"Safety-catch" anchoring linkages and protecting groups in solid-phase peptide synthesis

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Introduction

The C-terminal amide functionality which is found in many natural peptides often serves as a focal point for the development of synthetic methodology. The recent advances in peptide synthesis have opened vast new ideas for the intelligently designed use of anchoring linkages and protecting groups in synthesis of complex peptides [1]. In particular, there is growing interest in the use of linkages and protecting groups, the cleavage of which is based on the "safety-catch" principle [2,3,4]. Depending on the structure and cleavage conditions, these "protected protecting systems" may be applicable to Boc-/Fmoc-/Allyl-chemistries since such anchoring and protection offer the benefit of enhanced dimension of orthogonality during the synthesis. Moreover, the advantage that side-chain deprotection may be accomplished prior to cleavage from the support makes this methodology even more attractive. In our preliminary communication we have reported on the "safety-catch" anchoring linkage (SCAL) for synthesis of peptide amides [5]. The present report summarizes the results obtained with the SCAL anchoring and introduces a new "safety-catch" 2-methoxy-4,4'-bis(methylsulfinyl)benzhydryl (Msbh) (Figure 1) and 4,4'-bis(methylsulfinyl)trityl (Strt) protecting groups which have been evaluated as potential side-chain amide and SH protecting groups, respectively (Figure 2).

Results and Discussion

All three protecting blocks studied incorporate an interconvertible sulfoxide/sulfide system attached in the para position of the aromatic ring. In the first

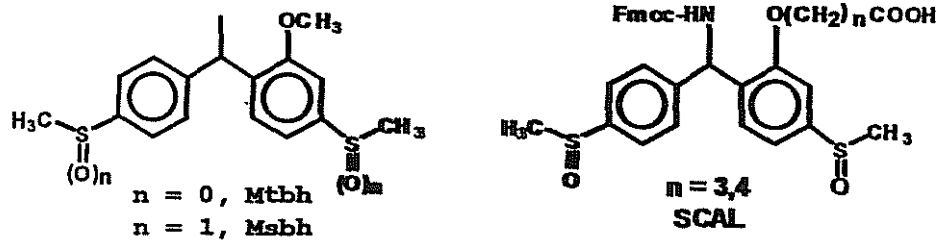

Fig. 1. Structures of Msbh/Mtbh protecting groups and SCAL linkage.

series of experiments we evaluated the stability/lability properties of Msbh and SCAL protecting groups under typical conditions for peptide synthesis. It was found that both the SCAL anchoring and the Msbh group are stable to anhydrous TFA (24h), 1M thioanisole/TFA (8h), TFA/thioanisole/EDT/phenol/water-82.5:5:2.5:5:5 (mixture K, 8h), anhydrous HF (0°C, 1h), 0.5M Ac_2O/NEt_3/ DCM (1h), and $Pd(PPh_3)_4$/AcOH/THF (1h). The 3.5M HCl/dioxane, 3M HBr/AcOH, and 1M Me_3SiBr/TFA, on the other hand, were found to cleave the benzhydrylamine C-N bond in SCAL skeleton within a few minutes by reductive acidolysis (one-step procedure). Alternatively, a two-step deprotection procedure using 1M Me_3SiCl/PPh_3/DCM (2h), 1M $PhSeSiMe_3$/DCM (1h), 20% $(EtO)_2P(S)SH$/DMPU (2h), 1M Me_3SiCl/PBu_3/DCM (2h) for reduction of the sulfoxide moieties, followed by the acidolytic cleavage with TFA/DCM/$^iBu_3SiH$ (90:8:2, 1h) offers the advantage of prior side-chain deprotection while the peptide chain remains attached to the carrier. A model peptide H-Tyr-Arg-Gln-Gly-NH_2 was synthesized on TentaGel (TG) resin using Fmoc-N^a-protected amino acids. The required N^a-fluorenylmethyloxycarbonyl-N^ω-2-methoxy-4,4'-bis(methylsulfinyl)benzhydryl-L-glutamine [Fmoc-Gln(Msbh)-OH] was prepared in three steps from Fmoc-Glu-O^{t}Bu and 2-methoxy-4,4'-bis(methylthio)benzhydrylamine. Oxidation of sulfur atoms to sulfoxides with $NaIO_4$ followed by the DCC/HOBt-mediated coupling afforded fully protected derivative Fmoc-Gln(Msbh)-O^{t}Bu. Subsequent treatment with 95% TFA/H_2O gave the Fmoc-Gln(Msbh)-OH in 37% overall yield. The sequence of reactions for the final cleavage of peptide from the support provides a basis for evaluation of acid stability/lability properties of both SCAL anchoring and Msbh protecting group. Firstly, after Fmoc group deprotection, the acid-labile ^{t}Bu and Pmc groups were removed from Tyr and Arg, respectively, by treatment with mixture K at 20°C for 2h. Secondly, the remaining Msbh group on the side chain of glutamine as well as the SCAL linker were reduced with 1M Me_3SiCl/PPh_3/DCM at 20°C for 2h to give the corresponding sulfide forms. Finally, cleavage of the peptide from the resin using a mixture of TFA/DCM/$^iBu_3SiH$ (90:8:2) afforded the pure peptide (95% by RPHPLC) in 84% yield. Further utility of the SCAL anchoring is illustrated in the preparation of the more complex peptide, Human Gastrin I (pGlu-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2). The synthesis was performed manually on TentaGel (PEG-PS) resin (Nle as internal standard) using Fmoc/^{t}Bu chemistry, DMF as a solvent, and DIC/HOBt for carboxyl activation. After completion of the synthesis, samples of peptidyl resin were treated with TFA/EDT/anisole (80:10:10, 1h) to remove all acid-labile side-chain protecting groups. Final treatment of peptidyl resin with Me_3SiBr/TFA/$^iBu_3SiH$/DCM (12:78:5:3, 2h, 0°C) afforded crude material which after purification by preparative RPHPLC furnished the title peptide in overall isolated yields of 10-12%. Purity and identity of product were assessed by RPHPLC (purified sample co-eluted with authentic Gastrin I), AAA, and FABMS.

The success of the synthesis of cysteine-containing peptides depends mostly on the protection chosen for the reactive thiol groups. Several thiol-protecting groups have been reported during the last decades, most of which are used in synthesis of simple monocyclic or more complex polycyclic peptides [6]. In the context of the "safety-catch" principle touched on above, a new "safety-catch" trityl protecting group was developed and evaluated as a potential S-protecting group (Figure 2). The synthesis of key intermediate, 4,4'-bis(methylsulfinyl)triphenylmethanol, involved initial treatment of 4,4'-bis(methylthio)-benzophenone with 1M PhMgBr/THF followed by the oxidation of sulfide moieties to sulfoxides with $NaIO_4$ (overall yield

91%). As a model system, N^{α}-fluorenylmethyloxycarbonyl-S-4,4'-bis(methylsulfinyl)trityl-L-cysteine [Fmoc-Cys(Strt)-OH] was prepared in 55% overall yield by the TFA-catalyzed reaction of L-cysteine with the derivative of trityl alcohol followed by the treatment with Fmoc-succinimide. In its oxidized form, the Strt group was found to be stable to 50% TFA/DCM (30min), 50% piperidine/DMF, iodine, $Tl(Tfa)_3$, and β-mercaptoethanol. On the other hand, the reduced form of this group can be easily removed by treatment with the mixture of 1% CCl_3COOH/DCM/scavenger. The applicability of Strt group was demonstrated by the synthesis of oxytocin (Fmoc/^{t}Bu chemistry) using SCAL anchoring to the TentaGel resin. After removal of Strt group (reduction with 1M Me_3SiCl/PPh_3/DCM (2h) followed by cleavage with CCl_3COOH/EDT/thioanisole/$^i Bu_3SiH$/DCM (1:3:3:5:88) for 1h), the disulfide bridge was formed on the resin under pseudo-dilution conditions using CCl_4/NEt_3/NMP (10 equiv, 5h, 35°C) [7]. Final cleavage of the cyclized peptide from the resin with TFA/DCM/$^i Bu_3SiH$ (90:8:2) followed by the preparative RPHPLC afforded pure oxytocin in 25% overall yield.

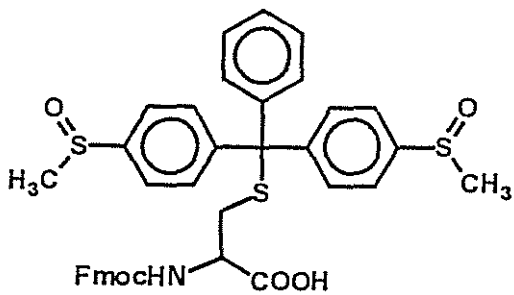

Fig. 2. Structure of Fmoc-Cys-(Strt)-OH.

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