Kinetic studies on depurination and detritylation of CPG-bound intermediates during oligonucleotide synthesis

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ABSTRACT

Fully protected CPG-immobilized monomer, dimer and trimer oligonucleotides were used to study depurination during the chemical synthesis of oligonucleotides. Disappearance of the oligonucleotide during acid exposure time relative to an internal thymidine standard not subject to depurination was monitored by reverse phase HPLC analysis. Depurination half-times obtained for dichloroacetic acid (DCA) and trichloroacetic acid (TCA) in methylene chloride were found to be 3% DCA >> 15% DCA > 3% TCA. In order to understand the implications of depurination during DNA synthesis, the detritylation kinetics of model compounds DMT-dG-pT dimer and DMT-[17mer] mixed-base sequence were also measured. These results improve our ability to properly balance the contradictory goals of obtaining maximum detritylation with minimum depurination in oligonucleotide synthesis.

INTRODUCTION

It is widely known that support-bound oligonucleotide intermediates exhibit some degradation under the acidic detritylation conditions of solid phase oligonucleotide synthesis (1–7). The most commonly observed acid-catalyzed events leading to artifacts in oligomer synthesis are cleavage of the glycosidic bond of N6-benzoyldeoxyadenosine residue, and to a lesser extent N2-isobutyryldeoxyguanosine (1–7).

Typically, the protic acids trichloroacetic acid (TCA, pKa = 0.7) or preferably (1–3) dichloroacetic acid (DCA, pKa = 1.5) are used at concentrations of 2–3% in dichloromethane to liberate free 5′-hydroxyl group prior to chain extension. While generally regarded as acceptable for small scale oligonucleotide synthesis, use of the above reagents has often provided far less effective results in large scale synthesis (>100 µmol). For example, use of the strongly acidic TCA leads to significant depurination, while use of less acidic DCA may lead to incomplete detritylation (<98–99%).

In order to overcome the problems associated with use of protic acids, methods have been reported in the literature for performing acid-catalyzed detritylation under non-protic conditions, such as with the Lewis acid zinc bromide (8,9). Alternatively, different exocyclic amine protecting groups such as N-methyl 2-pyridylamine (10) have been employed to reduce dA residue susceptibility to depurination. Others have suggested the abandonment of 5′-O-trityl derivative in favor of using acetal, acyl or silyl protection of 5′-hydroxyl group (reviewed in 11). Despite these attempts, use of dimethoxytrityl group and haloacetic acids remains the dominant combination of 5′-OH protecting group and deprotection reagent used in oligonucleotide synthesis.

As part of an effort to optimize large scale phosphoramidite-mediated DNA synthesis, it was discovered that DCA and TCA bind strongly to the immobilized oligonucleotide during the detritylation reaction (14). For the detritylation reaction to take place at a maximum rate, about one molecule of acid must be bound per monomer unit of oligonucleotide. Thus, more acid must be introduced as the oligomer chain length increases throughout the synthesis. It was further shown that use of a high DCA concentration (15% versus 3%) gave superior results as judged by product purity obtained during large scale synthesis of a 17mer. These results were unexpected because it is generally believed that such higher acid concentrations lead to excessive depurination.

For the above reason and because comparative data on the effects of variation in Deblock acid strength and concentration for a given X-CPG construct were in general lacking, a more thorough examination was warranted.

MATERIALS AND METHODS

CPG support materials and DNA synthesis reagents

Deblock reagents containing 3% (v/v) DCA and 3% (w/v) TCA, as well as other standard DNA synthesis reagents, were obtained from PerSeptive Biosystems (Framingham, MA). Other Deblock solutions (7% and 15% v/v) were formulated from DCA purchased from Aldrich Chemical (Milwaukee, WI) and HPLC grade dichloromethane from J. T. Baker (Phillipsburg, NJ).

The 5′-DMT-dN-CPG from PerSeptive Biosystems were either used directly in depurination studies or for preparation of dimer or trimer X-CPG materials on an Expedite model 8909 DNA synthesizer (PerSeptive Biosystems). Uncapped 5′-HO-dN-CPG materials (with free 5′-hydroxyl group) were synthesized using the standard Expedite 5′-O-acetylated) 5′-Ac-dN-CPG materials were prepared from 5′-DMT-protected starting materials using a modified final Deblock manual prime cycle.

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synthesizer protocol. In this protocol the total exposure time and volumes of Cap A and Cap B solutions used in the capping step were increased to ensure complete capping. Fully protected dimer supports 5'-DMT-dAp-T-CPG and 5'-DMT-dAp-T-CPG, as well as trimer support 5'-DMT-Tp-dAp-T-CPG, were prepared using standard diisopropylamino-β-cyanethyl phosphoramidite(s) and Expedite® 8909 synthesizer 'DNA 15 μmol' protocols. Capped dimer supports 5'-Ac-[dimer]-CPG were prepared from the corresponding 5'-DMT-[dimer]-CPG supports as described above for capped monomer support. A fully protected 17mer sequence (14) was prepared using Ultra High Load CPG and 8800 synthesizer (both from PerSeptive Biosystems). This 5'-DMT-d(TCAATGGACCTACGGT)-UHLC-CPG was used in detritylation studies only.

CPG support mixtures were prepared by weighing equal mass amounts of each dried CPG component into a tared brown glass bottle. Only one ternary CPG mixture was used in this work, that containing 5'-DMT-dG-CPG, 5'-DMT-dA-CPG and 5'-DMT-T-CPG components ('G/A/T Mix'). Binary and ternary CPG mixtures were thoroughly mixed before use in depurination reactions.

Measuring depurination kinetics

A simple assay format without induced experimental or theoretical bias was desired; one which is independent of any assumptions about extinction coefficients, hypochromicity or the sample aliquot size. From the literature, the depurination method of Froehler and Matteucci (6) appeared most amenable to systematic extension. Their work demonstrated the practical advantages of using a mixture consisting of DMT-dA-CPG support (susceptible to depurination) and an internal control consisting of DMT-T-CPG support (which is stable against glycosidic bond cleavage). After washing of a CPG time point sample to remove noncovalently attached nucleosidic material, the ratio of dA:T nucleoside after ammonia cleavage provides a precise estimate of depurination. In the present work, this internal reference CPG mixture format was employed with only minor modification.

For each kinetic run using a binary CPG mixture, 120 ± 10 mg of CPG mixture were weighed into a 10 ml volumetric flask. For the kinetic run using the ternary CPG mixture, 180 ± 10 mg of CPG were used. Duplicate ~1.0 ml CPG/Deblock mixture samples were taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to the kinetic run using the ternary CPG mixture, 180 ± 40 mg of CPG were used. Duplicate ~1.0 ml CPG/Deblock mixture samples were taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to samples taken with a disposable transfer pipette at appropriate intervals and each sample was separately added to each fraction.

The presence of DMT species was measured by absorbance at 495 nm with a GBC Scientific (Dandenong, Victoria, Australia) model 920 spectrophotometer. Samples with A495 >1.5 were diluted with 0.1 M toluenesulfonic acid in acetonitrile so that absorbance could be measured in the linear range. A495 and cumulative %A495 (i.e., sum of A495 [fractions (1 ton)]) divided by sum of A495 [all fractions]) versus fraction number were then plotted. The comparative endpoint was arbitrarily defined as the earliest fraction for which cumulative %A495 was ≥99%. The time required to achieve detritylation endpoint was then calculated as the endpoint fraction number multiplied by the number of pulses per fraction collected.

RESULTS AND DISCUSSION

The dA-support: monomer depurination

The observed depurination half-time for N-benzoyl protected 5'-DMT-dA-CPG support in the presence of 3% DCA (1.3 h) is consistent with the literature data for 2% DCA (6). Measured depurination half-time values for this material and the corresponding 5'-hydroxyl derivative are summarized in Table 1.

Several conclusions can be drawn from this data. There is no significant difference in observed depurination half-times between 5'-DMT and free 5'-OH constructs in the three Deblock reagents used. It may be concluded that in these experiments the presence of free and/or ion-paired DMT cation does not contribute to the depurination rate. Relative to 3% DCA, use of higher DCA
concentration (15%) resulted in 3-fold faster dA\textsuperscript{Bz} depurination, while use of the stronger acid 3% TCA exhibited 4-fold faster depurination. Although this appears to confirm the fears about stronger detritylation conditions, it also indicates that for DCA the rate of depurination increases more slowly than acid concentration. The data also indicate that the adenosine support is highly labile in acid, with a half-life in 3% DCA of 77 min and in 3% TCA of only 19 min. Large scale synthesis may require >10 min/cycle for detrbling, thus the use of dA-support presents a considerable risk even if the exposure time is effectively minimized.

**Table 1. Monomer depurination**

<table>
<thead>
<tr>
<th>Deblock</th>
<th>t\textsubscript{1/2} (h)</th>
<th>t\textsubscript{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% DCA</td>
<td>1.29 ± 0.11</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>15% DCA</td>
<td>0.42 ± 0.04</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>3% TCA</td>
<td>0.32 ± 0.04</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

Data for monomer species DMT-dA-CPG and its 5'-OH derivative obtained by detritylation.

**Dimer depurination**

The dimer depurination data shown in Table 2 indicate that separately, both 5'- and 3'-phosphotriester substitution of dA residue provide significant stabilization against depurination. The lowest depurination rates in the dimer studies were observed with a dA residue bearing both 3'-succinyl and 5'-phosphotriester substitution. The 5'-acetyl configurations of 5'-R-dApT-CPG and 5'-R-Tp-dA-CPG constructs can be viewed as simplified models of 5'-phosphotriester substitution in the homologous trimer species 5'-DMT-dNp-dApT-CPG and 5'-DMT-dNp-Tp-dA-CPG, respectively, wherein any non-electronic effects on depurination susceptibility would be expected to be absent due to the simple structure of the acetyl group. Indeed, these models exhibit depurination data consistent with a through-bond electron-withdrawing effect. Since the 5'-Ac-dApT-CPG depurination half-times are each 4-fold greater than those for the corresponding 5'-DMT material, it follows that 5'-DMT-dNp-dApT-CPG trimer species are expected to be significantly more stable to depurination than the dimer 5'-DMT-dApT-CPG. This is correct, as shown below in Table 3. In contrast, comparing 5'-DMT-Tp-dA-CPG with 5'-Ac-Tp-dA-CPG, there is no difference whatsoever in depurination half-times, as predicted based on the inability of a through-bond electron withdrawing effect to operate effectively across such a large number of bonds. These results suggest that only adjacent dNp- and/or -pN residues (i.e., 5'- and/or 3'-phosphotriester substitution of the dA residue) provide significant change in susceptibility to depurination. Further formal addition of nucleoside units in either direction from a dA site susceptible to depurination, e.g. that corresponding to chain extension during oligomer synthesis, should not significantly affect the depurination rate at that site.

The results in Tables 1 and 2 demonstrate that depurination occurs to the greatest extent when a dA residue is at the 5'-end of the nascent oligomer chain, particularly during initial detritylation of DMT-dA-CPG. Once the subsequent nucleotide is added the stability goes up markedly due to the electron withdrawing effect of the added phosphotriester group, as is evident upon formal phosphotriester substitution of dA monomer to produce dimer and trimer species (16). That this effect is primarily or exclusively electronic in nature is suggested by the fact that 5'-O-acetyl substituted dA-CPG (data not shown) and dApT-CPG constructs exhibit a similar stabilizing effect, with no apparent structural basis for exerting such effect by an alternate mechanism. To understand the depurination susceptibility of nucleic acids in aqueous environments, electrostatic and base stacking interactions as well as secondary and/or higher-order structure effects must be considered (16). In contrast, the depurination susceptibility of uncharged, fully-protected support-bound oligomer species in organic solvent-based Deblock mixtures is expected to be free of such structural effects.

**Table 2. Dimer depurination**

<table>
<thead>
<tr>
<th>Support Solvent</th>
<th>Dimer CPG</th>
<th>Deblock Solvent</th>
<th>t\textsubscript{1/2} (h)</th>
<th>t\textsubscript{1/2} (h)</th>
<th>AcO/DMT Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>dApT-CPG</td>
<td>3% DCA</td>
<td>3.4 ± 0.2</td>
<td>14.3 ± 2.3</td>
<td>4.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>7% DCA</td>
<td>2.5 ± 0.1</td>
<td>9.7 ± 1.7</td>
<td>3.9 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% DCA</td>
<td>1.9 ± 0.1</td>
<td>8.3 ± 3.5</td>
<td>4.4 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% TCA</td>
<td>1.2 ± 0.1</td>
<td>4.8 ± 1.3</td>
<td>4.1 ± 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TpdA-CPG</td>
<td>3% DCA</td>
<td>18.6 ± 1.2</td>
<td>17.6 ± 1.6</td>
<td>1.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>7% DCA</td>
<td>11.5 ± 2.1</td>
<td>11.8 ± 1.3</td>
<td>1.0 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% DCA</td>
<td>6.5 ± 0.5</td>
<td>7.6 ± 1.2</td>
<td>1.2 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% TCA</td>
<td>5.5 ± 1.0</td>
<td>4.9 ± 0.5</td>
<td>0.9 ± 0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*Ratios A, B and C are as defined in the Results and Discussion. Ratio A describes relative depurination for trimer versus dA monomer, Ratio B that for trimer versus 5'-DMT-dA-pT support, and Ratio C that for trimer versus 5'-DMT-Tp-dA.

**Table 3. Trimer DMT-Tp-dApT-CPG depurination**

<table>
<thead>
<tr>
<th>Deblock Solvent</th>
<th>t\textsubscript{1/2} (h)</th>
<th>Ratio A\textsuperscript{a}</th>
<th>Ratio B\textsuperscript{a}</th>
<th>Ratio C\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% DCA</td>
<td>49.3 ± 3.2</td>
<td>38.3 ± 6.3</td>
<td>14.6 ± 1.7</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>7% DCA</td>
<td>30.9 ± 1.8</td>
<td>–</td>
<td>12.3 ± 1.0</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>15% DCA</td>
<td>24.1 ± 3.6</td>
<td>57.4 ± 15</td>
<td>12.7 ± 3.0</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>3% TCA</td>
<td>15.5 ± 2.8</td>
<td>48.3 ± 17</td>
<td>13.3 ± 3.4</td>
<td>2.8 ± 1.2</td>
</tr>
</tbody>
</table>

\*Ratios A, B and C are as defined in the Results and Discussion. Ratio A describes relative depurination for trimer versus dA monomer, Ratio B that for trimer versus 5'-DMT-dA-pT support, and Ratio C that for trimer versus 5'-DMT-Tp-dA.

**Trimer depurination: positional dependence**

Trimer DMT-Tp-dApT-CPG is a model for the general case of a dA residue internal to the oligomer sequence. It has triester substitution on both 3'- and 5'-flanks, and the enhanced stabilization compared with that observed with single triester substitution (see Table B and Table C in data Table 3) is evidence for cooperative stabilization. Ratio A is the half-time of trimer depurination divided by that of the dA support (DMT-dA-CPG). This ratio represents the increased stability of an internal dA residue compared with the dA monomer on the support. Ratio B is the half-time of trimer depurination divided by that of the dimer DMT-dApT-CPG (in which dA is a 5'-terminal residue, although it is protected by a 3'-triester). An exposed
5′-terminal dA is more than one order of magnitude more labile than one in an internal position.

Ratio C is the half-time of trimer depurination divided by that of the dimer DMT-Tp-dA-CPG. An internal dA with triesters flanking both sides is about three times more stable than one at an ‘internal’ position with 5′-triester and 3′-succinyl groups. This indicates that oligonucleotides initiated from CPG-dA support remain significantly more vulnerable to depurination than other oligonucleotides, throughout the synthesis.

Depurination of adenosine versus guanosine

A direct comparison of relative acid lability was readily accomplished using a ternary 5′-DMT-dN-CPG mixture where dN was dA, dG and T (‘G/A/T Mix’). Results of this experiment are shown in Table 4.

Table 4. Comparing adenosine and guanosine

<table>
<thead>
<tr>
<th>Deblock Solvent</th>
<th>t(_{1/2}) (h)(^a) 5′-DMT-dA-CPG</th>
<th>t(_{1/2}) (h)(^a) 5′-DMT-dG-CPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% DCA</td>
<td>1.48 ± 0.17</td>
<td>9.85 ± 1.90</td>
</tr>
<tr>
<td>15% DCA</td>
<td>0.63 ± 0.08</td>
<td>2.98 ± 0.34</td>
</tr>
<tr>
<td>3% TCA</td>
<td>0.39 ± 0.07</td>
<td>4.64 ± 0.67</td>
</tr>
</tbody>
</table>

\(^a\)Depurination rates for dA and dG nucleosides were measured within the same experiment using ternary CPG mixture as described in Materials and Methods.

As expected, using the modified depurination assay format with the ternary CPG mixture, the observed dA depurination half-times are in good agreement with the corresponding values obtained using binary CPG mixture shown in Table 1. The corresponding dG depurination half-time values are 5–6-fold longer than those for dA depurination in DCA solutions, and ~12-fold longer in 3% TCA. From the data it can be seen that there should be no major problem from depurination of a dG residue at the 3′-terminal position of the sequence. It follows that dG depurination is likely to be insignificant in any other context, by analogy to the results presented for dA depurination as a function of CPG-oligomer structure.

Detritylation kinetics

Ideally, the time required to achieve an efficient detritylation yield (>99%) would be dependent only on the absolute kinetics of the detritylation reaction at a given acid concentration. In practice, all heterogeneous phase detritylation processes (including those in batch mode) have significant flow-through character, thus allowing the possibility that chromatography effects may substantially influence or control the overall results. Such chromatography effects include the retardation of detritylation due to Deblock acid binding by the oligomer (14) and recapture of ‘free’ DMT cation by 5′-OH group (retritylation). In consequence, rigorous kinetic analysis may be very complex, and results may be subject to considerable perturbation with apparently small changes in reactor column design. Rather than attempting a detailed kinetic analysis of this complex situation, the practical approach of employing a fixed experimental reactor column configuration with an endpoint defined by eluted DMT group absorbance was taken.

Small scale detritylation kinetics experiments employed two DMT-oligomer-CPG constructs. The first construct, the dimer support DMT-dG-pT-CPG loaded at ~30 µmol/g, was chosen as a model of an oligonucleotide early in its synthesis when minimal acid binding to the support would be expected. The second construct, a DMT-[17mer]-CPG made on higher loaded support (~100 µmol/g), was chosen to be representative of later syntheses when acid binding due to the increased mass of DNA on the CPG would be significant. The model CPGs were placed in column reactors, and then exposed to various acid concentrations in continuous flow mode as described in the Methods.

Plots of detritylation data for DMT-dG-pT-CPG and DMT-[17mer]-CPG supports are shown in Figure 1 (normalized absorbance [A495] versus time) and Figure 2 (cumulative A495 minus time). From the integrated DMT group elution data, the 99% detritylation endpoint values were derived. These endpoint values are summarized in Table 5.

As expected, for the CPG-dimer support model with low acid binding capacity, detritylation using 3% DCA was quite rapid and exhibited no significant tailing. Although the use of higher concentration of the same acid (15% DCA) or the use of a stronger acid (3% TCA) provided somewhat faster time to peak DMT absorbance and 99% endpoint, there is no compelling reason to use either reagent in place of 3% DCA. With 3% TCA, since detritylation must be at least 3-fold faster than that with 3% DCA to compensate for the increased depurination rates shown in Tables 1 and 3, it is clear that 3% TCA will not provide the highest purity or recovery of crude DNA.

However, most oligomer synthesis targets of interest are significantly longer than dimer. Thus the CPG-[17mer] support model with high acid binding capacity more closely represents
Figure 2. Detritylation kinetics in 3% DCA, 15% DCA and 3% TCA for CPG-dimer (DMT-dG-pT-CPG) and CPG-[17mer] model systems. For a complete description of 17mer construct, see Materials and Methods. Data are shown as cumulative A495 nm values, i.e., sum of A495 [fractions (1 to n)] divided by sum of A495 [all fractions]. Dimer detritylation: 3% DCA (□), 15% DCA (○) and 3% TCA (▲). 17mer detritylation: 3% DCA (□), 15% DCA (○) and 3% TCA (▲).

the general case in oligomer synthesis. Further, synthesizer Deblock cycles are optimized to reflect the worst-case situation, and no attempt is made to shorten early Deblock cycles even though such a maneuver would improve crude product purity by reducing depurination. Indeed, as is apparent by inspection of Figures 1 and 2 and Table 5, the use of 3% DCA incurs a significant penalty in time to reach the 99% endpoint with the UHL-CPG-[17mer] support (50% more) versus CPG-[2mer] support. An unanticipated result is that even greater relative retardation was exhibited with 3% TCA (100% more), apparently due to the presence of significant tailing in the more difficult, high acid binding capacity context. In contrast, use of 15% DCA exhibited the lowest retardation (29% increase) due to the burden of increased acid binding capacity. These results are consistent with and well rationalized by the acid binding phenomena reported in the companion paper (14).

Table 5. Detritylation endpoints

<table>
<thead>
<tr>
<th>Deblock solvent</th>
<th>SS-[2mer]</th>
<th>SS-[17mer]</th>
<th>17mer/2mer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t(99%) (s)</td>
<td>t(99%) (s)</td>
<td>Ratio</td>
</tr>
<tr>
<td>3% DCA</td>
<td>24</td>
<td>36</td>
<td>1.5</td>
</tr>
<tr>
<td>15% DCA</td>
<td>14</td>
<td>18</td>
<td>1.3</td>
</tr>
<tr>
<td>3% TCA</td>
<td>12</td>
<td>24</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Any chromatography effects observable in small scale experiments will clearly be understated relative to those which could be shown in large scale synthesis (14). However, it is evident from the small scale detritylation results discussed above that consideration of the chromatographic characteristics of the solid support material used is relevant to detritylation protocol optimization on any scale.

Are the depurination and detritylation data presented here consistent with the proposed large scale synthesis practice of using 15% DCA for deblocking (14), when there is significantly more depurination occurring at higher acid concentrations? The half-times for depurination with 15% DCA are significantly less than expected (relative to lower [DCA], e.g. 3%) if there was a linear relationship between half-time and [DCA]. In Figure 3, it is shown that log–log plots of depurination half-times versus [DCA] are linear for each CPG-oligomer model. It has been observed that a certain oligomer length-dependent stoichiometry of acid binding must be overcome in order to begin actual detritylation on CPG support, thus a high concentration of weak acid (e.g., 15% DCA) is more effective at driving detritylation toward completion than either low [weak acid] such as 3% DCA or low [strong acid] such as 3% TCA (14). Given that depurination is known to be acid-catalyzed (1–7) and based on pKₐ values the N7 position of dA residues present must be involved in the acid binding phenomena reported (14), it appears likely that depurination must occur during the acid-binding lag time which precedes the onset of detritylation. It follows that the optimal detritylation approach is to use the Deblock reagent which most effectively minimizes such lag time while supporting rapid detritylation, namely 15% DCA.

In summary, the use of well-defined fully protected CPG-monomer, CPG-dimer and CPG-trimer constructs as model systems allows us to understand and predict the susceptibility to depurination of any particular proposed target sequence in oligonucleotide synthesis by the standard phosphoramidite method. While this is of interest with regard to oligomer synthesis on any scale, it is of particular importance regarding the development of cost-effective high performance strategies for large scale oligonucleotide synthesis. A theoretical rationale for the surprising practical utility in large scale synthesis of using 15% DCA as Deblock reagent, and of the clear advantage of using...
15% DCA compared with 3% TCA as Deblock reagent, also are apparent in the results from this work.

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