used with RAMA, may change the position of the D-*threo*-*vic*-diol group, but does not change its absolute configuration, since the $nS,(n+1)R$ stereochemistry is retained on inversion of the sense of the carbon backbone. This strategy used with Fuc-1-P aldolase changes the absolute stereochemistry of the *vic*-diol unit from $3R,4R$ to $nS,(n+1)S$ by inverting the sense of the carbon backbone. Thus, the Fuc-1-P aldolase offers, in principle, a stereocontrolled synthetic route to two configurationally distinct -CHOHCHOH- units.

This work makes Fuc-1-P aldolase readily available for use in organic synthesis and establishes that this enzyme can be expected to have usefully broad specificity in its aldehyde reactant. The stereochemistry of the vicinal diol unit generated by Fuc-1-P aldolase is complementary to that generated by RAMA; it has the further advantage that its use with aldehydes of the type exemplified by **3** and **4** provides access to a third stereochemical unit. The availability of Fuc-1-P aldolase will significantly extend the range of application of aldolases as a class of enzymes in carbohydrate synthesis.

Work directed toward cloning and overexpression of a third stereochemically distinct aldolase, L-rhamnulose-1-phosphate aldolase (EC 4.1.2.19) (Scheme I), is now in progress.

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This paper is dedicated to Günther Ohloff.

Supplementary Material Available: Experimental procedure for the preparation of D-ribulose (3 pages). Ordering information is given on any current masthead page.

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Observation of Benzocyclobutadiene† by Flow Nuclear Magnetic Resonance[1]

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Benzocyclobutadiene (**1**) is a key molecule in the understanding of the aromaticity and antiaromaticity of cyclic delocalized π-electron systems.[2,3] Benzocyclobutadiene has eight π electrons

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and is the simplest bicyclic compound resulting from the fusion of one $(4n + 2)$ and one $(4n)$ π-electron system, the benzene and cyclobutadiene rings, respectively. The high reactivity of **1** has made it difficult to study, but it has been isolated in an Ar matrix at 20 K, and its IR, UV-visible,[4] and photoelectron[5] spectra have been obtained. Spectra,[6-11] including ^{1}H NMR,[7-11] of several substituted benzocyclobutadienes, some of which are stable at room temperature, have also been obtained. In this communication we report the ^{1}H NMR spectrum of **1** obtained by the technique of flow NMR.

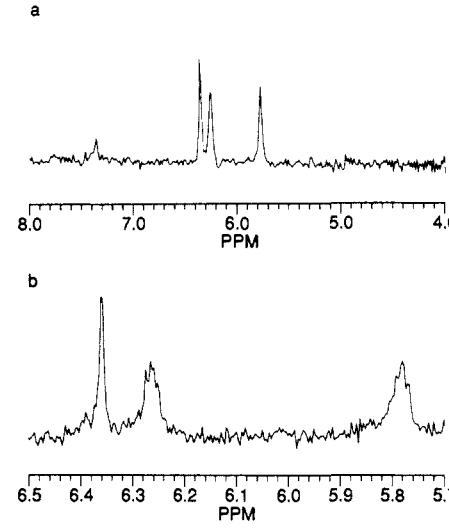
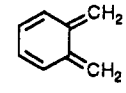


Figure 1. ^{1}H NMR spectra (300 MHz): (a) formed by mixing 10^{-3} M **4** in CD_3CN with 5×10^{-2} M TBAF in CD_3CN; flow rate, 45 mL/min; number of scans, 512; pulse interval, 0.127 s; (b) formed by mixing 10^{-3} M **4** in CD_3CN with 5×10^{-2} M TBAF in CD_3CN; flow rate, 3 mL/min; number of scans, 1105; pulse interval, 1.016 s.

Recently we reported the NMR spectra of *o*-xylylene (**2**) and other reactive *o*-quinodimethanes (*o*-QDMs).[12,13] These *o*-QDMs



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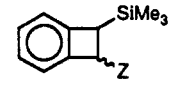
were generated by the fluoride ion induced 1,4-elimination from [*o*-((trimethylsilyl)methyl)benzyl]trimethylammonium halides, an excellent method for the generation of reactive molecules under flow NMR conditions because fast, quantitative formation of the transient species is desired.[12-16] Thus, in our pursuit of the ^{1}H NMR spectrum of **1**, we initially attempted to prepare **3** as a possible precursor of **1**. However, after failing to synthesize **3**, we prepared several analogues of **3** that have more readily accessible leaving groups and have found that the mesylate, **4**, is



3, Z = NMe_3I
4, Z = OSO_2Me

† The title compound is correctly named benzocyclobutene but commonly called benzocyclobutadiene. The systematic name for the compound commonly called benzocyclobutene is 1,2-dihydrobenzocyclobutene.

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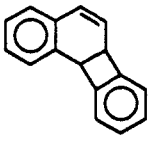
an excellent precursor of **1**. Mesylate **4**, as a ca. 5:1 mixture of the cis and trans isomers,[17] was prepared by starting from the corresponding α-(trimethylsilyl)cyclobutanone (**5**) prepared as described by Swenton et al.[18] Ketone **5** was reduced by AlH_3[19,20] to a ca. 5:1 mixture of the cis and trans isomers of the corresponding alcohol, which was converted to the mesylate by standard techniques.

The ^1H NMR spectrum of the solution formed by mixing an acetonitrile-d_3 (CD_3CN) solution of **4** (10^{-3} M) and a CD_3CN solution of tetrabutylammonium fluoride (TBAF)[16] (5×10^{-2} M) at a total flow rate of 45 mL/min[12] is shown in Figure 1a. The three strong peaks at δ 6.36, 6.26, and 5.78 are assigned to the reactive molecule benzocyclobutadiene (**1**). The spectrum shown in Figure 1b was obtained at a total flow rate of 3 mL/min and is plotted with smaller line broadening than the spectrum shown in Figure 1a. The spectrum in Figure 1b shows that the peaks at δ 6.26 and 5.78 are the AA'BB' multiplets resulting from the six-membered-ring protons of **1**. Thus the lowest field signal at δ 6.36 is assigned to the protons of the four-membered ring.

At slower flow rates, the signals for **1** are replaced with those of an intermediate, which relatively rapidly (within minutes) changes to dimer **6**, the known product of the dimerization of **1**.[21] Currently we are studying this transformation.



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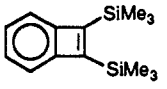
The positions of the six-membered-ring proton signals (δ 6.26 and 5.78) of **1** are very close to those reported (δ 6.30 and 5.75 in CD_3CN) for 1,2-bis(trimethylsilyl)benzocyclobutadiene (**7**).[11]



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It is interesting to note that these two signals are also similar to those of the ring protons of *o*-xylylene (**2**) which are at δ 6.3 and 6.0,[12] and to the signal of the six-membered-ring protons of 2,2-dimethylisoindene (**8**, 2,2-dimethyl-2*H*-indene) which is at δ 6.08.[22] The six membered ring proton signals of **1**, however, are significantly upfield from the benzene-ring protons of benzocyclobutene (δ 7.0)[23] and biphenylene (δ 6.72 and 6.62).[24]

The position of the four membered ring proton signal (δ 6.36) of **1** is very close to that of the olefinic-ring protons of cyclobutene (δ 6.0)[25] and 3,4-dimethylenecyclobutene (δ 6.7)[26] and to that of the five-membered-ring protons of **8** (δ 6.55).[22]

The picture of **1** that emerges from these comparisons is that it is a nonaromatic (polyolefinic) compound, not an aromatic or antiaromatic compound. The similarities of the spectra of **1**, **2**, and **8** are consistent with the view that it is an *o*-QDM, structure **9**. However, the X-ray structure of a highly substituted ben-

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zocyclobutadiene, 1,2-di-*tert*-butyl-3,4,5,6-tetramethylbenzocyclobutadiene, indicates that the benzocyclobutadiene framework is better represented by structure **1**.[27] Of course, the large substituents could be distorting the benzocyclobutadiene framework.

Although we have not studied the kinetics of the dimerization of **1**, it is evident from our flow NMR experiments that it is slightly less reactive than *o*-xylylene (**2**).

Acknowledgment. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Chemical Sciences Division, under Contract W-7405-ENG-82. The cooperation and excellent technical assistance of Robert David Scott, the operator of the NMR spectrometer, are greatly appreciated.

Registry No. **1**, 4026-23-7; *cis*-**4**, 126543-57-5; *trans*-**4**, 126543-58-6.

(17) **4** (5:1 mixture of cis and trans isomers): mp 38-47 °C; IR (KBr) 3024, 1362, 1173, 843 cm^{-1}; ^1H NMR (of major isomer) ($CDCl_3$) δ 7.31-7.01 (m, 4 H), 5.94 (d, J = 5.1 Hz, 1 H), 3.41 (d, J = 5.1 Hz, 1 H), 3.07 (s, 3 H), 0.07 (s, 9 H); ^{13}C NMR (of major isomer) ($CDCl_3$) δ 144.55, 141.52, 130.66, 126.43, 123.40, 121.44, 76.79, 42.26, 38.57, -2.02; HRMS calcd for $C_{12}H_{18}O_3SSi$ 270.0746, found 270.0743.

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Anomeric Specificity of 3-Deoxy-D-*manno*-2-octulosonate 8-Phosphate Phosphatase from *Escherichia coli*

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3-Deoxy-D-*manno*-2-octulosonate (KDO) is a specific constituent of the lipopolysaccharide (LPS) of most Gram-negative bacteria, and it provides the link between lipid A and the growing polysaccharide chain.[1] The synthesis and activation of KDO is a vital part of the assembly process of lipopolysaccharides in Gram-negative bacteria.[2] Indeed, it has been shown that an interruption of the production or utilization of KDO leads to a buildup of LPS precursors, and growth stasis.[3] There are at least four enzymes involved in the synthesis and utilization of KDO.[1-3] The KDO 8-phosphate produced from D-arabinose 5-phosphate is dephosphorylated by KDO 8-phosphate phosphatase, and the resulting KDO is then converted into cytidine monophosphate KDO by CTP:CMP-KDO cytidylyl transferase (CMP-KDO synthetase). This synthetase is considered to be the rate-limiting step in the biosynthetic incorporation of KDO into LPS.[2a] Recent studies have revealed that the β-pyranoside form of KDO, a minor form in solution, is the actual substrate of this enzyme.[4] Since the interconversion rates of the different anomers of KDO are slow, it has been suggested that the rate of KDO incorporation into lipopolysaccharides may be limited by the rate of formation of the β-pyranose form of KDO.[5] We have therefore investigated

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