Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 21 August 2007, Revised 8 October 2007, Accepted 9 October 2007 Published online 3 January 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1471

Radioiodine labelling of a small chemotactic peptide, utilizing two different prosthetic groups: A comparative study

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The use of iodobenzoates as pre-labelled prosthetic groups in the radioiodination of peptides is becoming increasingly popular. The utilization of an iodovinyl ester unit as an alternative conjugation agent for the preparation of a radioiodinated peptide conjugate (peptide-[^{123}I]I-PEA) was investigated in this study. A pre-labelled unit, containing a tetrafluorophenyl ester group, was purified on a small C18 column and the dimethylformamide eluate was used directly in the conjugation step. Similar methodology was applied for a comparative radiosynthesis of an iodobenzoate analogue (peptide-[^{123}I]IB), using a succinimidyl ester. The influences of various reaction parameters on conjugation yields were investigated while maintaining a fixed amount of peptide. At high activity levels (more than 200 MBq) the conjugation yield of peptide-[^{123}I]I-PEA was very sensitive to relatively large reaction volumes and increased amount of base. By optimizing these parameters, the formation of radiochemical impurities was minimized. Under similar conditions, peptide-[^{123}I]I-PEA formed much faster than peptide-[^{123}I]IB. Sep-Pak C18 purification afforded conjugates with radiochemical purities both in excess of 98% and free from unreacted peptide. Recovered conjugation yields of peptide-[^{123}I]I-PEA in 50% ethanol were in excess of 60%, while those for peptide-[^{123}I]IB were less than 40%.

Keywords: ^{123}I; peptide; conjugates; iodovinyl

Introduction

Radiolabelled monoclonal antibodies and peptides have become useful non-invasive diagnostic tools to detect malignant tumours and various types of infectious and inflammatory lesions. The presence of the tyrosine amino acid unit in many of their structures makes direct electrophilic radioiodination highly feasible. The lack of such a unit, as well as *in vivo* deiodination properties shown by directly labelled proteins, has prompted radiochemists to make use of pre-labelled prosthetic groups, such as the succinimidyl-iodobenzoates (SIB). The latter have been utilized especially in the labelling of monoclonal antibodies, but also recently in the labelling of a small chemotactic peptide.[1,2] The general consensus is that radioiodinated peptide conjugates are radiochemically more stable *in vivo*. There is, however, little clarity on the influence of the prosthetic groups on the biological behaviour of the peptide. Some investigators claimed that a ^{18}F-labelled peptide–succinimidyl-fluorobenzoate conjugate had a somewhat lower affinity than the parent peptide.[3] Changes observed in receptor-binding properties and the increase in hepatobiliary excretion could be the result of the increased lipophilicity of the labelled peptide, although biological activity is preserved.[2] The nature of the prosthetic group might also influence the overall biological behaviour of the conjugate.[1] The most suitable prosthetic group should therefore be selected in order to minimize alteration of the biological behaviour of the parent peptide.

The main purpose of this study was to investigate the utilization of the iodovinyl unit in peptide labelling. It has been used by Hadley and Wilbur[4] to prepare iodovinyl antibody conjugates, but has, to this author's knowledge, never been used to prepare iodovinyl peptide conjugates. The chemical and biological stabilities of vinyl iodides are comparable to those of aryl iodides. These features would therefore most likely not be sacrificed. One of the iodovinyl compounds prepared by Hadley and Wilbur[4] was selected for this study. The methodology to prepare labelled iodovinyl peptide conjugates was investigated, and a similar methodology was applied to prepare an iodobenzoate peptide conjugate in parallel. The effects of various conjugation reaction conditions on the different conjugation yields were compared. An alternative purification method for labelled peptide conjugates was also investigated. The biological evaluation of the conjugates will be described in a later study.

The small peptide model chosen for this study is a chemotactic peptide, *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys (fNleLFNleYK). It has been labelled in a few other studies with various radioisotopes such as 99m-technetium,[5] 111-indium,[6] 18-fluorine,[3] 131-iodine[2] and 123-iodine.[1] Radiolabelled chemotactic peptides generally show promise as imaging agents for bacterial infection.

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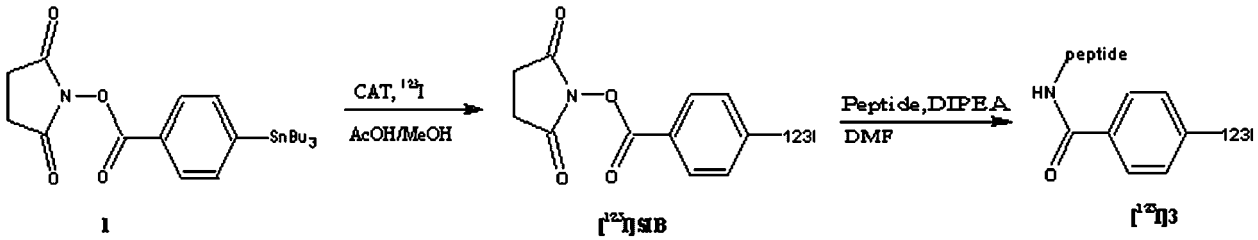
Results and discussion

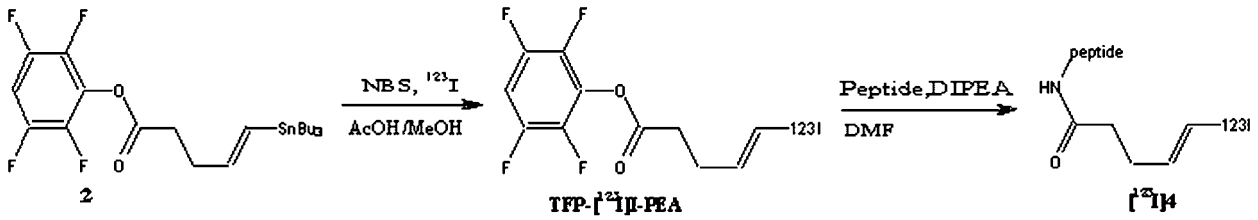
The reaction sequences for the radiosyntheses of the radiolabelled peptide conjugates are shown in Schemes 1 and Scheme 2. The first step comprises the radiosyntheses of the ^{123}I-labelled prosthetic groups or intermediates from their respective precursors. Reaction conditions were not deliberately or systematically optimized. It was found, however, that under the stated conditions of labelling, chloramine-T (CAT) proved to be a superior oxidant than *N*-bromosuccinimide (NBS) to synthesize *N*-succinimidyl-4-[^{123}I]iodobenzoate ([^{123}I]SIB) from its tributylstannyl precursor (N-suc-TBS-BA, **1**). Amounts of 20–40 μg of precursor **1** generally resulted in good yields of [^{123}I]SIB (50–75%). Owing to occasional erratic yields obtained with 20 μg precursor, no experiments were carried out using less than this amount. NBS proved to be a suitable oxidant for the synthesis of 2,3,5,6-tetrafluorophenyl 5-[^{123}I]iodo-4-pentenoate (TFP-[^{123}I]I-PEA) from 2,3,5,6-tetrafluorophenyl 5-(tri-*n*-butylstannyl)-4-pentenoate (TFP-TBS-PEA) **2**. Amounts of 5–40 μg of precursor **2** resulted in fair yields of TFP-[^{123}I]I-PEA (results not presented).

The work-up of TFP-[^{123}I]I-PEA reaction mixtures was slightly adapted from conventional methods due to the high volatility of the labelled product. (Hadley and Wilbur[4] only referred to volatile radioiodide.) Initially, they were purified on C18 mini-columns from which the pure product was eluted with methanol. Evaporation of the methanol solutions resulted in up to 70% loss in activity. Dimethylformamide (DMF), also being the solvent in the peptide conjugation step, was subsequently selected as eluant. Despite the much less volatile nature of [^{123}I]SIB, it was isolated similarly. It was presumed that labelling precursors could co-elute with the labelled intermediates and could consequently compete with them for coupling to the peptide. High-performance liquid chromatography (HPLC) purification of pre-labelled intermediates prior to their coupling to proteins is advisable,[3] especially when using an *N*-halosuccinimide as oxidant[7] or small quantities of protein (less than 100 μg).[8] The volatility of TFP-[^{123}I]I-PEA would also eliminate the possibility of HPLC purification of the labelled intermediate, and mini-column purification was therefore regarded as the only option. The results presented in Table 1 suggest that first-step labelling reactions containing up to 40 μg precursor did not have any significant effect on subsequent conjugation yields. Using excessive amounts, as illustrated for 100 μg TFP-TBS-PEA, could reduce conjugation yields significantly. Precursor contents in DMF eluates were not determined.

Conjugation reactions are normally conducted in small volumes (approximately 5 μl).[2,3] A mini-column containing a small amount of C18-packing material was therefore required, enabling a selective and quantitative washout of free radioiodide with water



Scheme 1



Scheme 2

Table 1. Effect of mass of pre-labelled precursors on radiochemical yields (RCY) of peptide conjugates

Mass precursor **1** (μg)	RCY[a] conjugate **3** (%)	Mass precursor **2** (μg)	RCY[b] conjugate **4** (%)
20	64.7 ± 0.6 ($n = 3$)	5	83 ($n = 1$)
40	65.0 ± 4.4 ($n = 3$)	10	81 ($n = 1$)
		40	82 ($n = 1$)
		100	28 ($n = 1$)

[a]RCY as assessed by analytical HPLC, ex-overnight. For peptide-[^{123}I]IB conjugation reactions: 40 μg peptide and 5 μl DIPEA per experiment. Reaction volume: 280–400 μl. Activity content: 96–388 MBq.
[b]RCY as assessed by analytical HPLC. For peptide-[^{123}I]I-PEA conjugation reactions: 40 μg peptide and 2 μl DIPEA per experiment. Reaction volume: 180–250 μl. Activity content: 300–400 MBq.

and optimal recovery of the labelled intermediate in the smallest possible volume of DMF. A mini-column containing 100 mg C18 was selected for this purpose. Elution of the main fraction was preceded by a pre-elution with 130 or 150 μl DMF in order to displace water. A minimum volume of 180–200 μl DMF was required for optimal elution of the labelled intermediate. The average radiochemical recovery yields of [^{123}I]SIB and TFP-[^{123}I]I-PEA were quite similar (63 and 65%, respectively). On average, 10 and 20% free radioiodide was eluted from mini-columns during purification of TFP-[^{123}I]I-PEA and [^{123}I]SIB, respectively, suggesting that more of the former was formed during labelling. Its lesser recovery was due to its higher lipophilicity, as also indicated by its stronger retention by the C18.

Conjugation reaction conditions were optimized by using a fixed, relatively small amount of peptide throughout and varying other reaction parameters. The rationale behind this was that peptides are often very expensive and/or difficult to remove from reaction mixtures, and that the use of excessive amounts (more than 50 μg) should be avoided. The authenticities of radiolabelled peptide conjugates were confirmed by comparing their HPLC retention times with those of authentic compounds, using UV detection. The effects of the reaction parameters on the formation of various radiochemical species in peptide-[^{123}I]I-PEA **4** conjugation reaction mixtures are presented in Table 2. Other than **4**, two unidentified, relatively more polar impurities, A and B, are formed, neither of the latter being the hydrolysed counterpart of TFP-[^{123}I]I-PEA. The size of the activity incorporated into conjugation reaction mixtures, as well as the reaction volume, which determines the peptide concentration, both have an impact on the formation of these species. At relatively low activity levels (74–137 MBq), less impurities are formed (entries 1, 3 and 9). At activity levels in excess of 200 MBq and volumes larger than 250 μl, higher amounts of especially the more polar impurity, A, are formed (entries 2, 7 and 8). Under these conditions increased amounts of *N,N*-diisopropylethylamine (DIPEA), used as the conjugation base, generate higher amounts of the more polar impurity (entries 8, 10 and 11). This becomes more pronounced at higher activity levels (entry 12). At higher activity levels, the optimal formation of **4** is favoured by reaction volumes of 250 μl or less and a DIPEA content of 2 μl (entries 4, 5 and 6). Less than 2 μl DIPEA could result in a too slow reaction rate, as suggested by entry 2. The nature of the formed impurities is debatable.

The reaction parameters that dictate peptide-[^{123}I]I-PEA conjugation yields do not have a similar strong impact on peptide-[^{123}I]IB **3** yields (Table 3). The [^{123}I]SIB-containing DMF eluates were dried with anhydrous $MgSO_4$ prior to peptide conjugation as inconsistent conjugation yields were obtained, under similar reaction conditions, using undried eluates. This suggests that the reaction is sensitive to the presence of moisture (results not presented). Peptide-[^{123}I]I-PEA **4** conjugation yields are unaffected by the drying of eluates (results not presented). Peptide-[^{123}I]IB conjugation reactions proceeded slower than peptide-[^{123}I]I-PEA reactions under similar conditions. Traces of unreacted [^{123}I]SIB were still present after 1 h, whereas TFP-[^{123}I]I-PEA was completely consumed within 30 min. The reason for this is not entirely clear, given the higher reactivity of *N*-hydroxysuccinimide esters.[4] (The TFP ester analogue of *N*-suc-TBS-BA was also synthesized, but this precursor yielded very poor radioiodination and subsequent peptide conjugation yields.) Unlike peptide-[^{123}I]I-PEA conjugation reactions, varying amounts of free radioiodine were also formed during the course of peptide-[^{123}I]IB reactions, together with *p*-[^{123}I]iodobenzoic acid and another unknown impurity. Extended reaction times led to increased formation of peptide-[^{123}I]IB with simultaneous consumption of *p*-[^{123}I]iodobenzoic acid. This suggests that the latter is sufficiently reactive for conjugation purposes, albeit very slow. Extended reaction periods for peptide-[^{123}I]I-PEA reactions did not result in the formation of significantly more product.

Conjugation reaction mixtures were purified on a Sep-Pak mini-column. The radiochemical yields of the conjugates are

Table 2. Effects of various reaction parameters on the formation of various radiochemical species in peptide-[^{123}I]I-PEA conjugation reaction mixtures

Entry no.	Activity (MBq)	Reaction volume (μl)	DIPEA (μl)	Radiochemical species in reaction mixture[a] (%) A	B	C
1	111	300	1	4	9	86
2	296	300	1	24	1	66[b]
3	137	200	2	6	5	86
4	296	200	2	15	3	81
5	300	250	2	11	5	83
6	333	180	2	12	6	80
7	322	290	2	28	6	64
8	226	300	3	34	5	59
9	74	250	5	7	4	86
10	226	300	5	37	7	52
11	259	250	5	30	6	62
12	474	250	5	53	3	41

40 μg peptide per experiment. Reaction times: 25–50 min. A, unknown radiochemical impurity, R_t 6 min; B, unknown radiochemical impurity, R_t 7.5 min; C, peptide-[^{123}I]I-PEA **4**, R_t 12.7 min.
[a]As assessed by analytical HPLC, balances made up by small amounts of unknown radiochemical impurities.
[b]Contains 9% unreacted TFP-[^{123}I]I-PEA.

Table 3. Effects of various reaction parameters on the formation of various radiochemical species in peptide-[^{123}I]IB conjugation reaction mixtures

					Radiochemical species in reaction mixture[b] (%)				
Entry no.	Activity (MBq)	Reaction volume[a] (μl)	DIPEA (μl)	Reaction time (min)	A	B	C	D	E
1	293	240	2	30	12	8	17	13	47
				60	13	7	18	2	58
				Overnight	15	9	6	1	66
2	107	270	5	25	2	40	4	21	24
				55	3	27	8	7	48
				150	3	7	10	1	74
3	130	390	5	25	2	33	6	27	25
				55	3	20	11	9	46
				Overnight	4	6	15	0	65
4	250	295	5	25	6	14	17	13	44
				60	6	7	20	2	59
				Overnight	9	10	6	1	70
5	389	370	5	25	8	33	6	14	29
				55	9	18	9	4	50
				Overnight	11	11	7	0	64
6	238	300	10	25	4	27	15	2	45
				70	4	8	20	1	64
				Overnight	4	4	21	0	67

40 μg peptide per experiment. A, free radioiodine, R_t 3.1 min; B, *p*-[^{123}I]iodobenzoic acid, R_t 8.9 min; C, unknown impurity, R_t 10.7 min; D, unreacted [^{123}I]SIB, R_t 13.7 min; E, Peptide-[^{123}I]IB **3**, R_t 18.8 min.
[a]$MgSO_4$-dried DMF eluates.
[b]As assessed by analytical HPLC, balances made up by varying small amounts of other unknown radiochemical impurities.

Table 4. Radiochemical yields (RCY) of peptide conjugates after Sep-Pak C18 purification

Conjugate	Final HPLC yield[a] (%)	50% EtOH eluant (ml)	RCY[b] (%)	Remaining activity on C18[c] (%)	Combined RCY[bc] (%)
Peptide-[^{123}I]IB **3**	48	5	36	13	49
	62	2.5	39	12	51
	59	5	35	22	57
Peptide-[^{123}I]I-PEA **4**	80	2.5	63	2	65
	80	2.5	68	4	72
	76	2.2	64	4	68
	75	3.0	62	2	64

[a]As assessed by analytical HPLC.
[b]Activity in 50% EtOH, expressed as a percentage of starting activity in conjugate reaction mixture.
[c]Expressed as a percentage of starting activity in conjugate reaction mixture.

presented in Table 4. The more polar radiochemical impurities as well as unreacted peptide were quantitatively and selectively eluted with mixtures of 0.1% trifluoroacetic acid (TFA) and acetonitrile (ACN). The conjugation products were eluted with 50% aqueous ethanol, thereby making it possible to fairly quickly convert the eluate into a radiopharmaceutical product. An added advantage of using this eluant was that lipophilic precursors, if still present, would not co-elute. UV-HPLC analyses of these eluates revealed the absence of any traces of precursor or unreacted peptide. The more lipophilic nature of peptide-[^{123}I]IB **3** resulted in a higher proportion of activity retained by C18 than in the case of peptide-[^{123}I]I-PEA **4**. The sum totals of activity, however, show that the overall radiochemical yield of compound **4** was still generally higher than that of **3**. This can be ascribed to the lesser amounts of radiochemical impurities formed under optimal conditions during the peptide-[^{123}I]I-PEA reaction. The radiochemical purities of all the conjugates after Sep-Pak purification were in excess of 98%.

Experimental

The chemotactic peptide was obtained from Sigma and GenScript Corporation (USA). All other reagents and chemicals were obtained from either Aldrich or Fluka. Analytical HPLC was performed on the following system: HPLC pump (Agilent 1100 Series, equipped with a HP 1100 Series Control Module for

binary gradient elution), analytical C18 column (Phenomenex Luna, 250 × 4.6 mm, 5 μm), injector (Rheodyne Model 7725), UV detector (Spectra Series UV100, set at 254 nm), radiation detector (Carroll&Ramsey Model 105S-1 with a CsI(Tl) detector probe). Chromatograms were recorded on a Chromatopac C-R8A from Shimadzu. HPLC-grade acetonitrile was used. Binary gradient elution was carried out for the analyses of all reaction mixtures and purified reaction products, using mixtures of 0.1% TFA and ACN as mobile phase. Elution was carried out at 1 ml/min, starting with 50% ACN over 15 min, followed by a 50–90% linear gradient of ACN over a period of 5 min and finally a 90–100% gradient of ACN over 5 min. Radioactivity measurements were carried out in a Vinten Isocal II Radionuclide Assay Calibrator. Electrospray ionization mass spectrometry was performed on a Waters API Q-TOF Ultima instrument. Strata C18 and Sep-Pak C18 mini-columns were obtained from Phenomenex and Waters, respectively.

N-succinimidyl 4-tri-n-butylstannylbenzoate (*N-suc-TBS-BA*), ***1***: This precursor was synthesized analogously to the method of Wilbur *et al.*,[8] except for using 4-bromobenzoic acid instead of the corresponding methyl ester as the starting material for the metal–halogen exchange reaction. Reaction intermediates and final product were purified by means of silica gel chromatography as described.

2,3,5,6-Tetrafluorophenyl 5-(tri-n-butylstannyl)-4-pentenoate (*TFP-TBS-PEA*), ***2***: This precursor was synthesized analogously to the method of Hadley and Wilbur,[4] with minor modifications. Methyl 4-pentynoate was synthesized by means of esterification of 4-pentynoic acid in refluxing methanol containing a catalytic amount of concentrated sulphuric acid. In short, a solution of 4-pentynoic acid (510 mg, 5.2 mmol) in methanol (2 ml) containing concentrated H_2SO_4 (35 μl) was refluxed for about 6 h. Diethyl ether (8 ml) was added and further work- up was conducted according to the described method. Methyl 5-(tri-*n*-butylstannyl)-4-pentenoate was also synthesized according to the described method, but the purification step was slightly modified by diluting the final reaction mixture with approximately 2.3 volumes of hexane prior to silica gel chromatography instead of evaporating the solvent. Hydrolysis of the methyl ester was conducted with NaOH instead of KOH, and the tetrafluorophenyl ester was synthesized as described. Purification of the ester was started by diluting the filtered reaction mixture with 2.3 volumes of hexane, followed by silica gel chromatography as described. This precursor was sometimes also further purified by means of HPLC, using the C18 column and 100% acetonitrile as eluant at a flow rate of 1 ml/min. The precursor appeared as a double peak (cis/trans mixture) with a retention time of 20–21 min. The fraction containing the peak was collected and evaporated to dryness under a flow of nitrogen.

fNleLFNleYK-p-iodobenzoate (*peptide-IB*), ***3***: This reference compound was synthesized from fNleLFNleYK and *N*-succinimidyl-4-iodobenzoate (SIB) [prepared from 4-iodobenzoic acid according to the method of Garg *et al.*[9]]. In short, a solution of the peptide in DMF (10 mM, 20 μl, 0.2 μmol) was mixed with a solution of SIB in DMF (145 mM, 1.4 μl, 0.2 μmol) and a solution of DIPEA (0.5 μl) in DMF (1 μl) was added. The mixture was stirred at room temperature for approximately 10 h, after which it was diluted with 16 μl methanol. The product peptide-IB was isolated by HPLC, using the elution conditions as described earlier. Retention times of the various components under these conditions were as follows: 4-iodobenzoic acid (8.3 min), unknown impurity (9.8 min), SIB (12.7 min), peptide-IB (18.5 min). The product peak fraction was collected during two separate runs (2 × 20 μl) and concentrated at 45°C under reduced pressure to approximately half of the original volume. The concentrate was loaded on to a Sep-Pak C18 mini-column (500 mg C18), the column was washed with water (4–5 ml) and the peptide-IB was eluted with methanol (0.8 ml). The structure was confirmed by mass spectral analysis. MS, *m/z* 1054.4 (M+H), 1076.4 (M+Na).

fNleLFNleYK-5-iodo-4-pentenoate (*peptide-I-PEA*), ***4***: This reference compound was synthesized from fNleLFNleYK and 2,3,5,6-tetrafluorophenyl 5-iodo-4-pentenoate (TFP-I-PEA) [prepared from TFP-TBS-PEA **2** according to the method of Hadley and Wilbur[4]]. In short, a solution of the peptide in DMF (10 mM, 12 μl, 0.12 μmol) was mixed with a solution of TFP-I-PEA in DMF (53.5 mM, 2.0 μl, 0.11 μmol) and a solution of DIPEA (0.4 μl) in DMF (1 μl) was added. The mixture was stirred at room temperature for approximately 2.5 h, after which it was diluted with 10 μl methanol. The product **4** was isolated by HPLC, using isocratic elution with 0.1% TFA/ACN (50:50) at 1 ml/min. The retention time of **4** was in the region of 12.1–12.5 min. Isolation of the product from the HPLC eluate was carried out as described for peptide-IB **3**. The structure for **4** was confirmed by mass spectral analysis. MS, *m/z* 1032.4 (M+H), 1054.4 (M+Na).

Radiochemical syntheses

No-carrier-added [^{123}I]iodine was produced by means of the ^{127}I(p,5n)^{123}Xe → ^{123}I route, using a 66 MeV proton beam, and recovered in 0.01 M NaOH solutions. These solutions were reduced in volume by a factor of 3–5 by means of evaporation, therefore effectively rendering a NaOH concentration of 0.03–0.05 M.

Peptide-[^{123}I]I-PEA, ***4***: The radiosynthesis of the pre-labelled intermediate, TFP-[^{123}I]I-PEA, was based on a literature method,[4] with few modifications. In short, to a solution of precursor **2** (5–40 μg) in 10% acetic acid/methanol (125–300 μl) was added a solution of ^{123}I in 0.03–0.05 M NaOH (50–80 μl, up to 600 MBq). A solution of NBS (30 μg) in methanol (30 μl) was added and the mixture was stirred at room temperature. After 10 min the reaction was quenched with a solution of $Na_2S_2O_5$ in water (0.72 mg/ml, 24 μl). The reaction mixture was diluted with water (0.5–1.0 ml, depending on the volume of the reaction mixture), and loaded on a pre-conditioned C18 mini-column (Strata C18-E, 100 mg, 55 μm, 1 ml). The C18 was washed with water (6 ml) and subsequently eluted with DMF (150 μl), followed by another portion of DMF (180–300 μl). The second DMF eluate, containing TFP-[^{123}I]I-PEA, was mixed with a solution of the peptide in DMF (10 mM, 5 μl), DIPEA (1–5 μl) was added and the mixture stirred at room temperature. After complete consumption of TFP-[^{123}I]I-PEA, the reaction mixture was purified on a pre-conditioned Sep-Pak C18 (500 mg) column after dilution with water (2 ml). Free radioiodide as well as the more polar radiochemical impurities were eluted with 0.1% TFA/acetonitrile = 70:30 (v/v) (14 ml). Subsequent elution with water (8 ml) and water/ethanol (50:50) (v/v) (1.5 ml) was followed by a final elution of **4** with water/ethanol (50:50) (v/v) (2.2–3 ml).

Peptide-[^{123}I]IB, ***3***: The radiosynthesis of the pre-labelled intermediate, [^{123}I]SIB was essentially similar to that of TFP-[^{123}I]I-PEA, but CAT was used as an oxidant instead of NBS. In short, to a solution of precursor **1** (20 or 40 μg) in 10 or 30% acetic acid/methanol (100–125 μl) was added a solution of ^{123}I in 0.03–0.05 M NaOH (50–60 μl, up to 600 MBq). A solution of CAT

(96 μg) in methanol (96 μl) was added and the mixture was stirred at room temperature. After 10 min the reaction was quenched with a solution of $Na_2S_2O_5$ in water (0.72 mg/ml, 48 μl). The reaction mixture was diluted with water (0.5) and loaded on a pre-conditioned C18 Strata C18-E. The C18 was washed with water (6 ml) and subsequently eluted with DMF (130 μl), followed by another portion of DMF (200–300 μl). The second DMF eluate, containing [^{123}I]SIB, was filtered through a small column containing anhydrous $MgSO_4$ (50 mg, pre-moistened with DMF). The column was washed with small amounts of DMF (40–80 μl). The combined dried eluate was mixed with a solution of the peptide in DMF (10 mM, 5 μl), DIPEA (2–10 μl) was added and the mixture stirred at room temperature. Reaction mixtures were purified on Sep-Pak C18 as described before, using the following eluants: 0.1% TFA/acetonitrile = 70:30 (v/v) (6 ml); 0.1% TFA/acetonitrile = 60:40 (v/v) (5 ml). Subsequent elution with water (8 ml) and water/ethanol (50:50) (v/v) (2 ml) was followed by a final elution of **3** with water/ethanol (50:50) (v/v) (2.5–5 ml).

Conclusion

A radiolabelled small peptide–iodopentenoate conjugate was successfully prepared in two radiochemical steps by employing the concept of pre-labelled intermediates previously reported. A similar methodology was applied to the radiosynthesis of the iodobenzoate analogue of the same peptide. An advantageous feature of the methodology used was the omission of an evaporation step after the work-up of the intermediates leading to the conjugation step, necessitated by the volatility of the labelled iodopentenoate intermediate. Results have shown that under similar conjugation reaction conditions the peptide–iodopentenoate conjugation reaction proceeds faster than its iodobenzoate counterpart. Under optimal conjugation reaction conditions radiochemical yields of the peptide–iodopentenoate conjugate were also higher than those of its iodobenzoate analogue. These features could make a radiolabelled iodovinyl intermediate an attractive alternative for the preparation of a radiolabelled peptide conjugate, provided that its biological properties remains intact.

Acknowledgement

The author wishes to thank Ms G. Visser for preparing some of the cold precursors used in this work.

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