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Microwave-assisted functionalization of solid supports: application in the rapid loading of nucleosides on controlled-pore-glass (CPG)☆

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Abstract—Rapid and efficient functionalization of solid-support by microwave-assisted procedures has been achieved. The functionalized solid-support can be readily loaded with nucleosides for use in oligonucleotide synthesis. This method can also be extended to rapid functionalization of other solid matrices for application in microarrays and combinatorial chemistry.

Over the past two decades, microwave-assisted procedures have been successfully employed in a number of synthetic transformations, resulting in rapid and efficient synthesis of different classes of organic compounds.[1] Several advantages have been claimed in the use of microwave-assisted organic synthesis:[2] (a) ultra fast reaction kinetics, (b) cleaner reactions with improved yields and reduced formation of side products, (c) ability to effect, chemo-, regio-, and stereoselective transformations, (d) flexibility to perform reactions with or without solvents, (e) economical and eco-friendly processes than the corresponding conventional reactions, and (f) successful product formations in reactions that fail under conventional conditions.

Some microwave-assisted reactions pose significant risk of explosion especially when conducted in closed vessels.[2] In such cases, reactions have been conducted following deposition of reactants on solid matrix such as alumina, silica gel, and clay. Interestingly, the supports by themselves were found to absorb very little microwave energy.[3] Examples of reactions carried out on matrix-bound reactants include transesterfication,[4] N-acylation,[5] Ugi four-component condensation,[6] and Suzuki coupling.[7]

However, there are only limited reports of application of microwave-assisted procedures in the solid-phase synthesis of nucleic acids.[1] As is well-known, besides the actual assembly of a polynucleotide on solid support, there are two other critical steps in nucleic acid synthesis[8] (a) preparation of solid support (usually controlled-pore-glass, [CPG]) loaded with the leader nucleoside, and (b) deprotection and cleavage of the assembled, support-bound oligonucleotide. Both steps involve prolonged, labor-intensive operations. Several improvements have been reported to facilitate rapid deprotection and cleavage of oligonucleotides[8] including a recent report of microwave-assisted procedures.[9] In contrast, there are only limited reports of improved procedures for loading of nucleosides on functionalized supports and their utility has not been fully explored.

Reported methodologies[10–12] for functionalization and loading of CPG are time-consuming, labor-intensive, and involve the use of toxic solvents. Figure 1 shows the reaction sequences for functionalization and loading of CPG[13] and involve (Path A): (a) reaction of CPG with 3-aminopropyltriethoxysilane to give the amino-functionalized CPG (b) reaction of aminated support **1** with succinic anhydride to give carboxy-terminated CPG **2**,[14] and (c) reaction of **2** with nucleoside **3** to give the nucleoside-loaded CPG **4**. Alternatively, reaction sequence in Path B has also been employed, but is less convenient especially when modified nucleosides are used because of tedious purification to obtain hemi-succinylated intermediate **5**.[13] Thus, starting from native CPG, complete set of sequence of reactions require 7 to

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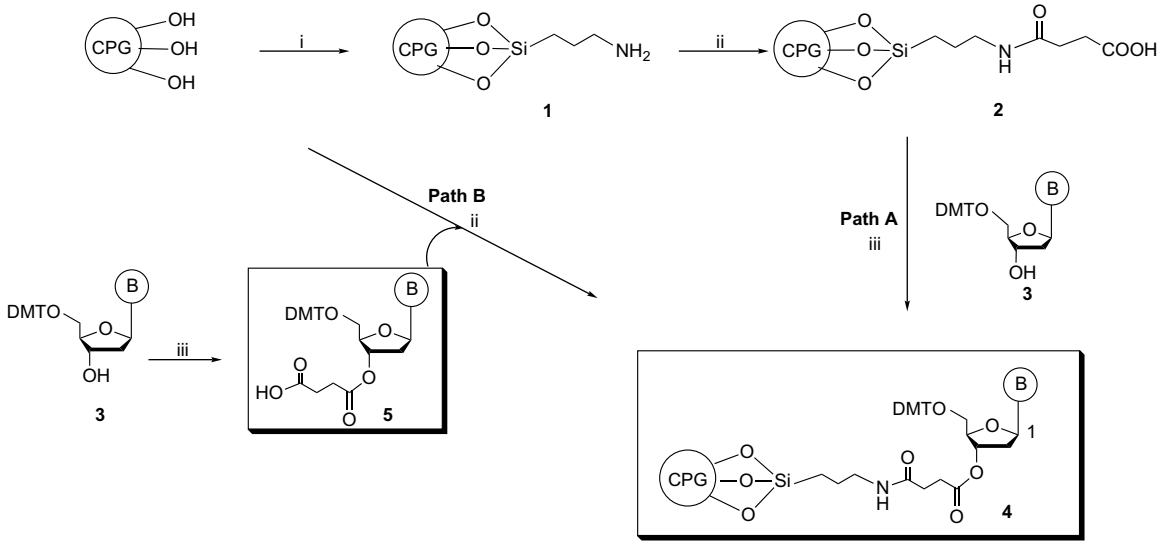

Figure 1. Functionalization and loading of nucleoside on CPG. (i) (3-aminopropyl)triethoxysilane, toluene, 48–72 h, reflux; (ii) succinic anhydride, py, DMAP, 48 h, rt; (iii) *N*-ethyl-*N*′-3-(dimethylaminopropyl)carbodimide hydrochloride (EDC), DMAP, py, rt, 24–48 h.

10 days to obtain nucleoside-loaded CPG **4**. Consequently, availability of nucleoside-bound supports presents a significant bottleneck in the synthesis and manufacture of oligonucleotides.

We report here the use of microwave-assisted procedures for ultra fast functionalization of solid supports that enable rapid loading of nucleosides on solid supports.

1. Microwave-assisted amination (MAA) of CPG

Using reported procedures,[10–12] we attempted the amination of CPG (500 A, Prime Synthesis) by treatment with (3-aminopropyl)triethoxysilane (APTES) in toluene for 48–72 h, followed by capping of unreacted hydroxy groups with trimethylsilyl chloride. Although aminopropyl-CPG **1** could be isolated from the reaction, the heterogeneous amination reaction had to be performed in refluxing toluene and was unmanageable. Also, on some occasions, after capping of unreacted hydroxyl groups with trimethylsilyl chloride, the product isolated was found to be devoid of amino group. This prompted us to investigate MAA of CPG for the preparation of **1** using a domestic microwave oven (800 W, high power setting).

For conducting MAA of CPG, (Fig. 1) a *specially fabricated heavy-walled glass chamber*[†] was employed. Typically, the MAA of slurry of CPG (50–100 g) in APTES gave the aminated product **1** within a few minutes. Following filtration and washing, aminated-CPG **1** with amino-loadings of 90–113 μmol/g was obtained.[‡] In order to ascertain if amino-loading of CPG could be further increased, the initially formed **1** was subjected to MAA with APTES. However, no further increase in loading resulted thereby suggesting that all available hydroxyl groups on the CPG had been functionalized during the first reaction. Thus, amination of CPG was completed within a few minutes under microwave conditions.

Table 1. Effect of solvents on microwave-assisted amination of CPG

Amination reagent	Solvent	Loading (μmol/g)
APTES	DMF	86
	DMSO	116
	Neat	113

We also evaluated the effect of different solvents on the MAA of CPG with APTES, and the results are given in Table 1. Although MAA could be conducted in DMF and DMSO, we found that reaction using slurry of CPG with APTES to be most convenient and also eco-friendly. Since APTES was used in excess in such a protocol, we attempted to use the recovered APTES in a subsequent MAA of CPG. However, MAA of CPG with recovered APTES was unsuccessful, probably due to the contamination of APTES with the liberated ethanol.

In attempts to further increase the amino-loading of CPG, we carried out MAA reactions (all neat) in

[†] The chamber was fitted with Teflon screw cap with a chemically resistant o-ring. *These microwave reactions should not be attempted in common laboratory glassware. All microwave reactions should be carried out behind safety shields.*

[‡] Typically 3.5 mL APTES/g of CPG was employed. In all cases, the desired aminopropyl CPG was isolated after washings with toluene, methanol, dichloromethane, and hexanes. Amino-loading was determined by standard protocol[13] by treating a known amount of support with DMTrCl and $Bu_4N^+ClO_4$ and 'trityl assay'[13] was carried out on the resulting DMTr derivative.

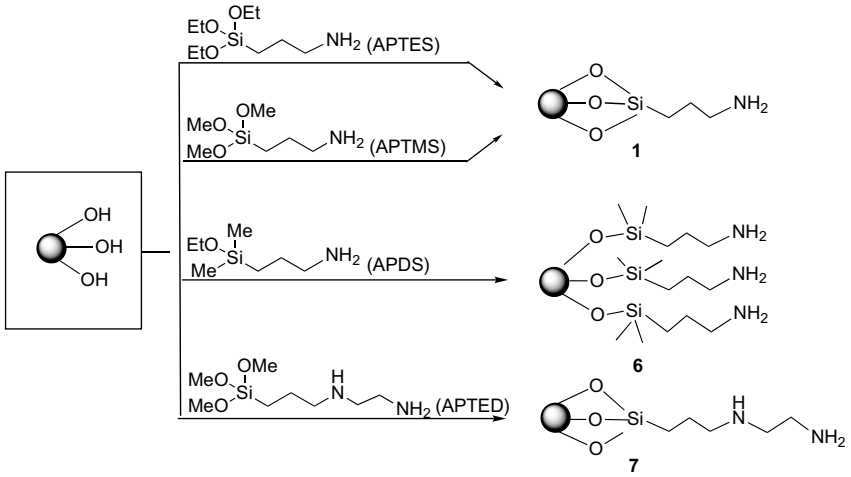

Figure 2. Microwave-assisted amination of CPG with various amination reagents.

conjunction with other amination agents such as (3-aminopropyl)trimethoxysilane (APTMS), and *N*-(3-trimethoxysilylpropyl)ethylenediamine (APTED) (Fig. 2). Although the corresponding aminated CPG **1** and **7** were obtained, loading was not increased beyond that obtained with APTES. It is believed that amination involves the condensation of three hydroxyl groups on the CPG matrix, with the three ethoxy groups of APTES to form **1**. We rationalized that if each of the hydroxyl groups of CPG could be engaged in reaction with a single molecule of a monoethoxysilane derivative, for example, 3-aminopropyldimethylsilane (APDS), amino-CPG **6** with increased amino-loading could result. However, although MAA reaction of CPG with (3-aminopropyl)dimethylsilane (APDS) gave the corresponding aminated product **6**, the loading was not increased beyond 96 μmol/g (Table 2).

In another experiment, following the first MAA with APDS, the resulting amino-CPG was further subjected to a second MAA using APTES, but there was no further improvement in loading suggesting that all available sites on the CPG had been functionalized at the first reaction itself as before.

We also evaluated the MAA of CPG with APTES using different additives (Table 3). Best results were obtained with *p*-toluene sulfonic acid and TFA giving **1** with amino loadings of 128 and 116 μmol/g, respectively. With boron trifluoride etherate as a catalyst, the product **1** had considerably reduced loading (~40 μmol/g).

CAUTION: *During the MAA of CPG with APDS, in the presence of tin (IV) chloride as a catalyst, an explosive reaction resulted.* It is interesting to note that various metallic halides such as $AlCl_3$,[15] CuBr,[16] $FeCl_3$,[17] $BiCl_3$,[18] $ZnCl_2$,[19,20] $InCl_3$,[21] and $TaCl_3$[22] have been apparently safely employed in microwave-assisted synthetic reactions. Our results suggest that additives and solvents influence the amino loading on CPG and hence the final loading of nucleosides. Further investigation is in progress to prepare amino-CPG with a predetermined loading level.

Table 2. Loading of CPG obtained using microwave-assisted amination

Amination reagent	Reaction conditions	Amino-loading (μmol/g)
EtO, OEt, Si, EtO, NH2 (APTES)	Condition (i), from Figure 1	73
EtO, OEt, Si, EtO, NH2	MAA, 5 min	98–113
MeO, OMe, Si, MeO, NH2 (APTMS)	MAA, 5 min	110
EtO, Me, Si, Me, NH2 (APDS)	MAA, 5 min	96
MeO, OMe, Si, MeO, H, N, NH2	MAA, 5 min	79

2. Microwave-assisted succinylation (MAS) of aminated CPG (1) with succinic anhydride

Encouraged by the success in MAA of CPG, the MAS of the functionalized support **1** (Fig. 1) were attempted under microwave conditions. Microwave-assisted reaction of **1** with succinic anhydride, without the aid of any solvent, resulted in a very dark yellow colored support, probably due to the formation of imide rather than the expected acid. Nevertheless, MAS of **1** (50–100 g scale) was achieved successfully to give **2**, in the presence

Table 3. Effect of additives on microwave-assisted amination of CPG

Reagent	Additive	Loading (μmol/g)
APTES	$BF_3{\cdot}Et_2O$	45
	PTSA	128
	TFA	116
	DMAP	110

of catalytic amount of DMAP in DMF as solvent, in less than 5 min.[§]. Completion of reaction was ascertained by testing for the absence of amino group on a sample of **1**. The use of DMF in place of pyridine under microwave conditions makes MAS procedure highly attractive for the preparation of succinylated CPG **2**. In addition to DMF, the succinylation can also be carried out using DMSO, dimethylacetamide, or CH_3CN.

3. Coupling of nucleoside to the succinylated CPG (2)

With the succinylated support **2** in hand, we carried out the loading of nucleosides using EDC, DMAP, and TEA (Fig. 1). When **2** was mixed with 5′-*O*-DMT-deoxyadenosine (**3**, B = dA), DMAP, TEA, and EDC (in that order) in anhydrous pyridine and shaken in an orbital shaker, CPG **4** with high nucleoside loadings of 70–75 μmol/g was obtained. Similar results were obtained with 5′-*O*-DMT-T (**3**, B = T).

In separate experiments, we have found that anhydrous DMF could be used instead of pyridine for the loading of nucleosides on **2**. The use of DMF provides an eco-friendly alternative to pyridine. In addition, high loading of nucleoside (high-loaded CPG, **4**) could be achieved when loading was carried out in a specially fabricated reactor, mounted on a rotary shaker, and with added provision for recycling of reactants.[¶] Thus, using recycling approach, significantly less molar excess of nucleoside could be used to achieve the same loading levels as compared to the conventional protocol. This could be due to efficient mixing of the solid and liquid phases brought about by the rotary motion in the reactor in conjunction with recycling process.

Microscopic examination revealed that microwave exposure did not affect the porosity, particle size or other physical characteristics of the CPG. To confirm this, automated synthesis of polynucleotides (using Expedite 8909 synthesizer) was carried out as below:

4. Synthesis of oligonucleotides

The CPG-loaded nucleoside **4** prepared as above and the corresponding CPG prepared by conventional method[10–13] was employed in the 10 μmol synthesis of di-, and 20-mer oligonucleotide (PO and PS) in an Expedite Synthesizer using phosphoramidite chemistry.[8] In both instances, the stepwise coupling yields was greater than 98% (as ascertained by trityl analysis). Following the synthesis, each of the CPG was treated with 28% NH_4OH at 55 °C for 12 h to isolate the fully deprotected di-, and polynucleotides. RP-HPLC analysis of crude mixture showed the profile of compounds prepared using both supports were similar.

In conclusion, a microwave assisted protocol for rapid and efficient functionalization of CPG has been reported whereby *CPG* **2** *carrying a carboxy-terminus could be obtained from native CPG within few hours, in contrast to the conventional procedures, which required several days*. Efficient processes for loading of nucleosides on the resulting functionalized support **2** are also described using anhydrous DMF as a solvent. Furthermore the use of a novel reactor in conjunction with recycling technology enables efficient loading of the nucleoside on support.

The methodologies described here can be applied for functionalization of other solid supports[23] and loading of supports with nucleosides, amino acids, small molecules, etc. for solid-phase synthesis of oligonucleotides, peptides, and ligands, respectively. The approach described here can also be potentially employed for rapid functionalization of other solid matrices in the form of beads, slides, pins for application in microarrays, combinatorial chemistry including medical diagnostics, environmental clean up (removal of toxic materials), Radio immunoassays, Fluorescent Immunoassays, ELISA, and Affinity Chromatography.

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[§] Typically 0.4 g of succinic anhydride and 50 mg of *N*,*N*-dimethylaminopyridine each per 1 g of aminated CPG was employed for MAS. Since this reaction was exothermic, each microwave exposure was carried out in 30 s cycles with intermittent cooling. After the reaction, the colored slurry was filtered, washed with dichloromethane, methanol, and hexanes, and dried. Similar results were obtained with Tentagel and PEGA resins.

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