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Solid-Phase Synthesis of 4(5),1′,5′-Trisubstituted 2,4′-Biimidazoles

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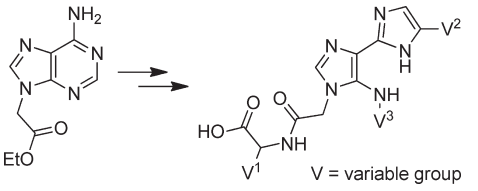

A method for the synthesis of libraries of 4(5),1′,5′-trisubstituted 2,4′-biimidazoles on a solid support was developed.[1] A trivalent scaffold, 2-(5′-amino-4(5)-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic acid, was first prepared in solution by a two-step synthesis from ethyl adenin-9-ylacetate and bromomalonaldehyde. The product was coupled to an amino acid loaded Wang resin and the formyl group was subsequently derivatized either by reductive amination, oximation, or hydrazone formation. The 5′-amino group of the resin-bound biimidazole was then acylated and the products were finally released from the resin and purified. 5′-Amino-2,4′-biimidazole offers a scaffold for lead compounds of drug discovery, possibly useful in finding leads for protein kinase inhibitors.

Introduction

Protein kinases (PK) mediate and regulate the majority of the signal transduction in cells and they, hence, play an important role in cell growth, metabolism, differentiation, and apoptosis.[2] A number of diseases, including cancer, diabetes, and inflammation, are linked to perturbation in these signaling pathways.[3] Thereby PKs are an attractive target for drug development[3,4] and they have recently become the most studied class of drug targets of the pharmaceutical industry.[5] PKs catalyze the transfer of the terminal phosphate group from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue of their protein substrate. The binding site for ATP is highly conserved among PKs and plenty of structural information of the catalytic domains is available.[2b,6] The majority of small-molecule kinase inhibitors that have been developed so far target the ATP binding site. The conformation of the kinase engaged in the protein-inhibitor complex usually closely resembles the conformation in the protein-ATP complex.[7] Although these competitive inhibitors of ATP exhibit high affinity and sometimes additionally interact with binding elements not involved in ATP binding, they often suffer from lack of sufficient selectivity.[8] To circumvent the selectivity issue, a growing number of small-molecule inhibitors that do not directly compete for the ATP-binding site have emerged.[9] These inhibitors may target the inactive conformation of the kinase, allosteric sites, or binding sites for substrate or a protein partner.[2b,6,7] Despite these extensions to the paradigm, the number of different molecular frameworks subjected to synthesis of combinatorial libraries still is rather limited. In fact, it appears that a top-heavy distribution of frameworks occurs in medicinal chemistry, i.e., the more often a framework has been used for synthesis of a library, the more likely it will be used for another combinatorial approach.[10] The selectivity issues together with highly congested intellectual property (IP) space resulting from the limited chemical space covered has increased the IP risk associated with the pursuit of protein kinase inhibitors.[11]

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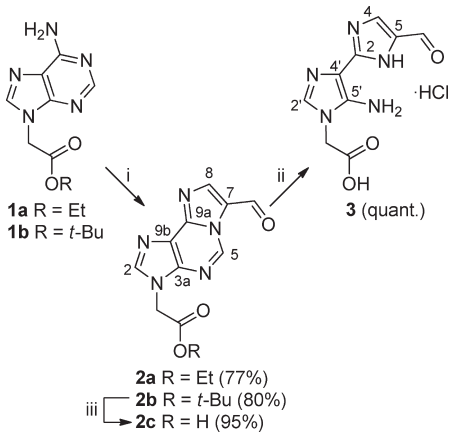
As a result, there is a permanent need for new scaffolds. 5′-Amino-2,4′-biimidazoles constitute a rarely studied group of compounds that may afford an interesting new scaffold for lead search. They obey well the 2−0 rule of kinase-likeness proposed by Aronov et al.,[12] they are easy to synthesize, and they contain six points of diversification. The structure of 5′-amino-2,4′-biimidazole is a rigid rod, but rotation around the 2,4′-bond connecting the imidazoles is possible, although hindered. Depending on substituents and pH, there is a possibility for intramolecular hydrogen bonding between the 5′-amino proton and the pyridine-like nitrogen of the second imidazole ring.[13] This hydrogen bond results in the formation of a pseudo-six-membered ring between the imidazoles thus making the ring system planar. Furet has successfully applied a similar pseudoring design approach to the kinase inhibitor scaffold search.[14] Different twisted conformations between the imidazole rings can be evoked by the choice of substituents. The multifaceted core structure makes it possible to design a spatially diverse set of compounds with a single scaffold. To exemplify the 5′-amino-2,4′-biimidazole scaffold synthesis, we have devised a method to produce 5,1′,5′-trisubstituted 2,4′-biimidazoles on a solid support.

Results and Discussion

The common core structure of the library, viz. 2-(5′-amino-5-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic acid (**3**, Scheme 1), was prepared as a hydrochloride from ethyl 2-(adenin-9-yl)acetate **1** and bromomalonaldehyde along a two-step route that did not contain chromatographic purifications. In the first step, an additional imidazole ring was formed by bridging *N*1 and *N*6 of the adenine moiety with a formyletheno group under the conditions originally developed for the solid supported synthesis of similar compounds.[15] The pyrimidine ring of the resulting 3*H*-imidazo[1,2-*i*]purine derivative (**2a**) could be opened by either a base-[16] or acid-catalyzed[17] hydrolysis. Attempted base-catalyzed hydrolysis led to a dark brown product mixture, while the acid-catalyzed hydrolysis gave a white or yellowish product that precipitated from the solution. The product obtained by the latter method was sufficiently pure to be used as such for further derivatization.

The pyrimidine ring could also be opened on a solid support. For that purpose, 2-(7-formyl-3*H*-imidazo[1,2-*i*]-purin-3-yl)acetic acid[18] (**2c**, Scheme 1) was coupled to an amino acid loaded Rink amide-PEG-PS resin (base-catalyzed hydrolysis) or HMBA-PS resin (acid-catalyzed hydrolysis). The base-catalyzed hydrolysis required overnight heating

SCHEME 1[a]



[a]Reagents and conditions: (i) $HCBr(CHO)_2$, HCO_2H, 2,6-lutidine, DMF; (ii) HCl (aq), (iii) TFA/DCM.

(55 °C) in a 1:1 mixture (v/v) of THF and aq NaOH, the overall hydroxide ion concentration being 0.08 mol L^{-1}. The acid-catalyzed hydrolysis was carried out as an overnight reaction at room temperature in 3.6 mol L^{-1} HCl in dioxane containing 10% water. However, part of the support-bound material was observed to be cleaved off during the hydrolytic ring-opening. Neither of these solid-supported reactions was, after all, applied to the library synthesis. Since **3** was a common precursor of all the library members, its preparation in solution phase followed by postsynthetic immobilization to commercially available supports loaded with various amino acids offered a simple way for parallel synthesis of several sublibraries.

Wang polystyrene resins loaded with Fmoc-protected amino acids (Leu, Asp, Trp, Ser) were used for the solid-supported library synthesis. Since **3** and its 1-hydroxybenzotriazole ester were sparingly soluble in DMF or DMF/diisopropylethylamine, compound **3** and 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), used as an activator, were dissolved in warm dry pyridine and in DMF, respectively. The combined solutions were added onto the deprotected solid support (Scheme 2) and the reaction was allowed to proceed several hours to ensure completion of the coupling. Extensive washing was carried out to remove the surpluses of reactants. The supports obtained, **4a**−**d**, were divided into three portions. The first portion was oximated with methoxylamine. The second portion was allowed to react with either 2,6-dinitrophenylhydrazine (DNPH) or phenylhydrazine. Unfortunately, the applicability of hydrazines as diversifying reagents turned out to be rather limited. Among the two products obtained, 2,6-dinitrophenylhydrazone nicely gave a single product, **6a**, upon subsequent acylation of the 5′-amino function, whereas the phenylhydrazone gave a product mixture. Evidently, the central nitrogen atom of the phenylhydrazone still was sufficiently nucleophilic to undergo acylation, in contrast to the dinitrophenyl (DNP) derivative that was deactivated by the strongly electron-withdrawing 2,6-dinitro groups. The resulting *N*-acylated phenylhydrazones were relatively labile and they were partially decomposed to formylbiimidazoles and 1-phenylhydrazides. Changing the order of these two reactions, hydrazone formation and acylation, did not

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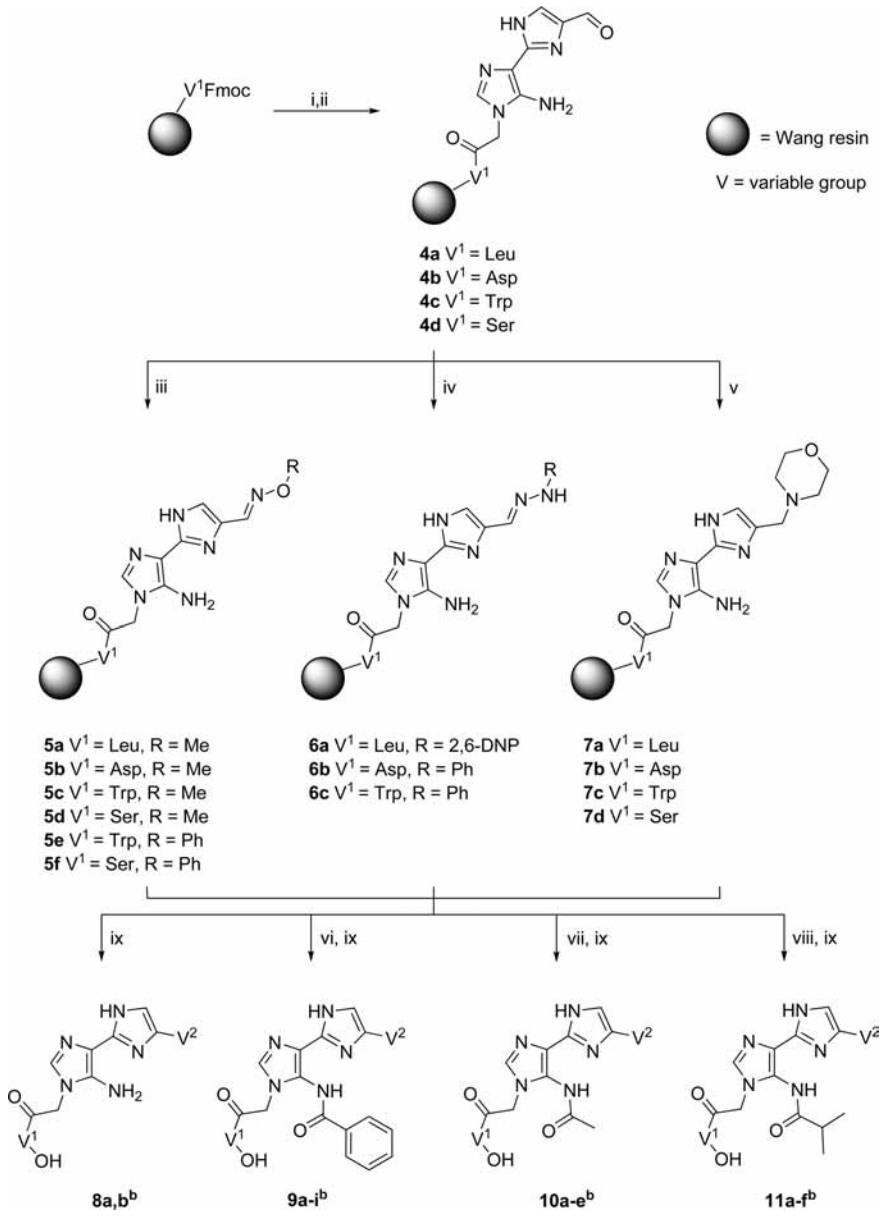
(18) See Supporting Information for synthesis and characterization.

SCHEME 2[a]



[a]Reagents and conditions: (i) 20% piperidine in DMF; (ii) **3**, HBTU, pyridine, DMF; (iii) $R^1ONH_2 \cdot HCl$, pyridine; (iv) R^2NHNH_2, AcOH, DMF, heating; (v) morpholine, HCO_2H, $NaCNBH_3$, MeOH, DMF; (vi) BzCl, pyridine; (vii) Ac_2O, pyridine; (viii) isobutyryl chloride, pyridine; (ix) 2.5% H_2O, 2.5% triisopropylsilane in TFA. [b]For annotation of variable groups see Table 1.

improve the situation, as the conditions needed for the hydrazone formation resulted in cleavage of the acyl group from the 5′-amino function. Accordingly, the synthesis was continued with **6a** and **6b**, but not with **6c**. Half of the remaining **4c** was instead oximated with phenoxyamine that circumvented the acylation problem. With **4d**, the hydrazone formation was replaced with a similar oximation. The third portion of **4a**−**d** was aminated with morpholine by using a method developed earlier.[15] The 5′-amino group of biimidazole is relatively unreactive which, taken together with the presence of other reactive groups in the molecules, limits the selection of chemistries applicable to the solid-supported library synthesis. Active ester approach and anhydride activation both failed to give good yields in preliminary experiments (data not included). For this reason, acyl halides and acetic anhydride were used in the library synthesis. Products **5**−**7** were divided into two portions (except **5e**, **5f**, and **7c**) which were acylated with benzoyl chloride, acetic anhydride, or isobutyryl chloride. Acyl halides reacted partially twice, but the second substituent was easily removed by a short ammonia treatment. Initially, the unsubstituted 5′-amino group was intended to be used as one of the diversity groups. For this purpose, compound **8a** was synthesized. Because the product remained brown, although homogeneous according to HPLC, recrystallization was attempted. Unfortunately the product decomposed upon recrystallization and therefore the free 5′-amino was abandoned as a diversity group.

TABLE 1. Members of the Synthesized 4(5),1′,5′-Trisubstituted 2,4′-Biimidazole Test Library and Isolated Overall Yields

compd	V^1	V^2	isolated yield (%)[a]
8a	Leu	CHNOMe	52
8b	Trp	CHNNHPh	n.p.
9a	Leu	CHNOMe	44
9b	Leu	2,6-DNPH	38
9c	Leu	morpholine	33
9d	Asp	CHNOMe	57
9e	Asp	CHNNHPh	6
9f	Asp	morpholine	17
9g	Trp	CHNOMe	8
9h	Ser	CHNOMe	53
9i	Ser	morpholine	44
10a	Leu	morpholine	29
10b	Asp	morpholine	21
10c	Trp	CHNOMe	8
10d	Ser	CHNOMe	71
10e	Ser	CHNOPh	47
11a	Leu	2,6-DNPH	36
11b	Asp	CHNOMe	45
11c	Asp	CHNNHPh	n.p.
11d	Trp	CHNOPh	7[c]
11e	Trp	morpholine	12
11f	Ser	morpholine	25

[a]According to ^{13}C NMR all as TFA salts except **9b**,**9e**,**10c**, and **11e**. [b]n.p. = not purified. [c]Combined yield of *E* and *Z* isomers.

Products **8**−**11** (Table 1) were cleaved from the support with TFA, using water and triisopropylsilane as scavengers. Regardless of the scavengers, the tryptophan derived compounds (**9g**, **10c**, **11d**−**e**) gave low yields. Although the majority of the products (see Figure 1 for illustrative HPLC traces) were relatively pure after cleavage and, in some cases, ether precipitation, they were still purified by HPLC for NMR analysis. Methoxyaminated products existed as *E*/*Z* isomers that could not be separated by the RP-HPLC method employed. The isomeric ratio was approximately 1:2, according to the ^1H NMR integrals. To identify the isomers, a nondecoupled ^{13}C spectrum of **10d** was recorded (^{13}C satellite signals were not visible in ^1HNMR). The $^1J_{CH}$ coupling constants of the oxyimino carbons of the isomers were 173 (δ 137.8 ppm) and 182 Hz (δ 133.9 ppm). Since a larger $^1J_{CH}$ coupling constant is expected for a hydrogen *syn* to the imine nitrogen lone pair,[19] it was concluded that the carbon with 182 Hz coupling constant referred to the *Z* isomer. Hence, it could be seen from the 2D spectra that *Z* was the major isomer of the methoxyaminated compounds. The isomeric ratio of the phenoxyamine analogues **11d** and **10e** was approximately 1:1. Isomers of **11d** were separated by HPLC. Because of the prototropic tautomerism and hindered rotation about the C4′−C2 bond adjoining the imidazole rings,[17b,13] the ^{13}C and ^1H NMR signals of the imidazole rings were broadened in most of the products. With several samples, some imidazole ring carbon signals were missing or weak. In those cases, the chemical shift assignment relied on the 2D spectra. In addition, signals referring to the morpholino group and methyl and methine groups connecting the outer imidazole ring to the second diversity group were broadened. No acid was added to the NMR samples to speed the exchange processes, but probe temperature was raised or lowered in many experiments to improve the spectral quality.

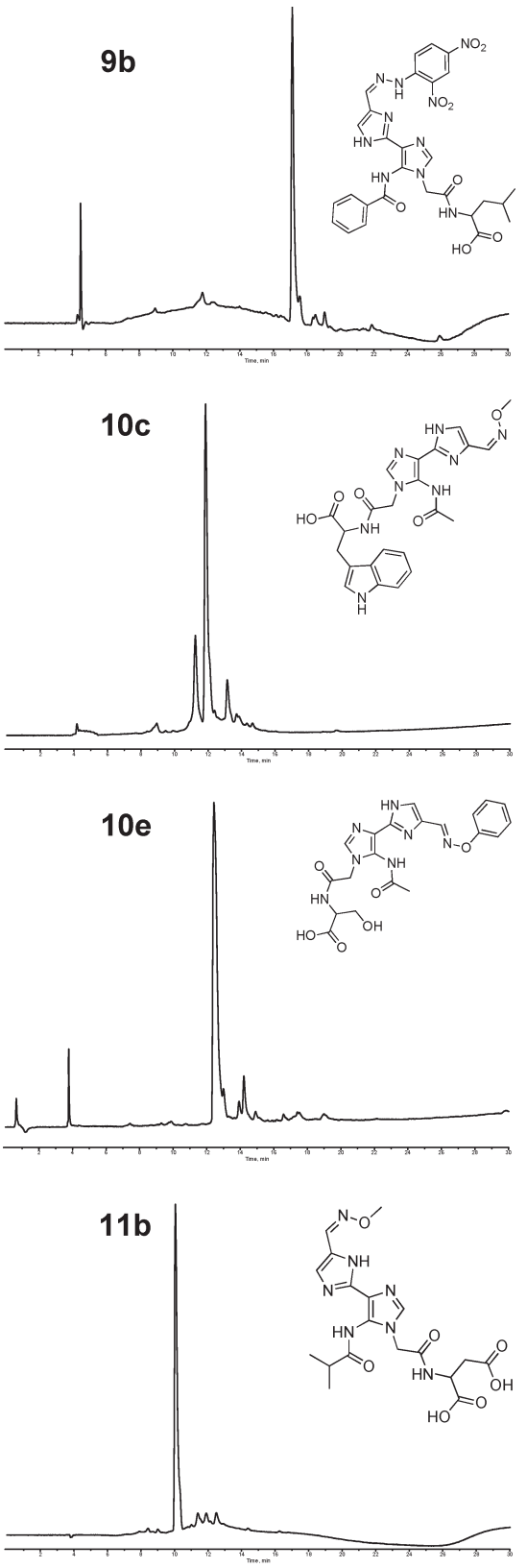


FIGURE 1. Illustrative examples of HPLC traces of crude products cleaved from the resin (RP HPLC 0−100% MeCN, 0.1% TFA, λ = 220 nm).

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Conclusion

A small 22 members library of 5,1′,5′-trisubstituted 2,4′-biimidazoles was synthesized on a solid support. With the developed method, larger libraries can easily be produced by increasing the selection of the diversity reagents used, namely the amino acid resin, *O*-substituted hydroxylamine, amine, and acid halide. By contrast, the use of hydrazines has limitations because of the side reactions. Nevertheless hydrazines having electron-withdrawing groups can be used as the second diversity reagent. Products isolated after cleavage reaction and ether precipitation are relatively pure, though in some cases additional purification is desirable. Nevertheless, the method is applicable for production of 2,4′-biimidazoles potentially useful for lead search of kinases.

Experimental Section

Ethyl 2-(7-Formyl-3*H*-imidazo[1,2-*i*]purin-3-yl)acetate (2a). Ethyl 2-(9*H*-adenin-9-yl)acetate (2.45 g, 11.5 mmol), bromomalonaldehyde (3.48 g, 23.1 mmol), and 3 Å molecular sieves were suspended into a mixture of formic acid (4.33 mL, 115 mmol), 2,6-lutidine (2.80 mL, 24.2 mmol), and DMF (20 mL). The reaction mixture was stirred 3 h with a magnetic stirrer at 60 °C in a closed bottle. The mixture turned to dark orange and a white precipitate formed during the reaction. The reaction was followed with TLC (10% H_2O/MeCN). After completion, the mixture was kept in a freezer overnight. The solidified reaction mixture was transferred to a Büchner funnel with water. The white crude product was washed additionally with water. The mother liquor was evaporated to dryness and the remaining deposit was suspended in water, suction filtered, and washed with water. The air-dried product was further dried over phosphorus pentaoxide in a vacuum desiccator. The product was recrystallized from 25% EtOH/EtOAc yielding 2.43 g (77%) of white fibrous product. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.22 (t, J = 7.1 Hz, 3 H, $CH_{3,Et}$), 4.20 (q, J = 7.1 Hz, 2 H, $CH_{2,Et}$), 5.34 (s, 2H, CH_2Im), 8.53 (s, 1H, H2), 8.59 (s, 1H, H8), 9.91 (s, 1H, H5), 10.00 (s, 1H, CHO) ppm; ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 14.0 ($CH_{3,Et}$), 44.9 (CH_2Im), 61.6 ($CH_{2,Et}$), 122.1 (C9b), 124.7 (C7), 136.8 (C5), 141.9 (C3a), 144.3 (C2), 144.8 (C9a), 147.9 (C8), 167.6 (CO_2), 179.1 (CHO) ppm. HRMS (ESI-TOF) calcd for $[MH^+]$ $C_{12}H_{12}N_5O_3^+$ 274.0935, found 274.0936.

2-(5′-Amino-4(5)-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic Acid Hydrochloride (3). 2a (1.00 g, 3.66 mmol) was stirred in 6 M hydrochloric acid (20 mL) until the reaction was completed according to ESI-MS. The white product precipitated during the reaction. The solution was cooled and suction filtered and washed with water. The mother liquor was concentrated and acetone was added until the mixture turned cloudy. After cooling, the precipitate was collected by suction filtration and washed with water. Product phases were combined and dried over phosphorus pentaoxide in a vacuum desiccator. Quantitative yield. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 4.96 (s, 2H, CH_2Im), 8.25 (s, 1H, H2′), 8.26 (s, 1H, H4), 9.73 (s, 1H, CHO) ppm; ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 45.6 (CH_2Im), 101.2 (C4′), 131.2 (C4), 133.2 (C2′), 133.8 (C5), 141.3 (C5′), 142.1 (C2), 168.3 (CON), 180.7 (CHO) ppm. HRMS (ESI-TOF) calcd for $[MH^+]$ $C_9H_{10}N_5O_3^+$ 236.0778, found 236.0782.

General Procedure for Coupling of 3 to a Solid Support (4). The Fmoc-protected amino acid Wang polystyrene resin (0.3 mmol) was deprotected with 20% piperidine in DMF for 20 min. Resin was successively washed with DMF, DCM, and MeOH and dried in vacuum. Compound **3** (421 mg, 1.5 mmol) was dissolved in warm pyridine (6 mL). The solution turned to orange and some undissolved **3** remained. HBTU (569 mg, 1.5 mmol) in DMF (3 mL) was added and the mixture was pipetted onto the deprotected resin. The reaction suspension was shaken in an orbital shaker for 7 h, filtered, and washed with pyridine, 30% DMF/pyridine, 10% water/pyridine, water, pyridine, 30% DMF/pyridine, DMF, DCM, 10% MeOH/DCM, and MeOH. Resin **4** was dried in suction and in a vacuum desiccator.

General Procedure for Oximation on a Solid Support (5). Methoxylamine hydrochloride (83.5 mg, 1.00 mmol) in pyridine (2 mL) was added onto resin **4** (0.1 mmol). The suspension was shaken for 1 h and then filtered and washed with pyridine, 10% water/pyridine, pyridine, DCM, 10% MeOH/DCM, and MeOH and dried as before.

Alternatively, phenoxyamine hydrochloride (36.4 mg, 0.25 mmol) in pyridine (1 mL) was added onto resin **4** (0.05 mmol). The suspension was shaken for 2 h and then filtered and washed subsequently with pyridine, 10% water/pyridine, pyridine, DCM, 10% MeOH/DCM, and MeOH and dried as before.

General Procedure for Hydrazone Formation on a Solid Support (6). 2,6-Dinitrophenylhydrazine (67% , 295.7 mg, 1.0 mmol) and acetic acid (250 μL) were dissolved in DMF (2.25 mL) and added onto resin **4** (0.1 mmol). The suspension was stirred 22 h with a magnetic stirrer at 88 °C and then filtered and washed with DMF, 10% AcOH/DMF, DMF, 10% MeOH/DMF, water, MeOH, 5% TEA/DCM, DCM, 10% MeOH/DCM, and MeOH and dried as before.

Alternatively, phenylhydrazine (98.5 μL, 1.0 mmol) and acetic acid (250 μL) were dissolved in DMF (2.25 mL) and added onto resin **4** (0.1 mmol). The suspension was stirred 22 h with a magnetic stirrer at 77 °C and then filtered and washed with DMF, 10% AcOH/DMF, DMF, 10% MeOH/DMF, water, MeOH, 5% TEA/DCM, DCM, 10% MeOH/DCM, and MeOH and dried as before.

General Procedure for Reductive Amination on a Solid Support (7). Morpholine (87 μL, 1.0 mmol) and formic acid (92 μL) dissolved in 1.00 mL of DMF were pipetted on resin **4** (0.1 mmol). After half an hour 85% $NaCNHB_3$ (37 mg, 0.4 mmol) and MeOH (138 μL) dissolved in 1.00 mL of DMF were added. The suspension was shaken for 18 h. The suspension was then filtered and washed with DMF, 10% MeOH/DMF, water, MeOH, DCM, 10% MeOH/DCM, and MeOH. The resin was dried as before.

General Procedure for Benzoylation on a Solid Support (9). Benzoyl chloride (29 μL 0.25 mmol) dissolved in 1.00 mL of pyridine was pipetted onto resins **5**−**7** (0.05 mmol). The suspension was stirred for 3 h, filtered, and washed with pyridine, 10% water/pyridine, and pyridine. The resin was then washed for 2 min with pyridine containing 10% water and 20% concentrated aqueous ammonia and finally with pyridine, 10% water/pyridine, water, pyridine, DCM, 10% AcOH/DCM, DCM, 10% MeOH/DCM, and MeOH. The resin was dried as before.

General Procedure for Acetylation on a Solid Support (10). Acetic anhydride (23.5 μL 0.25 mmol) dissolved in 1.00 mL of pyridine was pipetted onto resins **5**−**7** (0.05 mmol). The suspension was stirred for 3 h and then filtered and washed with pyridine, 10% water/pyridine, water, pyridine, DCM, 10% AcOH/DCM, DCM, 10% MeOH/DCM, and MeOH. The resin was dried as before. The reaction was repeated with 1 h reaction time if necessary.

General Procedure for the Isobutyrylation on the Solid Support (11). Isobutyryl chloride (26 μL 0.25 mmol) dissolved in 1.00 mL of pyridine was pipetted onto resins **5**−**7** (0.05 mmol). The suspension was stirred for 3 h, filtered, and washed with pyridine, 10% water/pyridine, and pyridine. The resin was then washed for 1 min with pyridine containing 10% water and 20% concentrated aqueous ammonia and finally with pyridine, 10% water/pyridine, water, pyridine, DCM, 10% AcOH/DCM, DCM, 10% MeOH/DCM, and MeOH. The resin was dried as before.

(*S*,*E*/*Z*)-2-{2-[5′-Benzamido-4(5)-(methoxyiminomethyl)-1*H*,1′-*H*-2,4′-biimidazol-1′-yl]acetamido}-4-methylpentanoic acid (9a): Yield as TFA salt 13.1 mg (44%). ^{1}H NMR (500 MHz, CD_3OD, 298 K) δ 0.82 (d, J = 6.1 Hz, 3 H, δ_{Leu}), 0.85 (d, J = 6.0 Hz, 3 H, Hδ_{Leu}), 1.51−1.61 (m, 3 H, Hβ_{Leu}, Hγ_{Leu}), 3.97 (s, 0.9 H, OMe_E), 4.05 (s, 2.1 H, OMe_Z), 4.41 (m, 1 H, Hα_{Leu}), 4.90 (s, $CH_{2,Ac}$), 7.51 (s, 0.7 H, $CHNO_Z$), 7.56 (m, 2 H, $H3_{Bz}$), 7.66 (m, 1 H, $H4_{Bz}$), 7.70 (s, 0.3 H, $H4_E$), 7.94 (s, 0.3 H, $H2'_E$), 7.96 (s, 0.7 H, $H2'_Z$), 8.03 (m, 2 H, $H2_{Bz}$), 8.03 (s, 0.7 H, $H4_Z$), 8.12 (s, 0.3 H, $CHNO_E$), 8.67 (m, NH) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 21.7 (Cδ_{Leu}), 23.3 (Cδ_{Leu}), 25.9 (Cγ_{Leu}), 41.5 (Cβ_{Leu}), 48.1 ($CH_{2,Ac}$), 52.4 (Cα_{Leu}), 63.1 (OMe_E), 63.6 (OMe_Z), 120.0 (C4′), 121.0 ($C4_E$), 124.6 ($C4_Z$), 124.8 ($C5_Z$), 128.2 ($C5_E$), 129.3 ($C2_{Bz}$), 129.9 ($C3_{Bz}$), 133.7 ($C1_{Bz,Z}$), 133.8 ($C1_{Bz,E}$), 134.0 ($ImCHN_Z$), 134.1 ($C4_{Bz}$), 137.7 ($ImCHN_E$), 140.4 (C2′), 140.9 ($C2_Z$), 142.3 ($C2_E$), 168.4 (CH_2CO), 169.1 ($CO_{Bz,E}$), 169.2 ($CO_{Bz,Z}$), 175.5 (CO_{Leu}) ppm. HRMS (ESI-TOF) calcd for [MH^+] $C_{23}H_{28}N_7O_5^+$ 482.2146, found 482.2126.

(*S*)-2-(2-{5′-Benzamido-4(5)-[2-(2,4-dinitrophenyl)hydrazonomethyl]-1*H*,1′*H*-2,4′-biimidazol-1′-yl}acetamido)-4-methylpentanoic acid (9b): Yield 12.1 mg (38%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$, 323 K) δ 0.79 (d, J = 6.1 Hz, 3 H, Hδ_{Leu}), 0.81 (d, J = 6.2 Hz, 3 H, Hδ_{Leu}), 1.49 (m, 2 H, Hβ_{Leu}), 1.54 (m, 1 H, Hγ_{Leu}), 4.27 (m, 1 H, Hα_{Leu}), 4.77 (d, 16.8 Hz, $CH_{2,Ac}$), 4.82 (d, J = 16.8 Hz, $CH_{2,Ac}$), 7.54 (m, 2 H, $H3_{Bz}$), 7.64 (m, 1 H, $H4_{Bz}$), 7.76 (s, 1 H, H4), 7.93 (s, 1 H, H2′), 8.04 (m, 2 H, $H2_{Bz}$), 8.18 (d, 9.6 Hz, 1 H, 6_{DNP}), 8.25 (dd, 2.5 Hz, 9.6 Hz, 1 H, 5_{DNP}), 8.42 (d, 7.9 Hz, 1 H, NH), 8.59 (s, 1 H, CHNN), 8.87 (d, 2.5 Hz, 3_{DNP}), 10.25 (b, 1 H, NH), 11.63 (s, 1 H, NH) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2SO$, 323 K) δ 21.1 (Cδ_{Leu}), 22.4 (Cδ_{Leu}), 24.0 (Cγ_{Leu}), 39.9 (Cβ_{Leu}), 46.7 ($CH_{2,Ac}$), 50.3 (Cα_{Leu}), 117.0 ($C6_{DNP}$), 121.7 (C4′), 122.6 ($C3_{DNP}$), 123.9 (C4), 127.8 ($C2_{Bz}$), 128.1 ($C3_{Bz}$), 128.9 ($C5_{DNP}$), 129.2 ($C2_{DNP}$), 130.3 (C5), 131.8 ($C4_{Bz}$), 133.1 ($C1_{Bz}$), 136.9 ($C4_{DNP}$), 137.5 (C2′), 139.4 (ImCHN), 142.6 (C2), 144.2 ($C1_{DNP}$), 165.7 (CH_2CO), 166.4 (CO_{Bz}), 173.2 (CO_{Leu}) ppm. Calcd for [MH^+] $C_{28}H_{29}N_{10}O_8^+$ 633.2164, found 633.2154.

(*S*)-2-{2-[5′-Benzamido-4(5)-(2-phenylhydrazonomethyl)-1*H*,1′*H*-2,4′-biimidazol-1′-yl]acetamido}succinic acid (9e): Yield 1.7 mg (6%). ^{1}H NMR (500 MHz, CD_3OD, 278 K) δ 2.83 (m, 2 H, Hβ_{Asp}), 4.78 (m, 1 H, Hα_{Asp}), 4.89 (d, J = 16.8 Hz, 1 H, $CH_{2,Ac}$), 4.93 (d, J = 16.8 Hz, 1 H, $CH_{2,Ac}$), 6.83 (m, 1 H, $H4_{Ph}$), 7.10 (m, 2 H, $H3_{Ph}$), 7.19 (m, 2 H, $H2_{Ph}$), 7.49 (s, 1 H, H4), 7.56 (m, 2 H, $H3_{Bz}$), 7.66 (m, 1 H, $H4_{Bz}$), 7.72 (s, 1 H, CHNN), 7.94 (s, 1 H, H2′), 8.07 (m, 2 H, $H2_{Bz}$) ppm; ^{13}C NMR (125 MHz, CD3OD, 278 K) δ 36.7 (Cβ_{Asp}), 48.3 ($CH_{2,Ac}$), 50.6 (Cα_{Asp}), 113.7 ($C2_{Ph}$), 117.8 (C4), 120.8 (C4′), 121.2 ($C4_{Ph}$), 123.6 (ImCHN), 129.4 ($C2_{Bz}$), 130.0 ($C3_{Bz}$), 130.1 ($C3_{Ph}$), 132.8 (C5), 133.8 ($C1_{Bz}$), 134.1 ($C4_{Bz}$), 140.0 (C2′), 141.4 (C2), 145.9 ($C1_{Ph}$), 168.5 (CH_2CO), 169.7 (CO_{Bz}), 173.7 (CO_{Asp}), 173.8 (CO_{Asp}) ppm. Calcd for [MH^+] $C_{26}H_{25}N_8O_6^+$ 545.1892, found 545.1897.

(*S*,*E*/*Z*)-2-{2-[5′-Acetamido-4(5)-(phenoxyiminomethyl)-1*H*,1′-*H*-2,4′-biimidazol-1′-yl]acetamido}-3-hydroxypropanoic acid (10e): Yield as TFA salt 13.3 mg (47%). ^{1}H NMR (500 MHz, CD_3OD, 273 K) δ 2.26 (s, 1.5 H, $CH_{3,Ac}$), 2.27 (s, 1.5 H, $CH_{3,Ac}$), 3.89 (dd, J = 3.6 Hz, J = 11.2 Hz,1 H, H$\beta_{Ser,E,Z}$), 3.96 (dd, J = 5.0 Hz, J = 11.2 Hz, 1 H, H$\beta_{Ser,E,Z}$), 4.56 (m, 1 H, H$\alpha_{Ser,E,Z}$), 4.90 (m, 2 H, $CH_{2,Ac,E,Z}$), 7.08 (m, 1 H, $H4_{Ph,E}$), 7.12 (m, 1 H, $H4_{Ph,Z}$), 7.29 (m, 1 H, $H3_{Ph,E}$), 7.35 (m, 1 H, $H3_{Ph,Z}$), 7.37 (m, 2 H, $H2_{Ph}$), 7.88 (s, 0.5 H, $CHNO_Z$), 7.88 (s, 0.5 H, $H4_E$), 7.93 (s, 0.5 H, H2′$_E$), 7.95 (s, 0.5 H, H2′$_Z$), 8.33 (s, 0.5 H, $H4_Z$), 8.55 (s, 0.5 H, $CHNO_E$) ppm; ^{13}C NMR (125 MHz, CD_3OD 273 K) δ 23.2 (Me_{Ac}), 48.0 ($CH_{2,Ac}$), 56.4 (Cα_{Ser}), 62.6 (Cβ_{Ser}), 115.4 ($C3_{Ph,E}$), 115.8 ($C3_{Ph,Z}$), 119.2 ($C4'_E$), 119.6 ($C4'_Z$), 122.8 ($C4_E$), 124.1 ($C4_{Ph,E}$), 124.4 ($C5_Z$), 124.6 ($C4_{Ph,Z}$), 126.0 ($C4_Z$), 127.3 ($C5_E$), 130.5 ($C2_{Ph,E}$), 130.6 ($C2_{Ph,Z}$), 137.2 ($ImCHN_Z$), 140.1 (C2′), 141.2 ($C2_Z$), 141.3 ($ImCHN_E$), 142.8 ($C2_E$), 160.2 ($C1_{Ph,Z}$), 160.4 ($C1_{Ph,E}$), 168.3 (CH_2CO_Z), 168.4 (CH_2CO_E), 173.1 (CO_{Ser}), 173.4 ($CO_{Ac,E}$), 173.7 ($CO_{Ac,Z}$) ppm. Calcd for [MH^+] $C_{20}H_{22}N_7O_6^+$ 456.1626, found 456.1624.

(*S*)-3-(1*H*-Indol-3-yl)-2-{2-[5′-isobutyramido-4(5)-(morpholinomethyl)-1*H*,1′*H*-2,4′-biimidazol-1′-yl]acetamido}propanoic acid (11e): Yield 3.5 mg (12%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$, 298 K) δ 1.05 (d, J = 6.6 Hz, 3 H, $CH_{3,i\text{-}Bu}$), 1.12 (d, J = 6.6 Hz, 3 H, $CH_{3,i\text{-}Bu}$), 2.73 (sep, J = 6.6 Hz, 1 H, $CH_{i\text{-}Bu}$), 3.22 (dd, J = 8.3 Hz, J = 15.0 Hz, 1 H, Hβ_{Trp}), 3.25 (br, 4 H, NCH_2), 2 H, 3.36 (dd, J = 4.6 Hz, J = 15.0 Hz, 1 H, Hβ_{Trp}), 3.89 (br, 4 H, OCH_2), 4.44 (br, 2 H, $ImCH_2N$), 4.71 (d, J = 16.7 Hz, 1 H, $CH_{2,Ac}$), 4.77 (d, J = 16.7 Hz, 1 H, $CH_{2,Ac}$), 4.82 (m, 1 H, Hα_{Trp}), 7.01 (m, 1 H, $H7_{Trp}$), 7.09 (m, 1 H, $H6_{Trp}$), 7.18 (s, 1 H, $H2_{Trp}$), 7.37 (m, 1 H, $H5_{Trp}$), 7.58 (m, 1 H, $H8_{Trp}$), 7.62 (s, 1 H, H4), 7.72 (s, 1 H, NH), 7.74 (s, 1 H, H2′), 9.97 (s, 1 H, NH), 10.26 (s, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $(CD_3)_2CO$, 298 K) δ 19.6 ($C3_{i\text{-}Bu}$), 28.1 (Cβ_{Trp}), 35.3 ($C2_{i\text{-}Bu}$), 48.3 ($CH_{2,Ac}$), 51.6 ($ImCH_2N$), 52.4 ($NCH_{2,morpholine}$), 53.8 (Cα_{Trp}), 65.0 ($OCH_{2,morpholine}$), 110.6 ($C1_{Trp}$), 112.2 ($C5_{Trp}$), 119.1 ($C8_{Trp}$), 119.6 ($C7_{Trp}$), 121.1 (C4′), 121.9 (C4), 122.2 ($C6_{Trp}$), 124.3 ($C2_{Trp}$), 126.3 (C5), 128.3 ($C9_{Trp}$), 137.4 ($C4_{Trp}$), 138.8 (C2′), 142.2 (C2), 166.7 (CH_2CO), 173.1 (CO_{Trp}), 178.6 ($CO_{i\text{-}Bu}$) ppm. Calcd for [MH^+] $C_{28}H_{35}N_8O_5^+$ 563.2725, found 563.2722.

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Supporting Information Available: General experimental methods, experimental procedures, and characterization of compounds **2b**,**c**, characterization of products not reported above, tables of ^{13}C NMR data of products **9**−**11**, and ^{1}H and ^{13}C NMR spectra of products. This material is available free of charge via the Internet at http://pubs.acs.org.