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Application of substituted 2-(trimethylsilyl)ethyl esters to suppress diketopiperazine formation

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Abstract—The use of differently substituted 2-(trimethylsilyl)ethyl esters for C-terminal protection in peptide synthesis has been investigated. While the use of the unsubstituted 2-(trimethylsilyl)ethyl ester resulted in a substantial amount of diketopiperazine at the dipeptide stage, use of the corresponding methyl-substituted silyl ester gave a significant reduction of this undesired pathway. Both esters could be deprotected by fluoride-induced cleavage under mild conditions.

A novel method for peptide manufacturing, called DioRaSSP—Diosynth rapid solution synthesis of peptides—has recently been introduced by Diosynth.[1,2] DioRaSSP combines the advantages of the homogeneous character of classical solution phase synthesis with the generic character and the amenability to automation of solid phase approaches. In the DioRaSSP approach, the growing peptide is essentially anchored in a permanent organic phase (generally EtOAc) by means of its hydrophobic C-terminal and side-chain protecting groups. Intermediates are not isolated, and excess reagents and by-products are intermittently removed by aqueous extractions. No organic waste streams are generated during the performance of the synthesis. Processes according to this highly efficient manufacturing method are easy to scale up and yield products of reproducibly high purity.

In a typical DioRaSSP process, the benzyloxycarbonyl (Z) function is applied for temporary amine protection, while *tert*-butyl type functions or functions of similar lability are generally applied for the semi-permanent protection of the C-terminal carboxylic function and functional side chains of the growing peptide. The Z protecting group is removed by hydrogenolysis in each cycle of the DioRaSSP process. In the case of peptides with sulfur-containing residues—which are not compatible with hydrogenolysis—and in the case of long peptides to reduce the risk of handling failures, a convergent synthetic approach using peptide fragments can be chosen. Such an approach requires the application of a C-terminal ester function, which is orthogonal with respect to both Z and *tert*-butyl type protection, that is the said function should be completely stable during hydrogenolysis—so all benzyl- and allyl-type functions cannot be used—and its cleavage should not give rise to premature loss of *tert*-butyl type protecting groups. Moreover, the conditions for its cleavage should be mild in order to preserve the integrity of the peptide chain. For instance, saponification to cleave primary alkyl esters cannot provide a general protocol, since this is likely to result in side reactions at incorporated Asp(OBu^t) residues.[3] Logically, the ester function should be stable under the conditions associated with the DioRaSSP process. Taking these specifications into account, the 2-(trimethylsilyl)ethyl (Tmse) ester was selected as an orthogonal C-terminal protecting group in our initial studies towards convergent DioRaSSP approaches. This ester can be deprotected under relatively mild conditions by fluoride-induced cleavage.[4] Its application, however, is associated with a serious drawback. While peptide fragments in a convergent approach are preferably selected to contain a Gly or Pro residue in the C-terminal position to ensure the enantiomeric purity of the ensuing product, such fragments

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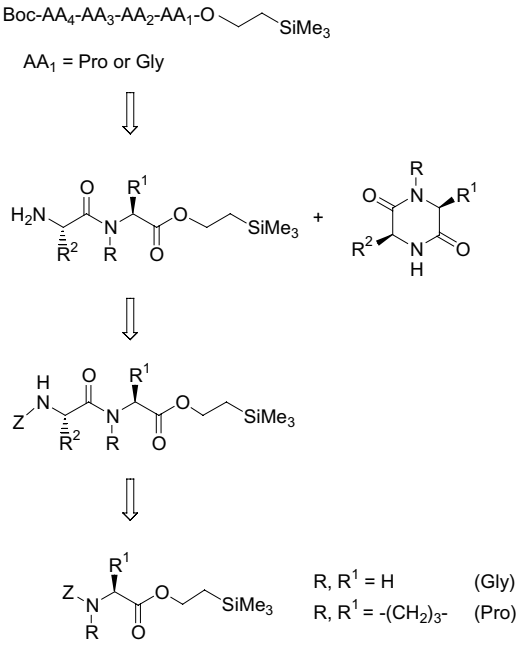
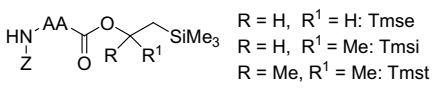


Scheme 1.

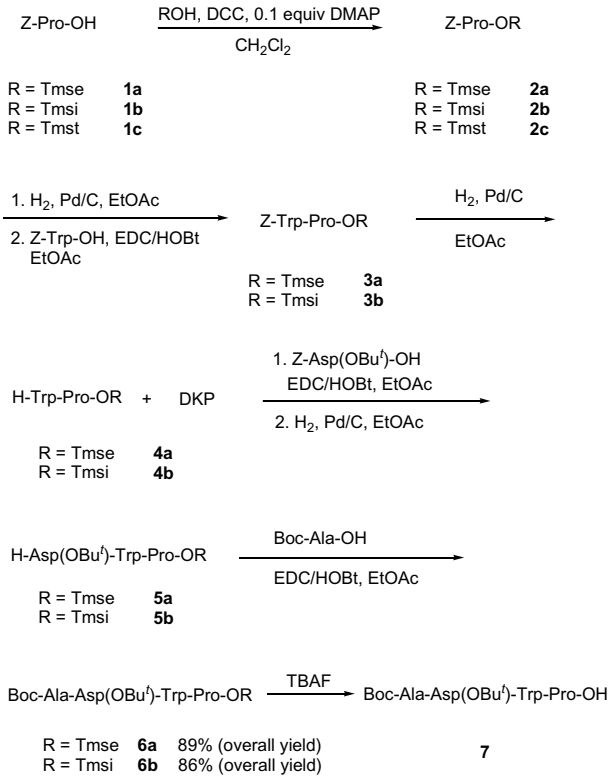
are extremely prone to diketopiperazine (DKP) formation at the dipeptide stage (Scheme 1).[5]

This propensity is most pronounced for primary esters. The problem was indeed encountered in the synthesis of the tripeptide Z-Asp(OBu^t)-Trp-Pro-OTmse according to the DioRaSSP protocol, but could be suppressed to a certain degree through addition of a molar equivalent of hydrochloric acid at the end of the hydrogenolysis of the intermediate dipeptide Z-Trp-Pro-OTmse. However, in order to arrive at a more robust protocol, the synthesis and application of secondary and tertiary analogues of the Tmse ester have been explored in our groups (Scheme 2).

For model studies, we selected *N*-benzyloxycarbonyl-L-proline (Z-Pro-OH), which was transformed into the corresponding esters (**2a**–**c**). These reactions were carried out using standard reaction conditions with the commercially available alcohol **1a** and the readily available alcohols **1b** and **c**,[6] dicyclohexylcarbodiimide (DCC) and a catalytic amount of DMAP[7] (Scheme 3). In the case of the Tmsi ester **2b**, a mixture of diastereoisomers was obtained in a 1:1.3 ratio, and used as such for further synthesis. The tertiary Tmst ester **2c** could be prepared, but was even cleaved under the mildly acidic conditions of HPLC analysis (0.1% TFA in the eluent). Consequently, we abandoned further investigations into the tertiary ester, since it would then also be too labile for use in the DioRaSSP methodology, which includes multiple acidic aqueous washing steps. Z-Pro-OTmsi **2b**



Scheme 2.



Scheme 3.

was then applied in an investigation regarding DKP formation. To this end, Boc-Ala-Asp(OBu^t)-Trp-Pro-OTmsi was prepared in an analogous fashion to the tetrapeptide Boc-Ala-Asp(OBu^t)-Trp-Pro-OTmse using the DioRaSSP protocol[8] (Scheme 3). During the hydrogenolysis at the dipeptide stage, the rate of DKP formation in the case of the Tmse and Tmsi esters **3a**, **b**, respectively, was studied and compared (Fig. 1). Clearly, in the latter case the extent of DKP formation was considerably reduced compared to the Tmse ester, which is probably due to the sterically somewhat more congested secondary ester function. Upon addition of a stoichiometric amount of HCl after completion of the hydrogenation reaction at ambient temperature, DKP formation was almost completely suppressed (Fig. 2).

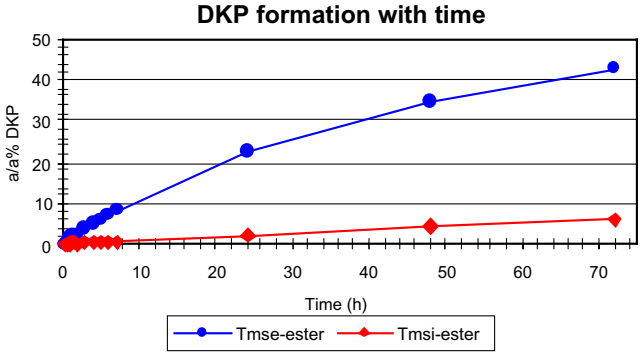


Figure 1.

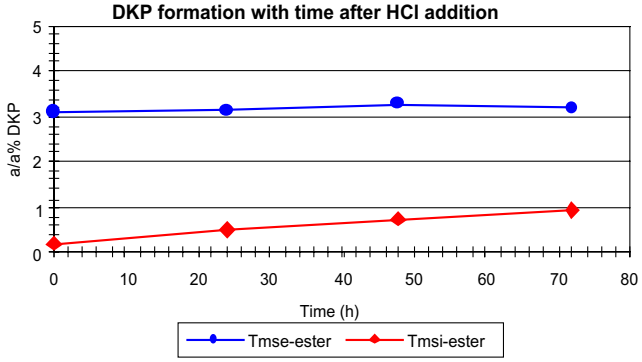


Figure 2.

We also investigated the deprotection of the Tmsi ester compared to the Tmse ester on the tetrapeptides **6b** and **6a**, respectively. As anticipated, the 2-(trimethylsilyl)ethyl and 2-(trimethylsilyl)isopropyl esters both readily underwent fluoride-induced cleavage upon treatment with an equimolar amount of tetra-*n*-butylammonium fluoride (TBAF) to afford the same tetrapeptide **7** with a free C-terminal carboxylic acid. The deprotection conditions for both esters were to some extent optimized (Table 1) using varying amounts of TBAF in combination with solvents that are compatible with the DioRaSSP procedure.

The reaction was relatively slow when carried out in a 1:1 mixture of EtOAc and THF containing 4 equiv of TBAF. The rate improved upon switching to pure THF and increasing the amount of TBAF. It was generally observed that deprotection occurred faster with the Tmse ester than with the Tmsi ester; however, both could be completely deprotected. Furthermore, deprotection of the Tmse ester occurred faster in pure THF than in EtOAc/THF, while no such rate enhancement was observed for the Tmsi ester. In all cases, the deprotection proceeded in a clean fashion without the formation of undesired side products.

In conclusion, we have investigated the application of differently substituted 2-(trimethylsilyl)ethyl esters as orthogonal C-terminal carboxylic acid protecting groups for peptide synthesis. The 2-(trimethylsilyl)isopropyl (Tmsi) ester proved most suitable for this purpose, giving rise only to very low amounts of diketopiperazine (DKP) formation in the peptide synthesis. The DKP formation could be suppressed even further by the addition of HCl after completion of the hydrogenolysis at the dipeptide stage. Additional advantages of the Tmsi group include its complete stability under the DioRaSSP conditions and its facile removal at the final stages of the desired sequence.

Table 1

Entry	Conditions	Reaction time for Tmse deprotection	Reaction time for Tmsi deprotection
1	TBAF (4 equiv) in ethyl acetate/THF 1:1 (v/v)	5–6 h	7–24 h
2	TBAF (4 equiv) in THF	3–4 h	7–24 h
3	TBAF (8 equiv) in THF	15–30 min	1–1.5 h

Acknowledgements

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References and notes

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8. Typical procedure for the preparation of tetrapeptide **6b** using the DioRaSSP protocol. *Z-Trp-Pro-OTmsi* **3b**: A solution of Z-Pro-OTmsi (1.75 g, 4.8 mmol) in a mixture of EtOAc (17 mL) and water (0.89 mL) at 20 °C was subjected to a H_2 atmosphere (1 bar) in the presence of 10% Pd/C (180 mg) and *N*-methylmorpholine (NMM, 528 μL, 4.8 mmol). Upon completion of the reaction, the catalyst was filtered off, and the residue was washed with EtOAc (5 mL). Then to the organic layer—containing the H-Pro-OTmsi derivative—were added 1-hydroxybenzotriazole (HOBt, 649 mg, 4.8 mmol), Z-Trp-OH (1.35 g, 4 mmol) and 1-(3′-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 843 mg, 4.4 mmol). After stirring the resulting solution for 1 h, an additional amount of EDC (84 mg, 0.44 mmol) was added. After stirring of the resulting solution until completion of the reaction, 3-dimethylamino-1-propylamine (254 μL, 2 mmol) was added. The mixture was stirred for 30 min and washed with 10% aqueous Na_2CO_3 (11 mL), 10% aqueous $KHSO_4$ (4×11 mL), 2×11 mL of 10% aqueous Na_2CO_3 (2×11 mL) and 30% aqueous NaCl (3×11 mL).
H-Trp-Pro-OTmsi **4b**: The organic layer containing the protected dipeptide Z-Trp-Pro-OTmsi was subjected to catalytic hydrogenolysis (H_2 gas) at 30 °C in the presence of 10% Pd/C (440 mg), water (1.14 mL) and NMP (0.69 mL). After completion of the reaction, 340 μL of 36% HCl (4 mmol) was added, the catalyst was filtered off and the catalyst was washed with EtOAc (5 mL).
H-Asp(OBu^t)-Trp-Pro-OTmsi **5b**: To the organic layer containing dipeptide **4b** were added HOBt (649 mg,

4.81 mmol), Z-Asp(OBu^t)-OH (1.55 g, 4.81 mmol) and EDC (843 mg, 4.41 mmol). The pH was adjusted to 5.2 using NMM (400 μL, 3.6 mmol). After stirring the resulting solution for 1 h at pH 5.2, an additional amount of EDC (84 mg, 0.44 mmol) was added. After stirring the resulting solution until completion of the reaction, H-β-Ala-OBzl *p*-tosylate (700 mg, 2.0 mmol) and NMM (244 μL, 2.2 mmol) were added. The mixture was stirred for 30 min and washed with 10% aqueous Na_2CO_3 (13 mL), 10% aqueous $KHSO_4$ (4×13 mL), 10% aqueous Na_2CO_3 (2×13 mL) and 30% aqueous NaCl (3×13 mL). The organic layer containing the protected tripeptide Z-Asp(OBu^t)-Trp-Pro-OTmsi was then subjected to catalytic hydrogenolysis (H_2-gas) at 20 °C in the presence of 10% Pd/C (280 mg) and water (1.4 mL). Upon completion of the reaction, 10% aqueous Na_2CO_3 (7.5 mL) was added and the resulting suspension was filtered. The residue was washed with EtOAc (5 mL) and the combined filtrates were washed with 10% aqueous Na_2CO_3 (7.5 mL and 15 mL) and 30% aqueous NaCl (3×15 mL).

Boc-Ala-Asp(OBu^t)-Trp-Pro-OTmsi **6b**: To the organic layer containing the tripeptide **5b** were added HOBt (649 mg, 4.81 mmol), Boc-Ala-OH (910 mg, 4.81 mmol) and EDC (843 mg, 4.41 mmol). After stirring the resulting solution for 1 h, an additional amount of EDC (84 mg, 0.44 mmol) was added. After stirring the resulting solution until completion of the reaction, 3-dimethylamino-1-propylamine (254 μL, 2.1 mmol) was added. The mixture was stirred for 30 min and washed with 10% aqueous Na_2CO_3 (15 mL), 10% aqueous $KHSO_4$ (2×15 mL), 10% aqueous Na_2CO_3 (2×15 mL) and of 30% aqueous NaCl (3×15 mL). The organic layer was evaporated to dryness to give the desired protected in 86% overall yield (2.52 g) based on the starting material Z-Trp-OH. Purity: 95.6 a/a % by reversed phase HPLC (2 to 75% MeCN in 0.1% trifluoroacetic acid in 48 min at 220 nm, 2.0 mL/min, 5 μ C18 column). Identity: *m/z* 758.6 $[M+H]^+$, 702.6 $[M-{}^t\mathrm{Bu}+H]^+$; 756.6 $[M-H]^-$, 802.6 $[M+HCOO]^-$ both by electrospray MS.