Pergamon

Tetrahedron Letters, Vol. 36, No. 38, pp. 6937-6940, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01374-1

Synthesis of 'Retro-Inverso' Peptide Nucleic Acids: 1. Characterization of the Monomers

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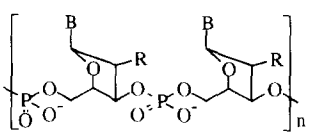
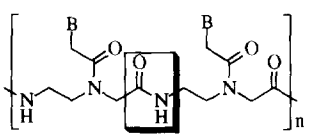
Abstract: Novel monomeric building blocks **4a** and **4b** of peptide nucleic acids with an *N*-(aminomethyl) β-alanine backbone ('retro-inverso' PNA) are conveniently synthesized *via* Hofmann rearrangement. In the major rotamer the side chain carbonyl points toward the C terminus.

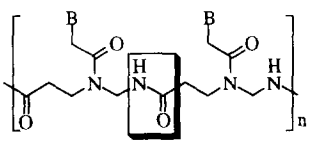
A great deal of interest is currently devoted to molecules that bind nucleic acids with high affinity and sequence specificity. Potential applications as novel therapeutics interfering with gene-expression on a transcriptional or translational level and also as tools in diagnostics and molecular biology make the investigation of DNA analogs an area of intense research.[1] In this context we described recently the synthesis[2] and the remarkable hybridization properties[3] of peptide nucleic acids (PNA) **A**, a novel class of nucleic acid binding DNA analogs with an achiral and charge neutral pseudo-peptide backbone consisting of *N*-(2-aminoethyl) glycine units to which the nucleobases are attached *via* methylenecarbonyl linkers. In addition, several PNA modifications have been investigated.[4]



DNA: R = H
RNA: R = OH



PNA **A**

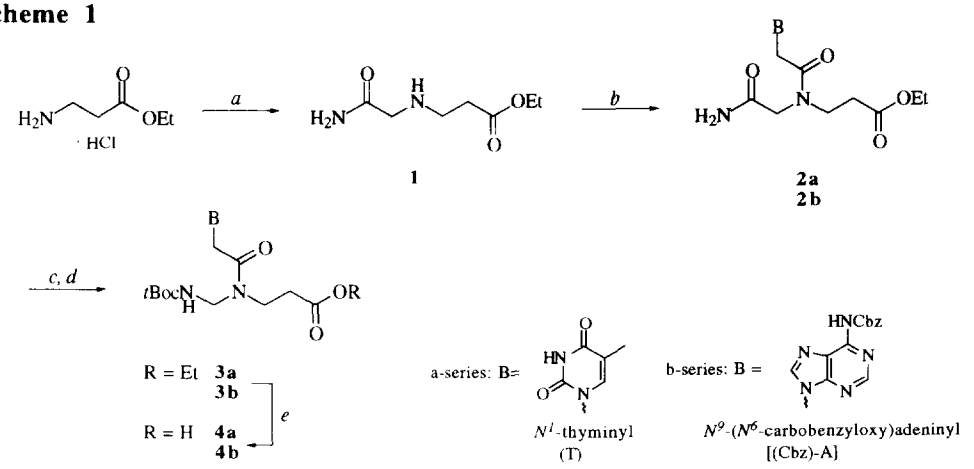


'Retro-inverso' PNA **B**

In our efforts to define the correlation between molecular structure[5a] and the ability of peptide nucleic acids to hybridize with DNA or RNA we designed a 'retro-inverso' PNA **B**[6] by interchanging the hydrogen bond donors and acceptors of the amide groups at the backbone while keeping the position of the nucleobases constant.[7] Thus the repeating motif of the backbone is the *N*-(aminomethyl) β-alanine unit.

In this communication, we describe a convenient synthesis of both a thymine (**4a**) and an adenine monomer (**4b**) of PNA **B**. We assign the structures of the major and minor rotamers at the tertiary amide group based on characteristic ^{1}H NMR chemical shift differences of the backbone methylene protons.

Scheme 1



1 2a 2b

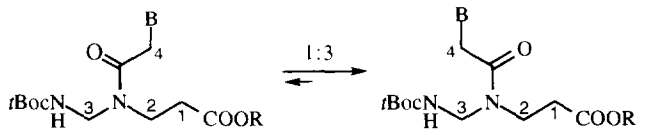
a, 2-chloroacetamide, K_2CO_3, DMF, 90°C, 24 h, 50%; b, BCH_2COOH, DhbtOH/DCC or HBTU, DMF, r.t.,1 h, 58-61%; *c*, $PhI(O_2CCF_3)_2$, CH_3CN/H_2O, r.t., 20 h; *d*, $(tBoc)_2O$, K_2CO_3, dioxane, r.t., 1 h, *c,d*: 64-75%); *e*, LiOH, THF/H_2O, 92%.

Scheme 1 depicts the synthetic route to the new PNA monomers **4a** and **4b**. As precursor of the labile *N*-(aminomethyl) amide moiety we chose to prepare a primary amide, as the amide-to-amine conversion *via* Hofmann rearrangement using Loudon's variant[8] is well established. First we alkylated β-alanine ethylester (4 eq) with 2-chloroacetamide in DMF in the presence of K_2CO_3. Unreacted β-alanine ethylester and solvent were easily recovered by distillation under reduced pressure and were reused. Flash chromatography on silica afforded **1** as a colorless oil (yield 50 %). N^1-thyminyl acetic acid was activated with 1,3-dicyclohexylcarbodiimide (DCC) and 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (DhbtOH) and coupled with amine **1** to furnish thymine derivative **2a**. Using N^6-benzyloxycarbonyladenin-9-yl acetic acid and *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HBTU) as activating agent we obtained **2b** (yield 58-61%). The ^1H NMR spectra ([D_6]DMSO, r.t.) of **2a** and **2b** showed two sets of resonances (ratio 1:1) of two degenerate rotamers at the tertiary amide group. Coalescence for the resonances of **2a** was observed at 85 °C. The subsequent Hofmann rearrangement[9] of **2a** and **2b** was carried out using *I*,*I*-bis(trifluoroacetoxy)-iodobenzene[8] in acetonitrile/water at room temperature. For stabilization of the N terminus, the labile rearrangement product was immediately reacted with di-*tert*-butyl dicarbonate in 1,4-dioxane in the presence of K_2CO_3 to afford **3a** and **3b**, respectively. The good overall yield for rearrangement/protection (64-75%) indicated that the unimolecular degradation of the N terminus was significantly slower than condensation with a reactive pyrocarbonate under the reaction conditions employed. Hydrolysis of **3a** and **3b** with LiOH in water/THF and acidic work-up afforded the Boc-protected monomeric building blocks **4a** and **4b**, respectively.

Two rotamers in a ratio 3:1 of **3a**, **3b**, **4a** and **4b** were detected by ^1H NMR spectroscopy ([D_6]DMSO, r.t.). The assignment of the conformation of the amide group of the major and minor conformers of **3** and **4** may be made on the basis of the ^1H NMR chemical shifts of the backbone methylene protons. NMR studies have shown that the side chain carbonyl group of the major rotamer of PNA **A** monomers with an *N*-2-(aminoethyl) glycine backbone points toward the C terminus[5b,c] which, in case of the thymine monomer, gives

rise to a downfield shift of about 0.1 ppm of the N terminal methylene protons compared to the minor rotamer, and an upfield shift of about 0.2 ppm of the C terminal methylene protons. The methylene protons adjacent to the nucleobase in the major rotamer are shifted 0.17 ppm to lower field. (Scheme 2)

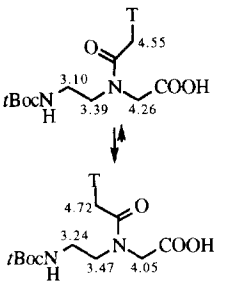
Scheme 2



CH_2	1		2		3		4	
	major	minor	major	minor	major	minor	major	minor
3a	2.50	2.72	3.50	3.59	4.61	4.58	4.74	4.6[1]
3b	2.6[1]	2.90	3.60	3.90	4.82	4.65	5.53	5.41
4a	2.52	2.73	3.54	3.64	4.69	4.64	4.82	4.7[1]
4b	2.51	2.78	3.57	3.75	4.83	4.65	5.54	5.44

[1] overlapping with solvent or other resonances.

For comparison:



(top) Rotamer equilibrium of **3a**, **3b**, **4a** and **4b** (incl. the atom numbering system). (bottom) Table of ^{1}H NMR chemical shifts (in ppm) of methylene protons of major and minor isomers ([D_6]DMSO, r.t.). (right) For comparison, ^{1}H NMR chemical shifts of methylene protons of major and minor isomers ([D_6]DMSO, r.t.) of the thymine*N*-(aminoethyl) glycine monomer.

The backbone protons of the two rotamers of the monomers of PNA **B** described here also exhibit characteristic chemical shift differences. (Scheme 2) The resonances of the C terminal methylene protons 1 and 2 of the major isomers of **3a**, **3b**, **4a** and **4b** are shifted about 0.1-0.3 ppm upfield and the N terminal methylene protons 3 are shifted downfield (ca. 0.05-0.15 ppm). This chemical shift difference between major and minor isomer is nucleobase dependent for protons 1 and 2 in the minor isomers (~0.1 ppm higher in the adenine series) and protons 3 in the major isomers and essentially nucleobase independent for protons 1 and 2 in the major isomer and protons 3 in the minor isomer. The side chain methylene protons 4 of the major isomers are shifted about 0.10-0.15 ppm to lower field. The differences in chemical shifts between major and minor isomers and the nucleobase dependence of some particular protons are caused by the magnetic anisotropy of the aromatic nucleobases and are consistent with an orientation of the side chain carbonyl group toward the C terminus in the major isomers of **3** and **4**. A stabilizing contribution from hydrogen bonding of the side chain carbonyl group to either N or C terminus in **2**, **3**, or **4** is not evident.[5d] X-ray crystal structure analysis[6] of **3a** revealed a conformation of the tertiary amide group as shown as the major isomer in Scheme 2.

In summary, the thymine and adenine monomers suitable for the synthesis[10] of a 'retro-inverso' PNA **B** have been synthesized. ^{1}H NMR spectroscopy indicates that the side chain carbonyl in the major rotamer of **3a**, **3b**, **4a** and **4b** points toward the C terminus.

Acknowledgement. This work was supported by the Danish National Research Foundation and The Danish Research Academy (a DANVIS fellowship to AHK).

References and Notes

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7. The reversal of peptide bonds (isomeric replacement) has been widely used in the design of peptidomimetics. In some cases significant differences in the conformational properties are observed, however, details of the effect of a retro-inverso modification on conformations are not well understood, for a review see Chorev, M.; Goodman, M. *Acc. Chem. Res.* **1993**, *26*, 266-273.

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9. A typical procedure for the Hofmann rearrangement/Boc protection is as follows:
A solution of amide **2** (5 mmol) and *I,I*-[bis(trifluoroacetoxy)iodo]benzene (6 mmol) in CH_3CN/H_2O (45 ml, 2:3, *v/v*) was kept for 20 h at r.t.. The mixture was filtered and the filtrate concentrated under reduced pressure. 1,4-Dioxane (45 ml) and Na_2CO_3 (4.5 g) were added, followed by addition of a solution of di-tert-butyl dicarbonate (7 mmol) in 1,4-dioxane (9 ml). The mixture was stirred at r. t. for 24 h, filtered and the filtrate was concentrated. Pure **3** was obtained by flash chromatography on silica (**3a**: hexane/acetone 1:1; **3b**: ethyl acetate/methanol 97:3), yield 64-75 %.

10. See following paper in this issue.

(*Received in UK* 11 *May* 1995; *revised* 17 *July* 1995; *accepted* 21 *July* 1995)