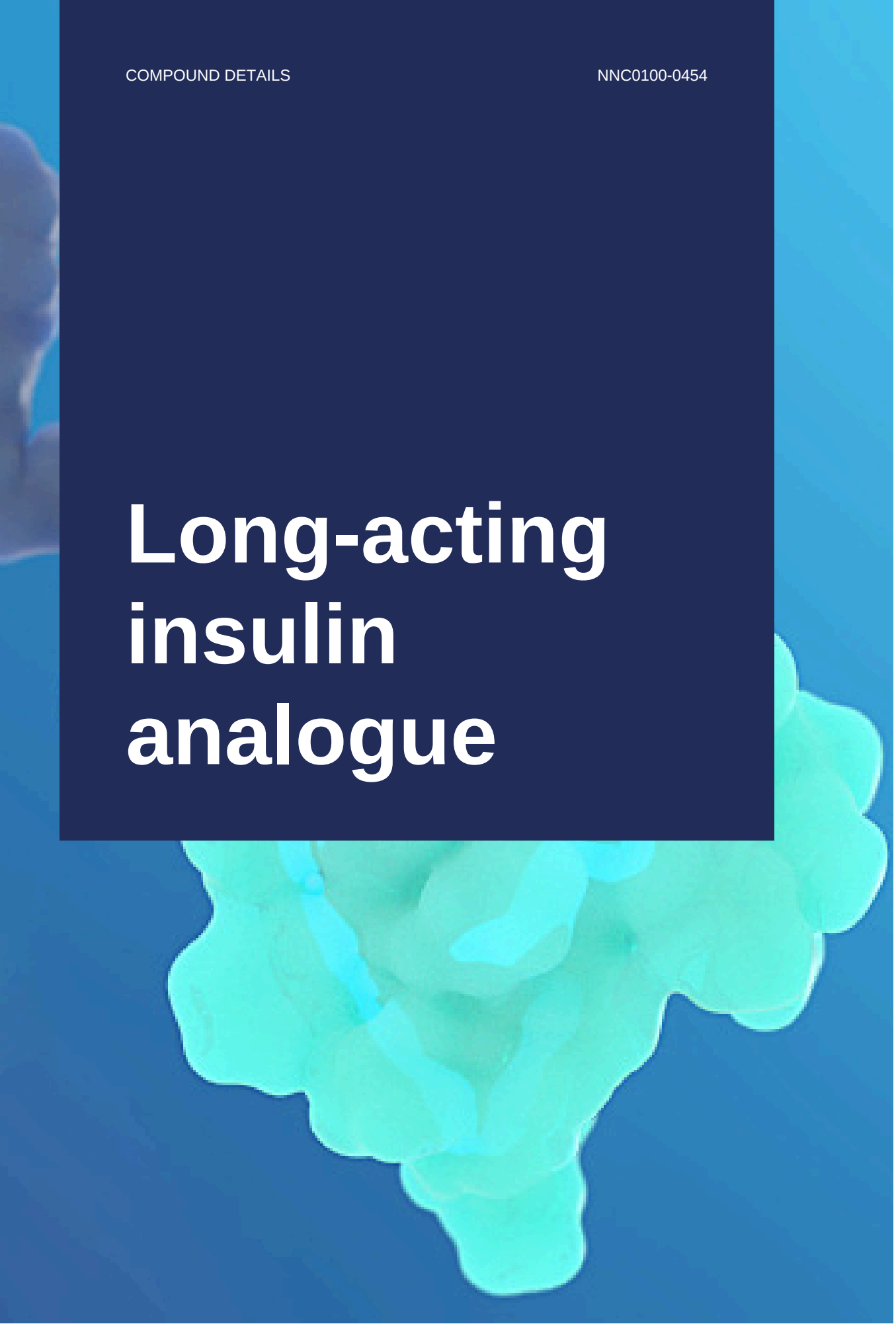
COMPOUND DETAILS NNC0100-0454

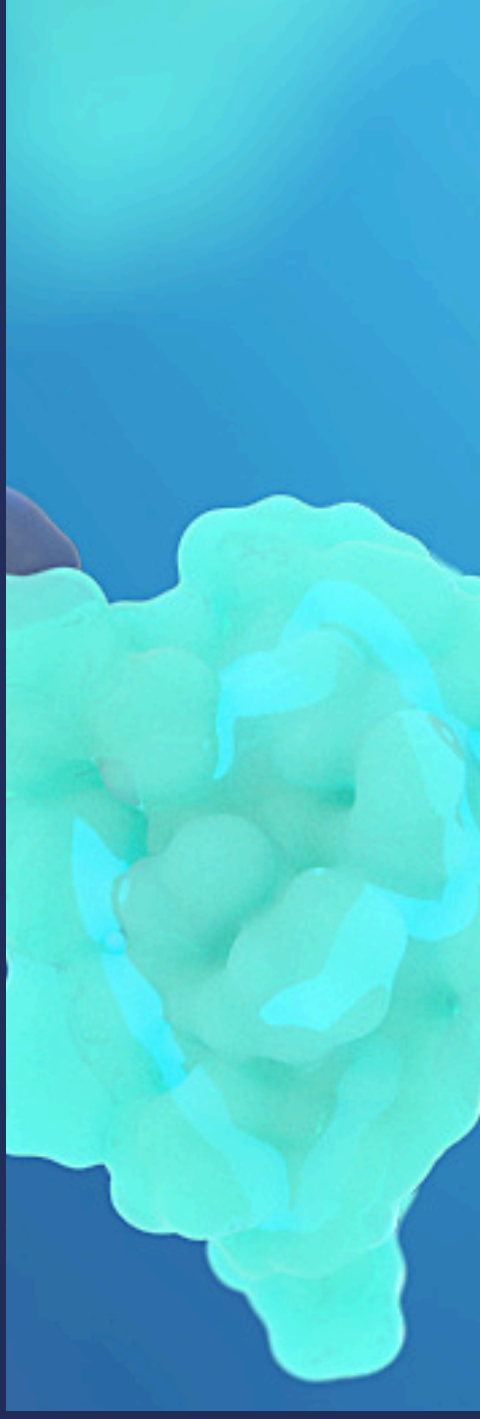
Long-acting insulin analogue



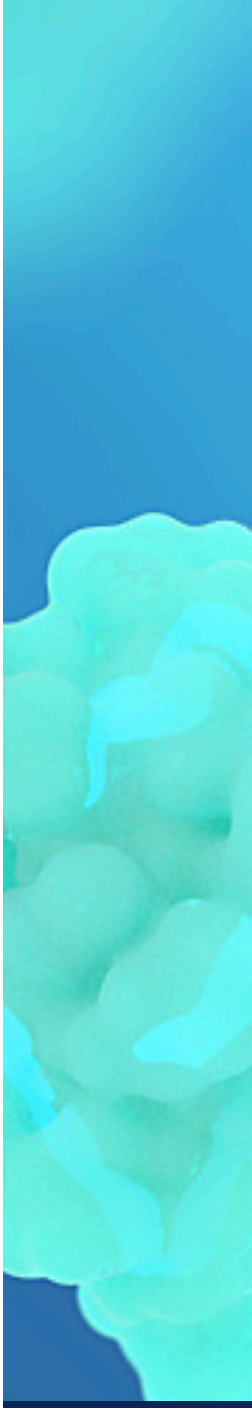
NOVO NORDISK COMPOUND SHARING

novo nordisk®

Content



Long-acting insulin analogue



Insulin is a peptide hormone acting as a key regulator of glucose homeostasis. 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 insulin receptor.

NNC0100-0454 (also known as insulin degludec) is a long-acting insulin analogue with a C16 fatty diacid attached to lysine at position B29 (B29K) via a -L-glutamic acid linker and is therefore able to reversibly bind to serum albumin. In the marketed formulation containing zinc and phenol, this insulin analogue forms a depot of soluble multi-hexamers in the subcutis after subcutaneous injection. Over time the multi-hexamers slowly dissociate into monomeric entities that are absorbed into the circulation and through the fatty acid bind to albumin. These properties contribute to the long PK profile of NNC0100-0454.

Category	Insulin
ID	NNC0100-0454
Amount pr. vial	1000 nmol (research grade purity)

Calculated properties

Property	NNC0100-0454	Human insulin
MW (Da)	6104	5808
pI	4.4	5.7
Fatty acid	C16 diacid	-
Linker	gGlu	-
Sequence substitutions	DesB30 (Thr at B30 is lacking)	-
Sum formula	C274 H411 N65 O81 S6	C257 H383 N65 O77 S6

Selected calculated properties for NNC0123-0327 and human insulin.

Structural information

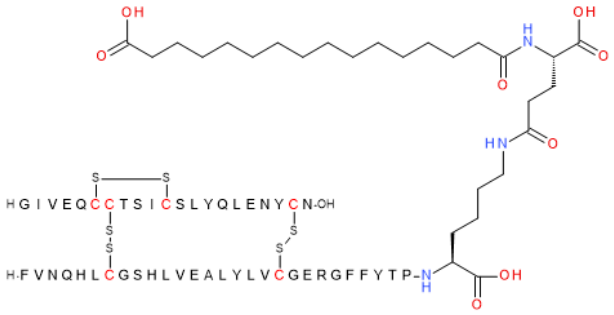


Figure 1

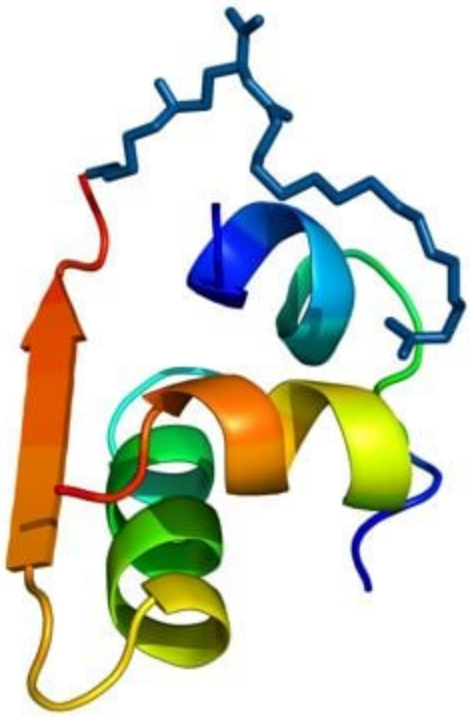


Figure 2

Figure 1

2D sketch of NNC0100-0454. The B chain is lacking Thr at position B30 (desB30) and a -L-glutamic acid linker connects B29 lysine with a C16 fatty diacid.

Figure 2

3D structure of a NNC100-0454 monomer modelled on the basis of pdb entries 4AK0 and 4AJX.

In vitro data

Receptor binding and lipogenic potency data for NNC0100-0454 (unpublished data). ;

The *in vitro;*receptor binding affinity of NNC0100-0454 relative to human insulin (HI) is shown in the table. The affinity was determined on human insulin receptor isoform A and B as well as on the IGF1R using solubilised holoreceptors in the presence of 0.1% (w/v) human serum albumin (HSA). The *in vitro;*lipogenic potency was measured in isolated primary rat adipocytes by assessment of the incorporation of 3-[^{3}H]-glucose into triglycerides (lipogenesis) in the presence of 0.1% (w/v) HSA. In all assays, NNC0100-0454 was a full agonist i.e. displayed the same maximal effect compared to that of human insulin. Since albumin binding is a key mechanism for the design of NNC0100-0454, be aware that the apparent affinity and potency will be very dependent on whether the *in vitro* assays contain albumin or not.

Assay	HSA concentration [%]	Relative affinity /potency (95% CI) [% of HI]	IC50 (95% CI) [nM]
Receptor binding, human InsR-A	0.1	9.2 (7.6 to 11)	0.015 (0.013 to 0.017)
Receptor binding, human InsR-B	0.1	11 (9.4 to 12)	0.016 (0.011 to 0.024)
Receptor binding, human IGF1R	0.1	2.6 (2.1 to 3.1)	1.2 (0.77 to 1.9)
Lipogenesis, rat adipocytes	0.1	3.6 (2.3 to 5.5)	-

CI: confidence interval; HI: human insulin; InsR-A: insulin receptor isoform A; InsR-B: insulin receptor isoform B; HSA: human serum albumin; IGF1R: IGF1 receptor

In vivo data

NNC0100-0454 displays a markedly protracted PK profile *in vivo* following a s.c. injection. Plasma PK parameters following i.v. dosing from a study in Sprague-Dawlay rats are given in the table below. For i.v. and s.c. studies in rats, dose levels of 1-5 nmol/kg and 5-30 nmol/kg, respectively, are recommended both for once and twice daily dosing studies. For the i.v. study in the table below, NNC0100-0454 was dissolved in 5 mM phosphate, 140 mM NaCl and 70 ppm polysorbate 20, pH 7.4. Please note that PK parameters for NNC0100-0454 is very dependent on the formulation used (e.g. the zinc content) due to its ability for form multi-hexamers upon subcutaneous injection under certain formulation conditions (see the Jonassen *et al.* 2012 reference listed in the reference section).

If using ELISA to detect NNC0100-0454 in your samples, ensure to use a kit that is able to detect this insulin analogue.

Parameter	Value
Dose (nmol/kg)	4.5
T1/2 (h)	2.3
MRT (h)	2.8
CL (mL/kg/min)	0.5
Vz (mL/kg)	10

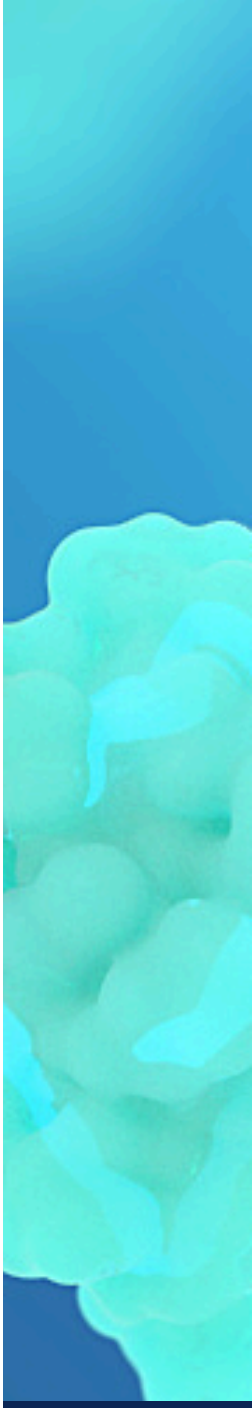
CL: clearance; MRT: mean residence time; T1/2: half-life; Vz: volume of distribution

Reference Compound

Human insulin (NNC0121-0308) is available as a reference compound to NNC0100-0454. 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, 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. 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 20oC. When thawed, the stock solution should be stable for up to three weeks at 4C. Recommended procedure for in vivo studies: NNC0100-0454 and human insulin (NNC0121-0308) can be dosed in vivo in a zinc-free and phenol/m-cresol-free formulation vehicle. For NNC0100-0454 it may contain 8mM sodium phosphate, 240mM glycerol, pH 7.4 and for human insulin it may contain 10mM sodium phosphate, 140mM sodium chloride, pH 7.4. If concentrations on the intended dosing formulations are very low (low uM to sub nM concentrations), adsorption to vials may affect the measurable concentration. In this case, considar adding 0.007% polysorbate 20.



Compound handling instructions

If insulin oligomeric/multimeric properties are of interest, zinc and phenol/m-cresol can be added. Zinc is typically added as zinc acetate between 0 and 6 moles/mole of insulin analogue. The resulting tonicity should be recalculated. Formulations should be used fresh but can be stored for up to one week refrigerated. The compound is research/laboratory grade purity.

References

1. Jonassen I et al.
Design of the novel protraction mechanism of insulin degludec, an ultra-long-acting basal insulin

Pharm Res. 2012;29(8):2104-14