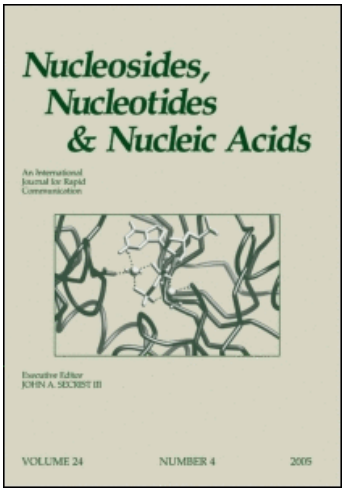


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Further Optimization of Detritylation in Solid-Phase Oligodeoxyribonucleotide Synthesis

Kha Tram[a]; Yogesh S. Sanghvi[b]; Hongbin Yan[a]
[a] Department of Chemistry, Brock University, St. Catharines, Ontario, Canada [b] Rasayan Inc., Encinitas, California, USA

Online publication date: 22 January 2011

To cite this Article Tram, Kha , Sanghvi, Yogesh S. and Yan, Hongbin(2011) 'Further Optimization of Detritylation in Solid-Phase Oligodeoxyribonucleotide Synthesis', Nucleosides, Nucleotides and Nucleic Acids, 30: 1, 12 — 19
To link to this Article: DOI: 10.1080/15257770.2010.537291
URL: http://dx.doi.org/10.1080/15257770.2010.537291

Nucleosides, Nucleotides and Nucleic Acids, 30:12–19, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2010.537291

Taylor & Francis
Taylor & Francis Group

FURTHER OPTIMIZATION OF DETRITYLATION IN SOLID-PHASE OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

Kha Tram,[1] Yogesh S. Sanghvi,[2] and Hongbin Yan[1]
[1]*Department of Chemistry, Brock University, St. Catharines, Ontario, Canada*
[2]*Rasayan Inc., Encinitas, California, USA*

□ *Various conditions for optimum detritylation (i.e., the removal of 5′-O-trityl protecting groups) during solid-phase synthesis of oligodeoxyribonucleotides were investigated. Di- and tri-chloroacetic acids of variable concentrations were used to study the removal of the 4,4′-dimethoxytrityl (DMTr) group. It was found that the DMTr group could be completely removed under much milder acidic conditions than what are currently used for automated solid-phase synthesis. The 2,7-dimethylpixyl (DMPx) is proposed as an alternative and more readily removable group for the protection of the 5′-OH functions both in solid- and solution-phase synthesis. The improved detritylation conditions are expected to minimize the waste and offer a protocol for incorporation of acid sensitive building-blocks in oligonucleotides.*

Keywords Detritylation; DMTr; pixyl; DMPx; solid-phase synthesis; oligonucleotides

INTRODUCTION

Since its introduction, 4,4′-dimethoxytrityl (DMTr **1**; Figure 1) group[1] has been used widely for the protection of 5′-OH functions in oligonucleotide synthesis.[2] Its removal is conveniently effected by treatment with a solution of di- and tri-chloroacetic acids. One possible side reaction that could arise during detritylation is depurination.[3] This is of particular concern when the sequence to be assembled contains nucleosides that are particularly prone to depurination, such as N^6-benzoyl-2′-deoxyadenosine and to a lesser degree N^2-isobutyryl-2′-deoxyguanosine. Of particular note, some modified nucleosides such as 8-aryl derivatives of 2′-deoxyguanosine are known to be extremely prone to depurination under acidic conditions.[4] Some of these modified nucleosides undergo depurination under acidic

Received 30 September 2010; accepted 1 November 2010.
The authors wish to thank the Natural Sciences and Engineering Research Council of Canada and the Research Corporation for funding this work. The authors also thank Professor Colin B. Reese for helpful discussions.
Address correspondence to Hongbin Yan, Department of Chemistry, Brock University, St. Catharines, ON, L2S 3A1, Canada. E-mail: tyan@brocku.ca

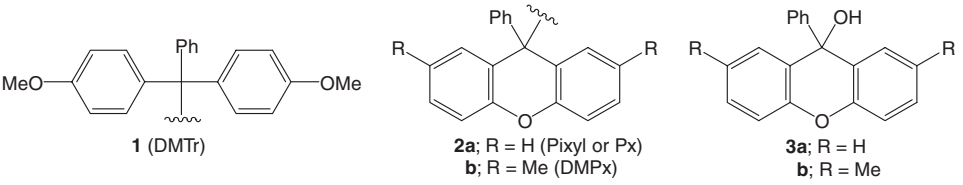


FIGURE 1 DMTr and pixyl protecting groups and their precursors.

conditions up to 200 times more readily than deoxyriboguanosine, leading to particular challenges in the chemical synthesis of oligonucleotides containing these modified nucleosides.[5] These abasic sites, once formed, are prone to chain cleavages under basic conditions, such as ammonolysis that is used to deprotect the oligonucleotides. It is clearly of importance to ensure that complete detritylation does not lead to depurination during acidic deprotection. Indeed, milder detritylation conditions, that is, use of weaker acids in detritylation and shorter acid contact times are very likely to be beneficial in the synthesis of such sequences. In this regard, dichloroacetic acid (DCA; pKa = 1.5) is to be preferred over trichloroacetic acid (TCA; pKa = 0.7).[6] In other cases, cation scavengers such as pyrrole,[7] alcohols,[8] silanes, anisole, thioanisole, benzyl mercaptan, and ethane-1,2-diol,[9] have been found to be useful in facilitating complete detritylation. It would be desirable to establish the "minimal" acid strength and acid contact time that are essential for complete detritylation during solid-phase oligonucleotide synthesis. We previously reported that when 3% TCA was used in the detritylation step, acid delivery time as short as 10 seconds (versus 110 seconds) did not compromise the yield of full-length product significantly.[10] We now report the effects of acid concentration and contact time on oligonucleotide quality during solid-phase synthesis.

RESULTS AND DISCUSSION

A model study involving thymidine decamer (T_{10}) was first undertaken. This oligothymidylic acid model was chosen because thymidine has been shown to be the most difficult nucleoside to detritylate compared to dC, dA and dG.[11] Syntheses of the T_{10}-mers on a 1.0 μmol scale were carried out on controlled pore glass (LCCA-CPG: 500 Å, 33 μmol/g) with an ABI 3400 DNA synthesizer using the ABI protocol for 1 μmol DNA (see Table 1 for cycle conditions). The pressure on the TCA bottle and the flow rate of acid to the column were the same as the values that the instrument manufacturer recommends. Phosphoramidites were prepared as 0.1 *M* solutions in anhydrous acetonitrile.

A solution of 3% TCA in dichloromethane (DCM) was used initially. Acid delivery time was varied at 110, 70, 60, 50, 30, and 20 seconds. The T_{10}

TABLE 1 Solid-phase synthesis cycle conditions

Step	Condition
Detritylation	as appropriate
Washing	3 × 12 seconds with reverse flush (7 seconds) in between
Coupling	20 seconds, 5-EtS-tetrazole (0.25 *M* solution in acetonitrile) as activator
Capping	12 seconds delivery followed by 6 seconds wait time
Oxidation	0.02 *M* I_2 in THF/Pyridine/H_2O
Washing	2 × 20 seconds with reverse flush (15 seconds) and block flush (3 seconds) in between
Trityl	Off

products were cleaved and unblocked by treatment with aqueous ammonium hydroxide for 16 hours at 55°C. After lyophilization, the fully-unblocked T_{10}–mers were analyzed by anion-exchange chromatography on a DNAPac PA-100 analytical column (4 × 250 mm) with a Dionex 3000 IC system. As can be seen from Table 2, shortened detritylation time (20 seconds versus 110 seconds) with TCA did not lower the overall yield. However, when a weaker acid solution, that is, DCA, was used for detritylation, marked differences

TABLE 2 Overall yields of full-length sequences as determined by anion-exchange HPLC

		5′-*O*-protecting group: DMTr			
			Yields of full-length sequence[c]		DMPx
Acid	Acid concentration (%)[a]	Detritylation time (s)[b]	T_{10}[d]	Mixed 21-mer sequence[e,f]	T_{10}
TCA	3.0	**20**	**88**	**87**	**90**
		30	–	86	–
		110	88	87	–
DCA	3.0	20	73	–	84
		30	82	–	87
		40	87	–	88
		50	92	86	–
		60	92	86	–
		110	**89**	**86**	**88**
	1.5	30	62	incomplete	89
		40	77	–	89
		50	81	–	88
		60	89	86	–
		70	90	–	–
	0.5	30	1	–	49
		50	–	–	89
		110	4	–	–

[a]Acid in dry dichloromethane, w/v for TCA and v/v for DCA.
[b]Continuous delivery to column.
[c]Yields were determined by anion-exchange HPLC.
[d]ESI-MS observed for T_{10}-mer $[M\text{-}H]^-$: 2978.7, $C_{100}H_{130}N_{20}O_{68}P_9^-$ requires 2978.96.
[e]Mixed 21-mer sequence: d(GCGTTTGCTCTTCTTCTTGCG).
[f]ESI-MS observed for the 21-mer $[M\text{-}H]^-$: 6360.0, $C_{204}H_{262}N_{63}O_{134}P_{20}^-$ requires 6360.08.
–: did not perform the experiment.

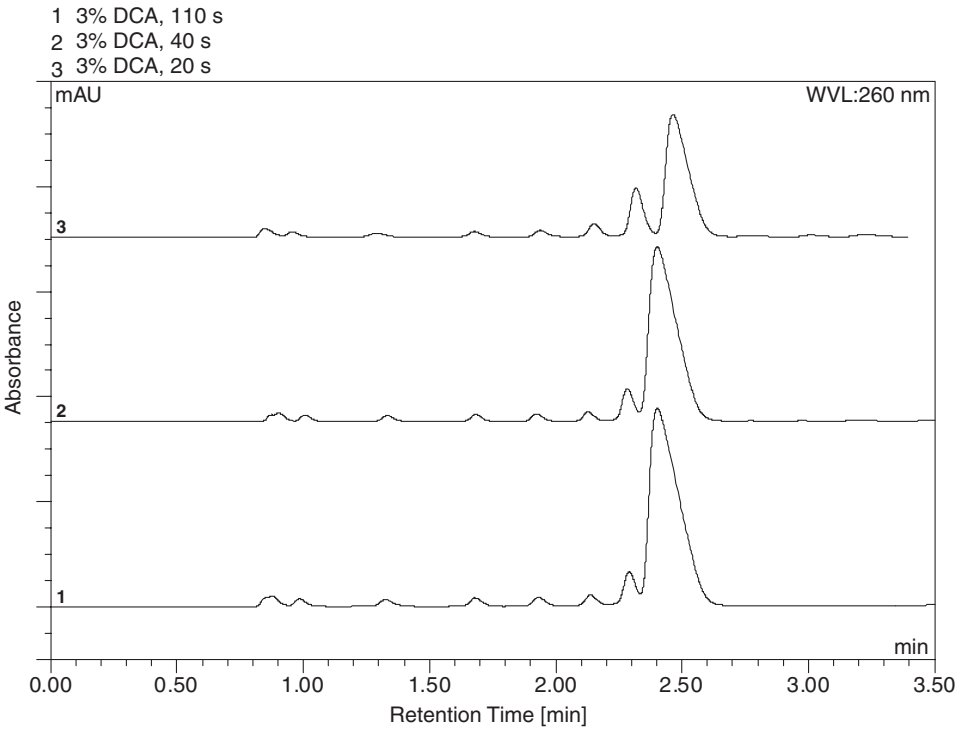


FIGURE 2 Anion-exchange HPLC profiles of T_{10}-mers. 1) Use of 3% DCA solution during detritylation with 110 seconds continuous acid delivery furnished an 89% yield of full-length T_{10}-mer; 2) use of 3% DCA solution during detritylation with 40 seconds continuous acid delivery furnished an 87% yield of full-length T_{10}-mer; 3) use of 3% DCA solution during detritylation with 20 seconds continuous acid delivery provided a 73% yield of full-length T_{10}-mer. Eluent A: 0.25 *M* Tris (constant at 10%), pH 8.0; eluent B: water; eluent C: NaCl (1.0 *M*). Gradient: convex gradient (curve 5) of 10% C to 55% in 20 minutes. Flow rate: 1.5 ml/min.

were observed (as shown in Figure 2). Thus, the times required for complete detritylation when 3.0 and 1.5% DCA were used were 50 and 60 seconds, respectively, and the use of 0.5% DCA did not lead to complete detritylation even after 110 seconds continuous acid delivery (Table 2).

Subsequently, a similar comparison was carried out with a 21-mer oligodeoxyribonucleotide mixed sequence [d(GCGTTTGCTCTTCTTC TTGCG)]. As can be seen from Table 2 and Figure 3, about 86% yields of full-length 21-mer product (which corresponds to an average stepwise yield of about 99.2%) were achievable when milder detritylation conditions were used.

From the comparisons shown above, it becomes apparent that if 3% TCA solution is used during detritylation, DMTr can be removed in as little as 20 seconds, which is close to the time required for the acid to reach the column in an ABI 3400 DNA synthesizer. Thus, use of a more acid-labile 5′-protecting group is not necessary. However, if a weaker acid, for example, DCA, is used

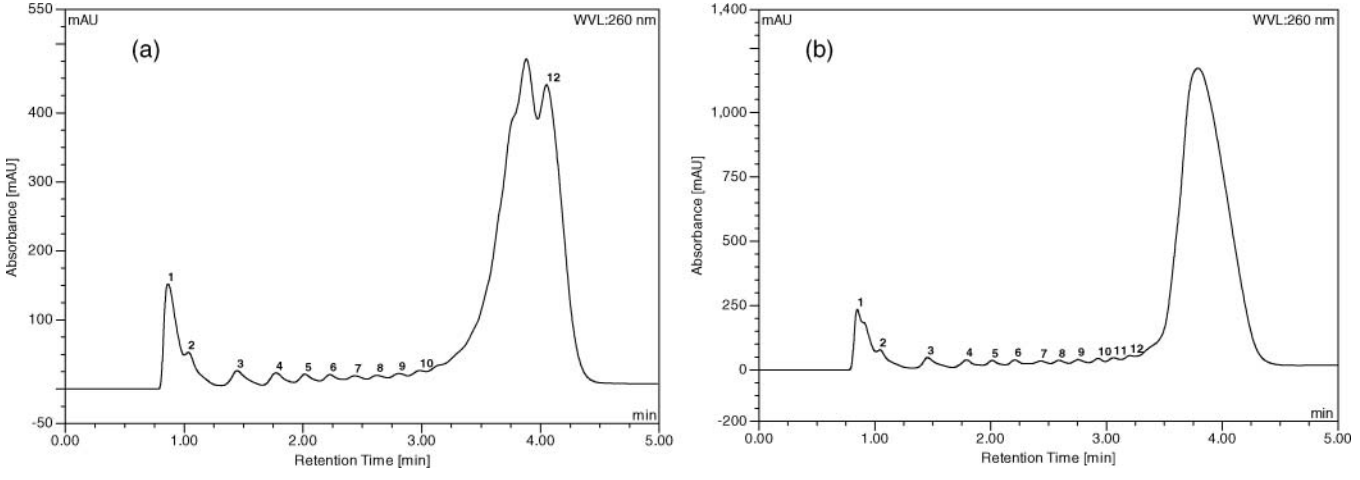
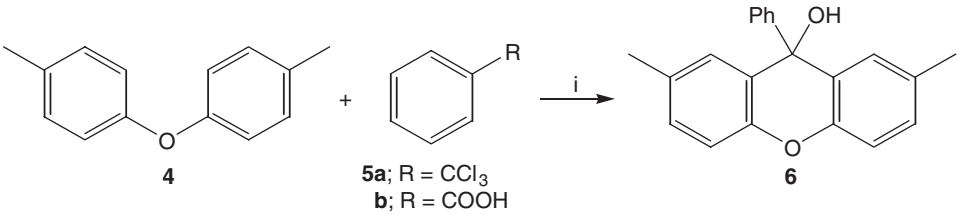


FIGURE 3 Anion-exchange HPLC profiles of the 21-mer mixed sequence. a) Detritylation with 1.5% DCA for 30 seconds. The peak with the second longest retention time was believed to be formed due to incomplete detritylation; b) detritylation with 3% TCA for 110 seconds. The HPLC profiles of the 21-mer assembled under the other six detritylation conditions as indicated in Table 2 (these experiments furnished 86–87% overall yields) were virtually identical to that shown in panel b. Eluent A: 0.25 *M* Tris (constantly at 10%), pH 8.0; eluent B: water; eluent C: $NaClO_4$ (1.0 *M*). Gradient: convex gradient (curve 5) of 10% C to 55% in 20 minutes. Flow rate: 1.5 ml/min.

in detritylation, a more acid-labile 5′-protecting group would be desirable in that it would further reduce the overall detritylation cycle time. For this reason, the use of the 5′-*O*-2,7-dimethylpixyl (DMPx) protecting group **2b** (Figure 1) was investigated.

As a pixyl (Px **2a**, Figure 1)[12] analogue,[13] DMPx (Figure 1)[14] was shown to be more acid-labile than the DMTr protecting group. Furthermore, the precursor reagent DMPx-OH **3b** is cheaper than Px-OH **3a** and is readily prepared (Scheme 1) in very high yield[14] by treating di-*p*-tolyl ether **4** with benzotrichloride **5a** or benzoic acid **5b** in the presence of zinc chloride and phosphorus oxychloride.



SCHEME 1 Reagents and conditions: i) $ZnCl_2$, $POCl_3$, 80–95°C, 1–2 hours.

The kinetic studies[15] were first conducted to determine the acid lability of the DMTr, Px-, and DMPx-group in solution. In all the kinetic experiments, 5′-protected-3′-*O*-acetylthymidine derivatives **7a–c** (Figure 4)[16] (0.025 *M* solution in DCM) were treated with 5 mol. equiv. of DCA in the presence of 15 mol. equiv. of pyrrole at 0°C. As expected, the results demonstrated that acid-lability increases in the order of DMTr, Px, and DMPx, as revealed by their half times of hydrolysis (Table 3).

When DMPx- was used as the 5′-protecting group in the synthesis of T_{10}-mers, it became apparent that shorter acid contact time could ensure its complete removal (Table 2). As an example, when 0.5% DCA was used as detritylating agent, a 50 seconds acid-delivery ensured complete removal of DMPx, whereas only 4% of full-length T_{10} was obtained under the same conditions when the DMTr protecting group was used.

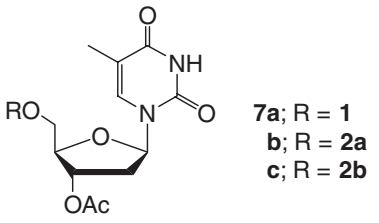


FIGURE 4 Substrate for hydrolysis kinetic experiments.

TABLE 3 Half times ($t_{1/2}$) of hydrolysis of DMTr-, Px-, and DMPx protecting groups

	$t_{1/2}$ (s)	
Substrate	Determined in this study	Literature values[17]
7a (DMTr-)	489	450
7b (Px-)	195	190
7c (DMPx-)	45	–

CONCLUSION

Using T_{10}-mer as a model system, the minimal detritylation conditions for the removal of DMTr in oligonucleotide synthesis were determined. These conditions also allowed successful assembly of a mixed sequence. Use of DMPx- as an alternative protecting group for the 5′-OH functions of nucleosides further reduces the required acid contact time during oligonucleotide synthesis. This feature may permit incorporation of acid labile nucleosides into oligonucleotides. We also believe that shorter acidic deprotection cycle described herein would lead to minimization of solvent waste generated during oligonucleotide synthesis.

REFERENCES

1. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. Studies on polynucleotides. XXIV. The stepwise synthesis of specific deoxyribopolynucleotides. Protected derivatives of deoxyribonucleosides and new syntheses of deoxyribonucleoside-3′ phosphates. *J. Am. Chem. Soc.* **1963**, 85, 3821–3827.
2. Seliger, H. Protection of 5′-hydroxy functions of nucleosides. In *Current protocols in nucleic acid chemistry*, eds. S.L. Beaucage, D.E. Bergstrom, G.D. Glick, R.A. Jones, Wiley, New York, 2001; v1, 2.3.1–2.3.34.
3. Suzuki, T.; Ohsumi, S.; Makino, K. Mechanistic studies on depurination and apurinic site chain breakage in oligodeoxyribonucleotides. *Nucleic Acids Res.* **1994**, 22, 4997–5003.
4. Schlitt, M.K.; Sun, K.M.; Paugh, R.J.; Millen, A.L.; Navarro-Whyte, L.; Wetmore, S.D.; Manderville, R.A. Concerning the hydrolytic stability of 8-aryl-2′-deoxyguanosine nucleoside adducts: implications for abasic site formation at physiological pH. *J. Org. Chem.* **2009**, 74, 5793–5802.
5. Richard Manderville, private communications.
6. Adams, S.P.; Kavka, K.S.; Wykes, E.J.; Holder, S.B.; Galluppi, G.R. Hindered dialkylamino nucleoside phosphite reagents in the synthesis of two DNA 51-mers. *J. Am. Chem. Soc.* **1983**, 105, 661–663.
7. Reese, C.B.; Serafinowska, H.T.; Zappia, G. An acetal group suitable for the protection of 2′-hydroxy functions in rapid oligoribonucleotide synthesis. *Tetrahedron Lett.* **1986**, 27, 2291–2294.
8. Habus, I.; Agrawal, S. Improvement in the synthesis of oligonucleotides of extended length by modification of detritylation step. *Nucleic Acids Res.* **1994**, 22, 4350–4351.
9. Ravikumar, V.; Andrade, M.; Mulvey, D.; Cole, D.J. Carbocation scavenging during oligonucleotide synthesis. US Patent 5,510,476, 1996.
10. Millar, S.; Yan, H.; Sanghvi, Y.S.; Pon, R. Some observations on detritylation in solid-phase oligonucleotide synthesis. *Nucleic Acids Symp. Ser.* **2008**, 52, 311–312.
11. Krotz, A.H.; McElroy, B.; Scozzari, A.N.; Cole, D.L.; Ravikumar, V.T. Controlled detritylation of antisense oligonucleotides. *Org. Proc. Res. Dev.* **2003**, 7, 47–52.
12. Chattopadhyaya, J.B.; Reese, C.B. The 9-phenylxanthen-9-yl protecting group. *J. Chem. Soc., Chem. Commun.* **1978**, 639–640.

13. Gaffney, P.R.J.; Liu, C.; Rao, M.V.; Reese, C.B.; Ward, J.G. Some substituted 9-phenylxanthen-9-yl protecting groups. *J. Chem. Soc., Perkin Trans 1*, **1991**, 1355–1360.
14. Song, Q.; Khammungkhune, S.; Ross, B.S.; Griffey, R.H. Substituted pixyl protecting groups for oligonucleotide synthesis. PCT Int. Appl. WO 2005077966 A1. 2005.
15. Experiments were conducted under identical conditions as reported in reference 17.
16. The 5′-*O*-DMPx protected nucleoside building blocks **2a–c** were prepared in the same fashion as described in R.T. Day, D. Williams, P. Soriano, and Y.S. Sanghvi, Large-scale synthesis of 5′-*O*-pixyl protected 2′-deoxynucleosides useful for oligonucleotide synthesis. *Nucleos. Nucleot. Nucleic Acids* **2005**, 24, 1135–1138.
17. Reese, C.B.; Yan, H. Alternatives to the 4,4′-dimethoxytrityl (DMTr) protecting group. *Tetrahedron Lett.* **2004**, 45, 2567–2570.