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Attachment of Histidine, Histamine and Urocanic acid to Resins of the Trityl-Type

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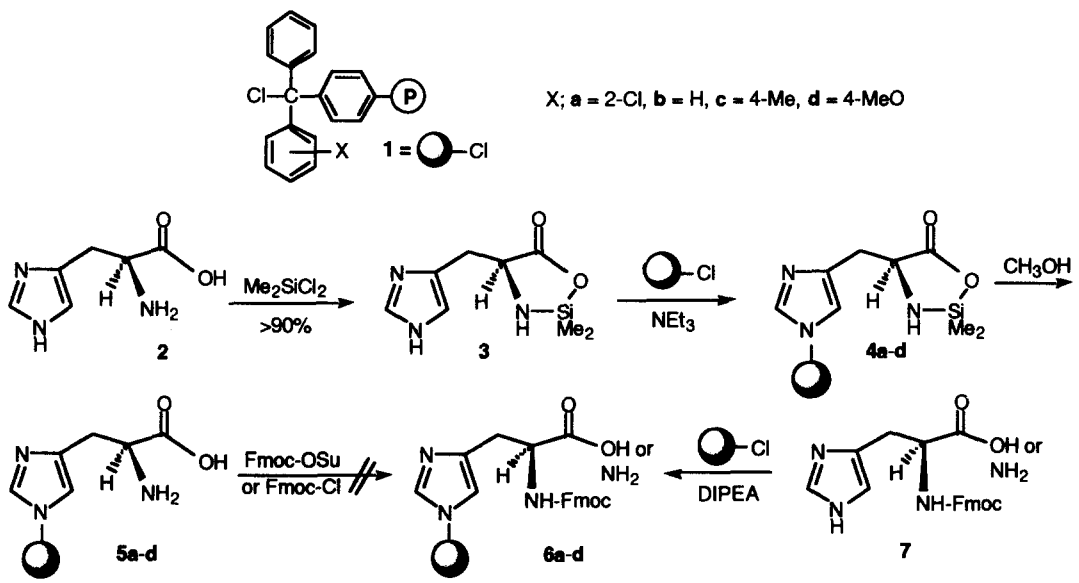
Summary: Histidine, histamine and urocanic acid were attached through the N^{im}-function on resins of the trityl type. The conditions of the cleavage of these derivatives from the resin were determined. Resin bound histamine was used in the solid phase synthesis of carcinine. © 1999 Elsevier Science Ltd. All rights reserved.

Histidine (**2**) and its degradation products histamine (**8**) and urocanic acid (**14**), are widely distributed in nature and are involved in several important biological functions [1-8]. Their attachment onto suitable solid supports will allow their application to combinatorial chemistry, aimed at the development of new drugs.

Scheme 1. Attachment of histidine and Fmoc-histidine to resins of the trityl type.



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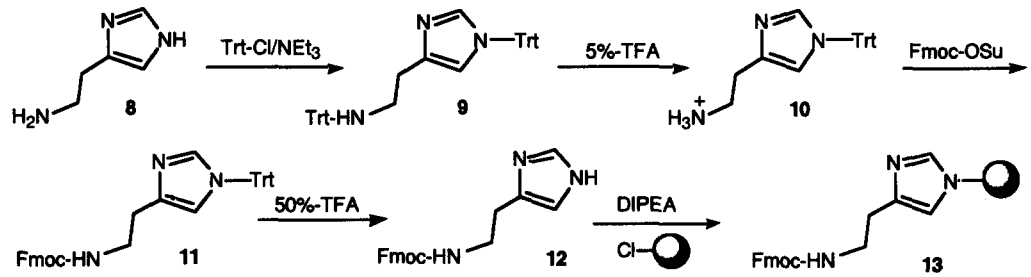
Scheme 2. Attachment of histamine to resins of the trityl-type

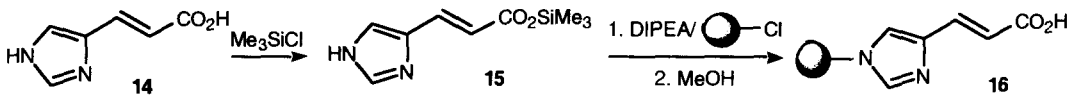


Groups of the trityl-type are well suited for the protection of the N^{im}-function of histidine [9-10]. Therefore the corresponding trityl-type [11-13] resins **1** were chosen for the attachment of these imidazolyl-derivatives. Attachment of histidine was performed analogously [9] to the method used for the side-chain tritylation of histidine (**2**). Thus, **2** applied in a twofold molar excess over resin **1**, was protected simultaneously at the N^{α}- and the carboxy-functions by its reaction with dichlorodimethylsilane in refluxing chloroform. Without isolation of the intermediate **3**, this was reacted with the trityl chloride resins **1** and triethylamine, according to Scheme 1. The dimethylsilyl group, was subsequently removed by treatment with dichloromethane (DCM)/diisopropylethylamine (DIPEA)/ methanol (85:5:15). Concurrently with the silyl-group removal, unreacted remaining trityl chloride groups were converted to the corresponding inert tritylmethyl ether. The yield of the histidine attachment, according to this procedure, is high and almost corresponds to the complete substitution of the trityl chloride groups of the resins. This was determined by the quantitative Kaiser-test. To obtain a suitably N^{α}-protected resin-bound His-derivative, we reacted **5** with excess Fmoc-OSu and DIPEA or Fmoc-Cl/DIPEA in dioxane or DMF for 24 h at 40°C. Under these conditions it was not possible to convert **5** completely to the corresponding Fmoc-derivative **6**. The latter was alternatively obtained in 50% attachment yield by the direct treatment of N^{α}-Fmoc-His with resins **1** and the 90% of the equimolar amount of DIPEA. The hydrogen chloride produced during the reaction cannot be neutralised completely by the DIPEA present in the reaction mixture. Under these slightly acidic reaction conditions, the attachment of Fmoc-His to the resin through its carboxy function is avoided. Similarly Fmoc-His-amide was attached to the resins.

To obtain a suitably protected derivative for the attachment of histamine to resins of the trityl type, we prepared ditrityl-histamine **9**, by the reaction of **8** with a twofold molar excess of Trt-chloride and triethylamine in chloroform. The *N*-blocked histamine **9** was then selectively deprotected at the primary amine function by treating it for 30 min at RT with 5%-TFA in DCM. The selective *N*-detritylation is possible, because the *N*-Trt-function is much more acid labile than the N^{im}-Trt-function. This different acid stability of imino- and amino-trityl was also observed for the corresponding ditrityl-histidine [14]. The selectively deprotected derivative **10** was then reacted with Fmoc-OSu in dioxane/10%-sodium carbonate (1:1) and the histamine derivative **11** detritylated by a 2h treatment with 50%-TFA in DCM/triethylsilane (95:5) at RT. The TFA salt of *N*-Fmoc-histamine **12**, was then reacted for 2 h at RT with resins **1** and DIPEA in DCM, to give the resin-bound histamine derivative **13**.

For attachment of urocanic acid (**14**), which is insoluble in aprotic organic solvents, onto resins of the trityl-type we converted it (Scheme 3) to the corresponding soluble trimethylsilyl ester **15**. This was achieved by reacting the acid with chlorotrimethylsilane in DCM [9]. That is similar to the procedure used

Scheme 3. Attachment of urocanic acid on resins of the trityl-type



for the preparation of polymer-bound tritylamino acids [15]. The resulting trimethylsilylester **15**, was then treated, without isolation, with resin **1** and excess DIPEA, to give resin-bound urocanic acid. The resin **16** was loaded with 0.2-0.5 mmol urocanic acid/g resin, corresponding to a substitution of the resin chloride of about 35%.

To determine the acid sensitivity of the N^{im}-resin-bond, we treated the new resins with TFA-solutions of various concentration in DCM, using triethylsilane (TES) as the scavenger of the resin-bound trityl-cations. Our results from histamine cleavage from the various trityl-type of resins under various conditions are presented in Table 1. These are typical for the cleavage of Fmoc-histidine and urocanic acid as well. We found that imidazolyl derivatives can be cleaved from the electron rich 4-methoxytrityl resin by treatment with 1%-TFA in DCM/TES (97:3) within 2 h at RT. Using 5%-TFA, cleavage is quantitative within 30 min at RT. In contrast, the cleavage from the much more acid stable 2-chlorotrityl resin is not complete even after 2 h treatment at RT using 65% TFA. It is interesting to note that the cleavage of the imidazolyl compounds from the 2-chlorotrityl resin using 5%-TFA was shown to be faster than the cleavage using 65%-TFA. This indicates that equilibrium is established and that reattachment of the imidazolyl derivatives onto the resin takes place (Scheme 4). Simultaneously the triethylsilane scavenger is attacked by concentrated TFA faster then diluted TFA and both, scavenger and cleavage agent are neutralized and removed from the equilibrium. The reattachment of the imidazolyl compounds to the resins is much more pronounced if the cleavage is performed in the absence of scavengers. Our results of the cleavage of urocanic acid from the trityl resin by treatment with a TFA solution in the absence of TES are presented in Table 2. We observed that 5 and 15% TFA lead to higher cleavage yield than 65% TFA.

The new resin bound derivatives were applied successfully to solid phase peptide synthesis. As an example, the N-Fmoc-derivative of the natural antioxidant [16] carcinine (β-alanylhistamine) was synthesised in 92% yield and 97% purity (Fig. 1). The Fmoc-group of **13d** was removed by treatment with 25% piperidine in DMF, followed by coupling of the *N*-deprotected resin bound histamine with Fmoc-β-alanine. The activation was performed by diisopropylcarbodiimide and 1-hydroxybenzotriazole. The cleavage of the Fmoc-carcinine from the resin was then mediated by treatment with 5% TFA in DCM/TES (97:3) for 15 min at RT.

Scheme 4. Reactions taking place during the TFA mediated cleavage of imidazolyl-compounds from resins of the trityl-type

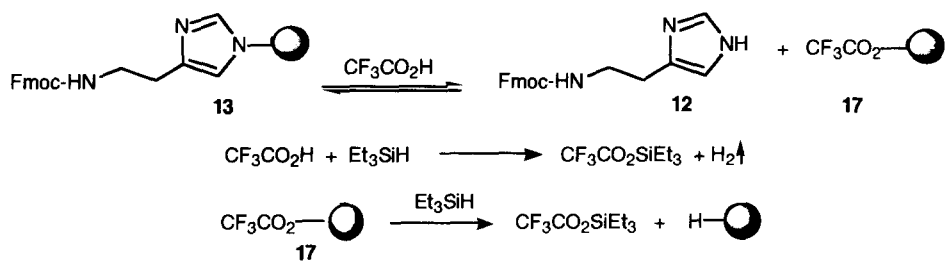


Table 1: % Cleavage of Fmoc-histamine from Trt-type resins with TFA in DCM/TES (97:3)

[min]	TFA [%]	Compound Nr. Cleavage from the resin [%]			
		13d	**13c**	**13b**	**13a**
5	1%	32	8	5	
30	1%	68	15	12	
60	1%	96	19	13	
120	1%	100	30	15	3
5	5%	77	13	11	
30	5%	100	36	37	46
60	5%		65	45	56
120	5%		91	80	70
5	65%	100	37	36	38
30	65%		98	62	49
60	65%			79	53
120	65%			98	60

Table 2: Cleavage of urocanic acid from the trityl resin with TFA in DCM

TFA [%]	**16b** Cleavage from the resin [%] time [min]			
	5	15	30	60
1%	11	14	18	17
5%	26	69	72	74
15%	67	73	67	69
65%	46	49	47	44

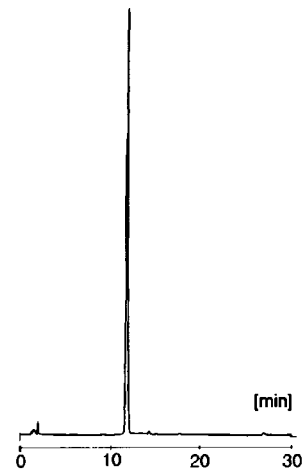


Figure 1. Analytical HPLC of crude Fmoc-carcinine.- Column: Nucleosil C8, 7μm, 125 X 4 mm; flow rate: 1 ml/min; 20 to 100% acetonitrile in water in 30 min; detection at 265 nm.

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