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Effect of Remote Trigonal Carbons on the Kinetics of Bergman Cyclization: Synthesis and Chemical Reactivity of Pyridazinedione-Based Enediynes

Amit Basak,*,† Subhendu Sekhar Bag,† Pooja Anjali Majumder,‡ Amit Kumar Das,‡ and Valerio Bertolasi§

Bioorganic Chemistry Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, India, Department of Biotechnology, Indian Institute of Technology, Kharagpur 721302, India, and Dipartimento di Chimica, Universita di Ferrara, Ferrara, Italy

absk@chem.iitkgp.ernet.in

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Abstract: The synthesis and chemical reactivity of pyridazinedione-based enediynes (**1**, **2**) are described. Both of these enediynes, namely the dihydro compound **1** and its corresponding tetrahydro analogue **2,** were prepared by double N,O-alkylation of the corresponding heterocyclic system with the acyclic enediynyl dibromide **8** in good yields. Their single-crystal X-ray structures revealed similar *c, d* distances (distance between the acetylenic carbons undergoing covalent connection in Bergman cyclization). Interestingly, these molecules undergo Bergman cyclization at different rates, and the reactivity is shown to be dependent upon the state of hybridization of C-4 and C-5 atoms of the parent heterocyclic ring.

Single electron-carrying species were long known to possess cytotoxic activity via DNA damage;[1] however, this property could not be utilized for the development of artificial antitumor agents as methods to generate radicals under mild ambient conditions were not known until Nature showed how to do that through the chemistry of naturally occurring enediynes.[2] The natural enediynes possess potent antitumor activity, but severe toxicity problems had prevented their direct medical use for a long time. Recently, the drug Mylotarg (a conjugate of monoclonal antibody and calicheamicin) has been approved by the U.S. Food and Drug Administration (FDA) to fight relapsed acute myeloid leukemia (AML), and a similar conjugate of esperamicin has entered phase II trials.[3] Despite these recent developments, the art of designing new enediynes[4] will continue as this paves the way of creating a large library of compounds with diverse potency in their DNA-cleaving activity. The most important aspect in designing enediynes is the so-called triggering device, which needs to be attached to the enediyne framework for its activation at the right moment. The incorporation of trigonal carbon atoms in bicyclic enediynes[5] to modulate the kinetics of BC is one of various strategies[6] adopted for designing new enediynes. In fact, the intramolecular addition of a thiol to the double bond in the bicyclic network of calicheamicin and esperamicin acts as a triggering mechanism to activate the enediyne system.[7] We became interested in dihydropyridazinedione-based enediynes as there is the possibility of modulation of electronic character by saturation of the double bond in the heterocyclic system.[8] Moreover, the construction of enediyne framework on to the pyridazine moiety should be possible by exploiting the nucleophilic character of the amide functionality. With these advantages in mind, we successfully synthesized a dihydropyridazinedione-based enediyne **1** and its corresponding tetrahydro analogue **2** and studied the chemical reactivity of both compounds. Our findings are presented in this paper.

As there was possibility of N- or O-alkylation, we decided to study the reactivity of a pyridazinedione moiety toward bis-alkylation. Thus, dihydropyridazine-

† Department of Chemistry, Indian Institute of Technology.
‡ Department of Biotechnology, Indian Institute of Technology.
§ Dipartimento di Chimica, Universita di Ferrara.

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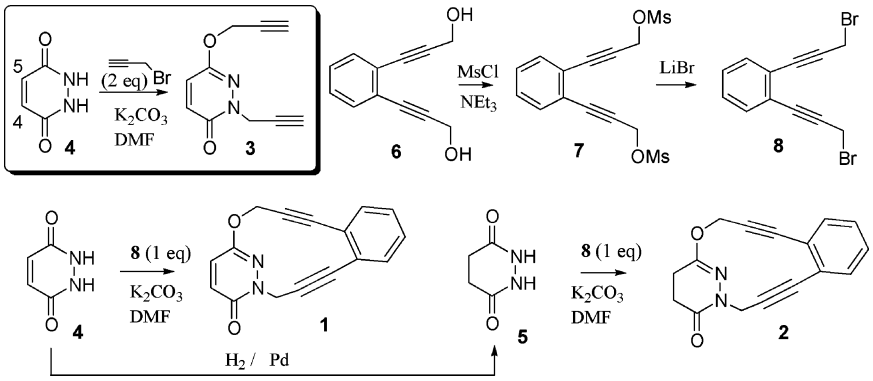
SCHEME 1. Synthesis of Pyridazinedione-Based Enediynes



dione **4** was reacted with propargyl bromide (2 equiv) in the presence of K_2CO_3 (2 equiv) and DMF at room temperature. Only one major product was isolated, whose structure **3** was confirmed by NMR as well as by single-crystal X-ray analysis. In the ^{1}H NMR, the two methylenes of the propargyl system appeared as separate doublets at δ 4.67 and 4.71, indicating N,O-bis-alkylation leading to an unsymmetrical structure. The single-crystal X-ray data confirmed the assigned structure. Having established that N,O-bis-alkylation occurred in a pyridazinedione system, we proceeded with the synthesis of the bicyclic enediyne **1** via the reaction of the heterocycle with enediynyl dibromide **8**[9] (1:1 ratio) under similar conditions (K_2CO_3 and DMF). The reaction afforded the expected product, which was isolated pure by Si gel chromatography in high yield (85%). The ^{1}H NMR showed the methylenes appearing at δ 5.13 and 5.01 again confirming the formation of an unsymmetrical structure. The ^{13}C NMR also showed the presence of 16 nonequivalent carbons. The structure was, however, confirmed beyond doubt by single-crystal X-ray analysis. The corresponding tetrahydropyridazinedione enediyne **2** was also prepared through a similar N,O-bis-alkylation (yield 80%) of **5** and was characterized by NMR and single-crystal X-ray studies (Scheme 1).

Having established the structure of the enediynes **1** and **2**, their reactivity toward Bergman cyclization was studied in both solid and solution phase. The solid-phase reactivity was determined by differential scanning calorimetry (DSC),[10] which showed strong exothermic peaks for both the enediynes (Figure 1). The onset temperature for Bergman cyclization for enediyne **1** was ~228 °C, whereas that for the other enediyne **2** the onset temperature was found to be ~196 °C after an initial endothermic dip due to melting. Thus, the enediyne lacking unsaturation at C-4 and C-5 (of the parent heterocyclic ring) has a lower activation barrier when heated in a neat state.

The solution-phase kinetics was determined by heating a solution of the enediynes **1/2** in a sealed tube at a preset temperature in $CHCl_3$ containing an excess of 1,4-cyclohexadiene and taking the ^{1}H NMR at different time points. In the NMR spectra, the signals for the two methylenes for the starting materials diminished over time, while two new singlets corresponding to the methylenes of the naphthalene system **9/10**, respectively, appeared and their signal intensity increased with time. For the tetrahydropydazinedione enediyne **2**, the half-life, determined at 130 °C, was found to be 120 h. The dihydropyridazinedione enediyne **1** failed to cyclize at 130 °C even after heating for 168 h. Finally, the half-life was determined at 150 °C and was found to be ~200 h. Thus, it was demonstrated that enediyne **2** is much more reactive than the corresponding unsaturated compound **1** both in a neat state and in the solution phase indicating the importance of the state of hybridization of the carbon atoms in the pyridazinedione framework (Scheme 2).

The observed difference in reactivity of the two enediynes is obviously not due to the distance factor as the *c*, *d* distances turned out to be almost the same (3.79 Å) by X-ray analysis. Inspection of the X-ray structure, however, showed less strain energy in the ground state of **2** because of the presence of flexible methylene chains, which allows the molecule to assume a more puckered structure, thereby releasing the strain. The participation of N-lone pair in delocalization with the carbonyl is more

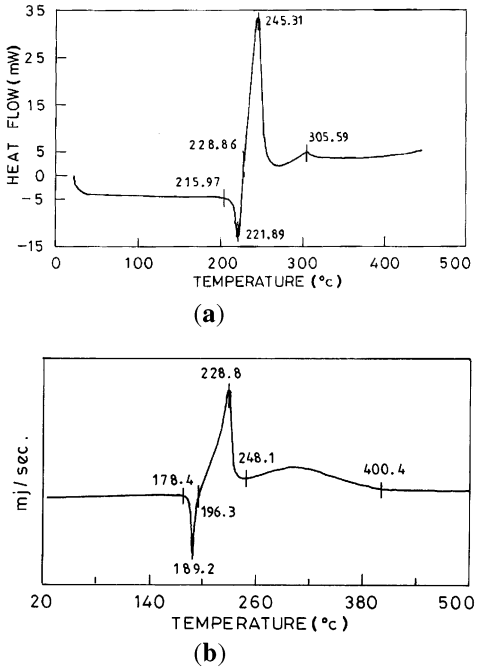


FIGURE 1. DSC curves for enediyne **1** and **2**.

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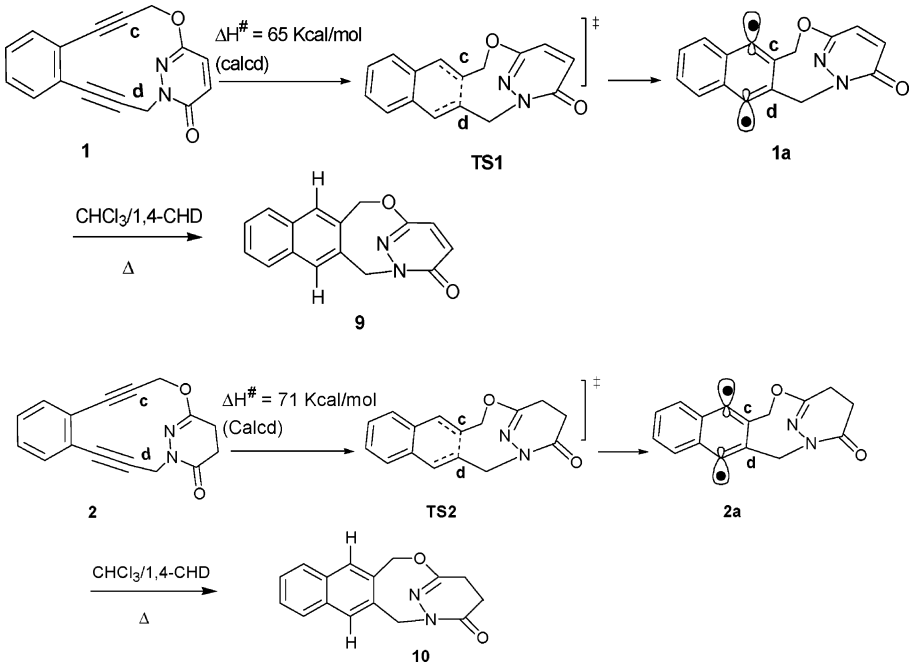
SCHEME 2. Formation of the Cyclized Products and TS



in **1** because of attainment of aromaticity. This is also reflected in the longer C−O bond (1.26 Å) in **1** (obtained from X-ray structure) as compared to that in **2** (1.23 Å). This greater delocalization makes the system in **1** more planar and consequently more strained. Although our argument is based on ground-state structure, similar differences may also be reflected in the structures of the TS. Semiempirical PM5 calculations[11] in the gas phase also indicated higher activation energy for compound **1** as compared to **2**.

Thus, we have successfully synthesized pyridazinedione-based enediynes and fully characterized their structures. We have also demonstrated the perturbation caused by a remote double bond in the diazine system toward Bergman cyclization. The observation that saturation of the double bond speeds up the reaction can be exploited to use this as a triggering mechanism. One can think of possible reduction of the enone or addition of any nucleophile in a Michael fashion to the enone for enediyne activation. Although we do not have any concrete evidence about the feasibility of such reactions at present, the fact that such systems do undergo cycloaddition reactions[12] suggests their behavior as an electron-deficient system. Similar effects may also exist in other heterocycles, especially in pyrimidine- and dihydropyrimidine-fused systems. Future work in our laboratory will address all these issues.

Experimental Section

1,2-Bis(1-hydroxyprop-2-ynyl)benzene (6) and 1,2-Bis-(1-methylsulfonatoprop-2-ynyl)benzene (7). Prepared according to the literature procedure.[13]

1,2-Bis(1-bromo-2-ynyl)benzene (8). Prepared by adopting a procedure different from that reported in the literature.[9] To a solution of mesylate (0.5 g, 1.46 mmol) in dry THF (20 mL) at 0 °C was added dropwise LiBr (0.38 g, 4.38 mmol) dissolved in THF for 5 min, and the solution was stirred at 0 °C for 4 h.[14] The mixture was poured into a saturated solution $NaHCO_3$ (25 mL), and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified via chromatography (Si-gel, hexane/Et_2O = 95:5) to yield the dibromide **8** as low melting pale brown solid (0.36 g, 80%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.12 (s, 4H), 7.18 (m, 2H), 7.33 (m, 2H). Spectral properties were identical with that reported in the literature.[9,14]

Synthesis of Tetrahydro-3,6-pyridazinedione (5). This was prepared by hydrogenation of **4** under balloon pressure using 30% Pd/C and obtained as a white crystalline solid (yield 95%): mp 274−276 °C (lit.[15] mp 277−278 °C).

General Procedure for the Synthesis of Enediynes 1 and 2. To a solution of 1,2-dihydropyridazine-3,6-dione (**4**)/tetrahydropyridazine-3,6-dione (**5**) (0.5 g, 4.46 mmol) in dry DMF were added K_2CO_3 (1.35 g, 9.81 mmol) and the dibromide **8** (1.39 g, 4.46 mmol), and the solution was stirred at room temperature for 12 h. The reaction mixture was extracted with EtOAc (2 × 50 mL), and the organic layer was washed with brine (100 mL). Evaporation of the solvent afforded an oily residue from which the title compounds (**1**, **2**) were isolated by column chromatography (Si-gel, hexane/EtOAc = 2:1) as white crystalline solids.

Synthesis of 14-oxa-1,19-diaza-tricyclo[13.3.1.0^{5,10}]nonadeca-5(10),6,8,15(19),16-pentaene-3,11-diyn-18-one (1): yield 85%; solid state; mp 222 °C; ν_{max} ($CHCl_3$) 3056, 2234, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40 (m, 2H), 7.28 (m, 2H), 6.96 (d, J = 9.7 Hz, 1H), 6.91 (d, J = 9.7 Hz, 1H), 5.14 (s, 2H), 5.01 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.8, 150.5, 132.6, 131.4, 130.3, 128.1, 127.3, 126.5, 126.4, 89.3, 87.9, 86.6, 85.6, 76.4, 56.2, 40.0; MS (ES+) m/z 263 (MH^+); HRMS calcd for $C_{16}H_{10}N_2O_2$ + H^+ 263.0821, found 263.0830.

Synthesis of 14-oxa-1,19-diazatricyclo[13.3.1.0^{5,10}]nonadeca-5(10),6,8,15(19)-tetraene-3,11-diyn-18-one (2): yield 75%;

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solid state; mp 190 °C; ν_{max} ($CHCl_3$) 2901, 2227, 1653 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.38 (m, 2H), 7.26 (m, 2H), 4.94 (s, 2H), 4.80 (s, 2H), 2.62 (dd, J = 4.0, 9.8 Hz, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 163.9, 156.7, 131.1, 130.2, 128.6, 127.9, 127.8, 126.5, 89.6, 88.2, 86.3, 84.4, 55.8, 37.6, 27.8, 23.6; MS (ES+) m/z 264 (M^+); HRMS calcd for $C_{16}H_{12}N_2O_2$ 264.0899, found 264.0895.

Synthesis of 2-Prop-2-ynyl-6-prop-2-ynyloxy-2*H*-pyridazin-3-one (3). The title compound **3** was synthesized following the same procedure as discussed for enediyne **1**, with 2 equiv of propargyl bromide: yield 80%; solid state; mp 95 °C; ν_{max} (neat) 2987 (CH), 2336, 1667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.87 (d, J = 9.8 Hz, 1H), 6.83 (d, J = 9.8 Hz, 1H), 4.71 (d, J = 2.4 Hz, 2H), 4.67 (d, J = 2.5 Hz, 2H), 2.39 (t, J = 3.6 Hz, 1H), 2.16 (t, J = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.3, 151.3, 133.2, 127.0, 77.1, 76.5, 75.5, 72.7, 54.8, 40.6; MS (EI) m/z 188 (M^+); HRMS calcd for $C_{10}H_8N_2O_2$ 188.0586, found 188.0588.

Procedure for Bergman Cyclization. To a solution of enediyne **1**/**2** in dry degassed $CHCl_3$ was added 100 equiv of 1,4-CHD, and the solution was kept in a sealed tube. The temperature of the solution was maintained at 130 °C (for **2**) and 150 °C (for **1**), and ^1H NMR was recorded taking the aliquots at different time points. However, the yields of the cyclized products were low (25−35%). The low yield is attributed to the formation of polynaphthalene type compounds via BC followed by polymerization[16] and the unreacted starting materials. It is to be noted that all the cyclizations have good mass balance.

Analytical data for 9: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.42−7.37 (m, 2H), 7.35 (m, 2H), 7.32−7.27 (m, 2H), 6.94 (d, J = 4.02 Hz, 2H), 5.05 (s, 2H), 5.02 (s, 2H); HRMS calcd for $C_{16}H_{12}N_2O_2 + H^+$ 265.0978, found 265.0970.

Analytical data for 10: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.38 (m, 2H), 7.29 (m, 2H), 7.27 (m, 2H), 4.76 (s, 2H), 4.51 (s, 2H), 2.71 (dd, J = 4.0, 9.6 Hz, 4H); HRMS calcd for $C_{16}H_{14}N_2O_2 + H^+$ 267.1134, found 267.1122.

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Supporting Information Available: General experimental, ^1H and ^{13}C NMR spectra of **1**−**3**, PM5-generated thermodynamic data (Table S1) and optimized geometries, crystallographic data (ORTEP structures of **1**−**3**), and solution kinetic profile. This material is available free of charge via the Internet at http://pubs.acs.org.

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