Design and Synthesis of Novel Pyrrolo[2,1-*c*][1,4]benzodiazepine−Lexitropsin Conjugates

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Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), a group of potent naturally occurring antitumor antibiotics from various *Streptomyces* species, are of considerable interest because of their potential as antitumor agents, gene regulators, and DNA probes.[1] Well-known members of this group include anthramycin, tomaymycin, the neothramycins A and B, sibiromycin, chicamycin, and DC-81.[2,4] The cytotoxicity and antitumor activity of these agents are attributed to their property of sequence-selective covalent binding to the N2 of guanine in the minor groove of duplex DNA via an acid-labile aminal bond to the electrophilic imine at the N10−C11 position. The N10−C11 carbinolamine form, **A**, may exist in the equivalent imine, **C**, or carbinolamine methyl ether form, **B**, depending on the precise structure of the compound and the method of isolation (Figure 1).[4b,16] The (S)-stereochemistry at the C11a position provides a right-handed molecular twist, when viewed from the C-ring toward the A-ring, which enables the PBD to assume a snug fit in the minor groove of DNA.[3] Molecular modeling, solution NMR, fluorimetry, and DNA footprinting experiments reveal that the PBDs recognize a three base-pair motif with a preference for 5′PuGuPu sequences.[1c]

Since the synthesis of the PBD ring system itself is problematic, due to the labile N10−C11 imine moiety (carbinolamine or methyl ether equivalent), much of the literature to date has focused on the development of new synthetic strategies.[4,5] In contrast, very little has been reported about modifications on the PBD ring system or conjugation with carriers.[6] Recently, a C8-linked PBD dimer was prepared[7] which forms a symmetric interstrand cross-link with duplex DNA involving a four-base pair bonding site but spanning six DNA base pairs overall.[8] Although some efforts to date have been directed at different modifications on the PBD ring system, no attempt has been made to link the PBD ring system with other well-established DNA groove binders such as distamycin (**1**) and netropsin (**2**). The latter agents bind to four or more consecutive A−T base pairs.[9] Agents that combine the features of minor groove binding and DNA sequence selectivity, together with the ability to form covalent attachment to the DNA, have proven to have clinical potential in the treatment of human malignancies.[10] We herein report the design, synthesis and characterization of a series of novel PBD−lexitropsin conjugates in order to probe the combined effect of both moieties on DNA sequence selective binding ability and cytotoxicity.

Molecular modeling studies suggested that C8-linked PBD dimers have greater isohelicity with the minor[11] groove of DNA compared with the C7-linked dimers. Thurston et al. reported that the C8-linked dimer, DSB-120 (**3**) (Figure 2) forms an irreversible interstrand cross-link between two guanine bases within the minor groove via their exocyclic N2 atoms, and it spans six base pairs, actively recognizing a central 5′-GATC sequence.[12] A variety of lexitropsin conjugates of other cytotoxic agents which are minor groove binders with improved selectivities have been reported and are summarized in a recent review.[13] In view of the commonly observed enhanced activity and selectivity of the parent drugs when conjugated with lexitropsins and the intrinsic activity of DSB-120, we attempted to conjugate certain lexitropsins with the PBD nucleus through the C8-position with a suitable linker. The natural product DC-81 (**4**) was chosen as the PBD unit, and an efficient synthetic pathway was developed (Scheme 1) to provide the various PBD−lexitropsin conjugates **5a**−**c**. The new compounds are designed to effect sequence-selective binding with duplex DNA.

The overall synthetic strategy is shown in Scheme 1. The lexitropsin unit was prepared according to the

(1) (a) Dervan, P. B. *Science* **1989**, *232*, 464. (b) Hurley, L. H. *J. Med. Chem.* **1989**, *32*, 2027. (c) Thurston, D. E.; Thompson, A. S. *Chem. Br.* **1990**, *26*, 767.

(2) Hurley, L. H. *J. Antibiot.* **1977**, *30*, 349. (b) Schimizu, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Fujimoto, K. *J. Antibiot.* **1982**, *35*, 992.

(3) (a) Thurston, D. E. *Advances in the study of pyrrolo[2,1-c]1, 4]-benzodiazepine (PBD) Antitumour Antibiotics* in *Molecular Aspects of Anticancer Drug−DNA Interactions*; Neidle, S., Waring, M. J., Eds.; The Macmillan Press: Riverside, NJ, 1993; Vol. 1, pp 54−88. (b) Remers, W. A. In *The chemistry of Antitumour Antibiotics*; Wiley: New York, 1988; Vol. 2, pp 28−92. (c) Hurley, L. H.; Pertrusek, R. L. *Nature* **1979**, *282*, 529.

(4) (a) Lown, J. W.; Joshua, A. V. *Biochem Pharmacol.* **1979**, *28*, 2017. (b) Thurston, D. E.; Bose, D. S. *Chem. Rev.* **1994**, *94*, 433. (c) Molina, P.; Diaz, I.; Tarraga, A. *Tetrahedron* **1995**, *51*, 5617. (d) Kamal, A.; Rao, N. V. *Chem. Commun.* **1996**, 385. (e) Kamal, A.; Reddy, B. S. P.; Reddy, B. S. N. *Tetrahedron Lett.* **1996**, *37*, 6803.

(5) (a) Kamal, A.; Damayanthi, Y.; Reddy, B. S. N.; Lakminarayana, B.; Reddy, B. S. P. *Chem. Commun.* **1997**, 1015. (b) Kamal, A.; Reddy, B. S. N.; Reddy, B. S. P. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1825.

(6) (a) Thurston, D. E.; Jones, G. B.; Davis, M. E. *J. Chem. Soc., Chem. Commun.* **1990**, 874. (b) Liegeois, J. F.; Rogister, F. A.; Bruhwyler, J.; Damas, J.; Nguyen, T. P.; Inarejos, M.; Chleide, E. M. G.; Mercier, M. G. A.; Delarge, J. E. *J. Med. Chem.* **1994**, *37*, 519.

(7) Bose, D. S.; Thompson, A. S.; Smellie, M.; Berardini, M. D.; Hartley, J. A.; Jenkins, T. C.; Neidle, S.; Thurston, D. E. *J. Chem. Soc., Chem. Commun.* **1992**, 1518.

(8) Jenkins, T. C.; Hurley, L. H.; Neidle, S.; Thurston, D. E. *J. Med. Chem.* **1994**, *37*, 4529.

(9) (a) Lown, J. W. *J. Mol. Recogn.* **1994**, *7*, 79. (b) Guo, D.; Gupta, R.; Lown, J. W. *Anti-Cancer Drug Design* **1993**, *8*, 369. (c) Fontana, M.; Lestingi, M.; Mondello, C.; Braghetti, A.; Montecucco, A.; Ciarrochi, G. *Anti-Cancer Drug Design* **1992**, *7*, 131.

(10) (a) Rao, K. E.; Lown, J. W. *Chem. Res. Toxicol.* **1990**, *3*, 262. (b) Rao, K. E.; Lown, J. W. *Biochemistry* **1992**, *31*, 12076. (c) Rao, K. E.; Shea, R. G.; Yadagiri, B.; Lown, J. W. *Anti-Cancer Drug Design* **1990**, *5*, 3. (d) Xie, G.; Gupta, R.; Lown, J. W. *Anti-Cancer Drug Design* **1995**, *10*, 389.

(11) Farmer, J. D.; Rudnicki, S. M. Jr.; Suggs, J. W. *Tetrahedron Lett.* **1988**, *29*, 5105.

(12) Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939.

(13) Sondhi, S. M.; Reddy, B. S. P.; Lown, J. W. *Curr. Med. Chem.* **1997**, *4*, 313.

(14) (a) Gupta, R. Xie, G.; Lown, J. W. *Gene* **1994**, *149*, 81. (b) Gupta, R.; Wang, H.; Huang, L.; Lown, J. W. *Anti-Cancer Drug Design* **1994**, *10*, 25. (c) Dervan, P. B.; Baird, E. E. *J. Am. Chem. Soc.* **1996**, *118*, 6141.

(15) Langley, D. R.; Thurston, D. E. *J. Org. Chem.* **1987**, *52*, 91.

(16) Kopka, M. L.; Goodsell, D. S.; Baikalov, I.; Grzeskowaik, K.; Cascio, D.; Dickerson, R. E. *Biochemistry* **1994**, *33*, 13593.

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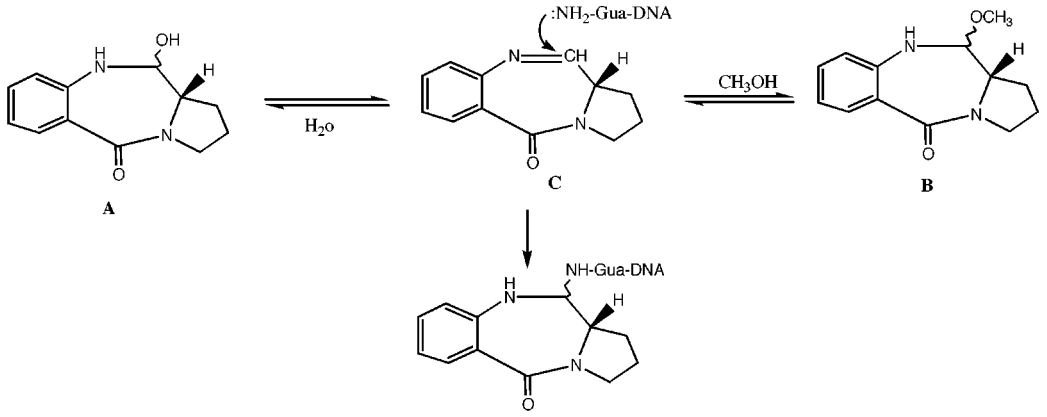


Figure 1. Equilibrium of N10−C11 carbinolamine form (**A**), imine (**C**), and carbinolamine methyl ether (**B**) and possible mechanism of the formation of PBD−DNA adduct.

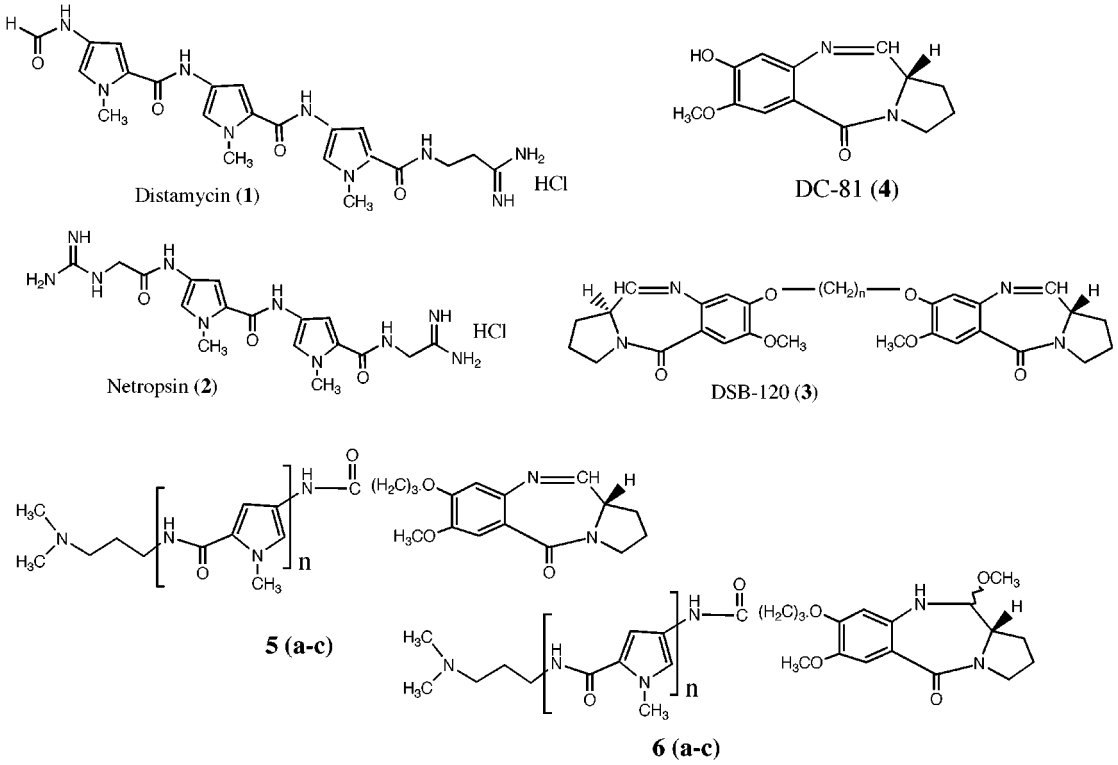
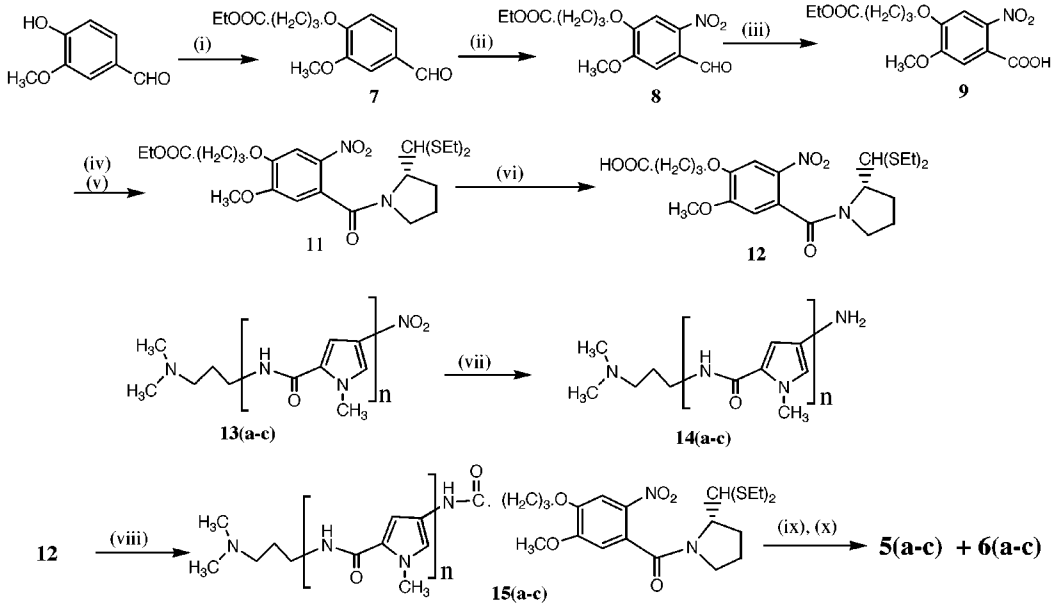


Figure 2.

previously described method.[14] A versatile approach has been developed to join the lexitropsin unit with the PBD unit, i.e., through an amidic linkage. The five-atom length of the spacer between lexitropsin and PBD unit is judged as optimal.[7] The deprotective cyclization method is adopted to obtain the conjugated PBD imines. The compounds **5a**−**c** were prepared to examine the effect of the number of pyrrole units (n = 1, 2, 3) on binding affinity and cytotoxicity of the PBD system.

The synthesis was started with coupling of an appropriate spacer with the commercially available vanillin (**6**) which serves as "A" ring of the PBD unit. The reaction of ethyl bromobutyrate with vanillin in the presence of K_2CO_3 in dry acetone under reflux conditions produced ester **7** which upon nitration gave the corresponding nitro compound **8** in about 85% yield. The aldehydic group of compound **8** was oxidized to the corresponding carboxylic acid **9** by sulfamic acid and sodium chlorite, and then coupled with (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**10**)[15] via the acid chloride. The resulting nitro ester **11** upon hydrolysis produced nitro acid **12** which was then coupled with the amine moiety of lexitropsin, (**14a**−**c**) with EDCI in dry DMF;[14] the latter amino compounds are prepared by hydrogenation of the corresponding nitro lexitropsins **13a**−**c**.[14] The DCC coupling resulted in poor yield, whereas the EDCI coupling resulted in 65−70% yield of **15a**−**c**. Compounds **15a**−**c** on reduction with the H_2/Pd−C followed by deprotection[4] with $HgCl_2$/HgO in aqueous acetonitrile at room temperature produced the corresponding imines **5a**−**c** in 40−45% yield. Since the conjugation of the lexitropsin moieties with PBD resulted in a high polarity of the imines, this necessitated the use of methanol in combination with chloroform as eluent during the purification of imines by column chromatography, and therefore the product was obtained as a mixture of imine and methyl ether. The presence of both the forms is confirmed by both NMR and mass spectra. Attempts were made to convert

Scheme 1[a]



[a] (i) K_2CO_3, THF, $Br(CH_2)_3COOEt$; (ii) $HNO_3/SnCl_4$, DCM; (iii) sodium chlorite/sulfamic acid, H_2O; (iv) $SOCl_2/C_6H_6$, rt, 2 h; (v) pyrrolidine-2-carboxaldehyde diethyl thioacetal (**10**), Et_3N, DCM, 0 °C, 1 h; (vi) 2 N NaOH solution, EtOH, 50 °C, 18 h; (vii) H_2/Pd−C, MeOH, 2 h; (viii) lexitropsin amine[14] (**14a**−**c**) (n = 1−3), HOBT, EDCI, dry DMF, rt, 14 h, (ix) H_2/Pd−C, MeOH; (x) $HgCl_2$/HgO, aq CH_3CN, rt, 14 h.

this mixture into either exclusively the methyl ether form or imine form, but the compounds are soluble only in a mixture of chloroform and methanol. The insolubility of the compounds in either of the solvents alone did not allow conversion into only one form. Attempts were made to isolate the imines by using chloroform and Hunig's base, but without use of methanol, the compounds do not elute by flash column chromatography. The methyl ether form **6a**−**c** may consist two diastereoisomers of 11*S*,11a*S* and 11*R*,11a*S*, as observed in the case of tomaymycin.[17] But, this diastereoisomer formation could not be determined by NMR because of the complexity of NMR spectra. The final compounds were isolated as pale yellow crystalline compounds in 28−30% overall yields.

In summary, we have described a versatile and convenient strategy for the design and synthesis of the first examples of PBD−lexitropsin conjugates bonded through the C8 position with a suitable linker of three carbons (overall five-atom spacer). The conjugation is achieved by amidic linkage by coupling amine of the lexitropsin unit with the acid moiety of the linker attached to the PBD system. We have synthesized the first members of PBD−lexitropsin conjugates in order to examine their sequence-selective binding affinity with duplex DNA. Efforts are underway to prepare complexes of these conjugates with DNA for X-ray diffraction analysis, and their results will be disclosed in due course.

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Supporting Information Available: Experimental procedures and compound characterization data for the total synthesis of **5a**−**c**; copies of NMR and mass spectra (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(17) Cheatham, S.; Kook, A.; Hurley, L. H.; Barkley, M. D.; Remers, W. *J. Med. Chem.* **1988**, *31*, 583.