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Synthesis of a novel ester analog of nucleic acids bearing a serine backbone

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Abstract—A novel analog of nucleic acids bearing an optically active serine ester backbone, serine-based nucleobase-linked polyester (SNE), was synthesized. Monomers containing a thymine base were synthesized from L- and D-serines. Furthermore, reaction conditions were thoroughly examined for the ester bond formation by using a new phosphonium-type condensing reagent on a solid support without racemization. The release of the dimer from the resin was also investigated using a new type of linker, which could be cleaved under neutral conditions.

The base pairing of nucleic acids is one of the most important events of molecular recognition in nature, which guarantees the storage, transfer, and expression of genetic information in living systems. The highly specific molecular recognition through the pairing of nucleobases has become increasingly important in diagnostic and therapeutic applications. Moreover, chiral recognition plays a critical role in most biological functions such as enzyme catalysis and ligand–receptor interactions. Recent years, the synthesis of peptide nucleic acids (PNAs), nucleic acid analogs based on an achiral polyamide backbone, and their biological studies as well as diagnostic applications were evaluated.[1]

Even though a wide variety of PNA analogs have been reported to date, polyester analogs of nucleic acids have never been reported because of the absence of the synthetic strategy. Therefore, we focused our attention on the synthesis of a novel polyester analog of nucleic acids bearing an optically active serine backbone (serine-based nucleobase-linked polyester: SNE) (Fig. 1) as a simple model of nucleic acids,[2] a potential primordial nucleic acid,[3] and a candidate for therapeutic agents.[4] The replacement of the amide linkages of PNA and of the phosphodiester linkages of nucleic acids by ester linkages is expected to change the chemical and physiological properties of these molecules, particularly solubility in water and backbone flexibility. Furthermore, the SNE has a shorter helical pitch compared with that of PNA,[5] which is identical for the hybridization with complementary nucleic acids. In addition, helicity induced on SNE oligomers by the chirality of the backbone might express an ability to recognize the chirality of other molecules. Because ester linkages are labile to strong acids or bases, compared with the amide linkages, the development of a new method for the synthesis of SNE oligomers is of great importance.[6]

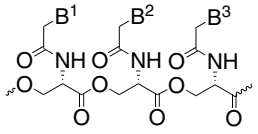

Figure 1. The structure of serine-based nucleobase-linked polyester (SNE).

Here, we describe the synthesis of optically pure monomer units containing a thymine base and the optimization of the reaction conditions for an ester bond formation on a solid support without racemization as well as for the release of a dimer from the solid support without the cleavage of the ester bond.

Keywords: Nucleic acid analog; Polyester; Serine; Solid-phase synthesis; Racemization; Condensing reagent; PNA.
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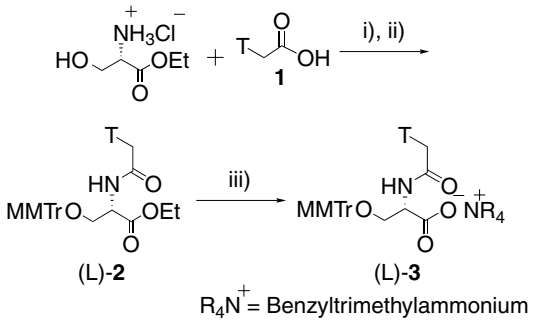
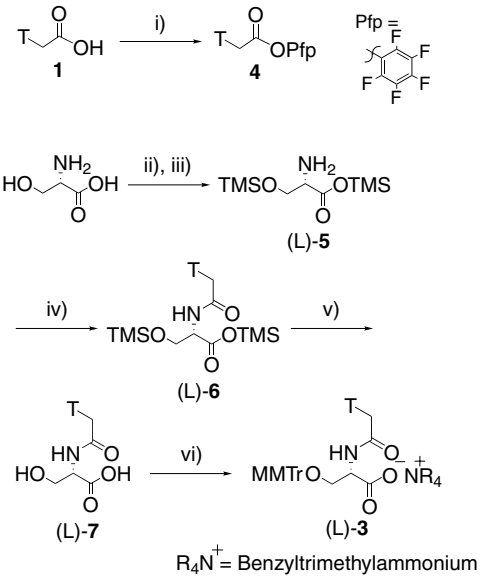


Scheme 1. Reagents and conditions: (i) DCC, Et_3N, CH_2Cl_2, DMF, rt, 12 h; (ii) MMTr-Cl, pyridine, rt, 12 h (87%, two steps); (iii) benzyltrimethylammonium hydroxide, methanol, H_2O, 0 °C, 1 h (92%). The monomer unit (D)-**3** was synthesized in a similar manner.

Thymine monomers were prepared from L- and D-serines as outlined in Scheme 1.[7] (Thymin-1-yl)acetic acid **1**[8] was condensed with (L)-serine ethyl ester in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and then the hydroxy group was protected with a monomethoxytrityl (MMTr) group. The resulting ester (L)-**2** was hydrolyzed by treatment with benzyltrimethylammonium hydroxide to give the monomer unit (L)-**3** in good yield. The monomer unit (D)-**3** was synthesized in a similar manner. However, the optical purities of the monomer units were found to significantly decrease to 66% ee for (L)-**3** and (D)-**3**.[9] Therefore, we decided to synthesize the monomer units by another route outlined in Scheme 2.

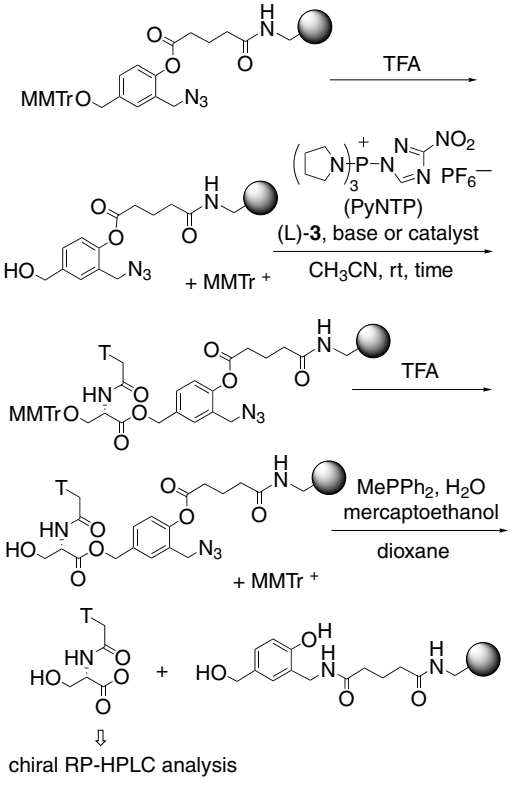
Pertrimethylsilylated serine, prepared by a method similar to the reported procedure,[10] was treated with an equimolar amount of anhydrous methanol to remove the amino-protecting group selectively. The crude (L)-**5** was then acylated with the thyminyl acetic acid pentafluorophenyl ester **4**.[11] After removal of the TMS groups, the hydroxy group was protected with an MMTr group. Finally, the resulting compound was converted to the corresponding benzyltrimethylammonium salt to give the monomer unit (L)-**3** in 97% total yield. The monomer unit (D)-**3** was synthesized in a similar manner. In these cases, the optical purities of (L)-**3** and (D)-**3** were estimated to be 98% ee and 97% ee, respectively.[9]



Scheme 2. Reagents and conditions: (i) DCC, DMF, rt, 17 h (37%); (ii) TMS-Cl, CH_2Cl_2, 60 °C, 1.5 h; (iii) MeOH, CH_2Cl_2, rt; (iv) **4**, CH_2Cl_2, rt, 12 h; (v) TFA, CH_2Cl_2, methanol, rt, 1 h; (vi) MMTr-Cl, pyridine, rt, 13 h (97%, four steps). The monomer unit (D)-**3** was synthesized in a similar manner.

Next, we investigated reaction conditions for an ester bond formation on a solid support and the effect of the conditions on racemization (Scheme 3). Similar to N-acylated amino acids, the monomer unit **3** would be susceptible to racemization, compared with N-carbamated amino acids. Therefore, a coupling reaction without racemization should be developed to obtain optically pure SNE oligomers. To evaluate the coupling efficiency for the ester bond formation, a novel linker developed by our group,[12] which could be cleaved by treatment with a phosphine reagent under reductive and neutral conditions, was attached to an aminomethylated highly cross-linked polystyrene (HCP) support.[13] The loading amount of the linker was estimated by an MMTr cation assay (24–26 μmol/g).

The coupling reaction of the monomer unit (L)-**3** (0.1 M) with the hydroxy group of the linker was performed by using PyNTP (0.2 M) as a coupling reagent[14] in the presence of a base or nucleophilic catalyst (0.3 M), and the coupling efficiency was verified by an MMTr cation



Scheme 3. The ester bond formation on a HCP support.

Table 1. The coupling efficiency and the effect of the coupling conditions on racemization of the monomer unit (L)-**3**

Entry	Base or catalyst	Coupling yield[a] (%)	Ee[b] (%)
1	—	21	96
2	Pyridine	92	84
3	Lutidine	Quant	68
4	Collidine	Quant	58
5	DMAP	98	7
6	DTBMP[c]	11	93
7	4-Methoxy pyridine *N*-oxide	72	30
8	Quinoline	66	97

[a] The yield was estimated by an MMTr cation assay.
[b] The enantiomeric excess of **3** was monitored by a chiral RP-HPLC.
[c] 2,6-Di-*tert*-butyl-4-methylpyridine.

assay (Table 1). All reactions were performed in acetonitrile at room temperature for 15 min. The residue attached to the resin was released from the solid support by treatment with 0.2 M methyldiphenylphosphine-mercaptoethanol in aqueous dioxane.[12] The released products were dissolved in water and washed with chloroform to remove phosphine oxide and excess phosphine. After concentration of the aqueous portion, the products were analyzed by chiral reversed-phase HPLC.[9]

As shown in Table 1, the coupling yield was very low, when the reaction was carried out without any base or catalyst (entry 1). In contrast, the reaction proceeded smoothly in the presence of an effective base or catalyst. However, the degree of racemization increased by increasing the basicity of the additive. As a result, the best result was realized by using quinoline as a base; the highest enantiomeric purity of the monomer unit (L)-**3** was achieved. Then, the coupling conditions were further optimized using PyNTP/quinoline to realize both the high coupling efficiency and the high optical purity of the product.

The concentration of the monomer unit (L)-**3** and reaction time for the ester bond formation were further investigated (Table 2). Prolonged reaction time increased the coupling yield, whereas the degree of racemization increased (entry 2). In contrast, the coupling efficiency was improved by increasing the concentration of the monomer unit (L)-**3** (0.2 M), and the racemization was at a low level. Finally, a double coupling protocol was found to be the most effective from the viewpoints of both the efficient coupling and the acceptable degree of racemization (entry 3).

Table 2. The coupling efficiency and the effect of the coupling conditions on racemization using PyNTP/quinoline as reagents

Entry	Concentration of monomer (M)	Reaction time	Coupling yield[a] (%)	Ee[b] (%)
1	0.1	15 min × 1	66	93
2	0.1	1 h	93	89
3	0.1	15 min × 2[c]	99	96
4	0.2	15 min	95	95
5	0.2	30 min	99	94

[a] The yield was estimated by an MMTr cation assay.
[b] The enantiomeric excess of **3** was monitored by a chiral RP-HPLC.
[c] A double coupling protocol.

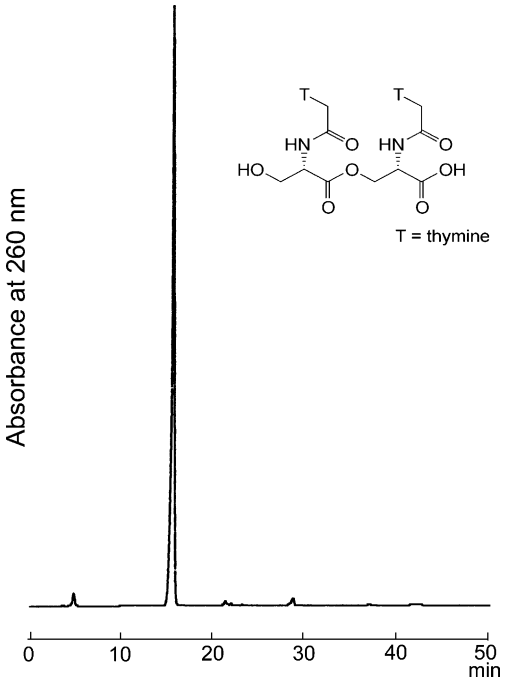


Figure 2. RP-HPLC profile of the crude dimer TT–SNE.

In the next stage, the dimer TT–SNE was synthesized on the solid support under the optimized conditions and detached from the resin. The released products were analyzed by reversed-phase HPLC (Fig. 2),[15] and the desired dimer TT–SNE was observed as a main peak. The result indicates that the dimer was synthesized efficiently on the solid support and successfully detached from the resin without decomposition of the product. After purification by RP-HPLC, the pure dimer TT–SNE was obtained in 52% yield.

In conclusion, optically pure monomers containing a thymine base were synthesized, and coupling conditions were optimized by using a new linker-attached solid support to form an ester bond with minimum racemization. Finally, the corresponding dimer was synthesized under the optimized conditions on the solid support and detached under almost neutral conditions to give the product without any decomposition. The solid-phase synthesis of long oligomers containing four nucleobases is now in progress.

Acknowledgments

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