# Affinity Mass Spectrometry from a Tailored Porous Silicon Surface

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## Supporting Information

**General.** DIOS-MS spectra were obtained on a PerSeptive Voyager STR time-of-flight mass spectrometer. A delayed extraction time of 50 nsec was used, and laser energy was slightly higher than is routinely used for MALDI. $^{1}H$ and $^{13}C$ NMR were recorded on a Varian Mercury-200 instrument with tetramethylsilane as an internal reference. ESI-MS was obtained on a Hewlett Packard 1100 LC-MS instrument. Infrared (FTIR) spectroscopic measurements were collected on a MIDAC FTIR spectrometer equipped with a diffuse reflectance accessory. All reagents for chemical synthesis were purchased from Sigma-Aldrich, Pierce and Acros. Flash chromatography was carried out using 200-400 mesh silica gel. Single polished n-type crystalline (100) silicon wafers, resistivities 0.005-0.02 and 0.5-2 Ω·cm, were purchased from Silicon Sense Inc. "PB" refers to 0.1 M potassium phosphate buffer, pH 7.4.

Porous silicon chips were prepared by electrochemical etching in a Teflon cell as described previously.[1] Briefly, a silicon wafer was cut to fit over the bottom of the Teflon etching cell chamber. A platinum wire positioned in the cell and a 0.1 mm thick gold foil placed under the silicon wafer was used as cathode and anode electrode, respectively. 24% w/v HF solution in absolute ethanol was added to the cell cavity. Two different sets of conditions, described in our previous paper,[2] give effective DIOS surfaces. Note that the higher-resistivity porous silicon chip contains a lower concentration of dopant and therefore can be examined by diffuse reflectance infrared spectroscopy, whereas the lower-resistivity wafers cannot.

**Preliminary experiments not shown in text.**

*Use of azide-alkyne cycloaddition to attach cleavable linker to the pSi surface (Figure S1).*

The 1,3,5-triazine unit is used to boost the detection efficiency of attached species, since triazines themselves are easily ionized in DIOS. To demonstrate the use of a click chemistry attachment method to porous silicon, alkyne-functionalized triazine **12** was used in a mixture with **13** and **14** (Figure S1). Freshly etched porous silicon was exposed to ozone for 30-60 sec, which has been shown independently to accomplish complete oxidation of the surface silicon hydride sites. The oxidized chip was heated with 20 mM 3-aminopropyldimethylethoxysilane in toluene at 60 $^{o}$C for 10 min and washed with toluene to give **10**. This material shows surface hydrophilic behavior consistent with the presence of amino groups,[2] The chip was then reacted with 40 mM 4-pentynoyl chloride in $CH_2Cl_2$ to give alkyne-coated pSi surface **11**. A representative diffuse-reflectance IR spectrum of such a chip prepared from the higher-resistivity (0.5-2 Ω·cm) pSi is shown at the bottom of Figure S1, showing the alkyne and amide carbonyl stretching vibrations at 2251 $cm^{-1}$ and 1649 $cm^{-1}$, respectively, and the broad silicon oxide band at 1105 $cm^{-1}$.

As reported earlier for materials derived from hydrosilylative attachment to freshly etched pSi,[3] the surface alkyne was addressed by copper-catalyzed [3+2] cycloaddition to azide **12**, giving **15**.[4] Thus, alkyne-coated chips **11** were incubated with a mixture of **12**, **13**, and **14** (20 μM each) in 5 mL solutions of 1:1 (v:v) acetonitrile:Tris buffer (pH 8) containing 12 μM copper sulfate and 24 μM L-ascorbic acid as catalyst. The reactions were allowed to run at room temperature for 12 hours in closed vials. The resulting silicon chips were subjected to DIOS-MS analyses before and after washing with acetonitrile, water and

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[1] Shen, Z.; Thomas, J. J.; Averbuj, C.; Broo, K. M.; Engelhard, M.; Crowell, J. E.; Finn, M. G.; Siuzdak, G. *Anal. Chem.* **2001**, *73*, 612-619.

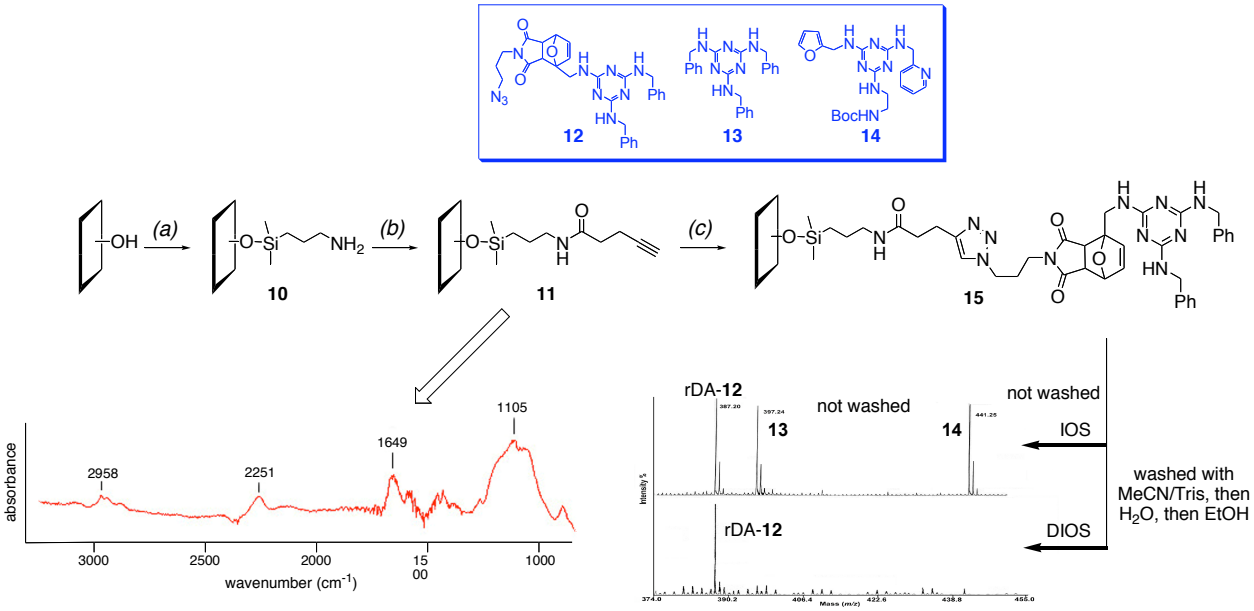
[2] Go, E.P.; Apon, J.V.; Credo, G.M.; Compton, B.J.; Finn, M.G.; Shen, Z.; Siuzdak, G., manuscript in preparation.

[3] Meng, J.-C.; Averbuj, C.; Lewis, W. G.; Siuzdak, G.; Finn, M. G. *Angew. Chem. Int. Ed.* **2004**, *43*, 1255-1260.

[4] (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599. (b) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192-3193.

ethanol. After washing, only rDA-**12** appeared in the DIOS-MS spectrum. Oxidized but underivatized pSi gave no signal in DIOS-MS after identical treatment with the **12**+**13**+**14** mixture. The azide-alkyne reaction represents the current best example of "click chemistry",[5] and the present application, which marries fragments at 20 μM concentration to a surface residue using 12 μM Cu(I) catalyst, is among the most impressive examples reported to date.

**Figure S1**. Covalent attachment and detachment to oxidized pSi chips for DIOS mass spectrometry. (a) $(EtO)Me_2Si(CH_2)_3NH_2$, toluene, 60 °C, 10 min; (b) 4-pentynoyl chloride, (c) **12**+**13**+**14** (20 μM each), $CuSO_4$ (12 μM), L-ascorbic acid (24 μM), MeCN-Tris (1:1, pH 8). The diffuse-reflectance IR spectrum of **11** (made with silicon of 0.5-2 Ω·cm resistivity) is shown at the lower left.



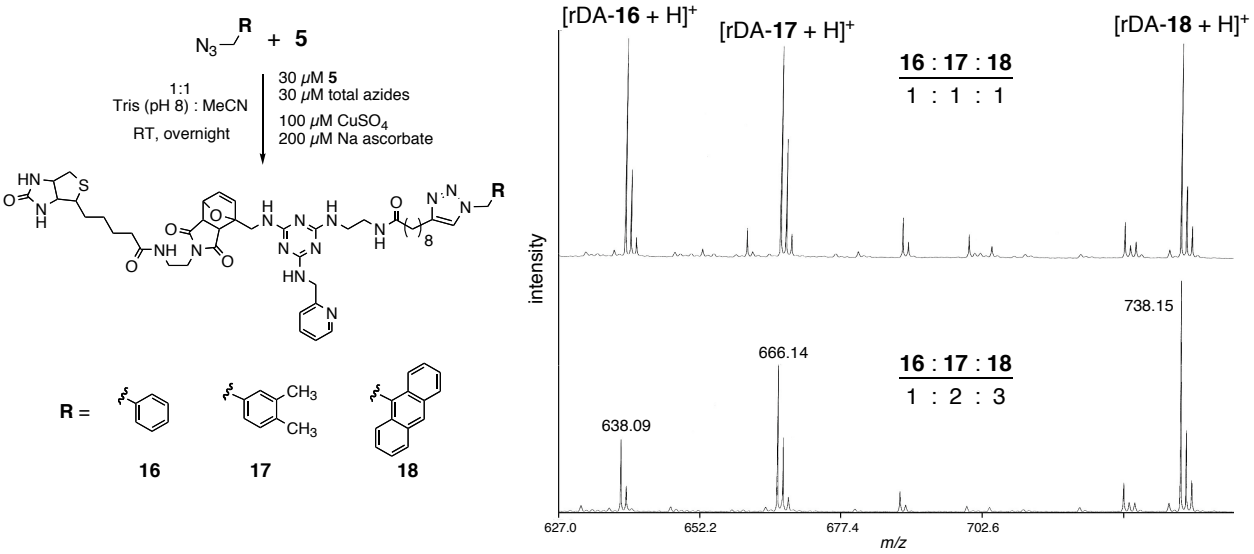

*Use of azide-alkyne cycloaddition to attach to biotin (Figure S2).*

To verify the capture of a compound in solution by click chemistry connection to biotin and then selective adsorption to an avidin DIOS surface, alkyne **5** was treated with mixtures of three azides in varying molar ratios and the copper(I) catalyst in a buffered aqueous-organic solvent (Figure S2). To a vial containing 30 μM **5** and 30 μM total azide (two mixtures of **16**:**17**:**18** were used, in molar ratios of 10:10:10 or 5:10:15 μM, respectively) in 10 mL Tris:MeCN (1:1, pH=8) was added 100 μM copper sulfate and 200 μM sodium L-ascorbate. After stirring overnight, 10 μL of each solution was deposited on chip **4** and incubated for 30 min at room temperature. After washing with PB:MeCN (3:1) and then water to remove the non-adsorbed species, the chips were subjected to DIOS-MS.

The resulting spectra showed three strong peaks corresponding to the retro-Diels-Alder release of the expected three triazoles derived from azide-alkyne cycloaddition. Furthermore, the relative intensities of the peaks tracked roughly with the differing ratios of azide reactants employed, suggesting that the bond-forming reaction, avidin-biotin binding, and rDA cleavage all proceed with equivalent (and presumably high) efficiencies for the three compounds.

[5] Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

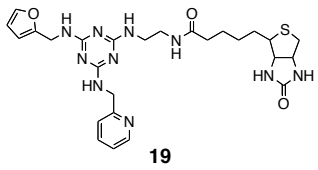
**Figure S2**. *Left*: reaction scheme for azide-alkyne cycloaddition of **5** with mixtures of the indicated three azides to give triazoles **16**–**18**. *Right*: DIOS spectra of **16**–**18** deposited on **4** for 20 min, and then washed with 3:1 PB:MeCN. The indicated ratios of **16**:**17**:**18** are the ratios of the starting azides used in the cycloaddition reaction.

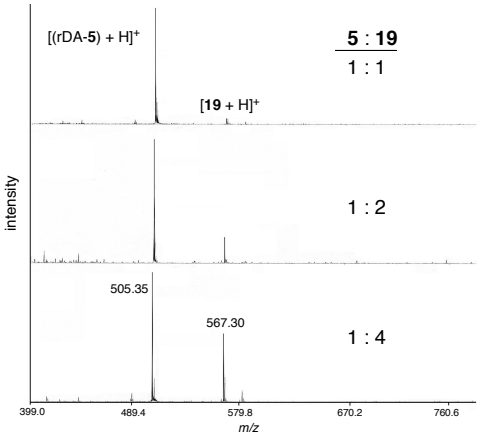



*Sensitivity enhancement by retro-Diels-Alder detachment (Figure S3).*

The detachment of triazine **5** from avidin-coated chips **4** by cycloreversion was tested against an analogous compound (**19**) lacking the Diels-Alder adduct moiety. 10 μL solutions containing **5** and **19** (in concentrations of 1:1, 1:2, and 1:4 μM, respectively) in MeCN:PB (1:3) were deposited on avidin-coated chip **4** and incubated for 30 min at room temperature. The chip was rinsed with MeCN:PB (1:3) solution, dried gently with nitrogen, and subjected to DIOS-MS analyses. Note that the 1:1 mixture of the triazines gave approximately 15 times stronger signal for **5**, and a four-fold excess of **19** was required to obtain a peak intensity approximately half that of **5**.

**Figure S3**. DIOS-MS of avidin-coated chips (**4**) incubated with varying ratios of **5** and **19** for 20 min, followed by thorough washing with 3:1 PB:MeCN, then water.

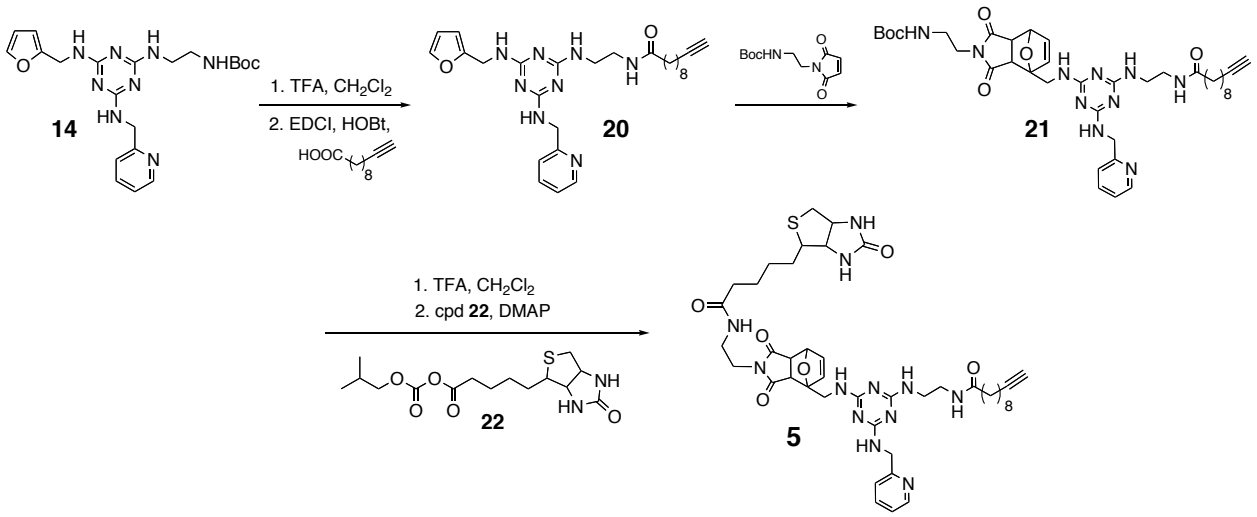



**Syntheses.**

**Triazine 5**. To a solution of **14** (880 mg, 2 mmol) in 10 mL $CH_2Cl_2$ was added 2 mL trifluoroacetic acid, and the reaction mixture was stirred at room temperature for 30 min. After removal of the solvent and TFA under high vacuum, the residue was dissolved in 2 mL dry DMF. This solution was added to a mixture containing hydroxybenzotriazole (HOBt, 405 mg, 3 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 576 mg, 3 mmol) and undec-10-ynoic acid (364 mg, 2 mmol) at 0 $^{o}$C. The reaction

was stirred at room temperature for 12 hours and then quenched with 5 mL saturated $Na_2CO_3$ solution. The mixture was extracted three times with EtOAc and the combined extract was washed with water, brine, and dried over $Na_2SO_4$. After removal of the solvent, the residue was chromatographed on silica gel to yield **20** as a white solid (858 mg, 85%). $^1$H NMR ($CDCl_3$, δ) 1.25 (br s, 10H), 1.49 (m, 4H), 1.93 (t, *J* = 2.6 Hz, 1H), 2.13 (m, 2H), 3.42 (m, 2H), 3.51 (m, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 4.68 (br s, 2H), 6.21 (br s, 1H), 6.30 (br s, 1H), 7.20 (m, 1H), 7.28 (d, *J* = 7.0 Hz, 1H), 7.33 (s, 1H), 7.65 (t, *J* = 7.0 Hz, 1H), 8.54 (br s, 1H). $^{13}$C NMR ($CDCl_3$, δ) 18.6, 25.9, 28.6, 28.9, 29.1, 2×29.4, 36.7, 37.9, 40.2, 40.7, 46.1, 68.5, 84.9, 107.1, 110.6, 2×122.4, 137.0, 141.9, 149.1, 152.6, 158.6, 3×165.0, 174.2. ESI-MS: m/z 505.3 ($M+H^+$) [M = $C_{27}H_{36}N_8O_2$, exact mass 504.30].




A mixture of Boc-aminoethylmaleimide (600 mg, 2.5 mmol) and **20** (1.0 g, 2 mmol) in 20 mL toluene was stirred at room temperature overnight. The organic solvent was evaporated and the residue was subjected to flash column chromatography (silica gel 200-400 mesh, eluting with EtOAc) to yield **21** as a white solid (891 mg, 60%). $^1$H NMR ($CDCl_3$, δ) 1.26 (br s, 10H), 1.41 (s, 9H), 1.53 (m, 4H), 1.94 (t, *J* = 2.6 Hz, 1H), 2.07-2.18 (m, 4H), 2.96 (m, 2H), 3.40 (s, 4H), 3.41 (s, 2H), 3.48 (m, 2H), 4.67 (s, 2H), 5.21 (s, 1H), 6.38 (m, 1H), 6.50 (m, 1H), 7.18 (t, *J* = 6.8 Hz, 1H), 7.33 (br d, *J* = 7.0 Hz, 1H), 7.65 (t, *J* = 7.2 Hz, 1H), 8.52 (br s,1H). $^{13}$C NMR ($CDCl_3$, δ) 3×18.5, 25.0, 28.5-28.9 (8 overlapping peaks), 36.8, 38.5, 38.9, 39.6, 46.1, 48.5, 50.5, 68.5, 79.5, 80.9, 84.9, 91.4, 2×122.4, 136.9, 2×139.0, 149.2, 156.1, 158.9, 166 (3 overlapping peaks), 174.0, 175.5, 173.3. ESI-MS: m/z 745.3 ($M+H^+$) [M = $C_{38}H_{52}N_{10}O_6$, exact mass 744.4071].

To a solution of **21** (745 mg, 1 mmol) in 10 mL $CH_2Cl_2$ was added 1.5 mL TFA and the reaction mixture was stirred at room temperature for 30 min. After removal of volatiles under high vacuum, the residue was dissolved in 2 mL dry DMF. A separate solution of 1.5 mmol biotin and 2 mmol diisopropylethylamine (300 μL) in 10 mL dry DMF was stirred at 0 °C. With vigorous further stirring, isobutylchloroformate (259 μL, 2 mmol) was added slowly by syringe. After stirring for 1h, the crude mixed anhydride **22** was treated with *N*,*N'*-4-dimethylaminopyridine (DMAP, 122 mg, 1 mmol) and the deprotected **21** in 2 mL dry DMF, added in dropwise fashion. The reaction was kept at room temperature for 30 min and quenched with 1 mL water. After removal the solvent, the residue was chromatographed on silica gel using $CH_2Cl_2$-MeOH as eluent to yield **5** as a white foam (626 mg, 72%). $^1$H NMR ($D_2O$, δ) 1.27 (br s, 10H), 1.41 (m, 4H), 1.58 (m, 4H), 2.11-2.19 (m, 7H), 2.20 (d, *J* = 12.6 Hz, 1H), 2.92 (dd, *J* = 8.8, 12.6 Hz, 1H), 3.18 (br s, 2H), 3.4-3.6 (m, 5H), 3.60 (s, 2H), 3.9-4.0 (m, 2H), 4.34 (m, 2H), 4.65 (br s, 4H), 5.14 (s, 1H), 6.42 (m, 1H), 6.53 (br s, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.43 (br d, *J* = 7.3 Hz, 1H), 7.78 (dt, *J* = 1.2, 7.4 Hz, 1H), 8.45 (br s, 1H). $^{13}$C NMR ($D_2O$, δ) 19.2, 26.7, 27.2, 8×29.9, 36.9, 37.4, 38.2, 39.6, 40.8, 41.1, 41.3, 46.9, 50.5, 51.9, 57.1, 61.8, 63.5, 69.7, 82.2, 85.4, 92.5, 122.9, 123.7, 138.5, 139.0, 139.7, 149.7, 160.1, 166.3, 3×167.5, 176.6, 2×177.5, 178.6. ESI-MS: m/z 871.5 ($M+H^+$) [M = $C_{43}H_{58}N_{12}O_6S$, exact mass 870.4323].

**Triazine 12** (Figure S1). Compound **12** was prepared by the procedure described in our previous paper.[1] Briefly, a mixture of 3-chloropropylmaleimide (347 mg, 2 mmol) and dibenzyl-furfuryl-[1,3,5]triazine-2,4,6-triamine (772 mg, 2 mmol) in 10 mL toluene was stirred at room temperature overnight. The organic solvent was evaporated and the residue subjected to flash silica gel column chromatography eluting with hexanes:EtOAc to yield a yellow gum (557 mg, 50%), $^1$H NMR ($CDCl_3$, δ) 1.86 (br s, 1H), 2.02 (t, *J* = 6.6 Hz, 1H), 2.87 (s, 2H), 3.39 (t, *J* = 6.6 Hz, 2H), 3.36 (m, 1H), 3.44 (t, *J* = 6.6 Hz, 2H), 3.60 (m, 1H), 4.53 (s, 4H), 5.10 (s, 1H), 6.25 (br s, 2H), 7.26 (br s, 10H-Ar). ESI-MS *m/z* 560.1 ($M+H^+$). Then the resulting gum was dissolved in 10 mL DMF, and sodium azide (650 mg, 10 mmol) was added. The reaction mixture was stirred at room temperature overnight followed by addition of 80 mL water. The mixture was extracted with ethyl acetate three times. Evaporation of organic solvent afforded **12** as a white solid (550 mg, 97%). $^1$H NMR ($CDCl_3$, δ) 2.19 (br s, 2H), 2.92 (s, 2H), 3.38 (t, *J* = 6.6 Hz, 2H), 3.42-3.45 (m, 2H), 3.58 (t, *J* = 6.6 Hz, 2H), 4.55 (s, 4H), 5.16 (s, 1H), 6.30 (br s, 2H), 7.26 (br s, 10H-Ar). $^{13}$C NMR ($CDCl_3$, δ) 27.3, 39.8, 40.4, 44.7, 49.0, 49.4, 50.6, 80.9, 91.6, 127.3, 127.7, 128.7, 136.6, 139.2, 139.9, 166.4, 175.5, 176.2. ESI-MS: m/z 567.2 ($M+H^+$).

**Triazine 13.** A mixture of cyanuric chloride (925 mg, 5 mmol) and benzylamine (3.21 g, 30 mmol) in 30 mL THF was refluxed overnight. The organic solvent was removed *in vacuo* and the residue was dissolved in 70 mL ethyl acetate, and washed successively with $KHSO_4$ (1 M, 30 mL), water (20 mL), 5% $NaHCO_3$ (10 mL) and brine. Evporation of the organic solvent under reduced pressure and drying over $P_2O_5$ afforded **13** as a white solid (1.77 g, 90%). $^1$H NMR ($CDCl_3$, δ) 4.53 (br d, *J* = 5.2 Hz, 6H), 7.26 (s, 15H); $^{13}$C NMR ($CDCl_3$, δ) 44.8, 127.3, 127.7, 128.7, 139.8, 166.5; ESI-MS: *m/z* 397.1 ($M+H^+$) [M = $C_{24}H_{24}N_6$, exact mass 396.21].

**Triazine 14.** A solution of cyanuric chloride (9.25 g, 50 mmol) in 80 mL acetone was added to 120 mL ice-water with vigorous stirring. A mixture of furfurylamine (4.85 g, 50 mmol) and $NaHCO_3$ (4.20 g, 50 mmol) in 80 mL of a acetone/water solution (1:1, v/v) was then added slowly. The reaction mixture was stirred at 0 °C for three hours. The resulting white precipitate was filtered, rinsed with water, and dried *in vacuo* to give furfuryl-4,6-dichloro-[1,3,5]triazine-2-amine as a white solid (11.3 g, 93%). $^1$H NMR ($CDCl_3$, δ) 4.68 (d, *J* = 5.8 Hz, 2H), 6.31-6.34 (m, 2H), 7.37 (d, *J* = 0.8 Hz, 1H); ESI-MS: m/z 245.0 ($M+H^+$) [M = $C_8H_6Cl_2N_4O$, exact mass 243.99].

To a 50 mL flask containing mono-Boc protected ethylenediamine (480 mg, 3 mmol) in 20 mL water was added a solution of furfuryl-4,6-dichloro-[1,3,5]triazine-2-amine (610 mg, 2.5 mmol) in 15 mL acetone at room temperature. The reaction mixture was heated at 60 °C for 2 hours. After the reaction mixture was cooled to room temperature, the resulting white precipitate was filtered, rinsed with water, and dried *in vacuo* to give Boc-aminoethyl-furfuryl-6-chloro-[1,3,5]triazine-2,4-diamine as a white solid, (864 mg, 94%). $^1$H NMR ($CDCl_3$, δ) 1.43 (s, 9H), 3.32 (m, 2H), 3.54 (m, 2H), 4.60 (d, *J* = 5.6 Hz, 2H), 6.24 (br s, 1H), 6.32 (br s, 1H), 7.36 (s, 1H). ESI-MS: m/z 369.1 ($M+H^+$) [M = $C_{15}H_{21}ClN_6O_3$, exact mass 368.14].

A solution of this triazine (736 mg, 2 mmol) and 2-aminomethylpyridine (1.08 g, 10 mmol) in 20 mL THF was refluxed for 6 hours. The organic solvent was evaporated and the residue was dissolved in 20 mL ethyl acetate, and then washed with $KHSO_4$ (1 M, 5 mL), water (10 mL), 5% $NaHCO_3$ (5 mL) and brine. Evaporation of the solvent and drying over $P_2O_5$ *in vacuo* afforded **14** (792 mg, 90%). $^1$H NMR ($CDCl_3$, δ) 1.41 (s, 9H), 3.28 (m, 2H), 3.45 (m, 2H), 4.56 (d, *J* = 5.8 Hz, 2H), 4.66 (br s, 2H), 6.19 (br s, 1H), 6.29 (br s, 1H), 7.13 (t, *J* = 6.6 Hz, 1H), 7.31 (d, *J* = 7.0 Hz, 1H), 7.36 (s, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 8.52 (br s, 1H). ESI-MS: m/z 441.2 ($M+H^+$) [M = $C_{21}H_{28}N_8O_3$, exact mass 440.23].

**Triazine 19:** To a solution of **14** (880 mg, 2 mmol) in 10 mL $CH_2Cl_2$ was added 2 mL TFA, and the reaction mixture was stirred at room temperature for 30 min. After removal of TFA under high vacuum, the residue was dissolved in 2 mL dry DMF. A solution of biotin (2 mmol) and diisopropylethylamine (380 μL, 2.2 mmol) in 10 mL dry DMF was stirred at 0 °C. With vigorous stirring, isobutylchloroformate (285 μL, 2.2 mmol) was added slowly via syringe. After stirring for 1h, DMAP (122 mg, 1 mmol) and the deprotected triazine residue in 2 mL dry DMF was added dropwise to the solution. The reaction was stirred for 30 min and quenched with 1 mL water. The solvent was evaporated and the product was purified by flash silica gel chromatography to give **19** as a white foam (560 mg, 50%). $^1$H NMR ($CDCl_3$, δ) 1.37 (m, 2H), 1.53 (m,

4H), 2.15 (m, 2H), 2.69 (d, *J* = 12.8 Hz, 1H), 2.90 (dd, *J* = 4.8, 12.7 Hz, 1H), 3.11 (m, 1H), 3.30 (m, 2H), 3.46 (m, 2H), 4.27 (dd, *J* = 4.4, 7.8 Hz, 1H), 4.45 (m, 1H), 4.48 (m, 2H), 4.65 (s, 2H), 6.30 (br s, 2H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.30 (br s, 1H), 7.43 (s, 1H), 7.77 (t, *J* = 7.2 Hz, 1H), 8.45 (d, *J* = 4.2 Hz, 1H). $^{13}$C NMR ($CDCl_3$, δ) 24.8, 27.4, 27.5, 34.9, 36.4, 38.7, 39.1, 44.6, 53.2, 54.9, 59.7, 61.3, 105.6, 109.3, 120.9, 121.5, 136.8, 140.9, 147.5, 152.4, 159.1, 4×166.5, 174.4. ESI-MS: m/z 567.2 ($M+H^+$) [M = $C_{26}H_{34}N_{10}O_3S$, exact mass 566.25].

***Manipulations in Figure 1.***

*(2-Pyridyldithiopropionamidopropyl)dimethylethoxysilane.* To a 50 mL flask containing 2,2'-bipyridyl disulfide (2.21g, 10 mmol) in 15 mL of ethanol was added 0.4 mL of glacial acetic acid at room temperature. A solution of 3-mercaptopropionic acid (0.53 g, 5 mmol) in 4 mL of ethanol was added dropwise with vigorous stirring. After stirring at room temperature overnight, the organic solvent was evaporated and the residue was subjected to column chromatography over neutral alumina. Elution with $CH_2Cl_2$:MeOH:AcOH (1:20:0.2) gave 2-pyridyl-2-carboxyethyl disulfide as a colorless oil (0.85 g, 80%). $^1$H NMR ($CDCl_3$, δ) 2.80 (t, *J* = 6.6 Hz, 2H), 3.07 (t, *J* = 6.7 Hz, 2H), 7.15 (m, 1H), 7.65 (m, 2H), 8.48 (d, *J* = 5.0 Hz, 1H).

This material was dissolved in 60 mL dichloromethane, and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (763 mg, 4 mmol) in 10 mL dichloromethane was added dropwise to the solution under a dry nitrogen atmosphere. After stirring for 30 min, 4-aminopropyldimethylethoxysilane (644 mg, 4 mmol) was added to the reaction mixture in dropwise fashion with vigorous stirring. The reaction was stirred at room temperature for 18 h, and then quenched with saturated $NaHCO_3$ solution. The organic layer was washed extensively with water and dried with anhydrous sodium sulfate. Evaporation of the organic solvent gave an oil which was further purified by silica gel column chromatography to yield the desired product (1.17 g, 81%). $^1$H NMR ($CDCl_3$, δ) 0.10 (s, 6H), 0.62 (t, *J* = 8.2 Hz, 2H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.58 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 3.11 (t, *J* = 6.6 Hz, 2H), 3.27 (dd, *J* = 7.0, 12.9 Hz, 2H), 3.65 (dd, *J* = 6.9, 13.0 Hz, 2H), 7.11 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.65 (m, 2H), 8.46 (d, *J* = 5.0 Hz, 1H). $^{13}$C NMR ($CDCl_3$, δ) 15.9, 20.6, 25.6, 36.9, 37.9, 44.5, 60.5, 111.8, 122.3, 123.0, 139.1, 151.6, 174.1. ESI-MS: m/z 359.0 ($M+H^+$) [M = $C_{15}H_{26}N_2O_2S_2Si$, exact mass 358.1205].

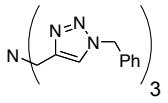
*Derivatization of the porous silicon.* An oxidized, silanol-terminated surface, obtained by exposing freshly etched samples to a stream of ozone for 30-60 sec, was immersed in a 10 mM toluene solution of (2-pyridyldithiopropionamidopropyl)dimethylethoxysilane and heated to reflux for 6 hours to give **1**. The material was washed with toluene and ethanol and subsequently incubated with 10 mM dithiothreitol (DTT) in 15% ethanol for 30 min at room temperature. After extensively rinsing with ethanol and PB, the chip was immersed in a 0.5 mg/mL solution of biotin-HPDP (**2**, obtained from Pierce) in MeCN:PB (1:1) for 1 h at room temperature and then washed (MeCN, MeCN:PB (1:1), and PB, in that order) to provide biotinylated surface **3**. Finally, avidin chips **4** were obtained by incubating biotin-covered surface **3** with 10 μM avidin in PB at room temperature for 1 hr, followed by washing with PB.

*Selective absorption of **5** on avidin-decorated pSi surface.* 5 μL of a solution containing **5** and the two peptides (ASTTTNYT and MKRSRGPSPRR, obtained from Sigma, 5 μM each) in MeCN:PB (1:3) was deposited on chip **4**. After incubation for 30 min at room temperature, the chip was rinsed three times with MeCN:PB (1:3) and water, and was then subjected to DIOS-MS analysis. As a control, a nonfunctionalized porous silicon chip was incubated with a mixture of **5** and the two peptides in same manner. DIOS-MS analyses showed none of these species after the same washing procedure.

***Procedure for in vitro active-site identification of GSTω protein (Figure 2).***

GSTω protein (40 μL of 4 mg/mL in PB, expressed in cos cells and kindly provided by A. Speers) was treated with **7** (10 μL of 100 μM stock solution in *t*-butanol) for 30-60 min at room temperature. The azide-labeled protein was diluted to 1 mL with deionized water and denatured at 90 °C for 30 min, and then digested with 80 μL 0.1 mg/mL trypsin at 37 °C for 12 hours. The solvent was removed at room temperature under vacuum, 40 μL water was added, and the mixture was briefly sonicated to mix.

Compound **5** was added (2.5 µL of a 1 mM stock solution in 1:1 *t*-butanol:water), followed by sodium L-ascorbate to (2.5 µL of 20 mM stock solution in deionized water, $CuSO_4$ (2.5 µL of 10 mM stock solution in deionized water) and tris(triazolyl)amine **22** (2.5 µL of 20 mM stock solution in 2:1 *t*-butanol:water), all added under nitrogen atmosphere. The mixture was allowed to react for 2 hours at room temperature in an inert-atmosphere drybox. After addition of 25 µL PB buffer (pH 7.4), the sample was briefly centrifuged and then 10 µL was removed from the top of the mixture and deposited onto an avidin-coated surface **4**. After incubation at room temperature for 30-60 min a wide-mouth jar containing a humid atmosphere, the chip was washed thoroughly with MeCN:PB (1:3, pH 7.4) and water, and then subjected to DIOS-MS analyses. For a heated control, GSTω protein was heated at 90 °C for 5 min prior to addition of **7**.




**22**