Zuschriften
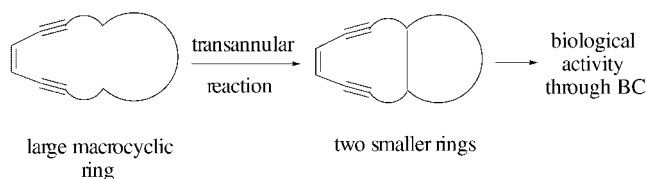
carried out to reduce the ring size of a large macrocyclic enediyne, which in turn should enhance the rate of the Bergman cyclization (Scheme 1).[9] Herein, we report the synthesis of two novel macrocyclic N-substituted enediynyl


Scheme 1. Design of macrocyclic enediynes.

Activation of Macrocyclic Enediynes by Transannular Cyclization**

Amit Basak,* Sandip Kumar Roy, and Subrata Mandal

Species containing single electrons were long known to possess cytotoxic activity through DNA damage; however, this property could not be utilized in the development of antitumor agents because methods to generate radicals under mild conditions were not known until Mother Nature revealed how it could be done through the chemistry of naturally occurring enediynes.[1] Although these enediynes possess potent antitumor activity, their extreme toxicity acted as a deterrent for their direct medical use. This prompted chemists to synthesize analogous molecules with the hope of reducing the toxicity without sacrificing the antitumor activity to a great extent. Designed enediynes can be broadly classified into two groups: one comprising intrinsically reactive enediynes stabilized by the use of a locking device. Fusion of small strained rings,[2] incorporation of sp²-carbon atoms,[3] tuning of redox characteristics,[4] and the ability to undergo allylic rearrangement[5] are some examples of locking devices. The other group comprises ambiently unreactive enediynes, usually acyclic, that are made active in situ by a suitable triggering reaction such as isomerization to eneyne allenes,[6] and ligation to metal ions.[7] Examples are also known in which a reactive enediyne core has been generated from a prodrug. As macrocyclic enediynes[8] with 11-membered rings or higher are stable at physiological temperature, it would be possible to broaden the scope of the second approach if an intramolecular transannular reaction were

ketones **1** and **2**. Ways to control their chemical and biological activity are also reported. To our knowledge, this is the first
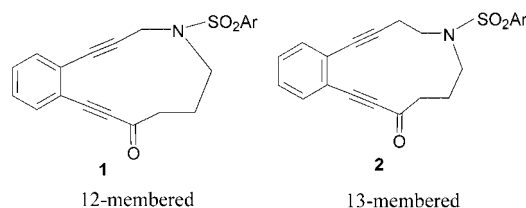
example of the use of a transannular reaction for enediyne activation,[10] although transannular reactions have been used to activate promitosene, a precursor to mitosene, under photoirradiation conditions.[11]

The retrosynthesis of the enediyne **1** (Scheme 2) involves three critical bond-forming reactions, namely the construction
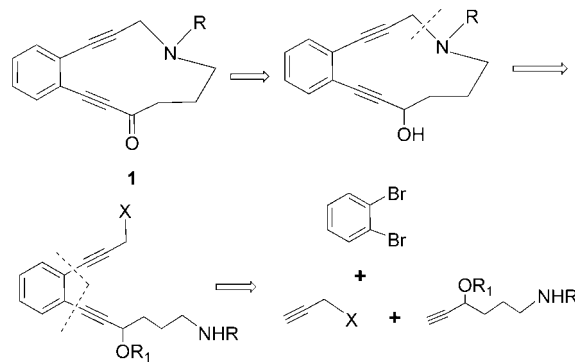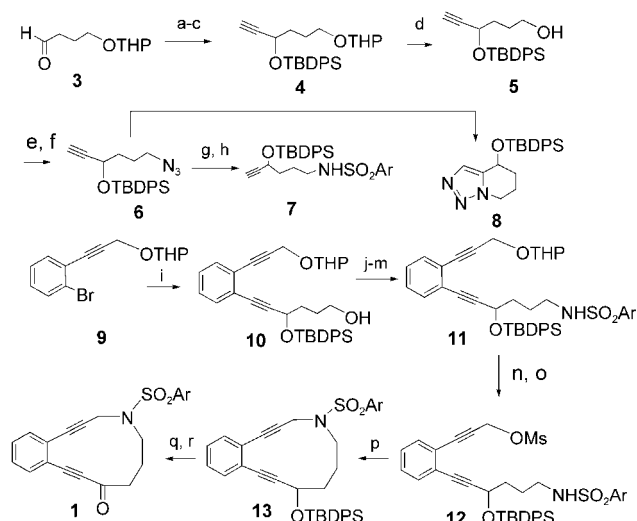

Scheme 2. Retrosynthesis of enediyne **1**.

of the enediyne framework by two successive Sonogashira couplings,[12] followed by macrocyclization by intramolecular N-alkylation.[13] The actual synthesis was, however, executed with a slight modification. Azide **6**, the precursor for the protected amine **7** proved to be unstable as it undergoes facile intramolecular cycloaddition with the alkyne functionality.[14] This forced us to do the second palladium(0)-mediated coupling with protected hydroxy alkyne **5** instead of **7** (Scheme 3). The resulting enediyne **10** was converted into the 4-nitrosulfonamide **11** by the following sequence of

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Scheme 3. Synthesis of enediyne **1**. a)TMS-acetylene, nBuLi, THF, 0°C; b) CsF, MeOH, RT; c) TBDPS-Cl, imidazole, DMAP, CH$_2$Cl$_2$, RT; d) PPTS, EtOH, 50°C; e) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0°C; f)NaN$_3$, DMF, RT; g) PPh$_3$, moist THF, RT; h) ArSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, 0°C; i) **5**, [Pd(Ph$_3$)$_4$], nBuNH$_2$, reflux; j) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0°C; k) NaN$_3$, DMF, RT; l) PPh$_3$, moist THF, RT; m) ArSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, 0°C; n) PPTS, EtOH, 50°C; o) MsCl, Et$_3$N, 0°C; p) K$_2$CO$_3$, DMF, RT; q) MeOH, 3% HCl, RT; r) PCC, CH$_2$Cl$_2$, RT. Ar = 4-nitrophenyl; THP = tetrahydropyranyl; TBDPS = *tert*-butyldiphenylsilyl; PCC = pyridinium chlorochromate.

reactions: mesylation, displacement with NaN$_3$,[15] reduction into the amine,[16] and protection with 4-nitrobenzene sulfonyl chloride. Removal of THP followed by treatment with mesyl chloride and Et$_3$N furnished the mesylate **12**. Intramolecular N-alkylation in K$_2$CO$_3$/DMF under high dilution worked quite well to give the 12-membered enediyne **13** as the only isolable product in about 60% yield.[17] This was then converted into the ketone **1**.

The synthesis of the 13-membered enediyne **2** could not be achieved in a similar way as intramolecular N-alkylation at a homopropargylic center is known to produce only the elimination product.[18] The synthesis was completed by intramolecular N-alkylation in the reverse direction (Scheme 4). As expected, this step was free from any elimination. The structures of the cyclic enediynes were confirmed by NMR and mass spectral studies. The appearance of peaks at *m/z* 431 [*M*−Na$^+$] for **1** and at *m/z* 423 [*M*−H$^+$] and 445 [*M*−Na$^+$] for **2** in the mass spectrum (ES + mode) confirmed that only intramolecular N-alkylation has occurred and ruled out the formation of any dimeric or oligomeric products during cyclization.

The sulfonamido ketone **1** was then deprotected by using thiophenol and K$_2$CO$_3$ in DMF[19] to generate the 12-membered free amino ketone **20**.[20] Compound **20** proved to be stable at room temperature and no significant decomposition was observed when a solution of **20** in CHCl$_3$ was kept at 37°C for seven days. Moreover, **20** could be converted back to the starting sulfonamide **1** upon treatment with 4-nitrobenzenesulfonyl chloride and triethylamine, thus demonstrating the inherent stability of **20**. Attempts to induce transannular cyclization to the aminol with catalytic amounts of *p*-toluenesulfonic acid (PTSA) also failed. On the contrary,
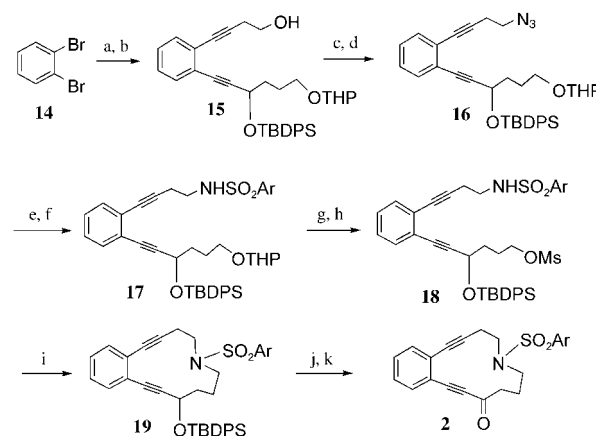
Scheme 4. Synthesis of enediyne **2**. a) 3-butyn-1-ol, [Pd(PPh$_3$)$_4$], nBuNH$_2$, reflux; b) **4**, [Pd(PPh$_3$)$_4$], nBuNH$_2$, reflux; c) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0°C; d) NaN$_3$, DMF, RT; e) PPh$_3$, moist THF, RT; f) ArSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, 0°C; g) PPTS, EtOH, 50°C; h) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0°C; i) K$_2$CO$_3$, DMF, RT; j) 3% HCl, MeOH/THF (4:1), RT; k) PCC, CH$_2$Cl$_2$, RT. Ar = 4-nitrophenyl; PPTS = pyridinium *p*-toluenesulfonate.

deprotection of the sulfonamido group in ketone **2** gave the product amine **21**, which remained mostly in the unstable aminol form **22** (as indicated by its inability to be reconverted to the starting sulfonamide **2**). The aminol, being a 10-membered enediyne, decomposes in CHCl$_3$ even at 4°C ($t_{1/2} \sim$ 40 h) to give the new product **24**.[21] The occurrence of a Bergman cyclization was indicated by the disappearance of all four acetylenic carbon atoms, which are now replaced by signals for aromatic carbon atoms. The probable mechanism of formation of **24** is shown in Scheme 5. That the mechanism
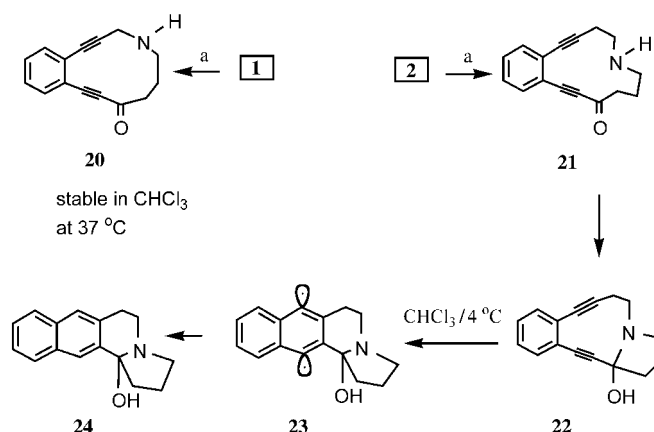
Scheme 5. Fate of aminoenediynes. a) PhSH, K$_2$CO$_3$, DMF, RT.

involves the generation of the diradical **23** is also supported by the interaction of the enediyne with supercoiled DNA. Thus **22** showed single-strand cuts to generate form II when incubated with double-stranded plasmid DNA (pBR 322) in the supercoiled form (Figure 1). The corresponding sulfonamido ketone **2**, under similar conditions, did not show any DNA damage, thus ruling out a possible cleavage mechanism by the Michael addition of nucleophilic bases of DNA to the
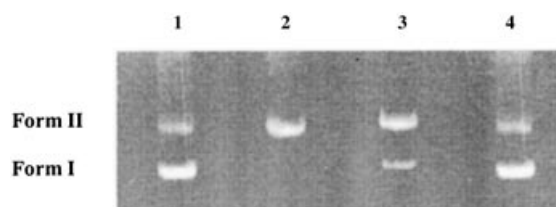
Figure 1. DNA cleavage studies. Lane 1: DNA in TAE buffer (pH 8, 0.4 µM/bp) (5 µL) + acetonitrile (15 µL) at 37°C; lane 2: DNA in TAE buffer (pH 8, 0.4 µM/bp) (5 µL) + aminol (**22**) (40 µM, 48 h) in acetonitrile (15 µL) at 37°C; lane 3: DNA in TAE buffer (pH 8, 0.4 µM/bp) (5 µL) + aminol (**22**) (40 µM, 24 h) in acetonitrile (15 µL) at 37°C; lane 4: DNA in TAE buffer (pH 8, 0.4 µM/bp) (5 µL) + sulfonamide (**2**) (40 µM, 48 h) in acetonitrile (15 µL) at 37°C.

unsaturated carbonyl system. The stability of the 12-membered amino ketone **20** is ascribed to its inability to undergo the transannular reaction, as it involves the formation of a nine-membered enediyne, which is a kinetically disfavored reaction.[22]

In conclusion, we have synthesized two novel N-based macrocyclic enediynes and have been able to activate one of these by a transannular reaction. The use of a 4-nitrophenylsulfonyl group as a deactivating device that renders the nitrogen non-nucleophilic has been demonstrated. Thiol-mediated deprotection can act as a triggering mechanism that activates the enediyne through an intramolecular cyclization reaction. When the protected amino ketone **2** was incubated with supercoiled DNA in the presence of glutathione, a biological thiol, at a pH of 8.0, it was able to cleave supercoiled DNA (Figure 2). This observation has proven
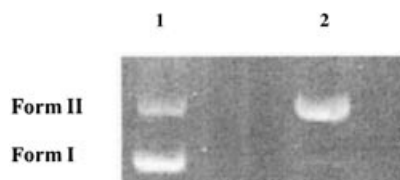

Figure 2. DNA cleavage studies. Lane 1: DNA in TAE buffer (pH 8, 0.4 µM/bp) (5 µL) + acetonitrile (15 µL) at 37°C; lane 2: DNA in TAE buffer (pH 8, 0.4 µM/bp) (7 µL) + glutathione (25 µM) in TAE buffer (pH 8, 20 µL) + sulfonamido ketone (**2**) (20 µm, 48 h) in acetonitrile (40 µL) at 37°C.

our design; namely, the activation by way of a biological thiol-mediated triggering of enediyne towards Bergman cyclization. Considering the repertoire of amine-protecting groups and different methods for their deprotection, our design offers a wide array of triggering mechanisms for the development of antitumor agents that work through generation of diradicals.

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[14] a) W. Lwowski in *1,3-Dipolar Cycloaddition Chemistry, Vol. 1* (Ed.: A. Padwa), Wiley Interscience, New York, **1984**, p. 559;

b) P. K. Kadaba, B. Stanovnik, M. Tisler in *Advances in Heterocyclic Chemsitry, Vol. 37* (Ed.: A. R. Kartrizsky), Academic Press, New York, **1984**, p. 217.

[15] The azide derived from **10** is stable and does not undergo intramolecular cycloaddition as the actylene is now part of the enediyne system.

[16] N. Knozvi, M. Vaultier, L. Toupet, R. Carrie, *Tetrahedron Lett.* **1987**, *28*, 1757.

[17] Experimental procedure for the intramolecular N-alkylation: Anhydrous K_2CO_3 (5 equiv) was added to a solution of acyclic enediyne **12/18** (0.35 mmol) in dry DMF (80 mL) under argon, and the mixture was stirred at room temperature for 13 h. The mixture was then partitioned between EtOAc and water ($2\times$ 200 mL each). The combined organic layers were washed with water (3×150 mL), dried (Na_2SO_4), and evaporated under vacuum at room temperature. The cyclized product was isolated by column chromatography by using hexane–EtOAc (4:1) as eluent (yield ~60%). Selected ^1H NMR data: For **1** (200 MHz, [D_6]DMSO): $\delta = 8.16$ (d, $^3J(H,H) = 8.8$ Hz, 2H; nitroaryl-H), 7.92 (d, $^3J(H,H) = 8.8$ Hz, 2H; nitroaryl-H), 7.49 (m, 1H; aryl-H), 7.28 (m, 3H; aryl-H), 4.28 (s, 2H; NCH_2CC), 3.19 (m, 2H; NCH_2CH_2), 2.46 (m, 2H; COCH_2), 2.01 ppm (m, 2H; NCH$_2CH_2$). For **2** (200 MHz, [D_6]DMSO): $\delta = 8.40$ (d, $^3J(H,H) = 8.6$ Hz, 2H; nitroaryl-H), 8.11 (d, $^3J(H,H) = 8.6$ Hz, 2H; nitroaryl-H), 7.68 (d, $^3J(H,H) = 7.5$ Hz, 1H; aryl-H), 7.57–7.41 (m, 3H; aryl-H), 3.28 (m, 4H; CH_2NCH_2CH_2CC), 2.92 (m, 2H; NCH$_2CH_2$CC), 2.60 (m, 2H; NCH$_2CH_2CH_2$), 2.30 ppm (m, 2H; NCH$_2CH_2$CH$_2$).

[18] J. C. Shain, Ph.D. Thesis, IIT, Kharagpur, **2000**, [Eq. (1)].

(1)

[19] T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373.

[20] Experimental procedure for the deprotection of the 4-nitrosulfonamido group: Thiophenol (1.1 equiv) and K_2CO_3 (2.2 equiv) were added to a solution of **1** or **2** (0.12 mmol) in DMF (5 mL), and stirred under argon for 1 h. The mixture was then partitioned between EtOAc and water (2×25 mL each). The combined organic layers were washed with water (3×25 mL), dried (Na_2SO_4), and evaporated under vacuum at room temperature. The free amine **20** or the aminol **22** was isolated by column chromatography using CH_2Cl_2–MeOH (10:1) as eluent (yield ~75%). Selected ^1H NMR data: For **20** (200 MHz, $CDCl_3$): $\delta = 7.22$–7.18 (bm, 1H; aryl-H), 7.04–6.90 (m, 3H; aryl-H), 6.87 (bs, 1H; NH), 3.74 (s, 2H; NCH_2CC), 2.95 (t, $^3J(H,H) = 6.3$ Hz, 2H; NCH_2CH_2), 2.65 (t, $^3J(H,H) = 6.1$ Hz, 2H; COCH_2), 2.05 ppm (m, 2H; NCH$_2CH_2$). For **22** (200 MHz, $CDCl_3$): $\delta = 7.46$ (m, 1H; aryl-H), 7.25–6.84 (m, 3H; aryl-H), 6.59 (bs, 1H; OH), 3.04–2.85 (m, 4H; CH_2NCH_2CH_2CC), 2.75 (m, 2H; NCH$_2CH_2$CC), 2.60 (m, 2H; C(OH)CH_2), 2.04 ppm (m, 2H; NCH$_2CH_2$CH$_2$).

[21] Cyclization of aminol **22**: The solution of aminol (40 mg) in $CHCl_3$ was kept at 4°C for seven days and periodically monitored by TLC. After evaporation of the solvent, the cyclized product **24** was isolated by column chromatography over silica gel using hexane–EtOAc (5:1) as eluent (17 mg, ~45%). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.70$ (dd, $^3J(H,H) = 8.5$, 1.1 Hz, 1H; aryl-H), 7.69 (dd, $^3J(H,H) = 8.5$, 1.1 Hz, 1H; aryl-H), 7.15 (m, 4H; aryl-H), 6.45 (s, 1H; OH), 3.03 (m, 3H; CHNCH_2), 2.82 (m, 2H; ArCH, NCH), 2.23 (m, 1H; ArCH), 1.95 (m, 1H; C(OH)CH), 1.68 ppm (m, 3H; C(OH)CH, C(OH)CH$_2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 135.9$ (quar-

ternary C), 133.7 (quarternary C), 133.4 (quarternary C), 133.1 (quarternary C), 129.5 (CH), 129.3 (CH), 128.3 (CH), 127.4 (CH), 126.6 (CH), 125.4 (CH), 68.9 (quarternary C), 50.6 (CH_2), 45.4 (CH_2), 36.9 (CH_2), 29.7 (CH_2), 22.8 ppm (CH_2); Mass (ES+): m/z: 239 [M^+], 221 [$M^+ - H_2O$]. Keeping a solution of the amine **22** in $CDCl_3$ gave a mixture of deuterated as well as monochloro products.

[22] Attempted intramolecular N-alkylation to produce a nine-membered enediyne failed. The reaction produced only the dimer [Eq. (2)] as shown below. (A. Basak, J. C. Shain, U. K. Khamrai, K. Rudra, A. Basak, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1955).

(2)