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Synthesis of the TT pyrimidine (6–4) pyrimidone photoproduct–thio analogue phosphoramidite building block

Sandra Karina Angulo Matus, Jean-Louis Fourrey and Pascale Clivio *
Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91190 Gif sur Yvette, France. E-mail: pascale.clivio@icsn.cnrs-gif.fr

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The phosphoramidite building block synthesis of the thio analogue at the 5,6-dihydropyrimidine C5 position of the thymidylyl(3′–5′)thymidine (6–4) photoproduct **1** is presented. This compound was readily obtained from the appropriately protected dinucleotide *P*-methyl-5′-*O*-dimethoxytritylthymidilyl(3′⟶5′)-4-thiothymidine **2** after irradiation at 366 nm, then *S*-sulfenylmethylation of the thiol function of the resulting (6–4) adduct, and phosphitylation of the 3′-hydroxyl group.

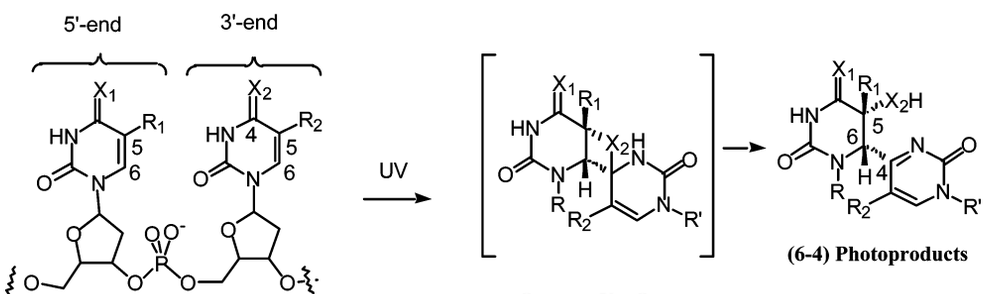
Introduction

Pyrimidine (6–4) pyrimidone photoproducts ((6–4)PPs) are one of the major causes of DNA damage. They are formed at dipyrimidine sites upon UV exposure (Fig. 1).[1] Due to their mutagenic properties[2] and their involvement in UV carcinogenesis,[3] these lesions attract considerable attention which is currently intensified by the recent discovery of polymerases that specialize in translesion synthesis.[4] In addition, the interference of (6–4)PPs with other metabolic processes such as transcription[5] is currently receiving great interest. Beside these natural lesions, their analogues in which the C5 hydroxyl or amino group is replaced by a thiol group (Fig. 1) have been proved to be valuable tools to investigate some of the properties of the natural adducts,[6] this thiol substituent conferring the unique ability to be in equilibrium with their thietane precursor. This particularity has been used to provide the first evidence of the formation mechanism of (6–4)PPs[7] and has contributed to the elucidation of the repair mechanism of (6–4) photolyases.[8,9] In addition, these thio analogues can afford h^5(6–5)PPs that could be suitable analogues for structure–mutagenicity relationship studies.[10] Thus s^5(6–4) analogues are attractive compounds and physico-chemical and biological studies using oligonucleotides containing the s^5(6–4) analogue of TT have therefore emerged.[9,11] So far, such oligonucleotides were prepared by direct photolysis of an oligonucleotide containing the Ts^{4}T sequence. However, despite the selective photoactivable properties of 4-thiothymine, this method does not afford exclusively the s^5(6–4) PP at Ts^{4}T sites.[7a] Furthermore, the 4-thionucleobase may also add photochemically to an adjacent 3′-pyrimidine.[12] In the natural series, the absence of specificity in the type and site of photoproduct formation has been circumvented by synthesizing and site specifically incorporating building blocks containing (6–4)PPs into oligonucleotides.[13]

Envisioning a similar strategy for the preparation of s^5(6–4) PP-containing oligonucleotides, we herein describe an efficient synthesis of the Ts^5(6–4)T photoproduct building block **1**.

Results and discussion

Having observed that the 5′-dimethoxytrityl (dmt) group was stable to the light used to photolyse 4-thiothymine (366 nm), we reasoned that this stability would shorten the synthetic route to **1** compared to the previously described syntheses of (6–4) phosphoramidite building blocks which required the removal of this protecting group, due to its instability at 254 nm, and its subsequent reintroduction.[13] Thus, we considered compound **2** as a key intermediate for the synthesis of the s^5(6–4) phosphoramidite photoproduct (**1**). We chose to protect the internucleotide phosphate linkage of **2** with a methyl group for ease



	X_1	X_2	R_1	R_2
TT	O	O	CH_3	CH_3
Ts^{4}T	O	S	CH_3	CH_3
TC	O	NH	CH_3	H
CT	NH	O	H	CH_3
CC	NH	NH	H	H

Fig. 1 Pyrimidine (6–4) pyrimidone photoproducts produced at bipyrimidine sites in DNA

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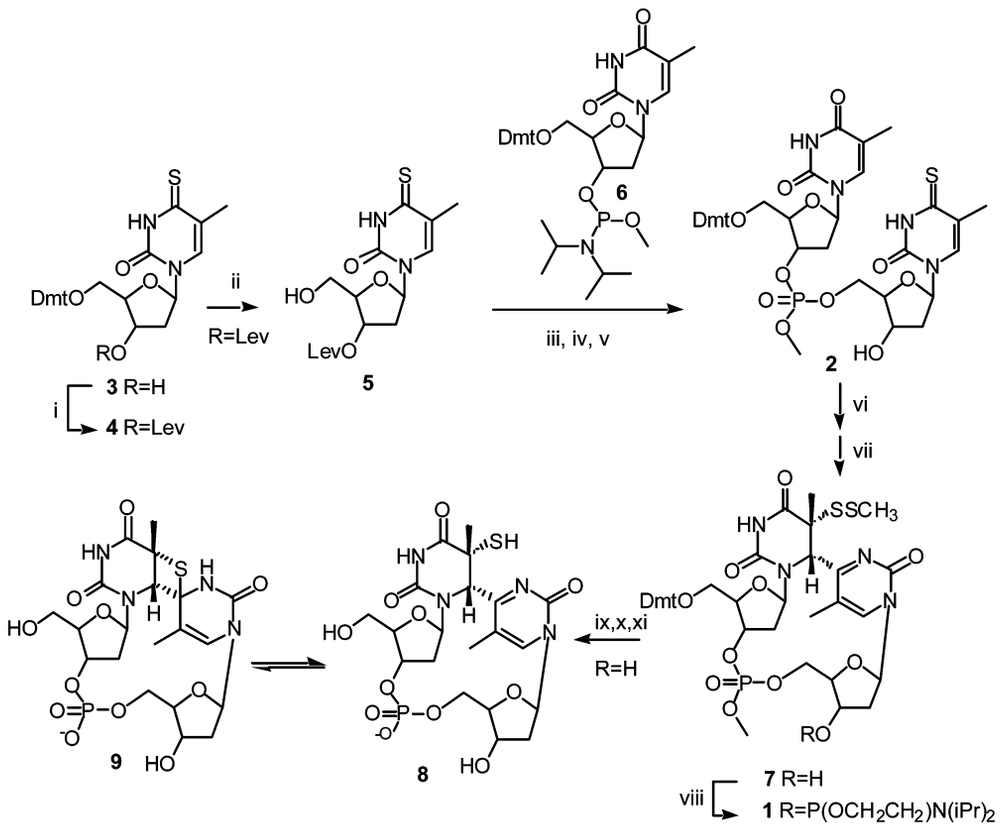

Scheme 1 Reagents and yields: i, Levulinic acid, DCC, 98%; ii, Trifluoroacetic acid/CH_2Cl_2, 85%; iii, 1H-Tetrazole; iv, I_2/THF/H_2O/lutidine; v, $NH_2NH_2.H_2O$,pyridine-AcOH, 74%; vi, *hv* 366 nm; vii, $CH_3SO_2SCH_3$, K_2CO_3, 43%; viii, $NCCH_2CH_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, 64%; ix, Conc. aq. ammonia; x, 40% CH_3COOH; xi, Dithiotreitol pH_8.

of interpretation of NMR spectra.[14] We also reasoned that the *P*-methoxy group was structurally close to the *P*-methyl group that was previously found not to interfere with the photochemical pathway leading to (6–4) PPs.[15]

The synthetic route envisaged to obtain **2**, compared to the corresponding method in the natural series, had to solve a major difficulty inherent to the presence of the sulfur atom. Thus, with regards to phosphoramidite chemistry, 4-thiothymine had to be incorporated either under a protected form (*S*-pivaloyloxymethyl or *S*-cyanoethyl)[16] for the formation of the internucleotide phosphotriester linkage or generated by thiol displacement of a pyrimidine activated at the 4-position[12,17] after the formation of the internucleotide phosphotriester linkage. However, in our case, deprotection of the methyl phosphotriester could be expected during the removal of the *S*-protecting group or the thiol introduction. Having previously observed that it is not always necessary to protect the thiol function of 4-thiothymine for the synthesis of dinucleotides at least for the H-phosphonate chemistry,[18] we decided to investigate the synthesis of **2**, using the phosphoramidite chemistry, without protection of the 4-thiothymine derivative sulfur atom.

Another concern was the choice of the 3′-hydroxyl transient protecting group of the 3′-unit. Removal conditions of this group had to be orthogonal with the thiocarbonyl function and the phosphate protecting group. We decided to use the levulinyl group as a 3′-protecting group due to its clean deprotection under mild conditions[19] that should not lead to the displacement of the sulfur,[20] and its compatibility with the methyl phosphate protection.[21]

Synthesis of compound **2** started from the known 5′-*O*-dimethoxytrityl-4-thiothymidine **3**[22] (Scheme 1) which was acylated with levulinic acid and DCC in the presence of DMAP to give **4** in 98%. This latter was subsequently detritylated on treatment with 3% trifluoroacetic acid in dichloromethane giving 3′-*O*-levulinyl-4-thiothymidine (**5**) in 85% yield. Condensation of **5** with 5′-*O*-dimethoxytritylthymidine 3′-methyl-*N*,*N*-diisopropylphosphoramidite (**6**)[23] in the presence of tetrazole followed by iodine oxidation and removal of the levulinyl protection with buffered hydrazine hydrate in pyridine-acetic acid resulted in the formation of **2** with an overall yield of 74% from **5** (3 steps).[24]

Photolysis of **2** at 366 nm in an aqueous/acetonitrile solution gave a mixture of photoproducts consisting mainly of the thietane in equilibrium with the (6–4)PP. Reverse phase (RP) HPLC separation of these compounds failed as they coeluted with the remaining starting material. Trapping the (6–4)PP/thietane equilibrium under a s[5](6–4) methyl sulfide yields **7**[7a] made its purification possible. In addition, the sulfenylmethyl group could provide a good protection for the C5 thiol function of the (6–4) adduct, in view of its functionalization into phosphoramidite and its incorporation into oligonucleotides, as this thiol protection is compatible with phosphoramidite-based oligonucleotide synthesis.[20b] Thus, treatment of the crude irradiation mixture of **2** with $CH_3SO_2SCH_3$ in the presence of K_2CO_3 afforded the two phosphorus diastereoisomers of compound **7** in pure form after RP HPLC purification (43 % yield from **2**). The stereochemistry at C5 and C6 of **7**, assigned from NOE correlations (H-6 Tp/H-3′ Tp; H-6 Tp/CH_3 Tp and H-6 Tp/CH_3 pT) was found to be C5 R and C6 S as in the unprotected series.[7a] It is worth mentioning that irradiation of **2** led to (6–4) photoproducts in a significant higher yield than in the natural series (16% for TT and 3.9% for TC).[13]

At this stage, the stability of **7** towards the deprotection conditions used during solid phase oligonucleotide synthesis was carefully examined by HPLC in the light of the known acid- and base-sensitivity of 2-pyrimidinone nucleosides.[20b,25] The conditions to remove the phosphate methyl group were also examined along with the stability of the internucleotide bond to alkaline conditions. Usually, this group is specifically removed by the action of strong nucleophiles such as thiophenate[26] or odorless disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate;[27] the use of a mixture of pyridine/aqueous ammonia has

also been reported.[28] Since this latter condition should also allow the deprotection of the nucleobases and the cleavage of the oligonucleotide from the support, its use would avoid the necessity to use an additional step for specifically removing the methyl group. Thus, treatment of a mixture of the two diastereoisomers **7** in a pyridine/concentrated aqueous ammonia solution (1/1) at room temperature for 20 h led, in 62% yield, to the formation of one single product of HPLC retention time (RT) 14 min whose mass spectrum (ESI^-: *m*/*z* 909) confirmed the removal of the methyl group. Therefore, we decided to utilize concentrated aqueous ammonia that had also been reported to deprotect methylphosphate groups.[29] Treatment of **7** with concentrated aqueous ammonia for 24 h at room temperature gave, in 90% yield, a single compound having by HPLC analysis a RT of 14 min. This established that the phosphate methyl group could be cleanly removed by the use of conc. aqueous ammonia and that the pyrimidinone motif would withstand the alkaline conditions necessary for the deprotection of the nucleobases and cleavage from the support. We then examined the stability of **7** towards aqueous and non aqueous acidic conditions. Treatment of **7** with a solution of 2% trichloroacetic acid in CH_2Cl_2 gave instantaneously the 5′-deprotected product (RT 14 min, ESI^+: *m*/*z* 645) as a major product. Prolonged treatment (1 h) did not lead to significant decomposition. The stability of the pyrimidinone motif of **7** in aqueous acidic conditions was then examined since it has been reported that 2-pyrimidinone deoxynucleosides and oligonucleotides containing a (6–4) PP could be unstable to this treatment.[30,31] Treatment of compound **7** deprotected at the P–OMe position with a 40% aqueous acetic acid solution gave instantaneously the detritylated product in quantitative yield. Finally the stability of the methylsulfenyl protecting group towards oxidative conditions was examined. Thus treatment of **7** with the 0.1 M iodine solution commonly used for oligonucleotide synthesis led to the formation of a more polar compound that was not further characterized. However, **7** was proved to be sufficiently stable upon treatment with a 0.02 M iodine solution.[32]

Finally, we studied the deprotection conditions of the thiol function. This last study also ascertained our synthesis for we transformed **7** into the known compound **8**.[7a] Removal of the methyl phosphate and dimethoxytrityl groups of **7** was successively achieved by a treatment with NH_4OH then 40% aqueous acetic acid. Dithiotreitol treatment in phosphate buffer (pH 8) of the resulting compound led instantaneously to a single product that co-eluted with an authentic sample of **8** prepared by photolysis of Tps^4T.[7a] This unambiguously confirmed the stereochemistry of the asymmetric centers of **7** at positions C5 and C6 indicating that the stereochemical course of the photochemical reaction involved the two pyrimidine bases in an *anti* glycosidic conformation as in the natural process. Interestingly, we did not detect any (6–4)PP derived from a 5′-base in a *syn* glycosidic bond conformation as observed in the methylphosphonate series.[7b,15]

Having established the identity and stability of **7** toward the solid phase oxidation and deprotection conditions, it was subsequently phosphitylated into the corresponding phosphoramidite **1** using Hunig's base and (2-cyanoethyl)-*N*,*N*-diisopropylchlorophosphoramidite in the presence of molecular sieves[33] in 64% yield. We have found it convenient to use **1** directly for its incorporation into oligonucleotides and the results will be reported in due course.

Conclusion

In this work, we have reported a straightforward and very efficient synthesis of the s^5(6–4) thymine–thymine phosphoramidite building block designed to allow the preparation of oligonucleotides containing, site specifically, this lesion analogue. Such attractive oligonucleotides should have various biophysical and biological applications. Moreover, in addition to the possible conversion of these oligomers into h^5(6–4)-containing oligonucleotides, functional tethered derivatives aimed at trapping DNA damage sensor proteins crucial for the signal transduction pathway leading to DNA repair could be obtained after thiol derivatization.

Experimental

(2-cyanoethyl)-*N*,*N*-diisopropylchlorophosphoramidite was obtained from Interchim (Montluçon, France). 5′-*O*-Dimethoxytrityl-4-thiothymidine **3** was prepared as previously described.[22] Compounds **5**, **6** and tetrazole were dried at room temperature over P_2O_5 under vacuum overnight prior to use. CH_3CN was dried by heating under reflux with P_2O_5. *N*,*N*-Diisopropylethylamine and CH_2Cl_2 were dried by distillation from calcium hydride and 1,4-dioxane by distillation from sodium/benzophenone. Thin-layer and column chromatography were carried out on silicagel 60 F_{254} 60–15 μm and silicagel 6–35 μm or 35–70 μm respectively from SDS (Peypin, France). ^1H and ^{13}C NMR spectra were recorded on Bruker AC250 or AM300 instruments. ^1H Chemical shifts (δ) are reported in ppm relative to residual solvent peak in CD_3OD (MeOD δ 3.30) or to TMS (δ 0.00) in $CDCl_3$. ^{13}C Chemical shifts are reported in ppm relative to solvent peak (CD^3OD δ 49.0, $CDCl_3$ δ 77.7). ^{31}P NMR spectra were recorded on a Bruker AC 300-P. Chemical shifts are reported relative to an external capillary standard of 85% phosphoric acid. FAB-HRMS (*m*-nitrobenzyl alcohol/glycerol matrix) and ESMS were carried out using a ZabSpec/T spectrometer (Micromass, Manchester, UK). CI HRMS (CH_4) was carried out using a Kratos MS 80 spectrometer.

5′-*O*-Dimethoxytrityl-3′-*O*-levulinyl-4-thiothymidine 4

5′-*O*-Dimethoxytrityl-4-thiothymidine **3**[22] (3.60 g, 6.43 mmol) was dissolved in anhydrous 1,4-dioxane (70 mL), and DMAP (0.06 g, 0.52 mmol) and DCC (3.31 g, 16.07 mmol) were added. To this solution, levulinic acid (1.50 g, 12.86 mmol) was added, and the mixture was stirred for 1 h at room temperature. The resulting mixture was concentrated, diluted with ethyl acetate and filtered on silica gel. After evaporation of the solvent, the residue was purified by flash chromatography using heptane–ethyl acetate (60:40) containing 0.1% of pyridine to give **4** (4.136 g, 98 %) as a yellow foam. ^1H NMR (300 MHz, $CDCl_3$): δ 1.52 (3H, s, CH_3), 2.19 (3H, s, CH_3 Lev), 2.48 (2H, m, H-2′, H-2″), 2.57 (2H, m, CH_2 Lev), 2.76 (2H, m, CH_2 Lev), 3.47 (2H, m, H-5′, H-5″), 3.79 (6H, s, OCH_3 Dmt), 4.16 (1H, br s, H-4′), 5.46 (1H, m, H-3′), 6.38 (1H, dd, J = 5.6; 8.8 Hz, H-1′), 6.82 (4H, d, J = 8.8 Hz, CH Dmt), 7.22–7.38 (9H, m, CH Dmt), 7.67 (1H, s, H-6), 9.68 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (CH_3), 28.5 (CH_2 Lev), 30.3 (CH_3 Lev), 38.3 (C-2′[a]), 38.8 (CH_2 Lev[a]), 55.8 (OCH_3 Dmt), 64.2 (C-5′), 76.0 (C-3′), 84.9 (C-4′[b]), 85.4 (C-1′[b]), 87.8 (CIV Dmt), 113.9 (CH Dmt), 120.5 (C-5), 127.8, 128.6, 128.7, 130.6 (CH Dmt), 132.2 (C-6), 135.7, 135.8, 144.7 (CIV Dmt), 148.5 (C-2), 159.3 (CIV Dmt), 172.8 (*C*OCH_2 Lev), 191.2 (C-4), 206.8 (*C*OCH_3 Lev). HRMS (FAB) $(M + Li)^+$ Calc. for $C_{36}H_{38}O_8N_2SLi$ 665.2509, found 665.2511.

3′-*O*-Levulinyl-4-thiothymidine 5

5′-*O*-Dimethoxytrityl-3′-*O*-levulinyl-4-thiothymidine **4** (3.88 g, 5.88 mmol) was dissolved in 50 mL of 3% CF_3COOH in CH_2Cl_2. After 1 h 30 min of stirring at room temperature, methanol (5 mL) containing 10% triethylamine was added. The mixture was concentrated and the crude product was purified by flash chromatography on silica gel using a gradient of methanol in CH_2Cl_2 (0–2%) to give compound **5** (1.78 g, 85 %) as a yellow foam. ^1H NMR (300 MHz, $CDCl_3$): δ 2.07 (3H, s, CH_3), 2.21 (3H, s, CH_3 Lev), 2.43 (2H, m, H-2′, H-2″), 2.59 (2H, m, CH_2 Lev), 2.81 (2H, m, CH_2 Lev), 3.91 (2H, m, H-5′, H-5″), 4.14 (1H, br s, H-4′), 5.37 (1H, m, H-3′), 6.24 (1H, t, J = 7.0 Hz,

H-1′), 7.70 (1H, s, H-6), 10.77 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8 (CH_3), 28.6 (CH_2 Lev), 29.7 (CH_3 Lev), 38.1 (C-2′[a]), 38.4 (CH_2 Lev), 62.8 (C-5′), 75.4 (C-3′), 86.0 (C-4′), 87.0 (C-1′), 120.3 (C-5), 133.3 (C-6), 148.6 (C-2), 173.2 (*C*OCH_2 Lev), 191.1 (C-4), 207.5 (*C*OCH_3 Lev). HRMS (CI) $(M + H)^+$ Calcd. for $C_{15}H_{21}O_6N_2S$ 357.1120, found 357.1152. Anal. calcd. for $C_{15}H_{20}O_6N_2S$: C, 50.55; H, 5.65; N, 7.85; O, 26.93; S, 8.99. Found: C, 50.27; H, 5.43; N, 7.71; O, 26.91; S, 8.92.

P-methyl-5′-*O*-dimethoxytritylthymidilyl(3′→5′)-4-thiothymidine 2

5′-*O*-Dimethoxytritylthymidine 3′-methyl-*N*,*N*-diisopropylphosphoramidite **6** (880 mg, 1.24 mmol) and 3′-*O*-levulinyl-4-thiothymidine **5** (296 mg, 0.83 mmol) were dissolved in anhydrous acetonitrile (12 mL), and 1H-tetrazole (194.5 mg, 2.77 mmol) was added. After 15 min of stirring at room temperature, a 0.2M iodine solution in tetrahydrofuran-2,6-lutidine-water (2:1:1, v/v/v) (8 mL) was added, and the mixture was stirred for 10 min. After concentration, the residue was diluted with CH_2Cl_2 and a saturated sodium thiosulfate solution was added until the solution became colorless, and water was added. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The residue was dissolved in pyridine (11 mL), and a 0.94 M hydrazine monohydrate solution in pyridine-acetic acid (3.25:2 v/v) (11 mL) was added. After stirring at room temperature for 40 min, the mixture was cooled in an ice bath and acetone (6 mL) was added. The mixture was diluted with CH_2Cl_2, washed with 2% aqueous NaHCO,[3] and dried with Na_2SO_4. After coevaporation with toluene, the residue was purified by chromatography on silica gel with a step gradient of 0–5% methanol in CH_2Cl_2 containing 0.1% triethylamine to give **2** (544 mg, 74%) as a yellow foam. ^{1}H NMR (300 MHz, CD_3OD): δ 1.86 (3H, s, CH_3 Tp), 2.06 (3H, s, CH_3 ps^{4}T), 2.21–2.42 (3H, m, H-2′ and H-2″ ps^{4}T, H-2′ Tp[a]), 2.50 (1H, m, H-2″ Tp[a]), 3.75 (6H, s, OCH_3 Dmt), 3.76 (2H, m, H-5′ and H-5″ Tp), 3.82 (3H, d, J = 11.8 Hz, $POCH_3$), 4.07 (1H, m, H-4′ ps^{4}T), 4.20 (1H, m, H-4′ Tp), 4.32 (2H, m, H-5′, H-5″ ps^{4}T), 4.41 (1H, m, H-3′ ps^{4}T), 5.07 (1H, m, H-3′ Tp), 6.20 (1H, m, H-1′ ps^{4}T), 6.27 (1H, m, H-1′ Tp), 6.81 (4H, d, J = 8.8 Hz, CH Dmt), 7.10–7.40 (9H, m, CH Dmt), 7.52 and 7.74 (1H, 2s, H-6 ps^{4}T), 7.76 (1H, br s, H-6 Tp). ^{13}C NMR (75 MHz, CD_3OD): δ 12.6 (CH_3 Tp), 17.4 (CH_3 ps^{4}T), 39.6 (C-2′ Tp), 40.7 (C-2′ ps^{4}T), 55.7 (P–OCH_3 and OCH_3 Dmt), 62.5 (C-5′ Tp), 68.7 (C-5′ ps^{4}T), 71.4 (C-3′ ps^{4}T), 80.5 (C-3′ Tp), 86.0 (C-1′ Tp, C-4′ ps^{4}T), 87.1 (C-1′ ps^{4}T, C-4′ Tp), 87.7 (CIV Dmt), 111.9 (C-5 Tp), 114.2 (CH Dmt), 120.6 (C-5 ps^{4}T), 127.7, 128.7, 129.3, 131.2 (CH Dmt), 133.5 (C-6 ps^{4}T), 137.3 (CIV Dmt), 137.7 (C-6 Tp), 146.3 (CIV Dmt), 149.7 (C-2 ps^{4}T), 152.2 (C-2 Tp), 159.9 (CIV Dmt), 166.3 (C-4 Tp), 192.7 (C-4 ps^{4}T). ^{31}P NMR (121 MHz, CD_3OD): δ 0.18. HRMS (FAB) $(M + Li)^+$ Calc. for $C_{42}H_{47}N_4O_{13}PSLi$, 885.2758; found, 885.2769.

Photolysis of 2

A 1mM, 50% aqueous acetonitrile solution of compound **2** (100 mL) was photolyzed at 366 nm at room temperature for 4 h under argon (bubbling) using an Original Hanau Quarzlampen Fluotest-Forte ref. 5261. The solution was then concentrated *in vacuo*. This experiment was repeated several times. The resulting residue from two experiments was dissolved in 1.0 mL of methanol and 1.5 mL of anh. CH_2Cl_2. To this solution, K_2CO_3 (30 mg, 0.22 mmol) then a 5% $CH_3SO_2SCH_3$ in methanol solution (200 µL) were added. The reaction was stirred under an argon atmosphere at room temperature and, after 5 min, the solvents were evaporated and the residue was purified by reverse-phase chromatography on a 25 × 100mm Prep Nova-Pak HR C18, 6 µm, 60 Å, PrePack® Cartridge using acetonitrile:water (40:60) as the eluent at a flow rate of 8 mL min^{-1} with detection set at 330 nm. One phosphorus diastereoisomer had a retention time of 57 min (isomer **α**, 43 mg) whereas the other eluted at 66 min (isomer **β**, 35 mg). The overall yield of **7** from **2** was 42%.

Photoproduct 7α

^{1}H NMR (400 MHz, CD_3OD): δ 1.42 (1H, ddd, J = 14; 8; 2 Hz, H-2′ Tp), 1.87 (3H, s, CH_3 Tp), 1.98 (3H, s, CH_3 pT), 2.16 (1H, dt, J = 14; 9 Hz, H-2″ Tp), 2.49 (3H, s, SCH_3), 2.54 (1H, m, H-2″ pT), 2.82 (1H, dd, J = 14; 7 Hz, H-2′ pT), 3.33 (2H, d, J = 4 Hz, H-5′ and H-5″ Tp), 3.52 (3H, d, J = 11 Hz, $POCH_3$), 3.62 (1H, q, J = 9 Hz, H-3′ Tp), 3.79 (6H, s, OCH_3 Dmt), 3.87 (1H, m, H-4′ pT), 3.99 (1H, td, J = 4; 9 Hz, H-4′ Tp), 4.09 (1H, br d, J = 11 Hz, H-5′ pT), 4.25 (1H, dd, J = 6; 11 hz, H-5′ pT), 4.55 (1H, m, H-3′ pT), 4.77 (1H, s, H-6 Tp), 6.14 (1H, dd, J = 2; 9 Hz, H-1′ Tp), 6.45 (1H, d, J = 8 Hz, H-1′ pT), 6.90 (4H, d, J = 9 Hz, CH Dmt), 7.20–7.53 (9H, m, CH Dmt), 7.84 (1H, s, H-6 pT). ^{13}C-NMR (50 MHz, CD_3OD): δ 15.9 (CH_3 pT), 25.2 (CH_3 Tp), 25.5 (SCH_3), 36.9 and 37.1 (C-2′ Tp and pT), 55.3 ($POCH_3$), 55.8 (OCH_3 Dmt), 57.7 (C-5 Tp), 58.9 (C-6 Tp), 65.1 (C-5′ Tp), 66.8 (C-5′ pT), 69.0 (C-3′ pT), 75.7 (C-3′ Tp), 81.5 (C-4′ Tp), 84.5 (C-1′ Tp), 85.8 (C-4′ pT), 87.6 (C-1′ pT), 88.0 (CIV Dmt), 114.3 (CH Dmt), 115.0 (C-5 pT), 128.0, 129.0, 129.3, 131.3, 131.4 (CH Dmt), 136.7 and 136.9 (CIV Dmt), 145.0 (C-6 pT), 146.0 (CIV Dmt), 153.9 (C-2 Tp), 157.4 (C-2 pT), 160.3 (CIV Dmt), 171.2 (C-4 Tp), 176.7 (C-4 pT). ^{31}P NMR (121 MHz, CD_3OD): δ 0.08. HRMS (FAB) $(M + Li)^+$ Calc. for $C_{43}H_{49}N_4O_{13}PS_2Li$, 931.2635; found, 931.2598.

Photoproduct 7β

^{1}H NMR (300 MHz, CD_3OD): δ 1.44 (1H, ddd, J = 14; 8; 3 Hz H-2′ Tp), 1.83 (3H, s, CH_3 Tp), 1.89 (3H, s, CH_3 pT), 2.13 (1H, m, H-2″ Tp), 2.41 (1H, dd, J = 14; 7 Hz, H-2″ pT), 2.48 (3H, s, SCH_3), 2.77 (1H, ddd, J = 14; 6; 3 Hz, H-2′ pT), 3.30 (1H, m, H-5′ Tp), 3.47 (1H, dd, J = 11; 2 Hz, H-5′ Tp), 3.65 (3H, d, J = 11 Hz, $POCH_3$; 1H, m, H-3′ Tp), 3.77 (6H, s, OCH_3 Dmt), 3.84 (1H, m, H-4′ pT[a]), 3.96 (1H, m, H-4′ Tp[a]), 4.04 (2H, m, H-5′ and H-5″ pT), 4.45 (1H, m, H-3′ pT), 4.74 (1H, s, H-6 Tp), 6.02 (1H, dd, J = 8; 3 Hz, H-1′ Tp), 6.41 (1H, dd, J = 7; 3 Hz, H-1′ pT), 6.87 (4H, d, J = 10 Hz, CH Dmt), 7.15–7.50 (9H, m, CH Dmt), 7.78 (1H, s, H-6 pT). ^{31}P NMR (121 MHz, CD_3OD): δ 0.47. HRMS (FAB) $(M + Li)^+$ Calc. for $C_{43}H_{49}N_4O_{13}PS_2Li$, 931.2635; found, 931.2627.

Stability essays

All the HPLC experiments were conducted at a flow rate of 1 mL min^{-1} and the effluent was monitored at 330 nm.

Alkaline conditions. *Pyridine/concentrated aqueous ammonia solution (1/1)*: A mixture of **7αβ** (0.4 mg in 400 µL) was allowed to stand at room temperature. Analysis was performed on a Nova-Pak® C18 3.9 × 150mm column, using a 20 min linear gradient of 0–50% CH_3CN in water followed by a 10 min plateau. Compound **7αβ** RT: 24 and 25 min. Phosphate deprotected **7**: RT: 14 min. *Concentrated aqueous ammonia*: A mixture of **7αβ** (0.4 mg in 400 µL) was allowed to stand at room temperature. Conditions of analysis are the same as described above.

Aprotic acidic conditions. *2% Trichloroacetic acid in CH_2Cl_2*: Isomer **7β** (1 mg mL^{-1}) was allowed to stand at room temperature. Analysis was performed on a Nova-Pak® Silica 3.9 × 150mm column using an isocratic eluent composed of 3% CH_3OH in CH_2Cl_2. Compound **7β** RT: 6 min; Compound **7β** detritylated RT: 14 min.

Protic acidic conditions. *40% Aqueous acetic acid*: Compound **7** deprotected at the P–OMe position (0.2mg/200 µL) was allowed to stand at room temperature. Analysis was performed on a Nova-Pak® C18 3.9 × 150mm column, using a 40 min

linear gradient of 0–50% CH_3CN in 0.05 M ammonium acetate. Compound **7** deprotected at the P–OMe position RT: 35 min. Compound **7** deprotected at the P–OMe position and detritylated RT: 14 min.

Oxidative conditions. Isomer **7β** (1 mg mL^{-1} of oxidative solution) was allowed to stand at room temperature. Analysis was performed on a Nova-Pak® C18 3.9 × 150 mm column, using 40% CH_3CN in water as eluent. **7β** RT: 21 min; Side product: 15 min.

Deprotection of 7 and its identity to 8

Compound **7** was phosphate deprotected then detritylated as described above. After evaporation of the acetic acid solution, the crude product was dissolved in a 100 mM dithiotreitol solution (0.02 M phosphate buffer, pH 8). Analysis was performed on a Nova-Pak® C18 3.9 × 150mm column, using a 40 min linear gradient of (0–50%) CH_3CN in 0.05 M ammonium acetate. Compound **7** deprotected at the P–OMe position and detritylated RT: 14 min; Completely deprotected **7** RT: 10 min; Compound **8** RT: 10 min.

Phosphoramidite 1

To photoproduct **7** (71 mg, 0.076 mmol) was added *N*,*N*-diisopropylethylamine (53 µL, 0.31 mmol), CH_2Cl_2 (1.1 mL) and 4 Å molecular sieve. Then chloro-(2-cyanoethyl)-bis-*N*,*N*′-diisopropylphosphoramidite (35 µL, 0.153 mmol) was added and the reaction stirred for 5 min. The mixture was then diluted with ethyl acetate containing 5% triethylamine washed with 2% aqueous $NaHCO_3$, and dried over Na_2SO_4. After evaporation of the solvent, the phosphoramidite **1** was purified by chromatography on silica gel eluted with ethyl acetate/heptane 95/5 containing 1% triethylamine. Compound **1** was obtained in 64% yield (55 mg) as a white amorphous solid that was directly used.

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