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O-(Carboxydifluoromethyl)- L-Tyrosine: Design and Synthesis of a Novel Non Phosphorous- Containing Phosphotyrosine Isostere

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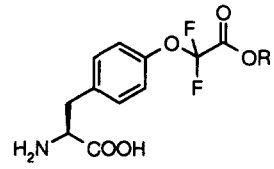
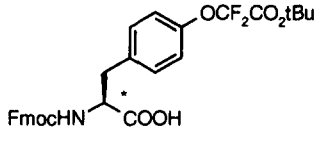
Abstract: *O*-(Carboxydifluoromethyl)- L-tyrosine **1** was designed as a non phosphorous-containing phosphotyrosine isostere. A synthesis leading to the racemic as well as a stereoselective preparation of the L-configurated amino acid is described. Conversion into the respective Fmoc derivatives DL-**2** and L-**2** afforded building blocks suitably protected for solid-phase peptide synthesis.

Introduction

Protein phosphorylation by kinases,[1] dephophosphorylation by phophatases[2] as well as protein-protein interactions through the recognition of phosphorylated protein sequences by Src homology (SH2) and phosphotyrosine binding (PTB) domains[3] are known to be crucial in intracellular signaling processes. Synthetic phosphopeptides have been widely used as valuable biological and biochemical tools to study these events at the molecular level. Eventually, specific inhibitors of these processes may lead to potential therapeutic agents for a variety of disease areas.[4] For cellular and in vivo studies compounds containing enzymatically (phosphatases) and chemically (hydrolysis) stable analogues of phosphotyrosine are a prerequisite.



1a R= H
1b R= tBu



L-**2**
DL-**2**

* indicates the location of the chiral center

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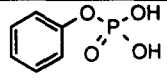
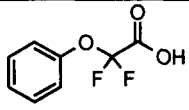
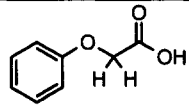
In the case of phosphotyrosine (pTyr), phosphonate analogues (CH_2-, CHF- and CF_2-phosphonates) have been widely explored as phosphatase stable mimics.[5] Poor cellular penetration due to the ionization of the phosphonate group at physiological pH has been proposed as a major drawback of phosphonate-containing inhibitors.[6] Non phosphorus-containing pTyr mimetics that utilized a malonyl or 2-fluoromalonyl structure in place of the parent phosphate group were developed in an effort to overcome this issue.[5b]

In order to overcome these drawbacks, our efforts were directed towards the development of mono charged, non phosphorous-containing pTyr mimetics.[7] Herein, we propose *O*-(carboxydifluoromethyl)-L-tyrosine **1a** as such a replacement. The design and a synthesis of racemic as well as of the enantiomerically pure L-configurated amino acid **2**, suitably protected for solid-phase peptide synthesis, are presented herein.

Results and Discussion

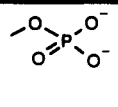
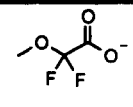
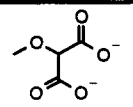
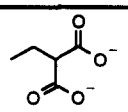
Several factors were considered important for the design of non-phosphorous containing phosphate mimics: (a) an acidic function with a pK_a ~2 for recognition; (b) a tetragonal arrangement of proton acceptors for optimal binding in the phosphate recognition pocket, as deduced from structural information;[8] (c) a volume of the van der Waals' sphere that is close to that known for the phosphate group. Size and the overall electrostatic potential of such a replacement seem to be of some importance, because it should fit into the phosphate binding pocket of an enzyme or a protein by forming the appropriate hydrogen bonds without causing major adjustments of the receptor molecule.

Table 1. A Comparison of the pK_a Values of Phenyl Phosphate **I** with the pK_a of Phenoxytrifluoroacetic Acid **II** and Phenoxyacetic Acid **III**.[9]

		
I	II	III
pK_{a1} 2.33±0.07 pK_{a2} 5.93±0.03	pK_a 1.88±0.09	pK_a 3.17

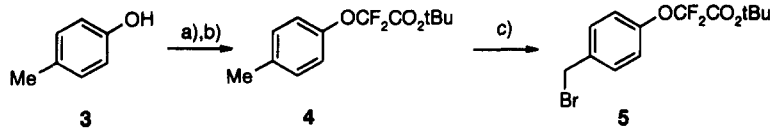
The difluoroacetic acid moiety was proposed as a phosphate isostere, because the two carbon bound fluorine atoms are thought to act as oxygen surrogates. Indeed, the van der Waals' radii of fluorine (1.47Å) and oxygen (1.57 Å) correlate very well. A limited hydrogen bonding ability was expected through the weaker electrostatic influence of fluorine compared to oxygen. The pK_a value of the phenoxydifluoroacetic acid moiety **II** was measured (pK_a =1.88) and found to be close to the pK_a of the corresponding phenylphosphate **I** (pK_a =2.33, Table 1). As expected, the two fluorine atoms lower the pK_a of the adjacent carboxylic acid compared to phenoxyacetic acid **III** (pK_a = 3.17). Whether or not the carbon bound fluorine atoms can act as hydrogen acceptors is still under debate. To date only few known examples of carbon bound fluorine are demonstrating H-acceptor capability as carefully elaborated in two recent review articles by Dunitz and Taylor,[10] and O'Hagan and Rzepa,[11] respectively.

Table 2. A Comparison of van der Waals' Volumes and Radii of Various Phosphate Mimics with those of the Phosphate Group (V(mimic)/ V(phosphate)).

	 IV	 V	 VI	 VII
van der Waals' volume [Å^3]	45.8	61.3 (x 1.33)	74.7 (x 1.63)	84.4 (x 1.84)
van der Waals' radius [Å]	2.44	2.69	2.88	3.00

The van der Waals' volumes of the various phosphate replacements **V-VII** were measured and compared with that of the phosphate group **IV** (Table 2). A more than 50% increase of the volumes was determined for the malonyl residues **VI** and **VII**, whereas the difluoroacetic acid **V** was found to be closer to the phosphate group. Most strikingly, the newly designed O-(carboxydifluoromethyl)-L-tyrosine **1a** fits perfectly into the positive charged phosphate binding pocket of the Grb2-SH2 protein.[8a]

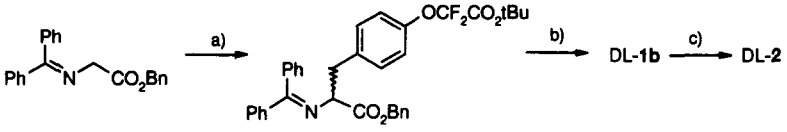
The synthesis of protected racemic as well as L-configurated phosphotyrosine mimic **2** requires the common alkylating agent **5**, which was prepared from p-cresol **3**, as outlined in Scheme 1. 4-Methylphenoxy-difluoroacetic acid, produced by the reaction of the sodium phenoxide with chlorodifluoroacetic acid,[12] was quantitatively converted into the *tert*-butyl ester **4**. Benzylic bromination of **4** by means of N-bromosuccinimide in the presence of $CaCO_3$ yielded the required benzyl bromide **5**.



3 4 5

Scheme 1. (a) NaH, $ClCF_2COOH$, dioxane; reflux 3h, quant. (b) $DMF(tBuO)_2$, toluene, 80°C, 4h, 86%; (c) N-bromosuccinimide, $CaCO_3$, dibenzoyl peroxide, CCl_4, reflux, 83%.

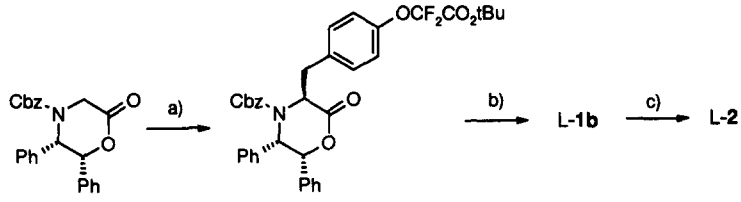
Racemic (CF_2CO_2tBu)Tyr (DL-**1b**) was prepared according to Scheme 2. In a first attempt, we performed the phase-transfer catalyzed alkylation of the Schiff base **6**, derived from the corresponding phenyl glycinate,[13] with benzyl bromide **5** in a biphasic mixture consisting of 10% aqueous sodium hydroxide in methylene chloride according to O'Donnell's procedure.[14] However, this protocol did not yield the desired alkylation product **7**. Eventually, alkylation could be achieved via enolate formation with lithium diisopropylamide/ DMPU in tetrahydrofuran at -78°C to give racemic compound **7** in 60% yield. Hydrogenation of **7** over 5% Pd/C yielded DL-**1b**, which then was condensed with FmocOSu affording the N^{α}-Fmoc protected building block DL-**2**.



6 7

Scheme 2. (a) LDA, DMPU, THF, -78°C, 60%; (b) H_2, 5% Pd/C, MeOH, 81%; (c) FmocOSu, $NaHCO_3$, 50% aqueous MeCN, 73.5%.

Among the different methods for a diastereoselective synthesis of α-amino acids,[15] we chose the stereoselective alkylation procedure of Williams using commercially available imino lactone **8**[16] as the chiral auxiliary.[17] This method provides the alkylation product generally in good yield and high diastereomeric excess. Treatment of the chiral glycine derivative **8** with sodium bis(trimethylsilyl)amide in THF at -78°C was followed by the alkylation of the enolate with benzyl bromide **5** providing diastereomerically pure **9** in 73% yield. HPLC and TLC analysis of the crude product **9** gave only a single peak/ spot. The proton NMR spectra recorded at room temperature displayed two sets of signals in a 11:5 ratio. Heating a DMSO solution to 120°C caused a collapse of the rotamer signals also suggesting that **9** was a single diastereomer.

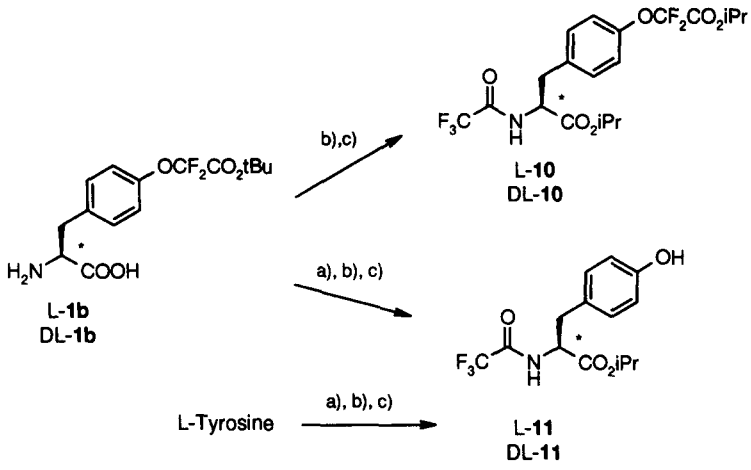


8 **9**

Scheme 3. (a) $Na(TMS)_2$, THF, -78°C, then **5**, 73%; (b) H_2, 10% Pd/C, THF/ EtOH 1:1, quant.; (c) FmocOSu, $NaHCO_3$, 50% aqueous MeCN, 86%.

Removal of the carbobenzyloxy and diphenylethylene groups was conveniently achieved by catalytic hydrogenation of purified **9** over 10% Pd/C to produce side chain protected amino acid L-**1b** in quantitative yield. Preparation of N^{α}-Fmoc protected derivative L-**2** was performed under the conditions described for the racemic material.

In order to verify the optical purity, crude product L-**1b** was derivatised in a two step reaction sequence involving first treatment with 1.25M HCl in isopropanol and then with trifluoroacetic acid anhydride (TFAA), as shown in Scheme 4. Derivative **10** was analyzed by GC using a chiral stationary phase. Two peaks were detected after 37.15min and 37.93 min, integrating in a 0.9: 99.1 ratio. A sample of L-**1b** was hydrolyzed prior to derivatisation, and then subjected to chiral GC analysis. Again, two compounds eluted at 25.93 min and 26.49 min which were identified as D- and L-tyrosine derivative **11** in a 1.3: 98.7 ratio. Subjecting L-tyrosine to the same conditions led to 0.4 % of D-tyrosine formation (peak 1: t_R= 25.98 min, 0.4 % D-**11**; peak 2: tR= 26.60 min, 99.6% L-**11**). The corrected value fully agrees with the value found for **10** accounting for >98% enantiomeric excess of the L-form. Converting DL-**1b** into DL-**10** and DL-**11** was performed under the conditions described above (Scheme 4). GC analysis DL-**10** revealed two peaks at 37.30 min and 37.82 min in a 1:1 ratio for D and L-**10**, respectively. The gas chromatogram of DL-**11** displayed two peaks in a 1:1 ratio at 26.04 min and 26.48 min for D and L-**11**, respectively. In addition, derivatives **10** and **11** were characterized by ESI MS and found to be the only products in their respective samples.



Scheme 4. (a) 6N HCl, 105°C, 24h; (b) 1.25N HCl, isopropanol, 105°C, 2h; (c) TFFA, CH_2Cl_2.

Conclusions

Herein, we have presented the design of O-(carboxydifluoromethyl)-L-tyrosine as a novel non-phosphate containing phosphotyrosine mimetic. The synthesis of racemic as well as L-configured amino acid is described. Incorporation of the Fmoc-protected building block into peptide sequences that bind to the Grb2-SH2 protein as well as a comparative study with peptides containing other phosphotyrosine mimetics is ongoing. Furthermore, it should be possible to derive information about the binding mode of the designed phosphotyrosine substitute in the phosphate binding pocket of the protein from structural investigations.

Experimental Section

General Methods

All reagents were commercially available and were used without further purification. Preparative flash chromatography was performed using Merck silica gel 60 (230-400 mesh). Medium-pressure liquid chromatography (MPLC) was achieved on a Büchi system, equipped with a Büchi 688 chromatography pump, a Büchi 687 gradient former, a Knauer variable wavelength monitor and a Büchi 684 fraction collector, using Merck ®LICHROPREP RP-18 column, 15-25 μm bead diameter, column length 46 cm, diameter 3.6 cm; flow rate 53.3 ml/min; detection at 215nm), eluting the products with an acetonitrile-water gradient containing 0.1% of TFA. Reactions were monitored by thin layer chromatography (TLC) using Merck 60 F254 precoated silica gel plates (0.25 mm thickness). The compounds were analyzed by reversed HPLC using a Merck-Hitachi system, equipped with a AS-2000 autosampler, a L-6200A intelligent pump, a L-4500 diode array detector, and a D-6000 interface, using a Nucleosil C_{18} column (125 x 3 mm, 3 μm, 100Å (AB), from Macherey-Nagel, Düren, FRG): linear gradient of acetonitrile/ 0.09% TFA and H_2O/ 0.1% TFA from 1:49 to 3:2 over 10 min.; flow rate 0.7 ml/min., detection at 215 nm. IR spectra were recorded with KBr plates or in

solution on a Bruker IFS 48 FT IR spectrophotometer. Optical rotations were measured using a sodium lamp (589 nm) and are reported in degrees, with conc. units of g/ 100 ml. The nuclear resonance spectra (^1H, ^{13}C and ^{19}F NMR) were recorded on a Bruker AM 500 or Varian Gemini 300 spectrometer using $CDCl_3$, CD_3OD or DMSO-d_6 as solvent. All ^1H NMR spectra are reported in δ units, ppm downfield from tetramethylsilane using residual solvent signal (δ 7.24 ppm for $CDCl_3$, δ 3.35 ppm for CD_3OD, δ 2.49 ppm for DMSO) as an internal standard. All ^{13}C NMR spectra are reported in ppm relative to the central line of the triplet for $CDCl_3$ at δ 77 ppm or of the septet for CD_3OD at 49.3 ppm. All ^{19}F NMR are reported in ppm referenced to CCl_3F in $CDCl_3$ (δ 0.0 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Electrospray mass spectra (ESI MS) were obtained with a Fisons Instruments VG Platform II, fast atom bombardment mass spectra (FAB MS) with a ZAB-HF instrument from VG Analytical (Manchester, UK). Gas chromatography (GC) analyses were perfomed on a Varian 3600 gas chromatograph, with a FID, and a Chirasil valine capillary column.

tert-Butyl Difluoro-(4-methyl-phenoxy)-acetate (4)

NaH (4.03g, 168 mmol) was added in portions to a stirred and cooled solution of p-cresol (**3**, 8.65g, 80 mmol) and $ClCF_2COOH$ (10.44g, 80 mmol) in dioxane under N_2 atmosphere. The resultant mixture was then heated under reflux for 3h. After cooling to room temperature, EtOH (5 ml) was added and the mixture was poured onto 1N HCl/ ice (500ml) and then extracted with EtOAc. The extracts were washed with H_2O and brine, dried (Na_2SO_4) and evaporated to give crude difluoro-(4-methylphenoxy)-acetic acid (16.5g, 100%), which was shown by HPLC to consist of a single compound (t_R = 6.87 min).

For analytical purposes, a sample of crude product was further purified by MPLC on a revesed phase C_{18} column (Lichroprep, 20-25μm, gradient H_2O/ MeCN containing 0.1% TFA) to yield difluoro-(4-methylphenoxy)-acetic acid as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) ppm: δ 7.16 (d, *J* = 8.7 Hz, 2H); 7.16 (d, *J* = 8.7 Hz, 2H); 4.93 (br. s, 3H, hydrate); 2.35 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) ppm: -77.35. FAB MS (m/z): 202 (M^+). p*K*a = 1.88.

A solution of crude difluoro-(4-methyl-phenoxy)-acetic acid (10.1g, 50mmol) and $DMF(tBuO)_2$ (15.2g, 75 mmol) in toluene were stirred at 80°C. After 2h another portion of $DMF(tBuO)_2$ (10.5g, 50 mmol) was added to the reaction mixture. After 4h the mixture was diluted with toluene, and the solution was washed with 5% aqueous Na_2CO_3 solution (2x), H_2O (3x), and brine (1x), dried (Na_2SO_4) and evaporated to dryness to yield crude ester **4** as a brownish liquid (11.1g, 86%). After flash chromatography on silica gel using hexane/ 2.5% ether, pure **3** (9.8g) was obtained in 76% yield. ^1H NMR (500 MHz, $CDCl_3$), ppm: δ 7.12 (d, *J* = 8.6 Hz, 2H); 7.07 (d, *J* = 8.6Hz, 2H); 2.32 (s, 3H); 1.49 (s, 9H). ^{13}C NMR (125.8 MHz, $CDCl_3$), ppm: 158.7 (t, $^2J_{C,F}$= 40.7 Hz), 147.5 (t, $^3J_{C,F}$= 2.1 Hz), 135.8, 129.9, 121.4, 114.0 (t, $^1J_{C,F}$= 271.9 Hz), 85.4, 27.6, 20.8. ^{19}F NMR (282 MHz, $CDCl_3$), ppm: -77.27. FAB MS (m/z): 258 (M^+).

tert-Butyl (4-Bromoethyl-phenoxy)-difluoro-acetate (5)

N-Bromosuccinimide (NBS, 1.83g, 97%, 10 mmol) was added in portions to a stirred solution containing **4**, (2.58g, 10 mmol), $CaCO_3$ (1.0g, 10 mmol), and dibenzoyl peroxide (DBP, 32.2mg, 75%, 0.1 mmol) in CCl_4 (75ml) at reflux. After addition of the final portion of NBS another 32 mg of DBP was added. Stirring at reflux was continued for another 3h. The reaction mixture was cooled to room temperature, washed with H_2O (3x), dried (Na_2SO_4), and evaporated. Flash chromatography of the crude product on silica gel using hexane/ 2.5% ether, yielded bromide **5** (2.79g, 83%) as a colorless liquid contaminated with about 15%

of dibrominated material as indicated by ^{1}H NMR. This material was used in the next step without further purification. TLC: R_f = 0.5 (hexane/ EtOAc 3:1). ^{1}H NMR (500 MHz, $CDCl_3$), ppm: δ 7.36 (d, J = 8.3 Hz, 2H); 7.16 (d, J = 8.3 Hz, 2H); 4.45 (s, 2H); 1.49 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$), ppm: 158.4 (t, $^2J_{C,F}$= 40.2 Hz), 149.7 (t, $^3J_{C,F}$= 1.9 Hz), 135.7, 130.3, 121.7, 113.9 (t, $^1J_{C,F}$= 273 Hz), 85.4, 32.2, 27.6. ^{19}F NMR (282 MHz, $CDCl_3$), ppm: -77.2. ESI MS (m/z): 336 and 338(M^+).

Benzyl 2-(Benzhydrylidene-amino)-3-[4-(*tert*-butoxycarbonyl-difluoro-methoxy)-phenyl]-propanoate (7)

A solution of lithium diisopropylamide (11 mmol) in THF (35 ml) was chilled to -78°C. Benzyl (diphenylmethylene)glycinate (**6**, 3.62 g, 11 mmol) in THF (25 ml) was added slowly. 1,3-Dimethyl-tetrahydro-pyrimidin-2-one (DMPU) was added dropwise. The mixture was stirred for 20 min and a solution of bromide **5** (11 mmol) in THF (40 ml) was added over a period of 40 min. The reaction mixture was maintaines at -78°C for 2 h, then warmed to room temperature over 2h. The reaction was quenched by adding saturated aqueous NH_4Cl solution (50 ml) and ether. After phase separation the aqueous phase was extracted with ether, the combined organic layers were washed with brine and dried (Na_2SO_4). Filtration and evaporation to dryness provided the crude product which was purified by flash chromatography on silica gel eluting with hexane/ ethyl acetate (10:1) to give **7** (3.9 g, 60%) as a colorless oil. IR (CH_2Cl_2): 2985, 2937, 1959, 1902, 1767, 1738, 1623, 1597, 1577, 1507, 1476, 1446, 1396, 1369, 1316, 1214, 1164, 1137. ^{1}H NMR (300 MHz, $CDCl_3$), ppm: δ 7.56 (d, J = 8.4 Hz, 2H); 7.42 -7.20 (m, 11 H); 7.02 (m, 4 H); 6.55 (br. s, 2H); 5.21 (d, J = 12 Hz, 1H); 5.16 (d, J = 12Hz, 1H); 4.26 (dd, 1H-C(α)); 3.26 (m, 2 H-C(β)); 1.50 (s, 9H). ^{19}F NMR (282 MHz, $CDCl_3$), ppm: -77.4. FAB MS: 586 $[M+H]^+$. Calcd for $C_{35}H_{33}F_2NO_5$: C; 71.78%, H; 5.68%, N; 2.39%, F; 6.49%. Found: C; 71.72%; H; 5.80%; N; 2.45%, F; 6.52%.

2-Amino-3-[4-(*tert*-butoxycarbonyl-difluoro-methoxy)-phenyl]-propanoic acid DL-(CF_2CO_2tBu)-Tyr (DL-1b)

Racemic **7** (2.293g, 2.4 mmol) was dissolved in MeOH (115 ml) and hydrogenated with 10% Pd/C at 5 bar of hydrogen for 4h. The solvent was evaporated and the residue triturated with diisopropyl ether. Phenylalanine derivative DL-**1b** (1.05 g, 81%) was obtained as a white powder, and found to be identical to L-**1b** with respect to HPLC, ^{1}H, ^{13}C, ^{19}F NMR, MS, IR and elemental analysis.

N^{α}-Fmoc-DL-(CF_2CO_2tBu)Tyr (DL-2)

Racemic phenylalanine derivative DL-**1** (450.6 mg, 1.36 mmol) was converted to its respective Fmoc derivative as described for L-**2**. Flash chromatography on silica using CH_2Cl_2/ 5% MeOH as eluent yielded DL-**2** (552mg, 73.5%), which was identical to L-**2** with respect to ^{1}H, ^{13}C, ^{19}F NMR, MS, TLC and HPLC.

Benzyl (2*R*,3*S*)-(+)-3-[4-(*tert*-Butoxycarbonyl-difluoro-methoxy)-benzyl]-2-oxo-5,6-diphenyl-morpholine-4-carboxylate (9)

Oxazinone **8** (1.88g, 4.4 mmol) was dissolved in THF (60ml) at 40°C. The clear, colorless solution was cooled to -70°C. A solution of 1M $NaN(TMS)_2$ (4.4 ml, 4.4 mmol) in THF was added via cannula under nitrogen. After stirring at -78°C for 15 min a solution of bromide **5** (1.35 g, 4 mmol) in THF (8 ml) was added slowly. The reaction mixture was subsequently stirred at -78°C for 7h. After quenching with EtOAc (70 ml) the mixture was washed with H_2O and brine, dried (Na_2SO_4) and the solvent evaporated. Flash

chromatography on silica gel using hexane/ EtOAc 3:1 yielded the desired product **9** (1.88 g, 73%) as a white powder. $[\alpha]^{20}_D$ = +44.6± 1.0 (c =1.029, $CHCl_3$). TLC: R_f = 0.33 (hexane/ EtOAc 3:1). HPLC: t_R = 11.4 min (single peak). ^1H NMR (500 MHz, $CDCl_3$); 2 conformers were observed in a 11:5 ratio: δ 7.38 (overlapping, m, 2H); 7.25-7.02 (overlapping, m, 10H); 6.79 (overlapping, d, *J* = 7.5 Hz, 2H); 6.57 (overlapping, m, 3H); 6.47 (overlapping, d, *J* = 7.5 Hz, 2H); major conformer: δ 5.28 (m, 1H-C(α)); 5.03 (d, *J* =12.5 Hz, 1H, OCH_2); 4.97 (d, *J* =12.5 Hz, 1H, OCH_2); 4.83 (d, *J* = 2.5 Hz, 1H); 4.39 (d, *J* = 2.5 Hz, 1H); 3.67 (dd, *J* =15 Hz, *J* =7.5 Hz, 1 H-C(β)); 3.39 (dd, *J* = 15 Hz, *J* = 4.5 Hz, 1H-C(β)); minor conformer: δ 5.18 (m, 1H-C(α)); 5.28 (d, 1H, OCH2); 5.09 (d, *J* = 12.5 Hz, 1H, OCH_2); 4.99 (d, *J* = 2.5 Hz, 1H); 4.57 (d, *J* =2.5 Hz, 1 H); 4.47 (dd, *J* =15 Hz, *J* = 7.5 Hz, 1 H-C(β)); 3.34 (dd. *J* = 15 Hz, *J* = 4.5 Hz, 1 H-C(β); 1.51 (overlapping, s, 9H). ^{19}F NMR (282 MHz, $CDCl_3$) ppm: -77.66. ESI MS (m/z): 644 $(M+H)^+$. ^1H NMR (500 MHz, DMSO-d_6/ D_2O, 120°C), ppm: 7.36-7.04 (m, 15H-arom.), 6.93 (d, *J*= 7.5 Hz, 2H, Phe), 6.59 (d, *J*= 7.5 Hz, 2H, Phe), 5.72 (br s, 1H), 5.22 (d, *J*=2.5 Hz, 1H), 5.11 (dd, *J*= 8.0 Hz, J= 5.0 Hz, 1H-C(α)), 5.01 (br s, 2 H-benzyl), 3.51 (dd, *J*= 15 Hz, *J*= 8.0Hz, 1H-C(β)), 3.42 (dd, J=15 Hz, *J*= 5.0 Hz, 1H-C(β)), 1.70 (s, 9H).

(2*S*)-(-)-2-Amino-3-[4-(*tert*-butoxycarbonyl-difluoro-methoxy)-phenyl]-propanoic acid L-(CF_2CO_2tBu)Tyr (L-1b)

Lactone **9** (1.61 g, 2.5 mmol) was dissolved in a 1:1 mixture of EtOH/ THF (20 ml) and hydrogenated with 10% Pd/C at 5 bar of hydrogen for 4h. All volatiles were removed in vacuo and the residue was triturated three times with hexane. The precipitate was isolated by filtration, washed with hexane and dried. The desired L-tyrosine derivative L-**1** (830.0 mg, quant.) was obtained as a white powder. HPLC: t_R = 6.43 (100%, single peak). $[\alpha]^{20}_D$ = -8.6 ± 1.0 (c = 1.023, CH_3OH). IR (DMSO): 2985, 1768, 1641, 1537, 1507, 1442, 1405, 1367, 1325, 1217, 1159, 1133. ^1H NMR (500 MHz, CD_3OD), ppm: δ 7.35 (d, *J* = 8.4 Hz, 2H). 7.18 (d, *J* = 8.4 Hz, 2H); 3.77 (dd, *J* = 8.6 Hz, *J* = 4.5 Hz, 1H); 3.05 (dd, *J* = 14.4 Hz, *J* = 8.6 Hz, 1H); 3.30 (dd, *J* = 14.4 Hz, *J* = 4.4 Hz, 1H); 1.50 (s, 9H). ^{13}C NMR (125 Mhz, CD_3OD), ppm: δ 173.5, 150.4, 135.7, 131.9, 122.9, 115.4 (t, $^1J_{C,F}$ = 270 Hz), 86.9, 57.4, 37.5, 27.8. ^{19}F NMR (282 MHz, CD_3OD), ppm: δ -74.76. FAB MS (m/z): 332 $(M+H)^+$. Calcd for $C_{15}H_{19}F_2NO_5$: C; 54.38%, H; 5.78%, N; 4.23%, F; 11.47%. Found: C; 54.28%, H; 5.94%, N; 4.39%, F; 11.37%.

N^{α}-Fmoc-L-(CF_2CO_2tBu)Tyr (L-2)

A solution of L-**1** (165.65 mg, 0.5 mmol) in 50% aqueous MeCN (10 ml) was cooled to 0°C before a solution of 10% $NaHCO_3$ (2.5 mmol) in H_2O (2.6 ml) was added. A solution of Fmoc-OSu (0.5 mmol) in MeCN (2.5 ml) was then added and stirring was continued at 0°C for 30 min, and then at room temperature for 2h. The reaction mixture was again cooled to 0°C and acidified with cold 0.5N HCl to pH = 2. The mixture was extracted with EtOAc, and the combined organic layers washed with H_2O and dried (Na_2SO_4). The solvent was removed in vacuo to give crude Fmoc-amino acid L-**2** (335.3 mg), which was then purified by MPLC on a reversed phase C_{18} column (Lichroprep 20-25mm, 5cm x 46cm) with 50% aqueous MeCN, containing 0.1% TFA. MeCN was removed by rotary evaporation, the remaining aqueous phase was extracted with EtOAc. The combined organic layers were washed with H_2O, dried (Na_2SO_4), and the solvent evaporated to dryness yielding L-**2** (236.9 mg, 86%) as a colorless foam. $[\alpha]^{20}_D$ = +23.8 ± 1.0 (c = 1.040, $CHCl_3$). TLC: R_f = 0.28 ($CHCl_3$/ MeOH 9:1). HPLC: t_R = 10.2 min (single peak). ^1H NMR (500 MHz, $CDCl_3$), ppm: δ 7.75 (d, *J*= 8.2 Hz, 2H), 7.54 (d, *J*= 8.2 Hz, 2H), 7.39 (dd, *J*= 8.2 Hz, 2H), 7.30 (dd, *J*= 8.2

Hz, 2H), 7.11 (d, *J*= 8.8 Hz, 2H, Phe), 7.08 (d, *J*= 8.8 Hz, 2H, Phe), 5.14 (d, *J*= 9.0 Hz, 1H-N$^{\alpha}$), 4.66 (m, 1 H-C(α)), 4.46 (m, 1H), 4.39 (m, 1H), 4.19 (t, *J*= 7.5Hz, 1H), 3.18 (dd, *J*= 14.5, *J*= 5 Hz, 1H-C(β)), 3.08 (dd, *J*= 14.5, *J*= 5.0 Hz, 1H-C(β)), 1.48 (s, 9 H, *tert*-butyl). ^{13}C NMR (125 MHz, $CDCl_3$), ppm: 175.1, 158.5 (t, $^2J_{C,F}$= 40.6 Hz), 155.7, 149.0, 143.5, 141.3, 133.4, 130.5, 127.8, 127.1 124.9 121.6, 120.0, 113.9(t, $^1J_{C,F}$= 272 Hz, 85.6, 67.0, 54.5, 47.1, 37.0, 27.6. ^{19}F NMR (282 MHz, $CDCl_3$), ppm: -77.31. ESI MS (m/z): 554 [M-H]$^+$.

Determination of the Enantiomeric Identity of the Substituted Tyrosine Derivative 1b

1 mg of amino acid **1** was hydrolyzed in 1.0 ml 6N HCl at 105°C for 24 h. The hydrolysat was dried in vacuo over KOH at room temperature for 24h. Derivatisation was performed in a two step procedure: Firstly the dry residue was treated with 1.25N HCl in isopropanol (0.3 ml) at 105°C for 2h. The reaction mixture was then dried in a stream of N_2. In the second step, the amino acid esters were N-acetylated with trifluoroacetic acid anhydride (TFAA, 0.2ml) in CH_2Cl_2 (0.3 ml) for 2h at room temperature. The volatiles were removed in a N_2 stream and the residue was dissolved in ethyl acetate (1 ml). The derivatized amino acid was injected (1 µl) on to a GC instrument equipped with a Chirasil valine capillary column at 75°C. The amino acids eluted with a temperature gradient (6 min at 75°C, then 75-180°C with 4°C/ min).

L-**1b** was converted to L-**10** and analyzed (GC), peak 1: t_R= 37.15 min (0.9%); peak 2: t_R= 37.93 min (99.1%).

DL-**1b** was converted to DL-**10** and analyzed (GC), peak 1: t_R= 37.35 min (50%); peak 2: t_R= 37.82 min (50%).

L-**1b** was hydrolyzed, then converted to L-**11** and analyzed (GC), peak 1: t_R= 25.93 min (2.3% identified as D-**11**); peak 2: t_R= 26.49 min (97.7%, identified as L-**11**).

DL-**1b** was hydrolyzed, then converted to DL-**11** and analyzed (GC), peak 1: t_R= 26.04 min (50% identified as D-**11**); peak 2: t_R= 26.48 min (50%, identified as L-**11**).

L-Tyrosine was hydrolyzed, then converted to L-**11** and analyzed (GC), peak 1: t_R= 25.98 min (0.4% identified as D-**11**); peak 2: t_R= 26.60 min (99.6%, identified as L-**11**). ESI MS of the samples containing derivative **10**, (m/z): 454 [M-H]$^-$ (455.38, calcd for $C_{19}H_{22}F_5NO_6$). ESI MS of the samples containing derivative **11**, (m/z): 318 [M-H]$^-$ (319.28, calcd for $C_{14}H_{16}F_3NO_4$).

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