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 iPrOH, 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 were first reported, designed enediynes have been synthesized and studied as DNA cleaving agents on the basis of the Bergman cyclization. Synthetic enediynes have a distinct advantage over the naturally occurring products as therapeutic agents because they may show reduced toxicity. The parameters for thermal enediyne activation by modification of 10-membered rings 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 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 (e.g., 25c). Despite the potential of the photo-Bergman cyclization in photodynamic therapy, little has been done to

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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. In this Letter we present the difference in the reactivity of pyrimidine enediyne 3 and enediyne 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 work. Coupling 6 with TIPS-protected acetylene using Cu and Pd(PPh₃)₄ as a catalyst in a mixture of diisopropylamine–THF at 70 °C under N₂ gave monocuppled 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(PPh₃)₄ in diisopropylamine followed by removal of the crude product was coupled with 5-hexyn-1-ol using catalytic Pd(PPh₃)₄ in diisopropylamine followed by removal of the TIPS protecting group. The crude product was isolated and purified by column chromatography. The purified product was then coupled with 5-hexyn-1-ol using catalytic Pd(PPh₃)₄ in diisopropylamine followed by removal of the crude product was coupled with 5-hexyn-1-ol using catalytic Pd(PPh₃)₄ in diisopropylamine followed by removal of the TIPS protecting group. The crude product was isolated and purified by column chromatography. 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Table 1. Thermal and Photochemical Bergman Cyclization of 3 and 4

<table>
<thead>
<tr>
<th>entry</th>
<th>compd</th>
<th>solvent</th>
<th>[concν] of temp, yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>PrOH (10)</td>
<td>reflux, Δ</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>PrOH (24)</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>PrOH (36)</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>PrOH (2)</td>
<td>0.005</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>PrOH (10)</td>
<td>0.020</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>PrOH (2)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Isolation yield. † By 1H NMR. ‡ Filter solution, [K₂CrO₄] = 0.01 M. § Filter solution, [K₂CrO₄] = 0.001 M. †† Remaining starting material. / By HPLC with internal standard, xylene.

photochemical conditions using a filter solution (aqueous K₂CrO₄), 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-life of 8 or 9 using I₂ and a base (morpholine, DBU, or DMAP) provided the product in low yield (10–20%). The key ring closure of 10 was carried out with a CrCl₂–NiCl₂ system. Oxidation of 3 with MnO₂ was used to prepare 4. Several unsuccessful attempts were also made to prepare pyrimidinone analogues 11 and 12 by TMSI cleavage of the methyl lactim ethers (Scheme 2).
life was 29 min ([3] = 0.005 M in PrOH, [K₂CrO₄] = 0.001 M, correlation = 0.997), while the thermal cyclization half-life at 60 °C was 58 h ([3] = 0.005 M in 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 PrOH, correlation = 0.996). However, compound 4 did not undergo efficient photochemical cyclization 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 products stem from photochemical processes involving enediyne 4 and not from decomposition of 4 after cyclization. This was accomplished by irradiating 4 (isolated from thermal cyclization) under the same photochemical conditions as used in the cyclization of 3 and 4. The experiment clearly demonstrated that ketone 4 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. 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 obtained after incubating 3 and 4 with 4X7 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 1e. Likewise, photochemical DNA cleavage was demonstrated for 3 and 4 (hv, 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 2c.

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, 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 G2/M DNA content accompanied by the appearance of cells with less G0/G1 DNA content (apoptotic cells?). At higher concentrations (4 μM) alcohol 3 caused accumulation of cells in G1/G0-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 G0/G1 DNA content accompanied by an accumulation of cells with late-S and G2/M DNA content. The number of cells with G0/G1 DNA content also increased. Alcohol 3 and ketone 4 both had IC₅₀ 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 (PrOH) λₘₐₓ (ε) 311 nm (7100), 260 nm (9200), 249 nm (10300); FT-IR (CHCl₃) 3590, 2352, 1572, 1525, 1378 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.68 (m, 1H), 4.02 (s, 3H), 3.98 (s, 3H); ²C NMR (100 MHz, CDCl₃) 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 C₁₉H₁₆N₅O₅ 325.0424, found 325.0894.

(11) 4: mp 110 °C (decomp); UV (PrOH) λₘₐₓ (ε) 323 nm (16100), 286 nm (14100), 249 nm (37000); FT-IR (CHCl₃) 2257, 1713, 1796, 1658, 1573, 1473, 1378 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.00 (s, 3H), 3.99 (s, 3H), 3.89 (s, 3H), 2.84 (t, 2H), J = 5.6 Hz), 2.63 (t, 2H, J = 5.6 Hz), 2.17 (m, 2H); ²C NMR (100 MHz, CDCl₃) δ 186.7, 169.9, 165.4, 161.1, 107.8, 101.6, 99.0, 90.6, 80.2, 55.4, 54.8, 46.3, 24.8, 22.1; HRMS (DEI) calcd for C₁₉H₁₆N₅O₅ 325.0484, found 325.0839.

(12) General procedure for the photochemical reaction: Photolysis experiments were carried out with a Rayonet photoreactor equipped with 16 3130 A lamps, and a 0.01 M potassium chromate (K₂CrO₄) filter solution was employed to filter out the 313 nm wavelength. A solution of 3 (2.58 mg, 0.01 mmol) in degassed 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 (SiO₂, hexane/ethyl acetate, 75:25), from which pure 13 was obtained (82%).


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 G0/G1 DNA content (possibly apoptotic cells), their effects on cell cycle traverse were different. While alcohol 3 caused accumulation of cells in G0/G1-early S-phase of the cell cycle, ketone 4 caused accumulation of cells in the G2/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 G2/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.\(^{15}\)


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

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