Journal of Peptide Science
J. Peptide Sci. **5**: 368–373 (1999)

im-Trityl Protection of Histidine

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Accepted 26 April 1999

Abstract: A rational attempt to prepare FmocHis(πTrt)OH regiospecifically gave in fact the well-known τ-trityl isomer, and experiments with model systems indicate that the prospects for access to π-trityl histidine derivatives, which would be of great value for the racemization-free synthesis of histidine-containing peptides, are poor. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: histidine; *im*-trityl protection; racemization

INTRODUCTION

The direct tritylation of histidine side-chains gives derivatives which are both very convenient for peptide assembly, and easily deprotected at the end of the synthesis [1], but which are not safe regarding racemization [2,3], because the trityl group is τ-located. If only reasonably straightforward methods of introducing π-trityl protection could be devised, this would doubtless provide a complete solution to the problems of peptide synthesis with histidine.

The recent report [4] from Novosibirsk that FmocHis(πTrt)OH (**1**) could be prepared as outlined in the Experimental section was therefore full of promise. The approach was entirely rational in concept. There is a well-established precedent [5] for the use, albeit under milder conditions, of τ-Boc temporary protection to achieve π-alkylation with concomitant loss of the Boc group. Unfortunately, the Nobosibirsk product has nevertheless been shown, by careful 500 MHz NMR comparison with commercial samples of FmocHis(τTrt)OH (**2**), to be indistinguishable from them, minor contaminants excepted. Neither the commercial samples nor the Novosibirsk product contained detectable amounts of π-isomer. This is not because of a false premise about the structure of the starting material BocHis(τBoc)OMe (**3**): the τ-location of the *im*-Boc group is quite certain, because **3** can be unambiguously related to BocHis(πBom)OH, for which a crystal structure has been determined [5,6]. It is not clear whether the unexpected τ-tritylation was the result of **3** decomposing to BocHisOMe (**4**) under the reaction conditions, or of its equilibration with the π-Boc-isomer **5** before reaction with the trityl chloride. The former is the simpler and perhaps more plausible hypothesis, but the latter cannot be ruled out under the vigorous conditions employed. In any case, the failure to introduce the trityl group at the required π-position is consistent with experience in Oxford [7] with model systems.

Manipulation of the reaction conditions was fruitless. Practically no tritylation took place on treatment of **3** with trityl chloride for several hours in refluxing toluene containing diisopropylethylamine (to take up HCl traces and prevent loss of Boc-protection). On the other hand, a few minutes sufficed for almost quantitative *im*-tritylation in refluxing pyridine. But it was exclusively τ-oriented.

Boc_2O reacts with 4-methylimidazole at room temperature (r.t.) to give an inseparable clean mixture of the *im*-Boc derivatives **6** and **7** in 4:1 proportions. Treatment of this mixture with one equivalent of trityl chloride gave exclusively 1-trityl-4-methylimidazole (**8**), in quantitative yield from the minor isomer **7** in the original mixture, leaving the major isomer **6** completely untouched (Schemes 1

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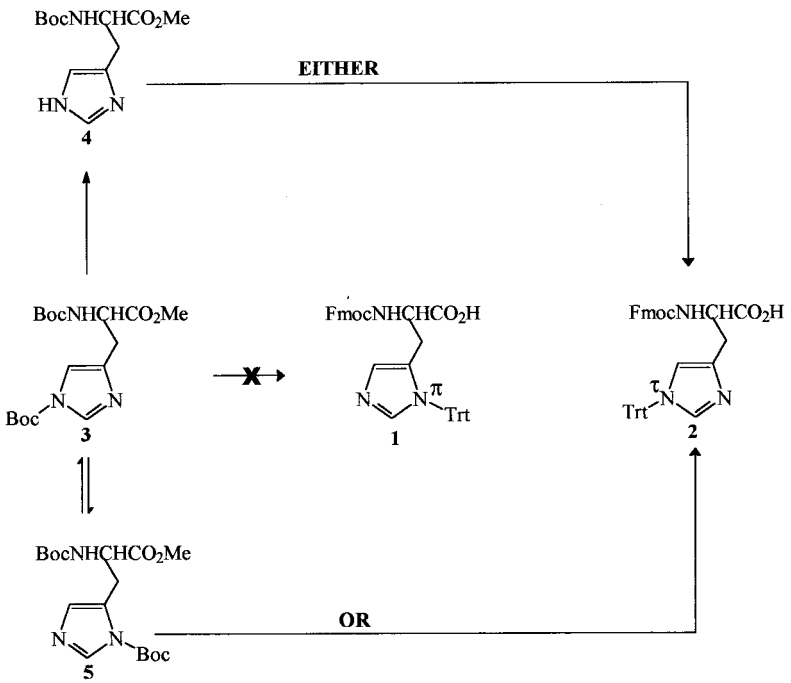
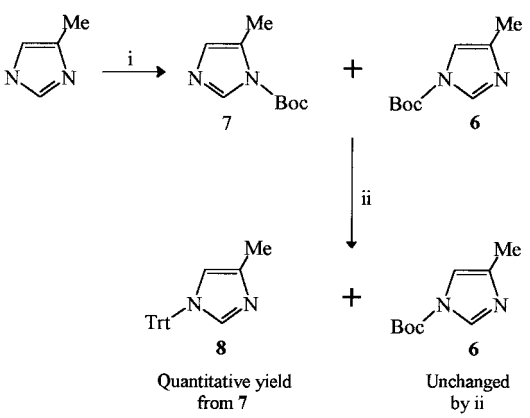


Scheme 1 Attempted synthesis of Fmoc(πTrt)OH. Conditions: (i) TrtCl/$ClCH_2CH_2Cl$/15 h/60°C; (ii) NaOH/aq. dioxane; (iii) HCl/AcOH; and (iv) FmocCl/Na_2CO_3/aq. MeCN.

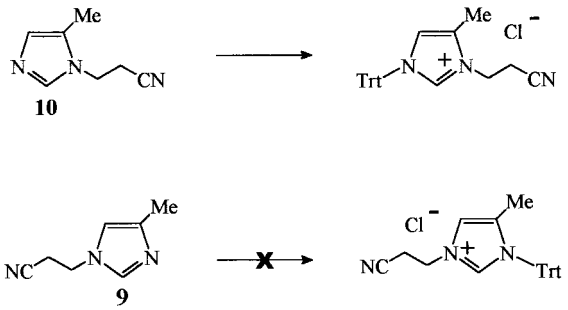
and 2). That the extreme selectivity demonstrated by this experiment was not due to the fact that the *im*-Boc group must be, wherever it is situated on the ring, electronically deactivating, is shown by experiments with 1-(2-cyanoethyl)-, 4- and 5-methylimidazoles (**9** and **10**). These can be separated after the reaction of 4-methylimidazole with acrylonitrile, by crystallization of their salts with oxalic acid. The reactivity of the 1,4-isomer **9** towards trityl chloride was explored as a model for a second possible regiospecific approach to π-trityl histidine derivatives, in which it was envisaged that after π-tritylation base would be used to remove the cyanoethyl temporary protection [8]. Unfortunately, although the 1,5-isomer **10** reacts easily with trityl chloride, the 1,4-isomer **9** does not react at all under the same conditions (Scheme 3).

Attempts to use *im*-acetyl or *im*-*t*-butyldimethylsilyl as temporary protection were also discouraging. In both cases the initial derivatization to introduce the temporary protecting group is τ-regiospecific, in the acetyl case probably because it is reversible [9]; but reaction with trityl chloride gave exclusively 1-trityl-5-methylimidazole (**8**) in both cases. Again, it is not clear whether this is because of τ–π equilibration before tritylation, or loss of the τ-temporary protection by mere decomposition before tritylation.

Inspection of physical and computer models of 1-trityl-5-alkyl-imidazoles does not seem to prohibit their existence, and the literature contains at least one well-characterized compound which would seem to be analogous. This is 1-trityl-2-methylimidazole, which has been prepared by methylation of 1-trityl-2-lithioimidazole [10] and also by heating silver 2-methylimidazolide with trityl chloride [11]. However, these conditions are not appropriate for the more delicate case of histidine side-chains, and



Scheme 2 The tritylation of 4-methylimidazole *im*-Boc derivatives. Conditions: (i) Boc_2O/CH_2Cl_2; and (ii) TrtCl/CH_2Cl_2/72 h/r.t.



Scheme 3 The tritylation of 4-methylimidazole *im*-(2-cyanoethyl)-derivatives. Conditions: TrtCl/$CHCl_3$/72 h/r.t.

our experience thus far indicates that the prospects for π-tritylation of histidine derivatives are poor.

EXPERIMENTAL

Microanalyses, NMR, and MS measurements were performed by Dyson Perrins Laboratory services.

Novosibirsk FmocHis(*im*-Trt)OH

BocHis(τBoc)OMe (**3**) [5] (1.85 g, 5 mmol) was dissolved in dry 1,2-dichloroethane (20 ml). TrtCl (1.4 g, 5 mmol) was added and the solution was maintained at 60°C for 15 h in a closed flask. The residual oil after evaporation was dissolved in dioxane (30 ml), and 2 M NaOH (10 ml) was added. The mixture was stirred for 1 h, diluted with water to a volume of 150 ml, acidified to pH 4–5 with 5% $KHSO_4$ and extracted with ethyl acetate. The extract was washed (water, brine), dried (Na_2SO_4), and the solvent was removed, 1 M HCl in 90% AcOH (15 ml) was added, followed after 20 min by 2 M NaOH (10 ml) and water (100 ml). Adjustment of the pH to 7 afforded His(*im*-Trt) 1.17 g (59%) after washing with ether. His(*im*-Trt) prepared in this way (4 g, 10 mmol) was dissolved with gentle warming in a 50% (v/v) mixture of acetonitrile and water (40 ml) containing 1.2 g (11 mmol) Na_2CO_3, and the solution was cooled to r.t. A solution of FmocCl (2.7 g, 10.5 mmol) in acetonitrile (20 ml) was added dropwise over 1–2 min. After a further 10 min, the reaction mixture was diluted with water to a volume of 150 ml and extracted twice with ether. The aqueous layer was acidified to pH 4 with 5% $KHSO_4$ and extracted with ethyl acetate. The extract was washed (water, brine), dried (Na_2SO_4), and the solvent was evaporated. Silica flash chromatography ($CHCl_3$–MeOH–AcOH) and trituration with hexane–ether gave FmocHis(*im*-Trt)OH (4.7 g, 76%). Rigorous 500 MHz NMR comparison of this material with FmocHis(τTrt)OH from Novabiochem (which was itself practically indistinguishable from the same compound as supplied by Bachem), showed them to be identical, trace impurities apart (Figures 1 and 2).

Reaction of 4-Methylimidazole with Boc_2O

4-Methylimidazole (0.781 g, 9.5 mmol) was added to a solution of Boc_2O (2.075 g, 9.5 mmol) in dry dichloromethane (20 ml). The solution was stirred at r.t. for 16 h, during which CO_2 was evolved. The solvent was evaporated and the product was investigated by NMR. Although apparently homogenous by TLC in a variety of systems, it was a clean mixture of 1-*t*-butoxycarbonyl-4-methylimidazole (**6**) [NMR ($CDCl_3$): δ 2.04 (3H, s, *im*-CH_3) 6.91 (1H, s, *im*-H5); 7.82 (1H, s, *im*-H2)] and 1-*t*-butoxycarbonyl-5-methylimidazole (**7**) [NMR ($CDCl_3$): δ 2.22 (3H, s, *im*-CH_3); 6.56 (1H, s, *im*-H5); 7.84 (1H, s, *im*-H2)] in 4:1 proportions.

Treatment of the Mixture of 6 and 7 with Trityl Chloride

The mixture obtained from the previous reaction (0.56 g, 3.07 mmol) was treated with a solution of trityl chloride (0.855 g, 3.07 mmol) in dichloromethane (10 ml). The solution was stirred at r.t. for 72 h. The solvent was evaporated, the sticky residue was taken up in the minimum volume of dichloromethane, and ether was added until a precipitate formed. The precipitate was separated and taken up in dichloromethane (10 ml). The solution was washed with aqueous $NaHCO_3$ (10 ml), dried and evaporated to give 1-trityl-4-methylimidazole (**8**) which was identical to authentic material prepared and characterized as described below (148 mg, quantitative yield based on the amount of **7** in the mixture).

1-Trityl-4-Methylimidazole (8)

The procedure is based on that of Buechel [12]. 4-Methylimidazole (8.17 g, 0.1 mol) and trityl chloride (13.9 g, 0.05 mol) were dissolved in acetonitrile (200 ml). The solution was heated under reflux for 3 h. The solvent was evaporated. The solid residue was recrystallized from toluene to give 1-trityl-4-methylimidazole as white crystals (9.3 g, 57%) of m.p. 222–224°C. NMR ($CDCl_3$): δ 2.21 (3H, s, CH_3); 6.53 (1H, s, *im*-H4); 7.10–7.39 (16H, m, Ph and *im*-H2); *m*/*z* (electrospray, CV = 10V): 325 (100%,

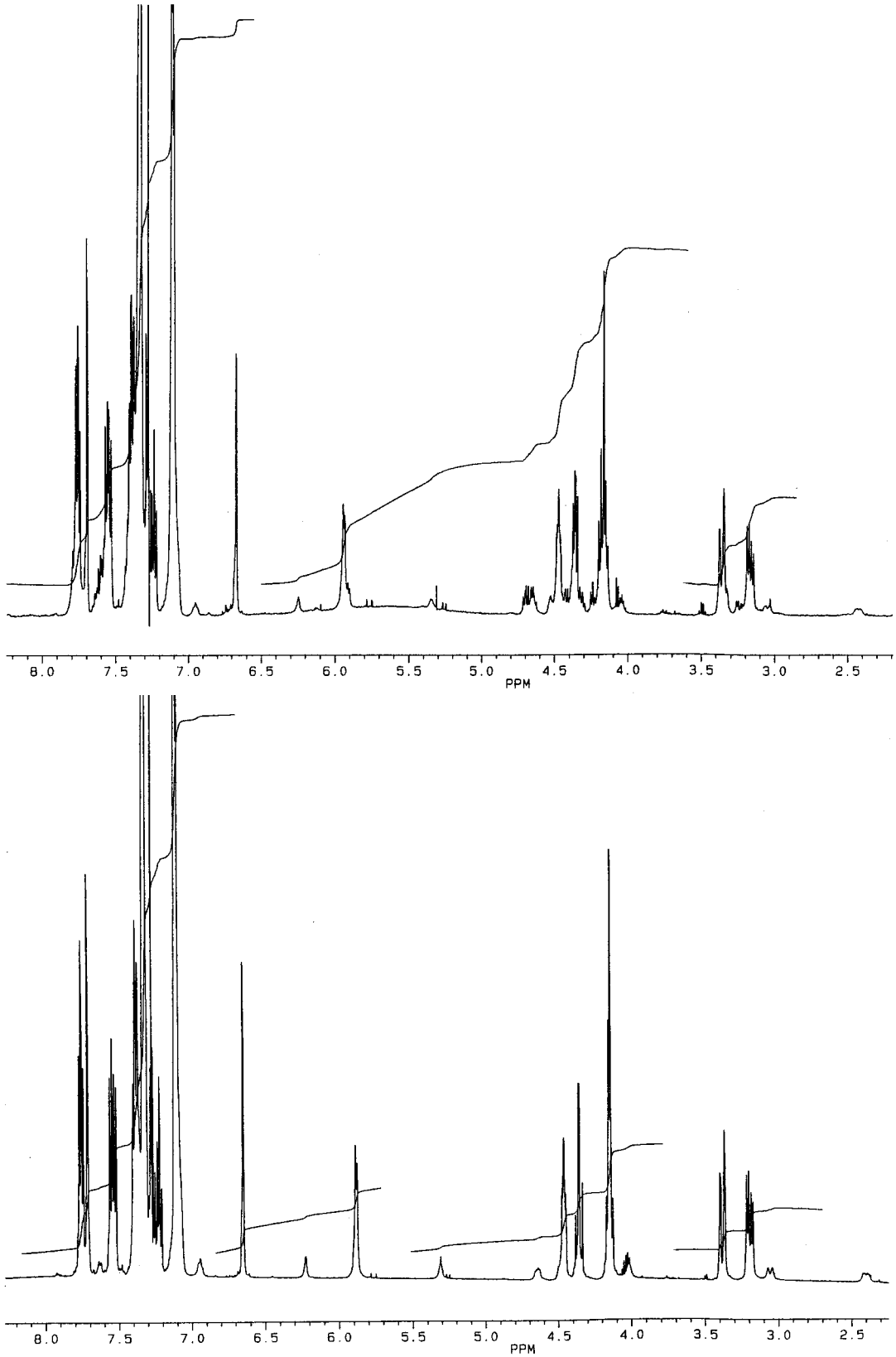


Figure 1 Comparison of the 500 MHz NMR spectra ($CDCl_3$) of Novosibirsk FmocHis(*im*-Trt)OH (above) and Novabiochem FmocHis(τTrt)OH (below).

MH^+); 243 (59%, Ph_3C^+). Calculated for $C_{23}H_{20}N_2$: M = 324. Found: C, 85.44; H, 6.33; N, 8.66%. Calculated for $C_{23}H_{20}N_2$: C, 85.19; H, 6.17; N, 8.64%. There can hardly be any doubt about the location of the trityl group in this compound, but it was in any case proved [7] to be as indicated by allylation with allyl bromide, detritylation with hot aqueous acetic acid and nOe measurements on the resulting 1-allyl-5-methylimidazole.

1-(2-Cyanoethyl)-4-Methylimidazole (9) and 1-(2-Cyanoethyl)-5-Methylimidazole (10)

The procedure is based on that of Horváth [8]. Acrylonitrile (16 g, 0.3 mol) was added to a stirred solution of 4-methylimidazole (24.5 g, 0.3 mol) in chloroform (300 ml). The solution was stirred at r.t. for 72 h. The chloroform was evaporated and the residue was taken up in methanol (50 ml). Oxalic acid (13.5 g, 0.15 mmol) was added and the methanol was evaporated. Investigation of the oily residue by NMR revealed the presence of three components: 4-methylimidazolium, 1-(2-cyanoethyl)4-methyl-imidazolium and 1-(2-cyanoethyl)-5-methyl-imidazolium oxalates, the cyanoethyl derivatives being present in 4:1 proportions. Fractional crystallization from methanol/ether was used to separate the two cyanoethyl derivatives (4-methylimidazolium oxalate remained in solution). The less soluble derivative was 1-(2-cyanoethyl)-4-methyl-imidazolium hemioxalate, which was obtained as white crystals of m.p. 146–148°C. NMR (CD_3OD): δ 2.35 (3H, s, *im*-CH_3), 3.12 (2H, t, $J = 6.3$ Hz, CH_2CN), 4.49 (2H, t, $J = 6.3$ Hz, CH_2N); 7.40 (1H, s, *im*-H5); 8.85 (1H, s, *im*-H2). Irradiation at δ 2.35 resulted in a 5.7% nOe at δ 7.40. Irradiation at δ 7.40 resulted in a 4.7% nOe at δ 4.49, a 2.6% nOe at δ 3.12 and a 5% nOe at δ 2.35. Irradiation at δ 8.85 resulted in a 3% nOe at δ 4.49 and a 0.9% nOe at δ 3.12; m/z (CI, NH_3): 136 (100%, M^+). Calculated for $C_7H_{10}N_3^+$: $M^+ = 136$. Found: C, 48.08; H, 4.64; N, 18.63%. Calculated for $C_9H_{11}N_3O_4$: C, 48.00; H, 4.89; N, 18.67%. The more soluble derivative was 1-(2-cyanoethyl)-5-methylimidazolium

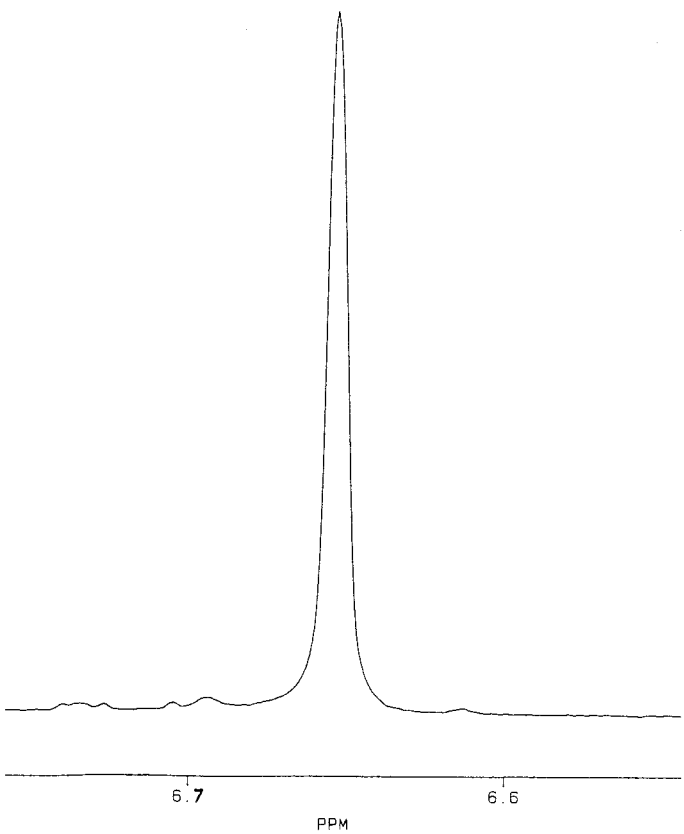


Figure 2 The signal at δ 6.65 (*im*-H4) from a 50:50 v/v mixture of the solutions whose spectra are shown in Figure 1, showing the absence of doubling. Similar scrutiny of other signals in the spectrum of the mixed solutions gave the same result.

oxalate, which was obtained as white crystals of m.p. 150–152°C. NMR (CD_3OD): δ 2.35 (3H, s, *im*-CH_3); 3.03 (2H, t, $J = 6.4$ Hz, CH_2CN); 4.42 (2H, t, $J = 6.4$ Hz, CH_2N); 7.08 (1H, s, *im*-H4); 8.41 (1H, s, *im*-H2). Irradiation at δ 2.35 resulted in a 3.6% nOe at δ 7.08, a 2.4% nOe at δ 4.42 and a 1% nOe at δ 3.03). Irradiation at δ 7.08 resulted in a 2.3% nOe at δ 2.35. Irradiation at δ 8.41 resulted in a 4.4% nOe at δ 4.42 and a 2% nOe at δ 3.03); *m*/*z* (CI, NH_3): 136 (100%, M^+). Calculated for $C_7H_{10}N_3^+$: $M^+ = 136$. Found: C, 53.37; H, 5.39; N, 23.31%. Calculated for $C_{16}H_{2O}N_6O_4$: C, 53.33; H, 5.56; N, 23.33%. Each salt was converted to the corresponding free-base form by distribution of the oxalate between aqueous $NaHCO_3$ (5 ml) and dichloromethane (5 ml). The organic phase was separated, dried and evaporated. The conversion proceeded in practically quantitative yield. 1-(2-Cyanoethyl)-4-methylimidazole (**9**) was obtained as a viscous yellow oil. NMR ($CDCl_3$): δ 2.18 (3H, s, CH_3); 2.75 (2H, t, $J = 6.5$ Hz, CH_2CN), 4.15 (2H, t, $J = 6.5$ Hz, CH_2N); 6.69 (1H, s, *im*-H5); 7.40 (1H, s, *im*-H2). The cross-ring coupling constant was $J = 1.1$ Hz, providing further corroboration [13] that the *im*-substituent was located on *N*-1. 1-2-Cyanoethyl-5-methylimidazole (**10**) was also obtained as a viscous yellow oil. NMR ($CDCl_3$): δ 2.23 (3H, s, CH_3); 2.74 (2H, t, $J = 6.5$ Hz, CH_2CN); 4.15 (2H, t, $J = 6.5$ Hz, CH_2N); 6.79 (1H, s, *im*-H4); 7.49 (1H, s, *im*-H2).

Treatment of 9 with Trityl Chloride

Trityl chloride (60 mg, 0.21 mmol) was added to a solution of **9** (30 mg, 0.21 mmol) in chloroform (5 ml). The solution was stirred at r.t. for 72 h. The solution was then filtered and evaporated. Investigation by NMR revealed that **9** had survived completely unchanged.

Treatment of 10 with Trityl Chloride

Trityl chloride (60 mg, 0.21 mmol) was added to a solution of **10** (30 mg, 0.21 mmol) in chloroform (5 ml). The solution was stirred at r.t. for 72 h. The solvent was evaporated, and the residue was taken up in $CDCl_3$ (1 ml). A white solid crystallized from the $CDCl_3$; this was separated and recrystallized from chloroform/ether to give 1-(2-cyanoethyl)-3-trityl-5-methylimidazolium chloride (40 mg, 44%) as a white powder of m.p. 128–131°C. NMR ($CDCl_3$): δ 2.48 (3H, s, CH_3); 3.37 (2H, t, $J = 6.4$ Hz, CH_2CN); 4.98 (2H, t, $J = 6.4$ Hz, CH_2N); 6.77 (1H, s, *im*-H4); 7.1–7.5 (15H, m, Ph_3C); 10.21 (1H, s, *im*-H2); *m*/*z* (electrospray): 378 (100%, M^+). Calculated for $C_{26}H_{24}N_3^+$: $M = 378$.

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