Preparation of *N*-Fmoc-Protected (*S*)-5-Amino-4,4-difluoro-7-methyloctanoic Acid, a Possible Dipeptide Isostere

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The title compound **1** was prepared from L-leucine. The key steps include a *Grignard* addition to Bn_2-leucinal, a CO/CF_2 replacement with Et_2NSF_3 (DAST) and use of a Ph group as synthetic equivalent of a COOH group. The difluoro-δ-amino acid **1** was incorporated into a peptide **8**; tests with various proteases showed no inhibition by this particular peptide.

Statine **B** [1], homostatine **D** [2], and ketomethylene analogs **C** [3] have been widely used as building blocks in peptides for inhibition of aspartic proteinases [4], notably of renin [5], and of aminopeptidases [6]. While the statines **B** are γ-amino-acid derivatives (providing five chain atoms), the 5-amino-4-oxo- and the 5-amino-4-hydroxy-carboxylic acid derivatives **C** and **D** contain six chain atoms, just like a dipeptide segment **A** of a 'normal peptide' ('dipeptide isosteres').

In spite of the fact that '*Organic Fluorine Hardly Ever Accepts Hydrogen Bonds*', [7] the literature is full of reports[2]) describing compounds containing C–F groups in positions where corresponding model compounds contain C–OH groups, with comparisons of physiological properties of the two types of compounds [8][9]. To provide another test case, we have prepared the *N*-protected difluoro amino acid **1** and incorporated it into a peptide for biological tests.

The starting point of the synthesis (*Scheme 1*) was (*S*)-2-(dibenzylamino)-4-methylpentanal (**2**), an aldehyde derived from L-leucine [10]. A Ph ring was used as a hidden COOH moiety [11][12] to avoid cyclizations of intermediates with formation of a lactone or lactam ring. Addition of 2-phenylethyl *Grignard* reagent to the aldehyde group gave a readily separated *ca.* 12:1 mixture of two crystalline diastereoisomeric amino alcohols (89% yield). By analogy with the literature [10], the major diastereoisomer **3** is assigned (3*R*,4*S*)-configuration ('non-chelation-controlled' approach of the nucleophile from the *Re*-face of the C=O group, relative topicity *ul*).

Swern oxidation [13] of the amino alcohol **3** gave the amino ketone **4** as an oil in 91% yield. Treatment of the ketone with eightfold excess of Et_2NSF_3 (DAST) [14] (CH_2Cl_2, -5 to $+20°$, 4 d) provided the difluoro amine **5** in 55% yield. The loss of one

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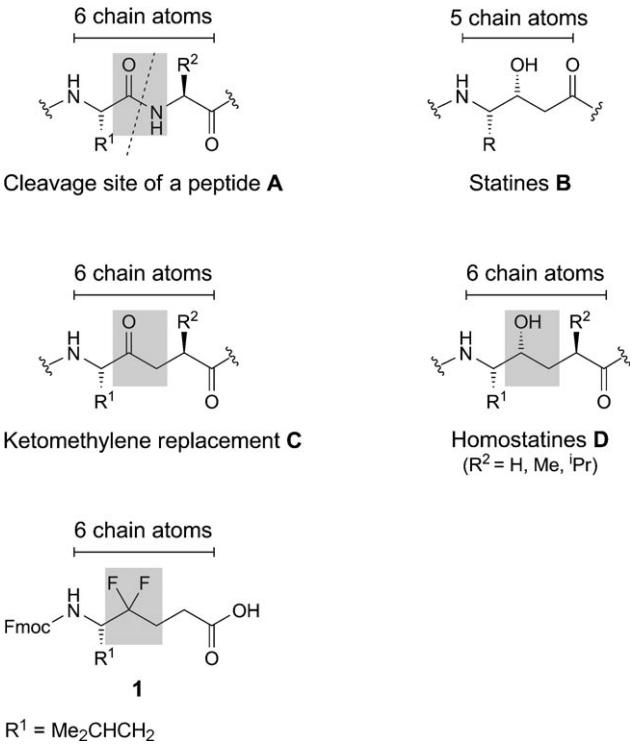
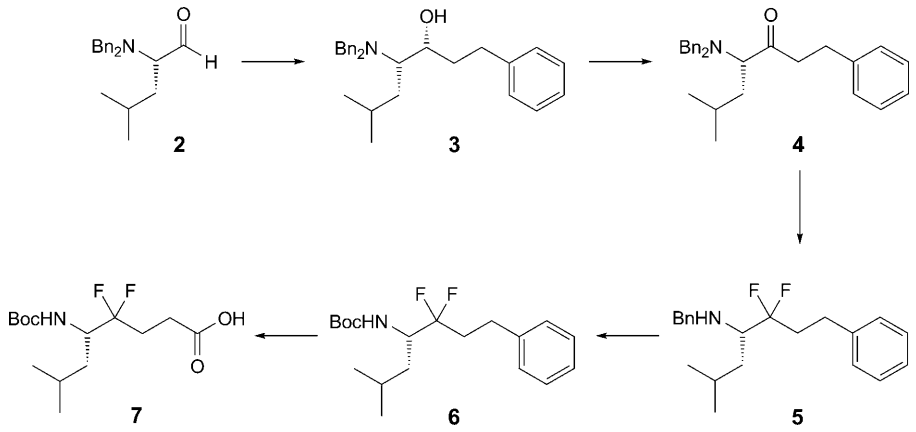
[2]) Especially in the patent literature describing pharmaceutical structure–activity relationships.



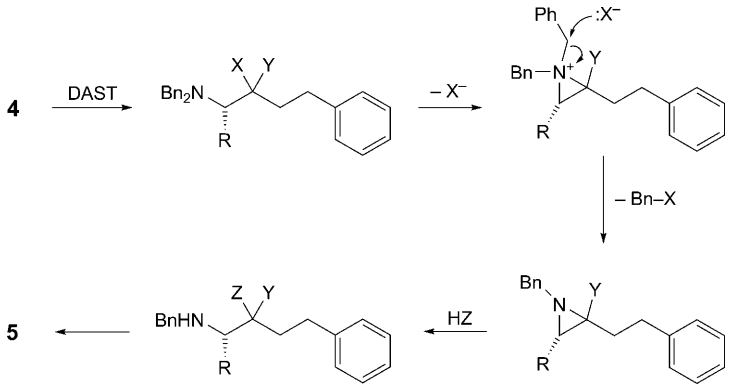
Scheme 1. *Preparation of the δ-Amino Acid Derivative* **1**



of the *N*-bound Bn groups in this process might be the result of the sequence of steps outlined in *Scheme 2*.

The remaining steps are functional-group manipulations: Bn/(*tert*-butoxy)carbonyl (Boc) exchange (H_2/Pd-C, $Boc_2O \rightarrow$ 87% **6**), oxidative degradation of the Ph ring to a

Scheme 2. *Possible Mode of Loss of a Benzyl Group in the Process of Replacement of CO by CF_2* (**4** → **5**) *with DAST* (Et_2NSF_3). Compare with the reports on OH/F-retention substitution in α-hydroxy-β-amino acid derivatives [15] and related works [16].



COOH group ($RuCl_3/NaIO_4$ → **7**) and direct Boc/(9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc) interchange (CF_3CO_2H, then Fmoc-OSu/Na_2CO_3 → **1**, 55% overall from **6**). Both the Boc- and the Fmoc-protected amino acids **7** and **1**, respectively, consisted of mixtures of rotamers as evident from temperature-dependent NMR spectra in (D_6)DMSO (see *Exper. Part*).

The 5-(Fmoc-amino)-4,4-difluoro-7-methyloctanoic acid **1**, thus obtained, has a sharp melting point and is optically active ($[\alpha]_D = -16.4$), like all the intermediates of the sequence of reactions leading to **1**. Although we did not determine the enantiomer purity of any of the compounds **1**–**7**, for instance by HPLC on chiral column materials or by NMR techniques, we have no reason to assume that there has been partial racemization on the way from L-leucine to the acid **1**.

To see whether a peptide containing the amino-difluoro-acyl moiety of **1** could possibly function as a dipeptide isostere (*cf.* ketomethylene in **C** with the CF_2-CH_2 segment in **1**) we have, so far, prepared only one peptide analog. We chose the octapeptide **E**, which, apart from replacement of the C-terminal His by a Tyr residue, is the 6–13 sequence of angiotensinogen, the substrate of human renin. Peptides of this kind had first been prepared in 1973, in the course of a search for renin inhibitors [17]. Thus, the peptide **8**, containing seven amino acids, was synthesized by the 'Fmoc strategy' [18] on *Wang* resin [19]. The crude product solution obtained by cleavage from the resin was lyophilized, and the residue purified (> 98%) by preparative HPL chromatography, and the pure peptide **8** was identified by NMR spectroscopy and high-resolution mass spectrometry.

Tests with enzymes hBAC, hCathepsin, hPepsin, and hRenin[3]) showed that there is no cleavage and no inhibition by peptide **8**. The use of the δ-amino-acid moiety of **1** in other peptides, especially those in which the ketomethylene replacement **C** was successful, will provide a further test of the viability of the CO/CF_2 replacement.

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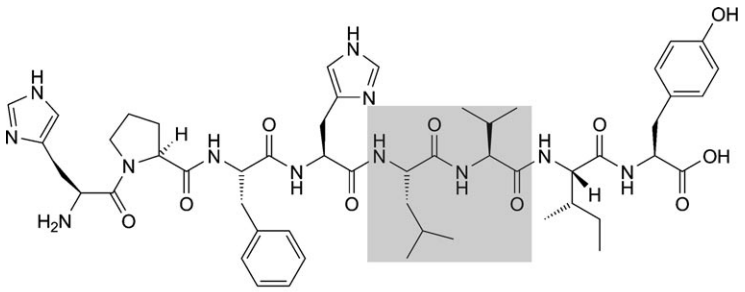
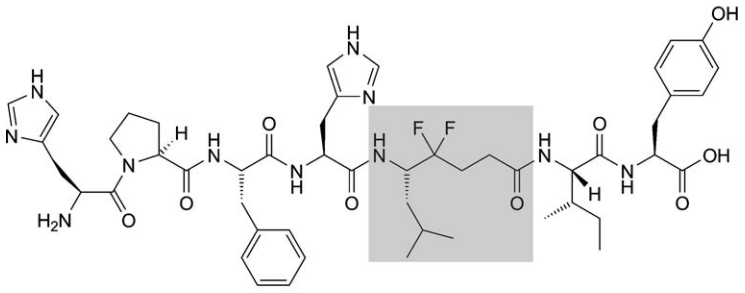
[3]) We thank Drs. *H. Sellner* and *O. Dreier* of *Novartis Pharma AG*, Basel, for carrying out these experiments.



E



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Furthermore, γ,γ-difluoro-acid analogs of **1** with an additional side chain in the α-position (*cf.* homostatines **D**, R = H) should be targets of future investigations.

Experimental Part

General. Abbreviations. DMAP: 4-(dimethylamino)pyridine, Bn: benzyl, DAST: Et_2NSF_3, Fmoc-OSu: *N*-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]oxy}succinimide, HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, TFA: CF_3COOH, TNBS: 2,4,6-trinitrobenzenesulfonic acid, MeIm: 1-methyl-1*H*-imidazole, MSNT: 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazol, HPLC: high-performance liquid chromatography, MALDI: matrix-assisted laser-desorption ionization.

Materials and Methods. All reagents were of synthetic grade and were used without further purification unless otherwise stated. Dry THF was distilled from Na and benzophenone; dry CH_2Cl_2 was distilled from CaH_2. All moisture-sensitive reactions were carried out under a positive pressure of N_2 in oven-dried glassware (140°). Org. extracts were dried over $MgSO_4$. TLC: *Merck* TLC silica gel *60* F_{254} aluminum plates; visualization by inspection under UV light (254 nm) or by the use of $KMnO_4$ stain, Mo-based stain, bromocresol green stain, or ninhydrin spray. Column chromatography (CC): silica gel *60* (40–63 μ) from *Fluka*. *Wang* resin was purchased from *Novabiochem*, amino acids were purchased from *Fluka*. Reversed-phase (RP) HPLC: *Merck/Hitachi* HPLC system (*LaChrom*, pump type *L-7150*, UV-detector *L-7400*, interface *D-7000*, HPLC-manager *D-7000*). Anal. HPLC: *Macherey-Nagel* C_{18} column (*Nucleosil 100-5 C18* (250 × 4 mm)) using a gradient of solvent *A* (MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 1 ml/min. Prep. HPLC: *Macherey-Nagel* C_{18} column (*Nucleosil 100–5 C18* (250 × 21 mm)) using a gradient of solvent *A* (MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 10 ml/min. Lyophilization was realized using a *Hetosicc* cooling condenser with high-vacuum pump to obtain peptides as their TFA salts. M.p.: *Büchi 510* melting-point apparatus; uncorrected. Optical rotations:

Perkin-Elmer 241 polarimeter (10 cm, 1-ml cell), $[\alpha]_D$ values are determined at 589 nm and given in 10^{-1} deg cm^2 g^{-1}. IR Spectra: neat (unless otherwise stated) on a *Perkin-Elmer precisely Universal ATR Sampling Accessory*; in cm^{-1}. NMR Spectra: either *Bruker AV-300* (^{1}H: 300.06 MHz, ^{13}C: 75.45 MHz, ^{19}F: 282.34 MHz), or *AMX-400* (^{1}H: 400.95 MHz, ^{13}C: 100.12 MHz), or *AV-600* (^{1}H: 600.13 MHz, ^{13}C: 150.91 MHz, ^{19}F: 376.33 MHz); chemical shifts δ are reported in ppm relative to internal standard Me_4Si for ^{1}H and ^{13}C, and to external standard $CFCl_3$ for ^{19}F; coupling constants J in Hz; ^{1}H-, ^{13}C-, and ^{19}F-spectroscopic data assigned on a routine basis by a combination of 1D and 2D experiments (COSY, HSCQ, HMBC, NOESY). High-resolution (HR) MS: *IonSpec Ultima 4.7 T FT Ion Cyclotron Resonance* (ICR, HR-MALDI-MS, in a 2,5-dihydrobenzoic acid matrix) mass spectrometer in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

Preparation of Compound **1**. *(3*R*,4*S*)-4-(Dibenzylamino)-6-methyl-1-phenylheptan-3-ol* (**3**). A soln. of (2-bromoethyl)benzene (4.06 ml, 30 mmol, 1.8 equiv.) in dry THF (30 ml) was added dropwise at r.t. into a round-bottom flask filled with N_2 and containing Mg turnings (778 mg, 32 mmol, 1.9 equiv.), one crystal of I_2, and dry THF (50 ml). The mixture was stirred at r.t. for 2 h, and a soln. of *N,N*-dibenzyl-L-leucinal (**2**) (prepared according to the literature procedure [10b]; 5 g, 17 mmol) in dry THF (20 ml) was added dropwise over 10 min. The mixture was stirred for an additional 2 h, and the reaction was quenched by the addition of sat. NH_4Cl soln. (80 ml). The product was extracted with AcOEt, and the combined org. phases were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by CC (hexane/AcOEt 15:1) to give the minor diastereoisomer (3*S*,4*S*)-4-(dibenzylamino)-6-methyl-1-phenylheptan-3-ol as a colorless crystalline solid, which eluted first (450 mg, 7% yield). The main product **3** was obtained as a colorless oil which slowly crystallized (5.58 g, 82% yield). M.p. 73–74° (hexane/CH_2Cl_2). R_f (hexane/AcOEt 10:1) 0.31. $[\alpha]_D^{20} = -3.1$ ($c = 1$, $CHCl_3$). IR: 3456 (br.), 3027*w*, 2951*m*, 1603*w*, 1495*s*, 1453*s*, 1365*w*, 1065*m*, 1028*m*, 746*s*, 698*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.73 (*d*, J = 6.5, Me); 0.88 (*d*, J = 6.5, Me); 1.23 (*td*, J = 6.9, 13.8, 1 H, CH_2); 1.57 (*td*, J = 6.9, 13.8, 1 H, CH_2); 1.63–180 (*m*, CH_2, Me_2CH); 2.31 (br. *s*, OH); 2.56 (*ddd*, J = 7.2, 9.2, 13.8, 1 H, PhCH_2); 2.74 (*dt*, J = 4.0, 7.0, CHN); 2.87 (*ddd*, J = 5.0, 9.0, 14.2, 1 H, PhCH_2); 3.63 (*s*, 2 PhCH_2N); 3.73 (*td*, J = 3.4, 9.8, C*H*OH); 7.14–7.31 (*m*, 15 arom. H); ^{13}C-NMR (75 MHz, $CDCl_3$): 22.9 (Me); 23.0 (Me); 24.8 (CH); 33.1 (CH_2); 34.5 (CH_2); 36.3 (CH_2); 55.2 (2 Ph*C*H$_2$N); 58.6 (CHN); 69.9 (CHOH); 125.7; 127.0 (2 C); 128.2 (4 C); 128.3 (2 C); 128.4 (2 C); 128.9 (4 C); 139.9 (2 C); 142.0. ESI-MS: 402.27942 ($[M+H]^+$, $C_{28}H_{36}NO^+$; calc. 402.27914 (+0.7 ppm)).

*(*S*)-4-(Dibenzylamino)-6-methyl-1-phenylheptan-3-one* (**4**). To a stirred soln. of oxalyl chloride (0.805 ml, 9.35 mmol, 1.5 equiv.) in dry CH_2Cl_2 (20 ml) cooled at −78° under N_2 was added dropwise DMSO (1.33 ml, 18.7 mmol, 3 equiv.). After stirring for 15 min, a soln. of **3** (2.5 g, 6.23 mmol) in dry CH_2Cl_2 (15 ml) was added dropwise at −78°. The mixture was stirred at this temp. for 30 min, and Et_3N (2.5 ml) was added. The mixture was then allowed to slowly warm to r.t. over 2 h, and the reaction was quenched with H_2O (20 ml). The product was extracted with CH_2Cl_2, and the combined org. phases were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by CC (hexane/AcOEt 20:1) to yield **4** (2.28 g, 91%). Colorless oil. $[\alpha]_D^{20} = -91.6$ ($c = 1$, $CHCl_3$). IR: 3027*w*, 2954*m*, 1713*s*, 1603*w*, 1495*s*, 1453*s*, 1368*m*, 1071*w*, 1028*w*, 746*s*, 698*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.71 (*d*, J = 6.4, Me); 0.80 (*d*, J = 6.4, Me); 1.34–1.48 (*m*, CH, 1 H of CH_2); 1.70 (*ddd*, J = 5.1, 8.8, 12.9, 1 H, CH_2); 2.62–2.70 (*m*, 1 H, CH_2CO); 2.74–2.92 (*m*, 1 H of CH_2CO, PhCH_2); 3.23 (*dd*, J = 4.2, 8.8, CHN); 3.50 (*d*, J = 13.7, 2 × 1 H, PhCH_2N); 3.67 (*d*, J = 13.7, 2 × 1 H, PhCH_2N); 7.08–7.31 (*m*, 15 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.5 (Me); 23.2 (Me); 25.5 (Me_2CH); 30.0 (PhCH_2); 32.2 (CHCH_2); 42.4 (CH_2CO); 54.6 (2 × PhCH_2N); 64.2 (CHN); 126.0; 127.2 (2 C); 128.3 (4 C); 128.4 (2 C); 128.5 (2 C); 129.0 (4 C); 139.7 (2 C); 141.3; 210.7 (CO). HR-MALDI-MS: 400.2635 ($[M+H]^+$, $C_{28}H_{34}NO^+$; calc. 400.2635 (+0.0 ppm)).

*(*S*)-Benzyl-3,3-difluoro-6-methyl-1-phenylheptan-4-amine* (**5**). To a soln. of **4** (2 g, 5 mmol) in dry CH_2Cl_2 (10 ml) stirred at −5° under Ar was added dropwise DAST (4.9 ml, 40 mmol, 8 equiv.). The mixture was allowed to slowly warm to r.t. and stirred for 4 d. The mixture was then cooled to 0°, and the reaction was quenched by the addition of sat. $NaHCO_3$ soln. (10 ml). The product was extracted with $CHCl_3$, the combined org. layers were evaporated under reduced pressure, and the residue was purified by CC (hexane/AcOEt 40:1) to give **5** (930 mg, 55% yield). Yellowish oil. $[\alpha]_D^{20} = -30.8$ ($c = 1$, $CHCl_3$). IR: 3028*w*, 2956*s*, 1496*m*, 1454*s*, 1379*m*, 1187*w*, 1123*m*, 1044*m*, 936*s*, 744*s*, 698*s*. ^{1}H-NMR (300 MHz,

$CDCl_3$): 0.81 (*d*, *J* = 6.5, Me); 0.91 (*d*, *J* = 6.6, Me); 1.16 (br. *s*, NH); 1.30 (*ddd*, *J* = 4.8, 9.6, 13.8, 1 H, CH_2); 1.40 (*ddd*, *J* = 3.5, 9.4, 13.4, 1 H, CH_2); 1.69–1.79 (*m*, Me_2CH); 2.05–2.21 (*m*, 1 H, CH_2CF_2); 2.24–2.40 (*m*, 1 H, CH_2CF_2); 2.79–2.94 (*m*, CHN, $PhCH_2$); 3.82 (*d*, *J* = 13.0, 1 H, $PhCH_2N$); 3.93 (*d*, *J* = 13.0, 1 H, $PhCH_2N$); 7.16–7.33 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 21.7 (Me); 23.8 (Me); 25.0 (Me_2CH); 27.9 (*t*, *J* = 5.0, $PhCH_2$); 35.0 (*t*, *J* = 24.7, CH_2CF_2); 39.2 (*t*, *J* = 3.4, CH_2); 53.0 ($PhCH_2N$); 59.1 (*t*, *J* = 25.4, CHN); 126.1; 126.47 (*t*, *J* = 245.7, CF_2); 127.1; 128.4 (2 C); 128.5 (4 C); 128.6 (2 C); 140.7; 141.3. ^{19}F-NMR (375 MHz, $CDCl_3$): −104.67 (*tdd*, *J* = 10.0, 26.7, 245.0, 1 F); −106.75 (*tdd*, *J* = 9.8, 26.5, 245.0, 1 F). ESI-MS: 332.2186 ($[M+H]^+$, $C_{21}H_{28}F_2N^+$; calc. 332.2184 (+0.45 ppm)).

*(*S*)-*N*-[(*tert*-Butoxy)carbonyl]-3,3-difluoro-6-methyl-1-phenylheptan-4-amine* (**6**). To a soln. of **5** (565 mg, 1.7 mmol) in MeOH (15 ml) was added Pd/C 10% (160 mg). The mixture was stirred at r.t. for 2 h under atmospheric pressure of H_2. Boc_2O (745 mg, 3.4 mmol, 2 equiv.) was added, and the black suspension was stirred for 24 h under H_2. The mixture was filtered over *Celite*® to remove Pd/C, and the solvent was evaporated under reduced pressure. The residue was taken in Et_2O (20 ml), and H_2O (4 ml) was added. The org. phase was separated, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by CC (100% hexane, then hexane/AcOEt 30:1) to give **6** (505 mg, 87% yield). Colorless crystalline solid. M.p. 76–77° (from hexane/AcOEt). $[\alpha]_D^{20} = -18.9$ (*c* = 1, $CHCl_3$). IR: 3364*m*, 2961*s*, 1701*s*, 1506*s*, 1367*s*, 1254*m*, 1166*s*, 1044*s*, 940*m*, 747*m*, 700*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (*d*, *J* = 6.5, Me); 0.96 (*d*, *J* = 6.6, Me); 1.45 (*s*, 3 Me); 1.36–1.60 (*m*, CH_2); 1.64–1.74 (*m*, Me_2CH); 2.09–2.25 (*m*, CH_2CF_2); 2.73–2.95 (*m*, $PhCH_2$); 4.06 (*m*, CHN); 4.47 (*d*, *J* = 10.2, NH); 7.17–7.30 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.3 (Me); 23.7 (Me); 24.5 (Me_2CH); 28.1 (*t*, *J* = 4.9, $PhCH_2$); 28.3 (3 Me); 36.1 (*t*, *J* = 24.5, CH_2CF_2); 37.0 (CH_2); 52.0 (*dd*, *J* = 24.4, 30.4, CHN); 79.9 (CMe_3); 123.9 (*t*, *J* = 246.2, CF_2); 126.1; 128.4 (2 C); 128.5 (2 C); 140.8; 155.6 (CO). ^{19}F-NMR (375 MHz, $CDCl_3$): −108.73 (*ddd*, *J* = 6.5, 18.7, 246.0, 1 F); −111.41 (*ddd*, *J* = 18.8, 31.1, 246.2, 1 F). ESI-MS: 364.20567 ($[M+Na]^+$, $C_{19}H_{29}F_2NNaO_2^+$; calc. 364.20586 (−0.52 ppm)). Anal. calc. for $C_{19}H_{29}NO_2F_2$: C 66.84, H 8.56, N 4.10; found: C 66.57, H 8.46, N 4.11.

*(*S*)-5-{[(*tert*-Butoxy)carbonyl]amino}-4,4-difluoro-7-methyloctanoic Acid* (**7**). Compound **6** (300 mg, 0.88 mmol) was dissolved in the biphasic solvent system CCl_4/MeCN/H_2O (8:8, 10 ml), and $NaIO_4$ (3.385 g, 15.8 mmol, 18 equiv.) was added. The mixture was then treated with $RuCl_3 \cdot x\ H_2O$ (36% Ru; 15 mg, 0.05 mmol, 6 mol-%), and the mixture was stirred vigorously at 25° in a water bath for 2 d. The mixture was then filtered through a large *Celite*® pad, and the solids were washed with AcOEt. The filtrate was concentrated to dryness to give a brownish residue that was used directly for the next step. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.5, Me); 0.95 (*d*, *J* = 6.5, Me); 1.37–1.54 (*m*, CH_2); 1.44 (*s*, 9 H); 1.61–1.75 (*m*, Me_2CH); 2.14–2.32 (*m*, CH_2CF_2); 2.52–2.73 (*m*, CH_2CO_2); 3.81–4.06 (*m*, CHN); 4.49 (*d*, *J* = 10.2, NH, major rotamer). ^{19}F-NMR (285 MHz, $CDCl_3$): −108.09 (*dm*, *J* = 249.0, minor rotamer); −109.13 (*dm*, *J* = 248.8, 1 F); −111.84 (*dm*, *J* = 249.2, minor rotamer); −112.92 (*dm*, *J* = 247.8, 1 F).

*(*S*)-5-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-4,4-difluoro-7-methyloctanoic Acid* (**1**). Crude **7** was treated at r.t. with TFA (8 ml), and the soln. was stirred at r.t. for 1 h. $CHCl_3$ was added, and the solvents were evaporated under reduced pressure. Final traces of TFA were removed under high vacuum, and the resulting residue was dissolved in 0.2M Na_2CO_3 (17 ml, 4 equiv.). A soln. of Fmoc-OSu (445 mg, 1.3 mmol, 1.5 equiv.) in acetone (8 ml) was then added dropwise, and the mixture was stirred at r.t. for 6 h. Acetone was evaporated under reduced pressure, the residual soln. was extracted with Et_2O (3 × 10 ml), and the aq. phase was acidified to pH 2 with 6N HCl before being extracted with AcOEt (3 × 10 ml). The org. extracts were combined, dried ($MgSO_4$), and evaporated. Purification by CC (hexane/AcOEt 3:1, 0.5% AcOH) afforded **1** (209 mg, 55% yield). Colorless solid. M.p. 130–132° (hexane/AcOEt). $[\alpha]_D^{20} = -16.4$ (*c* = 1, $CHCl_3$). IR: 2959*m*, 1713*s*, 1520*m*, 1450*m*, 1260*m*, 1216*s*, 1114*w*, 1056*m*, 947*m*, 757*s*. ^{1}H-NMR (400 MHz, (D_6)DMSO)[4]): 0.45 (*d*, *J* = 6.5, Me rotamer 10%); 0.74 (*d*, *J* = 6.5, Me rotamer 10%); 0.80 (*d*, *J* = 6.6, Me); 0.88 (*d*, *J* = 6.6, Me); 1.22–1.32 (*m*, 1 H, CH_2); 1.46–1.57 (*m*, Me_2CH, 1 H of CH_2); 2.02–2.17 (*m*, CH_2CF_2); 2.30–2.45 (*m*, CH_2CO_2); 3.77–3.90 (*m*, CHN); 4.21 (*t*, *J* = 6.9, CH of Fmoc); 4.37 (*d*, *J* = 6.9, CH_2O); 7.28–7.33 (*m*, 2 arom. H); 7.38–7.42 (*m*, 2 arom. H);

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[4]) NMR Measurement at 60° showed disappearance of the minor rotamer signals with concomitant broadening of the signals of the major rotamer.

7.60 (*d*, *J* = 9.4, NH); 7.69 (*dd*, *J* = 4.2, 7.1, 2 arom. H); 7.87 (*d*, *J* = 7.5, 2 arom. H); 12.05 (br. *s*, CO_2H). ^{13}C-NMR (100 MHz, (D_6)DMSO): 21.3 (Me); 23.7 (Me); 24.0 (Me_2CH); 26.5 (CH_2CO_2H); 28.3 (*t*, *J* = 24.7, CH_2CF_2); 35.6 (CH_2); 46.8 (CH of Fmoc); 52.7 (*t*, *J* = 27.6, CHN); 65.4 (CH_2O); 120.4 (2 C); 124.4 (*t*, *J* = 247.8, CF_2); 125.4 (2 C); 127.2 (2 C); 127.9 (2 C); 141.0 (2 C); 144.2 (2 C); 156.8 (*C*O); 173.5 (CO_2H). ^{19}F-NMR (375 MHz, (D_6)DMSO): −106.63 (*ddt*, *J* = 241.8, 18.3, 8.2, 1 F); −109.03 (*dm*, *J* = 241.8, 1 F). HR-MALDI-MS: 454.1800 ($[M+Na]^+$, $C_{24}H_{27}F_2NNaO_4^+$; calc. 454.18004 (1.32 ppm)).

Preparation of **2**·*TFA. H-(*S*)-His-(*S*)-Pro-(*S*)-Phe-(*S*)-His-(*S*)-3,3-difluoro-δ-h(F$_2$)hhLeu-(*S*)-Ile-(*S*)-Tyr-OH* (**8**). *Anchorage of* N-*Fmoc-Protected Amino Acid on* Wang *Resin.* Esterification of the *N*-Fmoc-Tyr(O^{t}Bu)-OH with *Wang* resin was performed by the MSNT/MeIm method [20]. The resin (75 mg, 1.1 mmol/g, 100–200 mesh) was placed in a dry manual reactor and swollen in CH_2Cl_2 (3 ml) for 30 min. A soln. of *N*-Fmoc-Tyr(O^{t}Bu)-OH (200 mg, 0.44 mmol, 5 equiv.), MeIm (26 µl, 0.33 mmol, 3.75 equiv.), and MSNT (130 mg, 0.44 mmol, 5 equiv.) in CH_2Cl_2 (3 ml) was then added to the swollen resin under N_2, and the suspension was mixed by N_2 bubbling for 4 h. The resin was filtered, washed with CH_2Cl_2, and dried under vacuum for 14 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene–piperidine adduct [18b] and was found to be 0.93 mmol/g (85%).

Capping Procedure. The peptide–resin was taken in a soln. of Ac_2O (66 µl, 10 equiv.) and DMAP (0.2 equiv.) in CH_2Cl_2 (3 ml), and the suspension was mixed by N_2 bubbling for 2 h. The resin was then washed by CH_2Cl_2 (5 × 5 ml, 1 min).

Deprotection of N-*Fmoc-Protected Amino Acids on* Wang *Resin* (*GP 1*). The Fmoc deprotection was performed using a soln. of 20% piperidine in DMF (2 ml/mmol; 3 × 10 min), with bubbling of N_2. After filtration, the resin was washed with DMF (3 × 1 min).

Coupling of Amino Acids on Wang *Resin* (*GP 2*). The resin was treated with a soln. of Fmoc-protected amino acid (3 equiv.), HATU (2.9 equiv.) and EtN(i-Pr)$_2$ (5 equiv.) in DMF (3 ml/mmol) for 1–4 h under bubbling of N_2. After complete coupling (visualized by the TNBS test), the resin was washed with DMF (5 × 1 min.).

Wang-*Resin Cleavage and Final Deprotection.* The dry peptide–resin was taken in a soln. of TFA/^{i}Pr$_3$SiH/H_2O (95 : 2.5 : 2.5, 3 ml/mmol) for 3 h under bubbling of N_2. The resin was removed by filtration and washed with TFA (2 × 2 ml). The combined filtrate was evaporated under reduced pressure, and the oily residue was treated with cold Et_2O. The precipitated crude peptide was filtered and dried under high vacuum. Purification by prep. RP-HPLC (2–40% in 50 min) and lyophilization yielded peptide **8** (29 mg, 35%) as TFA salt. Colorless solid. Anal. RP-HPLC (2–40% *A* in 35 min, t_R 28.1 min, purity >98%). ^{1}H-NMR (600 MHz, CD_3OD): 0.88–0.91 (*m*, 2 × Me of Ile + Me of h(F$_2$)hhLeu); 0.97 (*d*, *J* = 6.6, Me of h(F$_2$)hhLeu); 1.12–1.19 (*m*, 1 H, CH_2 of Ile); 1.44–1.50 (*m*, 1 H, CH_2 of Ile; 1 H, CH_2 of Leu); 1.54–160 (*m*, 1 H, CH_2 of Leu); 1.60–1.65 (*m*, CH of Leu); 1.78–1.85 (*m*, CH of Ile); 1.91–1.97 (*m*, 1 H, CH_2 of Pro); 1.99–2.10 (*m*, CH_2 of Pro, CH_2CF_2); 2.31–2.37 (*m*, 1 H, CH_2 of Pro); 2.41–2.49 (*m*, CH_2CO of h(F$_2$)hhLeu); 2.90 (*dd*, *J* = 8.8, 14.1, 1 H, CH_2 of Tyr); 3.03 (*dd*, *J* = 8.0, 13.6, 1 H, CH_2 of Phe); 3.09–3.15 (*m*, 1 H, CH_2 of Tyr; 1 H, CH_2 of Phe; 1 H, CH_2 of His4); 3.28 (*dd*, *J* = 7.2, 15.3, 1 H, CH_2 of His4); 3.41 (*d*, *J* = 5.7, CH_2 of His7); 3.54–3.58 (*m*, 1 H, CH_2N of Pro); 3.78–3.82 (*m*, 1 H, CH_2N of Pro); 4.21 (*d*, *J* = 7.7, H–C(α) of Ile); 4.25–4.33 (*m*, $CHCF_2$); 4.56 (*dd*, *J* = 6.4, 8.4, H–C(α) of Pro); 4.60–4.63 (*m*, H–C(α) of Tyr, H–C(α) of His7); 4.66–4.72 (*m*, H–C(α) of Phe, H–C(α) of His4); 6.69 (*d*, *J* = 8.7, 2 CH of Tyr); 7.06 (*d*, *J* = 8.7, 2 CH of Tyr); 7.20–7.24 (*m*, CH of Phe); 7.26–7.31 (*m*, 4 CH of Phe, CH of His4); 7.47 (CH, His7); 8.09 (*d*, *J* = 8.4, NH of Ile); 8.20 (*d*, *J* = 8.2, NH of Tyr); 8.34 (*d*, *J* = 9.4, NH of h(F$_2$)hhLeu); 8.50 (*d*, *J* = 7.8, NH of His4); 8.73 (*d*, *J* = 1.4, NH of His); 8.75 (*m*, NH of His); 8.80 (*d*, *J* = 7.0, NH of Phe). ^{13}C-NMR (150 MHz, CD_3OD): 11.3 (Me of Ile); 15.8 (Me of Ile); 21.6 (Me of h(F$_2$)hhLeu); 24.0 (Me of h(F$_2$)hhLeu); 25.4 (CH of h(F$_2$)hhLeu); 25.8 (CH_2 of Ile); 26.2 (CH_2 of Pro); 26.6 (CH_2 of His7); 28.5 (CH_2 of His4); 28.7 (*C*H_2CO of h(F$_2$)hhLeu); 30.5 (*t*, *J* = 24.3, *C*H_2CF_2 of h(F$_2$)hhLeu); 30.8 (CH_2 of Pro); 37.3 (CH_2 of Leu); 37.6 (CH_2 of Tyr); 37.9 (CH of Ile); 38.4 (CH_2 of Phe); 48.9 (CH_2N of Pro); 51.9 (C(α) of His7); 52.3 (*t*, *J* = 27.2, *C*HCF_2 of h(F$_2$)hhLeu); 53.8 (C(α) of His4); 55.2 (C(α) of Tyr); 56.4 (C(α) of Phe); 59.6 (C(α) of Ile); 61.8 (C(α) of Pro); 116.2 (2 × CH of Tyr); 118.6 (CH of His4); 120.4 (CH of His7); 128.8 (*t*, *J* = 245.4, CF_2); 127.9 (C of His7 + CH of Phe); 129.0 (C of Tyr); 129.5 (2 × CH of Phe); 130.3 (2 × CH of Phe); 130.5 (C of His4); 131.3 (2 × CH of Tyr); 134.9 (CH of His4); 136.2 (CH of His7); 137.9 (C of Phe); 157.2 (COH of Tyr); 167.6 (CO of His7); 172.0 (CO of His4); 173.1 (CO of Phe); 173.7 (CO of Ile); 174.2 (CO of h(F$_2$)hhLeu); 174.8 (2 × CO, Tyr +

Pro). ^{19}F-NMR (375 MHz, CD_3OD): -109.33 (*dm*, $J = 245.2$ Hz, 1 F); -111.10 (*dm*, $J = 245.2$ Hz, 1 F). HR-MALDI-MS: 1004.5164 ($[M+H]^+$, $C_{50}H_{68}F_2N_{11}O_2^+$; calc. 1004.51641 ($-1.89$ ppm)).

REFERENCES

[1] D. H. Rich, *J. Med. Chem.* **1985**, *28*, 263; J. Boger, L. S. Payne, D. S. Perlow, N. S. Lohr, M. Poe, E. H. Blaine, E. H. Ulm, T. W. Schorn, B. I. LaMont, T.-Y. Lin, M. Kawai, D. H. Rich, *J. Med. Chem.* **1985**, *28*, 1779; R. Guegan, J. Diaz, C. Cazaubon, M. Beaumont, C. Carlet, J. Clement, H. Demarne, M. Mellet, J.-P. Richaud, D. Segondy, M. Vedel, J.-P. Gagnol, R. Roncucci, B. Castro, P. Corvol, G. Evin, B. P. Roques, *J. Med. Chem.* **1986**, *29*, 1152; K. Y. Hui, W. D. Carlson, M. S. Bernatowicz, E. Haber, *J. Med. Chem.* **1987**, *30*, 1287; T. K. Sawyer, D. T. Pals, B. Mao, D. J. Staples, A. E. deVaux, L. L. Maggiora, J. A. Affholter, W. Kati, D. Duchamp, J. B. Hester, C. W. Smith, H. H. Saneii, J. Kinner, M. Handschumacher, W. Carlson, *J. Med. Chem.* **1988**, *31*, 18.

[2] H. Yanagisawa, T. Kanazaki, T. Nishi, *Chem. Lett.* **1989**, 687; H.-E. Radunz, H.-U. Reißig, G. Schneider, A. Riethmüller, *Liebigs Ann. Chem.* **1990**, 705; S. Atsuumi, M. Nakato, Y. Koike, S. Tanaka, H. Funabashi, K. Matsuyama, M. Nakano, Y. Sawasaki, K. Funabashi, H. Morishima, *Chem. Pharm. Bull.* **1992**, *40*, 3214.

[3] M. T. García-López, R. González-Muñiz, J. R. Harto, *Tetrahedron Lett.* **1988**, *29*, 1577; R. V. Hoffman, J. Tao, *Tetrahedron* **1997**, *53*, 7119.

[4] J. B. Cooper, S. I. Foundling, T. L. Blundell, J. Boger, R. A. Jupp, J. Kay, *Biochemistry* **1989**, *28*, 8596; N. S. Andreeva, L. D. Rumsh, *Protein Sci.* **2001**, *10*, 2439; P. T. Erskine, L. Coates, S. Mall, R. S. Gill, S. P. Wood, D. A. A. Myles, J. B. Cooper, *Protein Sci.* **2003**, *12*, 1741.

[5] M. G. Bock, R. M. DiPardo, B. E. Evans, K. E. Rittle, J. Boger, M. Poe, B. I. LaMont, R. J. Lynch, E. H. Ulm, G. P. Vlasuk, W. J. Greenlee, D. F. Veber, *J. Med. Chem.* **1987**, *30*, 1853; D. K. Stammers, J. G. Dann, C. J. Harris, D. R. Smith, *Arch. Biochem. Biophys.* **1987**, *258*, 413; V. Dhanaraj, C. G. Dealwis, C. Frazao, M. Badasso, B. L. Sibanda, I. J. Tickle, J. B. Cooper, H. P. C. Driessen, M. Newman, C. Aguilar, S. P. Wood, T. L. Blundell, P. M. Hobart, K. F. Geoghegan, M. J. Ammirati, D. E. Danley, B. A. O'Connor, D. J. Hoover, *Nature* **1992**, *357*, 466; J. Sueiras-Diaz, D. M. Jones, M. Szelke, B. J. Leckie, S. R. Beattie, J. J. Morton, *J. Pept. Res.* **1997**, *50*, 239.

[6] S. L. Harbeson, D. H. Rich, *Biochemistry* **1988**, *27*, 7301; S. L. Harbeson, D. H. Rich *J. Med. Chem.* **1989**, *32*, 1378.

[7] J. D. Dunitz, R. Taylor, *Chem.–Eur. J.* **1997**, *3*, 89; J. D. Dunitz, *ChemBioChem* **2004**, *5*, 614.

[8] D. O' Hagan, H. S. Rzepa, *Chem. Commun.* **1997**, *7*, 645.

[9] M. Hoffmann, J. Rychlewski, *Int. J. Quant. Chem.* **2002**, *89*, 419; H.-J. Boehm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Mueller, U. Obst-Sander, M. Stahl, *ChemBioChem* **2004**, *5*, 637; A. Wong, J. W. Munos, V. Devasthali, K. A. Johnson, H.-W. Liu, *Org. Lett.* **2004**, *6*, 3625; J. A. Hodges, R. T. Raines, *J. Am. Chem. Soc.* **2005**, *127*, 15923; J.-M. Zhou, H.-B. Zhang, X.-C. Dong, M.-B. Chen, *Carbohydr. Res.* **2006**, *341*, 2224.

[10] a) M. T. Reetz, M. W. Drewes, A. Schmitz, *Angew. Chem.* **1987**, *99*, 1186; b) P. L. Beaulieu, D. Wernic, *J. Org. Chem.* **1996**, *61*, 3635.

[11] P. H. J. Carlsen, T. Katsuki, V. S. Martin, K. B. Sharpless, *J. Org. Chem.* **1981**, *46*, 3936.

[12] G. Deniau, A. M. Z. Slawin, T. Lebl, F. Chorki, J. P. Issberner, T. van Mourik, J. M. Heygate, J. J. Lambert, L.-A. Etherington, K. T. Sillar, D. O' Hagan, *ChemBioChem* **2007**, *8*, 2265.

[13] K. Omura, D. Swern, *Tetrahedron* **1978**, *34*, 1651.

[14] R. P. Singh, J. M. Shreeve, *Synthesis* **2002**, *17*, 2561.

[15] F. Gessier, C. Noti, M. Rueping, D. Seebach, *Helv. Chim. Acta* **2003**, *86*, 1862; D. F. Hook, F. Gessier, C. Noti, P. Kast, D. Seebach, *ChemBioChem* **2004**, *5*, 691.

[16] J. E. Baldwin, R. M. Adlington, I. A. O'Neil, C. Schofield, A. C. Spivey, J. B. Sweeney, *J. Chem. Soc., Chem. Commun.* **1989**, 1852; M.-B. Giudicelli, D. Picq, D. Anker, *Tetrahedron* **1992**, *48*, 6033; M. D'hooghe, N. De Kimpe, *Synlett* **2006**, *13*, 2089; T.-X. Métro, D. G. Pardo, J. Cossy, *J. Org. Chem.* **2007**, *72*, 6556.

[17] K. Poulsen, J. Burton, E. Haber, *Biochemistry* **1973**, *12*, 3877; E. Haber, *Trans. Assoc. Am. Physicians* **1973**, *86*, 185; K. Poulsen, E. Haber, J. Burton, *Biochim. Biophys. Acta, Enzymol.* **1976**, *452*, 533; B.

Leckie, M. Szelke, A. Hallett, M. Hughes, A. F. Lever, G. McIntyre, J. J. Morton, M. Tree, *Clin. Exp. Hypertens.* **1983**, *5*, 1221; J. Boger, N. S. Lohr, E. H. Ulm, M. Poe, E. H. Blaine, G. M. Fanelli, T.-Y. Lin, L. S. Payne, T. W. Schorn, B. I. LaMont, T. C. Vassil, I. I. Stabilito, D. F. Veber, D. H. Rich, A. S. Bopari, *Nature* **1983**, *303*, 81.

[18] a) L. A. Carpino, G. Y. Han, *J. Org. Chem.* **1972**, *37*, 3404; b) 'Fmoc Solid Phase Peptide Synthesis: A Practical Approach', Eds. W. C. Chan, P. D. White, Oxford University Press, Oxford, 2000, Vol. 222; c) *Novabiochem* 2004/5 Catalogue: 'The Fine Art of Solid Phase Synthesis'.

[19] S. S. Wang, *J. Am. Chem. Soc.* **1973**, *95*, 1328.

[20] B. Blankemeyer-Menge, M. Nimtz, R. Frank, *Tetrahedron Lett.* **1990**, *31*, 1701.

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