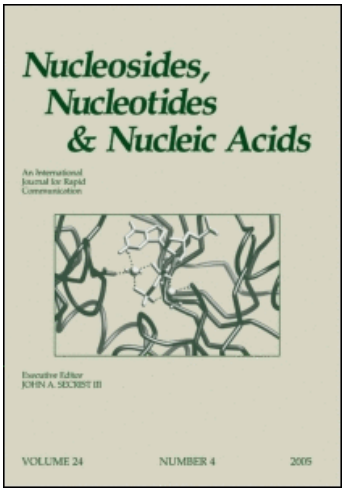

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DIMETHOXYTRITYL REMOVAL IN ORGANIC MEDIUM: EFFICIENT OLIGONUCLEOTIDE SYNTHESIS WITHOUT CHLORINATED SOLVENTS

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Oligonucleotides are finding widespread utility in various applications in diagnostics and molecular biology and as therapeutic agents. In standard synthesis of such oligonucleotides through phosphoramidite coupling, removal of the typical acid-labile 4,4'-dimethoxytrityl 5'-protecting group (DMTr), from the support-bound oligonucleotide plays a crucial role in each synthesis cycle in achieving high product yield and oligonucleotide quality. Although several reagents have been developed for this purpose, many have limited applicability to automated oligonucleotide synthesis on solid supports. The most commonly used reagents today are dilute solutions (2-15%) of an organic acid, typically trichloroacetic acid (TCA, pKa 0.8) or dichloroacetic acid (DCA, pKa 1.5) in dichloromethane. The high volatility (boiling point 40 ^{o}C) of dichloromethane and its high toxicity and carcinogenicity pose a hazard for personnel and the environment. In addition, as oligonucleotide synthesizers are now available to allow syntheses of up to 0.5 mole scale, the quantities of chlorinated waste generated have become quite large. In this context we became interested in replacing dichloromethane as deblocking reagent solvent with a less harmful solvent while preserving product yield and quality. We now report that it is not necessary to use halogenated solvents such as dichloromethane in the deblocking step of automated oligonucleotide synthesis in order to obtain high yields of high quality oligonucleotide product.

As initial screening, we performed a series of oligonucleotide syntheses (1 μmol) on an ABI 394 DNA/RNA Synthesizer using commercially available DNA synthesis columns charged with CPG loaded with the 5'-*O*-DMTr-protected 3'-terminal nucleoside of the desired oligonucleotide. As test sequences we chose phosphorothioate oligodeoxyribonucleotides with a homo-T sequence T_{19} (**1**) [as thymidine has the slowest detritylation kinetics among the four protected nucleosides (DMTr-dG^{iBu} > DMTr-dA^{Bz} > DMTr-dC^{Bz} > DMTr-T)], a deoxyadenosine rich sequence $(TdAdA)_6T$ (**2**) (in order to compare the extent of depurination, as it is well known that benzoyl deoxyadenosine is prone to depurination), and one mixed-sequence phosphorothioate oligodeoxyribonucleotide containing all four natural nucleobases, d(TCCCGCCTGTGACATGCATT) (ISIS 5132/CGP69846A, (**3**)). We compared oligonucleotides synthesized with 0.36M DCA (3%, *v/v*) or 0.18M TCA (3%, *w/v*) in chlorobenzene, benzotrifluoride, benzene, toluene and xylenes vs. syntheses using standard detritylation solutions at the same acid concentrations in dichloromethane. The efficiency of the syntheses and the quality of the oligonucleotide product were judged by the full length content of crude and purified oligonucleotide product as well as by the relative amount of (n-1)-mer formed (as determined by capillary gel electrophoresis (CGE) of DMTr-off oligonucleotide). Full length contents of 86% for the homopyrimidine sequence **1** and 75±1% for sequences **2** and **3** are typical when DCA (0.36M) in dichloromethane is used for DMTr removal. The relative (n-1)-mer content, defined as (n-1)/[(n-1)+n] x 100, of 3.2±0.3% in crude oligonucleotide product is due to incomplete coupling in the last coupling step and possibly incomplete detritylation during chain extension. Purification allows for separation of most of the shorter DMTr-off failure sequences, thus increasing the full length content to 93±2% and reducing the relative (n-1)-mer content to <2% for **1** and to 3±0.5% for **2** and **3**. Using TCA (0.18M), similar results are obtained for the homopyrimidine sequence **1**, reduced full length content and increased (n-1)-mer content are observed for **2** and **3**, possibly due to depurination. Using DCA (0.36M) in chlorobenzene, benzotrifluoride, benzene, toluene, and xylene, we obtained CGE traces of crude and purified oligonucleotide product almost identical to those when DCA/dichloromethane was used for DMTr removal. At a lower DCA concentration

(0.18M in toluene), a slightly increased (n-1)-mer content is observed in sequence **1**. Surprisingly, very low full-length and very high (n-1)-mer contents in both crude and purified oligonucleotide products were obtained when TCA was used in any of the aromatic solvents used. The high (n-1)-mer content seen in the DMTr-on fraction may be due to incomplete removal of the DMTr group. At higher acid concentration (0.36M TCA in toluene, the (n-1)-mer content was reduced, but was still unacceptable.

Solid support and reactor design play an important role in oligonucleotide synthesis. Currently, the preferred support for large scale synthesis (100-200 mmol) is a polystyrene-based matrix (Primer Support, Pharmacia) which is tightly packed in a steel column. To simulate those conditions, we synthesized **3** on an OligoPilot II synthesizer (Pharmacia) in a packed bed column reactor using Primer Support-T (170 μmol scale). The results are summarized below:

		crude oligonucleotide					purified oligonucleotide			
entry	solvent	yield $[OD/mg]_{260\ nm}$	DMTr-on [area %][a]	full length content [%][b]	(n-1)/(n-1)+n	PO content[c] [%]	full length content [%][b]	(n-1)/(n-1)+n	PS:PO:$(PO)_2$ ratio[d]	ES-MS[e] mol. mass
1	CH_2Cl_2	11.0±0.2	78±2	72±2	4.7%	0.60	93.7±1	2.4%	87.9:10.7:1.4	6349.6
2	toluene	10.9±0.2	76±2	69.0±2	4.6%	0.85	94.9±1	2.4%	83.7:14.2:2.1	6349.7
3	xylenes	11.0±0.2	80±2	72±2	4.6%	0.52	94.8±1	2.4%	88.6:10.0:1.4	6349.7
4	benzotrifluoride	11.2±0.2	77±2	69±2	4.7%	0.78	93.0±1	2.3%	86.7:11.6:1.7	6349.7

[a] by RP-HPLC (λ = 254 nm) [b] by CGE (λ = 260 nm) [c] by ^{31}P NMR [d] by SAX [e] calculated mass = 6349.6

As evident from the data presented above, a solution of dichloroacetic acid in toluene allows removal of dimethoxytrityl groups from support-bound oligonucleotides providing product oligonucleotides in high yield and purity.