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Facile formation of amide bonds between fragments containing unmasked imidazole-ring systems. Synthesis of *N*-[*N*-[[[(5-methyl-4-imidazolyl)methyl]thio]acetyl]-L-methionyl]histamine

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Abstract. The synthesis is reported of the optically pure title compound [α^{20} −2.31 $l \cdot mol^{-1} \cdot dm^{-1}$] from a sequence of reactions involving 5-methyl-4-imidazolemethanol, mercaptoacetic acid and (L-methionyl)histamine. The development of a suitable synthetic route, using *N*-benzyl-2-[[(5-methyl-4-imidazolyl)methyl]thio]acetamide as a model compound, and the problems associated with the presence of imidazole ring systems in the reagents are discussed. The main problem of the synthesis, *i.e.*, the formation of amide bonds between reagents that contain unmasked imidazole functions, is overcome by using an *N*,*N*′-dicyclohexylcarbodiimide-activated and *N*-hydroxybenzotriazole-moderated coupling procedure.
The present method seems generally applicable and yields gram quantities in an overall yield of more than 30%, without the need for chromatography techniques.

Introduction

One research project of our group comprises a study of the stereochemical aspects of d^{10} cations, especially Cu^I and Ag^I, in environments providing multiple nitrogen and sulphur hetero-atoms, with the ultimate aim of creating $M-N_2S_2$ chromophores.
Evolving from ligand systems, whose functional groups only consist of (thienylmethylene)amino moieties[1], the polydentate ligand *N*-[*N*-[5-methyl-2-thienyl)methylene]-L-methionyl]histamine (**1**, Figure 1) was developed[2]. Ligand **1** contains the potentially bidentate N,S-coordinating (thienylmethylene)amino unit, and two monodentate ligating functions, a thio-ether S and an imidazole N atom. As shown by X-ray structure determination, reaction of equivalent amounts of **1** and $Ag^IO_3SCF_3$, resulted in the stereoregular formation of a fascinating coordination polymer, exclusively having a Δ helix[3]. The polymeric structure is due to the fact that **1** is preorganized to be "stretched out" over three different metal cations, thus inducing a self-assembly process whose degree of self-organization is unprecedented in synthetic chemistry[4]. Each Ag^I centre in the polycationic coordination complex is predominantly coordinated by a thio-ether S, an imidazole N and an imine N atom; as expected, the thiophene S atom is not coordinated, although residing in the proximity of the metal cation due to the conformation of the ligand backbone.
In order to achieve genuine N_2S_2 coordination, we have recently designed the promising cation binder *N*-[*N*-[[[(5-methyl-4-imidazolyl)methyl]thio]acetyl]-L-methionyl]-histamine (**2**, Figure 1). Ligand **2** also contains the *N*-(L-methionyl)histamine subunit, but the (thienylmethylene)amino fragment of **1** is replaced by a [(5-methyl-4-imidazolyl)methyl]thio function. Various workers have shown the latter functional group to be a good bidentate N,S-coordinating entity[5].
Selecting a suitable method to create the desired amide bond between the two subunits, we used the synthesis of *N*-benzyl-2-[[(5-methyl-4-imidazolyl)methyl]thio]acetamide, (**3**, Figure 1) as a model reaction.

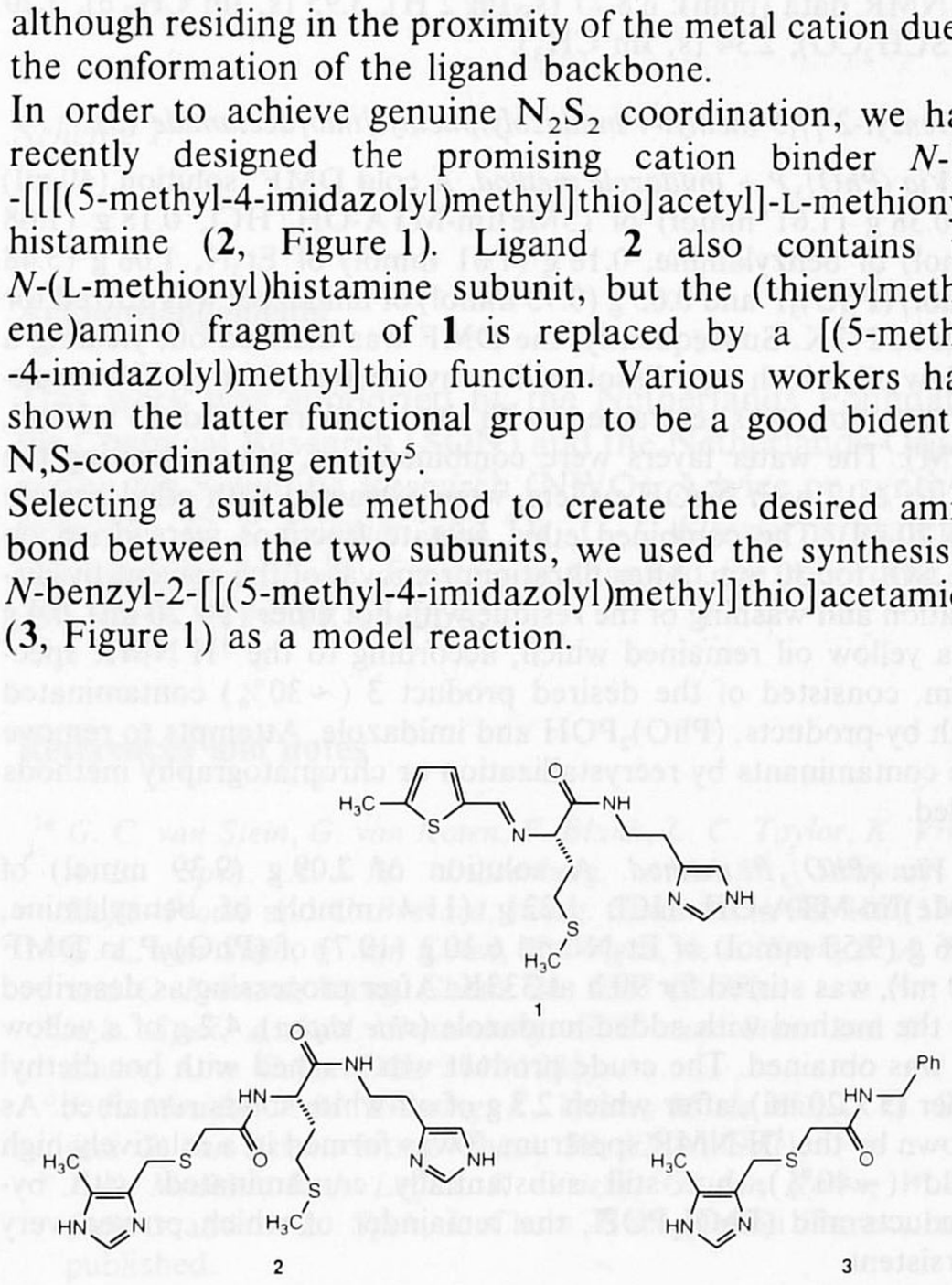

Figure 1

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In this paper, we will present the synthetic route developed to obtain **2** and **3** and discuss some aspects associated with reactions of unmasked imidazole derivatives.
Metal complexes of the type M(2) and $M(3)_2$ ($M = Ag^I$ or Cu^I) have been prepared and indicate the presence of the intended coordination, as discussed in detail elsewhere[6].

Experimental

Physical measurements

Melting points were measured on a Mettler apparatus model FP1. ^{1}H NMR spectra were recorded on Bruker AC100 and WM250 spectrometers in CD_3OD at room temperature, with tetramethylsilane being used as external reference. Optical rotation was measured on a Perkin Elmer 241 polarimeter in methanol of *p.a.* grade at 393K, $\lambda = 589$ nm, $l = 1$ dm.

Preparation of the compounds

Diethyl ether and hexane were freshly distilled and stored under nitrogen. Other solvents used in the preparation were of *p.a.* grade and used as such, unless denoted otherwise.
All organic chemicals used were purchased from Janssen Chimica and were used without further purification. *N*-(L-methionyl)-histamine dihydrochloride, abbreviated as H-Met-Histam · 2HCl, was prepared according to the method described by *Modder* et al.[2]. Elemental analyses were carried out by the Analytical Section of the Institute for Applied Chemistry, TNO, Zeist, The Netherlands.

2-[[(5-Methyl-4-imidazolyl)methyl]thio]acetic acid hydrochloride [(5Me)Im-MTA-OH · HCl]

A mixture of 11.26 g (75.8 mmol) of 5-methyl-4-imidazolemethanol hydrochloride and 50 ml (720 mmol) of mercaptoacetic acid was stirred for 12 h at 400K. After cooling to room temperature, diethyl ether (150 ml) was added to the viscous solution. Upon mixing thoroughly, a white solid separated. This crude product was filtered and subsequently purified by trituration with hot diethyl ether (3 × 100 ml). Drying *in vacuo* resulted in 13.32 g of (5Me)Im-MTA-OH · HCl (79%), a white and very hydroscopic solid. ^{1}H NMR data (ppm): δ 8.77 (s, Im 2 H), 3.95 (s, Im CH_2 δ), 3.20 (s, SCH_2CO), 2.34 (s, Im CH_3).

N-*Benzyl-2-[[(5-methyl-4-imidazolyl)methyl]thio]acetamide* (**3**)

(a) Via $(PhO)_3P$ + imidazole method. A cold DMF[7] solution (40 ml) of 0.38 g (1.61 mmol) of (5Me)Im-MTA-OH · HCl, 0.18 g (1.68 mmol) of benzylamine, 0.16 g (1.61 mmol) of Et_3N, 1.08 g (3.48 mmol) $(PhO)_3P$ and 0.05 g (0.73 mmol) of imidazole was stirred for 18 h at 273K. Subsequently, the DMF was distilled off, yielding a yellow oil, which was dissolved in ethyl acetate (30 ml). The resulting solution was extracted with hydrochloric acid (3 × 10 ml, 0.1M). The water layers were combined and, after adjusting the pH to ≥ 12 with NaOH pellets, were extracted with ethyl acetate (3 × 20 ml). The combined ethyl acetate fractions were dried on Na_2SO_4 for 30 min. After filtration, removal of the solvent by distillation and washing of the residue with hot ether (3 × 20 ml), 0.4 g of a yellow oil remained which, according to the ^{1}H NMR spectrum, consisted of the desired product **3** (~30%) contaminated with by-products, $(PhO)_2POH$ and imidazole. Attempts to remove the contaminants by recrystallization or chromatography methods failed.

(b) Via $(PhO)_3P$ method. A solution of 2.09 g (9.39 mmol) of (5Me)Im-MTA-OH · HCl, 1.22 g (11.4 mmol) of benzylamine, 0.96 g (9.50 mmol) of Et_3N and 6.10 g (19.7) of $(PhO)_3P$ in DMF (50 ml), was stirred for 90 h at 333K. After processing as described for the method with added imidazole (*vide supra*), 4.2 g of a yellow oil was obtained. The crude product was washed with hot diethyl ether (3 × 20 ml), after which 2.3 g of off-white solids remained. As shown by the ^{1}H NMR spectrum, **3** was formed in a relatively high yield (~40%), but still substantially contaminated with by-products and $(PhO)_2POH$, the remainder of which proved very persistent.

(c) Via DCC[7] + HOSu[7] method. Following the procedure[2] described for the coupling of *N*-(*tert*-butyloxycarbonyl)-L-methionine and histamine dihydrochloride, 1.87 g (8.40 mmol) of (5Me)Im-MTA-OH · HCl, 0.85 g (8.41 mmol) of Et_3N and 1.13 g (9.81 mmol) of HOSu in ethyl acetate (50 ml) were reacted with 2.20 g (10.7 mmol) of DCC. After the usual period, the mixture was filtered and then water (30 ml), 0.96 g (8.97 mmol) of benzylamine and 1.65 g (15.6 mmol) of Na_2CO_3 were added. Eventually, this procedure yielded 0.5 g of ivory solids. The ^{1}H NMR spectrum showed some product **3** had been formed (~10%) along with by-products. Recrystallization attempts, using various solvent mixtures, did not yield pure **3**.

(d) Via DCC[7] method. DCC (0.50 g, 2.42 mmol) was added to a cooled DMF solution (30 ml) of 0.47 g (2.11 mmol) of (5Me)Im-MTA-OH · HCl, 0.27 g (2.52 mmol) of benzylamine and 0.22 g (2.18 mmol) of Et_3N. The resulting mixture was stirred for 2 h at 273K, for 18 h at 298K and, finally, for 4 h at 313K. After cooling to ambient temperature, the solvent was distilled off and the residue was dissolved in ethyl acetate (30 ml). Subsequent processing as described for the $(PhO)_3P$ + imidazole method (*vide supra*), yielded 0.8 g of a yellow oil. Apart from high intensity cyclohexyl patterns, which are attributable to DCU[7], only traces of **3** were visible in the ^{1}H NMR spectrum.

(e) Via DCC[7] + HOBt[7] method. In a typical experiment, 5.65 g (25.4 mol) of (5Me)Im-MTA-OH · HCl, 2.59 g (24.2 mmol) of benzylamine, 2.57 g (25.4 mmol) of NEt_3, 3.26 g (24.4 mmol) of HOBt, 4.99 g (24.2 mmol) of DCC and 80 ml of ethyl acetate were heated at reflux for 18 h. After cooling to room temperature, hydrochloric acid (50 ml, 0.1M) was added. The resulting suspension was filtered, after which the residue was washed twice with hydrochloric acid (50 ml, 0.1M). The ethyl acetate and water layers of the filtrate were separated. After extraction with dichloromethane (3 × 50 ml), the water layer was cooled to 273K, brought to pH ≥ 12 by addition of NaOH pellets and extracted with ethyl acetate (4 × 50 ml). The combined ethyl acetate fractions were stirred with Na_2SO_4 for 30 min. Subsequent filtration, evaporation of the solvent and drying *in vacuo* gave 4.5 g crude product. Crystallization from a mixture of methanol (5 ml) and dichloromethane (15 ml), cooled to 278K for 15 h, allowed isolation of 4.33 g (65%) of pure **3**, as off-white solids; m.p. 123–124°C. Anal. $C_{14}H_{17}N_3OS$ calcd.: C 61.06, H 6.23, N 15.26, O 5.81, S 11.64; found: C 61.04, H 6.42, N 14.90, O 6.17, S 11.11%. ^{1}H NMR (ppm): δ 7.45 (s, Im 2 H), 7.29 (s, benzyl C_6H_5), 4.36 (s, benzyl CH_2), 3.73 (s, Im CH_2 δ), 3.15 (s, SCH_2CO), 2.12 (s, Im CH_3).

N-*[N-[[[(5-methyl-4-imidazolyl)methyl]thio]acetyl]-L-methionyl]histamine* (**2**)

Via DCC[7] + HOBt[7] method. In a typical experiment, 5.62 g (25.2 mmol) of (5Me)Im-MTA-OH · HCl, 5.67 g (18.0 mmol) of H-Met-Histam · 2HCl, 11.03 g (109.0 mmol) of Et_3N, 2.48 g (18.4 mmol) of HOBt, 3.71 g (18.0 mmol) of DCC were stirred in DMF (100 ml) at 223K for 72 h. After evaporation of the solvent, the remaining brown, sticky solid was stirred with hydrochloric acid (100 ml, 0.1M) for 24 h. Subsequently, the resulting suspension was filtered and the residue was washed twice with hydrochloric acid (50 ml, 0.1M). After extraction with dichloromethane (3 × 50 ml), the water layer was cooled to 273K, brought to pH ≥ 12 by addition of NaOH pellets and extracted with ethyl acetate (4 × 50 ml). The combined ethyl acetate fractions were stirred with Na_2SO_4 and active carbon for 30 min. Subsequent filtration, evaporation of the solvent and drying *in vacuo* gave 4.3 g crude product. Crystallization from a mixture of methanol (10 ml), diethyl ether (30 ml) and hexane (15 ml), cooled to 278K for 72 h, allowed isolation of 2.5 g of pure **2** (34%), as a light yellow solid: m.p. 145–150°C (decomp.). Optical rotation, α^{20} −2.31 $l \cdot mol^{-1} \cdot dm^{-1}$. Anal. $C_{17}H_{26}N_6O_2S_2$ calcd.: C 49.73, H 6.39, N 20.47, O 7.79, S 15.62; found: C 49.05, H 6.28, N 20.16, O 9.10, S 15.18%. ^{1}H NMR (ppm): δ 7.57 (s, Histam 2 H), 7.47 (s, Im 2 H), 6.84 (s, Histam 5 H), 4.42 (dd, NCHCO), 3.75 (s, Im CH_2 δ), 3.44 (t, Histam α-CH_2), 3.15 (s, SCH_2CO), 2.78 (t, Histam β-CH_2), 2.46 (m, Met 3-CH_2), 2.17 (s, Im CH_3), 2.05 (s, Met SCH_3), 1.85 (m, Met 2-CH_2).

Results and discussion

The title compound **2** can be obtained by condensation of 5-methyl-4-imidazolemethanol, mercaptoacetic acid, L-methionine and histamine. The synthetic approach taken was based on two considerations: *(i)* the accessibility of the dihydrochloride of *N*-(L-methionyl)histamine[2], being one half of the target molecule, and *(ii)* the availability of a promising lead to synthesize the other half, *viz.*, a published method to condense 5-methyl-4-imidazolemethanol hydrochloride and thiols by refluxing in acetic acid[8]. The latter method could successfully be adapted, by using mercaptoacetic acid both as reagent and solvent, to readily yield the other building block 2-[[(5-methyl-4-imidazolyl)methyl]-thio]acetic acid as its hydrochloride salt, in high yield and purity. This sequence of reactions (see Scheme 1) allowed the problems associated with (de)protection of the thiol functionality to be avoided[9].

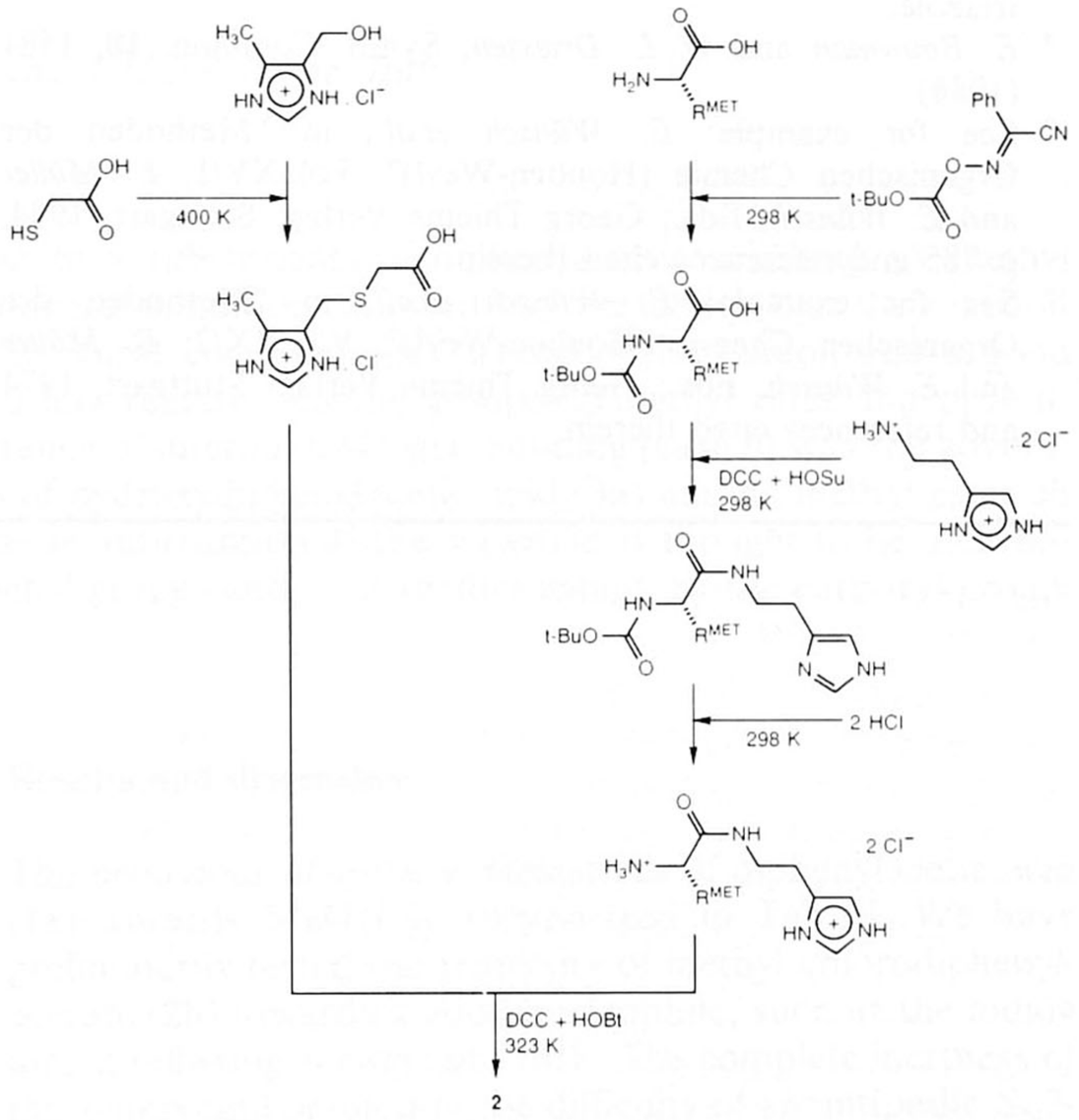
To complete **2**, the two halves of the molecule must be fused through an amide bond. Many rewarding methods to couple (pseudo) amino acid fragments have been published[10] but, unfortunately, information on whether a seemingly general amide formation method is applicable to imidazole-containing fragments has not always been provided. This is of particular interest, since it is known, from reactions with histidine and derivatives, that imidazole catalyzes racemization, ester and amide hydrolysis[11]. It is also prone to undergo ring opening and acylation[11]. These unwanted reactions can be circumvented by imidazole-ring protection during the crucial stages[12], but usually, since protection and deprotection of the imidazole unit and purification of the intermediate products involves even greater problems[12b], that is not a practical solution. Therefore, we decided to develop an amide bond formation procedure which could be applied to fragments containing unmasked imidazole rings and would allow relatively straightforward purification of the product. In testing the potential methods, synthesis of **3** served as a model reaction.

Because alkanoic acids and primary amines do not spontaneously react to give amide bonds, an activator, as a potent water abstracting agent, is often necessary. Most good activators, however, do not only activate the acid function, but also give rise to undesired reactions, notably racemization. To prevent/minimize unwanted reactions, moderating reagents may be added[13].

Various promising methods have been tested (see Experimental). Using a triphenyl phosphite activator and imidazole as moderator[14], **3** was formed in a yield of 30%, but it was contaminated with intractable by-products as well as by diphenyl phosphite and imidazole[15]. In view of the fact that **2** and **3** are intended as cation-binding agents, the presence of small amounts of by-products and phosphorous compounds (known as strongly coordinating ligands) was not acceptable.

As we had previously obtained good results with a DCC[7]-activated and HOSu[7]-moderated coupling procedure (synthesizing *N*-(L-methionyl)histamine, via pre-activation of *N*-(*tert*-butyloxycarbonyl)-L-methionine by conversion to its *N*-succinimide ester prior to addition of histamine; see Scheme 1)[2], we next tested this method to obtain **3**. However, in this case, the yield of **3** was very low, while dicyclohexylurea (DCU) and other products, most likely resulting from imidazole ring opening[16], were present as contaminants. Using only DCC activation also did not give satisfactory results[17], indicating that only a moderated DCC method would probably successful.

In scrutinizing other potential DCC moderators, we discovered that *N*-hydroxybenzotriazole (HOBt), which reportedly decreases ring opening when added as extra component in a DCC + HOSu approach[16], has recently been used as the only moderator in two patented amide formation reactions involving imidazole-containing subunits[18]. Applied to the synthesis of **3**, the DCC + HOBt method gave a very high yield and virtually no by-products. The use of ethyl acetate as reaction solvent allowed relatively easy isolation of the pure product. The title compound **2** could also be synthesized via the DCC + HOBt method (see Scheme 1 for the total synthesis). In this case, however, the procedure had to be modified slightly by using *N*,*N*-dimethylformamide (DMF) as reaction solvent because, in ethyl acetate, the reagents turned into an intractable gum, preventing efficient coupling.



Scheme 1

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