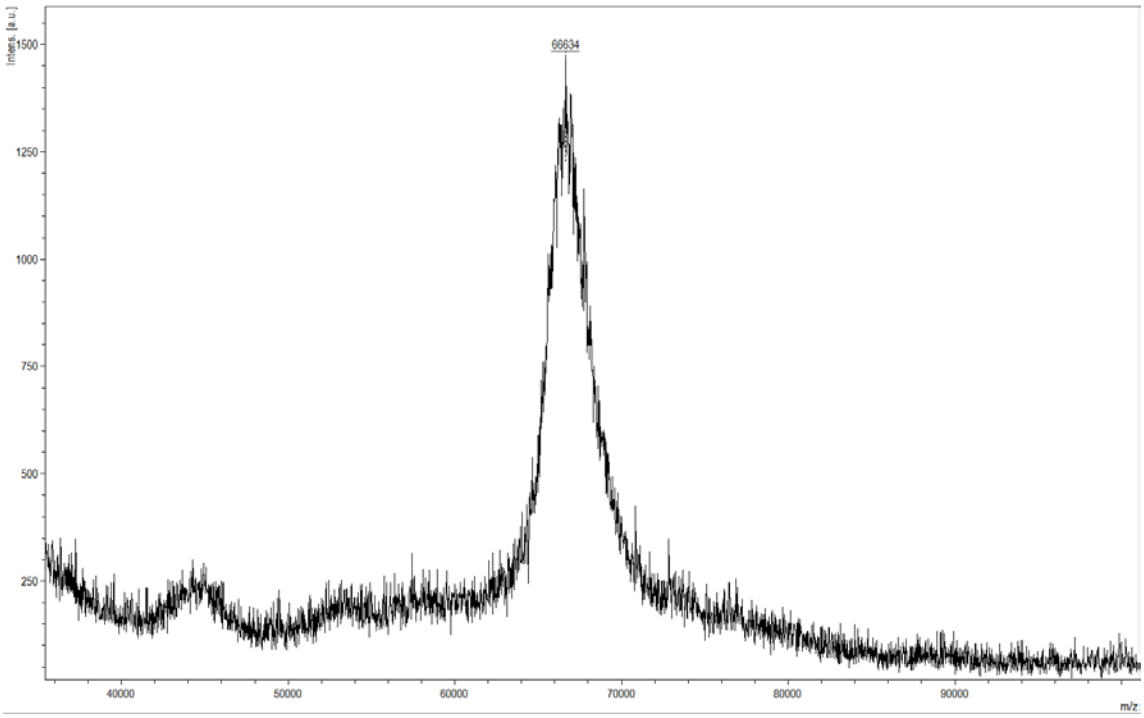


Figure 2.5. Control experiment: photoDIBOD, azido-BSA, and PEG_{5000}-azide incubated in the dark.

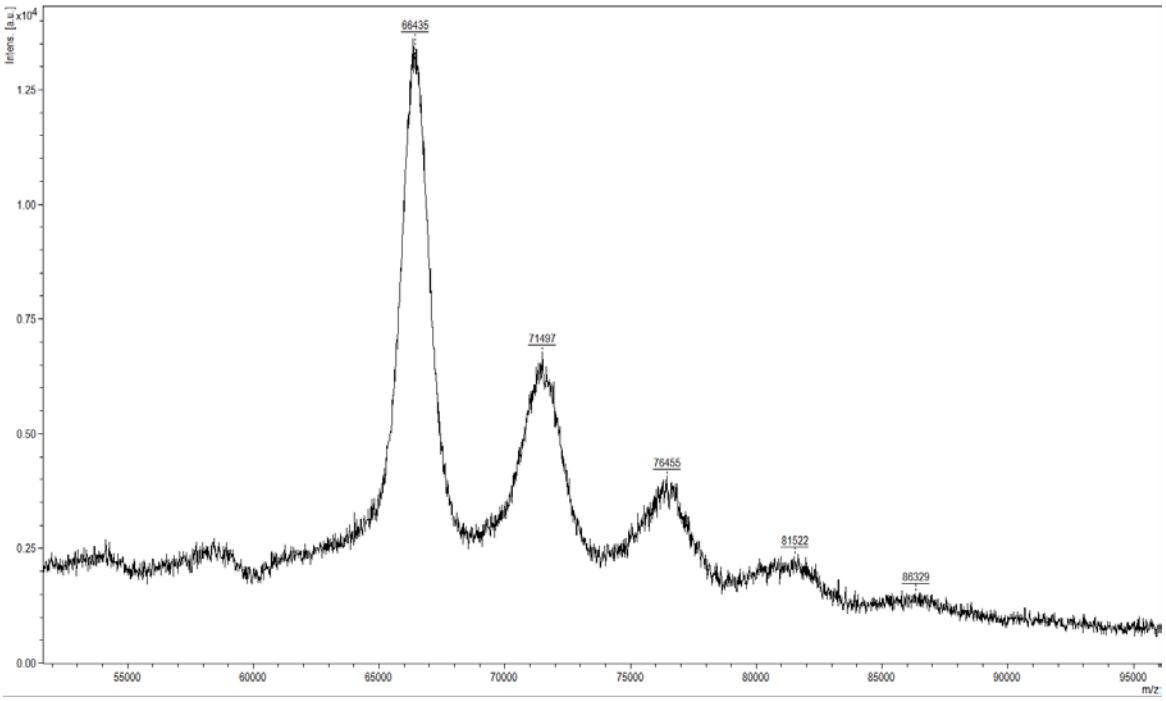


Figure 2.6. CuAAC-conjugation of azido-BSA with propargyl-PEG_{5000}

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1a	+	+	+	+	+	-	-	-	-
Irradiation (min)	5	-	8	5	-	-	-	-	-
Incubation (hours)	1	1	5	5	5	-	-	-	-
well 1	468	181	1080	1201	305	80	60	87	38
well 2	500	94	1312	758	254	42	52	53	61
well 3	543	125	1623	1110	253	57	41	75	116
well 4	658	121	1817	1193	409	95	54	28	36
well 5	685	83	2044	1089	153	51	64	59	51
well 6	597	95	2386	1472	239	49	52	35	58
well 7	788	106	3611	1893	476	64	30	38	56
well 8	703	236	1977	1376	257	44	61	5	64
Average	618	130	1981	1261	293	60	52	48	60

Table 2.1. Fluorescent intensity of wells on the Rhodamine B-azide derivatized 96-well plate

Functionalization of 96-well plates with Rhodamine-B azide. Amino binding 96-well plates were incubated overnight in a 46 mM solution of 3-azidopropylamine in MeOH. The wells in lanes 1-5 were loaded with 140 μL of 0.39 mM suspension of photo-DIBOD **1a** in MeOH and 140 μL of 0.03 mM Rhodamine B-azide in MeOH, irradiated for various time intervals with a 350 nm lamp, incubated for various intervals then thoroughly washed.

Flourescent readout at 580 nm was conducted in a plate reader using 510 nm excitation source. The values fluorescence intensity counts per well are presented in the Table 2.1.

2.7 References

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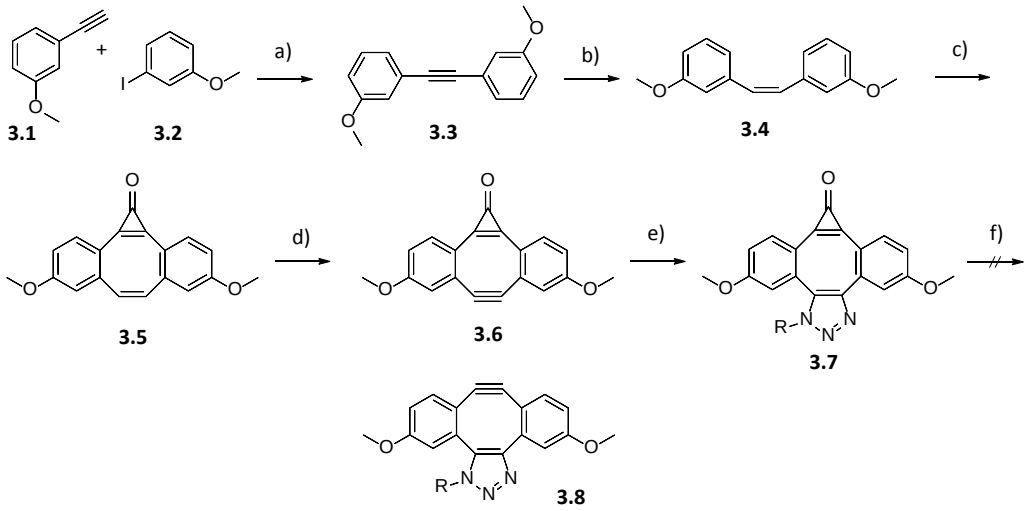
CHAPTER 3

MONO-CYCLOPROPENONE CAGED DIBENZOCYCLOOCTADIYNE (MOC-DIBOD): AN EFFICIENT PLATFORM FOR SEQUENTIAL CLICK LIGATIONS[1]

[1] Sutton D.A. and V.V. Popik, To be submitted to *J. Org. Chem.*

3.1 Introduction

As was mentioned in Chapter 2, we have developed bis-cyclopropenone caged dibenzo[*a*, *e*]cyclooctadiyne (photo-DIBOD) for photo-induced click crosslinking. For the full control of the sequential click ligation, however, a mono-caged species must be synthesized. Recently, Boons reported the synthesis of the mono-cyclopropenone caged dibenzocyclooctadiyne[1] **3.6** (Fl-DIBO) (Scheme 3.1). Fl-DIBO was obtained via a Friedel-Crafts alkylation strategy from acetylene **3.3**. Fl-DIBO was intended to be utilized as a sequential click platform, but unfortunately, subsequent photo-decarbonylation of the cyclopropenone moiety of **3.7** after the addition of one equivalent of azide did not proceed satisfactorily. The absence of photo-reactivity of the compound Fl-DIBO, was attributed to the highly efficient fluorescence of the resulting triazoles (Φ_F = 0.119). These results were unexpected, because the excited state lifetime of most cyclopropenones is extremely limited due to the facility of photoreaction. Indeed, this is the only cyclopropenone reported to date with even modest fluorescence efficiency. Furthermore, a better understanding of this unprecedented reactivity might help shed light on the somewhat controversial photochemistry of cyclopropenones.[2] To further probe the photochemistry and photophysics of this platform, we decided to synthesize a mono-caged diyne via our recently developed methodology.

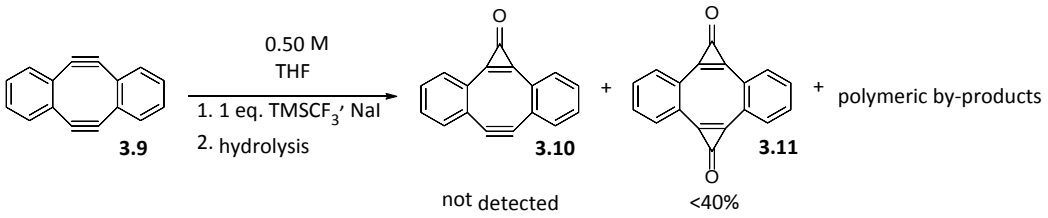


Reagents and conditions: a) $Pd(PPh_3)_4$, CuI, DIPEA, 97%; b) H_2/Lindlar, 89%; c) $AlCl_3$, C_3Cl_4, 62%; d) 1. Br_2, 87%; 2. KOH, EtOH, 76%; f) $h\nu$ 350 nm;

Scheme 3.1 Boons *et Al.* Synthesis of Fl-DIBO

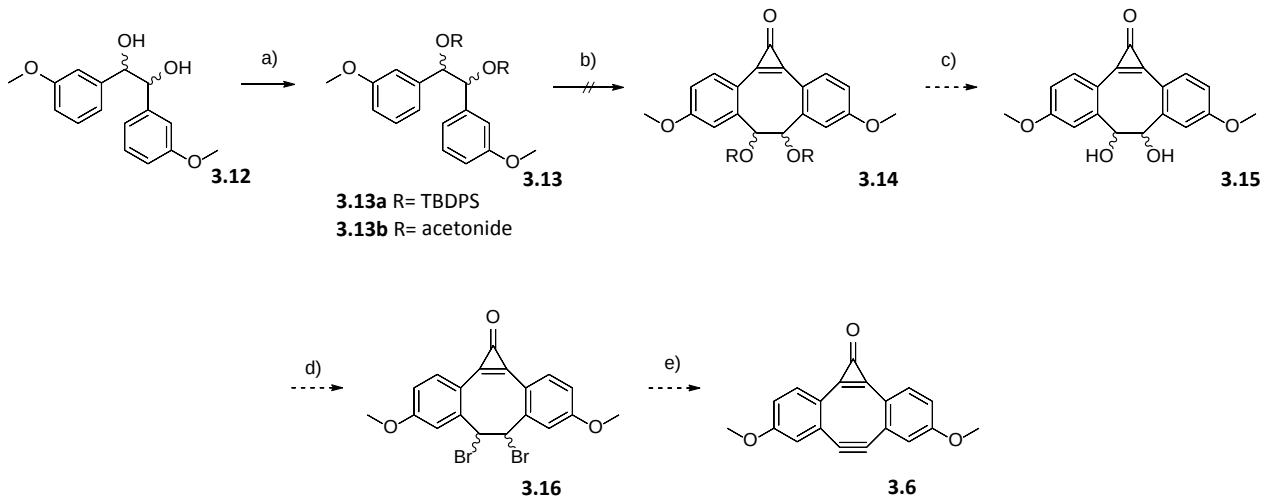
3.2 Synthesis of MOC-DIBOD

To begin this project, we attempted to synthesize mono-cyclopropenone caged dibenzocyclooctadiyne **3.10** (MOC-DIBOD) by Hu-Prakash reaction[3] of DIBOD **3.9** with 1 eq. of difluorocarbene (Scheme 3.2). Unfortunately, our initial attempts did not proceed. The major products produced were **3.11** (photo-DIBOD) and adducts resulting from the polymerization of the starting material. In view of these findings, we decided to instead synthesize Fl-DIBO for photochemical study.



Scheme 3.2 Attempted synthesis of MOC-DIBOD from DIBOD

Previous studies by our group have found significant difficulties in Friedel-Crafts alkylation of stilbenes. For this reason, we decided to synthesize **3.6** from pinacol adduct **3.12** (Scheme 3.3).

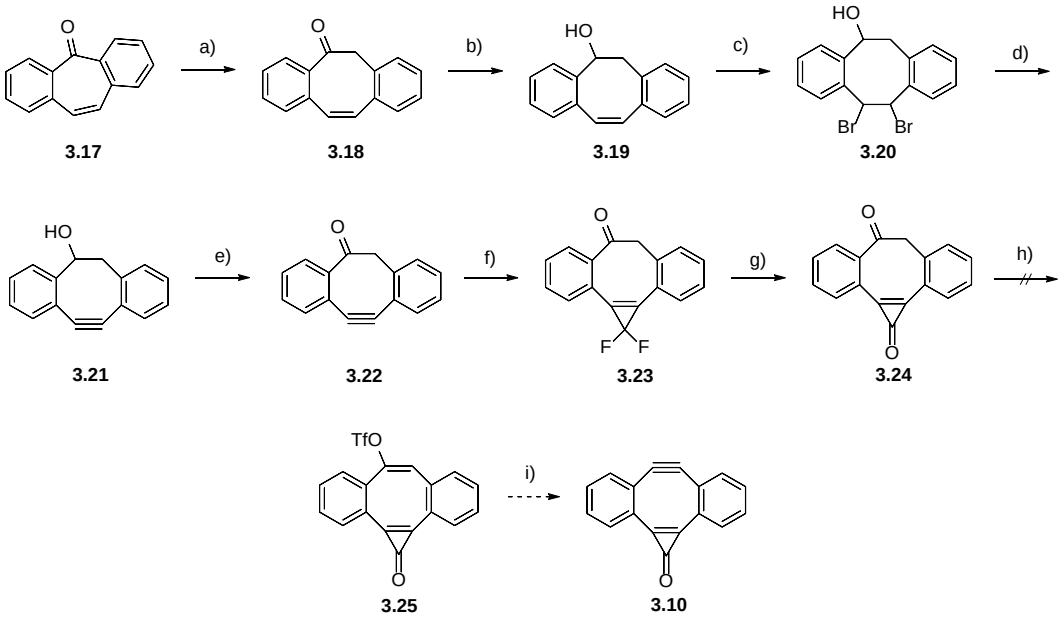


Reagents and conditions: a) TBDPSCl, imidazole (3.13a)' 90%; or 2,2-dimethoxypropane (3.13b), 81%; b) $AlCl_3$' C_3Cl_4' DCM; c) (**3.14a**) TBAF, or (**3.14b**) TsOH; d) PBr_3; e) K-tOBu;

Scheme 3.3 Attempted synthesis of Fl-DIBO via Friedel-Crafts Route

Precursor **3.12** was synthesized via pinacol coupling of *m*-anisaldehyde. Protection of the diol with TBDPSCl or 2,2-dimethoxypropane yielded **3.13a** and **3.13b** respectively. All attempts at Friedel-Crafts alkylation of these adducts failed, giving acrylic acid derivatives as the primary by-products. After several failed attempts at optimizing reaction conditions, we decided to pursue an alternative route.

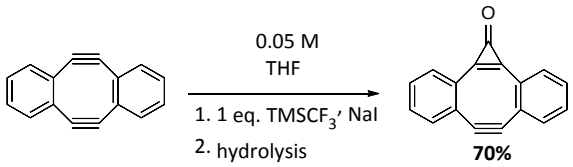
We envisaged that MOC-DIBOD **3.10** could be prepared from known compound **3.22** (DIBONE)[4] in a few simple steps (Scheme 3.4). Ring expansion of 5-dibenzosuberenone **3.17** provided cyclooctene derivative **3.18**. Borohydride reduction followed by bromination and elimination gave cyclooctyne **3.21**. Dess-Martin oxidation of **3.21** provided DIBONE **3.22**. Hu-Prakash reaction of **3.22** gave the difluorocyclopropene **3.23** in excellent yield, which was hydrolyzed on wet silica to provide the cyclopropenone-caged dibenzocyclooctynone **3.24** (photo-DIBONE). Unfortunately, attempts to trap the the enolate of photo-DIBONE as the vinyl triflate did not proceed, due to nucleophilic attack of the enolate on the cyclopropenone.



Reagents and conditions: a) $TMSCHN_2$, BF_3OEt_2, 51%; b) $NaBH_4$, 79%; c)Br_2, 79%; d) LDA, 77%; e) DMP, 56%;f) 1. $TMSCF_3$, NaI, 110°C, 90%; g) "wet" silica, 77%; h) KHMDS, Comin's reagent, i) K^-O*t*Bu

Scheme 3.4 Attempted synthesis of MOC-DIBOD from DIBONE

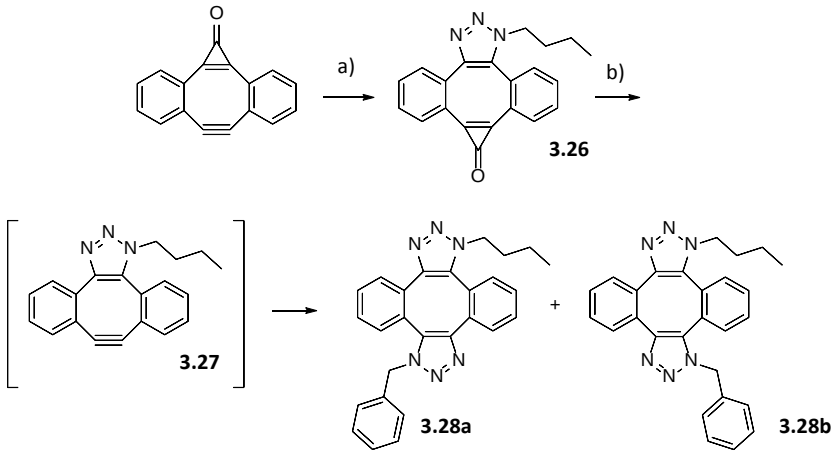
Concurrently with these studies, we attempted modification of reaction conditions of the Hu-Prakash reaction of DIBOD. Gratifyingly, reduction of reaction concentration led to isolation of the desired compound, MOC-DIBOD, in 70% yield (Scheme 3.5). With MOC-DIBOD in hand, we proceeded with photochemical analysis of this intriguing compound.



Scheme 3.5 Synthesis of MOC-DIBOD from DIBOD

3.3 Photochemistry and Kinetics of SPAAC with MOC-DIBOD

We initially examined the SPAAC reactivity of MOC-DIBOD (Scheme 3.6). Incubation with excess of butyl azide provided butyl mono-triazole **3.26** in 92% yield. To our delight, 300 nm irradiation of **3.26** in the presence of benzyl azide gave a mixture of the bis-triazoles **3.28a, b**.



Reagents and conditions: (a) BuN_3, MeOH, rt, 12h, 92%; (b) hv 300 nm, BzN_3, MeOH, rt, 2h, 78%.

Scheme 3.6 Sequential SPAAC Reaction of MOC-DIBOD

In the absence of azide, the intermediate alkyne **3.27** in the photolysate could be detected via ESI-MS in dilute solution, but isolation of the compound was not possible due to rapid decomposition upon concentration, presumably due to dimerization. EI-MS analysis of the concentrated product contained the molecular ion corresponding to calculated mass of the dimer.

Figure 3.1 shows the absorbance spectrum of MOC-DIBOD **3.10** before (solid line) and after (dashed line) incubation with butyl azide. MOC-DIBOD displays two close lying bands characteristic of cyclopropenones at 353 nm (log ε=3.92) and 340 nm (log ε=3.79). Upon formation of triazole **3.26**, these bands experience a hypsochromic shift to a single broad band centered at ca 310 nm (log ε=3.86). Irradiation with a 300 nm source bleaches the band at 310 nm, indicating formation of the alkyne **3.27** (Figure 3.2).

After characterization of the photo-click reaction products, the kinetics of the first and second click reactions were evaluated. Previous computation has suggested that the second addition of azide to the Sondheimer diyne proceeds much faster than the first. Mono-caging allows for the experimental evaluation of this suggestion. The second order rate constant of cycloaddition with butyl azide was determined for MOC-DIBOD **3.10** and mono-triazole **3.26**.

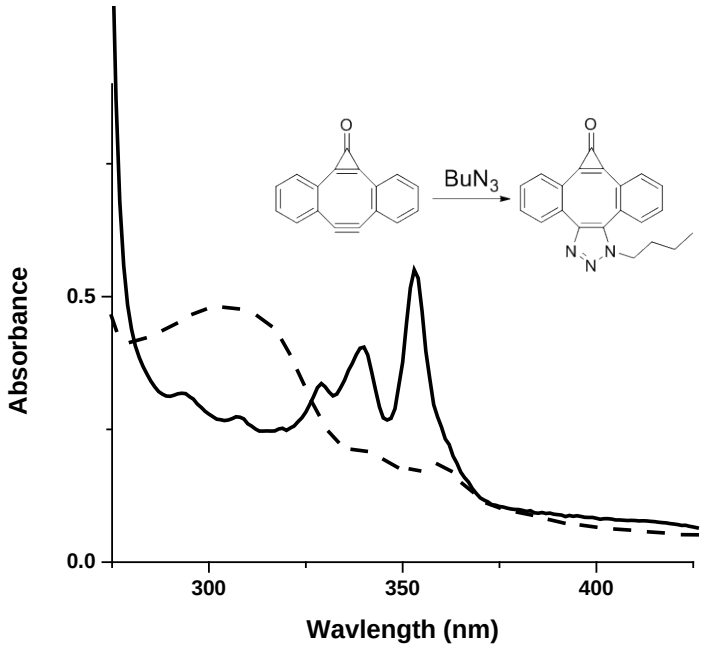


Figure 3.1 Absorbance spectrum of ca. 100 µM solution of MOC-DIBOD **3.10** in MeOH:DCM (9:1) before (solid line) and after (dashed line) incubation with BuN_3.

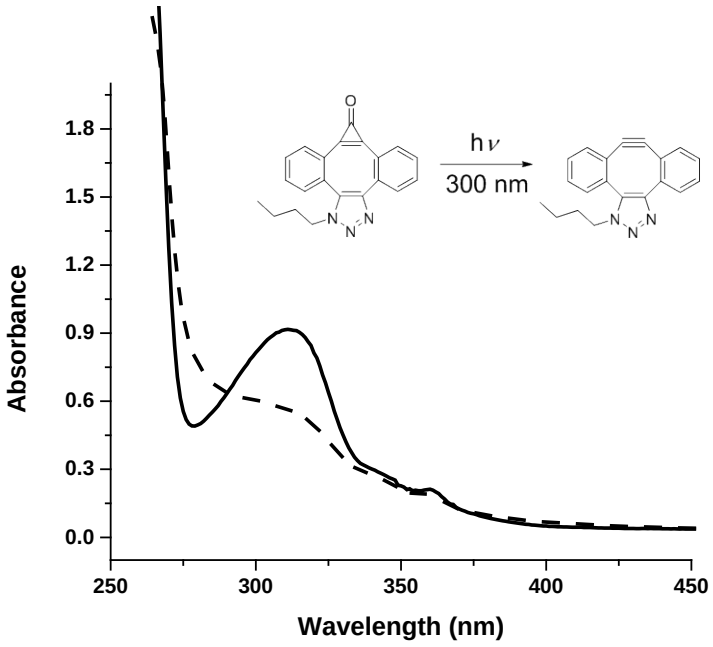


Figure 3.2 Absorbance spectrum of ca. 180 µM solution of triazole **3.26** in MeOH:DCM (9:1) before (solid line) and after (dashed line) irradiation at 300 nm.

The rate constants were evaluated in methanolic solution (9:1 MeOH:DCM) under pseudo first order conditions, with 20-fold or higher concentration of azide. For MOC-DIBOD, the rate constant was evaluated following the decay of the band at 354 nm (Figure 3.3). Interestingly, MOC-DIBOD reacts slightly slower with azides (0.0166±.0002 M^{-1} s^{-1}) than the saturated analogue DIBO (0.06 M^{-1} s^{-1})[5], suggesting reduced angle strain in the octyne ring.

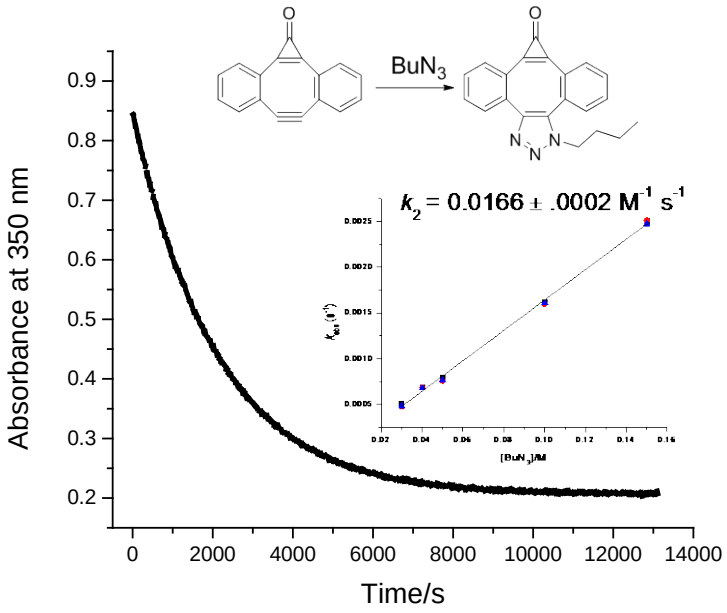


Figure 3.3 Reaction of ca. 0.088 mM MOC-DIBOD **3.10** with butyl azide in 1:1 DCM:MeOH at 25°C.

Following photo-decarbonylation of **3.26**, incubation with butyl azide bleaches a narrow absorption band of alkyne **3.27** centered at 266 nm (Figure 3.4). The second order rate constant was evaluated following the decay of this band (Figure 3.5). As anticipated, the addition of a second equivalent of azide proceeded much faster. The rate constant for addition of BuN_3 to the triazole was found to be 34 M^{-1} s^{-1}. In organic solution, this represents the fastest measured cyclooctyne-azide click reaction reported to date.

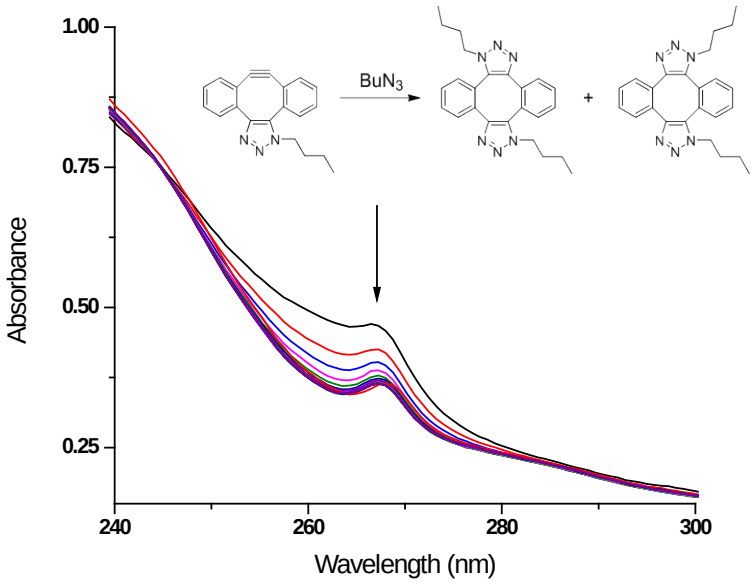


Figure 3.4 Absorbance spectrum of ca. 10 µM solution of alkyne **3.27** after incubation with butyl azide.

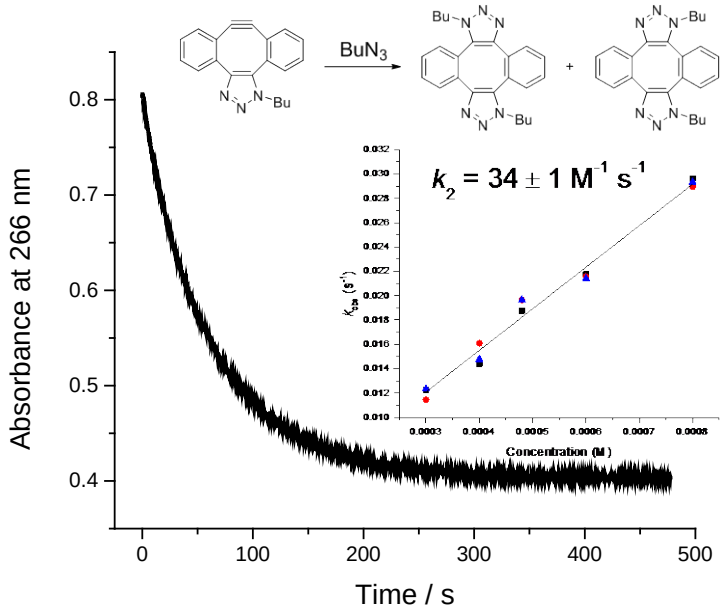
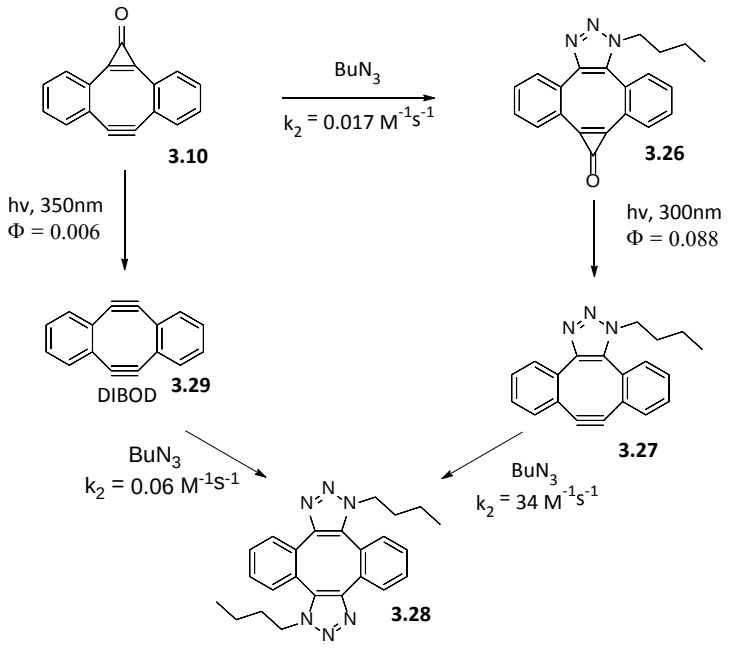


Figure 3.5 Reaction of ca. 0.029 mM **3.27** with butyl azide in 1:1 DCM:MeOH at 25°C.

We next studied the quantum yields for photoreaction of **3.10** and **3.26** (Scheme 3.7). The quantum efficiency of photo-decarbonylation is somewhat reduced in these compounds compared to acyclic cyclopropenones[6] ($\Phi \approx 0.3$-1.00) as well as photo-DIBO[5] ($\Phi = 0.33$) and photo-ODIBO[7] ($\Phi = 0.16$).

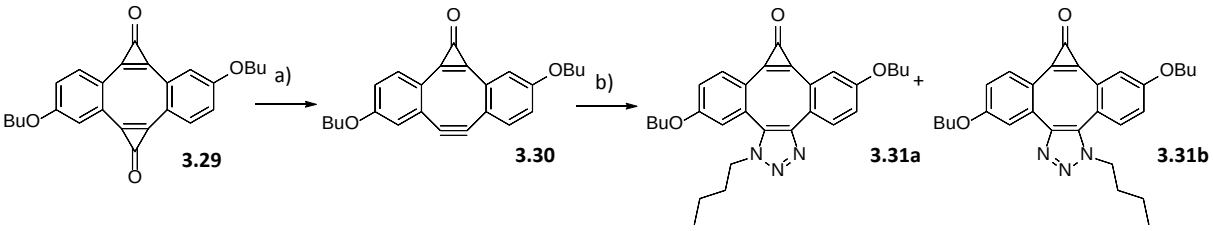


Scheme 3.7 Photochemistry and Kinetics of DIBOD Platform

The quantum yield of photolysis of triazole **3.26** is $\Phi = 0.088 \pm .001$, while the photolysis of MOC-DIBOD **3.10** has $\Phi = 0.006 \pm .001$. Surprisingly, this was almost an order of magnitude lower than the quantum yield of the bis-cyclopropenone analogue hν-DIBOD ($\Phi = 0.045 \pm .003$). These results suggested an efficient deactivation pathway was operable for these compounds.

Because triazoles of the dimethoxy analogue Fl-DIBO are known to be fluorogenic, we investigated the fluorescence emission of **3.10** and **3.26**. In DCM-MeOH (1:9) the butyl triazole **3.16** was very weakly fluorescent ($\Phi_F = 0.0039 \pm .0006$) with a broad emission band centered at ca. 500 nm. The parent alkyne **3.10** also displays emission in a similar range but the fluorescence efficiency was too weak to be measured. Emission was more efficient in MeOH:H_2O ($\Phi_F = 0.0119 \pm .0009$), suggesting a small external heavy atom effect from the chlorinated solvent. Surprisingly, this value remains an order of

magnitude lower than the fluorescence efficiency of Fl-DIBO. In view of these unexpected results, we decided to synthesize bis-butoxy MOC-DIBOD to see if electronic factors affected fluorescence (Scheme 3.8).



Reagents and Conditions: a) hν 350 nm, 73% b) BuN_3, MeOH, 58%

Scheme 3.8 Synthesis of bis-butoxy MOC-DIBOD **3.30** and corresponding butyl triazoles **3.31**

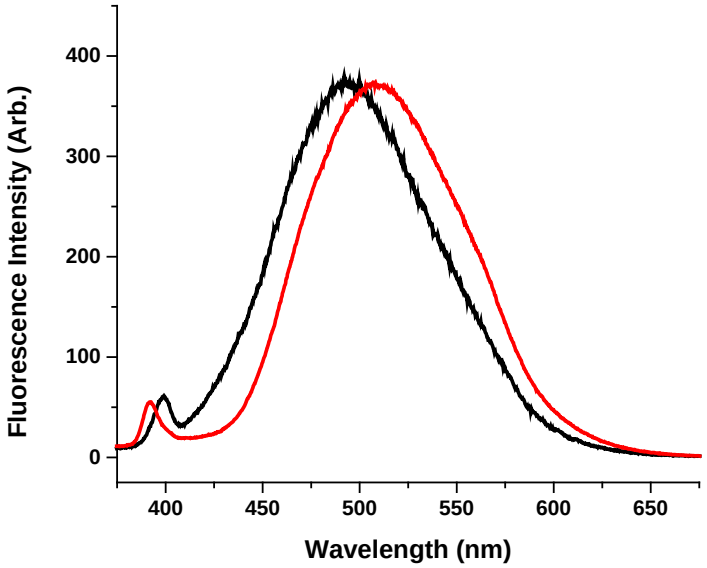
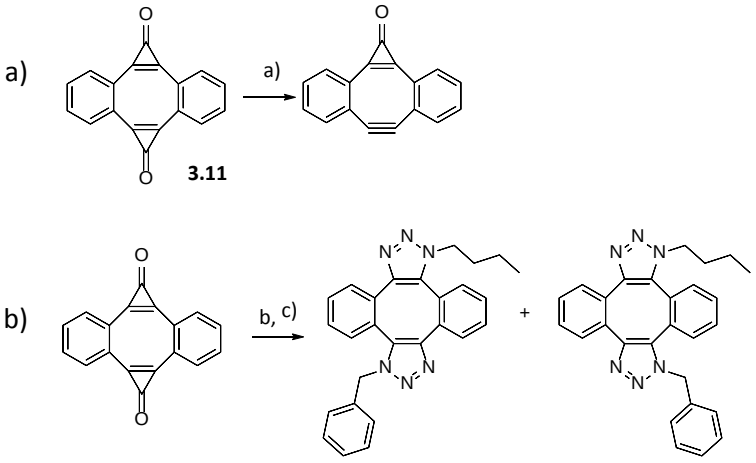


Figure 3.6 Emission spectrum (ex. 350 nm) in 1:4 MeOH:H_2O of **3.26** (black line) and **3.31a,b** (red line) at ca. 0.10 absorbance units (ca. 30 μM).

The measured fluorescence quantum yield of **3.31** in MeOH:H_2O was slightly lower than **3.26** (Φ_F= 0.0076±.0006), presumably due to increased vibrational freedom. The reduced quantum yields of photo-decarbonylation of **3.10** and **3.26** compared to their saturated analogue photo-DIBO and acyclic

phenyl cyclopropenones, as well as their negligible fluorescence, suggest these rigid compounds possess a very efficient internal conversion pathway.

As stated above, the photo-decarbonylation of the first cyclopropenone unit of bis-cyclopropenone hν-DIBOD is much more efficient than the second. This fortuitous difference in reactivity allows for selective photolysis of hν-DIBOD **3.32** to MOC-DIBOD (Scheme 3.9). Indeed, careful irradiation of photo-DIBOD at 350 nm gave MOC-DIBOD in excellent yield (92%). Selective activation of both reactive moieties adds an additional layer of control not accessible with other "photo-click" technologies. As an illustration of the high photolytic selectivity, we prepared a mixed bis-triazole in a one-pot procedure from hν-DIBOD, albeit at a reduced yield.



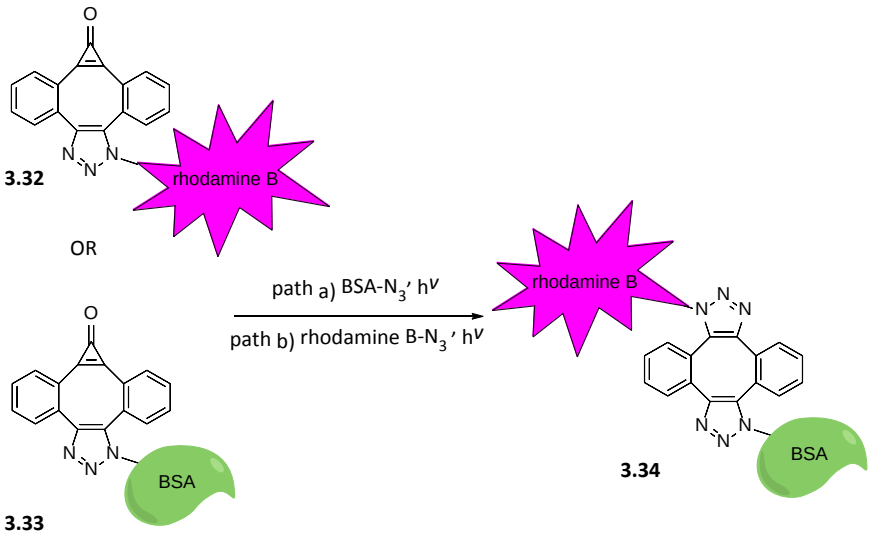
Reagents and conditions: (a) hv 350 nm,9:1 MeOH:DCM, 92%; (b) hv 350 nm, BuN_3, 9:1 MeOH:DCM, 12h; (c) hv 300 nm, BzN_3, 9:1 MeOH:DCM, 2h, 47%.

Scheme 3.9 One pot synthesis of mixed triazoles

3.4 Sequential Conjugation of Proteins with MOC-DIBOD

In a manner analogous to the ligations performed with photo-DIBOD, MOC-DIBOD's potential for bioorthogonal, sequential click cross-linking was showcased by functionalization of BSA as a model protein (Figure 3.7). MOC-DIBOD was incubated with either rhodamine B azide[8] or BSA-azide to form triazoles **3.32** and **3.33** respectively. The triazoles were then photolyzed at 300 nm, followed by addition

of the appropriate second azide. The irradiated samples (Lanes 2 and 4) gave excellent signal upon SDS-PAGE analysis versus dark controls (Lanes 3 and 5), which received identical treatment without irradiation.



Lane	1	2	3	4	5	1	2	3	4	5
3.32		+	+	-	-		+	+	-	-
3.33		-	-	+	+		-	-	+	+
hν		+	-	+	-		+	-	+	-
BSA-N_3		+	+	-	-		+	+	-	-
Rhod-N_3		-	-	+	+		-	-	+	+

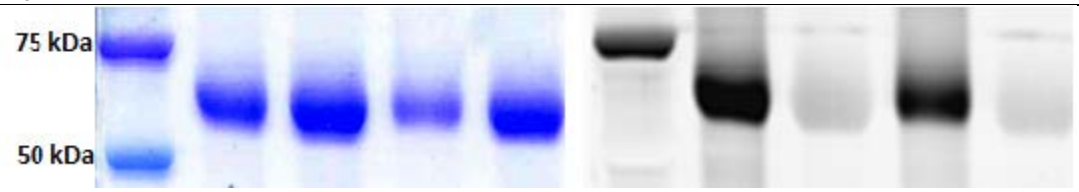
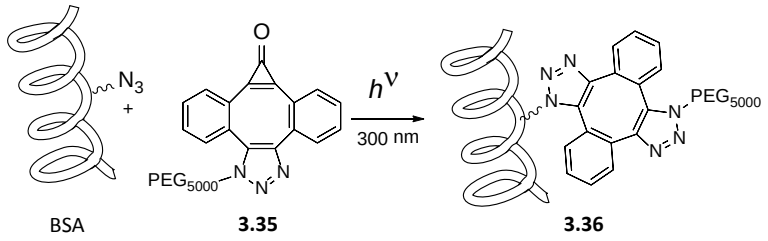


Figure 3.7 Synthesis and SDS-PAGE analysis of rhodamine-BSA conjugates. Coomassie Brilliant Blue stain (*left*). In gel fluorescence λ_{exc} = 510, λ_{em} = 580 nm (*right*).

In order to further illustrate the scope of this versatile sequential click platform, we have also employed MOC-DIBOD in the pegylation of BSA (Scheme 3.10). PEG_{5000}-triazole **3.35** was prepared from MOC-DIBOD and PEG_{5000}-azide[9], followed by photolysis in the presence of BSA-azide. A methanol solution of **3.35 (**2.2 mM**)** was added to azido-BSA (220 μM) in PBS, irradiated with 300 nm light for 8 min, and incubated overnight. The functionalized protein **3.36** was concentrated via spin filtration

(MWCO 10,000), purified on a Sephadex column, and lyophilized. The BSA-PEG conjugation experiments gave similar results as photo-DIBOD (Chapter 2), wherein azide modified BSA expressed varying degrees of functionalization. MALDI analysis of the conjugate revealed multi-functionalization of the irradiated protein, with peaks at ca. 66, 71, and 76 kDa. The dark control received the same treatment with the absence of irradiation, and produced a single mass peak at ca. 66 kDa.



Scheme 3.10 Conjugation of $BSA\text{-}N_3$ with $PEG_{5000}\text{-}N_3$

3.5 Conclusions

We have synthesized a mono-photocaged dibenzo[*a,e*]cyclooctadiyne **3.10** via two pathways: carbene insertion to Sondheimer diyne (DIBOD) and selective photolysis of a bis-cyclopropenone (photo-DIBOD). The resultant alkyne **3.10** undergoes facile click reaction with azides. The triazole product **3.26** displays negligible fluorescence, and is efficiently photo-decarbonylated at 300 nm (Φ = 0.088). The intermediate alkyne triazole **3.27** displays extraordinary reactivity with azides in organic solutions to form bis-triazoles, and possesses the fastest known rate constant of any cyclooctyne. We have demonstrated the applicability of this linchpin towards biological molecules by functionalization of azido-BSA with fluorescent and polymeric labels.

3.6 Experimental Procedures

General Methods

Tetrahydrofuran was freshly distilled from sodium/ benzophenone ketyl prior to use. Dichloromethane was freshly distilled from CaH_2 prior to use. Solutions were prepared using HPLC grade water and methanol. Flash chromatography was performed using 40-63 μm silica gel. Electronic spectra

were recorded using Cary 300 Bio UV-Vis spectrometer. Photolyses were conducted using a Rayonet photoreactor equipped with sixteen 4W 300 or 350 nm fluorescent lamps. The quantum yields of photolysis were measured against the 4-nitroveratrole actinometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer at a PMT voltage of 700V with excitation and emission slit widths of 5.0 and 2.0 nm respectively. All NMR spectra were recorded on a 400MHz spectrometer in deuterochloroform and referenced to TMS unless otherwise noted.

Materials

All reagents were purchased from Sigma Aldrich or VWR and used as received unless otherwise noted. DIBONE[4a] (**3.22**), 1,2-bis(3-methoxyphenyl)ethane-1,2-diol[10] (**3.12**), Rhodamine-B azide,[8] azido-BSA,[11] and PEG_{5000} azide[9] were prepared following the procedures reported previously.

Synthetic Procedures

(5R,6S)-5,6-bis(3-methoxyphenyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane and (5S,6S)-5,6-bis(3-methoxyphenyl)-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (3.13a). Imidazole (3.65g, 53.6 mmol) and TBDMS-Cl (8.46g, 56.1 mmol) were added to a solution of the diol (7g, 25.5 mmol) in DMF (128 mL). The reaction was stirred at 100°C for 20h, taken up in ether, and extracted with water and brine. After evaporation, the crude mixture was purified via flash chromatography (20% EtOAc/hexanes). The reaction yielded 11.51g (90%) of the desired product as a white solid (mixture of stereoisomers. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.18 (t, J = 7.8Hz, 2H), 7.07 (t, J= 7.8Hz, 2H), 6.92 (m, 4H), 6.80 (m, 2H), 6.72 (m, 4H), 6.63 (m, 2H), 4.71 (s, 2H), 4.45 (s, 2H), 3.79 (s, 6H), 3.66 (s, 6H), 0.88 (s, 9H), 0.72 (s, 9H), -0.08 (s, 6H), -0.19 (s, 6H), -0.33 (s, 6H), -0.37 (s, 6H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 159.08, 158.71, 144.71, 143.06, 128.32, 127.91, 120.37, 119.88, 113.15, 113.10, 112.86, 112.45, 80.09, 79.22, 55.11, 54.95, 25.88, 25.70, 25.66, 18.18, 17.98, -2.95, -4.98, -5.13, -5.18, -5.69.

(4R,5S)-4,5-bis(3-methoxyphenyl)-2,2-dimethyl-1,3-dioxolane and (4S,5S)-4,5-bis(3-methoxyphenyl)-2,2-dimethyl-1,3-dioxolane (3.13b). TsOH (0.437g, 2.3 mmol) and 2,2-dimethoxypropane (4.78g, 46 mmol) were added to a solution of the diols **3.12** (6.3g, 23 mmol) in DMF (115 mL) all at once. The mixture was stirred for 12h at r.t. The solution was diluted in ether and extracted with water and brine. The mixture was dried over $MgSO_4$ and evaporated *in vacuo*. The mixture was purified via flash chromatography (5% EtOAc/hexanes). The reaction yielded 5.88g (81%) as a clear oil (mixture of stereoisomers). ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.21 (m, 2H), 6.97 (m, 2H), 6.82 (m, 4H), 6.60 (m, 8H), 5.46 (s, 2H), 4.71 (s, 2H), 3.74 (s, 6H), 3.60 (s, 6H), 1.81 (s, 6H), 1.66 (s, 6H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 159.63, 158.97, 139.25, 138.54, 129.41, 128.48, 119.46, 119.07, 113.70, 113.24, 112.24, 112.11, 109.41, 108.83, 85.15, 81.31, 55.03, 55.02, 27.15, 26.80, 24.57.

Attempted synthesis of 6,7-bis((tert-butyldimethylsilyl)oxy)-4,9-dimethoxy-6,7-dihydro-1H-dibenzo[a,e]cyclopropa[c][8]annulen-1-one (3.14a). Tetrachlorocyclopropene (2.48g, 13.9 mmol) was added to a solution of the aluminum chloride (1.86g, 13.9 mmol) in anhydrous DCM (349 mL) at r.t. The mixture was stirred at r.t. for 20 min. then cooled to -78°C. The protected diol was added dropwise and the mixture was stirred at -78°C for 2h. The reaction was allowed to slowly warm to r.t., and the mixture was held at this temperature for 1h. The solution was quenched with ammonium chloride, taken up in DCM, and extracted with water and brine. After evaporation, the crude mixture was purified via flash chromatography (50% EtOAc/hexanes through 10% MeOH/DCM). Several byproducts were formed in the reaction, including acrylic acid derivatives. None of the desired product was detected.

(Z)-dibenzo[a,e][8]annulen-5(6H)-one (3.18). A solution of trimethylsilyl diazomethane (25 mL, 50 mmol) in 25 mL DCM was added dropwise over 1 hour to a suspension of dibenzosuberenone **3.17**

(6.87g, 33.3 mmol) and boron trifluoride diethyl etherate (7.10g, 50.0 mmol) in DCM (50 mL) at -10°C. The reaction mixture was stirred in ice bath for 2 hours and poured into ice water. The aqueous layer was extracted with DCM (3x100 mL) and the organic extracts were washed with brine and evaporated in vacuo. The crude solid was recrystallized with hexanes to give 3.75g (51%) of the product as a yellow solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.26 (m, 1H,), 7.10-7.14 (m 7H), 7.05 (m, 2H), 4.06 (s, 2H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 196.6, 136.9, 136.3, 135.4, 133.8, 133.1, 132.4, 131.4, 130.6, 129.3, 128.8, 128.0, 127.3, 126.9, 48.4.

(Z)-5,6-dihydrodibenzo[a,e][8]annulen-5-ol (3.19). Sodium brohydride (1.20g, 32mmol) was added to a suspension of the ketone **3.18** (3.49g, 15.8 mmol) in 1:1 THF/EtOH (160 mL) at r.t.. The reaction mixture was stirred for 8 hours until complete disappearance of the starting material via tlc. The reaction mixture was diluted in DCM, and washed with satd ammonium chloride, distilled water, and brine. The organic extract was evaporated in vacuo to give 3.31g (94%) of the product as a white solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.50-7.48 (d, J = 6.9 Hz, 1H), 7.28-7.13 (m, 6H), 6.87 (q, J = 8.8 Hz, 2H), 5.31 (m, 1H), 3.50-3.33 (m, 2H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 140.79, 136.83, 136.21, 134.48, 131.64, 131.56, 130.17, 129.91, 129.38, 128.67, 127.42, 127.17, 126.99, 125.93, 74.53, 42.59.

11,12-dibromo-5,6,11,12-tetrahydrodibenzo[a,e][8]annulen-5-ol (3.20). Bromine (1.53 mL, 30 mmol) was added to a solution of the alcohol **3.19** (3.31g, 14.89mmol) in chloroform (110 mL). The reaction mixture was stirred for 1 hour until complete disappearance of the starting material via tlc. The reaction mixture was diluted in chloroform, and washed with satd thiosulfate, distilled water, and brine. The organic extract was evaporated in vacuo and purified via flash chromatography (10% DCM/hexanes)to give 4.12g (73%) of the product as a yellow solid (mixture of isomers) and was used without further purification.

11,12-didehydro-5,6-dihydrodibenzo[a,e][8]annulen-5-ol (3.21). n-BuLi (19.69 mL, 2.3M, 45.3 mmol) was added to a solution of diisopropyl amine (6.76 mL, 47.4 mmol) in THF (75 mL).The solution was stirred at -78°C for 30min. and a solution of the dibromide **3.20** (4.12g, 10.78mmol) in THF (50 mL) was added dropwise. The mixture was slowly warmed to room temperature, quenched with ammonium chloride (10 mL) and diluted in DCM. The organic extract was washed with water and brine then evaporated in vacuo and purified via flash chromatography (1:1 DCM/hexanes) to give 1.45g (61%) of the product as a white solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.76-7.74 (d, J = 7.8Hz, 1H), 7.45-7.29 (m, 7H), 4.64 (s, 1H), 3.12-3.09 (d, J = 14.7 Hz, 1H), 2.96-2.92 (d, J = 18.3 Hz 1H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 155.56, 151.63, 129.64, 128.07, 128.00, 126.99, 126.85, 126.10, 126.09, 124.05, 123.76, 121.24, 112.91, 110.61, 75.26, 48.73.

11,12-didehydrodibenzo[a,e][8]annulen-5(6H)-one (3.22). Dess-Martin periodinane (4.17g, 9.87mmol) was added to a solution of the alkyne **3.21** (1.45g, 6.58 mmol) in DCM (66 mL, freshly distilled). The solution was stirred under argon for 4 hours. The solution was then diluted in DCM (200 mL) and worked up with satd. sodium thiosulfate, bicarbonate, and brine. The organic extract was washed with water and brine then evaporated *in vacuo* and purified via flash chromatography (1:1 DCM/hexanes) to give 1.16g (81%) of the product as a white solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.56-7.54 (d, J = 7.6 Hz, 1H), 7.49 (m, 2H), 7.41-7.30 (m, 5H), 4.17 (m, 1H), 3.66 (m, 1H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 200.22, 154.60, 148.11, 131.06, 131.04, 129.14, 128.10, 127.68, 126.15, 125.79, 123.64, 122.06, 110.95, 109.27, 49.16.

1,1-difluoro-1,7-dihydro-6H-dibenzo[a,e]cyclopropa[c][8]annulen-6-one (3.23). A mixture of DIBONE **3.22** (0.100g, 0.458 mmol), sodium iodide (0.151g, 1.01mmol), and Ruppert-Prakesh reagent (0.130g, 0.916mmol) in THF (5 mL, freshly distilled) was heated to 110°C in a pressure vessel for 2 hours. The

solution was cooled to r.t., diluted in DCM (150 mL), and worked up with bicarbonate and brine. The organic extract was evaporated in vacuo and purified via flash chromatography (1:1 DCM/hexanes)to give 0.111g (90%) of the product as a white solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.88-7.87 (d, J = 7.6 Hz, 1H), 7.84-7.82 (d, J = 6.8 Hz, 1H), 7.74-7.69 (m, 2H), 7.64-7.57 (m, 3H), 7.48 (td, J = 7.5 Hz, 1H), 3.99 (s, 2H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 198.36, 145.34, 138.41, 132.20, 132.11, 131.64, 131.40, 129.29, 128.89, 128.07, 124.99 (t, J = 12.4 Hz), 124.46, 123.69 (t, J = 12.4 Hz), 122.22, 100.95 (t, J = 276.6 Hz), 48.01. ESI HRMS cald. ($M+Na^+$): $C_{17}H_{10}F_2NaO$ 291.0597, found: 291.0593.

dibenzo[a,e]cyclopropa[c][8]annulene-1,6(7H)-dione (3.24). The difluorocyclopropene **3.23** was loaded onto a silica column with wet hexanes and allowed to stand for 72 hours. The column was flushed with 1:1 DCM/hexanes to remove any remaining starting material and then 5% MeOH/DCM was used to elute the cyclopropenone. The reaction yielded 0.500g (77%) of the product as a white solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.10-8.08 (d, J = 7.5, 1H), 7.89-7.73 (m, 6H), 7.58 (td, J = 7.5, 2.1 Hz 1H), 4.18 (s, 2H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 199.59, 152.03, 147.35, 145.99, 144.46, 139.08, 134.50, 134.12, 133.14, 133.02, 132.43, 132.16, 128.95, 128.75, 124.13, 121.23, 47.69. ESI HRMS cald. ($M+H^+$): $C_{17}H_{11}O_2$ 247.0759, found: 247.0755.

Attempted synthesis of (*E*)-1-oxo-dibenzo[a,e]cyclopropa[c][8]annulen-6-yl trifluoromethanesulfonate (3.25). KHMDS (0.736 mL, 0.487 mmol) was added to a suspension of the ketone **3.24** (0.100, 0.406 mmol) in THF (3.54 mL) at -78°C. The reaction mixture was stirred for 30 min, then a solution of Comin's reagent (0.223g, 0.569 mmol) in 10 mL THF was added slowly by hand. The reaction mixture was stirred at -78°C an additional 30 min and quenched with 5 mL of MeOH. The reaction mixture was evaporated *in vacuo* and purified via flash chromatography (1% MeOH/DCM). The only product detectable was an acrylic acid derived from attack on the cyclopropenone.

6,7-dehydrodibenzo[*a*,*e*]cyclopropa[*c*][8]annulen-1-one (3.10). $TMSCF_3$ (0.191ml, 1.29 mmol) was added to a solution of Sondheimer diyne (0.258g, 1.29 mmol) and NaI (0.232g, 1.55 mmol) in THF (24.3 mL) in a pressure vessel. The reaction mixture was heated at 110 °C for 2 h, cooled to r.t., and quenched by adding saturated bicarbonate solution (20 mL), diluted in DCM (200 mL) and extracted with deionized water and brine. The organic phase was dried over anhydrous K_2CO_3, concentrated, and onto a silica column (10% DCM/hexanes to 1% MeOH/ DCM). The difluorocyclopropene immediately hydrolyzed upon chromatography to give 0.195g (67%) of the title compound as a bright yellow solid (m.p. 204°C decomp.). ^{1}H NMR: 7.55 (d, J=7.4 Hz, 2H), 7.23 (m, 4H), 6.91 (d, J=8.3Hz, 2H) ^{13}C NMR: 154.28, 150.70, 135.16, 133.65, 132.14, 130.06, 127.18, 125.17, 106.78. IR: 1845cm^{-1} (C=O). ESI HRMS cald. (M+H$^+$): $C_{17}H_8O$ 229.0653, found: 229.0649.

1-butyldibenzo[3,4;7,8]cycloocta[1,2-*d*][1,2,3]triazol-8-one (3.26). Butyl azide (43mg, 0.438mmol) was added to a solution of MOC-DIBOD **3.10** (0.050g, .219mmol), in DCM/MeOH (1:4, 20ml). The mixture was stirred at r.t. overnight, concentrated, and purified via flash chromatography (0.5% MeOH/DCM) to give 0.066g (92%) of the title compound as an off-white solid (m.p. 153-156°C). ^{1}H NMR: 7.90 (d, J=7.7Hz, 1H), 7.73 (d, J=7.6Hz, 1H), 7.65-7.53 (m, 4H), 7.46 (t, J=7.6 Hz, 1H) 7.39 (d, J=7.6Hz, 1H), 4.35 (m 1H), 4.18 (m, 1H), 1.69 (m, 2H), 1.21 (m, 2H), .81 (m, 3H). ^{13}C NMR: 157.69, 154.20, 151.92, 142.87, 133.81, 133.07, 132.66, 132.16, 132.14, 131.68, 131.50, 131.31, 130.51, 129.29, 128.90, 126.93, 124.44, 49.15, 32.00, 19.56, 13.32. IR: 1852cm^{-1} (C=O). ESI HRMS cald. (M+H$^+$): $C_{21}H_{17}ON_3$ 328.1449, found: 328.1444.

1-benzyl-8-butyldibenzo[3,4;7,8]cycloocta[1,2-*d*:5,6-*d'*]bis([1,2,3]triazole) (3.28a) and **1-benzyl-10-butyldibenzo[3,4;7,8]cycloocta[1,2-*d*:5,6-*d'*]bis([1,2,3]triazole) (3.28b)**. A solution of the

cyclopropenone (0.030g, 0.092mmol), in DCM/MeOH (1:1, 510ml), was irradiated for 8 min. in a rayonet photoreactor equipped with 16 300nm lamps. After irradiation, benzyl azide (610mg, 4.58mmol) was added. The mixture was stirred at r.t. overnight, concentrated, and purified via flash chromatography (0.3% MeOH/DCM) to give 0.031g (78%) of a mixture of the bis-triazoles as a viscous oil. The 1,8-triazole could be partially separated for NMR characterization: ^{1}H NMR: 7.75 (d, J=6.7Hz, 1H), 7.68 (d, J=7.7Hz, 1H), 7.51 (m, 4H), 7.39 (t, J=8.2Hz, 1H) 7.26 (m, 3H), 7.10 (d, J=7.2Hz, 2H), 7.01 (m, 2H), 5.50-5.33 (dd, J=90.8, 15.3Hz, 1H) 4.32 (m 1H), 4.18 (m, 1H), 1.67 (m, 2H), 1.04 (m, 2H), .74 (m, 3H).^{13}C NMR: 145.18, 144.83, 135.11, 135.01, 134.44, 132.87, 132.67, 131.35, 131.26, 130.18, 130.02, 129.90, 129.43, 128.90, 128.83, 128.60, 128.25, 127.07, 126.97, 126.40, 52.20, 48.37, 31.79, 19.35, 13.17. ESI HRMS cald. ($M+H^{+}$): $C_{27}H_{25}N_{6}$ 433.2141, found: 433.2130.

N-(6-(diethylamino)-9-(2-((3-(8-oxodibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-*d*][1,2,3]triazol1(8*H*)-yl)propoxy)carbonyl)phenyl)-3*H*-xanthen-3-ylidene)-*N*-ethylethaneaminium chloride (3.32). MOC-DIBOD **3.10** (0.028g, 0.123 mmol) was added to a solution of rhodamine azide (0.050g, 0.094 mmol) in MeOH/DCM (9:1, 5 mL). The mixture was stirred overnight, evaporated and purified via flash chromatography (1% MeOH/DCM) to give 0.065g (91%) of the desired compound as a violet, amorphous solid. ^{1}H-NMR 8.03-8.02 (d, J = 7.7 Hz, 1H), 7.88 (d, J = 7.7Hz, 1H), 7.82-7.76 (m, 2H), 7.69-7.57 (m, 6H), 7.52-7.49 (t, J = 8.4 Hz, 2H), 7.10-7.05 (m, 2H), 7.02-6.99 (dd, J = 9.6, 2.3 Hz, 1H), 6.93-6.91 (dd, J = 9.6, 2.3 Hz, 1H), 6.81-6.79 (m, 2H), 4.46-4.41 (m, 1H), 4.31-4.28 (m, 1H), 4.05-3.94 (m, 2H), 3.64 (m, 8H), 1.95 (m, 2H), 1.33 (m, 12H). ^{13}C-NMR; 12.63, 28.68, 46.07, 46.11, 46.37, 62.13, 96.22, 113.38, 113.41, 113.59, 114.03, 114.27, 114.48, 124.30, 126.38, 128.17, 129.30, 129.37, 130.22, 130.38, 130.79, 131.18, 131.28, 131.56, 132.08, 131.16, 132.93, 133.10, 133.17, 133.22, 133.63, 133.71, 142.36, 142.87, 151.81, 152.05, 154.25, 155.47, 155.56, 157.38, 157.63, 157.67, 158.60, 164.45. ESI-MS (M+): Calculated: 754.3388 Found: 754.3391.

3,9-dibutoxy-6,7-dehydrodibenzo[a,e]cyclopropa[c][8]annulen-1-one (3.30). A solution of bis-butoxyphoto-DIBOD **3.29** (25mg, 0.098mmol) in DCM/MeOH (50/450 mL) was irradiated in a rayonet reactor fitted with 16 350nm bulbs following the disappearance of λ_{max} at 370nm (ca. 5 min irradiation). The solution was evaporated and the crude mixture was purified via flash chromatography (0.5-2% DCM/MeOH) to give 34 mg (73**%**) of the desired product as a yellow solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.45 (d, J = 8.5 Hz, 1H), 7.08 (d, J = 2.6 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.68 (dd, J = 8.5, 2.6 Hz, 1H), 6.58 (dd, J = 8.5, 2.6 Hz, 1H), 6.40 (s, 1H), 3.95 (q, J = 6.6Hz, 4H), 1.75 (m, 4H), 1.48 (m, 4H), .99 (t, J = 7.4 Hz, 6H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 163.46, 160.59, 154.07, 151.21, 145.80, 137.14, 133.80, 128.35, 127.92, 124.24, 121.23, 118.51, 116.00, 114.46, 113.37, 107.95, 104.67, 68.37, 68.24, 31.05, 30.95, 19.10, 13,78, 13.75.

6,11-dibutoxy-1-butyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-8-one (3.31a) and 5,10-dibutoxy-1-butyldibenzo[3,4:7,8]cyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-8-one (3.31b). Butyl azide (11 mg, 0.110 mmol) was added to a solution of the mono-cyclopropenone (34 mg, 0.091 mmol) in MeOH/DCM (0.5/0.5 mL). The mixture was stirred for 12 h, evaporated, and purified via flash chromatography (1% MeOH/DCM), evaporated, and recrystallized from DCM/hexanes to give (0.025g, 58%) of the desired product as a yellow solid (mixture of isomers). ESI HRMS [M+H]: Cald: 472.2600 Found: 472.2593.

Kinetics

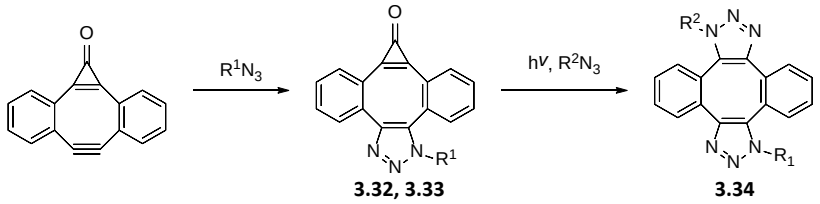
Rate measurements in organic solvent were performed using a Cary-300 Bio UV-Vis spectrometer. The temperature was set to 25.0°C and controlled to 0.1 °C accuracy. The reaction of mono-caged diyne with excess of butyl azide was monitored by following the decay of the characteristic 350 nm absorbance of the starting material. The reaction of photo-generated alkyne triazole was monitored by following decay

of absorbance at 266 nm. Rates of the cycloaddition reactions were measured under pseudo-first order conditions, using 20 fold or higher excess of azide. The experimental data fits the single exponential equation well (Figure 3.3 and 3.5). Linear dependence of the observed pseudo-first order rate constants on azide concentration was analyzed by the least squares method to obtain the bimolecular rate constant (Inserts in Figures 3.3 and 3.5).

Fluorescence quantum yields

The quantum yield of fluorescence for compounds **3.10**, **3.26**, and **3.31** was calculated by evaluation of the gradients of integrated fluorescence intensity vs. absorbance at the excitation wavelength. The gradients were compared to quinine sulfate actinometer (Φ_F = 0.55).[12]

Preparation of mono-cyclopropenone 3.10 from photo-DIBOD 3.32. A solution of photo-DIBOD (25mg, 0.098mmol) in DCM/MeOH (44 / 399 mL) was irradiated in a rayonet reactor fitted with 16 350nm bulbs following the disappearance of λ_{max} at 363nm (ca. 4 min irradiation). The solution was evaporated and the crude mixture was purified via flash chromatography (0.5-2% DCM/MeOH) to give 20mg (90**%**) of the desired product as a yellow solid.



3.32, 3.33 **3.34**

$R^{1=}$ Rhodamine B, R^{2} = BSA **3.32**
$R^{1=}$ BSA, R^{2} = Rhodamine B **3.33**

Scheme 3.11. Sequential Conjugation of BSA with Rhodamine B.

Photo-conjugation of azido-BSA with Rhodamine B–triazole, preparation of 3.34. To a solution of the mono-cyclopropenone (41mg, 0.180mmol) in 1:3 DCM/MeOH (8 mL) was added rhodamine B azide (95 mg, 0.180 mmol). The mixture was stirred overnight at r.t. and evaporated *in vacuo*. The crude solid was used in the subsequent step without further purification. A solution of the triazole **3.32** (2 mg, 3.90 µmol) in MeOH (241µL) was added to a solution of azido-BSA (17.5 mg, 0.265 µmol), in PBS (10 mL). The solution was irradiated for 8 min at 300 nm and stirred overnight in the dark. The resulting protein was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The BSA-Rhodamine conjugate, as well as a dark control, were resolved on 12% SDS-PAGE gel and visualized through in-gel fluorescence using a GE Typhoon scanner with excitation wavelength fixed at 532 nm and emission wavelength fixed at 580 nm.

Photo-conjugation of BSA-triazole with Rhodamine B – azide. To a solution of azido-BSA (145mg, 2.19 µmol) in PBS (10 mL) was added the mono-cyclopropenone (5 mg, 0.022 mmol) in MeOH (2 mL). The mixture was stirred overnight at r.t., concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. A portion of the triazole (25 mg, 0.379 µmol) was reconstituted in PBS (2.5 mL), and rhodamine B azide (2 mg, 3.79 µmol) was added. The solution was irradiated for 8 min at 300 nm and stirred overnight in the dark. The resulting protein was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The BSA-Rhodamine conjugate, as well as a dark control, was resolved on 12% SDS-PAGE gel and visualized through in-gel fluorescence using a GE Typhoon scanner with excitation wavelength fixed at 532 nm and emission wavelength fixed at 580 nm.

Photo-crosslinking of BSA and PEG_{5000}. To a solution of the mono-protected diyne (0.023g, 0.100 mmol) in MeOH, was added PEG_{5000}-N_3 (0.500g, 0.100 mmol). The mixture was stirred at r.t. for 12 h and

concentrated to give 0.519g (99%) of the crude product as a white solid. Evaluation of the IR spectrum of the product revealed disappearance of (N=N=N) stretch at 2100 cm^{-1}. The crude solid was used in the subsequent step without further purification. A solution of the PEG-triazole (5mg, 0.956 µmol) in MeOH (1 mL) was added slowly to a solution of azido-BSA (21mg, 0.319 µmol) in PBS buffer (pH 7.4) (2 mL). The mixture was irradiated at 300 nm in a rayonet reactor for 8 min. and incubated overnight. The resulting protein was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The product was characterized by MALDI-TOF analysis and compared with a dark control (same treatment without irradiation). The MALDI-TOF analysis of the BSA-PEG_{5000} conjugate revealed multi-functionalization of BSA as previously reported (Figure 3.8). Incubation of the mixture containing the triazole, azido-BSA, and PEG_{5000}-azide in PBS overnight, didn't produce any BSA-PEG_{5000} conjugate (Figure 3.9).

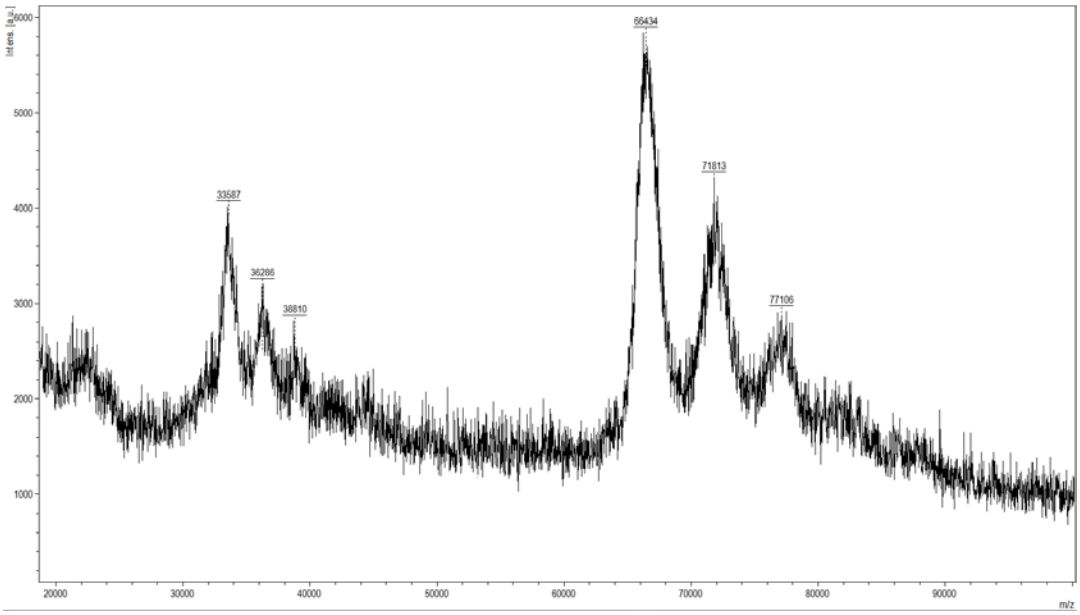


Figure 3.8 Photo-derivatization of azido-BSA-azide with PEG_{5000}-azide using PEG-triazole.

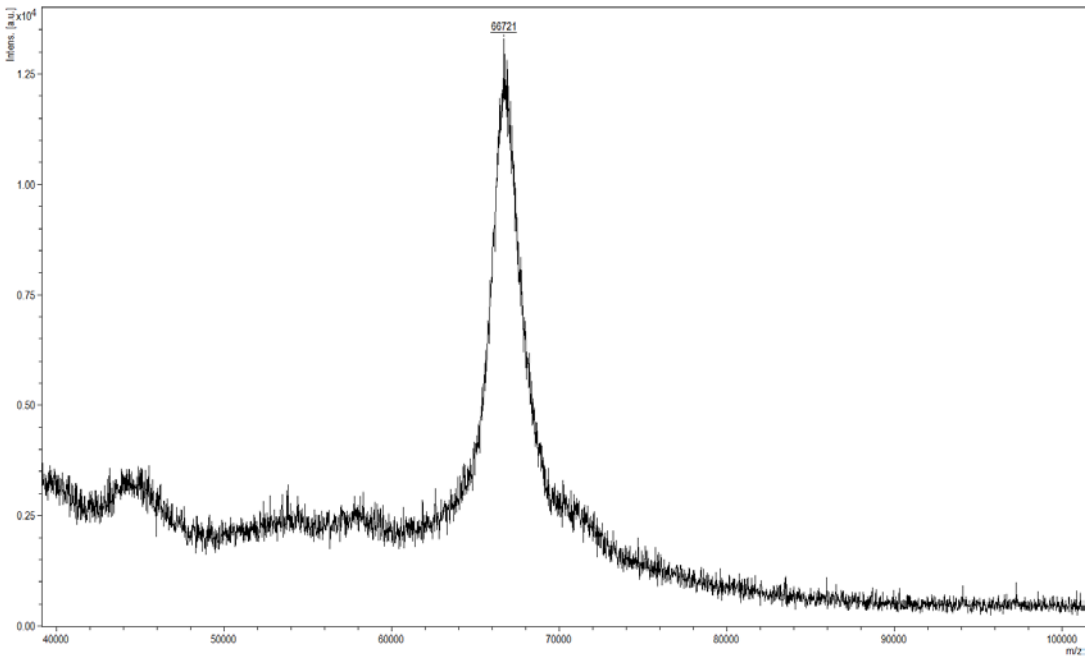


Figure 3.9 Dark control: azido-BSA-azide incubated with PEG_{5000}-triazole in the dark.

3.7 References

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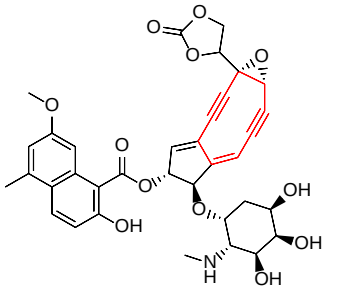
CHAPTER 4

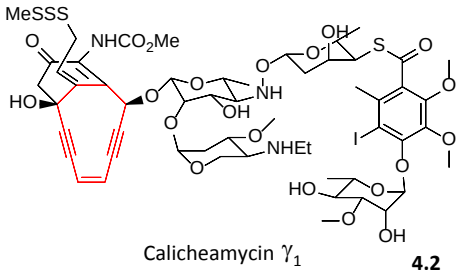
SYNTHESIS OF NAPHTHALENE-FUSED NINE-MEMBERED ENEDIYNES AND TEN-MEMBERED ENYNE-ALLENES

4.1 Introduction

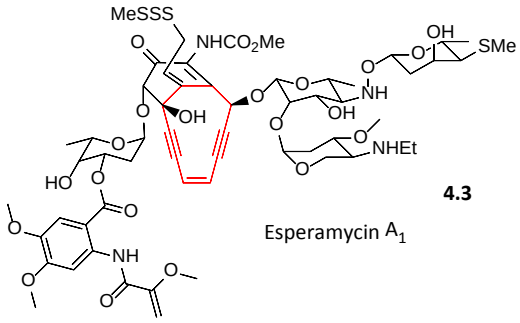
Naturally occurring enediyne antibiotics are among the most potent chemotherapeutic agents ever discovered.[1] The high antineoplastic activity of these compounds is due to their unique (Z)-3-ene-1,5-diyne cores, commonly referred to as the "warhead" fragment (Figure 4.1). The activity of this fragment is due to its ability to undergo triggered Bergman and Myers-Saito cyclizations.



Neocarzinostatin **4.1**



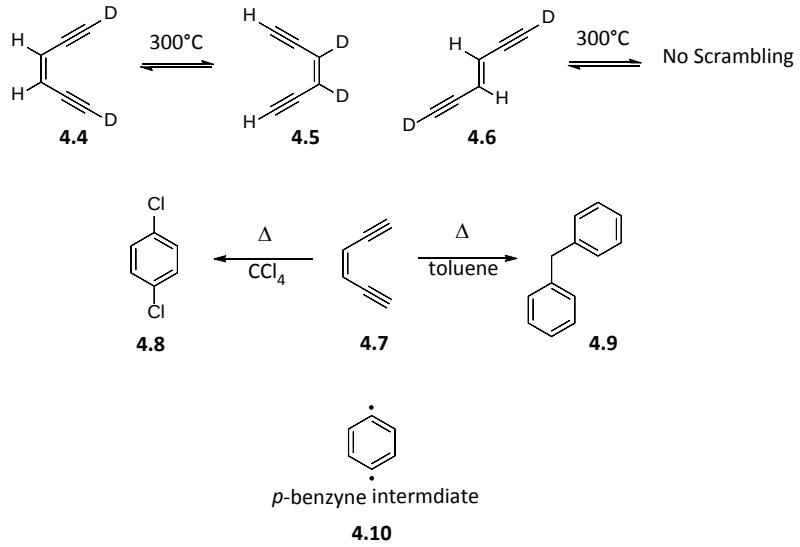
Calicheamycin γ_1 **4.2**



Esperamycin A_1 **4.3**

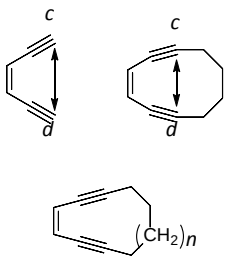
Figure 4.1 Structures of common enediyne antibiotics

In 1973, Bergman published the first report of the cycloaromatization of enediynes.[2] He found that pyrolysis of pure (Z)-hexa-3-en-1,5-diyne-1,6-d_2 **4.4** effectively scrambled the deuterium labels, providing a mixture of **4.4** and **4.5** (Scheme 4.1). Identical experiments with the *trans* isomer **4.6** didn't display this phenomenon. Additionally, heating **4.7** in CCl_4 or toluene yielded dichlorobenzene **4.8** and diphenylmethane **4.9** respectively, indicative of radical abstraction by a p-benzyne intermediate **4.10**.



Scheme 4.1 Bergman's initial studies of enediyne cycloaromatization

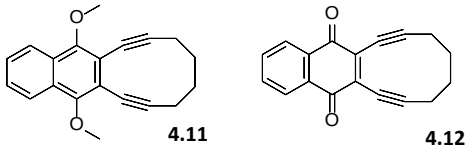
The activation barrier for Bergman cyclization in acyclic enediynes is relatively high, approximately 32 kcal mol^{-1} for **4.7**.[2a] The introduction of ring strain, as seen in enediyne antibiotics, effectively reduces the activation energy for cyclization. In 1988, Nicolaou noted that a critical factor in predicting the reactivity of cyclic enediynes is the "*cd* distance," the distance between the acetylenic termini (Scheme **4.2**).[3] It was noted that compounds with a *cd*< 3.20 Å undergo spontaneous cycloaromatization at room temperature.



n = 0 could not be synthesized (*cd*- 2.84 Å)
n = 1 cyclized at 25°C (*cd*- 3.25 Å)
n = 2-7 stable at 25°C (*cd*- 3.61-4.20 Å)

Scheme 4.2 Effect of *cd* distance on Bergman cyclization

The rate of Bergman cyclization in enediynes can also be modulated by electronic effects. Work by Nicolaou has shown that benzannulation of cyclic enediynes dramatically increases the activation barrier for cyclization.[4] Conversely, benzannulation of acyclic enediynes increases the rate of cyclization.[5] Substitution by electron withdrawing groups near the ene moiety can greatly increase cyclization rate. Semmelhack has reported that oxidation of naphthalene fused enediyne **4.11** to the quinone **4.12** dramatically decreases the half lifetime of cycloaromatization.[6]

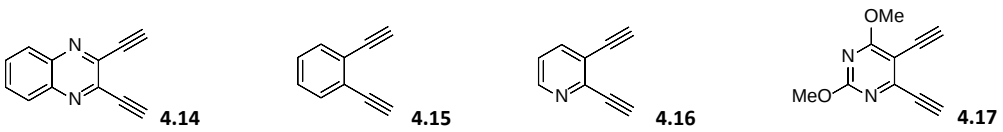


4.11 **4.12**

$t_{1/2}$ > 7d 120°C $t_{1/2}$ = 2h 55°C

Scheme 4.3 Effect of oxidation on cyclization rate

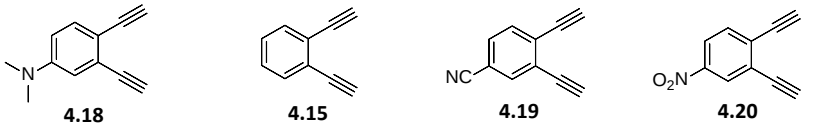
Fusion of heterocyclic rings to the enediyne core has a significant impact on reactivity. Russell has explored cyclization reactivity of a series of heteroarene fused acyclic enediyenes (Scheme 4.4).[7] Depending on the substitution, these analogues displayed increased or decreased activation energies for cyclization compared to benzannulated enediyne **4.15**.



4.14 E_a = 33.1 kcal mol^{-1} **4.15** E_a = 25.1 kcal mol^{-1} **4.16** E_a = 21.5 kcal mol^{-1} **4.17** E_a = 16.1 kcal mol^{-1}

Scheme 4.4 Activation energies of arene fused enediynes

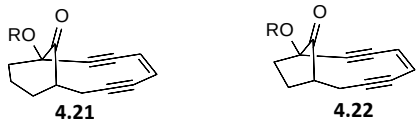
Calculations by Alabugin and co-workers suggest that substitution by electron withdrawing groups in the *ortho*- and *para*- positions of benzannulated enediynes generally reduce the activation barrier for cyclization.[8] Hammett analysis of a series of para-substituted benzannulated enediynes supports this conclusion (Scheme 4.5).[9]



4.18 $t_{1/2}$ = 15.6 min **4.15** $t_{1/2}$ = 11.8 min **4.19** $t_{1/2}$ = 5.2 min **4.20** $t_{1/2}$ = 4.3 min

Scheme 4.5 Half lifetime of enediynes incubated with 1,4-CHD at 170°C

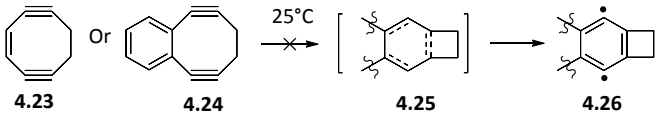
Another factor influencing the rate of Bergman cyclization is the difference in strain energy between the ground state and transition state. For example, bicyclic enediyne **4.21** has a rate 650 fold faster than its analog **4.22**, even though its *cd* distance is larger (Scheme 4.6).[10]



4.21 $k_{124°C}$ = 1.35 x 10^{-2} s^{-1} *cd* = 3.391 Å **4.22** $k_{124°C}$ = 2.08 x 10^{-5} s^{-1} *cd* = 3.368 Å

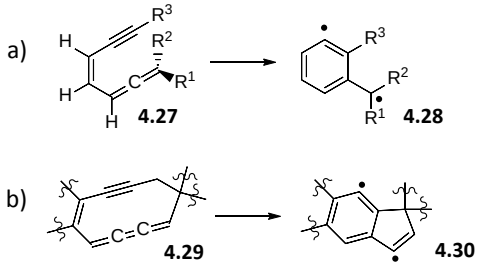
Scheme 4.6 Rate of cycloaromatization of bicyclic enediynes

The surprising stability of eight membered enediynes is an example of this effect (Scheme 4.7). Despite having a very small *cd* distance, **4.23** and **4.24** do not cyclize at room temperature due to high strain energy of the transition state **4.25**.[11]



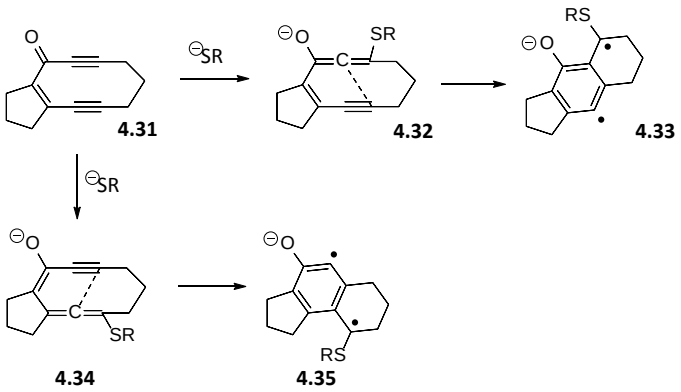
Scheme 4.7 Stability of eight membered enediynes

Some enediyne antibiotics, in particular members of the neocarzinostatin family[12], undergo a slightly different cycloaromatization pathway: Myers-Saito cyclization. First reported in 1989 independently by Myers[13] and Saito,[14] compounds possessing a (Z)-1,2,4-heptatrien-6-yne core undergo a facile C^2-C^7 cyclization to form an α, 3-didehydrotoluene biradical (Scheme 4.8a).



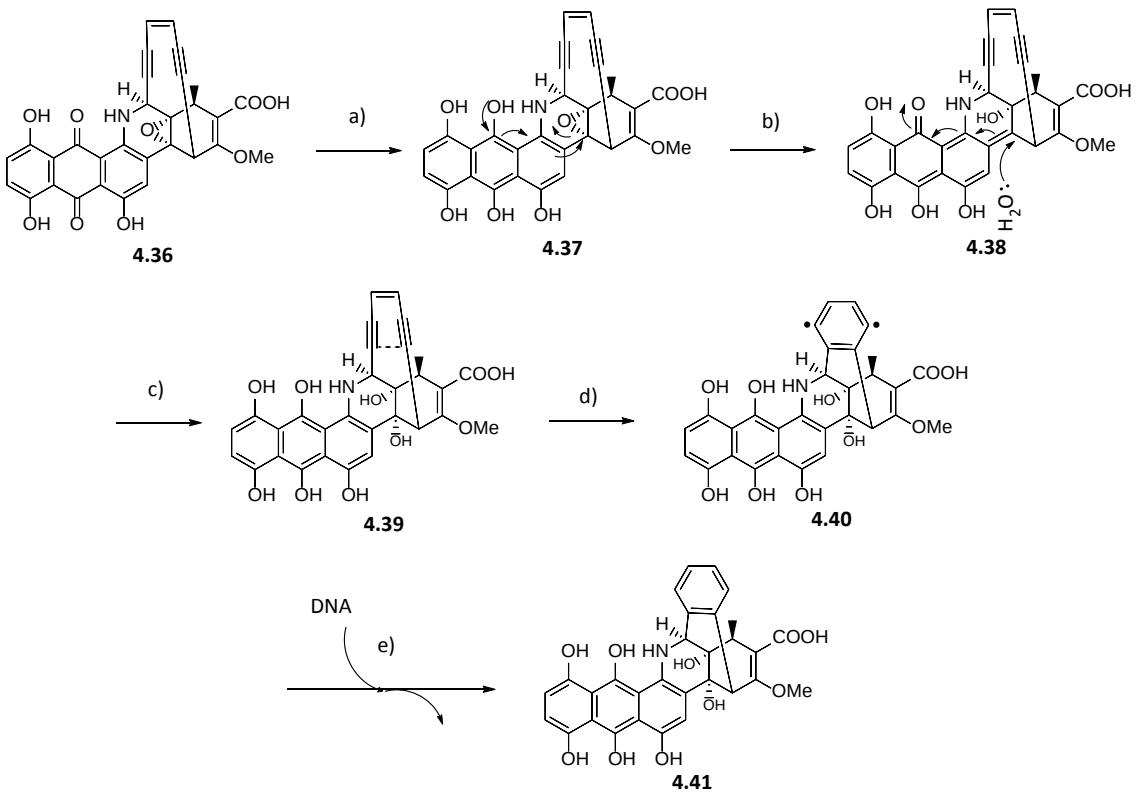
Scheme 4.8 Myers-Saito cycloaromatization

The activation barrier for Myers-Saito cyclization is much lower than that for Bergman, approximately 21-23 kcal mol^{-1} for unsubstituted **4.27**.[15] The Myers-Saito cyclization is also highly exothermic, with $\Delta H_{rxn} \approx$ -15 kcal mol^{-1} compared to the endothermic (+14 kcal mol^{-1})[2b] Bergman reaction of **4.7**. The favorable thermodynamics of the reaction is proposed to arise from conjugation of the methylene in biradical **4.28** to the aromatic ring.[13] The low activation barrier of Myers-Saito compared to Bergman cyclization is due to a more efficient overlap of the in-plane pi orbitals.[16] Cyclic enyne-allenes (Scheme 4.9) and enyne-cummulenes (Scheme 4.8b) are known to spontaneously cycloaromatize at room temperature.[17]



Scheme 4.9 Cycloaromatization of a cyclic enyne-allene

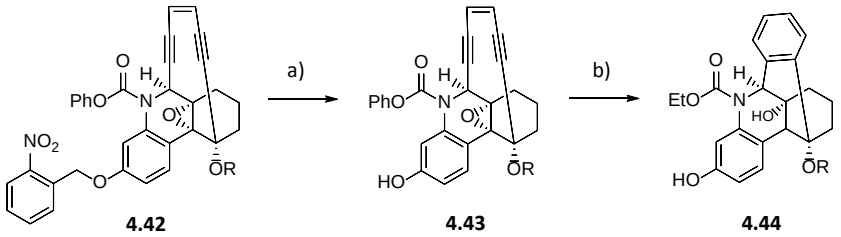
The mechanism of action of enediyne antibiotics is beautifully complex.[1, 12, 18] These compounds all share a common template of three sub-regions each with a specific role. A targeting region (typically an extended sugar moiety) allows for binding of the molecule to the minor groove of double-stranded DNA of the target. A molecular trigger then allows for activation of the enediyne "warhead." The mechanism for Dynemicin A is shown in Scheme 4.10.[18d] The anthraquinone unit of this molecule directs intercalation and binding to the DNA minor groove. In this case, the triggering mechanism is a bioreduction of the p-quinone moiety of **4.36** that is initiated by DNA binding. The next step in the cascade is epoxide opening to form **4.38**, concurrently introducing additional ring strain in the enediyne and causing spontaneous cycloaromatization. The resultant p-benzyne radical **4.40** abstracts hydrogen from the sugar backbone of the DNA, inducing dual strand cleavage.



Steps: a) bioreduction; b) epoxide opening; c) nucleophilic attack d) cycloaromatization; e) dNA dual strand cleavage

Scheme 4.10 Mechanism of dNA cleavage by Dynemicin A

Natural enediyne antibiotics lack tumor specificity, thus making them highly toxic. The high general toxicity of enediynes limits their clinical efficacy.[18b] Additionally, several mechanisms exist for development of tumor resistance, and multi-drug resistance (MDR) is a common problem in enediyne therapeutics.[19] To circumvent this issue, many efforts have focused on development of enediyne prodrugs, wherein directed triggering releases the active enediyne on demand. Several such strategies exist. Nicolaou has demonstrated photolabile protection of Dynemicin analogues (Scheme 4.11).[18c] Irradiation of **4.42** provides the corresponding phenol **4.43**. Bergman cyclization is then triggered by LiOH to provide **4.44**. Wender has developed a similar acid mediated strategy for activation of Dynemicin analogues.[20] Unfortunately, the requirement for acid or base activation makes this type of caging unsuitable for a potential prodrug.

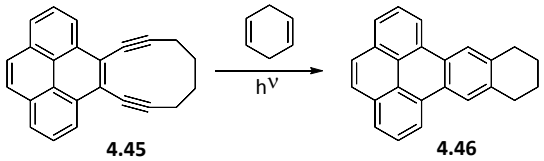


4.42 **4.43** **4.44**

Reagents and Conditions: a) h^{ν}, THF:H_2O, 83% ; b) 4 eq. LiOH in EtOH:H_2O, 56%

Scheme 4.11 Photochemical release of enediyne precursor

The Bergman cyclization can also be photochemically induced directly. Both acyclic[21] and cyclic[22] enediynes have been shown to undergo photoinitiated Bergman cyclization. Additionally, Funk and coworkers have designed a pyrene fused ten membered enediyne specifically for photoactivation (Scheme 4.12).[23] The compound **4.45** is stable at room temperature in the dark but cyclizes upon exposure to UV light in the presence of a hydrogen donor to form **4.46**. Irradiation of **4.45** in the presence of plasmid DNA induced dual strand scission. Unfortunately, the efficiency of the reaction is rather low, and long irradiation times are necessary for adequate conversions.

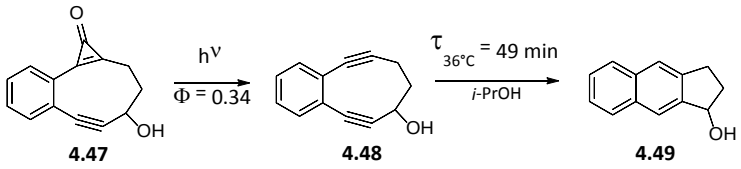


4.45 **4.46**

Scheme 4.12 Funk *et Al.* photochemical Bergman cyclization

An alternative strategy is to design a masked enediyne prodrug that will form a highly reactive enediyne upon irradiation. The unmasked compound should be labile enough to react spontaneously without the need for additional chemical reagents. The Popik lab has utilized this methodology extensively towards the development of enediyne prodrugs.[17b, 17c, 24]

Our group has recently reported the synthesis of a photocaged nine membered enediyne.[24a] Photolysis of cyclopropenone **4.47** efficiently (Φ = 0.34±.03) generates enediyne **4.48** (Scheme 4.13). At 36°C, **4.48** undergoes Bergman cyclization with a rate dependent upon H-donor concentration.



4.47 **4.48** **4.49**

Scheme 4.13 Bergman cyclization of photochemically generated enediyne

The ability of **4.48** to initiate DNA cleavage was evaluated (Figure 4.2). Solutions of **4.47** were irradiated and incubated with φX174 plasmid DNA for 16h. The samples were analyzed using agarose gel electrophoresis and compared against controls of DNA incubated alone and DNA incubated with **4.47** in the dark.

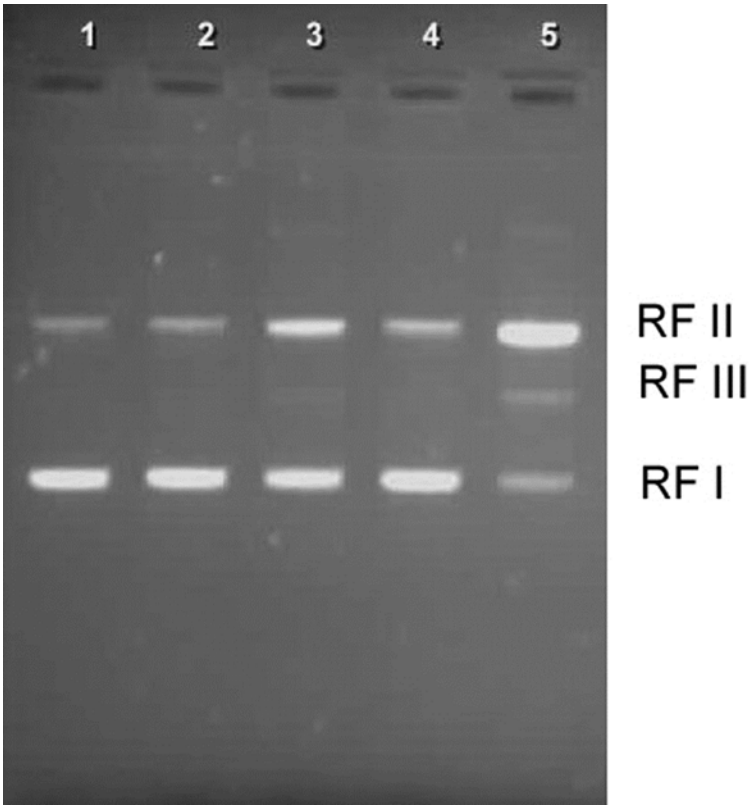


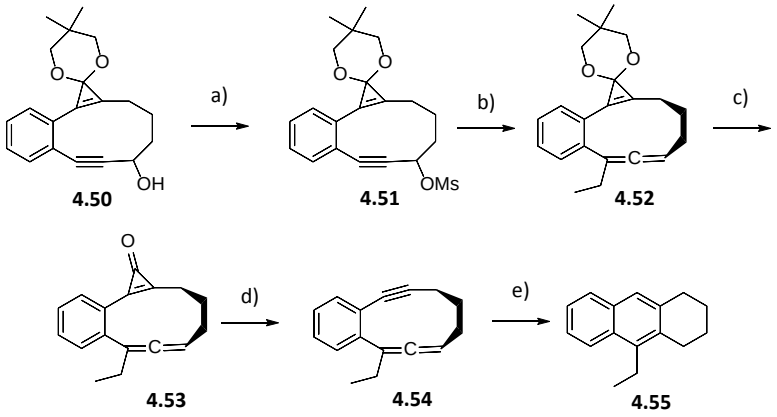
Figure 4.2 Gel electrophoresis of plasmid DNA scission produced by **4.47**.

Lane 1 features DNA incubated for 16h at 25°C. Lanes 2 and 4 show DNA incubated with 1 and 5 mM concentrations of **4.47** in the dark. Lanes 3 and 5 display DNA incubated with 1 and 5 mM concentrations of irradiated **4.47**. RF I shows uncleaved supercoiled plasmid DNA. RF II is the circular

relaxed form, the result of single strand scission. RF III is the linearized form, the result of dual strand scission. As shown, only the higher concentration of **4.48** displays dual strand scission, indicative of poor binding to the DNA.

Surprisingly, the nine membered enediyne was rather stable, the 49 minute lifetime being too long for good spatial and temporal control of the delivery of the p-benzyne radical. It was surmized that a photocaged ten membered enyne-allene could be developed from a similar platform, and the increased kinetics of Myers-Saito cyclization would allow for more precise targeting of DNA scission.

The synthesis of this photocaged, ten membered enyne-allene is shown in Scheme 4.14.[17b] Initially, cyclopropenone ketal **4.50** was treated with mesyl chloride and base to provide mesylate **4.51**. Treatment with an ethyl organocuprate followed by deprotection of the ketal yielded the photocaged enyne-allene **4.53**. Photolysis of **4.53** provided the enyne-allene **4.54** which cyclized in cyclohexadiene to **4.55** with a lifetime of 5 min.

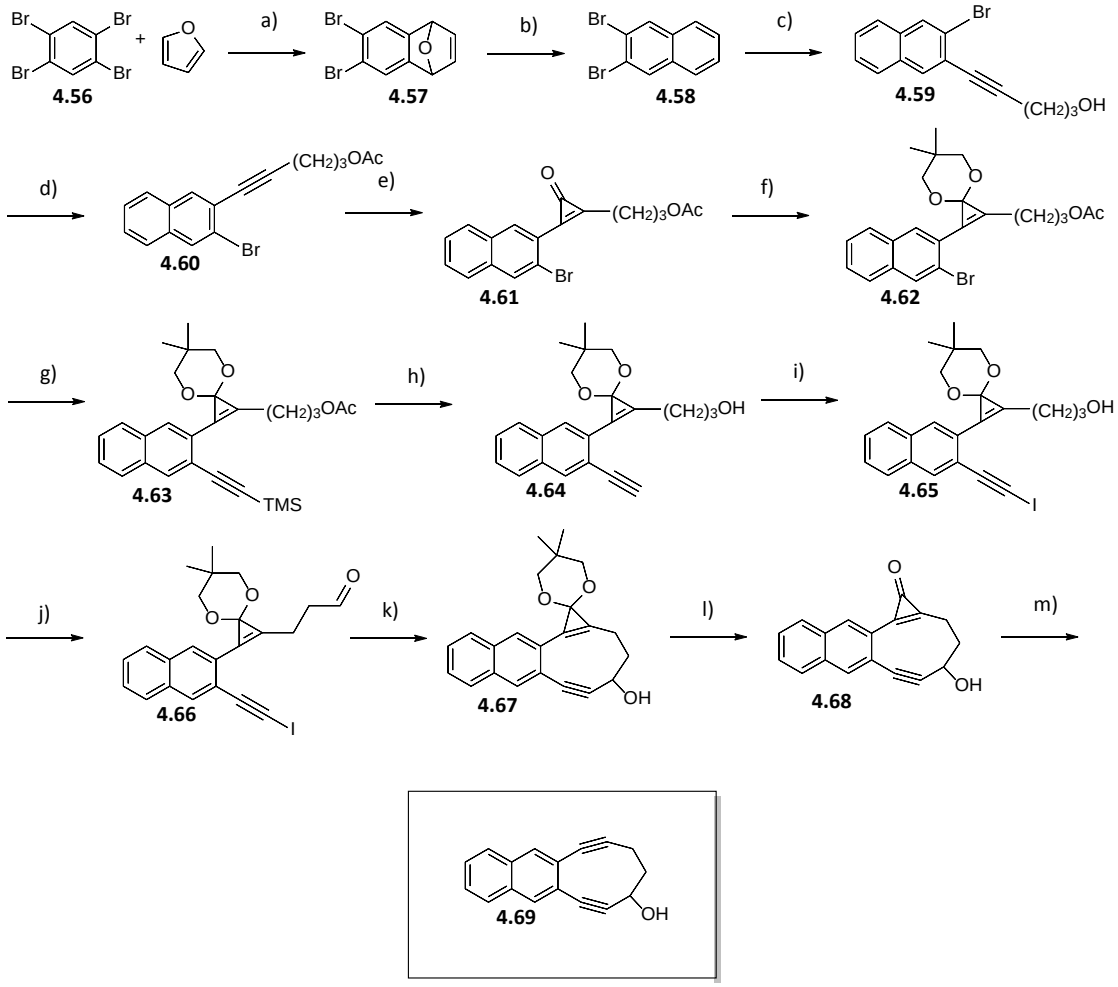


Reagents and conditions: a) MsCl, 90%; b) CuCN, LiCl, EtMgBr, 83%; c) Amberlyst-15, 75%; e) hν 300 nm; f) 1,4-CHD

Scheme 4.14 Synthesis and cyclization of photocaged enyne-allene

4.2 Synthesis of naphthalene fused nine membered enediyne

As shown above, dual strand DNA scission with 4.47 was not very efficient, presumably due to poor DNA binding. To improve DNA binding, we envisioned fusing a nine membered enediyne core to naphthalene. Substituted naphthalenes have been proven to be excellent DNA intercalators,[23, 25] and conjugation to the cyclopropenone chromaphore would allow for longer wavelength irradiation. The synthesis of this target is shown in Scheme 4.15.



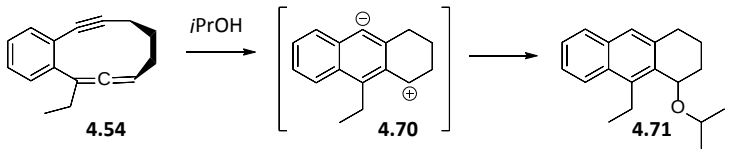
Reagents and conditions: a) n-BuLi, 73%; b) TMSI, NaI, 75%, c) 5-hexyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, NEt_2, 69%; d) Ac_2O, DMAP, 80%; e) $TMSCF_3$, NaI, 68%; f) neopentyl glycol, BF_4EtO_3, 72%; g) TMS-C_2SnBu_3, $Pd(PPh_3)_4$, 27%; h) K_2CO_3, MeOH, 72%; i) I_2, morpholine, 69%; j) DMP, 62%; k) $CrCl_2$, $NiCl_2$, 27%; l) Amberlyst-15, 88%; m) $h\nu$ 350 nm, 90%

Scheme 4.15 Synthesis of naphthalene-fused nine-membered enediyne

Initially, Diels-Alder reaction of benzyne and furan provided oxirane **4.57**. Treatment with *in situ*-formed TMSI gave 2,3-dibromonaphthalene **4.58**. Next, Sonogashira reaction with 5-pentyn-1-ol gave alcohol **4.59**. The alcohol moiety was capped as an acetate and subsequent Hu-Prakash reaction provided cyclopropenone **4.61**. The cyclopropenone was treated with neopentyl glycol and acid to form the ketal **4.62**, follwed by Stille coupling with TMS-acetylene to provide alkyne **4.63**. The yield of Stille coupling was rather low, probably due to deterioration of the sensitive Pd tetrakis catalyst. Simultaneous solvolysis of the silane and acetate provided terminal alkyne **4.64**. The compond was iodinated with I_2/morpholine followed by oxidation of the alcohol to give aldehyde **4.66**. Ring closure was conducted using an intramolecular NHK reaction, which gave **4.67** in low yield due to competing oligomerization. The ketal was removed by treatment with Amberlyst-15 resin in MeOH, yielding cyclopropenone **4.68**. Irradiation of the cyclopropenone at 350 nm provided the desired naphthalene fused enediyne **4.69** in excellent yield. To unambiguously prove the identity of the product, we hoped to prepare an X-ray quality crystal for diffraction. Unfortunately, when **4.69** was placed in chloroform for solvent diffusion, rapid polymerization occurred. The sample completely decomposed in ca. 8h, yielding a viscous, black oil.

4.3 Towards syntheis of a ten membered enyne-allene by enol-keto tautamerization.

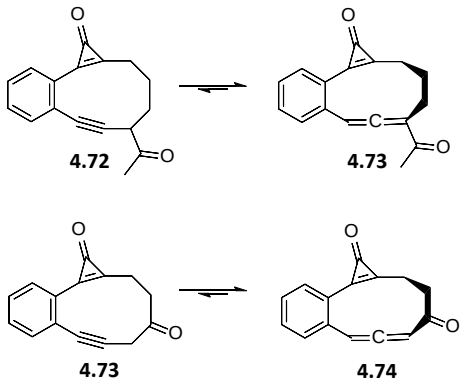
As stated in Section 4.1, our lab has prepared ten membered enyne-allenes and demonstrated their reactivity in Myers-Saito cyclizations. In polar solvents (alcohol/water), however, enyne-allene 4.54 cyclizes via polar mechanism, leading to solvent insertion product **4.71** (Scheme 4.16).



Scheme 4.16 Enyne-allene polar cyclization

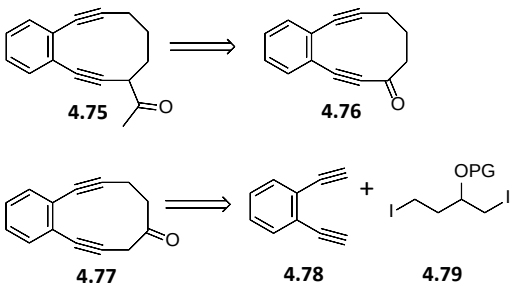
This dual reactivity makes these compounds unsuitable for potential therapeutic applications. Enyne-allenes featuring α-oxa-allenes, on the other hand, do not display this reactivity, providing Myers-Saito

adducts exclusively.[17c, 24f, 26] These compounds are readily accessed via keto-enol tautomerization of the corresponding β,γ-ketoacetylenes. We envisioned that cyclopropenone caged enyne-allenes could be prepared from either exo- or endocyclic ketoacetylenic precursors (Scheme 4.17).



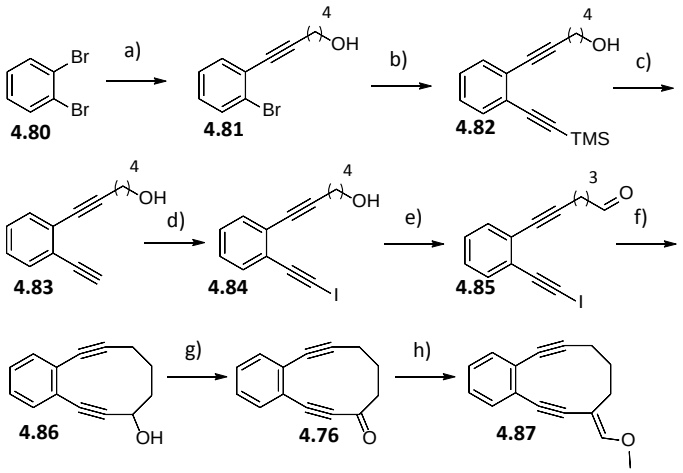
Scheme 4.17 Enyne-allene via keto-enol tautomerization

Initially, we decided to prepare the enediynes without photo-caging due to more straightforward synthesis to test the feasiblility of the project. We envisaged that both derivatives could be easily accessed (Scheme 4.18).



Scheme 4.18 Retrosynthetic analysis of desired precursors

Enediyne **4.76** was synthesized according to procedures previously developed by our lab (Scheme 4.19).[27] Sonogashira reaction of dibromobenzene **4.80** with 5-hexyn-1-ol provided acetylene **4.81**. A subsequent Sonogashira reaction with TMS-acetylene yielded bis-acetylene **4.82**. Solvolysis with MeOH followed by iodination with I_2 and morpholine gave the iodide **4.84**. Oxidation of the alcohol with Dess-Martin Periodinane provided aldehyde **4.85**.

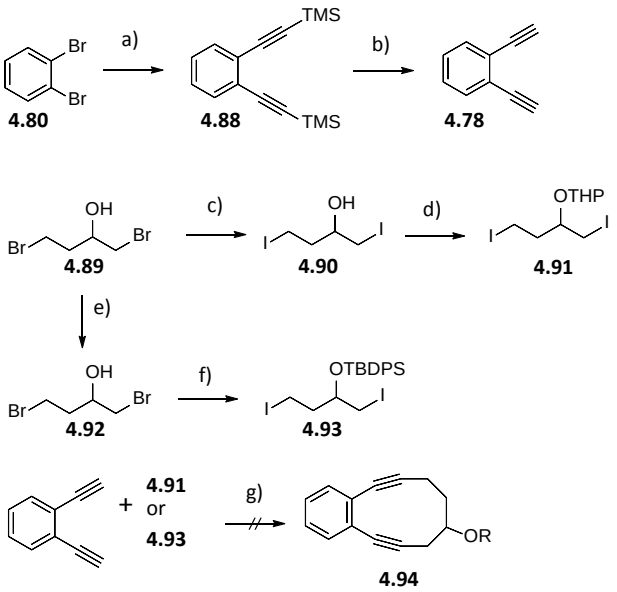


Reagents and conditions: a) 5-hexyn-1-ol, $Pd(PPh_3)_2Cl_2$, PPh_3, CuI, 64%; b)TMS-acetylene, $Pd(PPh_3)_2Cl_2$, PPh_3, CuI, 79%; c) K_2CO_3, MeOH, 82%; d) I_2, morpholine, 85%; e) DMP, DCM, 72%; f) $NiCl_2$, $CrCl_2$, THF, 25%; g) DMP, DCM, 63%; h) PPh_3CH_2OMe, n-BuLi, THF, 22%

Scheme 4.19 Synthesis of enediyne **4.87**

NHK conditions led to ring closure, albeit in low yield, to the enediyne **4.86**. A second Dess-Martin oxidation provided **4.76**. Wittig reaction of **4.76** with PPh_3CH_2OMe gave **4.87**, although the yield was extremely poor. Compound **4.87** proved to be extremely unstable, decomposing on storage even at low temperature.

Concurrently with the previous experiments, we attampted the synthesis of the precursor to the endocyclic ketone (Scheme 4.20). Benzene fused enediyne **4.78** was readily prepared from dibromobenzene **4.80** via Sonogashira coupling and desilylation. Ring closure of **4.80** using n-BuLi in HMPA was attempted with two different linkers **4.91** and **4.93** using a wide variety of conditions. Unfortunately, none of the attempts were successful, yielding either unreacted starting material or dehydrohalogenated linker.



Scheme 4.20 Attempted synthesis of enediyne **4.94**

4.4 Conclusions and Future Directions

We have synthesized a naphthalene fused nine membered enediyne, and attempted the synthesis of ten membered enyne-allenes. The high amount of angle strain in these compounds makes them highly unstable, susceptible to cyclization as well as polymerization. Due to the instability of the intermediates, future synthetic efforts should include early-stage protection of at least one of the acetylene moieties.

4.5 Experimental Procedures

General Methods

Tetrahydrofuran was freshly distilled from sodium/ benzophenone ketyl prior to use. Dichloromethane was freshly distilled from CaH_2 prior to use. HMPA was vacuum distilled and dried for 24h with 3 Å molecular sieves prior to use. DMF was distilled from phosphorous pentoxide and dried with 3 Å molecular sieves prior to use. Flash chromatography was performed using 40-63 μm silica gel obtained from Sorbent technologies. Electronic spectra were recorded using Cary 300 Bio UV-Vis spectrometer. Photolyses were conducted using a Rayonet photoreactor equipped with sixteen 4W 300

or 350 nm fluorescent lamps. All NMR spectra were recorded on a 400MHz spectrometer in deuterochloroform and referenced to TMS unless otherwise noted.

Materials

All reagents were purchased from Sigma Aldrich or VWR and used as received unless otherwise noted. The following compounds were synthesized according to known literature procedures: 2,3-dibromonaphthalene **4.58**,[28] 4,5-benzocyclodeca-2,6-diynone **4.76**,[17c] 1,2-diethynylbenzene **4.78**,[29] tert-butyl((1,4-diiodobutan-2-yl)oxy)diphenylsilane **4.93**,[30] and 2-((1,4-diiodobutan-2-yl)oxy)tetrahydro-2H-pyran **4.91**.[31]

Synthetic Procedures

7-methoxymethylidene-5,6,11,12-tetradehydro-7,8,9,10-tetrahydro-benzo[10]annulene (4.87). A solution of n-BuLi (1.58 mL, 2.53mmol) was added dropwise to a solution of methoxymethyl(triphenylphosphonium)chloride (0.796g, 2.32 mmol) in 21mL THF cooled to -78°C under argon.. The solution was stirred for 30 min. A solution of the ketone **4.76** (0.410 g, 2.11 mmol) in 21mL THF was added dropwise. The reaction mixture was stirred at -78°C for one hour, slowly warmed to r.t., and stirred for 5 hours. The reaction mixture was quenched with 30 mL saturated ammonium chloride, diluted with ether (200 mL), and extracted with distilled water (3x50 mL) and brine (2x50 mL). All aqueous layers were back extracted with small portions of ether. The crude mixtre was purified via flash chromatography (15% EtOAc in Hex) to give 0.106 g (22%) of the desired product as a yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.95 (m, 2H,), 2.53 (m, 4H), 3.71 (s, 3H), 6.43 (s, 1H), 7.17 (m, 2H), 7.28 (m, 2H).

Attempted synthesis of tert-butyldiphenyl((5,6,11,12-tetradehydro-7,8,9,10-tetrahydrobenzo[10]annulen-8-yl)oxy)silane (4.94) A solution of n-BuLi (9.36 mL, 14.98 mmol) was

added all at once to a solution of diethynylbenzene **4.78** (0.90g, 7.13 mmol) in THF (150 mL) and HMPA (50 mL) at -78°C. The mixture was stirred for 1.5 hrs. The diiodide **4.93** was added dropwise via syringe pump over 1 hr. The reaction mixture was allowed to slowly warm to rt and stirred for 48 hr. The solution was quenched with NH_4Cl (75 mL) and taken up in ether (300 mL). The organic layer was extracted with LiCl (200 mL) and NaCl (200 mL). The mixture was purified via flash chromatography (10% EtOAc/hexanes). Only unreacted bis-alkyne and the elimination product of **4.93** could be recovered.

5-(3-bromonaphthalen-2-yl)pent-4-yn-1-ol (4.59). $Pd(PPh_3)_2Cl_2$ (0.75g, 1.22 mmol), PPh_3 (0.290 g, 1.22 mmol), and CuI (0.215g, 1.22 mmol) were added to a degassed solution of Et_2N (20 mL) and 2,3-dibromonapthalene (3.20 g, 12.20 mmol) and the temperature was raised to 60°C. 4-pentyn-1-ol (1.22 mL, 13.9 mmol) was added dropwise to the heated solution. The mixture was allowed to reflux for 20 hours. The reaction mixture was diluted in ether and worked up with satd. NH_4Cl and brine, then dried over $MgSO_4$. The crude mixture was evaporated to dryness and purified via flash chromatography (50% hexanes in EtOAc) to yield 3.23 g (66%) of yellow solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.06 (s, 1H), 7.95 (s, 1H), 7.7-7.6 (m, 2H) 7.5-7.3 (m, 2H) 3.91 (t, J = 5.8Hz, 2H), 2.65 (t, J= 5.8Hz, 2H), 1.93 (m, 2H).

5-(3-bromonaphthalen-2-yl)pent-4-yn-1-yl acetate (4.60). Acetic anhydride (4.89ml, 51.77mmol) was added to a solution of the alcohol **4.59** (3.74 g, 12.94 mmol), DMAP (0.316 g, 2.59 mmol), and triethylamine (50 mL), in DCM (100 mL) in an ice bath. The reaction mixture was stirred for 2 hours. After the disappearance of the starting material, the mixture was taken up in ether (300 mL) and extracted with bicarbonate (150 mL), NH_4Cl (150 mL), and brine (150 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified via flash chromatography (25% EtOAc/hexanes). The reaction yield **4.27 g** (76%) of the title compound as a yellow oil. ^{1}H-NMR ($CDCl_3$), δ

(ppm): 8.01 (s, 1H), 7.95 (s, 1H), 7.73 (m, 2H), 7.48 (m, 2H), 4.31 (t, 6.4Hz, 2H), 2.63 (t, J = 6.4Hz, 2H), 2.09 (s, 3H), 2.01 (q, J = 6.4Hz, 2H.)

3-(2-(3-bromonaphthalen-2-yl)-3-oxocycloprop-1-en-1-yl)propyl acetate (4.61). The acetylene 4.60 (0.200 g, 0.604 mmol), $TMSCF_3$ (0.172g, 1.21 mmol), NaI (0.199g, 1.38 mmol), and THF (3.0 mL) were mixed into a pressure tube at room temperature. Then the reaction mixture was heated at 110°C for 2 h. The reaction was quenched by adding saturated Na_2CO_3 solution (5 mL), followed by extraction with Et2O (20 mL x2). The organic phase was dried over anhydrous K_2CO_3. After the removal of solvent *in vacuo*, the residue was subjected to silica gel column chromatography with petroleum ether/EtOAc/Et_3N (100/10/1, v/v) to give 0.188g (73%) of the desired product as a yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.17 (s, 1H), 8.11 (s, 1H), 7.87 (d, 1H), 7.78 (d, 1H), 7.56 (m, 2H), 4.20 (t, 2H), 2.96 (t, 2H), 2.11-2.07 (m, 5H).

3-(2-(3-bromonaphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propyl acetate (4.62). The cyclopropenone **4.61** (9.55g, 26.6 mmol) and triethyloxonium tetrafluoroborate (15.15g, 80 mmol) were dissolved in dry DCM (40 mL) and stirred for 1.5 hr. at r.t. A solution of neopentyl glycol (5.53 g, 53.2 mmol) in DCM (20 mL) and Et_3N (8.0 g, 80 mmol) were added dropwise by hand. The mixture was stirred under argon overnight. The crude mixture was then passed through a short silica plug and purified via flash chromatography (20% EtOAc/hexanes). The compound was immediately taken up in toluene for the following reaction. The reaction yielded **8.55 g** (78%) of the title compound as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.18 (s, 1H), 8.10 (s, 1H), 7.88 (d, J = 7.1Hz, 1H), 7.78 (d, J = 7.1Hz, 1H), 7.34 (m, 2H), 4.21 (t, J = 6.0Hz, 2H), 3.10 (t, J = 6.0Hz, 2H), 2.23 (q, 2H) 2.08, (s, 3H).

3-(6,6-dimethyl-2-(3-((trimethylsilyl)ethynyl)naphthalen-2-yl)-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propyl acetate (4.63). Pd(PPh3)4 (2.21 g, 1.91 mmol) was added to a solution of the acetal **4.62** (8.55 g, 19 mmol) and 1-(tributylstannyl)-2-(trimethylsilyl)acetylene (14.0 mL, 38 mmol) in 380 mL of dry toluene and stirred at for 2 h at 90°C. The reaction mixture was concentrated and solid impurities were removed by filtration through a short silica gel column (ca. 5 cm). The solvent was removed *in vacuo*. Purification of the residue by chromatography (hexanes : ethyl acetate 9:1, containing 1.5% Et3N) gave 2.4 g, (27 %) of the title compound as a thick yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.79 (s, 1H), 7.74 (s, 1H), 7.53 (m, 1H), 7.45 (m, 1H), 7.20 (m, 2H), 3.92 (t, J = 6.0Hz, 2H), 3.51 (q, 4H), 2.77 (t, J = 6.0Hz, 2H), 1.81 (m, 2H), 1.75 (s, 3H), .94 (s, 3H), .75 (s, 3H), 0.00 (s, 9H).

3-(2-(3-ethynylnaphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propan-1-ol (4.64). potassium carbonate (0.714 g, 5.17 mmol) was added to a solution of the alkyne **4.63** (2.39 g, 5.17 mmol) in MeOH (32 mL) and DCM (11 mL) all at once. The reaction mixture was stirred at room temperature for 45min. The organic layer was concentrated *in vacuo* and purified via flash chromatography (1:1 EtOAc/hexanes) to give 1.29g (71%) of the title compound as a yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.10 (s, 1H), 8.05 (s, 1H), 7.84 (m, 1H, *J*= 7.01, 10.5 Hz), 7.77(m, 1H, *J*= 7.01, 10.5 Hz) 7.52 – 7.50 (m, 2H, *J*= 7.01, 10.5 Hz), 3.93-3.77 (t, 2H, *J*= 5.88 Hz) and (m, 4H), 3.37 (s, 1H), 3.01 (t, 2H, *J*= 5.88 Hz), 2.03 (quintet , 2H, *J*= 5.88 Hz), 1.23 (s, 3H), 1.05 (s, 3H).

3-(2-(3-(iodoethynyl)naphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propan-1-ol (4.65). A solution of iodine (1.46 g, 5.74 mmol) and morpholine (0.750 g, 8.16 mmol) in anhydrous benzene (30 mL) was stirred at 45°C for 45min. A solution of the starting compound **4.64** (1.00g, 2.87mmol) in 50 mL benzene was added by syringe all at once. The reaction mixture was stirred at 45°C for an hour. Thiosulfate (satd. 100 mL) was added and the organic layer was dried with brine and

Na_2SO4. The mixture was concentrated *in vacuo* and purified via flash chromatography (50% EtOAc/hexanes), yielding 0.947g (69%) as a yellow oil. ^{1}H NMR (400 MHz, CDCl3) δ 8.05 (s, 1H), 8.03 (s, 1H), 7.83 (m, 1H, *J*= 7.21, 9.32 Hz), 7.77 (m, 1H, *J*= 7.21, 9.32Hz) 7.51 – 7.49 (m, 2H, *J*= 7.21, 9.32 Hz), 3.86-3.78 (t, 2H, *J*= 5.64 Hz and m, 4H), 2.97 (t, 2H, *J*= 5.64 Hz), 2.04 (quintet , 2H, *J*= 5.64 Hz), 1.23 (s, 3H), 1.06 (s, 3H).

3-(2-(3-(iodoethynyl)naphthalen-2-yl)-6,6-dimethyl-4,8-dioxaspiro[2.5]oct-1-en-1-yl)propanal (4.66)

A solution of DMP (1.68 g, 3.96 mmol) in DCM (20 mL) was added all at once to a solution of the alcohol **4.65** (0.94 g, 1.98 mmol) and sodium bicarbonate (0.333 g, 3.96 mmol) in DCM (20 mL). The solution was stirred at room temperature for 3 hours. The reaction was quenched with bicarbonate (50 mL) and the organic extract was washed with sodium thiosulfate and brine. The organic layer was dried over Na_2SO4 and concentrated *in vacuo*. The crude mixture was purified via flash chromatography (40%EtOAc/Hex) yielding 0.580g (61%) of yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 9.80 (s, 1H), 8.37 (s, 1H), 8.03 (s, 1H) 7.84 (d, J = 7.3Hz, 1H), 7.74 (d, J = 7.3Hz, 1H), 7.54 (m, 2H), 3.52 (m, 2H), 3.42 (d, J = 7.0Hz, 2H), 3.02 (t, J = 6.5Hz, 2H), 2.12 (m, 2H), 1.11 (s, 3H), .65 (s, 3H).

Naphthalene fused cyclononyne cyclopropenone acetal (4.67). $NiCl_2$ (0.080 g, 0.614 mmol), $CrCl_2$ (0.453 g, 3.68 mmol) were added to a solution of degassed THF (125 mL, 10mM) and stirred under argon for 20 min. A solution of the aldehyde **4.66** (0.580g, 1.23 mmol) in THF (40 mL) was slowly added via addition funnel. The reaction mixture was stirred for 3 hours, partially concentrated, diluted in 50/50 hexanes/EtOAc (250 mL) and filtered through a short celite plug, then a sillica plug, and evaporated *in vacuo*. The crude mixture was purified via flash chromatography (50%EtOAc/hex), yielding 0.116g (27%). ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.44 (s, 1H), 7.84 (m, 1H), 7.74 (m, 2H), 7.54 (m, 2H), 4.77 (s, 1H), 3.45 (s, 4H), 3.28 (m, 1H), 2.78 (m, 1H), 2.29 (m, 1H), 2.09 (m, 1H), .85 (s, 6H).

Naphthalene fused cyclononyne cyclopropenone (4.68). Amberlyst-15 resin (150mg) was added to a solution of the acetal **4.67** (0.114g, 0.329mmol) in acetone (40 mL). The solution was stirred at r.t. for 2 hours, filtered, and evaporated *in vacuo*. The product mixture was purified via flash chromatography 100% EtOAc-20% MeOH/DCM (gradient mode). The reaction yield 0.75g (88%) of the title compound as a white solid. ^{1}H-NMR ($DMSO_{D6}$), δ (ppm): 8.46 (s, 1H), 8.10 (d, J = 7.3Hz, 1H), 8.05 (s, 1H), 7.94 (d, J = 8.3Hz, 1H), 7.63 (m, 2H), 4.64 (s, 1H), 3.20 (m, 1H), 2.69 (m, 1H), 2.15 (m, 1H), 1.87 (m, 1H).

6,7,11,12-tetradehydro-9,10-dihydro-8H-cyclonona[b]naphthalen-8-ol (4.69). The cyclopropenone was taken up in dilute MeOH (300 mL, 3.9 x 10^{-4} M) and irradiated at 350nm for 2 min. The mixture was evaporated and purified via flash chromatography (30% EtOAc/hexanes) to give 31mg (85%) of enediyne as a white solid. ^{1}H-NMR ($DMSO_{D6}$), δ (ppm): 8.00 (s, 1H), 7.91 (m, 3H), 7.54 (m, 2H), 5.69 (s, 1H), 4.75 (s, 1H), 2.82 (m, 1H), 2.63 (m, 1H), 2.16 (m, 2H).

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CHAPTER 5

A TARGETED, CLEAVABLE PHOTOAFFINITY LABEL FOR IMMUNOGLOBULIN G CONJUGATION

5.1 Synthesis of biotinylated aminobenzophenone-naphthyl quinone methide precursor probes

The development of improved cancer therapies is an overarching goal for the modern scientific community. One route toward this purpose is the development of targeting methods for conventional therapies, which suffer from issues caused by cytotoxicity. Several such strategies exist[1], and perhaps the most promising method involves the synthesis of antibody-drug conjugates (ADC's). The most commonly used antibody, immunoglobulin G (IgG), is a Y-shaped molecule (Fugure 5.1) which binds its target antigen (proteins, carbohydrates, nucleic acids, or haptens) with high specificity via its two tips: the fragment antigen binding (Fab), and the fragment variable (Fvar)[2]. The base region, or fragment crystallizable (Fc), serves to communicate with other immune components via a highly conserved glycosylation site and does not participate in antigen binding. The advent of phage display technology has allowed for rapid, on-demand construction of fully human IgG's with high binding affinity for myriad antigens[3]. Conjugation of these IgG's to form an ADC provides targeted delivery of a payload (typically a traditional chemotherapeutic) to cancer cells.

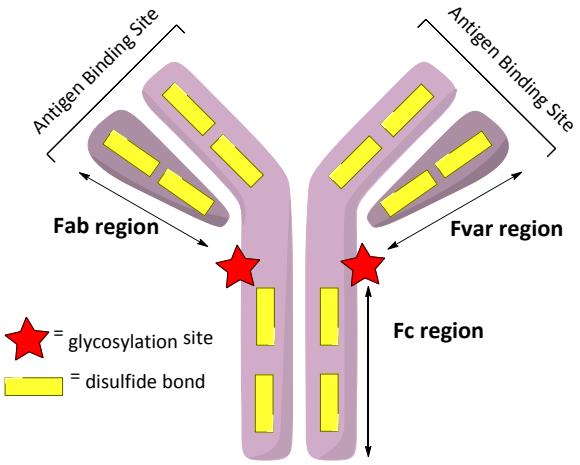


Figure 5.1 Structure of a typical imunnoglobulin G

Although the first ADC was synthesized over 50 years ago[4], only recently has their full potential been realized. The first ADC to be approved for clinical cancer therapy in the U.S. was rituximab in 1997[5]. Since then, seven more ADC's have followed as approved methods for cancer treatment[6].

Most existing strategies for ADC synthesis rely on alkylation of surface lysine[7] or cysteine[8] residues. Unfortunately, the conventional methods for synthesizing ADC's have shortcomings. Because there are numerous surface accessible nucleophilic residues, the antibodies experience varying degrees of conjugation, resulting in large variation in drug to antibody ratio (DAR). Studies have shown that ADC's which possess DAR's between 2 to 4 exhibit the highest clinical efficacy[9]. To improve the homogeneity of ADC's, many site-specific conjugation methods have been explored[10]. The simplest site-specific conjugation method involves reduction of all interchain disulfide bonds, followed by complete conversion with a thiol-reactive moiety[10]. This was demonstrated by Senter and coworkers in the development of auristatin[11], but the resultant ADC possessed a DAR of 8, and had a low half-lifetime of circulation.

Another method is to selectively modify the oligosaccharides that exist on the heavy chain region of most human IgG's. Because IgG's are glycosylated at a highly conserved, specific site[12] (Asn297) in their Fc region, modification of the glycan with a functional handle should in theory provide homogenous ADC's with little effect on antigen binding. In 2014, Boons and co-workers used

sialyltransferase to attach azide-tagged sialic acid derivatives to the glycan chain of human IgG[13]. Characterization of the conjugates revealed the antibodies were labelled with an average of four azide units due to promiscuous (at sites other than Asn297) glycosylation by the enzyme used for conjugation. The resultant conjugates also suffered from high heterogeneity. Similar experiments[14] have shown incomplete conversion and problems with heterogeneity.

Recently, a new approach has been developed which utilizes naturally occurring IgG binding domains derived from Staphylococcal proteins A and G[15]. A recombinant, 58 amino acid peptide dubbed protein Z[16] (Figure 5.2)[17] has been developed which selectively binds to the Fc region of IgG's. Because the peptide is non-covalently bound to IgG, the resultant complexes aren't stable in biological media. The work of Konrad[18], and Yu[19], has surmounted this problem by attachment of photoactivatable benzophenone crosslinkers to Z protein for post-binding covalent attachment to IgG. Konrad et Al utilized solid phase peptide synthesis to incorporate a benzoyl phenylalanine (BPA) residue into the Z domain, while Yu et Al decorated a cysteine modified Z protein via a benzophenone maleimide. Tsourkas and co-workers[20] later improved upon this methodology by genetically incorporating BPA into protein Z. Using an amber codon suppressor pair, BPA was incorporated site-specifically into an Fc facing region on the peptide, and the resulting recombinant protein Z was photo-crosslinked to a variety of IgG's with high efficiency.

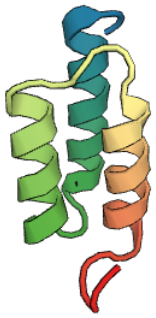
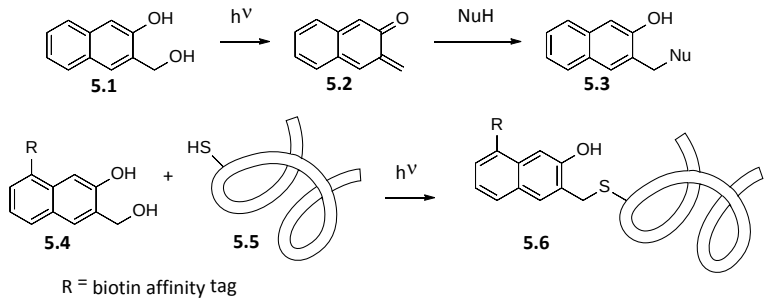


Figure 5.2 Staphylococcal protein A, Z-domain

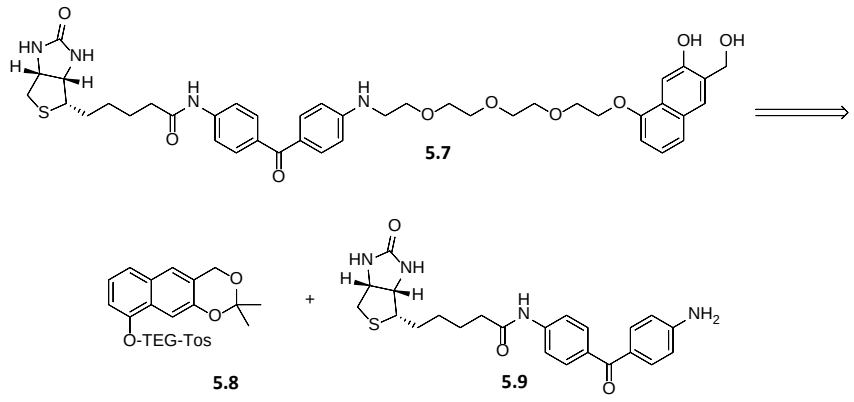
Because protein Z is derived from a bacterial protein, it has the potential for immunogenecity[21]. In light of this issue, it would be highly desirable to develop a method where protein Z could be used to direct crosslinking to a specific site on the heavy chain region, then cleaved from the antibody after the labelling event. To this end, our group has designed a strategy using two photoactivatable chromophores: a photoaffinity crosslinker and o-naphthyl quinone methide precursor **5.1** (NQMP), a well-studied excited state intramolecular proton transfer (ESIPT)[22] chromophore.[23] Upon irradiation in protic solvents, NQMP undergoes facile excited state dehydration to form a highly reactive quinone methide[24] (Scheme 5.1). The intermediate quinone methide **5.2** can then be attacked by nucleophiles such as thiols. Using this platform, our group has demonstrated reversible labelling of cysteine containing proteins **5.5** and peptides[25]. We envisioned that protein Z with an accessible cysteine residue could be reversibly conjugated to an NQMP derivative in a similar fashion. First, we would design a multi-functional NQMP analogue featuring a labelling moiety (fluorophore, affinity probe, etc.) as well as a photoactivatable crosslinker. This system would then be conjugated with protein Z via the photo-induced quinone methide. Site-selective conjugation to IgG can then be achieved by the photoactivatable crosslinker.



Scheme 5.1 Photo-conjugation of proteins via o-quinone methide

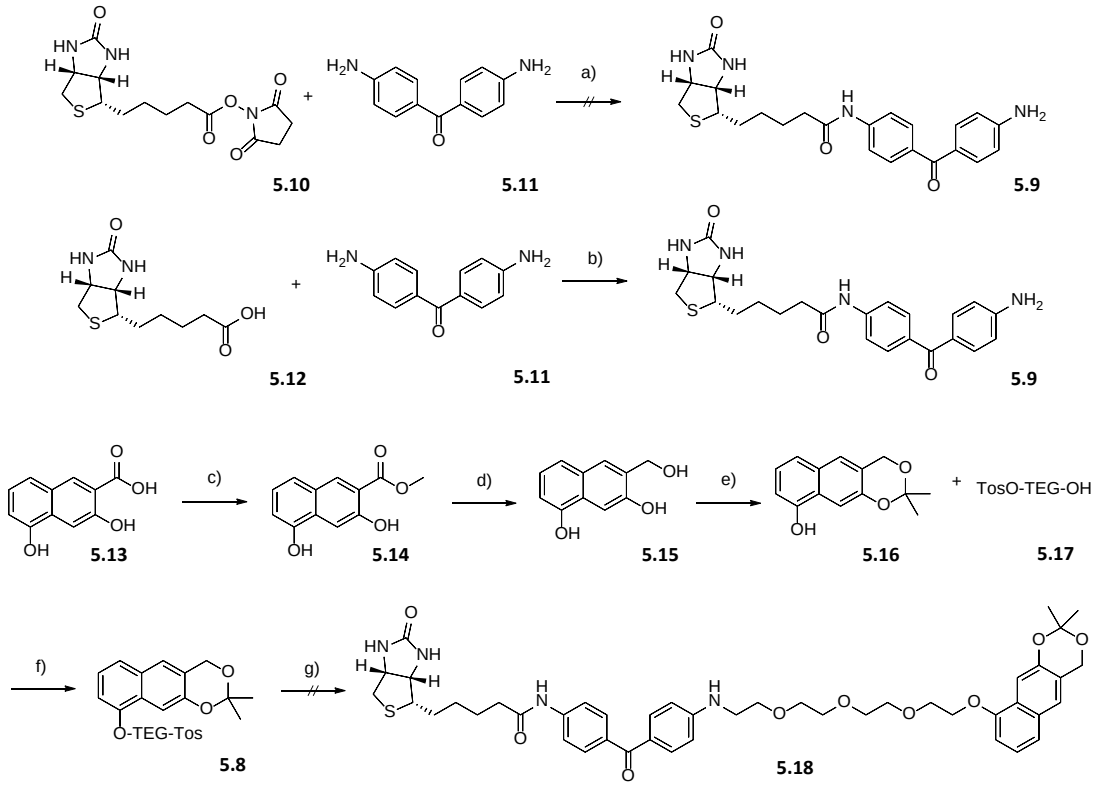
In designing this platform, it is imperative to select a crosslinker with a longer wavelength absorption band than the NQMP chromophore to achieve chromatic orthogonality. For this reason, we selected 4,4'-diaminobenzophenone (ABP) as our photo-crosslinker, which features a strong absorbance

band centered at 400 nm[26]. This should provide excellent orthogonality to NQMP, the absorbance of which drops sharply above 330 nm. We envisaged that an NQMP-ABP-Biotin probe **5.7** could be easily accessed via alkylation of biotinylated ABP[27] **5.9** with protected NQMP **5.3** (Scheme 5.2).



Scheme 5.2 Proposed synthesis of NQMP-ABP-Biotin **5.1**

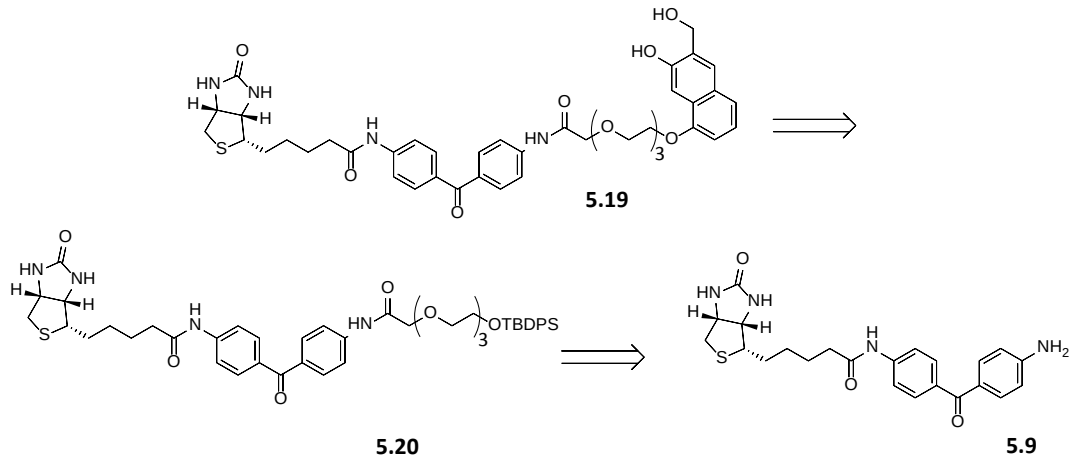
Our synthesis began with the preparation of ABP-biotin fragment **5.9** (Scheme 5.3). Initial attempts to acylate ABP **5.11** with biotin NHS-ester **5.10** did not proceed, presumably due to poor nucleophilicity of the aniline. Instead, fragment **5.9** was prepared via DCC coupling with biotin **5.12** according to literature procedure[27]. Synthesis of the NQMP fragment **5.8** began with Fischer esterification of naphthoic acid derivative **5.13**. Next, LAH reduction of ester **5.14** provided the corresponding alcohol **5.15**. Treatment with 2,2-dimethoxypropane gave acetonide **5.16**. Mitsunobu reaction of acetonide **5.16** with mono-tosylated tetraethylene glycol **5.17** yielded the desired NQMP fragment **5.8**. Unfortunately, alkylation of NQMP fragment **5.8** with ABP-biotin **5.9** did not proceed.



a) DMF, 80°C; b) DCC, aza-HOBT, DIPEA, 60%; c) H_2SO_4, MeOH, 98%; d) LAH, 81%;
e) 2,2-dimethoxypropane, TsOH, 72%; f) DIAD, PPh_3, DIPEA, 71%; g) **5.9**, DMF, K_2CO_3, 70°C

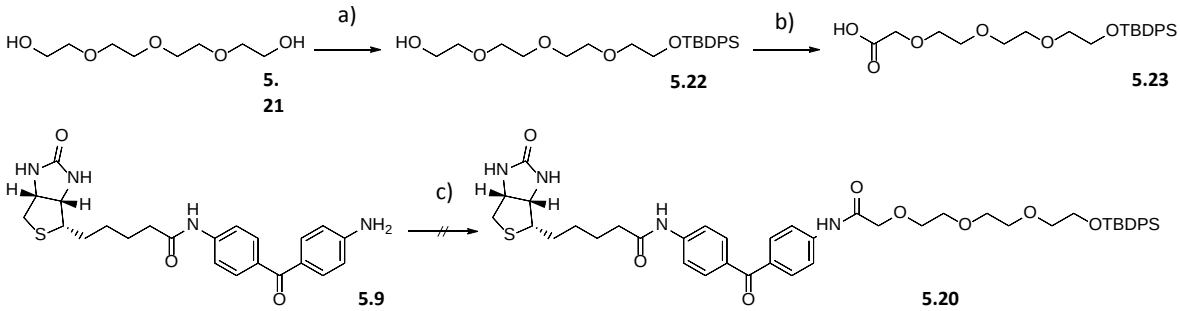
Scheme 5.3 Attempted synthesis of protected NQMP-ABP-biotin probe **5.18**

Because alkylation of the second aniline unit of **5.9** failed to proceed, we attempted an alternative route (Scheme 5.4). Attachment of a glycol linker to ABP-biotin **5.9** via DCC coupling would provide access to NQMP-ABP-biotin probe **5.19** after Mitsunobu reaction of the intermediate **5.20**.



Scheme 5.4 Proposed synthesis of NQMP-ABP-Biotin **5.19**

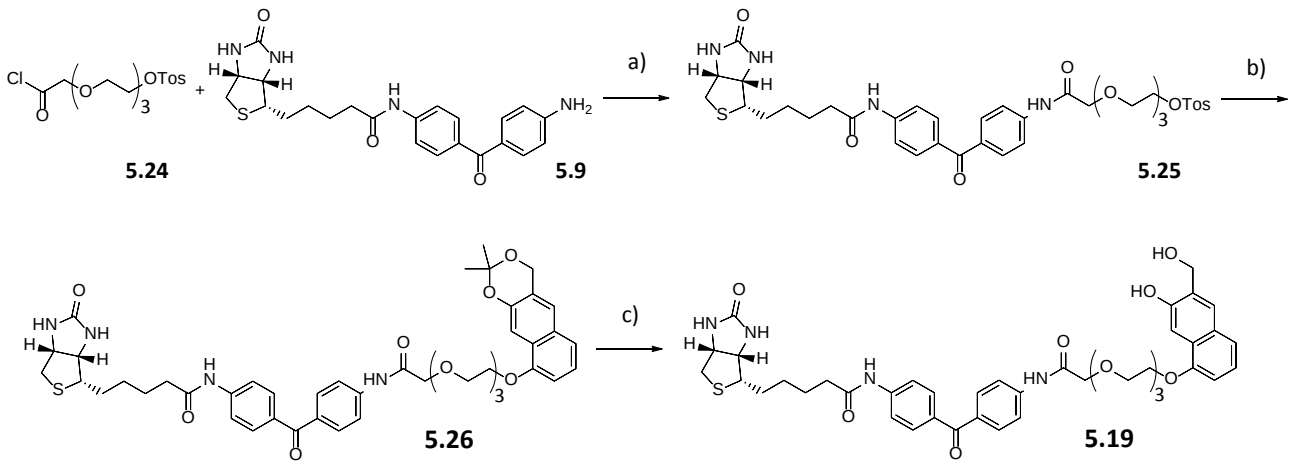
Initially, we prepared linker **5.23** by mono-silylation of tetraethylene glycol **5.21** followed by PDC oxidation (Scheme 5.5). Subsequently, no reaction occurred when ABP-biotin **5.9** was incubated with the glycol linker **5.23** under typical DCC coupling conditions.



a) TBDPSCl, imidazole, 40%; b) PDC, 20%; c) DCC, Aza-HOBT, **5.23**

Scheme 5.5 Attempted synthesis of ABP-biotin **5.20**

The failures of the two previous attempts demonstrated the low nucleophilicity of the aniline moiety of **5.9**. To circumvent this problem, we decided to use more reactive electrophiles to functionalize this platform. Towards this end, we treated ABP-biotin **5.9** with acyl chloride **5.24** which provided the tosylate **5.25** (Scheme 5.6). The reaction produced several byproducts due to partial alkylation of the biotin carbamate, which resulted in a very low yield.



a) NEt_3, 8%; b) **5.8**, K_2CO_3; c) Amberlyst-15, 30% over two steps

Scheme 5.6 Synthesis of NQMP-ABP-biotin probe **5.19**

Williamson ether synthesis of tosylate **5.25** with naphthol derivative **5.16** gratifying provided the acetonide-protected NQMP-ABP-biotin **5.26**, but ca. 10% of the starting material remained and could not be separated via chromatography. The crude mixture of **5.26** was treated with amberlyst-15 resin to provide diol **5.19**, albeit in low yield.

Unfortunately, analysis of the electronic spectrum of **5.19** revealed that the formation of the second amide moiety significantly blue shifts the absorbance of the benzophenone compared to **5.9** (Figure 5.3). The shift creates overlap in the absorbance of the two photo-active groups. Because orthogonality between the NQMP and benzophenone groups is critical to the success of our probe, bis-amide analogues are unsuitable.

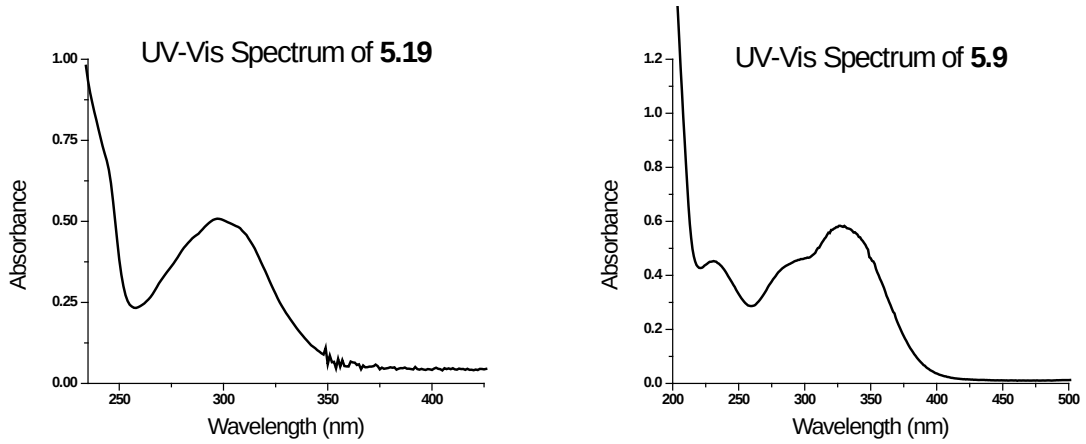
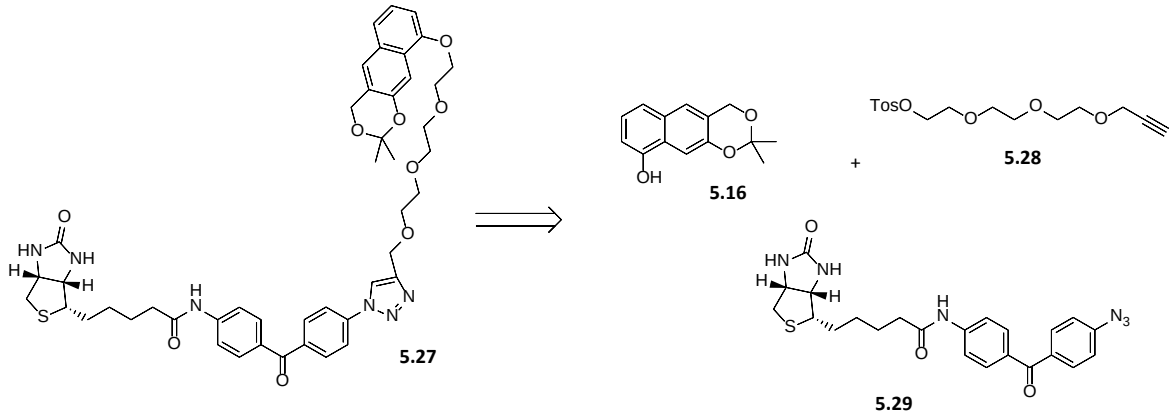


Figure 5.3 UV-Vis spectra of ca. 0.04 mM solutions of **5.19** and **5.9** in MeOH

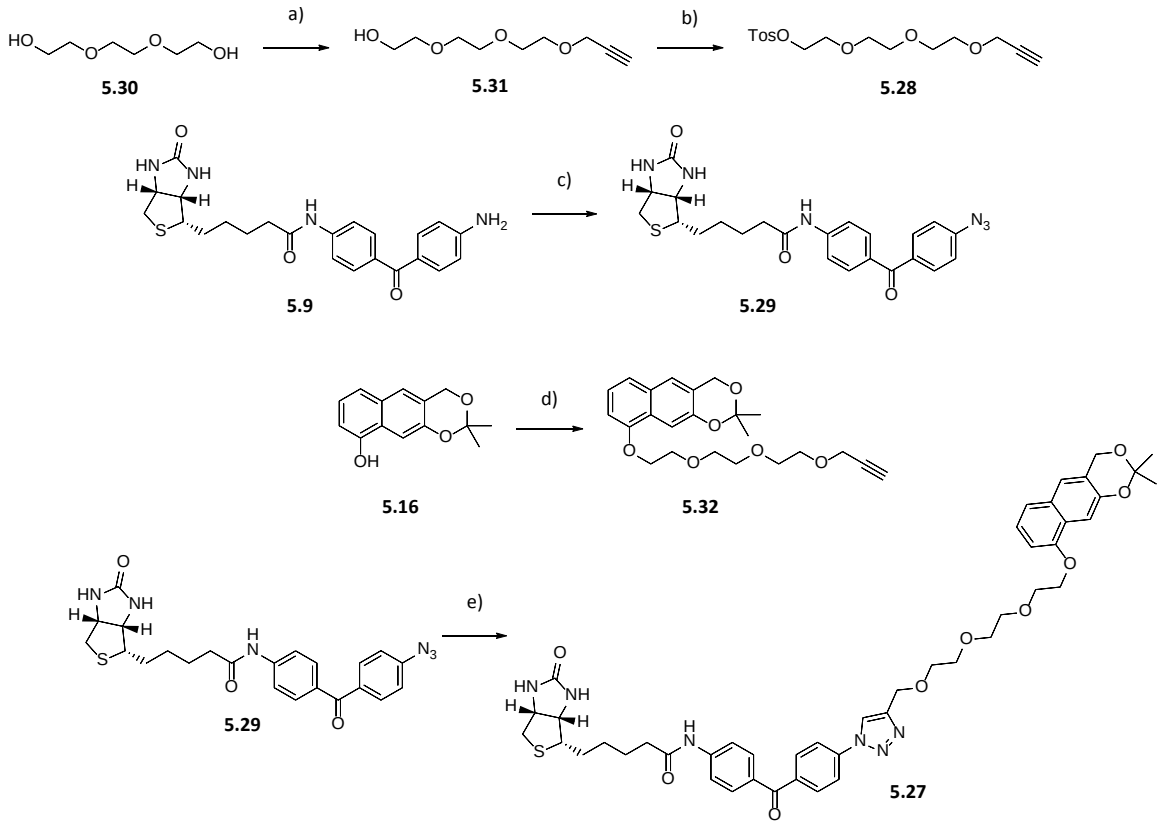
In light of these results, we formed another synthetic strategy to prevent this absorbance overlap (Scheme 5.7).



Scheme 5.7 Retrosynthetic scheme for triazole linked probe **5.27**

We believed that substituting the amide for a triazole linker, such as in **5.27** would preserve or possibly even red shift the benzophenone absorbance band due to extended conjugation. The necessary subunits could be easily assembled from simple synthons. This route aimed for convergent synthesis of probe **5.27** from three fragments: 1) acetonide protected NQMP **5.16**, 2) terminal acetylene linker **5.28**, and 3) azido ABP-biotin **5.29**.

The synthesis of probe **5.27** is shown in Scheme 5.8. Initially, triethylene glycol **5.30** was treated with propargyl bromide to give alkyne **5.31**[28], followed by addition of tosyl chloride to provide glycol linker fragment **5.28**[28]. Sandmeyer-type reaction of ABP-biotin **5.9** provided the azide fragment **5.29**. Williamson ether synthesis between naphthol **5.16** and the glycol linker **5.28** gave NQMP-acetylene **5.32**. Cu-mediated click reaction of acetylene **5.32** and the azide fragment **5.29** provided the desired target **5.27**.



a) NaH, propargyl bromide, 35%; b) TosCl, imidazole, 91%; c) $NaNO_2$, H_2SO_4, H_2O, NaN_3 52%;
d) **5.28**, K_2CO_3, DMF, 70°C 55%; e) **5.32**, $CuSO_4$, sodium ascorbate, NEt_3, 48%

Scheme 5.8 Synthesis of triazole linked probe **5.27**

With probe **5.27** in hand, we decided to check the electronic spectrum. Disappointingly, the benzophenone absorbance band was again red shifted to overlap with the NQMP band (Figure 5.4). The

red shift implies the triazole is perpendicular to the plane of the benzophenone and experiences little conjugation.

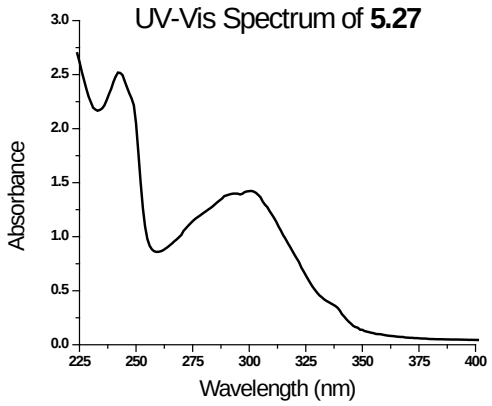
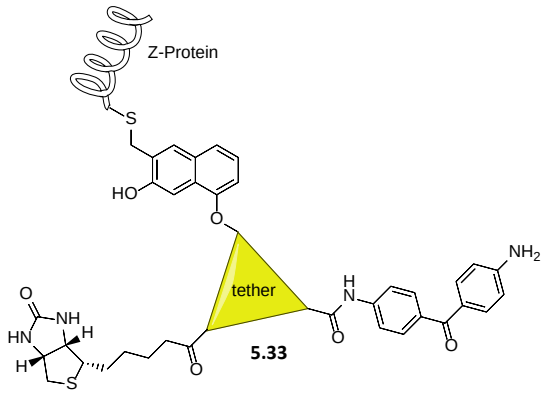


Figure 5.4 Electronic spectrum of ca. 0.1 mM **5.27** in MeOH

5.2 Conclusions and Future Directions

Both of the targets successfully synthesized were not viable candidates as photoaffinity probes due to absorbance overlap of their two chromophores. Although the project is not yet successful, there is positive progress that has made. We know now, for example, that at least one of the aniline units of the ABP chromophore must be preserved in order to maintain long wavelength optical density. Additionally, we have developed a variety of simple procedures and precursors that can be utilized for the functionalization of these molecules. It is also apparent that the ABP moiety must be used as an end group in the molecule rather than a bridge between functional groups. To this end, our future efforts will center on design and synthesis of a probe with the three functional subunits extending radially from a tether, rather than a linear arrangement (Scheme 5.9).



Scheme 5.9 General scheme for new target probe

5.3 Experimental Data

General Methods

Tetrahydrofuran was freshly distilled from sodium/ benzophenone ketyl prior to use. Dichloromethane was freshly distilled from CaH_2 prior to use. Solutions were prepared using HPLC grade water and methanol. Flash chromatography was performed using 40-63 μm silica gel. Electronic spectra were recorded using Cary 300 Bio UV-Vis spectrometer. All NMR spectra were recorded on a 400 MHz spectrometer in deuterochloroform and referenced to TMS unless otherwise noted. All reagents were purchased from Sigma Aldrich or VWR and used as received unless otherwise noted.

Experimental Procedures

N-(4-(4-aminobenzoyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (5.9). DCC (0.677g, 3.28mmol) was added to a solution of N,N'-bisaminobenzophenone (0.755g, 3.56 mmol), aza-HOBt (0.559g, 4.10mmol), DIPEA (0.667ml, 3.83mmol) and biotin (0.669g, 2.74mmol) in THF (25ml) at 25°C. The reaction mixture was warmed to r.t. and stirred for 12h. This mixture was evaporated in vacuo. The crude solid was purified via flash chromatography (70%

EtOAc/hexanes to 1% DCM/MeOH) to give 0.740g (62%) of the title compound (**5.9**) as a white solid. ^{1}H-NMR (DMSO), δ (ppm): 10.15 (s, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 6.58 (d, J = 8.7Hz, 2H), 6.42 (s, 1H), 6.34 (s, 1H), 6.06 (s, 1H), 4.30 (m, 1H), 4.10 (m, 1H), 3.10 (,, 1H), 2.81 (m 1H), 2.33 (t, 2H), 1.60 (m, 2H), 1.36 (m, 3H). ^{13}C-NMR (DMSO), δ (ppm): 192.86, 172.07, 163.13, 153.87, 142.52, 133.57, 132.80, 130.62, 124.57, 118.49, 112.91, 61.47, 59.63, 55.82, 36.75, 28.65, 28.53, 25.48.

methyl 3,5-dihydroxy-2-naphthoate (5.14). A solution of aq. H_2SO_4 (6.12ml, 110 mmol) was added to a solution of 3,5-dihydroxy-2-naphthoic acid (15g, 73.5 mmol) in MeOH (147 mL) all at once. The reaction mixture was refluxed overnight, diluted in ethyl acetate, and extracted with satd. sodium bicarbonate. The organic extract was dried over sodium sulfate and evaporated in vacuo to give 15.65g (98%) of the title compound as a yellow solid.

6-(hydroxymethyl)naphthalene-1,7-diol (5.15). LAH (2.75g, 70.3 mmol) was added to a solution of the ester (7.67g, 35.2 mmol) in THF (352 mL) slowly in small portions. The reaction mixture was stirred overnight and quenched with 1M HCl. The solvent was evaporated *in vacuo* and the crude residue was taken up in ethyl acetate. The solution was extracted with 1M HCl, distilled water, and brine. The organic extract was dried over sodium sulfate and evaporated *in vacuo* to give 5.43g (81%) of the title compound **5.15** as a yellow solid.

2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxin-9-ol (5.16). Toluenesulfonic acid (0.842g, 44.3 mmol) was added to a solution of the triol (8.42g, 44.3 mmol) and 2,2-dimethoxypropane (13.83g, 133 mmol) in DMF (221 mL) all at once. The reaction mixture was heated to 70°C and stirred 4h until disappearance of starting material on tlc. The solution was diluted in ethyl acetate and extracted with bicarbonate,

distilled water, and brine. The organic extract was dried over sodium sulfate and evaporated *in vacuo* to give 8.15g (80%) of the title compound **5.16** as a yellow oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.58(s, 1H), 7.42(s, 1H), 7.28 (d, J = 8.3 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 6.71 (d, J = 8.3 Hz, 1H), 5.34 (br s, 1H), 5.07 (s, 2H), 1.62 (s, 6H).

2-(2-(2-(2-((2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxin-9-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5.8). DIAD (4.47g, 22.97mmol) was added to a solution of the ketal **5.16** (5.29g, 22.97 mmol) and triphenylphosphine (6.63g, 25.3mmol) in THF (230ml) at 0°C. The reaction mixture was warmed to r.t. and stirred for 12h. This mixture was evaporated *in vacuo*. The crude solid was purified via flash chromatography (50% EtOAc/hexanes) to give 9.22g (72%) of the product **5.8** as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.76 (d, J = 8.3 Hz, 2H), 7.62 (s, 1H), 7.39 (s, 1H), 7.27 (m, 4H), 7.17 (t, J = 7.8 Hz, 1H), 6.70 (d, J = 7.5 Hz, 1H), 5.03 (s, 2H), 4.23 (t, J = 4.8 Hz, 2H), 4.10 (t, J = 4.8 Hz, 2H), 3.92 (t, J = 4.8 Hz, 2H), 3.74 (t, J = 4.8 Hz, 2H), 3.63-3.57 (m, 8H), 2.38 (s, 3H), 1.57 (s, 6H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 153.56, 149.34, 144.73, 132.92, 129.77, 129.40, 127.92, 126.08, 123.70, 123.10, 121.43, 119.80, 107.03, 104.49, 99.73.

Attempted synthesis of N-(4-(4-((2-(2-(2-(2-((2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxin-9-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)amino)benzoyl)phenyl)-4-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)butanamide (5.18). DBU (0.531g, 3.50mmol) was added to a suspension of the benzophenone **5.9** (0.740g, 1.74 mmol) and the ketal **5.8** (1.96g, 3.50mmol) in DMF (18ml) at 75°C. The reaction mixture was warmed stirred for 12h. No conversion occurred and all starting material was recovered.

2,2-dimethyl-3,3-diphenyl-4,7,10-trioxa-3-siladodecan-12-ol (5.22). TBDPSCl (19.33ml, 75mmol) and imidazole (6.70g, 98mmol) were added to a solution of tetraethylene glycol **5.21** (10ml, 75 mmol) in DCM (750ml). The reaction mixture was stirred for 12h, concentrated and purified via flash chromatography (50% EtOAc/hexanes) to give 10.47g (36%) of the product **5.22** as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.71 (m, 4H), 7.40 (m, 6H), 3.82 (t, J = 4.8 Hz, 2H), 3.66 (m, 10H), 2.75 (s br, 1H), 1.06 (s, 9H).

2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-oic acid (5.23). PDC (3.39g, 9.0mmol) and water (0.107g, 5.92mmol) were added to a solution of the alcohol **5.22** (1g, 2.57 mmol) in DMF (13ml). The reaction mixture was stirred for 12h, diluted in ethyl acetate (200ml), extracted with satd. NH_4Cl and brine, concentrated and purified via flash chromatography (1% DCM/MeOH) to give 0.130g (12%) of the product **5.23** as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.70 (m, 4H), 7.41 (m, 6H), 4.18 (s, 2H), 3.85 (t, J = 4.7 Hz, 2H), 3.76 (m, 2H), 3.74 (m, 2H), 3.68 (t, J = 4.8 Hz, 2H), 1.08 (s, 9H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 135.60, 133.39, 129.75, 127.73, 72.72, 71.68, 70.13, 63.17, 26.79, 19.18.

Attempted synthesis of 2,2-dimethyl-N-(4-(4-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)benzoyl)phenyl)-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-amide (5.20). DCC (0.364g, 1.76mmol) was added to a suspension of the benzophenone **5.9** (0.644g, 1.47 mmol), aza-HOBt (0.300g, 2.20mmol), DIPEA (0.359ml, 2.06mmol) and the acid **5.23** (0.853g, 1.91mmol) in DMF (12.66ml) at 25°C. The reaction mixture was warmed to r.t. and stirred for 12h. This mixture was evaporated *in vacuo*. The crude solid was purified via flash chromatography (70% EtOAc/hexanes to 1% DCM/MeOH). No conversion occurred and the starting material was recovered.

2-(2-(2-(2-oxo-2-((4-(4-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)benzoyl)phenyl)amino)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5.25). A solution of ABP-Biotin **5.9** (0.300g, 0.684mmol) was added to a suspension of the acid chloride **5.24** (0.521g, 1.37 mmol) and DIPEA (0.239ml, 1.37mmol) in DMF (3ml) at 0°C. The reaction mixture was warmed to r.t. and stirred for 2h. This mixture was diluted in n-butanol (150ml) and extracted with 5% sodium bicarbonate (100ml) and brine (100ml). The organic extracts were evaporated in vacuo. The crude solid was purified via flash chromatography (1% DCM/MeOH to 10% MeOH) to give 0.045g (8%) of the product **5.25** as a clear oil. ^{1}H-NMR (DMSO), δ (ppm): 10.27 (s, 1H), 10.00 (s, 1H), 7.81 (d, J=8.7Hz, 2H), 7.77 (d, J=7.8Hz, 4H), 7.73 (dd, J=8.7, 2.3Hz, 4H), 7.49 (d, J=8.1Hz, 2H), 6.46 (s, 1H), 6.38 (s, 1H), 4.31 (m, 1H), 4.15-4.05 (m, 6H), 3.65 (m, 2H), 3.58 (m, 4H), 3.48 (m, 4H), 3.16 (dd, J=12.4, 5.1Hz, 1H), 2.57 (d, J=12.4Hz, 1H), 2.38 (m, 5H), 1.63 (m, 3H), 1.51 (m, 1H), 1.38 (m, 2H). ^{13}C-NMR (DMSO), δ (ppm): 193.85, 172.28, 169.39, 163.17, 145.36, 143.63, 142.57, 131.41, 131.25, 130.59, 128.07, 119.24, 118.66, 70.81, 70.68, 70.42, 70.12, 70.06, 68.36, 61.51, 59.67, 55.66, 36.81, 28.68, 28.57, 25.47, 21.56. ESI HRMS cald. (M+Na+): $C_{38}H_{46}N_4NaO_{10}S_2$ 805.2553, found: 805.2550.

N-(4-(4-(2-(2-((2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxin-9-yl)oxy)ethoxy)acetamido)benzoyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (5.26). Potassium carbonate (0.117g, 0.843mmol) was added to a solution of the ABP-tosylate **5.25** (0.330g, 0.421 mmol) and the acetonide **5.16** (0.194g, 0.843mmol) in DMF (4ml). The reaction mixture was heated to 70°C and stirred for 12h. This mixture was diluted in n-butanol (150ml) and extracted with distilled water (100ml) and brine (100ml). The organic extracts were evaporated *in vacuo*. The crude solid was purified via flash chromatography (1% DCM/MeOH to 10% MeOH). After chromatography, 0.233g was isolated which was contaminated with ca. 10% of the starting material. The mixture was subjected to the following reaction without further purification.

N-(4-(4-(2-(2-((7-hydroxy-6-(hydroxymethyl)naphthalen-1-yl)oxy)ethoxy)acetamido)benzoyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (5.19).

Amberlyst-15 resin (0.100g) was added to a solution of the acetonide **5.26** (0.233g, 0.277 mmol) in DMF/acetonitrile (1mL/11mL). The reaction mixture was stirred for 12h. This mixture was diluted in n-butanol (150ml) and extracted with distilled water (100ml) and brine (100ml). The organic extracts were evaporated *in vacuo*. The crude solid was purified via flash chromatography (1% DCM/MeOH to 10% MeOH) to give 0.067g (30%) of the title compound **5.19** as a clear oil. ^{1}H-NMR (DMSO), δ (ppm): 10.27 (s, 1H), 10.03 (s, 1H), 9.81 (s, 1H), 7.82-7.69 (m, 10H), 7.43 (s, 1H), 7.33 (d, J=8.2Hz, 1H), 7.12 (t, J=7.9Hz, 1H), 6.79 (d, J=7.6Hz, 1H), 6.46 (s, 1H), 6.38 (s, 1H), 5.16 (t, J=5.6Hz, 1H), 4.61 (d, J=5.4Hz, 2H), 4.30 (m, 1H), 4.21 (m, 1H), 4.13 (m, 4H), 3.87 (m, 2H), 3.70-3.60 (m, 8H), 3.16-3.10 (m, 2H), 2.85 (m, 1H), 2.58 (d, J=12.4Hz, 1H), 2.37 (m, 2H), 1.63 (m, 3H), 1.52 (m, 1H), 1.39 (m, 2H). ^{13}C-NMR (DMSO), δ (ppm): 193.81, 172.26, 169.35, 163.15, 153.07, 153.04, 143.59, 142.55, 132.41, 131.97, 131.36, 131.21, 129.02, 125.42, 119.21, 118.62, 105.01, 103.06, 79.67, 70.77, 70.62, 70.48, 70.34, 70.08, 69.57, 67.83, 61.48, 59.63, 58.93, 55.83, 36.78, 28.65, 28.54, 25.44. ESI HRMS cald. (M+Na+): $C_{42}H_{38}N_4NaO_{10}S$ 823.2989, found: 823.2941.

2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol (5.31).

NaH (4.00g, 100 mmol) was added to a solution of tetraethylene glycol **5.21** (10g, 67 mmol) in DMF (333ml) at 0°C. The solution was stirred for 30 min and propargyl bromide (15 mL, 200 mmol) was added. The reaction mixture was warmed to r.t., stirred for 8h, concentrated, and purified via flash chromatography (50% EtOAc/hexanes) to give 4.42g (36%) of the product **5.31** as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 4.12 (s, 2H), 3.65-3.50 (m, 12H), 3.18 (s, 1H), 2.40 (m, 1H).

2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5.28).

Tosyl-Cl (4.02g, 21.1mmol) and triethylamine (2.31g, 22.9 mmol) were added to a solution of glycol **5.31** (3.31g, 17.6 mmol) in DCM (88ml). The reaction mixture was stirred for 20h, concentrated and purified via flash chromatography (50% EtOAc/hexanes) to give 5.45g (91%) of the product **5.28** as a clear oil. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.75 (d, J = 8.2Hz, 2H), 7.31 (d, J = 8.2Hz, 2H), 4.14 (d, J = 2.3Hz, 2H), 4.11 (t, J = 4.8Hz, 2H), 3.65-3.52 (m, 10H), 2.40 (s, 4H).

2,2-dimethyl-9-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)-4H-naphtho[2,3-d][1,3]dioxine (5.32). Glycol tosylate **5.28** (3.06g, 8.94 mmol) was added to a solution of acetonide **5.16** (2.06g, 8.94 mmol) and K_2CO_3 (2.47g, 17.87 mmol) in DMF (45 mL). The reaction mixture was heated to 70°C and stirred for 8h. This mixture was diluted in ethyl acetate (300ml) and extracted with distilled water (100ml) and brine (100ml). The organic extracts were evaporated *in vacuo*. The crude solid was purified via flash chromatography (50% EtOAc/hexanes) to give 1.96g (55%) of the title compound **5.32** as a light yellow amorphous solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.65 (s, 1H), 7.39 (s, 1H), 7.28 (d, J = 8.3Hz, 1H), 7.18 (t, J = 7.9Hz, 1H), 6.70 (d, J = 7.5 Hz, 1H), 5.03 (s, 2H), 4.24 (t, J = 4.8 Hz, 2H), 4.18 (m, 2H), 3.95 (t, J = 4.8 Hz, 2H), 3.78 (t, J = 4.8 Hz, 2H), 3.69 (m, 6H), 2.43 (s, 1H), 1.58 (s, 6H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 153.58, 149.35, 129.41, 126.10, 123.70, 123.10, 123.08, 121.43, 119.81, 107.07, 104.51, 99.74, 79.68, 74.60, 74.58, 70.95, 70.67, 70.44, 69.82, 69.10, 67.74, 61.15, 58.37, 24.96.

N-(4-(4-azidobenzoyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (5.29). A solution of sodium azide (1M, 2.44 mL, 2.44 mmol) and sodium nitrite (1M, 2.44 mL, 2.44 mmol) in water was added to a solution of ABP-biotin **5.9** (534mg, 1.22 mmol) in 6M H_2SO_4 (7 mL). The reaction mixture was stirred for 12h. This mixture was diluted in n-butanol (150ml) and extracted with 5% sodium bicarbonate (100ml) and brine (100ml). The organic extracts were

evaporated *in vacuo*. The crude solid was purified via flash chromatography (1% to 10% MeOH/DCM) to give 0.290g (51%) of the title compound **5.29** as a white solid. ^{1}H-NMR (DMSO-d_6), δ (ppm): 10.30 (s, 1H), 7.77 (m, 4H), 7.71 (m, 2H), 7.29 (d, J = 8.6Hz, 2H), 3.14 (m, 1H), 2.85-2.82 (m, 1H), 2.60 (m, 1H), 2.38 (m, 2H), 1.65 (m, 3H), 1.52 (m, 1H), 1.40 (m, 2H). ^{13}C-NMR (DMSO- d_6), δ (ppm): 193.64, 172.29, 163.15, 143.95, 143.83, 134.42, 132.02, 131.59, 131.45, 119.55, 118.65, 61.46, 59.63, 55.83, 36.77, 28.65, 28.53, 25.42.

N-(4-(4-(4-((2-(2-(2-((2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxin-6-yl)oxy)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)benzoyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (5.27). CuI (1.2mg, 6.24 μmol), and NEt_3 (0.174mL, 1.25 mmol) were added to a solution of ABP-biotin azide **5.29** (290mg, 0.624 mmol) and the acetonide-alkyne **5.32** (0.250g, 0.624 mmol) in THF (40mL). The reaction mixture was stirred for 18h. This mixture was diluted in n-butanol (150ml) and extracted with distilled water (100ml) and brine (100ml). The organic extracts were evaporated *in vacuo*. The crude material was purified via flash chromatography (1% to 10% MeOH/DCM) to give 0.258g (48%) of the title compound **5.27** as an off-white, amorphous solid. ^{1}H-NMR (DMSO-d_6), δ (ppm): 10.30 (s, 1H), 8.90 (s, 1H), 8.06 (d, J = 8.4Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 7.77 (m, 4H), 7.54 (s, 1H), 7.38 (s, 1H), 7.31 (m, 1H), 7.17 (t, 1H), 6.81 (d, J = 7.6Hz, 1H), 6.45 (s, 1H), 6.36 (s, 1H), 5.00 (s, 2H), 4.62 (s, 2H), 4.29 (m, 1H), 4.18- 4.12 (m, 4H), 3.85 (m, 2H), 3.64-3.50 (m, 8H), 3.10 (m, 1H), 2.82 (m, 1H), 2.36 (m, 2H), 1.61 (m, 3H), 1.58 (s, 6H), 1.35 (m, 2H). ^{13}C-NMR (DMSO- d_6), δ (ppm): 198.46, 177.07, 167.90, 158.06, 154.25, 150.67, 148.90, 144.11, 142.38, 136.32, 135.97, 134.06, 130.44, 128.99, 128.76, 126.82, 124.77, 123.46, 110.93, 110.01, 104.73, 100.60, 75.30, 75.06, 74.96, 74.38, 74.24, 72.76, 68.59, 66.23, 65.48, 64.40, 60.59, 53.79, 50.88, 41.55, 33.40, 33.29, 30.17, 29.89, 16.41.

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CHAPTER 6

CONCLUSIONS

This dissertation has discussed the design and synthesis of photo-caged, highly reactive molecules, for use in a wide range of biological and materials science applications. In Chapter 1, a literature review was presented discussing the copper-catalyzed alkyne azide cycloaddition (CuAAC) and the strain promoted alkyne azide cycloaddition (SPAAC). The pitfalls associated with each technique were presented, along with various strategies for the protection of strained alkynes. Methods for the synthesis of cyclopropenones were surveyed, along with a general overview of cyclopropenone photochemistry. Finally, the generation of strained alkynes through the utilization of the above-mentioned strategies was outlined.

Chapter 2 described the synthesis of bis-cyclopropenone caged Sondheimer diynes (photo-DIBODs). The photochemistry of these interesting and useful molecules was discussed, as well as methods for their utilization as photoactivatable crosslinkers. The utility of these compounds was demonstrated by conjugation of a model protein, bovine serum albumin (BSA) with a variety of functional moieties.

Chapter 3 described the synthesis of mono-cyclopropenone caged Sondheimer diynes (MOC-DIBODs). An order of magnitude difference in the photo-sensitivity between photo-DIBOD and MOC-DIBOD allows for the selective mono-decarbonylation of photo-DIBOD. MOC-DIBOD was also synthesized by selective mono-cyclopropenation of Sondheimer diyne. The alkynes generated from photolysis of MOC-DIBOD triazoles display extraordinary reactivity towards azides: the rate of addition of butyl azide in methanol (34 $M^{-1}s^{-1}$) represents the fastest ever reported SPAAC reaction of

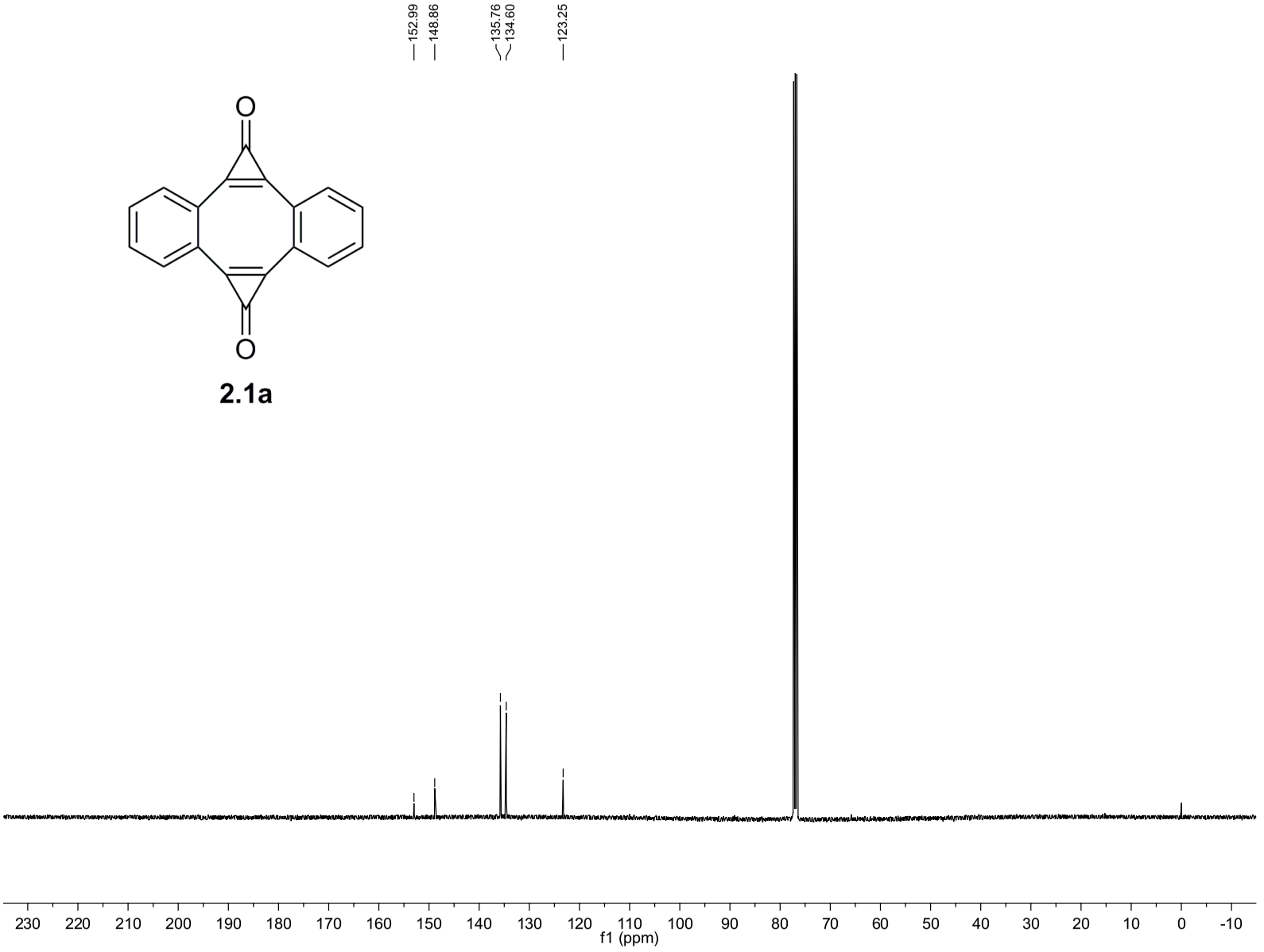
cyclooctynes in organic solvents. The utility of MOC-DIBOD was illustrated by one-pot preparation of mixed bis-triazoles, cross-conjugation of azido-derivatized protein and azido-terminated polymer, and by the efficient labelling of protein with fluorescent dye.

Chapter 4 described the synthesis of a photo-caged nine-membered cyclic enediyne and the progress toward synthesis of a photo-caged ten-membered enyne-allene. Optimization of these platforms would provide spatial and temporal control of Bergman and Myers-Saito cycloaromatization reactions respectively. This control could potentially lead to usage of these platforms in photo-dynamic therapies for various tumors.

Finally Chapter 5 outlined the progress toward the development of a photo-activatable, cleavable linker for immunoglobulin G. Successful development of such a compound would provide easy access to antibody-drug conjugates with complete homogeneity and low immunogenicity. Our results indicate that successful development of this linker requires preservation of at least one aniline moiety on the benzophenone core, and future work will focus efforts towards this end.

APPENDIX A

^{1}H-NMR AND ^{13}C-NMR OF ESSENTIAL COMPOUNDS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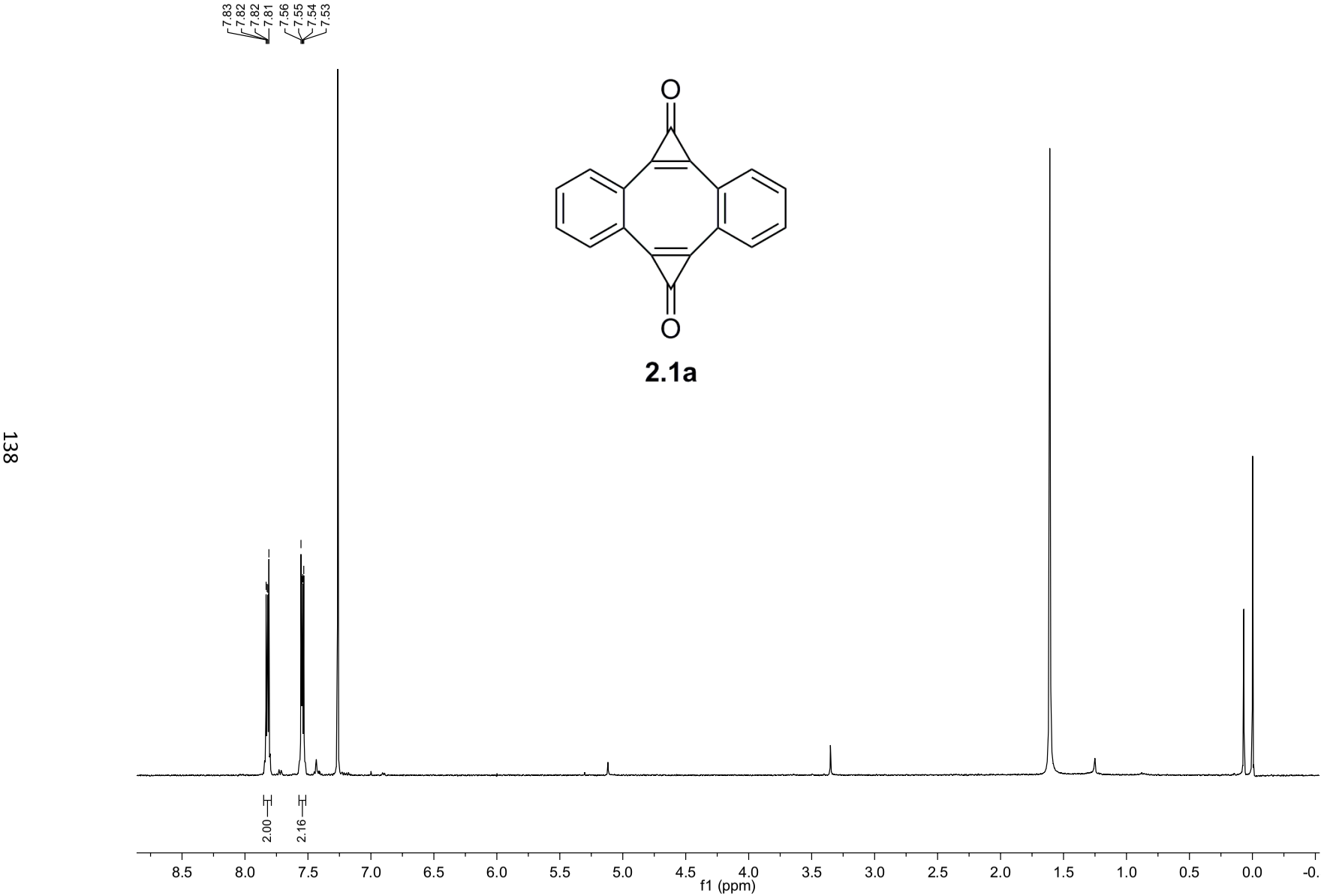
f1 (ppm)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8.0
7.5
7.0
6.5
6.0
5.5
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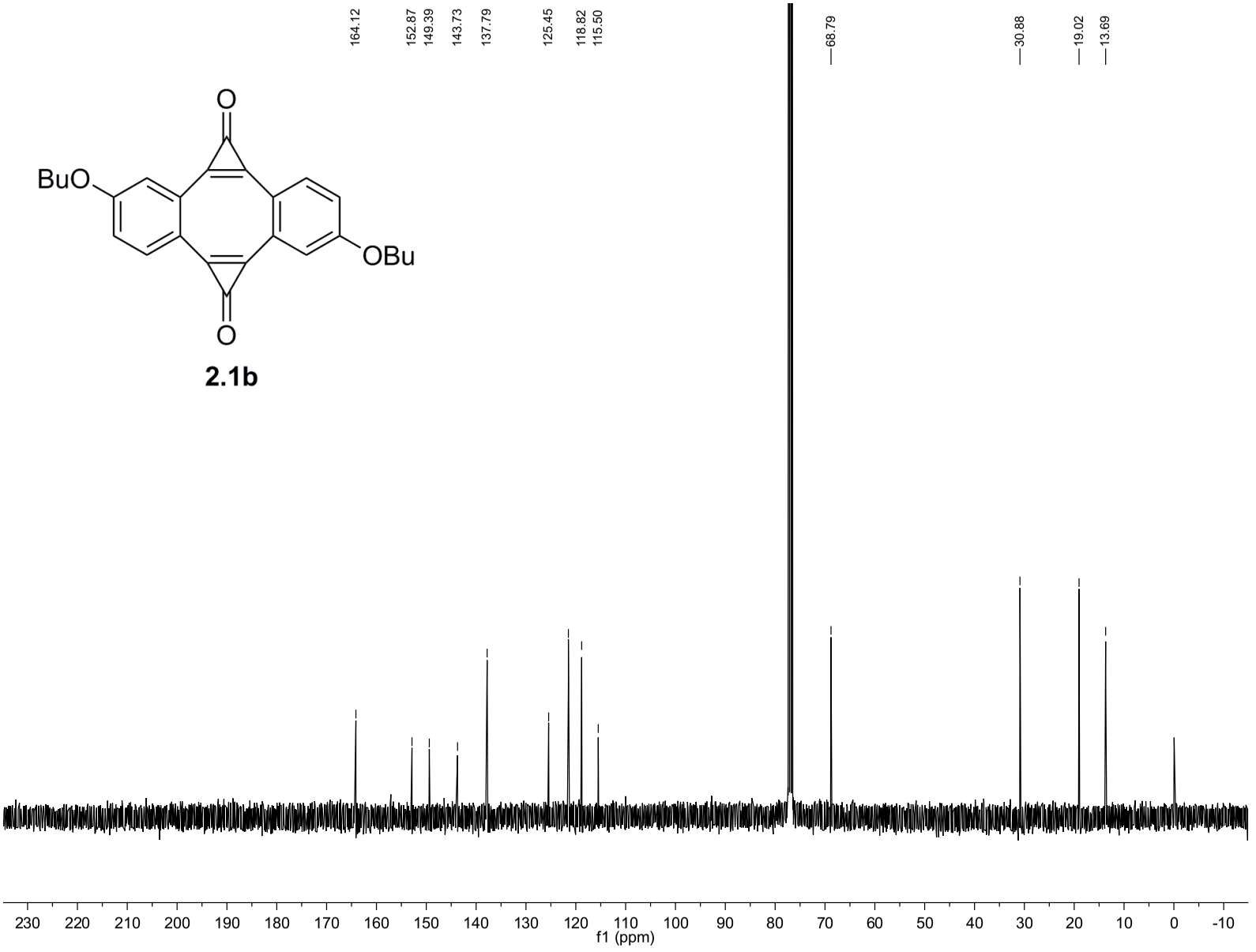
0.0
f1 (ppm)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125.45
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115.50
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30.88
19.02
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O
O
2.1b
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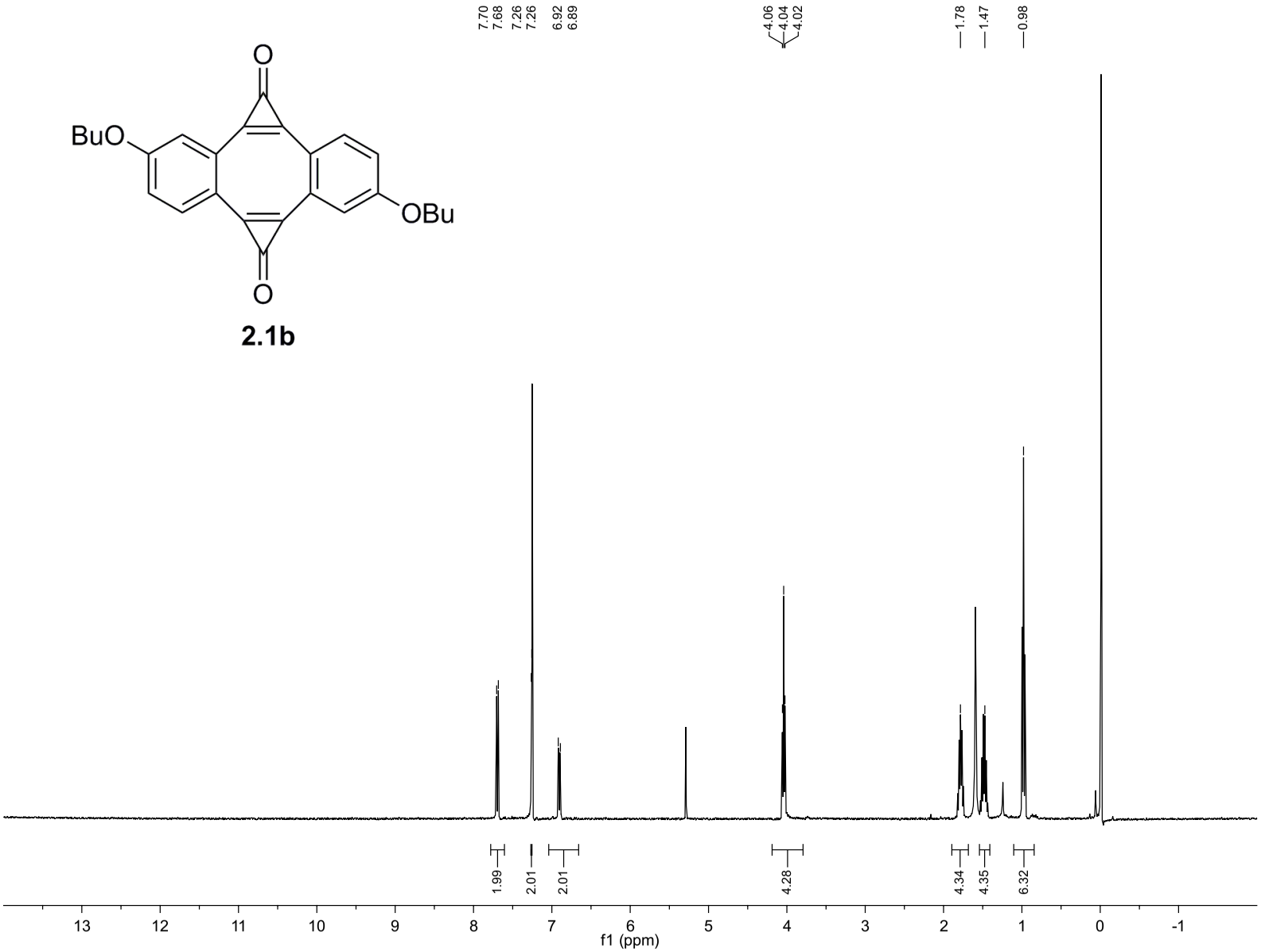
f1 (ppm)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7.26
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6.89
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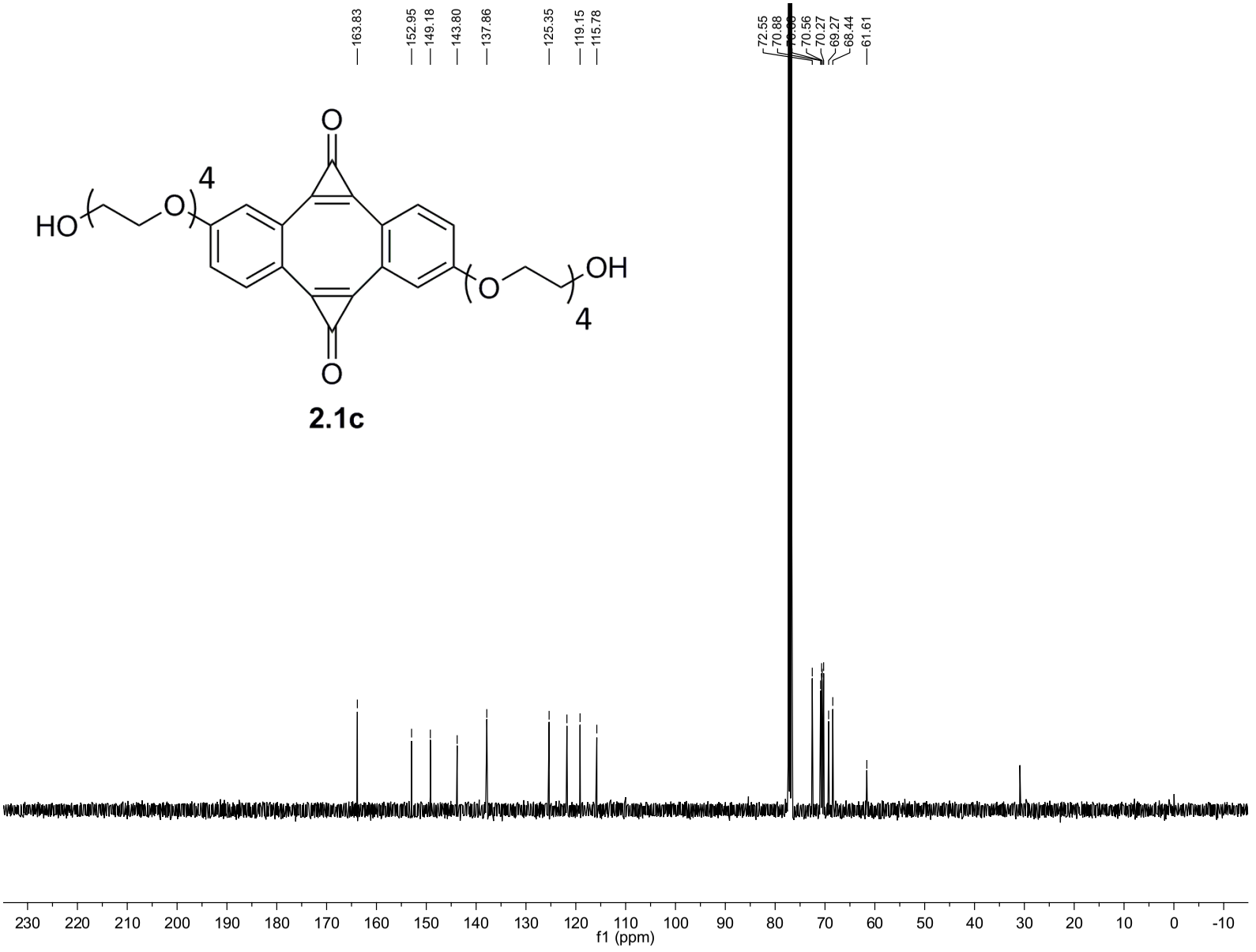
4.04
4.02
1.78
1.47
0.98
1.99
2.01
2.01
4.28
4.34
4.35
6.32
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)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69.27
68.44
61.61
HO
O
4
O
O
O
OH
4
2.1c
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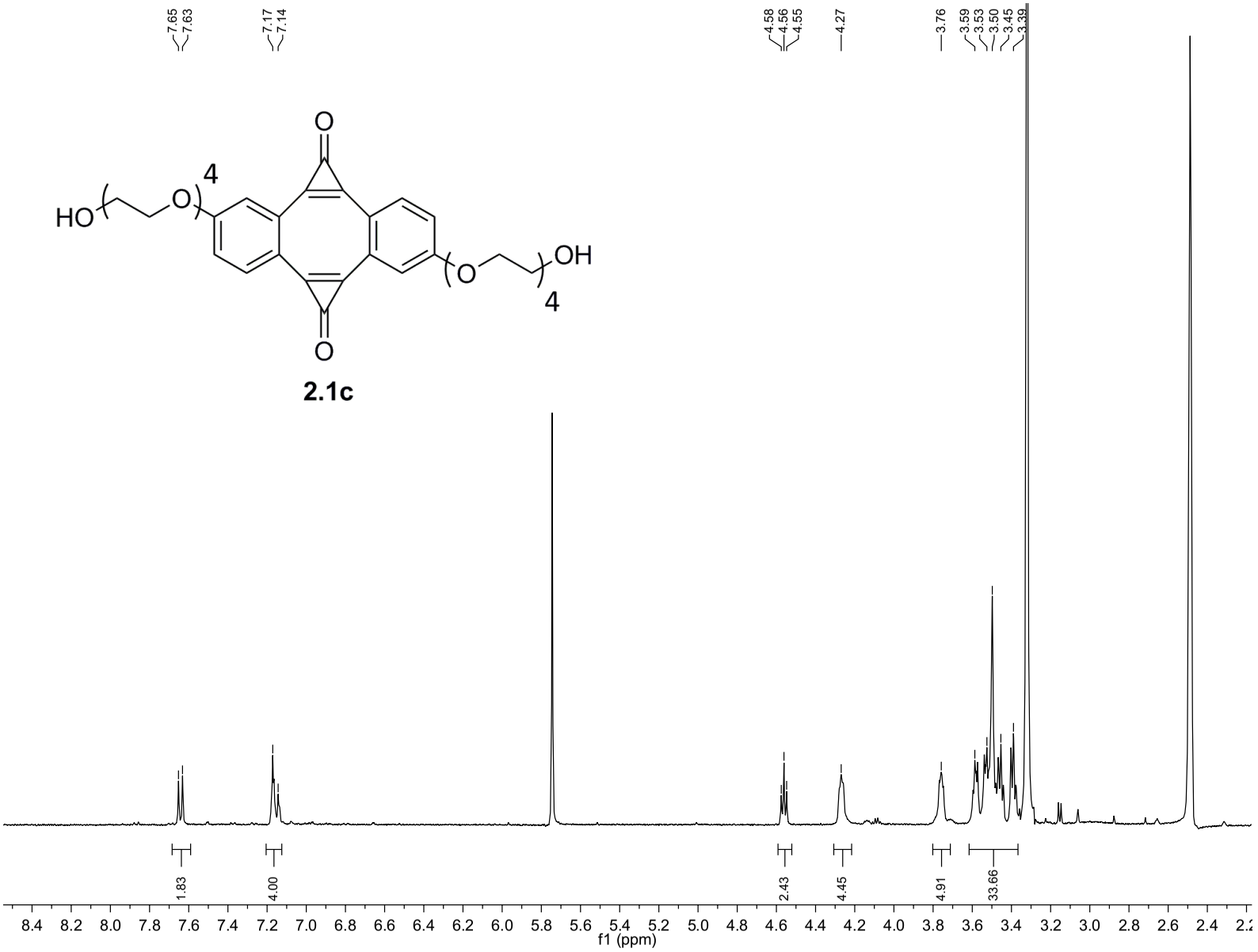
f1 (ppm)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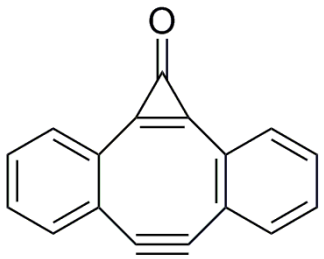
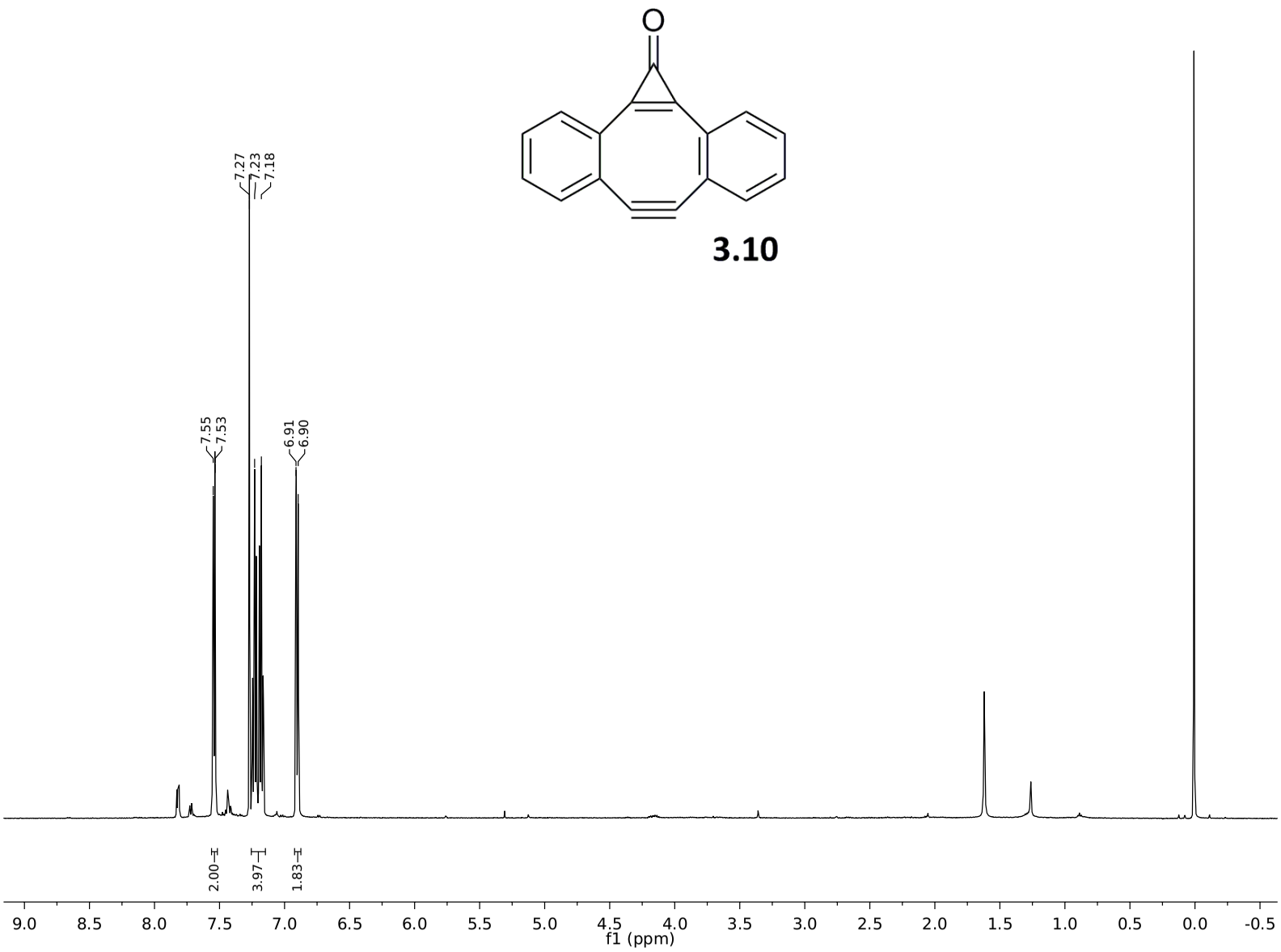
3.45
3.39
HO
O
4
O
O
O
OH
4
O
2.1c
1.83
4.00
2.43
4.45
4.91
33.66
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4
f1 (ppm)


O

3.10

154.28
150.70
135.16
133.65
132.14
130.06
127.18
125.17
106.78
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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7.23
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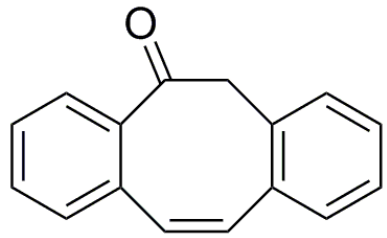
6.90
2.00
3.97
1.83
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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127.94
127.27
126.85
48.44
200
190
180
170
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110
100
90
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50
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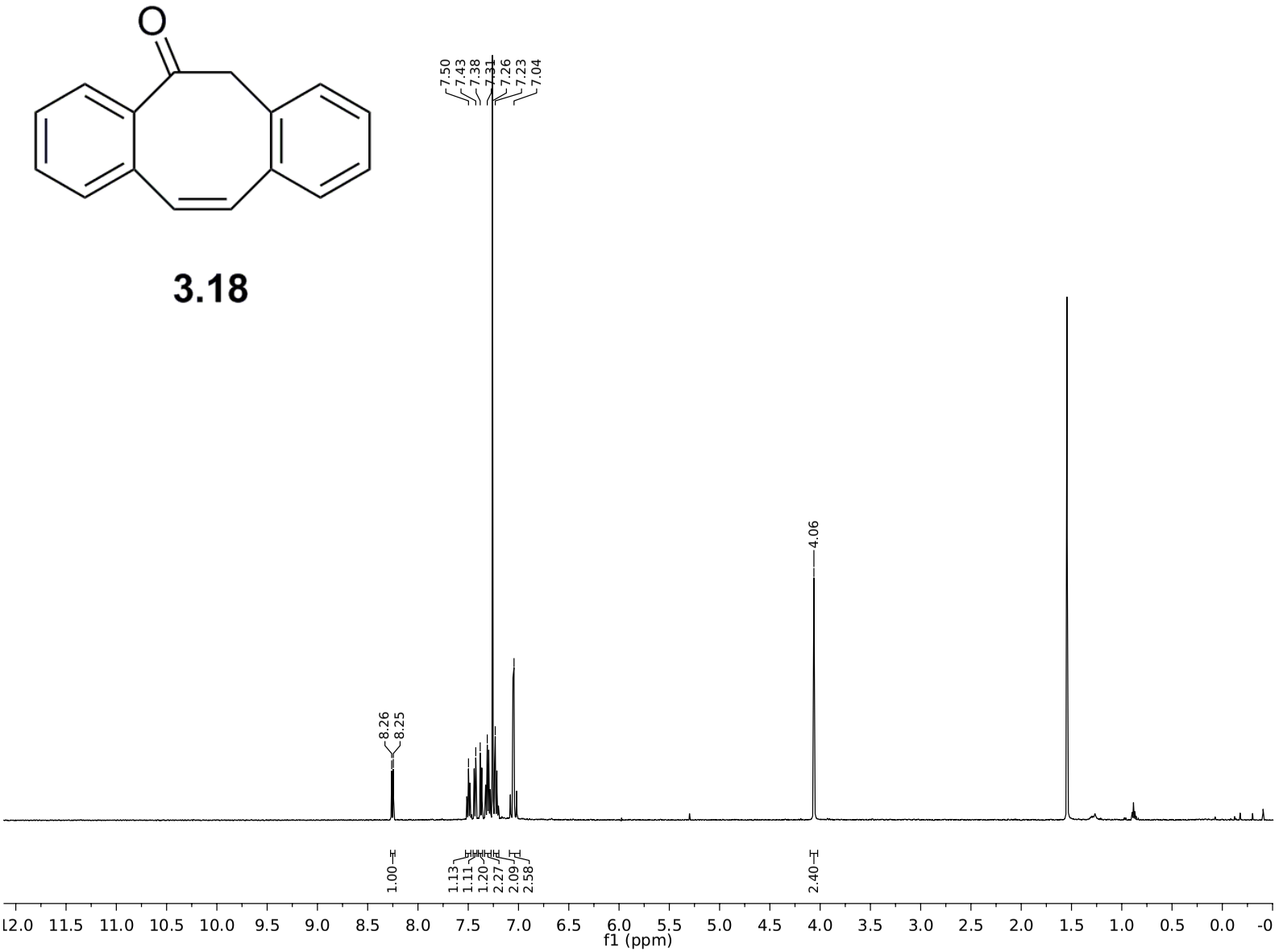
10
0
f1 (ppm)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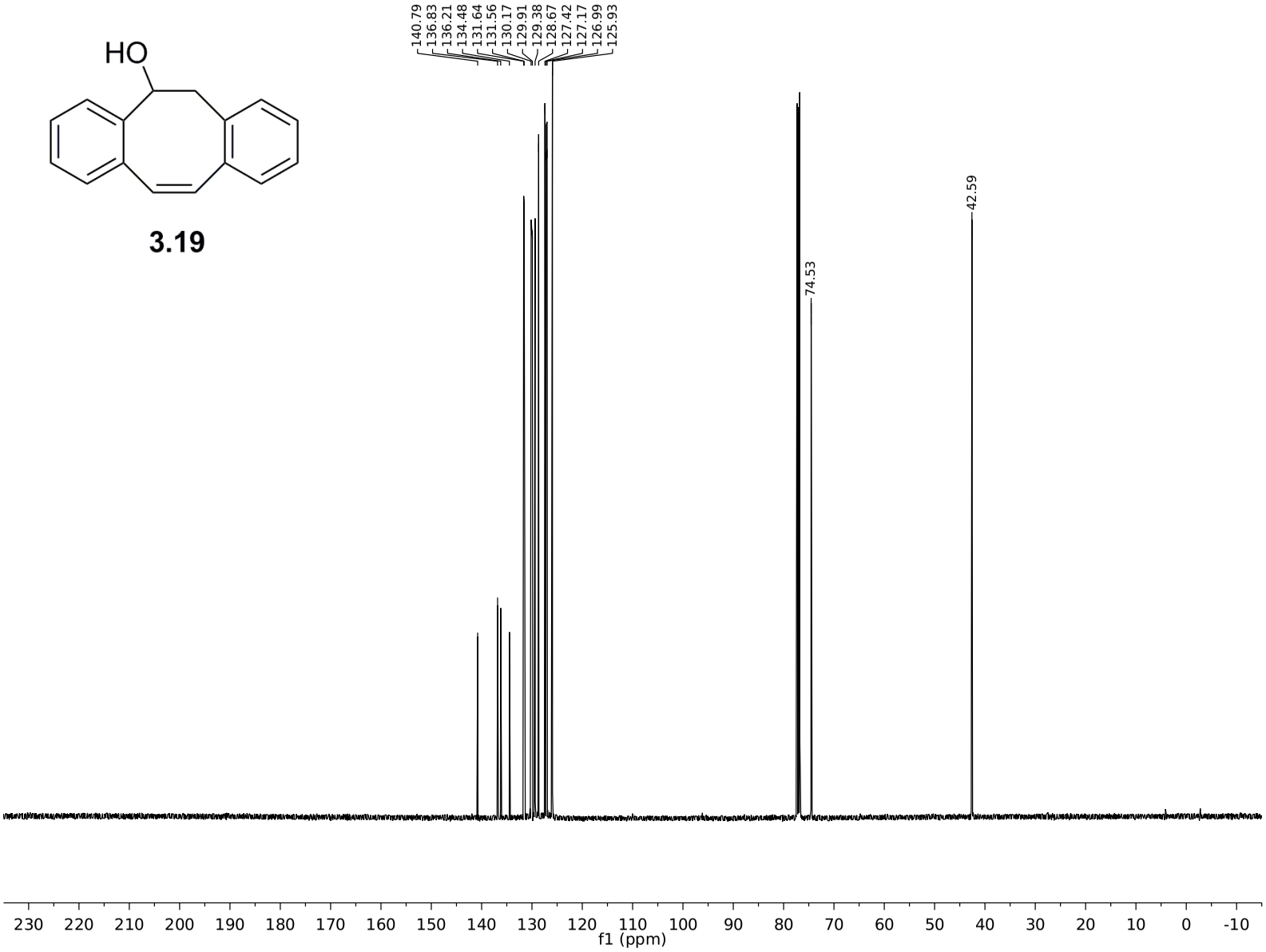
2.40
f1 (ppm)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128.67
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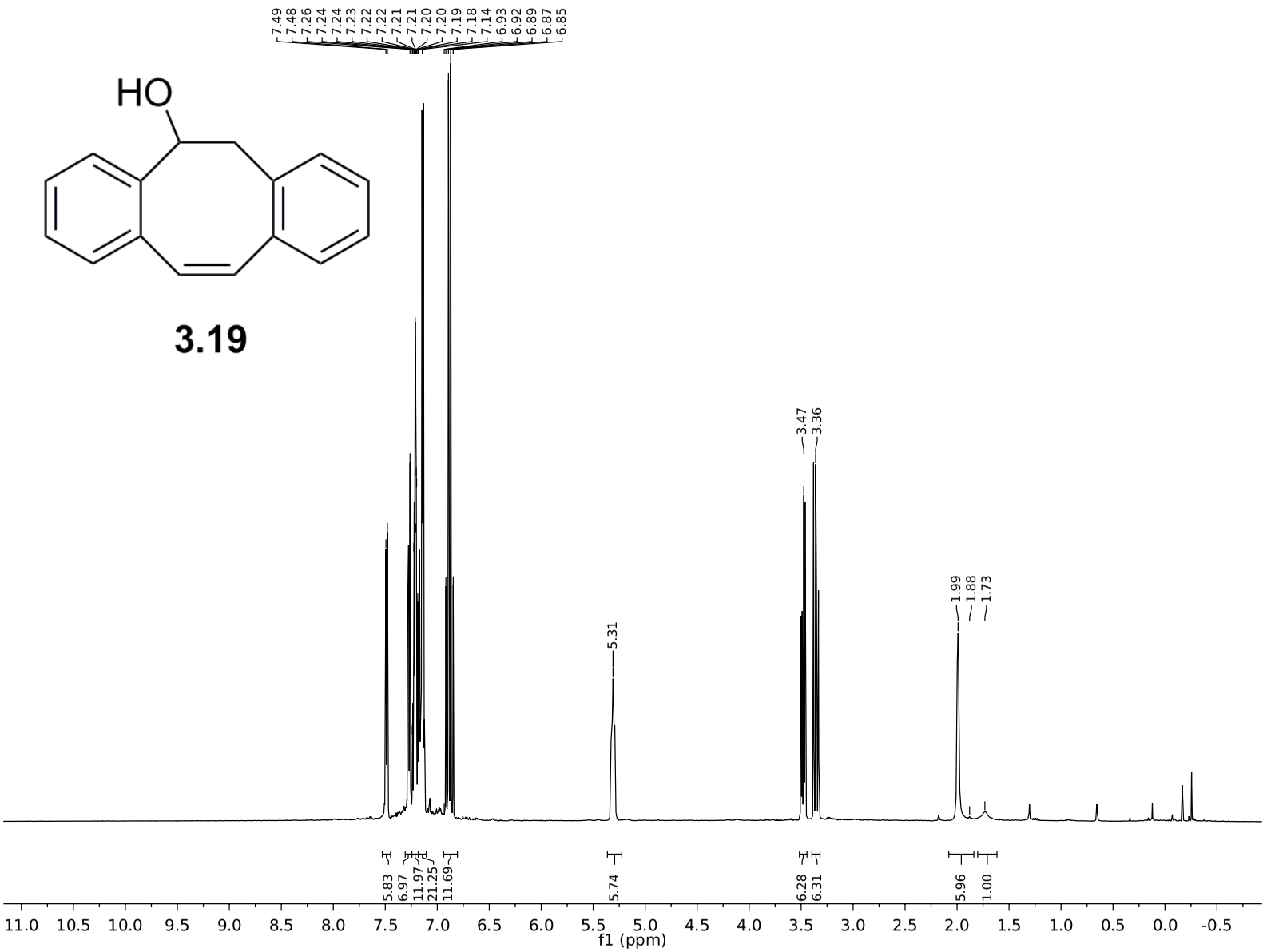
f1 (ppm)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7.21
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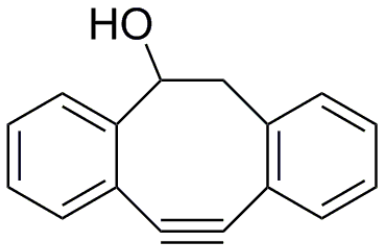
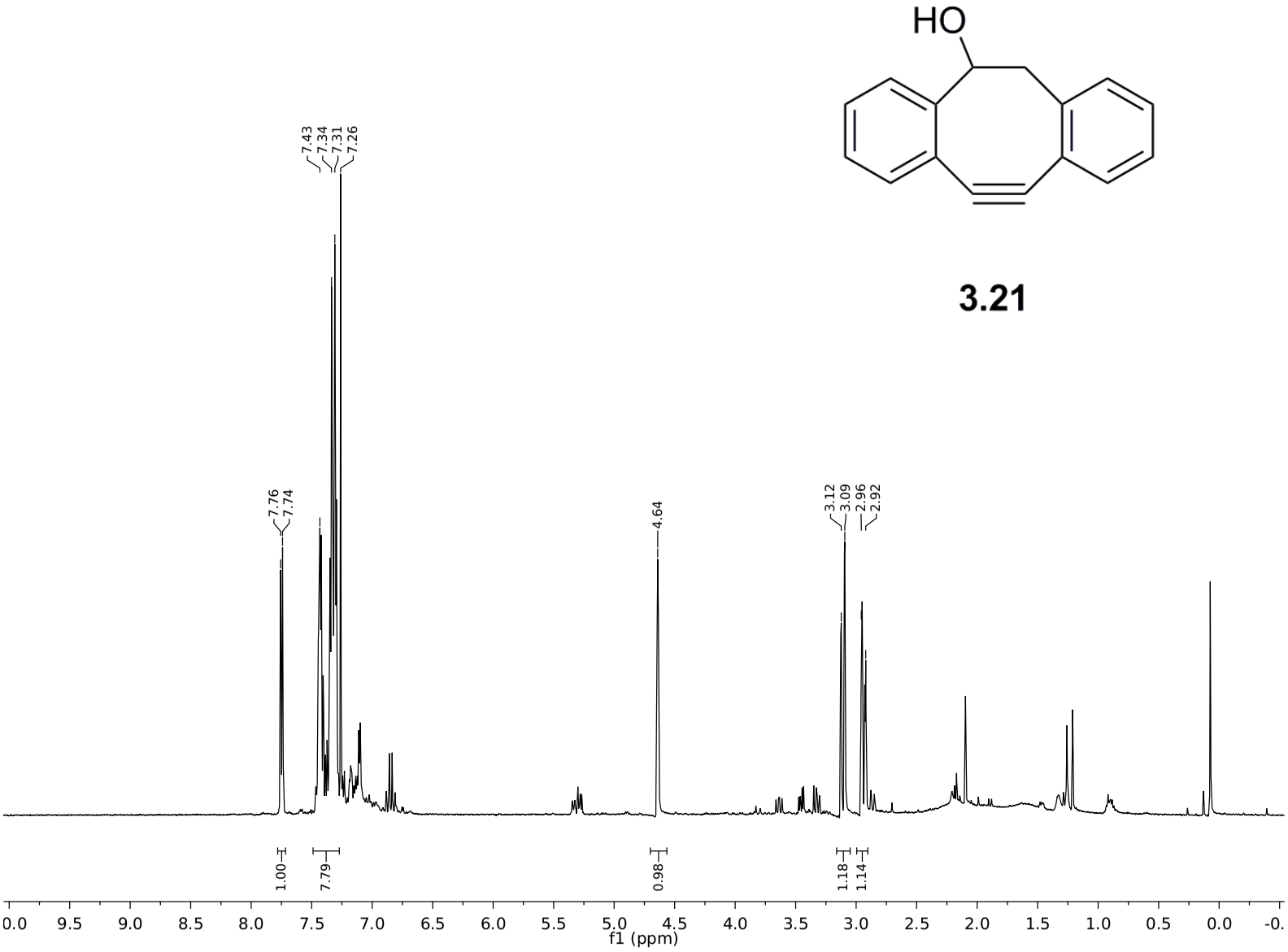
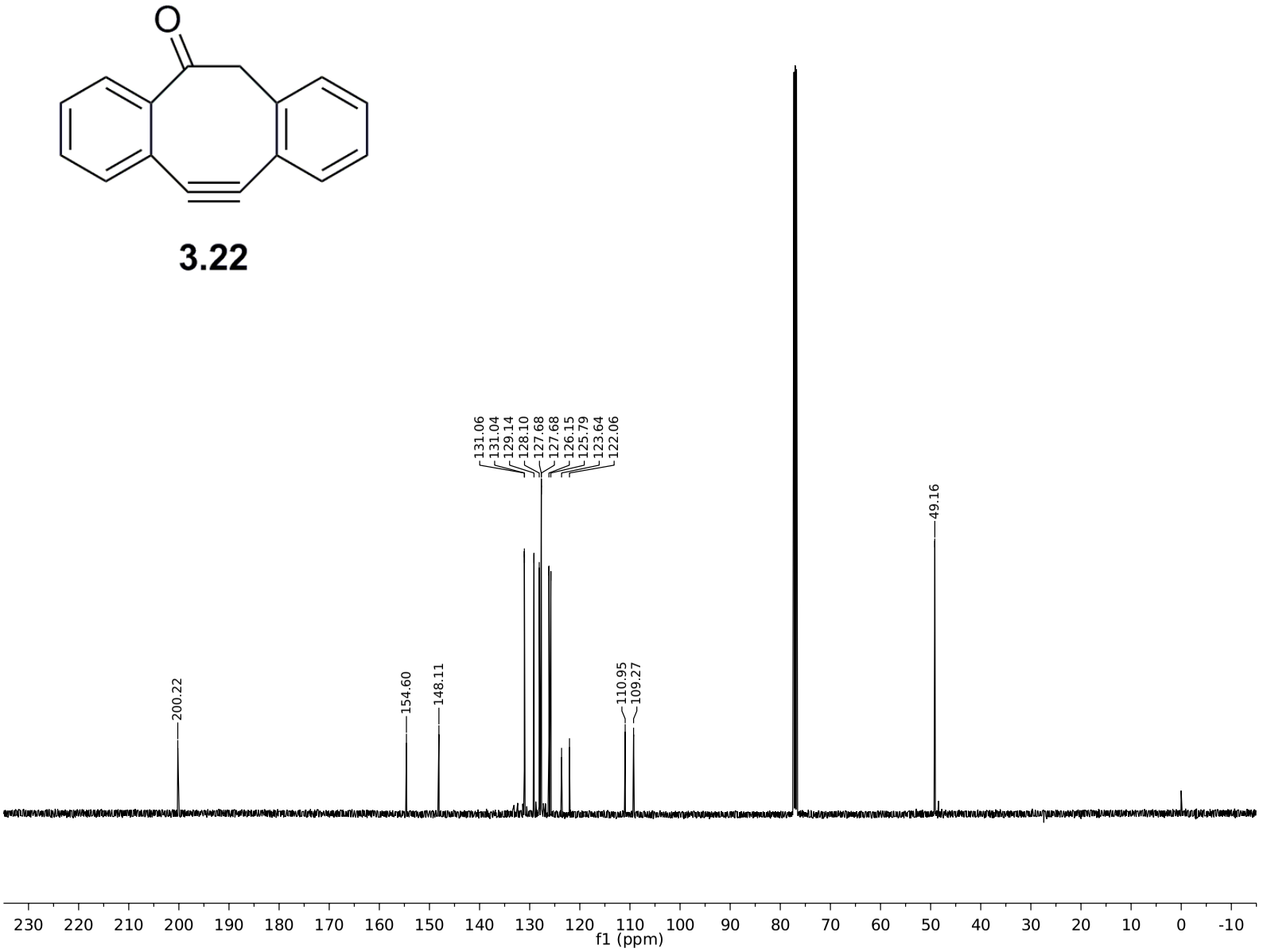
7.20
7.20
7.19
7.18
7.14
6.93
6.92
6.89
6.87
6.85
3.47
3.36
5.31
1.99
1.88
1.73
5.83
6.97
11.97
21.25
11.69
5.74
6.28
6.31
5.96
1.00
f1 (ppm)

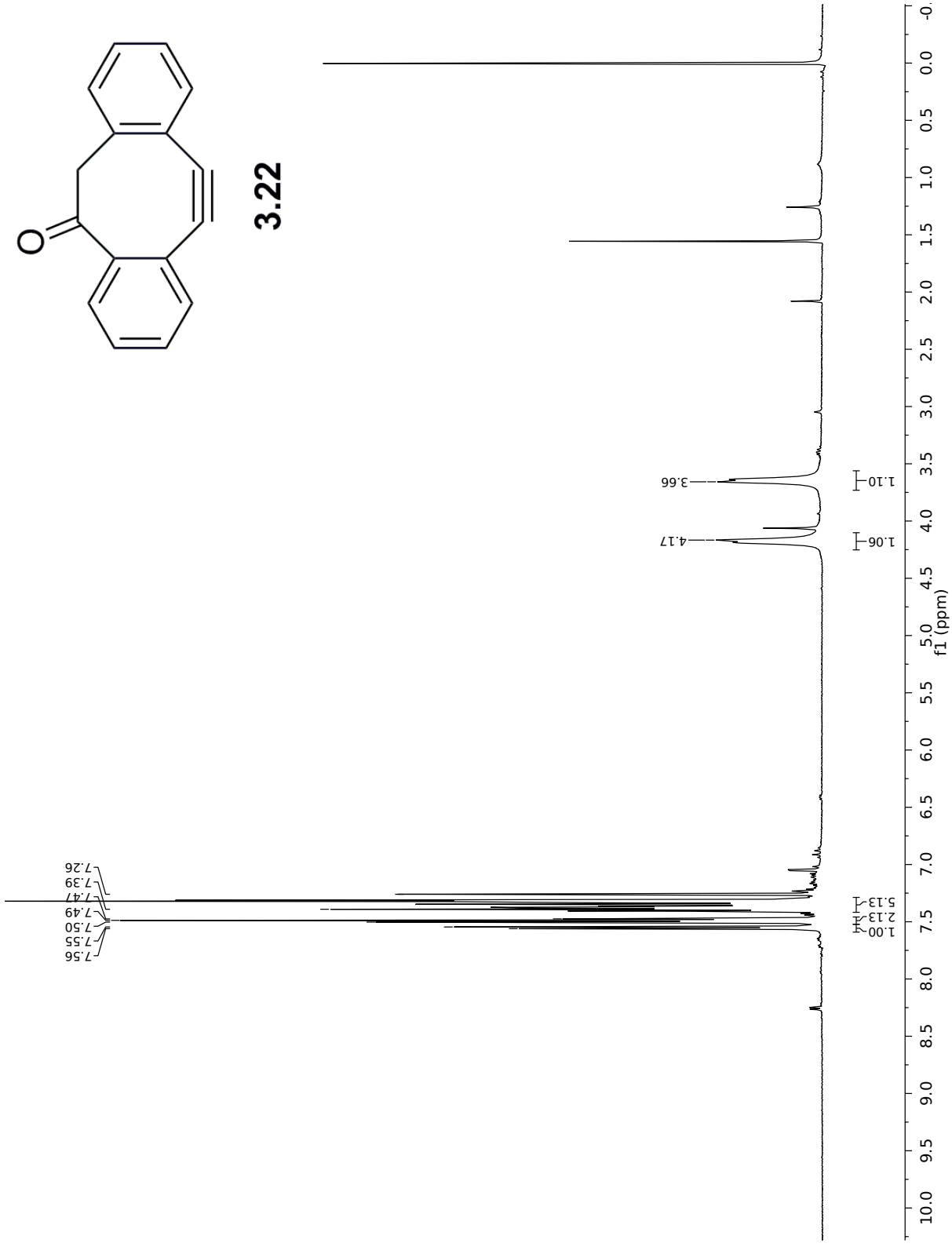

HO

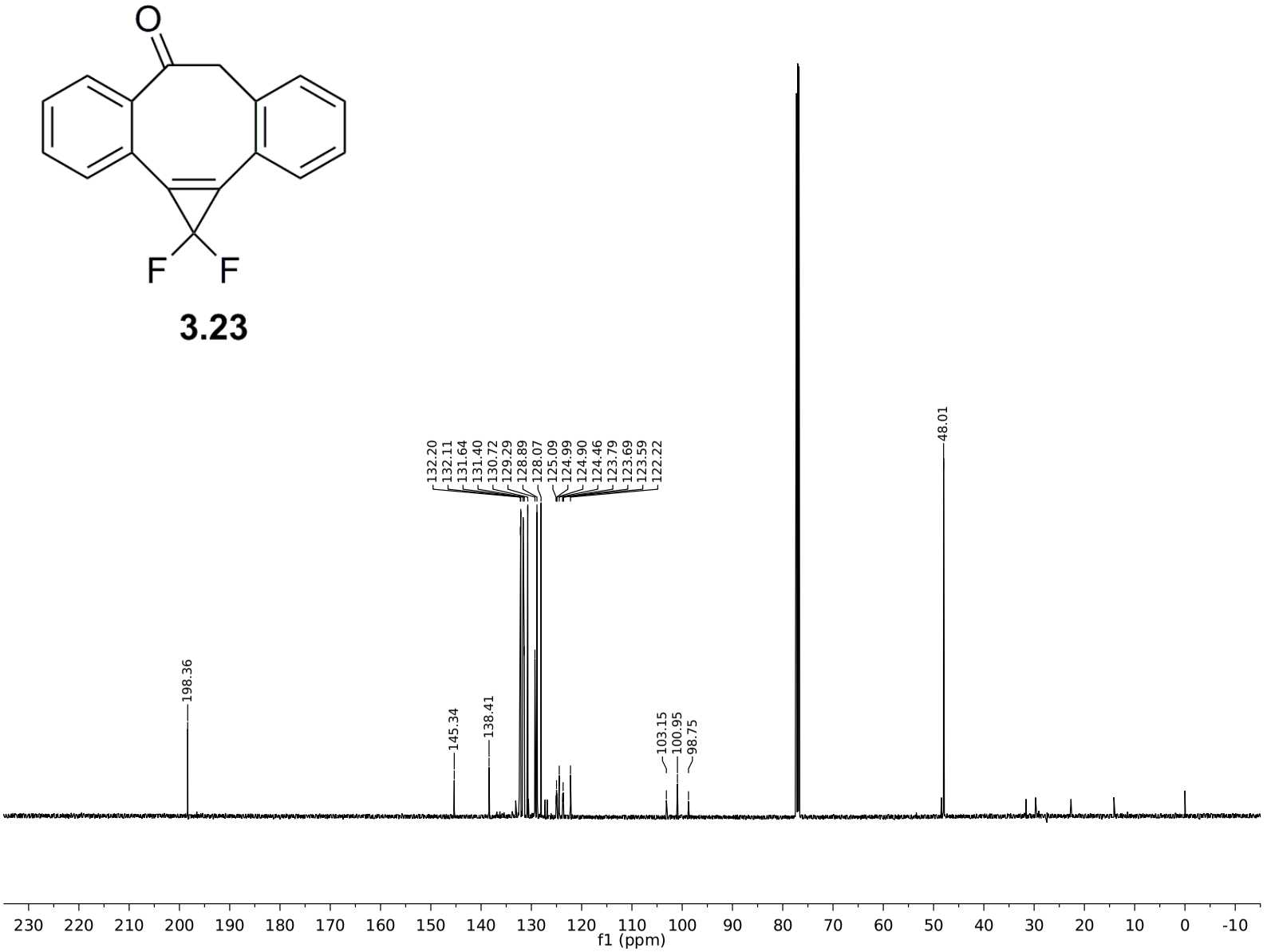
3.21

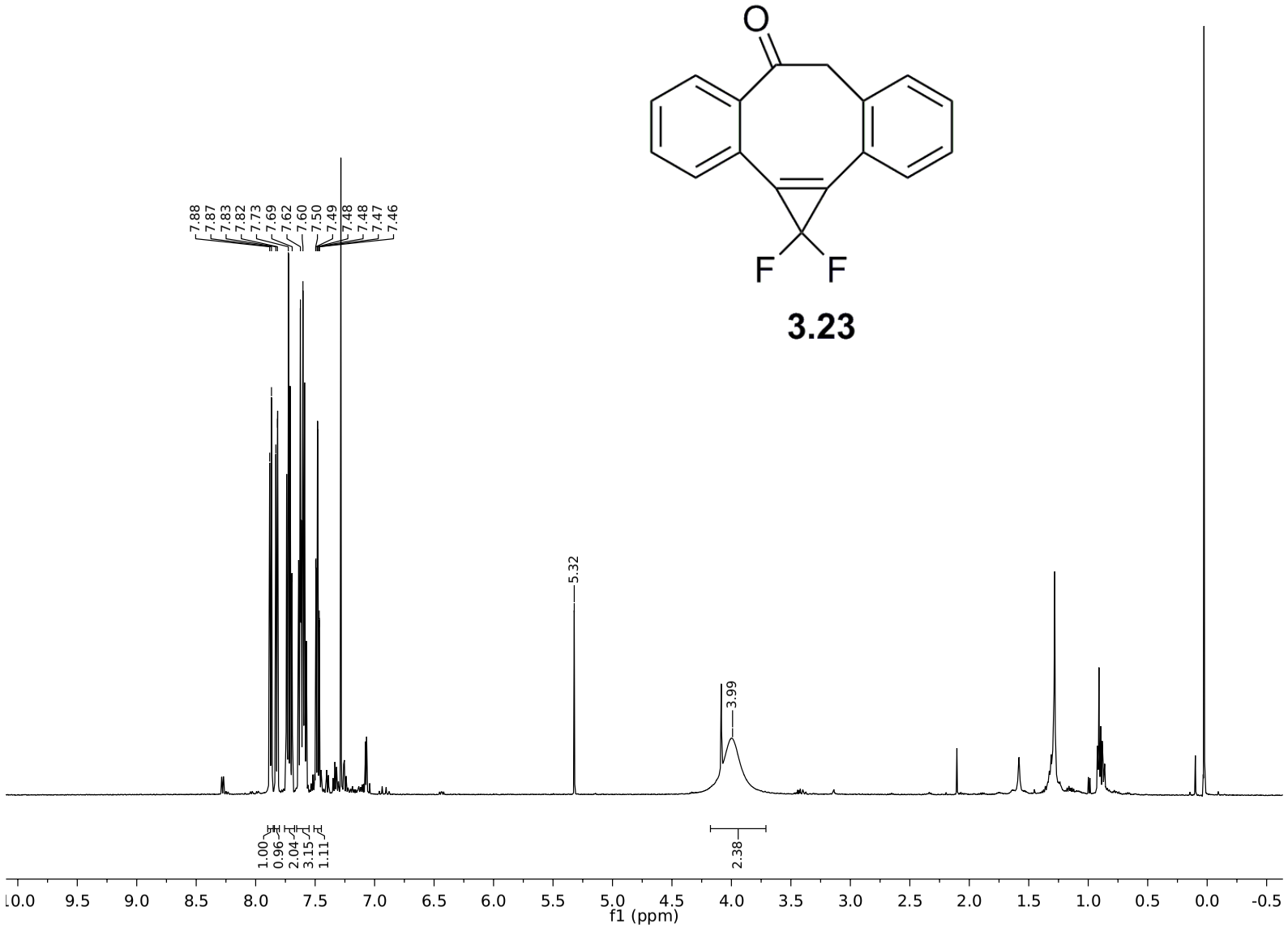
155.56
151.63
129.64
128.07
128.00
126.99
126.85
126.10
126.09
124.05
123.76
121.24
112.91
110.61
75.26
48.73
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

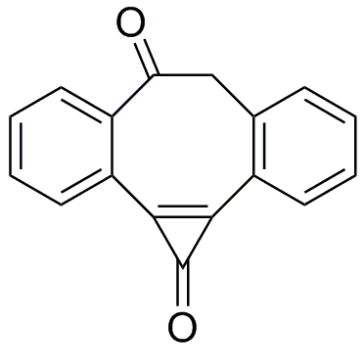

HO
3.21
7.43
7.34
7.31
7.26
7.76
7.74
4.64
3.12
3.09
2.96
2.92
1.00
7.79
0.98
1.18
1.14
f1 (ppm)


O
3.22
131.06
131.04
129.14
128.10
127.68
127.68
126.15
125.79
123.64
122.06
49.16
200.22
154.60
148.11
110.95
109.27
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


3.22
O
7.56
7.55
7.50
7.49
7.47
7.39
7.26
3.66
4.17
1.00
2.13
5.13
1.10
1.06
f1 (ppm)

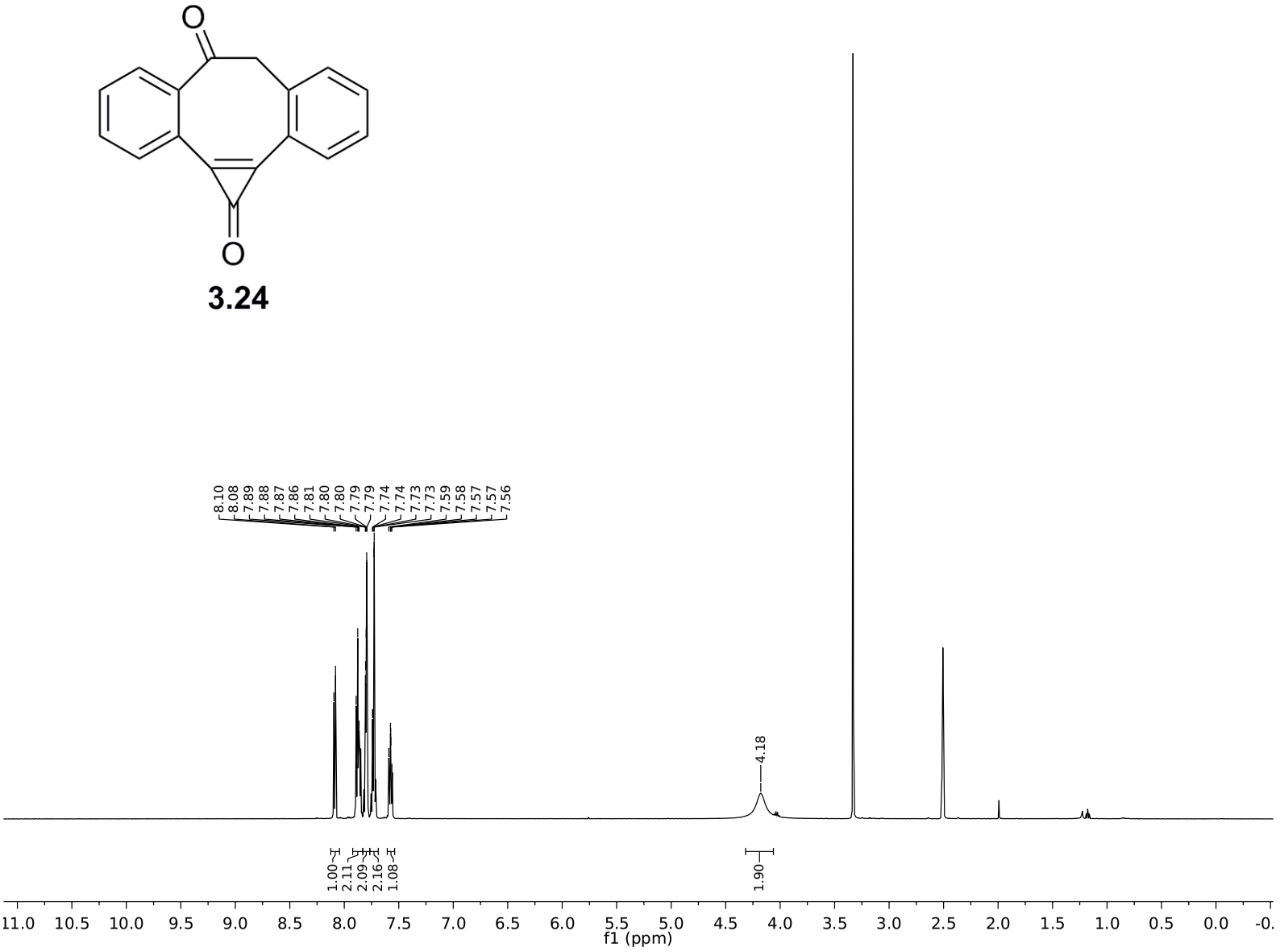

O
F
F
3.23
198.36
145.34
138.41
132.20
132.11
131.64
131.40
130.72
129.29
128.89
128.07
125.09
124.99
124.90
124.46
123.79
123.69
123.59
122.22
103.15
100.95
98.75
48.01
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

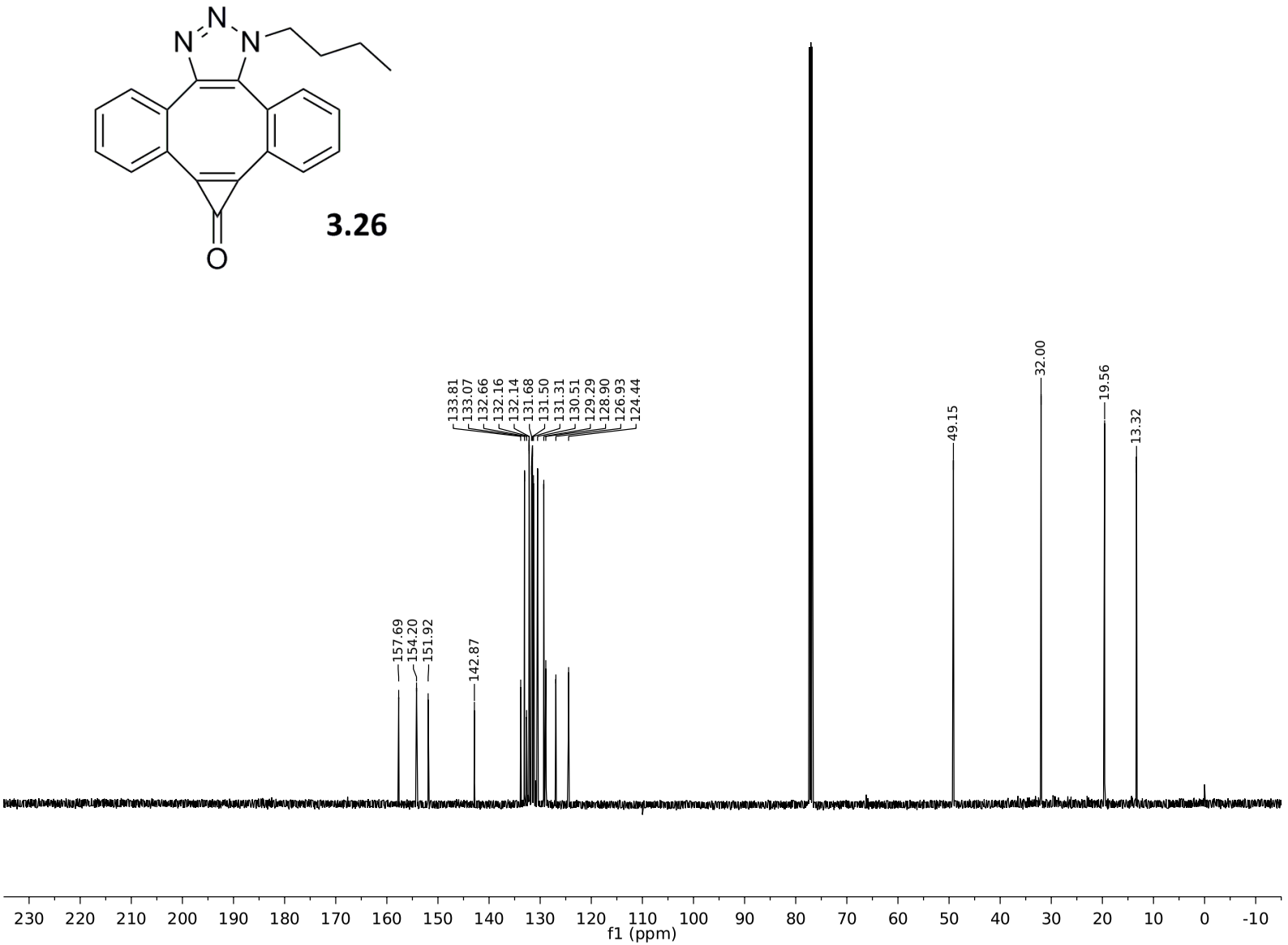

O
F
F
3.23
7.88
7.87
7.83
7.82
7.73
7.69
7.62
7.60
7.50
7.49
7.48
7.48
7.47
7.46
5.32
3.99
1.00
0.96
2.04
3.15
1.11
2.38
f1 (ppm)

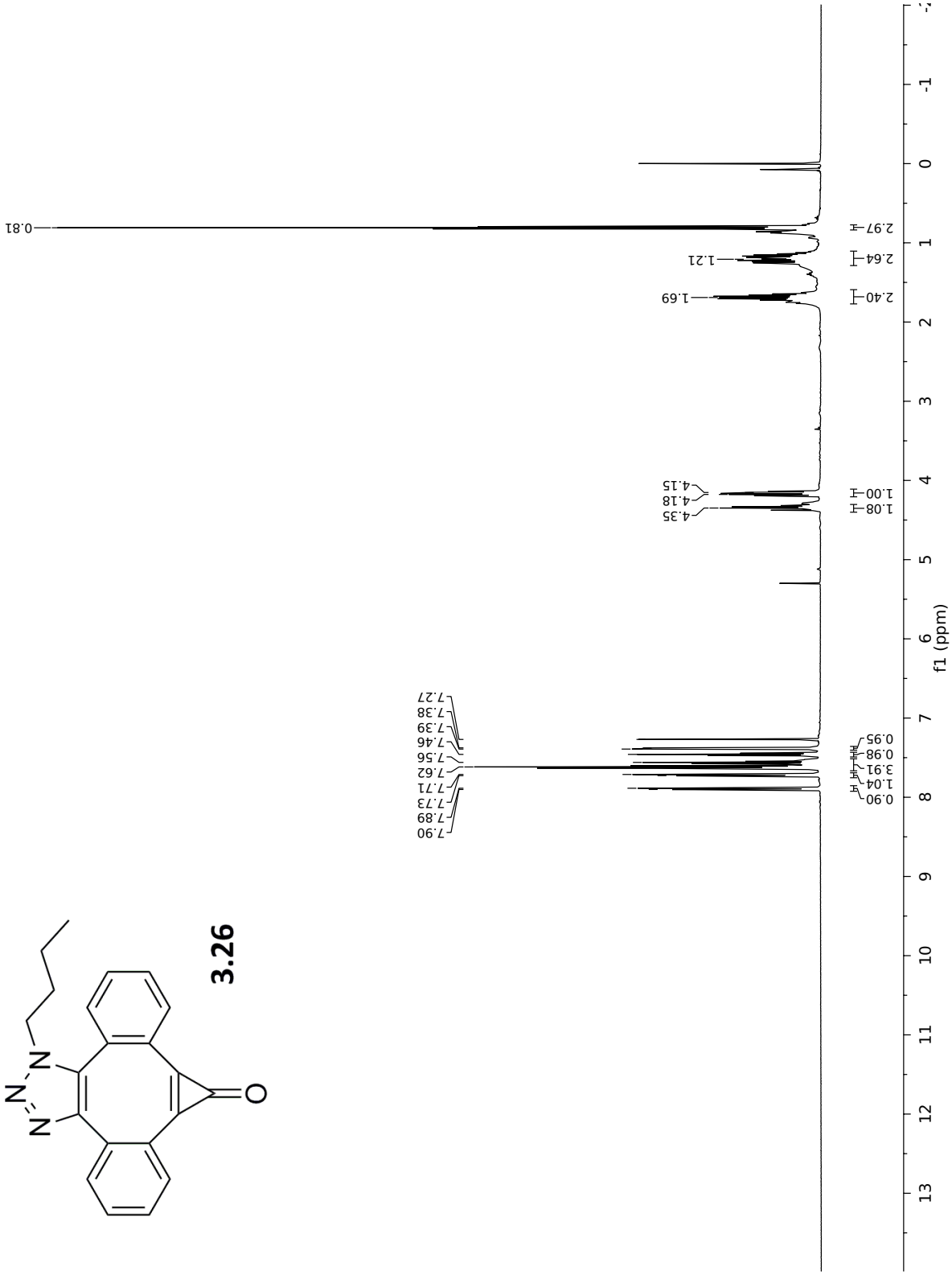

O
O

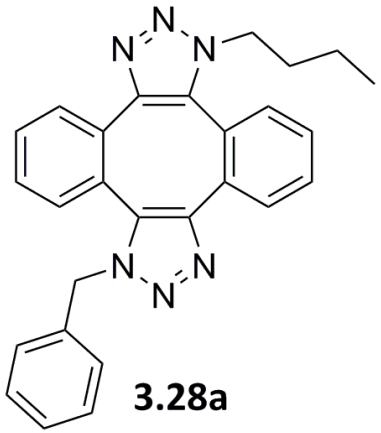
3.24

152.03
147.35
145.99
144.46
139.08
134.50
134.12
133.14
133.02
132.43
132.16
128.95
128.75
124.13
121.23
199.59
47.69
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
O
3.24
8.10
8.08
7.89
7.88
7.87
7.86
7.81
7.80
7.80
7.79
7.79
7.74
7.74
7.73
7.73
7.59
7.58
7.57
7.57
7.56
4.18
1.00
2.11
2.09
2.16
1.08
1.90
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.
f1 (ppm)

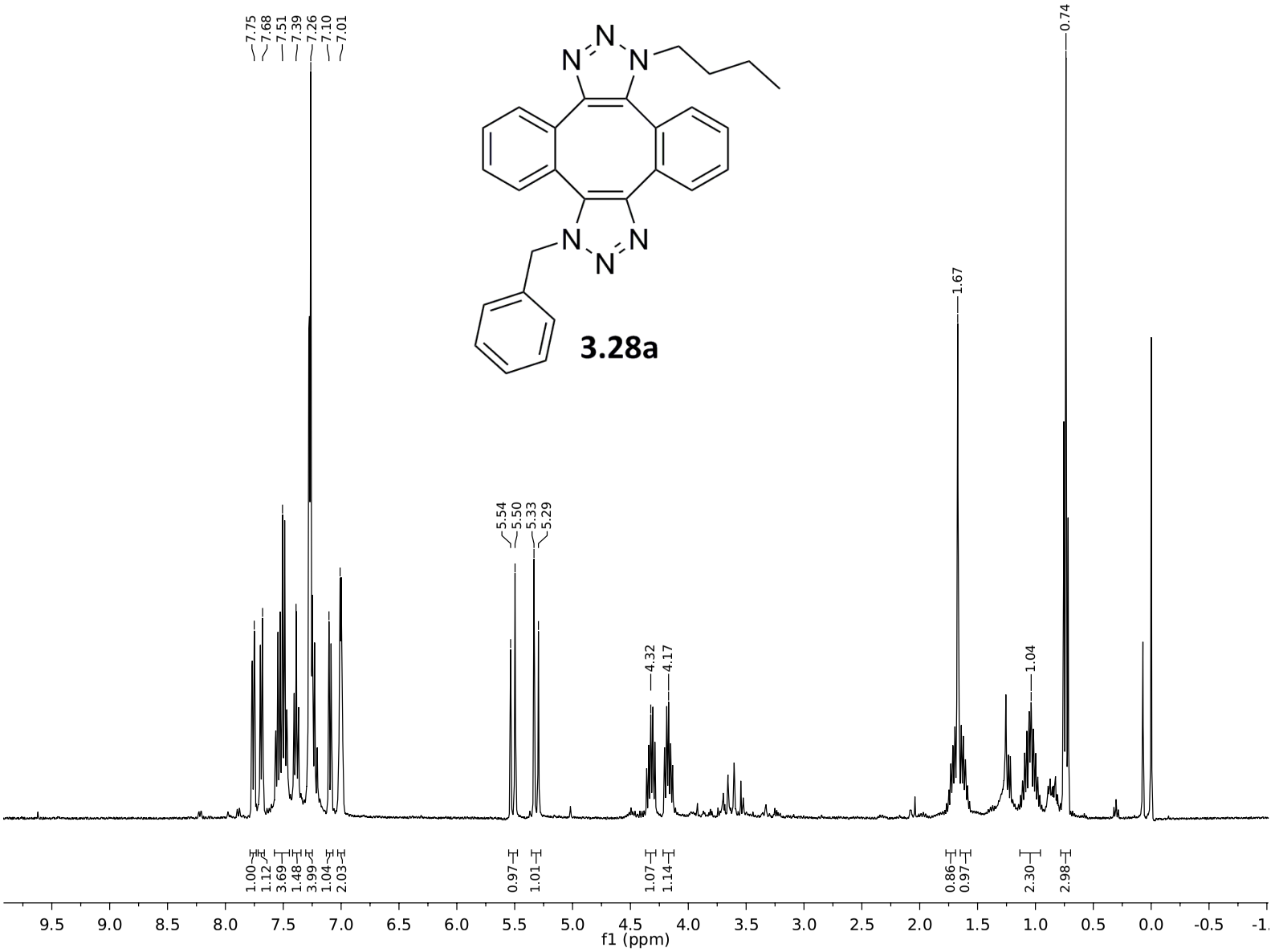

3.26
133.81
133.07
132.66
132.16
132.14
131.68
131.50
131.31
130.51
129.29
128.90
126.93
124.44
157.69
154.20
151.92
142.87
49.15
32.00
19.56
13.32
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

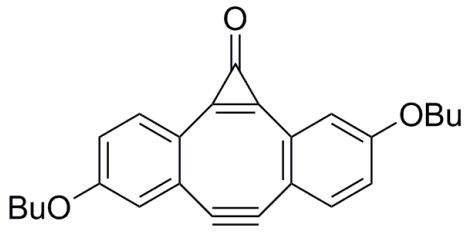

3.26
f1 (ppm)
0.81
1.21
1.69
4.15
4.18
4.35
7.27
7.38
7.39
7.46
7.56
7.62
7.71
7.73
7.89
7.90
0.90
1.04
3.91
0.98
0.95
1.00
1.08
2.40
2.64
2.97



3.28a

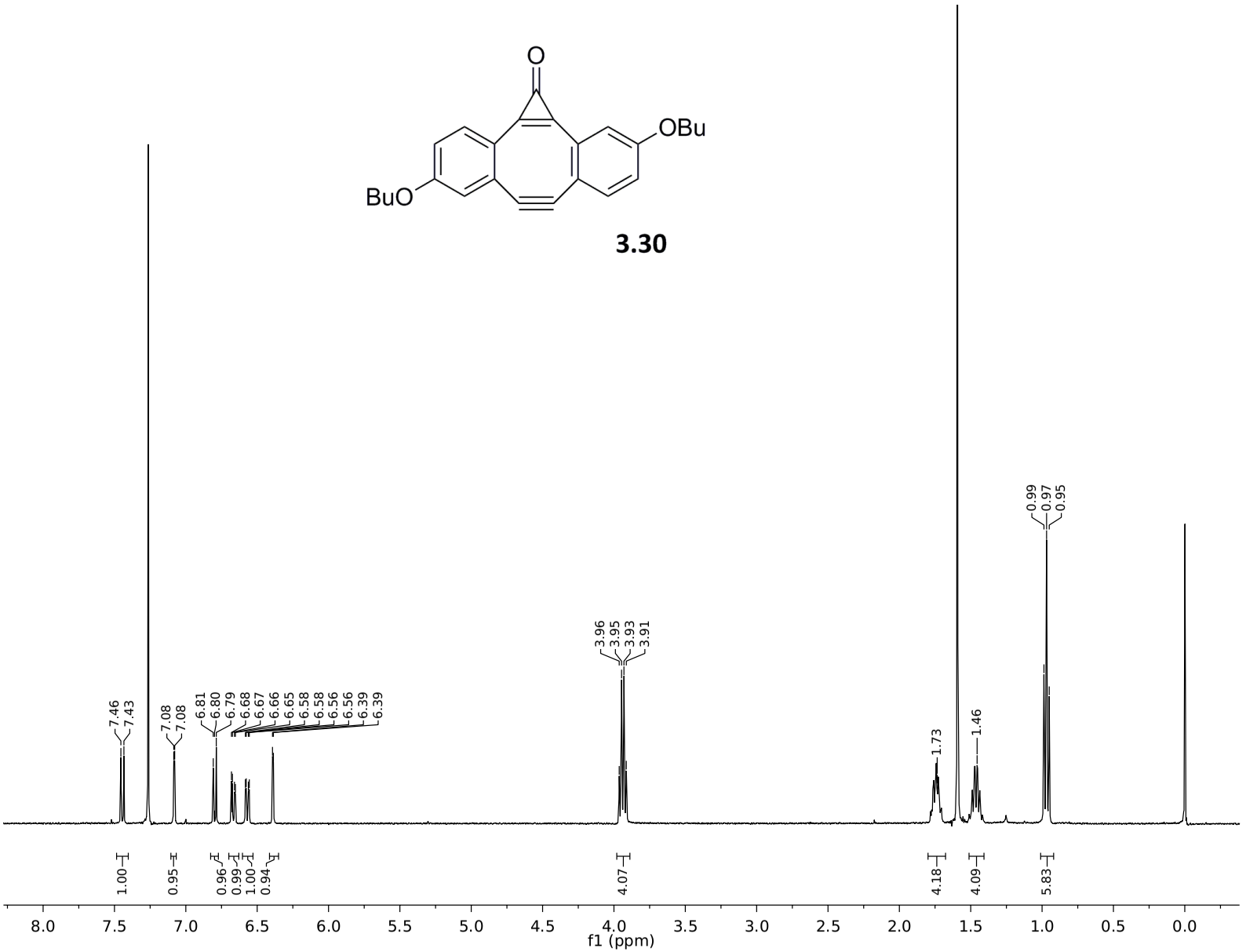
135.11
135.01
134.44
132.87
132.67
131.35
131.26
130.18
130.02
129.90
129.43
128.90
128.83
128.60
128.25
127.07
126.97
126.40
145.18
144.83
52.20
48.37
31.79
19.35
13.17
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


7.75
7.68
7.51
7.39
7.26
7.10
7.01
5.54
5.50
5.33
5.29
4.32
4.17
1.67
1.04
0.74
3.28a
1.00
1.12
3.69
1.48
3.99
1.04
2.03
0.97
1.01
1.07
1.14
0.86
0.97
2.30
2.98
f1 (ppm)

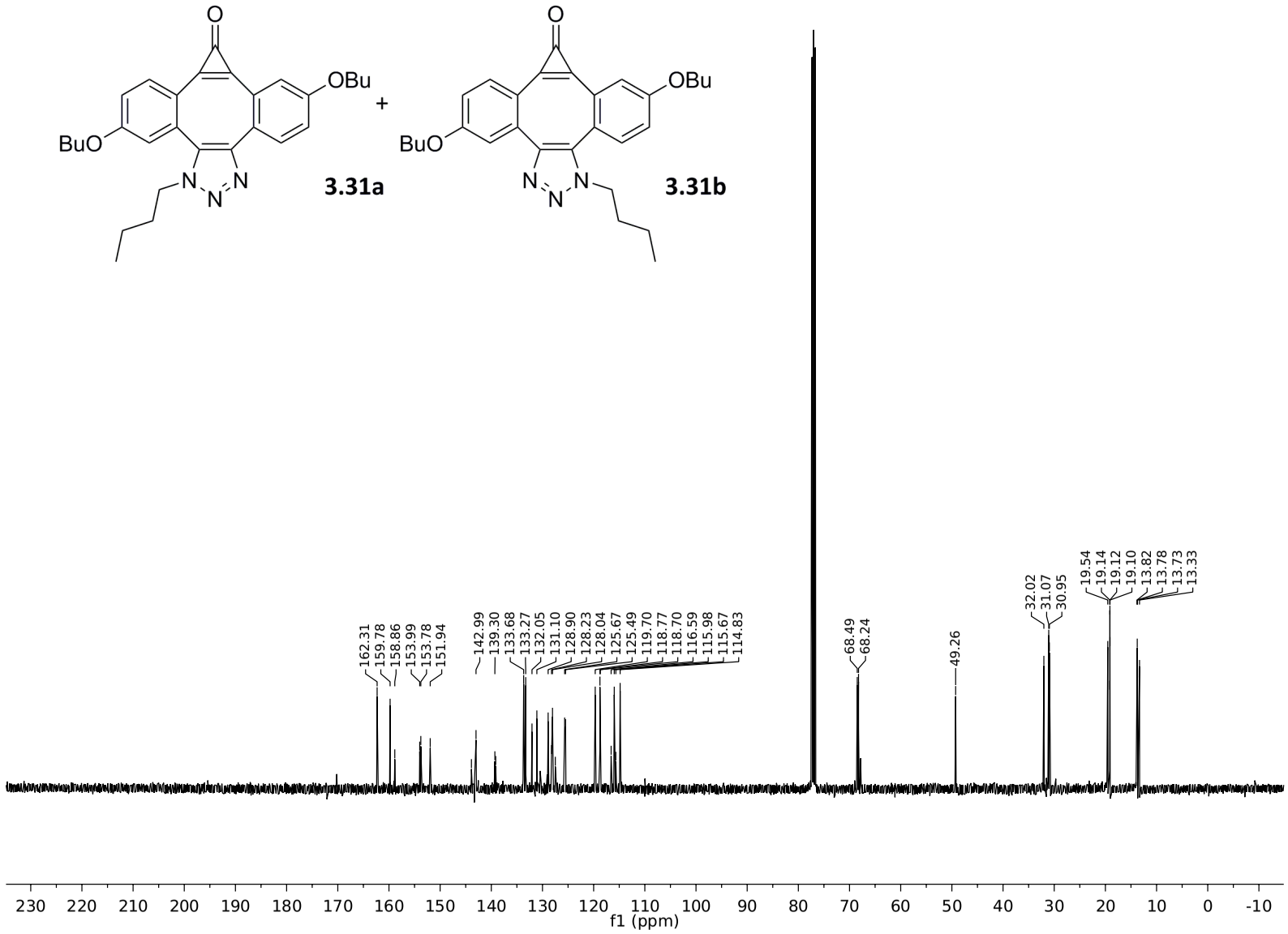

O
OBu
BuO

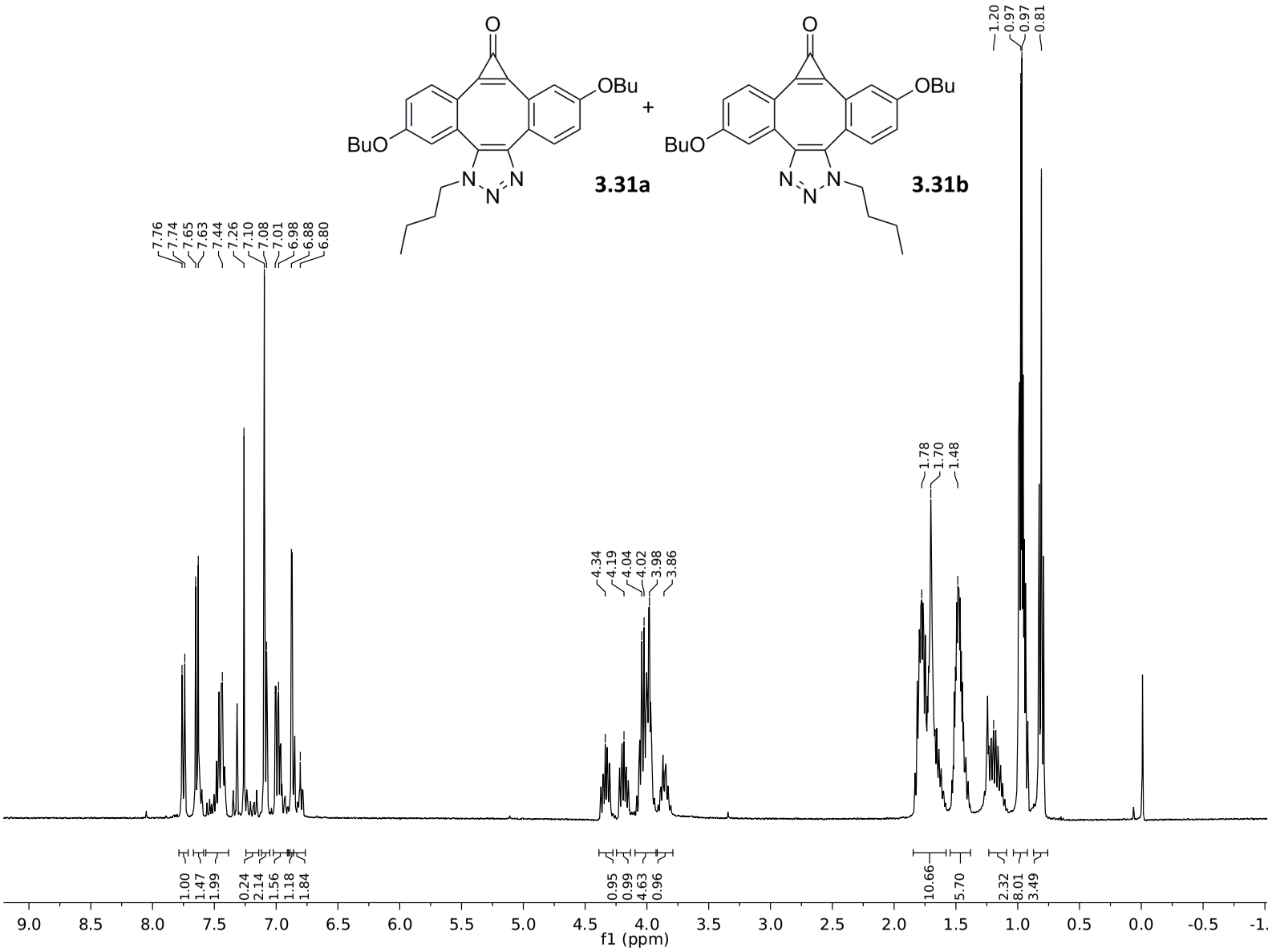
3.30

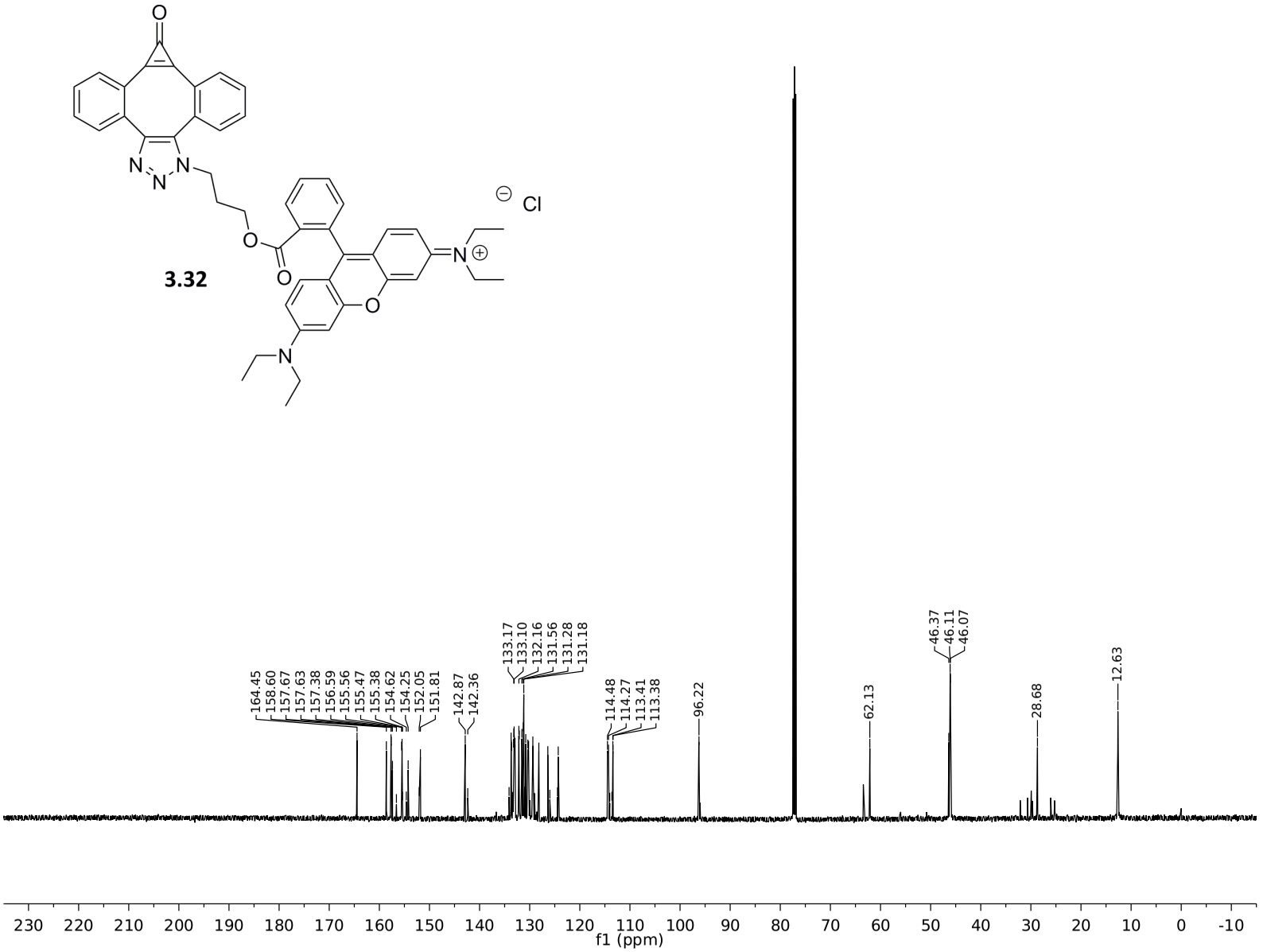
163.46
160.59
154.07
151.21
145.80
137.14
133.80
128.35
127.92
124.24
121.23
118.51
116.00
114.46
113.37
107.95
104.67
68.31
68.24
31.05
30.95
19.10
13.78
13.75
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
OBu
BuO
7.46
7.43
7.08
7.08
6.81
6.80
6.79
6.68
6.67
6.66
6.65
6.58
6.58
6.56
6.56
6.39
6.39
3.96
3.95
3.93
3.91
1.73
1.46
0.99
0.97
0.95
1.00
0.95
0.96
0.99
1.00
0.94
4.07
4.18
4.09
5.83
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

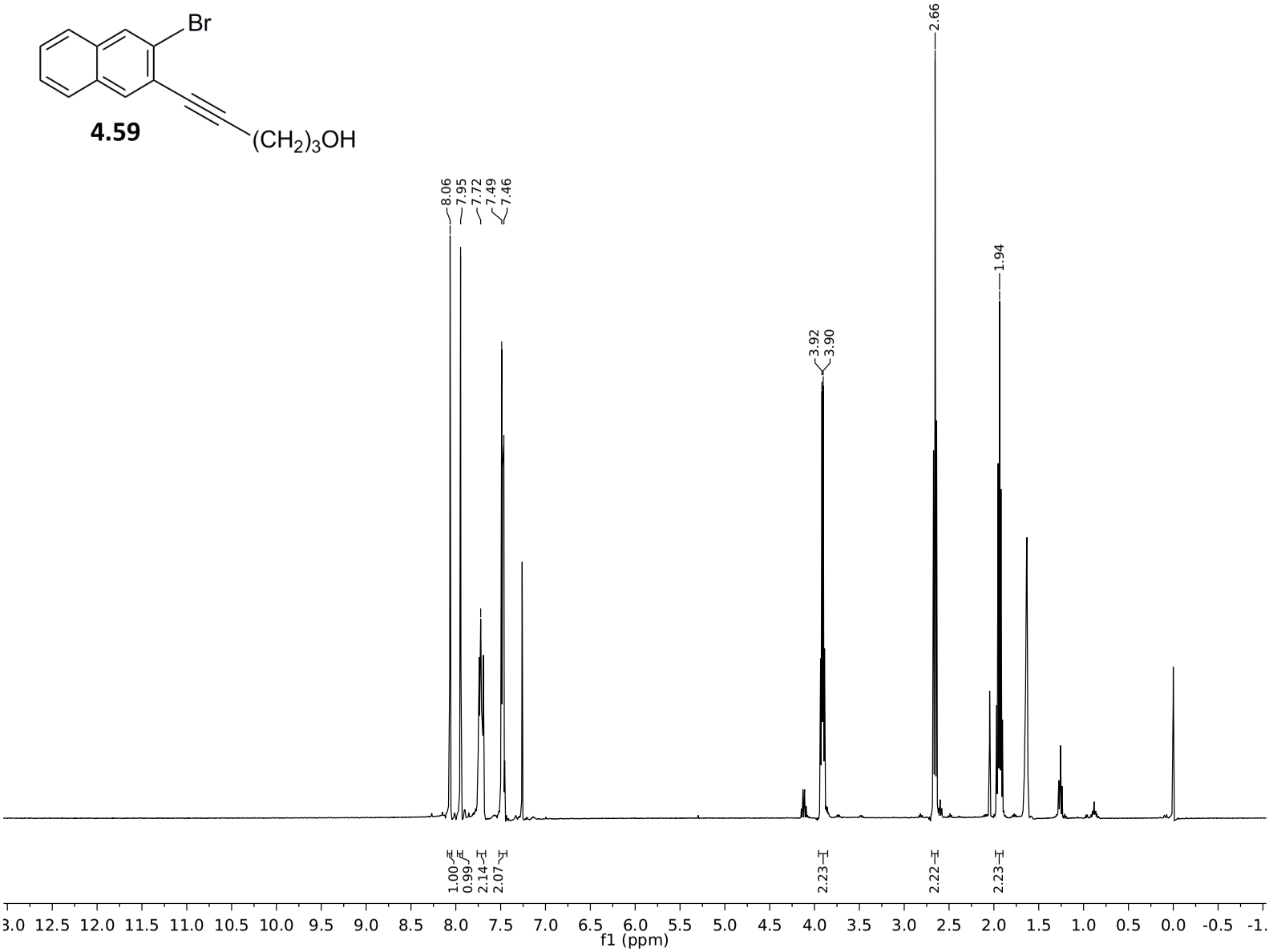
3.30

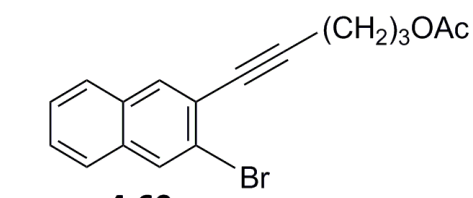

O
OBu
BuO
N
N
N
3.31a
+
3.31b
162.31
159.78
158.86
153.99
153.78
151.94
142.99
139.30
133.68
133.27
132.05
131.10
128.90
128.23
128.04
125.67
125.49
119.70
118.77
118.70
116.59
115.98
115.67
114.83
68.49
68.24
49.26
32.02
31.07
30.95
19.54
19.14
19.12
19.10
13.82
13.78
13.73
13.33
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
OBu
BuO
N
N
N
3.31a
+
3.31b
7.76
7.74
7.65
7.63
7.44
7.26
7.10
7.08
7.01
6.98
6.88
6.80
4.34
4.19
4.04
4.02
3.98
3.86
1.78
1.70
1.48
1.20
0.97
0.97
0.81
1.00
1.47
1.99
0.24
2.14
1.56
1.18
1.84
0.95
0.99
4.63
0.96
10.66
5.70
2.32
8.01
3.49
f1 (ppm)


O
N
N
N
O
O
O
N
Cl
3.32
164.45
158.60
157.67
157.63
157.38
156.59
155.56
155.47
155.38
154.62
154.25
152.05
151.81
142.87
142.36
133.17
133.10
132.16
131.56
131.28
131.18
114.48
114.27
113.41
113.38
96.22
62.13
46.37
46.11
46.07
28.68
12.63
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

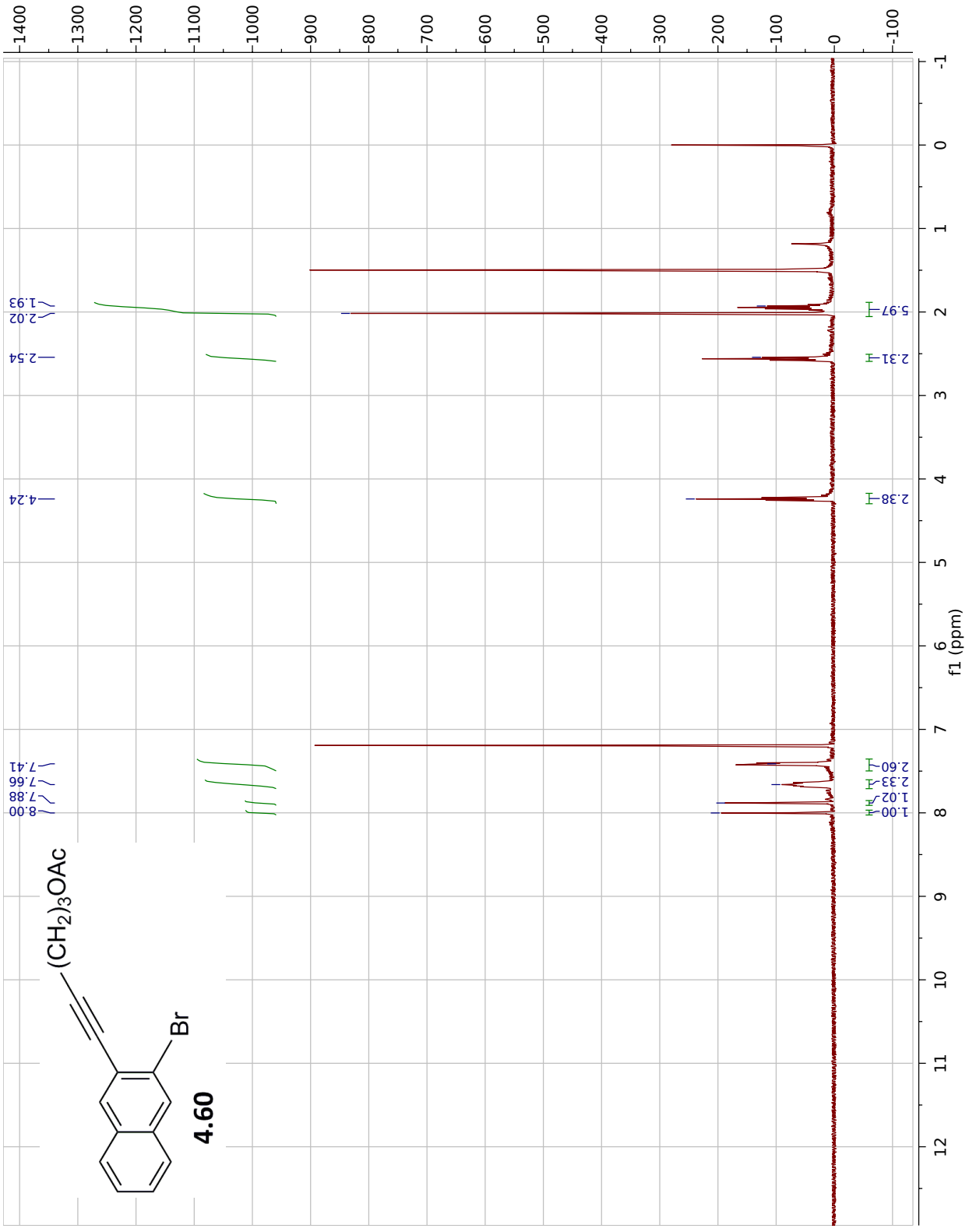
3.32
f1 (ppm)

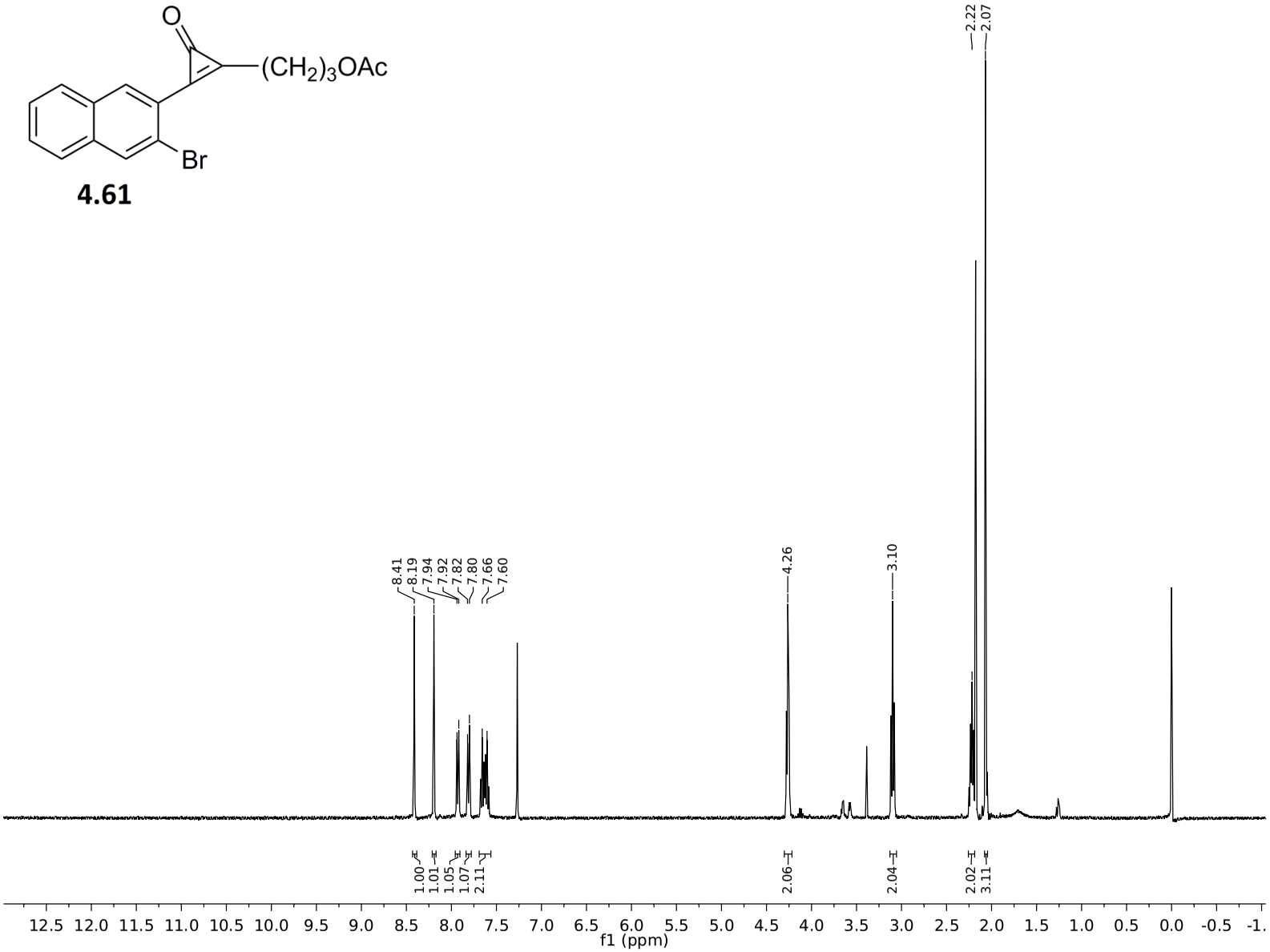

Br
4.59
(CH2)3OH
8.06
7.95
7.72
7.49
7.46
3.92
3.90
2.66
1.94
1.00
0.99
2.14
2.07
2.23
2.22
2.23
f1 (ppm)

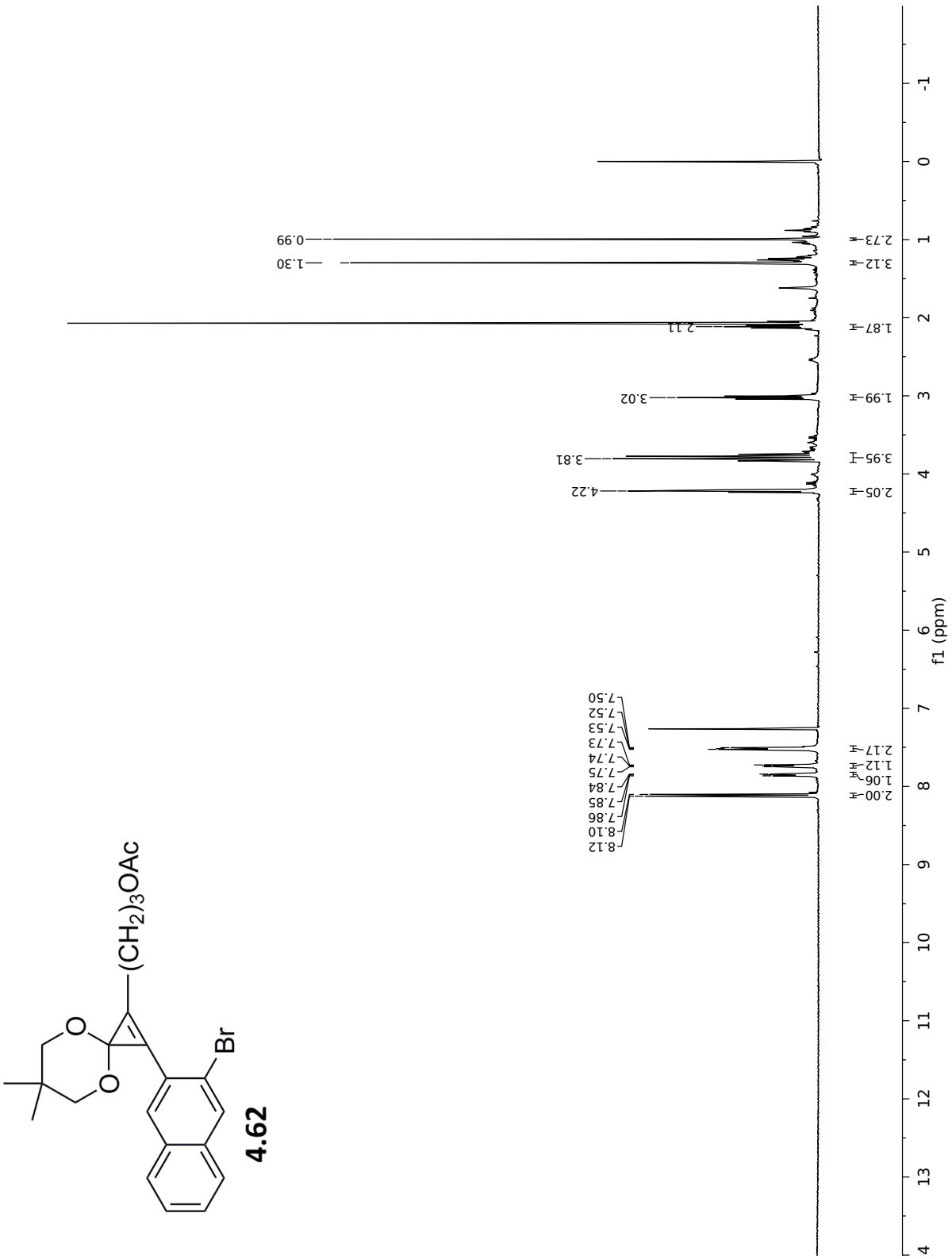

(CH2)3OAc
Br

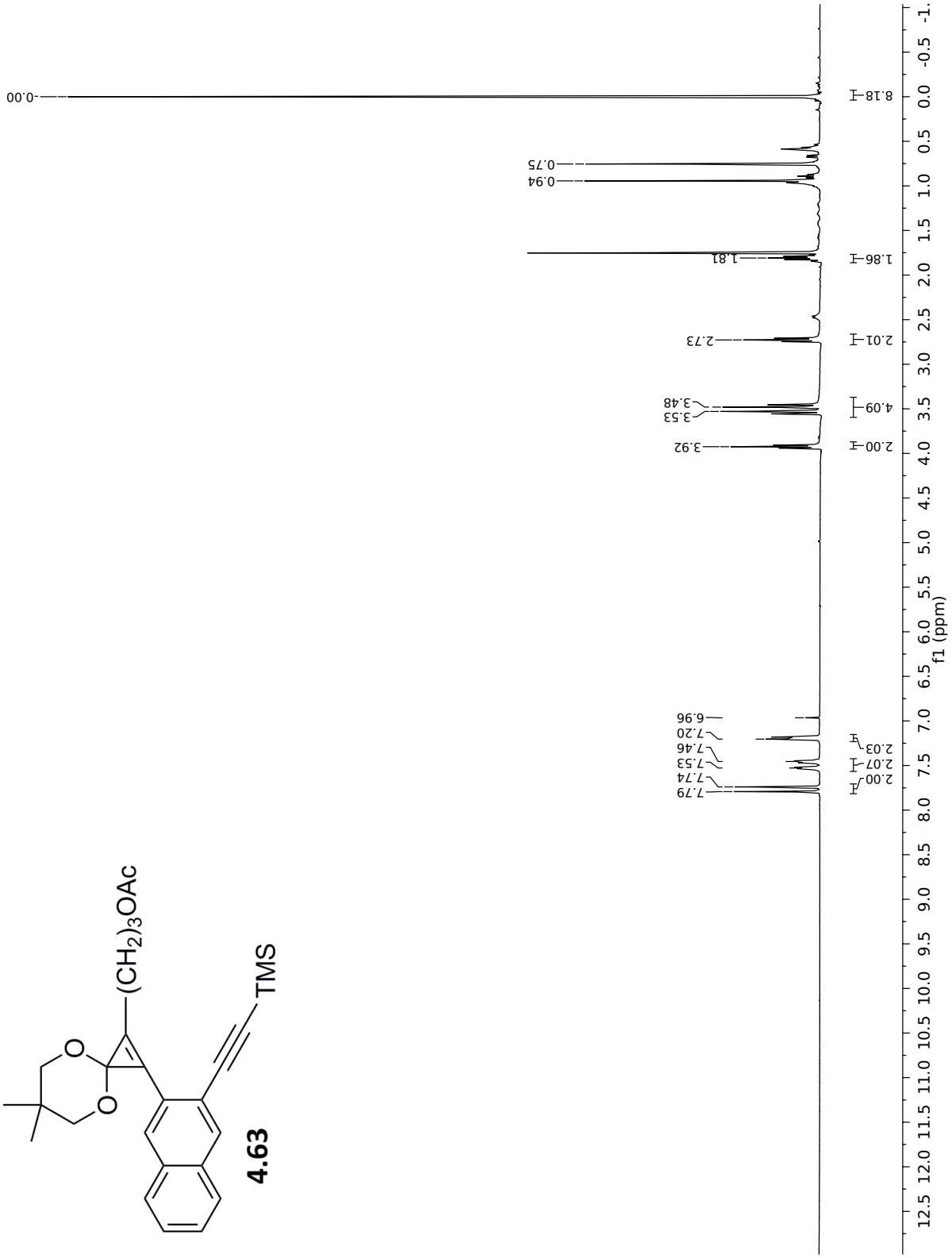
4.60

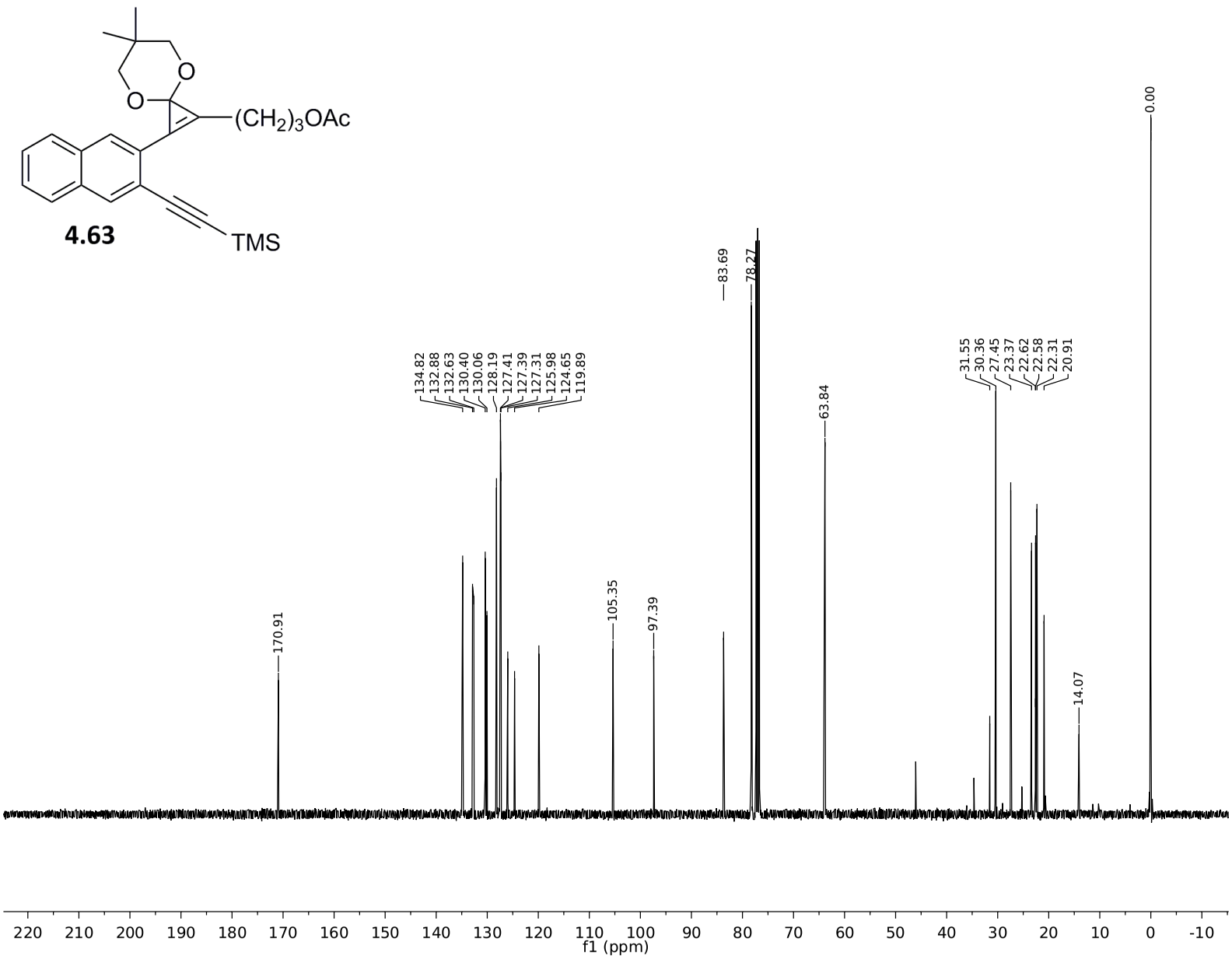
133.56
133.08
131.93
131.04
127.63
127.49
126.98
126.94
123.13
122.33
171.34
103.52
93.49
80.48
63.43
27.89
21.23
16.65
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

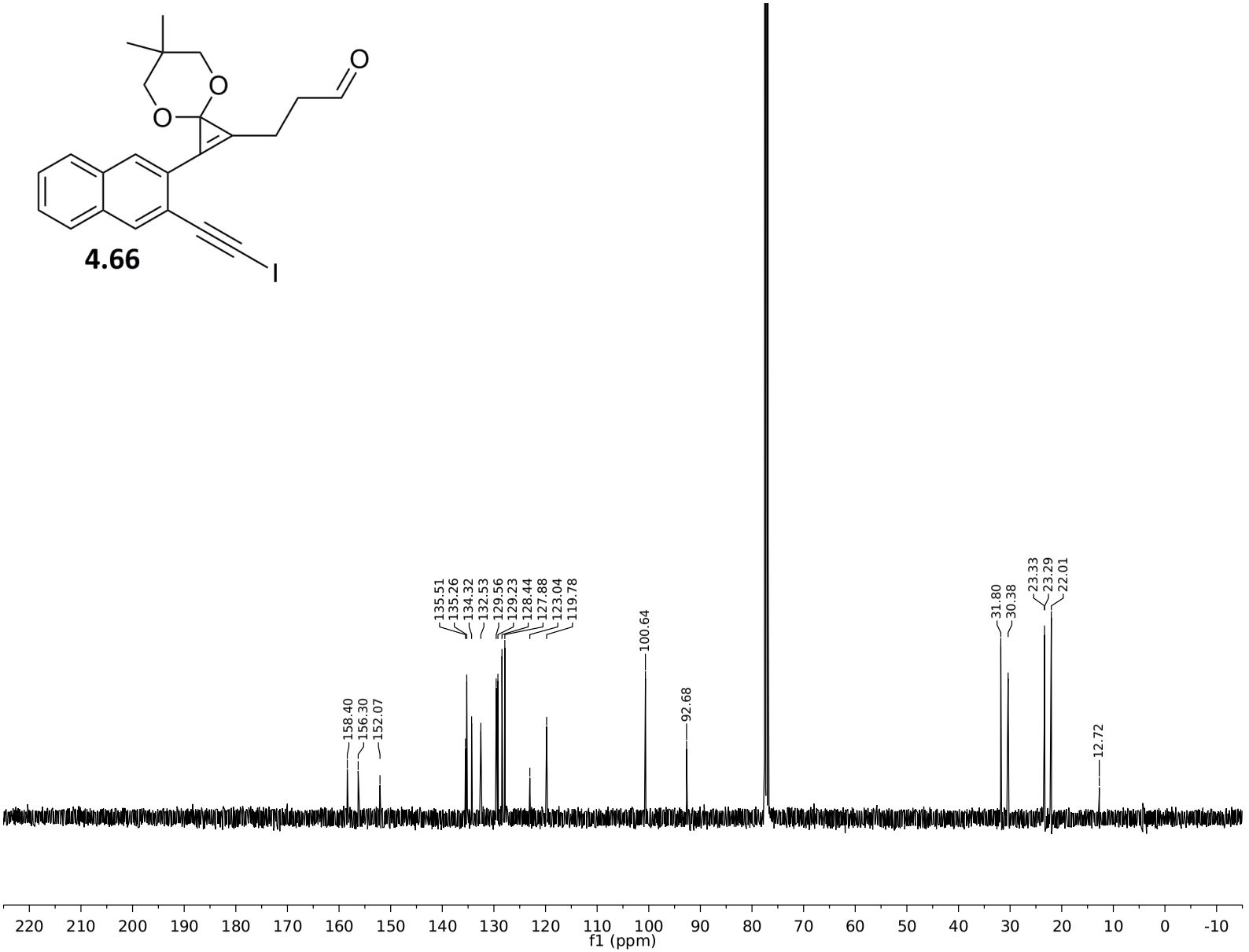

(CH2)3OAc
Br
4.60
f1 (ppm)

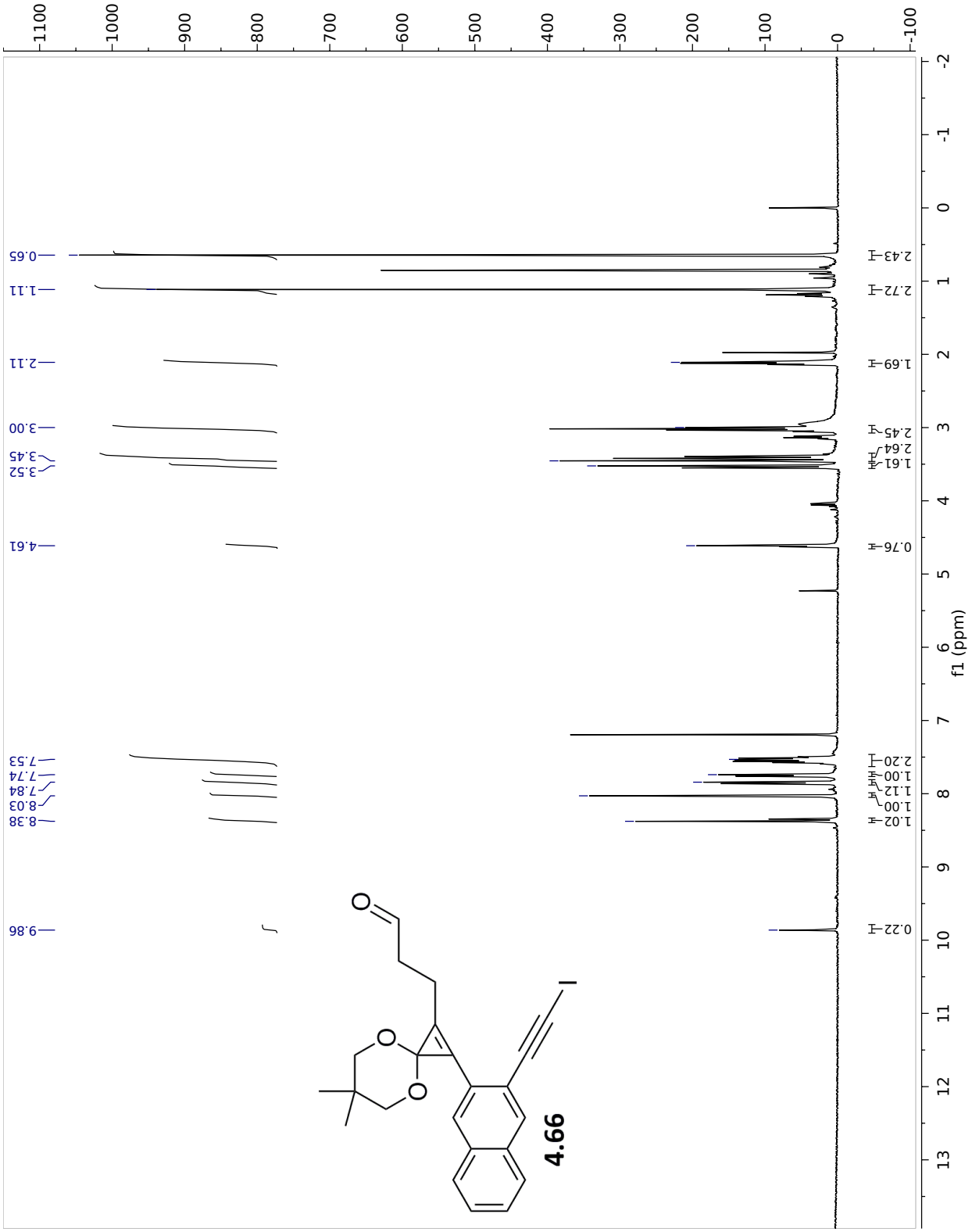

O
(CH2)3OAc
Br
4.61
2.22
2.07
8.41
8.19
7.94
7.92
7.82
7.80
7.66
7.60
4.26
3.10
1.00
1.01
1.05
1.07
2.11
2.06
2.04
2.02
3.11
f1 (ppm)

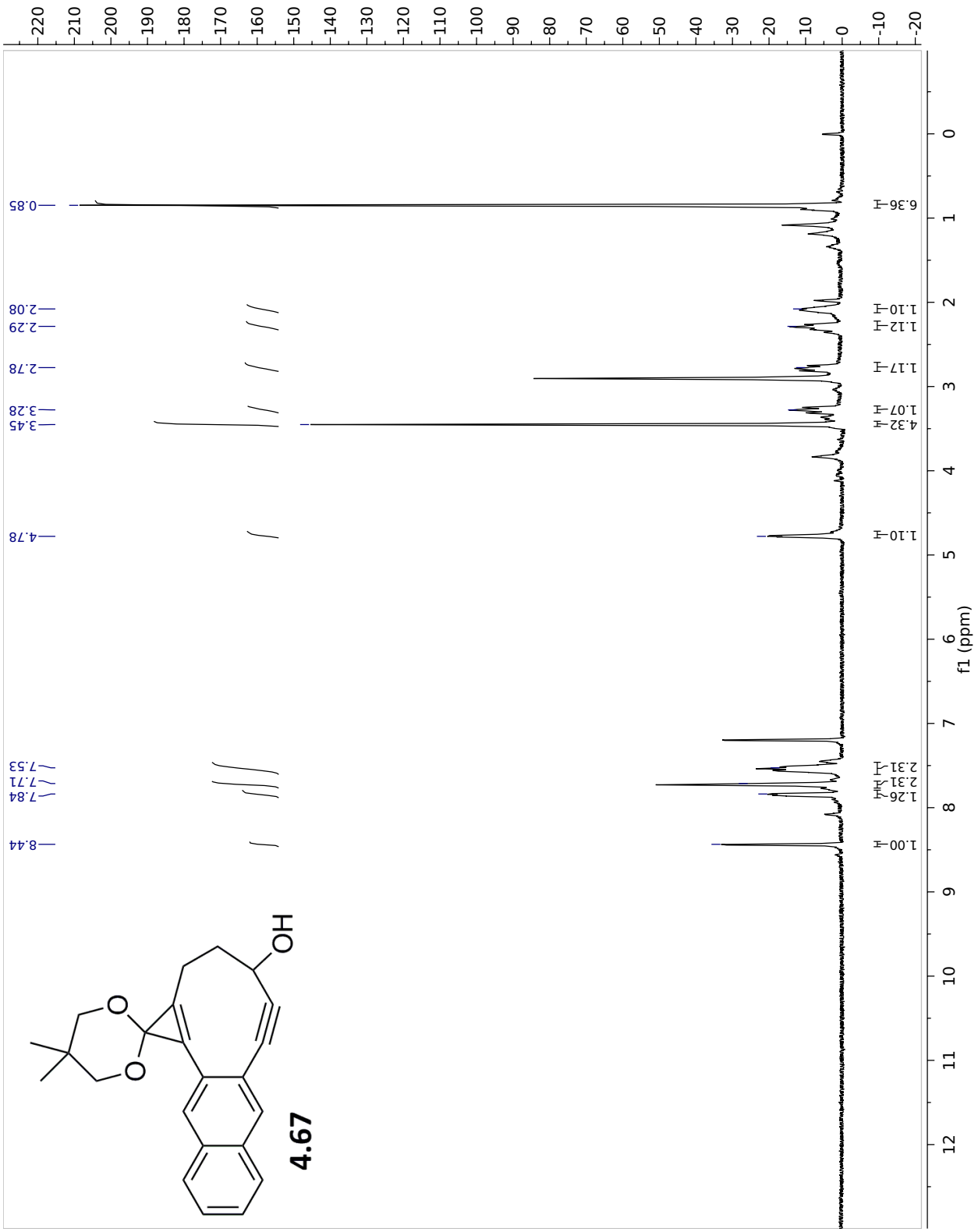

(CH2)3OAc
O
O
Br
4.62
8.12
8.10
7.86
7.85
7.84
7.75
7.74
7.73
7.53
7.52
7.50
4.22
3.81
3.02
2.11
1.30
0.99
2.00
1.06
1.12
2.17
2.05
3.95
1.99
1.87
3.12
2.73
f1 (ppm)

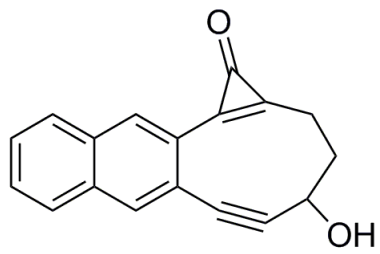

(CH2)3OAc
TMS
O
O
4.63
7.79
7.74
7.53
7.46
7.20
6.96
3.92
3.53
3.48
2.73
1.81
0.94
0.75
0.00
2.00
2.07
2.03
4.09
2.00
1.86
8.18
f1 (ppm)


O
O
(CH2)3OAc
TMS
4.63
170.91
134.82
132.88
132.63
130.40
130.06
128.19
127.41
127.39
127.31
125.98
124.65
119.89
105.35
97.39
83.69
78.27
63.84
31.55
30.36
27.45
23.37
22.62
22.58
22.31
20.91
14.07
0.00
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


4.66
O
O
O
I
158.40
156.30
152.07
135.51
135.26
134.32
132.53
129.56
129.23
128.44
127.88
123.04
119.78
100.64
92.68
31.80
30.38
23.33
23.29
22.01
12.72
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

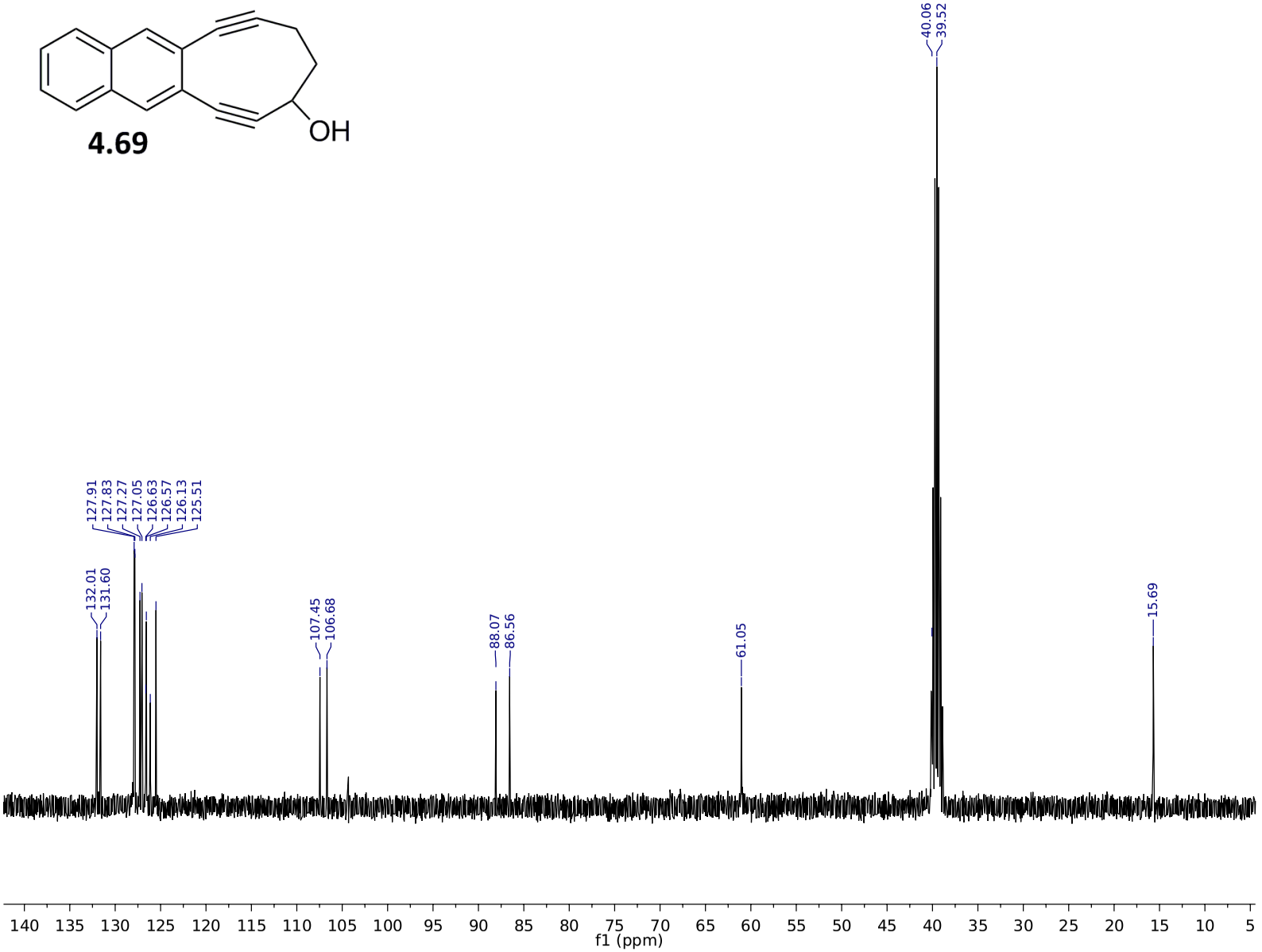

4.66
f1 (ppm)

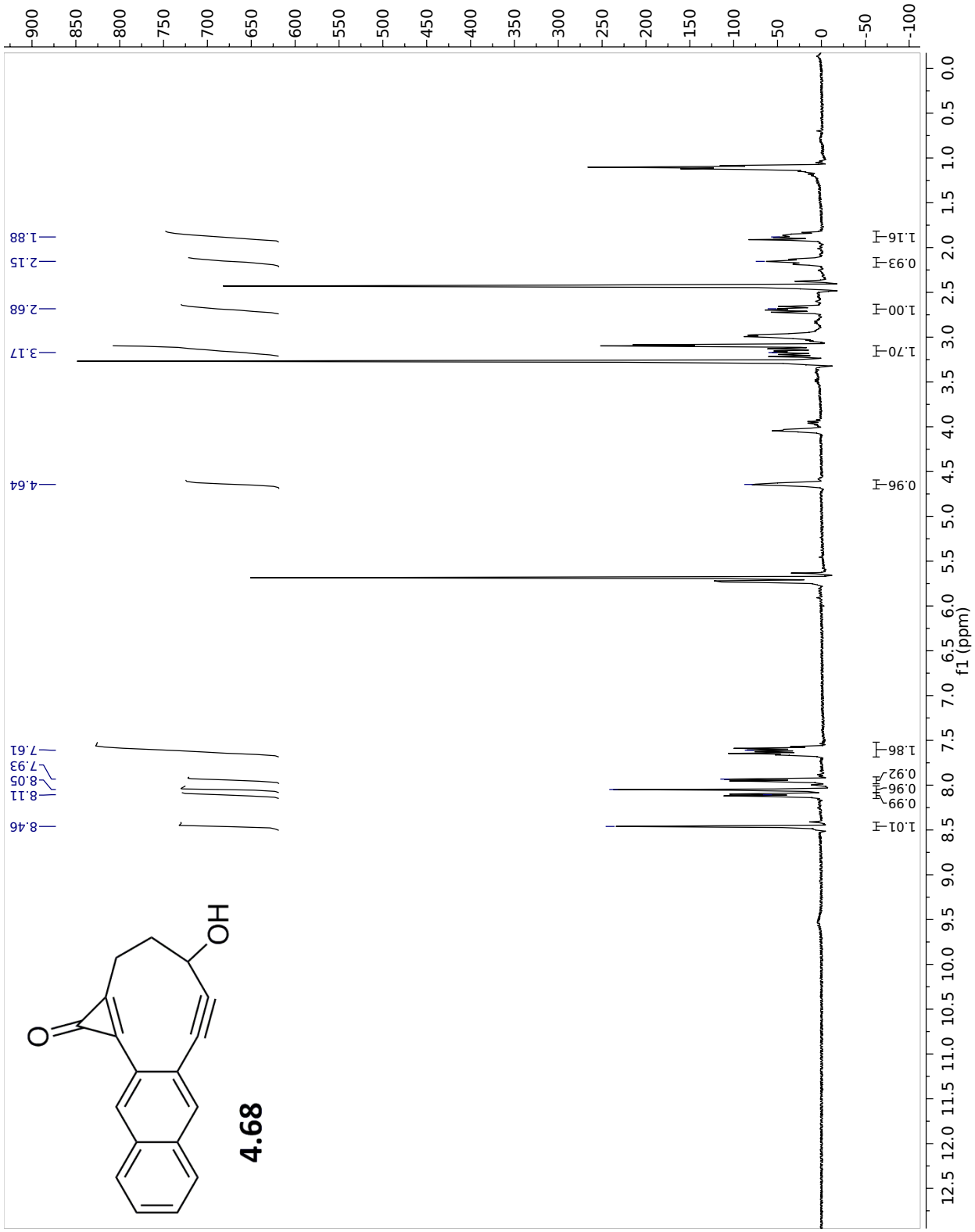

OH
4.67
f1 (ppm)

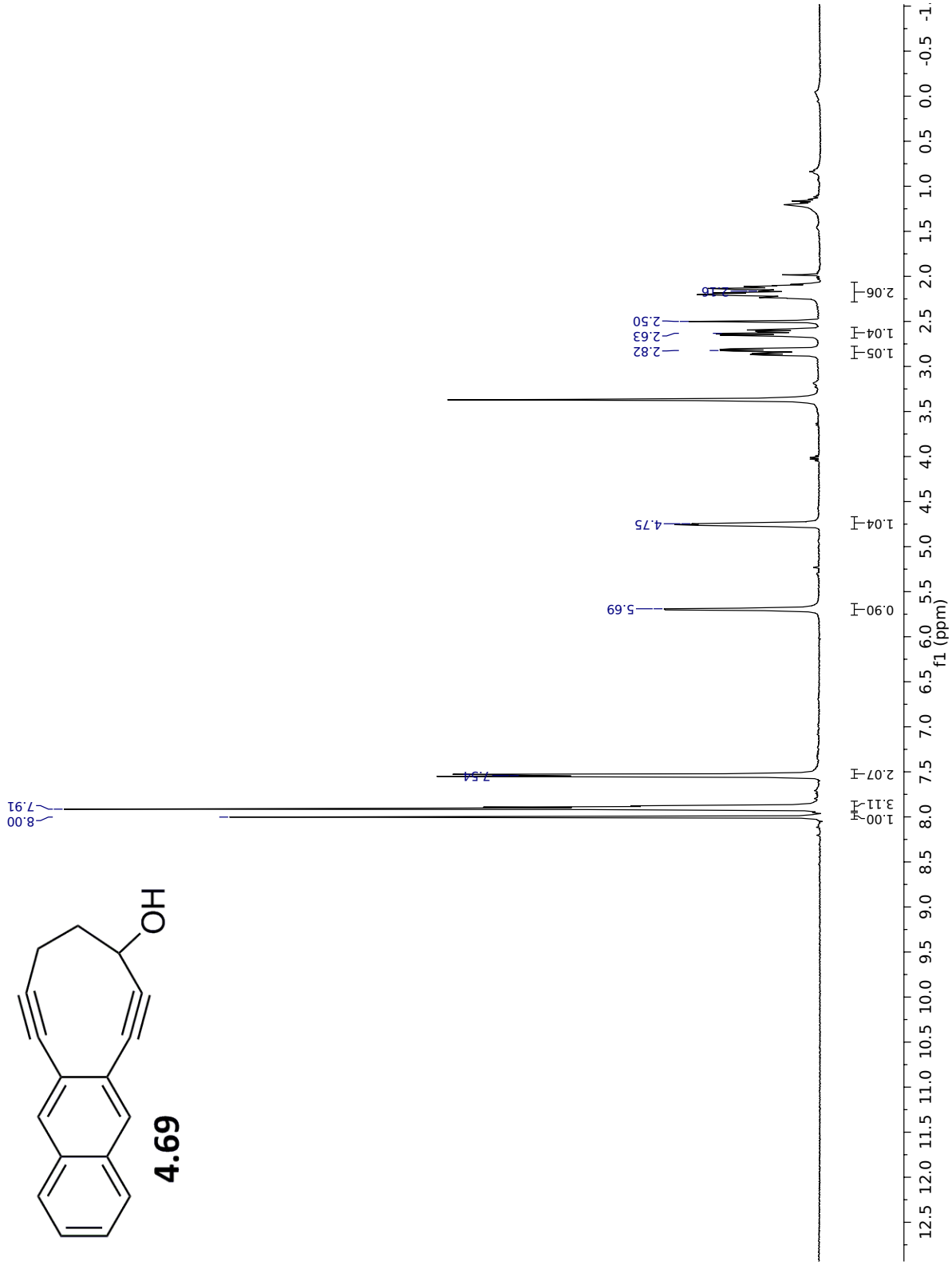

O
OH

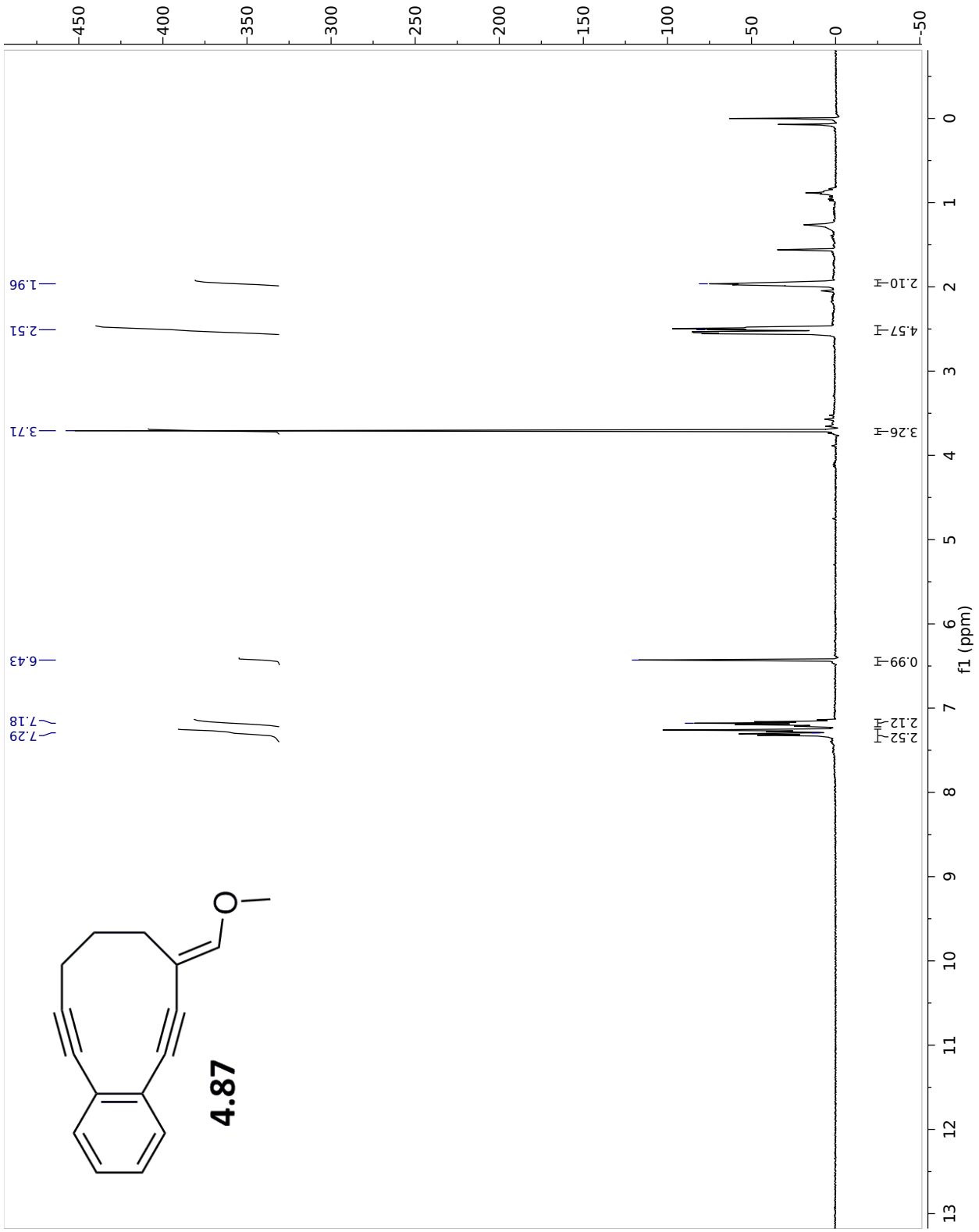
4.68

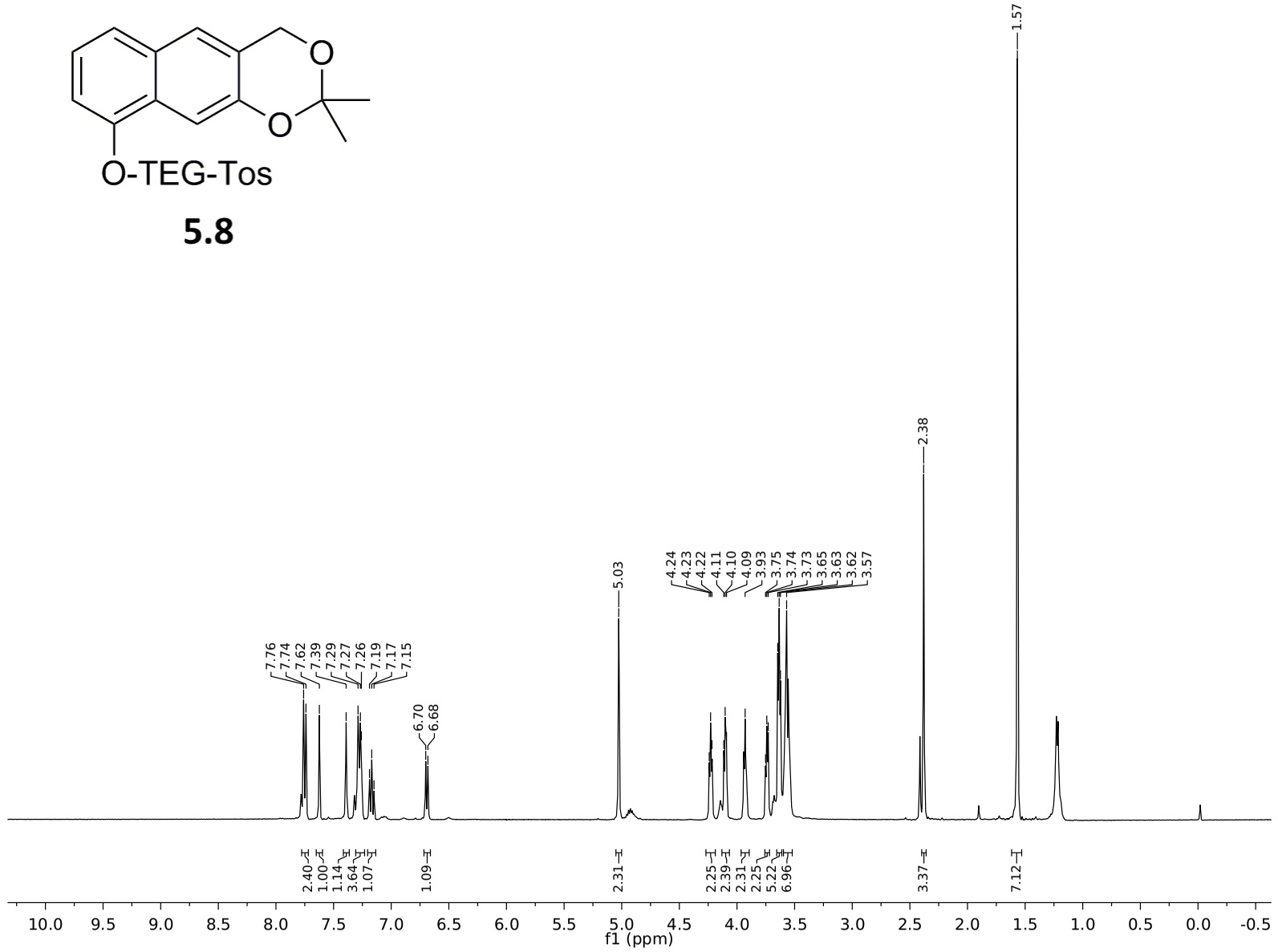
40.06
39.52
132.01
131.60
127.91
127.83
127.27
127.05
126.63
126.57
126.13
125.51
107.45
106.68
88.07
86.56
61.05
15.69
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


OH
4.69
40.06
39.52
127.91
127.83
127.27
127.05
126.63
126.57
126.13
125.51
132.01
131.60
107.45
106.68
88.07
86.56
61.05
15.69
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

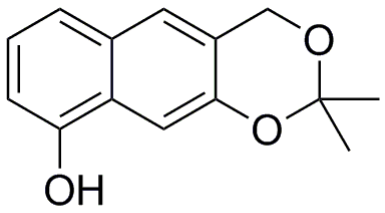

OH
O
4.68
f1 (ppm)


OH
4.69
8.00
7.91
7.54
5.69
4.75
2.82
2.63
2.50
1.00
3.11
2.07
0.90
1.04
1.05
1.04
2.06
f1 (ppm)


4.87
1.96
2.51
3.71
6.43
7.29
7.18
2.10
4.57
3.26
0.99
2.52
2.12
f1 (ppm)

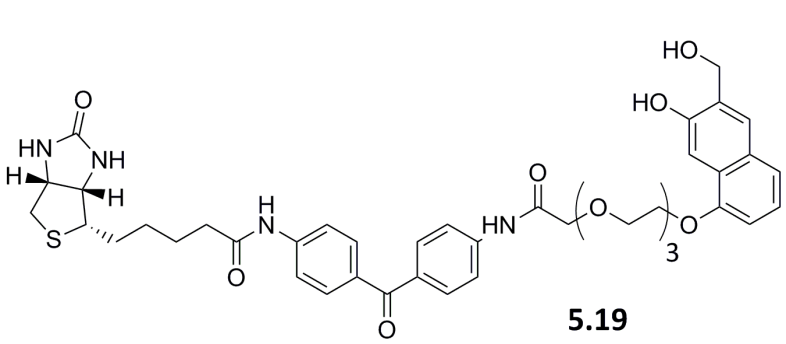

O
O
O-TEG-Tos
5.8
1.57
2.38
5.03
4.24
4.23
4.22
4.11
4.10
4.09
3.93
3.75
3.74
3.73
3.65
3.63
3.62
3.57
7.76
7.74
7.62
7.39
7.29
7.27
7.26
7.19
7.17
7.15
6.70
6.68
2.40
1.00
1.14
3.64
1.07
1.09
2.31
2.25
2.39
2.31
2.25
5.22
6.96
3.37
7.12
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

5.9
f1 (ppm)


O
O
OH

5.16

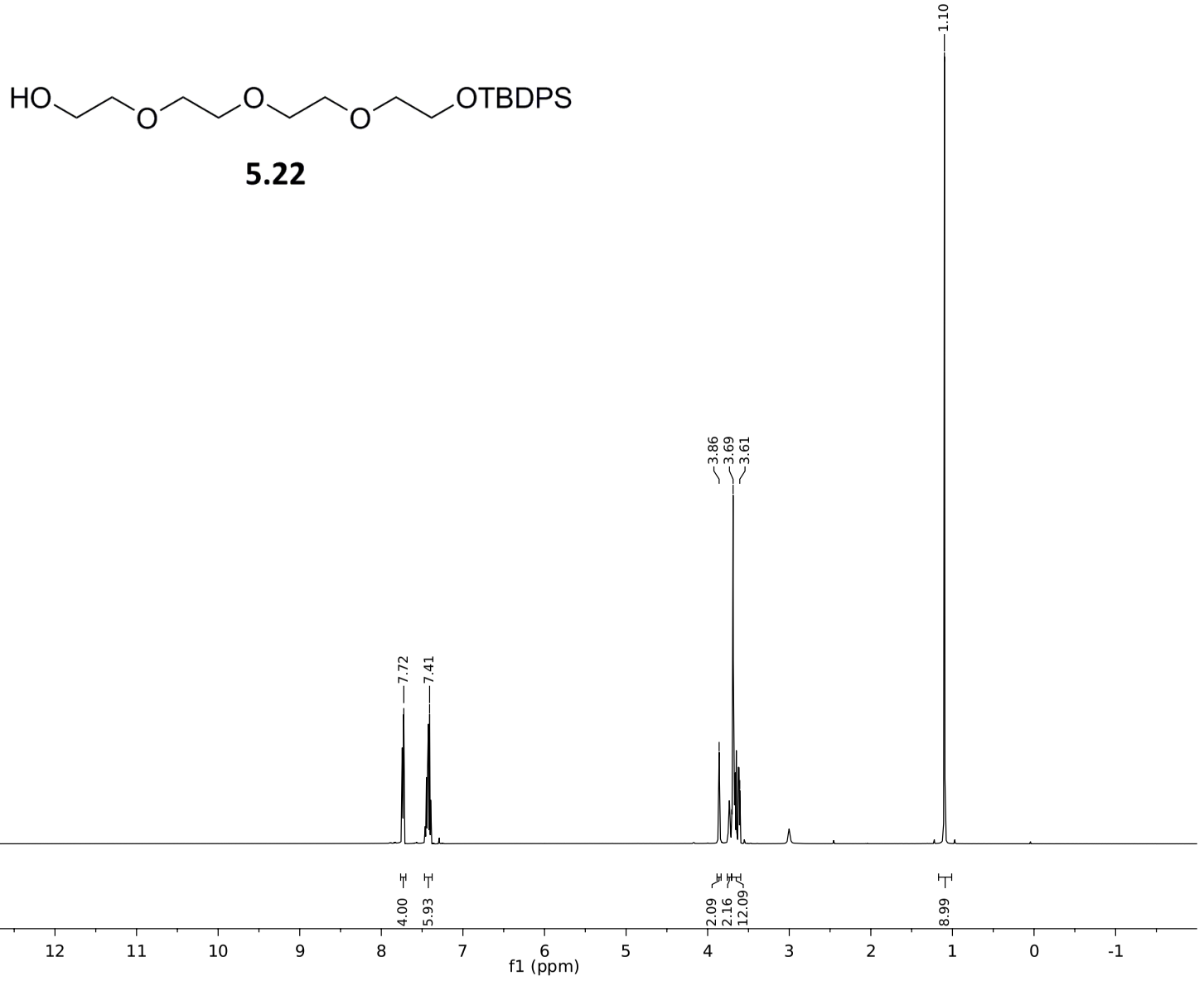
1.62
5.34
5.07
7.58
7.42
7.28
7.13
6.71
1.00
1.08
1.16
1.05
1.05
0.96
2.25
6.72
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

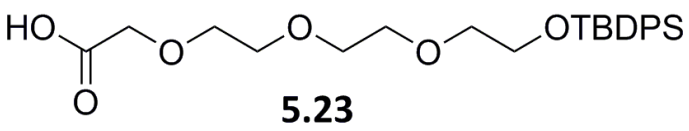

HO
HO
O
HN
NH
H
H
S
H
N
O
HN
O
O
O
3
O

5.19

193.81
172.26
169.35
163.15
153.07
153.04
143.59
142.55
132.41
131.97
131.36
131.21
129.02
125.42
119.21
118.62
105.01
103.06
79.62
70.48
70.34
70.08
61.48
59.63
58.93
55.83
36.78
28.65
28.54
25.44
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

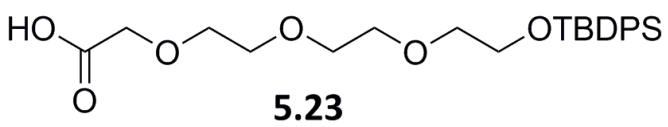
5.19
f1 (ppm)


HO
O
O
O
OTBDPS
5.22
1.10
3.86
3.69
3.61
7.72
7.41
4.00
5.93
2.09
2.16
12.09
8.99
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)


HO
O
O
O
O
OTBDPS

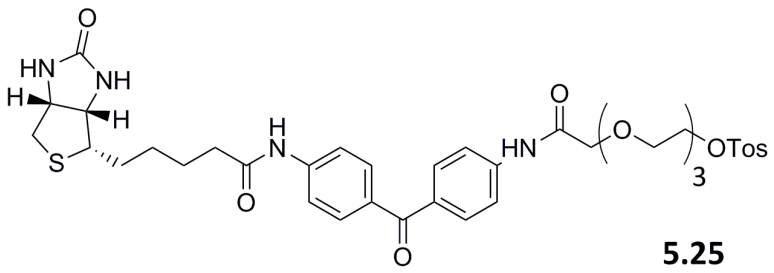
5.23

135.60
133.39
129.75
127.73
72.72
71.68
70.13
63.17
26.79
19.18
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


HO
O
O
O
O
OTBDPS

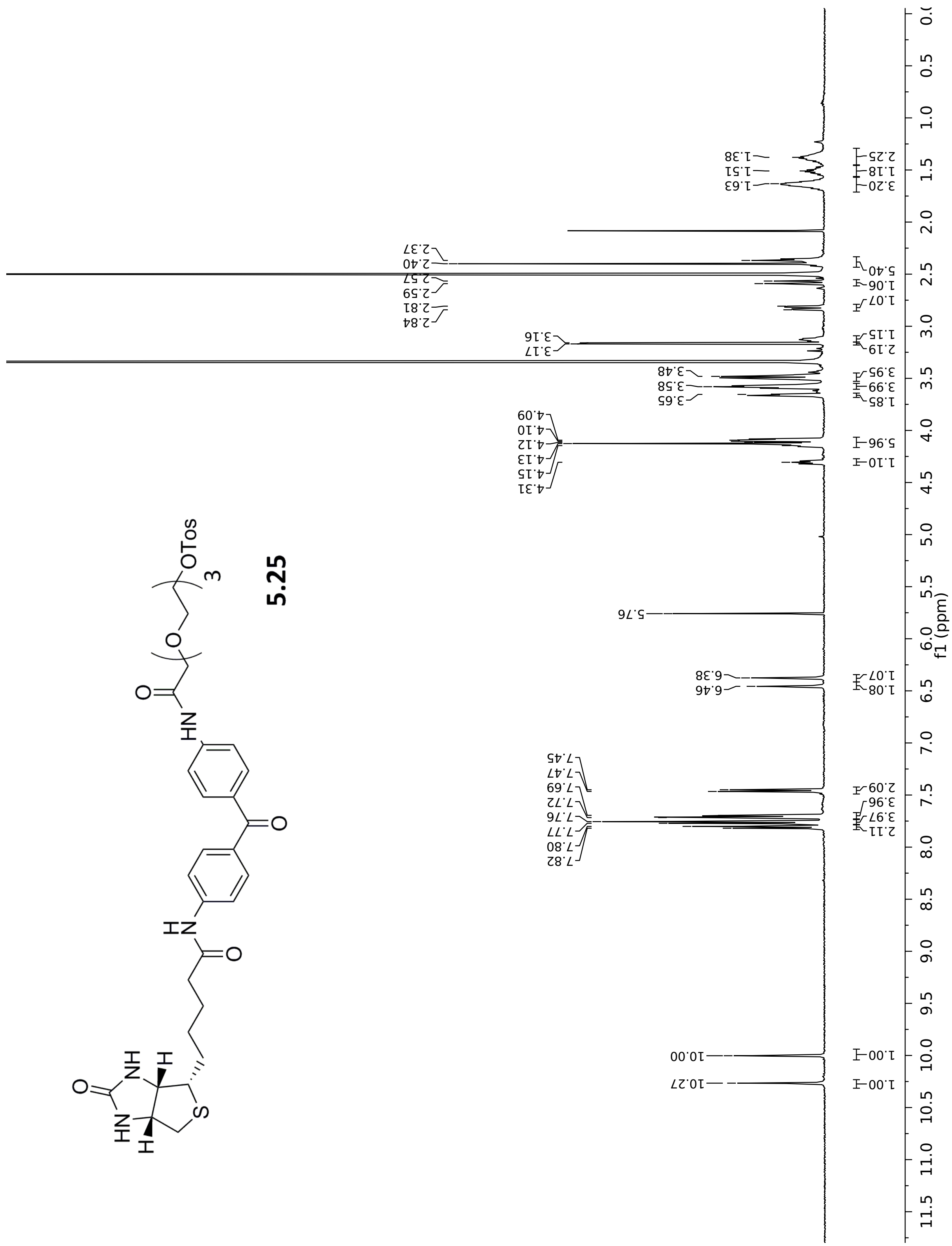
5.23

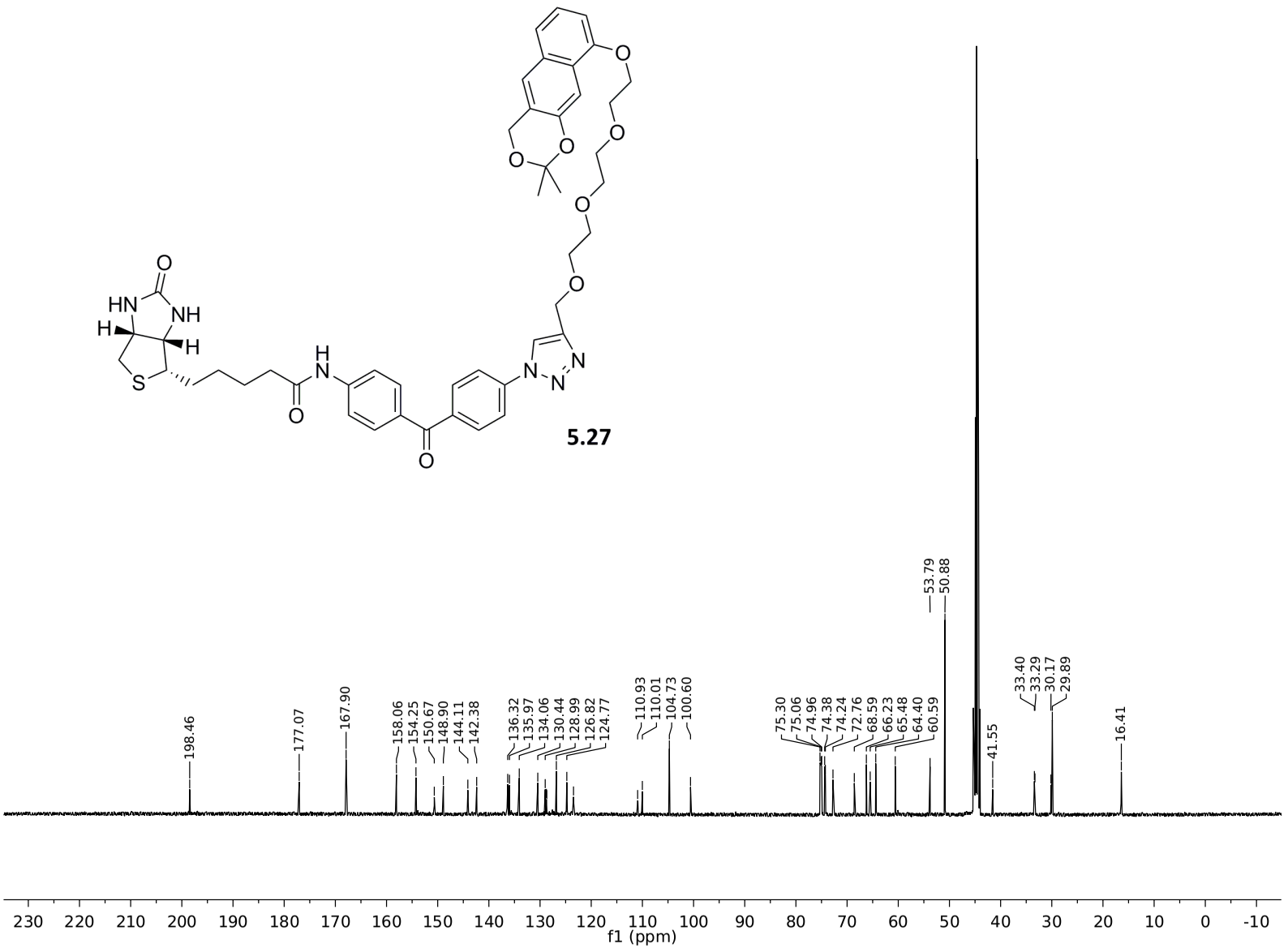
1.08
7.71
7.70
7.46
7.41
4.18
3.85
3.76
3.74
3.68
4.00
6.02
1.95
2.03
1.90
2.08
2.17
9.05
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


O
HN
NH
H
H
S
H
N
O
O
HN
O
O
OTos
3

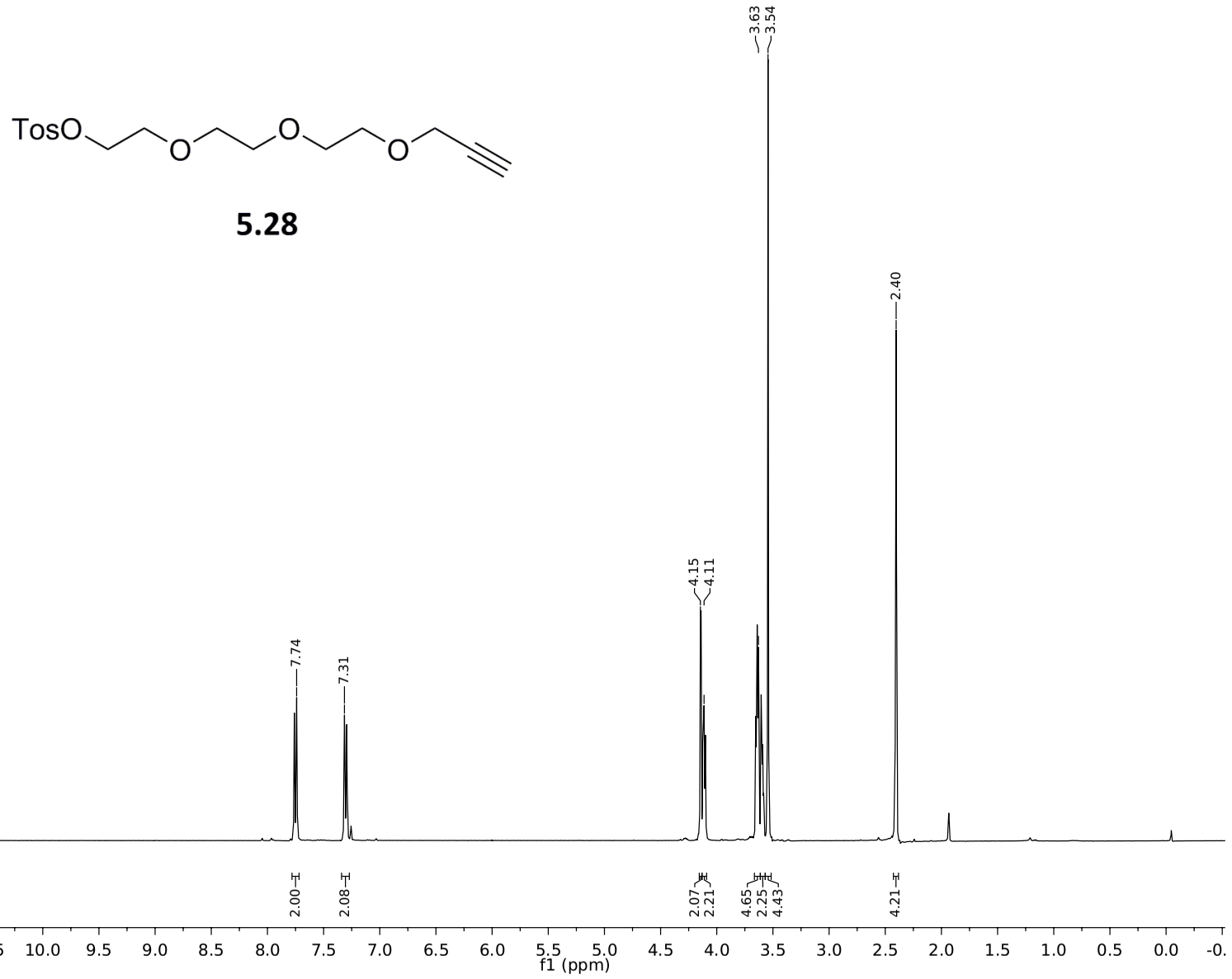
5.25

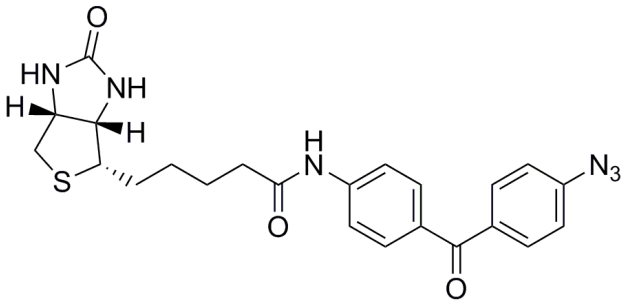
193.85
172.28
169.39
163.17
145.36
143.63
142.57
131.41
131.25
130.59
128.07
119.24
118.66
70.81
70.68
70.42
70.12
70.06
68.36
61.51
59.67
55.86
36.81
28.68
28.57
25.47
21.56
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


5.25
f1 (ppm)


5.27
198.46
177.07
167.90
158.06
154.25
150.67
148.90
144.11
142.38
136.32
135.97
134.06
130.44
128.99
126.82
124.77
110.93
110.01
104.73
100.60
75.30
75.06
74.96
74.38
74.24
72.76
68.59
66.23
65.48
64.40
60.59
53.79
50.88
41.55
33.40
33.29
30.17
29.89
16.41
f1 (ppm)

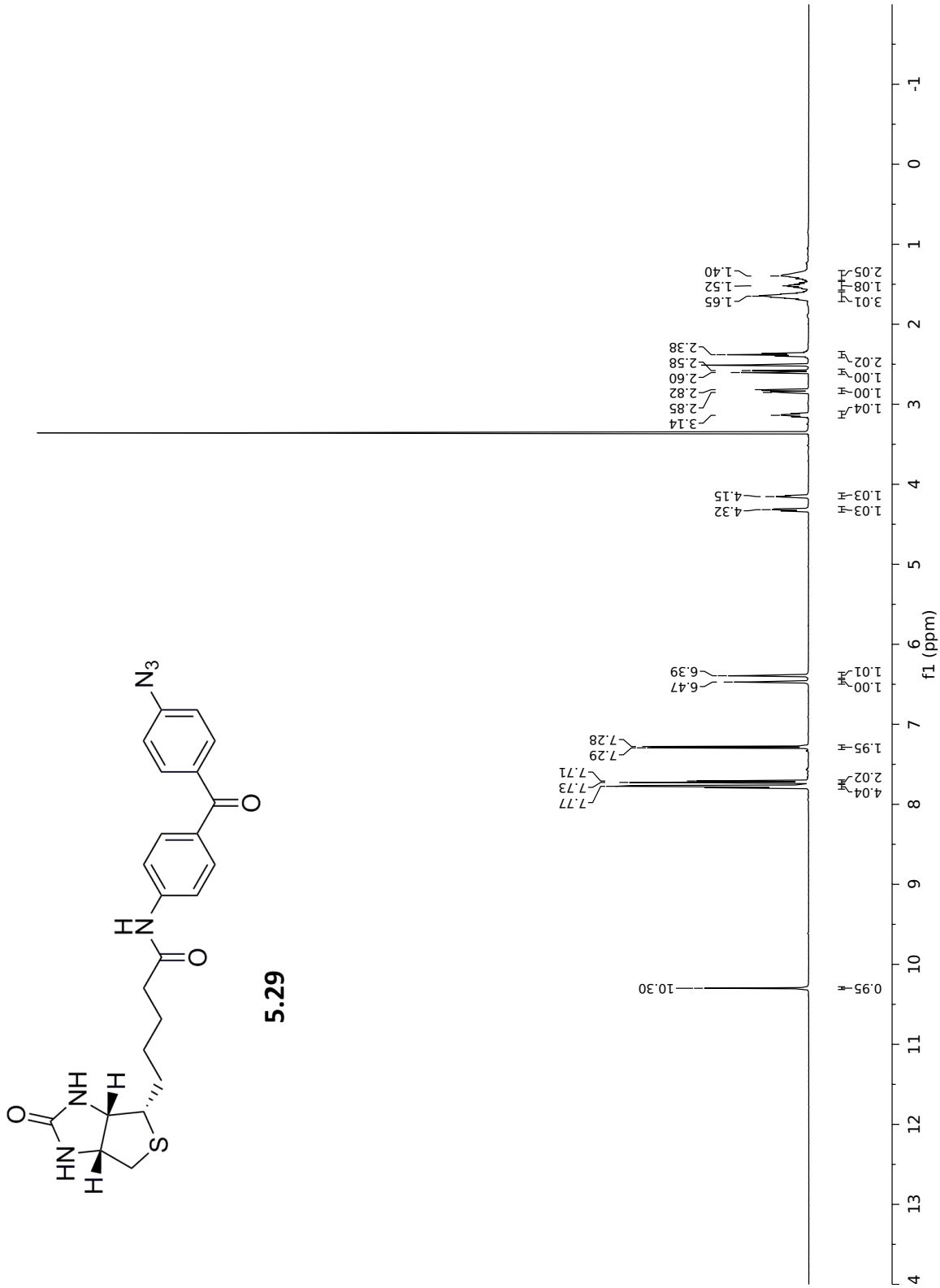
5.27
10.31
8.90
8.07
8.05
7.88
7.85
7.77
7.75
7.54
7.38
7.31
7.29
7.17
6.81
6.79
6.45
6.36
4.99
4.62
4.29
4.18
4.12
3.85
3.64
3.58
3.10
2.82
2.79
2.36
1.61
1.47
1.35
0.96
0.94
0.92
1.00
0.93
1.99
2.22
4.61
1.14
1.02
1.00
1.02
1.01
1.05
1.01
2.11
1.98
1.02
3.89
2.30
10.91
1.37
1.03
2.56
10.30
3.46
8.59
2.33
f1 (ppm)


TosO
O
O
O
5.28
3.63
3.54
2.40
4.15
4.11
7.74
7.31
2.00
2.08
2.07
2.21
4.65
2.25
4.43
4.21
f1 (ppm)

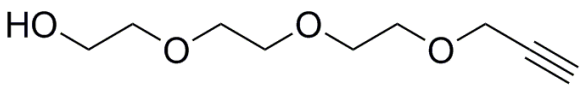

O
HN
NH
H
H
S
H
N
O
O
N3

5.29

193.64
172.29
163.15
143.95
143.83
134.42
132.02
131.59
131.45
119.55
118.65
61.46
59.63
55.83
36.77
28.65
28.53
25.42
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

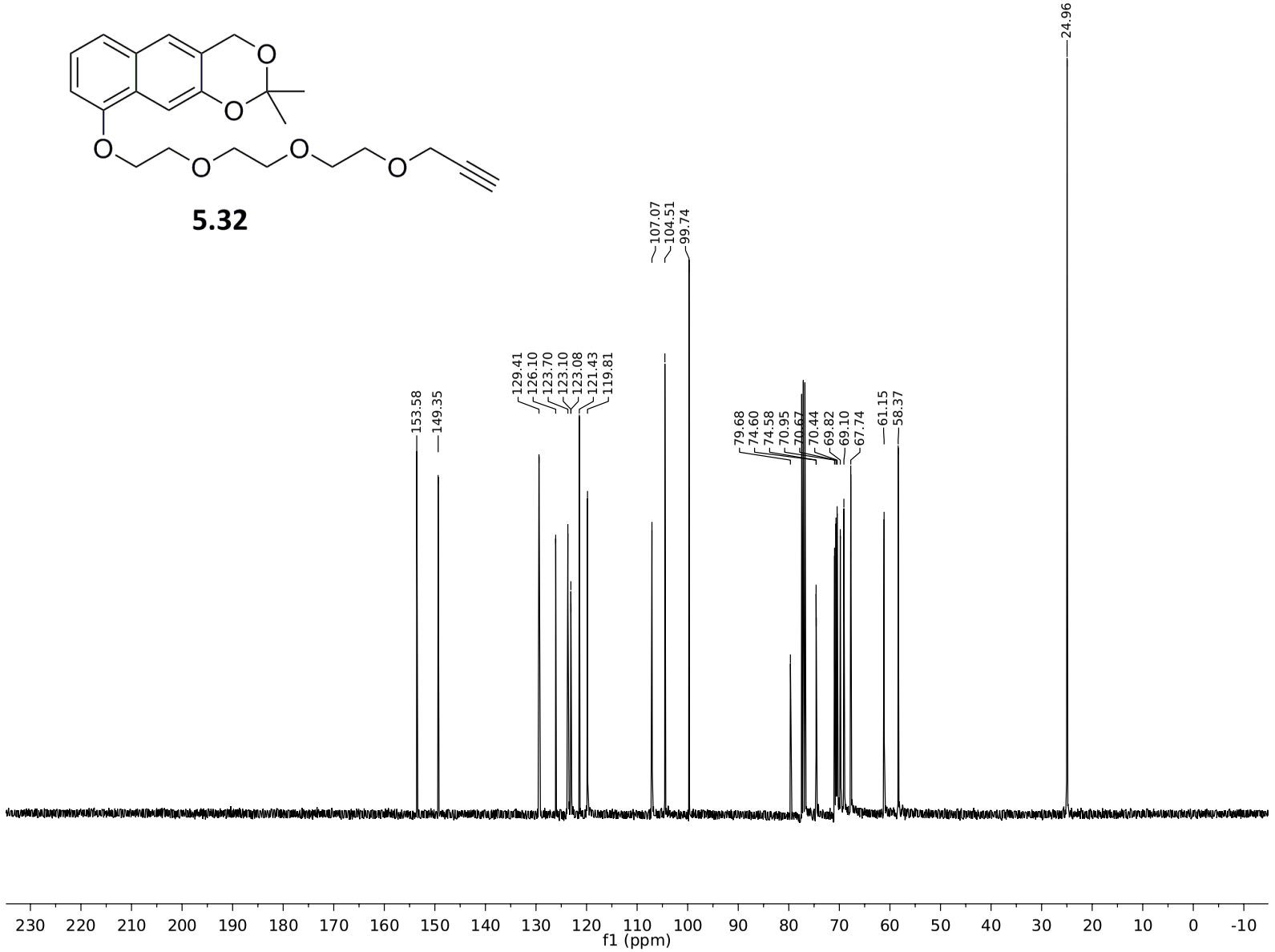

10.30
7.77
7.73
7.71
7.29
7.28
6.47
6.39
4.32
4.15
3.14
2.85
2.82
2.60
2.58
2.38
1.65
1.52
1.40
0.95
1.01
1.95
2.02
1.03
1.03
1.00
1.00
1.00
1.04
2.02
1.08
2.05
3.01
f1 (ppm)

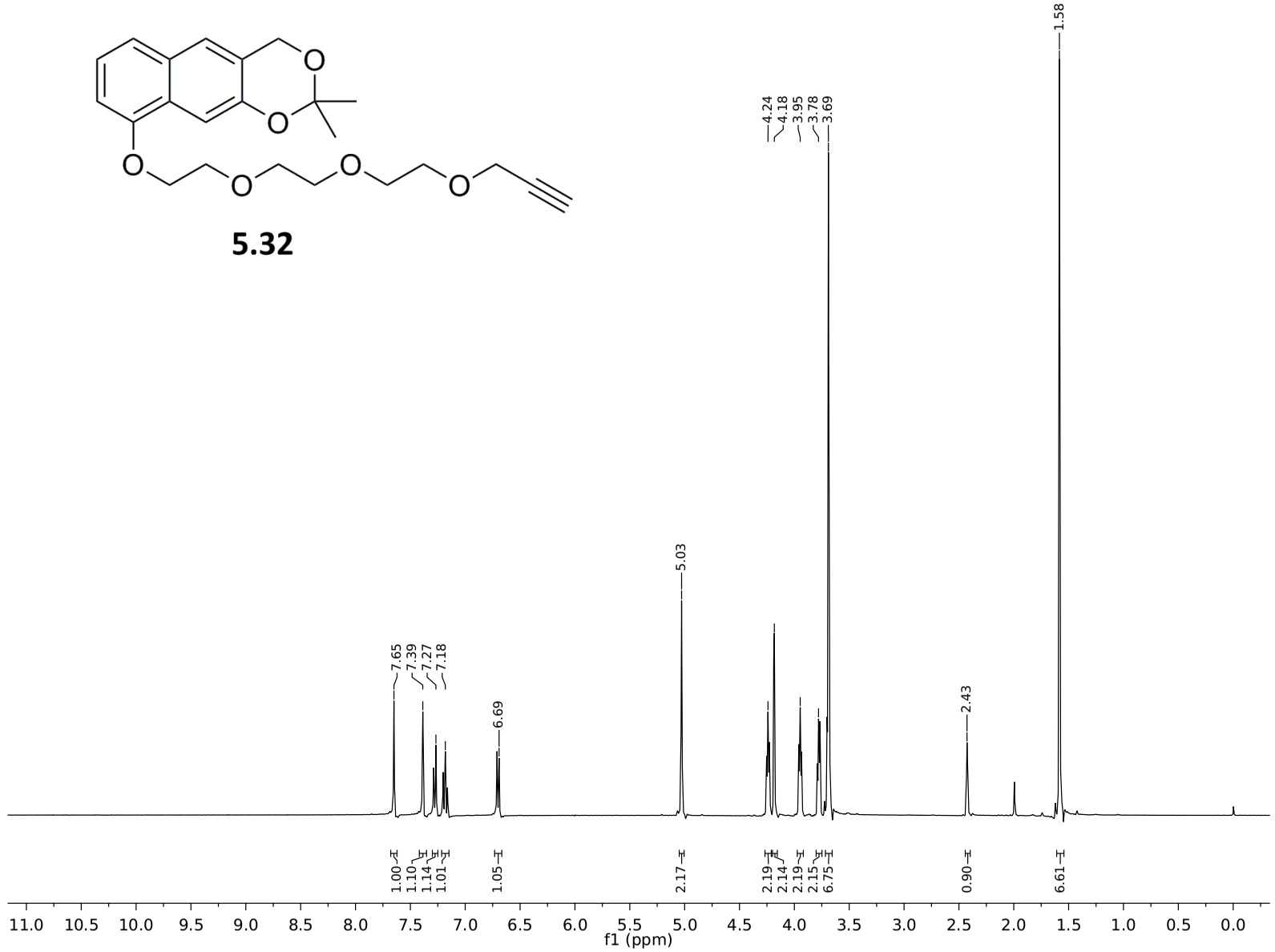
5.29


HO
O
O
O

5.31

3.62
3.59
3.52
4.12
4.12
3.18
2.88
2.80
2.72
2.40
2.05
10.45
2.34
1.00
0.90
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


5.32
153.58
149.35
129.41
126.10
123.70
123.10
123.08
121.43
119.81
107.07
104.51
99.74
79.68
74.60
74.58
70.95
70.67
70.44
69.82
69.10
67.74
61.15
58.37
24.96
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


5.32
7.65
7.39
7.27
7.18
6.69
5.03
4.24
4.18
3.95
3.78
3.69
2.43
1.58
1.00
1.10
1.14
1.01
1.05
2.17
2.19
2.14
2.19
2.15
6.75
0.90
6.61
f1 (ppm)