ORGANIC LETTERS

xxxx
Vol. 0, No. 0
A−D

Photochemical and Thermal Bergman Cyclization of a Pyrimidine Enediynol and Enediynone

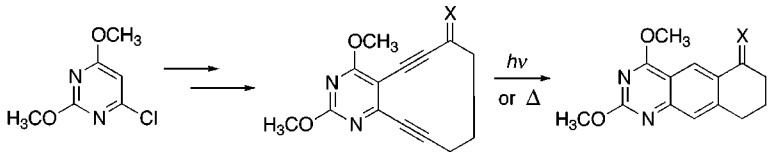
Nakyen Choy,[†] Beatriz Blanco,[†] Jenghai Wen,[‡] Awtar Krishan,[‡] and K. C. Russell*,[†]

Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33124, and Department Of Radiation Oncology (R-71), University of Miami, P.O. Box 016960, Miami, Florida 33101

krussell@umiami.ir.miami.edu

Received May 15, 2000 *(Revised Manuscript Received October 20, 2000)*

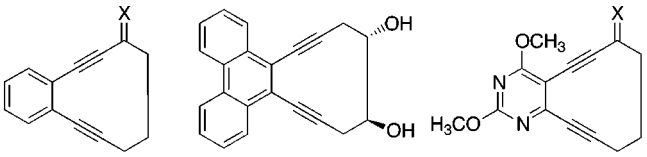
ABSTRACT



X = O or H, OH

Novel 10-membered pyrimidine enediynes (3 and 4) were synthesized in seven and eight steps, respectively. These compounds were compared for their abilities to undergo Bergman cyclization both thermally and photochemically. Alcohol 3 readily cyclized both thermally and photochemically in ^{i}PrOH, while ketone 4 only showed efficient thermal cyclization. Both compounds were also shown to cleave dsDNA under the appropriate conditions.

Since the natural enediyne anticancer antibiotics[1] were first reported, designed enediynes have been synthesized and studied as DNA cleaving agents on the basis of the Bergman cyclization.[2] Synthetic enediynes have a distinct advantage over the naturally occurring products as therapeutic agents because they may show reduced toxicity.[3] The parameters for thermal enediyne activation by modification of 10-membered rings[4] such as **1** have been well studied. Introduction of an alcohol (**1c**) adjacent to the triple bond tends to accelerate reactivity relative to that of **1b**. The ketone (**1d**) further activates cyclization by increasing the ring strain from



1a (X = H, CDPI$_3$)
1b (X = H$_2$)
1c (X = H, OH)
1d (X = O)

2

3 (X = H, OH)
4 (X = O)

the change in hybridization. It is also likely there is an electronic effect from the ketone, decreasing the electron density available to the enediyne. However, there are relatively few examples of photochemical Bergman cyclizations[5] (e.g., **2**[5c]). Despite the potential of the photo-Bergman cyclization in photodynamic therapy, little has been done to

* Phone (305)284-6856. Fax (305)284-1880.
[†] Department of Chemistry.
[‡] Department of Radiation Oncology.

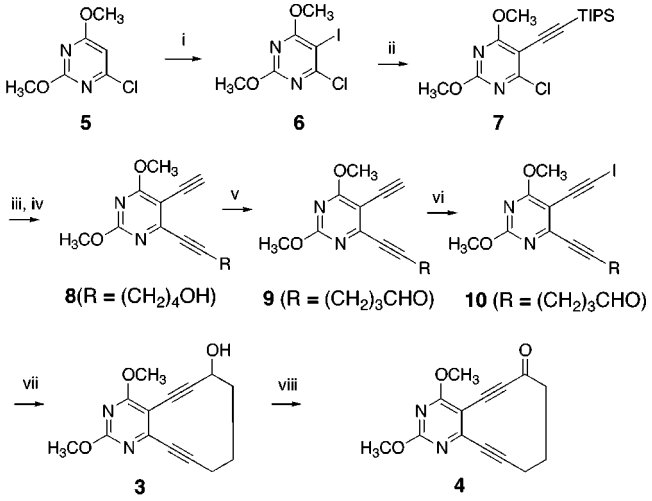
(1) (a) Long, B. H.; Golik, J.; Forenza, S.; Ward, B.; Rehfuss, R.; Dabrowiak, J. C.; Catino, J. J.; Musial, S. T.; Brookshire, K. W.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2. (b) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387.
(2) (a) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25. (b) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660.
(3) Nicolaou, K. C.; Dai, W. M.; Tsay, S. C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172.
(4) (a) Nicolaou, K. C.; Liu, A.; Zeng, Z.; McComb, S. *J. Am. Chem. Soc.* **1992**, *114*, 9279. (b) Boger, D. L.; Zhou, J. *J. Org. Chem.* **1993**, *58*, 3018. (c) Semmelhack, M. F.; Gallagher, J. J.; Ding, W.-d.; Krishnamurthy, G.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4357. (d) Dai, W. M.; Fong, K. C.; Lau, C. W.; Zhou, L.; Hamaguchi, W.; Nishimoto, S. I. *J. Org. Chem.* **1999**, *64*, 682.

10.1021/ol006061j CCC: $19.00
Published on Web 00/00/0000

explore how substitution on the 10-membered ring affects photoinduced cyclization. Our group has been interested in the examination of enediyne compounds where the double bond of the enediyne has been incorporated into biologically relevant heterocycles.[6] In this Letter we present the difference in the reactivity of pyrimidine enediynol **3** and enediynone **4** toward photochemical Bergman cyclization and contrast that with results from thermal cyclization under similar conditions. In addition, the DNA cleavage abilities of these new enediynes under thermal and photochemical conditions are reported.

The syntheses of enediynes **3** and **4** are depicted in Scheme 1. Compound **6** was prepared according to our previous

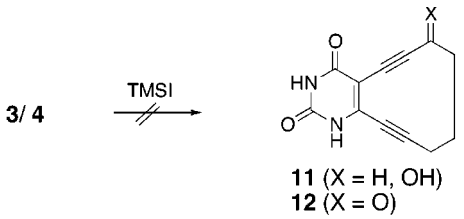
Scheme 1[a]



[a] Reagents: i, $(CF_3CO)_2O-CF_3CO_2H$ (1:4), NIS (1.5 equiv), reflux, 10 h, 95%; ii, TIPS−C≡C−H (1.2 equiv), $Pd(PPh_3)_4$ (10% w/w), CuI (1 equiv), $^iPr_2NH$−THF (1:1), 70 °C, 10 h, 85%; iii, H−C≡C$(CH_2)_4$OH, $Pd(PPh_3)_4$, $^iPr_2NH$, reflux, 15 h; iv, TBAF, THF, 0 °C, 0.5 h, 65% (two steps); v, PCC (3 equiv), CH_2Cl_2, rt, 2 h, 93%; vi, NIS (1.2 equiv), $AgNO_3$ (0.3 equiv), acetone, rt, 1 h, 91%; vii, $CrCl_2$ (10 equiv), $NiCl_2$ (0.1 equiv), THF, rt, 65%; viii, MnO_2 (10 equiv), CH_2Cl_2, rt, 5 h, 86%.

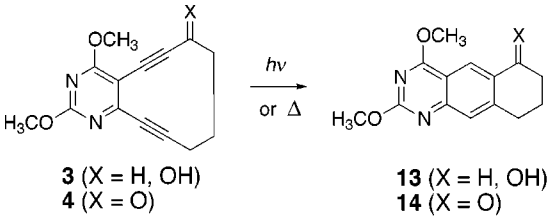
work.[6e] Coupling **6** with TIPS-protected acetylene using CuI and $Pd(PPh_3)_4$ as a catalyst in a mixture of diisopropylamine−THF at 70 °C under N_2 gave monocoupled product **7** and a trace of the dicoupled product (0.4% by GC). This crude product was coupled with 5-hexyn-1-ol using catalytic $Pd(PPh_3)_4$ in diisopropylamine followed by removal of the silyl group with tetrabutylammonium fluoride, affording **8** in good yield. The corresponding aldehyde **9** was prepared by PCC oxidation. Iodination of the terminal acetylene using NIS and silver nitrate[7] gave **10** in excellent yield. Iodination of **8** or **9** using I_2 and a base (morpholine,[4a] DBU,[8a] or DMAP[8b]) provided the product in low yield (10−20%). The key ring closure of **10** was carried out with a $CrCl_2$−$NiCl_2$ system.[9] Oxidation of **3**[10] with MnO_2 was used to prepare **4**.[11] Several unsuccessful attempts were also made to prepare pyrimidinone analogues **11** and **12** by TMSI cleavage of the methyl lactim ethers (Scheme 2).[6b]

Scheme 2



Alcohol **3** underwent both thermal and photochemical cyclization in isopropyl alcohol as a solvent as well as radical acceptor in excellent yield (Table 1, entries 1 and 2). Under

Table 1. Thermal and Photochemical Bergman Cyclization of **3** and **4**



entry	compd	solvent (time, h)	[concn] of **3** or **4**	temp, condn	**13**/**14** yield (%)
1	**3**	^{i}PrOH (10)	0.020	reflux, Δ	93[a]
2	**3**	^{i}PrOH (24)	0.001	40 °C, *hν*[c]	82[a]
3	**3**	^{i}PrOH (36)	0.001	40 °C, Δ	trace[b]
4	**3**	^{i}PrOH (2)	0.005	40 °C, *hν*[d]	(4[e])83[f]
5	**4**	^{i}PrOH (10)	0.020	80 °C, Δ	92[a]
6	**4**	^{i}PrOH (2)	0.005	40 °C, *hν*[d]	(34[e])10[f]

[a] Isolation yield. [b] By ^{1}H NMR [c] Filter solution, $[K_2CrO_4] = 0.01$ M. [d] Filter solution, $[K_2CrO_4] = 0.001$ M. [e] Remaining starting material. [f] By HPLC with internal standard, xylene.

photochemical conditions[12] using a filter solution (aqueous K_2CrO_4), **3** cyclized in good yield at 40 °C (entry 2), yet it cyclized extremely slowly thermally at the same temperature (entry 3). Under photochemical conditions at 40 °C, the half-

(5) (a) Kaneko, T.; Takahashi, M.; Hirama, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 1267. (b) Evenzahav, A.; Turro, N. J. *J. Am. Chem. Soc.* **1998**, *120*, 1835. (c) Funk, R. L.; Young, E. R. R.; Williams, R. M.; Flanagan, M. F.; Cecil, T. L. *J. Am. Chem. Soc.* **1996**, *118*, 3291. (d) Turro, N. J.; Evenzahav, A.; Nicolaou, K. C. *Tetrahedron Lett.* **1994**, *35*, 8089.

(6) (a) Choy, N.; Russell, K. C.; Alvarez, J. C.; Fider, A. *Tetrahedron Lett.* **2000**, *41*, 1515. (b) Kim, C. S.; Diez, C.; Russell, K. C. *Chem. Eur. J.* **2000**, 6, 1555. (c) Choy, N.; Russell, K. C. *Heterocycles* **1999**, *51*, 13. (d) Kim, C. S.; Russell, K. C. *Tetrahedron Lett.* **1999**, *40*, 3835. (e) Kim, C. S.; Russell, K. C. *J. Org. Chem.* **1998**, *63*, 8229.

(7) Elliott, M. R.; Dhimane, A.-L.; Malacria, M. *J. Am. Chem. Soc.* **1997**, *119*, 3427.

(8) (a) Rodriguez, G.; Rodriguez, D.; Lopez, M.; Castedo, L.; Dominguez, D.; Saa, C. *Synlett* **1998**, 1282. (b) Semmelhack, M. F.; Gu, Y.; Ho, D. M. *Tetrahedron Lett.* **1997**, *38*, 5583.

(9) (a) Takai, K.; Takashiro, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048. (b) Crevisy, C.; Beau, J.-M. *Tetrahedron Lett.* **1991**, *32*, 3171.

life was 29 min ([**3**] = 0.005 M in ^{i}PrOH, [K_2CrO_4] = 0.001 M, correlation = 0.997), while the thermal cyclization half-life at 60 °C was 58 h ([**3**] = 0.005 M in ^{i}PrOH, correlation = 0.995).

Ketone **4** underwent thermal cyclization in isopropyl alcohol in excellent yield (entry 5). Under thermal conditions at 40 °C, the half-life was 55 h ([**4**] = 0.005 M in ^{i}PrOH, correlation = 0.996). However, compound **4** did not undergo efficient photochemical cyclizaton under conditions identical to those of **3**. After a 2 h irradiation, only 10% of the cyclized product was observed (entry 6), unlike **3** which showed 83% of the product (entry 4). The reaction also showed a poor mass balance with only 34% of starting material remaining. We determined that the poor yields of cyclization product stem from photochemical processes involving enediyne **4** and not from decomposition of **14** after cyclization. This was accomplished by irradiating **14** (isolated from thermal cyclization) under the same photochemical conditions as used in the cyclization of **3** and **4**. The experiment clearly demonstrated that ketone **14** was stable over the time scale that led to poor cyclization of **4** and significant side products (entry 6).

The difference in reactivity between alcohol **3** and ketone **4** may arise from different excited states. Ketones are well-known to possess excited states and reactivity different from olefins and aromatics. Ketones readily form triplet excited states which can undergo hydrogen and electron abstraction processes.[13] If the photochemical Bergman cyclization is favored by a singlet excited state, then a triplet state ketone could interfere with the normal cyclization process and result in the poor yields and conversion observed for **4**.

Compounds **3** and **4** were also examined for their ability to cleave double-stranded DNA. Figure 1 shows the results

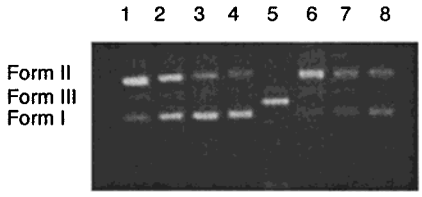


Figure 1. Supercoiled DNA interaction ΦX174. DNA was incubated for 70 h at 40 °C with compounds **3** and **4** in buffer (TE, pH 7.6) and analyzed by electrophoresis (1% agarose gel, ethinium bromide stain). Lanes 1−3: **3** (4000, 400, and 40 μM). Lane 4: DNA control. Lane 5: DNA control with restriction enzyme, Dra I. Lanes 6−8: **4** (4000, 400, and 40 μM).

obtained after incubating **3** and **4** with ΦX174 dsDNA for 70 h at 40 °C. The ketone shows significant DNA nicking (Form II) at concentrations as low as 40 μM and nearly complete nicking at 4 mM. The alcohol showed almost no reactivity at 40 μM but was able to nick DNA at 4 mM. While no double-strand (ds) cleavage (Form III) was observed at the lower concentrations, both **3** and **4** did show slight ds cleavage at 4 mM. The thermal cleavage properties of **3** are similar to those of alcohol **1c**.[4b]

Likewise, photochemical DNA cleavage was demonstrated for **3** and **4** ($h\nu$, 40 °C, 3 h; gel not shown). In this case, compound **3** showed superior DNA cleavage ability. At 40 μM, compound **3** showed significant DNA single-strand cleavage while compound **4** showed no discernible activity. At higher concentrations (4000 μM), both compounds showed signs of double-strand scission, again with **3** giving the more complete reaction. The photochemical DNA cleavage properties of **4** compare favorably with *O*-alkylated derivatives of pyrene **2**.[5c]

Compounds **3** and **4** were also examined for their anticancer activity. Human leukemic lymphoblasts of the CCRF-CEM cell line (log-phase cultures) were incubated with 2−40 μM of **3** or **4** for 24 h to test the effect on cell viability and cell cycle traverse. After staining with propidium iodide/hypotonic citrate,[14] aliquots were analyzed by laser flow cytometry. In cultures exposed to 2 μM of alcohol **3**, growth inhibition and cytotoxicity were indicated by the reduction in the number of cells with S and G_2/M DNA content accompanied by the appearance of cells with less G_0/G_1 DNA content (apoptotic cells?). At higher concentrations (4 μM) alcohol **3** caused accumulation of cells in G_0/G_1-early S-phase accompanied by a significant increase in the number of apoptotic cells. Ketone **4** at 2 μM did not have any significant effects on cell cycle traverse, but in cultures exposed at 4 μM, there was a pronounced reduction in the number of cells with the G_0/G_1 DNA content accompanied by an accumulation of cells with late-S and G_2/M DNA content. The number of cells with G_0/G_1 DNA content also increased. Alcohol **3** and ketone **4** both had IC_{50} values of ≈1.25 μM.

In conclusion, the first cyclic pyrimidine enediynes (**3** and **4**) were synthesized with 29% and 25% overall yields in seven and eight steps, respectively. Compound **3** cyclized with a half-life of 29 min at 40 °C under the photochemical conditions used here. The ketone does not efficiently cyclize under the same photochemical conditions. Both the ketone and the alcohol were able to effect DNA cleavage at reasonable concentrations at physiological temperatures. As

(10) **3**: mp 108−110 °C; UV (^{i}PrOH) λ_{max} (ϵ) 311 nm (7100), 260 nm (9200), 249 nm (10300); FT-IR (CH_2Cl_2) 3590, 2352, 1572, 1525, 1378 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (m, 1H), 4.02 (s, 3H), 3.98 (s, 3H); 2.52 (m, 2H), 2.29−1.84 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 163.9, 158.8, 107.6, 104.0, 103.3, 80.2, 79.1, 63.1, 55.3, 54.6, 37.5, 23.0, 21.3; HRMS (DEI) calcd for $C_{14}H_{14}N_2O_3$ 258.1004, found 258.0994.

(11) **4**: mp 110 °C (decomp); UV (^{i}PrOH) λ_{max} (ϵ) 323 nm (16100), 286 nm (14100), 249 nm (37000); FT-IR (CH_2Cl_2) 2257, 2173, 1793, 1656, 1567, 1520, 1473, 1378 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 3H), 3.99 (s, 3H), 2.84 (t, 2H, J = 5.6 Hz), 2.63 (t, 2H, J = 5.6 Hz), 2.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.7, 169.9, 165.4, 161.1, 107.8, 101.6, 99.0, 90.6, 80.2, 55.6, 54.8, 46.3, 24.8, 22.1; HRMS (DEI) calcd for $C_{14}H_{12}N_2O_3$. 256.0848, found 256.0839.

(12) **General procedure for the photochemical reaction:** Photolysis experiments were carried out with a Rayonette photochemical reactor equipped with 16 3100 Å lamps, and a 0.01 M potassium chromate (K_2CrO_4) filter solution was employed to filter out the 313 nm wavelength. A solution of **3** (2.58 mg, 0.01 mmol) in degassed ^{i}PrOH (10 mL) was stirred at 40 °C for 24 h. After the solvent was evaporated under reduced pressure, the reaction mixture was purified by column chromatography (SiO_2, hexane/ethyl acetate, 75/25), from which pure **13** was obtained (82%).

(13) Turro, N. J. In *Modern Molecular Photochemistry*; University Science Books: Sausalito, CA, 1991; p 628.

(14) Krishan, A. *J. Cell. Biol.* **1975**, *66*, 188.

expected from the cyclization studies, **3** gave better DNA cleavage under photochemical conditions while **4** was superior under thermal conditions alone. Flow cytometric studies showed that while both compounds caused an increase in the number of cells with less G_0/G_1 DNA content (possibly apoptotic cells), their effects on cell cycle traverse were different. While alcohol **3** caused accumulation of cells in G_0/G_1-early S-phase of the cell cycle, ketone **4** caused accumulation of cells in the G_2/M part of the cell cycle. The presence of apoptotic cells in cultures exposed to these compounds may suggest the involvement of the apoptotic mechanism in cytotoxicity while the presence of cells with G_2/M DNA content may indicate interference with DNA synthesis and repair mechanisms. The involvement of enediynes in apoptosis through specific receptor−ligand interactions has been previously proposed.[15]

(15) Nicolaou, K. C.; Stabila, P.; Esmaeli-Azad, B.; Wrasidlo, W.; Hiatt, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3142.

Investigations including detailed kinetic experiments on **3** and **4** and the synthesis of pyrimidinone analogues **11** and **12** are in progress.

Acknowledgment. The donors of the Petroleum Research Fund, administered by the American Chemical Society, are recognized for partial support of this project. We are also grateful for the financial assistance provided by the American Cancer Society, Florida Division, Inc. We thank Prof. C. Hoff, University of Miami, for helpful discussions and providing photochemistry equipment. Prof. M. S. Gaines, University of Miami, Biology Department, kindly supplied the electrophoresis equipment for the DNA cleavage studies. We are indebted to Prof. N. J. Turro, Columbia University, for his insightful comments. We also thank Mr. X Calyptratus for technical assistance.

OL006061J