Solid-Phase Synthesis of Tyrosine Peptide Aldehydes. Analogues of (*S*)-MAPI

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We report an efficient solid-phase synthesis of C-terminal tyrosine peptide aldehydes based on the HIV protease inhibitors (*S*)-MAPI and GE 20372 A. Our strategy consisted of anchoring the side chain of Dde-Tyrosinol **(5)** onto the brominated Wang linker derivative ((4-bromomethyl)-phenoxy-allyl acetate) **(6)** to give after ester hydrolysis the N^{α}-(Dde)-*O*-(4-methylphenoxyacetic acid)-L-Tyrosinol template **(8)**. This was attached to aminomethyl resin and elongated using standard Fmoc protocols. Importantly there was no evidence of esterification side reactions. The unsymmetrically substituted urea linkage of the (*S*)-MAPI family was incorporated using the N^{α}-(4-nitrophenyloxycarbonyl)amino acid *tert*-butyl esters following which the protected tetrapeptide alcohol immobilized on the solid support was oxidized to its corresponding aldehyde using sulfur trioxide−pyridine. The efficiency and reliability of the oxidation step was dramatically improved by the incorporation of a small PEG-spacer between the linker and the solid support. The tetrapeptides **12a** and **12b** were cleaved by acidolysis, purified by RP HPLC, and isolated in high yield and purity, demonstrating the success of the whole synthetic process.

Introduction

Serine[1,2] and cysteine[3,4] proteases are potently inhibited by peptide aldehydes[1] and peptide trifluoromethyl ketones.[5] Inhibition arises from the formation of hemiacetal or hemithioacetal tetrahedral adducts within the active sites of the proteases which mimic the proposed structure of the transition state/intermediate.[3] The C-terminal peptide aldehydes are thus an important class of transition-state analogues and have been the focus of considerable attention since their initial discovery in natural products.[6] Importantly, many serine and cysteine proteases have been implicated in a number of disease states,[7] and thus, their inhibition has been and continues to be widely exploited medicinally. For these reasons, recent literature provides many examples of the biological activity of peptide aldehydes toward a variety of biological targets. Thus, peptide aldehyde Ac-Leu-Ala-Ala-(*N*,*N*′-dimethylglutaminal) was found to be a reversible, slow-binding inhibitor for hepatitis-A virus (HAV) 3C proteinase[8] with a K_i of 42 nM. Whereas α-MAPI (or (*S*)-MAPI, $K_i = 1.3\ \mu$M) **(1)** was described by Stefanelli et al.[9] as a potent inhibitor of the human immunodeficiency virus (HIV) despite it being an aspartic acid protease.[10] This protease is essential for the processing of the viral polyproteins and plays a crucial role in the HIV life cycle. Related to MAPI is GE 20372 A which was recently isolated from *Streptomyces* sp. ATCC 55925 ($K_i = 18\ \mu$M), and which also demonstrates activity against HIV protease.[11] This family of tetrapeptides has two common features: a terminal aldehyde and a urea link between two amino acid residues.[12]

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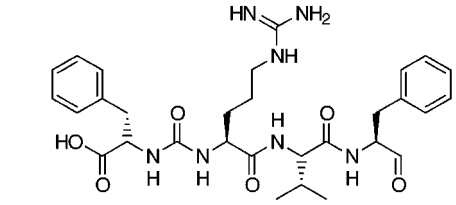
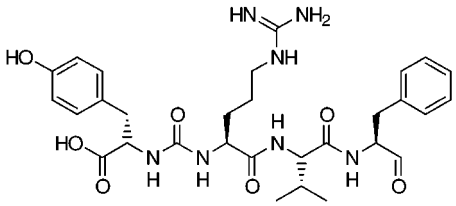


(1) α-MAPI or *S*-MAPI



(2) GE 20372 A

Figure 1. Human immunodeficiency virus (HIV) aspartic acid protease inhibitors GE 20372 and MAPI.

Results and Discussion

Ongoing work within our group on urea containing peptides as inhibitors of serine proteases attracted us to the (*S*)-MAPI and GE 20372 A (Figure 1) family of peptide aldehydes. Since the C-terminal residue was phenylalanine, we decided to use the corresponding hydroxylated residue tyrosine as this would allow side-chain anchoring to a solid support via an appropriate linker. The unsymmetrical substituted urea linkage of the (*S*)-MAPI family would be synthesized using N^{α}-(4-nitrophenyloxycarbonyl)amino acid esters,[13] although Pena et al.[10b] has recently published a solution synthesis of urea dipeptides of this class of compounds using carbonyldiimidazole. Several methods for the solid-phase synthesis of peptide aldehydes have been reported.[14−19] Bray et al.[14] described an oxazolidine linker based on threonine or serine, and Murphy et al.[15] developed a linker based on semicarbazone amino acid derivatives, although both of these methods required the use of protected amino acid aldehydes. Other linkers and techniques have also been devised, for example linkers have been developed on the basis of the Weinreb amide,[16] although they give low cleavage yields and the final products were contaminated with $LiAlH_4$ byproducts, applications were found in the synthesis of both peptide[17] and small molecule aldehydes.[18] Recently, Jouin et al.[19] reported the preparation of peptide aldehydes by copper salt-mediated neutral hydrolysis of the corresponding C-terminal thiazolidinyl peptides.

We decided to develop an efficient method based on a resin-supported oxidation of a peptide alcohol. The method, if successful, would be very versatile and applicable to any amino alcohol with a resin attachable side chain and followed the reported solid-phase oxidation methods of Kurth.[20a] Thus the overall strategy was based on the side-chain anchoring of a tyrosinol derivative onto the widely utilized Wang linker, providing an ideal template for attachment to aminomethyl resins, followed by subsequent Fmoc peptide chemistry, urea formation, alcohol oxidation, and finally compound cleavage from the solid support.

Our initial synthetic approach envisioned a Mitsunobu reaction between the Wang linker derivative (4-hydroxymethyl)phenoxyallyl acetate and Fmoc-Tyrosinol.[21] The allyl ester functionality could then be removed orthogonally to the Fmoc group prior to solid phase immobilization. Unfortunately, all attempts to carry out this Mitsunobu reaction, using a variety of coupling reagents such as DEAD/PPh_3, TMAD/PBu_3, and solvents (THF, DCM, and NMM) were unsuccessful, and no coupling products were isolated. Although formation of triphenyl phosphine oxide was observed after a few minutes when DEAD/PPh_3 were used as activating agents, purification of the crude reaction mixture on silica gel yielded pure starting materials. *O*-Alkylation reactions using the brominated Wang linker derivative[22] were also unsuccessful with the Fmoc-Tyrosinol derivative due to extensive Fmoc removal in the presence of bases such as cesium carbonate, sodium hydrogen carbonate, sodium hydride, or 1 equiv of potassium hexamethyl disilazane.

We therefore switched to the synthetic strategy shown in Scheme 1 which utilized as a key compound Dde-Tyrosinol **(5)**. This was prepared in 3 steps from commercially available Fmoc-Tyrosinol(tBu) **(3)** by Fmoc removal followed by *N*-reprotection with 2-acetyl dimedone[23] and tertiary butyl ether cleavage with trifluoroacetic acid in an overall yield of 86%. The N^{α}-(Dde)-*O*-(4-methylphenoxyacetic acid)-L-Tyrosinol scaffold **(8)** was prepared in high yield (91%) by treatment of **(5)** with the brominated Wang linker[22] derivative **(6)** in the presence of cesium carbonate in refluxing acetonitrile followed by ester hydrolysis, either by specific allyl ester removal with Pd(0) and dimedone[24] or by classical saponification which was obviously more appropriate for the production of the free carboxylic acid scaffold on a large scale.

The free carboxylic acid **(8)** was coupled to aminomethyl-(poly(ethylene glycol)-polystyrene-resin) (aminomethyl-PEG-PS-resin) **(9)** (which was synthesized in multigram quantities from aminomethyl-PS-resin in three steps as shown in Scheme 2), using diisopropylcarbodiimide (DIC)/hydroxybenzotriazole (HOBt) in dichloromethane/THF and gave an initial loading of 0.65 mmol/g (98% expected) as determined by quantitative Dde analysis.[23b] The Dde group was removed with a 10% solution of hydrazine in DMF to give the free amine.[23a]

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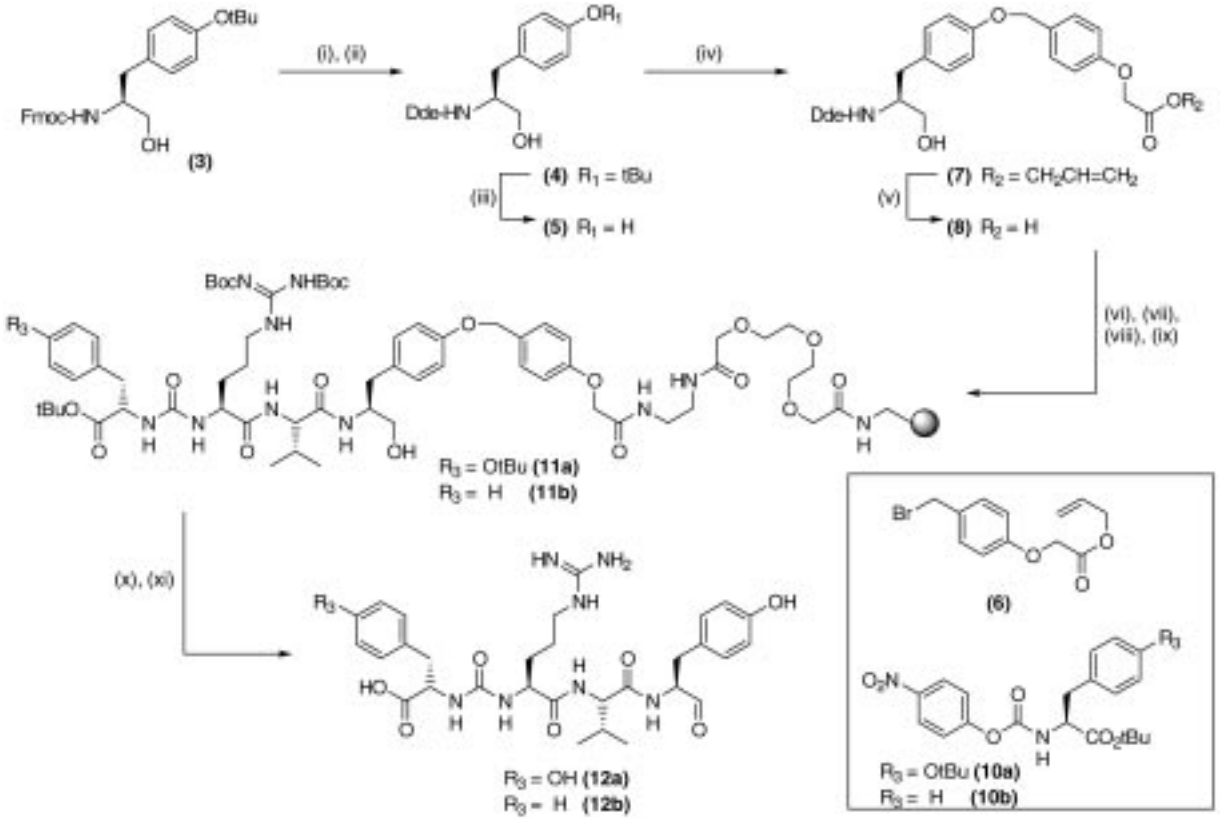
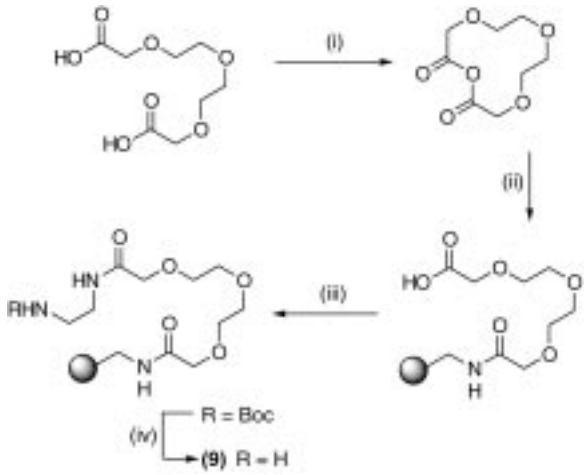
Scheme 1[a]



[a] Conditions: (i) 40% Et_2NH, DCM, 98%; (ii) Dde-OH, triethylamine, DMF, 90%; (iii) 50% trifluoracetic acid, DCM, 98%; (iv) **6**, Cs_2CO_3, acetonitrile reflux, 89%; (v) NaOH, THF/H_2O (9:1), 97% or $Pd(PPh_3)_4$, dimedone, THF/DCM (1:1), 94%; (vi) **9**, DIC, HOBt, DCM/THF; (vii) 10% NH_2NH_2/DMF; (viii) Fmoc peptide synthesis, Fmoc deprotection, 20% piperidine/DMF; sequential coupling with (a) Fmoc-Val-OH, DIC, HOBt, DCM/DMF; (b) Fmoc-Arg(Boc)$_2$-OH, DIC, HOBt, DCM/DMF; (ix) **10a** or **10b**, DIPEA, DMF; (x) SO_3−Pyr, triethylamine, anhydrous DMSO (×2); (xi) 95% trifluoracetic acid, 5% phenol or water.

Scheme 2. Preparation of Aminomethyl-PEG-polystyrene Resin (9)[a]



[a] Conditions: (i) DCC, DCM; (ii) aminomethyl polystyrene resin, DCM; (iii) Boc-NH$(CH_2)_2NH_2$, DIC, HOBt, DCM; (iv) 50% trifluoracetic acid/DCM.

At this stage a classical ninhydrin test[25] gave a loading of 25% of the expected value. Continuation of peptide synthesis (Fmoc-Val-OH) then gave Fmoc and ninhydrin values of the full, and expected, value, indicating that the poor ninhydrin value obtained previously related to poor quantification of the amines on the amino alcohol rather than to failed couplings. We attribute this to the poor cleavage of the ninhydrin imine formed when ninhydrin reacts with the amino alcohol.

Fmoc chemistry continued with the addition of Fmoc-L-Arg(Boc)$_2$-OH and Fmoc removal. The urea functionality was incorporated efficiently using 1.1 equiv of N^{α}-(4-nitrophenyloxycarbonyl)-*O*-(*tert*-butyl)-L-tyrosine-*tert*-butyl ester **(10a)** in DMF/diisopropyl ethylamine[13] and was monitored by both the quantitative ninhydrin test and by removal of small quantities of resin (ca. 5 mg), trifluoroacetic acid cleavage, and HPLC (shown in Figure 2a) and ES (electrospray) MS analysis.[26]

Conversion of the totally protected tetrapeptide alcohol immobilized on solid support **(11a)** to its corresponding aldehyde was achieved using a sulfur trioxide−pyridine mediated oxidation.[20] Of the various oxidation methods examined, which included Dess−Martin periodinane, activated manganese dioxide, and acetic anhydride/DMSO, 10 equiv of sulfur trioxide−pyridine in anhydrous dimethyl sulfoxide[20] in the presence of triethylamine turned out to be the most reliable. An important observation was that the oxidation failed when polystyrene resin was utilized but that the use of the aminomethyl-poly(ethylene glycol)-polystyrene resin **(9)** dramatically im-

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(26) Typical HPLC-ES MS procedure: A 5−8 mg sample of resin was preswollen in dichloromethane and subjected to treatment with trifluoracetic acid/water (95:5, 1 mL) for 1 h. After filtration and evaporation of the solvents, the product was analyzed by HPLC and ES MS.

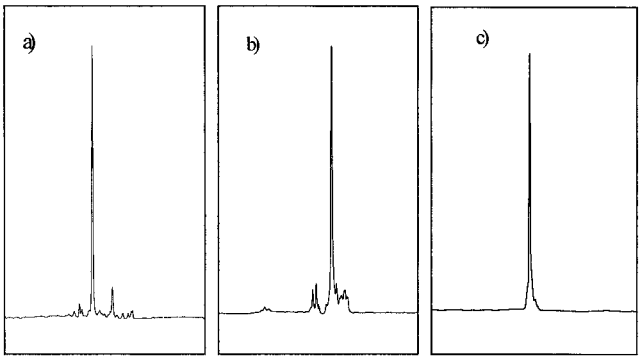

Figure 2. Crude RP-HPLC (C-18) of (a) tetrapeptide alcohol cleavage; (b) tetrapeptide aldehyde **(12a)**, and (c) purified tetrapeptide aldehyde **(12a)**.

proved the efficiency of the reaction. No tetrapeptide aldehyde **(12a)** was obtained when the corresponding primary alcohol **(11a)**, anchored to aminomethyl polystyrene resin was subjected to five treatments with 10 equiv of sulfur trioxide−pyridine/triethylamine in anhydrous dimethyl sulfoxide. The incorporation of the amino-PEG-spacer between the linker, and the solid support obviously reduces the steric hindrance in the environment of the primary alcohol functionality and improves the solvation of the solid-phase support. The use of TentaGel S aminomethyl resin was also successful and gave **(12a)** after one treatment with 10 equiv of sulfur trioxide−pyridine in anhydrous dimethyl sulfoxide in the presence of triethylamine and a few activated 4 Å molecular sieves. However, the substitution of our resin **(9)** (0.97 mmol/g) is much higher than TentaGel and thus was favored.

Compound **12a** was cleaved from the solid support using trifluoracetic acid/phenol (95:5) for 2 h. After evaporation of the solvents, precipitation with *tert*-butyl methyl ether and centrifugation, the crude product **(12a)** was dissolved in water/acetonitrile and lyophilized before purification by reverse-phase HPLC. The overall yield of the whole solid-phase synthetic process (9 steps, including anchoring and cleavage steps) was 55−70%, and the crude tetrapeptide **(12a)** was 68% pure as determined by HPLC analysis (and ES MS) (Figure 2b). There was no evidence of diastereoisomers by either ^{1}H or ^{13}C NMR analysis. Finally, the tetrapeptide **(12b)** was isolated using a similar procedure to that of **12a**, except the urea functionality was incorporated by means of N^{α}-(4-nitrophenyloxycarbonyl)-L-phenylalanine-*tert*-butyl ester **(10b)**.

In conclusion, we have devised a simple and very efficient solid-phase synthesis of C-terminal tyrosine peptide aldehydes. N^{α}-(Dde)-*O*-(4-methylphenoxyacetic acid)-L-Tyrosinol **(8)** provides an ideal scaffold that can be easily attached to solid supports and elongated using Fmoc protocols. Furthermore, we demonstrated that the use of immobilized amino-PEG-polystyrene resin **(9)** improves dramatically the efficiency of the oxidation reaction and that this resin is inexpensive and easy to prepare. Finally, the synthesis reported herein, for a C-terminal tyrosine analogue of the natural product (*S*)-MAPI may provide a novel template leading to therapeutic agents in the treatment of AIDS.

Experimental Section

General Procedures. Reactions in solution were monitored by thin-layer chromatography on precoated aluminum-backed plates (Merck silica gel 60 F_{254}). The chromatograms were visualized by UV light and staining with phosphomolybdic acid (PMA), bromocresol green, permanganate, or ninhydrin. All reagents and solvents were obtained from commercial suppliers and used without further purification. Specific sources of reagents were as follows: Fmoc-Tyrosinol-(tBu), Advanced ChemTech; 3,6,9-trioxaundecanedioic acid, Fluka; H-Tyr(tBu)-OtBu·HCl and H-Phe-OtBu·HCl, NovaBiochem; TentaGel S AM (0.26 mmol/g), Rapp Polymer; Merrifield resin (1.34 mmol/g) based on 1% cross-linked divinylbenzene styrene copolymer (100−200 mesh) was purchased from NovaBiochem and was treated with potassium phthalimide and hydrazine to give the corresponding aminomethyl polystyrene resin.[27] Analytical RP-HPLC was performed on a Phenomenex Prodigy ODS3 5 μm C18 column (3 × 150 mm) with a linear gradient of acetonitrile/0.043% trifluoracetic acid (0−100% over 20 min) in water/0.1% trifluoracetic acid at a flow rate of 0.5 mL/min and monitoring at 220 nm.

***N*$^{\alpha}$-(Dde)-L-Tyrosinol(tBu) (4).** To a solution of 40% diethylamine in DCM (50 mL) was added Fmoc-Tyrosinol(tBu) (2.57 g, 5.77 mmol), and the mixture was stirred for 3 h. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in diethyl ether (6 mL), and water (20 mL) was added. This organic/aqueous solution was concentrated under reduced pressure to remove the ether phase. The resulting aqueous layer was diluted with water (50 mL), and the precipitate was collected by filtration. The filtrate was lyophilized, giving a white solid which was redissolved in DCM (50 mL), dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo to give H-L-Tyrosinol(tBu) as a white foam (1.26 g, yield 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28 (9H, s), 2.47 and 2.68 (1H + 1H, AB part of ABX system, $J_{AX} = 6$ Hz, $J_{BX} = 7$ Hz $J_{AB} = 13$ Hz), 3.05 (1H, br m), 3.28 (3H, br m), 3.32 and 3.56 (1H + 1H, AB part of ABX system, $J_{AX} = 4$ Hz, $J_{BX} = 8$ Hz $J_{AB} = 11$ Hz), 6.85 (2H, d, $J = 8$ Hz), 7.02 (2H, d, $J = 8$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.97 (CH_3), 39.42 (CH_2), 54.42 (CH), 65.43 (CH_2), 78.48 (C), 124.46 (CH), 129.74 (CH), 133.19 (C), 154.03 (C). A solution of 2-acetyldimedone (1.16 g, 6.34 mmol) in DMF (10 mL) and triethylamine (1 mL, 6.92 mmol) were added to a solution of H-Tyrosinol(tBu) (1.26 g, 5.65 mmol) in DMF (5 mL), and the mixture was stirred overnight. The reaction mixture was poured into EtOAc (150 mL), the solution was washed with 2 M $KHSO_4$ (2 × 50 mL) and brine (50 mL), and the organic layer was dried over $MgSO_4$, filtered. The solvent was removed in vacuo to give an orange solid. The residue was purified by column chromatography on silica gel eluting with hexane/EtOAc (2:1) to give the title compound as a yellow-orange solid (2.01 g, yield 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (6H, s), 1.24 (9H, s), 2.15 (4H, s), 2.2 (3H, s), 2.7 and 2.9 (1H + 1H, AB part of ABX system m), 3.7 (2H, m), 3.97 (1H, m), 4.3 (1H, m), 6.83 (2H, d, $J = 7$ Hz), 6.99 (2H, d, $J = 7$ Hz), 13.49 (1H, m br). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.88 (CH_3), 28.27 (CH_3), 28.88 (CH_3), 30.0 (C), 37.86 (CH_2), 52.68 (CH_2), 57.82 (CH), 63.91 (CH_2), 78.65 (C), 107.88 (*C*), 124.56 (CH), 129.84 (CH), 131.95 (C), 154.27 (C), 173.66 (C), 197.98 (C). ES MS *m*/*e*: 388.4 $(M + H)^+$. HRMS for $C_{23}H_{33}O_4N$: calcd 387.2410, found 387.2427.

***N*$^{\alpha}$-(Dde)-L-Tyrosinol (5).** To a solution of compound **(4)** (2.0 g, 5.17 mmol) in DCM (20 mL) was added trifluoroacetic acid (20 mL), and the reaction was stirred at room temperature for 40 min. The solution was diluted with DCM (60 mL) and washed with brine (2 × 25 mL). The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed in vacuo to give a yellow oil. This oil was purified by column chromatography on silica gel eluting with DCM/MeOH (95:5) to give a colorless oil (1.68 g, yield 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (6H, s), 2.24 (4H, s), 2.28 (3H, s, CH_3), 2.68 and 2.88 (1H + 1H, AB part of ABX system m), 3.7 (3H, m), 3.98 (1H,

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m), 6.73 (2H, d, $J = 7$ Hz), 6.97 (2H, d, $J = 7$ Hz), 13.47 (1H, d, $J = 8$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.26 (CH_3), 28.24 (CH_3), 30.2 (C), 37.54 (CH_2), 52.52 (CH_2), 57.81 (CH), 63.66 (CH_2), 107.92 (*C*), 115.87 (CH), 127.67 (C), 130.47 (CH), 155.98 (C), 174.09 (C), 198.58 (C). ES MS *m/e*: 332.2 $(M + H)^+$. HRMS for $C_{19}H_{25}O_4N$: calcd 331.1785, found 331.1784.

***N*$^{\alpha}$-(Dde)-*O*-(4-methylphenoxy-allyl acetate)-L-Tyrosinol (7).** Dde-Tyrosinol **(5)** (1.68 g, 5.07 mmol), Cs_2CO_3 (2.34 g, 7.19 mmol) and the bromo linker derivative **(6)** (2.2 g, 7.7 mmol) in acetonitrile (100 mL) were refluxed for 3 h. The resulting solution was concentrated under reduced pressure, the residue redissolved in EtOAc (200 mL), washed with brine (2 × 60 mL), and filtered, and the solvent was removed in vacuo to give a yellow oil which was purified by column chromatography on silica gel eluting with DCM/MeOH (94:6) to give a colorless oil (2.42 g, yield 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (6H, s), 2.27 (4H, s), 2.29 (3H, s), 2.73 and 2.94 (1H + 1H, AB part of ABX system, $J_{AX} = 6$ Hz, $J_{BX} = 7$ Hz, $J_{AB} = 13.5$ Hz), 3.72 (2H, m), 3.90 (1H, m), 3.99 (1H, br m), 4.65 (2H, s), 4.69 (2H, d, $J = 5$ Hz), 4.92 (2H, s), 5.30 (2H, ddd, $J = 1.5$ Hz, $J = 11.5$ Hz, $J = 17$ Hz), 5.92 (1H, ddd, $J = 7$ Hz, $J = 11.5$ Hz, $J = 17$ Hz), 6.84 (2H, d, $J = 8$ Hz), 6.91 (2H, d, $J = 8$ Hz), 7.04 (2H, d, $J = 8$ Hz), 7.33 (2H, d, $J = 8$ Hz), 13.55 (1H, d, $J = 9$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0 (CH_3), 28.34 (CH_3), 30.15 (C), 37.59 (CH_2), 52.81 (CH_2), 57.73 (CH), 63.98 (CH_2), 65.49 (*C*H_2), 66.03 (*C*H_2), 69.73 (*C*H_2), 107.95 (C), 114.91 (CH), 115.17 (CH), 119.32 (*C*H_2), 129.14 (C), 129.4 (CH), 130.25 (C), 130.42 (CH), 131.52 (CH), 157.74 (C), 157.88 (C), 168.73 (C), 173.69 (*C*), 198.07 (C). ES MS *m/e*: 536.4 $(M + H)^+$. HRMS for $C_{31}H_{37}O_7N$: calcd 536.2648, found 536.2617.

***N*$^{\alpha}$-(Dde)-*O*-(4-methylphenoxyacetic acid)-L-Tyrosinol (8). Method A.** Allyl ester **(7)** (440 mg, 0.822 mmol) was dissolved in THF/DCM (1:1, 100 mL), and the solution was stirred and degassed for 1 h with a gentle flow of nitrogen through the solution. Dimedone (682 mg, 4.92 mmol) and Pd(PPh_3)$_4$ (144 mg, 0.124 mmol) were added, and the reaction was stirred under nitrogen for 3 h at room temperature. The solution was concentrated in vacuo, and the residue was redissolved in DCM (200 mL). The organic layer was washed with 2 M $KHSO_4$ (2 × 50 mL), brine (50 mL), and finally dried over $MgSO_4$ and concentrated in vacuo to give a yellow-orange residue. The residue was purified by column chromatography on silica gel eluting with DCM/MeOH (96:4) to give a white foam (398 mg, 97%).

Method B. Compound **(7)** (3.56 g, 6.65 mmol) was dissolved in THF (90 mL), and a solution of sodium hydroxide (293 mg, 7.32 mmol) in distilled water (10 mL) was added. The reaction mixture was stirred for 50 min at room temperature. The solution was concentrated under reduced pressure to ca. 10 mL, poured into water (20 mL), and acidified with 2 M $KHSO_4$. The aqueous phase was extracted with EtOAc (300 mL, 6 × 50 mL) and DCM (150 mL, 3 × 50 mL), the extracts were combined, washed (brine, 60 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo to give a white foam (3.1 g, yield 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0 (6H, s), 2.2 (3H, s), 2.28 (4H, s), 2.68 and 2.87 (1H + 1H, AB part of ABX system m), 3.69 (2H, m), 3.93 (1H, br m), 4.55 (2H, s), 4.85 (2H, s), 6.77 (2H, d, $J = 8$ Hz), 6.82 (2H, d, $J = 7$ Hz), 6.95 (2H, d, $J = 7$ Hz), 7.24 (2H, d, $J = 8$ Hz), 7.75 (1H, br s), 13.45 (1H, d, $J = 8$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.34 (CH_3), 28.29 (CH_3), 30.24 (C), 37.46 (CH_2), 52.41 (CH_2), 57.8 (CH), 63.67 (CH_2), 65.35 (*C*H_2), 69.7 (*C*H_2), 107.89 (C), 114.86 (CH), 115.29 (CH), 129.06 (C), 129.37 (CH), 130.07 (C), 130.44 (CH), 157.74 (C), 157.80 (C), 171.68 (C), 174.23 (C), 198.6 (C). ES MS *m/e*: 496.5 $(M + H)^+$, 534.5 $(M + K)^+$. HRMS for $C_{28}H_{33}O_7N$: calcd 496.2335, found 496.2369.

Preparation of Aminomethyl-PEG-polystyrene Resin (9). A solution of 3,6,9-trioxaundecanedioic acid (5 g, 22.5 mmol) and DCC (4.64 g, 22.5 mmol) was stirred in DCM (100 mL) for 6 h at room temperature. The reaction mixture was filtered, concentrated in vacuo (ca. 30 mL), and added to aminomethyl polystyrene resin (1 g, loading 1.38 mmol/g, preswollen for 30 min in DCM), and the mixture was shaken overnight. The resin was filtered and washed with DMF (3 × 30 mL) and DCM (3 × 30 mL). A ninhydrin test showed complete coupling. To this resin was added a solution of DIC (1.72 mL, 11.04 mmol) in DCM (15 mL), and the reaction mixture was shaken for 30 min before mono-Boc-protected ethylenediamine (1.8 g, 11.04 mmol) was added and the solution was shaken overnight. The resin was filtered and washed with DMF (3 × 30 mL) and DCM (3 × 30 mL). A BCG-test showed complete coupling. The resin was shaken in 50% trifluoracetic acid/DCM for 30 min, filtered, and washed with DCM (3 × 30 mL), 10% DIPEA/DMF (3 × 30 mL), DMF (3 × 30 mL), MeOH (3 × 30 mL), and ether (3 × 30 mL), and the resin was dried in vacuo overnight. A quantitative Fmoc test gave a loading of 0.97 mmol/g (93% expected).

Tetrapeptide (12a). Diisopropylcarbodiimide (61 μL, 0.388 mmol) was added to a solution of compound **(8)** (192 mg, 0.388 mmol) and HOBt (53 mg, 0.388 mmol) in 4 mL THF/DCM (1:1). The preactivated carboxylic acid scaffold was added to aminomethyl-PEG-polystyrene resin **(9)** (200 mg, 0.194 mmol, preswollen for 30 min in DCM), and the mixture was shaken for 4 h. The resin was filtered, washed with DMF (3 × 4 mL), DCM (3 × 4 mL), MeOH (3 × 4 mL), and ether (3 × 4 mL), and dried in vacuo. Resin substitution was determined by quantitative Dde analysis and found to be 0.65 mmol/g (98% expected). This resin was preswollen in DMF (3 mL) for 15 min and filtered, and a solution of 10% hydrazine monohydrate in DMF (4 mL) was added. The reaction mixture was shaken for 1 h. The resin was filtered, washed with DMF (3 × 4 mL), DCM (3 × 4 mL), and subjected to Fmoc-peptide synthesis using the following conditions: (i) Fmoc deprotection, 20% piperidine in DMF (4 mL) for 30 min, followed by washing with DMF (3 × 4 mL) and DCM (3 × 4 mL); (ii) coupling conditions, (a) Fmoc-Val-OH (132 mg, 0.388 mmol), HOBt (53 mg, 0.388 mmol), and DIC (61 μL, 0.388 mmol) in DCM/DMF (4 mL:10 drops), 4 h; (b) Fmoc-Arg(Boc)$_2$-OH (231 mg, 0.388 mmol), HOBt (53 mg, 0.388 mmol), and DIC (61 μL, 0.388 mmol) in DCM/DMF (4 mL:10 drops), 6 h; following all couplings the resin was filtered and washed with DMF (3 × 4 mL) and DCM (4 × 4 mL). All couplings were analyzed using the ninhydrin test.

N$^{\alpha}$-(4-Nitrophenyloxycarbonyl)-*O*-(*tert*-butyl)-L-tyrosine-*tert*-butyl ester[13] **(10a)** (100 mg, 0.218 mmol) and DIPEA (40 μL, 0.218 mmol) were dissolved in DMF (3 mL) and added to the peptide resin, and the reaction was shaken for 1 h. The resin was filtered, washed with DMF (3 × 4 mL), DCM (3 × 4 mL), MeOH (4 × 4 mL), and ether (3 × 4 mL), and dried overnight in vacuo. A ninhydrin test showed complete coupling. A solution of sulfur trioxide−pyridine (309 mg, 1.94 mmol) and triethylamine (270 μL, 194 mmol) in 4 mL of commercially available anhydrous DMSO (99.8% from Aldrich) was added to the resin (preswollen for 30 min in anhydrous DMSO), and the reaction mixture was shaken overnight. The resin was filtered, washed with anhydrous DMSO (3 × 4 mL), and followed by a second treatment with sulfur trioxide−pyridine and triethylamine in anhydrous DMSO. The resin was filtered and washed with DMSO (6 × 4 mL), DCM (3 × 4 mL), and ether (3 × 4 mL). The efficiency of the oxidation step was assessed by acid cleavage of a few milligrams of resin and LC/MS analysis. The product was cleaved from the solid support by treatment with trifluoracetic acid/phenol (95:5, 3 mL) or trifluoracetic acid/water (95:5, 3 mL) for 2 h. The resin was filtered and washed with trifluoracetic acid (2 × 2 mL), and the filtrate and washes were combined. The solution was concentrated in vacuo (ca. 0.5−1 mL), and 15 mL of *tert*-butyl methyl ether was added. After 15 min centrifugation (5000 rpm/ 25 °C), a white-yellow solid was isolated which was redissolved in water with a few drops of acetonitrile and lyophilized (67 mg, 55% yield from starting resin loading). The product was 68% pure as determined by analytical HPLC analysis. Purification was achieved using semipreparative RP HPLC on a Phenomenex Prodigy ODS 5 μm 250 × 10 mm column, eluting at 2.5 mL/min. The following gradient was used: $t = 0$ min, 100% A; $t = 30$ min, 25% A, 75% B; $t = 35$ min, 100% B (*A* = water, 0.1% trifluoracetic acid, B = acetonitrile, 0.043% trifluoracetic acid). Under these conditions

the product eluted at 21.8 min. Yield: 39 mg. ^{1}H NMR (360 MHz, DMSO-d_6) δ: 0.85 (6H, m), 1.52 (3H, m), 1.71 (1H, m), 2.00 (1H, m), 2.67 (1H, dd, J = 7 Hz, J = 13 Hz), 2.82 (2H, m), 2.97 (1H, dd, J = 5 Hz J = 13 Hz), 3.21 (2H, td, 2xJ = 6 Hz), 4.07 (1H, m), 4.11 (1H, dd, J = 7 Hz, J = 9 Hz), 4.26−4.40 (2H, m), 6.30 (1H, d, J = 8 Hz), 6.59 (1H, d, J = 9 Hz), 6.74 (2H, d, J = 8 Hz), 6.75 (2H, d, J = 8 Hz), 7.04 (2H, d, J = 8 Hz), 7.09 (2H, d, J = 8 Hz), 7.52 (1H, t, J = 6 Hz), 7.66 (1H, d, J = 8 Hz), 7.98 (1H, d, J = 9 Hz), 9.28 (1H, br s), 12.63 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 17.75 (CH_3), 19.15 (CH_3), 24.87 (CH_2), 30.33 (CH_2), 30.67 (CH), 35.78 (CH_2), 36.68 (CH_2), 40.35 (CH_2), 50.08 (CH), 52.25 (CH), 54.04 (CH), 58.37 (CH), 114.94 (CH), 114.95 (CH), 127.1 (C), 128.35 (C), 129.99 (CH), 130.11 (CH), 155.59 (C), 155.95 (C), 156.56 and 156.98 (Cx2), 170.32 and 171.94 (2xC), 173.74 (C), 200.23 (CH); ES MS m/e: 628.7 (aldehyde form) $(M + H)^+$, 646.7 $(M + H_2O + H)^+$.

Tetrapeptide (12b). The title compound was synthesized following the same procedure described for tetrapeptide **12a**. ES MS m/e: 612.8 (aldehyde form) $(M + H)^+$, 630.4 $(M + H_2O + H)^+$, 634.9 $(M + Na)^+$. HRMS for $C_{30}H_{43}O_8N_7$: calcd 630.3251, found 630.3199.

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