Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 8, pp. 1077-1080, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0104-M

PEPTIDE NUCLEIC ACID (PNA) WITH A CHIRAL BACKBONE BASED ON ALANINE[1]

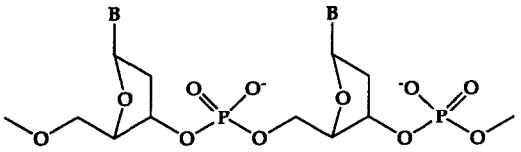
Kim L. Dueholm, Kenneth H. Petersen, Dorte K. Jensen, Michael Egholm, Peter E. Nielsen# and Ole Buchardt*

Research Center for Medical Biotechnology, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Ø, Copenhagen, Denmark

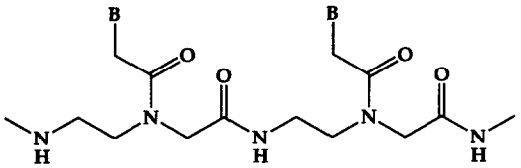
#Research Center for Medical Biotechnology, Department of Biochemistry B, The Panum Institute, Blegdamsvej 3c, DK-2200 N, Copenhagen, Denmark

Abstract: A synthesis of *N*-(2-Boc-aminoethyl)-*N*-(thymin-1-ylacetyl)alanine (**5**) is presented. It is demonstrated that chiral integrity can be preserved. PNA (Peptide Nucleic Acid) containing the D-form of **5** hybridizes to complementary DNA with higher affinity than PNA containing the L-form of **5**.

A number of recent reports from this laboratory have described how peptide nucleic acids (PNAs) bind to single-stranded and double-stranded DNA (ssDNA and dsDNA, respectively) as well as to RNA with unprecedented affinity and specificity[2-7]. PNA is a DNA mimic. In the PNA previously reported the entire ribose phosphate backbone of DNA was replaced by an achiral peptide-like backbone consisting of *N*-2-aminoethylglycine units with the nucleobases attached through methylenecarbonyl linkers[2-3]:



DNA

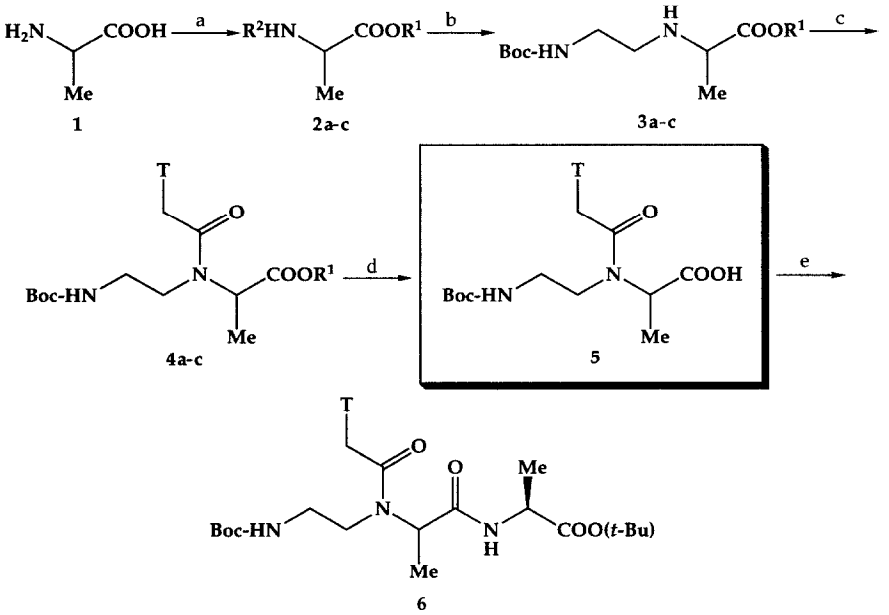


PNA

It has been demonstrated that such PNAs are capable of forming stable duplexes with complementary DNA and RNA in both the parallel orientation (PNA N-terminal opposite DNA 5′-terminal) and in the antiparallel orientation (PNA N-terminal opposite DNA 3′-terminal)[8]. PNAs are the first achiral oligomers shown to hybridize to DNA and RNA. In addition, monomer and oligomer synthesis is simplified considerably by the achirality of PNA

in comparison with chiral DNA analogues. However, we envisaged that by substituting the glycine moiety of the backbone with a chiral amino acid, e.g. alanine, the direction of binding might be controlled through the choice of chiral form, L or D. Such a modification might also serve as a model for the introduction of other amino acids which in turn could lead to modifications of such parameters as binding affinity, specificity and cell permeability. Hence, this communication describes the synthesis of the PNA monomer with L- or D-alanine in place of the glycine moiety and its incorporation into oligomers. It is demonstrated that chiral integrity can be preserved.

The target monomer *N*-(2-Boc-aminoethyl)-*N*-(thymin-1-ylacetyl)alanine (**5**) was prepared analogously to the corresponding glycine derivative[2-4,9]. Alanine methyl ester (**2a**) was reductively alkylated with Boc-aminoacetaldehyde. Attachment of the thymin-1-ylacetic acid was accomplished with DCC in the presence of DhbtOH. Finally, hydrolysis of the ester **4a** yielded the target monomer **5**.



a-series: R^1=Me, R^2=H,HCl; b-series: R^1=(S)-CH(Me)Et, R^2=Z; c-series: R^1=CH_2Ph, R^2=H
T=thymin-1-yl

Reagents and conditions: a) (S)-HOCH(Me)Et/DCC/DMAP (**2b**), $HOCH_2Ph/SOCl_2$ (**2c**); b) Boc-$HNCH_2CHO$ + H_2/10% Pd-C/NaOAc (**2a**) or H_2/10% Pd-C (**2b**) or $NaBH_3CN$/AcOH (**2c**); c) TCH_2COOH/DCC/DhbtOH (+DIEA (**3b**)); d) LiOH/H_2O/THF, 5 min., 0°C (**4a**), H_2/10% Pd-C (**4c**); e) (L)-$H_2NCH(Me)COO(t\text{-}Bu)$/TDBTU/DIEA.

In order to establish whether optical purity had been preserved through all the steps, the diastereomer **6** was prepared by coupling **5** to L-alanine t-butyl ester using TDBTU. This reagent was chosen, because DCC did not give a sufficiently clean reaction to allow unambiguous analysis by HPLC and because it has been shown to be superior to other coupling reagents in suppressing racemization of the acid component of the amide in peptide synthesis[10-11]. In this connection we noted that the presence of DIEA in the preparation of **6** was neccessary to suppress racemization of the acid component **5**. Experiments employing TDBTU alone or in the presence of DhbtOH led to significantly higher levels of racemization of **5**. An important

advantage of this approach is that this reaction served as a model for the ensuing oligomer synthesis. As **6** contained 6% of the undesired diastereomer, i.e., that resulting from racemization of **5**, when analyzed by HPLC, it was established that optical purity had not been preserved in the methyl ester series (i.e., the reactions from **2a** to **4a**).

In order to determine at what point the racemization had occurred, the ester **2b** of (S)-(+)-2-butanol was prepared from L-alanine (**1**)[12]. **2b** was subjected to the same operations as **2a** and analyzed by NMR at each step of the synthetic route. Since **4b** was obtained in an optically pure state, we conclude that chiral integrity is preserved in the reductive alkylation of **2a** and that the hydrolysis of **4a** to **5** caused the racemization.

In order to avoid the hydrolysis, the benzyl esters **2c** to **4c** were employed using hydrogenation to obtain **5** from **4c** quantitatively[13]. Using this procedure we did not observe a reduction of the 5,6 double bond of thymine as reported in the literature[14]. This strategy necessitated a change of the reducing agent used in the reductive alkylation. Thus, sodium cyanoborohydride was employed instead of catalytic hydrogenation[15-16]. Preparing **6** from the **c**- series showed that the ratio of the undesired diastereomer in this way could be reduced to 0.4%.

Both the L- and D-form of the monomer **5** were incorporated into the oligomer H-G(ala-T)A GA(ala-T) CAC (ala-T)-NH_2 in turn ((ala-T) is the alanine derivative **5** and C,A and G are the corresponding glycine derivatives with cytosine, adenine or guanine respectively, in place of thymine)[2-4,17]. The oligomer synthesis was carried out by standard solid phase peptide synthesis as previously described using DIPCDI as the coupling reagent[4], with the exception of the couplings involving **5** for which TDBTU was used in the presence of DIEA overnight.

The chiral oligomers were hybridized to their complementary ssDNA in both the parallel and antiparallel orientation and the melting temperatures (T_m) of the hybrids were measured as previously described (Table 1)[3].

Table 1. T_m(°C)[a] of hybrids between H-GXA GAX CAC X-NH_2 and ssDNA

	DNA		
X	antiparallel 5′-d(AGT GAT CTA C)	parallel 5′-d(CAT CTA GTG A)	alone[b]
T	51.0	38.0	41.0
L-ala-T	46.5	38.0	41.5
D-ala-T	50.0	41.0	40.5

[a]The solutions were 100 mM in NaCl, 10 mM in phosphate and 0.1 mM in EDTA, pH 7.
[b]Maximum of the first derivative of the sigmoidal melting curve, but not a T_m by definition.

The results indicate that the antiparallel orientation is preferred for both the L- and D-alanine containing oligomers, as it is for the corresponding glycine containing oligomer. The oligomer based on glycine and those containing the D-form of **5** have similar T_m′s in the antiparallel orientation, whereas a reduction is observed when the L-form of **5** is incorporated.

PNAs themselves appear to be highly organized at low temperature and a significant hyperchromicity is observed upon heating purine containing PNAs alone, representing the transition to a less organized state[8]. As the majority of this transition is observed at the same transition temperature as is observed for the parallel complexes, it is impossible to determine the T_m′s of these complexes by UV measurements. At present we are attempting to determine their T_m′s by means of circular dichroism.

Other chiral amino acids are being introduced into PNA at present. In addition, the incorporation of **5** into oligomers is currently being extended to other sequences. The results of

this work and further hybridization experiments will be reported in due course.

Acknowledgements:

The present investigation was carried out during tenure of an Alfred Benzon Investigator Fellowship to Kim L. Dueholm. Dr. Mogens H. Jakobsen is thanked for helpful discussions.

References and Notes

1. The following abbreviations are used: DCC (*N,N'*-dicyclohexylcarbodiimide); DhbtOH (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine); TDBTU (*O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate); DIEA (diisopropylethylamine); Z (benzyloxycarbonyl); Boc (*t*-butyloxycarbonyl); DMAP (4-dimethylaminopyridine); DIPCDI (*N,N'*-diisopropylcarbodiimide).
2. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
3. Egholm, M.; Buchardt, O.; Nielsen, P.E.; Berg, R.H. *J. Am. Chem. Soc.* **1992**, *114*, 1895-1897.
4. Egholm, M.; Nielsen, P.E.; Buchardt, O.; Berg, R.H. *J. Am. Chem. Soc.* **1992**, *114*, 9677-9678.
5. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Nucleic Acids Res.* **1993**, *21*, 197-200.
6. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Anti Cancer Drug Design* **1993**, *8*, 53-63.
7. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. Peptide Nucleic Acids (PNA): Oligonucleotide with a Peptide Backbone. In *Antisense Research and Applications*; Crook, S.; Lebleu, B. Eds.; CRC Press, Inc.; Florida, in press.
8. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S.M.; Driver, D.A.; Berg, R.H.; Kim, S.; Nordén, B.; Nielsen, P.E. *Nature*, in press.
9. Dueholm, K.L.; Egholm, M.; Buchardt, O. *Org. Prep. Proc. Int.* **1993**, *25*, 457-461.
10. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillesen, D. *Tetraheron Lett.* **1989**, *30*, 1927-1930.
11. Boomgarden, M.; Heinrich, G.; Henklein, P. *Pharmazie* **1992**, *47*, 710-711.
12. Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475-4478.
13. **5** was isolated as a mixture of two conformers due to restricted rotation around the amide bond. Consequently, some of the signals in the NMR spectra were split into a major (ma.) and a minor (mi.) component: ^{1}H-NMR (DMSO-d_6/TMS): δ = 1.42 (d, J=7.1Hz, ma. ala-Me); 1.44(s, mi. Boc); 1.46 (s, ma. Boc); 1.51 (d, J=7.0Hz, mi. ala-Me); 1.41-1.52 (12H); 1.83 (s, 3H, thymine-Me); 3.26 (m, 2H, NCH_2); 3.42 (m, 2H, NCH_2); 4.39 (q, J=7.0Hz, 1H, CH); 4.69 (s, 2H, acetyl-CH_2); 7.00 (s, 1H, thymine-H-6); 11.35 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6/TMS): δ = 12.0 (thymine-Me); 14.8 (ma. ala-Me); 15.9 (mi. ala-Me); 28.3 (Boc); 33.6 (CH); 43.8 (mi. acetyl-CH_2); 45.7 (ma. acetyl-CH_2); 48.0 (ma. NCH_2); 48.3 (mi. $NHCH_2$); 54.2 (mi. $NHCH_2$); 54.8 (ma. NCH_2); 77.9 (mi. Boc); 78.1 (ma. Boc); 108.1 (thymine-C-5); 142.2 (thymine-C-6); 151.0 (thymine-C-2); 155.8 (Boc-C=O); 164.4 (thymine-C-4); 166.9 (ma. acetyl-C=O); 167.2 (mi. acetyl-C=O); 172.6 (acid-C=O). MS(FAB-) m/z (%): 397 (100, M-H); 398 (21, M-H+1); 399 (5, M-H+2). *Anal.* Calcd for $C_{17}H_{26}N_4O_7$ H_2O: C, 49.03; H, 6.78; N, 13.45. Found: C, 48.67; H, 6.59; N, 13.12.
14. Watkins, B.E.; Kiely, J.S.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 5702-5708.
15. Booker, E.; Eisner, U. *J. Chem. Soc., Perkin I* **1975**, 929-931.
16. Szelke, M.; Leckie, B.; Hallett, A.; Jones, D.M.; Sueiras, J.; Atrash, B.; Lever, A.F. *Nature* **1982**, *299*, 555-557.
17. Egholm, M.; Behrens, C.; Christensen, L.; Berg, R.H.; Nielsen, P.E., Buchardt, O. *J. Chem. Soc., Chem. Commun.* **1993**, 800-801.

(*Received* 17 *August* 1993)