



HAL
open science

Synthesis of 2,6-Dimethyltyrosine-Like Amino Acids through Pinacolinamide-Enabled C–H Dimethylation of 4-Dibenzylamino Phenylalanine

Davide Illuminati, Anna Fantinati, Tiziano de Ventura, Daniela Perrone, Chiara Sturaro, Valentina Albanese, Erika Marzola, Virginia Cristofori, Julie Oble, Giovanni Poli, et al.

► **To cite this version:**

Davide Illuminati, Anna Fantinati, Tiziano de Ventura, Daniela Perrone, Chiara Sturaro, et al.. Synthesis of 2,6-Dimethyltyrosine-Like Amino Acids through Pinacolinamide-Enabled C–H Dimethylation of 4-Dibenzylamino Phenylalanine. *Journal of Organic Chemistry*, 2022, pp.acs.joc.1c02527. 10.1021/acs.joc.1c02527 . hal-03566662

HAL Id: hal-03566662

<https://hal.sorbonne-universite.fr/hal-03566662>

Submitted on 11 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Synthesis of 2,6-dimethyltyrosine-like aminoacids through pinacolinamide-enabled C–H dimethylation of 4-dibenzylamino phenylalanine

Davide Illuminati,^{†,§,‡} Anna Fantinati,^{‡,‡} Tiziano De Ventura,[†] Daniela Perrone,[‡] Chiara Sturaro,[‡] Valentina Albanese,[†] Erika Marzola,[†] Virginia Cristofori,[†] Julie Oble,[§] Giovanni Poli,^{*,§} and Claudio Trapella^{*,†}

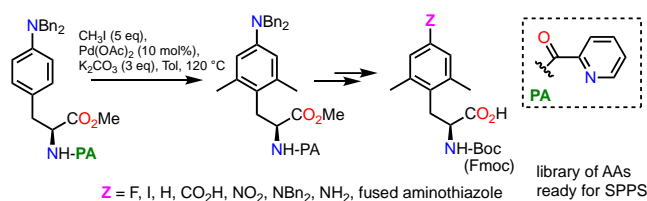
[†] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy.

[‡] Department of Environmental and Prevention Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy.

[§] Department of Neuroscience and Rehabilitation, Section of Pharmacology, University of Ferrara, Via Fossato di Mortara 17/19, Ferrara, 44121, Italy.

[§] Sorbonne Université, Faculté des Sciences et Ingénierie, CNRS, Institut Parisien de Chimie Moléculaire, IPCM, 4 place Jussieu, 75005 Paris, France

Supporting Information Placeholder



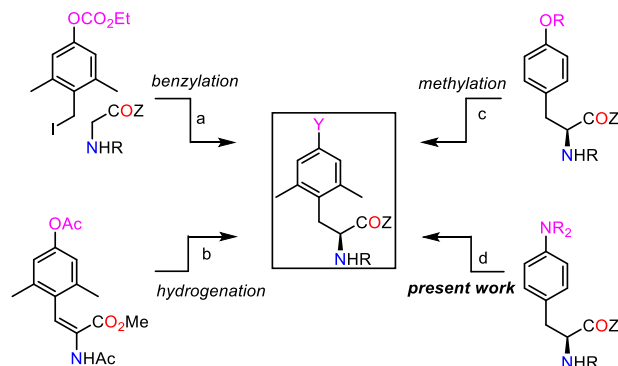
ABSTRACT: The synthesis of a small library of *N*-Boc or *N*-Fmoc protected (*L*)-phenylalanines carrying methyl groups at positions 2 and 6, and diverse functionalities at position 4 has been achieved. The approach, which took advantage of a Pd-catalyzed directed C–H dimethylation of picolinamide derivatives, allowed to alter the electronic/steric properties of the resulting amino acid derivatives by appending a variety of EWG, EDG, or bulky groups.

INTRODUCTION

The aromatic moiety in the *N*-terminal message domain of opioid peptides, commonly represented by Tyr1, Phe1, and Phe4, is fundamental in the binding and the activation processes of opioid receptors.¹ In particular, 2',6'-dimethyl-(*L*)-tyrosine (Dmt) has become one of the most popular non-natural amino acids to replace tyrosine in synthetic opioid peptides.² Indeed, the conformational restriction imparted by the two extra methyl groups affects the receptor affinity, selectivity and bioactivity of peptides incorporating this modified amino acid.³ In particular, peptides incorporating Dmt1 in lieu of Tyr1 in the message domain led to decreased selectivity profiles as MOP and NOP agonists, in addition to increased biological activities and stabilities. The co-activation of MOP/NOP or MOP/DOP receptors, in fact, tends to be characterized by strong analgesic potential, associated to low or no undesired effects, normally expressed by a single receptor activation.⁴

The important role of Dmt is underlined by the number of reported syntheses of tyrosine derivatives carrying two symmetrically disposed methyl groups on the aromatic ring.⁵ So far, three conceptually different approaches have been developed for preparing (*S*)-2,6-dimethyltyrosine and its derivatives. One approach is based on the enantio- or diastereoselective alkylation of glycine equivalents (Scheme 1, path a),⁶ while a second one is based on the asymmetric hydrogenation of *Z*-2-amido-3-(4-acetoxy-2,6-dimethylphenyl)-2-propanoates (path b).⁷

Scheme 1. Approaches to (*S*)-2,6-dimethyltyrosine and its derivatives



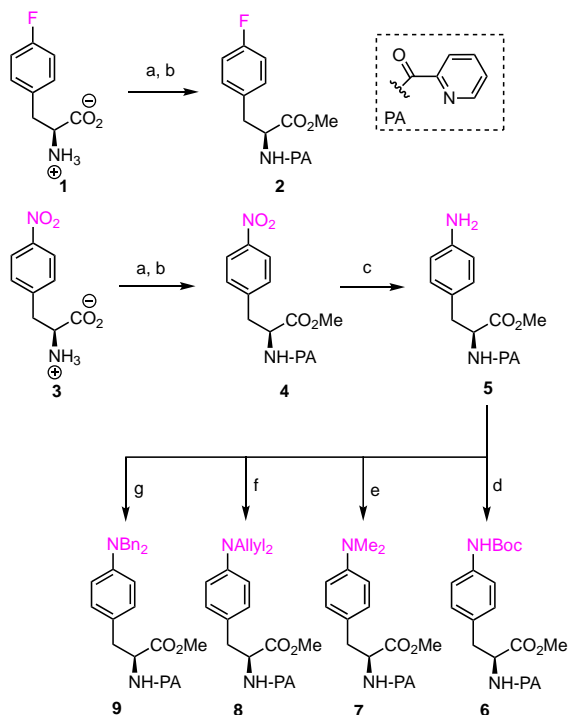
A third and more recent approach is based on the Pd-catalyzed dimethylation at positions 2 and 6 of the aromatic ring of tyrosine derivatives, as recently reported by Zhang and Ma (path c).⁸ This elegant non-racemizing C–H activation protocol plunges its roots in the pioneering work of Tremont on the Pd-promoted alkylation of acetanilides,⁹ which laid the foundation for the picolinamide-based strategy, to achieve the Pd-catalyzed γ - and δ -C–H activation of amines, as reported by Daugulis¹⁰ more than twenty years later.¹¹ This chemistry was further extended by Chen¹² in the alkylation of γ -*ortho*-C(sp²)-H bonds of benzylamides.¹³

Inspired by the above works, we envisioned to extend the study of the Pd-catalyzed δ -*ortho* C(sp²)-H activation strategy to differently 4-substituted (*L*)-phenylalanine picolinamides (path d), so as to obtain a small library of 2,6-dimethylated derivatives ready for incorporation in solid phase peptide synthesis (SPPS).

RESULTS AND DISCUSSION

We started our study by converting commercially available 4-fluorophenyl-*L*-alanine **1** into the corresponding methyl ester, which was subsequently submitted to a standard amidation protocol with picolinic acid, to afford the fluorinated picolinamide **2** (Scheme 2). Commercially available 4-nitro-*L*-phenylalanine **3** was analogously converted into the corresponding methyl ester picolinamide **4**. Treatment of **4** under hydrogen atmosphere in the presence of Pd/C (10%) in flow conditions, afforded the corresponding aniline derivative **5**. This latter was in turn converted into *NH*-Boc derivative **6**, *N,N*-dimethyl derivative **7**, *N,N*-diallyl derivative **8** and *N,N*-dibenzyl derivative **9**.

Scheme 2. Synthesis of the functionalized phenylalanine picolinamide methyl ester

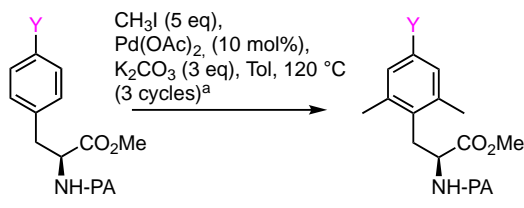


Conditions: a) SOCl₂, MeOH at 65 °C (99% from **1**, 99% from **3** without purification); b) picolinic acid, DIPEA, HATU, DCM at r.t. (91% from **1** Me ester, 91% from **3** Me ester); c) Pd/C (10%), AcOEt, 55 °C, H₂ 20 bar, flow conditions (97%); d) Boc₂O, NaOH 2N, H₂O/1,4-Dioxane at r.t. (75%); e) MeI, K₂CO₃, CH₃CN, 80 °C (87%) f) AllylBr, K₂CO₃, CH₃CN, 120 °C (80%) g) BnBr, K₂CO₃, CH₃CN, 120 °C (82%).

The study of the key dimethylation reaction was next tackled (Table 1). On the one hand, submitting the 4-fluoro-, 4-nitro, 4-*NH*-Boc, 4-*N*-Me₂ and 4-*N*-Allyl₂ derivatives **2**, **4**, **6**, **7**, **8** to the same reaction conditions as reported by Zhang and Ma⁸ [CH₃I, Pd(OAc)₂, (10 mol%), K₂CO₃, Toluene, 120 °C] gave no expected methylated product. On the other hand, and to our satisfaction, treatment of

the *N,N*-dibenzyl derivative **9** with the same reaction conditions as reported above gave the expected 2,6-dimethylated product **10** in 72 % yield, even if this step requires to be repeated three times to obtain the only dimethylated product (from 18.30 mmol of **9**).

Table 1. Tests of the Pd-catalyzed dimethylation

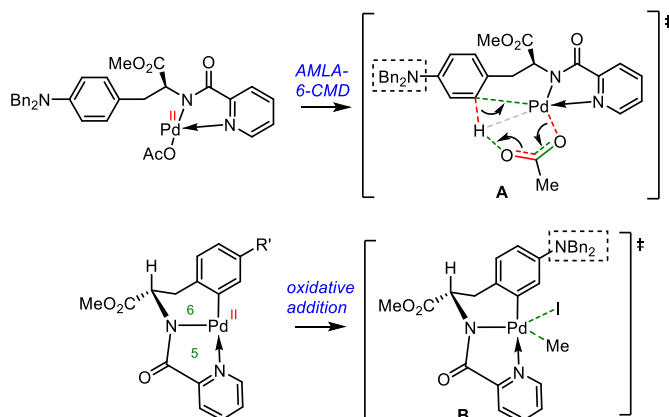


Y	Substrate	Product	Isolated yield (%)
F	2	-	-
NO ₂	4	-	-
NHBoc	6	-	-
NMe ₂	7	-	-
NAllyl ₂	8	-	-
NBn ₂	9	10	72%

a) All substrates (**2**, **4**, **6**, **7**, **8** and **9**) were treated with the same conditions, as reported.

These results reveal that the electronic situation of the aromatic ring of the picolinamide substrates is key for the success of the reaction. In particular, it is likely that the C–H activation¹⁴ step at the δ -*ortho* C(sp²)–H position (Figure 1, A), and/or the subsequent MeI oxidative addition step in the catalytic cycle are viable only when the aromatic ring is rich enough to enrich the palladium atom in turn (Figure 1, B).

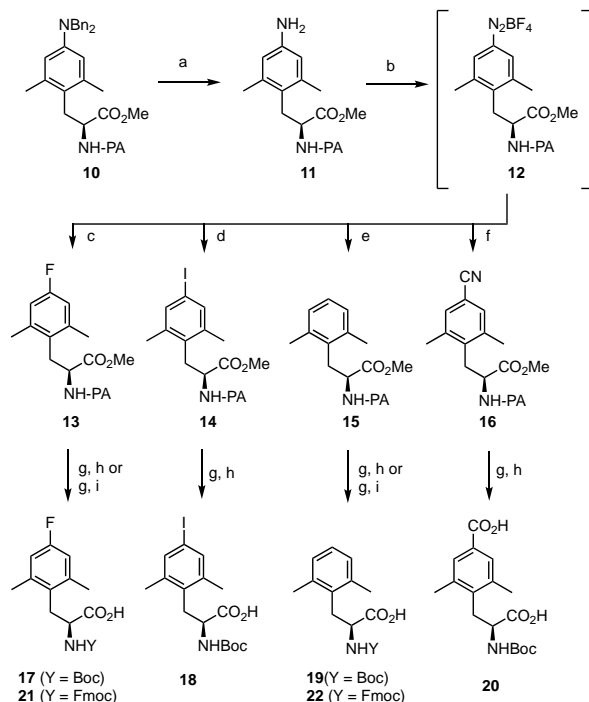
Figure 1. Postulated transition states for the δ -*ortho* C(sp²)–H palladation step (A) and the MeI oxidative addition step (B) in the dimethylation of **9 to **10**^{a,b}**



a) Green dashed bonds refer to forming bonds, red dashed bonds refer to breaking bonds, and the grey dashed one refers to an agostic interaction. b) A full catalytic cycle is proposed in the SI.

With the key 2,6-dimethylated aniline **10** in hand, we turned our attention to its conversion into a number of non-natural (*L*)-phenylalanine derivatives exploiting the rich chemistry of anilines (Scheme 3). Accordingly, debenylation of **10** gave the primary aniline **11**. This latter could be further transformed into the fluoride **13**,¹⁵ the iodide **14**, the protodeaminated product **15**,¹⁶ as well as the nitrile **16**¹⁷ passing through the common diazonium tetrafluoroborate intermediate **12**. Treatment of the four intermediates **13-16** with 6N HCl solution allowed ester and amide hydrolysis, and the resulting amino acids hydrochlorides were subsequently protected as *N*-tert-butyloxycarbonyl (*NH*-Boc) synthons, to afford the building blocks **17-20**. As expected, the acidic treatment of nitrile **16** brought also about hydrolysis of the nitrile function into the corresponding carboxylic acid. An alternative *N*-Fmoc protection was also carried out on the amino acids deriving from **13** and **15**, to afford the *N*-fluorenylmethyloxycarbonyl (*N*-Fmoc) protected modified amino acids **21** and **22**.

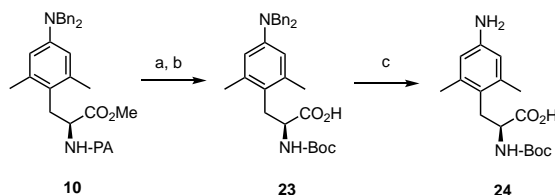
Scheme 3. Conversion of the key intermediate **10 into the selectively functionalized *NH*-Boc and *NH*-Fmoc 2,6-dimethyl-*L*-phenylalanine building blocks **17-22****



Conditions: a) Pd/C (10%), AcOEt, 80 °C, H₂ 45 bar, flow conditions (quantitative yield); b) HBF₄, isoamyl nitrite, THF, -10 °C (without purification and isolation); c) THF, 70 °C MW (70%); d) KI, CuI, acetone, 120 °C MW (35%); e) FeSO₄, DMF, r.t. (39%); f) TMSCN, Cu₂O, CH₃CN, 55 °C (without isolation); g) HCl 6N, at 110 °C (without purification); h) Boc₂O, NaOH 2N, H₂O/1,4-Dioxane at r.t. (**17**: 72%, **18**: 70%, **19**: 75%, **20**: 10%); i) FmocCl, NaOH 2N, H₂O/1,4-Dioxane at r.t. (**21**: 75%, **22**: 80%).

Furthermore, direct mild hydrolysis of the key intermediate **10** followed by standard Boc protection of the amine function afforded the *N,N*-dibenzyl 2,6-dimethylated *N*-Boc protected amino acid **23**, and subsequent catalytic hydrogenation gave the debenzylated aniline **24** (Scheme 4).

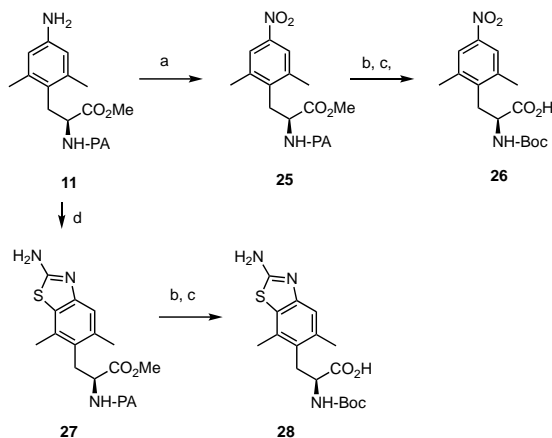
Scheme 4: Synthesis of the *N,N*-dibenzyl and free aniline *NH*-Boc derivatives



Conditions: a) HCl 3N at 60 °C for one week (without isolation); b) Boc₂O, NaOH 2N, H₂O/Dioxane at r.t. (70%); c) H₂, Pd/C (10%), AcOEt at r.t. (70%).

Finally, hydrogen peroxide oxidation¹⁸ of aniline **11** led to the corresponding nitro derivative **25**, which reversed the electron demand of the aromatic nucleus. Furthermore, anellation of **11** by treatment with potassium thiocyanate and bromine in acetic acid gave the corresponding aminobenzothiazole **27**, whose structure reminds the important Aba-Gly opioid scaffold shape.¹⁹ Once again, a standard two-step protocol allowed to convert the two picolinamides **25** and **27** into the corresponding *N*-Boc derivatives **26**, and **28** (Scheme 5).

Scheme 5. Conversion of intermediate 11 into selectively functionalized *NH*-Boc 2,6-dimethyl-*L*-phenylalanine building blocks 26 and 28

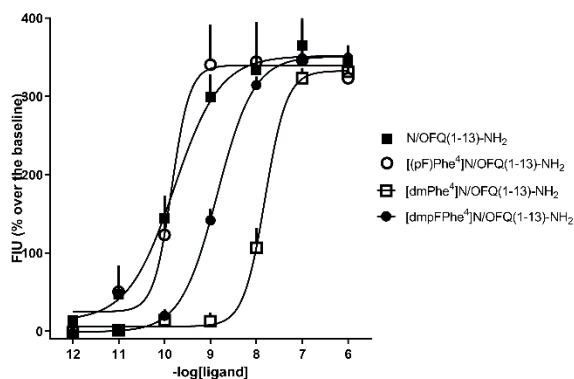


Conditions: a) EDTA, K₂CO₃, H₂O₂ (30%) CH₃CN at r.t. (56%); b) HCl 6N, at 110 °C; c) Boc₂O, NaOH 2N, H₂O/1,4-Dioxane at r.t. (**26**: 67%, **28**: 70%); d) KSCN, Br₂, AcOH at r.t. (30%).

PRELIMINARY BIOLOGICAL EVALUATIONS

We started the biological part of this project by SPPS mediated insertion of the newly synthesized amino acids **21** and **22** in N/OFQ(1-13)-NH₂, the shorter active fragment of Nocicpetin.²⁰ The resulting suitably methylated peptides [(pF)Dmp4](N/OFQ(1-13)-NH₂) and [Dmp4](N/OFQ(1-13)-NH₂) were then compared with the corresponding non-dimethylated ones, and we disclose here below our preliminary results.²² Specifically, these compounds were evaluated in an intracellular Ca²⁺ mobilization assay on CHO cells, transfected to express the recombinant NOP receptor, coupled to the chimeric protein Gαq15.²³ The results obtained are represented by the dose-response curves in Figure 2. All the examined peptides showed full-agonist behavior. In particular, it has to be noticed the beneficial effect of [p-EWG]4, and the loss of potency due to the presence of [Dmp]4.²⁶ Noteworthy, [(p-F)Dmp]4 showed a balanced potency profile due to the presence of both p-EWG group and the 2,6-dimethyl groups.

Figure 2. Calcium mobilization assay in CHO_{NOP+} Gαq15 cells.



Concentration-response curves to N/OFQ(1-13)-NH₂ and N/OFQ(1-13)-NH₂ analogues. N/OFQ(1-13)-NH₂ behaved as a NOP agonists with high potency (pEC₅₀ = 9.77 (9.36 – 10.18)) and efficacy (E_{max} = 352±15).²⁴

CONCLUSION

In summary, starting from 4-nitro-*L*-phenylalanine,²⁵ we have achieved the late stage synthesis of a small library of Dmt-like *NH*-Boc or *NH*-Fmoc protected (*L*)-phenylalanines whose aromatic ring is methylated at positions 2 and 6 and carry diverse functionalities at position 4. The appended groups can be strong as well as mild electron-withdrawing or releasing ones, bulky (such as *p,N,N*-dibenzyl), or having extended aromaticity (such as the aminothiazole fused ring). This goal, which has evident medicinal chemistry implications, has been achieved by merging the powerful picolinamide enabled Pd-catalyzed C–H 2,6-dimethylation of 4-dibenzylamino phenylalanine with the rich chemistry of anilines. As above mentioned, we started the biological phase of this project by inserting – via SPPS – two of the newly synthesized amino acids in the message domain of an active peptide fragment for biological assessment of their opioid-like properties.²⁶ More in-depth results will be published elsewhere.

EXPERIMENTAL SECTION

Chemistry

General Information

Reagents:

All commercial materials were purchased from Fluorochem and Sigma-Aldrich, and used as received unless otherwise noted. Pd(OAc)₂ (>98%, Fluorochem) was used in the Pd-catalyzed reactions. Reagents as 4-fluorophenyl-*L*-alanine **1** (CAS n. 64231-54-5) and 4-nitro-*L*-phenylalanine **3** (CAS n. 949-99-5) were purchased from Fluorochem (Hadfield, Derbyshire, UK).

Instruments:

Analytical RP-HPLC analyses were performed on a XBridge[®] C18 column (4.6 x 150 mm, 5 μm particle size) with a flow rate of 0.5 mL/min using a linear gradient of acetonitrile (0.1% TFA) in water (0.1% TFA) from 0% to 100% in 25 minutes. Retention times (Tr) from analytical RP-HPLC are reported in minutes. When necessary, compounds were purified on a reverse-phase Waters Prep 600 HPLC system equipped with a Jupiter column C18 (250 x 30 mm, 300 Å, 15 μm spherical particle size). Gradients used consisted of A (H₂O + 0.1% TFA) and B (40% H₂O in CH₃CN + 0.1% TFA) at a flow rate of 20 mL/min. UV detection wavelength for semi-preparative HPLC was 220 nm. All final products showed a degree of purity >95% at 220 and 254 nm. The mass spectra were recorded with a MICROMASS ZMD 2000. TLC were performed on pre-coated plates of silica gel F254 (Merck, Darmstadt, Germany). ¹H NMR and ¹³C, DEPT NMR analysis were obtained at ambient temperature using a Varian 400 MHz spectrometer and were referenced to residual ¹H signals of the deuterated solvents respectively (δ ¹H 7.26 for CDCl₃, δ ¹H 2.50 for DMSO-*d*₆, δ ¹H 3.31, 4.87 for CD₃OD); the following abbreviations were used to describe the shape of the peaks: s: singlet; d: doublet; dd: double doublet; t: triplet; m: multiplet. Optical rotations were obtained on a Jasco P-2000 Polarimeter instrument with a path length of 1 dm (589 nm), and reported as follows: [α]_T^D (c = g/100 mL, solvent). The infrared analyses were performed with a spectroscopy FT-IR spectrum 100 (Perkin Elmer Inc., Waltham, Massachusetts, USA). Hydrogenation reaction in AcOEt was performed under continuous-flow conditions in an H-Cube Pro[™] setup (Thalesnano, Hungary) equipped with a module for automatic control of operational parameters (reaction temperature in °C and pressure in bar, flow rates of liquid feed in mL/min and hydrogen). All microwave reactions were carried out using a Biotage Initiator + TM 2.0 apparatus (Biotage Sweden). The system can process reaction volumes between 0.2 and 20 mL at temperatures between 40 °C and 300 °C. The sealed vial is inserted into the microwave cavity and closed with the cavity lid, then high frequency microwaves (2.45 GHz), generated by magnetron, heat the reaction mixture. The reactor has an automated power control so that a constant reaction temperature can be automatically maintained throughout the reaction (indeed, the system has an external probe that measures the average temperature of reaction, given by the constant stirring of the mixture). After processing, the reaction mixture is immediately cooled with pressurized air.

(*S*)-methyl-3-(4-fluorophenyl)-2-(picolinamido)propanoate (**2**): To a solution of 4-fluoro-*L*-phenylalanine (2.28 mmol, 0.500 g) in anhydrous MeOH (20 mL) was added SOCl₂ (2.50 mmol, 0.182 mL) in a dropwise manner. The reaction mixture was heated using an oil bath at reflux overnight. The volatile substances were removed under vacuum to give the crude methyl-4-fluoro-phenylalanine hydrochloride, which was washed with 10 mL of saturated sodium bicarbonate aqueous solution (to pH ~ 8) and extracted with DCM. The organic layers were combined and evaporated under vacuum to give the corresponding ester as white solid (0.525 g, 99% yield), which was used directly for the next step.

A mixture of the previous crude amino product, picolinic acid (2.70 mmol, 0.333 g), HATU (2.70 mmol, 1.02 g) and DIPEA (5.64 mmol, 0.98 mL) in DCM (40 mL) was stirred at room temperature overnight. Then, the reaction was quenched with a saturated NH₄Cl aqueous solution and the two layers were separated. The aqueous layer was extracted with DCM (3 times), and the organic layers were combined, dried over Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash chromatography (1:1 petroleum ether/AcOEt) to afford the compound **2** (0.618 g, 91% yield) as white oil. HRMS *m/z*: [M+H]⁺ calcd for C₁₆H₁₆N₂O₃F 303.1139; found 303.1137. ¹H NMR (400 MHz, CDCl₃) δ 8.56 (ddt, J = 4.8, 1.8, 0.9 Hz, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.15 (dt, J = 7.8, 1.1 Hz, 1H), 7.84 (td, J = 7.7, 1.7 Hz, 1H), 7.43 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H), 7.18 – 7.10 (m, 2H), 6.96 (dd, J = 9.2, 8.3 Hz, 2H), 5.05 (dt, J = 8.4, 6.1 Hz, 1H), 3.73 (d, J = 0.9 Hz, 3H), 3.25 (dd, J = 13.9, 7.2 Hz, 1H), 3.17 (dd, J = 13.9, 7.2 Hz, 1H). ¹³C{¹H} NMR (101 MHz, CDCl₃) δ 171.8, 164.1, 163.4, 160.9, 149.3, 148.4, 137.5, 131.9, 130.9, 126.6, 122.4, 115.7, 115.5, 53.6, 52.5, 37.6. ¹⁹F NMR (376 MHz, CDCl₃) δ -115.8. IR 3384, 1740, 1673, 1506, 1218 cm⁻¹. [α]_D²² = -37.22 (c=0.039, MeOH).

(*S*)-methyl-3-(4-nitrophenyl)-2-(picolinamido)propanoate (**4**): To a solution of 4-nitro-*L*-phenylalanine (23.81 mmol, 5.00 g) in anhydrous MeOH (100 mL) was added SOCl₂ (26.19 mmol, 1.91 mL) in a dropwise manner. The reaction mixture was heated using an oil bath at reflux overnight. The volatile substances were removed under vacuum to give the crude methyl-4-nitro-phenylalanine hydrochloride, which was washed with 30 mL of saturated sodium bicarbonate aqueous solution (to pH ~ 8) and extracted with DCM. The organic layers were combined and evaporated under vacuum to give the corresponding ester as yellowish solid (6.17 g, 99% yield), which was used directly for the next step.

A mixture of the previous crude amino product, picolinic acid (28.56 mmol, 3.51 g), HATU (28.56 mmol, 10.86 g) and DIPEA (59.52 mmol, 10.37 mL) in DCM (150 mL) was stirred at room temperature overnight. Then, the reaction was quenched with a saturated NH₄Cl aqueous solution and the two layers were separated. The aqueous layer was extracted with DCM, and the organic layers were combined, dried over Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash chromatography (1:1 petroleum ether/AcOEt) to afford the compound **4** (7.12 g, 91% yield) as yellowish oil. HRMS *m/z*: [M+H]⁺ Calcd for C₁₆H₁₆N₃O₅ 330.1084; found 330.1087. ¹H NMR (400 MHz, CDCl₃) δ 8.57 – 8.54 (m, 1H), 8.51 (d, J = 8.4 Hz, 1H), 8.22 – 8.03 (m, 3H), 7.85 (td, J = 7.7, 1.7 Hz, 1H), 7.49 – 7.41 (m, 1H), 7.35 (d, J = 8.7 Hz, 2H), 5.12 (dt, J = 8.4, 6.2 Hz, 1H), 3.76 (s, 3H), 3.41 (dd, J = 13.8, 5.8 Hz, 1H), 3.30 (dd, J = 13.8, 6.5 Hz, 1H). ¹³C{¹H} NMR (101 MHz, CDCl₃) δ 171.3, 164.1, 148.4, 144.1, 137.7, 130.4, 126.8, 123.9, 122.6, 53.2, 52.8, 38.2. IR 3373, 1739, 1671, 1512, 1343 cm⁻¹. [α]_D²³ = -23.14 (c=0.028, MeOH).

(*S*)-methyl-3-(4-aminophenyl)-2-(picolinamido)propanoate (**5**): The compound **4** was dissolved in AcOEt (150 mL, 0.15 M) and set up in continuous-flow hydrogenator reactor H-Cube Pro Thales-Nano at the temperature of 55 °C, pressure of 20 bar, flow 0.3

mL/min with Pd/C (10 mol%) as catalyst. When the reaction was completed, monitored via mass spectrometry, the solvent was concentrated in vacuum to obtain the crude product **5** (6.70 g, 97% yield) as red-orange oil. HRMS m/z : $[M+H]^+$ Calcd for $C_{16}H_{18}N_3O_3$ 300.1342; found 300.1341. 1H NMR (400 MHz, DMSO- d_6) δ 8.79 – 8.55 (m, 2H), 8.07 – 7.89 (m, 2H), 7.67 – 7.51 (m, 1H), 6.83 (d, J = 8.4 Hz, 2H), 6.45 (d, J = 8.4 Hz, 2H), 4.90 (s, 2H), 4.68 (ddd, J = 8.1, 7.1, 6.0 Hz, 1H), 3.65 (s, 3H), 3.05 – 2.92 (m, 2H). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO) δ 171.8, 163.6, 149.1, 148.7, 147.3, 138.0, 129.6, 127.0, 123.5, 122.0, 114.0, 53.8, 52.1, 35.9. IR 3350, 1734, 1668, 1516, 831 cm^{-1} . $[\alpha]_D^{22} = +3.98$ ($c=0.034$, MeOH).

(S)-methyl-3-(4-((*tert*-butoxycarbonyl)amino)phenyl)-2-(picolinamido)propanoate (**6**): The compound **5** (1.13 mmol, 0.340 g) was solubilized in water/dioxane (1:2) solution (6 mL). The mixture was basified with NaOH 2N aqueous solution until pH 10/11 at 0 °C. Boc_2O (1.25 mmol, 0.273 g) was added and the reaction was left stirring at r.t. for 12 hours. The completion of the reaction was monitored per ESI mass spectrometry and TLC. The dioxane was removed under vacuum and HCl 1N aqueous solution was added at 0 °C to pH 1. The mixture was extracted with ethyl acetate (3 times) and the organic phases combined were dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by flash chromatography (1:1 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et_2O /petroleum ether to obtain the compound **6** as a white solid (0.340 g, 75% yield). HRMS m/z : $[M+H]^+$ Calcd for $C_{21}H_{26}N_3O_5$ 400.1867; found 400.1866. 1H NMR (400 MHz, $CDCl_3$) δ 8.55 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.15 (dt, J = 7.8, 1.1 Hz, 1H), 7.83 (td, J = 7.7, 1.7 Hz, 1H), 7.43 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H), 7.27 (d, J = 8.3 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 6.47 (s, 1H), 5.03 (dt, J = 8.4, 6.0 Hz, 1H), 3.72 (s, 3H), 3.24 – 3.13 (m, 2H), 1.50 (s, 9H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.9, 164.0, 152.9, 149.4, 148.4, 137.5, 130.7, 130.0, 126.5, 122.4, 118.7, 80.6, 53.7, 52.5, 37.7, 28.5, 27.6. IR 3315, 1721, 1667, 1515, 1155 cm^{-1} . MP 65-67 °C. $[\alpha]_D^{23} = +5.255$ ($c=0.0355$, MeOH).

(S)-methyl-3-(4-(dimethylamino)phenyl)-2-(picolinamido)-propanoate (**7**): To a solution of the aniline **5** (1.67 mmol, 0.500 g) in CH_3CN (20 mL) was added methyl iodide (4.17 mmol, 0.26 mL) and potassium carbonate (3.34 mmol, 0.46 g). The mixture was stirred and heated using an oil bath at 80 °C overnight. Then, the solvent was removed under vacuum and the residue was dissolved in AcOEt and washed with water. Once separated the layers, the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (2:3 AcOEt/petroleum ether) to afford **7** as a yellowish oil (0.47 g, 87% yield).

HRMS m/z : $[M+Na]^+$ Calcd for $C_{18}H_{21}N_3O_3Na$ 350.1475; found 350.1473.

1H NMR (400 MHz, $CDCl_3$) δ 8.56 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H), 8.53 – 8.39 (m, 1H), 8.16 (dt, J = 7.8, 1.1 Hz, 1H), 7.83 (td, J = 7.7, 1.7 Hz, 1H), 7.42 (ddd, J = 7.6, 4.7, 1.3 Hz, 1H), 7.14 – 6.96 (m, 2H), 6.69 (d, J = 8.2 Hz, 2H), 5.00 (dt, J = 8.3, 6.0 Hz, 1H), 3.73 (s, 3H), 3.15 (d, J = 6.0 Hz, 2H), 2.92 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.17, 164.12, 149.57, 148.44, 137.35, 130.11, 126.42, 122.38, 113.09, 53.85, 52.40, 40.96, 37.48. IR 3381, 1736, 1673, 1509, 1344 cm^{-1} . $[\alpha]_D^{22} = +0.978$ ($c=0.015$, MeOH).

(S)-methyl-3-(4-(diallylamino)phenyl)-2-(picolinamido)-propanoate (**8**): To a solution of the aniline **5** (1.67 mmol, 0.500 g) in CH_3CN (20 mL) was added allyl bromide (4.17 mmol, 0.36 mL) and potassium carbonate (3.34 mmol, 0.46 g). The mixture was stirred and heated using an oil bath at 120 °C overnight. Then, the solvent was removed under vacuum and the residue was dissolved in AcOEt and washed with water. Once separated the layers, the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (2:3 AcOEt/petroleum ether) to afford **8** as a yellowish oil (0.50 g, 80% yield).

HRMS m/z : $[M+Na]^+$ Calcd for $C_{22}H_{25}N_3O_3Na$ 402.1788; found 402.1774.

1H NMR (400 MHz, $CDCl_3$) δ 8.55 (ddt, J = 4.8, 1.9, 1.0 Hz, 1H), 8.46 (d, J = 8.2 Hz, 1H), 8.15 (dq, J = 7.8, 1.3 Hz, 1H), 7.90 – 7.75 (m, 1H), 7.46 – 7.38 (m, 1H), 7.01 (d, J = 8.1 Hz, 2H), 6.62 (s, 2H), 5.82 (d, J = 14.2 Hz, 2H), 5.21 – 5.09 (m, 4H), 4.98 (dt, J = 8.6, 6.0 Hz, 1H), 3.88 (d, J = 5.0 Hz, 4H), 3.72 (d, J = 2.0 Hz, 3H), 3.13 (d, J = 5.9 Hz, 2H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.15, 164.13, 149.57, 148.43, 148.28, 137.34, 134.19, 130.13, 126.42, 123.40, 122.37, 116.25, 112.68, 53.81, 52.89, 52.40, 37.47. IR 3387, 2979, 1741, 1674, 1509 cm^{-1} .

$[\alpha]_D^{22} = +1.52$ ($c=0.014$, MeOH)

(S)-methyl-3-(4-(dibenzylamino)phenyl)-2-(picolinamido)propanoate (**9**): To a solution of the aniline **5** (22.40 mmol, 7.32 g) in CH_3CN (150 mL) was added benzyl bromide (56.02 mmol, 6.66 mL) and potassium carbonate (44.81 mmol, 6.19 g). The mixture was stirred and heated using an oil bath at 120 °C overnight. The crude mixture was purified by flash chromatography (2:3 AcOEt/petroleum ether) to afford **9** as an orange yellowish solid (8.76 g, 82% yield). HRMS m/z : $[M+H]^+$ calcd for $C_{30}H_{30}N_3O_3$ 480.2281; found 480.2288. 1H NMR (400 MHz, $CDCl_3$) δ 8.54 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H), 8.46 (d, J = 8.3 Hz, 1H), 8.15 (dt, J = 7.8, 1.1 Hz, 1H), 7.83 (td, J = 7.7, 1.7 Hz, 1H), 7.41 (ddd, J = 7.6, 4.8, 1.3 Hz, 1H), 7.35 – 7.28 (m, 4H), 7.27 – 7.20 (m, 6H), 6.98 (d, J = 8.7 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 4.97 (dt, J = 8.3, 6.0 Hz, 1H), 4.61 (s, 4H), 3.70 (s, 3H), 3.17 – 3.05 (m, 2H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.9, 164.1, 149.5, 148.4, 137.4, 130.3, 130.1, 128.9, 128.8, 128.3, 127.9, 127.6, 127.1, 126.5, 122.4, 56.1, 53.7, 52.4, 37.5. IR 3374, 1725, 1665, 1516, 837, 737 cm^{-1} . MP 104-106 °C. $[\alpha]_D^{23} = -4.095$ ($c=0.0155$, MeOH).

(S)-methyl-3-(4-(dibenzylamino)-2,6-dimethylphenyl)-2-(picolinamido)propanoate (**10**): To a solution of compound **9** (18.30 mmol, 9.28 g) in toluene (150 mL) were added K_2CO_3 (54.86 mmol, 7.5 g), CH_3I (91.53 mmol, 5.69 mL), and $Pd(OAc)_2$ (1.83 mmol, 0.41 g). The mixture was stirred and heated using an oil bath at 120 °C overnight. After 24 hours the reaction was cooled to r.t. and filtered through celite pad, washed with AcOEt (50 mL). The filtrate was concentrated under vacuum to obtain the crude product, which was then use for other two catalytic reaction at same conditions. The final crude product was purified by flash chromatography (3:7 AcOEt/petroleum ether), to afford **10** (6.76 g, 72% yield) as a yellowish solid. HRMS m/z : $[M+H]^+$ calcd for $C_{32}H_{34}N_3O_3$ 508.2594; found 508.2597. 1H NMR (400 MHz, $CDCl_3$) δ 8.56 (ddd, J = 4.7, 1.7, 0.9 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.83 (td, J = 7.7, 1.7 Hz, 1H), 7.42 (ddd, J = 7.6, 4.8, 1.3 Hz, 1H), 7.33 – 7.26 (m, 4H), 7.26 – 7.20 (m, 6H), 6.44 (s, 2H), 4.89 (m, J = 7.9 Hz,

1H), 4.57 (s, 4H), 3.66 (s, 3H), 3.19 – 3.02 (m, 2H), 2.29 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 173.0, 164.1, 149.5, 148.4, 148.0, 138.9, 138.1, 137.4, 128.9, 128.7, 128.2, 126.9, 126.4, 122.3, 112.6, 53.8, 52.7, 52.4, 32.4, 20.8. IR 3384, 1738, 1676, 1602, 1494, 731, 700 cm^{-1} . MP 77-79 °C. $[\alpha]_D^{22} = -8.82$ (c=0.0085, MeOH).

(*S*)-methyl-3-(4-amino-2,6-dimethylphenyl)-2-(picolinamido)propanoate (**11**): The compound **10** was dissolved in AcOEt (186 mL, 0.05 M) and set up in continuous-flow hydrogenator reactor H-Cube Pro Thales-Nano at the temperature of 80 °C, pressure of 45 bar, flow 1 mL/min with the Pd/C (10 mol%) as catalyst. When the reaction was completed, monitored via mass spectrometry, the solvent was concentrated in vacuum to afford the crude product, which was purified by flash chromatography (1:1 AcOEt/petroleum ether) to obtain **11** (3.05 g, quantitative yield) as a yellowish oil. HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_3$ 328.1655; found 328.1657. ^1H NMR (400 MHz, DMSO) δ 8.78 (d, $J = 8.2$ Hz, 1H), 8.67 (dt, $J = 4.9, 1.2$ Hz, 1H), 8.05 – 7.93 (m, 2H), 7.62 (tdd, $J = 4.8, 2.5, 0.8$ Hz, 1H), 6.19 (s, 2H), 4.72 (s, 2H), 4.61 (td, $J = 8.4, 6.6$ Hz, 1H), 3.61 (s, 3H), 3.19 – 2.03 (m, 2H), 2.14 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) δ 173.7, 165.6, 149.9, 149.3, 145.7, 139.4, 138.7, 128.5, 124.1, 123.3, 116.5, 53.6, 53.6, 31.5, 20.8. IR 3360, 2952, 1734, 1670, 1511, 749 cm^{-1} . $[\alpha]_D^{23} = -17.97$ (c=0.011, MeOH).

(*S*)-4-(3-methoxy-3-oxo-2-(picolinamido)propyl)-3,5-dimethylbenzenediazonium tetrafluoroborate (**12**): To a solution of compound **11** (2.41 mmol, 0.79 g) dissolved in anhydrous THF (15 mL), cooled to -10 °C, isoamyl nitrite (4.83 mmol, 0.643 mL) and HBF_4 (9.66 mmol, 1.31 mL) were added. The reaction is stirred for 4 hours at -10 °C and a yellow precipitate is formed, which was directly used as wet crude for the next step. An IR analysis and a diazocoupling assay were performed on the compound **12** with positive results. IR peaks referable to diazonium salt: 2274 cm^{-1} .

(*S*)-methyl-3-(4-fluoro-2,6-dimethylphenyl)-2-(picolinamido)propanoate (**13**): Compound **12** (2.41 mmol, 1.02 g) was used directly as crude dissolved in anhydrous THF (15 mL) in a sealed reaction vessel of 10-20 mL. The mixture was heated to 70 °C for 5 minutes under microwave irradiations. The resulting brown solution was evaporated under vacuum and the crude was solubilized in AcOEt, washed with H_2O and a saturated aqueous solution of NaHCO_3 . The solvent of the organic phase was then removed under vacuum giving a crude product which was purified by flash chromatography (2:3 AcOEt/petroleum ether). The purified product **13** was obtained as a white solid (0.556 g, 70% yield). HRMS m/z : $[\text{M}+\text{H}]^+$ calcd $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_3\text{F}$ 331.1452; found 331.1455. ^1H NMR (400 MHz, CDCl_3) δ 8.62 – 8.51 (m, 2H), 8.10 (dt, $J = 7.8, 1.1$ Hz, 1H), 7.83 (td, $J = 7.7, 1.7$ Hz, 1H), 7.43 (ddd, $J = 7.6, 4.8, 1.3$ Hz, 1H), 6.70 (d, $J = 9.4$ Hz, 2H), 5.04 – 4.92 (m, 1H), 3.68 (s, 3H), 3.25 – 3.15 (m, 2H), 2.39 (d, $J = 0.6$ Hz, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 172.6, 164.0, 161.3 (d, $J = 244.2$ Hz), 149.3, 148.4, 139.4 (d, $J = 9$ Hz), 137.5, 129.0, 126.5, 122.4, 114.9 (d, $J = 21$ Hz), 52.5, 52.0, 32.6, 20.5. ^{19}F NMR (376 MHz, CDCl_3) δ -117.20 (t, $J = 9.4$ Hz). IR 3365, 2956, 1731, 1658, 1514, 1018 cm^{-1} . MP 117-119 °C. $[\alpha]_D^{23} = -17.22$ (c=0.01, MeOH).

(*S*)-methyl-3-(4-iodo-2,6-dimethylphenyl)-2-(picolinamido)propanoate (**14**): To a solution of crude compound **12** (0.704 mmol, 0.300 g) in anhydrous acetone (10 mL), CuI (0.070 mmol, 13.4 mg) and KI (1.76 mmol, 0.292 g) were added, in a sealed reaction vessel of 10-20 mL. The mixture was heated to 120 °C for 30 min under microwave irradiation. The resulting brown mixture was filtered through a celite pad and washed with acetone. The solvent was removed under vacuum until a dark solid was obtained, which was purified by flash chromatography (2:3 AcOEt/petroleum ether) to obtain compound **14** as a white oil (0.11 g, 35% yield). HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_3\text{I}$ 439.0513; found 439.0514. ^1H NMR (400 MHz, CDCl_3) δ 8.70 – 8.51 (m, 2H), 8.11 (d, $J = 7.9$ Hz, 1H), 7.91 – 7.78 (m, 1H), 7.50 – 7.43 (m, 1H), 7.35 (s, 2H), 4.98 (q, $J = 8.1$ Hz, 1H), 3.69 (s, 3H), 3.23 – 3.09 (m, 2H), 2.35 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 172.4, 164.3, 149.0, 148.4, 139.5, 137.7, 137.1, 133.1, 126.8, 122.6, 92.7, 52.7, 51.9, 32.9, 19.9. IR 3380, 2952, 1739, 1674, 1511, 1170 cm^{-1} . $[\alpha]_D^{23} = -18.67$ (c=0.0165, MeOH).

(*S*)-methyl-3-(2,6-dimethylphenyl)-2-(picolinamido)propanoate (**15**): To a solution of Fe_2SO_4 (0.884 mmol, 0.246 g) in 6 mL of DMF was added dropwise to the compound **12** (0.884 mmol, 0.3 g) solubilized in DMF (1.51 mL). The reaction was stirred at r.t. overnight. The solvent was removed under vacuum and the residue was dissolved in DCM. The organic layer was washed with water, dried over Na_2SO_4 , filtered and concentrated in vacuum. The crude orange oil was purified by flash chromatography (2:3 AcOEt/petroleum ether) obtaining the compound **15** as a yellow solid (0.107 g, 39% yield). HRMS m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{21}\text{N}_2\text{O}_3$ 313.1546; found 313.1544. ^1H NMR (400 MHz, CDCl_3) δ 8.58 (ddd, $J = 4.8, 1.8, 1.0$ Hz, 2H), 8.11 (dt, $J = 7.8, 1.1$ Hz, 1H), 7.82 (td, $J = 7.7, 1.7$ Hz, 1H), 7.43 (ddd, $J = 7.6, 4.8, 1.3$ Hz, 1H), 7.06 – 6.94 (m, 3H), 5.00 (q, $J = 8.1$ Hz, 1H), 3.67 (s, 3H), 3.32 – 3.21 (m, 2H), 2.41 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 172.8, 164.0, 149.4, 148.4, 137.4, 137.2, 133.3, 128.5, 126.9, 126.5, 122.4, 52.5, 52.1, 33.1, 20.3. IR 3389, 1736, 1673, 1508, 746 cm^{-1} . MP 94-96 °C. $[\alpha]_D^{23} = -18.19$ (c=0.0085, MeOH).

(*S*)-4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)-3,5-dimethylbenzoic acid (**20**): To a solution of compound **12** (1.76 mmol, 0.750 g) in acetonitrile (100 mL), were added trimethyl-silyl-cyanide (1.76 mmol, 0.220 mL) and Cu_2O (0.704 mmol, 0.100 g). The reaction was heated using an oil bath at 55 °C for 12 h leading to a crude orange solution. The solution was cooled to r.t., filtered through a celite pad and washed with DCM. The filtrate was concentrated under vacuum to reach an orange oil (**16**). The crude product was directly hydrolysed with HCl 6N aqueous solution at 110 °C to give the corresponding carboxylic acid. The crude was directly solubilized in water/dioxane (1:2) solution (30 mL), and basified with NaOH 2N aqueous solution until pH 10/11 at 0 °C. Boc_2O (2.11 mmol, 0.460 g) was added and the reaction was left stirring at r.t. for 12 hours. The completion of the reaction was monitored by ESI mass spectrometry and TLC. The dioxane was removed under vacuum and HCl 1N aqueous solution was added to the solution at 0 °C to pH 1. The mixture was extracted with ethyl acetate (3 times) and the organic phases were dried over Na_2SO_4 and concentrated under vacuum. The crude was purified per flash chromatography (1:1 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et_2O /petroleum ether to obtain compound **20**, as white solid (0.059 g, yield 10%). T_r : 15.92 min. HRMS m/z : $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{17}\text{H}_{22}\text{NO}_6$ 336.1453; found 336.1456. ^1H NMR (400 MHz, CD_3OD) (rotameric mixture) δ 7.65 (s, 2H), 4.40 (dd, $J = 9.5, 5.6$ Hz, 1H), 3.30 – 3.02 (m, 2H), 2.43 (s, 6H), 1.34 (s, 7H), 1.18 (s, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CD_3OD) (rotamers mixture) δ 175.4, 170.2, 157.6, 141.4, 138.8, 130.4, 129.6, 80.5, 54.4, 33.6, 28.6, 28.1, 20.5. IR 2924, 1687, 1163. MP 80-82 °C. $[\alpha]_D^{22} = -33.1$ (c=0.0145, MeOH).

(*S*)-methyl-3-(2,6-dimethyl-4-nitrophenyl)-2-(picolinamido)propanoate (**25**): The amino compound **11** (4.78 mmol, 1.56 g) was solubilized in CH₃CN (3.5 mL). An aqueous buffer solution of K₂CO₃ (0.6 M, 1.05 mmol, 0.14 g) and EDTA disodium salt (4x10⁻⁴ M; 0.7x10⁻³ mmol, 2.6 x 10⁻⁴ g) in 1.75 mL of H₂O was prepared and added to the mixture. Subsequently, 1.35 mL of H₂O₂ (30%) were added. The reaction was stirred at r.t. overnight, the formation of a yellow precipitate was observed. CH₃CN was removed under vacuum and the residue was dissolved in AcOEt. The organic layer was washed with water (10 mL x 3), dried over Na₂SO₄, filtered and concentrated in vacuum. The crude was purified by flash chromatography (1:1 AcOEt/petroleum ether) to obtain **25** (0.955 g, 56% yield), as a yellowish solid. HRMS *m/z*: [M+H]⁺ calcd for C₁₈H₂₀N₃O₅ 358.1397; found 358.1393. ¹H NMR (400 MHz, CDCl₃) δ 8.63 (d, *J* = 8.8 Hz, 1H), 8.61 – 8.54 (m, 1H), 8.07 (dt, *J* = 7.8, 1.1 Hz, 1H), 7.88 – 7.78 (m, 3H), 7.45 (ddd, *J* = 7.6, 4.7, 1.3 Hz, 1H), 5.07 (q, *J* = 8.2 Hz, 1H), 3.71 (s, 3H), 3.36 – 3.25 (m, 2H), 2.51 (s, 6H). ¹³C{¹H} NMR (101 MHz, CDCl₃) δ 172.0, 164.0, 149.0, 148.4, 146.4, 141.3, 139.1, 137.6, 126.7, 123.0, 122.5, 52.8, 51.4, 33.5, 20.6. IR 3389, 1737, 1637, 1508, 1342, 745 cm⁻¹. MP 132–134 °C. [α]_D²³ = -50.94 (c=0.0195, MeOH).

(*S*)-methyl-3-(2-amino-5,7-dimethylbenzo[d]thiazol-6-yl)-2-(picolinamido)propanoate (**27**): To a solution of compound **11** (1.07 mmol, 0.353 g) in AcOH (10 mL), KSCN (4.31 mmol, 0.419 g) and Br₂ (1.07 mmol, 55.6 μl) were added. The reaction was stirred for 48 h at room temperature. The solvent was removed under vacuum leading to an orange compound that was extracted with NaHCO₃ sat. and AcOEt (3 times). The organic phases were collected and dried over Na₂SO₄, filtered and concentrated under vacuum. The crude was purified by preparative HPLC obtaining the compound **27** (0.123 g, 30% yield), as a white solid. HRMS *m/z*: [M+H]⁺ calcd for C₁₉H₂₁N₄O₃S 385.1328; found 385.1329. ¹H NMR (400 MHz, DMSO) δ 8.94 (d, *J* = 8.3 Hz, 1H), 8.66 (dt, *J* = 4.8, 1.4 Hz, 1H), 8.06 – 7.89 (m, 2H), 7.62 (ddd, *J* = 6.8, 4.8, 2.0 Hz, 1H), 6.97 (s, 1H), 4.73 (td, *J* = 8.4, 5.8 Hz, 1H), 4.39 (s, 2H), 3.65 (s, 3H), 3.32 – 3.11 (m, 2H), 2.31 (s, 3H), 2.27 (s, 3H). ¹³C{¹H} NMR (101 MHz, DMSO) δ 171.6, 166.7, 163.7, 163.6, 149.1, 148.6, 137.9, 129.7, 129.2 (2CQ), 126.9, 126.6, 122.9, 122.0, 53.1, 52.1, 33.5, 17.9, 17.4. IR 2984, 1742, 1660, 1193, 1135 cm⁻¹. MP 83–85 °C. [α]_D²² = -2.67 (c=0.001, MeOH).

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(dibenzylamino)-2,6-dimethylphenyl)propanoic acid (**23**): Compound **10** (1.97 mmol, 1.0 g) was hydrolyzed in a milder way than other acidic hydrolysis described in the hydrolysis general procedure (as reported below). It was solubilized in HCl 3N (11 mL) aqueous solution and maintained under reflux at 60 °C using an oil bath for 1 week. The solution was concentrated under vacuum to reduce the volume (about 3 mL) and the crude was used for the protection of the amine as Boc. The previous amine hydrochloride solution was diluted in water/dioxane (1:2) solution (21 mL). The solution was basified with NaOH 2N aqueous solution to reach pH 10 at 0 °C. Boc₂O (2.36 mmol, 0.516 g) was added and the reaction was stirred at r.t. for 12 hours. The completion of the reaction was monitored by ESI mass spectrometry and TLC, and the dioxane was removed under vacuum. HCl 1N aqueous solution was added at 0 °C to reach pH 1, then the mixture was extracted with ethyl acetate (3 times) and the organic phases combined were dried over Na₂SO₄ and concentrated under vacuum. The crude was purified per flash chromatography (1:1 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford **23**, as a pale-yellow solid (0.576 g, 70% yield). T_r: 22.67 min. HRMS *m/z*: [M-H]⁻ calcd for C₃₀H₃₅N₂O₄ 487.2602; found 487.2606. ¹H NMR (400 MHz, DMSO) (rotamers mixture) δ 7.36 – 7.27 (m, 4H), 7.26 – 7.17 (m, 6H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.35 (s, 2H), 4.58 (s, 4H), 3.97 (td, *J* = 8.4, 5.2 Hz, 1H), 2.96 – 2.69 (m, 2H), 2.12 (s, 6H), 1.31 (s, 7H), 1.13 (s, 2H). ¹³C{¹H} NMR (101 MHz, DMSO) δ 173.9, 155.3, 146.5, 139.3, 137.2, 128.4, 126.7, 122.9, 112.1, 77.9, 53.9, 53.6, 30.6, 28.2, 27.6, 20.5. ¹H NMR (300 MHz, DMSO at 120 °C) δ 7.34 – 7.13 (m, 7H), 6.46 (s, 2H), 4.55 (s, 4H), 4.19 – 4.03 (m, 1H), 3.05 – 2.75 (m, 2H), 2.18 (s, 6H), 1.31 (s, 9H). IR 2980, 1713, 1603, 1494, 1158 cm⁻¹. MP 82–84 °C. [α]_D²³ = -133.6 (c=0.0515, MeOH).

(*S*)-3-(4-amino-2,6-dimethylphenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (**24**): The benzyl removal reaction was performed by solubilizing compound **23** (0.246 mmol, 0.118 g) in AcOEt (10 mL) with Pd/C (10%), at r.t., under H₂ atmosphere. The reaction was left stirring until complete consumption of the starting material, controlled by ESI mass spectrometry. A yellow crude oil was obtained, then purified by flash chromatography (4:1 AcOEt/Petroleum ether), then crystallized with 2:1 diethyl ether/petroleum ether until the compound **24** was obtained, as a white powder (0.052 g, 70% yield). T_r: 12.60 min. HRMS *m/z*: [M-H]⁻ calcd for C₁₆H₂₃N₂O₄ 307.1663; found 307.1666. ¹H NMR (400 MHz, DMSO) (rotamers mixture) δ 7.03 (d, *J* = 8.4 Hz, 1H), 6.18 (s, 2H), 3.94 (q, *J* = 7.6 Hz, 1H), 2.99 – 2.62 (m, 2H), 2.12 (s, 6H), 1.33 (s, 7H), 1.20 (s, 2H). ¹³C{¹H} NMR (101 MHz, DMSO) (rotamers mixture) δ 174.0, 155.3, 146.3, 136.9, 122.1, 113.9, 77.9, 54.1, 30.6, 28.2, 27.7, 20.0. IR 3361, 2984, 1694, 1363, 1171 cm⁻¹. MP 160–162 °C. [α]_D²¹ = +43.2 (c=0.009, MeOH).

General procedure for acidic hydrolysis deprotection:

Once purified the compound was dissolved in HCl 6N aqueous solution (17 equiv.) and heated using an oil bath at 110 °C for 24 hours. The obtained hydrolyzed crude product was concentrated under vacuum to reduce the volume and the crude solution was used directly for the subsequent protection step.

General procedure for Boc protection (**17**, **18**, **19**, **26**, **28**):

The HCl salt previously synthesized was directly used as crude, and diluted in water/dioxane (1:2) solution (0.2 M). The mixture was basified with NaOH 2N aqueous solution until reachment of pH value of 10/11 at 0 °C. Boc₂O (1.2 equiv.) was added and the reaction was left stirring at r.t. for 12 hours. The completion of the reaction was monitored per ESI mass spectrometry and TLC. The dioxane was removed under vacuum and HCl 1N aqueous solution was added at 0 °C to pH 1. The mixture was extracted with ethyl acetate (3 times) and the organic phases combined were dried over Na₂SO₄ and concentrated under vacuum. Each crude was purified by flash chromatography as reported below.

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-fluoro-2,6-dimethylphenyl)propanoic acid (**17**): The crude was purified by flash chromatography (3:2 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **17** as a white solid, (0.376 g, 72% yield). T_r: 20.05 min. HRMS *m/z*: [M-H]⁻ calcd for C₁₆H₂₁FNO₄ 310.1460; found 310.1459. ¹H NMR

(400 MHz, DMSO) (rotamers mixture) δ 12.61 (s, 1H), 7.16 (d, $J = 8.7$ Hz, 1H), 6.80 (d, $J = 9.8$ Hz, 2H), 4.06 (td, $J = 8.8, 6.1$ Hz, 1H), 3.07 – 2.80 (m, 2H), 2.28 (s, 6H), 1.31 (d, $J = 4.3$ Hz, 8H), 1.15 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) δ 173.5, 160.1 (d, $J = 240$ Hz), 155.2, 139.4, 131.1, 114.0 (d, $J = 20$ Hz), 78.0, 53.3, 30.7, 28.1, 27.6, 19.9. ^{19}F NMR (376 MHz, DMSO) δ -118.24 (t, $J = 9.7$ Hz). ^1H NMR (300 MHz, DMSO at 120 °C) δ 6.76 (d, $J = 9.8$ Hz, 2H), 4.17 (td, $J = 8.6, 6.4$ Hz, 1H), 3.18 – 2.85 (m, 2H), 2.32 (s, 6H), 1.32 (s, 9H). IR 2974, 1721, 1651, 1164, 1021 cm^{-1} . MP 133-135 °C. $[\alpha]_D^{23} = -30.4$ (c=0.025, MeOH).

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-iodo-2,6-dimethylphenyl)propanoic acid (**18**): The crude was purified by flash chromatography (3:2 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **18** as a white solid, (0.073 g, 70% yield). Tr: 22.60 min. HRMS m/z : $[\text{M-H}]^-$ calcd for C₁₆H₂₁INO₄ 418.0521; found 418.0524. ^1H NMR (400 MHz, DMSO) (rotamers mixture) δ 12.53 (s, 1H), 7.34 (s, 2H), 7.15 (d, $J = 8.6$ Hz, 1H), 4.04 (q, $J = 8.0, 7.5$ Hz, 1H), 3.07 – 2.80 (m, 2H), 2.24 (s, 6H), 1.30 (s, 8H), 1.15 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) δ 173.4, 155.2, 139.6, 136.1, 135.1, 92.0, 78.0, 53.0, 31.0, 28.1, 19.3. IR 3355, 2963, 1726, 1686, 1018, 793 cm^{-1} . MP 128-130 °C. $[\alpha]_D^{21} = -82.7$ (c=0.022, MeOH).

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(2,6-dimethylphenyl)propanoic acid (**19**): The crude was purified by flash chromatography (1:1 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **19** as a white solid, (0.075 g, 75% yield). Tr: 22.03. HRMS m/z : $[\text{M-H}]^-$ calcd for C₁₆H₂₂NO₄ 292.1543; found 292.1554. ^1H NMR (400 MHz, DMSO) (rotamers mixture) δ 12.55 (s, 1H), 7.15 (d, $J = 8.6$ Hz, 1H), 6.96 (d, $J = 1.7$ Hz, 3H), 4.07 (td, $J = 8.6, 6.1$ Hz, 1H), 3.11 – 2.85 (m, 2H), 2.28 (s, 6H), 1.31 (s, 8H), 1.14 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) (rotamers mixture) δ 173.7, 155.3, 136.7, 135.0, 127.9, 126.0, 78.0, 53.4, 31.3, 28.1, 27.6, 19.9. IR 3301, 2973, 1723, 1656, 1366, 1163, 765 cm^{-1} . MP 65-67 °C. $[\alpha]_D^{23} = +0.855$ (c=0.0055, MeOH).

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(2,6-dimethyl-4-nitrophenyl)propanoic acid (**26**): The crude was purified by flash chromatography (7:3 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **26** as a pale yellow solid, (0.605 g, 67% yield). Tr: 19.52 min. HRMS m/z : $[\text{M-H}]^-$ calcd for C₁₆H₂₁N₂O₆ 337.1405; found 337.1405. ^1H NMR (400 MHz, DMSO) (rotamers mixture) δ 12.75 (s, 1H), 7.87 (s, 2H), 7.26 (d, $J = 8.6$ Hz, 1H), 4.16 (td, $J = 9.1, 5.6$ Hz, 1H), 3.19 – 2.97 (m, 2H), 2.42 (s, 6H), 1.27 (s, 7H), 1.13 (s, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) (rotamers mixture) δ 173.0, 155.2, 145.4, 143.8, 139.3, 122.1, 78.1, 52.6, 31.6, 28.0, 27.5, 19.8. ^1H NMR (300 MHz, 120 °C, DMSO) δ 7.82 (s, 2H), 6.53 (s, 1H), 4.25 (q, $J = 8.2$ Hz, 1H), 3.31 – 3.02 (m, 2H), 2.45 (s, 6H), 1.37 – 1.25 (m, 9H). IR 3250, 2980, 1716, 1639, 1510, 1341, 1160 cm^{-1} . MP 170-172 °C. $[\alpha]_D^{23} = -164.2$ (c=0.05, MeOH).

(*S*)-3-(2-amino-5,7-dimethylbenzo[d]thiazol-6-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (**28**): The crude was purified by flash chromatography (4:1 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **28** as a white solid, (0.082 g, 70% yield). Tr: 13.64 min. HRMS m/z : $[\text{M-H}]^-$ calcd for C₁₇H₂₂N₃O₄S 364.1336; found 364.1339. ^1H NMR (400 MHz, CD₃OD) (rotamers mixture) δ 7.08 (s, 1H), 4.35 (dd, $J = 9.1, 6.0$ Hz, 1H), 3.29 – 2.99 (m, 2H), 2.43 (s, 3H), 2.42 (s, 3H), 1.33 (s, 7H), 1.11 (s, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CD₃OD) (rotamers mixture) δ 175.8, 169.4, 157.6, 149.6, 137.0, 130.9, 130.0, 129.6, 118.1, 80.4, 55.2, 33.3, 28.6, 28.1, 20.9, 19.6. IR 2927, 1692, 1513, 1158 cm^{-1} . MP 190-192 °C. $[\alpha]_D^{23} = -41.1$ (c=0.009, MeOH).

General procedure for Fmoc protection (**21**, **22**):

The HCl salt previously synthesized was used as crude. It was diluted in water/dioxane (1:2) solution (0.2 M) and basified with NaOH 2N aqueous solution until pH 10/11 at 0 °C. FmocCl (0.9 eq) was added and the reaction was left stirring at r.t. for 2 hours. The reaction was monitored by ESI mass spectrometry and TLC, at the complete consumption of starting material. The dioxane was then removed under vacuum and HCl 1N aqueous solution was added at 0 °C to pH 1. The mixture was extracted with ethyl acetate (3 times) and the organic phases combined were dried over Na₂SO₄ and concentrated under vacuum. Each crude was purified by flash chromatography as reported below.

(*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-fluoro-2,6-dimethylphenyl)propanoic acid (**21**): The crude was purified by flash chromatography (3:7 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford **21** as a white solid, (0.393 g, 75% yield). Tr: 23.33 min. HRMS m/z : $[\text{M+H}]^+$ calcd for C₂₆H₂₅NO₄F 434.1762; found 434.1762. ^1H NMR (400 MHz, CD₃OD) δ 7.79 (d, $J = 7.5$ Hz, 2H), 7.60 (d, $J = 4.2$ Hz, 2H), 7.38 (t, $J = 6.9$ Hz, 2H), 7.30 (q, $J = 6.9$ Hz, 2H), 6.70 (d, $J = 9.6$ Hz, 2H), 4.42 – 4.36 (m, 1H), 4.26 (dd, $J = 10.3, 6.9$ Hz, 2H), 4.14 (t, $J = 6.7$ Hz, 1H), 3.28 – 2.90 (m, 2H), 2.35 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CD₃OD) δ 175.3, 162.4 (d, $J = 243$ Hz), 158.3, 145.2, 142.5, 140.8, 131.5, 128.7, 128.1, 126.2, 120.9, 115.4 (d, $J = 21$ Hz), 67.9, 55.2, 46.9, 32.6, 20.5. IR 3313, 1716, 1696, 1533, 730 cm^{-1} . MP 197-199 °C. $[\alpha]_D^{22} = -3.32$ (c=0.006, MeOH).

(*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(2,6-dimethylphenyl)propanoic acid (**22**): The crude was purified by flash chromatography (7:3 AcOEt/petroleum ether/1% acetic acid) and crystallized with 2:1 Et₂O/petroleum ether to afford the product **22** as a white solid, (0.109 g, 80% yield). Tr: 23.30 min. HRMS m/z : $[\text{M-H}]^-$ calcd for C₂₆H₂₄NO₄ 414.1711; found 414.1715. $[\text{2M-H}]^-$ calcd for C₅₂H₄₈N₂O₈ 829.3494; found 829.3503. ^1H NMR (400 MHz, DMSO) δ 12.65 (s, 1H), 7.88 (d, $J = 7.6$ Hz, 2H), 7.83 (d, $J = 8.8$ Hz, 1H), 7.72 – 7.61 (m, 2H), 7.49 – 7.36 (m, 2H), 7.32 (td, $J = 7.5, 1.2$ Hz, 2H), 6.98 – 6.89 (m, 3H), 4.24 – 4.07 (m, 4H), 3.16 – 2.91 (m, 2H), 2.30 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO) δ 173.5, 155.9, 143.8, 140.7, 136.7, 134.9, 128.0, 127.6, 127.0, 126.1, 125.3, 120.1, 65.7, 53.9, 46.5, 31.3, 19.9. IR 3319, 2962, 1726, 1702, 1258, 1016, 794, 737 cm^{-1} . MP 153-155 °C. $[\alpha]_D^{23} = -284.7$ (c=0.04, MeOH).

Biology

Calcium mobilization assay: CHO cells stably coexpressing the human NOP and the C-terminally modified $G_{\alpha_{q15}}$ protein were used for calcium mobilization experiments. Cells were generated and cultured as described previously²⁷. Cells were maintained in DEMEM / F-12 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin, 100 μ g/ml hygromycin B and 200 μ g/ml G418, and cultured at 37 °C in 5% CO₂ humidified air. Cells were seeded at a density of 50,000 cells/well into 96-well black, clear-bottom plates. The following day, the cells were incubated with medium supplemented with 2.5 mM probenecid, 3 μ M of the calcium sensitive fluorescent dye Fluo-4 AM and 0.01% pluronic acid, for 30 min at 37 °C. After that time the loading solution was aspirated and 100 μ l/well of HBSS supplemented with 20 mM HEPES, 2.5 mM probenecid and 500 μ M Brilliant Black (Sigma Aldrich) was added. Concentrated solutions (1 mM) of N/OFQ(1-13)-NH₂ and analogues, were made in bidistilled water and kept at -20 °C. Peptides serial dilutions were carried out in HBSS / HEPES (20 mM) buffer (containing 0.02% bovine serum albumin fraction V). After placing both plates (cell culture and master plate) into the fluorometric imaging plate reader FlexStation II (Molecular Devices, Sunnyvale, CA), fluorescence changes were measured. On-line additions of the peptides were carried out in a volume of 50 μ l/well. To facilitate drug diffusion into the wells, the present study was performed at 37 °C. Maximum change in fluorescence, expressed as percent over the baseline fluorescence, was used to determine agonist response. All data are expressed as the mean \pm standard error of the mean (SEM.) of 3 experiments. Concentration-response curves to agonists were fitted to the classical four-parameter logistic nonlinear regression model: $\text{Effect} = \text{Baseline} + (\text{E}_{\text{max}} - \text{Baseline}) / (1 + 10^{(\text{LogEC}_{50} - \text{Log}[\text{compound}]) * \text{Hillslope}})$. Curves fitting were performed using PRISM 6.0 (GraphPad Software In., San Diego).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Further experimental procedures, compound characterization (PDF).

AUTHOR INFORMATION

Corresponding Author

* E-mail: trpcld@unife.it, giovanni.poli@sorbonne-universite.fr

ORCID

Julie Oble: 0000-0002-4002-255X.
Giovanni Poli: 0000-0002-7356-1568
Claudio Trapella: 0000-0002-6666-143X
Anna Fantinati : 0000-0003-0437-5670
Davide Illuminati : 0000-0002-0321-1941
Daniela Perrone: 0000-0001-5189-011X
Valentina Albanese: 0000-0002-1947-2644
Erika Marzola: 0000-0002-1428-1363
Chiara Sturaro: 0000-0002-2708-9223

Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡ These authors contributed equally: Davide Illuminati Anna Fantinati

ACKNOWLEDGMENT

JO and GP would like to acknowledge CNRS and Sorbonne Université for financial support. Support through the Marie Skłodowska-Curie Innovative Training Network European Training Networks (ITN-ETN) C–H Activation for Industrial Renewal is also gratefully acknowledged. CT would like to thanks FAR 2020 University of Ferrara (Fondo di Ateneo per la Ricerca 2020). The authors thanks Dr. Alberto Casolari and Giorgia Macedonio for NMR analysis.

REFERENCES

- (1) (a) Lemke, T. L.; Williams, D. A.; Roche, V. F.; Zito, S. W. Foye's Principles of Medicinal Chemistry, 7th ed. Kluwer ed. **2013**, Chapter 20. (b) Kastin, A. J. Handbook of biologically active peptides. **2013**, 7, Chapters 216 and 232. (c) Janecka, A.; Fichna, J.; Janecki, T. Opioid receptors and their ligands. *Curr. Top. Med. Chem.* **2004**, 4, 1–17.
- (2) Neumeyer, J. L.; Peng, X.; Knapp, B. I.; Bidlack, J. M.; Lazarus, L. H.; Salvadori, S.; Trapella, C.; Balboni, G. New opioid designed multiple ligand from Dmt-Tic and morphinan pharmacophores. *J. Med. Chem.* **2006**, 49, 5640-5643.
- (3) (a) Bryant, S. D.; Jinsmaa, Y.; Salvadori, S.; Okada, Y.; Lazarus, L. H. Dmt and opioid peptides: a potent alliance. *Biopolymers* **2003**, 71, 86–102. (b) Balboni, G.; Marzola, E.; Sasaki, Y.; Ambo, A.; Marczak, E. D.; Lazarus, L. H.; Salvadori, S. Role of 2',6'-dimethyl-L-tyrosine (Dmt) in some opioid lead compounds. *Bioorg. Med. Chem.* **2010**, 18, 6024–6030. (c) Sasaki, Y.; Suto, T.; Ambo, A.; Ouchi, H.; Yamamoto, Y. Biological properties of opioid peptides replacing Tyr in position 1 by 2,6-Dimethyl-Tyr1. *Chem. Pharm. Bull.* **1999**, 47, 1506–1509. (d) Sasaki, Y.; Ambo, A. 2',6'-Dimethylphenylalanine: A Useful Aromatic Amino Acid Surrogate for Tyr or Phe Residue in Opioid Peptides. *Int. J. Med. Chem.* **2012**, 498901.

- (4) (a) Pacifico, S.; Carotenuto, A.; Brancaccio, D.; Novellino, E.; Marzola, E.; Ferrari, F.; Cerelesi, M. C.; Trapella, C.; Preti, D.; Salvadori, S.; Calò, G.; Guerrini, R. Structure- and conformation-activity studies of nociceptin/orphanin FQ receptor dimeric ligands. *Sci Rep* **2017**, *7*, 45817. (b) Yamamoto, T.; Nair, P.; Largent-Milnes, T. M.; Jacobsen, N. E.; Davis, P.; Ma, S.; Yamamura, H. I.; Vanderah, T. W.; Porreca, F.; Lai, J.; Hruby, V. J. Discovery of a Potent and Efficacious Peptide Derivative for δ/μ Opioid Agonist/Neurokinin 1 Antagonist Activity with a 2',6'-Dimethyl-L-Tyrosine, in vitro, in vivo and NMR-Based Structural studies. *J. Med. Chem.* **2011**, *54*, 2029–2038. (c) Capasso A. Bioactivity of New Mu and Delta Opioid Peptides. *Med. Chem.* **2007**, *3*, 480–487.
- (5) Bender, A. M.; Griggs, N. W.; Gao, C.; Trask, T. J.; Traynor, J. R.; Mosberg, H. I. Rapid Synthesis of Boc-2',6'-dimethyl-L-tyrosine and Derivatives and Incorporation into Opioid Peptidomimetics. *ACS Med. Chem. Lett.* **2015**, *6*, 1199–1203.
- (6) For a selection, see: (a) He, K.; Zhu, F.; Li, H.; Tang, J.; Zhang, X. An Efficient and Highly Asymmetric Synthesis of (S)-2',6'-Dimethyltyrosine. *Org. Prep. Proc. Int.* **2020**, *52*, 510–516. (b) Tang, X.; Soloshonok, V. A.; Hruby V. J. Convenient asymmetric synthesis of enantiomerically pure 2',6'-dimethyltyrosine (DMT) via alkylation of chiral equivalent of nucleophilic glycine. *Tetrahedron: Asymmetry* **2000**, *11*, 2917–2925. (c) Balducci, D.; Contaldi, S.; Lazzari, I.; Porzi, G. A highly efficient stereocontrolled synthesis of (S)-2',6'-dimethyltyrosine [(S)-DMT]. *Tetrahedron: Asymmetry* **2009**, *20*, 1398–1401.
- (7) (a) Imamoto, T.; Tamura, K.; Zhang, Z.; Horiuchi, Y.; Sugiya, M.; Yoshida, K.; Yanagisawa, A.; Gridnev, I. D. Rigid P-Chiral Phosphine Ligands with tert-Butylmethylphosphino Groups for Rhodium-Catalyzed Asymmetric Hydrogenation of Functionalized Alkenes. *J. Am. Chem. Soc.* **2012**, *134*, 1754–1769. (b) Praquin, C. F. B.; de Koning, P. D.; Peach, P. J.; Howard, R. M.; Spencer, S. L. Development of an Asymmetric Hydrogenation Route to (S)-N-Boc-2,6-dimethyltyrosine. *Org. Process Res. Dev.* **2011**, *15*, 1124–1129.
- (8) Wang, X.; Niu, S.; Xu, L.; Zhang, C.; Meng, L.; Zhang, X.; Ma, D. Pd-Catalyzed dimethylation of Tyrosine-derived Picolinamide for synthesis of (S)-N-Boc-2,6-dimethyltyrosine and its analogues. *Org. Lett.* **2017**, *19*, 246–249.
- (9) Tremont, S. J.; Rahman, H. U. *Ortho*-Alkylation of acetanilides using alkyl halides and palladium acetate. *J. Am. Chem. Soc.* **1984**, *106*, 5760–5762.
- (10) (a) Zaitsev, V. G.; Shabashov, D.; Daugulis, O. Highly Regioselective Arylation of sp^3 C–H Bonds Catalyzed by Palladium Acetate. *J. Am. Chem. Soc.* **2005**, *127*, 13154–13155. (b) Nadres, E. T.; Santos, I. G.; Daugulis, D. Scope and Limitations of Auxiliary-Assisted, Palladium-Catalyzed Arylation and Alkylation of sp^2 and sp^3 C–H Bonds. *J. Org. Chem.* **2013**, *78*, 9689–9714.
- (11) For an excellent review, see: Sambiagio, C.; Schönbauer, D.; Blicke, R.; Dao-Huy, T.; Pototschnig, G.; Schaaf, P.; Wiesinger, T.; Zia, M. F.; Wencel-Delord, J.; Besset, T.; Maes, B. U. W.; Schürch, M. A comprehensive overview of directing groups applied in metal-catalysed C–H functionalisation chemistry. *Chem. Soc. Rev.* **2018**, *47*, 6603–6743.
- (12) Zhao, Y.; Chen, G. Palladium-Catalyzed Alkylation of *ortho*-C(sp^2)–H Bonds of Benzylamide Substrates with Alkyl Halides. *Org. Lett.*, **2011**, *13*, 4850–4853.
- (13) (a) Brandhofer, T.; García Mancheño, O. Site-Selective C–H Bond Activation / Functionalization of Alpha-Amino Acids and Peptide-Like Derivatives. *Eur. J. Org. Chem.* **2018**, 6050–6067. (b) Yun, Y.-I.; Yang, J.; Miao, Y.-h.; Sun, J.; Wang X.-j. Recent advances in Palladium(II)-catalyzed activation of aromatic ring C–H bonds. *J. Saudi Chem. Soc.*, **2020**, *24*, 151–185.
- (14) (a) Gensch, T.; Hopkinson, M. N.; Glorius, F.; Wencel-Delord. Mild metal-catalyzed C–H activation: examples and concepts. *J. Chem. Soc. Rev.* **2016**, *45*, 2900–2936. (b) Roudesly, F.; Oble, J.; Poli, G. Metal-catalyzed CH activation/functionalization: The fundamentals. *J. Mol. Catal. A*, **2017**, *426*, 275–296.
- (15) (a) Gardner Swain, C.; Rogers, R. J. Mechanism of formation of aryl fluorides from arenediazonium fluoborates. *J. Am. Chem. Soc.* **1975**, *97*, 799–800. (b) Yu, Z.-Q.; Lv, Y.-W.; Yu, C.-M.; Su, W.-K. Continuous flow reactor for Balz–Schiemann reaction: a new procedure for the preparation of aromatic fluorides. *Tetrahedron Lett.* **2012**, *54*, 1261–1263.
- (16) Wassmundt, F. W.; Kiesman, W. F. Efficient Catalysis of Hydrodediazoniations in Dimethylformamide. *J. Org. Chem.* **1995**, *60*, 1713–1719.
- (17) Xu, W.-B.; Xu, Q.-H.; Zhang, Z.-F.; Li, J.-Z. Copper(I)-Oxide-Mediated Cyanation of Arenediazonium Tetrafluoroborates with Trimethylsilyl Cyanide: A Method for Synthesizing Aromatic Nitriles. *Asian J. Org. Chem.* **2014**, *3*, 1062–1065.
- (18) Voutyritsa, E.; Theodorou, A.; Kokotou, M. G.; Kokotos, C. G. Organocatalytic oxidation of substituted anilines to azoxybenzenes and nitro compounds: mechanistic studies excluding the involvement of a dioxirane intermediate. *Green Chemistry* **2017**, *19*, 1291–1298.
- (19) (a) Maggiolo, A. The Reaction of Thiocyanogen with Nitrogen Heterocycles; Pyridines, Pyrimidines and Quinolines. *J. Am. Chem. Soc.* **1951**, *73*, 5815–5816. (b) Zhang, A.; van Vliet, S.; Neumeyer, J. L.; Synthesis of aminothiazole derived morphinans. *Tetrahedron Lett.* **2003**, *44*, 6459–6462. (c) Ballet, S.; Salvadori, S.; Trapella, C.; Bryant, S. D.; Jinsmaa, Y.; Lazarus, L. H.; Negri, L.; Giannini, E.; Lattanzi, R.; Tourwé, D.; Balboni, G. New 2',6'-dimethyl-L-tyrosine (Dmt) opioid peptidomimetics based on the Aba-Gly scaffold. Development of unique μ -opioid receptor ligands. *J. Med. Chem.* **2006**, *49*, 3990–3993.
- (20) Guerrini, R.; Calò, G.; Rizzi, A.; Bianchi, C.; Lazarus, L.H.; Salvadori, S.; Temussi, P.A.; Regoli, D. Address and Message Sequences for the Nociceptin Receptor: A Structure-Activity Study of Nociceptin-(1-13)-peptide amide. *J. Med. Chem.* **1997**, *40*, 12, 1789–1793.
- (21) Marastoni, M.; Trapella, C.; Scotti, A.; Fantinati, A.; Ferretti, V.; Marzola, E.; Gallerani, E.; Gavioli, R.; Preti, D. Naphtoquinone amino acid derivatives, synthesis and biological activity as proteasome inhibitors. *J. Enzyme Inhib. Med. Chem.*, **2017**, *32*, 865–877.
- (22) The pharmacological assay was performed by the research group directed by Prof. G. Calò at the Department of Medical Sciences of the University of Ferrara.
- (23) Camarda, V.; Calò, G. Chimeric G Proteins in Fluorimetric Calcium Assays: Experience with Opioid Receptors. *Calcium Signaling Protocols*. **2012**, *937*, 293–306.
- (24) Similar results were obtained with the [(pF)Phe4]N/OFQ(1-13)-NH₂ analogue (with commercial reagent CAS n. 169243-86-1). [dmpFPhe4]N/OFQ(1-13)-NH₂ (with compound **21**) and [dmPhe4]N/OFQ(1-13)-NH₂ (with compound **22**) displayed similar potency but lower efficacy (pEC₅₀ = 8.86 (8.65 – 9.06) and 7.85 (7.63 – 8.07), respectively) than N/OFQ(1-13)-NH₂. Data are the mean \pm sem of three separate experiments performed in duplicate.
- (25) Additional studies to assess if we could obtain the *o,o*-dimethylated aniline derivative **8**, not only from 4-nitro-*L*-phenylalanine **3**, but also from the less expensive *L*-tyrosine, were also undertaken. In particular, we envisioned that a Buchwald-Hartwig (BH) aromatic amination strategy from an appropriate tyrosine derivative might have enabled a second way of access to **8**. However, the BH couplings between *N,N*-dibenzylamine and *O*-triflyl *L*-tyrosine methyl ester PA (*o,o*-dimethylated at the aromatic ring, or not) met with failure, very likely due to incompatibility between the BH coupling and the PA group.
- (26) (a) Guerrini, R.; Calò, G.; Bigoni, R.; Rizzi, D.; Rizzi, A.; Zucchini, M.; Varani, K.; Hashiba, E.; Lambert, D. G.; Toth, G.; Borea, P. A.; Salvadori, S.; Regoli, D. Structure-Activity Studies of the Phe⁴ Residue of Nociceptin(1-13)-NH₂: Identification of Highly Potent Agonists of the Nociceptin/Orphanin FQ Receptor. *J. Med. Chem.* **2001**, *44*, 3956–3964. (b) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. Potent δ -Opioid Receptor Agonists Containing the Dmt-Tic Pharmacophore. *J. Med. Chem.* **2002**, *45*, 5556–5563.

(c) Sasaki, Y.; Sasaki, A.; Ariizumi, T.; Igari, Y.; Sato, K.; Kohara, H.; Niizuma, H.; Ambo, A. 2',6'-Dimethylphenylalanine (Dmp) can mimic the N-terminal Tyr in opioid peptides. *Biol. Pharm. Bull.* **2004**, *27*, 244–247.

(27) Camarda, V.; Fischetti, C.; Anzellotti, N.; Molinari, P.; Ambrosio, C.; Kostenis, E.; Rigoli, D.; Trapella, C.; Guerrini, R.; Salvadori, S.; Calò, G. Pharmacological profile of NOP receptors coupled with calcium signaling via the chimeric protein $G\alpha_{q5}$. *Naunyn-Schmied Arch. Pharmacol.* **2009**, *379*, 599-607.