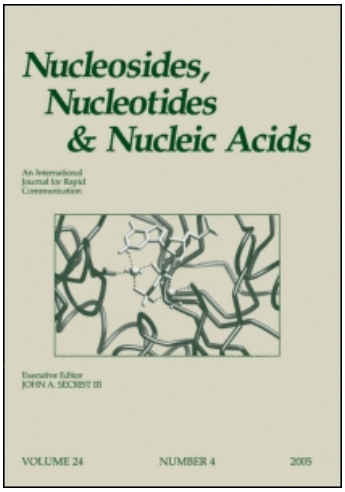


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

SYNTHESIS OF NEW CHIRAL PEPTIDE NUCLEIC ACID (PNA) MONOMERS

Bogdan Falkiewicz[a]; Wojciech Wiśniowski[a]; Aleksandra S. Kołodziejczyk[b]; Kazimierz Wiśniewski[c]
[a] Faculty of Biotechnology, University and Medical University of Gdańsk, Gdańsk, Poland [b] Faculty of Chemistry, University of Gdańsk, San Diego, California, U.S.A. [c] Ferring Research Institute, San Diego, California, U.S.A.

Online publication date: 31 March 2001

To cite this Article Falkiewicz, Bogdan , Wiśniowski, Wojciech , Kołodziejczyk, Aleksandra S. and Wiśniewski, Kazimierz(2001) 'SYNTHESIS OF NEW CHIRAL PEPTIDE NUCLEIC ACID (PNA) MONOMERS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1393 — 1397
To link to this Article: DOI: 10.1081/NCN-100002563
URL: http://dx.doi.org/10.1081/NCN-100002563

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1393–1397 (2001)

SYNTHESIS OF NEW CHIRAL PEPTIDE NUCLEIC ACID (PNA) MONOMERS

Bogdan Falkiewicz,[1,2,*] Wojciech Wiśniowski,[1] Aleksandra S. Kołodziejczyk,[2] and Kazimierz Wiśniewski[3]

[1]Faculty of Biotechnology, University and Medical University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland
[2]Faculty of Chemistry, University of Gdańsk
[3]Ferring Research Institute, San Diego, California

ABSTRACT

We have synthesised a series of new chiral type **I** peptide nucleic acid monomers in total yields of 36–53%, derived from Val, Ile, Ser(Bzl), Pro, and Trp, employing convenient procedure.

PNAs represent a relatively novel group of nucleic acid analogues in which the backbone of DNA or RNA is replaced by a polyamide backbone to which nucleobases are attached by appropriate linkers (1). Two main types of PNAs may be singled out (2): **I** - containing *N*-(aminoalkyl)aminoacid units, with nucleobases attached to secondary amine groups of the backbone, **II** - containing a backbone consisting of amino acid residues carrying the nucleobases in side chains.

The most widely known PNAs are based on the *N*-(2-aminoethyl)glycine type I backbone (1). They seem to have the most interesting properties and have been applied as tools in molecular biology and in antisense strategy (3–4). Both types of PNAs may also be expected to find interesting applications in chemistry (5). The main limitations of the usefulness of PNAs are poor solubility in physiological solutions and low permeability through cellular membranes. The PNA structure is easy to modify and it is probable that the synthesis of altered monomers would consequently lead to oligomers with improved properties, e.g. with better permeability

*Corresponding author. Fax: +48 58 3012807. E-mail: bogdanf@chemik.chem.univ.gda.pl

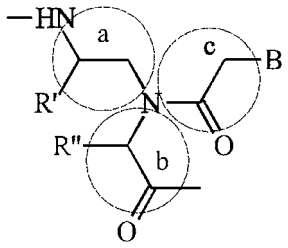


Figure 1. Unprotected type I chiral PNA monomer. Conventional parts: aminoalkyl (**a**), amino acid (**b**), linker (**c**), and nucleobase (**B**) (**d**). R′ or R″ (or both) are different from H.

through cellular membranes or with better solubility in physiological fluids (6–8). PNA hybridization characteristics may also be strongly modified by the employment of non-standard monomers incorporated into oligomeric structures (2). Chiral PNA monomers exhibit different properties from achiral units when incorporated into PNA oligomers (2). In general, oligomers containing chiral backbone ones retain strong hybridisation properties. The changes are dependent on the configuration of the chiral unit and on the dimension and chemical nature of the substituent. The incorporation of chiral monomers may enhance the sequence selectivity of oligomers during hybridisation, and functional groups of side chains might be sites for an attachment of ligands to PNA.

Four ideas of the PNA modification by the chirality introduction into the *N*-(2-aminoethyl)glycine backbone have been reported so far (Fig. 1). The chiralisation of the "amino acid" backbone part was accomplished either by the reductive amination of *N*-protected glycinal by chiral amino acid esters (6), or by the catalytic hydrogenation of enamido esters (7). The chiralisation of the "aminoalkyl" backbone part was accomplished by the reductive amination of *N*-protected chiral amino aldehydes by glycine esters (8), or using the Mitsunobu reaction for monomer backbone synthesis (8d). All reductive amination-based procedures need two purification steps: after reductive amination and after acylation. We report the synthesis of achiral and new chiral PNA monomers using our simplified and efficient procedure (8b).

RESULTS AND DISCUSSION

According to the procedure outlined in Figure 2 and described previously in details (8b) we have prepared a series of five new chiral protected PNA monomers comprising Val-, Ile-, Ser(Bzl)-, Pro-, and Trp-derived compounds (Boc-PNA[Val]-OMe - Boc-PNA[Trp]-OMe, respectively), and the Boc-PNA[Gly]-OMe monomer.

The products were obtained in satisfactory yields after preparative RP-HPLC [Table], but may also be effectively purified by low pressure liquid chromatography (not shown). Only the reactions carried out with Trp-derivatives were more laborious and challenging and our first attempts resulted in rather mediocre yields.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

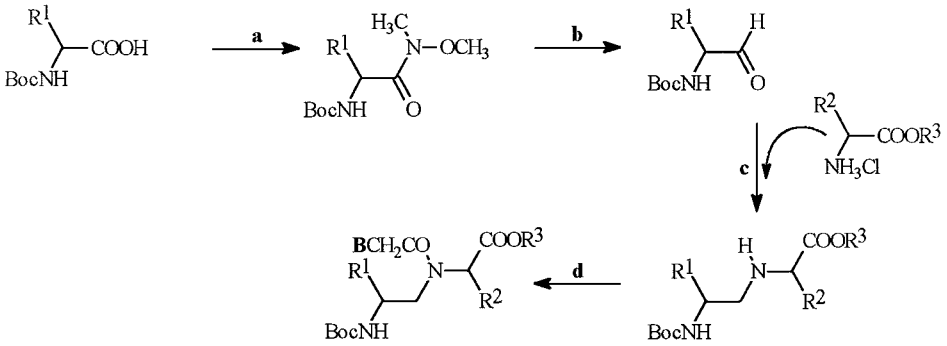


Figure 2. Scheme of the synthesis. R^1 = H or side chains of Val, Ile, Ser(Bzl), Pro (not shown), Trp; R^2 = H, but the procedure allows to use other side chains; R^3 = CH_3, **B** - nucleobase. **a** - $HN(OCH_3)CH_3$/TEA, 0°C, then TBTU/TEA; **b** - 0°C, $LiAlH_4$ (15 min), 10% citric acid; **c** - 3% AcOH/MeOH, then $NaBH_3CN$; **d** - ThyAcOH, EDC/DIEA/HODhbt (**A**) or TBTU/TEA (**B**).

Therefore, to ensure good results, we recommend the procedure in which all steps are performed under nitrogen and as quickly as possible. The structure of the products was confirmed by analysis of ^1H, ^{13}C NMR, heteronuclear Overhauser effect spectroscopy and MS.

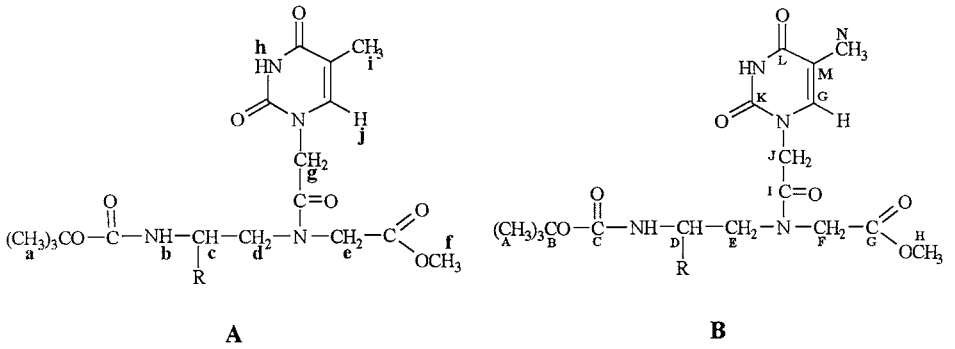
Boc-PNA[Gly]-OMe (A). MS, calc. (found): $(M+H^+)$ = 399 (399). ^1H NMR: 1.445 (Ha); 5.629 (Hb); 3.340 (Hc); 3.543 (Hd); 4.071 (He); 3.750 (Hf); 4.587 (Hg); 9.311 (Hh); 1.908 (Hi); 7.014 (Hj); ^{13}C NMR: 28.727 (C_A); 80.154 (C_B); 156.077 (C_C); 48.054 (C_D); 50.561 (C_E); 49.054 (C_E); 169.835 (C_G); 52.743 (C_H); 167.418 (C_I); 49.054 (C_J); 151.175 (C_K); 164.244 (C_l); 110.891 (C_M); 12.665 (C_N); 140.985 (C_O).

Boc-PNA[Val]-OMe (A). $[\alpha]^{r.t.}_{589nm} = 8^\circ$ (1, MeOH); MS, calc. (found): $(M+H^+)$ = 441 (441). ^1H NMR: 1.431 (Ha); 5.060 (Hb); 3.709 (Hc); 3.478 (Hd); 4.194 (He); 3.731 (Hf); 4.392 (Hg); 8.593 (Hh); 1.920 (Hi); 7.014 (Hj); 0.925 (Val,

Table . Yields of the Synthetic Steps during the Syntheses of PNA Monomers (%)

Protected Monomer	Boc-XaaN-(Me)-OMe	Boc-Xaa-H[a]	Boc-Xaa $\psi(CH_2NH)$ Gly-OMe[1]	Boc-PNA-[Xaa]OMe	Total[b]
Boc-PNA[Gly]-OMe	70	100	70	87	43
Boc-PNA[Val]-OMe	98	95	89	62	51
Boc-PNA[Ile]-OMe	95	100	76	73	53
Boc-PNA[Ser(Bzl)]-OMe	92	95	85	65	48
Boc-PNA[Pro]-OMe	85	100	99	43	36
Boc-PNA[Trp]-OMe	79	97	100	56	43

[a]As isolated, without extensive purification.
[b]Calculated from Boc-Xaa-OH used.



A B

Figure 3. Atom description for ^{1}H (**A**) and ^{13}C (**B**) NMR (400 MHz) chemical shifts of the monomers obtained (major rotamers only). Side chain atom description derived from the sceleton of the respective amino acid.

$\gamma(C\underline{H}_3)_2$); 1.791 (Val, $\beta C\underline{H}_2$); ^{13}C NMR: 28.704 (C_A); 79.50 (C_B); 156.236 (C_C); 48.592 (C_D); 50.902 (C_E); 49.47 (C_F); 169.396 (C_G); 52.584 (C_H); 168.017 (C_I); 49.645 (C_J); 150.826 (C_K); 163.941 (C_l); 110.854 (C_M); 12.642 (C_N); 140.901 (C_O); 18.355 & 18.779 (Val, $\gamma\underline{C}$); 30,674 (Val, $\beta\underline{C}$).

Boc-PNA[Ile]-OMe (B). $[\alpha]^{r.t.}_{589\,nm} = 10^{\circ}$ (1, MeOH). MS, calc. (found): $(M+H^+) = 455$ (455). ^{1}H NMR: 1.430 (Ha); 5.001 (Hb); 3.708 (Hc); 3.487 (Hd); 4.188 (He); 3.735 (Hf); 4.404 (Hg); 9.031 (Hh); 1.924 (Hi); 7.025 (Hj); 0.903 (Ile, $\delta C\underline{H}_3$); 0.921 (Ile, βCH-C$\underline{H}_3$); 1.140 (Ile, $\gamma C\underline{H}_2$); 1.530 (Ile, $\beta C\underline{H}$); ^{13}C NMR: 28.696 (C_A); 79.624 (C_B); 156.145 (C_C); 48.592 (C_D); 50.516 (C_E); 49.076 (C_F); 169.380 (C_G); 52.599 (C_H); 167.486 (C_I); 49.470 (C_J); 151.152 (C_K); 164.123 (C_l); 110.869 (C_M); 12.650 (C_N); 140.962 (C_O); 12.021 (Ile, δC); 16.051 (Ile, βC-$\underline{C}H_3$); 25.499 (Ile, γC); 37.220 (Ile, βC).

Boc-PNA[Ser(Bzl)]-OMe (B). $[\alpha]^{r.t.}_{589\,nm} = -2^{\circ}$ (1, MeOH). MS, calc. (found): $(M+H^+) = 519$ (519). ^{1}H NMR: 1.429 (Ha); 5.239 (Hb); 3.583 (Hc); 3.551 (Hd); 3.962 (He); 3.706 (Hf); 4.205 (Hg); 8.883 (Hh); 1.888 (Hi); 6.872 (Hj); 4.489 (Ser, $\beta C\underline{H}_2$); 4.576 ($O\underline{C}H_2C_6H_5$); 7.280–7.380 (Ph$\underline{H}$); ^{13}C NMR: 28.651 (C_A); 80.291 (C_B); 155.433 (C_C); 48.864 (C_D); 50.199 (C_E); 49.114 (C_F); 169.449 (C_G); 52.531 (C_H); 167.555 (C_I); 49.228 (C_J); 150.978 (C_K); 164.107 (C_l); 110.551 (C_M); 12.642 (C_N); 141.265 (C_O); 68.260 (Ser, βC); 73.730 ($O\underline{C}H_2$Ph); 127.862 (Ph, C4); 128.158, 128.218, 128.522, 128.688 (Ph, C2, C3, C5, C6); 137.340 (Ph, C1).

Boc-PNA[Pro]-OMe (A). $[\alpha]^{r.t.}_{589\,nm} = -20^{\circ}$ (c, MeOH). MS, calc. (found): $(M + H^+) = 439$ (439.9). ^{1}H NMR: 1.424 (Ha); 3.624 (Hc); 3.309 (Hd); 3.972 (He); 3.677 (Hf); 4.143 (Hg); 9.477 (Hh); 1.885 (Hi); 7.741 (Hj); 2.010 (Pro, $\gamma C\underline{H}_2$); 2.184 (Pro, $\beta C\underline{H}_2$); 3.287 (Pro, $\delta C\underline{H}_2$); ^{13}C NMR: 28.804 (C_A); 80.25 (C_B); 155.040 (C_C); 48.480 (C_D); 51.040 (C_E); 49.881 (C_F); 169.670 (C_G); 52.442 (C_H); 169.912

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(C_I); 49.025 (C_J); 151.025 (C_K); 164.692 (C_l); 110.750 (C_M); 12.630 (C_N); 141.494 (C_O); 23.826 (Pro, γC); 29.206 (Pro, βC); 46.805 (Pro, δC).

Boc-PNA[Trp]-OMe (A). $[\alpha]^{r.t.}_{589\,nm} = 13^\circ$ (1, MeOH). MS, calc. (found): ($M+H^+$) = 527 (527, 428 [- Boc]). ^{1}H NMR: 1.403 (Ha); 5.260 (Hb); 3.557 (Hc); 3.495 (Hd); 4.077 (He); 3.682 (Hf); 4.367 (Hg); 9.277 (Hh); 1.799 (Hi); 6.960 (Hj); 3.013 (Trp, βC$\underline{H}_2$); 7.036 (indolH5); 7.106 (indH6); 7.165 (indH2); 7.327 (indH7); 7.579 (indH4); 8.798 (indN$\underline{H}$); ^{13}C NMR: 28.681 (C_A); 78.555 (C_B); 155.857 (C_C); 48.887 (C_D); 51.546 (C_E); 49.774 (C_F); 169.540 (C_G); 52.562 (C_H); 167.592 (C_I); 48.069 (C_J); 151.387 (C_K); 164.289 (C_l); 110.732 (C_M); 12.551 (C_N); 141.144 (C_O); 28.75 (Trp, $\beta\underline{C}H_2$); 123.7 (indC5); 119.710 (indC6); 122.241 (indC2); 111.717 (indC7); 118.589 (indC4); 110.460 (indC3); 127.484 (indC8); 136.446 (indC9).

ACKNOWLEDGMENTS

Supported by the Polish State Committee of Scientific Research 3T09A08013 grant.

REFERENCES

1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
2. Falkiewicz, B. *Acta Biochim. Pol.* **1999**, *46*, 509–529.
3. Nielsen, P. E.; Egholm, M. (Eds.): Peptide Nucleic Acids: Protocols and Applications. Horizon Scientific Press, Wymondham **1999**, Norfolk, U.K.
4. Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem. Int. Ed.* **1998**, *37*, 2796–2823.
5. Szyrwiel, J.; Mlynarz, P.; Kozlowski, H.; Taddei, M. *J. Chem. Soc. Dalton Trans.* **1998**, 1263–1264. Brasuń J.; Ciapetti, P.; Kozlowski, H.; Oldziej, S.; Taddei, M.; Valensin, D.; Valensin, G.; Gaggelli, N. *J. Chem. Soc. Dalton Trans.* **2000**, 2639–2644.
6. Dueholm, K. L.; Petersen, K. H.; Jensen, D. K.; Egholm, M.; Nielsen, P. E.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1077–1080. Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem. Int. Ed.* **1996**, *35*, 1939–1942. Püschl, A.; Sforza, S.; Haaima, G.; Dahl, O.; Nielsen, P. E. *Tetrahedron Lett.* **1998**, *39*, 4707–4710. Sforza, S.; Haaima, G.; Marchelli, R.; Nielsen, P. E. *Eur. J. Org. Chem.* **1999**, 197–204.
7. Stammers, T. A.; Burk, M. J. *Tetrahedron Lett.* **1999**, *40*, 3325–3328.
8. a. Kosynkina, L.; Wang, W.; Liang, T. C. *Tetrahedron Lett.* **1994**, *35*, 5173–5176. b. Falkiewicz, B.; Kowalska, K.; Kolodziejczyk, A.; Wisniewski, K.; Łankiewicz, L. *Nucleosides Nucleotides* **1999**, *18*, 353–361. c. Falkiewicz, B.; Wiśniowski, W.; Kołodziejczyk, A.; Wiśniewski, K.; Łankiewicz, L. *Nucl. Acids Symp. Ser.* **1999**, *42*, 29–30. d. Falkiewicz, B.; Kolodziejczyk, A.; Liberek, B.; Wiśniewski, K. *Nucl. Acids Symp. Ser.* **1999**, *42*, 9–10. Wiśniewski, K.; Joswig, S.; Falkiewicz, B.; Kołodziejczyk, A. *Pol. J. Chem.* **1997**, *71*, 1506–1509. Wiśniewski, K.; Falkiewicz, B.; Kołodziejczyk, A. *J. Pept. Sci.* **1998**, *4*, 1–14.

Request Permission or Order Reprints Instantly!