Nucleic Acids Research Supplement No. 1 187-188

Synthesis of a new type of artificial nucleic acid derived from optically active serine

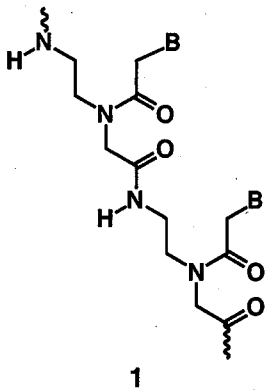
Takeshi Wada, Toshikazu Tsuneyama and Kazuhiko Saigo
Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, FSB-702, Kashiwanoha 5-1-5, Kashiwa 277-8562, Japan

ABSTRACT

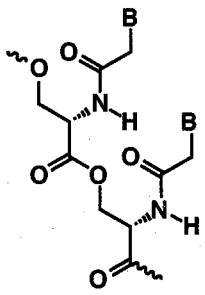
This paper describes the synthesis of a new type of nucleic acid analog derived from L-serine with ester linkages. The monomer unit bearing thymine base was synthesized from L-serine ethyl ester by a three-step reaction in good yield without racemization. 3-Nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP) was found to be highly effective for the rapid ester bond formation without racemization. 2-Phenyl-2-(trimethylsilyl)ethyl group was successfully employed as a carboxyl protecting group in solution-phase synthesis.

INTRODUCTION

Peptide nucleic acid (PNA, **1**), an oligonucleotide mimic, has an achiral polyamide backbone, to which nucleobases are attached.[1,2] Since PNA binds to single stranded DNA and RNA as well as to double stranded DNA, it is regarded as a potentially useful molecule for antisense strategy.[3] It is well known that the PNA-DNA and PNA-RNA duplexes are more stable than the corresponding DNA-DNA and DNA-RNA duplexes.[4]



1



2

However, the recent structural studies on PNA-DNA[5] and PNA-RNA[6] duplexes by NMR indicated that the conformation of PNA in such complexes was not optimal for the hybridization with the complementary DNA and RNA because the helical pitch of PNA is much larger than that of DNA and RNA. Here we propose a new type of DNA analog derived from L-serine with ester linkages (**2**). The present DNA analog bearing a polyester backbone is expected to have a shorter helical pitch compared with that of PNA, which is identical for the hybridization with complementary nucleic acids. In the present paper, we wish to describe a new strategy for the synthesis of a polyester-type nucleic acid analog consist of *N*-(thymin-1-yl)acetyl L-serine.

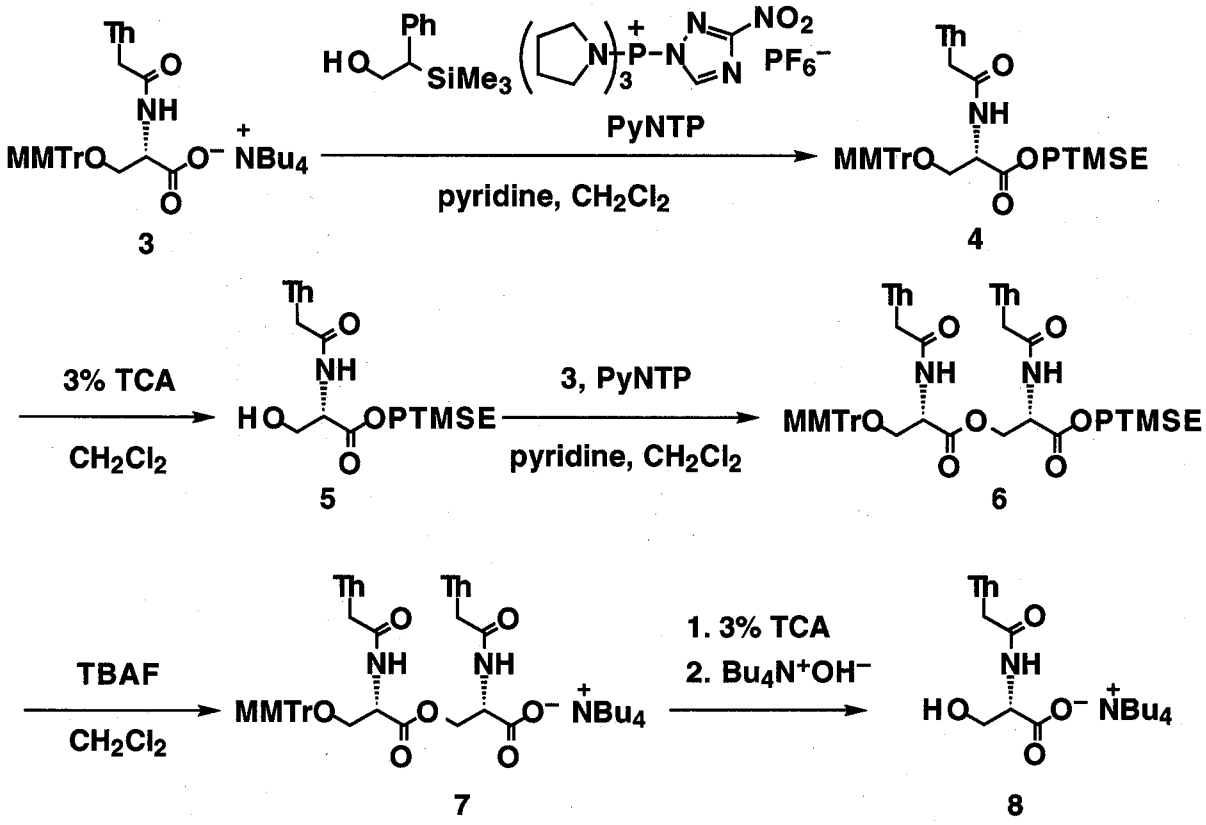
RESULTS AND DISCUSSION

Synthesis of the monomer building block

First, synthesis of the monomer building block bearing thymine base (**3**) was examined. (Thymin-1-yl)acetic acid was condensed with L-serine ethyl ester in the presence of DCC as a condensing reagent. The resulting *N*-(thymin-1-yl)acetyl L-serine ethyl ester was allowed to react with MMTr-Cl to give the fully protected monomer unit in 84% from the starting material. The ethyl ester was hydrolyzed by treatment with tetrabutylammonium hydroxide in aqueous MeOH at 0°C to give the monomer unit **3** in 88% yield. In order to clarify the optical purity of the monomer unit, the corresponding antipode **3** was synthesized from D-serine ethyl ester by the same manner. After deprotection of the MMTr group from both enantiomers by treatment with 3% dichloroacetic acid in CH_2Cl_2, the products were analyzed by a chiral HPLC using a conventional procedure. The results indicated that the optical purities of the products were higher than 99%.

Ester bond formation

The most important step in the synthesis of a polyester-type DNA analog is construction of the ester linkage. We have examined several condensing reagents known to be effective for the ester bond formation.[7] However, by using these reagents, the condensation of **3** with an alcohol proceeded very slowly. In contrast it was found that a new condensing reagent, 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP), was highly efficient for the rapid ester bond formation. For example, the monomer **3** was condensed with 9-fluorenylmethanol in the presence of PyNTP in



CH_2Cl_2-pyridine at rt. The reaction completed within 40 min to give the corresponding 9-fluorenylmethyl ester in 94% yield after silica gel column chromatography.

Synthesis of the dimer

In the solution-phase synthesis of a polyester-type DNA analog, the selection of a protecting group for the carboxyl terminal is of great importance. Quite recently, Kunz *et al.* have reported 2-phenyl-2-(trimethylsilyl)ethyl (PTMSE) as a new protecting group for the carboxyl function.[8] This protecting group can be removed by treatment of tetrabutylammonium fluoride under extremely mild conditions without cleavage of the esterlinkage. Therefore, we adopted PTMSE as a carboxyl terminal protection. The monomer unit **3** was condensed with 2-phenyl-2-(trimethylsilyl)ethanol in the presence of PyNTP to give the corresponding PTMSE ester **4** in 90% yield. After the quantitative removal of the MMTr group by TCA treatment, the resulting hydroxyl component **5** was condensed with **3**. The yield of the dimer **6** was 89% after purification. The PTMSE group of **6** was removed by treatment of TBAF in CH_2Cl_2 at rt for 15 min to give **7** in 80% yield. The product was further treated successively with TCA and tetrabutylammonium hydroxide to give the fully deprotected monomer **8**. The optical purity of the hydrolyzed product was estimated to be higher than 99% by use of a chiral HPLC. The result indicated that the condensation and deprotection proceeded without racemization.

In summary, the present method enabled us to synthesize a novel nucleic acid analog consist of optically active serine with a polyester backbone. The solid-phase synthesis of oligomers involving four kinds of nucleobases is now in progress.

REFERENCES

1. Nielsen, P. E., Egholm, M., Berg, R. H., Buchardt, O. (1993) *Science* **254**, 1497–1500.
2. Egholm, M., Buchardt, O., Nielsen, P. E., Berg, R. H. (1992) *J. Am. Chem. Soc.* **114**, 1895–1897.
3. Larsen, H. J., Bentin, T., Nielsen, P. E. (1999) *Biochim. Biophys. Acta* **1489**, 159–166.
4. Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S. M., Driver, D., Berg, R. H., Kim, S. K., Norden, B., Nielsen, P. E. (1993) *Nature* **365**, 566–568.
5. Rasmussen, H., Kastrup, J. S., Nielsen, J. N., Nielsen, J. M, Nielsen, P. E. (1997) *Nature Struct. Biol.* **4**, 98.
6. Eriksson, M., Kastrup, J. S., Nielsen, J. N., Nielsen, J. M, Nielsen, P. E. (1996) *Nature Struct. Biol.* **3**, 410–413.
7. Mulzer, J. (1991) In Winterfeldt, E. (ed.) *Comprehensive Organic Synthesis.* Pergamon Press, Vol. 6, pp. 323–380.
8. Wagner, M., Kunz, H. (2000) *Synlett* **3**, 400–402.