Direct Reductive Alkylation of Amino Acids: Synthesis of Bifunctional Chelates for Nuclear Imaging

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Abstract: A family of effective bifunctional chelators for technetium- and rhenium-based radiopharmaceuticals was conveniently synthesized in high yields through direct reductive N-alkylations of amino acids and their analogues with aldehydes, using $NaBH(OAc)_3$ as an efficient reagent. The mono-, di-, tetra- and even mixed alkylated amino acid derivatives were all prepared in one-pot synthesis.

Key words: reductive alkylation, amino acids, bifunctional chelators, radiopharmaceuticals, sodium triacetoxyborohydride

Radiolabeled bioactive peptides are attractive vectors for targeting a variety of diseases through interaction with specific cell surface receptors.[1] Introduction of a molecular entity that allows facile labeling with medically useful radionuclides such as Tc-99m for diagnosis and Re-186/188 for targeted therapy, without significant alteration of the biopolymer's structure and binding affinity, is desirable.[2] Current approaches make use of polypeptides that can form chelate complexes with the radiometals. While these peptidic chelates have been shown to provide a means of complexing both Tc and Re as the $\{M(V)\text{-}oxo\}^{+3}$ core,[3–8] they suffer from limitations related to metal complex instability and to the uncertainty of metal complex formation with the proposed metal binding sequence versus the sequence responsible for receptor binding. Alternatives to peptidic chelates, such as hydrazinonicotinic acid (HYNIC), which is introduced commonly through acylation of a lysine residue, provide more stable complexation of Tc over peptidic chelates.[9–11] However, the coordination chemistry of the Tc-HYNIC complex is such that metal binding sites are not completely saturated by HYNIC, allowing both multimodal binding as well as coligand lability and exchange which significantly influence the target and nontarget localization of the radiolabeled peptide.[12]

Technetium tricarbonyl chemistry has recently been the subject of considerable interest in radiopharmaceutical development.[13] Previous studies on the coordination chemistry of the $\{M(CO)_3\}^{+1}$ core has established amine, aromatic heterocyclic, and carboxylate donors as very effective chelating ligands.[14] These observations led to the design of tridentate chelators constructed as extensions of the side chain of amino acids. Such amino acid analogues provide a tridentate donor set for chelation and an amino acid functionality for attachment to biomolecules. We recently developed a family of such single amino acid chelates (SAAC) that serve this function[14,15] and can be readily incorporated into peptides via solid-phase synthesis techniques.[16]

This approach affords significant flexibility in the choice of donors for ^{99m}Tc coordination, combined with the considerable advantages provided by application of routine solid-phase synthetic techniques. Most significantly, the addition of a chelating moiety as a single amino acid substitution (or addition) to the peptide sequence should minimize the perturbation of structure and function compared to more elaborate chelating systems which are comprised of three to four amino acids in addition to chemical tethers or spacers. However, the exploitation of this approach to labeling requires facile, high yield syntheses of the derivatized amino acids, preferably without isomerization.

One synthetic approach involves reductive amination of aldehydes and ketones, a very useful reaction in modern organic preparations. In the case of amino acids, the reductive alkylation with aldehydes is generally performed after protection of the acid functionality as the ester,[17] rendering this a cumbersome three-step procedure. The reports that detail the reductive alkylation of amino acids with an open acid functionality (*direct*) are surprisingly few. The preferred reagent for this purpose is $NaCNBH_3$[18], while $NaBH_4$[19], H_2/C-$Pd(OH)_2$,[20] and NaHTe[21] have occasionally been used. Although $NaCNBH_3$ is an excellent reagent, it is relatively toxic and the isolated products are often contaminated with cyanide residue. One limitation is that the reactions are restricted to polar medium, which is sometimes sluggish and often mitigated by poor yields. Furthermore, the scope of the substrate is limited in heterogeneous hydrogenations as many functional groups, such as nitro, alkene, alkyne, OBn etc., are susceptible under the reaction condition. The use of $NaBH_4$ necessitates the preformed imine to avoid the reduction of aldehyde, and also alkaline medium, thereby limiting its practicality. Abdel-Magid et al.[22] have recently demonstrated that $NaBH(OAc)_3$ can effectively be used as a mild reagent in the reductive amination of aldehydes and ketones with shorter reaction time and excellent yields. It is surprising, that with the exception of a single example, there are no major reports of use of

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$NaBH(OAc)_3$ with free amino acids,[23e] even though there are several reports in the literature for the corresponding amino acid esters.[23] We sought to exploit the above method to provide direct access to ligands useful for applications to nuclear medicine.

As part of our ongoing efforts to develop practical preparative methods for a broad-spectrum of bifunctional ligands for nuclear imaging, we noted literature reports of reductive alkylation of phenylalanine with pyridine-2-carboxaldehyde for which a 21% yield with $NaCNBH_3$ had been obtained for the monoalkyl derivative.[18b] We have now investigated the dialkylation of the same system with $NaBH(OAc)_3$ in 1,2-dicholorethane (DCE) (Scheme 1). The reaction was complete within two hours, and the product **6** was isolated in 78% yield. Since DCE has been used in similar reactions, we employed the same medium, despite the insolubility of amino acids in DCE. It is noteworthy that the reaction was homogeneous in DCE despite the insolubility of amino acids while a suspension-like mixture was evident in the polar solvent MeCN. The rate of the reaction was significantly faster in chlorinated solvents, DCE and CH_2Cl_2 compared to THF and MeCN. The dipicolyl derivative of L-phenylalanine (compound **6**) was crystallized and structurally characterized by X-ray crystallography. Figure 1 shows an ORTEP view of the structure.[24]

The preparative results of the reductive alkylation of various amino acids and their derivatives with different aldehydes are summarized in Tables 1 and 2. The amino acids and heterocyclic aldehydes were chosen such that the products could ultimately be used as chelating agents in nuclear imaging with ^{99m}Tc. By appropriate stoichiometric manipulation of the amino acids and aldehydes, the mono-, di-, and even tetraalkylated amino acid derivatives could be obtained in good yields. In the case of tetraalkylation, the lysine dihydrochloride salt was treated with 4.5 equivalents of pyridine-2-carboxaldehyde and $NaBH(OAc)_3$ (5 equivalents) to afford the tetrapyridyl derivative **10** in 79% yield, along with a small amount of the reduced alcohol of the aldehyde. It is noteworthy that four alkylation steps are performed in one-pot in the case of such tetraalkyl amino acids, an observation consistent with the direct use of the amino acid hydrochloride salt as starting materials without any need for prior neutralization or base addition to the reaction, at the expense of a slight excess of aldehyde (0.5 equiv). In order to determine the generality of the protocol, we investigated the reductive amination of a noncoordinating aryl aldehyde. The required dialkylated product **11** was isolated in 74% yield, in addition to the monoalkylation product in 12% yield.

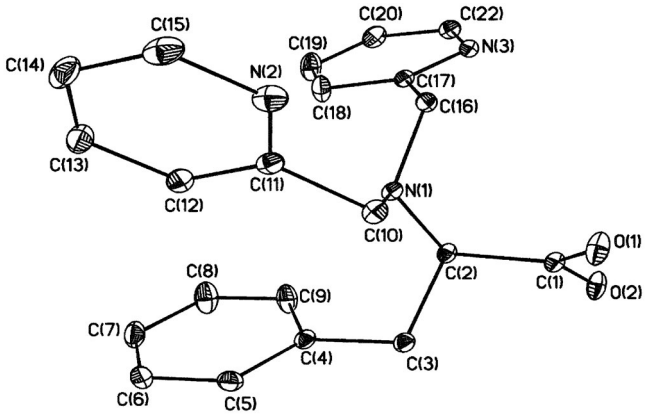


Figure 1 An ORTEP view of the structure of **6**, showing the atom-labeling scheme and 50% probability ellipsoids.

Table 1 Preparative Di- and Tetraalkylation

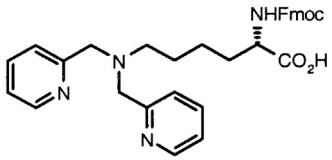
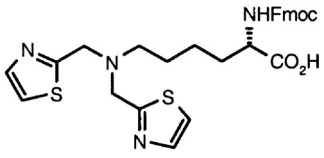
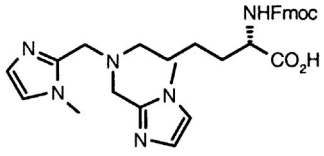
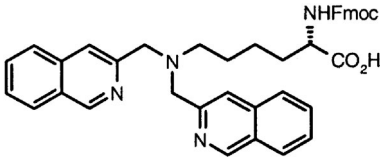
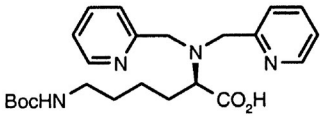
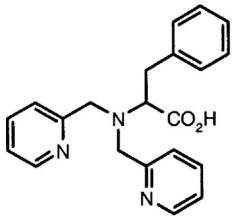
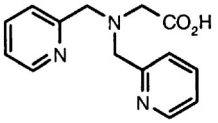
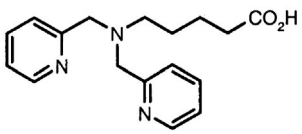
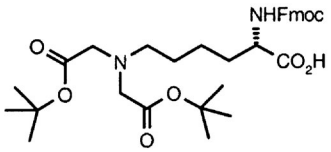
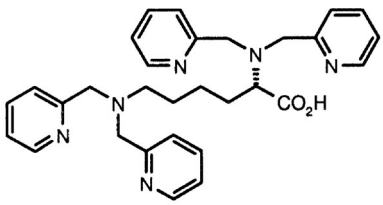
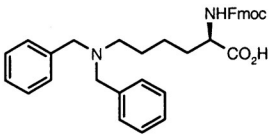
Product	Amino Acid	Aldehyde	Product[a]	Time (h)	Yield[b] (%)
1	N_α-Fmoc-L-lysine	A		1	86
2	N_α-Fmoc-L-lysine	B		1	84
3	N_α-Fmoc-L-lysine	C		2	82

Table 1 Preparative Di- and Tetraalkylation (continued)

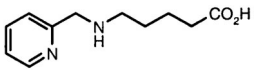
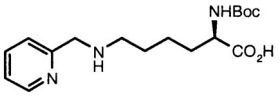
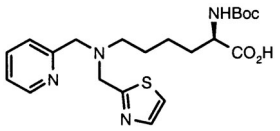
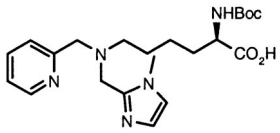
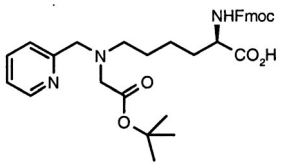
Product	Amino Acid	Aldehyde	Product[a]	Time (h)	Yield[b] (%)
4	N_α-Fmoc-L-lysine	E		2	86
5	N_ε-Boc-D-lysine	A		1	83
6	Phenylalanine	A		2	78
7	Glycine	A		2	76
8	Valeric acid	A		2	76
9	N_α-Fmoc-L-lysine	D		0.5	75
10	L-Lysine·2HCl	A		4	79[c]
11	Fmoc-D-lysine	F		2	74[d]

[a] New compounds gave satisfactory analytical data.
[b] Isolated yield.
[c] 12% of pyridin-2-methanol was also obtained.
[d] 10% of monoalkylated product was also isolated.

The simplified workup procedure involves the addition of water to quench the reaction followed by extraction into the organic phase. The methodology was also amenable to large scale synthesis, as we have successfully scaled up the reductive alkylation of amino acids (compound **1**) to a 10 g scale.

Encouraged by the above results, we then investigated the monoalkylation of similar systems with stoichiometric amounts of aldehyde and amine (compounds **12**, **13**). Unfortunately, the reaction resulted in the predominant formation of dialkylated product. However, it was subsequently found that the dialkylation could be significantly suppressed by forming the imine[25] in situ, i.e., re-

Table 2 Representative Monoalkylation and Mixed Dialkylation Products of This Study

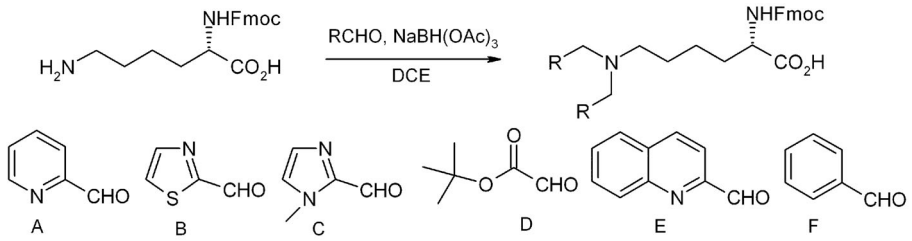
Product	Amino Acid	RCHO	R^1CHO	Product[a]	Time (h)	Yield[b] (%)
12	Valeric acid	A	-		0.5	71
13	N_α-Boc-D-lysine	A	-		0.5	73
14	N_α-Boc-D-lysine	A	B		2	75
15	N_α-Boc-D-lysine	A	C		2	73
16	N_α-Fmoc-D-lysine	A	D		2	70

[a] New compounds gave satisfactory analytical data.
[b] Isolated yield, the corresponding dialkylated product was isolated in 10–15% yields in all cases.

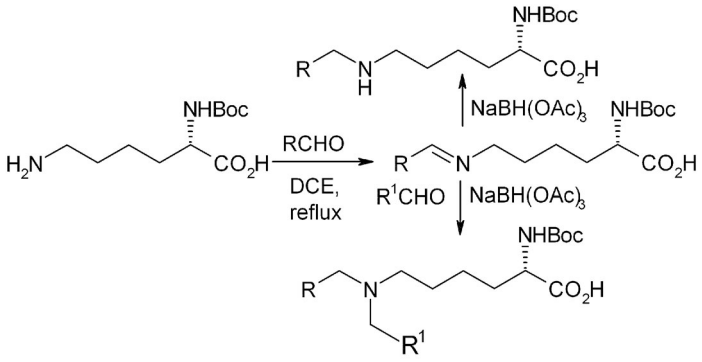
fluxing the mixture of amino acid and aldehyde in DCE for 10 minutes under inert atmosphere (Scheme 2), followed by treatment with $NaBH(OAc)_3$ at ambient temperature. This enabled us to isolate the monoalkylated products **12** and **13** in good yields with the formation of dialkylated products reduced to 10–15%. This small amount of dialkylation may be explained in light of the observation for amino esters by Abdel-Magid et al.[23c] that the monoalkylated product itself would add to the imine to form a dialkyliminium ion, which could then be reduced by $NaBH(OAc)_3$ to afford the dialkylated product. We found that other aprotic solvents CH_2Cl_2, THF and MeCN along with protic solvents such as methanol were equally effective for the formation of imine.

We next focused on mixed dialkylation of amino acids for which there were no direct and single-step methods available in the literature. For this endeavor, the amino acid was refluxed with the appropriate aldehyde in DCE for 10 minutes, followed by addition of $NaBH(OAc)_3$ and the second carboxaldehyde at ambient temperature in one pot to obtain the dialkyl derivatives in good yields (Table 2).

Representative rhenium complexes were prepared in quantitative yields by reacting $(NEt_4)_2[Re(CO)_3Br_3]$[26] with the ligands in methanol under reflux conditions.[14] The overall complex charges of the compounds of the general class $[Re(CO)_3L]^n$ (n = –1, 0, +1) were determined by the identity of the donor groups. Thus, the ligands **1**–**8**, **14** and **15** provided cationic complexes, while ligands **16** and **9,** after deprotection of *tert*-butyl group followed by reacting with $(NEt_4)_2[Re(CO)_3Br_3]$, yielded neutral and anionic metal complexes, respectively. The ^{99m}Tc analogues were prepared by the reaction of $[Tc(CO)_3(H_2O)_3]^+$ with the ligands.[27] The complex with ligand **1** was challenged in 1 mM cysteine and 1 mM histidine in PBS buffer (pH 7.4) for 24 hours at 37 °C to determine its stability against ligand exchange and



Scheme 1 Synthesis of the dialkyl derivatives of amino acids



Scheme 2 General procedure for the synthesis of mono- and mixed-alkylated (R …R^1) derivatives of amino acids.

decomposition. The sample was analyzed after 1, 4 and 24 hours. Minimal decomposition (< 2%) was observed even after 24 hours of incubation. The stable 'closed' shell octahedral coordination of $\{M(CO)_3\}^{+1}$, i.e., low spin d^6 Re(I)/Tc(I), renders substitution reactions via either dissociative or associative mechanisms unlikely. Furthermore, preliminary biodistribution experiments with the representative neutral, anionic and cationic complexes are even more encouraging as no significant localization of the complex in any organ of rat was observed.[28] These results suggested that this important family of bifunctional ligands can be functionalized with different biologically active molecules without significantly altering the biodistribution properties of the drug molecule. Our recent report[16b] demonstrated that the ligand could be conveniently incorporated into a small peptide sequences via solid phase synthesis while retaining its expected biodistribution properties. These latter results demonstrate the versatility and numerous advantage of using the single amino acid chelate system to prepare peptide-targeted Tc(I) and Re (I) radiopharmaceuticals.

In conclusion, a mild method has been developed for the direct reductive alkylation of amino acids and also for the rapid assembly of useful bifunctional chelators for application to nuclear medicine. The methodology offers numerous advantages: preparative convenience, relatively nontoxic reagents, base free workup, and mixed dialkylation in one pot. Our further efforts are directed towards rapid generation of a diverse set of bifuntional chelators to allow coordination for other metal radionuclides.

All reagents were weighed in air and the reactions were conducted under an atmosphere of air, unless otherwise indicated. Anhydrous DCE, pyridine-2-carbaldehyde, thiazole-2-carbaldehyde, 1-methyl-1*H*-imidazole-2-carbaldehyde, quinoline-2-carbaldehyde, benzaldehyde, $NaBH(OAc)_3$ were all obtained from Aldrich. All other reagents were purchased from commercial sources and used as received. Flash column chromatography was done with silica gel 60 (240–400 mesh). Analytical TLC was performed using Merck glass-backed 0.2 mm silica gel 60 F-254 plates and visualized by ultraviolet light, I_2, 5% phosphomolybdic acid or 1% ninhydrin in EtOH. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer, and all peak positions are relative to TMS. HSQC spectra were acquired on a Bruker Avance 500 MHz spectrometer, using standard pulse programs with gradients. HSQC spectra were collected with 4 scans per increment, or 64 scans per increment (depending on whether the natural abundance peaks were desired), into 2048×512 points with no sample spinning and no zero-filling. Electrospray mass spectrometry (ES-MS) was performed on a Fisons Platform quadrupole instrument where samples were dissolved in 1:1:1 mixture of MeOH–THF–MeCN. For compounds analyzed in the positive ion mode, 3% AcOH was added. For compounds run in negative mode, one drop of 0.10 M NaCl solution was added. High-resolution masses are within 5 ppm of theortical values. The crystal structure of ligand **6** was studied on a Bruker diffractometer equipped with the SMART CCD system using graphite-monochromated Mo-Kα radiation (λ = 0.71073Å). The data collection was carried out at 90(2) K. The data was corrected for Lorentz polarization effects and absorption corrections were made using SADABS. All calculations were performed using SHELXTL. The structure was solved by direct methods and all of the non-hydrogen atoms were located from the initial solution. After locating all the non-hydrogen atoms in the structure, the model was refined against F^2 initially using isotropic and later anisotropic thermal displacement parameters until the final value of Δ/σ_{max} was less than 0.001. At this point the hydrogen atoms were located from the electron density difference map and a final cycle of refinements was performed, until the final value of Δ/σ_{max} was again less than 0.001. No anomalies were encountered in the refinement of the structure.

Dialkylation Reactions; 6-[Bis(pyridin-2-yl)methyl]amino-2-(9*H*-fluoren-9-ylmethoxycarbonyl)amino-1-hexanoic Acid (1); Typical Procedure

To a mixture of Fmoc-L-lysine (10.0 g, 27.1 mmol) and $NaBH(OAc)_3$ (14.4 g, 67.95 mmol) in DCE (150 mL), was added 2-pyridinecarboxaldehyde (6.4 g, 57.0 mmol) in DCE (15 mL) at 0 °C under argon. The suspension was stirred at r.t. for 1 h. The reaction mixture was decomposed with H_2O (100 mL) and diluted with $CHCl_3$ (100.0 mL). The separated organic layer was washed with H_2O and brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified through a pad of silica gel using MeOH–$CHCl_3$ (1:6) as eluent to provide the dipyridylmethyl derivative of Fmoc-L-lysine (12.85 g, 86%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.85 (br s, 1 H), 8.50 (d, *J* = 5.10 Hz, 2 H), 7.70 (d, *J* = 7.24 Hz, 2 H), 7.55 (m, 4 H), 7.46 (m, 2 H), 7.32 (t, *J* = 7.72, 2 H), 7.22 (t, *J* = 7.52, 2 H), 7.09 (m, *J* = 6.20 Hz, 2 H), 6.0 (br s, 1 H), 4.29 (m, 3 H), 4.17 (t, *J* = 6.20 Hz, 1 H), 3.86 (br s, 4 H), 2.57 (t, 2 H), 1.90–1.20 (m, 6 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.67, 157.13, 155.93, 148.03, 143.75, 143.56, 140.87, 137.04, 127.36, 126.76, 124.92, 123.44, 122.35, 119.63, 66.40, 58.66, 54.34, 53.68, 46.85, 32.21, 25.70, 22.71.

HRMS: *m*/*z* calcd for $C_{33}H_{35}N_4O_4$ (M + H^+): 551.2652; found: 551.2672.

6-[Bis(thiazol-2-yl)methyl]amino-2-(9*H*-fluoren-9-ylmethoxycarbonyl)amino-1-hexanoic Acid (2)
Yield: 84%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.45 (br s, 1H, CO_2H), 8.20-7.60 (m, 4H), 7.59 (t, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.2 Hz, 2H), 7.30-7.10 (m, 4H), 5.86 (d, *J* = 8.1 Hz, 1H), 4.62-4.32 (m, 3H), 4.19 (t, *J* = 6.90 Hz, 1H), 4.17 (s, 4H), 2.60 (m, 2H), 2.00-1.30 (m, 6H, CH_2).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.04, 171.42, 156.14, 143.96, 143.76, 141.88, 141.23, 127.70, 127.07, 125.18, 119.96,119.82, 66.92, 55.33, 53.82, 47.15, 32.38, 26.73, 22.77.

HRMS: m/z calcd for $C_{29}H_{30}N_4O_4S_2Na$ (M+Na^+): 585.1600; found: 585.1653.

6-[Bis(1-methyl-1*H*-imidazol-2-yl)methyl]amino-2-(9*H*-fluoren-9- ylmethoxycarbonyl)amino-1-hexanoic Acid (3)
Yield: 82%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.73 (d, *J* = 7.20 Hz, 2 H), 7.61 (t, *J* = 7.35 Hz, 2 H), 7.36 (t, *J* = 7.35 Hz, 2 H), 7.27 (t, *J* = 6.90 Hz, 2 H), 6.95 (s, 2 H), 6.74 (s, 2 H), 5.96 (br s, 1 H), 4.40–4.15 (m, 3 H), 4.14 (t, *J* = 6.90 Hz, 1 H), 3.73 (q, *J* = 27.6, 14.1 Hz, 4 H), 3.46 (s, 6 H), 2.54 (m, 2 H), 2.00–1.20 (m, 6 H, CH_2).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 156.16, 145.05, 144.33, 144.13, 141.41, 127.80, 127.23, 125.47, 121.83, 120.07, 66.84, 54.77, 49.38, 47.40, 33.02, 32.31, 25.72, 22.61.

ESI-MS: *m*/*z* = 579.2 (M^+ + Na), 557.3 (M^+ + 1).

6-[Bis(quinolin-2-yl)]methyl]amino-2-(9*H*-fluoren-9-ylmethoxycarbonyl)amino-1-hexanoic Acid (4)
Yield: 86%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.23 (br s, 1 H), 8.07 (d, *J* = 8.4 Hz, 2 H), 8.01 (d, *J* = 8.7 Hz, 2 H), 7.75–7.15 (m, 16 H), 6.05 (br s, 1 H), 4.40–4.10 (m, 8 H), 2.81 (m, 2 H), 2.04–138 (m, 6 H, CH_2).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.85, 158.17, 156.19, 146.80, 144.06, 143.91, 141.23, 137.24, 131.59, 129.93,128.30, 127.64, 127.07, 126.63, 125.25, 121.30, 119.52, 66.81, 59.88, 54.67, 47.19, 32.50, 26.05, 22.88.

HRMS: *m*/*z* calcd for $C_{41}H_{38}N_4O_4$ (M + H^+): 651.2965; found: 651.2936.

2-[Bis(pyridin-2-yl)methyl]amino-6-(*tert*-butoxycarbonyl)amino-1-hexanoic Acid (5)
Yield: 83%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (d, *J* = 4.2 Hz, 2 H), 7.59 (t, *J* = 7.5 Hz, 2 H), 7.30 (d, *J* = 7.8 Hz, 2 H), 7.15 (t, *J* = 6.9 Hz, 2 H), 4.80 (br s, 1 H), 4.04 (q, *J* = 19.2, 14.9 Hz, 4 H), 3.39 (t, *J* = 7.2 Hz, 1 H), 3.07 (m, 2 H), 2.00–1.40 (m, 15 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.62, 159.27, 156.11, 148.30, 137.31, 123.15, 122.45, 79.00, 64.62, 56.29, 40.30, 29.84, 28.84, 28.50, 24.15.

2-[Bis(pyridin-2-yl)methyl]amino-3-phenyl-1-propionic Acid (6)
Yield: 78%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (d, *J* = 4.20 Hz, 2 H), 7.56 (t, *J* = 7.5 Hz, 2 H), 7.32–7.08 (m, 9 H), 4.10 (dd, *J* = 15.6 Hz, 4 H), 3.81 (dd, *J* = 5.6 Hz, 1 H), 3.39 (dd, *J* = 5.1 Hz, 1 H), 3.08 (dd, *J* = 9.9 Hz, 1 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 174.94, 159.28, 148.09, 139.40, 137.45, 129.68, 128.51, 126.46, 123.08, 122.52, 66.45, 56.36, 35.67.

HRMS: *m*/*z* calcd for $C_{21}H_{22}N_3O_2$ (M + H^+): 348.1707; found: 348.1721 and calcd for $C_{21}H_{21}N_3O_2$ + Na (M + Na^+): 370.1525; found: 370.1501.

2-[Bis(pyridin-2-yl)methyl]amino-1-ethanoic Acid (7)
Yield: 76%.

^{1}H NMR (300 MHz, MeOH-d_4): δ = 8.29 (d, *J* = 5.1 Hz, 2 H), 7.60 (t, *J* = 9.0 Hz, 2 H), 7.30 (d, *J* = 7.8 Hz, 2 H), 7.12 (t, *J* = 6.2 Hz, 2 H), 4.10 (s, 4 H), 3.39 (m, 2 H).

^{13}C NMR (300 MHz, MeOH-d_4): δ = 173.05, 156.10, 149.76, 139.31, 125.15, 124.77, 59.77, 57.77.

5-[Bis(pyridin-2-yl)methyl]amino-1-pentanoic Acid (8)
Yield: 83%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.51 (d, *J* = 4.8 Hz, 2 H), 7.64 (t, *J* = 7.5 Hz, 2 H), 7.51 (d, *J* = 7.8 Hz, 2 H), 7.13 (t, *J* = 5.1 Hz, 2 H), 3.78 (s, 4 H), 2.54 (t, *J* = 6.30 Hz, 2 H), 2.26 (t, *J* = 6.60 Hz, 2 H), 1.59 (m, 4 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 177.00, 159.45, 148.49, 137.12, 123.41, 122.34, 59.87, 54.12, 34.38, 26.49, 22.91.

6-[Bis(*tert*-butoxycarbonylmethylamino)]-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)hexanoic Acid (9)
Yield: 75%.

^{1}H NMR (300 MHz, MeOH-d_4): δ = 7.72 (d, *J* = 7.2 Hz, 2 H), 7.57 (d, *J* = 7.5 Hz, 2 H), 7.35 (t, *J* = 7.2 Hz, 2 H), 7.26 (t, *J* = 7.5 Hz, 2 H), 5.62 (d, *J* = 8.1 Hz, 1 H), 4.40–4.34 (m, 3 H), 4.18 (t, *J* = 6.9 Hz, 1 H), 3.39 (s, 4 H), 2.68 (t, *J* = 7.5 Hz, 2 H), 1.96–1.25 (m, 24 H).

^{13}C NMR (300 MHz, MeOH-d_4): δ = 174.57, 170.45, 156.08, 144.05, 143.91, 141.29, 127.70, 127.11, 125.25, 119.97, 81.12, 66.92, 55.62, 53.91, 53.75, 47.22, 32.34, 28.19, 27.38, 22.82.

HRMS: *m*/*z* calcd for $C_{33}H_{44}N_2O_8$ + Na (M + Na^+): 619.2989; found: 619.2960.

2,6-[Bis(pyridin-2-yl)methyl]diamino-1-hexanoic Acid (10)
Yield: 79%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.39 (m, 4 H), 7.72–7.40 (m, 6 H), 7.33 (d, *J* = 7.8 Hz, 2 H), 7.03 (m, 4 H), 4.0 (q, 4 H), 3.69 (s, 4 H), 3.30 (t, *J* = 7.2 Hz, 1 H), 2.43 (t, *J* = 7.2 Hz, 2 H), 1.94–1.16 (m, 6 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.45, 159.87, 159.64, 148.78, 148.24, 137.13, 136.61, 123.00, 122.28, 122.01, 64.21, 60.32, 56.54, 54.38, 29.51, 27.09, 24.66.

HRMS: *m*/*z* calcd for $C_{30}H_{35}N_6O_2$ (M + H^+): 511.2816; found: 511.2828.

6-Dibenzylamino-2-(9*H*-fluoren-9-ylmethoxycarbonyl)amino-1-hexanoic Acid (11)
Yield: 74%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.18 (br s, 1 H), 7.90 (d, *J* = 7.50 Hz, 2 H), 7.67 (t, *J* = 7.80 Hz, 2 H), 7.45–7.2 (m, 14 H), 6.24 (d, 1 H), 4.34 (m, 3 H), 4.21 (t, *J* = 6.20 Hz, 1 H), 4.06 (br s, 4 H), 2.74 (m, 2 H), 2.06–1.20 (m, 6 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 177.14, 156.31, 144.20, 143.99, 141.27, 131.92, 130.52, 129.94, 129.94, 128.62, 128.21, 127.69, 127.40, 127.11, 125.33, 119.96, 66.80, 56.13, 55.09, 51.47, 47.25, 32.67, 24.12, 22.77.

ESI-MS: *m*/*z* = 449.2 (M^+ + Na).

Monoalkylation Reactions; 2-[*tert*-Butoxycarbonyl]amino-6-[(pyridin-2-yl)methyl]amino-1-hexanoic Acid (13); Typical Procedure

A solution of Boc-D-lysine (2.0 g, 8.12 mmol) and 2-pyridinecarboxaldehyde (0.87 g, 8.12 mmol) in DCE (20 mL) was refluxed for 10 min under argon. The reaction mixture was cooled to 0 °C, and treated with $NaBH(OAc)_3$ (2.06 g, 9.74 mmol). The suspension was stirred at r.t. until the completion of reaction. The monopyridylmethyl derivative of Boc-D-lysine was obtained (2.73 g, 73%) along with 10% of bis(pyridyl) derivative as side product following the same purification procedure as mentioned earlier.

^{1}H NMR (300 MHz, MeOH-d_4): δ = 8.50 (d, *J* = 4.2 Hz, 1 H), 7.80 (t, *J* = 7.8 Hz, 1 H), 7.45 (d, *J* = 7.8 Hz, 1 H), 7.29 (t, *J* = 5.1 Hz, 1 H), 3.94 (m, 1 H), 3.88 (s, 2 H), 2.61 (t, *J* = 7.3 Hz), 1.85–1.3 (m, 15 H).

^{13}C NMR (300 MHz, MeOH-d_4): δ = 179.91, 160.09, 157.77, 149.98, 138.79, 124.23, 123.89, 80.02, 57.28, 55.20, 50.10, 34.36, 30.40, 28.93, 24.54.

5-[(Pyridin-2-yl)methyl]amino-1-pentanoic Acid (12)

Yield: 71%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 4.2 Hz, 1 H), 7.54 (t, *J* = 7.8 Hz, 1 H), 7.15 (d, *J* = 8.1 Hz, 1 H), 7.04 (t, *J* = 4.8 Hz, 1 H), 4.59 (s, 2 H), 3.21 (t, *J* = 5.7 Hz, 1 H), 2.32 (t, *J* = 5.4 Hz, 1 H), 1.69 (m, 4 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 170.14, 157.04, 148.82, 137.01, 122.33, 122.06, 52.03, 48.14, 32.18, 23.05, 21.18.

Mixed-Alkylation Reactions; 2-[*tert*-Butoxycarbonyl]amino-6-[(pyridin-2-yl)methyl,(thiazol-2-yl)methyl]amino-1-hexanoic Acid (14); Typical Reaction

A solution of Boc-D-lysine (2.0 g, 8.12 mmol) and 2-pyridinecarboxaldehyde (0.87 g, 8.12 mmol) in DCE (10 mL) was refluxed for 10 min under argon. The reaction mixture was cooled to 0 °C, and treated sequentially with $NaBH(OAc)_3$ (4.30 g, 20.3 mmol) and 2-thiazolecarboxyaldehyde(0.91 g, 8.12 mmol). The reaction mixture was stirred at r.t. until the completion of reaction and purified as mentioned above to obtain the mixed derivative of Boc-D-lysine (3.39 g, 75%) along with 12% of the bis-alkyl derivative as a side product.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.55 (d, *J* = 5.1 Hz, 1 H), 7.75 (t, *J* = 7.5 Hz, 1 H), 7.68 (d, *J* = 3.3 Hz, 1 H), 7.64 (d, *J* = 7.8 Hz, 1 H), 7.25 (d, *J* = 3.3 Hz, 1 H), 7.22 (t, *J* = 6.3 Hz, 1 H), 5.3 (d, *J* = 8.7 Hz, 1 H), 4.28 (m, 1 H), 3.99 (s, 2 H), 3.87 (s, 2 H) 2.56 (t, *J* = 7.2 Hz, 2 H), 1.92–1.3 (m, 15 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.46, 171.67, 158.54, 155.68, 147.97, 142.23, 137.90, 123.59, 122.81, 119.63, 79.57, 59.29, 55.36, 54.20, 53.63, 32.81, 28.51, 26.73, 22.98.

HRMS: *m*/*z* calcd for $C_{21}H_{31}N_4O_4S$ (M + H^+): 435.2061; found: 435.2025 and calcd for $C_{21}H_{30}N_4O_4S$ + Na (M + Na^+): 457.1880; found: 457.1859.

2-[*tert*-Butoxycarbonyl]amino-6-[(1-methyl-1*H*-imidazol-2-yl)methyl,(pyridin-2-yl)methyl]amino-1-hexanoic Acid (15)

Yield: 73%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.19 (br s, 1 H), 8.52 (d, *J* = 4.2 Hz, 1 H), 7.62 (t, *J* = 7.5 Hz, 1 H), 7.31 (d, *J* = 7.8 Hz, 1 H), 7.14 (t, *J* = 6.2 Hz, 1 H), 6.94 (s, 1 H), 6.73 (s, 1 H), 5.42 (br s, 1 H), 4.17 (m, 1 H), 3.38–3.64 (m, 4 H), 3.55 (s, 3 H), 2.48 (m, 2 H), 1.95–1.20 (m, 15 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 176.41, 158.70, 155.67, 148.90, 145.07, 136.95, 124.84, 123.81, 122.42, 121.76, 79.13, 60.19, 54.44, 54.24, 49.68, 33.33, 32.78, 28.56, 25.81, 22.84.

HRMS: *m*/*z* calcd for $C_{22}H_{33}N_5O_4$ (M + H^+): 432.2605; found: 432.2583.

6-[(*tert*-Butoxycarbonyl)methyl,(pyridin-2-yl)methyl]amino-2-[9*H*-fluoren-9-ylmethoxycarbonyl]amino-1-hexanoic Acid (16)

Yield: 70%.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 13.43 (br s, 1 H), 8.57 (d, *J* = 7.5 Hz, 1 H), 7.74–7.59 (m, 6 H), 7.36 (t, *J* = 7.5 Hz, 2 H), 7.295 (t, *J* = 7.5 Hz, 2 H), 7.20 (t, *J* = 6.0 Hz, 1 H), 5.88 (d, *J* = 7.8 Hz, 1 H) 4.35 (m, 3 H), 4.20 (t, *J* = 6.2 Hz, 1 H), 4.02 (s, 2 H), 3.48 (s, 2 H), 2.72 (br t, 2 H), 2.05–1.49 (m, 15 H).

^{13}C NMR (300 MHz, $CDCl_3$): δ = 175.64, 170.15, 157.82, 156.14, 147.60, 144.10, 143.92, 141.28, 138.12, 127.69, 127.09, 125.26, 124.24, 122.90, 120.32, 81.41, 66.86, 58.37, 55.11, 54.33, 53.87, 47.23, 32.50, 27.92, 26.62, 22.77.

HRMS: *m*/*z* calcd for $C_{33}H_{40}N_3O_6$ (M + H^+): 574.2911; found: 574.2935 and calcd for $C_{33}H_{39}N_3O_6$ + Na (M + Na^+): 596.2731; found: 596.2739.

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(24) Table 1, Entry **6** (C_{22} H_{25} N_3 O_3, formula weight 379.45): Orthorhombic, space group $P2_12_12_1$ with a = 7.3392(4) Å, b = 15.0539(7) Å, c = 17.9286(9) Å, V = 1980.82(17) Å^3, Z = 4, $R1$ = 0.0556, w$R2$ = 0.1079 (F^2, all data). Full details on the crystal structure of **6** are available from the authors.

(25) The imine to aldehyde ratio was in the range of 1: 0.10–0.20, as determined by ^{1}H NMR spectrum.

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