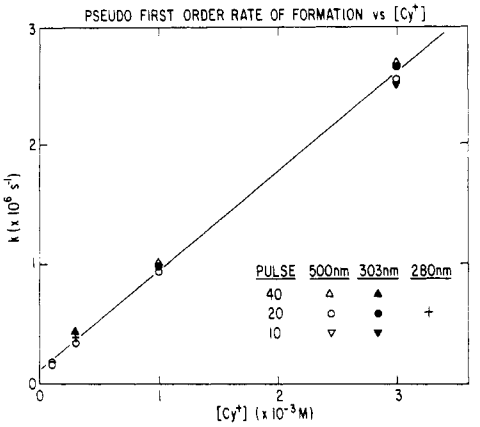

Figure 3. Pseudo-first-order rates of formation of Cy_2^+ vs. $[Cy_+]$ at different wavelengths and pulse widths (in nanoseconds).

spectrum is first order in the cation and first order in the primary reduction product. On the basis of these kinetics, we can write eq 1 and 2, where Cy^+ is the trimethyl-

$$Cy^+ + e^-_{aq} \xrightarrow{k_1} Cy\cdot \quad (1)$$

$$Cy\cdot + Cy^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Cy_2^+ \quad (2)$$

cyclopropenium cation and Cy· is the cyclopropenyl radical. The rate of electron capture at 25 °C is diffusion controlled; $k_1 = 3.2 \times 10^{10}$ M^{-1} s^{-1} (at zero ionic strength). The reversibility of reaction 2 is clearly established from the concentration study, and the values of $k_2 = 8.3 \times 10^8$ M^{-1} s^{-1} and $k_{-2} \approx 3 \times 10^5$ s^{-1} are taken from the slope and intercept of a plot of the apparent rate constant of formation of Cy_2^+ vs. $[Cy_+]$ (Figure 3). This equilibrium corresponds to $\Delta G = -4.7$ kcal/mol for the formation of the complex.

The rate of disappearance of Cy_2^+ is mostly second order with some small first-order component. A high concentration of Cy^+ retards the rate, indicating that the Cy_2^+ disappears via dissociation (eq 3–6). The major product

$$Cy_2^+ \rightleftarrows Cy^+ + Cy\cdot \quad (3)$$

$$Cy\cdot + Cy\cdot \rightarrow HMB \quad (4)$$

$$Cy\cdot + Cy_2^+ \rightarrow HMB + Cy^+ \quad (5)$$

$$Cy\cdot + R\cdot \rightarrow Cy\text{-}R \quad (6)$$

is hexamethylbenzene (HMB, ~70%) which can be formed either via reaction 4 or 5. Reaction 6 in which R· is the *tert*-butyl alcohol radical is probably responsible for the less than quantitative yield of HMB. At present the mechanism for the formation of HMB is unclear.

At this point there is no information on the structure of the complex, although we believe a sandwich structure to be the most likely. The band at 500 nm is probably a charge-transfer transition promoting the odd electron from one ring to the other.

This complex differs from other known cation dimers of aromatic hydrocarbons[6] in which the electron-deficient half of the dimer is nonaromatic, while in the complex described here the aromatic component functions as the electron acceptor.

The spectrum of Cy· could be deduced from absorption measurements taken at very short times after the electron pulse although it was not obtained completely free of $Cy2^+$. A cleaner spectrum (Figure 4) was obtained in the pulse

(6) Badger, B.; Brocklehurst, B. *Trans. Faraday Soc.* **1970**, *66*, 2939.

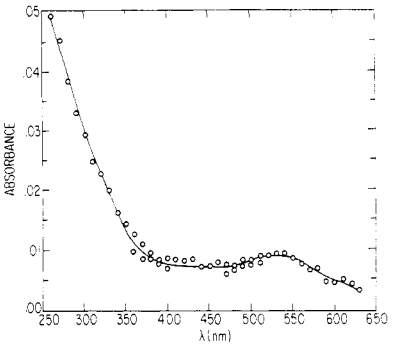

Figure 4. Composite transient spectrum produced by pulse radiolyzing a He-saturated solution of 0.001 M tri-*tert*-butylcyclopropenyl perchlorate (0.1 M *tert*-butyl alcohol; $[e^-_{aq}]_0 \approx 10^{-5}$ M.

radiolysis of tri-*tert*-butylcyclopropenium perchlorate. In this case, there is no evidence for complexation of the radical and the cation, presumably due to steric hindrance. The lifetime of this radical is substantially longer than that of the trimethyl derivative, and it disappears with mixed-order kinetics. The low extinction coefficient ($\epsilon \sim 200$ at 500 nm) and the steadily rising structureless feature of the spectrum suggests a substantial geometry change between ground and excited states.[7]

Acknowledgment. G.L.C. acknowledges support by NSF Grant CHE 7821789.

Registry No. Trimethylcyclopropenyl radical, 60528-77-0; trimethylcyclopropenyl cation, 26827-04-3; tri-*tert*-butylcyclopropenyl radical, 60528-80-5; tri-*tert*-butylcyclopropenyl perchlorate, 19985-80-9.

(7) After this manuscript had been submitted, another contribution on trimethyl cyclopropenyl radicals appeared: Sutcliffe, R.; Lindsay, D. A.; Griller, D.; Walton, J. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1982**, *104*, 4674.

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Enzyme-Catalyzed Synthesis of *N*-Acetyllactosamine with in Situ Regeneration of Uridine 5′-Diphosphate Glucose and Uridine 5′-Diphosphate Galactose

Summary: *N*-Acetyllactosamine has been synthesized on 80-mmol scale by an enzyme-catalyzed procedure starting from glucose 6-phosphate, *N*-acetylglucosamine, and phosphoenolpyruvate in a route requiring in situ (re)generation of UDP-galactose (Scheme I). UDP-galactose was generated from UDP-glucose by UDP-galactose epimerase catalyzed epimerization of UDP-glucose, which was in turn generated from UTP and glucose 6-phosphate with catalysis by phosphoglucomutase and UDP-glucose pyrophosphorylase. Pyrophosphatase was used to catalyze the hydrolysis of the inorganic pyrophosphate released to drive the reaction. UTP was regenerated from UDP and phosphoenolpyruvate with catalysis by pyruvate kinase.

(1) Supported by the National Institutes of Health, Grants GM-26543 and GM-30367.
(2) NSF Predoctoral Fellow.

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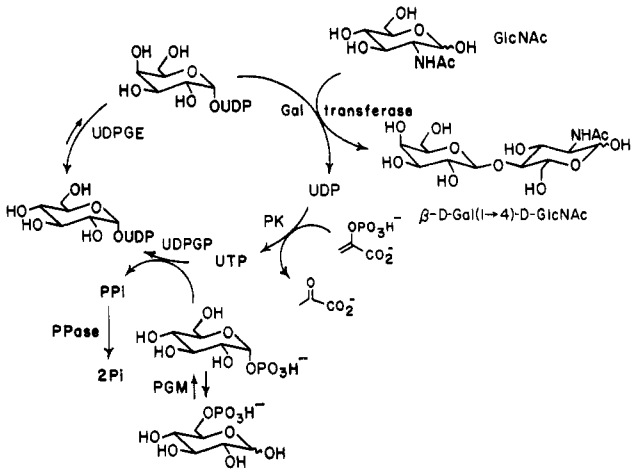
Scheme I. Synthesis of *N*-Acetyllactosamine[a]



[a] Abbreviations: UDP, uridine 5'-diphosphate; UTP, uridine 5'-triphosphate; UDPGE, UDP-galactose 4'-epimerase; Gal transferase, galactosyl transferase; UDPGP, UDP-glucose pyrophosphorylase; PGM, phosphoglucomutase; PK, pyruvate kinase; β-D-Gal(1→4)-D-GlcNAc, *N*-acetyllactosamine.

Sir: Oligosaccharides constitute an important class of targets for synthetic organic and medicinal chemistry because of their central role in immunology and biochemical recognition.[3] Chemical routes to these substances are highly developed but are complicated by multiple protection and deprotection steps and by difficult problems in regioselectivity.[4,5] Enzymatic syntheses of oligosaccharides might, in appropriate cases, circumvent these problems. The most general enzymatic routes to oligosaccharides[6] have not, however, been widely used in practical organic synthesis, because the required nucleoside diphosphate sugars and enzymes have not been readily available. Here we describe the enzyme-catalyzed synthesis of *N*-acetyllactosamine (β-D-Gal(1→4)-D-GlcNAc)[7] from glucose 6-phosphate and *N*-acetylglucosamine on a >10-g scale (Scheme I). *N*-Acetyllactosamine is a representative disaccharide, which is itself important as a core component in oligosaccharides of glycoproteins. This synthesis establishes the practicality of enzymatic procedures for in situ regeneration and reaction of preparatively useful quantities of UDP-glucose and UDP-galactose under conditions required for enzyme-catalyzed oligosaccharide synthesis. The principles underlying these procedures should be applicable to the several different nucleoside diphosphate sugars required in other polysaccharide synthesis.[8]

For the synthesis of *N*-acetyllactosamine, an 800-mL solution containing glucose 6-phosphate[9] (G-6-P, 40 mmol), UDP (0.5 mmol), *N*-acetylglucosamine (GlcNAc, 40 mmol), phosphoenolpyruvate (42 mmol, monopotassium salt),[10] $MnCl_2$ (2 mmol), and $MgCl_2$ (4 mmol) was deoxygenated with argon. Separately, PAN-immobilized[11] galactosyltransferase (Gal transferase, EC 2.4.1.22, 35 U, 15 mL of gel), UDP-galactose 4'-epimerase (UDPGE, EC 5.1.3.2, 32 U, 6 mL of gel), UDP-glucose pyrophosphorylase (UDPGP, EC 2.7.7.9, 40 U, 1 mL of gel), phosphoglucomutase (PGM, EC 2.7.5.1, 101 U, 1 mL of gel), inorganic pyrophosphatase (PPase, EC 3.6.1.1, 120 U, 1 mL of gel), and pyruvate kinase (PK, EC 2.7.1.40, 140 U, 1 mL of gel) were added.[12] The total volume of the mixture was adjusted to 1 L,[13] and the reaction was conducted at room temperature over the course of 4 days under argon with the pH controlled at 8.0 (2 N HCl). After separation of the enzyme-containing gels, the solution was stirred with 150 g of a mixture of Dowex 1 and Dowex 50 for 10 min to remove most of the charged species. The solution (pH 7.0) was concentrated under reduced pressure at 35 °C to a volume of 50 mL, and lyophilized to obtain a solid material (17.6 g), which contained 75% of *N*-acetyllactosamine (34 mmol, 85% yield based on G-6-P, determined by HPLC).[14] Further purification was performed by gel-permeation chromatography on Bio-Rad P-2 (100–200 mesh) with water as the mobile phase.[15] The purified disaccharide (70% overall yield based on GlcNAc) showed a single peak on HPLC.[14] The enzymatic activities recovered at the conclusion of the reaction were as follows: Gal transferase, 86%; PPase, 91%; UDPGP, 88%; PK, 90%; PGM, 91%; UDPGE, 82%. The turnover number for UTP was 80. These recovered enzymes were used for a second reaction under the same conditions as described, and another 35 mmol of *N*-acetyllactosamine was obtained.[16]

Pyruvate kinase/phosphoenolpyruvate was used in this scheme rather than acetate kinase/acetyl phosphate for regenerating UTP from UDP because phosphoenol-

(3) Montreuil, J. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 157–223. Sharon, N.; Lis, H. *Chem. Eng. News* **1981**, *58* (13), 21–44.

(4) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155–73. Flowers, H. M. *Methods Enzymol.* **1978**, *50*, 93–121.

(5) Lemieux, R. U.; Hendricks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4069. Frechet, J. M.; Nuyens, S. E. *Ibid.* **1979**, *101*, 432–6.

(6) Neufeld, E. F.; Hassid, W. Z. *Adv. Carbohydr. Chem.* **1963**, *18*, 309–56. Nikaido, H.; Hassid, W. Z. *Adv. Carbohydr. Chem. Biochem.* **1971**, *26*, 351–483.

(7) A striking illustration of enzyme-catalyzed oligosaccharide synthesis on a 100-μmol scale is that of: Rosevear, P. R.; Numez, H. A.; Barker, R. *Biochemistry* **1980**, *19*, 489–95.

(8) UDP-glucose is itself central to oligosaccharide synthesis, since it is the precursor to other nucleoside diphosphate sugars: UDP-galactose, UDP-glucuronic acid, UDP-xylose, UDP-rhamnose, and UDP-2-acetamido-2-deoxy-D-glucuronic acid.[6]

(9) Wong, C. H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890–9.

(10) Hirschbein, B. L.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765.

(11) Immobilizations were preformed according to the procedure described previously: Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324–36. The following components were added during the immobilization (immobilization yield). PPase: $MgCl_2$, 10 mM; PPi, 2 mM (51%). Gal transferase: UDP-Gal, 2 mM; GlcNAc, 2 mM (44%). UDPGE: UDP-Glc, 2 mM (42%).

(12) Enzymes were obtained from Sigma and assayed by using standard procedures: Bergmeyer, H. U. "Methods of Enzymatic Analysis"; Academic Press: New York, 1974. Gal transferase was assayed by HPLC analysis of the formation of *N*-acetyllactosamine from UDP-galactose and *N*-acetylglucosamine.

(13) The concentration of reactants in the synthesis of disaccharide were low (40 mM) because Gal transferase is subject to both substrate inhibition and product inhibition: Brew, K.; Vanaman, T. C.; Hill, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 491–7.

(14) Performed by using a Waters μ-Bondapak/carbohydrate column (0.4 × 30 cm), with refractometer detection and aqueous acetonitrile (H_2O/CH_3CN, 25:75 v/v) as solvent. For a flow rate of 2 mL/min the retention times were as follows: *N*-acetyllactosamine, 4.6 min; *N*-acetylglucosamine, 3.4 min; sodium pyruvate, 4.2 min.

(15) In a typical example, 1 g of synthetic crude *N*-acetyllactosamine was applied to a column (2.7 × 42 cm) of Bio-Rad P-2 equilibrated with water and eluted with water at a rate of 0.6 mL/min. The elution (6 mL/fraction) was monitored by absorbance at 230 nm. The fractions with an elution volume of 110–140 mL were collected and lyophilized, *N*-acetyllactosamine (0.7 g) showing a single peak on HPLC was obtained. The ^{13}C spectrum (in parts per million downfield from DSS) was essentially the same as that reported:[7] ^{13}C NMR (68 MHz, D_2O) δ 105.5 (Gal C_1), 73.6 (C_2), 75.2 (C_3), 71.1 (C_4), 78.0 (C_5), 63.6 (C_6), 93.1, 97.4 (GlcNAc C_1 α,β), 56.3, 58.8 (C_2 α,β), 71.9, 75.8 (C_3 α,β), 81.5 (C_4 α,β), 72.9, 77.4 (C_5 α,β), 62.6, 62.7 (C_6 α,β), 24.6, 24.9 (CH_3 α,β), 177.0, 177.3 (C=O α,β); ^1H NMR (270 MH_3, D_2O) δ 2.04 (s, 3 H, CH_3), 3.07–3.18 (m, 6 H), 4.46 (d, 1 H, Gal C_1 H, $J_{1,2}$ = 8 Hz), 4.72 (d, GalNAc C_1 H, $J_{1,2}$ = 6 Hz, β form), 5.20 (d, GlcNAc C_1 H, $J_{1,2}$ = 2 Hz, β form).

(16) If crude UDP-glucose prepared from RNA was used instead of pure UDP, UTP, or UDP-glucose (Wong, C. H.; Haynie, S.; Whitesides, G. M., submitted for publication in *J. Am. Chem. Soc.*), a similar result was obtained, but the reaction rate was slower (~80%), probably because other nucleoside triphosphates inhibit some reaction in the system.

pyruvate is the more stable compound in solution and is, as a result, the easier to handle.[10] PPase-catalyzed hydrolysis of pyrophosphate was employed to drive the UDPGP-catalyzed reaction. Glucose 6-phosphate was used as a starting material, instead of glucose 1-phosphate, because G-6-P is more stable and more readily available than G-1-P, and because PGM is inexpensive and stable. The good stability and high recovery observed for the enzymes used in the system and the satisfactory turnover number for UTP render the costs of these components acceptable for practical-scale synthesis.

The value of this synthesis lies in its demonstration that enzymatic catalysis can be used to prepare substantial quantities of a representative disaccharide on starting from unprotected sugars and utilizing the enzymes of the Leloir pathway.[17] Although a number of nucleoside diphosphate sugars are required to satisfy all the requirements for syntheses based on this pathway and although the enzymes required for any particular synthesis of interest will be more or less available, the nucleoside triphosphate cofactors involved in all of these syntheses can now be considered to be readily available, and the regeneration schemes for these cofactors function well. The general area of practical-scale, polysaccharide synthesis based on cofactor-requiring enzymes thus now seems amenable for development by synthetic organic chemists.

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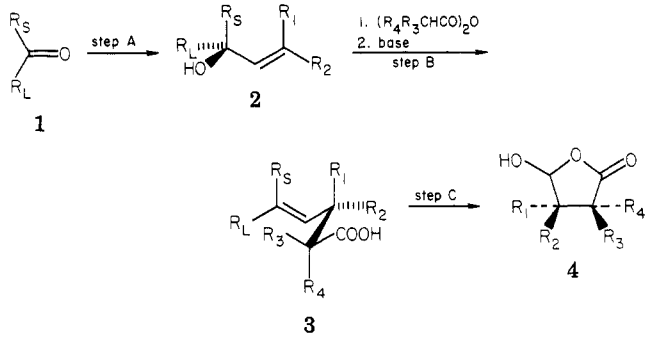
A Nonresolutive Approach to the Preparation of Configurationally Pure Difunctional Molecules

Summary: Treatment of menthone (**5**) with organometallic reagents affords axial tertiary alcohols of type **6** with high diastereoselectivity. Propionate **12**, derived from *trans*-**11**, undergoes an enolate Claisen rearrangement with complete transfer of chirality. The use of these two observations in the nonresolutive synthesis of configurationally pure difunctional molecules such as **15**, **16**, and **19** is discussed.

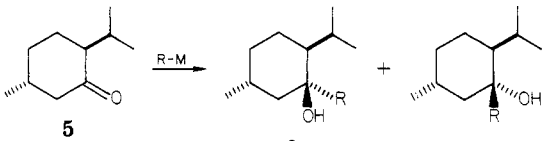
Sir: The phenomenon of transfer of chirality has been documented for a number of [2,3] and [3,3] sigmatropic rearrangements.[1] Until recently, one of the major obstacles to using these rearrangements in asymmetric synthesis was the inavailability of configurationally pure allylic fragments possessing the structural features required to observe transfer of chirality.[2] In this communication we describe the initial results of an approach to the nonresolutive synthesis of tertiary allylic alcohols that transfer chirality in enolate Claisen rearrangements.[3a] In addition, our progress toward establishing a protocol for the synthesis of configurationally pure 1,4-difunctional molecules will be discussed.

Scheme I



1 2 3 4

Table I. Preparation of Configurationally Pure Allylic and Propargyllic Alcohols



5 6 7

entry	R-M/solvent	major product[f]	% yield of adduct (6/7)[c]
1	$HC{\equiv}CLi/NH_3$ [a]	8	53 (9:1)[d]
2	$CH_3C{\equiv}CLi/NH_3$ [a]	9	25 (7:1)[d]
3	$CH_2{=}CHMgBr/THF$	10	77 (6 only)
4	$CH_3CH{=}CHMgBr/THF$ [b]	11	76 (6 only)[e]
5	$CH_3CH{=}CHLi/Et_2O$ [b]	11	90 (6 only)[e]

[a] Enolization of 5 was a competitive process. [b] A mixture of cis and trans organometallic reagents was used. [c] Isolated yields of 6 + 7 after separation. [d] The stereoisomers were easily separated by column chromatography. [e] Obtained as a mixture of cis and trans isomers. [f] In all cases the major product had the stereochemistry depicted by general structure **6**.

The general approach we have pursued is outlined in Scheme I. We hoped to identify readily available configurationally pure carbonyl compounds **1** that would react with appropriate organometallic reagents to give allylic alcohols **2** of defined absolute stereochemistry and olefin geometry (step A). In evaluating candidates for **1** it was important that R_L and R_S differ in size such that transfer of chirality would be observed in subsequent Claisen rearrangements (step B).[3b] Finally, if these requirements could be met, we felt it would be a simple task to liberate configurationally pure 1,4-difunctional molecules of type **4** by oxidative cleavage of rearrangement products **3** (step C).

Although a number of candidates for carbonyl compound **1** were considered, we soon focused on menthone (**5**) for several reasons. First, configurationally pure menthone was readily available on a large scale from Brown oxidation of menthol.[4] In addition, there was

(1) For a lead reference on intramolecular transfer of chirality, see: Scott, J. W.; Valentine, D. *Synthesis* **1978**, 329 and references cited therein.

(2) Recent reports that alkyl alkynyl ketones can be reduced enantioselectively in principle now render optically active allylic alcohols readily available: Brinkmeyer, R. S.; Kapoor, V. M. *J. Am. Chem. Soc.* **1977**, *99*, 8339. Vigneron, J.-P.; Bloy, V. *Tetrahedron Lett.* **1979**, 2683. Midland, M. M.; McDowell, D. C.; Hatch, R. L.; Tramontano, A. *J. Am. Chem. Soc.* **1980**, *102*, 867. For other relevant studies, see: Mori, K.; Akao, H. *Tetrahedron* **1980**, *36*, 91. Mukaiyama, T.; Suzuki, K.; Soai, K.; Sato, T. *Chem. Lett.* **1979**, 447. Terashima, S.; Tanno, N.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1980**, 1026. Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237.

(3) (a) This work is taken in part from the M.S. thesis of D. K. Hutchinson, The Ohio State University, Columbus, OH, 1980. (b) For a discussion that outlines criteria needed to observe transfer of chirality in Claisen rearrangements, see: Perrin, C. L.; Faulkner, D. J. *Tetrahedron Lett.* **1969**, 2783.

(4) Brown, H. C.; Garg, C. P.; Liu, K. T. *J. Org. Chem.* **1971**, *36*, 387.

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