Mass Spectrometry

Cleavable Linkers for Porous Silicon-Based Mass Spectrometry**

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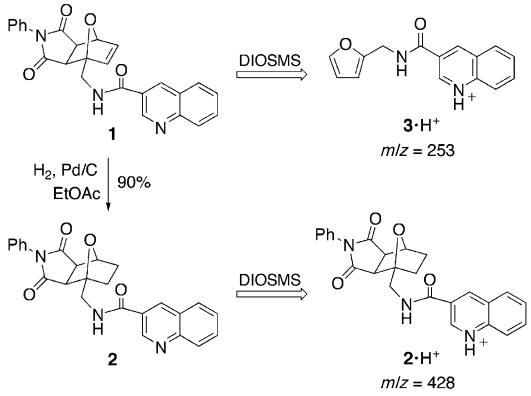
Desorption/ionization on silicon mass spectrometry (DIOSMS) uses porous silicon (pSi) to generate gas-phase ions of small (< 3000 Da) molecules without a matrix by using standard MALDI (matrix-assisted laser desorption/ionization) instrumentation.[1] The unique laser desorption/ionization surface properties of DIOSMS allow for the simultaneous detection of a broad range of small molecules as their molecular ions, and the chemical properties of DIOSMS facilitate the attachment of a variety of organic fragments through surface Si–H[2] and Si–OH groups.[3] We aim to combine these advantages with the proven ability of covalent immobilization to facilitate combinatorial chemistry and the extraction of molecular information from complex mixtures.[4] Previous reports in this vein include the use of self-assembled monolayers of functionalized alkanethiols to purify mixtures (usually of proteins) by specific molecular interactions or properties such as hydrophobicity or charge complementarity prior to MALDI analysis.[5] DIOS has been similarly employed to detect small molecules bound to pSi-immobilized proteins.[6] We report herein that Diels–Alder adducts undergo retro-[4+2] fragmentation in DIOSMS analysis, thus providing a convenient way to covalently attach and detach probe structures for chip-based mass analysis.

The most useful covalent linkers for our purposes should be stable under organic synthetic conditions, yet cleavable during desorption/ionization. While photocleavable groups are commonly used in solid-phase organic synthesis,[7] most (but not all[8]) of these systems require a workup step after irradiation to complete the cleavage, and are therefore poorly suited to detachment in the MALDI laser pulse. The retro-Diels–Alder (rDA) reaction was one of the first dissociation pathways to be investigated in mass spectrometry,[9] and has since been characterized with a variety of ionization methods.[10] Some studies showed that the fragmentation is highly stereospecific for *cis*-annelated systems, which suggests a concerted mechanism.[11]

We have found that Diels–Alder adducts undergo [4+2] cycloreversion readily upon desorption/ionization by the laser pulse in DIOS analyses. Thus, the maleimide–furan adduct **1** bearing a 3-quinoline carboxamide moiety showed only a single intense peak in the DIOSMS spectrum, which corresponds to the protonated diene $[\mathbf{3}\cdot H]^+$ (Scheme 1). The



Scheme 1. Retero Diels–Alder cleavage of a furan–maleimide adduct.

maleimide was not detected because it is poorly ionized, as verified by control experiments. Hydrogenation of **1** into **2** eliminated fragmentation in the DIOS mass spectrum, thus supporting the notion that cycloreversion is the cleavage mechanism. A survey of a set of Diels–Alder adducts suggested that the ease of thermal cycloreversion in solution may be correlated with retro-Diels–Alder fragmentation in DIOSMS (see Supporting Information). Furan–maleimide adducts were found to be conveniently accessible, of sufficient chemical stability,[12] and to undergo uniformly clean rDA cleavage, and were therefore used in subsequent studies.

The use of a Diels–Alder moiety as a connector allows small molecules to be both covalently attached to the porous silicon surface and detected by mass spectrometry when desired. This was illustrated by the derivatization of freshly-etched pSi by hydrosilylative attachment of *N*-(4-vinylphenyl)maleimide to give **4** (Scheme 2 and Supporting Information). It should be noted that many of the hydrosilylation reactions described here were performed at room temperature in the absence of air, which is in contrast to the higher temperatures (ca. 100 °C) that appear to be standard in previous cases.[1d,13] Under our mild conditions, it is assumed that only the most reactive surface silicon-hydride sites are derivatized giving rise to incomplete surface coverage, but a sufficient density for DIOSMS detection was invariably achieved. 1,3-Diphenylisobenzofuran was then introduced in a CH_2Cl_2 solution, followed by extensive washing with organic solvent. DIOSMS analysis showed a strong signal for the expected rDA peak at *m*/*z* 270. Control samples, involving noncovalently deposited diene and dienophile, as well as the diene alone, showed no signal after they had been washed (Scheme 2).

As in all mass-spectrometry techniques, the strength of the DIOS signal is affected by many factors, especially the

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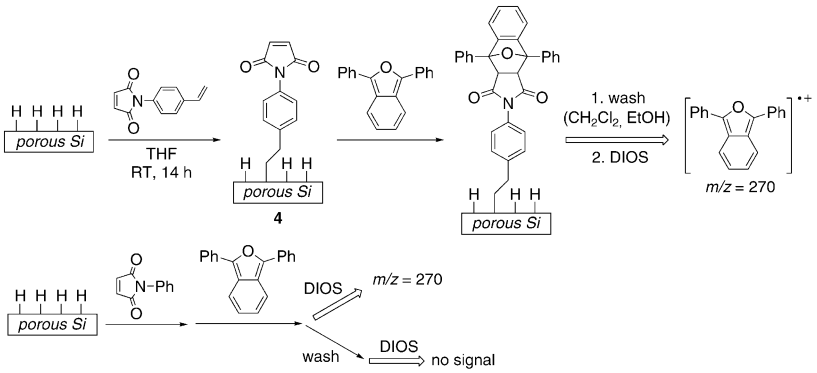
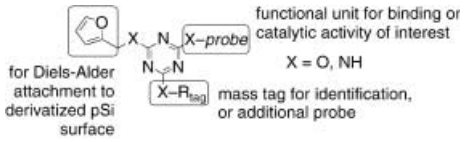


Scheme 2. Attachment and detachment of isobenzofuran by using a maleimide-derivatized porous-silicon surface.

ionization efficiency of the analyte. The combination of a retro-Diels–Alder linkage with an easily ionizable spacer should provide a useful platform for the analysis of a number of chip-based phenomena. The 1,3,5-triazine unit proved to be an effective scaffold, since it provides a strong DIOSMS signal regardless of the attached species and can be addressed sequentially in three positions from trihalide (cyanuric) derivatives.[14] The general design of such a system is shown in Scheme 3, and Figure 1 depicts an example of the synthetic manipulations performed.



Scheme 3. Trifunctional 1,3,5-triazine core.

Furfurylamine addition to cyanuric chloride at low temperature afforded **5**, which was subsequently converted into the fully substituted triazine–diene **6**. Compounds **7** and **8** were prepared analogously. Diels–Alder attachment to *N*-phenethylmaleimide (giving **9a**) and to the maleimide-decorated porous silicon surface **4** (giving **9b**) occurred under mild conditions. Both **9a**, deposited on a DIOS chip, and modified pSi **9b** gave a single dominant MS peak at *m*/*z* 387, indicative of clean cycloreversion to **6** upon laser desorption/ionization. Furthermore, immersion of a *p*Si-maleimide chip in a toluene solution of equimolar amounts of **6**, **7**, and **8** (10 μmol each in 2 mL), or deposition of a drop of this solution on the chip, showed all three species in approximately equal intensity. When the immersed chip was subsequently rinsed thoroughly with toluene and ethanol, only **6** appeared, presumably held by covalent attachment to the surface, whereas the other species were washed away. A nonfunctionalized pSi wafer retained none of these species after identical analyte deposition and washing procedures.

The sequence shown in Figure 2 illustrates the application of an alternative pSi-attachment strategy that incorporates a preformed oxanorbornene structure. Hydrosilyation of 1,6-heptadiyne with freshly prepared pSi afforded the terminal alkyne surface **10**. In spite of the presence of unconverted surface Si–H sites, at least some terminal alkyne residues remained available, as established by IR spectroscopy and subsequent reactivity; samples prepared by thermal or photochemical[15] hydrosilylation methods behaved very similarly. Attachment of **10** to azide **11** was then performed by using the Cu^{I}-catalyzed procedure recently reported by Fokin and Sharpless,[16] in a mixture of acetonitrile and pH 8 buffer at room temperature. The capture of azide at 10 μM concen-

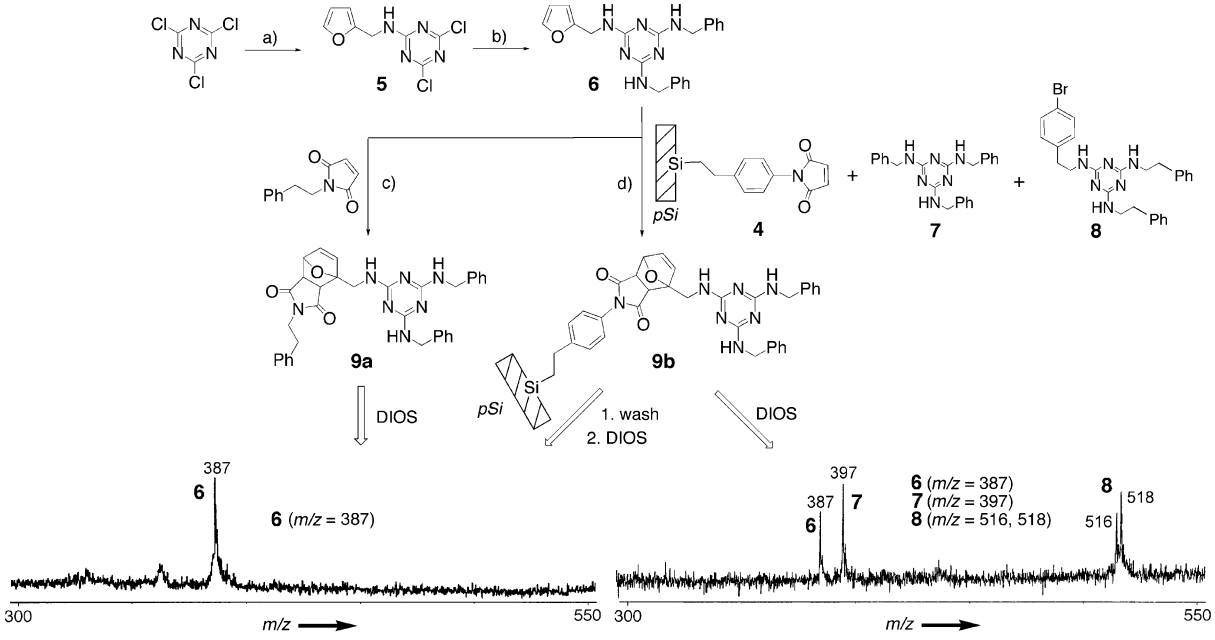


Figure 1. Detection of deposited and covalently attached compounds by DIOSMS. a) Furfurylamine (1 equiv), $NaHCO_3$, 2:3 acetone/H_2O, 93%. b) Benzylamine (10 equiv), THF, reflux, 90%. c) Toluene (25 °C) or benzene (reflux), 75–80%. d) Toluene, 25 °C, 12 h.

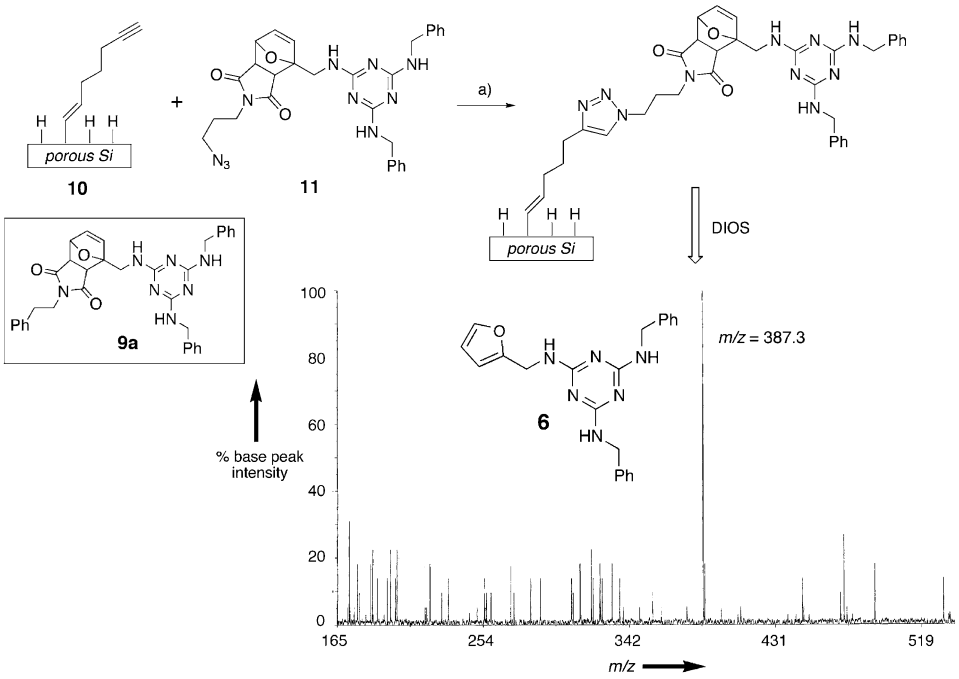


Figure 2. a) (I) 10 μM **11**, 1.0 μM $CuSO_4$, 2.0 μM L-ascorbic acid; 1:1 (v:v) MeCN:Tris buffer (pH 8.0), room temperature, 8 h. (II) Wash with THF and ethanol.

tration by the surface-attached alkyne by using 1 μM copper catalyst is among the most impressive examples of this "click reaction"[17] so far reported,[18] thus illustrating the extraordinary ability of the process to join appropriate pieces at low concentration.[19] After the product had been washed, DIOS analysis showed the expected rDA product **6** as the dominant signal. The corresponding control compound **9a** gave no MS signal after incubation with **10** and washing of the product under identical conditions.

The capture of a set of azides by an immobilized alkyne was demonstrated as shown in Figure 3. In this case, the alkyne was brought to the pSi surface as part of the triazenyl

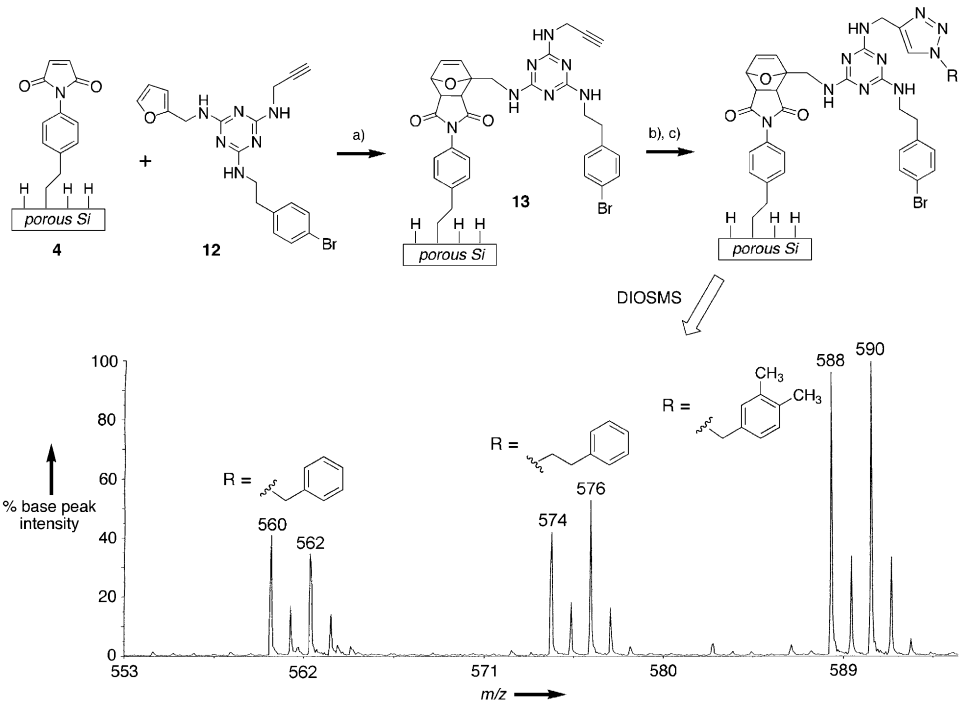
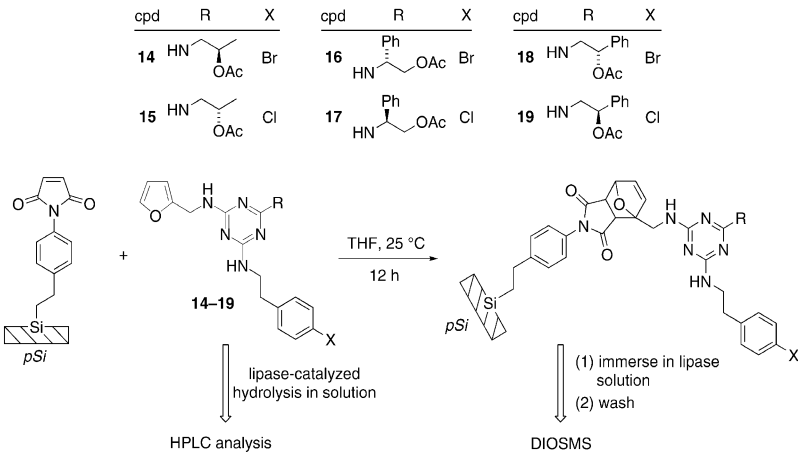


Figure 3. Capture and detection of solution-phase azides by DIOSMS. a) Toluene, 25 °C, 12 h. b) Mixture of indicated R–N_3 (10 μM each), 3.0 μM $CuSO_4$, 6.0 μM L-ascorbic acid, 1:1 (v/v) MeCN:Tris buffer (pH 8.0), RT, 8 h. c) Wash with THF, then ethanol.

furan dienophile **12**. The resulting wafer, **13**, was then exposed to an equimolar mixture of three azides, chosen to differ in mass by successive methylene units. Washing of the product followed by DIOSMS analysis gave a clean set of signals for the three $[M+H]^+$ species, each showing the expected bromine isotope pattern arising from the Br label introduced to the triazine core. As before, the negative controls (allyl in place of propargyl in **12**, nonderivatized pSi in place of **4**) gave no MS signals, thus demonstrating that both the covalent connections (Diels–Alder and [3+2] cycloaddition) were made.



Scheme 4. Lipase-mediated hydrolysis of soluble and pSi-bound acetate esters.

To show that the covalent attachment and detachment events enabled by the Diels–Alder reaction can be useful in catalyst screening, we explored the enantioselective hydrolysis of acetate esters by two lipase enzymes.[20] Triazines **14**–**19** comprise three pairs of pseudoenantiomers (Scheme 4). Each member of a pair has the opposite absolute configuration of the chiral component and was tagged with a bromide or chloride substituent to encode this information into the MS signal.[21]

Pseudoracemic mixtures of triazine–acetates (equimolar amounts of **14**+**15**, **16**+**17**, and **18**+**19**) were subjected to hydrolysis by lipases from *Pseudomonas cepacia* (PCL) and *Candida rugosa* (CRL) in aqueous buffer and the results were analyzed by reversed-phase HPLC (Figure 4). A range of outcomes was observed. In two cases, the enzymes differed dramatically in their reactivity: for the **14**/**15** pair, PCL gave excellent resolution ($k_{rel} > 50$) and CRL was nonselective ($k_{rel} = 1$), whereas for **18**/**19**, CRL catalyzed a moderately successful resolution ($k_{rel} = 16$) and PCL was inactive. In the case of **16**/**17**, the lipases were more similar in their activity, with PCL being somewhat more selective ($k_{rel} = 5.1$ versus 1.1). The good performance of PCL with **14**/**15** and the absolute configuration of the faster-reacting compound are consistent with previous results involving structures that are related to the substrates used here.[22] All the other cases are sufficiently far removed from prior reports as to constitute new results, although it was expected that the primary acetates **16**/**17** would undergo less efficient kinetic resolution than secondary acetates, which have the chiral center of interest closer to the reactive bond.[23] Few lipase-catalyzed transformations of alcohols and esters bearing nitrogen substituents in the α position have been reported.[24]

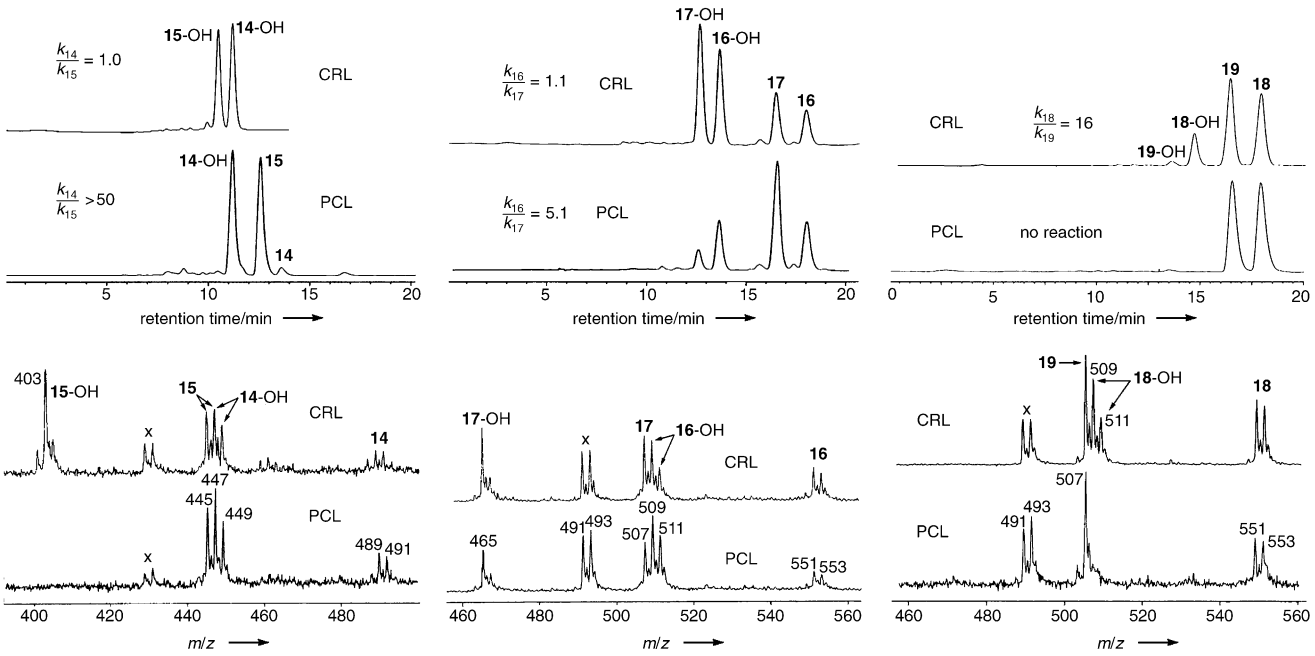


Figure 4. HPLC (top; I = relative intensity arbitrary units) and DIOSMS (bottom; %I = percentage base peak intensity) analyses of hydrolysis reactions using CRL and PCL on acetates **14**–**19**.

The pseudoenantiomeric mixtures of acetates were also separately incubated with maleimide-functionalized porous silicon as described above and the chips were then thoroughly rinsed with toluene and ethanol. Alternatively, each pair of acetates was attached to a different spot of the same pSi chip. The resulting wafers were swirled together in an aqueous buffer solution containing either CRL or PCL (12 h at room temperature), washed with water and ethanol, and then analyzed directly by DIOSMS. The resulting spectra, shown in Figure 4, reproduced the solution-phase/HPLC findings quite well. For example, with PCL, only the alcohol derived from **14** was observed, and no hydrolysis product from **15** was evident, whereas both alcohols were detected in comparable amounts with CRL. Similarly, in all other cases the relative reactivity observed by HPLC were clearly reproduced in the chip-based format. As a control, undecorated porous silicon was incubated with the same mixtures of substrates **14**–**19** and lipase in phosphate buffer under identical conditions. The substrates and the hydrolyzed products were poorly detected by DIOS, and washing of the products removed the signals entirely.

Analytical methods based on mass spectrometry have the general advantage over optical and radiolabel assays in that the installation of chromophoric or radioactive tags is not required and that detection of mass provides a general means to monitor chemical changes in the analytes of interest. The combination of the ability to detect analytes of different mass in a single spectrum with the rapid data acquisition provided by the chip-based format makes both DIOSMS and MALDI well suited to high-throughput screening. Covalent attachment of analytes further enhances these approaches by enabling spatially addressable and multistep synthesis on the chip. Most importantly, the making of a cleavable covalent link between the analyte/probe and the surface allows the user to wash the chips vigorously and thus overcome problems of nonspecific adsorption and signal suppression. Mrksich and Su have described the use of MALDI in this fashion to analyze the conversion of surface-bound substrates by a galactosyltransferase enzyme by using functionalized alkanethiols, which relies on the ability of the MALDI laser pulse to dissociate the Au–thiol bond.[5a]

Here we have shown Diels–Alder adducts to be cleavable covalent linkers compatible with the DIOSMS technique, useful in probing the reactivity of species attached to the porous silicon surface. The triazine unit is a well-ionized tripodal spacer, which allows for the attachment of tagging residues or other components. Enzyme-catalyzed transformations on pSi-immobilized substrates were found to proceed with similar relative activity and selectivity as those observed in solution. Our methodology allows covalent attachment and detachment to be implemented in the mass spectrometer without added matrix material, and should be applicable to a wide variety of analytes, chemical transformations, and washing conditions.

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