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Easy saponification by metal silanolates: application in SPPS and in (*S*)-5-hydroxynorvaline preparation

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Abstract—Alkali metal trimethylsilanolates, $TMSO^-$, M^+, has been used for efficient conversion of methyl esters into their corresponding anhydrous acid salts under mild non-aqueous conditions. This strategy has been applied to SPPS for the preparation of neurotoxin cyclic analogues and in (*S*)-5-hydroxynorvaline synthesis.

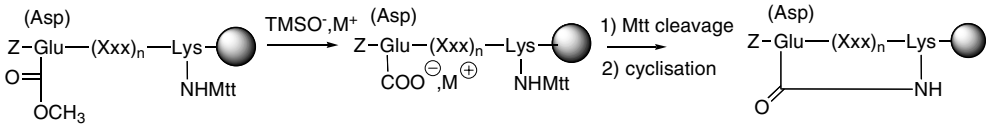
Many efforts are currently devoted to the solid phase synthesis of cyclic peptides.[1] An important part of our on-going research on peptide synthesis is directed towards a neurotoxin isolated from funnel web spider *Agenelopsis aperta* venom, and especially on one of its loops as a minimal active sequence. The aim of our efforts is the discovery of new pharmacological tools for cognition studies.[2,3] We undertook the preparation of cyclic peptides, analogues of this important loop, by performing the replacement of carefully chosen residues by an alanine in order to determine the key structural features for the toxin activity.

We envisioned a new solid phase cyclisation strategy that could be accomplished with relatively mild deprotection protocols. After molecular modelling studies, peptide cyclization by amide formation between the lateral chains of a native lysine and a glutamic acid or an aspartic acid has been considered.[4] The synthesis was carried out on various acid-labile resins (BARLOS chlorotrityl, Rink amide or Wang resins), by Fmoc strategy. The side chain of the glutamic acid or the aspartic acid derivative had to be orthogonally protected as methyl ester to avoid reaction on the lateral carboxylic acid function during the last coupling step (Scheme 1).

To prove the feasibility of the solid phase methyl ester selective removal, in a first step, N-protected H-Glu(OMe)-OH or H-Asp(OMe)-OH were anchored on the selected resins by classical procedures.[5] In a second step, and it is the aim of this report, methyl ester removal was studied using alkali metal trimethylsilanolates (TMSONa and TMSOK). The advantage of silanolates salts over other oxygen anions consists in the fact that they have a good solubility in organic solvents (diethylether, THF, toluene, methylene chloride).[6] Substrates were converted into their alkali salts in mild anhydrous conditions and in different organic solvents (THF, DMF, DCM) (Scheme 2).[7]

Best conditions of using Na silanolates or K silanolates were listed in Table 1.

In the standard procedure,[7] yields were evaluated by HPLC by comparison between P-Xxx-OH and P-Xxx(OCH_3)-OH peaks areas in the reaction mixture.



Scheme 1.

Keywords: Sodium and potassium silanolates; SPPS; Aminoacids; Glu; Asp; Selective deprotection.
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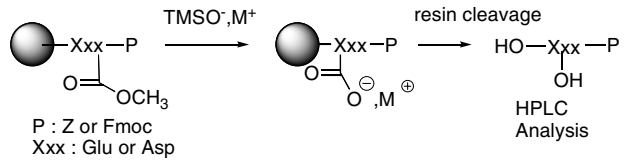
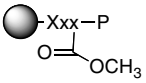
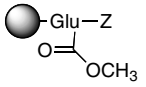
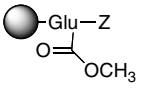
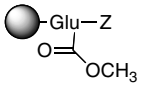
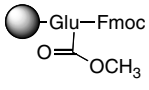
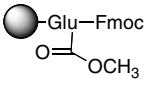
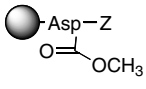
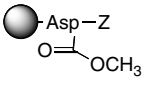


Scheme 2.

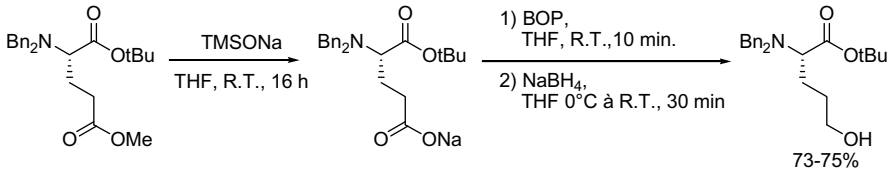
The best yield was observed with the couple TMSONa/THF with Z-Glu(OMe)-anchored on Barlos and Wang resins. However the kinetics were low (24 h) due to the steric hindrances of support and protecting group. The DMF was a bad solvent with both silanolates, Na silanolate gave better yields compare to the K silanolate, K being too bulky.

When the ZGlu(OCH_3)-OH was anchored on Rink amide resin, the hydrolysis gave an unchanging conversion rate of about 40% after 2 h whatever the solvent/silanolate system.

Table 1.

Resin: 	Silanolates	Reaction time (h)	Solvent	Hydrolysis yield in % (under microwave irradiations)
Barlos: 1.5 mmol/g				
	TMSOK	24	THF	88 (/)[a]
	TMSONa	24	THF	100 (/)[a]
Wang: 0.72 mmol/g				
	TMSOK	24	DMF	40 (55)
	TMSONa	24	THF	61 (80)
Rink amide: 0.7 mmol/g				
	TMSOK or Na	2	DMF	44 (/)[a]
	TMSOK or Na	2	THF	40 (/)[a]
Barlos: 1.5 mmol/g	TMSOK	2	DCM	43
	TMSOK	2	THF	35
	TMSONa	2	DCM	40
	TMSONa	2	THF	28
Wang: 0.72 mmol/g	TMSOK	2	DCM	27
	TMSOK	2	THF	By-products
	TMSONa	2	DCM	20
	TMSONa	2	THF	By-products
Barlos: 1.5 mmol/g	TMSOK	24	THF	41
	TMSONa	24	DCM	12
	TMSONa	24	THF	68
Wang: 0.72 mmol/g	TMSOK or Na	24	DCM	100
	TMSOK or Na	24	THF	100
				
Z-Glu(OMe)-OH	TMSONa	20	THF	100
Z-Asp(OMe)-OH	TMSONa	20	THF	100
Fmoc-Glu(OMe)-OH	TMSONa	24	THF	57 + by-products
Fmoc-Asp(OMe)-OH	TMSONa	24	THF	70 + by-products

[a] (/) under microwave irradiations without success (by-products and Resin degradation).



Scheme 3.

With Fmoc-Xxx(OMe)-OH, whatsoever the nature of the resin, dibenzofulvene appeared in HPLC before cleavage and has been identified by ^{1}H NMR after isolation by silicagel column chromatography. This can be explained by the fact that TMSONa or TMSOK are too basic to preserve Fmoc protecting group. The methyl ester was still present on the lateral chain of Glu or Asp showing that the kinetics of the Fmoc removal were much faster than the methyl ester hydrolysis.

To exclude the hypothesis that the different reaction rates were linked to the fact that the experiments were carried out on solid support, we performed silanolate-mediated saponifications in homogenous conditions, obtaining the same results.

Microwave irradiations[8,9] were used for hydrolysis activation on solid phase but without any success: by-products and support degradations were observed after 5 min at 600 W. As described in[9] only preloaded Wang resin resisted to microwave irradiations.

Because of the interest of this method, it has been applied on the selective deprotection of the side chain carboxylic function of Bn_2-Glu(OMe)-O*t*Bu, followed by a one pot reduction using the BOP/$NaBH_4$ system.[10] In this way, the anhydrous sodium salt obtained in the first step was directly reduced in alcohol (Scheme 3). The overall yield was 73–75% and the *t*Butyl ester was not affected by the whole procedure.[11]

The use of silanolates was a potent system for selective removal of methyl esters on the lateral chain of Glu or Asp when their NH_2 were protected by Z or Bn_2 group, on solid support and homogenous conditions. Further studies involving a Boc or a phthalimido group as N-protection are under way in our laboratory.

For SPPS applications the last Glu or Asp derivatives were protected by Z group and after cleavage of the Mtt group by 1% TFA, the cyclisation smoothly occurred on solid support and will be reported in due course.

References and notes

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7. Standard methyl ester removal procedure: the protected anchored aminoacid was stirred in a reactor with 1.5 equiv of alkali silanolate in different solvents. The kinetic was evaluated after acidic cleavage from a few beads (conditions for Barlos: 1% TFA in DCM + 1% scavenger TIPS ; for Wang: 50%TFA in DCM + 1% scavenger TIPS; for Rink-amide 95%TFA + 1% scavenger TIPS) and HPLC analysis at different time (30 min, 1, 2, 4, 20, 24 h, on nucleosil C18 reverse phase Symmetry ShieldTM column, 0–100% CH_3CN). The rate of hydrolysis was calculated by comparaison of Xxx(OMe)-OH and Xxx(OH)-OH peaks area by HPLC, after LC–MS identification by electrospray on a micromass ESI Platform II. The reaction was stopped at 24 h. Then the Xxx(OMe)-OH and Xxx(OH)-OH were cleaved from the resin.
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9. Collins, J. M.; Collins, M. J.; Steorts, R. C. *Peptides* **2004**, Standard hydrolysis procedure in the microwave oven: same conditions as in Ref. 7 but placed in a microwave vessel and taken up in different solvents with 1.5 equiv silanolates (DCM, DMF, THF). The vessel was sealed and heated to 180 °C for 5 min at 600 W in a CEM Explorer microwave oven.
10. McGeary, R. P. *Tetrahedron Lett.* **1998**, *39*, 3319–3322.
11. Bn_2-Glu(OMe)-O*t*Bu (1 g, 2.52 mmol, 1 equiv) was dissolved in anhydrous THF (15 mL). Commercially available TMSONa solution in DCM (1 M, 2.8 mL, 2.8 mmol, 1.1 equiv) was added and the mixture was stirred at rt during 16 h. Bop reagent (1.45 g, 3.27 mmol, 1.3 equiv) was added at 0 °C. The suspension was stirred 20 min at rt, become clear brown and $NaBH_4$ was slowly added (190 mg, 5.03 mmol, 2 equiv) at 0 °C. The yellow mixture was stirred 40 min at rt. The mixture was evaporated under reduced pressure and white residue was extracted by ethyl acetate and distilled water (20 mL). The aqueous layer was extracted again with ethyl acetate (2×20 mL). The organic layers were washed with brine (20 mL) dried over Na_2SO_4 and the solvent was eliminated by evaporation under reduced pressure to give a colourless oil. This oil was purified by chromatography on silicagel with petroleum ether/diethylether (6/4) (v/v) as solvent system. Overall yields: 73%. ^{1}H NMR, DMSO d^6, δ, ppm: 7.2–7.4 (10H, m, H_{arom}), 3.85 and 3.53 (2*2H, 2*d, H_{benzyl}, $J_{H-H'benzyl}$ = 14 Hz), 3.3 (2H, m, H_δ, $J_{\delta\text{-OH}}$ = 5.1 Hz), 3.07 (1H, t, H_α, $J_{\alpha-\beta}$ = 7.6 Hz), 1.67 (2H, m, H_β), 1.32 and 1.52 (2H, m, H_γ). ^{13}C NMR d^6, δ, ppm: 171,39 (C=O), 139.42 ($C_{arom\,1}$), 128.18 and 127.96 ($C_{arom\,m}$ and $C_{arom\,o}$), 126.89 ($C_{arom\,p}$), 80.28 ($C_{quat\,tBu}$), 64.88 (C_α), 62.86 (C_δ), 53.72 ($CH_{2benzyl}$), 29.17 (C_γ), 28.19 (CH_{3tBu}), 25.25 (C_β).