Synthesis of Nucleoside 3'-Alkylphosphonates: Intermediates for Assembly of Carbon-Bridged Dinucleotide Analogues

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Several 3'-modified nucleosides have been prepared, through an initial Wittig or Horner–Wadsworth–Emmons condensation with an adenosine 3'-ketone, followed by catalytic hydrogenation of the resulting olefin. Subsequent reaction of the 3'α-methylene carboxylate with the lithium salt of diethyl ethylphosphonate gave a β-keto phosphonate, while reaction of the methylene carboxylate with LDA, diethyl chlorophosphite, and O_2 gives the corresponding α-phosphono ester. These new nucleoside phosphonates can be viewed as analogues of natural phosphates and also can serve as synthetic intermediates for preparation of carbon-bridged dinucleotide analogues. To give the first such example, the β-keto phosphonate **13** was allowed to react with a nucleoside 5'-aldehyde, affording the dinucleoside enone **23**.

The synthesis and biological evaluation of antisense nucleotides, synthetic oligomers of defined sequence intended to bind with complementary, natural polynucleotides, is an area of substantial current interest.[1] While many details of the biological properties and therapeutic potential of antisense oligomers are yet to be determined, it is clear that significant problems of in vivo stability must be overcome. In particular, stability to endogenous nucleases must be improved if synthetic oligomers are to have a lifetime sufficient to demonstrate significant biological activity.

Many different approaches have been initiated to attain synthetic oligonucleotide analogues with enhanced resistance to nuclease activity. These strategies often involve substitution of various functional groups for the natural phosphodiester linkage, including both phosphorus-containing (e.g. phosphorothioates[2] and methylphosphonates)[3] and non-phosphorus-containing groups (e.g. carbonates,[4] carbamates,[5] and sulfur-based groups[6]). Oligonucleotides constructed entirely of repeating units other than the natural phosphates might achieve stability at the price of unattractive hybridization and/or solubility properties. However, oligonucleotides "capped" with but two terminal methylphosphonate groups,[7a] or a hydroxylamine linkage,[7b] have greatly enhanced stability with respect to important nucleases, while maintaining a structure based primarily on the natural phosphate diesters. Still another possibility, one not yet extensively explored, is to incorporate selected linkages capable of inhibiting nuclease activity through reaction with active site amino acids.

Our continuing interest in the chemistry of β-keto phosphonates,[8] and α-phosphono esters and lactones,[8e,9] has led us to attempt assembly of new dinucleotide analogues based on reactions of nucleoside phosphonates. In particular, the potential for incorporation of 3'-alkylphosphonate groups on a nucleoside template appeared to allow for the possibility of constructing new all-carbon chains bridging two nucleosides. In this report, synthesis of a number of new 3'-modified nucleosides is described, along with the preparation of the first dinucleotide analogue bridged by an enone system.

Results and Discussion

The keto phosphonate central to this strategy, compound **13**, requires extension of a carbon chain from the nucleoside 3'-carbon. The nucleoside ketone **1** represents an attractive starting material for that operation for several reasons. Ketone **1** is available in just two steps from commercially available adenosine,[10] and its use should lead to the appropriate enantiomer without need for either glycosidic bond formation or resolution. At the same time, it must be recognized that 3'-keto nucleosides are prone to elimination of the purine or pyrimidine base, leading to enone formation.

Introduction of a carbon chain at C-3' of nucleoside **1** has been accomplished through a Wittig reaction and also might be possible through a more convenient Horner–Wadsworth–Emmons (HWE) condensation (Scheme I). In fact, upon treatment with the sodium enolate of phosphono ester **2**, ketone **1** underwent the desired

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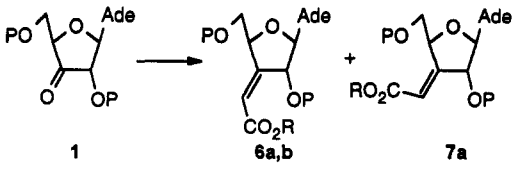
Scheme I



1 6a,b 7a

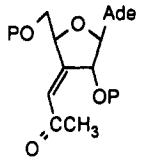
P = tBDMS
a R = CH_3
b R = CH_2CH_3

2 $(CH_3O)_2P(O)CH_2C(O)CH_3$ 62% (5:1 **6a**:**7a**)

3a, b $(C_6H_5)_3P{=}CHC(O)R$ 88% (**6** only)

4 $(C_6H_5)_3P{=}CHC(O)CH_3$ 10%

5 $(EtO)_2P(O)CH_2P(O)(OEt)_2$ —



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condensation without substantial purine elimination. Even though this condensation gives about a 5:1 mixture of olefin stereoisomers **6a** and **7a**, since the olefin stereochemistry is lost upon reduction in the next step, this issue is not critical. On the other hand, methoxycarbonyl phosphorane **3a** reacted as expected[11,12] with ketone **1** and gave only stereoisomer **6a** in good yield. Phosphorane **3b** gave the corresponding ethyl ester **6b**.

The success of these reactions prompted attempted condensations of ketone **1** with both the keto phosphorane **4** and methylenebis(phosphonate) **5**. However, attempted reaction of **4** with ketone **1** gave only a low yield (ca. 10%) of an olefin product (**8**), and treatment of the anion of bis(phosphonate) **5** with ketone **1** resulted only in loss of the purine, verifying literature cautions.[13] Even though the methodology exists to convert the methyl ketone **8** to the desired β-keto phosphonate **13**, through formation of the kinetic enolate, carbon–phosphorus bond formation,[8e] and catalytic hydrogenation, this route was not pursued due to the low yield of the condensation.

The stereochemistry of olefins **6** and **7** was tentatively assigned as *Z* and *E*, respectively, on the basis of their NMR spectra. In the ^1H NMR spectrum of **6a**, the 4′-hydrogen appeared at δ 5.43 (m), and the methoxy group appeared at δ 3.76. In contrast, the 4′-hydrogen of isomer **7a** was observed at δ 5.80 and the methoxy group at δ 3.69. Similar phenomena have been reported for modified carbohydrates such as the (*E*)- and (*Z*)-(alkoxycarbonyl)-methylene derivatives of a hexofuranose,[14] presumably resulting from a deshielding effect of a carbonyl group *cis* to C-4′.

Hydrogenation of these olefins, either compound **6a**, **6b**, or a mixture of compounds **6** and **7**, over 10% Pd/C gave a single reduced product, and this compound was initially assigned as the desired α-isomer **9** on the basis of its spectral data (Scheme II). Surprisingly, when the olefin **6b** was treated with H_2 and a wet Pd/C catalyst (Aldrich, Degussa type), the only observed product was the 5′-deprotected olefin **10**.[15] This fortuitous discovery turned out to be very helpful for confirmation of the 3′-stereochemistry of compound **9**. Reduction of the partially deprotected olefin **10** with excess $NaBH_4$ and subsequent reaction with tBDMSCl gave a major product identical to compound **9b** prepared by hydrogenation. This stereoselectivity is consistent with initial reaction of the free 5′-hydroxy group with borohydride and intramolecular delivery of hydride from the more hindered β-face of this complex.[16] In contrast, direct reduction of olefin **6a** with excess $NaBH_4$ in methanol afforded a different methyl ester, assigned as the 3′-β-isomer **12**, along with some by-products assumed to be primary alcohols.[12] Presumably, in this series hydride attack from the less hindered α-face leads to the 3′-β-isomer.

Once the stereochemistry of the 3′-modified nucleoside **9** was established unequivocally, phosphonate **13** was prepared from this ester according to the procedure of Mathey and Savignac.[17] Thus reaction of ester **9a** with the anion of diethyl methylphosphonate gave the expected β-keto phosphonate **13** in good yield (85%). This keto phosphonate undergoes HWE condensation with benzaldehyde under standard reaction conditions (K_2CO_3/18-crown-6/THF), producing enone **14** in 94% yield and with exclusively *E* stereochemistry (J = 16.3 Hz). As a final confirmation of structure, catalytic hydrogenation of enone **14** afforded the corresponding ketone **15**.

The 3′-modified nucleoside **9** also offered entry to a second series of nucleoside phosphonates, for conversion of ester **9** to the 3′-α-phosphono ester **16** could be envisioned by application of our newest method for C–P bond formation (Scheme III).[8e,9b] Direct treatment of ester **9a** with lithium hexamethyldisilizide and diethyl phosphorochloridite, followed by air oxidation, afforded a small amount of the desired phosphono ester **16** (<5%) while an N^6-phosphorylated product was isolated as the major product. To minimize formation of this byproduct, ester **9** was protected as its *N*,*N*-dibenzoyl derivative **17**. Phosphorylation of **17** using the same chlorophosphite/oxidation sequence provided the desired phosphonate **18** in better yield. Subsequent deprotection of compound **18** by treatment with methanolic ammonia gave phosphonate **16**, identical with material prepared by direct phosphonylation of compound **9**. The α-phosphono esters **18** and **16** can be viewed both as 3′-modified nucleosides and as potential synthetic intermediates for HWE condensations and other phosphono ester reactions.

Finally, to demonstrate that highly functionalized β-keto phosphonates can be used in assembly of dinucleotide analogues, the known nucleoside 5′-aldehyde **22** was prepared as a counterpart of phosphonate **13** in an HWE condensation. Although in most cases the 5′-aldehyde **22**

(11) The methyl esters were prepared to simplify subsequent displacement by phosphonate anions and minimize overlap in the midfield region of the ^1H NMR spectra.

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(15) This selective 5′-desilylation was found to be applicable to some other nucleoside bis(tBDMS)-ethers and will be reported in more detail elsewhere.

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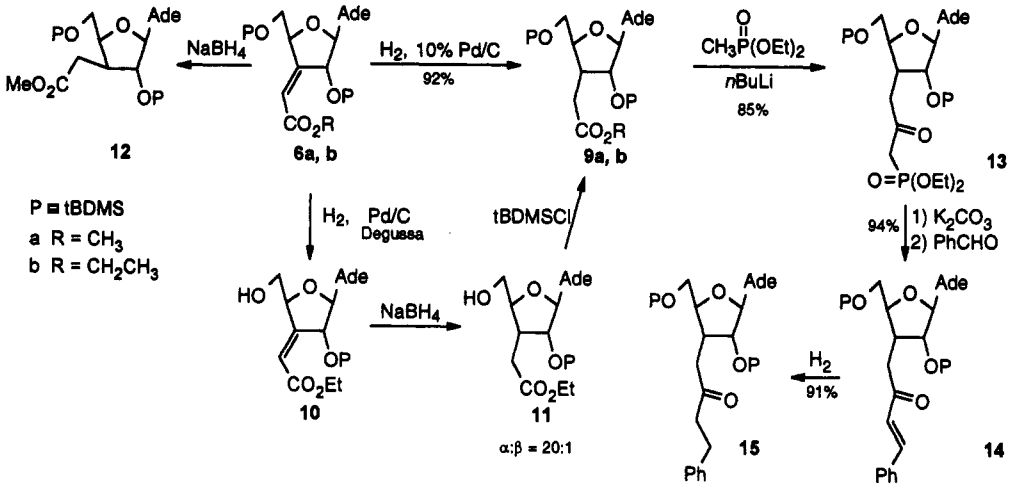
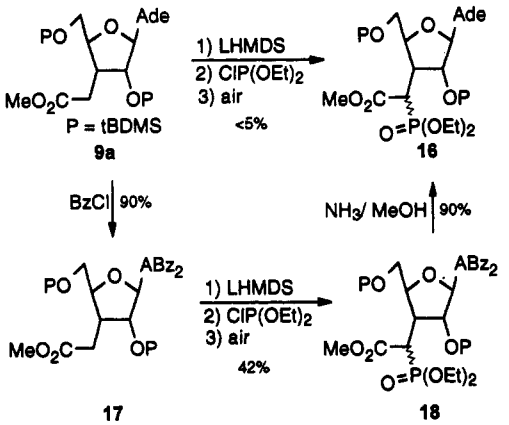
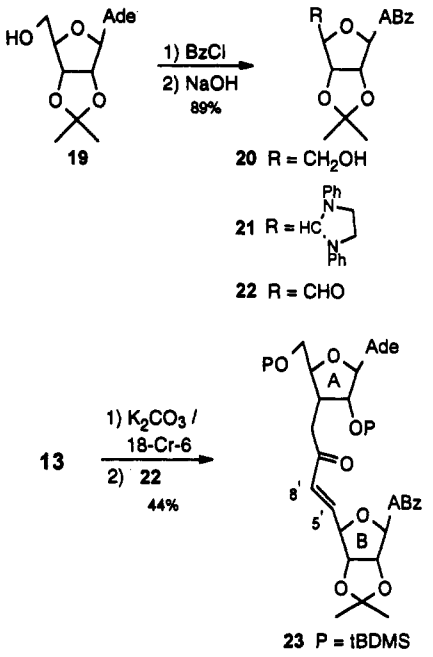
Scheme II



Scheme III



Scheme IV



has been prepared in situ from the parent 5′-alcohol **19**,[18] we chose to use aldehyde derived from 5′-imidazolidine **21**.[19] The N^6-benzoyl compound **20** was prepared from 2′,3′-*O*-isopropylideneadenosine (**19**) based on the method of Chládek and Smrt.[20] Conversion of compound **20** to the 5′-aldehyde **22** was achieved by a straightforward route involving oxidation, reaction with *N*,*N*′-diphenylethylenediamine, purification of this intermediate, and hydrolysis of protected aldehyde **21**, as described by Jones *et al.*[19]

The targeted dinucleotide analogue **23** was obtained through an HWE condensation of phosphonate **13** with aldehyde **22** (Scheme IV). Treatment of phosphonate **13** with K_2CO_3 in the presence of 18-crown-6, followed by addition of aldehyde **22** generated from diamine **21** and used without further purification, gave the desired enone **23**. This product was obtained as a single stereoisomer, assigned a trans configuration on the basis of ^{1}H NMR data (J = 15.9 Hz). Although the yield for this final step was only 44%, this probably reflects the difficulty of working with nucleoside 5′-aldehydes because it has been shown that condensation with simple aldehydes proceeds in good yield (vide infra).

In this report we have described the synthesis of some new nucleoside phosphonates, compounds **13** and **16**, as well as a new type of dinucleotide analogue, the enone **23**. Compounds such as **23** should be of interest for the potential stability they could contribute to oligonucleotides if incorporated as 3′-terminal residues, as well as for their potential to react with nucleases through Michael addition. Furthermore, intermediates reported here can themselves be considered to be nucleotide analogues, including the 3′-methylene carboxylate **9** that can be viewed as an analogue of a 3′-nucleotide and the 3′-keto phosphonate **13** that could be viewed as an analogue of a 3′-diphosphate. Studies on the biological activity of these compounds, and synthesis of related nucleoside phosphonates, will be reported in due course.

Experimental Section

Tetrahydrofuran (THF) was distilled from sodium/benzophenone and pyridine was distilled from calcium hydride immediately prior to use. All reactions in these solvents were conducted under a positive pressure of an inert gas. Column chromatography was done on Merck grade 62A silica gel (100–200 mesh), while radial chromatography was performed with a Chromatotron apparatus

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and Merck PF254 silica gel with $CaSO_4 \cdot 0.5H_2O$. NMR spectra (^1H and ^{13}C) were recorded with $CDCl_3$ as solvent and residual $CHCl_3$ as internal standard; ^{31}P chemical shifts are reported in ppm relative to 85% H_3PO_4 (external standard). Elemental analyses were performed by Atlantic Microlab, Inc.

9-(2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-((methoxycarbonyl)methylidene)-β-D-ribofuranosyl)adenine (6a). Procedure A. A solution of ketone **1**[10] (493 mg, 1 mmol) and (carbomethoxymethylene)triphenylphosphorane (401 mg, 1.2 mmol) in THF (15 mL) was heated at reflux for 7 h. The solvent was removed in vacuo and the brown oily residue was suspended in a 1:1 mixture of EtOAc and hexane. The precipitated triphenylphosphine oxide was removed by filtration and the filtrate was concentrated. Purification by flash chromatography (silica gel, 39:1 $CHCl_3$ and CH_3OH) gave pure compound **6a** (485 mg, 88%): ^1H NMR δ 8.33 (s, 1H), 8.31 (s, 1H), 5.96 –5.93 (m, 2H), 5.90 (br s, 2H), 5.43 (m, 1H), 5.13 (dm, 1H, $J_{1',2'}$ = 7.6 Hz), 4.20 (dd, 1H, $J_{5'a,5'b}$ = 11.0 Hz, $J_{5'b,4'}$ = 1.9 Hz), 3.97 (dd, 1H, $J_{5'a,5'b}$ = 11.0 Hz, $J_{5'b,4'}$ = 2.0 Hz), 3.76 (s, 3H), 0.92 and 0.76 (2s, 18H), 0.10, 0.06, –0.13, and 0.55 (4s, 12H); ^{13}C NMR δ 165.6, 160.0, 155.8, 153.1, 150.4, 138.3, 119.3, 113.0, 85.4, 80.3, 78.5, 64.4, 51.5, 25.9 (3C), 25.3 (3C), 18.3, 17.7, –5.2, –5.5, –5.61, –5.69. Anal Calcd for $C_{25}H_{43}N_5O_5Si_2$: C, 54.61; H, 7.88; N, 12.74. Found: C, 54.42; H, 7.89; N, 12.66.

Procedure B. Sodium hydride (60% dispersion in mineral oil; 13.2 mg, 0.33 mmol) was washed with pentane and then suspended in THF (3 mL) at 0 °C. A solution of trimethyl phosphonoacetate (60.1 mg, 0.33 mmol) in THF (2 mL) was added, and the mixture was stirred for 30 min at rt. The resulting solution was cooled to 0 °C, and a solution of ketone **1** (147.9 mg, 0.3 mmol) in THF (2 mL) was added dropwise. After the mixture was stirred overnight at rt, the reaction was quenched by addition of water (1 mL). The organic layer was separated, and the aqueous layer was extracted with ether (5 mL). The combined organic extract was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (9:1 $CHCl_3$ and CH_3OH) gave a 5:1 mixture (102 mg, 62%) of **6a** and the isomeric **7a** as a single spot on TLC. Selected ^1H NMR for **7a**: δ 8.30 (s, 1H), 7.96 (s, 1H), 6.24 (br s, 2H), 6.13 (br s, 1H), 6.03 (m, 1H), 5.80 (br s, 1H), 4.93 (m, 1H), 3.69 (s, 3H).

9-(2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-((methoxycarbonyl)methylene)-β-D-ribofuranosyl)adenine (9). A mixture of **6a** (549 mg, 1 mmol) and 10% Pd–C (825 mg) in CH_3OH (10 mL) was stirred for 3 days under H_2. After completion of the reaction, the solution was filtered through Celite and the pad was rinsed with CH_3OH. The filtrate was concentrated in vacuo and the residue was suspended in CH_2Cl_2 (20 mL) with vigorous stirring. The insoluble solid was removed by filtration and the filtrate was concentrated in vacuo to give compound **9a** as a white solid (484 mg, 88%): ^1H NMR δ 8.34 (s, 1H), 8.31 (s, 1H), 6.02 (d, 1H, $J_{1',2'}$ = 1.0 Hz), 5.83 (br s, 2H), 4.64 (dd, 1H, $J_{1',2'}$ = 1.0 Hz, $J_{2',3'}$ = 4.9 Hz), 4.08 (d, 1H, $J_{3',4'}$ = 9.6 Hz), 4.08 (dd, 1H), 3.77 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'b,4'}$ = 3.3 Hz), 3.63 (s, 3H), 2.82–2.74 (m, 1H), 2.69 (dd, 1H, $J_{6'a,6'b}$ = 16.4 Hz, $J_{6'a,3'a}$ = 9.6 Hz), 2.37 (dd, 1H, $J_{6'a,6'b}$ = 16.4 Hz, $J_{6'b,3'a}$ = 4.3 Hz), 0.93 and 0.90 (2s, 18H), 0.03, 0.21, 0.11, and 0.12 (4s, 12H); ^{13}C NMR δ 172.2, 155.2, 152.7, 149.4, 138.8, 120.0, 90.6, 84.2, 77.8, 62.5, 51.7, 38.1, 29.5, 26.0 (3C), 25.7 (3C), 18.5, 18.0, –4.4, –5.3, –5.5, –5.6. Anal Calcd for $C_{25}H_{45}N_5O_5Si_2$: C, 54.41; H, 8.22; N, 12.69. Found: C, 54.38; H, 8.00; N, 12.63.

The ethyl derivative **9b** was prepared from **6b**[12] in the same manner as above: selected ^1H NMR δ 4.13–4.06 (m, 4H, H-4', H-5'a, $-CO_2CH_2-$), 1.22 (t, 3H, $-CO_2CH_2CH_3$, J = 7.2 Hz).

9-(2-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-((ethoxycarbonyl)methylidene)-β-D-ribofuranosyl)adenine (10). A mixture of **6b** (140.5 mg, 0.25 mmol) and 5% Degussa-type Pd–C (60 mg) in CH_3OH (1 mL) was stirred overnight under H_2. The resulting solution was filtered, the catalyst was rinsed with CH_3OH, and the filtrate was concentrated in vacuo. Purification by flash chromatography (19:1 $CHCl_3$:CH_3OH) gave unreacted **6b** (56 mg) and deprotected compound **10** (63 mg, 56%, 90% based on recovered starting material): ^1H NMR δ 8.31 (s, 1H), 7.78 (s, 1H), 6.38 (br s, 2H), 5.90 (dd, 1H, J = 2.3, 2.1 Hz), 5.57–5.47 (m, 3H), 4.18 (q, 2H, J = 7.1 Hz), 4.06 (br d, 1H, J = 11.5 Hz), 3.98 (dd, 1H, $J_{5'a,5'b}$ = 11.5 Hz, $J_{5'b,4'}$ = 1.9 Hz), 1.29 (t, 3H, J = 7.1 Hz), 0.78 (s, 9H), –0.12 and –0.60 (2s, 6H). Anal Calcd for $C_{20}H_{33}N_5O_5Si$: C, 53.19; H, 7.37; N, 15.51. Found: C, 53.38; H, 7.34; N, 15.71.

Preparation of 9b from 10. Ester **10** (45 mg, 0.1 mmol) was dissolved in CH_3OH (1 mL), $NaBH_4$ (38 mg, 1.0 mmol) was added, and the mixture was stirred for 3 h at rt. The resulting solution was quenched by addition of a few drops of 50% aqueous acetic acid, diluted with EtOAc (5 mL), and washed with brine (1 mL). After treatment with $MgSO_4$ and concentration in vacuo, the resulting white solid was dissolved in pyridine (0.5 mL) containing tBDMSCl (22.5 mg, 0.15 mmol). The mixture was stirred overnight, and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 (3 mL), washed with water (0.5 mL), and purified by flash chromatography (1:2 EtOAc and hexane) to give a mixture of **9** and **12** (21.2 mg, 37%) in a ratio of 12:1 as measured by ^1H NMR. The major component was identical with compound **9b** prepared by direct hydrogenation of **6b**.

9-(2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-((methoxycarbonyl)methylene)-β-D-xylofuranosyl)adenine (12). Ester **9a** (110 mg, 0.2 mmol) was dissolved in CH_3OH (2 mL), $NaBH_4$ (76 mg, 2.0 mmol) was added, and the mixture was stirred for 48 h at rt. The resulting solution was quenched by addition of 1 N acetic acid in ether (0.5 mL) and the solvent was removed in vacuo. The residue was suspended in CH_2Cl_2 (10 mL) with vigorous stirring and the insoluble solid was removed by filtration through Celite. The filtrate was concentrated in vacuo to give a mixture of unreacted **9a**, saturated ester **12**, and alcohol derivatives[12] as observed by ^1H NMR. Purification by flash chromatography (silica gel, 39:1 to 19:1 $CHCl_3$ and CH_3OH) gave pure compound **12** (23 mg, 21%): ^1H NMR δ 8.33 (s, 1H), 8.23 (s, 1H), 5.95 (d, 1H, $J_{1',2'}$ = 6.5 Hz), 5.88 (br s, 2H), 4.58 (dd, 1H, $J_{1',2''}$ = 6.5 Hz, $J_{2',3'}$ = 9.5 Hz), 4.44 (dm, 1H, $J_{3',4'}$ = 8.1 Hz), 3.92 (dd, 1H, $J_{5'a,5'b}$ = 11.8 Hz, $J_{5'b,4'}$ = 3.1 Hz), 3.70 (dd, 1H, $J_{5'b,4'}$ = 2.1 Hz), 3.69 (s, 3H), 3.00–2.89 (m, 1H), 2.74 (dd, 1H, $J_{6'a,6'b}$ = 16.7 Hz, $J_{6'a,3'}$ = 10.6 Hz), 2.37 (dd, 1H, $J_{6'a,6'b}$ = 16.7 Hz, $J_{6'b,3'}$ = 4.9 Hz), 0.96, 0.74 (2s, 18H), 0.15, 0.13, –0.12, –0.59 (4s, 12H); ^{13}C NMR δ 172.5, 155.4, 153.1, 150.5, 138.9, 119.5, 87.5, 79.5, 78.4, 63.7, 51.9, 43.9, 31.7, 26.1 (3C), 25.5 (3C), 18.4, 17.6, –4.8, –5.3, –5.45, –5.48. Anal. Calcd for $C_{25}H_{45}N_5O_5Si_2$: C, 54.41; H, 8.22; N, 12.69. Found: C, 54.59; H, 8.00; N, 12.68.

9-(2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-((((diethoxyphosphinyl)methyl)carbonyl)methylene)-β-D-ribofuranosyl)adenine (13). To a solution of diethyl methylphosphonate (183 mg, 1.2 mmol) in THF (3 mL) was added dropwise a solution of *n*-BuLi in hexane (2.5 N, 5.2 mL) at –78 °C, and the mixture was stirred for a further 40 min. The resulting solution was transferred to a cooled solution of ester **9a** (22.4 mg, 0.4 mmol) via cannula at –78 °C, and the mixture was allowed to warm to rt over 2 h. After the reaction was quenched by addition of 1 N acetic acid in ether (2 mL), the resulting suspension was filtered through Celite. The filtrate was concentrated in vacuo and purified by flash chromatography on silica gel (19:1 $CHCl_3$ and hexane) to yield the desired phosphonate **13** (229 mg, 85%): ^1H NMR δ 8.32 (s, 1H), 8.30 (s, 1H), 6.00 (d, 1H, $J_{1',2'}$ = 1.7 Hz), 5.86 (br s, 2H), 4.67 (dd, 1H, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 4.6 Hz), 4.16–4.02 (m, 6H), 3.75 (dd, 1H, $J_{5'a,5'b}$ = 12.3 Hz, $J_{5'b,4'}$ = 3.1 Hz), 3.05 (dd, 1H, $J_{7'a,P}$ = 40.9 Hz, $J_{7'a,7'b}$ = 22.8 Hz), 3.05 (dd, 1H, $J_{7'b,P}$ = 68.1 Hz, $J_{7'a,7'b}$ = 22.8 Hz), 1.30 (td, 6H, J_{HP} = 2.1 Hz, J = 7.1 Hz), 0.92 and 0.86 (2s, 18H), 0.14, 0.11, 0.10, and –0.03 (4s, 12H); ^{13}C NMR δ 200.0 (J_{CP} = 6.1 Hz), 155.3, 152.7, 149.5, 138.8, 119.9, 90.3, 84.4, 77.6, 62.7, 62.6 (d, J_{CP} = 6.9 Hz), 62.5 (J_{CP} = 6.9 Hz), 42.7 (d, J_{CP} = 127.4 Hz), 39.7, 37.0, 26.0 (3C), 25.7 (3C), 18.5, 17.9, 16.4, 16.3, –4.6, –5.4 (3C); ^{31}P NMR ($CDCl_3$) +20.9. Anal. Calcd for $C_{29}H_{54}N_5O_7PSi_2$: C, 51.84; H, 8.10; N, 10.42. Found: C, 51.85; H, 8.14; N, 10.34.

Horner–Wadsworth–Emmons Condensation of Phosphonate 13 with Benzaldehyde. A mixture of compound **13** (67 mg, 0.1 mmol), 18-crown-6 (27 mg, 0.11 mmol), and potassium carbonate (152 mg, 1.1 mmol) in THF (2 mL) was stirred for 1 h at rt. A solution of benzaldehyde (16 mg, 0.15 mmol) in THF (0.3 mL) was added dropwise and the resulting mixture was stirred overnight at rt. After the reaction was quenched by addition of a few drops of saturated aqueous NH_4Cl, the resulting solution was filtered through a $MgSO_4$ pad. Concentration of the filtrate, followed by purification by radial chromatography (39:1 $CHCl_3$ and CH_3OH) gave enone **14** (58.5 mg, 94%): ^1H NMR δ 8.34 (s, 1H), 8.32 (s, 1H), 7.54 (d, 1H, $J_{8',9'}$ = 16.3 Hz), 7.54–7.37 (m, 5H),

6.71 (d, 1H, $J_{8',9'}$ = 16.3 Hz), 6.05 (d, 1H, $J_{1',2'}$ = 1.5 Hz), 6.03 (br s, 2H), 4.74 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, $J_{2',3'}$ = 4.8 Hz), 4.13 (dt, 1H, $J_{3',4'}$ = 8.9 Hz, $J_{4',5'}$ = 2.6 Hz), 4.03 (dd, 1H, $J_{5'a,5'b}$ = 11.5 Hz, $J_{5'a,4'}$ = 2.7 Hz), 3.77 (dd, 1H, $J_{5'a,5'b}$ = 11.5 Hz, $J_{5'b,4'}$ = 2.6 Hz), 3.11 (dd, 1H, $J_{6'a,6'b}$ = 17.6 Hz, $J_{6'a,3'}$ = 8.7 Hz), 2.99–2.90 (m, 1H), 2.74 (dd, 1H, $J_{6'a,6'b}$ = 17.6 Hz, $J_{6'b,3'}$ = 4.6 Hz), 0.92 and 0.86 (2s, 18H), 0.16, 0.11, and –0.05 (3s, 12H). Anal. Calcd for $C_{32}H_{49}N_5O_4Si_2$: C, 61.59; H, 7.92; N, 11.22. Found: C, 61.54; H, 8.02; N, 11.14.

Catalytic Hydrogenation of 14. A mixture of compound **14** (58.5 mg) and 10% Pd–C (10 mg) in CH_3OH (1 mL) was stirred for 2 h under H_2. After completion of the reaction, the solution was filtered through Celite and the catalyst was rinsed with CH_3OH. The combined filtrate was concentrated in vacuo and the residue was purified by radial chromatography (19:1 $CHCl_3$ and CH_3OH) to give compound **15** as a white solid (53.4 mg, 91%, 86% overall): ^{1}H NMR δ 8.31 (s, 1H), 8.29 (s, 1H), 7.27–7.12 (m, 5H), 6.08 (br s, 2H), 5.99 (d, 1H, $J_{1',2'}$ = 1.5 Hz), 4.67 (m, 1H), 4.02–3.98 (m, 2H), 3.75 (dd, 1H, $J_{5'a,5'b}$ = 12.4 Hz, $J_{5'b,4'}$ = 3.4 Hz), 2.89–2.40 (m, 7H), 0.91 and 0.86 (2s, 18H), 0.16, 0.09, 0.09, and –0.05 (4s, 12H); ^{13}C NMR δ 207.4, 155.4, 152.7, 149.5, 140.7, 138.7, 128.5 (2C), 128.2 (2C), 126.2, 119.9, 90.4, 84.3, 77.6, 62.9, 44.3, 38.3, 37.4, 29.6, 26.0 (3C), 25.7 (3C), 18.5, 17.9, -4.5, –5.4, –5.5 (2C). Anal. Calcd for $C_{32}H_{51}N_5O_4Si_2$: C, 61.40; H, 8.21; N, 11.19. Found: C, 61.63; H, 8.27; N, 11.16.

6-*N*,*N*-Dibenzoyl-9-(2,5-bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-((methoxycarbonyl)methylene)-β-D-ribofuranosyl)adenine (17). Benzoyl chloride (211 mg, 1.5 mmol) was added to a stirred solution of compound **9a** (225 mg, 0.5 mmol) in pyridine (3 mL), and the mixture was stirred for a further 5 h at rt. After the solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (6 mL) and washed with brine (1 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash chromatography (silica gel, 39:1 $CHCl_3$ and CH_3OH) gave compound **17** (350 mg, 92%): ^{1}H NMR δ 8.63 (s, 1H), 8.50 (s, 1H), 7.87–7.32 (m, 10H), 6.05 (d, 1H, $J_{1',2'}$ = 1.0 Hz), 4.74 (d, 1H, $J_{2',3'}$ = 3.5 Hz), 4.17–4.02 (m, 1H), 4.03 (dd, 1H, $J_{5'a,5'b}$ = 11.6 Hz, $J_{5'a,4'}$ = 2.9 Hz), 3.78 (dd, 1H, $J_{5'a,5'b}$ = 11.6 Hz, $J_{5'b,4'}$ = 3.0 Hz), 3.66 (s, 3H), 2.81–2.73 (m, 1H), 2.71 (dd, 1H, $J_{6'a,6'b}$ = 15.6 Hz, $J_{6'a,3'a}$ = 9.5 Hz), 2.39 (dd, 1H, $J_{6'a,6'b}$ = 15.6 Hz, $J_{6'b,3'a}$ = 3.6 Hz), 0.90 (s, 18H), 0.16, 0.09, 0.04 (3s, 12H); ^{13}C NMR δ 172.2, 172.0, 152.4, 151.9, 151.5, 143.2, 134.2, 132.7, 129.4, 128.6, 128.1, 90.9, 84.5, 77.6, 62.8, 51.7, 38.7, 29.6, 26.0 (3C), 25.7 (3C), 18.4, 17.9, –4.5, –5.4, –5.5, –5.6. Anal. Calcd for $C_{39}H_{53}N_5O_7Si_2$: C, 61.63; H, 7.03; N, 9.21. Found: C, 62.04; H, 7.25; N, 9.28.

Preparation of Phosphono Ester 18. To a stirred solution of ester **17** (190 mg, 0.25 mmol) in THF (1.5 mL) was added dropwise 1 N lithium hexamethyldisilizide in THF (0.275 mL, 0.275 mmol) at –78 °C. After the solution was stirred for 1.5 h, HMPA (0.047 mL) was added and the mixture was stirred for an additional 30 min. Diethyl phosphorochloridite (0.041 mL, 0.275 mmol) was added dropwise to the resulting enolate, and the reaction mixture was allowed to warm to rt over 2 h. The reaction was quenched by addition of acetic acid in ether (1 N, 0.3 mL) and the mixture was filtered through Celite. After concentration to a small volume, the oily residue was stirred overnight in a reaction vessel open to air. Purification by flash chromatography (silica gel, 1:4 to 1:1 EtOAc and hexane) afforded unreacted **17** (52 mg, 27% recovery) and compound **18** (93 mg, 42%) as a mixture of phosphonate diastereomers in a ratio of approximately 4:1 as measured by ^{1}H NMR: ^{1}H NMR major diastereomer, δ 8.60 (s, 1H), 8.44 (s, 1H), 7.83–7.28 (m, 10H), 6.02 (d, 1H, $J_{1',2'}$ = 5.9 Hz), 4.93 (m, 1H), 4.70 (dd, 1H, $J_{1',2'}$ = 5.9 Hz, $J_{2',3'}$ = 8.2 Hz), 4.19–4.08 (m, 4H), 3.97 (dd, 1H, $J_{5'a,5'b}$ = 11.2 Hz, $J_{5'a,4'}$ = 1.5 Hz), 3.81 (s, 3H), 3.77 (dd, 1H, $J_{5'a,5'b}$ = 11.2 Hz, $J_{5'b,4'}$ = 2.4 Hz), 3.43 (dd, 1H, J_{HP} = 24.3 Hz, $J_{6',3'a}$ = 4.1 Hz), 3.07–2.99 (m, 1H), 1.29 (dt, 6H, J_{HP} = 7.1 Hz, J = 7.1 Hz), 0.92 and 0.75 (2s, 18H), –0.03, –0.04, –0.13, and –0.46 (4s, 12H); ^{13}C NMR δ 172.1, 168.9 (d, J_{CP} = 8.6 Hz), 153.2, 152.1, 151.5, 143.1, 134.1, 132.7, 129.3, 128.5, 88.5, 81.6 (d, J_{CP} = 4.0 Hz), 77.8 (d, J_{CP} = 11.6 Hz), 64.7, 62.9 (d, 2C, J_{CP} = 6.7 Hz), 52.5, 41.7 (d, J_{CP} = 133.3 Hz), 40.4, 26.0 (3C), 25.4 (3C), 18.4, 17.7, 16.3 (d, 2C, J_{CP} = 5.1 Hz), –5.3 (2C), –5.49, –5.52; ^{31}P NMR ($CDCl_3$) +22.3; HR FAB MS calcd for $C_{43}H_{63}N_5O_{10}PSi_2$ 896.3854 (M^+ + H), found 896.3859.

Preparation of Phosphono Ester 16. A solution of compound **18** (67.1 mg, 0.075 mmol) in CH_3OH (1 mL) saturated with ammonia was stirred overnight at rt. The solvent was removed in vacuo to give a white foam (69.8 mg, 100%) consisting of 2:1 mixture of benzamide and deprotected phosphonate **16** as observed by ^{1}H NMR. Purification by flash chromatography (4:1 EtOAc and hexane) afforded phosphonate **16** as a mixture of diastereomers (46.2 mg, 90%): ^{1}H NMR major diastereomer, δ 8.28 (s, 1H), 8.27 (s, 1H), 6.07 (br s, 2H), 5.95 (d, 1H, $J_{1',2'}$ = 5.2 Hz), 4.84 (m, 1H), 4.71 (dd, 1H, $J_{1',2'}$ = 5.2 Hz, $J_{2',3'}$ = 7.6 Hz), 4.18–4.06 (m, 4H), 3.87 (dd, 1H, $J_{5'a,5'b}$ = 11.2 Hz, $J_{5'a,4'}$ = 1.6 Hz), 3.79 (s, 3H), 3.71 (dd, 1H, $J_{5'a,5'b}$ = 11.2 Hz, $J_{5'b,4'}$ = 2.2 Hz), 3.44 (dd, 1H, J_{HP} = 23.8 Hz, $J_{6',3'a}$ = 4.6 Hz), 3.05–2.98 (m, 1H), 1.27 (dt, 6H, J_{HP} = 10.2 Hz, J = 7.1 Hz), 0.93 and 0.78 (2s, 18H), 0.12, 0.10, –0.11, and –0.32 (3s, 12H); ^{13}C NMR δ 168.8 (d, J_{CP} = 5.0 Hz), 155.4, 152.9, 150.1, 138.9, 119.5, 88.6, 81.7 (d, J_{CP} = 5.6 Hz), 78.1 (d, J_{CP} = 10.6 Hz), 64.7, 62.9 (d, 2C, J_{CP} = 6.1 Hz), 52.5, 41.d (d, J_{CP} = 133.3), 40.5, 26.0 (3C), 25.5 (3C), 18.4, 17.8, 16.3 (d, 2C, J_{CP} = 5.6 Hz), –5.34, –5.39 (2C), -5.46; ^{31}P NMR ($CDCl_3$) +23.5; HR FAB MS calcd for $C_{29}H_{55}N_5O_8PSi_2$ 688.3327 (M^+ + H), found 688.3316.

Preparation of Enone 23. Dowex 50X8 resin (150 mg) was added to a solution of imidazolidine **21**[19] (100 mg, 0.17 mmol) in 50% aqueous THF (1.5 mL), and the mixture was stirred for 1 h at rt. The resin was removed by filtration and the filtrate was concentrated in vacuo and then dried under high vacuum overnight at 80 °C. The resulting foamy solid **22** was added in one portion to a stirred mixture of phosphonate **13** (33.6 mg, 0.05 mmol), oven-dried K_2CO_3 (11.4 mg), and 18-crown-6 (21.8 mg) in THF (1 mL), and then the mixture was stirred overnight at rt. The resulting solution was diluted with EtOAc (3 mL) and washed with brine (0.5 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated in vacuo to give a yellow gum. Purification by radial chromatography (4:1 EtOAc and hexane, followed by 39:1 $CHCl_3$ and CH_3OH) afforded dinucleotide analogue **23** as a pale yellow solid (20.4 mg, 44%): ^{1}H NMR δ 9.12 (br s, 1H), 8.77 (s, 1H), 8.30 (s, 1H), 8.28 (s, 1H), 8.08 (s, 1H), 8.01–7.48 (m, 5H), 6.87 (dd, 1H, $J_{B5',A8'}$ = 15.9 Hz, $J_{B5',B4'}$ = 5.9 Hz), 6.20 (dd, 1H, $J_{B5',A8'}$ = 15.9 Hz, $J_{A8',B4'}$ = 1.4 Hz), 6.17 (d, 1H, $J_{1',2'}$ = 2.0 Hz), 6.00 (d, 1H, $J_{1',2'}$ = 1.5 Hz), 5.74 (br s, 2H), 5.51 (dd, 1H, $J_{2',3'}$ = 6.3 Hz, $J_{1',2'}$ = 2.0 Hz), 5.10 (dd, 1H, $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 4.2 Hz), 4.78 (m, 1H), 4.65 (dd, 1H, $J_{2',3'}$ = 4.8 Hz, $J_{1',2'}$ = 1.5 Hz), 4.01 (m, 2H), 3.73 (dd, 1H, $J_{5'a,5'b}$ = 11.2 Hz, $J_{5'b,4'}$ = 3.4 Hz), 2.80–2.95 (m, 2H), 2.47 (dd, 1H, $J_{6'a,6'b}$ = 17.3 Hz, $J_{6'b,3'}$ = 3.6 Hz), 1.64 and 1.40 (2s, 6H), 0.88 and 0.85 (2s, 18H), 0.14, 0.07, 0.06, and –0.09 (4s, 12H). Anal. Calcd for $C_{45}H_{62}N_{10}O_9Si_2 \cdot 2H_2O$: C, 56.11; H, 6.91; N, 14.54. Found: C, 56.29; H, 6.70; N, 14.39.

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