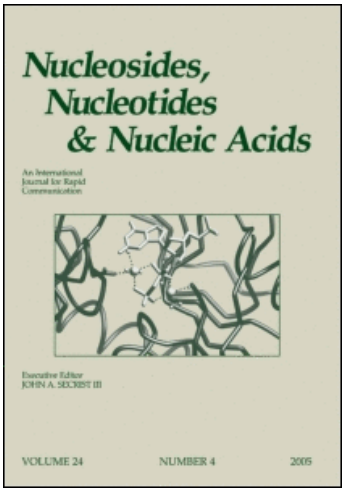

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APPLICATION OF MITSUNOBU REACTION TO SOLID-PHASE PEPTIDE NUCLEIC ACID (PNA) MONOMER SYNTHESIS*

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ABSTRACT

PNA type I monomer backbone with a reduced peptide bond was synthesized on a Merrifield resin in Mitsunobu reaction of Boc-aminoethanol with resin-bound *o*-nitrobenzenesulfonylglycine. The pseudodipeptide secondary amine group was deprotected by thiolysis and acylated with thymin-1-ylacetic acid. The monomer was released as a methyl ester. The procedure seems to be of general applicability and allows various modifications of PNA structure by using diverse alcohols and amino acid esters.

PNAs are nucleic acid analogues, which can mimic DNA or RNA forming heteroduplexes with complementary natural nucleic acids (NNA).[1] In PNAs the phosphodiester pentose backbone of DNA or RNA is replaced by a polyamide or peptide backbone. Depending on the manner of attachment of the nucleobase to the polyamide backbone, two main groups of PNAs can be singled out:[2] type I, PNAs containing a backbone consisting of N-(aminoalkyl)aminoacid units to whose secondary amine group nucleobases are

*Dedicated to Professor Bogdan Liberek on his 50th anniversary of scientific work.

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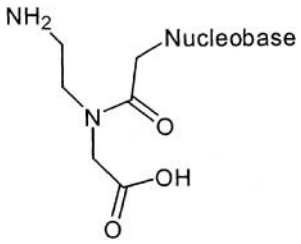

Figure 1. Structure of *N*-(2-aminoethyl)glycine-based PNA monomer (Type **I**).

attached by an alkylcarbonyl linker (Fig. 1); type II: PNAs containing a backbone consisting of amino acid residues carrying nucleobases in their side chains.

The most widely known PNAs are based on a *N*-(2-aminoethyl)glycine backbone (Fig. 1).[1] These PNA molecules prove to be better ligands of DNA or RNA than NNA; they can serve as NNA-targeted compounds with antigene and antisense properties or excellent molecular probes and already have numerous applications in the fields of molecular biology and experimental medicine.[3] Various PNAs also inspire the hope for interesting applications in chemistry[4] and technology.[5]

The synthesis of a protected pseudopeptide with a reduced peptide bond $\psi(CH_2NH)$ most frequently serves as the key step in the synthesis of type I PNA monomers. The most common method of reduced peptide bond synthesis is the reductive amination of *N*-protected amino aldehydes with amino esters; however, *N*-protected α-amino aldehydes are both chemically and chirally unstable, and their reductive amination gives rise to concern due to the recemization and possible branching of the peptide backbone during the reaction.[6] Recently we elaborated a new method of synthesizing pseudopeptide bond[7] based on the Mitsunobu reaction[8] between *N*-*t*-butoxycarbonyl-aminoethanol (BocGly-ol, **2**) and *N*-tosyl-protected (Tos) amino acid esters (the Tos group was first introduced for amine alkylation under the Mitsunobu conditions by Henry et al.[9] and Edwards et al.,[10] and then used for *N*-alkylation of amino acids by Papaioannou et al.[11]). Since the removal of the Tos group needs harsh conditions, modifications of the procedure have recently been devised, employing 2,2,5,7,8-pentamethylochromanyl-6-sulfonyl (Pmc),[12] *o*-, *p*-nitrobenzenesulfonyl (*o* or *p*Nbs) protective groups.[13] To the best of our knowledge, the Mitsunobu reaction has already been used in PNA chemistry, but (except our earlier attempts)[7] only in the attachment of the nucleobases to the side chains of the hydroxyamino acids or for the inversion of the configuration of carbinol carbon in some PNA monomer building blocks.

Here a procedure of solid-phase synthesis (SPS) of pseudopeptides with a reduced peptide bond, as well as PNA monomers, employing the

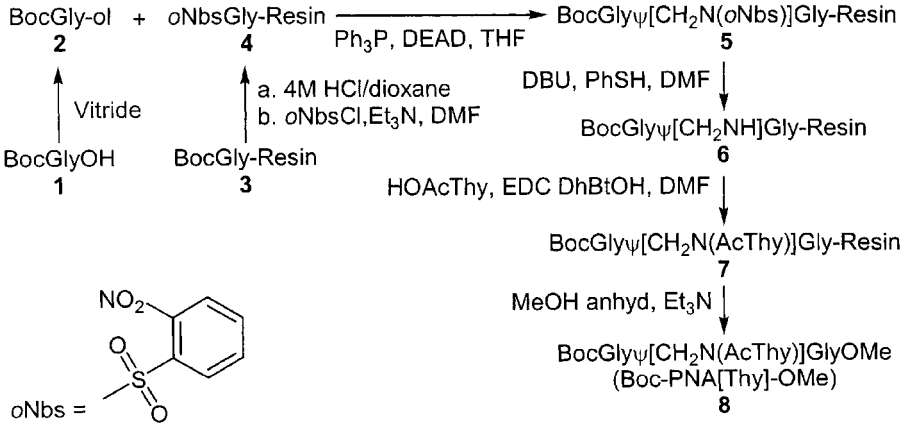

Figure 2. Synthesis of the monomer.

Mitsunobu reaction with the use of a Merrifield resin-bound *o*Nbs-protected amino acid was used (Fig. 2). The procedure is different from the other method of PNA monomer and oligomer SPS proposed.[14] The procedure may be useful for the synthesis of chiral PNA monomers: utilizing similar procedure in solution we obtained protected monomers of higher or comparable optical activity as compared to these obtained using reductive amination of α-amino aldehydes.[15]

RESULTS AND DISCUSSION

The key step of the synthesis was the Mitsunobu reaction with *o*NbsGly-substituted Merrifield resin (**4**), used as an acidic, and BocGly-ol (**2**), used as an alcoholic component. **2** was synthesized by direct reduction of BocGlyOH under mild neutral conditions by Vitride ($NaAlH_2(OCH_2CH_2OCH_3)_2$), which (to the best of our knowledge) has not been previously used for synthesis of amino alcohols from amino acids. The Mitsunobu reaction was carried out with the use of triphenylphosphine (Ph_3P) and diethyl azodicarboxylate (DEAD) and resulted in protected pseudodipeptide **5**. The *o*Nbs group was removed by thiolysis using thiophenol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF, yielding **6**. Thymin-1-yl-acetic acid (HOAcThy) was coupled to the peptidic part of PNA monomer with the use of EDC ([1-ethyl-3-3′-dimethylaminopropyl]carbodiimide) and HODhbt (1-oxo-2-hydroxydihydrobenzotriazine). The protected resin-bound PNA monomer **7** was cleaved from the resin as a methyl ester **8** by transesterification catalyzed by Et_3N in dry MeOH[16] and obtained in a total 65% yield after RP-HPLC purification (procedure employing catalysis by NaOMe may also be used, as we tested). NMR and MS data of the obtained monomer were in agreement with the expectations.

The obtained compound may be subjected to saponification procedure to yield Boc-protected monomer with a free carboxylic group. The procedure described may easily also be applied to the synthesis of protected type I PNA monomers containing chiral amino acid residues in the backbone, as we tested in the analogous synthesis in solution.[16] The procedure can also be used for the submonomer-based SPS of PNA oligomers, containing either the same nucleobase or various nucleobases, or for the SPS of respective chiral PNA oligomers, containing either the same or various nucleobases in the sequence.

EXPERIMENTAL

***o*NbsGly-Resin (4).** 0.429 g (0.7 mmol/g, 0.3 mmol) of BocGly-Merrifield resin **3** (NovaBiochem) was suspended in dioxane for 2 h at r.t., in a vessel for manual SPS. Then dioxane was removed by filtration and the resin was treated with a solution of 4 M HCl in dioxane (20 mL, 20 min). The resin was washed twice with dioxane and twice with DMF and treated with a solution of *o*Nbs-chloride (0.133 g, 0.6 mmol) and Et_3N (0.084 mL, 0.6 mmol) in DMF over 2 h. The solvent was removed by filtration and **5** was subsequently washed twice with DMF and twice with dry THF.

BocGly-ol (2).[17] To a stirred, ice-cooled solution of Vitride in toluene (Lancaster, 4.3 mL, 15 mmol), a solution of **1** (1.24 g, 7 mmol) in dry THF (5 mL) was added dropwise over 30 min and stirred for an additional 2 h at 0°C. MeOH (1.5 mL) was added dropwise (**CAUTION**! a strong evolution of gas was observed), followed by 25 mL of saturated potassium sodium tartarate. The resulting mixture was stirred over 2 h at r.t., then the water layer was extracted with EtOAc (3×70 mL), the organic layers were washed with water (3×30 mL) and brine (3×30 mL), dried over anh. $MgSO_4$ and the solvent was evaporated, yielding 0.87 g (5.4 mmol, 77.1%) of homogenous oily **2**.

The Mitsunobu Reaction (5). To **4**, a solution of BocGly-ol, **2** (0.1 g, 0.6 mmol), and Ph_3P (0.157 g, 0.6 mmol) in dry THF (20 mL) was added under anhydrous conditions; then DEAD (0.104 g, 0.6 mmol, Aldrich) was added in two portions. After overnight shaking, the solvent was removed by filtration and the resin was washed twice with THF and twice with DMF, yielding **5**.

Thiolysis of the *o*Nbs Group (6). To **5**, a solution of DBU (0.33 mmol), and tiophenol (0.9 mmol) in DMF (20 mL) was added. After overnight shaking, the solvent was removed by filtration and the resin was washed twice with DMF and twice with anhydrous MeOH, yielding **6**.

Acylation of the Pseudopeptide 6 (7). To an ice-cooled, stirred solution of HOAcThy (0.166 g, 0.9 mmol) and HODhbt (0.147 g, 0.9 mmol) in DMF (20 mL), EDC, 0.140 g, 0.9 mmol) was added portion-wise, and the stirred mixture was allowed to warm to r.t. over 30 min. The resin **5** was treated for 2 h with a pre-formed active derivative of HOAcThy, then washed twice with DMF and twice with anhydrous MeOH, yielding **7**.

Protected PNA Monomer (8). The resin **7** was treated with Et_3N (0.0014 mL, 0.01 mmol) in dry MeOH, as described.[16] The crude product **8** was purified on a preparative RP-HPLC Kromasil C_8, yielding **8** (0.026 g, 0.065 mmol, 65% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz), two rotational isomers: $\delta = 1.433$ (mi) 1.445 (ma) (2 s, 9H), 1.908 (ma) 1.918 (mi) (2 s, 3H), 3.261 (mi) 3.334 (ma) (2 m, 2H), 3.543 (m, 2H), 3.750 (ma) 3.812 (mi) (2 s, 3H), 4.071 (ma) 4.233 (mi) (2 s, 2H), 4.436 (mi) 4.587 (ma) (2 s, 2H), 5.629 (t, 1H), 6.986 (ma) 7.043 (mi) (2 s, 1H), 9.311 (ma) 9.367 (mi) (2 s, 1H). **^{13}C NMR** ($CDCl_3$, 400 MHz) : $\delta = 12.635$ (mi) 12.665 (ma); 28.681 (mi) 28.727 (ma); 39.053; 47.963 (mi) 48.054 (ma); 49.054 (mi) 49.205 (ma); 50.561; 52.743 (ma) 53.153 (mi); 77.427 (mi) 77.548 (ma); 110.891 (ma) 110.952 (mi); 140.985; 151.175; 156.077; 164.244; 167.418 (ma) 167.789 (mi); 169.835 (ma) 170.078 (mi). **FAB-MS** $m/z = 399$ $(M + H)^+$, 299 $(M - C_5H_9O_2$ (Boc) $+ H)^+$, **HR-MS**, calculated (found): $(M + H^+) = 399.18799$ (399.18806).

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17. As suggested by Dr. Regina Kasprzykowska, Faculty of Chemistry, University of Gdańsk (personal communication), with minor modifications.

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