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PAPER

Radical arylation of tyrosine and its application in the synthesis of a highly selective neurotensin receptor 2 ligand†‡

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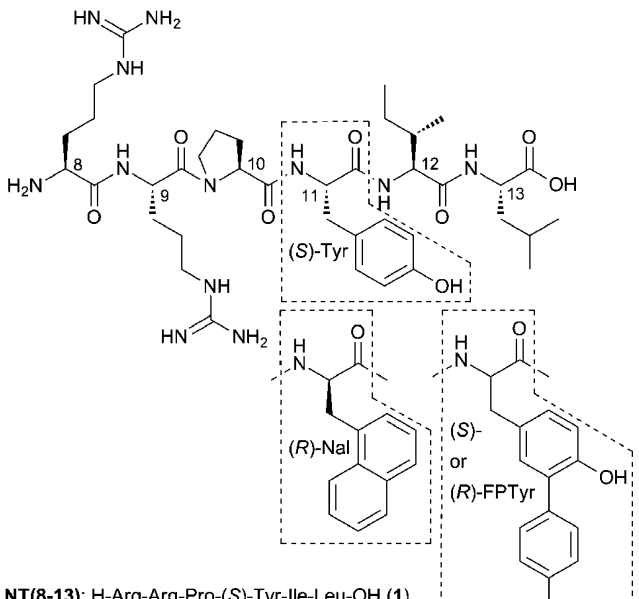
A small library of Fmoc-protected 3-arylated tyrosines was created by radical arylation. The new building blocks were successfully applied in the synthesis of two novel neurotensin receptor ligands. Both isomers showed high affinity for the human NTS2 receptor with K_i values in the nanomolar range. Interestingly, subtype selectivity strongly depends on the configuration of the peptide in position 11. Isomer (11*R*)-**3** displayed an excellent preference for NTS2 compared to NTS1.

Introduction

In recent years, the tridecapeptide neurotensin NT, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) has received increasing attention due to its strong modulatory effects in the central nervous system and the periphery.[1] The physiological modulations induced by the direct administration of NT to the brain[2] include hypothermia, antipsychotic effects[3] and μ-opioid-independent antinociception.[4] As an antinociceptive agent, NT was found to be even more potent than morphine.[5] Early SAR studies displayed that most, probably all, of the activity of NT is mediated by the *C*-terminal 6 amino acid fragment NT(8–13) (**1**) (Fig. 1).[6] For this reason, the hexapeptide **1**, which does not occur *in vivo*, became a widespread lead-structure for the development of new NTS ligands.[7–11] Especially, the differentiation between the NT receptor subtypes NTS1 and NTS2,[12] both belonging to the family of GPCRs, turned out to be an important task. While NTS2 is apparently involved in antinociceptive activity and hypothermia, NTS1 is considered to be largely responsible for the control of dopamine-mediated, neuroleptic effects.[13,14] While NTS1-selective ligands are therefore potential drug candidates for the treatment of schizophrenia and Parkinson's Disease, NTS-selective compounds might be a promising approach for the treatment of pain.

Due to the very limited number of X-ray crystal structures available until now,[15] a structure based design of specific GPCR ligands is difficult. Based on the structure of rhodopsin, we recently reported on a homology modeling study for the NTS1-NT(8–13) complex.[16] This investigation included validation by site-directed mutagenesis and clearly showed an aromatic binding pocket specifically interacting with the tyrosine residue in position 11 of the neuropeptide. Similar structural investigations for the NTS2 subtype have not been performed, yet. Based on previously described structure–activity relationship studies, however, ligand based design and an optimization of NTS2 affinity and selectivity over NTS1 is possible. These relationships clearly indicate that the orientation of the binding pocket interacting with the residue in position 11 is different for NTS2. It was shown that inversion of the configuration and an extension of the π-system is tolerated by NTS2 but not by the NTS1 subtype.[17] We envisaged synthesizing



NT(8-13): H-Arg-Arg-Pro-(*S*)-Tyr-Ile-Leu-OH (**1**)
H-Arg-Arg-Pro-(*R*)-Nal-Ile-Leu-OH (**2**)
H-Arg-Arg-Pro-(*S*)-FPTyr-Ile-Leu-OH [(11*S*)-**3**]
H-Arg-Arg-Pro-(*R*)-FPTyr-Ile-Leu-OH [(11*R*)-**3**]

Fig. 1 NT(8–13) (**1**), naphthylalanine (Nal) analogue (**2**) and the fluorophenyltyrosine (FPTyr) derivatives (**3**).

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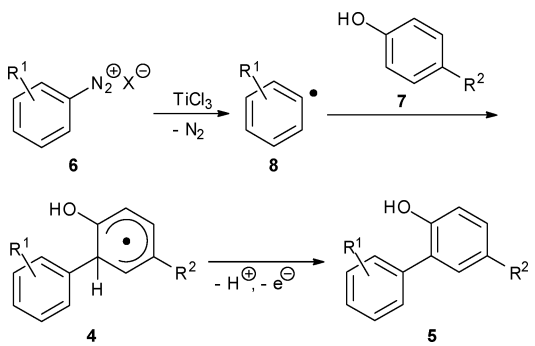
and evaluating compound **3** because it fulfils three requirements: firstly, the phenol substructure of the tyrosine residue of the parent peptide has been conserved, secondly, the π-system has been extended, and finally, a fluorine substituent has been incorporated that should prevent cytochrome P450 promoted metabolism.

A major advance towards the goal of NTS2-selectivity[18] has been reported by Richelson,[17] who found that a replacement of (*S*)-tyrosine for (*R*)-1-naphthylalanine [(*R*)-Nal] in NT(8–13) leads to a 104-fold binding preference of the new ligand **2** to NTS2 over NTS1 (Fig. 1).[19] To verify whether an extension of the aromatic system of tyrosine in combination with a change of the absolute configuration from (*S*) to (*R*) might be a more general concept to achieve NTS2-selectivity, we planned the synthesis and biological evaluation of the ligands (11*R*)-**3** and (11*S*)-**3**. The results from our study, in which the original (*S*)-tyrosine in NT(8–13) (**1**) was exchanged for both enantiomers of 3-(4-fluorophenyl)tyrosine (FPTyr), are reported in this article.

Results and discussion

Synthesis of 3-aryltyrosines as building blocks for peptides

The radical arylation of phenols using arenediazonium salts as aryl radical sources offers a straightforward access to various 2-hydroxybiphenyls by a reaction type being by its overall result comparable to popular CH-activations (Scheme 1).[20–24] Due to the stabilizing effect of the hydroxy group on the cyclohexadienyl radical intermediate **4**, the desired hydroxybiphenyls **5** are obtained with reasonable regio-selectivities from the simple starting materials **6** and **7** under reductive conditions.[25] While iron(II)-salts were found to be ideal reductants for the generation of aryl radicals **8** from **6** in *Meerwein*-type arylations and vinylations,[26] titanium(III)-chloride is so far our preferred reagent for the purpose of biaryl synthesis.



Scheme 1 Radical arylation of phenols **7** with arenediazonium salts **6** as radical precursors.

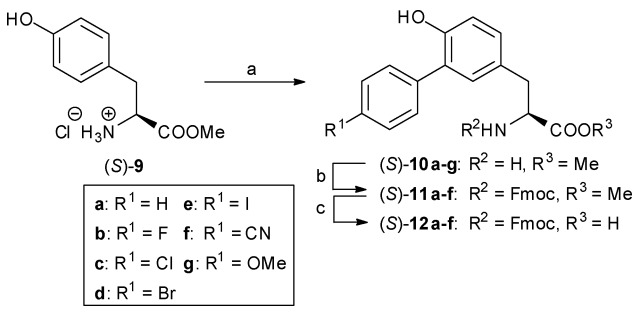
As evidenced by the successful arylations of tyramine and tyrosine,[23] this reaction type is not sensitive towards the presence of unprotected functional groups in the substrate, such as free carboxylic acids or amines. Given the advantage that an additional protective group strategy is not required, we wanted to apply our methodology in the synthesis of a small library of 3-aryltyrosines suitable for peptide synthesis. For the reason of simplified product isolation, methyl (*S*)-tyrosinate [(*S*)-**9**] was chosen as starting material instead of (*S*)-tyrosine. In the first step, the arylation reaction with a set of seven arenediazonium salts **6a–6g** provided

Table 1 Preparation of **10a–g**, **11a–f** and **12a–f**

	Yield **10** (%)[a]	Yield **11** (%)	Yield **12** (%)
a: R^1 = H	22 (31)[b]	42	66
b: R^1 = F	22 (34)[c]	45	67
	23 (36)[c,d]	42[c]	55[c]
c: R^1 = Cl	41 (56)[c]	52	57
d: R^1 = Br	32 (45)[c]	64	52
e: R^1 = I	21 (33)[c]	53	46
f: R^1 = CN	28 (35)[b]	65	69
g: R^1 = OMe	19 (27)[b]	—	—

[a] Yields after purification by column chromatography, calculated from all fractions containing the product (in brackets), from pure fractions. [b] Method A: diazonium tetrafluoroborate. [c] Method B: diazonium chloride. [d] Synthesis from methyl *R*-tyrosinate [(*R*)-**9**] leading to (*R*)-**12b**.

the biphenyls (*S*)-**10a–g** (Scheme 2 and Table 1). The formation of minor amounts of the regioisomers along with the desired products (selectivity ~ 5 : 1) however complicated purification by column chromatography. Protection of (*S*)-**10** with the 9-fluorenylmethyloxy-carbonyl (Fmoc) group using the oxysuccinimide (Fmoc-OSu) gave the esters (*S*)-**11**, which were finally converted to the acids (*S*)-**12** by mild saponification employing sodium carbonate in acetonitrile.[27]



Scheme 2 *Reagents and conditions*: (a) diazonium tetrafluoroborate (method A) or diazonium chloride (method B) **6a–g**, $TiCl_3$, CH_3CN/H_2O, r.t.; (b) Fmoc-OSu, Na_2CO_3, dioxane; (c) Na_2CO_3, CH_3CN.

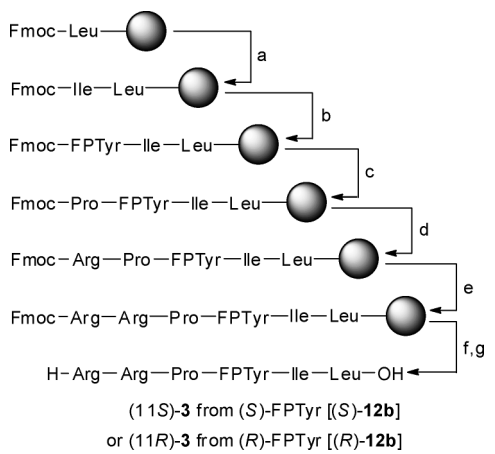
An important factor in radical biaryl coupling reactions with diazonium salts is the rate of addition of the salt **6** to the reaction mixture containing the substrate (*e.g.* phenol, tyrosinate) and the reductant (*e.g.* titanium(III)-chloride).[28] Since the conditions for the preparation of **10** have so far been only partially optimized using the *para*-chlorophenyl-diazonium chloride **6c**, it was not surprising to find the best yield in this step for **10c**. To obtain better results for the other compounds, too, a variation of addition rate and reaction temperature is required. In this context, the use of arenediazonium chlorides instead of tetrafluoroborates usually produces slightly better results. Within the set of the seven salts, only two reactions showed special difficulties. The preparation of the unsubstituted salt **6a** as chloride under standard conditions was significantly complicated by azo coupling, so that the salt had to be replaced by its tetrafluoroborate. For the biaryl synthesis with the iodinated salt **6e**, 1,4-diiodobenzene was isolated as by-product pointing to undesired iodine-transfer processes.[29] Compared to alternative methods such as Suzuki-type couplings,[30] the radical arylation is generally not sensitive to additional halogen atoms (especially Cl and Br) on the aromatic cores, which could otherwise

cause multiple substitutions.[31] A determination of the optical purity, as exemplified for compounds (*R*)- and (*S*)-**12b** (ee >98% for (*R*)-**12b**, ee >99% for (*S*)-**12b**), showed that the radical arylation does not lead to a loss of stereochemical information (*e.g.* by hydrogen abstraction from the α-carbon atom of the amino acid).

For the purpose of preparing the desired NT receptor ligands, and probing the applicability of the new tyrosine-derived building blocks **12** in peptide synthesis, we submitted the 4-fluorophenylated tyrosines (*S*)-**12b** [(*S*)-FPTyr] and (*R*)-**12b** [(*R*)-FPTyr] to standard amino acid coupling conditions.

Peptide synthesis

The synthesis of the neurotensin receptor ligands (11*S*)-**3** and (11*R*)-**3** was carried out using Fmoc-Leu-Wang resin in combination with conventional preparative methods (Fmoc strategy). The reagents and conditions applied for each coupling step are shown in Scheme 3. Fmoc deprotection of Leu/Ile and coupling of the sterically hindered Fmoc-Ile-OH was accelerated by microwave irradiation. Since the hydroxy group of the fluorophenyltyrosines **12b** had indicated its low reactivity in an unsuccessful attempt to protect it as *tert*-butyl ether,[32] we decided to submit (*S*)- and (*R*)-**12b** to the peptide synthesis without the *O*-protection usually required for tyrosine derivatives, however, all further Fmoc-deprotection and acylation steps were performed without microwave irradiation in order to circumvent potential tyrosine *O*-acylation. During the attachment of the building blocks **12b** (step b) as well as in the following coupling reactions (steps c–e), we indeed observed no negative effects arising from the unprotected hydroxy functionality.



Scheme 3 *Reagents and conditions*: (a) 1. piperidine/DMF (1 : 4), μ~: 5 × 5 s, 100 W, 5 × cooling to −10 °C; 2. Fmoc-Ile-OH, PyBOP (benzotriazol-1-yloxy)tri-pyrrolidino-phosphonium hexafluorophosphate), HOBt (1-hydroxybenzotriazole), *i*Pr_2NEt, DMF, μ~: 15 × 10 s, 50 W, 15 × cooling to −10 °C; (b) 1. Fmoc deprotection (see a), 2. FPTyr [(*S*)-**12b** or (*R*)-**12b**], DIC (diisopropyl carbodiimide), HOAt (1-hydroxy-7-azabenzotriazole), DMF, r.t., 10 h; (c) 1. 2 × piperidine/DMF (1 : 4), rt, 2 min; 2. Fmoc-Pro-OH, PyBOP, HOBt, *i*Pr_2NEt, DMF, rt, 2 h; (d) 1. Fmoc deprotection (see c), 2. 2 × coupling of Fmoc-Arg(Pbf)-OH (see c); (e) see d, single coupling of Fmoc-Arg(Pbf)-OH, (f) 1. Fmoc deprotection (see c), 2. CF_3COOH, phenol, H_2O, triisopropylsilane 88 : 5 : 5 : 2, 2 h; (g) RP-HPLC.

Table 2 Receptor binding properties of the test compounds (11*S*)-**3**, (11*R*)-**3** as well as the reference agents NT(8–13) (**1**) and **2** at the human NTS1 and NTS2 receptor (K_i values in nM)

	K_i ± SEM [nM][a]		
Compound	HumanNTS1 [^{3}H]neurotensin	HumanNTS2 [^{3}H]NT(8–13)	Selectivity ratio[b] NTS1/NTS2
NT(8–13) (**1**)	0.23 ± 0.029	1.3 ± 0.17[c]	0.18
2	4700 ± 990	65 ± 6.9	72
(11*S*)-**3**	39 ± 5.0[d]	11 ± 6.1[d]	3.5
(11*R*)-**3**	18000 ± 2800[d]	63 ± 10	290

[a] Mean values of 3 to 10 independent experiments each done in triplicate. [b] Ratio of K_i(NTS1)/K_i(NTS2). [c] K_D value. [d] K_i ± SD derived from two experiments.

After cleavage from the resin purification of (11*S*)-**3** and (11*R*)-**3** was achieved by preparative HPLC.

Biological evaluation

The binding properties of the synthesized fluorophenyltyrosine derivatives (**3**) and that of the reference structures NT(8–13) (**1**) and **2** were performed in competition binding experiments utilizing human NTS1 and NTS2 receptors.[33] Membranes of Chinese hamster ovary (CHO) cells stably expressing hNTS1[34] and preparations of human embryonic kidney (HEK) cells transiently expressing hNTS2[33] were incubated with the radioligands [^{3}H]neurotensin and [^{3}H]NT(8–13), respectively to measure the ability of the test compounds to displace the radioligand in a dose-dependent manner. The resulting K_i values are depicted in Table 2 indicating high affinity but nearly unselective binding behaviour for (11*S*)-**3** with K_i values of 39 nM and 11 nM for NTS1 and NTS2, respectively. Interestingly, the diastereomer (11*R*)-**3** displayed strongly reduced NTS1 binding (K_i = 18000 nM) while NTS2 affinity (K_i = 63 nM) was only slightly attenuated. The data result in a 290-fold selectivity to NTS2 over NTS1. In comparison to the binding properties of the reference compound **2**[17] showing a 72-fold selectivity in our test system. The *in vitro* binding data for the fluorophenyltyrosine peptide (11*R*)-**3** indicate superior subtype selectivity.

Conclusions

Taking advantage of the quick access to substituted tyrosines by radical arylation, a small library of Fmoc-protected 3-aryltyrosines has been synthesized. Although the methodology is insensitive to chloro- or bromophenyl substituents, which would be difficult to introduce by conventional cross-coupling protocols, we realized that a careful optimization for each substrate or diazonium salt is probably necessary to obtain good yields. Since the phenolic hydroxy functionality of the 3-aryltyrosines proved comparably unreactive, peptide synthesis could successfully be performed without a further protective group.

While the (11*S*)-configured **3** showed an unselective binding behaviour towards the NTS1 and NTS2 receptor, the diastereomer (11*R*)-**3** containing the configuration of an unnatural amino acid displayed a 290-fold selectivity to NTS2 over NTS1 which is superior when compared to the reference compound. Therefore,

(11*R*)-**3** can be considered as the most selective NTS2 ligand which is hitherto reported.

Experimental

General remarks

Reagents, building blocks and dry solvents were obtained from commercial sources and were used as received. Unless otherwise noted, reactions were conducted without inert atmosphere. The microwave assisted (Discover® microwave oven, CEM Corp.) peptide coupling step was carried out in a silanized glass tube loosely sealed with a silicon septum. Remark: the development of overpressure was avoided by using DMF as the solvent. In between each irradiation step, intermittent cooling of the reaction mixture to a temperature of −10 °C was achieved by sufficient agitation in an ethanol–ice bath. ^{1}H-NMR were recorded on 360 and 600 MHz spectrometers using $CDCl_3$ and CD_3OD as solvents referenced to TMS (0 ppm), $CHCl_3$ (7.26 ppm) or CHD_2OD (3.31 ppm). Chemical shifts are reported in parts per million (ppm). Coupling constants are in Hertz (*J* Hz). The following abbreviations are used for the description of signals: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). ^{13}C-NMR were recorded at 91 and 151 MHz in $CDCl_3$ and CD_3OD using $CDCl_3$ (77.0 ppm) or CD_3OD (49.5 ppm) as standard. Chemical shifts are given in parts per million (ppm). Mass spectra were recorded using electron impact (EI). Analytical TLC was carried out on Merck silica gel plates using short wave (254 nm) UV light to visualise components. Silica gel (Kieselgel 60, 40–63 μm, Merck) was used for flash column chromatography. The enantiomeric purity of compounds (*S*)- and (*R*)-**12b** was determined by chiral analytical HPLC (Varian 940-LC, column: Daicel Chiralpak® IC, 4.6 mm × 250 mm, 5 μm, flow rate 0.8 mL min^{-1}, hexane/*iso*-propanol = 90 : 10, detection wavelength 254 nm and 280 nm). Preparative RP-HPLC was performed using Agilent 1100 preparative series (column: Zorbax Eclipse XDB-C8, 21.2 × 150 mm, 5 μm particles [C8]; flow rate 10 mL min^{-1}, detection wavelength 220 nm) and solvent systems as specified below. Purity and identity were assessed by analytical RP-HPLC (Agilent 1100 analytical series, column: Zorbax Eclipse XDB-C8 analytical column, 4.6 × 150 mm, 5 μm, flow rate 0.5 mL min^{-1}, detection wavelength 220 nm) coupled to a Bruker Esquire 2000 mass detector equipped with an ESI-trap. Solvent systems are specified below.

General procedure for the radical arylation[22]

Preparation of the arenediazonium tetrafluoroborate (method A). A mixture of the aniline (40.0 mmol), tetrafluoroboric acid (50%, 80.0 mmol, 14.0 mL) and water (15 mL) was cooled down to 0–5 °C. A pre-cooled solution of sodium nitrite (42.0 mmol, 2.90 g) in water (6.5 mL) was dropwise added keeping the temperature below 5 °C. After stirring for 30 min at this temperature the diazonium salt was filtered off and washed with cold diethyl ether. The solid was dried *in vacuo* and could be stored for a few weeks at −18 °C.

Preparation of the arenediazonium chloride (method B). To an ice-cooled degassed solution of the aniline (20.0 mmol) in hydrochloric acid (3 N, 20 mL) and water (20 mL) a degassed solution of sodium nitrite (1.38 g, 20.0 mmol) in water (10 mL) was dropwise added over a period of 10 min. After stirring for 20 more minutes at 0 °C, the clear solution was used for the biaryl coupling reactions (20 mmol/50 mL = 0.4 M).

Biaryl coupling. A solution of the arenediazonium tetrafluoroborate (method A, 2.00 mmol) in water and acetonitrile (5 mL, ratio depends on the solubility of the diazonium salt) or a 5 mL aliquot of the 0.4 M arenediazonium chloride solution (method B, 2.00 mmol) was added dropwise by a syringe pump to a vigorously stirred, degassed solution of methyl tyrosinate hydrochloride (6.00 mmol, 1.39 g) in water (6 mL), titanium(III)-chloride (4 mL, *ca.* 1 M solution in 3 N hydrochloric acid, 4.00 mmol) under nitrogen atmosphere within 10–15 min. After the addition was complete, the mixture was left to stir for 10 more minutes. Before threefold extraction with diethyl ether (3 × 75 mL), satd. aqueous sodium carbonate was used to adjust the pH of the crude mixture to a value of 8–9. The combined organic phases were washed with satd. aqueous sodium chloride and dried over sodium sulfate. Concentration *in vacuo* and purification by column chromatography (silica gel, CH_2Cl_2/MeOH = 10 : 1) gave the desired arylated tyrosine derivatives.

General procedure for the Fmoc protection[27]

To an ice-cooled solution of the arylated tyrosine derivative (1.00 mmol) in aqueous sodium carbonate (10%, 2 mL) and *p*-dioxane (1 mL) a solution of *N*-(9*H*-fluoren-2-ylmethoxy-carbonyloxy)succinimide (Fmoc-OSu) (1.18 mmol, 395 mg) in *p*-dioxane (2 mL) was added dropwise. After stirring for 24 h with gradual warming to room temperature, the solution was poured in ice/water and extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with satd. aqueous sodium chloride and dried over sodium sulfate. Concentration *in vacuo* and purification by column chromatography (silica gel, CH_2Cl_2/MeOH/HCO_2H = 99 : 1 : 0.5) gave the desired Fmoc-protected tyrosine derivatives.

General procedure for the selective cleavage of the Fmoc-protected tyrosine methyl esters[27]

A solution of the Fmoc-protected methyl tyrosinate derivative (1.00 mmol) in aqueous sodium carbonate (3%, 90 mL) and acetonitrile (60 mL) was stirred for 24 h under nitrogen atmosphere. The reaction mixture was then washed with hexane (2 × 50 mL). Before threefold extraction with chloroform (3 × 100 mL), hydrochloric acid (2 N) was used to adjust the pH of the crude mixture to a value of 3–4. The combined organic phases were washed with satd. aqueous sodium chloride, dried over sodium sulfate and concentrated *in vacuo*.

The syntheses of compounds (*S*)-**10b**, (*S*)-**11b** and (*S*)-**12b** from 4-fluorophenyldiazonium chloride and (*S*)-tyrosine methyl ester hydrochloride [(*S*)-**9**] are described below. The preparation and analytical data of all other compounds is included in the electronic supplementary information.

Methyl (*S*)-2-amino-3-(4′-fluoro-6-hydroxybiphen-3-yl)propanoate [(*S*)-10b]

Compound (*S*)-**10b** was prepared from methyl (*S*)-tyrosinate hydrochloride [(*S*)-**9**] and 4-fluorophenyldiazonium chloride

(method B) according to the general procedure for the radical arylation described above. Methyl (*S*)-2-amino-3-(4′-fluoro-6-hydroxybiphen-3-yl)propanoate [(*S*)-**10b**] (0.44 mmol, 127 mg, 22%) was obtained as colorless oil. R_f 0.4 (CH_2Cl_2/MeOH = 10 : 1) [UV]; ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) = 2.80 (dd, J = 7.7 Hz, J = 13.7 Hz, 1 H), 3.05 (dd, J = 3.9 Hz, J = 13.6 Hz, 1 H), 3.72 (s, 4 H), 6.74 (d, J = 8.1 Hz, 1 H), 6.95–7.01 (m, 2 H), 7.09 (t, J = 8.8 Hz, J_{HF} = 8.8 Hz, 2 H), 7.42 (dd, J_{HF} = 5.4 Hz, J = 8.9 Hz, 2 H); ^{13}C-NMR (91 MHz, $CDCl_3$): δ = 39.7 (CH_2), 52.1 (CH_3), 55.5 (CH), 115.5 (d, J_{CF} = 21.0 Hz, 2 × CH), 116.4 (CH), 127.7 (C_q), 128.5 (C_q), 129.6 (CH), 130.8 (d, J_{CF} = 8.1 Hz, 2 × CH), 131.1 (CH), 133.6 (d, J_{CF} = 2.9 Hz, C_q), 152.1 (C_q), 162.1 (d, J_{CF} = 245.8 Hz, C_q), 175.2 (C_q); MS (EI) m/z (%): 290 (29) [M^+ + H], 289 (92) [M^+], 231 (25), 230 (100), 229 (30), 228 (14), 213 (15), 203 (49), 202 (100), 201 (100), 200 (32), 199 (45), 195 (32),187 (12), 186 (17), 185 (22), 184 (17), 183 (70), 182 (15), 181 (60), 173 (11), 172 (15), 171 (33), 170 (40), 165 (15), 159 (25), 157 (19), 153 (21), 152 (45), 151 (12), 147 (11), 146 (27), 133 (42), 127 (11), 120 (12), 115 (74), 114 (38), 107 (22), 89 (52), 88 (94), 77 (15), 74 (19); HRMS (EI): calcd. for $C_{16}H_{16}FNO_3$ [M^+]: 289.1114, found: 289.1114.

Methyl (*S*)-2-*N*-(fluorenylmethoxycarbonyl)-3-(4′-fluoro-6-hydroxybiphen-3-yl)propanoate [(*S*)-11b]

Compound (*S*)-**11b** was prepared from methyl (*S*)-2-amino-3-(4′-fluoro-6-hydroxybiphen-3-yl)propanoate [(*S*)-**10b**] (0.74 mmol, 213 mg) and Fmoc-OSu (0.87 mmol, 291 mg) according to the general procedure for the introduction of the Fmoc protecting group described above. Methyl (*S*)-2-*N*-(fluorenylmethoxycarbonyl)-3-(4′-fluoro-6-hydroxybiphen-3-yl)-propanoate [(*S*)-**11b**] (0.33 mmol, 169 mg, 45%) was obtained as white solid. R_f 0.1 (CH_2Cl_2/MeOH/HCO_2H = 99 : 1 : 0.5) [UV]; ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) = 3.01 (dd, J = 6.2 Hz, J = 14.0 Hz, 1 H), 3.10 (dd, J = 5.2 Hz, J = 14.0 Hz, 1 H), 3.70 (s, 3 H), 4.14 (t, J = 6.9 Hz, 1 H), 4.29–4.41 (m, 2 H), 4.65 (dd, J = 5.9 Hz, J = 13.9 Hz, 1 H), 5.40 (d, J = 8.3 Hz, 1 H), 5.82 (s, 1 H), 6.82 (d, J = 8.2 Hz, 1 H), 6.90–6.98 (m, 2 H), 7.05 (t, J = 8.7 Hz, J_{HF} = 8.7 Hz, 2 H), 7.20–7.26 (m, 2 H), 7.32–7.41 (m, 4 H), 7.51 (t, J = 8.1 Hz, 2 H), 7.72 (d, J = 7.6 Hz, 2 H); ^{13}C-NMR (91 MHz, $CDCl_3$): δ = 37.5 (CH_2), 47.1 (CH), 52.3 (CH_3), 54.9 (CH_2), 67.0 (CH), 115.7 (d, J_{CF} = 21.2 Hz, 2 × CH), 116.2 (CH), 119.9 (2 × CH), 120.0 (2 × CH), 125.0 (CH), 127.0 (2 × CH), 127.4 (CH), 127.7 (2 × CH), 129.8 (C_q), 130.7 (d, J_{CF} = 8.2 Hz, 2 × CH), 131.2 (C_q), 133.1 (d, J_{CF} = 3.0 Hz, C_q), 141.2 (2 × C_q), 143.6 (2 × C_q), 151.8 (C_q), 155.7 (C_q), 162.3 (d, J_{CF} = 246.9 Hz, C_q), 172.1 (C_q); MS (EI) m/z (%): 230 (12), 202 (42), 201 (100), 179 (49), 178 (100), 177 (17), 176 (28), 165 (11), 152 (15), 151 (11), 89 (17), 88 (24), 76 (13); HRMS (EI): calcd. for $C_{31}H_{26}FNO_5$ [M^+]: 511.1795, found: 511.1796.

(*S*)-2-*N*-(Fluorenylmethoxycarbonyl)-3-(4′-fluoro-6-hydroxybiphen-3-yl)propionic acid [(*S*)-12b]

Compound (*S*)-**12b** was prepared from methyl (*S*)-2-*N*-(fluorenylmethoxycarbonyl)-3-(4′-fluoro-6-hydroxybiphen-3-yl)propanoate [(*S*)-**11b**] (0.33 mmol, 169 mg) according to the general procedure for the selective saponification described above. (*S*)-2-*N*-(Fluorenylmethoxycarbonyl)-3-(4′-fluoro-6-hydroxybiphen-3-yl)-propionic acid [(*S*)-**12b**] (0.22 mmol, 110 mg, 67%) was obtained as yellow oil. R_f 0.1 (CH_2Cl_2/MeOH = 10 : 1) [UV]; ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) = 3.00–3.15 (m, 2 H), 4.10–4.18 (m, 1 H), 4.24–4.42 (m, 2 H), 4.60–4.68 (m, 1 H), 5.36 (d, J = 8.2 Hz, 1 H), 6.79–6.86 (m, 1 H), 6.94–7.11 (m, 4 H), 7.20–7.27 (m, 2 H), 7.32–7.41 (m, 4 H), 7.46–7.55 (m, 3 H), 7.73 (t, J = 7.4 Hz, 2 H); ^{13}C-NMR (91 MHz, $CDCl_3$): δ = 37.5 (CH_2), 47.1 (CH), 54.9 (CH_2), 67.1 (CH), 115.9 (d, J_{CF} = 21.0 Hz, 2 × CH), 116.2 (CH), 120.0 (4 × CH), 125.0 (CH), 127.0 (2 × CH), 127.4 (CH), 127.7 (2 × CH), 129.9 (C_q), 130.7 (d, J_{CF} = 7.9 Hz, 2 × CH), 131.2 (C_q), 133.1 (d, J_{CF} = 3.1 Hz, C_q), 141.2 (2 × C_q), 143.6 (2 × C_q), 151.8 (C_q), 156.0 (C_q), 162.3 (d, J_{CF} = 246.8 Hz, C_q), 175.3 (C_q); MS (EI) m/z (%): 208 (19), 196 (8), 179 (23), 178 (100), 177 (7), 176 (11), 166 (18), 165 (26), 88 (6), 76 (6); HRMS (EI): calcd. for $C_{30}H_{24}FNO_5$ [M^+]: 497.1638, found: 497.1638. Chiral analytical HPLC: t_R = 15.3 min; ee > 99%.

Microwave assisted Fmoc deprotection/acylation procedure for the synthesis of (11*S*)-3 and (11*R*)-3

The peptide synthesis was achieved starting from commercially available Fmoc-Leu-Wang resin (loading 0.41 mmol g^{-1}). Amino acids were incorporated as their commercially available derivatives in the following order: Fmoc-Ile-OH, Fmoc-FPTyr-OH [(*S*)-**12b** or (*R*)-**12b**], Fmoc-Pro-OH and two times Fmoc-Arg(Pbf)-OH. Fmoc deprotection was done by applying a *freshly prepared* solution of 20% piperidine in dry DMF and repetitive cycles of microwave irradiation (5 × 5 s, 100 W) in case of the resin bound Fmoc-Leu residue and after coupling of Fmoc-Ile-OH. For the other Fmoc deprotection steps, the resin was treated 2 × with the piperidine/DMF mixture for 2 min at rt without microwave irradiation. Thereafter, five washings with DMF were performed. Subsequent peptide coupling employing 5 eq of the corresponding Fmoc-amino acid/PyBOP/diisopropylethylamine and 7.5 eq HOBt, dissolved in a minimum amount of DMF was done. For the coupling of Fmoc-Ile-OH, irradiation with 15 × 10 s, 50 W was performed, for the other amino acids, the coupling reaction was performed at rt for 2 h. For the coupling of (*S*)-**12b** or (*R*)-**12b**, we employed building block/diisopropylcarbodiimide/1-hydroxy-7-azabenzotriazole (HOAt), each 3 eq, without microwave irradiation and a coupling time of 10 h. Acylation was monitored with the help of the Kaiser test. In case of the first Fmoc-Arg(Pbf)-OH introduction, the coupling procedure was performed two times. After the last acylation step the N-terminal Fmoc-residue was deprotected, the resin was ten-fold rinsed with CH_2Cl_2 and dried *in vacuo*. The cleavage from the resin was performed using a mixture of TFA/phenol/H_2O/triisopropylsilane 88 : 5 : 5 : 2 for 2 h. After evaporation of the solvent and precipitation in *tert*-butylmethylether, the crude peptide was purified using preparative RP-HPLC: eluent: MeOH (A) + 0.1% HCO_2H H_2O (B) applying a linear gradient 10–55% A in 90–45% B in 18 min, (11*S*)-**3**: t_r: 15.9 min, (11*R*)-**3**: t_r: 17.0 min. Subsequent lyophilization was performed to afford the peptide as the formate salt. Peptide purity and identity was assessed by analytical HPLC. System 1: 10–55% CH_3OH in 90–45% H_2O + 0.1% HCO_2H in 18 min, then 45–95% CH_3OH in 55–5% H_2O + 0.1% HCO_2H in 2 min, finally 95–95% in 5–5% H_2O + 0.1% HCO_2H in 2 min, system 2: 3–40% CH_3CN in 97–60% H_2O + 0.1% HCO_2H in 26 min, purity (11*S*)-**3**: system 1: > 99% (t_r: 19.1 min); system 2: purity > 99% (t_r: 19.1 min); MS (ESI): m/z calcd for $C_{44}H_{67}N_{12}O_8$: 911.5 [M + H]$^+$; found: 911.6. Purity (11*R*)-**3**: system 1: 98.6% (t_r: 20.4 min); system 2: purity >

99% (t_r: 21.0 min); MS (ESI): m/z calcd for $C_{44}H_{67}N_{12}O_8$: 911.6 $[M + H]^+$; found: 911.6.

Receptor binding experiments

Receptor binding data were determined according to protocols as described previously.[33–35] In detail, hNTR1 binding was measured using homogenates of membranes from Chinese hamster ovary (CHO) cells stably expressing the human NTR1 receptor at a final concentration of 2–4 μg/well and the radioligand [^{3}H]neurotensin (specific activity 116 Ci mmol^{-1}; Perkin Elmer, Boston, MA) at a concentration of 0.5 nM.[34] Specific binding of the radioligand was determined at a K_D value of 0.47–0.54 nM and a B_{max} of 5050–7025 fmol mg^{-1} protein. NTS2 binding was done using homogenates of membranes from human embryonic kidney cells (HEK 293), which were transiently transfected with the pcDNA3.1 vector containing the human NTS2 gene (UMR) by the calcium phosphate method. Membranes were incubated at a final concentration of 20 μg/well together with 0.5 nM of [^{3}H]NT(8–13) (specific activity 136 Ci mmol^{-1}; custom synthesis by GE Healthcare, Freiburg, Germany) at a K_D value of 0.86–1.14 nM and a B_{max} of 665–1380 fmol mg^{-1} protein.[33] Specific binding of the radioligand was determined at a K_D value of 0.47–0.54 nM and a B_{max} of 5050–7025 fmol mg^{-1} protein. Unspecific binding was determined in the presence of 10 μM neurotensin (for NTS1) or NT(8–13) (for NTS2) and the protein concentration was established by the method of Lowry using bovine serum albumin as standard.[36] Data analysis of the resulting competition curves was accomplished by non-linear regression analysis using the algorithms in PRISM (GraphPad Software, San Diego, CA). EC_{50} values derived from the resulting dose response curves were transformed into the corresponding K_i values utilizing the equation of Cheng and Prusoff.[37]

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