



(51) International Patent Classification:

C07F 9/38 (2006.01) *C07B 59/00* (2006.01)
C07F 9/40 (2006.01)

(21) International Application Number:

PCT/EP2012/051368

(22) International Filing Date:

27 January 2012 (27.01.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11075017.1 31 January 2011 (31.01.2011) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

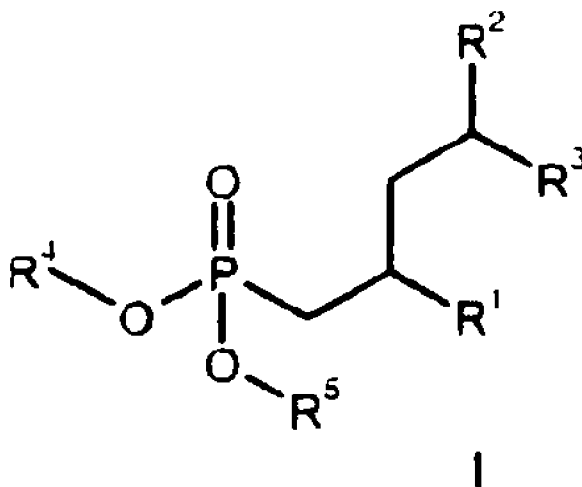
Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))

(54) Title: DERIVATIZATION OF RADIOPHARMACEUTICALS



(57) Abstract: This invention relates to methods for analyzing radiopharmaceuticals wherein the radiopharmaceutical is derivatized beforehand. This method is suitable for compounds labeled with radioisotope or with cold counterpart e.g. ^{18}F or ^{19}F , compositions comprising such compounds, kits comprising such compounds or compositions and uses of such compounds, compositions or kits for analyzing radiopharmaceuticals. wherein R^1 is $\text{C}(=\text{O})\text{OR}^6$; R^2 is $\text{C}(=\text{O})\text{OR}^7$; R^3 is ^{19}F or ^{18}F R^4 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl; R^5 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl; R^6 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl; R^7 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl; with the proviso that at least one of R^4 , R^5 , R^6 , and R^7 is hydrogen

Derivatization of Radiopharmaceuticals

Field of Invention

This invention relates to methods for analyzing radiopharmaceuticals wherein the radiopharmaceutical is derivatized before the said analyses are performed. This method is suitable for compounds labeled with radioisotope or with cold counterpart e.g. ^{18}F or ^{19}F , compositions comprising such compounds, kits comprising such compounds or compositions and uses of such compounds, compositions or kits for analyzing radiopharmaceuticals.

Background

The invention relates to the subject matter referred to in the claims, i.e. rapid and quantitative derivatization of radiopharmaceuticals to allow better analysis of the said radiopharmaceuticals.

Molecular imaging has the potential to detect disease progression or therapeutic effectiveness earlier than most conventional methods in the fields of oncology, neurology and cardiology. Of the several promising molecular imaging technologies having been developed as optical imaging and MRI, PET is of particular interest for drug development because of its high sensitivity and ability to provide quantitative and kinetic data.

Positron emitting isotopes include carbon, nitrogen, and oxygen. These isotopes can replace their non-radioactive counterparts in target compounds to produce tracers that function biologically and are chemically identical to the original molecules for PET imaging. On the other hand, ^{18}F is the most convenient labeling isotope due to its relatively long half life (109.6 min) which permits the preparation of diagnostic tracers and subsequent study of biochemical processes. In addition, its high β^+ yield and low β^+ energy (635 keV) are also advantageous.

Due to its short 20minute half-life ^{11}C containing radiotracers require an on-site cyclotron, whereas ^{18}F PET tracers, considering a half-life of 109 minutes, allow for off-site production and regional distribution.

For the simultaneous detection, separation and quantification of trace amounts of chemicals, within the pharmaceutical industry, food industry and water industry etc, are of relevance with respect to toxicity, environmental issues etc. Typically these trace chemicals are difficult to detect with high sensitivity due to a number of reasons:

1. Highly polar (high hydrophilicity),
2. No UV chromophore,

3. Mixture of isomers, i.e. stereoisomer and regioisomers,
4. Non-volatile.

The detection of carboxylic acids, phosphonic acids are of particular interest within many fields, i.e. pharmaceutical industry, water industry and food industry. as many compounds which are difficult to detect can be toxic, i.e. nerve agents like alkyl methyl phosphonates. Many derivatization methods are known for carboxylic acids and phosphonic acids, however, all the derivatization methods suffer from the following disadvantages:

1. Anhydrous conditions as the derivatizing agents react with water and create detrimental artefacts in the analysis.
2. Require long reaction times to ensure that the trace amounts of the acids are fully converted to their detectable derivatives.
3. The products of the derivatization are not stable or cannot be isolated, thus, limiting the options available to carry out the analysis, e.g. silylation derivation methods use gas chromatography (GC) only.

Known derivatizations methods for carboxylic acids include:

- 1) Amide formation with dehydrating agents and highly sensitive amines.
 - a) Chiral carboxylic acids detected using "Levobase" or "Dextrobase" with dicyclohexylcarbodiimide under anhydrous conditions within 10-15 mins followed by TLC or HPLC detection (TLC: Slégel *et al.*, *J. Pharmaceutical & Biomedical Analysis*, 1987, 5, 665-673; HPLC: Ladányi *et al.*, *Chromatographia*, 1987, 24, 477-481)
 - b) Multi-step conjugation reaction to detect various carboxylic acids, with some unsuccessful examples, by HPLC in aqueous conditions using 2-chloro-1-methylpyridinium iodide and tris(2,4,6-trimethoxyphenyl)phosphonium propylamine in over 35 minutes (Cartwright *et al.*, *Rapid Commun. Mass Spectrom.*, 2005, 19, 1058-1062)
- 2) Ester formation with alkylating agents:
 - a) Fatty acids were detected by HPLC using p-bromophenacyl bromide with potassium bicarbonate and 18-crown-6 in over 45 mins at 85°C with a work up step, HPLC detection (Püttmann *et al.*, *Clin. Chem.*, 1993, 39, 825-832)
 - b) Quinic acid derivatives were detected by HPLC using p-bromophenacyl bromide with potassium fluoride under anhydrous conditions at 40°C for 10 mins (Nagels *et al.*, *J. Chromatogr.*, 1980, 190, 411-417)
 - c) Polar carboxylic acids were detected by HPLC using 2-bromoacetyl-6-methoxynaphthalene with TEA under anhydrous conditions or with surfactant in aqueous solutions in 65 mins at 70°C (Gatti *et al.*, *Biomed. Chrom.*, 1996, 10, 19-24; *Chromatographia*, 1992, 33, 13-18; Gatti *et al.*, *Biomed. Chrom.*, 1997, 11, 11-15)
 - d) Fatty acids were detected by HPLC using 2-(2,3-naphthalimino)ethyl

trifluoromethanesulfonate and potassium fluoride in anhydrous conditions for over 30 mins at RT (Yasaka *et al.*, *J. Chromatogr.* 1990, 508, 133-140)

3) Ester formation with esterification methods:

a) Esterification of various carboxylic acids and phosphonic acids with trialkyl orthoacetate in ionic liquids under anhydrous conditions at 100°C in 0.5-5h (Yoshino *et al.*, *Tetrahedron*, 2006, 62, 1309-1317)

b) Fatty acids and steroids using acetyl chloride and methanol under anhydrous conditions at 37 – 50°C for 5 – 60mins followed by GC analysis (Lillington *et al.*, *Clinica Chimica Acta*, 1981, 111, 91-98)

c) >30 carboxylic and phosphonic acids were detected by HPLC using O-(4-nitrobenzyl)-N,N'-diisopropylisourea (PNDBI) under <10% aqueous homogenous conditions at 80°C for 60mins followed by complicated work-up (Badoud and Pratz, *J. Chromatogr.* 1986, 360, 119-136)

d) Fatty acids and some amino acids were detected by GC using N,N-dimethylformamide dimethylacetal (DMF-DMA) in various solvents under anhydrous conditions at 60-100°C for 10-15 mins (Thenot *et al.*, *Anal. Letters*, 1972, 5, 217-223 and 519-529)

4) Silylation of carboxylic acids:

a) Silylation of three carboxylic acids were detected by GC using N,O-bis(trimethylsilyl)acetamide under anhydrous conditions at 80°C for 5 mins (Sennello and Argoudelis, *Anal. Chem.*, 1969, 41, 171-173)

Known derivatizations methods for phosphonic acids include:

1) Ester formation with alkylating agents:

a) Alkyl methylphosphonic acids detected using p-bromophenacyl bromide with potassium bicarbonate and 18-crown-6 in over 60 mins at 60°C with a work up step, HPLC detection (Bossle *et al.*, *J. Chromatogr.*, 1983, 267, 209-212)

b) Alkyl phosphonic acids detected by HPLC using p-(9-anthroyloxy)phenacyl bromide under anhydrous conditions at 40°C in 2h (Roach *et al.*, *Anal. Chem.*, 1987, 59, 1056-1059)

2) Ester formation with esterification methods:

a) Esterification of various carboxylic acids and phosphonic acids with trialkyl orthoacetate in ionic liquids under anhydrous conditions at 100°C in 0.5-5h (Yoshino *et al.*, *Tetrahedron*, 2006, 62, 1309-1317)

b) >30 carboxylic and phosphonic acids detected by HPLC using O-(4-nitrobenzyl)-N,N'-diisopropylisourea (PNDBI) under <10% aqueous homogenous conditions at 80°C for 60mins followed by complicated work-up (Badoud and Pratz, *J. Chromatogr.* 1986, 360, 119-136)

c) Two step procedure to derivatize amino-phosphonic acids where the second step

uses diazobutane (explosive and not commercially available) under anhydrous conditions at RT in 15 mins (Rueppel *et al.*, *Biomedical Mass Spectrometry*, 1976, 3, 28-31)

- d) Two step procedure to derivatize amino-phosphonic acids where the second step uses diazomethane (toxic, explosive and has to be freshly synthesized prior to each use) under anhydrous conditions at RT (Korn *et al.*, *J. Biol. Chem.*, 1973, 248, 2257-2259)

Trimethylsilyldiazomethane (TMSCHN₂) has been reported in the literature to be an excellent alternative to the explosive and toxic diazomethane for the formation of methyl esters (Presser and Hufner, *Monatshefte Für Chemie*, 2004, 135, 1015-1022; Shiori and Aoyama, *Adv. Use Synthons Org. Chem.*, 1993, 1, 51-101). The reported methods with TMSCHN₂ are all carried out in non-aqueous system, i.e. methanol with or without toluene. The main drawback with this TMSCHN₂ reagent is the formation of trimethylsilylmethyl ester by-products. However, these by-products could be suppressed by the addition of tetrafluoroboric acid (HBF₄, Kühnel *et al.*, *Angew. Chem. Int. Ed.*, 2007, 46, 7075-7078). TMSCHN₂ has also been applied to phosphonic acids to give the corresponding methyl phosphonate esters (Cullen and Rovis, *Org. Lett.*, 2008, 10, 3141-3144). However, all these methods are time consuming with typically reaction times of 30 mins or longer and have either no or negligible amounts of water, which are not compatible for analyzing aqueous solutions for trace amounts of compounds.

Problem to be solved by the invention and its solution

Despite the aforementioned advances in derivatization methods for determining trace amounts of chemicals, these methods typically require anhydrous conditions, as water gives sub-optimal derivatization, and require relative long reactions time which are not compatible with the half-life of short-lived radioisotopes, e.g. C-11 (20 mins), F-18 (110 mins), Tc-99m (6 h), I-123 (13.2 h), etc.

The methods disclosed in the present invention allow for a surprisingly rapid and quantitative derivatization of aqueous radiopharmaceuticals solutions and their subsequent analysis, these analysis even allow for chiral analysis of the product which is not possible in the underivatized form.

Summary

The invention relates to the subject matter referred to in the claims, i.e. rapid and quantitative derivatization of radiopharmaceuticals to allow better analysis of the said radiopharmaceuticals.

Figures

Figure 1: C18 HPLC chromatogram of the underivatized compound **9**.

Figure 2: C18 HPLC chromatogram of the derivatization reaction mixture showing complete conversion of the underivatized compound **9** to the derivatized compound **10**.

Figure 3: Chiral HPLC chromatogram of the derivatized compound **10**.

5 Figure 4: Chiral HPLC chromatogram of the racemic compound **8** (four isomers).

Figure 5: C18 HPLC chromatogram of the attempted derivatization using Dimethylformamide dimethyl acetal (DMF-DMA)

Figure 6: C18 HPLC chromatogram of the attempted derivatization using trimethylorthoacetate and ionic liquid [bmim][PF₆].

10 Figure 7: C18 HPLC chromatogram of the attempted derivatization using acetyl chloride (AcCl) and methanol.

Figure 8: C18 HPLC chromatogram of the attempted derivatization using trimethylsilyl diazomethane.

15 Figure 9: C18 HPLC chromatogram of the attempted derivatization using boron trifluoride dimethanol complex.

Figure 10: HPLC chromatogram of the purified compound **12**.

Figures 11: HPLC chromatograms of the derivatized compound **13** with 25% aqueous solution.

20 Figures 12: HPLC chromatograms of the derivatized compound **13** with 90% aqueous solutions.

Description

In a **first aspect**, the invention is directed to a method for the derivatization of radiopharmaceutical being soluble in an aqueous solution. Additionally, the
25 radiopharmaceutical comprises one or more carboxylic acid groups and/or one or more phosphonic acid groups. The method is for obtaining derivatized radiopharmaceuticals.

The invention is directed to a method for the derivatization of radiopharmaceuticals comprising one or more carboxylic acid groups and/or one or more phosphonic acid groups and being soluble in an aqueous solution for obtaining a derivatized radiopharmaceutical.

30 The novel method for the derivatization of radiopharmaceutical results in improved analytical characterizations.

The novel method of the present invention is suitable for determining different stereoisomers of a radiopharmaceutical as described above and wherein the underivatized radiopharmaceutical is preferably polar.

35 The functional groups carboxylic acid and phosphonic acid of the radiopharmaceutical are unprotected or partially unprotected.

The radiopharmaceutical is an imaging suitable radiopharmaceutical e.g. PET or SPECT imaging comprising radioactive atom, preferably ¹⁸F or the radiopharmaceutical is a cold

radiopharmaceutical comprising a non-radioactive counterpart (cold isotope), preferably ^{19}F .

In a **first** embodiment the invention concerns a method for the derivatization of radiopharmaceutical comprising one or more carboxylic acid groups and being soluble in an aqueous solution.

Preferably, the radiopharmaceutical comprises one (1) to five (5) carboxylic acid groups. More preferably, the radiopharmaceutical comprises one (1) to two (2) carboxylic acid groups. Even more preferably, the radiopharmaceutical comprises two (2) carboxylic acid groups.

In a **second** embodiment the invention concerns a method for the derivatization of radiopharmaceutical comprising one or more phosphonic acid groups and being soluble in an aqueous solution.

Preferably, the radiopharmaceutical comprises one (1) to five (5) phosphonic acid groups. More preferably, the radiopharmaceutical comprises one (1) to two (2) phosphonic acid groups. Even more preferably, the radiopharmaceutical comprises one (1) phosphonic acid group.

In a **third** embodiment the invention concerns a method for the derivatization of radiopharmaceutical comprising one or more carboxylic acid groups and one or more phosphonic acid groups and being soluble in an aqueous solution.

Preferably, the radiopharmaceutical comprises one (1) to five (5) carboxylic acid groups and one (1) to five (5) phosphonic acid groups. More preferably, the radiopharmaceutical comprises one (1) to two (2) carboxylic acid groups and one (1) to two (2) phosphonic acid groups. Even more preferably, the radiopharmaceutical comprises two (2) carboxylic acid groups and one (1) phosphonic acid group.

Description of the Method for the derivatization applicable to embodiments 1, 2 and 3:

Preferably, the method for the derivatization of the radiopharmaceuticals as described above comprises of the step:

- Alkylation of at least one carboxylic acid groups and/or phosphonic acid groups of the radiopharmaceutical as described above.

The method allows the synthesise of derivatized radiopharmaceuticals (i.e. alkylated radiopharmaceuticals).

Alkylation is obtained by reacting the radiopharmaceutical with unsubstituted or substituted diazoalkane in presence of an acid preferably selected from the group of strong acid, Lewis acid and Brønsted acid.

The method comprises of the step of alkylation of at least one carboxylic acid groups and/or phosphonic acid groups of a radiopharmaceutical wherein alkylation is obtained by reacting radiopharmaceutical with unsubstituted or substituted diazoalkane in presence of an acid preferably selected from the group of strong acid, Lewis acid and Brønsted acid.

Additionally, the method comprises of the step for the analytical characterization of the obtained derivatized radiopharmaceuticals by suitable methods such as HPLC, preferably using a HPLC column with a solid-phase.

The subsequent analytical characterization allows chiral separation i.e separation of stereoisomers and determination of the ratio of stereoisomers of the radiopharmaceutical.

The above described method is preferably for determination of the ratio of stereoisomers of radiopharmaceutical as described above.

Preferably, diazoalkane is diazo-[C₁-C₆-alkane] wherein C₁-C₆-alkane is preferably Methane, Ethane, Propane, Butane, t-Butane. More preferably, diazoalkane is diazomethane or diazoethane. Even more preferably, diazoalkane is diazomethane.

Preferably, unsubstituted diazoalkane is a compound with the general formula R⁸R⁹C=N₂ wherein R⁸ is Hydrogen or lower alkyl and R⁹ is Hydrogen or lower alkyl. More preferably, R⁸ is methyl and R⁹ is Hydrogen i.e. diazoethane. More preferably, R⁸ and R⁹ are Hydrogen i.e. diazomethane.

Preferably, substituted diazoalkane is an diazoalkane as defined above wherein R⁸ is Hydrogen, lower alkyl or aryl-alkyl (C₇-C₁₆) and R⁹ is trialkylsilyl or aryl-alkyl (C₇-C₁₆). Preferably, trialkylsilyl is trimethylsilyl. More preferably, R⁸ is Hydrogen and R⁹ is trimethylsilyl.

More preferably, substituted diazoalkane is trimethylsilyldiazomethane or diazomethylbenzene. Even more preferably, substituted diazoalkane is trimethylsilyldiazomethane.

Preferably, unsubstituted or substituted diazoalkane is diazomethane, diazoethane trimethylsilyldiazomethane or diazomethylbenzene.

Preferably, Brønsted acid is tetrafluoroboric acid.

Preferably, alkylation occurs for

at least one carboxylic acid groups present in radiopharmaceutical,

at least one phosphonic acid groups present in radiopharmaceutical, or simultaneously at least one carboxylic acid groups and at least one phosphonic acid groups present in radiopharmaceutical.

More preferably, alkylation occurs for

- 5 all carboxylic acid groups present in radiopharmaceutical, all phosphonic acid groups present in radiopharmaceutical, or simultaneously for all carboxylic acid and phosphonic acid groups present in radiopharmaceutical.
- 10 Description of the radiopharmaceutical for the derivatization applicable to embodiments 1, 2 and 3:
- The radiopharmaceutical comprising one or more carboxylic acid groups and/or one or more phosphonic acid groups and being soluble in an aqueous solution, optionally the radiopharmaceutical is polar.
- 15 The radiopharmaceutical is soluble in an aqueous solution wherein the aqueous solution comprises
- water
 - water miscible solvent(s), and
 - optionally salt(s), buffering agent(s), acid(s) or base(s).
- 20 Preferably the aqueous solution comprises
- water and
 - water miscible solvent(s), and
 - acids.
- 25 Preferably, the water miscible solvents is selected from the group of tetrahydrofuran, acetonitrile, dimethylformamide, dimethylsulfoxide, methanol, ethanol, glycol, pegylated solvents, acetone, dioxane, n-propanol, and isopropanol. More preferably, the water miscible solvents is ethanol.
- 30 Optionally, the radiopharmaceutical comprises further functional groups such as amino, hydroxyl (O) and/or mercapto (S) groups.
- Preferably, amino, hydroxyl (O) and/or mercapto (S) groups of the radiopharmaceutical are partially or fully protected with the proviso that hydroxyl (O) group is not contained into the carboxylic acid group(s) or phosphonic acid group(s) to be alkylated.
- 35 More preferably amino group(s) of the radiopharmaceutical is/are protected with a suitable amino protecting group. Suitable amino protecting group are well known in the art. Preferably, amino protecting groups are selected from the group comprising Carbobenzyloxy

(Cbz), *tert*-Butyloxycarbonyl (BOC) and 9-Fluorenylmethyloxycarbonyl (Fmoc).

More preferably hydroxyl group(s) of the radiopharmaceutical is/are protected with a suitable hydroxyl protecting group. Suitable hydroxyl protecting group are well known in the art. Preferably, hydroxyl protecting groups are selected from the group comprising Methyl, Ethyl, Propyl, Butyl, *t*-Butyl, *para*-Methoxybenzyl or Benzyl.

More preferably mercapto group(s) of the radiopharmaceutical is/are protected with a suitable mercapto protecting group. Suitable mercapto protecting group are well known in the art. Preferably, mercapto protecting groups are selected from the group comprising Trityl, Benzyl, *t*-Butyl, *para*-Methoxybenzyl, 2,4,6-Trimethoxybenzyl or 9-Fluorenylmethyl.

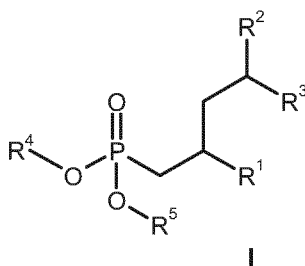
In a sub-embodiment, the invention is directed to radiopharmaceuticals selected from the group of

[¹¹C]Acetate, [¹⁸F]Fluoroacetate, 2-[¹⁸F]Fluoropropionic acid, (2RS,4S)-2-[¹⁸F]Fluoro-4-phosphonomethyl-pentanedioic acid [¹⁸F](2RS,4S)PMPA, 2-[¹⁸F]Fluoro-4-phosphonomethyl-pentanedioic acid [¹⁸F]PMPA, 2-(5-[¹⁸F]Fluoro-pentyl)-2-methyl-malonic acid, [¹¹C]Lactate, [¹¹C]labeled fatty acids, [¹⁸F]labeled fatty acids, [^{99m}Tc]MDP, [^{99m}Tc]DMSA, [^{99m}Tc]HMDP, and [^{99m}Tc]HEDP.

Preferably, the radiopharmaceutical is [¹⁸F]Fluoroacetate, 2-[¹⁸F]Fluoropropionic acid, (2RS,4S)-2-[¹⁸F]Fluoro-4-phosphonomethyl-pentanedioic acid [¹⁸F](2RS,4S)PMPA, 2-[¹⁸F]Fluoro-4-phosphonomethyl-pentanedioic acid [¹⁸F]PMPA or 2-(5-[¹⁸F]Fluoro-pentyl)-2-methyl-malonic acid.

More preferably, the radiopharmaceutical is (2RS,4S)-2-[¹⁸F]Fluoro-4-phosphonomethyl-pentanedioic acid [¹⁸F](2RS,4S)PMPA, or 2-(5-[¹⁸F]Fluoro-pentyl)-2-methyl-malonic acid.

In a further sub-embodiment, the invention is directed to radiopharmaceuticals of the formula (I)



wherein

R¹ is C(=O)OR⁶;

R² is C(=O)OR⁷;

R³ is ¹⁹F or ¹⁸F;

R⁴ is hydrogen, Methyl, Ethyl, Propyl, Butyl, *t*-Butyl or Benzyl;

R⁵ is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R⁶ is hydrogen. Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R⁷ is hydrogen. Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

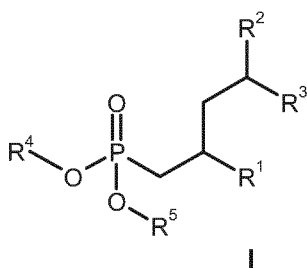
with the proviso that at least one of R⁴, R⁵, R⁶, and R⁷ is hydrogen;

5 and stereoisomers, stereoisomeric mixtures, and suitable salts thereof.

More preferably, the radiopharmaceutical is a PET radiopharmaceutical wherein R³ is ¹⁸F

More preferably, the radiopharmaceutical is a cold radiopharmaceutical useful as standard compound wherein R³ is ¹⁹F.

10 More preferably, the radiopharmaceuticals are radiopharmaceuticals of the formula (I)



wherein

R¹ is C(=O)OR⁶ ;

R² is C(=O)OR⁷;

15 R³ is ¹⁹F or ¹⁸F;

R⁴ is hydrogen;

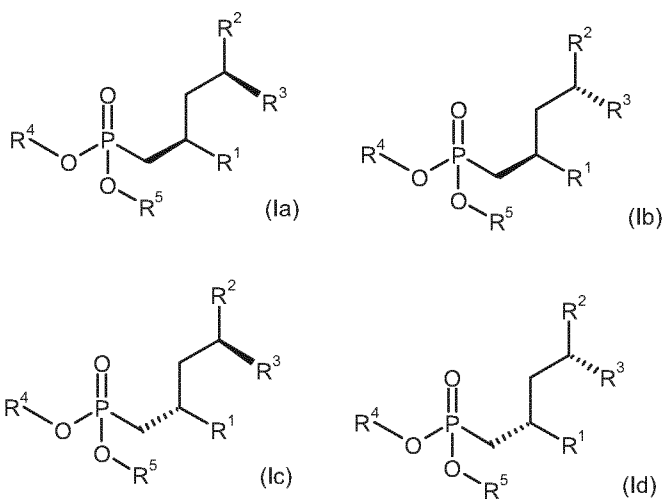
R⁵ is hydrogen;

R⁶ is hydrogen;

R⁷ is hydrogen;

20 and stereoisomers, stereoisomeric mixtures, and suitable salts thereof.

More preferably, the radiopharmaceuticals are radiopharmaceuticals of the formula (Ia), (Ib), (Ic) or (Id)

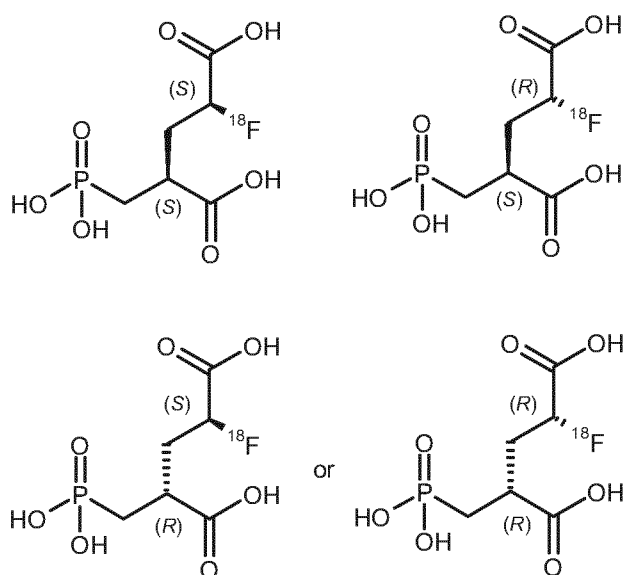


wherein

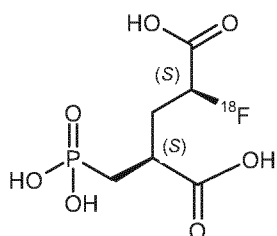
R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , and R^7 are as described above for the radiopharmaceutical of the formula (I).

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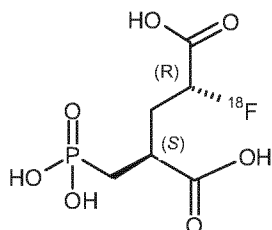
Radiopharmaceuticals suitable for the invention method i.e. derivatization are selected from but not limited to



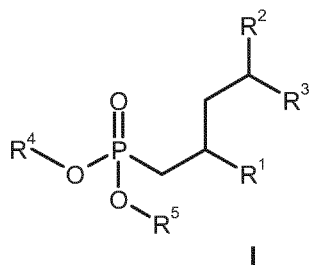
10 Preferably, the radiopharmaceutical is



Preferably, the radiopharmaceutical is



Preferably, the method for the derivatization of radiopharmaceuticals of the formula (I)



5

wherein

R^1 is $C(=O)OR^6$;

R^2 is $C(=O)OR^7$;

R^3 is ^{19}F or ^{18}F ;

10 R^4 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R^5 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R^6 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

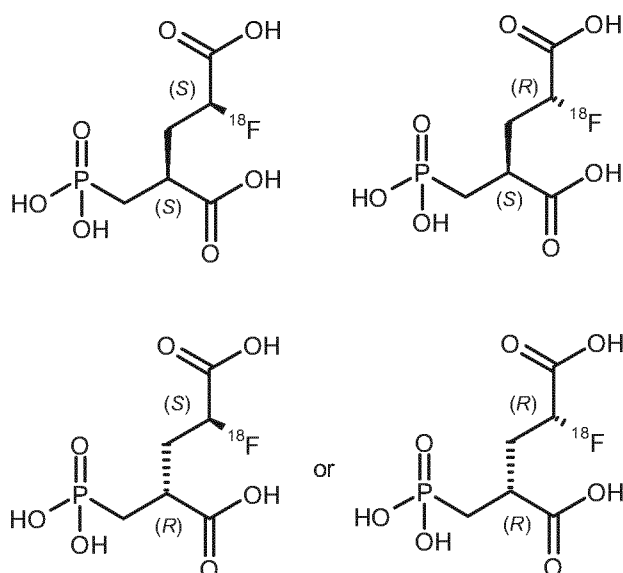
R^7 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

with the proviso that at least one of R^4 , R^5 , R^6 , and R^7 is hydrogen;

15 and stereoisomers, stereoisomeric mixtures, and suitable salts thereof;

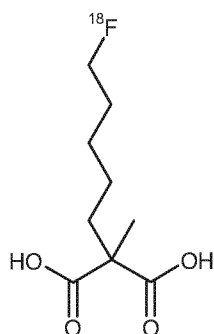
comprises the step of alkylation of at least one carboxylic acid groups or phosphonic acid groups of the radiopharmaceutical of the formula (I) by reacting radiopharmaceutical of the formula (I) with unsubstituted or substituted diazoalkane in presence of an acid.

20 More preferably, the method for the derivatization is applied to the radiopharmaceuticals



The radiopharmaceuticals here are fully unprotected at carboxylic acid groups and phosphonic acid groups.

- 5 Preferably, the method for the derivatization of the radiopharmaceutical 2-(5-[^{18}F]Fluoro-pentyl)-2-methyl-malonic acid



and stereoisomers, stereoisomeric mixtures, and suitable salts thereof;

comprises the step of alkylation of at least one carboxylic acid groups by reacting radiopharmaceutical with unsubstituted or substituted diazoalkane in presence of an acid.

The invention is directed also to cold radiopharmaceutical i.e. radiopharmaceutical comprising a counter part isotope (cold isotope) instead of a radioisotope.

- 15 Description of the derivatized radiopharmaceutical applicable to embodiments 1, 2 and 3:
See second aspect.

The invention is directed also to cold derivatized radiopharmaceutical i.e. radiopharmaceutical comprising a counter part isotope (cold isotope) instead of a radioisotope.

In a **second aspect**, the invention is directed to derivatized radiopharmaceuticals which are alkylated at one (1) or more carboxylic acid groups and/or one (1) or more phosphonic acid groups of the radiopharmaceutical as described above. Preferably, the derivatized radiopharmaceutical is alkylated at all carboxylic acid groups and/or all phosphonic acid groups of the radiopharmaceutical. Preferably, the derivatized radiopharmaceutical allows better subsequent analytical characterization, e.g. chiral HPLC with a solid-phase, allowing separation of stereoisomers and/or determination of the ratio of different stereoisomers in the final solution. This derivatization is extremely useful for polar compounds in which this polarity does not allow the ratio of stereoisomers to be determined.

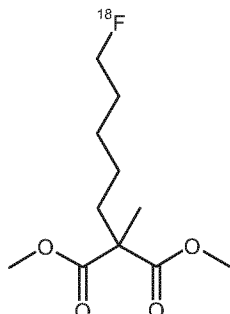
In a sub-embodiment, the invention is directed to derivatized radiopharmaceuticals which are obtained by the method described under first aspect wherein radiopharmaceutical as described above is alkylated at one (1) or more carboxylic acid groups and/or one (1) or more phosphonic acid groups.

In a further sub-embodiment, the invention is directed to derivatized radiopharmaceuticals which are alkylated at one (1) or more carboxylic acid groups and/or one (1) or more phosphonic acid groups wherein the radiopharmaceuticals are selected from the group of [^{11}C]Acetate, [^{18}F]Fluoroacetate, 2-[^{18}F]Fluoropropionic acid, (2RS,4S)-2-[^{18}F]Fluoro-4-phosphonomethyl-pentanedioic acid [^{18}F](2RS,4S)PMPA, 2-[^{18}F]Fluoro-4-phosphonomethyl-pentanedioic acid [^{18}F]PMPA, 2-(5-[^{18}F]Fluoro-pentyl)-2-methyl-malonic acid, [^{11}C]Lactate, [^{11}C]labeled fatty acids, [^{18}F]labeled fatty acids, [$^{99\text{m}}\text{Tc}$]MDP, [$^{99\text{m}}\text{Tc}$]DMSA, [$^{99\text{m}}\text{Tc}$]HMDP, and [$^{99\text{m}}\text{Tc}$]HEDP.

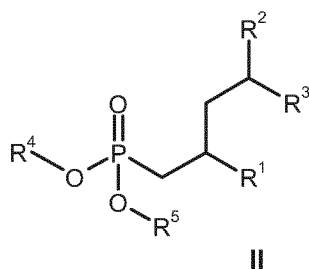
Preferably, the radiopharmaceutical is [^{18}F]Fluoroacetate, 2-[^{18}F]Fluoropropionic acid, (2RS,4S)-2-[^{18}F]Fluoro-4-phosphonomethyl-pentanedioic acid [^{18}F](2RS,4S)PMPA, 2-[^{18}F]Fluoro-4-phosphonomethyl-pentanedioic acid [^{18}F]PMPA or 2-(5-[^{18}F]Fluoro-pentyl)-2-methyl-malonic acid.

More preferably, the radiopharmaceutical is (2RS,4S)-2-[^{18}F]Fluoro-4-phosphonomethyl-pentanedioic acid [^{18}F](2RS,4S)PMPA, or 2-(5-[^{18}F]Fluoro-pentyl)-2-methyl-malonic acid.

Derivatized radiopharmaceutical isomer is selected from but not limited to

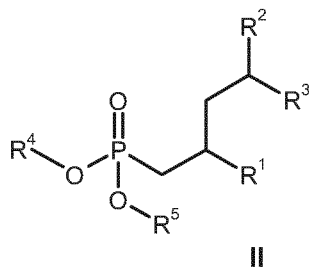


In a further sub-embodiment, the invention is directed to derivatized radiopharmaceuticals of the formula (II)



wherein

- 5 R^1 is $C(=O)OR^6$;
 R^2 is $C(=O)OR^7$;
 R^3 is ^{19}F or ^{18}F ;
 R^4 is hydrogen, alkyl or aryl-alkyl;
 R^5 is hydrogen, alkyl or aryl-alkyl;
- 10 R^6 is hydrogen, alkyl or aryl-alkyl;
 R^7 is hydrogen, alkyl or aryl-alkyl;
 with the proviso that R^4 , R^5 , R^6 , and R^7 are never simultaneously hydrogen;
 and stereoisomers, stereoisomeric mixtures, and suitable salts thereof.
- 15 Alkyl is lower alkyl i.e. C_1 - C_6 alkyl. Preferably, C_1 - C_6 alkyl is Methyl, Ethyl, Propyl, Butyl, t-Butyl. More preferably, C_1 - C_6 alkyl is Methyl.
- Aryl-alkyl is C_7 - C_{16} alkyl carbon chain moiety. Preferably, aryl-alkyl is benzyl.
- 20 Preferably, the derivatized radiopharmaceuticals are PET derivatized radiopharmaceuticals when R^3 is ^{18}F
 Preferably, the derivatized radiopharmaceuticals are cold derivatized radiopharmaceuticals useful as standard compound when R^3 is ^{19}F .
- 25 More preferably, the derivatized radiopharmaceuticals are derivatized radiopharmaceuticals of the formula (II)



wherein

R^1 is $C(=O)OR^6$;

R^2 is $C(=O)OR^7$;

R^3 is ^{19}F or ^{18}F ;

5 R^4 is alkyl or aryl-alkyl;

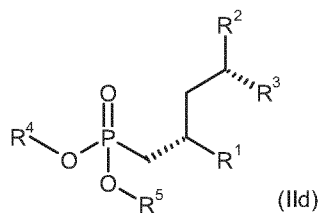
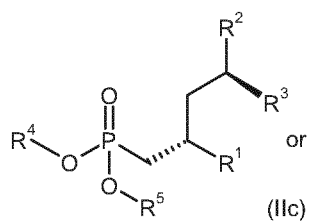
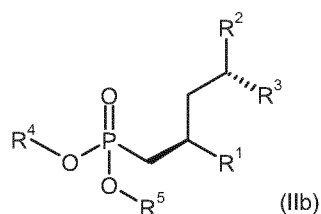
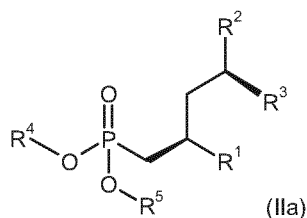
R^5 is alkyl or aryl-alkyl;

R^6 is alkyl or aryl-alkyl;

R^7 is alkyl or aryl-alkyl;

and stereoisomers, stereoisomeric mixtures, and suitable salts thereof.

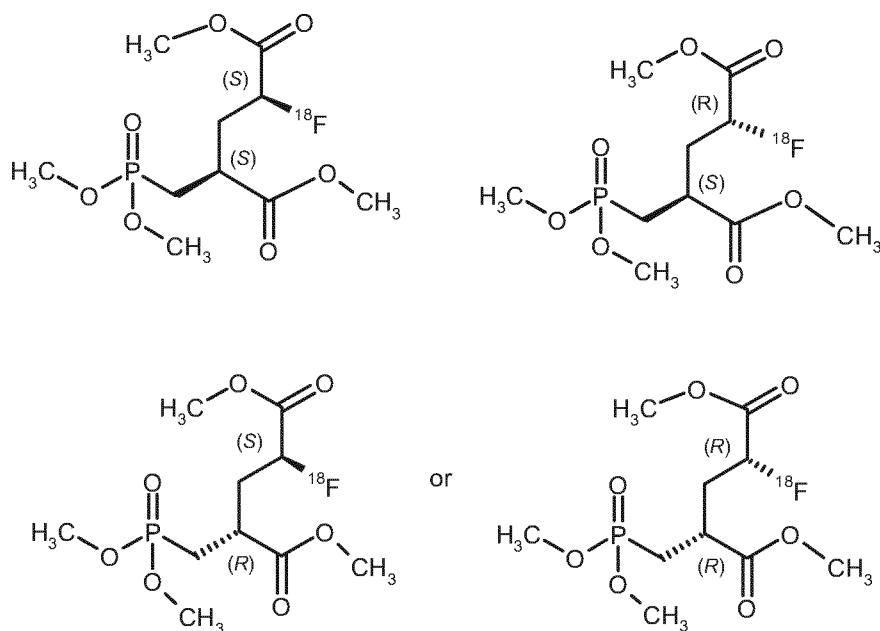
10 More preferably, the derivatized radiopharmaceuticals are derivatized radiopharmaceuticals of the formula (IIa), (IIb), (IIc) or (IId)



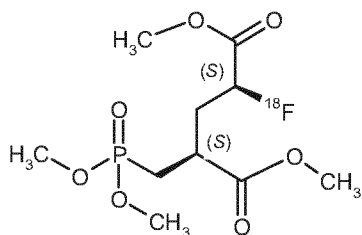
wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , and R^7 are as described above for derivatized radiopharmaceutical (II).

15

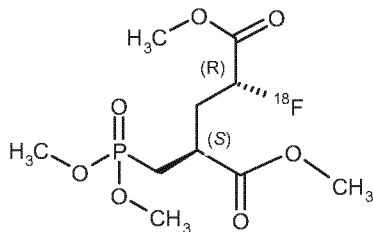
Derivatized radiopharmaceutical isomers are selected from but not limited to



Preferably, the derivatized radiopharmaceutical isomer is



Preferably, the derivatized radiopharmaceutical isomer is



5

The invention is directed also to cold derivatized radiopharmaceutical i.e. radiopharmaceutical comprising a counter part isotope (cold isotope) instead of a radioisotope.

10

In a **third aspect**, the invention is directed to the use of derivatized radiopharmaceuticals for subsequent analytical characterization of the said radiopharmaceutical, e.g. chiral HPLC with a solid-phase, allowing separation of stereoisomers and/or determination of the ratio of different stereoisomers in the final solution. This derivatization is extremely useful for polar compounds in which this polarity does not allow the ratio of stereoisomers to be determined. Preferably, the use refers to derivatized radiopharmaceuticals of the formula (II), (IIa), (IIb),

15

(IIc) or (IId), more preferably, derivatized radiopharmaceuticals of the formula (IIa) or (IIb).

The preferred features and embodiments disclosed above for radiopharmaceutical, radiopharmaceutical of the formula (II), (IIa), (IIb), (IIc) and (IId) are herein incorporated.

- 5 In a **fourth aspect**, the invention is directed to a kit comprising radiopharmaceutical as defined above. Preferably, the radiopharmaceutical is of the formula (I). Such kits may contain at least one sealed vial containing a radiopharmaceutical as defined above I, preferably of the formula (I), (Ia), (Ib), (Ic) or (Id), more preferably (Ia) or (Ib). The kit may also contain reagents suitable to perform the herein invention disclosed reaction, preferably
10 unsubstituted or substituted diazoalkane and an acid. The reagents disclosed herein may be also included in such kit and may be stored in a sealed vial. Furthermore, the kit may contain instructions for its use.

A kit comprises at least one sealed vial comprising radiopharmaceutical as defined above.

- The preferred features and embodiments disclosed above for radiopharmaceutical, radiopharmaceutical of the formula (I), (Ia), (Ib), (Ic) and (Id) unsubstituted or substituted
15 diazoalkane and acid are herein incorporated.

Definitions

- The terms used in the present invention are defined below but are not limiting the invention
20 scope.

- If chiral centers or other forms of isomeric centers are present in a compound according to the present invention, all forms of such stereoisomers, including enantiomers and diastereoisomers, are intended to be covered herein. Compounds containing chiral centers may be used as racemic mixture or as an enantiomerically enriched mixture or as a
25 diastereomeric mixture or as a diastereomerically enriched mixture, or these isomeric mixtures may be separated using well-known techniques, and an individual stereoisomer maybe used alone. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form.

- 30 In the context of the present invention, preferred salts are pharmaceutically acceptable salts of the compounds according to the invention. The invention also comprises salts which for their part are not suitable for pharmaceutical applications, but which can be used, for example, for isolating or purifying the compounds according to the invention.

- 35 Pharmaceutically acceptable salts of the compounds according to the invention include acid addition salts of mineral acids, carboxylic acids and sulphonic acids, for example salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid,

ethanesulphonic acid, toluenesulphonic acid, benzenesulphonic acid, naphthalene disulphonic acid, formic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

Pharmaceutically acceptable salts of the compounds according to the invention also include salts of customary bases, such as, by way of example and by way of preference, alkali metal salts (for example sodium salts and potassium salts), alkaline earth metal salts (for example calcium salts and magnesium salts) and ammonium salts, derived from ammonia or organic amines having 1 to 16 carbon atoms, such as, by way of example and by way of preference, ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylamine, N-methylmorpholine, arginine, lysine, ethylenediamine and N-methylpiperidine.

Those skilled in the art will further recognise that acid addition salts of the claimed compounds may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods. Alternatively, alkali and alkaline earth metal salts of acidic compounds of the invention are prepared by reacting the compounds of the invention with the appropriate base via a variety of known methods.

The present invention includes all possible salts of the compounds of the present invention as single salts, or as any mixture of said salts, in any ratio.

The term "buffering agents" as employed herein include but are not limited to potassium metaphosphate, dipotassium phosphate, sodium acetate, sodium citrate anhydrous and sodium citrate dehydrate.

The term "alkyl" as employed herein by itself or as part of another group refers to a C₁-C₁₀ straight chain or branched chain alkyl group such as, for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, isopentyl, neopentyl, heptyl, hexyl, decyl. Preferably, alkyl is C₁-C₆ straight chain or branched chain alkyl (lower alkyl) or C₇-C₁₀ straight chain or branched chain alkyl. Lower alkyl is a C₁-C₆ straight chain or branched chain alkyl.

The term "arylalkyl" as employed herein by itself or as part of another group refers to alkyl substituted with an aryl wherein arylalkyl is C₇-C₁₆ alkyl carbon moiety that is optionally substituted as listed below. Preferred arylalkyl is benzyl.

The term "aryl" as employed herein by itself or as part of another group refers to monocyclic or bicyclic aromatic groups containing from 6 to 12 carbons in the ring portion, preferably 6-10 carbons in the ring portion, such as phenyl, naphthyl or tetrahydronaphthyl.

- 5 Whenever the term "substituted" is used and unless explicitly defined, it is meant to indicate that one or more hydrogens on the atom indicated in the expression using "substituted" is / are replaced by one or multiple moieties from the group comprising halogen, nitro, C₁-C₆-alkylcarbonyl, cyano, trifluoromethyl, C₁-C₆-alkylsulfonyl, C₁-C₆-alkyl, C₁-C₆-alkoxy and C₁-C₆-alkylsulfanyl, provided that the regular valency of the respective atom is not exceeded, and
10 that the substitution results in a chemically stable compound, *i. e.* a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into a pharmaceutical composition.

- The term "diazalkane" is defined as a compound wherein two hydrogen atoms of an alkane
15 molecule have been replaced by a diazo group. Diazalkane has the general formula R⁸R⁹C=N₂ wherein R⁸ and R⁹ are independently from each other Hydrogen or lower alkyl, trialkylsilyl.

- The term "alkane" as employed herein refers to C₁-C₆-alkane e.g. Methane, Ethane,
20 Propane, Butane, t-Butane. More preferably, C₁-C₆ alkane is Methane.

The term "aryl-alkyl" as employed herein refers to C₇-C₁₆-aryl-alkane, e.g. benzyl, diphenylmethane.

- 25 A Brønsted acid is any substance that can donate a hydrogen ion (proton) to a base. Brønsted acids are H⁺-ion or proton donors and are known to those skilled in the art. Not-limiting examples are hydriodic acid, iodic acid, periodic acid, hydrochloric acid, perchloric acid, chloric acid, hydrobromic acid, bromic acid, perbromic acid, sulphuric acid, nitric acid, tetrafluoroboric acid, toluenesulfonic acid, trifluoromethanesulfonic acid,

- 30 Derivatization is the process in which a substance (chemical compound) is chemically modified to another chemical compound, i.e. derivative, to improve its chromatographic resolution, for example. Typically, a specific functional group of a compound, .e.g. carboxylic acid, amine, thiol, hydroxyl, phosphonic acid, reacts in the derivatization reaction to give a
35 new compound, i.e. derivate, which has different physical and chemical properties, e.g. different ultraviolet absorption, different solubility, different boiling point, different melting point, different aggregate state or different chemical composition. These new chemical properties resulting from the derivatizing reaction can be used for quantification or separation

process of the original substance. This derivatization reaction should be reliable and proceed to completion.

An aqueous solution is any solution in which water (H₂O) is the solvent or co-solvent.

- 5 Preferably, the amount of water in this aqueous solution can range from 1 to 100%. More preferably in the range 10% to 100%, even more preferably in the range 50% to 100%.

A compound is polar when an electric charge is not symmetrically distributed, so that there is a separation of charge or partial charge and formation of definite positive and negative poles.

- 10 Polar compounds are defined as having a log D (partition coefficient determined with octanol and water at pH 7.4) in the range of 0 to 1, and very polar compounds are defined as having a log D <0.

The term "halide" as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, and means fluoro, chloro, bromo, and iodo.

15

The term "alkylation" as employed herein refers to a process in which an alkyl group is added to or substituted in a compound.

20 The term "amine protecting group" as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, which is chosen from but not limited to a class of protecting groups namely carbamates, amides, imides, N-alkyl amines, N-aryl amines, imines, enamines, boranes, N-P protecting groups, N-sulphenyl, N-sulfonyl and N-silyl, and which is chosen from but not limited to those described in the textbook Greene and
25 Wuts, Protecting groups in Organic Synthesis, third edition, page 696-926, included herewith by reference.

The term "hydroxyl (O) protecting group" as employed herein as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, which is chosen
30 from but not limited to a class of protecting groups namely carbonates, esters, imides, O-alkyl ethers, O-aryl ethers, O-sulphenyl, O-sulfonyl and O-silyl, and which is chosen from but not limited to those described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, third edition, page 16-298, included herewith by reference.

35 The term "mercapto (S) protecting group" as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, which is chosen from but not limited to a class of protecting groups namely thioesters, S-alkyl thioethers, S-aryl thioethers, thiocarbonates, thiocarbamates and disulfides, and which is chosen from but not limited to those described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis,

third edition, page 647-695, included herewith by reference.

The term "Radiopharmaceutical" as employed herein refers to a drug that contains at least one radioactive atom(s), preferably one radioactive atom. Radiopharmaceuticals are administered to patient as diagnostic tracer for the diagnosis and/or treatment of diseases. Radioactive atom(s) are ^{18}F -fluorine, ^{124}I -, ^{124}I -, ^{123}I -, ^{125}I -, ^{131}I -iodine, ^{68}Ga -Gallium, C-11 Carbon 11 and $^{99\text{m}}\text{Tc}$ -Technetium (list not exhaustive). Preferably, the radioactive atom is ^{18}F -fluorine, ^{124}I -, ^{124}I -, ^{123}I -, ^{125}I -, or ^{131}I -iodine.

Present invention is directed to any radiopharmaceuticals falling within the scope of the invention. Preferably the radiopharmaceutical contains fluorine or iodine radioisotope.

For the purpose of the invention, radiopharmaceutical includes also drug comprising the non-radioactive counterpart (cold isotope).

In a sub-embodiment, the radioisotope is selected from the group comprising carbon-11 (^{11}C), nitrogen-13 (^{13}N), oxygen-15 (^{15}O), bromine-75 (^{75}Br), bromine-76 (^{76}Br), iodine-124 (^{124}I) and fluorine-18 (^{18}F). Preferably, the radioisotope is selected from the group comprising bromine-75 (^{75}Br), bromine-76 (^{76}Br), iodine-124 (^{124}I) and fluorine-18 (^{18}F). More preferably, the radioisotope is fluorine-18 (^{18}F).

In a sub-embodiment, the metal radioisotope complexed to a chelator is selected from the group comprising ^{177}Lu , ^{90}Y , $^{133\text{m}}\text{In}$, $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{52}Fe , ^{68}Ga , ^{72}As , ^{111}In , ^{97}Ru , ^{203}Pb , ^{62}Cu , ^{64}Cu , ^{51}Cr , $^{52\text{m}}\text{Mn}$, and ^{157}Gd . Preferably, the metal radioisotope is selected from the group comprising $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{68}Ga , and ^{111}In . More preferably, the metal radioisotope is ^{68}Ga .

The term "derivatized radiopharmaceutical" as employed herein means radiopharmaceutical (hot or cold) wherein at least one alkylation of a carboxylic or phosphonic group is carried out.

The term "water miscible solvent" as employed herein is selected from acetonitrile, methanol, ethanol, isopropanol, propanol, chloroform, dichloromethane, or dimethyl sulfoxide, preferably ethanol.

^{11}C]labeled fatty acids are well known in the art and have at least one carboxylic acid, see Kihlberg et al., Nucl. Med. Biol. 1994 Nov, 21(8): 1053-65 and Elmaleh et al., International Journal of Nucl. Med. and Bio. 10 issue 4, 1983, 181-187.

^{18}F]labeled fatty acids are well known in the art and have at least one carboxylic acid, see Pandey et al., Heart Metab (2011) 51:15-19.

MDP means Methyl diphosphonate.

DMSA means dimercaptosuccinic acid.

5

HMDP means hydroxymethylene diphosphonate.

HEDP means hydroxyethylidene diphosphonate.

10 As used herein, the term "one or more times", *e.g.* in the definition of the substituents of the compounds of the general formulae of the present invention, is understood as meaning "one, two, three, four or five times, particularly one, two, three or four times, more particularly one, two or three times, even more particularly one or two times".

15 Where the plural form of the word compounds, salts, polymorphs, hydrates, solvates and the like, is used herein, this is taken to mean also a single compound, salt, polymorph, isomer, hydrate, solvate or the like.

Unless otherwise specified, when referring to the compounds of formula the present
20 invention per se as well as to any pharmaceutical composition thereof the present invention includes all of the hydrates, salts, and complexes.

General synthesis of F-18 compounds

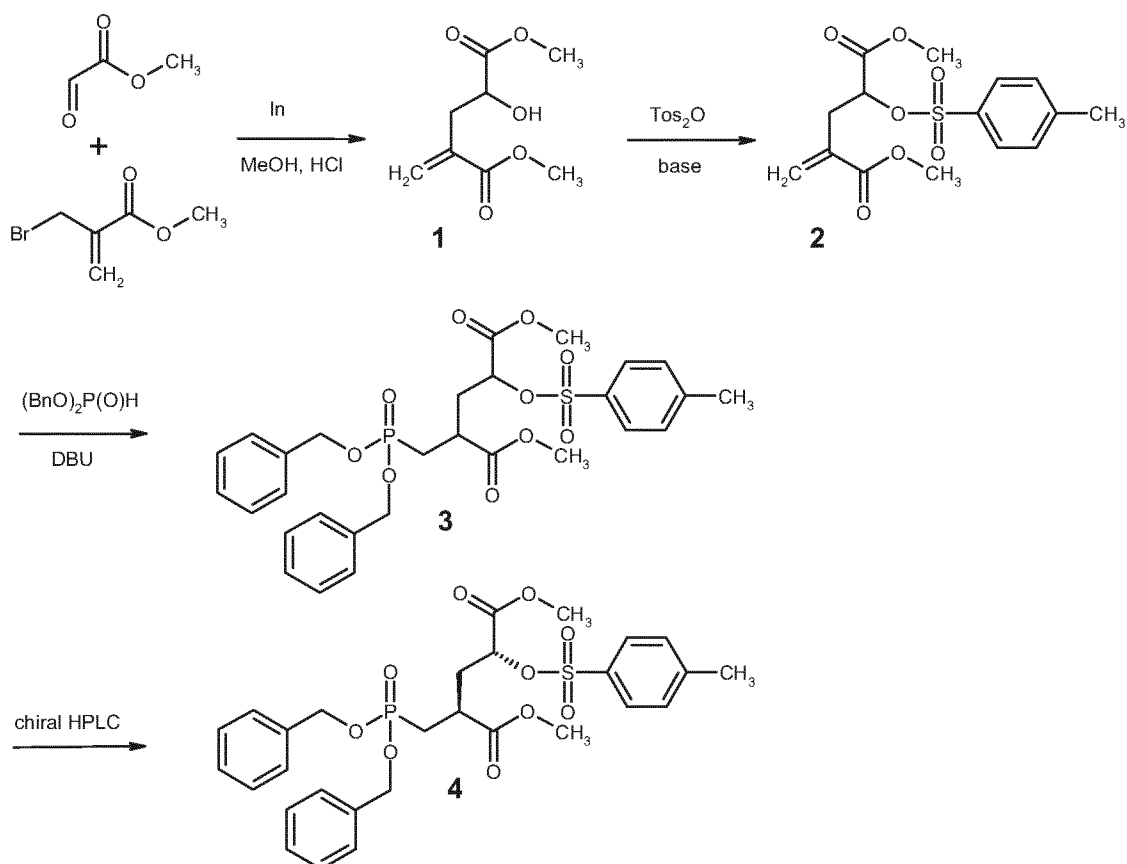
The radiofluorination reaction can be carried out, for example in a typical reaction vessel
25 (e.g. Wheaton vial) which is known to someone skilled in the art or in a microreactor. The reaction can be heated by typical methods, e.g. oil bath, heating block or microwave. The radiofluorination reactions are carried out in dimethylformamide with potassium carbonate as base and "kryptofix" as crown-ether. But also other solvents can be used which are well known to experts. These possible conditions include, but are not limited to: dimethylsulfoxide
30 and acetonitrile as solvent and tetraalkyl ammonium and tetraalkyl phosphonium carbonate as base. Water and/or alcohol can be involved in such a reaction as co-solvent. The radiofluorination reactions are conducted for one to 60 minutes. Preferred reaction times are five to 50 minutes. Further preferred reaction times are 10 to 40 min. This and other conditions for such radiofluorination are known to experts (Coenen, Fluorine-18 Labeling
35 Methods: Features and Possibilities of Basic Reactions, (2006), in: Schubiger P.A., Friebe M., Lehmann L., (eds), PET-Chemistry - The Driving Force in Molecular Imaging. Springer, Berlin Heidelberg, pp.15-50). The radiofluorination can be carried out in a "hot-cell" and/or by use of a module (review: Krasikowa, Synthesis Modules and Automation in F-18 labeling

(2006), in: Schubiger P.A., Friebe M., Lehmann L., (eds), PET-Chemistry - The Driving Force in Molecular Imaging. Springer, Berlin Heidelberg, pp. 289-316) which allows an automated or semi-automated synthesis.

5

PMPA Analog Precursor synthesis:

The following Scheme 1 shows the synthesis of PMPA analog precursors **3** and **4**.

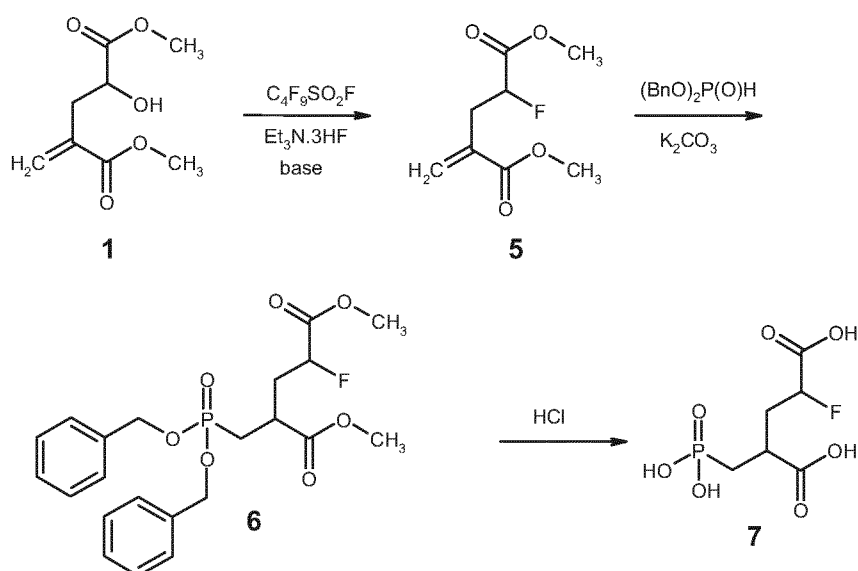


Scheme 1 shows the synthesis of PMPA analog precursor **3** and **4**.

10

Cold PMPA analog synthesis [¹⁹F] racemic:

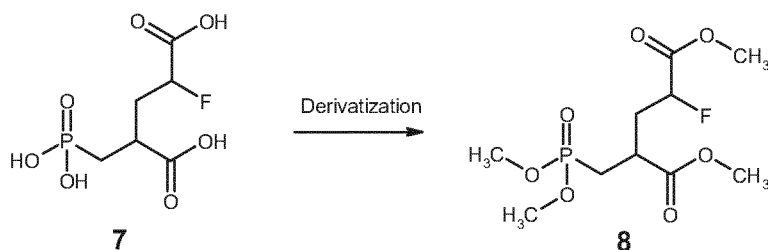
The following Scheme 2 shows the synthesis of the cold F19 PMPA analog compound **7**.



Scheme 2 shows the synthesis of the cold F19 PMPA analog compound 7.

Cold PMPA analog derivatization [^{19}F] racemic:

- 5 The following Scheme 3 shows the synthesis of the cold F19 PMPA analog derivatized compound 8.

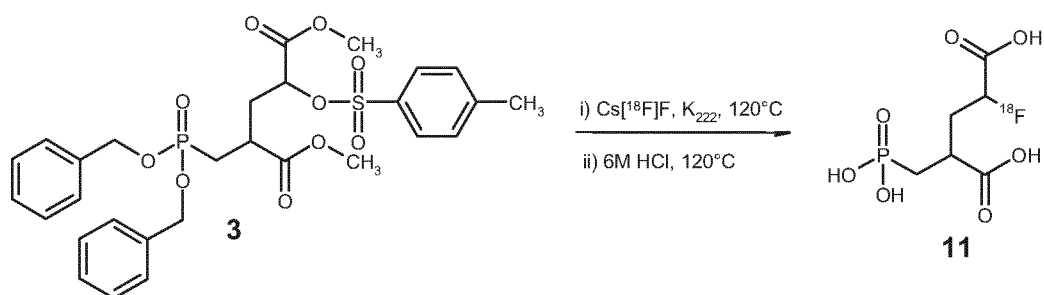


Scheme 3: Synthesis by derivatization of the cold F19 PMPA analog derivatized compound 8.

10

Hot PMPA analog synthesis [^{18}F] racemic:

The following Scheme 4 shows the radiosynthesis of the racemic hot F18 PMPA analog compound 11.

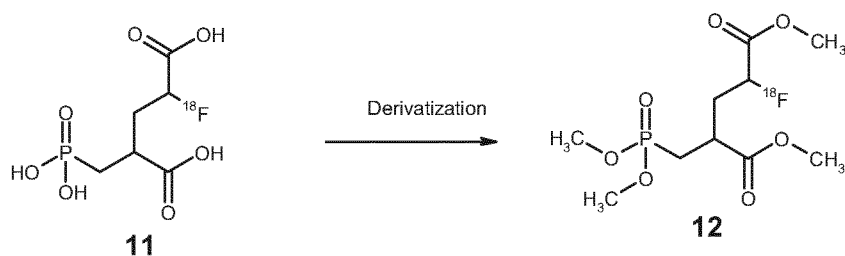


15

Scheme 4: Radiosynthesis of racemic hot F18 PMPA analog compound 11.

Hot PMPA analog derivatization [^{18}F] racemic:

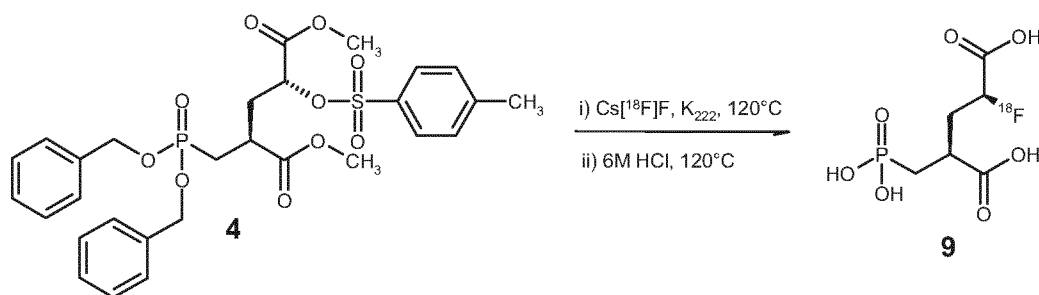
The following Scheme 5 shows the synthesis of the racemic hot F18 PMPA analog derivatized compound **12**.



Scheme 5: synthesis by derivatization of the racemic hot F18 PMPA analog derivatized compound **12**.

Hot PMPA analog synthesis [^{18}F] (2S,4S):

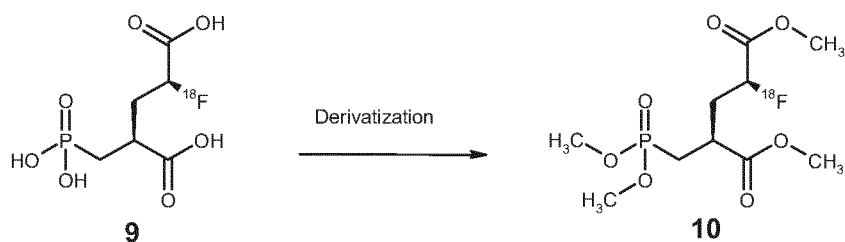
Scheme 6 shows the radiosynthesis of (2S,4S) hot F18 PMPA analog compound **9**.



Scheme 6: Radiosynthesis (2S,4S) hot F18 PMPA analog compound **9**.

Hot PMPA analog derivatization [^{18}F] (2S,4S):

The following Scheme 7 shows the synthesis of (2S,4S) hot F18 PMPA analog derivatized compound **10**.



Scheme 7 shows the radiosynthesis by derivatization of (2S,4S) hot F18 PMPA analog derivatized compound **10**.

The derivatization as shown in Schemes 5 and 7 surprisingly gave quantitative conversion of four acidic functionalities despite the derivatization solution containing a high content of water. In addition, the different chiral isomers could be clearly separated by chiral HPLC allowing the determination of the ratio of the different isomers generated in a radiosynthesis.

Experimental Section

Abbreviations

AcCl	Acetyl Chloride
[bmim][PF ₆]	1-Butyl-3-methylimidazolium hexafluorophosphate
Cs ₂ CO ₃	Cesium carbonate
DMF	<i>N,N</i> -Dimethylformamide
DMF-DMA	Dimethylformamide dimethyl acetal
DMSO	Dimethylsulphoxide
EtOAc	Ethyl Acetate
EtOH	Ethanol
GBq	GigaBequerel
h	Hour
HBF ₄	Tetrafluoroboric acid
HPLC	High Pressure Liquid Chromatography
K ₂ CO ₃	Potassium carbonate
K ₂₂₂	Kryptofix 2.2.2
Min(s)	Minute(s)
MBq	MegaBequerel
MTB	Methyl t-butyl ether
MeCN	Acetonitrile
MeOH	Methanol
MS	Mass Spectrometry
N ₂	Nitrogen
NMR	Nuclear Magnetic Resonance
PET	Positron Emission Technology
PMPA	2-(Phosphonomethyl) pentanedioic acid
RT	Room Temperature

SPE	Solid Phase Extraction
TMOA	Trimethylorthoacetate
TMSCHN ₂	Trimethylsilyldiazomethane
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

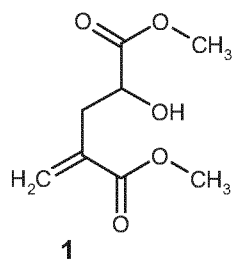
General: All solvents and chemicals were obtained from commercial sources and used without further purification. Anhydrous solvents and inert atmosphere (nitrogen or argon) were used if not stated otherwise. The preceding table lists the abbreviations used in this paragraph and in the Intermediates and Examples sections as far as they are not explained within the text body. NMR peak forms are stated as they appear in the spectra, possible higher order effects have not been considered.

Reactions were monitored by methods known to the person skilled in the art, such as thin-layer chromatography on suitable stationary phases, such as silica gel coated plates of aluminium or glass or HPLC or HPLC/MS analyses.

The compounds and intermediates produced according to the methods of the invention may require purification. Purification of organic compounds is well known to the person skilled in the art and there may be several ways of purifying the same compound. In some cases, no purification may be necessary. In certain cases, the compounds may be purified by crystallization. In some cases, impurities may be removed by trituration using a suitable solvent. In some cases, the compounds may be purified by column chromatography, or preparative HPLC according to the preparative HPLC methods listed below.

Column chromatography, as used hereinafter, typically refers to preparative liquid chromatography on a suitable stationary phase, such as commercial silica gel or prepacked silica gel cartridges, e.g. from Separtis such as Isolute® Flash silica gel or Isolute® Flash NH₂ silica gel in combination with e.g. an automated column chromatography system, and eluents such as gradients of hexane/EtOAc or dichloromethane/ethanol. Said automated chromatography systems are known to the person skilled in the art and are commercially available (e.g. FlashMaster II® by Argonaut/Biotage, SP4® by Biotage, Isolera Four® by Biotage, ISCO Companion®, and the likes).

Example 1: Dimethyl (*RS*)-2-hydroxy-4-methylenepentanedioate



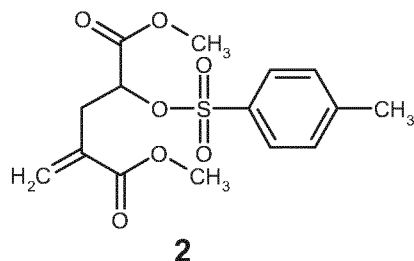
In a three-necked flask equipped with a powerful mechanical stirrer, to a cooled (+5°C, ice-water bath) mixture of methyl glyoxylate (10.0 grams, 114 mmol), methyl (2-bromomethyl) acrylate (22.5 g, 1.10 eq), methanol (80 mL), and 0.3 N aqueous hydrochloric acid (80 mL) was added powdered Indium (100 mesh, 13.0 g, 1.00 eq.) in several portions maintaining the temperature below 35 °C (which did no longer require full cooling during the addition of the later portions). The cooling bath was then completely removed, several freshly broken glass sherds (from a Pasteur pipette, to prevent clotting of the Indium) were added and the mixture was stirred vigorously for 4 h, during which the mixture cooled down to room temperature. The mixture was decanted off all solids and the supernatant was concentrated *in vacuo*. The residue was saturated with solid sodium chloride and then extracted by MTB; the residue remaining hereafter was loaded on Celite and was washed with MTB (4x) and EtOAc (1x). The combined organic layers were dried over sodium sulfate, passed over a plug of Celite, and evaporated. The residue was purified by column chromatography over silica to give 16.8 g of the target compound in 94 % purity (74 % yield).

¹H NMR (300 MHz, CDCl₃) δ ppm 2.65 (dd, 1 H) 2.87 (dd, 1 H) 3.07 (s br, 1 H) 3.77 (s, 3 H), 3.78 (s, 3 H) 4.34 - 4.44 (m, 1 H) 5.74 (m, 1 H) 6.30 (m, 1 H).

MS (CI): [M + H]⁺ = 189.

MS (CI): [M + NH₄]⁺ = 206.

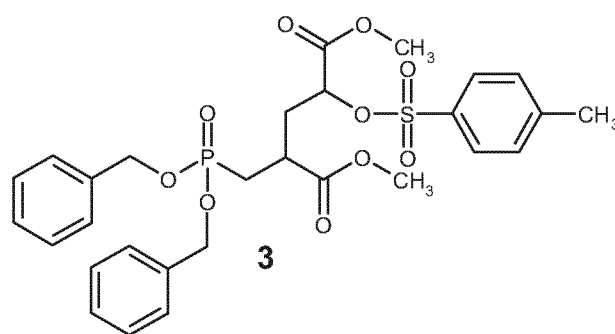
Example 2: Dimethyl (RS)-2-methylene-4-(tosyloxy)pentanedionate



To a cooled (0°C) solution of the starting alcohol **1** (1.00 g, 5.31 mmol) in pyridine (100 mL) was added *para*-toluenesulfonyl anhydride (2.00 eq). and the mixture was stirred for a period ranging from 2 h at 0°C. The reaction mixture was then partitioned between water and dichloromethane, the organic layer was then washed with water and brine, dried over sodium sulfate, and evaporated. The crude product was purified by column chromatography over silica to give 1.80 g of the target compound (99 % yield).

¹H-NMR (400 MHz, CDCl₃): δ ppm = 2.45 (s, 3 H) 2.69 (dd, 1 H) 2.90 (dd, 1 H) 3.67 (s, 3 H) 3.68 (s, 3 H) 5.06 (dd, 1 H) 5.67 (s, 1 H) 6.21 (s, 1 H) 7.32 (d, 2 H) 7.75 (d, 2 H).

Example 3: Dimethyl *rac*-2-[[bis(benzyloxy)phosphoryl]methyl]-4-(tosyloxy)-pentanedioate



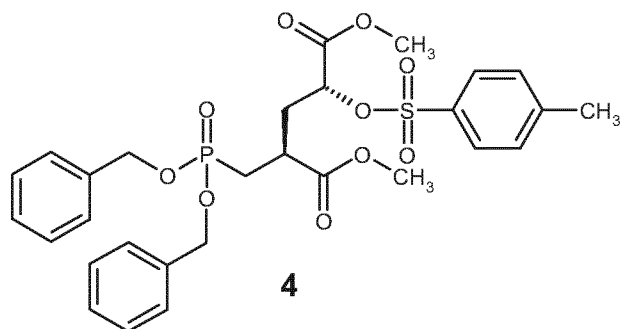
To a solution of compound **2** (1.32 g, 3.81 mmol) in THF (40 mL) was added DBU (1,8-Diazabicyclo(5.4.0)undec-7-en, 717 µL, 1.25 eq) and dibenzyl phosphite (1.70 mL, 2.00 eq.) and the mixture was stirred at room temperature for 1.5 h. The mixture was concentrated *in vacuo* and then partitioned between EtOAc and 5 % aqueous citric acid. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was purified by column chromatography on silica (hexane / EtOAc) to give the desired product (1.42 g, 61 % yield) as a mixture of stereoisomers.

¹H-NMR (400 MHz, CDCl₃): δ ppm 1.83 - 2.07 (m, 1 H) 2.10 - 2.33 (m, 3 H) 2.43 (s, 3 H) 2.81 - 2.98 (m, 1 H) 3.51 (s, 3 H, minor diastereomer) 3.53 (s, 3 H, major diastereomer) 3.57 (s, 3 H, major diastereomer), 3.62 (s, 3 H, minor diastereomer) 4.88 - 5.05 (m 5 H) 7.28 - 7.41 (m, 12 H) 7.75 - 7.81 (m, 2 H).

MS (ESI): $[M + H]^+ = 605$.

Example 4: Dimethyl (2*S*,4*R*)-2-[[bis(benzyloxy)phosphoryl]methyl]-4-(tosyloxy)-pentanedioate

5



The compound 3 was submitted to chiral HPLC purification, the compound 4 was the first peak and had a retention time (t_R) of 9.1 min using the following method:

10 SYSTEM: Dionex: Pump 680, ASI 100, Knauer: UV-Detektor K-2501

COLUMN: Chiralcel OD-H 5 μ m 150x4.6 mm

SOLVENT: Hexane / Ethanol 85:15 (isocratic)

FLOW: 1.0 mL/min

TEMPERATURE: 25 °C

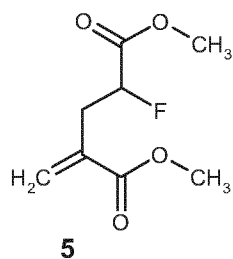
15 DETECTION: UV 210 nm

^1H NMR (400 MHz, CDCl_3) δ ppm 1.83 - 1.95 (m, 1 H) 2.10 - 2.31 (m, 3 H) 2.43 (s, 3 H) 2.81 - 2.95 (m, 1 H) 3.51 (s, 3 H) 3.62 (s, 3 H) 4.88 - 5.05 (m, 5 H) 7.28 - 7.40 (m, 12 H) 7.77 (d, 2 H).

20

Cold Standard Synthesis

Example 5: Dimethyl (*RS*)-2-fluoro-4-methylenepentanedioate

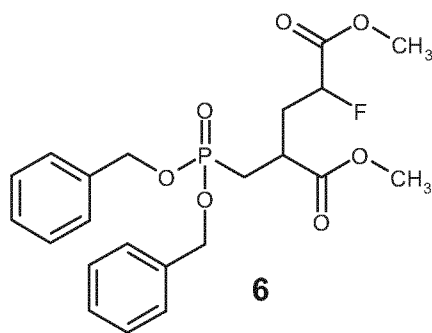


To a solution of compound **1** (6.00 g, 31.9 mmol) in THF (60 mL) was added at room temperature perfluorobutanesulfonic acid fluoride (11.7 mL, 2.00 eq), triethylamine trihydrofluoride (10.4 mL, 2.00 eq.) and triethylamine (26.7 mL, 6.00 eq.). After stirring for 20 h at room temperature, the reaction mixture was partitioned between water and dichloromethane. The organic layer was separated and washed with a brine/water mixture (1:1), dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, hexane / EtOAc) to give compound **5** (4.37 g, 72 % yield).

¹H-NMR (400 MHz, CDCl₃): δ ppm 2.76 - 2.88 (m, 1 H) 2.94 - 3.06 (m, 1 H) 3.79 (s, 3 H) 3.80 (s, 3 H) 5.18 (ddd, 1 H) 5.78 (s, 1 H) 6.35 (s, 1 H).

MS (ESI): [M + H]⁺ = 191.

Example 6: Dimethyl *rac*-2-[[bis(benzyloxy)phosphoryl]methyl]-4-fluoropentanedioate



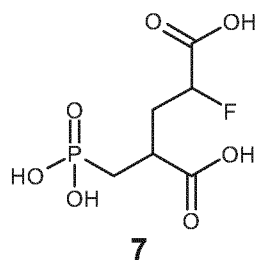
To a solution of compound **5** (50 mg, 0.26 mmol) and dibenzyl phosphite (86 mg, 1.25 eq) in DMF (3 mL) was added finely powdered potassium carbonate (mesh 325, 55 mg, 1.50 eq), and the resulting mixture was heated to 50 °C for 4 h in a microwave oven. After cooling to room temperature, the mixture was partitioned between EtOAc and 5% aqueous citric acid. The organic layer was washed with half-concentrated brine, dried over sodium sulfate, and

evaporated. The residue was purified by column chromatography over silica gel (hexane / EtOAc) to give the target compound as a mixture of stereoisomers (99 mg, 84 % yield).

¹H NMR (400 MHz, CDCl₃) δ ppm 1.88 - 2.04 (m, 1 H) 2.10 - 2.39 (m, 3 H) 2.91 - 3.08 (m, 1 H) 3.55 (s, 3 H, minor diastereomer) 3.58 (s, 3 H, major diastereomer) 3.76 (s, 3 H, minor diastereomer) 3.77 (s, 3 H, major diastereomer) 4.82 - 5.08 (m, 5 H) 7.29 - 7.41 (m, 10 H).

MS (ESI): [M + H]⁺ = 453.

10 Example 7: *rac*-2-Fluoro-4-(phosphonomethyl)pentanedioic acid



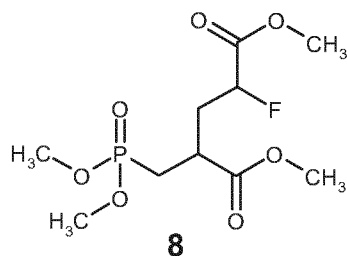
A mixture of compound **6** (100 mg, 221 μmol) in 6 N hydrochloric acid (1.33 mL) was stirred at 100 °C in a microwave oven. After cooling to room temperature, the mixture was diluted with water and evaporated by lyophilization to give the crude target compound which was not purified otherwise (100 mg, >100 % crude yield).

¹H NMR (400 MHz, d₆-DMSO) δ ppm 1.63 - 1.80 (m, 1 H) 1.85 - 2.29 (m, 3 H) 2.64 - 2.80 (m, 1 H) 4.81 - 5.11 (m, 1 H). CO₂H and PO₃H₂ protons form a broad peak with adventitious water at 5.4 ppm.

MS (ESI): [M - H]⁻ = 243.

Derivatization cold standard

Example 8: Dimethyl *rac*-2-[(dimethoxyphosphoryl)methyl]-4-fluoropentanedioate



To a solution of compound **7** (220 mg, 1.16 mmol) in DMF (3 mL) was added dimethyl phosphite (135 μ L, 1.25 eq.), and finely powdered potassium carbonate (325 mesh, 240 mg, 1.50 eq.), and the mixture was stirred for 2 h at 50 °C in a microwave oven. After cooling to room temperature, the mixture was partitioned between dichloromethane and 5% aqueous citric acid. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was purified by column chromatography over silica gel (dichloromethane / methanol) to give the target compound as a mixture of stereoisomers (210 mg, 54 % yield).

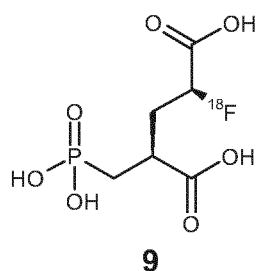
^1H NMR (400 MHz, CDCl_3) δ ppm 1.92 - 2.08 (m, 1 H) 2.17 - 2.48 (m, 3 H) 2.97 - 3.10 (m, 1 H) 3.71 - 3.83 (m, 12 H) 4.97 (ddd, 1 H, major diastereomer) 5.03 (ddd, 1 H, minor diastereomer).

MS (ESI): $[\text{M} + \text{H}]^+ = 300$.

Hot Standard Synthesis

Example 9

(2S,4S)-2- ^{18}F Fluoro-4-phosphonomethyl-pentanedioic acid **9**



[¹⁸F]Fluoride (7,7 GBq) was immobilized on a preconditioned QMA (Waters) cartridge (preconditioned by washing the cartridge with 5ml 0.5M K₂CO₃ and 10 ml water), The [¹⁸F]fluoride was eluted using a solution of Cs₂CO₃ (2,3 mg) in 500 µl water and K₂₂₂ (5 mg) in 1500 µl acetonitrile. This solution was dried at 120°C with stirring under vacuum, with a stream of nitrogen. Additional acetonitrile (1 ml) was added and the drying step was repeated. A solution of precursor **4** (4mg) in DMSO (500 µl) was added and heated at 120°C for 15 min. The mixture was diluted with 20ml water and passed through a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water). The SPE was washed with 5ml water and eluted with 1ml MeCN. The eluted solution was diluted with 3ml water and purified over prep. HPLC (ACE 5µ C18, 250 x 10mm, isocratic 60% MeCN in 40% water + 0.1%TFA, flow: 4ml/min). The product peak was collected and diluted with 20ml water and passed through a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water). The SPE was washed with 5ml water and was eluted with 1ml ethanol. The ethanol solution was dried under gentle N₂-stream for 10min at 90°C. 1000µl 6M HCl were added and the mixture was incubated for 15min at 120°C. After cooling the reaction mixture was diluted with 1ml water and passed through a cartridge containing AG11A8 resin (ion retardation resin, ~11g) connected in series with a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water), the cartridges were then washed with saline (5ml) and eluents were collected to give the desired product, 760 MBq (23% d.c.).

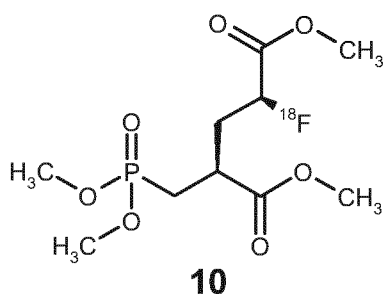
Figure 1 shows the chromatogram of the final compound **9** after acidic deprotection

Column:	ACE 3µ C18 50 x 4,6 mm	
Mobile Phase:	A: H ₂ O + 0.1%TFA, B: MeCN + 0.1%TFA	
Gradient:	00:00	05%B
	07:00	95%B
	07:10	100%B
	08:80	100%B
	09:00	05%B
	12:00	05%B
Flow:	2 ml/min	
Detection:	γ-detection	

Derivatization hot radiopharmaceutical

Example 10:

Dimethyl-(2S,4S)-2-[¹⁸F]Fluoro-[(dimethoxyphosphoryl)methyl]-pentanedioate **10**



To [^{18}F]PMPA (Compound **9**) final formulated product (100 μl) add HBF_4 (10 μl).

Stir the solution vigorously.

Add trimethylsilyldiazomethane solution (3 x 400 μl) dropwise (Care: exothermic reaction)

5 Stir for 2 min at RT.

Quench reaction with acetic acid (10 μl). Figure 2 shows complete conversion

Concentrate under a gentle N_2 stream at RT for 5 min.

Dilute with 1 ml water and load solution onto tC18 (preconditioned with 5 ml ethanol and with 10 ml water)

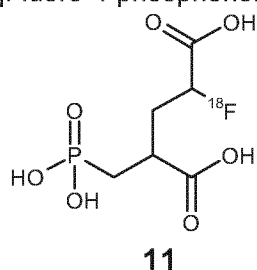
10 Wash with 3 ml water.

Elute with 1 ml ethanol and inject 5 μl onto chiral HPLC. Figure 3 shows the chiral HPLC of compound **10**, the derivatized product of compound **9**.

Hot Standard Synthesis

15 **Example 11:**

(rac)-2- ^{18}F Fluoro-4-phosphonomethyl-pentanedioic acid **11**



[^{18}F]Fluoride (4,6 GBq) was immobilized on a preconditioned QMA (Waters) cartridge (preconditioned by washing the cartridge with 5ml 0.5M K_2CO_3 and 10 ml water), The [^{18}F]fluoride was eluted using a solution of Cs_2CO_3 (2,3 mg) in 500 μl water and K_{222} (5 mg) in 1500 μl acetonitrile. This solution was dried at 120 $^\circ\text{C}$ with stirring under vacuum, with a stream of nitrogen. Additional acetonitrile (1 ml) was added and the drying step was repeated. A solution of precursor **3** (4mg) in DMSO (500 μl) was added and heated at 120 $^\circ\text{C}$ for 15 min. The mixture was diluted with 20ml water and passed through a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water). The SPE was washed with 5ml water and eluted with 1ml MeCN. The eluted solution was diluted with 3ml water and purified over prep. HPLC (ACE 5 μ C18, 250 x 10mm, isocratic 60% MeCN in 40% water + 0.1%TFA, flow:

4ml/min). The product peak was collected and diluted with 20ml water and passed through a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water). The SPE was washed with 5ml water and was eluted with 1ml ethanol. The ethanol solution was dried under gentle N₂-stream for 10min at 90°C. 1000µl 6M HCl were added and the mixture was incubated for 15min at 120°C. After cooling the reaction mixture was diluted with 1ml water and passed through a cartridge containing AG11A8 resin (ion retardation resin, ~11g) connected in series with a C18 light SPE (preconditioned with 5ml ethanol and with 10ml water), the cartridges were then washed with saline (5ml) and eluents were collected to give the desired product, 430 MBq (25% d.c.).

Derivatization Methods (Invention).

This derivatization method was surprisingly found to be rapid (<10 mins) and quantitative (>95%) despite the F18 labeled compound **9** being in a aqueous solution. In addition, this derivatization method allows for the chiral analysis of the derivatized product which was not possible with the underivatized compound **9** or **11**.

1. Trimethylsilyldiazomethane in ether with tetrafluoroboric acid

Materials:

Final formulated [¹⁸F]PMPA product (Compound **9**). Figure 1 shows underivatized [¹⁸F]PMPA (Compound **9**).

48% HBF₄ in Water (~8 M).

Trimethylsilyldiazomethane solution (2 M in diethyl ether).

tC18 light SPE-cartridge.

HPLC Method used to determine conversion for the derivatization:

Column:	ACE 3µ C18 50 x 4,6 mm
Mobile Phase:	A: H ₂ O + 0.1%TFA, B: MeCN + 0.1%TFA
Gradient:	00:00 05%B
	07:00 95%B
	07:10 100%B
	08:80 100%B
	09:00 05%B
	12:00 05%B
Flow:	2 ml/min
Detection:	γ-detection

HPLC Method used to determine ratio of the different isomer after derivatization:

Mobile Phase: A: EtOH, B: Hexane
 Injection volume: 5µl injection volumes as ethanol solutions
 5 Column: Lux 5µ Amylose-2, 150 x 4.6 mm, Phenomenex
 Flow: 1 ml/min
 Detection: UV (210-220 nm), γ-detection

Preparation :

- 10 To [¹⁸F]PMPA (Compound **9**) final formulated product (100µl) add HBF₄ (10µl).
 Stir the solution vigorously.
 Add trimethylsilyldiazomethane solution (3 x 400µl) dropwise (Care: exothermic reaction)
 Stir for 2 min at RT.
 Quench reaction with acetic acid (10µl). Figure 2 shows complete conversion
 15 Concentrate under a gentle N₂ stream at RT for 5 min.
 Dilute with 1 ml water and load solution onto tC18 (preconditioned with 5 ml ethanol and with
 10 ml water)
 Wash with 3 ml water.
 Elute with 1 ml ethanol and inject 5 µl onto chiral HPLC. Figure 3 shows the chiral HPLC of
 20 compound **10**, the derivatized product of compound **9**. Figure 4 shows a chiral HPLC of the
 racemic mixture **8**.

Derivatization Methods (Prior art).

- 25 **1. Dimethylformamide dimethyl acetal (DMF-DMA) (See background: Thenot *et al.*, *Anal. Letters*, 1972, 5, 217-223 and 519-529)**

Materials:

Final formulated [¹⁸F]PMPA product (Compound **9**). Figure 1 shows underivatized [¹⁸F]PMPA (Compound **9**).

- 30 N,N-Dimethylformamide dimethyl acetal (DMF-DMA), derivatization grade: Aldrich.

HPLC Method used to determine conversion for the derivatization:

- Column: ACE 3µ C18 50 x 4,6 mm
 35 Mobile Phase: A: H₂O + 0.1%TFA, B: MeCN + 0.1%TFA
 Gradient: 00:00 05%B
 07:00 95%B
 07:10 100%B
 08:80 100%B

09:00 05%B
12:00 05%B
Flow: 2 ml/min
Detection: γ -detection

5

Preparation :

To [^{18}F]PMPA (Compound **9**) final formulated product (100 μl) was added DMF-DMA (1000 μl). (Care: exothermic reaction)

Solution stirred at 100°C for 10 mins.

10 Quenched reaction with water (1000 μl). Figure 5 shows no conversion.

2. Trimethylorthoacetate and ionic liquid [bmim][PF₆] (See background: Yoshino *et al.*, *Tetrahedron*, 2006, 62, 1309-1317)

Materials:

15 Final formulated [^{18}F]PMPA product (Compound **9**). Figure 1 shows underivatized [^{18}F]PMPA (Compound **9**).

[bmim][PF₆]: Aaron Chemistry

Trimethylorthoacetate (TMOA): Fluka

20 HPLC Method used to determine conversion for the derivatization:

Column: ACE 3 μ C18 50 x 4,6 mm
Mobile Phase: A: H₂O + 0.1%TFA, B: MeCN + 0.1%TFA
Gradient: 00:00 05%B
25 07:00 95%B
07:10 100%B
08:80 100%B
09:00 05%B
12:00 05%B

30 Flow: 2 ml/min
Detection: γ -detection

Preparation :

To [^{18}F]PMPA (Compound **9**) final formulated product (100 μl) was added [bmim][PF₆] (100 μl)
35 followed by TMOA (400 μl).

Solution stirred at 100°C for 10mins.

Quenched reaction with water/MeOH (1:1; 1000 μl). Figure 6 shows no conversion.

3. Acetyl chloride (AcCl) and Methanol (MeOH) (See background: Lillington *et al.*, *Clinica Chimica Acta*, 1981, 111, 91-98)

Materials:

Final formulated [^{18}F]PMPA product (Compound **9**). Figure 1 shows underivatized [^{18}F]PMPA (Compound **9**).

Acetyl Chloride p.A.: Merck:

HPLC Method used to determine conversion for the derivatization:

Column:	ACE 3 μ C18 50 x 4,6 mm
Mobile Phase:	A: H ₂ O + 0.1%TFA, B: MeCN + 0.1%TFA
Gradient:	00:00 05%B
	07:00 95%B
	07:10 100%B
	08:80 100%B
	09:00 05%B
	12:00 05%B
Flow:	2 ml/min
Detection:	γ -detection

Preparation :

To [^{18}F]PMPA (Compound **9**) final formulated product (100 μ l) was added MeOH (900 μ l) followed by AcCl (60 μ l – Care: exothermic reaction).

Solution stirred at RT for 10mins.

Quenched reaction with water (1000 μ l). Figure 7 shows conversed to multiple products.

4. Trimethylsilyl diazomethane (See background: Korn *et al.*, *J. Biol. Chem.*, 1973, 248, 2257-2259)

Materials:

Final formulated [^{18}F]PMPA product (Compound **9**). Figure 1 shows underivatized [^{18}F]PMPA (Compound **9**).

Trimethylsilyldiazomethane solution (2 M in hexane).

HPLC Method used to determine conversion for the derivatization:

Column:	ACE 3 μ C18 50 x 4,6 mm
Mobile Phase:	A: H ₂ O + 0.1%TFA, B: MeCN + 0.1%TFA
Gradient:	00:00 05%B
	07:00 95%B

	07:10	100%B
	08:80	100%B
	09:00	05%B
	12:00	05%B
5	Flow:	2 ml/min
	Detection:	γ -detection

Preparation :

To [^{18}F]PMPA (Compound **9**) final formulated product (100 μl) was added
 10 trimethylsilyldiazomethane in hexane complex (900 μl).
 Solution stirred at RT for 10mins.
 Reaction quenched by the addition of AcOH (10 μl). Figure 8 shows multiple peaks.

5. Boron trifluoride dimethanol complex15 *Materials:*

Final formulated [^{18}F]PMPA product (Compound **9**). Figure 1 shows underivatized [^{18}F]PMPA
 (Compound **9**).

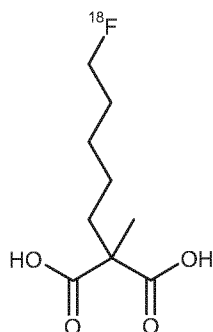
Boron trifluoride dimethanol complex: ACROS

20 HPLC Method used to determine conversion for the derivatization:

	Column:	ACE 3 μ C18 50 x 4,6 mm	
	Mobile Phase:	A: H ₂ O + 0.1%TFA, B: MeCN + 0.1%TFA	
	Gradient:	00:00	05%B
25		07:00	95%B
		07:10	100%B
		08:80	100%B
		09:00	05%B
		12:00	05%B
30	Flow:	2 ml/min	
	Detection:	γ -detection	

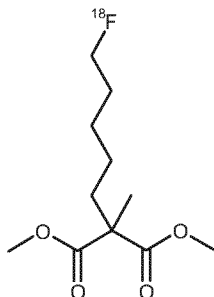
Preparation :

To [^{18}F]PMPA (Compound **9**) final formulated product (100 μl) was added boron trifluoride
 35 dimethanol complex (400 μl).
 Solution stirred at 100°C for 10mins.
 Quenched reaction with water (400 μl). Figure 9 shows multiple peaks.

Hot Standard Synthesis**Example 12**2-(5-[¹⁸F]Fluoro-pentyl)-2-methyl-malonic acid **12****12**

[¹⁸F]Fluoride (2514 MBq) was immobilized on a preconditioned QMA (Waters) cartridge (preconditioned by washing the cartridge with 5ml 0.5M K₂CO₃ and 10 ml water), The [¹⁸F]fluoride was eluted using a solution of 5mg K₂₂₂ in 0,95ml ACN / 1mg K₂CO₃ in 50μl water. This solution was dried at 120°C with stirring under vacuum, with a stream of nitrogen. Additional acetonitrile (1 ml) was added and the drying step was repeated. A solution of 2-methyl-2-[5-(toluene-4-sulfonyloxy)-pentyl]-malonic acid di-tert-butyl ester (2mg) in acetonitrile (300 μl) was added and heated at 120°C for 15 min. To the reaction mixture was added 300μl 6M HCl and the reaction heated at 120°C for 10min. The reaction mixture was diluted with 4ml 20% acetonitrile in water + 0.1% TFA and subjected to purification by semi-prep HPLC (ACE 5μ C18 250 × 10mm, Solvent A: water + 0.1% TFA; Solvent B: acetonitrile + 0.1% TFA; Gradient: 20% B to 90% B in 22 mins; Flow: 4 mL/min). The product peak was collected and diluted with 25ml water and passed through a C18 Plus Environmental (preconditioned with 10ml ethanol and with 10ml water). The SPE was washed with 5ml water and was eluted with 1.5 ml ethanol to give the desired product **12**, 418 MBq (51% d.c.).

Figure 10 shows the HPLC chromatogram of the purified compound **12**.

Derivatization hot radiopharmaceutical**Example 13:****13**

Derivatization 1 (25% aqueous solution):

To the ethanolic solution of compound **12** final (150µl) was added water (50 µL).

To this solution was added HBF₄ (20µl).

Stir the solution vigorously.

Add trimethylsilyldiazomethane solution (3 x 400µl) dropwise (Care: exothermic reaction)

5 Stir for 2 min at RT.

Quench reaction with acetic acid (20µl). HPLC showed >99% conversion

Concentrate to remove diethyl ether under a gentle N₂ stream at RT for 5 min.

Add 10µL aliquot to 50µl methanol. HPLC analysis showed >99% conversion.

10 Figures 11 shows the HPLC chromatograms of the derivatized compound **13** with 25% aqueous solution.

Derivatization 2 (50% aqueous solution):

To the ethanolic solution of compound **12** final (100µl) was added water (100 µL).

To this solution was added HBF₄ (20µl).

15 Stir the solution vigorously.

Add trimethylsilyldiazomethane solution (3 x 400µl) dropwise (Care: exothermic reaction)

Stir for 2 min at RT.

Quench reaction with acetic acid (20µl). HPLC showed >99% conversion

Concentrate to remove diethyl ether under a gentle N₂ stream at RT for 5 min.

20 Add 10µL aliquot to 50µl methanol. HPLC analysis showed >99% conversion.

Derivatization 3 (75% aqueous solution):

To the ethanolic solution of compound **12** final (50µl) was added water (150 µL).

To this solution was added HBF₄ (20µl).

25 Stir the solution vigorously.

Add trimethylsilyldiazomethane solution (3 x 400µl) dropwise (Care: exothermic reaction)

Stir for 2 min at RT.

Quench reaction with acetic acid (20µl). HPLC showed >99% conversion

Concentrate to remove diethyl ether under a gentle N₂ stream at RT for 5 min.

30 Add 10µL aliquot to 50µl methanol. HPLC analysis showed >99% conversion.

Derivatization 4 (90% aqueous solution):

To the ethanolic solution of compound **12** final (20µl) was added water (180 µL).

To this solution was added HBF₄ (20µl).

35 Stir the solution vigorously.

Add trimethylsilyldiazomethane solution (3 x 400µl) dropwise (Care: exothermic reaction)

Stir for 2 min at RT.

Quench reaction with acetic acid (20µl). HPLC showed >99% conversion

Concentrate to remove diethyl ether under a gentle N₂ stream at RT for 5 min.

Add 10µL aliquot to 50µl methanol. HPLC analysis showed >99% conversion.

Figures 12 shows the HPLC chromatograms of the derivatized compound 13 with 90% aqueous solutions.

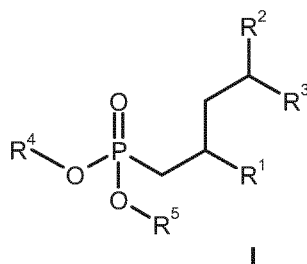
5 Column: ACE 3µ C18 50 x 4,6 mm
 Mobile Phase: A: H₂O + 0.1%TFA, B: MeCN + 0.1%TFA
 Gradient: 00:00 05%B
 07:00 95%B
 07:10 100%B
10 08:80 100%B
 09:00 05%B
 12:00 05%B
 Flow: 2 ml/min
 Detection: γ-detection
15

Claims:

1. A method for the derivatization of radiopharmaceuticals comprising one or more carboxylic acid groups and/or one or more phosphonic acid groups and being soluble in an aqueous solution for obtaining derivatized radiopharmaceuticals.

2. The method according to claim 1 comprising the step of alkylation of at least one carboxylic acid groups and/or phosphonic acid groups of a radiopharmaceutical wherein alkylation is obtained by reacting radiopharmaceutical according to claim 1 with an unsubstituted or substituted diazoalkane in presence of an acid, preferably selected from the group of strong acid, Lewis acid and Brønsted acid.

3. The method according to claims 1 and 2 wherein independently from each other radiopharmaceutical is a radiopharmaceutical of the formula (I)



wherein

R^1 is $C(=O)OR^6$;

R^2 is $C(=O)OR^7$;

R^3 is ^{19}F or ^{18}F ;

R^4 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R^5 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

R^6 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

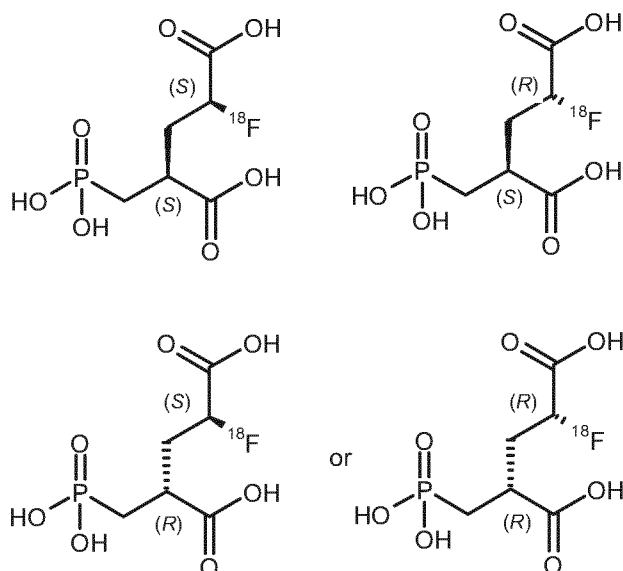
R^7 is hydrogen, Methyl, Ethyl, Propyl, Butyl, t-Butyl or Benzyl;

with the proviso that at least one of R^4 , R^5 , R^6 , and R^7 is hydrogen;

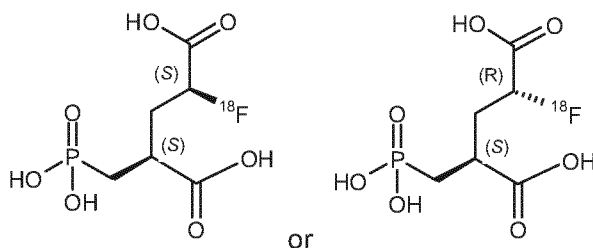
and stereoisomers, stereoisomeric mixtures, and suitable salts thereof and

unsubstituted or substituted diazoalkane is diazomethane, diazoethane trimethylsilyldiazomethane or diazomethylbenzene.

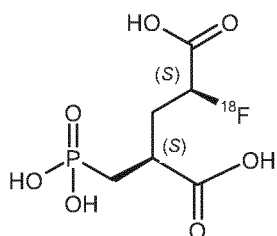
4. The method according to claims 1, 2 or 3 wherein the radiopharmaceutical is one of the following isomers



5. The method according to claim 4 wherein the radiopharmaceutical is one of the following isomers



6. The method according to claim 5 wherein the radiopharmaceutical is the isomer



7. The method according to the claims 1 to 6 comprises additionally the step of analytical characterization of the obtained derivatized radiopharmaceutical by suitable method such as HPLC, preferably using a HPLC column with a solid-phase.

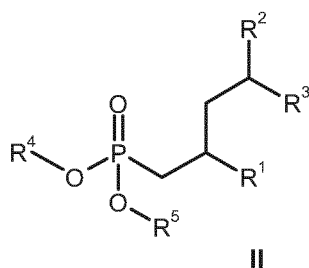
8. The method according to claim 7 for chiral separation, and determination of the ratio of stereoisomers of the radiopharmaceutical as described in all preceding claims.

9. The method according to claim 8 for determining the ratio of stereoisomers of the radiopharmaceutical as described in all preceding claims.

10. A derivatized radiopharmaceutical which is alkylated at one (1) or more carboxylic acid groups and/or one (1) or more phosphonic acid groups of a radiopharmaceutical as defined in claims 1 to 6 for subsequent analytical characterization of radiopharmaceutical as described in the preceding claims.

5

11. The derivatized radiopharmaceutical according to claim 10 of the formula (II)



wherein

R¹ is C(=O)OR⁶ ;

10 R² is C(=O)OR⁷;

R³ is ¹⁹F or ¹⁸F;

R⁴ is hydrogen, alkyl or aryl-alkyl;

R⁵ is hydrogen, alkyl or aryl-alkyl;

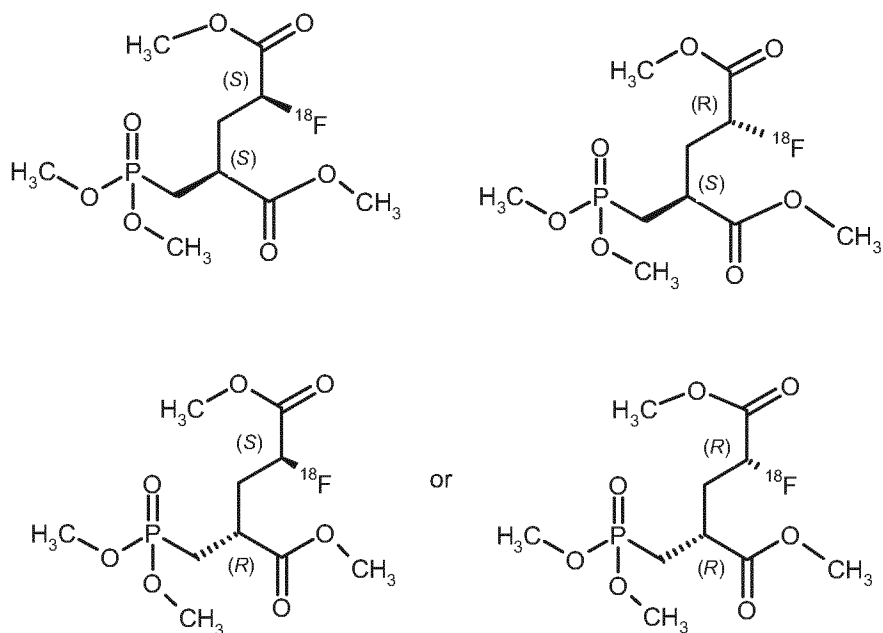
R⁶ is hydrogen, alkyl or aryl-alkyl;

15 R⁷ is hydrogen, alkyl or aryl-alkyl;

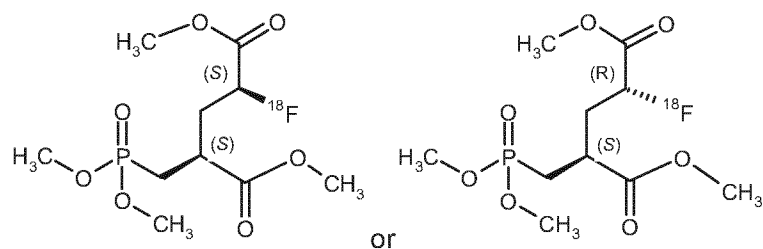
with the proviso that R⁴, R⁵, R⁶, and R⁷ are never simultaneously hydrogen;

and stereoisomers, stereoisomeric mixtures, and suitable salts thereof.

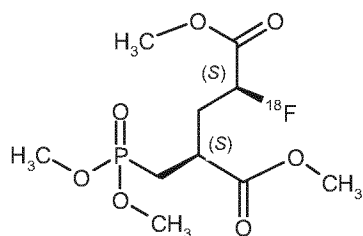
12. The derivatized radiopharmaceutical according to claim 11 that is one of the following
20 isomers



13. The derivatized radiopharmaceutical according to claim 12 that is one of the following isomers



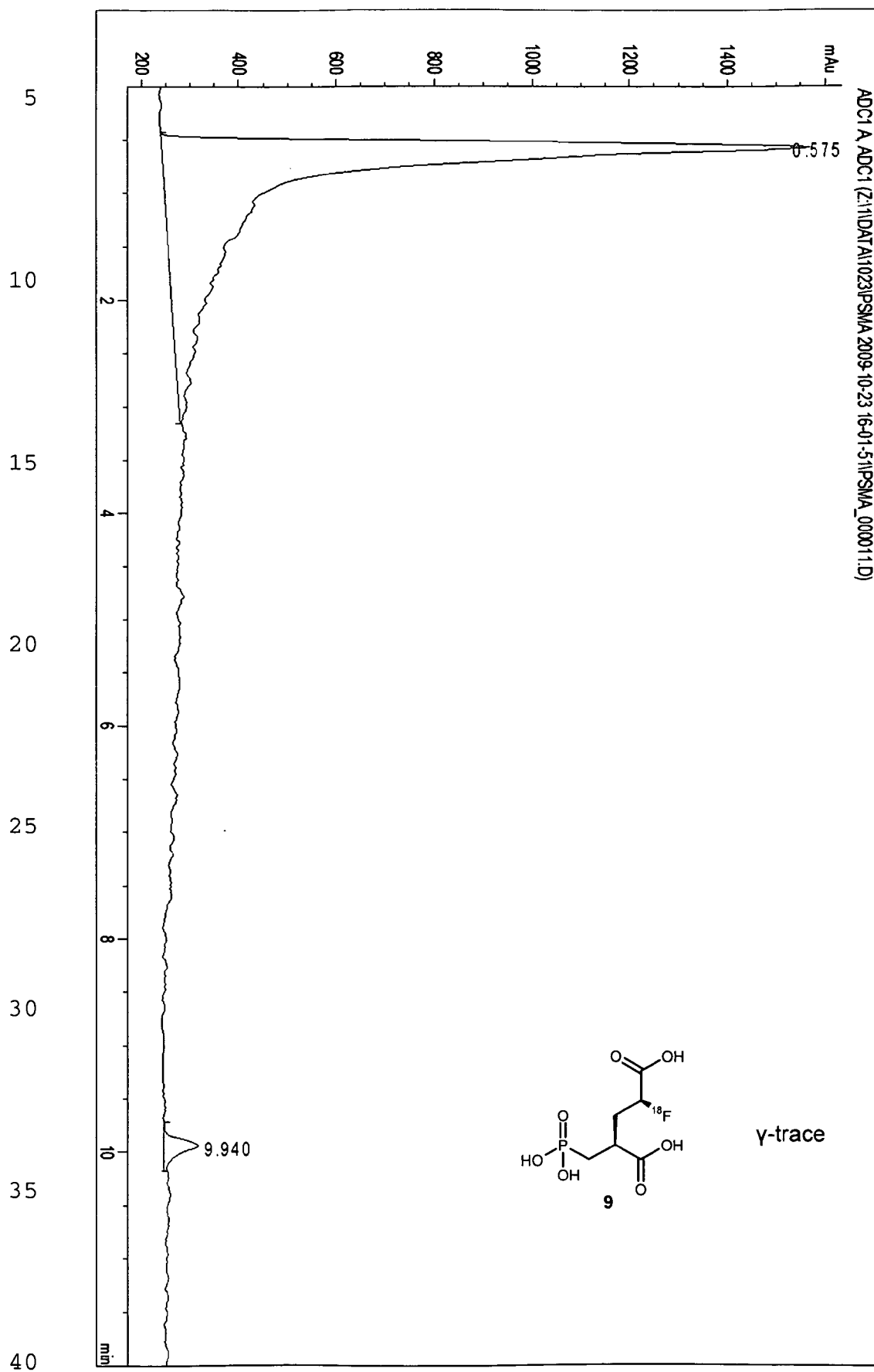
14. The derivatized radiopharmaceutical according to claim 13



15. A kit comprising at least one sealed vial comprising radiopharmaceutical as defined in claims 1 to 6.

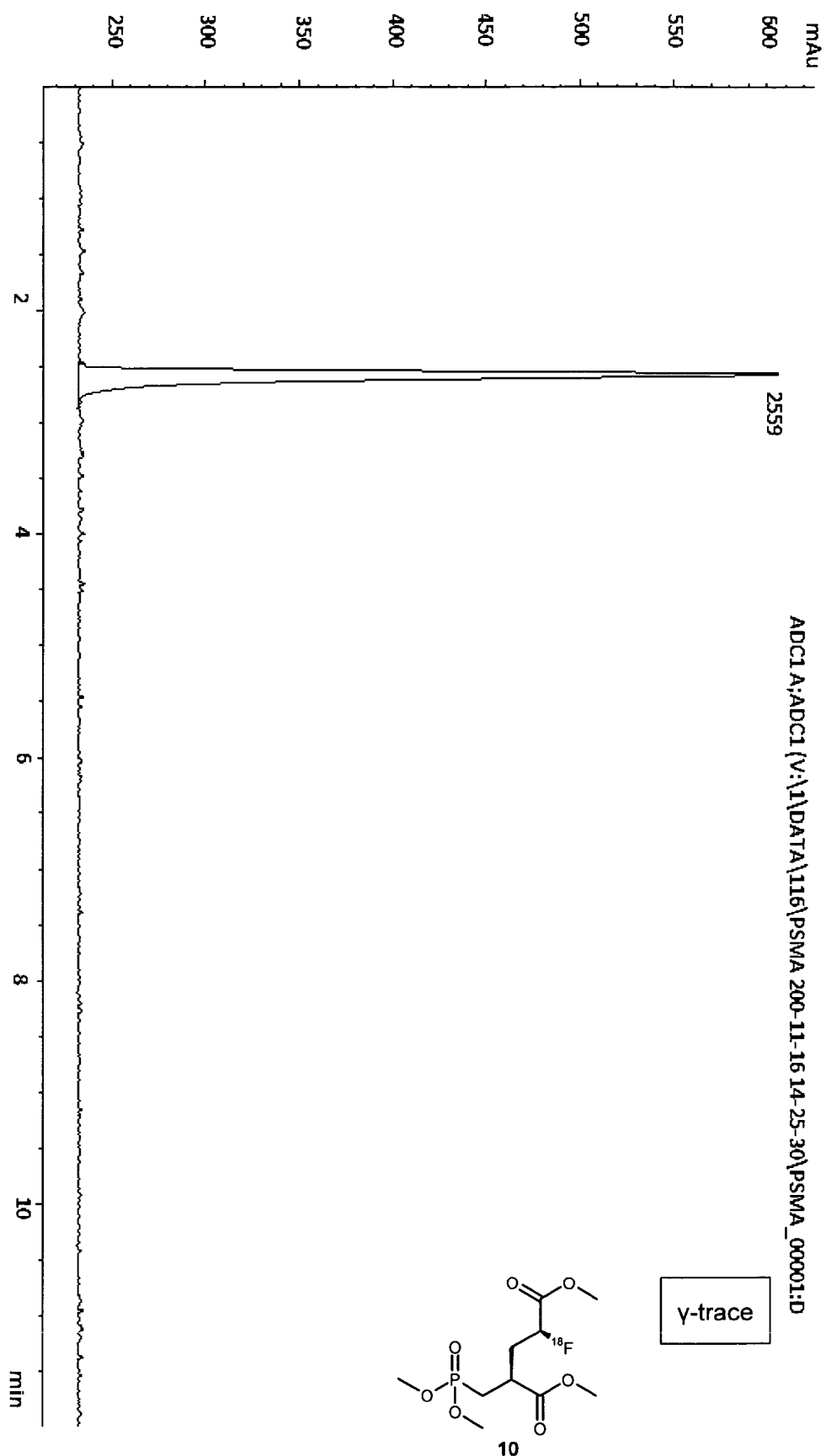
1/13

Figure 1:



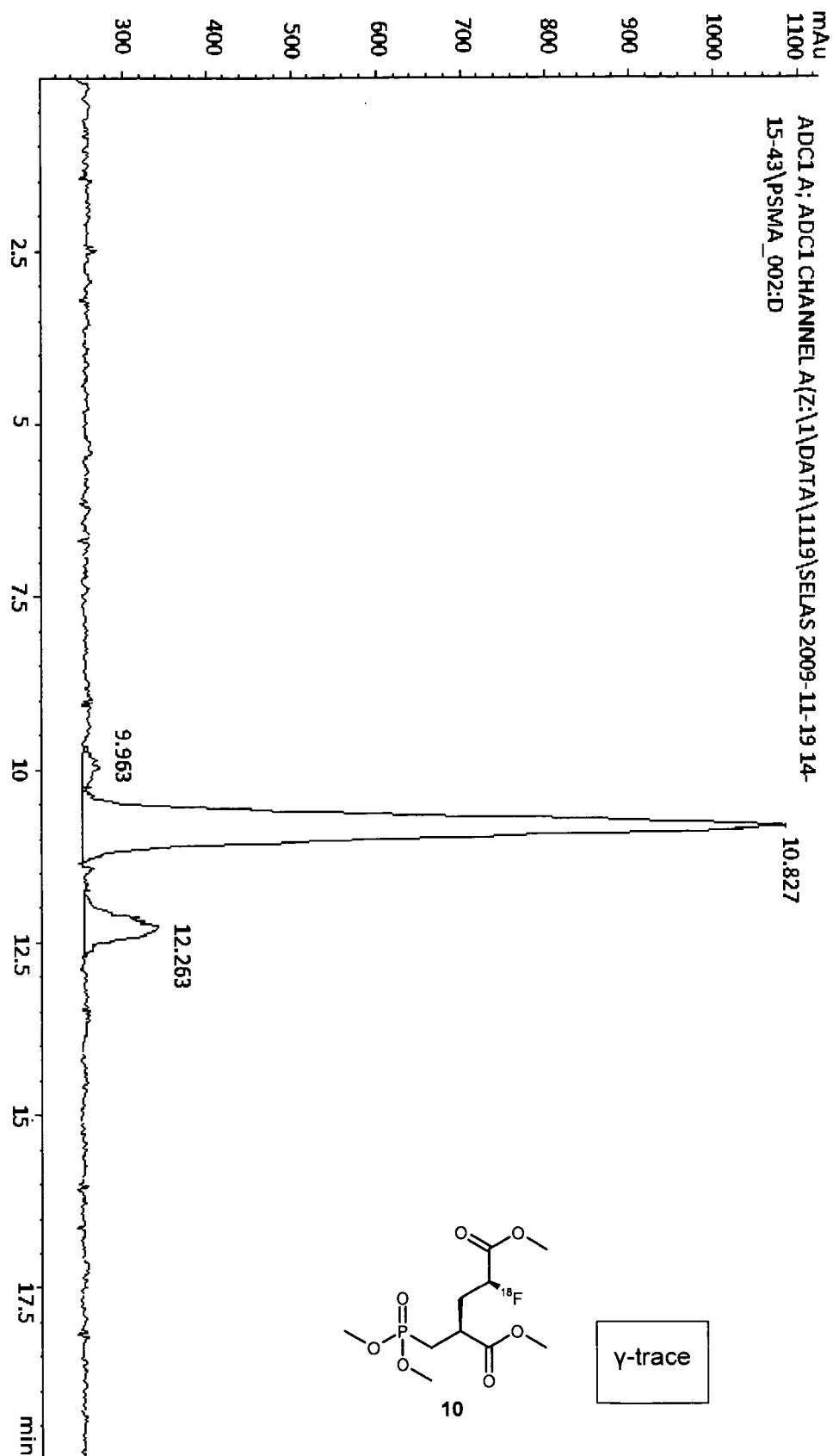
2/13

Figure 2:



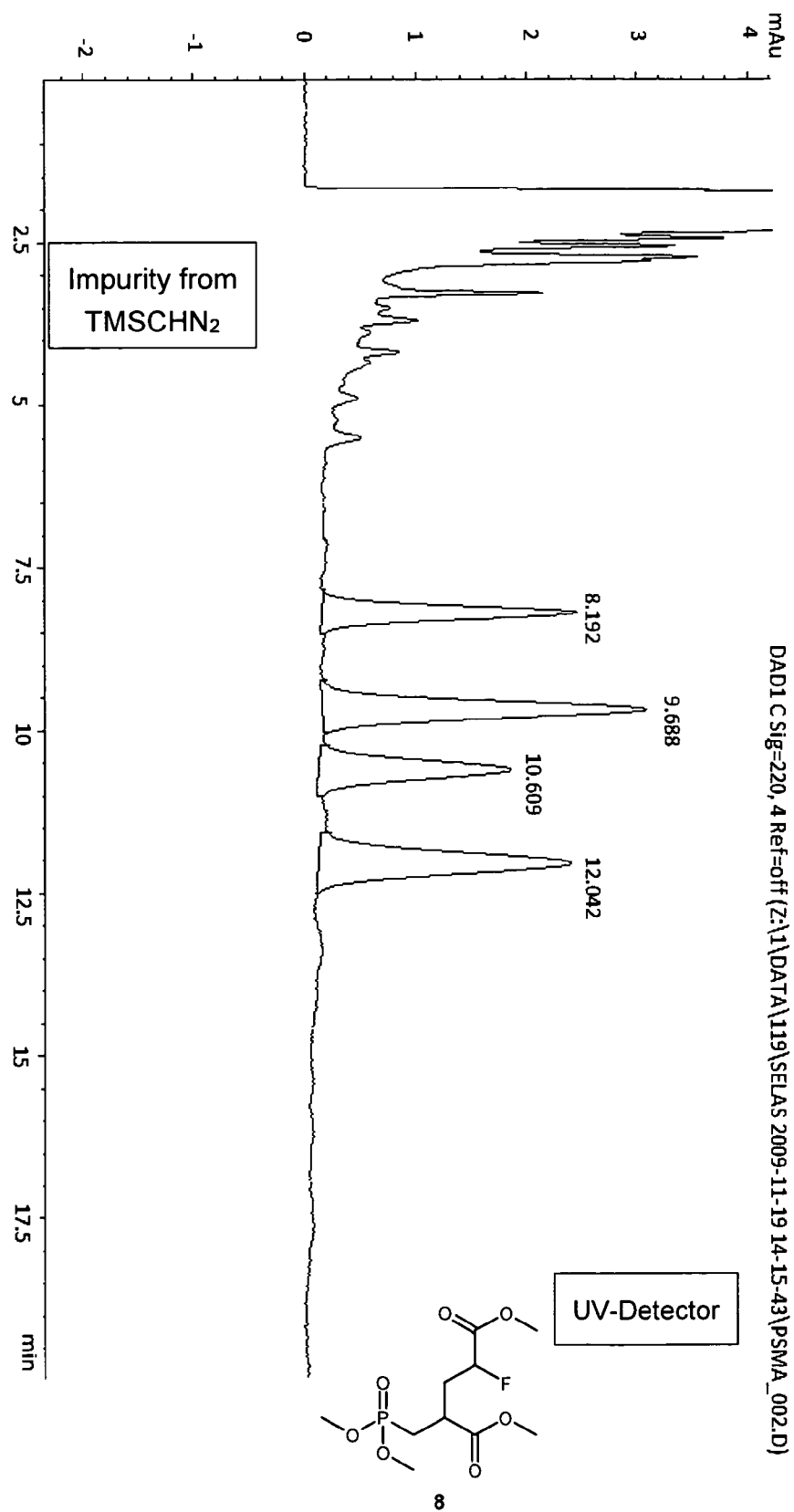
3/13

Figure 3:



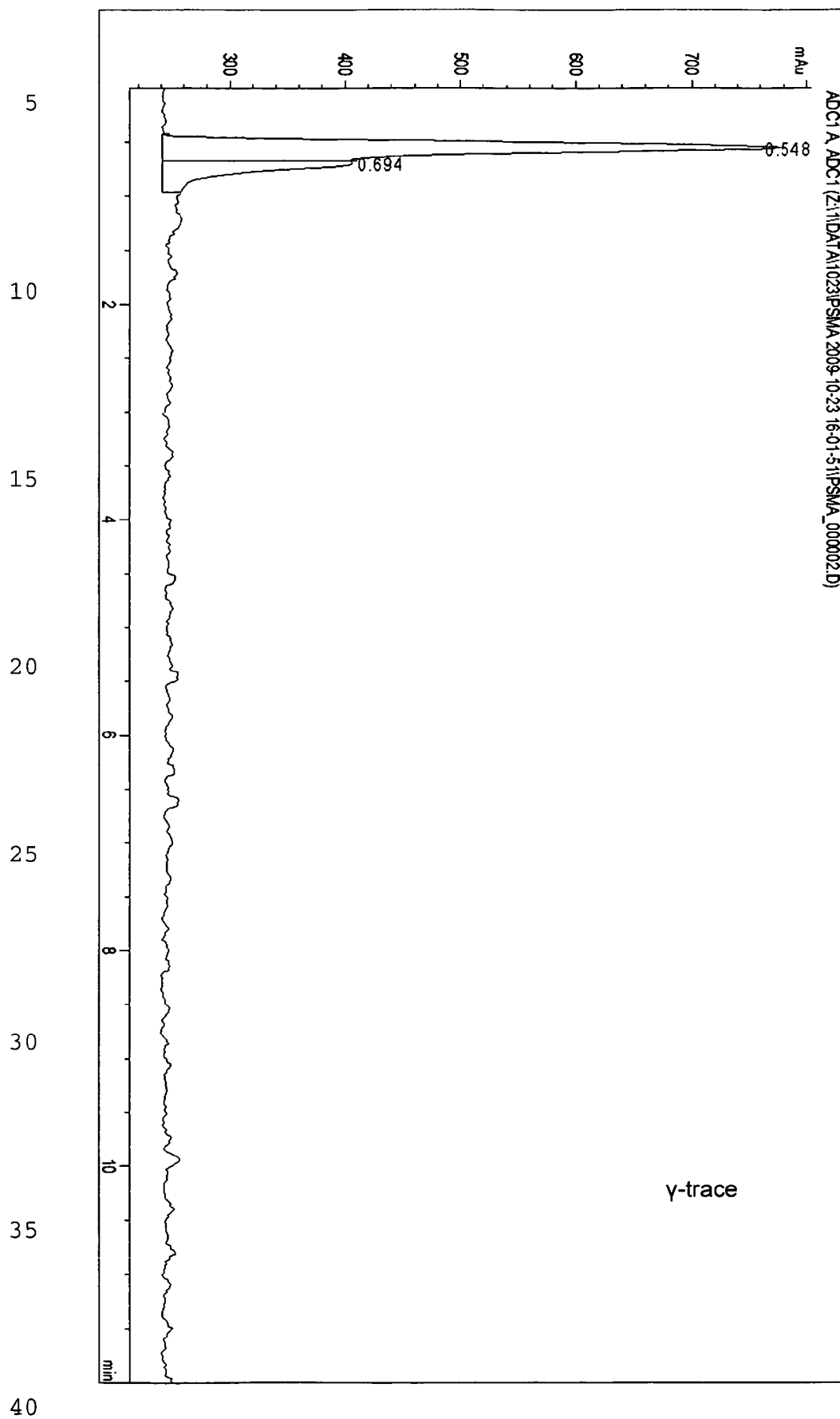
4/13

Figure 4 :



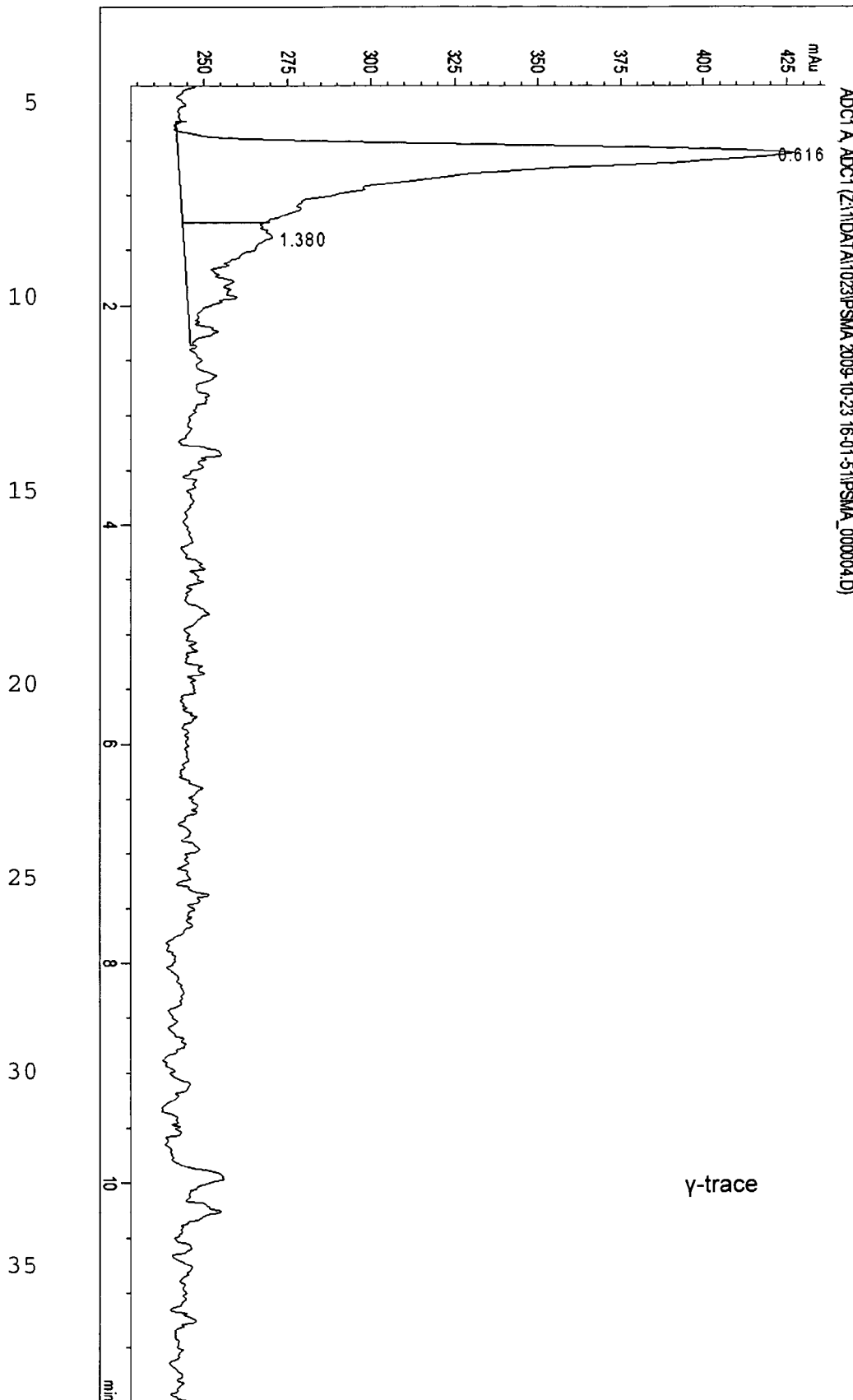
5/13

Figure 5:



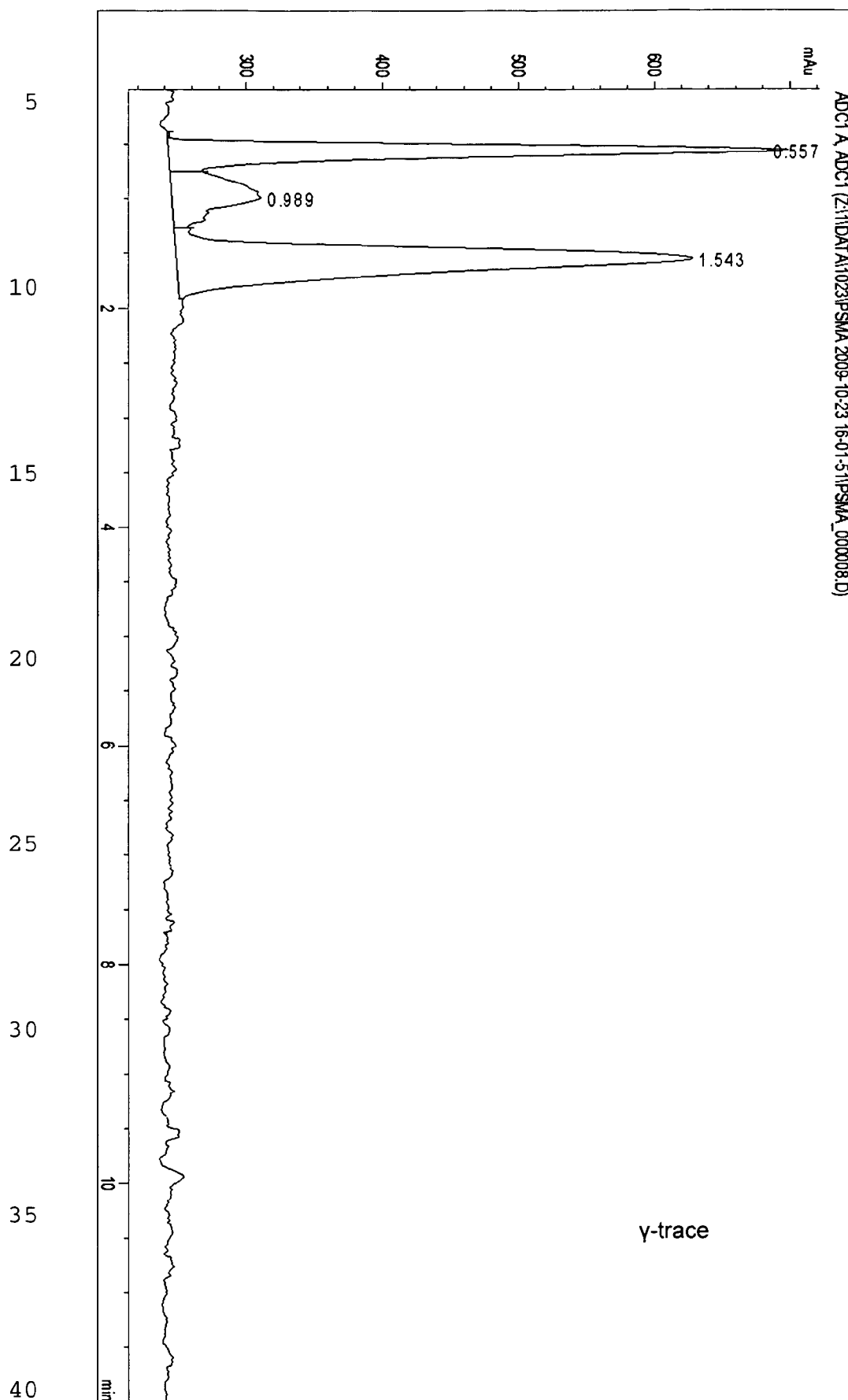
6/13

Figure 6:



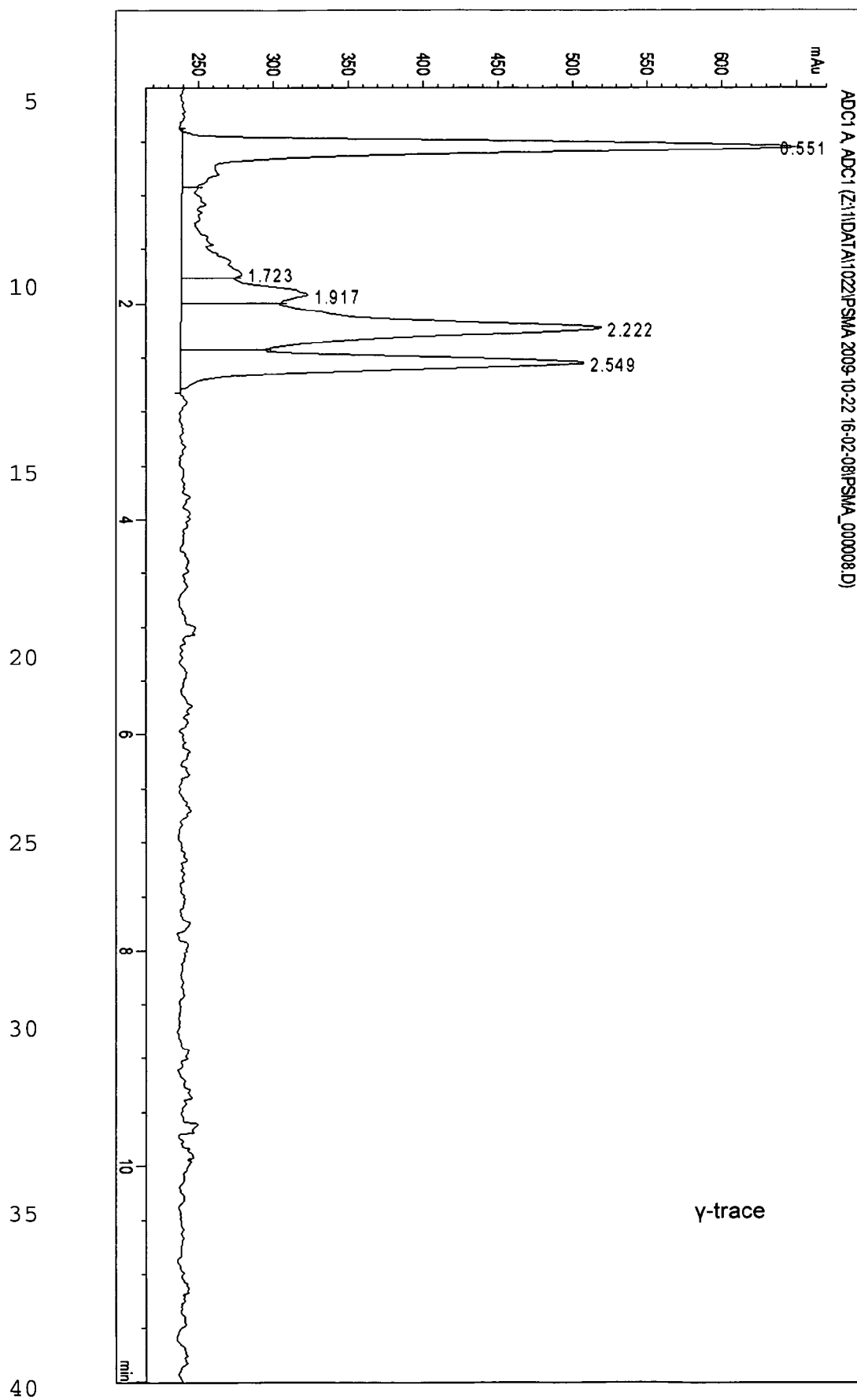
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Figure 7:



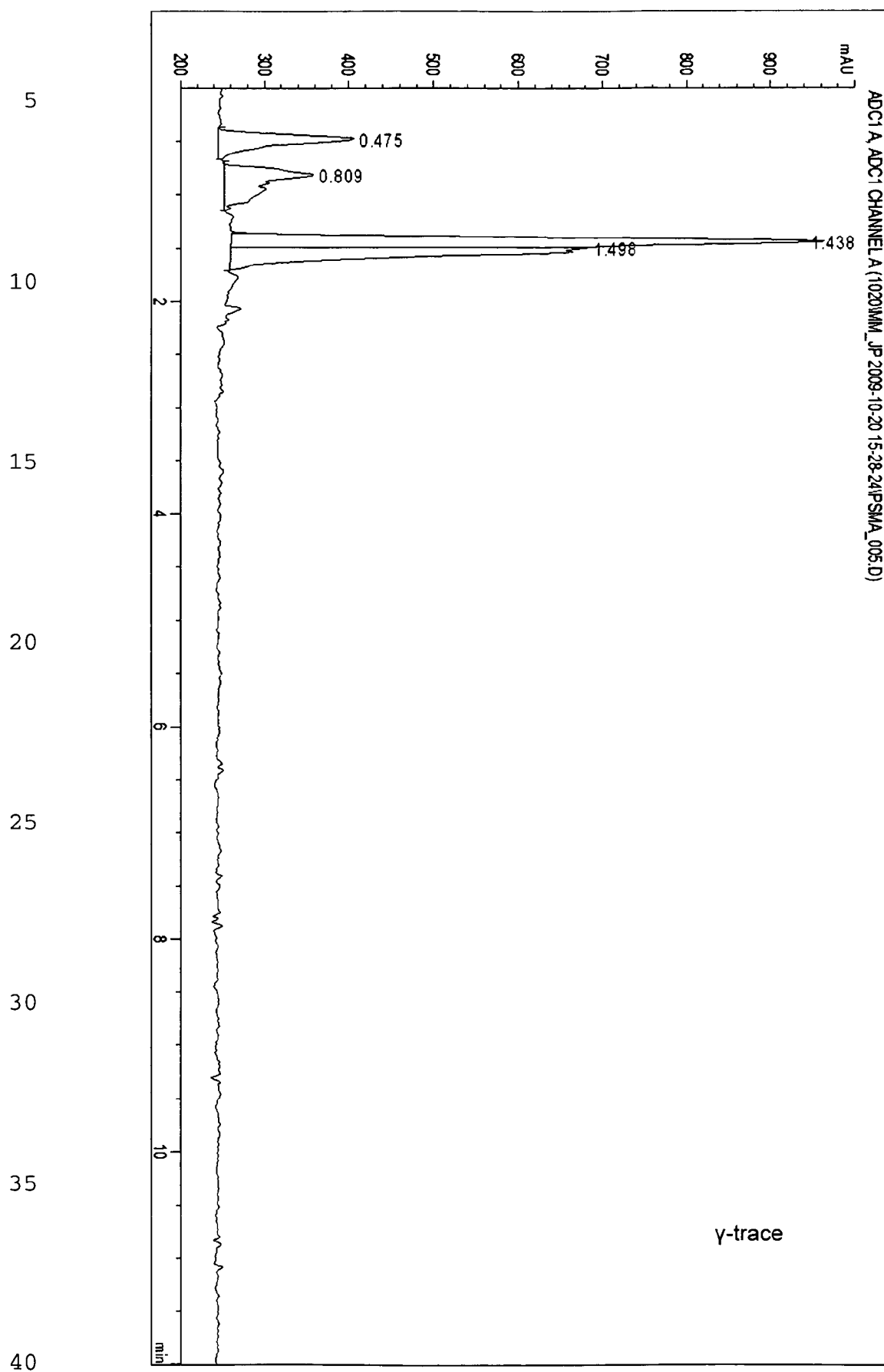
8/13

Figure 8:



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Figure 9:



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Figure 10

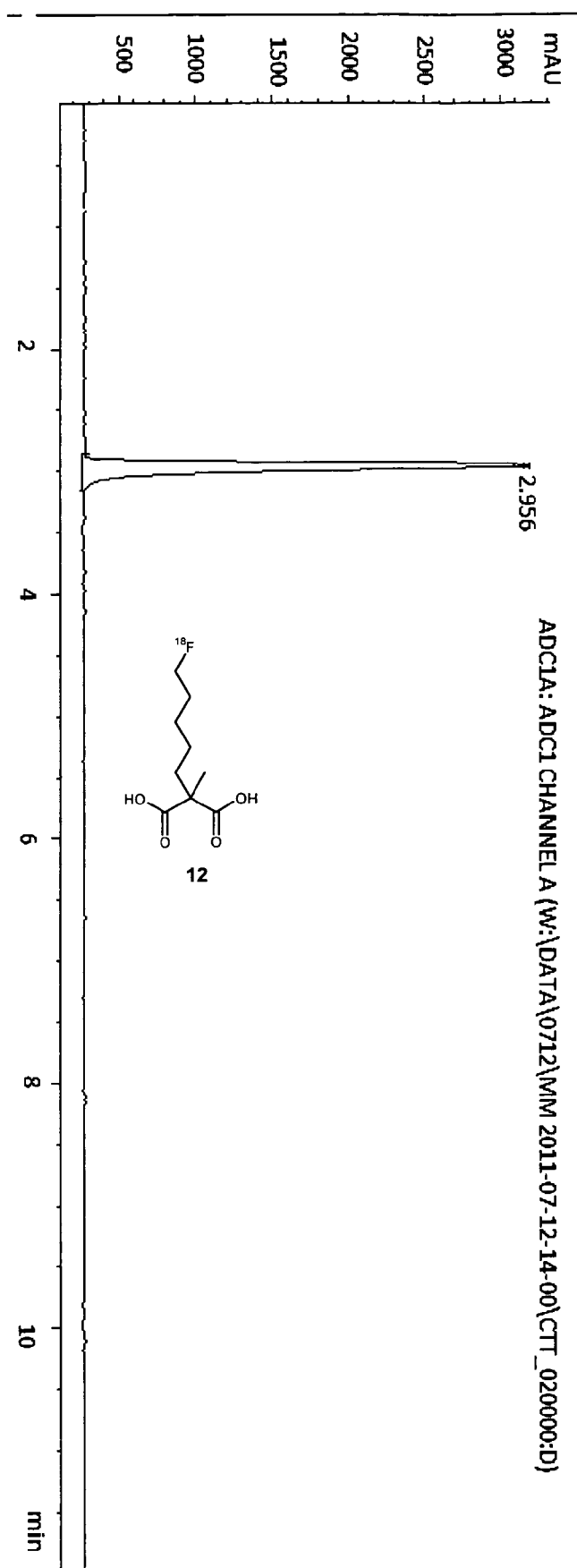
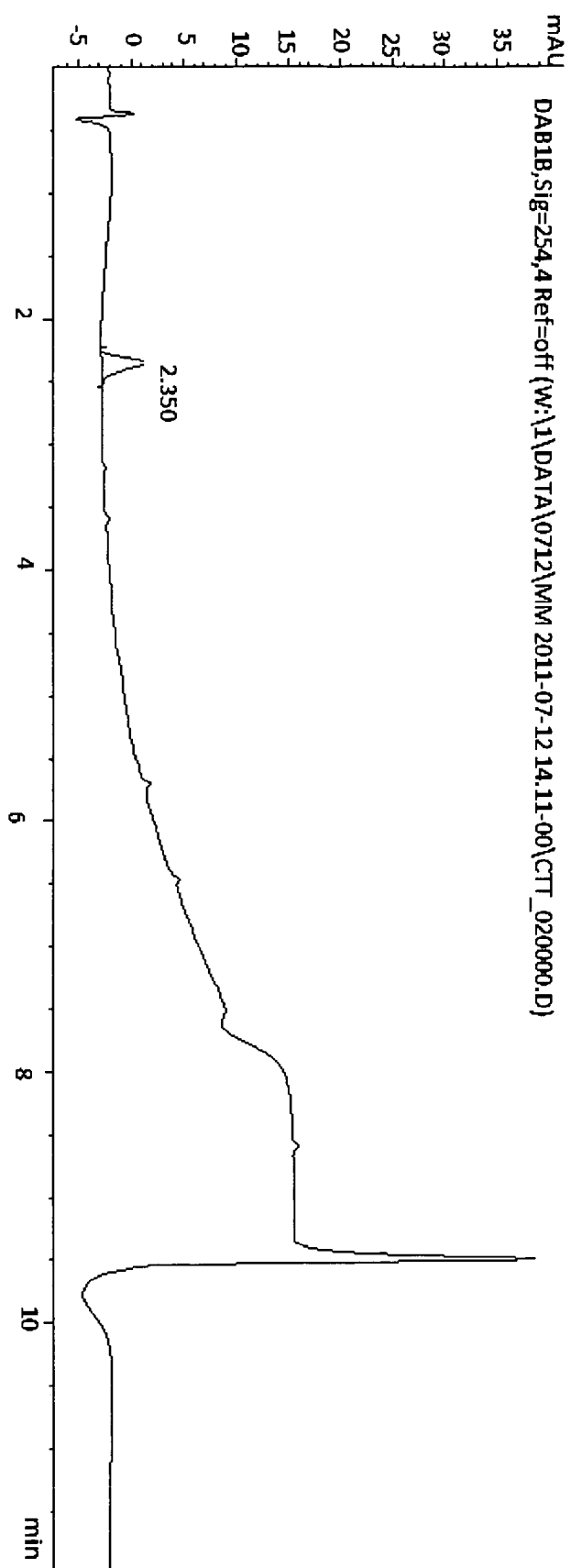


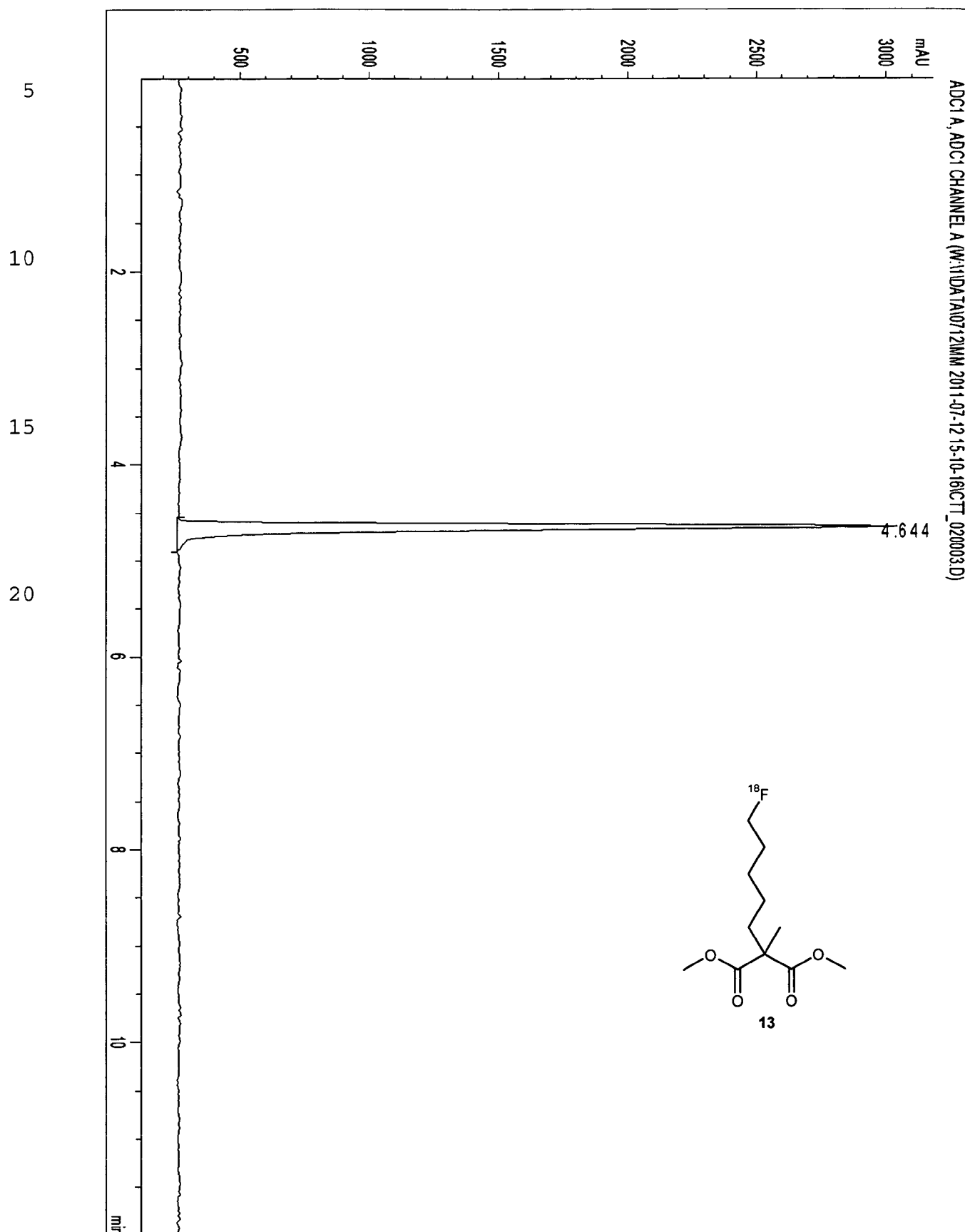
Figure 10 - continued

11/13



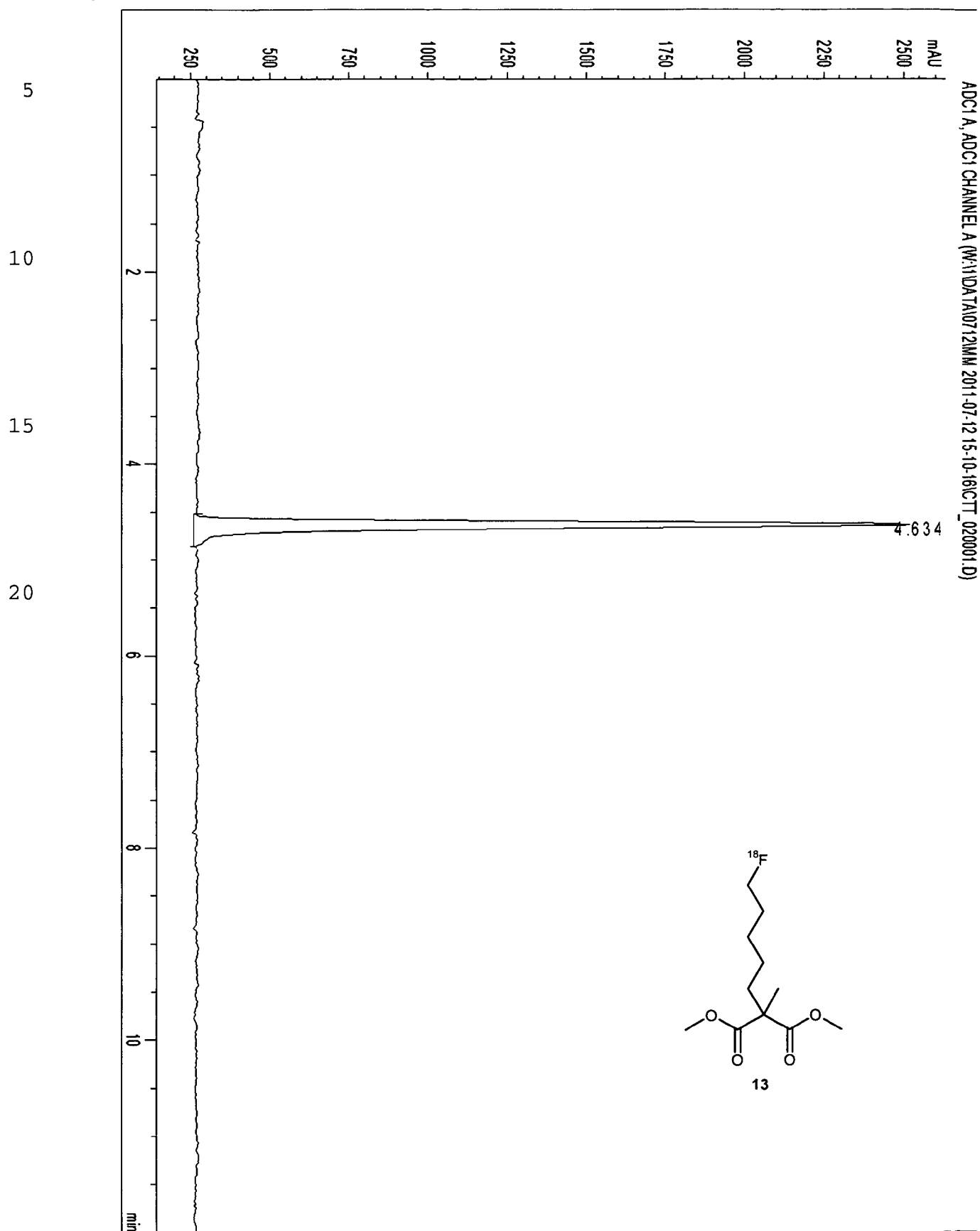
12/13

Figure 11:



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Figure 12:



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051368

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07F9/38 C07F9/40 C07B59/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLAUS O. MEESE ET AL: "Dimethyl 3,3,4,4,.5,.5,6,6-(2H8)-6-methoxycarbonyl-2-oxo-hexanephosphonate, a versatile reagent for the synthesis of deuterated [omega]-carboxy prostanoids", JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, vol. 23, no. 2, 1 February 1986 (1986-02-01), pages 175-185, XP55020551, ISSN: 0362-4803, DOI: 10.1002/jlcr.2580230208	1,2,10, 15
A	last paragraph - paragraph [0001] compounds 1a,8a ----- -/--	3-9, 11-14



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

1 March 2012

Date of mailing of the international search report

13/03/2012

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Authorized officer

Eberhard, Michael

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/051368

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	* scheme 2 * compounds 10, 11 page 308, paragraph 2	3-9, 11-14
X	----- DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1987, MIFTAKHOV, M. S. ET AL: "Prostanoids . XIX. Synthesis of C-14-labeled cloprostenol", XP002670490, retrieved from STN Database accession no. 109:37636 abstract & MIFTAKHOV, M. S. ET AL: "Prostanoids . XIX. Synthesis of C-14-labeled cloprostenol", ZHURNAL ORGANICHESKOI KHIMII , 23(6), 1185-90 CODEN: ZORKAE; ISSN: 0514-7492, 1987,	1,2,10, 15
X	----- EDWARD J. O'CONNOR ET AL: "Synthesis of (1, 2-13C2) 2-phosphoglycolic acid", JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, vol. 34, no. 8, 1 August 1994 (1994-08-01), pages 735-740, XP55020428, ISSN: 0362-4803, DOI: 10.1002/jlcr.2580340806 * scheme 1 *	1,10,15
X	----- SALLY FREEMAN ET AL: "Phosphonate biosynthesis: the stereochemical course of phosphoenolpyruvate phosphomutase", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 111, no. 26, 1 December 1989 (1989-12-01), pages 9233-9234, XP55020436, ISSN: 0002-7863, DOI: 10.1021/ja00208a018 compounds 3a,3b,4a,4b * scheme I * ----- -/--	1,10,15

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/051368

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIESEWETTER D O ET AL: "Enantiomeric radiochemical synthesis of R and S (1-(6-amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)methylphosphonic acid (FMPA)", JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, JOHN WILEY, CHICHESTER, GB, vol. 51, 13 March 2008 (2008-03-13), pages 187-194, XP002487665, ISSN: 0362-4803, DOI: 10.1002/JLCR.1505 [retrieved on 2008-03-13]	1,10,15
A	figure 4	2-9, 11-14
X	----- D BENDER: "Metabolites of 18F-FDG and 3-O-11C-Methylglucose in Pig Liver", JOURNAL OF NUCLEAR MEDICINE, vol. 42, no. 11, 1 January 2001 (2001-01-01), pages 1673-1678, XP55020457, figure 4 compounds 18FD-PG1, 18FD-PGL	1,10,15
A	----- DING P ET AL: "Design, synthesis and pharmacological activity of novel enantiomerically pure phosphonic acid-based NAALADase inhibitors", ORGANIC & BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 5, 1 January 2007 (2007-01-01), pages 826-831, XP002585048, ISSN: 1477-0520, DOI: 10.1039/B615603G [retrieved on 2007-01-19] table 1	1-15
X,P	----- EP 2 338 892 A1 (BAYER SCHERING PHARMA AG [DE]) 29 June 2011 (2011-06-29) paragraph [0233] - paragraph [0236] paragraph [0207] - paragraph [0232]	1,10-15
X,P	----- EP 2 322 514 A1 (BAYER SCHERING PHARMA AG [DE]) 18 May 2011 (2011-05-18) claim 5 paragraph [0155] - paragraph [0170]	1,10,15
X,P	----- JOHN T. WHITTECK ET AL: "On the Stereochemistry of 2-Hydroxyethylphosphonate Dioxygenase", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 133, no. 12, 30 March 2011 (2011-03-30), pages 4236-4239, XP55020548, ISSN: 0002-7863, DOI: 10.1021/ja1113326 column 2, paragraph 2	1,2,10, 15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/051368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
EP 2338892	A1	29-06-2011	EP 2338892 A1	29-06-2011
			WO 2011073286 A1	23-06-2011

EP 2322514	A1	18-05-2011	NONE	
