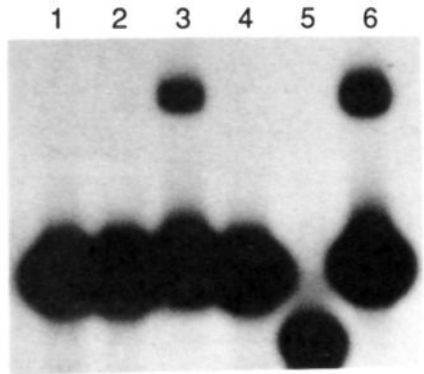
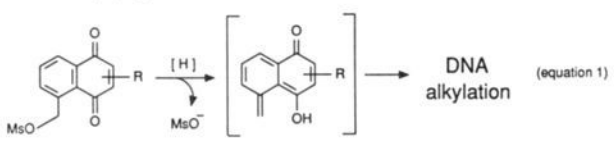


Figure 2. Enzyme dependence and sequence specificity of an oligonucleotide-based alkylating agent. All incubations were maintained at room temperature and buffered at pH 7 with 100 mM potassium phosphate. Product analysis was carried out in parallel with that described in Figure 1. Lane 1: **4** + **5** (2.2 μM each) were incubated in the absence of reducing agent for 30 min. Lane 2: **4** (2.2 μM) was pretreated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) for 2 h before **5** was added and further incubated for 30 min. Lanes 3 and 4: **4** and **5** (2.2 μM each) were incubated for 30 min and then treated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) in the absence (lane 3) and presence of 10 mM glutathione (lane 4) for an additional 30 min. Lane 5: **4** and a noncomplementary oligonucleotide 5′-[^{32}P]-d(CATGCGCTACCCGTG) were incubated for 30 min and then treated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) for an additional 30 min. Lane 6: the 5-methylnaphthoquinone derivatives of **4** and **5** (2.2 μM each) were incubated for 30 min and photolyzed for 2 min.[5]

ability to react with the electrophilic intermediate of alkylation (see below).

Most important to our goal of future study in vivo, the annealed duplex **4** + **5** was also reduced enzymatically with cytochrome *c* reductase in the presence of a low concentration of NADH (100 μM). This yielded (14%) a cross-linked product equivalent to that above (lane 1 vs lane 6, Figure 1). No cross-linking was observed in the presence of either enzyme or 100 μM NADH alone (lanes 5 and 7, Figure 1B); however, a high concentration of NADH did allow for reaction (8%) (lane 8, Figure 1B) with an efficiency much greater than that of NMNH (2%). The extent of cross-linking was unaffected by the addition of 10 mM methyl viologen. If reduction of the quinone had occurred at some distance from the enzyme, then addition of methyl viologen might have been expected to facilitate electron transfer and thus to stimulate cross-linking of DNA. Since no effect was detected, cytochrome *c* reductase likely reduced the quinone directly even though this moiety was held adjacent to the duplex.

The reactivity and selectivity of our model reagent **4** suggests that target alkylation proceeds through the rapid formation and depletion of an electrophilic intermediate such as a quinone methide (eq 1).[4] No reaction was detected in the absence of a



R = site directing appendage

reductant or when **4** was reduced prior to the addition of its target sequence **5** (lanes 1 and 2, Figure 2). The transient intermediate generated upon reduction was then likely trapped by solvent to produce a derivative that was incapable of later alkylation.[9] Furthermore, reaction between **4** + **5** was suppressed in the presence of 10 mM glutathione, a competing nucleophile (lane 4, Figure 2). Cross-linking was also specific for a complementary sequence of DNA; enzymatic reduction of a mixture of **4** and a noncomplementary sequence, 5′-[^{32}P]-d(CATGCGCTACCCGTG), did not produce any detectable covalent complexation (lane 5, Figure 2). Finally, the entire nucleotide sequence of **4** + **5** was likely present in the high molecular weight product of alkylation since its electrophoretic mobility was equivalent to that generated and characterized in a related system[5] in which **5** and the 5-methyl derivative of **4** were labeled alternatively (lane 3 vs 6, Figure 2).

Enzymatic control of target alkylation has now been demonstrated by integrating the principles of bioactivation and antisense technologies.[10] In this manner, the equivalent of an extremely reactive intermediate can be delivered to a chosen site without loss of target selectivity. Logical extension of this work should enhance both the cell and gene specificity of drugs that act upon nucleic acids.

Acknowledgment. This research was supported in part by the University Exploratory Research Program of The Procter and Gamble Company.

Supplementary Material Available: Preparation of oligonucleotides, syntheses of **1**–**4**, and a model reductive alkylation (3 pages). Ordering information is given on any current masthead page.

(9) A preliminary description of a model reaction is included in the supplementary material.

(10) For recent reviews, see: (a) Melton, D. A., Ed. *Antisense RNA and DNA*; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1988. (b) Symons, R. H. *Nucleic Acids Probes*; CRC press: Boca Raton, 1989. (c) Cohen, J. S., Ed. *Oligonucleotides*; CRC Press: Boca Raton, 1989. (d) Brakel, C. L., Ed. *Discoveries in Antisense Nucleic Acids*; Portfolio Publishing Company: The Woodlands, 1989. (e) Stein, C. A.; Cohen, J. S. *Cancer Res.* **1988**, *48*, 2659–2668. (f) Toulmé, J.-J.; Hélène, C. *Gene* **1988**, *72*, 51–58. (g) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. (h) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165–187.

Cyclization Protocols for Controlling the Glycosidic Stereochemistry of Nucleosides. Application to the Synthesis of the Antiviral Agent 3′-Azido-3′-deoxythymidine (AZT)

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As a class, nucleosides have proven to be particularly effective against a variety of viral infections. For example, 3′-azido-3′-deoxythymidine (AZT, **2**) is a potent inhibitor of HIV reverse transcription and is presently the only drug that has been approved by the FDA for the treatment of AIDS. The synthesis of nucleosides, such as AZT, can be categorized under two broad classifications: those that modify intact nucleosides[1,2] and those that couple a modified carbohydrate to a nucleoside base.[3] The former suffers from the high cost associated with using nucleosides as starting materials, while the latter is complicated by the general inability to control the glycosidic stereochemistry during base coupling.[4] Both approaches are schematically illustrated in Scheme I.

Given the problems cited above, we set the goal of developing a synthetic protocol that permits the stereoselective construction

(1) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076.
(2) For many examples of this, see: *Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques, Part I*; Townsend, L. B., Tipson, R. S., Ed.; Wiley-Interscience: New York, 1978.
(3) Chu, C. K.; Beach, J. W.; Ullas, G. V.; Kosugi, Y. *Tetrahedron Lett.* **1988**, *29*, 5349.
(4) As a general rule, most anomeric mixtures of nucleosides require sophisticated chromatographic separations, thereby making these approaches not amenable to scaleup. For a general description of base/carbohydrate coupling reactions, see: Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.

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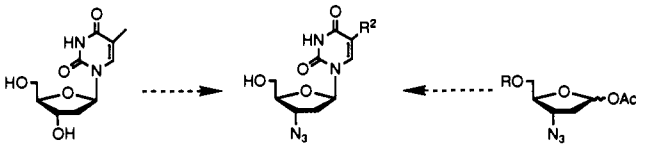
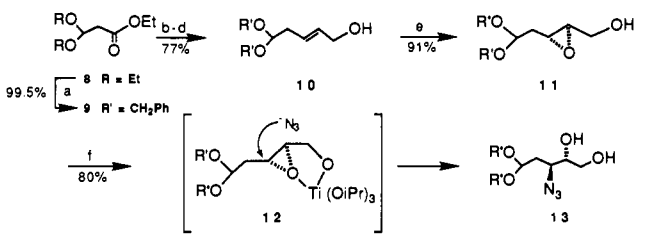
Scheme I



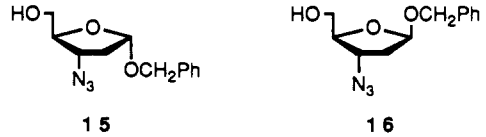
Scheme II[a]



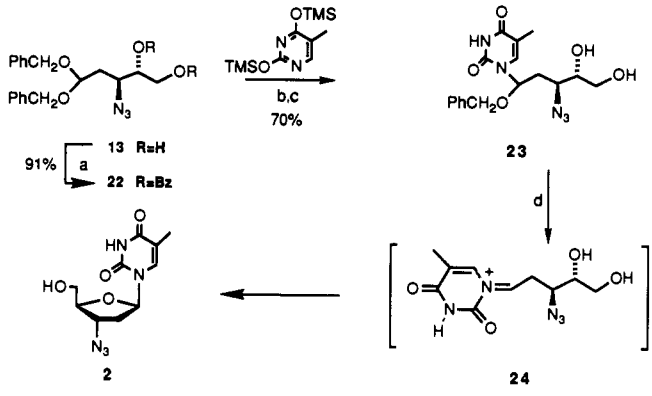
[a] (a) $PhCH_2OH$, TsOH, benzene, 80 °C: (b) DIBAL-H Et_2O, −78 °C; (c) $(iPrO)_2P(O)CH_2CO_2Et$, NaH, THF; (d) DIBAL-H (2 equiv), Et_2O, −78 °C; (e) $Ti(OiPr)_4$, (−)-DET, tBuOOH, 4-Å sieves, CH_2Cl_2, −20 °C; (f) $Ti(OiPr)_4$, Me_3SiN_3, benzene.

of AZT from inexpensive, non-carbohydrate precursors. A starting material that meets this criterion is ethyl 3,3-diethoxypropanoate (**8**), which was prepared on a multigram scale from ethyl vinyl ether and trichloroacetyl chloride in a two-step process.[5] For convenience in subsequent workup procedures, the hydrophilic diethoxypropanoate was converted to the dibenzyloxy derivative **9** in quantitative yield. Ethyl 3,3-bis(benzyloxy)propanoate (**9**) was reduced to the corresponding aldehyde, which was then exposed to Horner–Emmons conditions to yield an α,β-unsaturated ester with a greater than 98:2 selectivity for the *E* isomer.[6] Upon treatment with 2.2 equiv of DIBAL-H, the α,β-unsaturated ester was reduced to the prochiral allylic alcohol **10**. Under Sharpless asymmetric epoxidation conditions, the allylic alcohol was oxidized to the 2(*S*),3(*R*)-epoxy alcohol **11** in >97% ee.[7,8] The azido group was introduced regioselectively (C-3 to C-2 ratio of 16:1) by treatment of the epoxy alcohol with $Ti(O\text{-}i\text{-}Pr)_4/Me_3SiN_3$ (Scheme II).[9,10] The reaction undoubtedly proceeds via the bidentate intermediate **12**, in which the C-3 position is activated toward nucleophilic attack. This route permits a great deal of flexibility in controlling the absolute stereochemistry of the stereogenic centers of azido diol **13**. In principle, by variation of either the olefin geometry or the absolute configuration of the tartrate, all possible combinations of absolute configurations at these centers can be accessed.

The azido diol **13** cyclized to the carbohydrate derivatives **15** and **16** under acidic conditions presumably via an oxonium intermediate. Although anomeric mixtures were obtained at temperatures above 0 °C, only one product, the β-anomer, was observed under kinetic conditions (−5 °C) using either protic acid or mild Lewis acid conditions.[11]



Scheme III[a]



[a] (a) PhCOCl (2.2 equiv), NEt_3, DMAP, CH_2Cl_2; (b) $(CH_3)_3SiOTf$, $ClCH_2CH_2Cl$; (c) NaOH (2 equiv), MeOH; (d) 4.7 N H_2SO_4 in MeOH.

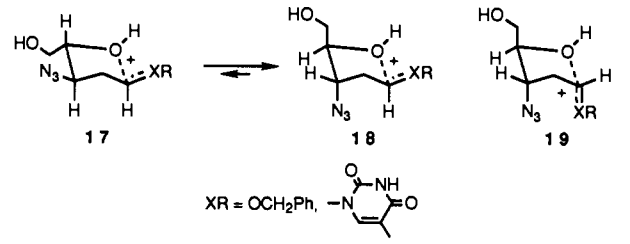


Figure 1.

Since this approach exhibited high selectivity for the formation of a single anomer, this cyclization strategy should be applicable to substrates that ultimately lead to the formation of the β-nucleosides. For example, incorporating the thymine base in the azido diol **13** at the benzyloxy acetal should form a substrate that is capable of undergoing cyclization similarly to the acetal system discussed above. Thus, azido diol **13** was protected as the dibenzoate **22**, and under Vorbruggen conditions, the silylated thymine base was coupled to the acetal.[6] Deprotection with sodium methoxide afforded a diastereomeric mixture of animal azido diol **23**. Even though the coupling conditions yield a mixture of animal diastereomers, the final selectivity of the cyclization should not be affected since both diastereomers presumably cyclize via iminium ion intermediate **24**. When exposed to the conditions used for the acetal cyclizations, no cyclization of animal substrate **23** was observed. However, under more concentrated acidic conditions (4.7 N H_2SO_4 in MeOH), **23** cyclized to give exclusively the β-anomer of AZT in 67% yield, based on recovered starting material (see Scheme III).

Although, at present, the factors that control the selectivity of this cyclization have not been determined, the observed selectivity can be rationalized by using the "gauche effect".[12] In this case the "gauche effect" predicts that transition-state **18** would be favored over **19** because, in **18**, the C–C bond containing the azido group and the partially charged hydroxyl group have a gauche arrangement. Once the rotamer population around this bond is fixed, then transition-state **18** should dominate over **19** since the latter is disfavored due to a severe 1,3-interaction between the azido and XR groups (Figure 1).

(5) Tietze, L. F.; Voss, E.; Hartfiel, U. *Org. Synth.* **1990**, *69*, 238.
(6) (a) Marshall, J. A.; DeHoff, B. S.; Cleary, D. G. *J. Org. Chem.* **1986**, *51*, 1735. (b) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873.
(7) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
(8) The enantiomeric excess (ee) was determined by GC analysis of the Mosher ester of the epoxy alcohol. The Mosher ester was formed with the (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.
(9) (a) Caron, M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1557. (b) Sutowardoyo, K. I.; Emziane, M.; Lhoste, P.; Sinou, D. *Tetrahedron* **1991**, *47*, 1435. (c) Maruako, K.; Sano, H.; Yamaoto, H. *Chem. Lett.* **1985**, 599.
(10) The assignment of the regioselectivity was based on the ^1H NMR (300-MHz) analysis of the impure reaction mixture after peracetylation.
(11) As a method of characterization, the carbohydrates **15** and **16** were transformed into the final product AZT under Vorbruggen coupling conditions. The 5-benzoates of **15** and **16** were separately treated with silylated thymine in the presence of $TiCl_4$ to yield a mixture of α- and β-anomers of AZT benzoates. The benzoate groups were removed by sodium methoxide to afford AZT in 55% yield, in a 2/1 (α/β) ratio from **15** and 1/1 ratio from **16**.
(12) Gauche rotamers are preferred electronically over their anti counterparts when at least two of the substituents are highly electronegative groups. For some examples of this, see: (a) Labelle, M.; Morton, H. E.; Guindon, Y.; Springer, J. P. *J. Am. Chem. Soc.* **1988**, *110*, 4533. (b) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.

In summary, we report a synthetic route to the biologically active β-anomer of AZT that utilizes inexpensive, non-carbohydrate starting materials.[13] Further studies have shown that this route is not limited only to the formation of AZT. Similar coupling of uracil with acetal **13** yields a substrate that cyclizes to AzddU. In addition, this synthetic protocol should be amenable to the preparation of other 3′ analogues of nucleosides by changing the nucleophile employed in the opening of epoxide **11**. Therefore, this approach should also prove useful in syntheses of other nucleosides with biological significance.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (AI-28731). D.C.L. also acknowledges stimulating discussions with Yvan Guindon, Grace Jung, and Christiane Yoakim (BioMega, Inc.) in which they suggested the possibility that the observed stereoselectivity was a consequence of the "gauche effect".

Supplementary Material Available: Full experimental details for the procedures described herein (9 pages). Ordering information is given on any current masthead page.

(13) While this manuscript was being reviewed, an alternative approach for constructing the carbohydrate portion of AZT was reported by Jung and Gardiner. See: Jung, M. E.; Gardiner, J. M. *J. Org. Chem.* **1991**, *56*, 2614.

Microscopic Observation of a Polyaphron Transforming into a Microemulsion

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Organic reactions are usually conceptualized in terms of single molecules or pairs of molecules. However, many reacting systems, and virtually all physical properties, require consideration of multimolecular assemblages in order to model their behavior.[1] A living cell is a wondrous example of a system that operates via a molecular cooperation that cannot be understood by extrapolating the properties of individual species. Indeed, there is a growing suspicion that the collective and holistic features of complex systems can display new and unforseen modes of behavior that are not captured by the Newtonian and thermodynamic approaches.[2] Widespread interest in self-assembling systems illustrates the desire to explore multimolecular phenomena at a relatively simple level. We ourselves have in the past studied molecular communities such as micelles,[3a] vesicles,[3b] films,[3c] pools,[3d] and laminates.[3e] This work led us to examine, by optical microscopy, the transformation of one molecular assemblage, a polyaphron, into another, a microemulsion.

A polyaphron (also called a high internal phase ratio emulsion) has been likened to a gas-in-liquid foam in which the gas has been replaced by a second liquid.[4] In fact, polyaphrons are commonly obtained by first making a foam and then exchanging the gas for a liquid. In a typical preparation, 1 mL of water containing 25–150 mg of surfactant is foamed with a stream of nitrogen. Dodecane (25 mL) is then added slowly with continuous shaking to produce a viscous and opaque "oil-in-water" polyaphron. The photomicrograph in Figure 1A shows densely packed oil globules separated by a thin film of water.[5]

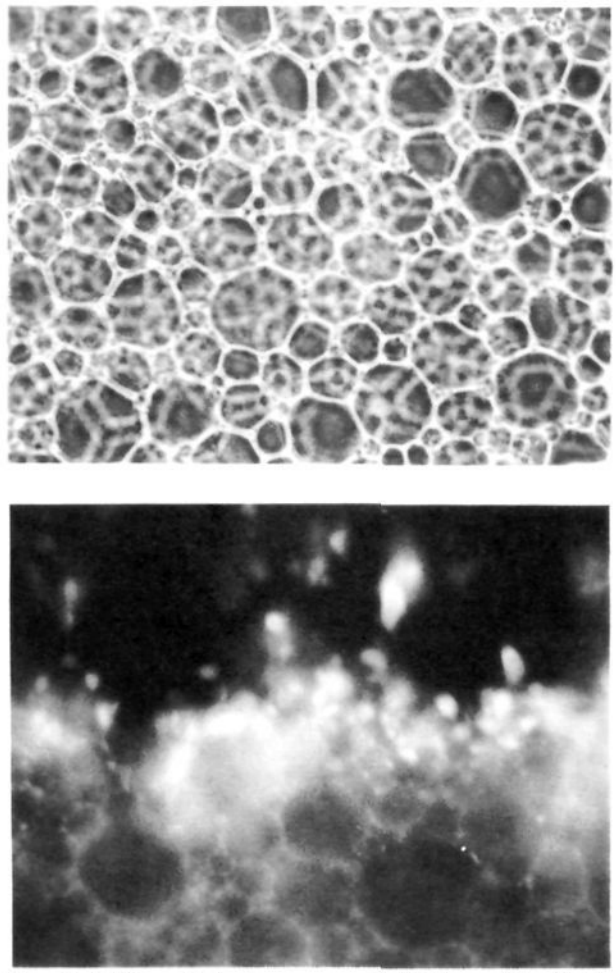

Figure 1. (A) Top: Polyaphron composed of dodecane (25 mL) dispersed in water (1 mL) with 25 mg of cetyltrimethylammonium bromide. The water film contains pyranine, a fluorescent dye. 290× magnification. (B) Bottom: Polyaphron exposed to *n*-hexanol. Within 1 min, water droplets are ejected (fluorescent spots). These gradually disappear as the isotropic microemulsion is formed.

A water-in-oil microemulsion is a fluid, optically transparent dispersion of water in hydrocarbon.[6] Such systems form spontaneously when water is added, for example, to a large excess of *n*-dodecane containing a surfactant and *n*-hexanol (a "cosurfactant"). Note that the water-in-oil microemulsion differs in gross composition from the oil-in-water polyaphron in that only the former possesses *n*-hexanol. Thus, a polyaphron should transform into the thermodynamically stable microemulsion upon exposure of the polyaphron to the alcohol. It was this phase-inversion process that we observed by optical microscopy.

A polyaphron (30–50 mg consisting of water, dodecane, and cetyltrimethylammonium bromide along with 1 mM pyranine, a fluorescent dye, in the water) was placed in the center of a hanging-drop slide resting on the stage of a Leitz Labrolux-S microscope equipped with a UKL condenser and an epifluorescence attachment. With the aid of a Narishige micromanipulator, we placed 5 μL of *n*-hexanol about 5 μm from the edge of the

(1) Menger, F. M. *Angew. Chem.*, to be published.
(2) Davies, P. *The Cosmic Blueprint*; Simon and Schuster: New York, 1988.
(3) (a) Menger, F. M. *Acc. Chem. Res.* **1979**, *12*, 111. (b) Menger, F. M.; Davis, D. S.; Persichetti, R. A.; Lee, J.-J. *J. Am. Chem. Soc.* **1990**, *112*, 2451. (c) Menger, F. M.; Wood, M. G., Jr.; Richardson, S.; Zhou, Q.; Elrington, A. R.; Sherrod, M. J. *J. Am. Chem. Soc.* **1988**, *110*, 6797. (d) Menger, F. M.; Donohue, J. A.; Williams, R. F. *J. Am. Chem. Soc.* **1973**, *95*, 286. (e) Menger, F. M.; Lee, J.-J.; Hagen, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 4017.
(4) Sebba, F. *Foams and Biliquid Foams-Aphrons*; Wiley: New York, 1987.
(5) We estimate that the water film is about 1-μm thick.
(6) Prince, L. M., Ed. *Microemulsions. Theory and Practice*; Academic Press: New York, 1974.

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