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Synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepines and their conjugates by azido reductive cyclization strategy as potential DNA-binding agents

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Abstract—Synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepines via azido reductive cyclization process employing $FeCl_3$–NaI reagent system. This methodology has been extended for the preparation of new nicotinamido-pyrrolobenzodiazepine hybrids linked through piperazino-alkane-oxy spacers that exhibit good DNA binding affinity.

The compounds that bind within the minor groove of DNA have attracted much attention due to their involvement in carcinogenesis and used as antitumour agents, as well as probes of DNA structure.[1] The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) class of DNA binding antitumour antibiotics has been investigated extensively to develop new antitumour agents.[2] The PBDs are produced naturally from various *Streptomyces* species. These molecules exert their biological activity by covalently binding to N2 of guanine in the minor groove of DNA through the imine or imine equivalent functionality at N10–C11 position of the PBD ring system and thus interfere with DNA function. A number of these molecules interact with DNA in a sequence selective manner and as such have potential as gene targeted drugs.[3,4]

In the last decade a number of synthetic routes have been developed for the preparation of these antibiotics and have met with varying degrees of success having some limitations.[5–9] The introduction of imine at N10–C11 position has been generally problematic because of its high reactivity. The reduction of azido functionality to the corresponding amino group employing different type of reagent systems have been investigated in this laboratory and recently $FeCl_3$–NaI reagent system has been utilized for the synthesis of fused quinazolinone[10] and pyranoquinoline systems.[11]

In conjunction with these efforts, we herein report the use of this reagent system for the preparation of pyrrolobenzodiazepines via azido reductive cyclization process.

The starting materials **1a**–**d** were prepared from their nitro counterparts by the known literature methods,[12] these upon reduction with DIBAL-H (2 equiv) gave the corresponding aldehydes **2a**–**d** in 55–61% yield. The reaction of **1a**–**d** with $FeCl_3$–NaI reagent system gave the corresponding dilactams **3a**–**d** in 80–85% yield, while the reaction of **2a**–**d** with the same reagent system has resulted in their imine forms **4a**–**d**[16] in 70–80% yield (Scheme 1).

This methodology has been further extended towards the synthesis of nicotinamido-pyrrolobenzodiazepine hybrids. Recently, a series of *N*-substituted nicotinamides has been identified as a new class of potent inducers of apoptosis and their potential as anticancer agents.[13] Additionally, nicotinamides could effect the DNA binding potential of a molecule because of the presence of a carbonyl and pyridyl functionality. Therefore, nicotinamide has been linked to the PBD ring system through a piperazino-alkane-oxy spacer chain at C8 position to unravel the DNA binding potential of such hybrids.

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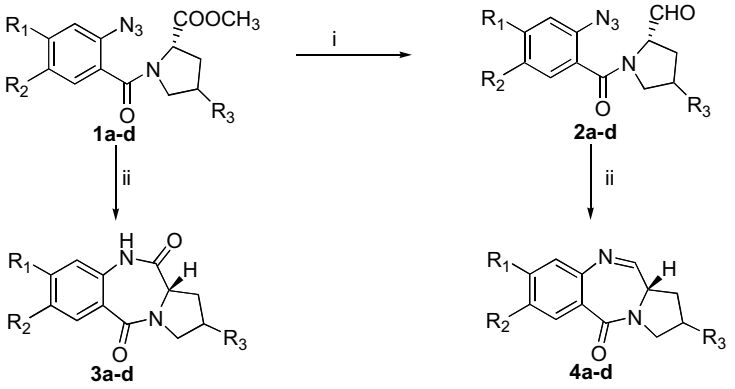
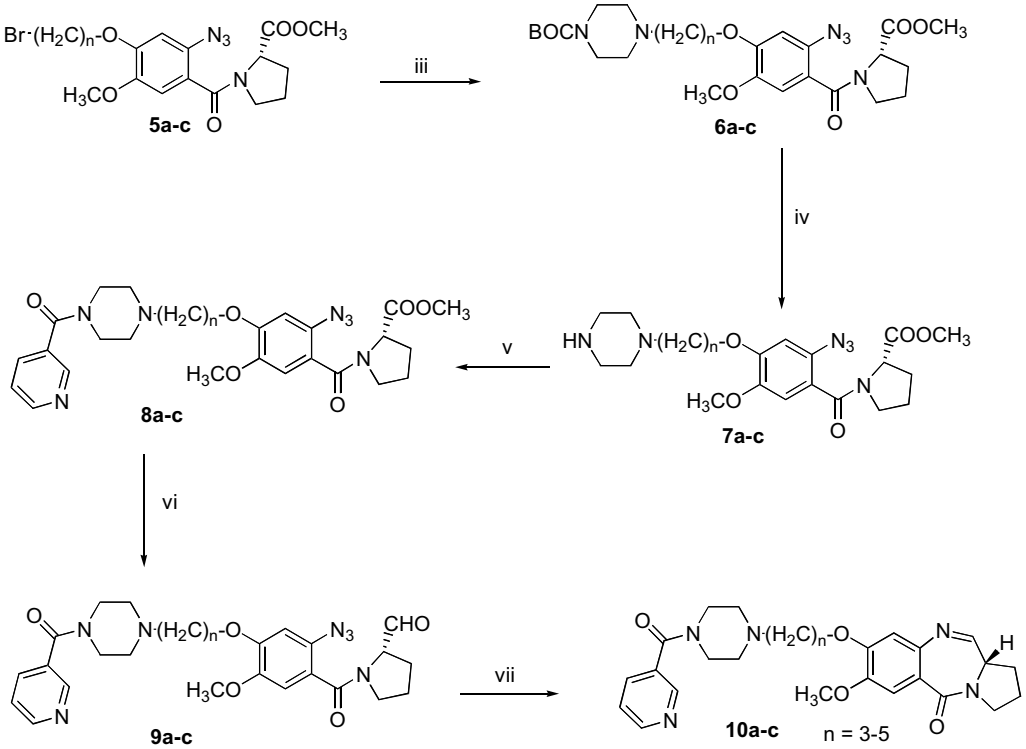


Scheme 1. Reagents and conditions: (i) DIBAL-H, −78 °C, CH_2Cl_2, 45 min; (ii) $FeCl_3$–NaI, MeCN, rt, 15 min.

The precursors **5a**–**c** have been prepared by the methods reported earlier.[12] This was further treated with *N*-Boc piperazine to give **6a**–**c**, which on Boc deprotection yielded **7a**–**c**. This is coupled with nicotinoyl chloride followed by reduction with DIBAL-H and on treatment with $FeCl_3$–NaI reagent system produced the compounds **10a**–**c**[17] in 50–60% yield (Scheme 2).

The DNA binding ability for these nicotinamido-PBD hybrids has been determined by thermal denaturation studies using calf thymus (CT)-DNA. It is interesting to observe that in this assay all the three compounds elevate the helix melting temperature of CT-DNA up to 4.5 °C (**10c**) after incubation for 18 h at 37 °C, whereas DC-81 exhibits a ΔT_m of 0.7 °C after incubation under similar conditions (Table 1). Thus demonstrating that these PBD hybrids posses good DNA binding ability.

The restriction endonuclease inhibition studies carried out on these molecules also confirm the relative binding affinity of these PBD hybrids. The experimental protocol described in the previous study has been



Scheme 2. Reagents and conditions: (iii) *N*-Boc-piperazine, MeCN, K_2CO_3, reflux, 24 h, 80–85% yield; (iv) TFA, CH_2Cl_2, 0 °C, 8 h, 75–80% yield; (v) nicotinoylchloride hydrochloride, MeCN, K_2CO_3, reflux, 24 h, 75–80% yield; (vi) DIBAL-H, −78 °C, CH_2Cl_2, 45 min, 55–60% yield; (vii) $FeCl_3$–NaI, MeCN, rt, 15 min, 50–60% yield.

Table 1. Thermal denaturation data of C8 linked PBD-nicotinamide hybrids with CT-DNA

PBD hybrid	PBD/DNA molar ratio[b]	ΔT_m (°C)[a] after 18 h incubation at 37 °C
10a	1:5	3.5
10b	1:5	3.8
10c	1:5	4.5
DC-81	1:5	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.2 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.

[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH = 7.00 ± 0.01].

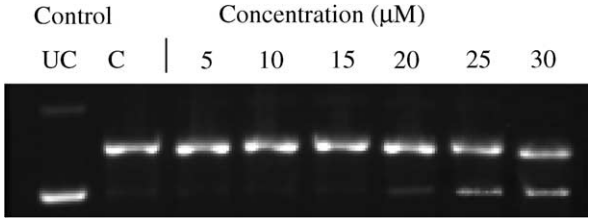


Figure 1. RED_{100}-restriction endonuclease digestion assay for *A*-C8 linked PBD with CT-DNA inhibitory activity of **10c** on the cleavage of plasmid pBR322 by restriction endonuclease *Bam*H1 (20 units in 2 μL) for 1 h at 37 °C. The cut (C) and uncut (UC) products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining under UV illumination. Lane 1: control pBR322; lane 2: complete digest of pBR322 by *Bam*H1; lane 3–8: increasing concentration of **10c**.

employed.[14,15] The results of this experiment for a representative compound **10c** is shown in Figure 1, suggesting the inhibition of *Bam*H1 by this PBD hybrid.

In conclusion, a practical and useful azido reductive cyclization of the PBD ring system has been developed employing $FeCl_3$–NaI reagent system. This methodology is not only spontaneous, which takes place under mild conditions but also employs less expensive reagents used for such a reductive cyclization. Furthermore, this reductive cyclization process has also been extended for the preparation of nicotinamido-PBD hybrids that exhibited good DNA binding affinity. The detailed biological studies for these compounds are under investigation.

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16. Typical procedure for the synthesis of **4a**: To a stirred solution of **2a** (244 mg, 1 mmol) in acetonitrile (10 mL) NaI (1350 mg, 9 mmol) was added at room temperature. After stirring for 5 min, ferric chloride (243 mg, 1.5 mmol) was added at same temperature and the stirring was continued for 15 min. After completion of the reaction indicated by TLC, ethyl acetate (20 mL) was added and the reaction mixture was washed with 10% sodium thiosulfate. The organic layer was dried over sodium sulfate, evaporated under reduced pressure and then purified by column chromatography on silica gel (ethyl acetate–hexane 9:1) as eluent to give the pure compound **4a** in 80% yield. ^{1}H NMR ($CDCl_3$): δ 1.56–2.48 (m, 4H); 3.20–3.90 (m, 3H); 7.10–7.59 (m, 3H); 7.65 (d, 1H, J = 4.2 Hz); 8.02 (d, 1H, J = 5.8 Hz); EIMS *m/z* 200 (M^+ 100).
17. Spectral data for compound **10a**: ^{1}H NMR ($CDCl_3$): δ 1.15–1.25 (m, 10H); 1.95–2.05 (m, 6H); 2.48–2.56 (m, 6H); 3.87 (s, 3H); 4.05–4.10 (m, 3H); 6.77 (s, 1H); 7.18–7.21 (d, 1H, J = 6.04 Hz); 7.45 (d, 1H, J = 5.2 Hz); 7.60 (s, 1H); 7.68–7.73 (t, 1H, J = 6.04 Hz); 7.70 (d, 1H, J = 7.55 Hz); 8.60 (s, 1H); MS (FAB) 506 $[M+1]^+$.