COMPOUND DETAILS NNC0403-0954

Long-acting GLP-1 with Cy3 for imaging

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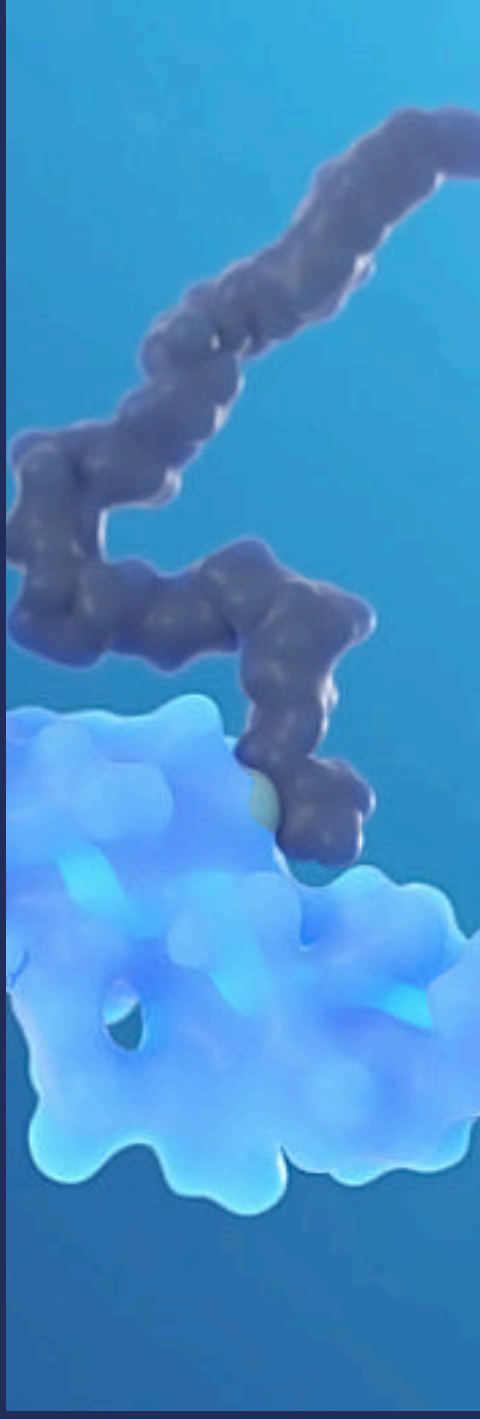
Content



Long-acting GLP-1 with Cy3 for imaging

NNC0403-0954 is a protracted GLP-1 analogue useful for imaging studies. NNC0403-0954 is a Cy3 modified version of NNC0113-0217 (also known as semaglutide). NNC0113-0217 is a GLP-1 analogue with 94% sequence homology to human GLP1 that binds and activates the GLP-1 receptor. Compared to native GLP-1, NNC0113-0217 have been designed to be long-acting *in vivo*. The main mechanism for the extended half-life is albumin binding. Furthermore, the NNC0113-0217 has a delayed uptake from the subcutis. Also, NNC0113-0217 is fully stable against dipeptidyl-peptidase 4 (DPP-4).

GLP-1 action is mediated via a specific interaction with GLP-1 receptors, leading to an increase in cyclic adenosine monophosphate (cAMP). GLP-1 stimulates insulin secretion in a glucose-dependent manner. Simultaneously, GLP-1 lowers inappropriately high glucagon secretion, also in a glucose-dependent manner. Thus, when blood glucose is high, insulin secretion is stimulated and glucagon secretion is inhibited. Conversely, during hypoglycaemia GLP-1 diminishes insulin secretion and does not impair glucagon secretion. GLP-1 is a physiological regulator of appetite and food intake and the GLP-1 receptor is widely expressed in the brain.

Category	GLP-1
ID	NNC0403-0954
Amount pr. vial	100 nmol

Calculated properties

Property	NNC0403-0954	GLP-1 (7-37)-OH (reference)
MW [Da]	4954.61	3355.67
pI	3.97	5.54
Sequence substitutions (compared to reference)	8Aib, 26K(C18diacid-gGlu-2xOEG),34R,37Cys(Cy3 maleimide)	

Selected calculated properties for NNC0403-0954 and GLP-1(7-37)-OH are listed in the table.

Structural information

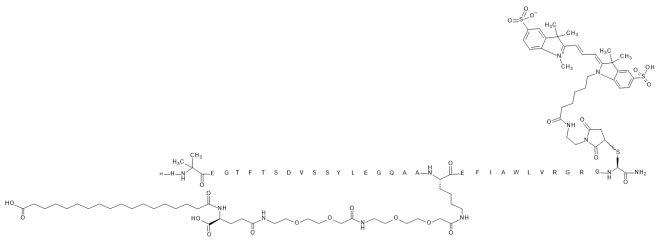


Figure 1

Figure 1

Figure 1. NNC0403-0954 has a C18 diacid chain which is attached via a glutamic acid linker plus a hydrophilic spacer (2xOEG) to lysine at position 26. In addition, lysine is replaced with arginine at position 34. The fatty acid side chain enables reversible binding to serum albumin in the blood stream, which increases the half-life of the molecule. Furthermore, alanine at position 8 is substituted with 2-aminoisobutyric acid (Aib) to increase stability against DPP-IV cleavage. For imaging studies the molecule has in addition a C-terminal cysteine which is attached to a Cy3 moiety via maleimide chemistry.

In vitro data

The *in vitro* potency data below are based on assays using cloned human GLP-1 receptors (hGLP-1R) co-expressed with a CRE-Luciferase reporter gene system in baby hamster kidney (BHK) cells. The assays were carried out essentially as described in the Lau et al. reference listed in the 'References' section using cells suspended in the absence or presence of human serum albumin. All data below are from the same experiments with NNC0403-0954 where NNC0113-0007 and NNC0113-0217 were used as internal reference compounds.

Since albumin binding is a key mechanism for the design of NNC0403-0954, the apparent affinity and potency will be very dependent on whether the assays contain albumin or not.;

Compound	hGLP-1R potency in the absence of HSA Mean EC_{50} (95% CI) [pM]	hGLP-1R potency in the presence of 1% HSA Mean EC_{50} (95% CI) [pM]
NNC0403-0954	90 (75 to 109)	1629 (1064 to 2493)
NNC0113-0217	3.0 (2.3 to 4.1)	110 (86 ro 139)
GLP-1 (7-37)-OH	3.1 (2.4 to 4.0)	1.1 (0.9 to 1.5)

CI: Confidence interval; hGLP-1R: human GLP-1 receptor; HSA: human serum albumin

In vivo data

Please see find the *in vivo;*data in the Gabery S et al. reference in the ‘References’ section in this table. Please note that NN403-0954 has a very minor modification compared to the compound in the paper but it is assumed that it does not affect the compound properties.

Reference Compound

Protracted GLP-1 analogue NNC0113-0217 (listed as ‘Long-acting GLP-1 analogue #2´ in the table with available compounds) is available as a reference compound to NNC0403-0954. Please indicate (with a check mark at ‘Please add the reference compound if available) during your compound request if you would like to have NNC0113-0217 included in your shipment.

Compound handling instructions

When handling Fluorophore labeled peptides in general, the hydrophobic nature most often will be shifted to more hydrophobic. Therefore, special attendance to handling is required, eg sterile filtration is not recommended without thorough evaluation of potential compound loss to filters. Peptides and proteins have a tendency to adhere to glass and plastic surfaces. This may at low concentration impact the actual amount in solution. To minimize this unspecific adherence, adding detergents or inert proteins like e.g., ovalbumin or other serum albumins to the solution can minimize this phenomenon. In case albumins are added to peptide/protein solutions, ensure that the albumins are free of any proteases, but be aware that it will affect the apparent potency and affinity in in vitro assays in case a fatty acid is attached to the compound. For in vitro studies, NNC0403-0145 can be dissolved in 80/20% DMSO/MilliQ water (e. g. at a concentration of 300 uM). NNC0403-0145 can be dosed in vivo in a formulation vehicle containing 50mM sodium phosphate, 70mM sodium chloride, (0.007% polysorbate 20 if concentrations are so low that adsorption to vials may affect the concentration), pH 8.0. Formulations should be used fresh, but can be stored for up to one week refrigerated. The compounds is at research grade purity.

References

1. Gabery et al.
Semaglutide lowers body weight in rodents via distributed neural pathways

JCI Insight, 5(6), 2020

2. Lau J et al.
Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide

J Med Chem, 2015, 58, 7370-7380