DEPBT as Coupling Reagent To Avoid Racemization in a Solution-Phase Synthesis of a Kyotorphin Derivative

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Abstract: The synthesis of IbKTP-NH_2 with reduction of racemization up to no detectable level has been achieved using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT). Among all coupling systems tested, only DEPBT-mediated amide bond formation between N^{α}-acylated tyrosine and arginine without loss of optical purity.

Key words: amide bond formation, coupling reagent, DEPBT, kyotorphin, racemization

Kyotorphin (KTP) is an endogenous dipeptide (Tyr-Arg)[1] found in the brain of several mammals, including humans.[2] Kyotorphin is a potent analgesic only when it is delivered directly to the central nervous system,[3] due to its reduced ability to cross the blood-brain barrier (BBB). As a part of our research oriented to enhance the BBB crossing properties of KTP, new kyotorphin derivatives were designed and synthesized. Unlike original KTP, these new peptides revealed to be highly analgesic following systemic administration,[4,5] and displayed safer side effects profile,[6] which make them a promising alternative to opioid drugs.

The compound IbKTP-NH_2 (**1**) was one of the kyotorphin derivatives that gave the best results. This new peptide **1** combines C-terminal amidation with chemical conjugation to ibuprofen at N-terminal position. It was synthesized at gram-scale following standard solution-phase peptide synthesis as depicted in Scheme 1. Briefly, the synthetic strategy started with the coupling reaction between (*S*)-ibuprofen and protected methyl L-tyrosinate followed by saponification of compound **2** to give the free carboxylic acid **3**. The latter was then coupled with L-arginine amide and the *tert*-butyl protecting group in the peptide **4** side chain was removed by treatment with TFA followed by repetitive lyophilization in aqueous HCl (3 times) in order to get the final IbKTP-NH_2 (**1**) as the hydrochloride salt. However, compound **1** was obtained as a diastereomeric mixture caused by the partial racemization at the tyrosine stereogenic carbon during the arginine coupling reaction step. As previously reported, the racemization was reduced until a diastereomeric ratio of 87:13 using the coupling reagent benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and a large excess of 1-hydroxybenzotriazole (HOBt) (Figure 1) as racemization suppressor at low temperature.[5] Nevertheless, we aimed to improve the optical purity of IbKTP-NH_2 (**1**) for further studies. In the present work, we describe our synthetic efforts to solve the racemization problem of compound **1**.

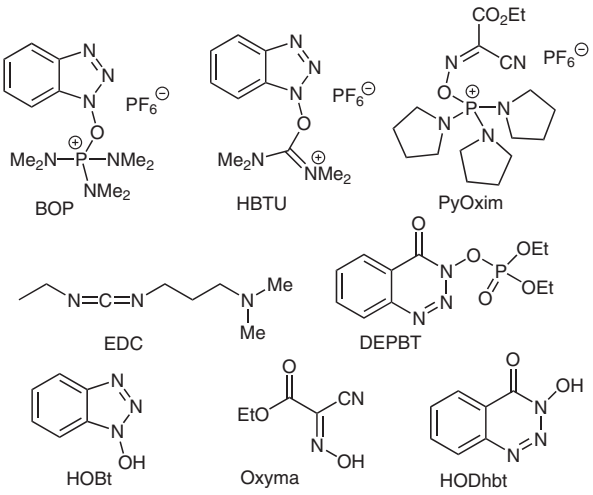

Figure 1 Coupling reagents and racemization suppressant agent structures

It is well established that during amide coupling reactions, racemization is prone to occur using N^{α}-acyl-protected amino acids than their N^{α}-carbamate-protected counterparts.[7] The significant amount of racemization produced during the amide bond formation between amino acid **3** and amidated arginine was probably due to the N^{α}-acyl group (ibuprofen) of the compound **3** (Scheme 1). In fact, in our previous synthesis of endogenous kyotorphin (KTP) and amidated kyotorphin (KTP-NH_2) derivative, no racemization was detected in the amide bond formation using BOP as coupling reagent. In this case, tyrosine was N^{α}-protected with Boc.[4]

The racemization pathway of N^{α}-acyl protected amino acids is well studied and it has been attributed to the facile formation of optically labile azlactone intermediates through the mechanism illustrated in Scheme 2. This mechanism involves a base-catalyzed ring closure to azlactone. The α-proton in the azlactone is rather acidic and in the presence of a base can be deprotonated and reprotonated causing its racemization. Both enantiomers of azlactone can acylate amines, but the resulting product

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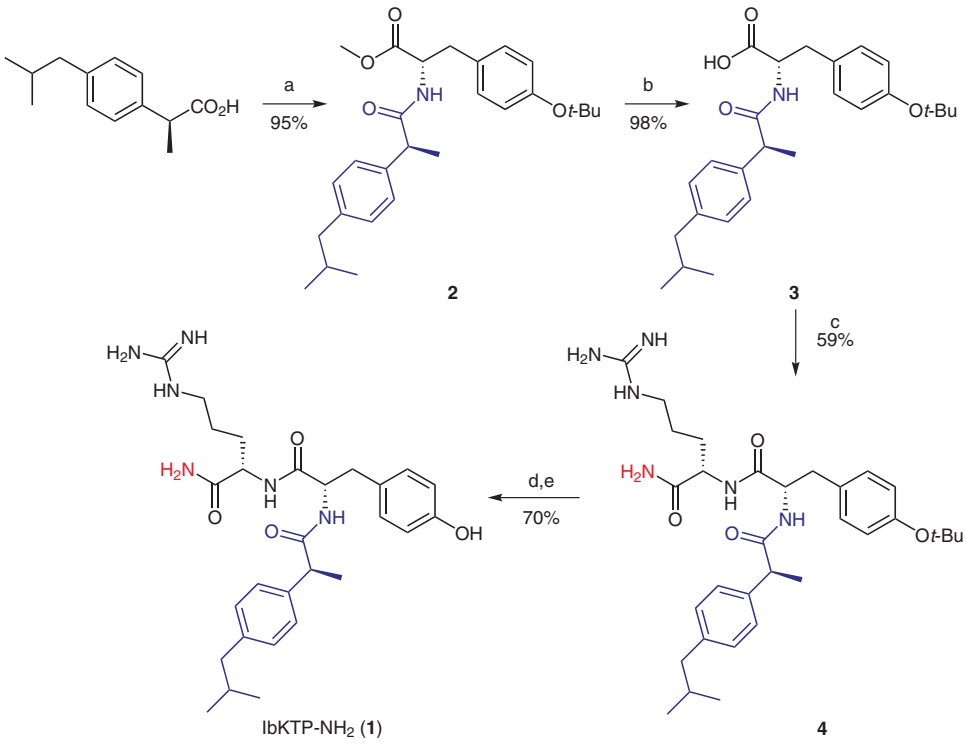
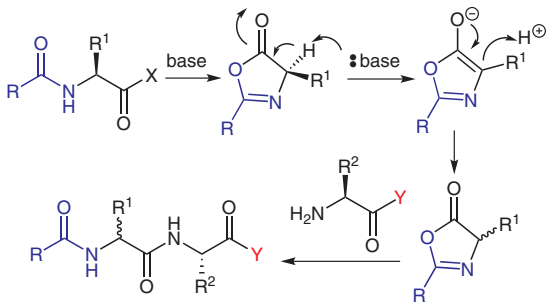


Scheme 1 Synthetic strategy for the preparation of IbKTP-NH_2 (**1**). *Reagents and conditions*: (a) H-Tyr(*t*-Bu)OMe (1 equiv), BOP (1.1 equiv), *N*-methylmorpholine (NMM, 3 equiv), DMF, r.t., 6 h; (b) LiOH (2.5 equiv) THF–MeOH–H_2O (1:2:2), r.t., overnight; (c) H-Arg-NH_2·2HCl (1 equiv), BOP (1 equiv), HOBt (6 equiv), NMM (3 equiv), DMF, –15 °C to r.t., 21 h; (d) TFA–DMF (1:1), r.t., 2 h; (e) HCl (1 M), lyophilization (3 times).

will be a mixture of epimeric peptides. Carbamate type N^{α}-protecting groups decrease the likelihood of azlactone formation.[7] However, in the intermediate **3**, the α-acyl group derivate from ibuprofen does not play the role of a protecting group because it is part of the compound structure and, consequently is not possible to change the acyl group by a carbamate group such as Boc. To circumvent the loss of optical integrity in the amide bond formation of intermediate **4**, we had to find the adequate coupling reaction, which could suppress completely the racemization.

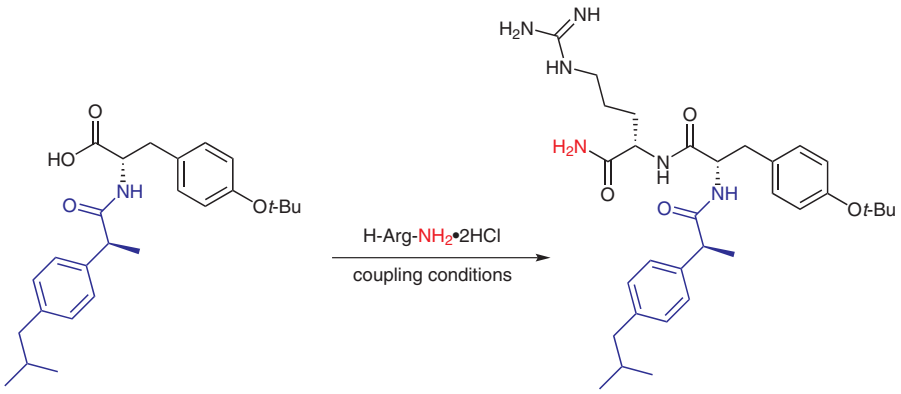


Scheme 2 Racemization of N^{α}-acylamino acid derivatives

As mentioned above, in a previous work, racemization of compound **4** was reduced until 87:13 ratio using the combination BOP/HOBt (Figure 1) at low temperature (Table 1, entry1).

Taking this result as a starting point, the use of HBTU and EDC (Figure 1) instead of BOP as coupling reagents was first examined in combination with the racemization suppressant HOBt. Both HBTU, an uronium-type coupling reagent, and EDC, a carbodiimide type coupling reagent, are commonly used in solution- and solid-phase peptide synthesis, and are remarkably efficient in terms of reactivity and racemization minimization.[8,9] Moreover, EDC has been largely used in solution synthesis of complex natural products; this reagent and its urea by-product are water soluble and facilitate product purifications.[10] In our hands, the use of these reagents gave compound **4** in good yield, but the racemization was more pronounced than observed previously (entries 2 and 3). Next, we investigated the use of 2-cyano-2-(hydroxyimino)acetate (Oxyma, Figure 1) as racemization suppressant instead of HOBt. Oxyma has been recently reported as a high efficient additive mainly in combination with carbodiimide-type coupling reagents for peptide bond formation both in solution and solid-phase synthesis.[11] Compared with HOBt, Oxyma showed greater capacity to suppress racemization and enhanced coupling rates. In addition, Oxyma is less hazardous than HOBt.[12] Thus, EDC in combination with Oxyma was examined as coupling reagent for the amide bond formation of dipeptide **4**. Nevertheless, the optical purity obtained for compound **4** was lower compared to our best result with BOP/HOBt and just a little bit better than those obtained with EDC/HOBt even when the reaction was performed at low temperature (entries 4 and 5).

Table 1 Study of the Coupling Conditions between Compound **3** and H-Arg-NH_2



3 **4**

Entry	Coupling reagent	Temp (°C)	Time (h)	Yield (%)[a]	dr[b]
1[c]	BOP (1 equiv) HOBt (6 equiv)	–15	21	59	87:13
2[d]	HBTU (1 equiv) HOBt (1 equiv)	0 to r.t.	1	73	68:32
3[d]	EDC (1 equiv) HOBt (1 equiv)	0 to r.t.	12	71	70:30
4[d]	EDC (2 equiv) Oxyma (2 equiv)	0 to r.t.	4	70	78:22
5[d]	EDC (2 equiv) Oxyma (2 equiv)	–15	12	71	80:20
6[d]	PyOxim (1equiv) Oxyma (1 equiv)	0 to r.t.	2	78	78:22
7[d]	DEPBT (1.1 equiv)	0 to r.t.	2	76	>98

[a] Yields of isolated products.
[b] Determined by ^{1}H NMR spectroscopy.
[c] Reaction was performed in DMF using NMM (3 equiv) as base.
[d] Reaction was performed in DMF using *i*-Pr_2NEt (DIPEA, 3 equiv) as base.

We also tested the viability of *O*-[(cyano(ethoxycarbonyl)methyliden)amino]yloxytri(pyrrolidino)phosphonium hexafluorophosphate (PyOxim, Figure 1) a phosphonium-type coupling reagent derived from Oxyma that was reported to exhibit higher control of optical purity over their HOBt counterparts such as BOP, in solution- as well as in solid-phase peptide synthesis.[13] In our case, the combination PyOxm/Oxyma as coupling reagent led to compound **4** in good yield, but with similar optical purity as the other tests (entry 6,). Later, our attention was focused on 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) developed by Ye et al. in the nineties.[14,15] DEPBT is a phosphonate-type coupling reagent derived from the racemization suppressant 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HODhbt, Figure 1).[16] This latter was developed contemporaneously with HOBt and other benzotriazole-type additives, but although proved to be superior to all in terms of coupling efficiency and inhibit racemization capacity,[17,18] its widespread use was limited due to ring-opening side reaction.[19,20] Even so, DEPBT demonstrated remarkable reactivity and reduces dramatically the degree of racemization in amide bond formation, even when a strong base is required,[21] especially in the solution synthesis of complex and racemization-sensitive natural products.[22,23] When our target coupling reaction was run with DEPBT in the absence of additives, dipeptide **4** was obtained in good yield. Comparison of ^{1}H NMR spectrum of compound **4** synthesized using BOP/HOBt with the one obtained using DEPBT clearly shows the elimination of signal splitting (Figure 2), and confirmed that coupling had occurred with no detectable epimerization (entry 7). This clearly shows the advantageous role of DEPBT on chiral integrity control of compound **4** over the other coupling systems tested. This significant performance of DEPBT in amide peptide bond formation and control on chiral integrity of compound **4** has to be related with the higher reactivity of the intermediate HODhbt and the nature of the diethyl phosphite ester involved in its formation, in agreement with the proposed DEPBT-mediated coupling mechanism.[15]

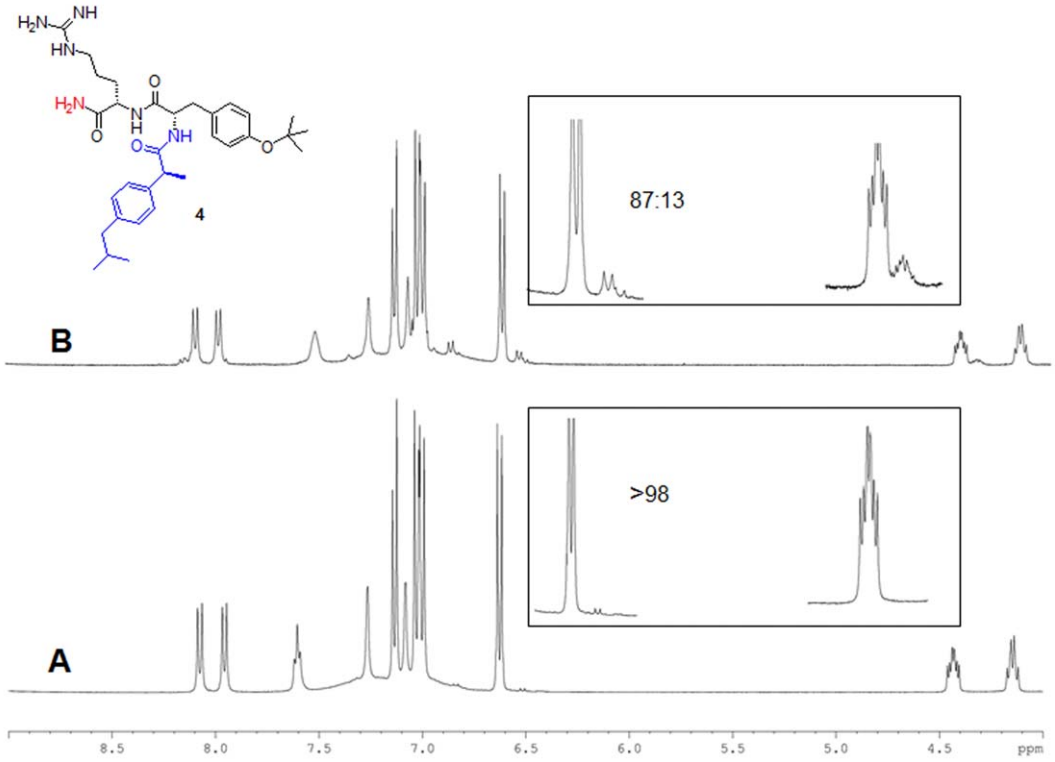
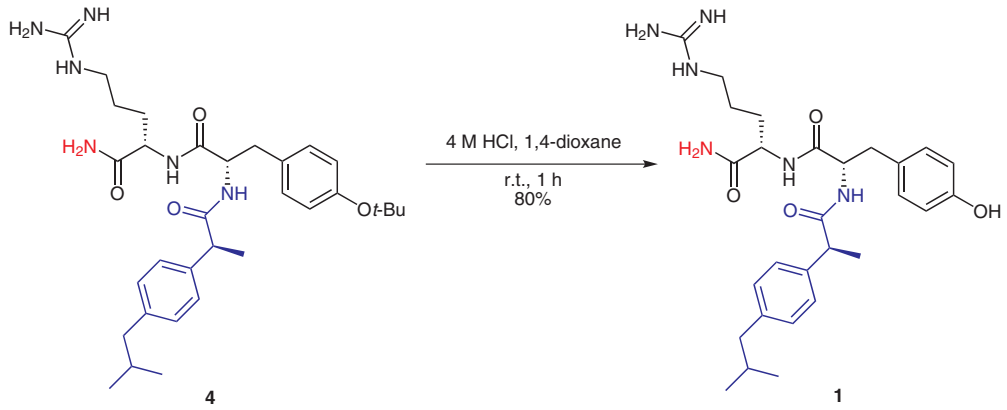


Figure 2 (A) ^{1}H NMR spectrum (region 4–9 ppm) of **4** synthesized with DEPBT. (B) ^{1}H NMR spectrum (region 4–9 ppm) of **4** synthesized with BOP/HOBt.

Finally, removal of *tert*-butyl protecting group of **4** by treatment with 4 M HCl in 1,4-dioxane instead of TFA (Scheme 1) directly provided the target kyotorphin derivative **1** as its hydrochloride salt in 80% yield (Scheme 3). This deprotection procedure improved the reaction yield and eliminated repetitive lyophilization steps with HCl necessary to exchange trifluoroacetate counterion from the final dipeptide **1**.

In conclusion, we have improved the synthesis of kyotorphin derivative **1** by reducing the racemization up to no detectable level at its key coupling reaction step; among all coupling reagent systems tested only DEPBT afforded the desired result. ^{1}H NMR spectra of kyotorphin derivative confirms the racemization-free coupling. Additionally, the final deprotection step of compound **4** has also been improved using 4 M HCl in 1,4-dioxane instead of TFA.

All commercially available chemicals were used as purchased without further purification. NMR spectra were recorded on a Bruker Ultrashield Avance III 400 spectrometer. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra and DEPT experiments were determined at 100 MHz. Spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for proton and 77.0 ppm for carbon. Spectra recorded in DMSO-d_6 were referenced to residual DMSO at 2.50 ppm for proton and 39.5 ppm for carbon. High resolution mass spectra (HRMS) were determined under conditions of



Scheme 3 Removal of *tert*-butyl group of compound **4**

electrospray ionization (ESI) on a Bruker Micro Q-Tof instrument using a hybrid quadrupole time-of-flight mass spectrometer. Analytical TLC was performed on precoated TLC plates, silica gel 60 F254 (Merck). The spots on the TLC plates were visualized with UV/visible light (254 nm) and/or with a solution of $KMnO_4$ (1.5 g/100 mL H_2O). HPLC analyses were carried out using a Dionex P680 instrument. Separations were achieved on an analytical C18 Kromasil reversed phase column (4.6 mm × 40 mm; 3.5 µm particle size). The compounds were eluted using a linear gradient of 0–100% MeCN in 0.1% TFA at flow rate of 1.0 mL/min. The absorbance was measured at 220 nm. The HPLC retention time of each compound was determined when the peak was at its maximum height.

Methyl (*S*)-2-[(*S*)-2-(4-Isobutylphenyl)propanamido]-3-(4-*tert*-butoxyphenyl)propanoate (2)

To a solution of (*S*)-ibuprofen (206 mg, 1 mmol) in DMF (4 mL) was added 0.33 mL (2 mmol) of NMM, and the resulting mixture was allowed to stir for 0.5 h at r.t. Next, BOP (442 mg, 1.1 mmol) and H-Tyr(*t*-Bu)-OMe·HCl (288 mg, 1 mmol) were added successively. The resulting reaction mixture was stirred overnight at r.t. Upon completion of the reaction (TLC, eluent: *n*-hexane–EtOAc, 1:1), the reaction mixture was diluted with EtOAc (15 mL). The resulting solution was washed with sat. aq $NaHCO_3$ (3 × 7 mL), H_2O (10 mL), 1 M aq $KHSO_4$ (3 × 7 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by SiO_2 flash chromatography (*n*-hexane–EtOAc, 10:1 to 5:1) to afford compound **2**; yield: 417 mg (95%); colorless oil; R_f = 0.41 (*n*-hexane–EtOAc, 1:1); HPLC: t_R = 10.16 min; 99.0% pure.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 [d, *J* = 6.4 Hz, 6 H, $CH(CH_3)_2$], 1.32 [s, 9 H, $C(CH_3)_3$], 1.48 (d, *J* = 7.2 Hz, 3 H, CH_3-$\beta_{ibuprofen}$), 1.85 [nonet, *J* = 6.7 Hz, 1 H, $CH(CH_3)_2$], 2.45 (d, *J* = 7.2 Hz, 2 H, $ArCH_{2ibuprofen}$), 2.94 (dd, *J* = 13.9 Hz, *J*′ = 6.2 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 3.02 (dd, *J* = 13.9 Hz, *J*′ = 5.7 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 3.52 (q, *J* = 7.2 Hz, 1 H, CH-$\alpha_{ibuprofen}$), 3.64 (s, 3 H, OCH_3), 7.75 (app q, *J* = 5.9 Hz, 1 H, CH-$\alpha_{tyrosine}$), 5.76 (d, *J* = 7.7 Hz, 1 H, NH), 6.79–6.84 (m, 4 H, CH_{aryl}), 7.09 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}), 7.14 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.1 (q), 22.3 (q, 2 C), 28.8 (q, 3 C), 30.1 (d), 37.0 (t), 45.0 (t), 46.6 (d), 52.1 (q), 53.1 (d), 78.3 (s), 124.1 (d, 2 C), 127.3 (d, 2 C), 129.52 (d, 2 C), 129.53 (d, 2 C), 130.5 (s), 137.6 (s), 140.7 (s), 154.3 (s), 171.8 (s), 174.0 (s).

HRMS (ESI): *m*/*z* calcd for $C_{22}H_{37}N_3O_6$ + Na $[M + Na]^+$: 462.2575; found: 462.2577; *m*/*z* calcd for $C_{22}H_{38}N_3O_6$ $[M + H]^+$: 440.2755; found: 440.2739.

(*S*)-2-[(*S*)-2-(4-Isobutylphenyl)propanamido]-3-(4-*tert*-butoxyphenyl)propanoic Acid (3)

To a solution of compound **2** (395 mg, 0.9 mmol) in THF–MeOH–H_2O (1:2:2, 7 mL) was added LiOH·H_2O (94.5 mg (2.25 mmol) and the resulting mixture was stirred for 1 h at r.t. Upon completion of the reaction (TLC, eluent: *n*-hexane–EtOAc, 1:1), the pH of the solution was adjusted to 2 with the addition of aq 1 M HCl. The resulting solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude material was triturated with *n*-pentane (4 mL), filtered, washed with *n*-pentane (1 mL), and dried to obtain compound **3**; yield: 375 mg (98%); colorless solid; mp 57–58 °C; R_f = 0.18 ($CHCl_3$–MeOH–NH_3, 12:2:0.2); HPLC: t_R = 9.41 min, 99.9% pure.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.84 [d, *J* = 6.6 Hz, 6 H, $CH(CH_3)_2$], 1.14 (d, *J* = 7.0 Hz, 3 H, CH_3-$\beta_{ibuprofen}$), 1.26 [s, 9 H, $C(CH_3)_3$], 1.79 [nonet, *J* = 6.7 Hz, 1 H, $CH(CH_3)_2$], 2.39 (d, *J* = 7.1 Hz, 2 H, $ArCH_{2ibuprofen}$), 2.80 (dd, *J* = 13.7 Hz, *J*′ = 9.9 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 3.02 (dd, *J* = 13.8 Hz, *J*′ = 4.7 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 3.58 (q, *J* = 7.0 Hz, 1 H, CH-$\alpha_{ibuprofen}$), 4.39 (ddd, *J* = 9.7 Hz, *J*′ = 8.5 Hz, *J*″ = 4.7 Hz, 1 H, CH-$\alpha_{tyrosine}$), 6.84 (d, *J* = 8.5 Hz, 2 H, CH_{aryl}), 7.04 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}), 7.08 (d, *J* = 8.5 Hz, 2 H, CH_{aryl}), 7.15 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}), 8.21 (d, *J* = 8.4 Hz, 1 H, NH), 12.63 (br, 1 H, OH).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 18.7 (q), 22.2 (q, 2 C), 28.5 (q, 3 C), 29.6 (d), 36.1 (t), 44.2 (t), 44.3 (d), 53.2 (d), 77.6 (s), 123.4 (d, 2 C), 127.1 (d, 2 C), 128.6 (d, 2 C), 129.7 (d, 2 C), 132.3 (s), 139.0 (s), 139.1 (s), 153.4 (s), 173.0 (s), 173.2 (s).

HRMS (ESI): *m*/*z* calcd for $C_{26}H_{35}NO_4$ + Na $[M + Na]^+$: 448.2458; found: 448.2476; *m*/*z* calcd for $C_{26}H_{36}NO_4$ $[M + H]^+$: 426.2639; found: 426.2653.

Ibu-Tyr(*t*-Bu)-Arg-NH_2 (4)

To a solution of compound **3** (340 mg, 0.8 mmol) in DMF (4 mL) was added DIPEA (0.43 mL, 2.5 mmol) and the resulting mixture was allowed to stir for 0.5 h at 0 °C. Next, DEPBT (264 mg, 0.88 mmol) and H-Arg-NH_2·2HCl (197 mg, 0.8 mmol) were added successively. The resulting reaction mixture was stirred 1 h at 0 °C and the stirring was continued at r.t. for another hour. Upon completion of the reaction (HPLC monitoring), the reaction mixture was diluted with EtOAc (15 mL). The resulting solution was washed with aq 1 M HCl (3 × 7 mL), sat. aq $NaHCO_3$ (3 × 7 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to obtain compound **4**; yield: 353 mg (76%); colorless solid; mp 122–124 °C; HPLC: t_R = 7.96 min, 98.0% pure.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.84 [d, *J* = 6.6 Hz, 6 H, $CH(CH_3)_2$], 1.10 (d, *J* = 7.0 Hz, 3 H, CH_3-$\beta_{ibuprofen}$), 1.25 [s, 9 H, $C(CH_3)_3$], 1.35–1.55 (m, 3 H of $CH_2CH_{2arginine}$), 1.61–1.66 (m, 1 H of $CH_2CH_{2arginine}$), 1.78 [nonet, *J* = 6.7 Hz, 1 H, $CH(CH_3)_2$], 2.37 (d, *J* = 7.0 Hz, 2 H, $ArCH_{2ibuprofen}$), 2.72 (dd, *J* = 13.9 Hz, *J*′ = 9.9 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 2.99–3.08 (m, 3 H, CH_2NH and 1 H of the CH_2-$\beta_{tyrosine}$), 3.59 (q, *J* = 7.0 Hz, 1 H, CH-$\alpha_{ibuprofen}$), 4.14 (q, *J* = 7.8 Hz, 1 H, CH-$\alpha_{arginine}$), 4.48–4.54 (m, 1 H, CH-$\alpha_{tyrosine}$), 6.83 (d, *J* = 8.4 Hz, 2 H, CH_{aryl}), 7.00–7.06 (m, 2 H, D_2O exch. and 2 H, CH_{aryl}), 7.08 (br, 2 H, D_2O exch.), 7.13–7.16 (m, 4 H, CH_{aryl}), 7.35 (br, 1 H, D_2O exch.), 7.68 (t, *J* = 5.2 Hz, 1 H, NH, D_2O exch.), 8.03 (d, *J* = 8.0 Hz, 1 H, NH, D_2O exch.), 8.21 (d, *J* = 8.2 Hz, 1 H, NH, D_2O exch.).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 18.5 (q), 22.2 (q, 2 C), 24.9 (t), 28.5 (q, 3 C), 29.2 (t), 29.6 (d), 36.8 (t), 39.9 (t), 44.2 (t), 44.4 (d), 51.9 (d), 53.9 (d), 77.5 (s), 123.3 (d, 2 C), 127.1 (d, 2 C), 128.6 (d, 2 C), 129.8 (d, 2 C), 132.7 (s), 139.0 (s), 139.1 (s), 153.3 (s), 156.9 (s), 171.1 (s), 173.1 (s), 173.3 (s).

HRMS (ESI): *m*/*z* calcd for $C_{32}H_{49}N_6O_4$ $[M + H]^+$: 581.3810; found: 581.3811.

Ibu-Tyr-Arg-NH_2 (1)

Compound **4** (320 mg, 0.55 mmol) was dissolved in 4 M HCl in 1,4-dioxane (15 mL) and stirred for 1 h at r.t. After completion of the reaction (HPLC monitoring), the solvent was evaporated in vacuo and the resulting residue was triturated with Et_2O (15 mL), filtered, washed with Et_2O (2 × 10 mL) and *n*-pentane (2 × 10 mL), and dried to obtain compound **1**; yield: 230 mg (80%); colorless solid; mp 140–142 °C; HPLC: t_R = 7.32 min, 99.9% pure.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.84 [d, *J* = 6.8 Hz, 6 H, $CH(CH_3)_2$], 1.15 (d, *J* = 7.2 Hz, 3 H, CH_3-$\beta_{ibuprofen}$), 1.34–1.52 (m, 3 H of the $CH_2CH_{2arginine}$), 1.60–1.68 (m, 1 H of the $CH_2CH_{2arginine}$), 1.78 [nonet, *J* = 6.7 Hz, 1 H, $CH(CH_3)_2$], 2.37 (d, *J* = 7.1 Hz, 2 H, $ArCH_{2ibuprofen}$), 2.65 (dd, *J* = 13.8 Hz, *J*′ = 9.5 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 2.91 (dd, *J* = 13.8 Hz, *J*′ = 4.3 Hz, 1 H of the CH_2-$\beta_{tyrosine}$), 3.03 (app q, *J* = 6.5 Hz, 2 H, CH_2NH), 3.60 (q, *J* = 6.9 Hz, 1 H, CH-$\alpha_{ibuprofen}$), 4.15 (app q, *J* = 7.6 Hz, 1 H, CH-$\alpha_{arginine}$), 4.41–4.46 (m, 1 H, CH-$\alpha_{tyrosine}$), 6.63 (d, *J* = 8.4 Hz, 2 H, CH_{aryl}), 7.0 (d, *J* = 8.4 Hz, 2 H, CH_{aryl}), 7.02 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}), 7.08 (br, 1 H, D_2O exch.), 7.13 (d, *J* = 8.1 Hz, 2 H, CH_{aryl}), 7.27 (br, 2 H, D_2O exch.), 7.61 (t, *J* = 5.5 Hz, 1 H, NH, D_2O exch.), 7.95 (d, *J* = 8.0 Hz, 1 H, NH, D_2O exch.), 8.07 (d, *J* = 8.3 Hz, 1 H, NH, D_2O exch.), 9.20 (br, 1 H, D_2O exch.).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 18.7 (q), 22.2 (q, 2 C), 25.0 (t), 29.3 (d), 29.7 (t), 36.6 (t), 40.4 (t), 44.3 (d), 44.4 (t), 51.9 (d), 54.1 (d), 114.8 (d, 2 C), 127.1 (d, 2 C), 127.9 (s), 128.7 (d, 2 C), 130.2 (d, 2 C), 139.1 (s), 139.2 (s), 155.8 (s), 156.8 (s), 171.2 (s), 173.2 (s), 173.5 (s).

HRMS (ESI): *m*/*z* calcd for $C_{28}H_{41}N_6O_4$ $[M + H]^+$: 525.3184; found: 525.3188.

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