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SYNTHESIS OF FLUORINE-18 LABELED GLUCOSE-Lys-Arg-Gly-Asp-D-Phe AS A POTENTIAL TUMOR IMAGING AGENT

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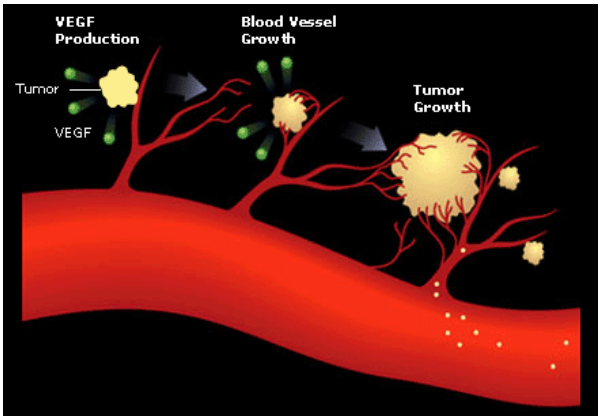
1. Introduction

The $\alpha_v\beta_3$ integrin is an important receptor affecting tumor growth, metastatic potential on proliferating endothelial cells as well as on tumor cells of various origin, tumor-induced angiogenesis could be blocked by antagonizing the $\alpha_v\beta_3$ integrin with RGD. Therefore, $\alpha_v\beta_3$ integrin is a target for angiogenesis imaging that might be useful in assessing tumor-induced angiogenesis and identifying tumor metastasis. To design potent radiotracer for imaging angiogenesis containing a cRGD moiety should include low hepatic uptake in vivo[1].



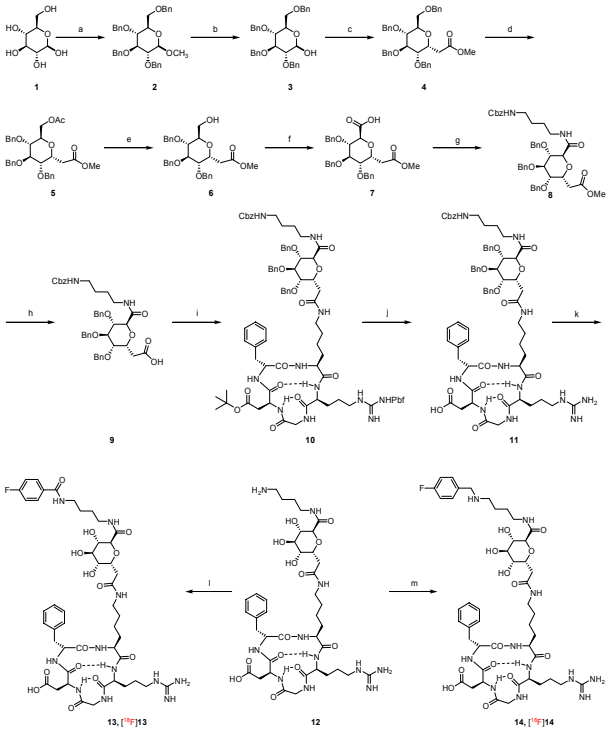
Tripeptide Arg-Gly-Asp (RGD), naturally existed in extracellular matrix proteins, is known to be the primary binding site of the $\alpha_v\beta_3$ integrin. The imaging of $\alpha_v\beta_3$ receptor expression will give the information of the metastatic ability of the tumor which is not available by [^{18}F]FDG.

Our interest in developing new radiopharmaceuticals for in vivo visualization of angiogenesis has led us to synthesize derivatives of cRGD (cyclic arginine-glycine-aspartic acid) that contains glucose moiety. Because sugar-protein interaction is a key step in metastasis and angiogenesis[2], it has also been proposed to play an intriguing role in imaging of tumor.

We designed and synthesized two fluorine-18 labeled RGD glycopeptides – *N*-fluorobenzyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzyl-glucose-KRGDf, **14**) and *N*-fluorobenzoyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzoyl-glucose-KRGDf, **13**) from same precursor as a diagnostic tumor imaging agent for positron emission tomography (PET). Fluorine-18 labeled cRGD glycopeptides were prepared using two different simple labeling methods: one is reductive alkylation of an amine with [^{18}F]fluorobenzaldehyde and the other is amide condensation with [^{18}F]fluorobenzoic acid.

2. Methods and Results

The precursor for **12** (G3) was prepared in 11 steps from D-glucose (**1**) as shown in scheme 1.



Scheme 1: a) i) AcCl, MeOH, rt, 12 h; ii) NaH, benzyl bromide, DMF, rt, 12 h, 60%; b) AcOH, 2 M trifluoromethanesulfonic acid, 80 ^{o}C, 12 h, 40%; c) NaH, trimethylphosphonoacetate, THF, rt-50 ^{o}C, 12 h, 25%; d) TMSOTf, acetic anhydride, CH_3CN, rt, 30 min, 90%; e) NaOMe, MeOH, rt, 30 min, 96%; f) Jones reagent, acetone, 30 ^{o}C, 1 h, 70%; g) *N*-(benzyloxycarbonyl)butane-1,4-diamine, TBTU, HOBT, DIEA, DMF, rt, 12 h, 62%; h) *aq*.0.4 N NaOH, MeOH,

50 °C, 4 h, 76%; i) cK(NH_2)RGDf, TBTU, HOBT, DIEA, DMF, rt, 12 h, 65%; j) TFA:H_2O: tributylsilane = 95:2.5:2.5, rt, 24 h, 89%; k) 10% Pd/C, AcOH:H_2O = 7:3, rt, 12 h, 36%; l) 2,5-dioxopyrrolidin-1-yl 4-fluorobenzoate, 0.1M sodium borate, rt, 30 min, 35%; m) 4-fluorobenzaldehyde, $NaBH_3CN$, AcOH, MeOH, rt, 12 h, 32%; **Labeling Method**; 2,5-dioxopyrrolidin-1-yl-[^{18}F]4-fluorobenzoate, 0.1M sodium borate, rt, 15min, 21%; **Labeling Method**; [^{18}F]4-fluorobenzaldehyde, $NaBH_3CN$, AcOH, MeOH, 85°C, rt, 40%.

Methyl 2-(2,3,4,6-tetra-o-benzyl-α-D-glucopyranosyl)acetate (**4**) was synthesized by the reaction of NaH and trimethylphosphonoacetate in THF at rt-50 °C for 12 h, isolated by column chromatography. But this step's isolation was very difficult because when reacted this step prepared 2 type isomers such as α and β form. After repeated several times isolation, obtained 25% yield pure compound. Also other steps were too hard for handling but each compounds obtained highly yield.

Radiochemical syntheses of *N*-fluorobenzoyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzoyl-glucose-cKRGDf) and *N*-fluorobenzyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzyl-glucose-cKRGDf). The preparation of fluorine-18 labeled *N*-fluorobenzoyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzoyl-glucose-cKRGDf (**13**)) with 2,5-dioxopyrrolidin-1-yl-[^{18}F]4-fluorobenzoate in 0.1M sodium borate at rt for 15 min and preparation of fluorine-18 labeled *N*-fluorobenzyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzyl-glucose-cKRGDf (**14**)) with [^{18}F]4-fluorobenzaldehyde, $NaBH_3CN$ and AcOH,in methyl alcohol at 85 °C for 20 min. The isolation of the labeled compounds was performed by HPLC using a semi-preparative column (Econosil C-18, 10 μ, 7.9 x 250 mm; 0 min - H2O : 0.1% TFA/ACN = 8:2, 30 min-4:6, 40 min - 0:10, 218 nm, 2 mL/min, *R*t =16.84 min, [^{18}F]**13** and *R*t = 14.15 min, [^{18}F]**14**).

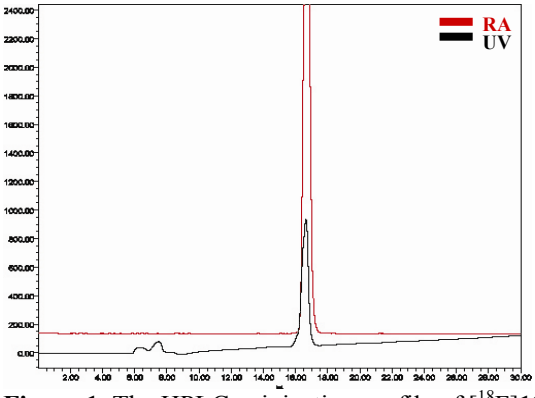


Figure 1. The HPLC coinjection profile of [^{18}F]**13** and cold authentic compound **13**

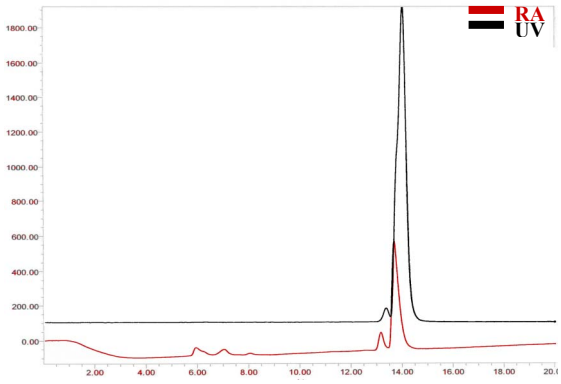


Figure 2. The HPLC coinjection profile of [^{18}F]**14** and cold authentic compound **14**

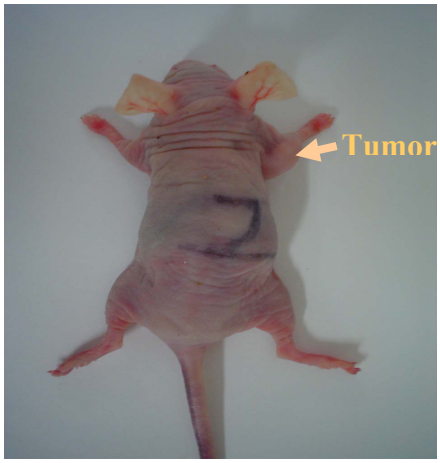


Figure 3. 9L tumor bearing nude mice (2 weeks)

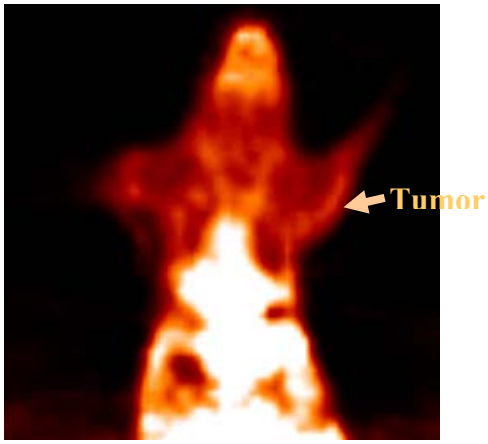


Figure 4. 300 mCi of **[^{18}F]14** tail vein Injection, 20 min uptake, and 20 min emission imaging
Image reconstruction with OSEM 2D method Using microPET R4 (Concode)

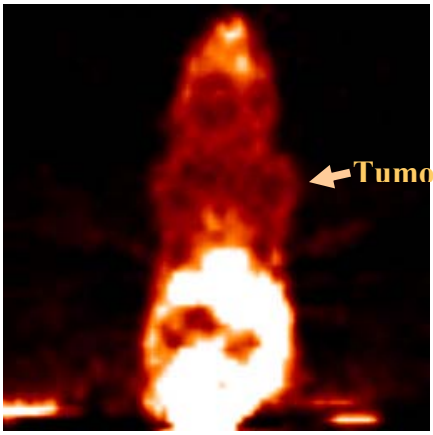


Figure 5. After injected cRGDyV I.P. injection (0.5 mg/head), 1 h Uptake, and 300 mCi of **[^{18}F]14** tail vein Injection

3. Conclusion

We designed and synthesized two fluorine-18 labeled cRGD glycopeptides–*N*-fluorobenzoyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzoyl-glucose-cKRGDf (**13**)) and *N*-fluorobenzyl-diaminobutane-*N'*-glucose-Lys-Arg-Gly-Asp-D-Phe ([^{18}F]fluorobenzyl-glucose-cKRGDf (**14**)) from the same precursor as diagnostic tumor imaging agents for positron emission tomography (PET). [^{18}F]**13** was prepared by amide condensation using 2,5-dioxopyrrolidin-1-yl-[^{18}F]4-fluorobenzoate in 21% radiochemical yield. [^{18}F]**14** was also prepared by reductive alkylation using 4-[^{18}F]fluorobenzaldehyde in 40% radiochemical yields. Then purified by HPLC at a flow rate of 2 mL/min (20-60% CH_3CN/0.1% TFA in H_2O, 30 min). The desired compounds eluted at 16.8 and 14.5 min were collected and matched with cold compounds. The radiolabeling conditions of the precursor are currently being optimized. Predominantly MicroPET studies of 9L tumor bearing mice with or without inhibition of unlabeled cRGDyV were performed.

REFERENCES

1. Haubner R, Wester H-J, Weber WA, Mang C, Zigler SI, Goodman SL, Senekowitsch-Schmidtke R, Kessler H, Schwaiger M. *Cancer Res* 2001;61:1781-1785.
2. Muramatsu T. *Glycobiology* 1993;3:291-296.