Synthesis and Reactions of a Chemical Model of the Urocanase Reaction

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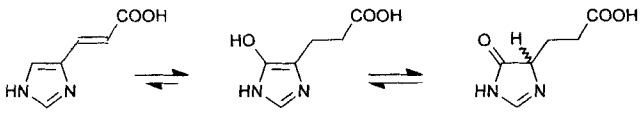
Dedicated to the memory of Wolfgang Oppolzer

Abstract: Using a convergent synthetic strategy starting from nicotinic acid and imidazole, we have prepared the (*E*) and (*Z*) isomers of 1-benzyl-3-carbamoyl-5-[2-(ethoxycarbonylmethylene)-2-(1-(*p*-tolylsulfamoyl)imidazol-4-yl)ethyl]pyridinium bromide (**21**) as models of the urocanase reaction. Domino reactions of both (*E*)-**21** and (*Z*)-**21** led to the same spirocyclic compound, (*3aRS*)-11-[9-([D_7]benzyl)-5-ethoxy-1-(*p*-tolylsulfamoyl)-1*H*,9*H*-furo[2,3-g]imidazo[5,4-f]isoquinolyl]carboxamide (**33**), which was isolated and spectroscopically characterised. A possible sequence of reactions leading to **33** shows a number of analogies to the conversions catalysed by the enzyme urocanase. Removal of the *p*-tolylsulfamoyl protecting group of (*E*)-**21** and (*Z*)-**21** under mild conditions led to the highly reactive model compounds (*E*)-**4** and (*Z*)-**4**, which were identified by ^{1}H NMR spectroscopy, but could not be isolated, owing to their instability. To facilitate the monitoring of the reaction cascade by NMR spectrocopy (*Z*)-**21** was prepared in which the benzyl group was fully deuterated. Its deprotection to (*Z*)-**4** started a reaction cascade, which led, after purification, to a main product. According to investigations by UV and ^{1}H NMR spectroscopy it seems very likely that 1-([D_7]benzyl)-3-carbamoyl-7-(ethoxycarbonylmethyl)-imidazo[4,5-f]isoquinolinium bromide (**27**) was formed. The presumed mechanism of its formation again shows similarities with the postulated mechanism of action of urocanase.

Keywords
enzyme catalysis · nicotinamide–adenine dinucleotide · reaction mechanisms · urocanase · urocanic acid

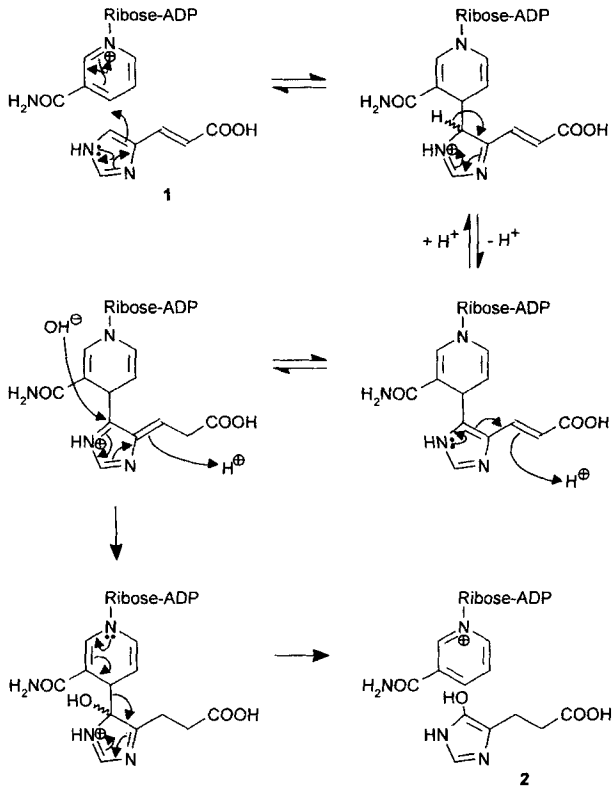
Introduction

The enzyme urocanase catalyses the second step of histidine degradation in most organisms. In this process an unusual hydration of urocanic acid (**1**) to imidazolone propionic acid (**3**) takes place (Scheme 1).



Scheme 1. Hydration of urocanic acid (**1**) to imidazolone propionic acid (**3**).

After the identification of tightly bound nicotinamide–adenine dinucleotide (NAD^+) as the catalytically essential prosthetic group of urocanase,[1] research focused on its role in the reaction mechanism. By using the competitive inhibitor imidazolylpropionate combined with specific ^{13}C labelling and NMR spectroscopy, both the structure of the NAD^+ inhibitor adduct and the mechanism of action of urocanase could be elucidated (Scheme 2).[2] The key step in the mechanism is the electrophilic



Scheme 2. Postulated mechanism of action of urocanase [2].

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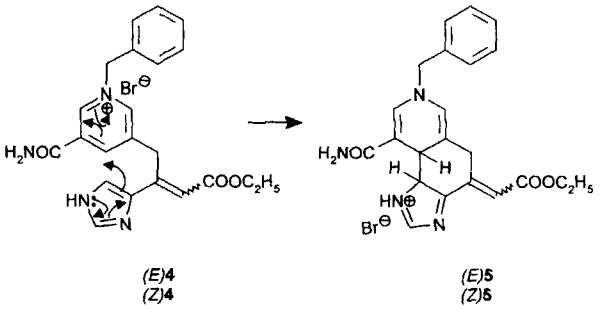
attack of the C-4 atom of pyridinium nucleus at C-5 of the imidazole ring of the substrate. This covalent bond seems to be crucial for the following proton transfers and tautomerisations, which are made possible by the correct positioning of the participating basic and acidic groups of the enzyme protein.

Although nucleophiles like cyanide are able to attack NAD^+ nonenzymically,[1] the spontaneous addition of imidazole to NAD^+, as postulated in Scheme 2, has not yet been observed. Though electrophilic substitution at the imidazole nucleus is facile, intermolecular substitution by a C-electrophile (Friedel–Crafts reaction) has, as yet, only been observed at an N but not a C position of imidazole.

To facilitate the first step in the model urocanase reaction, we devised a compound in which the two reaction partners, the pyridinium ring and the urocanic acid (or its ethyl ester), were connected by a methylene bridge (Scheme 3). Thus, in the target



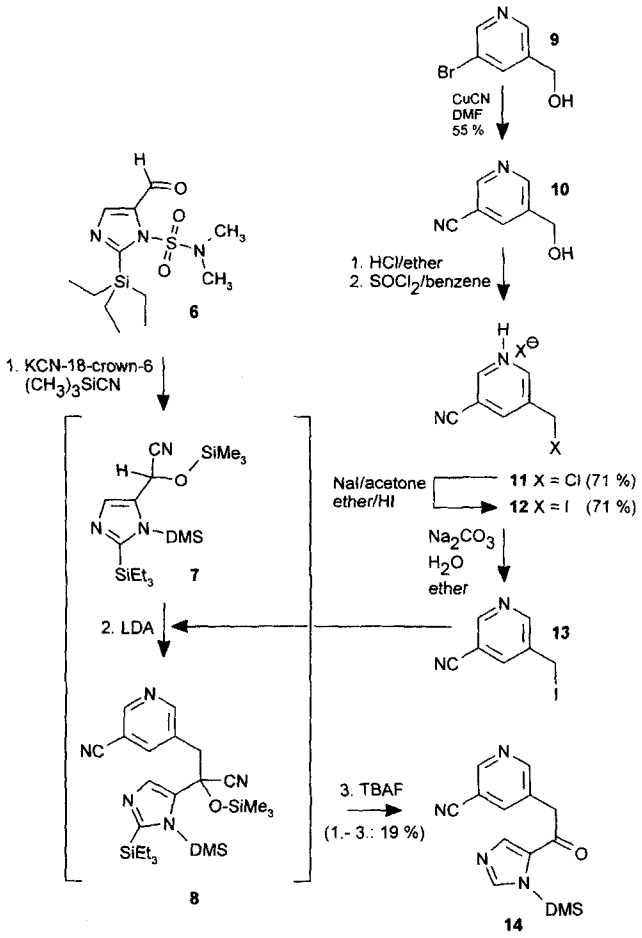
Scheme 3. Model for the urocanase reaction.

compound **4** the first step of the urocanase reaction would be entropically favoured by formation of a six-membered ring. The subsequent reactions of the presumably unstable intermediate **5** cannot be predicted because of the lack of enzymic control and the possible intervention of the methylene bridge. The introduction of an inert dimethylmethylene bridge was considered, but abandoned because of synthetic and solubility problems. A longer bridge leading to 7- or 8-membered rings would decrease the probability of an intramolecular nucleophilic attack. Also, since a free carboxylic group in the urocanate moiety led to decomposition of intermediates during the synthesis, the esterified model compound **4** was preferred.

Results and Discussion

Synthesis of the models: Retrosynthetic analysis suggested the synthesis of key intermediate **14**. This was achieved as shown in Scheme 4. Several other strategies, for example, via the labile, nonisolable (3-brompyrid-5-yl)acetaldehyde, failed.

The starting material for the imidazole moiety of **14** was the previously described[3] doubly protected imidazole carbaldehyde **6**, whose umpolung was achieved by addition of trimethylsilylcyanide followed by treatment with LDA.[4] Umpolung by other methods led only to low yields (0–5%) in the following coupling reaction.[5] The pyridine moiety of **14** was prepared for the coupling reaction starting from 3-bromo-5-(hydroxymethyl)pyridine (**9**).[6] Substitution of the bromide by

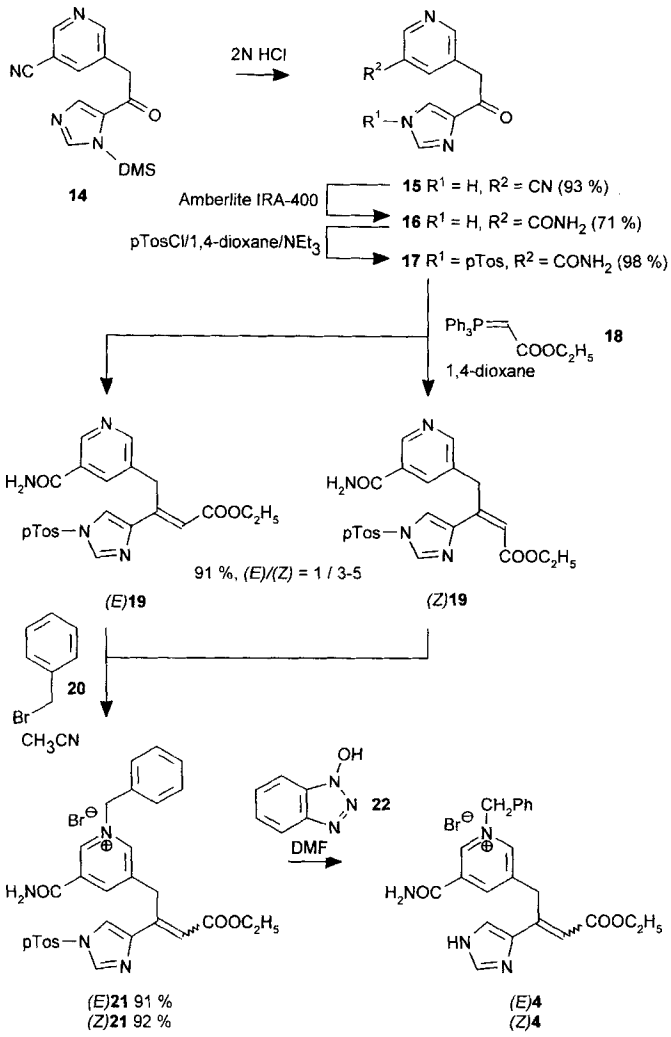


Scheme 4. Synthesis of the key intermediate **14** (DMS = *N,N*-dimethylsulfamoyl, LDA = lithium diisopropylamide, TBAF = tetrabutylammonium fluoride).

cyanide yielded the cyanopyridine **10**, which was precipitated as hydrochloride from a solution in diethyl ether. As a hydrochloride it was protected against decomposition during the following conversion of the hydroxymethyl group into chloromethyl by treatment with $SOCl_2$ to give **11**. The substitution of the chloride by iodide to yield **12** was carried out with NaI in acetone (**warning**: both the hydrohalides and the free unstable bases are extremely aggressive and cause irritation and severe damage upon skin contact).

The unstable and highly reactive base was set free from **12** and treated in situ with anion **7**, prepared from **6** by addition of trimethylsilylcyanide and LDA. The key intermediate **14** was thus obtained in 19% yield. This unusual experimental procedure had to be strictly observed because 3-(iodomethyl)-5-cyanopyridine (**13**) is only stable in diethyl ether for a short time. Cooling is not possible, since the compound crystallises, and attempts to redissolve it lead to decomposition.

Removal of the sulfamoyl protecting group from **14** by treatment with 2N HCl[7] afforded crystals of **15** (Scheme 5). Exploratory experiments suggested that the cyano group should be converted into an amide at this early stage. This was achieved by heating in water in the presence of Amberlite IRA-400 as catalyst.[8] Both **15** and **16** are insoluble in most solvents. The N_γ imidazole atom of **16** was protected by a *p*-tosyl group, and the resulting ketone **17** converted with (triphenylphosphoranyl-



Scheme 5. Synthesis of the model compounds **21** und **4** (DMS = *N*,*N*-dimethylsulfamoyl).

idene)acetic acid ethyl ester (**18**) into the corresponding olefin **19**. 1,4-Dioxane was the only solvent in which the desired products were obtained. The olefin **19** could be separated into its (*E*) and (*Z*) isomers [(*E*)/(*Z*) ≈ 1:4]. The configurations were assigned by means of NOE measurements. Attempts to isomerise (*Z*)-**19** to (*E*)-**19** have not yet been successful.

The diastereomers (*Z*)-**19** and (*E*)-**19** were each treated with unlabelled and [D_7]benzylbromide (**20**) leading to quaternisation of the pyridine moiety.[9] The resulting pyridinium salts (*Z*)-**21** and (*E*)-**21** are the protected forms of the target model compounds for the urocanase reaction. The mildest method for removing the *p*-tolylsulfamoyl protecting group turned out to be reaction with 1-hydroxybenzotriazole (**22**).[10] The deprotection reactions were monitored by NMR spectroscopy; this showed that the model compounds (*Z*)-**4** und (*E*)-**4** are most stable in dry dimethylformamide. Even so, significant decomposition occurred after 7 h at room temperature. In order to facilitate the interpretation of the ^{1}H NMR spectra the benzyl substituent at the pyridine nitrogen was fully deuterated, and the reagent **22** applied in moderate (less than optimum) excess.

Both the NMR and the preparative experiments showed a quantitative formation of the model compounds (*Z*)-**4** und (*E*)-**4** in pure form. However, they were so unstable that attempts to separate them from the excess reagent **22** and its *p*-tosyl derivative failed. We were therefore unable to fully characterise (*Z*)-**4** and (*E*)-**4** and concentrated our efforts on the isolation of the secondary products formed during the purification experiments.

Reactions of the unprotected model compounds: The eluent, the packing material and the temperature were found to be decisive in determining the number of secondary products that were formed during column chromatography. In the presence of even small amounts of water, the complexity of the product mixture increased dramatically. The best results were achieved by using dry, heat-treated (120 °C), neutral aluminium oxide as packing material and acetonitrile/methanol (8/1, *v*/*v*; dried over molecular sieves, 3 Å) as eluent. Nevertheless, the ratio of the two main compounds varied; this was useful for the assignment of the ^{1}H NMR signals (vide infra). The product mixture was examined by UV spectroscopy and, after lyophilisation and dissolution in [D_7]dimethylformamide to slow down further decomposition, by NMR spectroscopy.

The ^{1}H NMR signals corresponding to (*Z*)-**4** were assigned by conducting several experiments in which the relative amounts of the products differed. The signals whose relative integrals remained constant clearly belonged to the same product. The ^{13}C NMR signals could not be interpreted in the same way. Analysis of the products by NMR and UV spectroscopy indicated the presence of isoquinolinium salt **27**, in addition to the model compound (*Z*)-**4** (Scheme 6).

The first steps of the model reaction are analogous to those catalysed by urocanase. Addition of imidazole to C-4 of the pyridinium moiety is followed by abstraction of the now activated 5′ proton and tautomerisation induced by protonation of the exocyclic double bond. As a consequence, an umpolung of the imidazole ring takes place, which might react further in either of two ways:

a) A proton could be abstracted from the methylene bridge, with the imidazolium ion operating as an electron sink.
b) Addition of a methanolate ion (from the solvent) to the 5′ position could be followed by a 1,4-elimination.

Both pathways lead to the 1,4-dihydroisoquinoline derivative **25**, which yields, after oxidation by air, the observed isoquinolinium compound **27**. So as to provide a complete analogy with the urocanase reaction, we attempted to prevent the spontaneous oxidation by using rigorous anaerobic conditions (under argon, including during workup), but to no avail. The step that would have completed the urocanase model reaction, pathway c, involving cleavage of the bond between the pyridine and imidazole rings, could not be observed for obvious reasons.

Unfortunately, a complete characterisation of the isoquinolinium salt **27** could not be achieved, since its instability precluded its isolation as a pure compound. However, its NMR and UV spectra strongly support the postulated structure and hence the mechanistic pathway leading to it.

Reactions of the protected model compounds: In the hope of finding a compound whose reactions would more completely model the urocanase reaction, we turned to the more stable model compounds (*E*)-**21** and (*Z*)-**21**. Heating them in an aqueous buffer (pH = 7.3, K_2HPO_4/KH_2PO_4) to 90 and 75 °C,

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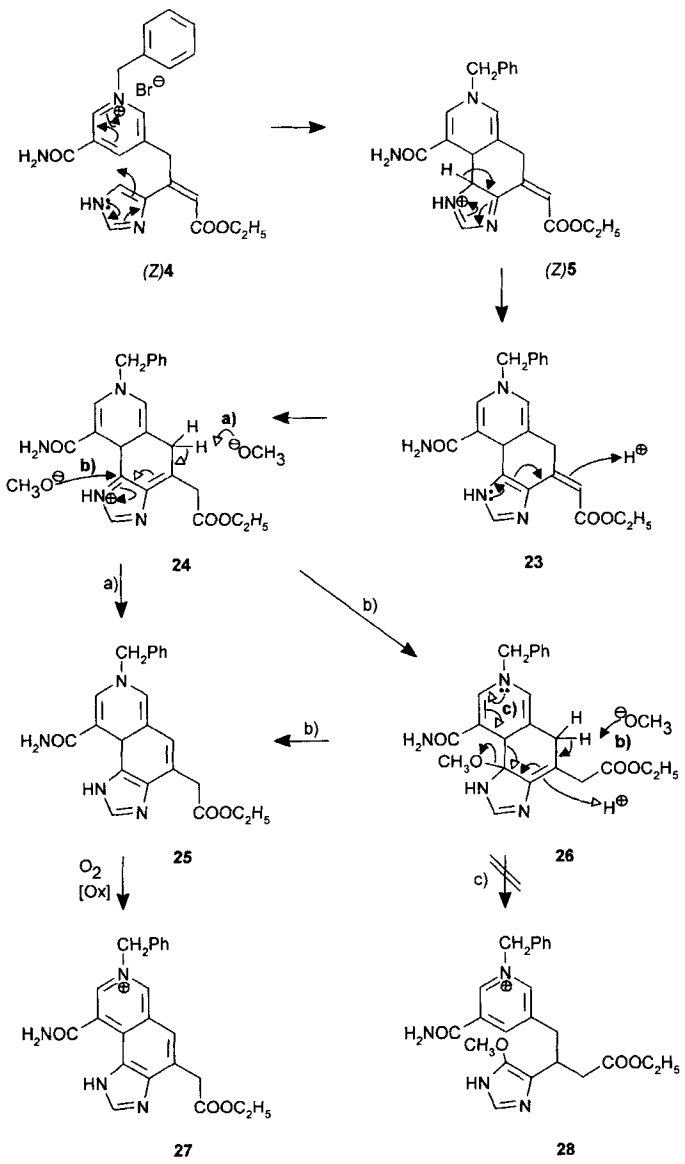
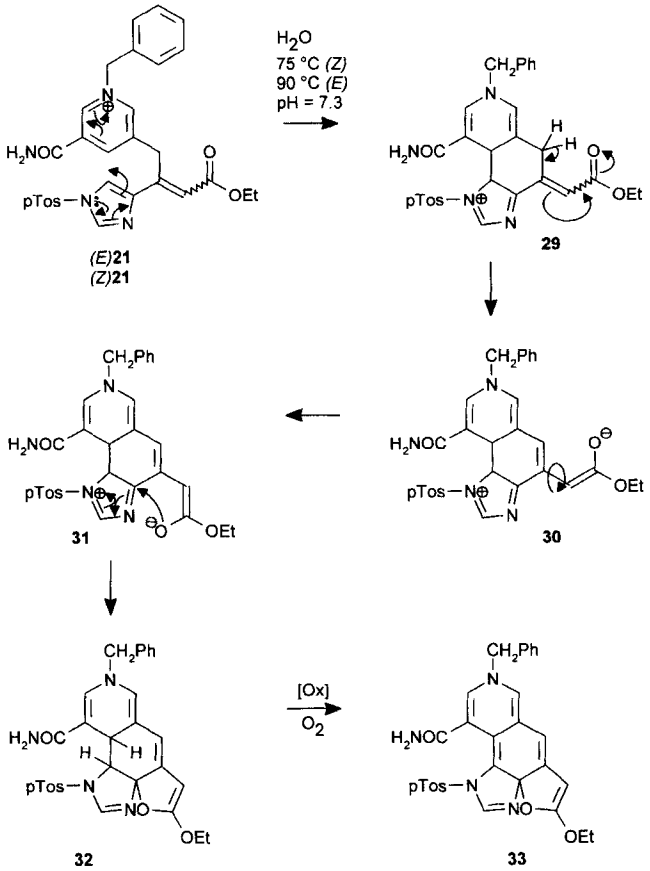


Scheme 6. Probable mechanism for the formation of **27** from (*Z*)-**4**. a), b) and c), see comments in text.

respectively, led in both cases to an orange, water-insoluble compound, which could be isolated in crystalline form. Its NMR, UV/Vis and mass spectra identified it as the spiro compound **33**. A plausible mechanism for its formation is delineated in Scheme 7.

As in the reaction of the unprotected model compound, the first step is again analogous to that in the urocanase reaction. This is followed by a vinylogous enolisation of the ester function. The enolate is set up for an intramolecular nucleophilic attack at the C-4′ atom of the imidazolium ion. This step resembles the attack by the OH^- ion at the imidazole 5′ position in the course of the urocanase reaction. In both cases an umpolung of the imidazole ring is followed by the attack of an O nucleophile. The model reaction and enzymic one differ, however, in their regiospecificity (C-4′ vs. C-5′ attack). The final oxidation probably occurred during workup, since the reaction was conducted under argon. A precedence for the formation of spirocyclic product **33** has been reported,[11] although the spirocentre was at C-2 rather than C-4 of the imidazole ring.



Scheme 7. Probable mechanism for the formation of the spiro compound **33**.

Conclusion

Some features of the urocanase reaction could be modelled by our synthetic system. By inserting a methylene bridge so as to favour the intramolecular first steps, we introduced an additional base-sensitive site. This interfered with the tautomerisations, so that the postulated steps of the enzymic reaction could not be reproduced in the correct sequence or regiospecificity. However, the enzyme should easily be able to control regiospecificity. The key step of the urocanase reaction, the electrophilic attack of the pyridinium ion at the imidazole 5′ position, was successfully modelled in the present system.

Experimental Section

General: Melting points were determined in open capillaries using a Büchi apparatus according to Dr. Tottoli or a Büchi 535 apparatus, and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker WH250, AM250, AM400 or DRX500 spectrometers at room temperature. Novel compounds were also characterised by their DEPT and CH correlation spectra. IR spectra were recorded on Beckman Aculab 8 or Bruker IFS88 spectrometers. Mass spectra and high-resolution mass spectra (HRMS) were measured on Varian MAT711 or Finnigan MAT90 spectrometers. UV/Vis spectra were recorded using a Perkin-Elmer Lambda 2 spectrometer. Glass apparatus were heated (120 °C) before use.

Materials: Solvents were dried by standard methods and stored over molecular sieves.[12] The concentrations of the commercial solutions of organolithium compounds were determined by titration with diphenylacetic acid. For column chromatography [height (cm) × inside diameter (cm)] silica gel (30–

60 μm = I) or (63–200 μm = II) was used. Commercial reagents were used without further purification. When necessary, reactions were conducted under argon or nitrogen. 3-Bromo-5-(hydroxymethyl)pyridine (**9**) and 1-(*N*,*N*-dimethylsulfamoyl)-2-(triethylsilyl)imidazole-5-carbaldehyde (**6**) were prepared according to ref. [6] and [3], respectively, and **6** was additionally purified by column chromatography (60 × 4.5, I, ethylacetate/cyclohexane 1/1 (*v*/*v*), $R_f = 0.73$). KCN/[18]crown-6 was prepared as described in ref. [13].

3-(Hydroxymethyl)-5-cyanopyridine (10): 3-Bromo-5-(hydroxymethyl)pyridine (**9**) (35 g, 186.2 mmol) and CuCN (41.65 g, 449.2 mmol) in abs. DMF (175 mL) were heated at 170 °C with stirring for *exactly* 105 min. The mixture was then cooled as quickly as possible to 50 °C or less. After addition of aqueous ammonia (25%, 70 mL) and a saturated solution of NH_4Cl (210 mL) stirring was continued for 1 h. This was followed by addition of chloroform (350 mL) and stirring for another hour. The precipitated salt was then filtered off under suction and the filter cake washed with chloroform. After separation of the layers the aqueous layer was extracted with chloroform (5 × 100 mL) and the combined organic layers dried over $MgSO_4$. Removal of the solvent gave a dark red oil, which was dried in vacuo. The resulting brown solid was heated with diethyl ether (400 mL) under reflux, and the hot solvent decanted. The solid residue was extracted with diethyl ether (5 × 50 mL, boiling for 30 min under reflux) and decanted extracts were collected separately. Upon cooling the diethyl ether extracts to −28 °C crystals appeared and were collected and dried. From the mother liquors a further crop of crystals could be obtained. Yield: 13.8 g (55%), colourless or light orange crystals: m.p. 80–82 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.73$ (chemical shift varied, s, 1H, -OH), 4.82 (s, 2H, $-CH_2-$), 8.06 (s, 1H, H-4), 8.77 (m, 2H, H-6/2); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 61.19$ ($-CH_2-$), 109.88 (-CN), 116.49 (C-5), 137.43 (C-3), 137.71 (C-4), 150.95 (C-6), 151.31 (C-2); IR (KBr): $\tilde{\nu} = 2235\ cm^{-1}$ (-CN); MS (70 eV, EI): *m*/*z* (%): 134.1 (100) [M^+]; HRMS: calculated for $C_7H_6N_2O$ 134.0480; found 134.0469.

3-(Chloromethyl)-5-cyanopyridinium hydrochloride (11): Dry gaseous HCl was introduced at room temperature into a solution of **10** (24 g, 178.8 mmol) in abs. diethyl ether (1800 mL) until saturation was reached (≈10 min), and the hydrochloride of **10** precipitated. After removal of the solvent the stirred crystalline residue was treated with $SOCl_2$ (120 mL), which had previously been cooled to −28 °C. Once the reaction mixture had reached room temperature and *all* the material had dissolved, it was heated under reflux for 2 h. The solution was then cooled to 0 °C and treated with dry benzene (810 mL). The precipitate was filtered off under suction and dried in vacuo. Yield: 24.0 g (71%), yellowish crystals: m.p. 133–135 °C; recrystallisation from abs. ethanol at 2 °C afforded colourless or light yellow crystals: m.p. 140–142 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 4.88$ (s, 2H, $-CH_2-$), 8.43 (t, $J = 1.4$ Hz, 1H, H-4), 8.95 (d, $J = 1.4$ Hz, 1H, H-6), 9.00 (d, $J = 1.4$ Hz, 1H, H-2), 9.59 (br, 1H, N-H); ^{13}C NMR (62.5 MHz, [D$_6$]DMSO): $\delta = 41.71$ ($-CH_2-$), 109.27 (-CN), 116.25 (C-5), 134.22 (C-3), 140.47 (C-4), 151.40 (C-6), 152.69 (C-2); IR (KBr): $\tilde{\nu} = 2248\ cm^{-1}$ (-CN); MS (70 eV, EI): *m*/*z* (%): 152.0 (45) [$C_7H_5ClN_2^+$], 117.1 (100); HRMS: calculated for $C_7H_5ClN_2$ 152.014; found 152.0124.

3-(Iodomethyl)-5-cyanopyridinium hydroiodide (12) (warning: extreme irritant): NaI (38.55 g, 257.2 mmol; dried at 105 °C in vacuo for 10 h) was dissolved at room temperature under nitrogen in abs. acetone (375 mL). 3-(Chloromethyl)-5-cyanopyridinium hydrochloride (**11**) (15 g, 79.3 mmol) was added to this solution in one portion with stirring and in the dark. Stirring was then continued for 14 h in the dark. The resulting suspension was rapidly filtered under suction and added with stirring to a mixture of Na_2CO_3 (19.5 g, 184 mmol) in water (187.5 mL) and diethyl ether (187.5 mL). After separation of the organic layer, the aqueous phase was extracted with diethyl ether (4 × 150 mL). The combined organic layers were dried over Na_2SO_4 (max. for 1 min). The solution was filtered, and an aqueous solution of HI (57%, 11.55 mL, 87.2 mmol) added with stirring. The yellow precipitate **12** was isolated by filtration under suction and dried *in the dark* in vacuo. As long as it is not exposed to moisture, dry **12** is not sensitive to light. Yield: 20.9 g (71%), yellowish powder: m.p. 191–193 °C; IR (KBr): $\tilde{\nu} = 2248\ cm^{-1}$ (-CN); MS (70 eV, EI): *m*/*z* (%): 244.1 (12) [$C_7H_5IN_2^+$], 117.2 (100); HRMS: calculated for $C_7H_5IN_2$ 243.9496; found 243.9509. Owing to the instability of **12**, NMR measurements in polar solvents were only feasible for 3-(iodomethyl)-5-cyanopyridine. This was generated by adding **12** (372 mg, 1 mmol) to a stirred mixture of Na_2CO_3 (159 mg) in water (2 mL) and $CDCl_3$ (1 mL). The chloroform layer was separated, dried over Na_2SO_4 for 2 min and filtered. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 4.37$ (s, 2H, $-CH_2-$), 7.92 (t, $J = 1.4$ Hz, 1H, H-4), 8.68 (d, $J = 1.4$ Hz, 1H, H-6), 8.75 (d, $J = 1.4$ Hz, 1H, H-2); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = -2.10$ ($-CH_2-$), 109.98 (-CN), 116.02 (C-5), 133.73 (C-3), 139.21 (C-4), 151.09 (C-6), 152.68 (C-2).

[1-(*N*,*N*-Dimethylsulfamoyl)imidazol-5-yl] [(3-cyanopyrid-5-yl)methyl] ketone (14): For coupling the pyridine and imidazole moieties two reagents had to be prepared separately.

Solution A—3-(iodomethyl)-5-cyanopyridine in diethyl ether: Compound **12** (6.5464 g, 17.6 mmol) was added to a vigorously stirred mixture of Na_2CO_3 (3.73 g, 35.2 mmol) in water (60 mL) and diethyl ether (60 mL). After separation, the aqueous layer was extracted with diethyl ether (4 × 60 mL), and the combined organic layers were dried over Na_2SO_4 (1 min). Molecular sieves (4 Å) were added to the filtered solution, and the mixture stirred very slowly in the dark for 22 h.

Solution B—TBAF/THF: Tetrabutylammonium fluoride trihydrate (TBAF, 12.62 g, 40 mmol) was dissolved in abs. THF (50 mL) under argon.

Aldehyde **6** (5.08 g, 16 mmol) and KCN/[18]crown-6 (120 mg) were added to a flask under argon. Trimethylsilylcyanide (2.25 mL, 16.8 mmol) was added to the stirred mixture, which was cooled so that the reaction temperature did not exceed 25 °C. After 15 min abs. THF (16 mL) was added, and the mixture cooled to −78 °C. Lithium diisopropylamide in THF (LDA, 16 mmol) was added dropwise by means of a syringe at a rate that allowed the temperature to be maintained below −70 °C. Solution A was then added as fast as possible while keeping the temperature between −75 and −80 °C by cooling with liquid nitrogen. The mixture was then stirred for a further 15 min at −78 °C, before being allowed to warm up to room temperature. Solution B was added 1 h after removal of the cold bath, and the suspension stirred for 2 h at room temperature. Water (120 mL) was then added over 5 min with stirring. Separation of the organic layer was followed by extraction of the aqueous phase with diethyl ether (1 × 125 mL and 4 × 50 mL). The combined organic layers were washed with a saturated solution of NaCl (25 mL) and dried over Na_2SO_4. Filtration and removal of the solvent led to the isolation of a dark oil, which was dried in vacuo. After addition of abs. THF (5 mL) and diethyl ether (25 mL) the solution was concentrated at 40 °C and 800 mbar until dark crystals appeared. The mixture was stored overnight at −28 °C and then the mother liquor removed by decantation. The solid residue was recrystallised twice from *n*-hexane/acetone by cooling to −28 °C. The combined mother liquors were purified by flash column chromatography [75 × 8, II, acetone/chloroform 2/1 (*v*/*v*), $R_f = 0.69$]. Yield: 1.0 g (19%), colourless crystals: m.p. 110–111 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.05$ (s, 6H, $N-CH_3$), 4.24 (s, 2H, $-CH_2-$), 7.94 (t, $J = 1.2$ Hz, 1H, Py H-4), 7.97 (s, 1H, Im H-4), 8.25 (s, 1H, Im H-2), 8.72 (d, $J = 1.2$ Hz, 1H, Py H-2), 8.83 (d, $J = 1.2$ Hz, 1H, Py H-6); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 38.80$ ($N-CH_3$), 42.58 ($-CH_2-$), 109.90 (-CN), 116.41 (Py C-5), 129.69 (Im C-4), 130.38 (Py C-3), 140.54 (Py C-4), 140.59 (Im C-5), 145.97 (Im C-2), 151.17 (Py C-2), 154.04 (Py C-6), 183.35 (C=O); IR (KBr): $\tilde{\nu} = 2236\ cm^{-1}$ (-CN), 1687 (C=O); MS (70 eV, EI): *m*/*z* (%): 319.1 (11) [M^+], 202.0 (100); HRMS: calculated for $C_{13}H_{13}N_5O_3S$ 319.0739; found 319.0715.

[Imidazol-4-yl] [(3-cyanopyrid-5-yl)methyl] ketone (15): Ketone **14** (3.79 g, 11,9 mmol) was added to 2N HCl (200 mL) and stirred for 1.5 h at room temperature. The pH of the mixture was adjusted to 10 with 5N NaOH. After stirring for a short time, a flocky precipitate appeared. The pH was subsequently brought to 6 by addition of 2N HCl, and the solution maintained at 2 °C for *at least* 48 h. The precipitate was then filtered by suction and dried in vacuo. The solid was recrystallised and again stored at 2 °C for *at least* 48 h. The precipitate was filtered by suction and again dried in vacuo. Recrystallisation of the mother liquor from water yielded some more crystals. Total yield: 2.3 g (93%), white powder or colourless, glossy leaflets: m.p. 231–232 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 4.41$ (s, 2H, $-CH_2-$), 7.89 (s, 1H, Im H-2), 8.03 (s, 1H, Im H-5), 8.22 (t, $J = 1$ Hz, 1H, Py H-4), 8.78 (d, $J = 1$ Hz, 1H, Py H-2), 8.91 (d, $J = 1$ Hz, 1H, Py H-6), 12.91 (br, 1H, Im N-H); ^{13}C NMR (62.5 MHz, [D$_6$]DMSO): The imidazole C-4 signal is not discernible, owing to its relaxation on the NMR timescale, $\delta = 41.32$ ($-CH_2-$), 108.51 (-CN), 116.88 (Im C-5), 125.07 (Py C-3), 131.75 (Py C-5), 137.70 (Im C-2), 140.75 (Py C-4), 150.28 (Py C-6), 154.45 (Py C-2), 189.80 (C=O); IR (KBr): $\tilde{\nu} = 2239\ cm^{-1}$ (-CN), 1721 (C=O); MS (70 eV, EI): *m*/*z* (%): 212.1 (12) [M^+], 95.0 (100); HRMS: calculated for $C_{11}H_8N_4O$ 212.0698; found 212.0675.

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[Imidazol-4-yl] [(3-carbamoylpyrid-5-yl)methyl] ketone (16): Amberlite IRA-400 (991 mg) was stirred in 2N NaOH (50 mL) for 10 min under nitrogen. The colourless resin was then repeatedly washed with CO_2-free water (10 mL each time) until the eluted water was neutral (pH ≈ 5–6). CO_2-free water (100 mL) and ketone **15** (0.95 g, 4.51 mmol) were added to the washed resin. The mixture was then heated with an oil bath (110 °C) for 2 h under reflux. The resin was subsequently removed by filtration and washed with hot CO_2-free water. The combined filtrates were concentrated and further dried in vacuo. The residue was recrystallised from dry methanol at −28 °C. Yield: 838 mg (71%), **16**·MeOH, white powder: m.p. 231–232 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.21 (s, 3H, CH_3OH), 4.33 (s, 2H, -CH_2-), 7.57 (br, 1H, -(C=O)-NH), 7.88 (s, 1H, Im H-2), 8.02 (s, 1H, Im H-5), 8.12 (t, J = 1 Hz, 1H, Py H-4), 8.15 (br, 1H, -(C=O)-NH), 8.62 (d, J = 1 Hz, 1H, Py H-2), 8.92 (d, J = 1 Hz, 1H, Py H-6), 12.79 (br, 1H, Im N-H); ^{13}C NMR (62.5 MHz, [D$_6$]DMSO): The imidazole C-4 signal is not discernible, owing to its relaxation on the NMR timescale, δ = 41.75 (-CH_2), 48.63 (CH_3OH), 126.38 (Im C-5), 129.26 (Py C-5), 130.87 (Py C-3), 136.86 (Py C-4), 137.87 (Im C-2), 146.70 (Py C-2), 152.87 (Py C-6), 166.54 (-(C=O)-NH_2), 190.28 (C=O); IR (KBr): $\tilde{\nu}$ = 1683 cm^{-1} (C=O); MS (70 eV, EI): m/z (%): 230.1 (18) [M^+], 95.0 (100); HRMS: calculated for $C_{11}H_{10}N_4O_2$ 230.0804; found 230.0818.

[1-(*p*-Tolylsulfamoyl)imidazol-4-yl] [(3-carbamoylpyrid-5-yl)methyl] ketone (17): Well-dried, finely powered **16**·MeOH (1 g, 3.81 mmol) was placed in a flask under argon. Abs. 1,4-dioxane (205 mL) was added, followed by triethylamine (6.05 mL) and then *p*-toluenesulfonyl chloride (2.47 g, 13.0 mmol) in one portion. The resulting mixture was stirred for 4–6 h at room temperature. Subsequently the *entire* reaction mixture was transferred to a chromatography column (24 × 8, II, acetone/ chloroform 2/1 (v/v), R_f = 0.33). The fractions containing **17** were collected and concentrated, and the residue was dried in vacuo. Yield: 1.4 g (98%), yellowish plates which, owing to their insolubility in most solvents, were directly used in the next step. A small sample was submitted to a tedious purification procedure (three recrystallisations from ethanol, column chromatography) which yielded a white powder: m.p. 148–149 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, * = Py H-4 and/or Im H-2 and/or Im H-5): δ = 2.43 (s, 3H, -CH_3), 4.38 (s, 2H, -CH_2), 7.54 (d, J = 8.3 Hz, 2H, tolyl 2,6-H), 7.60 (br, 1H, -NH), 8.10 (s, 1H, *), 8.10 (d, J = 8.3 Hz, 2H, tolyl 3,5-H), 8.15 (s, 1H, -NH), 8.57 (m, 2H, *), 8.66 (d, J = 1.2 Hz, 1H, Py H-6), 8.93 (d, J = 1.9 Hz, 1H, Py H-2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 21.15 (-CH_3), 42.00 (-CH_2-), 122.67 (Im C-5), 127.75 (tolyl 2,6-C–H), 129.27 (Py C-5), 130.16 (Py C-3), 130.66 (tolyl 3,5-C–H), 133.40 (Im C-4), 136.72 (Py C-4), 137.62 (Im C-2), 141.75 (tolyl *C*-CH_3), 146.57 (Py C-2), 147.07 (tolyl *C*-SO_2-), 152.82 (Py C-6), 166.26 (-(C=O)-NH_2), 191.19 (C=O); IR (KBr): $\tilde{\nu}$ = 1698 cm^{-1} (C=O); MS (FAB, glycerol, CsCl): m/z (%): 385 (56) [M^+], 93.0 (100); HRMS: calculated for $C_{18}H_{17}N_4O_4S$ 385.0971; found 385.0871.

Ethyl 3,3-[1-(*p*-tolylsulfamoyl)imidazol-4-yl][(3-carbamoylpyrid-5-yl)methyl]-acrylate [(*E*)-**19** and (*Z*)-**19**]: Ketone **17** (1.26 g, 2.97 mmol) and (triphenylphosphoranylidene)acetic acid ethyl ester (**18**) (4.58 g, 13.15 mmol) were placed in a flask, and, *immediately* after addition of 1,4-dioxane (107 mL), heated under reflux (temperature of the preheated oil bath was 110 °C) for 3 h. The oil bath was removed, and after 10 min the cooled mixture was treated with water (107 mL) and chloroform (231 mL) and vigorously stirred. After separation of the layers the aqeous phase was extracted with chloroform (5 × 70 mL). The combined organic layers were dried over Na_2SO_4 and the filtrate concentrated. The residue was dried in vacuo and purified by flash column chromatography (59 × 4, II, acetone/chloroform 1/1 (v/v); (*E*)-**19**: R_f = 0.59, (*Z*)-**19**: R_f = 0.51), 1.2 g (91%, (*E*):(*Z*) ≈ 1:3–5). The chromatography fraction containing (*E*)-**19** was recrystallised from acetone/chloroform 1/1 (v/v) at −28 °C to give a white, fine powder: m.p. 181–182 °C. The chromatography fraction containing (*Z*)-**19** was concentrated, and the solid washed with acetonitrile (1 mL) until the washings appeared colourless. The residue was dried in vacuo to give a white, fine powder: m.p. 147 °C.

(*E*)-**19**: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.30 (t, J = 7.1 Hz, 3H, -CH_2-CH_3), 2.44 (s, 3H, tolyl CH_3), 4.20 (q, J = 7.1 Hz, 2H, -CH_2-CH_3), 4.43 (s, 2H, Py-CH_2-), 5.88 (br, 1H, -(C=O)-NH), 6.23 (s, 2H, -(C=O)-NH), 6.76 (s, 1H, α-H), 7.35 (d, J = 8.5 Hz, 2H, tolyl 2,6-H), 7.37 (s, 1H, Im H-5), 7.78 (d, J = 8.5 Hz, 2H, tolyl 3,5-H), 7.94 (s, 1H, Im H-2), 8.01 (d, J = 1 Hz, 1H, Py H-4), 8.65 (d, J = 1 Hz, 1H, Py H-6), 8.82 (d, J = 1 Hz, 1H, Py H-2); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 14.19 (-CH_2-CH_3), 21.75 (tolyl CH_3), 31.50 (Py-CH_2-), 60.37 (-CH_2-CH_3), 116.00 (Im C-5), 117.84 (α-C), 127.40 (tolyl 2,6-C–H), 128.77 (*C*=C-(C=O)-), 130.64 (tolyl 3,5-C–H), 134.26 (Im C-4), 134.66 (Py C-3), 135.13 (Im C-2), 136.86 (Py C-4), 143.24 (tolyl *C*-CH_3), 145.25 (Py C-5), 146.32 (Py C-2), 146.83 (tolyl *C*-SO_2-), 152.85 (Py C-6), 166.40 (-(C=O)-O-), 167.38 (-(C=O)-NH); MS (70 eV, EI): m/z (%): 454.1 (1) [M^+], 91.1 (100); HRMS: calculated for $C_{22}H_{22}N_4O_5S$ 454.1311; found 454.1324.

(*Z*)-**19**: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.25 (t, J = 7.1 Hz, 3H, -CH_2-CH_3), 2.41 (s, 3H, tolyl CH_3), 4.00 (s, 2H, Py-CH_2-), 4.15 (q, J = 7.1 Hz, 2H, -CH_2-CH_3), 5.87 (s, 1H, α-H), 6.45 (br, 1H, -(C=O)-NH), 6.73 (br, 1H, -(C=O)-NH), 7.35 (d, J = 8.5 Hz, 2H, tolyl 2,6-H), 7.84 (d, J = 8.5 Hz, 2H, tolyl 3,5-H), 7.93 (s, 1H, Im H-2), 8.02 (t, J = 1 Hz, 1H, Py H-4), 8.33 (s, 1H, Im H-5), 8.60 (d, J = 1 Hz, 1H, Py H-6), 8.85 (d, J = 1 Hz, 1H, Py H-2); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 14.13 (-CH_2-CH_3), 21.74 (tolyl CH_3), 39.98 (Py-CH_2-), 60.46 (-CH_2-CH_3), 119.12 (α-C), 120.99 (Im C-5), 127.49 (tolyl 2,6-C–H), 128.95 (*C*=C-(C=O)-), 130.52 (tolyl 3,5-C–H), 134.52 (Py C-3 + Im C-4), 135.08 (Im C-2), 136.07 (Py C-4), 138.69 (tolyl *C*-CH_3), 144.67 (tolyl *C*-SO_2-), 146.47 (Py C-5), 146.56 (Py C-2), 153.19 (Py C-6), 165.69 (-(C=O)-O-), 167.63 (-(C=O)-NH); IR (KBr): $\tilde{\nu}$ = 1698 cm^{-1} (C=O); MS (70 eV, EI): m/z (%): 454.2 (0.4) [M^+], 91.1 (100); HRMS: calculated for $C_{22}H_{22}N_4O_5S$ 454.1311; found 454.1335.

1-Benzyl-3-carbamoyl-5-[2-((*Z*)-ethoxycarbonylmethylene)-2-(1-(*p*-tolylsulfamoyl)imidazol-4-yl)ethyl]pyridinium bromide [(*Z*)-21]: (*Z*)-**19** (266.9 mg, 587.2 μmol) was dissolved in as little acetonitrile as possible by refluxing (oil bath temperature, 88 °C) under argon. Then either unlabelled or [D_7]benzylbromide (**20**) (76.5 μL, 645.9 μmol) were added. The mixture was heated under reflux, and after about 5 min a precipitate appeared. After 30 min the reaction was cooled in a water bath. Abs. diethyl ether was added, and the mixture filtered with suction. After washing with abs. diethyl ether (10 mL) the product was dried in vacuo, and then thoroughly washed with abs. acetone (max. 1.5 mL for each wash) until the washings appeared colourless. The product was finally dried in vacuo. Yield: 336 mg (92%), white powder: m.p. 177–178 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, J = 7.1 Hz, 3H, -CH_2-CH_3), 2.41 (s, 3H, tolyl CH_3), 4.09 (q, J = 7.1 Hz, 2H, -CH_2-CH_3), 4.16 (s, 2H, Py-CH_2-), 5.87 (s, 2H, N-CH_2-), 6.13 (s, 1H, α-H), 7.38–7.57 (m, 5H, Ph-H), 7.50 (d, J = 8.5 Hz, 2H, tolyl 2,6-H), 7.96 (d, J = 8.5 Hz, 2H, tolyl 3,5-H), 8.15 (s, 1H, -(C=O)-NH), 8.32 (s, 2H, Im H-2 + Im H-5), 8.54 (s, 1H, -(C=O)-NH), 8.85 (s, 1H, Py H-4), 9.27 (s, 1H, Py H-6), 9.52 (s, 1H, Py H-2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 13.88 (-CH_2-CH_3), 21.18 (tolyl CH_3), 38.15 (-CH_2-CH_3), 60.05 (Py-CH_2-), 63.41 (N-CH_2-), 119.80 (Im C-5), 120.42 (α-C), 127.33 (tolyl 2,6-C–H), 128.86, 129.11, 129.37 (Ph C-H), 130.69 (tolyl 3,5-C–H), 133.58 (Im C-4), 133.73 (Py C-5), 133.93 (Py-C-3), 136.30 (Im C-2), 138.20 (*C*=C-(C=O)-), 140.04 ((C$_5$H$_5$)-*C*-CH_2-), 141.25 (tolyl *C*-CH_3-), 142.81 (Py C-2), 144.02 (Py C-4), 146.28 (Py C-6), 146.81 (tolyl *C*-SO_2-), 162.48 (-(C=O)-NH), 165.45 (-(C=O)-O-); MS (FAB, glycerol): m/z (%): 545 (100) [$C_{29}H_{29}N_4O_5S^+$]; HRMS: calculated for $C_{29}H_{29}N_4O_5S$ 545.1859; found 545.1801.

1-Benzyl-3-carbamoyl-5-[2-((*E*)-ethoxycarbonylmethylene)-2-(1-(*p*-tolylsulfamoyl)imidazol-4-yl)ethyl]pyridinium bromide [(*E*)-21]: Starting from [(*E*)-**19**] (266.9 mg, 587.2 μmol), the same procedure was applied as described for the (*Z*) diastereomer with the following changes: abs. acetonitrile (25 mL), unlabelled or [D_7]benzylbromide (**20**) (133 μL, 1.29 mmol). The mixture was refluxed for 1 h. After about 50 min a precipitate appeared. Yield: 333 mg (91%), white powder: m.p. 195–196 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.20 (t, J = 1 Hz, 3H, -CH_2-CH_3), 2.41 (s, 3H, tolyl CH_3), 4.11 (q, J = 1 Hz, 2H, -CH_2-CH_3), 4.53 (s, 2H, Py-CH_2-), 5.88 (s, 2H, Ph-CH_2-), 6.83 (s, 1H, α-H), 7.38–7.58 (m, 5H, Ph), 7.49 (d, J = 8.2 Hz, 2H, tolyl 2,6-H), 7.98 (d, J = 8.2 Hz, 2H, tolyl 3,5-H), 8.19 (s, 1H, -(C=O)-NH), 8.44, 8.45 (s, 2H, Im H-5 + Im H-2), 8.61 (s, 1H, -(C=O)-NH), 8.82 (s, 1H, Py H-4), 9.18 (s, 1H, Py H-6), 9.52 (s, 1H, Py H-2); ^{13}C NMR (100 MHz, [D$_6$]DMSO, D_7-labelled): δ = 13.48 (-CH_2-CH_3), 20.68 (tolyl CH_3), 30.29 (-CH_2-CH_3), 59.52 (Py-CH_2-), 116.91 (Im C-5), 117.83 (α-C), 126.93 (tolyl 2,6-C–H), 130.11 (tolyl 3,5-C–H), 133.12 (Im C-4), 133.21 (Py C-5), 137.60 (Im C-2), 139.65 (Py C-3), 141.35 (*C*=C-(C=O)-), 142.15 (Py C-2), 142.87 (Py C-4), 142.97 (tolyl *C*-CH_3), 144.83 (Py C-6), 146.28 (tolyl *C*-SO_2-), 162.05 (-(C=O)-NH), 165.17 (-(C=O)-O-); MS (70 eV, EI, D_7-labelled): m/z (%): 552.2 (41) [$C_{29}H_{22}D_7N_4O_5S^+$]; HRMS: calculated for $C_{29}H_{22}D_7N_4O_5S$ 552.2298; found 552.2332.

(3aRS)-11-[9-([D_7]Benzyl)-5-ethoxy-1-(*p*-tolylsulfamoyl)-1*H*,9*H*-furo[2,3-g]-imidazo[5,4-f]isoquinolyl]carboxamide (33):

Buffer: K_2HPO_4 (1.23 g) was dissolved in water (100 mL). Then a solution of KH_2PO_4 (0.907 g) in water (100 mL) was added until the pH was adjusted to 7.3 (≈0.07 M).

D_7-labelled (*Z*)-**21** or (*E*)-**21** (50 mg, 79.0 μmol) was treated with the buffer solution (2 mL), and the suspension heated with stirring and exclusion of light to 75 °C (90 °C for (*E*)-**21**) for 3 h. At the end of the reaction a yellow oily layer appeared on the wall of the reaction flask. Upon addition of chloroform the oily product dissolved. The layers were separated, and the aqueous phase was extracted several times with chloroform. The combined organic layers were concentrated *at room temperature*. The residue was dried in vacuo and purified by column chromatography (21 × 1.4, II, acetone/chloroform 1/1 (*v*/*v*), $R_f = 0.66$). The relevant fractions were concentrated *at room temperature* and the residue dried in vacuo. Yield: 10 mg (23%, only partial conversion), orange crystals: m.p. 110 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.92$ (t, $J = 7.1$ Hz, 3H, O-CH_2-CH_3), 2.41 (s, 3H, tolyl CH_3), 4.01 (q, $J = 7.1$ Hz, 2H, O-CH_2CH_3), 6.86 (s, 1H, H-2), 7.33 (d, $J = 8.3$ Hz, 2H, tolyl 2,6-H), 7.43 (d, $J = 1.2$ Hz, 1H, H-6), 7.83 (d, $J = 8.3$ Hz, 2H, tolyl 3,5-H), 8.02 (d, $J = 1.2$ Hz, 1H, H-7, H-8 or H-10), 8.18 (d, $J = 1.4$ Hz, 1H, H-7, H-8 or H-10), 8.29 (d, $J = 1.5$ Hz, 1H, H-7, H-8 or H-10); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.73$ (O-CH_2-CH_3), 21.68 (tolyl CH_3), 60.61 (O-CH_2-CH_3), 99.56 (C_q), 105.10 (C–H), 114.34 (C–H), 114.74 (C_q), 127.21 (C–H), 127.36 (tolyl 2,6-C–H), 130.38 (tolyl 3,5-C–H), 130.85 (C_q), 132.97 (C_q), 134.47 (C_q), 135.02 (C_q), 135.54 (C–H), 135.79 (C–H), 136.40 (C_q), 141.24 (tolyl *C*-CH_3), 146.22 (tolyl *C*-SO_2-), 167.02 (C=O); UV/Vis (CH_3-CN): $\lambda_{max} = 345$ nm; MS (70 eV, EI, $^2H_7$-labelled): *m*/*z* (%): 549 (0.7) [M^+], 98 (100); HRMS: calculated for $C_{29}H_{19}D_7N_4O_5S$ 549.2063; found 549.2167.

Preparation of 1-([D_7]benzyl)3-carbamoyl-5-[2-(ethoxycarbonylmethylene)-2-(imidazol-4-yl)ethyl]pyridinium bromide [(*E*)-4 and (*Z*)-4], and conversion to 1-([D_7]benzyl)-3-carbamoyl-7-(ethoxycarbonylmethyl)imidazo[4,5-f]isoquinolinium bromide (27): D_7-labelled (*E*)-**21** or (*Z*)-**21** (50 mg, 79.9 μmol] and 1-hydroxybenzotriazole (**22**) (172.8 mg, ≈1.28 mmol) were dissolved under argon in abs. dimethylformamide (1 mL) and stirred for 2 h in the dark. Abs. acetonitrile (1 mL) was then added, and the mixture transferred to a chromatography column (aluminium oxide, neutral, heat-treated (120 °C); acetonitrile/methanol 8/1 (*v*/*v*), dried over molecular sieves, 3 Å; cooling to −28 °C; detection by TLC: acetonitrile/water 2/1 (*v*/*v*), $R_f = 0.31$, in spite of decomposition during TLC the product is easy to detect). Immediately after column chromatography UV spectra were recorded. After combining the relevant fractions they were lyophilised in the dark. **27:** ^{1}H NMR (500 MHz, [D_7]DMF): $\delta = 1.20$ (t, $J = 7.1$ Hz, 3H, -CH_2-CH_3), 3.81 (s, 2H, -CH_2-COOEt), 4.13 (q, $J = 7.1$ Hz, 2H, -CH_2-CH_3), 7.71 (br, 1H, -(C=O)-NH), 7.82 (s, 1H, H-8), 8.14 (s, 1H, H-5), 9.58 (s, 1H, H-9), 9.62 (br, 1H, -(C=O)-NH), 10.42 (s, 1H, H-2); UV/Vis (acetonitrile/methanol 8/1 (*v*/*v*), dried over molecular sieves, 3 Å): $\lambda_{max} = 266$, 315 nm.[14]

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[1] a) R. M. Egan, A. T. Phillips, *J. Biol. Chem.* **1977**, *252*, 5701–5707; b) V. Keul, F. Kaeppeli, C. Gosh, T. Krebs, J. A. Robinson, J. Rétey, *ibid.* **1979**, *254*, 843–851.
[2] a) J. Klepp, A. Fallert-Müller, K. Grimm, W. E. Hull, J. Rétey, *Eur. J. Biochem.* **1990**, *192*, 669–676; b) C. Schubert, Y. Zhao, J.-H. Shin, J. Rétey, *Angew. Chem.* **1994**, *106*, 1331–1332; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1279–1280.
[3] J. Winter, J. Rétey, *Synthesis* **1994**, 245–246.
[4] K. Deuchert, U. Hertenstein, S. Hünig, G. Wehner, *Chem. Ber.* **1979**, *112*, 2045–2061.
[5] D. Seebach, E. J. Corey, *J. Org. Chem.* **1975**, *40*, 231–237.
[6] T. Kauffmann, H. Fischer, *Chem. Ber.* **1973**, *106*, 220–227.
[7] A. J. Carpenter, D. J. Chadwick, *Tetrahedron* **1986**, *42*, 2351–2358.
[8] A. Galat, *J. Am. Chem. Soc.* **1948**, *70*, 3945.
[9] M. A. Abdallah, J.-F. Biellmann, J.-P. Samama, A. D. Wrixon, *Eur. J. Biochem.* **1976**, *64*, 351–360.
[10] T. Fujii, S. Sakakbara, *Bull. Chem. Soc. Jpn.* **1974**, *47*, 3146–3151.
[11] H.-J. Knölker, R. Boese, D. Döring, A.-A. El-Ahl, R. Hitzemann, P. G. Jones, *Chem. Ber.* **1992**, *125*, 1939–1951.
[12] D. D. Perrin, W. L. F. Armargo, *Purification of Laboratory Chemicals*, 3rd ed., Pergamon, Frankfurt, **1988**.
[13] D. A. Evans, L. K. Truesdale, *Tetrahedron Lett.* **1973**, *49*, 4929–4932.
[14] UV spectra of benzylisoquinolinium bromide: D. W. Brown, S. F. Dyke, W. G. D. Lugton, A. Davis, *Tetrahedron* **1968**, *24*, 2517–2525.