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Research Article

Fluorine-18 labelling of PNAs functionalized at their pseudo-peptidic backbone for imaging studies with PET

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Summary

Peptide nucleic acids (PNAs) form a unique class of synthetic macromolecules, originally designed as ligands for the recognition of double-stranded DNA, where the deoxyribose phosphate backbone of original DNA is replaced by a pseudo-peptide *N*-(2-aminoethyl)glycyl backbone, while retaining the nucleobases of DNA. We have previously developed an original method to label oligonucleotide-based macromolecules with the short-lived positron-emitter fluorine-18 ($t_{1/2}$: 109.8 min) using the *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide reagent. Using this method, we herein report the fluorine-18-labelling of 13 decameric PNAs (**OLP_1-13**), of the same sequence (CTCATACTCT), but presenting selected modification of the pseudo-peptidic backbone at two or three of the thymine residues (positions 2, 5 and 8). Structural characteristics of these backbone modifications include either an amino acid side chain (L-Lys, L-Glu, L-Leu and L-Arg) or a glycosyl moiety (mannose, galactose, fucose, *N*-Ac-galactosamine and *N*-Ac-glucosamine) attached via an appropriate spacer. *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was synthesized in three radiochemical steps from 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate and HPLC-purified in 85–90 min (typical production: 3.7–4.8 GBq starting from a batch of 29.6–31.4 GBq of [^{18}F]fluoride). Conjugation of the fluorine-18-labelled bromoacetamide reagent with the PNAs was performed in a mixture of acetonitrile and HEPES buffer (0.1 M, pH 7.9) for 10 min at 60°C and gave the corresponding pure labelled

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conjugated PNAs ([^{18}F]**c-OLP_1-13**) after RP-HPLC purification. The whole synthetic procedure, including the preparation of the fluorine-18-labelled reagent, provides up to 0.9 GBq (25 mCi) of HPLC-purified [^{18}F]**c-OLP_1-13** in 160 min with a specific radioactivity of 45–65 GBq/μmol (1.2–1.7 Ci/μmol) at the end of synthesis starting from 29.6 to 31.4 GBq (800–850 mCi) of [^{18}F]fluoride.

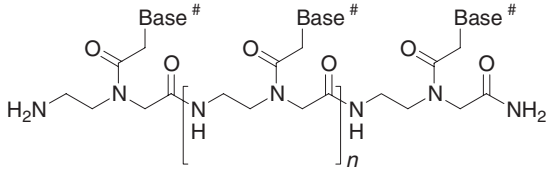
Key Words: fluorine-18; peptide nucleic acids; positron emission tomography

Introduction

Peptide nucleic acids (PNAs) are a unique class of synthetic DNA analogs, characterized by the replacement of the sugar–phosphate backbone of DNA or RNA by an uncharged and flexible pseudo-peptidic *N*-(2-aminoethyl)glycyl backbone, while retaining the nucleobases of DNA[1,2] (Figure 1). PNAs were originally designed as ligands for the recognition of double-stranded DNA, and have also showed promising therapeutic potential as antisense and antigene agents[3–8] as well as inspiring the development of a variety of research and diagnostic assays,[7,8] including their use as imaging tools.[9,10]

Positron emission tomography (PET) is a high-resolution, sensitive and non-invasive imaging technique that can be used in humans. It is the most advanced imaging technology currently available for studying *in vivo* molecular interactions and represents the method of choice to assess the pharmacokinetics of new potent therapeutic agents such as PNAs. Furthermore, today's access to high-resolution small-animal PET cameras provides new opportunities in drug development and *in vivo* direct screening of drug candidates, including oligonucleotide-based macromolecules.

Because of the increasing interest in labelled oligonucleotides and derivatives as radiopharmaceuticals for PET, we have developed an original method to label this class of macromolecules with the radioactive short-lived positron-emitter fluorine-18 ($t_{1/2}$: 109.8 min). The method gives access to the labelled macromolecules by conjugation of a prosthetic group[11] provided by (*N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide), carrying the radioisotope, with a selected reactive function added to the oligonucleotide (a phosphorothioate monoester group at its 3′- or 5′-end). Besides natural phosphodiester DNA



Base : # A (Adenine), C (Cytosine), G (Guanine), T (Thymine).

Figure 1. Design and general formula of a PNA without backbone modifications

oligodeoxyribonucleotides, this methodology has already been reliably and routinely applied to all the popular chemical modifications of oligonucleotides, such as full-length phosphorothioate diester internucleosidic-bond deoxyribonucleotides,[11–13] hybrid methylphosphonate/phosphodiester internucleosidic-bond deoxyribonucleotides and 2′*O*-methyl-modified oligoribonucleotides.[12,14] Pharmacokinetics of most of these fluorine-18-labelled oligonucleotides have then been established with PET and their metabolism also partially evaluated.[15] More recently, the methodology has also been successfully applied to the fluorine-18-labelling of Spiegelmers,[16] single-stranded mirror-image oligonucleotides consisting of L-ribose (L-RNA) or L-2′-deoxyribose (L-DNA) units.[17,18] It has so far been used once for the labelling of a selected PNA with fluorine-18.[19] Several groups have prepared radiometal-labelled PNAs or fluorescent chimerical PNA-peptide conjugates.[9,10] The strategies employed use all chemical properties of the pseudo-peptidic backbone allowing the functionalization of the C- and N-terminus end, as well as internal amino-acid-C-α functionalization by introduction of non-glycine residues or reactive linkers.[20] In the only example of fluorine-18-labelling, a cysteine residue was added to the original PNA structure (at the N-terminus end), allowing the conjugation of this modified PNA with the *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide reagent via the thiol function.

In the present study, we have extended our investigations to the fluorine-18-labelling of various decameric PNAs (**OLP_1-13**) using the *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide reagent based on previously developed methodology[19] and report that this strategy can routinely and efficiently be applied to the labelling of this particular class of oligonucleotide-like macromolecules. Thirteen PNAs, of the same sequence but presenting selected modifications of the pseudo-peptidic backbone were labelled with fluorine-18 ([^{18}F]**c-OLP_1-13**) (Figure 2).

Results and discussion

Chemistry

Table 1 summarizes the principal characteristics of the 13 decameric PNAs (**OLP_1-13**) used as starting material in the present study. All PNAs (**OLP_1-13**) present the same base sequence (CTCATACTCT), and were all functionalized at their N-terminus end with a cysteine residue whereas their C-terminus end were all amidified ((HS)Cys-CTCATACTCT-NH_2) (see also Figure 2).

Besides the original PNA sequence (**OLP_1**), 12 decameric PNA analogues, showing the same sequence, but presenting selected modification of the pseudo-peptidic backbone were used (**OLP_2-13**). Within this original PNA sequence (**OLP_1**), modified thymine residues (T̲) were introduced at positions

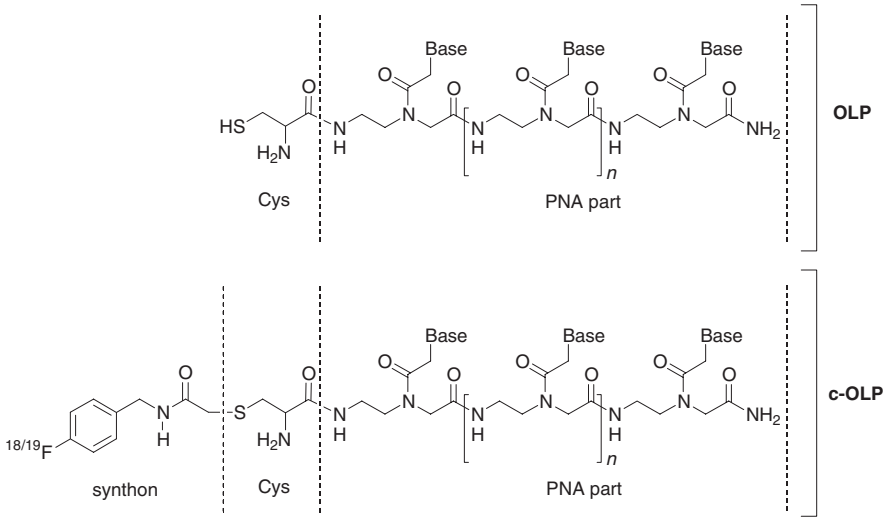


Figure 2. Structure and prosthetic [*radio*]labelling of an N-terminus-modified PNA (OLP)

Table 1. Sequences and analytical data for OLP_1-13/c-OLP_1-13

Name	Sequence[a]	X[b]	Y[c]	HPLC[d,e] (min)	MS calculated/ found[d,f]	
OLP_1/c-OLP_1	(HS)Cys-C T C A T A C T C T-NH_2	—	—	13.1	2905	2903
OLP_2/c-OLP_2	(HS)Cys-C T C A **T** A C **T** C T-NH_2	X_2	Y_1	12.3	3455	3452
OLP_3/c-OLP_3	(HS)Cys-C T C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_1	Y_2	11.7	3341	3340
OLP_4/c-OLP_4	(HS)Cys-C T C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_1	Y_1	11.8	3341	3342
OLP_5/c-OLP_5	(HS)Cys-C T C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_2	Y_3	13.1	3421	3423
OLP_6/c-OLP_6	(HS)Cys-C **T** C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_3	Y_2	11.2	3474	3473
OLP_7/c-OLP_7	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_1	Y_5	11.3	3684	3683
OLP_8/c-OLP_8	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C T C T-NH_2	X_1	Y_3	11.9	3309	3306
OLP_9/c-OLP_9	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C **T** C T-NH_2	X_1	Y_4	11.5	3684	3687
OLP_10/c-OLP_10	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_4	—	11.3	3110	3121
OLP_11/c-OLP_11	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_5	—	12.4	3122	3123
OLP_12/c-OLP_12	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_6	—	17.3	3073	3071
OLP_13/c-OLP_13	(HS)Cys-C $\underline{T}$ C A $\underline{T}$ A C $\underline{T}$ C T-NH_2	X_7	—	13.6	3203	3200

[a] A: adenine; C: cytosine; G: guanine; T: thymine; **T**: modified thymine; see Figure 3(A) and 3(B).
[b] X_{1-7}: see Figure 3(B).
[c] Y_{1-5}: see Figure 3(B).
[d] Analytical data for the conjugated PNAs (**c-OLP_1-13**).
[e] HPLC R_t (see HPLC C).
[f] Mass spectrometry data (Cary Spectrometer, Varian).

5 and 8 (**OLP_2-5**) or 2, 5 and 8 (**OLP_6-13**). Structural characteristics of the backbone modifications are indicated in Table 1 and illustrated in Figure 3(A) and (B) and include either (1) an amino acid side chain: L-Lys, L-Glu, L-Leu or L-Arg (respectively coded X_4, X_5, X_6 and X_7) or (2) a glycosyl moiety: mannose, galactose, fucose, *N*-Ac-galactosamine or *N*-Ac-glucosamine

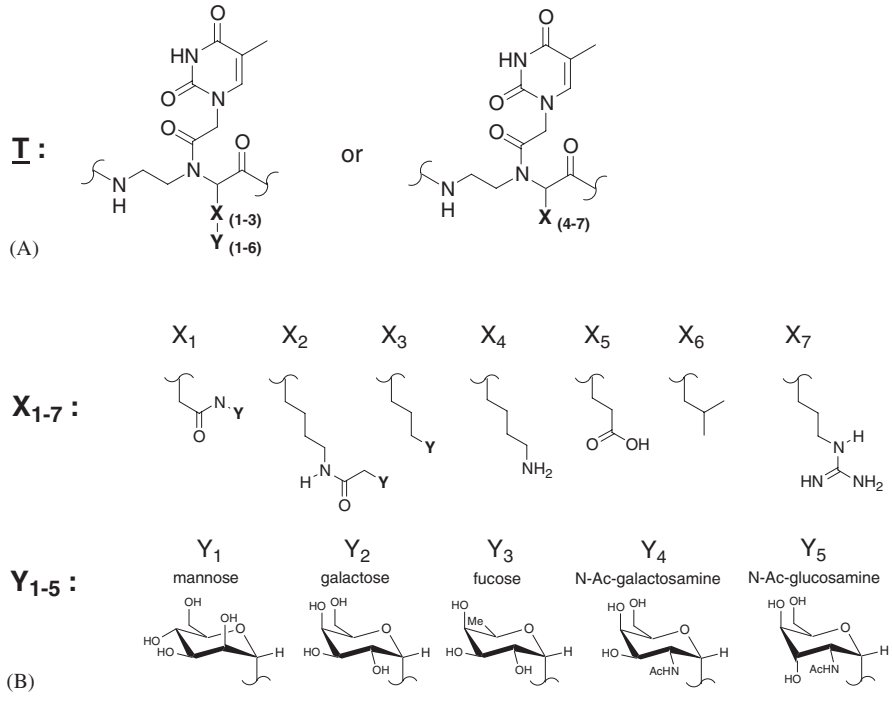


Figure 3. Modified thymine residues (T): nature of the chemical modifications

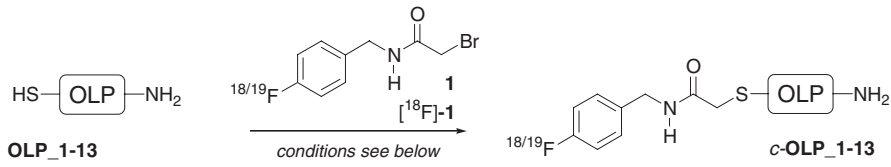
(respectively coded Y_1, Y_2, Y_3, Y_4 and Y_5), attached via an appropriate alkyl-based spacer (X_1, X_2, X_3).

N-(4-Fluorobenzyl)-2-bromoacetamide (**1**) was prepared in 60–65% non-optimized yields from commercially available 4-fluorobenzylamine and bromoacetyl bromide in the presence of *N*-methylmorpholine.[11]

The conjugation of the *N*-(4-fluorobenzyl)-2-bromoacetamide (**1**, about 10 equivalent excess) to the PNAs (**OLP_1-13**), bearing a cysteine at the N-terminus end, was carried out in a mixture of acetonitrile and phosphate buffer saline (PBS, 0.1 M aq. pH 8.75), at 60°C for 20 min (Scheme 1). These conditions gave the desired non-labelled conjugated PNAs (**c-OLP_1-13**) that were purified by semi-preparative HPLC (Table 1). Mass spectrometry analysis confirmed the mono-conjugation of the *N*-(4-fluorobenzyl)-2-bromoacetamide with the PNAs (Table 1).

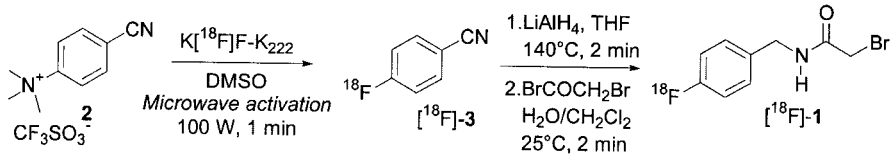
Radiochemistry

*Fluorination of the conjugating reagent: preparation of N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**1**).* *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**1**, Scheme 2), was synthesized in three steps from 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (**2**, prepared from commercial 4-dimethylaminobenzonitrile[11]). The first radiochemical step, the introduction of fluorine-18 into the benzonitrile ring, was performed in hot DMSO, using the



Conditions
for fluorine-19 : PBS 0.1 M, pH 8.75 / ACN (1/1 v:v), 20 min, 60°C
for fluorine-18 : HEPES 0.1 M, pH 7.9 (or PBS 0.1 M, pH 8.75) / ACN (1/1 v:v), 10 min, 75°C

Scheme 1. Synthesis of conjugated PNAs (c-OLP_1-13)



Scheme 2. Preparation of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-1)

K[^{18}F]F-K_{222} complex by microwave activation at 100 W for 1 min, giving the desired 4-[^{18}F]fluorobenzonitrile ([^{18}F]-**3**). The second step, the reduction of the cyano function, was performed with $LiAlH_4$ in refluxing THF (120°C) for 2 min, giving the desired labelled 4-[^{18}F]fluorobenzylamine. The final step, the condensation with bromoacetyl bromide, occurred cleanly in 2 min at room temperature in a 10/1 (v/v) mixture of CH_2Cl_2/H_2O. Semi-preparative HPLC gave pure *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**1**). Typically, starting from a batch of 29.6–31.4 GBq (800–850 mCi) of [^{18}F]fluoride, we produced 3.7–4.8 GBq (100–130 mCi) of HPLC-purified *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**1**) in about 90 min.

Conjugation of the N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide with the PNAs. Conjugation of the PNAs (**OLP_1-13**) with the HPLC-purified *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide [^{18}F]-**1** (Scheme 1) was carried out in a mixture of acetonitrile and HEPES buffer (0.1 M, pH 7.9) for 10 min at 75°C, according to a slightly modified procedure already published.[19] Phosphate buffer saline (PBS, 0.1 M aq. pH 8.75) was also used in the present study without showing notable yield differences. The labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**) were then purified by RP-HPLC. The procedure has been fully automated on our Zymate robot system.

*Quality control of the fluorine-18-labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**).* As demonstrated by HPLC analysis, radiosynthesized labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**) co-eluted with the authentic synthesized reference compounds. The fluorinated conjugated PNAs ([^{18}F]**c-OLP_1-13**) were found to be >95% chemically and radiochemically pure. The

preparations were shown to be free of non-radioactive starting PNAs and radiochemically stable for at least 120 min.

Experimental

General

Chemicals. Chemicals were purchased from Aldrich, Sigma and Fluka and were used without further purification. Thirteen PNAs (**OLP_1-13**) (10 mers) were used in the present study and were synthesized at the University of Copenhagen, Denmark.

Chromatography systems. Thin-layer chromatography (TLC) was run on precoated plates of silica gel $60F_{254}$ (Merck). The compounds were localized at 254 nm using a UV lamp. Flash chromatography was conducted on silica gel 63–200 μm (Merck) at 0.3 bar (compressed air). The following HPLC systems were used:

HPLC A: semi-preparative normal-phase HPLC: column Prep Nova-Pak® HR Silica Waters (7.8 × 300 mm, 60 Å, 6 μm), UV detector 440 Waters, Geiger-Müller detector; solvents: CH_2Cl_2/EtOAc (95/5 : v/v); isocratic elution, flow rate: 5 ml/min.

HPLC B: semi-preparative RP-HPLC: column C18 μBondapak® Waters (300 × 7.8 mm, porosity 10 μm), 600 Controller Gradient system Waters, UV detector multiwavelength 490E Waters (254 nm); solvents: *X*: 0.1% TFA in water and *Y*: 0.1% TFA in 90% ACN/10% H_2O; gradient elution: linear 30 min from 90/10 (*X*/*Y*) to 50/50 (*X*/*Y*), then wash-out 10 min at 50/50 (*X*/*Y*), flow rate: 5 ml/min.

HPLC C: analytical RP-HPLC: column Partisil ODS3 Waters (4.6 × 150 mm, porosity 5 μm), 600 Pump and 600 Controller Waters, UV detector Series 1100 (254 nm) Hewlett Packard, Flow One Scintillation Analyzer Packard equipped with a positron-dedicated cell for radioactivity monitoring; solvents: *X*: 0.1% TFA in water and *Y*: 0.1% TFA in 90% ACN/10% H_2O; gradient elution: linear 30 min from 90/10 (*X*/*Y*) to 50/50 (*X*/*Y*), then wash-out 10 min at 50/50 (*X*/*Y*); flow rate: 1.3 ml/min.

Spectroscopy. NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuterated solvents (DMSO-d_6, $\delta = 2.50$ ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents (DMSO-d_6, $\delta = 39.5$ ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (^{1}H and ^{13}C) (s, d, t, q, dd, m, b for singlet, doublet, triplet, quadruplet, doublet of doublet, multiplet (or multi sharp-peak system) and broad, respectively). The mass spectra were measured on a Cary Spectrometer (Varian) and a Quattro VG (Fison, Manchester, UK).

Miscellaneous. Radiosyntheses using fluorine-18 were performed in a 7.5 cm lead-shielded cell using a computer-assisted Zymate robot system (Zymark Corporation, USA). Microwave activation was performed with a MicroWell 10 oven (2.45 GHz), Labwell AB (Sweden). Specific radioactivity was determined as follows: the area of the absorbance peak corresponding to the radiolabelled product was measured on the HPLC chromatogram and compared to a standard curve relating mass to absorbance.

Radioisotope availability. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CGR-MeV 520 cyclotron by irradiation of a 2 ml water target using a 17 MeV proton beam on 95%-enriched [^{18}O]water by the [^{18}O(p,n)^{18}F] nuclear reaction and was transferred to the appropriate hot cell. Typical production: 29.6–31.4 GBq (800–850 mCi) of [^{18}F]F$^-$ at the end of bombardment for a 20 μA, 45 min (56 000 μC) irradiation. A complete description of the target hardware and operation can be found in reference Dolle *et al.*[11]

Chemistry

*N-(4-fluorobenzyl)-2-bromoacetamide (**1**)*. Synthesized from commercially available 4-fluorobenzylamine according to Dolle *et al.*[11] Rf (heptane/EtOAc : 50/50) : 0.35. ^{1}H NMR (DMSO-d$_6$, 300.0K): δ: 8.80 (bt, 1H); 7.32 (dd, *J*: 8.1, 5.70 Hz, 2H); 7.15 (t, *J*: 8.1Hz, 2H) ; 4.31 (d, *J*: 6 Hz, 2H); 3.92 (s, 2 H). ^{13}C NMR (DMSO-d$_6$, 300.0 K): δ: 166.1 [C]; 161.3 [C, d, *J*: 249 Hz]; 135.0 [C, d, *J*: 2 Hz]; 129.2 [CH, d, *J*: 8 Hz]; 115.1 [CH, d, *J*: 23 Hz]; 41.9 [CH$_2$]; 29.4 [CH$_2$]. MS (DCI/NH_4^+): C_9H_9BrFNO: 265 [M + NH_4^+]; 263 [M + NH_4^+]; 248 [M + H^+]; 246 [M + H^+].

*Conjugation of PNAs with N-(4-fluorobenzyl)-2-bromoacetamide (**1**). General procedure for preparation of non-labelled conjugated PNAs (**c-OLP_1-13**)*. All non-labelled conjugated PNAs (**c-OLP_1-13**) were prepared according to a slightly modified procedure described by Hamzavi *et al.*[20] Briefly, to a solution of PNAs (**OLP_1-13**, 1.3 mg) in phosphate buffer saline (0.5 ml, 0.1 M, pH 8.75) was added an excess of *N*-(4-fluorobenzyl)-2-bromoacetamide (10 equivalent) in acetonitrile (0.5 ml) and the mixture was heated at 60°C for 20 min (Scheme 1). Solvents were removed under reduced pressure and the conjugated PNAs (**c-OLP_1-13**) were purified by semi-preparative RP-HPLC before characterization by mass spectrometry. Analytical HPLC data (HPLC C) of **c-OLP_1-13** and mass spectrometry analyses are summarized in Table 1.

Radiochemistry

Preparation of the K[^{18}F]F-K_{222}-complex. In order to recover and recycle the [^{18}O]water target, the 2 ml of aqueous [^{18}F]fluoride from the target holder were

passed through an anion exchange resin (Sep-Pak® Light Waters AccellTM Plus QMA Cartridge in the chloride form, washed with 5 ml 1 M aq. $NaHCO_3$ and then rinsed with 50 ml of water) by helium pressure (1.5–2.0 bar). Helium was blown through the column to maximally extract the last traces of [^{18}O]water. See References[21,22] for more practical details. The [^{18}F]fluoride ion was then eluted from the resin using 1.0 ml of a 4.5 mg/ml aqueous K_2CO_3 solution into a Vacutainer® tube containing 12.0–15.0 mg of Kryptofix®$_{222}$ (K_{222}: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane). The resulting solution was then gently concentrated to dryness at 145–150°C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.[23]

*N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**1**)*. Synthesized in three steps starting from 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate according to the procedure described in references.[11,12] *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was purified using HPLC (HPLC A: Rt: 10.0–10.5 min). Typically, 3.7–4.8 GBq (100–130 mCi) of pure *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide could be obtained in 85–95 min starting from a 29.6–31.4 GBq (800–850 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch.

*General procedure for the preparation of fluorine-18 conjugated PNAs ([^{18}F]**c-OLP_1-13**)*. 1 mg of OLP (**OLP_1-13**) dissolved in a mixture of 200 µl of acetonitrile and 500 µl of HEPES buffer (0.1 M, pH 7.9) or 500 µl of phosphate buffer saline (PBS, 0.1 M aq. pH 8.75) was mixed with the HPLC purified *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide, redissolved in 300 µl of acetonitrile, and heated without stirring under a slight flow of nitrogen for 10 min at 75°C (Nitrogen flow caused concentration of the reaction mixture). 1 ml of deionized water was finally added and labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**) were separated from starting PNAs (**OLP_1-13**) and unreacted *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide by semi-preparative RP-HPLC (HPLC B).

Formulation and quality control. The HPLC-fraction containing the labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**) was concentrated to dryness under reduced pressure, taken up with physiological saline and finally filtered on a 0.22 µm GS-Millipore filter (vented). As demonstrated by HPLC analysis (HPLC C), radiosynthesized labelled conjugated PNAs ([^{18}F]**c-OLP_1-13**) co-eluted with the authentic synthesized reference compounds. The radiolabelled products were found to be >95% chemically and radiochemically pure. The preparations were shown to be free of non-radioactive precursor and radiochemically stable for at least 120 min.

Conclusion

In the present study, we report the fluorine-18 labelling of 13 decameric PNAs (**OLP_1-13**) with fluorine-18 using our *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide reagent. The whole synthetic procedure, including the preparation of the fluorine-18-labelled reagent, provides up to 0.9 GBq (25 mCi) of HPLC-purified labelled PNAs ([^{18}F]**c-OLP_1-13**) in 160 min with a specific radioactivity of 45–65 GBq/μmol (1.2–1.7 Ci/μmol) at the end of synthesis starting from 29.6 to 31.4 GBq (800–850 mCi) of [^{18}F]Fluoride.

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