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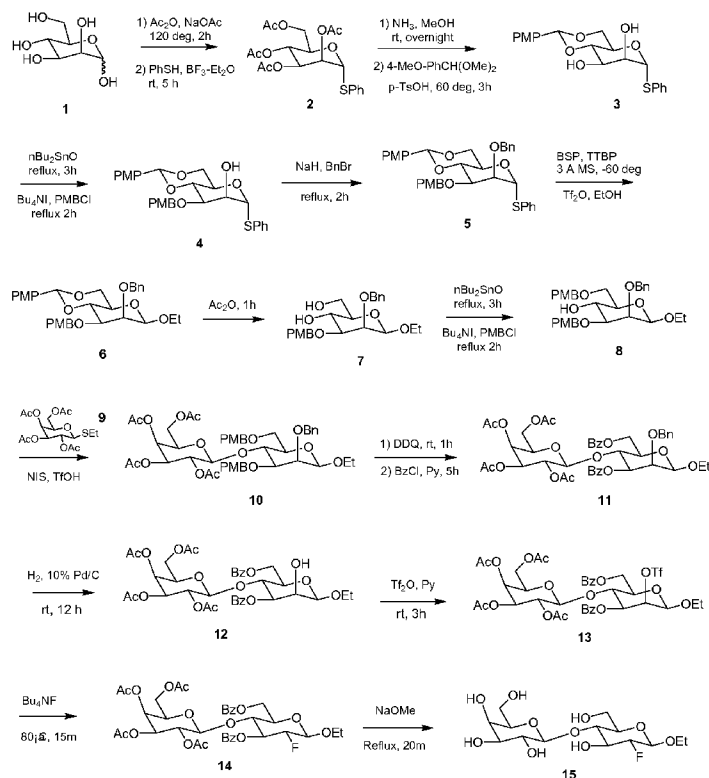
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(54) Title: RADIOHALOIMATINIBS AND METHODS OF THEIR SYNTHESIS AND USE IN PET IMAGING OF CANCERS



(57) Abstract: We disclose methods of synthesizing radiohalidated organic compounds and their use in positron emission tomography (PET) imaging of cancer cells.



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RADIOHALOIMATINIBS AND METHODS OF THEIR SYNTHESIS AND USE IN PET IMAGING OF CANCERS

5 BACKGROUND OF THE INVENTION

The present invention relates generally to the field of radiolabeled markers for positron emission tomography (PET) imaging. More particularly, it concerns methods of synthesizing various radiolabeled markers and methods for their use.

10 A large number of lactose-binding proteins are known, in both humans and other animals. Expression of particular lactose-binding proteins can vary in a number of cancers. For example, lectin is overexpressed in several types of tumors, including pancreatic carcinomas and colon carcinomas.

15 The ability to detect over- or underexpression of various lactose-binding proteins would be useful in detecting tumors and their extent and type.

SUMMARY OF THE INVENTION

20 One embodiment of the present invention relates to a method of synthesizing an alkyl 2'-radiohalodeoxylactose, comprising treating an alkyl 2'-triflate deoxylactose with n-Bu₄Nradiohalide, to yield the alkyl 2'-radiohalodeoxylactose.

25 One embodiment of the present invention relates to a method of positron emission tomography (PET) imaging of cancer cells in a mammal to detect the levels of expression or activity of a lactose-binding protein by the cancer cells, comprising administering to the mammal a composition containing an alkyl 2'-radiohalodeoxylactose, and imaging the mammal with PET.

BRIEF DESCRIPTION OF THE DRAWINGS

30 The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 shows the synthesis scheme for ethyl 2'-fluorodeoxylactose.

Figure 2 shows the synthesis scheme for ethyl 2'-[¹⁸F]fluorodeoxylactose.

Figure 3 shows a chromatogram of a protected ethyl 2'-fluorodeoxylactose precursor and an identically-protected ethyl 2'-[¹⁸F]fluorodeoxylactose precursor.

5 Figure 4 shows a chromatogram of ethyl 2'-[¹⁸F]fluorodeoxylactose.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

“Radiohalide,” as used herein, refers to any halogen isotope which decays by emitting
10 a positron. Examples of radiohalides include, but are not limited to, ¹⁸F, ¹²⁴I, ¹²⁵I, and ¹³¹I.

One embodiment of the present invention relates to a method of synthesizing an alkyl
2'-radiohalodeoxylactose, comprising treating an alkyl 2'-triflate deoxylactose with n-
Bu₄Nradiohalide, to yield the alkyl 2'-radiohalodeoxylactose.

An alkyl 2'-triflate deoxylactose is a deoxylactose having an -O(alkyl) group at the 1'
15 position and an -O(triflate) group at the 2' position. The alkyl 2'-triflate deoxylactose can
have further substituents, such as alkyl acid ethers or aryl acid ethers at other positions. The
alkyl 2'-triflate deoxylactose can be synthesized by a scheme such as that shown in Figure 1,
with routine variations in the steps, the reagents, and the reaction conditions shown therein
being a matter of routine experimentation for the ordinary skilled artisan.

20 The treatment of the alkyl 2'-triflate deoxylactose with n-Bu₄Nradiohalide can be
performed under any appropriate conditions. Anhydrous solvents, such as dry MeCN, are
most useful. The treatment can be performed at any appropriate temperature, such as from
about 50°C to about 120°C, for example, at about 80°C, for a duration sufficient to produce
the alkyl 2'-radiohalodeoxylactose in high yield, such as from about 5 min to about 60 min,
25 such as for about 15 min.

In one embodiment, the method further comprises (starting with an alkyl 3',6'-
dibenzoate deoxylactose, the synthesis of which can be performed by the technique described
in Example 1, below) triflation of an alkyl 3',6'-dibenzoate deoxylactose, to yield an alkyl 2'-
triflate, 3',6'-dibenzoate deoxylactose; using the alkyl 2'-triflate, 3',6'-dibenzoate
30 deoxylactose as the alkyl 2'-triflate deoxylactose in the treating step, to yield alkyl 2'-
radiohalo, 3',6'-dibenzoate deoxylactose; and deprotection of the alkyl 2'-radiohalo, 3',6'-
dibenzoate deoxylactose, to yield alkyl 2'-radiohalo deoxylactose.

Triflation can be performed by the use of ditrifyl oxide ($(CF_3SO_2)_2O$) in the presence of a suitable solvent, such as pyridine, under appropriate conditions of temperature (such as from about $0^\circ C$ to about $40^\circ C$, such as at about room temperature) and duration, to yield the alkyl 2'-triflate, 3',6'-dibenzoate deoxylactose.

5 The deprotection of the alkyl 2'-radiohalo, 3',6'-dibenzoate deoxylactose can be performed using any reactants and solvents known for use in replacing benzoate protecting groups with protons, such as NaOMe in MeOH.

10 In PET, a short-lived radioactive tracer isotope, such as ^{18}F (half life ≈ 110 min), which decays by emitting a positron, is chemically incorporated into a metabolically active molecule and injected into a living subject. Injection into blood circulation is the most common, but PET is not limited thereto. After injection, the metabolically active molecule becomes concentrated in tissues of interest that contain molecules, enzymes, or other structures which interact with the metabolically active molecule and the subject is placed in
15 the imaging scanner. Decay of the short-lived isotope emits a positron. After traveling a short distance (typically no more than a few millimeters) the positron annihilates with an electron, producing a pair of annihilation photons (similar to gamma rays) moving in opposite directions. These are detected when they reach a scintillator material in the scanning device, creating a burst of light which is detected by photomultiplier tubes.

20 The most significant fraction of electron-positron decays result in two 511keV photons being emitted at almost 180 degrees to each other, allowing localization of their source along a straight line of coincidence. Using statistics collected from tens-of-thousands of coincidence events, a set of simultaneous equations for the activity of each parcel of tissue along many lines of coincidence can be solved, and thus a map of locations and
25 radioactivities in the body may be plotted. The resulting map shows the tissues in which the molecular probe has become concentrated, and can be interpreted by nuclear medicine physician or radiologist in the context of the patient's diagnosis or treatment plan.

30 One embodiment of the present invention relates to molecular probes analogous to anti-cancer drugs which target given proteins expressed by given alleles of various oncogenes, which probes can be used in PET to determine whether the given proteins are targeted by the molecular probe and hence whether the anti-cancer drug would be likely to be

efficacious against the cancer characterized by activity of the given allele of the particular oncogene.

The short half-lives of many radionuclides useful in PET make necessary the rapid incorporation of the radionuclides into metabolically active molecules.

5 One embodiment of the present invention relates to a method of positron emission tomography (PET) imaging of cancer cells in a mammal to detect the levels of expression or activity of a lactose-binding protein by the cancer cells, comprising administering to the mammal a composition containing an alkyl 2'-radiohalodeoxylactose, and imaging the mammal with PET.

10 In one embodiment, the lactose-binding protein is lectin.

In one embodiment, the cancer cells are pancreatic carcinoma cells.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in
15 the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the
20 invention.

Example 1

Lactose-binding proteins, like lectin, are overexpressed in several types of tumors, like pancreatic carcinomas, colon carcinomas. Radiolabeled lactose was synthesized as a
25 molecular probe for the PET imaging of lactose-binding protein expression in pancreatic carcinomas. Here we describe the improved methods of cold synthesis, radiolabeling and in vivo tissue distribution in rats.

Synthesis of ethyl 2'-fluorodeoxylactose

30 As shown in Figure 1, the synthesis of ethyl 2'-fluorodeoxylactose **15** was carried out as shown in Scheme starting from commercial available D-Mannose **1**. Treatment of D-mannose with acetic anhydride and sodium acetate at 100-120°C for 2 h gave pentaacetate of

D-mannose quantitatively. Further treatment of pentaacetate with thiophenol, followed by $\text{BF}_3 \cdot \text{Et}_2\text{O}$ yielded **2** in 94% yield. **2** was deprotected by treatment with 7 N NH_3 in MeOH overnight. The thus formed crude phenyl 1-thio- α -D-mannopyranoside was treated with 4-methoxybenzaldehyde dimethyl acetal, and trace amount of p-TsOH in DMF to give **3** in 65% yield. Selective protection of 3-OH in **3** was carried out by treatment **3** with Bu_2SnO , followed by PMBCl and Bu_4NI . **4** was formed in 78% yield. Further protection of **4** with NaH and BnBr in THF gave **5** quantitatively. **6** was subsequently obtained from **5** by activation with 1-benzenesulfinyl piperidine and triflic anhydride in the presence of 2,4,6-tri-butylpyrimidine in 70% yield. Treatment of **6** with glacial acetic acid gave diol **7** in 97% yield. Reaction of **7** with dibutyltin oxide in toluene at reflux, followed by treatment with PMBCl and Bu_4NI provided **8** in 80% yield. Glycosylation of **9**, which was prepared from β -D-galactose pentaacetate and ethanethiol in 80% yield, with **8** in the presence of N-iodosuccinimide and TfOH gave **10** in 89% yield. Selective removal of two PMB protection group in **10** using DDQ, followed by protection by treatment with BzCl and pyridine provided **11** in 70% yield. Hydrogenation over 10% Pd/C of **11** in EtOAc-Et₂O (1:2) yielded **12** in 94% yield. Further treatment of **12** with Tf₂O in the presence of pyridine at rt provided the precursor **13** in 69% isolation yield.

The precursor **13** was then converted to the corresponding fluoride **14** by treatment with Bu_4NF in CH_3CN at 80 °C for 10 min in a 10% yield after HPLC separation. The quantitative deprotection of **14** with NaOMe in MeOH completed the cold version synthesis of target compound **15**.

Radiolabeling to yield ethyl 2'-[¹⁸F]fluorodeoxylactose

As shown in Figure 2, radiofluorination was performed on the triflate precursor in dry MeCN using n- $\text{Bu}_4\text{N}^{18}\text{F}$ at 80 °C for 15 min. The reaction mixture was passed through silica gel Sep-Pack cartridge and eluted with EtOAc. Solvent was evaporated and the crude product was purified by HPLC to isolate ¹⁸F-labeled protected lactose in 65% MeCN in water. Solvent was partially evaporated and the product was passed through a reverse phase cartridge, and eluted with MeOH (2 mL) then hydrolyzed with NaOMe by refluxing for 5 min. The product was neutralized with HCl. MeOH was evaporated and re-dissolved in saline. The product was filtered through a Millipore filter for biological studies.

The radiochemical yields were 65-70% (d.c.) with an average of 68% in 3 runs. Radiochemical purity was > 99% with specific activity > 74 GBq/ μ mol at the end of synthesis. Synthesis time was 110-115 min from the end of bombardment.

5 *Chromatography*

Figure 3 shows HPLC chromatography results for coinjection of purified 16 with its cold version (14). Figure 4 shows a chromatogram of final compound 17.

10 All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More
15 specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by
5 reference.

1. Yun, M.; Chun, K. H.; Shin, J. N.; Oh, J. *Bull. Korean Chem. Soc.* **2002**, *23*, 177-178.

WHAT IS CLAIMED IS:

1. A method of synthesizing an alkyl 2'-radiohalodeoxylactose, comprising:
treating an alkyl 2'-triflate deoxylactose with n-Bu₄Nradiohalide, to yield the alkyl 2'-
5 radiohalodeoxylactose.
2. The method of claim 1, further comprising:
triflation of an alkyl 3',6'-dibenzoate deoxylactose, to yield an alkyl 2'-triflate, 3',6'-
dibenzoate deoxylactose, wherein the alkyl 2'-triflate, 3',6'-dibenzoate deoxylactose is the
10 alkyl 2'-triflate deoxylactose in the treating step and the treating step yields alkyl 2'-
radiohalo, 3',6'-dibenzoate deoxylactose; and
deprotection of the alkyl 2'-radiohalo, 3',6'-dibenzoate deoxylactose, to yield alkyl
2'-radiohalo deoxylactose.
- 15 3. A method of positron emission tomography (PET) imaging of cancer cells in a
mammal to detect the levels of expression or activity of a lactose-binding protein by the
cancer cells, comprising:
administering to the mammal a composition containing an alkyl 2'-
radiohalodeoxylactose, and
20 imaging the mammal with PET.
4. The method of claim 3, wherein the lactose-binding protein is lectin.
5. The method of claim 3, wherein the cancer cells are pancreatic carcinoma cells.

Figure 1

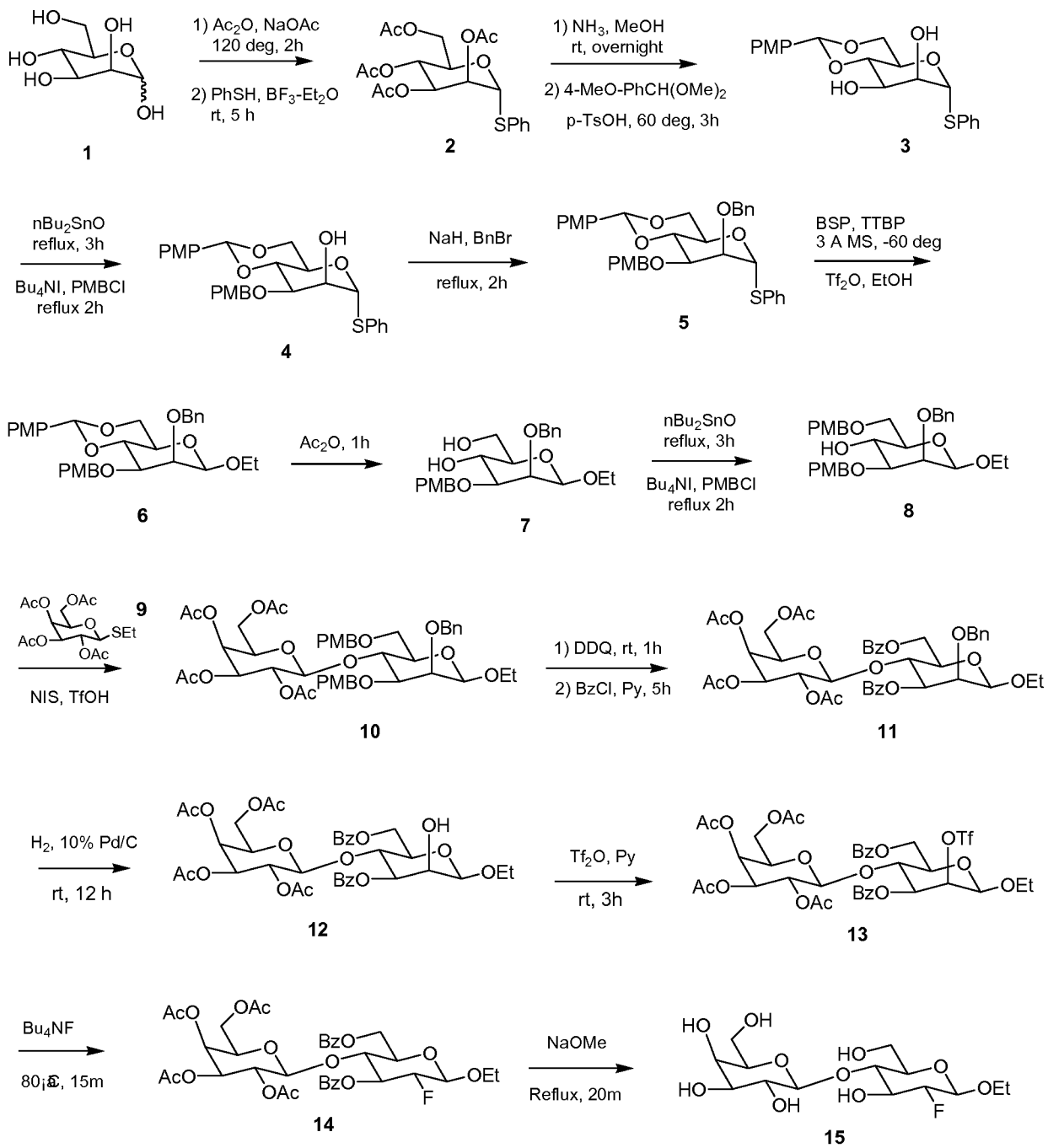
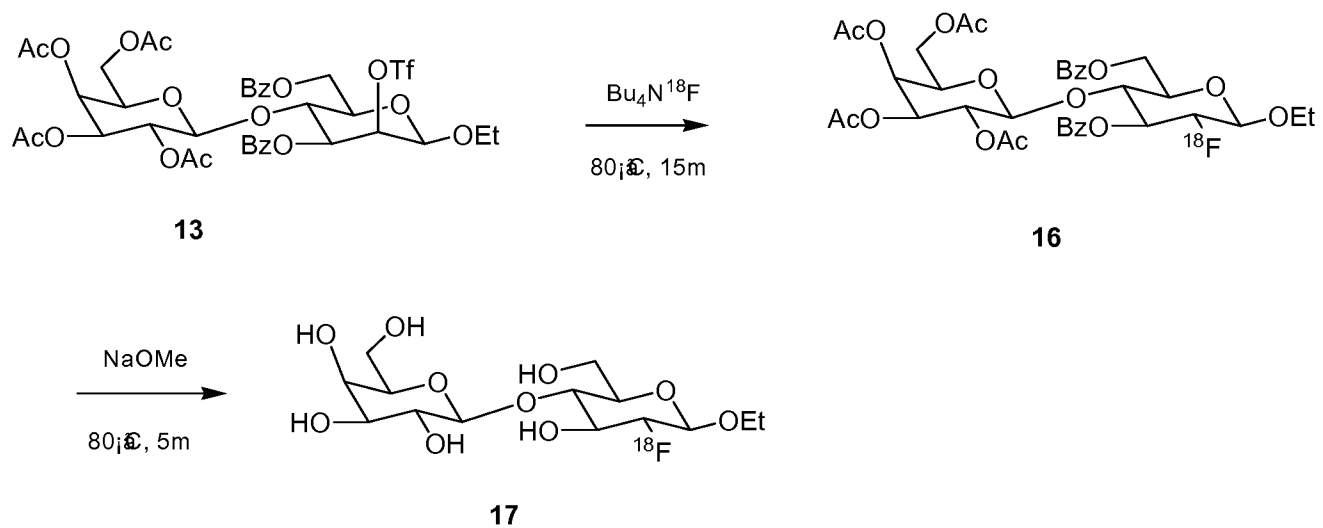
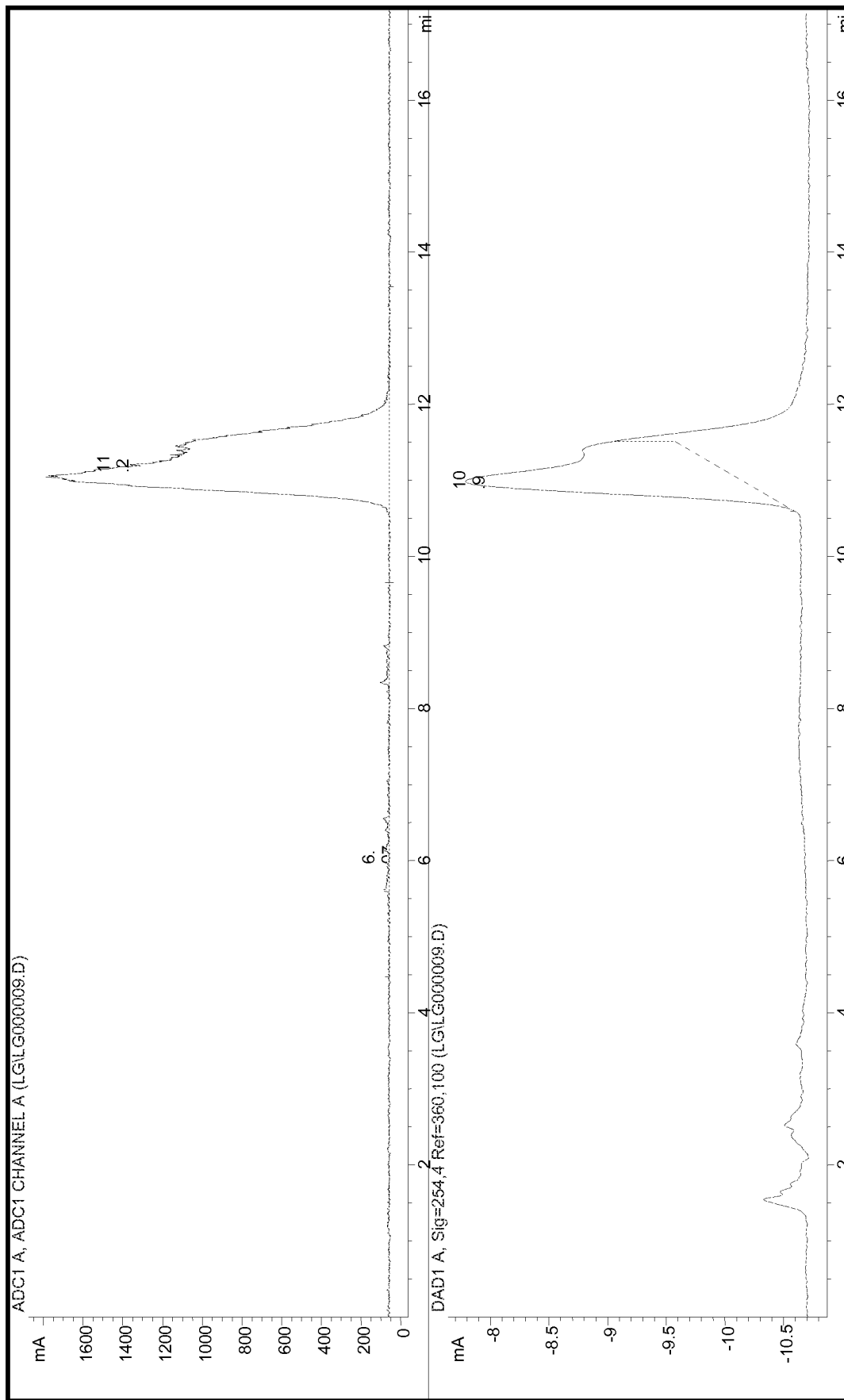


Figure 2



3

Figure 3



4

Figure 4

