Synthesis of L-Octaarginine through Microencapsulated Palladium-Catalyzed Allyl Ester Deprotection

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Abstract: Octaarginine has been described as a molecular transporter. We report a useful synthesis of orthogonally protected L-octaarginine by using a method based on a microencapsulated palladium catalyst. Known palladium-based methods for allyl ester deprotection have been modified to facilitate purification of the unprotected intermediates. This improvement in the purification step has also been tested with a variety of allyl α-amino esters and allyl α,β-unsaturated esters.

Key words: peptides, palladium, protecting groups, esters

The unprotected octamer of L-arginine[1] has been described as a molecular transporter derived from the $Tat_{49–57}$ sequence of HIV-1.[2] Although a convergent synthesis of the unprotected R_8 has been previously reported by Wender,[3] this successful approach suffers from the need to maintain a protected pro-guanidinium scaffold throughout the entire convergent procedure. Here, we describe an alternative synthesis that allows this drawback to be circumvented while also simplifying the purification of the intermediates.

Allyl ester was selected as a convenient protecting group of the α-amino acids and peptides. Palladium-based deprotection has been previously established,[4] although chromatography purification could be tedious and may reduce both the yield and the scale of the entire procedure. In this context, heterogeneous recyclable catalysts offer a number of advantages with respect to safety and to environmental and economy concerns. Easily available palladium(II) acetate microencapsulated in polyurea, [PdEnCat],[5] has been demonstrated to be a useful recyclable catalyst that is active in Suzuki, Heck, Stille, Sonogashira, and carbonylation reactions.[6] This catalyst has also been successfully used in the hydrogenation and transfer hydrogenation of a range of functional groups.[7]

However, few examples on the use of microencapsulated palladium for functional group deprotection can be found[7d] and none are available on allyl esters. Recently, a report was published on allyloxycarbonyl (Alloc) removal catalyzed by palladium immobilized on a silicon surface.[8]

We wished to develop a methodology involving supported catalysis that could be applied to general allyl ester removal with the aim of simplifying or, ideally, removing the chromatographic purification step. Here, we report the results of our investigation and demonstrate that, once optimized, the approach could be used in the synthesis of orthogonally protected L-octaarginine.

The conditions used for microencapsulated palladium catalyzed cleavage of allyl esters were first optimized and the scope of the methodology was then assessed with a range of allyl esters of N-protected α-amino acids, α,β-unsaturated carboxylic acids, and various representative commercial carboxylic acids. A water-soluble phosphine ligand was also used to simplifying the purification procedure, which involved straightforward filtration of the palladium nanocapsules and extraction with water. In this sense, the quaternary ammonium derivative 2-(dicyclohexylphosphino)ethyltrimethylammonium chloride (DCHT) served effectively. Other water-soluble phosphine ligands were tested, giving similar results to those obtained with DCHT; the ultimate choice of reagent was therefore based on cost. Consistent with the idea of aqueous workup, phenylsilane was chosen as the allyl acceptor nucleophile.

Allyl esters were prepared by coupling with allylic alcohol mediated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP). Ester cleavage using microencapsulated palladium catalysis proceeded as summarized in Table 1.[9]

Short reaction times were sufficient for consumption of the starting materials at room temperature, and the yields were higher than 60% (Table 1). The only exception to this general trend was found for tryptophan (**1b**), likely caused by the free indole nitrogen. For the purpose of the synthesis described below, the 82% yield for allyl ester deprotection of Fmoc/Pbf-protected L-arginine (**1a**) is notable. The allyl deprotection conditions were compatible with Fmoc and Boc carbamates, and with *tert*-butyl esters **1a**–**f**. These results suggest that this methodology is useful for the selective cleavage of allyl esters from aliphatic and α,β-unsaturated carboxylic acids bearing *tert*-butyl and fluorenyl protecting groups.

Workup of the reaction was performed by simple filtration of the catalyst followed by liquid–liquid extraction. How-

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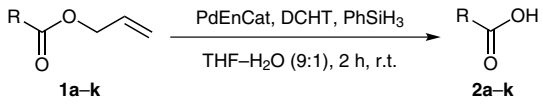
Table 1 [Pd EnCat] Catalyzed Deprotection of Allyl Esters



1a–k 2a–k

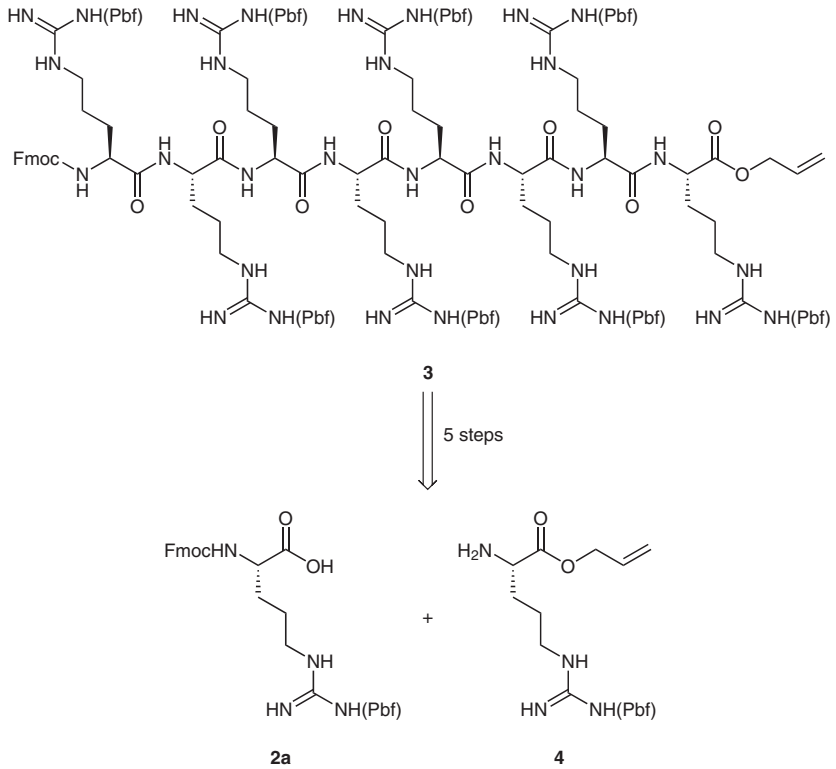
	1	2	Yield (%)
a	Fmoc-L-Arg(Pbf)-OAll	Fmoc-L-Arg(Pbf)-OH	82
b	Boc-L-Trp-OAll	Boc-L-Trp-OH	52
c	Fmoc-L-Phe-OAll	Fmoc-L-Phe-OH	84
d	Fmoc-L-Glu(O^{t}Bu)-OAll	Fmoc-L-Glu(O*t*-Bu)-OH	87
e	Fmoc-L-Asp(O^{t}Bu)-OAll	Fmoc-L-Asp(O*t*-Bu)-OH	62
f	Fmoc-L-Pro-OAll	Fmoc-L-Pro-OH	80
g	Ph_2CHCO_2All	Ph_2CHCO_2H	100
h	$PhOCH_2CO_2All$	$PhOCH_2CO_2H$	70
i	(2-furyl)CH=$CHCO_2All$	(2-furyl)CH=$CHCO_2H$	61
j	PhCH=$CHCO_2All$	PhCH=$CHCO_2H$	100
k	$C_6H_{11}CH_2CH_2CO_2All$	$C_6H_{11}CH_2CH_2CO_2H$	94

ever, the final carboxylic acids were found to contain traces of a polymeric residue released from the matrix that supports the catalyst. This drawback has been previously described.[10] Such impurities were only detected by ^1H NMR analysis and they could be removed by rapid solid-phase extraction. Attempts to stabilize the matrix by using different reaction solvents were unsuccessful.

A synthesis of the protected L-arginine octamer, R_8 (**3**; Scheme 1), was developed with the aim of obtaining bioavailable conjugates of biologically active peptides. The solution-phase synthesis approach for protected octaarginine (retrosynthetic analysis in Scheme 1) was based on the segment-doubling strategy reported by Wender et al.[3] This methodology is based on successive coupling and selective deprotection steps, which are alternated to prepare reactive free-amino-end and free-carboxy-end fragments, then coupled to give double-sized homopolymers.

In contrast to Wender's procedure,[3] the orthogonally protected amino acids Fmoc-L-Arg(Pbf)-OH (**2a**) and H_2N-L-Arg(Pbf)-OAll (**4**; Scheme 1) were selected as starting materials. The perguanidinylation step was avoided by the use of 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) guanidine protecting group. The orthogonal protecting strategy also included a base-labile Fmoc group on the amino terminus, and the allyl group on the carboxylic terminus. All deprotection conditions were compatible with each intermediate synthetic structure. Although allyl removal on the carboxylic ends was first attempted using soluble tetrakis(triphenylphosphine)palladium, the removal of the catalyst during the purification work-up proved to be tedious throughout the different synthetic steps. Instead, the optimized method based on PdEnCat did not suffer from this problem.

Having optimized the allyl group removal methodology, the synthesis of octamer **3** was fully accomplished



Scheme 1 Structure and retrosynthetic analysis of protected R_8

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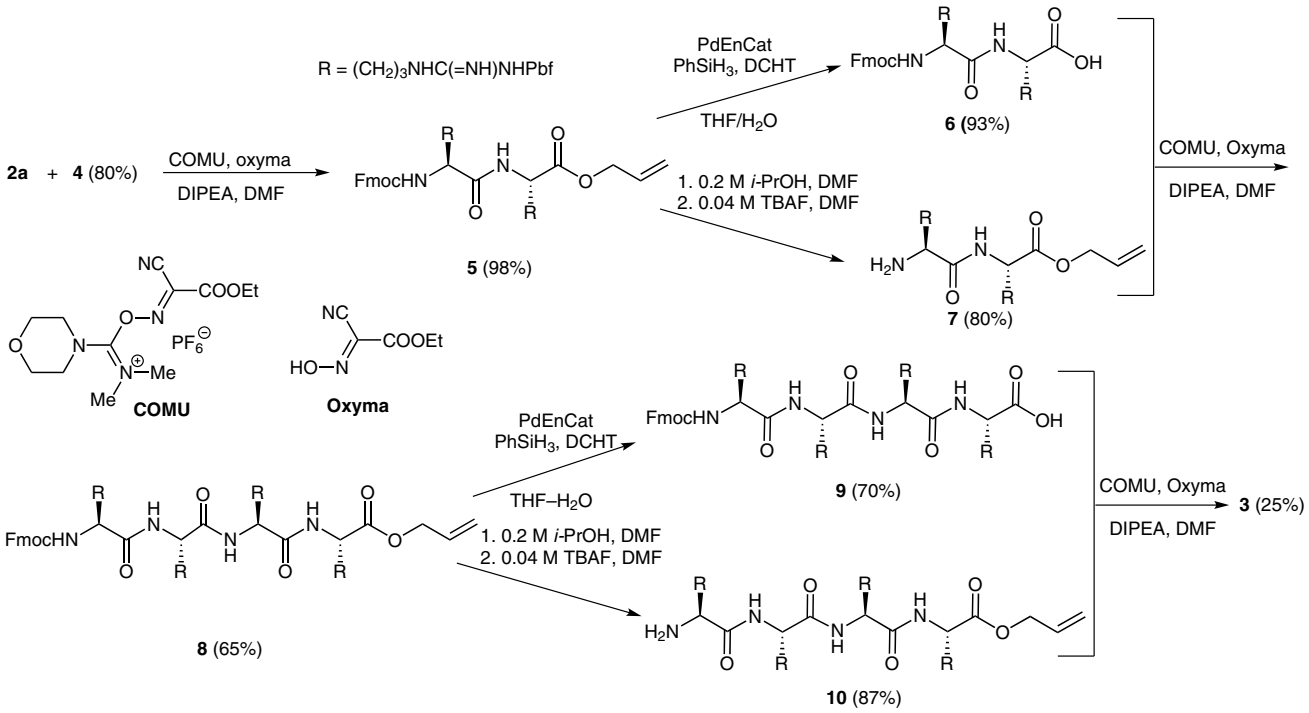

Scheme 2 Synthesis of the L-arginine octamer

(Scheme 2). Thus, free-carboxy-end segments (**6** and **9**) were obtained by microencapsulated palladium promoted allyl removal, although longer reaction times were needed for tetramer **9**. Free-amino-end segments **4**, **7**, and **10** were obtained by Fmoc removal with tetrabutylammonium fluoride (TBAF).[11] This reagent was chosen instead of piperidine to simplify purification. The same methodology used on the monomer **1a** for the preparation of **4** was also successfully applied with the Fmoc-[L-Arg(Pbf)]$_n$-OAll dimer and tetramer **5** and **8** along the synthetic route. These conditions afforded the free amines in high yields and in short reaction times.

Coupling reactions initially worked satisfactorily with the coupling reagent EDCI and the additive 7-aza-1-hydroxybenzotriazole (HOAt). Thus, once dimer **5** was obtained (71%), it was divided in two portions, which were respectively deprotected to afford the amino or the carboxylic free-ends. Subsequent coupling of the two portions gave the corresponding tetramer **8** (60%). However, when the reactions were conducted with (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)[12] and ethyl (hydroxyimino)cyanoacetate (Oxyma) (Scheme 2), a significant increase in yield of dimer **5** was observed (98%), but little improvement was found for the synthesis of tetramer **8** (65%). After deprotection of **8**, intermediates **9** and **10** were subjected to a coupling using COMU to afford protected octamer **3** (25%). Although different conditions were carried out by using EDCI or COMU, no improvement was found during the synthesis of the protected octamer **3**.

In summary, a useful synthesis of the orthogonally and conveniently protected molecular transporter L-octaarginine is reported. The method developed for the deprotection of allyl esters in the solution phase using solid-supported catalyst has been modified to simplify purification, and the approach has been used in the synthesis of octaarginine. The scope of this method has been validated on a series of allyl esters of aliphatic and α,β-unsaturated carboxylic acids, including α-amino acids, with yields of more than 60%. The conditions are compatible with other protecting groups that are frequently used such as *tert*-butyl and fluorenyl carbamates. The results of this study add further support for the utility of solution-based methods for the synthesis of peptide and peptide derivatives in which the availability of orthogonal protecting groups is essential.

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Supporting Information for this article is available online at http://www.thieme-connect.com/products/ejournals/journal/10.1055/s-00000083.

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(9) **Microencapsulated Palladium Catalyzed Cleavage of Allyl Esters; General Procedure:** A suspension of PdEnCat 30 (Aldrich; 0.4 mmol/g loading, 0.05 equiv) in THF–H_2O (9:1, 5 mL) was bubbled with argon for 10 min. After this time, DCHT (0.15 equiv) and the corresponding allyl ester **1** (1.0 equiv, 70 mg) were added under positive pressure. Finally, $PhSiH_3$ was injected (2.0 equiv), and the mixture was allowed to react for 2 h at r.t. under an argon atmosphere. After this time, the reaction was filtered through Celite and the filtrate was evaporated under reduced pressure. The remaining solid was dissolved with EtOAc (20 mL) and washed with H_2O (3 × 10 mL) and brine (3 × 10 mL). The organic layer was dried ($MgSO_4$) and evaporated under vacuum. Purification by solid-phase extraction (C18 reverse-phase chromatography, H_2O/MeOH) gave pure carboxylic acids **2** (yields shown in Table 1). Purity was determined by HPLC, ^1H NMR and ^{13}C NMR analyses. All the carboxylic acids are commercially available. The recorded ^1H and ^{13}C NMR spectra of the synthesized compounds were consistent with those registered for commercial samples.

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