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An improved protocol for the SmI_2-promoted *C*-alkylation of peptides: degradation and functionalization of serine residues in linear and cyclic peptides

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Abstract—The utility of the samarium diiodide promoted *C*-alkylation of peptides for the introduction of new side chains on peptide strands is dramatically enhanced by the initial oxidative degradation of serine residues in small peptides. In this way, cyclic peptides may also be included in this repertoire as a method for the preparation of peptide libraries.

In 2000, we reported a novel approach for the selective incorporation of non-natural side chains directly onto glycine residues of a peptide backbone as a potential route to peptide libraries.[1] As the incorporation of the side chain only requires a two-step procedure, this method allows for the preparation of a series of analogs from a single and intact peptide, without having to repeat the peptide synthesis for each modification. Our approach relies on the reductive samariation of α-pyridylsulfenyl glycyl units generating selectively a glycine enolate,[2] which is subsequently captured by the presence of a carbonyl electrophile. As the existing chiral centers of the peptide exert little effect on the diastereoselective outcome of the alkylation step, the method becomes suitable for the preparation of non-natural peptide libraries.

The method does, however, suffer from one serious drawback, which is related to the preparation of the α-pyridylsulfenyl glycyl moieties. The sulfenyl group is introduced onto the peptide via the selective bromination of glycine with *N*-bromosuccinimide[3] followed by nucleophilic displacement of the halide with the pyridylthiolate. Whereas this reaction works well with dipeptides, the yields of the bromination step are reduced significantly with larger peptides,[1b] thereby restricting the utility of this alkylation sequence for peptide modification.[4] In this paper, we provide a viable solution to this problem turning our attention instead towards the oxidative degradation of serine residues as a means of initial functionalization of the peptide backbone. The method considerably enhances the utility of the samarium diiodide promoted *C*-alkylation protocol of small peptides and is shown to be adaptable to the functionalization of a cyclic tetrapeptide.

Steglich and co-workers have previously published a remarkably simple and efficient procedure for the conversion of serine- or threonine-containing peptides into their α-acetyloxy glycine counterparts by treatment with a stoichiometric amount of lead tetraacetate in refluxing ethyl acetate.[5] Taking into consideration the known ability of these glycyl acetates to participate in nucleophilic displacement reactions, makes this oxidative degradation of serine units a promising route to incorporate the 2-pyridyl sulfide group into a peptide.

In order to evaluate this procedure, the Boc-protected dipeptide **1** was treated with 1.5 mol equiv of $Pb(OAc)_4$ in refluxing dry ethyl acetate (Scheme 1). After complete conversion to the acetate after just 30 min, simple filtration and extraction of the lead salts with aqueous citric acid afforded the desired α-acetyloxy derivative **2** in essentially quantitative yield. This crude acetate was smoothly converted into the 2-pyridyl sulfide derivative **3** as a 1:1 diastereomeric mixture by treatment with 2-mercaptopyridine and diisopropylethylamine (DIPEA) in dichloromethane at −78 °C to 20 °C in a rewarding

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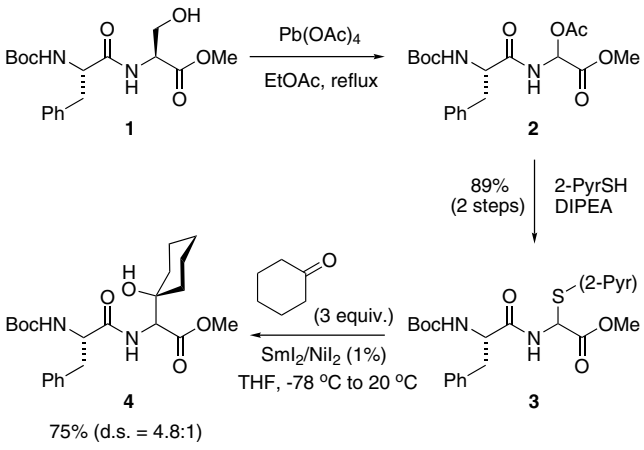
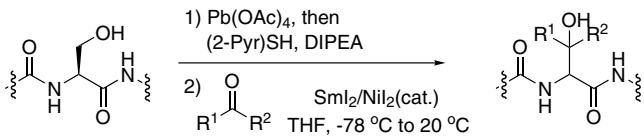


Scheme 1.

Table 1. Oxidative degradation and reductive coupling of serine residues[a]



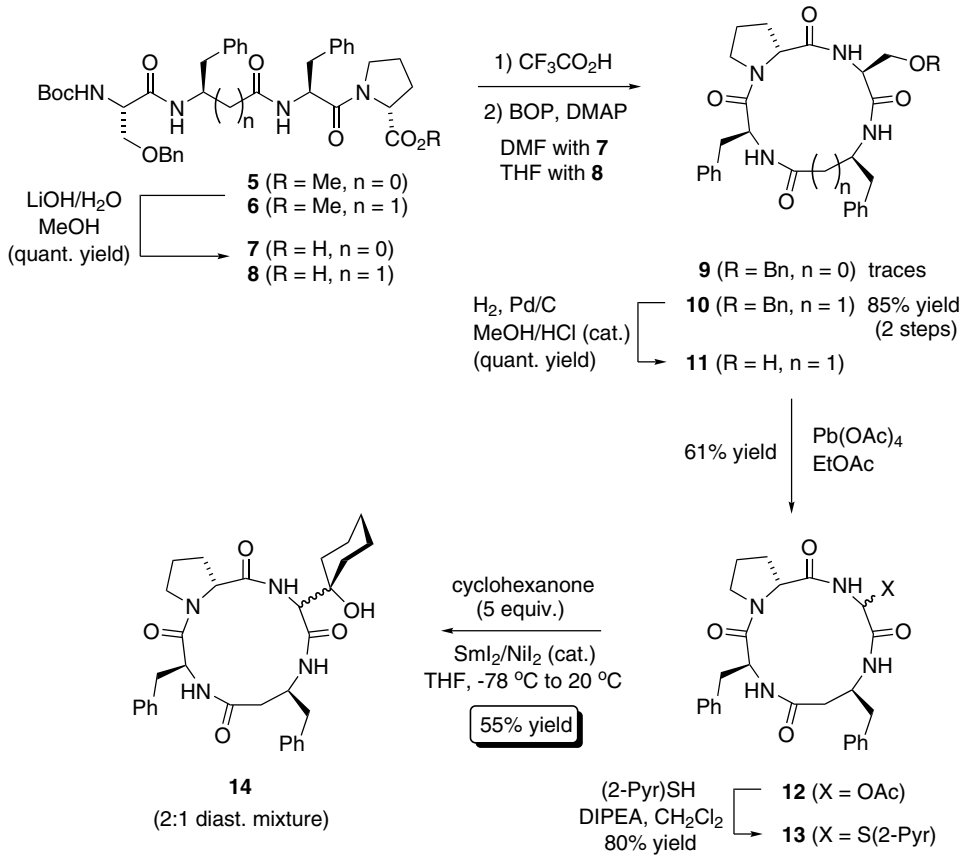
Entry	Yield of pyridyl sulfide (%)	*C*-Alkylation product (yield and diast. ratio)
1	78	(75%, 1:1)
2	56	(76%, 1.6:1)
3	89	(68%, 1:1)
4	62	(71%, 1.8:1)
5	70	(58%, 1:1)
6	83	(70%, 1:1)
7	82	(90%, 1:1)
8	74	(82%, 1:1)
9	73	(49%, 1:1)

[a] Isolated yields after column chromatography.

89% yield. It should be noted again, that this peptide could not have been converted to the 2-pyridyl sulfide derivative by means of bromination with NBS because of the labile Boc-protecting group.[1b] SmI_2-promoted alkylation of **3** with cyclohexanone under NiI_2 catalysis provided the coupling product **4** in a good yield of 75%.[6,7]

With these encouraging results in hand, a small series of seryl peptides were successfully subjected to the same conditions. The results listed in Table 1 reveal the viability of this method for the introduction of the 2-pyridyl sulfide and that the reductive coupling with cyclohexanone catalyzed by nickel(II) performs well. The three tripeptides in entries 7–9 contain either an *O*-benzyl protected serine and threonine residue, thereby demonstrating that selective modification at a specific serine residue is permitted. This is of course an advantage compared to the bromination approach where it is not possible to selectively distinguish between two glycine residues in the same peptide.

A few other ketones were also tested as illustrated in Table 2, where modest to good yields were obtained for the coupling step. In entry 3, all four diastereomers of the *C*-glycopeptide prepared were identified by NMR, providing a small library of non-natural *C*-glycosyl tripeptides.



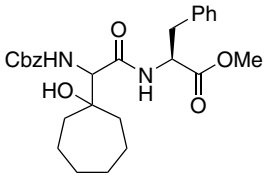
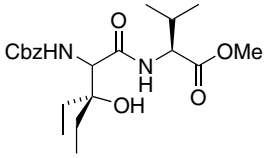
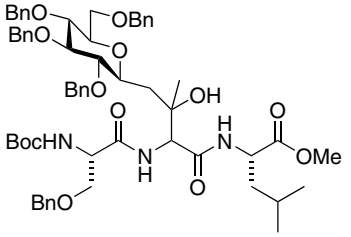
Scheme 2.

Finally, the high interest in small cyclic peptides as therapeutic agents against a variety of diseases,[8] prompted us to test the *C*-alkylation approach on a cyclic tetrapeptide, related to the histone deacetylase inhibitor, trapoxin A.[9] Cyclic tetrapeptides are generally difficult to prepare due to the high ring strain resulting from the transoid nature of the amide bonds.[8d] Hence, the ability to prepare a single cyclic peptide and thereafter introduce side chains at selected positions would represent a more direct method for the preparation of a large body of peptide analogs.

To examine the feasibility of this approach, the linear tetrapeptide **5** possessing a protected serine residue was prepared using standard peptide coupling techniques (Scheme 2). However, after hydrolysis of the terminal ester and Boc removal, the use of standard cyclization conditions in DMF as earlier reported for the ring closure of similar tetrapeptides failed to provide the requisite cyclic peptide **9**.[9] Recently, Fairlie and coworkers have demonstrated how the replacement of one of the phenylalanine moieties in a similar tetrapeptide with its corresponding β-amino acid substantially increases the cyclization yield due to increased flexibility of the 13-membered cyclic peptide.[10]

Hence, we synthesized the more flexible homolog **6** containing a β-L-Phe unit. Subsequent cyclization in THF provided the cyclic tetrapeptide **10** in a satisfactory yield of 85% (two steps). Liberation of the hydroxyl group to **11** followed by oxidation with $Pb(OAc)_4$ provided the acetate **12** in a good yield (61%, two steps). Transformation to the pyridyl sulfide **13** then gave a 1:1 epimeric

Table 2. Reductive coupling with other ketones[a]

Entry	Coupling yield (%) (diast. ratio)	Product
1	67 (1:1)	
2	35 (1.2:1)	
3	78 (4 diast.)	

[a] Isolated yields after column chromatography.

mixture in an 80% yield. Finally, coupling of this compound to cyclohexanone with SmI_2 led to the *C*-alkylated cyclic tetrapeptide **14** as a 2:1 mixture of diastereomers in a good 55% yield.[11] This result clearly illustrates the potential of this concept for the introduction of side chains onto cyclic peptides.

In conclusion, we have developed an alternative approach for the functionalization of serine residues in small peptides which has been extended to a cyclic peptide. Further work is in progress to test other cyclic peptides and for the introduction of more relevant side chains.

Acknowledgements

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11. **Data for compound 14 (major diastereomer)**. ^{1}H NMR ($CDCl_3$, 400 MHz) (major diastereomer) δ (ppm) 8.01 (d, 1H, J = 10.4 Hz), 7.30–7.12 (m, 10H), 6.40 (br s, 1H), 6.16 (br s, 1H), 4.97 (dt, 1H, J = 5.6, 10.0 Hz), 4.67 (d, 1H, J = 6.8 Hz), 4.19 (d, 1H, J = 9.6 Hz), 4.11 (br s, 1H), 3.95 (br s, 1H), 3.89 (dt, 1H, J = 3.6, 9.6 Hz), 3.18 (t, 1H, J = 9.6 Hz), 3.15 (t, 1H, J = 10.8 Hz), 2.99 (t, 1H, J = 12.8 Hz), 2.89 (dd, 1H, J=5.2, 12.8 Hz), 2.80 (dd, 1H, J = 6.0, 13.6 Hz), 2.78 (quint, 1H, J = 9.2 Hz), 2.41 (dd, 1H, J = 4.4, 14.4 Hz), 2.36–2.32 (m, 1H), 2.06 (sextet, 1H, J = 9.6 Hz), 1.80–1.25 (m, 11H) (the OH signal is missing). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 172.6, 172.4, 171.2, 170.2, 138.2, 136.4, 129.4 (2C), 129.3 (2C), 128.9 (2C), 128.8 (2C), 127.3, 127.0, 72.5, 58.2, 57.3, 53.1, 52.8, 47.1, 40.1, 39.7, 36.8, 35.8, 34.3, 25.7, 25.5, 24.8, 22.3, 21.8. ES-HRMS $C_{32}H_{40}N_4O_5$ [M + Na^+]; calculated: 583.2896 found: 583.2893.