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Synthesis and reactivity of enediyne–nucleobase hybrids: effect of intramolecular π-stacking

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ABSTRACT

Three distinct classes of nucleobase-containing enediynes **1**–**9** with varying nature of the linker have been synthesized to explore the effect of π-stacking interaction in accelerating the rate of Bergman cyclization (BC). Chemical reactivity study, both experimental and computations demonstrated the important role that aromatic π-stacking interactions between the appended nucleobases within an enediyne frame play in lowering the activation barrier of Bergman cyclization.

1. Introduction

Bergman cyclization[1] of enediyne to the 1,4-benzene diradical[2] and related reactions have remained a research area of contemporary interest. Many artificial synthetic structural motifs have been explored till date to affect the Bergman cyclization kinetics through intramolecular non-covalent interactions,[3] such as hydrogen bonding,[4] π–π stacking,[5] electrostatic[6] and charge transfer interactions.[7] One important natural component, namely the purine and pyrimidine bases present in the beautifully self-assembled DNA,[8] can show these weak interactions and hence are attractive motifs for enediynes in perturbing the kinetics of BC. In DNA, the two helical strands are connected to each other through weak hydrogen bonding interactions, though the stability of this complex molecular aggregation is mainly attributed to comparatively strong non-covalent π–π stacking interaction between aromatic bases of the two parallel helices.[9] According to Nicolaou's distance theory,[10] the spatial distance (*d*) between the terminals of two acetylenic arms of enediyne can dictate its reactivity. The value of *d* should range from 3.20 to 3.31 Å[11] for smooth cyclization at ambient temperature. If two different nucleobases are attached to the two acetylenic arms of the enediyne moiety through a spacer, they may show the complementary base pairing via hydrogen bonding and/or π-stacking interaction, depending on the conformation adopted. For identical nucleobases attached to the enediyne terminals, stacking interactions are mainly expected. In order to find out which interactions (H-bonding or π stacking) are predominant, we have synthesized three distinct classes of nucleobase-containing enediynes **1**–**9** with varying nature of the linker (Fig. 1). Their synthesis along with chemical reactivity and detailed structural analysis are reported in this paper.

2. Results and discussions

The synthesis of class **A** molecules with a methylene linker was done via double N-alkylation. For the AA or TT enediynes, the double N-alkylation was carried out in a single step on the bromide **2c** using A or T (Scheme 2). For the synthesis of **1** with different bases, alkylation was done in two steps (Scheme 1). The first alkylation was carried out on the monobromide **1d** with bis-Boc protected adenine to provide **1f**. Conversion to the bromide **1h** followed by alkylation with Boc-thymine to provide the tris-Boc hybrid **1i**, which was deprotected with TFA to furnish **1**. Protected bases had to be employed because of competing intramolecular N-alkylation. Similarly, class **B** and **C** compounds were prepared by using slightly modified protocol. For class **B** molecules, the dibromoacetyl enediyne **5b** was employed for the synthesis of AA or TT enediynes (**5** and **6**) (Scheme 4) while stepwise alkylation starting from mono bromoacylated enediyne **4f** was used for the synthesis of **4** with both A and T (Scheme 3). First alkylation was carried out with bis-Boc protected adenine. The product was deprotected and coupled directly with carboxymethyl thymine using EDC·HCl and HOBt. The triazole based enediynes belonging to class **C** were prepared via click reaction as shown in Schemes 5 and 6. Final compounds **1**–**9** were characterized by NMR and mass spectrometry, in which structures of **1i** and **3a** were unambiguously confirmed by X-ray crystallography study (Fig. 2).[12]

With the target compounds in hand, their thermal reactivity was studied, specifically to identify the BC products, if any. We were disappointed in not isolating any distinct product from the solution phase reactions (heating in DMSO within 150–180 °C for

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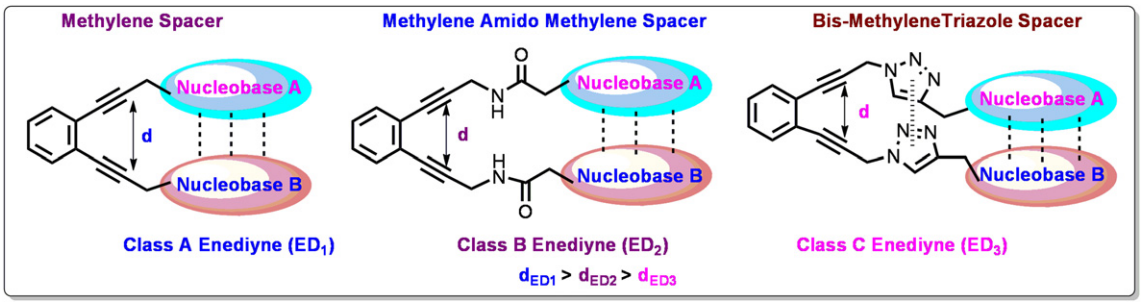
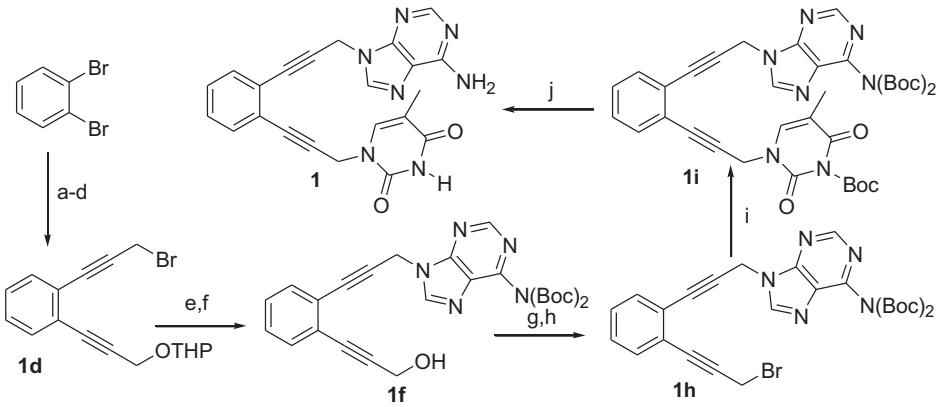
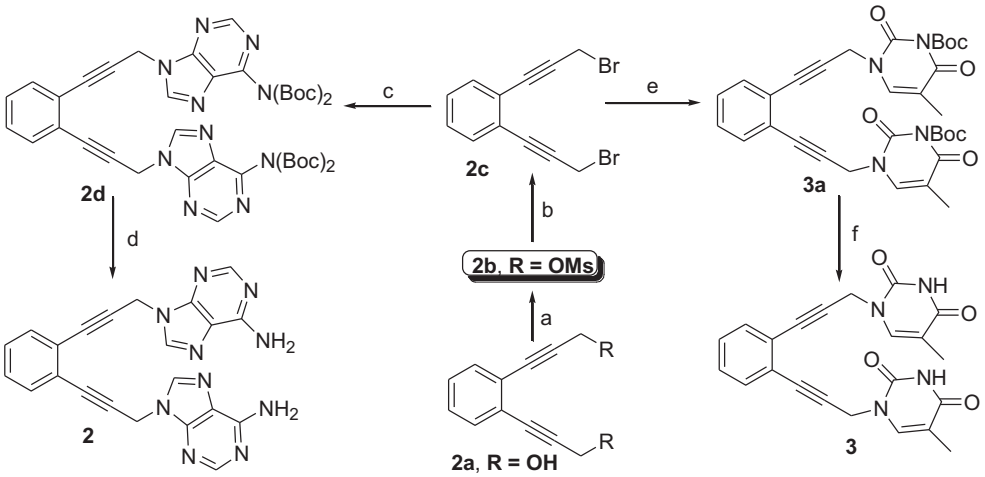


Fig. 1. Design of enediyne–nucleobase hybrids.



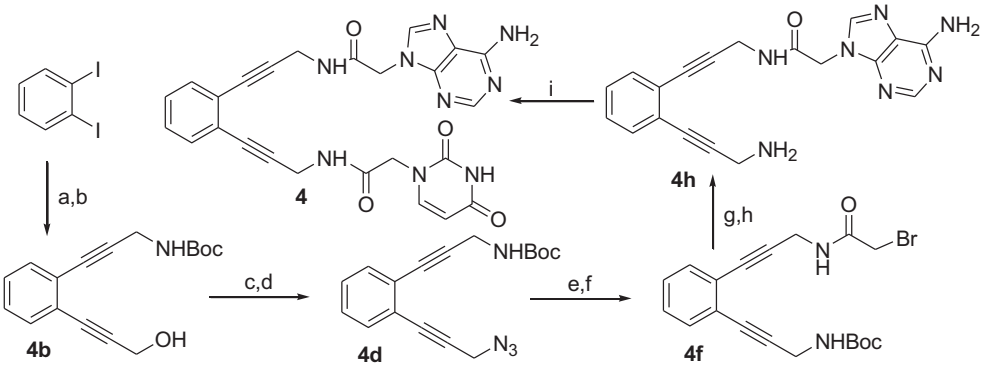
Reagents and conditions: (a) THP-protected propargyl alcohol, $Pd(PPh_3)_4$, *n*-$BuNH_2$, 80 °C to 85 °C, 3 h (82%); (b) Propargyl alcohol, $Pd(PPh_3)_4$, *n*-$BuNH_2$, 80 °C to 85 °C, 14 h (80%); (c) MsCl, Et_3N, DCM, 0 °C, 15 min (92%); (d) LiBr, THF, r.t., 8 h (72%); (e) Bis-Boc-adenine, Cs_2CO_3, CH_3CN, r.t., 7 h (60%); (f) PPTS, EtOH, r.t., 6 h (78%); (g) MsCl, Et_3N, DCM, 0 °C, 15 min (80%); (h) LiBr, THF, r.t., 10 h (80%); (i) N^3-Boc-thymine, Cs_2CO_3, CH_3CN, r.t., 7 h (54%); (j) TFA, DCM, 0 °C to r.t., 4 h (45%).

Scheme 1. Synthesis of compound **1** of class **A**.



Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C, 15 min (85%); (b) LiBr, THF, r.t., 10 h (72%); (c) Bis-Boc-adenine, Cs_2CO_3, CH_3CN, r.t., 8 h (58%); (d) TFA, DCM, 0 °C to r.t., 4 h (52%); (e) N^3-Boc-thymine, Cs_2CO_3, CH_3CN, r.t., 6 h (60%); (f) TFA, DCM, 0 °C to r.t., 4 h (54%).

Scheme 2. Synthesis of compound **2** and **3** of class **A**.

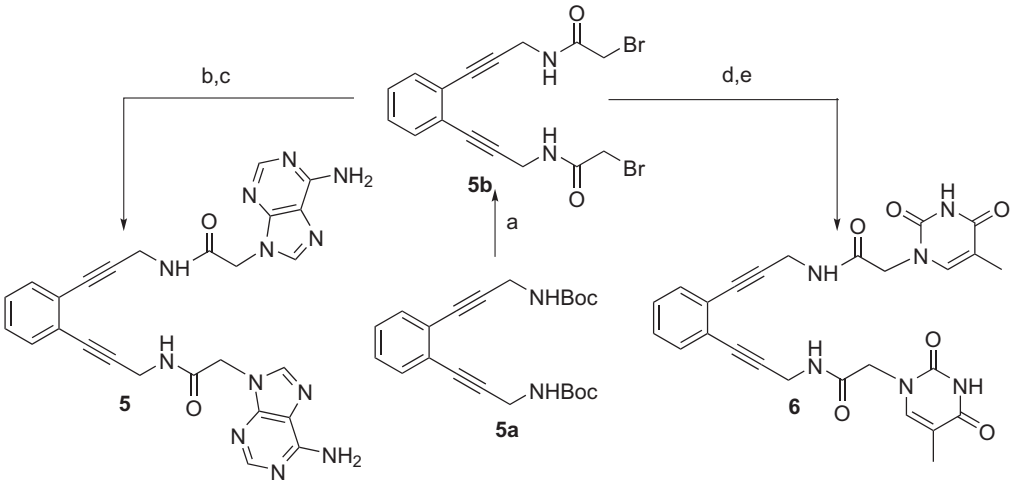


Reagents and conditions: (a) *N*-Boc-propargylamine, $Pd(PPh_3)_4$, CuI, Et_3N, r.t., 8 h (54%); (b) Propargyl alcohol, $Pd(PPh_3)_4$, CuI, Et_3N, r.t., 6 h (65%); (c) MsCl, Et_3N, DCM, 0 °C, 10 min (90%); (d) NaN_3, DMF, r.t., 6 h (70%); (e) PPh_3, THF, H_2O, r.t., 6 h (78%); (f) bromoacetyl chloride, Et_3N, DCM, 0 °C, 15 min (65%); (g) Bis-Boc-adenine, Cs_2CO_3, CH_3CN, r.t., 6 h (56%); (h) TFA, DCM, 0 °C to r.t., 3 h (50%); (i) 1-(carboxymethyl)thymine, EDC.HCl, HOBt, DCM, DMF, 0 °C to r.t., 10 h (52%).

Scheme 3. Synthesis of compound **4** of class **B**.

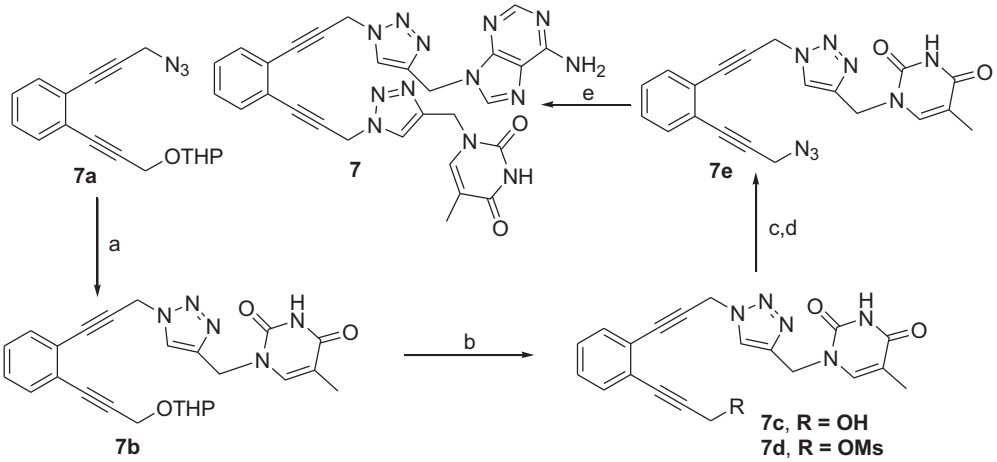
15–30 h) although there was disappearance of the starting enediynes. It appeared that the compounds underwent decomposition with formation of polymeric products.[13] ^{1}H NMR of the polymer indicated appearance of new peaks in the aromatic region thus pointing out the occurrence of BC. Also, the diradical mediated polymerization was confirmed by the MALDI mass analysis[14b] of the resultant DMSO solution of enediyne–nucleobase hybrids. The mass spectrum of compound **3** clearly showed the formation of different oligomers, dimer, trimer, tetramer etc. by exhibiting their corresponding *m*/*z* signals at 829 (with an added Na^+), 1231 (with an added Na^+), 1609, respectively (Fig. 3). The building block of all these oligomeric species was the intermediate 1,4-diradical.[14a] Solution phase kinetic experiment was performed with class **A** enediynes (**1**–**3**) maintaining the concentration of each compound at $\sim 4\times10^{-3}$ M in DMSO solvent using naphthalene[15] as inert internal standard. The solutions were heated at 170 °C and the rate of disappearance of the substrates was measured by recording the HPLC profile of aliquots at different time points (Zorbax SB C-18 column, 9.4 mm×25 cm, Solvent system=a mixture of methanol and water (85:15, v/v), Flow rate=1 mL/min). It showed a pseudo first order kinetics and the corresponding kinetic graph plots are shown in Fig. 4. The rate constants (k_{obs}) as mentioned in Table 1 showed the following order: A–A system **2**>A–T system **1**≈T–T system **3**.

The solid state reactivity was next studied by recording the onset temperatures for BC using the Differential Scanning Calorimetric measurements (DSC)[16] under neat conditions (DSC curves, vide SI, Fig. S3). The various onset temperatures as shown in Table 2 revealed that the reactivity of the enediynes depends not only on the nature of base pairs but also on the type of spacers. However, like the solution phase kinetics, a definite trend in reactivity could be observed. For example, in all the three classes of enediynes, the reactivity of the A–A pairs (**2**, **5** and **8**) turned out to be highest (as revealed by their corresponding lower onset temperatures for BC) followed by the A–T pairs (**1**, **4** and **7**) with the T–T pairs (**3**, **6** and **9**) showing the lowest reactivity (Table 2). Thus the solid phase reactivity is similar to what has been observed for class **A** molecules in solution phase.



Reagents and conditions: (a) i) TFA, DCM, 0 °C to r.t., 3 h; ii) bromoacetyl chloride, Et_3N, DCM, 0 °C, 10 min (67%); (b) Bis-Boc-adenine, Cs_2CO_3, CH_3CN, r.t., 10 h (58%); (c) TFA, DCM, 0 °C to r.t., 5 h (60%); (d) N^3-Boc-thymine, Cs_2CO_3, CH_3CN, r.t., 8 h (62%); (e) TFA, DCM, 0 °C to r.t., 5 h (65%).

Scheme 4. Synthesis of compound **5** and **6** of class **B**.



Reagents and conditions: (a) N^1-Propargyl thymine, $CuSO_4.5H_2O$, sodium ascorbate, ^tBuOH, H_2O, r.t., 10 h (65%); (b) PPTS, EtOH, r.t., 8 h (86%); (c) MsCl, Et_3N, DCM, 0 °C, 15 min (85%); (d) NaN_3, DMF, r.t., 6 h (78%); (e) N^9-propargyl adenine, $CuSO_4.5H_2O$, sodium ascorbate, ^tBuOH, H_2O, r.t., 14 h (52%).

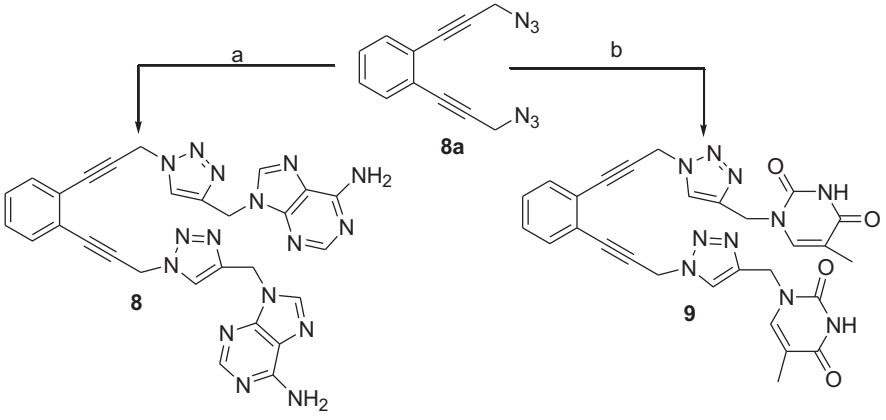
Scheme 5. Synthesis of compound **7** of class **C**.

Regarding the role of spacer, it can be seen that the reactivity for A–A pairs **2**, **5** and **8** increased as the spacer is changed from a simple methylene to a methylene amido methylene and ultimately to a bis-methylene triazole moiety. There was substantial reduction of onset temperature for BC. Similar trend was observed for the A–T pairs (**1**, **4** and **7**). However, the effect of change of spacer on the reactivity in case of the T–T pairs (**3**, **6** and **9**) was not so significant.

To find out which factor, namely the π–π stacking or intramolecular H-bonding, is playing a more important role on the observed reactivity pattern, variable temperature NMR (between 30 °C and 65 °C) were recorded as this is a well-known method to elucidate the intramolecular hydrogen bonding as well as the π-stacking interactions in solution phase (SI, Figs. S5–14).[17] The variations of different chemical shifts, as determined at concentration below 1 mM (to avoid intermolecular associations) are shown in Table 3. The $\Delta\delta/\Delta T$ values of A-NH_2 and T-NH for all compounds were beyond the Kessler limit,[18] thus proving the absence of any significant intramolecular hydrogen bonding between the bases under this condition.

Interestingly, the chemical shift of A-2H, A-8H, T-Me and T-6H also showed some degree of temperature dependence, which is an indication[17b] of existence of π-stacking interaction between the nucleobases. It has earlier been shown[19] that the π-stacking between the nucleobases follows the order A–A>A–T>T–T. Since π-stacking can be expected to bring the acetylenic arms to come closer, which should result in increase in reactivity towards BC, the reactivity should also follow the same order as of the π-interactions. This is indeed the case in all the classes of enediynes.

Regarding the dependence of reactivity on the nature of spacer, the following explanation seems reasonable. In class **B** and **C**, additional intramolecular stabilization forces were operative. For class **B**, the amide NH has a temperature coefficient close to the Kessler limit and thus showing a strong intramolecular H-bond between the amide carbonyl and the amide NH of opposing strands. The formation of H-bonded network between the amide carbonyl and the amide NH of opposing strands is also evident from the transition state optimized geometries of all the compounds of class **B**. For class **C**, the two triazole moieties in the opposite strands have additional π-stacking interaction as is evident from the temperature



Reagents and conditions: (a) N^9-Propargyl adenine, $CuSO_4.5H_2O$, sodium ascorbate, ^tBuOH, H_2O, r.t., 24 h (54%); (b) N^1-propargyl thymine, $CuSO_4.5H_2O$, sodium ascorbate, ^tBuOH, H_2O, r.t., 20 h (70%).

Scheme 6. Synthesis of compound **8** and **9** of class **C**.

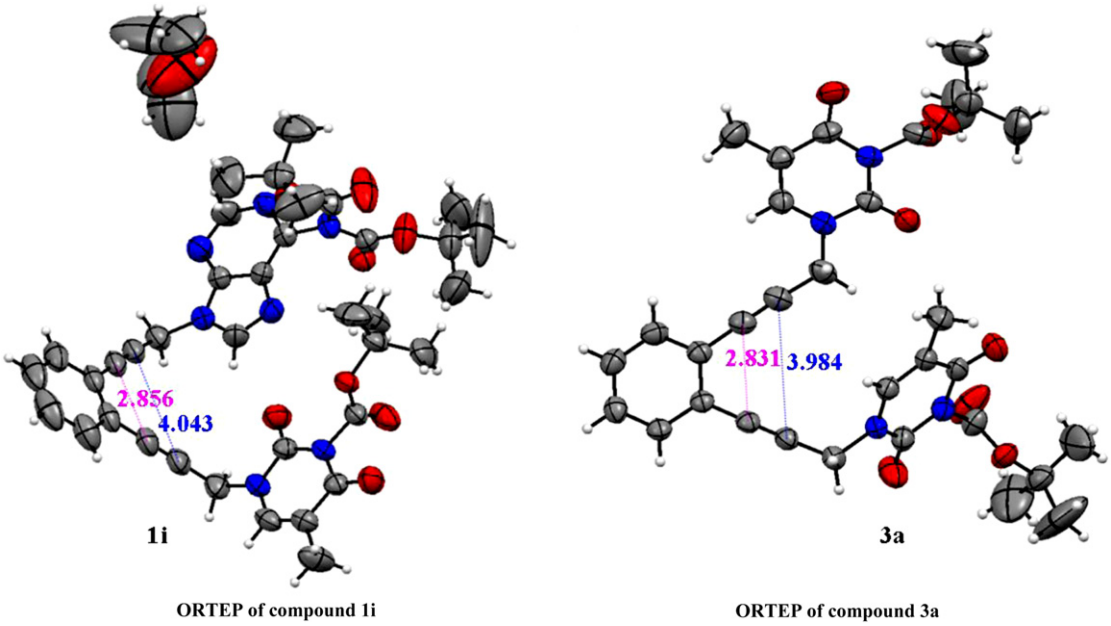


Fig. 2. ORTEP diagram of compound **1i** and **3a** with the thermal ellipsoids shown at 50% probability.

dependent chemical shift of the triazole-H. The cooperative effect of additional interstrand forces between the spacers along with the π-stacking between the base pairs enhanced the reactivity of enediynes with amide and triazole linkers.[20]

3. Computational study

We have also carried out theoretical calculations on molecules belonging to class **A** and **B** using Gaussian 03 program package.[21] Thus, a gas phase optimized geometries of the starting enediynes, transition states and products for the formation of naphthalene biradical derivatives were obtained using semi-empirical theory (PM3) for class **A** and the density functional theory (DFT) with the B3LYP exchange correlation functional and the 6-31G (d) basis set for both class **A** and **B**.[22] A restricted approach was used in the computational analysis for the closed-shell structure while for the transition state and the product biradical optimization, an unrestricted broken-symmetry approach has been used.[23] Optimization of all the structures at DFT level of theory was done using B3LYP method with the same basis set. The nature of stationary

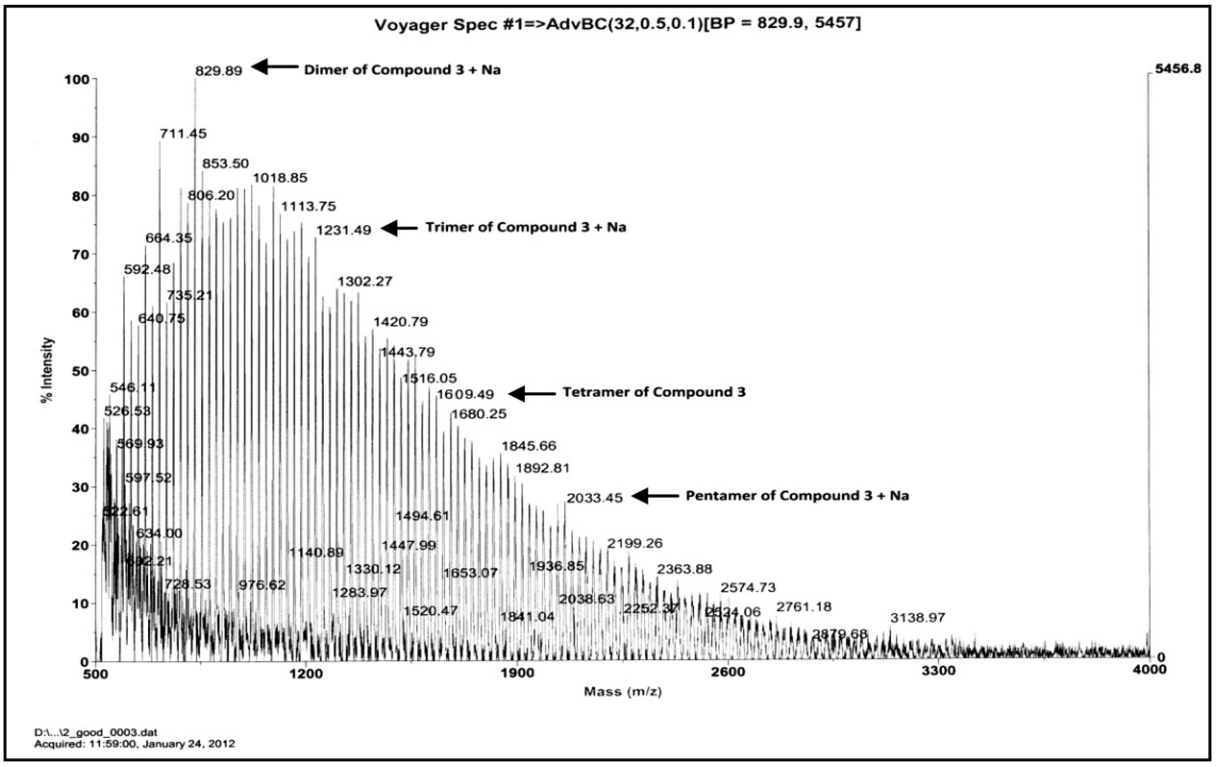
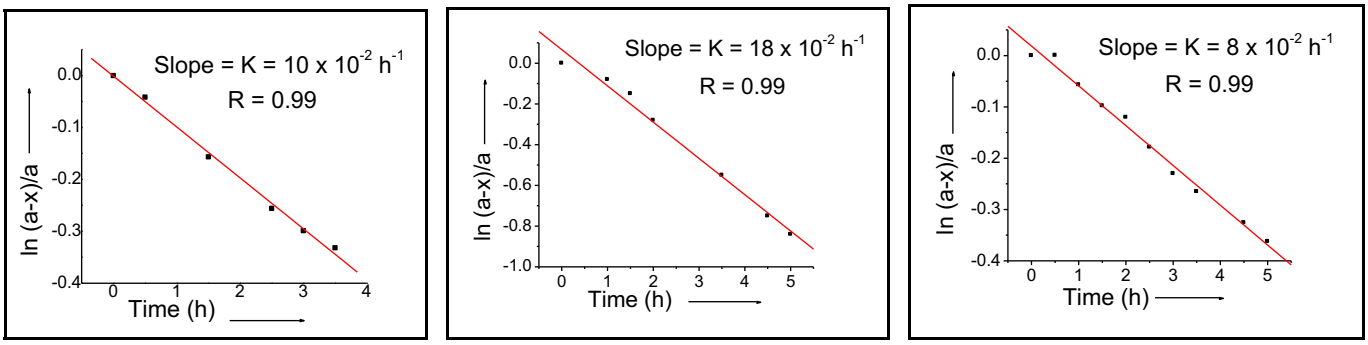


Fig. 3. MALDI mass spectrum of compound **3** heated at 170 °C in DMSO for 22 h.



Compound 1 **Compound 2** **Compound 3**

Fig. 4. Solution phase kinetic plots for class **A** enediynes.

Table 1
Solution phase (DMSO) reactivity of enediyne–nucleobase hybrids of class **A**[a]

Compound no.	Compound **1**	Compound **2**	Compound **3**
Solution phase kinetic rate constant (h^{-1})	10×10^{-2}	18×10^{-2}	8×10^{-2}

[a] Experimental error is within ±5%.

Table 2
Solid state reactivity of enediyne–nucleobase hybrids

Enediyne–nucleobase	DSC onset temperature (°C)		
	A–T pair	A–A pair	T–T pair
Class **A**	230 (for compound **1**)	207 (for compound **2**)	235 (for compound **3**)
Class **B**	177 (for compound **4**)	165 (for compound **5**)	240 (for compound **6**)
Class **C**	161 (for compound **7**)	151 (for compound **8**)	195 (for compound **9**)

points was characterized by vibrational frequency calculations. There are no imaginary frequencies in frequency analysis of all the starting enediynes and product biradicals, therefore each calculated structure is a local energy minimum and for all the transition states we observed a single imaginary frequency in the frequency analysis.

The relative energy profiles at all levels of theory for class **A** and **B** molecules (along with their imaginary frequencies in the parentheses) are shown in Figs. 5 and 6, respectively. Thus, the activation barriers for the cyclization step of enediyne **1**, **2** and **3** of class **A** are calculated to be 68, 67 and 70 kcal/mol, respectively, at PM3 level of theory, which supported the observed reactivity order A–A>A–T>T–T. At the B3LYP/6-31G (d) level of theory, the activation barrier also follows the same trend. Enediyne **5** of class **B** with two A bases appended at the two arm via short amide linkage, the energy barrier for the formation of product biradical **5p** from **5** is calculated as 33.7 kcal/mol at the B3LYP/6-31G* level of theory. The corresponding values for the other two compounds **4** and **6** were found to be higher. While the energy barrier (39.3 kcal/mol) for formation of product biradical **4p** from enediyne **4** is found to be higher than **5p**, it is almost similar in reactivity as that of **6p** whose activation barrier of formation from **6** is 38.4 kcal/mol.[24] The reaction profiles for class **A** and **B** molecules are shown in Fig. 7. Thus, computational results are more or less in agreement with the experimental observations. From the computations, it is clear that the activation barrier for the enediynes of class **B** with amide spacer is somewhat lower than that of the enediyne class **A**. This is again because of π-stacking as well as H-bonding interactions both of which are playing an important role in accelerating the reactivity of class **B** enediynes compared to that of the class **A** enediynes and thus supporting our experimental observation.

In conclusion, we have for the first time synthesized enediyne–nucleobase hybrids. Solution and solid phase studies indicated some degree of influence of the nucleobase pair on the reactivity of the enediynes towards BC. This influence originated from the π-stacking interactions between the base pairs, confirmed by VT-NMR studies. Interestingly, contrary to expectations, no significant intramolecular H-bonds are present between the base pairs. Current studies are aimed towards design of similar hybrids with reactivity profile under ambient conditions.

Table 3
Temperature coefficients ($\Delta\delta/\Delta T\times10^3$) of chemical shift of different hydrogens[a]

Compound no.	A-NH_2	T-NH	A-8H	A-2H	T-6H	T-Me
1	−7.99	−7.40	−1.13	+0.33	−1.56	+0.57
4	−6.74	−6.07	+0.52	−0.47	−1.22	+0.67
7	−6.50	−5.50	−0.94	−1.45	−1.73	+0.50

Compound no.	A-NH_2	A-8H	A-2H	Amide NH[b]	Triazole CH[c]
2	−7.25	−1.73	No change	—	—
5	−5.36	+0.34	−0.54	−3.86	—
8	−6.68	−1.13	−1.40	—	+0.35

Compound no.	T-NH	T-6H	T-Me	Amide NH	Triazole CH
3	−6.19	−1.28	+0.55	—	—
6	−6.32	−1.40	+0.61	−4.87	—
9	−5.99	−1.84	+0.51	—	−1.17

[a] Concentration of each compound was 5×10^{-3} M in DMSO-d_6.
[b] Temperature coefficients of chemical shift of two amide –NH of compound **4** are=−3.56 and -3.89×10^{-3} ppm/K.
[c] Temperature coefficients of two triazole CH of compound **7** are=−1.17 and $+2.97\times10^{-3}$ ppm/K.

4. Experimental

4.1. General

All reactions were conducted with oven-dried glassware under an atmosphere of argon (Ar) or nitrogen (N_2). All common reagents were commercial grade reagents and used without further purification. The solvents were dried by standard methods and purified by distillation before use. All crude products were purified by silica-gel column chromatography (60–120 mesh) with petroleum ether/ethyl acetate (P.E/E.A) as eluent and characterized by IR, NMR and mass spectrometry unless otherwise mentioned. ^1H and ^{13}C NMR spectroscopes of all compounds were recorded in 400 and 100 MHz, respectively, using $CDCl_3$ solvent unless otherwise noted. Infrared (FTIR) spectra were recorded as thin films on potassium bromide plates (solid sample), or in chloroform solvent (liquid sample) using Perkin Elmer Spectrum Rx1 spectrometer and are

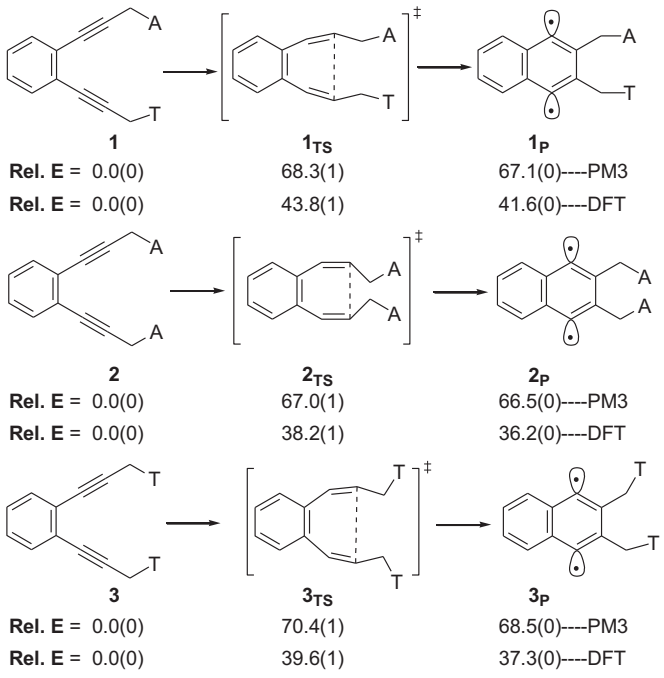


Fig. 5. Relative energies and the number of imaginary frequencies (in parentheses) at PM3 and B3LYP/6-31G* level of theories for the enediyne class **A**.

expressed as %-transmission (cm^{-1}). ESI-MS and HRMS were recorded using a Waters LCT mass spectrometer. The single crystal data was collected on Bruker APEX-2 CCD X-ray diffractometer that uses graphite monochromated MoKα radiation (λ=0.71073 Å) by hemisphere method. The structures are solved by direct methods and refined by least square methods on F^2 using SHELX-97. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were fixed at calculated positions and refined using a riding model.

4.2. General procedure for Sonogashira coupling of bromobenzene derivative (1a, 1b and 2a)

In an oven-dried flask dry *n*-butyl amine (10–20 mL) was taken and enough nitrogen gas was purged to degasify the solvent. Tetrakis-(triphenylphosphine) palladium (0) [$Pd(PPh_3)_4$] (0.03 equiv) catalyst was added carefully under nitrogen atmosphere. After 10 min of stirring, dibromobenzene/**1a** (3.36 mmol) and propargyl alcohol/THP-protected propargyl alcohol (1.1 equiv) were added successively. The reaction mixture was refluxed at 80–85 °C for 3–10 h (depending on mono or di coupling) under inert atmosphere. Progress of the reaction was monitored by TLC checking. On completion, it was quenched with water and extracted with EtOAc (3×15 mL). The combined organic layers were washed with a HCl solution (0.05 N, 25 mL), water and brine and then dried over Na_2SO_4 and concentrated. The compounds **1a**[25c] and **2a**[3c] are reported compounds and their spectral data are in agreement with those reported in literature.

*4.2.1. 3-{2-[3-(Tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-phenyl}-prop-2-yn-1-ol (**1b**).* Yellow viscous oil; R_f (P.E/E.A=3:1) 0.50; yield 80%; δ_H (200 MHz): 7.39–7.33 (m, 2H), 7.21–7.16 (m, 2H), 5.12 (br s, 1H), 4.49 (s, 2H), 4.45 (s, 2H), 3.86–3.74 (m, 1H), 3.57–3.52 (m, 1H), 1.76–1.51 (m, 6H); δ_C (50 MHz): 131.7, 131.5, 128.2, 128.0, 125.9, 125.3, 95.6, 92.2, 88.8, 85.1, 83.7, 61.5, 54.5, 51.2, 30.0, 25.4, 18.5.

4.3. General procedure for Sonogashira coupling of iodobenzene derivative (4a, 4b and 5a)

$Pd(PPh_3)_4$ (0.03 equiv) was added to degasified triethylamine (15 mL) followed by the addition of diiodobenzene/**4a** (3.06 mmol). After 15 min of stirring, copper iodide (0.10 equiv) and *N-tert-*

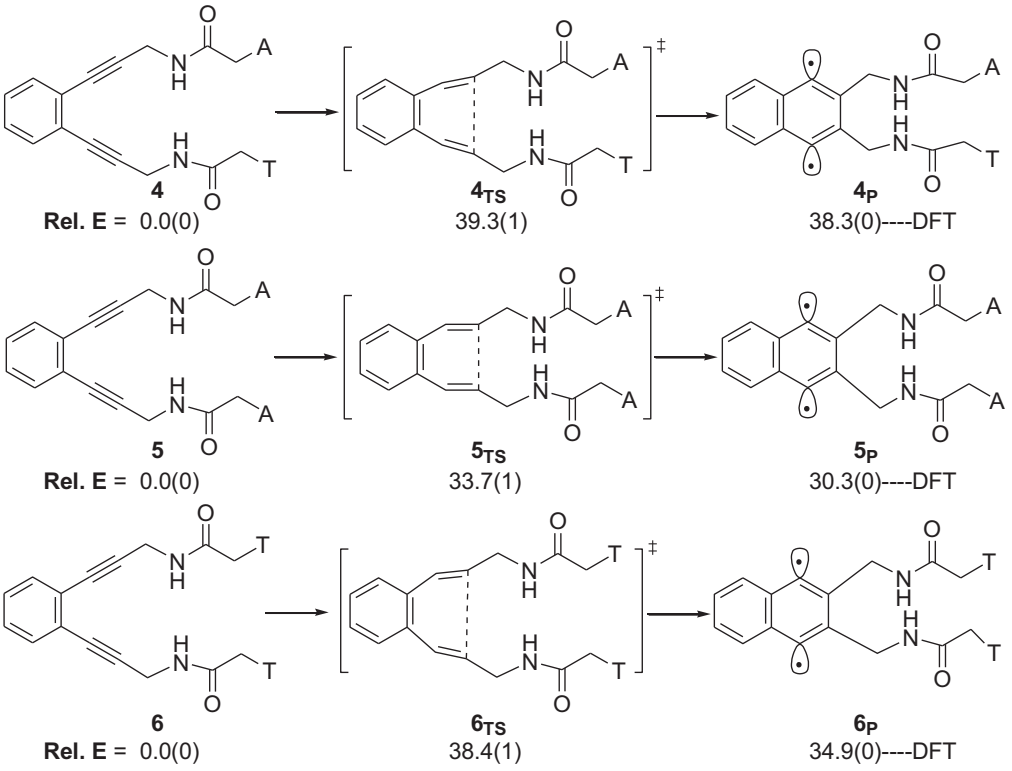


Fig. 6. Relative energies and the number of imaginary frequencies (in parentheses) at B3LYP/6-31G* level of theory for the enediyne class **B**.

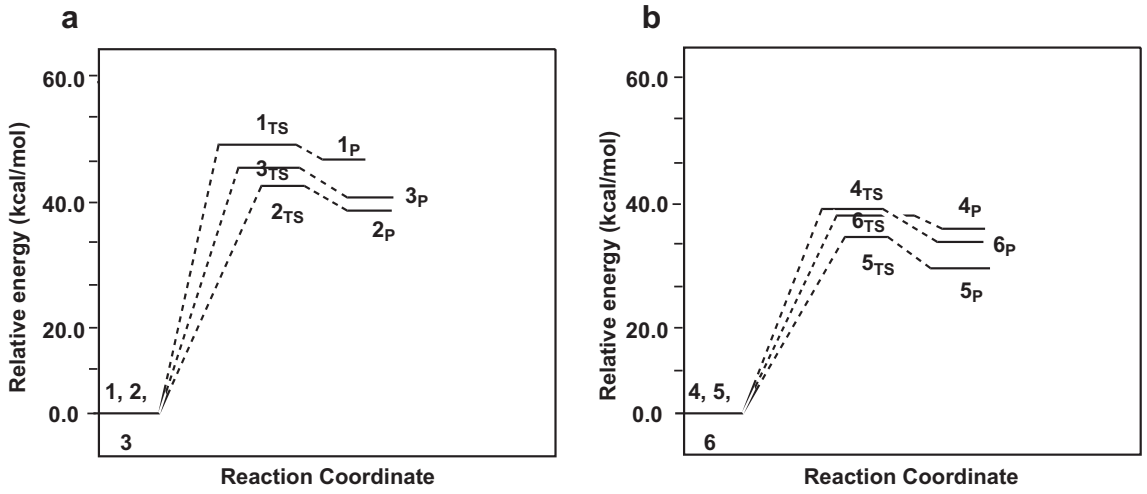


Fig. 7. Relative energy wise graphical representation of the enediynes reactants, transition states and product biradicals of class **A** and **B** enediynes.

butoxycarbonylprop-2-ynylamine (1.1 or 2.0 equiv, depending on mono or di coupling)/propargyl alcohol were added to it with 5 min interval and stirred for 8–12 h at room temperature. The reaction mixture was quenched with saturated aqueous solution of NH_4Cl (15 mL), and extracted with EtOAc (3×10 mL), dried over Na_2SO_4 and concentrated. The compound **4a**[25b] and **4b**[25b] are reported earlier and their spectral data are in agreement with those reported in literature.

*4.3.1. {3-[2-(3-tert-Butoxycarbonylamino-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (**5a**).* Brown gummy liquid; R_f (Purification, P.E/E.A=5:1) 0.40; yield 60%; δ_H (200 MHz): 7.39–7.35 (m, 2H), 7.25–7.19 (m, 2H), 5.20 (br s, 2H), 4.18–4.15 (m, 4H), 1.46 (br s, 18H); δ_C (50 MHz): 155.7, 131.9, 128.1, 125.8, 90.0, 81.8, 80.2, 31.6, 28.6.

4.4. General procedure for mesylation (1c, 1g, 2b, 4c and 7d)

Primary alcohol (**1b**/**1f**/**2a**/**4b**/**7c**) (2.52 mmol) was dissolved in dry DCM (10–20 mL) and cooled to 0 °C. Mesyl chloride (1.1 equiv) and triethylamine (1.5 equiv) were successively added to it dropwise and stirred for 15 min and monitored by TLC. On completion, checked by TLC, the reaction mixture was washed with brine solution, dried over Na_2SO_4 and concentrated. The compounds **1c**, **1g**, **2b**, **4c**, **7d** were synthesized according to the above general procedure among which the compounds **2b**[3c] and **4c**[25b] are reported compounds and their spectral data are in agreement with those reported in literature. Full characterization data of unknown compounds are listed here.

*4.4.1. Methanesulfonic acid 3-{2-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-phenyl}-prop-2-ynyl ester (**1c**).* Yellow oil; R_f (Purification, P.E/E.A=3:1) 0.45; yield 92%; δ_H (200 MHz): 7.43–7.37 (m, 2H), 2.27–7.21 (m, 2H), 5.07 (s, 2H), 4.89–4.87 (m, 1H), 4.47–4.45 (m, 2H), 3.86–3.75 (m, 1H), 3.55–3.47 (m, 1H), 3.15 (s, 3H), 1.77–1.51 (m, 6H); δ_C (50 MHz): 132.3, 132.2, 129.1, 128.3, 125.7, 123.8, 96.7, 89.8, 87.8, 84.6, 83.9, 61.9, 58.4, 54.5, 39.2, 30.2, 25.3, 18.9.

*4.4.2. Compound **1g**.* Yellow oil; R_f (P.E/E.A=1:1) 0.50; yield 80%; δ_H (200 MHz): 8.76 (s, 1H), 8.38 (s, 1H), 7.33–7.29 (m, 2H), 7.19–7.15 (m, 2H), 5.23 (s, 2H), 4.95 (s, 2H), 3.04 (s, 3H), 1.31 (s, 18H); δ_C (50 MHz): 152.8, 152.1, 150.5, 150.1, 132.3, 132.1, 129.2, 128.9, 128.6, 124.4, 124.0, 87.2, 85.4, 85.2, 84.5, 83.7, 58.1, 38.8, 34.2, 27.7; HRMS: found 582.2020. $C_{28}H_{31}N_5O_7S+H^+$ requires 582.2023.

*4.4.3. Methanesulfonic acid 3-(2-{3-[4-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-ylmethyl)-[1,2,3]triazol-1-yl]-prop-1-ynyl}-phenyl)-prop-2-ynyl ester (**7d**).* Yellow oil; R_f (3% MeOH in DCM as eluent) 0.25; yield 85%; δ_H (200 MHz): 10.03 (s, 1H), 8.04 (s, 1H), 7.45–7.38 (m, 2H), 7.33–7.27 (m, 3H), 5.42 (s, 2H), 5.10 (s, 2H), 4.95 (s, 2H), 3.11 (s, 3H), 1.83 (s, 3H); δ_C (50 MHz): 164.6, 151.2, 142.5, 140.4, 132.4, 132.3, 129.2, 129.0, 124.5, 124.2, 123.8, 111.2, 87.2, 85.3, 85.0, 84.7, 58.3, 43.0, 40.8, 39.0, 12.2; HRMS: found 454.1183. $C_{21}H_{19}N_5O_5S+H^+$ requires 454.1185.

4.5. General procedure for the synthesis of bromide (1d, 1h and 2c)

To a dry THF (10–20 mL) solution of mesylate (**1c**/**1g**/**2b**) (2.11 mmol) LiBr (1.5 and 3 equiv for mono and di substitution, respectively), dissolving in another fraction of dry THF (5 mL), was added dropwise for 5 min at 0 °C and stirred for 8–10 h. On completion, whole reaction mixture was poured into a saturated solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, concentrated. The bromo derivative **2c**[3c] is reported compound and its spectral data is in agreement with that reported in literature.

*4.5.1. 2-{3-[2-(3-Bromo-prop-1-ynyl)-phenyl]-prop-2-ynyloxy}-tetrahydro-pyran (**1d**).* Yellow oil; R_f (Purification, P.E/E.A=10:1) 0.40; yield 72%; δ_H (200 MHz): 7.41–7.36 (m, 2H), 7.24–7.17 (m, 2H), 4.96 (s, 1H), 4.52 (s, 2H), 4.17 (s, 2H), 3.90–3.79 (m, 1H), 3.56–3.50 (m, 1H), 1.89–1.53 (m, 6H); δ_C (50 MHz): 132.0, 128.5, 128.1, 125.7, 124.8, 96.5, 89.6, 88.2, 85.3, 84.1, 61.8, 54.6, 30.3, 25.4, 19.0, 15.2.

*4.5.2. Compound **1h**.* Yellow viscous oil; R_f (P.E/E.A=2:1) 0.40; yield 80%; δ_H (200 MHz): 8.79–8.78 (m, 1H), 8.44–8.43 (m, 1H), 7.33–7.30 (m, 2H), 7.20–7.16 (m, 2H), 5.24–5.23 (m, 2H), 4.06–4.05 (m, 2H), 1.35–1.34 (m, 18H); δ_C (50 MHz): 152.8, 152.1, 150.4, 150.3, 144.3, 132.1, 131.9, 128.9, 128.7, 128.6, 125.0, 124.1, 88.6, 85.3, 84.9, 84.7, 83.7, 34.4, 27.7, 14.9; HRMS: found 566.1402. $C_{27}H_{28}BrN_5O_4+H^+$ requires 566.1403.

4.6. General procedure for the synthesis of Boc-protected nucleobase attached enediyne (1e, 1i, 2d, 3a, 4g, 5c and 6a)

To a dry CH_3CN (15–20 mL) solution of bis-Boc-adenine/N^3-Boc thymine (1.0 or 2.0 equiv, depending on mono or di substitution) caesium carbonate (1 equiv or 2 equiv) was added and stirred for 0.5 h at room temperature. A colourless solution was obtained to which the bromide (**1d**/**1h**/**2c**/**4f**/**5b**) (0.50 mmol) dissolving in

5 mL dry CH_3CN solvent, was added dropwise and stirred for next 6–10 h at room temperature. Progress of the reaction was checked by TLC and on completion it was diluted with water and extracted with EtOAc (3×10 mL). Afterwards the combined organic layers were washed with water and brine, dried over Na_2SO_4 followed by evaporation in vacuum.

*4.6.1. Compound **1e**.* Yellow gummy oil; R_f (P.E/E.A=2:1) 0.50; yield 60%; δ_H (200 MHz): 8.88 (s, 1H), 8.54 (s, 1H), 7.50–7.42 (m, 2H), 7.35–7.27 (m, 2H), 5.32 (s, 2H), 5.00–5.98 (m, 1H), 4.54–4.53 (m, 2H), 3.92–3.80 (m, 1H), 3.59–3.49 (m, 1H), 1.86–1.62 (m, 5H), 1.45 (s, 19H); δ_C (50 MHz): 152.7, 151.9, 150.3, 150.1, 144.4, 132.1, 131.8, 128.7, 128.6, 128.2, 125.5, 124.0, 96.4, 89.6, 85.4, 84.4, 84.1, 83.4, 61.5, 54.4, 34.2, 30.1, 27.6, 25.2, 18.7; HRMS: found 588.2820. $C_{32}H_{37}N_5O_6+H^+$ requires 588.2822.

*4.6.2. Compound **1i**.* Colourless crystal; R_f (with 2% MeOH in DCM as eluent) 0.30; yield 54%; mp 98–100 °C; δ_H: 8.90 (s, 1H), 8.53 (s, 1H), 7.48–7.44 (m, 2H), 7.33–7.31 (m, 2H), 7.26 (s, 1H), 5.34 (m, 2H), 4.71 (m, 2H), 1.94 (s, 3H), 1.59 (s, 9H), 1.46 (s, 18H); δ_C: 161.5, 152.8, 152.2, 150.5, 150.3, 148.5, 147.9, 144.3, 138.5, 132.5, 132.0, 128.9, 128.8, 128.6, 124.5, 124.3, 111.1, 86.9, 85.7, 85.1, 84.8, 84.2, 83.8, 38.2, 34.2, 27.7, 27.4, 12.4; ν_{max} (KBr, cm^{-1}): ν 2926, 2854, 2344, 1784, 1717, 1667, 1602, 1581, 1457, 1371, 1338, 1258, 1146; HRMS: found 712.3096. $C_{37}H_{41}N_7O_8+H^+$ requires 712.3095.

*4.6.3. Compound **2d**.* Yellow oil; R_f (Flash column with silica-gel, 230–400 mesh using 1% MeOH in DCM as eluent) 0.25; yield 58%; δ_H: 8.93 (s, 2H), 8.52 (s, 2H), 7.48–7.45 (m, 2H), 7.35–7.33 (m, 2H), 5.31 (s, 4H), 1.47 (s, 36H); δ_C (50 MHz): 152.9, 152.3, 150.6, 150.4, 144.3, 132.2, 129.0, 128.7, 124.2, 85.1, 85.0, 83.8, 34.3, 27.8; ν_{max} (KBr, cm^{-1}): ν 2982, 2931, 2854, 2345, 2234, 1748, 1602, 1501, 1481, 1455, 1409, 1394, 1255, 1145; HRMS: found 821.3738. $C_{42}H_{48}N_{10}O_8+H^+$ requires 821.3735.

*4.6.4. Compound **3a**.* Colourless crystalline solid; R_f (P.E/E.A=1:2) 0.45; yield 60%; mp 138–140 °C; δ_H: 7.46–7.44 (m, 2H), 7.33–7.30 (m, 4H), 4.78 (s, 4H), 1.96 (s, 6H), 1.59 (s, 18H); δ_C (50 MHz): 161.6, 148.7, 148.0, 138.6, 132.3, 128.9, 124.8, 111.2, 86.9, 85.8, 84.3, 38.2, 27.5, 12.4; ν_{max} (KBr, cm^{-1}): ν 3439, 2092, 1781, 1709, 1659, 1443, 1371, 1235, 1145; HRMS: found 603.2454. $C_{32}H_{34}N_4O_8+H^+$ requires 603.2455.

*4.6.5. Compound **4g**.* Yellow oil; R_f (4% MeOH in DCM as eluent) 0.40; yield 56%; δ_H (200 MHz): 8.81 (s, 1H), 8.27 (s, 1H), 7.90 (br s, 1H), 7.34 (m, 2H), 7.23–7.21 (m, 2H), 5.42 (br s, 1H), 5.07 (s, 2H), 4.34–4.32 (m, 2H), 4.17–4.14 (m, 2H), 1.46 (s, 9H), 1.40 (s, 18H); δ_C (50 MHz): 165.6, 156.3, 153.5, 152.0, 150.4, 150.3, 145.9, 131.5, 128.4, 128.1, 125.6, 90.0, 88.4, 83.8, 82.1, 80.5, 45.8, 30.3, 28.4, 27.8; HRMS: found 660.3145. $C_{34}H_{41}N_7O_7+H^+$ requires 660.3146.

*4.6.6. Compound **5c**.* Yellow oil; R_f (Flash column with silica-gel, 230–400 mesh using 2% MeOH in DCM as eluent) 0.30; yield 58%; δ_H (200 MHz): 8.76 (s, 2H), 8.25 (s, 2H), 7.96 (br s, 2H), 7.35–7.30 (m, 2H), 7.21–7.17 (m, 2H), 4.91 (s, 4H), 4.23 (d, J=6.0 Hz, 4H), 1.41 (s, 36H); δ_C (50 MHz): 166.2, 153.7, 152.0, 150.6, 149.9, 146.3, 131.6, 128.3, 128.1, 125.5, 88.7, 84.1, 81.5, 45.5, 31.5, 27.7; ν_{max} (KBr, cm^{-1}): ν 2982, 2293, 1787, 1611, 1584, 1457, 1395, 1371, 1251, 1112; HRMS: found 935.4167. $C_{46}H_{54}N_{12}O_{10}+H^+$ requires 935.4164.

*4.6.7. Compound **6a**.* Colourless gummy oil; R_f (P.E/E.A=1:2) 0.45; yield 62%; δ_H (200 MHz): 7.67–7.64 (m, 2H), 7.35–7.30 (m, 2H), 7.24–7.17 (m, 2H), 7.06 (s, 2H), 4.39 (s, 4H), 4.24 (d, J=6.0 Hz, 4H), 1.87 (s, 6H), 1.55 (s, 18H); δ_C (50 MHz): 166.8, 161.6, 149.4, 148.1, 140.6, 131.6, 128.2, 125.4, 110.7, 88.6, 87.0, 81.5, 50.5, 30.2, 27.4, 12.2; ν_{max} (KBr, cm^{-1}): ν 3448, 2090, 1782, 1662, 1534, 1447, 1371, 1241, 1147; HRMS: found 717.2885. $C_{36}H_{40}N_6O_{10}+H^+$ requires 717.2884.

4.7. General procedure for the synthesis of azide (4d, 7a, 7e and 8a)

To a dry DMF (10–15 mL) solution of the mesylate (**4c**/**1c**/**7d**/**2b**) (0.32 mmol) sodium azide (1.5 or 3.0 equiv, depending on mono or di substitution) was added and stirred for 6–8 h at room temperature. Afterwards, it was quenched by water and extracted with EtOAc (3×10 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4 and concentrated under vacuum. The compounds **4d**[25b] and **8a**[25a] are reported compounds and their spectral data are in agreement with those reported in literature.

*4.7.1. 2-{3-[2-(3-Azido-prop-1-ynyl)-phenyl]-prop-2-ynyloxy}-tetrahydro-pyran (**7a**).* Yellow oil; R_f (Purification, P.E/E.A=10:1) 0.55; yield 82%; δ_H (200 MHz): 7.49–7.45 (m, 2H), 7.30–7.25 (m, 2H), 4.99–4.98 (m, 1H), 4.55 (s, 2H), 4.19 (s, 2H), 3.95–3.83 (m, 1H), 3.62–3.54 (m, 1H), 1.91–1.60 (m, 6H); δ_C (50 MHz): 132.2, 128.5, 128.1, 125.6, 124.6, 99.6, 89.7, 85.8, 85.0, 84.1, 61.8, 54.7, 40.6, 30.3, 25.4, 19.0.

*4.7.2. 1-(1-{3-[2-(3-Azido-prop-1-ynyl)-phenyl]-prop-2-ynyl}-1H-[1,2,3]triazol-4-ylmethyl)-5-methyl-1H-pyrimidine-2,4-dione (**7e**).* Yellow gummy oil; R_f (2% MeOH in DCM as eluent) 0.30; yield 78%; δ_H (200 MHz): 9.88 (s, 1H), 8.02 (s, 1H), 7.46–7.41 (m, 2H), 7.33–7.26 (m, 3H), 5.41 (s, 2H), 4.95 (s, 2H), 4.18 (s, 2H), 1.85 (s, 3H); δ_C (50 MHz): 164.5, 151.1, 142.4, 140.2, 132.4, 132.3, 129.0, 128.6, 124.9, 124.0, 123.6, 111.3, 85.6, 85.5, 85.4, 84.0, 42.8, 40.9, 40.6, 12.2; HRMS: found 401.1471. $C_{20}H_{16}N_8O_2+H^+$ requires 401.1475.

4.8. General procedure for THP-deprotection (1f and 7c)

THP-protected alcohol (**1e**/**7b**) (0.37 mmol) was dissolved in commercial grade EtOH (10–20 mL) and pyridinium *p*-toluene sulfonate (PPTS) (0.1 equiv) was added. Resulting reaction mixture was stirred for 6 h at room temperature. The solvent was removed under vacuum and the crude product was subjected to column chromatography without any further washing.

*4.8.1. Compound **1f**.* Pale yellow gummy oil; R_f (P.E/E.A=1:1) 0.60; yield 78%; δ_H (200 MHz): 8.81–8.80 (m, 1H), 8.67–8.66 (m, 1H), 7.30–7.28 (m, 2H), 7.15 (m, 2H), 5.21 (s, 2H), 4.40 (s, 2H), 1.37 (s, 18H); δ_C (50 MHz): 152.7, 152.1, 150.5, 150.2, 145.1, 132.2, 131.6, 128.8, 128.6, 128.1, 125.7, 123.8, 92.7, 86.0, 84.3, 84.0, 83.2, 50.8, 34.6, 27.7; HRMS: found 504.2248. $C_{27}H_{29}N_5O_5+H^+$ requires 504.2247.

*4.8.2. 1-(1-{3-[2-(3-Hydroxy-prop-1-ynyl)-phenyl]-prop-2-ynyl}-1H-[1,2,3]triazol-4-ylmethyl)-1H-pyrimidine-2,4-dione (**7c**).* White solid; R_f (3% MeOH in DCM as eluent) 0.20; yield 86%; mp 165 °C; δ_H (200 MHz, $CDCl_3$ and DMSO-d_6): 10.84 (s, 1H), 8.08 (s, 1H), 7.29 (m, 2H), 7.26 (s, 1H), 7.20–7.16 (m, 2H), 5.36 (s, 2H), 4.85 (s, 2H), 4.34 (s, 2H), 1.72 (s, 3H); δ_C (50 MHz, $CDCl_3$ and DMSO-d_6): 169.5, 156.0, 147.4, 145.0, 136.7, 133.7, 132.8, 130.6, 128.6, 128.5, 115.4, 98.2, 90.4, 89.0, 87.3, 55.3, 47.4, 34.3, 17.0; ν_{max} (KBr, cm^{-1}): ν 2931, 2264, 1672, 1479, 1431, 1388, 1364, 1253, 1123; HRMS: found 376.1412. $C_{20}H_{17}N_5O_3+H^+$ requires 376.1410.

*4.8.3. {3-[2-(3-Amino-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (**4e**).* {3-[2-(3-Azido-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid *tert*-butyl ester (**4d**) (700 mg,

2.26 mmol) was dissolved in THF (25 mL). 3–4 drops water and triphenylphosphine (1.18 g, 4.51 mmol) were added to the solution. The reaction mixture was stirred for 6 h at room temperature and then concentrated. The crude product was purified by column chromatography (10% MeOH in DCM as eluent).

4.9. General procedure for bromoacylation of amine (4f and 5b)

Primary amine (**4e**/bis-Boc-protected diamine **5a** after Boc deprotection with TFA) was dissolved in dry DCM (30 mL) at 0 °C under nitrogen atmosphere. Bromoacetyl chloride (1.2 and 2.2 equiv for mono and di substitution, respectively) and triethylamine (1.4 or 2.5 equiv) were successively added and stirred for next 10–15 min maintaining 0 °C temp. On completion, it was quenched with water and extracted with DCM (2×15 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4 and concentrated. It is to be noted that rapid quenching of the reaction mixture can diminish the formation of unwanted side products.

*4.9.1. 2-Bromo-N-(3-{2-[3-(2-bromo-acetylamino)-prop-1-ynyl]-phenyl}-prop-2-ynyl)-acetamide (**5b**).* Brown liquid; R_f (Purification, P.E/E.A=1:1) 0.45; yield 67%; δ_H (200 MHz): 7.43–7.39 (m, 2H), 7.29–7.24 (m, 2H), 4.36–4.34 (m, 4H), 3.95 (s, 4H); δ_C (50 MHz): 166.2, 132.0, 128.5, 125.6, 88.3, 82.2, 31.1, 28.9.

4.10. General procedure for Boc deprotection (1, 2, 3, 5 and 6)

To an ice cold dry DCM (10–20 mL) solution of *N*-Boc protected enediynyl amine (**5a**)/*N*-Boc protected nucleobase-linked enediyne (**1i**/**2d**/**3a**/**4g**/**5c**/**6a**) (0.20 mmol) trifluoroacetic acid (TFA) (25–50 equiv) was added dropwise. The whole reaction mixture was stirred for 2 h at 0 °C and for another 2 h at room temperature for complete deprotection. After that the reaction mixture was concentrated under reduced pressure by liquid nitrogen while the excess TFA was evaporated out. Resulting gummy mass was washed by dry benzene (3×10 mL) and subjected to column chromatography (silica-gel, 60–120 mesh, MeOH in DCM as eluent) to get free amine in pure form.

*4.10.1. 1-(3-{2-[3-(6-Amino-purin-9-yl)-prop-1-ynyl]-phenyl}-prop-2-ynyl)-5-methyl-1H-pyrimidine-2,4-dione (**1**).* White solid; R_f (Flash column with silica-gel, 230–400 mesh using 15% MeOH in DCM as eluent) 0.35; yield 45%; mp 250 °C (decomposition); δ_H (DMSO-d_6): 11.42 (s, 1H), 8.28 (s, 1H), 8.18 (s, 1H), 7.57 (s, 1H), 7.48 (br s, 2H), 7.38–7.37 (br s, 2H), 7.27 (s, 2H), 5.29 (s, 2H), 4.71 (s, 2H), 1.75 (s, 3H); δ_C (DMSO-d_6): 164.2, 156.0, 152.8, 150.5, 149.1, 140.2, 140.0, 132.0, 131.9, 129.1, 129.0, 124.2, 124.1, 118.5, 109.6, 88.3, 88.0, 82.4, 82.2, 37.2, 33.0, 12.0; ν_{max} (KBr, cm^{-1}): ν 3363, 3216, 2922, 2851, 2363, 1673, 1604, 1475, 1387, 1332, 1294; MS: m/z=434.39 [MNa^+], 412.39 [MH^+]; HRMS: found 412.1518. $C_{22}H_{17}N_7O_2+H^+$ requires 412.1522.

*4.10.2. Compound **2**.* Light brown solid; R_f (Flash column with silica-gel, 230–400 mesh using 15% MeOH in DCM as eluent) 0.35; yield 52%; mp 270 °C (dec); δ_H (DMSO-d_6): 8.31 (br s, 2H), 8.20 (br s, 2H), 7.48–7.46 (m, 2H), 7.38–7.34 (m, 2H), 7.28 (s, 4H), 5.26 (s, 4H); δ_C (DMSO-d_6): 156.5, 153.3, 149.6, 140.7, 132.5, 129.6, 124.5, 119.0, 88.4, 82.9, 33.5; ν_{max} (KBr, cm^{-1}): ν 2921, 2851, 2365, 1687, 1638, 1578, 1474, 1420, 1384, 1332; MS: m/z=443.38 [MNa^+], 421.41 [MH^+]; HRMS: found 421.1632. $C_{22}H_{16}N_{10}+H^+$ requires 421.1638.

*4.10.3. Compound **3**.* Light brown solid; R_f (Flash column with silica-gel, 230–400 mesh using 6% MeOH in DCM as eluent) 0.30; yield 54%; mp 230 °C (dec); δ_H (DMSO-d_6): 11.4 (s, 2H), 8.35 (s, 2H), 7.66 (s, 2H), 7.49–7.47 (m, 2H), 7.39–7.36 (m, 2H), 4.75 (s, 4H), 1.76 (s, 3H); δ_C (DMSO-d_6): 164.2, 150.5, 140.2, 132.0, 129.1, 124.3, 109.5, 88.4, 82.2, 37.2, 12.0; ν_{max} (KBr, cm^{-1}): ν 2953, 2843, 2080, 1774, 1654, 1528, 1450, 1422, 1355, 1245, 1016, 759; HRMS: found 403.1400. $C_{22}H_{18}N_4O_4+H^+$ requires 403.1406.

*4.10.4. 2-(6-Amino-purin-9-yl)-N-[3-(2-(6-amino-purin-9-yl)-acetylamino]-prop-1-ynyl}-phenyl)-prop-2-ynyl]-acetamide (**5**).* White solid; R_f (Flash column with silica-gel, 230–400 mesh using 15% MeOH in DCM as eluent) 0.35; yield 60%; mp 250 °C (dec); δ_H (DMSO-d_6): 8.85 (br s, 2H), 8.08–8.06 (m, 4H), 7.44–7.43 (br s, 2H), 7.37–7.36 (br s, 2H), 7.19 (br s, 4H), 4.88 (s, 4H), 4.23–4.22 (m, 4H); DEPT-135 (DMSO-d_6): 152.3 (CH), 141.6 (CH), 131.7 (CH), 128.5 (CH), 44.7 (CH_2), 28.8 (CH_2); ν_{max} (KBr, cm^{-1}): ν 3461, 2078, 1734, 1718, 1654, 1542, 1474, 1208; HRMS: found 535.2065. $C_{26}H_{22}N_{12}O_2+H^+$ requires 535.2067.

*4.10.5. 2-(5-Methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-N-[3-(2-{3-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetylamino]-prop-1-ynyl}-phenyl)-prop-2-ynyl]-acetamide (**6**).* White solid; R_f (Flash column with silica-gel, 230–400 mesh using 10% MeOH in DCM as eluent) 0.25; yield 65%; mp 270 °C (dec); δ_H (DMSO-d_6): 11.26 (s, 2H), 8.69–8.67 (m, 2H), 7.42 (br s, 4H), 7.36–7.34 (m, 2H), 4.31 (s, 4H), 4.21–4.20 (m, 4H), 1.72 (s, 6H); δ_C (DMSO-d_6): 166.9, 164.5, 151.0, 142.3, 131.8, 128.6, 124.7, 108.1, 90.6, 80.3, 49.3, 28.8, 11.9; ν_{max} (KBr, cm^{-1}): ν 3432, 2922, 2378, 1720, 1710, 1678, 1550, 1474, 1435, 1310, 1268, 1212; HRMS: found 517.1832. $C_{26}H_{24}N_6O_6+H^+$ requires 517.1836.

4.11. Synthesis of 2-(6-amino-purin-9-yl)-*N*-[3-(2-{3-[2-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetylamino]-prop-1-ynyl}-phenyl)-prop-2-ynyl]-acetamide (4)

1-(Carboxymethyl) thymine (50 mg, 0.29 mmol) was taken in a mixture of dry DCM (15 mL) and dry DMF (3 mL) solution for the uniform solubility. It was cooled to 0 °C followed by the addition of EDC·HCl (67 mg, 0.35 mmol) and HOBt (48 mg, 0.35 mmol) under N_2 atmosphere. After 1 h of stirring, the free amine **4h** (0.30 mmol) and DMAP (15 mg, 0.12 mmol) were successively added and continued the stirring for 10 h at room temperature. On completion, it was extracted with EtOAc (3×15 mL), washed with water and brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash column chromatography.

*4.11.1. 2-(6-Amino-purin-9-yl)-N-[3-(2-{3-[2-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetylamino]-prop-1-ynyl}-phenyl)-prop-2-ynyl]-acetamide (**4**).* White solid; R_f (230–400 mesh, 8% MeOH in DCM as eluent) 0.40; yield 52%; mp 230 °C (dec); δ_H (DMSO-d_6): 11.27 (s, 1H), 8.98 (br s, 1H), 8.81 (br s, 1H), 8.08–8.07 (m, 2H), 7.43 (m, 3H), 7.36–7.35 (m, 2H), 7.18 (s, 1H), 4.91 (s, 2H), 4.32 (s, 2H), 4.22 (m, 4H), 1.70 (s, 3H); DEPT-135 (DMSO-d_6): 152.3 (CH), 142.1 (CH), 141.6 (CH), 131.7 (CH), 128.5 (CH), 49.2 (CH_2), 44.7 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 11.7 (CH_3); ν_{max} (KBr, cm^{-1}): ν 3412, 2922, 2346, 1723, 1710, 1680, 1638, 1550, 1474, 1310, 1268; HRMS: found 512.1791. $C_{25}H_{21}N_9O_4+H^+$ requires 512.1795.

4.12. General procedure of click reaction (7b, 7, 8 and 9)

In a degassed aqueous (10 mL) solution of $CuSO_4.H_2O$ (10 mol %) sodium ascorbate (20 mol %) was added and stirred for 10 min followed by the addition of N^1-propargyl thymine/N^9-propargyl adenine (0.48 mmol) under inert condition. Another degassed solution of azide (**7a**/**7e**/**8a**) (1.1 equiv) in ^{t}BuOH (10 mL) was added to this solution after 10 min and stirred for 10–24 h at room temperature. On completion, it was quenched with saturated NH_4Cl solution (15 mL) and extracted with EtOAc (3×10 mL), washed with water, brine, dried over Na_2SO_4 and concentrated under vacuum.

*4.12.1. 5-Methyl-1-[1-(3-{2-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-phenyl}-prop-2-ynyl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-pyrimidine-2,4-dione (**7b**).* White solid; R_f (P.E/E.A=1:3) 0.38; yield 65%; mp 157 °C; δ_H (200 MHz): 10.03 (s, 1H), 8.05 (s, 1H), 7.43–7.36 (m, 2H), 7.32 (s, 1H), 7.26–7.21 (m, 2H), 5.40 (s, 2H), 4.94–4.92 (s, 2H), 4.49–4.48 (s, 2H), 3.85–3.74 (m, 1H), 3.52–3.47 (m, 1H), 1.65–1.49 (m, 6H); δ_C (50 MHz): 164.6, 151.1, 140.2, 132.2, 132.1, 128.9, 128.2, 125.7, 123.9, 123.6, 111.1, 96.5, 89.7, 85.9, 84.1, 83.6, 61.8, 54.6, 42.7, 40.9, 30.2, 25.3, 18.9, 12.2; HRMS: found 460.1983. $C_{25}H_{25}N_5O_4+H^+$ requires 460.1985.

*4.12.2. 1-{1-[3-(2-{3-[4-(6-Amino-purin-9-ylmethyl)-[1,2,3]triazol-1-yl]-prop-1-ynyl}-phenyl)-prop-2-ynyl]-1H-[1,2,3]triazol-4-ylmethyl}-5-methyl-1H-pyrimidine-2,4-dione (**7**).* Brown solid; R_f (10% MeOH in DCM as eluent) 0.40; yield 52%; mp 135 °C (dec); δ_H (DMSO-d_6) 11.31 (s, 1H), 8.24 (s, 2H), 8.19 (s, 2H), 8.14 (s, 2H), 7.62 (s, 1H), 7.53–7.46 (m, 2H), 7.42–7.40 (m, 4H), 5.60 (s, 4H), 5.47 (s, 2H), 4.91 (s, 2H), 1.71 (s, 3H); δ_C (DMSO-d_6): 164.3, 155.9, 152.5, 150.8, 149.3, 143.0, 142.9, 141.3, 140.8, 132.1, 129.4, 123.9, 123.7, 118.6, 109.0, 86.9, 83.4, 42.3, 39.8, 38.0, 12.0; ν_{max} (KBr, cm^{-1}): ν 2925, 2298, 1680, 1654, 1580, 1474, 1412, 1310; HRMS: found 574.2175. $C_{28}H_{23}N_{13}O_2+H^+$ requires 574.2176.

*4.12.3. Compound **8**.* Brown solid; R_f (10% MeOH in DCM as eluent) 0.35; yield 54%; mp 125 °C (dec); δ_H (DMSO-d_6): 8.25 (s, 2H), 8.23 (s, 2H), 8.12 (s, 2H), 7.51–7.49 (m, 2H), 7.42–7.39 (m, 2H), 7.30 (br s, 4H), 5.59 (s, 4H), 5.47 (s, 4H); δ_C (DMSO-d_6): 155.7, 152.3, 149.3, 142.9, 140.9, 132.0, 129.3, 123.9, 123.7, 118.6, 86.9, 83.4, 39.8, 38.0; ν_{max} (KBr, cm^{-1}): ν 3335, 2925, 2853, 2362, 1686, 1654, 1604, 1578, 1479, 1420, 1384, 1303, 1245, 1129, 1053; HRMS: found 583.2290. $C_{28}H_{22}N_{16}+H^+$ requires 583.2292.

*4.12.4. Compound **9**.* White solid; R_f (6% MeOH in DCM as eluent) 0.38; yield 70%; mp 155–158 °C; δ_H (DMSO-d_6): 11.28 (s, 2H), 8.19 (s, 2H), 7.62 (s, 2H), 7.53–7.51 (m, 2H), 7.42–7.40 (m, 2H), 5.59 (s, 4H), 4.92 (s, 4H), 1.72 (s, 6H); δ_C (DMSO-d_6): 164.3, 150.8, 142.9, 141.3, 132.1, 129.4, 123.9, 123.7, 108.9, 86.9, 83.4, 42.3, 39.8, 12.0; ν_{max} (KBr, cm^{-1}): 3356, 2924, 2354, 1686, 1650, 1612, 1594, 1468, 1356, 1324; HRMS: found 565.2058. $C_{28}H_{24}N_{10}O_4+H^+$ requires 565.2060.

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Supplementary data

Crystallographic data and crystallographic information files (CIFs) for compound **1i** and **3a**, DSC graphs, solution phase kinetics plots, VT NMRs for all compounds, Cartesian coordinates for all optimized geometries, copies of ^1H and ^{13}C NMR spectra. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tet.2012.07.093.

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12. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 871969 and 872945 for **1i** and **3a**, respectively. The difference in c, d-distance is also reflected in their thermal reactivity.
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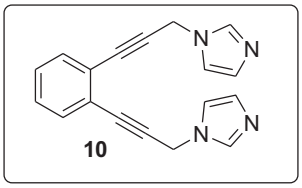
20. In order to clarify our doubt arising out of the assumption that decomposition of triazole moiety might cause the decrease in the resultant onset temperatures for triazole spacer containing enediyne–nucleobase hybrids (**7**–**9**), compound **10** was separately prepared. The onset temperature of compound **10** was found to be 165 °C whereas that of compounds **7**–**9** varied from 161–195 °C. Thus the considerable difference in onset temperatures between enediyne–nucleobase derivatives and compound **10** clearly proves the existence of some interstrand effect induced by the appended nucleobases on BC. DSC curve of compound **10** is included in SI, Fig. S3.



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24. While the DSC onset temperature deals with the solid state aggregation of the crystal, the DFT calculation deals with the gas phase. Therefore, it may be possible that the reactivity may slightly differ due to differences in molecular association between these two states. Thus, we observed a difference of 0. 9 kcal/mol in activation energy for compound **4** and **6** in our gas phase calculation [(vide Ref. Zeidan, T. A.; Kovalenko, S. V.; Manoharan, M.; Alabugin, I. V. *J. Org. Chem.* **2006**, *71*, 962)].
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