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A new safety-catch protecting group and linker for solid-phase synthesis

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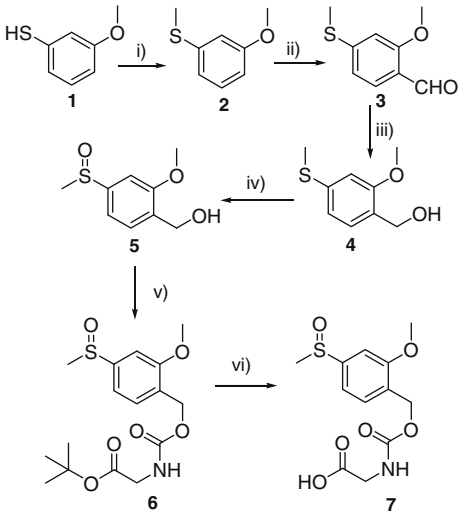
ABSTRACT

The use of two derivatives of 2-methoxy-4-methylsulfinylbenzyl alcohol is demonstrated as a safety-catch protecting group and linker for solid-phase peptide synthesis. The protecting group and linker are stable to TFA and are readily removed under reductive acidolytic conditions.

Orthogonal protecting groups are widely used in the solution and solid-phase synthesis of natural products, small molecules and complex peptides.[1] Consequently, various protecting groups that can be removed under selective conditions have been developed.[2] For instance, the two widely used solid-phase peptide synthesis methods involve, respectively, Fmoc/*t*-Bu and Boc/Bzl orthogonal protection strategies.[3] Incomplete coupling due to reduced solubility of the growing peptide chain and inter- and intra-chain interactions are major drawbacks encountered during Fmoc/*t*-Bu-based peptide synthesis.[4] These difficulties can be overcome by employing the Boc/Bzl method and solvents with high solvating parameter such as CH_2Cl_2, $(CF_3)_2CHOH$ and TFA.[5] In the case of the Boc/Bzl strategy, the use of acids such as hazardous HF and trifluoromethanesulfonic acid (TFMSA) precludes the synthesis of side-chain-protected peptides required for head-to-tail cyclization and convergent synthesis. Semipermanent protecting groups and linkers based on the TFA-stable *p*-methylsulfinylbenzyl ester have previously been reported.[6] Reductive acidolysis conditions have been employed for removal of the protecting group and cleavage of the peptide from the resin. However, the Bzl-type protecting groups are also removed under the reported conditions.[7] Selective removal of Bzl-type protecting groups while the peptide is attached to the resin, and selective cleavage of the peptide ester bond while the Bzl-type side-chain protecting groups are intact, would be useful in head-to-tail cyclization and convergent synthesis.

In this Letter, we report the potential of two derivatives of 2-methoxy-4-methylsulfinylbenzyl alcohol or Mmsb-OH, **7** and **14**, for the protection of amine and carboxylic acid groups, respectively. The protecting groups are structurally related to 4-methylsulfinylbenzyl alcohol-based Msib[8] and Msz[9] which were developed for the protection of hydroxy groups. Since the sulfoxide moiety *para* to the hydroxymethyl group is known to impart exceptional acid-stability to the ester bond,[6b,8] we envisioned that the presence of a methoxy group *ortho* to the hydroxymethyl group would render the ester bond more labile, especially during reductive acidolysis. As a logical extension of this proposition we also evaluated a carbamate-based amine protecting group, which would be useful for the preparation of N-protected peptide acids using the Fmoc/*t*-Bu strategy.

2-Methoxy-4-methylsulfinylbenzyl alcohol **5** was readily prepared in four steps as shown in Scheme 1. Commercially available 3-methoxythiophenol was alkylated using an equimolar quantity of MeI and a slight excess of triethylamine. Formylation of **2** using DMF–$POCl_3$ at 50 °C gave 2-methoxy-4-methylthiobenzaldehyde **3** as the major product owing to the strong *p*-directing ability of the thioether. The major isomer was isolated using column chromatography. Reduction of **3** with sodium triacetoxyborohydride gave the corresponding alcohol **4** which was subsequently oxidized using



Scheme 1. Synthesis of Mmsb-OH **5** and Mmsz-Gly-OH. Reagents and conditions: (i) MeI, Et_3N, CH_2Cl_2, rt, 6 h; (ii) DMF–$POCl_3$, DMF, 50 °C, 15 h, 55% for two steps; (iii) $NaB(OAc)_3$, Et_2O, 0 °C to rt, 3–5 h, 80%; (iv) *m*CPBA, CH_2Cl_2, 0 °C, 2–3 h, 80%; (v) (a) CDI, DMF, 3 h and (b) H-Gly-O*t*Bu, DMF, 6 h, 48%; (vi) TFA–CH_2Cl_2 (1:1), rt, 0.5 h, 71%.

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*m*CPBA to the corresponding sulfoxide **5**. The oxidation was carried out under controlled conditions whereby small portions of wet *m*CPBA (50–55%) were added over a period of 2–3 h to a stirred solution of **4** in ice-cold conditions to give only the sulfoxide product **5** in a very good yield.

For the preparation of Mmsz-protected glycine **6**, a slight excess of CDI was reacted with 1 equiv of Mmsb-OH **5** for 3 h and then with 2 equiv of glycine *t*-butyl ester. The Mmsz-protected glycine ester **6** was treated with 50% TFA/CH_2Cl_2 to liberate the carboxylic acid group. The overall yield involving all the steps in Scheme 1 was 12%. The NMR and mass spectral data were in agreement with the proposed structure (see Supplementary data).

In order to establish the suitability of the Mmsz-group for the safety-catch semipermanent protection of an amine group, a model hexapeptide corresponding to human Histone H3 (130–135) was synthesized on Wang resin using the Fmoc/*t*-Bu strategy (Scheme 2). Mmsz-Gly-OH was coupled to the resin-bound peptide using HBTU and DIEA.

The final cleavage was achieved with TFA/H_2O (97.5:2.5) (25 °C, 1 h) or TFA/(*i*-Pr)$_3$SiH/H_2O (92.5:5.0:2.5) (25 °C, 6 h). While the former cleavage conditions gave Mmsz-protected peptide **8** in excellent purity, the latter rendered the completely deprotected peptide **9** (Fig. 1). It is imperative to note that Mmsz protection of the amine group is stable to TFA but can be deprotected completely under reductive acidolysis conditions without requiring the use of a strong acid such as HF or TFMSA. For peptides containing Trp, Tyr and Cys residues, phenol and mercaptoethanol can be added as scavengers to obtain Mmsz-protected peptides. Considering the different acidolytic conditions reported in the literature[8] and the simple cleavage mixture used in this study, the Mmsz protective group appears to be a potential alternative to other benzyloxycarbonyl-based protecting groups, especially in solid-phase synthesis.

We also explored the possibility of using Mmsb-OH as a safety-catch linker vis-à-vis a semipermanent protecting group for carboxylic acids. A derivative of Mmsb-OH **14** was prepared for this purpose as shown in Scheme 3, and its utility as a linker and semipermanent protecting group for the carboxylic end of a peptide was demonstrated as shown in Scheme 4. First, ethyl bromoacetate was attached to the free thiol **1** to give ester **10**. Vilsmeier formylation of **10** gave two isomers and the major isomer **11** was isolated by column chromatography. After reduction of the aldehyde **11** to **12** and oxidation to the corresponding sulfoxide **13**, the carboxylic acid **14** was liberated by saponification using 2 N NaOH and subsequent acidification with 2 N HCl. The overall yield was 30%. The Mmsb-OH derivative **14** was anchored to the commercially

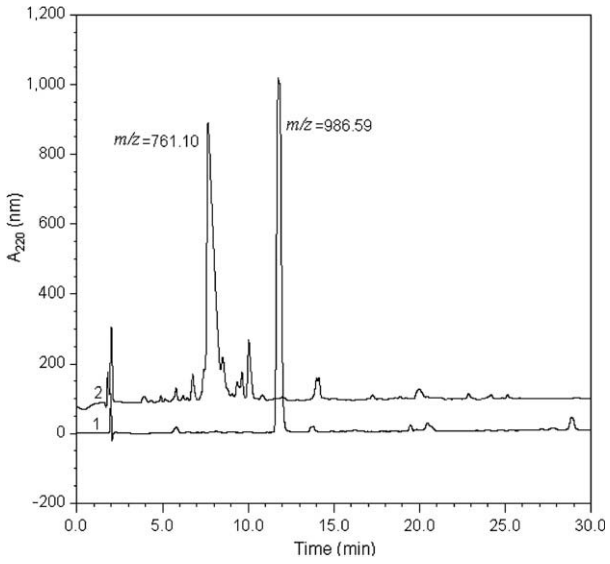
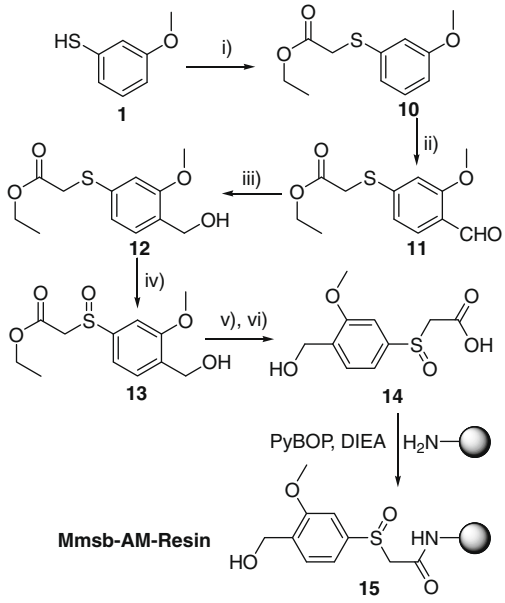
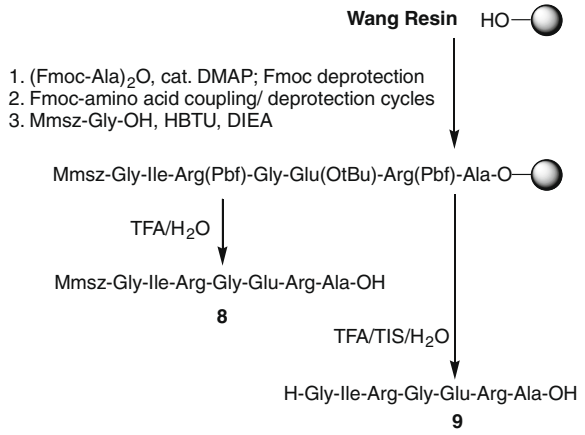


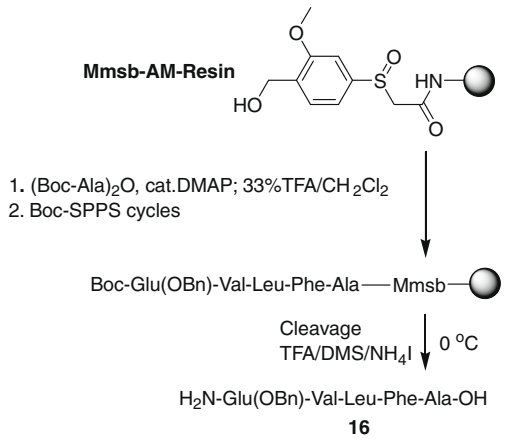
Figure 1. RP HPLC profiles of peptide **8** (trace 1) and peptide **9** (trace 2).



Scheme 3. Synthesis of an Mmsb linker for solid-phase synthesis. Reagents and conditions: (i) $BrCH_2COOEt$, Et_3N; (ii) DMF–$POCl_3$; (iii) $NaB(OAc)_3H$; (iv) *m*CPBA; (v) 2 N NaOH; (vi) 2 N HCl. See Supplementary data for experimental details.



Scheme 2. Synthesis of a model peptide in Mmsz-protected or deprotected form under different cleavage conditions.



Scheme 4. Boc solid-phase synthesis of a small model peptide using Mmsb resin.

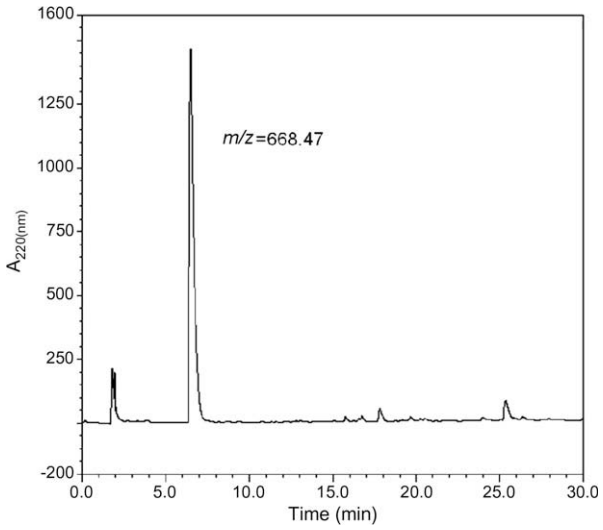


Figure 2. RP HPLC profile of the TFA/DMS/NH_4I-cleaved peptide **16**.

available aminomethyl polystyrene resin using PyBOP and DIEA. The resin was treated with 2% hydrazine in DMF to regenerate any benzyl alcohol that was acylated in the previous step.

To test the suitability of the Mmsb-OH derivative as a protecting group and linker in peptide synthesis, a model pentapeptide was synthesized following standard Boc-chemistry protocols. Boc-Alanine was attached to the linker using the symmetric anhydride of Boc-Alanine and catalytic amounts of DMAP. The Boc-protecting group was removed using 33% TFA in DCM. Subsequent amino acids were coupled using HBTU as the coupling reagent. The presence and absence of free amino groups were monitored during each step by the Kaiser test, which also indicated the stability of the peptide ester bond to repetitive treatment with 30% TFA in CH_2Cl_2. The peptide ester bond was finally cleaved from the resin using TFA/DMS/NH_4I[10] at 0 °C (2 × 30 min) and the crude products were analyzed by HPLC and ESI-MS. The yield of the crude peptide after lyophilization was about 64%. The cleavage cocktail TFA/TIS/H_2O was also capable of cleaving the peptide from the safety-catch linker at room temperature and took 6–7 h for complete cleavage. On the other hand, treatment of the peptidyl resin with TFA/H_2O (9.5:0.5) mixture did not give any detectable amounts of the peptide. It is clear from Figure 2 that the side-chain benzyl ester (protecting group) on Glu in peptide **16** remained intact under the conditions used for cleavage of the peptide ester bond with Mmsb-AM-Resin.

We envision that the Mmsz-group would be useful as a semipermanent protecting group for selected amines, while the Mmsb-ester would serve as a safety-catch carboxylic acid-protecting group which could be exploited as a linker in solid-phase synthesis of complex molecules that require selective removal of the side-chain protecting groups during synthesis. This work provides a new addition to the repertoire of orthogonal protecting groups that are available for organic synthesis.

Acknowledgements

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Supplementary data

Supplementary data (details on the synthesis of Mmsz-protected glycine and the Mmsb-OH derivative (linker), and NMR and mass spectrometric measurements) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.047.

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