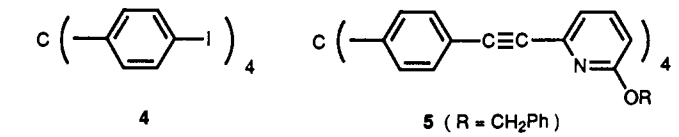
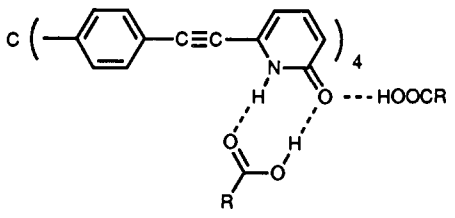
tected tetrapyridone **5** in 44% yield.[8] Deprotection (CF_3COOH)[10]



4 **5** (R = CH_2Ph)

then provided tecton **3** in 100% yield.[8] Crystallization of compound **3** could be achieved only in mixtures containing significant amounts of carboxylic acids. Use of acetic acid or propionic acid in hexane or CH_3OH/hexane consistently produced needles of composition **3**·8RCOOH (R = CH_3, CH_3CH_2) in high yield. Unfortunately, an X-ray crystallographic study of **3**·8CH_3CH_2COOH revealed that self-assembly of a diamondoid network had been thwarted by association of the sticky pyridone sites with propionic acid, producing adduct **6**.[11]



6 (R = CH_2CH_3)

In contrast, crystallization of tecton **3** from butyric acid/CH_3OH/hexane provided plates of approximate composition **3**·2$CH_3CH_2CH_2COOH$ in 88% yield. In this case, an X-ray crystallographic study has confirmed that the sticky pyridone sites interact in the expected way[12] to induce self-assembly of the remarkable diamondoid network shown in Figure 1a.[13] Since the tetrahedral centers of adjoining tectons are separated by 19–20 Å, the network defines enormous chambers and interconnecting windows. The chambers enclathrate only butyric acid, even though crystallization occurred in a mixed solvent. The interstitial guests are surprisingly well ordered and form two parallel columns in channels aligned with the *b* axis (Figure 1b). Since the columns are retained within a porous host framework by van der Waals forces alone, loss of butyric acid occurs when the crystals are placed under vacuum.

Crystallization of tecton **3** from valeric acid/CH_3OH/hexane provided plates of approximate composition **3**·1$CH_3CH_2CH_2CH_2COOH$ in 76% yield. Again, an X-ray crystallographic study has confirmed that self-assembly occurs to give a closely similar diamondoid network with cavities that enclathrate only valeric acid.[14] In addition, crystals of tecton **3** obtained from isobutyric acid and isovaleric acid proved to have similar compositions. This indicates that the self-assembly of a diamondoid network is a phenomenon of considerable generality, not merely a curiosity limited to the case of butyric acid. Further investigation will reveal what other interstitial guests can be accommodated and whether or not the ordered diamondoid framework remains intact when the guests are removed or exchanged. The stickiness of the sites that create the hydrogen-bonded network can easily be amplified by using pyridones connected in series,[1b] so we are optimistic that the framework can be further strengthened to resist forces favoring close packing.

Self-assembly of the diamondoid network **2** suggests that cleverly designed tectons can give chemists the elements of a powerful molecular-scale construction set. We believe that this strategy can be used to build predictably ordered materials with useful properties, including selective enclathration, microporosity, high ratios of strength to density, and catalytic activity. A principal advantage of this strategy is that complex structures with specific architectural or functional features are formed reversibly by spontaneous self-assembly, not by tedious bond-by-bond syntheses.

Acknowledgment. This work was financially supported by the Natural Sciences and Engineering Research Council of Canada and by the Ministère de l'Éducation du Québec.

Supplementary Material Available: Spectroscopic and analytical data for compounds **4**, **5**, **3**·8CH_3CH_2COOH, and **3**·2$CH_3CH_2CH_2COOH$ and tables of crystallographic data, descriptions of the structure determinations, and tables of atomic coordinates and isotropic thermal parameters, bond lengths and angles, anisotropic thermal parameters, and refined and calculated hydrogen atom coordinates for compounds **3**·8CH_3CH_2COOH and **3**·2$CH_3CH_2CH_2COOH$ (20 pages); observed and calculated structure factors for **3**·8CH_3CH_2COOH and **3**·2$CH_3CH_2CH_2COOH$ (31 pages). Ordering information is given on any current masthead page.

(8) The structure assigned to this new compound is consistent with its elemental analysis and its IR and NMR spectra. These data are included in the supplementary material.

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(11) Crystals of **3**·8CH_3CH_2COOH belong to the tetragonal space group $P4_2/n$ with $a = b = 21.977$ (2) Å, $c = 7.7866$ (9) Å, $V = 3760.7$ (6) Å^3, $D_{calcd} = 1.220$ g cm^{-3}, and $Z = 2$. A full description of the structure is provided in the supplementary material.

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(13) Crystals of **3**·2$CH_3CH_2CH_2COOH$ belong to the monoclinic space group $C2/c$ with $a = 31.249$ (7) Å, $b = 7.350$ (4) Å, $c = 23.145$ (6) Å, $\beta = 104.69$ (2)°, $V = 5142$ (3) Å^3, $D_{calcd} = 1.247$ g cm^{-3}, and $Z = 4$. A full description of the structure is provided in the supplementary material.

(14) Crystals of **3**·1$CH_3CH_2CH_2CH_2COOH$ belong to the monoclinic space group $P2_1/n$ with $a = 31.137$ (8) Å, $b = 7.290$ (2) Å, $c = 23.006$ (5) Å, $V = 5064$ (2) Å^3, $D_{calcd} = 1.303$ g cm^{-3}, and $Z = 4$.

Enzyme-Catalyzed Synthesis of Sialyl Oligosaccharide with in Situ Regeneration of CMP-Sialic Acid[1]

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Sugar nucleotide dependent glycosyltransferases have great potential for the stereocontrolled synthesis of oligosaccharides.[2,3] All glycosyltransferases in mammalian systems utilize nucleoside diphosphate sugars as activated donors with the exception of sialyl transferase, which requires CMP-sialic acid (or CMP-*N*-acetylneuraminic acid, CMP-NeuAc). Although small-scale (milligrams) enzymatic synthesis of oligosaccharides based on the stoichiometric reaction of a sugar nucleotide and a mono- or oligosaccharide acceptor has been well documented,[3,4] the procedure usually requires a separate preparation of expensive sugar nucleotides and often suffers from product inhibition caused by the released nucleoside di- or monophosphates.[4] A practical solution to these problems is to utilize catalytic amounts of sugar nucleotides and nucleoside phosphates that are regenerated in situ in glycosyltransferase reactions. Regeneration of nucleoside diphosphate sugars (UDP-Glc and UDP-Gal) has been developed by Wong et al.[5] for a large-scale (35–70 mmol) synthesis of

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Scheme I. Synthesis of Sialyl-*N*-acetyllactosamine with in Situ Regeneration of CMP-NeuAc and Nucleoside Phosphate Cofactors

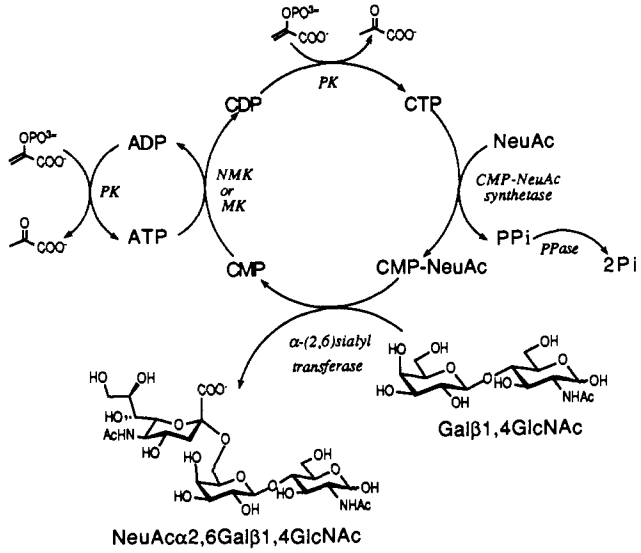


Table I. The Designed Primer Sequences Containing an Additional Decapeptide Tag Sequence and Restriction Sites for the Amplification of CMP-NeuAc Synthetase Gene

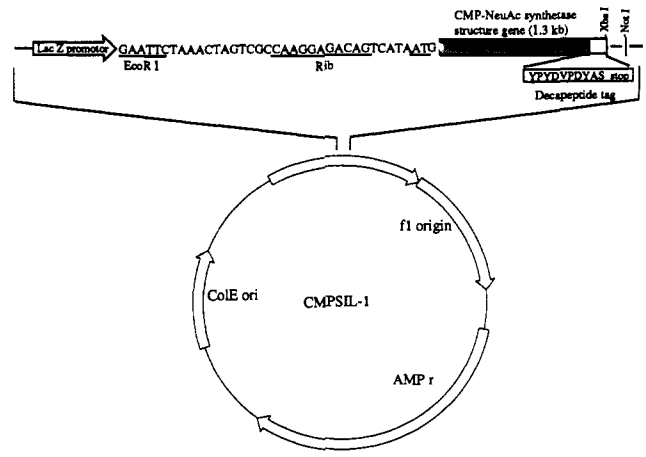
Primer CMP5
5'- ATATTGAATTCTAAACTAGTCGCCAAGGAGACAGTCATAATGAGA
EcoR I — Shine Dalgarno sequence — Start
ACAAAAATTATTGCG
gene N-terminal

Primer CMP3
5'-GCGCTCTAGACTATTAAGAACCGTAGTCCGGAACGTCGTACGGG
Xba I — Stop — Decapeptide tag
TATTTAACAATCTCCGCTATTTC
gene c-terminal

N-acetyllactosamine. Regeneration of nucleoside monophosphate sugars such as CMP-sialic acid, however, is not available for the direct incorporation of sialic acid into oligosaccharides.[6] We report here the first high-yield (97%) synthesis of sialyl-*N*-acetyllactosamine starting from NeuAc, *N*-acetyllactosamine Galβ1,4GlcNAc), phosphoenolpyruvate (PEP), and catalytic amounts of ATP and CMP, with in situ regeneration of CMP-NeuAc, CTP, ATP and ADP (Scheme I). This synthesis demonstrates a new strategy for the practical synthesis of sialyl oligosaccharides, a class of biologically important molecules often found as terminal structure of cell-surface glycoproteins and glycolipids.[7]

In this enzymatic synthetic system, CMP was converted to CDP catalyzed by nucleoside monophosphate kinase (NMK, EC 2.7.4.4) in the presence of ATP, which was regenerated from its byproduct ADP catalyzed by pyruvate kinase (PK, EC 2.7.1.40) in the presence of PEP.[8] CDP was further converted to CTP with PEP catalyzed by PK. CTP was then reacted with NeuAc catalyzed by CMP-NeuAc synthetase (EC 2.7.7.43) to produce CMP-NeuAc. The byproduct inorganic pyrophosphate was decomposed by pyrophosphatase (PPase, EC 3.6.1.1). Sialylation

Scheme II. Construction of Phagemid CMPSIL-1 Containing CMP-NeuAc Synthetase Gene



of Galβ1,4GlcNAc was accomplished by CMP-NeuAc and α(2,6) sialyltransferase (EC 2.4.99.1). The released CMP was again converted to CDP, to CTP, and to CMP-NeuAc. The turnover number for each of these cofactors was $10–10^2$ and that for ATP was $10^2–10^3$.

The gene encoding CMP-NeuAc synthetase from *Escherichia coli*[9] was prepared via PCR amplification in the presence of two designed primers (Table I), one of which contains a decapeptide tag. The amplified gene was cloned into λ ZAP vector[10] to construct a phagemid (Scheme II) for expression of the enzyme in *E. coli*. The peptide tag was incorporated to facilitate the selection of positive clones (using the antidecapeptide antibody conjugated with alkaline phosphatase) and the affinity purification of the enzyme, both based on the antipeptide monoclonal antibody. This overexpression *E. coli* strain produces approximately 100 units/L of CMP-NeuAc synthetase compared to <0.1 unit/L for the wild-type strain, corresponding to a >1000-fold increase of enzyme activity.

For the synthesis of Neuα2,6Galβ1,4GlcNAc, NeuAc (0.92 g, 3 mmol), GAlβ1,4GlcNAc (1.1 g, 3 mmol),[5] CMP (30–300 μmol), ATP (3–30 μmol) (we found that replacement of ATP with CTP resulted in a very slow formation of the product), PEP monosodium salt (2.8 g, 6 mmol), $MgCl_2 \cdot 6H_2O$ (0.61 g, 3 mmol), $MnCl_2 \cdot 4H_2O$ (0.15 g, 0.8 mmol), KCl (0.22 g, 3 mmol), NMK or MK (450 units), PK (6000 units), PPase (300 units), CMP-NeuAc synthetase (24 units), and α(2,6) sialyltransferase (4 units) were added to 150 mL of HEPES buffer (0.2 M, pH 7.5), and the reaction was conducted at room temperature for 2 days under argon.[11] The reaction mixture was reduced to 20 mL by lyophilization and applied to a Bio Gel P2 (200–400 mesh) column with water as the mobile phase. The trisaccharide-containing fractions were collected and lyophilized to give pure Neuα2,6Galβ1,4GlcNAc (2 g) in 97% yield.[12]

This synthesis demonstrates the direct incorporation of NeuAc into oligosaccharide on a large scale with in situ regeneration of

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(11) Monitored by silica gel TLC with 1 M NH_4OAc/2-propanol (1:2.4, v/v) as the developing solvent: R_f Galβ1,4GlcNAc, 0.63; NeuAc, 0.31; Neuα2,6Galβ1,4GlcNAc, 0.30; CMP-NeuAc, 0.19.

(12) ^{1}H NMR: δ 1.701 (1 H, t, J = 12.5 Hz, H-3$_{ax}$ of NeuAc), 2.007 (3 H, s, NHAc of GlcNAc), 2.044 (3 H, s, NHAc of NeuAc), 2.649 (1 H, dd, J = 5.0 and 12.5 Hz, H-3$_{eq}$ of NeuAc), 4.43 (1 H, d, J = 8.0 Hz, H-1 of Gal), 4.73 (0.5 H, d, J = 8.0 Hz, H-1b of GlcNAc), and 5.178 (0.5 H, d, J = 2.5 Hz, H-1a of GlcNAc). The only ^{1}H NMR data reported were the methyl glycoside of Neuα2,6Galβ1,4GlcNAc (see Sabesan and Paulson reference in footnote 6).

CMP-NeuAc and five other cofactors. Five enzymes act under the same conditions without product inhibition, and a separate synthesis of CMP-NeuAc is not necessary. The enzymes might be immobilized and recovered for reuse. The system should be applicable to many other sialyltransferase-catalyzed syntheses of sialosides. Since stereocontrolled sialylation is still a difficult problem in synthetic carbohydrate chemistry,[13] the enzymatic method based on sialyltransferases will obviously become an effective and practical option. With the increasing availability of glycosyltransferases through cloning techniques,[14] enzymatic methods for oligosaccharide synthesis will obviously complement the chemical methods that have already been well established and vigorously practiced by many elegant approaches.[15]

Acknowledgment. We thank Dr. J. C. Paulson at Cytel for kindly providing a sample of sialyltransferase and Professor Richard Lerner for the antidecapeptide antibody.

Supplementary Material Available: Experimental details of cloning, expression, and isolation of CMP-NeuAc synthetase and ^{1}H NMR spectrum of Neuα2,6Galβ1,4GlcNAc (5 pages). Ordering information is given on any current masthead page.

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Steric Course of the Reduction of Ethyl Coenzyme M to Ethane Catalyzed by Methyl Coenzyme M Reductase from *Methanosarcina barkeri*

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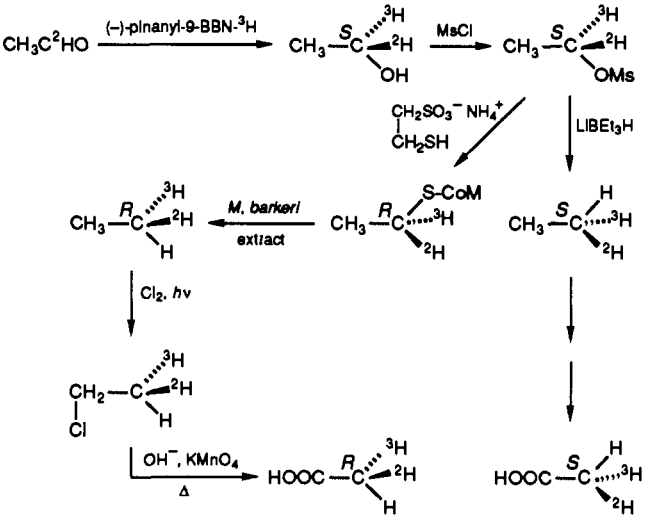
Methanogenic bacteria derive their energy from the reduction of CO_2 with hydrogen gas to methane.[1,2] The terminal step in this sequence, the reduction of methyl coenzyme M to methane, is catalyzed by methyl coenzyme M reductase,[2,3] a highly complex, multicomponent enzyme system[2,4] containing several new cofactors, including the novel nickel-containing tetrapyrrole, cofactor F_{430}[5] (cf. ref 4). Model studies[6] suggest that cleavage of the methyl–sulfur bond of CH_3S-CoM by attack of reduced (Ni^I) F_{430} leads to transfer of the methyl group to the nickel of the cofactor, leaving behind a heterodisulfide with component B of methylreductase (7-mercaptoheptanoyl threonine phosphate), from which coenzyme M is reductively regenerated.[3,7] Methane then arises by protonolysis of the methylated cofactor (CH_3-Ni-F_{430}).[6] The methylreductase complex seems to be located in a membrane-associated particle, the methanoreductosome,[8] which couples reductive methane formation to the generation of a transmembrane proton gradient used by the cell for ATP synthesis.

Scheme I



To test the proposed mechanism for the methylreductase reaction, we decided to determine the steric course of this process. Doing so with methyl-CoM as substrate presents an obvious problem: Four isotopes of hydrogen would be required to generate an isotopically chiral version of methane, but only three hydrogen isotopes are known. Consequently, a different group must be introduced to take the place of the fourth hydrogen isotope. We opted for a methyl group, i.e., we decided to use as substrate ethyl-CoM, which is known to be reduced to ethane at about 20% of the rate of methane formation from methyl-CoM.[9] (*R*)- and (*S*)-[1-$^2H_1$,^{3}H]ethyl coenzyme M were synthesized as shown in Scheme I. Reduction of [1-$^2H_1$]ethanal (98% ^{2}H) with tritiated (+)- and (−)-*B*-(3-pinanyl)-9-borabicyclo[3.3.1]nonane[10] (sp radioact. 5 mCi/mmol, >99% ee) gave (*R*)- and (*S*)-[1-$^2H_1$,^{3}H]-ethanol, respectively.[13] Conversion of each sample to the mesylate was followed by reaction with a solution of 2-thioethanesulfonic acid (coenzyme M) in dilute ammonium hydroxide[9] to produce (*S*)- and (*R*)-[1-$^2H_1$,^{3}H]ethyl coenzyme M (sp radioact. 1.4 mCi/mmol and 0.8 mCi/mmol, respectively) in 17.4 and 12.4% of the theoretical overall yield. An aliquot of the intermediate (*S*)-[1-$^2H_1$,^{3}H]ethyl mesylate was reacted with Super-Hydrid, and the resulting ethane was degraded to [2-$^2H_1$,^{3}H]acetic acid as described below. Configurational analysis[14,15] of this acetic acid sample gave an *F* value[16] of 28.1, corresponding to 75% ee *S* isomer,[17] establishing a maximum value for the optical purity of

[†] University of Washington.
[‡] The Ohio State University.

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