putative 1,4-diyl intermediate **2**. Presuming further a nonrandom orientation of the activated **1** at the TCCT cleavage sites of these oligonucleotides, deuterium transfer to the calicheamicin ϵ (**3**) product should be site specific at C-1 or C-4 and assayable by ^{1}H NMR to reveal the general organization of the drug/DNA complex.

A control experiment was carried out first with unlabeled dodecamer **4** in a deuteriated medium as described previously.[9] No deuterium was observed to be incorporated, and hence, **2** abstracted hydrogens solely from nonexchangeable positions of the DNA dodecamer, consistent with analogous experiments carried out earlier with sonicated calf thymus DNA.[8] Next, the specifically labeled dodecamers **4** and **5** were incubated in the presence of CLM in a nondeuteriated medium.[8,9] Integration of the aromatic proton signals in the NMR spectrum of calicheamicin ϵ (**3**) from both experiments indicated that deuterium was transferred *only* to the C-4 position of the newly formed aromatic ring (see Scheme I).

However, inspection of the ^{1}H NMR spectra revealed that the deuterium incorporations from the isotope-transfer experiments were not complete (62 ± 5% from dodecamer **4**, 82 ± 5% from dodecamer **5**[12]). Scanning densitometry of high-resolution sequencing gels provided the relative proportions of the cleavage products from dodecamers **4** and **5**, as shown in Chart I. Cleavage at the expected cytidine in dodecamer **4** amounted to 79 ± 3% of the observed products and 92 ± 3% in dodecamer **5**. To test the possibility that a kinetic isotope effect in the 5′-deuterium abstraction step might result in isotope-induced branching[13] or solvent abstraction, two further experiments were conducted. With respect to the latter possibility, dodecamer **5** was incubated as above but in a deuteriated medium. The extent of deuterium transfer was unchanged within experimental error (77 ± 5%[12]). With regard to the former, deuterium-labeled and unlabeled dodecamer **4** was 5′-^{32}P-end labeled and treated with CLM in parallel incubations. The fragmentation products were analyzed on sequencing gels where, remarkably, essentially *no* difference was observed between the cleavage patterns exhibited by the labeled and unlabeled oligonucleotide, indicating little or no isotope-induced branching in the distribution of the DNA cleavage products.[14] The origin of the remaining 5–20% hydrogen content in the spent drug presumably either owes to experimental error in NMR integration and densitometry or involves alternate modes of hydrogen abstraction from the 5′-deuterium-labeled deoxycytidine residue itself for it to fail to be readily distinguished on a sequencing gel.[15] Resolution of this issue will have to await additional experiments.

In conclusion, however, the major DNA cleavage process by far (>80%) within the TCCT sequence is initiated by discrete deuterium transfer from the C-5′-labeled deoxycytidine (C) to the proposed activated form of calicheamicin, the diradical **2**. Unlike the NCS-chrom, however, this process unexpectedly is accompanied by no apparent isotope-induced branching, indicating that alternate reaction pathways are not kinetically accessible or that no kinetic isotope effect exists. The deuterium from dodecamers **4** and **5** is transferred to the acetylene nearer the glycosidic linkage in CLM and thus emerges at C-4 in the spent drug leaving an imprint of the minor groove on the aglycon portion of calicheamicin. Given the absolute configuration of calicheamicin γ_1^1, the aryl-linked carbohydrate segment will, therefore, be directed to the 3′-side of the TCCT cleavage site in these oligomers.[16] While we have no direct evidence for the location of this side chain, it presumably extends along the minor groove to establish hydrophobic, electrostatic, and hydrogen-bonding interactions[9,17] that would account, at least in part, for the specificity of the drug for its cognate DNA receptor,[18] as discussed previously.[17]

The atom-transfer method described herein is a discriminating and direct means to overcome the pseudo-C_2 symmetry of the DNA helix and the inherent geometric ambiguity of drug/DNA interactions. For the growing class of diynene antibiotics, application of this method in principle can not only reveal the orientation of the drug at any cleavage site, but also identify the hydrogen(s) that are abstracted to initiate DNA cleavage events, whether or not isotope-induced branching is observed.[14]

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(12) These deuterium incorporations are corrected for the actual deuterium contents of **4** and **5** (95 ± 1%) accurately determined by mass spectrometry of stable intermediates in the respective chemical syntheses. Drug/DNA ratios of 1:2–1:1 gave identical extents of deuterium transfer. The latter ratio was used, therefore, in the experiments described herein.

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A New Approach to Cyclitols Based on Rabbit Muscle Aldolase (RAMA)[1]

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The synthesis of cyclitols having well-defined stereochemistry starting with nonchiral precursors is a current challenge in organic synthesis. Most synthetic strategies either start from chiral precursors (e.g., carbohydrates),[3-5] or resolve the racemic adduct formed in a Diels–Alder reaction.[6,7] Here we report the application of rabbit muscle aldolase (RAMA; EC 4.1.2.13) to the preparation of cyclitols and *C*-glycosides (Scheme I).

RAMA catalyzes the aldol condensation of dihydroxyacetone phosphate (DHAP) and aldehydes and forms products with the

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(2) "Erwin Schrödinger" Postdoctoral Fellow of the "Fonds zur Förderung der wissenschaftlichen Forschung in Österreich" (Austrian Science Foundation).

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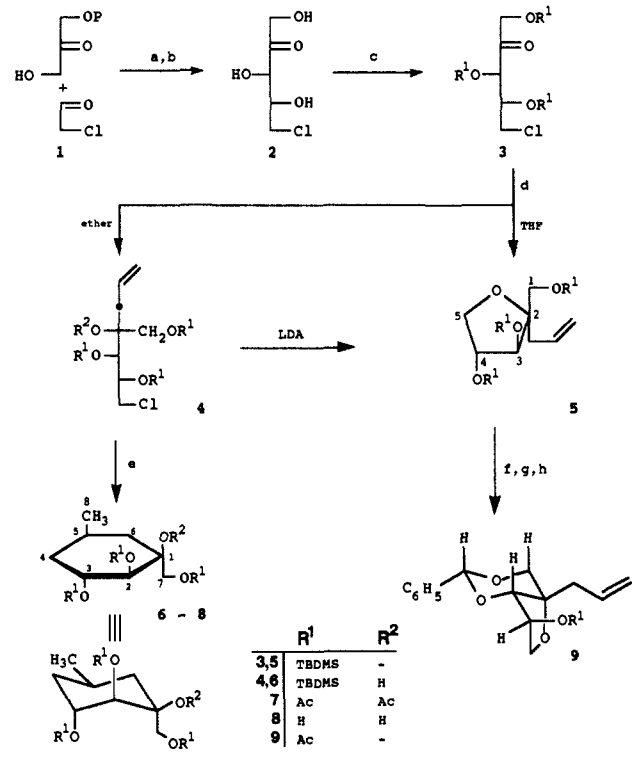
Scheme I[a]



[a] Reagents and conditions: (a) RAMA; (b) acid phosphatase, 50%; (c) TBDMSiOTf/Et_3N, 67%; (d) allylmagnesium bromide, 79%; (e) Bu_3SnH/AIBN, 75%, Δ; (f) TBAF, 96%; (g) $C_6H_5CH(OCH_3)_2$/TsOH; (h) Ac_2O/Pyr/DMAP.

D-threo (3*S*,4*R*) stereochemistry.[8-10] The aldol condensation between DHAP and chloroacetaldehyde **1** catalyzed by RAMA proceeds rapidly[9] and conveniently generates 5-deoxy-5-chloro-*threo*-pentulose (**2**) on a gram scale. Enzymatic dephosphorylation of **2** in situ with acid phosphatase (AP; EC 3.1.3.2) and protection of the hydroxyl groups as *tert*-butyldimethylsilyl ethers leads to **3**. Reaction of **3** with allylmagnesium bromide shows an interesting solvent dependence: in dry tetrahydrofuran (THF), the Grignard addition leads to an easily separable 2.7:1 mixture of *threo*-pentulose-*C*-allylglycoside **5** and the branched chain alditol **4**; in dry diethyl ether, this reaction gives **4** exclusively in 79% yield. Radical ring closure[11] starting from **4** forms the cyclitol **6**.[12]

We assigned the stereocenters in **4** and **6** in several ways. First, we transformed the alditol **4** into the *C*-glycoside **5** by treatment with LDA; this transformation establishes that the stereochemistry generated by the Grignard reaction is the same in **4** and **5**. Since the branched-chain alditol **4** can be converted to the cyclitol derivative **6**, the stereochemistry at the quaternary center in **6** must be the same as that of the anomeric center in **5**. This assignment was supported by NOE studies on **9**,[13] which showed a syn relationship between the hydrogen at C-3 and the allyl moiety at the "anomeric" center. The conformation shown in Scheme I is consistent with $J_{3,4} \sim 0$ Hz for **9**. ^{1}H NMR and NOE experiments on **6** showed $J_{2,3} = 3.3$ Hz and indicated a trans diaxial arrangement of the silyloxy groups at C-2 and C-3. The axial attachment of the hydrogen at C-5 was assigned on the basis of the large coupling constant of this proton to the proton H-6ax ($J = 12.5$ Hz), and because there was a significant NOE effect (4.2%) between H-5 and the CH_2 protons at C-7. These observations define the conformation of **6** unambiguously.[14] Since RAMA-catalyzed aldol condensations produce vicinal diols having only the 3*S*,4*R* stereochemistry, we were thus able to assign all the stereocenters.

The synthetic route outlined in Scheme I demonstrates an efficient approach to both cyclitols and *C*-glycosides based on catalysis by RAMA. Other aldolases generate other stereochemistries in the original aldol adduct.[15] Investigations directed toward expansion of these strategies are under way.

Supplementary Material Available: Experimental procedures for all compounds, ^{1}H and ^{13}C NMR data for **2-7**, **8** (^{1}H), and **9**, high-resolution mass spectra for **3**, **5**, and **6**, and elementary analysis for **3-6** (7 pages). Ordering information is given on any current masthead page.

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Thermal Rearrangement of Fluorinated Dioxoles[1]

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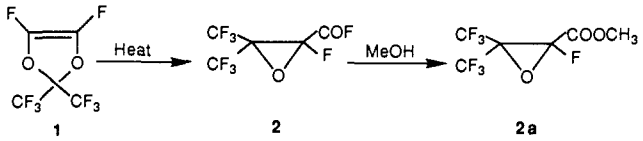
Du Pont has recently developed Teflon-AF,[2] a family of amorphous copolymers of 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole[3] (**1**) and tetrafluoroethylene. These polymers are similar to poly(tetrafluoroethylene) in chemical and thermal stability but have superior physical properties and optical clarity and lower dielectric constants. In addition, they are soluble in selected fluorinated solvents, thus allowing the preparation of thin cast films.[4] The dioxole monomer **1** and related dioxoles undergo a number of surprising reactions. We report the unusual thermal rearrangement of these dioxoles to substituted acyl epoxides.



Perfluoro-2,3-epoxy-3-methylbutyryl fluoride (**2**) was isolated in greater than 85% yield when **1** was heated at 250 °C over glass beads in a flow system. The acyl fluoride **2** was characterized by infrared absorption bands at 1887 (C=O) and 1462 cm^{-1} (oxirane) and by its ^{19}F NMR spectrum (Table I). Treatment of **2** with methanol yielded methyl perfluoro-2,3-epoxy-3-methylbutyrate (**2a**), characterized by infrared absorption bands at 1792 (C=O) and 1466 cm^{-1} (oxirane) and also by its ^{19}F NMR spectrum (Table I).

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