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3-(4-Hydroxymethylphenylsulfanyl)propanoic acid (HMPPA) as a new safety catch linker in solid phase peptide synthesis

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Abstract—A new safety catch linker, 3-(4-hydroxymethylphenylsulfanyl)propanoic acid (HMPPA), is described for use in solid phase peptide synthesis. The linker is readily synthesized from commercially available chemicals in a more cost efficient way compared to similar reported linkers. The HMPPA linker is easily attached to an amino derivatized solid support followed by on-resin oxidation of the thioether to sulfoxide, thereby making the linker very stable towards strong acid treatment. Final resin cleavage is performed by reductive acidolysis.

The synthesis of complex peptides often requires protective groups that can be selectively removed. For a number of reasons, final resin detachment and side-chain deprotection should be performed using anhydrous acid.[1] However, this requirement excludes many acid-cleavable protective groups, as the resin linkage could be jeopardized. The use of a safety catch linker is an attractive method to circumvent these problems, as most safety catch linkers are stable towards the reaction conditions commonly used in solid phase peptide synthesis. A safety catch linker is cleaved in a two-step process where the first step activates the linker and the second step involves the actual cleavage. Safety catch linkers based on TFA-stable arylalkyl sulfoxides have previously been reported.[2]

Marshall and Leiner coupled 4-mercaptophenol to Merrifield resin, with subsequent anchoring of the first amino acid to the phenolic oxygen and repetitive solid phase synthesis.[3] Oxidation of the thioether to the sulfone dramatically increased the linker's sensitivity towards nucleophiles. Treating the resin with the sodium salt of an amino acid resulted in both linker cleavage and C-terminal extension. This resin was later used to synthesize cyclic peptides on solid support.[4] A conceptually similar approach was later presented by Getman, who anchored 4-mercaptobenzylalcohol to Merrifield resin.[5] The resulting *p*-alkylthiobenzyl alcohol resin can be regarded as a sulfur analogue of the original Wang resin.[6] When oxidizing the thioether to the sulfoxide, the linker's stability towards acids is dramatically increased. The linker was finally cleaved by the 'low-TFMSA' method.[7] Another arylalkyl sulfoxide based linker for the synthesis of peptide acids is the DSB-linker described by Kiso et al.[8] Other linkers, such as the SCAL- and DSA-linkers, were developed for the synthesis of peptide amides.[9]

In projects related to the synthesis of various cyclic peptides, we came to the conclusion that the arylalkyl sulfoxide safety catch concept had many advantages but the currently described linkers had some specific disadvantages. The phenolic linkage of the Marshall linker cannot be expected to be stable during the repetitive nucleophile treatments needed for Fmoc protecting group removal. The linker described by Getman offers a simple route to introduce the linker on a solid support, but the starting material is extremely expensive. Anchoring the first residue to the DSB linker requires acylation of a secondary alcohol, which increases the risk of racemization.

This led us to develop a new arylalkyl sulfoxide safety catch linker that could be synthesized in high yields using inexpensive reagents in a few simple manipulations. A sulfur analogue of the frequently used

Abbreviations: AcN, acetonitrile; EDT, 1,2-ethanedithiol; HMPPA(O), 3-(4-hydroxymethylbenzenesulfanyl)propanoic acid; *p*-ether, petroleum ether; TFAA, trifluoroacetic anhydride; TFE, trifluoroethanol; TMSBr, bromotrimethylsilane.

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3-(4-hydroxymethylphenoxy)propionic acid linker is particularly attractive as this, and similar linkers, can be introduced onto a solid support by simple acylation of amino derivatized resins.[10]

Starting from the inexpensive and commercially available 4-(methylthio)benzaldehyde **1**, the linker 3-(4-hydroxymethylphenylsulfanyl)propanoic acid (HMPPA, **5**) was synthesized in three simple and high yielding steps, as outlined in Scheme 1.[11] It is clear that a more direct route to **5** would involve direct alkylation of commercially available 4-mercaptobenzaldehyde or 4-mercaptobenzyl alcohol. However, the cost of these reagents forced us to start from **1**.

An important intermediate in the synthesis of the HMPPA linker is the α-trifluoroacetoxy sulfide moiety **3**. The intermediate can be obtained through a Pummerer rearrangement by treating **2** with trifluoroacetic anhydride, yielding **3**, which in turn is readily hydrolyzed to the corresponding 4-mercaptobenzaldehyde.[12] However, under these conditions, the aldehyde moiety in **3** is simultaneously transformed into a geminal bistrifluoroacetate. This intermediate is quite unstable, and may undergo polymerization through intermolecular thioacetalization.[13] Polymerization can be avoided by performing the Pummerer rearrangement under mild reaction conditions and by adding the electrophile, 3-iodopropanoic acid, during hydrolysis.[14] This also provides for a simple work-up, as **4** can be separated by simple extraction. The HMPPA linker is finally obtained by reducing **4** with $NaBH_4$ in EtOH, which, after work-up, crystallizes readily from EtOAc/petroleum ether.

The linker is easily attached to an amino derivatized solid support in the form of an active ester.[15] The thioether is oxidized to the corresponding sulfoxide by treating the solid supported linker with 10 equiv of H_2O_2 in TFE/DCM (2:1) for 4 h.[16] TFE prevents further oxidation of the sulfoxide by forming strong hydrogen bonds, thereby diminishing the nucleophilicity of the sulfoxide for further oxidation by H_2O_2.[17] This was also confirmed experimentally by treating the model peptide Boc-K(Boc)FAFA-HMPPA(O)-AFK(Fmoc)LG-Merrifield resin, with 100 equiv of H_2O_2 in TFE/DCM (2:1) for 7 days. After cleaving the model peptide from the solid support by a 24 h treatment with neat TFA, RP-HPLC revealed that only 60% of the product was the sulfone and the remaining 40% was the sulfoxide.

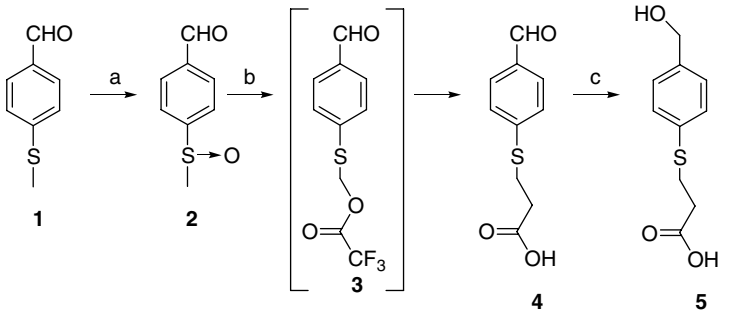
Attachment of the first residue to the linker can be achieved using standard protocols for derivatizing hydroxymethyl resins.[6]

A valuable application of the HMPPA safety catch linker is in the synthesis of side-chain-to-side-chain cyclic peptides. This was demonstrated by synthesizing the model peptide Fmoc-K(Boc)F-D-APE(O*t*Bu)G-HMPPA(O) on aminomethyl resin. This model peptide has previously been used in on-resin lactamization studies.[18] The peptide was treated for on-resin side-chain lactamization after acidolytic cleavage of the side-chain protective groups on Lys and Glu. Subsequent resin cleavage and analysis by RP-HPLC and MALDI-TOF revealed that both the linear and the cyclic peptides had been synthesized in very good purity. The only by-product detected after on-resin lactamization was the cyclic dimer.

Peptides synthesized on the oxidized HMPPA linker can be resin cleaved in a one-pot reductive acidolysis reaction.[19] By adding 0.2 M EDT/0.1 M TMSBr in TFA to the resin bound peptide, the linker sulfoxide is reduced to the corresponding thioether, thereby making the ester linkage susceptible for acidolysis.[20] The reducing agent is the bromine anion and EDT functions as a bromine scavenger.

Resin cleavage studies showed that cleavage efficiency is about 90% as measured by quantitative ninhydrin test.[21] Furthermore, the overall yield in the synthesis of the linear peptide KF-D-APGE-OH was 52% as calculated from resin loading.[22]

During reductive acidolysis, benzyl-type protective groups commonly used in solid phase peptide synthesis are also cleaved.[23] This is especially interesting, as it would open the possibility of using the Boc/Bzl synthetic strategy when synthesizing peptides on a solid support, yet avoiding the use of hazardous super acids for final resin cleavage. However, more acid resistant side-chain protective groups, in particular Arg(Tos) and Cys(MeBzl), are not cleaved by reductive acidolysis.[24] We therefore speculated that these protecting groups could be removed via a high-acid S_N1 mechanism on-resin,



Scheme 1. Synthesis of the HMPPA linker. Reagents and conditions: (a) H_2O_2 (2 equiv) in TFE/DCM (2:1), 4 h; (b) 3 equiv TFAA in AcN/ 2 equiv 2,6-lutidine, 1 h, 0 °C → rt, then 1.0 equiv 3-iodopropanoic acid in TEA/MeOH (1:1); (c) $NaBH_4$ in EtOH.

without cleaving the product from the HMPPA(O) linker, as the linker should be stable towards even very strong acid treatment.[25] Studies on model peptides containing the oxidized HMPPA linker in homogeneous solution suggest that the linker is very stable towards strong anhydrous acids and could potentially be used when cleaving side-chain protective groups through an S_N1 mechanism in Boc chemistry. Relatively low levels of linker cleavage (14%) were observed after a 3 h treatment with TFMSA/TFA (1:9) at 0 °C. However, common scavengers had a negative effect on the linker stability and integrity. When treating the model peptides with TFMSA/TFA and *p*-cresol or anisole (1:9:1), a single by-product was formed. Although the structure of this side-product was not determined, it is likely that it represents S-arylation of the HMPPA linker by the scavenger as previously reported.[26] S-arylation prevents subsequent resin cleavage by reductive acidolysis. The scavengers thioanisole and thiophenol, on the other hand, resulted in reduction of the HMPPA sulfoxide and subsequent cleavage of the linker.[27] These initial results were somewhat disappointing but suggested that if a scavenger can be identified that does not react with the linker under strong acidic conditions, it should be possible to selectively cleave most side-chain protecting groups commonly used in the Boc/Bzl protective groups strategy, while the peptide is still attached to solid support. Final resin cleavage is performed by a relatively mild method; hence, this approach would circumvent many of the problems associated with the use of super acids for final resin cleavage in Boc chemistry.

Relatively few studies have been carried out on side-reactions associated with the reductive acidolysis method presented here. It can, however, be expected that the indole side-chain functional group in Trp residues would be the most vulnerable residue for modifications during reductive acidolysis, in particular to bromination. Therefore, the model peptide Fmoc-K(Boc)W-(Boc)FAK(Boc)PVA-HMPPA(O)-aminomethyl resin was synthesized using standard Fmoc/*t*Bu protective group strategy and subsequently resin cleaved by reductive acidolysis. Analysis by RP-HPLC and MALDI-TOF revealed that the KWFAKPVA-OH peptide was obtained as the major product (87%). Two minor by-products were detected, that is, the 2-indolylindoline dimerization product (7%) and EDT scavenger addition products (6%).[28] However, it was noted that higher amounts of by-products were isolated if the removal of TFA was slow during work-up. On the other hand, it is noteworthy that no brominated by-products could be observed. It can, therefore, be suggested that the side-reactions observed are more a reflection of the use of EDT and a relatively weak acid during final cleavage, rather than the reductive acidolysis method.

In conclusion, although a large number of linkers are available for the solid phase synthesis of peptides, relatively few of these are safety-catch linkers. The HMPPA linker might, therefore, prove to be a valuable new tool in the synthesis of a number of complex peptides, which require selective removal of the side-chain protecting groups during synthesis.

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References and notes

1. Barany, G.; Merrifield, R. B. In *The peptides*; Erhart, G., Meienhofer, J., Eds.; Solid-phase Peptide Synthesis; Academic Press: New York, 1980; Vol. 2, pp 1–284.
2. Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091–2157.
3. Marshall, D. L.; Liener, I. E. *J. Org. Chem.* **1970**, *35*, 867–868.
4. Flanigan, E.; Marshall, G. R. *Tetrahedron Lett.* **1970**, *27*, 2403–2406.
5. Getman, D. P.; Heintz, R. M. *Chem. Abstr.* 110:213351, Eur. Pat. Appl. **1988**.
6. Wang, S.-S. *J. Am. Chem. Soc.* **1973**, *95*, 1328–1333.
7. Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1986**, *108*, 5242–5251.
8. Kiso, Y.; Fukui, T.; Tanaka, S.; Kimura, T.; Akaji, K. *Tetrahedron Lett.* **1994**, *35*, 3571–3574.
9. Pátek, M.; Lebl, M. *Tetrahedron Lett.* **1991**, *32*, 3891–3894; Kimura, T.; Fukui, T.; Tanake, S.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1997**, *45*, 18–26.
10. Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1985**, *26*, 92–97.
11. 4-Methylthiobenzaldehyde (5 g, 33 mmol) was dissolved in TFE/DCM (2:1, 75 ml), the solution was cooled on ice and then 30% H_2O_2/H_2O (7.45 ml, 66 mmol) added. The reaction was left on ice for 30 min and then at ambient temperature for 4 h. Subsequent TLC analysis (EtOAc/*p*-ether 1:1) indicated complete oxidation. Excess $H_2O_2$was decomposed by adding Pd/C. When the gas evolution had stopped, the catalyst was filtered and the solvents removed in vacuo. The resulting white solid (**2**, 5.5 g, 33 mmol) was dissolved in AcN (100 ml) containing 2,6-lutidine (11.5 ml, 99 mmol), and then cooled on ice under a blanket of N_2. TFAA (9.14 ml, 66 mmol) in AcN (25 ml) was added dropwise. Upon completion, the reaction was allowed to reach rt and then left for 30 min. The yellowish solution was added to a pre-cooled solution of TEA/MeOH (1:1, 100 ml), containing 3-iodopropanoic acid (6.6 g, 33 mmol) and then left at ambient temperature for 4 h. The solvents were removed in vacuo and the resulting oil was taken up in a saturated Na_2CO_3 solution (100 ml) and extracted with EtOAc (3×75 ml). The solution was acidified (concd HCl) resulting in voluminous precipitation. The precipitate was filtered, washed with cold 1 M $HCl_{(aq)}$ and dried in vacuo, yielding **4** (5.73 g, 83%). The crude product was dissolved in ethanol (50 ml) and cooled on ice. $NaBH_4$ (3.1 g, 82 mmol) was added and the reaction left for 5 h. Water (100 ml) was added, the ethanol removed in vacuo and the pH adjusted to 3 by adding concd HCl. The product was filtered and washed with 1 M HCl and dried, yielding **5** (5.06 g, 88%). A sample of the linker was crystallized from EtOAc/*p*-ether for NMR analysis. ^{1}H NMR **5**: (400 MHz, DMSO-d_6): δ 7.28, 7.26 (2d, 4H, $J = 5.8, 8.6$), 4.44 (s, 2H), 3.08 (t, 2H, $J = 7.1$), 2.48 (t, 2H, $J = 3.8$). ^{13}C NMR: (100 MHz, DMSO-d_6): 28.3, 33.9, 39.1, 62.5, 127.4, 128.9, 133.4, 140.7 and 172.8.
12. Pummerer, R. *Chem. Ber.* **1910**, *43*, 1401–1412; Young, R. M.; Gauthier, J. Y.; Coombs, W. *Tetrahedron Lett.* **1984**, *25*, 1753–1756.

13. Gryko, D. T.; Clausen, C.; Lindsey, J. S. *J. Org. Chem.* **1999**, *64*, 8635–8647.
14. Sugihara, H.; Tanikaga, R.; Kaji, A. *Synthesis* **1978**, *12*, 881.
15. Rich, D. H.; Singh, J. In *The Peptides*; Gross, E., Meinenhofer, J., Eds.; The Carbodiimide Method; Academic Press: New York, 1979; Vol. 1, pp 241–261.
16. Ravikumar, K. S.; Kesavan, V.; Crousse, B.; Bonnet-Delpon, D.; Begue, J.-P. *Org. Synth.* **2003**, *80*, 184–189.
17. Curci, R.; DiPrete, R. A.; Edwards, J. O.; Modena, G. *J. Org. Chem.* **1970**, *35*, 740–745.
18. Cavallaro, V.; Thompson, P.; Hearn, M. *J. Pept. Sci.* **1998**, *4*, 335–343.
19. Beck, W.; Jung, G. *Lett. Pept. Sci.* **1994**, *1*, 31–37.
20. Landini, D.; Modena, G.; Montanari, F.; Scorrano, G. *J. Am. Chem. Soc.* **1970**, *92*, 7168–7174.
21. Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147–157.
22. The model peptide was synthesized using the standard Fmoc/*t*Bu protecting group strategy on the HMPPA linker, attached on aminomethyl polystyrene. In brief: The HMPPA linker was coupled to 1 g of aminomethyl polystyrene (0.62 mmol/g), by HOBt ester activation (preformed by mixing 2.3 equiv HMPPA linker, HOBt and DIC, respectively, in DCM 30 min at 0 °C) for 4 h. After washing and drying, gravimetrical analysis gave a resin loading of 0.553 mmol/g resin. The thioether was oxidized on-resin, by adding 10 equiv of H_2O_2 (0.63 ml) in TFE/DCM (2:1, 18 ml) for 4 h. 200 mg of the resin was transferred to another vessel and the first residue was attached by OAt ester activation (formed by premixing 10 equiv of Boc-Gly, HOAt and DIC, respectively, and 0.05 equiv DMAP in DCM for 30 min at 0 °C). The Boc group was removed by treatment for 20 min with TFA/DCM (1:1). Next, N-termini Fmoc protected residues were coupled by OBt ester activation. The Fmoc groups were removed by treatment for 20 min with 20% piperidine in DMF. 50 mg of the resin bound model peptide was treated for side-chain-to-side-chain lactamization by adding 2 equiv of BOP and HOBt, respectively, and 4.2 equiv of DIPEA in NMP to the side-chain deprotected peptide. The peptide was washed after 4 h and the procedure repeated once. The N-terminal Fmoc group was removed and the cyclic peptide, and 50 mg of the starting linear peptide, was resin cleaved by reductive acidolysis (0.2 M EDT and 0.1 M TMSBr in 3 ml TFA) for 2 h. The solution was filtered and washed with TFA (×3) and the solvents then removed in vacuo. The peptides were taken up in water and extracted with diethyl ether (×4). Both peptides were analyzed by RP-HPLC and MALDI-TOF. To estimate overall yields, the linear peptide was partially purified by semi-preparative RP-HPLC, lyophilized and analyzed gravimetrically, giving a total, overall yield of 52% as measured from initial resin loading.
23. Yajima, H.; Fujii, N.; Funakoshi, S.; Watanabe, T.; Muryama, E.; Otaka, A. *Tetrahedron* **1988**, *44*, 805–819.
24. Hughes, J. L.; Leopold, E. J. *Tetrahedron Lett.* **1993**, *34*, 7713–7716.
25. Sakakibara, S.; Shimonishi, Y.; Kishida, Y.; Okada, M.; Sugihara, H. *Bull. Chem. Soc.* **1967**, *40*, 2164–2167.
26. Funakoshi, S.; Fujii, N.; Akaji, K.; Irie, H.; Yajima, H. *Chem. Pharm. Bull.* **1979**, *27*, 2151–2156.
27. Yajima, H.; Fujii, N. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Acidolytic Deprotection Procedures in Peptide Synthesis; Academic Press: New York, 1983; Vol. 5, pp 65–109.
28. Fontana, A.; Toniolo, C. *Fort. Chem. Org. Nat.* **1976**, *33*, 309–449.