Reactions of 3′-*C*-Halomethyl and 3′-*C*-Sulfonylmethyl Uridines with Phosphinic Acid Derivatives − Synthesis of Building Blocks for Oligonucleotides Containing 3′-*C*-Methylenephosphonate Linkages

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An efficient method for the synthesis of nucleoside 3′-*C*-methylenephosphinates, building blocks for oligo(ribonucleoside methylenephosphonate)s, has been developed. Treatment of bis(trimethylsilyl)hypophosphite (BTSP) with a 3′-deoxy-3-*C*-(iodomethyl)uridine resulted both in substitution to give the corresponding 3′-*C*-methylenephosphinate and in reduction of the iodomethyl group to afford the uridine 3′-deoxy-3′-*C*-methyl derivative. Optimisation of solvent and temperature gave a substitution/reduction ratio of 5:4 at best. Through the use of a trifluoromethanesulfonyl leaving group and further optimisation of reaction conditions, however, the triethylammonium 2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytriphenylmethyl)uridine-3′-*C*-methylenephosphinate was obtained in 93% isolated yield.

Introduction

In the past decade there has been a tremendous increase in interest in modified nucleic acid fragments. This is largely due to the development of antisense therapy[1−3] and the use of modified oligonucleotides (and nucleosides) in investigations of enzymatic mechanisms, especially in studies on catalytic RNA.[4−6] As a part of our program on the synthesis of modified oligonucleotides we are investigating 3′-*C*-branched analogues. An interesting minimal modification that introduces 3′-*C*-branching is internucleoside 3′-*C*-methylenephosphonate (MP) linkages, with the 3′-oxygen of the phosphodiester linkage replaced by a methylene group (Figure 1). After demonstrating efficient coupling of methylphosphinate with a nucleoside to give the corresponding phosphonate and thiophosphonate,[7] we considered a nucleoside 3′-*C*-methylenephosphinate as a potential building block for MP linkages. During the course of our studies, oligodeoxyribonucleotides containing MP linkages at selected positions were reported, albeit in little detail, with the indication that this modification stabilizes the complex with a target RNA.[8] Little is currently known about the effect of MP linkages in oligodeoxyribonucleotides, and the corresponding oligoribonucleotide analogues have to the best of our knowledge, not been studied. The development of a methodology to produce these analogues should probably also be extendable to 2′-*O*-alkyl derivatives, which are of interest as potential antisense drugs.

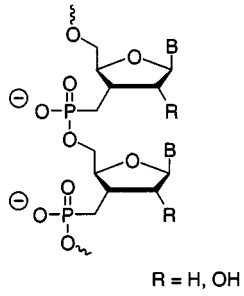

Figure 1. Internucleoside linkages with the 3′-oxygen replaced with a methylene group in the phosphodiester backbone

To investigate oligoribonucleotides in which the 3′-oxygens are replaced by methylene groups, the development of an efficient method for synthesis of building blocks was necessary. In analogy with the H-phosphonate approach,[9−14] phosphinate derivatives can be coupled with alcohols and can subsequently be oxidised to give the corresponding phosphonate derivatives.[7,8] Collingwood et al.[8] have reported on the synthesis of a phosphinate building block for the synthesis of oligodeoxyribonucleotide analogues. A temporary acid-labile protection of the phosphinate centre was used in the synthesis, which required additional deprotection and 5′-hydroxyl reprotection steps prior to oligomer synthesis. An et al.[15,16] have since also reported on the synthesis of phosphinate building blocks from the corresponding iodomethyl derivatives;[15,16] only low yields (15 to 37%) were obtained, however. In this paper, substitution of 3′-*C*-halomethyl and 3′-*C*-(sulfonylmethyl)uridines with some phosphinic acid derivatives is investigated. These studies enabled us to develop an efficient

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synthesis of triethylammonium 2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytriphenylmethyl)uridine 3′-*C*-methylenephosphinate.

Results and Discussion

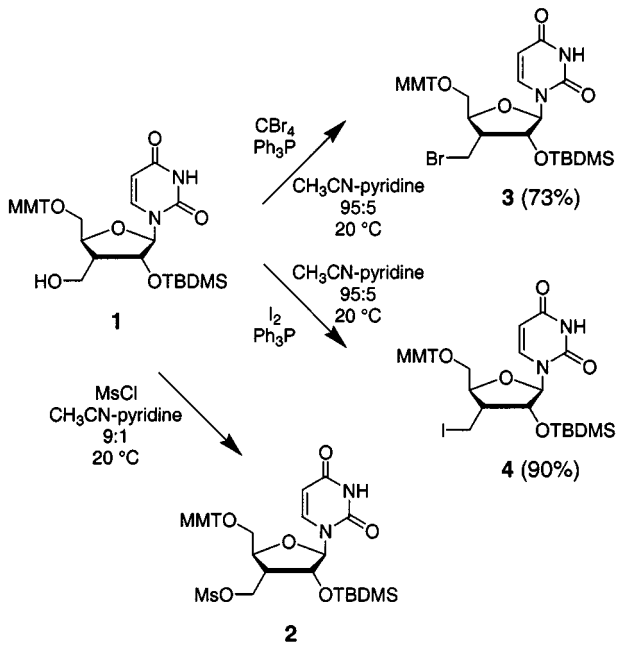
Few substitution methods with phosphinic acid derivatives as nucleophiles have been developed. Substitution has mostly been carried out with alkyl halides, in particular bromides and iodides.[17,18] As leaving groups we decided to explore not only halides, but also sulfonates such as methanesulfonate (mesylate) and trifluoromethanesulfonate (triflate). Nucleoside derivatives already bearing the 5′-*O*-(4-methoxytriphenylmethyl) (MMT) and 2′-*O*-(*tert*-butyldimethylsilyl) (TBDMS) protecting groups, which are the most commonly used in RNA synthesis, were selected. Of the nucleophiles that have been evaluated in the past, we considered the investigation of the use of salts of isopropyl[18] and *tert*-butyl phosphinate,[19] as well as bis(trimethylsilyl)hypophosphite[20] (BTSP). Initially, reactions were carried out with potassium salts of alkyl phosphinates at room temperature in THF. The potassium salts of isopropyl and *tert*-butyl phosphinate were not stable and disproportionated. In addition, the reactions with halides resulted mostly in reduction, and incomplete consumption of the halide affording less than 5% of the desired product.

Synthesis of 3′-*C*-Branched Uridine Precursors with Different Leaving Groups: Mesylate, Iodo and Bromo

We have recently developed an efficient method for the synthesis of 1-[2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-*C*-(hydroxymethyl)-5-*O*-(4-methoxytriphenylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (**1**).[21,22] This and similar 3′-*C*-hydroxymethyl nucleosides had previously been converted into the corresponding halomethyl[8,15,16,22] and methanesulfonyl[21,22] derivatives for the synthesis of variously functionalised 3′-*C*-branched uridine analogues. We initially considered the previously reported mesylate derivative **2**, and also the bromo and iodo derivatives **3** and **4**, which were synthesized from hydroxymethyl derivative **1**. Treatment of **1** with triphenylphosphane and carbon tetrabromide in acetonitrile/pyridine (95:5, v/v) afforded bromo derivative **3** (73%). Similarly, treatment of **1** with triphenylphosphane and iodine in acetonitrile/pyridine (95:5, v/v) gave the iodo derivative **4** (90%).

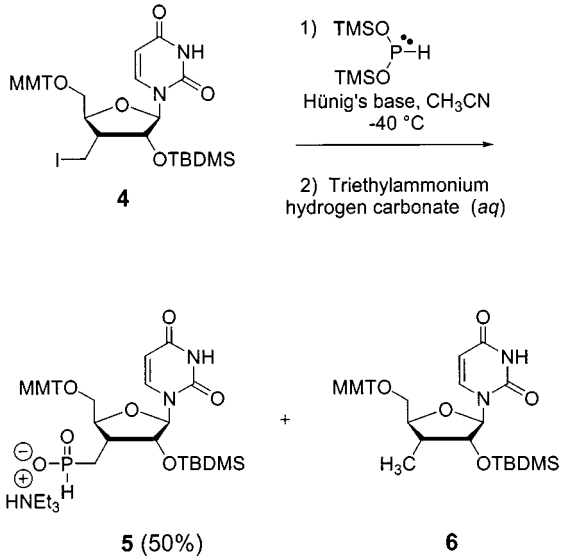
Treatment of Derivatives 2, 3 and 4 with BTSP

Bis(trimethylsilyl)hypophosphite[17,20,23] (BTSP) has previously been used for substitution in various alkyl halides,[8,15,16,24,25] and we first investigated nucleoside derivatives **2**, **3** and **4** (Scheme 1). Treatment with BTSP was carried out in the presence of base (Hünig's base, diisopropylethylamine) to avoid loss of the MMT group.



Scheme 1

The mesylate and bromo derivatives gave no detectable product on treatment with BTSP (**2** or **3** were treated with 0.3 M BTSP in acetonitrile at room temperature for 20 hours). TLC and NMR analysis showed only remaining starting material. The iodo derivative **4** was considerably more reactive, but compound **6** was formed along with the phosphinate derivative **5** (Scheme 2). This kind of competing reduction by BTSP has been reported previously.[15,16,26]



Scheme 2

In order to assist substitution over reduction, the influence of different solvents and temperatures was investigated for the iodo derivative **4**. The polarity of the solvent ranged

from toluene to acetonitrile, and the temperature was varied between room temperature and −42 °C.

In general, a polar solvent at low temperature favoured substitution, to give less formation of **6**. The ratio of **5** to **6** at 0 °C in toluene or dichloromethane, for example, was 14:86, in pyridine 18:82 and in acetonitrile 31:69. In acetonitrile at 20 °C the ratio was 19:81, whereas at −42 °C it was 56:44. In THF at 20 °C the ratio was 5:95, and at 0 °C 10:90. The best conditions (use of 0.3 M BTSP in acetonitrile at −42 °C) gave **5** in 50% isolated yield. Other attempts to affect the selectivity, by addition of various salts (sodium iodide and cadmium tosylate) or a radical scavenger, had little effect.

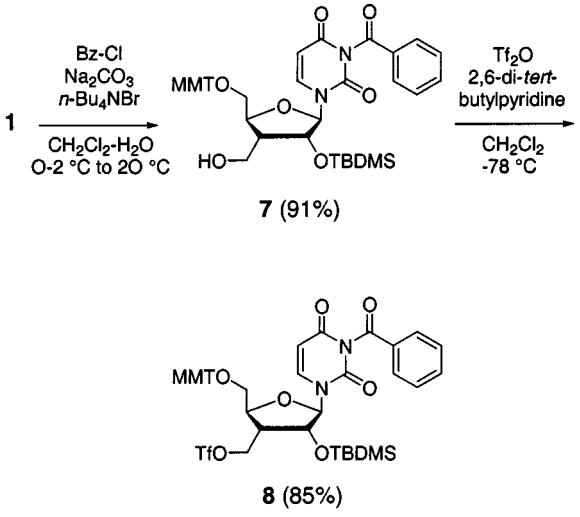
Finally, **4** was treated with neat BTSP at −42 °C. Products **5** and **6** were again obtained, in a ratio of 56:44 (entry 14). Thus, the use of neat BTSP at −42 °C is an alternative to acetonitrile, since the selectivity is similar. To improve the yield of phosphinate **5** further, by avoiding the competing reduction, we turned to a more reactive sulfonyl leaving group.

Synthesis of a 3′-*C*-Branched Uridine with a Triflate Leaving Group

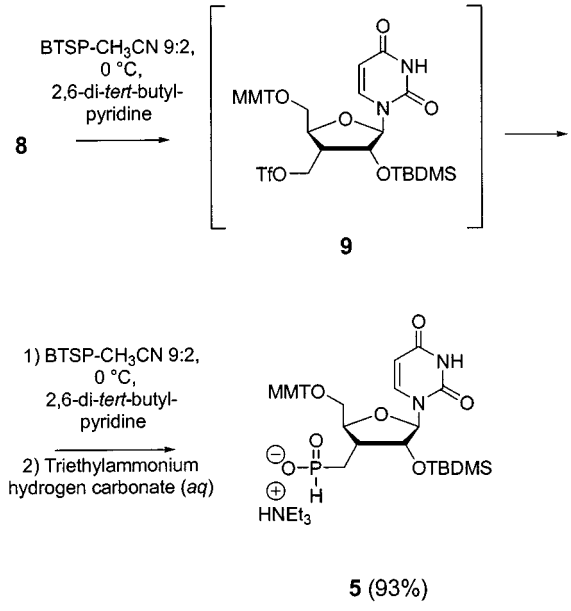
When **1** was treated with triflic anhydride (1.3 equiv.) in dichloromethane at −78 °C in the presence of 2,6-di-*tert*-butylpyridine, both the uracil moiety and the hydroxymethyl group were sulfonylated, thus producing a mixture of three products and remaining starting material. The N^3-position in the uracil moiety was therefore protected with a benzoyl group prior to treatment with triflic anhydride. Benzoylation was performed essentially according to the phase-transfer procedure of Sekine,[27] to give derivative **7** in 91% yield (Scheme 3). Treatment of **7** with triflic anhydride and 2,6-di-*tert*-butylpyridine in dichloromethane at −78 °C gave compound **8** (85%).

Treatment of Triflate 8 with BTSP

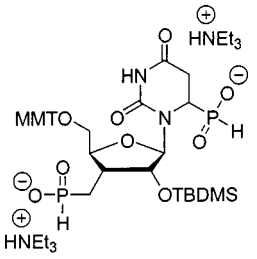
Treatment of triflate **8** with BTSP was carried out in the presence of 2,6-di-*tert*-butylpyridine, to avoid loss of the MMT group. Treatment of triflate **8** with BTSP in acetonitrile caused loss of the N^3-benzoyl group in situ. The cleavage reaction was significantly faster than the substitution, and the de-N^3-benzoylated compound **9** was observed as an intermediate (identified by MS) that reacted further to afford phosphinate **5** (Scheme 4). Triflate **8** was less reactive than the iodide **4** and required a longer reaction time. Treatment of **8** with 0.3 M BTSP in acetonitrile at 20 °C for 20 h. in the presence of 2,6-di-*tert*-butylpyridine (6 equiv.) afforded phosphinate **5** in an isolated yield of 52%. Several minor by-products were formed, one of which was probably the *C*-phosphinate-linked nucleoside dimer, as judged by MS analysis. Some of the other unidentified side products were possibly caused by degradation of remaining **9** during workup. An extended reaction time did not improve the yield, which may have been due, at least partially, to decomposition of BTSP. Attempts to carry out the reaction with an increased (double) concentration of BTSP (0.6 M in acetonitrile) resulted in the formation of an additional product. MS and ^{1}H NMR analyses of this by-product were consistent with structure **10**, which could be formed by Michael addition of BTSP to the uracil double bond of **5**. Unlike reactions with thiosulfate,[28,29] this reaction was not reversible under basic conditions. Addition either of Hünig's base (6 equiv.) or of DBU (6 equiv.) to the above reaction mixtures prior to quenching with triethylammonium bicarbonate (aq.) did not influence the ratio of products within 1 hour.



Scheme 3



Scheme 4



10

We finally explored whether the use of a lower temperature would reduce the various side reactions that limited the yield of the reaction. A successful approach was indeed achieved by cooling the reaction mixture and using a high concentration of BTSP, the latter ensuring a reasonable reaction time. Treatment with **8** was performed in BTSP/acetonitrile (9:2, v/v) at 0 °C for 50 h, in the presence of 2,6-di-*tert*-butylpyridine (6 equiv.). The reaction proceeded smoothly and was virtually quantitative, giving the phosphinate **5** in a yield of 93% after purification.

Concluding Remarks

We have shown here that substitution reactions with phosphinic acid derivatives can be adjusted to reduce side reactions in the synthesis of triethylammonium 2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytriphenylmethyl)-uridine 3′-*C*-methylenephosphinate (**5**). The reactions were studied in detail, and the yield of the phosphinate has been substantially improved. For substitution of the iodomethyl derivative **4** with BTSP, a polar solvent such as acetonitrile at a low temperature (−42 °C) was most efficient in suppressing the competing side reaction (reduction), and gave **5** in a reasonable yield (50%). A bulky neighbouring protecting group (TBDMS) was present, which was probably a disadvantage in the substitution reaction. It is therefore possible that an even higher selectivity might be obtained with less hindered compounds such as 2′-deoxy or 2′-*O*-methyl nucleosides.

In addition, we have introduced the use of an alternative leaving group, triflate, in this reaction. By using the corresponding triflate derivative **8** with BTSP we avoided the competing reduction and obtained **5** in an excellent yield (93%). We now clearly have a high-yielding method for the synthesis of a 3′-*C*-methylenephosphinate monomer that can be used as building blocks for oligoribonucleotides containing methylenephosphonate linkages. This makes the use of this modification more attractive for the development of potential antisense drugs (as *O*-alkyl derivatives), for studies of enzymatic mechanisms and for co-crystallization of enzymes/ribozymes with their RNA substrates. A study to evaluate and optimise the reactions crucial for oligoribonucleotide synthesis with these building blocks (condensation with alcohols, oxidation, and potential side reactions) is currently in train in our laboratory.

Experimental Section

General Remarks and Methods: NMR spectra were recorded on a Bruker AVANCE DRX-400 instrument (400.13 MHz in ^{1}H, 100.62 MHz in ^{13}C and 162.00 MHz in ^{31}P). Chemical shifts are given in ppm, downfield from external TMS. Most ^{1}H and ^{13}C NMR spectral assignments were based on standard ^{1}H-^{1}H COSY and ^{1}H-^{13}C-HMQC experiments; uridine numbering is used. Silica gel column chromatography was carried out on Matrex silica, 60Å (35−70 μm, Amicron). TLC analysis was carried out on precoated plates, Silica Gel 60 F_{254} (Merck), with detection by UV light and/or by charring with 8% sulfuric acid in methanol. Solutions were concentrated under reduced pressure at temperatures not exceeding 40 °C. Reagents and solvents were of ordinary commercial grade unless otherwise stated. THF was distilled at atmospheric pressure over a mixture of sodium and potassium prior to use. Acetonitrile was dried over 3Å molecular sieves, pyridine and CH_2Cl_2 were dried over 4Å molecular sieves, toluene was dried over sodium wire. In the substitution reactions with phosphinic acid derivatives, the solvents were purged with nitrogen for 20 minutes prior to use. Bis(trimethylsilyl)hypophosphite (BTSP) was prepared as a 4 molar crude reagent solution according to Boyd et al.[25] prior to use (the concentration was determined by ^{1}H NMR, with benzene as internal standard). *tert*-Butyl phosphinate was prepared by condensation of phosphinic acid and *tert*-butyl alcohol essentially according to Gallagher et al.,[18] but with *tert*-butyl alcohol in place of 2-propanol. The reaction mixture was concentrated to give a stock solution of *tert*-butyl phosphinate (66% w/w) in benzene and *tert*-butyl alcohol (1:1, v/v). The concentration was determined by ^{1}H NMR spectroscopy. Cadmium tosylate was prepared as follows: $CdCO_3$ (1 g, 5.8 mmol) was dissolved in a solution of *para*-toluenesulfonic acid (2.21 g, 11.6 mmol, 2 equiv.) in water (20 mL). When gas evolution had ceased, the solution was concentrated and further dried by evaporation of added acetonitrile (4 × 30 mL). The solid product was ground into fine particles and further dried at reduced pressure (80 mbar). 1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-*C*-hydroxymethyl-5-*O*-(4-methoxytriphenylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (**1**) and 1-[2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-5-*O*-(4-methoxytriphenylmethyl)-3-*C*-(methylsulfonylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (**2**) were synthesized as described earlier.[21]

1-[3-*C*-(Bromomethyl)-2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-5-*O*-(4-methoxytriphenylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (3): A solution of compound **1**[21,22] (50 mg, 0.078 mmol) in dry acetonitrile/pyridine (95:5 v/v, 1 mL) under nitrogen was cooled to 0−2 °C. Triphenylphosphane (29 mg, 0.11 mmol, 1.4 equiv.) and carbon tetrabromide (33 mg, 0.10 mmol, 1.3 equiv.) were added, and the mixture was left on a melting ice bath and then stirred at room temperature for 40 h. The reaction mixture was diluted with toluene (10 mL) and then concentrated. The residue was purified by silica gel column chromatography (toluene/ethyl acetate, 4:1, v/v) to give **3** (40 mg, 73%). ^{1}H NMR (CD_3CN, 20 °C): δ = 0.19 (s, 3 H, $SiCH_3$), 0.23 (s, 3 H, $SiCH_3$), 0.91 [s, 9 H, $SiC(CH_3)_3$], 2.66 (m, 1 H, 3′-H), 3.11 (dd, ^3J = 4.7 Hz, ^2J = 10.0 Hz, 1 H, CH_2Br), 3.37 (dd, $J_{5'b,4'}$ = 3.5 Hz, 1 H, 5′b-H), 4.03 (dt, 1 H, 4′-H), 3.42 (t, ^3J = ^2J = 10.0 Hz, 1 H, CH_2Br), 3.59 (dd, $J_{5'a,5'b}$ = 11.7 Hz, $J_{5'a,4'}$ = 2.2 Hz, 1 H, 5′a-H), 3.77 (s, 3 H, OCH_3), 4.47 (d, $J_{2',3'}$ = 4.2 Hz, 1 H, 2′-H), 5.24 (d, 1 H, 5-H), 5.62 (s, 1 H, 1′-H), 6.90 and 7.26−7.46 (14 H, MMT), 7.92 (d, $J_{5,6}$ = 8.1 Hz, 1 H, 6-H), 9.26 (br. s, 1 H, NH). ^{13}C NMR (CD_3CN, 20 °C): δ = −5.13 ($SiCH_3$), −3.94 ($SiCH_3$), 18.8 [Si*C*$(CH_3)_3$], 26.3 [$SiC(CH_3)_3$], 28.2 (CH_2Br), 46.1 (C-3′), 56.0 (OCH_3), 63.1 (C-5′), 78.6 (C-2′), 83.1 (C-4′), 88.2, 92.2 (C-1′), 101.9 (C-5), 114.3, 128.3, 129.1, 129.30, 129.34, 131.5,

135.8, 141.1 (C-6), 145.2, 151.5, 160.0, 164.3. MS (ES+/TOF) [M + Na]$^+$ calcd. for $C_{36}H_{43}BrN_2O_6SiNa$ 729.1971; found 729.1943. $C_{36}H_{43}BrN_2O_6Si$: calcd. C 61.10, H 6.12, N 3.96; found C 61.30, H 6.17, N 3.83.

1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-*C*-(iodomethyl)-5-*O*-(4-methoxytriphenylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (4): A solution of compound **1**[21,22] (400 mg, 0.620 mmol) in dry acetonitrile/pyridine (95:5 v/v, 8 mL) under nitrogen was cooled to 0−2 °C. Triphenylphosphane (228 mg, 0.87 mmol, 1.4 equiv.) and iodine (158 mg, 0.68 mmol, 1.1 equiv.) were added, and the mixture was left on a melting ice-water bath and then stirred at room temperature for 40 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with 0.5 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ (50 mL). The water layer was extracted with CH_2Cl_2 (2×20 mL). The combined organic layers were dried (Na_2SO_4), and activated carbon (DARCO G-60, 100 mesh, powder) was added. The solution was filtered through Celite, and remaining product was extracted with dichloromethane (50 mL). The filtrate was diluted with toluene (10 mL) and concentrated. The residue was purified by silica gel column chromatography (toluene/ethyl acetate, 5:1, v/v) to give **4** (422 mg, 90%). ^{1}H NMR (CD_3CN, 20 °C): δ = 0.24 (s, 3 H, $SiCH_3$), 0.25 (s, 3 H, $SiCH_3$), 0.91 [s, 9 H, $SiC(CH_3)_3$], 2.63 (m, 1 H, 3′-H), 2.84 (dd, ^3J = 4.0 Hz, ^2J = 9.9 Hz, 1 H, CH_2I), 3.10 (t, ^3J = ^2J = 9.9 Hz, 1 H, CH_2I), 3.36 (dd, $J_{5'b,4'}$ = 3.4 Hz, 1 H, 5′b-H), 3.58 (dd, $J_{5'a,5'b}$ = 11.7 Hz, $J_{5'a,4'}$ = 1.7 Hz, 1 H, 5′a-H), 3.78 (s, 3 H, OCH_3), 3.98 (m, 1 H, 4′-H), 4.41 (d, $J_{2',3'}$ = 3.9 Hz, 1 H, 2′-H), 5.24 (d, 1 H, 5-H), 5.61 (s, 1 H, 1′-H), 6.91 and 7.26−7.46 (14 H, MMT), 7.92 (d, $J_{5,6}$ = 8.1 Hz, 1 H, 6-H), 9.19 (br. s, 1 H, NH). ^{13}C NMR (CD_3CN, 20 °C): δ = −4.80 ($SiCH_3$), −3.74 ($SiCH_3$), −0.93 (CH_2I), 18.8 [Si*C*$(CH_3)_3$], 26.4 [SiC(*C*$H_3)_3$], 46.7 (C-3′), 56.0 (OCH_3), 62.8 (C-5′), 79.5 (C-2′), 83.3 (C-4′), 88.1, 91.9 (C-1′), 101.9 (C-5), 114.3, 128.3, 129.09, 129.11, 129.27, 129.31, 129.35, 131.5, 135.7, 141.1 (C-6), 145.2, 151.5, 160.0, 164.4. MS (ES+/TOF) [M + Na]$^+$ calcd. for $C_{36}H_{43}IN_2O_6$-SiNa 777.1833; found 777.1812. $C_{36}H_{43}IN_2O_6Si$: calcd. C 57.29, H 5.74, N 3.71; found C 57.40, H 5.80, N 3.59.

3-*N*-Benzoyl-1-[2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-*C*-(hydroxymethyl)-5-*O*-(4-methoxytriphenylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (7): Compound **1**[21,22] (230 mg, 0.357 mmol), Na_2CO_3 (302 mg, 2.85 mmol, 8 equiv.) and tetra-*n*-butylammonium bromide (6 mg, 0.019 mmol, 0.05 equiv.) were dissolved in a two-phase system of CH_2Cl_2 (8 mL) and H_2O (15 mL). The mixture was cooled on an ice-water bath and vigorously stirred while a solution of benzoyl chloride (54 μL, 0.46 mmol, 1.3 equiv.) in dry CH_2Cl_2 (2 mL) was added dropwise over 30 minutes. The reaction was stirred vigorously at 0−2 °C (ice-water bath) for an additional 2.5 h. TLC analysis showed remaining starting material, and therefore more benzoyl chloride (17 μL, 0.15 mmol, 0.4 equiv.) dissolved in dry CH_2Cl_2 (0.5 mL) was added dropwise over 10 minutes. The reaction mixture was kept on an ice-water bath, and vigorous stirring was continued for an additional 2 hours. TLC analysis showed complete conversion into one product (the O^4-benzoylated product[27]). The ice-water bath was removed, and the mixture was stirred for an additional 17 hours at room temperature in order to effect $O^4 \rightarrow N^3$ benzoyl migration[27] to give the N^3-benzoylated product **7**. The reaction mixture was transferred to a separation funnel, and the layers were separated. The water layer was washed with CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was purified by silica gel column chromatography (stepwise gradient of 1−2% methanol in CH_2Cl_2) to obtain compound **7** (244 mg, 91%). ^{1}H NMR (CD_3CN, 20 °C): δ = 0.08 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.87 [s, 9 H, $SiC(CH_3)_3$], 2.57 (m, 1 H, 3′-H), 3.48 (dd, $J_{5'b,4'}$ = 3.4 Hz, 1 H, 5′b-H), 3.53 (dd, $J_{5'a,5'b}$ = 11.3 Hz, $J_{5'a,4'}$ = 2.4 Hz, 1 H, 5′a-H), 3.56 (dd, ^3J = 6.1 Hz, ^2J = 10.7 Hz, 1 H, CH_2OH), 3.72 (dd, ^3J = 6.9, ^2J = 10.7 Hz, 1 H, CH_2OH), 3.78 (s, 3 H, OCH_3), 4.18 (m, 1 H, 4′-H), 4.53 (d, $J_{2',3'}$ = 4.5 Hz, 1 H, 2′-H), 5.26 (d, 1 H, H-5), 5.62 (s, 1 H, 1′-H), 6.92 (2 H, MMT), 7.26−7.51 (12 H, MMT), 7.57 (2 H, Bz), 7.75 (1 H, Bz), 7.99 (2 H, Bz), 8.17 (d, $J_{5,6}$ = 8.2 Hz, 1 H, 6-H). ^{13}C NMR (CD_3CN, 20 °C): δ = −5.12 ($SiCH_3$), −4.24 ($SiCH_3$), 18.7 [Si*C*$(CH_3)_3$], 26.2 [SiC(*C*$H_3)_3$], 45.1 (C-3′), 56.0 (OCH_3), 58.9 (*C*H_2OH), 63.8 (C-5′), 78.6 (C-2′), 83.6 (C-4′), 88.0, 92.9 (C-1′), 101.6 (C-5), 114.2, 128.2, 128.3, 129.06, 129.08, 129.5, 130.5, 131.4, 131.6, 132.6, 136.0, 136.5, 142.0 (C-6), 145.2, 145.5, 150.5, 159.9, 163.4, 170.8. MS (ES+/TOF) [M + Na]$^+$ calcd. for $C_{43}H_{48}N_2O_8SiNa$ 771.3078; found 771.3059. $C_{43}H_{48}N_2O_8Si$: calcd. C 68.96, H 6.46, N 3.74; found C 68.85, H 6.60, N 3.62.

3-*N*-Benzoyl-1-[2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-5-*O*-(4-methoxytriphenylmethyl)-3-*C*-(trifluoromethanesulfonylmethyl)-β-D-*ribo*-pentofuranosyl]uracil (8): A solution of compound **7** (160 mg, 0.21 mmol) and 2,6-di-*tert*-butylpyridine (115 μL, 0.51 mmol, 2.4 equiv.) in dry CH_2Cl_2 (6 mL) under nitrogen was cooled to −78 °C (dry ice-acetone), and a solution of trifluoromethanesulfonic anhydride (43 μL, 0.26 mmol, 1.2 equiv.) in dry CH_2Cl_2 (0.5 mL) was added dropwise over 30 min. The reaction mixture was stirred at −78 °C for an additional 3 h, and was then allowed to reach 0−2 °C (ice-water bath). The mixture was diluted with CH_2Cl_2 (15 mL) and washed with aqueous $NaHCO_3$ (15 mL). The water layer was washed with CH_2Cl_2 (2 × 15 mL) and the combined organic layers were dried (Na_2SO_4). The organic phase was concentrated under reduced pressure (<25 °C) and the residue was immediately purified by silica gel column chromatography (stepwise gradient of 0.5−2% ethyl acetate in CH_2Cl_2). If the product (**8**) was obtained as an oil, it readily turned orange in colour (probably due to detritylation). The product (**8**) was more stable in a solid form, which was obtained by lyophilization from benzene immediately after concentration of the pure fractions. This procedure gave **8** as a white powder (160 mg, 85%). The product was stored at −80 °C (it slowly decomposed if stored in room temperature). ^{1}H NMR (CD_3CN, 20 °C): δ = 0.06 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.85 [s, 9 H, $SiC(CH_3)_3$], 3.01 (m, 1 H, 3′-H), 3.43 (dd, $J_{5'b,4'}$ = 3.2 Hz, 1 H, 5′b-H), 3.55 (dd, $J_{5'a,5'b}$ = 11.5 Hz, $J_{5'a,4'}$ = 2.3 Hz, 1 H, 5′a-H), 3.76 (s, 3 H, OCH_3), 4.06 (m, 1 H, 4′-H), 4.53 (d, $J_{2',3'}$ = 4.9 Hz, 1 H, 2′-H), 4.64 (dd, ^3J = 6.0, ^2J = 10.0 Hz, 1 H, CH_2OTf), 4.77 (dd, ^3J = 7.9 Hz, ^2J = 10.0 Hz, 1 H, CH_2OTf), 5.31 (d, 1 H, H-5), 5.63 (s, 1 H, 1′-H), 6.92 (2 H, MMT), 7.25−7.46 (12 H, MMT), 7.54 (2 H, Bz), 7.73 (1 H, Bz), 7.97 (2 H, Bz), 8.00 (d, $J_{5,6}$ = 8.3 Hz, 1 H, 6-H). ^{13}C NMR (CD_3CN, 20 °C): δ = −5.45 ($SiCH_3$), −4.08 ($SiCH_3$), 18.7 [Si*C*$(CH_3)_3$], 26.1 [SiC(*C*$H_3)_3$], 42.9 (C-3′), 56.0 (OCH_3), 63.0 (C-5′), 65.4 (*C*H_2OTf), 77.8 (C-2′), 82.1 (C-4′), 88.2, 92.9 (C-1′), 101.9 (C-5), 114.3, 119.0 (q, $^1J_{C,F}$ = 319 Hz, CF_3), 128.4, 129.1, 129.2, 129.3, 130.5, 131.4, 131.6, 132.5, 135.7, 136.6, 141.7 (C-6), 144.9, 145.3, 150.5, 160.1, 163.3, 170.7. MS (ES+/TOF) [M + Na]$^+$ calcd. for $C_{44}H_{47}F_3N_2O_{10}SSiNa$ 903.2571; found 903.2592. $C_{44}H_{47}F_3N_2O_{10}SSi$: calcd. C 59.99, H 5.38, N 3.18, S 3.64; found C 60.12, H 5.48, N 3.02, S 3.40.

Triethylammonium 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytriphenylmethyl)uridine 3′-*C*-Methylenephosphinate (5). Method A: A solution of compound **3** (260 mg, 0.344 mmol) in dry acetonitrile (14 mL, nitrogen-purged), under nitrogen in a flask fitted with a septum, was cooled to −42 °C (dry ice/acetonitrile), and Hünig's base (360 μL, 2.07 mmol, 6 equiv.) was added by syringe. BTSP (4 M reagent solution prepared as described above, 1.0 mL, 4 mmol, 12 equiv.) was added dropwise by syringe (concentrated

BTSP may ignite in contact with air), and the reaction mixture was stirred at −42 °C for 8 h. The reaction mixture was allowed to reach room temperature and was then diluted with CH_2Cl_2 (50 mL) and washed with 2 M aqueous triethylammonium bicarbonate (pH 7.5, 30 mL). The water layer was washed with CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (a stepwise gradient of 0−25% methanol in $CHCl_3$ containing 0.1% triethylamine) to obtain **5** (134 mg, 50%).

Method B: BTSP (4 M reagent solution prepared as described above, 5.85 mL, 23.4 mmol, 140 equiv.) and 2,6-di-*tert*-butylpyridine (190 µL, 0.85 mmol, 5 equiv.) were added by syringe to a 25 mL flask (fitted with a septum and purged with nitrogen gas prior to use, as concentrated BTSP may ignite in contact with air). The mixture was cooled to 0−2 °C (ice-water bath), and a solution of compound **8** (150 mg, 0.17 mmol) and 2,6-di-*tert*-butylpyridine (40 µL, 0.18 mmol, 1 equiv.) in dry acetonitrile (1.35 mL, nitrogen-purged) was added dropwise by syringe. The reaction mixture was kept at 0 °C (ice-water bath kept in a refrigerator) for 50 h. The mixture was diluted with dry CH_2Cl_2 (20 mL, syringe), and the resulting solution was slowly added, with vigorous stirring, to 2 M aqueous triethylammonium bicarbonate (pH 7.5, 30 mL). The water layer was washed with dichloromethane (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (a stepwise gradient of 5−25% methanol in chloroform containing 0.1% triethylamine). The product still contained traces of salt impurities, which were removed by partitioning the residue between CH_2Cl_2 (15 mL) and 1 M aqueous triethylammonium bicarbonate (20 mL). The water layer was washed with CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give **5** (126 mg, 93%). ^{1}H NMR (CD_3OD, 20 °C): δ = 0.23 (s, 3 H, $SiCH_3$), 0.28 (s, 3 H, $SiCH_3$), 0.94 [s, 9 H, $SiC(CH_3)_3$], 1.31 [t, ^3J = 7.4 Hz, 9 H, $N(CH_2CH_3)_3$], 1.38 (m, 1 H, CH_2P), 1.87 (m, 1 H, CH_2P), 2.68 (m, 1 H, 3′-H), 3.20 [q, ^3J = 7.4 Hz, ^2J = 14.6 Hz, 6 H, $N(CH_2CH_3)_3$], 3.43 (dd, $J_{5'b,4'}$ = 2.7 Hz, 1 H, 5′b-H), 3.64 (dd, $J_{5'a,5'b}$ = 11.4 Hz, 1 H, 5′a-H), 3.79 (s, 3 H, OCH_3), 4.07 (m, 1 H, 4′-H), 4.57 (dd, $J_{2',3'}$ = 3.9 Hz, 1 H, 2′-H), 5.15 (d, 1 H, 5-H), 5.72 (s, 1 H, 1′-H), 7.12 (d, ^1J = 495 Hz, 1 H, PH), 6.90 and 7.24−7.48 (14 H, MMT), 8.28 (d, $J_{5,6}$ = 8.1 Hz, 1 H, 6-H). ^{13}C NMR (CD_3OD, 20 °C): δ = −4.9 ($SiCH_3$), −3.76 ($SiCH_3$), 9.22 [$N(CH_2CH_3)_3$], 19.0 [$SiC(CH_3)_3$], 26.5 [$SiC(CH_3)_3$], 27.9 (d, $^1J_{C,P}$ = 91.2 Hz, CH_2P), 38.0 (C-3′), 47.7 [$N(CH_2CH_3)_3$], 55.8 (OCH_3), 62.4 (C-5′), 78.8 (C-2′), 86.0 (C-4′, $^3J_{4',P}$ = 16.0 Hz), 88.5, 92.8 (C-1′), 101.5 (C-5), 114.3, 128.2, 128.3, 129.0, 129.7, 129.8, 131.8, 142.5 (C-6), 151.9, 160.4, 166.4. ^{31}P NMR spectroscopic data (CD_3OD, co-axial inner-tube with 2% v/v H_3PO_4 in D_2O, 20 °C): δ = 22.5. MS (ES+/TOF) $[M + H]^+$ calcd. for $C_{42}H_{60}N_3O_8PSi$ 794.3966, found 794.3950.

General Procedures for Test Treatments of 2, 3 and 4 with BTSP to Investigate Temperature- and Solvent-Dependence: Hünig's base (14 µL, 80 µmol, 6 equiv.) and BTSP (4 M reagent solution, 40 µL, 160 µmol, 12 equiv.) were added to solutions of the nucleoside derivatives (13 µmol), in given solvents (0.54 mL nitrogen-purged THF, toluene, CH_2Cl_2, pyridine or acetonitrile) under nitrogen at given temperatures (20 °C, 0 °C or −40 °C). The reaction mixtures were stirred for 1−9 h., depending on operating temperatures. Aqueous triethylammonium bicarbonate (2 M, 100 µL) was added to each reaction mixture, and the mixtures were concentrated and further dried by evaporation of added acetonitrile. Test reactions with higher ionic strengths or other reagents [NaI, cadmium tosylate (1.1 equiv.) or 2,6-di-*tert*-butyl-4-methylphenol (2 equiv.)] were carried out in a similar fashion. The residues were dissolved in CD_3OD (insoluble particles were removed by filtration in the cases of mixtures containing cadmium tosylate or NaI) and analysed by ^{1}H NMR (the ratios of products **5** and **7** were determined by comparison of integrals for the 2′-H signals).

General Procedures for Test Treatments of 2, 3 and 4 with *tert*-Butyl Phosphinate/Potassium *tert*-Butoxide: *tert*-Butyl phosphinate (66% w/w in benzene and *tert*-butyl alcohol, 1:1, v/v), prepared as described above (45.5 mg solution, 0.25 mmol of the reagent, 10 equiv.) and compound **2**, **3** or **4** (0.025 mmol) were dissolved in dry THF (322 µL) under nitrogen. A solution of potassium *tert*-butoxide (27.6 mg, 0.25 mmol, 10 equiv.) in dry THF (492 µL) was added dropwise. The reaction mixture was stirred for 1 h at room temperature, diluted with CH_2Cl_2 and washed with 2 M aqueous triethylammonium bicarbonate. The organic layer was dried (Na_2SO_4) and concentrated. The residue was analysed by ^{1}H NMR.

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[1] A. De Mesmaeker, R. Häner, P. Martin, H. E. Moser, *Acc. Chem. Res.* **1995**, *40*, 366−374.
[2] S. M. Freier, K-H. Altmann, *Nucleic Acids Res.* **1997**, *25*, 4429−4443.
[3] E. Uhlmann, A. Peyman, *Chem. Rev.* **1990**, *90*, 543−584.
[4] S. A. Strobel, *Current Opinion in Biotechnology* **1999**, *9*, 346−352 (and references cited therein).
[5] M. Warashina, Y. Takagi, W. J. Stec, K. Taira, *Current Opinion in Biotechnology* **2000**, *11*, 354−362 (and references cited therein).
[6] A. Yoshida, S. Shan, D. Herschlag, J. A. Piccirilli, *Chem. Biol.* **2000**, *7*, 85−96 (and references cited therein).
[7] J. Stawinski, R. Stömberg, T. Szabo, *Coll. Czech. Chem. Commun.* **1990**, *55*, 145−148.
[8] S. P. Collingwood, M. E. Douglas, F. Natt, U. Pieles, *Phosphorus Sulfur Silicon* **1999**, *144−146*, 645−648.
[9] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Chem. Scr.* **1985**, *25*, 280−282.
[10] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Chem. Scr.* **1986**, *26*, 59−62.
[11] B. C. Froehler, M. D. Matteucci, *Tetrahedron Lett.* **1986**, *27*, 469−472.
[12] P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, C. Henrichson, *Tetrahedron Lett.* **1986**, *27*, 4051−4054.
[13] P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, C. Henrichson, *Tetrahedron Lett.* **1986**, *27*, 4055−4058.
[14] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucl. Acids Res.* **1986**, *14*, 5399−5407.
[15] H. An, T. Wang, P. D. Cook, *Tetrahedron Lett.* **2000**, *41*, 7813−7816.
[16] H. An, T. Wang, M. A. Maier, M. Manoharan, B. S. Ross, P. D. Cook, *J. Org. Chem.* **2001**, *66*, 2789−2801.
[17] E. A. Boyd, A. C. Regan, *Tetrahedron Lett.* **1992**, *33*, 813−816.
[18] M. J. Gallagher, M. G. Ranasinghe, I. D. Jenkins, *Phosphorus Sulfur Silicon* **1996**, *115*, 255−259.
[19] A. W. Schwabacher, A. D. Stefanescu, A. Rehman, *J. Org. Chem.* **1999**, *64*, 1784−1788.
[20] M. G. Voronkov, L. Z. Marmur, O. N. Dolgov, V. A. Pestunovich, E. I. Pokrovskii, Y. I. Popel, *J. Gen. Chem. USSR (Engl. Trans.)* **1971**, *41*, 2005.

[21] A. Winqvist, R. Strömberg, *Eur. J. Org. Chem.* **2001**, *22*, 4305−4311.
[22] A. Winqvist, R. Strömberg, *Nucleosides, Nucleotides Nucleic Acids* **2001**, in press.
[23] E. A. Boyd, M. Corless, K. James, A. C. Regan, *Tetrahedron Lett.* **1990**, *31*, 2933−2936.
[24] P. Majewski, *Phosphorus Sulfur Silicon* **1989**, *45*, 151−154.
[25] E. A. Boyd, K. James, A. C. Regan, *Tetrahedron Lett.* **1994**, *35*, 4223−4226.
[26] B. Zeng, K. K. Wong, D. L. Pompliano, S. Reddy, M. E. Tanner, *J. Org. Chem.* **1998**, *63*, 10081−10086.
[27] M. Sekine, *J. Org. Chem.* **1989**, *54*, 2321−2326.
[28] R. Shapiro, R. E. Servis, M. Welcher, *J. Am. Chem. Soc.* **1970**, *92*, 422−424.
[29] H. Hayatsu, Y. Wataya, K. Kai, *J. Am. Chem. Soc.* **1970**, *92*, 724−726.

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