Synthetic Methods

DOI: 10.1002/ange.200700833

Tetraarylphosphonium Salts as Soluble Supports for the Synthesis of Small Molecules**

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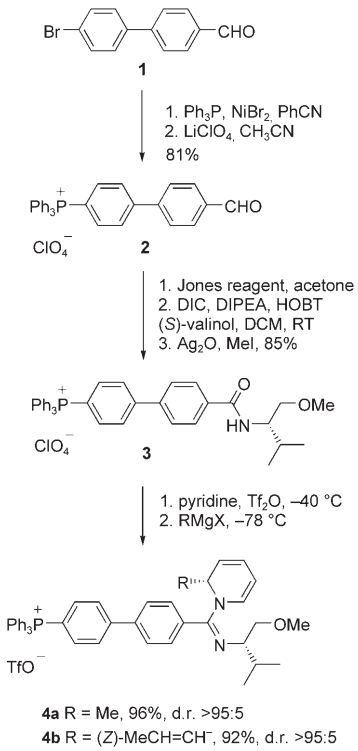
Several methods are currently available for the synthesis of small-molecule and peptide libraries on solid supports.[1] Since the pioneering work of Merrifield,[2] many researchers have exploited the use of solid supports for small-library synthesis.[3] Significant improvements have been made in this area, especially in the design of new supports and linkers to increase the scope of this technology.[4,5] Historically, a major challenge associated with solid-phase synthesis has been to merge homogeneous reaction conditions with the practical operational advantages of solid-phase chemistry. Some contributions towards this end include soluble polymer supports (for example, linear polyethylene glycol (PEG)),[6,7] fluorous-phase systems,[8] ionic-liquid supports,[9] and other tags.[10]

We have recently become interested in using tetraarylphosphonium salts as new solubility-control groups (SCGs) for reagents, ligands, and catalysts.[11] The main advantage of these groups is their lower molecular weight (≈ 450) when compared to polymeric materials (≥ 3000). This results in loadings that can be at least ten times higher than those with other traditional supports. The solubility of these salts is predictable with several types of reagents that are attached to them. We sought to exploit the solubility properties of the salts for the development of a complementary liquid-phase small-molecule synthesis that is cost-effective and versatile. We have previously shown that SCGs are soluble in polar solvent systems (CH_2Cl_2, CH_3CN, DMSO, DMF) and their precipitation is induced upon adding less polar solvents (hexane, Et_2O, toluene). Herein, we demonstrate that tetraarylphosphonium salts can be used as new soluble supports for small-molecule synthesis. The proof of concept will be illustrated by the synthesis of 2-substituted piperidine derivatives, which involves Grignard addition to a supported chiral pyridinium salt. Finally, the limits of the support will be established through peptide synthesis.

We have recently reported that the addition of Grignard reagents to a chiral pyridinium imidate salt led to 2-substituted dihydropyridine derivatives. As this chemistry involves the manipulation of relatively sensitive dihydropyridines, we envisioned that it would constitute a good test case for demonstrating the proof of concept of the phosphonium-supported chemistry. The supported chiral auxiliary[12] was prepared from 4-bromobiphenyl-4′-carboxaldehyde and triphenylphosphine (Scheme 1). Anion exchange, Jones oxidation, amide formation, and protection led to the desired supported chiral auxiliary in 69% overall yield and without any purification by chromatography. We next examined the amide electrophilic activation of **3** with triflic anhydride in the presence of pyridine under the previously reported conditions.[12] We were pleased to observe the complete formation of *N*-imidate pyridinium species within 3 h at room temperature. Subsequent nucleophilic addition of Grignard reagents led to the formation of the corresponding 1,2-dihydropyridine with high regio- and diastereocontrol. The products, which



Scheme 1. Synthesis of supported 2-substituted dihydropyridines. DIC: 1,3-diisopropylcarbodiimide; DIPEA: *N*,*N*-diisopropylethylamine; HOBT: 1-hydroxybenzotriazole; DCM: dichloromethane; Tf: trifluoromethanesulfonyl.

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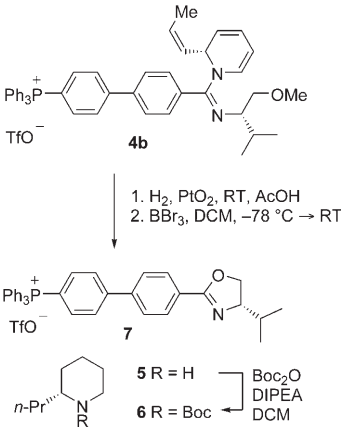
[**] This work was supported by NSERC (Canada), VRQ, Univalor, the Canada Research Chair Program, and the Université de Montréal. D.M. is grateful to the Université de Montréal and NSERC (ES D) for a postdoctoral fellowship.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

were isolated by precipitation/filtration upon adding diethyl ether, were obtained in higher yields than the corresponding unsupported examples that were reported previously[12] (96 vs. 77% when R = Me and 92 vs. 61% when R = (*Z*)-CH_3CH=CH). This improvement in yields can be accounted for by the difficulty of isolating relatively unstable, polar, unsupported dihydropyridine derivatives such as **4**.

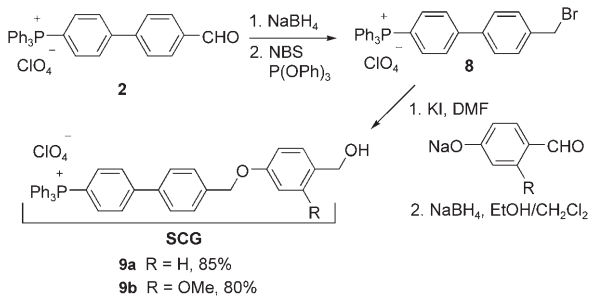
The cleavage of the substituted piperidine derivatives could be accomplished under the previously described conditions for the synthesis of unsupported product (Scheme 2). For example, *N*-Boc (−)-coniine (**6**) was readily accessed



Scheme 2. Cleavage of the support. Boc: *tert*-butoxycarbonyl.

from **4b** by hydrogenation using Adam's catalyst followed by cleavage of the amidine with BBr_3 and final Boc protection of the free amine (Scheme 2). The by-product oxazoline **7** could be easily removed by precipitation upon addition of diethyl ether and filtration. Flash chromatography of the crude filtrate gave **6** in 54% yield from amide **3**. The synthesis on the phosphonium support is more effective than the previously reported one (47 vs. 30% overall yield).

To test the efficiency of the phosphonium group in controlling the solubility of an attached substrate as a function of its molecular weight, a series of small peptides was synthesized. Two new SCG supports, based on the Wang (**9a**, SCG-W)[13] and Sasrin (**9b**, SCG-S)[14] resin structures, were synthesized starting from aldehyde **2** (Scheme 3).



Scheme 3. Synthesis of Wang (**9a**) and Sasrin phosphonium salts (**9b**). NBS: *N*-bromosuccinimide.

The main advantage of using these new supports in a reaction sequence is the ability to follow each step by traditional analytical techniques, in particular HPLC, mass spectrometry (MS), TLC, and NMR spectroscopy, and isolate the reaction product by a precipitation/filtration procedure. In this case, completion of the coupling event could be monitored and each intermediate of the growing peptide chain could be characterized. Furthermore, because each reaction was carried out under homogeneous conditions, the use of the large excess of reagents needed in solid-phase chemistry was not necessary. Usually a near-stoichiometric amount of the amino acid was used (1.2 equiv).

As the final cleavage was achieved under acidic conditions, 9-fluorenylmethoxycarbonyl/*tert*-butyl (Fmoc/*t*Bu) chemistry was successfully applied. Anchoring of the first Fmoc-protected amino acid (1.2 equiv) was performed in quantitative yield with a 4-(*N*,*N*-dimethylamino)pyridine (DMAP)/DIC[15] coupling in CH_2Cl_2. At the end of the reaction, Celite (1:5 w/w) was added and the product was precipitated upon addition of Et_2O. The product (absorbed on Celite) was then isolated by filtration, and washed with 1M HCl, water, toluene, and Et_2O. This workup gave quantitative recovery of the product in high purity (see Figure 1). The

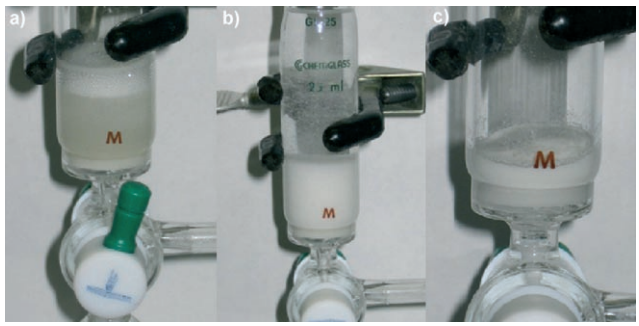


Figure 1. Typical reaction and workup for peptide synthesis. a) Coupling reaction (Celite added); b) precipitation of phosphonium-supported peptide on Celite upon ether addition; c) isolation of phosphonium-supported peptide (on Celite) after filtration and washing. The Celite-absorbed product is used directly in the next coupling step.

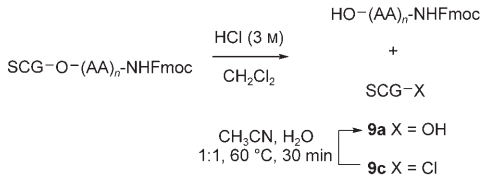
following steps were iterative: deprotection of the Fmoc group with piperidine (50% in CH_2Cl_2), then peptide coupling with an *N*-Fmoc-protected amino acid, mediated by HOBT/DIC/DIPEA.[16] Application of the standard workup afforded quantitative recovery of the product with acceptable purity.[17] It should be pointed out that the product on Celite could be carried throughout the entire sequence without purification.

The cleavage of the peptide from both SCG supports was considered separately. For the SCG-W, two reagents were used: trifluoroacetic acid (TFA; either 10% in CH_2Cl_2 or 100%) and HCl (3M in CH_2Cl_2). Under these conditions, *N*-Fmoc peptides were synthesized in good yields and purities (Table 1, entries 1–10). However, only the use of HCl allowed the isolation and recovery of a clean tetraarylphosphonium species that could be converted back to the starting SCG-W in >95% yield (Scheme 4). In the case of the SCG-S support,

Table 1: Synthesis of C-terminal peptides on phosphonium supports.

Entry	Product	Cleavage condition[a]	Yield [%][b]
1	Ala-Phe-Val-NHFmoc	10% TFA	80[c]
2	Ala-Ala-Ala-NHFmoc	10% TFA	75
3	Ala-$(Ala)_3$-Ala-NHFmoc	10% TFA	50
4	Ala-Phe-Ser-NHFmoc	100% TFA[d]	80[e]
5	Ala-Phe-Val-NHFmoc	HCl (3 M)	80[c] (70)[f]
6	Phe-Ala-Ala-NHFmoc	HCl (3 M)	67[e] (60)[f]
7	Ala-Phe-Ser-NHFmoc	HCl (3 M)[g]	70[e] (70)[f]
8	Ala-Ala-Ala-NHFmoc	HCl (3 M)	95 (95)[f]
9	Ala-Ala-Ala-Val-NHFmoc	HCl (3 M)	80 (73)[f]
10	$(Ala)_3$-Val-Gly-NHFmoc	HCl (3 M)	74[h] (70)[f]
11	Ala-Phe-Ser(Ot-Bu)-NHFmoc	1% TFA	81[e]
12	Ala-Ala-Ala-NHBoc	1% TFA	60[i]
13	Ala-Ala-Ala-NH_2·HCl	HCl (4 M)[j]	98[k] (80)[f]
14	Ala-Ala-Ala-NH_2·TFA	100% TFA[d]	96[k]
15	Locustakinin (six amino acids)[l]	100% TFA[m]	80[k]

[a] CH_2Cl_2 as solvent. [b] Purity >95% by HPLC. [c] After trituration with CH_3CN. [d] Anisole (5%) was added as a scavenger. [e] After chromatography on silica gel. [f] The yield in parentheses refers to that of the recovered phosphonium support. [g] Conditions for the cleavage: 40°C for 36 h. [h] After chromatography on C18. [i] Purity >95% by NMR spectroscopy. [j] Dioxane was used as solvent. [k] Phosphonium **9b** was used as soluble support. [l] Ala-Phe-Ser-Ser-Thr-Gly-NH_3·TFA. [m] Anisole (4%) and 1,2-diethanethiol (1%) were added as scavengers.



Scheme 4. Recovery of the soluble support. AA: amino acid.

very mild cleavage conditions (1% TFA in CH_2Cl_2) afforded fully protected *N*-Fmoc and *N*-Boc peptides in good yields (Table 1, entries 11 and 12). Harsher conditions with *N*-Boc peptides using **9a** (4 M HCl in dioxane or 100% TFA) afforded peptide salts in quantitative yields of isolated products (Table 1, entries 13–15). The peptides were characterized by reversed-phase HPLC.[18]

Low-polarity peptides (retention time >12 min on reversed-phase silica) were readily isolated by selective precipitation of the cleaved support (Table 1, entries 1, 4–7, and 11). The peptides were quantitatively recovered upon concentration of the filtrate by HPLC with an average purity of 90%. Further purification by silica-gel flash chromatography or trituration with CH_3CN gave a pure final product. Polar peptides (retention time <12 min) were purified by reversed-phase column chromatography after support cleavage (Table 1, entries 2 and 3). Finally, the amino acid salts were isolated simply by aqueous extraction and lyophilization (Table 1, entries 14 and 15). In many cases, the peptide precipitated upon cleaving the support, and thus purification by filtration was achieved (Table 1, entries 8–10 and 13). The scope of the methodology was demonstrated by the synthesis in high yield of the biologically active hexapeptide locustakinin (Table 1, entry 15).[19]

Although the synthesis of longer peptides was attempted, the solubility of the resulting species required the use of more polar solvents. For example, the syntheses of the phosphonium-supported hepta(alanine) and nona(alanine) could be accomplished, but DMSO had to be used to dissolve the phosphonium-supported peptide. Moreover, the precipitation/isolation sequence was not as straightforward, thus making this technique less practical for peptides containing seven amino acids or more.

In summary, we have developed a new practical synthesis that uses tetraarylphosphonium salts as solubility-control groups for small-molecule synthesis. This technique is a hybrid approach between solution- and solid-phase syntheses, which exploits the main advantages of both methodologies without the requirement of using special solvents. Advantages compared to known systems include higher loadings, ease of characterization, reagent compatibility of the support, and predictable solubility properties.

Experimental Section

General procedure for coupling: Support **9a** or **9b** (0.05–0.1 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (0.5–1 mL). *N*-Fmoc-protected amino acid (0.06–0.12 mmol, 1.2 equiv), DMAP (1.3–2.5 mg, 0.01–0.02 mmol, 0.2 equiv), and DIC (10–20 mL, 0.06–0.12 mmol, 1.2 equiv) were added. The solution was stirred at room temperature for 2 h. The reaction was monitored by electrospray MS and HPLC analysis. Celite (250–500 mg) was added and the product was precipitated by the addition of Et_2O (2.5–5 mL). The solid was isolated by filtration, and washed with Et_2O (5×10 mL), H_2O (2×10 mL), 1M HCl (2×10 mL; only in the case of **9a**), toluene (2×10 mL), and Et_2O (5×10 mL). The crude product absorbed on Celite was used directly in the next coupling step. The crude product could be extracted from Celite with MeOH/CH_2Cl_2 (1:9).

Received: February 23, 2007
Published online: May 22, 2007

Keywords: asymmetric synthesis · chiral auxiliaries · combinatorial chemistry · peptides · soluble supports

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