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Safety-catch anchoring linkage for synthesis of peptide amides by Boc/Fmoc strategy

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Summary: 2-Methoxy-4,4'-bis(methylthio)benzhydrylamine (**10**) and the corresponding disulfoxide were prepared and tested as a model amide protecting groups for their stability toward acidic conditions. Subsequently, the novel 4-[4,4'-bis(methylsulfinyl)-2-oxy-(9-fluorenylmethyloxycarbonyl) benzhydrylamino]butanoic acid (SCAL) handle (**9**) has been prepared and applied to solid-phase peptide synthesis of C-terminal peptide amide using both 9-fluorenylmethyloxycarbonyl (Fmoc) and tert-butyloxycarbonyl (Boc) groups for N^{α}-amino protection.

A range of naturally occurring peptides, especially hormones such as oxytocin, secretin, LHRH, and calcitonin, contain a C-terminal amide function. Synthesis of peptide amides is best carried out by application of appropriate handles, which are quantitatively coupled in a single step onto amino-functionalized supports to provide a general starting point for peptide chain assembly. Surprisingly, the development of amide-anchor groups which can be cleaved by relatively mild procedures (e.g., TFA), were established only recently.[1] Considerable attention has been focused on preparation of benzylamine[1,2] and benzhydrylamine[1,3] derivatives substituted with electron-donating alkoxy groups.

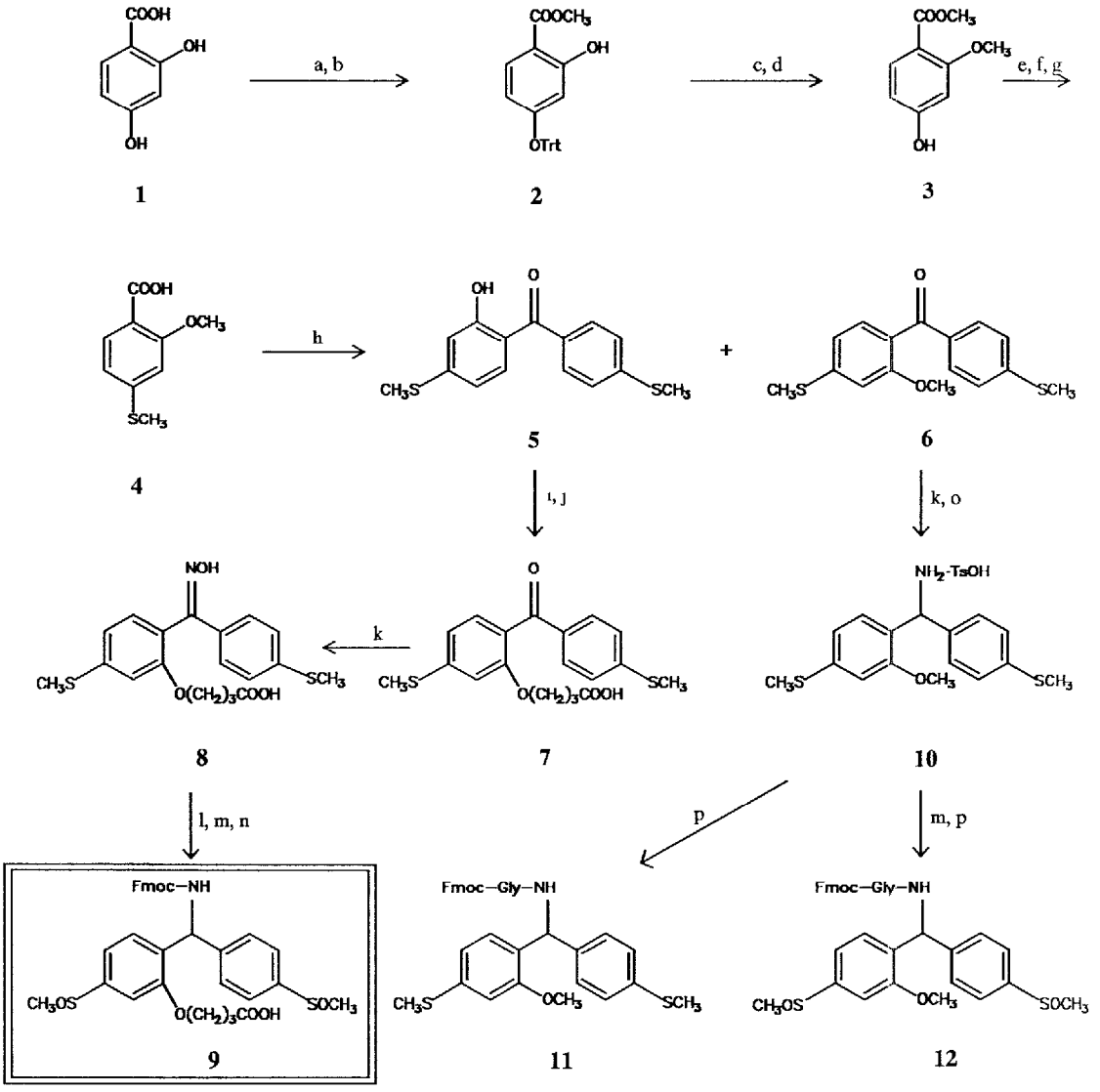
Earlier we have examined some benzhydrylamines containing *p*-methylsulfinyl or *p*-methylthio groups as a model amide protecting groups.[4] We have found that 4,4'-bis(methylsulfinyl)benzhydryl protecting group for amides is fully compatible with Fmoc and/or Boc protecting groups. We now describe the synthesis and application of a new type of safety-catch acid labile[5] (**SCAL**) handle **9** derived from 2-alkoxy-4,4'-bis(methylthio)benzhydrylamine, which extends the orthogonality[6] of currently used systems[7] in synthesis of C-terminal peptide amides and makes possible the combined use of both Fmoc and Boc groups in peptide synthesis. For a preliminary study of the suitability of this modified benzhydrylamine skeleton, we prepared in the first course new amide protecting group **12** and the corresponding sulfide **11**. The synthetic strategy chosen to achieve the overall transformation of *β*-resorcylic acid **1** both into compounds **11,12** and SCAL handle **9** is

outlined in Scheme 1.[8] 2-Methoxy-(4-methylthio)benzoic acid **4** as a key intermediate was prepared by modified procedure of Robertson and co-workers.[9,10] We have found that trityl group is more convenient then benzyl one for selective para protection of phenolic hydroxyl because of its easy introduction and mild removal. Not surprisingly, Friedel-Crafts acylation of thioanisole at 50°C afforded both required benzophenones **5,6** in 46% yield (mol ratio **5:6** = 1:1). Compounds **11** and **12** were prepared from **6** according to described procedure.[4] To determine the acid lability of **11** and **12** , a few deprotection experiments were performed. As a result, it was demonstrated that the sulfide **12** is extremely stable to conditions commonly used for removal of the Boc group (50% TFA/CH_2Cl_2-anisole). On the other hand, this sulfoxide undergoes fast one-pot reductive acidolysis when treated with $SiCl_4$/TFA/anisole[11] or Me_3SiBr/TFA/thioanisole[12] to give Fmoc-Gly-NH_2 within 30min. The corresponding sulfide **11** was shown to be considerable labile toward acidic cleavage mixture (1M-Me_2S/TFA, complete cleavage within 30min).

Since the results obtained with the pair **11/12** were encouraging, we advanced to the synthesis of analogous handle. Synthetic problems that were faced included the alkylation step that introduces an terminal carboxyl group for attachment to the support, and effective conditions for reduction of oxime into amine. Due to strong hydrogen interaction of 2-hydroxy group with benzophenone carbonyl, the alkylation of **5** carried out under common conditions (NaH, DMF) failed to give any product. To overcome this difficulty, we used the fluoride ion activation[13] for bridged phenolic function and the O-alkylated benzophenone ester was obtained in 64% yield. Saponification of this ester and subsequent reaction of **7** with hydroxylamine afforded the benzophenone oxime **8** in 94% yield. As regards the reduction of oxime **8**, the complex mixture was obtained upon acidic reduction conditions (Zn/AcOH). Therefore this oxime was reduced to the corresponding amine by zinc in ethanol/ammonia at 50°C.[14] However, the isolation of the pure amine was tedious due to the presence of salts in the reaction mixture. Therefore, the crude amine was directly oxidized into disulfoxide and then reacted with fluorenylmethylsuccinimidyl carbonate (FmocONSu) in one-pot reaction to give the SCAL handle **9** in 43% overall yield (based on oxime **8**).

Demonstration of the usefulness of SCAL anchoring was provided by the synthesis of segment with sequence related to human calcitonin. H-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-NH_2 was obtained in 95% purity (HPLC) after essentially quantitative cleavage with 1M-Me_3SiBr/thioanisole/TFA, for 2h at 0°C. As regards the peptide synthesis, SCAL handle was attached to the benzylamine resin and temporary N^{α}-amino protection was satisfactorily provided by Boc and Fmoc groups (Boc-Gly, Boc-Val, Boc-Ile; the other amino acids as Fmoc-derivatives).Controls during peptide synthesis proved that the deprotection and/or neutralization milieus did not cause either any premature cleavage or damage of handle.

Scheme 1



a) MeOH, $SOCl_2$; **b)** TrtCl, NEt_3, DMAP, DMF; **c)** MeI, NaH, DMF, **d)** DOWEX 50X2, MeOH; **e)** $ClCSNMe_2$, NaH, DMF; **f)** 235°C, aq NaOH, **g)** Me_2SO_4, NaH, DMF; **h)** $SOCl_2$ then 1,2-dichloroethane, $AlCl_3$, thioanisole, **i)** $Br(CH2)_3COOCH_3$, $Et_4NF\ 2H_2O$, DMF; **j)** aq NaOH, MeOH; **k)** AcONa, NH_2OH HCl, EtOH, **l)** Zn, aq NH_3, EtOH; **m)** $NaIO_4$, H_2O, MeOH, **n)** FmocONSu, THF, aq $NaHCO_3$, **o)** Zn, AcOH, TsOH, **p)** Fmoc-Gly-OH, HOBt, DCC, DMF

In conclusion, the new handle **9** seems to be very valuable tool for the preparation of peptide amides under mild conditions. The preparation of handle is achieved easily in good yield and the linker is fully characterized

before attachment to commercially available resin. Consequently, the anchor remains intact throughout the assembly of peptide chain by Fmoc and/or Boc chemistry and final cleavage from handle provides in high yield and purity peptide amide.

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