Pergamon

Tetrahedron Letters 39 (1998) 5129–5132

TETRAHEDRON LETTERS

SYNTHESIS OF NEW BUILDING BLOCKS: TOWARDS THE ANALOGS OF PEPTIDE NUCLEIC ACIDS (PNAs)[1a]

Carol Dallaire and Prabhat Arya[1b]

Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario, K1A 0R6, Canada

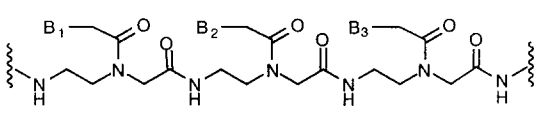
Received 16 April 1998; revised 8 May 1998; accepted 11 May 1998

Abstract: To obtain new analogs of peptide nucleic acids (PNAs), synthesis of the building block **14** has been achieved. Building block **14** has been derived from the coupling of the isothiocyanate derivative, **12** with **13**. Isothiocyanate derivative **12** was obtained from *S*-aspartic acid derivative **5** in a number of steps.

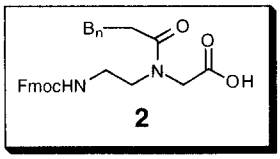
During the past several years, peptide nucleic acids (PNAs, Figure 1, **1**) have appeared to be useful mimics of DNA with promising applications in diagnostics and in the pharmaceutical area (e.g. antisense-antigene therapeutic agents).[2] Unlike DNA/RNA, PNA is an achiral, neutral molecule in which nucleobases are attached to the achiral backbone derived from N-(2-aminoethyl)glycine derivative (**2**). The synthesis of PNA could be easily achieved through peptide chemistry by using a building block methodology on solid phase.[3] PNA has shown to exhibit excellent hybridization and sequence specific properties to single-stranded (ss) DNA and RNA. It is also known to form stronger triplexes with double-stranded (ds) DNA. The strong binding of PNA with ssDNA, dsDNA and RNA has been attributed to its neutral character and to the flexible nature of the polyamide backbone, in combination with the rigidity of the amide bond in the backbone. Various studies on the flexibility of PNA have indicated that the N-(2-aminoethyl)glycine backbone provides optimal binding capabilities.[2,4a] However, very little has been studied to explore the importance of the rigidity of an amide bond within a backbone on binding properties of PNA.[2,4b-d]

Despite having several advantages, i.e. enzymatic stability, stronger and sequence-specific binding to ssDNA, dsDNA and RNA, ease of synthesis using solid phase methodology, its limited solubility at physiological *p*H, and passive transportation across the cell membrane are the two major limitations that are associated with PNA.[2,5] Moreover, hybridization of PNA with a ssDNA to form a PNA/ssDNA/PNA triplet (antigene strategy) at physiological salt concentration is not efficient. The formation of stable PNA/ssDNA/PNA triplex occurs only at low salt concentrations and is restricted to PNAs having a high pyrimidine content.[6]

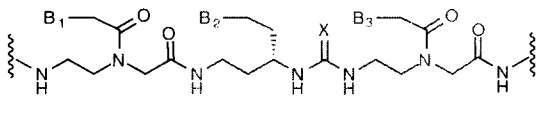
Figure 1:



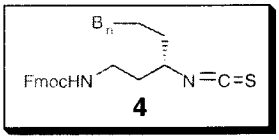
Peptide Nucleic Acid (PNA, **1**)



2



PNA analogs (**3**, X= S, NH^+)

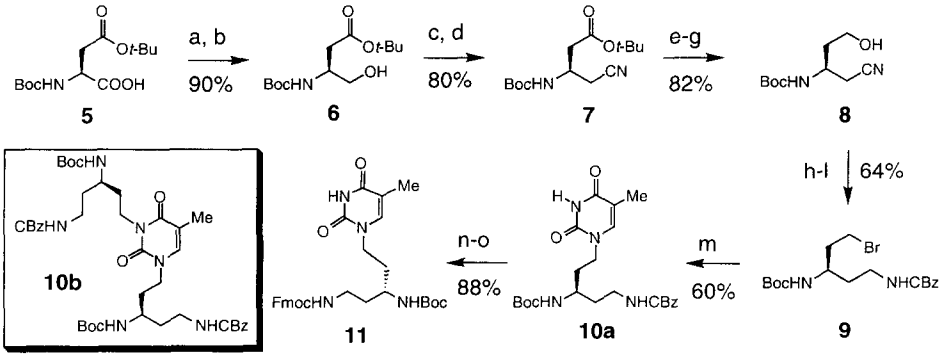


4

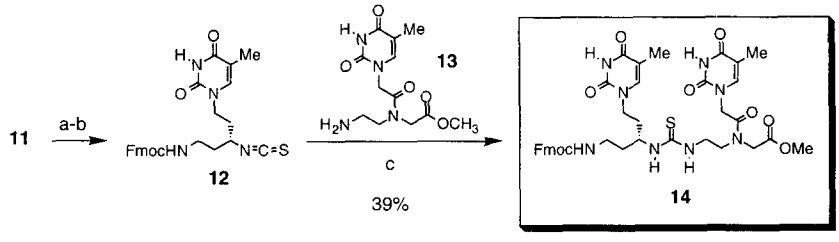
PII: S0040-4039(98)01007-7

To overcome the problems that are associated with PNA, the objective of our research is to incorporate modifications into the polyamide backbone of PNAs and explore its effects on the binding properties with DNA/RNA and on the transportation across the cell membranes. Our goal is to enhance the rigidity of the polyamide backbone by the incorporation of thiourea or guanidinium functional group (for PNA analogs, see Figure 1, **3**). Synthesis of PNA analogs, **3**, could be achieved using a building block **2** (required for the synthesis of PNA) and **4** on a solid phase. Using this approach, it would be possible to systematically control the rigidity of the polyamide backbone. Moreover, transformation of the thiourea to a guanidinium salt could easily be obtained within the backbone. Such a transformation would allow an enhancement in the solubility of the PNA analog at the physiological pH due to the incorporation of a positive charge into the backbone. Applications of cationic DNA analogs for stronger bindings with RNA or DNA are just appearing in the literature and a positively charged analog of PNA could open new opportunities in the area of antisense/antigene technology.[7]

Herein, we describe our approach for the synthesis of building blocks **12** and **14** (pyrimidine derivative as a base) required for the solid phase synthesis of PNA analogs. Building block **12** was obtained from a *S*-aspartic acid derivative, **5**, in the following steps. β-Amino alcohol **6** (**Scheme 1**) was derived from N-(butyloxycarbonyl)-*S*-aspartic acid mono-*t*-butyl ester (**5**) by the $NaBH_4$ reduction of the mixed anhydride (*iso*-



Scheme 1: (a) 1.1 eq iso-BuOCOCl, NMM, THF, 0 °C; (b) 3 eq $NaBH_4$, 0 °C MeOH; (c) TsCl, Pyr, rt; (d) 2.5 eq NaCN, DMSO, 80 °C; (e) 2M NaOH, MeOH, rt; (f) 1.1 eq iso-BuOCOCl, NMM, DME, -15 °C; (g) 3 eq $NaBH_4$, H_2O, -15 °C; (h) TBDMSCl, imidazole, rt; (i) 0.4 eq $CoCl_2.6H_2O$, MeOH, 10 eq $NaBH_4$, -15 °C; (j) 10% K_2CO_3, Dioxane, 1.2 eq CBzCl; (k) 1.2 eq Bu_4NF, THF, rt; (l) Ph_3P, CBr_4, THF, rt; (m) 3 eq Thymine, 3 eq K_2CO_3, Bu_4NI (cat), DMF, 80 °C; (n) H_2, 10% Pd/C, MeOH; (o) 1.2 eq FmocCl, 10% K_2CO_3, CH_3CN.



Scheme 2: (a) TFA, CH_2Cl_2, 0 °C; (b) DIPC, THF, CS_2, 0 °C; (c) CH_2Cl_2, DMF, 40 °C.

BuOCOCl, N-methylmorpholine inTHF) in 90% yield.[8] Alcohol derivative **6** was converted to the corresponding nitrile **7** in two steps (80% yield): (i) tosyl chloride (pyridine, CH_2Cl_2), and (ii) NaCN, DMSO at 80 °C.[9] Mild hydrolysis (2M NaOH, methanol) of **7**, followed by the $NaBH_4$ reduction of the mixed anhydride yielded **8** in 82% yield. Compound **8** was converted to **9** in the sequential order: (i) protection of the primary hydroxyl group (TBDMSCl, imidazole, rt); (ii) reduction of the nitrile group ($NaBH_4$, $CoCl_2 \cdot H_20$, MeOH);[10] (iii) protection of the amino group (CBzCl, K_2CO_3, Dioxane); (iv) deprotection of the hydroxyl group, and finally, (v) conversion of the primary hydroxyl group to the bromo derivative (Ph_3P, CBr_4, rt). Using alkylation reaction conditions as described by Taddei,[4c] nucleophilic substitution of a nucleobase (*i.e.*, thymine) gave the desired compound **10a** (60% yield) and a side product, **10b** (32% yield). The required building block, **11** was obtained from **10a** in two steps: (i) conversion of NHCBz to NH_2 (H_2, 10% Pd/C, MeOH), and (ii) the protection of NH_2 to NHFmoc (FmocCl, 10% K_2CO_3, CH_3CN). The thiourea dinucleotide building block **14** was obtained from the isothiocyanate derivative of **11** through several steps. The butyloxycarbamate protective group of **11** was removed by the treatment with TFA/CH_2Cl_2 and was reacted with CS_2, DIPC/THF that afforded the isothiocyanate derivative **12** (**Scheme 2**).[11] The thiourea dinucleotide methyl ester building block, **14** was obtained in 39%. isolated yield (purified by RP-HPLC) from the coupling of isothiocyanate derivative **12** with the PNA thymine monomer **13** (40 °C).[12] In a similar manner, the isothiocyanate **12** could also be coupled with other PNA monomers having different pyrimidine and purine bases to obtain new building blocks for the synthesis of PNA analogs.

To summarize, a successful synthesis of the methyl ester of building block **14**, required for the solid phase metholodology to obtain PNA anlogs, has been achieved. Further, incorporation of the building block **14** for various derivatives of modified PNAs to explore their properties are currently under investigation.

References and notes:

1. (a) NRCC publication no. 40869. (b) For information, contact PA: prabhat.arya@nrc.ca; (613) 993 7014 (Tel), 952 0068 (Fax).

2. (a) Nielsen, P. E.; Haaima, G. *Chem. Soc. Rev.*, **1997**, 73 and references therein. (b) Dueholm, K. L.; Nielsen, P. E. *New J. Chem.*, **1997**, *21*, 19 and references therein. (c) Nielsen, P. E. In *Perspectives in Drug Discovery and Design*; ESCOM Science Publishers, 1995, Vol 4, 76-84. (d) Nielson, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Anti-Cancer Drug Design*, **1993**, *8*, 53. (e) Egholm, M.; Buchardt, O.; Christensen, L.; Beherns, C.; Frier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Nordén, B.; Nielsen, P. E. *Nature*, **1993**, *365*, 566. (f) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.

3. Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.*, **1994**, *59*, 5767.

4. (a) Hyrup, B.; Egholm, M.; Nielsen, P. E.; Wittung, P.; Nordén, B.; Buchardt, O. *J. Am. Chem. Soc.* **1994**, *116*, 7964. (b) Kosynkina, L.; Wang, W.; Liang, T. C. *Tetrahedron Lett.* **1994**, *35*, 5173. (c) Lenzi, A.; Reginato, G.; Taddei, M. *Tetrahedron Lett.* **1995**, *36*, 1713. (d) Lenzi, A.; Reginato, G.; Taddei, M.; Trifilieff, E. *Tetrahedron Lett.* **1995**, *36*, 1717.

5. (a) Demidov, V.; Frank-Kamenetskii, M. D.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Nucleic Acid Res* **1993**, *21*, 2103. (b) Hanvey, J. C.; Peffer, N. J.; Bisi, J. E.; Thomson, S. A.; Cadilla, R.; Josey, J. A.; Ricca, D. J.; Hassman, C. F.; Bonham, M. A.; Au, K. G.; Carter, S. G.; Bruckenstein, D. A.; Boyd, A. L.; Noble, S. A.; Babiss, L. E. *Science* **1992**, *258*, 1481.

6. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Nucleic Acids Res* **1993**, *21*, 197. (b) Pardridge, W. M.; Boado, R. J.; Kang, Y.-S. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 5592. (c) Tomac, S.; Sarkar, M.; Ratilainen, T.; Wittung, P.; Nielsen, P. E.;

Nordén, B.; Gräslund, A. *J. Am. Chem. Soc.* **1996**, *118*, 5544.

7. Dempcy, R. O.; Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 6140.

8. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923.

9. Kaseda, T.; Kikuchi, T.; Kibayashi, C. *Tetrahedron Lett.* **1989**, *30*, 4539.

10. Satoh, T.; Suzuki, S. *Tetrahedron Lett.* **1969**, 4555.

11. Henichart, J. P.; Bernier, J. L.; Houssin, R. *Synthesis* **1980**, 311.

12. All the new compounds were well characterized by MS, ^{1}H NMR, ^{13}C NMR and $[\alpha]_D$. Compound **9**: R_f = 0.37 (CH_2Cl_2/ AcOEt, 9/1); $[\alpha]_D$ = +2.5 (c= 1.0, CH_3OH); ^{1}H nmr 200MHz ($CDCl_3$) δ ppm: 7.34 (s, 5H, Ph), 5.54 (m, 1H, NHCBz), 5.09 (s, 2H, CH_2Ph), 4.45 (br d, 1H, NHBoc), 3.78 (m, 1H, N-CH), 3.5-3.3 (m, 1H), 3.4 (t, 2H, CH_2Br), 3.0 (m, 1H), 2.1-1.9 (m, 2H), 1.9-1.7 (m, 2H), 1.42 (s, 9H, *t*-Bu); ^{13}C nmr 50MHz ($CDCl_3$) δ ppm: 156.4, 156.0, 136.5, 128.4, 127.9, 79.6, 66.4, 44.1, 38.5, 37.4, 35.5, 29.3, 28.2; MS (Fab+, LiCl) m/z reported for Br^{79} (%): 421.0 (100, MLi^+), 365 (37), 315 (23, M^+- Boc), 285 (27); EPMS m/z reported for Br^{79}: MH^+= 415.1. Compound **10a**: R_f = 0.35 (CH_2Cl_2/ acetone, 4/1); m.p.: 76-77°C; $[\alpha]_D$ = +19 (c= 1.0, $CHCl_3$); ^{1}H nmr 200MHz ($CDCl_3$) δ ppm: 8.7 (br s, 1H, NH thymine), 7.35 (s, 5H, Ph), 7.0 (s, 1H, CH thymine), 5.47 (br t, 1H, NHCBz), 5.09 (s, 2H, CH_2O), 4.64 (br d, 1H, J 9.6Hz, NHBoc), 3.9 (m, 1H), 3.37-3.3 (m, 3H), 3.0 (m, 1H), 1.9 (s, 3H, CH_3), 1.9-1.6 (m, 2H), 1.6-1.4 (m, 2H), 1.44 (s, 9H, *t*-Bu); ^{13}C nmr 50MHz ($CDCl_3$) δ ppm: 164.6, 156.4, 156.1, 151.1, 140.9, 136.5, 128.3, 127.9, 127.8, 125.1, 110.5, 79.6, 66.4, 46.2, 45.8, 37.3, 35.9, 34.1, 28.2, 12.2; MS (Fab+) m/z (%): 461 (11, MH^+), 405 (9, M^+- *t*-Bu), 362 (23), 361 (100); HRMS (Fab+, LiCl), MLi^+ observed: 467.2483, mass calc.: 467.2483. Compound **10b**: R_f = 0.59 (CH_2Cl_2/ acetone, 4/1); $[\alpha]_D$ = +20.0 (c= 1.0, CH_3OH); ^{1}H nmr 200MHz ($CDCl_3$) δ ppm: 7.36 (s, 10H, Ph), 7.0 (s, 1H, CH thymine), 5.7 and 5.5 (2 m, 2H, 2 NHCBz), 5.1 (m, 4H, 2 CH_2O), 4.65 (br d, 2H, 2 NHBoc), 4.0 (br t, 2H, 2 N-CH), 3.9-3.3 (3 m, 6H), 3.0 (m, 2H), 1.90 (s, 3H, CH_3), 1.9-1.4 (m, 4H), 1.42 (s, 18H, 2 *t*-Bu); ^{13}C nmr 50MHz ($CDCl_3$) δ ppm: 163.7, 156.5, 156.2, 151.4, 139.0, 136.7, 128.5, 128.1, 128.0, 109.9, 79.8, 79.4, 66.6, 66.5, 47.5, 46.2, 46.0, 38.5, 37.4, 36.2, 35.9, 34.2, 33.3, 28.3, 13.0; MS (Fab+) m/z (%): 794.3 (17, M^+), 694.7 (100, MH^+- Boc), 638.7 (50), 594.7 (13), 530.7 (7). Compound **11**: R_f = 0.33 (CH_2Cl_2/ acetone, 3/1); Rf = 0.33 (CH_2Cl_2/ toluene/ acetone, 5/2/3); m.p.: 101-102°C; $[\alpha]_D$ = +18 (c= 1.1, $CHCl_3$); ^{1}H nmr 600MHz ($CDCl_3$) δ ppm: 8.32 (s, 1H, NH thymine), 7.77 (d, 2H, J 7.3 Hz, 2 CH), 7.61 (d, 2H, J 7.3Hz, 2 CH), 7.40 (t, 2H, J 7.3Hz, 2 CH), 7.31 (t, 2H, J 7.3Hz, 2 CH), 7.01 (s, 1H, CH thymine), 5.44 (br s, 1H, NHFmoc), 4.58 (br d, 1H, NHBoc), 4.37 (d-d, 2H, J 14 and 6.8Hz, CH_2O), 4.22 (t, 1H, J 6.8Hz, CH furenyl), 3.91 and 3.59 (2 br s, 2H, H_2C-N thymine), 3.68 (br s, 1H, HC-NBoc), 3.45 and 3.05 (2 br s, 2H, H_2C-NHFmoc), 1.90 (s, 3H, CH_3), 1.90 and 1.75 (2 m, 2H, CH_2), 1.75 and 1.51 (2 m, 2H, CH_2), 1.46 (s, 9H, *t*-Bu); ^{13}C nmr 150MHz ($CDCl_3$) δ ppm: 164.0, 156.8, 156.2, 150.7, 144.0, 141.0, 141.3, 140.8, 127.7, 127.1, 125.1, 120.0, 110.8, 80.0, 66.8, 47.3, 46.3, 46.0, 37.5, 36.2, 34.5, 28.4, 12.3; MS (Fab+) m/z, %: 549.2 (9, MH^+), 492.3 (8), 449.2 (100); HRMS (Fab+), MH^+ observed: 549.2717, mass calc.: 549.2713. Compound **14**: R_f = 0.37 (CH_2Cl_2/ MeOH, 9/1); m.p. (white powder): 125-7°C; $[\alpha]_D$ = +65 (c= 0.77, $CHCl_3$); HPLC prep. (NovaPak C18 25 x 300mm, flow 20.0 mL/ min), 40% acetonitrile for 10min then 100%, t= 11.0 to 13.3 min; ^{1}H nmr 600MHz ($CDCl_3$) δ ppm: 11.3 and 10.7 (2 br s, 2H, 2 NH thymine), 7.75 (d, 2H, J 7.2Hz, 2 CH), 7.64 (d, 2H, J 7.2Hz, 2 CH), 7.38 (t, 2H, J 7.2 Hz, 2 CH), 7.31 (m, 2H, 2 CH), 7.25-7.1 (m, 2H, 2 HN-C=S), 7.18 (s, 1H, CH thymine), 7.0 (s, 1H, CH thymine), 6.1 (br s, 1H, NHFmoc), 4.75 (m, 2H, 2 HC-NC=S), 4.5-4.25 (m, 3H, 3 HC-N), 4.2 (t, 1H, J 7.1Hz, CH furenyl), 4.08 (s, 2H, N-CH_2-C=O), 3.95-3.55 (m, 4H, 2 H_2C-N), 3.80 (s, 2H, H_2C-N thymine), 3.71 (s, 3H, OCH_3), 3.4 and 2.95 (2 m, 2H, H_2C-NFmoc), 2.15 (m, 1H), 1.86 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 1.75 (m, 2H), 1.5 (m, 1H); ^{13}C nmr 150MHz ($CDCl_3$) δ ppm: 183.2, 169.4, 167.3, 165.1, 164.9, 156.5, 152.2, 151.8, 144.14, 144.07, 141.8, 141.5, 141.3, 127.8, 127.7, 127.1, 120.0, 111.2, 110.4, 66.7, 53.0, 52.6, 50.9, 48.9, 48.0, 47.3, 42.7, 36.9, 36.8, 33.8, 12.3, 12.1; MS (Fab+) m/z: 789.4 (MH^+).