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Functionalization of Mono- and Oligonucleotides with Phosphane Ligands by Amide Bond Formation

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Seven phosphane-functionalized deoxyuridines have been prepared by amide bond formation between aminodeoxyuridines and phosphanylcarboxylic acids. X-ray crystal structures for two of these new modified nucleosides have been obtained. The same coupling method has been extended to oligonucleotides. The phosphane containing strands have been purified and characterized by MALDI-TOF and, for the first time, ^{31}P NMR spectrometry. Coordination of a phosphane-modified 15-mer to a [PdCl(η^3-allyl)] moiety has been confirmed by ^{31}P NMR spectroscopy.

Introduction

During the past decades there has been an enormous interest in both academia and industry in the synthesis of optically pure compounds. One of the most efficient methods to obtain enantiopure compounds is through asymmetric homogeneous catalysis.[1] A countless number of ligands have been prepared and screened in the search of catalysts achieving the selectivity and reactivity needed in several reactions.[2] Despite all the effort and knowledge gained over the years, many reactions in industry still lack a proper catalytic system, forcing the use of stoichiometric amounts of reagents. Catalysts are also present in Nature in the form of enzymes. Enzymes have high selectivity and specificity in the many biochemical reactions they catalyze. Hybrid catalysts aim to merge the recognizing and chiral properties of biomolecules with a transition metal active site that gives the desired reactivity.[3–5] Several research groups have combined the molecular recognition of proteins with transition metal catalysis[6–8] and in more recent years the focus has also spread to the use of DNA combined with transition metals. Two methods have been used to attach transition metal fragments to DNA: they are the covalent[9–11] and non covalent[12–16] approaches. Both Jäschke[9] and Roelfes and Feringa[17] groups have found that the catalytic results were sequence dependent. This discovery is a great advantage of DNA as ligand because simple changes in the structure of the DNA such as the sequence of the complementary strands can give a complete new catalyst that can place the substrate of interest in a very different environment.

Our aim is to functionalize DNA strands with phosphane units at specific locations,[18–20] with the objective of applying them as ligands in homogeneous catalysis. A method to modify DNA strands with phosphane units via amide bond formation[21] in mono- and oligonucleotides is described in this paper. This method is compatible with water as solvent and results in high conversions.

Results and Discussion

For our strategy we required nucleosides modified with aliphatic amino groups to react with activated phosphanylcarboxylic acids. Our synthetic route was based on nucleoside **1**, prepared by Sonogashira coupling between 5-iodo-2′-deoxyuridine (IdU) and *N*-propynyltrifluoroacetamide (Scheme 1). The amino group has to be protected to avoid interference with the catalytic process.

There are a number of reports on the preparation of compound **1** under different conditions.[22–24] Although the reaction was successful under the conditions of Scheme 1, we found that during column chromatography purification triethylammonium iodide coeluted with the desired product. This problem has been solved by changing to a polymer-supported base (Amberlite Ira-67) and use of Pd/C as heterogeneous catalyst[24] but in our hands low conversions were found even after prolonged reaction times. Therefore, we decided to use triethylamine and optimize the purification method. Treatment of the crude reaction mixture with solid potassium carbonate in THF neutralized the ammonium salt back to triethylamine, which could be removed under vacuum. Addition of methanol allowed us to separate most of the palladium species as insoluble solids. The

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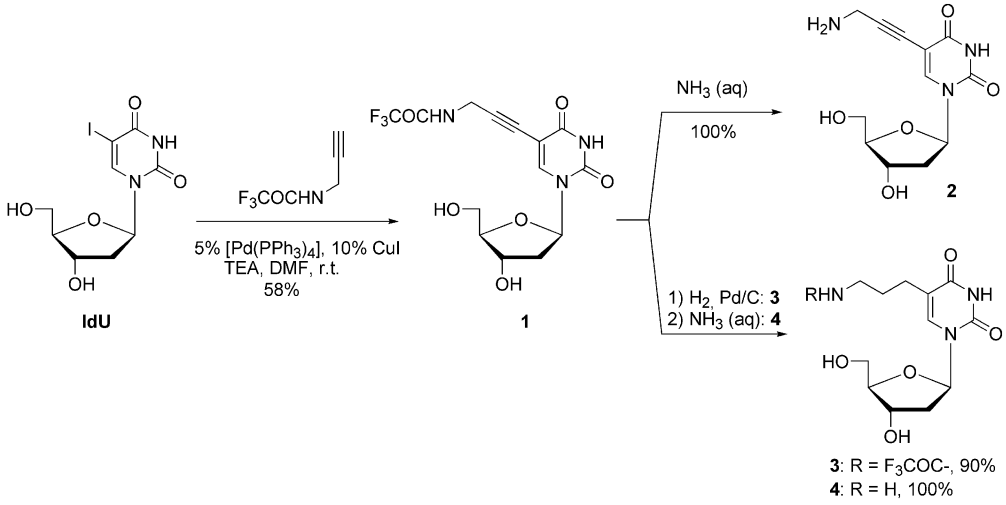


Scheme 1. Preparation of amino-modified nucleosides **2** and **4**.

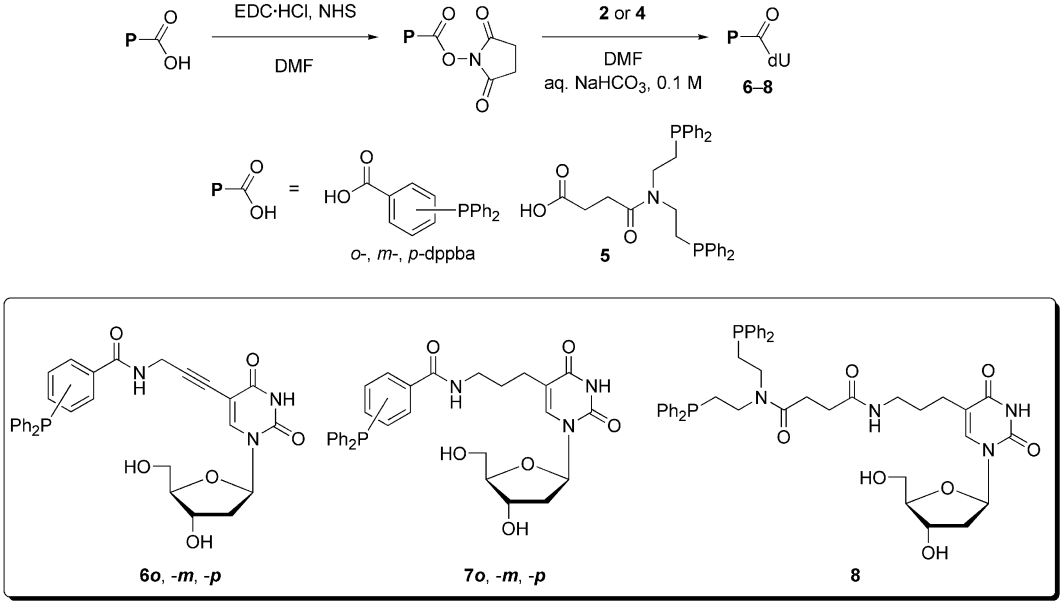
final residue was recrystallised from cold acetonitrile[23] to render pure **1** in moderate yield with no need of column chromatography.

In order to have a more flexible and reactive amino group, we considered the hydrogenation of **1**. This reaction has been described before in literature. Grasby and co-workers[25] prepared this compound by two stepwise reductions with $NiCl_2/NaBH_4$ isolating the (*Z*)-alkene intermediate. We used an excess of reducing reagent and longer reaction times in order to carry on a one-pot reduction to the alkane, but only starting material and alkene were isolated. The procedure of Williams and co-workers,[23] was also tested with 20% PtO_2 (Adam's catalyst) and 10 bar of H_2 in methanol at room temperature. After 12 h mainly compound **3** was obtained, but contaminated with small amounts of unidentified impurities. The best results were obtained by hydrogenation with 5% Pd/C for 24 h. With this method, we isolated compound **3** in high purity after simply removing the Pd/C by filtration.

The deprotection of the amine group was carried out in concentrated aqueous ammonia, rendering the compounds **2** and **4** as confirmed with ^{1}H NMR spectroscopy.

With the modified nucleosides in hand, we turned our attention to the coupling with phosphanylcarboxylic acids. As our ultimate goal is to perform these couplings with DNA strands in an aqueous environment, we sought a suitable method under these conditions and we applied the method of Jäschke and co-workers.[21] This method is depicted in Scheme 2 and consists of the activation of the carboxylic acid group with EDC in the presence of NHS to form a succinimidyl ester,[26] which is stable under aqueous conditions but reactive towards primary amines.

The phosphanylcarboxylic acids were activated with EDC/NHS in DMF and reacted in situ with degassed solu-



Scheme 2. Preparation of phosphane-modified nucleobases **6**–**8**.

tions of **2** or **4** in aqueous $NaHCO_3$. After extractive work-up and column chromatography we obtained the pure compounds **6**–**8**. The couplings with **2** resulted in much lower yields compared to **4**, possibly due to the reduced reactivity of the propargylamino group. All these compounds were fully characterized, including the crystal structures for **7*o*** and **7*m*** (Figure 1).

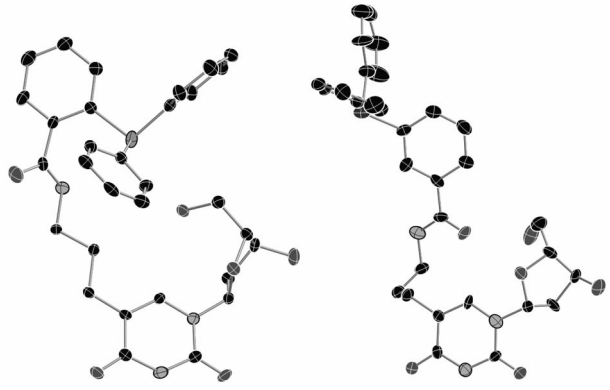


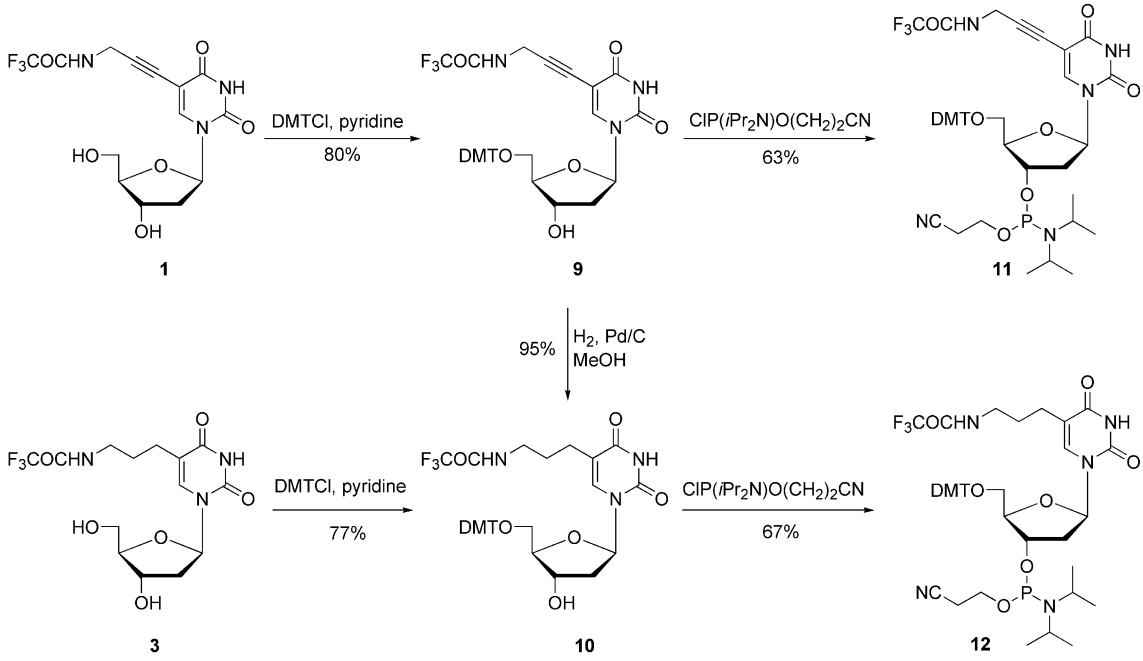
Figure 1. Ortep representation of **7*o*** (left) and **7*m*** (right). The thermal ellipsoids are drawn at 50% probability. Only one of the independent molecules of **7*m*** is represented.

The crystal structures proved the identity of the compounds and confirmed the expected absolute configurations of the stereogenic carbons on the ribose ring. Bonding distances and angles are similar to those found in a related compound reported by us.[10] In spite of that, **7*m*** presents a more open structure than **7*o***, with the diphenylphosphane unit displaced far away from the ribose ring and the electron pair at the phosphorus atom pointing outwards the molecule. This conformation is probably enforced by an intramolecular hydrogen bond between the OH at the 5' position of the ribose ring and the carbonyl oxygen atom of the amide group.

Following standard procedures,[27,28] the 5′-hydroxy group of nucleosides **1** and **3** was protected with DMTCl (4,4′-dimethoxytrityl chloride) to afford the compounds **9** and **10** respectively (Scheme 3). Interestingly, we could also obtain **10** by hydrogenation of **9** in excellent yield without affecting the DMT group. Standard phosphitylation with 2-cyanoethoxy-(*N*,*N*-diisopropylamino)chlorophosphane of **9** and **10** finally afforded **11** and **12** respectively, ready to be used in the oligonucleotide synthesis.[27,28]

As a model compound, the trimer T**X**G (X = **12**) was synthesized in solution according to a recently published procedure.[29] After FPLC purification, MALDI-TOF analysis confirmed the synthesis of the desired compound. The phosphane introduction was carried out with 4 equiv. of *ortho*-diphenylphosphanylbenzoic acid under the same conditions used for the mononucleosides. After FPLC purification with degassed solvents, MALDI-TOF and electrospray were inconclusive because no peaks of the expected product, oxidized product or starting material could be found. $^{31}P\{^{1}H\}$ NMR showed two peaks at 0 and +36 ppm in approximately 2:1 ratio, likely to correspond to the oxidized product. Apparently, the degassing of eluents for FPLC did not prevent product oxidation. At this point we turned our attention to longer strands, which can be purified by precipitation under more oxygen-free conditions.

DNA strands, 15 and 16 bases long, containing the bases **11** and **12** were synthesized on 10 μmol scale by automatic solid-supported synthesis, cleaved from the solid support and purified by HPLC. The prepared sequences and their MALDI-TOF analysis results are listed in Table 1.



Scheme 3. Synthesis of phosphoramidites **11** and **12**.

Table 1. DNA strands with a modified base.

Strand	Sequence[a]	Molecular weight	Found mass
1	5′-TAAGCCA**X**CATCCGC-3′	4536	4534
2	5′-ATTCGGT**X**GTAGGCG-3′	4678	4676
2H	5′-ATTCGGT**Y**GTAGGCG-3′	4682	4682
3H	5′-CGCCTAC**Y**ACCGAAT-3′	4540	4540
4	5′-GCGGATG**X**TGGCTTA-3′	4678	4677
5	5′-**X**ATTCGGTAGTAGGCG-3′	4991	4989
6H	5′-**Y**TTCGGTAGTAGGCG-3′	4682	4680
7[b]	5′-GCTGATGACTATCGA**Z**-3′	4729	4730

[a] **X** = **11**, **Y** = **12**, **Z** = $-(CH_2)_3NH_2$ at the terminal phosphate group of dA. [b] This strand (1 μmol) was purchased from Ocimum Biosolutions but cleaved from the solid support and purified by HPLC by us.

Cleavage from solid support was performed in aqueous ammonia at 55 °C overnight. This procedure also released the free amine from the trifluoroacetyl protecting group. After purification by preparative HPLC, MALDI-TOF analysis showed an extra peak in most of the strands with a mass 53 units higher than expected. This peak corresponds to strands whose aliphatic amino group has been attacked by the acrylonitrile released from the deprotection of the phosphates. Treatment of monobase **1** with ammonia in the presence of acrylonitrile confirmed the high affinity of the amino group towards acrylonitrile. We were able to minimise this problem by deprotecting the strands with aqueous solutions of the more nucleophilic methylamine.

Several attempts were made to couple strands 1, 4 and 5 to *o*-, *p*-(diphenylphosphanyl)benzoic acid (dppba) and **5**. The purified strands were coupled with 250–500 equiv. of activated phosphanylcarboxylic acids under the same conditions discussed above for the monomers and the trimer. Unfortunately, the coupling reactions with the propargylic amines exhibited bad reproducibility. Therefore, we discontinued our efforts with propargylic amine-modified strands 1, 2, 4 and 5 and decided to use strand 7, whose amino group should be more reactive. Gratifyingly, *p*-dppba was successfully coupled. The same activated phosphane was then successfully coupled to strands 2H and 3H with good reproducibility. The method is also applicable to the important class of diphosphane ligands as illustrated by the successful coupling of **5** to strand 6H.

Several methods for purification of the modified oligonucleotides were tested and ethanol precipitation proved to be the most efficient one. After work-up and purification the strands were analyzed by MALDI-TOF, which gave the molecular weight corresponding to the phosphane oxides of the coupled strands. Phosphane oxidation usually occurs under the conditions of MALDI-TOF analysis.

The coupled strand 4 with *o*-dppba was analyzed also with electrospray mass spectroscopy, giving the mass of the unoxidized oligonucleotide confirming that oxidation of the strands takes place during the MALDI-TOF analysis. Another way to prove that phosphane containing strands were not oxidized after purification is ^{31}P NMR spectroscopy although it proved challenging due to the extremely small amounts of oligonucleotide (less than 1 μmol). Under our best conditions (800 nmol, 16 h of acquisition time, 49,000 scans) we recorded the $^{31}P\{^1H\}$ NMR spectrum of strand 2H coupled to *p*-dppba (Figure 2).

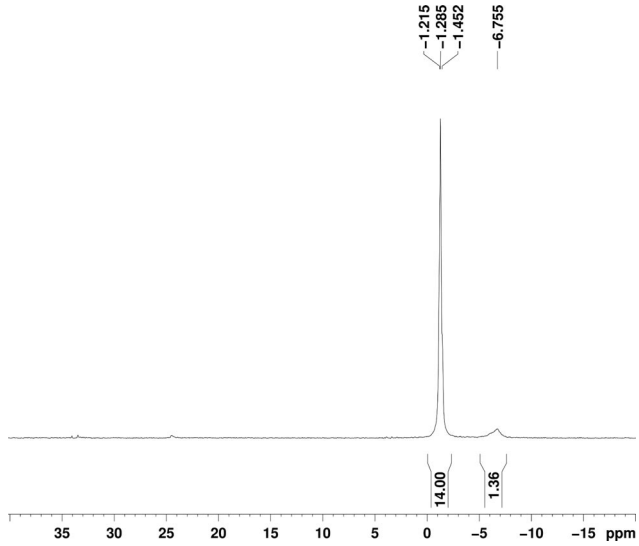


Figure 2. $^{31}P\{^1H\}$ spectrum of strand 2H coupled to *p*-dppba.

We assigned the peak at δ = –6.8 ppm to the coupled phosphane and the peaks around –1.3 ppm to the phosphates of the backbone. The difference in relaxation times between the two kinds of phosphorus atoms and the low concentration of the sample account for the 1.4:14 ratio.

The MALDI-TOF analysis of the same strand is represented in Figure 3 and shows the masses corresponding to the oxidized strand (expected at *m*/*z* = 4986 units) with the additional peaks due to multiple Na cations.

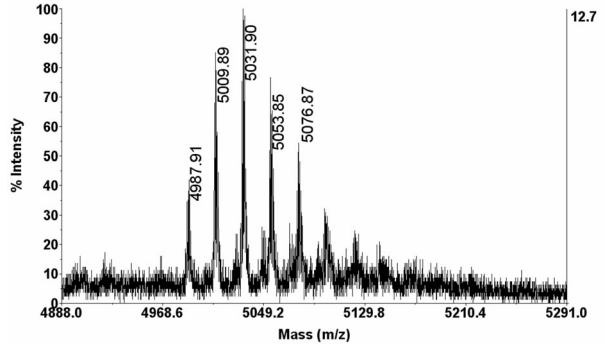


Figure 3. MALDI-TOF analysis of phosphane-modified strand 2H.

In situ complexation of a crude sample of a 15-mer with 0.5 equiv. of the dimer $[PdCl(\eta^3\text{-allyl})]_2$ resulted in a broad peak at δ = +28 ppm in the $^{31}P\{^1H\}$ NMR spectrum indicating that Pd complexation is feasible.

A preliminary catalytic study was performed with ligands **6** and **7**. Allylic alkylation at 1,3-diphenylallyl acetate with dimethyl malonate and allylic amination with benzylamine as nucleophile resulted in 100% conversion for ligands

6*p*, **-*m*** and **7*p***, **-*m*** while ligands **6*o*** and **7*o*** gave less than 10% conversions. Unfortunately, no enantioselectivity was obtained.

We are currently exploring the applicability of this synthetic method to other DNA strands and their use in catalysis.

Conclusions

In summary, we have shown that amide bond formation is an efficient tool to functionalize nucleosides with phosphane moieties. The strategy is also applicable to oligonucleotides, but utmost care has to be taken to avoid oxidation of the phosphane groups. We also coordinated a [PdCl(η^3-allyl)] to one of the phosphane-modified 15-mers.

Experimental Section

General: Chemicals were purchased from Aldrich, Acros, Fluka, Glen Research and Link Technologies. Triethylamine was distilled from sodium, acetonitrile over CaH_2, THF over sodium/benzophenone and ethanol and methanol over Mg/I_2; all under nitrogen. DMF was purchased "Extra dry" and kept over molecular sieves (4 Å) under argon. Aqueous solutions were degassed by three freeze/thaw cycles under vacuum and kept under argon. Air and water sensitive reactions were carried out in dry (from oven) glassware using standard Schlenk techniques under atmosphere of purified argon. Thin layer chromatography (TLC) was performed on silica plates (Polygram 0.2 mm with fluorescent indicator UV_{254}). Silica gel 60, particle size 0.063–0.2 mm from Fluka was used for flash chromatography purification. Oligonucleotides were synthesized at the University of Leiden with a AKTA-oligopilot DNA synthesizer. DNA concentration was determined spectrophotometrically when needed. Purification by ion exchange and size exclusion chromatography and desalting were carried out with a FPLC AktaBasic 100 (P-901) monitored by a UV-900 detector. Purification of modified oligonucleotides was carried out on a Hi-Load 16/10 Q Sepharose HP with the following buffers: buffer A (1 M NaCl/10 mM NaOH) and buffer B (10 mM NaOH). The column for the desalting was a HiPrep 26/10 Desalting with a linear gradient of 0.15 M NH_4HCO_3 buffer. HPLC purification of oligonucleotides was carried out in a Waters apparatus equipped with a Waters 2700 sample manager, Waters 600 controller and Waters 2487 dual λ absorption detector. The reverse phase column used was a Phenomenex, Clarity® C18 5 µ-oligo-RP, 250 × 21.20 mm. NMR spectra were recorded on Bruker Avance spectrometers (300, 400 and 500 MHz). Positive chemical shifts (δ values, ppm) are given for higher frequency shifts relative to tetramethylsilane (for ^1H and ^{13}C), 85% aqueous H_3PO_4 (^{31}P) and CCl_3F (^{19}F). ^{13}C, ^{31}P and ^{19}F spectra have been recorded ^1H-decoupled. The following abbreviations have been used in the description of the spectra: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were recorded in a Perkin–Elmer Spectrum GX spectrometer in KBr pellets, with a window between 4000 and 400 cm^{-1}. MALDI-TOF mass spectra were recorded in a 4800 Plus MALDI TOF/TOF™ Analyzer using a 9:1 3-hydroxypicolinic acid (HPA): ammonium citrate matrix. This matrix was made with 50 mg/mL of HPA in 50:50 acetonitrile/water and 50 mg/mL of ammonium citrate in water. The samples for MALDI-TOF analysis were desalted with a GE Healthcare Illustra micron G-25 column and Millipore C18 tips. Chiral HPLC analyses were carried out on an Agilent Technologies 1200 Series apparatus equipped with a UV/Vis photodiode array detector. The measurements were performed at 254 nm, with an injection volume of 1 µL. Compounds **9**,[27] **11**[27] and **12**[28] were prepared according to literature procedures.

CCDC-763240 (for **7*m***) and -763241 (for **7*o***) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request.cif.

5-[3-*N*-(Trifluoroacetyl)aminopropynyl]-2′-deoxyuridine (1): This compound was prepared by a slightly modified procedure than those previously published.[23,24,30,31] 5-Iodo-2′-deoxyuridine (2.00 g, 5.65 mmol) and CuI (108.6 mg, 0.57 mmol, 0.1 equiv.) were suspended in 30 mL of anhydrous DMF. The mixture was stirred for 30 min, protected from light. To this mixture triethylamine (1.60 mL, 11.50 mmol), *N*-propynyltrifluoroacetamide (2.60 g, 17.20 mmol)[32] and tetrakis(triphenylphosphane)palladium(0) (330 mg, 0.28 mmol, 0.05 equiv.) were added in this order. The yellow clear solution was stirred for 18 h, protected from light, giving an orange solution. The solvents were then removed in vacuo and the residue was dissolved in 70 mL of THF. Solid potassium carbonate (approximately 4 g) was added and the suspension stirred in an open flask for 2 h. The salts were filtered and the liquid was concentrated to dryness. The gummy residue was treated with 30 mL of methanol causing the precipitation of a yellow solid. This solid was filtered and discarded. The remaining liquid was concentrated to dryness and the methanol treatment was repeated until no more solid precipitated (usually two or three times). The filtrate was concentrated under vacuum again and the residue was recrystallised from 30 mL of acetonitrile. After several hours in the freezer, the desired product was obtained as a yellow powder; yield 1.2 g (58%). Characterization of the product fully agreed with the literature.[23,24,30,31]

5-(3-Aminopropynyl)-2′-deoxyuridine (2): The protected base **1** (377 mg, 1.0 mmol) was dissolved in 20 mL of concentrated commercial ammonia and stirred for 12 h. Evaporation of the liquid furnished the desired deprotected base **2** in quantitative yield as a yellowish oil. This product was used without any purification for the couplings with the phosphanylcarboxylic acids. However, $^{19}F\{^1H\}$ NMR spectroscopy showed the presence of the trifluoroacetate anion at $\delta = -75$ ppm. The compound could be used further as such or alternatively the impurity could be removed by column chromatography (SiO_2, CH_2Cl_2/MeOH, 70:30), to yield the pure product as a yellow solid. The yield was quantitative. Characterization of the product fully agreed with data from literature.[28]

5-[3-*N*-(Trifluoroacetyl)aminopropyl]-2′-deoxyuridine (3): The monocleoside **1** (2.0 g, 5.3 mmol) and 10% Pd/C (564 mg, 0.53 mmol Pd) were suspended in 30 mL of dry methanol and hydrogenated at 10 Bar of H_2 for 26 h at room temperature. The resulting suspension was filtered through a pad of celite twice and concentrated in vacuo. If needed, the crude was purified via a short column (SiO_2, EtOAc/MeOH, 90:10) to yield, after evaporation of the solvent, the title product as a pale yellow solid; yield 1.88 g (90%). Characterization of the product fully agreed with data from the literature.[23,25]

5-(3-Aminopropyl)-2′-deoxyuridine (4): The protected base **3** (115 mg, 0.3 mmol) was dissolved in 6 mL of concentrated ammonia and stirred for 12 h. Evaporation of the liquid furnished the desired deprotected base **4** in quantitative yield as a yellowish oil. This product was used without any purification for the couplings with the phosphanylcarboxylic acids. The yield was quantitative. Characterization of the product fully agreed with data from the literature.[28]

6*o*: *o*-(Diphenylphosphanyl)benzoic acid (306 mg, 1.2 mmol), EDC·HCl (230 mg, 1.2 mmol) and NHS (173 mg, 1.5 mmol) were dissolved in 20 mL of dry degassed DMF and stirred for 2 h. The nucleoside **2** (281 mg, 1.0 mmol) was dissolved in 10 mL of previously degassed 0.1 M $NaHCO_3$ aqueous solution and the activated phosphane was added via a syringe. A white precipitate formed but redissolved after a few minutes, giving a clear orange mixture, which was stirred for 12 h. Water (50 mL), $CHCl_3$ (50 mL) and brine (ca. 10 mL) were added to the reaction mixture giving a biphasic system. The organic phase was separated and the aqueous layer extracted twice with $CHCl_3$ (50 mL). The combined organic layers were washed with water (100 mL) and dried with Na_2SO_4. After filtration, the solvent was removed under vacuum (heating to 60 °C was necessary to remove the last traces of DMF) giving a gummy white residue. This crude product was purified column chromatography (SiO_2, $CHCl_3$/MeOH, 90:10) to give the title product as a white foam after solvent removal; yield 171 mg (30%). IR (KBr): $\tilde{\nu}_{max}$ = 3411, 3054, 1700 ν(C=O), 1653 ν(C=O), 1584, 1465, 1434, 1282, 1093, 747, 697 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 8.34 (s, 1 H, =CH), 7.60 (ddd, J = 7.6, 3.6, 1.0 Hz, 1 H, Ar), 7.43 (td, J = 7.5, 1.1 Hz, 1 H, Ar), 7.27–7.37 (m, 11 H, Ar), 7.01 (ddd, J = 7.6, 4.1, 0.7 Hz, 1 H, Ar), 6.27 (t, J = 6.6 Hz, 1 H, CHOC*H*), 4.43–4.41 (m, 1 H, C*H*OH), 4.13 (s, 2 H, NHCH_2), 3.97 (dd, J = 6.5, 3.2 Hz, 1 H, C*H*OCH), 3.83 (dd, J = 12.0, 3.1 Hz, 1 H, CH_2OH), 3.75 (dd, J = 12.0, 3.5 Hz, 1 H, CH_2OH), 2.36–2.31 (m, 1 H, CHCH_2), 2.26–2.21 (m, 1 H, CHCH_2) ppm. ^{13}C NMR (126 MHz, CD_3OD): δ = 171.4 (C), 164.8 (C), 151.2 (C), 145.5 (CH), 142.0 (d, J_{CP} = 24.5 Hz, 1 C), 138.9 (d, J_{CP} = 8.6 Hz, 1 C), 138.8 (d, J_{CP} = 13.6 Hz, 1 C), 135.2–128.6 (CH Ar), 100.0 (C), 89.8 (C), 89.2 (CH), 87.1 (CH), 75.6 (C), 72.1 (CH), 62.7 (CH_2), 41.8 (CH_2), 31.0 (CH_2) ppm. ^{31}P NMR (162 MHz, CD_3OD): δ = –9.5 ppm. MS (ES^+): m/z = 592.24 $[M + Na]^+$. HRMS: calcd. for $C_{31}H_{28}N_3NaO_6P$, 592.1613; found 592.1615.

6*m*: The same method as for **6*o*** was employed using *m*-(diphenylphosphanyl)benzoic acid, starting from 0.3 mmol (84.4 mg) of mononucleoside **2**; yield 107 mg (63%). IR (KBr): $\tilde{\nu}_{max}$ = 3423, 1686 ν(C=O), 1542, 1459, 1434, 1283, 1093, 746, 696 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 8.31 (s, 1 H, =CH), 7.90 (s, 1 H, Ar), 7.86 (dt, J = 8.1, 1.5 Hz, 1 H, Ar), 7.82 (dt, J = 7.7, 1.3 Hz, 1 H, Ar), 7.45 (tdd, J = 7.7, 1.3, 0.6 Hz, 1 H, Ar), 7.39–7.26 (m, 10 H, Ar), 6.23 (t, J = 6.6 Hz, 1 H, CHOC*H*), 4.39–4.37 (m, 1 H, C*H*OH), 4.31 (s, 2 H, NHCH_2), 3.93 (dd, J = 6.6, 3.3 Hz, 1 H, C*H*OCH), 3.80 (dd, J = 12.0, 3.1 Hz, 1 H, CH_2OH), 3.72 (dd, J = 12.0, 3.5 Hz, 1 H, CH_2OH), 2.33–2.27 (m, 1 H, CHCH_2), 2.24–2.219 (m, 1 H, CHCH_2) ppm. ^{13}C NMR (101 MHz, CD_3OD): δ = 169.3 (C), 164.7 (C), 151.1 (C), 145.5 (CH), 140.0 (d, J_{CP} = 13.5 Hz, 1 C), 138.0 (d, J_{CP} = 10.9 Hz, 1 C), 137.7 (d, J_{CP} = 15.5 Hz, CH), 135.4 (d, J_{CP} = 7.5 Hz, 1 C), 134.9–128.7 (CH Ar), 99.9 (C), 90.0 (C), 89.1 (CH), 87.0 (CH), 75.2 (C), 72.0 (CH), 62.6 (CH_2), 41.7 (CH_2), 31.0 (CH_2) ppm. ^{31}P NMR (162 MHz, CD_3OD): δ = –5.5 ppm. MS (ES^+): m/z = 592.03 $[M + Na]^+$. HRMS: calcd. for $C_{31}H_{28}N_3NaO_6P$, 592.1613; found 592.1605.

6*p*: The same method as for **6*o*** was employed but using *p*-(diphenylphosphanyl)benzoic acid; yield 171 mg (30%). IR (KBr): $\tilde{\nu}_{max}$ = 3394, 3054, 2963, 1691 ν(C=O), 1535, 1465, 1434, 1263, 1094, 802, 746, 696 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 8.32 (s, 1 H, =CH), 7.81–7.78 (m, 2 H, Ar), 7.39–7.26 (m, 12 H, Ar), 6.23 (t, J = 6.6 Hz, 1 H, CHOC*H*), 4.40–4.35 (m, 3 H, C*H*OH, NHCH_2), 3.93 (dd, J = 6.6, 3.3 Hz, 1 H, C*H*OCH), 3.80 (dd, J = 12.0, 3.1 Hz, 1 H, CH_2OH), 3.72 (dd, J = 12.0, 3.5 Hz, 1 H, CH_2OH), 2.34–2.20 (m, 2 H, CHCH_2) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 169.3 (C), 164.7 (C), 151.1 (C), 145.5 (CH), 144.0 (d, J_{CP} = 8.9 Hz, 1 C), 137.8 (d, J_{CP} = 6.0 Hz, 1 C), 135.2–128.3 (CH Ar), 99.9 (C), 90.0 (C), 89.2 (CH), 87.0 (CH), 75.2 (C), 72.0 (CH), 62.6 (CH_2), 41.7 (CH_2), 31.0 (CH_2) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –5.5 ppm. MS (ES^+): m/z = 592.02 $[M + Na]^+$. HRMS: calcd. for $C_{31}H_{28}N_3NaO_6P$, 592.1613; found 592.1617.

7*o*: The same method as for **6*o*** was employed but starting from 0.3 mmol (85.8 mg) of mononucleoside **4**; yield 80 mg (46%). IR (KBr): $\tilde{\nu}_{max}$ = 3403, 3053, 2930, 1671 ν(C=O), 1475, 1434, 1275, 1055, 746, 698 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 7.85 (s, 1 H, =CH), 7.52 (ddd, J = 7.6, 3.6, 1.3 Hz, 1 H, Ar), 7.42 (dt, J = 7.8, 1.3 Hz, 1 H, Ar), 7.36–7.23 (m, 11 H, Ar), 6.96 (ddd, J = 4.1, 3.6, 0.9 Hz, 1 H, Ar), 6.27 (t, J = 6.8 Hz, 1 H, CHOC*H*), 4.41–4.36 (m, 1 H, C*H*OH), 3.90 (dd, J = 6.9, 3.5 Hz, 1 H, C*H*OCH), 3.77 (dd, J = 12.0, 3.1 Hz, 1 H, CH_2OH), 3.68 (dd, J = 12.0, 3.8 Hz, 1 H, CH_2OH), 3.18 (t, J = 6.7 Hz, 2 H, NHCH_2), 2.28–2.21 (m, 4 H, CHCH_2CH, CH$_2$CH_2CH), 1.63–1.55 (m, 2 H, CH$_2$CH_2CH$_2$) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 172.2 (C), 166.0 (C), 152.3 (C), 138.9 (CH), 138.6–128.4 (C, CH), 114.8 (C), 88.9 (CH), 86.4 (CH), 72.2 (CH), 62.9 (CH_2), 41.2 (CH_2), 39.7 (CH_2), 28.9 (CH_2), 25.0 (CH_2) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –10.1 ppm. MS (ES^+): m/z = 572.19 [M – H]; 573.20 [M]. HRMS: calcd. for $C_{31}H_{31}N_3O_6P$, 573.1950; found 572.1942. Crystals suitable for X-ray diffraction were grown from an acetonitrile solution at room temperature.

7*m*: The same method as for **6*m*** was employed but starting from 0.3 mmol (85.8 mg) of mononucleoside **4**; yield 142 mg (82%). IR (KBr): $\tilde{\nu}_{max}$ = 3421, 3054, 2926, 1675 ν(C=O), 1539, 1465, 1434, 1275, 1093, 744, 696 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 7.87 (s, 1 H, =CH), 7.85–7.77 (m, 2 H, Ar), 7. 44 (td, J = 7.6, 0.7 Hz, 1 H, Ar), 7.37–7.26 (m, 11 H, Ar), 6.27 (t, J = 6.7 Hz, 1 H, CHOC*H*), 4.41–4.37 (m, 1 H, C*H*OH), 3.90 (dd, J = 6.7, 3.4 Hz, 1 H, C*H*OCH), 3.78 (dd, J = 12.1, 3.1 Hz, 1 H, CH_2OH), 3.69 (dd, J = 12.1, 3.7 Hz, 1 H, CH_2OH), 3.35 (t, J = 6.8 Hz, 2 H, NHCH_2), 2.37 (t, J = 7.2 Hz, 2 H, CH$_2$CH_2CH), 2.25–2.21 (m, 2 H, CHCH_2CH), 1.83–1.74 (m, 2 H, CH$_2$CH_2CH$_2$) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 169.9 (C), 166.1 (C), 152.2 (C), 138.8 (CH), 139.9–128.6 (C, CH), 114.8 (C), 88.9 (CH), 86.4 (CH), 72.2 (CH), 62.8 (CH_2), 41.3 (CH_2), 49.1 (CH_2), 29.2 (CH_2), 25.1 (CH_2) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –5.4 ppm. MS (ES^+): m/z = 596.11 $[M + Na]^+$; 612.11 $[M + O + Na^+]$. HRMS: calcd. for $C_{31}H_{32}N_3NaO_6P$, 596.1926; found 596.1908. Crystals suitable for X-ray diffraction were grown from an acetonitrile solution at room temperature.

7*p*: The same method as for **6*p*** was employed but starting from 0.3 mmol (85.8 mg) of mononucleoside **4**; yield 103 mg (60%). IR (KBr): $\tilde{\nu}_{max}$ = 3423, 3054, 2927, 1686 ν(C=O), 1475, 1434, 1276, 1093, 746, 697 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 7.89 (s, 1 H, =CH), 7.79 (d, J = 1.4 Hz, 1 H, Ar), 7.76 (d, J = 1.4 Hz, 1 H, Ar), 7.39–7.26 (m, 12 H, Ar), 6.27 (t, J = 6.8 Hz, 1 H, CHOC*H*), 4.42–4.38 (m, 1 H, C*H*OH), 3.90 (dd, J = 6.7, 3.4 Hz, 1 H, C*H*OCH), 3.80 (dd, J = 12.1, 3.1 Hz, 1 H, CH_2OH), 3.71 (dd, J = 12.1, 3.7 Hz, 1 H, CH_2OH), 3.40 (t, J = 6.7 Hz, 2 H, NHCH_2), 2.39 (t, J = 7.2 Hz, 2 H, CH$_2$CH_2CH), 2.25–2.22 (m, 2 H, CHCH_2CH), 1.82 (quint, J = 6.9 Hz, 1 H, CH$_2$CH_2CH$_2$) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 170.0 (C), 166.1 (C), 152.2 (C), 138.8 (CH), 135.1–128.2 (C, CH), 114.8 (C), 88.9 (CH), 86.3 (CH), 72.1 (CH), 62.8 (CH_2), 41.3 (CH_2), 40.0 (CH_2), 29.2 (CH_2), 25.2 (CH_2) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –5.6 ppm. MS (ES^+): m/z = 596.02 $[M + Na]^+$. HRMS: calcd. for $C_{31}H_{32}N_3NaO_6P$, 596.1926; found 596.1915.

8: The same method as for **7*o*** was employed but using 4-[bis(2-diphenylphosphanyl)ethyl]amino-4-oxobutanoic acid (**5**). Yields in the 10–20% range were obtained. IR (KBr): $\tilde{\nu}_{max}$ = 3423, 3054,

2923, 1671 ν(C=O), 1475, 1434, 1276, 1096, 742, 698 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 7.85 (s, 1 H, =CH), 7.39–7.29 (m, 20 H, Ar), 6.25 (t, J = 6.8 Hz, 1 H, CHOC*H*), 4.39–4.37 (m, 1 H, C*H*OH), 3.89 (dd, J = 6.3, 3.2 Hz, 1 H, C*H*OCH), 3.79 (dd, J = 12.1, 3.0 Hz, 1 H, CH_2OH), 3.69 (dd, J = 12.1, 3.5 Hz, 1 H, CH_2OH), 3.39–3.31 [m, 4 H, (CH_2)$_2$N], 3.20–3.12 (m, 2 H, NHCH_2), 2.37 (s, broad, 4 H, CCH_2CH_2C), 2.30 (m, 4 H, CH$_2$CH_2CH), 2.25 (t, J = 7.8 Hz, 2 H), 2.19–2.16 (m, 2 H, CHCH_2CH), 1.71–1.564 (m, 2 H, CH$_2$CH_2CH$_2$) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 174.8 (C), 173.6 (C), 165.9 (C), 152.2 (C), 139.4 (d, J_{CP} = 12.6 Hz, 1 C), 139.0 (d, J_{CP} = 12.2 Hz, 1 C), 138.8 (CH), 133.9–133.6 (CH), 130.3–129.6 (CH), 114.8 (C), 88.9 (CH), 86.4 (CH), 72.2 (CH), 62.9 (CH$_2$), 46.6 (d, J_{CP} = 24.7 Hz, CH$_2$), 45.0 (d, J_{CP} = 24.1 Hz, CH$_2$), 41.4 (CH$_2$), 39.2 (CH$_2$), 31.8 (CH$_2$), 29.5 (CH$_2$), 29.2 (CH$_2$), 28.5 (d, J_{CP} = 14.6 Hz, CH$_2$), 27.3 (d, J_{CP} = 13.8 Hz, CH$_2$), 24.9 (CH$_2$) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –21.0, –21.7 ppm. MS (ES^+): m/z = 831.02 $[M + Na]^+$; 847.02 $[M + O + Na]^+$. HRMS: calcd. for $C_{44}H_{50}N_4NaO_7P_2$, 831.3052; found 831.3040.

5-[3-*N*-(Trifluoroacetyl)aminopropyl]-5′-*O*-(4,4′-dimethyltrityl)-2′-deoxyuridine (10): The monocleoside **9** (2.0 g, 2.94 mmol) and 10% Pd/C (313 mg, 0.294 mmol of Pd) were suspended in 30 mL of dry methanol and hydrogenated under 10 bar of H_2 for 24 h at room temperature. The resulting suspension was filtered through a pad of celite twice and concentrated in vacuo. The crude product was purified via a short column (SiO_2, CH_2Cl_2/MeOH/NEt_3, 89:10:1) to yield, after removal of the solvent, the title product as a pale yellow solid; yield 1.91 g (95%). Characterization of the product fully agreed with data in the literature.[28]

Example of Phosphane Modification of a 15-mer DNA Strand: The DNA strand (15 nmol) was dissolved in a 300 μL of a 0.1 M $NaHCO_3$ aqueous solution. One of the diphenylphosphanylbenzoic acids (2.3 mg, 7.5 μmol, 500 equiv.), EDC·HCl (1 mg, 9 μmol, 600 equiv.), and NHS (1.4 mg, 7.5 μmol, 500 equiv.) were dissolved in 600 μL of DMF and stirred for 3 h. The activated phosphane solution was added to the DNA solution and stirred for 12 h. 600 μL of water were added to the reaction mixture, which was washed with dichloromethane (3 × 2 mL). The aqueous layer was concentrated in vacuo and redissolved in 1 mL of water. 200 μL of this solution were placed in an Eppendorf vial. 20 μL of a 3 M NaOAc aqueous solution and 700 μL of cold EtOH were added to the DNA solution which was cooled to 0 °C for 1 h. It was centrifuged for 30 min at 4 °C at 13000 rpm. The supernatant was removed and discarded, a cold 70% aqueous EtOH solution was added and the sample was centrifuged for another 30 min. Removal of the supernatant left a pellet of purified DNA.

General Procedure for Allylic Alkylation of 1,3-Diphenylallyl Acetate with Dimethyl Malonate: A mixture of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (0.5 μmol) and ligand (2 μmol) were dissolved in DCM, and stirred for 30 min. A trace of KOAc, 1,3-diphenylallyl acetate (100 μmol), diphenyl ether (100 μmol), dimethyl malonate (300 μmol) and BSA (300 μmol) were added to the metal complex solution in this order. The reaction mixture was stirred for 24 h. Each solution was filtered through SiO_2 and left to evaporate overnight. A CHIRACEL OD-H chiral column (Daicel Chemical Industry Ltd.) was employed using a 99:1 (hexane/*i*PrOH) isocratic mixture with a flow of 1 mL/min; $t_R(S)$ 23.6 min, $t_R(R)$ 24.3 min.

General Procedure for Allylic Amination of 1,3-Diphenylallyl Acetate with Benzylamine: A mixture of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (0.5 μmol) and ligand (2 μmol) were dissolved in DCM, and stirred for 30 min. 1,3-Diphenylallyl acetate (100 μmol), diphenyl ether (100 μmol) and benzylamine (300 μmol) were added to the metal complex solution in this order. The reaction mixture was stirred for 24 h. Each solution was filtered through SiO_2 and left to evaporate overnight. A CHIRACEL OD-H chiral column (Daicel Chemical Industry Ltd.) was employed using a 99.5:0.5 (hexane/*i*PrOH) isocratic mixture with a flow of 0.5 mL/min; $t_R(R)$ 28.4 min, $t_R(S)$ 30.3 min.

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