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# New functionalised silicas for highly selective cation exchange SPE purification in medicinal chemistry

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ABSTRACT

Functionalised silicas **2** and **3** are effective for the cation exchange SPE purification of basic molecules containing acid-sensitive functionalities, the selective separations of mixtures of basic compounds and accelerated reaction work-ups/product isolations.

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Recent years have seen a continuing drive for increased productivity from Medicinal Chemistry groups as one of many initiatives to optimise the chances of new medicines emerging from pharmaceutical company pipelines. New technologies need to be adopted to overcome bottlenecks within the drug discovery process, the most significant of which for medicinal chemists is compound purification, which can be tackled by specialist purification groups or more directly by the use of a number of continually emerging easy-to-use laboratory purification techniques.

Solid phase extraction (SPE) has rapidly become an essential tool in Discovery Medicinal Chemistry,[1] offering considerable operational benefits and time savings over traditional extractive work-up and chromatographic methods. Whilst utilised initially for high-throughput applications in lead discovery, this simple technique has now become widely-used for the purification of both high value single compounds and small focused compound arrays at both hit-to-lead and lead optimisation project stages. It is a technique ideally and equally suited to purifying milligrams through to multiple grams of product.

Strong cation exchange (SCX) is the most widely used method of SPE purification. Basic reaction products are typically retained by the SCX material, allowing non-basic impurities to be washed away, before the desired basic product is eluted ('catch and release'). Product purification can also be performed by the retention of basic impurities by the SCX material, with the non-retained, non-basic reaction product washed off and collected.

Products can be typically isolated in purities of >90%, generally rendering additional purification unnecessary before further synthetic steps. For screening compounds, SCX can deliver compounds of sufficiently high purity (97–>99%) for direct submission to screens; however, if the compound purity specification is not reached, the technique will generally have cleaned up the reaction mixture sufficiently to greatly facilitate additional purification by other techniques such as preparative HPLC.

Cation exchange materials based on both polystyrene[2] and silica are commercially available in easy-to-use cartridge formats. Silica adsorbents show excellent stability and flexibility to a wide range of both organic and aqueous solvents and do not swell; as such silicas have generally become the materials of choice for Medicinal Chemistry.

Commercially available cation exchange materials typically feature sulfonic acid functionalities attached to the support. Aryl sulfonic acids ($pK_a$ <1) are slightly more acidic than the alkyl variants ($pK_a \sim 1$). Alkyl carboxylic acids ($pK_a$ 4–5) are described as weak cation exchangers, though these materials have received little attention. Materials of this latter type add a degree of selectivity to the existing SCX technology, and our paper elaborates this

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aspect of cation exchange methodology with the introduction and application of new immobilised acids (Fig. 1).

Limitations of existing cation exchange technology include the strongly acidic nature of the sulfonic acids, which although responsible for the key ionic exchange with the basic products or reaction components to be retained, also have the effect of limiting the range of molecules which can be purified effectively—the presence of any acid-sensitive functionalities renders the technology unsuitable. In fact, SCX adsorbents are effective for the removal of *tert*-butoxycarbonyl (Boc) groups from a diverse range of protected amines[3] and alkyl/aryl sulfamides,[4] and *tert*-butyldimethylsilyl (TBDMS) groups from protected 1° and 2° alkyl ethers.[5]

Used in catalytic amounts, alkyl sulfonic acid functionalised silica has also been reported as a general method for the preparation of Boc-protected derivatives of amines, amino-esters and sulfonamides.[6] Sulfonic acid functionalised materials are also capable of catalysing additional reactions, such as the formation of pyrazoles from enol ketones and substituted hydrazines.[7] The potential for the sulfonic acid functionality present on SCX adsorbents to cleave acid-sensitive moieties and catalyse additional reactions has led to small compound arrays, involving purification by this method, being relatively limited in terms of additional functionalities.[7,8]

Existing SCX materials can also fail to differentiate adequately between different basicities of reaction components, for example, molecules containing a 2° amine and a 3° amine. Such mixtures are commonplace after a variety of standard synthetic steps including amine deprotections and reductive alkylation reactions.

We have developed the addition of free radicals to vinyl trialkoxysilanes to yield highly functionalised trialkoxysilanes, which are grafted onto existing silica frameworks, as depicted in Scheme 1, to provide novel functionalised materials of use for solving a variety of key industrial problems, including scavenging of metal and organic impurities from active pharmaceutical ingredients (APIs)[9] and as heterogeneous catalysts for chemical processes including oxidations and metal-mediated cross couplings.[10]

Figure 1 shows a selection of immobilised acids which were prepared using this approach, and which feature sulfonic acid **1**,[11] phosphonic acid **2** and carboxylic acid functionalities, leading to coverage of a p$K_a$ range from approximately 1–6. For this Letter, our efforts were focused on the use of immobilised phosphonic **2** and mercaptosuccinic **3** acids, for cationic exchange purifications.

Commonly used protecting groups include TBDMS and *tert*-butyl ($^t$Bu) for alcohols and phenols, and Boc for amines. Attempts to purify basic molecules containing these groups with immobilised sulfonic acids can result in partial or complete removal of the protecting group. Phosphonic acid **2** and mercaptosuccinic acid **3**, are however, effective for the catch and release of molecules such as those shown in Figure 2, all of which were caught and then released without any protecting group loss. Molecules containing tetrahydropyran (THP) groups have also been eluted through **3** without protecting group removal, allowing non-retained purification operations to be considered.

Amide formation, featuring Boc and $^t$Bu protecting groups on the amino and phenolic groups, respectively, proceeded smoothly with *N*-methylpiperazine as shown in Scheme 2. Use of excess amino acid **4** ensured complete consumption of the *N*-methylpiperazine and cation exchange treatment of the reaction mixture, in this case with **2**, resulted in only the desired amide product **5** being retained, with the excess amino acid, HOBt and *N*,*N'*-diisopropylurea by-product from the DIC coupling agent all being washed off with methanol. Piperazine amide **5** was eluted from the immobilised acid cleanly, in high yield and without loss of either of the

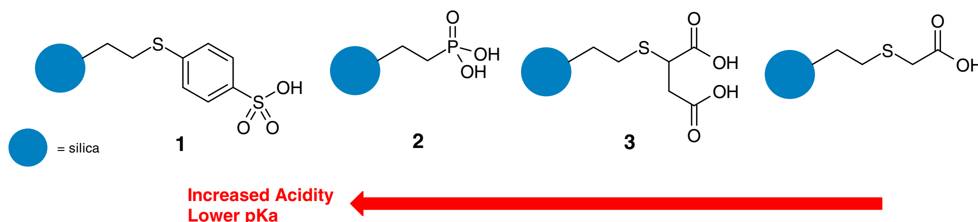
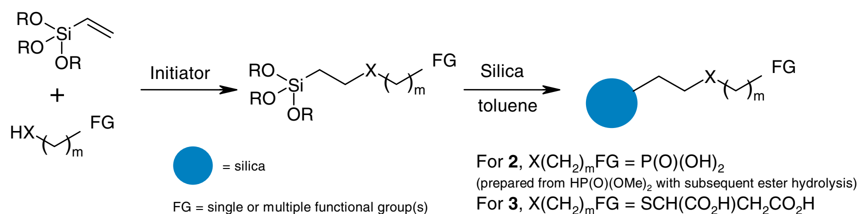



**Figure 1.**




**Scheme 1.**

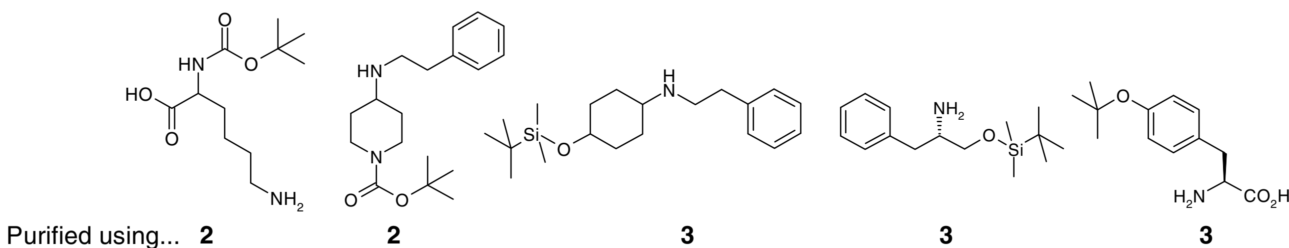
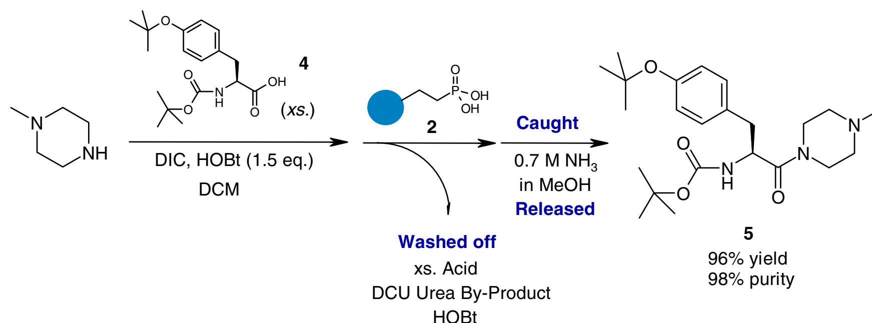



**Figure 2.**




**Scheme 2.**

protecting groups, by use of ca. 0.7 M ammonia in methanol solution.[12] Treatment of the amide product with immobilised sulfonic acids resulted in partial loss of both protecting groups almost immediately and complete removal of both protecting groups upon shaking overnight.

Preliminary screening of a range of basic compounds, including heterocycles and 1° and 2° amines, covering a p$K_a$ range of 5–11, revealed that **2** retained all compounds in the range, including pyridines and anilines. At lower p$K_a$, basic compound release from the cation exchange material was possible with ca. 0.35 M triethylamine in methanol solution,[13] whereas ca. 0.7 M ammonia in methanol solution was required as the p$K_a$ of the retained basic compound increased beyond 9. Mercaptosuccinic acid **3** failed to catch basic compounds including anilines and pyridines of lower p$K_a$ (5–6), but basic compounds above p$K_a$ 7–8 were retained. As with **2**, as the p$K_a$ of the caught basic component moved from lower to higher p$K_a$, the use of ammonia in methanol[12] was required for adequate compound elution rather than triethylamine in methanol.[13] We hoped to exploit these observations to perform selective separations of basic compounds and report here some of our initial results. A full investigation of these functionalised silicas for the separation of basic molecules will be reported in due course.

The separation of some 2° and 3° amine mixtures is possible using these cationic exchange materials of reduced acidity. *N*-Benzylaniline was readily separated from *N*-benzylethanolamine using **3**. *N*-Benzylaniline was not retained by **3** but was washed off within the methanolic loading solution, whereas *N*-benzylethanolamine was retained and only subsequently eluted with triethylamine in methanol solution. A weight recovery of >95% for the caught and separated compound was achieved. Acids **2** and **3** can also be used for the separation of basic product mixtures including cyclic 2° and 3° amines, and saturated and unsaturated heterocycles.

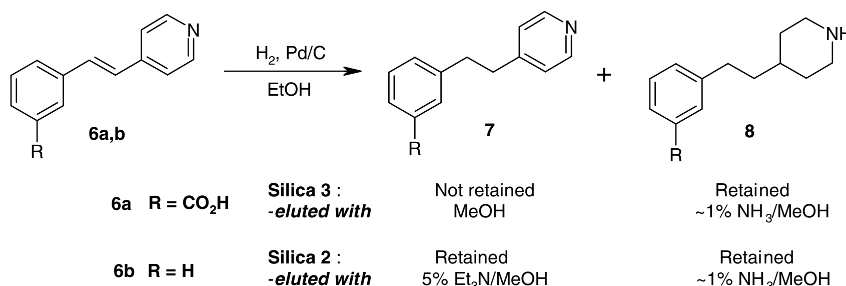
Hydrogenation of the central olefin along with the pyridyl moiety of the phenyl vinyl pyridine substrates **6a,b** shown in Scheme 3 did not always proceed to completion to give the desired phenethyl piperidine **8**, and this was often accompanied by significant amounts of the unwanted phenethyl pyridine **7**. Further treatment of the mixture was resisted due to the potential for reduction of the phenyl ring. Sulfonic acid-based SCX resulted in both products co-eluting, and no separation could be achieved.

Treatment of the R = H product mixture (from **6b**) with **2** resulted in both basic compounds being retained; however, the less basic pyridine product **7** was eluted selectively with triethylamine in methanol, allowing the more basic piperidine product **8** to be eluted cleanly with ammonia in methanol. With acid **6a**, use of **3** resulted solely in the more basic piperidine product **8** being retained, with the pyridine product **7** not retained but instead washed through with methanol. Piperidine **8** was eluted with ammonia in methanol solution. A 1:1 mixture of the two reaction products was used in these studies, and compound recoveries and purities were >95%.

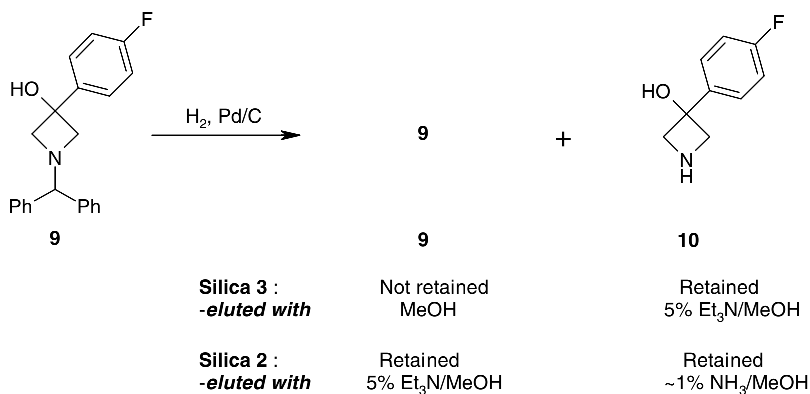
Deprotection of cyclic amines is another widely used and generally reliable hydrogenative synthetic step. Access to the synthetically-attractive 3-hydroxy-3-aryl azetidine building block **10** shown in Scheme 4 is best achieved through formation of the corresponding benzhydryl-protected azetidine **9** followed by hydrogenation of the amino protecting group. Unfortunately, the deprotection step sometimes failed to go to completion resulting in a mixture of 2° and 3° amines, which are extremely difficult to separate by conventional column chromatography or with SCX sulfonic acids.

Treatment of the mixture with either **2** or **3** allowed clean and high-yielding separation of the 2° and 3° amines. Both basic materials were caught by **2**, from which selective elution could again be achieved, using first triethylamine in methanol to release exclusively the starting material **9** followed by ammonia in methanol solution to release the desired 2° amine reaction product **10**. Both compounds were isolated in >95% purity.

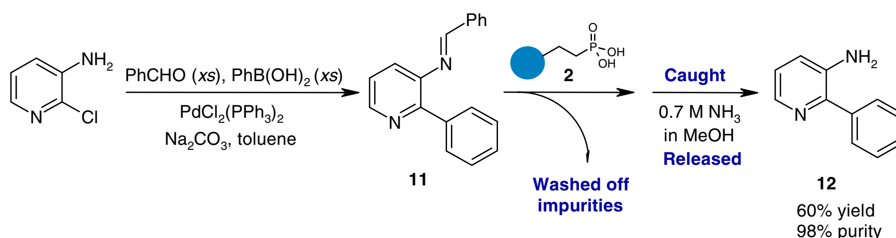
These functionalised silicas are also useful to simplify reaction work-ups and allow quicker access to purified products. For example, the synthesis of 2-phenyl-3-aminopyridine **12** is reported to proceed most efficiently from 2-chloro-3-aminopyridine by the use of an imine protecting group, which facilitates the desired Suzuki coupling step before being removed.[14] Product work-up




**Scheme 3.**




**Scheme 4.**




**Scheme 5.**

was achieved by extractive aqueous work-up, involving initial imine **11** cleavage with aqueous hydrochloric acid, followed in some cases by a crystallisation step. We have found that this intensive work-up protocol can be replaced by simply loading the crude Suzuki reaction product onto **2**, as shown in Scheme 5, resulting in the imine **11** being retained, purified, deprotected and finally released as the desired 1° aminopyridine reaction product **12** using ammonia in methanol solution as eluant. The product was isolated in a promising, unoptimised yield of 60% and in high purity, with no additional purification steps required.

A typical protocol for reaction purification using these functionalised cation exchange silicas is provided.[15]

In conclusion, we have shown the potential of functionalised silicas with modified acidities,[16] in comparison to the traditionally-employed sulfonic acid materials, for catch and release SPE purifications. These materials allow acid-sensitive functionalities such as Boc, TBDMS and $O^tBu$ to be retained during the purification of basic compounds. Use of the materials for the separation of basic products such as 2° and 3° amines and saturated and unsaturated nitrogen heterocycles has also been demonstrated, and further elaboration of this work is ongoing.

## Supplementary data

Example HPLC traces for the separations of the basic mixtures described are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.05.087.

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12. Commercially available 7 N ammonia in methanol solution (Aldrich) was diluted ×10 with methanol (~1% v/v solution).
13. Triethylamine (5 mL) was added to methanol (95 mL) to give a 5% v/v solution (~0.35 M).
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15. *Typical purification procedure using functionalised silicas for cation exchange*: The appropriate SPE cartridge containing 1 g of functionalised silica was conditioned by passing 10 mL of MeOH through it, either under gravity or with positive pressure or under controlled vacuum. The reaction mixture was next loaded onto the top of the cartridge in the solvent of choice (~1–2 mL solvent/0.050 g product). Loading onto the cartridge should typically be 5% by weight (reaction mixture: functionalised silica), though higher loadings are sometimes possible. Once the reaction mixture has been eluted through, a further 5 mL of methanol was passed through the cartridge. This 'load' fraction may contain unretained reaction components. The cartridge was washed with 2 × 10 mL of MeOH and fractions were collected. These 'wash' fractions should contain any further traces of unretained reaction components. *Basic product elution is dependent on the functionalised silica used.* The cartridge was eluted with either triethylamine in MeOH solution[13] (5% v/v; ~0.35 M, 10 mL) or ammonia in MeOH solution[12] (~1.2% v/v; ~0.7 M, 10 mL), or both, and the fractions collected. Fractions from these elution steps should contain any retained reaction components. The cartridge was finally rinsed using 10 mL of MeOH, which was also collected. Fractions were analysed to confirm that the desired purification had been achieved. The desired fractions were concentrated in vacuo to afford the purified product(s). In some cases, solvent quantities and wash steps may be reduced as the user deems most suitable for their particular purification process.
16. PhosphonicS™ cation exchange SPE materials POH1d **2** and STMAd **3** are available loose as well as in easy-to-use, pre-packed polypropylene 1 g or 10 g cartridges. Cartridges are also available for metal SPE purifications and organic scavenging—see www.phosphonics.com for further information.