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ORIGINAL ARTICLE

Synthetic strategy for side chain mono-N-alkylation of Fmoc-amino acids promoted by molecular sieves

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Abstract A new synthetic strategy to alkylate amino groups under mild conditions has been developed. It utilizes only 4 Å molecular sieves as base in order to promote the N-alkylation reaction, in presence of the appropriate alkyl halide. The methodology was validated by the simple and efficient side-chain N-alkylation of *o*-Ns-protected Fmoc-amino acid. One of them was introduced as building block into a peptide sequence, thus allowing the preparation of site-specific alkylated peptide molecules.

Keywords N-alkylation · Molecular sieves · N-alkylated amino acids · Peptidomimetics

Introduction

Therapeutically, relevant peptides of themselves are seldom used in the clinic because of their poor biostability, unfavorable absorption properties, as well as poor receptor subtype selectivity. Nevertheless, they can be used as a template in order to synthesize modified peptides, called peptidomimetics, which are endowed with increased pharmacological activities.

In a successfully designed peptidomimetic there are two structural factors: (1) a favorable docking in the binding site of the receptor target; if necessary the conformation can be stabilized by rigid bridges of various lengths between different parts of the molecule, and (2) the spatial orientation of certain structural motifs (e.g., functional groups, polar, and hydrophobic moieties) must correspond to the display in the bioactive conformation of the peptide so that the required interactions (e.g., hydrogen bonds, electrostatic or hydrophobic interactions) occur (Gante 1994).

One possible strategy to favor the formation of bioactive conformations is modifying the side chain of amino acids (Hanessian et al. 1997). In fact, the field of amino acid modification has gained a big relevance in recent years, particularly with the emergence of new building blocks that allow introducing chemical and functional diversity into molecules with therapeutic potential.

With these ideas in our mind, we focused on the development of an efficient synthetic route to obtain modified on their side chain, via N-alkylation reaction, N^{α}–Fmoc amino acids suitable to be introduced into a peptide sequence. The aim has been to set out a mild and general N-alkylation procedure, which does not affect the stereochemistry of the amino acids chiral center, neither other sensitive functional/protective groups (i.e., the α-COOH and Fmoc/Boc groups) also present on the substrate.

Many different strategies (Olsen et al. 2005; Sasaki and Coy 1987; Gazal et al. 2003; Yang and Chiu 1997; Dankwardt et al. 1997) have been developed to obtain N^{α}-alkyl amino acids, including reductive methods, Mitsunobu condition, direct alkylation by halides in the presence of strong bases like DBU (Reichwein and Liskamp 1998; Biron et al. 2006), NaOH (Biron and Kessler 2005), NaH (Biron and Kessler 2005; Stodulski and Mlynarski 2008), LiOH (Cho and Kim 2002), and by use of diazomethane in some cases of methylation (De Gioia et al. 2005). To the best of our knowledge, there are only few examples in literature that describe the side chain N-alkylation of amino acids (Huang et al. 2006,

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Demmer et al. 2008). The Yan-Mei Li method employs reductive conditions in order to obtain the N^{γ}-methyl lysine. The most cited Kessler protocol employs alkyl halides in the presence of DBU or Mitsunobu condition in order to N^{δ}-alkylate the ornithine in solid phase. Both Kessler's procedures require the protection of the ornithine N^{α}–NH_2 with an Alloc group, and this is performed by an additional synthetic step.

Most of the cited protocols protect the amino group as sulfonamide, by using the nitrobenzensulfonyl group (Ns) (Fukuyama et al. 1997; Toshiyuki and Fukuyama 2004; Greene and Wuts 2007) in order to synthesize mono-N-alkylated peptides (Rew and Goodman 2002). In fact, the Nosyl group requires mild conditions for its de-protection and is compatible with Fmoc solid-phase synthesis (Miller and Scanlan 1998), due to its stability under acidic (HCl) as well as basic (NaOH) conditions.

We earlier described an efficient synthetic strategy for preparing *o*-Ns-protected Fmoc-amino acids rapidly without purification under mild conditions. The products obtained [Fmoc–Lys(Ns)–OH, Fmoc–Orn(Ns)–OH, Fmoc–Dab(Ns)–OH, and Fmoc–Dap(Ns)–OH] were successfully employed as building blocks in solid-phase synthesis (De Luca et al. 2005). With these results, we next focused on the development of a synthetic procedure for N-alkylation of the Fmoc-amino acid derivatives mentioned above. In this context, it is worth noting that the reaction conditions of the cited N-alkylation protocols are not compatible with the Fmoc-group, since they employ quite strong basic conditions. Hence, we tried the traditional approach (K_2CO_3, halide, DMF) on the above-mentioned amino acid derivatives previously synthesized (De Luca et al. 2005). This method hardly provided the mono-N-alkylated amino acids; moreover, the separation of the product from salts was very unsuccessful.

Therefore, we developed an alternative and more practical synthetic route, which employed only 4 Å molecular sieves in order to promote the N-alkylation with halides in the absence of any other base.

Materials and methods

Chemicals and equipment

All purchased chemicals and solvents were used without further purification unless otherwise stated. Solid-phase peptide synthesis was performed on a fully automated Multisyntech Syro I synthesizer. Molecular sieves, type 4 Å (beads, diameter 1.6 mm) were activated by heating at 280°C for 4 h under vacuum and atmosphere of Ar.

LC/MS analysis

Analytical RP-HPLC runs were carried out using a C18 column, 4.6 × 250 mm with a flow rate of 1.0 mL min^{-1}. Preparative RP-HPLC was carried out using a C18 column, 22 × 250 mm with a flow rate of 20 mL min^{-1}. For all the RP-HPLC procedures, the system solvent used was: H_2O 0.1% TFA (A) and CH_3CN 0.1% TFA (B), with a linear gradient from 5 to 70% B in 30 min (gradient 1) or from 40 to 95% B in 30 min (gradient 2) and detection at 210 and 280 nm. LC–ES–MS data were obtained using a single quadrupole electrospray ionization mass spectrometer coupled with an HPLC apparatus. HRMS was run on a Micromass QTOF mass spectrometer.

NMR analysis

^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a spectrometer equipped with a *z*-gradient 5 mm triple-resonance probe head. Samples were prepared in tubes with a diameter of 5 mm using 0.5 ml of deuterated solvent. The chemical shifts are reported in units of ppm on the β scale relative to the solvent signal used ($CDCl_3$, 7.26 ppm for ^1H NMR; and 77.00 ppm for ^{13}C NMR). ^1H NMR assignments were based on homo-decoupling experiments.

Optical rotations were measured using a 1 mL cell with a 10 mm path length.

Polyalkylation promoted by molecular sieves

To a solution of Fmoc–Dap–OH (0.10 mmol) in DMF (1 mL) were added under argon atmosphere 4 Å molecular sieves (2 g) previously activated. The solution was stirred for 5 min at RT under argon flow. Benzyl bromide (0.15 mmol) was added to the solution and the reaction was continued at RT for 15 h. The reaction mixture was analyzed via HPLC–ES–MS and revealed the presence of mono (M), di (D), and tribenzylated (T) compound. HPLC: t_R(M) = 8.65 min, t_R(D) = 11.14 min, t_R(T) = 14.85 min; ES–MS: calcd ($M+H^+$), 416.18; found (M), *m/z* 416.00, calcd ($M+H^+$), 506.02; found (D), *m/z* 505.69, calcd ($M+H^+$), 596.08; found (T), *m/z* 595.57; HRMS (ESI) calcd for $C_{25}H_{24}N_2O_4$, 416.1832; found, 416.1844; calcd for $C_{32}H_{30}N_2O_4$, 506.0272; found, 505.0281; calcd for $C_{39}H_{37}N_2O_4$, 596.0811; found, 595.0829.

General procedure for N-alkylation promoted by molecular sieves

A solution of Fmoc–Xaa(Ns)–OH in DMF (50 mg/ml), previously synthesized by using the already published

protocol (De Luca et al. 2005), was added under Ar atmosphere in a round-bottom flask containing 4 Å molecular sieves previously activated at 280°C for 4 h under vacuum. After few minutes the alkylating reagent was added (1.5 equiv.). The reaction was stirred at room temperature and followed by analytical RP-HPLC until disappearance of the starting compound. The mixture was centrifuged and the precipitate was washed with DMF. Afterwards the supernatant was concentrated under vacuum and the crude product was purified by HPLC to be fully characterized by mass spectrometry and NMR spectroscopy.

General procedure for peptides synthesis

The peptides synthesis was carried out by solid-phase method using the standard Fmoc procedure. The first amino acid was preloaded on the used Wang resin. The α-amino acids were activated in situ by the standard PyBop/HOBt// DIPEA protocol. Fmoc-de-protection was performed with 20% piperidine in DMF for 5 + 10 min. Amino acid couplings were monitored by Kaiser et al. (1970).

The peptides were cleaved from the solid support and simultaneously all protecting groups were removed by suspending the fully protected compound-resins in TFA/ H_2O/TIS (97:2:1) for 3 h followed by filtration. The solution was then concentrated and the crude product was isolated by precipitation into cold diethyl ether.

N_α-(9-fluorenylmethoxycarbonyl)-N_β-benzyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1a, 1k** HPLC: t_R = 23.581 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): β 8.07 & 7.81 (2d, J = 7.1 Hz, 2H, Ns-$H_{3,6}$), 7.77 & 7.58 (2d, J = 7.6 Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.68 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.32 (2t, J = 7.2 Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.37 (m, 5H, Ph–$H_{2,3,4,5,6}$), 5.23 (m, 2H, CH_2–Ph), 4.48 (br, 1H, Dap–CH^α), 4.36 (d, J = 6.8 Hz, 2H, Fmoc–CH_2), 4.20 (t, J = 6.8 Hz, 1H, Fmoc–CH), 3.58 (br, 2H, Dap–CH_2^β); ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.40; 47.48; 54.48; 67.97; 68.66; 120.50; 125.59; 126.01; 127.64; 128.28; 126.01; 127.64; 128.28; 129.06;129.25; 131.50; 133.39; 134.27; 141.77; 144.10; 156.59; 169.87. ES–MS: calcd (M+H$^+$), 601.15; found, m/z 601,35; calcd (M+Na$^+$), 623.15; found, m/z 623,89; HRMS (ESI) calcd for $C_{31}H_{27}N_3O_8S$, 601.1557; found, 601.1561; $[\alpha]_D^{25} = -15.7$ (c = 0.3, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-2-phenylethyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1b** HPLC: t_R = 23.97 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): β 8.07 & 7.83 (2d, J = 6.8 Hz, 2H, Ns–$H_{3,6}$), 7.78 & 7.60 (2d, J = 7.6 Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.42 & 7.23 (2t, J = 7.2 Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.32 (m, 5H, Ph–$H_{2,3,4,5,6}$), 4.42 (m, 3H, Dap–CH^α & CH_2–CH_2–Ph), 4.37 (d, J = 6.8 Hz, 2H, Fmoc–CH_2), 4.21 (t, J = 6.8 Hz, 1H, Fmoc–CH), 3.51 (br, 2H, Dap–CH_2^β), 3.00 (t, J = 6.8 Hz, 2H, CH_2–CH_2–Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.31; 43.88; 47.48; 53.92; 67.12; 67.53; 120.51; 125.47; 125.69; 127.35; 127.63; 156.43; 172.32. ES–MS: calcd (M+H$^+$), 615.17; found, m/z 615,52, calcd (M+Na$^+$), 627.17; found, m/z 627,67; HRMS (ESI) calcd for $C_{32}H_{29}N_3O_8S$, 615.1749; found, 615.1754. $[\alpha]_D^{25} = -6.2$ (c = 0.4, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-3-phenylpropyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1c, 1l** HPLC: t_R = 25.48 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 & 7.82 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.59 (2d, J = 7.6 Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.19 (2t, J = 7.2 Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.31 (m, 5H, Ph–$H_{2,3,4,5,6}$), 4.39 (m, 3H, Dap–CH^α & Fmoc–CH_2), 4.21 (m, 3H, Fmoc–CH and CH_2–CH_2–CH_2–Ph), 3.54 (br, 2H, Dap–CH_2^β), 2.70 (t, J = 6.8 Hz, 2H, –CH_2–CH_2–CH_2–Ph), 2.01 (m, 2H, –CH_2–CH_2–CH_2–Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.34; 32.57; 45.40; 47.49; 54.37; 66.38; 67.92; 120.52; 125.57; 126.01; 126.65; 127.65; 128.30; 128.88; 129.02; 131.51; 133.40; 134.28; 141.79; 144.12; 156.02; 169.65. ES–MS: calcd (M+H$^+$), 629.18; found, m/z 629.41, calcd (M+Na$^+$), 651.18; found, m/z 651.80; HRMS (ESI) calcd for $C_{33}H_{31}N_3O_8S$, 629.1867; found, 629.1895. $[\alpha]_D^{25} = -5.8$ (c = 0.3, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-propyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1d** HPLC: t_R = 22.14 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.78 & 7.60 (2d, J = 7.6 Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.71 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.33 (2t, J = 7.2 Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.45 (br, 1H, Dap–CH^α), 4.37 (d, J = 7.2 Hz, 2H, Fmoc–CH_2), 4.22 (t, J = 6.8 Hz, 1H, Fmoc–CH), 4.18 (t, J = 6.8 Hz, 2H, –CH_2–CH_2–CH_3), 3.58 (br, 2H, Dpr–CH_2^β), 1.71 (m, 2H, –CH_2–CH_2–CH_3), 0.96 (t, J = 7.6, 3H, –CH_2–CH_2–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.76; 22.31; 45.51; 47.50; 67.95; 68.56; 120.53; 125.61; 126.03; 127.65; 128.31; 131.53; 133.42; 134.29; 141.79; 144.14; 156.79; 170.06. ES–MS: calcd (M+H$^+$), 553.15; found, m/z 553.21, calcd (M+Na$^+$), 575,15 found, m/z 575,75; HRMS (ESI) calcd for $C_{27}H_{27}N_3O_8S$, 553.1498; found, 553.1503. $[\alpha]_D^{25} = -8.3$ (c = 0.1, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-1-heptenyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1e** HPLC: t_R = 26.20 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.59 (2d, J = 7.6 Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.71 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.33 (2t, J = 7.2 Hz, 4H, Fmoc–$H_{2,3,6,7}$), 5.77 (m, 1H, –CH_2–CH=CH_2), 5.00 & 4.95 (2m, 2H, –CH_2–CH=C$H_{a,b}$), 4.44 (m, 1H, Dap– + –CH^α), 4.36 (d, J = 7.2 Hz, 2H, Fmoc–CH_2), 4.21 (m, 3H, Fmoc–CH and –CH_2–$(CH_2)_4$–CH=CH_2), 3.57 (br, 2H, Dpr–CH_2^β), 2.06

(m, 2H, –$(CH_2)_4$–CH_2–CH=CH_2),1.69 & 1.40 (2m, 8H, –$(CH_2)_4$–CH_2–CH=CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.67; 28.72; 28.83; 33.96; 45.45; 47.46; 54.38; 67.032; 67.93; 115.09; 120.49; 125.58; 125.97; 127.61; 128.27; 128.84; 128.98; 131.48; 133.38; 134.24; 139.04; 141.76; 144.11; 156.33; 170.04. ES–MS: calcd (M+H^+), 607.20; found, *m/z* 607.64, calcd (M+Na^+), 629.20; found, *m/z* 630.00; HRMS (ESI) calcd for $C_{31}H_{33}N_3O_8S$, 607.1988; found, 607.1996. $[\alpha]_D^{25} = -6.9$ ($c = 0.9$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-diphenylmethyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1f** HPLC: $t_R = 26.09$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 & 7.79 (2d, $J = 7.1$ Hz, 2H, Ns–$H_{3,6}$), 7.76 & 7.57 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.66 (m, 2H, Ns–$H_{4,5}$), 7.42–7.31 (m, 14H, Fmoc–$H_{2,3,6,7}$ and CH–$(Ph)_2$–H), 6.90 (br, 1H, CH–$(Ph)_2$), 4.55 (br, 1H, Dap–CH^α), 4.35 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.19 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.59 (br, 2H, Dap–CH_2^β); ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.37; 47.48; 54.54; 68.07; 79.76; 120.51; 125.64; 126.03; 127.53; 127.65; 128.31; 128.85; 128.92; 129.19; 129.28; 131.57; 133.38; 134.27; 139.52; 141.78; 144.12; 156.42; 169.29. ES–MS: calcd (M+H^+), 677.18; found, *m/z* 677,54; calcd (M+Na^+), 699.18; found, *m/z* 700,02; HRMS (ESI) calcd for $C_{37}H_{31}N_3O_8S$, 677.1831; found, 677.1847. $[\alpha]_D^{25} = -6.4$ ($c = 0.5$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-naphthylmethyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1g** HPLC: $t_R = 25.87$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 & 7.84 & 7.76 & 7.64 & 7.56 & 7.50 & 7.45 & 7.39 & 7.30 (9m, 19H, Ns–$H_{3,4,5,6}$, Fmoc–$H_{1,2,3,4,5,6,7,8}$, Naph–$H_{2,3,4,5,6,7,8}$), 5.38 (m, 2H, CH_2–Naph), 4.50 (br, 1H, Dap–CH^α), 4.36 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.19 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.58 (br, 2H, Dap–CH_2^β); ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.40; 47.49; 54.37; 66.38; 67.92; 120.59; 125.39; 125.90; 126.57; 127.23; 127.54; 128.27; 128.56; 129.07; 131.53; 133.20; 133.99; 141.80; 144.35; 156.57; 170.11. ES–MS: calcd (M+H^+), 651.17; found, *m/z* 651,32; calcd (M+Na^+), 673.17; found, *m/z* 673,89; HRMS (ESI) calcd for $C_{35}H_{29}N_3O_8S$, 651.1764; found, 651.1783. $[\alpha]_D^{25} = -2.4$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-t-butylacetyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1h** HPLC: $t_R = 22.998$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.63 (1d & 1m, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.68 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.33 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.87 & 4.40 (2d, $J = 14$ Hz, 2H, C$H_2CO_2C(CH_3)_3$), 4.58 (d, $J = 6.4$ Hz, 2H, Fmoc–CH_2), 4.38 (m, 1H, Dap–CH^α), 4.25 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.69 (br, 2H, Dap–CH_2^β), 1.51 (s, 9H, $CH_2CO_2C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.44; 45.73; 47.45; 54.25; 62.28; 68.01; 84.55; 120.40; 125.71; 127.59; 128.19; 131.28; 133.14; 133.97; 141.69; 144.11;148.12; 156.29; 167.81; 169.76. ES–MS: calcd (M+H^+), 625.17; found, *m/z* 625,70; calcd (M+Na^+), 647.17; found, *m/z* 647,68; HRMS (ESI) calcd for $C_{30}H_{31}N_3O_{10}S$, 625.1730; found, 625.1761; $[\alpha]_D^{25} = -6.5$ ($c = 0.4$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-methyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1i** HPLC: $t_R = 18.088$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 & 7.84 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.59 (2d, $J = 7.2$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.71 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.33 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.44 (m, 1H, Dap–CH^α), 4.35 (d, $J = 6.4$ Hz, 2H, Fmoc–CH_2), 4.21 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.81 (s, 3H, N_β–CH_3), 3.56 (br, 2H, Dap–CH_2^β), ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.32; 47.43; 53.60; 54.29; 67.90; 120.46; 125.52; 125.97; 127.59; 128.24; 131.45; 133.37; 134.25; 141.73; 144.02; 157.29; 169.75. ES–MS: calcd (M+H^+), 525.12; found, *m/z* 525,52; calcd (M+Na^+), 547.12; found, *m/z* 547,88; HRMS (ESI) calcd for $C_{25}H_{23}N_3O_8S$, 525.1206; found, 525.1210; $[\alpha]_D^{25} = -5.9$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-ethyl–N'_β-2-nitrobenzensulfonyl-L-2,3-diaminopropionic acid **1j** HPLC: $t_R = 19.518$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.59 (2d, $J = 7.2$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.32 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.43 (m, 1H, Dap–CH^α), 4.36 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.25 (m, 3H, Fmoc–CH, N_β–CH_2CH_3), 3.57 (br, 2H, Dap–CH_2^β), 1.32(t, $J = 6.4$ Hz, 3H, N_β–CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.58; 30.19; 45.46; 47.49; 54.38; 63.07; 67.93; 120.51; 125.59; 126.00; 127.64; 128.29; 131.50; 133.41; 133.94; 134.27; 141.78; 144.11; 148.49; 156.33; 169.96. ES–MS: calcd (M+H^+), 539.14; found, *m/z* 539,44; calcd (M+Na^+), 561.14; found, *m/z* 561,38; HRMS (ESI) calcd for $C_{26}H_{25}N_3O_8S$, 539.1362; found, 639.1398; $[\alpha]_D^{25} = -6.3$ ($c = 0.5$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_γ-benzyl–N'_γ-2-nitrobenzensulfonyl-L-2,4-diaminobutirric acid **2a** HPLC: $t_R = 23.41$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 & 7.78 (2br, 2H, Ns–$H_{3,6}$), 7.67 & 7.58 (2md, 6H, Fmoc–$H_{1,4,5,8}$, Ns–$H_{4,5}$), 7.41–7.32 (m, 9H, Fmoc–$H_{2,3,6,7}$ Ph–$H_{2,3,4,5,6}$), 5.15 (m, 2H, CH_2–Ph), 4.47 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.41 (m, 1H, Dab–CH^α), 4.18 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.32 & 3.02 (2m, 2H, Dab–CH_2^γ), 2.12 & 1.75 (2m, 2H, Dab–CH_2^β); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.12; 40.38; 47.64; 51.79; 67.68; 68.27; 120.52; 125.56; 125.76; 127.62; 128.28; 129.03; 129.23; 131.15; 133.16; 133.89; 135.33; 141.83; 144.00; 144.24; 156.94; 172.05. ES–MS: calcd (M+H^+), 615.65; found, *m/z* 616.16, calcd (M+Na^+), 637.65; found, *m/z* 637.85; HRMS (ESI) calcd for $C_{32}H_{29}N_3O_8S$, 615.6523; found, 616.6542. $[\alpha]_D^{25} = -17.7$ ($c = 0.3$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{δ}-benzyl–N'_{δ}-2-nitrobenzensulfonyl-L-ornithine **3a** HPLC: $t_R = 23.77$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.80 (2br, 2H, Ns–$H_{3,6}$), 7.78 & 7.59 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.69 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.32 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.37 (m, 5H, Ph–$H_{2,3,4,5,6}$), 5.18 (m, 2H, CH_2–Ph), 4.41 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.37 (m, 1H, Orn–CH^{α}), 4.20 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.08 (m, 2H, Orn–CH^{δ}_2), 1.70 (m, 2H, Orn–CH^{β}_2), 1.55 (m, 2H, Orn–CH^{γ}_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.95; 30.22; 43.60; 47.66; 53.78; 67.52; 67.96; 1203.52; 125.57; 125.89; 127.62; 128.27; 128.94; 129.23; 131.53; 133.27; 134.07; 135.56; 141.84; 144.15; 144.32; 148.56; 156.43; 172.32. ES–MS: calcd (M+H$^+$), 629.18; found, m/z 629.52, calcd (M+Na$^+$), 651.18; found, m/z 651.84; HRMS (ESI) calcd for $C_{33}H_{31}N_3O_8S$, 629.1812; found, 629.1822. $[\alpha]_D^{25} = -1.3$ ($c = 0.8$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{ε}-benzyl–N'_{ε}-2-nitrobenzensulfonyl-L-lysine **4a, 4k** HPLC: $t_R = 24.20$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.82 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.60 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.31 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.35 (m, 5H, Ph–$H_{2,3,4,5,6}$), 5.18 (m, 2H, CH_2–Ph), 4.41 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.38 (br, 1H, Lys–CH^{α}), 4.22 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.04 (m, 2H, Lys–CH^{ε}_2), 1.82 & 1.65 (2m, Lys–C$H^{\beta}_{a,b}$), 1.52 (m, 2H, Lys–C$H^{\gamma}_{a,b}$) 1.32 (m, 2H, Lys–C$H^{\delta}_{a,b}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.40; 29.40; 32.60; 43.87; 47.64; 53.97; 67.59; 67.84; 120.49; 125.59; 125.90; 127.59; 128.23; 128.93; 129.19; 131.56; 133.22; 134.02; 141.82; 144.24; 156.46;172.56. ES–MS: calcd (M+H$^+$), 643.20; found, m/z 643.11, calcd (M+Na$^+$), 665.20; found, m/z 665.81; HRMS (ESI) calcd for $C_{34}H_{33}N_3O_8S$, 643.2031; found, 643.2048. $[\alpha]_D^{25} = -6.1$ ($c = 0.4$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{ε}-2-phenylethyl–N'_{ε}-2-nitrobenzensulfonyl-L-lysine **4b** HPLC: $t_R = 24.73$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.60 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.21 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.30 (m, 5H, Ph–$H_{2,3,4,5,6}$), 4.48–4.28 (m, 5H, Lys–CH^{α}, Fmoc–CH_2, CH_2–CH$_2$–Ph), 4.21 (t, 1H, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.03 (m, 2H, Lys–CH^{ε}_2), 2.96 (t, 2H, $J = 6.8$ CH$_2$–CH_2–Ph), 1.72 (m, 1H, Lys–CH^{β}_2), 1.55–1.44 (m, 3H, Lys–CH^{β}_2 and Lys–CH^{γ}_2), 1.24 (m, 2H, Lys–CH^{δ}_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.33; 29.41; 32.57; 35.41; 43.87; 47.64; 53.94; 66.40; 67.52; 120.50; 125.60; 125.93; 127.24; 127.59; 128.25; 129.07; 129.39; 131.57; 133.25; 134.05; 137.83; 141.82; 144.26; 148.60; 156.41; 172.61. ES–MS: calcd (M+H$^+$), 657.21; found, m/z 657.61, calcd (M+Na$^+$), 679.21; found, m/z 679.81; HRMS (ESI) calcd for $C_{35}H_{35}N_3O_8S$, 657.2162; found, 657.2182. $[\alpha]_D^{25} = -6.8$ ($c = 0.3$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{ε}-3-phenylpropyl–N'_{ε}-2-nitrobenzensulfonyl-L-lysine **4c, 4l** HPLC: $t_R = 26.22$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.82 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.60 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.20 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.29 (m, 5H, Ph–$H_{2,3,4,5,6}$), 4.41 (d, 2H, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.32 (m, 1H, Lys–CH^{α}), 4.21 (t, 1H, $J = 6.8$ Hz, 1H, Fmoc–CH), 4.16 (m, 2H, CH_2–CH$_2$–CH$_2$–Ph) 3.09 (m, 2H, Lys–CH^{ε}_2), 2.68 (t, 2H, $J = 7.6$ CH$_2$–CH$_2$–CH_2–Ph), 1.98 (m, 2H CH$_2$–CH_2–CH$_2$–Ph), 1.79 (m, 1H, Lys–CH^{β}_2), 1.68–1.54 (m, 3H, Lys–CH^{β}_2 and Lys–CH^{γ}_2), 1.38 (m, 2H, Lys–CH^{δ}_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.32; 22.55; 29.47; 30.52; 32.59; 43.90; 47.67; 54.01; 65.51; 67.56; 120.50; 125.61; 125.91; 126.65; 127.61; 128.25; 128.89; 129.03; 131.56; 133.24; 134.05; 141.36; 141.84; 144.28; 148.60; 156.53; 172.76. ES–MS: calcd (M+H$^+$), 671.23; found, m/z 671.11, calcd (M+Na$^+$), 693.23; found, m/z 693.81; HRMS (ESI) calcd for $C_{36}H_{37}N_3O_8S$, 671.2319; found, 671.2340. $[\alpha]_D^{25} = -5.7$ ($c = 1.1$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{ε}-propyl–N'_{ε}-2-nitrobenzensulfonyl-L-lysine **4d** HPLC: $t_R = 22.95$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 & 7.82 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.60 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.71 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.32 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.41 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.33 (br, 1H, Lys–CH^{α}), 4.21 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 4.10 (t, $J = 6.4$ Hz, 2H CH_2–CH2–CH$_3$) 3.95 (m, 2H, Lys–CH^{ε}_2), 1.83 (m, 1H, Lys–CH^{β}), 1.73–1.50 (m, 5H, Lys–CH^{β}, CH$_2$–CH_2–CH$_3$, Lys–CH^{γ}_2) 1.38 (m, 2H Lys–CH^{δ}_2), 0.94 (t, $J = 7.2$ Hz, CH$_2$–CH$_2$–CH_3). ^{13}CNMR(100 MHz, $CDCl_3$): δ 10.26; 22.25; 22.31; 29.74; 32.34; 45.51; 47.43; 54.49; 67.95; 68.56; 120.83; 125.31; 126.03; 127.45; 128.31; 131.84; 133.42; 134.79; 141.80; 144.23; 156.76; 170.26. ES–MS: calcd (M+H$^+$), 595.20; found, m/z 595.31, calcd (M+Na$^+$), 617.20; found, m/z 617.45; HRMS (ESI) calcd for $C_{30}H_{33}N_3O_8S$, 595.2022; found, 595.2056. $[\alpha]_D^{25} = -7.4$ ($c = 0.5$, $CHCl_3$).

N_{α}-(9-fluorenylmethoxycarbonyl)-N_{ε}-diphenylmethyl–N'_{ε}-2-nitrobenzensulfonyl-L-lysine **4f** HPLC: $t_R = 27.25$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 & 7.79 (2br, 2H, Ns–$H_{3,6}$), 7.76 & 7.58 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.68 (m, 2H, Ns–$H_{4,5}$), 7.39 & 7.39–7.30 (m, 9H, Fmoc–$H_{2,3,6,7}$, CH–(Ph)$_2$), 6.88 (s, 1H, CH–(Ph)$_2$), 4.46 (m, 1H, Lys–CH^{α}), 4.40 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.19 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 2.99 (m, 2H, Lys–CH^{ε}_2), 1.66–1.26 (4m, Lys–C$H^{\beta}_{a,b}$, Lys–CH^{γ}_2, Lys–CH^{δ}_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.27; 29.44; 32.54; 43.89; 47.62; 54.00; 67.59; 66.40; 78.71; 120.48; 125.58; 125.90; 127.45; 127.59; 127.78; 128.23; 128.68; 128.85; 129.14; 131.57; 133.21; 134.02; 139.70; 141.80; 144.25; 156.73; 171.80. ES–MS: calcd (M+H$^+$), 719.23; found, m/z 719.57, calcd (M+Na$^+$), 741.23; found, m/z 741.66; HRMS (ESI) calcd

for $C_{40}H_{37}N_3O_8S$, 719.2378; found, 719.2394. $[\alpha]_D^{25} = -12.9$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_ε-naphthylmethyl–N'_ε-2-nitrobenzensulfonyl-L-lysine **4g** HPLC: $t_R =$ 26.92 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 & 7.85 (2br, & Ns–$H_{3,6}$), 7.83 & 7.76 & 7.66 & 7.59 & 7.50 & 7.39 & 7.29 (7m, 17H, Ns–$H_{4,5}$, Fmoc–$H_{1,2,3,4,5,6,7,8}$, Naph–$H_{2,3,4,5,6,7,8}$), 5.29 (m, 2H, CH_2–Naph), 4.55–4.40 (m, 3H, Fmoc–CH_2, Lys–CH^α), 4.20 (t, 1H, $J = 6.8$ Hz, 1H, Fmoc–CH), 2.98 (m, 2H, Lys–CH_2^ε), 1.66 (m, 1H, Lys–CH_2^β), 1.52–1.46 (m, 3H, Lys–CH_2^β and Lys–CH_2^γ), 1.30 (m, 2H, Lys–CH_2^δ); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.40; 29.40; 32.59; 43.82; 47.63; 54.01; 67.58; 68.02; 120.48; 123.15; 125.58; 125.89; 126.37; 126.98; 127.58; 128.22; 128.51; 129.07; 131.53; 133.20; 133.99; 141.80; 144.13; 156.13; 170.25. ES–MS: calcd (M+H$^+$), 693.21; found, m/z 693.07, calcd (M+Na$^+$), 715.21; found, m/z 715.73; HRMS (ESI) calcd for $C_{38}H_{35}N_3O_8S$, 693.2152; found, m/z 693.2169. $[\alpha]_D^{25} = -3.5$ ($c = 0.4$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_ε-t-butylacetyl–N'_ε-2-nitrobenzensulfonyl-L-lysine **4h** HPLC: $t_R = 23.774$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 & 7.81 (2br, 2H, Ns–$H_{3,6}$), 7.76 & 7.60 (2d, $J = 7.2$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.31 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.68 & 4.44 (2d, $J = 10$ Hz, 2H, CH_2CO$_2$C(CH$_3$)$_3$), 4.56 (d, $J = 6.4$, 2H, Fmoc–CH_2), 4.41 (m, 1H, Lys–CH^α), 4.21 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.11 (m, 2H, Lys–CH_2^ε), 1.89 & 1.73 (2m, 2H, Lys–C$H_{a,b}^\beta$), 1.58 (m, 2H, Lys–CH_2^γ), 1.47 (m, 11H, CH$_2$CO$_2$(CH_3)$_3$, Lys–CH_2^δ). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.17; 28.45; 29.24; 32.29; 43.69; 47.54; 53.79; 62.11; 67.59; 83.46; 120.42; 125.53; 125.75; 127.53; 128.17; 131.50; 133.11; 133.92; 141.74; 144.13; 148.51; 156.47; 166.79 172.14. ES–MS: calcd (M+H$^+$), 667.22; found, m/z 667.46, calcd (M+Na$^+$), 689.22; found, m/z 689.81; HRMS (ESI) calcd for $C_{33}H_{37}N_3O_{10}S$, 667.2200; found, 667.2233. $[\alpha]_D^{25} = -6.3$ ($c = 0.4$, $CHCl_3$).

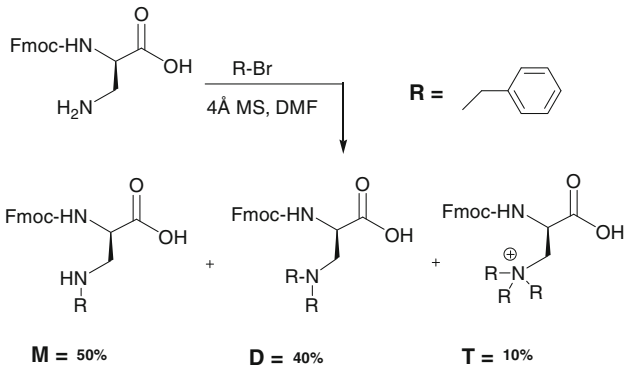
N_α-(9-fluorenylmethoxycarbonyl)-N_ε-methyl–N'_ε-2-nitrobenzensulfonyl-L-lysine **4i** HPLC: $t_R = 19.577$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.76 & 7.60 (2d, $J = 7.2$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.70 (m, 2H, Ns–$H_{4,5}$), 7.40 & 7.31 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.40 (d, $J = 7.2$ Hz, 2 H, Fmoc–CH_2), 4.31 (m, 1H, Lys–CH^α), 4.21 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.74 (s, 3H, N_ε–CH_3), 3.09 (m, 2 H, Lys–CH_2^ε), 1.80 & 1.63 (2m, 2H, Lys–C$H_{a,b}^\beta$), 1.56 (m, 2H, Lys–CH_2^γ) 1.37 (m, 2H, Lys–CH_2^δ). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.42; 29.35; 32.45; 43.78; 47.54; 53.01; 53.91; 67.57; 120.44; 125.51; 125.83; 127.54; 128.19; 131.48; 133.19; 134.01; 141.74; 144.14; 148.49; 156.56; 173.19. ES–MS: calcd (M+H$^+$), 567.17; found, m/z 567.11, calcd (M+Na$^+$), 589.17; found, m/z 589.26; HRMS (ESI) calcd for $C_{28}H_{29}N_3O_8S$, 567.1675; found, 567.1802. $[\alpha]_D^{25} = -6.7$ ($c = 0.5$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_ε-ethyl–N'_ε-2-nitrobenzensulfonyl-L-lysine **4j** HPLC: $t_R = 20.94$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 & 7.83 (2br, 2H, Ns–$H_{3,6}$), 7.77 & 7.60 (2d, $J = 7.2$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.71 (m, 2H, Ns–$H_{4,5}$), 7.41 & 7.32 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 4.41 (d, $J = 6.8$ Hz, 2H, Fmoc–CH_2), 4.31 (m, 1H, Lys–CH^α), 4.21 (m, 3H, Fmoc–CH, N_ε–CH_2CH$_3$), 3.10 (m, 2H, Lys–CH_2^ε), 1.80 & 1.64 (2m, 2H, Lys–C$H_{a,b}^\beta$), 1.58 (m, 2H, Lys–CH_2^γ) 1.39 (m, 2H, Lys–CH_2^δ), 1.29 (t, $J = 7.2$ Hz, 3H, N_β–CH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.64; 22.47; 29.43; 32.61; 43.87; 47.61; 53.99; 62.23; 67.62; 120.50; 125.58; 125.90; 127.59; 128.24; 131.56; 133.24; 134.05; 141.81; 144.21; 148.57; 156.62; 172.78. ES–MS: calcd (M+H$^+$), 581.18; found, m/z 581.39, calcd (M+Na$^+$), 603.18; found, m/z 603.61; HRMS (ESI) calcd for $C_{29}H_{31}N_3O_8S$, 581.1832; found, 581.1864. $[\alpha]_D^{25} = -7.1$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-benzyl–N'_β-t-butoxycarbonyl-L-2,3-diaminopropionic acid **5a** HPLC: $t_R = 24.608$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 & 7.60 (2d, $J = 7.6$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.40 & 7.30 (2t, $J = 7.6$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.35 (m, 5H, Ph–$H_{2,3,4,5,6}$), 5.20 (m, 2H, CH_2–Ph), 4.38 (m, 3H, Fmoc–CH_2, Dap–CH^α), 4.24 (t, $J = 6.8$ Hz, 1H, Fmoc–CH), 3.59 (br, 2H, Dap–CH_2^β), 1.44 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.70; 42.66; 47.57; 55.61; 67.59; 68.03; 80.53; 120.40; 125.58; 127.50; 128.15; 128.82; 128.98; 129.08; 141.74; 144.16; 156.45; 170.73. ES–MS: calcd (M+H$^+$), 516.23; found, m/z 516,49; calcd (M+Na$^+$), 538.23; found, m/z 538,73; HRMS (ESI) calcd for $C_{30}H_{32}N_2O_6$, 516.2260; found, 516.2291; $[\alpha]_D^{25} = -8.3$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-benzyl–N'_β-methylthrityl-L-2,3-diaminopropionic acid **6a** HPLC: $t_R =$ 29.639 min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 & 7.58 (1d & 1m, $J = 8$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.33–7.13 (m, 23H, Mtt–(Ph)$_3$, Ph–$H_{2,3,4,5,6}$, Fmoc–$H_{2,3,6,7}$), 5.07 (dd, 2H, CH_2–Ph), 4.30 (m, 1H, Dap–CH^α), 4.22 (d, $J = 6.4$ Hz, 2H, Fmoc–CH_2), 4.05 (t, $J = 6.4$ Hz, 1H, Fmoc–CH), 3.47 & 3.29 (2br, 2H, Dap–CH_2^β), 2.35 (s, 3H, Mtt–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.45; 41.46; 47.19; 52.41; 68.20; 68.66; 82.43; 120.34; 125.53; 127.61; 128.16; 128.32; 128.71; 128.99; 129.06; 134.88; 137.38; 141.62; 143.89; 147.42; 157.25; 169.35. ES–MS: calcd (M+H$^+$), 672.30; found, m/z 672,54; calcd (M+Na$^+$), 694.30; found, m/z 694,08; HRMS (ESI) calcd for $C_{31}H_{27}N_3O_8S$, 672.2988; found, 672.3005; $[\alpha]_D^{25} = -11.6$ ($c = 0.3$, $CHCl_3$).

N_α-(9-fluorenylmethoxycarbonyl)-N_β-benzyl–N'_β-1-(4,4-dimethyl-2,6-dioxocycloex-1-ylidene)-3-methylbutyl-L-2,3-diaminopropionic acid **7a** HPLC: $t_R = 26.381$ min (gradient 2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (br, 1H, N$H\alpha$), 7.76 & 7.60 (2d, $J = 8$ Hz, 4H, Fmoc–$H_{1,4,5,8}$), 7.40 & 7.30 (2t, $J = 7.2$ Hz, 4H, Fmoc–$H_{2,3,6,7}$), 7.35 (m, 5H,



Scheme 1 N-alkylation reaction of Fmoc–Dap–OH

Ph–$H_{2,3,4,5,6}$), 5.25 (br, 2H, CH_2–Ph), 4.64 (d, $J = 6.4$ Hz, 2H, Fmoc–CH_2) 4.39 (m, 1H, Dap–CH^{α}), 4.22 (t, $J = 6.4$ Hz, 1H, Fmoc–CH), 3.97 (br, 2H, Dap–CH_2^{β}), 2.91 (m, 2H, Dde–CH_2CH(CH$_3$)$_2$), 2.42 (s, 4H, Dde–CH_2C(CH$_3$)$_2$), 1.89 (m, 1H, Dde–CH(CH$_3$)$_2$), 1.04 (s, 6H, Dde–C(CH_3)$_2$, 0.92 (d, $J = 6.8$ Hz, 6H, Dde–CH(CH_3)$_2$); ^{13}C NMR (100 MHz, CDCl$_3$): δ 22.87; 28.54; 29.59; 30.45; 37.96; 45.51; 47.40; 52.65; 54.19; 68.09; 68.85; 107.68; 120.46; 125.52; 125.58; 127.56; 128.24; 129.19; 129.23; 129.40; 134.81; 141.72; 143.98; 156.21; 169.32; 178.87; 199.43. ES–MS: calcd (M+H$^+$), 622.30; found, *m/z* 622,19; calcd (M+Na$^+$), 644.30; found, *m/z* 644,69; HRMS (ESI) calcd for $C_{38}H_{48}N_2O_6$, 622.3043; found, 622.3083; $[\alpha]_D^{25} = -9.8$ ($c = 0.4$, CHCl$_3$).Fmoc–Dap (Ns, Bn)–Ile–OH **8a** HPLC: $t_R = 22.35$ min (gradient 2); ES–MS: calcd (M+H$^+$), 714.23; found, *m/z* 714.08; H–Dap(Ns, Bn)-Ile-Gly-Gly-Asp-Gly-Asp-Gly-OH **9a** Yield: 35%; HPLC: $t_R = 11.92$ min (gradient 1); ES–MS: calcd (M+H$^+$), 950.36; found, *m/z* 950.71.

Results and discussion

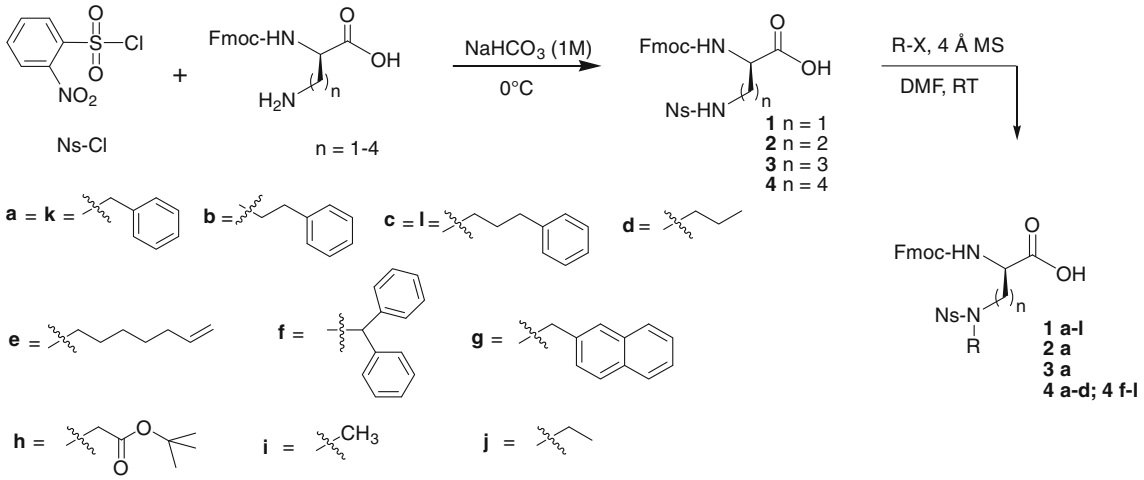
As first step, we investigated the efficiency of our synthetic method performing the reaction on Fmoc-amino acid free on its side chain (Fmoc–Dap–OH). Benzyl bromide was used as an alkylating agent only in the presence of activated 4 Å molecular sieves. A high yield of mono (M), di (D), and tribenzylated (T) products was obtained after stirring the reaction mixture in DMF for 15 h (Scheme 1).

This result confirmed the hypothesized role of molecular sieves as base (Hasegawa et al. 2008), which are supposed to capture the proton of the amino group.

Next, we performed the alkylation reaction on several Fmoc-amino acids previously protected on their side chain with Ns group (Fmoc–Lys(Ns)–OH, Fmoc–Orn(Ns)–OH, Fmoc–Dab(Ns)–OH Fmoc–Dap(Ns)–OH) (De Luca et al. 2005). As shown in Scheme 2, each amino acid derivative was dissolved in DMF at room temperature and treated with the appropriate alkyl halide in presence of activated 4 Å molecular sieves.

The optimized N-alkylation procedure was carried out on a wide range of alkyl halides; the reaction time and the relative yield for each halide are summarized in Table 1.

In particular, the N-alkylation reactions with benzyl and 3-phenylpropyl groups were performed by using the appropriate bromide and then repeated by using the corresponding chloride. As expected, the employed halides did not react equally (Andersen and Stromgaard 2004; Hahn and Schepers 2008). After 24 h we could recover compound **1k** and **4k** with a yield of around 50% (Table 1), while **1a** and **4a** were obtained in almost quantitative yield (>95%). Concerning **1l** and **4l**, the chlorides promoted a minimal conversion, which was characterized by a reaction



Scheme 2 Synthetic strategy of mono-N-alkylated amino acids

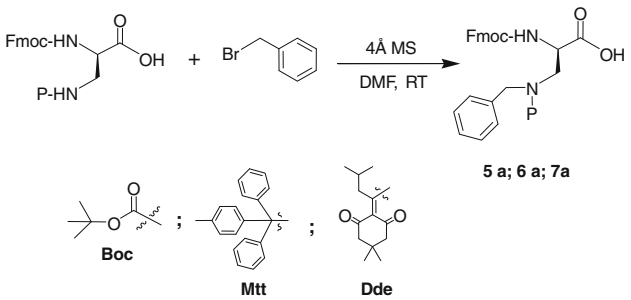
Table 1 Mono-N-alkylation efficiency of each Fmoc-amino acid with respect to the employed halides

Compound	Time (h)	Yield (%)	R–X
1a	15	>95	Br–Bn
1b	15	95	Br–$(CH_2)_2$–Ph
1c	15	95	Br–$(CH_2)_3$–Ph
1d	24	90	Br–$(CH_2)_2$–CH_3
1e	35	60	Br–$(CH_2)_5$–CH_2=CH_2
1f	38	70	Br–$CH(Ph)_2$
1g	18	95	Br–CH_2–Naph
1h	15	>95	Br–CH_2–CO_2–$C(CH_3)_3$
1i	15	>95	I–CH_3
1j	15	95	I–CH_2–CH_3
1k	24	50	Cl–Bn
1l	24	<5	Cl–$(CH_2)_3$–Ph
2a	18	>95	Br–Bn
3a	18	>95	Br–Bn
4a	22	>95	Br–Bn
4b	22	90	Br–$(CH_2)_2$–Ph
4c	24	<70	Br–$(CH_2)_3$–Ph
4d	28	75	Br–$(CH_2)_2$–CH_3
4f	48	50	Br–$CH(Ph)_2$
4g	22	90	Br–CH_2–Naph
4h	18	95	Br–CH_2–CO_2–$C(CH_3)_3$
4i	18	95	I–CH_3
4j	22	80	I–CH_2–CH_3
4k	24	50	Cl–Bn
4l	24	<5	Cl–$(CH_2)_3$–Ph

yield of <5% (Table 1), whereas the bromides were successful in producing **1c** and **4c** (yield 90–95%).

It is known that while the side chain pKa of Lys, Orn, and Dab is very similar, this value for Dap is lower by one unit (Isidro-Llobet et al. 2009). Nevertheless, as suggested by the similar reaction yield obtained for all amino acid derivatives (Lys, Orn, Dab, and Dap) alkylated by the same halides, the same nucleophilicity toward the same alkylating agent has been shown by Lys, Orn, and Dab as well as by Dap (in Table 1; compare yields of **1a**, **2a**, **3a**, **4a**; **1b** and **4b**; **1g** and **4g**; **1h** and **4h**; **1i** and **4i**).

It is worth noting that a decrease in reaction rate corresponded with an increase of the amino acid side chain length, for instance, the substitution reaction for compounds like **4c**, **4d**, and **4f** proceeded so slowly that by-products were formed. Several effects are likely to contribute to a great decrease in reaction rate and yield (**4c** < **4d**, **4f** < **1f**), like the presence of not particularly electron-rich (**4d**) or sterically hindered (**4f**) substituents on the alkyl bromide central carbon. Therefore, the reaction yields observed for these compounds were lower than what was expected.



compound	time (h)	yield (%)	P
5a	24	75	Boc
6a	24	75	Mtt
7a	24	95	Dde

Fig. 1 N-benzylation of differently protected Fmoc-diaminopropionic acid derivatives and relative reaction times and yields

In general, the highest yield values occurred for the most electron-rich alkyl bromides (see Table 1: yield of **1a**, **1b**, and **1c** is **1g**; yield of **2a**, **3a**, **4a**, and **4b** is **4g**). However, for around 90% yield, the compound obtained could be used as a crude product for the subsequent peptide synthesis following centrifugation that eliminated the molecular sieves; however, for all compounds that yielded <75%, it was necessary to purify the final product by RP-HPLC. In these cases, the purification allows for the recovery of any unreacted amino acid, which can be re-employed for alkylation reactions.

In order to further study the developed N-alkylation procedure promoted by molecular sieves, we performed the N-benzylation of the following Fmoc-diaminopropionic acid derivatives, Fmoc–Dap(Boc)–OH, Fmoc–Dap(Mtt)–OH and Fmoc–Dap(Dde)–OH (Fig. 1), which are among the most used in peptide solid-phase synthesis. As shown in Fig. 1, the reaction was successful for each of these differently protected diaminopropionic acid derivative, even though it proceeded slower (24 h) than the same N-benzylation performed on Fmoc–Dap(Ns)–OH (15 h). Moreover, the formation of **5a** and **6a** is also characterized by a slightly lower yield (75%), compared with **1a** (>95%). In fact, the synthesis of **5a** was incomplete, whereas the synthesis of **6a** produced some by-products; consequently, for these building blocks, a purification step is required before being employed in peptide synthesis. Overall, the proposed N-alkylation methodology resulted validated for different amino protecting groups (Fig. 1).

In order to demonstrate the practical applicability of the functionalized amino acids as building blocks, we decided to introduce **1a**, into a peptide sequence by using the standard Fmoc-based solid-phase protocol (Scheme 3).

As preliminarily shown by Kaiser test result and confirmed by LC–MS analysis on the crude products obtained

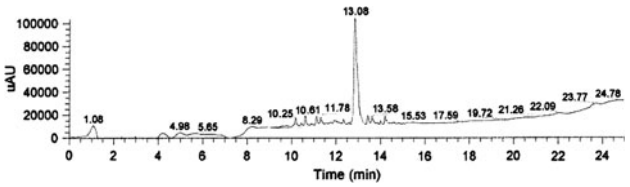
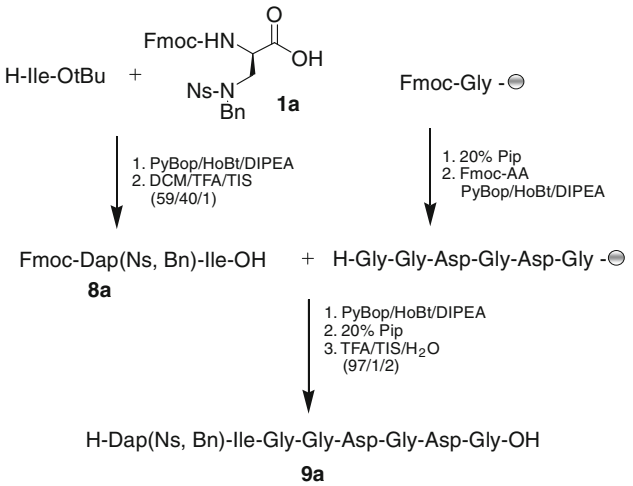


Fig. 2 HPLC profile of **9a** crude product



Scheme 3 Introduction of N-alkylated amino acids into solid supported generic peptide (Fmoc-Gly Wang Resin, 0.8 mmol g^{-1}. *TFA* trifluoroacetic acid, *TIS* triisopropylsilane)

upon cleavage from the resin, the desired alkylated peptide was found with a low yield (data not shown). It is likely that the sterical hindrance of the introduced substituent and the nitrobenzensulfonyl (nosyl) groups on the side amino group makes the coupling of N-alkylated amino acid with the peptide sequence on the resin difficult. Therefore, a solution phase coupling, in DMF with standard carboxyl activation PyBop/HOBt/DIPEA, of **1a** with H–Ile–OtBu was performed (Scheme 3). The corresponding dipeptide **8a** was recovered with quantitative yield and high purity, after treatment with trifluoroacetic acid in order to remove the tBu group. As a subsequent step, **5a** was introduced into a peptide sequence in solid phase using standard Fmoc chemistry (Scheme 3). After cleavage from resin, the alkylated peptide **9a**, fully characterized by LC–MS analysis, was obtained with a yield of around 70% (Fig. 2). This result confirmed the reliability of the alkylation method developed, since it was proven to fit well with Fmoc chemistry.

Conclusion

In conclusion, by using only molecular sieves to promote the reaction, a mild, scalable and efficient solution phase procedure for side chain N-alkylation of Fmoc-amino acids was developed. It results completely innovative, since in literature, at the best of our knowledge, it is not described any example of N-alkylation reaction performed with halides and molecular sieves as base. The proposed N-alkylation methodology was validated for different amino protecting groups, but the best results were obtained by using *o*-Ns-protected Fmoc-amino acids, which represent readily available starting materials. In fact, the employed Fmoc-amino acids are rapidly and efficiently protected with Ns group and afford the desired mono-N-alkylated compound in high yield.

For the majority of the employed halides, the procedure is a one-pot synthesis, which avoids the purification after each reaction step. Moreover, it allowed the production of the desired compound in synthetically useful yield for the subsequent peptide chain assembly. Therefore, the developed methodology enables the introduction of different substituents on the side chain of peptides and, due to the very mild reaction conditions required, promises general applicability in the field of organic synthesis.

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