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Synthesis of nucleobase-functionalized β-peptoids and β-peptoid hybrids

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Abstract—The solid-phase synthesis of a new class of nucleobase-modified peptide-mimetic oligomers is described. β-Peptoids and β-peptoid hybrids bearing thymine on the side chain are prepared from *N*-Fmoc-*N*-[2-(thymin-1-yl)ethyl]-β-alanine.

Over the past 25 years, the highly specific recognition through the natural pairing of nucleobases has prompted the design of nucleic acid mimetics as promising drug candidates in molecular biology and diagnostics.[1] Of the many structural motifs examined, peptide nucleic acids (PNAs) have emerged as the most successful oligonucleotide surrogates.[2] PNAs stand out in terms of their structural simplicity and ability to bind sequences specifically to ssRNA and DNA. In these systems, the ribose phosphate backbone is replaced by a repeating *N*-(2-aminoethyl)glycine unit to which the naturally occurring nucleobases are attached via a methylene carbonyl linkage. Despite their excellent properties, there are some limitations for the use of PNAs such as their low solubility and poor cellular uptake. Many efforts have been made to circumvent these drawbacks and to optimize the properties of PNAs, which have led to the synthesis of a wide number of new structures.[3] Recently, peptoid nucleic acids have been described, which showed good hybridization with DNA.[4] Moreover, nucleobase-modified β-peptides have been reported as suitable scaffolds for the construction of well-defined tertiary structures organized by nucleobase pair recognition.[5] Based on the interesting properties displayed by peptoid nucleic acids and nucleobase-modified β-peptides, we became interested in studying compounds which combine both structural features. In particular, we planned to study the preparation of nucleobase-functionalized β-peptoids. The synthesis of these compounds requires a β-alanine monomer bearing nucleobases.

Here, we report the synthesis of *N*-Fmoc-*N*-[2-(thymin-1-yl)ethyl]-β-alanine (**1**) as monomer for the preparation of nucleobase-functionalized β-peptoids and β-peptoid hybrids (Fig. 1).

The synthesis of monomer **1** was accomplished as shown in Scheme 1 using N^1-(2-aminoethyl)thymine (**2**) as the key compound. There are several reports for the synthesis of **2**, most of which based on the direct alkylation of thymine.[6] However, bis-alkylated products are usually obtained along with the desired mono-alkylated compound. In order to avoid the bis-alkylation, N^3-benzoylthymine (**3**) was prepared following a protocol described previously by Reese.[7] Thymine (1 equiv) was treated with benzoyl chloride (4 equiv) in pyridine/acetonitrile (1:2.5) at room temperature for 46 h, yielding N^1,N^3-dibenzoylthymine, which was converted into N^3-benzoylthymine (**3**) under basic hydrolysis. Using alkylation

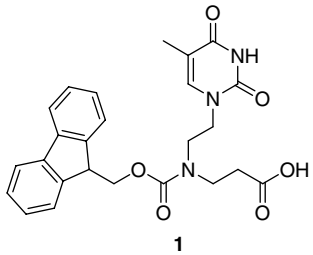

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Figure 1. Structure of the Fmoc-protected β-alanine monomer **1**.

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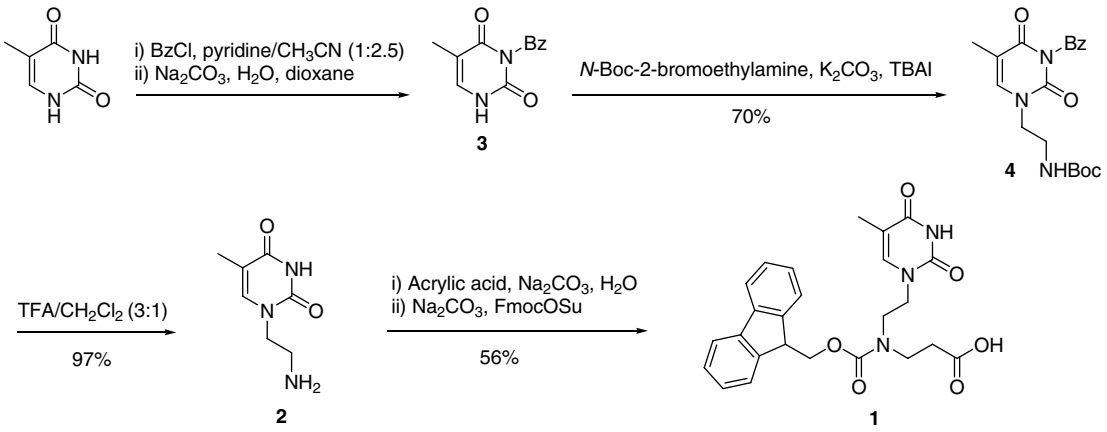


Scheme 1. Synthesis of the Fmoc-protected β-alanine monomer **1**.

reaction conditions described by Taddei,[8] thymine **3** (1 equiv) was reacted with *N*-Boc-2-bromoethylamine (0.8 equiv) at 70 °C for 4 h in the presence of K_2CO_3 (1 equiv) and tetrabutylammonium iodide (TBAI) (0.1 equiv) in DMF to give N^3-benzoyl-N^1-(*N*-Boc-2-aminoethyl)thymine (**4**) in a 70% yield. The treatment of **4** with TFA/CH_2Cl_2 (3:1) at room temperature for 12 h prompted the removal of both benzoyl and Boc groups, providing N^1-(2-aminoethyl)thymine (**2**) in a 97% yield. The spectroscopic data of **2** were in good agreement with those reported already.[6]

The free amino group of thymine derivative **2** (1 equiv) was alkylated with acrylic acid (1.3 equiv) in H_2O in the presence of Na_2CO_3 (1 equiv) at 100 °C for 23 h. The reaction mixture was then cooled to 0 °C and treated with Na_2CO_3 (1 equiv) and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (FmocOSu) (1 equiv). After stirring at room temperature for 23 h, *N*-Fmoc-*N*-[2-(thymin-1-yl)ethyl]-β-alanine (**1**) was obtained in a 56% yield. This compound was characterized by NMR and mass spectrometry.[9]

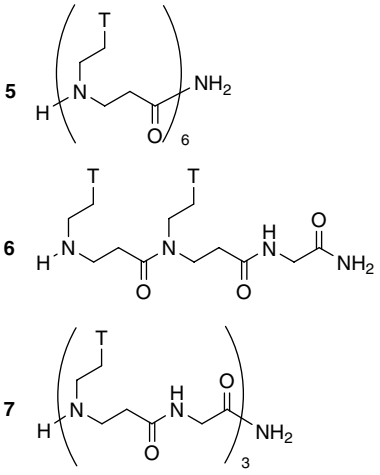
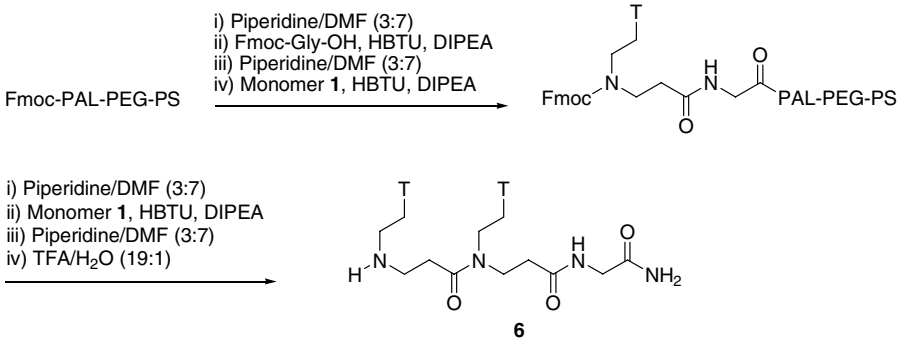


Figure 2. Structure of nucleobase-functionalized β-peptoid **5** and β-peptoid hybrids **6** and **7** (T stands for nucleobase thymine).

With the Fmoc-protected β-alanine monomer **1** in hand, the nucleobase-functionalized β-peptoid **5** and the β-peptoid hybrids **6** and **7** were prepared (Fig. 2, Scheme 2). Solid-phase synthesis was performed onto an Fmoc-PAL-PEG-PS[10] resin. The couplings of Fmoc-Gly-OH (3 equiv) or monomer **1** (3 equiv) were mediated by *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (3 equiv) and DIPEA (3 equiv) in DMF for 3–5 h at 25 °C. Upon completion of couplings, ninhydrin or chloranil tests were negative.[11] Removal of the Fmoc group was carried out in piperidine/DMF (3:7, 2 + 10 min). The final treatment of the resin with TFA/H_2O (19:1, 2 h) afforded the expected peptoids which were analyzed by



Scheme 2. Synthesis of nucleobase-functionalized β-peptoid hybrid **6** (T stands for nucleobase thymine).

HPLC and characterized by ESI-MS.[12] Nucleobase-functionalized β-peptoid **5** was obtained with 90% HPLC purity, and β-peptoid hybrids **6** and **7** with 91% and 85% HPLC purities, respectively. These oligomers showed a low solubility in H_2O and in organic solvents such as CH_2Cl_2, and were completely soluble in $MeOH/H_2O$ mixtures. The overall synthetic process is summarized in Scheme 2 for the synthesis of β-peptoid hybrid **6**.

In summary, we describe an efficient procedure for the synthesis of *N*-Fmoc-*N*-[2-(thymin-1-yl)ethyl]-β-alanine and the application of this monomer for the solid-phase synthesis of β-peptoids and β-peptoid hybrids bearing thymine on the side chain. These compounds represent a new class of peptide-mimetic oligomers. Ongoing work is directed towards the synthesis of β-peptoids and β-peptoid hybrids bearing other nucleobases and to perform DNA or ssRNA binding studies with these new oligomers.

Acknowledgement

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9. Compound **1** was fully characterized: ^{1}H NMR (200 MHz, DMSO-d_6) $\delta = 1.64$ (s, 3H), 1.73 (s, 3H), 2.29–2.52 (m, 2H), 3.36–3.74 (m, 6H), 4.15–4.27 (m, 3H), 7.15 (s, 1H), 7.39–7.91 (m, 8H). ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 12.27$, 39.92, 45.96, 46.55, 46.67, 47.02, 67.18, 108.56, 120.53, 125.37, 128.08, 130.42, 141.14, 141.73, 144.20, 151.34, 153.54, 164.70, 171.18. ESI-MS (*m*/*z*): 463.6 $[M+H]^+$, 485.7 $[M+Na]^+$.
10. PAL, 5-(4-amino)-methyl-3,5-dimethoxy-phenoxy valeric acid handle (Peptide Amide Linker); PEG-PS, poly(ethylene glycol)-polystyrene (graft resin support).
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12. Mass spectrometry analysis of **5**, **6**, and **7**. β-Peptoid **5**: MALDI-MS (*m*/*z*): 1357.0 $[M+H]^+$, 1379.0 $[M+Na]^+$, 1395.0 $[M+K]^+$. β-Peptoid hybrid **6**: MALDI-MS, (*m*/*z*): 521.2 $[M+H]^+$. β-Peptoid hybrid **7**: MALDI-MS, (*m*/*z*): 881.0 $[M+Na]^+$, 897.0 $[M+K]^+$.