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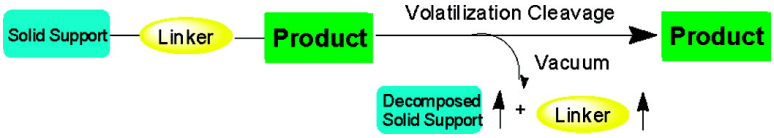

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"Volatilizable" Supports for High-Throughput Organic Synthesis

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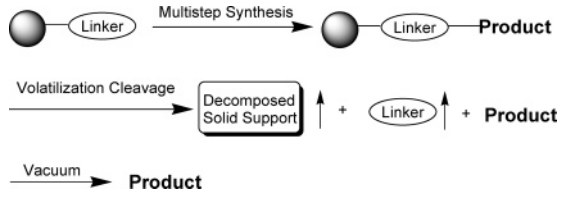
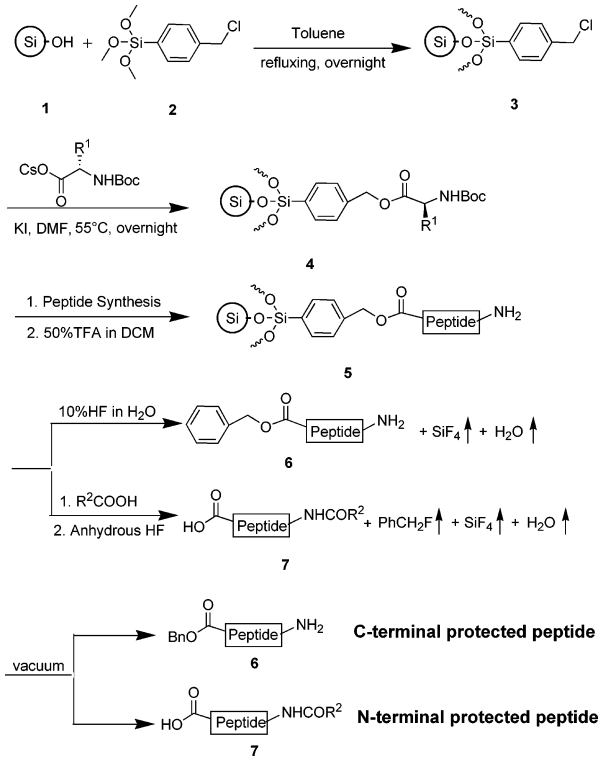
Solid-phase synthetic approaches, pioneered by Bruce Merrifield in 1963,[1] in conjunction with combinatorial methods developed over the past 20 years are highly efficient means for the production of tens of thousands of individual compounds and/or mixture-based libraries made up of millions of compounds.[2,3] In conjunction with high-throughput biological screening, combinatorial approaches are now routinely used in the search for new pharmaceutical lead compounds. All currently utilized solid-phase organic synthetic supports require that the desired synthetic compound(s), whether they are low-molecular weight acyclic or heterocyclic compounds, peptidomimetics, peptides, or oligonucleotides, etc., must be cleaved from the support and, as a final step, separated from the spent support. This final separation step in the synthesis of very large numbers of compounds, typically exhaustive extraction followed by filtration or centrifugation, inherently results in reduced yields and increased costs. We herein report one solution to this problem. This is exemplified by the solid-phase synthesis on "volatilizable" solid supports and linkers that can be completely removed by their ultimate decomposition and complete "volatilization" during the final cleavage step of the synthetic process. This approach eliminates the final extraction step and yields solely the desired synthetic product(s) in the reaction vessel (Scheme 1).

While many possible fully "volatilizable" solid supports can be envisioned, silica gel, one of the first solid supports used for the synthesis of peptides,[4] is used herein to illustrate these concepts. Silica gel is a well characterized, widely available, and inexpensive solid support that can be functionalized at excellent substitution levels.[5] In the example presented, silica gel was first functionalized with *p*-chloromethylphenyltrimethoxysilane in refluxing toluene to form the desired chloromethylbenzyl-functionalized silica gel **3** (see Supporting Information). The initial Boc-amino acid was introduced by the treatment of this functionalized support with the cesium salts of Boc amino acids in DMF in the presence of KI at 55 °C overnight to yield the support-bound Boc-amino acid **4** (loading: 0.7 mmol/g). Selective C-terminal and N-terminal protected peptides were then synthesized on this "volatilizable" support as shown in Scheme 2. Parallel solid-phase synthesis was carried out using the "teabag" approach.[6] Following removal of the N-terminal Boc group with 50% trifluoroacetic acid in dichloromethane, protected amino acids **4** were sequentially coupled using standard Boc-peptide synthesis chemistry (Boc/TFA/DIC)[7] to afford the resin-bound peptide **5**. The silica gel portion of the benzyl ester-linked peptide **5** was completely decomposed by treatment with 10% hydrofluoric acid (pH 4.3) for 1 h at room temperature. This yielded the C-terminal benzyl ester-protected peptide **6**, tetrafluorosilane, and water. Following solvent removal by either rotary evaporation or lyophilization (HF and tetrafluorosilane are readily trapped and/or decomposed by in-line traps containing solid CaO), the desired protected peptides were

Scheme 1. Concept of Solid-Phase Organic Synthesis on "Volatilizable" Support and Linker



Scheme 2. Selective Synthesis of C- and N-Terminal Protected Peptides on "Volatilizable" Support and Linker



obtained as the sole products remaining in the reaction vessel in excellent yields and purities (Table 1, **6a**−**e**). In a separate experiment, support-bound **6** was N-acylated with phenyacetic acid. The silica gel was volatilized in this instance by treatment with anhydrous HF for 1.5 h at 0 °C. Following evaporation of the anhydrous HF with a gaseous nitrogen stream, the N-terminal modified peptide **7** was obtained in excellent yield and purity following lyophilization (Table 1, entries **7a**−**c**). The central feature of this strategy is the ease in which functionalized silica gel is decomposed through Si−O−Si and Si−Aryl bond cleavage into volatile constituents (SiF_4 and water) under the influence of aqueous or anhydrous hydrogen fluoride. Thus, this flexible approach enables one to rapidly produce either C- and N-terminal protected peptide

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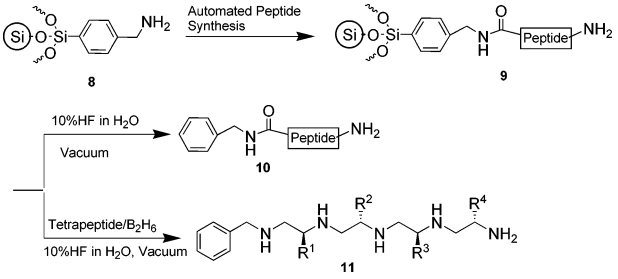
Table 1. C-Terminal and N-Terminal Protected Peptides and Chiral Polyamines Synthesized on "Volatilizable" Supports

	product (%)	yield[a] (%)
6a	Ala-Phe-OBzl	93
6b	Leu-Phe-OBzl	95
6c	Leu-Ala-Phe-OBzl	91
6d	Val-Ala-Phe-OBzl	92
6e	Vla-Leu-Ala-Phe-OBzl	94
7a	Phenylacetyl-Val-Ala-OH	96
7b	Phenylacetyl-Phe-Val-Ala-OH	98
7c	Phenylacetyl-Asn-Leu-Vla-Ala-OH	92
10a	Phe-Gly-Arg(Pbf)-Ala-benzyl amide	89
10b	Ala-Gly-Gly-Arg(Tos)-benzyl amide	90
11	reduced (Tyr-Tyr-Phe-Pro-benzyl amide)	81

[a] Percent yields are based on the weight of crude material and are relative to the loading of the first amino acid on silica gel resin.

Scheme 3. "Volatilizable" Solid Support Synthesis of Amide Protected Peptides and Chiral Polyamines



fragments by the selective treatment of the bound compounds with either aqueous or anhydrous HF. The majority of commonly used amino acid side chains, as well as C- and N-terminal protecting groups, including O-benzyl, benzyloxycarbonyl, Fmoc, tosyl, etc., but not Boc, are stable to aqueous HF. This enables fully protected peptides to be generated for use in the synthesis of larger peptides and proteins by segment coupling and/or the preparation of libraries of peptidomimetics.

The automated synthesis of individual compound arrays in a 96-well format using silica gel as the volatilizable solid support was also examined. Utilizing an automated centrifuge-based synthesizer,[8] Fmoc amino acids were coupled to benzylamine-linked silica gel support **8** using standard Fmoc peptide chemistry (Fmoc/piperidine/DIC)[7] to yield resin-bound peptide **9** Scheme 3). Following volatilization with 10% aqueous hydrofluoric acid and lyophilization, the desired C-terminal and side-chain-protected peptides were obtained as the sole products in each well (Table 1, **10 a**−**b**). The 96- or 384-well plates used can serve not only as the reaction vessels for combinatorial array synthesis but also as "mother" plates for later storage and/or distribution.

Chiral polyamines can be obtained by the exhaustive reduction of support-bound peptides (see ref 9 and citations therein). These, in turn, have been used as templates for the diversity oriented synthesis (DOS) of low-molecular weight acyclic and heterocyclic compounds.[9] We examined the reduction of silica gel-bound peptides to their corresponding chiral polyamines. The silica gel-bound L-Tyr-L-Tyr-L-Phe-L-Pro-benzyl amide was reduced with borane to yield the desire mono N-benzylated chiral pentaamine **11** in excellent yield and purity following volatilizable cleavage with 10% aqueous hydrofluoric acid and lyophilization (Table 1, entry 11).

In summary, we have presented here an innovative solid-phase approach for the synthesis of low-molecular weight acyclic, heterocyclic compounds, and peptides on functionalized silica gel as an example illustrating the concept of "volatilizable" solid supports and linkers. The solid support and linker were completely removed by their decomposition and ultimate "volatilization" during the final cleavage step in the synthetic process to yield solely the desired synthetic product(s) in the final reaction vessel.

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Supporting Information Available: Experimental methods; LC-MS of representative products. This material is available free of charge via the Internet at http:/pubs.acs.org.

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