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Supporting Information

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Conformationally rigid trifluoromethyl-substituted a-amino acid designed for peptide structure analysis by solid state ^{19}F NMR

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Abbreviations

TMS: $(CH_3)_4Si$;

TIS: triisopropylsilane;

Fmoc: 9-fluorenylmethyloxycarbonyl;

Boc: *t*buthoxycarbonyl;

DIEA: *N,N*-diisopropylethylamine;

DMF: *N*,*N*-dimethylformamide;

HCTU: 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;

6-*Cl*-HOBt: 6-chloro-*N*-hydroxybenzotriazole;

TFA: trifluoroacetic acid.

Experimental

General: All air- and moisture-sensitive reactions were performed under an argon atmosphere using standard Schlenk technique. Solvents were purified accoding to standard procedures. 1,1-dibromo-2,2-bis(chloromethyl)cyclopropane (**8**), tricyclo[1.1.1.0^{1,3}]pentane (**9**), and 1-iodo-3-(trifluoromethyl)bicyclo[1.1.1]pentane (**10**) were prepared according to the procedures described in the literature.[S1] All other starting

materials were purchased from Acros, Merck and Fluka. Melting points are uncorrected. Analytical TLC was perfomed using Polychrom SI F_{254} plates. Column chromatography was performed using Kieselgel Merck 60 (230-400 mesh) as the stationary phase. Analytical RP-HPLC was done on an Agilent 1100 HPLC device using Zorbax Eclipse® XDB-C_8 column (4.6 mm x 150 mm) and MeCN/H_2O (60:40 v/v) as the eluent. Peptides were analyzed by RP-HPLC (Jasco, Japan) equipped with a diode-array detector and a Vydac C18-column (4.6 mm x 250 mm). ^{1}H NMR spectra were recorded either on a Varian Unity Plus 400 spectrometer at 400.4 ??z or on a Bruker Avance 500 spectrometer at 499.9 MHz. ^{13}C NMR and ^{19}F NMR spectra were recorded on a Varian Unity Plus 400 spectrometer at 100.7 and 376.7 ??z, respectively. Chemical shifts are reported in ppm downfield from TMS (^{1}H, ^{13}C) or C_6F_6 (^{19}F) as internal standards. IR spectra were obtained on a Hewlett Packard UR 20 spectrometer. The n_{max} (cm^{-1}) values of the IR spectra are given for the main absorbtion bands. Mass spectra were recorded either on an Agilent 1100 LCMSD SL instrument by chemical ionization (CI), or on a Bruker Biflex IV instrument (MALDI-TOF). MALDI samples were co-crystallized with a matrix of 3,5-dihydroxy-benzoic acid from acetonitrile/water solutions onto a stainless steel target. Elemental analysis: Mikroanalytisches Labor, Institute of Organic Chemistry , University of Karlsruhe. Optical rotation values were measured on a PerkinElmer 341 polarimeter. The models of amino acids were built using our own X-Ray data for **14**, and from the data deposited in the Cambridge Crystallographic Database.[S2] The physical properties of the amino acids in Table 1 were calculated by QikProp (Schrödinger software), www.schrodinger.com.

3-(Trifluoromethyl)bicyclo[1.1.1]pentane-1-carbaldehyde (11) and methoxy[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]methanol (12).

A solution of *t*BuLi in Et_2O (2.0 ml of 1.6 M, 3.2 mmol) was added dropwise to the stirred solution of **10** (424 mg, 1.6 mmol) in Et_2O (10 ml) at -78ºC. The addition was completed in 10 min, and the mixture was stirred for an ?dditional 30 min at -78ºC. The cooled to -78ºC reaction mixture was added dropwise to the stirred solution of HCOOMe (0.40 ml, 6.8 mmol) in Et_2O (15 ml) at -78ºC over 10 min. Once the addition was complete, the stirred mixture was allowed to warm to room temperature. It was extracted with H_2O (3 x 10 ml), and the water phase was discarded. The organic layer was separated, dried over $MgSO_4$ and concentrated in vacuum at 0ºC to ~10 ml (the products are very volatile!). The resulting solution of **11** and **12** in Et_2O was used in the next step without further purification. Compound **11**: ^{1}? NMR (400 MHz, ?D?l_3): *d*=9.61 (s, 1H; C*H*O), 2.25 (s, 6H;

3 x CH_2); ^{19}F NMR (377 MHz**?** D?I_3): 88.24 (s, CF_3). Compound **12**: ^{1}? NMR (400 MHz**?** ? D?I_3): *d*=4.51 (s, 1H; C*H*); 3.41 (s, 3H; OCH_3), 1.90 (s, 6H; 3 x CH_2); ^{19}F NMR (377 MHz, CD?I_3): 88.24 (s, CF_3). The ratio of **11** to **12** was ~1/1 according to the NMR data. When the NMR spectra were measured in $CDCl_3$ in the presence of CH_3OH (2-3 drops of CH_3OH in 0.6 ml of $CDCl_3$) both ^{1}? NMR and ^{19}F NMR spectra showed only the signals of compound **12**.

2(*S*)-{[(1*R*)-2-hydroxy-1-phenylethyl]amino}-2-[3-(trifluoromethyl)bicyclo[1.1.1] pent-1-yl]acetonitriles (14) and 2(*R*)-{[(1*R*)-2-hydroxy-1-phenylethyl]amino}-2-[3-(trifluoromethyl)bicyclo[1.1.1] pent-1-yl]acetonitriles (13)

A solution of (*R*)-2-phenylglycinol (222 mg, 1.6 mmol) in MeOH (20 ml) was added to the solution of **11** and **12** in Et_2O (10 ml), obtained in the previous step, and the resulting mixture was stirred for 2 hours at room temperature. The reaction mixture was then cooled (0ºC), $(CH_3)_3SiCN$ (634 mg, 6.4 mmol) was added, and the mixture was stirred at room temperature for 10 h. Evaporation of the solvent in vacuum gave a residue, which was submitted to column chromatography. Elution with hexane-ethyl acetate (3:2) afforded **14** first (244 mg, 0.78 mmol, 49%) as a colourless solid. R*f* = 0.7; m.p. 99-100ºC; $[a]_D$ = -133.1 (*c* = 0.26 in MeOH); ^{1}? NMR (400 MHz, CD?I_3): *d*=7.38-7.30 (m, 5H; Ph); 4.80 (dd, *J*=9.6; 4 Hz, 1H; CH-CH_2); 3.80 (dd, *J*=10.4; 4 Hz, 1H; CH-CH_2), 3.58 (dd, *J* = 10.4; 9.6 Hz, 1H; CH-CH_2), 3.42 (s, 1H; C*H*(CN)-N), 2.35 (bs, 2H; O*H*+N*H*), 2.06-2.00 (AB system, *J* = 9.6 Hz, 6H; 3 x CH_2); ^{19}F NMR (377 MHz**?** D?I_3): *d*=88.66 (s, CF_3); ^{13}C NMR (101 MHz, **?** D?I_3): *d*=137.97 (s, *C*, Ph), 129.21 (s, *C*H, Ph), 128.73 (s, *C*H, Ph), 127.27 (s, *C*H, Ph), 122.65 (q, *C*F_3, ^{1}*J*(H, F)=274 Hz), 117.86 (s, *C*N); 67.53 (s, N-*C*H-CH_2-OH); 63.19 (s, *C*H_2); 48.67 (s, *C*H_2); 47.52 (q, *C*H(CN)-N, ^{5}*J*(H, F)=2 Hz); 38.87 (s, CH_2-*C*-CH); 37.40 (q, CF_3-*C*, ^{2}*J*(H, F)=40 Hz); IR (KBr), *n* (cm^{-1})=3487, 3306, 3003, 2950, 2232, 1960, 1900, 1830, 1780, 1503, 1393, 1195, 1175, 1120, 1067, 753, 710, 553; MS (CI): *m/z*: 311.2 (M^+), 284.4 (M^+ - CH_2OH), 282 (M^+ - HCN), 164, 138, 124, 121, 106, 99, 95; Anal. calcd for $C_{16}H_{17}F_3N_2O$: C, 61.93; H, 5.52; N, 9.03. Found: C, 61.63; H, 5.45; N, 8.90. Further elution with the same solvent yielded **13** (202 mg, 0.62 mmol, 40%) as a colourless solid. R*f* = 0.6; m.p. 101-102ºC. $[a]_D$ = -21.4 (*c* = 0.25 in MeOH); ^{1}? NMR (400 MHz, ? D?I_3): *d*=7.34-7.26 (m, 5H; Ph), 3.92 (dd, *J*=8; 4 Hz, 1H; CH-CH_2), 4.00 (s, 1H; C*H*(CN)-N), 3.72 (dd, *J*=11.2; 4 Hz, 1H, CH-CH_2), 3.63 (dd, *J*=11.2; 8 Hz, 1H, CH-CH_2), 3.2-2.3 (bs, 2H, O*H*+N*H*), 2.00-1.95 (AB system, *J*=9.6 Hz, 6H, 3 x CH_2); ^{19}F NMR (377 MHz, CD?I_3): *d*=88.64 (s, CF_3); ^{13}C NMR (101 MHz**?**[D_6]DMSO): *d*=141.64 (s, *C*, Ph), 128.71 (s, *C*H, Ph), 128.22 (s, *C*H, Ph), 127.87 (s, *C*H, Ph), 122.83 (q, *C*F_3, ^{1}*J*(H, F)=274 Hz), 119.07 (s,

*C*N), 66.55 (s, N-*C*H-CH_2-OH), 63.75 (s, *C*H_2), 49.19 (bs, *C*H_2), 47.61 (q, *C*H(CN)-N, ^5J(H, F)=2 Hz), one signal (*C*H_2-CH) is overlapped with the residual peak of [D_6]DMSO, 36.76 (q, CF_3-*C*, ^2J(H, F)=38 Hz); IR (KBr), *n* (cm^{-1})=3750-3000, 3300, 3273, 3003, 2924, 2851, 2240, 1955, 1905, 1830, 1785, 1482, 1359, 1197, 1172, 1130, 701; MS (CI): *m/z*: 311.2 (M^+), 284.4 (M^+ - CH_2OH), 282 (M^+ - HCN), 164, 138, 124, 121, 106, 99, 95. Anal. calcd. for $C_{16}H_{17}F_3N_2O$: C, 61.93; H, 5.52; N, 9.03. Found: C, 61.70; H, 5.40; N, 8.95.

Isomerization of (2*R*)-2-{[(1*R*)-2-hydroxy-1-phenylethyl]amino}-2-[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]ethanenitrile (13)

A solution of **13** (202 mg, 0.62 mmol) in ? ?? ? (5 ml) was refluxed (~3 h) until a constant ratio of **14** to **13** (~4:1) was observed (analysis by HPLC). Evaporation of the solvent in vacuum gave a residue, which was submitted to column chromatography as described above. Compound **14** (145 mg, 0.47 mmol) and **13** (36 mg, 0.12 mmol) were obtained. Overall yield of **14** is 349 mg (80% calculating on **10**).

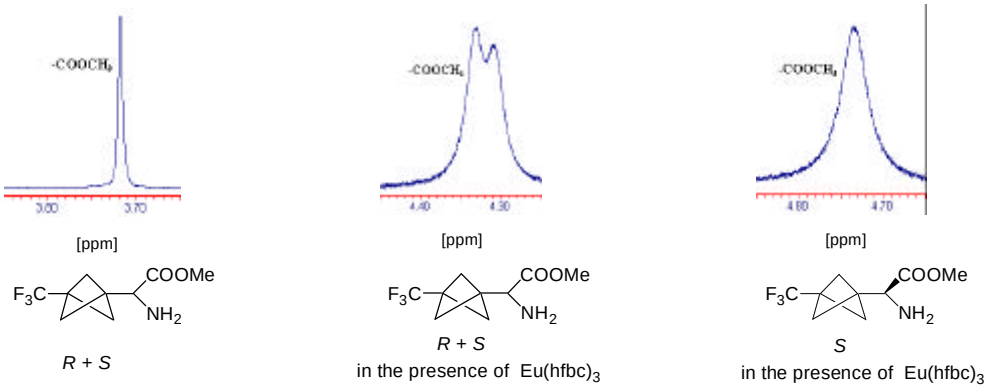
(2*S*)-2-Amino-2-[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]ethanoic acid (7)

$Pb(OAc)_4$ (0.200 mg, 0.45 mmol) was added to a solution of **14** (100 mg, 0.32 mmol) in CH_2Cl_2-MeOH (20 ml, 1/1, v/v) stirred at 0ºC. After being stirred at this temperature for 5 min, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ (5 ml). The resulting insoluble material was removed by filtration and washed with CH_2Cl_2 (10 ml). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 ml). The combined organic layers were evaporated in vacuum to give the yellow oil. The residue was dissolved in aqueous ? ? l (6M, 20 ml) and refluxed for 2 h. After cooling, the reaction mixture was washed with Et_2O (3 x 5 ml), and the aqueous layer was evaporated to give a white solid. The residue was dissolved in H_2O (~5 ml), neutralized with aqueous NaOH (0.3M) and submitted to ion exchange resin chromatography (Dowex® 50 x 400 cation-exchange). Elution with water and then with aqueous NH_3 (10%) afforded **7** (47 mg, 0.22 mmol, 70%); m.p. 210-215ºC; $[a]_D$ = +14.0 (*c* = 0.84 in H_2O); ^{1}? NMR (400 MHzD$_2$?): *d*=3.70 (s, 1H; C*H*), 1.94 (s, 6H; 3 x CH_2); ^{19}F NMR (377 MHzD$_2$?): *d*=87.66 (s, CF_3); ^{13}C NMR (101 MHz, D_2?): *d*=171.64 (s, *C*OOH), 122.61 (q, *C*F_3, ^1J(H, F)=274 Hz), 54.71 (s, *C*H_2), 47.75 (q, *C*H, ^5J(H, F)=2 Hz), 37.35 (q, CH_2-*C*-CH, ^4J(H, F)=2 Hz), 36.05 (q, CF_3-*C*-CH, ^2J(H, F)=40 Hz); IR (KBr), *n* (cm^{-1})=3700-2000, 3695, 3327, 3019, 2950, 2900, 2560, 1982, 1650, 1493, 1390, 1344, 1248, 1202, 1142, 1121, 1038, 998, 857, 775, 728, 699, 511. Anal. calcd. for $C_8H_{10}F_3NO_2$: C, 45.94; H, 4.82; N, 6.70. Found: C, 45.78; H, 4.53; N, 6.59.

(2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-2-[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]ethanoic acid (Fmoc-L-CF_3-Bpg-OH)

A solution of Fmoc-*Cl* in dioxane (1.30 g, 5.01 mmol) was added dropwise over 15 min to a solution (stirred at 0ºC) of **7** (1.00 g, 4.77 mmol) and Na_2CO_3 (2 g) in dioxane-water (50 ml, 2/3, v/v). After being stirred at this temperature for 30 min, the reaction was left overnight at RT. Water (500 ml) was added and the formed mixture was extacted with Et_2O (2 x 50 ml). Organic layer was discarded, water phase was acidified with aqueous HCl to pH 1 and extracted with EtOAc (3 x 100 ml). After being dried ($MgSO_4$) the solvent was evaporated to produce 1.87 g of a white solid (4.29 mmol, 90%). The compound was pure according to HPLC (the sole peak was observed). ^{1}? NMR (500 MHz, ?D?I_3): *d*=7.71 (dd, *J*=7.5; 2.5 Hz, 2H), 7.52 (d, *J*=7.5 Hz, 2H), 7.34 (m, 2H), 7.25 (dd, *J*=12; 7.5 Hz, 2H), 5.19 (d, *J*=8.5 Hz, 1H, N*H*), 4.48 (d, *J*=8.5 Hz, 1H, C*H*-COOH), 4.45 (dd, *J*=11.5; 6.5 Hz, 1H, C*H*-O), 4.37 (dd, *J*=11.5; 6.5 Hz, 1H, C*H*-O), 4.45 (t, *J*=6.5 Hz, 1H, C*H*-CH_2-O), 1.89 (AB system, *J*=9.7 Hz, 6H, 3 x CH_2).

Determination of optical purity of (2S)-2-Amino-2-[3-(trifluoromethyl) bicyclo[1.1.1]pent-1-yl]ethanoic acid (7)



Relevant regions of the ^{1}H NMR spectra of **7**-OMe (racemate and optically active samples, ~10^{-1}M solutions in $CDCl_3$, 20ºC) without and in the presence of Eu(hfbc)$_3$ (~$5\cdot10^{-2}$M), hfbc=3-heptafluorobutyryl-(*R*)-camphorate.

Table 1. Calculated physical properties for compounds 1-7 and some hydrophobic natural amino acids.

Molecule	SASA [Å^2][a]	FOSA [Å^2][b]	Volume [Å^3][c]	QlogP$_{o/w}$
CF_3-Bpg	384.8	136.2	624.9	-0.72
4CF_3-Phg	405.3	15.7	647.0	-0.53
4F-Phg	359.7	15.6	561.4	-1.23
4F-Phe	382.6	48.9	613.6	-0.89
5F-Trp	424.1	43.3	698.5	-0.73
F_3-Ala	276.9	20.1	407.7	-2.0
F_3-Aib	301.3	69.4	454.1	-1.73
Met	357.8	167.4	549.5	-1.63
Ile	338.6	204.5	527.5	-1.65
Leu	334.5	205.7	522.9	-1.64
Val	311.8	176.1	474.0	-1.96
Ala	260.8	105.3	368.5	-2.63
Pro	301.6	175.8	449.0	-2.04
Phe	364.9	60.7	592.3	-1.06
Trp	416.3	43.3	683.8	-0.92

[a] total solvent-accessible surface area; [b] hydrophobic component of SASA; [c] total solvent-accessible volume; [d] octanol/water partition coefficient.

Solid phase peptide synthesis

The fluorinated amino acid L-CF_3-Bpg was incorporated in the antimicrobial peptide PGLa (GMASKAGAIAGKIAKVALKAL-NH_2) at the position of either Ile9, Ala10, Ile13, or Ala14. Four labeled peptides were thus produced, namely PGLa-Ile9/CF_3-Bpg (**15**), PGLa-Ala10/CF_3-Bpg (**16**), PGLa-Ile13/CF_3-Bpg (**17**), PGLa- Ala14/CF_3-Bpg (**18**). All peptides were synthesized on an Applied Biosystems (Foster City, CA) 433A instrument, using standard solid phase Fmoc-protocols.[S3] While incorporating Lys or Ser into the peptide sequence, side chain protected Fmoc-(Boc)Lys and Fmoc-(*t*Bu)Ser were used. A MBHA polystyrene resin was employed to obtain the peptides in the amidated form. Deprotection was carried out with 20% piperidine in DMF, and pre-activation was performed with HCTU and 6-*Cl*-HOBt in the presence of the base DIEA. In some cases the deprotection and coupling efficiency was monitored by performing a Kaiser test.[S4] The peptides were cleaved from the resin at room temperature by treatment with a cocktail of TFA (95%),

water (2.5%) and TIS (2.5%) for 3-5 h with occasional shaking. The resin was filtered off and washed with pure TFA twice. The combined filtrates were evaporated under a gentle stream of nitrogen, and the products were precipitated with cold diethyl ether. After centrifugation the supernatant (diethyl ether) was decanted. The solid precipitate was re-dissolved in H_2O and lyophilized. Peptides were purified by semi-preparative RP-HPLC (Jasco, Japan; 10 x 250 mm Vydac C18-column). The H_2O/MeCN gradients were individually optimized for each peptide at 30-40 °C. The crude peptides were loaded on the column as 7 mg/ml solution in MeOH. To remove TFA and to avoid any ^{19}F NMR background, 5 mmol HCl was used as ion-pairing agent instead of conventional TFA.[S3c] After purification, all peptides were of >95% purity according to analytical HPLC, and they were stored as lyophilized powders at -40^{o}C until use.

PGLa-Ile9/CF_3-Bpg (GMASKAGA-CF_3-Bpg-AGKIAKVALKAL-NH_2) (15)

MALDI-TOF: *m/z*: 2045.9 $[M]^+$, calcd mass 2046.49.

PGLa-Ala10/CF_3-Bpg (GMASKAGAI-CF_3-Bpg-GKIAKVALKAL-NH_2) (16)

MALDI-TOF: *m/z*: 2088.1 $[M]^+$, calcd mass 2088.57.

PGLa-Ile13/CF_3-Bpg (GMASKAGAIAGK-CF_3-Bpg-AKVALKAL-NH_2) (17)

MALDI-TOF: *m/z*: 2046.1 $[M]^+$, calcd mass 2046.49.

PGLa- Ala14/CF_3-Bpg (GMASKAGAIAGKI-CF_3-Bpg-KVALKAL-NH_2) (18)

MALDI-TOF: *m/z*: 2087.8 $[M]^+$, calcd mass 2088.57.

Circular dichroism spectroscopy.

Circular dichroism spectra were recorded on a Jasco 800 instrument with a 1 mm cuvette in aqueous buffer of 10 mmol NaH_2PO_4/Na_2HPO_4, pH 7.0. The peptides (0.25 mg) were dissolved in $CHCl_3$/MeOH 1:1 (v:v), the solvent was removed with a gentle N_2 stream, followed by vacuum overnight. To the dried peptide films either pure buffer or buffer containing SDS (5 mmol) was added. The solutions were transferred into the cuvettes and equilibrated 30 min at 20^{o}C prior to measurements.

Minimal inhibitory concentration

Antimicrobial activity was characterized by the standard minimal inhibitory concentration (MIC) assay,[S5] carried out with gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC10240, *Kocuria rhizophila* ATCC 9341, and the gram-negative bacteria *Escherichia coli* ATCC 25922, *Escherichia coli* DH5a, *Acinetobacter sp.* ATCC 33304. Bacterial cells were first grown (overnight, 37°C) in standard Luria-Bertani-Medium (LB). The cultures were then used as an inoculum for further cultivation in LB (3-4 h, until OD_{550} = 1-2). The cells were then diluted to the inoculum range with salt-free LB.[S6] Microtiter plates (Nunc No. 167008, 96 wells of 200 µl/Well) were filled with 50 µl of salt-free LB medium, and serial 2-fold dilutions of peptides (512 µg/ml stocks in salt-free LB) were arranged in quadruple. Two rows of each plate were used for the positive (no peptide) and negative controls (not inoculated). 50 µl of bacterial suspension were added to the wells (except for the negative control) to give a final concentration of 10^5-10^6 colony forming units per ml. The plates were incubated at 37°C for 24 h, and the MIC was determined visually on the basis of turbidity as the lowest (among four repetitions) concentration that reduced growth by more than 50%.

Supplementary references

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