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Dependence of reactivity of a novel 2,6-diamino pyridine-based enediyne on the extent of salt formation with external acids: a possible implication in pH based drug design

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Abstract—A novel pyridine diamine-based cyclic enediyne **1** was synthesized by bis-N-alkylation of 2,6-bis-sulfonamido pyridine **6** followed by deprotection with thiophenol. A variety of salts **1a**–**c** of the parent amine **1** were prepared. Thermal reactivity studies indicated a dependence of the reactivity upon the extent of salt formation. This observation may be useful in pH-based design of enediynes.

Selective cytotoxic activity against tumor cells has been a major challenge in anticancer chemotherapy.[1] Although a large repertoire of anticancer drugs exists, their side effects arising from their action against normal cells in addition to the cancer cells puts a serious limitation to their use. Apart from the targeted delivery of antibody conjugate of an anticancer drug like calicheamicin,[2] attempts have also been made to exploit the lower pH of cancer cells as compared to normal cells by drugs,[3] which show higher cytotoxic activity at higher acidity. In the area of enediynes, the azaenediynes synthesized by Kerwin and David[4] provides a unique example where the abstraction of H radical from a suitable donor has been shown to be pH dependent due to large singlet to triplet barrier.[5] Although enediynes are activated by electron withdrawal,[6] no attempt has so far been made to prepare an organo base-containing enediyne and study its properties under salt forming conditions (to mimic the variation of pH by using a variety of acid). In this communication we report the synthesis of such a molecule, namely, a novel pyridine diamine-based cyclic enediyne **1**. We have also compared its thermal reactivity with those of its salts prepared from various acids. The aim of our study was to determine the effect of salt formation, which happens at acidic pH on the activation barrier to Bergman cyclization (BC).

At the outset, we realized the need to protect the two primary amines in order to achieve the synthesis of our target. Our initial plan was to use *t*-Boc as the protecting group because of its easy deprotection under acid conditions. Thus we synthesized N,N′-di-*t*-Boc-2,6-diamino pyridine **3** using standard conditions (Boc-anhydride in EtOAc). Attempted alkylation with the enediyne dimesylate **4** in the presence of NaH in DMF or DMSO was not successful; the expected dialkylation product could never be isolated. Having failed in the initial strategy, we thought of using 4-nitrobenzene sulfonamide as the protecting group.[7] The latter offers several advantages, which include the ease of alkylation and deprotection under mild conditions. Thus we prepared the bis-sulfonamide **6** and double alkylation with the dibromide in the presence of K_2CO_3 and DMF worked out nicely under normal dilution to afford the cyclic enediyne as a brown solid. The free diamino enediyne was then obtained by deprotection of the sulfonamido groups with thiophenol and K_2CO_3 in DMF. It was isolated pure as a brown solid by chromatography over Si-gel using hexane–ethyl acetate (5:1) as eluent. The ^{1}H NMR and mass spectral data fully corroborated its structure. The synthesis is shown in Scheme 1.

Having successfully prepared enediyne **1**, we first studied its salt forming abilities with various acids. Three different acids of varying pK_a, namely *p*-toluene sulfonic acid, pyridine 2,6-dicarboxylic acid and 3,5-dinitrosalicylic acid were used. These acids were chosen because of their reported salt formation with pyridine diamine.[8]

Keywords: Enediyne; Bergman cyclization; Calorimetry; Drug; Design.
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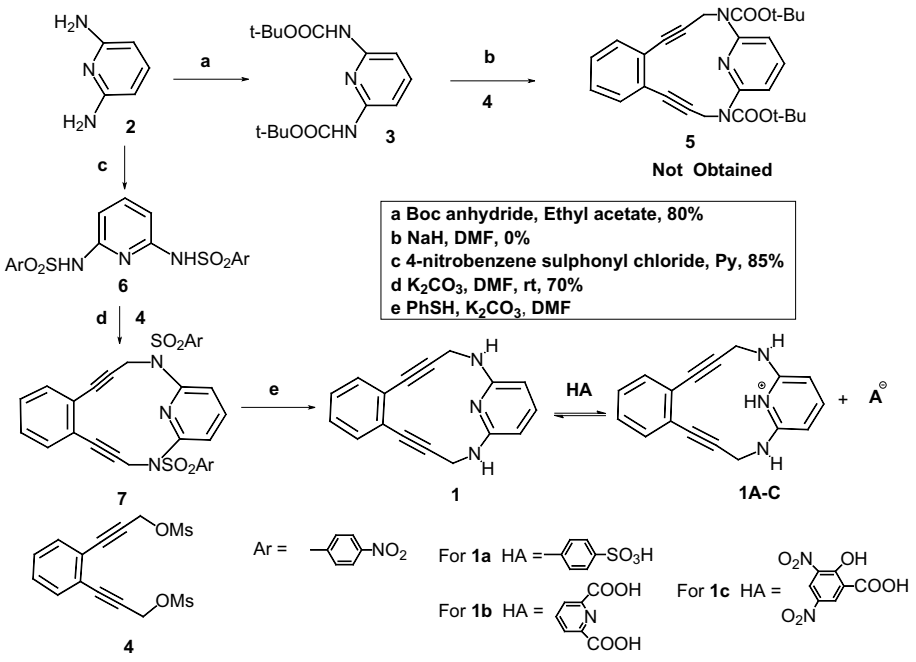

Scheme 1. Synthesis of enediyne **1** and its salts.

The salts were prepared by adding a DMSO solution of the acid to a DMSO solution of 1 equiv of the diamine. The solution was stirred at rt for 12 h and then lyophilized. Addition of CH_2Cl_2 precipitated the salts, which were then dried under vacuum. The extent of salt formation was checked by ^1H NMR. The signal for the methylene protons as well as for the pyridine 3,5-hydrogens underwent downfield shifts of varying degrees (Table 1).[9]

With a strong acid like *p*-toluene sulfonic acid, the amount of shift was dependent upon the time of interaction between the enediyne and the acid. The NMR taken immediately after the addition of sulfonic acid showed a shift of methylene and pyridine 3,5-hydrogens, which ultimately reached a limiting value after 12 h of stirring. A similar result was obtained with 3,5-dinitrosalicylic acid, which is sufficiently strong for complete conversion into the pyridinium salt. With a weaker acid like pyridine 2,6-dicarboxylic acid, the shift of the above signals was just marginal indicating little salt formation (Fig. 2). The shift for the NH hydrogens could not be determined as the signal was obscured by the aromatic signals (Fig. 1).

The thermal reactivity towards BC for the parent enediyne and its salts were next studied by Differential Scanning Calorimetry, which has now become a useful tool for studying such a reaction.[10] The onset temperature for BC for the three compounds along with change in chemical shift values are shown in Table 1. From the data presented in the table, it is clear that the salts were undergoing BC at a lower temperature than the parent enediyne. This is most likely due to the electron withdrawal from the enediyne network by the positively charged pyridine nucleus. The lowering of onset temperature is highest for the salts with toluene sulfonic acid and 3,5-dinitrosalicylic acid. This can be explained on the basis of lower pK_a of both the acids in comparison to pyridine dicarboxylic acid. The salt formation being more, there will be greater withdrawal of electrons from the enediyne network in the case of the tosylate and the salicylate.[12] With negligible salt formation, there is only a marginal decrease in the onset temperature for cyclization for the case with pyridine dicarboxylic acid.

In conclusion we have demonstrated a novel way of activation of a basic pyridine diamine based enediyne via salt formation with organic acids. Varying the pK_a of the acid can also regulate the extent of activation. This may have implication in the design of pH based anticancer agents. Current efforts in our laboratory are aimed towards bringing down the activation further so that BC takes place under ambient conditions.

Selected spectral data

Pyridine diamine bis-sulfonamide 7

δ_H (200 MHz, d_6-DMSO) 4.74 (2H, br s), 7.17–7.24 (4H, m), 7.61 (2H, dd, $J = 2.0$, 6.0 Hz), 7.82 (4H, d,

Table 1. NMR shifts and DSC results

Compd no.	$\Delta\delta$ for CH_2	pK_a of acids[11]	$\Delta\delta$ for pyridine H-3 and H-5	Onset temp (T_2, °C)	ΔT ($T_1 - T_2$)	Onset temp for **1** (T_1, °C)
1A	0.43	−6.5	0.33	215	19	
1B	~0.00	4.3	~0.00	232	2	234
1C	0.39	1.53	0.32	214	20	

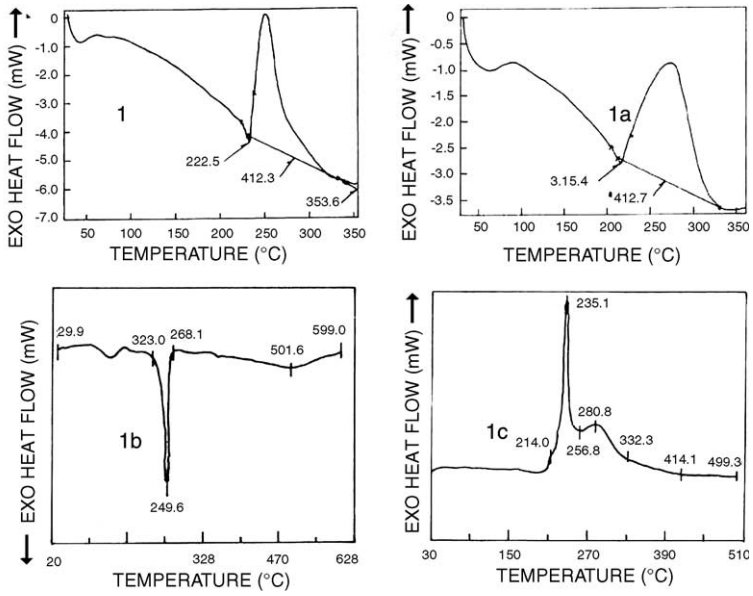

Figure 1. DSC curves of parent enediyne **1** and its salts.

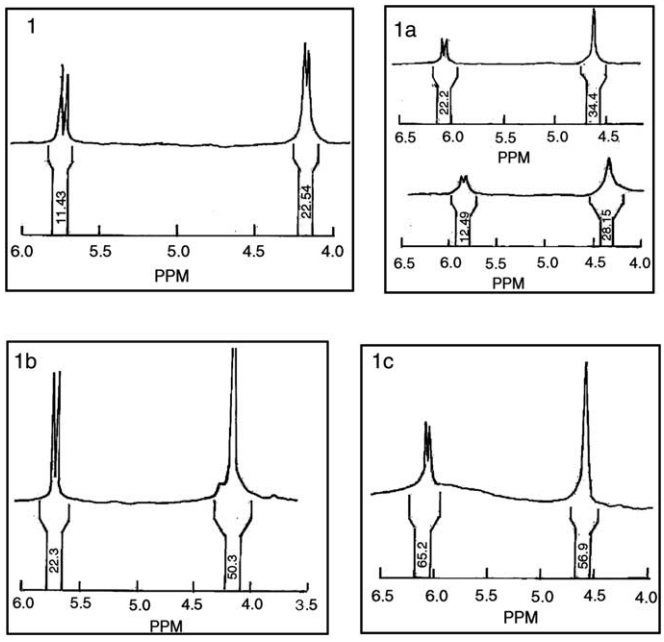

Figure 2. NMR spectra of parent enediyne and its salts.

J = 8.8 Hz), 8.09 (4H, d, J = 8.8 Hz), 8.12 (1H, m, obscured); δ_C (50 MHz, d_6-DMSO) 83.0, 86.5, 121.5, 123.9, 124.3, 128.4, 129.2, 130.9, 141.4, 143.4, 149.6, 150.5; Mass (ES+) 652 (MNa^+), 630 (MH^+).

Enediyne 1

δ_H (200 MHz, d_6-DMSO) 4.18 (4H, d, J = 5.5 Hz), 5.75 (2H, d, J = 9.1 Hz), 6.68 (2H, t, J = 5.5 Hz), 7.08 (1H, t, J = 9.1 Hz), 7.42 (4H, m); Mass (ES+) 260 (MH^+).

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12. It may be argued that the salt is in equilibrium with the free amine in DMSO solution. Thus the lyophilized material will be either pure salt or a mixture of salt and free amine and acid depending upon the pK_a of the acid used. The $\Delta\delta$ values for the protons mentioned in the table are indications of the extent of salt formation. Greater the extent of salt formation, the more will be the lowering of onset temperature for BC. Thus, there is good correlation between the pK_a, $\Delta\delta$ and ΔT. Positive ion ES mass spectral analysis of the salts with toluene sulfonic acid and 3,5-dinitrosalicylic acid showed no peak corresponding to the free acid (but showed the peak corresponding to the protonated base). Their existence as anions could clearly be detected when the mass spectra were recorded in negative mode ES. For the pyridine-2,6-dicarboxylic acid salt, the free acid peak at *m/z* 167 was present in the spectrum recorded under ES (+) mode while in the negative ion mode the peak at *m/z* 166 corresponding to the anion was also detected. This proved incomplete salt formation in agreement with the NMR study.