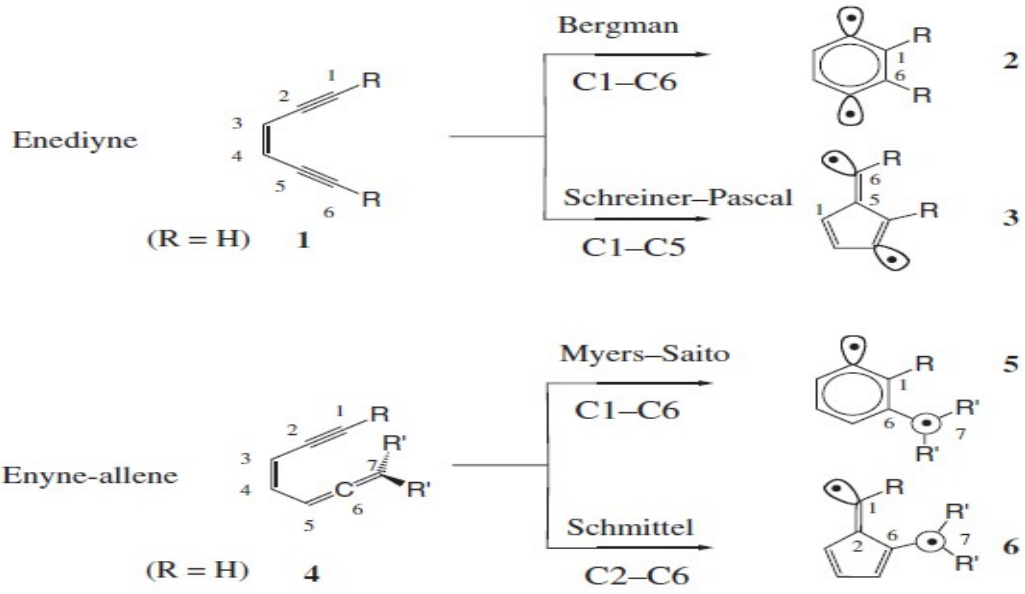
Bergman Cyclization Thalidomide Precursors

By: Robert B. Login rloginconsulting.com

I became interested in the Bergman Cyclization(BC) because it can produce diradical intermediates that are of interest to medicinal chemists. As I looked into the BC literature, I realized that because natural products obtained from microorganisms used the BC to kill by apopthosis, after damaging target cells DNA, then some of these structures seemed to be applicable, I envisioned to thalidomide and its analogs. Could thalidomide analogs be weaponized by BC, first as a transitory diradical warhead then as a thalidomide derivative? This then would be a double attack on cancer cells.

But let me start by reviewing BC and related chemistry for those who are unfamiliar with it.



Bergman, Schreiner–Pascal, Myers–Saito, and Schmittel reaction of enediynes and enyne-allenes.

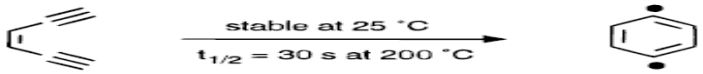
Kraka, E., & Cremer, D. (2014). Enediynes, enyne-allenes, their reactions, and beyond. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *4*(4), 285-324.

The BC is the most important to this proposal even though the Myers-Saito cyclization has been found in some warheads(diradicals) but will not be discussed. The diradicals shown above are reactive intermediates that will abstract protons. In the case of DNA these lost protons can cause cell death.

Can abstraction of protons from cancer cell survival proteins damage them and lead to apopthosis?

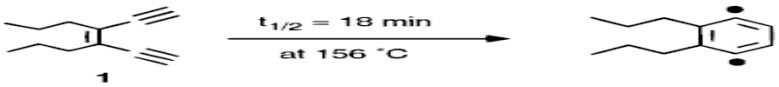
II. The Bergman Cyclization of Enediynes

In 1972, Bergman and Jones reported cycloaromatization of the parent (Z)-3-hexene-1,5-diyne to 1,4-didehydrobenzene biradical (eq 1).[1a] At 200 °C, the



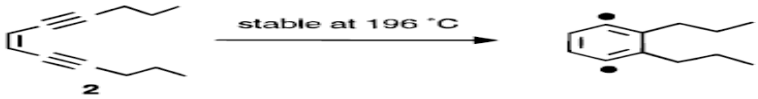
(1)

reaction was estimated to have a half-life of ~30 s. Since then a wealth of information concerning the effects of substitution patterns and structural features on the rate of cycloaromatization has become available.[8] A more careful measurement of the reaction rates of **1** allowed the determination of its activation energy (E_a) to be 27.4 ± 0.5 kcal/mol with a half-life of 18 min at 156 °C (eq 2).[1b,d] Placing



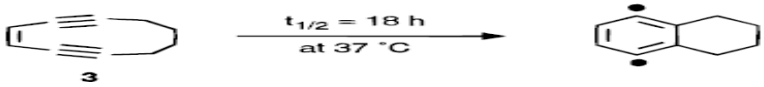
1 (2)

substituents at both ends of the enediyne system, such as **2**, reduces the rate of reaction, and **2** exhibits little propensity for cyclization at 196 °C (eq 3).[1b] By



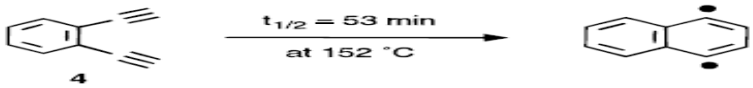
2 (3)

tethering the two acetylenes into a 10-membered ring as shown in **3**, the activation energy is greatly reduced, and cycloaromatization of **3** occurs at 37 °C with a half-life of 18 h (eq 4).[8a] Incorporation of the



3 (4)

central carbon-carbon double bond of acyclic enediynes into a benzene ring has a minimal effect on the rate of the Bergman cyclization (eq 5).[8m] Aro-



4 (5)

Wang, K. K. (1996). Cascade radical cyclizations via biradicals generated from enediynes, enyne-Allenes, and enyne-ketenes. *Chemical reviews*, *96*(1), 207-222.

Why the differences in reactivity?

Proximity Effect

"Nicolaou and co-workers[52,55] derived an empirical rule stating that the critical distance *R*(*C*1*C*6) between the two enediyne carbon atoms forming the new bond (see Figure 3) should be in the range of 3.31–3.20 A° for a spontaneous Bergman cyclization at body temperature (*proximity rule*)."

Note that the word spontaneous doesn't mean instantaneous but could be hours or days depending on structure. Not being an MD, biochemist or molecular biologist, I have no idea how long said enediyne drugs would take to reach target cancer cells but BC half lives of many hours or days seems like that would not be a problem and enough would reach the cancer cell? Would there be enough BC diradical warheads left to do the job and would said enediyne drugs not attack normal cells?

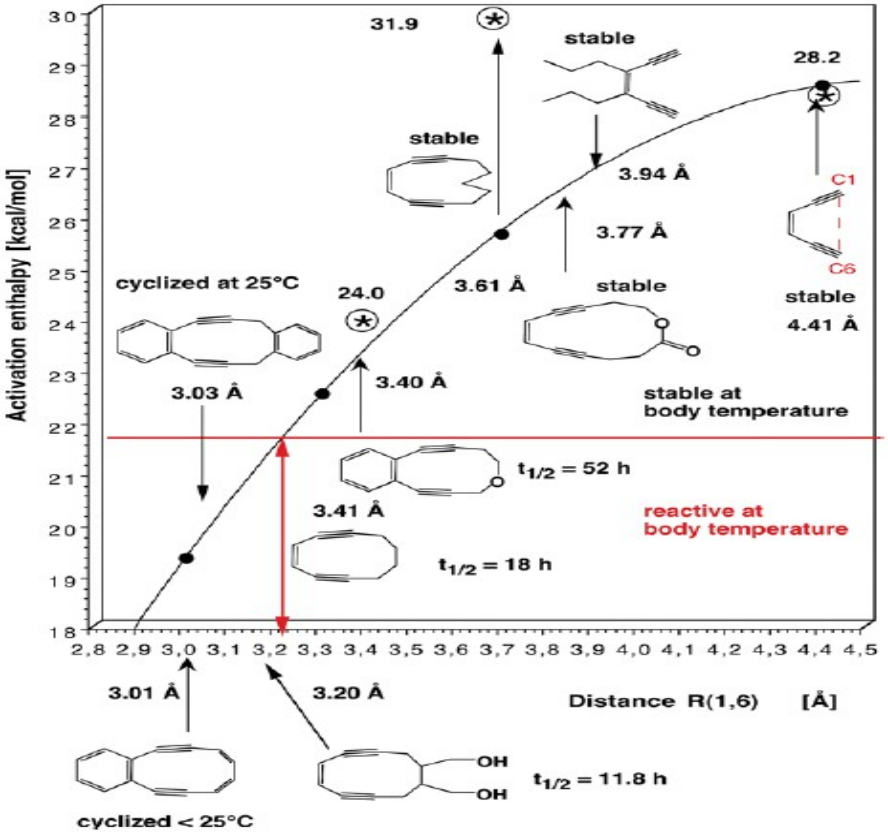
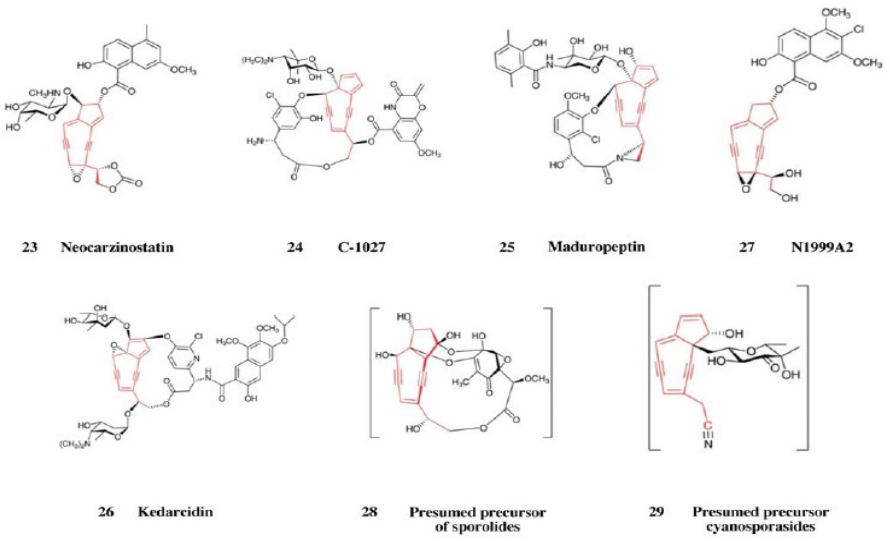
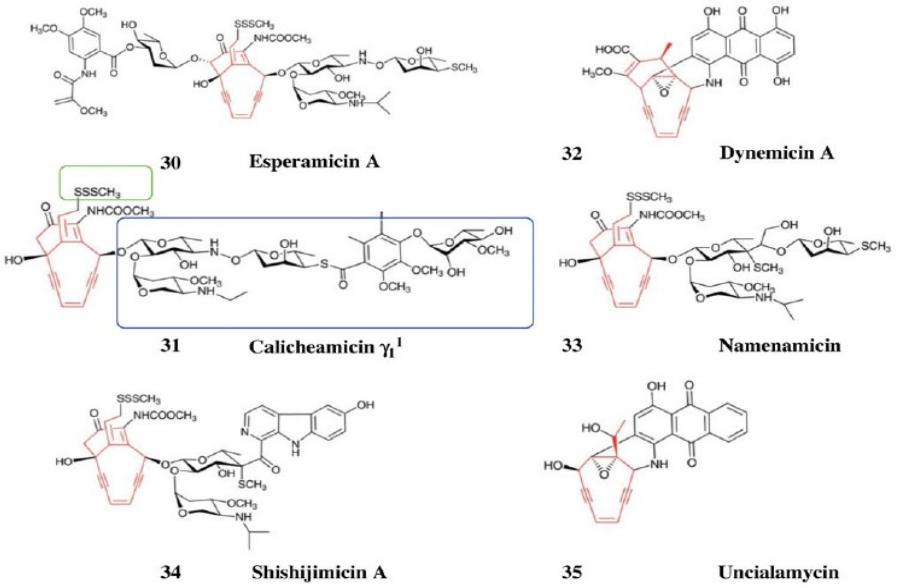


FIGURE 3 | Dependence of the activation enthalpy of the Bergman cyclization of (Z)-hex-3-ene-1,5-diyne on the critical distance between carbon atoms C1 and C6 according to *ab initio* calculations (black dots).[56] Known C1C6 distances, measured activation enthalpies (starred values), half-life times $t_{1/2}$, and cyclization temperatures are also given.[55] The deviations of the starred values indicate the limitations of the *ab initio* relationship. (Reproduced with permission from Ref 56. Copyright 1994, American Chemical Society.)

The natural enediynes produced by microorganisms contain a trip-wire(or trigger) structure that when tripped allow the BC reaction to take place. The structures are complex with segments designed to fit in the victims DNA where the warhead can do the most damage. The problem with the natural enediynes is that they were indiscriminate and destroyed normal cells. The following are typical versions.



(a) Nine-memberedenediynes.



(b) 10-Membered enediynes.

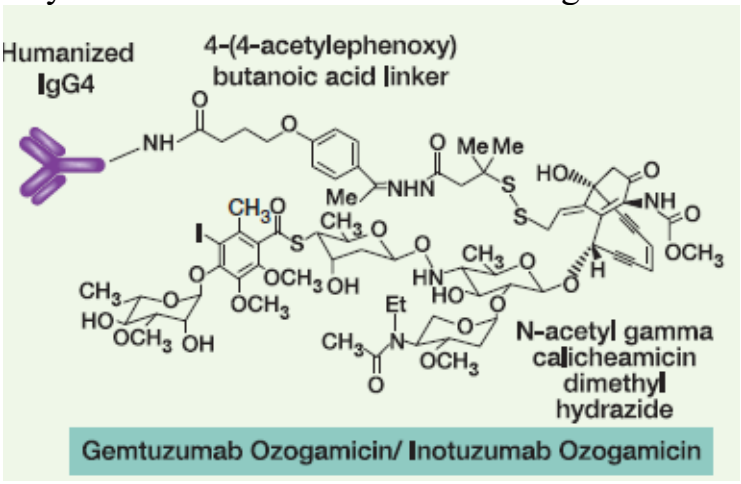
FIGURE 11 | Naturally occurring enediynes. For each enediyne, the warhead is given in red. For calicheamicin γ_1^1, the docking (blue box) and the triggering device (green box) are also indicated.

Kraka, E., & Cremer, D. (2014). Enediynes, enyne-allenes, their reactions, and beyond. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *4*(4), 285-324. All of the above illustrations are from this excellent reference. Another older ref. is;

Kar, M., & Basak, A. (2007). Design, synthesis, and biological activity of unnatural enediynes and related analogues equipped with pH-dependent or photo-triggering devices. *Chemical reviews*, *107*(7), 2861-2890. This one goes over most if not all of the synthetic enediynes up to that date.

The easiest triggers to visualize are the epoxides. Opening of the epoxides frees the enediyne to undergo BC by relieving ring strain. In fact ring strain relief seems to be the most popular trigger.

The enediyne chemistry has been used in medicinals to fight cancer.



Ricart, A. D. (2011). Antibody-drug conjugates of calicheamicin derivative: gemtuzumab ozogamicin and inotuzumab ozogamicin. *Clinical Cancer Research*, *17*(20), 6417-6427.

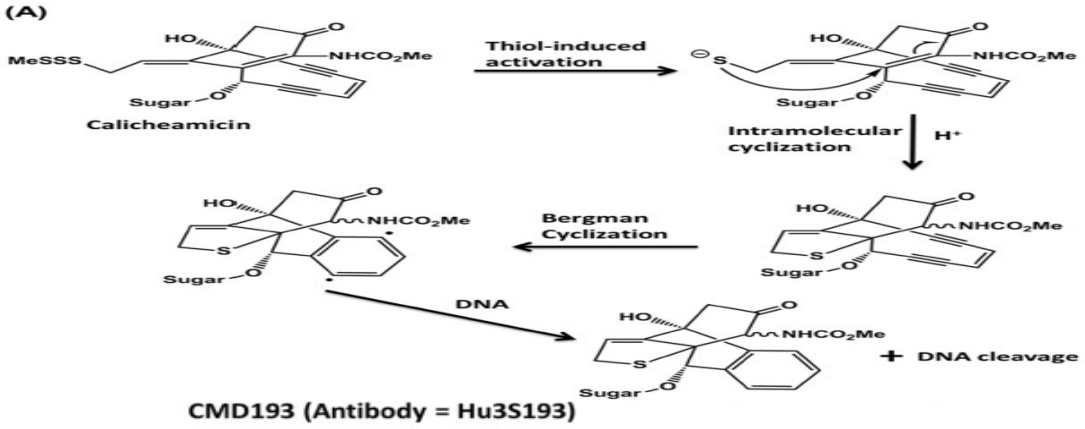
Some Toxicity problems with the above did emerge.

Lambert, J., Pautas, C., Terré, C., Raffoux, E., Turlure, P., Caillot, D., ... & Rubin, S. D. (2019). Gemtuzumab ozogamicin for de novo acute myeloid leukemia: final efficacy and safety updates from the open-label, phase III ALFA-0701 trial. *Haematologica*, *104*(1), 113-119.

The monoclonal antibody will conduct the enediyne to the cancer cell avoiding attack on healthy cells.

My question is if everything on the calicheamicin structure is necessary for its activity? The answer is apparently yes as the sugars etc are claimed to afford docking, to the targeted DNA.

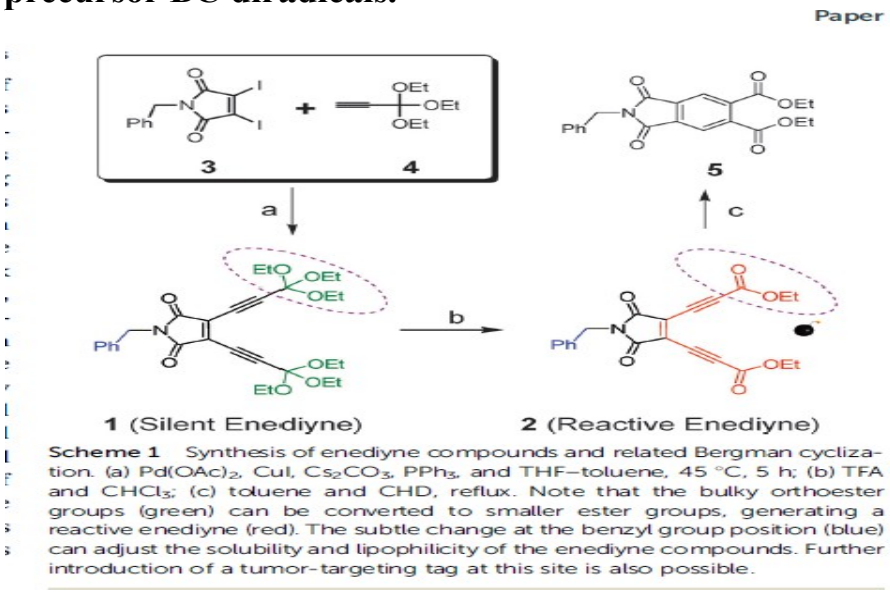
Mechanism:



"In brief, the methyl trisulfide undergoes reductive bond cleavage by intracellular reducing components (e.g. glutathione). After spontaneous cyclization and Bergman cycloaromatization, 1,4-dehydrobenzene diradicals are generated, which subsequently abstract hydrogen atoms fromDNA, resulting in a double-strand diradical. In the presence of oxygen, DNA double strands are cleaved, followed by cell death."

Fu, Y., & Ho, M. (2018). DNA damaging agent-based antibody-drug conjugates for cancer therapy. *Antibody therapeutics*, *1*(2), 43-53.

Thalidomide precursor BC diradicals.



Scheme 1 Synthesis of enediyne compounds and related Bergman cyclization. (a) $Pd(OAc)_2$, CuI, Cs_2CO_3, PPh_3, and THF–toluene, 45 °C, 5 h; (b) TFA and $CHCl_3$; (c) toluene and CHD, reflux. Note that the bulky orthoester groups (green) can be converted to smaller ester groups, generating a reactive enediyne (red). The subtle change at the benzyl group position (blue) can adjust the solubility and lipophilicity of the enediyne compounds. Further introduction of a tumor-targeting tag at this site is also possible.

Song, D., Sun, S., Tian, Y., Huang, S., Ding, Y., Yuan, Y., & Hu, A. (2015). Maleimide-based acyclic enediyne for efficient DNA-cleavage and tumor cell suppression. *Journal of Materials Chemistry B*, *3*(16), 3195-3200.

They claim that the reactive enediyne is formed at acidic cancer cell pH and undergoes the BC at body temperature. Replacing the above imide nitrogen derivative with glutarimide would produce a thalidomide analog derivative.

The same workers also recently proposed a drug-conjugate based on another imide enediyne. Apparently this imide enediyne is stable but does BC at body temperature.

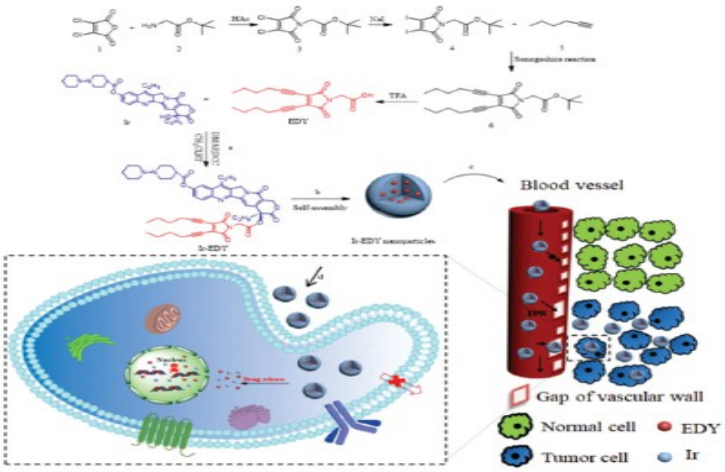
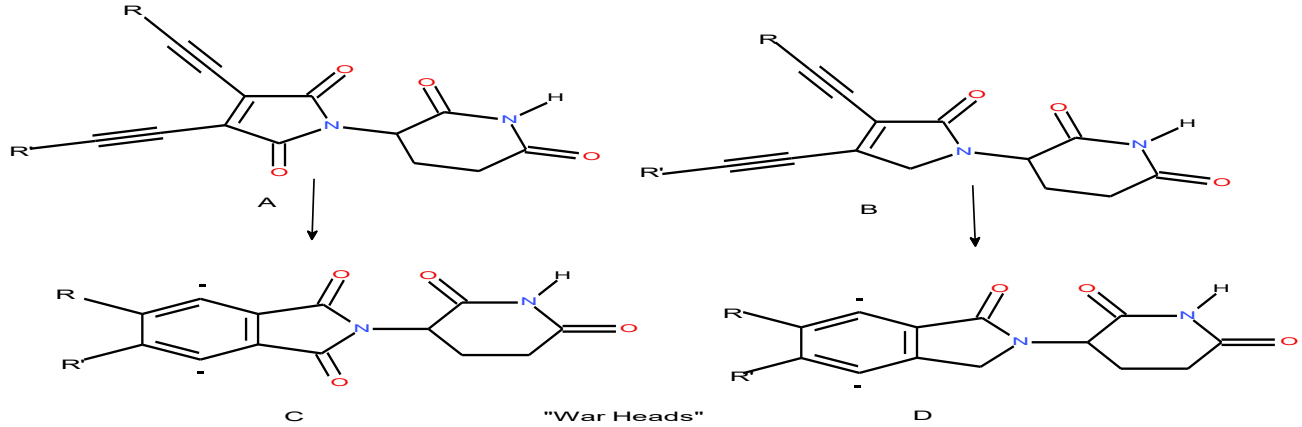


Fig. 1 Schematic illustration of amphiphilic drug–drug conjugate (ADDC) from synthesis, self-assembly and self-delivery. (a) Synthesis of **Ir–EDY** ADDC through esterification in the DCC/DMAP-catalyzed system. (b) **Ir–EDY** ADDC self-assembles into nanoparticles in water. (c) **Ir–EDY** ADDC nanoparticles access tumor tissues by means of their leaky vasculatures (EPR effect). (d) **Ir–EDY** ADDC nanoparticles enter tumor cells by endocytosis.

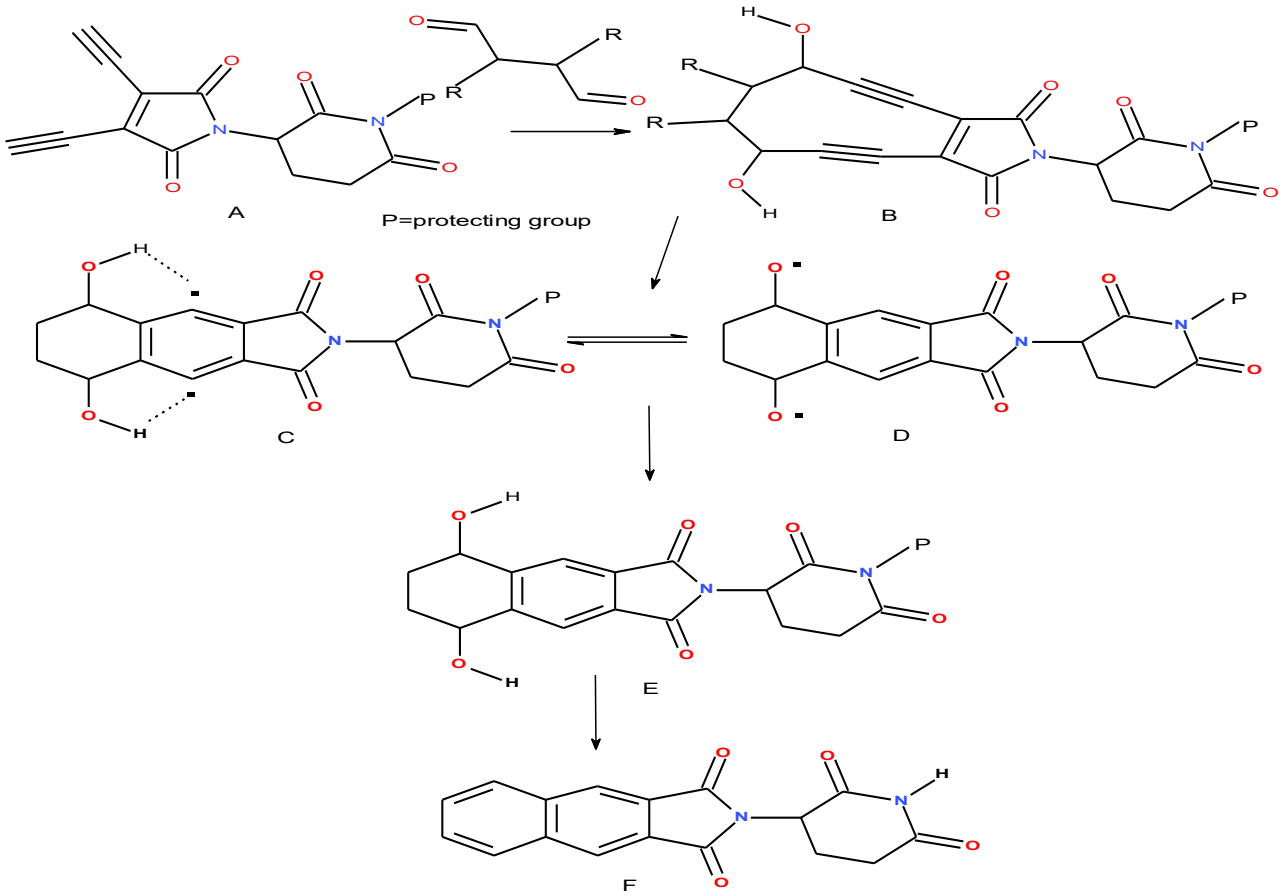
"After entering the tumor cell through endocytosis, the ester linkage in Ir–EDY NPs would break due to the acidic microenvironment and release the Ir and EDY respectively. The released free Ir and EDY further damage tumor cells synergistically through different mechanisms, and finally induce tumor cell death through the cell apoptosis pathway efficiently. The EDY based drug self-delivery nanoparticles are suggested to have potential application for chemotherapy in future cancer therapy"

Li, J., Li, B., Sun, L., Duan, B., Huang, S., Yuan, Y., ... & Hu, A. (2019). Self-delivery nanoparticles of an amphiphilic irinotecan–enediyne conjugate for cancer combination chemotherapy. *Journal of Materials Chemistry B*, *7*(1), 103-111.



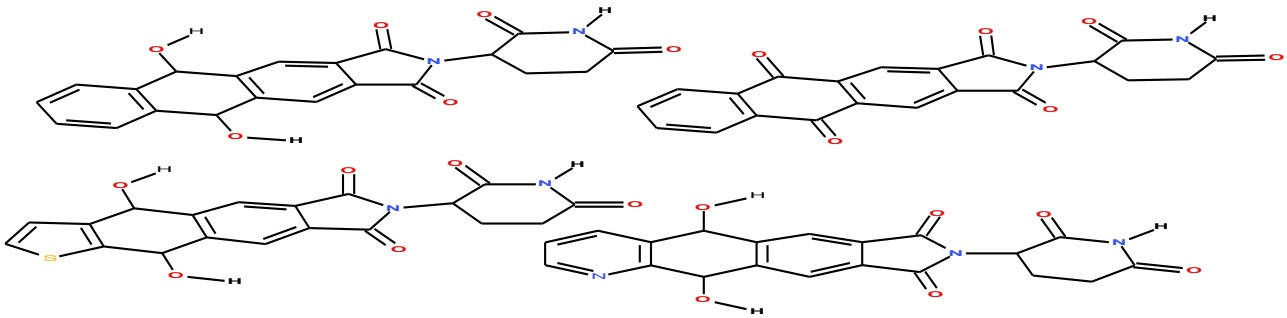
Scheme 1: It's not too far a reach to view Prof Hu's ideas directed to thalidomide analogs. Obviously, the R groups or group in this and other ideas could be linked to a mono-clonal antibody or PROTACs(proteolysis targeting chimeras) designed for example for multiple myeloma.

Prof. Hu's group has shown that these maleimide-based compounds will undergo the BC reaction at body temperature(37C) but can be handled at RT.

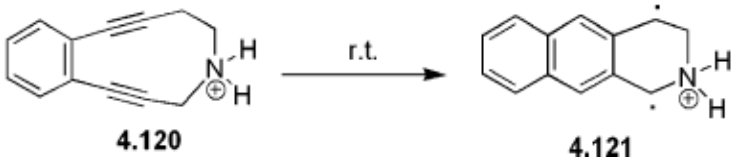


Scheme 2: My idea for a 10membered warhead precursor. The R groups are as needed for linkage to mono-clonal antibodies or PROTACs. This idea is lacking a trip-wire (or trigger) as the enediyne is ready to do the BC reaction(note that this BC might not proceed at RT but could occur at body temperature after insertion into a tumor cell). It was not obvious to me how to design a scheme 2 trip-wire that would work in a target cancer cell but it might not be necessary because the rate of BC might be slow but adequate at body temperature.

Kraka, E., & Cremer, D. (2000). Computer design of anticancer drugs. A new enediyne warhead. *Journal of the American Chemical Society*, *122*(34), 8245-8264.



Scheme 3: Possible modified derivatives from scheme 2 BC. These oxygen rich thalidomide analogs might exhibit greater affinity to target proteins because after BC they form electron rich thalidomide IMid analogs.

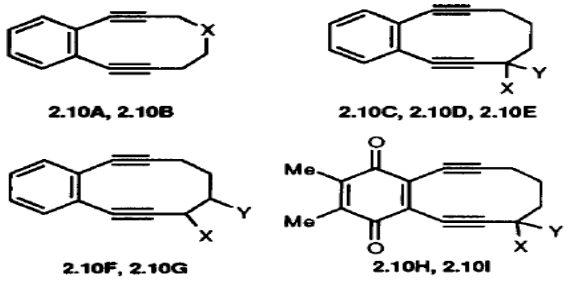


4.120 4.121

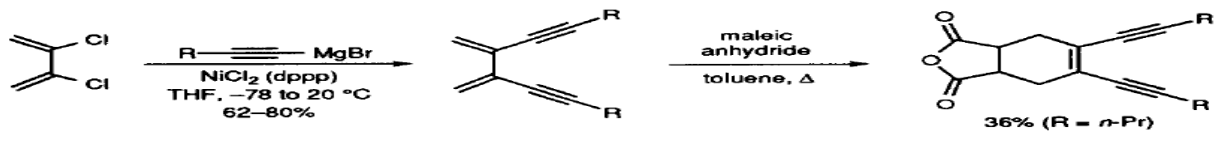
Kar, M., & Basak, A. (2007). Design, synthesis, and biological activity of unnatural enediynes and related analogues equipped with pH-dependent or photo-triggering devices. *Chemical reviews*, *107*(7), 2861-2890.

This reference suggests that the above BC takes 30 days at RT and might be triggered when the stable free amine is protonated in acidic cancer cells? Structure 4.120 can modified and visualized as a thalidomide analog.

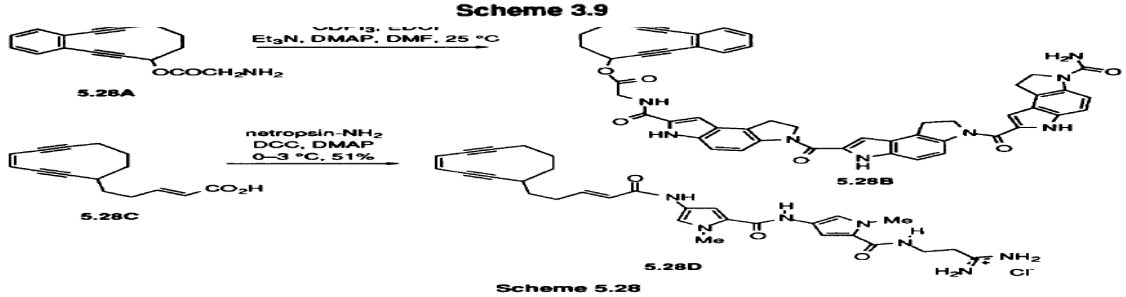
Nicolaou, K. C., Smith, A. L., & Yue, E. W. (1993). Chemistry and biology of natural and designed enediynes. *Proceedings of the National Academy of Sciences*, *90*(13), 5881-5888.



Cmpd	X	Y	$t_{1/2}$ (h)	T (°C)	Ref.
2.10A	CH_2	-	24	84	25b
2.10B	O	-	52	37	30
2.10C	H	H	24	84	25b
2.10D	H	OH	4.5	84	25b
2.10E	O		<1	84	25b
2.10F	OH	H	4.5	84	25b
2.10G	H	OH	14	84	25b
2.10H	H	OH	2	55	25b
2.10I	O		2	55	25b



Scheme 3.9



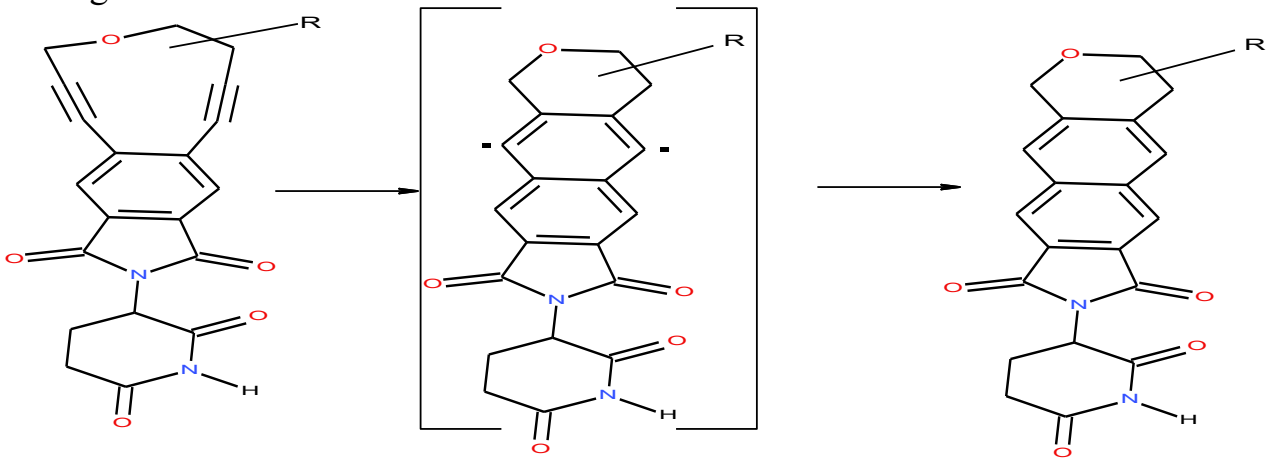
Scheme 5.28

The above are from the following excellent reference.

Grissom, J. W., Gunawardena, G. U., Klingberg, D., & Huang, D. (1996). The chemistry of enediynes, enyne allenes and related compounds. *Tetrahedron*, *52*(19), 6453-6518.

This reference shows what would now be called PROTACs (see above 528B&D). (See PROTACT definition below.)

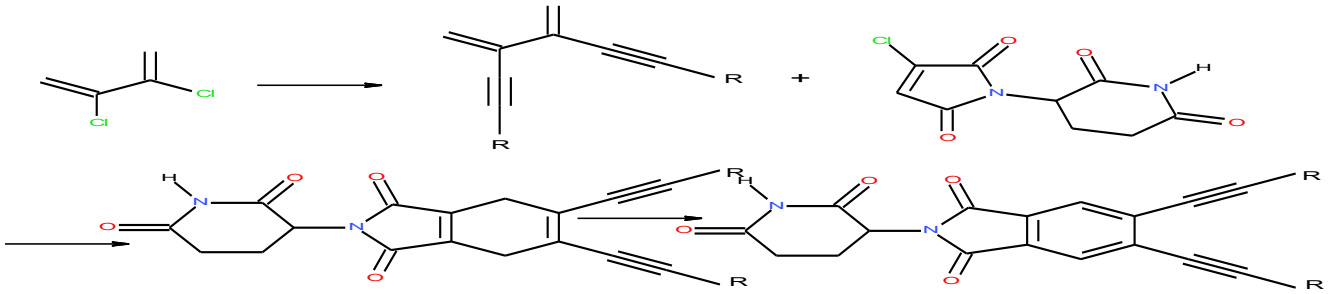
Compound 210B has a long half-life at body temperature suggesting that as a thalidomide analog it would be stable enough to reach its target with enough warhead to do damage to cancer cells.

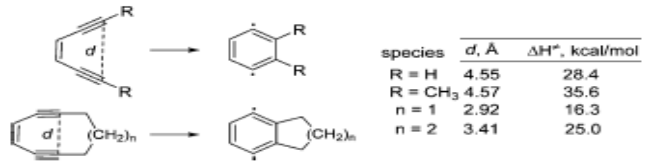


Scheme 4: R can be the rest of a PROTAC derivative etc.

Singh, R., & Just, G. (1990). The synthesis of a 10-membered benzo-oxadiyne ring. *Tetrahedron letters*, *31*(2), 185-188.

"Scheme 3.9" above, could be substituted with chloromaleic anhydride to produce a diene easily converted to the benzene derivative.





species	d, Å	$\Delta H^{\ddagger}$, kcal/mol
R = H	4.55	28.4
R = CH_3	4.57	35.6
n = 1	2.92	16.3
n = 2	3.41	25.0

Figure 15. The effect of strain-induced change in C_1-C_6 distances (Å) and the corresponding effect on $\Delta H^{\ddagger}$ (kcal/mol) of Bergman cyclization of cyclic and acylic enediynes calculated at BLYP/6-311+G**//BLYP/6-31G* level.

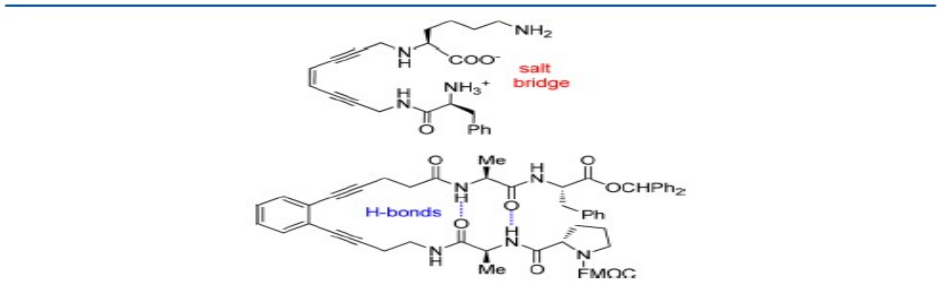


Figure 21. Supramolecular interactions manipulate the distance between the terminal carbons of enediyne moiety, facilitating cyclization.

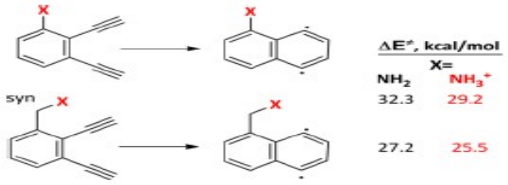
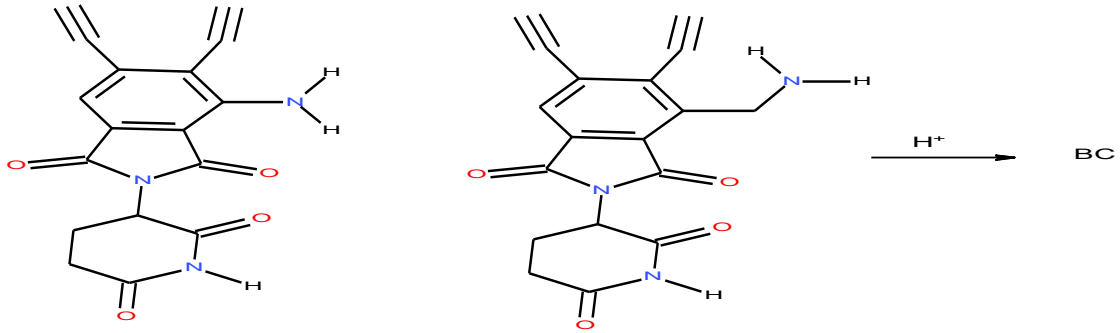


Figure 31. B3LYP activation energies for the Bergman cyclization of amino enediynes and their salts.

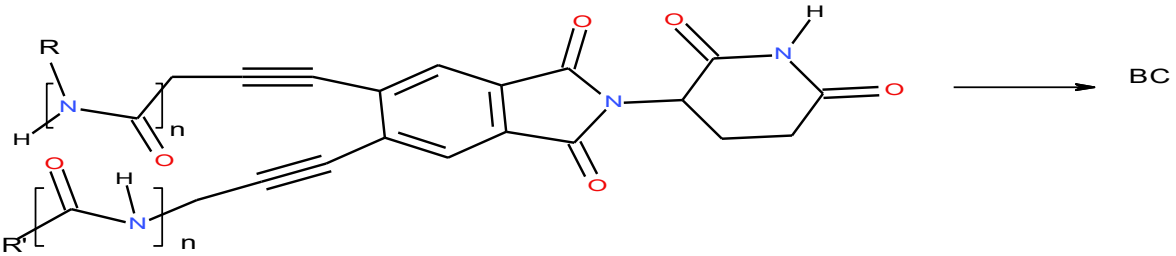
The above are from the following detailed accounting of BC reactions.

Mohamed, R. K., Peterson, P. W., & Alabugin, I. V. (2013). Concerted reactions that produce diradicals and zwitterions: Electronic, steric, conformational, and kinetic control of cycloaromatization processes. *Chemical reviews*, *113*(9), 7089-7129.

Could Figs. 21 and 31 above be incorporated into thalidomide analogs?



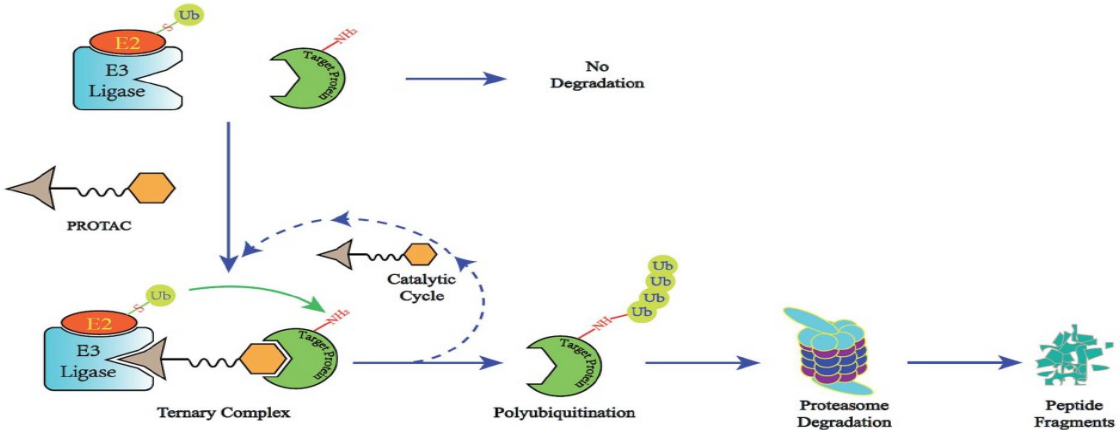
Scheme 5: Would the acid level and temperature in cancer cells be enough to do BC? Lenalidomide types are not shown but implied. The above is either or.



Scheme 6: H-bonding like fig. 21 above. Said H-bonding brings the terminal acetylenes together encouraging BC.

PROTACs

"Selective degradation of proteins by proteolysis targeting chimeras (PROTACs) offers a promising potential alternative to protein inhibition for therapeutic intervention. Current PROTAC molecules incorporate a ligand for the target protein, a linker, and an E3 ubiquitin ligase recruiting group, which bring together target protein and ubiquitinating machinery."
Lebraud, H., Wright, D. J., Johnson, C. N., & Heightman, T. D. (2016). Protein degradation by in-cell self-assembly of proteolysis targeting chimeras. *ACS central science*, *2*(12), 927-934.



Pei, H., Peng, Y., Zhao, Q., & Chen, Y. (2019). Small molecule PROTACs: an emerging technology for targeted therapy in drug discovery. *RSC Advances*, *9*(30), 16967-16976.

"Structural studies have shown that cereblon modulators bind to cereblon through the glutarimide moiety, which docks into a hydrophobic tritryptophan pocket formed by Trp380, Trp386, and Trp400.9 This binding mode leaves the insoindolinone/ phthalimide ring of the compounds exposed on the cereblon surface, and it was predicted that this would form a hotspot of unsatisfied hydrogen bonds that would mediate substrate binding via direct protein−protein interactions. Subsequent structural and mutagenesis studies on cereblon−substrate complexes have confirmed this hypothesis and surprisingly revealed that unrelated proteins CK1a and GSPT1 bind to cereblon through a common structural feature, thereby defining a degron. The enhancement of protein−protein interactions by a small molecule is highly reminiscent of the "molecular glue" mechanism described for the plant hormones auxin and jasmonate. This mechanism contrasts with the linker-based approaches, where heterobifunctional ligands recruit substrates to a ligase via distinct small molecule binding events on each side of a linker."
Matyskiela, M. E., Zhang, W., Man, H. W., Muller, G., Khambatta, G., Baculi, F., ... & Lu, C. C. (2017). A cereblon modulator (CC-220) with improved degradation of Ikaros and Aiolos. *Journal of medicinal chemistry*, *61*(2), 535-542.

These Celgene workers should know how their thalidomide analogs attach to cereblon. I

take this to mean that my thalidomide BC precursors would also bond and then attack in two ways. First by biradical attack on the close or attached protein target and then by thalidomide “glue” by the ubiquitin–proteasome system.

Since the problem with BC type warheads was their indiscriminate attack on normal cells, attaching the warhead to a mono-clonal antibody that would deliver it to the cancer cell where because of acidity(scheme 5) the BC would be initiated killing the cancer cell by two mechanisms, diradical DNA cleavage or protein damage and then thalidomide UPS “glue”.

Another possible criticism is that my proposals would result in very little BC eventually in the target cancer cell, but the natural enediyne types work at the pico-mole concentration, and hence very little diradicals would apparently do the job. Hence even a very small amount of the docked diradical would fatally damage targeted proteins with the ubiquitin–proteasome system eventually killing the cancer cell.

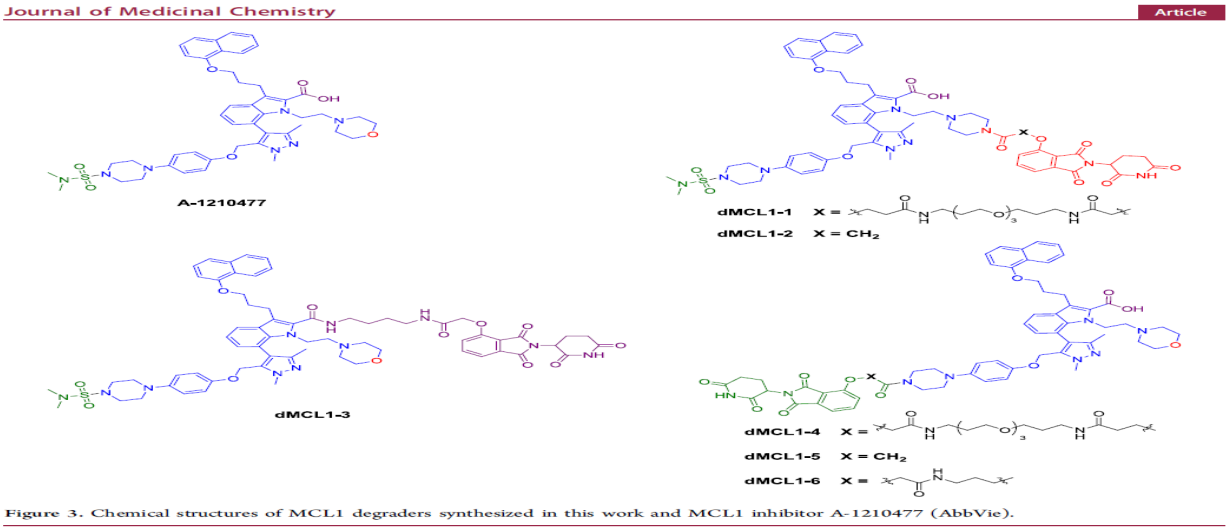


Figure 3. Chemical structures of MCL1 degraders synthesized in this work and MCL1 inhibitor A-1210477 (AbbVie).

“Myeloid cell leukemia 1 (MCL1) is a prosurvival protein over expressed in a variety of different cancers and is of tremendous therapeutic interest.[1,2] MCL1 is involved in complex protein−protein interactions (PPIs) involving proapoptotic factors Bim, Bak, and Bax.[3] These antiapoptotic interactions prevent the activation of caspase cascades, promoting cell survival. Due to this antiapoptotic nature, MCL1 has been recognized as a vital survival factor in human cancers, such as lymphoma, leukemia, breast cancer, and multiple myeloma (MM), wherein levels of MCL1 directly correlate to disease progression.[4] The ability of MCL1 to silence apoptotic pathways allows it to circumvent the typical clearance mechanisms of cells and is therefore often over expressed by tumor cells to gain a survival advantage.”

Papatzimas, J. W., Gorobets, E., Maity, R., Muniyat, M. I., MacCallum, J. L., Neri, P., ... & Derksen, D. J. (2019). From Inhibition to Degradation: Targeting the Anti-apoptotic Protein Myeloid Cell Leukemia 1 (MCL1). *Journal of*

medicinal chemistry.

Although I'm repeating my idea, it is the basis of this proposal.
I understand that each of the above PROTAC's job is to attach the thalidomide derivative to the cereblon(E3 ligase CUL4A-DDB1 cereblon (CRBN) ubiquitination pathway), where the tethered MCL1 protein because of its proximetry can be distroyed. My idea to weaponize the thalidomide derivative to also damage this MCL1 enough to result in ubiquitination by two routes. The BC diradical could attack the MCL1 and then be converted to the PROTAC's thalidomide derivative.

I also thought that cereblon might also be damaged by BC diradicals and enter the UPS. This would be a problematic result as thalidomide analogs depend on the availability of cereblon to work. Apparently loss of cereblon in multiple myeloma cancer cells is a mechanism for these cells to defeat thalidomide and its analogs. However, other references illustrate degrading cereblon and other UPS types as a cancer treatment.

Neri, P., Maity, R., Keats, J. J., Tagoug, I., Simms, J., Auclair, D., ... & Bahlis, N. J. (2016). Cereblon splicing of exon 10 mediates IMiDs resistance in multiple myeloma: clinical validation in the CoMMpass trial. *Blood*, *128*(22), 120-120.

Zhu, Y. X., Shi, C. X., Bruins, L. A., Wang, X., Riggs, D. L., Porter, B., ... & Stewart, A. K. (2019). Identification of lenalidomide resistance pathways in myeloma and targeted resensitization using cereblon replacement, inhibition of STAT3 or targeting of IRF4.*Blood cancer journal*, *9*(2), 19.

Franssen, L. E., Nijhof, I. S., Couto, S., Levin, M. D., Bos, G. M., Broijl, A., ... & Bloem, A. C. (2018). Cereblon loss and up-regulation of c-Myc are associated with lenalidomide resistance in multiple myeloma patients. *Haematologica*, *103*(8), e368-e371.

On the other hand, there is value in eliminating cereblons.
"We describe Homo-PROTACs as an approach to dimerize an E3 ligase to trigger its suicide-type chemical knockdown inside cells."

Maniaci, C., Hughes, S. J., Testa, A., Chen, W., Lamont, D. J., Rocha, S., ... & Ciulli, A. (2017). Homo-PROTACs: bivalent small-molecule dimerizers of the VHL E3 ubiquitin ligase to induce self-degradation. *Nature communications*, *8*(1), 830.

"We hypothesized that the E3 ligases themselves might be hijacked against one another using a PROTAC approach, thus inducing E3 ligase degradation as opposed to E3 blockade. In 2017, we disclosed the first report of a small molecule dimerizer of an E3 ligase as a means to induce its own degradation, an approach that we called "homoPROTAC". We designed bifunctional molecules made up of the same ligand for the ubiquitously expressed VHL protein, connected via a linker, that would induce VHL dimerization as the key step to trigger VHL ubiquitination and subsequent degradation."

Girardini, M., Maniaci, C., Hughes, S. J., Testa, A., & Ciulli, A. (2019). Cereblon versus VHL: Hijacking E3 ligases against each other using PROTACs. *Bioorganic & medicinal chemistry*, *27*(12), 2466-2479.

Wang, Y., Jiang, X., Feng, F., Liu, W., & Sun, H. (2019). Degradation of protein by PROTAC and other strategies. *Acta Pharmaceutica Sinica B*.

Thank you for reading these proposals!
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