Synthesis of a New Trialdehyde Template for Molecular Imprinting

Deborah C. Tahmassebi and Tomikazu Sasaki*

*Department of Chemistry, University of Washington, Seattle, Washington 98195*

A recent approach in the area of molecular recognition involves template-assisted imprinting[1] of the surface of rigid polymers such as silica gel. Siloxy-Schiff bases of structurally rigid dialdehydes have been used as templates to introduce two appropriately spaced amino groups for substrate binding on the solid surface. The distance between the two amino groups is defined by the structure of the dialdehydes. Moderate selectivity has been reported in rebinding of the dialdehydes to the modified surface.[2] Current objectives in this quest involve the development of multidentate template molecules to expand the repertoire of functional groups available at the binding site.[3] We report herein the synthesis of a trialdehyde and its Schiff bases as a new template for molecular imprinting of solid surfaces.

The reaction of cyanuric chloride with 3 equiv of *p*-hydroxybenzaldehyde in benzene gave the desired trialdehyde, TRIPOD (1) (Scheme 1), in a single step. Trialdehyde 1 was then reacted at room temperature with (aminopropyl)triethoxysilane (APTES) or *p*-(methoxy-dimethylsilyl)aniline (MODSA)[4] to afford the corresponding silane–TRIPODs, APTES TRIPOD (2), and MODSA TRIPOD 4 (Scheme 2), respectively. Model compounds PROPYL TRIPOD (3) and ANILINE TRIPOD (5) were also synthesized for future mechanistic studies of Schiff base formation. Acid hydrolysis of all the Schiff bases regenerated the intact TRIPOD.

Molecular imprinting was carried out with silane–TRIPOD molecules 2 and 4 on silica gel to yield modified silicas 7 and 8, respectively. Porous silica gel, Fractosil 500, was refluxed in dry toluene for 2 days[2a] with an appropriate amount of the silane–TRIPODs to get a low substitution level on the silica and ensure site isolation (Scheme 3). Initial experiments with APTES TRIPOD (2) indicated that a partial degradation of 2 took place during the imprinting reaction. Refluxing of the model compound 3 in toluene for 2 days produced *p*-hydroxy-benzaldehyde, suggesting that the degradation was caused by the partial hydrolysis of the Schiff base and subsequent attack on the ether linkage by the amine. On the other hand, MODSA TRIPOD (4) did not decompose, presumably due to the greater strength of the aromatic Schiff
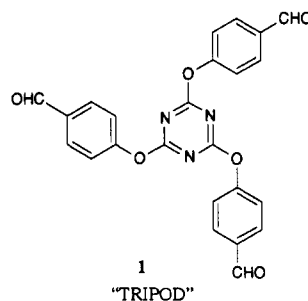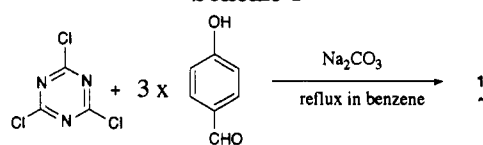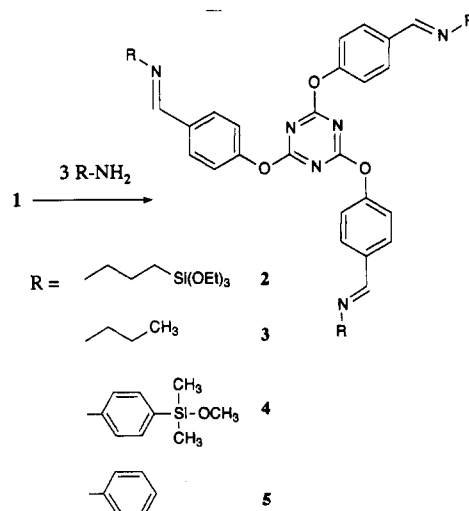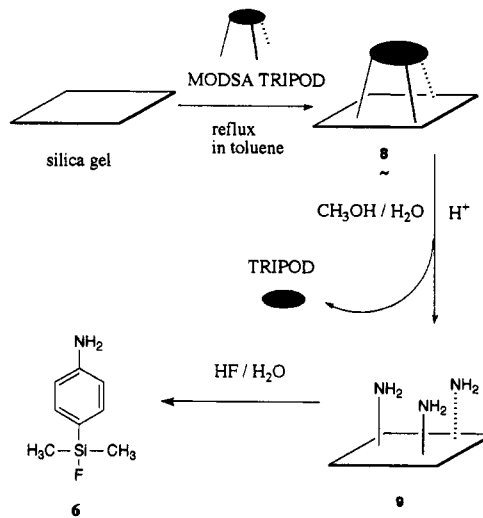
(1) (a) Wulff, G. In *Polymeric Reagents and Catalysts*; Washington DC, 1986; p 186. (b) Pirkle, W. H.; Pochapsky, T. C. *Chem. Rev.* **1989,** *89,* 347.

(2) (a) Wulff, G.; Heide, B.; Helfmeier, G. *React. Polym.* **1987,** *6,* 299. (b) Wulff, G.; Heide, B.; Helfmeier, G. *J. Am. Chem. Soc.* **1986,** *108,* 1089. (c) Shea, K. J.; Dougherty, T. K. *J. Am. Chem. Soc.* **1986,** *108,* 1091. (d) Tao, Y.-T.; Ho, Y.-H. *J. Chem. Soc., Chem. Commun.* **1988,** 417.

(3) (a) Sellergren, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988,** *110,* 5853. (b) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1989,** *111,* 3442. (c) Wulff, G.; Minarik, M. *J. Liquid Chromatogr.* **1990,** *13,* 2987. (d) Wulff, G.; Dhal, P. K. *Macromolecules* **1990,** *23,* 4525. (e) Wulff, G.; Wu, Y. *Macromol. Chem.* **1990,** *191,* 2993. (f) Wulff, G.; Wu, Y. *Macromol. Chem.* **1990,** *191,* 3005. (g) Wulff, G.; Schauhoff, S. *J. Org. Chem.* **1991,** *56,* 395. (h) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1991,** *113,* 4109. (i) Dhal, P. K.; Arnold, F. H. *J. Am. Chem. Soc.* **1991,** *113,* 7417.

(4) Weisenfeld, R. B. *J. Org. Chem.* **1986,** *51,* 2434.

Scheme 1

Scheme 2

Scheme 3

base linkage as well as the poor nucleophilicity of aniline compared to alkylamines. TRIPOD was released from the modified silica gel, 8, by aqueous acid hydrolysis to yield silica gel with free amines on the surface, 9. The integrity of the recovered TRIPOD was confirmed by TLC and HPLC. No *p*-hydroxybenzaldehyde was detected in the hydrolysis products. The amine-modified silica gel,

9, was treated with aqueous hydrogen fluoride[5] to analyze the surface-bound molecules. GC-MS analysis of the product indicated that 4-(fluorodimethylsilyl)aniline was released by the treatment, confirming the attachment of the dimethylsilylaniline group on the silica surface.

In conclusion, this note illustrates the synthesis of a new tridentate template for molecular imprinting of silica surface. The imprinted surface provides a site of three organized amino groups which can be further modified with various functional groups. Aldehyde-modified peptides can, for example, be attached to assemble protein monolayers on the surface. Molecular modeling showed the average distance between two formyl carbons in TRIPOD to be approximately 10 Å, which is reported[6] to be an appropriate spacing for the construction of 3-helix bundle proteins. Reaction of cyanuric chloride with different hydroxy aldehydes would give structurally diverse template molecules. A series of such templates should prove to be useful in creating geometrically well-defined sites to which various functional groups can be attached to construct specific substrate binding sites on a solid surface.

Experimental Section

All reagents were obtained from commercial suppliers and used without further purification. MeOH was freshly distilled. Fractosil 500 was purchased from EM Science. *p*-(Methoxy-dimethylsilyl)aniline was synthesized according to the published procedure:[4] IR (neat) ν 3461, 3357, 3226, 2956, 2831, 1621, 1590, 1506, 1071, 838 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.34 (s, 6H), 3.41 (s, 3H), 3.75 (s, 2H), 6.70 (d, 2H, J = 8.3 Hz), 7.37 (d, 2H, J = 8.3 Hz); ^{13}C NMR (CDCl$_3$ + DMSO) δ -2.3, 49.6, 113.3, 133.6, 134.2, 149.8; MS(GC) m/e = 181 (M$^+$).

^{1}H NMR spectra were recorded on a Bruker AF-300 at 300 MHz and on a Bruker AC-200 at 200 MHz using TMS as internal standard. ^{13}C NMR spectra were recorded on a Bruker AF-300 at 75 MHz using TMS as internal standard. Low-resolution mass spectra were recorded on a KRATOS Profile HV3 mass spectrometer in GC mode using electron ionization (EI). High-resolution fast atom bombardment (FAB) mass spectra were recorded on a VG70SEQ double-focusing mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR.

2,4,6-Tris(*p*-formylphenoxy)-1,3,5-triazine, TRIPOD (1). *p*-Hydroxybenzaldehyde (1.6 g, 1.31 × 10^{-2} mol (1.3× excess)) and cyanuric chloride (0.6 g, 3.25 × 10^{-3} mol) were added to a suspension of Na$_2$CO$_3$ (10 g) in 50 mL of benzene. The mixture was refluxed for 20 h. The reaction mixture was then cooled and the solid was removed by filtration and washed with hot AcOEt twice. The filtrate was extracted with 10% Na$_2$CO$_3$ twice and with H$_2$O once. The organic layer was dried over anhydrous Na$_2$SO$_4$ and then concentrated. The white powder was recrystallized from 20 mL of AcOEt to afford 0.87 g of a white fluffy precipitate (61%): IR (KBr) ν 2833, 1702, 1567, 1361, 1211, 842 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.32 (d, 6H, J = 8.5 Hz), 7.92 (d, 6H, J = 8.5 Hz), 10.00 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 122.2, 131.3, 134.5, 155.7, 173.2, 190.6; MS(FAB) m/e = 442 (M$^+$ + 1). Anal. Calcd for C$_{24}$H$_{15}$N$_3$O$_6$: C, 65.31; H, 3.43; N, 9.52. Found: C, 65.00; H, 3.43; N, 9.49.

APTES TRIPOD (2). A solution of 1 (0.018 g, 4.17 × 10^{-5} mol) in 1 mL of CHCl$_3$ was added to a solution of APTES (0.028 g, 1.25 × 10^{-4} mol) in 1 mL of CHCl$_3$, and the mixture was stirred for 1 h at room temperature over 3-Å molecular sieves. The reaction mixture was then concentrated and applied to a Sephadex LH-20 column (1.1 cm × 8.5 cm) that had been equilibrated in CHCl$_3$ to remove a minor colored impurity. The column was eluted with CHCl$_3$, and the major fractions were collected. Evaporation of the solvent yielded a yellow oil, 2 (0.033 g, 75%): IR (neat) ν 2974, 1646, 1574, 1392, 1387, 1080, 957 cm^{-1}; ^{1}H NMR

(CDCl$_3$) δ 0.67 (t, 6H, J = 8.3 Hz), 1.23 (t, 27H, J = 6.9 Hz), 1.83 (m, 6H), 3.62 (t, 6H, J = 6.7 Hz), 3.82 (q, 18H, J = 6.8 Hz), 7.19 (d, 6H, J = 8.6 Hz), 7.74 (d, 6H, J = 8.6 Hz), 8.25 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 8.0, 18.3, 24.2, 58.4, 64.1, 121.7, 129.4, 160.1, 173.5; MS(FAB) m/e = 1052 (M$^+$ + 1).

PROPYL TRIPOD (3). The synthesis was identical to 2 using *n*-propylamine instead of APTES: yield 69%, yellow oil; IR (neat) ν 2961, 2931, 1646, 1571, 1367, 1208, 842 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.95 (t, 9H, J = 7.3 Hz), 1.74 (m, 6H), 3.59 (t, 6H, J = 6.7 Hz), 7.18 (d, 6H, J = 8.4 Hz), 7.74 (d, 6H, J = 8.4 Hz), 8.24 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 11.8, 24.0, 63.4, 121.6, 129.3, 131.3, 159.5, 159.6 ; MS(FAB) m/e = 565 (M$^+$ + 1).

MODSA TRIPOD (4). A suspension of 1 (0.010 g, 2.3 × 10^{-5} mol) in 500 μL of MeOH was added to a solution of *p*-(methoxydimethylsilyl)aniline[4] (0.012 g, 6.9 × 10^{-5} mol) in 500 μL of MeOH, 2.5 μL of 1 N HCl /MeOH (2.5 × 10^{-6} mol), and 3-Å molecular sieves. The mixture was stirred at room temperature for 1 h. After 10 min, the suspension became thicker as a white fluffy precipitate formed. IR indicated the disappearance of aldehyde. The mixture was centrifuged and the solvent was pipetted off. The product was washed with MeOH. After being dried under vacuo, a white solid, 4, was obtained (0.020 g, 94%): IR (KBr) ν 2958, 1571, 1382, 1362, 1210, 840 cm^{-1}; ^{1}H NMR (CDCl$_3$ + DMSO) δ 0.36 (s, 18H), 3.40 (s, 9H), 7.21 (d, 6H, J = 7.7 Hz), 7.36 (d, 6H, J = 8.0 Hz), 7.55 (d, 6H, J = 7.7 Hz), 7.98 (d, 6H, J = 8.0 Hz), 8.57 (s, 3H); ^{13}C NMR (CDCl$_3$ + DMSO) δ -2.3, 50.1, 120.3, 121.84 129.9, 134.2, 152.6, 153.6, 159.5, 173.0; MS(FAB) m/e = 931 (M$^+$ + 1).

ANILINE TRIPOD (5). The synthesis was identical to 4 using aniline instead of MODSA: yield >95%, white solid; IR (KBr) ν 3133, 1569, 1503, 1373, 1206, 844 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.24 (m, 9H), 7.39 (m, 6H), 7.41 (d, 6H, J = 8.7 Hz), 7.99 (d, 6H, J = 8.7 Hz), 8.61 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 120.9, 121.9, 126.1, 129.2 130.1, 158.8; MS(FAB) m/e = 667 (M$^+$ + 1). Anal. Calcd for C$_{42}$H$_{30}$N$_6$O$_3$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.29; H, 4.57; N, 12.56.

p-(Fluorodimethylsilyl)aniline (6). A 50% aqueous HF solution (50 μL) was added to *p*-(methoxydimethylsilyl)aniline (13 mg, 2.8 × 10^{-5} mol) dissolved in 1 mL of CH$_3$CN, and the mixture was stirred at room temperature for 30 min. After 30 min chloroform (1 mL) and 20% NaHCO$_3$ (1 mL) were added. The organic layer was separated and washed twice with H$_2$O. The organic layer was dried with MgSO$_4$ and then evaporated to yield a yellow oil, 6 (8.5 mg, 70%): IR (neat) ν 3473, 3385, 2960, 1622, 1600, 1122, 843 cm^{-1}; ^{1}H NMR (DMSO) δ 0.42 (d, 6H, J = 7.3 Hz), 3.91 (br s, 2H), 6.69 (d, 2H, J = 8.3 Hz), 7.38 (d, 2H, J = 8.3 Hz); ^{13}C NMR (DMSO) δ -1.2 (d, J = 16.4 Hz), 114.5, 129.2, 134.6, 148.5; MS(GC) m/e = 169 (M$^+$).

Molecular Imprinting of Fractosil 500[2a] with MODSA TRIPOD 4 to give 8. In order to increase the concentration of surface hydroxyls, Fractosil 500 was refluxed in 5% HNO$_3$ for 1 h and then washed with H$_2$O thoroughly and dried in a vacuum oven (120 °C) for approximately 4 h before use. Into a suspension of oven-dried Fractosil 500 (2.0 g) in 10 mL of dry toluene was added MODSA TRIPOD (4) (12.0 mg, 1.3 × 10^{-5} mol) dissolved in a few drops of dry CHCl$_3$. The suspension was refluxed in a nitrogen atmosphere. After 48 h, the mixture was filtered and the silica was washed thoroughly with toluene and then CH$_2$Cl$_2$ and dried under vacuum at room temperature overnight. The modified silica gel, 8, was stirred in a solution of 4 μL of 5 N HCl/MeOH in 2 mL of 50% MeOH to remove TRIPOD and leave silica gel with free amines on the surface, 9. After 3 h, the silica gel was filtered, washed with MeOH, H$_2$O, 20% NaHCO$_3$, and then H$_2$O again, and dried under vacuum. The recovered TRIPOD co-eluted with an authentic sample of 1 by reverse phase HPLC (Vydac C4 analytical, flow rate = 1.0 mL/min, CH$_3$-CN–H$_2$O containing 0.1% trifluoroacetic acid); t_R = 12.4 min with a linear gradient from 42% to 70% CH$_3$CN in 20 min. The substitution level of the modified silica gel was determined on the basis of the amount of TRIPOD recovered using naphthalene as internal standard.[7]

The modified silica gel, 9, (30 mg) was treated with 50% aqueous HF (50 μL) in 1 mL of CH$_3$CN. After 30 min, CHCl$_3$ (1 mL) and H$_2$O (1 mL) were added and the organic layer was

(5) Newton, R.; Reynolds D. *Tetrahedron Lett.* **1979**, *41*, 3981.
(6) Lieberman, M.; Sasaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 1470.

removed. The organic layer was washed twice with H_2O to remove traces of acid and then concentrated to approximately 5 μL. The

organic layer was then analyzed by GC-MS (splitless): *m/e* = 169 (M^+ for *p*-(fluorodimethylsilyl)aniline, **6**).

Supplementary Material Available: ^{1}H NMR spectra of 1–6 (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(7) (a) Three batches of modified silica gels were prepared with the following substitution levels; 16.7, 11.9, and 9.24 μmol amine/g. Direct quantification of aniline groups on the surface was also attempted with the 2-hydroxy-1-naphthaldehyde method[7b] after the TRIPOD removal. This method, however, gave significantly lower substitution levels, 2.99, 2.45, and 1.44 μmol amine/g, respectively, due in part to incomplete coupling of 2-hydroxy-1-naphthaldehyde to the modified surface. The MODSA–silica linkage is stable under the conditions used for TRIPOD removal since the second HCl treatment of the modified silica shows the same substitution levels. Since it is also possible that there is incomplete coupling of MODSA TRIPOD to the silica surface, work is in progress to further characterize the modified silica and to optimize the surface imprinting with MODSA TRIPOD. (b) Esko, K.; Karlsson, S. *Acta Chem. Scand.* **1970**, *24*, 1415.