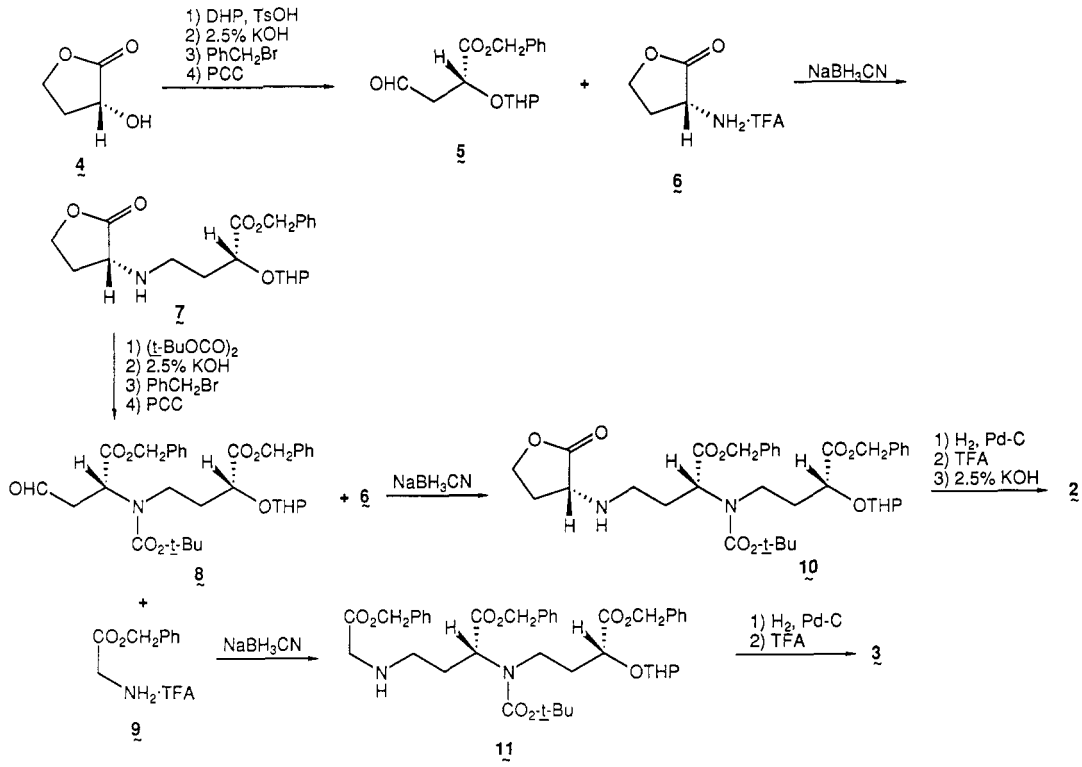
Scheme I



meaning that no racemization took place during the course of the syntheses.

D- and L-AA complexes with Fe(III) gave identical ESR spectra (g = 7.66, 4.26; $S = {}^5/_2$), indicating that the structural configurations are mirror images of each other. The two isomers of DDA also exhibited identical ESR characteristics (g = 7.69, 4.24; $S = {}^5/_2$) for the Fe(III) complex in aqueous solution (pH 7). These g values are very close to those (g = 7.66, 4.26; $S = {}^5/_2$) for the MA-Fe(III) complex.[7,8] In addition, a chromatographic study using ^{59}Fe showed that essentially all of the iron present was the chelated form with both D and L ligands. These results clearly suggest that in both AA and DDA the D form as well as the L form makes an Fe(III) coordination complex similar to that of MA, namely, a nearly octahedral configuration in which two amine nitrogens and both terminal carboxylate oxygens bind to the metal ion as basal planar atoms and hydroxyl oxygen and intermediate oxygen coordinate as axial donors.[8]

Figure 1 shows the iron-uptake activities of the L and D forms of the phytosiderophore for the rice plant (*Oriza sativa* L. var. Koshihikari), which is susceptible to iron chlorosis because of the very limited ability to excrete its own phytosiderophore. The natural phytosiderophore L-AA remarkably stimulated ^{59}Fe uptake in the leaves of the water-cultured rice plant at pH 7. In contrast, the biological effect of its optical isomer, D-AA, was <30% of the L form. In the case of DDA, the L form also demonstrated high iron-uptake activity. On the other hand, the activity of the D form dramatically decreased and was comparable to that of the control. Thus the D form clearly differs from the L form in iron-uptake activity, although their metal coordination properties are very similar. It is most likely that there is a strict stereospecific recognition system (function) for the Fe(III) complex molecule on the membrane. Probably, a certain receptor protein that is able to bind selectively only for natural phytosiderophores exists on the root's membrane. In the plant kingdom, especially gramineous plants, the iron uptake may be regulated by both the excretion of phytosiderophores such as MA and the mobilization of the receptor protein for the iron complex molecule. A similar mechanism of iron regulation mediated by microbial siderophores such as enterobactin (catecholate type) and aerobactin (hydroxamate type) has been proposed for some microorganisms.[15,16]

In conclusion, the optical isomers of phytosiderophores reveal a significant distinction in iron-uptake ability. The present result indicates stereospecific iron-uptake mediated by phytosiderophore in gramineous plants.

Acknowledgment. We are grateful to Dr. Y. Ohfune for helpful suggestions in the synthesis of the phytosiderophores. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

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New Phosphite Method: The Synthesis of Oligodeoxyribonucleotides by Use of Deoxyribonucleoside 3′-[Bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphites] as New Key Intermediates

Tetsuo Watanabe, Hirotaka Sato, and Hiroshi Takaku*

Laboratory of Bioorganic Chemistry
Department of Industrial Chemistry
Tsudanuma, Narashino, Chiba 275, Japan

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Recently, the developments of phosphite, phosphoramidite, and H-phosphonate approaches by Letsinger[1], Caruthers[2], and Matteucci[3] have enabled the rapid chemical synthesis of oligo-

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Scheme I

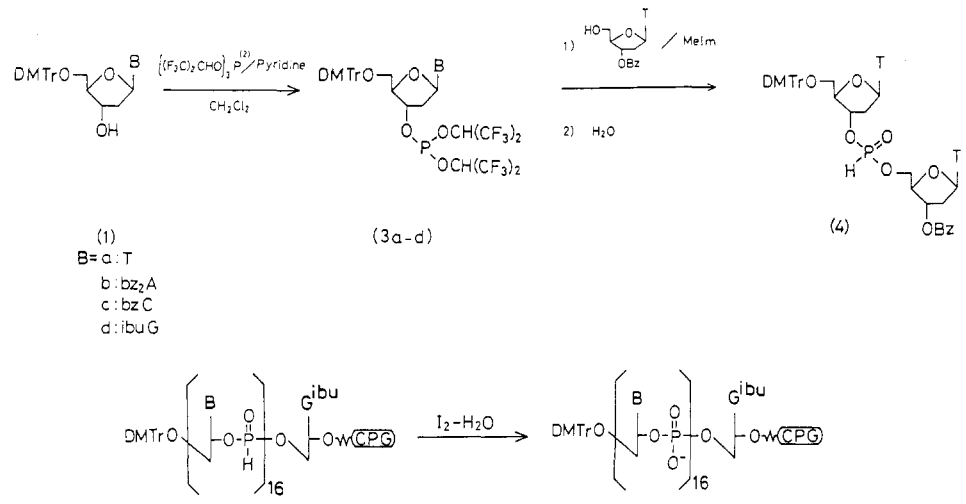

Table I. Isolated Yields and ^{31}P NMR Spectra Analysis of Compounds **3a–d**[a]

compd	yields (%)	^{31}P NMR chemical shift (ppm)
3a	88	140.2
3b	85	141.2
3c	83	140.2
3d	81	141.1

[a] The chemical shifts are reported relative to 85% H_3PO_4 in $CDCl_3$ as an external standard.

and polydeoxyribonucleotides on solid supports. The phosphoramidite approach has been especially accessible and more successful in application to molecular biology.[4] However, the phosphoramidite approach requires a phosphate protecting group and a capping and oxidation (an oxidation reaction is performed at the end of each coupling reaction) reaction during the course of synthesis compared with the H-phosphonate approach. On the other hand, the H-phosphonate approach also has some disadvantages; instability of the coupling agent (pivaloyl chloride) and a necessity for a large excess of the phosphitylating reagent to prepare the H-phosphonate units.

In this paper, we wish to report a much more efficient approach to oligodeoxyribonucleoside synthesis by use of deoxyribonucleoside 3′-[bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphites] as key intermediates.

Recently, we have reported[5] a simple method for the synthesis of deoxyribonucleoside 3′-[H-phosphonates] using the transesterification of a new reagent, bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphonate. This reagent was easily activated by pyridine to give the reactive *N*-phosphonylpyridine intermediates.

In order to investigate the utility of nucleoside 3′-[bis-(1,1,1,3,3,3-hexafluoro-2-propyl) phosphites] as the starting units for oligodeoxyribonucleotide synthesis, several experiments have been tested. First, we examined the synthesis of nucleoside 3′-[bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphites] (**3a–d**) by reaction of nucleoside derivatives (**1a–d**) with tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite (**2**).[6] A typical procedure follows: Compound **1** (1 molar equiv) was treated with **2** (2 molar equiv) in the presence of pyridine (2 molar equiv) in CH_2Cl_2. After 15 min the solution was washed with phosphate buffer (pH 7.0). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. The residue was applied to a column of silica gel and eluted with ether. The appropriate fractions were pooled and evaporated to give the phosphite units **3a–d** in 81–88% yields (Table I). Compounds **3** can be stored unchanged in a screw-cap vial at −30 °C for several months.

It was found that *N*-methylimidazole (MeIm) was much more effective for the activation of **3** than (dimethylamino)pyridine (DMAP) and pyridine. Compound **3a** was treated with 3′-*O*-benzoylthymidine in the presence of MeIm in CH_3CN for 10 min. After the coupling reaction was completed, the mixture was quenched with a small amount of water. After the usual workup followed by silica gel column chromatography, the protected dithymidine (3′-5′)phosphonate (**4**) (^{31}P NMR, δ 7.336, 8.682) was isolated in 89% yield. In the above reaction, when DMAP and pyridine were used in place of MeIm, the rate of the coupling reaction was considerably slower.

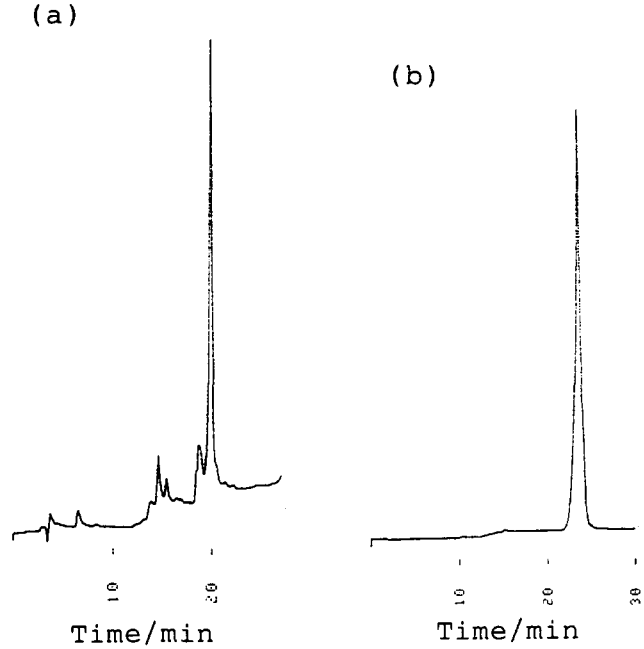

Figure 1. Purification and analysis of the 17-mer. (a) The 17-mer was purified by anion-exchange HPLC with a TSKgel DEAE-2SW column with a linear gradient of ammonium formate (from 0.1 to 0.75 M during 30 min) in 20% aqueous acetonitrile. (b) The purified 17-mer was analyzed by reversed-phase HPLC with a TSKgel oligo-DNA RP column with a linear gradient of acetonitrile (from 5 to 25% during 30 min) in 0.1 M triethylammonium acetate (pH 7.0).

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To demonstrate the utility of the present new phosphite approach, heptadecamer, d-AGACTTCTCCTCAGGAG on a solid support was synthesized. The reaction was carried out on controlled pore glass (15 mg, 36 μmol/g) with a Biosearch Model 381 A DNA synthesizer. We showed the following elongation cycle to be effective: treatment with (1) washing [CH_3CN, 20 s], (2) 5′-unblocking [3% Cl_3CCOOH in CH_2Cl_2, 90 s], (3) washing [CH_3CN, 30 s], (4) coupling [19 μmol phosphite unit (**3**), 95 μmol MeIm in CH_3CN, 10 min], (5) washing [CH_3CN, 30 s], (6) hydrolysis [THF–pyridine–H_2O, 4:3:1, v/v, 2 min], (7) washing [CH_3CN, 30 s]. The extent of coupling in each cycle was monitored by the spectrophotometric assay of DMTr cations; it was estimated each averaged ca. 96%. When the assembly of the oligonucleotide chain was completed, the solid support was treated with 0.1 M I_2 in THF–pyridine–H_2O (4:3:3, v/v) for 15 min. After the usual deprotection, isolation of the desired oligomer, d-AGACTTCTCCTCAGGAG, was performed by TSKgel DEAE-2SW (Figure 1a). The main peak was found to be homogeneous by reversed phase ^{18}C HPLC (Figure 1b) and by gel electrophoresis. The proportions of four nucleosides were analyzed by reversed phase ^{18}C HPLC after hydrolysis with snake venom phosphodiesterase and alkaline phosphatase.

This result and those shown above clearly demonstrate that transesterification of a new type of phosphite unit (**3**) could prove to be very effective for the synthesis of deoxyribooligonucleotides on a solid support. They are readily activated by *N*-methylimidazole under very mild conditions. It is noteworthy that this operation involves a one-step reaction, which is an advantage over both the phosphite and H-phosphonate approaches. The syntheses of phosphorothioylated oligonucleotides and other modified DNA fragments are now in progress.

Podand Ionophores. A New Class of Nonmacrocyclic Yet Preorganized Hosts for Cations

Takamasa Iimori and W. Clark Still*

Department of Chemistry, Columbia University
New York, New York 10027

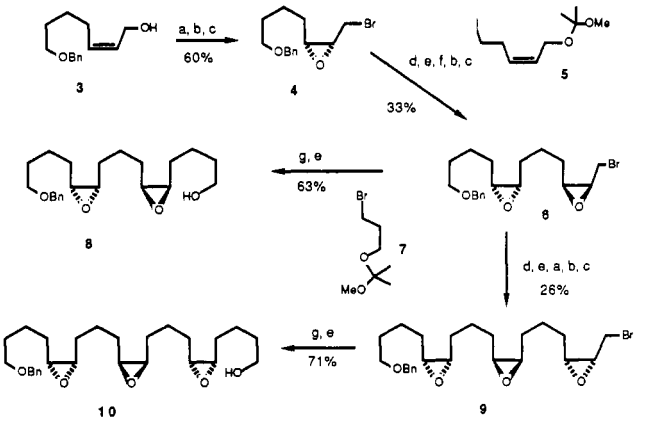
Arnold L. Rheingold and Donna L. Staley

Department of Chemistry, University of Delaware
Newark, Delaware 19716

Received November 8, 1988

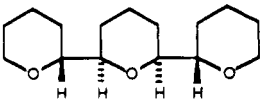
Nonmacrocyclic host molecules (podands) are traditionally regarded as poor ligands when compared with analogous monomacrocycles (coronands) and bridged polymacrocycles (cryptands).[1] The weak binding properties of the podands stem from the conformational freedom of their component acyclic chains. This freedom disfavors binding both entropically and enthalpically because effective binding conformations are usually both few in number and high in energy. On the other hand, the naturally occurring polyether antibiotics are podand-like structures which bind cations rather well. The cation-binding properties of these natural ionophores result in part from their incorporation of an anionic carboxylate and in part from the stereochemically reinforced preorganization of the array of ligating oxygens.[2] In this communication, we describe the synthesis and properties of two neutral podands which structurally resemble the poly-THF/THP substructures of the polyether antibiotics. These novel podands are preorganized by connectivity and stereochemistry into binding conformations and form molecular complexes with small, cationic guests.

Scheme I[a]

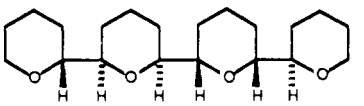


[a] a. (L)-Diethyl tartrate, $Ti(OiPr)_4$, *t*-BuOOH, CH_2Cl_2; b. TsCl, Et_3N, DMAP, CH_2Cl_2; c. LiBr, acetone; d. **5** + *t*-BuLi/Et_2O; $MgBr_2$, Li_2CuCl_4, 4:1 THF–HMPA; e. Pyr·HOTs, MeOH; f. (D)-diethyl tartrate, $Ti(OiPr)_4$, *t*-BuOOH, CH_2Cl_2; g. **7** + Li/Et_2O, $MgBr_2$, Li_2CuCl_4, 4:1 THF–HMPA.

The substances we have prepared (**1** and **2**) are shown below and are derivatives of the acyclic ethers diglyme and triglyme



1



2

dimethyl ether. Compared with the corresponding acyclic glyme ethers, however, **1** and **2** have substantially fewer low-energy conformations (see below). This reduction in the number of possible conformations is analogous to the restriction in conformational space which is effected by the macrocyclization of the glyme ethers to form the ionophoric crown ethers. In our podands, decreased conformational freedom follows from the highly restricted conformational nature of all bonds except those linking the chairlike tetrahydropyran[3] rings. The three-dimensional properties of the low-energy conformations of **1** and **2** depend critically upon the stereochemistry at the ring junctures, and different diastereomers will favor different geometrical arrangements of the cation-ligating oxygens. We therefore expect binding properties to vary with the diastereomer studied. We chose the stereochemistry shown above because, according to molecular mechanics, it preorganizes the podands into low-energy conformers resembling parts of the potassium-binding conformation of 18-crown-6.[4]

The synthesis of **1** and **2** was planned around a polyepoxide cyclization[5] and is summarized in Scheme I. The stereochemistry of the product is controlled by a combination of stereoselective olefin formations and enantioselective[6] epoxidations. Here, in-

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