Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z 52803

69451 Weinheim, Germany

Cleavable Linkers for Porous Silicon-Based Mass Spectrometry

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General experimental details. DIOS-MS spectra were obtained on PerSeptive Biosystems Voyager DE and PerSeptive Voyager STR time-of-flight mass spectrometers. Typically, a delayed extraction time of 50 nsec was used, and the laser intensity was slightly higher than is usually used for MALDI. ^{1}H and ^{13}C NMR were recorded on a Varian Mercury-200 instrument in $CDCl_3$ with tetramethylsilane as an internal reference. ESI-MS was obtained on Hewlett Packard 1100 LC-MS instrument. HPLC was recorded on Hewlett Packard 1100 instrument. Lipases from *Pseudomonas cepacia* and *Candida rugosa* were purchased from Sigma. All reagents for chemical synthesis were purchased from Aldrich Chemical Co. 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. Note that the higher-resistivity material contains a lower concentration of dopant and therefore can be examined by diffuse reflectance infrared spectroscopy, whereas the lower-resistivity wafers cannot.

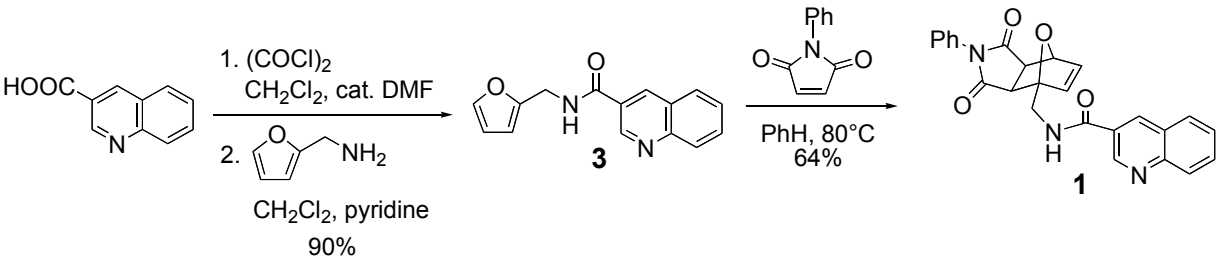
Porous silicon chips was prepared by electrochemical etching in a Teflon cell as described previously.[1] Briefly, a silicon wafer was cut to fit over the bottom of the Teflon etching cell chamber. A platinum wire positioned in the cell and a 0.1 mm thick gold foil placed under the silicon wafer was 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 were used to give effective DIOS surfaces.

Procedure A: 0.5-2.0 Ω-cm resistivity chips were etched under illumination with white light at ca. 0.5 mW/cm^2, at a current density of 31 mA/cm^2 for 5 min. Thorough washing with absolute ethanol and drying in a stream of nitrogen provides a "single-etched" high-resistivity chip. These chips are amenable to analysis by diffuse-reflectance IR, which was performed on a MIDAC spectrometer with attenuated total reflectance accessory (Pike Instruments).

Procedure B: 0.005-0.02 Ω-cm resistivity chips were etched under illumination with white light at ca. 0.5 mW/cm^2, at a current density of 4.4 mA/cm^2 for 2 min. Thorough washing with absolute ethanol and drying in a stream of nitrogen provides a "single-etched" low-resistivity chip. If desired, a grid of porous silicon spots may be created by interposing a transparency or slide with the desired pattern in the light beam. The pSi was exposed to ozone for several seconds and immersed in a 5% aqueous HF solution for 1 min to remove the oxidized layer so generated. Such "double etched" porous silicon chips are more effective and more uniform than those for which the ozone/HF treatment is omitted.[1] The chips were stored in absolute ethanol

before use. These low-resistivity chips cannot be analyzed by IR due to their relatively high dopant content.

*Syntheses of **1** and **2** (Scheme 1).*

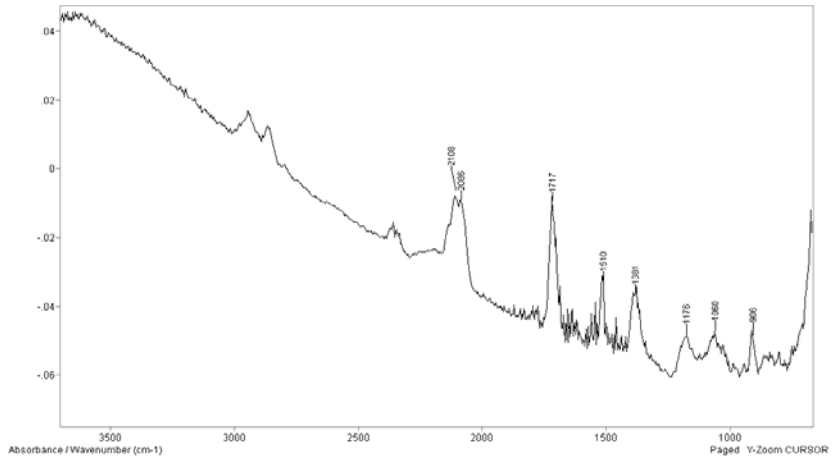


To a flask containing 3-quinoline carboxylic acid (52 mg, 0.3 mmol) in 20 mL dry CH_2Cl_2 was added oxalyl chloride (27 μL, 0.3 mmol) dropwise at room temperature. Then a drop of DMF in approximately 0.5 mL CH_2Cl_2 was added. After stirring for one hour the solvent and remaining volatiles were removed under vacuum. The residue was dissolved in 20 mL CH_2Cl_2, and furfurylamine (39 mg, 0.4 mmol) in 2 mL CH_2Cl_2 and 50 μL pyridine were added. The mixture was stirred for 3 hours and then washed with water three times. The organic layer was concentrated to afford compound **3** (68 mg, 90%). ^{1}H NMR ($CDCl_3$, δ) 4.72 (d, J = 5.5 Hz, 2H), 6.36 (s, 2H), 7.39 (s, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.76 (dd, J = 1.2, 8.0 Hz, 1H), 7.87 (t, J = 7.8 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.60 (d, J = 1.9 Hz, 1H), 9.28 (s, 1H).

To a round-bottomed flask was added amide **3** (50 mg, 0.2 mmol) and *N*-phenylmaleimide (138 mg, 0.8 mmol) in 15 mL benzene. The mixture was refluxed overnight. After removal of organic solvent under vacuum, the residue was purified by silica gel column chromatography (9:1 hexane:EtOAc as eluent), giving the adduct **1** (55 mg, 64%). ^{1}H NMR ($CDCl_3$, δ) 9.39 (br s, 1H, NH), 8.65 (d, J < 2 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 7.86 (m, 2H), 7.65 (d, J = 7.0 Hz, 1H), 7.45 (m, 4H), 7.27 (apparent dd, 2H), 6.71 (d, J = 5.8, 1H), 6.60 (dd, J = 1.8, 5.8 Hz, 1H), 5.39 (d, J = 1.5 Hz, 1H), 4.38 (dd, J = 6.6, 13.6 Hz, 1H), 4.11 (dd, J = 6.6, 13.6 Hz, 1H), 3.16 (dd, 2H); ^{13}C NMR ($CDCl_3$, δ) 39.6 (CH_2), 48.9 (CH), 50.5 (CH), 81.6 (CH), 91.4 (C), 126.7 (CH), 128.0 (CH), 129.3 (CH), 129.1 (CH), 129.5 (CH), 131.6 (CH), 132.0 (C), 136.4 (C), 137.7 (CH), 148.2 (C), 165.6 (C), 175.1 (C), 175.3 (C).

A suspension of Pd/C (8 mg, 150 μmol) in ethyl acetate (10 mL) was purged with hydrogen and then stirred with a baloon of H_2 for one hour. The adduct **1** (33 mg, 78 μmol) was added and the mixture was allowed to react overnight. The reaction mixture was filtered and concentrated. Flash column chromatography of the residue (using hexane-ethyl acetate 9:1 as eluent) yielded compound **2** (28 mg, 90%). ^{1}H NMR ($CDCl_3$, δ) 1.85 (m, 4H), 3.18 (s, 2H), 3.90 (dd, J = 6.0, 14.0 Hz, 1H), 4.40 (dd, J = 6.0, 14.0 Hz, 1H), 5.02 (d, J = 4.3 Hz, 1H), 7.20-7.50 (m, 5H), 7.65 (t, J = 8.0 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.85 (dd, J = 7.5, 8.2 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 8.57 (d, J = 2.3 Hz, 1H), 9.34 (d, J = 2.4 Hz, 1H).

*Preparation of maleimide-decorated pSi (**4**, Scheme 2 and Figure 1)*
A freshly etched chip was dipped in a solution of *N*-(4-vinylphenyl)maleimide (4 mg, 20 μmol) in 1 mL anhydrous THF which was purged thoroughly with nitrogen. The reaction mixture was shaken in an inert-atmosphere drybox for 14 hours at ambient temperature under room light illumination. The chip was rinsed with THF, toluene and absolute ethanol, and allowed to dry under nitrogen atmosphere. Such chips should be stored in absolute ethanol if necessary. An IR spectrum of **4** derived from higher-resistivity silicon is shown below, showing Si-H bands at 2100 and 905 cm^{-1}, as well as the imide band at 1717 cm^{-1}.



*Preparation of alkyne-decorated pSi (**10**, Figure 2)*. Freshly etched porous silicon was refluxed under nitrogen for three hours in 20 mL toluene containing 1 mL 1,6-heptadiyne or incubated with neat 1,6-heptadiyne at room temperature under room light illumination in an nitrogen-atmosphere drybox overnight, followed by thorough washing with toluene and ethanol.

*Preparation of alkyne-decorated pSi (**13**, Figure 3).* The maleimide-decorated pSi **4** was shaken with **12** (6.4 mg, 15 μmol) in 2 mL degassed toluene under nitrogen overnight. The chip was washed with toluene and ethanol and used immediately.

Cu-mediated azide-alkyne cycloadditions on DIOS chips (Figures 2, 3). Alkyne-decorated chips **10** and **13** were incubated in 2 mL solutions of 1:1 (v:v) acetonitrile:Tris buffer (pH 8) containing the indicated concentrations of azides (10 μM **11** for Fig. 2; 10 μM each of three azides for Fig. 3), 10 mol% (relative to total azide) $CuSO_4$, and 20 mol% (relative to total azide) of L-ascorbic acid. The reactions were allowed to run for 8 hours at room temperature in closed vials under air atmosphere. Then silicon chips were then washed with THF and ethanol.

Synthesis of triazines. Dichlorotriazine **5** (Figure 1). 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 **5** 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].

Triazine **6** (Figure 1). To a 50 mL flask containing benzylamine (2.67 g, 25 mmol) in 20 mL THF was added a solution of **5** (610 mg, 2.5 mmol) in 15 mL THF at room temperature. The reaction mixture was heated at reflux for 6 hours, after which the organic solvent was removed by rotary evaporation. The residue was dissolved in 50 mL ethyl acetate and washed successively with $KHSO_4$ (1 M, 20 mL), water (20 mL), 5% $NaHCO_3$ (10 mL) and brine. Removal of the organic solvent and drying over P_2O_5 *in vacuo* afforded the product **6** (865 mg, 90%). ^{1}H NMR ($CDCl_3$, δ) 4.53 (br s, 6H), 5.58 (br s, NH), 6.12 (s, 1H), 6.26 (dd, J = 1.6, 3.2 Hz, 1H), 7.27 (s, 11H); ^{13}C NMR ($CDCl_3$, δ) 37.9 (CH_2), 44.8 (CH_2), 107.7 (CH), 110.5 (CH), 127.2 (CH), 127.7 (CH), 128.7 (CH), 139.7 (C), 142.0 (CH), 152.9 (C), 166.4 (C-triazine); ESI-MS: *m/z* 387.1 (M+H$^+$) [M = $C_{22}H_{22}N_6O$, exact mass 386.19].

Phenethylmaleimide[2] (reaction c, Figure 1). To a flask containing maleic anhydride (4.9 g, 50 mmol) in 20 mL dry benzene was added dropwise a solution of phenethylamine (6.05 g, 50 mmol) in 20 mL dry benzene at room temperature. The resulting suspension was stirred at room temperature for another hour and then zinc chloride (6.75 g, 50 mmol) was added in one portion. The reaction mixture was heated to 80 °C, and a solution of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (15.8 mL, 75 mmol) in 10 mL dry benzene was added slowly over a period of 30 min. The reaction was refluxed for another 90 min. and then cooled to room temperature. The precipitated product was obtained as a white solid by filtration (3.05 g, 30%). ^{1}H NMR ($CDCl_3$, δ) 2.89 (dd, J = 8.1, 6.4 Hz, 2H), 3.75 (dd, J = 8.0, 6.4 Hz, 2H), 6.64 (s, 2H), 7.21-7.25 (m, 5H); ^{13}C NMR ($CDCl_3$, δ) 34.8 (CH_2), 39.4 (CH_2), 126.9 (CH), 128.8 (CH), 129.0 (CH), 134.2 (CH), 138.1 (C), 170.7 (C); ESI-MS: *m/z* 224.1 (M+Na$^+$) [calculated exact mass 224.07].

Adduct 9a (Figure 1). A mixture of phenethylmaleimide (201 mg, 1 mmol) and **6** (386 mg, 1 mmol) in 5 mL toluene was stirred at room temperature for 20 hours. The organic solvent was evaporated and the residue was subjected to flash column chromatography (silica gel 200-400 mesh, eluting with hexanes:EtOAc 2:1) to yield **9a** as a white solid (457 mg, 78%). ^{1}H NMR ($CDCl_3$, δ) 2.85 (t, J = 7.0 Hz, 2H), 2.89 (s, 2H), 3.71 (dd, J = 4.6, 7.8 Hz, 2H), 3.83 (m, 1H), 3.90 (m, 1H), 4.55 (s, 4H), 5.12 (s, 1H), 5.23 (s, 1H), 6.30 (br s, 2H), 7.17-7.28 (m, 15H); ^{13}C NMR ($CDCl_3$, δ) 33.9 (CH_2), 39.6 (CH_2), 40.4 (CH_2), 44.8 (CH_2), 48.4 (CH), 50.5 (CH), 80.8 (CH), 91.5 (CH), 126.9 (CH), 127.3 (CH), 127.6 (CH), 128.7 (CH), 129.2 (CH), 136.6 (CH), 137.9 (C), 139.3 (C), 139.8 (CH), 166.5 (C-triazine), 175.1 (C), 176.1 (C); ESI-MS: *m/z* 588.1 (M+H$^+$) [M = $C_{34}H_{33}N_7O_3$, exact mass 587.26].

Triazine 7 (Figure 1). 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 product **7** (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 (CH_2), 127.3 (CH), 127.7 (CH), 128.7 (CH), 139.8 (C), 166.5 (C-triazine); ESI-MS: *m/z* 397.1 (M+H$^+$) [M = $C_{24}H_{24}N_6$, exact mass 396.21].

Triazine 8 (Figure 1). To 30 mL well-stirred ice-water, a solution of cyanuric chloride (925 mg, 5 mmol) in 20 mL acetone was added. This mixture was then treated slowly with a solution of 4-bromophenethylamine (485 mg, 5 mmol) in 10 mL acetone and sodium bicarbonate (420 mg, 5 mmol) in 10 mL water. The reaction mixture was stirred at 0 °C for three hours. The resulting white precipitate was filtered off and rinsed with water and then dried *in vacuo* over P_2O_5 to yield 4-bromophenethyl-4,6-dichloro-[1,3,5]triazine-2-amine (1.5 g, 86%). ^{1}H NMR ($CDCl_3$, δ) 2.88 (t, J = 7.0 Hz, 2H), 3.74 (dt, J = 6.6 Hz, 2H), 7.08 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H); ESI-MS: *m/z* 350.9, 348.9 (M+H$^+$).

A solution of this dichlorotriazine (348 mg, 1 mmol) and phenethylamine (726 mg, 6 mmol) in 15 mL THF was refluxed for 6 hours. The organic solvent was evaporated and the residue was dissolved in 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 **8** (480 mg, 93%). ^{1}H NMR ($CDCl_3$, δ) 2.74 (br s, 6H), 3.47 (br s, 6H), 5.24 (br s, NH), 6.92 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.07-7.33 (m, 10H); ^{13}C NMR ($CDCl_3$, δ) 35.8 (CH_2), 36.4 (CH_2), 42.3 (CH_2), 42.4 (CH_2), 120.4 (C), 126.6 (CH), 128.8 (CH), 129.1 (CH), 130.8 (CH), 131.8 (CH), 138.7 (C), 139.6 (C), 166.1 (C-triazine); ESI-MS: *m/z* 519.1, 517.1 (M+H$^+$) [M = $C_{27}H_{29}N_6Br$, exact mass 516.16].

Triazine 11 (Figure 2). A mixture of 3-chloropropylmaleimide (347 mg, 2 mmol) and **6** (772 mg, 2 mmol) in 10 mL toluene was stirred at room temperature overnight. The organic solvent was evaporated and the residue was 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 produced gum was dissolved in 10 mL DMF, and sodium azide (650 mg, 10 mmol) was added. The reaction mixture was stirred at rt overnight followed by addition of 80 mL water. The mixture was extracted with ethyl acetate three times. Evaporation of organic solvent afforded white solid **11** (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 (CH_2), 39.8 (CH_2), 40.4 (CH_2), 44.7 (CH_2), 49.0 (CH_2), 49.4 (CH), 50.6 (CH), 80.9 (CH), 91.6 (CH), 127.3 (CH), 127.7 (CH), 128.7 (CH), 136.6 (CH), 139.2 (C), 139.9 (CH), 166.4 (C-triazine), 175.5 (C), 176.2 (C). ESI-MS: *m/z* 567.2 (M+H$^+$).

Triazine 12 was synthesized in the same manner. ^{1}H NMR ($CDCl_3$, δ) 2.19 (s, 1H), 2.81 (s, 2H), 3.56 (s, 2H), 4.18 (s, 2H), 4.56 (s, 2H), 6.21 (s, 1H), 6.30 (s, 1H), 7.08 (d, J = 7.0 Hz, 2H), 7.33 (s, 1H), 7.40 (d, J = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, δ) 30.6 (CH_2), 35.6 (CH_2), 37.9 (CH_2), 42.2 (CH_2), 71.3 (CH), 81.3 (C), 107.2 (CH), 110.6 (CH), 120.3 (C), 130.8 (CH), 131.7 (CH), 138.6 (C), 142.1 (CH), 152.7 (C), 165.7 (C-triazine). ESI-MS: *m/z* 429.0, 427.0 (M+H$^+$).

Representative procedure for synthesis of 14–19 (Scheme 4).[3] A suspension of **5** (490 mg, 2 mmol) and Na_2CO_3 (233 mg, 2.2 mmol) in 15 mL acetone was added to a solution of 4-bromophenethylamine (440 mg, 2.2 mmol) in 45 mL water at room temperature. After stirring at 65 °C for 5 hours, the white precipitate was filtered off and washed with water, then dried *in vacuo* over P_2O_5 to yield 4-bromophenethyl-furfuryl-6-chloro-[1,3,5]triazine-2,4-diamine (760

mg, 94%). ^{1}H NMR ($CDCl_3$, δ) 2.84 (m, 2H), 3.67 (m, 2H), 4.60 (d, J = 6.6 Hz, 2H), 6.23 (s, 1H), 6.33 (s, 1H), 7.06 (d, J = 8.2 Hz, 2H), 7.36 (s, 1H), 7.40 (d, J = 8.2 Hz, 2H); ESI-MS: *m/z* 409.9, 408.0 (M+H^+). 4-Chlorophenethyl-furfuryl-6-chloro-[1,3,5]triazine-2,4-diamine (650 mg, 90%) was synthesized by the analogous procedure. ^{1}H NMR ($CDCl_3$, δ) 2.89 (m, 2H), 3.73 (m, 2H), 4.62 (m, 2H), 6.23 (s, 1H), 6.33 (s, 1H), 7.14 (d, J = 7.0 Hz, 2H), 7.28 (s, 1H), 7.35 (d, J = 7.8 Hz, 2H); ESI-MS: *m/z* 366.0, 364.0 (M+H^+).

A solution of 4-bromophenethyl-furfuryl-6-chloro-[1,3,5]triazine-2,4-diamine (612 mg, 1.5 mmol) and (*R*)-1-amino-2-propanol (338 mg, 4.5 mmol) in 20 mL THF was refluxed for 10 hours. The organic solvent was evaporated and the residue dissolved in EtOAc; the resulting solution was washed successively with $KHSO_4$ (1 M, 20 mL), water (20 mL), 5% $NaHCO_3$ (10 mL) and brine. The organic solvent was removed and the residue was purified by flash column chromatography (silica gel, 200-400 mesh) eluting with hexanes:ethyl acetate (1:1.5) to yield trisubstituted triazine **14-OH** (596 mg, 89%). ^{1}H NMR ($CDCl_3$, δ) 1.04 (d, J = 5.2 Hz, 3H), 2.68 (t, J = 6.6 Hz, 2H), 3.42 (s, 4H), 3.83 (br s, 1H), 4.43 (s, 2H), 6.10 (s, 1H), 6.19 (dd, J = 2.0, 2.8 Hz, 1H), 6.94 (d, J = 8.0 Hz, 2H), 7.21 (s, 1H), 7.28 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, δ) 21.1 (CH_3), 35.6 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 48.7 (CH_2), 68.4 (CH), 107.1 (CH), 110.6 (CH), 120.3 (C), 130.8 (CH), 131.7 (CH), 138.5 (C), 142.1 (CH), 152.6 (C), 165.8 (C-triazine); ESI-MS: *m/z* 449.0, 447.0 (M+H^+) [M = $C_{19}H_{23}O_2N_6Br$, exact mass 446.11).

To a solution of **14-OH** (447 mg, 1 mmol) in 10 mL CH_3CN was added acetic anhydride (510 mg, 5 mmol) at room temperature, followed by the slow addition of a solution of *N,N*-dimethylaminopyridine (12.2 mg, 0.1 mmol) in 3 mL CH_3CN, and triethylamine (151 mg, 1.5 mmol). The reaction mixture was stirred at room temperature for 1 hour and the organic solvent was then evaporated. The resulting residue was subjected to flash column chromatography eluting with hexanes:EtOAc (1:1) to afford compound **14** (445 mg, 91%). ^{1}H NMR ($CDCl_3$, δ) 1.22 (d, J = 6.4 Hz, 3H), 2.01 (s, 3H), 2.79 (t, J = 7.2 Hz, 2H), 3.55 (s, 4H), 4.53 (s, 2H), 5.03 (s, 1H), 6.19 (d, J = 3.0 Hz, 1H), 6.29 (dd, J = 2.0, 3.2 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 7.31 (s, 1H), 7.38 (d, J = 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, δ) 17.8 (CH_3), 21.6 (CH_3), 35.7 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 45.1 (CH_2), 70.4 (CH), 107.0 (CH), 110.6 (CH), 120.3 (C), 130.8 (CH), 131.8 (CH), 138.5 (C), 142.1 (CH), 152.8 (C), 166.0 (C-triazine), 170.9 (C=O); ESI-MS: *m/z* 491.0, 489.1 (M+H^+) [M = $C_{21}H_{25}O_3N_6Br$, exact mass 488.12]. Compounds **15**–**19** were synthesized by the same procedures.

15-OH. ^{1}H NMR ($CDCl_3$, δ) 1.11 (d, J = 6.2 Hz, 3H), 2.76 (s, 2H), 3.49 (s, 4H), 3.91 (s, 1H), 4.51 (s, 2H), 6.17 (s, 1H), 6.26 (s, 1H), 7.05 (d, J = 7.4 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.18 (s, 1H); ^{13}C NMR ($CDCl_3$, δ) 21.1 (CH_3), 35.5 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 45.0 (CH_2), 68.5 (CH), 107.1 (CH), 110.6 (CH), 128.7 (CH), 130.4 (CH), 132.2 (C), 138.1 (C), 142.0 (CH), 152.6 (C), 165.8 (C-triazine); ESI-MS: *m/z* 405.1, 403.1 (M+H^+) [M = $C_{19}H_{23}O_2N_6Cl$, exact mass 402.16].

15. ^{1}H NMR ($CDCl_3$, δ) 1.14 (d, J = 6.2 Hz, 3H), 1.93 (s, 3H), 2.73 (t, J = 6.6 Hz, 2H), 3.47 (s, 4H), 4.46 (s, 2H), 4.95 (s, 1H), 6.11 (d, J = 1.2 Hz, 1H), 6.21 (dd, J = 1.6, 2.8 Hz, 1H), 7.02 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 7.14 (s, 1H); ^{13}C NMR ($CDCl_3$, δ) 17.8 (CH_3), 21.5 (CH_3), 35.6 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 45.0 (CH_2), 70.3 (CH), 107.1 (CH), 110.6 (CH), 128.8 (2×CH), 130.3 (2×CH), 132.3 (C), 138.0 (C), 142.1 (CH), 152.6 (C), 165.5 (C-triazine), 170.9 (CH_3*C*OO); ESI-MS: *m/z* 447.1, 445.1 (M+H^+) [M = $C_{21}H_{25}O_3N_6Cl$, exact mass 444.17].

16-OH. ^{1}H NMR ($CDCl_3$, δ) 2.62 (s, 2H), 3.40 (s, 2H), 3.77 (s, 2H), 4.40 (s, 2H), 5.12 (br s, 1H), 6.09 (s, 1H), 6.20 (br s, 1H), 6.91 (d, J = 7.0 Hz, 2H), 7.21-7.31 (m, 8H); ^{13}C NMR ($CDCl_3$, δ) 35.5 (CH_2), 37.9 (CH_2), 42.1 (CH_2), 57.4 (CH), 66.8 (CH_2OH), 107.1 (CH), 110.6 (CH), 120.3 (C), 127.0 (CH), 127.6 (CH), 128.8 (CH), 130.8 (CH), 131.7 (CH), 138.6 (C), 140.6 (C), 142.0 (CH), 152.7 (C), 165.8 and 166.1 (C-triazine); ESI-MS: *m/z* 511.1, 509.0 (M+H^+) [M = $C_{24}H_{25}O_2N_6Br$, exact mass 508.12].

16. ^{1}H NMR ($CDCl_3$, δ) 1.96 (s, 3H), 2.71 (br s, 2H), 3.48 (br s, 2H), 4.32 (d, J = 3.6 Hz, 2H), 4.47 (s, 2H), 5.40 (d, J = 6.6 Hz, 1H), 6.15 (d, J = 3.2 Hz, 1H), 6.25 (d, J = 1.6 Hz, 1H), 7.00 (d, J = 8.0 Hz, 2H), 7.19-7.36 (m, 8H); ^{13}C NMR ($CDCl_3$, δ) 21.1 (CH_3), 35.6 (CH_2), 37.9 (CH_2), 42.0 (CH_2), 53.3 (CH), 66.9 (CH_2OH), 107.0 (CH), 110.6 (CH), 120.3 (C), 127.0 (CH), 127.8 (CH), 128.8 (CH), 130.7 (CH), 131.7 (CH), 138.6 (C), 139.9 (C), 142.0 (CH), 152.9 (C), 165.9 (C-triazine), 171.1 (C=O); ESI-MS: *m/z* 553.0, 551.0 (M+H^+) [M = $C_{26}H_{27}O_3N_6Br$, exact mass 550.13].

17-OH. ^{1}H NMR ($CDCl_3$, δ) 2.67 (s, 2H), 3.42 (s, 2H), 3.80 (s, 2H), 4.43 (s, 2H), 5.11 (d, J = 5.2 Hz, 1H), 6.09 (s, 1H), 6.22 (br s, 1H), 7.00 (d, J = 7.4 Hz, 2H), 7.15-7.25 (m, 8H); ^{13}C NMR ($CDCl_3$, δ) 35.5 (CH_2), 37.9 (CH_2), 42.1 (CH_2), 57.4 (CH), 67.2 (CH_2OH), 107.1 (CH), 110.6 (CH), 127.0 (CH), 127.6 (CH), 128.8 (CH), 130.3 (CH), 132.2 (C), 138.0 (C), 140.5 (C), 142.0 (CH), 152.7 (C), 165.8 (C-triazine); ESI-MS: *m/z* 467.1, 465.1 (M+H^+) [M = $C_{24}H_{25}O_2N_6Cl$, exact mass 464.17].

17. ^{1}H NMR ($CDCl_3$, δ) 1.97 (s, 3H), 2.73 (s, 2H), 3.49 (s, 2H), 4.34 (s, 2H), 4.47 (s, 2H), 5.40 (d, J = 6.6 Hz, 1H), 6.15 (s, 1H), 6.24 (s, 1H), 7.05 (s, 2H), 7.17-7.29 (m, 8H); ^{13}C NMR ($CDCl_3$, δ) 21.1 (CH_3), 35.5 (CH_2), 37.9 (CH_2), 42.1 (CH_2), 53.7 (CH), 66.9 (CH_2OH), 107.1 (CH), 110.6 (CH), 127.0 (CH), 128.7 (CH), 128.8 (CH), 130.3 (CH), 132.2 (C), 138.0 (C), 139.8 (C), 142.0 (CH), 152.7 (C), 165.7 (C-triazine), 171.1 (C=O); ESI-MS: *m/z* 509.1, 507.0 (M+H^+) [M = $C_{26}H_{27}O_3N_6Cl$, exact mass 506.18].

18-OH. ^{1}H NMR ($CDCl_3$, δ) 2.76 (s, 2H), 3.52 (s, 4H), 3.68 (s, 1H), 4.52 (s, 2H), 4.84 (s, 2H), 6.17 (s, 1H), 6.28 (s, 1H), 7.04-7.39 (m, 10H); ^{13}C NMR ($CDCl_3$, δ) 35.6 (CH_2), 38.0 (CH_2), 42.2 (CH_2), 49.4 (CH_2), 74.8 (CH), 107.2 (CH), 110.6 (CH), 120.4 (C), 126.1 (CH), 127.7 (CH), 128.6 (CH), 128.6 (CH), 130.8 (CH), 138.4 (C), 142.1 (CH), 142.8 (C), 152.4 (C), 165.6 (C-triazine); ESI-MS: *m/z* 511.1, 509.0 (M+H^+) [M = $C_{24}H_{25}O_2N_6Br$, exact mass 508.12].

18. ^{1}H NMR ($CDCl_3$, δ) 2.06 (s, 3H), 2.80 (t, J = 6.2 Hz, 2H), 3.56 (s, 4H), 3.84 (s, 1H), 4.55 (s, 2H), 6.19 (d, J = 2.8 Hz, 1H), 6.29 (dd, J = 1.6, 3.0 Hz, 1H), 7.05 (d, J = 8.6 Hz, 2H), 7.31-7.40 (m, 8H); ^{13}C NMR ($CDCl_3$, δ) 21.4 (s, CH_3), 35.7 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 45.9 (CH_2), 75.0 (CH), 107.1 (CH), 110.6 (CH), 120.4 (C), 126.6 (CH), 128.5 (CH), 128.8 (CH), 130.8 (CH), 131.8 (CH), 138.4 (C), 138.5 (C), 142.1 (CH), 152.6 (C), 165.5 (C-triazine), 170.4 (C=O); ESI-MS: *m/z* 553.0, 551.0 (M+H^+) [M = $C_{26}H_{27}O_3N_6Br$, exact mass 550.13].

19-OH. ^{1}H NMR ($CDCl_3$, δ) 2.78 (s, 2H), 3.53 (s, 4H), 3.69 (s, 1H), 4.53 (s, 2H), 4.84 (s, 2H), 6.17 (s, 1H), 6.28 (s, 1H), 7.09-7.30 (m, 10H); ^{13}C NMR ($CDCl_3$, δ) 35.5 (CH_2), 38.0 (CH_2), 42.2 (CH_2), 49.4 (CH_2), 74.8 (CH), 107.2 (CH), 110.6 (CH), 126.1 (CH), 127.7 (CH), 128.6 (CH), 128.8 (CH), 130.4 (CH), 132.3 (C), 137.9 (C), 142.1 (CH), 142.9 (C), 152.6 (C), 165.8 (C-triazine); ESI-MS: *m/z* 467.1, 465.0 (M+H^+) [M = $C_{24}H_{25}O_2N_6Cl$, exact mass 464.17].

19. ^{1}H NMR ($CDCl_3$, δ) 2.05 (s, 3H), 2.80 (t, J = 6.2 Hz, 2H), 3.55 (s, 4H), 3.83 (s, 1H), 4.54 (s, 2H), 6.18 (d, J = 3.0 Hz, 1H), 6.28 (dd, J = 1.6, 3.2 Hz, 1H), 7.07 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.30 (m, 6H); ^{13}C NMR ($CDCl_3$, δ) 21.4 (s, CH_3), 35.6 (CH_2), 38.0 (CH_2), 42.2 (CH_2), 45.9 (CH_2), 75.0 (CH), 107.1 (CH), 110.6 (CH), 126.6 (CH), 128.5 (CH), 128.8 (CH), 130.4 (CH), 132.3 (C), 138.0 (C), 138.6 (C), 142.1 (CH), 152.7 (C), 165.5 (C-triazine), 170.4 (C=O); ESI-MS: *m/z* 509.1, 507.1 (M+H^+) [M = $C_{26}H_{27}O_3N_6Cl$, exact mass 506.18].

Representative procedure for lipase-catalysed hydrolysis of triazines 14–19 in aqueous buffer.[4] A mixture of 5 μmol **14** (2.45 mg) and 5 μmol **15** (2.23 mg) in 0.5 mL 20 mM phosphate buffer (pH 7.2) containing 50% DMSO (v/v) was mixed by ultrasonic dispersion and then lipase (2 mg) was added. The reaction mixture was shaken at room temperature for 12 hours. The reaction mixture was extracted with EtOAc (0.5 mL). After removal of the solvent, the residue was dissolved in methanol and analyzed by HPLC (using methanol-H_2O 80:20 (v/v) as eluent) on a Supelco C-18 column to determine the conversion and the extent of reaction selectivity (kinetic resolution).

Representative procedure for lipase-catalysed hydrolysis of 14–19 attached to pSi chips. Triazines **14** (2.45 mg, 5 μmol) and **15** (2.23 mg, 5 μmol) were dissolved in 1 mL toluene. A maleimide-decorated pSi wafer was immersed in this solution under a nitrogen atmosphere in the drybox overnight. The wafer was rinsed with toluene (3x) and ethanol (3x). Alternatively, 0.6 μL aliquots (in three 0.2 μL portions) of 5 mM toluene solution of each mixture (**14**+**15**, **16**+**17**, **18**+**19**) were deposited on separate positions of a pSi chip photopatterned with 2 mm-wide etched spots. The solvent was allowed to evaporate after each 0.2 μL deposition, creating an analyte spot of 1-1.5 mm in diameter). The chip was allowed to stand at room temperature for several hours, and then was rinsed with toluene and ethanol as above. The functionalized chips were then immersed in a solution of lipase (2 mg) in 1 mL 20 mM phosphate buffer (pH 7.2) and DMSO (1/1, v/v) in the drybox for 12 hours at room temperature. Each chip was washed with 20 mM phosphate buffer, water, and ethanol, blow-dried with nitrogen, and analyzed by DIOS-MS.

Preliminary survey of fragmentation of Diels-Alder adducts in DIOS-MS.

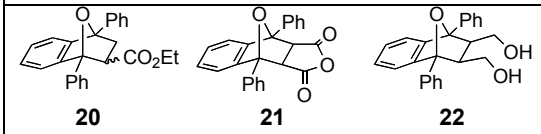
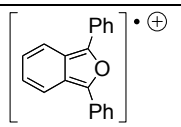
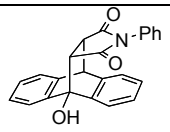
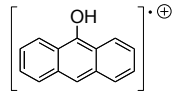
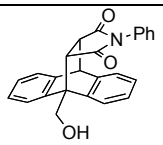
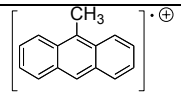
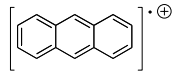
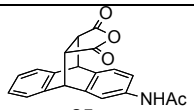
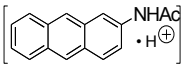
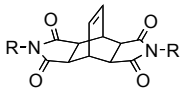
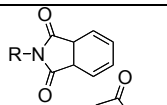
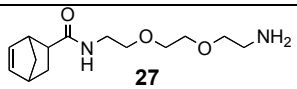
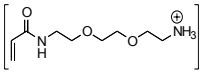
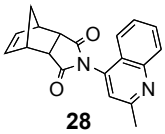
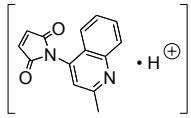
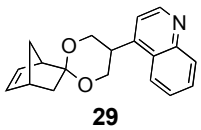
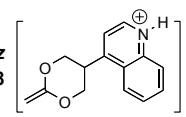
Table S1 summarizes the DIOS-MS behavior of a selection of Diels-Alder adducts. These results support the hypothesis that [4+2] cycloreversion in DIOS-MS is a thermal process, since the efficiency of cleavage was found to be inversely proportional to the solution-phase thermal stability of the adduct.

In addition to the furan adducts described in the text, clean retro-Diels-Alder fragmentation was observed for isobenzofuran adducts[5] (**20**–**22**), anthrone adduct[6] (**23**), anthracene adduct **25**, and the symmetric bis(maleimide) **26**. The latter is of course cleaved to two different fragments, both of which are detected with high sensitivity. Anthracene adducts appear to be at the border of stability for DIOS-MS cleavage, suggested by the case of anthracenemethanol adduct **24**, which gives rDA cleavage but also cleavage at the benzylic position.

Isobenzofuran adducts **20**–**22** were examined because they span a wide range of retro-Diels-Alder reaction temperatures. Thus, in solution-phase experiments, **20** and **21** were observed to undergo cycloreversion in refluxing xylenes (140 °C) within hours, but the reduced compound **22** completely resisted cleavage under these conditions. In the last case, we believe that the observation of a dominant signal corresponding to cleaved isobenzofuran in the DIOS

spectrum does not reflect complete cleavage of **22**, since the isobenzofuran radical cation is likely to be detected with far greater efficiency than the parent Diels-Alder adduct.

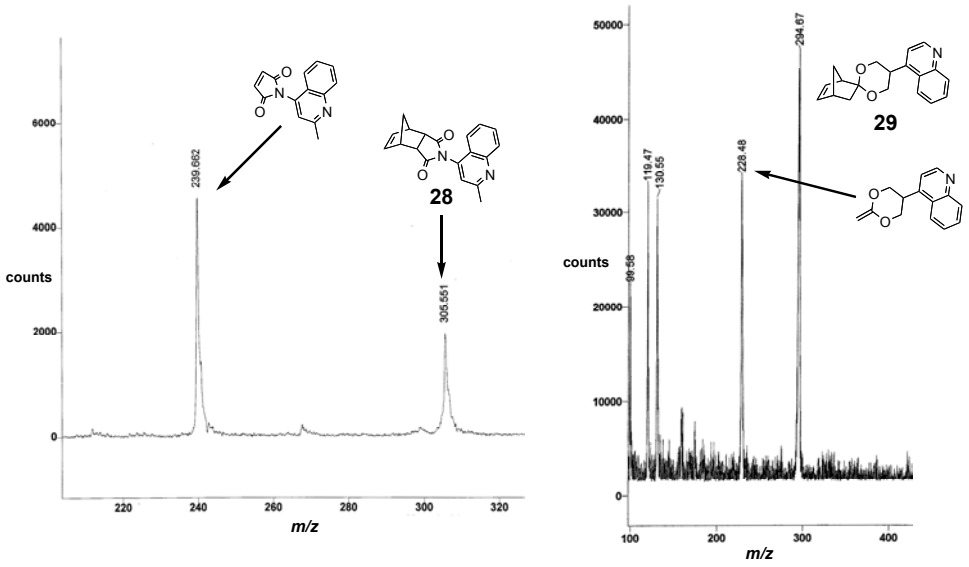
Table S1. Diels-Alder adducts analyzed by DIOS-MS.

Adduct(s)	Peak in DIOS-MS	Comment
 **20** **21** **22**	**m/z 270** 	rDA cleavage
 **23**	**m/z 194** 	rDA cleavage [a]
 **24**	**m/z 192**  **m/z 178** 	rDA cleavage and fragmentation [b]
 **25**	**m/z 236** 	rDA cleavage
 R = $(CH_2)_5COOH$ **26**	**m/z 286**  **m/z 234**	rDA cleavage
 **27**	**m/z 203** 	rDA cleavage
 **28**	**28** and **m/z 239** 	partial rDA cleavage [c]
 **29**	**29** and **m/z 228** 	partial rDA cleavage [c]

a) m/z = 193 (alkoxide anion) observed as base peak in negative-ion MS mode
b) benzylic C-O cleavage (formal loss of OH^-) and aromatic C-C cleavage (formal loss of $H_2C{=}O$)
c) see Figure S1.

Cyclopentadiene-derived compounds **27**–**29** bracket the range of stabilities at which Diels-Alder adducts undergo rDA cleavage. Since maleimide adducts are particularly stable, we expect **27** to undergo cycloreversion somewhat more easily than **28**. Compound **29**, lacking an activating carbonyl group on the dienophile fragment, is the most resistant to thermolysis. These structures show the same trend in DIOS-MS fragmentation (Figure S1): **27** showed complete rDA cleavage, **28** more than 50% cleavage, and **29** somewhat less than 50% (assuming that ionization efficiency is dominated by the isoquinoline moiety).

Figure S1. Incomplete rDA cleavage of cyclopentadienyl adducts **28** and **29** by DIOS. All species are observed in their $[M+H]^+$ protonated form.



Examination of porous silicon surface after DIOS; speculation on DIOS mechanism.

Scanning electron microscopy (SEM) was performed on pSi chips following DIOS analysis. Figure S2 shows an image that includes an undisturbed region of the pSi plate with its regular pore structure, adjacent to two spots irradiated in the DIOS experiment. The latter areas appear as though the surface has melted and bubbled up, perhaps reflecting the release of volatile material trapped in the pores. We propose that the 337 nm light pulse is efficiently absorbed by the silicon substrate beneath the porous layer, resulting in very rapid and localized heating. This thermal energy induces efficient retro-Diels-Alder fragmentation, which is expected to be a thermal, not a photochemical, process. A reviewer has also correctly pointed out that photoionization of the Diels-Alder adduct may well precede retro-Diels-Alder dissociation.

We are grateful to Dr. Mark Englehard of the Environmental Molecular Sciences Laboratory, Department of Energy Office of Biological and Environmental Research, Pacific Northwest National Laboratory, for this SEM image.

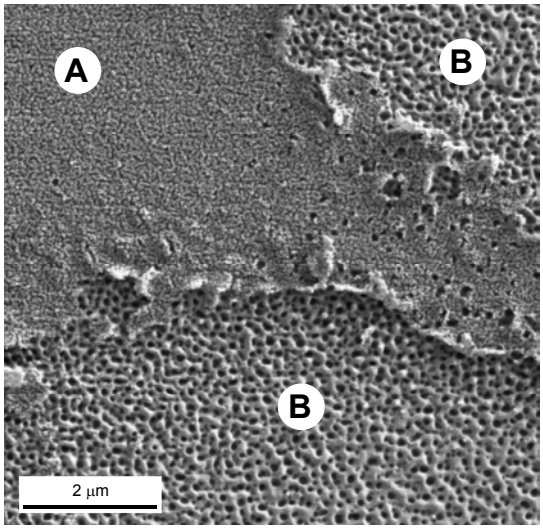


Figure S2. SEM image of a porous silicon chip after DIOS-MS analysis of a deposited analyte. (A) Normal region of the pSi wafer, identical in morphology to freshly-etched material.[1] (B) Irradiated regions of the surface.

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