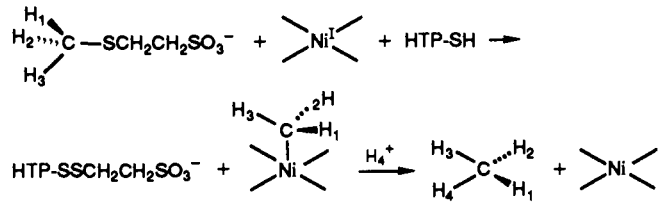
Scheme II[a]



[a] HTP-SH = 7-mercaptoheptanoyl threonine phosphate.

the isotopically chiral methylene group of the ethyl coenzyme M samples.[18]

The samples of (*R*)- and (*S*)-[1-$^2H_1$,^3H]ethyl coenzyme M (1.6 μCi and 2.8 μCi, 2 mM) were each incubated under H_2 gas with an anaerobically prepared cell-free extract[19] of *Methanosarcina barkeri* (1 mL) and 12 mM ATP in crimp-sealed 10-mL vials overnight at 37 °C on a water bath shaker. GC analysis of the head space revealed the formation of 564 nmol of ethane per vial from the *R* samples and 206 nmol from the *S* isomers. The samples were stored frozen prior to workup.

To convert the ethane samples into acetic acid for configurational analysis, the liquid was removed from the thawed reaction vessels with a syringe, and the vessels were rinsed twice with 1 mL of water each. Carrier ethane gas (2.2 mL) and chlorine gas (22 mL) were then introduced into each vial with a gas-tight syringe, and the vials were illuminated for 20 h at room temperature with a UV lamp (254 nm). KOH (0.4 g) dissolved in 5 mL of 5% aqueous $KMnO_4$ solution was added to each vial, and the vials were heated to 120 °C for 24 h. The excess $KMnO_4$ was destroyed with sodium bisulfite. Steam distillation of the alkaline solution then removed impurities, followed by recovery of the acetic acid by steam distillation of the acidified (10% H_2SO_4) solution. The second steam distillate was adjusted to pH 9 with NaOH and evaporated to dryness to give sodium acetate in about 2-3% radiochemical yield.[20] The samples were then subjected to configurational analysis by the method of Cornforth et al.[14] and Arigoni and co-workers,[15] using a procedure employed routinely in this laboratory.[17]

The analyses gave an *F* value of 38.1 for the acetate from (*S*)-[1-$^2H_1$,^3H]ethyl coenzyme M, corresponding to 41% ee *S* configuration; conversely, the acetate from (*R*)-[1-$^2H_1$,^3H]ethyl coenzyme M gave an *F* value of 64.8, corresponding to 51% ee *R* configuration. It follows that the reductive replacement of the sulfur of coenzyme M by a hydrogen in the methylreductase reaction proceeds with net inversion of configuration. This result is consistent with and supports the proposed sequence of events at the alkyl group in the reductive methane formation catalyzed by this enzyme (Scheme II). Displacement of the sulfur from the methyl (ethyl) group by Ni^I presumably proceeds with inversion of configuration, and the subsequent protonolytic cleavage of the alkyl-metal bond, on the basis of existing precedent,[21] would be expected to proceed in a retention mode.

Acknowledgment. We are indebted to Dr. Lynne D. Zydowsky for her contributions in the early stages of this project and to Dr. Sungsook Lee for assistance with the configurational analyses of the acetate samples. Financial support by the National Institutes of Health (Grant GM 32333 to H.G.F.) and by the National Science Foundation (Grant DCB 8904099 to J.A.K.) is gratefully acknowledged.

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(18) The low optical purity is probably due to the presence of traces of [^3H]$NaBH_4$ in the [^3H]-9-BBN preparation. When benzaldehyde was reduced with another preparation of (+)-[^3H]pinanyl-9-BBN and the alcohol converted to the (*S*)-mandelate ester, ^3H NMR revealed the presence of (*R*)- and (*S*)-benzyl alcohol in the reduction product in a ratio of 79.4:20.6 (58.8% ee *R*), whereas the reduction of [7-$^2H_1$]benzaldehyde with unlabeled pinanyl-9-BBN is completely stereospecific within the limits of detection.[13]

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Molecular Recognition of Bivalent Sialosides by Influenza Virus

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Infection by influenza virus is initiated by the binding of virus to cell-surface glycoproteins and glycolipids that terminate in sialic acid (SA).[1,2] This interaction is mediated by the trimeric viral protein hemagglutinin (HA).[3] Crystallographic studies of the complex between sialyllactose and hemagglutinin have defined the binding site and confirmed that HA simply recognizes the terminal sialoside of cell-surface oligosaccharides;[3] yet monovalent sialosides bind only weakly, whether to whole virus as measured by the inhibition of erythrocyte agglutination[2] or to bromelain-released HA (BHA) as studied by ^1H NMR.[4] Indeed, there are no reported monovalent α-sialosides with K_d values <2 mM. The binding of virus to cells presumably involves many HA trimers and many sialoside ligands.[5-7] We have investigated two families of bivalent sialosides and find that bis-sialosides of appropriate length bind tightly, not to isolated BHA, but to intact virus. *These ligands evidently bind intermolecularly to adjacent hemagglutinin trimers on the viral surface*, illustrating the energetic consequences of multivalent binding.[5-7]

The binding to BHA of bidentate sialosides having polyethylene glycol linkers (Figure 1A) was shown by NMR titration to be no better than that of the prototypical monovalent ligand, α-methyl-*N*-acetylneuraminic acid (Neu5Acα2Me), which has a K_d of 2.8 mM.[4] Even the longest of these bivalent species, P(5,5) (Figure 1A), which model building suggests is long enough to span two sites on an HA trimer, shows no decrease in K_d. This low affinity has one of two causes. Either the linker is too short (or conformationally too inflexible) to permit both SA residues si-

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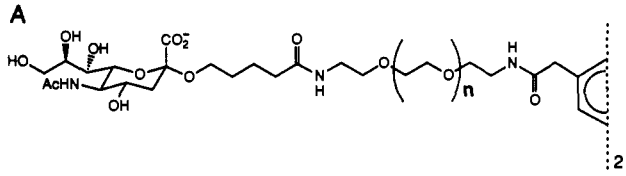
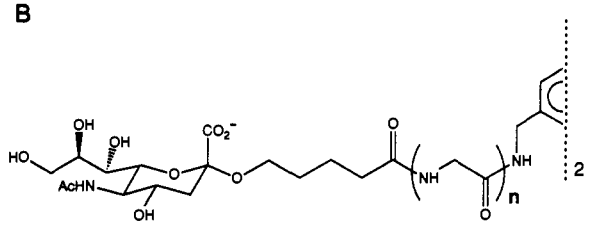
A



SPACER (n)	SPAN (Å)	DESIGNATION
1	49	P(3,3)
2	57	P(4,4)
3	65	P(5,5)

B



SPACER (n)	SPAN (Å)	DESIGNATION
0	27	G(0,0)
1	34	G(1,1)
2	41	G(2,2)
3	49	G(3,3)
4	57	G(4,4)

Figure 1. Structure of the bivalent sialosides. Distances were measured (between the carbonyl carbons of the *N*-acetyl groups) for molecules in an extended conformation[8] by using the MACROMODEL software package[9] and then minimizing the energy with the AMBER united atom force field.[10] Each compound was purified by gel filtration followed by reverse-phase HPLC and gave ^{1}H NMR and high-resolution FAB mass spectra consistent with the assigned structure. Synthetic details will be reported elsewhere.[11] A: Inhibitors with spacers derived from polyethylene glycol (the P series). B: Inhibitors using oligoglycine as spacer (the G series). The poor solubility of compounds possessing more glycine units than G(4,4) precluded their evaluation.

multaneously to reach their sites (indeed, the length of linker required cannot be defined precisely because of uncertainty in the location of a disordered oligosaccharide on asparagine-165, which lies between two SA sites on the HA surface), or alternatively, the entropic loss when the linker is constrained by *bi*dentate binding may be so great that binding at a second site is energetically disfavored.[5]

The second possibility is ruled out by the fact that bidentate ligands of proper length do bind more tightly to intact virus. Compound P(4,4) (Figure 1A) inhibits erythrocyte agglutination by virus[12] more effectively than the other bivalent sialosides in the P series, suggesting that P(4,4) bridges two HA trimers and that the distance between SA binding sites on adjacent trimers is $\lesssim$55 Å (Figure 1A).

On the basis that the inhibition of hemagglutination observed with P(4,4) is due to bidentate binding,[13] reducing the flexibility of the linker could reduce the loss in conformational entropy on binding and afford a better inhibitor. This possibility was examined with inhibitors containing oligoglycine links (the G series: Figure 1B), which are more rigid than the P-series inhibitors, having the planar constraint of peptide bonds within the spacer arms. The binding to BHA of the longest inhibitor in the G series, G(4,4), was measured by NMR, and no decrease in K_d relative to Neu5Acα2Me was observed. In the inhibition of hemagglutination by whole virus, however, G(3,3) is 2-fold better than P(3,3), and G(4,4) binds 5-fold tighter than P(4,4) and 100-fold tighter than Neu5Acα2Me (Figure 2). A more rigid linker evidently yields an inhibitor of greater potency. The finding that P(4,4) and G(4,4) are the best inhibitors in their respective series suggests that the two compounds have a similar effective length. The dependence of binding strength on linker length seen in Figure 2 indicates a defined distance between sialic acid binding loci and is consistent with earlier work showing low rotational and lateral mobility of HA trimers on the viral surface below 30 °C.[14-16]

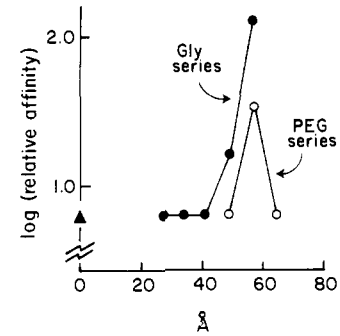


Figure 2. Inhibition of hemagglutination by the P series (○) and G series (●) of bivalent sialosides using influenza virus X-31 at 4 °C (measured relative to Neu5Acα2Me, K_i = 2.5 mM). ▲ represents an analogue of P(4,4) that only has one SA. Phosphate-buffered saline (50 μL) was added to each well of a 96-well v-shaped microtiter plate. The inhibitor (50 μL, pH 7.0) was added to the first well of a row and serially diluted 2-fold into each successive well. Virus (50 μL of freshly diluted suspension, 2–4 hemagglutinating units) was added to each well, and the plate was incubated for 30 min. Fresh chicken erythrocytes (<2 days old) from 21-week-old chickens (SPAFAS Inc., Storrs, CT; 100 mL of a 0.5% (v/v) solution) were then added to each well. The end point of the titration is defined as the minimum concentration of compound required for complete inhibition of hemagglutination after 2 h.

Influenza virus also displays neuraminidase, which has an SA binding site.[18,19] However, this enzyme does not participate in hemagglutination or its inhibition, since the addition of a specific neuraminidase inhibitor (2-deoxy-2,3-dehydro-Neu5Ac, to 4mM: K_i = 30 μM) does not alter the inhibitory potency of the compounds shown in Figure 1.[20,21] That the observed inhibition of hemagglutination derives from the cross-linking of virus by the bidentate ligand is also unlikely: the virus concentration used in the hemagglutination inhibition studies is low, and this explanation is inconsistent with the fall in potency from P(4,4) to P(5,5). In agreement with these findings, synthetic polymers carrying pendant α-sialosides interact strongly with intact virus and inhibit the

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hemagglutination of erythrocytes 10^3–10^4 times more than Neu5Acα2Me.[22]

In summary, it appears that bidentate binding occurs to different HA trimers on influenza virus. From the relative binding affinities of our bivalent compounds, the distance between SA binding sites on different HA trimers must be less than about 55 Å. Interestingly, the effects we have observed are not unique to influenza virus: G(4,4) inhibits the agglutination of erythrocytes by polyoma virus some 500-fold better than Neu5Acα2Me.[23] While we do not know if inhibition of hemagglutination correlates with blockade of viral infectivity, further experiments with defined polyvalent ligand analogues should clarify the nature of viral recognition and may suggest routes to new antiviral agents.

Acknowledgment. This work was supported by the National Institutes of Health as a collaboration among the groups of M. Karplus, J. R. Knowles, G. M. Whitesides, and D. C. Wiley and by the Howard Hughes Medical Institute. We are especially grateful to P. L. Toogood for practical help and discussions, to J. E. Hanson and N. K. Sauter for assistance with the NMR binding assay, and to J. J. Skehel for generous supplies of BHA and virus.

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Chemical Reactivity of a Metallabenzene[1]

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In addition to its effects on molecular structure and spectroscopy, aromaticity strongly influences chemical reactivity. Studies of reduction, oxidation, and substitution reactions involving benzene and heterobenzene derivatives form a central component of organic chemistry.[2] However, related studies on metallabenzenes have not been pursued because of the scarcity of metal-containing aromatic systems.[3] We recently reported the synthesis of a rare, stable metallabenzene complex, (IrCH=C(Me)CH=C(Me)CH)(PEt$_3$)$_3$ (**1**).[4] Structural and spectroscopic data for **1** clearly indicated the presence of an aromatic ring system. We now report initial findings on the reactivity of **1**, which parallels in some respects the reactivity of conventional benzene derivatives, and in others does not.

Unlike organic arenes, **1** reacts with H_2 at room temperature and 1 atm of pressure, generating the partially hydrogenated iridacyclohexadiene complex *mer*-(IrCH$_2$C(Me)=CHC(Me)=CH)(PEt$_3$)$_3$(H) (**2**)[5] (see Scheme I). This reaction probably proceeds via dissociation of a PEt$_3$ ligand from **1**, producing 16 e$^-$ (IrCH=C(Me)CH=C(Me)CH)(PEt$_3$)$_2$. Oxidative addition of H_2, followed by hydride migration from the iridium center to the ortho carbon of the ring and readdition of PEt$_3$, yields the observed product.

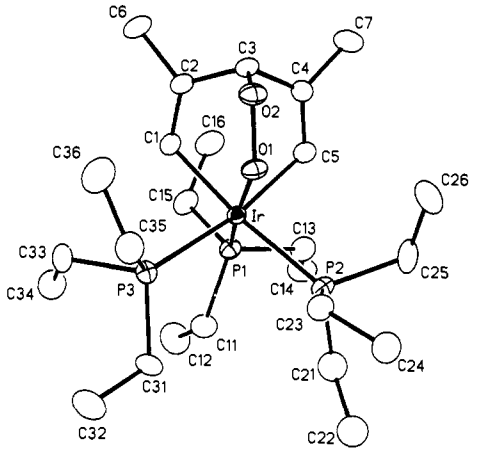
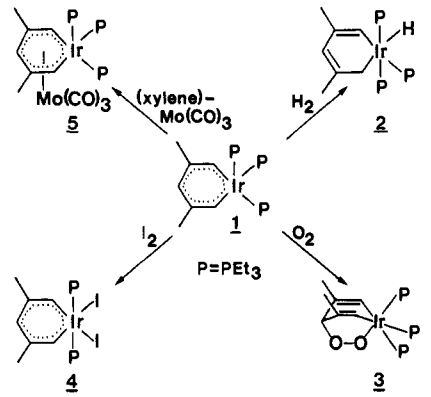


Figure 1. ORTEP drawing of (IrCH=C(Me)CHC(Me)=CH)(PEt$_3$)$_3$ (**3**). Selected bond distances: Ir–P(1), 2.279 (3) Å; Ir–P(2), 2.387 (2) Å; Ir–P(3), 2.395 (2) Å; Ir–C(1), 2.075 (8) Å; Ir–C(5), 2.062 (8) Å; Ir–O(1), 2.111 (6) Å; C(1)–C(2), 1.315 (11) Å; C(2)–C(3), 1.512 (11) Å; C(3)–C(4), 1.521 (13) Å; C(4)–C(5), 1.339 (12) Å; O(1)–O(2), 1.466 (7) Å; O(2)–C(3), 1.435 (10) Å.

Scheme I



Compound **1** in pentane solution reacts with atmospheric oxygen to produce the novel dioxygen-bridged species (IrCH=C(Me)CHC(Me)=CH)(PEt$_3$)$_3$ (**3**) (see Scheme I and ORTEP drawing, Figure 1).[6,7] The iridacyclohexa-2,5-diene ring in **3** is boat-shaped with Ir and C(3) residing 0.92 and 0.65 Å, respectively, out of the C(1)/C(2)/C(4)/C(5) plane. This reaction is reminiscent of the reaction of certain polycyclic aromatic compounds (e.g., anthracene) with O_2, which lead to internal peroxides.[8] However, unlike these organic reactions which require singlet oxygen, **1** reacts with ground-state (triplet) oxygen. Furthermore, unlike the reaction of **1** with H_2 (which requires PEt$_3$ loss and therefore proceeds relatively slowly), the O_2 reaction

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(6) Spectroscopic data for **3**: ^{1}H NMR (C_6D_6, 17 °C, 300 MHz) δ 7.37 (m, 2, H1/H5), 4.59 (s, 1, H3), 2.32 (s, 6, ring CH$_3$'s), 1.95–1.77 (m, 6, PEt$_3$ CH$_2$'s), 1.71–1.53 (m, 12, PEt$_3$ CH$_2$'s), 1.10–0.97 (m, 18, PEt$_3$ CH$_3$'s), 0.82–0.69 (m, 9, PEt$_3$ CH$_3$'s); ^{13}C{^{1}H} NMR (C_6D_6, 17 °C, 75 MHz) δ 132.1 (d of t, J_{C-P} = 89.4, 9.2 Hz, C1/C5), 129.5 (s, C2/C4), 92.3 (s, C3), 26.5 (t, J_{C-P} = 4.7 Hz, ring CH$_3$'s), 19.8 (d, J_{C-P} = 32.2 Hz, PEt$_3$ CH$_2$'s), 16.1 (d, J_{C-P} = 22.8 Hz, PEt$_3$ CH$_2$'s), 8.8 (virtual t, J_{C-P} = 5.1 Hz, PEt$_3$ CH$_3$'s), 8.6 (d, J_{C-P} = 5.1 Hz, PEt$_3$ CH$_3$'s); ^{31}P{^{1}H} NMR (C_6D_6, 17 °C, 121 MHz, referenced to external H_3PO_4) δ −29.5 (t, J_{P-P} = 9.5 Hz, 1, PEt$_3$), −35.8 (d, J_{P-P} = 9.5 Hz, 2, equivalent PEt$_3$'s).

(7) Crystal data for **3**: monoclinic, space group $P2_1/c$, a = 16.713 (5) Å, b = 10.454 (3) Å, c = 17.609 (4) Å, β = 103.99 (2)°, V = 2985.3 (14) Å^3, Z = 4.

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