Pople, *J. Chem. Phys.* **1972**, *56*, 2257; M. S. Gordon, *Chem. Phys. Lett.* **1980**, *76*, 163) were carried out on the super-computer NEC-SX4 of the Höchstleistungs-Rechenzentrum Stuttgart. The starting point was the structure coordinates of the two compounds (Figure 1a and 2a), in which the vectors of the electron pairs on N were turned perpendicular to the connecting axes SiN while controlling the interatomic distances on-line to minimize additional structural changes (Figures 1b and 2b, c). Removal of the molecular Si centers (SiH or SiH_2) by H saturation of the ruptured bonds of the substituent groups (Figures 1c, d and 2d, e) allows an estimate of the van der Waals energy changes in the periphery of the molecule. After subtraction of the total energy from these estimations (Figures 1a and 2a), approximate sums of the energy contributions due to the long-range SiN interactions result. The accuracy of the B3LYP procedure has been statistically evaluated for relative energies to be about 0.4 kcal mol^{-1} (J. B. Foresmanm, A. Frisch in "Exploring Chemistry with Electronic Structure Methods", 2nd ed., GAUSSIAN Inc., Pittsburg, **1996**), and, therefore, deviations of only 1–2 kJ mol^{-1} are assumed for the basis sets used. In addition, substantial changes in the Si tetahedral geometry due to N→Si hyperconjugation can be excluded by comparison with the average bond angle $\varphi(CSiC) = 111^\circ$ in triphenylsilane (J. Allemand, R. Gerdi, *Cryst. Struct. Commun.* **1979**, *8*, 927): both the smaller average angle of 108° in the compound with the seven-coordinate Si center (Figure 2) as well as the larger one of 113° for eightfold coordination (Figure 3) can be straightforwardly rationalized by the different central SiH or SiH_2 units and the phenyl group substituents of considerably different bulk.

[7] The total energy potential curve for the model adduct chosen, H_3NA, has been calculated using fourth-order Møller-Plesset perturbation theory (MP4) and a correlation consistent basis set with polarized valence "double-zeta" functions(aug-cc-pVDZ) (D. E. Woon, T. H. Dunning, Jr., *J. Chem. Phys.* **1993**, *98*, 1358; R. A. Kendall, T. H. Dunning Jr., R. J. Harrison, *J. Chem. Phys.* **1992**, *96*, 6796). The same basis set also was employed in the geometry optimization at the MP2 level.

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Synthesis of Immobilized CMP-Sialic Acids and Their Enzymatic Transfer with Sialyltransferase**

Yasuhiro Kajihara,* Takashi Ebata, and Hisashi Kodama

The enzymatic synthesis of oligosaccharides and their analogues with glycosyltransferases (GTases) has progressed remarkably as a result of the ability to synthesize the donor substrates.[1] Sialyltransferase (STase)[2] and fucosyltransferase[3] reactions have also been used to study the structure and function of oligosaccharides on glycoproteins and on cell surfaces by use of modified sugar nucleotides. We have developed a concise synthetic method for the preparation of CMP-sialic acid (CMP-*N*-acetylneuraminic acid, CMP-Neu-Ac; CMP = cytidinmonophosphate) and its analogues.[4] Since then we have begun investigating possible applications of STase reactions with use of synthetic CMP-NeuAc analogues (Figure 1).

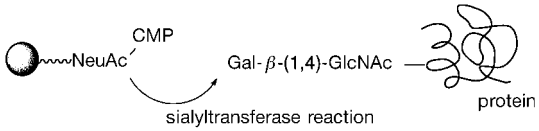

Figure 1. Schematic representation of the novel immobilization method for glycoproteins.

Many enzymes and lectins are glycoproteins containing N- or O-linked oligosaccharides. When these proteins have several oligosaccharides on their surfaces, this layer of oligosaccharides exhibits dynamic fluctuation.[5] Therefore, increasing the number of oligosaccharide chains also increases the surface area covered. With most immobilization methods[6] used for such glycoproteins, this carbohydrate layer hinders the approach to the amino or carboxyl groups on the surface of the protein. Unfortunately, if the reagent attaches to amino acids located close to the catalytic site, the immobilized enzyme may lose its activity. Therefore, we wanted to synthesize a CMP-NeuAc derivative in which the 9″-position is attached to the solid phase, and examine its sialyltransfer ability as part of a novel immobilization procedure. The nonreducing end of oligosaccharides are frequently galactosides which serve as acceptors in STase

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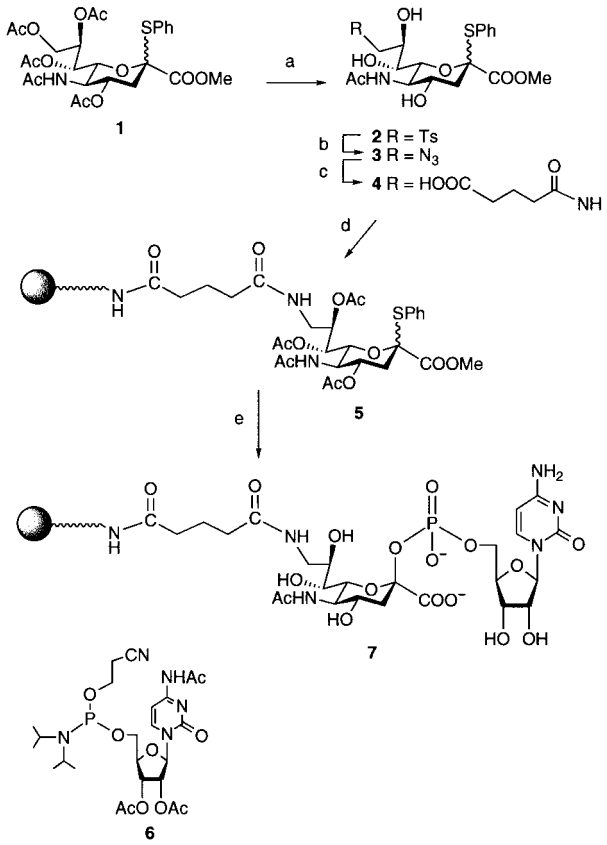
reactions. If STase successfully catalyzes the transfer of immobilized NeuAc to these terminal galactosides, a novel and mild immobilization method will have been discovered. Such a method would have two advantages. First, since the STase reaction generally occurs at the galactosides most remote from the protein, the immobilization reaction should smoothly occur in an area which avoids the steric hindrance of the carbohydrate layer. Second, since sialylation of galactosides at the nonreducing end of bioactive glycoproteins or enzymes may not depend on their activity in vitro,[7] sialoside can be used as a new type of linker between bioactive glycoproteins and the solid-phase support.

Here we report the solid-phase synthesis of CMP-NeuAc and its transfer reaction to both a corresponding oligosaccharide acceptor and an asialoglycoprotein (that is, a nonsialylated glycloprotein). The chemical synthesis of immobilized CMP-NeuAc is shown in Scheme 1. The NeuAc **4**,[8] which is modified in the 9-position and can be obtained by conventional methods from **1**,[9] was coupled with long-chain alkylamino controlled pore glass (CPG, Sigma).[10] The residual amino functionalities on the CPG were then capped by acetylation. Quantification by the periodate/resorcinol method[11] after O-deacetylation and saponification of **5** indicated 576 nmol of the immobilized NeuAc derivative per 1 g of CPG. By our previously developed method[4] the immobilized NeuAc **5** was converted into immobilized CMP-NeuAc **7**, which was identified by comparison of its ^{31}P NMR[12] spectrum with that of CMP-NeuAc (Figure 2).



Scheme 1. a) 1. NaOMe, MeOH, 25 °C; 2. TsCl, py, 25 °C, 48 % (2 steps); b) NaN_3, DMF, 80 °C, 92 %; c) 1. H_2S, py/NEt_3/H_2O (5/1/2), 25 °C; 2. glutaric anhydride, MeOH, 25 °C, 83 %, (2 steps); d) 1. CPG, 1-ethyl-3-(3-diaminopropyl)carbodiimide (HCl salt), NEt_3, DMF, 25 °C; 2. Ac_2O, py, DMAP, 25 °C, 3 – 5 % (estimated by the periodate/resorcinol method); e) 1. NBS, acetone/H_2O (10/1), 25 °C; 2. **6**, 1*H*-tetrazole, MeCN, 25 °C; 3. *t*BuOOH, MeCN, 25 °C; 4. DBU, THF, 25 °C; 5. NaOMe, MeOH/H_2O (2/1), 25 °C. Ts = *p*-toluenesulfonyl; py = pyridine; DMAP = 4-dimethylaminopyridine; NBS = *N*-bromosuccinimide; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

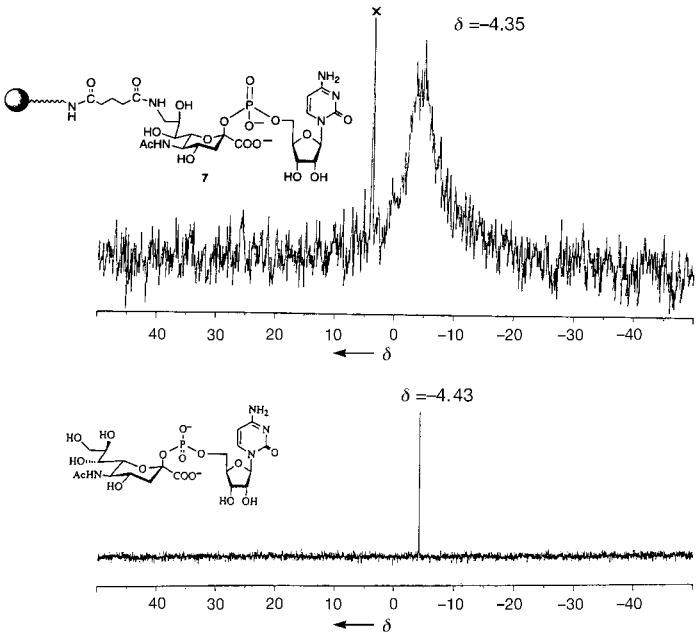


Figure 2. ^{31}P NMR spectra of immobilized CMP-NeuAc **7** (top) and CMP-NeuAc (bottom). X: impurity.

The transfer assay[13] was performed in a solution of HEPES buffer containing **7**, [U-^{14}C]-Gal-β-(1 →4)-GlcNAcβ-OMe([^{14}C]-LacNAc[14]), BSA (bovine serum albumin), and STase. If the immobilized NeuAc was transferred to [^{14}C]-LacNAc, sialyl-[^{14}C]-LacNAc formed on the surface of CPG. After incubation at 37 °C, isotope counts on CPG were measured with a liquid scintillation counter.[15] For the transfer reaction, we used both enzymes α-(2 →3)-STase (3STase, EC 2.4.99.6, Cytel) and α-(2 →6)-STase (6STase, EC 2.4.99.1, Boeringer-Mannheim). The results of the assays are presented in Figure 3 and Table 1 . Panel A (3STase) indicates that the transfer activity of NeuAc in the form of immobilized CMP-NeuAc **7** was elevated with increasing amounts of the enzyme. Transfer activities were also raised by increases in either the acceptor concentration or the incubation time (panels B and C, 3STase). In the case of 6STase (panels D – F), the reaction dynamics are similar to those of 3STase. When STase was first boiled under reflux for 5 min and then added to the reaction mixture, no transfer activity was observed (panel G). These results indicate that the immobilized CMP-NeuAc **7** can act as the sialyl donor. When the incubation time was extended to 24 h (panel B), the amount transferred at 500 mM [^{14}C]-LacNAc increased to 721 pmol. These data indicate that more than 721 pmol of CMP-NeuAc is immobilized on 1 mg of CPG. We also compared the relative transfer rates of CMP-NeuAc and **7**. The transfer rate for **7** was estimated to be 3 % of that for CMP-NeuAc.[16]

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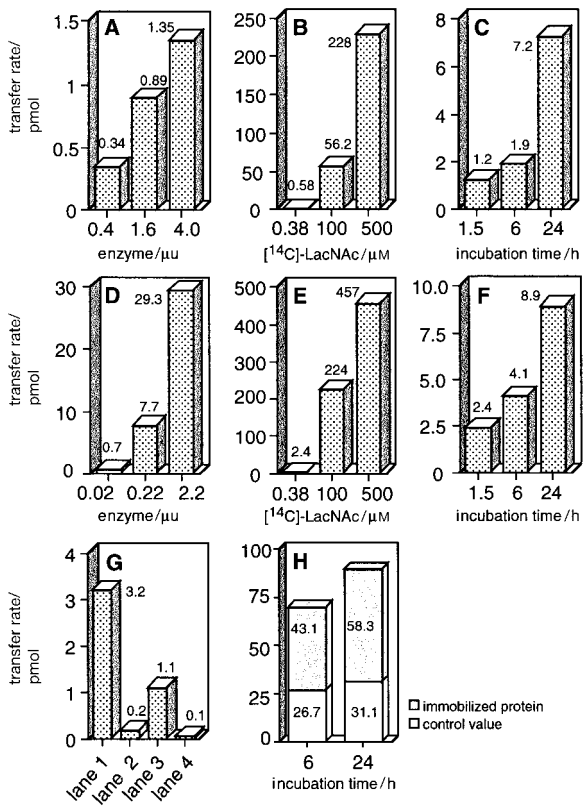

Figure 3. Amount [pmol] of immobilized CMP-NeuAc (**7**) transferred with α-(2→3)- and α-(2→6)-sialyltransferases. The assay conditions are given in Table 1.

Table 1. Assay conditions for the experiments presented in panels A – H of Figure 3.[a]

Panel	**7**	[^{14}C]-LacNAc	STase [μU]		Volume	Incubation time
	[mg]	[μg]	2,3	2,6	[μL]	[h]
A	2	0.4	[b]	–	32	5
B	1	[b]	0.8	–	32	3
C	1	1.0	0.8	–	35	[b]
D	2	3.8	–	[b]	32	4
E	1	[b]	–	2.2	32	3
F	1	1.0	–	2.0	34	[b]
G	2	0.7	[c]	[c]	34	3
H	1	[d]	0.8	–	27[e]	[b]

[a] Transfer assays with CMP-Neu5Ac (**7**) were carried out at 37 °C in 1.5-mL eppendorf tubes containing methyl *N*-acetyl-[1′-6′-^{14}C]-lactosaminide ([^{14}C]-LacNAc), ca 1.15 Kbq per 100 pmol to 1.15 KBq per 16 nmol by dilution with LacNAc, STase, HEPES buffer (0.1M, pH 7.0), and BSA (1 mg per 1 mL of assay mixture). All assays (except in G) were performed without enzyme system as control experiments. [b] Various amounts. [c] Enzyme quantities: lane 1 (6.6 μU of 6STase), lane 2 (6.6 μU of boiled 6STase), lane 3 (2.0 μU of 3STase), lane 4 (2.0 μU of boiled 3STase). [d] [^{14}C]-asialoglycoprotein, ca. 1.0 nmol. [e] The buffer solution contains 0.1 % nonidet P-40 and $MnCl_2$ (5 mM).

In addition, we examined whether **7** is active toward an asialoglycoprotein acceptor (α_1-acidic asialoglycoprotein containing [U-^{14}C]-gal[18]) by use of 3STase. As shown in panel H, the isotope counts for the asialoglycoprotein increased upon extention of the incubation time. However, isotope counts measured in the control assay (without enzyme) indicated as much as half the level of the actually immobilized glycoprotein. When [^{14}C]-LacNAc was used in the assays (panels A – F), the control values were less than 5 % of the value determined for the actually immobilized [^{14}C]-LacNAc. The control values for the assay in panel H are due to nonspecific binding of asialoglycoprotein to CPG. Therefore, the difference in control values between 6 h and 24 h is only 4.4 pmol. On the other hand, the difference in immobilized asialoglycoprotein between 6 h and 24 h is 15.2 pmol. Since the value for the immobilized protein increased independent of the control value upon simple extention of the incubation time, the data in panel H show that the immobilized CMP-NeuAc actively immobilizes asialoglycoprotein. In addition, the transfer activity is reproducible over several experiments. Since 58 pmol of α_1-acidic asialoglycoprotein was incorporated into CPG, about 10 % of the CMP-NeuAc was consumed during immobilization. Furthermore, these data also suggest that about 2.5 mg of the glycoprotein can be immobilized on 1 g of CPG. Since the α_1-acidic asialoglycoprotein used in this assay is estimated to have one equivalent of [U-^{14}C]-galactoside on its surface,[18] this glycoprotein may be a poor sialyl acceptor. Therefore, a glycoprotein containing several galactosides might be a good sialyl acceptor due to the multivalency effect, and, as a result, a practical quantity of glycoprotein might be immobilized.

Our methodology is currently limited to the immobilization of glycoproteins having galactosides at the nonreducing terminus. Other glycoproteins having fully sialylated oligosaccharides or high mannose-type oligosaccharides are more difficult to immobilize. In the former case, the immobilization can be performed after sialidase digestion. As shown in panel H, the overall efficiency[19] and yield[20] of immobilization are still not satisfactory. Work is in progress to solve the above difficulties in order to enable more efficient immobilization.

In conclusion, we have described a novel finding in which immobilized CMP-NeuAc reacts with sialyltransferase. This sialoside can be used as a new type of linker to covalently bond a labile biomolecule to a solid-phase support.

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H-9b), 2.51 (dd, $J = 4.8$, 13.8 Hz, 1H; H-3eq), 1.90, 1.88, 1.80, 1.77 (each s, 3H; Me); HR-FAB-MS: calcd for $C_{29}H_{38}N_2O_{13}SNa$ [M+Na$^+$]: 677.1993; found: 677.1997.

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[15] After incubation (panels A – H) the mixture was filtered, and the CPG remaining on the filter paper was washed with water twice (3 mL). The filter paper and CPG were inserted into a scintillation vial for counting. The radioactivity on the CPG was measured by a liquid scintillation counter, and the amount of [^{14}C]-LacNAc incorporated on the CPG was estimated. The transfer rates after subtraction of control values are summarized in Figure 3 (panels A – F). The data given (panels A – H) are averages values.

[16] The transfer assays were performed by one of the following two methods. The assays of CMP-NeuAc were carried out at 37 °C with a solution of HEPES buffer (total 35 μL) containing CMP-[U-^{14}C]-NeuAc (12.1 KBq nmol^{-1}, 20 μM), LacNAc (1 μM), BSA, and 3STase (0.8 μU). The amounts that were transferred were estimated by the conventional method.[17] For immobilized CMP-NeuAc, a solution of HEPES buffer (total 35 μL) containing **7** (1 mg: the concentration of CMP-NeuAc corresponds to 20 μM based on 721 pmol of CMP-NeuAc on 1 mg of CPG), [^{14}C]-LacNAc (1 μM), BSA, and 3STase (0.8 μU) was incubated at 37 °C, and the transferred amount was estimated with the above method.[15]

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[20] Immobilization yields are estimated to be about 4.5% and 5.8% in the case of LacNAc and asialoglycoprotein, respectively, based on the sialylacceptors added.

Cationic Homoleptic Vanadium(II), (IV), and (V) Complexes Arising from Protonolysis of $[V(NEt_2)_4]$

Robert Choukroun,* Pierre Moumboko, Sandrine Chevalier, Michel Etienne, and Bruno Donnadieu

Although the chemistry of cations containing Group 4 metals is very well documented[1] and a subject of ongoing investigations, with regard to the modeling of the Ziegler – Natta polymerization, the chemistry of cationic vanadium complexes has not been as thoroughly studied as that of their neutral complexes.[2–4] The recent interest in complexes in which amido and imido groups are directly bound to a Group 4 or 5 metal atom has led to new catalysts in alkene polymerization.[5] Hydridotris(pyrazolyl)borate imidovanadium(V) in the presence of a methylaluminoxane (MAO) activator shows modest ethylene polymerization activity.[6] We reported that the protonolysis of $[Cp_2VMe_2]$ or $[Cp_2Zr(BH_4)_2]$ can lead to a disproportionation redox reaction resulting in cationic vanadium(III) or zirconium(III) complexes.[7,8] In this context we report here the formation of new cationic vanadium(II), (IV), and (V) species when $[V(NEt_2)_4]$ (**1**) is treated with the ammonium salt $[NHMe_2Ph][BR_4]$ (R = Ph, C_6F_5).

Treatment of **1** with one equivalent of $[NHMe_2Ph][BPh_4]$ in thf at room or low temperature (− 78 °C) causes precipitation of the unexpected air-sensitive, dicationic, heteroleptic dialkylamidovanadium(IV) complex **2** with one thf molecule of

$[V(NEt_2)_4]$ **1**

$[V(NEt_2)_2(thf)_4][BPh_4]_2 \cdot thf$ **2**

crystallization (yield: 26%). This crystalline red product was characterized by X-ray structure analysis (Figure 1).[9] The environment around the metal is octahedral and the V−N and V−O distances (2.054 and 2.11 Å (average), respectively) are comparable to those in other vanadium compounds containing the bis(trimethylsilyl)amide ligand and a coordinated thf molecule.[10] The sum of the angles about each N center is close to 360°, indicating that the amide ligands are probably acting as three-electron donors. We also observe that a small, nonquantifiable amount of an uncharacterized green precipitate is present among the crystals of **2**, providing evidence that the reaction is not simple.

Reconsidering the formation of **2**, we repeated our experiment with **1** and two equivalents of $[NHMe_2Ph][BR_4]$ (R = Ph or C_6F_5) in thf at room temperature. Compound **1** was treated with two equivalents of $[NHMe_2Ph][B(C_6F_5)_4]$ in thf, and pentane was allowed to diffuse slowly into the solution to give a mixture of crystalline, highly air-sensitive, red products.

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