Chemistry and stereochemistry of internucleotide bond formation by the *H*-phosphonate method†

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The appropriately protected ribonucleoside 3′-*H*-phosphonates react with nucleosides and alcohols in the presence of condensing agents under mild conditions, with notable stereoselectivity. The chemistry and stereochemistry of this reaction were investigated using ^{31}P NMR spectroscopy. A plausible mechanism for the asymmetric induction observed was identified as Dynamic Kinetic Asymmetric Transformation (DYKAT), operating due to different esterification rates of rapidly equilibrating P-epimers of the reactive intermediates. This diverse reactivity was tentatively attributed to steric demands of the *H*-phosphonic moiety located in the vicinity of the bulky protecting group in the 2′-position. The role of nucleophilic and base catalysis was analysed and the absolute configuration of the intermediates involved in the reaction pathways was tentatively assigned using ^{31}P NMR stereochemical correlation analysis. Under optimal reaction conditions, the diastereomers of dinucleoside *H*-phosphonate diesters could be obtained usually in >90 : 10 ratio.

Introduction

Stereocontrolled formation of P-chiral analogues of nucleotides and oligonucleotides has attracted the interest of chemists and biochemists since the early work by Eckstein, who showed that R_P and S_P epimers of nucleoside phosphorothioates differ in their substrate specificity in enzymatic reactions.[1,2] Unfortunately, none of the standard methods of oligonucleotide synthesis could provide direct access to pure diastereomers of phosphorothioate derivatives, while isolation of individual diastereomers from their mixture is limited to relatively short sequences. For instance, chromatographic purification of diastereomers of short oligonucleotides containing one or two phosphorothioate moieties is a relatively simple task,[2,3] while for longer oligonucleotides with an internal phosphorothioate modification, separation of diastereomers by means of HPLC could not be achieved.[4] The limit of HPLC separation capability seems to lie in the range of 16 diastereomers for phosphorothioates[5] and even less (8 diastereomers) for phosphoramidites.[6] High-performance capillary electrophoresis might be useful for analysis of diastereomeric composition of oligo(nucleoside phosphorothioate)s;[7] however, it remains an analytical tool only since in more productive slab electrophoresis (PAGE) all diastereomers of a given sequence migrate as a single spot.[8] The limitations of separation techniques and the demand of oligonucleotide phosphorothioates with defined configuration at the chiral phosphorus centre called for stereocontrolled methods for their preparation.

Since nucleoside α-(S_P)-thiotriphosphates are good substrates for DNA/RNA polymerases (which invert configuration at the phosphorus),[9] protocols for template-directed preparation of all-(R_P)-oligo(nucleoside phosphorothioate)s were developed.[10] Nevertheless, biochemical methods cannot be considered as a general tool for the preparation of P-chiral oligonucleotide analogues, and more flexible and scalable chemical approaches are desired for this purpose.

The first reported attempts of stereocontrolled chemical synthesis of oligo(deoxynucleoside phosphorothioate)s date back to 1984, when Stec and Zon separated diastereomers of a protected cytidine phosphoramidite and attempted to condense them stereospecifically with nucleosidic units.[11] Unfortunately, complete epimerization at the phosphorus centre was observed,

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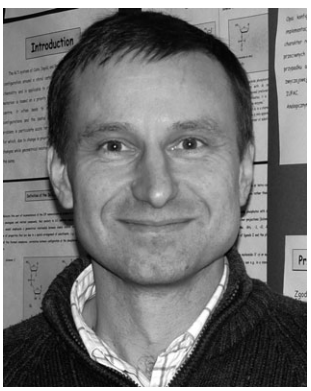
† Dedicated to Professor Wojciech J. Stec on the occasion of his 70th birthday. This article is part of a themed issue on Biophosphates.



Michal Sobkowski

Michał Sobkowski graduated from the Faculty of Chemistry of the Adam Mickiewicz University in Poznań and received his PhD in 1997 in organic chemistry in the Institute of Bioorganic Chemistry, Polish Academy of Sciences, under the supervision of Prof. A. Kraszewski. After a post-doctoral stay (1998–2000) in Prof. H. Seliger's lab at the Universität Ulm (Germany), he returned to IBCh in Poznań where he became an Assistant Professor. His research activities are focused on the organophosphorus chemistry of natural products; in particular, synthetic, mechanistic and stereochemical investigations into phosphorus-modified analogues of nucleotides and oligonucleotides.

which was attributed to rapid multiple exchange of the tetrazole moiety in the intermediate phosphorotetrazolidites. Since P-epimerization seemed to be unavoidable in the phosphoramidite approach to oligonucleotide synthesis, several other strategies for desymmetrization of the internucleotide linkage were proposed in the following years. Those included stereospecific transesterification of phosphorothioate triesters with nucleosides activated by *t*BuMgCl,[12] stereoselective deacylation/sulfurization of dinucleoside acylphosphonates in the presence of DBU,[13] and an adaptation of the Ohtsuka method[14] for stereoselective phosphotriester formation, applied to phosphorothioate chemistry.[15] Unfortunately, all those approaches suffered from various disadvantages, such as low yields, moderate stereoselectivity, or poor reproducibility.[16]

The landmark in the field was an idea to involve the phosphorus centre in a configurationally restricted phospholane ring, which was formulated in 1991 by Stec *et al.*[17] Diastereomers of such synthons could be isolated chromatographically and applied to phosphorothioate diester formation. To this end, individual diastereomers of appropriately protected deoxyribonucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholane) were reacted with nucleosides activated by DBU,‡ yielding dinucleoside phosphorothioates in a stereospecific manner. The stereochemistry of the reaction (retention) indicated one pseudorotation step at the level of the intermediate phosphorane, which collapsed subsequently with endocyclic P–S bond scission, followed by elimination of ethylene sulfide. This mechanism was confirmed in studies on model compounds[18] and *ab initio* calculations.[19] Various applications of the "oxathiaphospholane" or "Stec method" are summarized in a review paper.[20]

The concept of configurationally arresting a phosphorus centre by engaging it in a ring structure was developed further in several laboratories in order to relieve some limitations of the oxathiaphospholane approach, *e.g.* the need for chromatographic separation of diastereomers of the synthons or necessity of using strong base (DBU) for the condensation. These studies were initiated by the groups of Agrawal and Just in 1995. In both strategies phosphoramidite synthons were prepared in a stereoselective manner using readily available chiral amino alcohols. In the Agrawal approach,[21] nucleoside oxazaphospholidines were activated by weakly acidic tetrazole, while Just used nucleoside oxazaphosphorinanes in conjunction with basic DBU[22] or mildly acidic dicyanoimidazole derivatives[23] (for a recent review, see ref. 24). The oxazaphospholidine approach was developed also in the Beaucage laboratory[25] and, more recently, by the Wada group, who showed its application *e.g.* for stereocontrolled solid-phase synthesis of oligo(deoxyribonucleoside phosphorothioate)s[26] and *H*-phosphonates[27] of defined configuration.[28]

Reports on other approaches, not engaging phosphocyclic motifs in the nucleotidic synthons, are scarce in the recent literature. One of the few is a variant of the phosphotriester method, in which a derivative of pyridine oxide bound to the phosphorus moiety served as an intramolecular nucleophilic catalyst, securing complete stereospecificity of condensation.[29] In another approach, nucleoside phosphorothioate diesters were shown to react stereospecifically with nucleoside under Mitsunobu reaction conditions (interestingly, the R_P and S_P synthons could be obtained from a common precursor in a stereospecific manner).[30]

The methods mentioned above were designed for oligodeoxyribonucleotide derivatives, while stereocontrolled synthesis of P-chiral oligoribonucleotide derivatives was investigated to a much lesser extent. In an early attempt, *t*BuMgCl-promoted stereospecific transesterification of ribonucleoside 3′-phosphorothioate triesters provided internucleotide linkages of defined configuration; however, the yields were rather poor (15–25% per linkage).[31] The oxathiaphospholane method was significantly more effective, but nevertheless, the yields approached those achieved in the deoxy series only for uridine derivatives.[32] The intramolecular catalyst[29b] and oxazaphospholidine[33] approaches were successful in solid-phase synthesis of all-(R_P)- and all-(S_P)-oligo(ribonucleoside phosphorothioate)s. Nevertheless, the recent rapidly increasing interest in oligoribonucleotide synthesis[34] indicates that better access to a stereocontrolled preparation of their P-chiral analogues may be required.

In this context, another approach, based on stereoselective condensations of ribonucleoside 3′-*H*-phosphonates, may appear as a convenient alternative for the formation of P-chiral oligoribonucleotide derivatives due to its two main unique advantages: (i) commercial accessibility of the synthons and (ii) a variety of analogues that can be readily generated stereospecifically from the *H*-phosphonate diester intermediate product.

Historically, the first observation of some stereoselectivity (S_P/R_P 2 : 1 for phosphite triester) during chemical internucleotide bond formation was reported in 1980 by Marlier and Benkovic for the phosphite triester method.[35] A decade later, Stawinski *et al.*[36] and Battistini *et al.*[37] independently published papers on ribonucleoside *H*-phosphonates, noting high stereoselectivity during their condensations with nucleosides. Since similar reactions in the deoxy series are practically not stereoselective, this phenomenon was attributed to steric effects induced by bulky silyl moieties located on the neighbouring hydroxy groups. Further studies by Almer *et al.* resulted in a method for synthesis of all-(R_P)-oligo(ribonucleoside phosphorothioate)s;[38,39] however, little was known on the underlying mechanism of the stereoselectivity observed.

In this paper we present an overview of our investigations focused on elucidation of the mechanism of asymmetric induction during ribonucleoside *H*-phosphonate diester formation, and optimization of the reaction conditions.

Results and discussion

During condensations of fully protected ribonucleoside 3′-*H*-phosphonates (which are prochiral at the phosphorus centre) with nucleosidic components having an unprotected 5′-OH

‡ Abbreviations: DABCO, 1,4-diazabicyclo[2.2.2]octane; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP, 4-(*N*,*N*-dimethylamino)-pyridine; DMA, *N*,*N*-dimethylaniline; DMTr, dimethoxytrityl; MMTr, monomethoxytrityl; NMI, *N*-methylimidazole; PhCl, *p*-chlorophenyl; $PhNO_2$, *p*-nitrophenyl; Pv, pivaloyl (trimethylacetyl); TEA, triethylamine; $dmtU_{PH}$, 5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-uridine 3′-*H*-phosphonate.

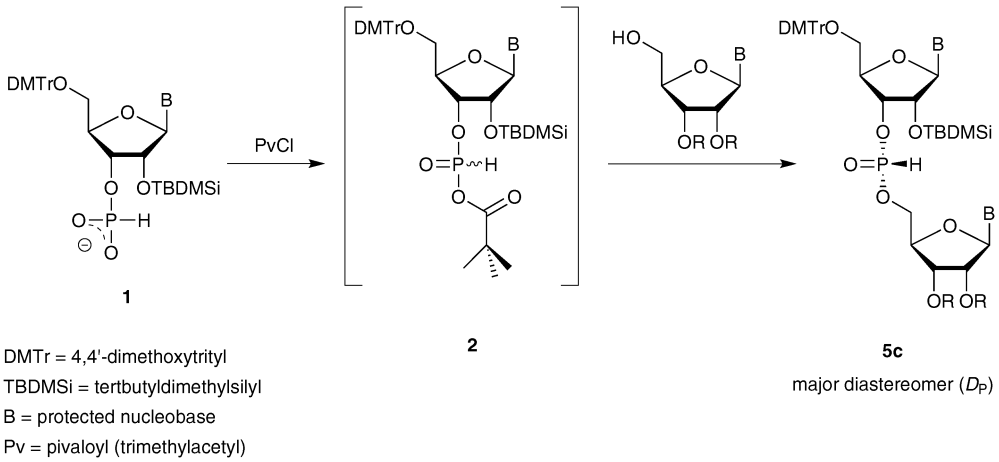
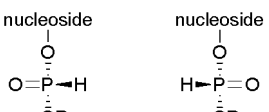


Fig. 1 Stereoselective condensation of ribonucleoside *H*-phosphonates (**1**) with nucleosides.

group, formation of the D_P diastereomer§ of the *H*-phosphonate diester was favoured (Fig. 1). The diastereoselectivity of this reaction under non-optimized conditions, expressed as a diastereomeric excess (*de*) of the D_P product, was estimated to be *ca.* 70% for all nucleoside 3′-*H*-phosphonates, with an exception for a cytidine derivative (B = Cyt^{Bz}, *de ca.* 40%).[39,41] The fraction of each diastereomer could be conveniently assigned by integration of their ^{31}P NMR signals. This technique was also applied to analysis of the reaction mixtures, since the signals of the substrates, the intermediates and the products could be identified according to their chemical shifts and coupling constants. Additionally, for most ribonucleoside 3′-*H*-phosphonates, the signal located at the lower field corresponded to the D_P diastereomer, while the signal at higher field corresponded to the L_P diastereomer. This rule of thumb held for the vast majority of compounds in this class; however, it should be noted that the relative positions (higher *vs.* lower field) of the signals could be inverted in some solvents (*e.g.* in toluene) and for some sequences of nucleobases (*e.g.* for $G_{PH}U$ linkage). Therefore, such correlation must be treated with care and as a provisional guide only.[42]

In a typical condensation, a triethylammonium salt of the nucleoside 3′-*H*-phosphonate of type **1** and a nucleoside (both appropriately protected) are dissolved in pyridine and *ca.* 3 equiv. of pivaloyl chloride (PvCl) as a condensing agent is added. This causes a rapid (less than 1 min, the time required to record the first ^{31}P NMR spectrum) formation of *H*-phosphonate diester, accompanied by the products of decomposition of the condensing agent, *i.e.* hydrochlorides of amines, pivalic acid and/or pivalic anhydride. Despite the apparent simplicity, the reaction is a multi-step process involving reactive species whose structure was only deduced indirectly. The reaction sequence starts with an attack of nucleoside 3′-*H*-phosphonate anion at the carbonyl carbon of PvCl, followed by an attack of the 5′-OH group of nucleoside at the chiral phosphorus atom of an activated *H*-phosphonic moiety. Apart from *H*-phosphonic pivalic mixed anhydride, formation of several other intermediates is possible. For instance, it is generally believed that in this reaction pyridine acts not only as base but also as a nucleophilic catalyst,[43] giving rise to extremely reactive pyridinium derivatives of *H*-phosphonates. These intermediates may rapidly equilibrate before reacting with an alcohol or nucleoside to form an *H*-phosphonate diester. Substitution at the phosphorus centre in these reactions is usually considered to be an $S_N2(P)$ process, although addition–elimination (implying a possibility of pseudorotation) or $S_N1(P)$ (*via* metaphosphites)[44] mechanisms cannot be excluded in the sterically demanding environment of protected ribonucleoside derivatives. In pyridine none of these species could be detected; however, it was possible to generate uridine 3′-*H*-phosphonic–pivalic mixed anhydride **2** quantitatively using 1.2 equiv. of PvCl in neutral solvents containing small amounts of amines.¶ The mixed anhydride **2** could not be isolated in pure form but it remained stable under nearly

§ Recently, we introduced a D_P/L_P stereochemical notation for describing stereochemistry of reactions involving nucleotides.[40] Since these descriptors refer to the sense of chirality rather than to priority of substituents of a chiral centre (as in the Cahn–Ingold–Prelog convention), we will use this notation through this paper to facilitate the stereochemical correlation analysis. Briefly, for nucleoside *H*-phosphonates, the D_P descriptor refers to a structure in which the P–H bond is directed to the right in the Fischer projection, while the L_P descriptor refers to a structure in which it is directed to the left:



D_P L_P

¶ Formation of carboxylic–*H*-phosphonic mixed anhydride **2** (eqn (1)) does not require basic conditions, and theoretically no acidification of the reaction mixture should occur during the reaction. However, formation of small amounts of HCl and carboxylic acid is unavoidable due to hydrolysis of the acyl chloride by spurious water. These acids were neutralized with amines (0.2–0.5 equiv.) to suppress protonation of *H*-phosphonate monoester that halted its reaction with acyl chlorides (eqn (2)) after *ca.* 50% conversion.

$$ROP(H)O_2^- \; HNR_3^+ + R'COCl \rightarrow ROP(H)O_2OR' + HNR_3^+ \; Cl^- \quad (1)$$

$$ROP(H)O_2H + R'COCl \rightarrow (\text{no reaction}) \quad (2)$$

neutral reaction conditions for *ca.* 1 h and could be subjected to mechanistic studies.

The mechanism of asymmetric induction

Since the phosphorus centre in *H*-phosphonate monoesters is achiral and is configurationally stable in *H*-phosphonate diesters,[16] the asymmetric induction must occur in between these two stages and may result from (i) preferential formation of one diastereomer of the mixed anhydride **2**, followed by its stereospecific esterification, (ii) preferential esterification of one diastereomer of the mixed anhydride **2** (which would have to be configurationally labile), (iii) pseudorotation of one diastereomer of an intermediate phosphorane formed by the addition of a nucleoside to the mixed anhydride **2**, or (iv) different geometry of an attack/departure during esterification of the two diastereomers of **2** (in the last two mechanisms the mixed anhydride **2** could be configurationally stable or labile).

The first hypothesis is in agreement with the *dynamic thermodynamic resolution* type of asymmetric induction. It implies that the mixed anhydride **2** is configurationally stable (or its esterification much faster than P-epimerisation), and the diastereomeric ratio of **2** is preserved in the final *H*-phosphonate diester **5**. This was not the case: in the ^{31}P NMR spectrum the signal ratio of *ca.* 2 : 1 for the intermediate **2** was different from that of *ca.* 4 : 1 for the product **5**. Moreover, under the optimized reaction conditions the latter ratio was $>9:1$,[45] proving the lack of coincidence with the 2 : 1 ratio for **2**. Since the pivalic moiety in the mixed anhydride **2** undergoes rapid and reversible displacement with other nucleophiles (carboxylates or phenols),[46] it may be concluded that diastereomers of the mixed anhydride **2** exist in rapid equilibrium and there must be another mechanism governing the stereoselectivity of the reaction.

In the second scenario (*dynamic kinetic asymmetric transformation*), two conditions had to be fulfilled to yield *H*-phosphonate diester **5**-D_P as the main product (the Curtin–Hammett principle): diastereomers of the mixed anhydride **2** must exist in a rapid equilibrium and one of them (**2**-L_P) must react significantly faster than the other P-epimer (**2**-D_P). The first requirement, *i.e.* the existence of **2**-D_P $\rightleftharpoons$ **2**-L_P equilibration, was confirmed by showing that the mixed anhydride **2** rapidly exchanged its substituents with carboxylates and phenols added to the reaction mixture. Another evidence of equilibration of the mixed anhydride **2** was gained when **2** was kinetically quenched with a large excess of methanol. Under such conditions the rate of esterification apparently exceeded that of **2**-D_P $\rightleftharpoons$ **2**-L_P equilibration and, in contrast to the standard reaction, the L_P diastereomer of the methyl uridine *H*-phosphonate diester (**5a**) was formed as a predominant product (Fig. 2). Assuming S_N2(P) type of the esterification, this would imply a D_P configuration of the main diastereomer of the mixed anhydride **2** and an L_P configuration of the minor diastereomer, which appeared to be significantly more reactive (Fig. 3, reaction B).

More precise ^{31}P NMR analysis of transformations of the mixed anhydride **2** were hampered by high reaction rates, which limited the recorded data to the final stages of the processes. Since the attempts to decrease the reaction rate (for instance by lowering temperature or using less reactive condensing agents) were unsuccessful, we replaced the acyl group in **2** with an aryl moiety (Fig. 3, reaction C). Aryl nucleoside 3′-*H*-phosphonate derivatives (**4**) are active esters, which are transesterified with alcohols in a similar manner as *H*-phosphonic–pivalic mixed anhydride (**2**) is esterified, while their reactivity may be adjusted by an appropriate choice of substituents in the phenyl ring.[47]

Thus, several aryl uridine 3′-*H*-phosphonates of type **4** were prepared *in situ* and studied in transesterifications with alcohols and nucleosides (Fig. 4). In preliminary experiments, combinations of phenols and alcohols that fitted well to the time scale of the ^{31}P NMR experiment and did not give rise to by-products, were established. As a result, reactions of

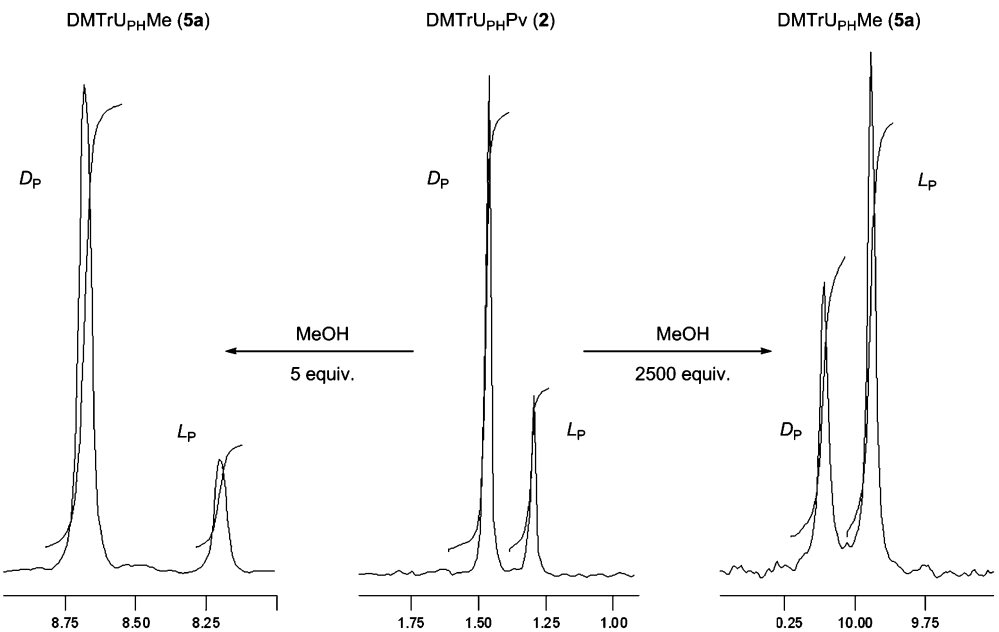


Fig. 2 Stereoselectivity in reactions of dmtU$_{PH}$Pv (**2**) with MeOH.

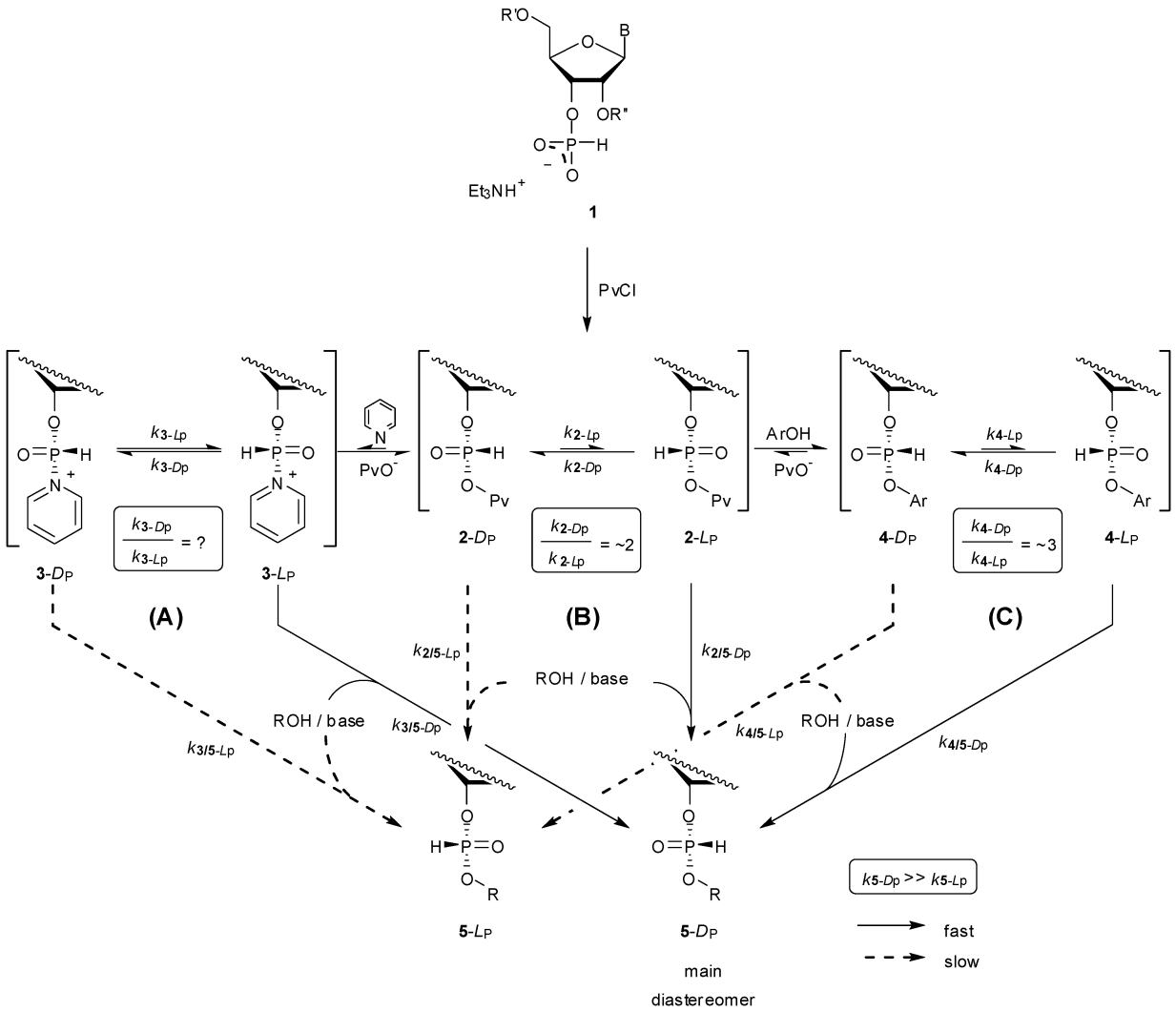


Fig. 3 The dynamic kinetic asymmetric transformation (DYKAT) mechanism as a source of asymmetric induction during formation of ribonucleoside *H*-phosphonate diesters of type **5**. (A) Reaction in the presence of a nucleophilic catalyst (pyridine); (B) direct esterification of the mixed anhydride **2**; (C) transesterification of aryl nucleoside *H*-phosphonate **4**.

p-nitrophenyl and *p*-chlorophenyl esters (hereinafter referred to as dmtU$_{PH}$PhNO$_2$ (**4a**) and dmtU$_{PH}$PhCl (**4b**), respectively) with MeOH and EtOH, were chosen as the most suitable to study the kinetics of transesterification.

Fig. 5 shows changes in diastereomeric ratio of alkyluridine 3′-*H*-phosphonate of type **5** formed during transesterification of dmtU$_{PH}$PhNO$_2$ (**4a**) with alcohols of different steric hindrance. For promptly-reacting primary alcohols, a stepwise decrease of D_P-**5** fraction due to progressive accumulation of the L_P isomer of the product is clearly seen. Those changes coincided with a rapid disappearance of the ^{31}P NMR signal of the minor diastereomer of dmtU$_{PH}$PhNO$_2$, *e.g.* in the first minute of its the reaction with EtOH (Fig. 4). This suggested that the D_P isomer of dmtU$_{PH}$Et (**5b**) was formed in the early

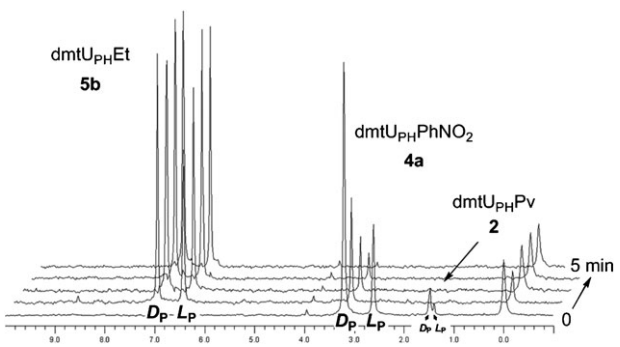


Fig. 4 ^{31}P NMR traces for the time course of transesterification of *p*-nitrophenyl uridine *H*-phosphonate **4a** with EtOH (5 equiv.). Note the immediate consumption of L_P-**4a** in the first minute of the reaction.

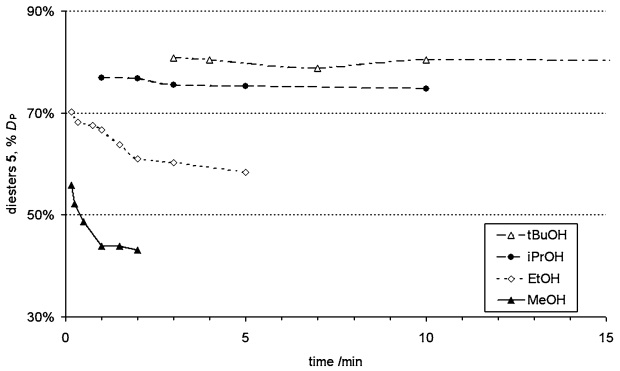


Fig. 5 Changes in fractions of D_P diastereomers of alkyl uridine *H*-phosphonates of type **5** formed during transesterification of $dmtU_{PH}PhNO_2$ **4a** with alcohols. Starting point, *ca.* 50% conversion. Approx. completion times: MeOH, 1.5 min; EtOH, 5 min; *i*PrOH, 10 min; *t*BuOH, 30 min.

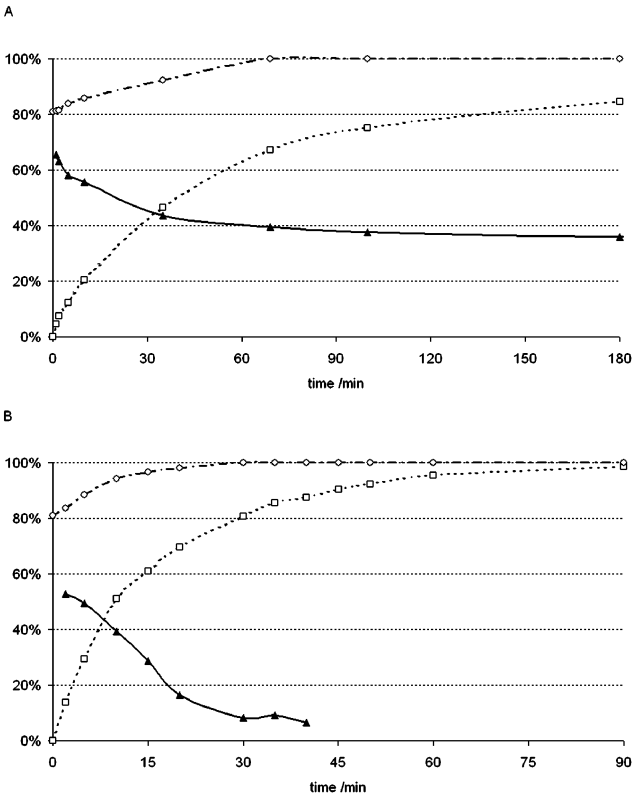


Fig. 6 Time course of the reaction of $dmtU_{PH}PhCl$ (**4b**) with (A) 5 equiv. and (B) 20 equiv. of MeOH. –○– fraction of D_P-**4b** in total **4b**; –□– % of the product $dmtU_{PH}Me$ (**5a**); —▲— fraction of D_P-**5a** in **5a** formed during the preceding interval of time between two recorded ^{31}P NMR spectra.

stages of the reaction from the minor isomer of nitrophenyl derivative **4a** (indicating in that way its L_P configuration) and, after its consumption, formation of L_P-**5b** from the remaining D_P-**4a** was a dominating pathway.

For secondary and tertiary alcohols the reaction rate decreased and the changes in diastereomeric ratios gradually vanished, indicating that the D_P-**4** ⇋ L_P-**4** equilibration was significantly more rapid than transesterification, and that the concentration of L_P-**4** remained at a level sufficient for a dominant formation of L_P diastereomers of alkyl uridine *H*-phosphonate diesters of type **5** (Fig. 5).

The above findings were further confirmed in the reaction of $dmtU_{PH}PhCl$ (**4b**) with MeOH, for which a clear-cut correlation between L_P-**4b** depletion in the reaction mixture and an inversion of stereoselectivity during the reaction was observed (Fig. 6). It is worth noting that in the late stages of the reaction with a small excess of MeOH (5 equiv.), despite very low amounts of L_P-**4b** diastereomer (below the detection limit of ^{31}P NMR), *ca.* 40% of the newly formed $dmtU_{PH}Me$ (**5a**) had a D_P configuration. *i.e.* derived apparently from L_P-**4b** (Fig. 6A). When transesterification rate was increased by using 20 equiv. of MeOH, after *ca.* 30 min of reaction the formation of D_P-**5a** almost ceased, apparently due to lack of its precursor, **4b**-L_P (Fig. 6B). Finally, in kinetic quenching experiments (pouring MeOH into the reaction mixture), the D_P/L_P ratios of aryl nucleoside *H*-phosphonate diesters of type **4** were accurately preserved in the diastereomeric composition of the product **5a** (Table 1), as a result of a change in the asymmetric transformation pathway from DYKAT (observed in reaction with small excess of alcohol) to DYTR (*i.e.* stereospecific esterification of the aryl *H*-phosphonate **4** intermediate).[46]

The attempts to determine kinetic parameters of this reaction (Fig. 3, reaction C) were only partly successful. Under pseudo-first-order conditions (20 equiv. of MeOH, Fig. 6B), the initial pseudo-first-order rate constant k'_{obs} was 1.2×10^{-3} s^{-1} in the first few minutes of the reaction, while after *ca.* 10 min it decreased to 7.0×10^{-4} s^{-1} (Fig. 7), apparently due to consumption of the more reactive isomer L_P-**4b**, the amount of which dropped to <5% of **4b** in that period of time. Analogous rate constants observed in the presence of 5 equiv. of MeOH (8.0×10^{-4} s^{-1} and 2.3×10^{-4} s^{-1}, respectively) were higher than might be expected from the 4-fold lowering of the MeOH concentration, revealing apparently a significant participation of D_P-**4b** ⇋ L_P-**4b** equilibration in the kinetics of the reaction. Unfortunately, the rate constants for this equilibrium could not be determined reliably, mainly because the D_P/L_P ratios of *in situ*-generated and equilibrated $dmtU_{PH}PhCl$ (**4b**) were very similar (81 : 19 → 72 : 28), and their ^{31}P NMR signals partially overlapped. This in turn precluded calculation of the rate constants for transesterification.

Table 1 Kinetic quenching of pivaloyl (**2**) and aryl (**4a**, **4b**) uridine *H*-phosphonates with MeOH compared to the standard reaction with 5 equiv. of MeOH

		5a ($dmtU_{PH}Me$) (% of D_P)	
Substrate	Substrate (% of L_P)	Substrate + 5 equiv. of MeOH	Substrate + 2500 equiv. of MeOH
2 ($dmtU_{PH}Pv$)	30	81	36
4a ($dmtU_{PH}PhNO_2$)	25	42	30
4b ($dmtU_{PH}PhCl$)	19	41	23

Concluding this part of the discussion, the presented experimental data supported the scenario in which diastereomers of

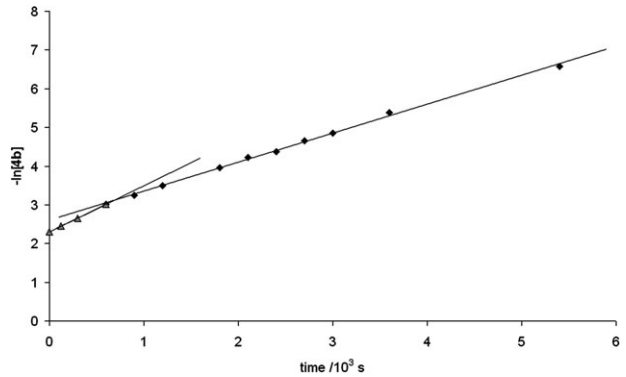


Fig. 7 A plot of reaction of dmtU$_{PH}$PhCl (**4b**) with 20 equiv. of MeOH.

the reactive intermediates exist in a dynamic equilibrium, and one of them, being significantly more reactive than the other, gives rise to a kinetic product of the process (dynamic kinetic asymmetric transformation, DYKAT). Upon decreasing the rate of the equilibrium and increasing the rate of the esterification, a reversal of stereoselectivity could be achieved due to the prevalence of dynamic thermodynamic resolution (DYTR).‖ Model experiments on aryl nucleoside *H*-phosphonates and kinetic quenching of the reaction mixtures indicated that the more reactive diastereomer of pivalic or aryl uridine 3′-*H*-phosphonate of type **2** or **4**, respectively, had an L_P configuration. These conclusions are valid also for other ribonucleoside 3′-*H*-phosphonates.[46]

The other proposed mechanisms of asymmetric induction, *i.e.* pseudorotation of phosphorane intermediates or other rules of formation and collapsing of trigonal bipyramid (apical entry–equatorial departure or *vice versa*) in general imply significant retention of configuration during esterification of one diastereomer of the mixed anhydride **2**, and inversion for the second diastereomer. Unfortunately, experimental verification of those scenarios is difficult. It seems, however, that involvement of energetically unfavourable structures (*e.g.* trigonal bipyramids with apical hydrogen or phosphoryl oxygen)[46] is unavoidable in such instances, and thus those mechanisms were not considered as likely; however, their participation in asymmetric induction cannot be excluded.

Interestingly, preliminary experiments on ribonucleoside 3′-*H*-phosphonothioates pointed toa different mechanism of asymmetric induction. For instance, in the reaction of *p*-chlorophenyl uridine 3′-*H*-phosphonothioate (dmtU$_{PHS}$PhCl, the thio analogue of **4b**) with MeOH (5 equiv.) the major diastereomer of dmtU$_{PHS}$PhCl (not the minor one as in the oxo series, *cf.* Fig. 6) was the more reactive isomer and it was consumed preferentially in the reactions with alcohols (Fig. 8). This might indicate that the DYTR mechanism was operating; however, some experimental data suggested that retention of configuration could occur. Further studies on these phenomena are in progress in this laboratory.

‖ This may be exploited practically for the synthesis of alkyl nucleoside *H*-phosphonate diesters enriched in the L_P diastereomers; however, this method seems to be limited to simple alkyl derivatives, preferably primary ones.

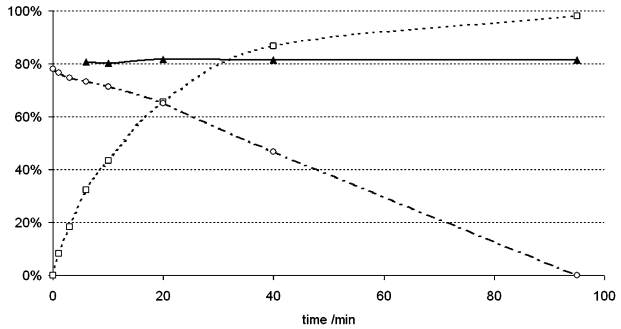


Fig. 8 Time course of reaction of dmtU$_{PHS}$PhCl with 5 equiv. of MeOH. –○– fraction of the initially major (presumably D_P) diastereomer of dmtU$_{PHS}$PhCl in total D_P + L_P mixture; –□– % of the product dmtU$_{PHS}$Me; —▲— fraction of the major (presumably D_P) diastereomer of dmtU$_{PHS}$Me in the total D_P + L_P mixture.

Influence of the kind of catalysis on chemistry and stereochemistry of condensations

For the sake of simplicity, the participation of potential organocatalysis was omitted in the mechanistic considerations presented so far. However, the amines used in condensations of *H*-phosphonates may act not only as acid scavengers and base catalysts activating alcohols (general base catalysis) but also as nucleophilic catalysts operating on the condensing agent or on the mixed anhydride of type **2**, while their salts, formed as by-products in the course of condensation, as acid catalysts operating on the mixed anhydride **2** (general acid catalysis). Since each of these types of catalysis may have an impact on the mechanism of condensation and possible side reactions, their involvement in the condensation of *H*-phosphonates deserves some comments.

Powerful nucleophilic catalyst used in carbon[48] and P(V)[49] chemistries, *e.g.* DMAP, DABCO, and NMI, were found to be inefficient in condensation of *H*-phosphonates.[50,51] While under mild reaction conditions used in the current experiments (3 equiv. of amine in neutral solvent at r.t.), the formation of P-acylated by-products reported previously was largely or completely eliminated, and an increased demand of pivaloyl chloride (>3 equiv. instead of 1.2 equiv. in the presence of pyridine) for completion of the condensation was observed.[52,53] There may be several reasons for this. Strong nucleophilic catalysts can operate efficiently on acyl chlorides used as condensing agents, forming very reactive N-acyl onium salts. Such species lack selectivity towards *H*-phosphonate centre and are able to react rapidly with the hydroxylic components of the condensation mixture[54,55] and, presumably, with the pivalate anions released to the reaction mixture as side products of condensation, leading to undesired consumption of the condensing agent and depletion of alcohol/nucleoside. Poor yields of esterification of the mixed anhydride **2** in the presence of NMI or DMAP was also observed,[53] and could be due to lack of chemoselectivity of attack of the nucleophilic catalyst on P *vs.* C=O centres in dmtU$_{PH}$Pv (**2**) with partial formation of the *N*-acyl onium salt (which may give rise to several side products, *vide supra*) and regeneration of the substrate **1**.

Apart from problems with efficiency, the lower stereoselectivity of condensations in the presence of strong nucleophilic catalysts was observed (*e.g.* *de* 53% for DMAP *vs.* 62% for pyridine or 70% for 2,6-lutidine[52]). This could be a result of relatively low $k_{equilibration}/k_{esterification}$ ratio for P–N^+ adducts of type **3** (*cf.* Fig. 3, reaction A).

In contrast to the unsatisfactory results obtained with powerful nucleophilic catalysts, pyridine and most of its alkyl derivatives were able to promote quantitative and stereoselective condensations of ribonucleoside 3′-*H*-phosphonates. The participation of nucleophilic catalysis during *H*-phosphonate condensation is a well-established phenomenon,[43,51,56] which clearly may have an effect on the mechanism of asymmetric induction. Despite this, in condensations performed in the presence of nucleophilic catalysts the same trend of stereoselectivity was observed as in their absence (*i.e.* predominant formation of D_P diastereomers of *H*-phosphonate diesters).[52] This suggested that in the presence of nucleophilic catalysts the DYKAT process operates at the level of phosphonopyridinium adducts of type **3**, which apparently equilibrate and are esterified (Fig. 3, reaction A), with the $k_{equilibration}/k_{esterification}$ ratio being comparable to those for the mixed anhydride **2** (reaction B).

Significant differences between nucleophilic and non-nucleophilic amines were observed in kinetic quenching experiments, in which a dramatic decrease of stereoselectivity of *H*-phosphonate condensations was observed when mild nucleophilic catalysts were used (*e.g.* pyridine), while for non-nucleophilic amines only minor variations in stereoselectivity occurred. This indicated that the DYKAT was less efficient when nucleophilic catalysis operated, presumably due to decreasing equilibration rates of intermediate **3** in comparison to its esterification ($k_{\mathbf{3}\text{-}L_P}/k_{\mathbf{3/5}\text{-}D_P} < k_{\mathbf{2}\text{-}L_P}/k_{\mathbf{2/5}\text{-}D_P}$; Fig. 3).[52] Such an interpretation was in line with the previously assumed reasons for lower stereoselectivity observed for strong nucleophilic catalysts (*vide supra*).

The above conclusions indicated that condensations of ribonucleoside 3′-*H*-phosphonates in the presence of non-nucleophilic amines could exhibit particularly high stereoselectivity. To verify this, a set of tertiary amines differing in basicity and structure were tested as bases in condensations of $dmtU_{PH}$ (**1**) with ethanol using PvCl as a condensing agent.[53] Despite the lack of nucleophilic catalysis, the reactions were rapid and in the majority of cases, no by-products could be observed in the ^{31}P NMR spectra. However, a close-to-stoichiometric amount of PvCl (1.2 equiv.), which was usually adequate for quantitative condensations in the presence of pyridines, was often insufficient in the case of tertiary amines, particularly for those of $pK_a > 7$, which required > 2.5 equiv. of the condensing agent. Since pivaloylation of nucleoside moieties and hydroxy functions was found to be rather sluggish in the absence of a nucleophilic catalyst,[55] two processes were considered to explain the increased need for PvCl: (i) formation of pivalic anhydride (Pv_2O) in reaction of PvCl and PvO^-, and (ii) formation of Pv_2O in reaction of PvO^- and the mixed anhydride **2**. Both of the above mechanisms were in agreement with the increased demand of the condensing agent in the presence of amines of higher pK_a (higher extent of ionisation of PvOH)[57] and for reactions performed in less polar solvents.** However, since pre-formed mixed anhydride **2** was esterified almost quantitatively in the presence of various tertiary amines,[53] deacylation of **2** by PvO^- could not be the main pathway of formation of Pv_2O during regular condensations (nevertheless, it was significant when the rate of esterification of **2** was substantially decreased due to low basicity of an amine and large steric hindrance of the attacking nucleophile).

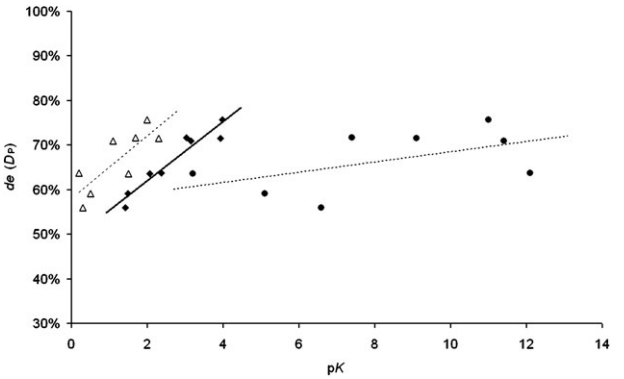


Fig. 9 A plot of *de* of condensation of $dmtU_{PH}$ (**1**) with EtOH in the presence of various amines *vs.* their basicities.[53] △, H-bonding basicity (pK_{HB}; $R^2 = 0.657$); ●, Brønsted basicity (pK_a; $R^2 = 0.286$); ◆, "DYKAT basicity" ($pK_{DK} = 0.18\,pK_a + pK_{HB}$; $R^2 = 0.916$). Amines (pK_a): Tröger's base (3.2); dimethylaniline (5.1); diethylaniline (6.6); *N*-methylmorpholine (7.4); N^1,N^1,N^2,N^2-tetramethylethylenediamine (9.1); triethylamine (11.0); diisopropylethylamine (11.4); Proton Sponge (12.1).

While the yields of condensations could be correlated with Brønsted basicity of the tertiary amine used (with several exceptions, mainly due to specific steric or electronic factors) and the rates of reaction of PvCl with PvO^-, for stereoselectivity such a relationship could not be found (Fig. 9). However, when hydrogen bond basicities (pK_{HB}) of the amines were plotted against *de*, a trend of increasing *de* could be noticed. It was interesting to see that upon combining these two types of basicity in a form of a single parameter tentatively called "DYKAT basicity", given by empirical eqn (3):

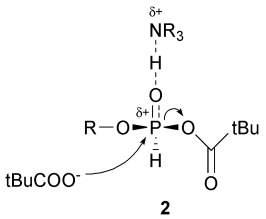
$$pK_{DK} = 0.18\,pK_a + pK_{HB} \quad (3)$$

a fit of $R^2 = 0.916$ was achieved for a trend line (the solid line in Fig. 9) expressed by eqn (4):

$$de = 0.0661\,pK_{DK} + 0.4875 \quad (4)$$

The contribution of Brønsted basicity in eqn (3) may be explained in terms of increasing the rate of D_P-**2b** ⇋ L_P-**2b** equilibration by more efficient ionisation of pivalic acid. The essential role of H-bonding basicity is less obvious. Since this parameter may reflect a propensity of a conjugate acid of an amine to act as a general acid catalyst, amines of high H-bonding basicity may be expected to facilitate equilibration of **2** by increasing electrophilicity of *H*-phosphonate moiety in the mixed anhydride **2** (Fig. 10). Alternatively, the generated acid catalyst may facilitate the departure of the leaving group.

** Side reactions involving charged molecules are expected to be slowed down in polar environments, while esterification should be accelerated; the latter was confirmed experimentally.[53]



2

Fig. 10 A putative role of general acid catalysis on the increased rate of P-epimerisation of the mixed anhydride **2**.

Preliminary attempts to employ tertiary amines in the synthesis of dinucleoside *H*-phosphonate were only partially successful. A significantly lower rate of esterification of the mixed anhydride **2** with nucleoside than that of EtOH made competitive P-acylation a significant side reaction (≥10%). On the other hand, a ratio of $D_P/L_P > 95:5$ of the produced diester **5c** could be easily achieved, and called for further studies on optimisation of this approach. At present stage of investigations, heterocyclic amines able to act as mild nucleophilic catalysts may be recommended as basic components for the *H*-phosphonate condensation. According to a series of model experiments, the reaction conditions that provided the highest yield and stereoselectivity consisted of using 25 mM concentration of *H*-phosphonate **1** in DCM containing 2,6-lutidine (*ca.* 7% v/v) and 1.2 equiv. of an alcohol/nucleoside, with 2–3 equiv. of PvCl as a condensing agent.[45]

Structural backgrounds for DYKAT

According to the discussion presented so far, a critical role of the rates of isomerization of the reactive *H*-phosphonic intermediates (**2**, **3**, or **4**) was manifested in every variant of the process (Fig. 3, reactions A, B, and C). Moreover, in all instances the L_P diastereomer of the intermediate of type **2–4** was significantly more reactive than its D_P congener, and this was also the case for other types of the condensing agents used.[45] The structure of a hydroxylic component seems to be of minor importance for the stereoselectivity observed for various alcohols and nucleosides[41,46] and presumably reflected different rates of esterification, *e.g.* due to accessibility of the

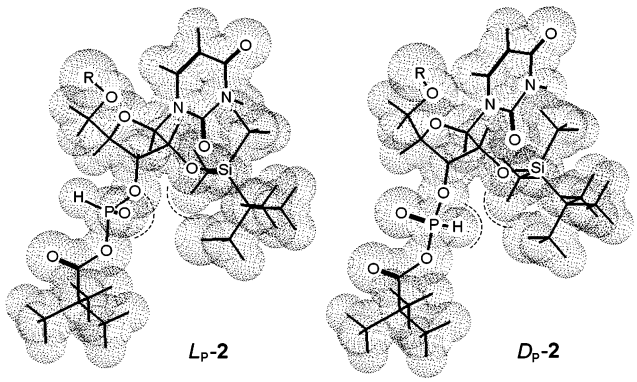


Fig. 12 3D models of the mixed anhydride **2** with *H*-phosphonate moiety in the *ap* conformation (structures of the low energy conformation family generated in HyperChem;[58] Polak–Ribiere algorithm in CHARMM force field). A putative repulsion between the *H*-phosphonate and the 2′-groups is indicated with dotted lines. The P–$O^{2'}$ distances in the above models are 4.2 Å and 3.9 Å, while the 2′-H–C–O–P dihedral angles are 43.4° and 13.0° for **2**-L_P for **2**-D_P, respectively.

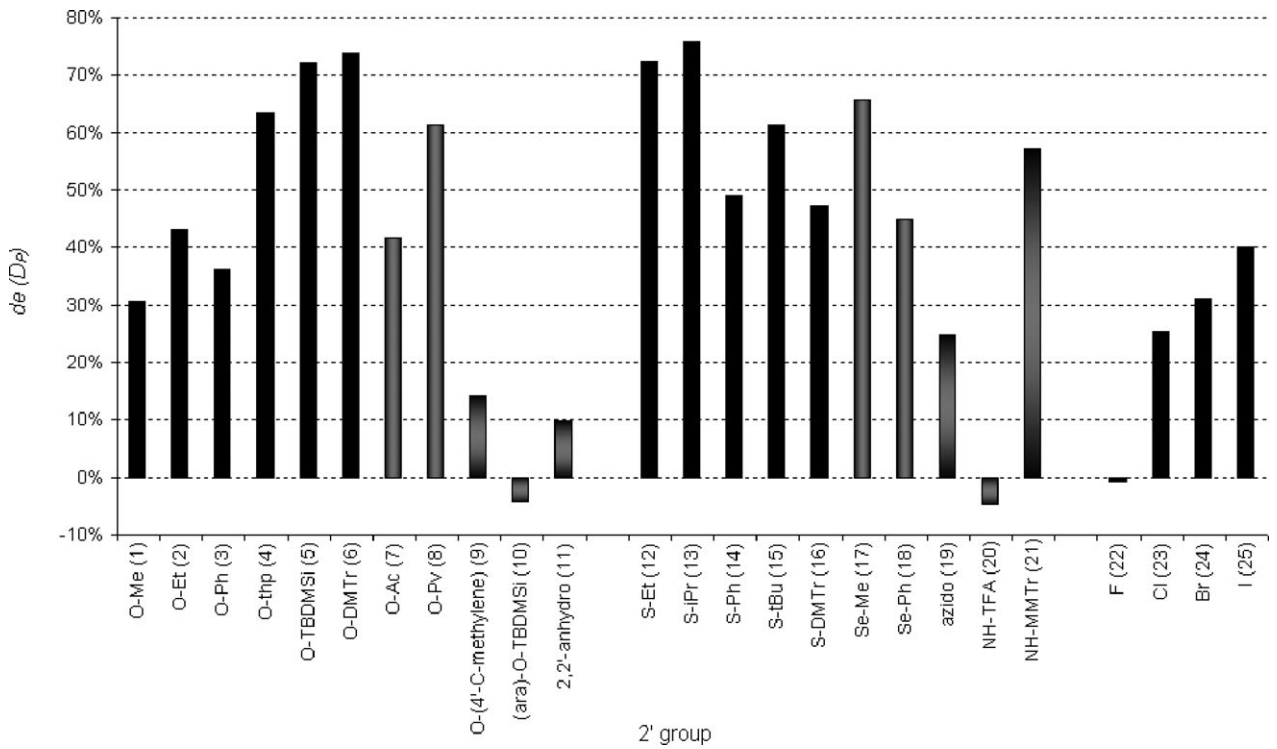


Fig. 11 The influence of 2′-modification on the stereochemistry of condensations of derivatives of uridine 3′-*H*-phosphonates of type **1** with EtOH. LNA-T (entry 9): a derivative of 2′-*O*-(4′-*C*-methylene)thymidine.

hydroxy group, concentration, or diffusion rate. This indicates that the steric features of the incoming nucleophiles and the leaving groups are less important in the context of the underlying mechanism of asymmetric induction. Thus, a question arises, what are the structural backgrounds for the observed diverse reactivity of D_P and L_P diastereomers of ribonucleoside 3′-*H*-phosphonates?

While the most obvious answer indicates steric factors introduced by the bulky protecting group in the 2′-O position,[36,37] electronic reasons, *e.g.* electrostatic repulsion or hydrogen bonding between atoms in the *H*-phosphonic moiety and the 2′-O oxygen, cannot be excluded. The A P–H···O$^{2'}$ hydrogen bond may be expected to be rather weak; however, in the crowded hydrophobic environment of ribonucleoside protecting groups its formation may be favoured. This would constrain the conformational freedom of the *H*-phosphonic moiety in a 6-membered quasi-ring structure in which the phosphorus atom in one P-diastereomer could be better accessible for a nucleophile than in another one.

In order to get insight into this problem, a number of derivatives of 5′-(dimethoxytrityl)uridine *H*-phosphonate **1** bearing various modifications at the 2′ position were prepared and studied in condensations with EtOH (Fig. 11). A good correlation between the size of the substituent in the 2′-O position and the stereoselectivity observed was found (entries 1–8), with no significant differences between alkyl and acyl groups [*e.g.* ethyl (entry 4) and acetyl (entry 7)] that could be expected if the hydrogen bond was involved (the case of 2′-*O*-phenyl derivative is discussed later in the text). Also in a series of 2′-deoxy-2′-(β-halogeno)uridines (entries 22–25), the

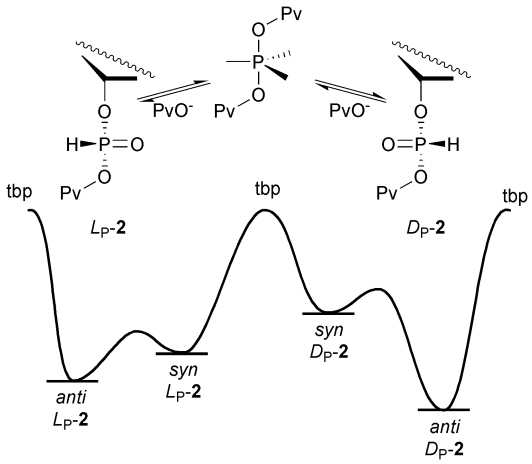


Fig. 13 A putative energy diagram for PvO$^-$ + **2**-D_P ⇋ **2**-L_P + PvO$^-$ isomerization.

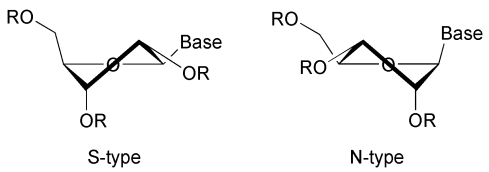


Fig. 15 S- and N-types of conformation of the ribose ring.

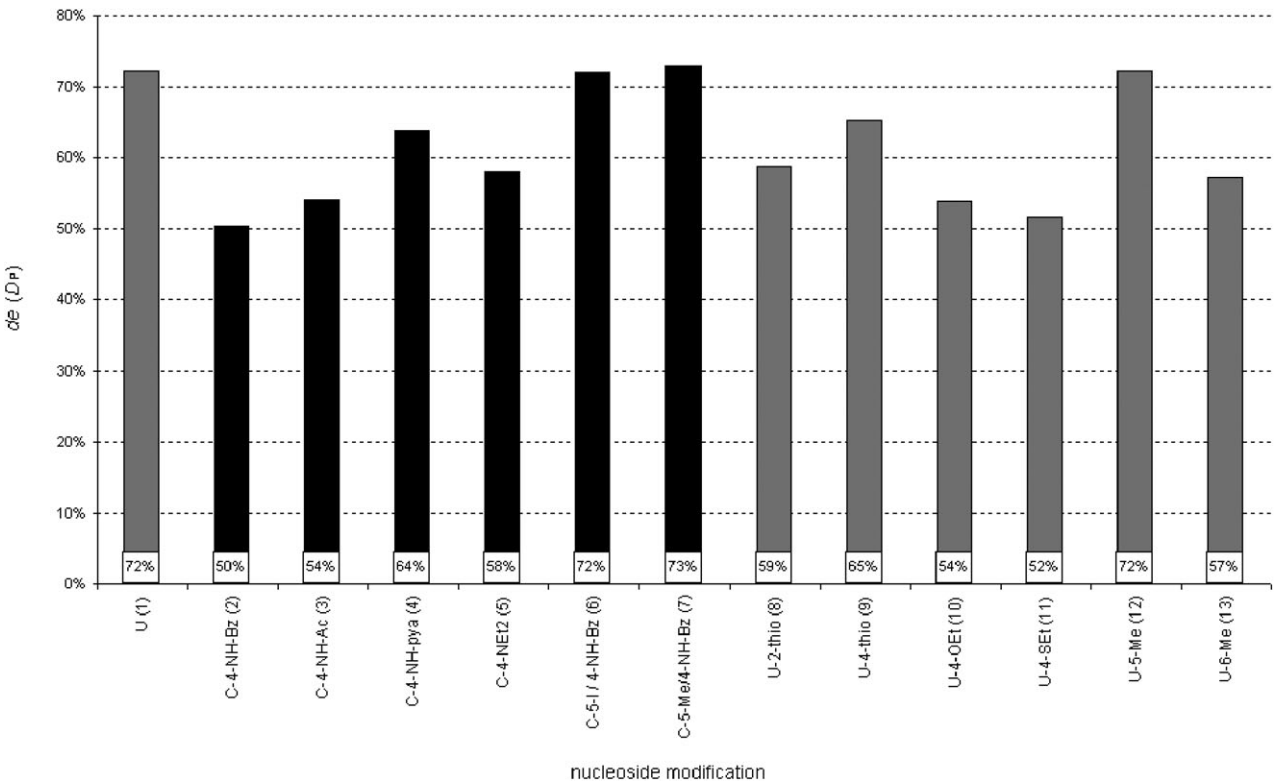


Fig. 14 The influence of modifications in pyrimidine moiety on the stereochemistry of condensations of 5′-(DMTr)-2′-(TBDMSi)nucleoside 3′-*H*-phosphonates of type **1** with EtOH. Grey bars, uridine derivatives; black bars, cytidine derivatives. "C-4-NH-pya", a derivative of N^4-(*N*-methylpyrrolidin-2-ylidene)cytidine.

stereoselectivity gradually increased with the growing size of a halogen atom, the *de* reaching 40% for the iodo derivative.

It is worth noting however, that the ^{1}H NMR signals of the P–H proton in 5′-*O*-(dimethoxytrityl)-2′-deoxy-2′-fluorouridine *H*-phosphonate were split into doublets (*J* 1.5 Hz) that might be a result of formation of a P–H···F hydrogen bond. If this was the case, the complete lack of stereoselectivity during condensation of this derivative (entry 22) might indicate that the hydrogen bonding plays only a supportive role, while the primary reason for the asymmetric induction observed were the steric interactions. Indeed, the condensations of 2′-thio and 2′-seleno derivatives, for which the contribution of P–H···X bonding was expected to be negligible, showed particularly high stereoselectivity (entries 12, 13 and 17). Interestingly, further increasing the size of the substituent in the 2′-X position ultimately led to decrease of stereoselectivity (X = S, entries 15 and 16; X = S *vs.* X = Se, entries 12 and 14 *vs.* 17 and 18, respectively). This somewhat surprising change in the previous trend might indicate that a steric hindrance that is too large could compromise P-epimerisation or that a new reaction pathway, so far unfavourable, could become energetically accessible when the steric hindrance exceeded some threshold value.

Among the investigated compounds, unusual stereochemical results were obtained for 2′-deoxy-2′-(trifluoroacetylamino)-uridine 3′-*H*-phosphonate (entry 20), which shows a small inverted stereoselectivity during condensation with EtOH. This could hardly be explained on steric grounds, since condensation of a sterically similar *O*-acetyl derivative was clearly a stereoselective reaction (entry 7, *de* 42%). However, in this case strong hydrogen bonding of reversed polarity, P=O···H–N, could develop, and the electrostatic and steric effects could work in opposite directions, yielding a net lack of stereoselectivity. When a large trityl group was introduced into the 2′-N position, the steric effect apparently prevailed (entry 21). A common trend of decreased stereoselectivity observed for 2′-X-phenyl derivatives (entries 3, 14 and 18 for X = O, S, Se, respectively) might be a result of 'staking' interactions between phenyl and pyrimidine rings, which could pull out the 2′-group from the vicinity of the *H*-phosphonate moiety. Marginal stereoselectivity of condensation was also observed when the 2′-O group was bonded in a distant 4′-position (entry 9) or pointed 'upwards' as in the *arabino* configuration (entries 10 and 11).

Thus, apart from several exceptions, for most of the cases the observed variations in stereoselectivity of condensations could be associated with steric features of the substituent in the 2′-position. In contrast to this, modifications at the 5′-position were insignificant for the stereochemistry of condensations, and even 5′-deoxyuridine *H*-phosphonate reacted with the same stereoselectivity as 5′-*O*-DMTr, 5′-*O*-Pv, or 5′-*O*-Ac derivatives (*de* 70 ± 1.5%).

Similar qualitative conclusions about the impact of steric hindrance at the 2′ and 5′ positions on stereoselectivity can be drawn from inspection of 3D models. Thus, for D_P diastereomers of the disubstituted *H*-phosphonate moiety (*e.g.* in the mixed anhydride **2**) placed in an antiperiplanar conformation, the P–H hydrogen points towards the bulky 2′-moiety, while the P=O oxygen points towards the relatively

Table 2 ^{1}H (in $CDCl_3$ unless otherwise noted) and ^{31}P NMR (in DCM) data for the 2′-derivatives of 5′-(dimethoxytrityl)uridine-3′-yl *H*-phosphonates of type **1**

Entry	2′-group (solvent)	δ_H/ppm[a]								J/Hz					δ_P/ppm	Other signals/ppm
		1′	2′	3′	4′	5′	5″	5	6	P–H	1′–2′	2′–3′	3′–4′	4′–5′	4′–5″	5′–5″	5–6	($^1J_{PH}$, $^3J_{PH}$/Hz)	
1	O-Me	5.99 d	4.04 dd	4.91 ddd	4.29 dt	3.52 m	3.52 m	5.16 d	7.92 d	6.97 d	2.7	5.1	7.2	nr[b]	nr	nr	8.1	1.64 dd (626.4, 7.3)	2′-OCH_3: 3.57, s, 3 H
2	O-Et	5.97 d	4.13 dd	4.86 ddd	4.29 dt	3.53 dd	3.47dd	5.14 d	7.89 d	6.95 d	7.5	4.5	0.0	2.1	1.7	11.0	8.1	2.91 dd (628.2, 8.2)	2′-OCH_2CH_3: 1.18, t, *J* 7.0 Hz, 3 H; 2′-OCH_2CH_3: 3.76 & 3.79, 2× q, *J* 7.0 Hz, 2× 1 H
3	O-Ph	6.14 d	5.07 m	5.10 m	4.50 br dt	3.60 br	3.59 br	5.17 d	7.97 d	6.95 d	2.7	nr	6.1	nr	nr	nr	8.1	2.33 dd (630.0, 9.1)	Ar: 6.81–7.40 m, 18 H
4	O-THP[c] (Py-d_5)	6.88 d & 6.64 d	5.18 t & 5.11 t	5.64 m	4.84 m & 4.80 m	3.95 m & 3.81 dd	3.89 m & 3.73 dd	5.69 d & 5.61 d	8.25 d & 8.19 d	7.64 d & 7.56 d	6.6 & 3.6	nr	nr	nr & 3.3	nr & 2.1	nr & 10.5	8.1 & 8.1	2.32 dd (620.9, 10.0) & 1.81 dd (620.0, 9.2)	2′-O-THP: 5.10 & 5.36 br, 1 H; 3.7 m, 2 H; 1.75 m, 2 H; 1.29 m, 4 H
5	O-TBDMSi	5.94 d	4.45 t	4.76 dt	4.36 br dt	3.57 dd	3.53 dd	5.15 d	7.89 d	6.93 d	4.2	4.5	4.8	2.3	2.3	11.5	8.2	3.16 dd (623.6, 9.2)	$SiCH_3$: 0.11 & 0.15, 2× s, 2× 3 H; $SiC(CH_3)_3$: 0.87, s, 9 H
6	O-DMTr	8.53 d	5.23 dd	4.77 m	4.30 m	3.62 dd	3.52 dd	5.13 d	7.74 d	7.14 d	6.6	nr	nr	2.2	1.8	10.9	8.1	2.16 dd (620.6, 8.2)	Ar: 6.69–7.50 m, 26 H; OMe (O-DMTr): 3.80, 2× s, 2× 6 H
7	O-Ac	6.23 d	5.57 t	5.08 dt	4.39 br dt	3.54 dd	3.49 dd	5.22 d	7.78 d	6.90 d	6.0	4.5	3.3	2.7	2.3	11.0	8.1	2.99 dd (628.8, 10.0)	2′-O-Ac: 2.22 s, 3 H

Table 2 (*continued*)

Entry	2′-group (solvent)	δ_H/ppm[a] 1′	2′	3′	4′	5′	5″	5	6	P–H	*J*/Hz 1′–2′	2′–3′	3′–4′	4′–5′	4′–5″	5′–5″	5–6	δ_P/ppm ($^1J_{PH}$, $^3J_{PH}$/Hz)	Other signals/ppm
8	O-Pv	6.20 d	5.51 t	5.03 dt	4.39 br d	3.56 dd	3.49 dd	5.21 dd	7.79 d	6.88 d	5.7	4.8	3.3	2.6	2.4	10.5	8.1	3.59 dd (627.3, 10.1)	2′-O-Pv: 1.23 s, 9 H $^4J_{H3-H5}$ 1.5 Hz
9	LNA-T	5.63 br	4.69 br	4.75 br d	—	3.97 br d	3.82 br d	—	7.59 s	6.92 d	nr	nr	nr	nr	nr	7.8	—	1.64 dd (635.6, 7.3)	C^5–CH_3: 1.61 s, 3 H; $C^{4\prime}$–CH_2: 3.51 s, 2 H
10	α-*O*-TBDMSi	6.21 d	4.29 m	4.31 m	4.44 dd	3.36 d	3.29 d	5.50 d	7.41 d	6.89 d	2.9	nr	3.9	nr	nr	9.8	8.1	1.61 dd (618.6, 10.1)	$SiCH_3$: −0.21 & −0.05, 2× s, 2× 3 H; $SiC(CH_3)_3$:0.58, s, 9 H
11	2,2′-anhydro	6.22 d	4.41 m	4.67 m	4.08 br dt	3.93 dd	3.88 d	5.63 d	7.81 d	6.90 d	5.1	4.5	5.0	3.1	2.5	12.3	8.1	2.06 dd (620.8, 9.2)	—
12	S-Et	6.20 d	3.64 m	5.05 m	4.40 br	3.58 dd	3.47 dd	5.18 d	7.78 d	7.00 d	7.1	nr	nr	nr	nr	10.4	7.9	3.40 (632.8, 10.1)	2′-SCH_2CH_3: 1.24, t, *J* 7.1 Hz, 3 H; 2′-SCH_2CH_3: m, 2 H
13	S-iPr	5.16 d	3.59 dd	5.02 ddd	4.41 br	3.63 d	3.46 dd	5.16 d	7.80 d	7.01 d	8.1	4.8	3.0	nr	2.4	10.8	8.1	3.57 dd (627.3, 10.1)	$CH(CH_3)_2$: 1.23 & 1.28, 2× d, *J* 6.6 Hz, 2× 3H; $CH(CH_3)_2$: overlapped by $TEAH^+$
14	S-Ph	6.41 d	3.93 dd	5.17 dd	4.43 br	3.56 dd	3.39 dd	4.87 d	7.34 d	7.03 d	9.3	4.8	nr	2.6	2.6	10.8	8.2	2.37 (618.2, 10.1)	Ar: 6.74–7.46 m, 18 H
15	S-*t*Bu	6.16 d	3.55 2× dd	4.99 dd	4.47 br	3.67 dd	3.50 dd	5.20 d	7.93 d	7.11 d	9.9	4.5	10.5	1.7	3.0	10.8	8.1	3.08 dd (622.7, 10.1)	$C(CH_3)_3$: 1.36, s, 9 H
16	S-DMTr	6.24 d	3.30 2× dd	4.50 m	3.7 br	3.25 dd	3.13 dd	5.17 d	7.69 d	7.24 d	10.2	4.7	nr	1.8	2.8	10.9	8.1	2.50 dd (619.6, 8.2)	Ar: 6.63–7.39 m, 26 H; OMe (O-DMTr): 3.69 s, 6 H; OMe (S-DMTr): 3.72 2× s, 2× 3 H
17	Se-Me	6.34 d	3.67 dd	5.03 ddd	4.40 br d	3.57 dd	3.47 dd	5.17 d	7.76 d	6.99 d	6.9	5.7	3.1	2.3	2.7	10.8	8.1	3.59 dd (626.4, 9.2)	2′-Se-CH_3: 2.05 s, 3 H
18	Se-Ph	6.55 d	3.89 dd	5.16 dd	4.45 br	3.56 dd	3.39 dd	4.80 d	7.28 d	7.02 d	9.6	4.7	nr	2.6	2.6	10.5	8.1	2.41 dd (617.2, 9.9)	Ar: 6.73–7.57 m, 18 H
19	azido	5.87 d	4.36 dd	5.00 ddd	4.26 br d	3.55 dd	3.49 dd	5.22 d	7.91 d	6.96 d	2.5	5.4	7.3	1.5	2.1	11.5	8.1	3.72 (627.7, 9.3)	—
20	NH-TFA (DMSO-d_6)	5.99 d	4.55 dt	4.68 dd	4.13 br t	3.38 dd	3.28 dd	5.44 dd	7.58 d	6.64 d	8.4	5.7	4.0	2.1	3.9	10.7	8.1	3.57 (628.3, 10.1)	2′-NH: 11.45 d, $^3J_{H2'-NH}$ 5.9 Hz, 1 H; N3H: 11.32 d, $^4J_{H3-H5}$ 1.9 Hz, 1 H
21	NH-MMTr	6.34 d	3.62 m	3.52 m	4.56 br s	3.23 d br	3.08 dd	5.13 d	7.88 d	6.43 d	8.8	nr	nr	nr	2.7	11.0	8.1	6.02 (629.2, 6.4)	2′-NH: *ca.* 3.0; overlapped by $TEAH^+$; OMe (O-DMTr): 3.74 & 3.75, 2× s, 6 H; OMe (N-MMTr): 3.71 s, 3 H
22	F	6.08 d	5.21 dd	4.95 ddt	4.28 br d	3.60 dd	3.53 dd	5.19 d	7.99 d	7.01 dd	<1 Hz	3.9	8.7	2.1	2.7	11.3	8.1	1.05 (628.2, 9.2)	^{19}F NMR: δ_F −124.87 ddd, $^2J_{F-2'H}$ 51.9 Hz, $^3J_{F-1'H}$ 15.6 Hz, $^3J_{F-3'H}$ 21.9 Hz
23	Cl	6.13 d	4.68 dd	5.04 ddd	4.38 dt	3.56 br	3.56 br	5.17 d	7.94 d	7.02 d	2.7	4.7	7.2	2.1	2.4	11.1	8.1	1.90 dd (629.1, 8.2)	—
24	Br (Py-d_5)	6.84 d	5.42 dd	5.50 m	4.79 dt	3.87 dd	3.78 dd	5.58 d	8.21 d	7.61 d	1.5	5.1	7.8	2.1	2.7	11.1	7.8	1.79 dd (608.5, 10.1)	—
25	I	6.19 d	4.67 dd	4.27 ddd	4.20 br	3.36 m	3.22 dd	5.38 d	7.67 d	6.78 d	2.7	4.8	7.2	1.8	1.8	11.0	8.0	1.81 dd (626.4, 7.3)	—

[a] The signals of dimethoxytrityl group are omitted. [b] nr – not resolved. [c] Two diastereomers.

Table 3 ^{1}H (in $CDCl_3$) and ^{31}P (in DCM) NMR data for the pyrimidine-derivatives of 5′-(dimethoxytrityl)-2′-(*tert*-butyldimethylsilyl)nucleosid-3′-yl *H*-phosphonates of type **1**

		δ_H/ppm[a]									*J*/Hz							δ_P/ppm	
Entry	Nucleobase	1′	2′	3′	4′	5′	5″	5	6	P–H	1′–2′	2′–3′	3′–4′	4′–5′	4′–5″	5′–5″	5–6	($^{1}J_{PH}$, $^{3}J_{PH}$/Hz)	Other signals/ppm
1	N^4-Ac-Cyt	5.87 d	4.42 dd	4.68 m	4.39 br dt	3.61 dd	3.49 dd	6.98 d	8.35 d	6.84 d	1.5	4.0	7.8	1.8	2.8	11.3	7.5	2.06 dd (620.8, 9.9)	C(O)–CH_3: 2.20 s, 3 H
2	N^4-Pya-Cyt[b]	6.00 d	4.44 br	4.67 m	4.38 br	3.53 br d	3.47 br d	5.61 d	7.96 d	6.87 d	3.0	nr[c]	nr	nr	nr	13.6	7.1	1.71 dd (612.7, 9.9)	NCH_2CH_2: 3.39 t, *J* 7.1 Hz, 2 H; CCH_2CH_2: 3.15 t, *J* 7.8 Hz, 2 H; NCH_3: 2.99 s, 3 H $CH_2CH_2CH_2$: 1.99 dt, 2 H
3	N^4,N^4-Diethyl-Cyt	5.91 d	4.39 br	4.67 m	4.33 br d	3.59 br d	3.44 dd	7.46 d	7.99 d	6.82 d	1.8	nr	6.8	nr	2.2	10.9	7.8	2.75 dd (624.5, 9.8)	$N^4CH_2CH_3$: 1.10, br, 6 H; $N^4CH_2CH_3$: 3.18 & 3.65, 2× br, 4 H
4	5-Iodo-Cyt^{Bz}	6.05 d	4.60 t	4.78 m	4.44 br	3.47 br	3.47 br	—	8.30 s	6.88 d	5.6	4.5	nr	nr	nr	nr	—	2.30 dd (626.4, 8.3)	—
5	5-Methyl-Cyt^{Bz}	6.09 d	4.57 t	4.77 dt	4.41 br	3.54 dd	3.46 dd	—	7.86 s		5.7	4.5	3.0	2.0	1.9	10.9	—	3.43 (625.5, 10.0)	5-CH_3: 1.39 s, 3 H
6	2-Thio-Ura	6.58 d	4.56 t	4.78 ddd	4.39 br dt	3.57 br	3.53 br	5.52 d	7.71 d	5.85 d	3.3	4.3	5.9	nr	nr	nr	7.6	2.18 dd (626.3, 9.7)	—
7	4-Thio-Ura	5.83 d	4.44 t	4.75 dt	4.13 br dt	3.58 br	3.30 br	6.01 d	7.74 d		3.3	7.4	5.9	2.4	2.7	10.8	7.6	1.80 dd (622.7, 9.2)	—
8	O^4-Ethyl-Ura	5.91 d	4.42 m	4.68 m	4.42 m	3.60 dd	3.49 dd	5.38 d	8.17 d	6.85 d	2.3	nr	nr	2.0	3.0	11.0	7.5	2.91 dd (620.9, 10.1)	$O^4CH_2CH_3$: 1.32 t, *J* 7.1 Hz, 3 H; $O^4CH_2CH_3$: 4.37 q, *J* 7.1 Hz, 2 H
9	4-Ethylthio-Ura	5.79 d	4.41 dd	4.66 ddd	4.37 br dt	3.59 dd	3.46 dd	5.60 d	8.10 d		1.3	4.2	8.1	1.5	2.8	11.1	7.2	1.52 dd (615.4, 10.1)	$S^4CH_2CH_3$: 1.28, t, *J* 7.4 Hz, 3 H; $S^4CH_2CH_3$: 3.10 & 3.08, 2× dd, ^{2}J 14.7 Hz, ^{3}J 3.3 Hz, 2× 1 H
10	5-Methyl-Ura	6.06 d	4.56 br dd	4.8 ddd	4.40 br dt	3.52 dd	3.46 dd	—	7.69 d		6.0	4.6	2.6	2.2	2.0	11.0	—	2.55 dd (616.3, 9.2)	5-CH_3: 1.22 d, $^{4}J_{C5Me-H6}$ 1.0 Hz, 3 H
11	6-Methyl-Ura	5.68 d	4.61 t	5.02 m	4.16 br	3.87 dd	3.73 dd	5.52 s	—	6.96 d	4.3	7.0	nr	3.1	3.1	12.8	—	2.76 dd (639.3, 10.1)	6-CH_3: 2.30 s, 3 H

[a] The signals of dimethoxytrityl group are omitted. [b] N^4-pya – N^4-(*N*-methylpyrrolidin-2-ylidene. [c] nr – not resolved.

open space on the 4′-side of ribose. For the L_P isomer in similar *ap* conformation, the crowded vicinity of the 2′-group is occupied by the large P═O oxygen atom, making this diastereomer energetically less favourable (Fig. 12). However, in the *anti* conformation of the *H*-phosphonate moiety, an in-line attack opposite to the pivaloyl moiety is hampered by the atoms located above the ribose ring. Upon adopting a *syn* conformation, both approaching and leaving groups are free and easily accessible both for small alcohols and large nucleoside molecules. The *syn* conformation is expected to be energetically more favourable for the L_P diastereomer for the same reasons as the *anti* configuration is for the D_P diastereomer. In consequence, concentration of the L_P diastereomer of the mixed anhydride **2** in the equilibrium state is lower that of D_P, but the larger population of **2**-L_P is in an *anti* conformation, which is more prone to nucleophilic attack, *e.g.* by alcohol. This is qualitatively depicted in Fig. 13.

Its is probable that other factors also significantly affect the stereochemical outcome of condensations of ribonucleoside 3′-*H*-phosphonates, since the above model does not explain why N^4-benzoylcytidine *H*-phosphonate of type **1** reacts with substantially lower stereoselectivity than that observed for A, G, and U.[39,41] However, preliminary studies on condensations of nucleoside *H*-phosphonates derivatised in the 4-, 5- and 6-positions of the pyrimidine ring revealed several regularities (Fig. 14). For instance, high stereoselectivity was observed for the 5-substituted cytidines (entries 6 and 7) and decreased stereoselectivity for the 6-substituted uridine (entry 13) and the 4-alkylated uridines (entries 10 and 11). According to the ^{1}H NMR data of the synthons (Table 3), an increased population of the N-type of conformation (Fig. 15) of the ribose moiety (showing a low $J_{1'-2'}$ value and a high $J_{3'-4'}$ value)[59] usually coincided with a lower stereoselectivity of condensation. It may be speculated that if the conformational preferences of a nucleobase permit adopting the N-type of conformation by the sugar, the *H*-phosphonate moiety is more easily accessible and the stereoselectivity decreases. However, this problem requires further, quantitative, studies on ribonucleoside 3′-*H*-phosphonate condensations, and will be addressed in a separate paper.

Conclusions

This paper summarises the studies into stereoselective condensations of ribonucleoside 3′-*H*-phosphonates with alcohols and nucleosides that have been conducted in our laboratory in recent years.[41,42,45,46,52,53,60] The collected data indicate that these reactions owe their stereoselectivity to a dynamic kinetic asymmetric transformation (DYKAT) type of asymmetric induction, which is based on diverse reactivity of D_P and L_P diastereomers of the reactive intermediate, *H*-phosphonic–pivalic mixed anhydride, towards alcohols.

This process has its source apparently in steric interactions between the 3′-*H*-phosphonic moiety and a bulky group in the 2′-position. It seems that the less reactive D_P diastereomer easily adopts a low-energy *anti* conformation with the phosphoryl group pointing to the bulk of solvent, while for an analogous arrangement of the O═P–H group, the L_P diastereomer requires a *syn* conformation. Since in the *syn*

Table 4 ^{1}H (in $CDCl_3$) and ^{31}P (in DCM) NMR data for the 5′-derivatives of 2′-(*tert*-butyldimethylsilyl)uridine-3′-yl *H*-phosphonates of type **1**

		δ_H/ppm[a]								J/Hz						δ_P/ppm		
Entry	5′-group	1′	2′	3′	4′	5′	5″	5	6	P–H	1′–2′	2′–3′	3′–4′	4′–5′	4′–5″	5′–5″	5–6	($^1J_{PH}$, $^3J_{PH}$/Hz)	Other signals/ppm
1	O-Pv	5.70 d	4.24 t	4.77 m	4.22 br dt	4.40 dd	4.34 dd	5.74 d	7.52 d	6.88 d	3.2	5.5	6.5	3.9	2.6	11.7	8.1	3.09 dd (634.6, 10.0)	5′-O-Pv: 1.24 s, 9 H
2	O-Ac	5.77 d	4.31 t	4.70 m	4.17 br	4.38 br	4.37 br	5.91 d	7.86 d	6.79 d	2.9	5.5	nr[b]	nr	nr	nr	8.1	2.47 dd (615.4, 10.1)	5′-O-Ac: 2.20 s, 3 H
3	5′-Deoxy	5.67 d	4.23 br dd	4.18 m	4.33 quin-tet	1.42 d	—	5.61 d	7.35 d	6.98 d	1.7	7.3	5.9	6.6	—	—	8.1	2.31 dd (624.6, 9.8)	—

[a] The signals of the *tert*-butyldimethylsilyl group are omitted. [b] nr – not resolved.

conformation the phosphorus atom of the mixed anhydride seems to be readily accessible for the attacking alcohol, the L_P diastereomer is more prone to nucleophilic substitution.

Apart from elucidation of a mechanism underlying the asymmetric induction, systematic investigations of the factors influencing chemistry and stereochemistry of ribonucleoside 3′-*H*-phosphonates allowed for developing reaction conditions, under which the D_P diastereomer of *H*-phosphonate diester is formed usually with *de* better than 85%.

Experimental section

Methods and materials

The chemicals, instrumentation, and procedures were similar to those reported earlier.[52] NMR spectra were collected on a Bruker Avance II 400 MHz and Varian Unity 300 MHz spectrometers. ^{1}H and ^{13}C NMR signal assignments were based on 2D correlation spectra. 1 M triethylammonium hydrogencarbonate (TEAB) buffer was prepared by bubbling CO_2 through aqueous TEA at 0 °C until pH 7.0 was reached.

Derivatives of uridine [2′-*O*-alkyl,[61] 2′-*O*-dimethoxytrityl,[62] 2′-*O*-deoxy-2′-*S*-alkyl,[63] 2′-*O*-deoxy-2′-*S*-dimethoxytrityl,[64] 2′-*O*-deoxy-2′-*Se*-methyl,[65] 2′-*O*-deoxy-2′-*Se*-phenyl,[66] 2′-*O*-deoxy-2′-halogeno,[67] 2′-*O*-deoxy-2′-azido,[68] 2,2′-anhydro,[69] arabinouridine,[70] 5′-*O*-acyl,[71] 5′-deoxy,[72] 2-thio,[73] 4-thio,[74] O^4-ethyl,[75] 4-(ethylthio),[75] 5-methyl[76] and cytidine [N^4-acetyl,[77] N^4-(*N*-methylpyrrolidin-2-ylidene,[78] N^4,N^4-diethyl,[79] 5-methyl,[75] and 5-iodo[80]] were prepared according to published methods. 2′-*O*-(Tetrahydropyranyl)uridine[81] and 2′-*O*-deoxy-2′-(trifluoroacetyl)aminouridine[82] were a gift from Dr Ryszard Kierzek, and 6-methyluridine[83] was a gift from Dr Elżbieta Sochacka. LNA-T-CE phosphoramidite was purchased from Link technologies and dephosphitylated[84] to 5′-*O*-(dimethoxytrityl)-2′-*O*-(4′-*C*-methylene)thymidine.

Analytical properties of all the nucleoside derivatives were in agreement with the published data.

The nucleosides were protected in a standard way (a 4,4′-dimethoxytrityl group for the 5′-OH and a *tert*-butyldimethylsilyl group for the 2′-OH position; a benzoyl group for the 4-NH_2 position of cytidine derivatives).[85] The 2′-amino group in 5′-(dimethoxytrityl)-2′-deoxy-2′-aminouridine[82] was protected with a 4-monomethoxytrityl group. The nucleosides were phosphonylated with diphenyl *H*-phosphonate yielding 3′-*H*-phosphonates of type **1**.[86] Their ^{1}H and ^{31}P NMR data are given in Tables 2–4.

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