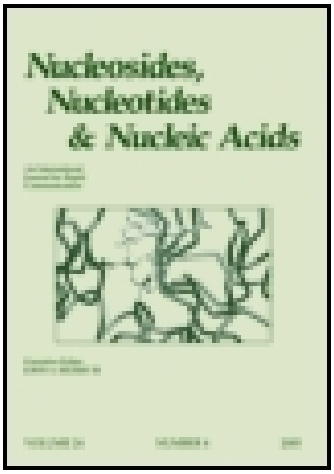

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STEREOCHEMISTRY OF INTERNUCLEOTIDE BOND FORMATION BY THE *H*-PHOSPHONATE METHOD. 5. THE ROLE OF BRØNSTED AND H-BONDING BASE CATALYSIS IN RIBONUCLEOSIDE *H*-PHOSPHONATE CONDENSATION—CHEMICAL AND STEREOCHEMICAL CONSEQUENCES

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□ *Efficiency and stereoselectivity of condensations of ribonucleoside 3′-*H*-phosphonates with ethanol promoted by pivaloyl chloride were investigated as a function of tertiary amines used. Side reactions leading to an increased demand for the condensing agent were identified as derived from an attack of the pivalate anion at carbonyl centers of reactive pivaloyl derivatives. The conditions that secured quantitative yields of* H*-phosphonate diester condensations were assessed. Several tertiary amines promoted condensations with stereoselectivity higher than that observed for pyridine derivatives. A correlation between diastereoselectivity of the product formation and Brønsted and H-bonding basicities of the amine used was found.*

Keywords H-phosphonates; stereochemistry; P-chirality; organocatalysis; reaction mechanism

INTRODUCTION

While *H*-phosphonate chemistry was introduced to nucleotide field by Sir Todd in 1957,[1] the modern approach to internucleotide bond formation by *H*-phosphonate method dates back to 1985–1986 and papers by Stawinski[2] and Froehler.[3] Due to these works, *H*-phosphonates emerged as particularly efficient and versatile precursors for preparation of many biologically active phosphorus compounds.[4,5] Despite this significant broadening of the scope of applications of *H*-phosphonate esters, the initially devised conditions for *H*-phosphonate diester formation, that is, condensations of nucleoside *H*-phosphonate with an alcohol (or nucleoside) in pyridine (which acted both as a base and as nucleophilic catalyst) promoted pivaloyl chloride

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(ca. 3 equiv.), remain practically unaltered in routine laboratory practice.[6] Moreover, the underlying mechanism was recognized only superficial, since the early findings of Stawinski, Froehler and Matteucci, and Efimov were focused mainly on practical aspects of nucleoside 3′-*H*-phosphonate condensations, that is, increasing the yields and avoiding side reactions. Thus, it was established that (i) acyl chlorides (preferentially pivaloyl chloride; PvCl) were efficient activators for nucleoside *H*-phosphonate monoesters,[3,7,8] (ii) *H*-phosphonic—carboxyl acid mixed anhydrides were putative reactive intermediates of the condensations,[9,10] and (iii) pyridine and quinoline significantly enhanced the rate of *H*-phosphonate diester formation, acting as nucleophilic catalysts,[11,12] Pyridine or pyridine-acetonitrile (Py-ACN; in a ratio >1:1) were established as best media for nucleoside *H*-phosphonate condensations, while the use of dichloromethane (DCM) or tetrahydrofuran (THF) as co-solvents was found to be disadvantageous.[10]

The relatively voluminous knowledge on the chemistry of simple alkyl *H*-phosphonates[13] is of limited use when comes to the chemistry of natural product derivatives and mechanical aspects of nucleoside *H*-phosphonate formation promoted by acyl chlorides. A complete chemoselectivity of a nucleophilic attack at the phosphorus center of *H*-phosphonic-acyl mixed anhydrides is a unique and still intriguing feature of *H*-phosphonate chemistry as many other acyl derivatives of phosphorus compounds, for example, acyl *C*-phosphonates and acyl phosphates, show reverse chemoselectivity.

Only recently, the subject of pyridines-catalyzed reactions of nucleoside 3′-*H*-phosphonates was resumed in several mechanistic and computational papers by Sigurdsson and Strömberg,[14–17] while our group focused on stereochemical aspects of nucleoside 3′-*H*-phosphonate condensations.[18–23] For instance, it was found that by decreasing contents of pyridines in the reaction mixture, stability of the reactive *H*-phosphonate intermediates involved was increased by orders of magnitude, while the condensations with nucleophilic species were still rapid and effective.[15,18,20,21,24] An advantage of low concentration of a base in the reaction mixture is suppression of the rate of side reactions associated with an overactivation of *H*-phosphonate monoesters. The amount of condensing agent could be reduced to almost quantitative value (1.1 equiv.).

It was also found that condensation of ribonucleoside *H*-phosphonates **1** with alcohols and nucleosides owes its stereoselectivity to the dynamic kinetic asymmetric transformation (DYKAT) that favors formation of $D_P(S_P)$[64] diastereomer of *H*-phosphonate diesters **3** (Figure 1).[21]

According to this model, diastereomers of nucleoside *H*-phosphonic—pivalic mixed anhydrides exist in a rapid equilibrium, with one diastereomer, namely the $L_P(S_P)$, being significantly more reactive toward nucleosides or alcohols than the other one. A variety of pyridine derivatives promote efficient and highly stereoselective condensations of *H*-phosphonate **1**, most

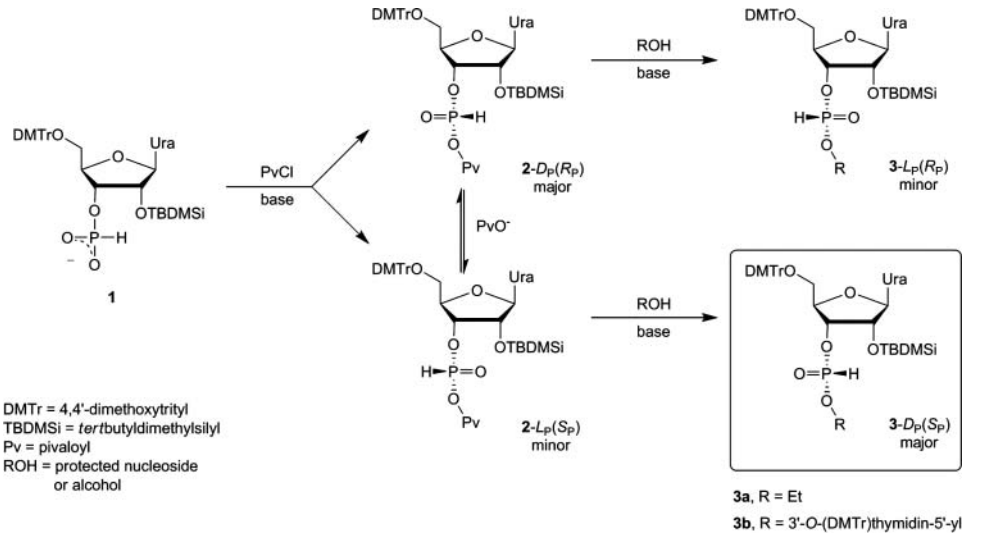

FIGURE 1 Stereoselective formation of $D_P(S_P)$ *H*-phosphonate diesters **3** from ribonucleoside *H*-phosphonate **1**.

probably due to their ability to act as mild nucleophilic catalysts.[22] In contrast, the analogous reactions performed in the presence of more powerful nucleophilic catalysts, for example, 4-dimethylaminopyridine (DMAP) or *N*-methylimidazole (NMI), suffered from decreased stereoselectivity and low yields that were attributed to side reactions at nucleobases and the phosphorus center.[11,22,29–31] Interestingly, in the corresponding condensations in which tertiary aliphatic amines (triethylamine, TEA; diisopropylethylamine, DIPEA) were used as bases, the reactions were rapid, and stereoselectivity was usually higher than that observed for nucleophilic catalysts; however, the yields were not quantitative.[22,23] This unusual observation prompted us to study in more detail *H*-phosphonate condensations proceeding in the presence of non-nucleophilic amines. In this article, we present mechanistic investigations on the influence of general base and acid catalysis on chemistry and stereochemistry of ribonucleoside *H*-phosphonate condensations.

RESULTS AND DISCUSSION

Usually, tertiary aliphatic amines are not considered as suitable basic components of the condensation reactions of *H*-phosphonates since they are unlikely to act as nucleophilic catalysts, and their rather high basicity may result in several side reactions, for example, transesterification of the produced *H*-phosphonate diester,[32] disproportionation,[33] P-acylation,[11] and bis-activation of *H*-phosphonates.[9,17] Nevertheless, we anticipated that if these amines would be used in a small excess (for instance 3 molar equiv.),

the above side reactions might be significantly reduced. Indeed, condensations of uridine 3′-*H*-phosphonate (**1**; c = 0.1 M) with ethanol (5–6 equiv.) using pivaloyl chloride (1.6 equiv.) as a condensing agent, in the presence of various amines (3 equiv.)[65] yielded the desired *H*-phosphonate diester **3a** without detectable P-acylation or bis-activation (^{31}P NMR).[66]

Moreover, despite the lack of nucleophilic catalysis, the condensations appeared to be appreciably rapid in many cases. Both formation of the mixed anhydride **2**, and its subsequent esterification to produce *H*-phosphonate diester **3a** were accelerated not only due to nucleophilic catalysis, but probably also due to base catalysis. For example, the reaction of the mixed anhydride **2** with 3′-*O*-(dimethoxytrityl)thymidine (T_{DMTr}, 1 equiv.) in DCM in the presence of *N,N*-dimethyl aniline (DMA; pK_a 5.1; 10 equiv.) required several hours for completion, while for pyridine (pK_a 5.1) it was complete after ca. 10 minutes. The same reaction in the presence of non-nucleophilic *N*-methylmorpholine, (NMM; pK_a 7.4) was complete after ca. 2 minutes, and for more basic amines the reaction was complete before the first ^{31}P NMR spectrum was recorded (ca. 1 minute).

Yield of Condensation

In contrast to many pyridine derivatives,[15,22] for most of the tertiary amines investigated, including these of relatively low basicity, the condensations were not quantitative and usually ca. 5–30% of the starting *H*-phosphonate monoester **1** remained unreacted (Table 1). From the data collected, a general trend could be noticed, namely that the yield of *H*-phosphonate diester **3a** was lower for more basic amines. The distinct exceptions were tertiary amines able to act as nucleophilic catalysts (hexamethylenetetramine; HMTA, 1,4-diazabicyclo[2.2.2] octane; DABCO), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) that is outstandingly basic in organic solvents.

The presence of significant amounts of unreacted starting material (*H*-phosphonate **1**) in the reaction mixtures suggested that pivaloyl chloride was partly consumed due to side reactions and its amount (1.6 equiv.) was not sufficient for complete esterification of the substrate **1**. Actually, upon increasing the excess of the condensing agent the yield of diester increased proportionally and reached quantitative value when >2.5 equiv. of PvCl were used (Figure 2).

There are several possible reasons for the decreased yields of condensations in comparison to those observed in the presence of pyridine derivatives: (i) reaction of PvCl with residual water, (ii) reaction of PvCl with alcohols and nucleosides, (iii) reaction of PvCl with pivalic acid, and (iv) deacylation of the mixed anhydride **2** by pivalic acid. Other side reactions known in *H*-phosphonate chemistry, for example, dealkylation of the produced *H*-phosphonate diester **3**, for example, by amines or chloride anions,[61,62] were

TABLE 1 The yields of the *H*-phosphonate diester **3a** and diastereomeric excess (*de*) of its D_P(S_P) diastereomer formed from *H*-phosphonate monoester **1** (0.1 M in DCM) and EtOH (6 equiv.) upon activation with 1.6 equiv. of PvCl in the presence of various amines

	Amine[a]	pK_a (H_2O)[a]	pK_a (DMSO)	pK_a (ACN)	pK_a (THF)	pK_{HB}[b]	pK_{ip}[c]	*de* (D_P)[d] /%	Yield of diester[d] /%
Pyridine derivatives[22]									
1	2,6-Di*tert*butyl-pyridine	5.0[34]	1.0[35]					47	70
2	Pyridine	5.2	3.2[36]	12.5[37]	8.3[38]	1.9[39]		62	100[f]
3	2,4,6-Collidine	7.5		15.0[37]		2.3[39]		68	100[f]
4	DMAP	9.7	7.9[36]	17.7[37]		2.8[39]		53	85
Tertiary amines									
5	Tröger's base[f]	3.2[g,40]				1.5[41]		64	100[e]
6	Dimethylaniline (DMA)	5.1	2.5[42]	11.4[37]	7.4[38]	0.5[41]		60	95
7	HMTA[g]	5.2				1.9[43]		57	66
8	*N,N*-diethylaniline (DEA)	6.6				0.3[41]		56	95
9	*N′,N′,N²,N²*-tetramethylphenylenediamine (TEMPhD)	6.7[44]						65	100[e]
10	*N,N*-diisopropylaniline (DIPA)	7.4						53	100[e]
11	*N*-methylmorpholine (NMM)	7.4		15.6[45]	8.5[46]	1.7[43]		72	87
12	*N*-ethylmorpholine	7.7						72	84
13	*N,N*'-dimethylpiperazine	8.3[47]						73	79
14	DABCO[g]	8.7	8.9[48]	18.3[49]		2.6[43]	0.8	53	42
15	*N′,N′,N²,N²*-tetramethylethylenediamine (TEMED)	9.1				2.3[43]		72	80
16	*N′,N′,N²,N²*-tetramethylpropylenediamine (TEMPrD)	9.8						73	74
17	N-methylpiperidine	10.1						72	76
18	*N′,N′,N²,N²*-tetramethylbutylenediamine (TEMBuD)	10.3[50]						71	74
19	*N*-methylpyrrolidine	10.4		18.4[51]				69	79
20	TEA	11.0	9.0[52]	18.8[37]	13.7[38]	2.0[43]	2.1	75	71
21	DIPEA	11.4			10.0[46]	1.1[43]		70	71
22	DBU	11.6		24.3[37]	20.0[38]	3.8[53]	−3.8	73	13
23	(−)− or (+/−)−Sparteine[f]	12.0		21.7[54]				70	72
24	Proton Sponge[f]	12.1[55]	7.5[48]	17.3[56]		0.2[41]	2.2	56	90

[a]Aqueous pK_a data, unless otherwise indicated, are taken from refs.[57,58]

[b]Hydrogen bonding basicity, $pK_{HB} = \log K_{(\text{formation of HB complex})}$, larger values correspond to greater basicity.[59]

[c]Ion pair basicity, $pK_{ip} = -\log K_{ip}$, smaller values correspond to greater basicity.[60]

[d]Determined via integration of the corresponding ^{31}P NMR signals.

[e]No signal of the starting monoester **1** could be detected in the spectrum.

[f]For structure, see Chart 1.

[g]In aqueous EtOH.

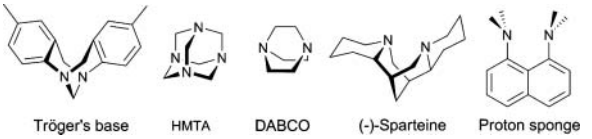

CHART 1

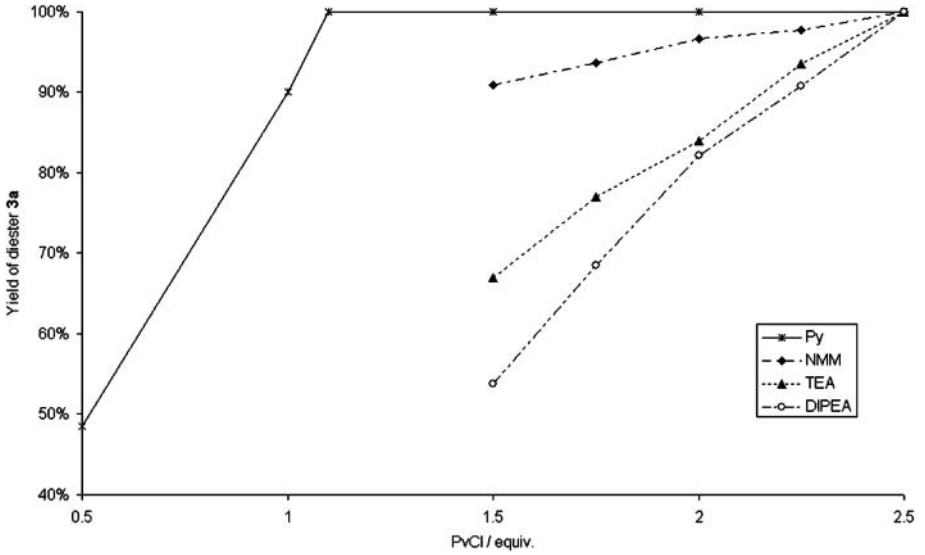

FIGURE 2 Yields of condensations of uridine *H*-phosphonate **1** (0.05 mmol in 0.5 mL of DCM) with EtOH (6 equiv.) promoted by PvCl (0.5–2.5 equiv.) in the presence of different amines (3 equiv.).

rejected as being very unlikely under the mild reaction conditions and short reaction times used in this work.

In order to verify the possibility that water present in the reaction mixture was responsible for lower yields of *H*-phosphonate condensations, the reaction of *H*-phosphonate **1** with EtOH (6 equiv.) in the presence of various amines (3 equiv.) was repeated in ACN with controlled amounts of water added (0.1–6.0 equiv.) to the reaction mixture, or in DCM saturated with water (corresponding to ca. 1 equiv. of H_2O) prior to pivaloyl chloride addition (1.6 equiv.). For weakly basic amines (pyridine and DMA, pK_a ~5) surprisingly high tolerance to water was observed, while for NMM and TEA (pK_a 7.4 and 11.0, respectively) even small amounts of water affected the yield of condensation (Figure 3). Nevertheless, even relatively large amounts of water did not deteriorate the yields dramatically, and the 1.6 equiv. of PvCl used should to be sufficient to compensate for 0.3 equiv. of water in the reaction mixture and preserve a quantitative yield also in the presence of NMM and TEA. Thus, while spurious water might account for some variations in the yields of the condensations investigated, it could not be considered as the reason for a substantially increased demand for PvCl during condensations of *H*-phosphonates in the presence of tertiary amines.

The second possible reason for incomplete condensations in the presence of tertiary amines could be consumption of pivaloyl chloride in acylation of the hydroxyl group and/or N3H function of uridine. While indeed, the condensing agent was found to react rapidly for pivaloylation of a nucleoside in the presence of strongly nucleophilic amines (e.g., DMAP) or

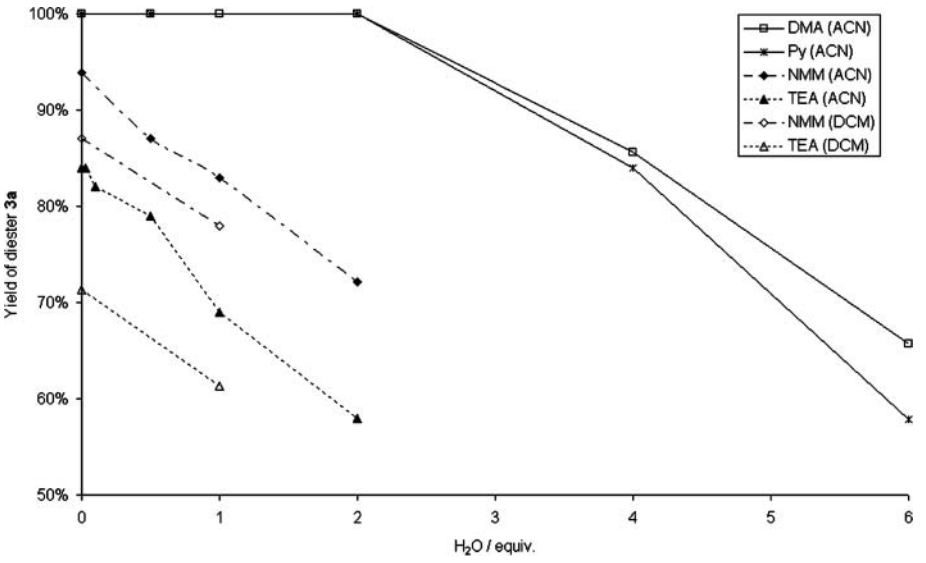

FIGURE 3 Yields of condensations of uridine *H*-phosphonate **1** (0.05 mmol in 0.5 mL of ACN or DCM) with EtOH (6 equiv.) promoted by PvCl (1.6 equiv.) in the presence of water (0–6 equiv.) and different amines (3 equiv.).

TEA-pyridine mixtures, these reactions were negligible when tertiary non-nucleophilic amines were used alone, and could not account for the observed low yields.[29,30]

Thus, the requirement of using relatively large excess of PvCl (>2.5 equiv.) to achieve a complete coupling in the presence of most tertiary amines was tentatively assigned to an attack of the pivalic acid formed as a side product during condensation at carbonyl center of one or both reactive species present in the reaction mixture, that is, pivaloyl chloride and the *H*-phosphonic-pivalic mixed anhydride **2**, yielding pivalic anhydride (Pv_2O) that is unable to act as a condensing agent for nucleoside *H*-phosphonates[63] (Figure 4). The observed general trend for an inverse relationship between the pK_a of an amine and the yield of diester **3a** (Table 1) was in line with this assumption and might be attributed to an increased ionization of the pivalic acid, and the associated enhanced reactivity toward a carbonyl group. Moreover, the differences in yields of the condensations performed in ACN and DCM (Figure 3) were consistent with the assumption that the side reaction involved a pivalate anion (Figure 4, paths c and d), since this reaction might be expected to be slowed down in polar solvent, while the rate of esterification of the mixed anhydride **2** (which is believed to be of $S_N2(P)$ type and involves uncharged species), should be enhanced in polar acetonitrile. Indeed, k_{obs} for the reaction of the mixed anhydride **2** (c = 0.1 M) with T_{DMTr} (1 equiv.)[15] in the presence of DMA (10 equiv.) was found to be 0.014 $M^{-1}s^{-1}$ in DCM and 0.028 $M^{-1}s^{-1}$ in ACN. For pyridine and

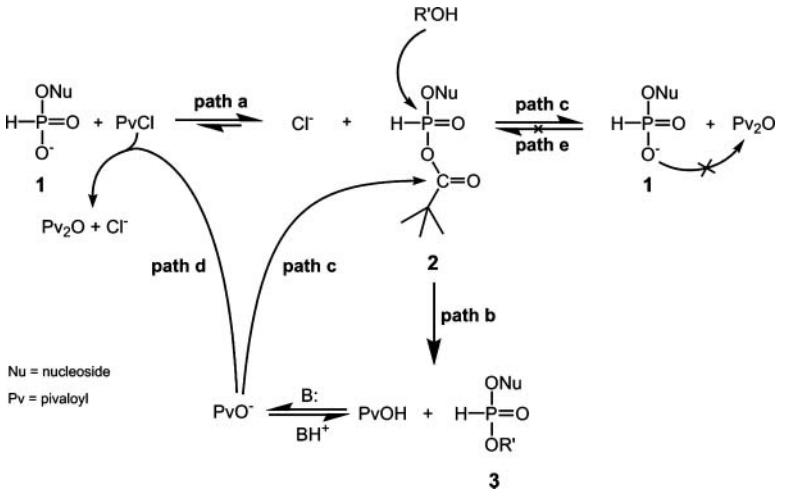

FIGURE 4 Putative reaction pathways induced by pivalate anions released during esterification (path **b**) of the mixed anhydride **2**. Path **a**, formation of **2** (presumably reversible but shifted quantitatively to the right, ^{31}P NMR). Path **b**, reaction with an alcohol yielding diester **3** and pivalic acid. Path c, deacylation of **2** with pivalate. Path d, deactivation of the condensing agent with a simultaneous release of Cl^- anions that may shift the putative equilibrium in path a partly to the left. Path e, a hypothetic acylation of *H*-phosphonate **1** by Pv_2O (not observed[63]).

NMM, the reactions were too rapid for determination of the rate constants, nevertheless, they were significantly faster in ACN than in DCM, as it could be estimated when the reactions were performed in the presence of only 3 equiv. of the same bases.

The influence of the pivalate anion on the condensations of *H*-phosphonate **1** was investigated in further experiments. Thus, when the reactions of **1** with ethanol were performed in the presence of increasing concentration of pivalic acid salts (Table 2), only pyridinium pivalate did not affect the quantitative yield of *H*-phosphonate diester **3**, irrespective of the amount used. Pivalate of DMA caused a moderate reduction in the yield of

TABLE 2 The yield of *H*-phosphonate diester **3a** and the diastereomeric excess of the D_P (S_P) diastereomer of **3a** formed during condensations promoted by PvCl (1.6 equiv.) performed in the presence of pivalic salts of amines

	Equivalents of pivalate			
	0	1.0	3.0	5.0
	yield of **3a** (*de* of **3a**-D_P)/%			
Py•PvOH	100 (62)	100 (58)	100 (55)	100 (48)
DMA•PvOH	95 (56)	92 (53)	88 (49)	81 (47)
TEA•PvOH	70 (75)	58 (69)	17 (65)	8 (59)

TABLE 3 The fraction of *H*-phosphonate monoester **1** formed from the mixed anhydride **2** during a stepwise addition of pivalic salts of amines

		Equivalents of pivalate					
		0.4	0.8	1.2	1.6	2.0	3.0
Salt	pK_a of amine	monoester **1** formed/%					
Py•PvOH	5.2	0	0	0	14	39	58
DMA•PvOH	5.1	0	0	0	0	3	5
TEA•PvOH	11.0	34	77	100	100	100	100

3,[67] while in the presence of triethylammonium pivalate, the yield deterioration was dramatic. In additional experiments it was found that the mixed anhydride **2** was hardly deacylated by DMA•PvOH (Table 3) and that formation of the mixed anhydride **2** in the presence of DMA was rather sluggish (~5 minutes with 2.5 equiv. of PvCl). Thus, for weakly basic tertiary amines, the lower yield of condensations could be attributed to the competitive reaction of PvCl with pivalic acid (resulting Pv_2O) instead of *H*-phosphonate **1** (Figure 4, path d). Since the formation of the mixed anhydride **2** in the presence of TEA was very rapid and deacylation of **2** by TEA•PvOH was very efficient,[68] it could not be excluded that in this case the last reaction was a significant route leading to low yield of *H*-phosphonate diesters formation (Figure 4, path c).

To resolve this ambiguity, the mixed anhydride **2** (prepared in situ) was treated with EtOH in the presence of various amines (pyridine, TEA, DIPEA, DMA, NMI, or DMAP). Under such conditions ethyl nucleoside *H*-phosphonate diester **3a** was formed quantitatively for pyridine and DMA, and almost quantitatively (96–97%) for TEA and DIPEA. In contrast, in the presence of strong nucleophilic catalysts, NMI and DMAP, the yield of **3a** was comparable to that of during routine condensations without preactivation of *H*-phosphonate **1**. (*de* 80% vs. 73% for NMI, and *de* 48% vs. 46% for DMAP).[69] High yields in the reactions in the presence of non-nucleophilic amines indicated that deacylation of the mixed anhydride **2** was too slow to compete significantly with its esterification. However, when the mixed anhydride **2** was reacted with 1 equiv. of T_{DMTr} in the presence of 10 equiv. of DMA and 3 equiv. of DMA•PvOH, the main product of the reaction was monoester **1**, indicating that the main pathway under such conditions was deacylation of the mixed anhydride **2** by pivalate anions.

To conclude this part, during routine condensations of *H*-phosphonates in the presence of tertiary amines, formation of pivalic anhydride was the main side reaction responsible for increased demand for pivaloyl chloride in comparison to condensations done in the presence of pyridine derivatives. Most probably, the pivalic anhydride was formed mainly via the reaction of pivaloyl chloride and the pivalate anion (path d in Figure 4). Deacylation of

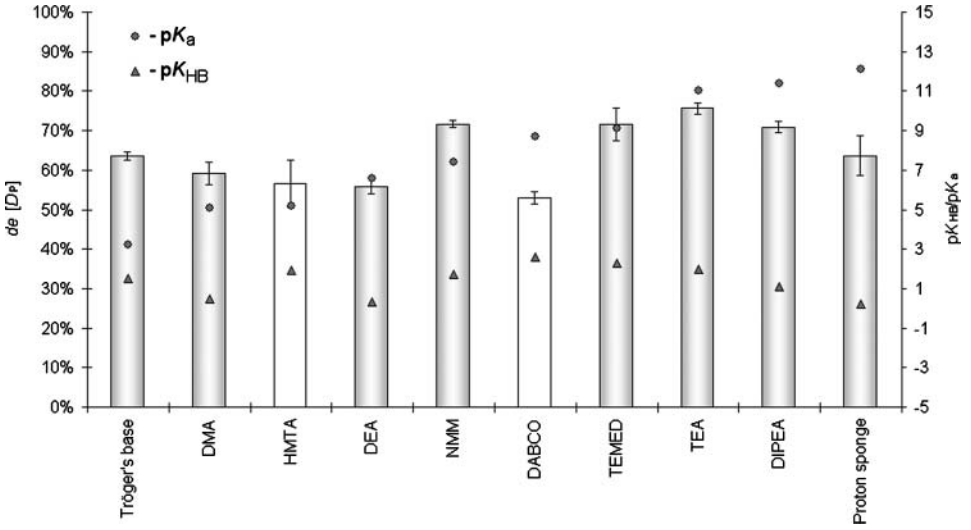

FIGURE 5 Diastereomeric excess (*de*) of the D_P (S_P) diastereomer of *H*-phosphonate diester **3a** in the presence of chosen tertiary amines (for which the pK_{HB} values are known). The data are arranged according to pK_a values of the amines used. The reaction conditions: 0.05 mmol of **1** + EtOH (6 equiv.) + amine (3 equiv.) + PvCl (1.6 equiv.) in DCM (0.5 mL). The tertiary amines able to act as nucleophilic catalysts are shown as empty bars.

the mixed anhydride **2** by the pivalate anion (path c) might be a significant route only when the esterification pathway was retarded.

Stereoselectivity of Condensation

The stereoselectivity of condensations of *H*-phosphonate **1** with ethanol in the presence of various tertiary amines differed in the range of *de* 53–75% what corresponds to the ratio of diastereomers from 3:1 to 7:1. No clear-cut correlation between the observed stereoselectivity and various parameters of basicity could be found (Table 1). However, the stereoselectivity of *H*-phosphonate condensations showed some positive correspondence with both the pK_a and pK_{HB}[70] values of the amines used (Figure 5).

This might indicate that stronger bases can increase rates of the **2**-$D_P \rightleftharpoons$ **2**-L_P equilibrium responsible for the DYKAT efficiency (Figure 1), possibly due to base–promoted ionization of the pivalic acid (the influence of pK_a) and, presumably independently, due to general acid catalysis by the conjugate acids of the amines operating on the mixed anhydride **2** (the influence of pK_{HB}). A possible catalytic action of a conjugate acid of an amine is shown in Figure 6.

We found that a possible contribution of both pK_a and pK_{HB} basicities of the amines to the stereoselectivity of ribonucleoside 3′-*H*-phosphonates condensations could be expressed in a form of a single parameter which we tentatively called "DYKAT basicity" (pK_{DK}). This is a weighted sum of pK_a

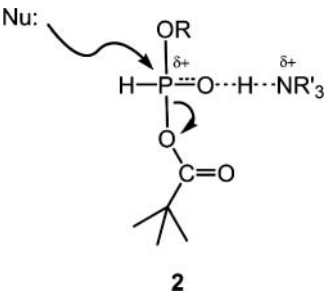

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FIGURE 6 A possible influence of general acid catalysis on reactivity of the mixed anhydride **2**.

and pK_{HB} basicities, given by Eqaution (1), where "n" is a proportionality constant.

$$pK_{DK} = n * pK_a + pK_{HB} \quad (1)$$

The regression analysis of *de* versus pK_{DK} for various investigated amines gave the best linear fit ($R^2 = 0.916$) for $n = 0.18$ (Figure 7).[71] The empirically found relationship between *de* and pK_{DK} has a form of a linear

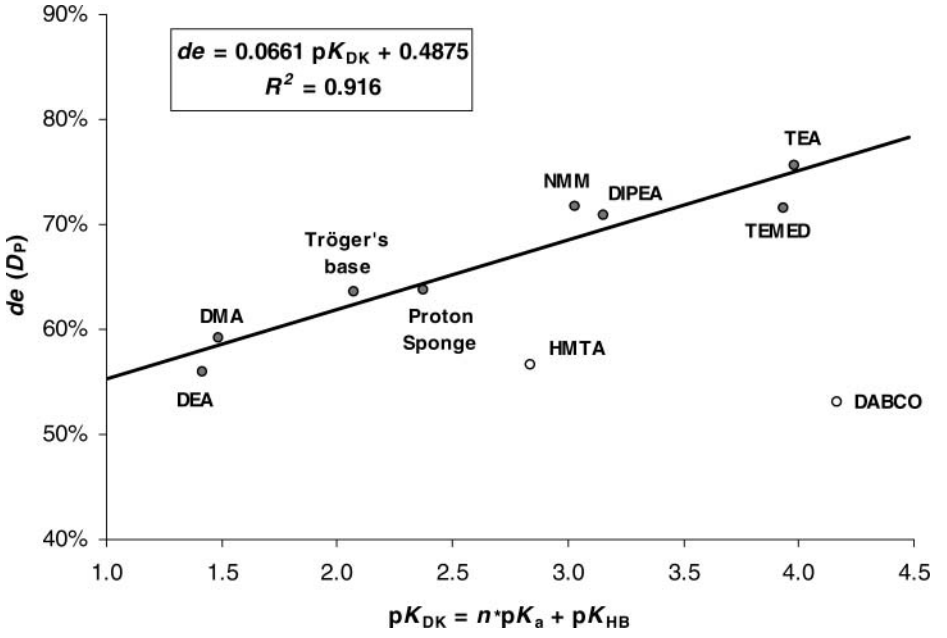

FIGURE 7 A correlation between "DYKAT basicity" (pK_{DK}) and the stereoselectivity of condensations of uridine 3′-*H*-phosphonate **1** with EtOH in the presence of various amines. The data points for HMTA and DABCO were excluded from calculation of the trend line.

equation (2).

$$de = 0.0661 * \mathrm{p}K_{\mathrm{DK}} + 0.4875 \quad (2)$$

The numerical value of $\mathrm{p}K_{\mathrm{DK}}$ does not have an obvious physical sense, but it may be a measure of acid-base properties of an amine that are essential for stereoselectivity of the reaction investigated.

In contrast to $\mathrm{p}K_{\mathrm{a}}$ and $\mathrm{p}K_{\mathrm{HB}}$, we did not find any correlation of the yield and stereoselectivity of condensations of ribonucleoside 3′-*H*-phosphonates with the ion pair basicity[60] of the several amines investigated ($\mathrm{p}K_{\mathrm{ip}}$, Table 1).

It should be noted that the stereoselectivity of condensations performed in the presence of additional amounts of pivalic acid salts decreased irrespective of the amine used (Table 2). This was rather a surprising result since pivalic acid was expected to increase stereoselectivity by accelerating isomerization of the mixed anhydride **2** (Figure 1). To get more insight into the influence of pivalic acid on reactivity of **2** we measured the rate constant of its reaction with $\mathrm{T}_{\mathrm{DMTr}}$ in the presence of DMA•PvOH (3 equiv.) and DMA (10 equiv.). It was found that k_{obs} under such conditions was ca. half of that without the pivalate added (k_{obs} 0.0061 vs. 0.014 $\mathrm{M}^{-1}\mathrm{s}^{-1}$ respectively). These results suggested that high concentrations of pivalic acid in the reaction mixture decelerated both esterification and, presumably to higher extent, P-epimerization of the mixed anhydride **2**. Similar effect of deteriorated stereoselectivity was observed when amine salts of other acids (tosylates and hydrochlorides) were present in the reaction mixture. A possible reason for this phenomenon could be decreased basicity of the reaction mixture due to introduced salts and the following less efficient base catalysis. A practical conclusion from those experiments is that in order to achieve the highest stereoselectivity of the condensation of *H*-phosphonates of type **1**, one should avoid increased concentration of salts in the reaction mixtures.

The stereoselectivity of condensation of *H*-phosphonate **1** with ethanol in the presence of TEA remained at the same high level (*de* 76% ^{31}P NMR) when instead of 1.6 equiv. of PvCl, 2.5 equiv. (or more) were used, while the yield of condensation became quantitative under these conditions (^{31}P NMR). This prompted us to check if tertiary amines could be used as basic components for highly stereoselective and efficient internucleotide bond formation. In initial experiments uridine *H*-phosphonate **1** (c = 0.1 M) was reacted with $\mathrm{T}_{\mathrm{DMTr}}$ (3 equiv.) in the presence of TEA (3 equiv.) using PvCl (3 equiv.) as a condensing agent. The expected D_{P} (S_{P}) diastereomer of *H*-phosphonate diester **3b** was formed with *de* >90% (i.e., the ratio of $D_{\mathrm{P}}/L_{\mathrm{P}}$ isomers was >95:5) and the starting monoester could not be detected in ^{31}P NMR spectrum. Unfortunately, the yield of the desired *H*-phosphonate diester was rather low (ca. 70%) due to formation of P-acylated compounds (ca. 30%); also acylation of N3 position in pyrimidine moiety (up to 50%) was

observed. These side reactions could be reduced (ca 10% of P-acylation, no N-acylation) upon dilution of the reactants. Thus, we expect that achieving quantitative yield and very high stereoselectivity of condensation of ribonucleoside *H*-phosphonates with nucleosides could be possible and fine-tuning of this approach is a subject of further investigations in our laboratory.

CONCLUSIONS

Condensations of ribonucleoside *H*-phosphonates with alcohols can be performed efficiently using tertiary, non-nucleophilic amines as base catalysts. The reactions subject to general base catalysis and for amines having $pK_a \geq 7.4$ (*N*-methylmorpholine) are significantly more rapid than those in the presence of pyridine. In order to achieve quantitative yields of the condensations, >2.5 equiv. of the condensing agent (pivaloyl chloride) should be used for most of tertiary amines (particularly for those of high pK_a), and non-hindered alcohols should be used in an excess of >3 equiv. to prevent P-acylation. The necessity of using the condensing agent in excess arises from its competitive reaction with pivalic acid released to the reaction mixture as a side product of the condensations, and, in the cases when the esterification rate was retarded, due to deacylation of nucleoside *H*-phosphonic-pivalic mixed anhydride (the reactive intermediate) by pivalate anion.

Stereoselectivity of the condensations showed a positive trend with Brønsted basicity (pK_a) and H-bond basicity (pK_{HB}) of the amines present in the reaction mixture. This trend can be expressed in a form of an empirical equation that correlates these parameters with diastereomeric excess of D_P (S_P) diastereomer of ethyl uridine *H*-phosphonate diester **3a** formed.

The highest stereoselectivity (*de* 75%) and quantitative yield of diester **3a** (^{31}P NMR) was achieved in the presence of triethylamine. In the preparation of dinucleoside *H*-phosphonate diester **3b** the stereoselectivity was exceptionally high, *de* >90%, however, ca. 10% of P-acylated products were formed (the conditions are currently a subject of further optimization).

EXPERIMENTAL

The chemicals, instrumentation, and procedures were similar to those reported earlier.[22] ^{31}P NMR spectra were recorded at 121 MHz on a Varian Unity BB VT spectrometer and the ratios of compounds were based upon integration of the corresponding ^{31}P NMR signals. The yield and diastereomeric excess for the data in Table 1 are an average of three experiments, while for the data in Figures 5 and 7, of five repetitions.

General Procedure for Condensation of *H*-Phosphonates of Type 1 with Alcohols

Nucleoside *H*-phosphonate **1** (0.05 mmol) was rendered anhydrous by repeated evaporation (3 × 5 mL) of the added toluene and dissolved in 0.5 mL of DCM. An amine (3 equiv.) and EtOH (6 equiv.) were added, followed by PvCl (1.6 equiv.), and the reaction mixture was transferred to an NMR tube and the ^{31}P NMR spectra were recorded.

Procedure for the In Situ Preparation of the Mixed Anhydride 2

Nucleoside *H*-phosphonate **1** (0.1 mmol) was rendered anhydrous by repeated evaporation (3 × 5 mL) of the added toluene and dissolved in DCM or ACN (0.6 mL), then amine (0.2 equiv. of TEA or NMM, or 0.5 equiv. of DMA) and pivaloyl chloride (1.2 equiv) were added successively. Such a reaction mixture was analyzed by ^{31}P NMR spectroscopy and used for further transformations within 1 hour (no degradation products formation was observed within that period of time).

Kinetic Measurements

To a solution of 3′-(dimethoxytrityl)thymidine[30] (0.05 mmol) in DCM (1 mL), toluene (5 mL) was added and the solvents were evaporated to dryness under reduced pressure; the procedure was repeated twice. The residue was dissolved in DCM or ACN (0.2 mL) containing an appropriate amine (3 or 10 equiv.) and the solution of the mixed anhydride **2** (prepared as described above, 0.3 mL; 0.05 mmol) was added. The reaction mixture was transferred to an NMR tube and ^{31}P NMR spectra were recorded immediately.

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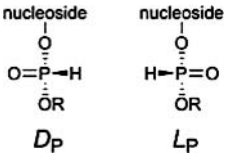
64. For the compounds presented in this paper the D_P descriptor refers to a structure in which the P-H bond is directed to the right in the Fischer projection, while for the L_P one, to the left. The full D_P/L_P notation is described in refs.[25–28]



D_P L_P

65. The same conditions as used in previous studies.[22]
66. When the amount of an alcohol was reduced to 3 equiv., the above mentioned by-products appeared as trace impurities (1–2%), while for stoichiometric amounts of an alcohol, these by-products accounted for ca. 20% of the phosphorus-containing compounds present in the reaction mixtures, most likely due to decreased rates of the condensations.
67. When the reaction was repeated in the presence of 10 instead of 3 equiv. of DMA, a significant deacylation of the mixed anhydride 2 was observed, demonstrating the importance of the basicity of the reaction mixture on the course of reaction.
68. Because analogous experiments revealed lack of deacylating activity of tosylates and hydrochlorides of the same amines, the observed deacylation of 2 should be attributed to rather unique properties (in discussed context) of the studied pivalates, while their deacylating power could be correlated with pKa values of the conjugated amines. Thus, amines of higher pKa apparently might increase nucleophilicity of pivalic acid via its more efficient deprotonation.[15]
69. The reasons for low yields of esterification of the mixed anhydride 2 in the presence of powerful nucleophilic catalysts (NMI, DMAP) remain uncertain. One may speculate that the *H*-phosphonic onium salts formed by those nucleophilic amines during the course of the reaction[11] may react partly with the pivalate anion forming acyl onium salts (and *H*-phosphonate monoester 1), which subsequently might rapidly form unreactive pivalic anhydride with the next pivalate molecule. In contrast, all reactions during condensations of nucleoside *H*-phosphonates in the presence of moderate nucleophilic catalyst (pyridine) were evidently fully chemoselective toward *H*-phosphonate diester formation.
70. pK_{HB} is a measure of a relative strength of the acceptor in hydrogen-bonded complex formed with a reference acid (H-bonding basicity) and may indicate on propensity of a conjugate acid of an amine to act as a general acid catalyst. pK_a and pK_{HB} may be unrelated.[59]

71. HMTA and DABCO are strong nucleophilic catalysts and the outstandingly low stereoselectivity found for these compounds were similar to those observed for *N*-methylimidazole and DMAP.[22] It seems that these nucleophilic catalysts, efficient for reactions at carbon and P^V centers, are poor nucleofuges in *H*-phosphonate chemistry, and the relative stability of $P\text{-}N^+$ intermediates makes room for side reactions and slows down their epimerization, making the DYKAT process of asymmetric induction less efficient.