and Mgm1 have been demonstrated and involve the outer membrane fusion protein Ugo1 (*7*, *8*). The exact nature of the interactions between Fzo1, Ugo1, and Mgm1 and their specific roles in mitochondrial fusion remain largely unknown. However, Ugo1 functions as an adaptor between Fzo1 and Mgm1 (*18*). Fzo1 interactions with inner membrane components may be required in a mechanical manner for the formation of regions of close inner and outer membrane contact within mitochondria. Such regions of contact would function to bring inner membranes into closer proximity after outer membrane fusion and also perhaps to eliminate cristae in the vicinity of fusion. Indeed, by EM analysis, no cristae are observed at sites of inner membrane contact (Fig. 4B). Alternatively, but not exclusively, Fzo1 may function in a regulatory manner by stimulating, by means of GTP cycle–dependent conformations, events in the inner membrane required for fusion.

Fzo1 is a key player in the evolution of mitochondrial fusion. Based on a phylogenetic analysis, Fzo1 is derived from the eubacterial endosymbiotic precursor of mitochondria (*10*, *11*). Our data showing that Fzo1 plays essential and fundamental roles in the fusion of both outer and inner membranes are consistent with this idea. Phylogenetic analysis of Fzo1 also identifies it as a member of the dynamin-related GTPase family (*11*). The similarity of Fzo1 to DRPs suggests the intriguing possibility that DRPs evolved from a eubacterial progenitor, and that Fzo1, like DRPs, functions to remodel membranes through self-interaction and assembly. An additional evolutionary connection between DRPs and endosymbiotic organelles is that their division also has evolved to require the action of a DRP (*25*).

DRPs most commonly have been shown to function in membrane fission events, such as mitochondrial and chloroplast division and endocytosis (*26*). However, the actions of two DRPs, Fzo1 on the outer membrane and Mgm1 on the inner membrane, are required for mitochondrial membrane fusion. In a fusion event, Fzo1 and Mgm1 may possess modified activities and function through self-assembly only to tubulate, and not divide, regions of outer and inner membrane, thereby creating a bending stress, which can be harnessed for membrane fusion. The utilization of DRPs to drive membrane fusion events mechanistically distinguishes mitochondrial fusion from other fusion events in eukaryotic cells. Understanding their exact mode of action will enhance our understanding of the fundamental principles that underlie membrane fusion events.

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Materials and Methods
Figs. S1 and S2
References
Movies S1 to S3

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REPORTS

Two-Step Synthesis of Carbohydrates by Selective Aldol Reactions

Alan B. Northrup and David W. C. MacMillan*

Studies of carbohydrates have been hampered by the lack of chemical strategies for the expeditious construction and coupling of differentially protected monosaccharides. Here, a synthetic route based on aldol coupling of three aldehydes is presented for the de novo production of polyol differentiated hexoses in only two chemical steps. The dimerization of α-oxyaldehydes, catalyzed by L-proline, is then followed by a tandem Mukaiyama aldol addition-cyclization step catalyzed by a Lewis acid. Differentially protected glucose, allose, and mannose stereoisomers can each be selected, in high yield and stereochemical purity, simply by changing the solvent and Lewis acid used. The reaction sequence also efficiently produces ^{13}C-labeled analogs, as well as structural variants such as 2-amino– and 2-thio–substituted derivatives.

Hexose carbohydrates play vital roles in biological processes as diverse as signal transduction, cognition, and the immune response. However, the study of this fundamental class of bioarchitecture has been hindered by the paucity of chemical methods for the efficient synthesis and coupling of hexose systems to form polysaccharides and other derivatives (*1*). Specifically, the challenge in selectively linking and functionalizing these monosaccharides lies in distinguishing among their five chemically similar hydroxyl groups. During the last century, chemists have focused on using iterative alcohol protection-deprotection strategies, an approach that typically requires 8 to 14 chemical steps (*1*, *2*). While the abundant and inexpensive supply of native carbohydrates may render such a strategy intuitively attractive, we felt that a de novo enantioselective synthesis of differentially protected hexoses might provide a more efficient approach (*3*–*10*). The appeal of this strategy is that fragments of the hexose can be independently derivatized (isotopically or

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functionally) before assembly of the molecule; thus, there is no longer a need to chemically discriminate between similar groups on a hexose framework.

In this context, the aldol reaction has been applied to the synthesis of carbohydrates on a few occasions; however, the need for iterative oxidation-state adjustments has thus far precluded a broadly used or step-efficient protocol. From a conceptual standpoint, a two-step carbohydrate synthesis can be envisioned based on an iterative aldol sequence using simple α-oxyaldehydes. While the approach is attractive in theory, the practical execution of this synthetic strategy requires two unproven aldol applications: (i) an enantioselective aldol union of α-oxyaldehyde substrates (Aldol Step 1, Fig. 1A) and (ii) a diastereoselective aldol coupling between tri-oxy–substituted butanals and an α-oxyaldehyde enolate (Aldol Step 2, Fig. 1B). In this report, we describe the successful development of both such aldol reactions for the two-step synthesis of enantioenriched, polyol-differentiated hexoses.

The first step in our synthetic scheme (Aldol Step 1) is a stereoselective α-oxyaldehyde dimerization. Beyond the traditional demands of enantio- and diastereocontrol, the reaction requires that the α-oxyaldehyde reagent **1** participate as both a nucleophile and an electrophile, whereas the product **2**, also an α-oxyaldehyde, must not perform as either (Fig. 1A). Recently, we disclosed an organocatalytic strategy that uses L-proline for the regio-, diastereo-, and enantioselective aldol cross-coupling of α-alkyl–bearing aldehydes (*11*–*16*). Notably, the aldehyde-containing products of this reaction do not participate in further aldol chemistry. With this in mind, we attempted to extend this methodology to the coupling of α-oxygenated aldehydes (Fig. 1A).

As shown in Fig. 2, the proline catalyzed α-oxy aldol (Step 1 results) does provide direct and enantioselective access to differentially protected *anti*-1,2 triols. Specifically, exposure of α-triisopropylsilyloxy-acetaldehyde to 10 mol% L-proline at room temperature readily provides enantioenriched [95% enantiomeric excess (ee)] α,γ-oxy–protected L-erythrose **3**, whereas the corresponding reaction of α-benzyloxyacetaldehyde leads to the dimeric aldol adduct **4** in 98% ee. As required, the α-oxyaldehyde products of this new aldol protocol are inert to further proline-catalyzed enolization or enamine addition. We recently published a report outlining the scope and limitations of this α-oxyaldehyde dimerization (*17*).

Having succeeded in our first step, we next focused on adding the third aldehyde building block and achieving cyclization. Given that β-hydroxy aldehydes (the products of Step 1) are relatively inert to enamine addition, we focused instead upon Lewis acid activation for the second aldol coupling. Specifically, we reasoned that a Mukaiyama aldol reaction between an α-oxy-enolsilane **5** and a trioxy-aldehyde **2** (the product of Aldol Step 1) might generate a hexose-oxocarbenium intermediate **6** that would rapidly undergo cyclization to form the carbohydrate ring system (Fig. 1B). This tandem aldol addition and cyclization presents two selectivity issues: (i) The chemoselective preference for the oxocarbenium **6** to undergo cyclization in lieu of further aldol addition and (ii) the diastereoselective construction of two new oxy-stereocenters in the carbon-carbon bond–forming step, which ultimately defines the extent to which one carbohydrate isomer is generated in preference to another (e.g., allose versus altrose versus glucose versus mannose; see Fig. 2).

We first examined the use of the triisopropylsilyl (TIPS)-protected β-oxy aldehyde **3** (Aldol Step 1 product) and the α-acetoxy-

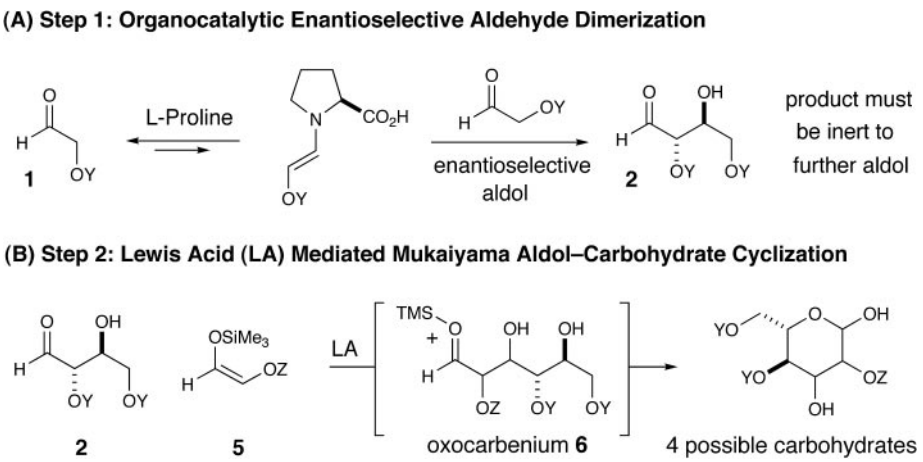


Fig. 1. (**A**) Step 1: Proline-catalyzed enantioselective dimerization of α-oxyaldehydes. (**B**) Step 2: Mukaiyama aldol-carbohydrate cyclization.

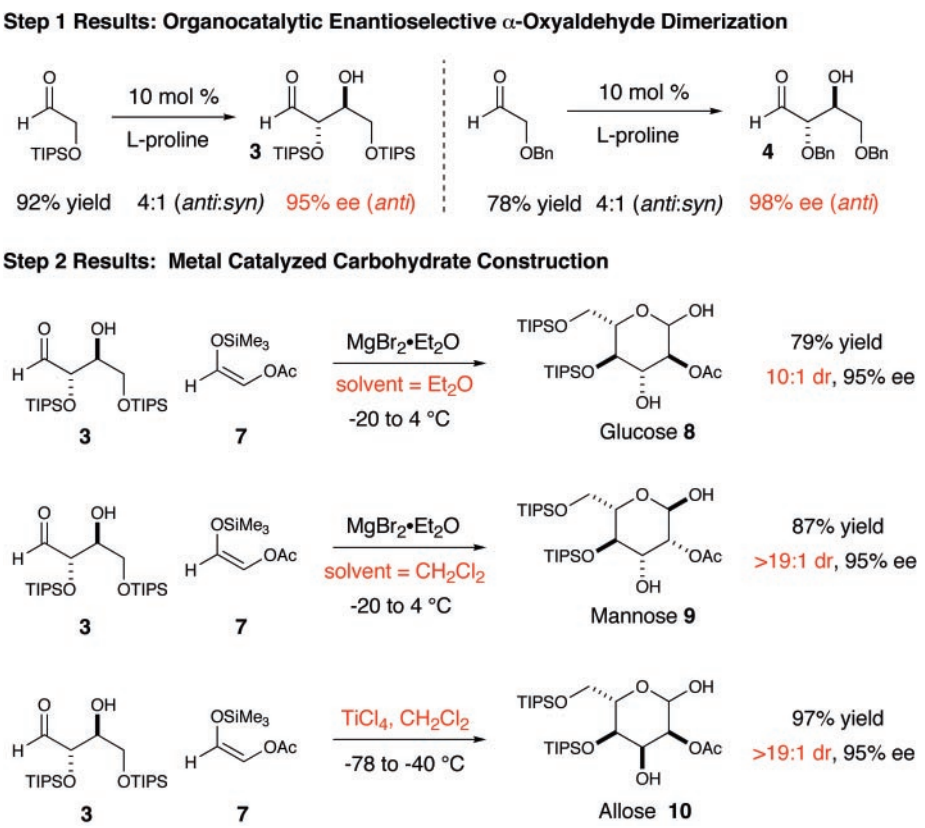


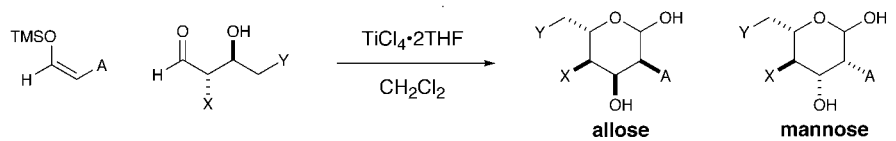
Fig. 2. Step 1 Results: The enantioselective dimerization of α-oxyaldehydes. Step 2 Results: The Lewis acid–mediated Mukaiyama aldol-carbohydrate cyclization.

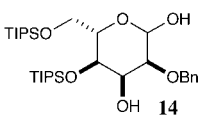
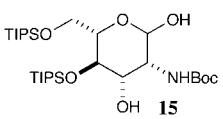
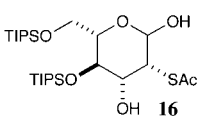
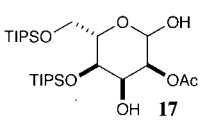
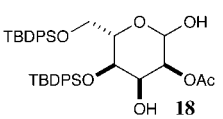
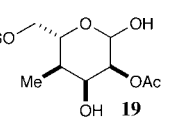
enolsilane **7** in the presence of Lewis acidic salts such as $MgBr_2{\cdot}OEt_2$ or $TiCl_4$ (Fig. 2, Step 2 results). Preliminary studies revealed that this second aldol reaction does provide polyol-differentiated hexose carbohydrates in excellent yields and diastereoselectivities (dr) (*18*). More important, selective access to either glucose, mannose, or allose can be accomplished by judicious choice of Lewis acid and reaction solvent.

For example, the use of $MgBr_2{\cdot}OEt_2$ in solvents such as ether, toluene, or pentane affords high levels of selectivity for glucose **8** (8:1 to 10:1), whereas the analogous reaction in dichloromethane is selective for mannopyranose **9**. Using optimized conditions, we obtained a 79% yield and a 10:1 preference for glucose in diethyl ether, whereas we observed an 87% yield and >19:1 selectivity for mannose in dichloromethane (Fig. 2). The origins of this dramatic change in isomer selectivity as a function of reaction solvent reflect the capacity of the reaction medium to dictate which face of the enolsilane reacts with the aldehyde. Furthermore, exposure of the same aldehyde and enolsilane components **3** and **7** to $TiCl_4$ leads to the selective formation of the allose carbohydrate isomer in >19:1 selectivity, 97% yield, and 95% ee. In this latter case, we have determined that the enolsilane undergoes transmetallation to generate a titanium-enolate before the Aldol Step 2 event. We propose that this metalloenolate participates in a cyclic (closed) transition state with the Felkin diastereoface of the aldehyde, whereas the magnesium reactions involve addition of the enolsilane to the opposite (non-Felkin) aldehyde face. We note that the unnatural (L) form of carbohydrates **8**, **9**, and **10** could be accessed selectively by using the alternate (D) enantiomer of proline in the Aldol Step 1.

Having developed this methodology, we applied our reaction sequence to the preparation of $^{13}C_6$-labeled hexoses. Specifically, we produced fully ^{13}C-labeled, differentially protected D-glucose **11**, D-mannose **12**, and D-allose **13** derivatives in only four linear steps from $^{13}C_2$-ethylene glycol (*19*), in overall yields of 33%, 35%, and 43%, respectively.

Table 1. Representative two-step enantioselective carbohydrate synthesis. Temperature refers to the final temperature of the reaction mixture after being warmed from –78°C. Yield refers to the combined yield of diastereomers. Diastereoselectivity (dr) was determined by proton nuclear magnetic resonance (^1H NMR) integration of the reaction mixture. Entry 4 was performed with $TiCl_4$.



Entry	A	X	Y	Major isomer	Temp (°C)	% yield	dr	%ee
1	OBn	OTIPS	OTIPS	 **14**	–30	83	>19:1	95
2		OTIPS	OTIPS	 **15**	–40	74	10:1 (mannose)	95
3	SAc	OTIPS	OTIPS	 **16**	–20	71	19:1 (mannose)	95
4	OAc	OTIPS	OTIPS	 **17**	–40	96	>19:1	95
5	OAc	OTBDPS	OTBDPS	 **18**	–20	86	>19:1	96
6	OAc	Me	OTBDPS	 **19**	–30	68	>19:1	99

Our route to differentiated hexoses is also amenable to considerable structural variation in both the enolsilane reagent and the β-oxyaldehyde component (Table 1). This critical feature in reaction versatility allows the rapid construction of hexoses that can be directly used in the synthesis of di- or polysaccharides. For example, carbohydrates that contain participating or nonparticipating groups at the C(2) position are readily accessed by using the respective acyloxy- or benzyloxy-substituted enolsilanes (Table 1, entry 1, A = OBn, 83% yield, >19:1 allose selective; entry 4, A = OAc, 96% yield, >19:1 allose selective). Such hexose systems have established utility as either α- or β-coupling partners in polysaccharide synthesis (*1*, *2*). The modular nature of the Aldol Step 1 also allows for broad diversification of substituents at the carbohydrate C(4) and C(6) positions (*10*, *16*). For example, the incorporation of TIPS-protecting and tertiary-butyldiphenylsilyl (TBDPS)–protecting groups at these sites is readily accomplished (Table 1, entries 4 and 5, 86 to 96% yield, >19:1 dr, ≥95% ee). These protecting groups can be selectively removed from the C(6) position, thereby affording carbohydrates that are differentially protected at each hydroxyl site. As such, these versatile saccharide monomers can be rapidly manipulated to expose the C(2), C(3), C(4), or C(6) hydroxyl groups, an important consideration for di- or polysaccharide couplings.

The reaction sequence also allows rapid access to a wide variety of unnatural carbohydrates that substitute carbon, nitrogen, and sulfur groups for the native hydroxy constituents. The analogous reactions using amino- and thio-substituted enolsilanes provide the mannose architecture in high selectivity, affording the 2-*tert*-butylcarba-moylmannose derivative **15** (Table 1, entry 2) in 74% yield and 10:1 diastereocontrol and the 2-acetylmercaptomannose product **16** (Table 1, entry 3) in 71% yield and >19:1 mannose selectivity. Carbogenic substituents can also be introduced at the saccharide C(4) position in the case where α-alkyl and α-oxy aldehydes were cross-coupled in the Step 1 Aldol event (Table 1, entry 6, 68% yield, >19:1 dr, 99% ee). The capacity to selectively build known carbohydrates with single-point atomic mutations will enable medicinal chemists to rapidly study structure activity relationships (SAR) on mono-, di-, and polysaccharide templates.

Our strategy for the synthesis of differentially protected hexoses thus provides rapid enantioselective access to key building blocks in saccharide and polysaccharide synthesis. Furthermore, our approach efficientlyyields isotopic and functional variants of the hexoses that have not been readily accessible for pharmaceutical study.

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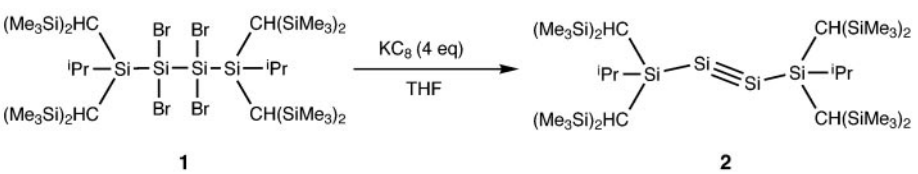
A Stable Compound Containing a Silicon-Silicon Triple Bond

Akira Sekiguchi,* Rei Kinjo, Masaaki Ichinohe

The reaction of 2,2,3,3-tetrabromo-1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyltetrasilane with four equivalents of potassium graphite (KC_8) in tetrahydrofuran produces 1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1,4-diisopropyl-2-tetrasilyne, a stable compound with a silicon-silicon triple bond, which can be isolated as emerald green crystals stable up to 100°C in the absence of air. The Si≡Si triple-bond length (and its estimated standard deviation) is 2.0622(9) angstroms, which shows half the magnitude of the bond shortening of alkynes compared with that of alkenes. Unlike alkynes, the substituents at the Si≡Si group are not arranged in a linear fashion, but are trans-bent with a bond angle of 137.44(4)°.

Hydrocarbons containing C=C double bonds (alkenes) and C≡C triple bonds (alkynes) form an abundant and structurally diverse class of organic compounds. However, the ability of heavier congeners of carbon (where element E is Si, Ge, Sn, and Pb) to form double bond of the type >E=E< and triple bond of the type -E≡E- was for a long time doubted (*1–4*). The first attempts to generate such species were unsuccessful, resulting in the formation of polymeric substances. This led to the oftencited "double-bond rule": Those elements with a principal quantum number equal to or greater than three are not capable of forming multiple bonds because of the considerable Pauli repulsion between the electrons of the inner shells (*5–7*). Such a viewpoint prevailed despite the accumulation of a vast amount of experimental data supporting the existence of multiply bonded species as reactive intermediates (*1–4*). This conflict was resolved nearly 30 years ago, when Lappert and Davidson reported the synthesis of the stable distannene $[(Me_3Si)_2CH]_2Sn=Sn[CH(SiMe_3)_2]_2$, where Me is methyl, which has a Sn=Sn double bond in the solid state (*8*). The next important discoveries came from two research groups in 1981: West and colleagues reported the synthesis of a stable compound with a Si=Si double bond, tetramesityldisilene (*9*), and Brook *et al.* synthesized a compound with a Si=C double bond (*10*). As for triple bonds, Power and co-workers recently prepared alkyne analogs of the heavier group 14 elements: germanium, tin, and lead (*11–13*). However, despite bearing nominal triple bonds, these compounds actually exhibited a highly pronounced nonbonding electron density character at the central atoms, resulting in a decrease in the bond order on descending group 14 (*14*, *15*). In light of these results, isolation of the silicon analog of alkynes has been a compelling goal. Although the theoretical analysis predicted the experimental accessibility of disilynes with a silicon-silicon triple



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Reaction 1.

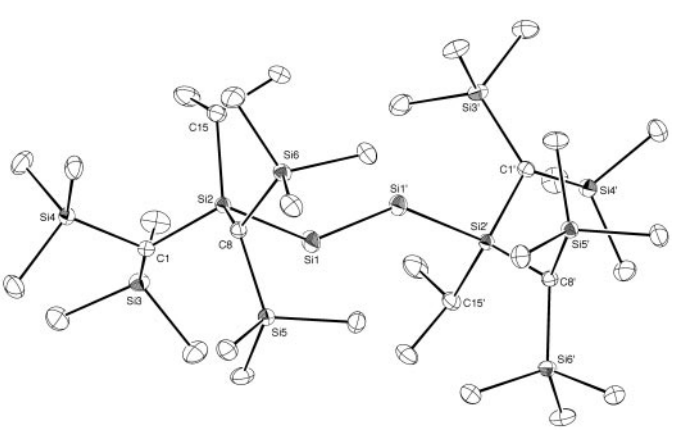

Fig. 1. Molecular structure of 1,1,4,4-tetrakis[bis(trimethylsilyl)methyl]-1, 4-diisopropyl-2-tetrasilyne (**2**) (30% probability ellipsoids for Si and C). Selected bond lengths (Å): Si1–Si1′ = 2.0622(9), Si1–Si2 = 2.3698(6), Si2–C1 = 1.9119(15), Si2–C8 = 1.9120(15), and Si2–C15 = 1.9180(16). Selected bond angles (°): Si1′–Si1–Si2 = 137.44(4), Si1–Si2–C1 = 108.97(5), Si1–Si2–C8 = 108.38(5), Si1–Si2–C15 = 106.47(5), C1–Si2–C8 = 106.83(6), C8–Si2–C15 = 114.77(7), and C1–Si2–C15 = 111.30(7). Estimated standard deviations are in parentheses.

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