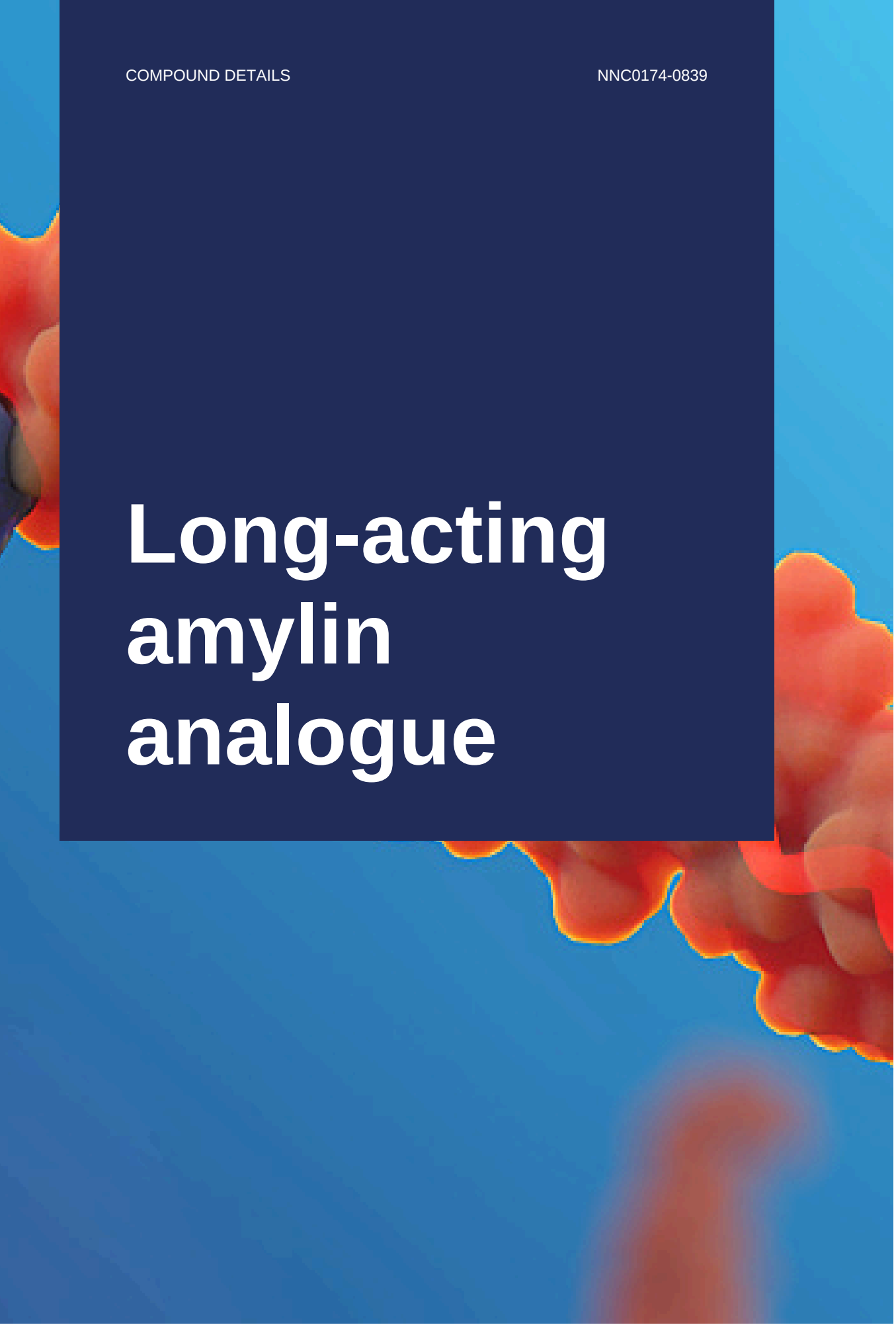
COMPOUND DETAILS NNC0174-0839

Long-acting amylin analogue



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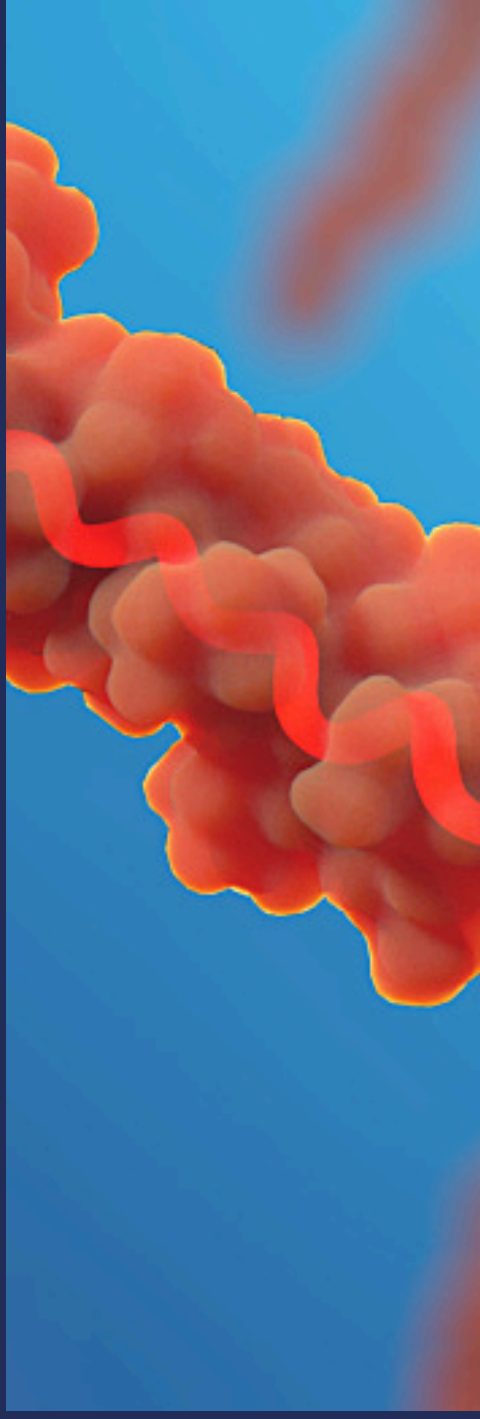
Content



Long-acting amylin analogue

Amylin is a 37-amino acid peptide that is stored in pancreatic beta cells and co-secreted with insulin. Amylin is an important regulator of energy metabolism and belongs to the calcitonin peptide family. Amylin acts via amylin receptors have mainly been identified in the hind brain. The amylin receptor consists of a calcitonin receptor (several splice variants) which heterodimerizes with one of three receptor activity-modifying protein (RAMP) splice variants thus generating many possible amylin receptor subtypes with different pharmacological properties.

NNC0174-0839 is a dual-acting amylin analogue with agonistic effects on the amylin receptor as well as the calcitonin receptor. NNC0174-0839 have been designed to be long-acting *in vivo*. The main mechanism for the extended half-life is albumin binding.

Category	Amylin
ID	NNC0174-0839
Amount pr. vial	1000 nmol

Calculated properties

Property	NNC0174-0839	Rat amylin (NNC0174-4010)
MW [Da]	4479.1	3920.4
pI	7.0	13.3
Fatty acid	C20 diacid	-
Linker	gGlu	-
Extinction coefficient 280 nm	1615	1615
Sum formula	C200 H310 N54 O59 S2	C167 H272 N52 O53 S2

Selected calculated properties for NNC0174-0839 and rat amylin (NNC0174-4010)

Structural information

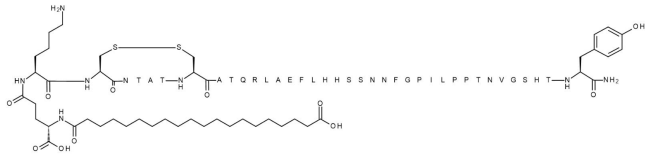


Figure 1

Figure 1

2D sketch of NNC0174-0839. NNC0174-0839 has a C20 fatty diacid side chain which is attached via a glutamic acid linker to the N-terminal. The fatty acid side chain enables reversible binding to serum albumin in the blood stream, which increases the half-life of the molecule.

In vitro data

The *in vitro* data for NNC0174-0839 in the table are based on cellular assays using cloned receptors in Baby Hamster Kidney (BHK) cells. A BHK cell line which was stably transfected with the human calcitonin (a) receptor (hCTR) and a cAMP responsive element (CRE) luciferase reporter gene. The cell line was further transfected with receptor modifying protein 3 (RAMP3) thus generating the human amylin 3(a) receptor (hAMYR3). The *in vitro* potency assays with hAMYR3 and hCTR were performed in the absence or presence of 1% (w/v) human serum albumin (HSA) in a buffer containing 0.1% ovalbumin. Since albumin binding is a key mechanism for the design of NNC0174-0839, be aware that the apparent affinity and potency will be very dependent on whether the i*n vitro* assays contain albumin or not.

	hAMYR3 potency (EC50, pM) Mean (95% confidence interval)	
Compound	**No HSA**	**1% HSA**
NNC0174-0839	6.6. (5.0 to 8.6)	522 (422 to 631)
Pramlintide	2.1 (1.4 to 3.2)	2.3 (1.8 to 3.0)
Salmon calcitonin	1.4 (1.0 to 1.9)	1.0 (0.8 to 1.2)
	hCTR potency (EC50, pM) Mean (95% confidence interval)	
NNC0174-0839	7.4 (5.4 to 9.7)	249 (199 to 311)
Pramlintide	28.3 (13.8 to 57.9)	32.9 (17.0 ot 63.7)
Salmon calcitonin	3.5 (1.5 to 8.4)	1.7 (0.6 to 4.9)

hAMYR3: human amylin receptor with RAMP3; hCTR: human calcitonin receptor; HSA: human serum albumin

In vitro potency data of NNC0174-0839, pramlintide and salmon calcitonin (unpublished data)

In vivo data

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of amylin can be assessed in various animal models; however, pigs are not a suitable model for PD studies.

Unlike other known amylin receptor agonists such as salmon calcitonin and pramlintide, NNC0174-0839 can bind to serum albumin and therefore displays a significantly longer half-life. In mini-pigs, NNC0174-0839 displays a terminal half-life (T1/2) of 110 h following a s.c. administration of a single dose of 10 nmol/kg.

The pharmacodynamic properties of amylin such as appetite reduction and reduction of body weight can be studied in rats. The effect on food intake (FI) in lean Sprague Dawley rats after s.c. administration of a single dose of NNC0174-0839 given prior to onset of the dark phase is shown in the table below. The reduction in accumulated food intake is reported relative to vehicle-treated rats within the same study.

Dose	FI reduction 0-24 h (%)	FI reduction 24-48 h (%)	FI reduction 0-48 h (%)
3 nmol/kg	49	25	37

Adverse effects

Amylin analogues can cause gastro-intestinal side effects such as nausea and feeling of malaise. Therefore, dose escalation should be introduced if doses higher than 10 nmol/kg is being investigated in rats. Doses can be escalated every third day.

NNC0174-0839 also induce acute hypocalcaemia in young rodents, especially in rats. This can potentially lead to serious side effects and even death, so stress should be avoided prior to dosing and Sprague Dawley rats from Janvier Labs should not be used for *in vivo* experiments since these are very sensitive to the hypocalcaemia-inducing properties of NNC0174-0839.

Reference Compound

Rat amylin (NNC0174-4010) is available as a reference compound to NNC0174-0839. Please indicate (with a check mark at ‘Please add the reference compound if available) during your compound request if you would like to have NNC0174-4010 included in your shipment.

Compound handling instructions

Peptides and proteins tend 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, NNC0174-0839 and NNC174-4010 (rat amylin) can be dissolved in 80/20% DMSO/MilliQ water (e. g. at a concentration of 300 uM). NNC0174-0839 can be dosed in vivo in a formulation vehicle containing 5mM sodium acetate, 240mM propylene glycol, (0.007% polysorbate 20 if concentrations are so low that adsorption to vials may affect the concentration), pH 4.0. It is always recommended to evaluate compound stability over the entire length of the experiment. Formulations can be used fresh for up to one week if stored refrigerated. Formulations can also be aliquoted and frozen for up to three months.

References

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