STEREOCHEMISTRY OF INTERNUCLEOTIDE BOND FORMATION BY THE *H*-PHOSPHONATE METHOD. 2. TRANSESTERIFICATION OF ARYL RIBONUCLEOSIDE *H*-PHOSPHONATE DIESTERS WITH ALCOHOLS

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Aryl ribonucleoside 3'-*H*-phosphonates were used as model compounds in investigations of stereochemistry of an internucleotide bond formation. For all four ribonucleoside 3'-*H*-phosphonates studied the major diastereomers of the produced *H*-phosphonate diesters were found to be formed from the minor diastereomers of nucleoside aryl *H*-phosphonate diesters. These studies indicate the importance of an equilibrium between substrate diastereomers and can be relevant to the stereoselectivity observed in condensation reactions of ribonucleoside *H*-phosphonates promoted by condensing agents, *e.g.* pivaloyl chloride.

INTRODUCTION

Ribonucleoside *H*-phosphonate monoesters show surprisingly high stereoselectivity during condensing agents-promoted formation of an internucleotidic bond[1,2]. The activation of ribonucleoside *H*-phosphonates of type **1** with pivaloyl chloride yields two diastereomers (**A** and **B**, Fig. 1) of mixed anhydrides **2**. These isomers have to exist in a rapid equilibrium to regenerate the more reactive diastereomer, as the ratio of the *H*-phosphonate diesters diastereomers produced (e.g. **3**) is usually different from that of mixed anhydrides **2**. Unfortunately, due to high reactivity of **2** it is not possible to isolate them nor to follow their esterification by ^{31}P NMR technique. In consequence, a stereochemical correlation between diastereomers **A**/**B** and the products formed remains elusive.

To investigate this problem we turned our attention to aryl nucleoside *H*-phosphonates of type **5/6** (Fig. 2), that can be perceived as models of activated *H*-phosphonate monoesters[3,4]. Preliminary studies indicated that it was the minor diastereomer of aryl nucleoside *H*-phosphonates of type **5/6** that afforded the major diastereomer D_P (S_P)[5] of the *H*-phosphonate diesters formed. Taking into account a similar reactivity of **2** and **5/6**, we assumed that probably a similar stereochemical correlation should be valid for mixed anhydrides of type **2**, and thus, D_P (R_P) configuration was assigned

for the major diastereomer **A,** and the L_P (S_P) configuration for the isomer **B** [6]. To substantiate these findings and to get a deeper insight into a stereochemical relation between aryl nucleoside *H*-phosphonate diastereomers and the products of their transesterification, a similar studies were performed on all four ribonucleoside *H*-phosphonate monoesters.

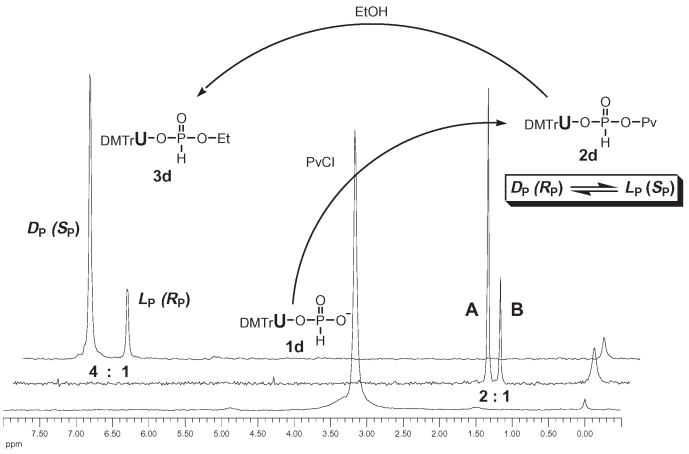

FIG. 1
^{31}P NMR spectra showing generation of mixed anhydride **2d** and its esterification with ethanol (DMTr**U** = 5'-*O*-dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-uridin-3'-yl; Pv = pivaloyl; **A** and **B** stand for major and minor diastereomers of **2**, respectively)[5]

RESULTS AND DISCUSSION

As model compounds for this study 4-nitrophenyl and 4-chlorophenyl 5'-*O*-dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-nucleosid-3'-yl *H*-phosphonate diesters (**5a-d** and **6a-d**, respectively; **a**, B=Ade^{Bz}; **b**, B=Cyt^{Bz}; **c**, B=Gua^{ibu}; **d**, B = Ura) were chosen. These were generated *in situ* from the corresponding suitably protected nucleoside *H*-phosphonate monoesters (0.05 mmol) and 4-nitrophenol (or 4-chlorophenol, 1.2 equiv.) dissolved in 0.5 mL of dichloromethane containing 2.5% of pyridine, using pivaloyl chloride (1.6 equiv.) as a condensing agent. The ratios of D_p/L_p diastereomers of the produced diesters **5**/**6** were in the range of 65:35 to 75:25. To such reaction mixtures, an alcohol (5 equiv.) was added and the transesterifications were monitored by ^{31}P NMR.

For all 4-nitrophenyl derivatives **5a-d** investigated, the reactions with ethanol resulted first in a rapid disappearance of the minor diastereomer of the substrate (2-3 min), followed by a slower conversion of the major

diastereomer of **5** into the products **3** (6–9 min; Fig. 2a). Although these results were in agreement with our previous findings[6] and showed notably higher reactivity of the minor isomer of **5**, a short reaction time limited the number of ^{31}P NMR spectra that could be acquired. Probably due to this, we could not observe the expected changes in the ratios of the produced nucleoside ethyl *H*-phosphonate diastereomers during the course of reactions, although transesterifications seemed to be much faster than the rates of equilibration of diastereomers of substrates **5**.

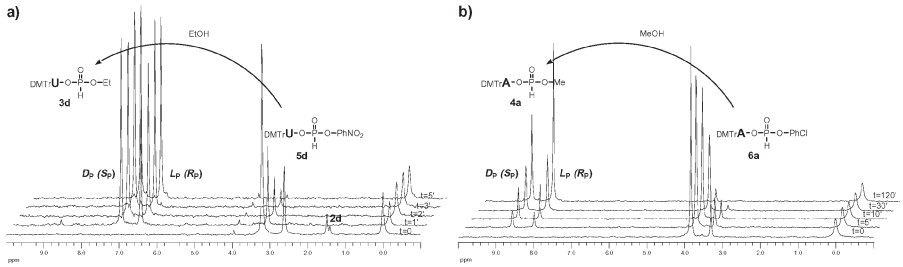

FIG. 2
Exemplary ^{31}P NMR spectra of the courses of transesterifications of (a) 4-nitrophenyl uridin-3'-yl *H*-phosphonate **5d** with ethanol and (b) 4-chlorophenyl adenin-3'-yl *H*-phosphonate **6a** with methanol (DMTr**U** = 5'-*O*-dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-uridin-3'-yl; (DMTr**A** = 5'-*O*-dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyl-6-*N*-benzoyl-adenin-3'-yl; $PhNO_2$ = 4-nitrophenyl; PhCl = 4-chlorophenyl)[5]

As to the transesterification with other alcohols, one ought to expect that isopropanol and *tert*-butanol should react slower and thus the equilibrium between diastereomers of **5** could be maintained during the course of the reaction. This should result in increased stereoselectivity of the reactions with sterically hindered alcohols.

Indeed, the rate of transesterification of 4-nitrophenyl uridin-3'-yl *H*-phosphonate diester with *t*-BuOH **5d** changed from 6 min for EtOH to 35 min, while the stereoselectivity increased from **3d**-D_P/**3d**-L_P = 55:45 to 80:20, respectively. The changes in the ratio of diastereomers **5d** during the course of the reaction were less pronounced for iPrOH than for EtOH, while for *t*-BuOH it remained constant.

Since methanol was expected to cause rapid transesterification, we tried to use this alcohol in combination with less reactive 4-chlorophenyl derivatives **6** (Table I). As expected, the reactions were much slower than those of 4-nitrophenol *H*-phosphonates **5**. However, in contrast to ethanololysis investigated earlier,[6] during methanololysis of **6a–d** in all instances there was a clear increase in the ratio of diastereomers of **6** in favour of the major

one, indicating significantly higher reactivity of the minor diastereomer. Moreover, during the course of reactions there were also distinct changes in the ratio of the diastereomers of the products formed. At the initial stages, the usual predominance of the D_P isomers of corresponding nucleoside methyl diesters was observed. However, when the reactions progressed and the concentration of the minor diastereomer of the substrate was depleted, the L_P diastereomer of the products started to dominate and ultimately became the major product (see Fig. 2b and Table 1).

TABLE I
Transesterification of nucleoside 4-chlorophenyl *H*-phosphonates **6a-d** with methanol

Substrate	Reaction time min	Substrate consumption, %	Substrate $D_P(R_P):L_P(S_P)$	Product $D_P(R_P):L_P(S_P)$
6a (B = Ade^{Bz})	1	3	81:19	55:45
	30	56	93:7	44:56
	180	98	100:0	37:63
6b (B = Cyt^{Bz})	1	5	69:31	n.d.[a]
	30	45	82:18	n.d.[a]
	180	95	98:2	39:61[b]
6c (B = Gua^{ibu})	1	5	80:20	56:44
	30	53	83:17	50.5:49.5
	180	98	100:0	41:59
6d (B = U)	1	5	81:19	64:36
	30	43	92:8	52:48
	180	91	100:0	42:58

[a] Diastereomers ratio not determined due to signals overlapping. [b] Ratio determined after methylene chloride evaporation and dissolving the residue in toluene.

To conclude, we have developed two model systems: 4-nitrophenyl nucleoside *H*-phosphonates (**5a-d**) + ethanol and 4-chlorophenyl nucleoside *H*-phosphonates (**6a-d**) + methanol, that clearly indicated a considerably higher reactivity of the minor diastereomers of aryl nucleoside diesters (from which the major diastereomers of the products were formed). These results seem to be relevant to the stereoselectivity observed in condensation reactions of ribonucleoside *H*-phosphonates promoted by condensing agents and may permit to extend the stereochemical correlation established for aryl *H*-phosphonates, also to ribonucleoside *H*-phosphonic pivalic mixed anhydrides **2a–d**.

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5. An outline of the D_P/L_P notation is presented in the accompanying paper in this issue as well as in: Sobkowski M., Stawinski J., Kraszewski A.: *Nucleosides Nucleotides Nucleic Acids* **2005**, *25*, accepted.
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