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Research Article

Fluorine-18- and iodine-125-labelling of spiegelmers

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Summary

Spiegelmers are high-affinity L-enantiomeric oligonucleotide ligands (aptamers) that display high resistance to enzymatic degradation compared to D-oligonucleotides. Spiegelmers belong to the third generation of aptamers, and are currently extensively investigated as potential therapeutic agents. We have previously developed an original method to label natural oligonucleotides with radiohalogens and particularly with fluorine-18, the most widely used positron-emitter, $t_{1/2}$: 109.8 min. Using the same strategy, we herein report the labelling of Spiegelmers, both with fluorine-18 for positron emission tomography imaging and iodine-125 for high resolution autoradiography. Three 25-mer L-oligonucleotides have been used, differing (a) by the position of the terminal phosphorothioate monoester group (3′- or 5′-end, and therefore differing by the position of the labelling on the macromolecule) and (b) by the nature of the backbone sugar moiety (2′-OH or 2′-H, therefore covering the RNA and DNA series, respectively). *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was synthesized in three radiochemical steps from 4-cyano-*N*,*N*,*N*-

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trimethylanilinium trifluoromethanesulfonate and HPLC-purified in 90 min (typical production: 2.2–2.4 GBq starting from a batch of 22–24 GBq of [^{18}F]fluoride). *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide was synthesized from the corresponding trimethylsilyl derivative (one pot, two radiochemical steps) and HPLC-purified in 60 min (typical production: 24 MBq starting from 37 MBq of Na[^{125}I]I). Coupling of the Spiegelmers with the appropriate HPLC-purified [*radiolabelled*]-halobenzyl-2-bromoacetamide (MeOH/PBS (0.1 M, pH 8), 10 min, 120°C) gave the corresponding labelled conjugated Spiegelmers after RP-HPLC purification. For fluorine-18, the whole synthetic procedure yields up to 1.1 GBq of pure labelled Spiegelmers in 160 min with a specific radioactivity of 37–74 GBq/μmol at the end of synthesis starting from 22–24 GBq of [^{18}F]fluoride. For iodine-125, the whole synthetic procedure allows producing up to 7.4 MBq of pure labelled Spiegelmers in 100 min with a specific radioactivity of 11–37 GBq/μmol starting from 37 MBq of Na[^{125}I]I.

Key Words: fluorine-18; iodine-125; Spiegelmer; positron emission tomography; autoradiography

Introduction

Oligonucleotide ligands, termed aptamers, with high affinities for a variety of molecular targets have been identified from combinatorial libraries.[1] As a new class of man-made affinity ligands, aptamers are meeting an increasing variety of applications as target recognition agents. However, their use *in vivo* is still restricted by the low biostability of nucleic acids. Spiegelmers are single-stranded mirror-image aptamers consisting of L-ribose (L-RNA) or L-2′-deoxyribose (L-DNA) units (Figure 1). The chiral inversion of the pentose results in high stability *in vitro* and *in vivo* compared with D-oligonucleotide ligands[2,3] (natural RNA or DNA), suggesting that Spiegelmers may present potential for therapeutic and diagnostic applications.[4–8] Positron Emission Tomography (PET), a high-resolution, sensitive and non-invasive imaging technique that can be used in humans, is the most advanced technology currently available for studying *in vivo* molecular interactions and represents a method of choice to assess the pharmacokinetics of new therapeutic agents such as Spiegelmers.

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 radioactive

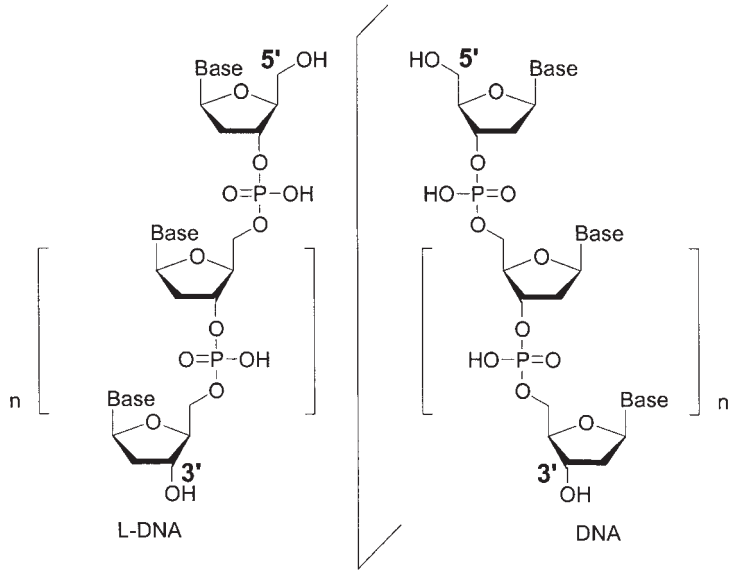


Figure 1. Design and general formula of a DNA-Spiegelmer (L-DNA)

isotopes of halogens, such as fluorine-18 (the most widely used positron-emitter, $t_{1/2}$: 109.8 min), bromine-76 (another positron-emitter with a relatively long half life, $t_{1/2}$: 16.1 h) and iodine-125 (electron capture with Auger-electron emission used for high resolution autoradiography, $t_{1/2}$: 59.9 days). This method gives access to the labelled macromolecules by conjugation of a prosthetic group, carrying the radioisotope, with a reactive function of the oligonucleotide. This strategy allows for the use of different chemical routes and possibly drastic chemical conditions for the preparation of the labelled prosthetic group, followed by the conjugation of the latter with an oligonucleotide under mild conditions preserving its integrity. *N*-(4-halobenzyl)-2-bromoacetamide was therefore designed as a general and radiochemically feasible reagent, the benzyl function carrying the radioactive halogen, and the 2-bromoacetamide moiety offering the opportunity of an efficient and regioselective conjugation with an oligonucleotide provided with a phosphorothioate monoester group at its 3′- or 5′-end.[9,10] Besides natural phosphodiester DNA oligodeoxyribonucleotides,[9,11] the strategy has already been reliably and routinely applied to all the popular chemical modifications of oligonucleotides, such as full-length phosphorothioate diester internucleosidic-bond deoxyribonucleotides, hybrid methylphospho-

nate/phosphodiester internucleosidic-bond deoxyribonucleotides and 2′*O*-methyl-modified oligoribonucleotides.[10,12,13]

In the present study, we have investigated the labelling of Spiegelmers using the radiohalogenated reagents, *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide and *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide and report that the method developed can also be applied efficiently to the labelling of this new class of macromolecules. Three 25-mer L-oligonucleotides, differing (a) by the position of the terminal phosphorothioate monoester group (3′- or 5′-end, and therefore differing by the position of the labelling on the macromolecule) and (b) by the nature of the backbone sugar moiety (2′-OH or 2′-H, thus covering the RNA and DNA series, respectively), were labelled.

Results and discussion

Chemistry

Table 1 summarizes the principal characteristics of the Spiegelmers (**1**-**3**) used as starting material in the present study and the conjugated Spiegelmers (C-**1**-C-**3**) we have synthesized, especially the nature of the halogen on the prosthetic group (fluorine-18 for C-**1**, C-**2a** and C-**3**, iodine-125 for C-**2b**). Sequences, lengths, chemistry (L-RNA or L-DNA) and position of the terminal phosphorothioate group (5′ or 3′) are indicated.

Figure 2 presents the general chemical structure of the Spiegelmers **1** (L-RNA, 5′-phosphorothioate), **2** (L-RNA, 3′-phosphorothioate) and **3** (L-DNA, 3′-phosphorothioate). Note that the Spiegelmer **2** (L-RNA, 3′-phosphorothioate) is bearing a deoxyribose instead of a ribose at the 3′-end to circumvent the known and described cyclization between the 2′-OH function and the phosphorothioate group during the conjugation in basic conditions.

The conjugation of the *N*-(4-halobenzyl)-2-bromoacetamide (**4a** or **4b**) with the Spiegelmers **1**, **2** or **3** bearing a phosphorothioate

Table 1. Sequences, lengths and chemistry of the Spiegelmers used

Name	Length	Sequence	Chemistry	Halogen	Conjugated
1	25 mer	UAAUAUGACUCACUAUAGGUAACUU	5′ps-L-RNA	Fluorine	C-**1**
2	25 mer	UAAUAUGACUCACUAUAGGUAACUT	L-RNA-3′ps	Fluorine	C-**2a**
				Iodine	C-**2b**
3	25 mer	TAATATGACTCACTATAGGTAACTT	L-DNA-3′ps	Fluorine	C-**3**

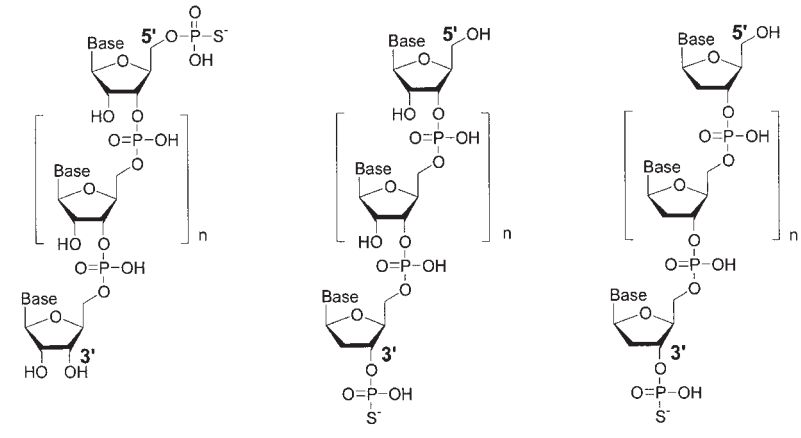
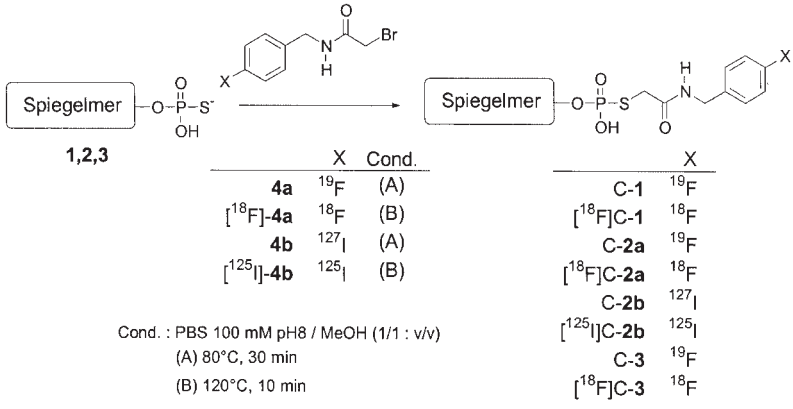


Figure 2. Chemical structure of a 5′-end phosphorothioate L-RNA, of a 3′-end phosphorothioate L-RNA and a 3′-end phosphorothioate L-DNA



Scheme 1. Synthesis of conjugated L-RNA (C-1, C-2a, C-2b) and L-DNA (C-3)

function at their 3′- or 5′-end was carried out in a mixture of methanol and 0.1 M phosphate buffer saline (PBS, pH 8), at 80°C for 30 min (Scheme 1).

This procedure gave the non-labelled conjugated Spiegelmers (C-**1**, C-**2a**, C-**2b** or C-**3**) in a 70–80% yield (determined by HPLC). They (C-**1**, C-**2a**, C-**2b** or C-**3**) were separated from the non-reacted material by semi-preparative RP-HPLC and desalted using a Sephadex® Cartridge before characterization.

The regioselectivity of the coupling was verified by ^{31}P-NMR. The chemical shift of the 3′- or 5′-end phosphorus atom was moved from +46.00 ppm to +15.40 ppm for C-**1**, from +46.12 ppm to +15.72 ppm for C-**2a**, from +46.12 ppm to +15.26 ppm for C-**2b** and from +41.26 ppm to +14.69 ppm for C-**3**, after alkylation (Table 2). These results are in agreement with published ^{31}P-NMR tables[14,15] and previously reported data for oligonucleotides having a phosphodiester- or a chemically modified backbone.[9,12,13] Furthermore, mass spectrometry confirmed the conjugation with just one *N*-(4-halobenzyl)-2-bromoacetamide (**4a** or **4b**) molecule (Table 2).

Radiochemistry

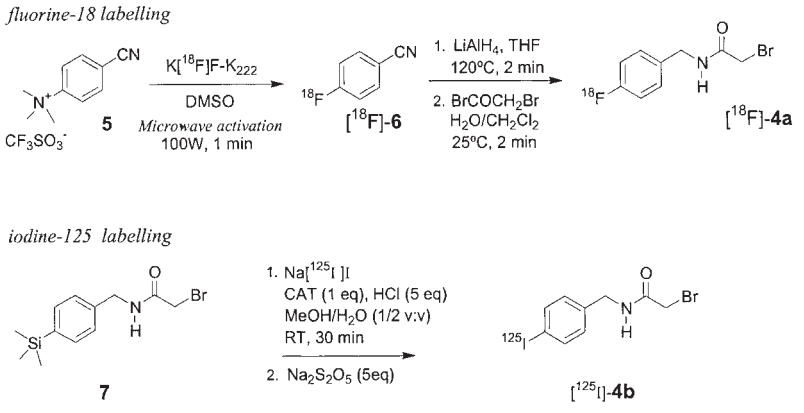
Labelling of the conjugating reagents.

Fluorination. *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide [^{18}F]-**4a** (Scheme 2), was synthesized in three steps from 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (**5**, prepared from commercial 4-dimethylaminobenzonitrile).[9] The first radiochemical step, the introduction of fluorine-18 into the benzonitrile ring, was performed in hot DMSO, using the 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]-**6**). The second step, the reduction of the cyano function, was performed with $LiAlH_4$ in refluxing THF (140°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]-**4a**). Typically,

Table 2. Chromatographic and spectrometric characterizations of non-labelled conjugated Spiegelmers

Conjugate	Halogen	HPLC Rt[a] (min)	MS Found (calc)[b]	^{31}P NMR[c]
C-**1**	Fluorine	22.5–23.0	8175.3 (8174.0)	δ: +15.40
C-**2a**	Fluorine	22.5–23.0	8175.0 (8172.0)	δ: +15.72
C-**2b**	Iodine	22.0–22.5	8281.5 (8279.9)	δ: +15.26
C-**3**	Fluorine	21.5–22.0	8286.3 (8284.2)	δ: +14.69

[a] HPLC column and conditions: C18 μBondapak® Waters (300 × 3.9 mm, porosity 10 μm); triethylammonium acetate, 100 mM, pH 7 (TEAA) and acetonitrile; gradient elution: linear 30 min from 99/1 to 70/30 (TEAA/acetonitrile), wash-out 10 min at 50/50, flow rate: 1.5 ml/min.
[b] Quattro VG (Fison, Manchester, UK), negative mode.
[c] NMR Bruker AMX (300 MHz) apparatus; TMP as internal standard, spectra recorded in water at 298 K.



Scheme 2. Preparation of the *N*-(4-[*labelled*]halobenzyl)-2-bromoacetamides [^{18}F]-4a and [^{125}I]-4b

starting from a batch of 22–24 GBq of [^{18}F]fluoride, we produced 2.2–2.4 GBq of HPLC-purified *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**4a**) in about 90 min.

Iodination. *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**) was synthesized in two radiochemical steps starting from *N*-(4-trimethylsilylbenzyl)-2-bromoacetamide (**7**) (prepared according to procedures described in Kuhnast *et al.*[12]) on treatment with Na[^{125}I]I and chloramine T at room temperature for 30 min (Scheme 2). RP-HPLC purification of the crude gave the desired *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**) in a 60–70% yield. Typically, starting from 37 MBq (1 mCi) of Na[^{125}I]I, we routinely produced up to 24 MBq (630 μCi) of pure *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**) in about 60 min.

Conjugation of the [*radiolabelled*]*halobenzylbromoacetamides to the Spiegelmers*. Conjugation of the Spiegelmers (**1**, **2** or **3**) with the appropriate HPLC-purified [*radiolabelled*]-halobenzyl-2-bromoacetamide ([^{18}F]-**4a** or [^{125}I]-**4b**) (Scheme 1) was carried out in a mixture of MeOH/PBS (0.1 M, pH 8) for 10 min at 120°C. The labelled conjugated Spiegelmers ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b** or [^{18}F]C-**3**) were then purified by RP-HPLC (followed by NAP-10 desalting procedure) to provide pure labelled conjugated Spiegelmers ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b** or [^{18}F]C-**3**). For fluorine-18, the whole synthetic procedure

allows obtaining up to 1.1 GBq (30 mCi) of pure labelled Spiegelmer ([^{18}F]C-**1**, [^{18}F]C-**2a** or [^{18}F]C-**3**) in 160 min with a specific radioactivity of 37–74 GBq/μmol (1–2 Ci/μmol) at the end of synthesis starting from a 20.3 to 24.0 GBq (550–650 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch. The procedure has been fully automated on our Zymate robot system. For iodine-125, the whole synthetic procedure allows producing up to 7.4 MBq (200 μCi) of [^{125}I]C-**2b** in 100 min with a specific radioactivity of 11–37 GBq/μmol (0.3–1 Ci/μmol), starting from 37 MBq (1 mCi) of Na[^{125}I]I. The decay corrected radiochemical yields of the conjugation of the Spiegelmers (**1**, **2** or **3**) with either [^{18}F]-**4a** or [^{125}I]-**4b** varied between 30 and 50%.

Quality control of the [*radiolabelled*] *conjugated Spiegelmers*. As demonstrated by HPLC analysis, radiosynthesized labelled conjugated Spiegelmers co-eluted with the authentic synthesized reference compounds. The fluorinated and iodinated conjugated Spiegelmers ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b** or [^{18}F]C-**3**) 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.

Experimental

General

Chemicals. Chemicals were purchased from Aldrich, Sigma and Fluka and were used without further purification. The 25mer-oligonucleotides were synthesized by NOXXON Pharma AG (Germany).

Analytical methods. Thin Layer Chromatography (TLC) was run on precoated plates of silica gel $60F_{254}$ (Merk). 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). HPLC systems: HPLC A: semi-preparative RP-HPLC: column C18 μBondapak® Waters (300 × 7.8 mm, porosity 10 μm), 600 Controller Gradient system Waters, UV detector (254 nm) multiwavelength 490E Waters, Geiger-Müller detector; solvents: triethylammonium acetate, 100 mM, pH 7 (TEAA) and acetonitrile; gradient elution: linear 30 min from 99/1 to 70/30 (TEAA/acetonitrile) and wash-out

10 min at 50/50, flow rate: 6 ml/min. HPLC B: 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 C: semi-preparative RP-HPLC: column C18 μBondapak® Waters (300 × 7.8 mm, porosity 10 μm), gradient pump (Merck), UV detector (254 nm) (Merck), radioactivity detector LB 2040 (Berthold); solvents: aqueous NaH_2PO_4 (0.01 M) and acetonitrile (60/40: v/v); isocratic elution, flow rate: 6 ml/min. HPLC D: analytical RP-HPLC: column C18 μBondapak® Waters (300 × 3.9 mm, porosity 10 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: triethylammonium acetate, 100 mM, pH 7 (TEAA) and acetonitrile; gradient elution: linear 30 min from 99/1 to 70/30 (TEAA/acetonitrile) then wash-out 10 min at 50/50, flow rate: 1.5 ml/min. 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; CD_2Cl_2, $\delta = 5.32$ ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents (DMSO-d_6, $\delta = 39.5$ ppm; CD_2Cl_2, $\delta = 53.8$ ppm) and/or TMS as internal standards for ^{13}C NMR and TMP as internal standard for ^{31}P NMR. The chemical shifts are reported in ppm, downfield from TMS (^{1}H and ^{13}C) or TMP (^{31}P) (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 Nermag R10-10 apparatus and a Quattro VG (Fison, Manchester, UK) for electrospray ionization (negative mode).

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). Iodine-125 radiosyntheses were performed in a glovebox. 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: 20.3–24.0 GBq (550–650 mCi) of [^{18}F]F^- at the end of bombardment for a 20 μA, 30 min (36 000 μC) irradiation. A complete description of the target hardware and operation can be found in Dollé *et al.*[16]

No-carrier-added Na[^{125}I]I, 37 MBq (1 mCi), was purchased from Amersham Pharmacia Biotech (France).

Chemistry

*N-(4-fluorobenzyl)-2-bromoacetamide (***4a***)*. Synthesized from commercially available 4-fluorobenzylamine according to Dolle *et al.*[9] R_f (heptane/EtOAc: 50/50): 0.35. ^{1}H NMR (DMSO-d_6, 300.0 K): δ: 8.80 (bt, 1H); 7.32 (dd, *J*: 8.1 Hz and 5.70 Hz, 2H); 7.15 (t, *J*: 8.1 Hz, 2H); 4.31 (d, *J*: 6 Hz, 2H); 3.92 (s, 2H). ^{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 [$M + H^+$].

*N-(4-iodobenzyl)-2-bromoacetamide (***4b***)*. Synthesized in two steps from commercially available 4-bromobenzylamine according to Kuhnast *et al.*[12] R_f (heptane/EtOAc 50/50): 0.27. ^{1}H NMR (CD_2Cl_2, 298 K): δ: 7.67 (d, *J*: 8.1 Hz, 2H); 7.04 (d, *J*: 8.1 Hz, 2H); 6.80 (bs, $w_{1/2}$ 23.5 Hz, 1H); 4.38 (d, *J*: 6 Hz, 2H); 3.89 (s, 2H). ^{13}C NMR (CD_2Cl_2, 298 K): δ: 165.8 [C]; 165.0 [C]; 138.1 [CH]; 129.9 [CH]; 93.1 [C]; 43.8 [CH_2]; 29.57 [CH_2]. MS(DCI/NH_4^+): C_9H_9BrINO: 373 [$M + NH_4^+$]; 371 [$M + NH_4^+$].

*Conjugation of Spiegelmers with N-(4-halobenzyl)-2-bromoacetamide. General procedure for preparation of non-labelled conjugated Spiegelmer (*C-**1**, C-**2a**, C-**2b**, C-**3***)*. 1 mg of Spiegelmer (**1**, **2** or **3**) dissolved in 1 ml of PBS (100 mM, pH 8) was mixed with an excess (3 equivalent) of *N*-(4-halobenzyl)-2-bromoacetamide (**4a** or **4b**), dissolved in 1 ml of methanol, and reacted for 30 min at 80°C. The solvents were evaporated and non-labelled conjugated Spiegelmers (C-**1**, C-**2a**, C-**2b**, C-**3**) were purified by semi-preparative RP-HPLC (HPLC A). All non-labelled conjugated Spiegelmers were obtained in 70–80% yield (determined by

HPLC). Conjugated Spiegelmers were desalted using a NAP-10® G25 Sephadex Column (Amersham Pharmacia Biotech) before characterisation.

*N-(4-fluorobenzyl)-2-(5′ps-L-RNA)-acetamide (*C-**1**). R_t(C-**1**): 22.5–23.0 min and R_t(**1**): 19.5–20.0 min (HPLC D). MS (electrospray): 8175.3 (theor.), 8174.0 (exp.). ^{31}P NMR (D_2O, 298.0 K): δ: +15.40 (-OP(O)(OH)(*S*-acetamide)).

*N-(4-fluorobenzyl)-2-(L-RNA-T-3′ps)-acetamide (*C-**2a**). R_t(**C-2a**): 22.5–23.0 min and R_t(**2**): 20.5–21.0 min (HPLC D). MS (electrospray): 8175.0 (theor.), 8172.0 (exp.). ^{31}P NMR (D_2O, 298.0 K): δ: +15.72 (-OP(O)(OH)(*S*-acetamide)).

*N-(4-iodobenzyl)-2-(L-RNA-T-3′ps)-acetamide (*C-**2b**). R_t(C-**2b**): 22.0–22.5 min R_t(**2**): 20.5–21.0 min (HPLC D). MS (electrospray): 8281.5 (theor.), 8279.9 (exp.). ^{31}P NMR (D_2O, 298.0 K): δ: +15.26 (-OP(O)(OH)(*S*-acetamide)).

*N-(4-fluorobenzyl)-2-(L-DNA-3′ps)-acetamide (*C-**3**). R_t(C-**3**): 21.5–22.0 min and R_t(**3**): 19.0–19.5 min (HPLC D). MS (electrospray): 8286.3 (theor.), 8284.2 (exp.). ^{31}P NMR (D_2O, 298.0 K): δ: +14.69 (-OP(O)(OH)(*S*-acetamide)).

Radiochemistry

Preparation of the K[^{18}F]F-K_{222}-complex. In order to recover and recycle the [^{18}O]water target, 2 ml of aqueous [^{18}F]fluoride from the target holder were passed through an anion exchange resin (Sep-Pak® Light Waters Accell™ Plus QMA Cartridge in the chloride form, washed with 5 ml 1 M aqueous $NaHCO_3$ and then rinsed with 50 ml of water) by He pressure (1.5–2.0 bar). Helium was blown through the column to extract maximum of [^{18}O] water. See Dollé *et al.*[16] and Dolci *et al.*[17] 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.[18]

*N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**4a**)*. Synthesized in 3 steps starting from 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (**5**) according to the procedure described in Dollé *et al.*[9] and Kuhnast *et al.*[10,12,13] *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**4a**) was purified using HPLC B: R_t([^{18}F]-**4a**): 10.0–10.5 min. Typically, 2.2–3.3 GBq (60–90 mCi) of pure *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**4a**) could be obtained in 85–95 min starting from a 20.3–24.0 GBq (550–650 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch.

N-(4-(^{125}*I)iodobenzyl)-2-bromoacetamide ([*^{125}*I]-**4b**)*. Synthesized in two radiochemical steps from *N*-(4-trimethylsilylbenzyl)-2-bromoacetamide (**7**) according to the procedure described in Kuhnast *et al.*[12] The *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**) was purified by semi-preparative RP-HPLC, HPLC C: R_t([^{125}I]-**4b**): 8.5–9.0 min. Typically, up to 24 MBq (630 µCi) of pure *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**) were produced starting from 37 MBq (1 mCi) of Na[^{125}I]I.

General procedure for preparation of fluorine-18- and iodine-125-labelled conjugated Spiegelmers ([^{18}*F]*C-**1**, *[*^{18}*F]*C-**2a**, *[*^{125}*I]*C-**2b**, *[*^{18}*F]*C-**3**)*. 1 mg of Spiegelmer (**1**, **2** or **3**) dissolved in 500 µl of PBS (0.1 M, pH 8) was mixed with the corresponding *N*-(4-[*labelled*]halobenzyl)-2-bromoacetamide (*N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-**4a**) or *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide ([^{125}I]-**4b**)), dissolved in 500 µl of methanol, and reacted for 10 min at 120°C in a sealed vial. The solvents were evaporated and labelled conjugated Spiegelmers ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b**, [^{18}F]C-**3**) were separated from starting Spiegelmers (**1**, **2** or **3**) and unreacted *N*-(4-[*labelled*]halobenzyl)-2-bromoacetamide ([^{18}F]-**4a** or [^{125}I]-**4b**) by semi-preparative RP-HPLC. RP-HPLC systems, elution conditions and retention times were similar to those described for the preparation of the non-labelled conjugated Spiegelmers.

For fluorine-18 labelling, the whole synthetic procedure allows producing up to 1.1 GBq (30 mCi) of fluorine-18-labelled Spiegelmers, [^{18}F]C-**1**, [^{18}F]C-**2a** or [^{18}F]C-**3**, in 160 min with a specific radioactivity of 37–74 GBq/µmol at the end of synthesis, starting from a 20.3–24.0 GBq (550–650 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch.

For iodine-125, the whole synthetic procedure allows producing up to 7.4 MBq (200 μCi) of [^{125}I]C-**2b** with a specific radioactivity of 11–37 GBq/μmol, starting from 37 MBq (1 mCi) of Na[^{125}I]I.

Formulation and quality control. The HPLC-fraction containing the labelled conjugated Spiegelmer ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b** or [^{18}F]C-**3**) was concentrated under reduced pressure and transferred in a volume of 1 ml onto a NAP-10® G25 Sephadex Column (Amersham Pharmacia Biotech). Pure labelled and desalted labelled conjugated Spiegelmers ([^{18}F]C-**1**, [^{18}F]C-**2a**, [^{125}I]C-**2b** or [^{18}F]C-**3**) were eluted with 1.5 ml of water or buffer according to the manufacturer's instructions.

As demonstrated by HPLC analysis (HPLC D), radiosynthesized labelled conjugated Spiegelmers 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 labelling of Spiegelmers, L-enantiomeric oligonucleotide aptamers, both with fluorine-18 for positron emission tomography imaging and iodine-125 for high resolution autoradiography, using our radiohalogenated reagents, *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide and *N*-(4-[^{125}I]iodobenzyl)-2-bromoacetamide, respectively. Three 25-mer L-oligonucleotides were efficiently labelled at their 3′- or 5′-end. For fluorine-18, the whole synthetic procedure allows obtaining up to 1.1 GBq of pure labelled Spiegelmer in 160 min with a specific radioactivity of 37–74 GBq/μmol at the end of synthesis starting from 22–24 GBq of [^{18}F]fluoride. For iodine-125, the whole synthetic procedure allows producing up to 7.4 MBq of pure labelled Spiegelmer in 100 min with a specific radioactivity of 11–37 GBq/μmol starting from 37 MBq of Na[^{125}I]I.

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