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for

Sequence-Specific DNA Recognition by Monomeric bZIP-Basic Regions Equipped with a Tripyrrole Unit on the N-Terminal Side: Towards the Development of Synthetic Mimics of Skn-1

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Peptide synthesis was performed by using standard Fmoc solid-phase methods on a Rink-MBHA amide resin (~0.62 mmol/g), with mixtures of HBTU/HOBt as coupling agents, DIEA as base and DMF as solvent. The cleavage/deprotection step was performed by treatment of the resin-bound peptide with the following mixture: 830 µL TFA, 25 µL EDT, 50 mg PhOH, 50 µL thioanisole and 50 µL H_2O (300 µL of this mixture for each 10 mg of resin). The amino acids used in the synthesis were standard protected, except the Fmoc-Lys(alloc)-OH introduced at position 232.

CD measurements were made in a 2 mm-cell at 4ºC. Samples contained 10 mM phosphate buffer (pH 7.5), 5-10 mM Tris (pH 7.5), 100 mM NaCl, 5 µM peptide and 5 µM ds-oligo when present. The peptide-DNA mixtures were incubated for 15 min before registering. The spectra are the average of 5 scans and were processed using the "smooth" macro implemented in the program *Kaleidagraph* (v 3.5 by Synergy Software). Spectra of the peptides in the presence of DNA were calculated as the difference between the spectra of the peptide-DNA mixture and the spectrum of free DNA.

For **gel mobility shift assays**, binding reactions were performed over 30 min using ~45 pM labeled dsDNAs (unless otherwise stated) in a binding mixture (20 µL) containing 38 mM Tris (pH 7.5), 90 mM KCl, 1.8 mM $MgCl_2$, 1.8 mM EDTA, 9% glycerol, 0.11 mg/mL BSA and 2.2% NP-40. Products were resolved by PAGE using a 10% nondenaturing poliacrylamide gel and 0.5X TBE buffer, and analyzed by autoradiography.

Synthesis of the solid phase-bound peptides 3 and 7: Selective removal of the allyl group on the acetylated peptides was carried out by treating the resin (160 mg) with H_2O/CH_2Cl_2 (6 mL, 2%), morpholine (0.42 mL, 4.84 mmol) and $Pd(PPh_3)_4$ (29 mg, 0.025 mmol). The mixture was smoothly shaken under N_2 for 10 h, and the resin was filtered and washed with DMF (3 x 5 mL x 5 min), *i*PrOH (2 x 5 mL x 5 min) and CH_2Cl_2 (5 mL x 5 min). The formation of the resin-bound peptides **3** and **7** was confirmed by cleavage/deprotection of an aliquot and MS analysis of the majoritary HPLC peak. **3**: (Merck 300 column at a flow rate of 1 mL min^{-1}, 220 nm, gradient 10? 35 % B 1/2 h, A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t=18.1 min): MS MALDI-TOF [*M*+H]: calculated for $C_{110}H_{199}N_{44}O_{32}$ 2648.5297, observed 2647.8. **7**: (Merck 300 column at a flow rate of 1 mLmin^{-1}, 220 nm, gradient 10->35% B 1/2 h, A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t=19.8 min) calcd. for $C_{117}H_{204}N_{45}O_{33}$ 2767.5668, observed 2767.6.

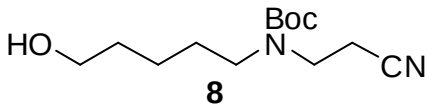
Coupling of tripyrrole 2 to the resin-bound peptide 3. Synthesis of hybrid 5: Resin-bound peptide **3** (25 mg, Eppendorf tube) was suspended in DMF (1 mL) and the mixture was shaken for 1 h to ensure a good resin swelling. The DMF was removed and a solution of HATU in DMF (2,9 mg in 200 µL), DIEA (61 µL, 0.5 M in DMF) and acid **2** (6.8 mg) previously shaken for 5 min were added. The reaction mixture was shaken for 2 h, and the resin washed with DMF (3 x 0.6 mL, for 5 min), and Et_2O (2 x 0.5 mL). Cleavage/deprotection of the bound peptide under standard conditions afforded a major product that was purified by RP-HPLC (Merck 300 column at a flow rate of 1 mLmin^{-1}, 304 nm, gradient 10? 35% B 1/2 h, A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t=23.5 min) and identified as the desired hybrid **5**: MS MALDI-TOF [*M*+H]: calcd. for $C_{144}H_{250}N_{54}O_{37}$ 3327.9341, found 3326.9. Approximate 11% yield, also considering the peptide synthesis.

Coupling of the tripyrrole 1 to the resin-bound peptide 3. Synthesis of hybrid 4: Resin-bound peptide **3** (25 mg, Eppendorf tube) was suspended in DMF (1 mL) and

the mixture was shaken for 1 h to ensure a good resin swelling. The DMF was removed and a solution of disuccinimydil carbonate in DMF (17 mg in 170 μL), DIEA (30 μL, 0.5 M in DMF) and DMAP in DMF (4.4 mg in 20 μL) were added. The resulting mixture was shaken for 2 h and the solution was removed. The resin was washed with DMF (3 x 0.6 mL), and a solution of the tripyrrole **1** (10 mg in 300 μL of DMF), DIEA (30 μL, 0.5 M in DMF) and DMAP (a solution of 4.4 mg in 20 μL of DMF) were added. The reaction mixture was shaken for 2 h, and the resin washed with DMF (3 x 0.6 mL, for 5 min), and Et_2O (2 x 0.5 mL). Cleavage/deprotection of the bound peptide under standard conditions afforded a major product that was purified by RP-HPLC (Merck 300 column at a flow rate of 1 mLmin^{-1}, 304 nm, gradient 10->35% B 1/2 h, A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t=24.5 min), and identified as hybrid **4**: MS MALDI-TOF [*M*+H]: calcd. for $C_{143}H_{248}N_{54}O_{37}$ 3313.9184, found 3312.6). Approximate 15% yield, also considering the peptide synthesis.

Synthesis of the Boc-protected derivative of benzyl 2-[3-(5-iodopentylamino)-propylamino]acetate

tert-*butyl 2-cyanoethyl(5-hydroxypentyl)carbamate (**8**)*

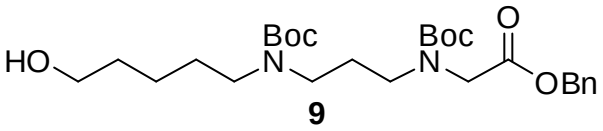


8

Acrylonitrile (6.38 mL, 96.9 mmol) was added dropwise to an ice-cooled solution of 5-aminopentanol (10 g, 96.9 mmol) in EtOH (100 mL). The mixture was allowed to reach room temperature and stirred overnight. The solvent were removed under reduced pressure and the residue was redissolved in CH_2Cl_2 (100 mL). Et_3N (27 mL, 0.194 mol) and Boc_2O (21.1 g, 96.9 mmol) were added to this solution, and the mixture was stirred at room temperature. After 1 h the mixture was poured into aqueous HCl (60 mL, 3%) and extracted with CH_2Cl_2 (2x60 mL). The organic layers were collected, dried over Na_2SO_4 and concentrated under vacuo. The resulting oily residue was observed by NMR to consist majoritarily of the desired addition product **8**, and was used in the next step without further purification. ^{1}H NMR (250.13 MHz, $CDCl_3$, 25ºC, TMS)**:** *d* 1.23-1.32 (m, 2H), 1.38 (s, 9H), 1.44-1.52 (m, 4H), 2.52 (br s, 2H), 3.17 (t, *J*= 7.3 Hz, 2H), 3.37 (t, *J*= 6.7 Hz, 2H), 3.52 (t, *J*= 6.4 Hz, 2H). ^{13}C NMR (62.89 MHz, $CDCl_3$, 25ºC, TMS)**:** *d* 16.7 (CH_2), 17.2 (CH_2), 22.7 (CH_2), 27.7 (CH_2),

28.1 (CH_3), 28.3 (CH_2), 31.9 (CH_2), 43.1 (CH_2), 43.6 (CH_2), 47.3 (CH_2), 48.2 (CH_2), 62.0 (CH_2), 80.0 (C), 80.3 (C), 117.8 (C), 118.2 (C), 154.6 (C), 155.1 (C). MS (CI+): *m/z* 257 (1), 201 (26), 184 (53), 157 (100), 139 (36), 116 (72). HRMS**:** calcd for $C_{13}H_{25}N_2O_3$ 257.1865, found 257.1876.

Di-tert*butoxycarbonyl derivative of benzyl 2-(3-(5-hydroxypentylamino)propylamino)-acetate (9)*



9

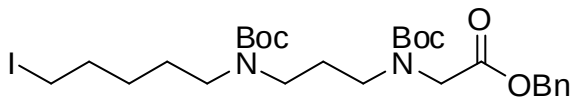
TMSCl (43.5 mL, 0.343 mol) was slowly added to a suspension of $LiBH_4$ (3.66 g, 0.168 mol) in THF (50 mL). The solution was stirred at room temperature for 45 min and cooled in an ice-water bath. A solution of the above nitrile **8** (24.8 g, 96.9 mmol) in THF (50 mL) was added dropwise to this mixture. After 12 h the reaction mixture was cooled again and quenched by the slow addition of MeOH. Solvents were partially removed under vacuo and the residue poured into aqueous KOH (30 mL, 10 %). The resulting suspension was extracted with CH_2Cl_2 (4 x 40 mL), and the organic layers were dried and concentrated to give a residue (20.3 g, 81 %) which was submitted to the next step without further purification.

To a solution of the resulting amine (1.7 g, 6.5 mmol) in dry CH_3CN (50 mL) was added K_2CO_3 (3.5 g) and the suspension was cooled in a ice-water bath. Benzyl bromoacetate (1.3 g, 5.77 mmol) was added and the mixture was stirred for 12 h and allowed to reach room temperature. Solvents were partially removed and the solution was poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). Organic phases were collected, dried over Na_2SO_4, concentrated in a rotary-evaporator and the residue was purified by silica gel column chromatography (10 % MeOH/CH_2Cl_2).

The crude product was dissolved in CH_2Cl_2 (20 mL), and Et_3N (2.71 mL, 19.5 mmol) and Boc_2O (2.84 g, 13 mmol) were added. The mixture was stirred for 1 h, poured into aqueous HCl (20 mL, 3%) and extracted with CH_2Cl_2 (2 x 20 mL). The organic phases were collected, dried and concentrated and the product purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) and identified as the title alcohol **9** (1.68 g, 51 %, viscous oil). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25 °C, TMS)**:** *d* 1.19 (m, 4H), 1.28-1.31 (2s, 18H), 1.36-1.60 (m, 4H), 2.11 (s, 1H), 2.87-3.16 (m, 6H), 3.45 (t, *J*=

6.45 Hz, 2H), 3.75-3.84 (2s, 2H), 5.0 (s, 2H), 7.19 (s, 5H). RMN ^{13}C (62.89 MHz, $CDCl_3$, 25ºC, TMS)**:** *d* 22.8 (CH_2), 28.0 (CH_3), 28.3 (CH_3), 32.2 (CH_2), 44.6 (CH_2), 46.1 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 48.9 (CH_2), 49.4 (CH_2), 62.3 (CH_2), 66.6 (CH_2), 79.1 (C), 80.2 (C), 128.1 (CH), 128.2 (CH), 128.4 (CH), 135.3 (C), 155.0 (C), 155.5 (C), 169.8 (C). MS (FAB+, M+H): *m/z* 509 (35), 409 (100), 353 (97), 309 (69). HRMS: calcd for $C_{27}H_{44}N_2O_7$ 508.3149, found 508.3134.

Di-tert*butoxycarbonyl derivative of benzyl 2-(3-(5-hydroxypentylamino)propylamino)-acetate (10)*



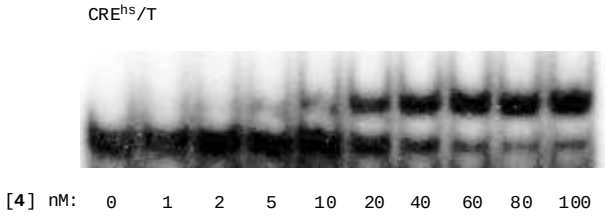
10

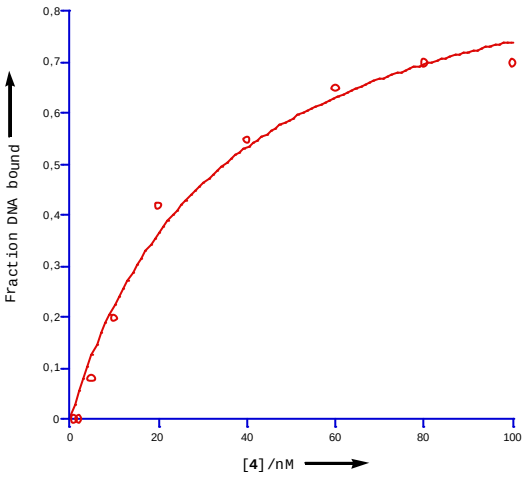
I_2 (840 mg, 3.31 mmol) was added to a solution of Ph_3P (955 mg, 3.64 mmol), imidazol (337 mg, 4.96 mmol) and the above alcohol **9** (1.68 g, 3.31 mmol) in CH_2Cl_2 (40 mL). After 10 min at room temperature the mixture was poured into an aqueous solution of sodium bisulfite (20 mL, 5 %) and extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were collected, dried and concentrated and the residue chromatographed in EtOAc/hexane to give the iodide as an oily liquid (1.94 g, 95%). ^1H NMR (250.13 MHz, $CDCl_3$, 25ºC, TMS)**:** *d* 1.20 (m, 4H), 1.29-1.32 (2s, 18H), 1.50-1.72 (m, 4H), 2.98-3.14 (m, 8H), 3.76-3.85 (2s, 2H), 5.01 (s, 2H), 7.19 (s, 5H). ^{13}C NMR (62.89 MHz, $CDCl_3$, 25 °C, TMS)**:** *d* 6.57 (CH_2), 27.4 (CH_2), 27.8 (CH_3), 28.2 (CH_3), 32.8 (CH_2), 44.4 (CH_2), 45.9 (CH_2), 46.4 (CH_2), 48.8 (CH_2), 49.2 (CH_2), 66.4 (CH_2), 78.8 (C), 79.9 (C), 127.8 (CH), 128.0 (CH), 128.2 (CH), 135.2 (C), 154.7 (C), 155.1 (C), 169.6 (C). MS (FAB+, M+H): *m/z* 619 (49), 519 (61), 419 (40). HRMS: calcd. for $C_{27}H_{43}IN_2O_6$ 618.2166, found 618.2135.

Synthesis of the tripirrole 2: K_2CO_3 (300 mg) and iodide **10** (500 mg, 0.8 mmol) were added to a solution of tripyrrole **6** (100 mg, 0.2 mmol) in dry acetone (6 mL). The reaction mixture was refluxed for 8 h and the resulting suspension was filtered through celite. The filtrate was concentrated and the residue purified by RP-HPLC (Scharlau 120 column at a flow rate of 4 mL min^{-1}, 304 nm, gradient 5? 95 % B 1/2 h, A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t=24.5 min) to afford the expected coupled product as a pale-yellow solid (167 mg, 84 %).

A solution of this tripyrrole (160 mg, 0.16 mmol) in MeOH (10 mL) was hydrogenated over 10% palladium on charcoal (130 mg) at room temperature for 2 h (balloon pressure). The catalyst was removed by filtration through celite, and the filtrate concentrated under reduced pressure to afford the desired acid **2** (133 mg, 93%). ^{1}H NMR (250.13 MHz, CD_3OD, 25ºC)**:** *d* 1.13-1.25 (m, 2H), 1.30-1.33 (2s, 18H), 1.39-1.74 (m, 6H), 1.97 (s, 3H), 2.97 (s, 6H), 3.09-3.28 (m, 14H), 3.77-3.82 (m, 8H), 6.73-6.92 (m, 3H), 7.07-7.11 (m, 3H). HRMS (FAB+, M+H): *m/z* calcd. for $C_{44}H_{69}N_{10}O_{10}$ 897.5198, found 897.5182.

PAGE Titration of peptide **4** with ^{32}P-labelled CRE^{hs}/T.





Data were fitted to the equation $q = P_T/(P_T+K_d)$, where q is the fraction of DNA bound and P_T is the total concentration of the peptide. We calculate a K_d of 35±2 X 10^{-9} M.