Supplementary Information for: Chemoselective enrichment for natural products discovery

Antoinette Y. Odendaal,[a‡] Darci J. Trader[a‡] and Erin E. Carlson[a,b]*

Departments of Chemistry[a] and Molecular and Cellular Biochemistry[b], Indiana University, 212 S. Hawthorne Dr., Bloomington, IN 47405

[‡] These authors contributed equally to this work.

Supplementary Information Contents

Methods:

Standard resin wash protocol.

Determination of resin loading capacity.

Demonstration of resin regeneration.

Protocols for acquisition and analysis of mass spectral data.

Supplementary Information Table S1. Loading capacities of synthesized resins.

Supplementary Information Figure S1. Illustration of the regenerative properties of the developed capture resins.

Supplementary Information Figure S2. Representative TIC and EIC traces of enriched alcohols.

Supplementary Information Table S2. Ratios of enrichment of captured compounds compared to non-captured compounds.

Supplementary Information Table S3. Ratios of enrichment of alcohols subjected to activated resin versus deactivated resin.

Supplementary Information Figure S3. Comparison of TIC data for *Streptomyces griseolus* extract material prior to and following alcohol-mediated enrichment.

Supplementary Information Figure S4. Demonstration of chemoselectivity during alcohol enrichment experiment from *Streptomyces griseolus* extract.

Supplementary Information Figure S5. Representative standard curve used for alcohol capture quantification.

Supplementary Information Figures S6-S9. ^{13}C NMR and IR characterization of the generated resins.

Methods

Standard Resin Wash Protocol.

Resin is transferred to a fritted vessel and rinsed as follows: CH_2Cl_2 (suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), THF (suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), DMSO/ CH_2Cl_2 (1:1, suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), THF (suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), DMSO/ CH_2Cl_2 (1:1, suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), CH_2Cl_2 (suspend resin in 2 mL for 10 min, rinse 2 x 2 mL), toluene (suspend resin in 2 mL for 5 min, rinse 2 x 2 mL), DMF (suspend resin in 2 mL for 5 min, rinse 2 x 2 mL), CH_2Cl_2 (suspend resin in 2 mL for 5 min, rinse 2 x 2 mL), hexanes (suspend resin in 2 mL for 5 min, rinse 2 x 2 mL), CH_2Cl_2/MeOH (3:1, suspend resin in 2 mL for 5 min, rinse 2 x 2 mL), $CHCl_3$ (3 x 2 mL; ensure that solvent has not become acidic or premature cleavage of the resin could result), CH_2Cl_2 (3 x 2 mL).

Determination of resin loading capacity.

For each of the resins, 200 mg was placed in a dry 20 mL scintillation vial. The resin was swollen with 4 mL of anhydrous CH_2Cl_2 under Ar. Next, 14 equiv of triethylamine relative to the initial loading capacity of the resin was added, followed by 10 equiv of the dichlorodialkylsilane. 4-Dimethylaminopyridine (1.1 equiv) was added and the vial was capped and allowed to agitate at room temperature for 4 h. The resin was transferred to a 10 mL biospin vial and rinsed under Ar with anhydrous CH_2Cl_2 (2 x 2 mL). Next, 3 mL of anhydrous CH_2Cl_2 was added to the resin and allowed to swell for 1 min. The resin was aliquoted into five 2 mL vials (40 mg of resin into each). One aliquot acted as a control, which was hydrolyzed as described previously in the manuscript Experimental section. To each of the vials was added 8.0

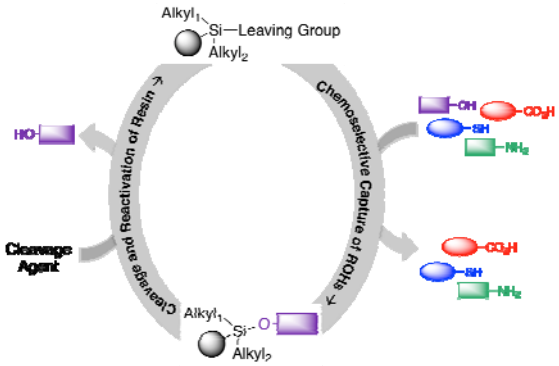
equiv of triethylamine and 3.0 equiv of Fmoc-aminopropanol dissolved in 1 mL of anhydrous THF. The reactions were agitated overnight at room temperature. Resin was transferred to a 2 mL biospin vessel and subjected to the standard wash protocol. The resins were dried overnight at room temperature in a vacuum desiccator at 30 mmHg. Coupled resin was transferred to polypropylene vials (5 mL). To the resin was added 500/50/50 µL (v/v) of a freshly prepared solution of THF/HF•pyridine(70/30 wt%)/pyridine (1.6 mmol of HF, 28 equiv relative to Si) and the reaction was gently agitated at room temperature for 3 h. To this was added 500 µL of TMSOMe (3.6 mmol, 83 equiv relative to Si) to quench excess HF and the resin was agitated for an additional 30 min at room temperature. The resin was washed with THF (3 x 1 mL x 10 minutes) and filtered over a 1 mL fritted polypropylene column into a 20 mL scintillation vial. The THF wash was concentrated under reduced pressure with no additional heating and the sample was redissolved in 10 mL of 2:1:1 H_2O/THF/MeOH. Analysis was performed by injection of 1 µL of this solution onto a LC-MS-TOF and comparing the observed peak area to that of standard curve data. The average of the three replicates was used as the loading capacity for all subsequent coupling experiments. Dimethylbenzyl siloxane resin loading capacity 0.15 mmol/g; diethylbenzyl siloxane resin loading capacity 0.18 mmol/g (**Supplementary Table S1**).

	First Capture Loading Capacity	**Second Capture Loading Capacity**
Dimethylbenzyl siloxane resin 1	0.15 mmol/g	0.16 mmol/g
Dimethylbenzyl siloxane resin 2	0.16 mmol/g	0.12 mmol/g
Dimethylbenzyl siloxane resin 3	0.14 mmol/g	0.13 mmol/g
Dimethylbenzyl siloxane resin Average	0.15 mmol/g	0.14 mmol/g
Diethylbenzyl siloxane resin 1	0.19 mmol/g	0.23 mmol/g
Diethylbenzyl siloxane resin 2	0.18 mmol/g	0.19 mmol/g
Diethylbenzyl siloxane resin 3	0.17 mmol/g	0.21 mmol/g
Diethylbenzyl siloxane resin Average	0.18 mmol/g	0.21 mmol/g

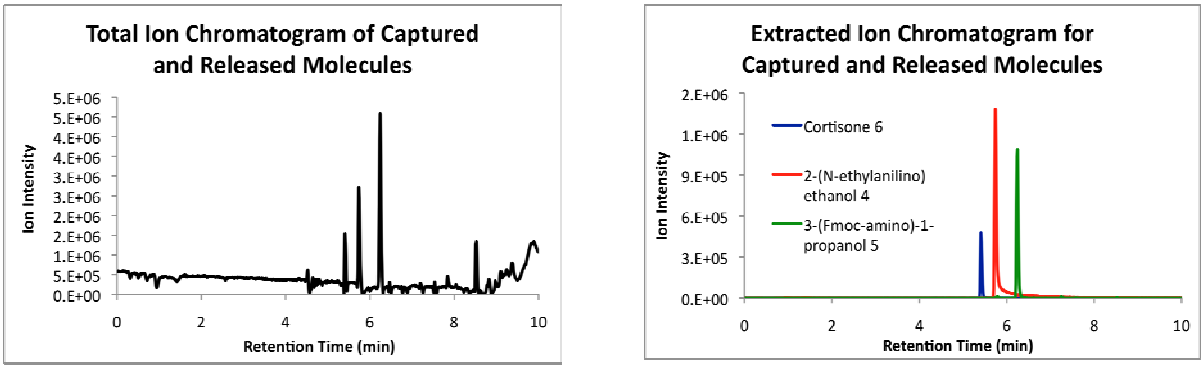
Supplementary Information Table S1. Loading capacities of synthesized resins.

Demonstration of resin regeneration.

Each resin was coupled as in the *Loading Capacity Experiment section*, with the exception that these resins had previously been coupled to alcohols and subjected to cleavage conditions resulting in regeneration of the benzyl alcohol. Averaging of three replicates gave the following loading capacities which were equivalent to those seen after the first round of alcohol capture and release: Dimethylbenzyl siloxane resin loading capacity 0.14 mmol/g; diethylbenzyl siloxane resin loading capacity 0.21 mmol/g (**Supplementary Table S1**).



Supplementary Information Figure S1. Illustration of the regenerative properties of the developed capture resins. Regeneration enables reuse of the solid-supported reagent in multiple cycles of alcohol enrichment.



Supplementary Information Figure S2. Representative TIC and EIC traces of enriched alcohols. Cortisone, 2-(*N*-ethylanilino)ethanol and 3-(Fmoc-amino)-1-propanol were subjected to enrichment with diethylchloro-benzylsiloxane resin in this experiment. **Left**. Total ion chromatogram of released molecules. Peak at t_R~8.5 min is a column contaminant. **Right**. Extracted ion chromatogram of the compounds that were captured and released.

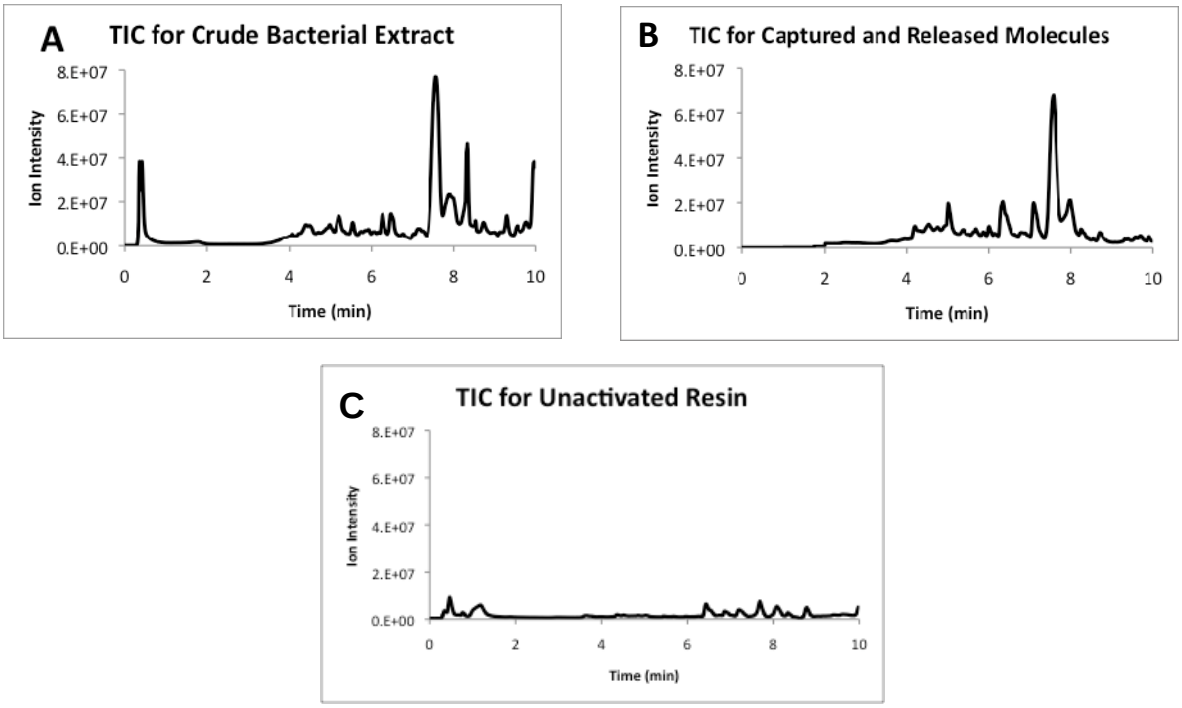
	Peak Area Prior to Capture	Peak Area after Capture
Androsterone	767815	579571
Cortisone	1091718	879095
Fmoc-Val-OH	6999770	165291
Tryptamine	5915145	12432
Cys-methyl ester	1109245	34786

	Peak Ratio Before Capture	Normalization Factor	Peak Ratio After Capture	Normalized Ratio After Capture
Androsterone/Fmoc-Val-OH	0.1097	9.1165	3.5064	31.9657
Androsterone/Trypamine	0.1298	7.7039	46.6193	359.1488
Androsterone/Cys-methyl ester	0.6922	1.4447	16.6610	24.0698
Cortisone/Fmoc-Val-OH	0.1560	6.4117	5.3185	34.1004
Cortisone/Trypamine	0.1846	5.4182	70.7123	383.1332
Cortisone/Cys-methyl ester	0.9842	1.0161	25.2715	25.6772

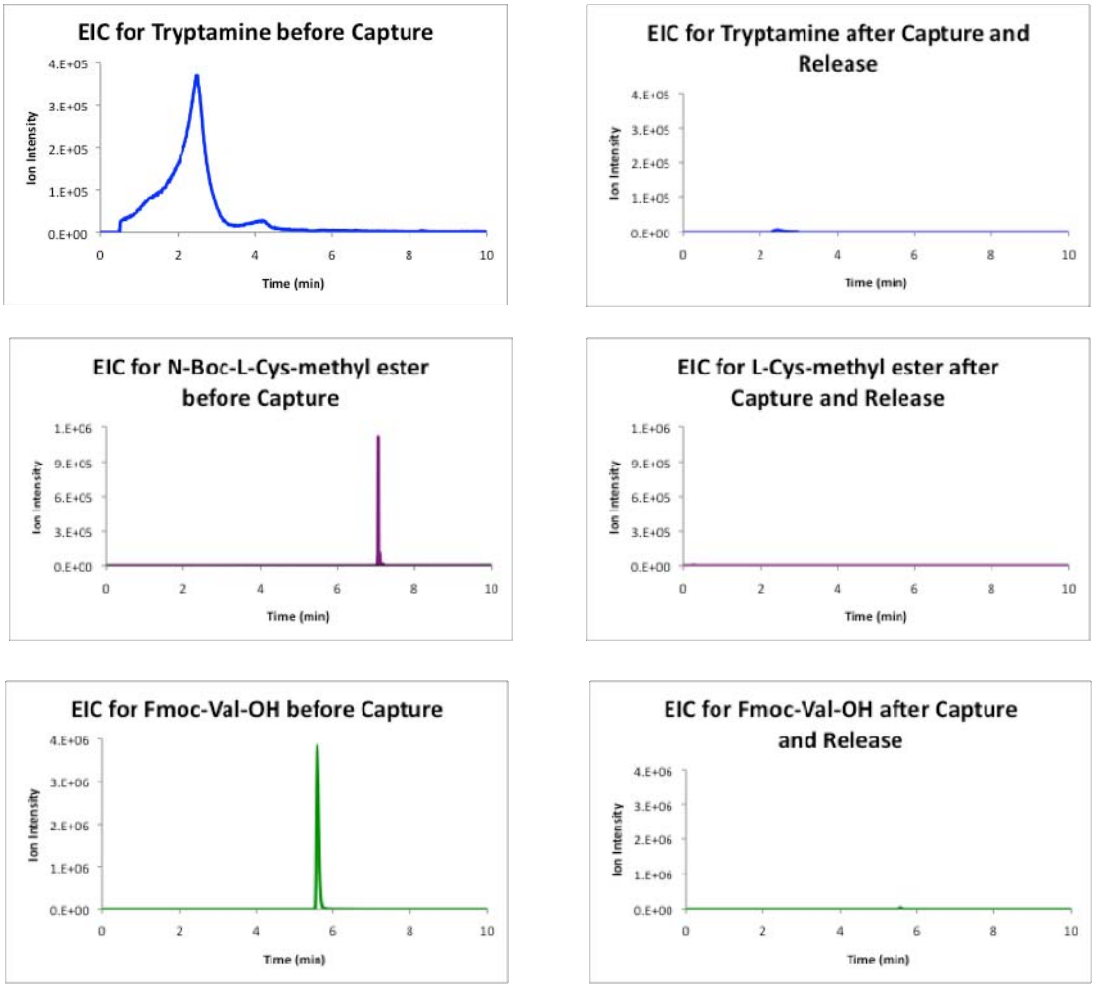
Supplementary Information Table S2. Ratios of enrichment of captured compounds compared to non-captured compounds. To illustrate that the alcohol-containing compounds are being dramatically enriched in comparison to the molecules containing other functional groups, we calculated the ratio of enrichment. The ratio of each alcohol to the chemoselective set of compounds was calculated and normalized. Normalization is required given that although an equivalent number of moles of each compound was used, the ionization efficiency of each compound is unique, making the observed peak areas dramatically different. Accordingly, the initial ratios were normalized to a 1:1 ratio and this factor was applied to the post-capture data. In all cases, following the capture and release protocol at least a 24-fold enrichment of the alcohols was seen in comparison to the non-alcohol compounds. These data were obtained from the *Streptomyces griseolus* extract enrichment experiment.

	Peak Area Following Exposure to Activated Resin	**Peak Area Following Exposure to Deactivated Resin**	**Ratio of Enrichment (Activated/Deactivated resin)**
Anisomycin	21199738	8185	2590
***trans*-Androsterone**	579571	5846	99.14
Cortisone	879095	5680	154.8

Supplementary Information Table S3. Ratios of enrichment of alcohols subjected to activated resin versus deactivated resin. The *Streptomyces griseolus* extract (containing the full chemoselective set and two additional known alcohols) was subjected separately to an activated resin and a deactivated control. The peaks areas obtained following performance of the release protocol from both resin samples are shown above. These data clearly illustrate that the observed enrichment of the alcohols is only a result of specific capture by the activated silane and not due to non-specific binding to the resin.



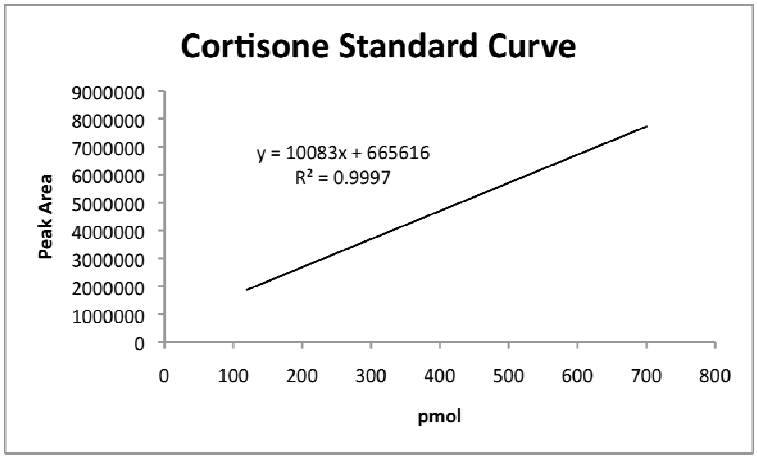
Supplementary Information Figure S3. Comparison of TIC data for *Streptomyces griseolus* extract material prior to and following alcohol-mediated enrichment. **A**. Total ion chromatogram for the extracted bacterial broth containing anisomycin. This bacterial extract was also spiked with a set of model compounds. **B**. Total ion chromatogram for the compounds enriched from the bacterial extract in panel A. Extracted ion chromatographs for captured alcohols are provided in the paper body (**Figure 2**). **C**. Total ion chromatogram following extract exposure to deactivated resin illustrating that alcohol enrichment results from specific interactions with the siloxyl-functionalized resin.



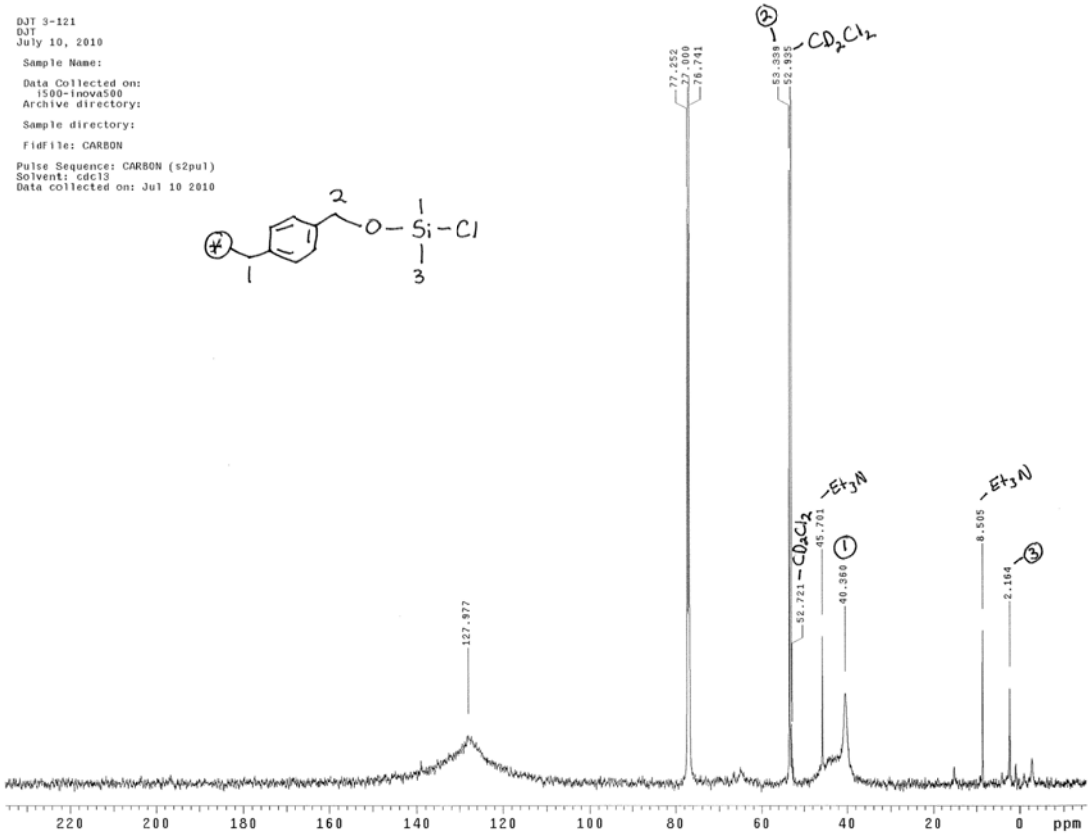
Supplementary Information Figure S4. Demonstration of chemoselectivity during alcohol enrichment experiment from *Streptomyces griseolus* extract. The left column represents the amount of each compound that was spiked into the bacterial broth. As the right column shows, these molecules were not captured, demonstrating that in a biological setting the diethylbenzyl siloxane resin retains its chemoselectivity for alcohols.

Protocol for acquisition of enrichment yields using LC-MS-TOF standard curve analysis.

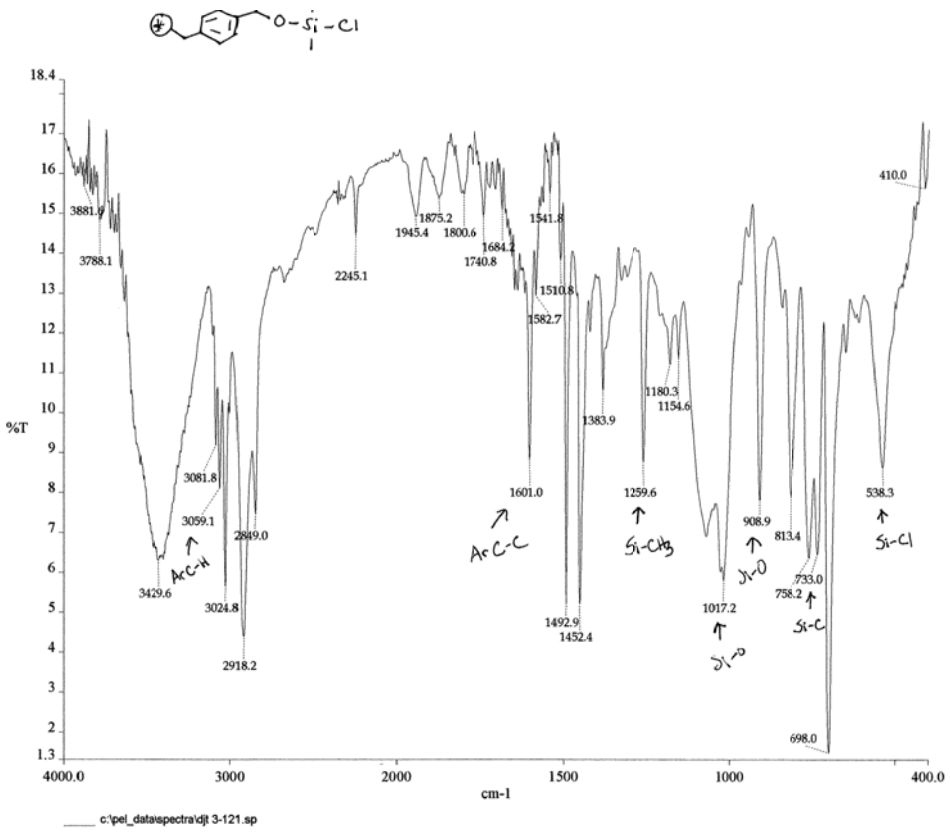
Each compound was dissolved in 2:1:1 H_2O:MeOH:THF to yield the concentrations required to provide 1000 pmol, 700 pmol, 560 pmol, 420 pmol, 280 pmol, 140 pmol, and 1 pmol in separate 1 µL injections into the LC-MS-TOF. For all compounds, the optimal fragmentation voltage was determined by assessment of the 700 pmol injection at 50V, 100V, 125V, 150V, 175V, 200V, 225V, and 250V. The fragmentation voltage yielding the highest ion intensity was selected. Following this analysis, all samples were run at each optimal voltage determined for each compound included in the sample. Standard curves were generated for all model compounds by running two independent sets of samples. A representative graph is shown for cortisone **(Supplementary Information Figure S5).** Unknown samples were quantified by comparison to the generated standard curves. All reactions were run in triplicate. An internal standard was added to each reaction mixture after the TMSOMe quench of the HF and used to standardize across the triplicate set (phenol, detected in UV trace at 220 nm, t_R = 3.2 min).



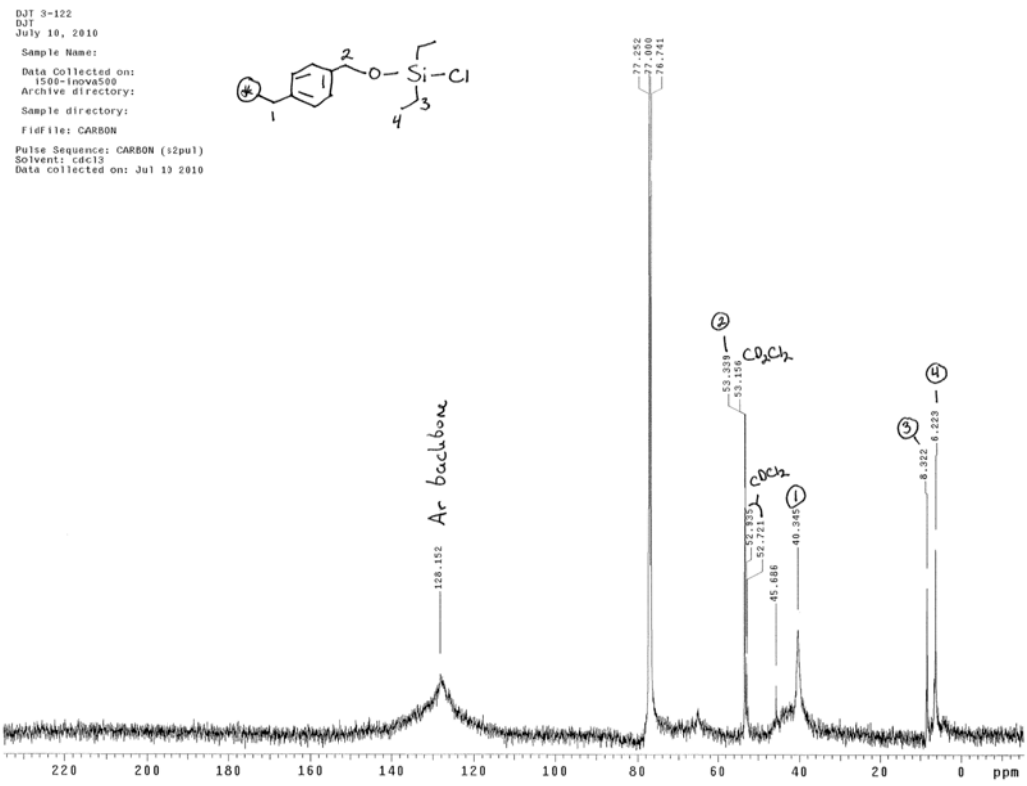
Supplementary Information Figure S5. Representative standard curve for alcohol capture quantification. Unknowns were quantified using the generated equation from each graph.



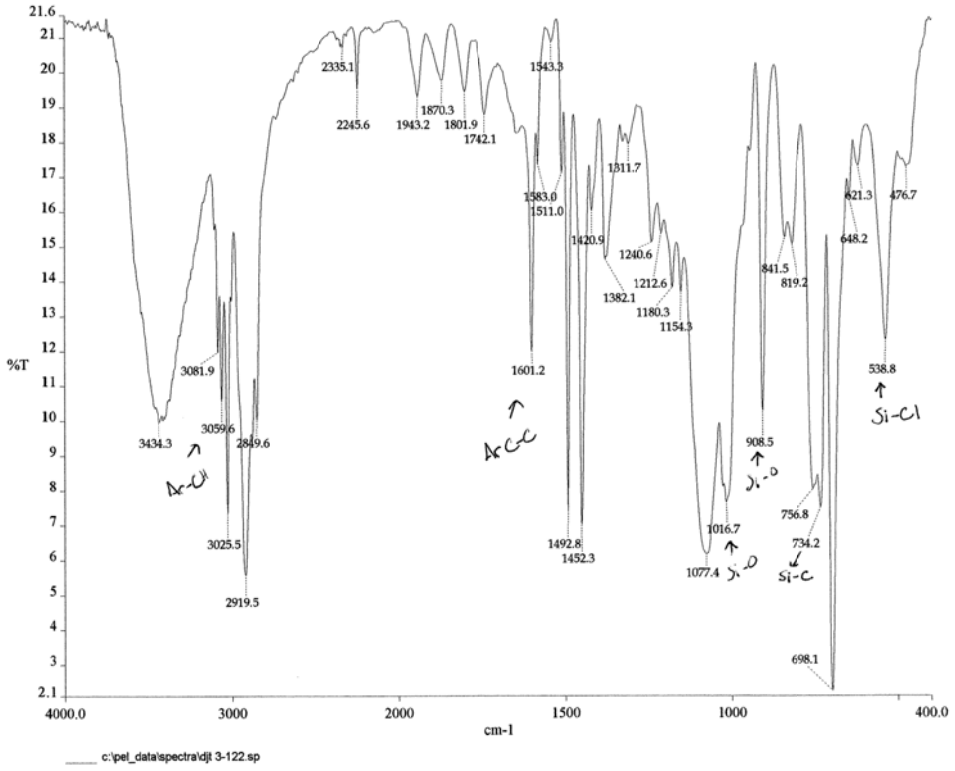
Supplementary Information Figure S6. ^{13}C gel-phase NMR of dimethylchloro-benzylsiloxane resin. Et_3N and CD_2Cl_2 present in spectra as complete drying of the resin results in hydrolysis. Both reagents are used in subsequent alcohol capture reactions making residual base from the activation inconsequential during enrichment protocols.



Supplementary Information Figure S7. FT-IR (KBr pellet) of dimethylchloro-benzylsiloxane resin.



Supplementary Information Figure S8. ^{13}C gel-phase NMR of diethylchloro-benzylsiloxane resin.



Supplementary Information Figure S9. FT-IR (KBr pellet) of diethylchloro-benzylsiloxane resin.