Polymerization on Solid Supports**

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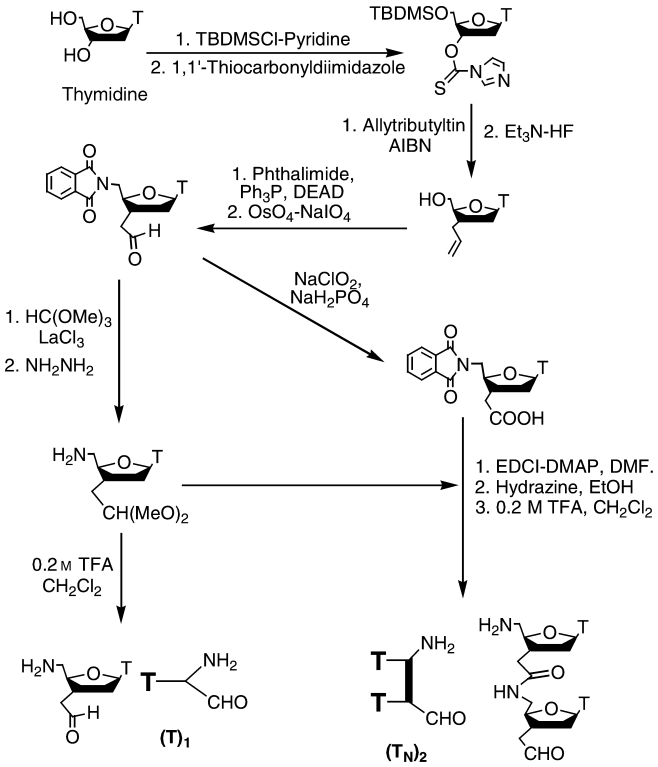
Dedicated to Fredric M. Menger and Albert Padwa on the occasion of their 65th birthdays

Solid-phase synthesis methods have made possible the construction of chain-length- and sequence-specific polymers, which have revolutionized biopolymer research.[1–8] Likewise, incorporating catalysts on solid supports has dramatically improved catalyst lifetimes and enabled facile catalyst recycling.[9–17] Here we combine these strategies, by using solid-supported DNA for both catalysis and product capture, to enable multiple rounds of chain-length-specific polymerizations along a DNA template.

With oligonucleotide or peptide solid-phase synthesis, one "monomer" is added with each reaction cycle. Such a linear, iterative process ensures sequence specificity, but with longer polymers the lengthy reaction time and the overall yield often limit synthetic success. Moreover, the density of sites/solid-support area limits the synthetic scale with supports that typically cannot be recycled. To overcome these limitations, a DNA homopolymer, 5′-dApApApApApApApA-3′, was prepared by solid-phase methods on oligoaffinity-supports–polystyrene/polyethyleneglycol copolymer support (OAS-PS) beads and deprotected in such a way as to retain the polymer on the support, giving S(dAp)$_8$. A small number of beads were cleaved from the polymer and the purity of the resulting DNA polymer was confirmed by analysis, and the remaining beads were used as catalysts for the polymerization of the amino aldehydes 5′-H_2N-T-3′-CH_2CHO ((T)$_1$, T = thymine) and 5′-H_2N-T_NT-3′-CH_2CHO ((T$_N$)$_2$).

As outlined in Scheme 1, (T)$_1$ and (T$_N$)$_2$ can both be constructed efficiently from 5′ and 3′ functionalization of thymidine.[18,19] The differentially protected amine and carboxylic acid products can be condensed and deprotected to give (T$_N$)$_2$, where the subscripted N signifies an amide-backbone linkage replacing the native phosphate of this thymidine dinucleotide analogue.

The product (T$_N$)$_2$ showed no reaction under aqueous reductive amination conditions in the absence of the supported DNA template S(dAp)$_8$. However, when allowed to react at a 4:1 molar excess relative to the template (Figure 1, R2), classic step-growth polymerization ensued. MALDI-TOF mass spectroscopy analyses and HPLC comparisons confirmed the accumulation of an intermediate tetramer product prior to conversion into the octamer, similar to the kinetics seen with soluble catalysts.[18] When the reaction was complete, the beads were washed to remove excess reagent, and the polymer product was recovered with a hot MeOH elution. Two pure products were present in the methanol eluent as



Scheme 1. Syntheses of the substrates (T)$_1$ and (T$_N$)$_2$. TBDMS = *tert*-butyldimethylsilyl; AIBN = azobisisobutyronitrile; DEAD = diethylazodicarboxylate; EDCI = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; DMAP = 4′-aminopyridine; TFA = trifluoroacetic acid.

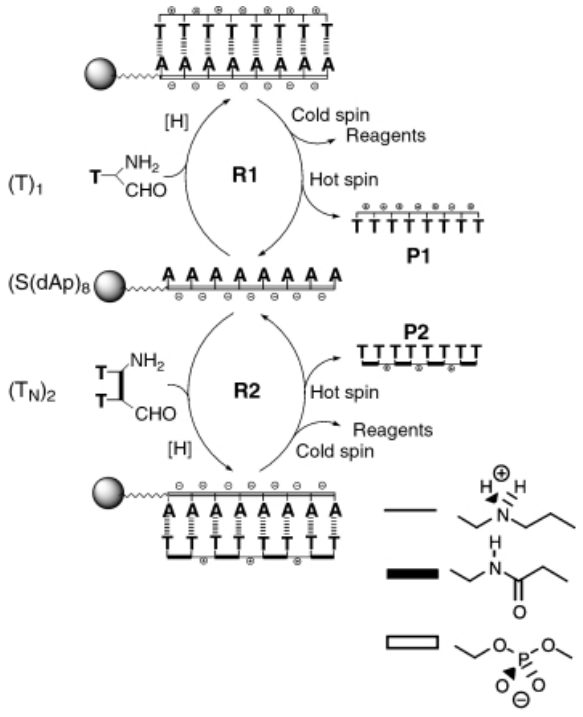


Figure 1. DNA-templated polymerization of (T)$_1$ and (T$_N$)$_2$ on solid-supports.

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[**] This work is partial fulfillment of the Doctor of Philosophy requirements (X.L.) in the Department of Chemistry, University of Chicago, Chicago IL, 60637. We gratefully acknowledge the NIH RR12723 and Emory University for financial support.

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seen in the HPLC chromatogram (Figure 2). The MALDI-TOF molecular ion analyses and induced fragmentation patterns were consistent with the $(T_N)_2$ octamer, 5′-H_2N-$(T_N)_2$-[NH-$(T_N)_2$]$_3$-3′-CHO, as the major product and the

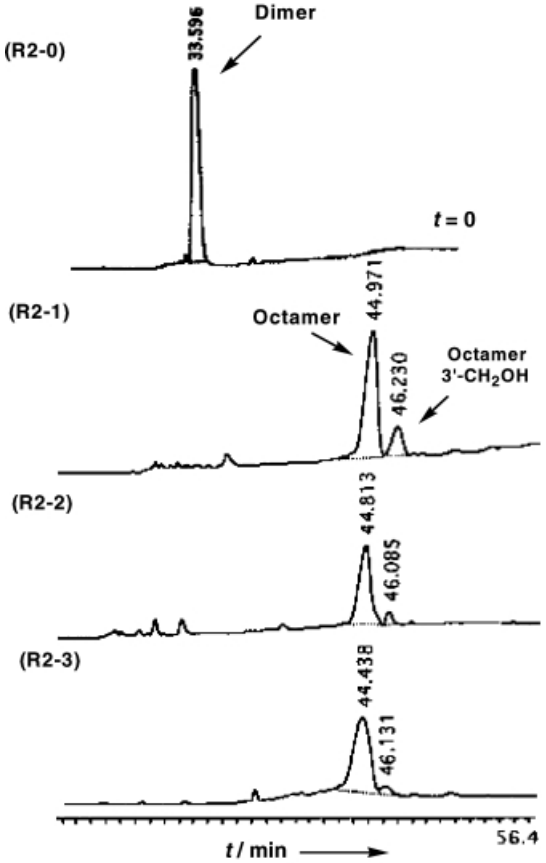

Figure 2. HPLC analysis of S(dA$_p$)$_8$-directed polymerization of $(T_N)_2$. Profiles of (R2-0) at $t = 0$, $(T_N)_2$ only, and the first to third reaction cycle, (R2-1) to (R2-3).

octamer alcohol, 5′-H_2N-$(T_N)_2$-[NH-$(T_N)_2$]$_3$-3′-CH_2OH as the minor product. The beads were reused for additional rounds of synthesis as shown in Figure 2 and the yields of each cycle are given in Table 1, R2. Each subsequent reaction gave a similar product distribution; a total yield of $\approx 90\%$ for each cycle. While each individual reaction occurs on a small scale, the efficient sequential reactions and facile purification suggests that scale-up syntheses could be automated.

The S(dAp)$_8$-catalyzed polymerization of $(T)_1$, in this case at an 8:1 monomer molar excess under the same reductive amination conditions (Figure 1, R1), again showed clear

Table 1. Yield of octomer and octomer-3′-ethanol from each indicated reaction cycle of Figure 1. All yields [%] are calculated by HPLC peak integration following template purification.

Reaction Cycles	Octamer	Octamer 3′-CH_2OH
R2-1	78.9	11.0
R2-2	83.2	4.6
R2-3	87.7	5.8
R1-1	85.1	None
R1-2	88.6	None
R1-3	89.4	None

evidence for step-growth polymerization in the early stages. Again the yields of the octamer product approached 90% at the end of the reaction (Figure 3, Table 1, R1). In each cycle, seven new bonds were formed and at the end of the reaction no octamer alcohol could be detected, which suggests that the octamer product still had reactive ends. Therefore, S(dAp)$_8$ functions as a general catalyst for efficiently templating chain-length-specific polymerization, and offers efficient cycle-dependent amplification of specific DNA templates.

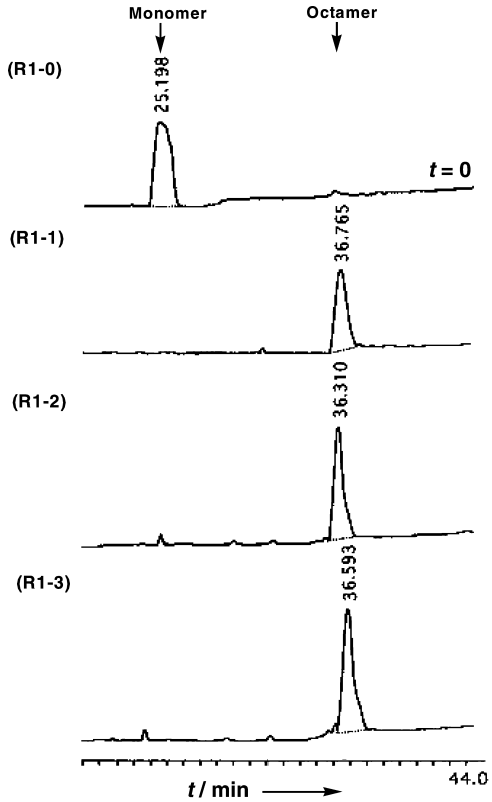

Figure 3. HPLC analysis of S(dA$_p$)$_8$-directed polymerization of $(T)_1$. Profiles of (R1-0) at $t = 0$, $(T)_1$ only, and the first to third reaction cycle, (R1-1) to (R1-3).

In conventional solid-phase synthesis, the formed products are covalently bound to solid supports. In contrast, S(dAp)$_8$ associates with the product through hydrogen bonds, which enables convenient removal of reactants and byproducts, as well as temperature-controlled recovery of the chain-length-specific product. This noncovalent association with the solid support avoids the additional reagents traditionally required for cleavage of the product from the support.

The reaction appears to follow step-growth-polymerization kinetics exclusively, consistent with those seen for soluble oligonucleotide catalysts.[18] For the $(T_N)_2$ reaction, only the tetramer intermediate is observed, for $(T)_1$, only dimer and tetramer intermediates. Polymerization does not proceed past the length of the template, S(dAp)$_8$ in this case, which is consistent with a mechanism in which the template becomes saturated with the thermodynamically most stable duplex and no intermediate ternary complexes (for example, S(dAp)$_8$/P1/P2 see Figure 1) accumulate to a sufficient concentration to be reduced. This observation is significant because the reactive

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5′-amino and 3′-acetaldehyde groups remain at the end of the reaction.

As in the polymerase chain reaction (PCR),[20–22] $S(dAp)_8$ templates the ligation of multiple monomers in a single reaction cycle. Unlike PCR, where both strands of the DNA duplex are amplified to give exponential growth, the DNA-templated polymerization reactions employ only a single strand as the template and growth is presumably linear with each reaction cycle. In addition, the requirement for primers compatible with the double-strand-binding polymerase is avoided, and short sequences of DNA are amplified efficiently.

Finally, the reaction does not synthesize native DNA, but a backbone analogue. Therefore, solid-supported oligomeric DNA can be used to catalyze the rapid synthesis of polymers containing different backbones simply by changing the structure of the reactant, in this case $(T)_1$ and $(T_N)_2$, Quite unlike other solid-supported syntheses, $S(dAp)_n$ can be used repeatedly both to catalyze the polymerization as well as purify the product, which greatly reduces the time and effort for the synthesis of modified DNA-analogues. The extension of this chemistry to mixed-sequence templates should enable the rapid amplification of DNA sequence information into specific backbone-modified analogues. Moreover, this general strategy for solid-phase synthesis can now be extended more broadly, through other molecular recognition elements, to accomplish chain-length-specific polymerizations.

Experimental Section

DNA Synthesis: All native DNA oligomers were prepared by the Emory University Microchemical Facility on a PE-Biosystems 394 DNA Synthesizer. The DNA $S(dAp)_8$ template was synthesized on OAS-PS (Glen Research, Batch No. G008062, Cat. No. 26-4001) solid supports by standard cyanoethyl phosphoramidite chemistry. The linker of OAS-PS is stable to the last step of ammonium hydroxide deprotection treatment in the automated synthesis. To confirm purity, the DNA oligomers were removed from the resin and analyzed by Rainin HPXL RP-HPLC: Phenomenex Prodigy 5 analytical ODS(2) C18; Rainin Dynamax UV detector at 260 nm, and eluted with MeOH in H_2O (0–100 % in 50 min), and the structure confirmed by MALDI-MS: $C_{80}H_{97}N_{40}O_{38}P_7$ calcd m/z: 2443.69 $[M+H^+]$; found 2444.87.

Polymerization: The substrates, 8 mM for $(T_N)_2$ and 16 mM for $(T)_1$, were mixed with $S(dAp)_8$ at the indicated stoichiometry, heated to 75 °C for 2–3 min, and cooled to 4 °C for 3 h.[23] $NaBH_3CN$ (20 equiv) was added at room temperature and the mixture stirred vigorously in a vortex mixer for 72 h, with the addition of more $NaBH_3CN$ (10 equiv) after 48 h to ensure complete reaction. The mixture was subjected to centrifugation in the Ultrafree-MC (Millipore Corp.) centrifugal filter tube at room temperature to remove the reagents, and the beads resuspended in MeOH, heated to 75 °C, and subjected to centrifugation whilst hot, with 3 × 1 mL MeOH. The MeOH washes were combined, evaporated to dryness, and analyzed by reverse-phase HPLC (same system as above), and eluted with MeOH in H_2O: 0–5 % from 0–8 min, 5–20 % from 8–9 min, 20–35 % from 9–24 min, 35–60 % from 24–25 min, 60–100 % from 25–50 min. The product assignments were justified by co-injection with standard samples and by mass spectrometry. MALDI-TOF (2′,4′,6′-trihydroxyacetophenone monohydrate (THAP)/citrate): $(T_N)_2$-[NH-$(T_N)_2]_3$-CHO, $C_{96}H_{128}N_{24}O_{29}$ calcd m/z: 2080.9279 $[M^+]$; found 2082.85 $[M+2H]^+$; $(T_N)_2$-[NH-$(T_N)_2]_3$-CH_2OH, $C_{96}H_{130}N_{24}O_{29}$ calcd m/z: 2082.9436 $[M^+]$; found 2083.84 $[M+H]^+$; $(T)_1$-[NH-$(T)_1]_7$, $C_{96}H_{136}N_{24}O_{25}$, calcd m/z: 2025.0109 $[M^+]$; found 2026.12 $[M+H]^+$.

For resin recovery, the beads were washed 3 × 1 mL with deionized H_2O and resuspended in H_2O overnight under vortex mixing before being used for the next reaction cycle.

Received: June 5, 2002 [Z19473]

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Asymmetric Syntheses of Pectenotoxins-4 and -8, Part I: Synthesis of the C1–C19 Subunit**

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The first members of the pectenotoxin family of marine natural products were isolated off the northeastern coast of Japan in 1985. Subsequently, ten members of this group have been identified.[1] The structural diversity within the pectenotoxins stems from variations in the oxidation state at C43, as well as the differing configurations of the AB spiroketal portion of the structures. Pectenotoxin-2 (C43 = Me) is cytotoxic towards human lung, colon, and breast cancer cell lines with LC_{50} values in the nanomolar range.[1c] Pectenotox-

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[**] Financial support has been provided by the National Institutes of Health (GM33327-16). A BASF-Forschungsstipendum Postdoctoral Fellowship to D.S. and a Howard Hughes Medical Institute Predoctoral Fellowship to H.A.R. are gratefully acknowledged. The NIH BRS Shared Instrumentation Grant Program 1-S10RR04870 and the NSF (CHE 88-140019) are acknowledged for providing NMR facilities.

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