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Efficient Synthesis of Nicotinic Acid Based Pseudopeptides Bearing an Amidoxime Function

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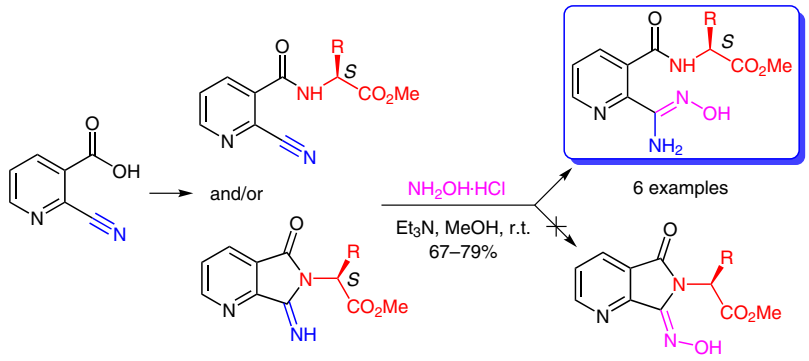

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Abstract The synthesis of nicotinic acid based amino acid units bearing an amidoxime function on the pyridine ring has been developed. The starting 2-cyanonicotinic acid was efficiently coupled with methyl esters of L-α-amino acids to afford intermediate 2-cyanopyridin-3-yl-containing pseudopeptides together with the tautomeric methyl esters of (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acids. Regioselective pyrrolidine ring opening of these esters occurred upon hydroxylamine hydrochloride treatment, giving rise to the open-chain pyridin-3-yl 2-amidoxime pseudopeptides bearing the same structure as amidoximes obtained by direct hydroxyamination of the corresponding cyano esters.

Key words amino acids, coupling, ring opening, amidoximes, pyridines, pseudopeptides, prodrugs

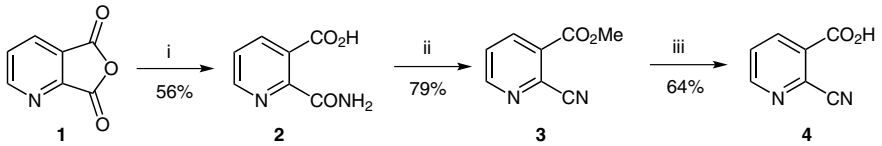
The amidine moiety is known to effectively replace the guanidine group of arginine that is used in the synthesis of arginine mimetics for the treatment of blood-coagulation disorders.[1] Other substrates containing basic groups, such as guanidines and amidinohydrazones, are also known as thrombin inhibitors. To enhance their oral bioavailability, the prodrug principle was developed.[2,3] Certain amidoximes have been demonstrated to be reduced *in vitro* and *in vivo* to the amidines,[4,5] and this approach has been successfully applied to the oral thrombin inhibitor Ximelagatran, an amidoxime prodrug.[6]

Amidoximes have been shown to possess a wide range of biological activities, and they are important intermediates in organic synthesis.[7] They are also useful as versatile building blocks for the synthesis of pharmaceutically important 1,2,4-oxadiazoles and oxadiazole-derived peptidomimetics.[8–10]

In our attempts to develop a new variety of arginine peptidomimetics with an amidinoheteroaroyl motif in a peptide chain, we have elaborated the synthesis of nicotinic acid based amino acid units bearing an amidoxime function on the pyridine ring as precursors of the corresponding amidine-containing pseudopeptides.

We envisioned that the coupling of 2-cyanonicotinic acid (**4**) with methyl esters of L-α-amino acids with further conversion of the cyano residue into an amidoxime group might be employed as a basic strategy for the synthesis of the target pyridine-based pseudopeptides.

Preparation of the starting acid **4** was perfomed according to a known literature procedure;[11,12a,b] the synthetic route is depicted in Scheme 1. Thus, commercially available quinolinic anhydride (**1**) was first quickly hydrolyzed with aqueous ammonia (28%), similar to the method developed for phthalamic acid,[11] to give 2-carbamoylnicotinic acid (**2**). Then, ester **3** was obtained by treatment of acid **2** with methyl chloroformate, which was followed by hydrolysis with sodium hydroxide (1 M) to give the corresponding 2-cyanonicotinic acid (**4**) in 28% overall yield.[12a,b]

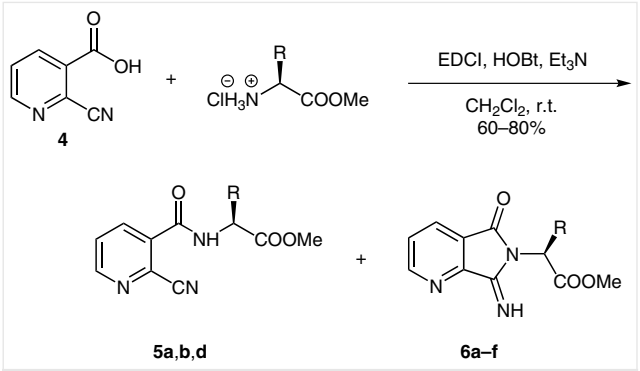


Scheme 1 *Reagents and conditions*: (i) NH_4OH (28%), 70 °C, 10 min; (ii) methyl chloroformate, Et_3N, CH_2Cl_2, 0 °C, 8 h; (iii) 1 M NaOH, MeOH, r.t., 8 h.

The amination reaction in a series of α-cyanopyridinecarboxylic acids has been studied previously,[12b,c] and it was shown that conversion of the methyl or ethyl ester of 2-cyanonicotinic acid (**4**) into the corresponding cyano amide was unsuccessful, and produced only cyclic 7-imino-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridin-5-one upon reaction with ammonia. Similar bicyclic imines were obtained by treatment of cyano esters with primary amines.[12c]

To investigate the scope of this novel method, the coupling of 2-cyanonicotinic acid (**4**) with several commercially available methyl esters of L-α-amino acids (such as Gly, Ala, Val, Leu, Phe, Pro and Trp) was examined (Scheme 2). As activating agent, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in the presence of 1-hydroxybenzotriazole (HOBt) and triethylamine was used.[13] ^{1}H NMR analysis of the reaction products confirmed that, in most cases, the formation of open-chain methyl esters of (2*S*)-*N*-(2-cyanopyridin-3-yl)carbonyl-substituted amino acids **5** was followed by further intramolecular cyclization giving a pyrrolidine ring and production of the tautomeric methyl esters of (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acids **6**.

When the valine, alanine and leucine methyl esters were used, the formation of both products **5** and **6** (**a**,**b**,**d**) in different ratios, with an increasing amount of the cyclic form **6**, was observed while in the case of glycine, phenylalanine and tryptophan, the individual cyclic esters **6c**,**e**,**f** were exclusively isolated. The total yield of the amino acid derivatives **5** and **6** was 60–80% after purification by column chromatography (Table 1).

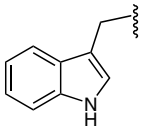


Scheme 2 Synthesis of the methyl esters of (2*S*)-*N*-(2-cyanopyridin-3-yl)carbonyl-substituted amino acids **5a**,**b**,**d** and (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acids **6a**–**f**

We also succeeded in separation of the open **5** and cyclic **6** forms of the valine, alanine and leucine derivatives by flash column chromatography, and in identification of the individual components by means of LC/MS, and ^{1}H NMR, ^{13}C NMR and IR spectroscopy. The assignment of the synthesized compounds to the open-chain or closed structure was possible on the basis of comparative analysis of their ^{1}H NMR spectra (Table 1) and X-ray crystallography data.

The only product formed from the reaction of methyl glycinate with acid **4** was analyzed by single-crystal X-ray diffraction. It was shown that this product has the cyclic structure **6c** (Figure 1).

Table 1 Yield and ^{1}H NMR Data for Compounds **5a**,**b**,**d** and **6a**–**f**

		δ, ppm (*J*, Hz)				
Ester	R	$H2_{Ar}$, d (*J* = 4.8 Hz)	$H3_{Ar}$, dd (*J*, Hz)	$H4_{Ar}$, d (*J*, Hz)	NH	Yield[a] (%)
5a	*i*-Pr (Val)	8.81	7.62 (9.0, 4.8)	8.18 (9.0)	6.97	37
6a		8.87	7.58 (9.0, 4.8)	8.15 (9.0)	9.33	34
5b	Me (Ala)	8.79	7.61 (8.1, 4.8)	8.15 (8.1)	7.15[b]	15
6b		8.86	7.57 (8.1, 4.8)	8.14 (8.1)	9.34	58
6c	H (Gly)	8.87	7.58 (7.8, 4.8)	8.17 (7.8)	9.33	58
5d	*i*-Bu (Leu)	8.78	7.61 (8.1, 4.8)	8.15 (8.1)	7.06[c]	9
6d		8.81	7.54 (m)	8.09 (6.6)	9.37	76
6e	Bn (Phe)	8.81	7.51 (7.8, 4.8)	8.06 (7.8)	9.29	76
6f	 (Trp)	8.69	7.36 (7.8, 4.8)	7.91 (7.8)	9.33	83

[a] Isolated yield.
[b] (d, *J* = 5.7 Hz).
[c] (d, *J* = 6.9 Hz).

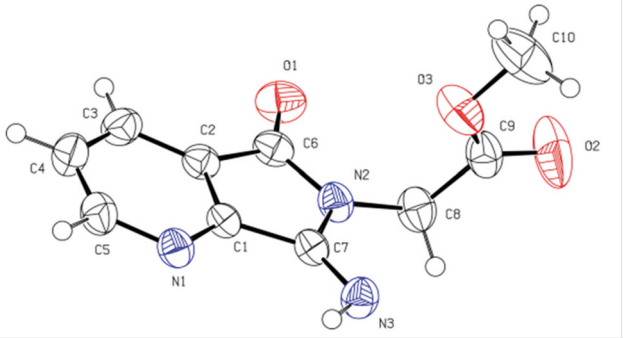


Figure 1 Molecular structure of compound **6c** according to results of the X-ray diffraction study, with the atom numbering used in the crystallographic analysis; thermal ellipsoids are shown at 50% probability level

In the ^{1}H NMR spectrum of this cyclic glycine derivative **6c**, the NH proton resonance appeared at δ = 9.33 ppm. The individual phenylalanine **6e** and tryptophan **6f** derivatives also displayed NH signals in the same region (Table 1). Thus, the NH proton peaks at δ = 6.97–7.15 ppm should correspond to derivatives **5** with an open chain. Correspondingly, the open **5** or closed **6** structures in other cases were assigned based on the NH proton chemical shifts in their ^{1}H NMR spectra.

As follows from the X-ray crystallography data, compound **6c** adopts the *E* configuration at the C=N bond and *syn* orientation of the NH proton to the aromatic ring, for steric reasons. The substituent at the N2 atom is almost planar and has orthogonal orientation with respect to the rings (the C6–N2–C8–C9 and N2–C8–C9–O3 torsion angles are 91.93(16)° and 3.24(19)°, respectively; atom numbering as used in the crystallographic analysis). The structures of other compounds in this series were assigned by analogy with **6c**.

In the isolated pyrrolopyridines **6a–f** the amino acid residue is attached to the endocyclic nitrogen atom, and not to the exocyclic imine nitrogen atom as was assumed for the structure of the major products in the similar reaction of ester **3** with ethyl or benzyl amine.[12c] Two products were also isolated when ester **3** was reacted with benzylhydrazine, and their structures were assigned as isomeric pyrrolopyridines possessing a benzylamino substituent at either the imino or the pyrrole nitrogen atom.[12d] Along with tryptophan derivative **6f**, a very small amount of crystals of the unexpected substance **6f′** was isolated from the reaction mixture (Figure 2); the crystal structure of **6f′** was reported previously.[14] In contrast to the major cyclic product **6f**, this unexpected material has an open structure that is regioisomeric to the nonisolated open form of **6f**. Even though the starting acid **4** was obtained as an individual compound of 100% purity (according to LC/MS data), the presence of the isomeric ester **6f′** could result from an undetected 3-cyanopicolinic acid impurity in the 2-cyanonicotinic acid (**4**).

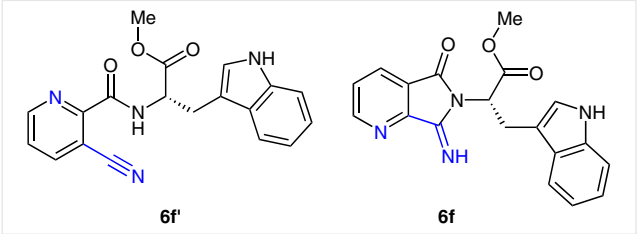


Figure 2 Structures of **6f′** and **6f**

Under the LC/MS analysis conditions (gradient elution of H_2O + 0.1% formic acid and MeCN + 0.1% formic acid), each of the individual samples **5a**,**b**,**d** and **6a–c**,**e**,**f** (according to ^{1}H and ^{13}C NMR spectra) yielded two peaks, in variable ratios, which corresponded to the same molecular ion (Table 2). It was found that these major and minor peaks appear as a result of reversible intramolecular cyclization/recyclization of compounds **5** and **6** during analysis. For each compound, the major peak value was around 70–100% and the minor peak was less than 30%.

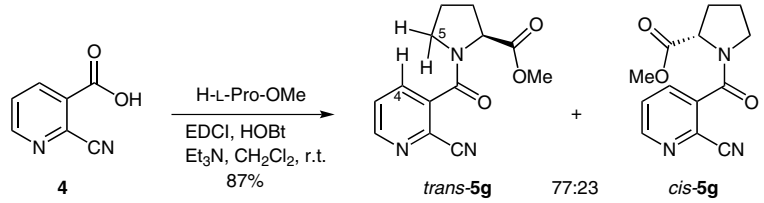
Table 2 LC/MS Data for Esters **5a**,**b**,**d** and **6a–c**,**e**,**f**[a]

Ester	[M + 1]	Major peak (%)	Minor peak (%)
5a	262	78–100	0–22
6a	262	87–98	2–13
5b	234	68–96	4–32
6b	234	74–100	0–26
6c	220	78–94	6–22
5d	276	73	27
6e	310	87	13
6f	349	80–88	12–20

[a] The values were determined on the basis of three LC/MS analyses for each of the compounds **5a**,**b** and **6a–c**,**f**, and one LC/MS analysis each for **5d** and **6e**.

The proline derivative **5g** was prepared similarly from acid **4** in 87% yield. Unlike other amino acids, in the case of L-proline the formation of only the open-chain product, methyl (2*S*)-1-[(2-cyanopyridin-3-yl)carbonyl]pyrrolidine-2-carboxylate (**5g**), was possible (Scheme 3).

Most amide bonds in peptides exist almost exclusively in the *trans* configuration. The *cis* form is energetically unfavorable, largely due to steric hindrance. The cyclic structure of proline lowers the steric hindrance, making both the *cis* and *trans* forms nearly equally energetically stable.[15] Due to this fact, two sets of signals were observed in the ^{1}H NMR spectrum of proline derivative **5g**. From the ^{1}H NMR, ^{13}C NMR and NOESY spectra of **5g**, it was possible to estimate the approximate ratio of the rotamers. Cross-peaks corresponding to the spatial interaction between the proton at the C4 position of the pyridine ring and the protons



Scheme 3 Synthesis of methyl (2*S*)-1-[(2-cyanopyridin-3-yl)carbonyl]pyrrolidine-2-carboxylate (**5g**)

at the C5 position of the pyrrolidine residue indicated that the major product (ca. 77%) was the *trans*-isomer; accordingly, the minor product (ca. 23%) was the *cis*-isomer.

The open-chain methyl esters of (2*S*)-*N*-(2-cyanopyridin-3-yl)carbonyl-substituted amino acids **5a**,**b**,**d**,**g** exhibited a characteristic cyano group IR absorption band at around 2237 cm^{-1}. The band intensity was weak, similar to the almost undetectable absorption of the reference compound, 2-cyanonicotinic acid (**4**).[12b]

The next step in the synthesis of amidine prodrugs consisted of transformation of the cyano group into an amidoxime function. Methyl esters of (2*S*)-*N*-[2-(*N*′-hydroxycarbamimidoyl)pyridin-3-yl]carbonyl-substituted amino acids **7** were obtained as individual products in 67–79% yield from the corresponding methyl esters **5a**,**b**,**d** and/or **6a**,**b**,**d**–**f** by treatment with hydroxylamine hydrochloride in methanol (Table 3).[16]

The corresponding proline analogue **7g** was synthesized similarly from the cyano derivative **5g** in 70% yield (see Scheme 4). The cyano transformation reaction proceeded upon stirring the mixture at room temperature overnight, and the crude products were purified by flash column chromatography. For these amidoximes the LC/MS analytical data showed a high purity of 98% (**7e**) and 100% (**7a**,**d**,**g**) in some cases, while for others (**7b**,**f**) about 4% and 13% of impurities were detected that are assumed to be a result of substance degradation under the LC/MS analysis conditions (gradient elution of H_2O + 0.1% formic acid and MeCN + 0.1% formic acid).

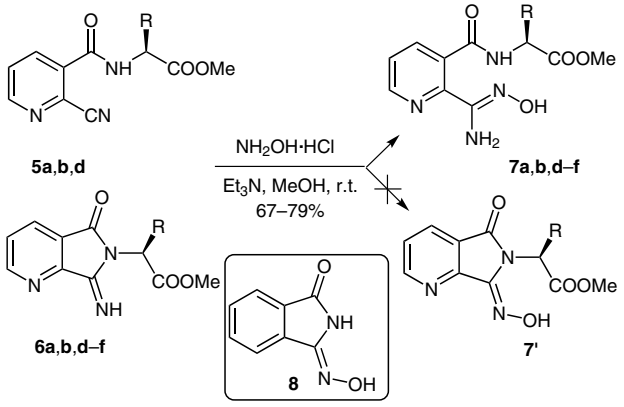
NMR and X-ray diffraction analysis of the representative pseudopeptide **7e** (Figure 3) confirmed that it consisted of only one open-chain structural isomer of nicotinic acid. Thus, the reaction of ester **6e** with hydroxylamine proceeded with unexpected selective ring opening at the C–N bond closest to the imino group moiety. The same regiochemistry has been generalized for all other products **7a**,**b**,**d**,**f** formed from this reaction.

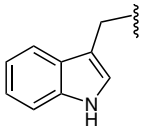
The ^1H NMR spectrum of the phenylalanine derivative **7e** revealed a chemical shift of δ = 5.49 ppm for the NH_2 protons. The other amidoximes **7a**,**b**,**d**,**f** and proline derivative **7g** also gave amino group protons with a signal in the same region, at δ = 5.46–5.50 ppm.

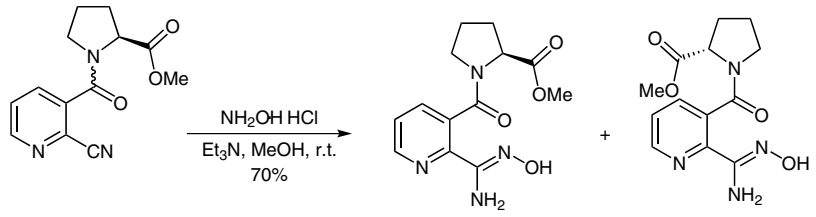
A predominance of the *trans* form for the proline-containing amidoxime **7g** was observed. The ^1H and ^{13}C NMR spectra displayed two sets of resonance signals, indicating a *trans*/*cis* isomeric ratio of about 68:32 (Scheme 4).

Both open-chain **5** and the corresponding cyclic **6** amino acid derivatives gave the same open-chain amidoximes **7** as the sole product. No cyclic amidoximes **7′**, the formation of which could be expected according to a previously reported condensation of the benzene analogue 3-imino-1-oxoisoindoline with hydroxylamine that led to 3-(hydroxyimino)-1-oxoisoindoline (**8**),[17] were found (see Table 3). The formation of pseudopeptides **7** from esters of type **6** proceeds via pyrrolidine ring opening by hydroxylamine to afford the same open-chain amidoximes as those obtained from the corresponding cyano esters **5**.

Table 3 Synthesis of the Methyl Esters of (2*S*)-*N*-[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl-Substituted Amino Acids **7a**,**b**,**d**–**f**



5, 6	R	7	Isolated yield (%)
5a, **6a**	*i*-Pr	**7a**	79
5b, **6b**	Me	**7b**	68
5d, **6d**	*i*-Bu	**7d**	70
6e	Bn	**7e**	77
6f		**7f**	67



5g *trans/cis* **7g** *trans*/cis = 68:32

Scheme 4 Synthesis of methyl (2*S*)-1-[[2-(*N*′-hydroxycarbamimidoyl)pyridin-3-yl]carbonyl]pyrrolidine-2-carboxylate (**7g**)

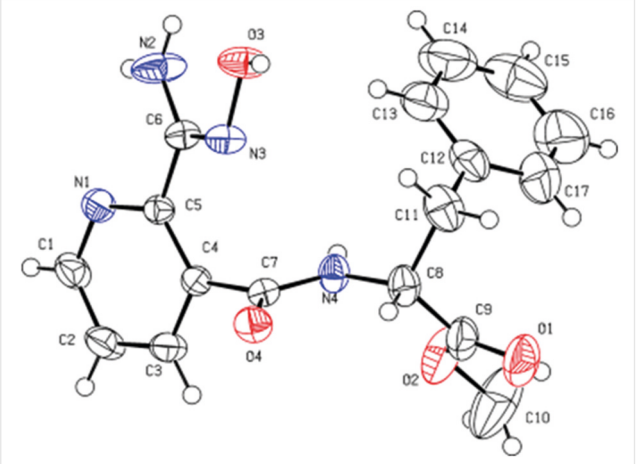


Figure 3 Molecular structure of compound **7e** according to results of the X-ray diffraction study, with the atom numbering used in the crystallographic analysis; thermal ellipsoids are shown at 50% probability level

In summary, we have found that 2-cyanonicotinic acid reacts with methyl esters of different L-α-amino acids to afford methyl esters of (2*S*)-*N*-(2-cyanopyridin-3-yl)carbonyl-substituted amino acids with an open structure. These esters undergo further intramolecular pyrrolidine ring closure leading to the tautomeric methyl esters of (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acids. Reaction of the latter with hydroxylamine hydrochloride resulted in pyrrolidine ring opening to afford open-chain amidoximes bearing the same structure as amidoximes obtained by direct hydroxyamination of the corresponding cyano esters.

This readily available variety of nicotinic acid based pseudopeptides with an amidoxime function on the pyridine ring might be considered as intermediates towards the synthesis of the corresponding amidines as arginine peptidomimetics and/or as potential amidine prodrugs. Further studies of similar amidoximes are ongoing in our laboratories.

Melting points were measured on a Buchi M-560 melting point apparatus. Elemental analyses (C, H, N) were conducted using a Vario Micro Cube analyzer. LC/MS spectra were recorded using a system that consisted of a high-performance liquid chromatograph (Agilent 1100 Series) equipped with a diode-matrix and mass-selective detector (Agilent LC/MSD SL). Parameters for LC/MS analyses: Zorbax SB-C18 column (1.8 µm), 4.6 × 15 mm; gradient elution of solvent A = H_2O + 0.1% formic acid and solvent B = MeCN + 0.1% formic acid; eluent flow: 1 mL/min; volume of injected sample: 1 µL; UV detectors operating at 215, 254 and 265 nm; ionization method: chemical ionization under atmospheric pressure (APCI); ionization mode: simultaneous scanning of positive and negative ions in the mass range *m*/*z* 80–1000. IR spectra were recorded with a Perkin–Elmer Spectrum BX FTIR spectrometer. NMR spectra were measured with Bruker Avance (300 MHz for ^{1}H, 75 MHz for ^{13}C) and Varian Mercury 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometers with tetramethylsilane as standard. Preparative column chromatography was carried out with silica gel 60 (40–63 µm).

2-Carbamoylnicotinic Acid (2)[11]

Acid **2** was prepared from quinolinic anhydride (**1**) and aqueous ammonia according to a literature procedure.[11] A suspension of anhydride **1** (7.45 g, 50 mmol) in 28% NH_4OH (37 mL) was stirred at 70 °C for 10 min, whereupon the reactant had dissolved. After the mixture was cooled to 0 °C, a precipitation was performed by adding 12 M HCl (dropwise) while cooling. The precipitate was collected by filtration and dried.

White solid; yield: 4.65 g (56%); mp 176 °C (Lit.[12b] 175 °C).

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 13.26 (s, 1 H, COOH), 8.67 (dd, *J* = 4.8, 1.4 Hz, 1 H, H_{Ar}), 8.04 (dd, *J* = 7.8, 1.3 Hz, 2 H, 2 × H_{Ar}), 7.72–7.44 (m, 2 H, NH_2).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 168.1, 167.1, 150.4, 149.6, 136.7, 129.2, 125.2.

Methyl 2-Cyanonicotinate (3)[12a,b]

To a stirred suspension of 2-carbamoylnicotinic acid (**2**; 7.47 g, 45 mmol) in CH_2Cl_2 (50 mL) at 0 °C were added Et_3N (13.08 mL, 94 mmol) and methyl chloroformate (7.65 mL, 99 mmol). After the reaction mixture was stirred at r.t. for 8 h, it was diluted with $CHCl_3$ (50 mL) and washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The product was washed with hexane and dried.

Pale-yellow solid; yield: 5.76 g (79%); mp 80–83 °C (Lit.[12b] 89 °C).

IR (KBr): 3090, 2959, 2241, 1726, 1578, 1287 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 8.94 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.48 (d, *J* = 8.1 Hz, 1 H, H_{Ar}), 7.88 (dd, *J* = 8.1, 4.8 Hz, 1 H, H_{Ar}), 3.95 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 163.0, 153.9, 138.8, 132.4, 129.4, 127.5, 116.2, 53.1.

2-Cyanonicotinic Acid (4)[12b]

A mixture of ester **3** (3.16 g, 19.5 mmol), MeOH (60 mL) and 1 M NaOH (22 mL) was stirred at r.t. for 8 h, and then concentrated in vacuo. The residue was diluted with H_2O (20 mL) and CH_2Cl_2 (50 mL), and then the solution was acidified with 1 M HCl under cooling. The resulting precipitate was collected, washed with H_2O and hexane, and dried in vacuo.

Pale-yellow solid; yield: 1.84 g (64%); mp 186 °C [Lit.[12b] 214 °C, 185 °C (sublimation)].

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 14.25 (s, 1 H, COOH), 8.92 (dd, *J* = 4.8, 1.5 Hz, 1 H, H_{Ar}), 8.47 (dd, *J* = 8.1, 1.5 Hz, 1 H, H_{Ar}), 7.86 (dd, *J* = 8.1, 4.8 Hz, 1 H, H_{Ar}).

^{13}C NMR (100 MHz, DMSO-d_6 + CCl_4, 1:1, 25 °C): δ = 164.2, 153.5, 139.1, 133.1, 130.9, 127.2, 116.4.

Anal. Calcd for $C_7H_4N_2O_2$: C, 56.76; H, 2.72; N, 18.91. Found: C, 56.74; H, 2.73; N, 18.88.

Compounds 5a,b,d,g and 6a–f;[13] General Procedure

To a solution of 2-cyanonicotinic acid (**4**; 740 mg, 5 mmol) in CH_2Cl_2 (30 mL) were added Et_3N (1.39 mL, 10 mmol), the corresponding methyl ester of an amino acid hydrochloride (5 mmol) and HOBt (0.675 g, 5 mmol). The mixture was stirred at 0 °C and EDCI (0.967 g, 5.05 mmol) was added. Then, the mixture was stirred at r.t. overnight. The mixture was diluted with CH_2Cl_2 (50 mL); then, the solution was washed with 0.1 M HCl (3 × 15 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated in vacuo.

Methyl (2*S*)-2-{[(2-Cyanopyridin-3-yl)carbonyl]amino}-3-methylbutanoate (5a)

Purification by flash column chromatography (EtOAc–petroleum ether, 1:1) gave **5a** as a white solid; yield: 0.488 g (37%); mp 142–143 °C.

IR (KBr): 3270, 3081, 2972, 2238 (CN), 1730, 1645, 1549, 1205 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.81 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.18 (d, *J* = 9.0 Hz, 1 H, H_{Ar}), 7.62 (dd, *J* = 9.0, 4.8 Hz, 1 H, H_{Ar}), 6.97 (br m, 1 H, NH), 4.79 (m, 1 H, C*H*NH), 3.78 (s, 3 H, OCH_3), 2.39–2.28 (m, 1 H, C*H*$(CH_3)_2$), 1.07 (d, *J* = 6.0 Hz, 3 H, CH_3), 1.03 (d, *J* = 6.0 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.5, 164.0, 153.1, 137.9, 135.8, 131.6, 127.4, 116.7, 58.9, 53.2, 32.2, 19.7, 18.5.

LC/MS: *m*/*z* = 262 $[M + H]^+$.

Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.55; H, 5.81; N, 16.12.

Methyl (2*S*)-2-[(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-3-methylbutanoate (6a)

Purification by flash column chromatography (EtOAc–petroleum ether, 1:1) gave **6a** as a white solid; yield: 0.438 g (34%); mp 141 °C.

IR (KBr): 3271, 2968, 1734, 1668, 1406, 1212, 1096 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.33 (br s, 1 H, NH), 8.87 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.15 (d, *J* = 9.0 Hz, 1 H, H_{Ar}), 7.58 (dd, *J* = 9.0, 4.8 Hz, 1 H, H_{Ar}), 4.80 (d, *J* = 9.0 Hz, 1 H, CHN), 3.68 (s, 3 H, OCH_3), 2.92–2.80 (m, 1 H, C*H*$(CH_3)_2$), 1.20 (d, *J* = 6.0 Hz, 3 H, CH_3), 0.88 (d, *J* = 6.0 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 170.3, 166.2, 160.2, 154.9, 150.1, 132.2, 127.2, 125.3, 58.7, 52.9, 28.8, 21.8, 20.1.

LC/MS: *m*/*z* = 262 $[M + H]^+$.

Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.57; H, 5.80; N, 16.04.

Methyl(2*S*)-2-{[(2-Cyanopyridin-3-yl)carbonyl]amino}propanoate (5b)

Purification by flash column chromatography (EtOAc–CH_2Cl_2, 1:1) gave **5b** as a white solid; yield: 0.177 g (15%); mp 135–137 °C.

IR (KBr): 3273, 3080, 2997, 2941, 2237 (CN), 1739, 1648, 1581, 1548, 1226 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.79 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.15 (d, *J* = 8.1 Hz, 1 H, H_{Ar}), 7.61 (dd, *J* = 8.1, 4.8 Hz, 1 H, H_{Ar}), 7.15 (d, *J* = 5.7 Hz, 1 H, NH), 4.80 (m, 1 H, C*H*NH), 3.78 (s, 3 H, OCH_3), 1.56 (d, *J* = 7.2 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.4, 163.6, 153.0, 137.4, 135.53, 132.0, 127.3, 116.5, 53.4, 49.7, 18.7.

LC/MS: *m*/*z* = 234 $[M + H]^+$.

Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.48; H, 4.76; N, 17.95.

Methyl (2*S*)-2-[(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]propanoate (6b)

Purification by flash column chromatography (EtOAc–CH_2Cl_2, 1:1) gave **6b** as a white solid; yield: 0.671 g (58%); mp 90 °C.

IR (KBr): 3294, 3238, 1743, 1731, 1665, 1403, 1410, 1258 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.34 (br s, 1 H, NH), 8.86 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.14 (d, *J* = 8.1 Hz, 1 H, H_{Ar}), 7.57 (dd, *J* = 8.1, 4.8 Hz, 1 H, H_{Ar}), 5.24 (q, *J* = 7.2 Hz, 1 H, CH), 3.73 (s, 3 H, OCH_3), 1.74 (d, *J* = 7.2 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 171.1, 165.9, 159.6, 154.8, 150.4, 132.1, 127.1, 125.5, 53.3, 48.6, 15.7.

LC/MS: *m*/*z* = 234 $[M + H]^+$.

Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.45; H, 4.77; N, 17.96.

Methyl [(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]acetate (6c)

Acetate **6c** was obtained as a white solid of analytical purity without further purification; yield: 0.63 g (58%); mp 160 °C.

IR (KBr): 3198, 3057, 2937, 1736, 1669, 1593, 1440, 1281, 1267, 1248 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.33 (s, 1 H, NH), 8.87 (dd, *J* = 4.8, 1.5 Hz, 1 H, H_{Ar}), 8.17 (dd, *J* = 7.8, 1.5 Hz, 1 H, H_{Ar}), 7.58 (dd, *J* = 7.8, 4.8 Hz, 1 H, H_{Ar}), 4.66 (s, 2 H, CH_2), 3.77 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 168.5, 166.0, 159.8, 154.8, 150.4, 132.1, 127.0, 125.6, 53.1, 40.0.

LC/MS: *m*/*z* = 220 $[M + H]^+$.

Anal. Calcd for $C_{10}H_9N_3O_3$: C, 54.79; H, 4.14; N, 19.17. Found: C, 54.57; H, 4.15; N, 19.10.

Methyl (2*S*)-2-{[(2-Cyanopyridin-3-yl)carbonyl]amino}-4-methylpentanoate (5d)

Purification by flash column chromatography (EtOAc–petroleum ether, 1:1) gave **5d** as a white solid; yield: 0.117 g (9%); mp 117–118 °C.

IR (KBr): 3280, 3080, 2956, 2871, 2237 (CN), 1740, 1666, 1645, 1584, 1545, 1407, 1205 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.78 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.15 (d, *J* = 8.1 Hz, 1 H, H_{Ar}), 7.61 (dd, *J* = 8.1, 4.8 Hz, 1 H, H_{Ar}), 7.06 (d, *J* = 6.9 Hz, 1 H, NH), 4.87–4.79 (m, 1 H, C*H*NH), 3.76 (s, 3 H, OCH_3), 1.85–1.66 (m, 3 H, CH + CH_2), 0.99–0.95 (2 × d, 6 H, $(CH_3)_2$).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.5, 163.9, 153.0, 137.5, 135.49, 131.8, 127.3, 116.5, 53.2, 52.4, 42.0, 25.5, 23.4, 22.5.

LC/MS: *m*/*z* = 276 $[M + H]^+$.

Anal. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 60.89; H, 6.23; N, 15.22.

Methyl (2*S*)-2-[(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-methylpentanoate (6d)

Purification by flash column chromatography (EtOAc–petroleum ether, 1:1) gave **6d** as a light-yellow oil; yield: 1.046 g (76%).

IR (KBr): 3276, 2956, 1734, 1665, 1593, 1405, 1257, 1209 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.37 (br s, 1 H, NH), 8.81 (s, 1 H, H_{Ar}), 8.09 (d, *J* = 6.6 Hz, 1 H, H_{Ar}), 7.54 (m, 1 H, H_{Ar}), 5.23–5.16 (m, 1 H, CHN), 3.64 (s, 3 H, OCH_3), 2.44–2.36 (m, 1 H, C*H*H), 1.95–1.90 (m, 1 H, CH*H*), 1.44 (m, 1 H, C*H*$(CH_3)_2$), 0.90 and 0.85 (2 × d, *J* = 5.7 Hz, 6 H, $(CH_3)_2$).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 171.0, 166.1, 159.8, 154.7, 150.1, 132.0, 127.0, 125.3, 53.0, 51.6, 37.7, 25.6, 23.7, 21.7.

LC/MS: *m*/*z* = 276 $[M + H]^+$.

Anal. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 60.85; H, 6.24; N, 15.21.

Methyl (2*S*)-2-[(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-3-phenylpropanoate (6e)

Purification by flash column chromatography (EtOAc–petroleum ether, 7:3) gave **6e** as a light-yellow solid; yield: 1.174 g (76%); mp 72–73 °C.

IR (KBr): 3275, 3252, 3201, 3062, 3030, 2955, 2923, 2853, 1741, 1731, 1666, 1408, 1256 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.29 (s, 1 H, NH), 8.81 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.06 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.51 (dd, *J* = 7.8, 4.8 Hz, 1 H, H_{Ar}), 7.20–7.14 (m, 5 H, 5 × H_{Ar}), 5.47 (dd, *J* = 10.8, 5.4 Hz, 1 H, CH), 3.78 (s, 3 H, OCH_3), 3.72–3.59 (m, 2 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 170.2, 166.0, 159.7, 154.7, 149.9, 137.6, 132.0, 129.5, 129.0, 127.3, 127.0, 125.1, 54.2, 53.4, 35.1.

LC/MS: *m*/*z* = 310 $[M + H]^+$.

Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 65.81; H, 4.91; N, 13.53.

Methyl (2*S*)-2-[(7*E*)-7-Imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-3-(1*H*-indol-3-yl)propanoate (6f)

Purification by flash column chromatography (CH_2Cl_2–MeCN, 8:2) gave **6f** as a yellow solid; yield: 1.436 g (83%); mp 119–121 °C.

IR (KBr): 3396, 3266, 3067, 1732, 1664, 1429, 1407, 1260 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.33 (s, 1 H, NH), 8.69 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 8.29 (s, 1 H, NH_{Ar}), 7.91 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.58 (d, *J* = 7.5 Hz, 1 H, H_{Ar}), 7.36 (dd, *J* = 7.8, 4.8 Hz, 1 H, H_{Ar}), 7.18 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.09–6.98 (m, 3 H, 3 × H_{Ar}), 5.58 (dd, *J* = 10.2, 4.8 Hz, 1 H, CH), 3.95–3.72 (m, 2 H, CH_2), 3.77 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 170.6, 166.1, 159.8, 154.5, 149.8, 136.6, 131.8, 127.8, 126.9, 125.0, 123.3, 122.4, 119.8, 119.0, 111.7, 111.6, 53.6, 53.3, 25.1.

LC/MS: *m*/*z* = 349 $[M + H]^+$.

Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found: C, 65.29, H, 4.64; N, 16.02.

Methyl (2*S*)-1-[(2-Cyanopyridin-3-yl)carbonyl]pyrrolidine-2-carboxylate (5g)

Purification by flash column chromatography (EtOAc–CH_2Cl_2, 1:1) gave **5g** as a pale-yellow oil; yield: 1.127 g (87%).

IR (KBr): 2952, 2237 (CN), 1736, 1634, 1428, 1407, 1175 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.74 and 8.70 (2 × d, *J* = 4.5 Hz, 1 H, H_{Ar}), 7.90 and 7.79 (2 × d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.62–7.51 (m, 1 H, H_{Ar}), 4.71 and 4.28 (2 × m, 1 H, CH), 3.77 and 3.55 (2 × s, 3 H, OCH_3), 3.51–3.38 (m, 2 H, CH_2N), 2.42–2.31 (m, 1 H, C*H*H), 2.21–1.77 (m, 3 H, CH*HCH*$_2$).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.4, 165.2, 164.8, 152.1, 151.9, 138.2, 137.9, 136.4, 136.2, 131.3, 127.4, 127.2, 116.0, 61.4, 59.7, 53.3, 53.1, 49.6, 47.5, 31.8, 29.9, 25.5, 23.3.

LC/MS: *m*/*z* = 260 $[M + H]^+$.

Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 59.98; H, 5.07; N, 16.15.

Compounds 7a,b,d–g;[16] General Procedure

To a solution of the methyl ester of a (2*S*)-*N*-(2-cyanopyridin-3-yl)carbonyl-substituted amino acid **5** and/or the methyl ester of a (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acid **6** (2 mmol) in MeOH (15 mL) was added Et_3N (0.56 mL, 4 mmol) followed by hydroxylamine hydrochloride (0.278 g, 4 mmol), and the reaction mixture was stirred at r.t. overnight. The mixture was then concentrated to dryness. The residue was dissolved in EtOAc (100 mL) and the solution was washed with H_2O (20 mL). The organic layer was dried with $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by column chromatography.

Methyl (2*S*)-2-({[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}amino)-3-methylbutanoate (7a)

Purification by flash column chromatography (CH_2Cl_2–EtOH, 95:5) gave **7a** as a white solid; yield: 0.465 g (79%); mp 120–121 °C.

IR (KBr): 3297, 1739, 1646, 1534, 1198, 1149 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.57 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 7.92 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.50 (d, *J* = 8.1 Hz, 1 H, NH), 7.33 (dd, *J* = 7.8, 4.8 Hz, 1 H, H_{Ar}), 5.46 (s, 2 H, NH_2), 4.62 (dd, *J* = 8.1, 5.1 Hz, 1 H, C*H*NH), 3.69 (s, 3 H, OCH_3), 2.22–2.12 (m, 1 H, CH), 0.94 (d, *J* = 6.9 Hz, 3 H, CH_3), 0.91 (d, *J* = 6.9 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.9, 168.3, 151.4, 150.1, 147.2, 138.4, 131.9, 124.5, 58.8, 52.8, 31.9, 19.4, 18.7.

LC/MS: *m*/*z* = 295 $[M + H]^+$.

Anal. Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 52.92; H, 6.18; N, 18.98.

Methyl (2*S*)-2-({[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}amino)propanoate (7b)

Purification by flash column chromatography (CH_2Cl_2–EtOH, 91:9) gave **7b** as a white solid; yield: 0.362 g (68%); mp 74–75 °C.

IR (KBr): 3470, 3322, 3067, 2918, 2849, 1739, 1731, 1641, 1582, 1565, 1547, 1538, 1454, 1216 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.60 (d, *J* = 4.5 Hz, 1 H, H_{Ar}), 7.92 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.46 (d, *J* = 7.2 Hz, 1 H, NH), 7.36 (dd, *J* = 7.8, 4.8 Hz, 1 H, H_{Ar}), 5.47 (s, 2 H, NH_2), 4.75 (m, 1 H, CH), 3.75 (s, 3 H, OCH_3), 1.48 (d, *J* = 7.2 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 174.1, 168.3, 151.2, 150.0, 147.3, 137.9, 131.7, 124.3, 53.0, 49.3, 18.2.

LC/MS: *m*/*z* = 267 $[M + H]^+$.

Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.48; H, 5.32; N, 20.96.

Methyl (2*S*)-2-({[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}amino)-4-methylpentanoate (7d)

Purification by flash column chromatography (CH_2Cl_2–EtOH, 9:1) gave **7d** as a white solid; yield: 0.431 g (70%); mp 114–115 °C.

IR (KBr): 3479, 3377, 3236, 3076, 2955, 1746, 1638, 1581, 1547, 1248 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.62 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 7.95 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.41–7.35 (m, 2 H, NH + H_{Ar}), 5.46 (br s, 2 H, NH_2), 4.84–4.76 (m, 1 H, CH), 3.76 (s, 3 H, OCH_3), 1.78–1.64 (m, 3 H, CH_2 + CH), 0.98 (d, *J* = 6.3 Hz, 3 H, CH_3), 0.96 (d, *J* = 6.3 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 174.1, 168.3, 151.4, 150.1, 147.0, 138.4, 131.9, 124.5, 53.0, 52.2, 42.1, 25.5, 23.4, 22.7.

LC/MS: *m*/*z* = 309 $[M + H]^+$.

Anal. Calcd for $C_{14}H_{20}N_4O_4$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.36; H, 6.55; N, 18.10.

Methyl (2*S*)-2-({[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}amino)-3-phenylpropanoate (7e)

Ester **7e** was obtained as a white solid of analytical purity without further purification; yield: 0.527 g (77%); mp 172 °C.

IR (KBr): 3359, 3201, 3020, 2886, 1723, 1672, 1644, 1586, 1575, 1537, 1276, 934 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.60 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 7.88 (d, *J* = 7.8 Hz, 1 H, H_{Ar}), 7.63 (d, *J* = 7.5 Hz, 1 H, NH), 7.37–7.19 (m, 6 H, 6 × H_{Ar}), 5.49 (br s, 2 H, NH_2), 5.11 (dd, *J* = 13.5, 6.0 Hz, 1 H, CH), 3.72 (s, 3 H, OCH_3), 3.24–3.21 (m, 2 H, CH_2).

^{13}C NMR (100 MHz, DMSO-d_6 + CCl_4, 1:1, 25 °C): δ = 171.9, 167.5, 149.9, 148.7, 147.4, 137.4, 136.8, 131.7, 129.5, 128.4, 126.6, 123.3, 54.2, 51.9, 37.5.

LC/MS: *m*/*z* = 343 $[M + H]^+$.

Anal. Calcd for $C_{17}H_{18}N_4O_4$: C, 59.64; H, 5.30; N, 16.37. Found: C, 59.51; H, 5.32; N, 16.31.

Methyl (2*S*)-2-({[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)propanoate (7f)

Purification by flash column chromatography (CH_2Cl_2–MeOH, 94:6) gave **7f** as a white solid; yield: 0.507 g (67%); mp 129–130 °C.

IR (KBr): 3331, 3043, 1730, 1641, 1582, 1214 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.90 (s, 1 H, NH), 8.46 (d, *J* = 4.8 Hz, 1 H, H_{Ar}), 7.72 (dd, *J* = 4.8, 1.2 Hz, 1 H, H_{Ar}), 7.50 (d, *J* = 7.5 Hz, 1 H, NH), 7.33–7.24 and 7.18–7.02 (2 × m, 6 H, 6 × H_{Ar}), 5.49 (s, 2 H, NH_2), 5.07 (dd, *J* = 12.9, 6.0 Hz, 1 H, CH), 3.62 (s, 3 H, OCH_3), 3.32 (m, 2 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.1, 168.6, 151.5, 150.1, 147.0, 137.9, 136.8, 131.7, 128.1, 124.5, 122.4, 119.9, 119.0, 112.1, 109.9, 54.1, 53.1, 28.0.

LC/MS: *m*/*z* = 382 $[M + H]^+$.

Anal. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.84; H, 5.02; N, 18.36. Found: C, 59.65; H, 5.04; N, 18.29.

Methyl (2*S*)-1-{[2-(*N*′-Hydroxycarbamimidoyl)pyridin-3-yl]carbonyl}pyrrolidine-2-carboxylate (7g)

Ester **7g** was obtained as a white solid of analytical purity without further purification; yield: 0.409 g (70%); mp 135–136 °C.

IR (KBr): 3521, 3159, 3117, 3080, 2886, 2867, 2835, 1732, 1651, 1608, 1537, 1471, 1433, 1368, 1199, 1177 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.57 (m, 1 H, H_{Ar}), 8.08 (br s, 1 H, OH), 7.69 and 7.61 (2 × dd, *J* = 7.8, 1.5 Hz, 1 H, H_{Ar}), 7.39–7.26 (m, 1 H, H_{Ar}), 5.50 (br s, 2 H, NH_2), 4.74 and 4.11 (2 × dd, *J* = 8.6, 4.3 Hz, *J* = 6.1, 2.7 Hz, 1 H, CH), 3.79 and 3.48 (2 × s, 3 H, OCH_3), 3.75 (m) and 3.25 (t, *J* = 6.5 Hz, 2 H, CH_2), 2.38–2.20 and 1.96–1.86 (2 × m, 4 H, CH_2CH_2).

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.6, 173.3, 169.5, 169.2, 150.6, 150.3, 149.2, 145.7, 145.2, 137.2, 136.5, 131.5, 131.1, 124.4, 124.0, 61.2, 59.3, 52.9, 52.7, 48.9, 47.4, 31.5, 30.3, 25.2, 23.8.

LC/MS: *m*/*z* = 293 $[M + H]^+$.

Anal. Calcd for $C_{13}H_{16}N_4O_4$: C, 53.42; H, 5.52; N, 19.17. Found: C, 53.25; H, 5.54; N, 19.10.

X-ray Diffraction Study of Compounds 6c and 7e

The structures were solved by direct methods using the SHELXTL package.[18] Positions of the hydrogen atoms were located from electron-density difference maps and refined by 'riding' model with U_{iso} = nU_{eq} of the carrier atom (n = 1.5 for methyl groups, n = 1.2 for other hydrogen atoms). Full-matrix least-squares refinement of the structures against F^2 in anisotropic approximation for non-hydrogen atoms using 3209 (**6c**) and 4673 (**7e**) reflections was converged to wR_2 = 0.133 (R_1 = 0.047 for 2332 reflections with $F > 4\sigma(F)$, *S* = 1.03) for structure **6c** and wR_2 = 0.096 (R_1 = 0.035 for 4279 reflections with $F > 4\sigma(F)$, *S* = 1.02) for structure **7e**. The final atomic coordinates and crystallographic data for molecules **6c** and **7e** have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44(1223)336033; E-mail: deposit@ccdc.cam.ac.uk] and are available on request quoting the deposition numbers CCDC 991411 for **6c** and CCDC 991412 for **7e**.

Compound **6c** ($C_{10}H_9N_3O_3$): monoclinic crystals; at 293 K, *a* = 5.0866(4) Å, *b* = 9.0455(7) Å, *c* = 21.760(1) Å, β = 92.030(7)°, *V* = 1000.6(1) $Å^3$, M_r = 219.20, *Z* = 4, space group $P2_1/n$, D_{calc} = 1.455 g/cm^3, μ(Mo Kα) = 0.111 mm^{-1}, *F*(000) = 456. Intensities of 10110 reflections (3209 independent, R_{int} = 0.022) were measured on an Xcalibur-3 diffractometer (graphite monochromated Mo Kα radiation, CCD detector, ω-scanning, $2\Theta_{max}$ = 64°).

Compound **7e** ($C_{17}H_{18}N_4O_4$): monoclinic crystals; at 293 K, *a* = 8.5563(3) Å, *b* = 10.2899(3) Å, *c* = 10.1182(3) Å, β = 104.315(3)°, *V* = 863.19(5) $Å^3$, M_r = 342.35, *Z* = 2, space group $P2_1$, D_{calc} = 1.317 g/cm^3, μ(Mo Kα) = 0.096 mm^{-1}, *F*(000) = 360. Intensities of 9410 re-

flections (4673 independent, R_{int} = 0.013) were measured on an Xcalibur-3 diffractometer (graphite monochromated Mo Kα radiation, CCD detector, ω-scanning, $2\Theta_{max}$ = 64°).

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1379917.

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