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Synthesis of HBED–CC–tris(*tert*-butyl ester) using a solid phase and a microwave reactor

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ABSTRACT

N,N′-bis-[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N,N′*-diacetic acid (HBED-CC) belongs to the acyclic, bifunctional complexing compounds used mainly for radiolabeling with gallium-68 (^{68}Ga). Due to the high stability of the $^{68}Ga^{3+}$complex, HBED-CC is well known for its rapid and efficient labeling at ambient temperature and the high stability of the complexes *in vivo*. The HBED-CC chelator in combination with a PSMA (Prostate Specific Membrane Antigen) inhibitor and labeled with isotope of gallium is an important tool for diagnosing the stage of cancer in patients with prostate cancer. Many HBED-CC derivatives have been described in the literature, but one of the most commonly used is 3-(3-{[(2-{[5-(2-*tert*-butoxycarbonylethyl)-2-hydroxybenzyl]-*tert*-butoxycarbonylmethylamino}-ethyl)-*tert*-butoxycarbonylmethylamino]-methyl}-4-hydroxyphenyl)propionic acid (HBED–CC–tris(*tert*-butyl ester)). This compound is very expensive and commercially limited. Therefore this work describes an innovative method of synthesis on solid phase using of a microwave reactor. Optimization of the reaction allowed to obtain HBED-CC-tris(*tert*-butyl ester) with high purity and yield.

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1. Introduction

N,N′-bis(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (HBED) ligand and its derivatives are very well known substances frequently used in agricultural research and radiopharmacy [1,2]. Research shows that by modifying the lead structure of HBED, new molecules with better physical properties (e.g. better water solubility) were obtained [3]. In literature we can find a lot HBED analogues such as (3-(3-{[(carboxymethyl)[2-({[5-(2-aminoethyl)-2- hydroxyphenyl]methyl}(carboxymethyl)amino)ethyl]amino]methyl}-4-hydroxyphenyl)propionic acid) (HBED-CA), (3-(3-{[(carboxymethyl){2-[(carboxymethyl){[2-hydroxy-5-(2-thiocyanatoethyl)-phenyl]methyl}amino]ethyl}amino]methyl}-4-hydroxyphenyl)-pionic acid (HBED-Cl), (({[5-(2-aminoethyl)-2-hydroxyphenyl]methyl}[2-({[5-(2-aminoethyl)-2-hydroxyphenyl]methyl}(carboxymethyl)amino)ethyl]amino)acetic acid (HBED-AA), 3-(3-{[(carboxy-methyl){2-[(carboxymethyl)({2-hydroxy-5-[2-(2,3,5,6-tetrafluoro-phenoxycarbonyl)ethyl]phenyl}methyl)amino]ethyl}amino]methyl}-4-hydroxyphenyl)propionic acid (HBED-CC-TFP), [({2-hydroxy-5-[2-(2,3,5,6-tetrafluorophenoxycarbonyl)ethyl]phenyl}-methyl){2-[(carboxymethyl)({2-hydroxy-5-[2-(2,3,5,6-tetrafluoro- phenoxycarbonyl)ethyl]phenyl}methyl)amino]ethyl}amino]acetic acid (HBED-CC-TFP2) but the most popular derivative is HBED-CC [3].

N,N′-bis-[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N,N′*-diacetic acid (HBED-CC) is a bifunctional ligand that forms complexes with divalent and trivalent metal ions like as Cu^{2+} [4,5], Fe^{3+} and radioisotopes such as ^{68}Ga and ^{99m}Tc [6,7]. HBED-CC with metals creates structures, in which phenolic groups are responsible for coordinating the central metal ion. Furthermore, nitrogen and oxygen atoms (three carboxylic groups) are necessary to form complexes (for example hexadentate complex) [5,8]. HBED-CC is attached to other chemical molecules and/or antibodies by one of carboxylic group, which is connected to phenol rings.

HBED-CC is most commonly used as a highly effective chelator for ^{68}Ga labeling due to the high thermodynamic stability. The labeling conditions are mild due to the acyclic structure of HBED-CC, and the given Ga complex has high kinetic inertness at physiological pH which is essential for *in vivo* applications [9,10]. Last years showed that [^{68}Ga]Ga-PSMA-HBED-CC ([^{68}Ga]Ga-PSMA-11) can be

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considered as the one of standard agents in diagnostic prostate cancer (PET imaging) [10–12].

This work presents a new efficient synthesis method of HBED–CC–tris(*tert*-butyl ester), which is the most useful molecule for coupling with PSMA among the HBED-CC analogues. Our previous experiments showed that the use of HBED–CC–di(*tert*-butyl ester) for conjugation with PSMA led to the formation of the undesirable product as dimer: $PSMA_2$-HBED-CC. On the other hand, the use of HBED–CC–monomethyl-di(*tert*-butyl ester) chelator was also unfavorable due to the problem with removal of the methyl group in the final product (PSMA-11).

In the literature there is only one method of synthesis of HBED–CC–tris(*tert*-butyl ester) and it is based on a 10 step reaction starting from 3-(4-hydroxyphenyl) propanoic acid [13]. Other publications present methods of synthesis analogues of HBED-CC. One of these method describe synthesis of (HBED-CC)TFP based on the reaction of HBED-CC with Fe^{3+} ions and esterification with 2,3,5,6-tetrafluorophenol. The final product as a monoester is conjugated to e.g. antibody [11]. The next method of synthesis of HBED–CC–di(*tert*-butyl ester) is based on a 5 step reaction starting from 3-(4-hydroxyphenyl) propanoic acid [10].

The aim of our work was to develop a new way of synthsis HBED–CC–tri(*tert*-butyl ester).

In this work, we employed the previously described methods of synthesis [10,13]. We adopted and modified them in order to develop a better, more effective synthesis method. We planned the preparation of HBED–CC–tri(*tert*-butyl ester) using a solid phase and a microwave reactor.

2. Results and discussion

The synthesis of the HBED-CC-tris (tert-butyl ester) chelator consisted of two main experimental parts, and the full structural characterization of the intermediates and main product is illustrated in Scheme 1 and Scheme 2. The first part of the experiments were performed basing on previously described procedures (with some minor modifications) starting with the esterification of 3-(4-hydroxyphenyl) propionic acid resulting compounds **2** and **5** [10,14,15]. Methyl 3-(4-hydroxyphenyl)propanoate **2** was obtained with 86% yield after purification by column chromatography (dichloromethane) and with above 98% purity (HPLC-method A). Noteworthy, the synthesis of **2** was already described by Zha Z. at al. [10], but lower yield (83,5%) was reported. Using $SOCl_2$ instead of $BF_3 \cdot Et_2O$ allowed to increase the yield up to 86%.

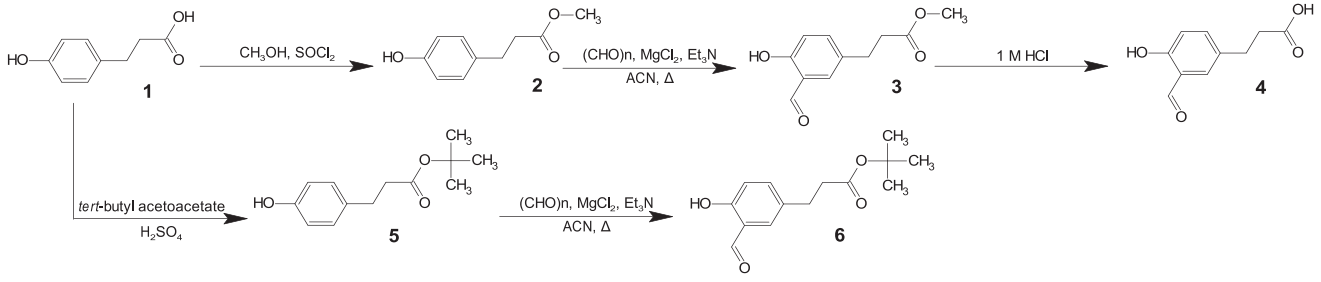
The synthesis of compound **5** was very demanding. During the development work, few methods of synthesizing *tert*-butyl esters were tested [16–18]. For example, syntheses using *N,N′*-carbonyl diimidazole DBU as base and transesterification methods using potassium *tert*-butoxide in THF were tried. The most satisfying results have been obtained with *tert*-butyl acetoacetate, sulfuric acid and dry anhydrous magnesium sulfate (powder). Product **5** was purified by column chromatography (dichloromethane/ethyl acetate, 10/0.1) and obtained with 23% yield and with purity exceeding 95% (HPLC-method A).

In two next steps, the formyl group was introduced into the aromatic rings of compounds **2** and **5** to give products **3** and **6**. The crude products were purified by column chromatography (dichloromethane) and obtained compound **3** in 70% yield and purity exceeding 98% (HPLC-method B) and compound **6** in 48% yield and purity exceeding 97% (HPLC-method A). The 3-(3-formyl-4-hydroxyphenyl)propanoic acid **4** was prepared by hydrolysis of **3** using 1 M HCl and was obtained in 85.6% yield after purification by crystallization from acetonitrile and with above 97% purity (HPLC – method C). Compounds **4** and **6** were prepared from 3-(4-hydroxyphenyl) propionic acid **1** in overall yield: 46% for **4** and 10% for **6**.

The second part of the experiments was carried out in accordance with the previously described procedures but the introduction of certain modifications allowed to increase the yield of the HBED–CC–tris(*tert*-butyl ester) synthesis [13,19–21]. First of all, the novelty was the application of a solid support synthesis strategy using 2-chlorotrityl resin. This strategy greatly simplifies the synthesis steps by allowing work at excessive molar ratios and eliminates the need to separate unreacted substrates, reducing the overall time required for synthesis.

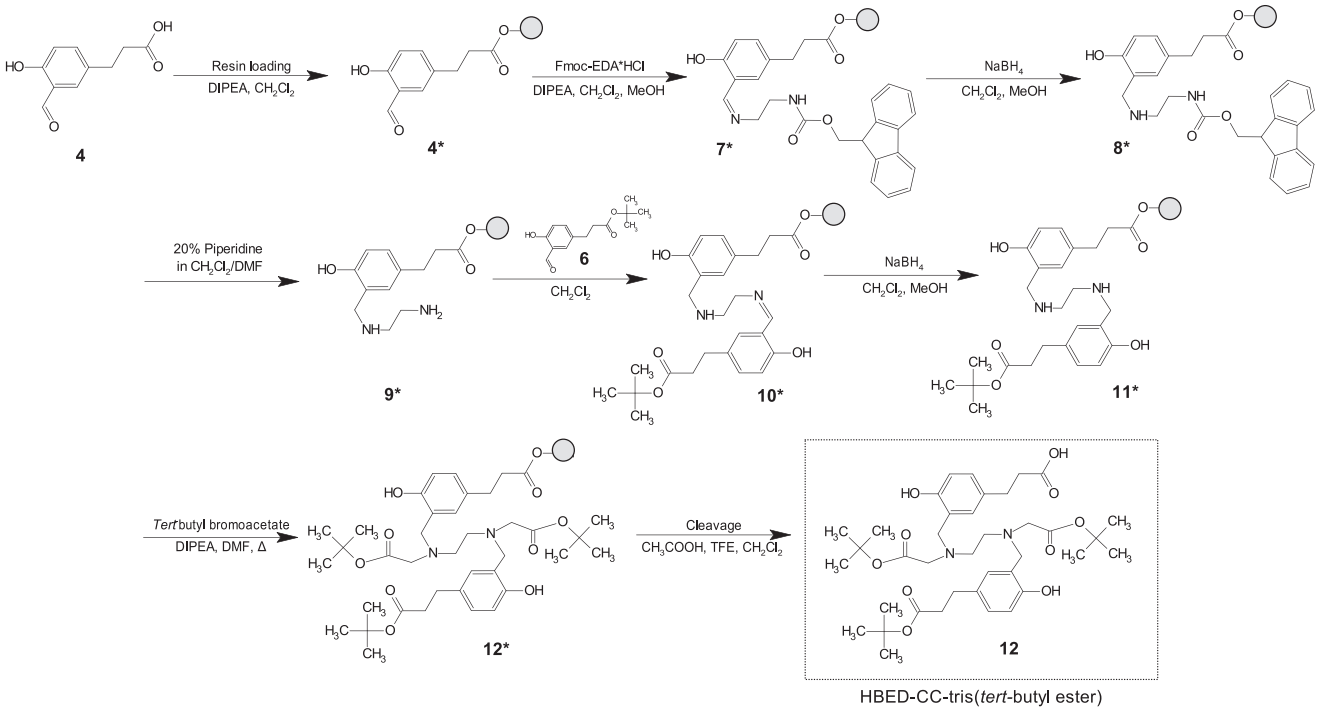
Solid phase synthesis started with attachment of 3-(3-formyl-4-hydroxyphenyl) propanoic acid **4** to the resin and then reaction with mono-Fmoc ethylenediamine hydrochloride (Fmoc-EDA*HCl) resulting compound **7***. In the initial experiments, ethylenediamine and N-Boc-ethylenediamine were used instead of Fmoc-EDA*HCl. In the case of using ethylenediamine, it turned out that after cleavage from the resin and confirming the identity of the obtained compound, the main by-product of the reaction (produced in large amount) was a dimer with the mass: 412 g/mol (MS: $[M+H]^+ = 413$ g/mol). Therefore, further experiments using ethylenediamine were abandoned, and a modification involving the use of Boc-protected ethylenediamine was proposed. Due to the type of the resin used, the Boc group could not be deprotected in the traditional way (HCl, dioxane) [22], but only with iodine [23]. Unfortunately, trials to deprotection the Boc group with iodine failed (as shown by MS results), therefore it was decided to use ethylenediamine protected with one-sided Fmoc group.

In the next two steps the reduction of compound **7*** using $NaBH_4$ and the deprotection of the Fmoc protecting group from



$SOCl_2$= thionyl chloride
$(CHO)_n$=paraformaldehyde
Et_3N= triethylamine
$MgCl_2$ = magnesium chloride

Scheme 1. Synthesis of 3-(3-formyl-4-hydroxyphenyl)propanoic acid (**4**) and *tert*-butyl 3-(3-formyl-4-hydroxyphenyl)propanoate (**6**).



= 2-Chlorotrityl chloride resin
Fmoc-EDA*HCl=mono-Fmoc ethylene diamine hydrochloride
DIPEA= *N,N*-Diisopropylethylamine
TFE = 2,2,2-trifluoroethanol

Scheme 2. Synthesis of HBED−CC−tris(*tert*-butyl ester) (**12**).

compound **8*** using 20% piperidine in dichloromethane/*N,N*-dimethylformamide was performed. During these experiments, there was also a problem with the order of the reduction step and the deprotection of the Fmoc group. When an attempt was made to deprotect the Fmoc group from the unreduced imine **7*** (using 20% piperidine in *N,N*-dimethylformamide/dichloromethane), the compound was turned out be unstable. When the order of the reaction was changed (first reduction followed by deprotection of the Fmoc group), a series of subsequent steps were successfully performed.

Product **11*** was obtained by reacting **9*** with *tert*-butyl 3-(3-formyl-4-hydroxyphenyl) propanoate and then reduction of compound **10*** using $NaBH_4$. The N-alkylation reaction **11*** was carried out using a microwave reactor, which was another innovation in this synthesis. Final compound **12** was prepared by deprotection from resin of **12*** using mixture of CH_3COOH: CF_3CH_2OH:CH_2Cl_2 (1:2:7) [24]. HBED−CC−tris(*tert*-butyl ester) **12** was obtained in 36 % overall yield (from compound **4**) after purification by preparative HPLC and with above 97% purity (HPLC - method D). The yield achieved is more than that (28%) reported by Makarem at al. [13]. The HPLC chromatogram of HBED−CC−tris(*tert*-butyl ester) **12** is shown in Fig. 1. All the described analytical methods (A-F) and methods of purification of compounds were new and prepared especially for this study.

All synthesized compounds were characterized by mass spectroscopy or ^1H and ^{13}C NMR spectroscopy. Only compounds **7*** and **10*** could not be separated from the resin for identity confirmation due to the low stability of substance after deprotection. A typical mass spectrum confirming the identity of HBED−CC−tris(*tert*-butyl ester) **12** is shown in Fig. 2.

To optimize the N-alkylation reaction in a microwave reactor, several experiments were performed to determine the effect of the microwave process parameters (time and temperature) on the chromatographic purity of the obtained HBED−CC−tris(*tert*-butyl ester). During each experiment, the temperature (40−150°C) and time (7−30 min) of the reaction were selected. In all studies, the same power 60 W was used. The results are presented in Fig. 3.

It was observed that there is an optimal temperature range from 60°C to 120°C, where the purity of raw product was increased above 70% (HPLC - method D) depending on the heating time. However, the findings indicate that a reaction time of 30 min and a microwave heating temperature of 80°C give the product with the highest chromatographic purity above 73% (HPLC - method D). Fig. 3 shows that a significant portion of compound **11*** was not reacted at temperatures below 60°C and in less than 30 min. On the other hand, when the temperature rose above 120°C, and the heating time was longer than 7 min, there was an increase one of impurities (base on MS spectrum results is predicted that it might be HBED−CC−di(*tert*-butyl ester), MS: $[M+H]^+ = 645$) reducing the purity of the chelator. The N-alkylation reaction was carried out on a large scale under the following conditions: time 30 min, temperature 80°C, power 60 W.

The use of a microwave reactor allowed for a significant reduction in the synthesis time (reduction of the N-alkylation reaction time from several hours to 30 min) [5,6], and an increase in the yield and purity of the final product. The undoubted advantages of solid phase synthesis include: no need to isolate intermediate products for cleaning, increasing the speed of chemical processes (which is related to the possibility of using excess substrates and the ease of washing them from the resin after given reaction

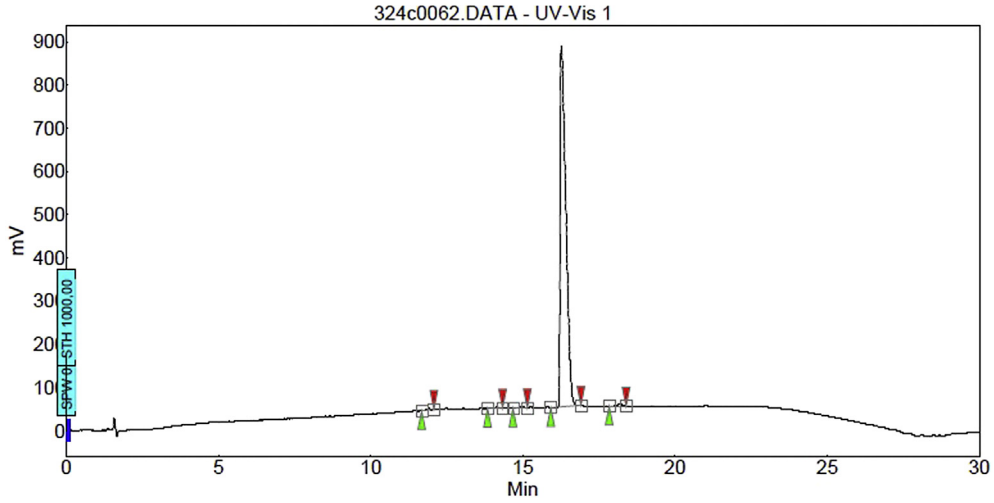


Fig. 1. HPLC typical analytical chromatogram (method D) of HBED–CC–tris(*tert*-butyl ester) **12**.

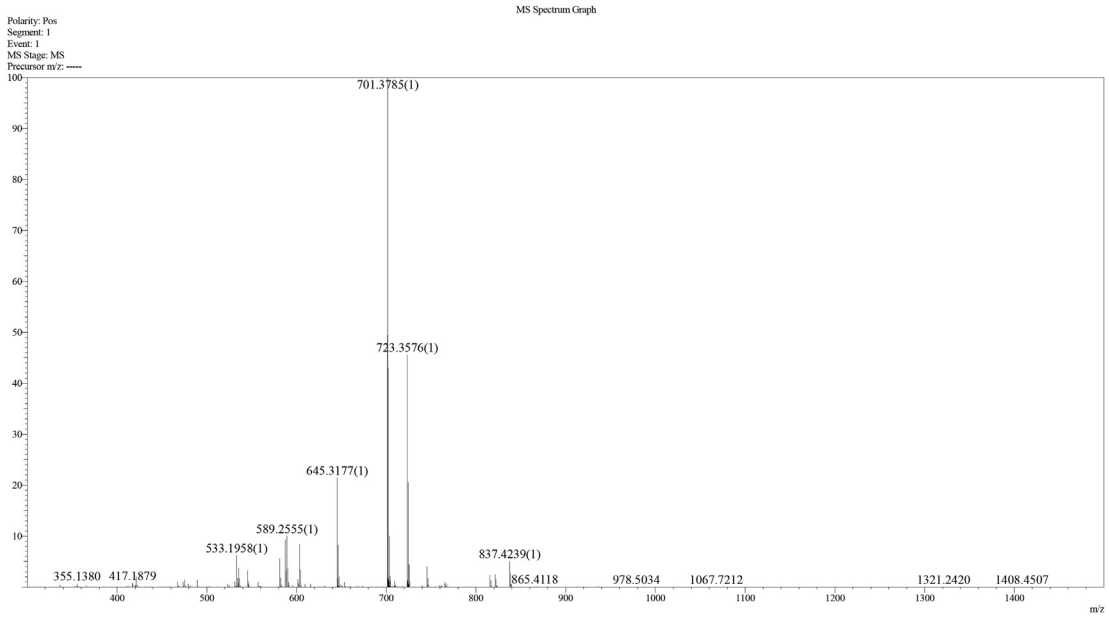


Fig. 2. Mass spectrum confirming the identity of HBED–CC–tris(*tert*-butyl ester) **12**.

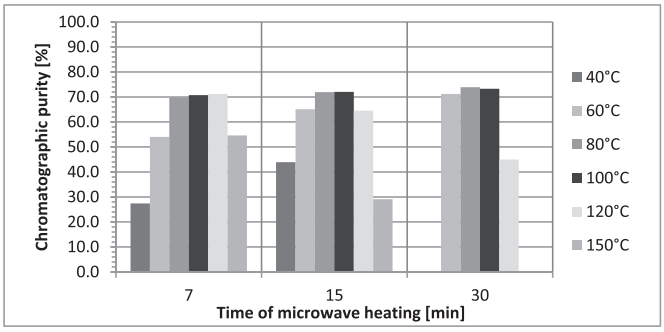
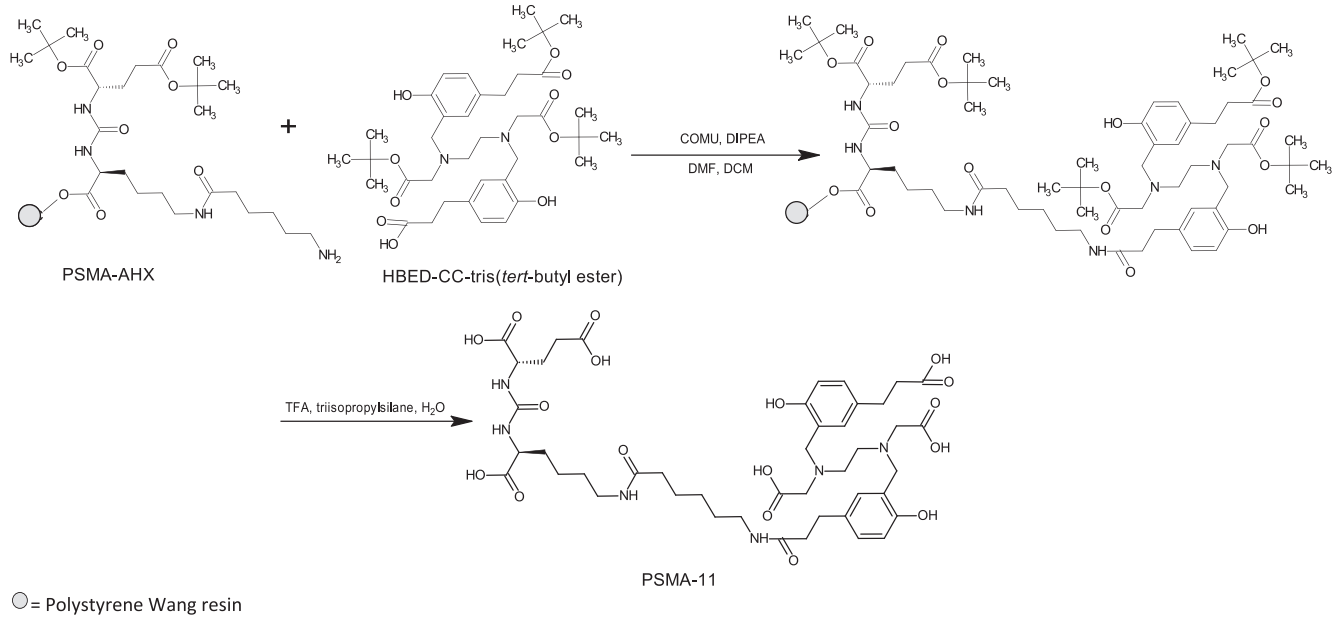


Fig. 3. Comparison between heating time and reaction temperature vs. the chromatographic purity of HBED–CC–tris(*tert*-butyl ester) **12**.

stages), reducing the formation of impurities. The possibility to wash out excess of unreacted compound from the resin is particularly important for the product **6** which may be used in the next solid phase synthesis.

In order to confirm the usefulness of the synthesized HBED–CC–tris(*tert*-butyl ester) chelator, small-scale tests were performed (see Scheme 3). The experiments consisted of attaching the final product **12** to PSMA-Ahx, deprotecting from the resin and purifying by preparative HPLC. The obtained PSMA-11 with a purity of more than 99% (HPLC - method E) was labeled with ^{68}Ga. The identity of PSMA-11 was confirmed by comparison with PSMA-11 standard. A typical HPLC chromatogram and mass spectrum for PSMA-11 are shown in Figs. 4 and 5. The quality control of the preparation of [^{68}Ga]Ga-HBED–CC–PSMA ([^{68}Ga]Ga-PSMA-11) was performed by TLC and HPLC system (method F) and the radiochemical purity was >97%.



= Polystyrene Wang resin
DCM= dichloromethane
DIPEA= *N,N*-Diisopropylethylamine
DMF = *N,N*-dimethylformamide
COMU= 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylaminemorpholino)]uroniumhexafluorophosphate

Scheme 3. Synthesis of PSMA-11.

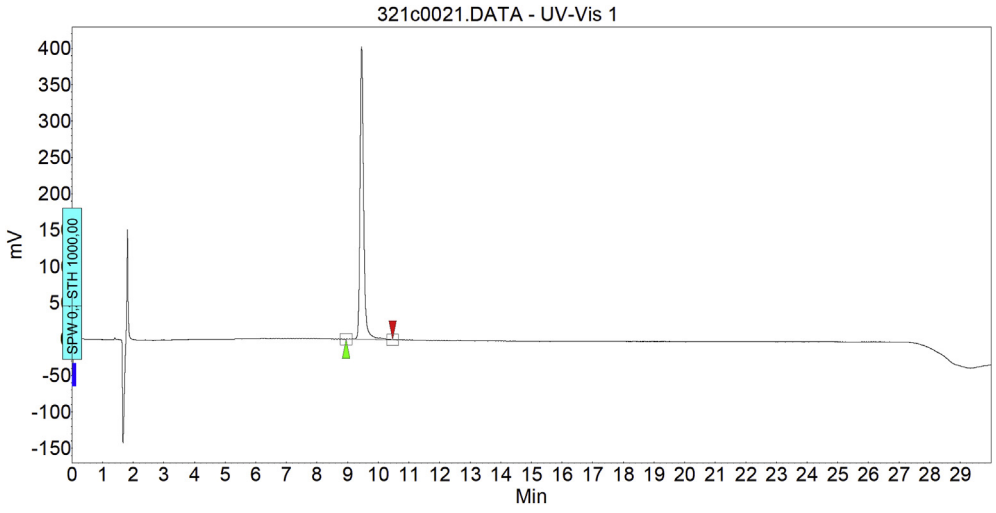


Fig. 4. HPLC typical analytical chromatogram (method E) of PSMA-11.

3. Conclusion

The synthesis of HBED–CC–tris(*tert*-butyl ester) using 2-chlorotrityl chloride resin and microwave oven was successfully developed. Performing the reaction on a solid resin required no purification of the intermediates and reduced the formation of impurities.

The use of a microwave radiation helped to reduce the time of the N-alkylation reaction – from several hours to 30 min and achieve better purity of the chelator. HBED–CC–tris(*tert*-butyl ester) was obtained with better overall yield (36%) than is described in the literature and in high chemical purity. The results presented will be a good starting point for the large-scale synthesis of PSMA-HBED-CC (PSMA-11) ligand for ^{68}Ga radiolabeling.

4. Experimental section

4.1. Materials and methods

Materials. All reagents and solvents used were purchased from Sigma-Aldrich Co. (USA), Avantor Performance Materials Poland S.A. (Poland), Fluorochem Ltd. (UK) and Iris Biotech GmbH (Germany). 2-chlorotrityl chloride resin (1.60 mmol Cl-/g resin, 100–200 mesh), polystyrene Wang resin (0.59 mmol/g resin, 100–200 mesh) and COMU were purchased from Iris Biotech GmbH (Germany). In the second part of the synthesis, anhydrous organic solvents (dichloromethane, *N,N*-dimethylformamide) were used, which were prepared by drying over molecular sieves.

Methods. Column chromatography was used for routine

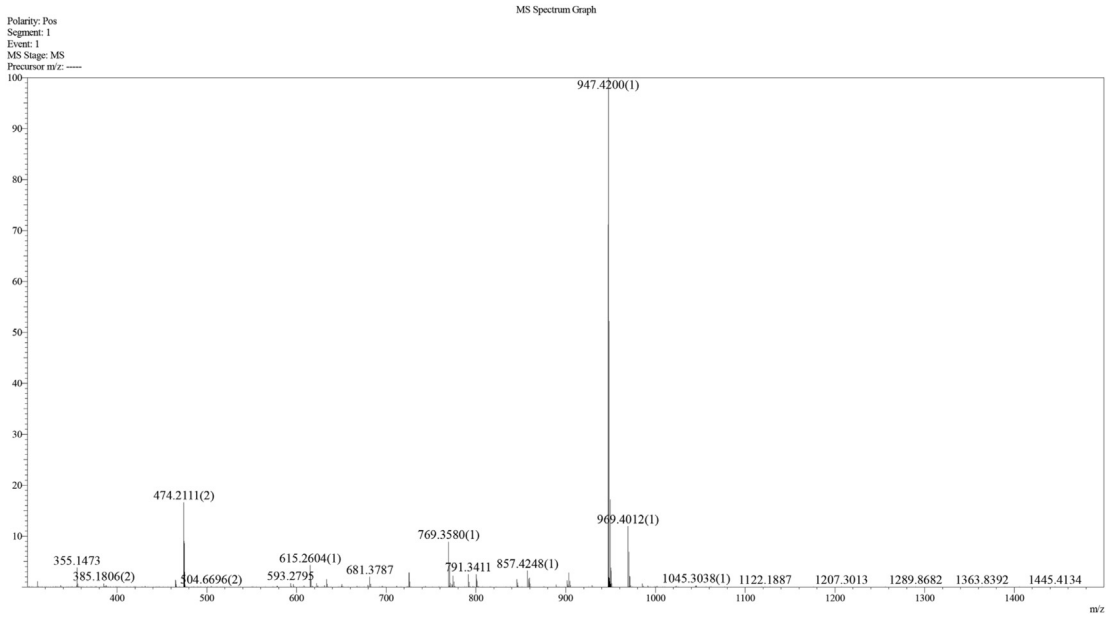


Fig. 5. Mass spectrum confirming the identity of PSMA-11.

purification of reaction products using silica gel (Kiesegel, 60 Å, 230–400 mesh, Sigma-Aldrich).

Preparative HPLC. Preparative HPLC was run on Gilson system, consisting of a Gilson 306 pump, a Gilson 151 UV/VIS detector, a Gilson 506C controller system, and the Unipoint software. Purification of HBED–CC–tris(*tert*-butyl ester) **12** was performed using Phenomenex Luna C18(2) column (15 μm, 250 × 30 mm, 100 Å). The gradient method using the following two mobile phases was used: A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile. The gradient profile and timing were as follows: (i) 0–15 min: 60% B, (ii) 15–45 min linear gradient from 60% to 70% B, (iii) 45–55 min: 70% B, (iv) 55–65 min: linear gradient from 70% to 60%, and finally, (v) 65–75 min: 60% B. A maximum of 150 mg substance dissolved in acetonitrile/water (60%/40%, 3.5 mL) was loaded onto the column.

Purification of PSMA-11 was performed using Phenomenex Luna C18(2) column (10 μm, 250 × 15 mm, 100 Å). A gradient method using the following two mobile phases was used: A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile. The gradient profile and timing were as follows: (i) 0–8 min: 10% B, (ii) 8–45 min linear gradient from 10% to 30% B, (iii) 45–55 min: linear gradient from 30% to 10%, and finally, (iv) 55–65 min: 10% B. A maximum of 20 mg substance dissolved in acetonitrile/water (10%/90%, 1 mL) was loaded onto the column. Each purification process (for compound **12** and PSMA-11) lasted 75 min, flow rate was 10 mL/min and UV detection was measured at 220 nm.

4.2. Analytical methods

Analytical HPLC was run on Varian ProStar system consisting of 2 pumps working in high pressure setup ProStar type 210, UV–Vis 345 detector, digital-analog converter Star 800, rheodyne injector type 7725i (Cotati, USA) and the Galaxy software. Chemical purity analyzes of compounds **2**, **3**, **4**, **5** and **6** were carried out using methods A, B, C. Chemical purity of HBED–CC–tris(*tert*-butyl ester) **12** was confirmed using method D. Chemical purity analysis of PSMA-11 was performed using method E. Quality control of [^{68}Ga]Ga-HBED–CC–PSMA ([^{68}Ga]Ga-PSMA-11) was performed by RP-HPLC (Shimadzu, Japan) using method F (see Table 1).

^{1}H and ^{13}C NMR spectra were recorded using Bruker 500 MHz and Varian 500 MHz spectrometers with use of $CDCl_3$ or CD_2Cl_2 as solvent. Mass spectra were obtained on LCMS-IT-TOF tandem mass spectrometer (Shimadzu, Japan).

The radiochemical purity was tested by TLC (ITLC SG; 1 M Ammonium acetate/MeOH, 50/50). The distribution of radioactivity on developed TLC strips was acquired by Phosphor Imager System (PerkinElmer, USA).

4.3. Chemistry

*Methyl 3-(4-hydroxyphenyl)propanoate (**2**).* 3-(4-hydroxyphenyl) propanoic acid (**1**, 3.0 g, 18.1 mmol) was dissolved in methanol (50 mL), and $SOCl_2$ (1.5 mL) was added. The solution was stirred at room temperature for 2 h. Then, the reaction mixture was concentrated *in vacuo* to afford 3.2 g (17.8 mmol) of crude product **2** as oil. The obtained crude product 2 was purified by silica gel column chromatography (dichloromethane). The process was monitored by TLC on silica gel plates (dichloromethane:ethyl acetate, 4:1, Rf = 0,79) with compound detection accomplished in an iodine chamber. The eluate was evaporated on a rotary evaporator to give compound **2** (2.8 g, 15.5 mmol, 86%) as an oil and with purity >98% (HPLC, method A, UV 220 nm). MS: $[M+H]^+$= 181; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.59–2.62 (t, 2H, CH_2); 2.86–2.89 (t, 2H, CH_2); 3.67 (s, 3H, O–CH_3); 5.11 (s, 1H, Ar), 6.73–6.76 (d, 2H, Ar); 7.03–7.05 (d, 2H, Ar).

*Methyl 3-(3-formyl-4-hydroxyphenyl)propanoate (**3**).* Methyl 3-(4-hydroxyphenyl)propanoate (**2**, 2.8 g, 15.5 mmol) was dissolved in acetonitrile (75 mL) followed by addition of $MgCl_2$ (2.9 g, 30.5 mmol), paraformaldehyde (3.7 g, 123.2 mmol) and triethylamine (6.2 g, 61.3 mmol). The reaction mixture was heated under reflux for 8 h, cooled to room temperature, diluted with water (25 mL) and acidified with 5% HCl (100 mL). Then, the resulting solution was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford 3.3 g of crude product **3** as yellow oil. Purification by column chromatography (dichloromethane) on silica gel and process control by TLC (dichloromethane:ethyl acetate, 9.8:0.2, Rf= 0,49) on silica gel plates gave compound **3** (2.2 g, 10.7 mmol, 70%) as colorless oil of >98% purity (HPLC, method B, UV

Table 1
HPLC methods used to confirm the chemical purity of compounds 2,3,4,5,6,12, PSMA-11 and the radiochemical purity of [^{68}Ga] Ga-HBED−CC−PSMA ([^{68}Ga]Ga-PSMA-11).

Method	A	B	C	D	E	F
Column	Phenomenex Kinetex® EVO C18(2) (5 μm, 250 × 4.6 mm, 100 Å)			Phenomenex Kinetex® EVO C18(2) (5 μm, 150 × 4.6 mm, 100 Å)	Phenomenex Kinetex® C18 (5 μm, 150 × 4.6 mm, 100 Å)	ACE 3C18-300 (150 mm × 3.0 mm)
Mobile phases	A: 0.1% HCOOH in H_2O B: 0.1% HCOOH in ACN			A: 0.1% TFA in H_2O B: 0.1% TFA in ACN		
% B	Isocratic			Gradient		
	60% B	70% B	30% B	0−10 min: from 10% to 50% B; 10−20 min: 50% B; 20−25 min: from 50% to 10% B, 25−30 min: 10% B.	0−25 min: from 15% to 30% B; 25−26 min: from 30% to 15% B 26−30 min: 15% B	0−10 min: from 5% to 40% B
Flow rate	1 mL/min					0.6 mL/min
Injection	5 μL					20 μL
Detection	UV, 220 nm; radiometric					

220 nm). MS: $[M+H]^+$= 209; ^{1}H NMR (500 MHz, CD_2Cl_2) δ: 2.61−2.64 (t, 2H, CH_2); 2.91−2.94 (t, 2H, CH_2); 3.64 (s, 3H, O−CH_3); 6.90−6.92 (d, 1H, Ar); 7.38−7.42 (m, 2H, Ar); 9.87 (s, 1H, CHO); 10.85 (s, 1H, Ar-OH); ^{13}C NMR (500 MHz, CD_2Cl_2) δ: 30.0; 35.8; 51.8; 117.8; 132.7; 133.5; 137.6; 197.1.

*3-(3-formyl-4-hydroxyphenyl)propanoic acid (**4**).* Methyl 3-(3-formyl-4-hydroxyphenyl)propanoate (**3**, 2.0 g, 9.6 mmol) was dissolved in 1 M HCl (70 mL). The mixture was heated under reflux for 1 h and then cooled in the refrigerator (2−8°C) for 5 h. After this time, crystallized solid was filtered and dried under vacuum for 3 h to afford 1.7 g of crude product. Purification by crystallization from acetonitrile gave compound **4** (1.6 g, 8.2 mmol, 86%) as a white solid and with purity >97% (HPLC, method C, UV 220 nm). MS: $[M-H]^-$ = 193; ^{1}H NMR (500 MHz, CD_2Cl_2) δ: 2.68−2.71 (t, 2H, CH_2); 2.93−2.96 (t, 2H, CH_2); 6.91−6.93 (d, 1H, Ar); 7.40−7.43 (m, 2H, Ar); 9.87 (s, 1H, CHO); 10.41 (s, 1H, −COOH); 10.86 (s, 1H, Ar-OH); ^{13}C NMR (500 MHz, CD_2Cl_2) δ: 29.7; 35.6; 117.9; 132.2; 133.5; 137.6; 197.1.

*Tert-butyl 3-(4-hydroxyphenyl)propanoate (**5**).* 3-(4-hydroxyphenyl)propanoic acid (**1**, 4.5 g, 27.1 mmol), anhydrous sodium sulfate powder (11.0 g, 77.4 mmol) and *tert*-butyl acetoacetate (14.3 g, 15 mL, 90.4 mmol) were added to 50 mL round flask. Then, concentrated sulfuric acid (0.8 g, 0.45 mL, 8.2 mmol) was added to mixture and reaction was stirring at room temperature for 30 min. After this time, the sodium sulfate was filtered out. Filtrate was extracted with diethyl ether and 1.5% aqueous solution of NaOH. The organic phase was dried with using anhydrous sodium sulfate and concentrated *in vacuo* to afford about 13.0 g of crude product **5** as a yellow oil. Purification performed by chromatography (dichloromethane:ethyl acetate, 10:0.1) on silica gel column under TLC (dichloromethane:ethyl acetate, 10:0.4, Rf = 0,54) process control gave compound **5** (1.4 g, 6.3 mmol, 23%) as a slightly yellow or colorless oil and with purity >95% (HPLC, method A, UV 220 nm). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.41 (s, 9H, 3 x CH_3); 2.49−2.52 (d, 2H, CH_2); 2.81−2.83 (d, 2H, CH_2); 6.72−6.74 (d, 2H, Ar); 7.04−7.06 (d, 2H, Ar);^{13}C NMR (500 MHz, $CDCl_3$) δ: 28.0; 30.2; 37.4; 80.5; 115.2; 129.4; 132.6; 154.0; 172.7.

*Tert-butyl 3-(3-formyl-4-hydroxyphenyl)propanoate (**6**).* $MgCl_2$ (1.0 g, 10.5 mmol), paraformaldehyde (1.3 g, 43.3 mmol) and triethylamine (2.2 g, 21.7 mmol) were added to solution of *tert*-butyl 3-(4-hydroxyphenyl)propanoate (**5**, 1.2 g, 5.4 mmol) in acetonitrile (40 mL). Mixture was heated under reflux for 1.5 h. Color of reaction mixture changed from white to yellow. After this time, reaction mixture was cooled to room temperature, diluted with water (20 mL) and acidified with 5% HCl (80 mL). Then, the mixture was extracted with diethyl ether and dried over anhydrous Na_2SO_4. Organic phase was evaporated *in vacuo* to afford about 2.5 g of crude product **6** as a yellow oil. The obtained crude product **6** was purified by silica gel column chromatography (dichloromethane). The process was monitored by TLC on silica gel plates (dichloromethane:ethyl acetate, 9.8:0.2, Rf = 0,74) and compound detection was accomplished in an iodine chamber. The eluate was evaporated on a vacuum evaporator to give compound **6** (650 mg, 2.6 mmol, 48%) as a slightly yellow or colorless oil and with purity >97% (HPLC, method A, UV 220 nm). ^{1}H NMR (500 MHz, CD_2Cl_2) δ = 1.39 (s, 9H, 3 x CH_3); 2.50−2.53 (t, 2H, CH_2); 2.87−2.90 (t, 2H, CH_2); 6.89−6.91 (d, 1H, Ar); 7.38−7.41 (dd, 2H, Ar); 9.86 (s, 1H, CHO); 10.84 (s, 1H, Ar-OH); ^{13}C NMR (500 MHz, CD_2Cl_2) δ: 28.1; 30.2; 37.2; 80.6; 117.7; 120.8; 132.9; 133.4; 137.7; 160.3; 172.0; 197.0; 197.1.

*3-(3-formyl-4-hydroxyphenyl) propionic acid coupled on the resin (**4***).* 2-chlorotrityl chloride resin (1.0 g, 1.6 mmol) was swelled with dichloromethane (25 mL) for 0.5 h. After this time, dichloromethane was removed from the glass reaction vessel. Then, 3-(3-formyl-4-hydroxyphenyl) propionic acid (**4**, 0.8 g, 4.1 mmol) in dichloromethane (45 mL) and DIPEA (1.0 g, 7.7 mmol) in dichloromethane (5 mL) were added to the resin. The reaction mixture was stirred by bubbling argon for 4 h. The reaction solution was again removed from the glass reaction vessel and the 3-(3-formyl-4-hydroxyphenyl) propionic acid coupled with resin **4*** was washed in sequence with: dichloromethane (50 mL), *N,N*-dimethylformamide (20 mL), mixture of dichloromethane/methanol/DIPEA (8mL:1.5mL:0.5mL) by 10 min and dichloromethane (50 mL).

*3-[3-({2-[2-(9H-Fluoren-9-yl)acetylamino]ethylimino}methyl)-4-hydroxyphenyl]propionic acid coupled on the resin (**7***).* Fmoc-EDA*HCl (1.0 g, 3.1 mmol) in methanol (20 mL) and DIPEA (0.8 g, 6.2 mmol) in dichloromethane (30 mL) were added to the **4***. The reaction mixture was stirred by bubbling argon for 4 h. After 2 h, dichloromethane (30 mL) was added to the reaction mixture. Then, the reaction solution was removed from the reaction vessel and the yellow product coupled with resin **7*** was washed in sequence with dichloromethane (35 mL), methanol (15 mL) and dichloromethane (35 mL).

*3-[3-({2-[2-(9H-Fluoren-9-yl)acetylamino]ethylamino}methyl)-4-hydroxyphenyl]propionic acid coupled on the resin (**8***).* Mixture of dichloromethane and methanol (9:1, 40 mL) was added to the **7***. Then, sodium borohydride (0.3 g, 7.9 mmol) in methanol (10 mL) was added in portions and the reaction mixture was stirred by bubbling argon for 2 h. After this time, the reaction solution was

removed from the reaction vessel and the white compound coupled with resin **8*** was washed in sequence with methanol (40 mL) and dichloromethane (50 mL). MS: $[M+H]^+ = 461$.

*3-(3-{[(2-aminoethyl)amino]methyl}-4-hydroxyphenyl)propanoic acid coupled on the resin (**9***).* Solution of 20% piperidine in dichloromethane and *N,N*-dimethylformamide (1:1, 16 mL) was added to the 8*. The reaction mixture was stirred by using argon for 0.5 h. After this time, solution was removed from the reaction vessel. 20% piperidine in dichloromethane (16 mL) was added to the 8* again and the mixture was stirred by bubbling argon for 0.5 h. Then, the solution was removed from the glass vessel. White compound coupled with the resin **9*** was washed in sequence with *N,N*-dimethylformamide (40 mL) and dichloromethane (40 mL). MS: $[M+H]^+ = 239$.

*3-{3-[(2-{(Z)-[2-hydroxy-5-(2-tertbutoxycarbonylethyl)phenyl] methylideneamino}ethylamino)methyl]-4-hydroxyphenyl}propionic acid coupled on the resin (**10***).* *Tert*-butyl 3-(3-formyl-4-hydroxyphenyl) propanoate (**6**, 1.2 g, 4.8 mmol) was dissolved in dichloromethane (50 mL), and added to the **9***. The reaction mixture was stirred by bubbling argon for 4 h. After this time, the solution was removed from the glass reaction vessel and the yellow product coupled with resin **10*** was washed with dichloromethane (50 mL).

*3-(4-hydroxy-3-{[2-({[2-hydroxy-5-(2-tertbutoxycarbonylethyl) phenyl]methyl}amino)ethylamino]methyl}phenyl)propionic acid coupled with resin (**11***).* Mixture of dichloromethane and methanol (9:1, 40 mL) was added to the **10***. Then, sodium borohydride (0.3 g, 7.9 mmol) in methanol (10 mL) was added in portions and reaction mixture was stirred by bubbling argon for 2 h. After this time, the reaction solution was removed from the reaction vessel and the white compound coupled with resin **11*** was washed with methanol (40 mL) and dichloromethane (50 mL). MS: $[M+H]^+ = 473$.

*3-(3-{[(2-{[5-(2-tert-butoxycarbonylethyl)-2-hydroxybenzyl]-tert-butoxycarbonylmethylamino} ethyl)-tert-butoxycarbonyl-methylamino]-methyl}-4-hydroxyphenyl) propionic acid coupled on the resin (**12***).* *Tert*-butyl bromoacetate (2.5 g, 12.8 mmol) in *N,N*-dimethylformamide (25 mL) and DIPEA (3.3 g, 25.6 mmol) in *N,N*-dimethylformamide (25 mL) were added to the **11***. The N-alkylation reaction was carried out in a microwave oven where the power, temperature and time were appropriately selected after the optimization of the process. The reaction was finally carried out under the following conditions: power 60 W, temperature 80°C, time 30 min, stirred by bubbling argon. Then, resin with compound **12*** was filtrated and washed with *N,N*-dimethylformamide (40 mL) and dichloromethane (40 mL).

3-(3-{[(2-{[5-(2-tert-butoxycarbonylethyl)-2-hydroxybenzyl]-tert-butoxycarbonylmethylamino}-ethyl)-tert-butoxycarbonyl-methylamino]-methyl}-4-hydroxyphenyl) propionic acid [HBED-CC-tris (tert-butyl ester)] (**12**). Mixture of CH_3COOH:CF_3CH_2OH:CH_2Cl_2 (1:2:7, 60 mL) was added to compound coupled with resin **12***. The cleavage step was carried out at room temperature, stirring for 1 h on a shaker. After this time, the resin was filtered off. The filtrate was then concentrated on a vacuum evaporator and dried under vacuum for 24 h to afford 610 mg (54%) of crude product **12** as an orange oil. The obtained crude product **12** was purified by preparative HPLC. The fractions containing the desired product were collected, combined, frozen on dry ice and lyophilized (24 h) to afford purified compound **12** (400 mg, 0.57 mmol, 36%) as white solid and with purity >97% (HPLC, method D, UV 220 nm). MS: $[M + H]^+ = 701$; ^{1}H NMR (500 MHz, CD_2Cl_2) δ: 1.40 (s, 9H, 3 x CH_3); 1.48–1.49 (ss, 18H, 6 x CH_3); 2.46–2.49 (t, 2H, CH_2); 2.60–2.62 (t, 2H, CH_2); 2.78–2.86 (2t, 4H, 2 x CH_2); 3.37–3.43 (d, 4H, 2 x CH_2–N); 3.62–3.65 (d, 4H, 2 x CH_2); 4.12–4.13 (d, 4H, 2 x CH_2–N); 6.80–7.02 (m, 4H, Ar); 7.11–7.12 (s, 2H, Ar); ^{13}C NMR (500 MHz, CD_2Cl_2) δ: 27.9; 27.9; 28.1; 30.1; 30.3; 36.0; 37.4; 49.7; 50.1; 54.0; 54.2; 54.6; 54.7; 80.9; 85.1; 85.2; 115.1; 116.0; 116.6; 117.0; 117.0; 117.4; 131.9; 132.0; 132.3; 132.5; 132.6; 133.0; 155.0; 155.1; 161.2; 161.5; 166.6; 167.1; 172.8; 176.2;

The identity of the small amount of compounds **8*, 9*, 11*** was confirmed by MS analysis after cleavage from the resin by using a mixture of CH_3COOH:CF_3CH_2OH:CH_2Cl_2 (1:2:7, 6 mL, stirring for 1 h).

Small Scale Tests - proof of applicability

PSMA-11. Glu–CO–Lys-Ahx fragment (further referred to as PSMA-Ahx [PSMA-Aminohexanoic acid]) coupled with the Polystyrene Wang resin was swelled in dichloromethane for at least 20 min. At the same time equimolar amounts of HBED–CC–tris(*tert*-butyl ester) **12** and COMU were placed in a separate vessel and stirred until dissolved in *N,N*-dimethylformamide [25,26]. Then *N,N*-diisopropylethylamine was added to the solution (stirred for 5 min) and dichloromethane (reaction environment). The solution of activated amino acid prepared in such a way was transferred into the vessel with swollen resin and stirred overnight. PSMA-11 coupled with the resin was washed: *N,N*-dimethylformamide, 50% *N,N*-dimethylformamide solution in dichloromethane and dichloromethane and then dried in vacuum.

In a separate vessel, the solution of trifluoroacetic acid with addition of triisopropylsilane and water was prepared (98:2:2) in order to detach the product from the resin and to remove the protective groups. The ready solution was transferred to the vessel comprising the resin and stirred for 5 h. The product was precipitated with diethyl ether. The precipitate was centrifuged and washed with diethyl ether and then dissolved in a solution of acetonitrile:water (2:8) and transferred to a flask. The precipitate was frozen on dry ice and lyophilized. The obtained crude product (PSMA-11) was purified by preparative HPLC. The fractions containing the desired product were collected, combined, frozen on dry ice and lyophilized to afford PSMA-11 as a white solid and with purity >99% (HPLC, method E, UV 220 nm). MS: $[M + H]^+ = 947.4$.

5. Radiochemistry

Gallium-68 was obtained from a ^{68}Ge/^{68}Ga generator (IRE Elit Belgium). A stock solution of ligand (PSMA-11, 1 mg in 1 mL H_2O) was prepared and used for the radiolabeling studies. ^{68}Ga labeling was performed in aq. NaOAc buffer (1 mL, 60 mg/mL) by combining the ligand solution (20 μL) and ^{68}Ga solution (5 mL, 0.1 M HCl, 370 MBq). The mixture was heated at 95°C for 15 min and the final pH of this solution was 4–4.5. The radiochemical purity of [^{68}Ga]Ga-HBED–CC–PSMA ([^{68}Ga]Ga-PSMA-11) was >97% and determined by TLC and HPLC system (method F).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2021.132018.

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