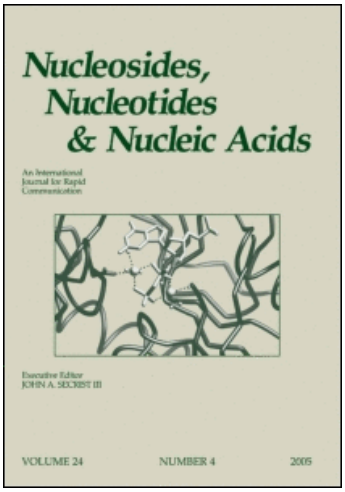

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MICROWAVE ASSISTED HIGH YIELDING PREPARATION OF *N*-PROTECTED 2′-DEOXYRIBONUCLEOSIDES USEFUL FOR OLIGONUCLEOTIDE SYNTHESIS

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ABSTRACT

A rapid and high yielding method for the synthesis of precursors of synthons for DNA synthesis, *N*-protected 2′-deoxyribonucleosides is described, which occur under mild conditions using microwave irradiation. The desired material, *N*-protected nucleosides, was obtained in 93–96% yield in few minutes. The final products were then characterized by ^{1}H-NMR and MALDI-TOF and compared with the standard samples. The method is amenable to small to moderate scale of synthesis.

INTRODUCTION

Synthetic oligonucleotides are finding numerous applications in the field of molecular biology, genetics and other related areas. These are required in minute (micrograms) to large quantities depending upon the type of applications. In particular, the potential of synthetic oligonucleotides as future

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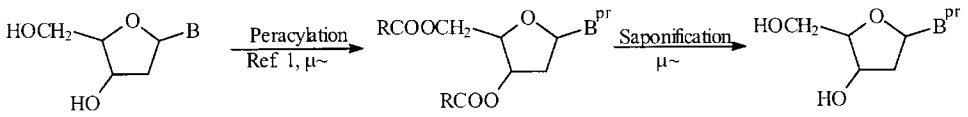
drugs has thrown new challenges before the organic chemists to develop rapid and economical processes for the synthesis of these molecules. *N*-Protected nucleosides are one of the key intermediates in the preparation of synthons required for oligonucleotide synthesis. Basically, two approaches are being followed for the synthesis of *N*-protected nucleosides. The first one, developed by Khorana et al.,[1,2] is based on peracylation of nucleosides with an appropriate acylating reagent followed by selective hydrolysis of ester functions to obtain the desired *N*-acylated nucleosides in (60–70%) yields. In the other protocol, Jones et al.[3] have proposed a new approach based on 'transient protection' for the synthesis of these molecules. The method involves the use of trimethylsilyl chloride for transient protection of hydroxyl groups of nucleosides followed by *in situ* protection of exocyclic amino functionalities with an acylating reagent. The reaction proceeds in one pot and yields are generally high. However, large-scale synthesis following this protocol is not economical, as it involves the use of large excess of the reagents and yields are not as high as one expects at small scale. In another modified method, McGee et al.[4] have reported one pot, high yielding process for the synthesis of *N*-isobutyryl-2′-deoxyguanosine based on the transient protection approach. All of these methods[1–4] are either quite time consuming or requiring a large excess of the reagents, making them economically unviable at large scale synthesis.

Recently, a number of researchers have reported organic reactions under microwaves.[5–14] The yields obtained are generally high in relatively shorter times. The technique has been used both in the presence of solvent as well as in dry conditions using different types of supporting materials, viz., silica gel, alumina, montmorillonite etc. These reactions are carried out in polar solvents having high dielectric constant as well as boiling points, such as, dimethylformamide (DMF), dimethylacetamide (DMA), acetic acid (AcOH), *N*-methylpyrrolidone (NMP).

In this communication, we describe a rapid and economical protocol, which utilizes the fast reaction kinetics under microwave irradiation to obtain *N*-protected nucleosides in shorter times as well as in high yields.[15] Both the steps, i.e. peracylation and saponification, were carried out under microwaves. Selective protection of 2′-deoxycytidine was also achieved in higher yield.

RESULTS AND DISCUSSION

The demand of synthetic oligonucleotides is growing at exponential rate for various biological applications where these are required in large numbers as well as in large quantities. In order to meet this growing demand of synthetic oligonucleotides, it is necessary that suitable methods be developed to synthesize *N*-protected-2′-deoxynucleosides, precursors of building blocks



R = benzoyl (bz) for dA / dC and isobutyryl (ibu) for dG
pr = protection (bz for A / C and ibu for G)
μ~ = microwave

Scheme 1. Scheme for the preparation of *N*-protected-2′-deoxyribonucleosides.

used for oligonucleotide synthesis, rapidly and economically. In the proposed study, the following points were kept in mind, (a) the process should be straightforward and high yielding, (b) it should not involve specialized chemicals and reagents as well as equipments, and (c) the synthesized products must match the corresponding standard *N*-protected-2′-deoxynucleosides.

Keeping these factors into consideration, a rapid and high yielding process has been developed for the preparation of *N*-protected-2′-deoxyribonucleosides under microwaves. The method involves a two step process. The first one involves *N*- and *O*-acylation of 2′-deoxyribonucleosides (Scheme 1) followed by the second one, i.e. saponification, to yield the desired *N*-protected-2′-deoxyribonucleosides.

2′-Deoxycytidine was *N*- and *O*-acylated with benzoic anhydride (5 eq.) in dimethylformamide in the presence of an acylating hypernucleophilic catalyst, DMAP, under microwaves. The progress of the reaction was monitored on tlc and no exposure was given longer than 8s (525 W). After every exposure, the contents were allowed to cool to room temperature to avoid overheating and evaporation of the solvent. Reaction proceeded almost quantitatively. After usual work-up, the desired *N*- and *O*-protected-2′-deoxycytidine was isolated and compared with the standard sample.

The other two deoxyribonucleosides were also peracylated under identical conditions except that benzoic anhydride (7.5 eq.) in case of deoxyadenosine and isobutyric anhydride (10 eq.) in case of deoxyguanosine were employed. The fully protected compounds were compared with respect to their R_fs on tlc with standard samples.

The next step involves the selective saponification of peracylated nucleosides. The optimum concentration of the alkali for saponification was determined following kinetic studies under microwaves. The above mentioned step was found to be complete in few minutes in a homogeneous mixture of pyridine:methanol:water (1:1:1, v/v). The proposed method was studied at three different scales, viz., 0.5, 10 and 25 mmol. The irradiation time required under microwaves in each case was found to be different for selective saponification. After complete saponification, as monitored on tlc, the reaction mixture was neutralized followed by evaporation of the solvent.

Table 1. ^{1}H-NMR and Mass Spectra of *N*-Protected-2′-deoxyribonucleosides

N-Protected-2′-Deoxyribonucleosides	^{1}H-NMR (DMSO-d^{6}) δ (ppm)	MALDI-TOF (m/z); matrix: 2,5-dihydroxybenzoic acid
N-Benzoyl-2′-deoxycytidine	3.7 (m, 3H), 3.9 (m, 1H), 4.3 (m, 1H), 6.1 (m, 1H), 7.3–8.0 (m, 5H), 8.1 (s, 1H), 8.4 (d, 1H)	332 (M+H) (Expected 331)
N-Benzoyl-2′-deoxyadenosine	1.25 (s, 1H), 2.5 (s, 1H), 3.6 (m, 2H), 4.1 (s, 1H), 4.3 (d, 1H), 6.5 (m, 2H), 7.3–7.9 (m, 5H), 8.1 (d, 1H)	356.6 (M+H) (Expected 355)
N-Isobutyryl-2′-deoxyguanosine	1.2 (d, 6H), 2.3 (m, 1H), 2.6 (m, 1H), 2.8 (m, 1H), 3.7 (m, 2H), 6.3 (t, 1H), 8.1 (s, 1H)	338.5 (M+2H) (Expected 336)

The residual mass was triturated with diethylether to get rid of acidic impurities (benzoic acid released from peracylated deoxyadenosine and deoxycytidine and isobutyric acid from deoxyguanosine). Inorganic impurities including the remaining sodium benzoate, isobutyrate or acetate present in the product was taken care of by a silica gel column chromatography. The eluted fractions containing desired products were pooled together and concentrated on a rotary evaporator to obtain the desired *N*-protected-2′-deoxyribonucleosides in high yields. These compounds were characterized by ^{1}H-NMR and MALDI-TOF (Table 1) and compared with the corresponding standard sample of *N*-protected-2′-deoxyribonucleosides. Figure 1(a,b,c) shows mass spectra of *N*-protected-2′-deoxyribonucleosides.

Taking advantage of the better nucleophilicity of exocyclic amino group of cytidine, a simple and rapid method has also been developed for its selective protection under microwaves. An overall yield of 84% of *N*-benzoyl-2′-deoxycytidine was obtained in just 125s.

EXPERIMENTAL PART

General: All solvents and reagents used in the present investigation were purified prior to their use. Benzoic anhydride, isobutyric anhydride, diisoproylethylamine and 4-dimethylaminopyridine were obtained from Sigma Chemical Company, USA. 2′-Deoxyribonucleosides (dA, dC, dG) were purchased from Yuki Gosei Kogyo Co., Japan. Other solvents and reagents were procured from local suppliers.

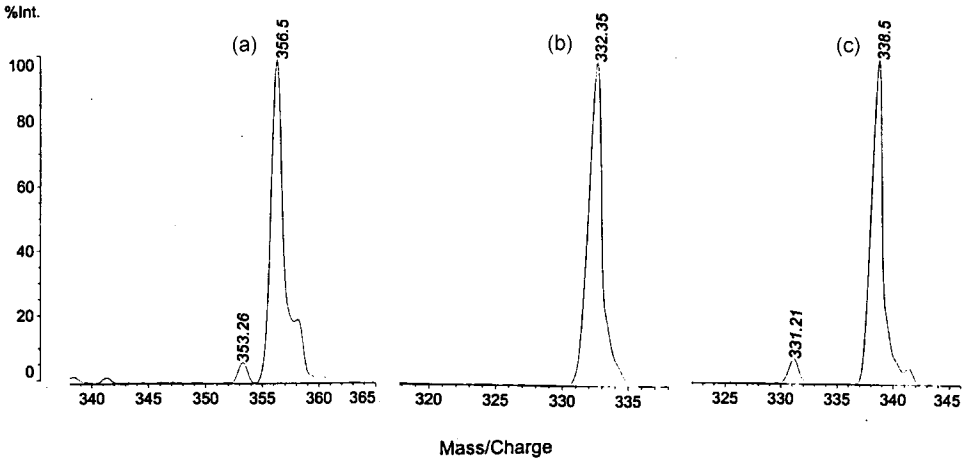


Figure 1. MALDI-TOF spectra of (a) N-benzoyl-dA, (b) N-benzoyl-dC and (c) N-isobutyryl-dG. Matrix used: 2,5-dihydroxybenzoic acid; Laser power : 104; Mode : Linear; positive ion.

The domestic microwave oven (BPL, India, 800 W) was used in the present study. The oven was set at 525 W (2450 MHz for each reaction) and the reaction mixture was irradiated for a particular duration, followed by cooling down the reaction mixture to room temperature and re-irradiated until the reaction was found to be complete on tlc. The frequency and duration of exposures are mentioned in the parenthesis against each reaction.

N-Protected-2′-deoxyribonucleosides prepared in the present investigation were characterized by ^{1}H-NMR (300 MHz, Bruker-Avance DPX 300) and MALDI-TOF (SEQ IV, Kratos, UK, Nitrogen laser operating at 337 nm). Standard samples of *N*-protected 2′-deoxyribonucleosides were prepared following either of the reported procedures [1–4].

Synthesis of *N*-Benzoyl-3′, 5′-Di-*O*-benzoyl-2′-deoxycytidine

2′-Deoxycytidine (2.27 g, 10 mmol) was taken in a conical flask and suspended in 25 mL of anhydrous N,N-dimethylformamide (DMF). Diisopropylethylamine (8.71 mL, 50 mmol) and 4-dimethylaminopyridine (DMAP) (610 mg, 5 mmol) were added to the reaction flask followed by the addition of an acylating reagent, benzoic anhydride (11.3 g, 50 mmol). The reaction mixture was irradiated for 64s (8×8s). The reaction was monitored on tlc after each exposure till completion. The solvent was removed on a rotary evaporator under vacuum to obtain a syrupy material, which was taken up in chloroform (100 mL) and subjected to washings with cold 5% aq. sodium bicarbonate (4×50 mL) and water (2×50 mL), respectively. The organic phase was separated, dried over anhydrous sodium sulfate and concentrated to get the desired *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxy-

cytidine in almost quantitative yield (5.28 g, 98%). The sample was compared with the authentic sample with respect to Rf value on tlc.

2′-Deoxyguanosine (10 mmol) and 2′-deoxyadenosine (10 mmol) were also derivatized in similar manner except the following changes. In case of 2′-deoxyguanosine, the acylating reagent was isobutyric anhydride (16 mL, 100 mmol) with irradiation time of 120s (15×8s), while 2′-deoxyadenosine required benzoic anhydride (16 g, 75 mmol) and irradiation time of 96s (12×8s). The fully protected nucleosides were obtained in 97% and 98%, respectively. The triacylated deoxyribonucleosides were compared with standard samples.

General Procedure for Synthesis of *N*-Protected-2′-deoxynucleosides

The peracylated material, *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxycytidine, (5.39 g, 10 mmol), was dissolved in a mixture of pyridine (100 mL) and methanol (100 mL) and 0.3 N sodium hydroxide (100 mL, 30 mmol) in a conical flask and subjected to irradiation under microwaves. The progress of the reaction was monitored on tlc. The reaction was found to be complete in 36s (6×6s). The reaction mixture was then neutralized by adding glacial acetic acid (1.8 mL) and the resulting solution concentrated to a syrupy mass, which was triturated with diethyl ether (4×50 mL) to get rid of benzoic acid. The residual mass, containing the desired material and the inorganic salts, such as sodium acetate and sodium benzoate, was dissolved in methanol (100 mL) and adsorbed slowly on to a silica gel (100 g) column packed in methanol. The column was eluted slowly with methanol and the fractions containing the desired product were pooled together. The title compound was obtained by concentrating the methanol solution in 96% yield (3.17 g). The title compound was characterized by ^{1}H-NMR and MALDI-TOF (matrix assisted laser desorption ionization-time of flight).

Similar procedure was used for 2′-deoxyadenosine and 2′-deoxyguanosine derivatives (10 mmol of each) except the following changes. The irradiation time required for the completion of hydrolysis of 3′,5′-diester functions of 2′-deoxyadenosine and 2′-deoxyguanosine were 24s (4×6s) and 30s (5×6s), and the yields were found to be 95.8% and 93.4%, respectively after passing through silica gel column. These were also characterized by ^{1}H-NMR and MALDI-TOF.

Large Scale Preparation of *N*-Protected-2′-deoxynucleosides (25 mmol)

The selective hydrolysis of 3′,5′-diester functions in *N*-acyl-3′,5′-di-*O*-acyl-2′-deoxynucleosides at 25 mmol was achieved using the following conditions. In case of *N*-isobutyryl-3′,5′-di-*O*-isobutryl-2′-deoxyguanosine,

0.05M sodium hydroxide was used with irradiation time of 54s (6×9s), *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxycytidine required 0.1 M sodium hydroxide concentration with irradiation time of 36s (4×9s) and 0.05 M sodium hydroxide was employed for *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxyadenosine with irradiation time of 27s (3×9s). The yields of the *N*-protected nucleosides were, however, the same as obtained at 10 mmol scale.

Small Scale Preparation of *N*-Protected-2′-deoxynucleosides (0.5 mmol)

Selective hydrolysis of 3′,5′-ester functions in *N*-acyl-3′,5′-di-*O*-acyl-2′-deoxynucleosides at 0.5 mmol was achieved using the following conditions. In case of *N*-isobutyryl-3′,5′-di-*O*-isobutryl-2′-deoxyguanosine, 0.3M sodium hydroxide was used with irradiation time of 30s (5×6s), *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxycytidine required 0.2 M sodium hydroxide concentration with irradiation time of 12s (2×6s) and 0.3 M sodium hydroxide was employed for 36s (6×6s) for *N*-benzoyl-3′,5′-di-*O*-benzoyl-2′-deoxyadenosine. The yields of the *N*-benzoyl-2-deoxyadenosine (dA^{bz}), *N*-isobutyryl-2′-deoxyguanosine (dG^{ibu}) and *N*-benzoyl-2′-deoxycytidine (dC^{bz}) nucleosides were obtained in 98%, 98.5% and 98%, respectively.

Selective *N*-Benzoylation of 2′-Deoxycytidine

2′-Deoxycytidine (2.27 g, 10 mmol) taken in pyridine (40 mL) in a conical flask was charged with diisopropylethylamine (3.48 mL, 20 mmol) and benzoic anhydride (5.65 g, 25 mmol). The reaction mixture was subjected to microwave irradiation (800 W). The progress of the reaction was monitored on tlc plate after each exposure. The reaction was found to be complete in 125s (5×25s). The mixture was then concentrated on a rotary evaporator to obtain a syrupy mass, which was taken up in water (100 mL) and extracted with dichloromethane (3×50 mL). The organic phase was separated and discarded. The aqueous phase was concentrated to obtain the title compound in 84% yield.

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