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A Novel Catechol-Based Universal Support for Oligonucleotide Synthesis

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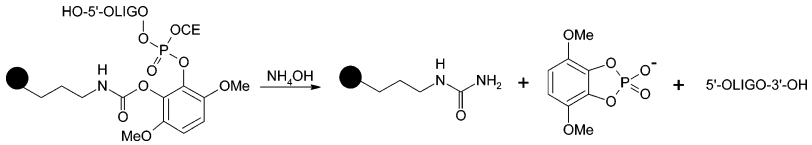

A novel universal support for deoxyribo- and ribonucleic acid synthesis has been developed. The support, constructed from 1,4-dimethoxycatechol, represents an improvement over existing universal supports because of its ability to cleave and deprotect under mild conditions in standard reagents. Because no nonvolatile additives are required for cleavage and deprotection, the synthesized oligonucleotides do not require purification prior to use in biochemical assays. Using reverse phase HPLC and electrospray mass spectroscopy, it was determined that oligonucleotides synthesized on the universal support (UL1) 3′-dephosphorylate quickly (9 h in 28−30% ammonium hydroxide (NH_4OH) at 55 °C, 2 h in 28−30% NH_4OH at 80 °C, or <1 h in ammonium hydroxide/methylamine (1:1) (AMA) at 80 °C). Oligonucleotides used as primers for the polymerase chain reaction (PCR) assay were found to perform identically to control primers, demonstrating full biological compatibility. In addition, a method was developed for sintering the universal support directly into a filter plug which can be pressure fit into the synthesis column of a commercial synthesizer. The universal support plugs allow the synthesis of high-quality oligonucleotides at least 120 nucleotides in length, with purity comparable to non-universal commercial supports and ∼50% lower reagent consumption. The universal support plugs are routinely used to synthesize deoxyribo-, ribo-, 3′-modified, 5′-modified, and thioated oligonucleotides. The flexibility of the universal support and the efficiency of 3′-dephosphorylation are expected to increase the use of universal supports in oligonucleotide synthesis.

Introduction

Oligonucleotides are typically synthesized on a solid support such as controlled pore glass (CPG) or polystyrene (PS).[1] The 3′-oxygen of the first nucleoside is preattached to the solid support by a base-labile tether such as a succinate or hydroquinone.[2,3] These linkers were adopted early in the development of oligonucleotide synthesis because standard phosphoramidite monomers contain a protected 3′-phosphorus which must be removed postsynthesis for the oligonucleotide to be biologically active. Finding a convenient method for complete 3′-dephosphorylation proved difficult. Thus, solid supports are preloaded with the first nonphosphorylated nucleoside to eliminate the need for downstream 3′-dephosphorylation.

Laboratories that produce synthetic oligomers, therefore, must manage a library of solid supports to enable the synthesis of commonly produced oligomers, including standard deoxyribo-, 3′-modified, 5′-modified, and ribooligomers. In addition, many laboratories routinely use several nucleobase protection strategies, so they must maintain large libraries of compatible supports including isobutyryl (*i*Bu),[4] acetyl (Ac),[5] dimethylformamidyl (DMF),[6,7] phenoxyacetyl (PAC),[8] and *tert*-butyl phenoxyacetyl

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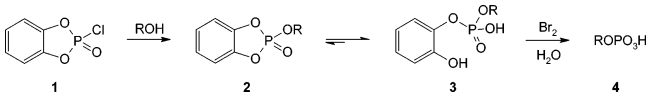
(TAC).[9,10] Managing libraries is difficult and necessitates accurate handling or the use of an automated system so that supports can be tracked and correctly loaded into the appropriate position on a synthesizer. Incorrect loading results in an oligomer with a 3′-error that has reduced or no biological functionality.[11] A universal support is particularly important for use in high-throughput oligonucleotide synthesis where manually loading supports in high-density titer plates is time-consuming, error-prone, and cumbersome.[12−14] Universal supports are also expected to reduce side reactions that take place on the preattached nucleoside of standard supports[15] because the universal support does not have a preattached nucleoside.

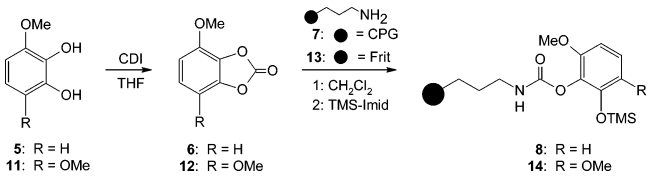
The need for a universal synthesis support that increases flexibility and reduces 3′-sequence errors has drawn the attention of several groups.[16−22] A recent review reported that today's commercially available universal supports have not met the promise of increased flexibility and error reduction due to inefficient dephosphorylation or the requirement for cleavage and deprotection steps that are incompatible with established production lines.[20] For example, one universal support requires the addition of lithium chloride (LiCl) for efficient dephosphorylation, which must be removed by precipitation or purification prior to use.[21] Another support[16] requires harsh treatment with AMA for 17 h at 55 °C, thus limiting its compatibility with production pipelines that produce material more sensitive to harsh alkali treatment.

One promising universal support (Universal Support II) is commercially available. This support does not require nonvolatile additives for 3′-dephosphorylation; therefore, desalting or other purification is unnecessary. One disadvantage of this universal support is incomplete cleavage of oligonucleotides from the support matrix caused by a side reaction that occurs during dephosphorylation.[23] This side reaction produces a minor (∼20%) phosphorylated, uncleaved species. Though it is possible to compensate for reduced yield by increasing the amount of starting material, larger reagent excesses and increased reaction times for coupling may be necessary to prevent reduced oligo quality. Another problem is that cleaving the linker from the support is favored by an organic base (such as 2−3 M methanolic ammonia), but removing protecting groups from the nucleobases (deprotection) is favored by an aqueous base (such as NH_4OH). Since cleavage and deprotection steps are favored under slightly orthogonal conditions, they cannot be easily combined into a single procedure using a standard reagent such as NH_4OH or AMA.

SCHEME 1



SCHEME 2



The catechol-based universal support described in this work has several characteristics that should enable greater incorporation into academic and industrial laboratories: (1) the oligonucleotide and linker cleave from the support quickly (<1 h); (2) dephosphorylation reactions are quantitative in standard cleavage and deprotection reagents; (3) the linker is compatible with a variety of synthesis chemistries, especially the synthesis of DNA, most 5′- and 3′-modifications, and RNA; and (4) the linker enables the synthesis of high-quality oligonucleotides that are functional in biochemical reactions without any postsynthesis cleanup or purification.

Results and Discussion

(a) Initial Strategy. After screening several unsuccessful candidates for a universal support, we chose to produce a dephosphorylator prepared from commercially available catechols.[24] Catechol (1,2-benzenediol or pyrocatechol) and its derivatives, such as catechin and urushiols, are found naturally in woody plants, including mahogany and poison oak leaves. Purified by distillation of catechin, catechols are used industrially as dyeing agents, as a photographic developing chemical, and in electroplating.[25] Investigating catechol derivatives as universal dephosphorylators was not an obvious choice since catechol-based agents such as *o*-phenylene phosphorochloridate **1** (Scheme 1) are known to be highly reactive phosphorylators.[26] Compound **1** reacts with aliphatic alcohols to yield cyclic triesters **2** which are readily hydrolyzed into the corresponding ring-opened phosphate diester **3**.[26] The *o*-hydroxyphenyl esters **3** were found to be stable under acidic and basic conditions, although the catechol groups could be oxidatively removed. Treated with an excess of bromine in a neutral aqueous buffer, the diester **3** is converted to a monoester **4**.

Initial attempts to produce a universal linker prepared from commercially available catechols, such as catechol, 3-fluoro-

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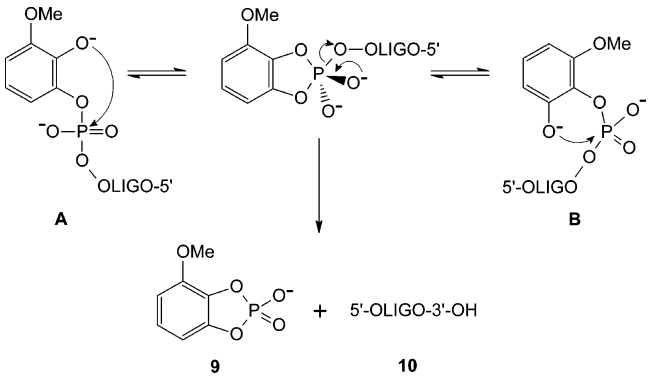
SCHEME 3



catechol, 3-nitrocatechol, and 3-methylcatechol, confirmed previous observations[26] regarding the base stability of species **3** when tethered to an oligonucleotide. Early results from universal supports based on these compounds were discouraging, showing little or no catechol-mediated 3′-phosphate elimination under standard deprotection conditions. However, investigation into methoxy catechols produced encouraging results.

(b) Reactions with 3-Methoxycatechol and 1,4-Dimethoxycatechol. 3-Methoxycatechol **5** (Scheme 2), when tethered to an aminopropyl support **7**, was the first catechol-based compound to show any dephosphorylation. Cleavage and deprotection of oligonucleotides synthesized using this linker tethered to CPG **7** produced ~80% release of 3′-hydroxyoligonucleotides in 3 h at 80 °C in NH_4OH.

The first steps in the sequence of events were concomitant and rapid deblocking of the cyanoethyl protection groups and cleavage of the carbamate tether. HPLC analysis showed that the ratio of the two resulting phosphate diesters **A** and **B** was roughly 1:1. These compounds underwent an intramolecular cyclization followed by release of 3′-hydroxyoligonucleotide **9** and elimination of the cyclic catecholphosphate **10** (Scheme 3). One of the isomers, presumably **A**, disappeared rapidly (by HPLC analysis) with a proportional appearance of the corresponding 3′-native oligonucleotide **9**. After deprotection for 1 h, equilibrium was reached between species **A** and **B**, found respectively in about a 2:5 ratio. Complete disappearance of species **A** and **B** took 3 h. The faster disappearance of species **A** led to the next step of investigating 1,4-dimethoxycatechol for a quicker release of native oligonucleotides.

A series of reactions was therefore performed using the 1,4-dimethoxycatechol-based universal linker **14** (Scheme 2). 1,4-Dimethoxycatechol **11** was synthesized from 2,5-dimethoxybenzaldehyde in four steps, according to published literature.

(c) Utilization of the Universal Linker on a Synthesizer. We chose to sinter the support directly into the filter matrix (frit) of the reaction column. Sintering the CPG in the synthesis plugs gave several advantages, including improved control over CPG "splashing" out of wells when used on multiwell synthesizers[27] and improved reagent retention for long reaction times due to the high density of the filter matrix. Filters were made from a slurry of aminopropyl CPG **7** and polypropylene powder that were mixed, poured into a cylindrical mold, rapidly sintered at 190 °C, then cooled and demolded. Once the bare CPG was embedded in the filter matrix, 1,4-dimethoxypyrocatechol **12** was reacted with embedded aminopropyl CPG **13** which yielded **14** for testing in oligonucleotide synthesis.

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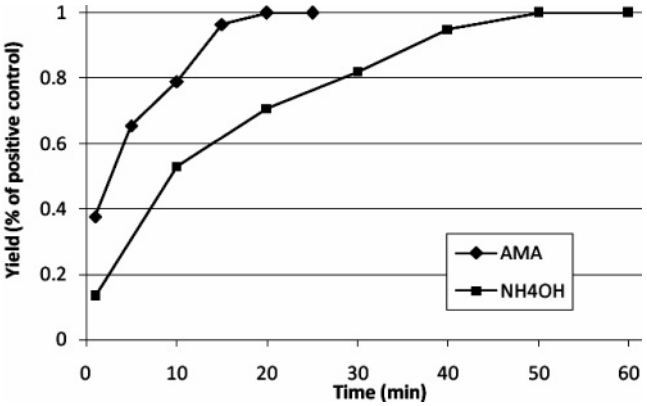


FIGURE 1. Time required for complete cleavage of a deoxythymidine T9 homopolymer from the catechol-based universal support.

TABLE 1. Summary of LC-MS Data Showing the Time Required to Fully Dephosphorylate the T9 Homopolymer

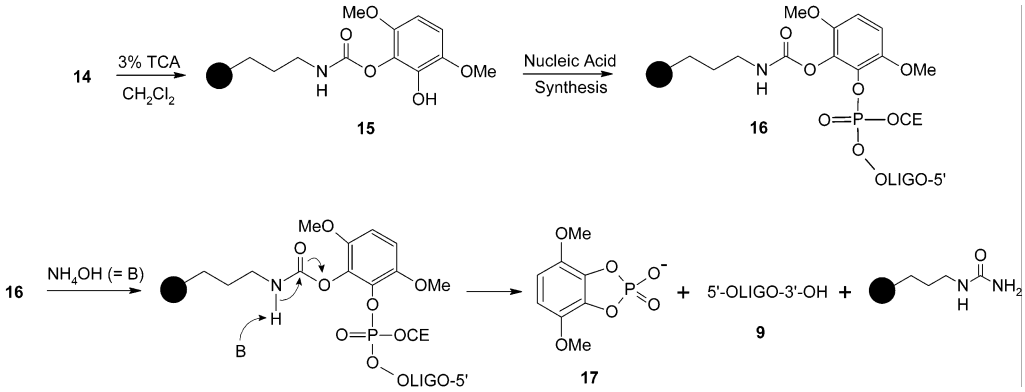
reagent	temp (°C)	time (h)
NH_4OH	55	9
	65	6
	80	2
AMA	55	5
	65	2
	80	<1

To test amenability of the plug and universal support to oligonucleotide synthesis, a 9mer thymidine homopolymer (T9) was synthesized and cleaved from the support at room temperature in standard cleavage/deprotection reagents, NH_4OH and AMA. Complete cleavage (relative to the same oligo synthesized on a standard CPG support) was achieved in about 15 min in AMA. In the milder cleavage reagent (NH_4OH), complete cleavage was achieved in about 45 min. These cleavage times were indistinguishable from those obtained when using a standard long-chain alkyl amine (LCAA) CPG support. A plot of full length product yield versus time is shown in Figure 1.

(d) Characterization of Dephosphorylation Kinetics on the Universal Support. We used another T9 oligonucleotide to empirically determine the rate and extent of dephosphorylation in standard cleavage/deprotection reagents. T9 oligos were synthesized and cleaved from the support at room temperature for 15 min in AMA and for 45 min in NH_4OH, then deprotected in the cleavage reagent in sealed tubes at the times and temperatures indicated in Table 1. If dephosphorylation was not complete, it was clearly identified by an increase of 232 atomic mass units (amu) on a mass spectrometer (MS traces are available in the Supporting Information). Relative abundance of catecholated and dephosphorylated oligonucleotide was determined by measuring the peak amplitude of the two species on LC-MS. Example LC data are shown in the Supporting Information for a cleavage reaction in NH_4OH at 55 °C for 1 and 17 h. Confirmation of the mass of the catecholphosphate T9 homopolymer during the kinetic studies, described in greater detail in the next section, supports the putative structure of **14**.

Identity of the T9 oligomer was obtained by electrospray mass spectroscopy. Identity traces showed the predicted monoisotopic half-mass of phosphorylated and dephosphorylated species. Shown in the Supporting Information is the electrospray MS trace showing the monoisotopic half-mass of the T9 homopolymer after dephosphorylation in NH_4OH for 1 h. The expected monoisotopic half-mass for a T9 oligomer is 1337.2 amu and increases to 1453.9 amu for a T9 homopolymer with 3′-catechol

SCHEME 4



phosphate still attached. Because 3′-phosphorylated contaminants can be identified and quantitated using this method, a profile was easily generated showing the amount of time required to fully dephosphorylate oligonucleotides in common deprotection reagents. A summary of the mass results for each deprotection time point is shown along with the time needed to fully dephosphorylate in the corresponding reagent in Table 1. The time required for dephosphorylation is similar to the time required for complete removal of dG(ibu) protection groups,[4] demonstrating that the universal support will not present a bottleneck during deprotection.

(e) Selection of Hydroxyl Protection. Oligonucleotide monomers and supports are typically protected with a dimethoxytrityl (DMT) or monomethoxytrityl (MMT) group. These protection groups are typically favored due to their quick removal in mild acid, thus reducing possible side reactions such as depurination. Another advantage during synthesis is the colorimetric assays that can be performed on the orange (DMT) or yellow (MMT) cation, which is used to monitor reaction progress. In this case, however, both DMT and MMT protection were not suitable because of their lability on the aromatic catechol. Instead, TMS protection was found to be more stable and is compatible with automated synthesizer cycles. TMS protection can take over 1 min to remove, although depurination is not a concern during its removal because of the non-nucleosidic nature of the support.

(f) Biological Compatibility of Synthesized Oligonucleotides. To test for the presence of any undesired side products or inhibition of enzymatic processes, the UL1 universal support was used to synthesize ~20mer oligonucleotides for use as PCR primers. Cyclic 1,4-dimethoxycatechol phosphate **17** (Scheme 4), if not completely removed from the primer, can act as an inhibitor in subsequent biological reactions. This is because enzymes such as *Taq* polymerase require a 3′-hydroxyl to initiate primer extension.

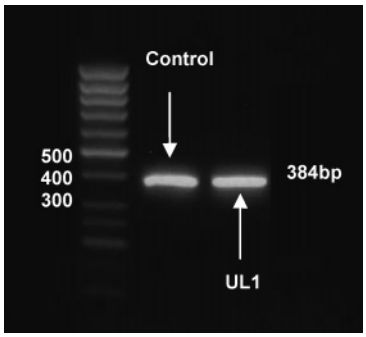


FIGURE 2. Nondenaturing agarose gel image of PCR products obtained from oligos produced using standard supports (control) and the same PCR product obtained using oligos produced on the universal support **14**.

PCR was performed using primers synthesized on the UL1 universal support. Following cleavage of the oligonucleotides from the support in NH_4OH, the primers were deprotected overnight at 55 °C, lyophilized, normalized to 3.2 μM, and used in PCR. Product bands were visualized on an agarose gel, shown in Figure 2.

The similar band intensities for the two PCR amplicons demonstrate that the amount of product obtained with the UL1 support-bound oligonucleotide is identical to that obtained with desalted control forward and reverse primers synthesized by a commercial oligonucleotide supplier. Since further purification or dephosphorylation is unnecessary, the oligonucleotides may be used directly in biological reactions.

It should also be noted that the proposed mechanism for oligonucleotide cleavage from the support (to produce **9** and **17** in Scheme 4) was tested to determine whether cleavage followed the proposed route on the *N*-alkylphenoxycarbamate or whether cleavage was driven through nucleophilic attack on the adjacent carbonyl. When the *N*-alkylphenoxycarbamate is replaced with a *N*,*N*-dialkylphenoxycarbamate tether, there is little or no cleavage of oligonucleotide, even when treated for up to 16 h at 80 °C in NH_4OH. This gives more evidence in support of the proposed mechanism.

(g) Characterization of Longmer Synthesis on UL1 Support. Due to the emergence of assays that rely heavily on high-quality, long oligonucleotides such as molecular inversion probes[28,29] (MIPs) and gene synthesis,[30−32] the linker was tested to determine suitability for the routine production of 40, 80, and 120 nt long oligonucleotides. The oligonucleotides were synthesized on the UL1 support and were compared to another commercially available nucleoside-specific support. Synthesis on the commercially available support was performed using the synthesis cycle recommended by the column manufacturer,

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whose reagent consumption was roughly 50% higher than that required by the UL1 universal support. The quality of the oligonucleotides was analyzed by HPLC (chromatograms are available in the Supporting Information). Little or no difference in quality was detected using the two different supports, although comparable quality was achieved on the UL1 universal support with ~50% lower reagent consumption. Synthesis of 120 nt oligonucleotides required ~9 h on both UL1 and the standard support.

(h) Synthesis of RNA on UL1 Supports. In general, the synthesis of RNA oligomers presents a special challenge due to the need for protection of the 2′-hydroxyl. In addition to possible steric hindrance during monomer coupling, 2′-protection with 2′-*O*-*tert*-butyldimethylsilyl (TBDMS) or triisopropylsilyloxymethyl (TOM) may also cause problems during deprotection of the nucleobases, resulting in strand cleavage.[33−35] Several approaches have been taken to alleviate this problem, most notably by substituting aqueous NH_4OH or AMA with a mixture of NH_4OH and ethanol (3:1), methylamine, or anhydrous ethanolic or methanolic ammonia. Another approach is to substitute standard nucleobase protection with more labile groups such as acetyl, dimethylformamidyl, phenoxyacetyl, or *tert*-butyl phenoxyacetyl. This reduces the time required for nucleobase deprotection, making premature removal of silyl protection groups less problematic. The nucleobase deprotection procedure used in our laboratories for routine RNA synthesis represents a successful tradeoff between deprotection time and the quality of finished products. For routine RNA synthesis, we have interchangeably used 2′-TBDMS and TOM-protected monomers and have removed nucleobase protecting groups using NH_4OH and ethanol (3:1) for 10 h at 55 °C.

Conclusion

Universal supports have moved the industry incrementally closer to adopting universal chemistries. However, the benefits of universal supports have not been fully realized because previously published universal supports require cleavage and dephosphorylation conditions that are cumbersome and/or limit the biological activity of the oligonucleotide. Because no widely adoptable universal support is available, laboratories are still forced to maintain a library of 3′-specific supports, to manually load them onto the correct position of a synthesizer, or to use a robotic liquid handler to load them into synthesis columns. These workarounds can be time-consuming, expensive, and error-prone.

The universal support presented in this paper is compatible with DNA, RNA, most 3′- and 5′-modifications, and phosphorthioate synthesis, without the need for additives to achieve efficient dephosphorylation. Thus, deoxyribo- and ribonucleic acids can be used directly after synthesis without the need for purification. Utilizing the UL1 support will also circumvent quality problems caused by *n*-branched oligonucleotides since branching is reported to originate from reactivity of the preattached nucleoside on a standard support. UL1 also makes it possible to produce 3′-degenerate oligonucleotides without having to manually add 2, 3, or 4 different 3′-specific supports into synthesizer columns. HPLC traces of oligonucleotides produced on the UL1 show that the oligonucleotides are of comparable purity to the control sequences synthesized on other commercially available synthesis columns (chromatograms are shown in the Supporting Information). The heat-sintered CPG filters also greatly reduced reagent consumption, reduced problems with the support "splashing" during synthesis, increased flexibility in synthesizer setup, and caused less error when loading synthesizer columns.

Experimental Section

Preparation of Aminopropyl CPG. Aminopropyl CPG **7** was prepared by reacting CPG (75/200, 1000 Å pore size) with aminopropyltriethoxysilane (APTEOS) following published procedures.[36]

Preparation of Cyclic 1,4-Dimethoxycatechol. 1,4-Dimethoxycatechol **11** was synthesized following published procedures[24] from commercially available catechol. Cyclic 1,4-dimethoxycatechol **12** was prepared from 1,4-dimethoxycatechol following published procedures,[37] except *N*,*N*′-carbonyldiimidazole was substituted for phosgene. Product **12** was confirmed on a TLC plate eluted in dichloromethane (DCM). The differential migration of **12** (retention factor (R_f) = 0.9) compared to **11** allowed easy confirmation of the product. The product was also confirmed by RP-HPLC.

Preparation of 100 nmol 1,4-Dimethoxycatechol-Based Cylindrical Frits. Cylindrical frits **13** were prepared by embedding aminopropyl CPG **7** in polypropylene. First, high-density polypropylene (125 g) and aminopropyl CPG **7** (85 g, 10 mol/g, 1000 Å pore size, 75/200 micron particle size) were mixed thoroughly, allowing uniform dispersion of the inorganic material into the thermoplastic resin. The mixture was poured into cylindrical cavities in an aluminum plate and milled to produce the desired size and dimensions. The plate was heated at 190 °C for 15 min and cooled prior to releasing the frits from the mold. The density of the frit prevented reagents from diffusing into the frit prior to application of a pulse of inert gas. Therefore, during coupling and capping steps, where two reagents were added to the frit, a hold step before chamber pressurization allowed premixing of the two reagents prior to entry into the resin.

A 1 L two-neck flask was equipped with a flush-closing PTFE drain valve and was loaded with 1000 100 nmol frits **13** in suspension with 100 mL of DCM. A solution of 200 mg of 1,4-dimethoxypyrocatechol **12** in 10 mL of tetrahydrofuran (THF) was added, and the flask was agitated overnight. A ninhydrin test[38] was used to detect disappearance of a primary amine, and additional 1,4-dimethoxypyrocatechol **12** was added until the primary amine was undetectable. The frits were drained and washed successively with acetone and DCM, then resuspended in a solution of 100 mL of DCM and 0.8 mL of TMS/imidazole. After shaking for 4 h, the frits were drained, washed with methanol, and dried under vacuum.

Prior to synthesis, a blunt-ended steel pin was used to insert the frits into open-ended polypropylene synthesis columns. After completing the synthesis, the frits were removed from the column by applying top pressure on the plug with a steel pin.

Synthesis of Oligonucleotides on UL1 Universal Supports. Synthesis of oligonucleotides was performed on a commercially available 48-position oligonucleotide synthesizer. Empty support columns and 100 nmol synthesis supports **14** were provided by CTGen Inc. Synthesis supports were pressure fit by hand into empty columns, which were then inserted into the carousel of the synthesizer. Deblocking solution, 3% trichloroacetic acid (TCA) in DCM, was used for removal of the TMS protecting group. Two

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150 μL aliquots were dispensed into each column, followed by a 1 s pressurization at 5 psi with a 60 s hold time after each dispensation to ensure complete TMS removal prior to the first coupling step. TCA was then drained from the column by applying a short (5 s) burst of argon. Next, a single wash was performed with 180 μL of dry acetonitrile. Coupling was performed by a single, near-simultaneous dispensation of 30 μL of 5-benzylthio-1*H*-tetrazole activator (BTT) and 35 μL of phosphoramidite. Therapure phosphoramidites, 5′-dimethoxytrityl-*N*-benzoyl-2′-deoxyadenosine-3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite, 5′-dimethoxytrityl-*N*-benzoyl-2′-deoxycytidine-3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite, 5′-dimethoxytrityl-*N*-isobutyryl-2′-deoxyguanosine-3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite, and 5′-dimethoxytrityl-2′-deoxythymidine-3′-[(2-cyanoethyl)-(*N*,N-diisopropyl)]phosphoramidite were purchased in 2 g bottles and dissolved in dry acetonitrile to a final concentration of 50 mM. Coupling was allowed to proceed for 20 s to ensure stepwise coupling efficiencies of at least 99%. Phosphoramidites and BTT were drained from the column. Next, the newly formed phosphite linkage was oxidized to a pentavalent phosphate using 0.02 M iodine in THF/H_20 followed by a 10 s hold. Following a drain step, capping (acylation) of truncated species was performed by dispensing 25 μL of Cap A (acetic anhydride, pyridine, THF (10:10:80)) and 25 μL of Cap B (16% *n*-methylimidazole in THF), followed by a 10 s hold step and a 5 s drain. The columns were washed with one aliquot of 180 μL of dry acetonitrile. The synthesis cycle was repeated for the introduction of each new base. For subsequent base additions, the only modification to the synthesis cycle was shortening the hold step after each TCA addition since subsequent 5′-deblocking steps remove the more labile 5′-dimethoxytrityl (DMT) group.

Synthesis of Long Oligonucleotides on Commercially Available Synthesis Supports. Synthesis of oligonucleotides on commercially available synthesis columns was performed using the synthesis cycles recommended by the column and instrument manufacturer. Oligonucleotides with respective sequences 5′-CCTTA CTGCC CGGGA AATAG GGAGA CCATC CGATG TCTAA-3′, 5′-ATCAC AATAA AGGTT GAAAC GGTGC AACAG TATGA TCCGA CCTTA CTGCC CGGGA AATAG GGAGA CCATC CGATG TCTAA-3′, and 5′-TCCAG TATTT AGTTG TTATG CTACG ACCAA TTCTT ACTAC ATCAC AATAA AGGTT GAAAC GGTGC AACAG TATGA TCCGA CCTTA CTGCC CGGGA AATAG GGAGA CCATC CGATG TCTAA-3′ were synthesized on a commercially available 48-position oligonucleotide synthesizer and were analyzed by RP-HPLC.

Measurement of Oligonucleotide Yield versus Cleavage Time. Oligonucleotides (5′-TTTTT TTTT-3′) were cleaved from universal supports using NH_4OH (28−30% ammonia in water). Optimal cleavage times were determined by exposing supports to NH_4OH in 5 min increments between 0 and 60 min. Columns were placed on a vacuum manifold and sealed with caps to prevent ammonia evaporation during incubation. A vacuum was applied to each column so that the cleaved oligonucleotide could be collected in a 1.5 mL polypropylene tube. Samples were immediately placed in a vacuum centrifuge for lyophilization. Oligonucleotides were resuspended in 100 μL of deionized water and 1 μL was taken and further diluted in 99 μL of diH_2O for absorbance measurements. Optical density at 260 nm was measured from 2 μL of diluted sample in triplicate on a fiber-optic spectrophotometer pedestal. Measurements were averaged, and absorbances were converted to initial concentration (μM) according to Beer's Law.

Quantification of Uncleaved Linker Following Deprotection. To check for the presence of oligonucleotides with uncleaved catechol linker or 3′-phosphate, the atomic mass of the cleaved product versus time was measured at three different temperatures, 55, 65, and 80 °C. For each temperature, deprotection was allowed to proceed for up to 17 h with measurements taken at 1 h increments from 0 to 8 h, the last time point was checked after 17 h. Deprotection was carried out in NH_4OH and then separately in AMA. Samples from early time points were kept frozen at −80 °C until deprotection was complete for all conditions. Oligonucleotides were then lyophilized in a vacuum centrifuge on medium heat, resuspended in 100 μL of diH_2O, and quantitated using the method previously described. Samples were analyzed for purity and mass by LC-MS (performed at the Stanford University Mass Spec Core Facility). The mass values obtained for samples run on the mass spectrometer were within 0.1% of the expected values, corresponding to the error typically seen in electrospray LC-MS.

Dephosphorylation kinetics were determined by measuring the relative sizes of peaks that correspond to the phosphorylated and dephosphorylated species over the time course specified above. Relative abundance was measured by peak amplitude, and identity was determined by the monoisotopic half-mass ($m/2z$). Phosphorylated species could also be readily distinguished on RP-HPLC chromatograms since these species have longer retention times than dephosphorylated species.

PCR with Catechol-Synthesized Oligonucleotide Primers. Forward (L_Primer_1719_pUC19_384; 5′-GATAC GGGAG GGCTT ACCAT-3′) and reverse primer (R_Primer_2102_pUC19_384; 5′-GATAA CACTG CGGCC AACTT-3′) were made on the universal linkers and compared with control primers synthesized on standard supports.

PCR was carried out on a 96-well thermal cycler using the following temperature profile: [94 °C 10 min], 30 cycles of [94 °C 30 s, 55 °C 45 s, 72 °C 2 min], [72 °C 7 min]. The reaction was performed in a total volume of 20 μL using 1× PCR Buffer II, 1.25 mM $MgCl_2$, 5U AmpliTaq gold DNA polymerase, 1 ng pUC19 plasmid, and 3.2 pmol of each forward and reverse primer. Primers were synthesized in-house at Stanford Genome Technology Center (SGTC) using a commercially available 48-position synthesizer using the chemistry cycle already described. PCR products were analyzed on a nondenaturing 0.9% agarose gel. PCR amplification produced a 384 bp band on the gel. Control primers were purchased from a commercial oligonucleotide house. A 50 bp DNA sizing ladder was used to determine amplicon size.

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Supporting Information Available: MS traces and LC chromatograms showing identity and purity of dephosphorylated oligos and quality of longmer synthesis on UL1 supports are shown. This material is available free of charge via the Internet at http://pubs.acs.org.

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