The Preparation and Properties of Partially Protected 4-Amino-1-methylimidazole-2-carboxylic Acids to be Used as Intermediates in the Synthesis of Analogues of Distamycin A

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Partially protected 4-amino-1-methylimidazole-2-carboxylic acid derivatives have been prepared by a convenient route from the corresponding nitro analogue. Such derivatives, blocked on the amino function with *tert*-butyloxycarbonyl (**4**) or formyl groups (**8**) and on the carboxy function with benzyl (**6**) or ethyl groups (**2**), should serve as suitable precursors for the synthesis of oligoamides related to distamycin A. In addition, several intermediates and side-products have been characterized.

Distamycin A (DA, Fig. 1) is a heterocyclic oligoamide with interesting antibiotic properties, isolated from the fermentation broth of *Streptomyces distallicus*. Its structure was elucidated by spectroscopic methods of the parent compound as well as by its degradation products.[1–3] Several other structurally related oligoamides such as netropsin, kikumycin and anthelvencin have also been described and subsequently studied with respect to pharmaceutical properties.[4,5]

DA exhibits a broad spectrum of antibiotic properties. In particular, its antiviral and oncolytic activities have received considerable attention and over the last two decades, numerous structure–activity relationship investigations have been carried out.[6–9] It appears that this drug, at least partly, exerts its biological effects by blocking the template function by binding to specific nucleotide sequences in the minor groove of double-stranded DNA.[10,11]

The structure of DA was confirmed early on by total synthesis.[3] In addition, owing to the pharmaceutical potential of this compound, a wide range of DA analogues was prepared and screened with respect to antimicrobial activity. The first derivatives were modified predominantly at the aliphatic amidine function or at the formamide end, but it was soon established that these structural elements were essential to high potency.[6] Later synthetic studies also gave rise to analogues where various aromatic and heterocyclic moieties such as pyridine, thiophene, thiazole and isoxazole rings were exchanged for the pyrrole ring.[5,9,12,13] However, with few exceptions, the compounds thus prepared did not display enhanced biological activity in a variety of different test systems.

Some years ago, we designed a novel efficient synthesis of DA, in which very mild reaction conditions suitable for more sensitive structures were used.[14] This convenient route was exploited in the preparation of several new analogues. Some of these derivatives lacking a methyl substituent on one pyrrole nitrogen were found to possess elevated antiviral activity against herpes simplex virus type 1 in comparison with DA.[15,16]

The preparation of DA congeners containing the corresponding imidazole residue in the pyrrole backbone has been reported.[17] Furthermore, a paper describing the synthesis of 1-methyl-4-nitro-2-trichloroacetylimidazole appeared recently. This compound is claimed to be a suitable

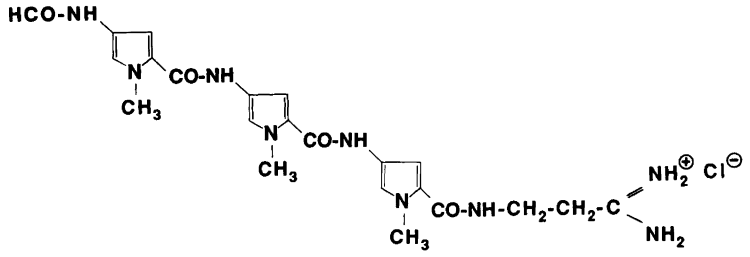

Fig. 1.

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precursor for obtaining DA related analogues.[18] Together with the assumption that the interaction of DA analogues with DNA could be facilitated by a stabilizing hydrogen bond to additional azole nitrogens in the DA molecule,[19] these interesting studies led us to consider the incorporation of imidazole moieties in DA. In order to adhere to our earlier synthetic scheme, various specifically protected aminoazolecarboxylic acids (**4**, **6** and **8**) would be required as building blocks.

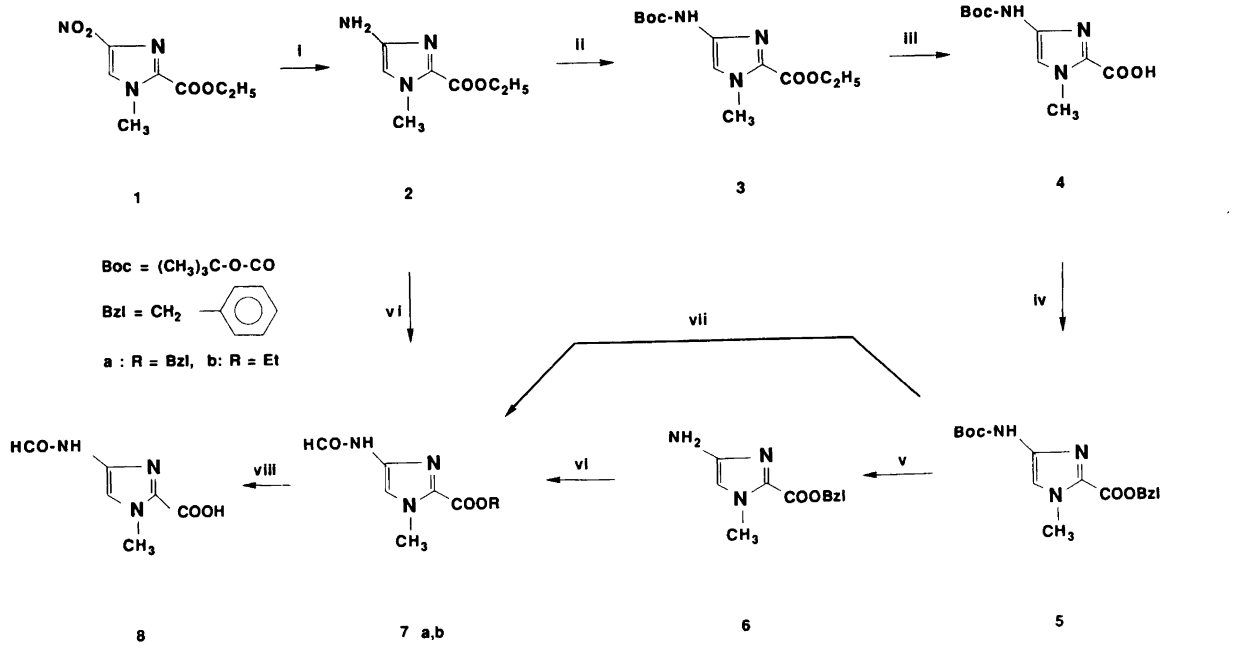
Results and discussion

The preparation of key compounds **4**, **6** and **8** is outlined in Scheme 1. Thus, the preferential reduction of the nitro group of **1**[17] was accomplished by catalytic hydrogenation in EtOH, the yield being essentially quantitative. Apparently, this reduction method is superior to procedures involving Sn/aq. HCl which are known to give chlorinated side-products in a similar case.[17,20]

The amine **2** was readily *tert*-butyloxycarbonylated with Boc-F/Et_3N in Et_2O-MeCN and **3** was isolated in an excellent yield. The use of Boc_2O in CH_2Cl_2, THF, MeCN or DMF was less efficient. The reaction proceeded slightly faster in pyridine but in this case, an impure product was obtained. However, the reaction took a different course when **2** was subjected to Boc_2O/Et_3N in MeCN maintaining similar conditions and **10** (Fig. 2) could be isolated as the main product. Only small quantities of the desired **3**, severely contaminated with **2** and other substances could be found in the remaining fractions. The reason for the formation of **10** is not well understood. Presumably the more basic conditions in this case favoured the formation of the intermediary isocyanate to which a second molecule of amine is then finally added.

It is also worth emphasizing the importance of working with rigorously purified **1** in this synthesis. When **1** contaminated with ethyl 1-methyl-4,5-dinitroimidazole-2-carboxylate[17] was used, minor amounts of **9** (Fig. 2) could be collected in the chromatographic work-up of **3**. The formation of this compound from the dinitroimidazole derivative could be given a rational explanation. It is well known that a substituent in disubstituted imidazoles can be displaced by nucleophiles, when one or both of them are electron-withdrawing. Furthermore, in 4,5-disubstituted derivatives, the 5-substituent is preferentially exchanged under such conditions.[21] In this case EtOH, used as a solvent, could serve as nucleophile to furnish the 5-ethoxy analogue of **1**. Subsequent reduction and *tert*-butyloxycarbonylation would then give rise to **9**.

Alkaline hydrolysis of the ethyl ester **3** afforded the pivotal acid **4** in good yield after a simple work-up. This compound is, however, rather unstable when stored under normal laboratory conditions (see the Experimental). Thus, spontaneous decarboxylation occurred even at room temperature and furnished **12a** (Fig. 2) as the sole decomposition product. The 4-nitro analogue was reported to be sensitive to decarboxylation under similar conditions.[17] When the Cs salt of **4** was subjected to reaction with BzlBr in DMF under anhydrous conditions, the corresponding benzyl ester **5** was produced in satisfactory yield.[14,22] This esterification method appears to be very suitable in this case as no side-products due to alkylation of azole nitrogens were observed.



Scheme 1. Reagents: i, H_2(Pd/C), 3 h; ii, Boc-F/NEt_3, MeCN/Et_2O, 30 h; iii, aq. NaOH (1.2 equiv.); iv, 1: Cs_2CO_3, aq. EtOH; 2: BzlBr (1.0 equiv.), DMF, 20 h; v, 80 % aq. CF_3COOH, 1 h; vi, $HCOOC_6F_5$, $CHCl_3$, 2 h; vii, HCOOH, 26 h; viii, a: H_2(Pd/C), DMF/EtOH, 2 h; b: as for iii.

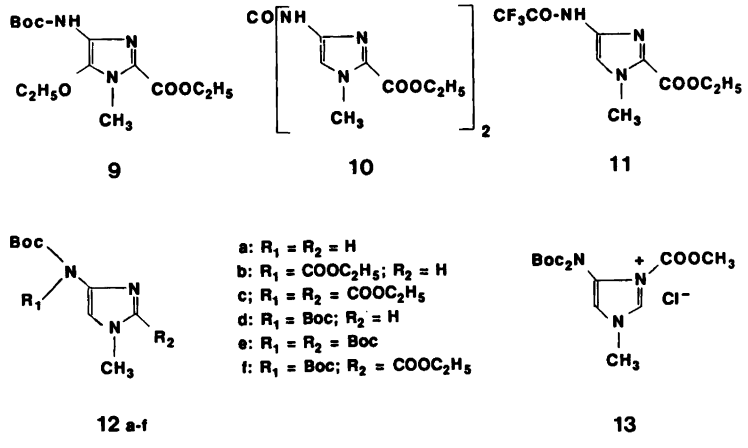


Fig. 2.

Treatment of **5** with 80% aq. CF_3COOH at ambient temperature removed the Boc group to give the corresponding amine **6**.[23] Preliminary investigations indicated that significant amounts (>10%) of the trifluoroacetamide analogue **11** (Fig. 2) were formed together with other substances when neat CF_3COOH or mixtures with CH_2Cl_2 under anhydrous conditions were used as the deprotecting agent.

Initial attempts employing **2** indicated that the formylation of such aminoimidazole derivatives could be accomplished with HCOOH/dicyclohexylcarbodiimide (DCC) according to a standard method.[24] Although an acceptable yield of **7b** was achieved under these conditions, the removal of the simultaneously formed dicyclohexylurea required time-consuming chromatography. Explorative small-scale experiments also revealed that **2** was slowly converted into **7b** when dissolved in 99% HCOOH at room temperature. This reaction proceeded significantly faster when pentafluorophenyl formate in $CHCl_3$ was used as acylating agent.[25] Thus, the transformations of **6** and **2** into **7a** and **7b**, respectively, were readily accomplished in a very high yield without noticeable formation of by-products. This efficient and practical formylation is the method of choice when more acid-sensitive amino derivatives are involved. It was also discovered that **7a** could be obtained in excellent yield by a one-pot procedure from the Boc analogue **5**. Excess HCOOH at ambient temperature efficiently removed the Boc group and the intermediate **6** was then formylated to give **7a** of high purity. It is interesting to note that such direct formylations were not reported when Boc amino acid derivatives were subjected to this deprotection procedure.[26]

The cleavage of the ester function in **7a** and **7b** was readily achieved with only one problem. Catalytic hydrogenation of the former compound smoothly removed the benzyl group but, due to the low solubility of the acid **8** in most organic solvents, complete recovery of the product from the solid catalyst support was rather difficult. However, the alkaline hydrolysis of **7b** was more practical and **8** was obtained in quantitative yield after a convenient work-up. Obviously, no detectable cleavage of the formamide moiety occurred with this procedure. Paralleling the behaviour of **4**, **8** also lost CO_2 on attempted recrystallization from EtOH and the expected 4-formylamino-1-methylimidazole was isolated from the mother liquor.

Alternative approaches for the synthesis of suitably designed 4-amino-1-methylimidazole-2-carboxylic acid derivatives were also sought but none of these have so far been successful. In one sequence of experiments, the introduction of the 2-carboxy function was attempted as the final step; conventional nitration of 1-methylimidazole furnished a product mixture from which the 4-nitro isomer was isolated.[27] The reduction of the nitro group was effected by catalytic hydrogenation and the resulting unstable 4-amino-1-methylimidazole was immediately treated with Boc-F/K_2CO_3 under inert conditions to give **12a** in moderate yield. When **12a** was allowed to react for an extended period of time with a large excess of EtOCOCl/Et_3N in MeCN, essentially mimicking the conversion of 1-methylimidazole into ethyl 1-methylimidazole-2-carboxylate,[17] a complex mixture resulted from which **12b** and **12c** could be isolated in low yields. Evidently, the urethane nitrogen is more susceptible to electrophilic attack than the 2-position in the imidazole nucleus as no trace of **3** could be detected. Therefore, the blocking of this reactive nitrogen site seemed desirable. Exhaustive *tert*-butyloxycarbonylation using Boc_2O in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) afforded **12d** in acceptable yield.[28] Although **12d** was the main product in this reaction, traces of **12e** were also formed under these conditions as judged from the ^1H NMR spectrum of a chromatographed fraction. Similar DMAP-catalysed electrophilic substitution reactions of imidazoles have been observed recently.[29] In a preliminary experiment using MeOCOCl as the electrophile and the fully protected **12d** as the substrate, only traces of the methyl analogue of **12f** were formed as could be judged from the ^1H NMR spectrum of the reaction mixture before work-up. A ^1H NMR study also revealed that reaction between **12d** and MeOCOCl in CD_3CN in the absence of base furnished a transient adduct

to which was tentatively assigned the structure **13** (a general discussion of various aspects of such imidazolium derivatives has been published[30]). Migration of the N-3 substituent to the 2-position would give rise to the methyl analogue of **12f** but instead this imidazolium salt slowly reverted to **12d** with the concomitant release of MeCl and CO_2. This decomposition also appeared to be facilitated by the presence of organic bases such as DMAP and Et_3N. The reference substance **12f** was synthesized by exhaustive *tert*-butyloxycarbonylation of **3** according to a standard procedure.[28]

Perspectives

An exploration of the synthetic potential of the new imidazole derivatives has been initiated and preliminary results indicate that these compounds fit well into our previous scheme. Thus, the condensation of acid **4** with amine **2** using DCC/DMAP in CH_2Cl_2 by analogy with a known procedure,[16] afforded the corresponding dimeric amide fragment in satisfactory yield (84 %). Furthermore, the formylated analogue **8** has been successfully incorporated into a novel DA related compound.

Experimental

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. All solvents used as reaction media were of analytical grade unless otherwise stated and dried over molecular sieves (4A, activated at 320 °C/0.01 mmHg for 10–15 h). TLC analyses were performed on 0.25 mm thick precoated UV-sensitive silica plates (Merck DC-Fertigplatten Kieselgel 60 F_{254}) and the mobile phases used were CH_2Cl_2:MeOH 9:1 (A); toluene:MeCN 2:1 (B); petroleum ether (b.p. 40–65 °C):Et_2O 2:1 (C); CH_2Cl_2:Me_2CO:HOAc 40:10:1 (D); EtOAc : Me_2CO : HOAc : H_2O 5 : 3 : 1 : 1 (E) and CH_2Cl_2:Me_2CO 4:1 (F). TLC spots were visualized by inspection under UV-light at 254 nm or, preferentially, after brief heating, by exposure to Cl_2 followed by dicarboxidine spray (violet–blue spots).[31] Preparative chromatography was carried out using silica gel 60 (70–230 mesh). NMR spectra were routinely recorded with a JEOL FX90Q or a JEOL FX100 instrument at 90/100 MHz (^1H) or 22.5/25 MHz (^{13}C). The ^1H NMR signals were tentatively assigned by comparison of chemical shifts and peak multiplicities. Elemental analyses were carried out by *Mikro Kemi AB*, Uppsala, Sweden. Yields, melting points, spectroscopic data and other information about most compounds discussed are compiled in Table 1.

Ethyl 4-amino-1-methylimidazole-2-carboxylate (**2**). Finely ground **1**[17] (2.99 g, 15.0 mmol, recrystallized from CCl_4:$CHCl_3$ 7:1, 40 ml g^{-1}, until free from ethyl 1-methyl-4,5-dinitroimidazole-2-carboxylate) was suspended in 99 % EtOH (150 ml) and hydrogenated at atmospheric pressure and ambient temperature in the presence of Pd (5 % on C, 0.30 g). After 5 h, TLC (A, B) indicated complete reaction and the catalyst was removed by filtration. The ethanol was evaporated off at room temperature and the remaining pale yellow solid was thoroughly triturated with dry Et_2O and the solvent was stripped off at reduced pressure. This procedure was repeated once. After removal of the last Et_2O portion, the crispy residue was again treated with dry cold Et_2O (25 ml). The insoluble part was collected by filtration and carefully rinsed with small portions of cold Et_2O (3×10 ml) and meticulously dried under high vacuum. The yield of crude chromatographically pure (A) **2** was 2.51 g. This pale yellow powder which was suitable for further work darkened slowly on exposure to air.

Ethyl 4-tert-butyloxycarbonylamino-1-methylimidazole-2-carboxylate (**3**). Finely ground, dried **2** (1.35 g, 8.00 mmol, freshly prepared) was suspended in dry MeCN (20 ml) containing Et_3N (1.67 ml, 12.0 mmol, freshly distilled from CaH_2). A solution of Boc-F* (1.0 M in Et_2O, 10 ml, 10 mmol) was slowly introduced with rapid stirring under N_2 at room temperature over a period of 10 min. The reaction mixture was heated gently with agitation (40 °C, 10 min) until a clear, yellow solution was obtained and the stirring was continued at ambient temperature. The reaction was monitored by TLC (B) and after 5 h more Et_3N (0.84 ml, 6.00 mmol) was added followed by Boc-F solution (5 ml, 5 mmol). After a further 20 h reaction, TLC still indicated traces of **2** and a final portion of Boc-F solution (2.5 ml, 2.5 mmol) was added and the resulting mixture was stirred 4 h at 40 °C. The solvent was stripped off at reduced pressure below 35 °C and the resulting dirty yellow solid residue was partitioned between Et_2O (300 ml) and 1 M aq. $NaHCO_3$ (150 ml). The bright yellow aqueous phase was discarded and the pale yellow Et_2O extract was washed with $NaHCO_3$ and satd. NaCl (3×75 ml each), dried over $MgSO_4$ and treated with decolourizing carbon. Removal of the solvent afforded chromatographically pure (B, C) **3** as a colourless solid weighing 2.02 g and suitable for further work. An analytical specimen was obtained by chromatography in CH_2Cl_2:Et_2O 15:1 and crystallization of the chromatographed material. Pure **3** precipitated as white glittering flakes.

When **2** was treated with excess Boc_2O/Et_3N in MeCN by analogy with the above procedure until all starting material was consumed, a dark reaction mixture resulted. Removal of the solvent and addition of Et_2O/1 M aq. $NaHCO_3$ gave a slurry from which essentially pure **10** (B) could be collected by filtration. Thus from **2** (592 mg, 3.50 mmol), a white solid weighing 320 mg was obtained after rinsing with H_2O and Et_2O and vacuum drying. The Et_2O phase in the combined filtrate contained **3** together with impurities.

* For the preparation of Boc-F: See Ref. 14, footnote 19.

Table 1. Properties of imidazole intermediates.

Comp.	Formula (mol. wt.)[a]	Yield (%)[b]	Solvent for recrystallization[c]	M.p./°C	^{1}H NMR ($CDCl_3$, δ/ppm rel. TMS)
2	$C_7H_{11}N_3O_2$ (169.18)	99	–[d]	–[e]	6.37 (s, 1 H, H-5), 4.38 (q, 2 H, CH_2CH_3), 3.91 (s, 3 H, NCH_3), 3.69 (br s, 2 H, NH_2), 1.40 (t, 3 H, CH_2CH_3)
3	$C_{12}H_{19}N_3O_4$ (269.30)	94	Hexane–Et_2O 6:1 (30 ml g^{-1})	138–138.5	7.21 (s, 1 H, H-5), 7.06 (br s, ca. 1 H, NH), 4.40 (q, 2 H, CH_2CH_3), 3.97 (s, 3 H, NCH_3), 1.50 (s, 9 H, Boc CH_3), 1.41 (t, 3 H, CH_2CH_3)
4	$C_{10}H_{15}N_3O_4$ (241.25)	94	CCl_4–CH_2Cl_2 3:1 (30 ml g^{-1})	201–202 (decomp.)	10.98 (s, 1 H, COOH), 7.17 (s, ca. 2 H, H-5, NH), 4.14 (s, 3 H, NCH_3), 1.55 (s, 9 H, Boc CH_3)
5	$C_{17}H_{21}N_3O_4$ (331.37)	89	Hexane–EtOAc 3:1 (80 ml g^{-1})	174–175 (decomp.)	7.27–7.48 (complex signal, 5 H, $CH_2C_6H_5$), 7.22 (s, 1 H, H-5), 7.06 (br s, ca. 1 H, NH), 5.37 (s, 2 H, $CH_2C_6H_5$), 3.95 (s, 3 H, NCH_3), 1.49 (s, 9 H, Boc CH_3)
6	$C_{12}H_{13}N_3O_2$ (231.26)	89	–[d]	–[e]	7.25–7.53 (complex signal, ca. 5 H, $CH_2C_6H_5$), 6.34 (s, 1 H, H-5), 5.35 (s, 2 H, $CH_2C_6H_5$), 3.88 (s, 3 H, NCH_3), 3.58 (br s, 2 H, NH_2)
7a	$C_{13}H_{13}N_3O_3$ (259.27)	95[f] (98[g])	CCl_4–CH_2Cl_2 4:1 (50 ml g^{-1})	195–196	10.79 (br s, ca. 1 H, NH), 8.28 (d, 1 H, $J_{NH,HCO}$ 1.5 Hz, HCO), 7.57 (s, 1 H, H-5), 7.39 (perturbed signal, ca. 5 H, $CH_2C_6H_5$), 5.37 (s, 2 H, $CH_2C_6H_5$), 3.98 (s, 3 H, NCH_3)
7b	$C_8H_{11}N_3O_3$ (197.19)	97[f]	Hexane–CH_2Cl_2 3:1 (300 ml g^{-1})	230–230.5 (decomp.)	10.41 (br s, ca. 1 H, NH), 8.45 (d, 1 H, $J_{NH,HCO}$ 1.6 Hz, HCO), 7.58 (s, 1 H, H-5), 4.42 (q, 2 H, CH_2CH_3), 4.01 (s, 3 H, NCH_3), 1.41 (t, 3 H, CH_2CH_3)
8	$C_6H_7N_3O_3$ (169.14)	100[h] (52[i])	–[j]	ca. 155 (decomp.)	10.65 (br s, ca. 1 H, NH), 8.18 (d, 1 H, $J_{NH,HCO}$ 1.6 Hz, HCO), 7.52 (s, 1 H, H-5), 3.90 (s, 3 H, NCH_3)[k]
9	$C_{14}H_{23}N_3O_5$ (313.35)	–[l]	Hexane–CCl_4 5:1 (30 ml g^{-1})	76–76.5	6.18 (br s, ca. 1 H, NH), 4.36 (q, 2 H, $CO_2CH_2CH_3$), 4.29 (q, 2 H, 5-OCH_2CH_3), 3.77 (s, 3 H, NCH_3), 1.46 (s, 9 H, Boc CH_3), 1.38 (t, 3 H, $CO_2CH_2CH_3$), 1.37 (t, 3 H, 5-OCH_2CH_3)
10	$C_{15}H_{20}N_6O_5$ (364.36)	50	EtOH (30 ml g^{-1})	225–226 (decomp.)	9.20 (br s, 2 H, NH), 7.30 (s, 2 H, H-5), 4.20 (q, 4 H, CH_2CH_3), 3.96 (s, 6 H, NCH_3), 1.19 (t, 6 H, CH_2CH_3)
11	$C_{14}H_{12}F_3N_3O_3$ (327.26)	–[m]	Et_2O (20 ml g^{-1})	121–121.5	9.95 (br s, ca. 1 H, NH), 7.55 (s, 1 H, H-5), 7.30–7.46 (complex signal, 5 H, $CH_2C_6H_5$), 5.34 (s, 2 H, $CH_2C_6H_5$), 4.01 (s, 3 H, NCH_3)[n]
12a	$C_9H_{15}N_3O_2$ (197.24)	61	Et_2O (10 ml g^{-1})	196–198 (decomp.)	9.24 (br s, ca. 1 H, NH), 7.14 and 6.98 (2×d, together 2 H, $J_{2,5}$ ca. 1 Hz, H-2, H-5), 3.63 (s, 3 H, NCH_3), 1.52 (s, 9 H, Boc CH_3)
12d	$C_{14}H_{23}N_3O_4$ (297.38)	87	Et_2O (30 ml g^{-1})	118–118.5	7.34 and 6.79 (2×d, together 2 H, $J_{2,5}$ 1.2 Hz, H-2, H-5), 3.67 (s, 3 H, NCH_3), 1.48 (s, 18 H, Boc CH_3)
12f	$C_{17}H_{27}N_3O_6$ (369.42)	96	Hexane–Et_2O 10:1 (100 ml g^{-1})	134.5–135	6.95 (s, 1 H, H-5), 4.40 (q, 2 H, CH_2CH_3), 4.00 (s, 3 H, NCH_3), 1.47 (s, 18 H, Boc CH_3), 1.40 (t, 3 H, CH_2CH_3)

[a]Satisfactory microanalyses obtained for recrystallized specimens: C ± 0.4, H ± 0.2, N ± 0.3. [b]Crude, essentially pure by TLC or ^{1}H NMR. [c]Decolourizing carbon if necessary. [d]Not recrystallized. Decomposes slowly on standing. [e]Not recorded. [f]Formylation with pentafluorophenyl formate. [g]One-pot procedure. [h]Alkaline hydrolysis of **7b**. [i]Catalytic hydrogenation of **7a**. [j]Sparingly soluble in all common solvents. [k]DMSO-d_6. Minor resonances (<10 %) at δ = 8.58 (d, HCO, $J_{NH,HCO}$ 11.1 Hz) and 7.12 (s, H-5) indicated the presence of *trans* conformers. For a brief discussion see the following paper and references therein. [l]Isolated in small amounts by chromatography of crude **5** when impure **3** containing ethyl 1-methyl-4,5-dinitroimidazole-2-carboxylate was used as the starting material. [m]Small amounts formed in the preparation of **6**. [n]^{19}F NMR: δ (CF_3CO) −76.05 [δ($CFCl_3$) = 0].

*4-*tert*-Butyloxycarbonylamino-1-methylimidazole-2-carboxylic acid* (**4**). Recrystallized, finely ground **3** (2.69 g, 10.0 mmol) was suspended in water (15 ml) and 1.00 M NaOH (12 ml, 12 mmol) and the resulting slurry was heated to 60 °C for 15 min with vigorous stirring whereupon all solid material dissolved completely. The resulting pale yellow solution was stirred for 1 h at room temperature and cautiously acidified to pH ca. 2 with 3 M HCl whilst being cooled in ice. The resulting jelly was diluted with ice–water (20 ml) and after ca. 1 h, the gelatinous solid was collected by filtration, rinsed with ice–water (3×5 ml) and dried *in vacuo*. The crispy solid weighing 2.27 g was sufficiently pure for synthetic work. TLC (D, E) gave one spot. Recrystallisation caused partial decarboxylation and **12a** could be detected in the mother liquor. The crystallized material also decomposed when allowed to stand. Thus, a very pure sample contained ca. 10 % of **12a** after 2 months at room temperature. Compound **4** was even more unstable in solution and about half remained in a 5 % solution in DMSO-d_6 after 2 weeks.

*Benzyl 4-*tert*-butyloxycarbonylamino-1-methylimidazole-2-carboxylate* (**5**). Crude **4** (1.04 g, 4.31 mmol) was converted into the corresponding Cs salt according to a previous procedure.[14] The dried salt was suspended in dry DMF and treated with BzlBr essentially as described earlier.[14] When the reaction was complete, most of the solvent was stripped off at room temperature (oil pump) and the semi-solid residue was taken up in Et_2O:EtOAc 4:1 (200 ml). The extract was washed and dried as described above for **3**. Removal of the solvent left a white solid weighing 1.27 g after repeated trituration with small portions of cold petroleum ether. TLC (B, C) showed one spot. Recrystallization gave an analytical specimen as white fluffy crystals.

Benzyl 4-amino-1-methylimidazole-2-carboxylate (**6**). Recrystallized **5** (1.66 g, 5.00 mmol) was dissolved in 80 % v/v aq. CF_3COOH (25 ml) and the resulting almost colourless solution was stirred under N_2 for 1 h at room temperature. The reaction mixture was then quenched in an ice-cold mixture of 30 % aq. K_2CO_3 (300 ml), brine (100 ml) and CH_2Cl_2 (100 ml) in a stream of N_2 and the pale yellow organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (2×50 ml) and the combined extracts were washed with satd. NaCl (2×50 ml), dried over Na_2SO_4 and evaporated to complete dryness. The yellow semi-solid residue was taken up in dry Et_2O (25 ml) and again taken to dryness. This was repeated once. The partly solidified residue was again triturated with dry Et_2O (10 ml) and the suspension was left in the cold overnight. The yellow insoluble powder was collected by filtration, rinsed with cold dry Et_2O (3×2 ml) and throroughly dried. This crude material weighing 1.03 g was pure enough for further work. TLC (A, B) gave one spot. [From the yellow ether washings, minor amounts of **11** could be isolated by chromatography in CH_2Cl_2:Me_2CO 6:1].

Benzyl 4-formylamino-1-methylimidazole-2-carboxylate (**7a**). *(A) Formylation of* **6**. A solution of crude **6** (1.03 g, 4.44 mmol) in dry $CHCl_3$ (13 ml) was treated with pentafluorophenyl formate[25] (1.89 g, 8.91 mmol) in one portion with rapid stirring at ambient temperature with exclusion of moisture. After a few minutes, the reaction mixture became dark and turbid and soon a thick precipitate appeared. TLC (B) indicated complete reaction after 2 h and the solvent was removed at reduced pressure. The brownish oily residue was treated with dry Et_2O (50 ml) and again evaporated to dryness. This was repeated once to remove the last traces of $CHCl_3$ and the remaining solid was triturated with dry Et_2O (30 ml). After being chilled overnight, the tan, fine-grained product was collected by filtration, rinsed with cold dry Et_2O (3×5 ml) and dried at reduced pressure. The yield of crude, chromatographically pure (A, B) product was 1.09 g. Recrystallization afforded an analytical sample as small white needles.

(B) One-pot procedure from **5**. Recrystallized **5** (994 mg, 3.00 mmol) was dissolved in 99 % HCOOH (25 ml) and the clear solution was stirred under N_2 overnight. Complete reaction was ascertained by TLC (A) and the solvent was stripped off at room temperature. The remaining light yellow solid was redissolved in dry CH_2Cl_2 (25 ml) and again taken to dryness. This was repeated until the smell of HCOOH was no longer detectable. The solid residue was treated with dry ether and worked up as described in the previous procedure, thus yielding 761 mg of crude **7a** in all respects identical with the sample obtained above.

Ethyl 4-formylamino-1-methylimidazole-2-carboxylate (**7b**). Crude **2** (1.18 g, 7.00 mmol) was formylated with pentafluorophenyl formate according to the procedure for **7a** from **6**. Thus crude, chromatographically pure (A, B) **7b** weighing 1.34 g was obtained after a similar work-up.

4-Formylamino-1-methylimidazole-2-carboxylic acid (**8**). *(A) Hydrogenolysis of* **7a**. A solution of **7a** (380 mg, 1.47 mmol) in DMF:EtOH 4:1 (40 ml) was stirred under H_2 at atmospheric pressure and room temperature in the presence of Pd (5 % on C, 75 mg). After 2 h, TLC (E) indicated complete reaction and the catalyst was filtered off and the filter was thoroughly washed with DMF (15×10 ml). Evaporation of the combined washings at room temperature left a greyish solid which was treated with dry Et_2O (25 ml) and again taken to dryness. The residue was thoroughly triturated with dry Et_2O (5 ml) and the insoluble grey powder was collected, repeatedly rinsed with dry Et_2O and dried *in vacuo*. The crude product, essentially pure by TLC (E), weighed 130 mg.

(B) Hydrolysis of **7b**. Finely ground **7b** (986 mg, 5.00 mmol) was suspended in 30 % aq. EtOH (15 ml) and 1.00 M NaOH (6.0 ml, 6.0 mmol), was added. The mixture was stirred at 45 °C for 20 min whereupon all of the solid

dissolved completely and the stirring was continued for 30 min at ambient temperature. After filtration, the clear solution was cautiously acidified with 3 M HCl to pH ca. 2. A white precipitate appeared and after several hours in the cold, the fine-grained solid was filtered off, rinsed with ice–water (5×3 ml) and sucked dry. The powder was repeatedly washed with dry Et_2O (5×3 ml) and meticulously dried *in vacuo*. The crude, chromatographically pure (E) **8** weighed 846 mg. Attempted recrystallization from hot EtOH caused extensive decarboxylation and essentially pure (E) 4-formylamino-1-methylimidazole could be isolated by filtering the cooled solution and evaporating the filtrate. ^{1}H NMR ($CDCl_3$): δ 11.15 (br s, ca. 1 H, NH), 8.32 (s, 1 H, HCO), 7.35 and 7.24 (perturbed signals, 1 H + 1 H, H-2, H-5), 3.68 (s, 3 H, NCH_3).

*4-*tert*-Butyloxycarbonylamino-1-methylimidazole* (**12a**). Recrystallized, finely ground 1-methyl-4-nitroimidazole[27] (1.27 g, 10.0 mmol) in 99% EtOH (100 ml) was hydrogenated at 1 atm over Pd (5% on C, 0.40 g). The reaction was monitored by TLC (A, B) and after 4 h only traces of the nitro compound remained. A solution of Boc-F (1.0 M in Et_2O, 20 ml, 20 mmol) and K_2CO_3 (2.76 g, 20.0 mmol) was added in portions with rapid stirring under N_2 over a period of 3 h and the stirring was continued overnight. TLC then indicated essentially complete reaction and the catalyst was removed by filtration. After evaporation, the dark semi-solid residue was partitioned between EtOAc (160 ml) and 1 M $NaHCO_3$ (40 ml). The pale yellow organic extract was washed with 1 M $NaHCO_3$ (2×20 ml) followed by 0.2 M aq. citric acid (3×20 ml). The combined bright yellow citric acid phase was washed with Et_2O (20 ml) and then made alkaline (pH ca. 11.5) with 30% aq. K_2CO_3 (ca. 40 ml). The resulting milky suspension was extracted with EtOAc (3×60 ml) and the combined yellow extract was washed and dried as for **3**. Evaporation left a pale yellow solid weighing 1.21 g after trituration with a small portion of cold ether. TLC (A) displayed one spot. Recrystallization furnished an analytical specimen as tiny white crystals.

Reaction of **12a** *with EtOCOCl/Et_3N.* A suspension of **12a** (196 mg, 1.00 mmol) in dry MeCN (5 ml) was treated with 9 equiv. of EtOCOCl/Et_3N in three portions over a period of 24 h whilst being stirred at room temperature under dry conditions. The resulting brown mixture was left for two days and then worked up essentially as described above. The dark viscous mass thus obtained (ca. 0.3 g) was subjected to column chromatography in CH_2Cl_2:Me_2CO 9:1. First eluted was **12c** as a yellow oil (40 mg) ^{1}H NMR ($CDCl_3$): δ 6.96 (s, 1 H, H-5), 4.41 (q, 2 H, ester CH_2CH_3), 4.22 (q, 2 H, $NCO_2CH_2CH_3$), 4.02 (s, 3 H, NCH_3), 1.47 (s, 9 H, Boc CH_3), 1.41 (t, 3 H, ester CH_2CH_3), 1.25 (t, 3 H, $NCO_2CH_2CH_3$). Continued elution with CH_2Cl_2:Me_2CO 4:1 afforded a brown oil consisting of **12b** (75 mg), ^{1}H NMR ($CDCl_3$): δ 7.32 (d, 1 H, H-2), 6.79 (d, 1 H, $J_{2,5}$ 1.6 Hz, H-5), 4.23 (q, 2 H, CH_2CH_3), 3.68 (s, 3 H, NCH_3), 1.47 (s, 9 H, Boc CH_3), 1.26 (t, 3 H, CH_2CH_3). Both fractions gave one spot on TLC (F).

*4-[Bis(*tert*-butyloxycarbonyl)amino]-1-methylimidazole* (**12d**). A suspension of recrystallized, well dried **12a** (1.07 g, 5.43 mmol) and DMAP (66 mg, 0.54 mmol, recrystallized from EtOAc) in dry CH_2Cl_2 (8 ml) was treated with Boc_2O (1.48 g, 6.80 mmol) and the resulting mixture was stirred at ambient temperature with exclusion of atmospheric moisture. After 2 h, all of the solid had dissolved completely and stirring was continued overnight. TLC (A, B) indicated incomplete reaction and the brownish solution was taken to dryness at reduced pressure. The semi-solid residue was redissolved in dry CH_2Cl_2 (5 ml) and more Boc_2O (370 mg, 1.70 mmol) was added. The resulting mixture was refluxed gently until TLC only showed traces of **12a**. Removal of the solvent afforded a dirty yellow semi-solid residue which was partitioned between Et_2O (200 ml) and 1 M aq. $NaHCO_3$ (100 ml). The pale yellow ether extract was washed and dried as described for **3**. Removal of the solvent afforded a waxy solid which was chromatographed using CH_2Cl_2:Me_2CO 4:1 as the mobile phase. First was eluted a small quantity of **12e** (ca. 80 mg of a yellowish oil). ^{1}H NMR ($CDCl_3$): δ 6.90 (s, 1 H, H-5), 3.95 (s, 3 H, NCH_3), 1.60 (s, 9 H, C-Boc), 1.48 (s, 18 H, Boc CH_3). Continued elution gave chromatographically pure (A, B) **12d** weighing 1.13 g. Recrystallization gave an analytical specimen as pale yellow flakes.

Reaction of **12d** *with MeOCOCl. (A) ^{1}H NMR experiment.* A 0.2 M solution of **12d** in CD_3CN was treated with a slight excess of MeOCOCl (1.2 equiv.) and the reaction was studied by ^{1}H NMR at room temperature. After 6 min, a new set of signals indicating compound **13**, appeared in the spectrum: δ 10.82 (dd, 1 H, $J_{2,5}$ 1.7 Hz, J_{2,CH_3} 0.7 Hz, H-2), 7.83 (d, 1 H, $J_{2,5}$ 1.7 Hz, H-5), 4.15 (d, 3 H, J_{2,CH_3} 0.7 Hz, NCH_3), 4.11 (s, 3 H, OCH_3), 1.42 (s, 18 H, Boc CH_3). Gas was evolved when the reaction was allowed to stand at ambient temperature and after 5 h ^{1}H NMR spectroscopy showed that **13** had completely reverted to **12d**. ^{1}H NMR (CD_3CN). δ 7.27 and 6.83 (2×d, $J_{2,5}$ 1.5 Hz, 2 H, H-2, H-5), 3.63 (s, 3 H, NCH_3), 1.43 (s, 18 H, Boc CH_3) and a new peak at δ 3.02 indicating MeCl emerged in the spectrum.

(B) Small-scale preparative experiment. To a solution of **12d** (99 mg, 0.33 mmol) in dry MeCN (2.5 ml) was added dry Et_3N (0.10 ml) and the mixture was chilled to −25 °C. With exclusion of moisture, MeOCOCl (45 µl, 0.58 mmol) was introduced dropwise with rapid stirring over a period of 10 min and the resulting light brown solution was allowed slowly to reach room temperature. A precipitate appeared briefly but this redissolved within a few minutes with concomitant evolution of gas. The brownish mixture was stirred overnight at ambient temperature and worked up as described in the procedure for **12d**. A white solid (100 mg) was obtained and ^{1}H NMR of this crude product showed

that it largely consisted of **12d**. Minor peaks at δ 6.97 (s, 1 H, H-5), 4.01 (s, 3 H, NCH_3), 3.92 (s, 3 H, OCH_3) indicated 2–3 % of the methyl analogue of **12f**.

*Ethyl 4-[bis(*tert*-butyloxycarbonyl)amino]-1-methylimidazole-2-carboxylate* (**12f**). To a solution of recrystallized **3** (49.2 mg, 0.182 mmol) and DMAP (2.3 mg, 0.018 mmol) in dry MeCN (1.0 ml) was added Boc_2O (50 mg, 0.23 mmol) and the resulting mixture was stirred at ambient temperature with exclusion of moisture. After 1 h TLC (B) indicated complete reaction and stirring was continued overnight to decompose excess Boc_2O. The solvent was removed at reduced pressure and the remaining yellow oil was taken up in Et_2O (30 ml). The extract was washed in turn with 0.2 M citric acid, 1 M $NaHCO_3$ and satd. NaCl (3×10 ml each) and dried over $MgSO_4$. Evaporation to complete dryness left chromatographically pure (B) **12f** (65.1 mg) as a white solid. Recrystallization gave an analytical specimen as tiny colourless needles.

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