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A fast entry to the novel medicinally-important 9-anilinoacridine peptidyls by solid phase organic synthesis (SPOS)

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ABSTRACT

A new approach to the synthesis of novel medicinally-important mono- and bis-9-anilinoacridine (9-AnA) peptidyl derivatives is described. The method generates efficiently 9-AnAs with variable spacer lengths and charged, polar or hydrophobic residues at desired positions, which can increase binding affinity, conformational stability, intracellular transport and/or biological activity. The synthetic routes reported in this work are both general and applicable, and significantly expand the scope of potential 9-aminoacridine (9-AA) anticancer candidates.

Many antitumour agents, including the anthracyclines, epipodophyllotoxins and mitoxantrone target DNA topoisomerase II arresting cell proliferation.[1] Important 9-anilinoacridine (9AnA) drugs (e.g., Amsacrine, AHMA) and their derivatives also play an important role in medicine and are successful candidates for the treatment of cancer, viral, and prion diseases.[2a] These compounds have been classified as powerful DNA intercalators inhibiting DNA replication by forming topoisomerase II–DNA complexes, causing DNA strand breakdown and subsequent apoptosis. Evidence has been presented showing that 9-aminoacridine based reagents are capable of targeting DNA without inducing DNA damage and additional mutations in cellular genes. This phenomenon reduces side effects and the risk of developing secondary cancers, thereby supporting the use of nongenotoxic DNA-binding small molecules as anticancer therapies.[2b] The traditional solution syntheses of 9AnA analogs involve several steps and harsh reaction conditions, requiring laborious purification of intermediates and final compounds.[3] Solid phase organic synthesis (SPOS) is a fast and efficient technique that has become a productive approach in the discovery of bioactive hits.

Recently, several research groups have reported the solid phase synthesis of novel DNA binding molecules by chemically modifying known 9-anilinoacridine intercalating moieties with a variable peptide appendage[4] (e.g., **I**, Fig. 1). In such molecules the intercalation domain will direct the peptide into a groove of the double helical secondary structure where sequence-specific contacts at minor and major grooves are possible. Moreover, several approaches based on dual amino-acridines (e.g., **II**, Fig. 1) have been reported in the literature.[5] These bisintercalators, including 9-aminoacridine (9AA) moieties, exhibited both higher affinity[6] and selectivity[7] in binding compared to their mono counterparts. The improved bioactivity of the covalent dimers of aminoacridine can be attributed to the increased local concentration of the active moiety. It is noteworthy that all the methods mentioned above utilized preformed 9-AA or 9AnA units.

We have previously demonstrated a new, highly-efficient, one-pot derivatization of 9-AAs at the 9-amine position by simple S_NAr reactions yielding a series of substituted *N*(9)-anilinoacridines in solution.[8] However, purification of the products was problematic in several of the syntheses. This encouraged us to develop a short and efficient SPOS method for the rapid generation of new 9-AnA core based compounds. In the context of exploring the rapid derivatization of the 9-AnA scaffold we found that assembly of a 9-AnA core on a solid support was a useful methodology, generating relatively diverse and pure products without any purification step, and, therefore, enhancing greatly their availability for examination in biological systems.

This Letter describes the facile solid phase synthesis of novel mono- and bis-9-anilinoacridine (9-AnA) peptidyl derivatives using Fmoc chemistry compatible with the protocol developed by us.[8] The synthetic strategy generates rapidly 9-AnAs with variable spacer lengths and charged, polar or hydrophobic residues at desired positions, which can lead to increased binding affinity, conformational stability, intracellular transport, and biological activity. The results described here should permit advanced

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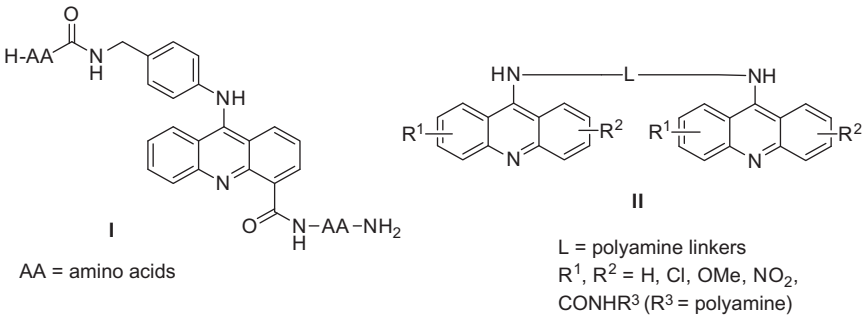
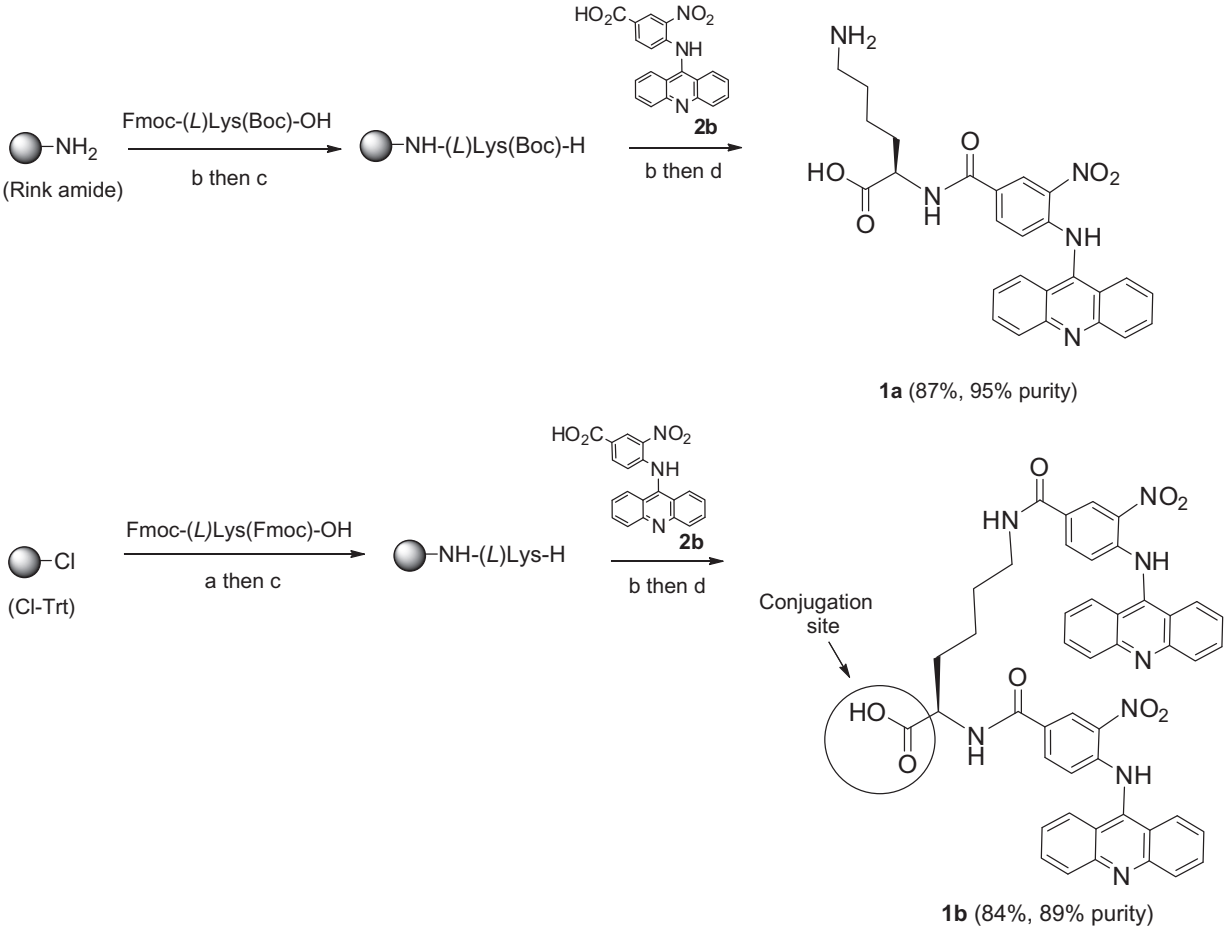


Figure 1. Examples of known potent 9-anilinoacridine peptides and polyamino bis-9-aminoacridines.

conjugation of 9-AnAs with biomolecules, such as peptides, antibodies, and proteins, modulating their activity, bioavailability, and applicability.

Initially we decided to try coupling preformed 9-AnA **2b**[8] with preloaded Fmoc-(L)Lys(Boc)-OH on Rink amide MBHA and Cl-Trt resins as shown in Scheme 1. The purpose of this experiment was to examine the straightforward coupling of a preformed 9AnA building block versus the 9AnA core assembly approach on both resins. Thus, after applying the standard Fmoc chemistry protocol (PyBoP, NMM in DMF), **2b** was successfully coupled to the α-amino group of the preloaded (on Rink amide MBHA) lysine, affording, after cleavage the corresponding Lys-9AnA peptide, **1a** in good yield and purity. When preloaded bifunctional Fmoc-(L)Lys(Fmoc)-OH on Cl-Trt resin was used, the corresponding bis-Lys(9AnA)$_2$ **1b** was successfully obtained, confirming the compatibility of carboxylated 9-AnA under standard peptide coupling conditions. At this stage we decided to move to a solid phase 'assembly' approach toward 9-AnAs, enabling diverse derivatization of the 9-AnA core.

Notably, the carboxylic acid group on **1b**, released after cleavage from the Cl-Trt acid-sensitive resin, can act as an anchor point for conjugation chemistry. Cleavage of all compounds in this work was carried out under strong acidic conditions [TFA/H_2O/1,2-ethanedithiol (EDT) (95:2.5:2.5)] due to the low solubility of the products in most organic solvents. Previously, we had shown that the amine (NH_2) at C-9 of the 9-AA was nucleophilic enough to take part in nucleophilic aromatic substitution (S_NAr) yielding 9-anilinoacridines.[8] Most electrophilic haloaryl reagents involved in this reaction bear two strongly electron-withdrawing (EW) groups, such as CF_3, CN, CO_2H, and NO_2 at *ortho* and *para* positions, favoring



Scheme 1. Synthesis of mono and bis-Lys-9-anilinoacridine derivatives **1a**,**b** from **2b**. Reaction conditions: (a) NMM, CH_2Cl_2, 40 min, rt; (b) PyBOP, NMM, DMF, 40 min, rt; (c) 20% piperidine/DMF (2 × 20 min); (d) TFA/H_2O/EDT (95:2.5:2.5).

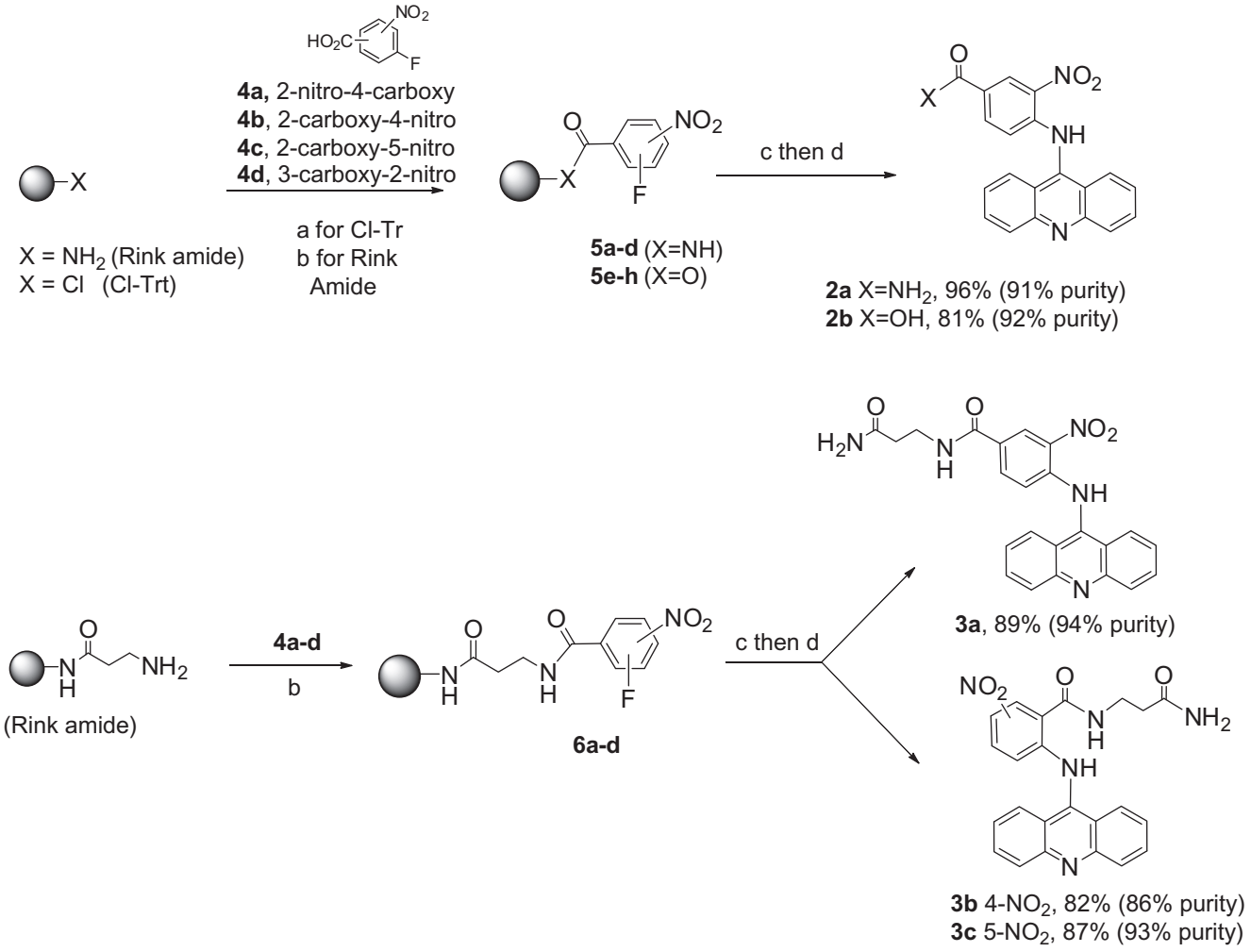
stabilized resonance structures.[9] The NO_2 group was found to be the most effective, affording products in good yield and high purity. Following our preliminary biological results,[10] in which compound **2b** exhibited remarkable anticancer activity in vitro, we decided to investigate the influence of the positions of the NO_2 and CO_2H groups in halobenzene substrates **4a–d** (Scheme 2) on the effectiveness of S_NAr reactions with 9-AA to form 9-AnAs on a solid support, and on their structure–activity relationship (SAR).

Thus Rink amide[11] and Cl-Trt[12] preloaded fluoronitrocarboxylic acids **5a–d** and **5e**–**h**, respectively, were submitted to S_NAr reactions with 9-AA (3 equiv) and excess Cs_2CO_3 in DMF at room temperature for 24 h (Scheme 2). Unfortunately, only **4a** yielded the corresponding 9-AnAs **2a** and known **2b** in good yields and purity, probably due to the bulkiness of the resin (for **5b**,**f**) and unfavorable resonance effects (for **5c,d,g,h** with an EW group at the *meta* position). Hence, we decided to distance the electrophilic site from the resin by spacer hopping to eliminate the steric problems. Thus, β-Ala preloaded on Rink amide was coupled successfully to benzoic acids **4a–d** under standard conditions to give amides **6a–d** which underwent further S_NAr reactions with 9-AA. In this case, the three starting reagents **4a, 4b**, and **4c** afforded smoothly the corresponding β-Ala-9-AnAs **3a**, **3b**, and **3c**. Warming the reaction mixtures up to 90 °C did not lead to better results. Apparently, steric problems can be overcome potentially enabling the incorporation of **4a–c** in the planned 9-AnA peptidyl assemblies.

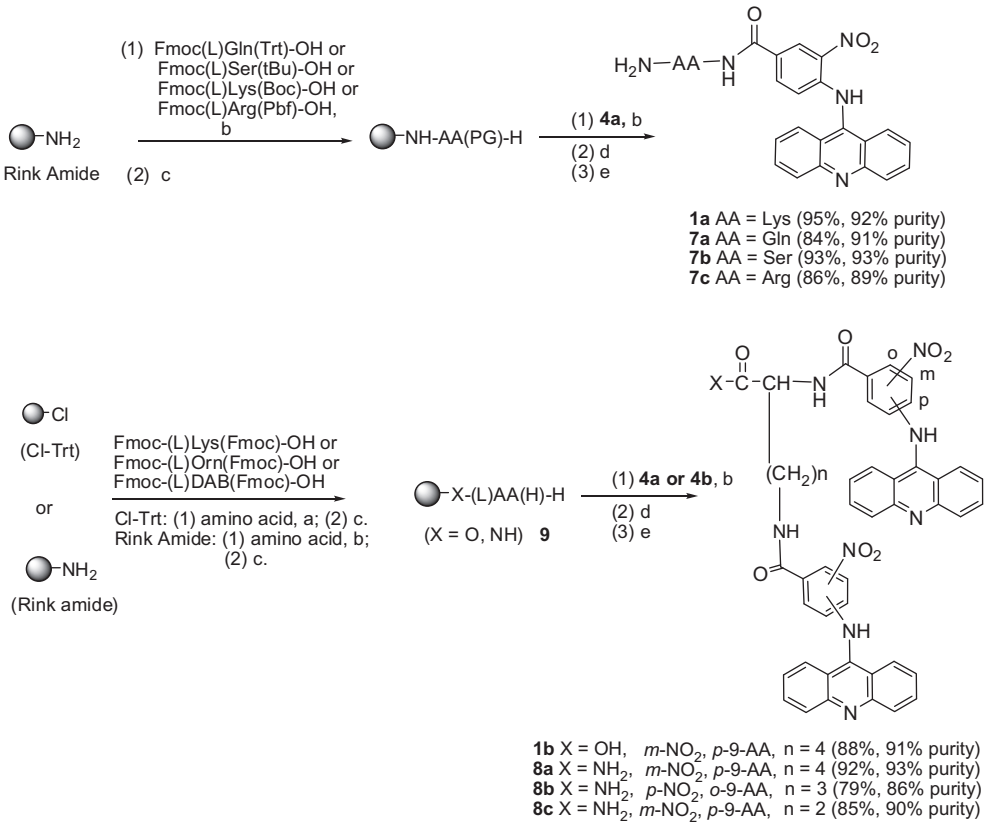
Amino acid prodrugs usually impart improved pharmacological characteristics such as enhanced transport and higher resistance to metabolism, exhibiting slower rates of enzymatic activation for suspended release of a drug.[13]

Based on these facts we attempted the assembly of 9-AnA on several representative amino acids as possible minor and major groove contact spacers[4] (and potential prodrug moieties) on Rink amide for the synthesis of peptidyls **1a** and **7a–c** as shown in Scheme 3. All the final compounds were prepared in good yields and high purity regardless of the nature of the amino acid linkers. Similarly, bis-9-AnAs were prepared from several di-Fmoc protected Lys analogs. A large variety of commercially available L and D diamino aliphatic acids can be used to synthesize rapidly bis-intercalators with variable distances between the two 9-AnAs that sandwich two base pairs, and thread their side chains through the helix to form hydrogen bonding contacts with the O6/N7atoms of guanine in the major or minor grooves.[14] It has been shown that bis(9-aminoacridine-4-carboxamides) bis-intercalate via a threading mode and that their complexes dissociate many orders of magnitude more slowly than simple intercalators.[15]

Moving forward, representative diFmoc protected L-Lys, L-Orn and L-DAB were loaded on resins using standard methods. After Fmoc release (Scheme 3) compounds **4a** and **4b** were coupled with the intermediate preloaded amino acids **9**, and the products submitted to S_NAr reactions with a large excess of 9-AA (4 equiv) and Cs_2CO_3 in DMF for 24 h at rt. Upon completion of the



Scheme 2. Synthesis of nitro-carboxy and carboxamide-9-anilinoacridine derivatives **2a** and **3a–c** by S_NAr reactions on solid phase. Reaction conditions: (a) NMM, CH_2Cl_2, 40 min, rt; (b) PyBOP, NMM, DMF, 40 min, rt; (c) 20% piperidine/DMF (2 × 20 min); (d) **9-AA**, Cs_2CO_3, DMF, 24 h, rt; (e) TFA/H_2O/EDT (95:2.5:2.5).



Scheme 3. Synthesis of mono and bis peptidyl-9-anilinoacridine derivatives **1a**, **7a–c**, **1b**, **8a–c** by S_NAr reactions on solid phase. Reaction conditions: (a) NMM, CH_2Cl_2, 40 min, rt; (b) PyBOP, NMM, DMF, 40 min, rt; (c) 20% piperidine/DMF (2 × 20 min); (d) **9-AA** (4 equiv), Cs_2CO_3, DMF, 24 h, rt; (e) TFA/H_2O/EDT (95:2.5:2.5).

solid-phase synthesis, the resin-bound bis-9-AnAs were cleaved by treatment with TFA/EDT/H_2O (95:2.5:2.5) to give **1b** (prepared earlier from preformed **2b**, see Scheme 1) and **8a–c** in good yields and purity, confirming that Rink amide MBHA and Cl-Trt resins were suitable for this synthetic route. The products were characterized by MS and NMR spectrometry (see Supplementary data). The purity of the synthesized compounds was determined by HPLC (MeCN/0.1% TFA in H_2O).

In conclusion, we have developed a new method for the efficient synthesis of mono- and bis-9-anilinoacridine (9-AnA) peptidyl derivatives via S_NAr reactions on solid phase. The resulting 9-AnA peptidyls were obtained in good yields rapidly affording novel potential DNA intercalators with variable spacer lengths and charged, polar or hydrophobic residues at desired positions. All the products were obtained from commercially available reagents. The in vitro anticancer activity of the synthesized compounds is under evaluation.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.05.023.

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10. Unpublished results.
11. *General procedure for the solid phase synthesis on Rink amide MBHA resin:* The procedure for the synthesis on Rink amide MBHA resin was identical to the synthesis on Cl-Trt resin except for the loading: Loading: The Fmoc protecting group from rink amide was removed by reaction with 20% piperidine in NMP (2 × 15 min, 5 mL each) and subsequent washing (2 × CH_2Cl_2, 2 × DMF, 5 mL each). Next, a preactivated solution of protected amino acid (0.78 mmol acid, 0.78 mmol, PyBOP, 2.34 mmol DIEA in 4.5 mL of DMF) was added to the resin and shaken for 2 h. The resin was washed with 2 × DMF and 2 × CH_2Cl_2 (3 mL each). Cleavage was performed as for Cl-Trt resin. *Data for* **7c**: yellowish powder (0.083 g, 86% yield), IR (KBr): 3200–2800 (br, s), 1650 (C=O), 1630, 1240 cm^{-1}; HRMS (CI, *m/z*) calcd for $C_{26}H_{27}N_8O_4$ (MH^+) 515.207, found 515.276; ^1H NMR (300 MHz, DMSO-d_6): 8.80 (m,1H), 8.63 (m,1H), 8.92–8.88 (m, 2H), 8.50–8.35 (m, 4H), 7.8 (d, *J* = 8 Hz, 2H), 7.60–7.45 (m, 4H), 7.14–6.95 (m, 3H), 4.21 (m, 1H), 3.26–3.02 (m, 4H), 1.55 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 168.2, 164.2, 160.1, 158.4, 153.3, 145.5, 143.1, 133.7, 131.0, 128.7, 128.6, 127.0, 124.1, 123.6, 121.9, 121.3, 57.8, 44.3, 25.0, 21.7.
12. *General procedure for the solid phase synthesis on Cl-Trt resin:* To 2-chlorotrityl resin (0.2 g, 0.28 mmol loading) in a jacketed fritted peptide vessel was added a solution of protected amino acid (0.26 mmol) in dry DMF (3.5 mL), and after

addition of diisopropylethylamine (DIEA, 185 mL, 1.04 mmol) the reaction mixture was shaken for 1.5 h. After completion of the loading, dry MeOH (1.5 mL) was poured into the vessel and shaking was continued for an additional 20 min. The solvent was removed by filtration and the following washings were performed sequentially: 2 × CH_2Cl_2/MeOH/DIEA (17:2:1), 2 × CH_2Cl_2, 2 × DMF, 2 × CH_2Cl_2, 2 × CH_2Cl_2/DMF (1:1) (3 mL each). The Fmoc protecting group was removed by reaction with 20% piperidine in NMP (2 × 15 min, 5 mL each) and subsequent washing (2 × CH_2Cl_2, 2 × DMF, 5 mL each). Next, a preactivated solution of fluoronitrobenzoic acid (0.78 mmol acid, 0.78 mmol PyBOP, 2.34 mmol DIEA in 4.5 mL of DMF) was added to the resin and shaken for 2 h. The resin was washed with 2 × DMF and 2 × CH_2Cl_2 (3 mL each) and the aromatic substitution using 9-AA (4 equiv) with 0.5 g of Cs_2CO_3 in 3 mL of DMF was performed for 24 h. After washing with 2 × H_2O, 2 × DMF, 2 × MeOH, 2 × CH_2Cl_2 and 2 × DMF (3 mL each) the resin was transferred to a vial for cleavage. *Cleavage*: a cold solution of 2.5% H_2O/2.5% triisopropylsilane in 95% TFA (2 mL) was added. After shaking for 1.5 h, the solution was collected and the resin washed with cold TFA (2 × 1 mL each). After combining the TFA solutions, the solvent was evaporated first under N_2 stream and then in vacuo to give after the usual work-up (fast purification using the solid-phase extraction pack RP-18, first washed with H_2O and then extracted with MeCN, 5 mL each). *Data for* **1b**: yellowish powder (0.141 g, 88% yield). IR (KBr): 3500–3080 (br, s), 1700 (C═O), 1650, 1180 cm^{-1}, HRMS (CI, *m*/*z*) calcd for $C_{46}H_{37}N_8O_8$ (MH^+) 829.273, found 829.285; ^1H NMR (300 MHz, DMSO-d_6): 8.83–8.65 (m,4H), 8.50–8.35 (m, 4H), 8.18–8.02 (m, 2H), 7.83–7.79 (m, 2H), 7.67–7.55 (m, 3H), 7.54–7.46 (m, 3H), 7.20–6.85 (m, 6H), 4.40 (m, 1H), 3.25–3.07 (m, 4H), 1.89–1.80 (m, 2H), 1.58-1.40 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): 178.0, 165.1, 163.2, 161.9, 160.1, 159.0, 158.4, 156.3, 153.2, 144.6, 144.1, 143.1, 136.8, 133.3, 133.0, 132.9, 130.0, 129.5, 127.6, 126.4, 125.3, 124.2, 120.6, 120.2, 119.5, 118.5, 115.4, 56.6, 50.1, 33.6, 30.2, 22.3.

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