JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 217–229.
DOI: 10.1002/jlcr.547

Research Article

C-Terminal ^{18}F-fluoroethylamidation exemplified on [Gly-OH^{9}] oxytocin†

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Summary

The no-carrier-added (n.c.a.) ^{18}F-fluoroethylamidation of the acid function of the protected nonapeptide Boc–Cys–Tyr(tBu)–Ile–Gln(Mtt)–Asn(Mtt)–Cys–Pro–Leu–Gly–OH forming the labelled peptide hormone derivative [Gly-(2-[^{18}F]fluoroethyl)NH^{9}]-oxytocin is described. The labelling conditions were elaborated using a protected tripeptide, identical to the C-terminal sequence of oxytocin. The prosthetic group n.c.a. 2-[^{18}F]fluoroethylamine was synthesised via cryptate mediated n.c.a. ^{18}F-fluorination of *N*-Boc-2-(*p*-toluenesulfonyloxy) ethylamine in DMSO (RCY: ca. 60%) and subsequent deprotection with a radiochemical yield of $46 \pm 5\%$. [^{18}F]Fluoroethylamine was reacted with Z–Pro–Leu–Gly–OH in presence of the coupling reagent TBTU or with activated esters of the model-tripeptide. The activated ester method as well as the condensation in presence of TBTU yielded $\geqslant 90\%$ of the ^{18}F-fluoroethyl-amidated tripeptide. TBTU-mediated condensation of n.c.a. 2-[^{18}F]fluoro-ethylamine with the C-terminal free acid group of protected oxytocin gave the radiochemical yield of about 75%. Deprotection under acidic conditions led to the formation of [Gly–(2-[^{18}F]fluoroethyl)NH^{9}]oxytocin within 75 min with a radiochemical yield of about 30% as measured by analytical HPLC. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: nucleophilic ^{18}F-fluorination; ^{18}F-prosthetic group; n.c.a. 2-[^{18}F]fluoroethylamine; ^{18}F-fluoroethylamidation; oxytocin

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Received 6 April 2001
Revised 15 October 2001
Accepted 27 October 2001

Introduction

Endogenous oxytocin acts as labour inducing and milk ejecting peptide hormone and it is the most significant pharmaceutical in obstetrics.[1] It plays an important role in the sexual and social behaviour of both sexes. Besides its responsibility for sexual receptivity, maternal behaviour and care of the brood, it also influences the choosing of a sexual partner and, in a general way, the development of social bindings.[2]

Our aim was to develop an efficient labelling method of this hormone by using a small prosthetic group and a labelling position outside the pharmacophoric group. A future goal will be to study non-invasively the pharmacokinetics and *in vivo* distribution of this hormone, especially the possibility of its uptake in the brain.

It is known, that a derivatisation of the N-terminus of the peptide results in a significant change of the biological activity[3] and that the cyclic ring system, generated by a disulfide bridge between the two cysteine-residues, is essential for the biological activity (see Figure 1).[4] Consequently a modification of the C-terminal amide function of oxytocin by a small prosthetic group appeared as the only attractive alternative for avoiding a major change of its biochemical behaviour. Therefore, it was decided to substitute the amide function by a [^{18}F]fluoroethylamide group. *N*-methylation with [^{11}C]methyl iodide was not chosen due to the short half-life of carbon-11 and the resulting limited time frame for pharmacokinetic studies. The radioiodination of

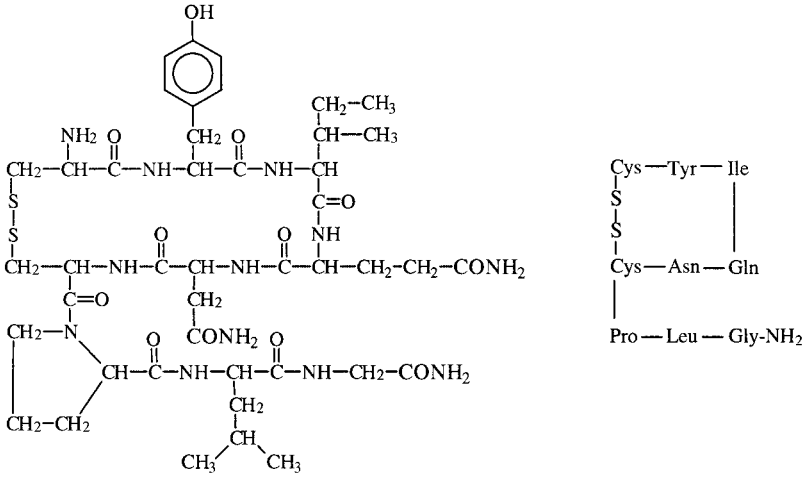


Figure 1. Formula of oxytocin

the tyrosine-residue was avoided since [*o*-iodo-Tyr^2]oxytocin is known as an inhibitor of the oxytocin-receptor.[4,5]

N-Boc-2-[^{18}F]fluoroethylamine has previously been synthesised by Guhlke[6] using *N*-Boc-2-bromoethylamine as precursor and by Gilissen *et al.*[7] using *N*-Boc-2-(*p*-toluenesulfonyloxy)ethylamine. Whereas Gilissen reached 2% of 2-[^{18}F]fluoroethylamine by deprotecting *N*-Boc-2-[^{18}F]fluoroethylamine and distilling the product, he achieved 39 $\pm$ 6% by producing the amine from *N*-(2-[^{18}F]fluoroethyl)phthalimide.[7] Tewson reported the ^{18}F-fluoroethylamidation of 2-nitroimidazole acetic acid via its tetrafluorophenyl ester with a radiochemical yield of 95%, whereas the reaction of the *N*-hydroxy succinyl ester of 2-nitroimidazole acetic acid resulted only in low radiochemical yields.[8] Unfortunately, no information was given about the radiochemical yield of 2-[^{18}F]fluoroethylamine.

In this work, the radiochemical yield of *N*-Boc-2-[^{18}F]fluoroethylamine via ^{18}F-fluorination of *N*-Boc-2-(*p*-toluenesulfonyloxy)ethylamine was optimised. Before labelling oxytocin, ^{18}F-fluoroamidation was performed using the model-tripeptide Z–Pro–Leu–Gly–OH with an identical C-terminal amino acid sequence. In context with this developmental work in the MBq range all radiochemical yields were determined on an analytical scale.

Experimental

Materials

DMSO (dimethyl sulfoxide), DMF (*N*,*N*-dimethylformamide), DMAP (4-dimethylaminopyridine), DMAA (*N*,*N*-dimethylacetamide) *p*-nitrophenol, pentafluorophenol, TBTU (*O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate), BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate), WSC (1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide), HOBT (*N*-hydroxybenzotriazole) and TFA (trifluoroacetic acid) were purchased from Fluka, *N*-hydroxysuccinimide, DCC (dicyclohexylcarbodiimide), *N*-Boc-2-aminoethanol and 2-fluoroethylamine hydrochloride from Aldrich. Acetonitrile, diethylether, dioxane, Kryptofix® 2.2.2. and triethylamine were delivered by Merck. Z–Pro–Leu–Gly–OH (Z: benzyloxy-carbonyl), Boc–Cys–Tyr(tBu)–Ile–Gln(Mtt)–Asn(Mtt)–Cys–Pro–Leu–Gly–OH (Boc: *tert*.-butoxycarbonyl, tBu: *tert*.-butyl,

Mtt: 4-methyltrityl) and Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly–*N*–(2-fluoroethyl)amide were obtained from Bachem (Heidelberg, Germany).

Dioxane was refluxed over lithium aluminum hydride for 30 min and distilled afterwards. Triethylamine was also purified by distillation. Pentafluorophenol was dissolved in DMF and dried over sodium sulfate. Thin layer chromatography was carried out on precoated plates of silica gel 60 F 254 (Merck). ^{1}H- and ^{19}F-NMR spectra were recorded on a Bruker Avance 200 apparatus at 200 MHz. Chemical shifts are reported in ppm relative to TMS ($\delta = 0$). Mass spectra were performed with a Finnigan mass spectrometer using Atmospheric Pressure Chemical Ionisation (APCI). IR spectra (KBr pellet) were recorded using a Shimadzu (IR-440) spectrometer. Analytical HPLC was performed on the following system: HPLC-pump (Merck, Hitachi 655A-11), UV/VIS-detector (Merck, Hitachi 655A) with variable wavelength and a radioactivity detector (EG&G Ortec, model 276). Melting points were determined on a Gallenkamp apparatus and are reported uncorrected.

N-Boc-2-(p-toluenesulfonyloxy)ethylamine[7]

N-Boc-2-ethanolamine (1.10 g, 6.80 mmol) was reacted with *p*-toluenesulfonyl chloride (1.41 g, 7.40 mmol). After stirring for 6 h at 0°C the reaction mixture was kept in the refrigerator overnight before mixing with 30 ml of ice-cold water. The aqueous phase was extracted with dichloromethane, the combined organic layers were washed with 0.25 N hydrochloric acid (2 × 10 ml) and water (2 × 10 ml) and dried over sodium sulphate. The solvent was evaporated and the residue was purified by column chromatography with hexane/diethylether (1 : 2 v/v) yielding 1.13 g (52.7%, Lit. 30.7%) of *N*-Boc-2-(*p*-toluenesulphonyloxy)ethylamine.

mp: 65°C; Lit. 63–65°C

^{1}H-NMR: s. Reference 7

IR (KBr, cm^{-1}): ν(N–H) 3410 (s); ν(H–C arom.) 3055 (w);ν(C=O) 1712 (s); ν(C=C) 1597 (m); ν_{as} (-SO_2O-) 1354 (s); ν(CH_3 Boc) 1267 (s); ν_{sym}(-SO_2O-) 1177 (s)

Mass spectrum (m/z): 216 $[M\text{-Boc} + H]^+$.

N-Boc-2-fluoroethylamine

Di-*tert*.-butyl dicarbonate (1.47 g, 6.78 mmol) was added to a solution of 2-fluoroethylamine hydrochloride (0.5 g, 5.02 mmol) and triethyla-

mine (1.53 g, 14.15 mmol) in DMF (20 ml). After stirring for 6 h at 60°C the reaction mixture was diluted with water (50 ml) and extracted with diethylether (4 × 20 ml). The combined organic layers were washed with 0.25 N hydrochloric acid (2 × 15 ml) and water (2 × 15 ml) and dried over sodium sulphate. The solvent was evaporated and the residue purified on silica gel with hexane/diethylether (1 : 1 v/v) to yield an oil, which was kept in the refrigerator over night for crystallization (0.62 g, 76.0%, Lit. 77%).

mp = 54–56°C

^{1}H-NMR: s. Reference 7

^{19}F-NMR ($CDCl_3$): δ −224.89 (tt, 1F); $^{2}J_{(1H/19F)}$ = 47 Hz, $^{1}J_{(1H/19F)}$ = 28 Hz

IR (KBr, cm^{-1}): ν(N–H) 3440 (s); ν(C=O) 1714 (s); ν(C–F) 1368 (s); ν(CH_3 Boc) 1250 (s)

Z–Pro–Leu–Gly–N–succinimidyl ester

N-Hydroxysuccinimide (90 mg, 0.72. mmol) was added to a solution of *Z*–Pro–Leu–Gly–OH (300 mg, 0.72 mmol) in dioxane (4 ml). While the solution was cooled with ice, dicyclohexylcarbodiimide (155 mg, 0.79 mmol) was added. After 15 min at 0°C stirring was continued at room temperature overnight. The dicyclohexylurea was filtered off, and the solvent was removed under reduced pressure. The colourless oil was crystallized from dichloromethane/petroleumether (250 mg, 67.7%).

mp: 184°C

^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 6H), 1.60 (m, 2H), 2.20 (m, 4H), 2.85 (s, 4H, succinimidyl), 3.60 (m, 4H), 3.90 (m, 2H), 4.38 (m, 3H), 4.25 (m, 1H), 5.18 (s, 2H), 6.70 (m, 2H), 7.39 (s, 5H)

IR (KBr, cm^{-1}): ν (N–H) 3495 (m); ν_{as}(N–H) 3285 (s); ν(C–H arom.) 3065 (w); ν(C=O, Ester) 1730 (s); ν(C=O, Pro) 1692 (s); ν(C=O, Gly) 1644 (s); ν(C–O) 1425 (s); ν(C–N) 1359 (s); ν_{as}(C–O–N) 1202 (s)

Mass spectrum (*m*/*z*): 517 $[M + H]^+$.

Z–Pro–Leu–Gly–p–nitrophenyl ester

Dicyclohexylcarbodiimide (165 mg, 0.80. mmol) was added to a cooled solution of Z–Pro–Leu–Gly–OH (300 mg, 0.72 mmol) and *p*-nitrophenol (110 mg, 0.80 mmol) in tetrahydrofuran (4 ml). After cooling for 15 min at 0°C stirring was continued at room temperature overnight.

The suspension was filtered, and the solvent was removed under reduced pressure. The product was crystallized from DMF/water (104 mg, 65%).

mp: 164°C

^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 6 H), 1.60 (m, 2H), 2.20 (m, 4H), 3.60 (m, 4H), 3.90 (m 2H), 4.38 (m, 3H), 4.25 (m, 1H), 5.18 (s, 2H), 6.70 (m, 2H), 7.37 (s, 7H), 8.30 (m, 2H);

IR (KBr, cm^{-1}): ν_{as}(N–H) 3285 (s); ν(C–H, arom.) 3070 (w); ν(C=O, Ester) 1764 (m); ν(C=O) 1704 (s); ν(C=O 1643 (s, Gly); ν(N–H) 1545 (s); ν(C–O) 1422 (s)

mass spectrum (m/z): 540 $[M + H]^+$.

Z–Pro–Leu–Gly–pentafluorophenyl ester

Dicyclohexylcarbodiimide (0,1 g, 0.49 mmol) was added to an ice cold solution of Z–Pro–Leu–Gly–OH (0.2 g, 0.48 mmol) and pentafluorophenol (95 mg, 0.52 mmol) in 1 ml DMF, and the reaction mixture was stirred for 1 h at room temperature. After filtration the solvent was removed under reduced pressure. The product was crystallized from hexane (243 mg, 87%).

mp = 138°C

^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 6 H), 1.60 (m, 2H), 2.20 (m, 4H), 3.60 (m, 4H), 3.90 (m 2H), 4.38 (m, 3H), 4.25 (m, 1H), 5.18 (s, 2H), 6.70 (m, 2H), 7.38 (s, 5H)

^{19}F-NMR ($CDCl_3$): $\delta -152.44$ (m, 2F), -157.82 (m, 2F), -162.41 (m, 1F)

IR (KBr, cm^{-1}): ν_{as}(N–H) 3290 (s); ν(C–H, arom.) 3065 (w); ν(C=O, Ester) 1796 (s); ν(C=O) 1706 (s); ν(C=O) 1639 (s, Gly); ν(N–H) 1543 (s); ν(C–O) 1418 (s); ν(C–F) 1311 (w)

mass spectrum (m/z): 586 $[M + H]^+$.

Z–Pro–Leu–Gly–2–fluoroethylamide

Z–Pro–Leu–Gly–OH (300 mg, 0.72 mmol) and TBTU (235 mg, 0.73 mmol) were dissolved in 1 ml DMF. Ethyldiisopropylamine (370 µl) and 2-fluoroethylamine hydrochloride (72 mg, 0.72 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The solvent was removed under reduced pressure and the oily residue was purified by preparative HPLC (RP-18, 15×230 mm, acetonitrile/water (acetic acid 0.1%, pH 5) (35/65) (v/v), flow: 10 ml/min). The solvent was removed under reduced pressure, and the product was

extracted with glacial acid and precipitated with diethylether to yield 191 mg (58%).

mp: 154–156°C

^{1}H-NMR (DMSO-d_6): δ–0.90 (m, 6H), 1.50 (m, 3H), 1.83 (m, 3H), 2.20 (m, 1H), 3.45 (m, 6H), 3.70 (m, 2H), 4.28 (m, 2H), 4.5 (m, 1H), 5.08 (s, 2H), 7.35 (s, 5H), 8.10 (m, 2H)

^{19}F-NMR (DMSO-d_6): δ–221.89 (tt, 1F); $^{2}J_{(1H/19F)} = 47$ Hz, $^{1}J_{(1H/19F)} = 28$ Hz

IR (KBr, cm^{-1}): ν_{as}(N–H) 3300 (s); ν(C–H, arom.) 3065 (w); ν(C=O) 1710 (s); ν(C=O) 1649 (s, Gly); ν(N–H) 1543 (s); ν(C–O) 1415 (s); ν(C–F) 1306 (w)

mass spectrum (m/z): 465 $[M + H]^+$.

N.c.a.2-[^{18}F]fluoroethylammonium trifluoroacetate

N.c.a. [^{18}F] fluoride was produced by standard procedures at the JSW-BC1710 Cyclotron of FZ-Jülich.[9,10] After azeotropic drying of the [^{18}F]fluoride containing the aminopolyether complex Kryptofix® [2.2.2.]/K_2CO_3 by anhydrous acetonitrile,[11] a solution of *N*-Boc-2-(*p*-toluenesulfonyloxy)ethylamine (5 mg) in anhydrous DMSO (250 µl) was added. The solution was heated 10 min at 140°C before adding DMAP (10 mg) in anhydrous DMSO (100 µl). The quaternisation was performed at 100°C for 15 min. After cooling at room temperature, the reaction mixture was diluted with 0.1 N hydrochloric acid (3.15 ml) and then passed through a C18 Sep Pak column (Waters). The column was first rinsed with 0.01 N hydrochloric acid (10 ml) and then washed with water (5 ml). The radioactive product was eluted with ether (2 ml). The solvent was removed under reduced pressure, trifluoroacetic acid (0.3 ml) was added and evaporated after 2 min reaction time.

N.c.a. Z–Pro–Leu–Gly–(2–[^{18}F]fluoroethyl)NH

Method A.

A 0.095 N solution (250 µl) of one of the above mentioned active esters of *Z*–Pro–Leu–Gly–OH in DMF and triethylamine (10 µl) was added to n.c.a. 2-[^{18}F]fluoroethylammonium trifluoroacetate and stirred at room temperature for 1 min.

Method B.

A solution of Z–Pro–Leu–Gly–OH (2.5. mg, 6 µmol), TBTU (2 mg, 6 µmol) and triethylamine (3 µl) in DMF (250 µl) was added to n.c.a.

2-[^{18}F]fluoroethylamine trifluoroacetate and stirred at room temperature. For optimising the reaction parameters, the type of coupling reagent, the concentration of the peptide and the reaction time were varied.

N.c.a. [Gly-(2-[^{18}F]fluoroethyl)NH^{9}]oxytocin

A given amount of Boc–Cys–Tyr(tBu)–Ile–Gln(Mtt)–Asn(Mtt)–Cys–Pro–Leu–Gly–OH (optimally 5 mg, 3 μmol), TBTU (1 mg, 3 μmol) and triethylamine (2 μl) were dissolved in DMF (250 μl), added to n.c.a. 2-[^{18}F]fluoroethylammonium trifluoroacetate and stirred at room temperature. After a given time the reaction mixture was diluted with 0.1 N hydrochloric acid and passed through a C-18 Sep Pak cartridge (Waters). The cartridge was washed with 0.01 N hydrochloric acid and water in order to remove residual 2-[^{18}F]fluoroethylamine. The labelled peptide was eluted with acetonitrile and evaporated to dryness. Deprotection of the oxytocin derivative was subsequently performed with trifluoroacetic acid (0.2 ml) within 1 min at room temperature.

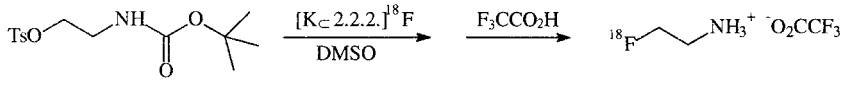
HPLC of Z–Pro–Leu–Gly–2-[^{18}F]fluoroethylamide and [Gly-(2-[^{18}F]fluoroethyl)–NH^{9}]oxytocin

The following HPLC-conditions were used for Z–Pro–Leu–Gly–(2-[^{18}F]fluoroethyl-amide: LiChrosorb RP 8−5μ, 250 × 4 mm, acetonitrile/phosphate-buffer pH7 (40/60) (v/v), 1 ml/min, k' (Z–Pro–Leu–Gly–2–[^{18}F]fluoroethylamide) = 2.13, k' (2-[^{18}F]fluoroethylamine) = 0.36. In the case of [Gly–(2-[^{18}F]fluoroethyl)–NH^{9}]oxytocin the trifluoroacetic acid was evaporated after the deprotection step and the residue dissolved in 0.2 ml of acetonitrile/water (10/90). An aliquot (50 μl) was analysed by gradient HPLC: Nucleosil 120−5μ C4, 250 × 4 mm, gradient: 1 min acetonitrile/water (10/90), 20 min 50% acetonitrile, k'[Gly-(2-[^{18}F]fluoroethyl)-NH^{9}]oxytocin = 4.24, k' (free acid) = 3.63.

Results and Discussion

Formation of 2-[^{18}F]fluoroethylamine

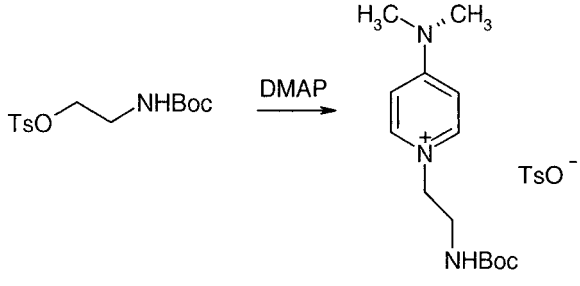
The preparation of n.c.a. 2-[^{18}F]fluoroethylamine was based on the cryptate mediated nucleophilic ^{18}F-fluorination[11] of *N*-Boc-2-(*p*-toluenesulfonyloxy)ethylamine with subsequent deprotection under acidic



Scheme 1. Radiosynthesis of 2-[^{18}F]fluoroethylammonium trifluoroacetate

conditions (Scheme 1). The labelling of this precursor, when first described by Gilissen *et al.*,[7] has been performed in acetonitrile leading to a radiochemical yield of only about 20% of *N*-Boc-2-[^{18}F]fluoroethylamine. Here, the yield could be increased up to 30% at 90°C by increasing the precursor concentration up to 0.4 mol/l and up to 61 ± 6 % when DMSO was used as solvent. With DMF a radiochemical yield of only about 15% was obtained even at higher temperatures. For comparison, the nucleophilic ^{18}F-fluorination of *N*-Boc-2-bromoethylamine in acetonitrile led to a radiochemical yield of 38 ± 9% at a temperature of 90°C.[6]

For simpler isolation of the product, the excess of precursor was reacted with DMAP after ^{18}F-exchange forming a quaternary ammonium salt (Scheme 2), as Guhlke has demonstrated in the case of the bromo-precursor.[6] This water soluble salt together with the



Scheme 2. Derivatisation of the excess precursor with DMAP

cryptate and residual [^{18}F]fluoride could easily be separated from the hydrophobic Boc-protected [^{18}F]fluoroethylamine via solid phase extraction using a C-18 Sep-Pak cartridge. The *N*-Boc-2-[^{18}F]fluoroethylamine was obtained by subsequent elution with ether and was deprotected quantitatively with trifluoroacetic acid at room temperature within 2 min after evaporation of the solvent. This resulted in a somewhat improved radiochemical yield of 46 ± 5% of 2-[^{18}F]fluoroethylamine compared to 39 ± 6% reached by producing the amine from *N*-(2-[^{18}F]fluoroethyl)phthalimide.[7]

C-Terminal ^{18}F-fluoroethylation of the tripeptide Z-Pro-Leu-Gly-OH

The tripeptide Z–Pro–Leu–Gly–OH represents the C-terminal amino acid sequence of oxytocin. Accordingly, it is a useful model for developing and optimising the ^{18}F-fluoroethylamidation of the neuropeptide either based on the condensation with active esters or starting with the corresponding free acid. The reaction of 2-[^{18}F]fluoroethylamine with various activated esters, i.e. the *N*-succinimidyl-, *p*-nitrophenyl- and pentafluorophenylester of Z–Pro–Leu–Gly–OH, could be performed in DMF at room temperature in the presence of triethylamine. In each case radiochemical yields $\geqslant$90% were obtained within 1 min.

However, due to the very expensive nonapeptide oxytocin and the losses when converting to the corresponding activated esters (chemical yields of esters were 65, 67, 87%, respectively) an attempt was made to perform the amidation starting with the free acid of the tripeptide in presence of a coupling reagent such as TBTU, BOP, WSC or HOBT (in situ activation). The synthesis of Z–Pro–Leu–Gly–(2-[^{18}F]fluoroethylamide in presence of an activating agent and a base such as triethylamine was performed in DMF at room temperature. Within 10 min and a precursor concentration of 0.024 mol/l, TBTU supported amidation gave a radiochemical yield of 91 $\pm$ 2%, which was comparable to those obtained by condensation using 2-[^{18}F]fluoroethylamine and the active esters. With the same reaction conditions a good radiochemical yield of 84 $\pm$ 8% was also obtained with BOP, whereas the other two coupling reagents gave much lower radiochemical yields: 27 $\pm$ 4% (WSC) and only 1% with HOBT.

In further optimisation experiments the dependence of the radiochemical yield on reaction time and precursor concentration was examined. The results are graphically depicted in Figure 2. The diagram shows, that a reaction time of 1–2 min is sufficient and that the maximum radiochemical yield of 90 $\pm$ 7% is achieved with a precursor concentration of $\geqslant$0.024 mol/l.

Thus, the best reaction conditions found for the synthesis of Z–Pro–Leu–Gly–2-[^{18}F]fluoroethylamide using the free acid and a coupling reagent are a precursor concentration of 0.024 mol/l, a reaction temperature of 20°C, a reaction time of 1 min and TBTU as activating agent. Compared with the active ester method, the synthesis via TBTU under comparable conditions results in the same radiochemical yield of $\geqslant$90% with high reliability. However, the expensive synthesis of the activated ester of a peptide is avoided when using a coupling reagent.

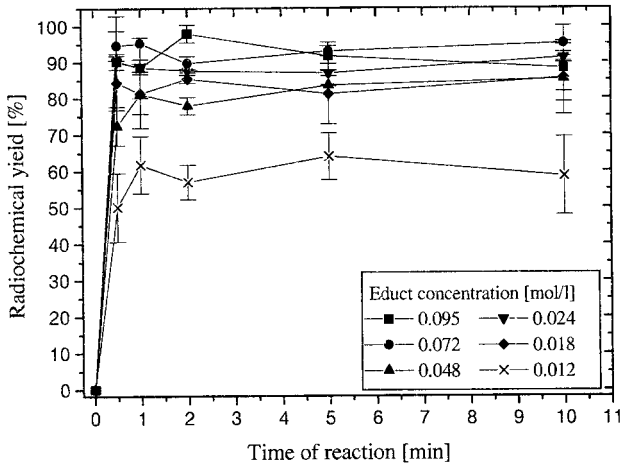


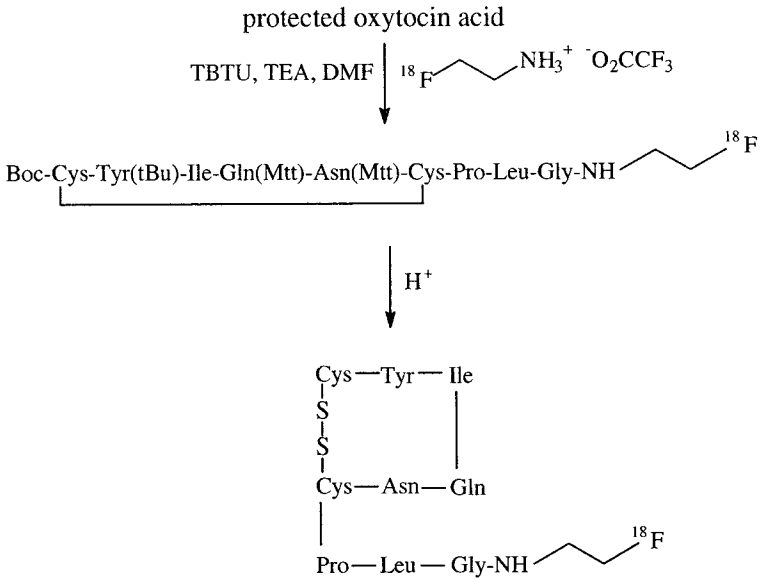
Figure 2. Time dependence of ^{18}F-fluoroethylamidation of Z–Pro–Leu–Gly–OH conditions: 250 μl DMF, 20°C, $n = 3–4$, Z–Pro–Leu–Gly–OH/TBTU/TEA = 1/1/2

[Gly-(2-[^{18}F]fluoroethyl)NH^{9}]oxytocin

Based on the model-studies, [Gly-(2-[^{18}F]fluoroethyl)NH^{9}]oxytocin with protected functional groups, i.e. Boc–Cys–Tyr(tBu)–Ile–Gln(Mtt)–Asn(Mtt)–Cys–Pro–Leu–Gly–(2-[^{18}F]fluoroethyl)NH, was correspondingly synthesised via condensation of [^{18}F]fluoroethylamine in presence of TBTU (Scheme 3).

Results of optimisation experiments with variation of reaction time and precursor concentration are summarised in Figure 3. Radiochemical yields of 75 ± 5% were obtained after 2 min with a peptide concentration of ⩾0.01 mol/l, whereas at 0.006 mol/l a radiochemical yield of about 60% was achieved.

Deprotection of the ^{18}F-fluoroamidated peptide with trifluoroacetic acid was quantitative at room temperature within 1–2 min. Attempts to remove the protecting groups after absorption of the ^{18}F-labelled peptide on a solid phase or dissolved in DMF failed. Thus, the solvent DMF had to be removed before treatment with TFA. The radiochemical yield of [Gly–(2-[^{18}F]fluoroethyl)-NH^{9}]oxytocin was determined at the end of deprotection with TFA via analytical HPLC to 29 ± 5% (based on starting [^{18}F]fluoride). The results now encourage the development of a large scale preparation for pharmacokinetic PET-studies, including determination of actual specific activity and examination of the biochemical properties of derivatized oxytocin.



Scheme 3. Reaction sequence of the synthesis of [Gly-(2-[^{18}F]fluoroethyl)NH^9]-oxytocin

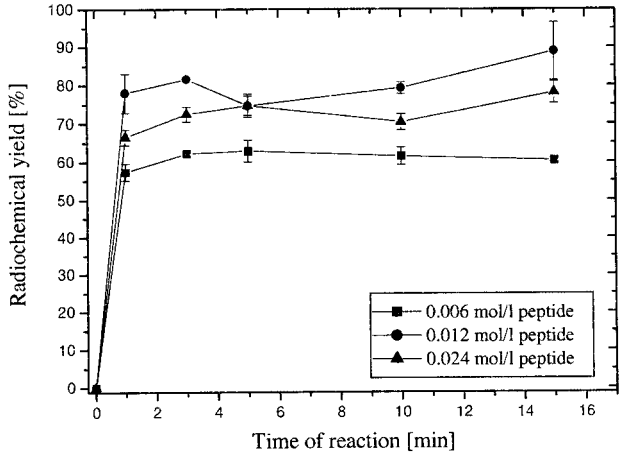


Figure 3. Time dependence of ^{18}F-fluoroethylamidation of protected [Gly–OH^9]oxytocin in presence of TBTU, $n = 3–4$, conditions: 250 μl DMF, 20°C, [Gly-OH^9]oxytocin/TBTU/TEA = 1/1/2

Conclusion

Since oxytocin has a C-terminal amide function, ^{18}F-labelling of the peptide hormone was performed via the ^{18}F-fluoroethylamidation of the free acid of oxytocin with the hope of no significant alteration of the biochemical properties. The radiochemical yield of the intermediate 2-[^{18}F]fluoroethylamine was somewhat increased in comparison to preparations previously described.[6,7] With the most suitable coupling reagent TBTU, 2-[^{18}F]fluoroethylamine can be efficiently condensed with the peptide acid to give [Gly–(2-[^{18}F]fluoroethyl)NH^{9}]oxytocin. Thus, expensive synthesis of activated esters can be avoided.

Acknowledgements

We would like to thank Dr. D.H. Hellhammer, University of Trier, Germany, for support and the team of the JSW BC 1710 cyclotron, FZ-Jülich, for producing [^{18}F]fluoride.

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