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A novel linker for solid-phase synthesis cleavable under neutral conditions

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Abstract—A novel linker cleavable under neutral conditions has been developed for the solid-phase synthesis of base-labile compounds. The linker is comprised of a 3-azidomethyl-4-hydroxybenzyl alcohol moiety, and the azidomethyl group in the linker is readily converted to an aminomethyl group by treatment with a phosphine reagent in the presence of water to result in an intramolecular cyclization to release the compounds. Using the linker, a base-labile dinucleoside methyl phosphate was synthesized on a highly cross-linked polystyrene (HCP) support and cleaved successfully from the resin without decomposition of the product.

Solid-phase methods have been used in organic synthesis over the past years, first for peptides,[1] then for nucleotides,[2] and recently for small molecules in combinatorial chemistry.[3–5] Accompanied with the development of the solid-phase synthesis of organic compounds, various linkers have been designed and synthesized for the attachment of molecules to solid supports.[6] These linkers can be cleaved by acids,[7,8] bases,[9] fluoride ion,[10] or photochemical reactions.[11] A class of linkers which have been used for solid-phase chemistry is based on so-called 'an assisted cleavage mechanism; the cleavage reaction occurs via the conversion of a masked auxiliary into a reactive nucleophile, followed by the intramolecular attack of the nucleophile on the neighboring reactive site'.[12] Although linkers cleavable through the assisted mechanism are fascinating, there have been few linkers, which can be cleaved under neutral conditions.

This circumstance has prompted us to develop a new type of linker, which can be cleaved under neutral and reductive conditions, based on this 'assisted cleavage' concept. The linker synthesized here is comprised of a 3-azidomethyl-4-hydroxybenzyl alcohol moiety, and the azide as a latent amino group was used for a masked auxiliary.[13,14] The benzylic hydroxy group in the linker would be functionalized with appropriate molecules bearing an acidic function, such as a carboxyl or phosphoryl group. Following the reduction of the azidomethyl group to the corresponding aminomethyl group by the Staudinger reaction,[15] an intramolecular cyclization should occur rapidly to release the product by a 1,6-elimination process (Scheme 1).

This cleavage reaction is expected to proceed under almost neutral conditions, so that the linker would be applied to the synthesis of compounds, which is unstable under acidic or basic conditions. In this letter, we describe the synthesis of a new linker, and the efficient cleavage of a base-labile 3′-phosphorylated oligonucleotide analog from the linker.

The linker **4**[16] was prepared from 2,4-bis(hydroxymethyl) phenol **1**[17] through five steps (Scheme 2). Following the selective protection of the phenolic hydroxy and 2-hydroxymethyl groups of **1** with tetraisopropyldisiloxane,[18] the remaining benzylic hydroxy group was protected with a monomethoxytrityl (MMTr) group. After cleavage of the 1,3-cyclic silyl ether by treatment with tetrabutylammonium fluoride (TBAF), the 2-hydroxymethyl group was converted into an azidomethyl group via the chloromethylation to give the linker **4** in good yield. Then the phenolic hydroxy group in the linker **4** was acylated by treatment with glutaric anhydride, and the resulting compound **5**[19]

Keywords: Linker; Solid-phase; Azidomethyl group; Staudinger reaction; Neutral conditions.
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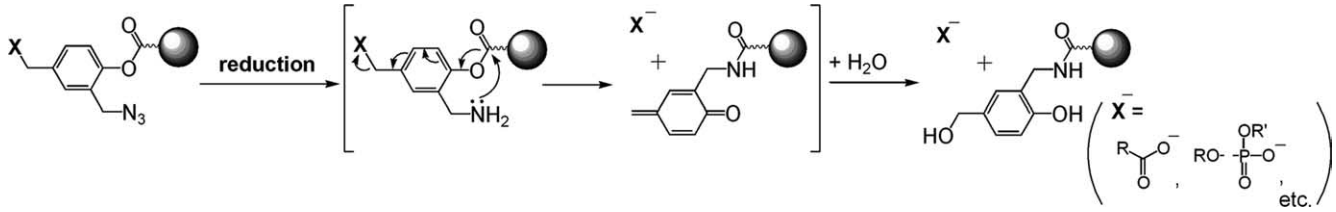
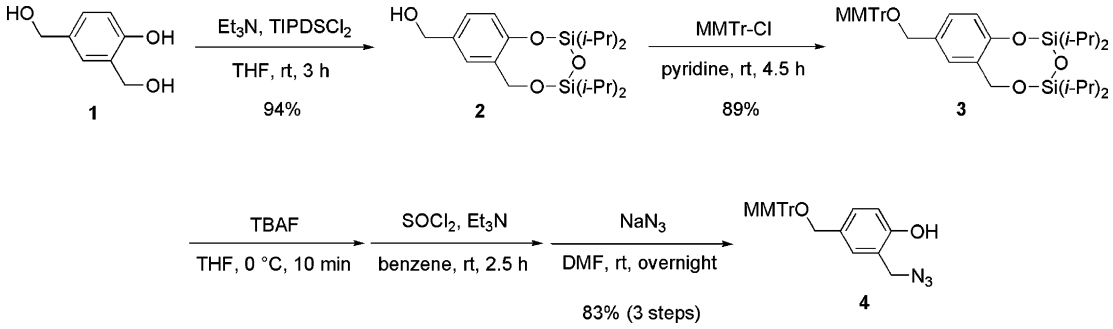


Scheme 1. A proposed reaction mechanism for the cleavage of attached compounds from the linker.

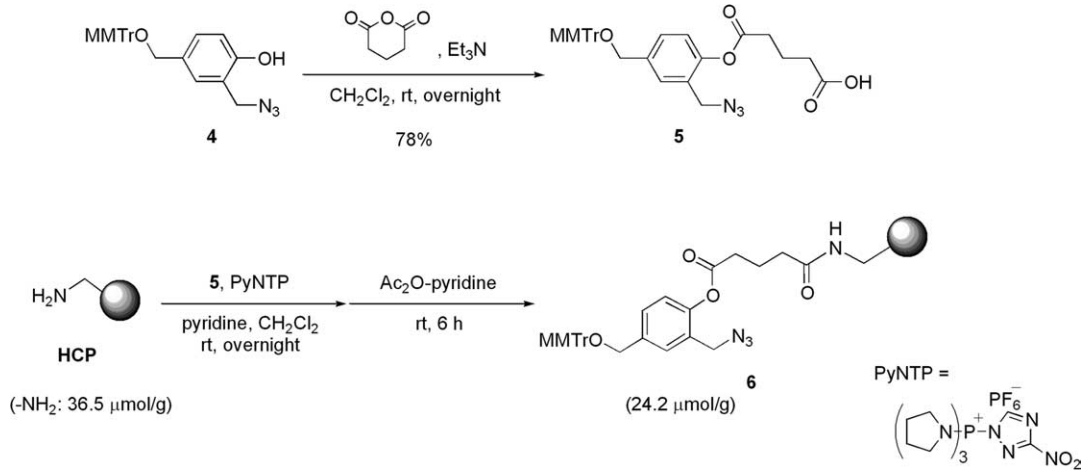


Scheme 2. Synthesis of the linker.

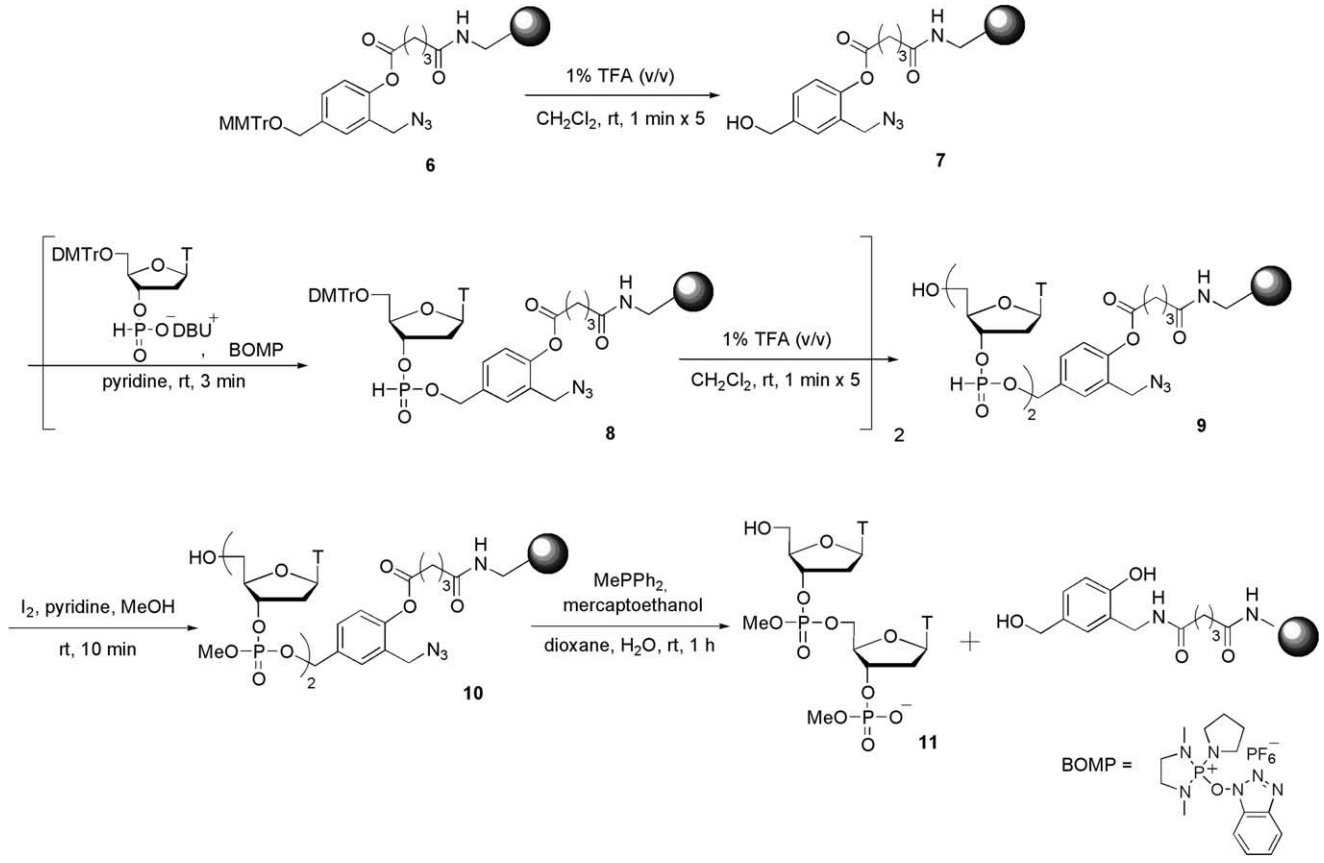
was successfully attached to a highly cross-linked aminomethylated polystyrene (HCP) support[20] through an amide bond by using a new condensing reagent, PyNTP[21] (Scheme 3). In contrast, when the acylation was carried out using succinic anhydride, the desired product was not obtained because of an intramolecular cyclization caused by the succinic carboxylate anion to give the starting material. The loading amount of the linker molecules to the solid support was estimated to be 24.2 μmol/g by a MMTr cation assay.

Next, the synthesis of a base-labile oligonucleotide analog was investigated by using the new linker-attached solid support. As a model compound, a dinucleoside methyl phosphotriester derivative was chosen. Dithymidine *H*-phosphonate was synthesized in 0.5 μmol scale on the solid support,[22] and was transformed to the corresponding methyl phosphate by treatment with 2% iodine (w/v) in methanol/pyridine (1/9, v/v). The resulting dimer was released from the solid support by treatment with 0.2 M methyldiphenylphosphine as a reducing reagent in aqueous dioxane[13] (Scheme 4). In the cleavage reaction, mercaptoethanol was found to be effective as a scavenger of quinone methide, which was generated in the course of the reaction. The released products were dissolved in water and washed with chloroform to remove excess phosphine and phosphine oxide. After concentration of the aqueous portion, the crude dimer was analyzed by RP-HPLC (Fig. 1). Two main peaks corresponding to the diastereomers of dithymidine methyl phosphate were observed. The result clearly shows the efficient cleavage of the linker without decomposition of the product. After purification by RP-HPLC, the pure dimer **11** was obtained in 46%.[23]

In conclusion, a novel linker was synthesized for solid-phase synthesis, which can be cleaved under neutral



Scheme 3. Attachment of the linker to a solid support.



Scheme 4. Synthesis of dithymidine methyl phosphate on the resin using the linker.

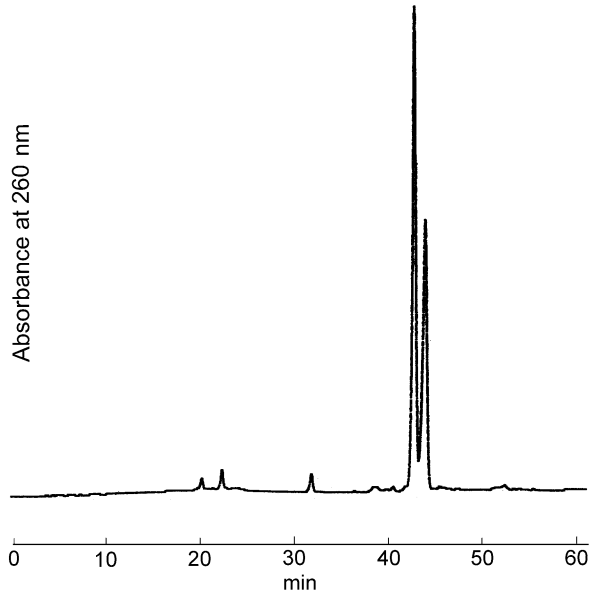


Figure 1. RP-HPLC profile of the crude dimer of dithymidine methyl phosphate.

and reductive conditions. Using the linker, a base-labile 3′-phosphorylated dithymidine methyl phosphate was synthesized and successfully cleaved from the solid support without decomposition of the product. The linker may be useful for the synthesis of other oligonucleotide analogs, peptides, and natural products as well as a wide variety of organic molecules, which are unstable under basic and acidic conditions.

Acknowledgements

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16. Selected data for **4**: ^{1}H NMR ($CDCl_3$): δ 7.50–7.13 (14H, m), 6.83 (2H, d, $J = 8.7$ Hz), 6.77 (1H, d, $J = 8.4$ Hz), 5.52 (1H, s), 4.38 (2H, s), 4.08 (2H, s), 3.78 (3H, s); ^{13}C NMR ($CDCl_3$): δ 158.4, 153.4, 144.5, 135.8, 131.7, 130.4, 129.1, 128.9, 128.4, 127.9, 126.9, 121.5, 115.9, 113.2, 86.8, 65.3, 55.4, 51.2; IR (KBr) 3398, 2103, 1608, 1509, 1446, 1250, 1179, 1032, 830, 767, 706 cm^{-1}; MALDI/TOF mass spectrometry, using 2,5-dihydroxybenzoic acid as a matrix. Calcd for $(M+Na)^+$ 474.18. Found: 474.06.
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23. The analysis and purification of the 3′-phosphorylated dithymidine methyl phosphate **11** were carried out on a Senshu-Pak C18 column, using 0.1 M TEAA buffer at a linear gradient from 0% to 20% MeCN in 60 min at a flow rate 0.5 ml/min. The characterization of **11** was established by MALDI/TOF mass spectrometry, using 2,5-dihydroxybenzoic acid as a matrix. Calcd for $[M]^-$; 653.13. Found: 652.87.