Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 24, pp. 3135-3138, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10172-X

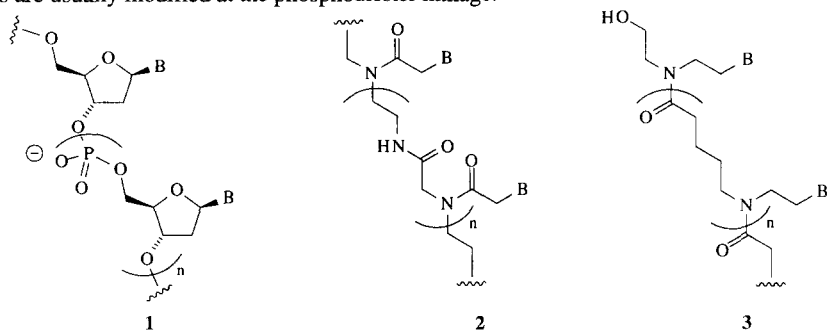
SYNTHESIS OF OLIGO(5-AMINOPENTANOIC ACID)-NUCLEOBASES (APN): POTENTIAL ANTISENSE AGENTS

Stephen C. Bergmeier* and Susan L. Fundy

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 W. 12 th Ave., Columbus, Ohio 43210-1291

Abstract: Oligomers of 5-amino pentanoic acid nucleobases have been prepared for use as antisense agents. The synthesis of the 5′-end starter unit and the 3′-end monomer unit, as well as the coupling procedures used for oligomer formation are described. © 1997 Elsevier Science Ltd.

The principle of antisense inhibition of gene expression requires the binding of a complementary oligonucleotide (the antisense agent) to either DNA (antigene) or RNA (antisense). This binding of the antisense agent to DNA or RNA will then inhibit transcription, translation, or RNA processing through a variety of mechanisms.[1] Due to severe limitations in the stability and utility of natural oligonucleotides as drugs, most antisense or antigene agents make use of modified oligonucleotides as the antisense agent. These modified antisense agents are usually modified at the phosphodiester linkage.[1]



1 2 3

Figure 1: The structures of DNA (**1**), PNA (**2**), and of the PNA analog, APN (**3**).

A unique type of antisense agent is the peptide nucleic acid (PNA, **2**), in which the purine or pyrimidine base is attached to a peptide type of backbone.[2] Several analogs of the PNA have been prepared and most of these PNAs rely upon ethylene diamine/glycine as the backbone of the molecule.[3] A major advantage of these PNA analogs is their stability both to nucleases as well as peptidases. These compounds have also shown excellent hybridization properties with both DNA and RNA.[4]

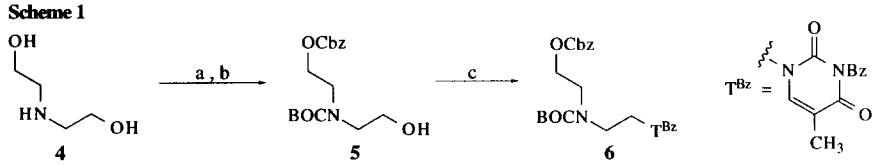
To further investigate the structural requirement of PNAs, we decided to prepare a PNA of the general structure **3** in which we have oligomerized 5-aminopentanoic acid nucleobase (APN) monomers. We chose these monomeric units for two reasons. First, we were aware that the preorganization of the PNA **2** makes hybridization to DNA and RNA thermodynamically favorable.[5] We thus wished to evaluate how little preorganization was required in a molecule to still obtain favorable thermodynamics of hybridization. Of particular note to our studies has been the preparation of a variety of very flexible PNA type molecules.[6] While some of these molecules have shown decreased hybridization ability, others have shown very similar hybridization properties to PNAs. We believe that the all carbon backbone of the APN will make modifications

(e.g., olefins, amino/carboxyl groups) relatively easy to explore from a synthetic point of view. Such modifications will allow us to further probe the structural requirements necessary for hybridization.

In order to obtain oligomer **3**, it was necessary to synthesize two separate pieces, the 5′-end starter unit **6** and the 3′-end monomer unit **11**. Pieces **6** and **11** could be deprotected and coupled together to form the APN oligomer. Once incorporated into an oligomer, the benzyl carbonate (Cbz) protecting group could be removed from the 5′-end of the APN giving the free hydroxyl. The free hydroxyl serves as a mimic of the 5′-end of natural DNA, and provides a handle for possible incorporation of the APN into a chimeric structure with DNA. The hydroxyl is also an appropriate site for attachment to a solid support which would be useful for synthesis of a large oligomer.

The synthesis of the 5′-end of the oligomer is shown in scheme one. Starting from diethanolamine (**4**), the nitrogen is protected as a *t*-butyl carbamate and one of the alcohols is protected as a benzyl carbonate to produce **5**.[7] The remaining alcohol is coupled to N^3-benzoylthymine[8] via Mitsunobu conditions[9] to give **6**.

Scheme 1

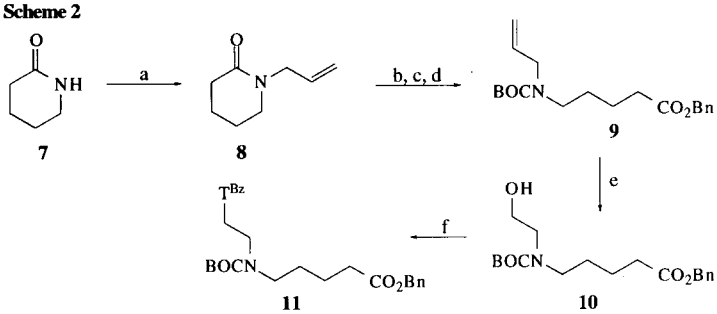


4 **5** **6**

(a) $(BOC)_2O$, THF, 0 °C→ rt, 24 h, 100% (b) Cbz-Cl, Et_3N, DMAP, CH_2Cl_2, 0 °C→ rt, 24 h, 43% (c) N^3-benzoylthymine, DEAD, Ph_3P, THF, 0 °C→ rt, 2 h, 65%.

Initial attempts to prepare the aminopentanoic acid monomer unit **11** from 5-aminopentanoic acid directly proved fruitless. The optimal route to **11** is shown in scheme two. Starting from δ-valerolactam (**7**), the nitrogen was *N*-allylated to give **8**. The lactam was hydrolyzed, the acid esterified and the nitrogen protected as the *t*-butyl carbamate derivative to give **9** in 62% yield from **8**. The olefin was then cleaved with O_3, and a reductive workup with $NaBH_4$ gave alcohol **10**. Finally the thymine was introduced via a Mitsunobu reaction as before to give **11**.

Scheme 2



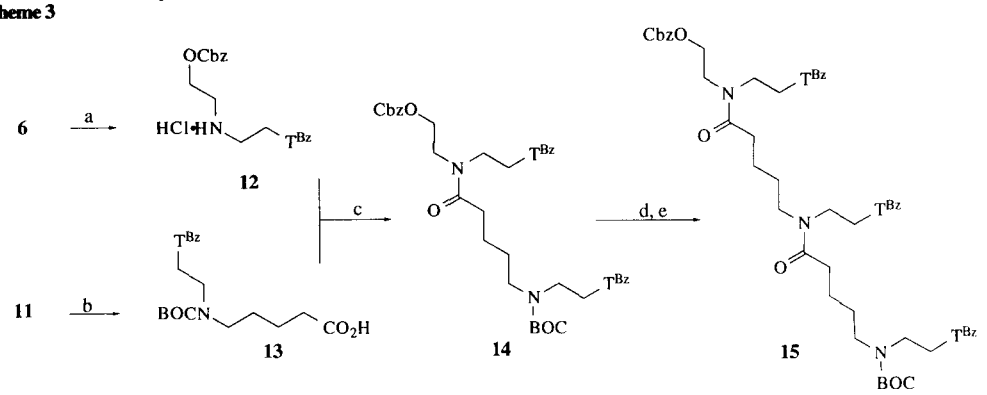
7 **8** **9** **11** **10**

(a) KH, allylbromide, THF, 0 °C→rt, 20 h, 89% (b) 7M HCl, reflux, 24 h (c) BnOH, *p*TSA, benzene, reflux, 24 h (d) $(BOC)_2O$, Et_3N, CH_2Cl_2, 0 °C→ rt, 48 h, 62% over 3 steps (e) O_3, $NaBH_4$, THF/ CH_2Cl_2, -78 °C→rt, 3.5 h, 79% (f) N^3-benzoylthymine, DEAD, Ph_3P, THF, 0°C→rt, 85%.

With starter unit **6** and monomer unit **11** in hand, the synthesis of oligomers was addressed as shown in scheme three. The starter unit was deprotected with HCl to form the amine hydrochloride **12**.[10] The benzyl ester of **11** was removed with 10% $Pd(OH)_2/C$ in THF to produce acid **13**. The choice of solvent for the

hydrogenolysis proved crucial. The use of EtOH or MeOH for hydrogenolysis gave ethyl or methyl esters along with the desired carboxylic acid.

Scheme 3



(a) HCl, EtOAc, rt, 48 h, 78% (b) H_2, 1 atm, 10% $Pd(OH)_2$ /C, THF, rt, 24 h, 90% (c) TBTU, iPr_2NEt, CH_3CN, 0°C→rt, 24 h, 67% (d) HCl EtOAc, rt, 48 h, 100% (e) TBTU, **13**, iPr_2NEt, CH_3CN, 0 °C→rt, 24 h, 51%.

We had originally sought to carry out oligomer formation using only the monomer units **13** and the N-BOC deprotected amine of **11**. All methods used to effect this coupling led to the formation of a δ-valerolactam derivative. Thus, we were required to initiate oligomer synthesis with monomer unit **12**. Our initial method for coupling **12** and **13** utilized carbonyldiimidazole and Et_3N, but no coupling product was obtained. Carbodiimides were then investigated as coupling reagents. Reaction with 1,3-diisopropylcarbodiimide and Et_3N resulted in formation of the N-acyl urea derivative of **13** as the major product in 19% yield. When DCC and Et_3N were tried, some product **14** was formed (29%), however the major product was still the N-acyl urea derivative of **13** (42%). We then examined benzotriazole based coupling reagents. Both 2-(1*H*-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU)[11] and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3 tetramethyl uronium tetrafluoroborate (TBTU)[12] were examined. The highest yields were obtained with TBTU/*i*Pr_2NEt (67%) as the coupling agent while HBTU coupling gave only 56% of **14**. Bromo-tris-pyrrolidino-phosphonium hexafluoro-phosphate (PyBroP®)[13] coupling was also examined and gave yields comparable to TBTU, but the significantly higher cost of PyBroP® relative to TBTU made it a less desirable coupling agent.[14] Chain extension was accomplished by repeating the same sequence of reactions. The BOC protecting group of dimer **14** was removed and the product was coupled to another monomer unit **13** to give trimer **15**.

In conclusion, we have developed an efficient synthesis of the monomeric units for a new class of peptide nucleic acids, in addition, conditions have been worked out to prepare oligomers of these compounds. We are currently evaluating the hybridization of these compounds with DNA as well as adapting the synthesis to solid phase for the preparation of larger oligomers. These studies will be reported in due course.

Acknowledgment
We wish to thank the American Cancer Society, Ohio Division, Inc., for support of this work.

References and Notes

1. (a) Hélène, C. *Anti-Cancer Drug Design.* **1991**, *6*, 569. (b) Thuong, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Eng.* **1993**, *32*, 666. (c) Kiely, J. S. *Annual Reports in Med. Chem.* **1994**, 297. (d) Nielsen, P. E.; Egholm, M.; Buchardt, O. *Bioconjugate Chem.* **1994**, *5*, 3. (e) Frank-Kamenetskii, M. D.; Mirkin, S. M. *Ann. Rev. Biochem.* **1995**, *64*, 65. (f) Stull, R. A.; Szoka Jr., F. C. *Pharm. Res.* **1995**, *12*, 465. (g) Crooke, S. T. *Med. Res. Rev.* **1996**, *16*, 319. (h) Miller, P. In *Bioorganic Chemistry,*

Nucleic Acids, Hecht, S. M., Ed.; Oxford University: New York, 1996, Chapter 12. (i) Akhtar, S.; Agrawal, S. *TIPS.* **1997**, *18*, 12.

2. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *245*, 1497. (b) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895. (c) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 9677. (d) 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**, *52*, 5767.
3. (a) Weller, D. D.; Daly, D. T.; Olson, W. K.; Summerton, J. E. *J. Org. Chem.* **1991**, *56*, 6000. (b) Haung, S. B.; Nelson, J. S.; Weller, D. D. *J. Org. Chem.* **1991**, *56*, 6007. (c) Lewis, I. *Tetrahedron Lett.* **1993**, *34*, 5697. (d) Kosynkina, L.; Wang, W.; Liang, T. C. *Tetrahedron Lett.* **1994**, *35*, 5173. (e) Dueholm, K. L.; Petersen, K. H.; Jensen, D. K.; Egholm, M.; Nielsen, P. E.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1077. (f) Lagriffoul, P. H.; Egholm, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1081. (g) Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179. (h) Will, D. W.; Breipohl, G.; Langner, D.; Jochen, K.; Uhlmann, E. *Tetrahedron* **1995**, *51*, 12069. (i) Ceulemans, G.; Kahn, K.; Van Schepdael, A.; Herdewijn, P. *Nucleosides Nucleotides* **1995**, *14*, 445. (j) Lenzi, A.; Reginato, G.; Taddei, M. *Tetrahedron Lett.* **1995**, *36*, 1713. (k) Lenzi, A.; Reginato, G.; Taddei, M.; Trifilieff, E. *Tetrahedron Lett.* **1995**, *36*, 1717. (l) Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6937. (m) Krotz, A. H.; Buchardt, O.; Nielsen, P. E.; *Tetrahedron Lett.* **1995**, *36*, 6941. (n) Habus, I.; Agrawal, S. *Nucleosides Nucleotides* **1995**, *14*, 1853. (o) Larsen, E.; Danel, K.; Pedersen, E. B. *Nucleosides Nucleotides* **1995**, *14*, 1905. (p) Diederichsen, U. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 445. (q) Farése, A.; Patino, N.; Condom, R.; Dalleu, S.; Guedj, R. *Tetrahedron Lett.* **1996**, *37*, 1413. (r) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1939. (s) Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. *Tetrahedron* **1996**, *52*, 15017. (t) Ciapetti, P.; Soccolini.; Taddei, M. *Tetrahedron* **1997**, *53*, 1167. (u) Cantin, M.; Schütz, R.; Leumann, C. J. *Tetrahedron Lett.* **1997**, *53*, 4211.
4. (a) Cherny, D. Y.; Belotserkovskii, B. P.; Frank-Kamenetskii, M. D.; Egholm, M.; Buchardt, O.; Berg, R. H.; Nielsen, P. E. *Proc. Natl. Acad. Sci.,U.S.A.* **1993**, *90*, 1667. (b) Peffer, N. J.; Hanvey, J. C.; Bisi, J. E.; Thomson, S. A.; Hassman, C. F.; Nobel, S. A.; Babiss, L. E. *Proc. Natl. Acad. Sci., U.S.A.* **1993**, *90*, 10648. (c) Kim, S. K.; Nielsen, P. E.; Egholm, M.; Buchardt, O.; Berg, R. H.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 6477. (d) Chen, S.; Mohan, V.; Kiely, J. S.; Griffith, M. C.; Griffey, R. H. *Tetrahedron Lett.* **1994**, *35*, 5105. (e) Nielsen, P. E.; Christensen, L. *J. Am. Chem. Soc.* **1996**, *118*, 2287. (f) Wittung, P.; Nielsen, P. E.; Nordén, B. *J. Am. Chem. Soc.* **1996**, *118*, 7049.
5. (a) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Frier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature (London)* **1993**, *365*, 566. (b) Almarsson, O.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci.,U.S.A.*. **1993**, *90*, 7518. (d) Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G. *Science* **1994**, *265*, 777. (e) Betts, L.; Josey, J. A.; Veal, J. M.; Jordan, S. R. *Science* **1995**, *270*, 1838. (f) Rasmussen, H.; Kastrup, J. S.; Nielsen, J. N.; Nielsen, P. E.; *Nature Struct. Biol.* **1997**, *4*, 98.
6. (a) Howarth, N. M.; Wakelin, L. P. G. *J. Org. Chem.* **1997**, *62*, 5441. (b) Tsantrizos, Y. S.; Lunetta, J. F.; Boyd, M.; Fader, L. D.; Wilson, M. -C. *J. Org. Chem.* **1997**, *62*, 5451. (c) Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083. (d) Jordan, S.; Schwemier, C.; Kosch, W.; Kretschmer, A.; Schwenner, E.; Stropp, U.; Mielke, B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 681. (e) Altmann, K.; Chiesi, C. S.; García-Echeverría, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1119. (f) García-Echeverría, C.; Hüsken, D.; Chiesi, C. S.; Altmann, K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1123.
7. All new compounds show satisfactory ^{1}H NMR, ^{13}C NMR, IR, and HRMS.
8. Cruickshank, K. A.; Jiricny, J.; Reese, C. B. *Tetrahedron Lett.* **1984**, *25*, 681.
9. Harnden, M. R.; Parkin, A.; Parratt, M. J.; Perkins, R. M. *J. Med. Chem.* **1993**, *36*, 1343.
10. Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216.
11. Dourtoglou, V.; Gross, B. *Synthesis* **1984**, 572.
12. Knorr, R.; Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1989**, *30*, 1927.
13. Coste, J.; Dufour, M. N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669.
14. For example, catalog prices (Calbiochem-Novabiochem) of PyBroP® ($3.28/mmol) are four times higher than TBTU ($0.80/mmol).

(*Received in USA* 11 *August* 1997; *accepted* 5 *November* 1997)