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Stereochemistry of internucleotide bond formation by the *H*-phosphonate method. Part 3: Investigations on a mechanism of asymmetric induction

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Abstract—The stereochemistry of *H*-phosphonate diester bond formation (including internucleotide ones) with ribonucleoside *H*-phosphonates as substrates has been investigated using ^{31}P NMR spectroscopy. It was found that the reactions investigated owe their stereoselectivity to a dynamic kinetic asymmetric transformation. The absolute configurations of the compounds involved in the reaction pathways were tentatively assigned on the basis of their ^{31}P NMR chemical shifts and their correctness was verified for the *H*-phosphonic–pivalic mixed anhydrides and *H*-phosphonate aryl esters.

1. Introduction

During condensation of deoxyribonucleoside 3′-*H*-phosphonate monoesters with nucleosides both diastereomers of the nascent diesters are usually formed in approximately equal amounts. In contrast, in an analogous reaction with ribonucleoside 3′-*H*-phosphonates, the formation of (D_P)[1–3] (S_P) diastereomers is favoured, and thus the reaction is stereoselective.[4,5] This stereoselectivity was found to be dependent mainly on structural elements of the reacting *H*-phosphonate (the nucleobase and its protective groups, sugar moiety protective groups), and also on the solvent composition.[4–6] Recently published papers have provided some practical solutions to attain a relatively high stereoselectivity, although no attempt has been made to elucidate the underlying mechanism of the observed phenomenon.[5,6]

Since the stereoselectivity of *H*-phosphonate condensation was expected to have its sources in the initial stages of the *H*-phosphonate diester bond formation, we investigated the stereochemical aspects of the activation of *H*-phosphonate monoesters and reactivity of the intermediates produced.

2. Results and discussion

The synthesis of ribonucleoside *H*-phosphonate diesters involves activation of nucleoside 3′-*H*-phosphonate monoesters of type **1** with pivaloyl chloride with the formation of diastereomeric mixtures of mixed anhydrides **2**-D_P(R_P) and **2**-L_P(S_P), which upon reaction with a hydroxylic component afford the corresponding diesters **3** (Fig. 1). These reactions are usually stereoselective and produce *P*-epimeric mixtures of *H*-phosphonates with the significant advantage of only one diastereomer, identified as having D_P(S_P) configuration.[4,5] However, due to the high reactivity of the mixed anhydrides of type **2**, the assignment of ^{31}P NMR signals **2A** and **2B** (Fig. 1b)[†] to individual diastereomers **2**-D_P(R_P) and **2**-L_P(S_P) cannot be done directly.

The observed stereoselectivity during the ribonucleoside *H*-phosphonate condensation may have various origins, which could arise from one of the following phenomena: (i) *dynamic thermodynamic resolution* (DYTR),[7,8] (ii) *diastereoconvergent transformation*,[8] or (iii) *dynamic kinetic asymmetric transformation* (DYKAT)[8,9] (for the relevant energy profiles see Fig. 2).

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[†] Due to a similar stereoselectivity being observed for the esterification with nucleosides and simple aliphatic alcohols,[6] the model reactions were performed with ethanol as a hydroxylic component.

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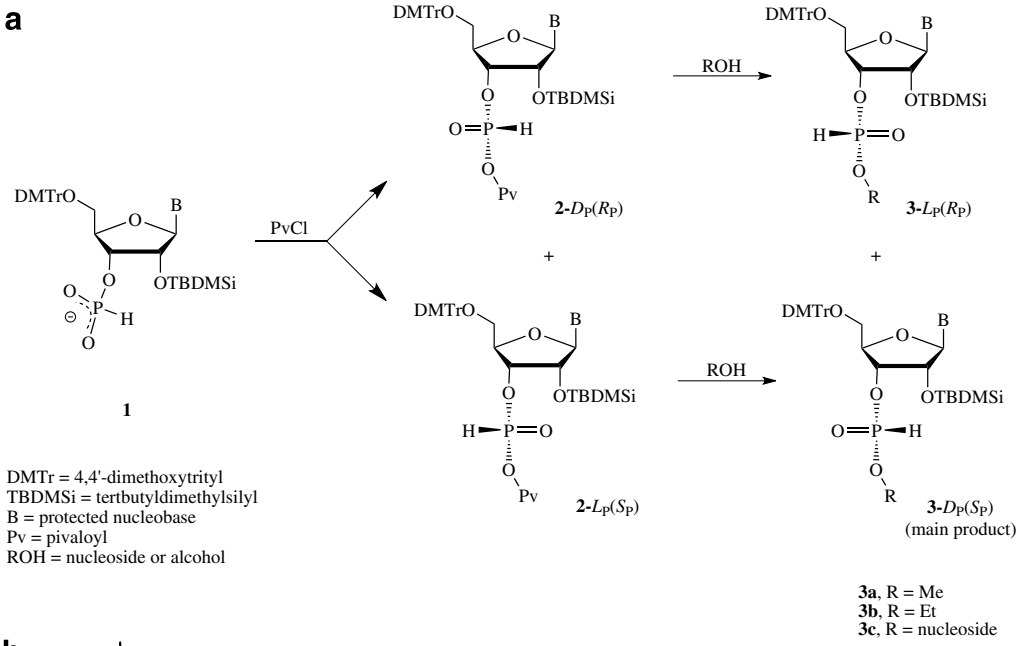

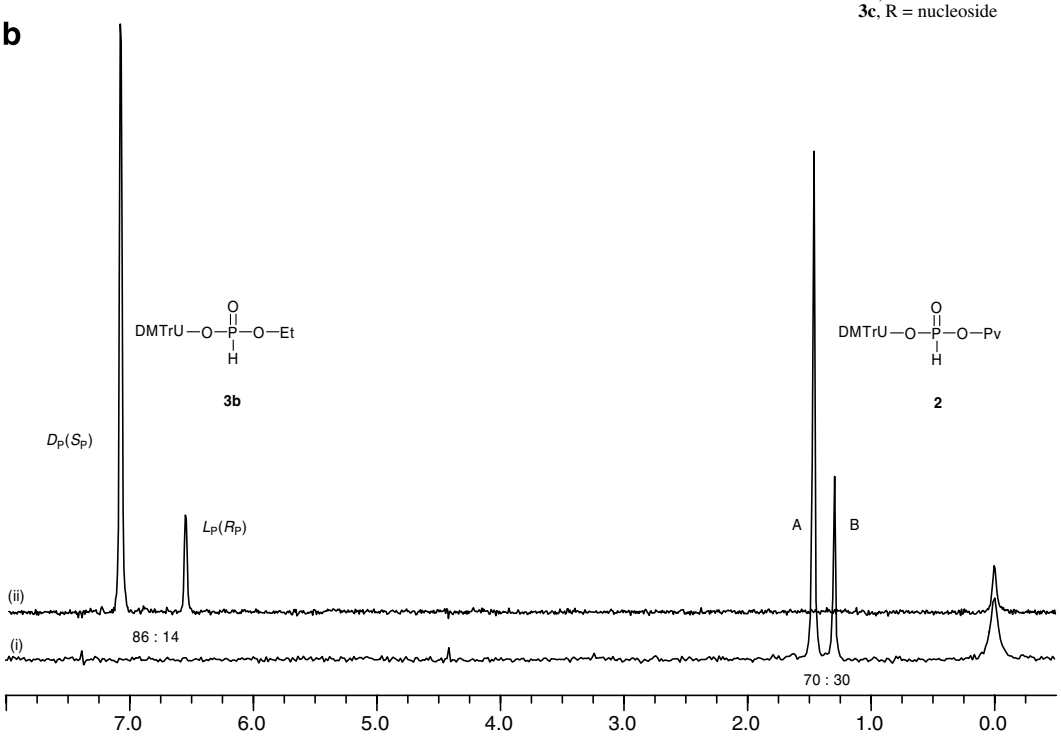

Figure 1. (a) A synthetic route from nucleoside *H*-phosphonate monoester **1** to diester **3**. (b) Exemplary ^{31}P NMR spectra of the reaction of the mixed anhydride **2** with ethanol in DCM containing 3 equiv of 2,6-lutidine; (A) the mixed anhydride **2** (B) after addition of 2 equiv of ethanol.

According to the first mechanism (*dynamic thermodynamic resolution*, Fig. 2a), the mixed anhydride diastereomers **2**-D_P and **2**-L_P are configurationally stable enough to permit their stereospecific esterification into the corresponding diesters **3**. Thus, the major $D_P(S_P)$ epimer of product **3** should descend from the main epimer **2A** of the intermediate mixed anhydride **2**, and the minor $L_P(R_P)$ epimer of product **3**, from the minor epimer **2B** of intermediate **2**.

However, integration of the ^{31}P NMR signals of intermediate **2** and product **3** revealed that the ratio of diastereomers for intermediate **2** was 70:30 (de = 40%) while that for ethyl nucleoside *H*-phosphonate diester **3b**, 86:14 (de = 72%; Fig. 1b). The significant difference in the diastereomeric ratio of intermediate **2** and product **3** indicated that the thermodynamic stability of the diastereomers of **2** does not dominate the observed stereoselectivity.

In the second scenario—*diastereoconvergent transformation* (Fig. 2b), esterification of either of the diastereomers of intermediate **2** [designated as **2**-$L_P(S_P)$ and **2**-$D_P(R_P)$] was expected to take a different course as shown in Figure 3.

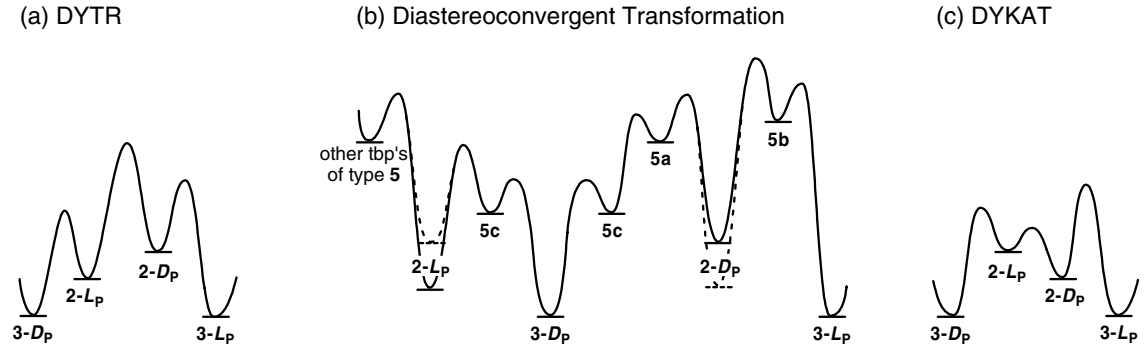

Figure 2. Possible energy profiles for stereoselective transformation of the mixed anhydride **2** into diester **3**. (a) *dynamic thermodynamic resolution*; (b) *diastereoconvergent transformation* (the dashed lines show alternative energies of intermediates **2**); (c) *dynamic kinetic asymmetric transformation*.

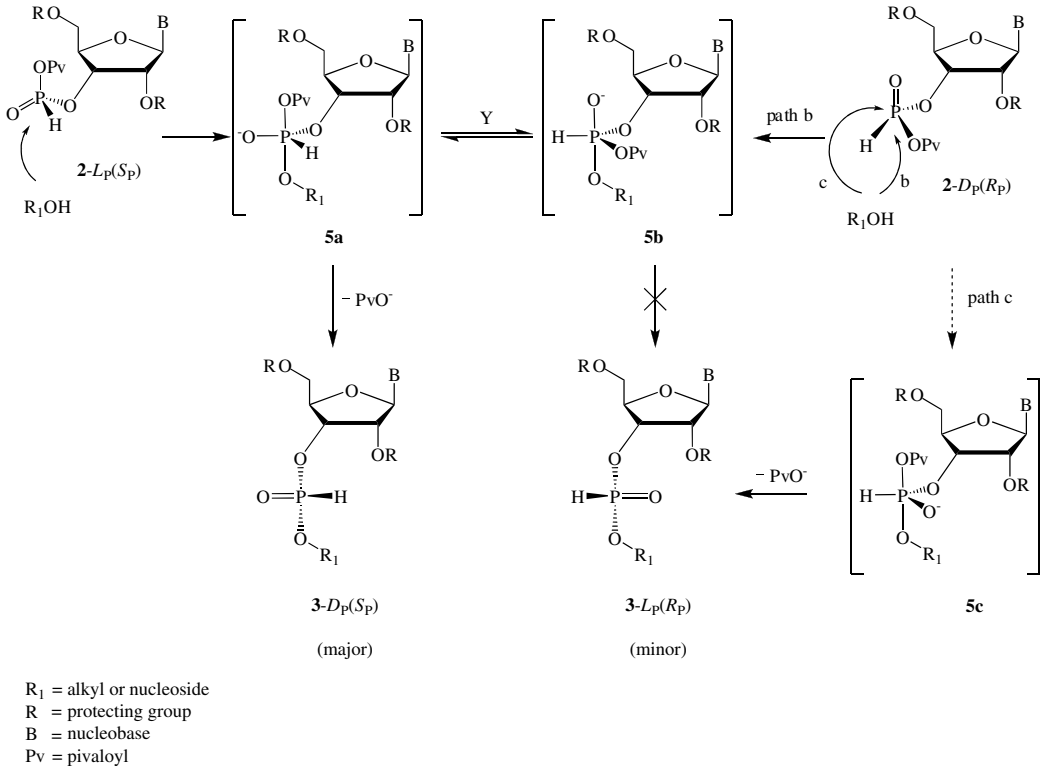

Figure 3. Putative mechanism of induction of stereoselectivity due to differences of phosphorane intermediates.

The main path of esterification of the mixed anhydride **2** having an $L_P(S_P)$-configuration should proceed via phosphorane intermediate **5a** (*tbp* with a pivaloyl residue in the apical position) that can preferentially collapse to diester **3**-$D_P(S_P)$ with inversion of configuration.

For the other diastereomer of **2** [$D_P(S_P)$], two reaction pathways involving *tbp* **5b** (path b) and *tbp* **5c** (path c) might be available. Path c should produce the $L_P(R_P)$ diastereomer of diester **3** (inversion of configuration), while path b, the same product as the one obtained from **2**-$L_P(S_P)$ [i.e., **3**-$D_P(S_P)$; retention of configuration]. To explain the observed stereoselectivity in the transformation investigated, one would have to assume that the activation barrier leading to *tbp* **5c** was higher (probably due to steric factors) than that leading from **2**-$D_P(R_P)$ to *tbp* **5b** (Fig. 2b) and the latter cannot collapse to diester immediately[10] but undergoes pseudorotation to **5a**. The assignment of the absolute configuration to **2A** and **2B** (Fig. 1b) is a complicated task in this scenario (this ambiguity is indicated by solid and dashed lines in Fig. 2b); however, for a reaction with high stereoselectivity one might expect the predominance of **2**-L_P versus **2**-D_P and thus, the assignment of signals of **2** should be similar as for a DYTR mechanism discussed above [i.e., major **2A** = **2**-$L_P(S_P)$ and minor **2B** = **2**-$D_P(R_P)$].

The major drawback of this hypothesis is that path b in Figure 3 would involve energetically unfavourable *tbp* **5b** (with apical negatively charged oxygen)[‡] and there is no obvious explanation why this route could predominate over path c.[§] However, if both paths b and c (and other possible ones) would be unfavourable, the epimer **2**-D_P could be relatively stable, and if it could interconvert into **2**-L_P, the intermediacy of pseudorotation could be omitted and the energy diagram would be identical as in the third proposed mechanism, *dynamic kinetic asymmetric transformation* (Fig. 2c).[7,8] In this scenario it is required that the energy profile of the transformation is subject to the Curtin–Hammett principle.[11] For the investigated reactions, the conditions require a rapid equilibrium between diastereomers of the mixed anhydride **2**, followed by the esterification of **2**-L_P and **2**-D_P to form the corresponding diastereomers of product **3** with different rates.

Since such an equilibrium between the diastereomers of the mixed anhydrides (**2**-$D_P \rightleftharpoons$ **2**-L_P) was crucial for the DYKAT mechanism, its existence had to be proven experimentally. To this end, *H*-phosphonic–pivalic mixed anhydride **2** (B = U) was prepared in situ from a suitably protected uridine 3′-*H*-phosphonate **1** and pivaloyl chloride (1.2 equiv). The ^{31}P NMR spectroscopy (^{1}H decoupled spectra) revealed two signals at 1.39 and 1.47 ppm in a ratio ca. 1:2, assigned to a diastereomeric mixture of **2**. The mixed anhydride **2** was quite stable under the reaction conditions [0.1 M solution in dichloromethane (DCM) containing 3 equiv of 2,6-lutidine, rt] and products of its degradation appeared in the ^{31}P NMR spectrum after ca. 1 h. No changes in the ratio of diastereomers of **2** were observed after several hours. However, the addition of triethylammonium adamantanecarboxylate (0.5 equiv) to the reaction mixture caused an immediate (ca. 1 min; time required for recording the first ^{31}P NMR spectrum) appearance of two new signals at 1.55 and 1.67 ppm (ca. 30%) due to the mixed nucleoside *H*-phosphonic–adamantanecarboxylic anhydride.[¶] In separate experiments, it was found that the ratio of the ^{31}P NMR signals of these two mixed anhydrides proportionally changed with the amount of the carboxylate (pivalate or adamantanecarboxylate) added. A similar equilibrium was observed when acetate anions were added to the reaction mixture containing the mixed anhydride **2** (data not shown); this allowed us to conclude that the mixed anhydrides of type **2** might undergo a fast exchange of the carboxylate moieties. As the solution of the in situ generated **2** always contained some quantity of pivalate anions (i.e., due to hydrolysis of PvCl by residual water), these may react with the mixed anhydride **2** causing rapid interconversion of the corresponding diastereomers (Eq. 1).[‖]

$$\mathbf{2}\text{-}D_P(R_P) + \mathrm{PvO}^- \rightleftharpoons \mathbf{2}\text{-}L_P(S_P) + \mathrm{PvO}^- \qquad (1)$$

2.1. Esterification of the mixed anhydrides of type 2

Since the fast equilibrium between the isomers of the mixed anhydride **2**-$D_P \rightleftharpoons$ **2**-L_P supported the DYKAT scenario, we tried to assess the relative rates of the equilibration and esterification as well as to find out which diastereomer of this intermediate (**2A** or **2B**, Fig. 1b) was more reactive and thus was a precursor from which the main diastereomer of the product, that is, the corresponding *H*-phosphonate diester **3**, was formed. To this end, the generated mixed anhydride **2** (B = U)[††] was treated with various aliphatic alcohols (MeOH, EtOH, *i*-PrOH, *t*-BuOH; 5 equiv), to see whether gradual changes in the ratio of diastereomers of **2** during the course of the reaction could be registered. Unfortunately, the formation of the respective *H*-phosphonate diesters of type **3** was completed before the first ^{31}P NMR spectrum could be recorded (ca. 1 min), even for sterically hindered alcohols (e.g., *t*-BuOH) and in the absence of a nucleophilic catalyst. The rates of the reactions remained beyond the time suitable for kinetic measurements by ^{31}P NMR spectroscopy also when the above alcohols were used in sub-stoichiometric amounts (0.5 equiv). Some insight into the composition of the reaction mixture at the initial stages of the reaction was gained by quenching experiment (addition of aqueous ACN). This revealed that the half-life of the reaction was ca. 13 s and the ratio of diastereomers of the produced *H*-phosphonate diester **3** was constant throughout the reaction (Fig. 4A).

When a nucleoside with a free 3′-hydroxyl function [5′-*O*-(4,4′-dimethoxytrityl)thymidine, 3 equiv] was used instead of simple alcohols as a hydroxylic component, the esterification became slow enough (ca. 8 min for completion) to record a series of the ^{31}P NMR spectra (Fig. 4B). From the collected data depicted in Figure 4, it is clear that the equilibrium between diastereomers of **2** (**2**-$D_P \rightleftharpoons$ **2**-L_P) had to be significantly faster than their esterification. As a consequence, no essential changes in the ratio of diastereomers of intermediate **2** and the products, *H*-phosphonate diesters, could be observed over the course of the reaction.

2.2. Experiments on kinetic quenching of the mixed anhydrides of type 2

The data collected so far showed that the equilibration between diastereomers of the mixed anhydride **2** is very fast; this made the measurement of kinetic parameters of particular *P*-epimers impossible. Since the efforts to reduce the rate of equilibration **2**-$D_P \rightleftharpoons$ **2**-L_P (e.g., by trimming

[‡] There are several other stereoretentive pathways leading from **2**-$D_P(R_P)$ to **3**-$D_P(S_P)$; however in each case the intermediacy of unfavourable *tbp*s is unavoidable.

[§] One should keep in mind that the above discussed pseudorotational mechanism, despite its rather low likelihood, could not be completely rejected and may contribute to some extent to the observed stereoselectivity.

[¶] The assignment of new signals to diastereomers of adamantanecarboxylic mixed anhydride was confirmed by spiking the reaction mixture with an authentic sample of this compound (prepared from *H*-phosphonate **1** with adamantanecarbonyl chloride).

[‖] The equilibrium between diastereomers of the mixed anhydride **2** may be also promoted by chloride anions present in the reaction mixture.

[††] Due to extremely high reactivity of mixed anhydrides of type **2**, all attempts to isolate these species in a pure form failed and the experiments were performed with the DCM solutions of compound **2** generated in situ.

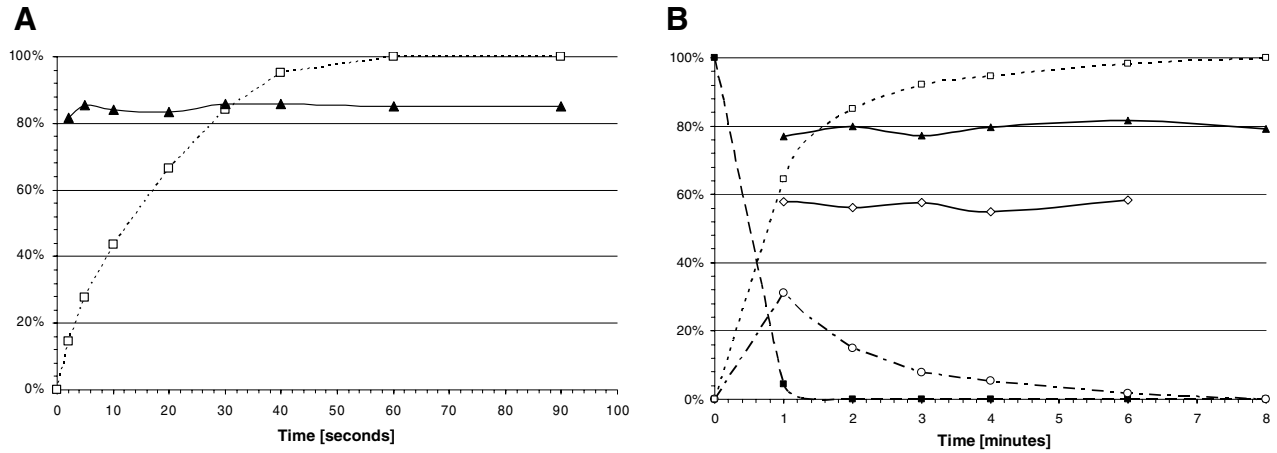

Figure 4. Reaction of uridine *H*-phosphonate **1** (B = U) with (A) EtOH (quenched with aqueous ACN) and (B) 5′-*O*-dimethoxytrityl-thymidine, promoted by pivaloyl chloride (1.5 equiv) in the presence of 3 equiv of 2,6-lutidine (^{31}P NMR data). ---■--- % of monoester **1**; ---○--- % of the mixed anhydride **2**; ---□--- % of the product [*H*-phosphonate diester **3b** (A) or 3′-3′ dinucleoside *H*-phosphonate (B), sum of diastereomers]; —◇—the fraction of the downfield signal of the mixed anhydride **2**; —▲— the fraction of D_P diastereomer of the product [$D_P(S_P)$ *H*-phosphonate diester **3b** (A) or $D_P(R_P)$ 3′-3′ dinucleoside *H*-phosphonate (B)].

down the concentration of pivalic or chloride anions that might be involved in the catalysis of the equilibration **2**-$D_P \rightleftharpoons$ **2**-L_P) were unsuccessful, experiments on kinetic quenching were attempted. We expected that the comparison of data from the regular and quenching experiments could help us in the assignment of the configuration of diastereomers **2A** and **2B**.

In preliminary experiments, stereospecific sulfurization[4] of the mixed anhydride **2** (B = U; the ratio **2A**: **2B** ≈ 2:1) yielded a configurationally stable mixture of *P*-epimers of phosphorothioic–pivalic mixed anhydride [δ_P 50.0 and 51.2 ppm], whose ratio depended significantly on the basicity of the medium. In the presence of 2,6-lutidine, this ratio was ca. 1:1, while when sulfurization was carried out in the presence of triethylamine (TEA), the upfield diastereomer dominated (ratio ca. 1:2). Since strong bases are known to speed up the sulfurization of *H*-phosphonates,[12] one could speculate that the stereochemistry observed in the presence of TEA resulted from kinetic quenching of the mixed anhydride **2**.

Unfortunately, phosphorothioic–pivalic mixed anhydrides were found to be of little use due to their limited stability. Nevertheless, the kinetic quenching observed during the fast sulfurization of the mixed anhydride **2** encouraged us to search for conditions under which a similar kinetic quenching could be achieved for the esterification of **2**.[‡‡] To achieve this goal, a DCM solution (0.3 mL) of the in situ prepared mixed anhydride **2** (B = U) was injected into methanol (5 mL, 2500 equiv). Analysis of the ^{31}P NMR spectra revealed that the esterification was quantitative and that the intensity of signals of the produced methyl nucleoside *H*-phosphonate **3a** (B = U) has been reversed, compared to the standard reaction in which only a few equivalents of methanol were used (Fig. 5U). It seems reasonable to assume that the very reactive alcohol used in large excess esterified both diastereomers of the mixed anhydride **2** with substantially higher rates than the rate of the equilibrium **2**-$D_P \rightleftharpoons$ **2**-L_P, and thus under such conditions the major $L_P(S_P)$-epimer of diester **3a** was formed from the major diastereomer **2A**, and consequently **3a**-$D_P(R_P)$, from **2B** (DYTR kinetics, Figs. 2 and 6a). As the esterification proceeds with the inversion of configuration, *P*-epimer **2A** should thus have $D_P(S_P)$-configuration, while *P*-epimer **2B**, $L_P(R_P)$-configuration.

In contrast to the above kinetic quenching experiment, under standard reaction conditions (small excess of an alcohol), the equilibration **2**-$D_P \rightleftharpoons$ **2**-L_P was presumably significantly faster than the esterification and therefore the major diastereomer $D_P(S_P)$ of the produced diester **3** should originate from the minor diastereomer **2B** of the mixed anhydride **2**, having an $L_P(R_P)$-configuration (Fig. 6b).

For other nucleotide derivatives (B = A^{Bz}, C^{Bz}, G^{ibu}) the kinetic quenching experiments gave less clear-cut results (Fig. 5A, C and G), but also in these cases the trend in changing the ratio of diastereomers of product **3a** was congruent with the postulated DYKAT mechanism.

2.3. Transesterification of aryl nucleoside *H*-phosphonates of type 4

The above experiments with *H*-phosphonic–pivalic mixed anhydrides of type **2** implied that the DYKAT mechanism was a source of stereoselectivity during the condensation of ribonucleoside *H*-phosphonates. Unfortunately, the very high rate of equilibration of the intermediate mixed anhydrides prevented a quantification of reactivities of their individual diastereomers, and the assumed DYKAT kinetics could not be proved directly. Thus, we have focused our attention on other types of active derivatives, also bearing electron-withdrawing ligands, namely aryl nucleoside

[‡‡] In mechanistic terms this meant that dynamic kinetic process (DYKAT) would be changed into dynamic thermodynamic one (DYTR).[7]

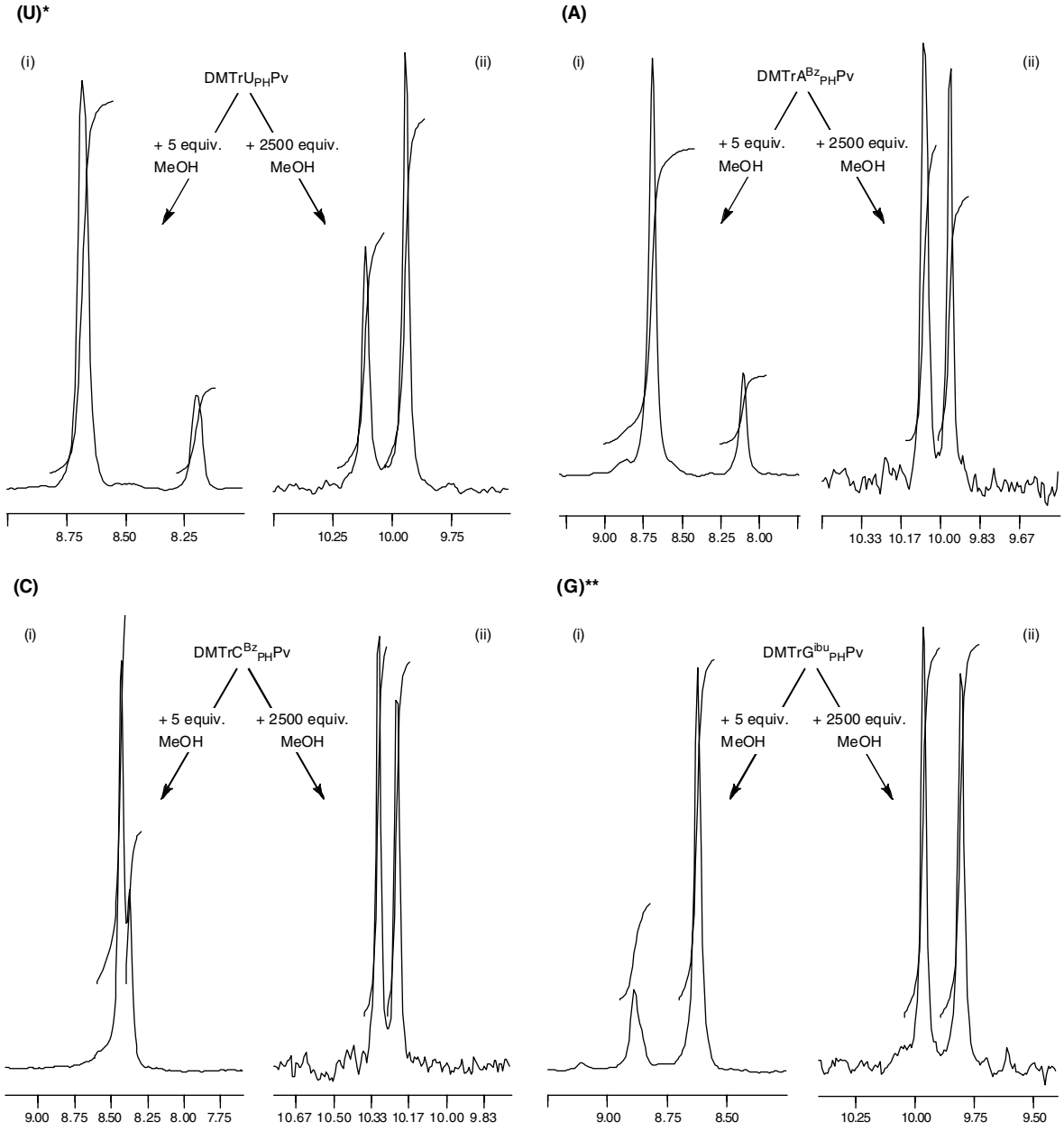

Figure 5. ^{31}P NMR spectra of methyl nucleoside *H*-phosphonates of type **3** prepared (i) under standard conditions [using 5 equiv of MeOH (10 μL)]; (ii) by quenching the reaction with 5 mL (ca. 2500 equiv) of MeOH. (A) B = A^{Bz}, (C) B = C^{Bz}, (G) B = G^{ibu}, (U) B = U. Different chemical shifts in panels (i) and (ii) are due to differences in solvent compositions.[6] *The reversal of product distribution for uridine derivative was confirmed by evaporating the solvent, dissolving the residue in DCM and mixing with an aliquot of reaction (i). **For guanosine derivative the signals of diastereomers in DCM have apparently inverted positions.[6]

H-phosphonates[13,14] of type **4** (B = U, Fig. 7), the reactivity of which can be controlled and adjusted.[15,16] We have chosen three aryl groups, phenyl, *p*-chlorophenyl and *p*-nitrophenyl, that clearly diversified the reactivity of the phosphorus centre in *H*-phosphonate diesters **4**.

From a stereochemical point of view, this approach consisted of three events: the formation of two diastereomers of the mixed anhydride **2** (i.e., **1**→**2**, Fig. 7A, discussed in previous sections), formation of aryl nucleoside *H*-phosphonate (i.e., **2**→**4**) and its transesterification with an alcohol (i.e., **4**→**3**). It is believed (and partially proven[13]) that the diastereomers of intermediate **4** exist in an equilibrium (Eq. 2), similarly as it was found for the mixed anhydrides **2** (Eq. 1).

$$\mathbf{4}\text{-}D_P(R_P) + \mathrm{ArOH} \rightleftharpoons \mathbf{4}\text{-}L_P(S_P) + \mathrm{ArOH}, \qquad (2)$$

We found that the esterification of the mixed anhydride **2** with phenol, *p*-chlorophenol and *p*-nitrophenol (3 equiv) was complete within <1–2 min (the time required for recording the first ^{31}P NMR spectrum). The ratios of the produced aryl nucleoside *H*-phosphonate diester diastereomers were 80:20, 81:19 and 71:29, for **4c**, **4b** and **4a**, respec-

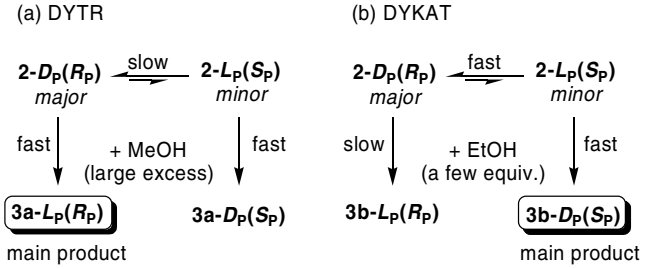

Figure 6. Postulated mechanisms of stereoselective esterification of the mixed anhydride **2**. (a) DYTR, with a large excess of methanol. The rate of equilibration is significantly lower than the rates of esterification. (b) DYKAT, with several equivalents of ethanol. The rate of esterification of **2**-$L_P(S_P)$ is comparable to the rate of equilibration and significantly higher than the rate of esterification of **2**-$D_P(R_P)$.

tively, and these were apparently equilibrium values as no changes were observed when reactions were left for several hours (^{31}P NMR). Sacrificing the advantages of pseudo-first order kinetics, we were able to slow down these reactions by reducing the excess of phenols from 3 equiv to 1.2 equiv. This manoeuvre allowed us to observe a gradual appearance of aryl *H*-phosphonates **4** at the expense of intermediate **2** during the 10 min required for the completion of the reaction. The ratio of the produced diastereomers of diesters **4** was constant during the progress of the reaction, and was the same as for the reactions with 3 equiv of phenols. Furthermore, the ratio of the diastereomers of **2** remained unchanged, and this precluded drawing tentative conclusions regarding a correlation of the ^{31}P NMR chemical shifts of the signals and configuration at the phosphorus centre during the formation of aryl diesters **4** (Fig. 8).

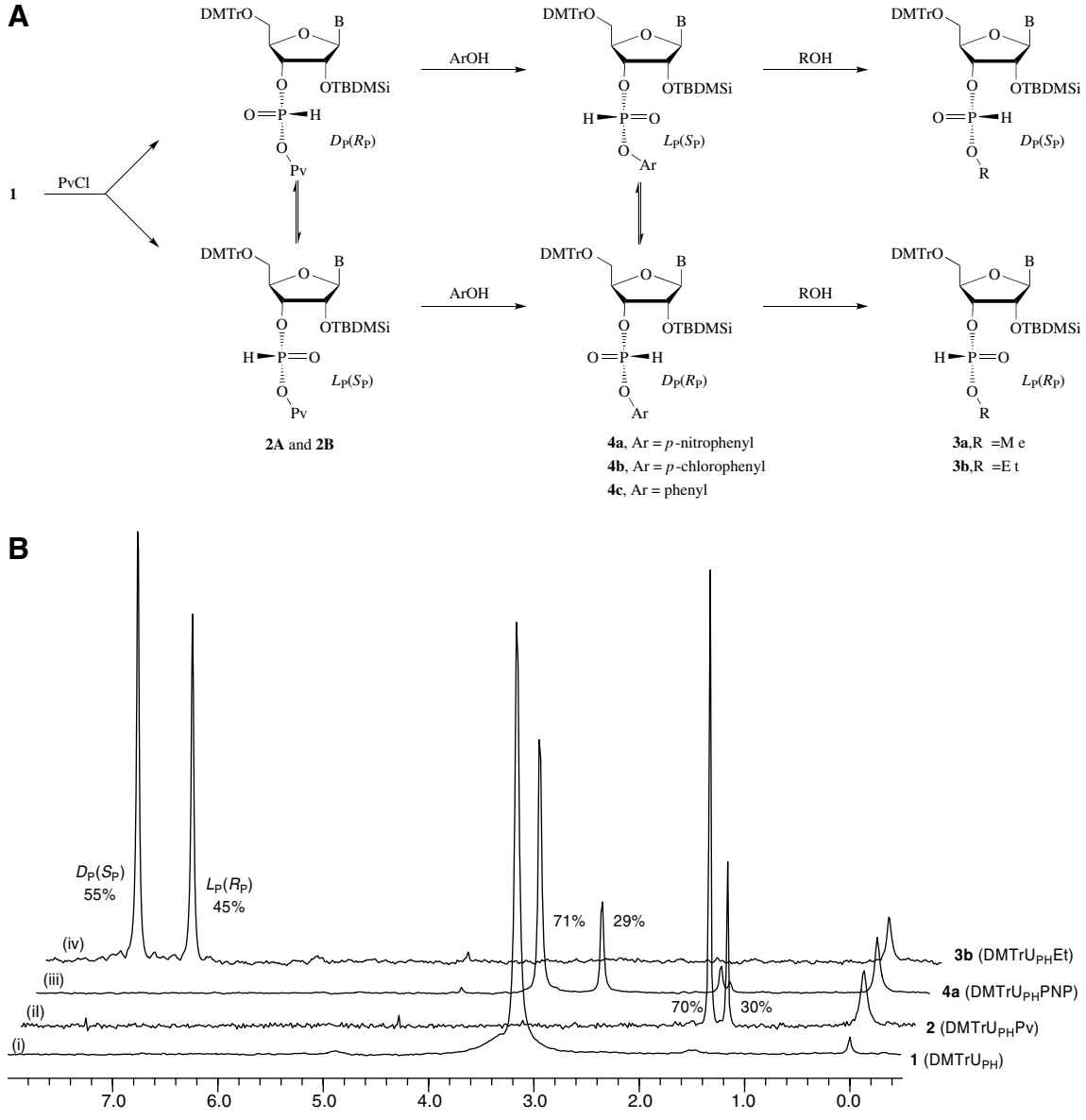

Figure 7. (A) Stereochemistry of reactions involving aryl nucleoside *H*-phosphonates. (B) An example of ^{31}P NMR traces of the above reactions leading to ethyl uridine *H*-phosphonate **3b**, using *p*-nitrophenyl (PNP) intermediate **4a** (minor signals about 1.5 ppm in spectrum (iii) are due to an equilibrium between **2** and **4a**). For abbreviations, see Figure 1.

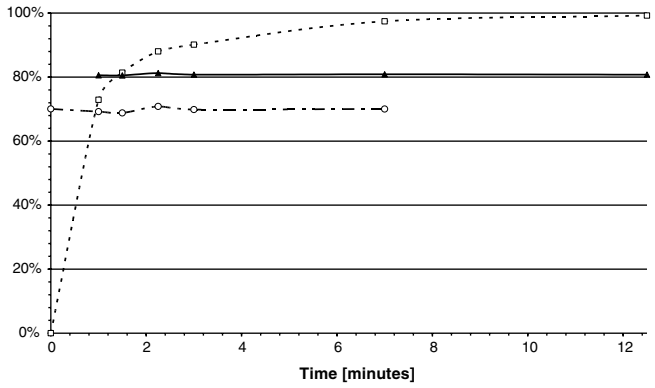

Figure 8. Reaction of the mixed anhydride **2** (generated in situ) with *p*-chlorophenol (1.2 equiv) in DCM containing 3 equiv of 2,6-lutidine (^{31}P NMR data). ---□--- % of the product (*p*-chlorophenyl nucleoside *H*-phosphonate **4b**; sum of diastereomers); ---○--- fraction of the downfield signal of the mixed anhydride **2a**; —▲— fraction of $D_P(R_P)$ diastereomer (downfield signal) of the product (*p*-chlorophenyl nucleoside *H*-phosphonate **4b**).

The initial experiments on transesterification of aryl *H*-phosphonates of type **4** (the last stage in Fig. 7A) were performed in order to find reactions having rates in a range that would enable tracking their progress with ^{31}P NMR spectroscopy. Very fast reactions could not be monitored by ^{31}P NMR (at least 1 min was required to register the spectrum), while the reactions lasting longer than one hour had to be excluded due to the possible subsequent side products formation (e.g., further transesterification[17,18] or disproportionation[13,19] of *H*-phosphonate diesters under basic conditions, or detritylation due to released HCl, PvOH and acidic phenols).

To this end, three aryl diesters **4a**, **4b** and **4c** were treated with various alcohols and the half-life times of the reactions were estimated by ^{31}P NMR (Table 1). The transesterification of phenyl *H*-phosphonate diester **4c** was too slow to be useful for our purposes and was abandoned. The convenient range of the reaction rates of several minutes was found for the transesterification of *p*-nitrophenyl uridine *H*-phosphonate **4a** with various alcohols, and for *p*-chlorophenyl uridine *H*-phosphonate **4b** with methanol. These combinations of the reactants were investigated in more detail.

2.4. Transesterification of *p*-nitrophenyl nucleoside *H*-phosphonates 4a

The progress of the reactions of *p*-nitrophenyl ester **4a** with alcohols is shown in Figure 9. In the case of methanol (Fig. 9A), the transesterification was practically over before the first spectrum could be recorded with no changes observed in the later spectra. In order to gain more insight into the composition of the reaction mixture during transesterification, the reaction was repeated and the aliquots taken 10–90 s after the addition of methanol, were quenched with aqueous acetonitrile (ACN). In this way, the reactive *H*-phosphonates **2** and **4a** were hydrolyzed rapidly to *H*-phosphonate monoester **1**, while *H*-phosphonate diester **3b** remained intact and its contents and diastereomeric composition could be analyzed. This revealed a gradual decrease in the fraction of the $D_P(S_P)$-diastereomer of product **3** during the progress of the reaction (56→43%), however, a correlation of these changes with those in the ratio of diastereomers of **4a**, was not possible.

Table 1. Estimated half-life times of transesterification of aryl uridine *H*-phosphonates **4a–c** (dmtU$_{PH}$Ar) with 5 equiv of alcohols (interpolation of ^{31}P NMR data)

Ar	+MeOH	+EtOH	+*i*-PrOH	+*t*-BuOH
	$t_{1/2}$	$t_{1/2}$	$t_{1/2}$	$t_{1/2}$
Ph	150 min	330 min	500 min	630 min
*p*Cl–Ph	35 min	90 min	180 min	270 min
*p*NO$_2$–Ph	10 s	20 s	55 s	170 s

Similar, but clearer results (longer reaction time) were obtained for the reaction of aryl *H*-phosphonate **4a** with ethanol (Fig. 9B). In this instance, the minor diastereomer of **4a** was consumed very quickly and after 2 min. in the ^{31}P NMR spectra only the major diastereomer of **4a** could be detected. Moreover, simultaneous changes in the ratio of diastereomers of the forming ethyl nucleoside *H*-phosphonate diester **3b** were observed. Initially (i.e., quenching after 10 s) the ratio of the product diastereomers was 70:30 while after 5 min, it decreased to 60:40. Such a trend indicated that it could be the minor diastereomer of the intermediate that was the precursor of the major isomer of product **3b**, and that the consumption of the minor diastereomer of **4a** was probably faster than its regeneration from the less reactive major diastereomer. This would explain why the minor diastereomer of **4a** disappeared quickly from the spectrum, and also why the ratio of diastereomers of product **3b** decreased. It should be noted that this interpretation is in line with the preliminary conclusions regarding reactivities of the mixed anhydrides of type **2** (vide supra) and strongly supports the DYKAT scenario for their esterification.

Reactions of aryl *H*-phosphonate **4a** with sterically hindered alcohols showed notably higher stereoselectivities than those with MeOH and EtOH, indicating that the equilibration rate of diastereomers of **4a** was significantly higher than the rate of their transesterification (DYKAT scenario). While for isopropanol, an increase of the ratio of diastereomers of **4a** was still perceptible (Fig. 9C), for *tert*-butanol it remained constant (Fig. 9D). In both these cases, the ratio of diastereomers of the products did not vary over the course of the reactions.

The best experiment with ethanol (Fig. 9B) was repeated for *H*-phosphonates bearing other nucleobases (Fig. 10) and the general trend in changes of the ratio of diastereomers was found to be similar in all the cases. Thus, the conclusions concerning the kinetics of transesterification drawn for *p*-nitrophenyl uridine *H*-phosphonate seem to be valid for all four *H*-phosphonates **4a**.[§§]

[§§] For cytidine derivative (Figs. 10C and 11C), the signals of diester **3** (B = C^{Bz}) overlapped, precluding their individual integration. The final fraction of $D_P(S_P)$ diastereomer (shown in the figures) was determined by changing the solvent of the quenched reaction mixture from DCM to toluene where the signals were well separated.[6]

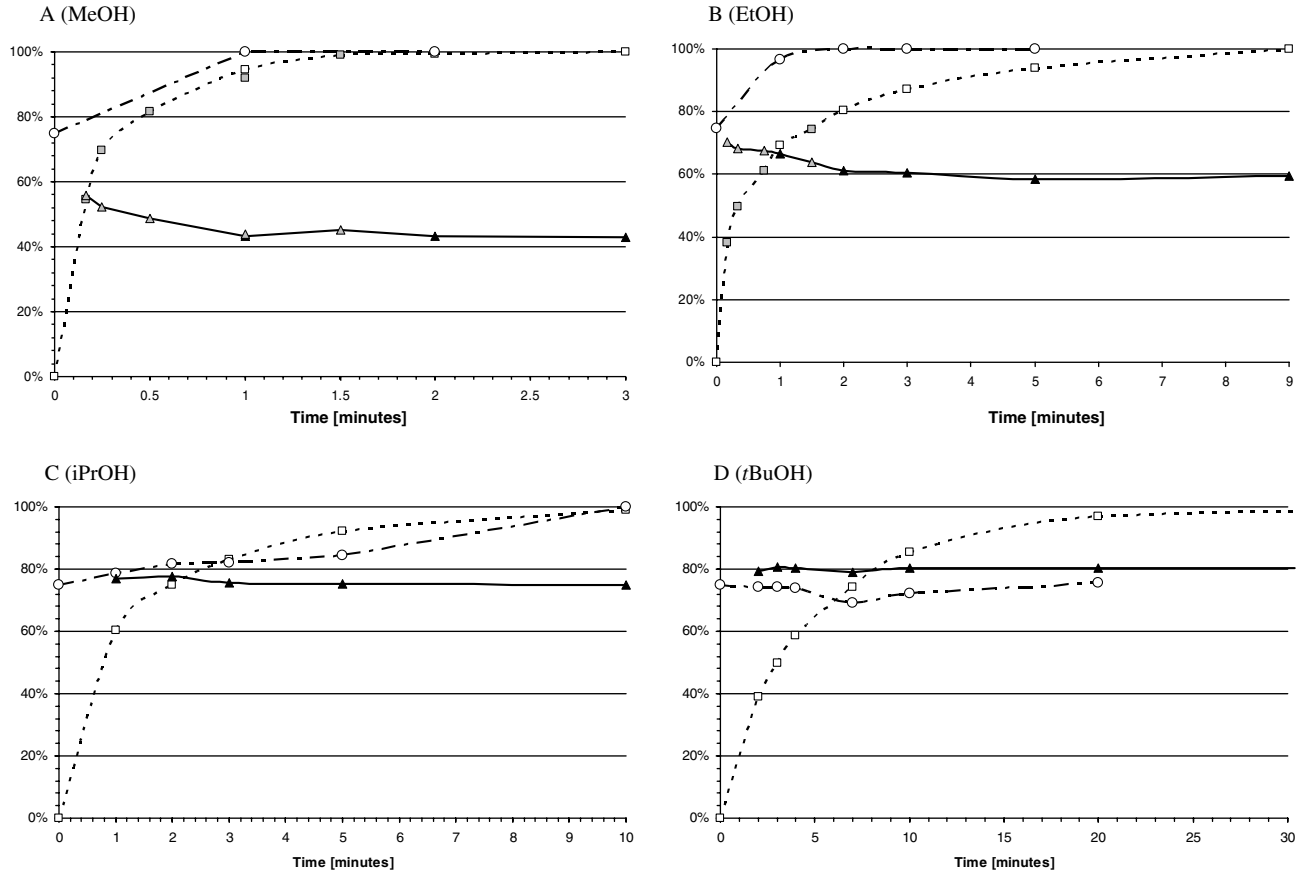

Figure 9. Reactions of *p*-nitrophenyl uridine *H*-phosphonate **4a** (B = U) with (A) MeOH, (B) EtOH, (C) *i*-PrOH and (D) *t*-BuOH (5 equiv) in DCM containing 3 equiv of 2,6-lutidine (^{31}P NMR data). Line description for Figures 9–11, ---○--- the fraction of the downfield signal of aryl nucleoside *H*-phosphonate of type **4**; ---□--- % of the product (alkyl nucleoside *H*-phosphonate of type **3**; a sum of diastereomers); —▲— the fraction of $D_P(S_P)$ diastereomer of product **3**. Grey-filled data points derived from reactions quenched with water.

2.5. Esterification of *p*-chlorophenyl nucleoside *H*-phosphonates 4b

According to the preliminary data (Table 1), the reactions of *p*-chlorophenyl nucleoside *H*-phosphonates of type **4b** with methanol were expected to give at least equally distinct results as for *p*-nitrophenyl ester **4a**. Indeed, we observed not only clear-cut changes in the ratio of diastereomers of aryl *H*-phosphonate **4b** (similarly as for **4a**, vide supra), but also a substantial decrease in the fraction of the $D_P(S_P)$-epimer of the produced *H*-phosphonate diester **3** (Fig. 11).[§§] This second phenomenon was probably caused by the fast consumption, in the early stages of the reaction, of the minor $L_P(S_P)$-diastereomer of aryl *H*-phosphonate **4b**, evidently the more reactive substrate for **3**-$D_P(S_P)$. As a result, after a relatively short period of time the reaction mixture contained a large excess of the $D_P(R_P)$-epimer of intermediate **4b**, which apparently underwent faster transesterification with reactive methanol [yielding **3**-$L_P(R_P)$] than transformation into its epimer **4b**-$L_P(S_P)$ [according to Eq. 2]. Fast transesterification of **4b**-$D_P(R_P)$ with methanol led to an accumulation of **3**-$L_P(R_P)$ that ultimately became the main epimer of the product.

Concluding the above set of experiments, the acquired data suggested that the minor diastereomers of aryl nucleoside *H*-phosphonate diesters of type **4** have an $L_P(S_P)$-configuration, are substantially more reactive than $D_P(R_P)$-diastereomers, and are usually precursors of the major $D_P(S_P)$ diastereomers of the produced alkyl nucleoside *H*-phosphonate diesters of type **3** according to the DYKAT mechanism (cf. Fig. 5A).[¶¶] However, when the rate of equilibration (Eq. 2) was not fast enough to keep the ratio of D_P/L_P diastereomers constant, the transesterification produced the respective epimers of **3** in a smaller or even in the reversed ratio due to a higher participation of the DYTR scenario. It may be expected that by increasing of the transesterification rate of the moderately reactive aryl esters further, this might result in achieving a kinetics of an almost pure DYTR.

2.6. Experiments on kinetic quenching of aryl nucleoside *H*-phosphonates of type 4

The above conclusions were confirmed by performing transesterification of aryl *H*-phosphonates **4a-b** (B = A^{Bz}, C^{Bz}, G^{ibu}, U) under the conditions of kinetic quenching with a large excess of methanol. Thus, when aryl

[¶¶] Attempts to obtain quantitative kinetic data were unsuccessful due to complexity of the reaction mixtures.

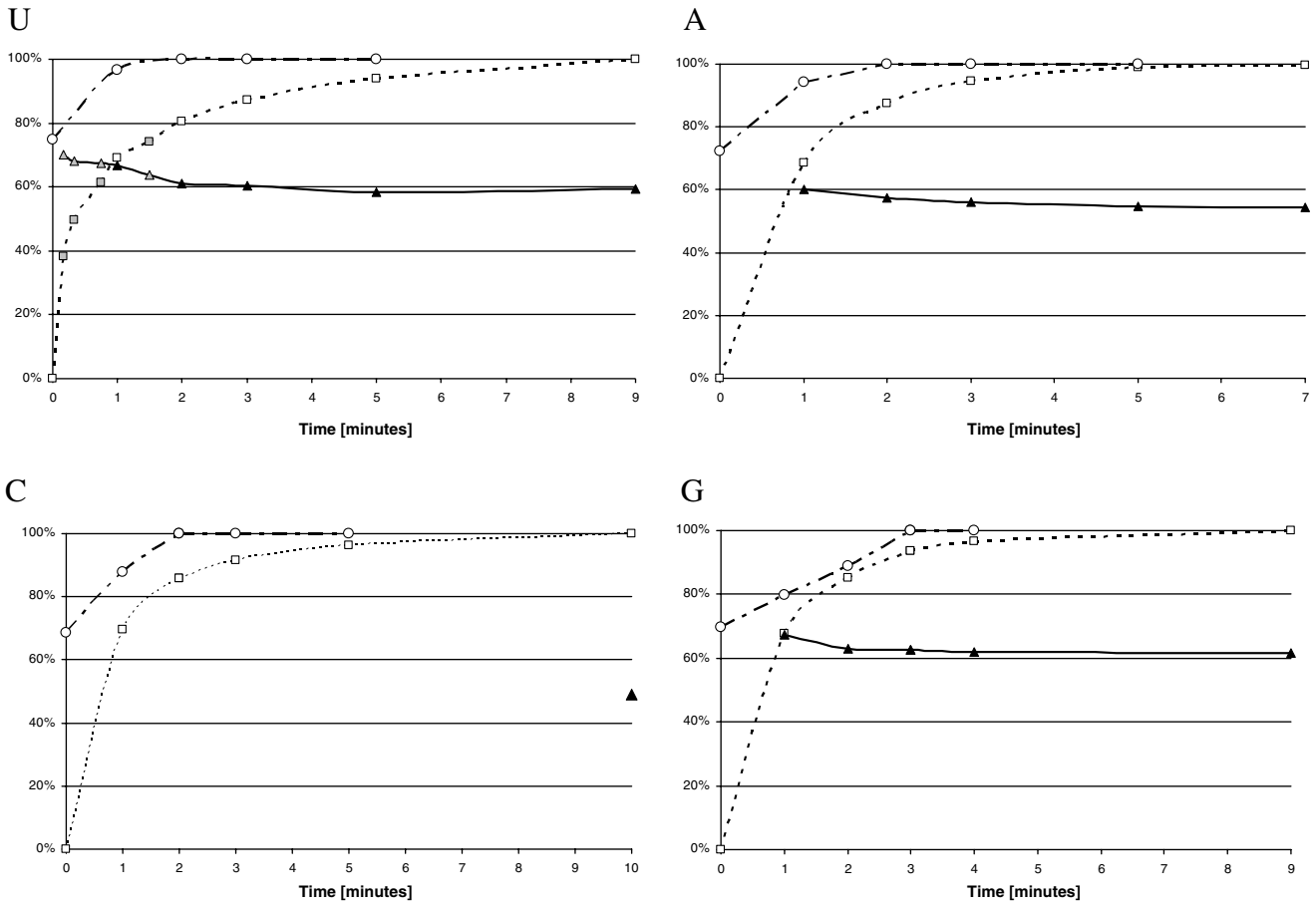

Figure 10. Reactions of *p*-nitrophenyl nucleoside *H*-phosphonates of type **4a** with ethanol (^{31}P NMR data). (U) B = U, (A) B = A^{Bz}, (C) B = C^{Bz} and (G) B = G^{ibu}. ---○--- downfield signal of **4a**; ---□--- % of product **3** ($D_P + L_P$); —▲— **3**-$D_P(S_P)$. For detailed description, see the legend for Figure 9.

H-phosphonates **4a**,**b** were treated with 2500 equiv of methanol, a clear-cut reversal of stereoselectivity was obtained [i.e., the predominant formation of the $L_P(R_P)$ diastereomer of produced **3a**, Table 2, column 5] for both aryl derivatives and all four nucleobases. In these cases, the diastereomeric composition of the produced *H*-phosphonate diester **3a** (Table 2, column 5) practically mirrored the population of diastereomers of the intermediate aryl *H*-phosphonates **4** (Table 2, column 2). Apparently, the minor diastereomer of intermediate **4**-$L_P(S_P)$ yielded the minor diastereomer of product **3a**-$D_P(S_P)$, while the major diastereomer **4**-$D_P(R_P)$ yielded the major diastereomer of the product, **3a**-$L_P(R_P)$. This could be rationalized by assuming a high rate of transesterification of aryl *H*-phosphonates **4** with methanol, and a moderate rate of their *P*-epimerization (DYTR kinetics).

A similar reactivity of nucleoside 3′-*H*-phosphonates activated by pivaloyl chloride and that of the corresponding aryl esters[13–16] allows us to extend the assignment of the configuration made for the diastereomeric aryl *H*-phosphonates **4** to pivalic mixed anhydrides **2**, that is, to assign the $D_P(R_P)$-configuration to the major diastereomer **A** and the $L_P(S_P)$, to the minor diastereomer **B** of the mixed anhydride **2** (Fig. 2). Importantly, this assignment is in full agreement with the preliminary conclusions drawn from the kinetic quenching experiments of the mixed anhydrides of type **2** (vide supra).

Table 2 contains additional data (some of them discussed previously), containing a comparison of stereochemistries of the reactions of *H*-phosphonates **2**, **4a** and **4b**. Thus, kinetic quenching of the mixed anhydride **2** with methanol caused, in general, a loss of stereoselectivity (Table 2, cf. column 2 and 5) that favoured the $D_P(S_P)$ epimer of product **3** in the regular reactions (columns 3 and 4). A similar stereochemical outcome was found for the reactions of *p*-nitrophenyl *H*-phosphonate intermediate **4a** with limited amounts of alcohols (columns 3 and 4), and for **4b** + EtOH (5 equiv). Most probably, the lack of stereoselectivity in these cases was due to an intermediate character of kinetics of the reactions, having the energetic profiles inbetween DYKAT and DYTR (cf. Fig. 2).

It should be noted that the stereoselectivities obtained in the regular esterification of the *H*-phosphonic–pivalic mixed anhydrides **2** were considerably higher than those found for aryl *H*-phosphonates **4a/b** (Table 2, columns 3 and 4), despite the reactivities of **2** and **4a** towards alcohols being comparable [$t_{1/2} = \sim$13 s (Fig. 4A) and ~20 s (Table 1), respectively]. In contrast, the stereoselectivity of transesterifications of **4a** and **4b** was similar (Table 2), despite

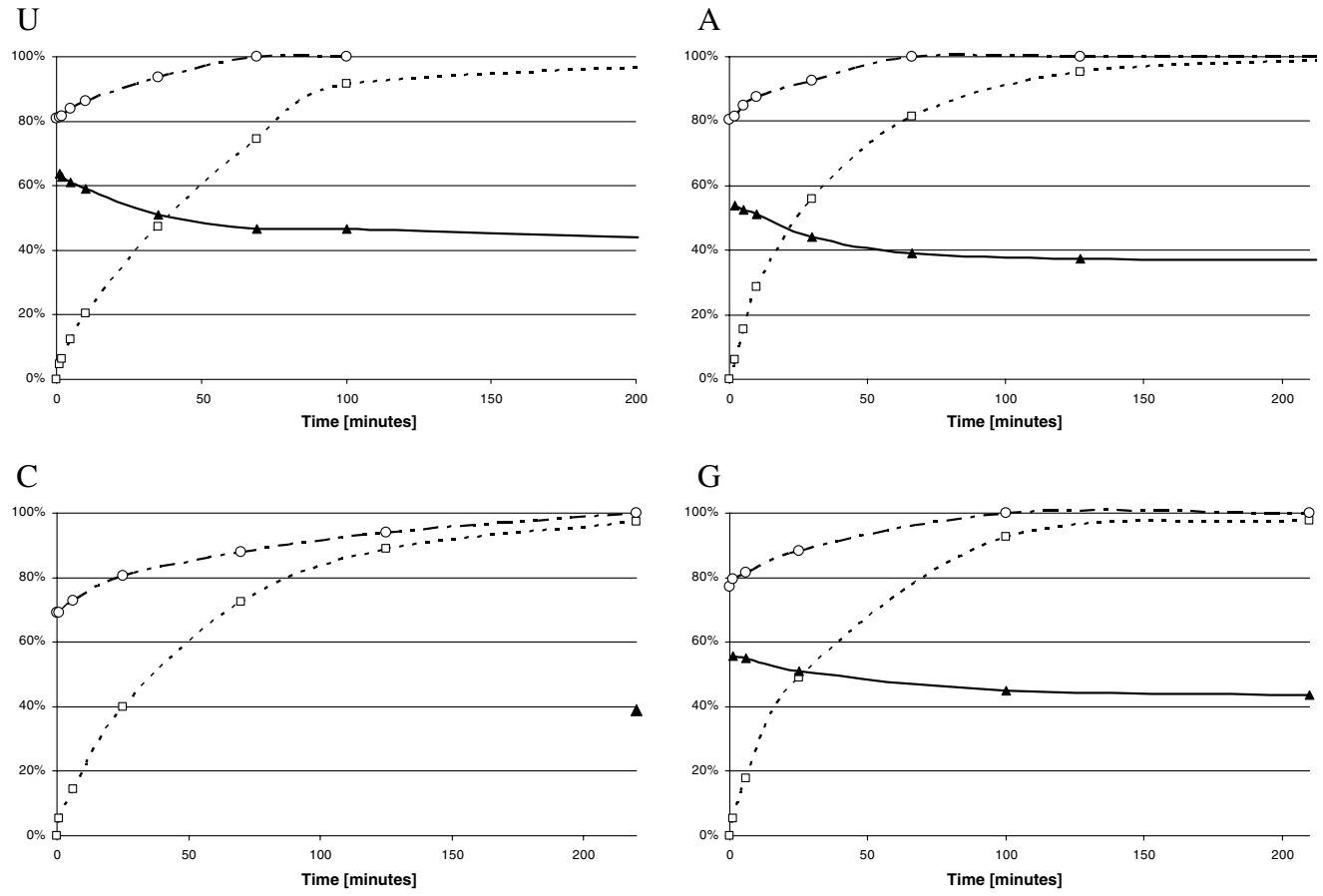

Figure 11. Reactions of *p*-chlorophenyl nucleoside *H*-phosphonates of type **4b** with methanol (^{31}P NMR data). (U) B = U, (A) B = A^{Bz}, (C) B = C^{Bz} and (G) B = G^{ibu}. ---○--- downfield signal of **4b**; ---□--- % of product **3** ($D_P + L_P$); —▲— **3**-$D_P(S_P)$. For detailed description, see the legend for Figure 9.

Table 2. Diastereomeric composition of diesters **3a** obtained from substrates **2**, **4a** and **4b** by treatment with 5 equiv of EtOH, or 5 or 2500 equiv of MeOH

Substrate	Substrate, % of $L_P(S_P)$	Product **3b**, % of $D_P(S_P)$ (substrate + 5 equiv of EtOH)	Product **3a**, % of $D_P(S_P)$ (substrate + 5 equiv of MeOH)	Product **3a**, % of $D_P(S_P)$ (substrate + 2500 equiv of MeOH)
1	2	3	4	5
2 (B = A^{Bz})	26.4	81.9	80.2	57.9
2 (B = C^{Bz})	38.9	74.6	75.7	47.1
2 (B = G^{ibu})	34.6	80.5	80.0	51.7
2 (B = U)	30.0	81.0	81.0	35.7
4a (B = A^{Bz})	27.6	54.1	48.1	29.7
4a (B = C^{Bz})	34.0	49.0	54.8	36.4
4a (B = G^{ibu})	30.5	62.0	51.0	37.6
4a (B = U)	25.2	54.7	42.0	29.6
4b (B = A^{Bz})	19.5	ca. 55[a]	32.5	20.8
4b (B = C^{Bz})	27.8	ca. 53[a]	39.0	28.4
4b (B = G^{ibu})	22.7	—[b]	43.5	25.3
4b (B = U)	19.0	ca. 55[a]	41.3	22.7

[a] Due to low rate of transesterification (several hours) a significant decomposition of *H*-phosphonate diesters took place before the completion of the reaction.

[b] Not determined due to decomposition and overlapping of the signals.

significant differences in their reactivities, (ca. two orders of magnitude, cf. Table 1). Thus, the equilibration rate is presumably governed by the nucleophilic character of the interchanging ligand ($PvO^- > pNO_2$–$PhO^- \approx p$Cl–PhO^-), while the esterification rate depends on the leaving group ability ($PvO^- \approx pNO_2$–$PhO^- > p$Cl–PhO^-). As a result, aryl *H*-phosphonate esters of type **4** are apparently more prone to the DYTR scenario than the mixed anhydrides **2**.[||||]

[||||] However, for the reactions with sterically hindered alcohols, *i*-PrOH and particularly *t*-BuOH, these differences were significantly smaller and the aryl *H*-phosphonates **4** reacted also with high stereoselectivity (for example, see Fig. 9C and D).

3. Conclusion

The aim of this work was to elucidate mechanistic and stereochemical aspects of the condensation of ribonucleoside *H*-phosphonates with nucleosides and alcohols. The results of the experiments performed supported a mechanism involving *dynamic kinetic asymmetric transformation* (DYKAT) which consists of a very fast equilibrium between diastereomers of the intermediate mixed anhydrides or aryl esters, followed by rate-diversified esterification of each active diastereomer. However, some participation of a *diastereoconvergent transformation*-type mechanism involving pseudorotation of the phosphorane intermediates could not be excluded. For several reactions in which a large excess of methanol was used (kinetic quenching), a reversal of stereoselectivity was found, indicating a kinetics of *dynamic thermodynamic resolution* (DYTR) under such conditions. The relative ease of reaching this kinetic quenching suggested that the rates of the equilibria involved and the esterification reactions are of the same order of magnitude. In that way the Curtin–Hammett principle might be followed only partly and the diastereomeric composition of the mixed anhydride (or aryl diester) could influence the ratio of diastereomers of the final diesters formed.

Under standard reaction conditions, amongst the pair of diastereomers of *H*-phosphonic–pivalic mixed anhydride **2** and aryl *H*-phosphonate **4** intermediates, the minor diastereomers were identified as the more reactive species yielding the major [$D_P(S_P)$] diastereomers of the produced *H*-phosphonate diesters. As a consequence, the configuration at the phosphorus centre in these more reactive and thermodynamically less stable intermediates was assigned as $L_P(S_P)$.

Interestingly, a literature survey indicates that the DYKAT mechanism may be an underestimated common phenomenon taking place in the transformations of several other types of *P*-chiral nucleotide derivatives. For example, DYKAT might explain the diastereoselective synthesis of $L_P(R_P)$ dinucleoside methylphosphonates[20,21] or the rather unusual retention of configuration during the $S_N2(P)$ reaction of chlorooxazaphospholidines.[22,23]

4. Experimental

^{31}P NMR spectra were recorded at 121 MHz on a Varian Unity BB VT spectrometer. ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 M concentrations of phosphorus-containing compounds in the appropriate solvents (0.5 mL) and the spectra were referenced to 2% H_3PO_4 in D_2O (external standard). Dichloromethane (POCh, Poland) and acetonitrile (Merck) were refluxed over P_2O_5 and distilled. Toluene (POCh) and 2,6-lutidine (Fluka) were distilled and after discarding ca. 30% of forerunnings the fractions containing below 20 ppm of water were collected. All solvents were stored over molecular sieves 4 Å and contained below 20 ppm of water (Karl Fischer coulometric titration, Metrohm 684 KF coulometer). Anhydrous triethylamine (POCh) was distilled and kept over CaH_2. Pivaloyl chloride (Fluka) was distilled and used within one month. Phenol (Merck), *p*-chlorophenol (Aldrich), *p*-nitrophenol (Aldrich), adamantanecarbonyl chloride (Fluka), acetic acid (POCh) were of commercial grade and used without purification. Nucleoside *H*-phosphonates[17] were obtained according to the published method.

Immediately prior to the reaction, all nucleosidic derivatives were rendered anhydrous by dissolving in DCM (0.5 mL/0.05 mmol) followed by toluene (3 mL/0.05 mmol) and the evaporation of these solvents under reduced pressure (pyridine was avoided in order not to contaminate the mixture with a nucleophilic catalyst). After drying under vacuum (15 min, 0.5 Torr), the flask was filled with air which has been dried by passing through Sicapent (Merck).

4.1. General procedure for the condensation of *H*-phosphonates of type 1 with alcohols and phenols

Nucleoside *H*-phosphonate **1** (0.05 mmol) was dissolved in 0.5 mL of the appropriate solvent (DCM or ACN containing 3 equiv of 2,6-lutidine) and dry alcohol (5 equiv) or phenol (3 equiv) was added, followed by pivaloyl chloride (1.2 equiv). For kinetic analysis, the reaction mixture was transferred immediately to an NMR tube and sets of 8 scans (ca. 40 s acquisition time) for each consecutive spectrum were registered.

4.2. General procedure for condensation of *H*-phosphonates with nucleosides

Nucleoside *H*-phosphonate **1** (0.05 mmol) and nucleoside [2′,3′-*O*-dibenzoyl-uridine or 5′-*O*-(4,4′-dimethoxytrityl)-thymidine; 0.06 mmol] were mixed together, dried and dissolved in 0.5 mL of DCM containing 3 equiv 2,6-lutidine followed by pivaloyl chloride (1.2 equiv), and the ^{31}P NMR spectrum was recorded.

4.3. General procedure for in situ preparation of the mixed anhydrides of type 2

Nucleoside *H*-phosphonate **1** (0.05 mmol) was rendered anhydrous by the evaporation of the added toluene (3 mL) under reduced pressure. After drying under vacuum (15 min, 0.5 Torr), the flask was filled with air which has been dried by passing through Sicapent (Merck). The residue was dissolved in 0.5 mL of the appropriate solvent (DCM, ACN, or toluene) and 2,6-lutidine (3.0 equiv) and pivaloyl chloride (1.2 equiv) were added successively. The ^{31}P NMR spectra showed that under such conditions, the mixed anhydrides of type **2** were stable for several hours without the appearance of detectable degradation products.

4.4. General procedure for esterification of the mixed anhydrides of type 2 or aryl nucleoside diesters of type 4

Typical procedure: to a solution of **2** or **4** (generated as described above) dry alcohol was added in the appropriate amount with vigorous stirring, and the mixture was analyzed by ^{31}P NMR spectroscopy. For the experiments

requiring the addition of <5 μL of reagents their 1 M solutions in DCM or ACN were used.

The composition of the reaction mixtures in the early stages of the fast reactions was determined by hydrolytic quenching. To this end, after defined time periods 170 μL aliquots of the reaction mixtures were taken and added to 336 μL of ACN–pyridine–H_2O, 300:18:18, and the mixture was analyzed by ^{31}P NMR spectroscopy.

4.5. General procedure for kinetic quenching experiments

The solution (0.3 mL) of intermediate **2** or **4** (0.5 mmol; generated as described above) was added dropwise by a syringe to a septum-sealed flask containing vigorously stirred alcohol (5 mL) and 2,6-lutidine (120 μL). After 5 min, a sample of 0.5 mL was used for recording a ^{31}P NMR spectrum. The remaining solution was concentrated under reduced pressure (without heating), dissolved in DCM (1 mL) and analyzed by ^{31}P NMR spectroscopy.

4.6. 5′-*O*-Dimethoxytrityl-2′-*O*-*t*-butyldimethylsilyl-uridin-3′-yl phosphorothioic–pivalic mixed anhydride

To a stirred solution of 5′-*O*-dimethoxytrityl-2′-*O*-*t*-butyldimethylsilyl- uridin-3′-yl *H*-phosphonate (413 mg, 0.5 mmol) in DCM (5 mL) containing 2,6-lutidine (120 μL) pivaloyl chloride (1.2 equiv, 0.6 mmol, 76 μL) was added. After 5 min, elemental sulfur was added (3 equiv, 1.5 mmol, 48 mg), optionally followed by triethylamine (10% v/v). After 15 min 1 M TEAB buffer (pH 7.2) was added and after work-up the organic layer was collected and evaporated (TLC and ^{31}P NMR analysis indicated stability of the compound during work-up). Attempted purification of the mixed anhydride on a silica-gel column failed due to the decomposition of the product. TLC (DCM–MeOH, 9:1 v/v): R_f 0.19; (DCM–MeOH, 8:2 v/v): R_f 0.54 (diastereomers not resolved); ^{31}P NMR (121 MHz, DCM): δ 50.07 (d, $^3J_{P,H3'} = 10.08$ Hz), 51.23 (d, $^3J_{P,H3'} = 11.91$ Hz).

4.7. Adamantanecarboxylic acid, triethylammonium salt

Adamantanecarboxylic acid (9.0 g, 50 mmol) and triethylamine (7.7 mL, 55 mmol) were mixed together in ACN (100 mL). The solvent was removed under reduced pressure and the remaining residue was recrystallized from ACN to yield white crystals (12.8 g; 91%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.17 (t, $J = 7.30$ Hz, 9H, $N^+CH_2CH_3$), 1.71 (m, 6H, adamantane), 1.90 (m, 6H, adamantane), 2.00 (m, 3H, adamantane), 2.95 (q, $J = 7.29$ Hz, 6H, $N^+CH_2CH_3$), 10.33 (br s, 1H, HN^+).

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