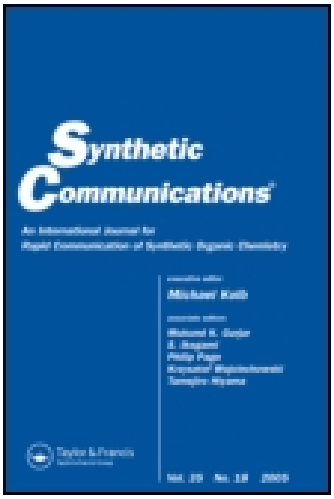

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Orthogonally Positioned Diamino Pyrrole- and Imidazole-Containing Polyamides: Synthesis of 1-(3-Substituted-propyl)-4-nitropyrrole-2-carboxylic Acid and 1-(3-Chloropropyl)-4-nitroimidazole-2-carboxylic Acid

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ORTHOGONALLY POSITIONED DIAMINO PYRROLE- AND IMIDAZOLE-CONTAINING POLYAMIDES: SYNTHESIS OF 1-(3-SUBSTITUTED-PROPYL)-4-NITROPYRROLE-2-CARBOXYLIC ACID AND 1-(3-CHLOROPROPYL)-4-NITROIMIDAZOLE-2-CARBOXYLIC ACID

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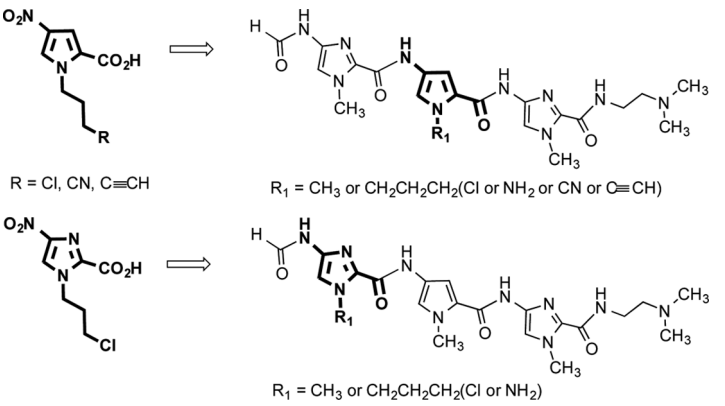
GRAPHICAL ABSTRACT



Abstract *Pyrrole- and imidazole-containing polyamides can be tailored to recognize the DNA 6–8 base pair sequence. We found that adding a second amino group via the N1-position of pyrrole or imidazole in polyamides could enhance their DNA binding affinity and water solubility while retaining sequence specificity. Synthesis of the key 1-substituted-4-nitropyrrole (and imidazole)-2-carboxylic acid building blocks are described.*

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications® *for the following free supplemental resource(s): Full experimental and spectral details.]*

Keywords Diamino polyamides; DNA binding; imidazole; pyrrole; sequence specificity

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INTRODUCTION

Many human diseases, including cancer, are caused by aberrant gene expression.[1] The transcription factors responsible for the growth and metastatic behavior of human cancers can be regulated by cell-permeable small molecules that disrupt transcription factor–DNA interactions that could stop aberrant gene expression pathways.[2] Pyrrole (P)-imidazole (I) polyamides (PAs) are analogs of distamycin (**1**, Fig. 1) that bind in the minor groove of DNA and they are capable of regulating the expression of specific genes.[3] PAs are also shown to control cancer growth in vivo.[4] The established pairing rules (I/P binds G/C and P/I binds C/G, P/P binds either to A/T or T/A,[5] and I/I binds T/G mismatched base pairs[6]) have enabled researchers to design and synthesize the P- and I-PAs to target virtually any DNA 6–8 base-pair sequence. Recently, our group has augmented the usefulness of PAs by reporting a new class of compounds called Hx-amides.[7] These are modified PAs that contain a fluorescent 4-methoxyphenylbenzimidazole or Hx moiety, and they bind in the minor groove with a similar mechanism as PAs. A stacked Hx motif behaves as two contiguous pyrrole units; thus, a stacked Hx/PP unit recognizes two A/T base pairs, and a stacked Hx/II unit recognizes CC/GG.[7] In addition to the ability to track Hx-amides in cells by fluorescence, Hx-amides have enhanced binding affinity over their N-formamido PA counterparts.[7]

Despite these advances, major challenges still limit the PA field. These include the need to increase the water solubility of PAs and their penetration into cells and

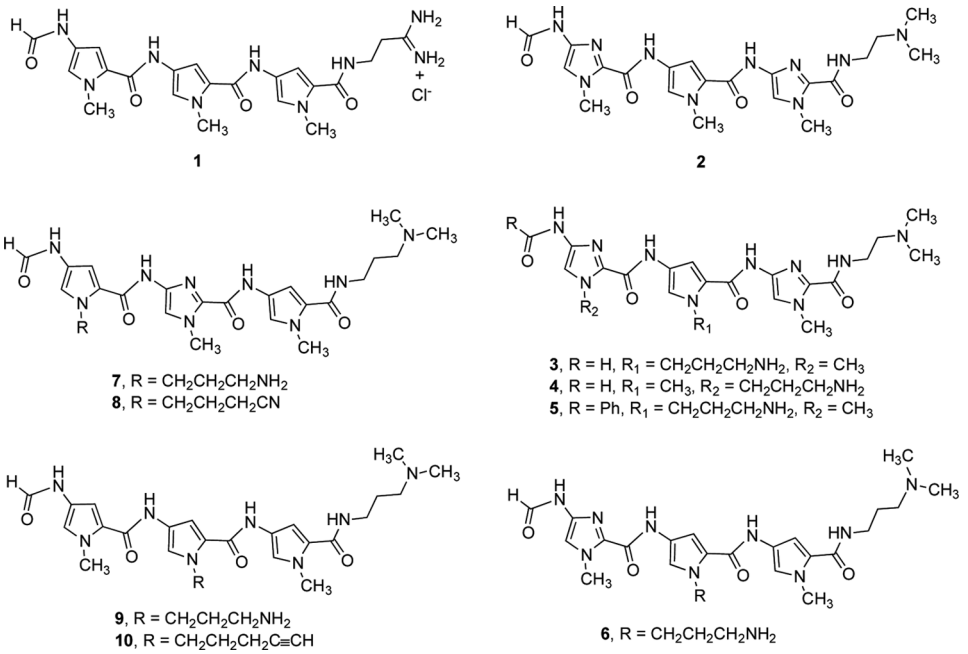


Figure 1. Structures of distamycin (**1**) as a hydrochloride salt, f-IPI (**2**), diamino f-I*P*I (**3**), diamino f-*I*PI (**4**), diamino Ph-I*P*I (**5**) [f = formamido, Ph = benzamido, *P* or *I* = site of 1-(3-aminoalkyl) moiety], f-I*P*P (**6**), f-*P*IP (**7**), f-P(CN)IP (**8**), f-P*P*P (**9**), and f-PP(alkyne)P (**10**).

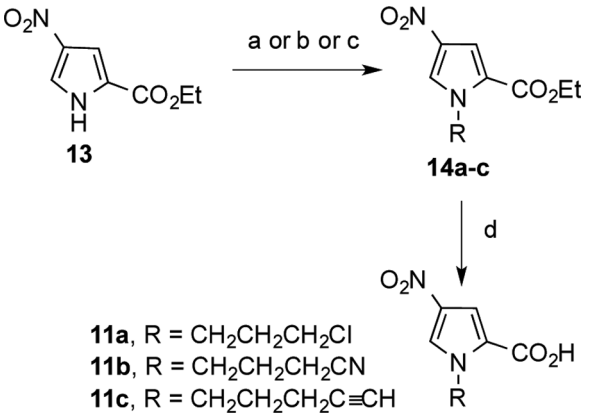
localization in the nucleus. Instead of focusing on larger, linked PAs, such as hairpins,[8] H-pins,[9] and cyclic PAs,[10] our group has focused on simpler PAs, such as tri- and tetraamides.[11] To overcome some of these challenges, our group has reported novel N1-modified PAs that include a second amino functionality. The basis for this design are as follows. First, N1-modified PAs are relatively underexplored.[12] Second, the second amino group can be protonated, thereby making the diamino PAs more water soluble than their respective monoamino counterparts. The second positively charged ammonium group could also enhance the electrostatic attraction between the PAs and the negatively charged phosphodiester backbone on DNA. This would enhance the binding affinity. Third, modification of N1 does not increase the molar mass and size of the PAs in any significant way, thereby giving the molecules a better chance of diffusing into cells and concentrating in the nucleus.

Accordingly, our group has reported the synthesis and DNA binding properties of a group of diamino PAs as shown in Fig. 1. Analogs f-IPI (or PA **2**),[13] diamino PAs f-I*P*I (**3**), f-*I*PI (**4**), and Ph-I*P*I (**5**) bind strongly to their cognate sequence 5′-ACGCGT-3′, a biological relevant site.[14] The latter three compounds have comparable sequence specificity as PA **2** and they have improved solubility in water. PAs **3** and **5** have superior binding affinity to their cognate sequence than their respective monoamino counterparts, with PA **4** showing comparable binding affinity to PA **2**. A similar water-solubility advantage was observed for PAs **6, 7**, and **9** compared to their respective monoamino counterparts[14] yet they gave similar sequence specificity and either stronger or comparable binding affinity to their respective monoamino PAs. As part of our studies, we have also synthesized PAs **8** and **10**, which contained a 3-cyanoalkyl- or 1-(4-pentynyl) pyrrole group,[15] respectively. Clearly, the N1-position of pyrrole and imidazole offers an opportunity for designing newer generations of PAs and Hx-amides. Even though several 1-alkylaminopyrrole PAs have been reported,[12] and only two 1-alkylaminoimidazole PAs have been described by us,[12,14] we felt the approach reported herein, which uses 1-substituted-4-nitropyrrole-(and imidazole)-2-carboxylic acid building blocks, is more efficient than the strategies previously reported. First, the number of reaction steps to synthesize the PAs isminimal. Second, the nitro-carboxylic acid structures of **11a–c** and **12** make them highly amenable to the Schotten–Baumann reaction, amine–acid chloride coupling approach, which offers advantages over other approaches in terms of chemical yields, costs, and purity.[16]

Accordingly, we hereby report the synthesis of novel 1-(3-modifiedpropyl)-4-nitro-pyrrole-2-carboxylic acids **11a–c** and 1-(3-chloropropyl)-4-nitroimidazole-2-carboxylic acid **12**. Upon incorporation of the chloroalkyl moiety in the PAs, the chlorine atom could be transformed into the amine either by direct displacement with ammonia in methanol[14,17] or in a stepwise manner (sodium azide, DMF, and heat; followed by catalytic hydrogenation).[14]

RESULTS AND DISCUSSION

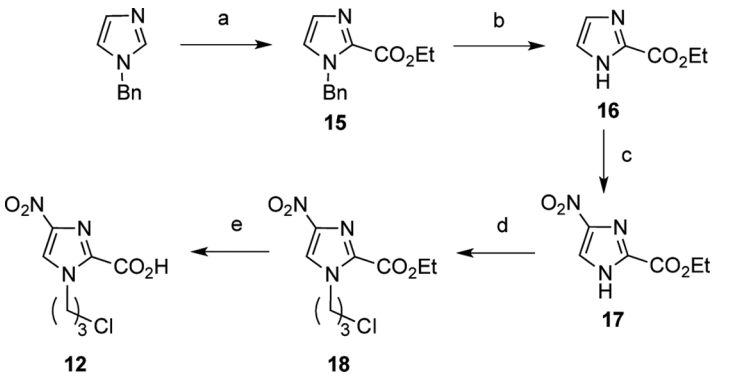
The synthesis of 1-(3-substitutedpropyl)-4-nitropyrrole-2-carboxylic acids **11a–c** is shown in Scheme 1. It involves an S_N2 reaction of ethyl 4-nitro-*1H*-pyrrole-2-carboxylate **13**[18] with 1-bromo-3-chloropropane in the presence of anhydrous potassium carbonate and potassium iodide and in dry acetone under reflux. The



Scheme 1. (a) 1-Bromo-3-chloropropane, K_2CO_3, KI, dry acetone, reflux, 16 h for **14a**. (b) 4-Bromobutyronitrile, K_2CO_3, KI, dry acetone, reflux, overnight for **14b**. (c) 5-iodo-1-pentyne, K_2CO_3, dry acetone, reflux, overnight for **14c**. (d) (i) 4.0 M NaOH, 15 min, reflux, (ii) 6.0 M HCl.

reaction yielded ethyl 1-(3-chloropropyl)-4-nitropyrrole-2-carboxylate **14a** as a yellow solid in 85% yield. Hydrolysis of the ester moiety in compound **14a** in aqueous sodium hydroxide solution under reflux conditions afforded the desired 1-(3-chloropropyl)-4-nitropyrrole-2-carboxylic acid **11a** in 86% yield. The conditions were optimized to reduce the amount of dimer formation due to substitution of two pyrrole-*NH* moieties of **13** at both ends of 1-bromo-3-chloropropane during the S_N2 reaction and over hydrolysis of the alkyl chloride moiety in the second step. The preparation of acids **11b** and **c** were achieved using the same process except 4-bromobutyronitrile/potassium iodide and 5-iodo-1-pentyne were used, respectively.

Our initial synthesis of 1-(3-chloropropyl)-4-nitroimidazole-2-carboxylic acid **12** utilized an approach we had reported earlier.[12a] That involved alkylation at N1 of imidazole with 1-bromo-3-chloropropane, followed by installation of the ethoxycarbonyl group at C2. These transformations were successful but subsequent attempts to introduce a nitro group at the C4 position using a wide range of methods[12,19] failed to give more than a tiny amount of the product **18**. A fruitful synthesis of acid **12** is given in Scheme 2. It required the synthesis of ethyl 4-nitro-1H-imidazole-2-carboxylate **17**,[20] using a similar strategy for the synthesis of acids **11a–c**. Imidazole ester **16** was synthesized by reaction of 1-benzylimidazole with ethyl chloroformate to give ester **15** in 50% yield.[20] Removal of the benzyl group by catalytic hydrogenation afforded ester **16** in quantitative yield, which upon nitration using fuming nitric acid and concentrated sulfuric acid gave the desired ester **17** in 86% yield.[20] Ester **17** was reacted with 1-bromo-3-chloropropane in the presence of anhydrous potassium carbonate and potassium iodide in dry dimethylformamide at 60–65 °C to yield the desired N1-(3-chloropropyl) product **18** in 74% yield, with minor admixture of the 3-bromopropyl derivative. Because of the mesomeric nature of imidazole, we needed to ascertain the exact position of the chloroalkyl group on the imidazole unit. That was unambiguously accomplished through a single-crystal X-ray diffraction study on ester **18**, and the structure is



Scheme 2. (a) Ethylchloroformate, dry Et_3N, dry MeCN, −20 °C for 15 min, rt for 16 h. (b) H_2, 10% Pd-C, cold ethanol, 16 h, rt. (c) Fuming HNO_3, conc. H_2SO_4, 60–65 °C for 3 h. (d) 1-Bromo-3-chloropropane, K_2CO_3, KI, dry DMF, 60–65 °C, 2.5 h. (e) (i) LiOH, THF:H_2O (1:1), rt, overnight, (ii) 6.0 M HCl.

shown in Fig. 2. Selective hydrolysis of ester **18** was achieved using lithium hydroxide in tetrahydrofuran (THF) and water at room temperature to furnish the desired 1-(3-chloropropyl)-4-nitroimidazole-2-carboxylic acid **12** in 88% yield.

In conclusion, modification of the N1-position of pyrrole and imidazole offers an opportunity for the design and synthesis of newer generations of DNA sequence–specific binding PAs. Reporting the synthesis of the key 1-substituted-4-nitropyrrole (and imidazole)-2-carboxylic acidsynthonswill enable further developmentinthe field of polyamide minor groove binding ligands.

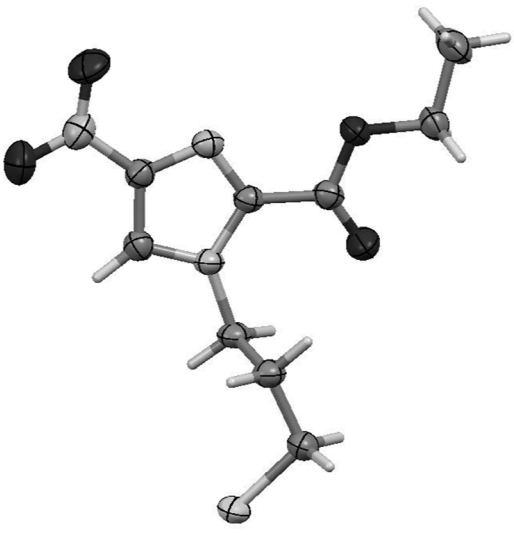


Figure 2. X-ray crystal structure of ethyl 1-(3-chloropropyl)-4-nitro-*1H*-imidazole-2-carboxylate **18** (50% thermal ellipsoid probability level). Minor disorder of Cl and Br omitted for clarity. The X-ray structural information has been deposited in the Cambridge Crystallographic Data Centre (CCDC 922340) and can be obtained via www.ccdc.cam.ac.uk/data_request/cif.

EXPERIMENTAL

The general experimental information as well as the syntheses and characterization of esters **14b, 14c**, and **18** as well as acids **11b, 11c**, and **12** are given in the supplementary materials section. Representative syntheses of ester **14a** and acid **11a** are given here.

Ethyl 1-(3-Chloropropyl)-4-nitropyrrole-2-carboxylate 14a

A solution of ethyl 4-nitro-*1H*-pyrrole-2-carboxylate **13**[18] (2.0 g, 10.8 mmol), anhydrous K_2CO_3 (4.5 g, 32.6 mmol), and KI (1.98 g, 11.95 mmol) in dry acetone (25 mL) was refluxed for 30 min. 1-Bromo-3-chloropropane (1.0 mL, 11.95 mmol) was added to the reaction mixture. The reaction mixture was refluxed for 16 h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure. The residue obtained was dissolved in $CHCl_3$ (20 mL) and washed with water (10 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the crude compound, which was purified by column chromatography using silica gel and $CHCl_3$ as the eluent. Ester **14a** was isolated as a yellow solid (2.41 g, 85%). Mp 74–76 °C, R_f 0.71 (2% MeOH/$CHCl_3$). IR (KBr): 3300–2500 br, 3113, 2960, 1676, 1541, 1511, 1480, 1414, 1368, 1308, 1282, 1254, 1216, 1191, 1155, 1106, 1089, 977, 914, 863, 818, 749, 721, 658.^{1}H NMR ($CDCl_3$): 7.70 (s, 1H); 7.46 (s, 1H); 4.55 (t, $J = 6.6$, 2H); 4.32 (q, $J = 7.0$, 2H); 3.51 (t, $J = 6.6$, 2H); 2.29 (quint, $J = 6.6$, 2H); 1.37 (t, $J = 7.0$, 3H). ^{13}C NMR ($CDCl_3$): 159.97; 135.56; 127.19; 122.40; 113.37; 61.09; 47.45; 41.16; 33.07; 14.24. MS (EI): 260 ($^{35}M^+$, 80%), 262 ($^{37}M^+$, 25%). HR-MS (EI): 260.0567 ($^{35}M^+$, $C_{10}H_{13}{}^{35}ClN_2O_4^+$; calc. 260.0564).

1-(3-Chloropropyl)-4-nitropyrrole-2-carboxylic Acid 11a

Aqueous NaOH solution (4.0 M, 15 mL) was added to a solution of ethyl 1-(3-chloropropyl)-4-nitropyrrole-2-carboxylate **14a** (3.0 g, 11.5 mmol) in MeOH (10 mL). The reaction mixture was refluxed for 15 min. The solvent was removed under reduced pressure. Water (10 mL) was added to the reaction mixture. The reaction mixture was cooled to 0–5 °C and acidified using 6.0 M HCl until pH was 1. The separated white solid was filtered and dried to obtain acid **11a** (2.29 g, 86%). Mp 190–194 °C. R_f 0.16 (5% MeOH/$CHCl_3$). IR (KBr): 3139, 3114, 2970, 2845, 1675, 1560, 1541, 1480, 1447, 1414, 1368, 1308, 1282, 1254, 1191, 1154, 1106, 1089, 914, 773, 658. ^{1}H NMR ($CDCl_3$): 7.77 (d, $J = 1.2$, 1H); 7.61 (d, $J = 1.2$, 1H); 4.57 (t, $J = 6.2$, 2H); 3.52 (t, $J = 6.2$, 2H); 2.31 (quint, $J = 6.2$, 2H). ^{13}C NMR ($CDCl_3$): 163.63; 135.87; 128.32; 120.89; 115.47; 47.61; 40.98; 32.97. MS (EI): 232 ($^{35}M^+$, 50%), 234 ($^{37}M^+$, 18%). HR-MS (EI): 232.0247 ($^{35}M^+$, $C_8H_9{}^{35}ClN_2O_4^+$; calc. 232.0251).

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SUPPLEMENTARY INFORMATION

Full experimental detail for esters **14b, 14c**, and **18** and acids **11b, 11c**, and **12**; and ^{1}H and ^{13}C NMR spectra of esters **14a–c** and **18** as well as acids **11a–c** and **12** are provided. This material can be found via the "Supplementary Content" section of this article's Web page.

REFERENCES

1. (a) Maher, B. ENCODE: The human encyclopaedia. *Nature* **2012**, *489*, 46–48; (b) Collins, F. S.; Green, E. D.; Guttmacher, A. E.; Guyer, M. S. A vision for the future of genomics research: A blueprint for the genomic era. *Nature* **2003**, *422*, 835–847.
2. Cai, X.; Gray Jr., P. J.; Von Hoff, D. D. DNA minor groove binders: Back in the groove. *Cancer Treat. Rev.* **2009**, *35*, 437–450.
3. (a) Gottesfeld, J. M. Small molecules affecting transcription in Friedreich ataxia. *Pharmacol. Ther.* **2007**, *116*, 236–248; (b) Dervan, P. B.; Poulin-Kerstien, A. T.; Fechter, E. J.; Edelson, B. S. Regulation of gene expression by synthetic DNA-binding ligands. *Top. Curr. Chem.* **2005**, *253*, 1–31; (c) Hochhauser, D.; Kotecha, M.; O'Hare, C.; Morris, P. J.; Hartley, J. M.; Taherbhai, Z.; Harris, D.; Forni, C.; Mantovani, R.; Lee, M.; Hartley, J. A. Modulation of topoisomerase IIα expression by a DNA sequence-specific polyamide. *Mol. Cancer Ther.* **2007**, *6*, 346–354.
4. (a) Chou, C. J.; Farkas, M. E.; Tsai, S. M.; Alvarez, D.; Dervan, P. B.; Gottesfeld, J. M. Small molecules targeting histone H_4 as potential therapeutics for chronic myelogenous leukemia. *Mol. Cancer Ther.* **2008**, *7*, 769–778; (b) Raskatov, J. A.; Nickols, N. G.; Hargrove, A. E.; Marinov, G. K.; Wold, B.; Dervan, P. B. Gene expression changes in a tumor xenograft by a pyrrole-imidazole polyamide. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16041–16045; (c) Minoshima, M.; Chou, J. C.; Lefebvre, S.; Bando, T.; Shinohara, K.; Gottesfeld, J. M.; Sugiyama, H. Potent activity against K562 cells by polyamide-*seco*-CBI conjugates targeting histone H4 genes. *Bioorg. Med. Chem.* **2010**, *18*, 168–174.
5. Dervan, P. B.; Edeleson, B. S. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. *Curr. Opin. Struc. Biol.* **2003**, *13*, 284–299.
6. (a) Lacy, E. R.; Cox, K. K.; Wilson, W. D.; Lee, M. Recognition of T*G mismatched base pairs in DNA by ed imidazole-containing polyamides: Surface plasmon resonance and circular dichroism studies. *Nucleic Acids Res.* **2002**, *30*, 1834–1841; (b) Rucker, V. C.; Foister, S.; Melander, C.; Dervan, P. B. Sequence-specific fluorescence detection of double strand DNA. *J. Am. Chem. Soc.* **2003**, *125*, 1195–1198.
7. Satam, V.; Patil, P.; Babu, B.; Gregory, M.; Bowerman, M.; Savagian, M.; Lee, M.; Tzou, S.; Olson, K.; Liu, Y.; Ramos, J.; Wilson, W. D.; Bingham, J. P.; Kiakos, K.; Hartley, J. A.; Lee, M. Design, synthesis, and DNA binding characteristics of a group of orthogonally positioned diamino, N-formamido, pyrrole-, and imidazole-containing polyamides. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1699–1702.
8. (a) Dervan, P. B.; Edelson, B. S. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. *Curr. Opin. Struc. Biol.* **2003**, *13*, 284; (b) Murty, M. S. R. C.; Sugiyama, H. Biology of N-methylpyrrole-N-methylimidazole hairpin polyamide. *Biol. Pharm. Bull.* **2004**, *27*, 468–474.
9. (a) Chen, Y.-H.; Lown, J. W. A new DNA minor groove binding motif cross-linked lexitropsins. *J. Am. Chem. Soc.* **1994**, *116*, 6995–7005; (b) Greenberg, W. A.; Baird, E. E.; Dervan, P. B. A comparison of H-Pin and hairpin polyamide motifs for the recognition of the minor groove of DNA. *Chem. Eur. J.* **1998**, *4*, 796–805; (c) Mackay, H.; Brown, T.; Uthe, P. B.; Westrate, L.; Sielaff, A.; Jones, J.; Lajiness, J. P.; Kluza, J.;

O'Hare, C.; Nguyen, B.; Davis, Z.; Bruce, C.; Wilson, W. D.; Hartley, J. A.; Lee, M. Sequence-specific and high affinity recognition of 5′-ACGCGT-3′ by rationally designed pyrrole-imidazole H-pin polyamides: Thermodynamic and structural studies. *Bioorg. Med. Chem.* **2008**, *16*, 9145–9153.

10. Li, B. C.; Montgomery, D. C.; Puckett, J. W.; Dervan, P. B. Synthesis of cyclic Py-Im polyamide libraries. *J. Org. Chem.* **2013**, *78*, 124–133.
11. Lacy, E. R.; Le, N. M.; Price, C. A.; Lee, M.; Wilson, D. W. Influence of a terminal formamido group on the sequence recognition of DNA by polyamides. *J. Am. Chem. Soc.* **2002**, *124*, 2153–2163.
12. (a) Lee, M.; Preti, C. S.; Vinson, E.; Wyatt, M. D.; Hartley, J. A. GC sequence-specific recognition by an N-formamido, C-terminus-modified, and imidazole-containing analogue of netropsin. *J. Med. Chem.* **1994**, *37*, 4073–4075; (b) Satz, A. L.; Bruice, T. C. Recognition in the minor groove of double-stranded DNA by microgonotropens. *Acc. Chem. Res.* **2002**, *35*, 86–95; (c) Moretti, R.; Donato, L. J.; Brezinski, M. L.; Stafford, R. L.; Hoff, H.; Thorson, J. S.; Dervan, P. B.; Ansari, A. Z. Targeted chemical wedges reveal the role of allosteric DNA modulation in protein–DNA assembly. *ACS Chem. Biol.* **2008**, *3*, 220–229; (d) Carlson, C. D.; Warren, C. L.; Hauschild, K. E.; Ozers, M. S.; Qadir, N.; Bhimsaria, D.; Lee, Y.; Cerrina, F.; Ansari, A. Z. Specificity landscapes of DNA binding molecules elucidate biological function. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4544–4549; (e) Fujimoto, J.; Bando, T.; Minoshima, M.; Kashiwazaki, G.; Nishijima, S.; Shinohara, K.; Sugiyama, H. Perylene-conjugated pyrrole polyamide as a sequence-specific fluorescent probe. *Bioorg. Med. Chem.* **2008**, *16*, 9741–9744.
13. Buchmueller, K. L.; Bailey, S. L.; Matthews, D. A.; Taherbhai, Z. T.; Register, J. K.; Davis, Z. S.; Bruce, C. D.; O'Hare, C.; Hartley, J. A.; Lee, M. Physical and structural basis for the strong interactions of the–ImPy-central pairing motif in the polyamide f-ImPyIm. *Biochemisry* **2006**, *45*, 13551–13565.
14. Chavda, S.; Babu, B.; Patil, P.; Plaunt, A.; Ferguson, A.; Lee, M.; Tzou, S.; Sjoholm, R.; Rice, T.; Mackay, H.; Ramos, J.; Wang, S.; Lin, S.; Kiakos, K.; Wilson, W. D.; Hartley, J. A.; Lee M. Design, synthesis, and DNA binding characteristics of a group of orthogonally positioned diamino, N-formamido, pyrrole-, and imidazole-containing polyamides. *Bioorg. Med. Chem.* **2013**, *21*, 3907–3918.
15. Babu, B.; Brien, K. A.; Satam, V.; Plaunt, A.; Pressler, M.; Shen, H.; Alger, S.; Ogilvie, R.; Sjoholm, R.; Tzou, S.; Sweat, O.; Rice, T.; Mackay, H.; Seymour, M. D.; Lee, M. Synthesis and DNA-binding properties of 1,2,3-triazole-linked H-pin pyrrole- and imidazole-containing polyamides formed by the Huisgen reaction. *Heterocycl. Commun.* **2012**, *18*, 79–85.
16. Lown, J. W.; Krowicki, K. Efficient total syntheses of the oligopeptide antibiotics netropsin and distamycin. *J. Org. Chem.* **1985**, *50*, 3774–3779.
17. Ramos, J. P.; Babu, B.; Chavda, S.; Liu, Y.; Plaunt, A.; Ferguson, A.; Savagian, M.; Lee, M.; Tzou, S.; Lin, S.; Kiakos, K.; Wang, S.; Lee, M.; Hartley, J. A.; Wilson, W. D. Affinity and kinetic modulation of polyamide-DNA interactions by N-modification of the heterocycles. *Biopolymers* **2013**, *99*, 497–507.
18. (a) Hale, W. J.; Hoyt, W. H. The constitution of the nitro-α-carbopyrrolic acids. *J. Am. Chem. Soc.* **1915**, *37*, 2538–2552; (b) Fanta, P. E. Sodium nitromalonaldehyde monohydrate. *Org. Synth. Collect. Vol.* **1963**, *4*, 844.
19. Krowicki, K.; Lown, J. W. Synthesis of novel imidazole-containing DNA minor groove binding oligopeptides related to the antiviral antibiotic netropsin. *J. Org. Chem.* **1987**, *52*, 3493–3501.
20. Zimmerman, H.; Brueckener, D.; Henninger, K.; Rosentreter, U.; Hendrix, M.; Keldenich, J.; Lang, D.; Radtke, M.; Paulsen, D.; Kern, A. Heterocyclyamide-substituted imidazoles. US Patent 2008/0176859 A1, July 24, 2008.