ORGANIC LETTERS

2002
Vol. 4, No. 3
359−362

Efficient Synthesis of 3-Furanosyl-6′-furanosylphosphinate through a Tandem Sequential Radical Process

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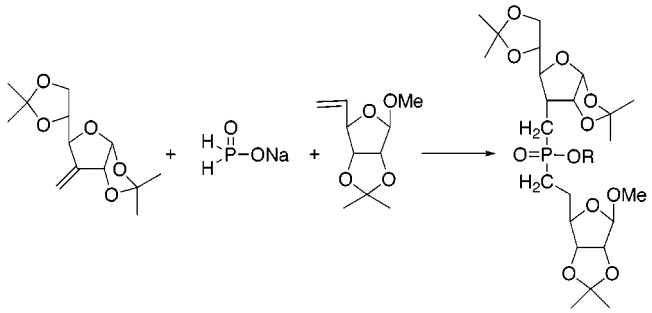
Received November 8, 2001

ABSTRACT



The sodium salt of hypophosphorous acid is shown to act as a double radical precursor in a double, sequential radical addition on 3-*exo*-methylenefuranose derivative 14 and 4-ethylenefuranose 10 to furnish phosphinates 18d in good overall yields. Unambiguous structural assignment establishes the high diastereoselection of the process.

In the recent past, intense efforts have been devoted to the preparation of modified oligonucleotides (ODNs) because of their interest in the context of the antisense strategy.[1] As a result, potential applications as antiviral (including HIV), anticancer, and antibacterial agents have blossomed, and recently the first modified ODN has been approved in the United States by the Food and Drug Administration (FDA) as a treatment against cytomegalovirus infection.[2] Most of the modified ODNs have the diesterified phosphates (**1**) replaced by other functional groups (Figure 1). Among these, the phosphorothioate group (**2**) is the most widespread as a result of both its structural similarity and its increased nuclease resistance.[3] Other modifications of the ODN backbone have involved the replacement of the phosphate units by phosphonates, phosphoramidates, amides, and others.[4] However, one of the major problems plaguing diesters of ODNs, as well as many of the above-cited

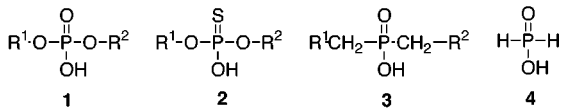


Figure 1.

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(1) (a) Uhlmann, E.; Peyman, A. *Chem Rev.* **1990**, *90*, 543−584. (b) Crooke, S. T.; Bennett, C. F. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 107−129. (c) Bennett, C. F. *Biochem. Pharmacol.* **1998**, *55*, 9−19. (d) Gee, J. E.; Robbins, I.; Van der Laan, A. C.; Van Boom, J. H.; Colombier, C.; Leng, M.; Paible, A. M.; Nelson, J. S.; Lebleu, B. *Antisense Nucleic Acid Drug Dev.* **1998**, *8*, 103−111. (e) Crooke, S. T. *Biochim. Biophys. Acta* **1999**, *1489*, 31−44.

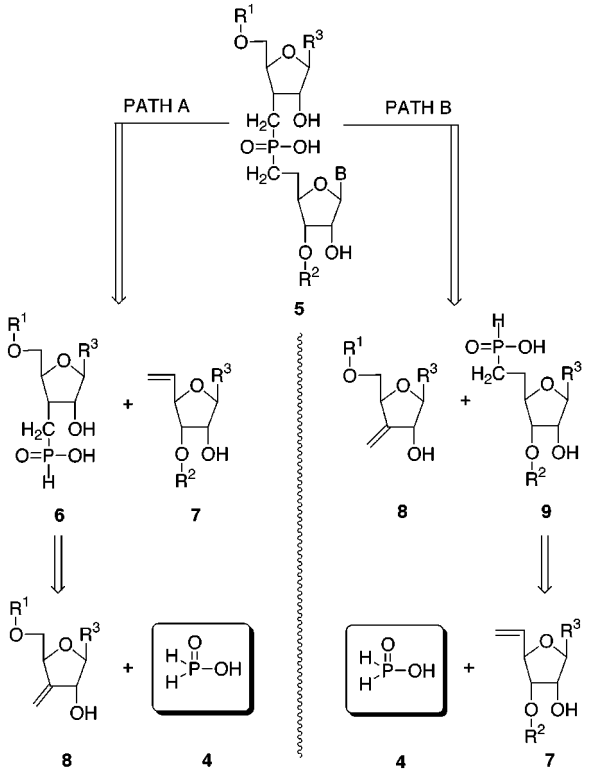
10.1021/ol017011x CCC: $22.00
Published on Web 01/09/2002

analogues, is their high propensity for rapid degradation by nucleases in the cell.[5] Moreover, use of a functional group (between two of the (deoxy)ribose units) that is structurally and electronically *too* different from the phosphate induces a loss of other properties (e.g., conformation) of ODNs, essential for the antisense activity.

To the best of our knowledge, the phosphinate function (**3**) has been used only once in modified ODNs, despite its close analogy with the parent functional group and its reported higher nuclease resistance: indeed, the replacement of the two esterified O−P bonds with two C−P bonds renders the modified ODNs more resistant to hydrolysis by the enzymes.[6] From a synthetic standpoint, however, the introduction of the phosphinate linkage required six steps and resulted in a 13% overall yield. Recent results from An and co-workers on 3′-nucleoside *H*-phosphonate derivatives prompt us to report our preleminary results toward the development of a more efficient approach.[7]

Carbon−phosphorus bonds have traditionally been constructed through interaction between a trivalent phosphorus and an electrophilic center (e.g., the Arbuzov and Pudovik reactions) or by addition of a phosphorus-centered radical on an sp^2 carbon.[8] Our own work in this latter field demonstrated the possibility of preparing 3-phosphonomethyl- and 3-phosphonothiomethylfuranosides from 3-*exo*-methylenefuranosyl derivatives.[9] The involvement of hypophosphorous acid (**4**) in the formation of phosphinates through a radical chain mechanism has been reported by Nifant'ev.[10] We herein report that this methodology is applicable to the stereocontrolled preparation of 3-furanosyl-6′-furanosyl phosphinates. Retrosynthetic analysis of the target molecule **5** indicates that a sequential, double radical addition of hypophosphorous acid (or one of its salts) may be achieved in two different manners, depending on the order of the sequence (Scheme 1). Thus, addition of hypophosphorous acid (**4**) might be conducted first on either the *exo*-methylene derivatives **8** of 3-deoxyfuranosides (path A) or **7** of 5-deoxyfuranosides (path B) to yield molecules of the type **6** or **9**, respectively. A second addition reaction of the phosphorus-centered radicals generated from **6** or **9** on alkenes **7** or **8**, respectively, would deliver the same target compound.

Scheme 1



Particular attention must be paid to the stereochemical outcome of the reaction in the creation of the new stereocenter in position 3. Data from the literature show that hydrogen quenching of a radical generated on carbon 3 of a furanose occurs mostly from the face opposite to a sterically hindering group in position 1, or 1 and 2.[9,11] For these reasons, the radical addition reactions were conducted on carefully chosen protected saccharides, and we thus elected the readily available ribofuranosyl and glucofuranosyl derivatives (**10** and **14**, respectively) as adequate substrates for the preparation of the target compound (Scheme 2).[12]

Heating an ethanol suspension of furanose derivative **10** and sodium hypophosphite **11** (2 equiv) in the presence of

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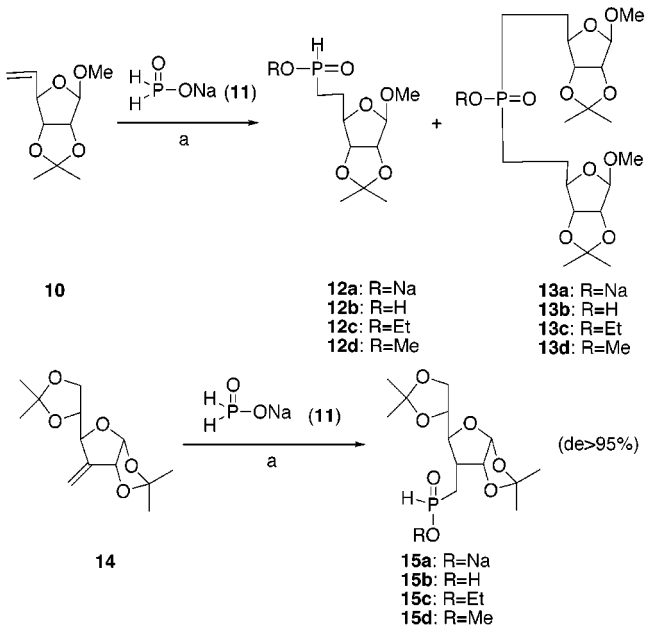
Scheme 2[a]



[a] (a) 1.0 equiv of $(t\text{-BuO})_2$, ethanol, sealed tube, 130 °C, 4 h.

di-*tert*-butyl peroxide at 130 °C (sealed tube) led to the clean formation of two products (4:1 ratio), quickly identified as the expected furanosylphosphonous acid salt **12a** and the sodium salt **13a** of bisfuranosylphosphinic acid. Increasing the amount of sodium hypophosphite to 5 equiv allowed us to maximize the formation of **12a** (>98%), the phosphinate **13a** being now detected only in trace amounts.

Similarly and under the same optimized conditions, 3-methylenefuranoside **14** cleanly and quantitatively furnished the desired salt **15a**. The diastereoselection was found to be over 95% in favor of the α-isomer (by ^{1}H and ^{31}P NMR spectrometries; see below), the result of a nearly exclusive hydrogen quenching of the radical-adduct **16** from the face opposite to that occupied by the 1,2-acetonide moiety (Figure 2). No dimeric structure **17a** (analogous to **13a**) could this time be detected by NMR spectrometry; apparently, the combined steric hindrances of both the phosphorus-centered

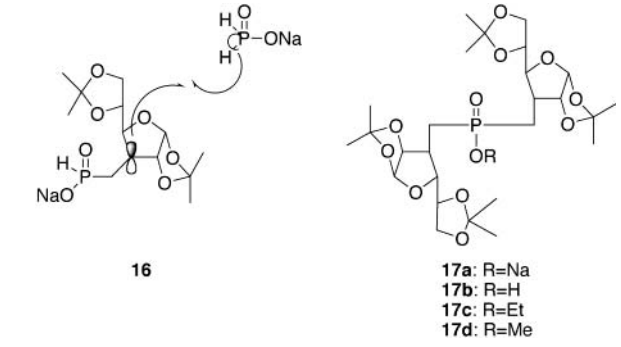


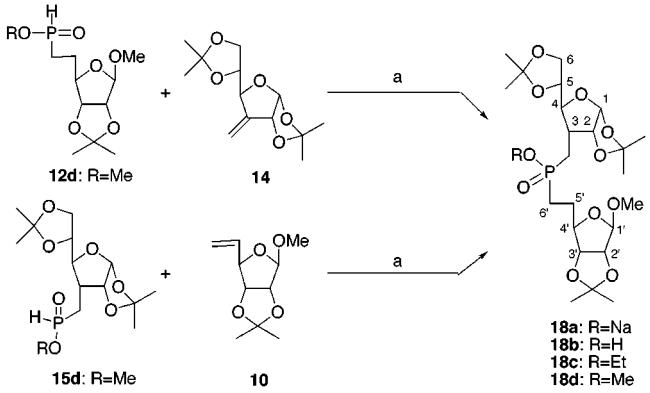
Figure 2.

radical derived from the salt **15a** of furanosylphosphonous acid and the alkene **14** are high enough to totally suppress the process.

To facilitate both the isolation in the pure form and their use as substrates for further reactions, furanosylphosphonous acid salts **12a** and **15a** were then transformed into the corresponding acids and esterified. The use of freshly prepared diazomethane in the presence of 2 equiv of trifluoroacetic acid led to a clean conversion to the methyl esters **12d** and **13d**; separation by chomatography led, this time, to the isolation of **12d** in a reproducible 83% yield (two steps). In a similar way, the sodium salt **15a** was cleanly transformed to the methyl ester **15d**, isolated in 70% yield.

Both esters **12d** and **15d** were then engaged in a second radical addition reaction with a slight excess (1.25 equiv) of their complementary alkene (**14** and **10**, respectively), in the presence of *tert*-butylperoxypivalate (55 °C, 16 h) (Scheme 3). In both cases, a 1:9 mixture of the expected unsym-

Scheme 3[a]



[a] (a) 0.6 equiv of $t\text{-BuCO}_3t\text{-Bu}$, toluene, 55 °C, 4 h.

metrical methyl phosphinate **18d** and the corresponding phosphinic acid **18b** was obtained. The mixture was then treated with TFA and diazomethane to allow isolation of the desired esters **18d** in 82% and 70% yield, respectively (a 1:1 diastereomeric mixture at the phosphorus center).

The formation of phosphinic acid **18b** may be explained by an intramolecular quench of radical adduct **19** by a hydrogen atom of the methyl ester group, loss of formaldehyde, quench of the resultant phosphinyl radical **21** by **12d** or **15d**, tautomerization and oxidation of the thereby-formed trivalent phosphorus atom during workup (Scheme 4).[14]

Various NMR spectrometry techniques allowed us to verify that the two unseparable diastereomers **18d** obtained from the esterification process were the result of a lack of stereoselection at the newly created stereogenic center (phosphorus atom). Thus the crucial stereochemical assignment of carbon 3 of the glucofuranosyl unit, as well as the

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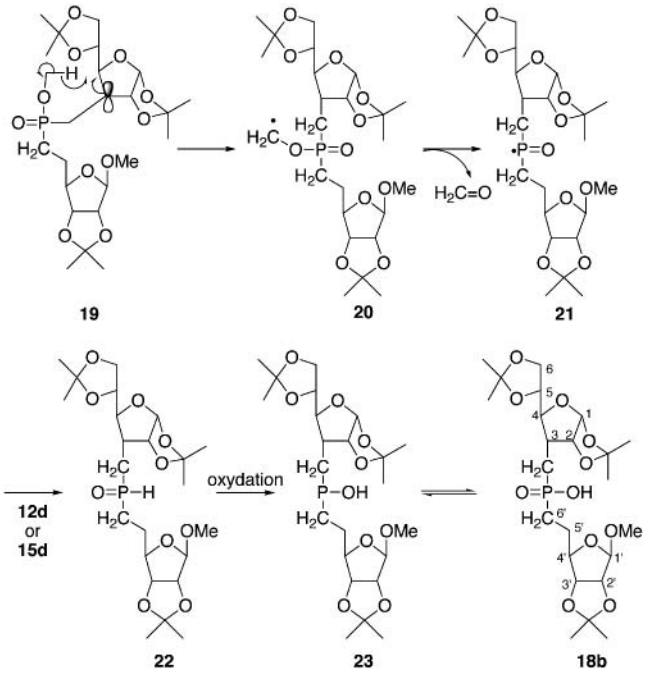
(14) For a related example of β-sission followed by oxidation, see: Piettre, S. R. *Tetrahedron Lett*. **1996**, *37*, 2233−2236.

Scheme 4



retention of configuration of carbon 1 of the ribofuranose unit, were ascertained by 1D,2D homonuclear and inverse heteronuclear NMR pulse sequences using pulsed field gradient. Sequential assignment of ^{1}H and ^{13}C signals was achieved from scalar interaction. Methodical and reiterative analyses of ^{1}H, $^{1}H\{^{31}P\}$, ^{13}C, ^{13}C SEFT,[15] $^{1}H-^{1}H$ COSY,[16] $^{1}H-^{13}C$ HSQC,[17] $^{1}H-^{13}C$ HMBC,[18] and $^{1}H-^{13}C$ HSQC-TOCSY[19] NMR data were carried out. Because of the small chemical shift differences between the signals of both diastereomers and the overlapping of many signals, the analysis was conducted on two fronts. COSY and HSQC experiments established most of the different spin systems of the molecule, and HMBC data revealed the connectivity of the respective fragments to one another and to the remaining quaternary carbons. An HSQC-TOCSY experiment was conducted to provide relayed magnetization transfer inside the spin system, thereby allowing the complete and unambiguous assignment of overlapped resonance signals such as those of the methylenes.

In addition, a more accurate strategy based on the comparison between the distances derived from NOESY and from modeling was carried out. ^{1}H spin-lattice relaxation time (T_1) of **18d** was evaluated using inversion-recovery sequence.[20] This was followed by recording several NOESY spectra with different mixing times (τ_m) in the range of T_1 for drawing a NOE buildup curve of the interresidue cross-peaks concerning the furanose structures. Interproton distances were obtained from volume integration of a NOESY spectrum with mixing time in the linear part of the NOE buildup (τ_m = 500 ms). Molecular mechanics calculations performed using Cerius2 gave the lowest energy conformation of the four possible stereoisomers after synthesis (3-*R* or 3-*S* and 1-*R* or 1-*S*).[21] Comparison of experimental distances with data from the models clearly supports the stereochemistry of the skeleton depicted in structures **18d** (Table 1).[22]

Table 1. Distances from Modeling versus Distances from NOESY Spectrum

		d_2 (Å)[b]	
label (i-j)	d_1 (Å)[a]	3-*R*	3-*S*
3-1	3.4	3.2 (6%)	4.2 (21%)
3-4	3.1	3.1 (0%)	2.4 (25%)
3-2	2.6	2.5 (3%)	2.8 (4%)
		1′-*R*	1′-*S*
1′-2′	3.0	3.0 (0%)	2.4 (22%)
1′-3′	4.2	3.9 (7%)	3.0 (33%)
1′-4′	2.9	2.6 (11%)	3.7 (24%)

[a] Distances from the NOESY spectrum. [b] Distances from the models and, between parentheses, the calculated deviation from experimental.

The work described above establishes an efficient synthetic route to molecules featuring two furanosides linked by a C-P(O)(OR)-C-C moiety in positions 3 and 4, respectively. The method of preparation is short, efficient, and easy to implement and addresses the crucial issue of the stereoselection at carbon 3, thereby opening the way to the synthesis of nonhydrolyzable modified oligonucleotides featuring a phosphinate linkage; work is currently under way to attain this goal.

Supporting Information Available: Experimental procedure and complete characterization for compounds **12d**, **15d,** and **18d** and NMR spectrometry and molecular modeling data for compound **18d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) These results are in complete agreement with the small coupling constants observed for the hydrogens on carbon 3 of the glucofuranose unit and on carbon 1 of the ribofuranose unit (see Supporting Information).