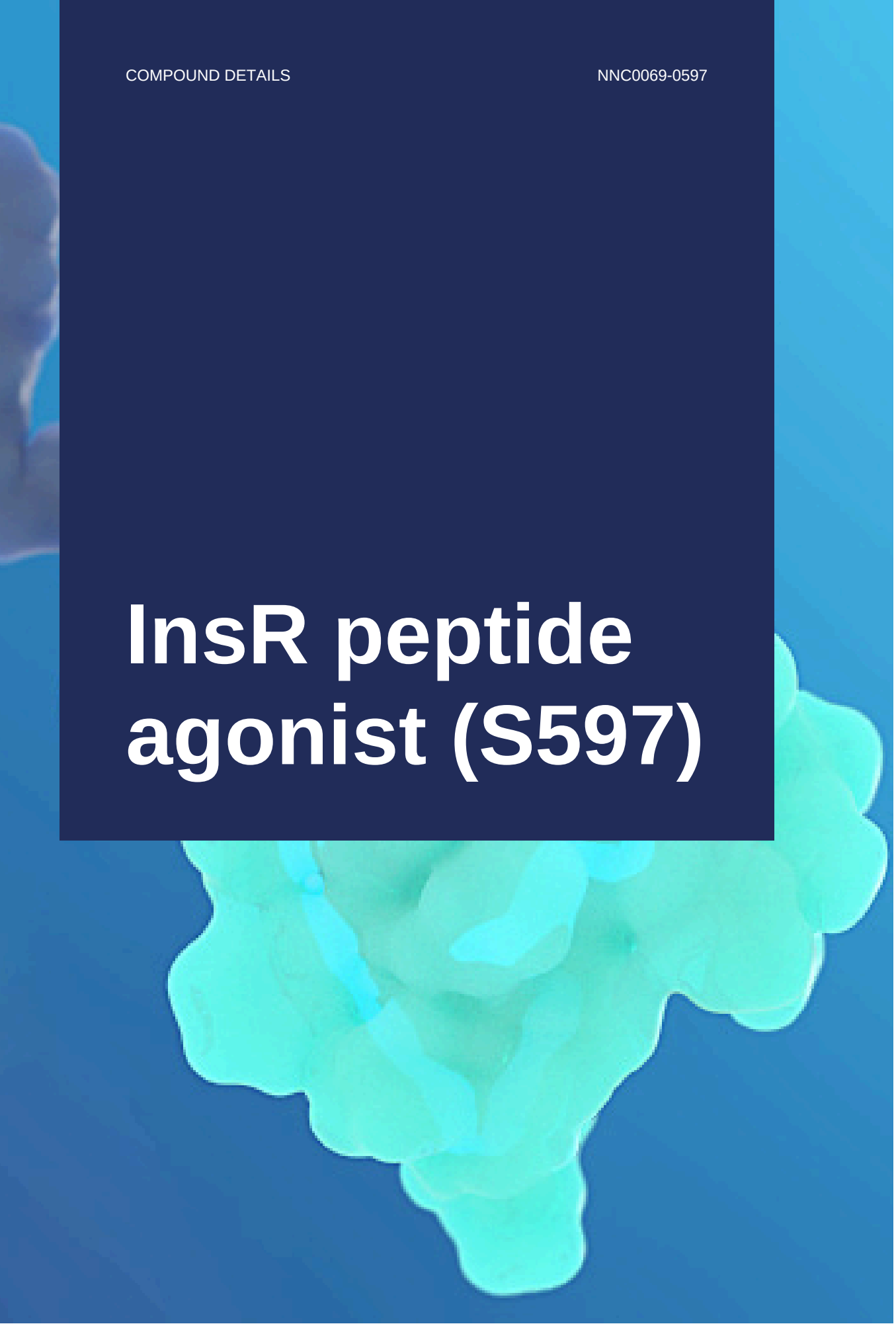
COMPOUND DETAILS NNC0069-0597

# InsR peptide agonist (S597)



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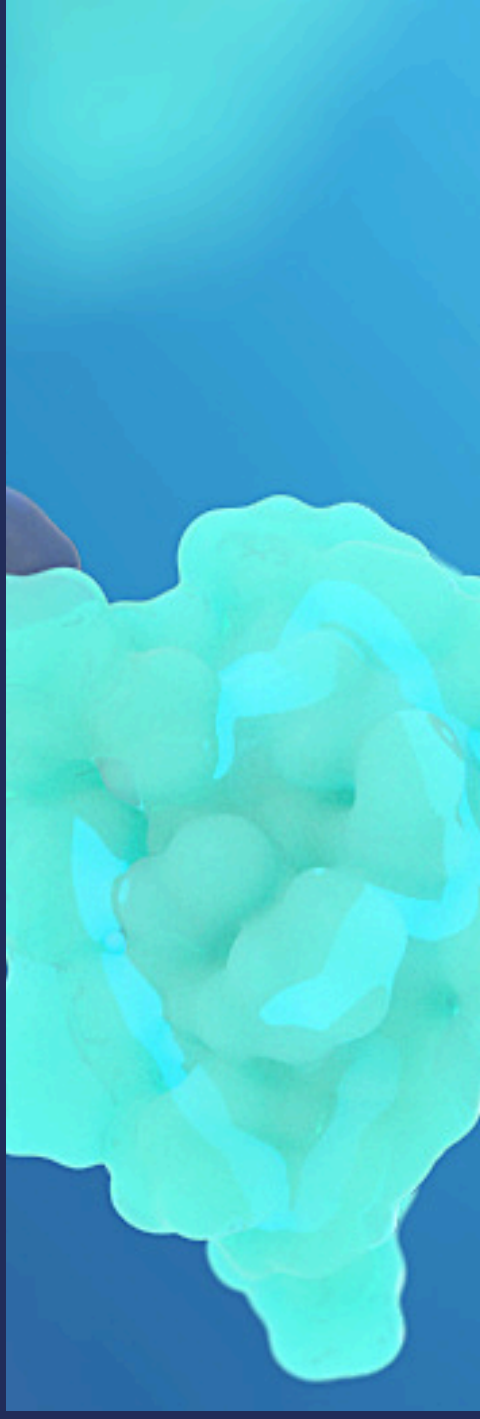
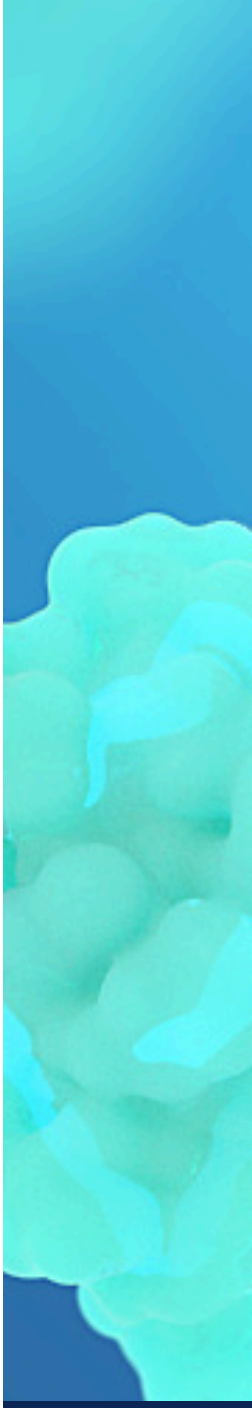
# Content





# InsR peptide agonist (S597)



Insulin binds to the insulin receptor (InsR), which exists in two alternatively spliced isoforms, InsR-A and InsR-B. The insulin receptor belongs to the same family of receptor tyrosine kinases as the IGF1 receptor (IGF1R) and insulin is therefore also able to bind to the IGF1R albeit with considerably lower affinity as compared to the InsR.

NNC0069-0597 (also known as S597) is a single chain peptide consisting of 31 amino acids, which has been shown to be a specific InsR agonist. The affinity of S597 for the human insulin receptor is comparable to that of human insulin, however, the signalling and consequently also cellular responses elicited by of S597 is different from that of human insulin. S597 can be used in both *in vitro* and *in vivo* experiments.

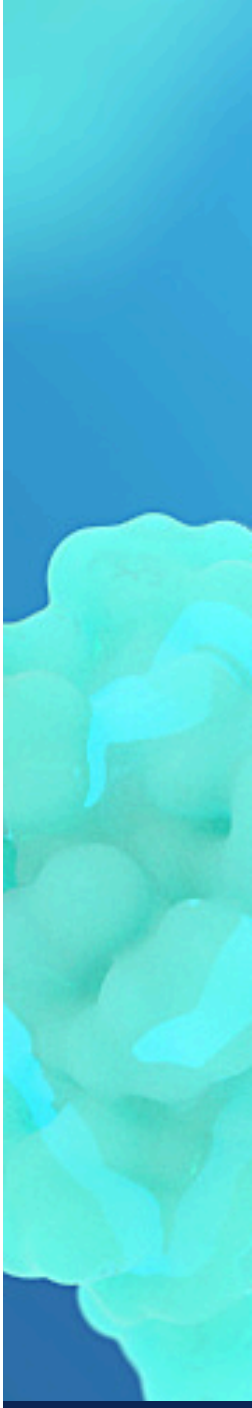


| | |
|---|---|
| Category | Insulin |
| ID | NNC0069-0597 |
| Amount pr. vial | 1000 nmol |

# Calculated properties

| Property | NNC0069-0597 | Human insulin |
|---|---|---|
| MW [Da] | 3826.1 | 5807.6 |
| pI (calculated) | 3.9 | 5.7 |
| Extinction coefficient (calculated, 280 nm) [1/cm x 1 /M] | 14105 | 6335 |

Selected calculated properties for NNC0069-0597 and human insulin.



# Structural information

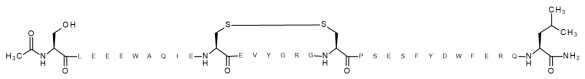


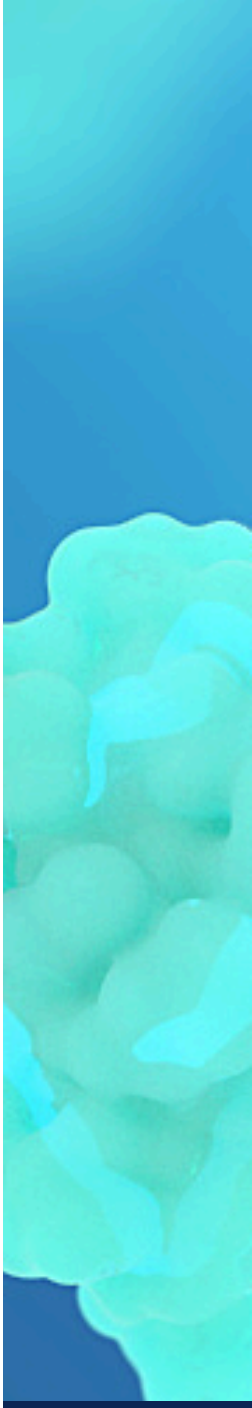
**Figure 1**

**Figure 1**

2D sketch of the structure of NNC0069-0597. The compound has an acetylation of the N-terminal, a C-terminal amide and contains one disulfide bridge.

# *In vitro* data

| Assay | NNC0069-0597; IC50/EC50 (nM) | Human insulin IC50/EC50;(nM) | % Maximal activation compared to human insulin (Emax) |
|---|---|---|---|
| Human InsR-A binding (IM9 cells) | 0.39 | 0.34 | - |
| Human InsR-A binding (L6-hIR cells) | 2.5 | 2.1 | - |
| InsR-A tyrosine phosphorylation (L6-hIR cells) | 2.4 | 2.6 | 58 |
| PKB phosphorylation (L6-hIR cells) | 3.2 | 0.11 | 94 |
| ERK1 phosphorylation (L6-hIR cells) | 3.5 | 0.55 | 18 |





The affinity of S597 (NNC0069-0597) for the human InsR is comparable to that of human insulin, but unlike human insulin, S597 does not bind to the IGF1R. S597 is also able to bind to the rat InsR and pig InsR, but with lower affinity (around 20% and 5%, respectively, compared to human insulin). Even though S597 and human insulin bind the human InsR with similar affinities, S597 interacts with the InsR in a different way than human insulin and the two agonists display clear differences in their abilities to stimulate InsR activation and downstream signalling pathways. S597 is able to phosphorylate the InsR, but to a lesser extent than human insulin (Emax around 60% compared to human insulin) which leads to full activation of PKB while the MAPK pathway (ERK1) is only weakly activated (Emax around 20% compared to human insulin). Consequently, S597 is very poor at eliciting cell proliferation (see the M. Jensen et al 2007 reference listed in

the reference section for further details).

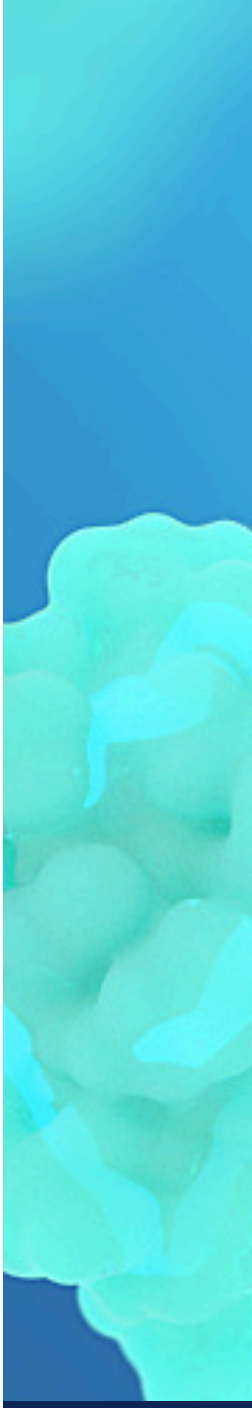


# *In vivo* data

| Species (administration route) | Dose (nmol/kg) | MRT (min) | T1/2 (min) |
|---|---|---|---|
| Wister rat (i.v.) | 2.5 | 50 | 49 |
| Sprague-Dawley rat (s. c.) | 30 | 63 | 53 |
| LYD pig (i.v.) | 0.3 | 97 | 104 |
| LYD pig (s.c.) | 1 | 204 | 140 |

*i.v.: intravenous; MRT; mean residence time; s.c. : subcutaneous;*

Since S597 is able to bind to other InsR species than human InsR, S597 can also be dosed in vivo to e.g. rats and pigs to lower blood glucose levels.

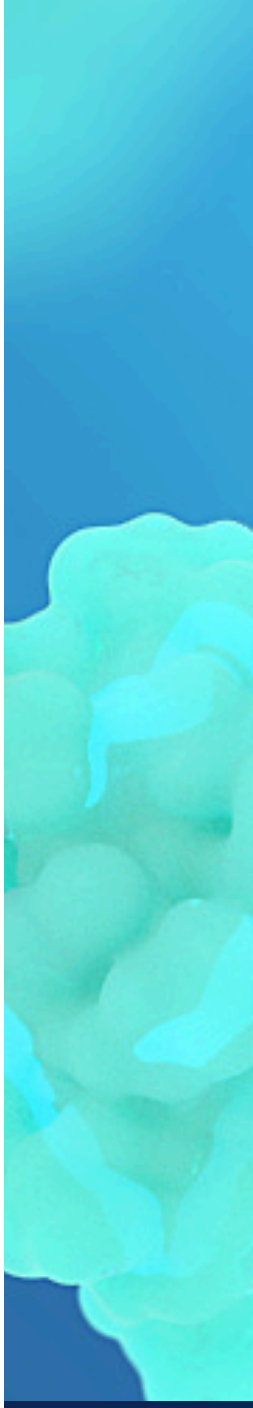


# Reference Compound

Human insulin (NNC0121-0308) is available as a reference compound to NNC0069-0597. Please indicate (with a check mark at ‘Please add the reference compound if available) during your compound request if you would like to have human insulin (NNC0121-0308) 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. Recommended storage of the freeze-dried compound is -20C. Recommended procedure for in vitro studies: dissolve the entire content of the vial by adding 4 mL 30 mM HEPES buffer pH 8. Gently rotate the vial until all content is dissolved. Avoid harsh shaking or stirring of the solution. Keep the stock solution at 4C overnight and make the desired number of aliquots (use low protein binding vials) of the stocks. Snap freeze the aliquots in liquid nitrogen and store them at minus 20C. When thawed, the stock solution should be stable for up to three weeks at 4C. Recommended procedure for in vivo studies: NNC0069-0597 can be dosed in vivo in a formulation vehicle containing 10mM sodium phosphate, 140mM sodium chloride, (0.007% polysorbate 20 if concentrations are so low that adsorption to vials may affect the concentration), pH 7.4. Formulations should be used fresh but can be stored for up to one week refrigerated.



# References

**1. Jensen M et al.**
Activation of the insulin receptor by insulin and a synthetic peptide leads to divergent metabolic and mitogenic signaling and responses

*J. Biol. Chem. 2007, 282 (48): 35179-35186*

**2. Jensen M et al.**
Activation of the insulin receptor (IR) by insulin and a synthetic peptide has different effects on gene expression in IR-transfected L6 myoblasts

*Biochem J. 2008; 412 (3): 435–445*

**3. Knudsen L et al.**
Agonism and antagonism at the insulin receptor

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**4. Frikke-Schmidt, H et al. Treatment**
Treatment of diabetic rats with insulin or a synthetic insulin receptor agonist peptide leads to divergent metabolic responses

*Diabetes 2015; 64(3):1057–1066*