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P-Cyclisations

Stereoselective *P*-Cyclisation and Diastereoisomeric Purification of 5-Phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine Formed from a Thermolabile Protecting Group

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Abstract: A one-pot, two-step synthesis of 5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine from linear precursor bis(diisopropylamino){2-[(pyridin-2-yl)amino]-1-phenylethoxy}phosphine is achieved using a stereoselective intramolecular cyclisation. Application of a pure enantiomer {1-phenyl-2-[(pyridin-2-yl)amino]ethanol} enabled partial diastereopurification by crystallisation. For all four diastereoisomers, the absolute configuration of the *P*-centre was determined using X-ray crystallography and correlative ^{31}P NMR data. Stereochemically pure **5a** was then used in nucleoside phosphitylation reactions with partial loss of stereopurity by retention of configuration on the phosphorus centre.

Introduction

Five-membered heterocycles[1] with a stereogenic phosphorus atom are broadly applicable in stereoselective syntheses of compounds involved in biopharmaceutical drug design efforts.[2] As potential therapeutic agents, oligodeoxyribonucleotide phosphorothioates (PS-ODNs)[3] and PS-nucleotides[4] require improved synthetic availability, hopefully by methods that provide defined chirality on the *P*-centre. Oxathiaphospholanes,[5,6] indoloxazaphosphorines,[7] xylose-oxazaphosphorinanes,[8] *N*-acylphosphoramidites,[9] and oxazaphospholidines[10] have all been used to form *P*-stereospecific backbones in nucleic acids. Application of stereocontrol in synthetic oligoribonucleotide phosphorothioates (PS-ORNs) has been achieved by the oxazaphospholidine approach[11] using highly nucleophilic azole activators.[12] PS-ORNs have been successfully synthesized on solid support with both sufficient coupling efficiencies (94–99 %) and acceptable diastereoselectivities (≥ 98:2).[13]

Compounds bearing the 1,3,2-oxazaphospholidine framework are promising agents in *P*-stereospecific synthesis. Such agents have been obtained by the reaction of trivalent phosphorus with substituted amino alcohols or other amine derivatives. 2-Octadecyl-1,3,2-oxazaphospholidine has been used in oxidative decyclisations with bromine to produce cationic amido diester phospholipids.[14] It is also well established that 2-chloro-3-methyl-1,3,2-oxazaphospholidine can serve as an alternative to synthons commonly employed in oligonucleotide synthesis. Moreover, 3-methyl-1,3,2-oxazaphopspholidine nucleoside phosphoramidites have been successfully used to install the internucleotidic diester phosphate bond in the dimer and 17-*mer* combination with complete removal of the base protecting groups under standard deprotection conditions (28 % NH_4OH, 55 °C, 12 h).[15]

However, 3-(pyridin-2-yl)-1,3,2-oxazaphospholidine is formed as a result of intramolecular cyclisation of 2-PyTPG (2-Pyridinyl Thermolabile Protecting Groups).[16,17] Consequently, 2-PyTPGs temporarily "lose" their thermolabile properties, but gain stability under ambient conditions. The presence of a pyridinyl moiety changes bond polarisation and facilitates its reopening and restoration of thermolability. This process is known as the "click-clack" approach, to denote the facile switching between two states: labile and stable.[18] It has been shown that 2-PyTPGs can support oxidation of *H*-phosphonates to phosphate diesters when using 1,3,2-oxazaphospholidine oxide.[19] On the basis of these realisations we reasoned that 2-(diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine might be a potentially convenient phosphitylating agent. The prospect that this reagent might offer some level of stereocontrol during the phosphitylation process enhanced our enthusiasm, especially in light of the importance of stereopure nucleosides and oligonucleotide analogues in both therapeutic and biological contexts.

Results and Discussion

Here, we report the stereoselective formation of 2-(diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2oxazaphospholidines (**5a**–**d**) resulting from a one-pot, two-step reaction of 1-phenyl-2-[(pyridin-2-yl)amino]ethanol (**3**) (Scheme 1) and their potential as phosphorylating agents. Also, studies of the crystal structure and partial diastereopurification of **5a**–**d** are presented. The presence of a phenyl ring had two significant effects: (i) it generated a stereogenic centre, which, after *P*-cyclisation, formed the diastereoisomers that are easily identifiable by ^{31}P NMR

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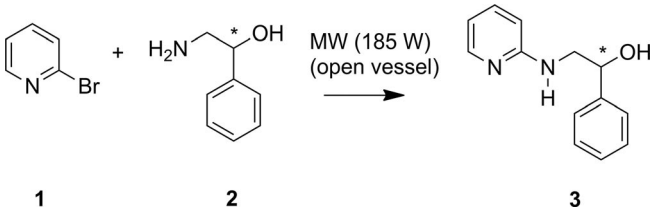
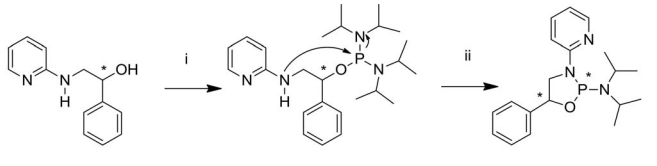
spectroscopy, and (ii) it introduced steric considerations that influence the stereochemistry at the phosphorus centre. *P*-Cyclisation of phosphorus-containing thermolabile protecting groups based on intramolecular attack of a nitrogen atom upon the phosphorus atom in bis(diisopropylamino){1-phenyl-2-[(pyridin-2-yl)amino]ethoxy}phosphine (**4**) afforded oxazaphospholidines **5a**–**d**. Consequently, the thermolabile properties of the protecting group were "frozen", and their recovery was not possible until the oxazaphospholidine ring was opened. *P*-Cyclisation of TPGs is possible in precursors bearing an H atom at the pyridine-conjugated exocyclic amino moiety. 1-phenyl-2-[(pyridin-2-yl)amino]ethanol (**3**) is such a precursor of TPGs and is readily synthesised in a microwave reactor with an open vessel using a waveguide-assisted platform to increase the yield. Purification of **3** was possible by crystallisation; its crystal structure has been previously published.[20] This preliminary study showed that the electron-withdrawing effect of the phenyl group depletes electron density of the asymmetric center (Scheme 1) and thereby influences N–P bond generation involved in cyclisation (Scheme 2).[20] The presented *P*-cyclisation process was induced by activating one nitrogen atom in the diisopropylamine substituent in **4**, either spontaneously in the presence of diisopropylamine hydrochloride, or by using a weak acid in the reaction mixture. Our preliminary observation based on ^{31}P NMR analysis showed that *P*-cyclisation of **4** gave unequal quantities of two products. However, we observed that both products were formed simultaneously. The one-pot, two-step synthesis started from 1-phenyl-2-[(pyridyn-2-yl)amino]ethanol (**3**) and chloro(diisopropylamino)phosphine to obtain **4** without final purification (Scheme 2). Stable oxazaphospholidine **5**, the result of *P*-cyclisation, was purified by chromatography and its structure rigorously elucidated.



Scheme 1. Microwave-assisted 1-phenyl-2-[(pyridin-2-yl)amino]ethanol synthesis.



No	C
3a	*R*,*S*
3b	*R*

No	C
4a	*R*,*S*
4b	*R*

No	C	P	^{31}P NMR	Ratio
5a	*R*	*S*	128 ppm	87%
5b	*S*	*R*	128 ppm	
5c	*R*	*R*	116 ppm	13%
5d	*S*	*S*	116 ppm	

Scheme 2. One-pot, two-step synthesis of **5** with mechanism of stereoselective intramolecular cyclisation and corresponding ^{31}P NMR chemical shifts indicated. (i) chloro(diisopropylamino)phosphine, benzene; (ii) benzene, spontaneously. Compound **4** was characterised by ^{31}P NMR spectroscopy but not isolated.

P-Cyclisation of a racemic mixture of **4a** proceeded with a high degree of selectivity leading to a diastereomeric product ratio of 87:13 (as determined on the basis of integration; Scheme 2). Subsequently, a mixture of all four possible stereoisomers (**5a**–**d**), grouped in two pairs of enantiomers, were obtained. In this arrangement, ^{31}P NMR analysis in an achiral solvent (benzene) showed two signals with different intensities and a chemical shift of δ = 128.4 ppm for one pair of enantiomers and δ = 116.7 ppm for the other enantiomer pair (Figure 2A). Correlating the specific and relevant diasteroisomers to concrete spectroscopic signals was not possible with any certainty. However, after purification, compounds **5a**–**d** were easily crystallised. The ^{31}P NMR analysis of redissolved crystals showed the presence of only one peak (δ = 128.4 ppm; Figure 2B), and crystallographic analysis confirmed the presence of both enantiomers **5a** and **5b** (Figure 1). This enabled us to assign absolute configurations of (R_C,S_P) and (S_C,R_P) to **5a** and **5b**, respectively.

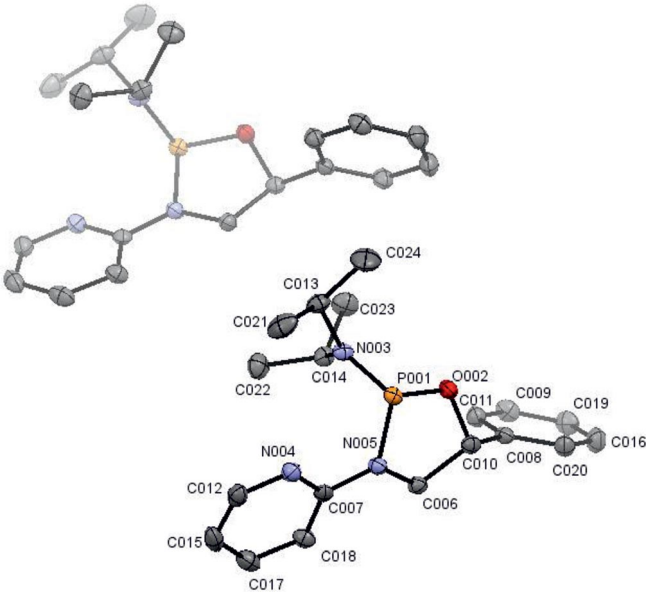


Figure 1. Crystal structure of enantiomers **5a** and **5b**. The molecule in the background is generated by a mirror plane. Ellipsoids are drawn at the 50 % probability level. Hydrogen atoms are omitted for clarity.

In order to simplify the analysis, the *P*-centre was protected by a TPG using precursor **3b** with a defined (*R*) configuration at the lone asymmetric center. ^{31}P NMR studies of **4b** generated in this way gave the same result as had the racemic mixture of **4a**; two peaks in a ratio of ca. 85:15 (Figure 3A) were clearly apparent. Using the (*R*)-configured substrate **3b** allowed us to eliminate enantiomer **5b** (S_CR_P) paired previously with **5a** (R_CS_P) in the crystal and observed as the same ^{31}P NMR signal (δ = 128.4 ppm; Figure 2B).

The products of *P*-cyclisation of **4b** were also obtained as crystals, but their crystallographic analysis[21] this time revealed a different composition of diastereoisomers: **5a** (R_CS_P) and **5c**

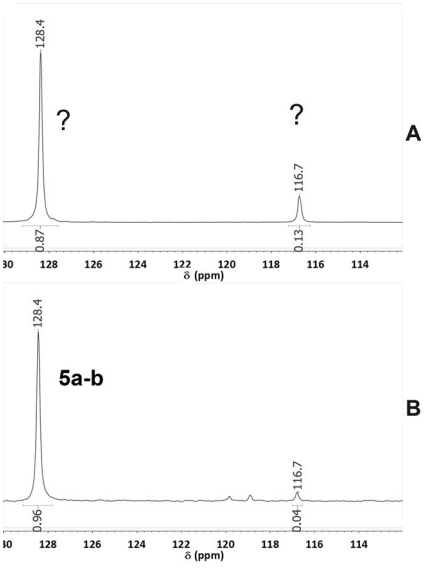


Figure 2. ^{31}P NMR analysis of **5a**–**d**: (A) reaction mixture; (B) redissolved crystals (presence of a peak at δ = 116 ppm is probably caused by the solvent residue, which contains some amount of **5c** and **5d**) at the surface of crystals.

(R_CR_P) in the crystals and the redissolved crystals showed two signals in an equal ratio upon ^{31}P NMR analysis (Figure 3B). Because **5c** was obtained in smaller quantity and crystallised completely with **5a**, it became clear that the obtained solution contained pure diastereoisomer **5a**.

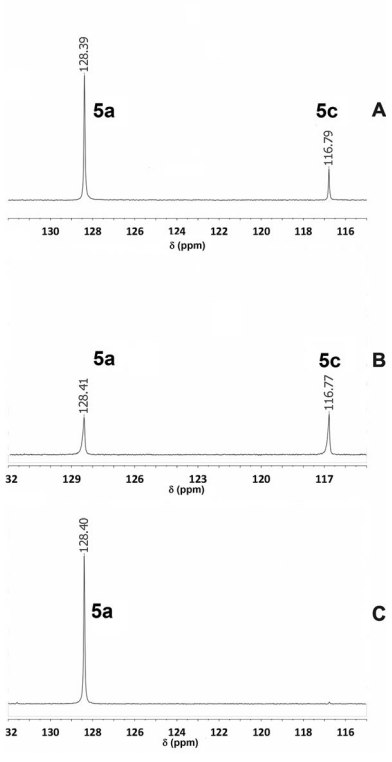


Figure 3. ^{31}P NMR analysis of **5a** and **5c**: (A) reaction mixture; (B) redissolved crystals; (C) solution after crystallisation.

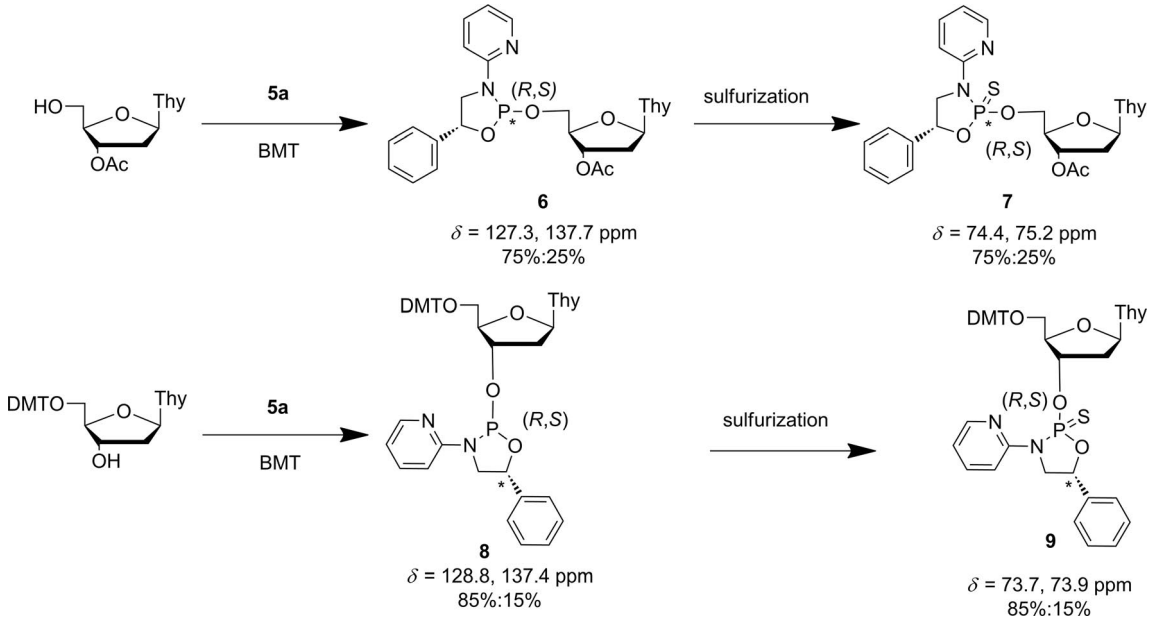
Thus, crystallisation is possible only between two matching diastereoisomeric forms (Figure 3B). Therefore, if **5a** and **5c** are initially present in the solution in an unequal ratio (Figure 3A), the solution after combining **5c** with the corresponding amount of **5a** during crystallisation contains only the remaining diastereoisomer **5a** (Figure 3C); **5a** is stable during weeks of storage, and no transformation to **5b** or other forms was observed. The discussed process, i.e. diastereoselective purification by crystallisation, may be referred to as "crystallisation by exclusion". This term denotes the fact that one diastereoisomer, which is present in smaller quantities, is excluded from solution by a crystallisation process that precedes dissolution in contrast to simple purification by crystallisation.

The experiments described above enabled us to determine the correlation between absolute configurations of **5a**–**d** and chemical shifts in their ^{31}P NMR spectra. As a result, we could confidently assign the ^{31}P resonance at δ = 128 ppm to the (R_CS_P) configuration and the resonance at δ = 116 ppm to the (R_CR_P) configuration. The obtained data reveal that the pair of enantiomers **5a** and **5b** correlate to one peak at δ = 128 ppm, and the second pair of enantiomers **5c** and **5d** gives rise to the NMR signal at δ = 116 ppm.

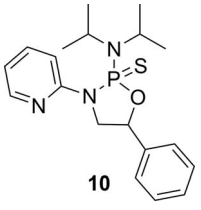
Crystallographic data and ^{31}P NMR studies also showed that the *syn* configuration of the phenyl group and phosphorus substituent (in relation to the oxazaphospholidine ring) is preferred among the *P*-cyclisation products identified. This stereoselectivity is probably dictated by steric interactions between all three spatially large substituents of the oxazaphospholidine ring.

The obtained fraction with a pure diastereoisomer allowed us to study the stereopurity of subsequent phosphitylation reactions and to exploit thermolabile properties in the formation of phosphate and thiophosphate[22] backbones in nucleic acid chemistry. To demonstrate this, a solution containing only one diastereoisomer (R_CS_P) (**5a**) was used in phosphitylation reactions with (i) a free primary hydroxy group of 3′-*O*-acetylthymidine, and (ii) a secondary hydroxy group of 5′-*O*-DMT-thymidine (Scheme 3). Unfortunately, the stereopurity was lost, and two kinetic products were formed in unequal proportions. It is known that phosphitylation reactions catalysed by weak acids, for example 1*H*-tetrazole, are performed with complete inversion of configuration. Based on studies of the kinetic and thermodynamic stability of the product formed,[23] it was concluded that phosphitylation by **5a** leads to the formation of two epimers in a ratio of 75:25 in the case of **6** and a ratio of 85:15 in the case of **8**; both ratios result from inversion of the P-atom configuration. Similar stereochemical outcomes were observed when the mixture of all four diastereoiseomers (**5a**–**d**) was applied to similar phosphitylation reactions.

Finally, we found that both **6** and **8** were amenable to sulfurisation and that this chemistry did not alter the diastereomeric ratio for each set of products. The ratio of diastereoisomers for both **6** and **7** was 75:25 and the ratio for both **8** and **9** was 85:15; in neither case did P–S bond formation impact the stereopurity of substrates **6** and **8**. Notably, 1,3,2-oxazaphospholidine 2-sulfides **10** (Scheme 4) are stable to acid hydrolysis and do not generally suffer ring-opening reactions.



Scheme 3. Reaction of (R_CS_P)-oxazaphospholidine (**5a**) with 3′-*O*-acetylthymidine and 5′-*O*-DMT-thymidine affording **6** and **8**. Both **6** and **8** display partial loss of stereopurity on the phosphorus centre. Phosphitylated **6** and **8** were then sulfurized to **7** and **9**, respectively.



10

Scheme 4. Compound **10** is useless as a phosphitylating agent (for more details see the Supporting Information).

Conclusions

In the case of the presented cyclisation of **4a** to **5**, diastereoselectivity was observed. Using substrate **4b** with a known configuration as well as correlating the results of ^{31}P NMR and crystallographic analyses allowed us to ascribe specific resonances to individual stereoisomers. Hence, we suggest that the *syn* configuration of substituents on the asymmetric carbon and phosphorus atoms is preferred. It was also possible to isolate, by crystallisation, a pure product **5a** with known absolute configuration. We attempted to use **5a** as a phosphitylating agent to stereoselectively obtain a thymidine oxazaphospholidine analogue that is stable during storage, yet easily convertible to 2-PyTPG. Because nucleoside phosphitylation by **5a** resulted in partial loss of P-atom stereopurity, we plan to continue studies to find an effective method for stereopure phosphitylation as a stereocontrolled method in nucleic acid chemistry. Further research will be focused on oxazaphospholidine ring-opening reactions using nucleophilic agents such as hydroxy, amino or phosphate groups as well as possible ways of recovering the thermolabile properties of TPGs.

Experimental Section

Materials and General Methods: Common chemicals and solvents were purchased from various commercial sources and used without further purification. Benzene was distilled from P_2O_5 and CaH_2, and stored over 3 Å molecular sieves prior to use. Anhydrous acetonitrile – commercially available (max. 30 ppm H_2O) – was stored over 3 Å molecular sieves to decrease the water level to ca. 5 ppm. Molecular sieves were also used for anhydrous amines. 3′-*O*-Acetylthymidine and 5-benzylmercaptotetrazole (BMT) were dried by lyophilisation from benzene. The progress of the reactions was monitored by thin layer chromatography (TLC) conducted on 2.5 cm × 7.0 cm glass plates coated with a 0.25 mm thick layer of silica gel 60 F_{254}. Chromatography on silica gel columns was performed using silica gel 60 (70–230 mesh).

Microwave Reactor: An ERTEC® open-vessel microwave reactor was used, power range 0–750 W with stepless adjustment, field frequency 2.45 GHz and reflected power monitoring. The measurement was performed using a pyrometer, and the reaction temperature was monitored in the range of 0–500 °C.

^{1}H, ^{13}C, ^{31}P NMR and Mass Spectrometer Parameters: The NMR spectra were obtained with a Bruker spectrometer (400 or 500 MHz for ^{1}H and 125 MHz for ^{13}C). For ^{31}P NMR analysis, a Varian 300 MHz and a Bruker 400 MHz spectrometer were used. Chemical shifts δ are reported in parts per million (ppm). The residual protonated solvent was used as internal standard ([D_6]DMSO at δ = 2.50 ppm). *J* values are given in Hz. Abbreviations used in the description of resonances are: s (singlet), d (doublet), t (triplet), br. (broad), dd (double doublet), ddd (double double doublet) and m (multiplet). The mass spectrometer was equipped with an electrospray ionisation source (ESI) and a q-TOF analyser. Source parameters are as follows: ESI source voltage of 3.2 kV, nebulisation with nitrogen at 0.4 bar, dry gas flow of 4.0 L/min at 220 °C or 50 °C.

X-ray Crystallography Studies: All crystals were obtained from hexane by slow concentration at room temperature until the first

crystals could be observed in the solution. Then, the solution was stored at 5 °C overnight. Data collections were performed at 130 K with a SuperNova diffractometer[24] using a mirror monochromator for Cu-K_α radiation (λ = 1.5418 Å). Corrections were made for the Lorentz-polarisation effect and for absorption. Unit-cell parameters were determined by a least-squares fit of 5558 (**A** = **5a**+**5b**) and 22781 (**B0** = **5a**+**5c**) reflections of highest intensity, selected from the whole experiment. SIR92[25] was used for structure solution. Refinement with the full-matrix procedure on F^2 was carried out in SHELXL97.[26] The function $\Sigma w(|F_o|^2 - |F_c|^2)^2$, where $w^{-1} = [\sigma^2(F_o)^2 + A \cdot P^2 + B \cdot P]$ and $P = [\max(F_o^2,0) + 2F_c^2]/3$, was minimised. All non-hydrogen atoms were refined anisotropically, while the positions of hydrogen atoms were calculated and refined as a riding model. CCDC 1435750 [for **A** (**5a**+**5b**)], and 1435740 [for **B0** (**5a**+**5c**)] contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

1-Phenyl-2-[(pyridin-2-yl)amino]ethanol (3): 2-Amino-1-phenylethanol (2 g, 14.6 mmol) was dissolved in 2-bromopyridine (1.152 g, 7.3 mmol). Triethylamine (0.96 g, 9.5 mmol) and a few drops of *N*-ethyldiisopropylamine were added to the mixture. The reaction was carried out in a microwave reactor (microwave power: 185 W), at a temperature range of 158–165 °C using a magnetic stirrer. Progress of the reaction was monitored using TLC (dichloromethane/methanol, 95:5). The reaction was terminated after 8 h. The resulting mixture was concentrated, and the product was isolated on a silica gel column, eluting with dichloromethane/methanol (methanol gradient 0→5 % in dichloromethane). The fractions were concentrated and dried to give the product as white powder (0.8 g, 51 % yield). ^{1}H NMR (500 MHz, DMSO): δ = 7.97 (dd, *J* = 5.0, 1.2 Hz, 1 H), 7.38–7.31 (m, 5 H), 7.25–7.22 (m, 1 H), 6.52 (dd, *J* = 11.4, 7.2 Hz, 2 H), 6.48–6.46 (m, 1 H), 5.67 (s, 1 H), 4.75 (dd, *J* = 7.7, 4.3 Hz, 1 H), 3.51 (ddd, *J* = 13.4, 6.7, 4.3 Hz, 1 H), 3.32–3.24 (m, 1 H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 158.8, 147.3, 144.3, 136.6, 127.9, 126.8, 126.0, 111.6, 108.6, 71.6, 49.3 ppm. HRMS (ESI-q-TOF): calcd. for $C_{13}H_{15}N_2O$ $[M + H]^+$ 215.1179, found 215.1178.

2-(Diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine (5a–d): PCl_3 (0.089 g, 0.65 mmol) was dissolved under an inert gas in anhydrous benzene (3.25 mL) in a flask with a magnetic stirrer, at 0 °C (ice cooling). Then, a mixture of anhydrous diisopropylamine (0.066 g, 0.65 mmol) and *N*-ethyldiisopropylamine (0.295 g, 2.27 mmol) was added in portions. The mixture was warmed to room temperature and left for 24 h. The level of conversion was monitored by ^{31}P NMR spectroscopy. After full conversion of diisopropylphosphoramidous dichloride (loss of signal δ = 168.8 ppm) to chlorobis(diisopropylamino)phosphine (δ = 134.5 ppm), the mixture was cooled to 0 °C in an ice bath, and then dry 1-phenyl-2-[(pyridin-2-yl)amino]ethanol (0.14 g, 0.65 mmol) was added in portions using a flow of inert gas for 3 h. After all alcohol had been added, the reaction was carried out at room temperature for 12 h, and the progress was monitored by ^{31}P NMR spectroscopy to obtain bis(diisopropylamino){1-phenyl-2-[(pyridin-2-yl)-amino]ethoxy}phosphine (**4**) with a ^{31}P NMR shift at δ = 113.1 ppm; after another 12 h without other operations, products of cyclisation (**5a–d**) were observed at δ = 115.9 and 128.5 ppm. Excess benzene was evaporated, and the product was isolated on a silica gel column (benzene/triethylamine, 95:5); the fractions were analysed by TLC. The mixture was dissolved in *n*-hexane (5 mL) and allowed to crystallise. After a few hours, the crystals were dried. The product was obtained with 65 % yield (0.144 g). The expected structure of the product was also confirmed by crystallographic analysis. ^{1}H NMR (500 MHz, DMSO): δ = 8.11 (dd, *J* = 4.9, 1.2 Hz, 1 H), 7.58–7.55 (m, 1 H), 7.49–7.48 (m, 2 H), 7.42 (dd, *J* = 14.3, 6.6 Hz, 2 H), 7.34 (t, *J* = 7.8 Hz, 1 H), 6.74 (d, *J* = 8.3 Hz, 1 H), 6.72 (dd, *J* = 6.9, 5.1 Hz, 1 H), 5.19 (dd, *J* = 9.9, 6.3 Hz, 1 H), 4.04 (ddd, *J* = 12.0, 10.1, 6.3 Hz, 1 H), 3.24 (td, *J* = 10.1, 1.5 Hz, 1 H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 156.7, 156.6, 148.1, 140.2, 137.7, 136.9, 126.7, 126.1, 114.4, 109.1, 77.2, 50.5, 44.9, 44.8, 25.1, 23.8 ppm. ^{31}P NMR (300 MHz, benzene): δ = 128.5, 116.9 ppm. HRMS (ESI-q-TOF): calcd. for $C_{19}H_{27}N_3OP$ $[M + H]^+$ 344.1886, found 344.1891.

3′-*O*-Acetyl-5′-*O*-[5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidin-2-yl]thymidine (6): 2-(Diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine (**5**) (0.19 g, 0.55 mmol) and 3′-*O*-acetylthymidine (0.156 g, 0.55 mmol) were dissolved in anhydrous acetonitrile (2.75 mL). Then, 5-benzylmercaptotetrazole (1.206 mL of 0.25 M solution in anhydrous acetonitrile, 0.35 mmol) was added. The reaction was monitored by ^{31}P NMR spectroscopy and finished after 24 h. The resulting mixture was concentrated, and the product was isolated on a silica gel column, eluting with dichloromethane/methanol (methanol gradient 0→5 % in dichloromethane). The fractions were concentrated and dried. The product was obtained with 25 % yield (0.08 g). ^{1}H NMR (500 MHz, DMSO): δ = 11.33 (s, 1 H), (t, 1 H), 7.6 (d, *J* = 11.4 Hz, 1 H), 7.49 (dd, *J* = 11.1, 8.1 Hz, 2 H), 7.45–7.35 (m, 4 H), 6.87–6.84 (m, 1 H), 6.73 (dd, *J* = 8.3, 4.3 Hz, 1 H), 5.84 (ddd, *J* = 13.1, 10.1, 6.4 Hz, 1 H), 5.20 (dd, *J* = 12.4, 5.6 Hz, 2 H), 4.20–3.99 (m, 4 H), 3.97 (d, *J* = 1.9 Hz, 2 H), 2.27 (dd, *J* = 8.5, 6.1 Hz, 2 H), 2.06 (s, 3 H), 1.78 (s, 3 H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 170.0, 163.7, 163.6, 150.5, 147.8, 138.5, 128.5, 128.3, 126.5, 126.4, 115.5, 109.9, 109.7, 107.8, 107.7, 84.6, 83.7, 74.7, 61.3, 36.5, 20.9, 12.3 ppm. ^{31}P NMR (300 MHz, acetonitrile): δ = 127.6, 132.6, 134, 137.8 ppm. HRMS (ESI-q-TOF): calcd. for $C_{25}H_{28}N_4O_7P$ $[M + H]^+$ 527.1690, found 527.1692.

3′-*O*-Acetyl-5′-*O*-[5-phenyl-3-(pyridin-2-yl)-2-sulfido-1,3,2-oxazaphosphosphlidin-2-yl]thymidine (7): 3′-*O*-Acetyl-5′-*O*-[5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidin-2-yl]thymidine (**6**) (50 mg, 0.95 mmol) was dissolved in anhydrous acetonitrile (4.75 mL), then sulfur (3 equiv., 9 mg, 0.28 mmol) was added. The reaction mixture was stirred under argon and monitored by ^{31}P NMR spectroscopy. After 15 min, the reaction was completed with a yield of ca. 90 %, estimated from ^{31}P NMR peak areas. ^{31}P NMR (300 MHz, acetonitrile): δ = 74.4, 75.2 ppm. HRMS (ESI-q-TOF): calcd. for $C_{25}H_{28}N_4O_7PS$ $[M + H]^+$ 559.1411, found 559.1439.

5′-*O*-(Dimethyltrityl)-3′-*O*-[5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidin-2-yl]thymidine (8): 2-(Diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine (**5**) (150 mg, 0.43 mmol) and 5′-*O*-(dimethyltrityl)thymidine (240 mg, 0.55 mmol) were dissolved in anhydrous acetonitrile (5.5 mL). Then, 5-benzylmercaptotetrazole (0.5 equiv., 41 mg, 0.22 mmol) was added. The reaction was monitored by ^{31}P NMR spectroscopy and finished after 24 h. The resulting mixture was concentrated, and the product was isolated on a silica gel column, eluting with dichloromethane/methanol (methanol gradient 0→5 % in dichloromethane). The fractions were concentrated and dried. The product was obtained with 52 % yield (180 mg). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 11.32 (s, 1 H), 8.06 (dd, *J* = 4.9, 1.8 Hz, 1 H), 7.63 (td, *J* = 7.8, 1.9 Hz, 1 H), 7.51 (s, 1 H), 7.47 (d, *J* = 7.1 Hz, 3 H), 7.42 (t, *J* = 7.3 Hz, 2 H), 7.40–7.31 (m, 4 H), 7.28 (t, *J* = 7.5 Hz, 2 H), 7.21 (d, *J* = 8.9 Hz, 4 H), 6.84 (pd, *J* = 8.8, 3.3 Hz, 6 H), 5.78 (dd, *J* = 10.0, 6.3 Hz, 1 H), 4.98 (ddt, *J* = 10.1, 6.8, 3.4 Hz, 1 H), 4.03 (d, *J* = 3.9 Hz, 1 H), 3.73 (d, *J* = 1.6 Hz, 2 H), 3.70 (d, *J* = 3.3 Hz, 6 H), 3.27–3.15 (m, 2 H), 2.44 (q, *J* = 7.1 Hz, 2 H), 1.49 (s, 3 H) ppm. ^{13}C NMR (500 MHz, DMSO): δ = 163.6, 158. 1, 158.1, 155.5, 155.4, 150.3, 150.3, 147.8, 144.6, 138.7, 138.7, 138.5, 138.4, 135.7, 135.3, 135.2, 135.2, 135.1, 129.7, 129.62, 128.5, 127.8, 127.6, 126.3, 126.3, 115.4, 115.2, 113.2, 109.7, 107.7, 107.6, 85.9, 84.0, 84.0, 83.7, 81.3, 81.2, 74.0, 73.9, 63.1, 55.0, 55.0, 55.0, 45.7, 11.7,

11.6 ppm. ^{31}P NMR (300 MHz, acetonitrile): δ = 137.36, 128.79 ppm. HRMS (ESI-q-TOF): calcd. for $C_{44}H_{43}N_4NaO_8P$ [M + Na]$^+$ 809.2711, found 809.2714.

5′-*O*-(Dimethyltrityl)-3′-*O*-[5-phenyl-3-(pyridin-2-yl)-2-sulfido-1,3,2-oxazaphospholidin-2-yl]thymidine (9): 5′-*O*-(Dimethyltrityl)-3′-*O*-[5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidin-2-yl]-thymidine (**8**) (50 mg, 0.64 mmol) was dissolved in anhydrous acetonitrile (3.25 mL), then sulfur (3 equiv., 6 mg, 0.28 mmol) was added. The reaction mixture was stirred under argon and monitored by ^{31}P NMR spectroscopy. After 30 min, the reaction was completed with a yield of ca. 95 %, estimated from ^{31}P NMR peak areas. ^{31}P NMR (300 MHz, acetonitrile): δ = 73.7, 73.9 ppm. HRMS (ESI-q-TOF): calcd. for $C_{44}H_{43}N_4NaO_7PS$ [M + Na]$^+$ 841.2431, found 841.2398.

2-(Diisopropylamino)-3-(pyridin-2-yl)-5-phenyl-1,3,2-oxazaphospholidine 2-Sulfide (10): 2-(Diisopropylamino)-5-phenyl-3-(pyridin-2-yl)-1,3,2-oxazaphospholidine (**5a–d**) (50 mg, 0.145 mmol) was dissolved in anhydrous acetonitrile (2.25 mL), then sulfur (3 equiv. 14 mg, 0.43 mmol) was added. The reaction mixture was stirred under argon and monitored by ^{31}P NMR spectroscopy. After 30 min, the reaction was completed with a yield of ca. 95, estimated from ^{31}P NMR peak areas. ^{31}P NMR (300 MHz, acetonitrile): δ = 68.9, 71.4 ppm. HRMS (ESI-q-TOF): calcd. for $C_{19}H_{26}N_3NaOPS$ [M + Na]$^+$ 398.1426, found 398.1429.

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[21] Extended crystallographic analyses can be found in the Supporting Information (Figure S4, **B0**, page 26).
[22] Compound **5** can be easily transformed into the corresponding sulfide. However, based on the results of the performed experiments it can be concluded that: (i) it cannot be used as a phosphitylating agent in a reaction with nucleotides, and (ii) it is resistant to hydrolysis under acidic conditions, which makes ring-opening difficult. More information can be found in the Supporting Information (Figure S3, page 4).
[23] The kinetic studies with **5** can be found in the Supporting Information (Scheme S1).
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