Synthetic methodology for the preparation of nucleic acid containing peptides

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Chapter 2

A Versatile One-Pot Procedure to Phosphate Monoesters and Pyrophosphates[1]

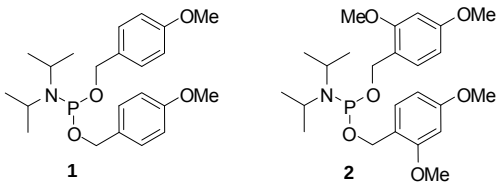
Introduction

Phosphorus containing functional groups are not only essential elements of nucleic acids but are also present in other naturally occurring compounds such as proteins[2], carbohydrates[3] and lipids.[4] Among these biomolecules phosphomono- and diesters[2,3], pyro- and triphosphates[5,6], phosphoramidates[7] and C-phosphonates[8] can be discerned. The pivotal role that these phosphoric acid derivatives play in a broad palette of biological processes has stimulated research into the synthesis of these classes of compounds.[9,10] In addition, the pharmacological potential of phosphate ester analogues[11-13] led to the development of artificial phosphate derivatives such as phosphorothioates[14] and phosphofluoridates.[15] Initially developed to meet the needs of automated nucleic acid synthesis, the phosphoramidite approach is one of the most applied synthetic methodologies towards phosphate ester containing molecules.[16-18] Introduction of phosphate esters is generally attained with the aid of amidites provided with protecting groups, which are often base-labile.[19,20] Upon activation with a weakly acidic azole the amidite reagent reacts with an alcohol to give a phosphite triester. Subsequent oxidation to the phosphotriester and deprotection using alkaline conditions furnishes the target phosphodi- or monoester.[21]

As part of ongoing efforts[22-24] aiming at the synthesis of naturally occurring phosphates and derivatives thereof via the phosphoramidite approach the phosphitylation properties of methoxybenzyl-*N*,*N*-diisopropylphosphoramidites **1** and **2** (**Figure 1**) were explored. One of the interesting envisioned properties of these substituted benzyl groups is their stability towards mild alkaline conditions and their lability towards mild acidic conditions. These reagents provide attractive entries to phosphomonoesters, since phosphitylation of the alcohol of choice with di-(*p*-methoxybenzyl)-*N*,*N*-diisopropyl phosphoramidite (**1**) followed by oxidation and

mild acidic removal of both *p*-methoxybenzyl groups would readily provide the corresponding phosphomonoester. Reagent **2** (**Figure 1**) equipped with *o*,*p*-dimethoxybenzyl groups would conceivably lead to a phosphotriester moiety convertible to a phosphomonoester under even milder conditions. An additional useful feature of reagents **1** and **2** is the potential of methoxybenzyl substituents to engage in Arbuzov-type reactions.[25] This would open the way to the synthesis of modified phosphates.

Figure 1

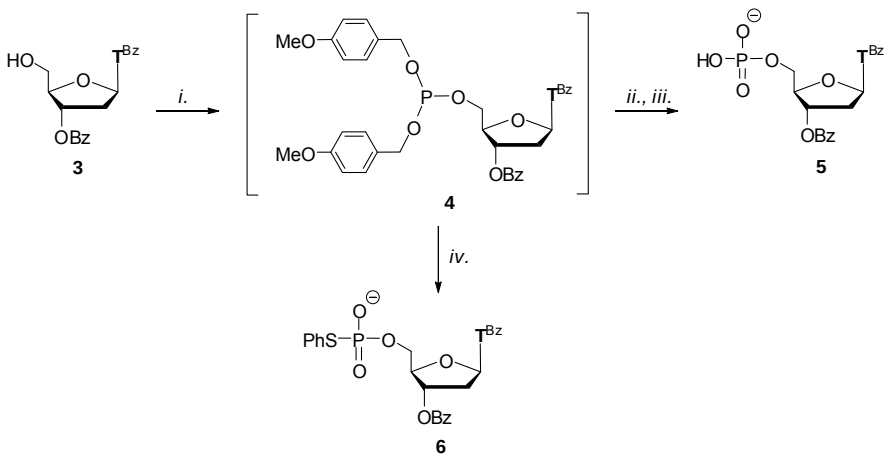


Methoxybenzyl phosphoramidites **1** and **2**

Results and Discussion

Phosphoramidites **1** and **2** were synthesized in good yields via a procedure reported for a related compound[26], comprising treatment of *p*-methoxybenzylalcohol and 2,4-dimethoxybenzylalcohol, respectively, with *N*,*N*-diisopropylaminodichlorophosphine in the presence of triethylamine. Reaction of reagent **1** (1.2 eq.) with thymidine **3** (1.0 eq.) using dicyanoimidazole (DCI) (1.2 eq.) as activator (**Scheme 1**) gave, as monitored by ^{31}P-NMR spectroscopy (δ: 141.8 ppm), formation of phosphite triester **4** as the major product together with some minor phosphonates (δ: 14.1, 9.1 ppm), caused by the hydrolysis of excess reagent **1**. In the subsequent steps **4** was used without isolation, since silica gel column chromatography yielded only 29% of **4**, presumably due to its instability. Addition of anhydrous *t*BuO_2H to oxidize phosphite **4**, followed by *p*-methoxybenzyl deprotection using 3% TFA in DCM and chromatographic purification yielded phosphate **5** (^{31}P-NMR, δ: 1.6 ppm) in 78% yield. In contrast, phosphitylation of **3** using agent **2** under identical conditions failed to yield the intended phosphite triester but instead produced unidentified C- and H-phosphonates in a 1:1 ratio (^{31}P-NMR, δ: 31.9, 14.5 ppm).

Scheme 1. Synthesis of phosphate **5** and phoshorothioate **6**



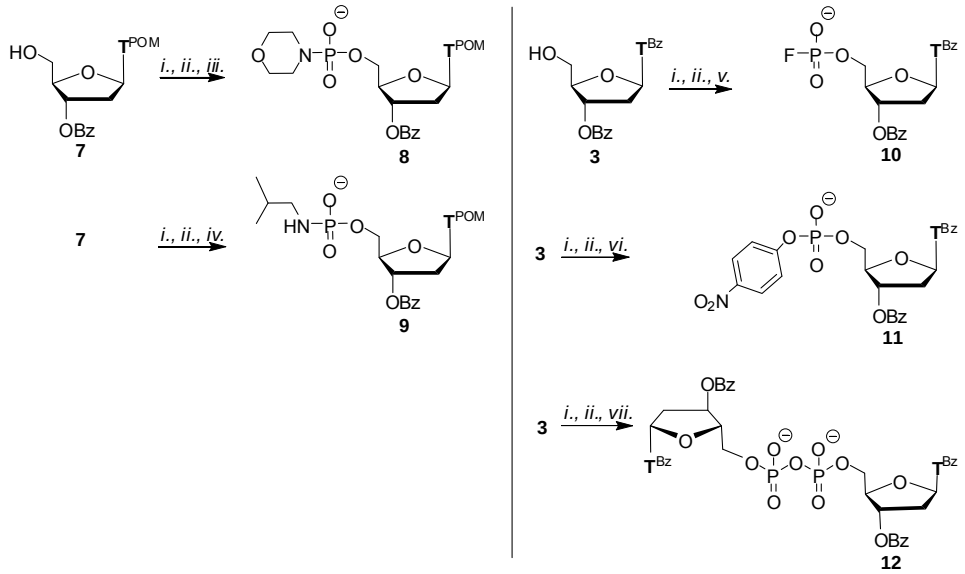
Reagents and conditions; *i.* **1**, DCI, MeCN, 0 ºC, 20 min, *ii*. 5.5 M *t*BuO_2H, 2 h, r.t., *iii*. 3% TFA in DCM, 5 min, r.t., 78% (over two steps), *iv*. $(PhS)_2$, MeCN, 16 h, r.t., 79%.

Having demonstrated the effectiveness of phosphitylating agent **1** to introduce phosphate monoesters under mild acidic (deprotection) conditions the susceptibility of di-(*p*-methoxybenzyl) protected phosphite triester **4** to undergo an Arbuzov-type reaction was explored. Thymidine **3** was converted into phosphite triester **4** as described above, but now the reaction mixture was treated with diphenyldisulfide at the time the transformation of **3** to **4** was complete (as revealed by NMR) leading to phosphorothioate diester **6** in 79% yield (**Scheme 1**). Surprisingly complete in situ cleavage of both *p*-methoxybenzyl protecting groups was observed when the reaction was monitored by ^{31}P-NMR spectroscopy, while it was expected to observe the corresponding triester still carrying one *p*-methoxybenzyl group. This one-pot procedure thus allows an immediate access to S-phenyl phosphorothioates, known precursors[27] in the synthesis of pyrophosphates. The question arose whether these results could be extended to other Arbuzov-type reactions using this one-pot procedure. The use of iodine as the oxidation reagent for **4** would allow introduction of a range of nucleophilic atoms at the phosphorus center via the putative phosphoryliodide.[28]

An interesting class of compounds are the phosphoromorpholidates, the most well-known intermediates en route to pyrophosphates.[29] The solution phase synthesis of thymidine phosphoromorpholidate derivative **8** (**Scheme 2**) was undertaken by adaptation of an iodine mediated procedure to create phosphoromorpholidates on a solid support.[30] Thymidine **7** was phosphitylated with **1** in presence of DCI under the same conditions as described above and the obtained di-(*p*-methoxybenzyl) phosphite triester was oxidized with iodine in pyridine and subsequently treated with morpholine for 1 hour. After silica gel column chromatography morpholidate **8** was isolated in 86% yield.[31] Following an analogous procedure using *iso*-butylamine instead of morpholine as nucleophile led to the clean formation of phosphoramidate **9** (79%).[31]

To broaden the scope of the one-pot procedure the use of non-nitrogen nucleophiles was investigated. Addition of DAST as fluoride source to the reaction mixture after iodine-mediated oxidation resulted in the formation of phosphofluoridate **10** (80%). Similarly, addition of the sodium salt of *p*-nitrophenol to the oxidized reaction mixture resulted in formation of the corresponding phosphodiester **11** (44%). Surprisingly, addition of water did not result in the formation of the expected phosphate monoester but furnished symmetric pyrophosphate **12** (78%).

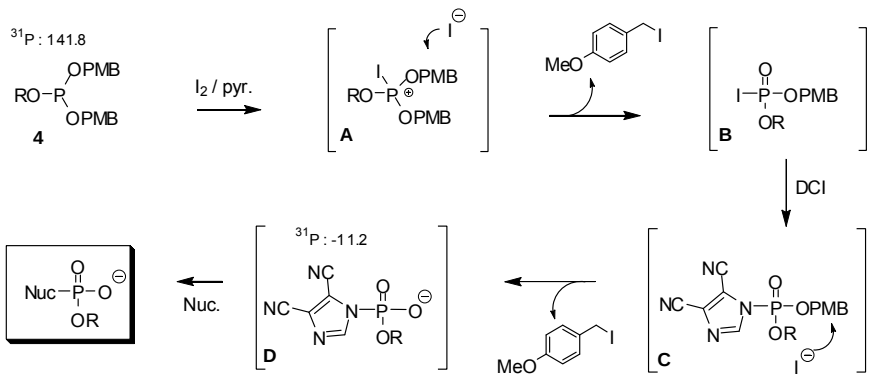
Scheme 2. Synthesis of phosphate monoesters **8** – **11** and symmetric pyrophosphate **12**



Reagents ands conditions: *i*. **1**, DCI, MeCN, 0 °C, 20 min, *ii*. I_2, pyridine, 30 min, r.t., *iii*. morpholine, 1 h, r.t. (86% over three steps), *iv*. *iso*-butylamine,1 h, r.t. (79% over three steps), *v*. DAST,16 h, r.t. (80% over three steps), *vi*. *p*-nitrophenolate sodium salt, 16 h, r.t. (44% over three steps), *vii*. H_2O,16 h, r.t. (78% over three steps).

This unexpected formation of the pyrophosphate **12** upon addition of water as well as the fact that no special treatment is needed to remove the second *p*-methoxybenzyl group in the remaining reactions (**Scheme 2**) put forward the question on the identity of the reactive intermediates involved in the reaction sequence of this one-pot procedure. On the basis of the data that resulted from the analysis of selected reactions by ^{31}P NMR spectroscopy the following mechanism is proposed (**Scheme 3**).

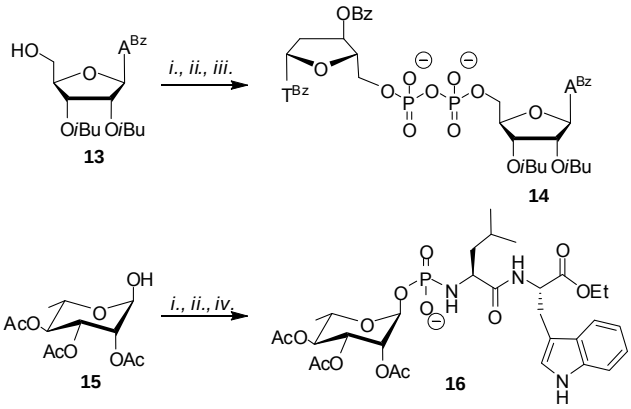
Scheme 3. Proposed reaction mechanism



Activation of amidite **1** (^{31}P-NMR, δ: 147.5) with DCI and subsequent coupling with alcohol **3** gave the phosphite triester **4** (^{31}P-NMR, δ: 141.8, see **Scheme 1**). Treatment of the phosphite triester **4** with iodine in pyridine (**Scheme 3**) would lead to phosponium species **A** and the subsequent phosphoryliodide **B**, having a resonance around δ: -44, as described.[28] However, this resonance could not be detected, but instead a signal at δ: -11.2 was observed. This indicated that the putative phosphoryliodide **B** was transformed immediately into another intermediate with concomitant cleavage of the second *p*-methoxybenzyl group (PMB). This loss of the protective group can be explained by nucleophilic attack of iodide generated in step **C** on the benzylic position of the PMB group. On the basis of literature data that comparable phosphorazolides have ^{31}P-NMR resonances at δ: -12.2 and -11.24 the resonance at δ: -11.2 was tentatively assigned to the dicyanoimidazolide species **D**.[29,32] Subsequent addition of a nucleophile would lead to displacement of dicyanoimidazole to give the corresponding phosphate derivative. The proposed dicyanoimidazolide **D** as the reactive intermediate would explain not only the removal of both *p*-methoxybenzyl groups but also the formation of pyrophosphate **12**. Addition of water will partly and slowly hydrolyze dicyanoimidazolide **D** to give the corresponding monophosphate that, in turn, reacts faster than water with **D** to give pyrophosphate (for example **12**, **Scheme 2**).

To further establish the applicability of this one pot procedure in the preparation of various phosphite derivatives, the syntheses of asymmetric pyrophosphate[33] **14** and phosphoramidon precursor[34] **16** (**Scheme 4**) were undertaken. Crude pyrophosphate **14** was obtained by phosphitylation of protected adenosine **13** with **1** under the influence of DCI, subsequent iodine/pyridine oxidation and, finally, addition of thymidine phosphate **5**. RP-HPLC and gel filtration yielded homogeneous pyrophosphate **14** in moderate yield (27%). Similarly phosphoramidon precursor **16** was synthesized in a moderate yield (19%) by phosphitylating β-rhamnose-triacetate **15** with **1**, iodine oxidation and addition of leucyltryptophane ethyl ester. Such modest yields, compared to synthesis of compounds **8 - 12** are probably caused by the losses in the purification process, as no major side reactions have been observed.

Scheme 4. Synthesis of asymmetric pyrophosphate **14** and phosphoramidon precursor **16**



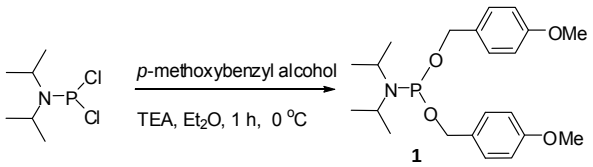
Reagents and conditions: *i.* **1**, DCI, MeCN, 0 °C, 20 min, *ii.* I_2, pyridine, 1 h, r.t., *iii.* **5 (Scheme 1**),16 h, r.t., 27% (over three steps), *iv.* H-Leu-Trp-OEt, 16 h, r.t. 19% (over three steps).

In conclusion, an efficient one-pot procedure to phosphoramidates, phosphorothioates, pyrophosphates and phosphofluoridates has been presented that comprises a sequence of reactions starting with phosphitylation of an alcohol with *di*-(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite **1**, followed by oxidation with iodine and treatment with a nucleophile of choice. The favourable properties of the *p*-methoxybenzyl groups allowed the use of reagent **1** to introduce phosphate monoesters under relatively mild acidic conditions. This feature as well as the potential to form asymmetric pyrophosphates will be further explored in the research as described in **Chapter 3**.

Experimental

General: All chemicals were used as received. Petroleum Ether with a boiling range of 40 - 60°C was used. THF and Et_2O were distilled over $LiAlH_4$ prior to use. DCM was distilled over CaH_2 prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4Å) except for methanol which was stored over 3Å molecular sieves. Solvents used for workup and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 20% H_2SO_4 in EtOH, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed using silica gel (0.04 – 0.063 mm, 60 Å, Screening Devices). For LC-MS analysis a JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C18 column (4.6 mmD × 50 mmL, 3µ particle size) in combination with eluents A: H_2O, B: MeCN and C: 0.5% aq. TFA was used and coupled to a PE/SCIEX API 165 single quadruple mass spectrometer (Perkin-Elmer). Alternatively a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer with an electrospray ion source coupled to Surveyor HPLC system (Thermo Finnegan) was used with the same analytical column. For reversed-phase HPLC purification of final compounds, an automated HPLC system supplied with a C18 column (10.0 mmD × 250 mmL, 5µ particle size) was used. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.2842) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ^{1}H- and ^{13}C-NMR spectra were measured on a Brüker AV-400 (400 MHz), a Brüker AV-500 (500 MHz) or a Brüker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) and coupling constants are given in Hz. Phosphitylation reactions were monitored using ^{31}P-NMR, by placing 400 µL of the crude reaction mixture in a NMR-tube. An acetone-cappilary filled with acetone-d_6 was used as external lock. Chemical shifts are given in ppm (δ) relative to 85% aq. H_3PO_4 (0 ppm). IR spectra of the neat compounds were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations are measured in $CHCl_3$ at a concentration of 10 mg/mL at 25 ^{0}C on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm).

Di-(*p*-methoxybenzyl)-*N, N*-diisopropylphosphoramidite (1)

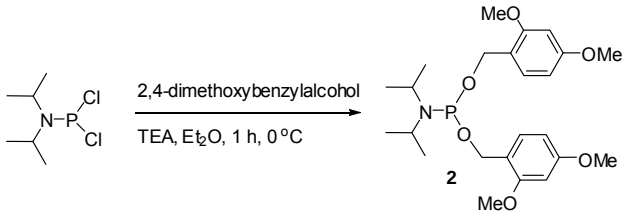


1

To a cooled (0 °C) solution of *N*, *N*-diisopropylphosphorodichloridite (2.4 g, 12.0 mmol) in Et_2O (50 mL) was added dropwise a solution of *p*-methoxybenzyl alcohol (3.8 mL, 30 mmol) and TEA (5.6 mL, 40 mmol) in Et_2O (50 mL). After 1 hour, the suspension was filtered and washed with sat. aq. $NaHCO_3$. The filtrate was dried using $MgSO_4$, concentrated and purified using silica gel column chromatography PE:TEA (99:1 → 23:2, v/v) as eluent to afford **1** as a colourless oil (3.98 g, 82%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.26 (d, *J* = 8.6 Hz, 4H, Arom. PMB), 6.85 (d, *J* = 8.7 Hz, 4H, Arom. PMB), 4.71 - 4.59 (m, 4H, CH_2 PMB), 3.78 (s, 6H, CH_3 PMB), 3.72 - 3.63 (m, 2H, CH *i*Pr), 1.20, 1.19 (2x s, 12H, CH_3 *i*Pr).^{13}C-NMR (100 MHz, $CDCl_3$), δ: 158.8, 131.7 (Cq Arom.), 128.5 (Arom.), 113.7 (Arom.), 65.1, 65.0 (CH_2

PMB), 55.2 (CH_3 PMB), 43.0, 42.9 (CH *i*Pr), 24.6, 24.5 (CH_3 *i*Pr). ^{31}P-NMR (162 MHz, $CDCl_3$), δ: 147.52. IR: 2965, 1612, 1512, 1244, 1172, 1027, 967, 818, 729, 514. HRMS $[C_{22}H_{32}NO_4P + H]^+$: calc. 406.2141, fnd. 406.2099.

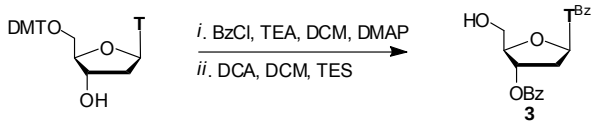
Di-(2,4-dimethoxybenzyl)-*N*, *N*-diisopropylphosphoramidite (2)



2

To a cooled (0 °C) solution of *N*, *N*-diisopropylphosphorodichloridite (0.5 g, 2.5 mmol) in Et_2O (10 mL) was added dropwise a solution of 2,4-dimethoxybenzylalcohol (0.9 g, 5.2 mmol) and TEA (1.2 mL, 8.3 mmol) in Et_2O (10 mL). The reaction was slowly warmed to room temperature and after 1 hour, the suspension was diluted in 40 mL pentane and filtered. The filtrate was concentrated and purified using silica gel column chromatography using Et_2O: PE:TEA as eluent (0:95:5 → 10:85:5, v/v) to afford **2** as a colourless oil (0.43 g, 0.9 mmol, 36%). ^{1}H-NMR (400 MHz, MeCN-d_3), δ: 7.25 (d, *J* = 8.3 Hz, 2H, Arom. DMB), 6.51 (d, *J* = 10.5, 2H, Arom. DMB),6.48 (s, 2H, Arom. DMB), 4.73 - 4.54 (m, 4H, CH_2 DMB), 4.04 - 3.54 (2x s, 12H CH_3 DMB), 3.74 - 3.56 (m, 2H, CH *i*Pr), 1.20, 1.19 (2x s, 12H, CH_3 *i*Pr).^{13}C-NMR (100 MHz, MeCN-d_3), δ: 161.6, 159.1 (Cq Arom.), 130.6 (Arom.), 121.0 (Cq Arom.), 105.2, 99.1 (Arom.), 61.2, 61.0 (CH_2 DMB), 56.1, 56.0 (CH_3 DMB), 43.9, 43.8 (CH *i*Pr), 25.1, 25.0 (CH_3 *i*Pr). ^{31}P-NMR (162 MHz, MeCN-d_3), δ: 148.59. HRMS $[C_{24}H_{36}NO_6P + H]^+$: calc. 466.2353, fnd. 466.2342.

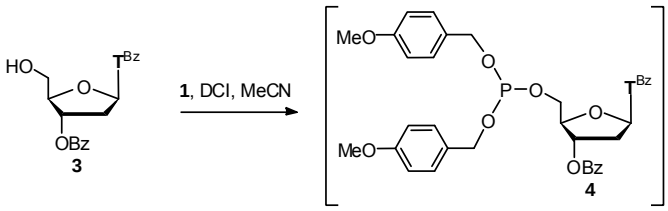
***N*^{3}-Benzoyl-3'-*O*-benzoylthymidine (3)**



3

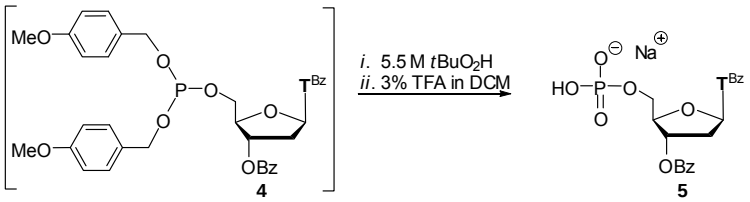
To a solution of 5'-(4',4-dimethoxytrityl)thymidine (5.45 g, 10 mmol) in DCM (50 mL) was added TEA (4.2 mL, 30 mmol), DMAP (0.24 g, 2 mmol) and benzoylchloride (3.0 mL, 26 mmol) after which the reaction mixture was stirred for 16 hours. The reaction mixture was concentrated and taken up in EtOAc followed by washing with sat. aq. $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was dissolved in DCM (60 mL) and dichloroacetic acid (2.5 mL, 30 mmol) was added. After 15 min triethylsilane (16.1 mL, 100 mmol) was added and the reaction mixture was stirred at r.t. for 30 min. After washing with sat. aq. $NaHCO_3$, drying using $MgSO_4$ and concentration the residue was purified using silica gel column chromatography (0:100 →10:90 MeOH/DCM v/v) to yield the title compound as a white foam (4.38 g, 9.7 mmol, 97%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ: 8.03 (d, *J* = 1.1 Hz, 2H, Arom. Bz), 7.94 (d, *J* = 1.1 Hz, 2H, Arom. Bz), 7.81 (d, *J* = 1.1 Hz, 1H, H6), 7.74 - 7.54 (2x m, 2H, Arom. Bz), 7.46 (2x t, *J* = 10.8, 4.9 Hz, 4H, Arom. Bz), 6.40 (dd, *J* = 8.3, 6.0 Hz, 1H, H1'), 5.58 (dt, *J* = 5.9, 2.0 Hz, 1H, H3'), 4.24 (q, *J* = 2.3 Hz, 1H, H4'), 3.99 - 3.93 (m, 2H, H5'), 2.84 (bs, 1H, OH), 2.63 - 2.44 (m, 2H, H2'), 1.96 (d, *J* = 1.0 Hz, 3H, CH_3 T).^{13}C-NMR (100 MHz, $CDCl_3$), δ: 168.9 (CO Bz), 166.1, 162.8 (C4, C2), 149.4 (CO Bz), 136.1 (C6), 135.1, 133.6 (Arom. Bz), 131.4 (Cq Bz), 130.4, 129.6, 129.1, 128.5 (Arom. Bz), 111.3 (C5), 85.7 (C1'), 85.3 (C4'), 75.3 (C3'), 65.2 (C5'), 37.6 (C2'), 12.6 (CH_3T). IR: 3417, 1756, 1696, 1661, 1652 1644, 1447, 1262, 1229, 1096. HRMS $[C_{28}H_{30}N_2O_7 + H]^+$: calc. 451.1499, fnd. 451.1499.

General procedure for synthesis of phosphite triester (4)



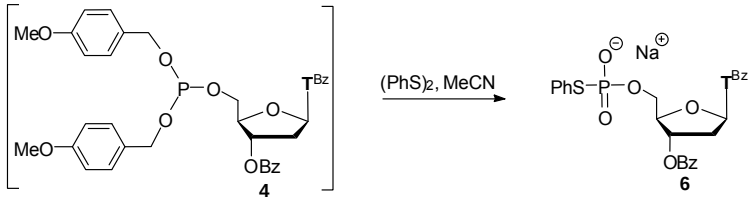
To a cooled (0 °C) solution of thymidine **3** (200 mg, 0.45 mmol) and **1** (0.20 mL, 0.50 mmol) in MeCN (3 mL) was slowly added a solution of DCI (60 mg, 0.5 mmol) in MeCN (1 mL). After 20 min. ^{31}P-NMR showed complete disappearance of **1** (148.5) and formation of triester **4** (^{31}P-NMR; 141.8). The solution thus obtained was used directly in the subsequent transformations. An analytical sample of **4** was concentrated and purified by column chromatography using a gradient of EA:PE:TEA 20:80:0.1 (v/v/v) to yield the title compound as a colourless oil (95 mg, 0.13 mmol, 29%). ^{1}H-NMR (400 MHz, CD_3CN), δ: 8.14 - 8.03 (m, 2H, Arom.), 8.03 - 7.94 (m, 2H, Arom.), 7.75 (s, 1H, H6), 7.67 – 7.64 (m, 1H, Arom.), 7.55 (2x t, *J* = 7.8 Hz, 4H, Arom.), 7.37 - 7.20 (m, 4H, Arom.), 6.91 (dd, *J* = 8.5, 1.2 Hz, 4H, Arom.), 6.36 (dd, *J* = 8.2, 6.0 Hz, 1H, H1'), 5.49 - 5.48 (m, 1H, H3'), 4.88 (d, *J* = 8.2 Hz, 4H, CH_2 PMB), 4.37 (q, *J* = 3.0 Hz, 1H, H4'), 4.23 - 4.03 (m, 2H, H5'), 3.86 - 3.71 (m, 6H, OMe PMB), 2.69 - 2.34 (m, 2H, H2'), 1.86 (s, 3H, CH_3 T).^{13}C-NMR (100 MHz, MeCN-d_3), δ: 170.7 (CO Bz), 166.7, 163.8 (C4, C2), 150.5 (CO Bz), 137.1 (C6), 136.3, 134.4 (Arom. Bz), 132.6, 131.2 (Cq Bz), 130.8 (Cq PMB), 130.5, 130.40, 130.37, 129.6 (Arom. Bz), 114.8 (Arom. PMB), 111.8 (C5), 86.2 (C1'), 84.8, 84.7 (C4'), 76.2 (C3'), 65.6, 65.4, 65.1, 65.0 (CH_2 PMB) , 63.3, 63.2 (C5'), 55.9 (CH_3 OMe), 38.2 (C2'), 12.7 (CH_3 T). ^{31}P-NMR (162 MHz, MeCN-d_3), δ: 141.87. IR: 2933, 1748, 170, 1659, 1612, 1515, 1449, 1246, 1175, 1096, 1026, 975, 713. HRMS $[C_{40}H_{39}N_2O_{11}P+H]^+$: calc. 755.2364, fnd. 755.2361.

***N*^{3}-Benzoyl-3'-*O*-benzoylthymidine-5'-phosphate sodium salt (5)**



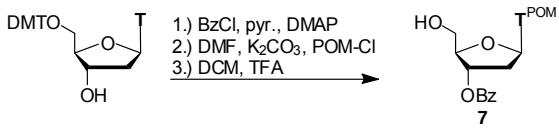
To a cooled (0 °C) solution of phosphite triester **4** (1.8 mmol) in MeCN (5 mL) was added *t*BuO_2H (1.0 mL, 5.5 M in nonane) and the reaction mixture was stirred for 2 hours. The reaction mixture was concentrated and coevaporated three times with toluene. The residue was taken up in anhydrous DCM (20 mL) and TFA (0.6 mL) was added. After 5 min, toluene (5 mL) was added and the mixture was concentrated. The crude product was purified by silica gel column chromatography using DCM:MeOH as eluent (1:0 → 17:3 v/v) and treated with Amberlite Na^+-resin to yield **5** as a sodium salt (0.91 g, 78 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ: 8.04 (s, 1H, H6), 8.03 (d, *J* = 7.5 Hz, 2H, Arom. Bz), 7.96 (d, *J* = 7.4 Hz, 2H, Arom. Bz), 7.81 - 7.37 (m, 6H, Arom. Bz), 6.56 - 6.31 (m, 1H, H1'), 5.68 (d, *J* = 5.2 Hz, 1H, H3'), 4.40 (s, 1H, H4'), 4.31 - 4.12 (m, 2H, H5'), 2.76 - 2.41 (m, 2H, H2'), 2.03 (s, 3H, CH_3 T). ^{13}C-NMR (100 MHz, MeOD-d_4), δ: 170.3 (CO Bz), 167.3, 164.9 (C2,C4), 151.1 (CO Bz), 138.4 (C6), 136.3, 134.6 (Arom. Bz), 133.0 (C_q, Bz), 131.5 (Arom. Bz), 130.6 (C_q Bz), 130.4,129.7, 129.1 (Arom. Bz), 112.5 (C-5), 86.6 (C1'), 85.9 (C4'), 78.1 (C3'), 66.3 (C5'), 38.5 (C2'), 12.6 (CH_3 T). ^{31}P-NMR (162 MHz, $CDCl_3$/MeOD-d_4), δ: 1.60. IR: 3390, 1747, 1699, 1661, 1635, 1600, 1450, 1318, 1270, 1178, 1099, 1061, 916, 712. HRMS $[C_{24}H_{23}N_2O_{10}P+H]^+$: calc. 531.1163, fnd. 531.1160.

N^3-Benzoyl-3'-*O*-benzoylthymidine-5'-(*S*-phenyl)-phosphorothioate sodium salt (6)



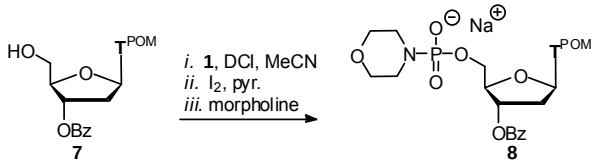
To a cooled (0 °C) solution of phosphite triester **4** (0.42 mmol) in MeCN (4 mL) was added diphenyldisulfide (0.22 g, 1.0 mmol) and the reaction mixture was stirred for an additional 16 hours. The reaction mixture was concentrated and the crude product was purified by silica gel column chromatography using DCM:MeOH as eluent (1:0 → 23:2 v/v) and treated with Amberlite Na^+-resin to yield **6** (0.24 g, 79 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ: 8.12 (s, 1H, H6), 8.04 (d, *J* = 7.8 Hz, 2H, Arom. Bz), 7.96 (d, *J* = 7.9 Hz, 2H, Arom. Bz), 7.79 - 7.24 (m, 6H, Arom. Bz), 7.20 - 7.12 (m, 5H, Arom. Ph), 6.44 (t, *J* = 7.6 Hz, 1H, H1'), 5.62 (d, *J* = 5.4 Hz, 1H, H3'), 4.30 (s, 1H, H4'), 4.03 - 3.88 (m, 2H, H5'), 2.73 - 2.44 (m, 2H, H2'), 2.00 (s, 3H, CH_3 T).^{13}C-NMR (100 MHz, MeOD-d_4), δ: 170.6 (CO Bz),167.4, 166.2 (C4, C2), 151.0 (CO Bz), 138.3 (C6), 136.3, 134.6 (CH Bz), 133.0 (C_q Bz), 131.5, 130.7, 130.4, 129.7 (Arom. Bz), 111.9 (C5), 87.1 (C1'), 86.8 (C4'), 77.0 (C3'), 63.0 (C5'), 38.7 (C2'), 12.5 (CH_3, T). ^{31}P-NMR (81 MHz, $CDCl_3$/MeOD-d_4), δ: 12.1. IR: 3406, 2925, 1760, 1700, 1661, 1644, 1447, 1267, 1096, 708. HRMS $[C_{30}H_{27}N_2O_9PS + H]^+$: calc. 623.1247, fnd. 623.1263.

3'-*O*-Benzoyl-N^3-Pivaloyloxymethylthymidine (7)



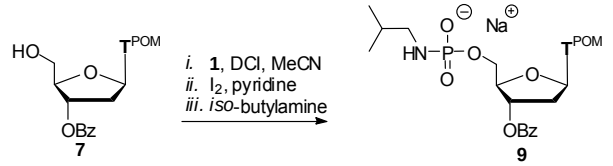
To a solution of 5'-(4',4-dimethoxytrityl)thymidine (8.17 g, 15 mmol) in pyridine (75 mL) was added a catalytic amount of DMAP and benzoylchloride (2.3 mL, 20 mmol) followed by stirring for 2 hours at room temperature. After concentration the residue was taken up in EtOAc and washed with an aqueous $CuSO_4$ solution and the organic phase was dried ($MgSO_4$) and concentrated. The crude residue was dissolved in dry DMF (50 mL) and K_2CO_3 (10.3 g, 75 mmol) and pivaloyloxymethylchloride (5.5 mL, 37.5 mmol) were added and the mixture was stirred for 16 hours at room temperature. After concentration the residue was dissolved in DCM (50 mL) and TFA (1.5 mL) was added. The reaction mixture was quenched by the addition of MeOH (5 mL) and neutralized using TEA (2.8 mL, 20 mmol) after 1 hour. The reaction mixture was washed with water and sat. aq. NaCl, dried and concentrated. The crude residue was purified using silica gel column chromatography using a gradient of PE:EtOAc (80:20 → 40:60 v/v) to yield the title compound as a white foam (5.11 g, 11.1 mmol, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.95 (d, *J* = 8.0 Hz, 2H, Arom. Bz), 7.80 (s, 1H, H6), 7.60 (t, *J* = 7.4 Hz, 1H, Arom. Bz), 7.46 (t, *J* = 7.7 Hz, 2H, Arom. Bz), 6.47 (dd, *J* = 8.4, 5.9 Hz, 1H, H1'), 6.08 - 5.84 (m, 2H, CH_2 POM), 5.69 - 5.57 (m, 1H, H3'), 4.30 (d, *J* = 2.0 Hz, 1H, H4'), 4.19 - 3.95 (m, 2H, H5'), 3.31 (bs, 1H, OH), 2.98 - 2.33 (m, 2H, H2'), 1.97 (s, 3H CH_3 T), 1.19 (s, 9H, *t*Bu). ^{13}C-NMR (100 MHz, $CDCl_3$), δ: 177.6 (CO POM), 166.0, 162.5 (C4,C2), 150.3 (CO Bz), 135.2 (C6), 133.7, 133.4 (Arom. Bz), 129.5 (Cq Bz), 129.8, 127.9 (Arom. Bz), 110.3 (C5), 86.0 (C1'), 85.1 (C4'), 75.4 (C3'), 64.9 (CH_2 POM), 62.4 (C5'), 38.7 (Cq *t*Bu), 37.5 (C2'), 26.8 (*t*Bu), 13.0 (CH_3 T). IR: 3489, 2976, 1714, 1652, 1465, 1267, 1096, 711. HRMS $[C_{23}H_{28}N_2O_8 + H]^+$: calc. 461.1918, fnd. 461. 1916.

3'-*O*-Benzoyl-N^3-Pivaloyloxymethylthymidine-5'-*O*-monophosphomorpholidate sodium salt (8)



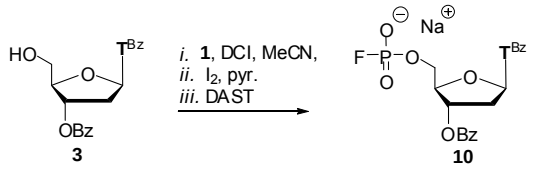
To a cooled (0 °C) solution of **7** (0.46 g, 1.0 mmol) and **1** (0.63 g, 1.3 mmol) in MeCN (15 mL) was slowly added a solution of DCI (0.15 g, 1.25 mmol) in MeCN (5 mL). After 20 min, a solution of iodine in pyridine (2 mL, 0.5 M) was added to the reaction mixture until the solution kept its brown colour. After 30 min of additional stirring, morpholine (0.26 mL, 3.0 mmol) was added and the mixture was stirred for an hour and then concentrated. The crude product was pre-purified by silica gel column chromatography using DCM:MeOH:TEA as eluent (98:0:2 → 8:2:0, v/v/v) to yield the slightly impure title compound (0.52 g, 86%). A part of this crude product was further purified using HPLC and treated with Amberlite Na^+-resin to afford the sodium salt. ^{1}H-NMR (500 MHz, MeOD-d_4), δ : 8.07 (d, J = 7.4 Hz, 2H, Arom. Bz), 7.98 (s, 1H, H6), 7.64 - 7.44 (m, 3H, Arom. Bz), 6.47 (dd, J = 8.6, 5.9 Hz, 1H, H1'), 5.94 - 5.92 (m, 2H, CH_2 POM), 5.65 (d, J = 5.4 Hz, 1H, H3'), 4.38 (s, 1H, H4'), 4.22 - 3.99 (m, 2H, H5'), 3.71 - 3.58 (m, 4H, CH_2 Morph.), 3.13 (dd, J = 9.0, 4.6 Hz, 4H, CH_2 Morph.), 2.70 - 2.47 (m, 2H, H2'), 2.01 (s, 3H, CH_3 T), 1.17 (s, 9H, *t*Bu POM).^{13}C-NMR (125 MHz, MeOD-d_4), δ: 179.0 (CO POM), 167.3, 164.5 (C4, C2), 152.0 (CO Bz), 137.3 (C6), 134.6 (Arom. Bz), 131.0 (C_q Bz), 130.6, 129.6 (Arom. Bz), 111.4 (C5), 87.1 (C1'), 85.7 (C4'), 77.8 (C3'), 68.6 (CH_2N morpholine), 66.3 (CH_2 POM), 65.8 (C5), 46.7 (CH_2O, morpholine), 39.8 (Cq POM), 38.4 (C2'), 27.4 (CH_3 POM), 13.2 (CH_3 T). ^{31}P-NMR (162 MHz, MeOD-d_4), δ: 6.42. IR: 1717, 1663, 1452, 1269, 1258, 1099, 712. HRMS $[C_{27}H_{36}N_3O_{11}P + H]^+$: calc. 610.2160, fnd. 610.2157.

3'-*O*-Benzoyl-3-*N*-Pivaloyloxymethylthymidine-5'-*O*-monophospho-*iso*butylamidate sodium salt (9)



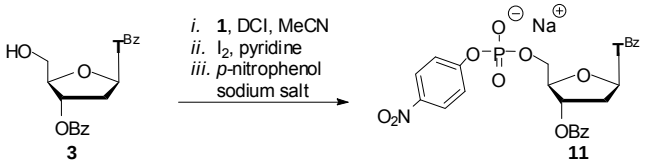
To a cooled (0 °C) solution of **7** (0.46 g, 1.0 mmol) and **1** (0.63 g, 1.3 mmol) in MeCN (15 mL) was slowly added a solution of DCI (0.15 g, 1.25 mmol) in MeCN (5 mL). After 20 min, a solution of iodine in pyridine (2.0 mL, 0.5 M) was added to the reaction mixture until the solution kept its brown colour. After 30 min of additional stirring, *iso*butylamine (0.3 mL, 3.0 mmol) was added and stirred for an hour. The reaction mixture was concentrated and the crude product was pre-purified by silica gel column chromatography using DCM:MeOH:TEA as eluent (98:0:2 → 8:3:0, v/v/v) to yield slightly impure **9** (0.24 g, 79 %). A part of this crude product was further purified using HPLC and treated with Amberlite Na^+-resin to afford the sodium salt. ^{13}C-NMR (125 MHz, MeOD-d_4), δ: 179.12 (CO POM), 167.4, 164.5 (C2, C4), 152.10 (CO Bz), 137.51 (C6), 134.58 (Arom. Bz), 130.93 (C_q, Bz), 130.64, 129.66 (Arom. Bz), 111.54 (C5), 87.08 (C1'), 85.73, 85.66 (C4', J_{CP} = 9.2 Hz), 77.74 (C3'), 66.25 (C5'), 65.50 (CH_2 POM), 49.52 (CH_2 *iso*butyl), 38.31 (C2'), 31.34 (CH *iso*butyl), 27.34 (CH_3 POM), 20.58, 19.37 (CH_3 *iso*butyl), 13.28 (CH_3 T). ^{31}P-NMR (80.7 MHz, MeOD-d_4), δ: 7.42. MS: 596.2 $[M + H]^+$. IR: 1716, 1666, 1469, 1446, 1311, 1269, 1257, 1111, 659, 644. HRMS $[C_{27}H_{38}N_3O_{10}P + H]^+$: calc. 596.2367, fnd. 596.2366.

N^3-Benzoyl-3'-*O*-benzoylthymidine -5'-phosphorofluoridate sodium salt (10)



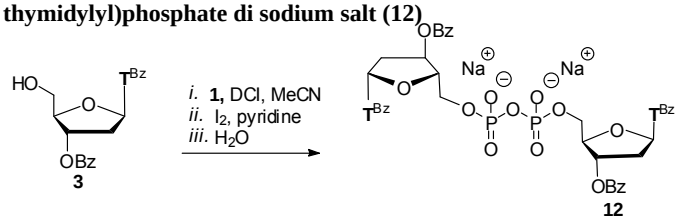
To a cooled (0 °C) solution of **3** (0.45 mmol) and **1** (0.59 mmol) in MeCN (4 mL) was slowly added a solution of DCI (0.59 mmol) in MeCN (1 mL). After 20 min, a solution of iodine in pyridine was added to the reaction mixture until the solution kept its brown colour. After 30 min of additional stirring, DAST (0.07 mL, 1.17 mmol) was added followed by 16 hours of stirring. The reaction mixture was concentrated and the crude product was purified by silica gel column chromatography using DCM:MeOH as eluent (1:0 → 98:2 v/v) and treatment with Amberlite Na^+-resin to yield **10** (0.19 g, 80 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ : 8.08 - 7.87 (m, 5H, Arom. Bz/ H6), 7.70 (t, J = 7.5 Hz, 1H, Arom. Bz), 7.56 - 7.44 (m, 3H, Arom. Bz), 7.25 - 6.99 (m, 2H, Arom. Bz), 6.43 (t, J = 7.3 Hz, 1H, H1'), 5.62 (s, 1H, H3'), 4.39 (s, 2H, H4'), 4.37 - 4.17 (m, 2H, H5'), 2.57 (dd, J = 6.9, 3.4 Hz, 2H, H2'), 2.00 (s, 3H, CH_3 T).^{13}C-NMR (100 MHz, MeOD-d_4), δ: 170.3 (CO Bz), 167.3,164.8 (C2, C4), 151.0 (CO Bz), 137.8 (C6), 136.3, 134.6 (Arom. Bz), 132.9 (Cq, Bz), 131.5 (Arom. Bz), 130.8 (Cq Bz), 130.4, 129.9, 129.7, 129.2 (CH, Bz), 112.4 (C5), 86.6 (C1'), 85.4 (C4'), 77.4 (C3'), 67.6 (C5'), 38.4 (C2'), 12.5 (CH_3 T). ^{31}P-NMR (162, MeOD-d_4), δ: -2.27, -8.01 (d, J_{PF} = 930 Hz). IR: 3391, 1747, 1698, 1652, 1656, 1599, 1449,1250, 1100, 1061, 823, 714, 489. HRMS $[C_{24}H_{22}FN_2O_9P +H]^+$: calc. 533.1119, fnd. 533.1118.

N^3-Benzoyl-3'-*O*-benzoylthymidine -5'-(*p*-nitrophenyl)phosphate sodium salt (11)



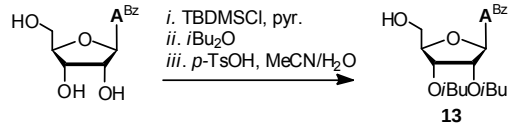
To a cooled (0 °C) solution of **3** (0.25 mmol) and **1** (0.33 mmol) in MeCN (2.5 mL) was slowly added a solution of DCI (0.33 mmol) in MeCN (1 mL). After 20 min, a solution of iodine in pyridine was added to the reaction mixture until the solution kept its brown colour. After 30 min of additional stirring, *p*-nitrophenol sodium salt (0.05 g, 0.33 mmol) was added followed by 16 hours of stirring. The reaction mixture was concentrated and the crude product was purified by silica gel column chromatography using DCM:MeOH as eluent (1:0 → 97:3 v/v) and treated with Amberlite Na^+-resin to yield **11** (73 mg, 44%). ^{1}H-NMR (400 MHz, MeCN-d_3), δ: 8.14 - 7.31 (m, 16H, Arom. NO_2Ph/Arom. Bz/H6), 6.42 (bs, 1H, H1'), 5.54 (bs, 1H, H3'), 4.38 (bs, 1H, H4'), 4.27 (bs, 2H, H5'), 2.46 (bs, 2H, H2'), 1.89 (bs, 3H CH_3 T). ^{13}C-NMR (100 MHz MeCN-d_3), δ: 170.7 (CO Bz), 166.7, 163.8 (C4, C2), 158.0 (Cq NO_2Ph), 150.4 (CO Bz), 143.8 (Cq NO_2Ph), 137.3 (C6), 136.3, 134.4 (Arom. Bz), 132.0 (C_q, Bz), 131.2, 130.5, 130.4, 129.5 (Arom. Bz), 126.2, 121.4 (Arom. NO_2Ph), 111.9 (C5), 85.9 (C1'), 84.4 (C4'), 76.6 (C3'), 65.0 (C5'), 37.9 (C2'), 12.9 (CH_3 T). ^{31}P-NMR (160 MHz, MeCN-d_3), δ: -6.32. IR: 1748, 1700, 1658, 1652, 1344, 1248, 1098, 897. HRMS $[C_{30}H_{26}N_3O_{12}P +H]^+$: calc. 652.1326, fnd. 652.1330.

5'-(N^3-Benzoyl-3'-*O*-benzoyl–thymidylyl-phosphoryl)-5'-(N^3-Benzoyl-3'-*O*-benzoyl – thymidylyl)phosphate di sodium salt (12)



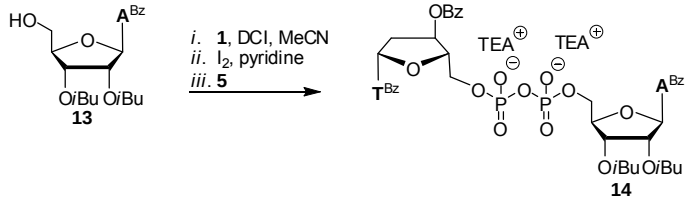
To a cooled (0 °C) solution of **3** (0.55 mmol) and **1** (0.71 mmol) in MeCN (5 mL) was slowly added a solution of DCI (0.71 mmol) in MeCN (1 mL). After 20 min, a solution of iodine in pyridine was added to the reaction mixture until the solution kept its brown colour. After 30 min of additional stirring, H_2O (70 µL, 3.5 mmol) was added followed by 16 hours of stirring. The reaction mixture was concentrated and the crude product was purified by silica gel column chromatography using DCM:MeOH:H_2O as eluent (1:0:0 → 6:3:1 v/v/v) followed by Amberlite Na^+ -resin treatment to yield **12** (0.45 g, 78 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ: 8.10 - 7.86 (m, 10H, Arom. Bz), 7.77 - 7.32 (m, 12H, Arom. Bz/H6), 6.54 - 6.37 (m, 2H, H1'), 5.74 (d, J = 4.4 Hz, 2H, H3'), 4.49 - 4.38 (m, 6H, H4', H5'), 2.86 - 2.46 (m, 4H, H2'), 2.05 (s, 6H, CH_3 T). ^{13}C-NMR (100 MHz, MeOD-d_4), δ: 169.9 (CO Bz), 167.1, 164.2 (C2, C4), 150.5 (CO Bz), 137.5 (C6), 136.0, 134.4 (Arom. Bz), 132.2 (C_q Bz), 130.3, 129.9, 129.3, 129.2 (Arom. Bz), 112.2 (C5), 86.1 (C1'), 84.9 (C4'), 76.9 (C3'), 66.5 (C5'), 37.7 (C2'), 12.6 (CH_3 T). ^{31}P-NMR (162 MHz, $CDCl_3$/MeOD-d_4), δ: -8.44. IR: 3384, 1748, 1699, 1661, 1645, 1635, 1448, 1249, 1108, 1066, 931, 712. HRMS $[C_{48}H_{44}N_4O_{19}P_2 + H]^+$: calc. 1043.2147, fnd. 1043.2157.

***N*^{6}-Benzoyl-2', 3'-di-*O*-*iso*-butyryladenosine (13)**



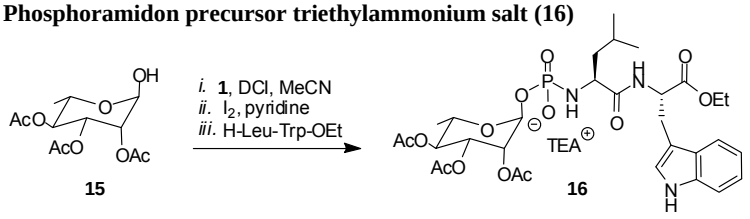
N^6-Benzoyladenosine[35] (1.8 g, 4.8 mmol) was dissolved in pyridine (25 mL) after coevaporation with pyridine. TBDMS-Cl (10 mmol, 1.5 g) was added and the reaction mixture was stirred for 1 hour at room temperature. After this *iso*-butyric anhydride (20 mmol, 3.7 mL) was added and the reaction mixture was stirred for 48 hours. The reaction mixture was concentrated and taken up in DCM. After washing with 5% citric acid and H_2O the organic phase was dried ($MgSO_4$) and concentrated. The residue was taken up in MeCN/ H_2O (50 mL, 4:1, v/v) and *p*-TsOH (5 mmol, 0.8 g) was added after which the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated and purified using silica gel column chromatography EA/PE (0:100 → 45:55 v/v) to afford the title compound as a white foam (1.9 g, 3.9 mmol, 78%). ^{1}H-NMR (400 MHz, $CDCl_3$); δ 9.26 (s, 1H, NH), 8.76 (s, 1H, H2), 8.14 (s, 1H, H8), 8.07 - 7.96 (m, 2H, Arom. Bz), 7.71 - 7.56 (m, 1H, Arom. Bz), 7.51 (dd, J = 10.4, 4.7 Hz, 2H, Arom. Bz), 6.13 (d, J = 7.5 Hz, 1H, H1'), 6.01 (dd, J = 7.5, 5.4 Hz, 1H, H2'), 5.88 (bs, 1H, OH), 5.71 (dd, J = 5.4, 1.3 Hz, 1H, H3'), 4.35 (d, J = 1.4 Hz, 1H, H4'), 4.10 (dd, J = 12.8, 1.6 Hz, 1H, $H5_A$'), 3.88 (d, J = 12.8 Hz, 1H, $H5_B$'), 2.65 (hept., J = 7.0 Hz, 1H, CH *i*Bu), 2.50 (hept., J = 7.0 Hz, 1H, CH *i*Bu), 1.24 (dd, J = 7.0, 2.2 Hz, 6H, CH_3 *i*Bu), 1.09 (dd, J = 14.0, 7.0 Hz, 6H, CH_3 *i*Bu).^{13}C-NMR (400 MHz, $CDCl_3$); δ 175.7, 175.1 (CO *i*Bu), 164.6 (CO Bz), 152.3 (C2), 150.9 (C6), 150.2 (C4), 142.2 (C8), 133.3 (Cq Bz), 132.8, 128.8, 127.9 (Bz), 124.4 (C5), 88.4 (C1'), 86.3 (C4'), 72.8 (C2'), 72.2 (C3'), 62.4 (C5'), 33.8, 33.5 (CH *i*Bu), 18.9, 18.8, 18.7, 18.6 (CH_3 *i*Bu). HRMS $[C_{25}H_{29}N_5O_7 + H]^+$; calc. 512.2135, fnd. 512.2139.

5'-(N^3-Benzoyl-3'-*O*-benzoyl-thymidylyl-phosphoryl)-5'-(N^6-Benzoyl, 2',3'-*O*-di-*iso*butyryladenylyl)-phosphate di triethylammonium salt (14)



To a cooled solution of adenosine **13** (0.18 mmol, 0.92 mg) in MeCN (5 mL) and **1** (0.25 mmol, 97 uL) was added DCI (0.22 mmol, 26 mg). After 20 min a solution of iodine in pyridine (0.5 M) was added to the reaction mixture and stirred for 1 hour. Then, phosphate **5** (0.25 mmol) in 2.5 mL MeCN was added and stirred at room temperature for 16 hours. RP-HPLC purification followed by gel filtration using a HW40 column (50 mM triethylammonium acetate in H_2O as eluent) afforded the title compound as a white solid (62 mg, 27 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ: 8.70 (s, 1H, H8-A), 8.50 (s, 1H, H2-A), 7.90 (d, J = 7.6 Hz, 2H, Arom. Bz), 7.76 (m, 3H, Arom. Bz/ H6-T), 7.68 (d, J = 7.2 Hz, 3H, Arom. Bz), 7.47 (dd, J = 13.4, 6.4 Hz, 4H, Arom. Bz), 7.38 (t, J = 7.5 Hz, 2H, Arom. Bz), 7.27 (d, J = 6.9 Hz, 2H, Arom. Bz), 6.29 (d, J = 5.8 Hz, 1H, H1'-A), 5.99 (d, J = 7.2 Hz, 1H, H1'-T.), 5.73 (d, J = 5.5 Hz, 1H, H2'-A), 5.63 (s, 1H, H3'-A), 5.40 (s, 1H, H3'-T), 4.49 (s, 1H, H4'-A), 4.26 (s, 1H, H4'-T), 4.19 (s, 4H, H5'-A, H5'-T), 2.72 - 2.56 (m, 1H, CH *i*Bu), 2.50 - 2.40 (m, 1H, CH *i*Bu), 2.36 (s, 2H, H2'-T), 1.87 (s, 3H, CH_3-T), 1.11 (dd, J = 9.2, 7.3 Hz, 6H, CH_3 *i*Bu), 0.91 (dd, J = 16.6, 6.9 Hz, 6H, CH_3 *i*Bu). ^{31}P-NMR (162 MHz, MeOD-d_4), δ: -10.69, -10.80, -10.96, -11.07 (dd, J = 18.3, 42.7 Hz). IR: 3358, 2927, 1746, 1699, 1661, 1652, 1455, 1247,1099. HRMS $[C_{49}H_{51}N_7O_{19}P_2 + H]^+$: calc. 1104.2787, fnd. 1104.2796.

Phosphoramidon precursor triethylammonium salt (16)



To a cooled solution of 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose[34] (0.5 mmol, 0.145 mg) in MeCN (4.5 mL) and **1** (0.6 mmol, 235 uL) was added DCI (0.55 mmol, 65 mg). After 20 min a solution of iodine in pyridine (0.5 M) was added and the reaction mixture was stirred for 20 min. Then, H-Leu-Trp-OEt[34] (0.50 mmol) in 1.0 mL MeCN was added and stirred at room temperature for 48 hours. RP-HPLC purification followed by gel filtration using a HW40 column (50 mM triethylammonium acetate in H_2O as eluent) afforded the title compound as a white solid (74 mg, 19 %). ^{1}H-NMR (400 MHz, MeOD-d_4), δ: 9.07 (bs, 1H, Trp-indole NH), 7.52 (d, J = 7.8 Hz, 1H, Trp-NH), 7.45 (d, J = 7.6 Hz, 1H, Trp ArH), 7.34 (d, J = 8.1 Hz, 1H, Trp ArH), 7.23 (s, 1H, Trp CH indole), 7.13 (t, J = 7.4 Hz, 1H, Trp ArH), 7.05 (t, J = 7.4 Hz, 1H, Trp ArH), 5.44 (d, J = 7.5 Hz, 1H, Rha-H1'), 5.37 - 5.25 (m, 2H, Rha-H2', Rha-H3'), 5.06 (t, J = 10.0 Hz, 1H, Rha-H4'), 4.79 (dd, J = 13.2, 5.7 Hz, 1H, Trp-CH), 4.25 - 3.99 (m, 3H, Rha-H5', Trp-OC$\underline{H}_2$CH), 3.78 - 3.69 (m, 1H, Leu-CH), 3.31 (dd, J = 5.2, 2.6 Hz, 2H, Trp-CH_2), 2.96 - 2.93 (m, 1H, Leu-NH), 2.12 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.83 - 1.68 (m, 2H, Leu-C$\underline{H}(CH_3)_2$, Leu-CH_2), 1.63 (s, 1H, Leu-CH_2), 1.25 - 1.12 (m, 6H, Trp-$OCH_2C\underline{H}_3$, Rha-CH_3), 0.88 (dd, J = 6.4, 2.5 Hz, 6H, Leu-$CH(C\underline{H}_3)_2$). ^{31}P-NMR (162 MHz, MeOD-d_4), δ: 3.16. HRMS $[C_{31}H_{44}N_3O_{13}P + H]^+$: calc. 698.2684, fnd. 698.2685.

References and Notes

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