Pergamon

Tetrahedron Letters 40 (1999) 4161–4164

TETRAHEDRON LETTERS

Solid Phase Synthesis of Protected Peptide Nucleic Acids

Oliver Seitz

Universität Karlsruhe, Institut für Organische Chemie, Richard-Willstätter-Allee 2, D-76128 Karlsruhe

Received 8 March 1999; accepted 6 April 1999

Abstract: Boc/Z-protected PNA oligomers were synthesised on solid phase. The use of the allylic HYCRON resin allowed for the application of both Boc- and Fmoc-protecting groups. Highest yields were obtained when the monomeric building block was synthesised on solid phase rather than loaded as preformed unit. The selective attachment of fluorescent labels at the C-terminal (3') end demonstrated for the first time that further manipulations on protected PNA fragments are feasible.

Peptide nucleic acid (PNA) is a DNA analogue, in which the entire sugar-phosphate backbone is replaced by a pseudopeptide backbone.[1] In spite of this substantial alteration of the backbone, PNA binds with remarkably high affinity and specificity to complementary DNA and RNA sequences. Moreover, PNA exhibits an increased stability against chemical and enzymatical degradation reactions. These properties make PNA an interesting tool for gene targeting applications and inspire considerable research efforts on the development of ever new classes of oligoamide-based antisense compounds. However, the synthesis of PNA and PNA conjugates is still not as efficient as DNA synthesis. As part of our research on new assays for DNA diagnostics, we were in need of *C*-terminally labelled PNA conjugates. Labelling of PNA is routinely carried out at the *N*-terminal amino group (5').[2] Although 3'-labelled oligonucleotides are used in gene diagnostics[3], the corresponding postsynthetic tagging of the *C*-terminus of PNA has not been described. Routine protocols of solid phase synthesis afford unprotected PNA. In presence of the exocyclic amino groups of the nucleobases, selective reactions on the *C*-terminal carboxyl group are difficult to achieve.[4] In this comunication it is demonstrated for the first time, that protected PNA-oligomers allow for the selective attachment of reporter groups to the *C*-terminal carboxyl group (3' in DNA), thus making a variety of PNA-conjugates accessible.[5]

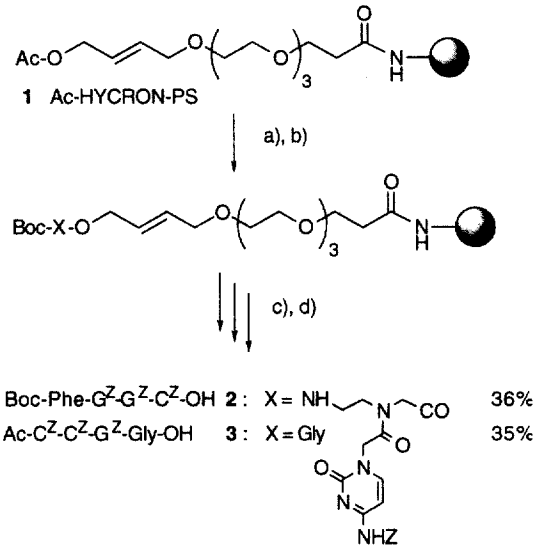
The linker-group between the polymeric support and the growing chain of the biooligomer plays a decisive role in the solid phase synthesis of protected oligomers. An excellent choice would be a linker of the allyl-type such as the HYCRON linker (Scheme 1).[6] This linker group provides orthogonal stability in combination with the commonly used protecting groups. Recently, a strategy was published, in which Boc/Z-protected PNA monomers were synthesised on the solid phase.[7] However, the disulfide linker used did not provide a sufficient stability for the synthesis of a full lenght oligomer. Herein, both the on-resin synthesis of a PNA-monomer and the subsequent extension to a protected oligomer is reported.

For the loading of the HYCRON resin **1**[6b] with the starting PNA-building block, the acetyl group was saponified before esterification of the liberated allylic alcohol (Scheme 1). The subsequent solid phase synthesis was performed according to the Boc/Z-strategy.[8] The Pd(0)-catalysed cleavage of the allylic ester liberated the protected PNA conjugate **2**, which was obtained in 36% overall yield after chromatographic purification.

Fax: Intl. + (0)721 6084825; e-mail: seitz@ochhades.chemie.uni-karlsruhe.de

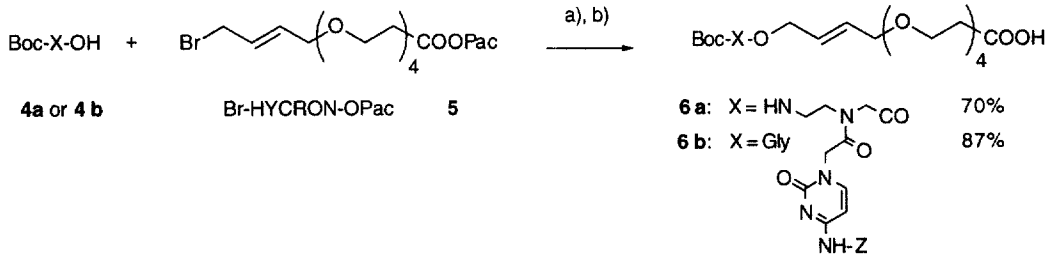
0040-4039/99/$ - see front matter
PII: S0040-4039(99)00717-0

Analogous esterification with Boc-protected glycine and subsequent solid phase synthesis yielded the protected PNA conjugate **3** in 35% overall yield. The overall yields obtained are based on the original loading of polymeric support **1** with acetyl groups, as determined by NMR quantification of the acetyl groups of the in-situ hydrolysed resin.



Scheme 1: a) 1N NaOH/dioxane (1:3); b) Boc-X-OH, DIC, DMAP, c) *i* TFA/m-cresol (95:5); *ii* Boc-B^Z-COOH, HBTU, DIPEA, Pyr; *iii* Ac_2O/Pyr; *iiii* repeat *i-iii*); d) [$Pd(PPh_3)_4$], morpholine, DMSO, DMF (DIC= *N,*N'-diisopropylcarbodiimide, DIPEA= ethyl-diisopropylamine, HBTU= *O*-(benzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium-hexafluorophosphate, PS= polystyrene).

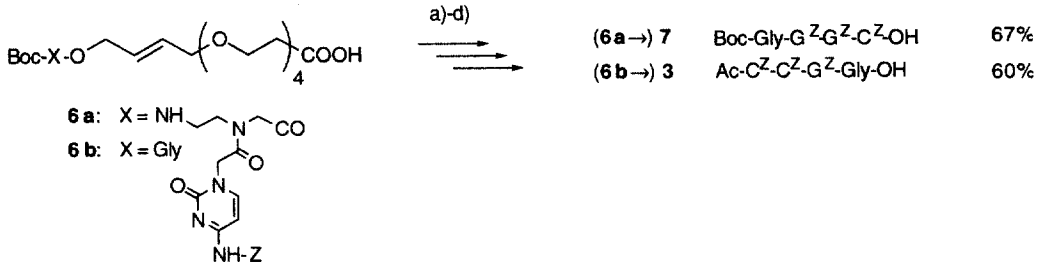
The esterification of the polymerbound alcohol appeared to be inappropriate to achieve quantitative loading levels and hence resulted in low overall yields. Higher loading can be obtained by acylating the polymerbound amines with preformed conjugates of the starting monomer and the HYCRON anchor. The synthesis of a HYCRON-conjugate such as the cytosine-HYCRON-conjugate **6a** was performed by reacting cytosine monomer **4a** with the allylic bromide **5** followed by the reductive removal of the phenacyl ester (Scheme 2). Analogous treatment of Boc-protected glycine **4b** yielded the Boc-Gly-HYCRON derivative **6b**.



Scheme 2: a) sat. $NaHCO_3$, Bu_4NBr, CH_2Cl_2; b) Zn, AcOH.

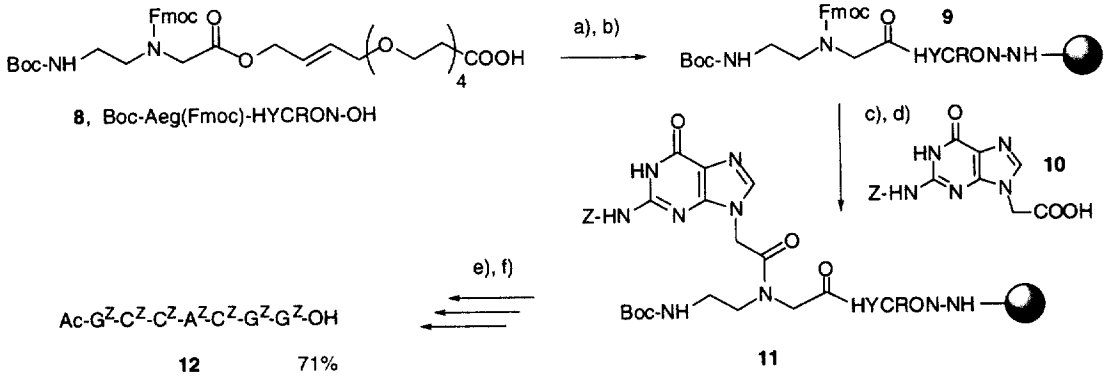
To assemble the protected PNA-conjugates **3** and **7** on a solid support, the HYCRON-conjugates **6** were coupled to aminomethyl-polystyrene (Scheme 3). The subsequent solid phase synthesis was performed as described. The liberation of the protected oligomers was accomplished by the Pd(0)-catalysed allyl transfer to morpholine. After the chromatographic purification the protected PNA-conjugate **3** and **7** were obtained in 60%

and 67% overall yield, respectively. This yield is based on the initial load of the resin with reactive amino groups and therefore illustrates the efficiency of this strategy.



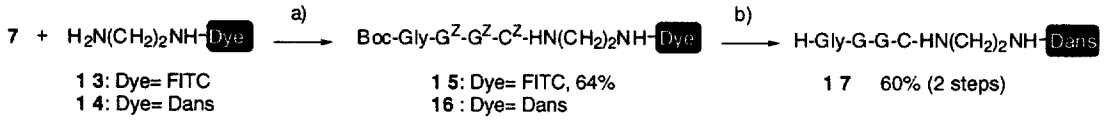
Scheme 3: a) aminomethyl polystyrene, HBTU, DIPEA, HOBt, DMF; b) Ac_2O, Pyr; c) Boc/Z-PNA-solid phase synthesis; d) $[Pd(PPh_3)_4]$, morpholine, DMSO, DMF.

The strategy outlined above gives rise to high overall yields but for the PNA-synthesis of a general applicability a set of at least four PNA-monomer-HYCRON conjugates would be required. More convenient is the use of a common precursor, which would allow for the loading of all nucleobases. Starting from Boc/Fmoc-protected aminoethylglycine[9], the HYCRON-linked precursor **8** was synthesised according to the method shown in Scheme 2 and attached to the resin (Scheme 4). Treatment with DMF/morpholine liberated the secondary amino group, which subsequently was subjected to a coupling reaction with the carboxymethylguanine **10**. In addition, the supported guanosine **11** formed was utilised to assemble the oligomer **12** according to the Boc/Z-strategy. For the final cleavage, the resin was treated with the Pd(0)-catalyst and morpholine. The chromatographic purification furnished the protected oligomer **12** in 71% overall yield based on the initial loading of **9** with Fmoc-groups. In the corresponding solution synthesis of the Boc/Z-protected guanosine[10] 70-80% yields was achieved, which illustrates the efficiency of both the on-resin-synthesis of the monomeric PNA-building block and the subsequent solid phase synthesis. It has to emphasised, that by simply changing the positions of the Boc- and the Fmoc-group of **8** the synthesis of Fmoc/Bhoc-protected monomers[11] would be feasible.



Scheme 4: a) HBTU, DIPEA, HOBt, DMF; b) Ac_2O, Pyr; c) DMF/morpholine; d) HATU, DIPEA, DMF; e) Boc/Z-PNA-solid phase synthesis; f) $[Pd(PPh_3)_4]$, morpholine, DMSO, DMF (HATU= = *O*-(1-oxy-7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate).

The palladium-(0)-catalysed cleavage reaction furnishes protected oligomers suitable for further selective manipulations on the *C*-terminal ends. To demonstrate, that protected PNA-oligomers can serve as acyl donor in peptide couplings, we activated conjugate **7** with EDC in presence of 1-hydroxy-7-azabenzotriazole and coupled it to fluoresceinyl- or dansylethylenediamine[12] (Scheme 5). The subsequent removal of the protecting groups furnished the *C*-terminally labelled PNA-conjugates such as the dansyl conjugate **17**[13]. The purity and identity of conjugates **15-17** and the previous PNA-oligomers were confirmed by RP-HPLC and FAB-MS or MALDI-TOF analysis.



Scheme 5: a) EDC, HOAt, DMF, CH_2Cl_2; b) m-cresol, MeSMe, TFA, TFMSA (1:1:10:1) (HOAt = 1-hydroxy-7-azabenzotriazole).

The use of the highly orthogonal HYCRON-linker allowed for the solid phase synthesis of protected peptide nucleic acids (PNA). The highest overall yields were obtained by the on-resin synthesis of the first PNA-monomer. Protected PNA-oligomers such as **7** are suitable for further functionalisation of the *C*-terminal carboxyl group as demonstrated by the synthesis of the *C*-terminally dansyl-labelled oligomer **17**.

This work was supported by the Fonds der Chemischen Industrie. O.S. is grateful for a gift of chemicals from Boehringer Mannheim GmbH.

References and Notes

1) a) B. Hyrup, P.E. Nielsen, *Biorg. Med Chem.* **1996**, *4*, 5; b) P.E. Nielsen, G. Haaima, *Chem. Soc. Rev.* **1997**, 73; c) P.E. Nielsen, *Chem. Eur. J.* **1997**, *3*, 505.

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

3) a) S.I. Beaucage, R.P. Iyer, *Tetrahedron* **1993**, *49*, 1925; b) P.D. Grossman, W. Bloch, E. Brinson, C.C. Chang, F.A. Eggerding, S. Fung, D.A. Iovannisci, S. Woo, E.S. Winn-Deen, *Nucleic Acids Res.* **1994**, *22*, 4527.

4) a) S.S Gosh, G.F. Musso, *Nucleic Acids Research* **1987**, *15*, 5353; b) M.-N. Erout, A. Troesch, C. Pichot, P. Cros, *Bioconjugate Chem.* **1996**, *7*, 568..

5) For analogous modifications of protected 3'-alkylamino-oligonucleotides: D.L. McMinn, M.M. Greenberg, *J. Am. Chem. Soc.* **1998**, *120*, 3289.

6) a) O. Seitz, H. Kunz, *Angew. Chem.* **1995**, *107*, 901; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 807; b) O. Seitz, H. Kunz, *J. Org. Chem.* **1997**, *62*, 813.

7) G. Aldrian-Herrada, A. Rabié, R. Wintersteiger, J. Brugidou, *J. Peptide Sci.* **1998**, *4*, 266. For a general "Submonomer Strategy": L.S. Richter, R.N. Zuckermann, *Bioorg. Med. Chem. Lett.* **1995**, *5*. 1159.

8) T. Koch, H.F. Hansen, P. Andersen, T. Larsen, H.G. Batz, K. Otteson, H. Ørum, *J. Pep. Res.* **1997**, *49*, 80.

9) D. Muller, I. Zeltser, G. Bitan, C. Gilon, *J. Org. Chem.* **1997**, *62*, 411.

10) a) K.L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H.F. Hansen, T. Vulpius, K.H. Petersen, R.H. Berg, P.E. Nielsen, O. Buchardt, *J. Org. Chem.* **1994**, *59*, 5767; b) J.M. Coull, M. Egholm, R.P. Hodge, M. Ismail, S.B. Rajur, US 96/09144, **1996**.

11) S.A. Thomson, J.A. Josey, R. Cadilla, M.D. Gaul, C.F. Hassman, M.J. Luzzio, A.J. Pipe, K.L. Reed, D.J. Ricca, R.W. Wiethe, S.A. Noble, *Tetrahedron* **1995**, *51*, 6179.

12) K. Brinkmann, H. Linnertz, E. Amler, E. Lanz, P. Herman, W. Schoner, *Eur. J. Biochem.* **1997**, *249*, 301.

13) MS (MALDI-TOF, DHB, pos): 1185 (1185 calc.)