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Improved Preparation of a Safety-Catch Linker for the Solid Phase Synthesis of Peptide Acids Finally Released into Aqueous Buffers

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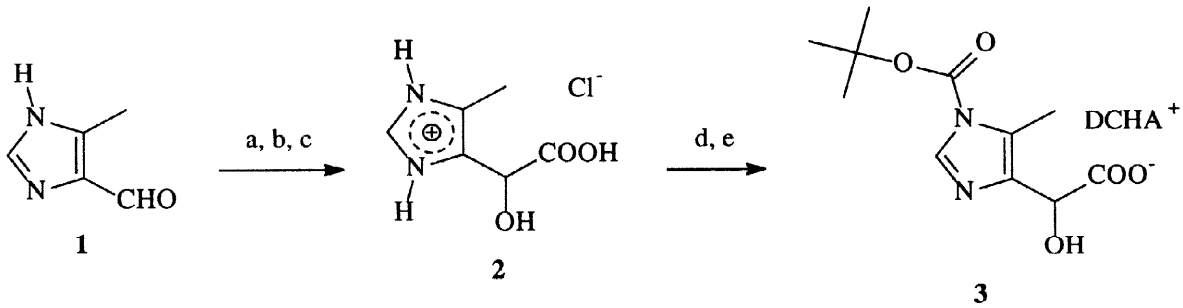
Abstract: We have substantially improved the synthetic route for the preparation of 2-(N^{im}-tert-butoxycarbonyl-5-methyl-imidazol-4yl)-2-hydroxyacetic acid, a safety-catch-linker recently developed by us, which allows the direct release of peptide acids into aqueous buffers after Fmoc solid phase synthesis.[1]

Recently we have described a new type of safety-catch linker for the solid phase synthesis of compounds under neutral to weak basic reaction conditions followed by a final acidic deprotection step.[1-3] Utilization of this linker results in compounds still attached to the synthesis support via an ester bond to the linker. The immobilized compounds then can be purified from chemicals by washing with a series of acidic solvent mixtures and buffers. The final release of the compounds from the support is achieved through an intramolecular catalyzed hydrolysis at neutral to weak basic pH directly into the aqueous medium of the bioassay. Such type of linker, thus, allows the simultaneous parallel solid phase synthesis, purification and direct solution phase assay of large numbers of different compounds or compound libraries. The efficiency of this linker was demonstrated for the preparation and solid phase purification of peptide C-terminal acids following the Fmoc route.

We were aware that the reported preparation of this linker compound includes some problematic steps, especially the isolation of the highly water soluble 2-(5-methyl-imidazol-4-yl)-2-hydroxyacetic acid or its hydrochloride **2** from large excesses of inorganic salts and have attempted to improve the procedure particularly with respect to larger scale preparations.

Both, the former and the novel synthesis start with a cyanohydrin reaction. For the isolation of the



Scheme 1: *Synthetic route for linker* **3**; a) KCN, HCl, acetic anhydride, H_2O; b) ethanol reflux, recrystallization from ethanol; c) 6N HCl reflux; d) $BocN_3$, triethylamine; e) dicyclohexylamine.

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formed hydrochloride **2** different methods are described,[2] but all turned out to be too laborious for the separation of the large amount of inorganic salts resulting from this reaction. We circumvented this problem by converting **2** into its ethyl ester which is readily obtained by extracting the solid residue of the evaporated reaction mixture with ethanol under reflux.[4] The ethyl ester is then hydrolyzed with aq. HCl to give **2** in a pure form.

Following the former synthetic route, **2** is then treated with bis-tert-butoxycarbonyl oxide (Boc_2O)/triethylamine or with tert-butoxycarbonyl azide ($BocN_3$)/triethylamine,[5] but all efforts to purify the free acid of **3** proved unsuccessful because it slowly decomposes during all crystallization conditions tested. Only the isolation of the corresponding dicyclohexylammonium salt yielded a pure and stable product. **3** is thus obtained in an acceptable yield of 61 % from **2** by acylation with $BocN_3$/triethylamine.[6] This synthetic procedure was then successfully carried out at a 50 to 100 mmol scale. To our surprise, the reaction of **2** with Boc_2O instead of $BocN_3$ generates **3** as a 1:1-mixture of the two possible regioisomers in quantitative yield.

We feel it is worth to communicate these easy and quite effective improvements in the large scale preparation of safety-catch linker **3** because of the wide interest in this compound from many colleagues working in the field of combinatorial chemistry.

REFERENCES AND NOTES

1. Hoffmann, S.; Frank, R. *Tetrahedron Lett.* **1994**, 35, 7763-7766.
2. Hoffmann, S. *New Convergent Strategies in the Synthesis of Complex Peptide Structures*,Technical University of Braunschweig 1995
3. Hoffmann, S.; Frank, R.; German pat. no. DP 43 19 475.3 / DP 43 20 260.8, PCT / EP 94 / 01 896.
4. Synthesis of 2-(5-methyl-1H-imidazol-4-yl)-2-hydroxy-acetic acid hydrochloride (**2**): To a suspension of 10 g (90.91 mmol) of 4-methyl-5-imidazolylcarbaldehyde (**1**) in 120 ml of water 13 g of potassium cyanide, 16 ml of conc. hydrochloric acid and 35 ml of acetic anhydride are added at 0 °C successively. The reaction mixture is stirred for 1 h at 0 °C and for 24 h at room temperature. After the addition of 280 ml of 6 N hydrochloric acid the solution is refluxed for 2 h followed by stirring for 24 h at 80 °C. The solvent is removed and the residue is dried in vacuo. Then it is refluxed with 500 ml of abs. ethanol for 10 min and filtered off after cooling. The sludge is refluxed again with 250 ml of abs. ethanol and the combined filtrates are evaporated. A saturated ethanolic solution is made and cooled to 0 °C. The precipitate is filtered off, the filtrate is evaporated and the residual solid is dried in vacuo. The obtained hydrochloride of the ethyl ester (10.87 g; 49.30 mmol; 54 %) is added to 460 ml of 6 N hydrochloric acid and is refluxed overnight. Removal of the solvent and drying in vacuo yield 9.27 g (48.16 mmol; 53 %) of **2** as a light brown solid. - The analytical data of compound **2** agree with those described in literature.[1,2]
5. For the preparation of $BocN_3$ see: Carpino, L. A. *J. Amer. Chem Soc.* **1957**, 79, 4427.
6. Synthesis of dicyclohexylammonium 2-(1-tert.-butoxycarbonyl-5-methyl-imidazol-4-yl)-2-hydroxy-acetate (**3**): In a dry reaction flask 500 mg (2.60 mmol) of hydrochloride **2** are dissolved under nitrogen atmosphere at 0 °C in 10 ml of abs. dimethylformamide and 1.08 ml (7.79 mmol) of triethylamine are added. After the addition of 743 mg (5.19 mmol) of tert-butoxycarbonyl azide the reaction mixture is stirred for 2 d at 4 °C. Then the dimethylformamide is removed in vacuo completely. The residue is suspended in dioxane and filtered off. 0.52 ml (2.60 mmol) of dicyclohexylamine are added to the filtrate before it is evaporated to dryness from the dioxane under reduced pressure. The residue is treated with excess ether. The precipitated product is filtered off and washed with ether. The filtrate and washing phase are combined, evaporated and the remaining residue is again submitted to the above described ether precipitation procedure. Collectively, 692 mg (1.58 mmol; 61 %) of product are obtained as a light brown solid. The same yield and purity is obtained at a twentyfold scale. - The analytical data of compound **3** agree with those described in literature with additional signals resulting from the dicyclohexylammonium ion.[1,2]