Preparation of Stilbene-Tethered Nonnatural Nucleosides for Use with Blue-Fluorescent Antibodies

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The synthesis of the first examples of stilbene-tethered hydrophobic C-nucleosides is described. Compounds of this type are targeted for use with our recently reported "blue-fluorescent antibodies" with the aim of probing native and nonnatural DNA. The nucleophilic addition of aryl Grignard reagents to either a protected 2′-deoxy-1′-chloro-ribofuranose or a protected 2′-deoxy-ribonolactone was the key synthetic step and afforded C-nucleosides in good yields. Both routes resulted in a final product that was ≥90% of the *β*-anomer. Amide- and ether-based linkers for attachment of *trans*-stilbene to the nucleobase were assessed for utility during synthesis and in binding of the ligands to a blue-fluorescent monoclonal antibody. X-ray structures of each complex were obtained and serve as a guideline for second-generation stilbene-tethered C-nucleosides. The development of these hydrophobic nucleosides will be useful in current native and nonnatural DNA studies and invaluable for investigations regarding novel, nonnatural genomes in the future.

The advent of the new millenium holds great promise for revolutions in genomic engineering. In the past few years, the sequences of the entire genomes of both prokaryotic and eukaryotic species have been published.[1,2] The pinnacle, the complete human genome, is almost in hand. However, full annotation of the human genome, even with continued advances in bioinformatics, presents a formidable task and will likely continue well into this century. While the genetic codes of known organisms are being solved, Schultz and Romesberg have also proposed an expansion of the genetic alphabet with ramifications for the coding of unknown life forms.[3] In light of the onset of these bold ventures, the analysis of both natural and nonnatural genomes will benefit from new sequencing, fingerprinting, and mapping technologies.

DNA-duplex stability and sequence specificity are founded on the complementary Watson−Crick hydrogen-bonding patterns of adenine with thymine and cytosine with guanine. However, over the past several years, the concept of hydrophobicity has been evaluated as a driving force in DNA structure and synthesis that could lead to an alternative mode of selective information storage and replication. Efforts to modify the genetic alphabet rely on the specific thermodynamic and kinetic parameters of nucleosides containing hydrophobic, nonnatural bases. The nonnatural bases should form stable pairs in B-form DNA with high selectivity relative to mispairing with the native bases. Furthermore, a DNA polymerase must be capable of efficiently utilizing both the nonnatural nucleosides and the DNA containing them, and must do so with high fidelity. Kool and co-workers[4] introduced the concept of nonpolar nucleoside isosteres (hydrophobic "shape analogues") and demonstrated stability in substituted DNA, as well as good substrate activity with DNA polymerases, providing surrogates for the natural base pairings. Schultz and Romesberg, et al.[3] have focused on the dominant role of hydrophobicity to achieve, with some nonnatural pairings, rates comparable to those for the synthesis of native DNA.[3b,c] The ability to replicate DNA containing hydrophobic, nonnatural nucleobases would also allow for the enzymatic incorporation of additional functional moieties as handles for analytical procedures.

We recently reported antibody−stilbene complexes, coined "blue-fluorescent antibodies," that emitted a bright-blue fluorescence of very high quantum yield.[5] Aside from important fundamental principles concerning protein−ligand dynamics engendered by these antibodies, a

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number of practical applications should also be possible and include uses in immunochemistry, in vitro and in vivo histological assays, and genomic studies. It is in regard to the last of these applications, that we have embarked on a program to create a novel set of aryl C-nucleosides possessing a tethered stilbene molecule for probing native and nonnatural DNA. The eventual goal is the assembly of nonnatural oligonucleotides by chemical means for use as primers and for hybridization experiments, and ultimately the construction of full length genomic transcripts by enzymatic runoff. In this way, blue-fluorescent antibody binding to gene sequences could be used to create markers that would be valuable for a variety of structural and functional genomic studies. Herein, we describe our initial efforts by synthesis of the simplest archetype of a stilbene-tethered hydrophobic C-nucleoside.

Results and Discussion

Two substituted benzene C-nucleosides were prepared that differed in the composition of the linker between stilbene and nucleobase. Each linker type could have unique advantages during polymer-supported oligonucleotide synthesis or in enzymatic reactions. In addition, two synthetic approaches were explored to assess their utility for future work. In the preparation of the first compound **9**, the key step depended on formation of the Grignard reagent derived from **2** and its coupling with 1,2-dideoxy-3,5-di-*O*-*p*-toluoyl-α-1-chloro-D-ribofuranose **10** (Scheme 1).[6, 7]

These substitution reactions do not proceed with inversion, but yield a mixture of isomers (anomers) in which the α-isomer is predominant.[7] Here, the nucleobase was installed to give **3** in 60% yield comprised of 78% of the α-anomeric configuration. Notably, the yield was better than generally observed for such couplings. Assignment of α- and *β*-isomers was carried out using ^{1}H NMR in conjunction with literature data. For aromatic C-nucleosides, α-isomers are characterized by an apparent triplet (J = 6−7 Hz) and *β*-isomers by a doublet of doublets (J = 10−11, 5−6 Hz) for proton 1′-H on the deoxyribose ring.[7] Since the α-isomer is undesirable for our DNA studies, it was necessary to isolate the *β*-isomer. However, upon examination of **3**, no separation was evident by thin-layer chromatography. To make the anomer ratio more favorable, the mixture was subjected to acid-catalyzed isomerization that resulted in a new mixture **4** now slightly enriched in the *β*-isomer. Finally, upon formation of the azido compound **6**, one chromatographic operation could be used to separate the two closely eluting anomers to yield pure material that was 95% *β*-isomer. Reduction of the azido group with triphenylphosphine followed by EDC-mediated amide bond formation with **12**, the original substrate for blue-fluorescent antibodies,[5] afforded the protected compound **8**. Hydrolysis of the *p*-toluate esters resulted in **9**, the first of our stilbene-tethered C-nucleosides.

A second C-nucleoside incorporating an alternative linker was similarly founded on the most fundamental structure involving a benzene nucleobase. In this case, the *para*-substituted aromatic ring was introduced using recent methodology developed by Woski and co-workers that utilized the ribonolactone **19** (Scheme 2).[8]

Unlike **10**, the lactone is a very shelf-stable reagent suitable for long-term storage. Furthermore, organometallic additions to **19** result in an anomeric mixture with a high percentage of the *β*-configuration. The drawbacks of the approach are that the initial addition gives a hemiketal which requires a silane reduction operation and that the overall yield for the C-nucleoside is generally lower. After coupling to obtain **15**, selective removal of the methoxymethyl ether with TMSBr afforded the deprotected alcohol.[9] For this synthesis, we used a benzylic alcohol as a nucleobase so that a glycol linker could be attached via conversion of **16** to the trifluoromethane-sulfonate followed by reaction with **23**. Although the yield was poor, no other methodology was successful for ether formation in these compounds.[10] Fluoride cleavage of the tetraisopropyldisiloxane of **17** afforded the C-nucleoside **18** that was 90% *β*-isomer. At no point in the synthesis could the anomers be distinguished by silica gel chromatography, and so the final product ratio was fixed by the addition to **19** and hydride reduction of the hemiketal. Whether the inability to separate isomers will be a general occurrence using this route, remains to be determined. However, several cases were reported[8] where the *β*-isomer was obtained as >90% of the mixture which bodes well for other C-nucleosides in our plans.

Each C-nucleoside was tested for the ability to bind to the blue-fluorescent monoclonal antibody (mAb) 19G2. Indeed, the bright, powder-blue fluorescence characteristic of the mAb 19G2-stilbene interaction was observed, and the quantum yield (ϕ_f) for each of the two complexes were comparable to that measured previously for 19G2-**12** with a value of $\phi_f \sim 0.80$.[5] Also, soaking a crystal of mAb 19G2 with **9** or **18** resulted in the blue emission. Subsequently, we acquired X-ray crystallographic data on both complexes primarily to obtain information regarding positioning of the nucleobase moiety with regard to the protein framework. The structures of the 19G2-**9** and 19G2-**18** complexes were determined to a resolution of 2.45 and 2.20 Å, respectively (Table 1). In both cases, the antibody structures differ by an RMSD of 0.61 and 0.50 Å from that of the previously determined 19G2-**12** complex.[5] The stilbene portion of both **9** and **18** is clear in 2σ density and could be readily modeled into the density as the *trans*-isomer (Figure 1, A and B). For both complexes, binding site amino acid residues within 5 Å of the stilbene are observed to be in the same conformation as in 19G2-**12**.

In 19G2-**9**, the electron density is clear for ∼7.5 Å from the stilbene out to the second amide group of the linker and then diverges in multiple directions. This indicates that the linker terminus and appended C-nucleoside assume multiple conformations at the outer rim of the antibody binding site. The two conformations most obvious from the electron density were modeled (Figure 1A)

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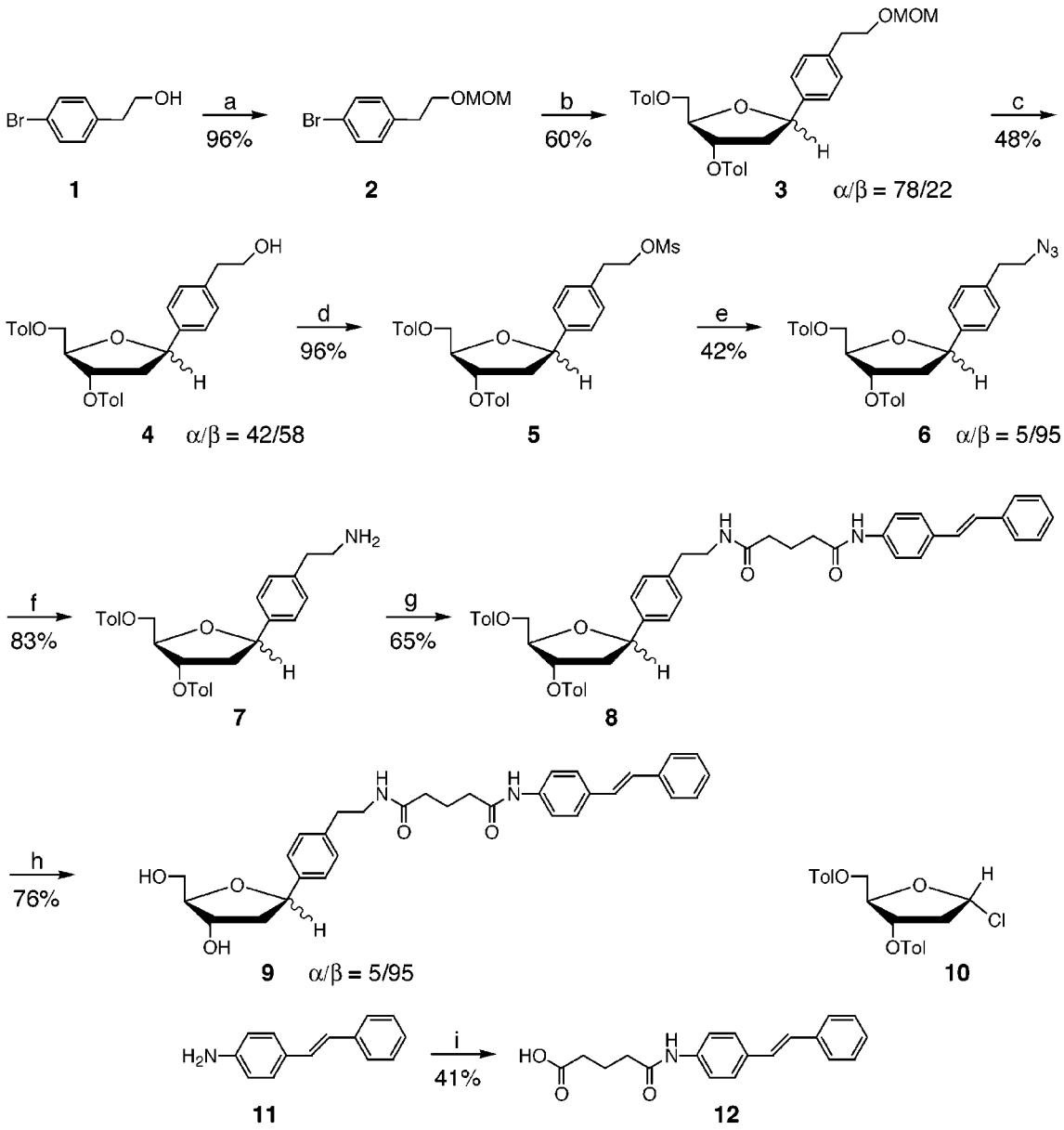
Scheme 1. Synthesis of a Stilbene-Tethered C-Nucleoside Using an Amide Linker[a]

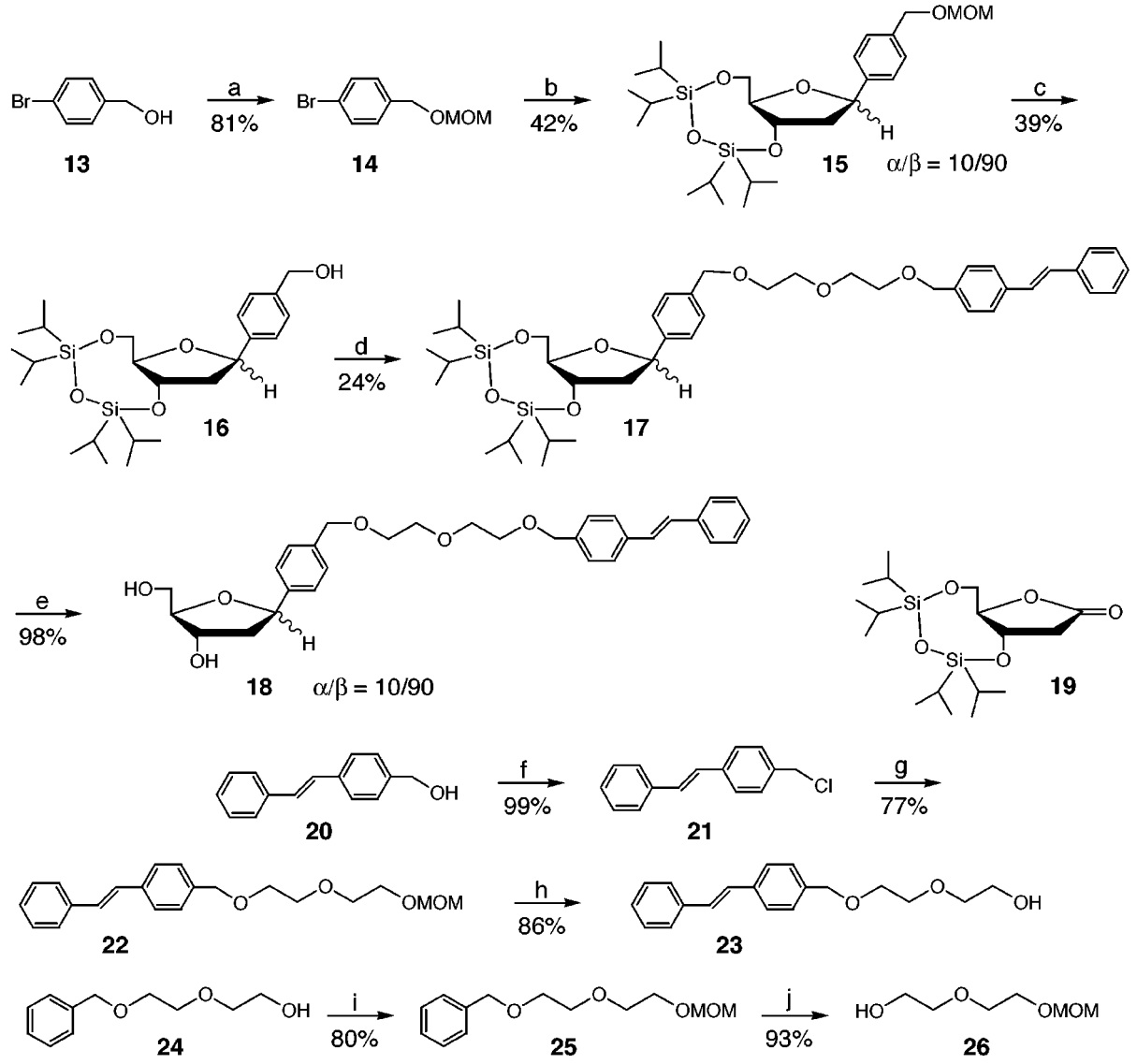


[a] (a) $CH_2(OMe)_2$, LiBr, TsOH; (b) (i) Mg, THF, (ii) **10**; (c) (i) concentrated HCl (cat.), MeOH, 65 °C, (ii) $PhSO_3H$/aq H_2SO_4 (cat.), toluene, reflux; (d) MsCl, NEt_3, CH_2Cl_2; (e) NaN_3, DMF, 40 °C; (f) PPh_3/H_2O, THF; (g) **12**, EDC, DMF; (h) NaOMe, MeOH; (i) glutaric anhydride, DMAP, CH_2Cl_2.

and diverge about 60° away from one another, but both bring the nucleoside analogue within close proximity of some parts of the complementarity-determining regions (CDRs). One conformation interacts almost exclusively with the heavy-chain CDR3 (H-CDR3), and the other interacts with H-CDR3, as well as H-CDR1 and H-CDR2. Both conformations form hydrogen-bonding interactions between the glutaric-amide linker and the antibody, either to the side-chain amide nitrogen of the H96 Gln in one mode, or to the backbone carbonyls of light-chain 91 (L91) Asn and L92 Leu in the other conformation. The C-nucleoside fragment is also within hydrogen bonding distance of antibody functional groups, but it is likely that the mobility generates conformations in addition to what is modeled. Although electron density exists for the C-nucleoside in either conformation, the density is not sufficient to confidently place the deoxyribose or phenyl groups into an exact orientation and the B-factors in this region are relatively high.

Similarly, in the 19G2-**18** complex the linker and C-nucleoside region can be modeled in at least two different conformations placed almost 180° away from one another (Figure 1B). One of the conformations assumes a position near H-CDR3 as observed in 19G2-**9**, whereas the other conformation interacts primarily with the L-CDR3 loop, which was not observed in 19G2-**9**. No hydrogen bonds are formed to the ligand prior to the divergence of the conformations. However, the first conformation forms a hydrogen bond between the last ether oxygen of the linker and the backbone oxygen of L92 Leu and both conformations bring the C-nucleoside within hydrogen-bonding distance of the antibody. The deoxyribose group can form a hydrogen bond to the guanidinium group of H94 Arg and the backbone oxygen

Scheme 2. Synthesis of a Stilbene-Tethered C-Nucleoside Using a Polyether Linker[a]



[a] (a) $CH_2(OMe)_2$, LiBr, TsOH; (b) (i) t-BuLi, THF, −78 °C, (ii) **19**, (iii) Et_3SiH/BF_3-Et_2O, CH_2Cl_2, −78 °C; (c) TMSBr, CH_2Cl_2, −30 °C; d) (i) TfO_2, 2,4,6-collidine, CH_2Cl_2, −70 °C, (ii) **23**; (e) TBAF, THF, 0 °C to room temperature; (f) MsCl, NEt_3, CH_2Cl_2; (g) **26**, NaH, THF, 60 °C; (h) concentrated HCl (cat.), MeOH, 65 °C; (i) $CH_2(OMe)_2$, LiBr, TsOH; (j) H_2, 10% Pd/C, $CHCl_3$.

of H96 Asn in one conformation, and in the other comes within hydrogen-bonding distance of the L92 Leu backbone oxygen. As was the case with 19G2-**9**, the high B-factors for the C-nucleoside are probably indicative of a wide range of possible conformations.

Our previous work on 19G2-**12** suggested that the linker lengths employed in **9** and **18** would allow complete immersion of the stilbene moiety in the binding site, while retaining the C-nucleoside portion at the precipice. The X-ray structures show this to be the case. This now serves as a guideline for our second-generation designs in which the linker length will be increased in order to ensure the complete emergence of *trans*-stilbene from the ~8.5 Å deep major groove of double-helical DNA enabling recognition by mAb 19G2. We anticipate that stilbene-tethered C-nucleosides will be compatible with duplex formation if the linker is of sufficient length and flexibility to avoid steric congestion of the appended stilbene with the DNA strands and/or the active site of a DNA-utilizing enzyme. In this way, an appropriate linker

Table 1. X-ray Data for Antibody Complexes

parameter	19G2-**9**	19G2-**18**
molecular		
space group	*C*2	*C*2
a (Å)	196.352	194.651
b (Å)	60.613	60.840
c (Å)	93.039	92.498
α (deg)	90	90
β (deg)	117.5	117.3
γ (deg)	90	90
data collection		
resolution (Å)	20−2.45 (2.54−2.45)	20−2.2 (2.28−2.20)
observations	33485	47669
completeness (%)	92.9 (90.8)	96.7 (92.5)
I/σ	23.1 (4.6)	22.4 (2.8)
R_{merge} (%)	6.1 (26.4)	7.0 (39.7)
refinement		
R_{work}	0.260	0.265
R_{free}	0.319	0.293
bond lengths (Å)[a]	0.007	0.015
bond angles (deg)[a]	1.3	1.8

[a] RMSD for both bond lengths and bond angles.

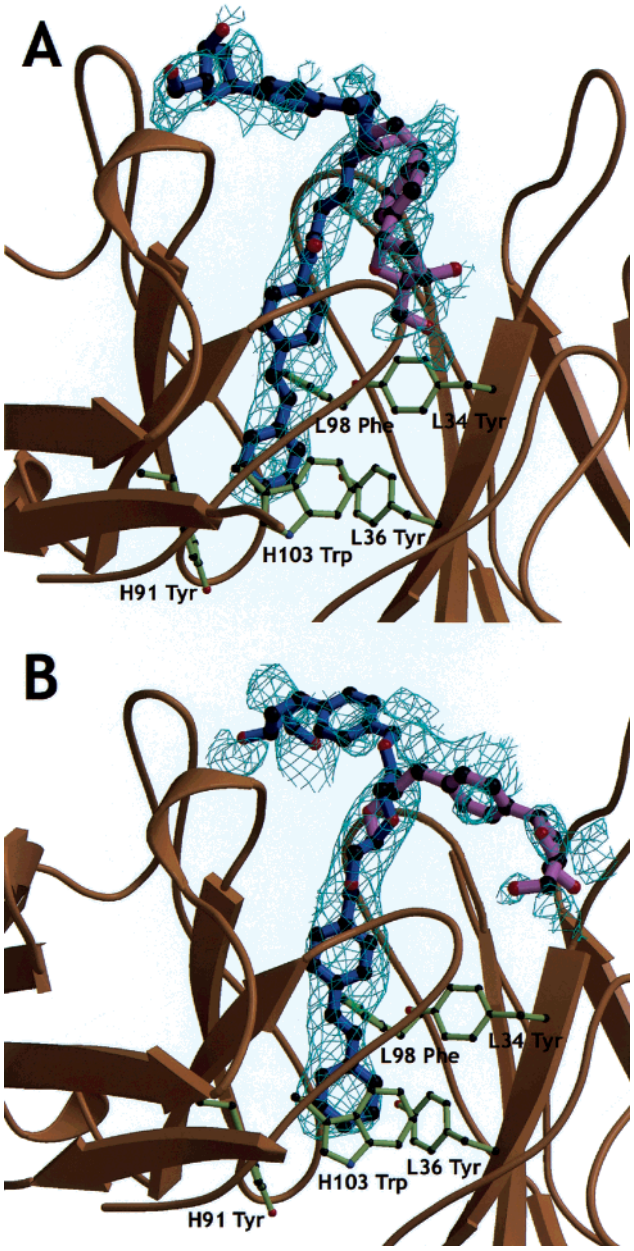


Figure 1. (A) A ribbon diagram of the 19G2-**9** complex with the Fo-Fc electron density for **9** contoured at 1.3σ. Residues that pack against the stilbene molecule, such as H103 Trp are shown explicitly. (B) A ribbon diagram of the 19G2-**18** complex with the Fo-Fc electron density for **18** contoured at 1.3σ. The primary conformational models for **9** and **18** are shown in blue, a second conformation is shown in pink.

might allow for base pairing and nucleoside ligation during DNA synthesis.

In the work described, we have provided a proof-of-principle for construction of a new class of compounds of potential value in both current and future DNA and genomic studies. The structures as presented are readily amenable to activation as the 2′-OH phosphoramidite for oligonucleotide synthesis or formation of the 5′-OH triphosphate for use as DNA polymerase or reverse transcriptase substrates using well-established methods.[11,12] Furthermore, it should be possible to prepare the C-nucleosides with a ribose,[13] rather than deoxyribose sugar, using a similar approach that would afford substrates for RNA polymerases. The exo-nuclease-deficient Klenow fragment of *E. coli* DNA polymerase I is able to efficiently recognize a large number of nonnatural hydrophobic bases and incorporate them into DNA. In this way, mapping and chain-termination sequencing could be used in a fashion similar to current protocols.[14] Hybridization, widely used in high-throughput genomics strategies, would also be feasible. Yet, a significant advance will come from polymerase-mediated extension of DNA containing the nonnatural base, at present a hurdle in most cases, for synthesis of read-through or runoff transcripts/reverse transcripts. Ultimately, with regard to both nonnatural DNA and genomes in the years to come, a sequencing methodology will be needed comparable to what is now routine with natural DNA. Finally, targeting DNA with a macromolecular marker has unique advantages associated with the ability to apply immobilization technology for fragment isolation and recovery. Continued developments in nonnatural nucleobase design and the protein engineering of polymerase substrate specificity and activity will eventually provide a unique set of tools for the investigation of genetic material.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were measured on a Brucker AMX-400 or Brucker AMX-500 spectrometer as indicated. Chemical sifts (ppm) were reported relative to internal $CDCl_3$ (^{1}H, 7.26 ppm and ^{13}C, 77.0 ppm), CD_3OD (^{1}H, 3.30 ppm and ^{13}C, 49.2 ppm), and DMSO-d_6 (^{1}H, 2.49 ppm and ^{13}C, 39.0 ppm). HRMS spectra were recorded using electrospray ionization (ESI) or MALDI techniques. Glassware and solvents were dried by standard methods. Flash chromatography was performed on silica gel 60 (230−400 mesh) and thin-layer chromatography on glass plates coated with a 0.02 mm layer of silica gel 60 F-254. All chemical reagents and solvents were from Aldrich Chem. Co., unless otherwise noted, and used without further purification.

4-[2-(Methoxymethoxy)ethyl]bromobenzene (2). To a solution of 4-bromophenethyl alcohol **1** (1.0 g, 5.0 mmol) in dimethoxymethane (10 mL) were added LiBr (87 mg, 1.0 mmol) and *p*-TsOH−H_2O (95 mg, 0.50 mmol) with stirring. The white suspension was stirred at room temperature for 2 h or until completion of the reaction (TLC; hexane/EtOAc, 4/1). Brine was added, and the mixture was extracted with ether. After evaporation of the solvent, the crude product was purified using flash chromatography (FC) (hexane/EtOAc, 4/1) to afford 1.17 g (96%) of **2** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (d, 2H, J = 8.0 Hz), 7.12 (d, 2H, J = 8.0 Hz), 4.60 (s, 2H), 3.74 (t, 2H, J = 7.0 Hz), 3.29 (s, 3H), 2.86 (t, 2H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.0, 131.4, 130.6, 120.0, 96.4, 68.0, 55.2, 35.7.

1,4-Anhydro-2-deoxy-1-*C*-[4-[2-(methoxymethoxy)ethyl]phenyl]-D-*erythro*-pentitol 3,5-Bis(4-methylbenzoate) (3). A solution of **2** (0.967 g, 3.95 mmol) in THF (4 mL) was added into a flask charged with Mg powder and a few crystals of iodine at room temperature under nitrogen. The mixture was stirred at 50 °C for 2 h to complete the formation of the Grignard reagent. A solution of chlorosugar **10** (1.23 g, 3.16

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mmol) in THF (8 mL) was added at 0 °C, and the reaction mixture was stirred at room temperature for 12 h. The mixture was concentrated to a small volume. The residue was purified by FC (hexane/EtOAc, 4/1) to give 1.0 g (60%) of **3** as an oil that was a mixture of isomers ($\alpha/\beta = 78/22$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93−7.71 (m, 4H), 7.36−7.32 (m, 2H), 7.28−7.17 (m, 6H), 5.61−5.58 (m, 1 H, α-isomer and β-isomer 3′-H), 5.34 (t, 1H, α-isomer 1′-H, J = 6.6 Hz), 5.23 (dd, 1H, β-isomer 1′-H, J = 11.4, 5.5 Hz), 4.69−4.52 (m, 5H), 3.77−3.73 (m, 2H), 3.30 (s, 3H), 2.96−2.88 (m, 3H, benzylic, α-isomer 2′-Hβ), 2.51 (dd, 1H, β-isomer 2′-Hα, J = 13.2, 4.4 Hz), 2.44−2.40 (m, 6H), 2.32−2.20 (m, 1H, α-isomer 2′-Hα, β-isomer 2′-Hβ). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3, 166.2, 166.1, 166.0, 165.9, 144.0, 143.8, 143.7, 143.6, 140.1, 138.8, 138.5, 138.4, 138.0, 138.8, 130.0, 129.6, 129.5, 129.1, 129.0, 128.9, 128.8, 128.7, 127.0, 126.9, 126.8, 125.9, 125.7, 125.6, 96.3, 82. 8, 81.9, 80.6, 80.0, 77.2, 76.3, 68.4, 68.2, 64.7, 64.5, 55.0, 41.6, 40.2, 35.8, 21.6, 21.5. MALDI−FTMS: calcd for M + Na^+ 541.2197, found 541.2211.

1,4-Anhydro-2-deoxy-1-*C*-[4-(2-hydroxyethyl)phenyl]-D-*erythro*-pentitol 3,5-Bis(4-methylbenzoate) (4). Compound **3** (1.0 g, 1.9 mmol) was dissolved in MeOH (25 mL) with one drop of 37% HCl. The mixture was stirred at 65 °C. After completion of the reaction in 6−8 h, the solvent was evaporated. The residue was purified by FC (hexane/EtOAc, 2:1) to afford 0.82 g (90%) of a colorless syrup (α/β= 73/27). The compound (0.82 g, 1.7 mmol) was epimerized in toluene (50 mL) with benzenesulfonic acid (30 mg, 0.17 mmol), concentrated H_2SO_4 (1 drop), and water (3 drops). The mixture was stirred vigorously and refluxed for 4 h. After concentration, the crude product was purified by FC (hexane/EtOAc, 2/1) to afford 0.39 g (48%) of **4** as an oil (α/β = 42/58). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00−7.71 (m, 4H), 7.39−7.34 (m, 2H), 7.29−7.34 (m, 2H), 7.29−7.17 (m, 6H), 5.62−5.58 (m, 1H), 5.34 (t, 1H, α-isomer 1′-H, J = 6.8 Hz), 5.23 (dd, 1H, β-isomer 1′-H, J = 11.2, 5.0 Hz), 4.70−4.52 (m, 3H), 3.87−3.82 (m, 2H), 2.97−2.84 (m, 3H), 2.52 (dd, 1H, β-isomer 2′-Hα, J = 13.8, 5.0 Hz), 2.44−2.40 (m, 6H), 2.34−2.20 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.4, 166.1, 166.0, 144.1, 144.0, 143.8, 140.4, 138.8, 138.2, 137.7, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 127.1, 127.0, 126.8, 126.2, 126.0, 125.9, 81.9, 80.0, 76.4, 64.8, 64.6, 63.6, 63.5, 40.3, 38.8, 21.7, 21.6. MALDI−FTMS: calcd for M + Na^+ 497.1934, found 497.1932.

1,4-Anhydro-2-deoxy-1-*C*-[4-[2-[(methylsulfonyl)oxy]-ethyl]phenyl]-D-*erythro*-pentitol 3,5-Bis(4-methylbenzoate) (5). Compound **4** (0.387 g, 0.82 mmol) was dissolved in CH_2Cl_2 (10 mL). Methanesulfonyl chloride (0.126 mL, 1.63 mmol) and then NEt_3 (0.262 mL, 1.88 mmol) were added at 0 °C under nitrogen. The mixture was stirred overnight while the temperature was allowed to rise to room temperature. The CH_2Cl_2 layer was washed with water and brine and then dried over Na_2SO_4. After evaporation of solvent, 0.435 g (96%) of the product **5** was obtained as a yellow oil and used in the next step without further purification.^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97−7.72 (m, 4H), 7.40−7.18 (m, 8H), 5.61−5.58 (m, 1H), 5.34 (t, 1H, α-isomer 1′-H, J = 7.0 Hz), 5.24 (dd, 1H, β-isomer 1′-H, J = 11.0, 5.2 Hz), 4.70−4.52 (m, 3H), 4.42−4.37 (m, 2H), 3.08−3.97 (m, 2H), 2.84 (s, 3H), 2.23 (dd, 1H, β-isomer 2′-Hα, J = 14.0, 5.2 Hz), 2.44−2.40 (m, 6H), 2.31−2.19 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3, 166.0, 144.1, 143.9, 143.8, 141.2, 140.0, 135.9, 135.4, 131.5, 129.6, 129.5, 129.1, 129.0, 128.9, 127.0, 126.9, 126.7, 126.2, 126.0, 82.9, 82.0, 80.4, 80.0, 77.1, 76.3, 70.0, 64.6, 64.5, 53.4, 41.6, 40.3, 37.2, 35.2, 31.4, 21.7, 21.6, 21.5. MALDI−FTMS: calcd for M + Na^+ 575.1710, found 575.1711.

1,4-Anhydro-2-deoxy-1-*C*-[4-(2-azidoethyl)phenyl]-D-*erythro*-pentitol 3,5-Bis(4-methylbenzoate) (6). Compound **5** (0.78 g, 1.43 mmol) was dissolved in anhydrous DMF (15 mL) under nitrogen, and then NaN_3 (0.186 g, 2.86 mmol) was added. The mixture was stirred at 40 °C and followed by TLC which showed completion in 4 h. After dilution with EtOAc, aqueous workup, and solvent evaporation, the residue was purified by FC (hexane/EtOAc, 3:1). The desired β-isomer of **6** (0.30 g, 42%) eluted first and was obtained as a syrup. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (d, 2H, J = 8.0 Hz), 7.94 (d, 2H, J = 8.0 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.27 (d, 2H, J = 8.0 Hz), 7.22 (d, 2H, J = 7.7 Hz), 7.18 (d, 2H, J = 7.7 Hz), 5.61 (d, 1H, J = 5.5 Hz), 5.24 (dd, 1H, J = 10.6, 4.8 Hz), 4.65−4.64 (m, 2H), 4.54−4.52 (m, 1H), 3.49 (t, 2H, J = 7.4 Hz), 2.88 (t, 2H, J = 7.4 Hz), 2.52 (dd, 1H, J = 14.0, 5.2 Hz), 2.44 (s, 3H), 2.41 (s, 3H), 2.27−2.20 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.3, 166.1 144.1, 143.8, 139.1, 137.6, 129.7, 129.6, 129.2, 129.1, 128.8, 127.0, 126.9, 126.2, 82.9, 80.6, 77.2, 64.7, 52.3, 41.6, 34.9, 21.7, 21.6. MALDI−FTMS: calcd for M + Na^+ 522.1999, found 522.1997.

1,4-Anhydro-2-deoxy-1-*C*-[4-(2-aminoethyl)phenyl]-D-*erythro*-pentitol 3,5-Bis(4-methylbenzoate) (7). Compound **6** (0.357 g, 0.71 mmol) was dissolved in THF (10 mL). Ph_3P (0.28 g, 1.06 mmol) and water (0.1 mL) were added. The reaction mixture was stirred at room temperature under nitrogen for 36 h until TLC showed the disappearance of starting material. The mixture was concentrated and the residue purified by FC (hexane/EtOAc, 1/2) to remove Ph_3P, Ph_3PO and byproducts, and then (CH_2Cl_2/MeOH, 3/1) to give 0.30 g (83%) of **7** as a yellow syrup. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, 2H, J = 8.2 Hz), 7.94 (d, 2H, J = 8.2 Hz), 7.33 (d, 2H, J = 8.2 Hz), 7.27 (d, 2H, J = 8.2 Hz), 7.22 (d, 2H, J = 7.9 Hz), 7.16 (d, 2H, J = 7.9 Hz), 5.62−5.60 (m, 1H), 5.23 (dd, 1H, J = 10.8, 5.0 Hz), 4.65−4.64 (m, 2H), 4.53 (brs, 1H), 2.95 (brs, 2H), 2.74 (t, 2H, J = 7.0 Hz), 2.51 (dd, 1H, J = 13.8, 5.0 Hz), 2.43 (s, 3H), 2.40 (s, 3H), 2.28−2.20 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.3, 166.1, 144.1, 143.8, 139.4, 138.4, 129.6, 129.1, 129.0, 128.9, 127.0, 126.9, 126.1, 125.9, 82.8, 80.7, 77.2, 64.7, 43.3, 41.6, 39.4, 21.7, 21.6. MALDI−FTMS: calcd for M + Na^+ 496.2094, found 496.2100.

***N*-[2-[4-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-D-*erythro*-pentofuranosyl]phenyl]ethyl]-*N*′-[4-[(1*E*)-2-phenylethenyl]phenyl]pentanediamide (8).** Into a mixture of **7** (163 mg, 0.345 mmol) and **12** (117 mg, 0.379 mmol) in DMF (3.5 mL) was added EDC−HCl (88 mg, 0.448 mmol) at room temperature. The mixture was stirred under nitrogen for 4 h. After concentration, the residue was purified by FC (EtOAC) to afford 172 mg (65%) of **8** as a syrup. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.50 (s, 1H), 7.97 (d, 2H, J = 8.2 Hz), 7.94 (d, 2H, J = 8.2 Hz), 7.56 (d, 2H, J = 8.5 Hz), 7.49−7.42 (m, 4H), 7.36−7.12 (m, 11H), 7.02 (dd, 2H, J = 20.0, 16.4 Hz), 5.91 (t, 1H, J = 6.2 Hz), 5.59 (d, 1H, J = 6.8 Hz), 5.20 (dd, 1H, J = 10.9, 5.0 Hz), 4.67−4.59 (m, 2H), 4.52−4.52 (m, 1H), 3.52−3.51 (m, 2H), 2.80 (t, 2H, J = 7.0 Hz), 2.50 (dd, 1H, J = 14.1, 5.9 Hz), 2.42 (s, 3H), 2.40 (s, 3H), 2.35−2.31 (m, 2H), 2.62−2.23 (m, 2H), 1.98−1.92 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 171.1, 166.4, 166.1, 144.2, 143.9, 138.7, 138.5, 137.6, 137.3, 133.0, 129.7, 129.2, 128.9, 128.6, 127.6, 127.4, 127.0, 126.9, 126.4, 126.3, 119.8, 82.9, 80.6, 77.1, 64.7, 41.4, 40.4, 36.1, 35.2, 35.0, 21.8, 21.6. MALDI−FTMS: calcd for M + Na^+ 787.3354, found 787.3334.

***N*-[2-[4-[2-Deoxy-D-*erythro*-pentofuranosyl]phenyl]ethyl]-*N*′-[4-[(1*E*)-2-phenylethenyl]phenyl]pentanediamide (9).** Compound **8** (172 mg, 0.225 mmol) was dissolved in MeOH/CH_2Cl_2 (3 mL/2 mL) at room temperature under nitrogen. A solution of 25% MeONa in MeOH (0.154 mL, 0.675 mmol) was added with stirring. After 30 min, a suspension developed and TLC indicated the disappearance of starting material. After stirring for an additional 1.5 h, solid NH_4Cl was added to quench the reaction followed by water (1 mL). The solid was collected by filtration, washed with water, and dried under vacuum in a desiccator to afford 94 mg (76%) of **9** as a white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.92 (brs, 1H), 7.86 (brs, 1H), 7.61 (d, 2H, J = 8.4 Hz), 7.56 (d, 2H, J = 7.4 Hz), 7.52 (d, 2H, J = 8.4 Hz), 7.35 (t, 2H, J = 7.7 Hz), 7.26−7.22 (m, 3H), 7.19−7.11 (m, 4H), 4.96−4.94 (m, 2H), 4.69 (brs, 1H), 4.16 (brs, 1H), 4.76−4.75 (m, 1H), 3.49−3.38 (m, 2H), 3.26−3.23 (m, 2H), 2.68 (t, 2H, J = 7.4 Hz), 2.31 (t, 2H, J = 7.4 Hz), 2.11 (t, 2H, J = 7.0 Hz), 2.03 (dd, 1H, J = 12.4, 5.5 Hz), 1.83−1.72 (m, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.5, 170.8, 140.3, 138.8,138.4, 137.2, 131.7, 128.6, 128.3, 128.0, 127.3, 126.8, 126.2, 126.1, 119.1, 87.7, 79.0, 72.4, 62.5, 43.4, 35.7, 34.9, 34.6, 21.1. MALDI−FTMS: calcd for M + Na^+ 551.2516, found 551.2524.

5-Oxo-5-[[4-[(1*E*)-2-phenylethenyl]phenyl]amino]pentanoic Acid (12). A solution of *trans*-4-aminostilbene **11** (0.53 g, 2.7 mmol) (TCI Chem. Co.) in CH_2Cl_2 (10 mL) was stirred at room temperature, and triethylamine (1.13 mL, 7.1 mmol) was added followed by glutaric anhydride (464 mg, 4.05 mmol) and 4-(dimethylamino)pyridine (DMAP) (2 mg). The solution was stirred at room temperature for 18 h, poured into water/EtOAc, shaken, and filtered, and the solid was washed with water, EtOAc, hexane, and then dried that afforded **12** as a white solid (340 mg, 41%).^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.96 (1H, s), 7.60 (2H, d, J = 8.8 Hz), 7.56 (2H, d, J = 7.4 Hz), 7.52 (2H, d, J = 8.8 Hz), 7.35 (2H, t, J = 7.4 Hz), 7.23 (1H, t, J = 7.4 Hz), 7.18 (1H, d, J = 16.5 Hz), 7.13 (1H, d, J = 16.5 Hz), 2.36 (2H, t, J = 7.3 Hz), 2.27 (2H, t, J = 7.3 Hz), 1.81 (2H, quin, J = 7.3 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 174.12, 170.69, 138.80, 137.22, 131.78, 128.65, 128.03, 127.30, 126.88, 126.84, 126.23, 119.10, 35.39, 32.96, 20.39. MALDI−FTMS: calcd for $C_{19}H_{19}NO_3$ 332.1263 (M + Na^+), found: 332.1256.

4-[(Methoxymethoxy)methyl]bromobenzene (14). To a solution of 4-bromobenzyl alcohol **13** (4.0 g, 21.4 mmol) in dimethoxymethane (40 mL) were added LiBr (0.37 g, 4.28 mmol) and *p*-TsOH−H_2O (0.41 g, 2.14 mmol). The white suspension was stirred at room temperature for 2 h and then quenched by addition of brine, followed by extraction of the mixture with ether. After evaporation, the residue was purified by FC (hexane/EtOAc, 4/1) to give **14** (4.0 g, 81%) as a colorless oil.^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz), 4.69 (s, 2H), 4.54 (s, 2H), 3.40 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.8, 131.4, 129.4, 121.4, 95.6, 68.3, 55.3.

1,4-Anhydro-2-deoxy-1-*C*-[4-[(methoxymethoxy)methyl]phenyl]-3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-D-*erythro*-pentitol (15). To a solution of compound **14** (0.23 g, 1.0 mmol) in dry THF (2.5 mL) at −78 °C under N_2 was added t-BuLi (1.7 M in pentane, 1.17 mL, 2.0 mmol). The mixture was stirred for 30 min and then transferred to a solution of **19** (0.224 g, 0.60 mmol) in dry THF (2.5 mL) at −78 °C. After 1 h, the reaction was quenched with saturated aqueous NH_4Cl and the mixture extracted with ether. The organic layer was washed with water and brine and dried over Na_2SO_4, and the solvent was evaporated to give a crude oil. To a solution of the oil at −78 °C in CH_2Cl_2 (5 mL) under N_2 were added Et_3SiH (0.288 mL, 1.8 mmol) and BF_3·Et_2O (0.227 mL, 1.8 mmol). The mixture was stirred at −78 °C for 6 h and then quenched by addition of sat. $NaHCO_3$ at −78 °C. The mixture was extracted with ether, and the ether layer was washed with water and brine and dried over Na_2SO_4. After evaporation, the crude oil was purified by FC (hexane/EtOAc, 8/1) to give product **15** (0.13 g, 42%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33 (s, 4H), 5.10 (t, 1H, J = 7.3 Hz), 4.70 (s, 2H), 4.58 (s, 2H), 4.56−4.52 (m, 1H), 4.15 (d, 1H, J = 8.4 Hz), 3.94−3.87 (m, 2H), 3.41 (s, 3H), 2.40−2.35 (m, 1H), 2.09−2.03 (m, 1H), 1.14−0.95 (m, 28H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.6, 137.0, 127.9, 125.8, 95.5, 86.3, 78.8, 73.2, 68.8, 63.6, 55.2, 43.1, 17.5, 17.4, 17.3, 17.2, 17.0, 16.9, 13.4, 13.3, 12.9, 12.5. MALDI−FTMS: calcd for M + Na^+ 533.2725, found 533.2725.

1,4-Anhydro-2-deoxy-1-*C*-[4-(hydroxymethyl)phenyl]-3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-D-*erythro*-pentitol (16). To a solution of compound **15** (0.121 g, 0.237 mmol) in CH_2Cl_2 (5 mL) at −30 °C under N_2 was added TMSBr (0.125 mL, 0.949 mmol). After stirring at −30 °C for 1 h, the reaction was quenched by addition of sat. $NaHCO_3$ and the mixture extracted with ether. After evaporation, the crude oil was purified by PTLC (hexanes/EtOAc, 1/1) to give **16** (43 mg, 39%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (s, 4H), 5.09 (t, 1H, J = 7.0 Hz), 4.68 (s, 2H), 4.55−4.51 (m, 1H), 4.13 (dd, 1H, J = 10.3, 2.1 Hz), 4.93−3.86 (m, 2H), 2.37−2.34 (m, 1H), 2.06 (dt, 1H, J = 12.9, 7.6 Hz), 1.12−0.94 (m, 28H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.6, 127.1, 126.1, 86.4, 78.8, 73.2, 65.2, 63.6, 43.1, 17.6, 17.4, 17.3, 17.2, 17.1, 17.0, 13.5, 13.4, 13.0, 12.5. MALDI−FTMS: calcd for M + Na^+ 489.2463, found 489.2457.

1,4-Anhydro-2-deoxy-1-*C*-[4-[[2-[2-[[4-[(1*E*)-2-phenylethenyl]phenyl]methoxy]ethoxy]ethoxy]methyl]] phenyl]-3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-D-*erythro*-pentitol (17). Triflic anhydride (0.0176 mL, 0.105 mmol) was added to dry CH_2Cl_2 (0.5 mL) at −70 °C under N_2 followed by a solution of **16** (46.7 mg, 0.10 mmol) and 2,4,6-collidine (0.0139 mL, 0.105 mmol) in CH_2Cl_2 (1 mL). After 30 min, a solution of **23** (29.8 mg, 0.10 mmol) and 2,4,6-collidine (0.0264 mL, 0.20 mmol) in CH_2Cl_2 (1 mL) was added with stirring. After 30 min, the mixture was allowed to warm to room temperature for an additional 3 h. The reaction mixture was concentrated and purified by PTLC (hexane/EtOAc, 2/1) to give **17** (17.9 mg, 24%) as a colorless oil.^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53−7.48 (m, 4H), 7.38−7.24 (m, 9H), 7.10 (s, 2H), 5.08 (t, 2H, J = 8.0 Hz), 4.58 (s, 2H), 4.56 (s, 2H), 4.53−4.50 (m, 1H), 4.13 (d, 2H, J = 8.0 Hz), 3.91−3.85 (m, 2H), 3.71−3.62 (m, 8H), 2.38−2.32 (m, 1H), 2.09−2.02 (m, 1H), 1.11−1.01 (m, 28H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.4, 137.7, 137.5, 137.3, 128.6, 128.5, 128.3, 128.1, 127.8, 127.6, 126.5, 125.9, 86.4, 78.9, 73.3, 73.0, 70.7, 69.4, 69.3, 63.7, 43.1, 17.6, 17.4, 17.4, 17.2, 17.1, 17.0, 13.5, 13.4, 13.0, 12.5. MALDI−FTMS: calcd for M + Na^+ 769.3926, 769.3914.

1,4-Anhydro-2-deoxy-1-*C*-[4-[[2-[2-[[4-[(1*E*)-2-phenylethenyl]phenyl]methoxy]ethoxy]ethoxy]methyl]] phenyl]-D-*erythro*-pentitol (18). To a solution of **17** (17.9 mg, 0.024 mmol) in THF (0.3 mL) at 0 °C under N_2 was added TBAF (1.0 M in THF, 0.072 mL, 0.05 mmol). The mixture was stirred for 2 h while the reaction temperature was allowed to warm to room temperature. After concentration, the crude oil was purified by PTLC (EtOAc/MeOH, 40/1) to give **18** (11.8 mg, 98%) as a white syrup. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53−7.48 (m, 4H), 7.38−7.24 (m, 9H), 7.10 (s, 2H), 5.16 (dd, 1H, J = 10.0, 5.0 Hz), 4.58(s, 2H), 4.57 (s, 2H), 4.42−4.40 (m, 1H), 4.01−3.99 (m, 1H), 3.81 (dd, 1H, J = 12.0, 4.0 Hz), 3.73−3.63 (m, 9H), 2.23 (ddd, 1H, J = 13.2, 5.5, 1.8 Hz), 2.05−1.98 (m, 1H), 1.93 (brs, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 140.9, 138.3, 138.1, 137.7, 137.1, 129.1, 129.0, 128.8, 128.6, 128.4, 128.0, 126.9, 126.5, 87.6, 80.3, 74.2, 73.4, 73.3, 71.1, 69.9, 63.8, 44.5. MALDI−FTMS: calcd for M + Na^+ 527.2404, found 527.2402.

4-Chloromethyl-*trans*-stilbene (21). To a solution of 4-hydroxymethyl-*trans*-stilbene **20** (0.557 g, 2.65 mmol) and triethylamine (0.85 mL, 6.1 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added MsCl (0.41 mL, 5.3 mmol) dropwise with stirring. The reaction mixture was stirred at room-temperature overnight. After workup, **21** (0.602 g, 99%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54−7.50 (m, 4H), 7.40−7.35 (m, 4H), 7.30−7.28 (m, 1H), 7.12 (d, 2H, J = 2.3 Hz), 4.61 (s, 2H).

11-[4-[(1*E*)-2-Phenylethenyl] phenyl]-2,4,7,10-tetraoxaundecane (22). The alcohol **26** (0.40 g, 2.65 mmol) was treated with 60% NaH (0.19 g, 4.75 mmol) in dry THF (10 mL) at room temperature for 10 min. To this mixture, a solution of **21** (0.602 g, 2.65 mmol) in THF (10 mL) and cat. NaI was added. The mixture was stirred at 60 °C overnight. The reaction was quenched by addition of water and the mixture extracted with ether. After evaporation, the crude oil was purified by FC (hexane/EtOAc, 2/1) to give **22** (0.7 g, 77%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 753−7.49 (m, 4H), 7.38−7.34 (m, 4H), 7.28−7.25 (m, 1H), 7.11 (s, 2H), 4.68 (s, 2H), 4.58 (s, 2H), 3.74−3.66 (m, 8H), 3.38 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.6, 137.2, 136.6, 128.6, 128.5, 128.3, 128.1, 127.6, 126.4, 96.5, 72.9, 70.6, 70.5, 69.4, 66.7, 55.1. MALDI−FTMS: calcd for M + Na^+ 365.1723, found 365.1716.

2-[2-[[4-[(1*E*)-2-Phenylethenyl]phenyl]methoxy]ethoxy]-ethanol (23). A solution of **22** (0.7 g, 2.05 mmol) in MeOH (10 mL) was treated with a catalytic amount of concentrated HCl at 65 °C. The reaction was followed by TLC until starting material disappeared (∼8 h). The mixture was concentrated and purified by FC (hexane/EtOAc, 1/1) to give **23** (0.524 g, 86%) as a pale white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54−7.50 (m, 4H), 7.39−7.34 (m, 4H), 7.29−7.26 (m,1H), 7.12 (s, 2H), 4.57 (s, 2H), 3.75−3.73 (m, 2H), 3.71−3.68 (m, 2H), 3.66−3.64 (m, 2H), 3.62−3.60 (m, 2H), 3.02 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.2, 137.0, 136.6, 128.5, 128.0, 127.9,

127.4, 126.3, 72.8, 72.3, 70.2, 69.2, 61.5. MALDI−FTMS: calcd for M + Na^+ 321.1461, found 321.1454.

Di(ethylene glycol)benzyl Methoxymethyl Ether (25). To a solution of di(ethylene glycol) benzyl ether **24** (2.0 g, 10.0 mmol) in dimethoxymethane (20 mL) were added LiBr (0.17 g, 2.0 mmol) and *p*-TsOH−H_2O (0.19 g, 1.0 mmol). The white suspension was stirred at room temperature for 3 h, and then the reaction was quenched by addition of brine and the mixture extracted with ether. After evaporation, the residue was purified by FC (hexanes/AcOEt 3/1) to give **25** (1.92 g, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.26 (m, 5H), 4.67 (s, 2H), 4.58 (s, 2H), 3.72−3.64 (m, 8H), 3.37 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.2, 128.3, 127.6, 127.5, 96.5, 73.2, 70.6, 70.5, 69.4, 66.8, 55.1.

Di(ethylene glycol) Methoxymethyl Ether (26). The benzyl ether **25** (1.92 g, 8.0 mmol) was dissolved in chloroform (10 mL) with 10% Pd/C (0.85 g, 0.1 mmol) and stirred under a hydrogen atmosphere provided by a balloon. The reaction was followed by TLC and was complete in 1 h. The mixture was filtered and the filtrate concentrated to give **26** (1.12 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.68 (s, 2H), 3.76−3.62 (m, 8H), 3.38 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 96.5, 72.4, 70.4, 66.9, 61.7, 55.2.

X-ray Crystallography. Crystals of mAb 19G2 were soaked overnight with a 0.25 mM solution of either **9** or **18** (DMF stock solutions) in mother liquor from the crystal growth (12% poly(ethylene glycol), 0.1 M sodium acetate pH 4.75, 0.3 M magnesium chloride) containing 5% DMF. Formation of the complex in the crystal was assayed by the appearance of blue fluorescence from soaked crystals when illuminated by a hand-held UV lamp at 312 nm (Spectronics Corp.; Westbury, NY). The crystals were soaked in a cryobuffer consisting of 20% glycerol, 0.25 mM **9** or **18**, mother liquor, and 5% DMF and flash frozen in liquid nitrogen. X-ray diffraction was collected in-house with an FRD X-ray generator and a RAXISIV^{2+} detector. Data were processed and scaled using the HKL software package.[15] The previously determined structure of the antibody (PDB ID CODE 1FL3) was used as a starting model for refinement. Multiple rounds of rigid body refinement, B-factor refinement, Powell minimization, simulated annealing in CNS and manual rebuilding in O were performed.[16, 17]

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Supporting Information Available: Copies of the ^{1}H NMR and ^{13}C NMR spectra for all prepared compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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