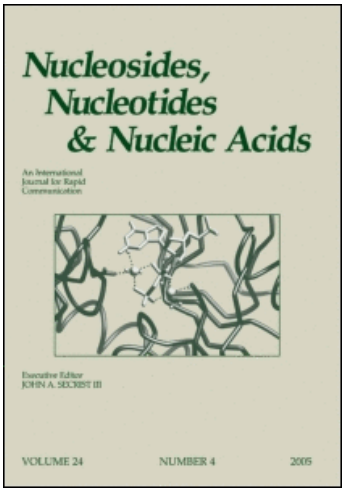

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Microwaves Synthesis of Solid Supports for the Synthesis of 3'-Aminoalkyl Oligodeoxynucleotides

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MICROWAVES SYNTHESIS OF SOLID SUPPORTS FOR THE SYNTHESIS OF 3′-AMINOALKYL OLIGODEOXYNUCLEOTIDES

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▫ *Phthalimido-alkyl alcohol solid supports were rapidly prepared from solid supported phthalic anhydride and amino alcohol condensation induced by microwaves. These supports were used to synthesize 3′-aminoalkyl oligodeoxynucleotides allowing a two step deprotection necessary to avoid aminolink alkylation.*

INTRODUCTION

3′-Aminoalkyl oligodeoxynucleotides (ODN) are required for labelling with fluorescent dyes or other molecules by means of their activated ester or also for the preparation of DNA arrays. ODN with 3′-amino alkyl arms are conveniently prepared through standard solid-phase synthesis on a 3′-amino protected support which releases the free 3′-amino during the final deprotection.[1–6] The 3′-amino support based on an immobilized phthalimido protective group is particularly attractive since it releases an enantiomerically pure ODN as compared to some amino link[2] giving rise to resolvable diastereoisomers and it cannot react with acetic anhydride during the capping step of the elongation process. Unfortunately its preparation requires three steps to obtain the paranitrophenyl active ester in 47%

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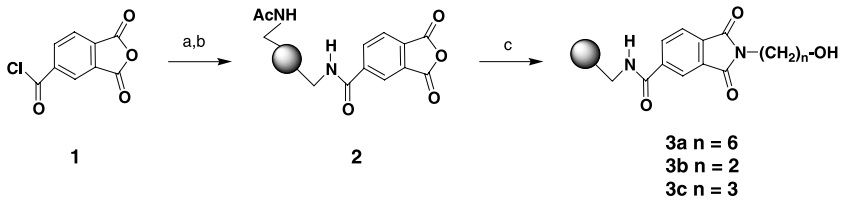

SCHEME 1 Synthesis of phthalimido alkyl solid support. a: LCAA CPG, 1,2-anhydrotrimellitic chloride, DCM, DIEA 3 h; b: CAP A: Ac_2O, pyridine, THF (10/10/80) + CAP B: 10% NMI, THF 2 h; c: amino alcohol, DMF, MW 150 s.

yield which then poorly reacts with LCAA CPG (Long Chain Amino Alkyl Controlled Pore Glass) to give a loading of only 20 μmol/g.[3]

In this communication, we report a very convenient, rapid and efficient synthesis of the phthalimido hexamethylene alcohol solid support. Then, this method has been extended to phthalimido-ethyl and-propyl alcohols. All the steps have been carried out using commercially available reagents on solid support allowing easy work up (*i.e.*, filtration and washes). The solid supports **3a–c** were prepared in two steps (Scheme 1). The first step was the reaction of 1,2-anhydrotrimellitic acid chloride **1** (0.3 mmol) on CPG-LCAA (1 g) to yield the phthalic anhydride CPG **2** within 3 h.[5,7] The resin was filtered off, washed, and dried. Unreacted amines were capped with commercial Cap A and Cap B solutions for 2 h. The resin was filtered off, washed, and dried. The key step was the formation of a phthalimido function which was performed under microwaves irradiation (MW). It should be noted that a partial reaction yielding an amide linkage must be avoid since its hydrolysis under basic condition is very long ($t_{1/2}$ = 17,500 h at 0.1 M NaOH at 30°C).[8] Reactions of phthalic anhydride with amine occurs with or without solvent by conventional heating (175–225°C)[3] or by MW.[9,10] Note that the reaction is chemoselective since it occurs specifically with the amino moiety. In addition it was reported that reaction of phthalic anhydride with glycine exhibits the same rate when it occurred by MW or conventional heating in DMF at the same temperature.[10] Theoretically, since 6-amino-1-hexanol is a solid with a low melting point (56–58°C), it could be used without solvent. In our case, to optimize the interaction between the solid supported phthalic anhydride and the 6-amino-1-hexanol, and later with the other amino DMF was selected as solvent. Reactions were performed with 0.3 mmol of amino alcohol per gram of solid supported phthalic anhydride **2** in 5 mL of dry DMF.

The reaction was first performed using a domestic MW oven (650 W full power) by 6 × 20 s with a cooling of the mixture between two runs (to avoid bumping and spillage). yielding the expected solid support **3a** with a loading of 49 μmol per gram of resin (Table 1, entry 1). Initially, the loading was determined by dimethoxytritylation of a support sample followed by its detritylation and assay of the trityl cation at 498 nm. This method was not expedient because it was time consuming and poorly reproducible. Thus a coupling with commercial thymidine

TABLE 1 Reagents and Conditions for the Preparation of Phthalimido Alkanols Solid-Support **3a–c** Using Microwaves

Entry	Reagents	Time (s)	Temperature or PW	Solid support & loading
1	**2**+ $NH_2(CH_2)_6OH$	120 (6 × 20 s)	650 watts	**3a** 49 μmol/g
2	**2**+ $NH_2(CH_2)_6OH$	150	200°C	**3a** 62 μmol/g
3	**2**+ $NH_2(CH_2)_6OH$	300	200°C	**3a** 59 μmol/g
4	**2**+ $NH_2(CH_2)_2OH$	150	150°C	**3b** 67 μmol/g
5	**2**+ $NH_2(CH_2)_2OH$	400	200°C	**3b** 75 μmol/g
6	**2**+ $NH_2(CH_2)_3OH$	150	150°C	**3c** 59 μmol/g
7	**2**+ $NH_2(CH_2)_3OH$	400	200°C	**3c** 57 μmol/g

cyanoethyl diisopropylphosphoramidite was preferred and the loading was determined from the cation released during a detritylation step. This method is fast and gives reproducible values. The reaction was ultimately performed in sealed tube using a microwave synthesizer (explorer CEM) setting the temperature at 200°C, two reaction times (150 and 300 s) were tested (Table 1, entries 2 and 3). Whatever the time used, a similar loading was obtained around 60 μmol/g demonstrating that reaction is fast and efficient. The reaction was extended to ethanolamine and 3-amino-1-propanol. Using a microwave synthesizer soft conditions (150°C for 150 s, entries 4 and 6) and harder ones (200°C for 400 s, entries 5 and 7) were applied. For ethanolamine the loading was higher with the "hard conditions." In contrast, no significant difference of loading was found for the 3-amino-1-propanol whatever the conditions used. In all the cases a high loading was obtained between 57 to 75 μmol/g. The use of a microwave synthesizer for the preparation of the phthalimidoalkanol solid support is convenient and gives reproducible results and high loading. However this solid support could also be prepared with a domestic MW oven with a lower but still high loading (49 μmol/g).

To evaluate the properties of the resins, few short ODN T_6-NH_2 were synthesized on these various supports (Figure 1A) and for each the same trend was observed in the HPLC profiles. Longer ODNs were synthesized as T_{15}-C_3-NH_2

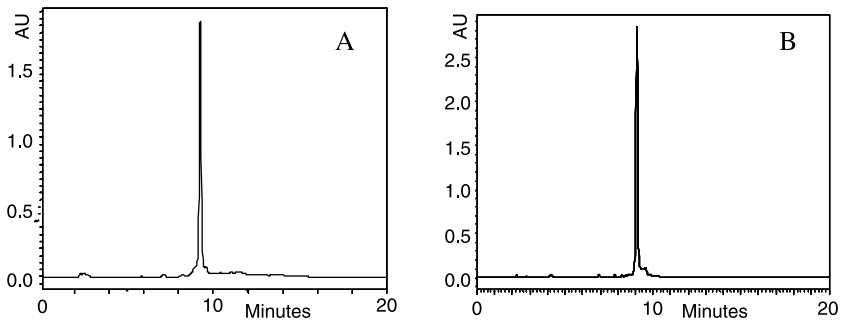

FIGURE 1 Crude HPLC profiles of (A) T_6-$(CH_2)_6$-NH_2 and (B) T_{15}-$(CH_2)_3$-NH_2 on column C_{18} nucleosil, gradient 5 to 36% acetonitrile in 50 mM TEAAc in 20 min.

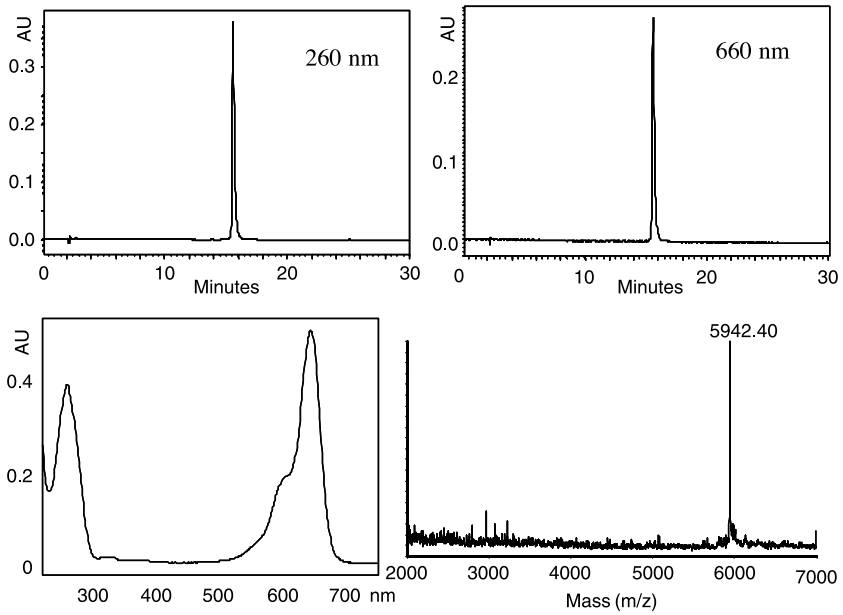

FIGURE 2 HPLC profiles at 260 and 660 nm of purified Cy5-GCG AAA AAA AAA AGC TC-$(CH_2)_6$-NH_2, UV/visible spectrum and MALDI-TOF mass spectrometry.

(Figure 1B) and also an hetero ODN [$^{5'}$Cy5-d(GCG AAA AAA AAA AGC $TC_{3'}$-$(CH_2)_6$-NH_2)], labelled with the cyanine Cy5 on its 5′ position (Figure 2). The syntheses were carried out on 1 μmol scale using 15 eq of phosphoramidite and regular reagents on an ABI 394 DNA synthesizer. The first detritylation step was skipped. The average yield was better than 98.5%. ODNs were deprotected by a two steps procedure. First cyanoethyl groups were removed by a piperidine treatment[11] (10% piperidine in acetonitrile for 10 min at room temperature and washing) to remove the resulting acrylonitrile that could react further with the amino function. Then on the partially deprotected ODN still attached to the CPG, a treatment with conc. ammonia at 55°C for 17 h for unmodified ODN or 16 h at room temperature for the Cy5 ODN yielded the fully deprotected 3′-amino ODN in solution* (Figures 1 and 2). With this method we prepared easily (within a day) phthalimido alkyl alcohol on CPG† for the synthesis of 3′ amino-alkyl-ODN.

*MALDI-TOF MS analysis: negative mode THAP as matrix T_6-C_6NH_2 m/z for $C_{66}H_{92}N_{13}O_{43}P_6$: Calc. 1941.38 Found 1941.82 T_{15}-C_3NH_2 m/z for $C_{153}H_{203}N_{31}O_{106}P_{15}$: Calc. 4637.08 Found 4633.78 Cy5-17mer-C_6NH_2: m/z for $C_{204}H_{258}N_{79}O_{98}P_{18}$: Calc. 5941.33 Found 5942.40.

†LCAA-CPG (1 g), pretreated 2 h with 3% TCA in CH2Cl2, was gently shaken with 63.2 mg (0.3 mmol) 1,2-anhydrotrimellitic chloride, 105 mL (0.6 mmol) diisopropyl-ethylamine, in 5 mL of CH2Cl2 for 3 h. The resin was filtered off, washed with CH_2Cl_2, ether, and dried under vacuum. The resulting resin was mixed with 0.3 mmol of amino alcohol in 5 mL of DMF and heated in a microwave synthesizer setting at 300 W and 200°C for 150 s or in a domestic MW oven at 650 watt for 6 × 20 s. The resin was filtered off, washed with CH_2Cl_2, ether, and dried.

CONCLUSION

Using this procedure, all the steps were performed on solid supports that allow rapid and easy workups. The use of MW speeds up the reaction and a new solid support was prepared in less than one day using inexpensive, commercially available reagents.

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