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Reaction of boranephosphonate diesters with amines in the presence of iodine: the case for the intermediacy of H-phosphonate derivatives

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Key words: boranephosphonates; boranophosphates; oxidation with iodine; mechanism; stereochemistry; phosphoramidates; nucleotide analogues

Abstract: Mechanistic and stereochemical aspects of the reaction of boranephosphonate diesters with amines promoted by iodine were investigated. This is a complex, multistep reaction that ultimately produces the corresponding phosphoramidate diesters *via* a formal replacement of the borane group by an amine moiety. We found by a stereochemical correlation analysis that contrary to a literature report, the whole transformation proceeded with total inversion of configuration at the phosphorus center. Our study also showed that instead of the postulated nucleophilic substitution by iodide at the phosphorus centre of the initially formed intermediate, the corresponding iodoboranephosphonate, the crucial step of the reaction involved intermediacy of H-phosphonate derivatives that reacted with iodine to afford ultimately phosphoramidate diesters. The reaction of the iodoboranephosphonate with the amine to produce an aminoboranephosphonate diester that rapidly dissociated into the corresponding H-phosphonate and the borane parts was apparently instrumental to the formation of an H-phosphonate diester intermediate.

INTRODUCTION

Boranephosphonates[1] are analogues of natural phosphate esters in which the -BH_3 group replaces one of the non-bridging oxygen atoms.[2] The borane group (-BH_3) is isoelectronic with oxygen in phosphate esters, and also with sulfur and the methyl group present in other phosphate analogues, *e.g.*, phosphorothioates and methylphosphonates, respectively.[3] Despite high similarities (*e.g.*, a tetrahedral structure, the presence of a negative charge), there are significant differences between boranephosphonates compared to phosphate diesters.[4] The phosphorus-boron bond in boranephosphonate diesters is a dative bond[5] formed *via* transfer of a lone electron pair from a phosphite diester to an electron deficient borane. This is reflected in formula **A** in Fig. 1 with a formal negative charge placed on the boron atom. However, since boron is less electronegative than oxygen, the negative charge resides mainly on the phosphoryl oxygen atom as shown in a mesomeric form **B**. Moreover, in **A** and **B** the formal negative charge on the boron atom is assumed to be spread on the hydrogens as partial charges. Such charge distribution was supported by X-ray analysis, and this bonding pattern is somewhat similar to that of phosphonium salts.[4,6] Alternatively, boranephosphonates can be presented as complex anions, analogously to $[BH_4]^-$, without assignment of a negative charge to the specific ligands (formula **C**).

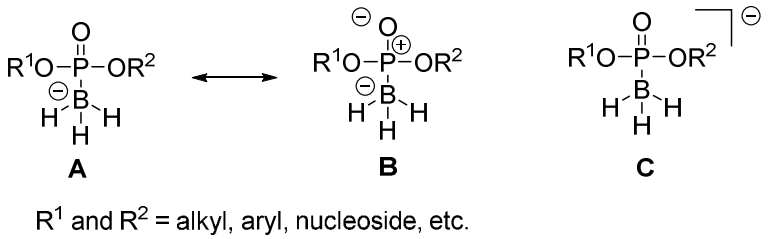


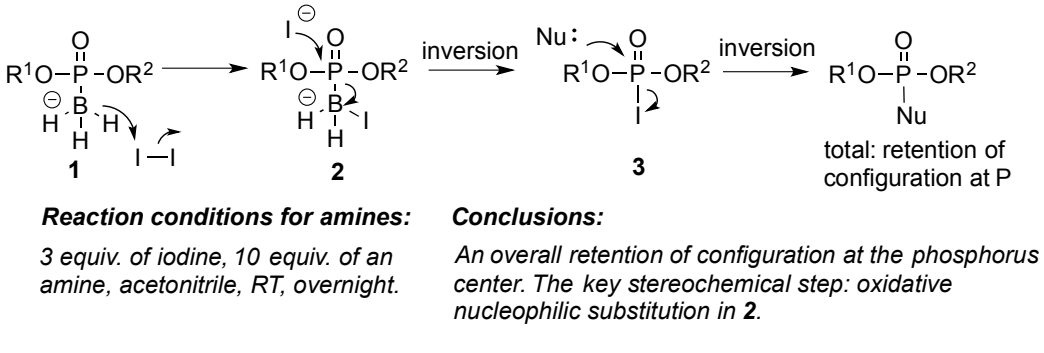
Fig. 1. Various structural formulas for boranephosphonate diesters.

The above makes clear that due to significant bonding differences compared to phosphodiesters, incorporation of boranephosphonates into DNA or RNA can impart new properties to these biomolecules, *e.g.,* chirality at the phosphorus centers, different bonds length, and disparate charge distribution in internucleotidic bonds. Indeed, it was found that the presence of the P-BH_3 group in oligonucleotides modulates their nuclease resistance, interaction with proteins, lipophilicity, hydrolytic stability, and secures generally low toxicity of the boron analogues.[3,7] These features constituted the basis for the development in the last two decades of boranephosphonate-modified nucleic acids as potential antisense/antigene and siRNA gene silencing agents.[2-3,8] Nucleoside boranephosphonates as phosphate analogues were introduced by Shaw, Spielvogel and Sood in the early 1990s,[9] and then further developed by Shaw,[10] Wada,[6a,11] Caruthers,[12] and others.[4,7f,13]

Recently, Caruthers *et al*.[14] expanded the scope of possible applications of boranephosphonates by developing the P-BH_3 group as a potential post-synthetic modification for nucleic acids. In this concept, the borane group serves as a stable chemical marker that can be targeted and labilized by a selective reagent to replace the -BH_3 group with another functionality,[14a] or to introduce a modification at the boron atom itself.[14b] The added value of such an approach is that due to chirality at the phosphorus center of boranephosphonates, one can in principle obtain nucleic acid mimics with stereochemically defined chiral internucleotide linkages.

The underlying chemistry for targeting and the replacement of the borane group by nucleophiles developed by Caruthers *et al*.[14] is shown in Scheme 1 and consists of an oxidative conversion of boranephosphonates to stereochemically defined phosphate diester derivatives, *e.g*., phosphoramidates, phosphotriesters, phosphorothiolates and C-phosphonates. This transformation, which bears high potential for post-synthetic

modification of oligonucleotides, was claimed to proceed with an overall retention of configuration at the phosphorus center and to involve as a crucial step a nucleophilic attack of iodide on the activated boranephosphonate intermediate **2** (Scheme 1).



R^1 and R^2 = nucleoside moieties; Nu = nucleophile, *e.g.* amine, alkoxide, carbanion, *etc.*

Scheme 1. A mechanism for the formal replacement of the -BH_3 group by a nucleophile in boranephosphonate diesters promoted by iodine postulated by Caruthers *et al.*[14a]

Our interest in the development of synthetic methods based on H-phosphonate chemistry for the preparation of biologically important phosphorus compounds and their analogues,[15] and some unusual features of the above reaction prompted us to take a closer look at the proposed mechanism. By analysing the reaction steps we found a possibly fundamental flaw in the mechanism that might affect the predicted stereochemistry of the reaction and also has a bearing for a general type of reactivity postulated for boranephosphonate derivatives. Taking into account the potential importance of this type of reaction in oligonucleotide chemistry and the claimed significance of the proposed oxidative nucleophilic substitution step at the boranephosphorus center to boron chemistry, we undertook these studies to clarify some chemical and stereochemical aspects of the above reaction. As model compounds for our studies we chose boranephosphonate diesters **1a** and **1b**, and *n*-butylamine as a nucleophile.

RESULTS AND DISCUSSION

Oxidation of a hydride in boranephosphonates **1** by iodine to produce monoiodoboranephosphonates **2** (and possibly di- or triiodo derivatives) that subsequently may undergo nucleophilic substitution at the phosphorus centre by

iodide are the initial steps in the mechanism postulated by Caruthers *et al.*[14a] (Scheme 1). While the first step, the oxidation of a borane hydride by iodine, is quite likely,[16] the second step, a nucleophilic substitution of the iodoborane group by iodide seemed very implausible on at least three counts. Firstly, iodide is not a particularly good nucleophile for the phosphorus.[17] As a soft base, it is unlikely that iodide would efficiently attack hard phosphonate diesters in accordance with the principle of hard and soft acids and bases.[18] Secondly, the boranephosphonate group in boranephosphonate diesters, similarly to phosphate diesters, is negatively charged and thus can hinder the approaching nucleophile. Thirdly, assuming a nucleophilic attack by iodide on the phosphorus center in **2** would necessitate the departure of the iodoborane group with an electron pair in the form of boryl dianion $[:BH_2I]^{2-}$ with two formal negative charges. This would be thermodynamically unfavorable given the high basicity and nucleophilicity of boryl dianions.[19] These are rather elusive species that are formed under highly reducing conditions as evidenced by ^{11}B NMR spectroscopy (for generic $[:BH_3]^{2-}$)[20] and their stability become manageable only upon extensive delocalization of the lone electron pair (casus $[B(CN)_3]^{2-}$).[21]

The authors predicted retention of configuration at the phosphorus center for the whole process and this claim was substantiated by the NOESY-experiments. In the later, the authors compared the NOE effects between protons of the BH_3 group and the ribose protons in the starting boranephosphonates of type **1** *vs* methyl and the ribose protons in the corresponding methyl phosphotriesters.[14a] However, since spatial arrangements of the BH_3 *vs* OCH_3 protons in these compounds may differ significantly, we considered that this NMR spectroscopy evidence warrants additional studies to firmly establish a stereochemical relationship between the substrates and the products in this reaction.

Stereochemical correlation analysis.

To establish a stereochemical outcome of the investigated reaction, we carried out a stereochemical correlation analysis. To this end dinucleoside H-phosphonate **5a**, enriched in one of the diastereomer (^{31}P NMR analysis; 2:1 mixture of the low field: the high field diastereomers; δ_P = 9.38 and 8.31 ppm), was synthesized and subjected to oxidative coupling with *n*-butylamine (Scheme 2, lower reaction path).[22] This reference reaction that is known to be stereospecific and to occur with inversion of

configuration at the phosphorus center,[22-23] produced two diastereomers of dinucleoside phosphoramidate **4a** in the expected ratio of 1:2 (the high field: the low field diastereomers, ^{31}P NMR analysis). In the second experiment, we converted the same diastereomeric mixture of H-phosphonate diesters **5a** into boranephosphonate diesters **1a** (see the Experimental Section), and subjected these to a reaction with iodine in the presence of *n*-butylamine, as described by Caruthers *et al*.[14a] We found that patterns of the signals in the ^{31}P NMR spectra of diastereomeric phosphoramidates **4a** obtained from H-phosphonate **5a** *via* boranephosphonate **1a** as an intermediate and that from a direct oxidative coupling of H-phosphonate **5a** with *n*-butylamine (the reference reaction), were identical (two signals between δ_P = 9-10 ppm, ratio 1:2; see Fig. 2), and thus both reactions had to proceed with the same overall stereochemistry, namely, with total inversion of configuration at the phosphorus center.

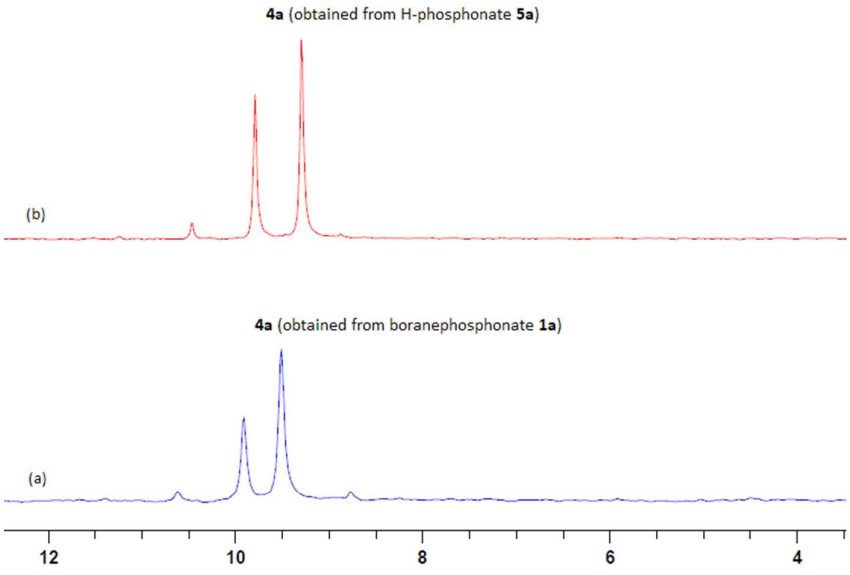
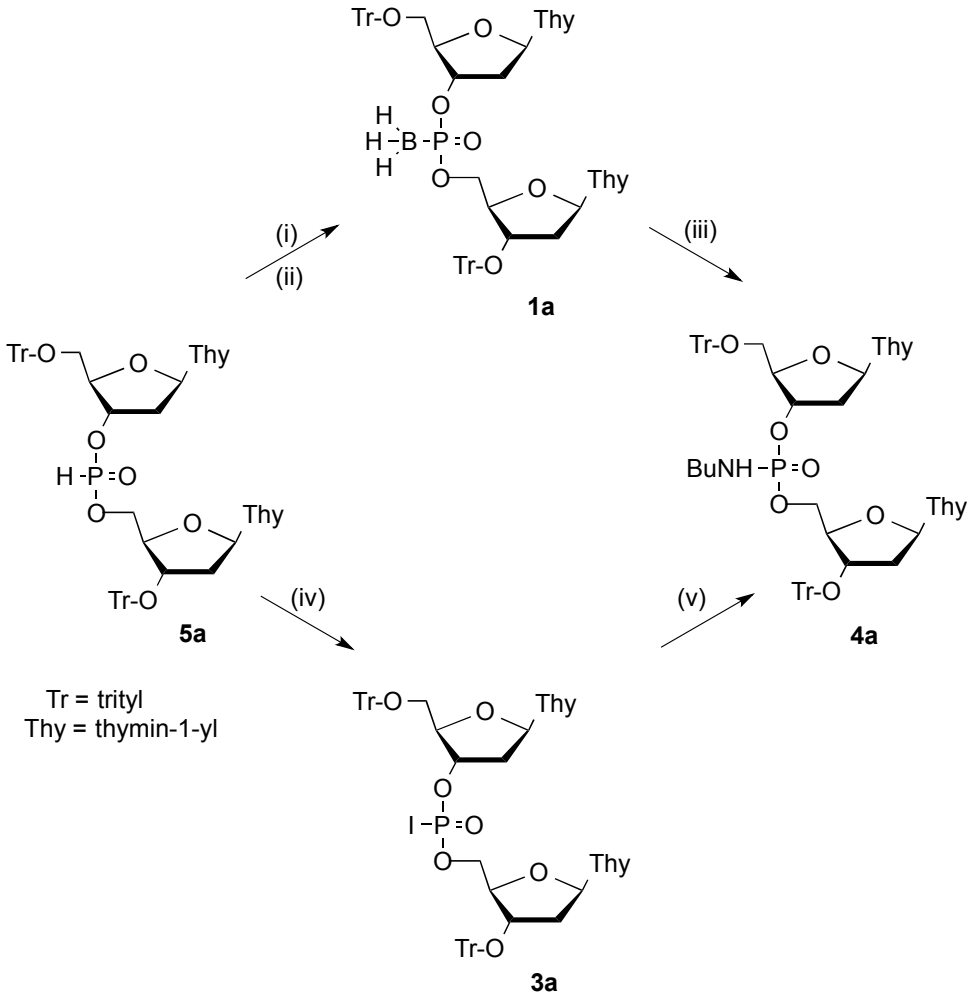


Fig. 2. Excerpts of ^{31}P NMR spectra of crude reaction mixtures of: (a) boranephosphonate **1a** + *n*-butylamine + iodine, and (b) H-phosphonate **5a** + *n*-butylamine + iodine (the reference reaction). For experimental details, see in the text.

Since transformation of H-phosphonate diesters into the corresponding boranephosphonates is known to occur with retention of configuration,[13d,24] it implied that a reaction of boranephosphonate **1a** with iodine to produce iodophosphate **3a** also has to occur with retention of the configuration at the phosphorus centre. This

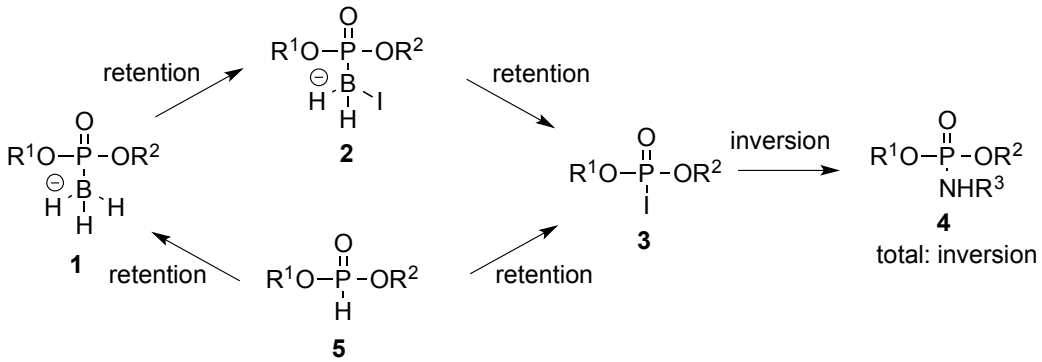
contradicted the finding by Caruthers *et al*.[14a] who claimed for the latter step inversion of the configuration for this process.



Scheme 2. Conversion of H-phosphonate diester **5a** into phosphoramidate **4a** *via* oxidative coupling with an amine (lower path) and *via* boranephosphonate intermediate **1a** (upper path), both promoted by iodine. Reaction conditions: (i) 5 equiv. TMS-Cl, 5 equiv. TEA in CH_2Cl_2; (ii) 10 equiv. $BH_3{\cdot}SMe_2$ in THF, followed by 25% aq. ammonia treatment; (iii) 3 equiv. I_2, 10 equiv. *n*-$BuNH_2$ in acetonitrile;[14a] (iv) 2 equiv. I_2, 4 equiv. TEA in acetonitrile;[22a] (v) 5 equiv. *n*-$BuNH_2$ in acetonitrile.[22b] Abbreviations: TMS-Cl – trimethylsilyl chloride; TEA – triethylamine; THF – tetrahydrofuran.

To clarify this point, all steps important for the stereochemical correlation analysis from Schemes 2 and 1 were summarized in Scheme 3. The correlation hinges on the common intermediacy of phosphoroiodidate **3.** This on one hand, can be stereospecifically converted in the presence of an amine to the corresponding

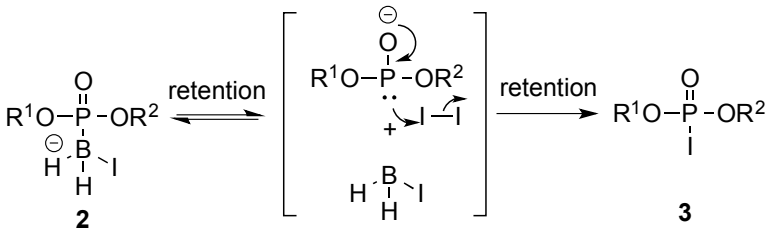
phosphoramidate **4** through inversion of configuration at the phosphorus,[22b] and on the other one, can be generated on two independent routes, bifurcated from H-phosphonate **5**. In the reference reaction, a tricoordinated form of H-phosphonate **5** reacted directly with iodine to afford with retention of configuration iodophosphate **3**,[22a] while for the investigated reaction, the same starting material **5** (after silylation) was used for the preparation of boranephosphonate **1**. Since transformation of H-phosphonate diesters into the corresponding boranephosphonates (**5** into **1**) proceeds with retention of configuration[13d,24] and the subsequent reaction with iodine (**1** into **2**) does not involve the phosphorus center (boron hydride oxidation), sense of chirality of iodoboranephosphonate **2** must be the same as that of H-phosphonate **5**. Thus, in Scheme 3 the stereochemistry of all the reaction steps but one, namely that from **2** to **3**, is known. To obtain agreement with the experimental data we had to assume that the conversion of iodoboranephosphonate **2** into the corresponding iodophosphate **3** occurred with retention of the configuration as indicated in Scheme 3. This, on stereochemical ground, in principle ruled out a nucleophilic substitution by iodide at the phosphorus centre suggested by Caruthers *et al.*[14a] Having said that, there could be a remote possibility for this step to be anyway a nucleophilic substitution, provided it would involve a pseudorotation of the initially formed trigonal bipyramid intermediate (*tbp*). However, in acyclic phosphorus compounds nucleophilic substitution occurs with inversion of configuration without intermediacy of *tbp* and a pseudorotation step is usually invoked to explain the stereochemical outcome of reactions when phosphorus is part of a ring system.[25] This adds another argument to those in the text above against an $S_N2(P)$ process and thus called for another mechanism for this step for this reaction.



1a - **5a**, R^1 and R^2 = nucleoside moieties as in Scheme 2: R^3 = *n*-butyl
1b - **5b**, $R^1 = R^2$ = ethyl: R^3 = *n*-butyl

Scheme 3. A stereochemical correlation scheme for the conversion of H-phosphonate diester **5** into phosphoramidate **4** *via* a direct oxidative coupling (the lower path) and *via* intermediacy of boranephosphonate **1** (the upper path).

To provide a mechanistic explanation for this crucial step of the transformation (step **2** to **3**) we propose that iodoboranephosphonate **2**, formed by a borane hydride oxidation of the boranephosphonate **1**, could undergo dissociation to a phosphite anion and iodoborane, followed by oxidation of the former by iodine to afford phosphoroiodidate **3** (Scheme 4).



2

3

2a, **3a**, R^1 and R^2 = nucleoside moieties as in Scheme 2.
2b, **3b**, $R^1 = R^2$ = ethyl

Scheme 4. A possible mechanism for transformation of iodoboranephosphonate **2** into phosphoroiodidate **3** with retention of configuration.

Since both reactions in Scheme 4, *i.e.* the dissociation of iodoboranephosphonate **2**[13d,24] and the oxidation of the phosphite anion,[22] do not involve any change in stereochemistry at the phosphorus atom, a total retention of configuration is expected for this step, as depicted in the stereochemical correlation diagram (Schemes 4 and 5).

One should note that in contradistinction to a mechanism proposed by Caruthers *et al*.[14a] where iodine acted only as an activator for the borane group of boranephosphonates of type **1** (*via* oxidation of a borane hydride), in our mechanism iodine plays a dual role, being also a stoichiometric oxidant for the *in situ* generated P(III) species (phosphite anion of an H-phosphonate diester; Scheme 4). Thus, the two mechanisms could in principle be also distinguished by the amount of iodine consumed during the reaction. For the oxidative nucleophilic substitution mechanism, only 1 molar equivalent of iodine was claimed to be necessary[14a] however, to get reproducible results, the authors routinely used 3 equivalents of iodine (see Scheme 1). In our hands, the reaction consistently required at least 2-3 equiv. of iodine to go to completion. Apparently, due to some subsequent reactions of iodine with an amine

present and with borane derivatives formed, its consumption varied, depending on the reaction conditions.

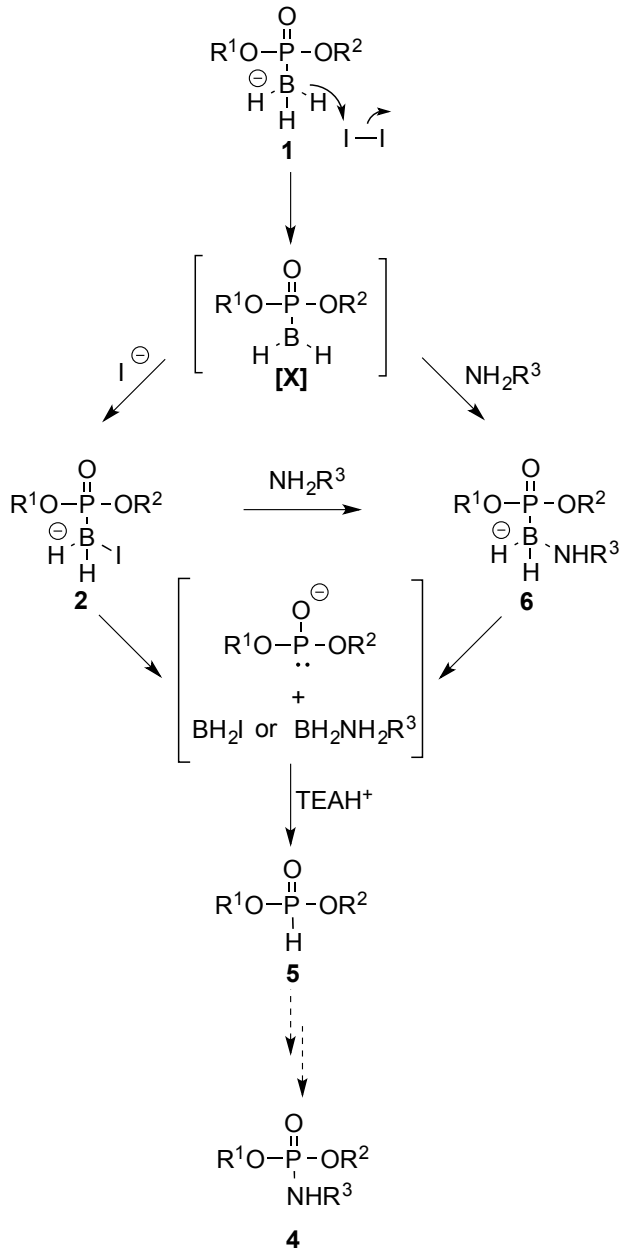
Formation of an H-phosphonate diester as a key intermediate.

The dissociation step that generates an H-phosphonate species (as a phosphite anion or its protonated form; Scheme 4) is critical for the discussed mechanism to be operative during the reaction of boranephosphonates with amines. Boranephosphonate diesters are stable compounds that can be stored and handled at ambient temperature without any particular precautions. In chemical terms this means that the P-B bond in these compounds is rather strong and does not undergo a spontaneous scission. Since boranephosphonates are Lewis acid–base complexes, their stability is controlled by acidity and basicity of the parent borane and the phosphonate parts.

Both our model compounds, dinucleoside boranephosphonate **1a** and diethyl boranephosphonate **1b**, afforded the corresponding phosphoramidates **4a** and **4b** when treated separately in dichloromethane or acetonitrile with *n*-butylamine (10 equiv.) and iodine (3 equiv.).[14a] The ^{31}P NMR experiments showed that the starting materials, boranephosphonates **1a** and **1b**, were stable in pyridine, acetonitrile, and dichloromethane in the presence of *n*-butylamine (50 equiv., 24 h, room temperature). Since upon addition of elemental sulfur (10 equiv.) or sulfur and trimethylsilyl chloride, (10 equiv. of each) to such solutions, no sulfurization products (formation of the corresponding phosphorothioate diesters) could be detected, we inferred that under these experimental conditions boranephosphonates **1** did not dissociate spontaneously to afford detectable equilibrium mixtures of the parent H-phosphonate derivatives and borane. Thus, it seemed that the generation of appreciable amounts of H-phosphonate diesters from boranephosphonates must apparently involve an initial activation of **1** by iodine[26] as was suggested by Caruthers *et al*.[14a] To unravel this point, first we wanted to know if we could observe, by ^{31}P- or ^{11}B NMR spectroscopy, the formation of the postulated initial intermediates of the reaction, namely, the corresponding iodoboranephosphonate derivatives **2**. This would permit further studies on susceptibility of intermediates **2** to dissociation into the Lewis acid - Lewis base components, a critical step in our mechanistic proposition. We considered two scenarios: (i) a direct fragmentation of iodoboranephosphonates **2** into the

corresponding H-phosphonate diesters **5**, and (ii) a possible formation of H-phosphonates **5** from **2** *via* other, unidentified yet boranephosphonate intermediates.

Scheme 5 depicts some possible pathways for generation of H-phosphonate diesters **5** during the course of the reaction of boranephosphonates **1** with amines promoted by iodine. Oxidation of a borane hydride in **1** by iodine might initially form a trigonal, electron-deficient borane intermediate **[X]** that coordinated iodide to afford iodoboranephosphonate **2**. In the presence of amine nucleophile during the hydride oxidation by iodine, it could intercept the incipient intermediate **[X]** to form aminoboranephosphonate **6**. Alternatively, both species **2** and **6** could also be formed directly from boranephosphonate **1** without the intermediacy of **[X]** *via* four-center transition states involving iodine (for iodoboranephosphonate **2**) or amine-iodine complexes[27] (for aminoboranephosphonate **6**). Irrespective how they were formed, **2** and **6** might afford H-phosphonate **5**, but their propensity to spontaneous dissociation should depend on the Lewis acid strength of the borane moieties in these compounds. By comparing the inductive *vs* the π-electron donating effects of iodo and amino substituents in trigonal borane derivatives,[28] we expected that the latter should significantly prevail in aminoboranes, making them weaker Lewis acids.



2a, **5a**, **6a**, R^1 and R^2 = nucleoside moieties as in Scheme 2; R^3 = *n*-butyl
2b, **5b**, **6b**, $R^1 = R^2$ = ethyl; R^3 = *n*-butyl

Scheme 5. Various possible pathways for the formation of H-phosphonate diester **5** intermediates.

By this token we anticipated that aminoboranephosphonates **6** would be more susceptible to dissociation into the parent Lewis acid – Lewis base components than iodoborane **2** derivatives, and thus a pathway to H-phosphonate **5** *via* aminoborane **6** intermediates (Scheme 5) should be thermodynamically favoured.

To find support for this hypothesis we carried out a reaction of boranephosphonates **1** with iodine under various experimental conditions by varying solvents, ratio, and kinds of reactants. When iodine (3 equiv.) was added to a solution of boranephosphonates **1** and *n*-butylamine (5-10 equiv.) in dichloromethane or acetonitrile, the reaction proceeded to completion within a few minutes to afford the expected phosphoramidates **4** without the formation of any detectable intermediates. This indicated that the first step of the reaction in Scheme 5, *i.e.* the oxidation of a borane hydride in **1** by iodine appeared to be the rate limiting step of the whole transformation.

To get some insight into the possible intermediates involved in this reaction, we first attempted to react diethyl boranephosphonate **1b** in dichloromethane with iodine alone (an activation process). Since amine is apparently involved in the very last step of the reaction, its absence should possibly cause accumulation, and thus detection by ^{31}P NMR spectroscopy, some of the postulated upstream intermediates. Indeed, after the addition of 1 equiv. of iodine to a dichloromethane solution of **1b**, the first ^{31}P NMR spectrum recorded revealed, besides the starting material **1b** (ca 29%, δ_P = 99.80 ppm) and diethyl phosphoroiodidate **3b** (ca 7%, δ_P = -41.88 ppm), also the presence of an intermediate we searched for, namely, diethyl H-phosphonate **5b** (ca 22%, δ_P = 8.21 ppm), and a signal from an unknown species, resonating at δ_P = 67.42 ppm (ca 35%). Significant broadening of this resonance indicated the presence of a boron atom and thus the signal was tentatively assigned to the postulated, initial intermediate of the reaction, diethyl iodoboranephosphonate **2b** (Scheme 5). The reaction with 2 equiv. of iodine proceeded analogously, except the starting material **1b** completely disappeared and the intermediate **2b** amounted to 68%. In both instances, addition of *n*-butylamine (10 equiv.) and more iodine (another 1-2 equiv) resulted in the immediate, complete disappearance of all the intermediates, and the formation of diethyl *n*-butylphosphoramidate **4b** (δ_P = 8.45 ppm) as the sole phosphorus-containing product (^{31}P NMR analysis). A similar course of the reaction was also observed in acetonitrile,[29] but since the activation of boranephosphonates **1** was more efficient in dichloromethane than in acetonitrile, the former solvent was used in further studies.

These results were consistent with the proposed reaction pathway of boranephosphonate diesters with amines promoted by iodine, depicted in Scheme 5. Firstly, they indicated that iodoboranephosphonate intermediate **2b** can, to some extent, spontaneously dissociate to afford some amounts of H-phosphonate **5b**, a stereochemically important type of intermediate for this reaction, and secondly, that all the intermediates observed, *i.e.* iodoboranephosphonate **2b**, H-phosphonate diester **5b**, and phosphoroiodidate **3b**, are possible or factual intermediates in the above reaction.

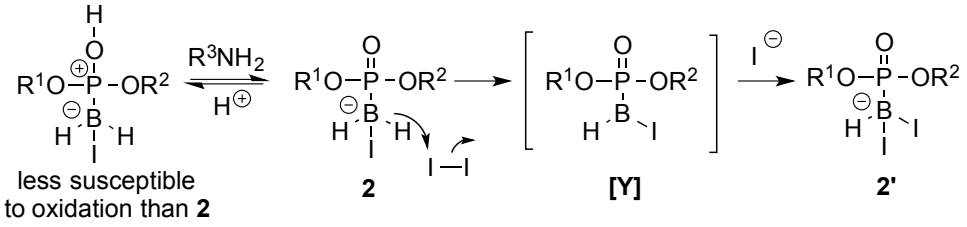
As mentioned above, addition of iodine as the last reagent (*i.e.* when boranephosphonate diester and amine were both present in the reaction mixture), could trigger a new reaction pathway. This concerns a putative aminoboranephosphonate **6** intermediate, which could be formed both *via* a direct coordination of an amine to an electron-deficient borane intermediate **[X]** (or directly from **1** *via* a four-center transition state), and a nucleophilic attack of an amine at the boron center of iodoboranephosphonate **2b** (Scheme 5) under these conditions.

Since these pathways are cryptic during the course of a regular reaction (*i.e.* when all reactants are present in sufficient amounts), we addressed this issue by (i) reacting **1a** with a limited amount of iodine in the presence of *n*-butylamine, and (ii) treating a generated iodoboranephosphonate **2b** with *n*-butylamine. To this end we added to a solution of boranephosphonate **1b** in dichloromethane containing *n*-butylamine (1 equiv.), 1 equiv. of iodine. In the ^{31}P NMR spectrum obtained the pattern of the signals was qualitatively similar to that of the reaction of **1b** with iodine alone (presence of signals from the starting material **1b**, the putative iodoboranephosphonate intermediate **2b**, H-phosphonate **5b**, and iodophosphate **3b**; *vide supra*), except for a new resonance from an unknown intermediate at δ_P = 42.63 ppm (ca 10%).[30] Quenching this reaction mixture with *n*-butylamine (10 equiv.) caused the signals from iodoboranephosphonate **2b** (at ca 69 ppm) and the unknown intermediate (at ca 42 ppm) to immediately disappear, and only the unreacted starting material **1b**, H-phosphonate **5b**, and a small amount of phosphoramidate **4b** (from iodophosphate **3b**), remained (^{31}P NMR analysis).

These experiments indicated that the signal at ca 42 ppm was not due to a side product formation but corresponded to a legitimate intermediate of the reaction, and

thus it was tempting to assign it to the postulated aminoboranephosphonate **6b**. Although the signal at ca 42 ppm was clearly observed when the activation of boranephosphonate **1b** was carried out in the presence of *n*-butylamine, we exercised some caution as it was rather broad and could be overlooked in noisy ^{31}P NMR spectra, especially at low concentration of the intermediate. Indeed, scrutiny of all our previous ^{31}P NMR spectra from the reaction of **1b** with iodine in the absence of *n*-butylamine showed that in a few instances we could discern a slight bump in the spectra at ca 42 ppm that might correspond to the sought intermediate. This was a compelling argument that an intermediate resonating at ca 42 ppm did not contain *n*-butylamine moiety as a structural fragment, and thus could not be assigned to aminoboranephosphonate **6b**.

In the light of the above we hypothesized that when the activation of boranephosphonate **1b** with iodine is carried out in the presence of a base (*n*-butylamine), the initially formed activation product, iodoboranephosphonate **2b**, can be prone to further borane hydride oxidation and form diiodoboranephosphonate **2′b** (Scheme 6). We assigned this structure to an intermediate that resonated at ca 42 ppm in the ^{31}P NMR spectra.



2a, 2'a, R^1 and R^2 = nucleoside moieties as in Scheme 2.
2b, **2'b**, $R^1 = R^2$ = ethyl.

Scheme 6. Formation of a putative diiodoboranephosphonate intermediate under basic conditions.

To substantiate this hypothesis we reacted boranephosphonate **1b** in dichloromethane with iodine (2 equiv.) in the presence of 2,6-lutidine (5 equiv.). The ^{31}P NMR spectrum (after 5 min.) revealed, apart from a resonance due to iodophosphate **3b** (δ_P = - 41.32 ppm, ca 13%), two, broad signals at 62.56 ppm (ca 60%) and 40.75 ppm (ca 27%). These were assigned to the two putative intermediates, namely, iodoboranephosphonate **2b** and diiodoboranephosphonate **2′b**, respectively. Since

both of them were converted into phosphoramidate **4a** upon the addition of *n*-butylamine, they were assumed to be potential intermediates involved in the investigated reaction.

We also probed the reactivity of iodoboranephosphonate **2b** towards *n*-butylamine as a possible step in the investigated reaction (step **2** to **6**, Scheme 5). The most revealing experiment consisted of an incremental addition of *n*-butylamine to the reaction mixture resulted from activation of boranephosphonate **1b** with a limited amount of iodine (Fig. 3). We tried to minimize the amount of iodine used so that in the reaction mixture after generation of iodoboranephosphonate intermediate **2b**, no iodine was left. [31]

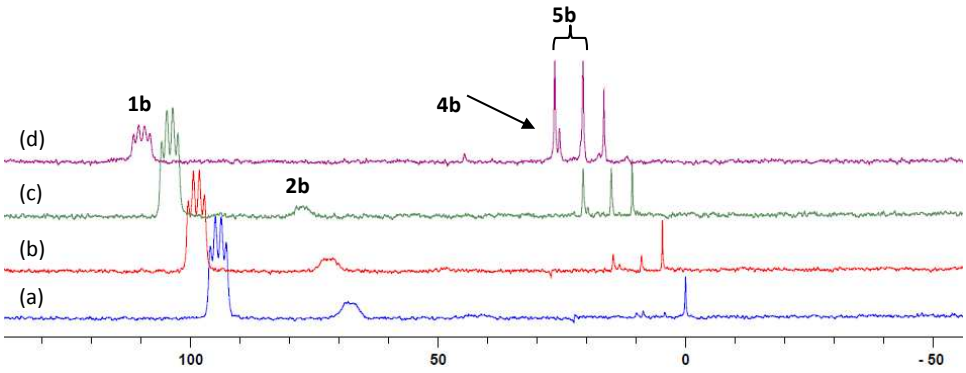


Fig. 3. ^{31}P NMR spectra of the reaction of iodoboranephosphonate **2b** with *n*-butylamine in dichloromethane: (a) the reaction of **1a** with *n*-butylamine (1 equiv) and iodine (0.8 equiv.), (b) after the addition of 1 equiv. of *n*-butylamine, (c) after the addition of another equiv. of *n*-butylamine (total 2 equiv.), (d) after the addition of total 10 equiv. of *n*-butylamine.

This would permit us to observe what species are formed upon reaction of **2b** with the added *n*-butylamine. Under such conditions (Fig. 3) no new intermediates could be detected, and only a gradual disappearance of **2b** and accumulation of H-phosphonate **5b** was observed (Fig. 3). This strongly suggested that in the presence of *n*-butylamine iodoboranephosphonate **2b** underwent a rapid nucleophilic substitution at the boron center to form aminoboranephosphonate **6b** that immediately collapsed to the corresponding H-phosphonate **5b** and the aminoborane. Apparently, due to inherent instability of aminoboranephosphonates **6**, the postulated intermediate **6b** did not accumulate and thus could not be detected under the reaction conditions.

The above experiment was also pertinent to the mechanism proposed by Caruthers *et al*.[14a] If iodoboranephosphonates **2** would be susceptible to nucleophilic substitution by iodide,[14a] they should even more readily react with *n*-butylamine to afford phosphoramidates **4**. Since this was not the case, the reaction of **2b** with *n*-butylamine lent support for the mechanism depicted in Scheme 5 that involves the intermediacy of H-phosphonate diester **5**. Additionally, in a separate experiment iodoboranephosphonate **2b**, generated from **1b** and iodine (1 equiv.) in dichloromethane, was subjected to a reaction with 10 equiv. of the added tetrabutylammonium iodide to probe its susceptibility to nucleophilic substitution. Since no measurable increase in the amount of phosphoroiodidate **3b** could be detected this, again, argued against a mechanism involving a nucleophilic attack of iodide on the phosphorus center. It seems that a mechanism in Scheme 5 is rather general for amine nucleophiles as analogous types of intermediates were observed in the reactions with aromatic amines (*e.g.*, aniline) and 2° amines (*e.g.*, diethylamine).

To sum up this part, all the above experiments indicate that various pathways are possible for the investigated reaction (Scheme 5 and 6). The reaction of boranephosphonate diesters **1** with *n*-butylamine promoted by iodine most likely commences with a borane hydride oxidation to produce a putative trivalent borane intermediate **[X]**. Under the reaction conditions, this may coordinate iodide and *n*-butylamine to produce two putative intermediates: iodoboranephosphonate **2** and aminoboranephosphonate **6**, respectively. The latter, due to its inherent instability, immediately collapsed to afford the corresponding H-phosphonate diester **5**, while the former, most likely reacted with the amine present to be converted into **5**, with the intermediacy of the corresponding aminoboranephosphonate **6**. Alternatively, **2** and **6** could be formed from boranephosphonate **1** directly *via* reactions involving four-center transition states. Under basic conditions, monoiodoborane intermediate **2** can partially be converted into diiodoboranephosphonate **2′** that is apparently also susceptible to the reaction with amine. H-Phosphonate **5** can probably be also formed directly from iodoboranephosphonate **2**, but this process is expected to be much slower than the reaction with the amine. All the above reaction steps occur with a retention of configuration and thus the sense of chirality of boranephosphonate **1** is preserved in the H-phosphonate diester **5** formed. There are two more reaction steps that follow this key intermediate formation, namely, the oxidation of **5** by iodine to

the corresponding phosphoroiodidate **3** (with retention of configuration) and its reaction with *n*-butylamine (with inversion of configuration). These furnished the final product, the corresponding phosphoramidate **4**, with a total inversion of configuration at the phosphorus center.

Finally, due to probably similar kinetics of the formation of **2** and **6** (directly from **1** or *via* intermediate **[X]**), and formation of **2′** from **2**, as well as conversion of **2** and **2'** into **6**, all these pathways can most likely be operative during the course of the reaction. Although changes in the reaction conditions can modulate their contributions this, however, should not affect the final stereochemical outcome of the whole transformation that is governed by stereochemistry of the key intermediate formed, namely the corresponding H-phosphonate diester.

CONCLUSIONS

We designed and carried out a stereochemical correlation analysis for the formation of phosphoramidate diesters from the corresponding boranephosphonate derivatives. Contrary to previous studies[14a] we found that the reaction of boranephosphonate diesters **1** with amines promoted by iodine proceeded with total inversion of configuration at the phosphorus center and involved the formation of the corresponding H-phosphonate as a stereochemically important intermediate. On this basis we could dismiss an oxidative nucleophilic attack on the phosphorus as a crucial step in this reaction. The role of iodine in the whole transformation was found to be twofold: (i) activation of the borane group *via* oxidation of the hydride anion to produce an iodoborane function (-BH_2I; intermediate **2** and possibly **2′**) and (ii) oxidation of the key intermediate, H-phosphonate diester **5**, to the corresponding phosphoroiodidate diester. The reaction of iodoboranephosphonates **2** with an amine to produce a putative aminoboranephosphonate **6** that apparently rapidly dissociated into the H-phosphonate **5** and the borane parts is most likely instrumental to the formation of the H-phosphonate intermediate. Since all the reaction steps leading to H-phosphonate diesters **5** occur with retention of configuration, the sense of chirality of boranephosphonates **1** is preserved in this intermediate. The two final steps, oxidation of H-phosphonate **5** by iodine, followed by the reaction with the amine present, furnished the final phosphoramidates **4** with total inversion of configuration.

EXPERIMENTAL SECTION

All reagents were of analytical grade, obtained from commercial suppliers and used without further purification. Anhydrous solvents used for reactions were stored over molecular sieves 4 Å. TLC analyses were carried out on Merck silica gel 60 F 254 precoated plates using CH_2Cl_2–MeOH 9:1 (v/v) solvent system. All evaporations were carried out on rotatory evaporators under reduced pressure at 40 °C.

H-Phosphonate diester **5a** was synthesized from the appropriate H-phosphonate monoesters[32] and alcohols according to standard coupling procedures.[33] The P-diastereomers of **5a** were separated using silica gel chromatography with ethyl acetate : toluene 99:1 (v/v) as a solvent system. These diastereomers are referred to as "slow" and "fast" (chromatographic mobility) or "low field" and "high field" (signals in the ^{31}P NMR spectra). For these studies **5a** was used as a 2:1 or 1:2 mixture of the diastereomers.

The NMR spectra were recorded using Bruker Avance II 400 MHz instrument. The chemical shifts are reported in ppm, relative to solvent peaks (^{1}H, ^{13}C NMR), 2% H_3PO_4 solution in D_2O (^{31}P NMR), and BF_3 diethyl etherate (^{11}B NMR). Assignment of the NMR signals was accomplished on the basis of the expected chemical shift values, splitting pattern of the signals, and 2D correlation experiments. High resolution mass spectra (HRMS) were recorded on Thermo Fisher Scientific Q-Exactive Orbitrap mass spectrometer. Identification of intermediates observed in the ^{31}P- and ^{11}B NMR spectra of the reaction mixtures was done on the basis of the expected chemical shifts, coupling constants, shapes of the multiples, and spiking of the reaction samples with compounds of known structures.

For compounds **1a** and **4a**, due to the presence of two P-diastereomers and similar chemical shifts of the protons in the 3′- and 5′-nucleoside units, multiplicity and overlapping of the resonances were observed in the ^{1}H NMR spectra. For this reason only selected diagnostic signals are listed. Purity of the isolated compounds was >97% (^{1}H NMR spectroscopy).

*Synthesis of 3′-O-tritylthymidin-5′-yl 5′-O-tritylthymidin-3′-yl boranephosphonate **1a**, triethylammonium salt*

3′-*O*-Tritylthymidin-5′-yl 5′-*O*-tritylthymidin-3′-yl H-phosphonate **5a** (0.1 mmol) was placed in a 100 mL pear-shaped flask and evaporated twice the added anhydrous dichloromethane (2 x 20 mL). The residue was dissolved in anhydrous dichloromethane (20 mL), and triethylamine (0.5 mmol), followed by trimethylsilyl chloride (0.5 mmol), were added. After stirring for 10 min., borane dimethyl sulfide complex in THF (1 mmol) was added to the generated silyl phosphite derivative. The reaction mixture was stirred for another 10 min. and progress of the reaction was monitored by ^{31}P NMR spectroscopy. After quenching the reaction mixture by the addition of an aqueous ammonia (25%, 2 mL) and the removal of the solvents, the product was purified by a silica gel column chromatography using a gradient of methanol in dichloromethane (0-5%, v/v) containing triethylamine (1%). Fractions containing the desired product were collected and evaporated, to yield **1a** as a white solid (96 mg). Yield 85%. R_f = 0.13 and 0.28. HRMS *m/z*: $[M\text{-}Et_3NH^+]^-$ Calcd for $C_{58}H_{57}BN_4O_{11}P$ 1027.3860; Found 1027.3878.

^{1}H NMR ($CDCl_3$, 400 MHz): (diagnostic signals) δ -0.20-0.80 (br m, 3H), 1.36 (s, 3H), 1.88 (s, 3H), 6.30-6.70 (m, 2H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 8.6, 11.6, 12.5, 39.1, 39.5, 45.6, 64.2, 74.9, 76.0, 84.9, 85.5, 85.9, 87.6, 87.8, 111.4, 127.4, 128.1, 128.7, 128.9, 135.79, 136.5, 143.3, 144.1, 144.3, 150.8, 164.2. ^{31}P NMR ($CDCl_3$, 162 Hz): δ 94.15 (br m). ^{11}B NMR ($CDCl_3$, 128.4 Hz): δ -38.47 (br).

*Synthesis of diethyl boranephosphonate **1b**, triethylammonium salt*

1b was obtained analogously to **1a** using diethyl H-phosphonate (0.1 mmol) as a starting material. The product was purified by a silica gel column chromatography using 0-10% gradient of methanol in dichloromethane containing triethylamine (1%). The separation was controlled by TLC chromatography by evocating spots in a chamber with iodine vapour. Compound **1b** was obtained as a colorless oil (23 mg). Yield 90%. HRMS *m/z*: $[M\text{-}Et_3NH^+]^-$ Calcd for $C_4H_{13}BO_3P$ 151.0701; Found 151.0689.

^{1}H NMR ($CDCl_3$, 400 MHz): δ -0.40-0.60 (br m, 3H), 0.15 (m, 3H), 1.06 (t, 6H, $^{3}J_{HH}$ = 7.1 Hz), 1.13 (t, 6H, $^{3}J_{HH}$ = 7.3 Hz), 2.9 (q, 4H, 7.3 Hz), 3.73 (m, 4H, $^{3}J_{HH}$ = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 8.1, 16.4 (d, $^{3}J_{PC}$ = 5.8 Hz), 45.4, 59.1 (d, $^{2}J_{PC}$ = 4.1 Hz). ^{31}P NMR ($CDCl_3$, 162 Hz) : δ 95.31 (br q, $^{1}J_{PB}$ = 131.0 Hz). ^{11}B NMR ($CDCl_3$, 128.4 Hz): δ -41.25 (d, $^{1}J_{PB}$ = 134.7 Hz).

*Transformation of 3′-O-tritylthymidin-5′-yl 5′-O-tritylthymidin-3′-yl H-phosphonate **5a** into 3′-O-tritylthymidin-5′-yl 5′-O-tritylthymidin-3’-yl n-butylphosphoramidate **4a** (the reference reaction)*

3′-*O*-Tritylthymidin-5′-yl 5′-*O*-tritylthymidin-3′-yl H-phosphonate **5a** (0.1 mmol; a ca 2:1 mixture of the low and high field diastereomers; δ_P = 9.38 and 8.31 ppm) was made anhydrous by evaporation of the added pyridine (2 x 20 mL), then acetonitrile (2 x 20 mL), and left overnight on the vacuum line. The residue was dissolved in anhydrous acetonitrile (20 mL), and *n*-butylamine (0.5 mmol), followed by iodine (0.3 mmol) dissolved in acetonitrile (0.5 mL), were added. After stirring for 5 min. the ^{31}P NMR spectrum was recorded. It showed two signals of intensities ca 1:2 at δ_P = 9.91 and 9.51 ppm corresponding to the expected phosphoramidate **4a**. The product was isolated by a silica gel column chromatography using a gradient of methanol in dichloromethane (0-5%). Compound **4a** was obtained as a white solid (80 mg). Yield 74%. R_f = 0.66. HRMS *m/z*: $[M\text{-}H]^-$ Calcd for $C_{62}H_{63}N_5O_{11}P$ 1084.4267; Found 1084.4277.

^{1}H NMR ($CDCl_3$, 400 MHz): (diagnostic signals) δ 1.42 (s, 3H), 1.84 (s, 3H), 6.27-6.45 (br m, 2H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 14.1, 14.8, 16.1, 22.1, 36.0, 41.5, 43.4, 65.9, 68.5, 86.8, 87.3, 87.7, 90.1, 90.5, 113.5, 114.0, 130.0, 130.5, 131.1, 131.2, 137.7, 138.2, 145.4, 146.3, 152.9, 166.4. ^{31}P NMR ($CDCl_3$, 162 Hz): δ 9.51, 9.91 (integration ca 2:1).

*Transformation of 3′-O-tritylthymidin-5′-yl 5′-O-tritylthymidin-3′-yl boranephosphonate **1a** into 3′-O-tritylthymidin-5′-yl 5′-O-tritylthymidin-3′-yl n-butylphosphoramidate **4a***

3′-*O*-tritylthymidin-5′-yl 5′-*O*-tritylthymidin-3′-yl boranephosphonate **1a** (0.1 mmol; obtained from ca 1:2 diastereomeric mixture of **5a** as described above) was dried by

evaporation of the added anhydrous acetonitrile (2 x 20 mL) and dissolved in the same solvent (20 mL). To this, *n*-butylamine (1 mmol), followed by iodine (0.3 mmol, dissolved in 0.5 mL of acetonitrile), were added. After stirring for 5 min. the ^{31}P NMR spectrum was recorded. It showed two signals of intensities 1:2 at δ_P = 9.66 and 9.30 ppm corresponding to the expected phosphoramidate **4a**. The pattern of the signals was the same as in the reference reaction, and after isolation of the product (74 mg) it was found to be identical to that from the direct oxidative coupling above. Yield 68%. R_f = 0.68. HRMS *m/z*: $[M-H]^-$ Calcd for $C_{62}H_{63}N_5O_{11}P$ 1084.4267; Found 1084.4276.

^{1}H NMR ($CDCl_3$, 400 MHz): (diagnostic signals) δ 1.43 (s, 3H), 1.85 (s, 3H), 6.30-6.45 (br m, 2H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.7, 12.4, 13.6, 14.1, 19.6, 19.7, 22.7, 29.7, 30.0, 31.9, 33.7, 39.1, 41.0, 63.6, 66.01, 74.4, 84.3, 85.4, 87.7, 88.1, 111.1, 111.7, 127.6, 128.09, 128.14, 128.7, 128.8, 135.2, 135.5, 143.0, 144.0, 150.3, 163.7. ^{31}P NMR ($CDCl_3$, 162 Hz): δ 9.30, 9.66 (integration ca 2:1).

*Synthesis of diethyl n-butylphosphoramidate **4b***

4b was obtained analogously to **4a** using diethyl H-phosphonate (0.1 mmol) as a starting material. The product was purified by a silica gel column chromatography using 0-10% gradient of methanol in dichloromethane containing triethylamine (1%). The separation was controlled by TLC chromatography by evocating spots in a chamber with iodine vapour. Compound **4b** was obtained as a colorless oil (17.5 mg). Yield 85%. HRMS *m/z*: $[M-H]^-$ Calcd for $C_8H_{19}NO_3P$ 208.1108; Found 208.1100.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, 3H, $^3J_{HH}$ = 7.3 Hz), 1.25 (m, 8H), 1.41 (m, 2H), 2.82 (m, 2H), 3.98 (m, 4H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 13.6, 16.1 (d, $^2J_{PC}$ = 7.1 Hz), 19.6, 33.7 (d, $^2J_{PC}$ = 6.1 Hz), 41.0, 62.0 (d, $^2J_{PC}$ = 5.3 Hz). ^{31}P NMR ($CDCl_3$, 162 Hz) : δ 9.33.

Supporting information

^{1}H, ^{13}C, ^{31}P, and ^{11}B NMR spectra.

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References and Notes

(1) Since boron is less electronegative than carbon or hydrogen, for this class of compounds we prefer a generic name "phosphonate" over "phosphate", analogously to C-phosphonate and H-phosphonate derivatives.

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electron pair to iodine. This would not affect stereochemistry of the formed phosphoroiodidate **3** (retention of configuration). However, due to the found stability of the P-B bond in boranephosphonate diesters, we deemed this reaction pathway as less likely.

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(29) In acetonitrile, the analogous reactions were less efficient. With 1 equiv. of iodine only formation of diethyl phosphoroiodidate (ca 14%) was observed. When the amount of iodine was increased to 2 equiv., formation of diethyl iodoboranephosphonate **2b** intermediate could also be detected (ca 22%).

(30) In this reaction formation of a minute amount (ca 1%) of the corresponding phosphoramidate **4b** was also observed. When the reaction was carried out with 2 equiv. of iodine and 4 equiv. of the amine, the resonance from the phosphoramidate became the major signal. In this instance, no signals from the starting material **1b** and H-phosphonate **5b** could be detected.

(31) The reaction of boranephosphonate **1b** with amines in the presence of iodine was very sensitive to the ratio of the reagents used. With 0.5 - 0.8 equiv. of iodine in the presence of 1 equiv. of *n*-butylamine only iodoboranephosphonate **2b** and the unreacted starting material **1b** could be observed in the ^{31}P NMR spectra, while with 1 equiv. of iodine, additional signals due to diiodoboranephosphonate **2b'** and iodophosphate **3b**, appeared. When the amount of *n*-butylamine was increased to 3 equiv., pattern of the signals was similar to that of the reaction with 1 equiv. of the amine, but the signal from **3b** was replaced by that of the corresponding phosphoramidate **4b**.

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Graphical abstract