After pivalation, it is often difficult to separate the pivalate esters from the remaining pivalic anhydride by column chromatography because of their similar polarity. In our protocol, methanol was added after consumption of the alcohol in order to convert the remaining anhydride to methyl pivalate. $Bi(OTf)_3$ effected the methanolysis of the pivalic anhydride quantitatively at 50 °C where methanol as well as CH_2Cl_2 was used without purification. The product pivalate esters could be readily isolated from the crude product mixture by simple filtration through a thin pad of silica gel with hexane as the solvent. The pivalate esters thus obtained were of sufficient purity. Methanolysis of the excess anhydride in the presence of $Sc(OTf)_3$ or Me_3SiOTf[9] under the same reaction conditions was not complete. Apparently, $Bi(OTf)_3$ is superior to the other metal triflates.

The pivalation of a mandelic ester proceeded with complete retention of the configuration (entry 8). Furthermore, this pivalation method was applicable to a sugar having an acetal as protecting group, which can not survive in the Me_3SiOTf protocol (entry 10).[2e] The acylation of a nucleoside with $Bi(OTf)_3/(t\text{BuCO})_2O$ led quantitatively to the triply pivalated nucleoside despite the existence of an amide function (entry 11). In all the trials for pivalation, CH_2Cl_2 could be used directly from the bottle without purification.

In conclusion, a powerful and versatile acylation method has been developed based on $Bi(OTf)_3$ and acid anhydride. This method has several advantages: the catalyst is cheap and easy to handle, a variety of primary, secondary, and tertiary alcohols can be transformed, and the operations are simple because neither dry reaction conditions nor lowering of the reaction temperature for tertiary alcohols is required. The reactivity of $Bi(OTf)_3$ can be modified, if necessary, by changing the coordinating character of the cosolvent.

Experimental Section

Representative procedure for pivalation (entry 8, Table 3): A CH_2Cl_2 solution (3 mL, unpurified and wet) of methyl (*R*)-mandelate (166.2 mg, 1.0 mmol) and $(t\text{BuCO})_2O$ (299.4 mg, 1.5 mmol) was stirred at 25 °C in the presence of $Bi(OTf)_3$ (21.8 mg, 3.0 mol %, calculated as the tetrahydrate) for 4 h. MeOH (10 mL, unpurified and wet) was added and the mixture was stirred at 50 °C for 7 h. The mixture was passed through a pad of silica gel with hexane as the solvent and the filtrate was evaporated. Ethyl acetate (30 mL) was added to the crude product, and the organic layer was washed three times with aqueous $NaHCO_3$ and dried ($MgSO_4$). Evaporation furnished the pure pivalate ester (97 % yield, 242.8 mg).

Received: April 14, 2000
Revised: June 5, 2000 [Z14996]

[1] W. Green, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., Wiley, New York, **1999**, p. 150.

[2] Acetylation of alcohols with Ac_2O in the presence of a) DMAP: W. Steglich, G. Höfle, *Angew. Chem.* **1969**, *81*, 1001; *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 981; b) Bu_3P: E. Vedejs, N. S. Bennet, L. M. Conn, S. T. Diver, M. Gingras, S. Lin, P. M. Oliver, M. J. Peterson, *J. Org. Chem.* **1993**, *58*, 7286; c) aminophosphane superbase: B. A. D'Sa, J. G. Verkade, *J. Org. Chem.* **1996**, *61*, 2963; d) $Sc(OTf)_3$: K. Ishihara, M. Kubota, H. Kurihara, H. Yamamoto, *J. Org. Chem.* **1996**, *61*, 4560; e) Me_3SiOTf: P. A. Procopiou, S. P. D. Baugh, S. S. Flack, G. G. A. Inglis, *J. Org. Chem.* **1998**, *63*, 2342; f) $In(OTf)_3$: K. K. Chauhan, C. G. Frost, I. Love, D. Waite, *Synlett* **1999**, 1743; g) distannoxane: A. Orita, K. Sakamoto, Y. Hamada, A. Mitsutome, J. Otera, *Tetrahedron* **1999**, *55*, 2899; h) cationic organotin dimer: K. Sakamoto, Y. Hamada, H. Akashi, A. Orita, J. Otera, *Organometallics* **1999**, *18*, 3555.

[3] A. Orita, A. Mitsutome, J. Otera, *J. Org. Chem.* **1998**, *63*, 2420.

[4] Both $Sc(OTf)_3$ and Sc_2O_3, which is a starting material for the triflate, can be purchased from Aldrich. The price of Sc_2O_3 is \$159.9 for 5 g (72.5 mmol). Bi_2O_3, the starting compound for $Bi(OTf)_3$, is also commercially available, and its price is cheaper: \$187.1 for 250 g (1.07 mol). $Bi(OTf)_3$ is accessible alternatively by the reaction of Ph_3Bi (\$57.3, 25 g, 56.8 mmol) and TfOH. In this study, $Bi(OTf)_3$ was prepared as a tetrahydrate according to this procedure. M. Labrouillere, C. Le Roux, H. Gaspard, A. Laporterie, J. Dubac, *Tetrahedron Lett.* **1999**, *40*, 285, and references therein.

[5] a) J. R. Desmurs, M. Labrouillere, C. Le Roux, H. Gaspard, A. Laporterie, J. Dubac, *Tetrahedron Lett.* **1997**, *38*, 8871; b) S. Répichet, C. Le Roux, J. Dubac, J. R. Desmurs, *Eur. J. Org. Chem.* **1998**, 2743.

[6] B. Garrigues, F. Gonzaga, H. Robert, J. Dubac, *J. Org. Chem.* **1997**, *62*, 4880.

[7] H. Laurent-Robert, C. Le Roux, J. Dubac, *Synlett* **1998**, 1138.

[8] For the acetylation of the 12α-hydroxy group of methyl cholate, prolonged heating in Ac_2O/pyridine or addition of an excess amount of 4-pyrrolidinopyridine is needed: G. Höfle, W. Steglich, *Synthesis* **1971**, 619.

[9] A similar methanolysis was reported in the Me_3SiOTf protocol,[2e] but we could not reproduce this result.

Remarkable *In*/*out* Inversions at Bridgehead Phosphorus Atoms**

Roger W. Alder* and David Read

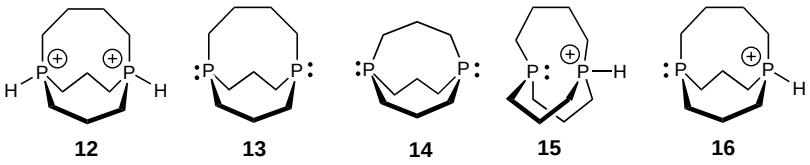
Phosphanes are more pyramidal and invert much less readily than amines. The C-P-C angles for trialkylphosphanes are typically about 100° and they have inversion barriers around 150 kJ mol^{-1},[1] hence temperatures of well over 100 °C are required for the racemization of chiral phosphanes. In medium-sized bicyclic ring systems[2] *in*/*out* isomerism[3] is possible, and bridgehead nitrogen atoms are known to adopt whichever arrangement is more stable.[4] Thus 1,4-diazabicyclo[2.2.2]octane is *out*,*out*, 1,6-diazabicyclo[4.4.4]tetradecane is *in*,*in*,[5] 1,5-diazabicyclo[3.3.3]undecane has nearly planar nitrogen atoms,[6] and 1,9-diazabicyclo[7.3.1]tridecane is *in*, *out*.[7] We expected that in related phosphorus compounds *in*,*out* and *in*,*in* isomers would become preferred to *out*,*out* isomers as the ring systems became larger, and also that unstable isomers might be isolated as a result of the high inversion barriers for phosphanes. We find however that these barriers are much lower than expected. We focus here on a

[*] Prof. R. W. Alder, Dr. D. Read
School of Chemistry
University of Bristol
Bristol BS8 1TS (UK)
Fax: (+44) 117-9298611
E-mail: Rog.Alder@bristol.ac.uk

[**] We thank Dr. J. M. Oliva (Institute of Materials Sciences of Barcelona, CSIC) for the calculations on **12** and **13**, the EPSRC for a quota studentship to D.R., and the EPSRC National Mass Spectrometry Service for high-resolution electrospray spectra.

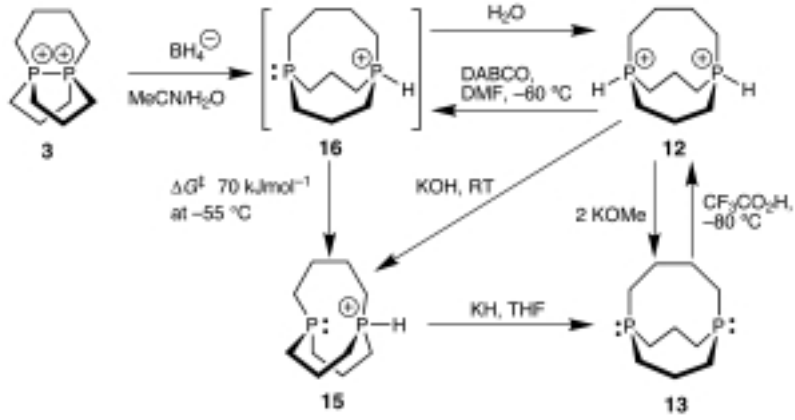
  1433-7851/00/3916-2879 $ 17.50+.50/0

case where phosphorus atoms not only invert readily, but do so under the control of an unexpected kinetic effect (inversion at the leaving group in a nucleophilic substitution), in which they go from *in*,*in* to *in*,*out* via a less-stable *out*,*out* structure.



The preparations of salts of dications **1**, **2**, and **3** have been described.[2] Reactions of **1** and **2** with borohydride lead to **4** and **5**, respectively, which are related to diphosphanes **6** and **8**, but clearly have *in*,*out* structures with significant P–P bonding. Deprotonation of **4** leads to **7** through an unexpected rearrangement, but deprotonation of **5** with strong base gives *out*,*out* diphosphane **8**, which is easily re-protonated to **5**,[2] with inversion at the nonprotonated phosphorus atom at ambient temperature. The protonation of **8** to give **5**, and its strong basicity, are reminiscent of prophosphatranes **9**, which are strong bases that lead to **10**.[8] Pascal and co-workers have shown that the reaction of **11** with sulfur to give the *out* sulfide involves a rate-limiting inversion of the phosphorus atom, but here the energy barrier (146 kJ mol^{-1}) is relatively normal.[9]

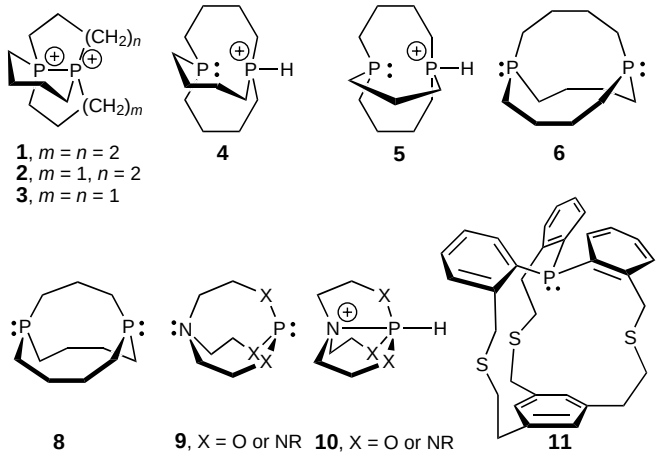
When **3**$(OTf)_2$ is treated with less than one equivalent of KBH_4 in wet CH_3CN, the product is **12**$(OTf)_2$, namely *out*,*out*-diprotonated **13**—both phosphorus atoms have inverted! The [3.3.3] diphosphane **14**[2] only forms *out*-mono- and *out*,*out*-diprotonated ions, and so the formation of **12** might simply reflect a preference for *out*,*out* structures in the [4.3.3] series too. However, deprotonation of **12**$(OTf)_2$ with one equivalent of base at room temperature immediately gives the *in*,*out* monoprotonated salt **15**(OTf), with a coupling constant $J_{P,P} = 253$ Hz observed in the ^{31}P NMR spectrum. This salt can be further deprotonated by strong base to **13**, which can also be prepared directly from **12** by treatment with two equivalents of KOMe in CH_3CN. Addition of more than two equivalents of trifluoroacetic acid at $-80\,^{\circ}C$ to **13** leads back to **12** (Scheme 1). Addition of acid to **15**, however, does not give **12**, but gives dication **3** and hydrogen, with the P–H acting as a hydride source.



Scheme 1.

It seems clear that **12** is formed in the borohydride reduction by protonation of *out*,*out* monoprotonated ion **16** by water (or borate-derived species). Compound **16** must be the kinetic product of the addition of hydride to **3**. When **12** is treated with one equivalent of 1,4-diazabicyclooctane (DABCO) in DMF at $-55\,^{\circ}C$ and the solution monitored by ^{31}P NMR spectroscopy, a new species is observed with broad ^{31}P lines at $\delta = -40$ and $+10$ (no P–P coupling in the spectrum, the broadening may be a consequence of slow exchange via **12** or **13**). This new species has a half-life of 2.5 h at $-55\,^{\circ}C$ for conversion into **15**. If this new species is **16**, then $\Delta G^{\ddagger}$ for inversion to **15** is 70 kJ mol^{-1} at $-55\,^{\circ}C$, which we believe to be the lowest measured barrier for a trialkylphosphane and is less than half the typical value.

We made strenuous efforts to obtain structural data on **12**, **13**, and **15** without success. B3LYP/6-311G(d,p)//B3LYP/6-31G(d) density functional calculations[10] predict that **15** is more stable than **16** by 24 kJ mol^{-1}, which is in agreement with our observations. The calculated structures for **12**, **13**, **15**, and **16** are interesting (Table 1). Diphosphane **13** has C-P-C angles which are approximately 7° larger than normal, and the C-P-C angles increase substantially on protonation of **12** (about 5° above a tetrahedral angle), which allows some reduction in the C-C-C angles. The *out*,*out* isomer **16** appears to be a normal protonated phosphane with a Natural Bond Orbital (NBO)[11] charge of $+0.02$ on the hydrogen atom, but has strongly flattened phosphorus atoms. The P(H) atom in the *in*,*out* isomer **15** has essentially trigonal pyramidal geometry, with the other phosphorus atom and the hydrogen atom as apical substituents. The apical



  1433-7851/00/3916-2880 $ 17.50+.50/0

Table 1. Calculated structural data for **12**–**16**.

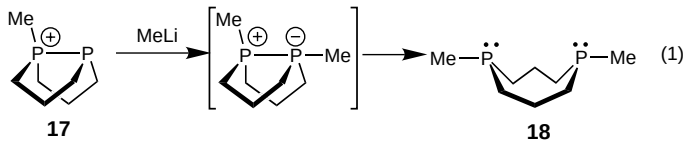
	Energy	P···P [Å]	Average P–H [Å]	Average C-P-C [°]	Average C-P(H)-C [°]
13	–1075.777927	4.39	–	107.1	–
15	–1076.184252	2.55	1.44	113.1	119.4
16	–1076.173587	4.06	1.41	107.9	115.3
12	–1076.442301	3.88	1.40	114.5	–

hydrogen atom shows an NBO charge of −0.10, which suggests it has hydridic character, and is in agreement with the chemical behavior of these species; thus **12** is also formed by reaction of **3** with **4** in CH_3CN/H_2O.

Two aspects of the chemistry involved in these apparently unnecessary journeys from *in*,*in* to *in*,*out* via *out*,*out* (Scheme 1) invite further comment: 1) Why are inversion barriers at these bridgehead phosphanes much lower than normal? 2) Why does the phosphorus atom distal from the borohydride invert in the reaction of **3**?

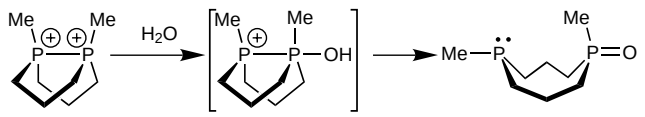
These bridgehead phosphanes are clearly much flatter than normal (Table 1), and so ground-state destabilization surely contributes to the lowered barriers. We only have actual X-ray structural data for **14**, which shows an average C-P-C angle of 105.7°,[2] but the larger angles in the calculated structure for **13** continue the expected trend.

The second question raised above is much more puzzling. Formation of **16** demands kinetically controlled inversion at the phosphorus atom, which is the leaving group in the $S_N2(P)$ reaction. There is little evidence concerning inversion or retention at the leaving group in S_N2 reactions, since stereochemical information can only be preserved if the leaving atom is tricoordinate, and nitrogen atoms invert too easily. A phosphorus leaving group may provide the best possibility of observing such a process, but the only examples we know of come from our own work. Reaction of monocations such as **17** with nucleophiles leads stereoselectively to *cis* products such as **18**,[12] presumably by a least-motion process [Eq. (1)] that



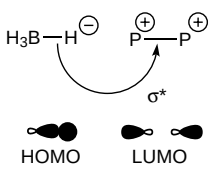
17 **18** (1)

corresponds to retention at the leaving group. Also, attack of water on **19** gives *trans*-monooxide **20** by single inversion [Eq. (2)]. The formation of **4** and **5** from **1** and **2** involves



19 **20** (2)

apparent retention at the leaving group, but we cannot rule out the fleeting formation of *out*,*out* ions such as **16**, which might invert too rapidly to be trapped by protonation. Even if the reaction of **3** to give **12** is an exceptional case, where the balance of strain on the *in*,*out* and *out*,*out* isomers is just right for observation of inversion at the leaving group, the cause of the non-least motion behavior must still be sought. A tentative suggestion can be based on frontier MO arguments. If electron transfer from the HOMO of the hydride donor to the P–P σ* orbital (LUMO) is extensive at the transition state, this could be enough to drive the phosphorus atoms apart, and lead to double inversion. This question could benefit from computational studies.



Experimental Section

All operations involving phosphanes were carried out under dry N_2.

12$(OTf)_2$: KBH_4 (45 mg; 0.83 mmol) was added to a stirred solution of **3**$(OTf)_2$ (832 mg; 1.66 mmol) in CH_3CN (15 mL). After one hour, the cloudy solution was filtered and the filtrate evaporated to give a sticky white solid, which was recrystallized from EtOH to give colorless needles (701 mg, 84%), m.p. 163–165 °C. ^{1}H NMR (400 MHz, CD_3NO_2, 25 °C): δ = 2.25–2.44 (4H, m), 2.50–2.73 (4H, m), 2.75–3.02 (12H, m), 6.58 (d, ^1J(P,H) = 519 Hz, 2H); ^{13}C NMR (100 MHz): δ = 11.71 (t, ^2J(P,C) = 7 Hz, C-8, C-11), 12.21 (d, ^1J(P,C) = 41 Hz, C-7, C-9, C-10, C-12), 14.70 (d, ^1J(P,C) = 40 Hz, C-2, C-5), 15.52 (d, ^2J(P,C) = 6 Hz, C-3, C-4); ^{31}P NMR (162 MHz) δ = 7.71 (d, ^1J(P,H) = 519 Hz); electrospray-MS: *m*/*z*: 501.0154 [M^- – H] ($C_{12}H_{21}P_2S_2F_6O_6$ requires 501.0159). The salt **12**$(PF_6)_2$ precipitated on addition of NH_4PF_6 (179 mg, 1.11 mmol) to a solution of **12**$(OTf)_2$ (251 mg; 0.50 mmol) in a minimum volume of water, and was recrystallized from EtOH as colorless plates (143 mg, 58%), m.p. 136–137 °C. Elemental analysis calcd for $C_{10}H_{22}P_4F_{12} \cdot C_2H_5OH$ C 26.68, H 5.22; found: C 26.16, H 4.89.

15(OTf): Methanolic KOH (55 μL, 1.04 M, 0.06 mmol) was added to a stirred solution of **12**$(OTf)_2$ (29 mg; 0.06 mmol) in CH_3CN (10 mL). After stirring the mixture for 10 min, the solvent was removed, the residue extracted into CH_2Cl_2 (3 × 10 mL), filtered and evaporated to give a colorless waxy solid (16 mg; 82%). ^{1}H NMR (400 MHz, CD_2Cl_2, 25 °C): δ = 1.65–2.40 (18H, m), 2.62–2.70 (2H, m), 6.15 (dd, ^1J(P,H) = 279 Hz, ^2J(P,H) = 83 Hz, 1H); ^{13}C NMR (100 MHz): δ = 19.07 (t, ^2J(P,C) = 8 Hz, 2C), 22.58 (d, ^2J(P,C) = 5 Hz, 1C), 22.78 (d, ^2J(P,C) = 6 Hz, 1C), 25.35 (d, ^1J(P,C) = 12 Hz, 1C), 28.34 (dd, ^1J(P,C) = 82 Hz, ^2J(P,C) = 66 Hz, 2C), 33.81 (d, ^1J(P,C) = 93 Hz, 1C), 35.19 (dd, ^1J(P,C) = 47 Hz, ^2J(P,C) = 41 Hz, 2C); ^{31}P NMR (162 MHz): δ = –91.49 (dd, ^1J(P,P) = 253 Hz, ^1J(P,H) = 279 Hz), –52.17 (dd, ^1J(P,P) = 253 Hz, ^2J(P,H) = 83 Hz); electrospray-MS: *m*/*z*: 203.1119 [M^+ – CF_3SO_3] ($C_{10}H_{21}P_2$ requires 203.1119). This compound is stable in the crystalline state, but decomposes slowly in solution to give a complex mixture of products (^{31}P NMR).

13: Methanolic $KOCH_3$ (0.33 M, 3.38 mL, 1.12 mmol) was added to stirred solution of **12**$(OTf)_2$ (280 mg, 0.56 mmol) in CH_3CN (5 mL). After stirring the mixture for 1 h, the volatiles were removed. The solid residue was then extracted with pentane (2 × 10 mL) and filtered through a glass sinter. Removal of the solvent furnished a waxy white solid (92 mg, 81%). ^{13}C NMR (100 MHz; C_6D_6): δ = 21.69 (t, ^2J(P,C) = 5 Hz, C-8, C-11), 22.30 (d, ^1J(P,C) = 28 Hz, C-2, C-5), 22.59 (d, ^1J(P,C) = 25 Hz, C-7, C-9, C-10, C-12), 24.46 (d, ^2J(P,C) = 5 Hz, C-3, C-4); ^{31}P NMR (162 MHz): δ = –25.24; CI-MS: *m*/*z*: 203.1119 [M^++H] ($C_{10}H_{21}P_2$ requires 203.1119). Diphosphane **13** can also be prepared from **15**(OTf) by treatment with KH in THF.

Received: February 11, 2000
Revised: May 2, 2000 [Z14687]

[1] R. D. Baechler, K. Mislow, *J. Am. Chem. Soc.* **1970**, *72*, 3090; A. Rauk, L. C. Allen, K. Mislow, *Angew. Chem.* **1970**, *82*, 453; *Angew. Chem. Int. Ed. Engl.* **1970**, *9*, 400.

[2] R. W. Alder, D. D. Ellis, R. Gleiter, C. J. Harris, H. Lange, A. G. Orpen, D. Read, P. N. Taylor, *J. Chem. Soc. Perkin Transactions 1* **1998**, 1657.

  1433-7851/00/3916-2881 $ 17.50+.50/0

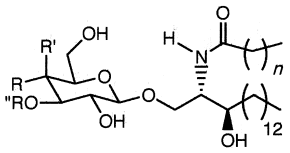
[3] R. W. Alder, S. P. East, *Chem. Rev.* **1996**, *96*, 2097.
[4] R. W. Alder, *Tetrahedron* **1990**, *46*, 683.
[5] R. W. Alder, A. G. Orpen, R. B. Sessions, *J. Chem. Soc. Chem. Commun.* **1983**, 999.
[6] R. W. Alder, N. C. Goode, T. J. King, J. M. Mellor, B. W. Miller, *J. Chem. Soc. Chem. Commun.* **1976**, 173; R. W. Alder, R. J. Arrowsmith, A. Casson, R. B. Sessions, E. Heilbronner, B. Kovac, H. Huber, M. Taagapera, *J. Am. Chem. Soc.* **1981**, *103*, 6137.
[7] R. W. Alder, E. Heilbronner, E. Honegger, A. B. McEwen, R. E. Moss, E. Olefirowicz, P. A. Petillo, R. B. Sessions, G. R. Weisman, J. M. White, Z.-Z. Yang, *J. Am. Chem. Soc.* **1993**, *115*, 6580.
[8] J. G. Verkade, *Acc. Chem. Res.* **1993**, *26*, 483; J. G. Verkade, *Coord. Chem. Rev.* **1994**, *137*, 233; X. Liu, J. G. Verkade, *J. Org. Chem.* **1999**, *64*, 8058, and references therein.
[9] Y. T. Chen, K. K. Baldridge, D. M. Ho, R. A. Pascal, Jr., *J. Am. Chem. Soc.* **1999**, *121*, 12082.
[10] Gaussian 98, Revision A.7, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**.
[11] A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899.
[12] R. W. Alder, C. Ganter, M. Gil, R. Gleiter, C. J. Harris, S. E. Harris, H. Lange, A. G. Orpen, P. N. Taylor, *J. Chem. Soc. Perkin Trans. 1* **1998**, 1643.

Gp120 Binds Cooperatively to Several Biologically Relevant Glycosphingolipids: Quantitative Measurements at Equilibrium by Total Internal Reflection Fluorescence Microscopy**

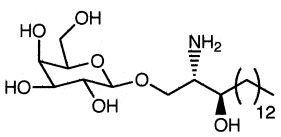
John C. Conboy, Katherine D. McReynolds, Jacquelyn Gervay-Hague, and S. Scott Saavedra*

Understanding the molecular events responsible for HIV-1 viral recognition and binding to receptors expressed on the host cell is critical to developing treatment strategies based on HIV-1 inhibition. The virus is encapsulated by a lipid bilayer which supports the envelope glycoprotein gp160. This protein is comprised of two noncovalently linked subunits: 1) a 41-kD transmembrane protein (gp41) which anchors the assembly in the viral membrane and 2) a 120-kD protein (gp120) which coats the outer surface of the viral particle and is responsible for initial recognition and attachment to the host cell.[1]

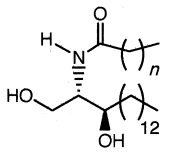
HIV-1 infection of a host cell can be initiated by gp120 binding to galactosyl ceramide (GalCer), a glycosphingolipid (GSL) expressed in human vaginal epithelial, colonic epithelial, and sperm cells.[2–7] The literature on gp120-GSL binding activity is surprisingly inconsistent. Harouse et al.[3, 4] and Bhat et al.[8] used high-performance thin-layer chromatography (HPTLC) and enzyme-linked immunosorbent assays (ELISA) to study the binding of several glycolipids to rgp120 including GalCer (**1**), galactosyl sulfatide (GalS, **2**), glucosyl ceramide (GlcCer, **3**), lactosyl ceramide (LacCer, **4**), psychosine (**5**), and ceramide (Cer, **6**), and found that **1**, **2**, and



R=H, R'=OH, R"=H: GalCer **1**
R=H, R'=OH, R"=SO_3^-: GalS **2**
R=OH, R'=H. R"=H: GlcCer **3**
R=*O*-β (1-4)-D-Gal, R'=H, R"=H: LacCer **4**



Psychosine **5**



Ceramide **6**

5 bound. Latov and co-workers used immunospot assays on nitrocellulose and TLC plates as well as ELISA to test the binding affinity of **1** and **2** for recombinant gp120 (rgp120).[5] They reported that in both the nitrocellulose immunospot assay and ELISA **2** bound to rgp120 but **1** did not. However, in the immunospot TLC assay, both **1** and **2** bound. Long et al.[9] studied interactions of rgp120 and liposomes doped with various glycolipids (**1**–**5** and sphingomyelin). They reported that **1** bound to rgp120 strongly, **2**–**4** were less efficient binders, and **5** was inactive. More recently, McReynolds et al.[10] found that rgp120 binds to **1**, **3**, and **4**, with **4** being the preferred receptor. In contrast, Hammache et al. have reported minimal binding to **3** and **4**.[11]

It is evident that considerable confusion exists regarding the relative affinities of gp120 for GalCer and similar lipids. Furthermore, quantitative studies of gp120–glycolipid binding have not been performed, and thus the structural variations among these GSLs that are responsible for the apparent differences in affinity for gp120 are not known. The confusion may be in part a result of the inherent limitations of the binding assays used to study gp120–GSL interactions to date. Employing a heterogeneous solid-phase assay (for example, ELISA) to quantitatively characterize protein ligand–receptor binding processes that occur in vivo at the extracellular surface of a plasma membrane is fraught with difficulties. Although such methods are useful in screening for such interactions, proper geometric presentation of a mem-

[*] Prof. Dr. S. S. Saavedra, Dr. J. C. Conboy, Dr. K. D. McReynolds, Prof. Dr. J. Gervay-Hague
Department of Chemistry
University of Arizona
Tucson, AZ 85721-0041 (USA)
Fax: (+1) 520-621-8407
E-mail: saavedra@u.arizona.edu

[**] This research was supported by the NIH (AI40359-02), the NSF (CHE-9726132 and CHE-9623583), Eli Lilly (JGH), and the Alfred P. Sloan Foundation (J.G.H.). K.D.M. gratefully acknowledges receipt of the University of Arizona Dean's Fellowship and the Department of Chemistry Carl S. Marvel Fellowship. We thank Ying-Mei Gu for performing the streptavidin adsorption measurements.

  1433-7851/00/3916-2882 $ 17.50+.50/0