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A BH_3 masked *H*-phosphonate for coupling in oligonucleotide synthesis

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ABSTRACT

A method of masking 3′-*H*-phosphonate group for the solution-phase synthesis of ribonucleotide by *H*-phosphonate approach was described. The phosphonic acid group was masked by bis-(2-cyanoethyl) boranophosphate during the reactions. After a successive demasking treatment by triethylamine, trityl cation, and triethylamine, the triethylammonium 3′-*H*-phosphonate nucleotide can be obtained efficiently ready for the coupling cycle in synthesis of oligonucleotide.

Solution-phase synthesis of oligonucleotide, compared to solid-phase synthesis, has the advantages of easy characterization of reaction intermediates and easy scale-up, which should be useful in the synthesis of large scale of specific sequence oligonucleotide for therapeutical application such as siRNA. In our previous work,[1] we reported the synthesis of siRNA targeting on human superoxide dismutase gene in solution by the phosphotriester approach,[2] which has successfully yielded siRNA showing same efficiency of the gene silencing effect as its solid phase phosphoamidite approach synthesized counterpart.[3,4] The phosphotriester approach generally gives quite efficient coupling in less than 14 mer oligoribonucleotides, while still needs to be optimized for longer than 20 mer synthesis.

H-Phosphonate approach,[5–18] which has high coupling efficiency, is regarded as an alternative approach for oligonucleotide synthesis in solution. As same as phosphotriester approach, the *H*-phosphonate approach can adopt 'block' condensation reactions in solution-phase synthesis.[10] During synthesis of those building block intermediates, a suitable protecting group for 3′ end is crucial. Levulinyl (Lev) ester group was used as 3′-OH protecting group in the synthesis of DNA by *H*-phosphonate approach in solution.[10,12] However, when using the same protecting group for the synthesis of RNA, demasking Lev group from 3′-OH would induce 2′→3′ migration of 2′-protecting group such as TBDMS.[19] It would be advantageous if we can develop an efficient masking–demasking protocol for 3′-*H*-phosphonate, in which 3′-*H*-phosphonate was masked during the coupling reaction, and demasked to give 3′-*H*-phosphonate monoester for the next nucleotide coupling.

BH_3 group was frequently used as the protecting group for phosphine derivatives,[20] and boranophosphate nucleotide was synthesized successfully.[21–23] Wada group has done some excellent work using BH_3 to protect phosphonic acid.[24,25] They have synthesized a boranophosphate-linked disaccharide, and converted it into *H*-phosphonate diester disaccharide. Inspired by their results, herein we have tested whether boranophosphotriester (compound **3** in Scheme 1) could be demasked to give *H*-phosphonate diester (compound **4** in Scheme 1), then further be demasked to give *H*-phosphonate monoester (compound **1** in Scheme 1) for coupling in oligonucletide synthesis. Several aromatic and alkyl groups can be used as a leaving group in the protection of phosphate.[26] Cyanoethyl group (CE), a frequently-used protecting group in oligonucleotide synthesis, has been chosen here as the protecting group for nucleotide 3′-phosphate. After BH_3 treatment, thus bis-(2-cyanoethyl) boranophosphate nucleotide (compound **2** in Scheme 1) was synthesized. Also, it is known that BH_3 group can be demasked by trityl cation in acidic solution,[24,25,27] in order to avoid the simultaneous deprotection on 5′-DMTr protecting group, the trityl group, which is much stable in acid than DMTr group, was used as we have found that when the reaction was carried out in 0.2% dichloroacetic acid and 2.5 equiv of TMTrOH in CH_2Cl_2 in 10 min, the coordinated BH_3 group could be demasked by TMTrOH rapidly without observed deprotection on 5′-trityl group. Fortunately, the 2-cyanoethyl group and the BH_3 group were successfully demasked successively to give 3′-*H*-phosphonate monoester nucleotide.

The nucleoside **5** in THF and 3-hydroxypropionitrile was added successively into a THF solution of PCl_3 and 2,6-dimethylpyridine at −78 °C under argon atmosphere. Followed by adding 1 M BH_3·THF in THF solution at −20 °C. The resulting solution was

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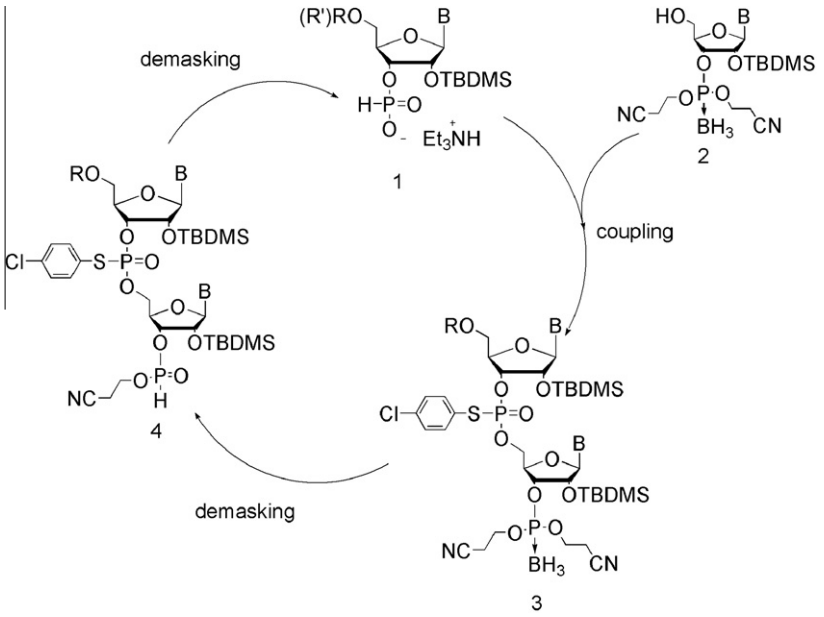
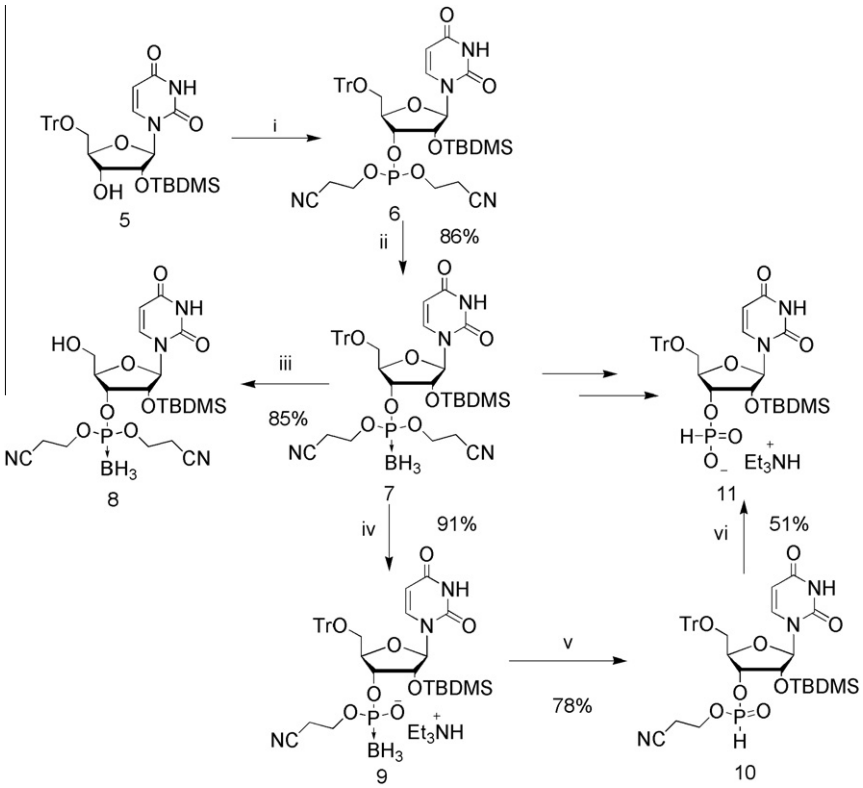


Scheme 1. BH_3 masked *H*-phosphonate for coupling cycle in oligonucleotide synthesis.

stirred at 0 °C for additional 30 min to give fully protected intermediate **7** in 86% yield (Scheme 2).

All other building blocks for *H*-phosphonate coupling reaction such as compounds **8** and **11** were obtained from the fully protected mononucleotide **7** by different demasking reaction. 5′-Trityl group was cleaved by 3% TFA in the presence of 50 mM BH_3·Py and 50% Et_3SiH as a trityl cation scavenger to avoid the demasking on BH_3 group.[24,27] 5′-OH nucleotide **8** was thus obtained in 85% yield.

The triethylammonium *H*-phosphonate monoester **11** was obtained by three sequential reactions: firstly, one of the two 2-cyanoethyl groups was demasked by the treatment with Et_3N/CH_3CN to give compound **9** in 91% yield; secondly, the coordinated BH_3 group in compound **9** was demasked to give *H*-phosphonate diester **10** in 78% yield by the treatment of 0.2% dichloroacetic acid and 2.5 equiv of TMTrOH in CH_2Cl_2 in 10 min; finally, the remaining 2-cyanoethyl group in compound **10** was



Scheme 2. Reagents and conditions: (i) PCl_3, 2,6-dimethylpyridine, 3-hydroxypropionitrile, THF, −78 °C; (ii) BH_3·THF/THF, −20 to 0 °C, 30 min; (iii) 3% TFA, BH_3·Py (50 mM), CH_2Cl_2/Et_3SiH (v:v = 1:1), 0 °C, 15 min; (iv) Et_3N/CH_3CN (v:v = 3:1), rt, 10 min; (v) 0.2% DCA, TMTrOH (12.5 mM), CH_2Cl_2, 0 °C, 10 min; (vi) Et_3N/CH_3CN (v:v = 4:1), rt, 5 min.

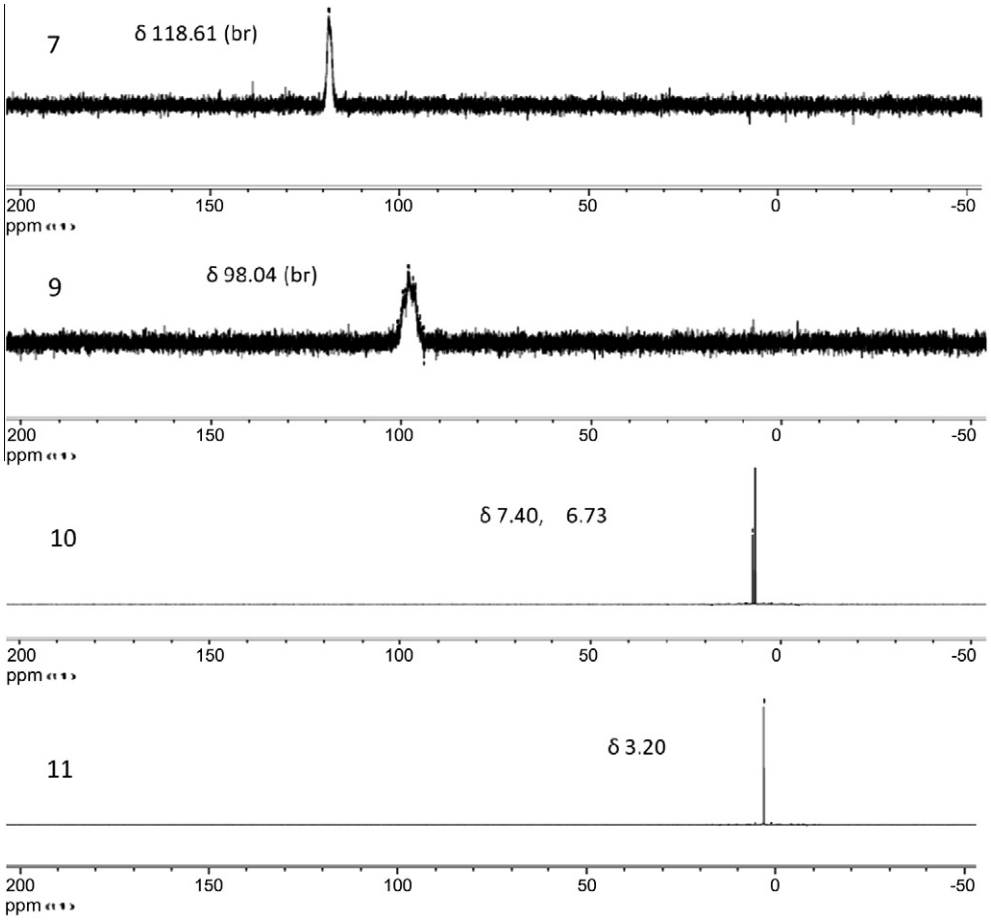
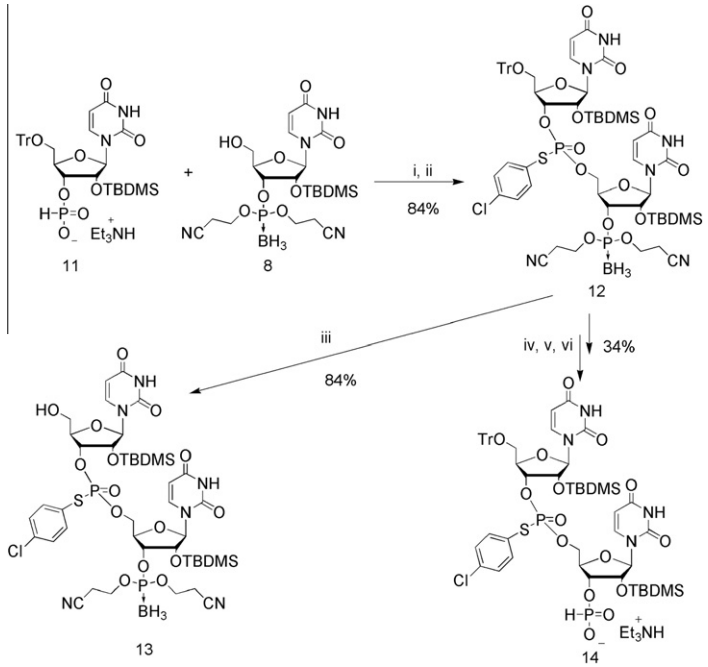


Figure 1. ^{31}P NMR of compound **7**, **9**–**11**.



Scheme 3. Reagents and conditions: (i) pivaloyl chloride, BH_3·Py (0.1 M), C_5H_5N, 0 °C, 20 min. (ii) *N*-(phenylsulfanyl)-succinimide, C_5H_5N, 0 °C, 20 min; (iii) 3% TFA, BH_3·Py (50 mM), CH_2Cl_2/Et_3SiH (v:v = 1:1), 0 °C, 15 min; (iv) Et_3N/CH_3CN (v:v = 3:1), rt, 10 min; (v) 0.2% DCA, TMTrOH (12.5 mM), CH_2Cl_2, 0 °C, 10 min; (vi) Et_3NH/CH_3CN.

demasked by Et_3N/CH_3CN to give compound **11** in 51% yield. The ^{31}P NMR (Fig. 1) clearly showed the transformation reaction from compound **7** to **11**.

By coupling triethylammonium 3′-*H*-phosphonate monoester **11** and 5′-OH nucleotide **8**, the dinucleotide **12** was synthesized. The coupling reaction was activated by pivaloyl chloride in the presence of 0.1 M BH_3·Py at 0 °C, then the thus formed *H*-phosphonate dinucleotide was reacted in situ with *N*-(phenylsulfanyl)-succinimide to give phosphorothioate triester **12** in 84% yield (Scheme 3). The presence of 0.1 M BH_3·Py in the coupling reaction can greatly reduce the partial demasking of P-BH_3.

The fully protected dinucleotide **12** was demasked to give 5′-OH dinucleotide **13** in 84% yield and triethylammonium *H*-phosphonate dinucleotide **14** in 34% yield, respectively, as described above for compound **7**. Thus obtained dinucleotide **13** and **14** can go on the coupling-demasking cycle for the longer oligonucleotide synthesis (Scheme 3).

In conclusion, a BH_3 masked *H*-phosphonate approach for coupling cycle in oligonucleotide solution synthesis was established herein successfully. This approach can not only take the advantage of high coupling efficiency by *H*-phosphonate in solution, but also avoid 2′ → 3′ migration by 2′-protecting group. This masking–demasking protocol for 3′-*H*-phosphonate, in which 3′-*H*-phosphonate was masked by BH_3 and CE during the coupling reaction, and demasked to give 3′-*H*-phosphonate monoester for next coupling, may find new applications in the oligo-phosphate synthesis. The further optimization of this BH_3 masked *H*-phosphonate approach for oligomer synthesis is under active investigation in our lab and will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization data and ^1H, ^{13}C NMR, ^{31}P NMR spectra and mass data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.02.054.

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