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Brief Communication

Fast Deprotection of Synthetic Oligodeoxyribonucleotides Using Standard Reagents under Microwave Irradiation

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ABSTRACT

Fast methods for the removal of permanent amide exo-cyclic protective groups widely used in phosphoramidite-method DNA synthesis are desirable for many genomics and proteomics applications. In this communication, we present a method for the deprotection of a range of N-acyl deoxyribonucleosides (T, dA^{Bz}, dC^{Bz}, dC^{Ac}, dG^{ibu}, dG^{PAC}) and synthetic oligodeoxyribonucleotides, ranging in length from 5-mer to 50-mer. Oligodeoxyribonucleotides were synthesized using standard amide protecting groups (dA^{Bz}, dC^{Bz}, dG^{ibu}) and phosphoramidite chemistry on *cis*-diol solid phase support. This deprotection method utilizes 29% aqueous ammonia solution at 170°C for 5 minutes under monomode microwave irradiation at a 20-nmole reaction scale. Reaction products were analyzed by TLC, RP-HPLC, CE, ESI-MS, real-time PCR, agarose gel electrophoresis, and by DNA uracil glycosylase (UDG) and phosphodiesterase I (PDE) enzymatic digestions.

INTRODUCTION

SINCE THE ACCOMPLISHMENTS of whole genome sequencing at the turn of this millennium and the large-scale use of oligodeoxyribonucleotides (ODNs) as sequencing aids, probes, primers, diagnostics, and continuing with their application as therapeutics, there exists a large-scale need for synthetic ODNs. Although synthesis is now automated, the removal of permanent N-acylamido protecting groups largely remains a tediously slow and manual task (Ti et al., 1982; Caruthers et al., 1987; Sonveaux, 1994). This dichotomy is at odds with the needs for ODN library preparation.

Numerous reports have sought to address the challenge through the development of more labile exocyclic amine protecting groups or the development of particular reagents or reaction conditions to enhance the removal of established protecting groups. The standard N-protective groups (dA^{Bz}, dC^{Bz}, dG^{ibu}) are widely used in ODN library synthesis and the typical deprotection conditions for these N-acylamido protective groups as set forth by Khorana et al. (Schaller et al., 1963) require a long reaction time (17 hours) at 55°C temperature.

Alternative methods utilizing more labile and, consequently, less stable and more expensive N-protective groups have been described (McBride et al., 1986;

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Schulof et al., 1987; Uzanski et al., 1989; Vu et al., 1990; Vinayak et al., 1992; Sinha et al., 1993; Theisen et al., 1993; Reddy et al., 1994; Chen et al., 2000; Ferreira et al., 2004; Ferreira and Morvan, 2005). Even with these, deprotection can still necessitate lengthy exposure to 29% aqueous ammonia solution, the standard hydrolytic reagent (Schulhof et al., 1987; Vu et al., 1990; Vinayak et al., 1992; Boal et al., 1996).

Efforts have also been placed into the development of new reagents for deprotection. These include variations on the theme of ammonia gas or ammonia solutions in ethanol or methylamine (Vinayak et al., 1992; Reddy et al., 1994, 1995; Boal et al., 1996; Iyer et al., 1997), alkali metal hydroxide-based reagents (Köster et al., 1981; Surzhikov et al., 2000; Kumar et al., 2002; Kumar and Gupta, 2003), or even more complex mixtures, such as hydrazine:ethanolamine:methanol (Polushin et al., 1994). However, many biological and biomedical applications are compromised by the presence of metal ions, which some of these reagents would incur. For ammonia or alkylamine gas deprotection, a purpose-built and formatted pressure vessel is needed for safe operation. Such considerations detract from the use of these newer reagents that can provide faster deprotection of established N-protective groups.

In light of recent reports of the use of domestic, multimode microwave ovens for rapid deprotection of ODNs using alkali metal hydroxide reagents and other nucleic acid reactions (Kumar and Gupta, 1996, 1997, 1999a, 1999b, 2003; Gupta and Kumar, 1998; Andrzejewska et al., 2002; Rao et al., 2002; Seetharamaiyer et al., 2005), we considered the possibility for the fast deprotection of standard protected ODNs (dA^{Bz}, dC^{Bz}, dG^{ibu}) using the standard deprotection reagent (29% aqueous ammonia) (Schaller et al., 1963) from *cis*-diol universal support. These materials represent the most commonly used and widely available ODN synthesis reagents in use today.

MATERIALS AND METHODS

All nucleosides, DNA phosphoramidites, and synthesis reagents were purchased from ChemGenes Corporation (Wilmington, MA). Universal Support 1000 *cis*-diol derivatized controlled-pore glass (CPG) was purchased from Glen Research (Stirling, VA). DNA oligodeoxyribonucleotides (ODN, 5-mer to 50-mer) were synthesized by the Atlantic Microarray Facility (Moncton, NB, Canada) at a 40-nmole scale. *Escherichia coli* DNA uracil glycosylase (UDG), *Crotalus adamanteus* phosphodiesterase I (PDE), and *E. coli* alkaline phosphatase (AP) were from Sigma-Aldrich (Oakville, ON, Canada). Twenty-nine percent aqueous ammonia solution was from Fisher Scientific (Napean, ON, Canada).

N-acyl mononucleoside and ODN deprotection conditions

N-acyl protected mononucleosides (T, dA^{Bz}, dC^{Bz}, dC^{Ac}, dG^{ibu}, dG^{PAC}) and synthetic ODNs were deprotected under standard condition **A** (55°C, 19 hours, 29% aqueous ammonia) and also under microwave irradiation (for sequences and conditions, see Table 1 and Results section). Mononucleosides and ODNs from deprotection condition **A** were cooled to room temperature before vacuum centrifugation to yield a dry pellet. Following dissolution in nuclease-free water (Ambion, Austin, TX), analysis was performed by negative-ion mode ESI-MS (Agilent VL1100MSD), RP-HPLC [Fig. 1; Agilent 1100; Zorbax Extend-C18 column, isocratic 15%A: 85%B (A was 1:1 water:acetonitrile with 0.1% acetic acid and 10 mM ammonium acetate and B was water), UV detection at 260 nm; flow rate was 0.50 mL/min] and capillary electrophoresis (CE) (Fig. 2, P/ACE System MDQ (Beckman, Allendale, NJ) using the eCAP ssDNA 100-R Kit (Beckman). Orange G was included in all samples as a reference standard. Detection at 254 nm).

For the deprotection of ODNs (5-mer to 50-mer) at an elevated temperature (condition **B**; 170°C, 5 minutes, 29% aqueous ammonia), a sand bath and a silicone oil bath on a hot plate-stirrer were calibrated using a 5-mL glass vial containing light mineral oil (0.5 mL) and a thermocouple temperature probe. Fully protected ODN on CPG support (McClean and Garman, 1997) was washed into a glass vial (5 mL) with 29% aqueous ammonia solution (0.5 mL) and the screw cap was secured. Following thermal exposure at 170°C for 5 minutes, the vials were cooled on ice and ammonia was removed from the reaction mixture by vacuum centrifugation (55°C, 10 minutes). The supernatant solution was transferred for ESI-MS analysis.

In a typical microwave experiment the N-acyl protected mononucleoside (0.5 mg) or 20 nmole of fully protected ODN linked to controlled-pore glass (CPG) along with 29% aqueous ammonia solution (300 μL), and a PTFE-coated magnetic flea was loaded into a thick-walled glass reaction vessel (0.2 mL, Biotage AB or CEM). The reaction vessel was sealed and placed into a focused monomode microwave synthesizer (Biotage Initiator or CEM Discover, 300 W, 2.45 GHz) for deprotection. Reactions, including cleavage from the solid-support in the case of ODNs, were performed under a range of highly defined temperature and time conditions for mononucleosides and ODNs (Table 1, Figs. 1–3).

Following reaction vessel cool down to room temperature, accelerated by compressed air at 0.2 MPa (typically 1–2 minutes), ammonia was vented in a fume hood, the reaction vessel was opened and vacuum centrifuged at 55°C for 10 minutes to ensure rapid elimination of all ammonia. The aqueous solution of deprotected mononucleoside or supernatant aqueous solution of deprotected

TABLE 1 ODN SEQUENCES AND IDENTITIES

ODN sequences	*Identity*
TCGATGGAGTAAACTATTTGATTGATTGTGAACGGCGTCTGGAGAGAGGC	50#1
GTGAAGCTGAAGCTGTTGGACACCTGCCTTCTCATGTGGACATCATCTGG	50#2
TTGAAGGCAACTGTGCTGAACAGGATGGATCTGGTTGGTGGATGAACAAG	50#3
CTGGAATGCTGGGTATGCTCTGTGACAAGGCTACGCTGACAATCAGTTAA	50#4
TGGCAATGTGGTGAAGCTGAGTTTCTGTCCAATGAGCAGGAAGATTCTGA	50#5
ATCCATGTGGCTGAGGTTACCTACAAACCCCTGAGGAACAAAGACTTCCA	50#6
CCAGAGGCAAGAGCTCCCCTTGTGGCAGCTTATCCG	36#1
CGGATAAGCTGCCACAAGGGGAGCTCTTGCCTCTGG	36#2
CTTGTGGCAGCTAGCCCGATTGTAC	25#1
TGCCTCGGACATTAAGTGGG	20#1
ACCGATGCAT	10#1
ACACACACAC	10#2
GTGTGTGTGT	10#3
AGTGCATTCT	10#4
CCCCCCCCCC	10#5
CCCCUCCCCC	10#6
UCCCCCCCCC	10#7
CCCCCCCCCU	10#8
UUUUUUUUUU	10#9
CCCCC	5#1
CCUCC	5#2
UCCCC	5#3
CCCCU	5#4
UUUUU	5#5
CCCCC†	5#6[a]
CAAAA	5#7
ACAAA	5#8
AACAA	5#9
AAACA	5#10
AAAAC	5#11
CAAAC	5#12
ACCCA	5#13
AAAAA	5#14
PCR primers	
CTGGGAGTGGGTGGAGGC	β-ACTIN FORWARD
TCAACTGGTCTCAAGTCAGTG	β-ACTIN REVERSE
TGTCCTCGGTGAGCACGGATTC	*Pax-5* EX5 sens
GCCCACAGAAAAGCAAGAAGGTATT	RTEX10AS

[a]ODN 5#6, N-acetyl exocyclic protecting group at all positions. Microwave heated at 150°C for 1 minute, reaction power level 30W. Description of Table 1 is in the Results section.

ODN was transferred to a glass vial for RP-HPLC, ESI-MS, and CE analyses (Figs. 1–3).

TLC analysis of mononucleosides was performed on normal phase silica plates with UV detection (254 nm; solvent systems: 9:1 $CHCl_3$:MeOH and 9:2 $CHCl_3$:MeOH). Commercial protected and unprotected nucleosides were used as reference materials.

Quantitative real-time PCR

PCR analyses were conducted using the following primer pairs β-Actin Forward and β-Actin Reverse, which amplify a 95-bp portion of the human β-Actin gene, and *Pax-5* EX5 sens and RTEX10AS (Table 1), which amplify a 650-bp portion of the human *Pax-5* gene.

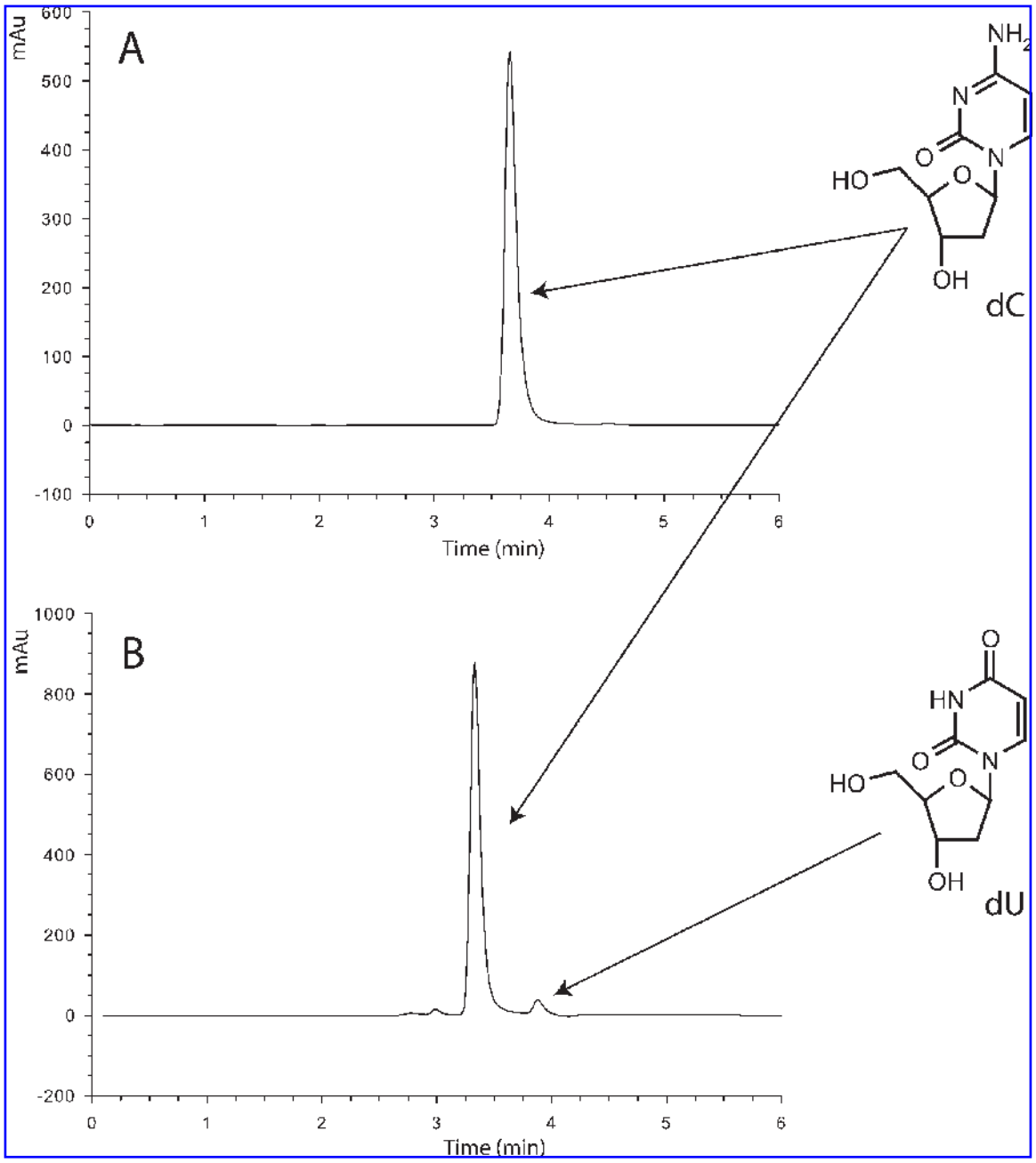

FIG. 1. RP-HPLC analysis of dC^{Bz} mononucleoside. Conventional thermal deprotection of dC^{Bz} mononucleoside (condition **A;** 55°C and 19 hours) (**A**). Microwave deprotection of dC^{Bz} mononucleoside with 265 W at 170°C and 13 bar pressure for 5 minutes (condition **B**) revealed 5% deamination to dU (**B**).

UDG enzymatic digestion

ODNs (10#5–10#9, 5#1–5#5, Table 1) dissolved in 50 mM KCl, 2.5 mM $MgCl_2$, and 10 mM Tris–HCl (pH 8.3) were exposed to DNA uracil glycosylase (UDG, *E. coli*). For ESI-MS analysis, samples (5 μL) were injected onto an Agilent Zorbax Extend-C18 precolumn to facilitate the separation of ODNs from UDG. The mobile phase consisted of acetonitrile:water (1:1, 125 mL) to which was added dibutylamine (300 μL) and EDTA (300 μL of a 1.6 mg/mL aqueous solution). Flow rate was 0.25 mL/min.

Phosphodiesterase I digestion and alkaline phosphatase treatment

ODNs (10#5, 5#1, 5#6–5#14, Table 1) were digested by snake venom phosphodiesterase I (*Crotalus adaman-*

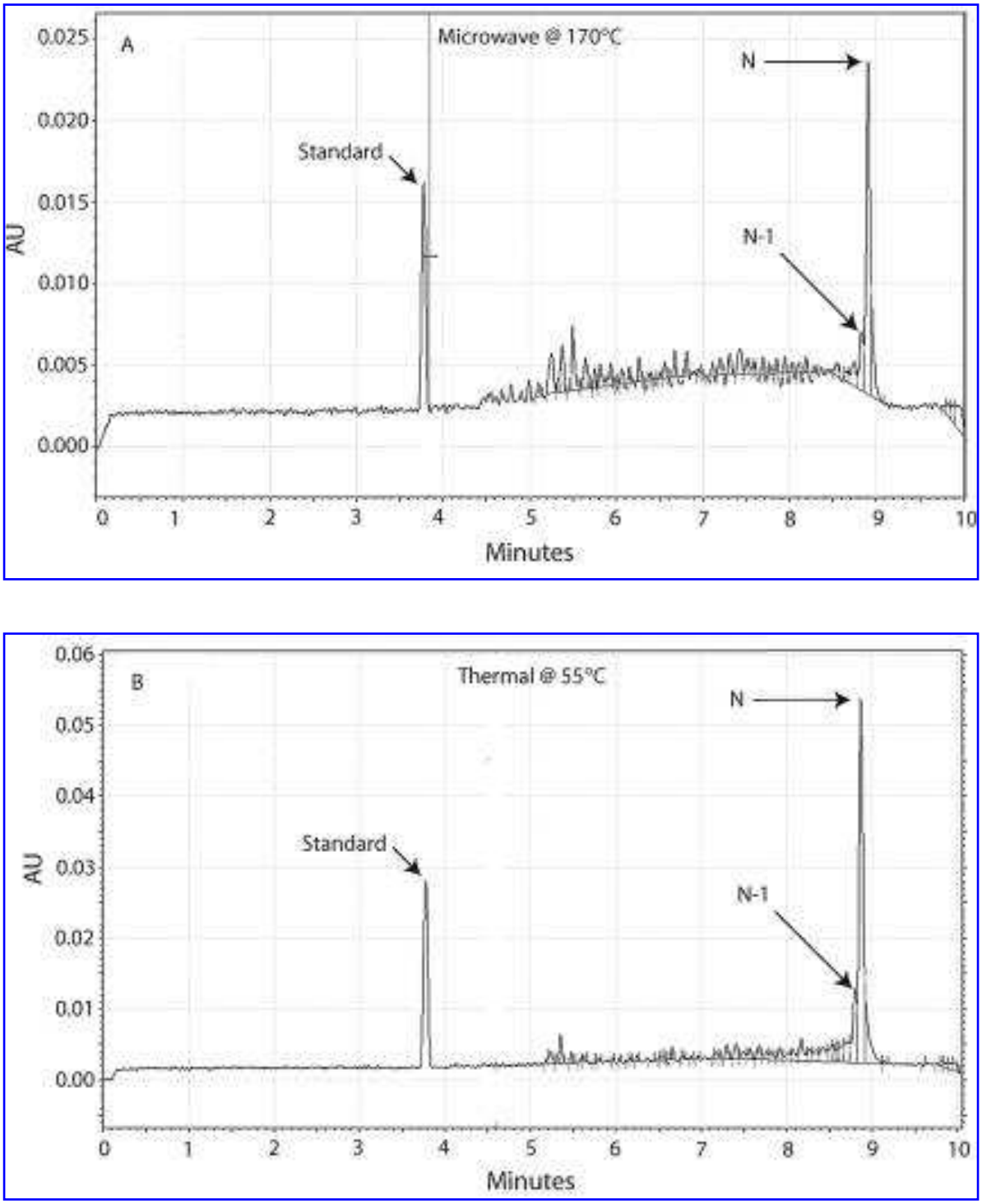

FIG. 2. Capillary electrophoretic (CE) analyses of ODN 50#2, a 50-mer, with 254 nm detection. Microwave deprotection, condition B (170°C, 5 min) (**A**) and thermal deprotection, condition A (55°C, 19 hours) (**B**) gave similar responses. N = full sequence peak; $N - 1$ = 49-mer peak; Standard = Orange G.

teus). Following 16 hours incubation at 37°C, 5′-phosphates were lysed by *E. coli* alkaline phosphatase. The resulting mononucleosides were analysed by RP-HPLC (conditions as above).

RESULTS

Monomode microwave deprotection conditions were developed to explore a rapid procedure for the cleavage

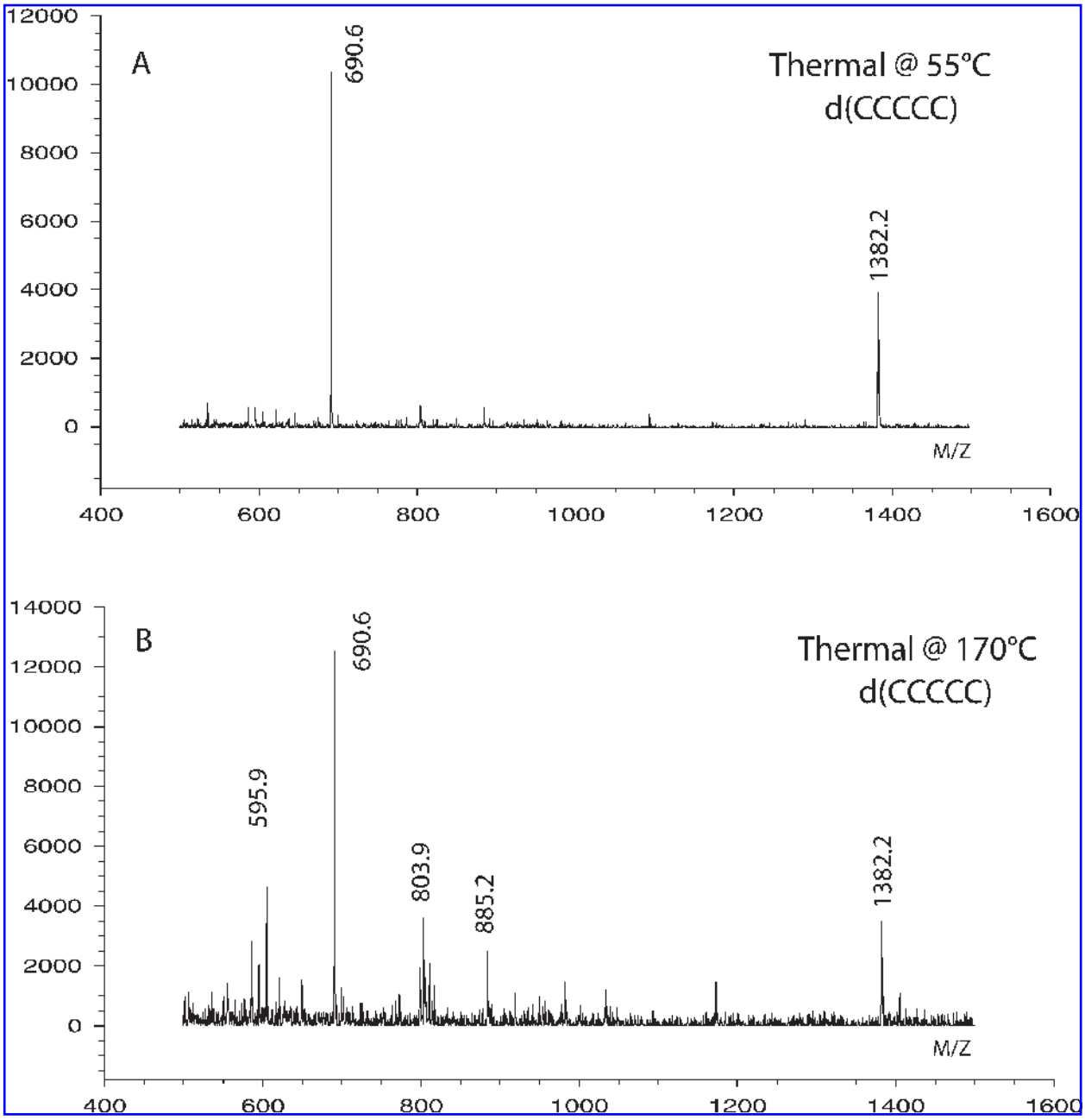

FIG. 3. Negative-ion mode electrospray mass spectra of deprotected dC_5 and dU_5 ODNs following DNA uracil glycosylase (UDG) digestion. Conventional thermal deprotection of $dC^{Bz}{}_5$ at 55°C for 19 hours—Condition **A** (**A**); conventional thermal deprotection of $dC^{Bz}{}_5$ at 170°C for 5 minutes—Condition **B** (**B**); Deprotection of $dC^{Bz}{}_5$ under microwave irradiation at 170°C for 5 minutes—Condition **B** (**C**); dU_5 subjected to conventional thermal deprotection conditions of 55°C for 19 hours—Condition **A** (**D**). $dC^{Bz}{}_5$ deprotected under microwave irradiation, Condition **B** (**C**) was subjected to UDG digestion to identify any occurrence of 2′-deoxycytidine deamination to 2′-deoxyuridine, which would be depyrimidinated from the ODN by UDG leading to the loss of this base from the sugar-phosphate backbone. No 2′-deoxycytidine deamination was observed. As an enzyme check, dU_5, subjected to conventional deprotection was also subjected to UDG digestion and uracil excision was observed by electrospray mass spectrometry (**D**).

and deprotection of ODN libraries using the established benzoyl and isobutyryl N-acyl protecting groups and standard 29% aqueous ammonia (Schaller et al., 1963).

In an initial deprotection kinetics and stability study, N-acyl mononucleosides (T, dA^{Bz}, dC^{Bz}, dC^{Ac}, dG^{ibu}, dG^{PAC}), containing both standard (Bz, ibu) and more labile exocyclic protecting groups (Ac, PAC), were reacted with 29% aqueous ammonia using a focused, monomode microwave synthesizer. All reactions were performed in duplicate. For dA^{Bz}, dGi^{bu}, and dG^{PAC} complete and clean

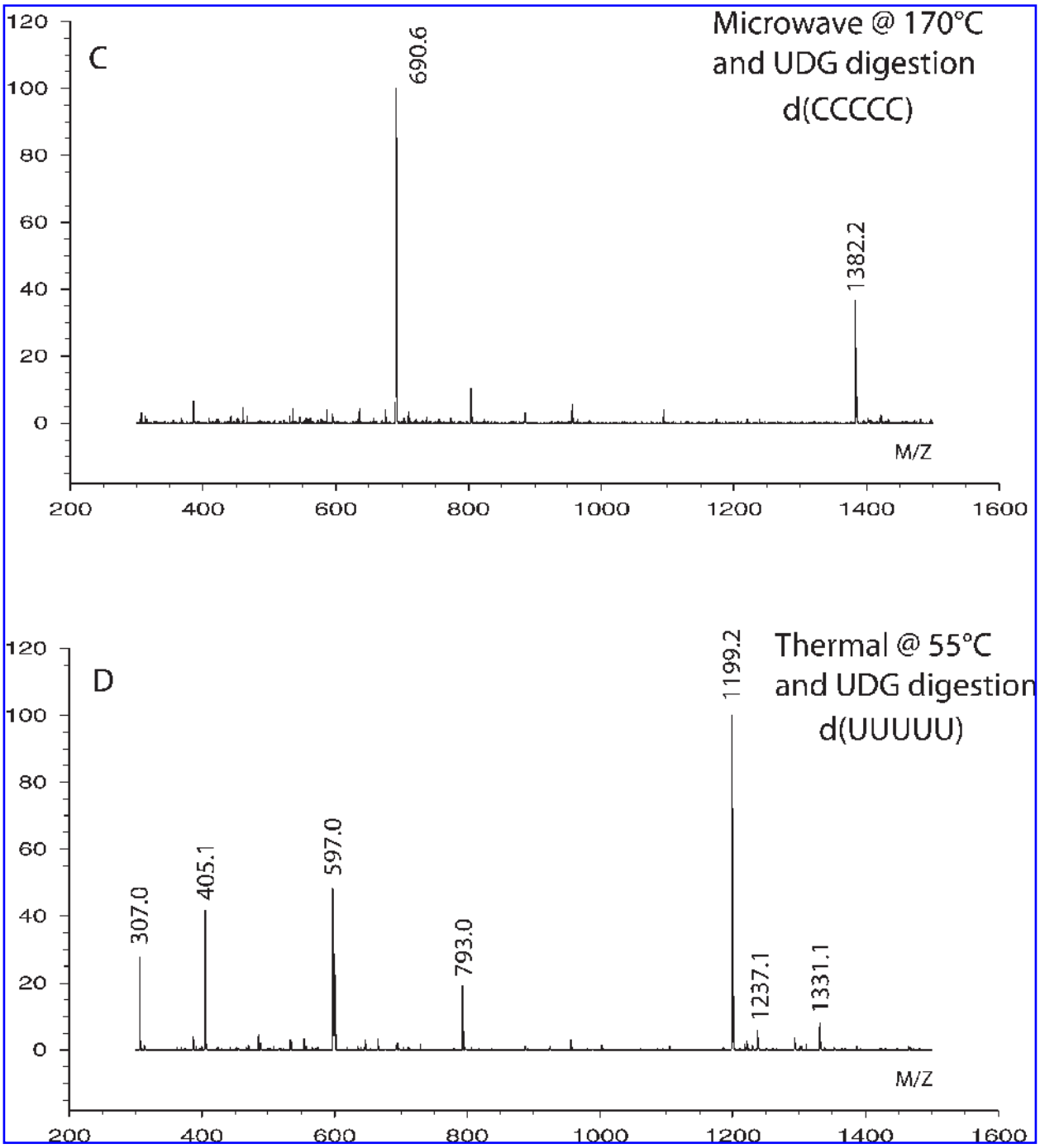

FIG. 3. (*Continued*)

deprotection was possible in 5 minutes at 150°C using 70 W (2.45 GHz) of microwave irradiation under 11 bar pressure, as analyzed by RP-HPLC. A higher temperature of 170°C using 130 W (2.45 GHz) under 13 bar pressure was necessary for complete dC^{Bz} deprotection in the 5-minute reaction time. However, this also resulted in a detectable dU contamination (Fig. 1). dU was confirmed by a spike-in of additional dU. In contrast, the more labile dC^{Ac} mononucleoside fully deprotected in only 1 minute at 150°C using 115 W (2.45 GHz) of microwave irradiation under 10 bar pressure, free of dU contamination. dA^{Bz} and dG^{ibu} were reacted at the higher temperature and deprotection was again complete giving the pure nucleoside as product. No structural changes were observed when dT was reacted for 10 minutes at 170°C under microwave irradiation. Hence, the minimum uniform

reaction condition necessary for deprotection of the standard N-protection groups (dA^{Bz}, dC^{Bz}, dG^{ibu}) (Schaller et al., 1963) was a 5-minute exposure of the N-acyl mononucleosides to 29% aqueous ammonia at 170°C.

A range of lengths (5-mer to 50-mer) and sequence motifs was chosen for the studied ODNs (Table 1). The exocyclic protecting groups used were dA^{Bz}, dC^{Bz}, and dG^{ibu}, except ODN, 5#6 where dC^{Ac} was used. Sequence 20#1 is a mixed base 20-mer consisting of binary combinations of the four DNA bases (Sindelar and Jaklevic, 1995). Sequences 50#1–50#6 were designed for DNA microarray applications for investigations of titin, nebulin, and obscurin muscle genes. ODNs 10#5–10#9 and 5#1–5#5 were used as model systems to investigate cytosine deamination by *E. coli* DNA uracil glycosylase exposure and electrospray mass spectrometric analysis. ODNs 10#5, 5#1, and 5#6–5#14 were used as model systems to investigate cytosine deamination by sequential *Crotalus adamanteus* phosphodiesterase I and *E. coli* alkaline phosphatase exposure and RP-HPLC analysis of resultant mononucleosides. Typical deprotection conditions used were Condition **A** (conventional) 55°C and 19 hours, 29% aqueous ammonia; Condition **B** (microwave), a 97 ± 29 s ramp time to reaction temperature, power ramp 285 ± 26W with reaction power level 142 ± 27W over 5 minutes. Pressure was 11.3 ± 0.9 bar or 170°C and 5 minutes with conventional heating.

All ODN deprotections were repeated in duplicate under conventional conditions (55°C, 19 hours: condition **A**); under microwave irradiation and in a sand bath and also a silicone oil bath at the same temperature for comparison (170°C, 5 minutes: condition **B**). ODN 5#6 ($d(CCCCC)^{Ac}$) with the labile N-acetyl group was deprotected at 150°C for 1 minute at 30 W power. Mass spectral, RP-HPLC and CE analyses were similar for all full-length ODNs deprotected under conditions **A** and **B** with conventional or microwave heating (Fig. 2, comparative CE traces of ODN 50#2). Peak area analysis of many CE traces revealed that the percentage area for the main ODN peak, in this case, ODN 50#2 (Table 1), relative to all the peaks picked by the CE instrument software was considered uninformative. More useful was the comparison of the main ODN peak and the largest deletion peak, the $n - 1$ peak (peak N and $N - 1$ respectively, Fig. 2). For the thermally deprotected ODN50#2, the $n - 1$ peak is 19% of the peak area of the full-length ODN peak by CE, whereas the same measure is 16% for the microwave deprotected ODN 50#2. ODNs deprotected by conventional heating at 170°C were degraded (data not shown), reflecting the known difficulty of temperature control for conventional heating (Kappe and Stadler, 2005; Tierney and Lidstrom, 2005).

Biological activity of microwave deprotected primers was tested for *β-actin* and *Pax-5* genes in the polymerase chain reaction. Melt curve and agarose gel electrophoretic analyses suggested that single amplicons were present. Experiments with mixed commercial ODNs/microwave deprotected ODNs yielded identical behaviour (data not shown).

Under RP-HPLC analytical scrutiny, for mononucleosides only, we observed about 5% deamination of dC^{Bz} to 2′-deoxyuridine (dU) under microwave condition **B,** whereas the same modification was 0.7% or less under conventional deprotection condition **A** (Fig. 1). Consequently, 2′-deoxycytidine and 2′-deoxyuridine-containing ODNs (10#5–10#9, 5#1–5#5, Table 1) prepared by microwave-assisted deprotection were used to investigate potential 2′-deoxycytidine deamination in ODNs under our conditions (Fig. 3). Results for 10-mer ODNs are not shown but yielded the same results. Full-length ODNs were exposed to DNA uracil glycosylase (UDG) (Varshney and van de Sande, 1991; Scharer and Jiricny, 2001). Any potentially depyrimidinated polyC ODNs would be easily observed by mass spectrometry. Uracil containing ODNs were used as a check of the correct functioning of the enzyme (Table 1 and Fig. 3).

In an alternate analysis approach to the study of potential 2′-deoxycytidine deamination, 2′-deoxycytidine-containing ODNs (10#5, 5#1, 5#6–5#14, Table 1) prepared by microwave-assisted deprotection were enzymatically hydrolyzed to mononucleosides by a combination of snake venom phosphodiesterase I (*Crotalus adamanteus*) (Williams et al., 1961; Bowman et al., 2001) and alkaline phosphatase (*E. coli*) (Reid and Wilson, 1971; McComb et al., 1979; Chandrasegaran et al., 1985; Marguet and Forterre, 2001; Gasan et al., 2002; Guerrero-Sanchez et al., 2006). Differential elution of the resultant mononucleosides by RP-HPLC was observed by UV-detection.

DISCUSSION

We have demonstrated a fast, microwave-assisted 5-minute deprotection of ODNs in 29% aqueous ammonia. The standard dA^{Bz}, dC^{Bz}, and dG^{ibu} N-acyl exocyclic amine protective groups (Schaller et al., 1963) were used and the ODNs were synthesized following the popular phosphoramidite method (Caruthers et al., 1987) on *cis*-diol solid phase support (MacMillan and Verdine, 1991; Hardy et al., 1994; Kumar and Gupta, 1999a, 2003; Kumar et al., 2002), as these are the most widely practised protocols.

We found that dC^{Bz} exhibited the slowest deprotection kinetics under microwave irradiation thereby dictating the deprotection reaction condition (condition **B**) necessary for synthetic ODNs. This order of deprotection for the mononucleosides may reflect a microwave dipolar heating effect (Kappe and Stadler, 2005; Tierney and Lidstrom, 2005). Our method has been successfully applied to ODNs up to 50-mer in length (Table 1). We have

illustrated biological activity by the use of microwave deprotected ODNs in the real-time PCR reaction and as substrates for the enzymes UDG, phospodiesterase I, and alkaline phosphatase (data not shown).

Experimental evidence for the high-temperature thermal decomposition characteristics of ODNs is limited in the literature. However, available sources indicate that degradation of the sugar–phosphate backbone occurs at temperatures of approximately 200°C when studied by differential scanning microcalorimetry (Gasan et al., 2002). The rate of ODN thermodegradation is reported to be accelerated at low pH and in the absence of metal ions, proceeding by a mechanistic route whereby depurination is followed by hydrolysis of the phosphodiester bond adjacent to the apurinic site (Marguet and Forterre, 2001). We did not observe ODN thermogradation under our conditions (170°C, 5 minutes, 29% ammonia) using CE, RP-HPLC, and ESI-MS analyses, either directly or following UDG and a combination of PDE/AP enzymatic degradations. For example, ODN 50#2 gave a similar CE response following either standard or microwave assisted deprotection (Fig. 2) and RP-HPLC and ESI-MS results were identical (data not shown). Although our reactions were conducted under high pressure, it has been shown that ODNs are not adversely affected by ammonia gas at a similar pressure (10 bar) (Boal et al., 1996; Iyer et al., 1997).

Kumar and Gupta (1997) found it necessary to use threefold more power (520 W, 2.75 GHz) in a multimode domestic microwave oven for a comparable 4-minute reaction time with 1 M NaOH in MeOH:H_2O (1:1) (Kumar and Gupta, 1997, 1999a 1999b). It should be noted that a similar reagent at a fivefold lower 0.2 M NaOH concentration has been shown to be 5 to 13 times faster than 29% aqueous ammonia in deprotection (Köster et al., 1981). In a modern monomode microwave instrument using 29% aqueous ammonia as the deprotective reagent, we have found that 110–170 W is sufficient for the preparation of biologically active ODNs for their intended purpose.

Furthermore, our procedure was performed on standard protective groups (Schaller et al., 1963). This contrasts with the fully labile N-protective strategy (dA^{PAC}, dC^{ibu}, dG^{PAC}) when using 1 M NaOH demonstrated by Kumar Gupta (1997, 1999a, 1999b) or the use of dC^{Ac} by Reddy et al. (1994, 1995, 1997) making our procedure more attractive for library production. Interestingly, Guerrero-Sanchez et al. (2006) have demonstrated the stability of CPG support material under more intense microwave irradiation (800 W).

We observed an increase in dC^{Bz} mononucleoside deamination to dU under microwave deprotection (about 5%, condition **B**, Fig. 1) in comparison to standard deprotection (about 0.7%, condition **A**, Fig. 1). It is recognized that the C4 reactivity of dC is a concern in deprotection of synthetic ODNs (Weber and Khorana, 1972; Miller et al., 1986; Hogrefe et al., 1993; Polushin et al. 1994; Reddy et al., 1997; Wang et al., 2002), and indeed, C4 reactivity of cytidine toward nucleophiles has been utilized to provide base-specific conjugation (MacMillan and Verdine, 1991). As dC and dU have equal nominal molecular mass, chromatographic analyses (TLC, RP-HPLC) of the mononucleoside was an effective method in making this minor structural base modification apparent. RP-HPLC and MS analyses of full-length ODNs did not resolve this biologically important dU modification (data not shown). By the use of 2′-deoxycytidine and 2′-deoxyuridine-containing ODNs (10#5–10#9, 5#1–5#5, Table 1) and a novel analysis strategy utilizing DNA uracil glycosylase (UDG) we did not observe any deamination within the limitations of the enzyme's specificity (Varshney and van de Sande, 1991). *E. coli* DNA uracil glycosylase used in these experiments does not excise dU from the last two positions of the 3′-terminus or the unphosphorylated 5′-terminus (Varshney and van de Sande, 1991). This means that a $d(C)_4$ ODN is the theoretical minimum requirement for observation of at least one base loss due to dC to dU conversion via deamination.

In a further approach to address the remaining terminal positions, we utilized phosphodiesterase I (PDE) digestion with subsequent alkaline phosphatase (AP) treatment to degrade ODNs to their respective mononucleosides (ODNs 10#5, 5#1, 5#6–5#14, Table 1) (Williams et al., 1961; Reid and Wilson, 1971; McComb et al., 1979; Chandrasegaran et al., 1985; Bowman et al., 2001). The mononucleoside mixture from each ODN was then subjected to RP-HPLC separation. Analysis of the results (data not shown) reveals that only a 5′-terminal dC (ODNs 5#11, 5#12, Table 1) undergoes any deamination, and then only to a small extent (<2%). 3′-Terminal dC^{Bz} and endo-dC^{Bz} do not undergo deamination in the 5-mer or 10-mer ODNs studied. Therefore, our rapid reaction conditions are valid with one reservation for sequences that possess a 5′-terminal 2′-deoxycytidine. However, if the very small extent of deamination (<2%) to 2′-deoxyuridine can be tolerated then even this qualification may be overlooked. It is speculated that the negative charge of the phosphodiester linkage offered some protection against dC deamination in ODNs. This protective effect is absent in dC mononucleoside. ODN 5#6, $d(CCCCC)^{Ac}$, when microwave deprotected at 150°C for 1 minute (Table 1) revealed a complete absence of deamination. This observation illustrates the practical utility of the initial mononucleoside study.

Comparative experiments performed under conventional and microwave heating illustrate the distinct advantage of performing reactions under microwave irradiation due to the very tight control of reaction temperature that is possible and the very rapid attainment of reaction temperature. An average of 90 seconds was necessary to

attain 170°C from ambient conditions in the microwave, whereas a longer period (>5 minutes) was necessary in conventionally heated experiments—the same as the reaction time itself (5 minutes). This is due to the fact that reaction mixtures absorb microwave radiation directly through a microwave-transparent glass or PTFE container, in contrast with conventional heating where there exists an inverse thermal gradient and the outside of the reaction vessel is hotter. This effect means that it takes a longer period of time for the reaction medium to reach reaction temperature, which is then, subsequently, more difficult to control (Kappe and Stadler, 2005; Tierney and Lidstrom, 2005; Guerrero-Sanchez et al., 2006). These effects are reflected in the poorer quality of the ODNs prepared by conventional heating at 170°C as evidenced by their ESI-MS spectra (Fig. 3) and RP-HPLC chromatograms (data not shown). In conclusion, we have demonstrated, through the use of monomode microwave irradiation, that DNA oligonucleotides bearing the standard N-groups are rapidly deprotected in 5 minutes using 29% aqueous ammonia.

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