Stability Studies of *N*-Acylimidazoles[‡]

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Studies of the stabilities of a series of *N*-acylimidazoles towards acidic and basic conditions of potential usefulness for the removal of common temporary protection in peptide and oligonucleotide synthesis are presented. *N*-Acylimidazoles with a variety of substituents in the acyl component were prepared and treated with 3% trifluoroacetic acid (TFA) in chloroform and with 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF), the extent of their degradation being determined by proton NMR. *N*-(2,4,6-Trimethylbenzoyl)imidazole (**1**) and *N*-(2,6-dimethoxybenzoyl)-imidazole (**2**) remained unaffected under the above acidic and basic conditions after 4 d and 2 d, respectively. In addition, **1** and **2** were resistant to treatment with a solution of 2% piperidine/2% DBU in DMF for 24 h. Under ammonolytic conditions, **2** was rapidly cleaved (less than 1 h), whereas **1** was 64% degraded after 48 h, as ascertained by reversed-phase HPLC.

Introduction

Imidazole groups are found not only in peptides/proteins, but also in various modified biomolecules, such as peptidomimetics,[1] nucleopeptides,[2] or oligonucleotide conjugates in which the conjugate may be a peptide[3] or another imidazole-containing structure.[4] Since imidazoles are known to play an essential role in many enzyme mechanisms, low-molecular-weight model compounds containing imidazole moieties are important tools in studies aimed at more detailed knowledge of enzyme catalysis.[5] Derivatives of imidazole have also been used in artificial enzymes.[6] As part of an ongoing program, we are interested in the synthesis of several of the above classes of molecules.

Oligonucleotide conjugates are both acid- and base-sensitive, due to the presence of the nucleic acid component. It is well established that native RNA and, especially, DNA are labile in strongly acidic media.[7] In addition, RNA strands are prone to degradation upon prolonged exposure to base. Since nucleic acids are rather sensitive to acids, the use of *tert*-butyl- or benzyl-type protecting groups in the synthesis of these conjugates is precluded. It has nevertheless been reported[8,9] that nucleopeptides can be prepared by employment of the 9-fluorenylmethyloxycarbonyl (Fmoc) system as an α-amino protecting group for the peptide component, yet maintaining standard techniques for the nucleic acid segment. It should be possible to incorporate imidazole-containing components in conjugates to oligonucleotides both prior to and subsequent to oligonucleotide synthesis, provided that the side-chain protection be adapted to that technique. A standard oligonucleotide procedure entails several repeated steps, such as the removal of mono- or dimethoxytrityl (MMTr and DMTr) groups from the 5′-hydroxy groups of RNA and DNA, respectively. Moreover, deprotection of the nucleobases by ammonolysis is also included. It would thus be preferable if an imidazole protecting group for the synthesis of nucleopeptides and oligonucleotide-imidazole conjugates were stable to repeated detritylation, but cleaved by ammonolysis. It would also be an advantage, and would make it more versatile, if it were stable to Fmoc removal conditions.

Most of the commonly used protecting groups for imidazole functions are of the alkyl,[10−14] urethane,[15,16] or sulfonyl types.[17] Protecting groups of the first type are usually removed under strongly acidic conditions, which are incompatible with the presence of acid-sensitive structures. Those of the second and third classes may be unstable to some reagents used in standard peptide and oligonucleotide chemistry.[15,17] We were curious to find out whether acylation could provide sufficient protection for imidazole functions. *N*-Acylimidazoles are generally considered to be mild acylating agents,[18] but their reactivity, like that of other acylating reagents, depends on steric and electronic factors. It is known that sterically hindered aliphatic *N*-acylimidazoles show better stability towards nucleophiles than unhindered ones do.[19] In addition, it has also been reported that electron-donating groups in substituted aromatic *N*-acylimidazoles exhibit enhanced stability towards hydrolysis.[20,21] Several stability studies of various *N*-acylimidazoles have already been published.[20−22] However, these

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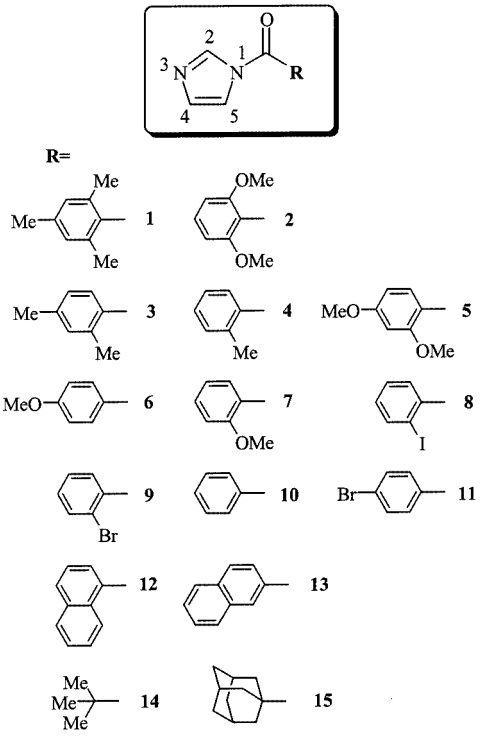
were limited, and the conditions employed were generally not those most suitable for the synthesis of acid- and base-sensitive compounds. As a first step towards the development of acyl-type protecting groups for the imidazole functions of oligonucleotide-imidazole-containing conjugates, we subjected a set of (a few) aliphatic and (mostly) aromatic derivatives to stability tests. They were exposed to acidic and basic conditions that can be used for the removal of MMTr and Fmoc, respectively, and to ammonolysis. This paper reports the results of these investigations, performed in order to provide a basis for the development of acyl-based imidazole protection.

Results and Discussion

It is well established that steric hindrance at the carbonyl function and the presence of electron-releasing groups render acylating agents less prone to nucleophilic attack.[23−26] The most stable compounds are not the best choice for all purposes, and so we decided to investigate a series of derivatives to provide a basis for choosing the most suitable for each application. For this purpose, *N*-benzoylimidazoles with methyl or methoxy groups in their *ortho* and/or *para* positions (compounds **1**−**7**) were synthesized, together with the naphthoyl (**12** and **13**), pivaloyl (**14**), and adamantoyl (**15**) derivatives. Bromo- and iodo-substituted benzoylimidazoles were also prepared (compounds **8**, **9**, and **11**). We reasoned that the bulkiness of a halogen atom in the *ortho* position might overcome the inductive effect and that, as a consequence, derivatives **8** and **9** could be more stable towards degradation than the parent *N*-benzoylimidazole (**10**). All the above-mentioned molecules were obtained in good yields by treatment of imidazole in twofold excess with the individual acyl chlorides at room temperature, except for compounds **1**, **2**, and **3**, which were synthesized from the corresponding acid and 1,1′-carbonylbis(1*H*-imidazole) in equimolar amounts.

We chose to follow the cleavage of *N*-acylimidazoles by proton NMR. The stability studies were performed in 3% (v/v) trifluoroacetic acid (TFA) in chloroform, conditions resembling those used for the removal of MMTr from the 5′-hydroxy moieties of nucleotides. Solutions such as 1% TFA or 2−3% dichloroacetic acid (advisable with DNA) in a chlorinated solvent are normally employed, and complete detritylation occurs within 2 min.[27,28] However, in order to provide a large safety margin and to stretch the limits of applicability to other groups that may require stronger acid for removal, we decided to use 3% TFA solution. It should be mentioned that concentrations of TFA much higher than this would be precluded due to rapid degradation of the oligonucleotide component of the conjugate. As a consequence, conditions commonly used for removal of Boc or *tert*-butyl ethers and esters are incompatible with most nucleic acids.

Initially, *N*-pivaloylimidazole (compound **14**) was treated with 3% TFA in chloroform containing 15 or 200 ppm water in order to evaluate the effect of water on the stability of the substrate. Derivative **14** was partially degraded (10%) within 30 min in deuterated chloroform containing 200 ppm water, compared with 2% degradation of the same substrate in 15 ppm of water. To circumvent possible partial degradation of the *N*-acylimidazoles, the water level in all further TFA experiments was monitored, and did not exceed 15 ppm.



The results of the experiments under anhydrous conditions (water content < 15 ppm) are reported in Table 1. Degradation of the aromatic derivatives was almost undetectable in the first 5−6 h and limited in most cases to a few percent after 4 d. Compounds **1** and **2** showed no cleavage at all within the detection limits of the instrument. As expected, substitution of the aromatic ring in *N*-benzoylimidazoles with electron-donating groups such as methoxy and methyl resulted in substantial stabilization (see compounds **1**−**7**) in comparison with the parent unsubstituted *N*-benzoylimidazole (**10**). The data presented in Table 1 also indicate that compounds bearing bulky substituents in their *ortho* positions (see compounds **1**−**5**, **7**−**9**, and **12**) were significantly more stable than *N*-benzoylimidazole and substantially more stable than the aliphatic *N*-acylimidazoles.

The results of the stability tests on the substituted *N*-acylimidazoles in diluted TFA prompted us to investigate whether they would also withstand basic conditions. In view of possible applications to peptide-containing molecules, such as nucleopeptides, we chose conditions that can be used for removal of the Fmoc group from α-amino functions of amino acids. In most cases, piperidine is employed to promote cleavage of Fmoc by β-elimination. Unfortunately, treatment with the nucleophilic piperidine resulted

Table 1. Stability of *N*-acylimidazoles in 3% (v/v) TFA in $CDCl_3$, at 25 °C; [substrate] = $1.3 \cdot 10^{-2}$ M; [TFA] = $4 \cdot 10^{-1}$ M

Substrate[a]	Substrate cleavage [%][b]				δ(^{1}H)
	23 h	48 h	70 h	96 h	[ppm][c]
2,4,6-Me_3BzIm (**1**)	n.d.[d]	n.d.	n.d.	n.d.	8.83 (2)
2,6-MeO_2BzIm (**2**)	n.d.	n.d.	n.d.	n.d.	7.87 (5)
2,4-Me_2BzIm (**3**)	< 1	< 1	< 1	< 1	7.84 (5)
2-MeBzIm (**4**)	< 1	< 1	< 1	1	7.86 (5)
2-IBzIm (**8**)	< 1	< 1	1	1	7.85 (5)
2-BrBzIm (**9**)	1	1	2	2	9.03 (2)
4-MeOBzIm (**6**)	< 1	1	2	3	9.19 (2)
1-NaphtIm (**12**)	1	2	3	3	9.14 (2)
2,4-MeO_2BzIm (**5**)	1	2	3	3	7.82 (5)
2-MeOBzIm (**7**)	1	2	3	5	7.86 (5)
BzIm (**10**)	2	4	7	10	9.23 (2)
2-NaphtIm (**13**)	2	6	9	10	9.30 (2)
4-BrBzIm (**11**)	7	20	20	40	9.25 (2)
AdCOIm (**15**)	30	60	80	–	7.92 (5)
PivIm (**14**)	70	–	–	–	7.91 (5)

[a] Substrate concentration for compounds **1**, **6**, **9**, **10**, and **11** was $1.4 \cdot 10^{-2}$ M. [b] Experiments are based on a single run, except for substrate **3** which was shown to be reproducible with a maximum deviation of 20%. [c] Chemical shifts of the substrate protons used for quantification; cleavage is referred to the 5-H of free imidazole at δ = 7.48 ppm, except for compounds **4** and **8**, which referred to the *o*-CH_3 signal at δ = 2.66 ppm and to the 2-H signal at δ = 8.73 ppm, respectively. [d] n.d. means not detected.

in rapid degradation of substrate **5** (20% cleavage after 60 min with 20% piperidine in DMF) even under anhydrous conditions. As a consequence, we turned our attention to the nonnucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), which has also been used for removal of Fmoc.[29] Rapid degradation of substrate **5** was observed in a solution of 2% DBU in DMF containing 350 ppm of water (70% cleavage in 60 min). In order to obtain a relatively anhydrous solvent, molecular sieves were added prior to use, reducing the water content to about 30 ppm. Table 2 summarizes the results of the stability tests towards DBU in anhydrous DMF. The order of stability was similar to that observed under acidic conditions; however, the relative stabilities of the halogenated compounds **8**, **9**, and **11** were not as high in this basic environment as under dilute TFA solutions. In most cases, a significant amount of cleavage had already occurred in the early stages of the experiments, due to the unavoidable presence of traces of water in the samples. Compounds **1** and **2**, however, were stable in this environment. In fact, no detectable cleavage was observed even after 2 d. In addition to the above tests, compounds **1** and **2** were subjected to treatment with a mixture of 2:2:96 (v/v/v) DBU/piperidine/DMF at room temperature. These conditions are applied in batch peptide synthesis, the additional piperidine being used to scavenge undesired dibenzofulvene.[30] In these experiments the substrates were unaffected after 24 h.

Ammonolysis tests were also conducted on compounds **1**–**3** and **5**. These tests were performed because ammonolysis is often used to effect final deprotection of different biomolecules, in particular oligonucleotides. To ascertain how quickly the acyl group could be removed from the imidazole residue, the reactions were monitored by reversed-phase HPLC. The results are summarized in Table 3 and indicate that removal of the acyl groups from the imidazole residue was particularly fast, except for the 2,4,6-trimethylbenzoyl group, which exhibited a substantial resistance to cleavage.

The data presented in Tables 1–3 show the expected stabilizing effect of an *ortho* substituent, which it is particularly evident in the bis(*ortho*)-substituted derivatives (compounds **1** and **2**). This effect of steric hindrance on nucleophilic attack at the carbonyl group is best illustrated by comparison of the stabilities of compounds **1** vs. **3**, **2** vs. **5**, **9** vs. **11**, and **12** vs. **13**. In the *ortho*-iodo and -bromo derivatives (**8** and **9**) the steric effect is clearly larger than the electron-withdrawing influence, rendering these compounds as stable as their methyl and methoxy counterparts (**4** and **7**).

The stability exhibited by compounds **1**–**9** and **12** in diluted TFA solution can be considered satisfactory, a fact that should make these acyl groups suitable for imidazole protection in association with the acid-labile MMTr and DMTr groups commonly used in oligonucleotide chemistry. The stability of these acyl groups should be sufficient to allow prolonged exposure to 3% TFA with negligible loss of imidazole protection.

In association with the Fmoc group, only the use of acyl groups in *N*-acylimidazoles **1** and **2** is recommended. The Fmoc group can be removed with DBU in less than 10 min; however, some procedures require repetition of the treatment and the overall time of contact with the cleaving solution can be rather long.[31] Because of their lability in basic solutions, compounds **3**–**15** should be excluded from application in procedures requiring repeated removal of the Fmoc group.

The fact that the 2,6-dimethoxybenzoyl group was completely removed in aqueous ammonia within 1 h should make it more suitable for general use in synthesis. The 2,4,6-trimethylbenzoyl residue required prolonged ammonolysis, conditions that may not be ideal for base-sensitive molecules.

Conclusion

From the results of the acid stability tests it is evident that *N*-acylimidazoles **3**–**15**, and in particular derivatives **1** and **2**, can survive prolonged exposure to anhydrous 3% TFA in chloroform. They should thus remain intact during the repeated removal of, for example, MMTr and DMTr. On the other hand, compounds **3**–**15** are not very stable in basic environments, a fact that precludes the application of their acyl groups in procedures that require repeated removal of, for example, Fmoc groups. However, compounds **1** and **2** are quite stable and their acyl groups could therefore be applied for imidazole protection in the synthesis of compounds by procedures that require mild basic conditions.

Table 2. Cleavage of *N*-acylimidazoles in 2% (v/v) DBU in [D_7]DMF, at 25 °C; [substrate] = $1.3\cdot10^{-2}$ M; [DBU] = $1.3\cdot10^{-1}$ M

Substrate[a]	Substrate cleavage [%][b]				Relative error	δ(^{1}H)
	15 min	30 min	60 min	120 min	[%]	[ppm][c]
2,4,6-Me_3BzIm (**1**)	n.d.	n.d.	n.d.	n.d.	–	7.20 (4)
2,6-MeO_2BzIm (**2**)	n.d.	n.d.	n.d.	n.d	–	7.15 (4)
2,4-Me_2BzIm (**3**)	1.3[d]	2.9	4.6	7.5	±15	7.19 (4)
2-MeBzIm (**4**)	1.7[e]	3.3	5.4	8.2	±15	7.20 (4)
2,4-MeO_2BzIm (**5**)	5.1	8.8	11	13	±5	6.84 (3′)
4-MeOBzIm (**6**)	9.3	9.9	11	12	±10	8.23 (2)
2-MeOBzIm[f] (**7**)	10	12	13	14	±5	7.34 (3′)
2-IBzIm (**8**)	6.3	8.8	12[e]	16	±15	7.65 (5)
2-BrBzIm (**9**)	6.1[g]	11	14	20[g]	±5	8.12 (2)
1-NaphtIm (**12**)	11	15	20	23	±5	7.22 (4)
BzIm (**10**)	14	17	23	37[h]	±5	7.21 (4)
2-NaphtIm (**13**)	16	19	26[g]	30[g]	±5	7.25 (4)
PivIm (**14**)	17	23	30	40	±15	8.53 (2)
4-BrBzIm (**11**)	28	37	48	59	±15	7.21 (4)

[a] Substrate concentration for compounds **3**, **4**, **10**, and **11** is $1.4\cdot10^{-2}$ M. [b] Experiments are based on duplicate runs. [c] Chemical shifts of the substrate protons used for quantification; cleavage relates to the 5-H of free imidazole at δ = 7.09 ppm, except for compounds **5** and **14**, in which it relates to the 2-H signal at δ = 7.72 ppm. [d] Relative error 55%. [e] Relative error 25%. [f] Cleavage of substrate **7** is somewhat overestimated due to overlapping of proton signals. [g] This value is based on a single measurement. [h] Value corresponding to the cleavage after 180 min.

Table 3. Cleavage of *N*-acylimidazoles in 3:1 (v/v) aq. NH_3/EtOH at room temperature; [substrate] = $4\cdot10^{-2}$ M

Substrate	Substrate cleavage [%][a]				Peak t_R
	0.5 h	1 h	4 h	21 h	[min]
2,4,6-Me_3BzIm (**1**)	16	20	31	46	28.1
2,6-MeO_2BzIm (**2**)	97	100	–	–	21.0
2,4-Me_2BzIm (**3**)	100	–	–	–	25.7
2,4-MeO_2BzIm (**5**)	100	–	–	–	24.1[b]

[a] Experiments are based on duplicate runs. [b] Gradient: 16% hold for 5 min, then 16–80% over 40 min (see for mobile phases in the General Remarks section).

The fact that *N*-acylimidazoles **3**–**15** are fairly stable to 3% TFA in chloroform but not very stable under basic conditions may be a useful combination, which could result in applications of such acyl groups as temporary or transient protecting groups in organic chemical procedures involving imidazole rings.

As mentioned, *N*-(2,4,6-trimethylbenzoyl)- and *N*-(2,6-dimethoxybenzoyl)imidazoles both have excellent stabilities in dilute TFA and DBU solutions. They exhibit different labilities when exposed to ammonolysis, upon which compound **1** is more stable than compound **2**. This feature implies that removal of the acyl group of **1** would require prolonged and/or higher temperatures during ammonolysis. This may compromise the structural integrity of base-labile molecules. To circumvent such degradation, the duration of ammonolysis should be limited as much as possible, thus making the 2,6-dimethoxybenzoyl group the protecting group of choice for the general synthesis of biologically active compounds of various biomolecules, in particular nucleopeptides, oligonucleotides, and oligonucleotide conjugates. On the other hand, the increased ammonolytic stability of **1** might prove advantageous in term of storage, handling, and workup, and so this may be the protection of choice for more base-stable compounds. Further evaluation of 2,6-dimethoxy- and 2,4,6-trimethylbenzoyl groups for imidazole protection in the synthesis of nucleopeptides, oligonucleotide conjugates, and other biomolecules is in progress. These investigations are also addressing the selective acylation of one of the two distinct nitrogen atoms of a substituted imidazole ring in different imidazole derivatives, including histidine.

Experimental Section

General Remarks: Imidazole (Lancaster) was recrystallized from chloroform. 1,1′-Carbonylbis(1*H*-imidazole) (Fluka) and all acyl chlorides (Fluka, Aldrich, and Lancaster) were of synthesis grade and used without further purification. THF was distilled at atmospheric pressure from a K/Na mixture. All other solvents used during syntheses and kinetic measurements were stored over 4 Å molecular sieves and kept under nitrogen. Solvents were evaporated under reduced pressure at a water bath temperature not exceeding 40 °C. TFA and piperidine were distilled at atmospheric pressure, and DBU was distilled in vacuo. All glassware, syringes, and needles used during syntheses and kinetic measurements were dried in an oven prior to use. The glassware was assembled while hot and cooled under a flow of dry nitrogen. *N*-Acylimidazoles were stored in the presence of a desiccant and kept cold. Sample preparation for kinetics was performed under a flow of dry nitrogen. NMR spectra were recorded with a Bruker Avance DRX 400 spectrometer at 400 and 100 MHz for proton and carbon-13, respectively. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane, with the residual nondeuterated solvent signal as an internal standard. The substrate concentration was set to be

$1.3 \cdot 10^{-2}$ M, so that a distinguishable signal could be obtained within a spectrum acquisition time of 2 min. The extent of degradation was quantified by integration of the proton signals of the imidazole ring (normally the 2-H or the 5-H signal in the acidic tests, and the 2-H or the 4-H signal in the basic tests) in the substrate and in the free imidazole, at different time intervals. The signals used for quantification were selected because they did not overlap with other signals. Chemical shifts of these signals are reported in Tables 1 and 2. Mass analysis of *N*-acylimidazoles was performed with a Micromass LCT (ES-TOF) mass spectrometer in positive ion mode. Reversed-phase HPLC analysis was carried out with a Jasco HPLC system equipped with a Jones Genesis C18 analytical column (250 × 4.6 mm). A gradient (16% hold for 5 min, then 16−80% over 30 min) of CH_3CN in 0.1 M triethylammonium acetate (pH = 6.5) was used with a flow rate of 0.8 mL/min, at 30 °C. Chromatograms were recorded at 254 nm.

Synthesis of the *N*-Acylimidazole Derivatives: Imidazole (2 mmol) was dissolved in dry CH_2Cl_2 (1 mL), and a solution of acyl chloride (1 mmol) in CH_2Cl_2 (1 mL) was slowly added to the stirred solution, resulting in the precipitation of imidazolium chloride. The mixture was stirred for 2−3 h at room temperature. The mixture was filtered, and the solution was rapidly washed with cold water (2 × 10 mL). The organic phase was dried with $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure, yielding the crude *N*-acylimidazole. Some of the crude products were successfully recrystallized from dichloromethane/*n*-hexane or cyclohexane mixtures. In an alternative method, the parent carboxylic acid (1 mmol) and 1,1′-carbonylbis(1*H*-imidazole) (1 mmol) were dissolved in anhydrous THF (2 mL) and stirred for 2−3 h. The THF was then evaporated, and the residue was dissolved in CH_2Cl_2 (10 mL), washed with water (2 × 10 mL), and dried with $MgSO_4$, and the solvents were evaporated to dryness. Compounds **1**, **2**, **5**, **6**, and **11**−**15** were isolated in crystalline form, whereas compounds **3**, **4**, and **7**−**10** were recovered as viscous oils and used without further purification. NMR analysis showed that the only organic impurities were traces of hydrolysis products (imidazole and parent carboxylic acids, some of which may have formed during NMR analysis) and the purity was > 99−99.5% (down to 95%, 97.5%, and 98% for **7**, **12**, and **14**, respectively). Furthermore, the synthetic method used precludes the formation of inorganic impurities. All *N*-acylimidazoles employed in this work have previously been reported in the literature,[18,32−37] except for compounds **3** and **12**. Compounds were characterized by high-resolution mass spectrometry and ^1H and ^{13}C NMR, and showed the expected spectra.

***N*-(2,4,6-Trimethylbenzoyl)imidazole (1):** ^1H NMR (400 MHz, $CDCl_3$): δ = 2.19 (s, 6 H), 2.36 (s, 3 H), 6.96 (s, 2 H), 7.14 (s, 1 H), 7.54 (br. s, 1 H), 7.72 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.5, 21.7, 116.5, 129.1, 131.0, 131.9, 135.2, 137.5, 141.1, 168.1 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{13}H_{15}N_2O$ [M + H]$^+$: 215.1184; found 215.1178.

***N*-(2,6-Dimethoxybenzoyl)imidazole (2):** ^1H NMR (400 MHz, $CDCl_3$): δ = 3.79 (s, 6 H), 6.65 (d, *J* = 8.4 Hz, 2 H), 7.09 (s, 1 H), 7.44 (t, *J* = 8.5 Hz, 1 H), 7.47 (s, 1 H), 7.83 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.4, 104.4, 112.0, 116.9, 131.2, 133.3, 138.3, 158.1, 163.6 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{12}H_{13}N_2O_3$ [M + H]$^+$: 233.0926; found 233.0930.

***N*-(2,4-Dimethylbenzoyl)imidazole (3):** ^1H NMR (400 MHz, $CDCl_3$): δ = 2.38 (s, 3 H), 2.41 (s, 3 H), 7.11−7.13 (m, 2 H), 7.17 (s, 1 H), 7.31 (d, *J* = 7.8 Hz, 1 H), 7.46 (s, 1 H), 7.90 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.9, 21.8, 117.8, 126.8, 129.0, 129.5, 131.4, 132.6, 138.2, 138.5, 142.9, 167.0 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{12}H_{13}N_2O$ [M + H]$^+$: 201.1028; found 201.1023.

***N*-(2-Methylbenzoyl)imidazole (4):** ^1H NMR (400 MHz, $CDCl_3$): δ = 2.39 (s, 3 H), 7.13 (s, 1 H), 7.32−7.37 (m, 2 H), 7.42 (d, *J* = 7.1 Hz, 1 H), 7.46 (s, 1 H), 7.47 (td, *J* = 7.7, *J* = 1.3, 1 H), 7.89 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.9, 117.7, 126.2, 128.6, 131.5, 131.8, 132.3, 132.5, 137.8, 138.4, 166.9 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{11}H_{11}N_2O$ [M + H]$^+$: 187.0871; found 187.0869.

***N*-(2,4-Dimethoxybenzoyl)imidazole (5):** ^1H NMR (400 MHz, $CDCl_3$): δ = 3.80 (s, 3 H), 3.90 (s, 3 H), 6.55 (d, *J* = 1.9 Hz, 1 H), 6.60 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.08 (s, 1 H), 7.46−7.48 (m, 2 H), 7.90 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.1, 56.2, 99.3, 105.6, 115.1, 117.6, 130.8, 132.7, 138.7, 159.5, 164.9, 165.0 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{12}H_{13}N_2O_3$ [M + H]$^+$: 233.0926; found 233.0927.

***N*-(4-Methoxybenzoyl)imidazole (6):** ^1H NMR (400 MHz, $CDCl_3$): δ = 3.92 (s, 3 H), 7.05 (d, *J* = 8.9 Hz, 2 H), 7.18 (s, 1 H), 7.55 (t, *J* = 1.3 Hz, 1 H), 7.82 (d, *J* = 8.9 Hz, 2 H), 8.10 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.1, 114.7, 118.7, 124.3, 131.1, 132.8, 138.6, 164.5, 165.9 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{11}H_{11}N_2O_2$ [M + H]$^+$: 203.0821; found 203.0827.

***N*-(2-Methoxybenzoyl)imidazole (7):** ^1H NMR (400 MHz, $CDCl_3$): δ = 3.83 (s, 3 H), 7.06 (d, *J* = 8.4 Hz, 1 H), 7.09−7.14 (m, 2 H), 7.49−7.45 (m, 2 H), 7.58 (td, *J* = 8.1, *J* = 1.6, 1 H), 8.16 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.2, 112.1, 117.4, 121.3, 122.6, 130.3, 131.1, 134.0, 138.6, 157.3, 165.4 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{11}H_{11}N_2O_2$ [M + H]$^+$: 203.0821; found 203.0828.

***N*-(2-Iodobenzoyl)imidazole (8):** ^1H NMR (400 MHz, $CDCl_3$): δ = 7.17 (d, *J* = 0.9 Hz, 1 H), 7.31 (td, *J* = 7.8, *J* = 1.7, 1 H), 7.43−7.45 (m, 2 H), 7.54 (t, *J* = 7.5 Hz, 1 H), 7.85 (s, 1 H), 7.99 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 92.7, 117.5, 128.7, 129.2, 132.0, 133.1, 138.4, 139.0, 140.5, 166.3 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{10}H_8N_2OI$ [M + H]$^+$: 298.9681; found 298.9669.

***N*-(2-Bromobenzoyl)imidazole (9):** ^1H NMR (400 MHz, $CDCl_3$): δ = 7.17 (s, 1 H), 7.46 (s, 1 H), 7.48−7.54 (m, 3 H), 7.74 (d, *J* = 8.0 Hz, 1 H), 7.87 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 117.3, 120.2, 128.1, 129.6, 132.0, 133.3, 134.1, 135.0, 138.3, 165.0 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{10}H_8N_2OBr$ [M + H]$^+$: 250.9820; found 250.9817.

***N*-Benzoylimidazole (10):** ^1H NMR (400 MHz, $CDCl_3$): δ = 7.17 (s, 1 H), 7.54 (s, 1 H), 7.56 (t, *J* = 7.8 Hz, 2 H), 7.69 (t, *J* = 7.5 Hz, 1 H), 7.80 (d, *J* = 8.6 Hz, 2 H), 8.07 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.4, 129.3, 130.4, 131.4, 132.3, 134.0, 138.6, 166.5 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{10}H_9N_2O$ [M + H]$^+$: 173.0715; found 173.0718.

***N*-(4-Bromobenzoyl)imidazole (11):** ^1H NMR (400 MHz, $CDCl_3$): δ = 7.20 (s, 1 H), 7.54 (s, 1 H), 7.70 (d, *J* = 8.6 Hz, 2 H), 7.75 (d, *J* = 8.6 Hz, 2 H), 8.08 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.4, 129.3, 131.1, 131.6, 132.8, 138.4, 165.7 ppm. HRMS (ES-TOF): *m*/*z* calcd. for $C_{10}H_8N_2OBr$ [M + H]$^+$: 250.9820; found 250.9827.

***N*-(1-Naphthoyl)imidazole (12):** ^1H NMR (400 MHz, $CDCl_3$): δ = 7.19 (s, 1 H), 7.57 (s, 1 H), 7.60−7.64 (m, 3 H), 7.73 (dd, *J* = 7.0, *J* = 0.7, 1 H), 7.97−8.03 (m, 3 H), 8.13 (d, *J* = 8.2 Hz, 1 H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.1, 124.8, 125.0, 127.6, 128.3, 128.6, 129.1, 130.1, 130.8, 131.6, 133.3, 134.0, 138.7, 166.5 ppm. HRMS (ES-TOF): *m/z* calcd. for $C_{14}H_{11}N_2O$ $[M + H]^+$: 223.0871; found 223.0878.

***N*-(2-Naphthoyl)imidazole (13):** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.23 (s, 1 H), 7.64 (s, 1 H), 7.65−7.72 (m, 2 H), 7.87 (dd, *J* = 8.6, *J* = 1.6, 1 H), 7.97 (d, *J* = 8.1 Hz, 1 H), 7.99 (d, *J* = 8.1 Hz, 1 H), 8.04 (d, *J* = 8.6 Hz, 1 H), 8.18 (s, 1 H), 8.34 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.6, 125.5, 128.0, 128.4, 129.5, 129.6, 129.7, 131.4, 131.9, 132.5, 135.9, 138.8, 166.7 ppm. HRMS (ES-TOF): *m/z* calcd. for $C_{14}H_{11}N_2O$ $[M + H]^+$: 223.0871; found 223.0872.

***N*-Pivaloylimidazole (14):** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (s, 9 H), 7.06 (d, *J* = 0.8 Hz, 1 H), 7.57 (t, *J* = 1.4 Hz, 1 H), 8.29 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4, 41.4, 117.9, 130.3, 137.7, 175.4 ppm. HRMS (ES-TOF): *m/z* calcd. for $C_8H_{13}N_2O$ $[M + H]^+$: 153.1028; found 153.1027.

***N*-(1-Adamantylcarbonyl)imidazole (15):** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.77−1.85 (m, 6 H), 2.14−2.16 (m, 9 H), 7.06 (s, 1 H), 7.64 (s, 1 H), 8.38 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3, 36.7, 39.6, 44.2, 117.9, 130.1, 137.6, 175.0 ppm. HRMS (ES-TOF): *m/z* calcd. for $C_{14}H_9N_2O$ $[M + H]^+$: 231.1497; found 231.1494.

General Procedures for Stability Studies, Demonstrated by Experiments with Compound 1

Studies in 3% (v/v) TFA in $CDCl_3$: A stock solution of **1** was prepared by dissolving the compound (14.3 mg) in $CDCl_3$ (0.5 mL), in a vial sealed with a septum. The stock solution was stored over 4 Å molecular sieves at 4 °C under nitrogen. An aliquot of the stock solution (50 μL) was withdrawn and transferred to an NMR tube, previously dried in an oven. $CDCl_3$ (450 μL) was added, and the first spectrum was recorded. Distilled TFA (15.5 μL) was rapidly added, and the tube was tightly sealed with Parafilm®. When out of the spectrometer, the sample was kept in a water bath at 25 °C.

Studies in 2% (v/v) DBU in [D_7]DMF: A stock solution of **1** was prepared by dissolving the substrate (14.3 mg) in deuterated DMF (0.5 mL), in a vial provided with a septum. The solution was stored over 4 Å molecular sieves at 4 °C under nitrogen. An aliquot of the stock solution (50 μL) was withdrawn and transferred to an NMR tube, previously dried in an oven, and deuterated DMF (450 μL) was then added. The first spectrum was recorded. DBU (10.2 μL) was then rapidly added, and the tube was tightly sealed with Parafilm®. When out of the spectrometer, the sample was kept in a water bath at 25 °C.

Studies in 2% (v/v) DBU/2% (v/v) Piperidine in [D_7]DMF: A stock solution of **1** was prepared by dissolving the substrate (14.3 mg) in deuterated DMF (0.5 mL), in a vial provided with a septum. The solution was stored over 4 Å molecular sieves at 4 °C under nitrogen. An aliquot of the stock solution (50 μL) was withdrawn and transferred to an NMR tube, previously dried in an oven. Deuterated DMF (450 μL) was added, and the first spectrum was recorded. DBU (10.2 μL) and piperidine (10.2 μL) were rapidly added, and the tube was tightly sealed with Parafilm®. When out of the spectrometer, the sample was kept in a water bath at 25 °C.

Studies in 3:1 (v/v) Aqueous Ammonia/Ethanol: Compound **1** (4.3 mg) was dissolved in a mixture of aq. ammonia and ethanol (3:1, v/v; 0.5 mL total volume) in a tightly sealed vial, and left at room temperature with occasional swirling. Aliquots of 20 μL each were withdrawn at time intervals and diluted in 50% CH_3CN in 0.1 M triethylammonium acetate at pH = 6.5 (1 mL). The pH was adjusted to 6.5 by addition of diluted acetic acid. Aliquots were analyzed by reversed-phase HPLC. The extent of substrate cleavage at a given time was determined from the ratio of products to *N*-acylimidazole. Differences in the extinction coefficients were corrected for by calibration with carboxylic acid/*N*-acylimidazole mixtures of known composition. Minor extinction coefficient differences between amide, ester, and acid were corrected by using the values obtained from benzamide, ethyl benzoate, and benzoic acid.

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