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Synthesis of *N*-protected *N*-methyl serine and threonine

Yue Luo, Ghotas Evindar, Dan Fishlock and Gilles A. Lajoie*

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

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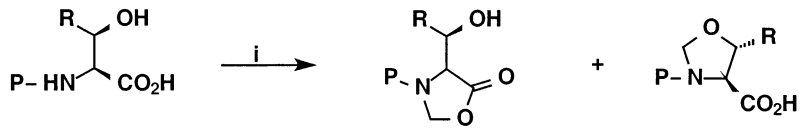
Abstract—Two efficient and convenient syntheses of *N*-Cbz and *N*-Fmoc *N*-methyl serine and threonine are described. The amino acid side-chain alcohol can be protected as a TBDMS ether in very good yield or left free, followed by the formation and subsequent reduction of the corresponding oxazolidinone. © 2001 Published by Elsevier Science Ltd.

N-Methyl amino acids are important constituents of many biologically active peptides[1] and are often critical for their biological activity. The assembly of such peptides depends on the availability of *N*-methyl amino acid precursors as direct *N*-methylation of peptide bonds is not selective.

There are a number of methods to prepare *N*-methyl amino acids with unfunctionalized side chains.[2] However, these methods are not adequate for the *N*-methylation of the β-hydroxy amino acids serine and threonine, giving mixtures of products including *O*-methylated and/or dehydrated derivatives and partial racemization.[2,3] Freidinger and co-workers reported a method in which *N*-Fmoc amino acids were reacted with paraformaldehyde in the presence of catalytic *p*-TSA to form oxazolidinones[4] that are subsequently reduced[5] to *N*-methylated amino acids with triethylsilane–TFA.[6] The *O*-benzyl derivative of Fmoc *N*-methyl serine was prepared in excellent yields by this method. However, the *O*-benzyl protection scheme requires commercially expensive *N*-protected *O*-benzylserine or threonine, and if further elaboration of the side-chain function is desired an additional deprotection step is required precluding the use of Cbz as the *N*-amino protecting group.

We report here the preparation of *N*-protected *N*-methyl serine and threonine by an analogous scheme but with the *t*-butyl dimethyl silyl (TBDMS) protection of the side-chain alcohol.[7] The (*O*-TBDMS) *N*-protected serine and threonine are reacted with formaldehyde and reduction of the corresponding oxazolidinone gives the desired *N*-methyl derivative with simultaneous removal of the TBDMS protecting group. We also describe an improved procedure to prepare the *O*-TBDMS derivatives of serine and threonine, giving excellent yields with both *N*-Cbz and *N*-Fmoc protecting groups.

Our first attempt to obtain *N*-methyl β-hydroxy amino acids involved the formation of the oxazolidinone with paraformaldehyde under standard conditions without protection of the hydroxyl function (Scheme 1). The desired *N*-Cbz oxazolidinone **1a** and **1b** is indeed obtained in 25–30% yield but the major product is the corresponding oxazoline **2a** and **2b** (60–65%), corre-



1a R=H P=Cbz 25%
1b R=CH_3 P=Cbz 30%
1c R=H P=Fmoc 42%
1d R=CH_3 P=Fmoc 45%

2a,b

Scheme 1. (i) $(CH_2O)_n$, *p*-TsOH (0.1 equiv.), reflux in toluene.

* Corresponding author. E-mail: glajoie@uwo.ca

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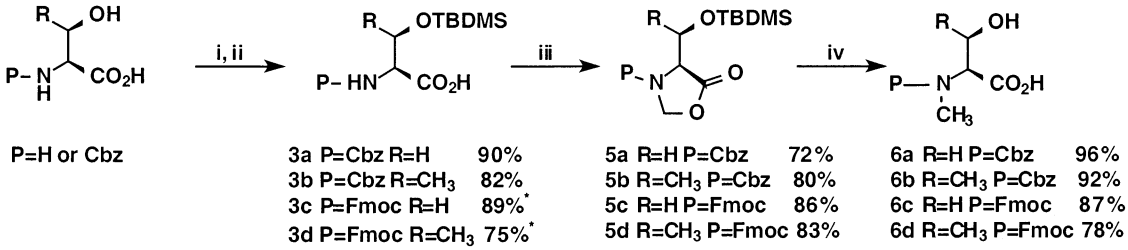

Scheme 2. (i) TBDMS-Cl (1.1 equiv.), imidazole (2 equiv.), DMF; (ii) (for **3c–d**) Fmoc-OSu (1 equiv.), Na_2CO_3, dioxane/H_2O (1:1) (*yield for two steps); (iii) $(CH_2O)_n$, TsOH (0.1 equiv.), reflux in toluene, (iv) Et_3SiH (3–10 equiv.), TFA/$CHCl_3$, rt, 3–5 days.

sponding to recently reported results.[3] With the *N*-Fmoc derivative the yield of the oxazolidinone **1c** and **1d** is better (40–45%). In each case the mixture of products can be separated by simple base/acid extraction. The oxazolidinones **1a–d** can be reduced in 90–95% yield using the triethylsilane/TFA conditions reported by Freidinger and co-workers.[6]

We have examined a similar reaction scheme with protection of the hydroxyl group as *O*-TBDMS ether. The *O*-TBDMS protecting group is introduced to Cbz-serine and Cbz-threonine to give the corresponding products **3a** (90%) and **3b** (82%) via reaction with TBDMS-Cl and imidazole in DMF for 2–3 days[8–10] (Scheme 2). The products are readily separated by simple base/acid extraction, and no chromatography is needed.[11,12]

In contrast, Fmoc-serine and Fmoc-threonine react extremely slowly under the same conditions and give a very low yield of product (20–40%) along with partial loss of Fmoc protecting group.[10] The crude products are difficult to purify by acid/base extraction and chromatography is necessary. Increasing the amount of TBDMS-Cl (5 equiv.) and imidazole (3 equiv.) does not improve the yield. The protection sequence was reversed such that the TBDMS group was first introduced to L-serine and L-threonine, followed by addition of the Fmoc group (Scheme 2). The reaction of L-serine with TBDMS-Cl (1.1 equiv.) and imidazole (2.2 equiv.) in DMF gives the ether in excellent yield (90%),[13] while L-threonine gives a very good yield (77%) with TBDMS-Cl (2 equiv.) and imidazole (3 equiv.). The Fmoc protecting group was subsequently introduced in a standard procedure to give the corresponding (*O*-TBDMS) protected Fmoc-serine **3c** and Fmoc-threonine **3d** with high yields (90–98%). This approach avoids column purification and provided higher overall yield than the literature method.[10]

The *O*-TBMDS amino acids **3a–d** were refluxed with paraformaldehyde and catalytic *p*-TsOH in toluene using a Dean–Stark apparatus to give the oxazolidinone **5a–d** in very good yields.[14] Deprotection of the hydroxyl groups and reduction of the oxazolidinone **5a–d** is achieved in one-step using TFA and triethylsilane giving protected *N*-methyl amino acids **6a–d** in excellent yields.[15]

Two alternative methods to the Freidinger procedure are described for the generation of *N*-protected *N*-methyl β-hydroxy amino acids. The first method, without protection of the hydroxyl group, provides a shorter route to the *N*-Fmoc protected substrates while the second method with *O*-TBDMS protection works best for the *N*-Cbz group, and provides the highest yields overall. In each case, purification is convenient at each step simplifying scale-up. The choice between the two methods will thus be dependent on the nature of the β-hydroxy amino acid and which *N*-protecting group is desired.

Acknowledgements

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2. *N*-Methylation of *N*-tosylated amino acids with MeI occurs in basic solutions at 65°C, but deprotection of the Tos group results in racemization in acidic conditions unless carried out in Na/NH_3.[2a] *N*-Benzyl protection is introduced via reductive amination of amino acids, then reductive alkylation with paraformaldehyde yields *N*-methyl amino acids,[2b] but Schiff base intermediates tautomerize causing racemization.[2e] *N*-Boc or Cbz protected amino acids react with methyl iodide and silver oxide to give *N*-methyl amino acid methyl esters[2c] which racemize during saponification of the methyl ester.[2d,e] The reaction with Boc-Ser and Boc-S-benzyl cysteine gave β-elimination by-products.[2c] Boc or Cbz amino acids have been *N*-methylated with NaH/CH_3I in THF at room temperature.[2d–i] Boc-Ser(Bzl)-OH undergoes β-elimination to room temperature under this condition:[2g] (a) Fisher, E.; Lipschitz, W. *Ber* **1915**, *48*, 360; (b) Quitt, P.; Hellerbach, J.; Vogler, K. *Helv. Chim. Acta* **1963**, *46*, 327; (c) Olsen, R. K. *J. Org. Chem.* **1970**, *35*, 1912; (d) McDermott, J.

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11. All compounds were characterized by ^{1}H and ^{13}C NMR, ES-MS and elemental analysis.
12. Sample procedure: Cbz-Ser (15 g, 62.7 mmol), TBDMSCl (1 equiv.) and imidazole (2 equiv.) were dissolved in dry DMF (150 mL) and stirred at rt for 48 h under argon. The reaction was concentrated, suspended in hexane and extracted with 5% $NaHCO_3$. The aqueous fraction was acidified to pH 3 using 1 M $KHSO_4$ then extracted into EtOAc. The combined EtOAc fractions were washed with brine, dried over $MgSO_4$ and evaporated to dryness. After 4 h under high vacuum **3a** was obtained as a white solid (20.50 g, 90% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 9.65–10.00 (bs, 1H), 7.23–7.34 (m, 5H), 5.58–5.61 (d, 1H, $J=8.1$ Hz), 5.10–5.13 (br m, 2H), 4.42–4.46 (bs, 1H), 4.00–4.10 (br m, 1H), 3.80–3.87 (br m, 1H), 0.84 (2, 9H), 0.02 (s, 6H).
13. Sample procedure: L-Ser (10.6 g, 0.1 mol) was suspended in DMF (100 mL). Imidazole (2 equiv.) and TBDMSCl (1.1 equiv.) were added and the mixture stirred at rt for 20 h. DMF was evaporated to give an oily residue. This was stirred with 1:1 H_2O:hexane for 4 h and the resulting white solid filtered off, rinsed with hexane and air dried to give L-Ser(OTBDMS)-OH (20.0 g, 91% yield). ^{1}H NMR (CD_3OD, 250 MHz): δ 3.84–3.98 (m, 2H), 3.49–3.55 (m, 1H), 0.82 (s, 9H), 0.01 (s, 6H).
14. Sample procedure: **3a** (10.16 g, 28.74 mmol), paraformaldehyde (5.08 g) and *p*-TsOH (0.27 g, 0.05 equiv.) were suspended in toluene (550 mL) and slowly heated to dissolve most of the starting material, then refluxed for 1 h using a Dean–Stark water removal apparatus. The reaction was cooled to rt and washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to yield **5a** as a yellow non-viscous oil (7.60 g, 72% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.24–7.39 (m, 5H), 5.47–5.54 (bs, 2H), 5.11–5.31 (m, 3H), 4.15–4.30 (bs, 1H), 3.90–4.10 (bs, 1H), 0.82 (s, 9H), −0.01 (s, 3H), −0.03 (s, 3H).
15. Sample procedure: **5a** (7.13 g, 19.51 mol) was dissolved in $CHCl_3$ (40 mL) under argon at rt in which TFA (40 mL) was added followed by Et_3SiH (3 equiv.). The mixture was stirred at rt and monitored by TLC (95:5 CH_2Cl_2:MeOH). Additional Et_3SiH (up to 10 equiv.) was periodically added. After 3 days the reaction was concentrated and re-dissolved in CH_2Cl_2 several times to remove most of the TFA and Et_3SiH. The crude oil was dissolved in hexane and extracted with 5% $NaHCO_3$. The aqueous fraction was acidified to pH 3 using 1 M $KHSO_4$, and extracted with EtOAc. The combined EtOAc fractions were washed with brine and dried over $MgSO_4$, and evaporated to yield **6a** as thick colorless oil (96% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.20–7.45 (m, 5H), 5.10–5.20 (br, 2H), 4.55–4.63 (m, 1H), 4.05–4.15 (m, 1H), 3.85–4.05 (m, 1H), 3.04 (s, 3H).