[^{11}C]octanoic acid as a potential labeling agent for Ghrelin

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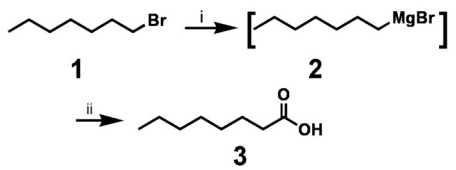
Abstract The recently discovered peptide Ghrelin, which is mainly expressed in the stomach, is a key regulator of food intake and energy balance. We developed a strategiy to radiolabel *pro*-Ghrelin by synthesizing [^{11}C]octanoic acid. For this, we developed efficient synthetic, analytical and formulation methods. Future experiments will focus on determining if Ghrelin can be radiolabeled *in vitro* or *in vivo* using [^{11}C]octanoic acid.

Introduction Obesity has become a more and more important issue in the health management of society, especially in the US. Recent research has linked an acylated 28 amino acid peptide called Ghrelin to various eating disorders, which often result in obesity.[i] Ghrelin was isolated from rat stomach and characterized as an endogenous ligand for the growth-hormone secretagogue receptor (GHS-R).[ii] Nakazato et al. showed the stimulating effect of Ghrelin in the pituitary, which induced growth-hormone (GH) secretion, food intake, body weight gain and inhibition of gastric emptying.[iii] In contrast to other peptides, Ghrelin is unique with regard to posttranslational acylation with ocatanoic acid.[iv] Research herein was focused on the preparation of [^{11}C]-octanoic acid as a potential precursor to [^{11}C]Ghrelin, which may provide insight into gut/brain communication though Positron Emission Tomography.

Results and Discussion Our approach to radiolabeling ghrelin focused on the potential for enzymes *in vivo* to append a carbon-11 labeled aliphatic acid on to the serine-3 residue of *pro*-Ghrelin. We surmised by administering [^{11}C]-octanoate to animals during a variety of dietary protocols, we might be able to elucidate whether the enzymes responsible for Ghrelin synthesis are activated at times coincident with Ghrelin release into the serum and the brain.[v] Our studies began with the synthesis of non-radioactive octanoic acid using CO_2. We synthesized **3** by Grignard preparation originated from 1-bromoheptane (Scheme 1).[vi] For this, iodine was used to activate the magnesium. Our attention at this point turned to developing rapid methods for analysis and purification of reaction mixtures. CO_2 (g) was bubbled through the Grignard solution and afforded the carboxylate as the product. With the crude reaction mixture in hand, we screened several separation methods including sodium salt precipitation and esterification followed by chromatography. We were able to develope a direct TLC method for octanoic acid, noteably uncommon for amphiphilic molecules. Visualization was accomplished by use of iodine staining followed or by an oxidation with potassium permanganate. With this robust analytical method, we proceeded to radiochemical synthesis. $^{11}CO_2$ (g) was passed through as solution of heptylmagnesium bromide under an inert atmosphere, Figure 2a. $^{11}CO_2$ (g) was trapped on molecular sieves and released into a cold trap that was

Scheme 1: Grignard and carboxylation reaction



Reagents and conditions: i) Mg, Et_2O, reflux, 1 *h ii*) CO_2, -10°C, 3 h

ultimately delivered to the reaction solution. We found that the crude reaction solution was quite pure radiochemically, (Figure 2b). In order to isolate **7**, we evaporated the solvent by heating under argon stream and then extracted into an aqueous buffer. While there were issues associated with the final formulation steps, (e.g. loss of product during sterile filtration) a sufficient yield was obtained for future animal studies.

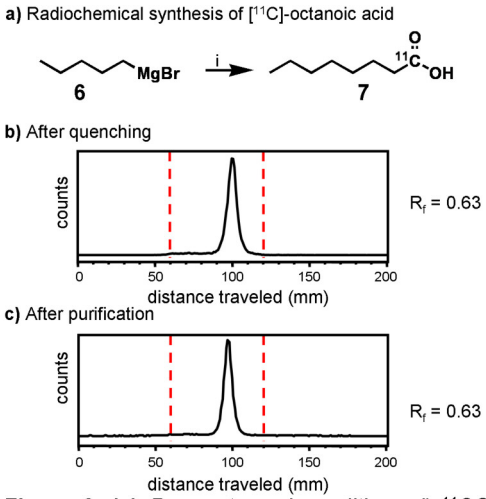


Figure 2. (a) *Reagents and conditions: i)* $^{11}CO_2$, -10°C, 5 min., under inert gas atmosphere. Radio-TLC-Analyses. Plates were pre-treated with 70% hex:30% EtOAc and 1% HCl in methanol. Staining in Iodide-chamber and Potassiumpermangante.TLC plates ran in the same solution Red lines represent the running distance of the compound from baseline to solvent front. **(b)** Spot of a crude reaction mixture after quenching with ether/HCl **(b)** Spot of the purified compound in bicarbonate-buffer/saline/2M NaOH

Conclusion The present results demonstrate the ability to make C-11 radiolabeled octanoic acid. The product is conveniently isolated for future PET imaging and studies on the gut brain communication path.

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