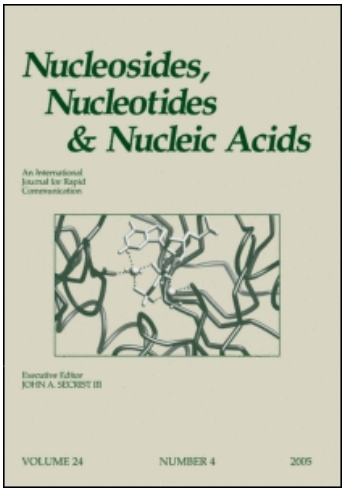

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A Solid Support with a Hydroxyallyl Linker, Full Parts of Which are Potentially Reusable for the Synthesis of Oligonucleotides

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A SOLID SUPPORT WITH A HYDROXYALLYL LINKER, FULL PARTS OF WHICH ARE POTENTIALLY REUSABLE FOR THE SYNTHESIS OF OLIGONUCLEOTIDES

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□ *This paper describes a solid support with a hydroxyallyl linker that is regenerated without loss of any parts after having used for the synthesis of nucleotides. Reproduction of the solid support can be achieved through detachment of the oligonucleotide by treatment with an organopalladium catalyst in the presence of triethylammonium acetate and subsequent methanolysis of the resulting allyl acetate.*

INTRODUCTION

In the solid-phase synthesis of oligonucleotides, a solid support (with a spacer and a linker) occupies a major cost of the synthetic process. Therefore, it is desirable, particularly in the large-scale synthesis, to be able to reuse the solid support multiple times. Recently, Pon and coworkers[1,2] reported the first reusable solid support with $HOCOCH_2CH_2CONH(CH_2)_6OCOCH_2OC_6H_4OCH_2COOH$ as a linker (Q linker), where $HOCOCH_2OC_6H_4OCH_2COOH$ is 1,4-hydroquinone-*O*,*O*′-diacetic acid. However, this support only partially meets the above requirement, because the 1,4-hydroquinone-*O*,*O*′-diacetyl is eliminated by treatment with the aqueous ammonia employed for detachment of the produced oligonucleotide. Therefore, in order to reuse the recovered solid as the support, the 1,4-hydroquinone-*O*,*O*′-diacetic acid function has to be re-introduced. Ideally, we would like to have a solid support, where whole parts are reusable; that is, a solid support that does not undergo any loss of parts throughout the synthetic

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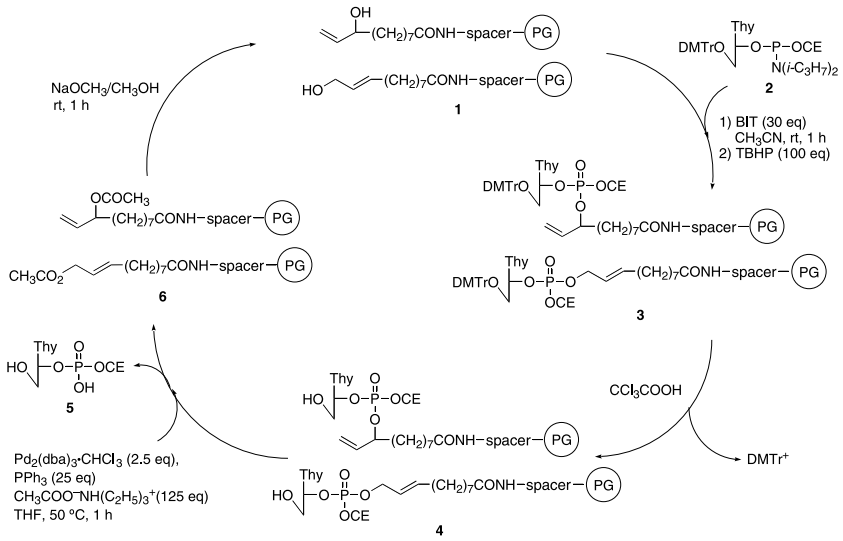

SCHEME 1 Reaction cycle of a reusable solid support with a hydroxyallyl linker.

processes, including detachment of the nucleotide product. We now report a fully reusable support, **1**, i.e., Porous Glass® (PG) with a hydroxyallyl linker.*

RESULTS AND DISCUSSION

According to the reaction cycle shown in Scheme 1, we examined the reusability of the solid support **1**. Thus, a mononucleotide was introduced to **1** by the reaction with the 5′-*O*-dimethoxytritylthymidine 3′-phosphoramidite **2** using benzimidazolium triflate (BIT)[7,8] as a promoter in acetonitrile, followed by oxidation with *tert*-butyl hydroperoxide (TBHP).[9] The resulting product **3** was exposed to a 3% solution of trichloroacetic acid in dichloromethane (5 min) for removing the DMTr protector to afford **4**. The amount of the released DMTr group was determined by a colorimetric method. The thymidine 3′-[(2-cyanoethyl) phosphate] moiety (**5**) was then detached by treatment with $Pd_2[(C_6H_5CH = CH)_2CO]_3 \cdot CHCl_3$ (2.5 equiv to the nucleotide estimated as above by the amount of the released DMTr group) and $P(C_6H_5)_3$ (25 equiv) in the presence of triethylammonium acetate (125 equiv) in THF (50°C, 1 h). This reaction simultaneously formed the solid support **6** with an allyl acetate structure at the terminal positions of the linker. Finally, the acetyl group was removed with a 0.02 M solution of sodium methoxide in methanol (1 h) to give back **1**. The recovered support **1** was subjected to the following series of four reactions; i.e., 1) introduction of 5′-O-DMTr-protected thymidine 3′-phosphate, 2) detritylation, 3) detachment of the thymidylate by the palladium reaction, and 4) deacetylation of the resulting allyl acetate anchored on the solid support. Table 1 shows the amount

*Several solid supports with allyl linkers have been reported, but all of them are not reused: see Refs. [3–6].

TABLE 1 Reusablity of the Support **1**

Cycle	1	2	3	4
Amount of the loaded nucleotide[a] (μmol/g)	39.16	50.32	32.31	42.69

[a]Determined by DMTr cation assay.

of the DMTr function released by the step (2) in each reaction cycle. Although the resulting values were not constant, and thus there is a problem with the reproducibility, the results indicated that the recovered **1** may serve as a full-structure reusable solid support.

The solid support **1** can be used for synthesis of oligonucleotides on an automated DNA/RNA synthesizer. For example, synthesis of thymidilyl 10 mer on **1** was achieved in a 97% average coupling yield on an Applied Biosystems model 392 DNA/RNA synthesizer using **2** as the monomer unit and BIT as a promoter.

In conclusion, we describe a novel solid support **1** with a hydroxyallyl linker, which can be regenerated via the procedure outlined in Scheme 1. The success of the support is based mainly on the use of π-allyl–palladium chemistry.[†] This new support has some advantages over the existing reusable support with the Q linker. As described above, the support with the Q linker undergoes partial degradation by the aqueous ammonia treatment for detaching the oligonucleotide product, and thus, for reuse, it is necessary to newly introduce the lost part before every synthesis. By contrast, support **1** is regenerated after every synthesis without loss of any parts, and thus, recovered material can be reused without any chemical modifications.

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[†]Representative reviews: see Refs. [10–16]. See also, Ref. [17].

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