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Application of t-Butyldimethylsilyl Ethers of Serine, Threonine and Tyrosine in Peptide Synthesis

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Abstract: The utility of Tbdms **(*t*-butyldimethylsilyl)** ethers, prepared conveniently in a one-pot procedure from **N^{α}-Fmoc (9-fluorenylmethoxycarbonyl)** and **N^{α}-Z (benzyloxycarbonyl) hydroxyamino** acids, is demonstrate& **peptide** bond formation and **esterification** to **4-alkoxybenzylalcohol** resin are achieved readily with these derivatives. The lability of the Tbdms ethers to various reagents enables selective deprotection of the hydroxyl side-chains after **peptide** chain assembly, desirable, e.g., for phosphorylation or glycosilation.

Traditionally, the side-chain **hydroxyl** functions in **Ser,** Thr and Tyr have been protected as **Bu^t** ethers in solid-phase **peptide** synthesis (SPPS) based on **N^{α}-Fmoc** protection and **CF_3COOH-labile** resin linkage agents. When a more acid-labile hydroxyl protecting group is required, the corresponding trityl ethers can be used'. Alternatively, side-chain unprotected **Fmoc-Ser-OH[2], Fmoc-Thr-OH[3]** and **Fmoc-Tyr-**OH' can be incorporated directly into **peptides** under certain conditions. The ***O*-trimethylsilyl** protecting group has been discussed' but is not suitable since it **is** removed under the conditions of **peptide** bond formation. The Tbdms ether is very important as a protecting group in general organic **synthesis[6]** but has not found general application in **peptide** chemistry.

Scheme 1

	i		ii	
R-Xaa-OR'	——— .	**R-Xaa(R'')-OR'**	——— .	**R-Xaa(R'')-OH**
la; **R=Z, R'=*p*-NO_2Bn**		**2a; R=Z, R'=*p*-NO_2Bn, R''=Bu^t**		**3a; R=Fmoc, R''=Bu^t**
lb; R=Fmoc, **R'=** Me/Et		2b; **R=Fmoc, R'=Me/Et, R''=Bu^t**		
lc; **R=Fmoc, R'=Bn**		**2c; R=Fmoc, R'=Bn, R''=Bu^t**		
1d; R=Fmoc, **R'=phenacyl (Pha)**		2d; **R=Fmoc, R'=Pha, R''=Bu^t**		
le; **R=Fmoc, R'=H**		**2e; R=Fmoc, R'=R''=Tbdms**		**3e; R=Fmoc, R''=** Tbdms
1f; R=Z, R'=H		**2f; R=Z, R'=R''=Tbdms**		**3f;** R=Z, **R''=Tbdms**

Reagents: i; a - d: **$(CH_3)_2C{=}CH_2/H_2SO_4$** or **$Cl_3(Bu^tO)C{=}NH/BF_3.Et_2O$,** e **& f: Tbdms-Cl, *N*-methylimidazole (MeIm), DMF; ii;** a, **H_2/Pd(C)** then **Fmoc-Cl/Et_3N,** b, 2 % **Na_2CO_3, H_2O/MeCN** then **Fmoc-Cl/Et_3N, c, H_2/Pd(C),** d, **Zn/AcOH;** e **& f, pH** 4 **(Xaa** stands for Ser, Thr or Tyr residue).

Although the Fmoc-amino acid **Bu^t** ethers 3a are used widely in **SPPS,** their preparation is inconvenient' and the commercial products are expensive. Temporary protection of both the amino and **carboxyl** functions in 1 is necessary during ***t*-butylation[8]**. Usually this is achieved with the aid of **benzyl** esters (e.g. la or c) which can subsequently be removed by **hydrogenolysis[9]**. Because of the uncertain stability of the Fmoc group in **2c** to hydrogenolytic **conditions[10],** the *Z* group **(2a)** is usually preferred for the **etherification** step before Pmoc-reprotection. Alternatively, carboxyl protection as an **alkyl** ester" is

effective and **lb** may be etherified directly, prov from **2b** during subsequent base hydrolysis". We find that carboxyl-protection as the phenacyl ester" **2d** is even more convenient because these esters can be prepared" easily from the Fmoc-amino acids **le** directly and, unlike saponification of the alkyl esters **2b, selective** deprotection with zinc in acetic acid is very effective and there is no danger of racemisation [15]. Silylation of **le** or **f** yielded the silyl ether-esters **2e** and **f,** which were transformed without isolation to the silyl ethers **3e** and **f** [16]. Apparently the silyl esters 2e are more acid-sensitive than usual" although even **non-acidid** solvolysis can remove some Tbdms esters". Unlike the Bu' ethers **3a, the** silyl ethers 3e can thus be obtained in a one-pot reaction sequence from **le** directly.

Even when the hydroxysmino acid residues are incorporated into growing **peptide** chains without side-chain protection, the conditions required for the esterification of the commonly used SPPS supports with Ser. Thr **and** Tyr derivatives obviously still necessitates masking of the hydroxyl function. For this reason the utility of **3e** for resin esterification was studied (Table 1). Both the Ser and Thr derivatives could be anchored successfully using the conventional dicyclohexylcarbodiimide **(DCC)/4-**dimethylaminopyridine **(DMAP)** method". The Tyr derivative, on the other hand, failed to react with DCC but could be **anchored** using a different **method**[20] with the aid of Castro's **reagent**[21]. The levels of racemisation were found to be comparable to results obtained with **3a**[22].

Table 1. ***Esterification of 4-alkoxybenzylalcohol peptide synthesis resin."***

Derivative used for esterification	% Incorporation[b]	Racemisation[c] (% *D*- or *D-allo* isomer)
Fmoc-Ser(Tbdms)-OH	98"	0.4
Fmoc-Thr(Tbdms)-OH	82[d]	1.5
Fmoc-Tyr(Tbdms)-OH	76"	1.2

[a] Aliquots of PepSyn KA resin (MilliGen; 0.1 meq/g) were esterified.
[b] Measured by amino acid analysis of esterified and Fmoc-deprotected resin samples and comparison with resin-internal Nle content.
[c] Measured by Fmoc-deprotected amino acid resin derivatisation with 1-fluoro-2,3-dinitrophenyl-5-*L*-alanine amide before resin detachment and chromatography of released dipeptides[23].
[d] Esterification with 5 eq preformed symmetrical anhydride in the presence of 0.5 eq DMAP and 5 eq N-methyl morpholiae (NMM) in DMF (2 x 30 min).
[e] Esterification with 5 eq amino acid derivative in the presence of 5 eq BOP (benzotriazole-1-yl-oxy-(dimethylamino)-phosphonium hexatluorophosphate), 0.5 eq DMAP and 5 eq NMM in DMF for 18 h.

Table 2 shows that the silyl ethers of Ser and Thr are somewhat more labile to acidolysis by CF_3COOH than the corresponding Bu' ethers. For the Tyr derivatives the situation is reversed, the silyl ether being almost resistant to dilute CF_3COOH in CH_2Cl_2 whereas the Bu' ether is removed efficiently. Selective deprotection of Tyr(Bu^t) in the presence of Tyr(Tbdms) should be possible if the conditions are chosen carefully. Under the hydrolytic conditions applied, the Bu^t ethers are completely stable, whereas the silyl ethers of Ser and Thr can be removed smoothly. The Tyr silyl ether is again more resistant. When fluoride ions[24] are used to effect Tbdms deprotection, the reactivities of the derivatives varies[25]. It can be seen that for the relatively acid-stable aromatic silyl ether the fluoride treatment is very effective. Therefore, if complete deprotection of Tyr(Tbdms)-containing peptides is desired, it is advisable to remove

the N-terminal Fmoc group[26] and the aromatic Tbdms silyl ester simultaneously with Bu^n_4NF prior to peptide resin detachment with CF_3COOH. The Tbdms ether groups were found to be stable to piperidine, as well as to other basic reagents used in SPPS, such as DMAP, 1,8-diazabicyclo[5.4.0]undec-7-ene and Pr^i_2NEt[27].

Table 2. *Deprotection of Tbdms ethers.*

Deprotection Conditions[a]	°C		% *O*-Tbdms or *O*-Bu^t Deprotection[b]					
			Serine		Threonine		Tyrosine	
			Tbdms	Bu^t	Tbdms	Bu^t	Tbdms	Bu^t
1% CF_3COOH, CH_2Cl_2	15	22	19					
25 %CF_3COOH, CH_2Cl_2	15	22		< 1	42	14	2	43
35 % CF_3COOH, CH_2Cl_2	30	22					3	> 99
anhydrous CF_3COOH			100	95	100	99	46	100
				100		100	80	
							> 99	
3:1:1AcOH/THF/H_2O			27		9		< 1	
			53		27		3	
			99	0	96	0	18	0
			100		80		17	
Bu^n_4NF,			47		2		100	
			72		6			
			91	0	8	0		0
			100		36			
					> 99			
20 % Piperidine, DMF	1080	22	0		0		0	

[a] [Substrate]=1 μM; were incubated with the mixtures shown prior to analysis.
[b] Determined by integration of RP-HPLC analysis peaks (Nova-Pak C,,, 3.9x150 cm, 1 mL/min, 40 to 80 % MeCN in 0.1 % CF_3COOH/H_2O over 10 min, λ=245 nm); the Fmoc-amino acid derivatives were used for the acidolysis/hydrolysis conditions and the Z-amino acid ethers for the other experiments.

The Z-hydroxyamino acid Tbdms ethers were reacted with one equivalent of H-Val-OEt.HCl in DMF using BOP/HOBt/NMM chemistry[28]. The reactions were monitored by TLC and found to be practically complete within minutes. The analytically pure dipeptides were obtained in > 90 % isolated yield after silica gel chromatography and no racemisation was detected upon RP-HPLC analysis of the *O*-deprotected dipeptides.

Table 3. *SPPS of peptide H-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-OH.*

Side-chain protecting groups	HPLC R_f[a]	Yield[b]	Purity[c]
Ser/Thr/Tyr(Bu^t), Asn(Trt)	0.32[d], 0.50[e]	77 %	96 %
Ser/Thr/Tyr(Tbdms), Asn(Trt)	0.32[d], 0.50[e]	84 %	93%

[a] Peak fractions were collected and authenticated variously usmg ammo acid analysis, gas-phase sequencing and FAB-MS. Chromatography at 1 mL/min with MeCN gradients in 0.1% CF_3COOH in H_2O, λ=220 nm.
[b] Determined by quantitative amino acid analysis of the products from known quantities of peptidyl rosin.
[c] Measured chromatographically.
[d] Aquapore butyl, 4.6x220 cm, 0 to 30 % MeCN over 30 min.
[e] Vydac 201TP54, 4.6x250 cm, 2.5 to 12 % MeCN over 30 min.

A peptide rich in hydroxyamino acid residues was synthesised using two different approaches. In one experiment commercial Fmoc-Tyr-OPepSyn KA resin and Fmoc-Xaa(Bu^t)-OH's were used, in the other experiment Fmoc-Tyr(Tbdms)-OPepSyn KA prepared as described above and the Fmoc-Xaa(Tbdms)-OH's were used. In each case 4 eq of the amino acid derivatives were coupled for 1 h using the above coupling

conditions and deprotection/resin cleavage was achieved with 5 % PhSMe in CF_3COOH for 2 h. In the Tbdms synthesis the last Fmoc-deprotection was effected for 15 min with 0.1 M Bu^n_4NF in DMF. The results in Table 3 show the efficacy of the Fmoc-amino acid Tbdms ethers in SPPS.

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14. The intermediates 1d are obtained from le with phenacyl bromide and Pr^i_2NEt in EtOAc. After treatment for 10 d with isobutylene in the usual fashion, 2d are obtained. Treatment with 10 eq Zn powder in AcOH overnight, evaporation and crystallisation from CH_2Cl_2/hexanes, afforded analytically pure 3a in 70 - 80 % overall yield.
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