Synthesis N. Schädel et al. Paper

Synthesis of Imidazole and Histidine-Derived Cross-Linkers as Analogues of GOLD and Desmosine

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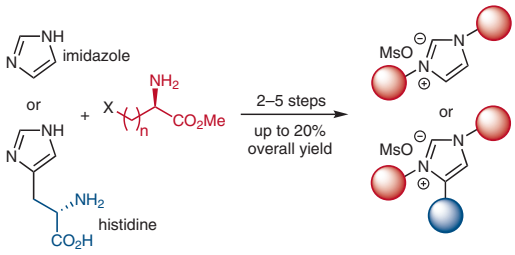
Dedicated to Prof. Horst Kunz on the occasion of his 80th birthday



Received: 16.11.2020
Accepted: 11.01.2021
Published online: 22.02.2021
DOI: 10.1055/s-0040-1706144; Art ID: ss-2020-z0588-op

Abstract Amino acid derivatives with a central cationic heterocyclic core (e.g., imidazolium) are biologically relevant cross-linkers of proteins and advanced glycation end (AGE) products. Here, imidazolium-containing cross-linkers were synthesized from imidazole or histidine by N-alkylation employing aspartate- and glutamate-derived mesylates as key step. Biological investigations were carried out to probe the biocompatibility of these compounds.

Keywords amino acid, cross-linker, desmosine, GOLD, biological activity, advanced glycation end-products

Heterocyclic amino acids gain increasing interest as cross-linkers for proteins and polysaccharides.[1] Pyridinium amino acids are important members of this compound class. For example, the cationic cross-linkers desmosine (**1**) and isodesmosine (**2**) (Figure 1) cause the unique tensile strength and elasticity of elastin, a major constituent of connective tissue.[2]

The structurally similar pyridinoline (**3a**) and deoxypyridinoline (**3b**) are found in collagen cross-links.[3] These tetrafunctional pyridinium amino acids are biosynthetically generated via cyclocondensation of four lysine residues. Several diseases lead to the destruction of elastin, and thus the free cross-linkers are relevant as biomarkers via LC-MS analysis for the diagnosis of chronic obstructive pulmonary disease (COPD), cystic fibrosis, α_1-antitrypsin deficiency (AATD), or bronchiectasis.[4]

In addition, imidazolium amino acids are also prominent members of heterocyclic amino acids, for example, glyoxal-lysine dimer (GOLD) **4** and methylglyoxal-lysine dimer (MOLD) **5**, are cross-linking advanced glycation end-products (AGEs).[5] Their biosynthesis originates in the Maillard reaction of proteins and carbohydrates.[6b] Imidazolium compounds **4**, **5**, and other AGEs are relevant in the

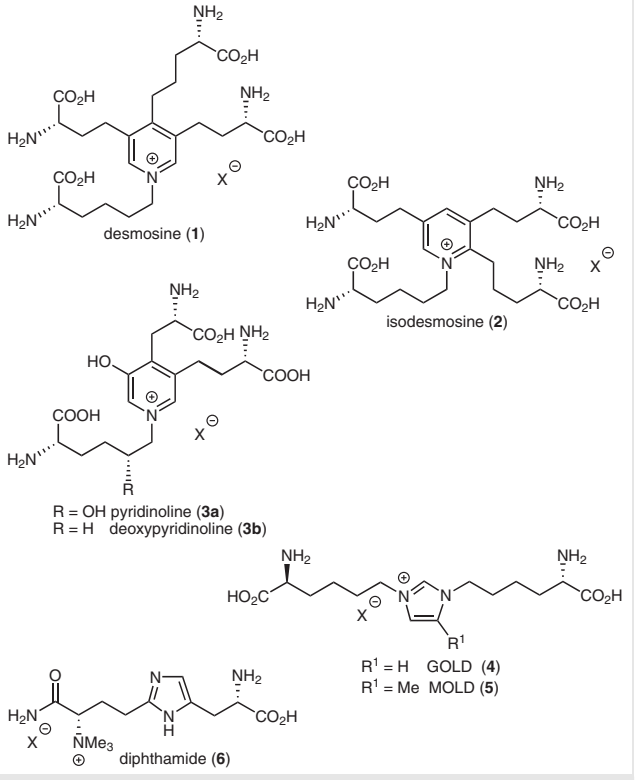


Figure 1 Prominent examples of known heterocyclic amino acid cross-linkers

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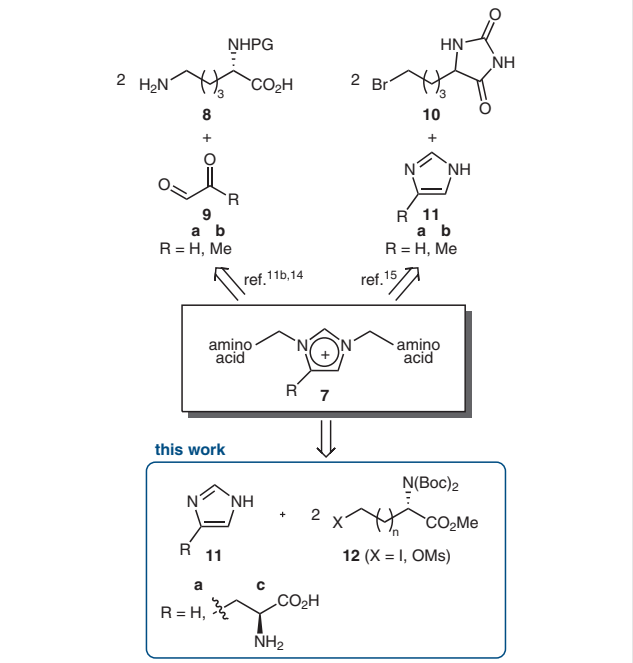
browning and processing of food[6] as well as the aging of tissue resulting in protein-degenerative diseases.[7] Another member of bifunctional imidazole amino acids is diphthamide (**6**), a post-translationally modified histidine residue of the elongation factor 2 (EF2), which plays a key role in cell proliferation.[8] Thus, elucidation of the various biological functions of these heterocyclic amino acid cross-linkers has motivated endeavors towards their synthesis.[3,9–12]

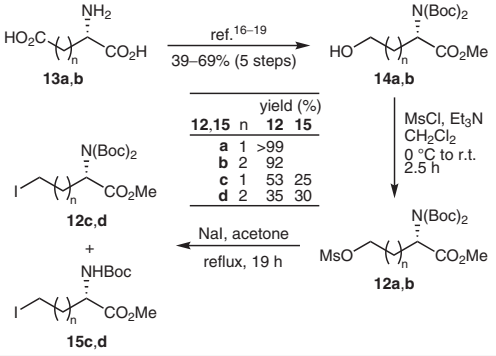
The majority of previous synthetic efforts has been devoted to the pyridinium containing cross-linkers.[3,9,10,13] Much less work has been carried out regarding the synthesis of imidazolium cross-linkers bearing amino acid subunits. While Brimble reported an acid-catalyzed condensation of the Fmoc-protected lysine derivatives **8** with glyoxal (**9a**) or methylglyoxal (**9b**) (Scheme 1),[11a,b] Cravotto independently performed the condensation under basic conditions with microwave irradiation.[14] Linetsky utilized racemic ω-bromohydantoins **10** and imidazole (**11a**) or *N*-methylimidazole (**11b**) for the two-fold N-alkylation and subsequent deprotection.[15] As an alternative attempt, we were interested to explore the alkylation of imidazoles **11a**,**c** with N-protected amino acid methyl esters **12**. Of particular interest was the reaction with L-histidine (**11c**), because the additional amino acid function may offer structural variability and thus access to more complex cross-linkers. In the current manuscript, we report on the successful realization of the N-alkylation approach. Considering the biological impact of these imidazolium bisamino acids **7** when cross-linked with the respective proteins,[11] it is surprising that the biological activity, for example, cytotoxicity of the imidazolium bisamino acids themselves, has not received much attention so far. Therefore, we performed preliminary biological screenings of the imidazolium target compounds **7** and their precursors. The results are discussed below.



Scheme 1 Previous synthetic strategies towards imidazolium bisamino acids and current work

Alkylation of Imidazole (11a)

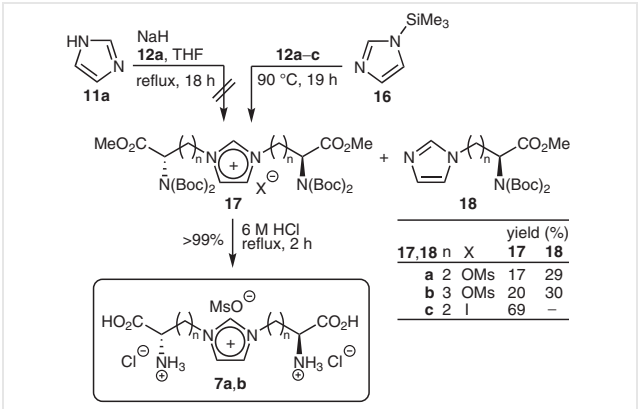
For the synthesis of bisamino acids **7** according to Scheme 1 either two-fold N-alkylation of imidazole (**11a**) with two alkylating agents **12** or N-alkylation of the imidazole moiety of histidine (**11c**) were planned as key steps. The required amino acid functionalities in the side chains should be introduced by using the respective amino acid derived alkylation agents **12**. Their synthesis was therefore accomplished from L-aspartic acid (**13a**) and L-glutamic acid (**13b**), respectively, as depicted in Scheme 2. The methyl 2-(*N*,*N*-di-Boc-amino)hydroxyalkanoates **14**[16–19] were accessible in five steps from **13a** and **13b** in 39 and 69% total yield, respectively, via a sequence of esterification, two-fold Boc-protection, and reduction with DIBAL-H followed by $NaBH_4$ according to literature procedures.[16–19] Mesylation of **14a**,**b** with MsCl in CH_2Cl_2–Et_3N yielded the desired alkylating reagents **12a** in >99% and **12b** in 92% yield, respectively. The Finkelstein reaction of mesylates **12** with NaI in acetone was accompanied by partial deprotection of the second Boc group and thus gave the *N*,*N*-di-Boc-protected iodides **12c**,**d** in 35–53% yield together with the mono-Boc-protected iodides **15c**,**d**.



Scheme 2 Synthesis of alkylating reagents **12** starting from L-aspartic acid (**13a**) and L-glutamic acid (**13b**) [for details, see Supporting Information (SI)]

In order to achieve the two-fold N-alkylation of imidazole (**11a**), mesylates **12a**,**b** were examined first (Scheme 3). In a preliminary experiment, imidazole (**11a**) was deprotonated with NaH in THF and heated with mesylate **12a** under reflux overnight, but no conversion could be detected. We therefore followed a protocol by Dyson and co-workers.[20] Trimethylsilylimidazole (**16**) was heated with 2.2 equivalents of mesylate **12a** at 90 °C for 19 hours without any solvent to give the desired imidazolium mesylate **17a** in 17% yield (Scheme 3). Despite the excess of alkylating agent

12a, the reaction could not be driven to completion and the corresponding monoalkylated imidazole **18a** was also isolated in 29% yield.[21]



17,18	n	X	yield (%) 17	18
a	2	OMs	17	29
b	3	OMs	20	30
c	2	I	69	–

Scheme 3 Synthetic route to imidazolium based bisamino acids **7** by alkylation of trimethylsilylimidazole (**16**) followed by acidic deprotection of **17** (for details see SI)

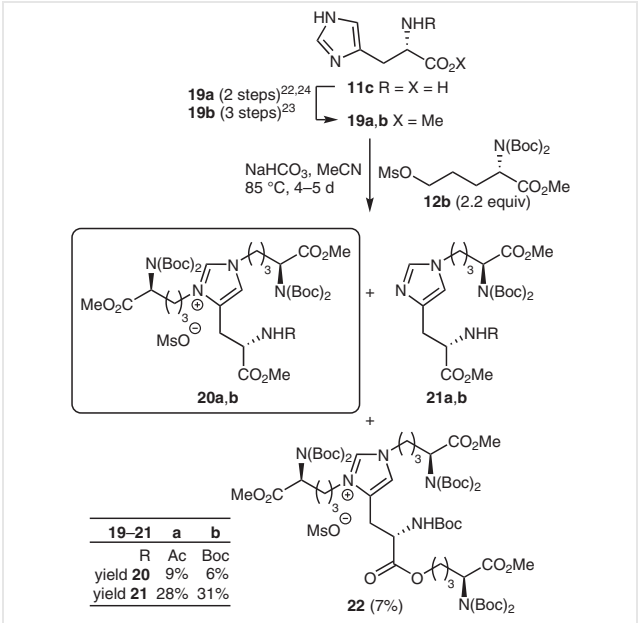
Similar results were obtained for the homologous mesylate **12b**, which gave **17b** and **18b** in 20 and 30% yield, respectively. When the aspartate-derived iodide **12c** was used instead, the dialkylated imidazolium iodide **17c** was isolated in 69% yield after chromatographic purification. Final deprotection of the imidazolium mesylates **17** with aqueous 6 M HCl under reflux gave the imidazole amino acids **7a**,**b** in quantitative yield.

Alkylation of Histidine Derivatives 19

The synthesis of histidine-based precursors **20** and **21** via N-protected histidine methyl esters **19a**,**b**,[22–24] which were prepared from L-histidine (**11c**), is shown in Scheme 4.

In order to test the alkylation reaction, first 1-iodohexane was chosen as a model alkylating reagent (Scheme S3, SI). When *N*-acetylhistidine methyl ester **19a** was treated with 1.1 equivalents of 1-iodohexane in acetonitrile in the presence of $NaHCO_3$ at 85 °C for 5 days following a method by Albrecht and co-workers,[24] methyl *N*-acetyl-1,3-dihexylhistidinate iodide (**S8a**) was obtained in 22% yield. The alkylation of the corresponding *N*-Boc-histidine methyl ester **19b** gave methyl *N*-Boc-1,3-dihexylhistidinate iodide (**S8b**) and the monoalkylated product **S9b** in 22 and 2% yield, respectively (Scheme S3, SI). In these experiments, dialkylation was preferred over monoalkylation, but neither the dialkylated nor monoalkylated product could be obtained exclusively.

When L-glutamate-derived mesylate **12b** (2.2 equiv) was employed in the alkylation of *N*-acetylhistidine methyl ester **19a**, the dialkylated histidinate mesylate **20a** could be

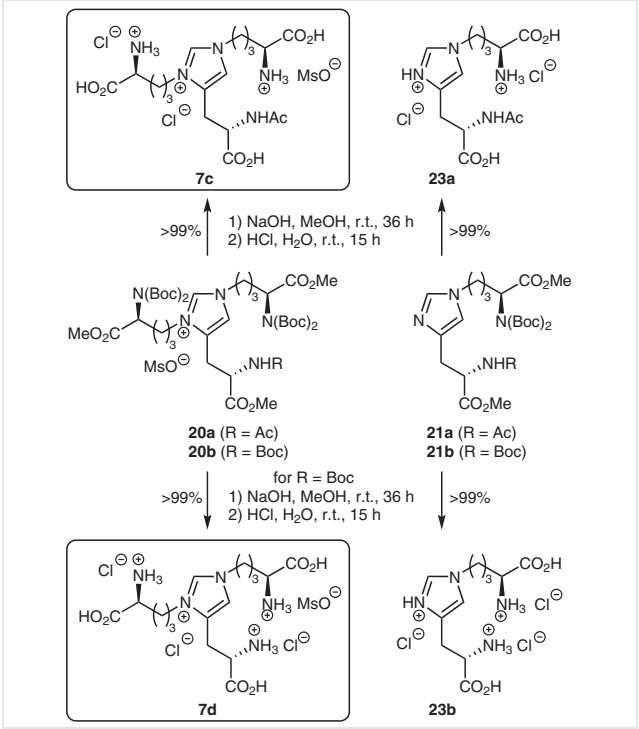


19–21	a	b
R	Ac	Boc
yield 20	9%	6%
yield 21	28%	31%

Scheme 4 Alkylation of N-protected histidine methyl esters **19** with mesylate **12b** to give histidine derivatives **20** and **21** (for details see SI)

obtained as the minor product (9%), whereas the monoalkylated derivative **21a** was isolated as the major product (28%) (Scheme 4). Under similar conditions, the alkylation of *N*-Boc-histidine methyl ester **19b** was also dominated by formation of monoalkylation product **21b** (31%). The dialkylated histidinate mesylate **20b** was isolated in 6% yield. Additionally, 7% of the mesylate **22** was isolated, resulting from a transesterification of the histidine methyl ester moiety. The mesylate **12b** was a less powerful alkylating agent under these conditions than iodohexane.

Finally, the synthesis of the histidine-based bisamino acids **7c**,**d**, and **23a**,**b** was accomplished by deprotection (Scheme 5). By a two-step strategy from basic hydrolysis of esters **20** and **21** with aqueous 1 M NaOH in MeOH[25] and subsequent treatment of intermediate carboxylates with aqueous 6 M HCl in H_2O, the trifunctional histidine compounds **7c**,**d** and monoalkylated derivatives **23** were obtained quantitatively together with 2.5–11.4 equivalents of NaCl.[26] For the bisamino acids **7c** and **23a** derived from *N*-acetylhistidine methyl ester **19a**, the ^1H and ^{13}C NMR spectra in D_2O showed signal doubling for several proton and carbon signals, whereas for **7d** and **23b** only one set of signals was obtained (see SI). Presumably, signal doubling was caused by conformational diastereomers (rotamers), which is in good agreement with previous experimental and theoretical studies on protected amino acids, small peptide derivatives,[27] and N-acetylated amino acids.[28]



Scheme 5 Deprotection of histidine methyl esters **20** and **21** to bis-**7c**,**d** and mono-amino acid alkylated histidine salts **23**. NaCl precipitated with the deprotected amino acids (for details see SI).

Evaluation of Biological Activity

The biological activities of bisamino acids and precursors were evaluated using the mammalian cell line L929 (murine fibroblast cell line) and bacteria (*Escherichia coli* ΔTolC and *Staphylococcus aureus*), respectively, as test organisms. They were incubated with defined concentrations of the test compounds for a limited period of time, and the remaining viability or growth of the cells was determined. The viability of mammalian cells was quantified via the resazurin reduction assay,[29] and growth of the bacteria was evaluated via measurements of the turbidity of the growth medium at 600 nm (for details of the experimental procedures, see SI). To obtain an overview of the activity, all compounds were used in the single rather high concentration of 100 μM. The L929 cells were incubated with the compounds for 72 hours to allow the cells to proliferate for at least 3 generations. In viable cells, the oxidized non-fluorescent blue resazurin is reduced to the strongly fluorescent red resorufin, of which the fluorescence was determined (λ_{ex} = 540 nm; λ_{em} = 590 nm). Subsequently, the compounds showing high activity (fluorescence of treated cells <50% fluorescence of non-treated cells) were selected and the concentration leading to 50% cell viability was determined (IC_{50} values). As shown in Table 1, most of the test compounds showed no biological activity, in particular the unprotected derivatives **7** and **23**. Only the *N*-Boc-protected two-armed and three-armed histidines **20b**, **21b**, and **22**, respectively, showed some cytotoxic effects, of which the *N*-Boc-protected three-armed histidine **22** displayed the most pronounced cytotoxicity with an IC_{50} value of 12 ± 7 μM. This compound also inhibited growth of *Staphylococcus aureus* (IC_{50} = 7.3 ± 2 μM).

In conclusion, a series of bisamino acid alkylated imidazole- and histidine-derived compounds have been conveniently synthesized in 2–5 steps from starting materials **16** and **11c**, respectively, and 2–20% overall yield utilizing a two-fold N-alkylation as the key step with alkylating agents synthesized from L-aspartic and L-glutamic acids **13a**,**b**. A preliminary biological screening revealed the absence of cytotoxic and antibiotic effects for the free imidazolium bisamino acids, which enables their use as mimics of desmosine and GOLD, because their interaction with proteins or other cellular components is not obscured by cytotoxic (or antibiotic) effects.

Table 1 Cytotoxicity and Antimicrobial Activity of Amino Acid Alkylated Histidine Derivatives[a]

Compound	L929 (% viability)	L929 IC_{50} (μM)	*E. coli* ΔTolC (% growth)	*E. coli* ΔTolC IC_{50} (μM)	*S. aureus* (% growth)	*S. aureus* IC_{50} (μM)
7c	78.3	n.d.	104.4	n.d.	109.3	n.d.
7d	97.3	n.d.	113.0	n.d.	116.6	n.d.
12b	102.1	n.d.	84.6	n.d.	n.d.	n.d.
20a	75	n.d.	96.8	n.d.	100.5	n.d.
20b	0.4	20.0 ± 5	88.4	n.d.	n.d.	n.d.
21a	97.3	n.d.	93.0	n.d.	n.d.	n.d.
21b	32.0	>>50	91.0	n.d.	88.1	n.d.
22	0.05	12.0 ± 7	96.1	n.d.	3.6	7.3 ± 2
23a	105.3	n.d.	97.1	n.d.	111.5	n.d.
23b	103.2	n.d.	100.9	n.d.	117.0	n.d.

[a] Cell viability was screened at a single concentration of 100 μM. IC_{50} >50 μM can be considered as non-active; n.d. = not determined.

Melting points were determined on a Stuart SMP10 apparatus or an Olympus BX50 microscope with a Linkam TP93 temperature control and are uncorrected. NMR spectra were recorded on Bruker Avance 500 (^{1}H 500 MHz, ^{13}C 125 MHz) and Avance III HD (^{1}H 700 MHz, ^{13}C 175 MHz) spectrometers, using $CDCl_3$, D_2O, and CD_3OD as solvents. Chemical shifts are given in ppm and were referenced to residual solvent signal. Coupling constants are given as frequencies in Hz. The signals were assigned by using additional HSQC, COSY, HMBC, and NOESY experiments. For easier comparison of NMR spectra, atom numbering may deviate from the IUPAC nomenclature. IR spectra were taken on a Bruker FT-IR spectrophotometer ALPHA with diamond ATR system (Platinum ATR) and a Bruker Vector 22 with MKII Golden Gate Single Reflection Diamant ATR system and are reported cm^{-1}. LRMS spectra and HRMS spectra were recorded via electrospray (ESI) ionization on a Bruker micrOTOF-Q. Column chromatography was performed using silica gel 60 M (Macherey-Nagel, grain size 40–63 µm). TLC was performed on Macherey-Nagel Alugram® Xtra SIL G/UV$_{254}$ plates and visualized with permanganate reagent (3.0 g $KMnO_4$, 20 g K_2CO_3, 5.0 mL of a 5% NaOH solution in 300 mL H_2O) or phosphomolybdic acid reagent (12 g [12 $MoO_3{\cdot}H_3PO_4{\cdot}xH_2O$] in 200 mL EtOH). All chemicals were used as purchased, unless otherwise stated. CH_2Cl_2 and NEt_3 were dried over CaH_2 by heating at reflux and subsequent distillation, THF was dried over potassium with benzophenone as an indicator. Hexanes (bp 30–70 °C), EtOAc, CH_2Cl_2, and MeOH for chromatography were distilled prior to use. Moisture-sensitive reactions were performed in oven-dried glassware under N_2 atmosphere.

Alkylation of TMS-Imidazole 16; General Procedure

Under N_2 atmosphere TMS-imidazole **16** (1.00 mmol) was added to the appropriate alkylation agent **12a–c** (2.20 mmol), and the reaction mixture was stirred under reflux overnight. The oil was washed with Et_2O (4 mL) and EtOAc (15 mL). The remaining oil was purified by column chromatography on SiO_2 with CH_2Cl_2/MeOH to give the corresponding imidazolium salts.

1,3-Bis{(3*S*)-3-[bis(*tert*-butoxycarbonyl)amino]-4-methoxy-4-oxobutyl}-1*H*-imidazol-3-ium Methanesulfonate (17a)

Yield: 135 mg (17%); orange oil; R_f = 0.49 (CH_2Cl_2/MeOH 9:1).

FT-IR (ATR): 3428 (w), 2981 (w), 2360 (w), 1789 (w), 1742 (m), 1696 (m), 1567 (w), 1457 (w), 1366 (s), 1314 (m), 1223 (m), 1164 (m), 1143 (s), 1115 (s), 1040 (m), 912 (m), 853 (m), 768 (m), 730 (m), 644 (w), 552 (w), 527 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.50 (s, 36 H), 2.38–2.50 (m, 2 H), 2.75 (s, 3 H), 2.73–2.83 (m, 2 H), 3.72 (s, 6 H), 4.29–4.40 (m, 2 H), 4.54–4.63 (m, 2 H), 4.85 (dd, *J* = 8.3, 5.7 Hz, 2 H), 7.36 (s, 2 H), 10.05 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.0, 31.1, 39.5, 47.2, 52.6, 54.8, 84.2, 121.9, 138.8, 152.2, 170.2.

MS (ESI): *m*/*z* = 699 [M]$^+$, 599 [M – Boc]$^+$.

HRMS (ESI): *m*/*z* [M]$^+$ calcd for [$C_{33}H_{55}N_4O_{12}$]$^+$: 699.3811; found: 699.3816.

Methyl (2*S*)-2-[Bis(*tert*-butoxycarbonyl)amino]-4-(1*H*-imidazol-1-yl)butanoate (18a)

Yield: 113 mg (29%); yellow oil; R_f = 0.71 (CH_2Cl_2/MeOH 9:1).

FT-IR (ATR): 2981 (w), 2360 (w), 1792 (w), 1745 (s), 1700 (m), 1507 (w), 1457 (w), 1367 (s), 1332 (m), 1168 (m), 1142 (s), 1116 (m), 1051 (w), 907 (w), 853 (w), 812 (w), 784 (w), 644 (w), 625 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.50 (s, 18 H), 2.26–2.35 (m, 1 H), 2.62–2.72 (m, 1 H), 3.73 (s, 3 H), 4.06 (t, *J* = 7.2 Hz, 2 H), 4.83 (dd, *J* = 8.6, 5.8 Hz, 2 H), 6.95 (s, 1 H), 7.07 (s, 1 H), 7.49 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.0, 31.9, 44.1, 52.5, 55.4, 83.8, 118.7, 129.8, 137.3, 152.0, 170.5.

MS (ESI): *m*/*z* = 383 [M]$^+$, 368 [M – CH_3]$^+$, 312, 283 [M – Boc]$^+$, 252, 226, 210, 196, 168 [M – 2 × Boc – CH_3]$^+$, 151, 124, 95, 82, 69 [imidazole]$^+$, 57, 41.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for [$C_{18}H_{30}N_3O_6$]$^+$: 384.2129; found: 384.2111.

1,3-Bis{(4*S*)-4-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}-1*H*-imidazol-3-ium Methanesulfonate (17b)

Yield: 163 mg (20%); yellow oil; R_f = 0.50 (CH_2Cl_2/MeOH 9:1).

FT-IR (ATR): 3434 (w), 2980 (w), 2231 (w), 1788 (w), 1743 (s), 1698 (m), 1564 (w), 1457 (w), 1367 (s), 1314 (m), 1250 (m), 1217 (s), 1169 (s), 1143 (s), 1040 (m), 1001 (w), 909 (w), 853 (w), 767 (w), 731 (w), 665 (w), 645 (w), 552 (w), 526 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.49 (s, 36 H), 1.84–1.94 (m, 2 H), 1.96–2.07 (m, 4 H), 2.14–2.23 (m, 2 H), 2.78 (s, 3 H), 3.71 (s, 6 H), 4.30–4.45 (m, 4 H), 4.84 (dd, *J* = 8.1, 5.9 Hz, 2 H), 7.24 (d, *J* = 1.7 Hz, 2 H), 10.2 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.8, 27.0, 28.0, 39.6, 49.3, 52.4, 57.1, 83.7, 121.3, 138.8, 152.2, 170.7.

MS (ESI): *m*/*z* = 727 [M]$^+$, 627 [M – Boc]$^+$.

HRMS (ESI): *m*/*z* [M]$^+$ calcd for [$C_{35}H_{59}N_4O_{12}$]$^+$: 727.4124; found: 727.4106.

Methyl {(2*S*)-2-[Bis(*tert*-butoxycarbonyl)amino]-5-(1*H*-imidazol-1-yl)}pentanoate (18b)

Yield: 119 mg (30%); yellow oil; R_f = 0.67 (CH_2Cl_2/MeOH 9:1).

FT-IR (ATR): 2979 (w), 2933 (w), 2360 (w), 1791 (w), 1741 (m), 1699 (m), 1508 (w), 1455 (w), 1366 (s), 1312 (m), 1250 (m), 1227 (m), 1166 (m), 1140 (s), 1035 (w), 906 (w), 852 (m), 810 (w), 783 (w), 764 (w), 732 (w), 664 (w), 624 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.48 (s, 18 H), 1.79–1.92 (m, 3 H), 2.07–2.16 (m, 1 H), 3.71 (s, 3 H), 3.91–4.05 (m, 2 H), 4.88 (dd, *J* = 8.7, 5.6 Hz, 1 H), 6.91 (s, 1 H), 7.05 (s, 1 H), 7.48 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.0, 27.9, 28.0, 46.4, 52.3, 57.2, 83.5, 118.7, 129.5, 137.1, 151.0, 170.8.

MS (ESI): *m*/*z* = 420 [M + Na]$^+$, 398 [M + H]$^+$, 354, 320, 298 [M – Boc]$^+$, 288, 270, 242, 227, 159.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for [$C_{19}H_{31}N_3O_6$]$^+$: 398.2286; found: 398.2300.

1,3-Bis{(3*S*)-3-[bis(*tert*-butoxycarbonyl)amino]-4-methoxy-4-oxobutyl}-1*H*-imidazol-3-ium Iodide (17c)

Yield: 86.0 mg (69%); yellow oil; R_f = 0.30 (CH_2Cl_2/MeOH 12:1).

FT-IR (ATR): 2980 (w), 2929 (w), 2360 (w), 1745 (s), 1698 (m), 1564 (w), 1456 (w), 1367 (s), 1313 (m), 1251 (m), 1144 (s), 1120 (s), 903 (s), 853 (w), 724 (s), 649 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.51 (s, 36 H), 2.43–2.52 (m, 2 H), 2.75–2.84 (m, 2 H), 3.72 (s, 6 H), 4.30–4.37 (m, 2 H), 4.56–4.64 (m, 2 H), 4.84 (dd, *J* = 8.4, 5.6 Hz, 2 H), 7.37 (br d, *J* = 1.6 Hz, 2 H), 9.99 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.0, 31.1, 47.2, 52.6, 54.8, 84.2, 121.9, 138.8, 152.2, 170.2.

MS (ESI): m/z = 699 $[M]^+$, 599 $[M - Boc]^+$.

HRMS (ESI): m/z $[M]^+$ calcd for $[C_{33}H_{55}N_4O_{12}]^+$: 699.3811; found: 699.3808.

Deprotection of Imidazolium Salts 17; General Procedure

The appropriate imidazolium salt **17** (1.0 equiv) and a 6 M solution of HCl in H_2O (8.1 equiv) were stirred at reflux for 2 h. After cooling to r.t., the solvent was evaporated under reduced pressure. The crude product was washed with acetone (1 mL) und Et_2O (1 mL) to give to the corresponding product **7**.

1,3-Bis[(3*S*)-3-ammonio-3-carboxypropyl]-1*H*-imidazol-3-ium Dichloride Methanesulfonate (7a)

Yield: 62.0 mg (>99%); brown oil.

FT-IR (ATR): 3360 (w), 2476 (m), 2216 (w), 2071 (m), 1646 (w), 1450 (w), 1215 (m), 1120 (m), 972 (s), 821 (w) cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 2.46–2.54 (m, 2 H), 2.54–2.63 (m, 2 H), 2.72 (s, 3 H), 4.11 (dd, *J* = 8.2, 5.5 Hz, 2 H), 4.49–4.62 (m, 4 H), 7.77 (d, *J* = 1.5 Hz, 2 H), 9.27 (s, 1 H).

^{13}C NMR (125 MHz, CD_3OD): δ = 31.9, 39.6, 47.3, 51.0, 124.2, 138.6, 170.1.

MS (ESI): m/z = 271 $[M]^+$, 155, 125.

HRMS (ESI): m/z $[M]^+$ calcd for $[C_{11}H_{19}N_4O_4]^+$: 271.1401; found: 271.1382.

1,3-Bis[(4*S*)-4-ammonio-4-carboxybutyl]-1*H*-imidazol-3-ium Dichloride Methanesulfonate (7b)

Yield: 79.0 mg (>99%); brown oil; $[\alpha]_D^{20}$ –25 (*c* 1.35, CH_2Cl_2).

FT-IR (ATR): 3359 (w), 2472 (m), 2215 (w), 2070 (m), 1730 (w), 1452 (w), 1166 (w), 1121 (m), 972 (s), 820 (w) cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 1.90–1.99 (m, 2 H), 1.99–2.13 (m, 4 H), 2.13–2.24 (m, 2 H), 2.72 (s, 3 H), 4.03–4.13 (m, 2 H), 4.31–4.41 (m, 4 H), 7.72 (d, *J* = 1.3 Hz, 2 H), 9.20 (s, 1 H).

^{13}C NMR (125 MHz, CD_3OD): δ = 27.0, 28.2, 39.6, 50.1, 53.2, 124.0, 137.8, 171.3.

MS (ESI): m/z = 360, 333, 316, 299 $[M]^+$, 232, 202, 184 $[M - C_5H_{10}NO_2]^+$, 153, 133, 116 $[C_5H_{10}NO_2]^+$.

MS (ESI–): m/z = 94.98 $[SO_3CH_3]^-$.

Alkylation of Histidine Derivatives; General Procedure

A solution of histidine derivative **19a** or **19b** (2.39 mmol), $NaHCO_3$ (9.56 mmol), and mesylate **12b** (5.26 mmol) in MeCN (40 mL) was stirred at 85 °C for 4–5 days. After evaporation of the solvent, the residue was taken up in CH_2Cl_2 (20 mL) and filtered. The filtrate was concentrated and the residue purified by column chromatography on MsOH-treated SiO_2 with CH_2Cl_2/MeOH (20:1 → 5:1) or EtOAc/MeOH (20:1 → 3:1) (for **20a**,**b**) and CH_2Cl_2/MeOH (40:1 → 5:1) or EtOAc/MeOH (10:1 → 5:1) (for **21a**,**b**) to give the corresponding products **20** and **21**.

Treatment of SiO_2: To MsOH (100 mL, 99%) diluted with demineralized H_2O (1.0 L) was added dry silica gel until a viscous suspension was obtained. After vigorous stirring and shaking for 1 h, the silica gel was filtered and washed several times with demineralized H_2O until free of residual acid. Subsequently the silica gel was washed with acetone several times and dried under N_2 flow or at 50 °C under reduced pressure.

Methyl *N*-Acetyl-1,3-bis{(4*S*)-4-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}histidinate Methanesulfonate (20a)

Yield: 0.21 g (9%); colorless solid; mp 57–60 °C; R_f = 0.39 (EtOAc/MeOH 3:1); $[\alpha]_D^{20}$ –5 (*c* 0.10, CH_2Cl_2).

FT-IR (ATR, $CDCl_3$): 2981 (w), 1740 (vs), 1697 (s), 1559 (w), 1456 (w), 1437 (m), 1367 (vs), 1311 (m), 1218 (s), 1170 (vs), 1141 (vs), 1117 (vs), 1040 (s), 915 (m), 852 (m), 768 (m), 727 (vs), 646 (m), 552 (m), 526 (m), 464 (w) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.47 (s, 36 H), 1.79–1.90 (m, 2 H), 1.90–1.99 (m, 4 H), 2.01 (s, 3 H), 2.09–2.20 (m, 2 H), 2.68 (s, 3 H), 3.12–3.20 (m, 1 H), 3.38–3.46 (m, 1 H), 3.69 (s, 6 H), 3.73 (s, 3 H), 4.09–4.32 (m, 4 H), 4.70–4.76 (m, 1 H), 4.78–4.84 (m, 2 H), 7.44–7.48 (m, 1 H), 8.43 (2 d, *J* = 7.9 Hz, 1 H), 8.76–8.80 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.8, 25.0, 25.1 (C-3′)*, 26.57, 26.62, 26.66 (C-4″, C-4‴)*, 26.83, 26.84, 26.87, 26.88, 26.96, 26.97, 27.03, 27.05 (C-3″, C-3‴)*, 28.1, 39.5, 46.8, 49.5, 50.8, 50.9 (C-2′)*, 52.4, 52.5, 52.9, 57.1, 83.78, 83.83, 120.99, 121.03, 121.09, 121.12 (C-5)*, 132.12, 132.15, 132.20 (C-4)*, 135.1, 152.3, 170.82, 170.84, 171.0, 171.7.

Signal doubling (marked with *) in NMR spectra presumably due to the presence of conformational diastereomers.

MS (ESI+): m/z = 870.48 $[M]^+$, 770.42 $[M - Boc]^+$, 714.36 $[M - Boc - tBu]^+$, 670.37 $[M - 2 \times Boc]^+$, 614.30 $[M - 2 \times Boc - tBu]^+$, 514.25 $[M - 3 \times Boc - tBu]^+$, 385.17.

HRMS (ESI+): m/z $[M]^+$ calcd for $[C_{41}H_{68}N_5O_{15}]^+$: 870.4706; found: 870.4756.

MS (ESI–): m/z = 94.98 $[SO_3CH_3]^-$.

Methyl *N*-Acetyl-1-{(4*S*)-4-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}-L-histidinate (21a)

Yield: 0.36 g (28%); colorless oil; R_f = 0.51 (EtOAc/MeOH 5:1); $[\alpha]_D^{20}$ –28 (*c* 0.10, CH_2Cl_2).

FT-IR (ATR, $CDCl_3$): 3276 (br), 2979 (w), 2952 (w), 1789 (w), 1742 (vs), 1698 (s), 1677 (s), 1534 (m), 1502 (m), 1437 (m), 1367 (vs), 1307 (m), 1250 (s), 1229 (s), 1169 (s), 1141 (vs), 1118 (vs), 1005 (w), 919 (w), 852 (m), 784 (w), 732 (w), 645 (w) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 1.48 (s, 18 H), 1.76–1.89 (m, 3 H), 2.03 (s, 3 H), 2.05–2.14 (m, 1 H), 2.97 (ddd, *J* = 14.9, 4.4, 1.9 Hz, 1 H), 3.08 (dd, *J* = 14.9, 5.5 Hz, 1 H), 3.67, 3.68 (2 s, 3 H)*, 3.70 (s, 3 H), 3.84–3.96 (m, 2 H), 4.75–4.80 (m, 1 H), 4.80–4.87 (m, 1 H), 6.68 (s, 1 H), 7.23 (t, *J* = 7.7 Hz, 1 H), 7.39 (s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.4, 27.0, 27.1 (C-3″)*, 27.8, 27.9 (C-4″)*, 28.1, 29.6, 46.65, 46.66 (C-5″)*, 52.34, 52.36, 52.45, 52.53 (2 × CO_2CH_3, C-2′)*, 57.3, 83.7, 116.6, 116.7 (C-5)*, 136.80, 136.83 (C-2)*, 137.7, 137.8 (C-4)*, 152.31, 152.33, 170.2, 170.9, 172.12, 172.14 ($C{=}O_{Ac}$, 2 × CO_2CH_3)*.

Signal doubling (marked with *) in ^1H and ^{13}C NMR spectra presumably due to the presence of conformational diastereomers. Additionally, a second signal set with low intensity was observed in the ^1H NMR spectrum, probably from the imidazolium-N3-alkylated regioisomer (as determined from ^1H-^1H-NOESY). Ratio major/minor regioisomer: 95:5 by integration of the ^1H NMR spectrum.

MS (ESI): m/z = 1103.54, 579.24 $[M + K]^+$, 563.27 $[M + Na]^+$, 541.29 $[M + H]^+$, 479.19 $[M - Boc + K]^+$, 463.21 $[M - Boc + Na]^+$, 441.23 $[M - Boc + H]^+$, 407.15 $[M - Boc - tBu + Na]^+$, 385.17 $[M - Boc - tBu + H]^+$, 363.16 $[M - 2 \times Boc + Na]^+$, 343.16, 325.15, 283.14.

HRMS (ESI): m/z $[M + Na]^+$ calcd for $[C_{25}H_{40}N_4O_9Na]^+$: 541.2868; found: 541.2866.

Methyl 1,3-Bis{(4*S*)-4-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}-*N*-(*tert*-butoxycarbonyl)-L-histidinate Methanesulfonate (20b)

Yield: 0.17 g (6%); colorless solid; mp 68 °C; R_f = 0.43 (EtOAc); $[\alpha]_D^{20}$ +4 (*c* 0.10, CH_2Cl_2).

FT-IR (ATR, $CDCl_3$): 3268 (br), 2980 (m), 2935 (w), 1787 (w), 1743 (vs), 1701 (vs), 1526 (w), 1456 (m), 1367 (vs), 1251 (s), 1221 (vs), 1168 (vs), 1144 (vs), 1121 (vs), 1041 (s), 918 (w), 854 (m), 769 (m), 731 (m), 646 (w), 553 (w), 527 (w) cm^{-1}.

^1H NMR (700 MHz, $CDCl_3$): δ = 1.38–1.41 (m, 9 H), 1.46, 1.47 (2 s, 36 H)*, 1.81–1.91 (m, 2 H), 1.91–2.02 (m, 4 H), 2.10–2.21 (m, 2 H), 2.71 (s, 3 H), 3.12–3.25 (m, 2 H), 3.68 (s, 6 H), 3.75 (s, 3 H), 4.14–4.34 (m, 4 H), 4.51 (br s, 1 H), 4.81 (br s, 2 H), 6.05–6.13 (m, 1 H), 7.20–7.25 (m, 1 H), 9.57 (s, 1 H).

^{13}C NMR (175 MHz, $CDCl_3$): δ = 26.2, 26.9, 27.0, 28.06, 28.07, 28.3 [5 × $C(CH_3)_3$]*, 39.6, 46.74, 46.78, 49.37, 49.40 (C-5″, C-5‴)*, 52.4, 53.0, 57.2, 80.6, 83.7, 120.1, 131.4, 137.2, 152.3, 155.8, 170.81, 170.84, 171.0.

Signal doubling (marked with *) in NMR spectra presumably due to the presence of conformational diastereomers.

MS (ESI+): *m*/*z* = 1243.85, 1143.76, 1043.68, 928.59 $[M]^+$, 872.52 [M – *t*Bu]$^+$, 828.52 [M –Boc]$^+$, 772.44 [M – Boc – *t*Bu]$^+$, 728.44 [M – 2 × Boc]$^+$, 672.37 [M – 2 × Boc – *t*Bu]$^+$, 572.30 [M – 3 × Boc – *t*Bu]$^+$, 472.24 [M – 4 × Boc – *t*Bu]$^+$.

HRMS (ESI+): *m*/*z* $[M]^+$ calcd for $[C_{44}H_{74}N_5O_{16}]^+$: 928.5125; found: 928.5108.

MS (ESI–): *m*/*z* = 94.98 $[SO_3CH_3]^-$.

Methyl 1-{(4*S*)-4-[Bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}-*N*-(*tert*-butoxycarbonyl)-L-histidinate (21b)

Yield: 0.48 g (31%); colorless oil; R_f = 0.43 (EtOAc); $[\alpha]_D^{20}$ –5 (*c* 0.28, CH_2Cl_2).

FT-IR (ATR, $CDCl_3$): 3355 (br), 2979 (w), 2952 (w), 2935 (w), 1790 (w), 1743 (s), 1702 (vs), 1496 (m), 1455 (m), 1437 (m), 1366 (vs), 1306 (m), 1249 (s), 1163 (vs), 1139 (vs), 1116 (vs), 1052 (m), 1025 (m), 915 (m), 853 (m), 782 (m), 730 (vs), 646 (m), 463 (w) cm^{-1}.

^1H NMR (700 MHz, $CDCl_3$): δ = 1.41 (s, 9 H), 1.46 (s, 18 H), 1.73–1.87 (m, 3 H), 2.04–2.11 (m, 1 H), 2.96 (dd, *J* = 14.7, 4.6 Hz, 1 H), 3.05 (dd, *J* = 14.7, 5.1 Hz, 1 H), 3.67 (2 s, 3 H)*, 3.69 (s, 3 H), 3.82–3.93 (m, 2 H), 4.47–4.54 (m, 1 H), 4.82–4.86 (m, 1 H), 5.93 (t, *J* = 9.8 Hz, 1 H), 6.65 (d, *J* = 2.7 Hz, 1 H), 7.34 (s, 1 H).

^{13}C NMR (175 MHz, $CDCl_3$): δ = 27.05, 27.08, 27.85, 27.89 (C-3″, C-4″)*, 28.1, 28.5, 30.31, 30.32 (C-3′)*, 46.5, 52.2, 52.4, 53.7, 57.3, 79.6, 83.6, 116.4, 116.5 (C-5)*, 136.91, 136.94 (C-2)*, 137.79, 137.83 (C-4)*, 152.3, 155.8, 170.9, 172.7.

Signal doubling (marked with *) in NMR spectra presumably due to the presence of conformational diastereomers.

MS (ESI): *m*/*z* = 621.32 $[M + Na]^+$, 599.34 $[M + H]^+$, 521.27 [M – Boc + Na]$^+$, 443.22 [M –Boc – *t*Bu + H]$^+$, 399.23 [M – 2 × Boc + H]$^+$, 365.15 [M – 2 × Boc – *t*Bu + Na]$^+$, 343.17 [M –2 × Boc – *t*Bu + H]$^+$, 321.16 [M – 3 × Boc + Na]$^+$, 283.15.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $[C_{28}H_{46}N_4O_{10}Na]^+$: 621.3106; found: 621.3233.

{(4*S*)-4-[Bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl} 1,3-Bis{(4*S*)-4-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-5-oxopentyl}-*N*-(*tert*-butoxycarbonyl)-L-histidinate Methanesulfonate (22)

Yield: 0.24 g (7%); colorless solid; mp 75–78 °C; R_f = 0.50 (CH_2Cl_2/MeOH 10:1); $[\alpha]_D^{20}$ –2 (*c* 0.10, CH_2Cl_2).

FT-IR (ATR, $CDCl_3$): 2979 (m), 2935 (w), 1789 (w), 1741 (vs), 1699 (vs), 1523 (w), 1456 (m), 1366 (vs), 1312 (m), 1223 (s), 1120 (vs), 1144 (vs), 1160 (vs), 1041 (s), 918 (w), 853 (m), 768 (m), 731 (m), 646 (w), 553 (w), 526 (w), 465 (w) cm^{-1}.

^1H NMR (700 MHz, $CDCl_3$): δ = 1.40 (s, 9 H), 1.47 (s, 54 H), 1.66–1.77 (m, 2 H), 1.83–1.92 (m, 3 H), 1.92–2.04 (m, 4 H), 2.11–2.20 (m, 3 H), 2.72 (s, 3 H), 3.08–3.17 (m, 1 H), 3.19–3.28 (m, 1 H), 3.69 (s, 9 H), 4.09–4.35 (m, 6 H), 4.50 (br s, 1 H), 4.79–4.87 (m, 3 H), 5.90–6.00 (m, 1 H), 7.20–7.24 (m, 1 H), 9.73 (s, 1 H).

^{13}C NMR (175 MHz, $CDCl_3$): δ = 25.4, 26.3, 26.5 (C-3′)*, 26.95, 26.98, 27.04, 28.1, 28.3, 39.6, 46.76, 46.80, 49.38, 49.43 (C-5″, C-5‴)*, 52.36, 52.43, 57.21, 57.24, 57.54, 57.56 (C-2″, C-2‴, C-2⁗)*, 65.68, 65.77 (C-5⁗)*, 80.6, 83.5, 83.7, 120.0, 131.4, 137.4, 152.3, 155.7, 170.6, 170.8, 170.9, 171.0 (3 × CO_2CH_3)*.

Signal doubling (marked with *) in ^1H and ^{13}C NMR spectra presumably due to the presence of conformational diastereomers.

MS (ESI+): *m*/*z* = 1458.80, 1243.68 $[M]^+$, 1143.63 [M – Boc]$^+$, 643.36, 587.30, 543.31, 340.68.

HRMS (ESI+): *m*/*z* $[M]^+$ calcd for $[C_{59}H_{99}N_6O_{22}]^+$: 1243.6807; found: 1243.6796.

MS (ESI–): *m*/*z* = 94.97 $[SO_3CH_3]^-$.

Deprotection of Histidine Derivatives 20a and 21a; General Procedure

To a solution of the appropriate **20a** or **21a** (1.0 equiv) in MeOH (3 mL) was added a 1 M solution of NaOH in H_2O (3.78 equiv for **20a**, 2.5 equiv for **21a**), and the reaction mixture was stirred at r.t. for 36 h. Then the solvent was evaporated, the residue was taken up in demineralized H_2O (1 mL), a 6 M solution of HCl in H_2O (74.3 equiv for **20a**, 50 equiv for **21a**) was added, and the mixture was stirred at r.t. for 15 h followed by evaporation of the solvent. To determine the pH value, the residue was taken up in demineralized H_2O.

***N*-Acetyl-1,3-bis[(4*S*)-4-ammonio-4-carboxybutyl]-L-histidine Dichloride Methanesulfonate (7c)**

Yield: 41.0 mg (>99%, with 3.75 equiv NaCl, calculated from the used amount of NaOH); colorless solid; $[\alpha]_D^{20}$ +12 (*c* 0.23, H_2O).

FT-IR (ATR, solid): 2935 (br s), 1730 (vs), 1638 (m), 1561 (m), 1417 (m), 1197 (vs), 1149 (vs), 1039 (vs), 840 (m), 778 (m), 622 (m), 533 (s), 523 (s) cm^{-1}.

^1H NMR (500 MHz, D_2O): δ = 1.87–2.17 (m, 8 H), 2.01 (s, 3 H), 2.80 (s, 3 H), 3.17 (dd, *J* = 16.0, 9.4 Hz, 1 H), 3.38 (dd, *J* = 16.0, 5.1 Hz, 1 H), 4.12–4.20 (m, 2 H), 4.21–4.33 (m, 4 H), 4.73–4.77 (m, 1 H), 7.45 (s, 1 H), 8.91 (s, 1 H).

^{13}C NMR (125 MHz, D_2O): δ = 21.8, 25.1, 25.19, 25.21, 25.24, 26.4, 26.5 (C-3′, C-3″, C-3‴, C-4″, C-4‴)*, 38.5, 46.1, 48.8, 51.0, 52.1, 120.66, 120.69 (C-5)*, 131.2, 135.7, 171.3, 173.1, 174.1.

In the ^{13}C NMR spectrum signal doubling (marked with *) presumably due to the presence of conformational diastereomers.

MS (ESI+): *m*/*z* = 450.19, 428.21 $[C_{18}H_{30}N_5O_7]^+$, 335.13, 313.15, 271.14, 198.09.

HRMS (ESI+): *m*/*z* [M]$^+$ calcd for $[C_{18}H_{30}N_5O_7]^+$: 428.2140; found: 428.2124.

MS (ESI–): *m*/*z* = 448.18, 426.20 $[C_{18}H_{28}N_5O_7]^-$, 311.13, 196.07.

HRMS (ESI–): *m*/*z* [M]$^-$ calcd for $[C_{18}H_{28}N_5O_7]^-$: 426.1983; found: 426.1951.

_N_-Acetyl-1-[(4_S_)-4-ammonio-4-carboxybutyl]-L-histidine Dichloride (23a)

Yield: 37.0 mg (>99%, with 2.5 equiv NaCl, calculated from the used amount of NaOH); colorless solid; $[\alpha]_D^{20}$ +11 (*c* 0.14, H_2O).

FT-IR (ATR, solid): 3361 (br s), 3244 (br s), 3130 (s), 2854 (vs), 1727 (vs), 1619 (vs), 1546 (vs), 1433 (m), 1376 (m), 1216 (s), 1128 (m), 1039 (w), 828 (w), 610 (m), 530 (m) cm^{-1}.

^{1}H NMR (500 MHz, D_2O): δ = 1.85–2.17 (m, 4 H), 2.00 (s, 3 H), 3.17 (dd, *J* = 15.5, 8.6 Hz, 1 H), 3.33 (dd, *J* = 15.5, 5.2 Hz, 1 H), 4.15 (t, *J* = 5.8 Hz, 1 H), 4.28 (t, *J* = 6.4 Hz, 2 H), 4.73 (dd, *J* = 8.6, 5.2 Hz, 1 H), 7.24 (s, 1 H), 8.72 (d, *J* = 1.1 Hz, 1 H).

^{13}C NMR (125 MHz, D_2O): δ = 21.7, 25.2, 25.3, 26.4, 48.4, 51.7, 52.1, 119.85, 119.87 (C-5)*, 129.8, 134.5, 171.4, 173.3, 174.0.

In the ^{13}C NMR spectrum, signal doubling (marked with *) presumably due to the presence of conformational diastereomers. Additionally, a second signal set with low intensity was observed in the ^{1}H NMR spectrum, probably from the imidazolium-N3-alkylated regioisomer (as determined from ^{1}H-^{1}H-NOESY).

MS (ESI–): *m*/*z* = 391.08, 333.12, 311.14 $[C_{13}H_{19}N_4O_5]^-$, 196.07.

HRMS (ESI–): *m*/*z* [M]$^-$ calcd for $[C_{13}H_{19}N_4O_5]^-$: 311.1361; found: 311.1358.

Deprotection of Histidine Derivatives 20b, 21b; General Procedure

To a solution of the appropriate **20b** or **21b** (1.0 equiv) in MeOH (2 mL) was added a 1 M solution of NaOH in H_2O (3.78 equiv for **20b**, 2.5 equiv for **21b**), and the reaction mixture was stirred at r.t. for 28 h. Then the solvent was evaporated, the residue was taken up in demineralized H_2O (1 mL) and a 6 M solution of HCl in H_2O (50.3 equiv for **20b**, 50.3 equiv for **21b)** was added. After stirring for 36 at r.t., the mixture was concentrated, taken up in H_2O (2 mL), again a 1 M solution of NaOH in H_2O (7.6 equiv for **20b**, 5 equiv for **21b**) was added and the mixture stirred for 36 h. Then a 6 M solution of HCl in H_2O (50.3 equiv for **20b**, 50.3 equiv for **21b**) was added, and the reaction mixture was stirred for 30 min followed by evaporation of the solvent.

1,3-Bis[(4_S_)-4-ammonio-4-carboxybutyl]-L-histidine Trichloride Methanesulfonate (7d)

Yield: 58.0 mg (>99%, with 7.5 equiv NaCl, calculated from the used amount of NaOH); yellowish solid; $[\alpha]_D^{20}$ +7 (*c* 0.15, H_2O).

FT-IR (ATR, solid): 3367 (br m), 2924 (br vs), 1733 (vs), 1603 (m), 1562 (m), 1509 (m), 1418 (m), 1200 (vs), 1153 (vs), 1041 (vs), 847 (w), 779 (m), 622 (w), 555 (s), 523 (m) cm^{-1}.

^{1}H NMR (500 MHz, D_2O): δ = 1.98–2.25 (m, 8 H), 2.86 (s, 3 H), 3.49 (dd, *J* = 16.5, 7.1 Hz, 1 H), 3.58 (dd, *J* = 16.5, 7.4 Hz, 1 H), 4.21–4.28 (m, 2 H), 4.32–4.40 (m, 4 H), 4.52 (t, *J* = 7.1 Hz, 1 H), 7.70 (s, 1 H), 9.05 (s, 1 H).

^{13}C NMR (125 MHz, D_2O): δ = 24.1, 25.2, 26.4, 26.5, 38.6, 46.4, 49.0, 51.4, 52.22, 52.24, 121.8, (C-5), 129.2, 136.3, 170.1, 171.3, 171.4.

MS (ESI+): *m*/*z* = 430.17, 408.18, 386.20 $[C_{16}H_{28}N_5O_6]^-$, 253.06, 187.02, 169.01.

HRMS (ESI+): *m*/*z* [M]$^+$ calcd for $[C_{16}H_{28}N_5O_6]^+$: 386.2034; found: 386.2019.

MS (ESI–): *m*/*z* = 384.19 $[C_{16}H_{26}N_5O_6]^-$, 328.80, 268.80, 210.85, 152.94.

HRMS (ESI–): *m*/*z* [M]$^-$ calcd for $[C_{16}H_{26}N_5O_6]^-$: 384.1878; found: 384.1903.

1-[(4_S_)-4-Ammonio-4-carboxybutyl]-L-histidine Trichloride (23b)

Yield: 68.0 mg (>99%, with 7.5 equiv NaCl, calculated from the used amount of NaOH); yellow solid; $[\alpha]_D^{20}$ +14 (*c* 0.20, H_2O).

FT-IR (ATR, solid): 3369 (br m), 3117 (br s), 2853 (br vs), 2627 (br s), 1731 (vs), 1617 (m), 1600 (m), 1548 (m), 1505 (m), 1417 (m), 1203 (vs), 824 (m), 623 (m), 520 (m) cm^{-1}.

^{1}H NMR (500 MHz, D_2O): δ = 1.95–2.23 (m, 4 H), 3.48 (dd, *J* = 16.0, 7.0 Hz, 1 H), 3.53 (dd, *J* = 16.0, 6.7 Hz, 1 H), 4.22 (t, *J* = 5.9 Hz, 1 H), 4.35 (t, *J* = 6.5 Hz, 2 H), 4.49 (t, *J* = 6.7 Hz, 1 H), 7.62 (s, 1 H), 8.85 (s, 1 H).

^{13}C NMR (125 MHz, D_2O): δ = 25.2, 25.3, 26.5, 48.7, 51.9, 52.2, 121.1, 127.4, 135.3, 170.1, 171.4.

MS (ESI–): *m*/*z* = 291.11, 269.13 $[C_{11}H_{17}N_4O_4]^-$.

HRMS (ESI–): *m*/*z* [M]$^-$ calcd for $[C_{11}H_{17}N_4O_4]^-$: 269.1244; found: 269.1258.

Funding Information

Generous financial support by the Fonds der Chemischen Industrie (Ph.D. fellowship for N.S.), the Ministerium für Wissenschaft, Forschung und Kunst des Landes Baden-Württemberg (project BioMatS-11 BiogelPlus), the Carl-Zeiss-Stiftung (Projekthaus NanoBioMater), and the Alfred-Kärcher-Stiftung are gratefully acknowledged.

Acknowledgement

The excellent technical assistance of Brigitte Pawletta, HZI, in the cell-culture lab is gratefully acknowledged.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1706144.

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