Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 6, pp. 681-686, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00079-6

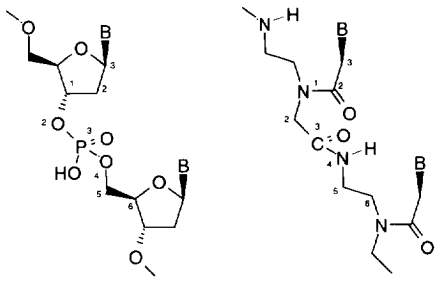
SYNTHESIS OF NEW BUILDING BLOCKS FOR PEPTIDE NUCLEIC ACIDS CONTAINING MONOMERS WITH VARIATIONS IN THE BACKBONE

Stephan Jordan,* Christoph Schwemler,* Winfried Kosch,# Axel Kretschmer,+ Eckhardt Schwenner, Udo Stropp, Burkhard Mielke

Bayer AG, Central Research, D-51368 Leverkusen, Germany

Abstract: New PNA monomers containing aminoprolines or pyrrolidines as backbones have been synthesized. Oligomerisation was carried out on a solid support. Resistance to enzymatic degradation was tested.

Since their discovery, PNAs[1] (peptide nucleic acids) have become an important class of DNA-analogues for antisense and molecular biological purposes. They show strong binding capacities to complementary DNA or RNA by Watson-Crick base-pairing. Due to their structure they are not substrates for enzymatic degradation. No undesired side effects as reported for other DNA-analogues are known[2], suggesting that hybridisation is strongly selective. In PNAs the complete sugar-phosphate backbone is replaced by a polyamide backbone containing N-(2-aminoethyl) glycine (aeg) units. The nucleobase is attached to the nitrogen of the glycine through an acetic acid linker, the number of atoms between the ends of one monomeric unit being the same as in DNA. Formally, the primary structure of a PNA is very similar to an oligonucleotide, although PNA binds more strongly to complementary oligonucleotides than DNA itself.

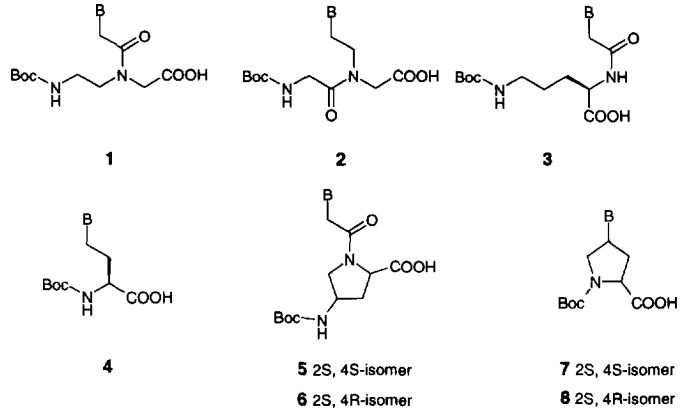


DNA PNA

Beside aeg building units **1** a number of different new PNA-buildung blocks have been developed (scheme 1), e.g. the glycylglycine-type **2**,[3] the ornithine-type **3**[4] and the aminobutyryl-type **4**.[5] In this paper, we wish to describe the synthesis and properties of new monomeric units for peptide nucleic acids. The monomers contain pyrrolidine derivatives (**5**, **6**) or different 4-aminoproline building blocks (**7**, **8**). Solid phase synthesis was used for oligomerisation. The hybridisation of these new compounds to complementary DNA and their resistance to enzymatic degradation was investigated.

* fax address: +49214/3050070

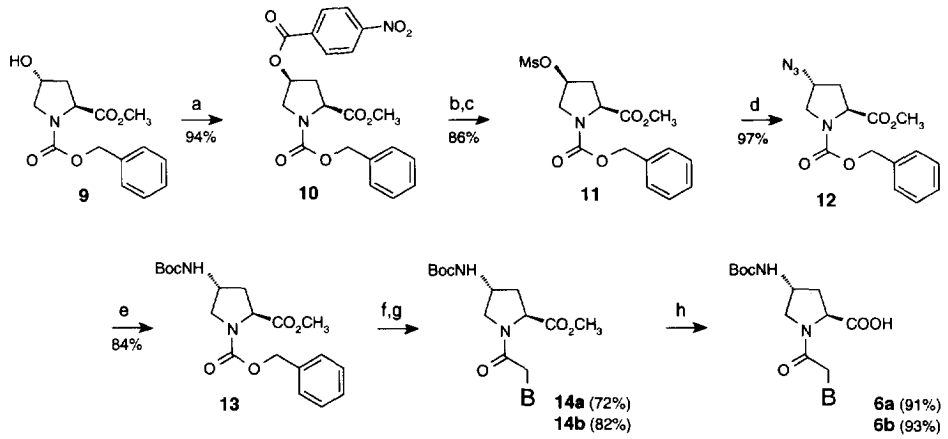
Scheme 1. Peptide nucleic acid building blocks



The preparation of Boc-protected aminoproline monomers is outlined in scheme 2. Starting from **9** the configuration at C-4 was inverted using the Mitsunobu[6] reaction with p-nitrobenzoic acid followed by hydrolysis of the corresponding ester. The hydroxy group was converted into an azido group via it's mesylate. After reduction to the amine and protection with di-*tert*-butyl dicarbonate the Cbz-group was removed by hydrogenation. Thymin-1-yl and N^4-Cbz-cytosin-1-yl acetic acid (synthesized as described in the literature)[7] were activated with EDC and HOBt and coupled with the amine to furnish fully protected derivatives. Finally the methyl ester was hydrolysed with LiOH.[8]

Starting from L-4-trans-hydroxyproline **9** mesylation followed by substitution and reduction of the azido-group affords the L-4-cis-aminoproline analogue with overall retention of the configuration at C-4.[5c]

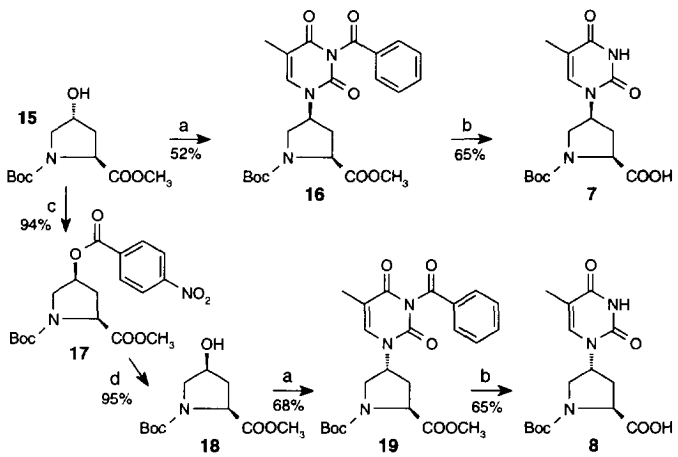
Scheme 2. Synthesis of the L-4-trans-aminoproline monomers



Reagents and conditions: **a)** $pNO_2(C_6H_4)CO_2H$/DEAD/$P(Ph)_3$; **b)** NaOMe/MeOH; **c)** Ms-Cl/pyridine; **d)** LiN_3; **e)** H_2S/pyridine, then Boc_2O/DIEA; **f)** H_2/10% Pd-C; **g)** BCH_2COOH/HOBt/EDC x HCl/NEt_3 (B = thymin-1-yl (a) or N^4-Cbz-cytosin-1-yl (b)); **h)** LiOH/H_2O/1,4-dioxan

Pyrrolidine-2-carboxylic acids are another new class of building blocks for PNAs (scheme 3). The key step in this synthesis is the introduction of the nucleobase by Mitsunobu reaction[9] at the 4-position of diprotected hydroxyproline. Removal of the benzoylic group of the heterobase and cleavage of the ester leads to compound **7**, the 2S,4S-isomer. Successive Mitsunobu reactions results in the 2S,4R-isomer **8** via a double inversion. To introduce cytosine as nucleobase we used N^4-Cbz-cytosine instead of N^3-Benzoyl-thymine during the Mitsunobu reaction (47.2 % isolated yield).

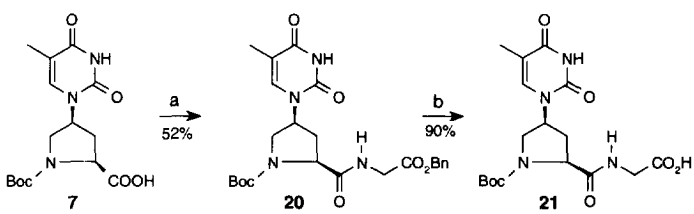
Scheme 3. Synthesis of the 2S,4S- and 2S,4R-N-Boc-4-(thymin-1-yl)-pyrrolidine-2-carboxylic acid



Reagents and conditions: **a)** N^3-Benzoyl-thymine/DEAD/$P(Ph)_3$/THF; **b)** NaOH/iPrOH then NH_3/MeOH; **c)** $pNO_2(C_6H_4)CO_2H$/DEAD/$P(Ph)_3$; **d)** NaOMe/MeOH.

Since the pyrrolidine-2-carboxylic acids lack three carbon atoms in their backbone in comparision with aeg, the new compounds are coupled to derivatives of glycine, e.g. compound **7** was coupled with glycine benzyl ester in the presences of HOBt and a carbodiimide (scheme 4). The last step in the synthesis is the hydrogenation of the ester to the carboxylic acid.[10] Using other amino acids than glycine allows backbone modification.[11]

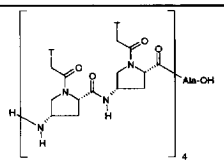
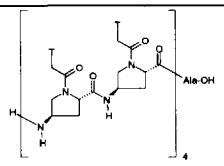
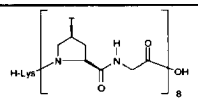
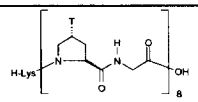
Scheme 4. Synthesis of protected pyrrolidine-2-carboxy-glycines



Reagents and conditions: **a)** Glycine benzylester/EDC/HOBt/DMF **b)** H_2/Pd/C/MeOH.

The monomers were then oligomerised by solid-phase peptide chemistry on a peptide synthesizer[12] using the standard Boc-strategy on PAM-resins. In order to suppress the tendency for aggregation in thymine PNA oligomers, resins pre-loaded with Boc-protected alanine were used. The monomers were coupled by activation with HOBt and DCC or DCC and pentafluorophenol in NMP. Oligomers containing up to 15 monomers have been constructed. After the final coupling, the Boc-group was removed by TFA and the product was cleaved from the resin with trifluoromethanesulfonic acid in TFA. The crude products were purified by reverse-phase HPLC using gradients of TFA in water and TFA in water/acetonitrile.

Table 1: Nuclease and protease stability of modified oligonucleotides

sequence	yield (%)	purity (HPLC)	stability against nucleases and proteases[13]
	20	>95%	+
	42	>95%	+
	74	>95%	+
	30	>95%	+

The resulting oligomers were tested for stability to enzymatic degradation with nucleases and proteinase K by known procedures.[13] No products of degradation were found following reaction mixture analysis by HPLC, demonstrating that the oligomers are not substrates for these enzymes. We have studied the hybridising properties of the modified oligomers with their complementary DNA by measuring UV-absorption to determine melting temperatures (T_m-values). Unfortunately, no hybridisation was found for our oligomers. Due to the impressive binding properties of the homo-oligomers containing aeg, we have sought explanations for these unexpected results. In the case of the ornithines (scheme 1), the lengthening of the side-chain from the backbone to the nucleobase may be responsible for the loss in binding capacity. For the other compounds, we have carried out molecular modelling experiments, allowing one to conclude that there is a pre-orientation of the oligomers containing an aeg backbone, leading to a pre-formed structure fitting very well to complementary DNA. In contrast, oligomers of the type described above show only random three-

dimensional structures. We plan to test this hypothesis by creating hetero-oligomers of our new building blocks and aeg.

In conclusion, new thymidine and cytidine monomers useful for the synthesis of modified PNAs for antisense research, as well as for diagnostic tools, have been synthesized. The new compounds are totally stable against enzymatic degradation. With these compounds the number of building blocks available for PNA synthesis has been enlarged. Although oligomers of the new monomers do not bind to complementary DNA when oligomerized as homo-oligomers, they may interact with DNA as hetero-oligomers in combination with various amounts of (2-aminoethyl) glycine. Work in this area is in progress.

Acknowledgement: We wish to thank Dr. A. Plant for helpful discussions and Dr. T. Fäcke for his help in interpreting the NMR spectra.

References and Notes

current address: Biotest AG, Landsteinerstr. 5, D-63303 Dreieich, Germany.

+ current address: Bayer AG, Pharma Research Center, D-42096 Wuppertal, Germany.

1 a) Nielsen. P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science*, **1991**, *254*, 1497; b) Egholm, M.; Buchardt, O; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.*, **1992**, *114*, 1895. c) Sarmieto, U. M.; Perez, J. R.; Becker, J. M.; Narayan, R. *Antisense Res. Dev.*, **1994**, *4*, 99.

2 a) De Mesmeaker, A.; Häner, R.; Martin, P.; Moser, H.E. *Acc. Chem. Res.*, **1995**, *28*, 366. b) De Mesmaeker, A.; Altmann, K.-H.; Waldner, A.; Wendeborn S. *Curr. Opin. Struc. Biol.*, **1995**, 343. c) Matteucci, M. *Persp. Drug Discov. Des.*, **1996**, *4*, 1.

3 Almarsson, Ö.; Bruice, T.C.; Kerr, J.; Zuckermann, R.N. *Proc. Natl. Acad. Sci.*, **1993**, *90*, 7518

4 a) Löbberding, A.; Mielke, B.; Schwemler, C.; Schwenner, E.; Stropp, U.; Springer, W.; Kretschmer, A.; Pötter, T. Europian Patent EP 0646596 A1 **1995**. b) Lioy, E.; Kessler, H. *Liebigs Ann.* **1996**, 201. c) Petersen, K.H.; Buchhardt, O.; Nielse, P.E. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 793.

5 a) Lenzi, A.; Reginato, G.; Taddei, M. *Tetrahedron Lett.*, **1995**, *36*, 1713. b) Ceulemans, G.; Khan, K.; Van Schepdeal, A.; Herdewijn, P. *Nucleosides & Nucleotides*, **1995**, *14*, 813. c) Löbberding, A.; Mielke, B.; Schwemler, C.; Schwenner, E.; Stropp, U.; Springer, W.; Kretschmer, A.; Pötter, T. Europian Patent EP 06465956 A1 **1995**.

6 a) Mitsunobu, O. *Synthesis* **1991**, 1. b) Martin, S.F.; Dogde, J.A. *Tetrahedron Lett.*, **1991**, *32*, 3017.

7 Egholm, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *J. Am. Chem. Soc.* **1992**, *114*, 1895.

8 ^{1}H-NMR (DMSO-d^6+MeOH-d^4) **8**: 7.44 (s, 1H, 6-Thy-H); 3.82 - 4.05 (m, 6H, CH_2); 3.55 (m, 2H, CH_2); 1.89 (s, 3H, CH_3-Thy); 1.49 (s, 9H,$(CH_3)_3C$). ^{13}C-NMR (DMSO-d^6+MeOH-d^4) **8**: 175.4 (C=O); 171.7 (C=O); 165.4 (4-C-Thy); 157.1 (C=O); 151.9 (2-C-Thy); 142.8 (6-C-Thy); 109.7 (5-C-Thy); 79.2 ($C(CH_3)_3$); 52.1, 46.9, 45.6, 41.8 (4x CH_2); 27.7 ($C(CH_3)_3$); 12.3 (CH_3-Thy). ^{1}H-NMR (DMSO-d^6) **6a** (main rotamer): 11.25 (s(br), 1H, COOH); 7.30 (s, 1H, 6-Thy-H); 7.04 (d(br), J = 7.8 Hz, 1H, NH); 4.49, 4.28 (AB-system, J = 16.5 Hz, 2H, NCH_2CO); 4.09 (dd, J = 8.7 Hz, 2.0 Hz, 1H, 2'-H-Pro); 3.98 (sextett, J = 7.9 Hz, 1H, 4'-H-Pro); 3.52, 3.05 (2dd, J = 11.8 Hz, 7.8 Hz, 2H, 5'-H-Pro); 2.25 (m, 1H, 3'-H-Pro); 1.98 (dt, J = 11.8 Hz, 8.6 Hz, 1H, 3'-H-Pro); 1.76 (s, 3H, CH_3-Thy); 1.48 (s, 9H, $(CH_3)_3C$). **6b**: 10.78 (s(br), 1H, COOH); 7.86 (s(br), 1H, NH), 7.45-7.30 (m, 6H, 5 Ar-H, 6-Cyt-H); 7.05 (d, J = 7.8 Hz, 1H, 5-Cyt-H); 6.92 (s(br) 1H, NH); 5.18 (s, 2H, Ar-CH_2O); 4.66, 4.37 (AB-system, J = 16.5 Hz,

2H, NCH_2CO), 4.14 (dd, J = 8.7 Hz, 1.0 Hz, 1H, 2'-H-Pro); 3.98 (sextett, J = 7.9 Hz, 1H, 4'-H-Pro); 3.52, 3.05 (2dd, J = 11.8 Hz, 7.8 Hz, 2H, 5'-H-Pro); 2.25 (m, 1H, 3'-H-Pro); 1.98 (dt, J = 11.8 Hz, 8.6 Hz, 1H, 3'-H-Pro), 1.49 (s, 9H, $(CH_3)_3C$).

[9] a) Jenny, T.F.; Previsani, N.; Benner, S.A. *Tetrahedron Lett.*, **1991**, *32*, 7029. b) Jenny, T.F. *Helv. Chim. Acta.*, **1993**, *78*, 248.

[10] ^{1}H-NMR (DMSO-d^6) **21**: 11.19 (s, 1H, NH), 8.22 (t, J = 7.0 Hz, 1H, NH), 7.53 (s, 1H, 6-Thy-H), 4.96 (p, J = 9.0 Hz, 1H, 4'-Pyr-H), 4.23 (t, J = 9.0 Hz, 1H, 2'-Pyr-H), 3.95 - 3.60 (m, 3H, 5'-Pyr-H, CH_2CO_2), 3.37 (dd, J = 11.9 Hz, 13.0 Hz, 1H, 5'-Pyr-H), 2.59, 2.02 (2dt, J = 17.0 Hz, 9.0 Hz, 2H, 3'-Pyr-H), 1.77 (s, 3H, CH_3), 1.35 (s, 9H, $(CH_3)_3C$).

[11] To introduce sarcosine instead of gylcine into compound **21,** compound **7** was coupled with H-Sar-OMe in the presence of BOP-Cl/Hünigs base followed by ester hydrolysis with NaOH/isopropanol (76% overall yield).

[12] An Applied Biosystem ABI 380B® synthesizer was used. Merrifield, R. B. *J. Am. Chem. Soc.*, **1963**, *85*, 2149; Barany, G.;
Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.*, **1990**, *30*, 705.

[13] Jordan, S.; Schwemler, C.; Kosch, W.; Kretschmer, A.; Stropp, U.; Schwenner, E.; Mielke, B. submitted.

(*Received in Belgium* 4 *December* 1996; *accepted* 6 *February* 1997)