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Synthesis and DNA Binding Properties of Saturated Distamycin Analogues

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Abstract—A series of saturated heterocyclic analogues of distamycin were prepared and examined. A fluorescent intercalator displacement (FID) assay conducted on p[dA]–p[dT] DNA to obtain C_{50} values and a hairpin deoxyoligonucleotide containing an A/T-rich binding site was used to evaluate DNA binding affinity. It is observed that saturated heterocycles greatly reduce the DNA binding relative to distamycin. © 2002 Elsevier Science Ltd. All rights reserved.

Polyamides composed of *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and a growing set of structural analogues bind in the DNA minor groove with predictable sequence selectivity and high affinities.[1] Several factors contribute to the success of polyamide binding including hydrogen bonding, curvature, Van der Waals contacts, and charged end groups. Each component has been explored through the preparation of analogous systems designed to probe the magnitude, requirements, and relative importance of each feature. Yet, the binding effectiveness for polyamides lacking the π-system of the integral heteroaromatic rings has been relatively unexplored. Herein we report the synthesis and evaluation of a series of saturated cyclic polyamides **1**–**5** (Fig. 1), based on the known, effective DNA minor groove binding agent distamycin.[2] Saturation removes the π-system, alters the conformation, and increases the thickness of the compound, yet maintains the relative structure of distamycin and was used to assess the importance of the π-system. A fluorescent intercalator displacement (FID) assay conducted on p[dA]–p[dT] DNA to obtain C_{50} values and a hairpin deoxyoligonucleotide containing an A/T-rich binding site was used to evaluate DNA their binding affinity.

A solution-phase synthesis of the polyamides was conducted using a series of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) mediated coupling reactions as described previously[3] where workup, isolation, and purification could be addressed principally by liquid–liquid acid–base extractions. Each series of analogues (**1**–**5**) are discussed separately below.

N-CBz pyrrolidine based system (1a and b). N-Boc deprotection of **6**[4] (HCl–EtOAc) provided the amine **7** as the hydrochloride salt and methyl ester hydrolysis of **6**[4](LiOH, THF–MeOH–H_2O) provided carboxylic acid **8** (Scheme 1).

After deprotection, **7** and **8** were coupled to produce dimer **9**[5] (EDCI, HOBt, DMF, 25 °C, 13–20 h, 66%)

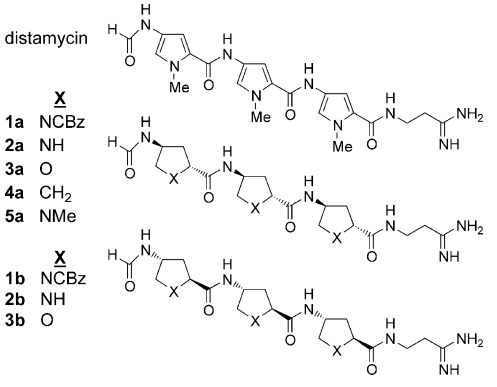

Figure 1. Distamycin and saturated heterocyclic polyamides **1**–**5**.

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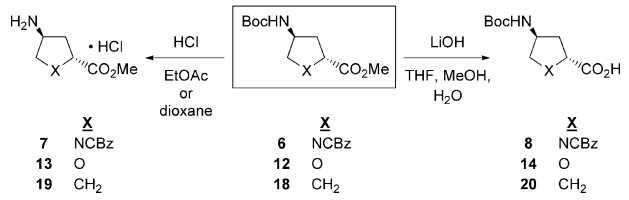


Scheme 1.

(Scheme 2). N-Boc deprotection of **9** (HCl–EtOAc, 25 °C, 1 h) and coupling with **8** (EDCI, HOAt, DMF, 25 °C, 20 h, 73%) provided trimer **10**.[6] The methyl ester of **10** was then hydrolyzed (2.5 equiv NaOH, THF–H_2O, 1.5 h, 91%) to give the free carboxylic acid which was coupled with 3-aminopropionitrile to afford **11**[7] (EDCI, HOAt, DMF, 25 °C, 20 h, 75%). End group functionalization of **11** was accomplished by simultaneous N-Boc deprotection and imidate formation (HCl–EtOH), amidine formation (NH_3–EtOH, 25 °C, 2–24 h), and *N*-formylation (*N*-formyl imidazole, THF–MeOH, −40 °C, 2 h) without purification of the intermediates yielding **1a**[8] in 56% over the three steps. The enantiomer **1b**[8] was produced in the same manner starting from the enantiomer of **6**.[4]

Pyrrolidine based system (2a and b). Several hydrogenolysis conditions for N-CBz deprotection of **1a** and **b** were examined to produce **2a** and **b** (Table 1, entries 1–5) before efficient conditions were found (H_2, 10% Pd/C, 10 equiv TFA, MeOH, 25 °C, 4 h, entry 6). Thus, although N-CBz deprotection was slow and typically problematic, acid catalysis with inclusion of TFA provided **2** in good conversions.

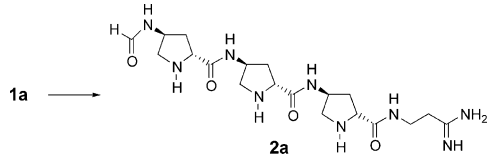
Furan based system (3a and b). Transformation of the monomer **12**[4] and its enantiomer into their respective functionalized trimers **3a**[9] and **b**[12] was performed using the strategy detailed above for **1a** and **b** (Schemes 1 and 2). However, the tetrahydrofuran-based compounds proved highly water soluble. Thus, removal of excess starting materials, reagents, and side products by simple acid–base extraction was not possible and chromatographic purifications were required.

Cyclopentane based system (4a). Transformation of the monomer **18**[4] into **4a** was performed uneventfully using the strategy detailed above (Schemes 1 and 2).

N-Me pyrrolidine based system (5a). N-Boc deprotection (HCl–dioxane) of **24**[4] provided the amine **25** as the hydrochloride salt and methyl ester hydrolysis of **24**[4] (LiOH, THF–MeOH) provided the lithium carboxylate **26**[13] (Scheme 3).

Following the strategy detailed above, the lithium salt **26** was used to provide trimer **28** (Scheme 4). However, coupling of the carboxylic acid derived from **28** with 3-aminopropionitrile afforded **29** in only moderate yield

Table 1. Hydrogenolysis conditions for **1a** using 10% Pd/C

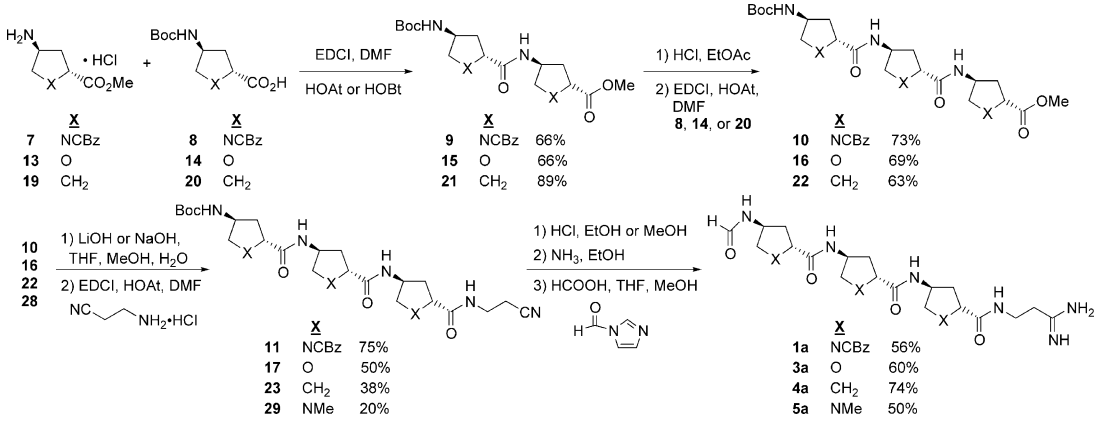


Entry	Conditions	Result
1	H_2, MeOH, 25 °C, 24–48 h	No reaction
2	H_2, MeOH, 2 N HCl, 25 °C, 24 h[10]	Decomposition
3	4% HCOOH, MeOH, 25 °C, 14 h[11]	Decomposition
4	H_2, acetone–MeOH, 25 °C, 24 h[12]	No reaction
5	H_2, 2 equiv HOAc, MeOH, 25 °C, 24 h	No reaction
6	H_2, 10 equiv TFA, MeOH, 25 °C, 4 h	**2a**, 64%

Table 2. C_{50} values

Compd	C_{50} (μM)
Distamycin	0.46
1a	4.70
1b	5.10
2a	6.37
2b	8.55
3a	5.40
3b	5.50
4a	7.91
5a	6.70

p[dA]–p[dT] 8.8 μM bp, EtBr 4.4 μM.



Scheme 2.

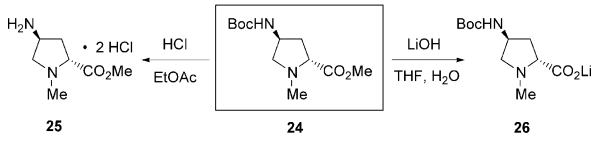
(20%, Scheme 2). Consequently, an alternative convergent synthesis was used to provide **29** (Scheme 4). Coupling **26** with 3-aminopropionitrile (HATU, *i*-Pr_2NEt, DMF, 25 °C, 80%) yielded nitrile monomer **30**, which was subsequently N-Boc deprotected (HCl–EtOAc) and coupled with the carboxylic acid derived from **27** (LiOH, THF–MeOH–H_2O) using standard coupling conditions (HATU, *i*-Pr_2NEt, DMF, 25 °C, 60%) to provide **29** in a more satisfactory yield. Treatment of **29** with freshly prepared 8 N HCl–EtOH led to N-Boc deprotection and imidate formation. Subsequent treatment of the imidate with NH_3–EtOH provided the corresponding amidine which was formylated using *N*-formyl imidazole freshly prepared by treating carbonyldiimidazole with formic acid to provide **5a**.[14]

Initial assessment of DNA binding. The compounds **1**–**5** were initially screened for DNA binding using p[dA]–p[dT] in a fluorescence intercalator displacement (FID) assay.[15] The relative binding affinities were established by monitoring the loss of fluorescence derived from titration displacement of prebound ethidium bromide from p[dA]–d[dT]. A comparison the compound concentration required for 50% displacement of the ethidium bromide (C_{50}) revealed that none of the saturated analogues compared well to distamycin (Table 2). The best of the analogues, N-CBz pyrrolidine **1a**, required a 10-fold greater concentration relative to distamycin (4.70 vs 0.46 μM), while the remaining analogues required even higher concentrations (5.10–8.55 μM). Interestingly, one stereochemical configuration, $(R)_{C\text{-ring}}(S)_{N\text{-ring}}$ (the **a** analogues), consistently performed better than their respective enantiomers.

Binding to a hairpin deoxyoligonucleotide containing an A/T-rich binding site. In an attempt to assess binding constant comparisons of **1**–**5**, a FID titration assay was performed using a DNA hairpin with an A/T-rich binding site of 5 bp. As above, the analogues exhibited low binding affinity toward the hairpin DNA, yielding flat fluorescence reduction curves too shallow for accurate Scatchard analysis.[16] Qualitatively, their behavior can be compared using the ratio of fluorescence present before ($F_{100\%}$) and after the addition of one equivalent of agent (F_1),[17] using the $F_1/F_{100\%}$ value (Table 3). With distamycin, a $F_1/F_{100\%}$ of 0.48 is observed, whereas much higher values are observed with the saturated systems (0.77–0.92). The trends for hairpin and p[dA]–p[dT] DNA binding ($F_1/F_{100\%}$ versus C_{50}) are similar with the N-CBz derivatives (**1a** and **b**) exhibiting the better binding properties than the remaining analogues. However, additional insights are difficult to extrapolate due to the low binding ability.

Discussion

One major impact of saturating the distamycin pyrrole rings with **2** or **5** is the introduction of three additional basic nitrogens. Those of **2** are present as secondary amines while those of **5** are tertiary amines. Although not observed, one might have suspected that pH 7 protonation of these amines might provide positively charged centers that would benefit from stabilizing electrostatic interactions with DNA enhancing their affinity. In addition, the comparable behavior of **2** and **5** with either **3** or **4**, suggest that the presence of the basic amines do not enhance or inhibit binding relative to the impact of introduction of the saturated versus unsaturated



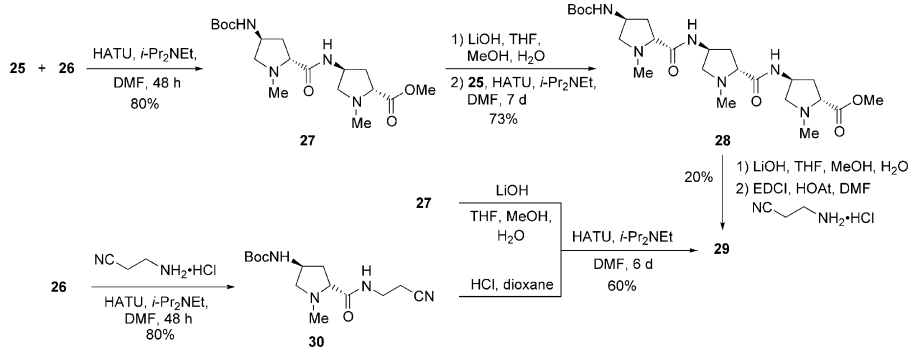
Scheme 3.

Table 3. $F_1/F_{100\%}$ values of distamycin agents **1**–**5** with hairpin DNA

5′ C·G·A·A·A·A·C·A·A
3′ G·C·T–T–T–T–T·G·A·A

Compd	F_1 / $F_{100\%}$[a]	Curve description
Distamycin	0.48	Good
1a	0.88	Flat
1b	0.77	Flat
2a	0.90	Flat
2b	0.88	Flat
3a	0.90	Flat
3b	0.90	Flat
4a	0.92	Flat
5a	0.83	Flat

[a]$F_1/F_{100\%}$ is the ratio of fluorescence before ($F_{100\%}$) and after the addition of 1 equivalent of agent (F_1).



Scheme 4.

heterocycles. Interestingly, the most effective analogue in the series is **1** bearing the hydrophobic N-CBz protecting group. However, even **1** would not be considered an effective DNA binding agent. Finally, the all carbon cyclopentyl-based analogue **4** emerged in the DNA binding assays as the poorest analogue in the series.

Although several features imparted by the structural changes could be responsible for the poor binding of **1**–**5**, the magnitude of the reductions is notable. From molecular modeling studies, a minor groove complementary shape, hydrogen bonding, and stabilizing electrostatic interactions analogous to those observed with distamycin are possible with **1**–**5** and the hydrophobic character covered by the series brackets that of distamycin. Nonetheless, no saturated analogue in the series came close to the DNA binding affinity observed with distamycin illustrating the importance of the unsaturated heterocycles.[18]

Acknowledgements

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References and Notes

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4. Experimental details and characterization for intermediates and final products can be provided upon request.
5. For **9a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.66 (m, 1H), 7.40–7.30 (m, 10H), 6.34 (brs, 1H), 5.13–4.95 (m, 4H), 4.47–4.24 (m, 4H), 3.79 and 3.39 (s, 3H), 3.75 and 3.38 (m, 4H), 2.20 (m, 4H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3315, 2975, 1700, 1644, 1531, 1417, 1356, 1169 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 647.2686 ($M+Na^+$, $C_{32}H_{40}N_4O_9$ requires 647.2687); $[\alpha]_D^{25}$ +23 (*c* 1, THF). For **9b**, $[\alpha]_D^{25}$ −22 (*c* 1, THF).
6. For **10a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.93 (m, 2H), 7.35–7.29 (m, 15H), 6.39 (m, 1H), 5.11–4.95 (m, 6H), 4.49–4.23 (m, 6H), 3.72 and 3.60 (s, 3H), 3.72 and 3.37 (m, 6H), 2.18 (m, 6H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3311, 2933, 1704, 1537, 1420, 1356, 1169 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 893.3688 ($M+Na^+$, $C_{45}H_{54}N_6O_{12}$ requires 893.3692); $[\alpha]_D^{25}$ +24 (*c* 1, THF). For **10b**, $[\alpha]_D^{25}$ −17 (*c* 1, THF).
7. For **11a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.76 (m, 1H), 7.58 (m, 2H), 7.35–7.30 (m, 15H), 6.32 (brs, 1H), 5.14–4.92 (m, 6H), 4.4–4.3 (m, 6H), 3.73 and 3.42 (m, 8H), 2.54 and 2.56 (m, 2H), 2.20 (m, 6H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3309, 2964, 2340, 1604, 1539, 1420, 1357, 1169 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 931.3929 ($M+Na^+$, $C_{47}H_{56}N_8O_{11}$ requires 931.3961); $[\alpha]_D^{25}$ +29 (*c* 0.1, THF). For **11b**, $[\alpha]_D^{25}$ −29 (*c* 0.1, THF).
8. For **1a**, ^{1}H NMR (methanol-d_4, 400 MHz) δ 8.31 (brs, 2H), 8.02 (brs, 1H), 7.33 (m, 15H), 5.12–4.88 (m, 6H), 4.5–4.3 (m, 6H), 3.81 (m, 3H), 3.43 (m, 5H), 2.67 (m, 2H), 2.19 (m, 6H): IR (NaCl, film) υ_{max} 3243, 1662, 1542, 1542, 1356, 1126 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 854.3834 ($M+H^+$, $C_{43}H_{51}N_9O_{10}$ requires 854.3831); $[\alpha]_D^{25}$ +26 (*c* 0.1, MeOH). For **1b**, $[\alpha]_D^{25}$ −27 (*c* 0.1, MeOH).
9. For **3a**, ^{1}H NMR (methanol-d_4, 500 MHz) δ 8.02 (s, 1H), 4.47 (m, 6H), 4.11 (m, 3H), 3.76 (m, 3H), 3.47 (m, 2H), 2.69 (m, 2H), 2.31 (m, 6H); IR (NaCl, film) υ_{max} 3128, 2851, 1668, 1630, 1085 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 455.2244 ($M+H^+$, $C_{19}H_{30}N_6O_7$ requires 455.2249); $[\alpha]_D^{25}$ −25 (*c* 0.1, MeOH). For **3b**, $[\alpha]_D^{25}$ +22 (*c* 0.1, MeOH).
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13. The lithium salt **26** was used instead of the carboxylic acid to avoid potential coupling problems with the zwitterionic form of the amino acid.
14. For **5a**, ^{1}H NMR (methanol-d_4, 400 MHz) δ 8.01 (s, 1H), 4.37 and 3.99 (m, 6H), 3.56 and 3.43 (m, 3H), 3.18 (m, 3H), 2.81 and 2.68 (m, 2H), 2.40 (m, 11H), 2.14 (m, 6H); IR (NaCl, film) υ_{max} 2939, 2852, 1643, 1659, 1580, 1256, 1107 cm^{-1}; MALDI–HRMS (DHB) *m*/*z* 516.3022 ($M+Na^+$, $C_{22}H_{39}N_9O_4$ requires 516.3017); $[\alpha]_D^{25}$ +88 (*c* 0.1, MeOH).
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16. Scatchard, G. *N.Y. Acad. Sci.* **1949**, *51*, 660.
17. Addition of one equivalent of agent from the titration curves was chosen arbitrarily for the comparisons.
18. **1a**, **1b**, **3a**, and **3b** were inactive in an L1210 cytotoxic assay (IC_{50} > 10 μM).