Glucagon binding nucleic acid molcules of Type A

Compounds	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	PD (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
257-A1-001	47	**GCACTG**GTGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**GCAGTGC**	<	143		
257-D4-001	47	**GCACTG**GTGAAATGGGAGGGCTATGTGGAAGGAATCTGAG**GCAGTGC**	<<			
257-F4-001	47	**GCACTGA**TGAAATGGGAGGGCTAGGTGGAAGGAATCTGAA**GCAGTGC**	<			
257-B3-001	47	**GCACTA**GGGAAATGGGAGGGCTAGGCGGAAGGAATCTGAG**GTAGTGC**	<<			
257-D3-001	47	**GCACTAA**CGAAATGGGAGGGCTAGGTGGAAGGAATCTAAG**GTAGTGC**	<<			
257-E4-001	47	**GCAGTGG**CGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**TCACTGC**	=	124		
257-E1-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	=	137	179	500-1000
257-C4-001	47	**GCATTACT**GAAATGGGAGGGCTAGGTGGAAGGAATCTGG**AGTAATGC**	<<			
257-C1-001	47	**GCGCTG**GGGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**GCAGTGC**	<			
257-H2-001	47	**GCGCCA**GCGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**TCGGCGC**	<			

nt.: number of nucleotides; any of G, C, T and A is a 2'-desoxyribonucleotide;

APM: aptamer; **SPM:** Spiegelmer

nucleotides edged by ☐ represents a glucagon-binding motif;

Fig. 1

Comp(APM): Molecules of the indicated sequence were tested as aptamers (D-nucleic acid) in pull-down competition binding assay vs. 257-E1-001 as reference
=: similar binding affinity as 257-E1-001; <: weaker binding affinity than 257-E1-001; <<: much weaker binding affinity than 257-E1-001
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;
PD(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated L-glucagon or competition with non-biotinylated L-glucagon;
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 1 continued

Glucagon binding nucleic acid molecules of Type A: 257-E1-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (APM)
257-E1-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	=
257-E1-002	45	**CAGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTG**	<
257-E1-003	45	**GAGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTC**	<
257-E1-004	43	**AGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACT**	<
257-E1-005	41	**GTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTAC**	<

nt.: number of nucleotides; any of G, C, T and A is a 2'-desoxyribonucleotide;

nucleotides edged by ☐ represents a glucagon-binding motif;

Comp(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. 257-E1-001 as reference
=: similar binding affinity as reference; <: weaker binding affinity than reference;

APM : aptamer

Fig. 2A

Glucagon binding nucleic acid molecules of Type A: 257-E1-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (SPM) K_D [nM]	Biacore(SPM) K_D [nM]	CHO_GR IC_{50} [nM]
257-E1-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	187		
257-E1-R9-001	47	**GCAGTGG**GrGAAATGGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	+		
257-E1-R15-001	47	**GCAGTGG**GGAAATGrGGAGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	33.5		
257-E1-R18-001	47	**GCAGTGG**GGAAATGGGArGGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	+		
257-E1-R19-001	47	**GCAGTGG**GGAAATGGGAGrGGCTAGGTGGAAGGAATCTGAG**CTACTGC**	+		
257-E1-R29-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGrAAGGAATCTGAG**CTACTGC**	64		
257-E1-R30-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGArAGGAATCTGAG**CTACTGC**	111		
257-E1-R15/29-001	47	**GCAGTGG**GGAAATGrGGAGGGCTAGGTGGrAAGGAATCTGAG**CTACTGC**	18.7*		
257-E1-R29/30-001	47	**GCAGTGG**GGAAATGGGAGGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	48.8**		
257-E1-R15/29/30-001	47	**GCAGTGG**GGAAATGrGGAGGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	20**		
257-E1-R18/29/30-001	47	**GCAGTGG**GGAAATGGGArGGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	35**		
257-E1-R15/18/29/30-001	47	**GCAGTGG**GGAAATGrGGArGGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	=**		
257-E1-6xR-001 (=257-E1-R9/15/18/19/29/30-001)	47	**GCAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	6.4***	4.82	2.8-3.1

Fig. 2B

nt.: number of nucleotides

nucleotides edged by ☐ represents a glucagon-binding motif;

any of rG and rA is a ribonucleotide; any of G, C, T and A is a 2'-desoxyribonucleotide;

Comp(SPM): Molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) in pull-down competition binding assay vs. 257-E1-001, vs. 257-E1-R15/29-001*, 257-E1-R15/29/30-001**, or vs. 257-E1-6xR-001*** as reference, wherein for some molecules the dissociation constant ($\mathbf{K_D}$) was determined

+: better binding affinity than reference =: similar binding affinity than reference

Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon

CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

SPM: Spiegelmer

Fig. 2B continued

Glucagon binding nucleic acid molecules of Type A: 257-E1-6xR-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (SPM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
257-E1-6xR-001	47	**GCAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	6.4	4.82	2.8-3.1
257-E1-6xR-003	45	**GAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACTC**	8.4		
257-E1-6xR-004	43	**AGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACT**	19		
257-E1-6xR-005	45	**GGGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACCC**	<		
257-E1-6xR-006	45	**GCGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACGC**	10		
257-E1-6xR-007	45	**GGGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTGCCC**	<		
257-E1-6xR-008	45	**GCGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTGCGC**	=		
257-E1-6xR-009	45	**GGGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGCCC**	7.8	4.91	2.1
257-E1-6xR-010	45	**GCGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGCGC**	5.9		3.3

nt.: number of nucleotides nucleotides edged by ☐ represents a glucagon-binding motif;
any of rG and rA is a ribonucleotide; any of G, C, T and A is a 2'-desoxyribonucleotide; SPM: Spiegelmer

Comp(SPM): Molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) in pull-down competition binding assay vs. reference 257-E1-6xR-001, wherein for some molecules the dissociation constant (**K_D**) was determined
=: similar binding affinity as reference <: weaker binding affinity than reference

Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon

CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 3A

Glucagon binding nucleic acid molecules of Type A: 257-E1-6xR-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (SPM) K_D [nM]
257-E1-6xR-001	47	**GCAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	=
257-E1-6xR-011	45	**GGGCCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGGCCC**	+
257-E1-6xR-012	45	**GCGCCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGGCGC**	=
257-E1-6xR-013	45	**GAGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGCTC**	=
257-E1-6xR-014	45	**GAGCCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGGCTC**	<
257-E1-6xR-015	45	**GAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCACTC**	<
257-E1-6xR-016	45	**GCGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCACGC**	6.2
257-E1-6xR-017	45	**GAGTCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGACTC**	<
257-E1-6xR-018	45	**GCGTCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGACGC**	=

nt.: number of nucleotides nucleotides edged by ☐ represents a glucagon-binding motif;
any of rG and rA is a ribonucleotide; any of G, C, T and A is a 2'-desoxyribonucleotide;
SPM: Spiegelmer
Comp(SPM): Molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) in pull-down competition binding assay vs. reference 257-E1-6xR-001
=: similar binding affinity as reference +: better binding affinity than reference <: weaker binding affinity than reference

Fig. 3B

Glucagon binding nucleic acid molecules of Type A: 257-E1-6xR-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (SPM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
257-E1-6xR-001	47	**GCAGTGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CTACTGC**	6.4*	4.82	2.8-3.1
257-E1-6xR-019	43	**GGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGCC**	7.7*	3.69	2.0-3.6
257-E1-6xR-020	43	**CGCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGCG**	6.3*		
257-E1-6xR-029	41	**GCGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCGC**	8.9*	4.61	2.97
257-E1-6xR-030	39	**GCG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CGC**	9.6*	5.02	1.6-4.6
257-E1-6xR-031	39	**CGG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CCG**	<*		
257-E1-6xR-032	37	**GG**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**CC**	<*		
257-E1-6xR-033	35	**G**GrGAAATGrGGArGrGGCTAGGTGGrArAGGAATCTGAG**C**	<*		
257-E1-7xR-023	45	**GCGCGG**GrGAAATGrGGArGrGGCTAGGrTGGrArAGGAATCTGAG**CCGCGC**	4.4*		
257-E1-7xR-037	39	**GCG**GrGAAATGrGGArGrGGCTAGGrTGGrArAGGAATCTGAG**CGC**	5.6**	1.88	1.7-2.5

nt.: number of nucleotides nucleotides edged by ☐ represents a glucagon-binding motif;
any of rG, rA and rT is a ribonucleotide; any of G, C, T and A is a 2'-desoxyribonucleotide; SPM: Spiegelmer

Comp(SPM): Molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) in pull-down competition binding assay vs. 257-E1-6xR-001* or 257-E1-7xR-037** as reference, wherein for some molecules the dissociation constant (K_D) was determined
=: similar binding affinity as reference +: better binding affinity than reference <: weaker binding affinity than reference

Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon

CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 3C

Glucagon binding nucleic acid molecules of Type B

Compound	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	CHO_GR IC_{50} [nM]
259-D5-001	50	**CGACTCGAG**AGGAAAGGTTGCTAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	<		
259-H6-001	50	**CGACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	=	33	300-400
259-B7-001	50	**CGACTCGAG**AGGAAAGGTTGGTATAGGTTCGGTTGGATTCA**CTCGAGTCG**	<		
259-B8-001	50	**CGACTCGAG**AGGAAATGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	<<		
259-A5-001	50	**CGACTCGAG**AGGAGAGGTTGGTAAAGATTCGGTTGGATTCA**CTCGAGTCG**	<<		
259-C8-001	50	**CGGCTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	=		
259-E5-001	50	**CGACTCGAG**ATGAAAGGTTGGCAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	<<		
259-E7-001	48	**CGAGTCGAT**A-G-AAGGTCGGTA-AGTTTCGGTAGGATCTG**CGACGAGACG**	<<		
259-F5-001	48	**CGAGTCGAT**A-G-AAGGTTGGTA-AGTTTCGGTTGGATCTG**CGACGAGACG**	<	180	

nt.: number of nucleotides; **APM:** aptamer

nucleotides edged by ☐ represents a glucagon-binding motif;

any of G, C, T and A is a 2'-desoxyribonucleotide;

Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. 259-H6-001

=: similar binding affinity as 259-H6-001; <: weaker binding affinity than 259-H6-001; <<: much weaker binding affinity than 259-H6-001

PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;

CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 4

Glucagon binding nucleic acid molecules of Type B: 259-H6-001 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
259-H6-001	50	**CGACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGTCG**	=	33		300-400
259-H6-002	46	**ACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	=	57	12.3	176
259-H6-005	44	**GTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAC**	=			
259-H6-003	42	**TCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGA**	<			
259-H6-004	38	**GAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTC**	<			
259-H6-006	45	**ACTCGAG**AGGA-AGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	<<			
259-H6-007	45	**ACTCGAG**AGGAAAGGTTGGTA-AGGTTCGGTTGGATTCA**CTCGAGT**	<<			
259-H6-008	44	**ACTCGAG**AGGA-AGGTTGGTA-AGGTTCGGTTGGATTCA**CTCGAGT**	<<			

nt.: number of nucleotides; **APM:** aptamer **SPM:** Spiegelmer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, T and A is a 2'-desoxyribonucleotide;
Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. references 259-H6-001 and 259-H6-002, respectively
=: similar binding affinity as reference; <: weaker binding affinity than reference; <<: much weaker binding affinity than reference;
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;
Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 5

Glucagon binding nucleic acid molecules of Type B: 259-H6-002 and derivatives thereof

Compund	nt.	Sequence: 5'->3'	PD (APM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
259-H6-002	46	**ACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	57	12.3	176
259-H6-002-R13	46	**ACTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	9	1.8	12.5
259-H6-002-R24	46	**ACTCGAG**AGGAAAGGTTGGTAAArGGTTCGGTTGGATTCA**CTCGAGT**		3.8	
259-H6-002-R36	46	**ACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGArUTCA**CTCGAGT**		5.1	
259-H6-002-R13/24	46	**ACTCGAG**AGGAArAGGTTGGTAAArGGTTCGGTTGGATTCA**CTCGAGT**		1.5	
259-H6-002-R13/36	46	**ACTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGArUTCA**CTCGAGT**		1.8	
259-H6-002-R24/36	46	**ACTCGAG**AGGAAAGGTTGGTAAArGGTTCGGTTGGArUTCA**CTCGAGT**		7.6	
259-H6-002-R13/24/36	46	**ACTCGAG**AGGAArAGGTTGGTAAArGGTTCGGTTGGArUTCA**CTCGAGT**		0.8	6.2

nt.: number of nucleotides **APM:** aptamer **SPM:** Spiegelmer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, T and A is a 2'-desoxyribonucleotide; any of rG, RA and rU is a ribonucleotide;
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;
Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 6A

Glucagon binding nucleic acid molecules of Type B: 259-H6-002 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
259-H6-002	46	**ACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**		57	12.3	176
259-H6-002-R13	46	**ACTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	=	9	1.8	12.5
259-H6-005-R12	44	**GTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAC**	+			
259-H6-009-R12	44	**TTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAA**	<			
259-H6-010-R12	44	**TGCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGCA**	=			
259-H6-011-R12	44	**GGCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGCC**	<			
259-H6-012-R12	44	**GGCCAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTGGCC**	=			

nt.: number of nucleotides **APM:** aptamer **SPM:** Spiegelmer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, T and A is 2'-desoxyribonucleotide; any of rA is a ribonucleotide;
Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. reference 259-H6-002-R13
=: similar binding affinity as reference +: better binding affinity than reference <: weaker binding affinity than reference
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;
Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 6B

Glucagon binding nucleic acid molecules of Type B: 259-H6-002 and derivatives thereof

Compound	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	Biacore (SPM) K_D [nM]	CHO_GR IC_{50} [nM]
259-H6-002	46	**ACTCGAG**AGGAAAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**		57	12.3	176
259-H6-013-R12	44	**GCGCAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTGCGC**	<			
259-H6-014-R12	44	**GCCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGGC**	+	10	1.07	7-19
259-H6-015-R12	44	**CTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAG**	=			
259-H6-016-R12	45	**CTCGAG**AGGAArAGGTTGGTAAAGGTTCGGTTGGATTCA**CTCGAGT**	<			
259-H6-014-R12/23/35	44	**GCCGAG**AGGAArAGGTTGGTAAArGGTTCGGTTGGArUTCA**CTCGGC**			0.72	4-8
259-H6-014-R12/23/29/35/38	44	**GCCGAG**AGGAArAGGTTGGTAAArGGTTCGrGTTGGArUTCrA**CTCGGC**			0.545	

nt.: number of nucleotides **APM:** aptamer **SPM:** Spiegelmer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, T and A is a 2'-desoxyribonucleotide; any of rG, RA and rU is a ribonucleotide;
Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. reference 259-H6-002-R13
=: similar binding affinity as reference +: better binding affinity than reference <: weaker binding affinity than reference
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon;
Biacore(SPM): Dissociation constant K_D of Spiegelmers (L-nucleic acid), molecules of the indicated sequence were tested as Spiegelmers (L-nucleic acid) measured as surface plasmon resonance on Biacore using direct binding to biotinylated L-glucagon
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 6C

Glucagon binding nucleic acid molecules of Type C

Compound	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]
258-D4-001	46	**CG**GCC**TA**GAAGGTAGGTAAGTTTCGGTTGGATCTACGGTCG**TA**ACA**CG**	=	3500
258-H1-001	46	**CGTCCTA**GAAGGTAGGTAAGTTTCGGTTGGATCTAGGATAG**TAG**CA**CG**	+	266

nt.: number of nucleotides **APM:** aptamer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, T and A is a 2'-desoxyribonucleotide;
Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. 258-D4-001
=: similar binding affinity as 258-D4-001; **+:** better binding affinity than 258-D4-001;
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon

Fig. 7

Further glucagon binding nucleic acid molecules of Type C

Compounds	nt.	Sequence: 5'->3'	Comp (APM)	PD (APM) K_D [nM]	CHO_GR IC_{50} [nM]
GLU-18-25-A3-001	46	**CGUGUG**UGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGC**CACACG**	=		
GLU-18-25-A3-002	52	**CGACGUGUG**UGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGC**CACACGUCG**	+		400
GLU-18-25-A3-003 (= NOX-G11stabi2)	54	**CAGACGUGUG**UGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGC**CACACGUCUG**	+	80	

nt.: number of nucleotides **APM:** aptamer
nucleotides edged by ☐ represents a glucagon-binding motif;
any of G, C, U and A is a ribonucleotide;
Comp(APM): Molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down competition binding assay vs. GLU-18-25-A3-001
=: similar binding affinity as GLU-18-25-A3; +: better binding affinity than GLU-18-25-A3;
PD(APM): Dissociation constant K_D of aptamers (D-nucleic acid), molecules of the indicated sequence were measured as aptamers (D-nucleic acid) in pull-down assay using direct binding to biotinylated D-glucagon or competition with non-biotinylated D-glucagon
CHO_GR: Half maximal inhibitory concentration (IC50), molecules of the indicated sequence were tested as Spiegelmers with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation;

Fig. 8

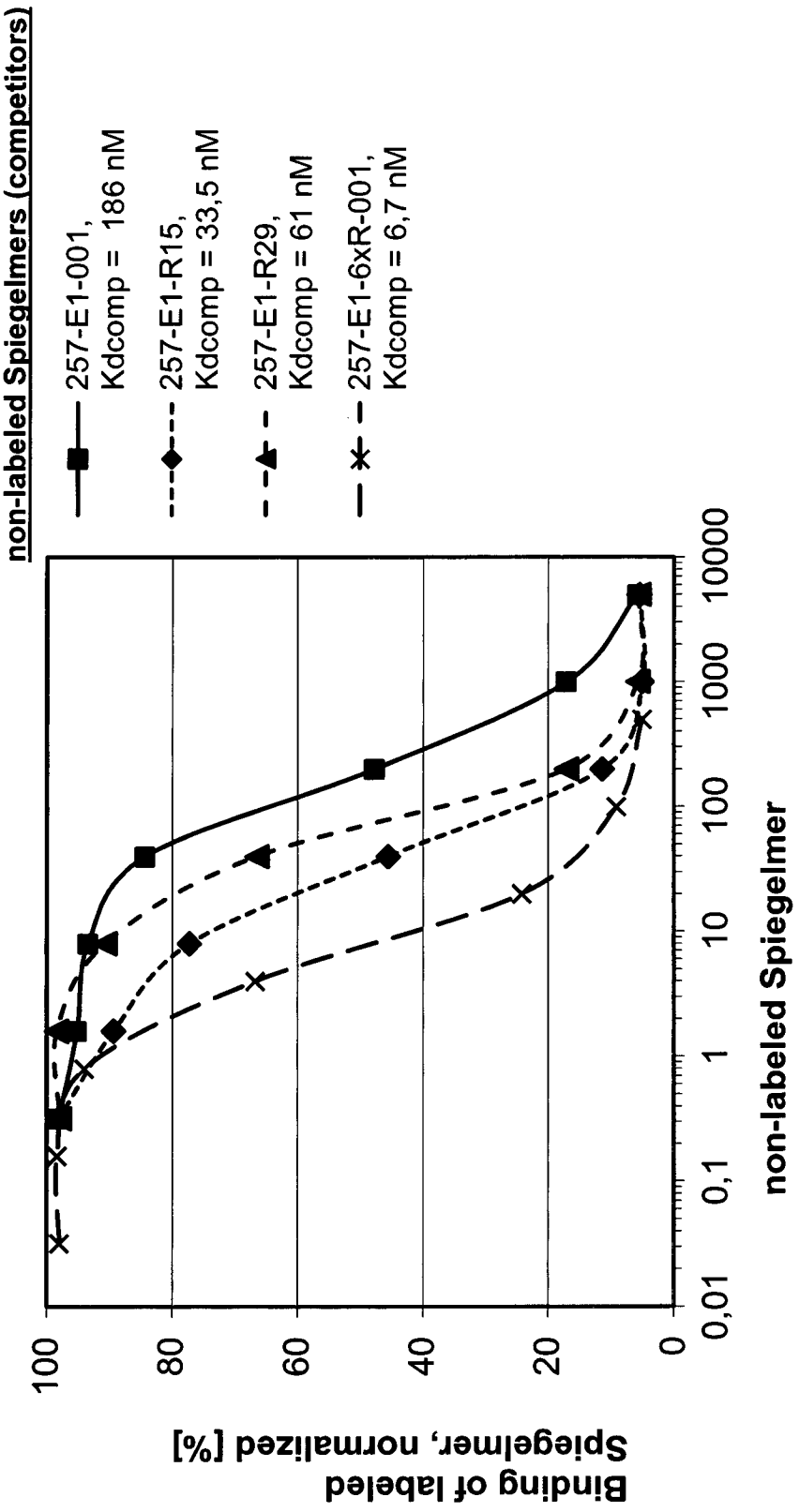
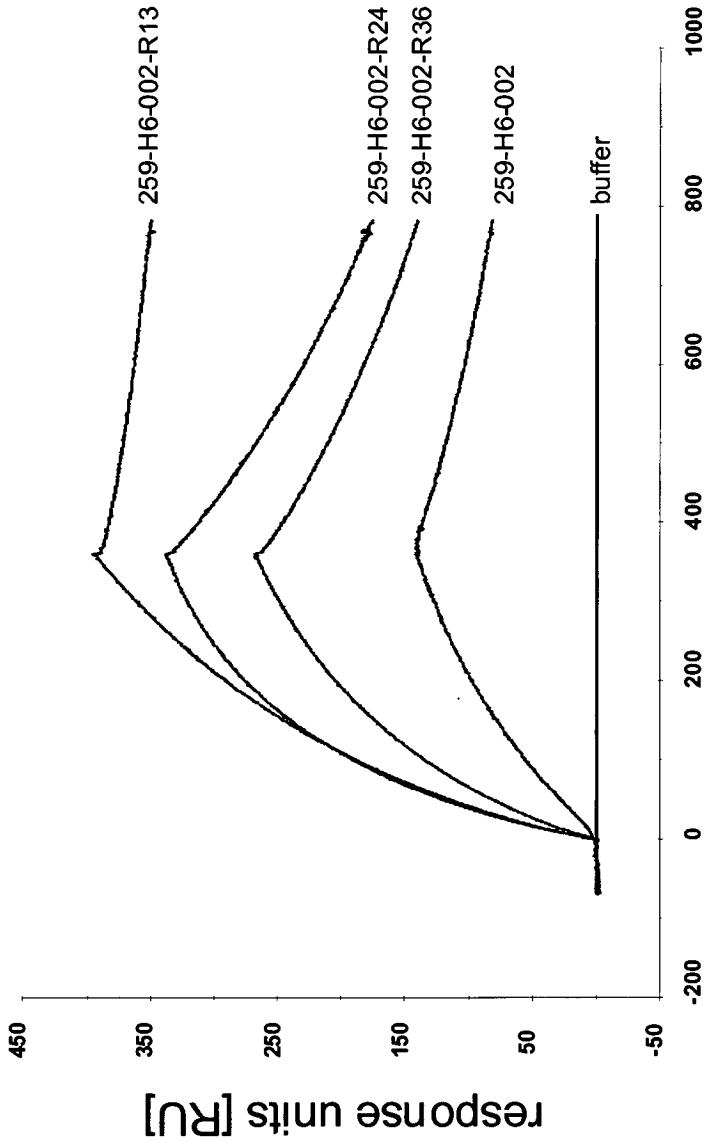
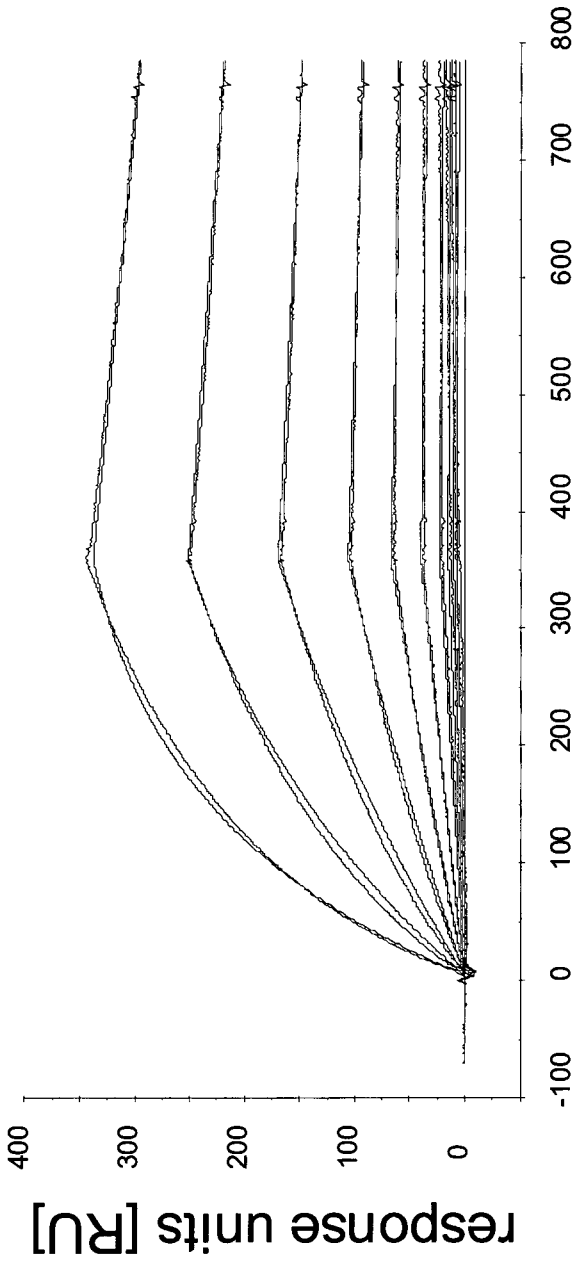

non-labeled Spiegelmers (competitors)
257-E1-001, Kdcomp = 186 nM
257-E1-R15, Kdcomp = 33,5 nM
257-E1-R29, Kdcomp = 61 nM
257-E1-6xR-001, Kdcomp = 6,7 nM
Binding of labeled Spiegelmer, normalized [%]
100
80
60
40
20
0
0,01
0,1
1
10
100
1000
10000
non-labeled Spiegelmer

Fig.9



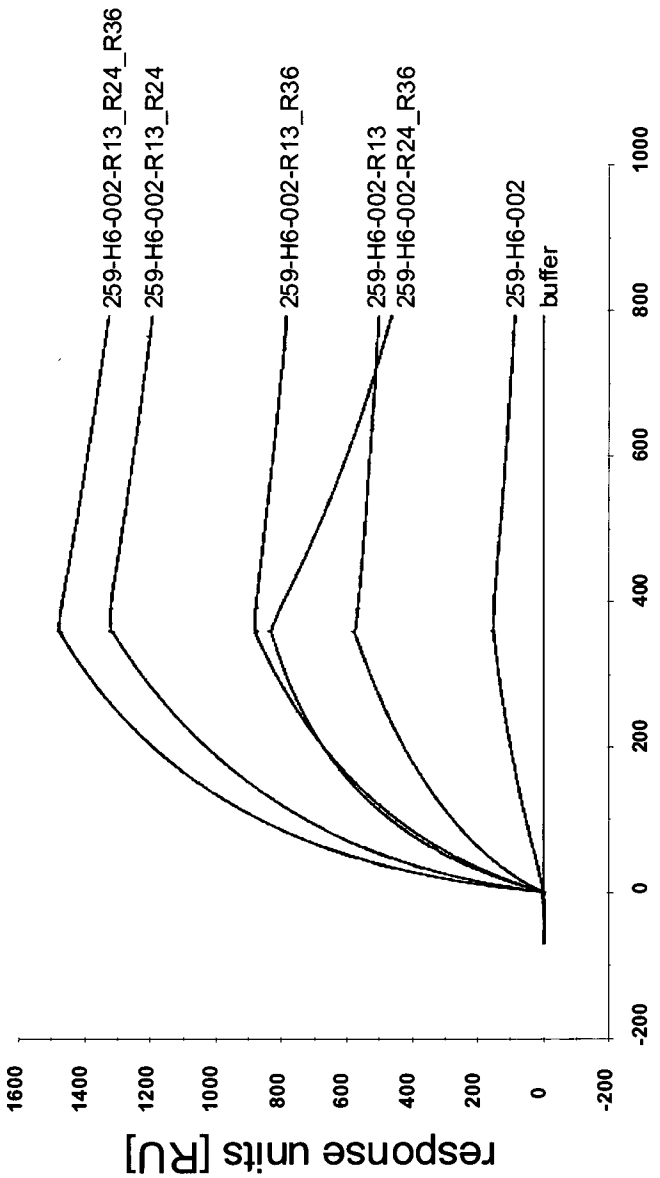
	k_a [1/M*s]	k_d [1/s]	K_D [nM]
259-H6-002 (reference)	8.73E+04	1.07E-03	12.3
259-H6-002R13	2.26E+05	4.01E-04	1.77
259-H6-002R24	3.21E+05	1.22E-03	3.80
259-H6-002R36	1.77E+05	9.02E-04	5.10

Fig. 10



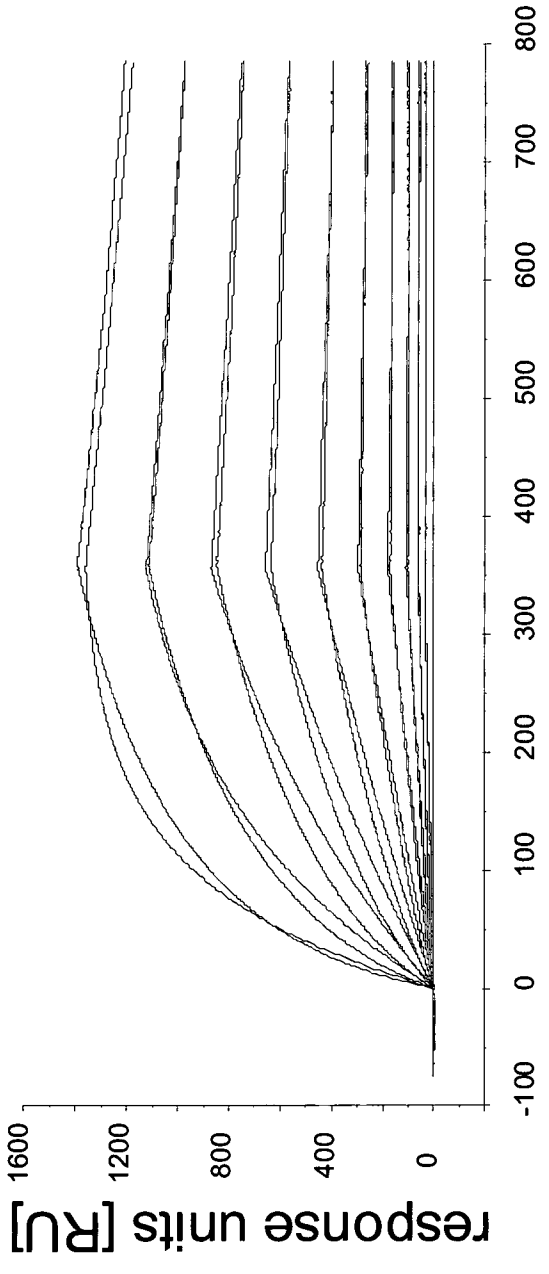
	k_a [1/M*s]	SE (k_a [1/M*s])	k_d [1/s]	SE (k_d [1/s])	K_D [nM]
NOX-G13	5.69E+05	1.10E+04	1.04E-03	1.29E-05	**1.83**

Fig. 11



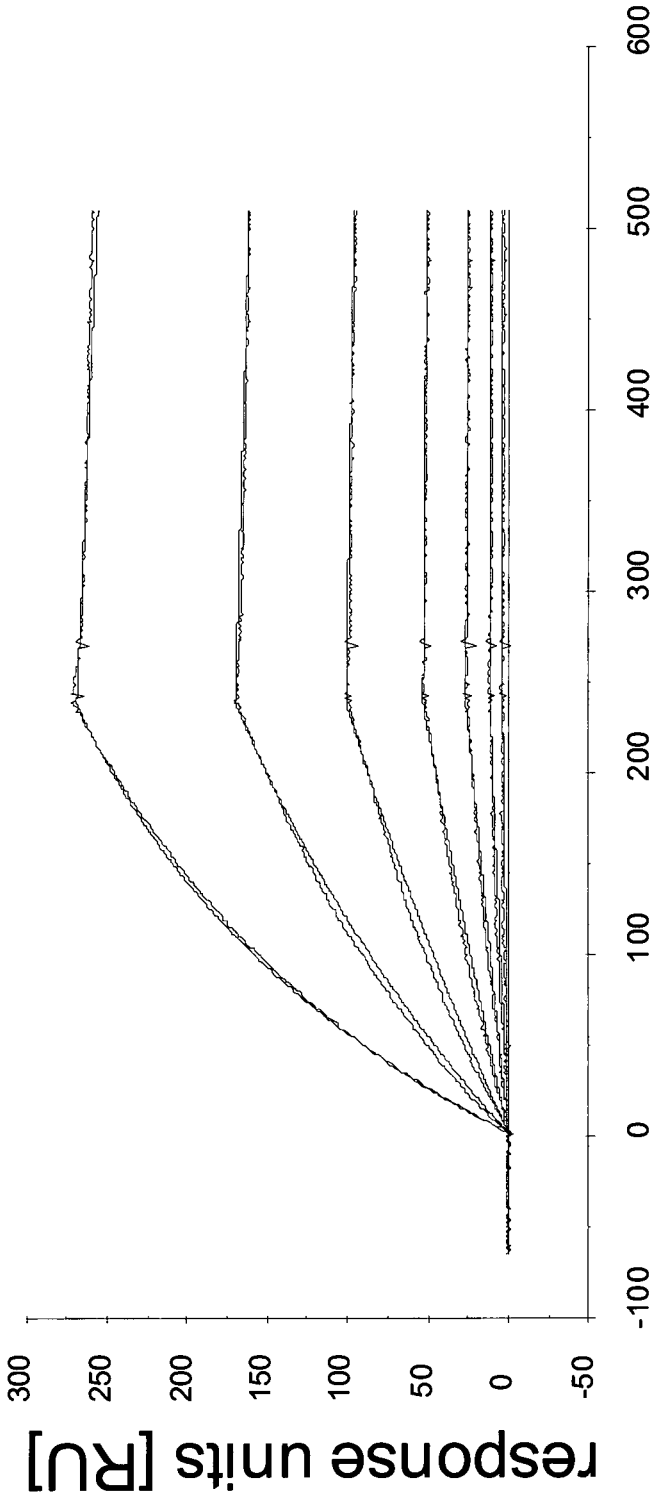
	k_a [1/M*s]	k_d [1/s]	K_D [nM]
259-H6-002	9.25E+04	1.18E-03	12.7
259-H6-002R13 (reference)	2.65E+05	8.84E-04	3.33
259-H6-002R13_R24	4.42E+05	6.54E-04	1.48
259-H6-002R13_R36	3.53E+05	6.40E-04	1.82
259-H6-002R13_R24_R36	5.57E+05	4.45E-04	0.798
259-H6-002R24_R36	2.56E+05	1.94E-03	7.59

Fig. 12



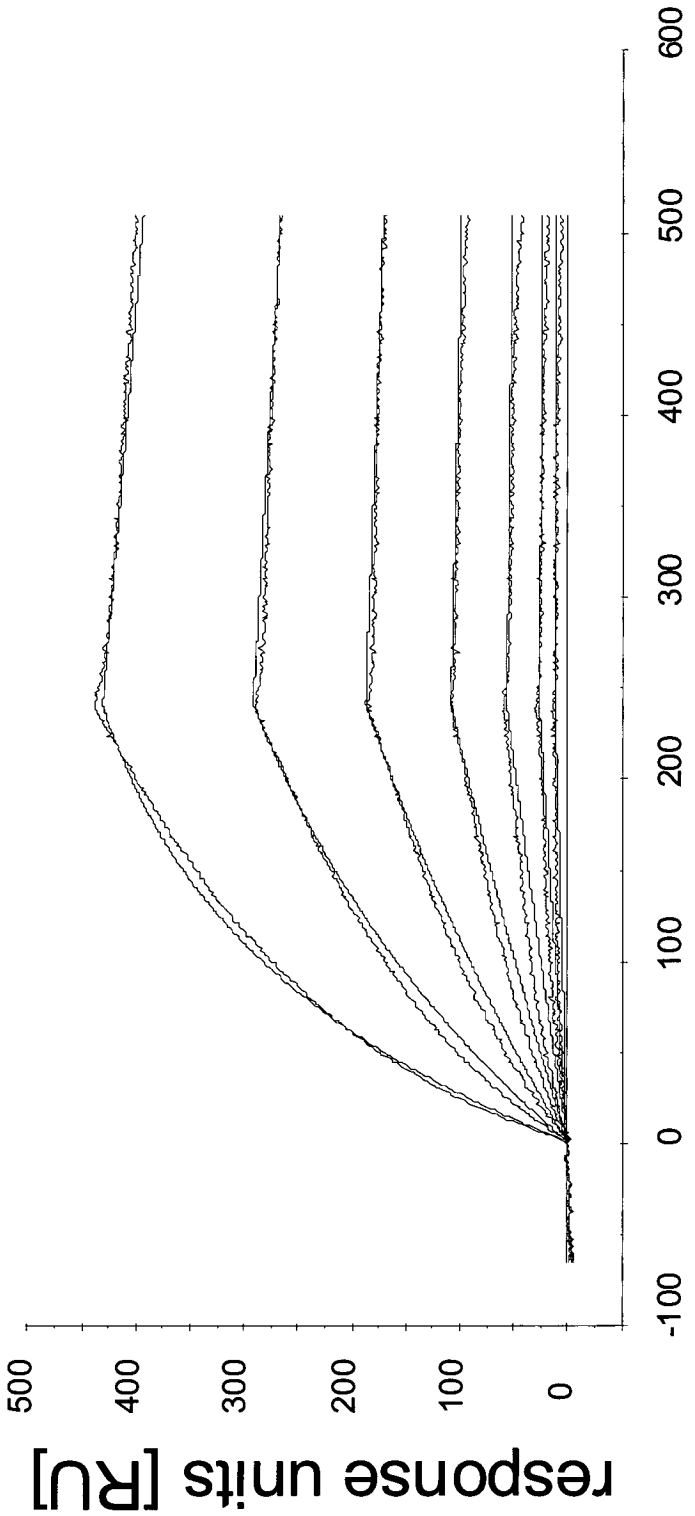
	k_a [1/M*s]	SE (k_a [1/M*s])	k_d [1/s]	SE (k_d [1/s])	K_D [nM]
NOX-G14	5.57E+05	2.72E+03	4.45E-04	2.08E-06	0.798

Fig. 13



	k_a [1/M*s]	SE (k_a [1/M*s])	k_d [1/s]	SE (k_d [1/s])	K_D [nM]
NOX-G15	4.65E+04	0.21E+02	1.80E-04	3.20E-06	**3.87**

Fig. 14



	k_a [1/M*s]	SE (k_a [1/M*s])	k_d [1/s]	SE (k_d [1/s])	K_D [nM]
NOX-G16	1.48E+05	2.11E+03	2.44E-04	8.86E-06	**1.65**

Fig. 15

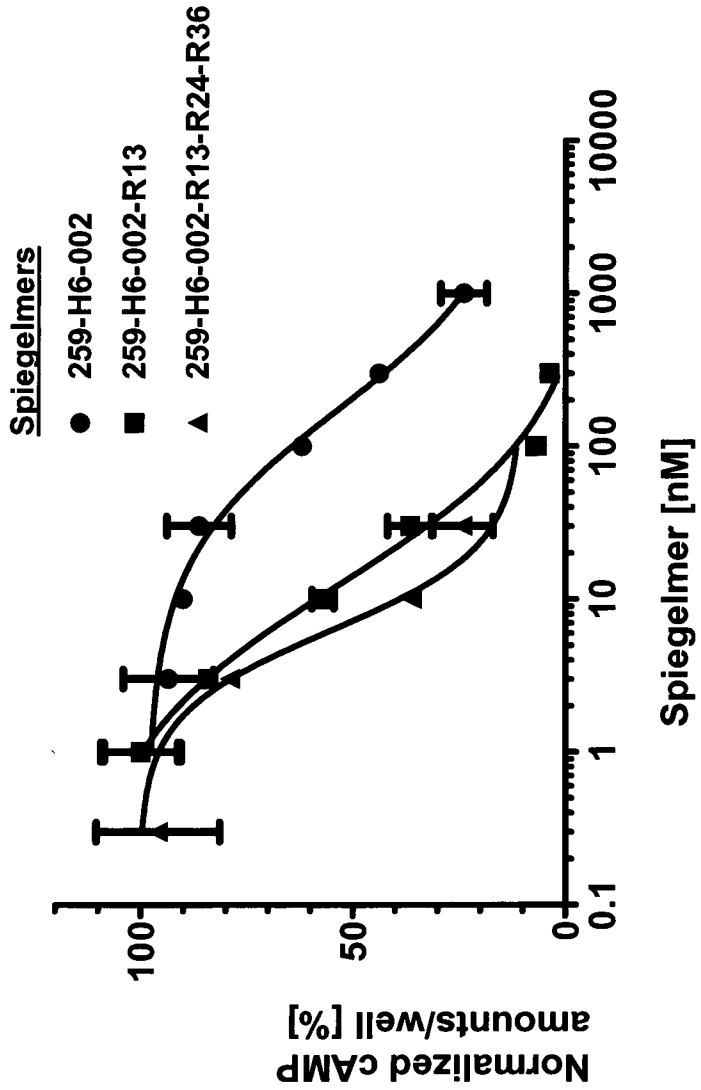

Spiegelmers
259-H6-002
259-H6-002-R13
259-H6-002-R13-R24-R36
Normalized cAMP amounts/well [%]
100
50
0
0.1
1
10
100
1000
10000
Spiegelmer [nM]

Fig.16

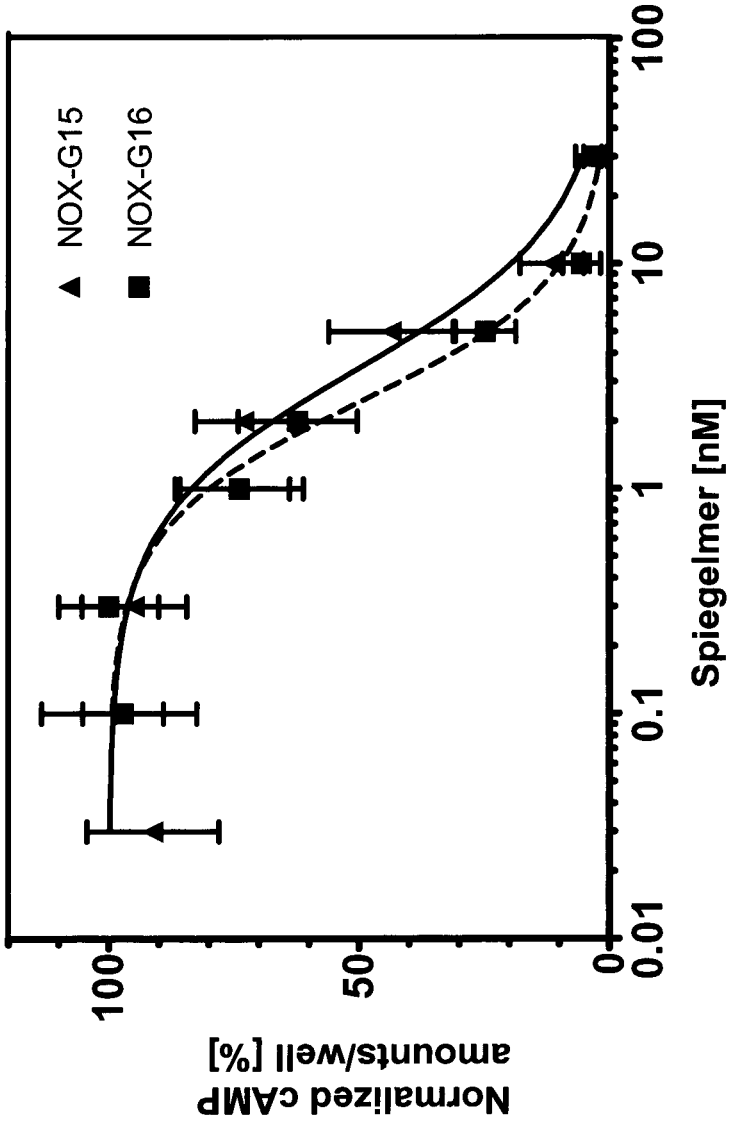

NOX-G15
NOX-G16
Normalized cAMP amounts/well [%]
100
50
0
0.01
0.1
1
10
100
Spiegelmer [nM]

Fig.17

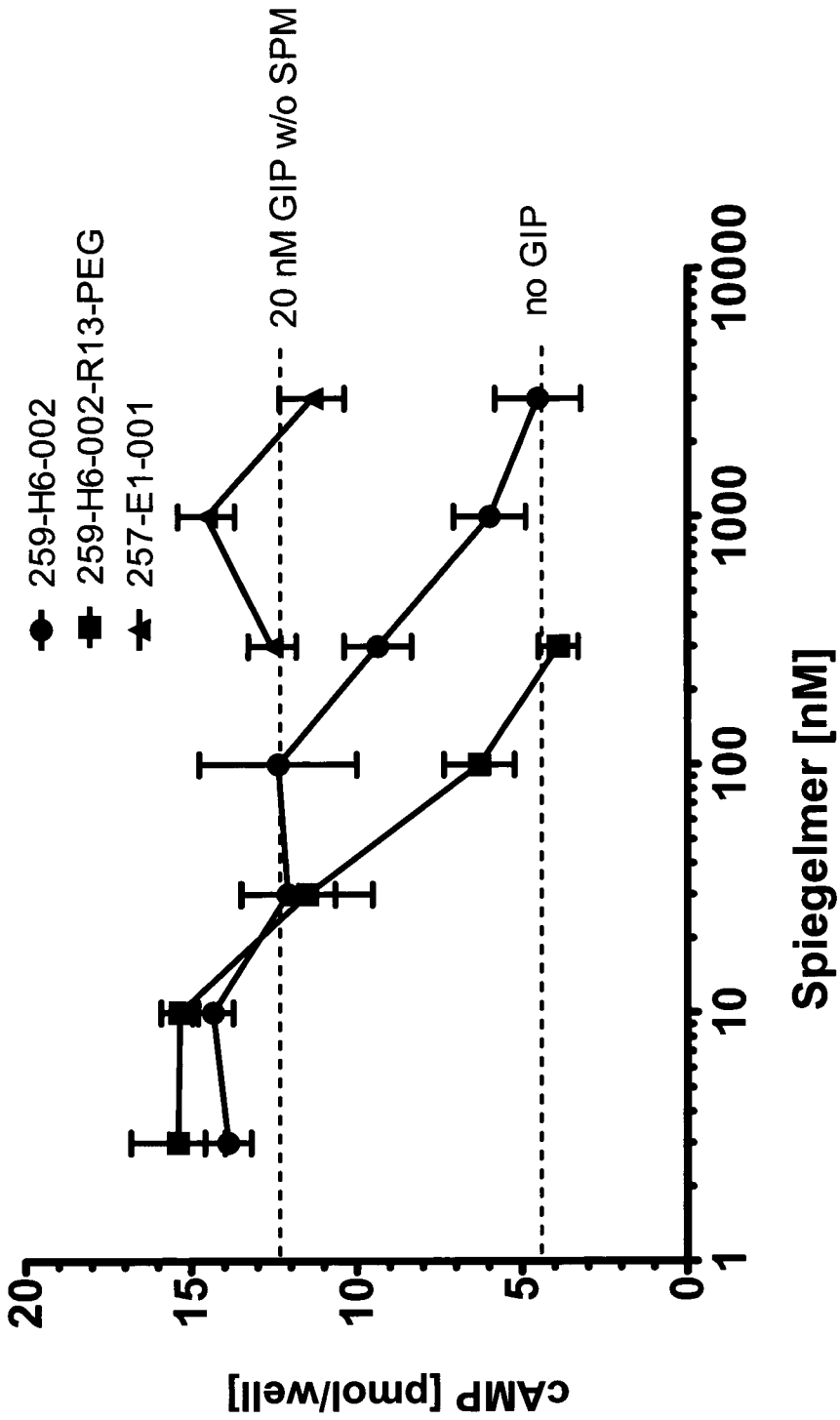
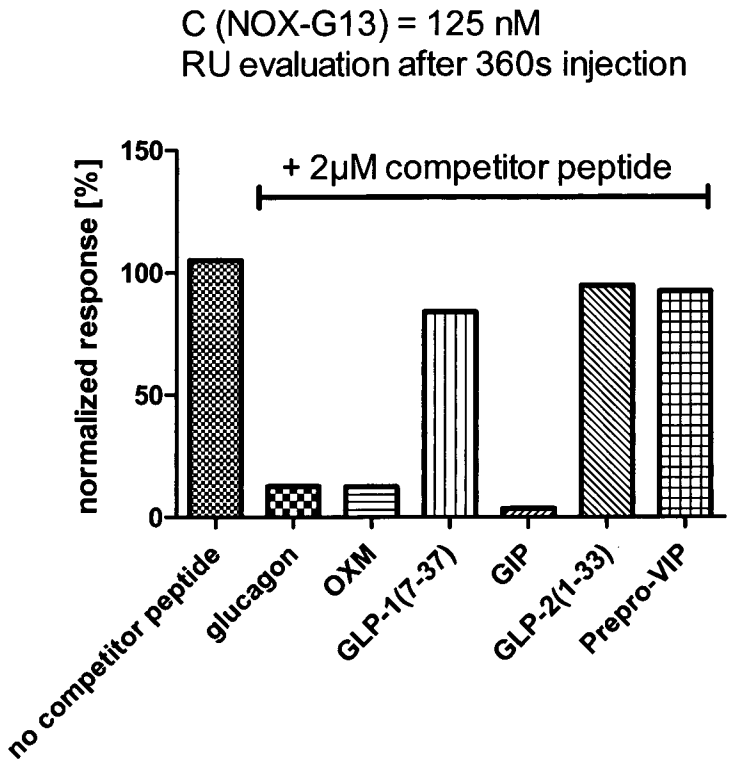

259-H6-002
259-H6-002-R13-PEG
257-E1-001
20 nM GIP w/o SPM
no GIP
cAMP [pmol/well]
Spiegelmer [nM]
0
5
10
15
20
1
10
100
1000
10000

Fig.18


C (NOX-G13) = 125 nM
RU evaluation after 360s injection
+ 2µM competitor peptide
normalized response [%]
150
100
50
0
no competitor peptide
glucagon
OXM
GLP-1(7-37)
GIP
GLP-2(1-33)
Prepro-VIP

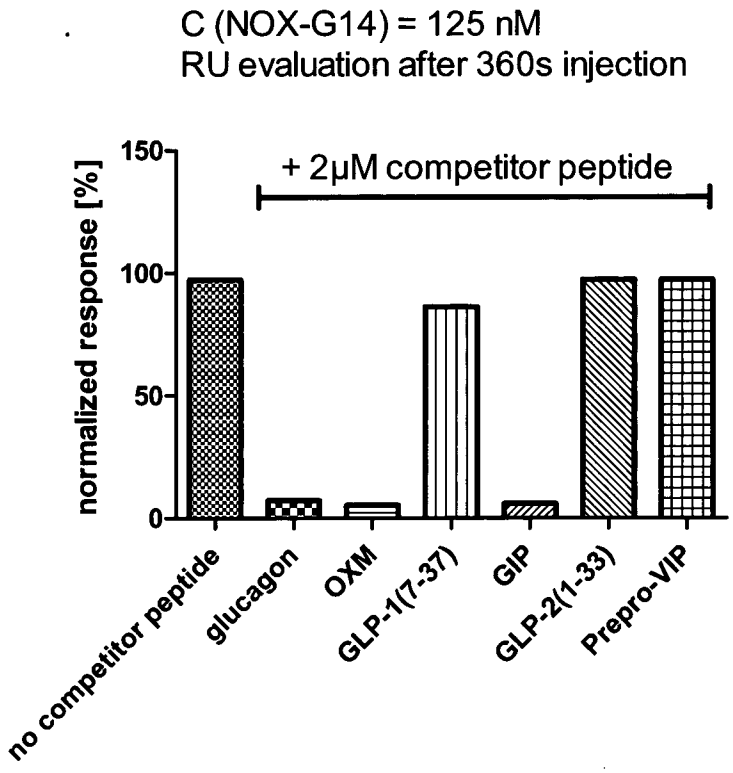
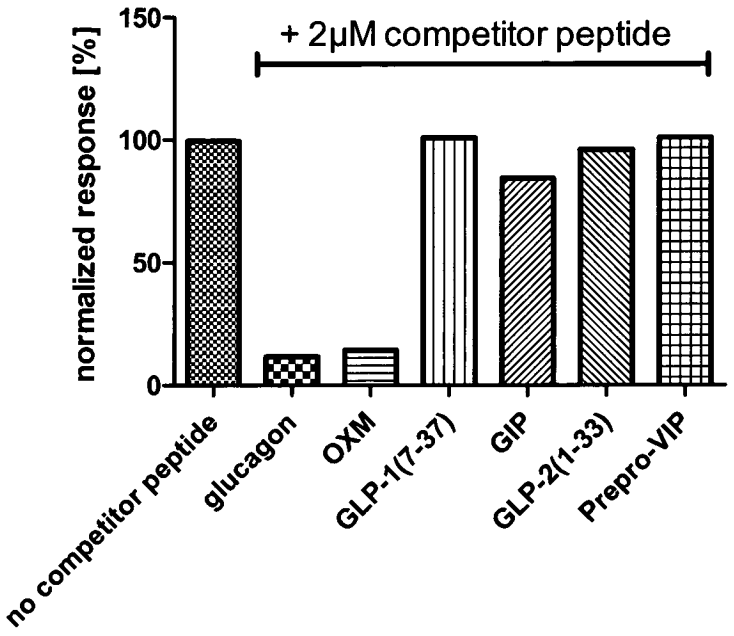

C (NOX-G14) = 125 nM
RU evaluation after 360s injection
+ 2µM competitor peptide
normalized response [%]
150
100
50
0
no competitor peptide
glucagon
OXM
GLP-1(7-37)
GIP
GLP-2(1-33)
Prepro-VIP

Fig. 19 A

C (NOX-G15) = 125 nM
RU evaluation after 360s injection



C (NOX-G16) = 125 nM
RU evaluation after 360s injection

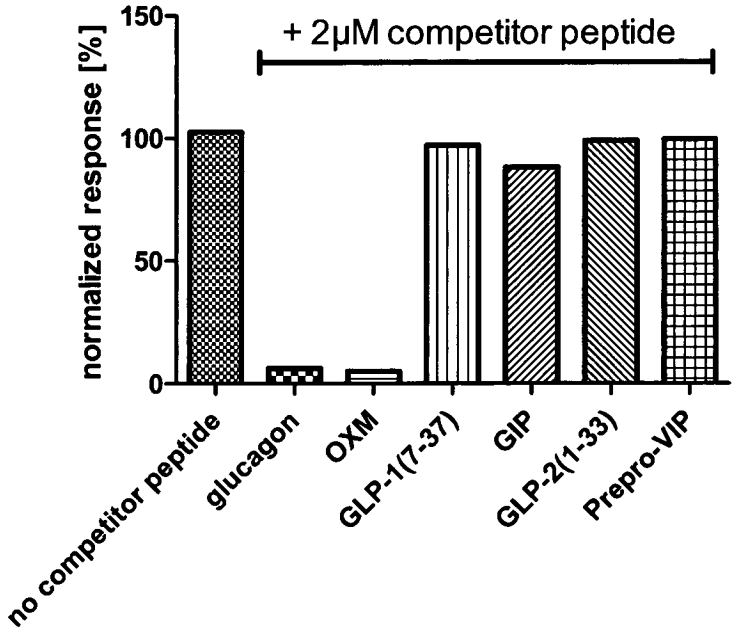


Fig. 19 B

Compound	Glucagon			Glucose-dependent insulinotropic polypeptide (GIP)		
	Comp (SPM) K_D [nM]	Biacore (SPM)	CHO_GR IC_{50} [nM]	Comp (SPM)	Biacore (SPM)	RIN-m5F IC_{50} [nM]
257-E1-6xR-001	5.4*	≈	3	n.c.*	n.c.	
257-E1-7xR-037	4.2*		2	n.c.*		
257-E1-6xR-030-5'-PEG (NOX-G15)	7.5**	≈	3.4		n.c.	>300
257-E1-7xR-037-5'-PEG (NOX-G16)	3.5***	≈	2.4		n.c.	>300
259-H6-002-R13-5'-PEG (NOX-G13)		≈	4.7		≈	~60
259-H6-014-R12/23/35-5'-PEG (NOX-G14)		≈	6.0		≈	~40

Comp(SPM): Spiegelmers (L-nucleic acid) were tested in pull-down competition binding assay vs. non-biotinylated peptides*, or vs. reference molecules 257-E1-6xR-030** and 257-E1-7xR-037***, respectively, wherein for some molecules the dissociation constant (K_D) was determined

Biacore(SPM): Spiegelmers (L-nucleic acid) were tested on Biacore using binding to biotinylated L-glucagon that is competed by an excess of non-biotinylated glucagon or GIP

CHO_GR: Half maximal inhibitory concentration (IC50), Spiegelmers (L-nucleic acid) were tested with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation

RIN-m5F: Spiegelmers were tested with RIN-m5F cells to inhibit GIP-induced cAMP formation

n.c.: no competition of Spiegelmer binding to biotinylated L-glucagon in competition pull-down assay or on Biacore

≈: Spiegelmers show the same affinity to non-biotinylated competitor peptide as to non-biotinylated glucagon on Biacore

Fig.20A

	Glucagon-like peptide-1 (GLP-1)			GLP-2	Oxyntomodulin (OXM)		Prepro Vasoactive intestinal peptide (Prepro VIP) [81-122]	
Compound	Comp (SPM)	Biacore (SPM)	CHO_GR$_{comp}$	Biacore (SPM)	Biacore (SPM)	CHO_GR IC_{50} [nM]	Biacore (SPM)	CHO_GR$_{comp}$
257-E1-6xR-001	n.c.	n.c.	n.c.	n.c.	≈	4.6		n.c.
257-E1-7xR-037	n.c.							
257-E1-6xR-030-5'-PEG (NOX-G15)		n.c.	n.c.	n.c.	≈		n.c.	
257-E1-7xR-037-5'-PEG (NOX-G16)		n.c.	n.c.	n.c.	≈		n.c.	
259-H6-002-R13-5'-PEG (NOX-G13)		n.c.	n.c.	n.c.	≈		n.c.	n.c.
259-H6-014-R12/23/35-5'-PEG (NOX-G14)		n.c.	n.c.	n.c.	≈	9.6	n.c.	n.c.

Comp(SPM): Spiegelmers (L-nucleic acid) were tested in pull-down competition binding assay vs. non-biotinylated GLP-1

Biacore(SPM): Spiegelmers (L-nucleic acid) were tested on Biacore using binding to biotinylated L-glucagon that is competed by an excess of non-biotinylated GLP-1, GLP-2, OXM, or Prepro VIP(81-122)

CHO_GR: Half maximal inhibitory concentration (IC50), Spiegelmers (L-nucleic acid) were tested with CHO cells stably transfected with the human glucagon receptor to inhibit oxyntomodulin induced cAMP formation

CHO_GR$_{comp}$: Spiegelmers were tested with CHO cells stably transfected with the human glucagon receptor to inhibit glucagon induced cAMP formation, whereas an excess of GLP-1 or Prepro VIP (81-122) competes with glucagon for Spiegelmer binding to abolish the inhibition of glucagon-induced cAMP formation

n.c.: no competition of Spiegelmer binding to biotinylated glucagon in competition pull-down assay or on Biacore; no competition of Spiegelmer action on glucagon induced cAMP formation

≈: Spiegelmers show the same affinity to non-biotinylated competitor peptide as to non-biotinylated glucagon on Biacore

Fig.20B

Glucagon Precursor Cleaved into the following 8 chains:

1. Glicentin
2. Glicentin-related polypeptide, Short name=GRPP
3. Oxyntomodulin, Short name=OXY,Short name=OXM
4. Glucagon
5. Glucagon-like peptide 1, Short name=GLP-1
6. Glucagon-like peptide 1(7-37), Short name=GLP-1(7-37)
7. Glucagon-like peptide 1(7-36), Short name=GLP-1(7-36)
8. Glucagon-like peptide 2, Short name=GLP-2

```
Glicentin                                 RSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA
GRPP                                      RSLQDTEEKSRSFSASQADPLSDPDQMNED
OXY/OXM                                                                 HSQGTFTSDYSKYLDSRRA-QDFVQWLMNTKRNRNNIA
Glucagon                                                                HSQGTFTSDYSKYLDSRRA-QDFVQWLMNT
GLP-1                                                                HDEFERHAEGTFTSDVSSYLEGQAA-KEFIAWLVKGRG
GLP-1(7-37)                                                             HAEGTFTSDVSSYLEGQAA-KEFIAWLVKGRG
GLP-1(7-36)                                                             HAEGTFTSDVSSYLEGQAA-KEFIAWLVKGR
GLP-2                                                                   HADGSF-SDEMNTILDNLAARDFINWLIQTKITD
GIP                                                                     YAEGTFISDYSIAMDKIHQ-QDFVNWLLAQKGKKNDWKHNITQ
Intestinal peptide PHV-42/ Prepro-VIP (81-122)                          HADGVFTSDFSKLLGQLSA-KKYLESLMGKRVSSNISEDPVPV
Intestinal peptide PHM-27                                               HADGVFTSDFSKLLGQLSA-KKYLESLM
```

Fig.21

Glucagon species alignment

Human	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Rat	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Mouse	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Squirrel Monkey	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Pig	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Guinea pig	HSQGTFTSDYSKYLDSRRAQQFLKWLLNV
Rabbit	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Hamster	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Chinchilla	HSQGTFTSDYSKHLDSRYAQEFVQWLMNT
Dog	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Sheep	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Chicken	HSQGTFTSDYSKYLDSRRAQDFVQWLMST
Bovine	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT

Fig.22

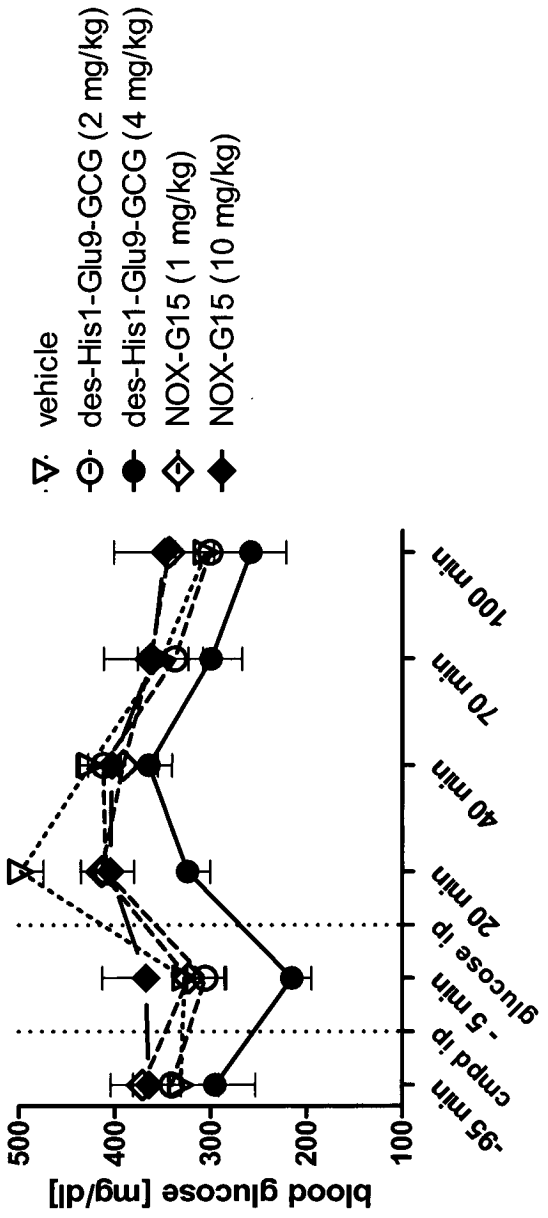

vehicle
des-His1-Glu9-GCG (2 mg/kg)
des-His1-Glu9-GCG (4 mg/kg)
NOX-G15 (1 mg/kg)
NOX-G15 (10 mg/kg)
blood glucose [mg/dl]
500
400
300
200
100
-95 min
cmpd ip
-5 min
glucose ip
20 min
40 min
70 min
100 min

Fig.23 A

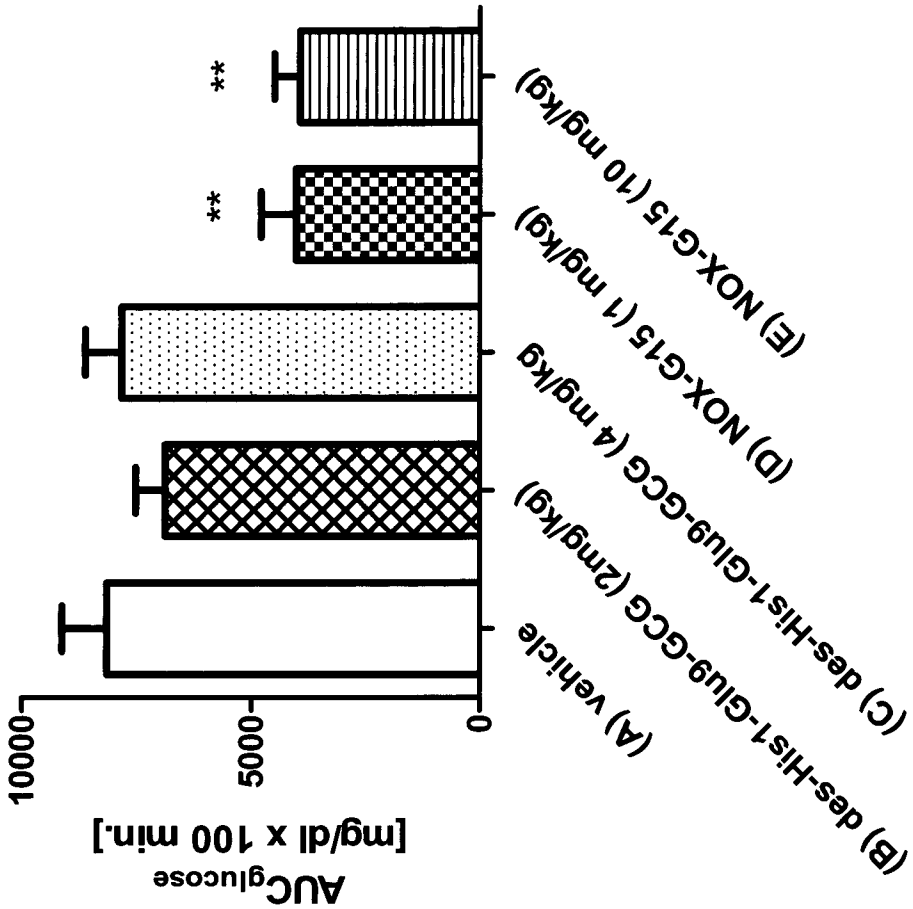

AUC glucose
[mg/dl x 100 min.]
10000
5000
0
(A) vehicle
(B) des-His1-Glu9-GCG (2mg/kg)
(C) des-His1-Glu9-GCG (4 mg/kg
(D) NOX-G15 (1 mg/kg)
(E) NOX-G15 (10 mg/kg)
**
**

Fig.23 B

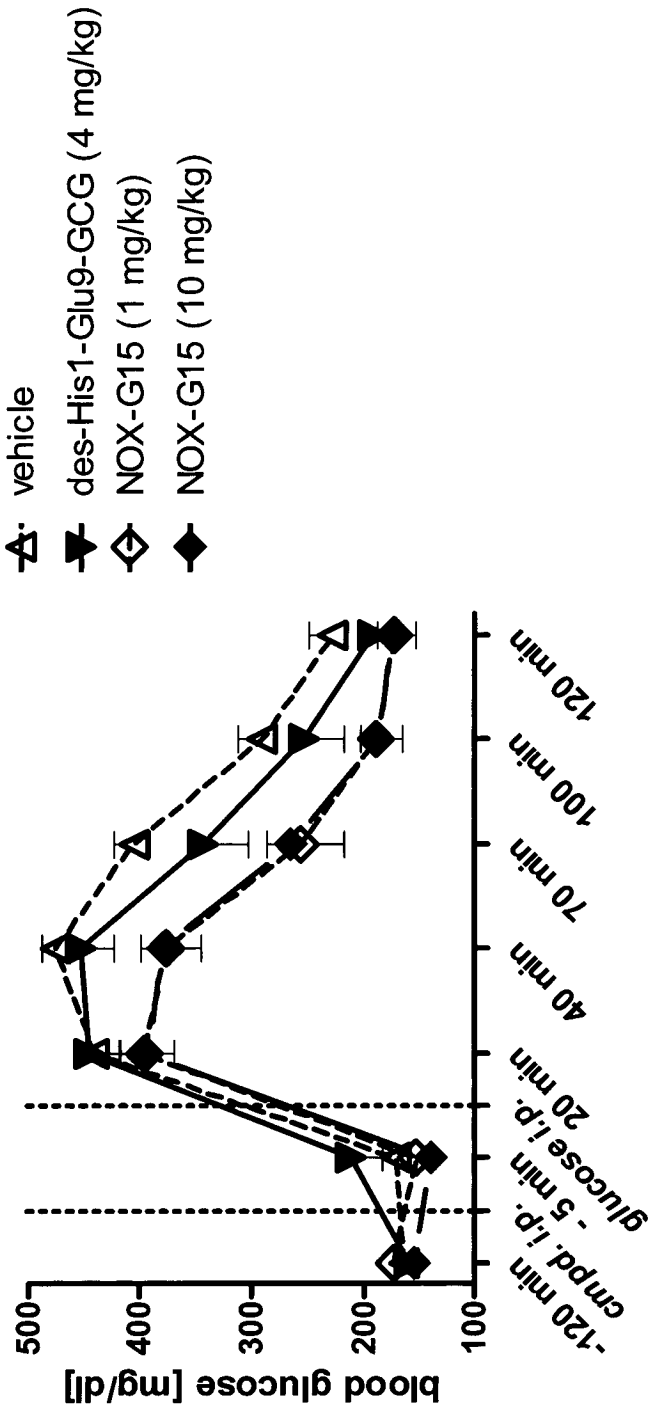

vehicle
des-His1-Glu9-GCG (4 mg/kg)
NOX-G15 (1 mg/kg)
NOX-G15 (10 mg/kg)
blood glucose [mg/dl]
500
400
300
200
100
-120 min
cmpd. i.p.
- 5 min
glucose i.p.
20 min
40 min
70 min
100 min
120 min

Fig.24 A

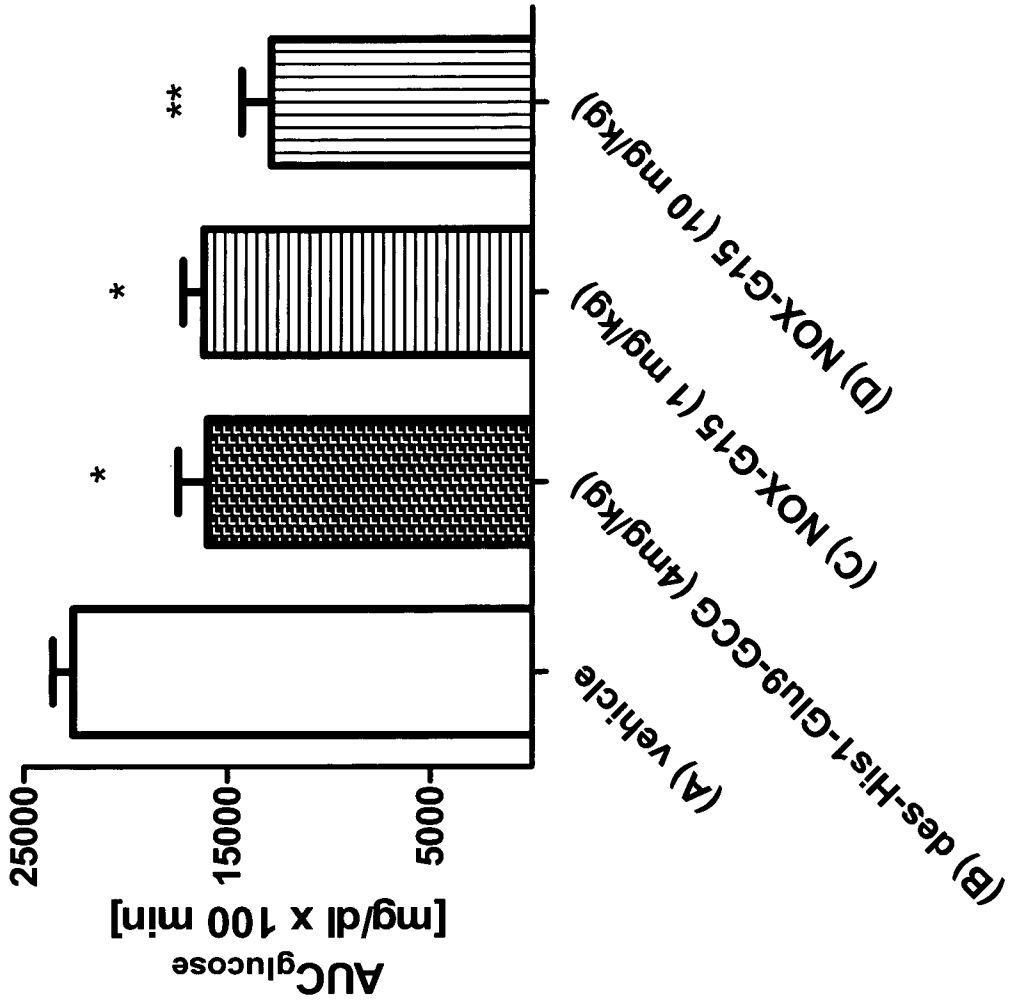

AUC glucose
[mg/dl x 100 min]
25000
15000
5000
(A) Vehicle
(B) des-His1-Glu9-GCG (4mg/kg)
(C) NOX-G15 (1 mg/kg)
(D) NOX-G15 (10 mg/kg)
*
*
**

Fig.24 B

Compound	Sequence: 5'->3'	K_D (SPM) [nM]
NOX-G11stabi2	CAGACGUGUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	**55.7**
NOX-G11-D05	CAGA**dC**GUGUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	18.1
NOX-G11-D07	CAGACG**dT**GUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	12.2
NOX-G11-D15	CAGACGUGUGUGGG**dT**AGAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	22.0
NOX-G11-D16	CAGACGUGUGUGGGU**dA**GAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	17.3
NOX-G11-D19	CAGACGUGUGUGGGUAGA**dT**GCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	15.5
NOX-G11-D20	CAGACGUGUGUGGGUAGAU**dG**CACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	29.4
NOX-G11-D21	CAGACGUGUGUGGGUAGAUG**dC**ACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	11.2
NOX-G11-D22	CAGACGUGUGUGGGUAGAUGC**dA**CCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	14.6
NOX-G11-D23	CAGACGUGUGUGGGUAGAUGCA**dC**CUGCGAUUCGCUAAAAAGUGCCACACGUCUG	27.4

any of dG, dC, dT and dA is a 2'-desoxyribonucleotide; any of G, A, C and U is a ribonucleotide;

Dissociation constant K_D of Spiegelmers was measured as surface plasmon resonance on Biacore using direct binding to immoblized human glucagon

SPM : Spiegelmer

Fig.25 A

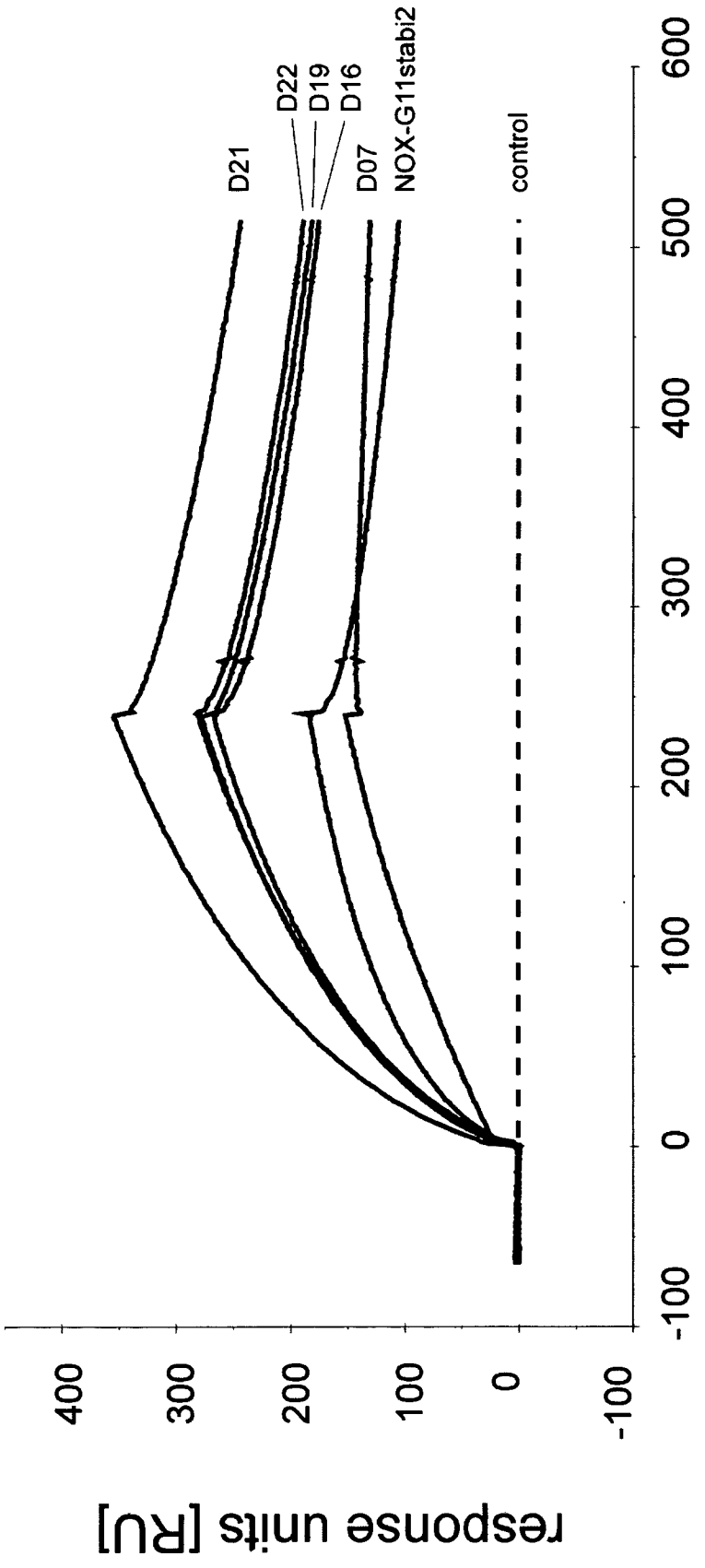
Compound	Sequence: 5'->3'	K_D (SPM) [nM]
NOX-G11stabi2	CAGACGUGUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCCACACGUCUG	**55.7**
NOX-G11-D24	CAGACGUGUGUGGGUAGAUGCAC**dC**UGCGAUUCGCUAAAAAGUGCCACACGUCUG	22.5
NOX-G11-D25	CAGACGUGUGUGGGUAGAUGCACC**dT**GCGAUUCGCUAAAAAGUGCCACACGUCUG	27.2
NOX-G11-D26	CAGACGUGUGUGGGUAGAUGCACCU**dG**CGAUUCGCUAAAAAGUGCCACACGUCUG	22.0
NOX-G11-D27	CAGACGUGUGUGGGUAGAUGCACCUG**dC**GAUUCGCUAAAAAGUGCCACACGUCUG	25.3
NOX-G11-D46	CAGACGUGUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCC**dA**CACGUCUG	23.9
NOX-G11-D48	CAGACGUGUGUGGGUAGAUGCACCUGCGAUUCGCUAAAAAGUGCCAC**dA**CGUCUG	33.8

any of dG, dC, dT and dA is a 2'-desoxyribonucleotide; any of G, A, C and U is a ribonucleotide;

Dissociation constant K_D of Spiegelmers was measured as surface plasmon resonance on Biacore using direct binding to immoblized human glucagon

SPM : Spiegelmer

Fig.25 B



D21 = NOX-G11-D21 D22 = NOX-G11-D22 D19 = NOX-G11-D19 D16 = NOX-G11-D16

D07 = NOX-G11-D07

Fig.26

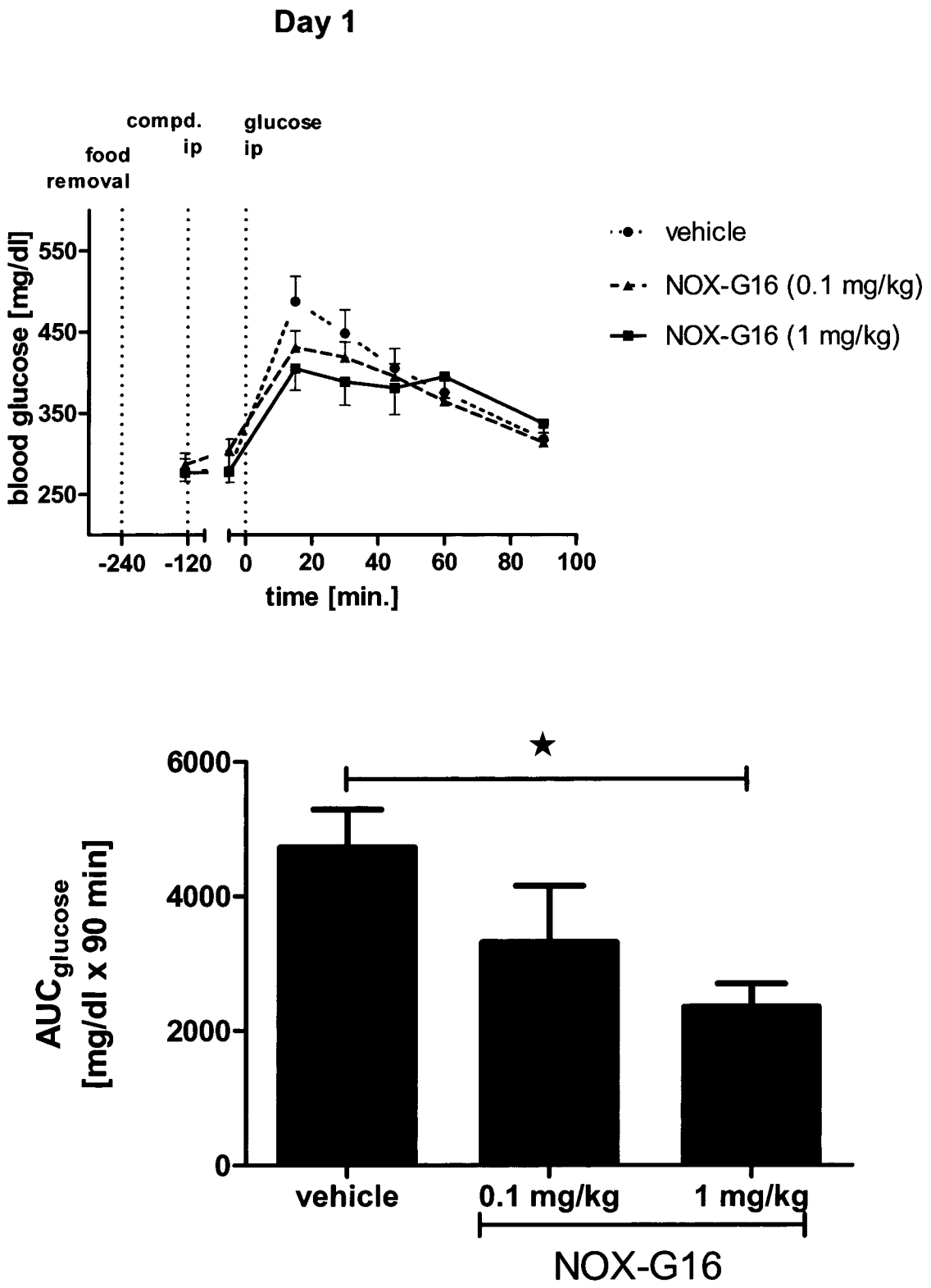

Day 1
compd. ip
glucose ip
food removal
blood glucose [mg/dl]
550
450
350
250
-240
-120
0
20
40
60
80
100
time [min.]
vehicle
NOX-G16 (0.1 mg/kg)
NOX-G16 (1 mg/kg)
AUC glucose [mg/dl x 90 min]
6000
4000
2000
0
vehicle
0.1 mg/kg
1 mg/kg
NOX-G16

Fig.27A

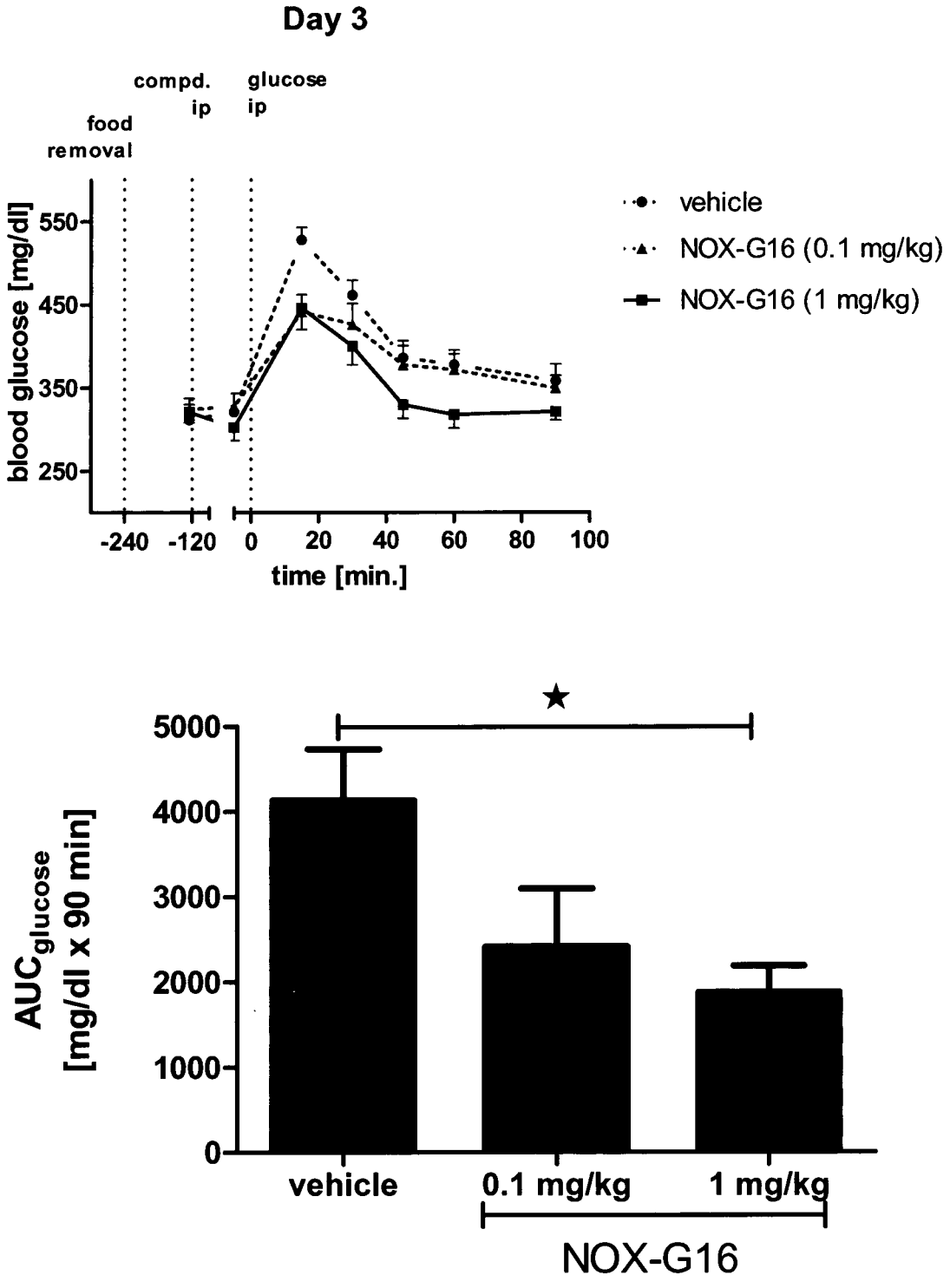

Day 3
compd. ip
glucose ip
food removal
blood glucose [mg/dl]
550
450
350
250
-240
-120
0
20
40
60
80
100
time [min.]
vehicle
NOX-G16 (0.1 mg/kg)
NOX-G16 (1 mg/kg)
AUC glucose [mg/dl x 90 min]
5000
4000
3000
2000
1000
0
vehicle
0.1 mg/kg
1 mg/kg
NOX-G16

Fig.27B

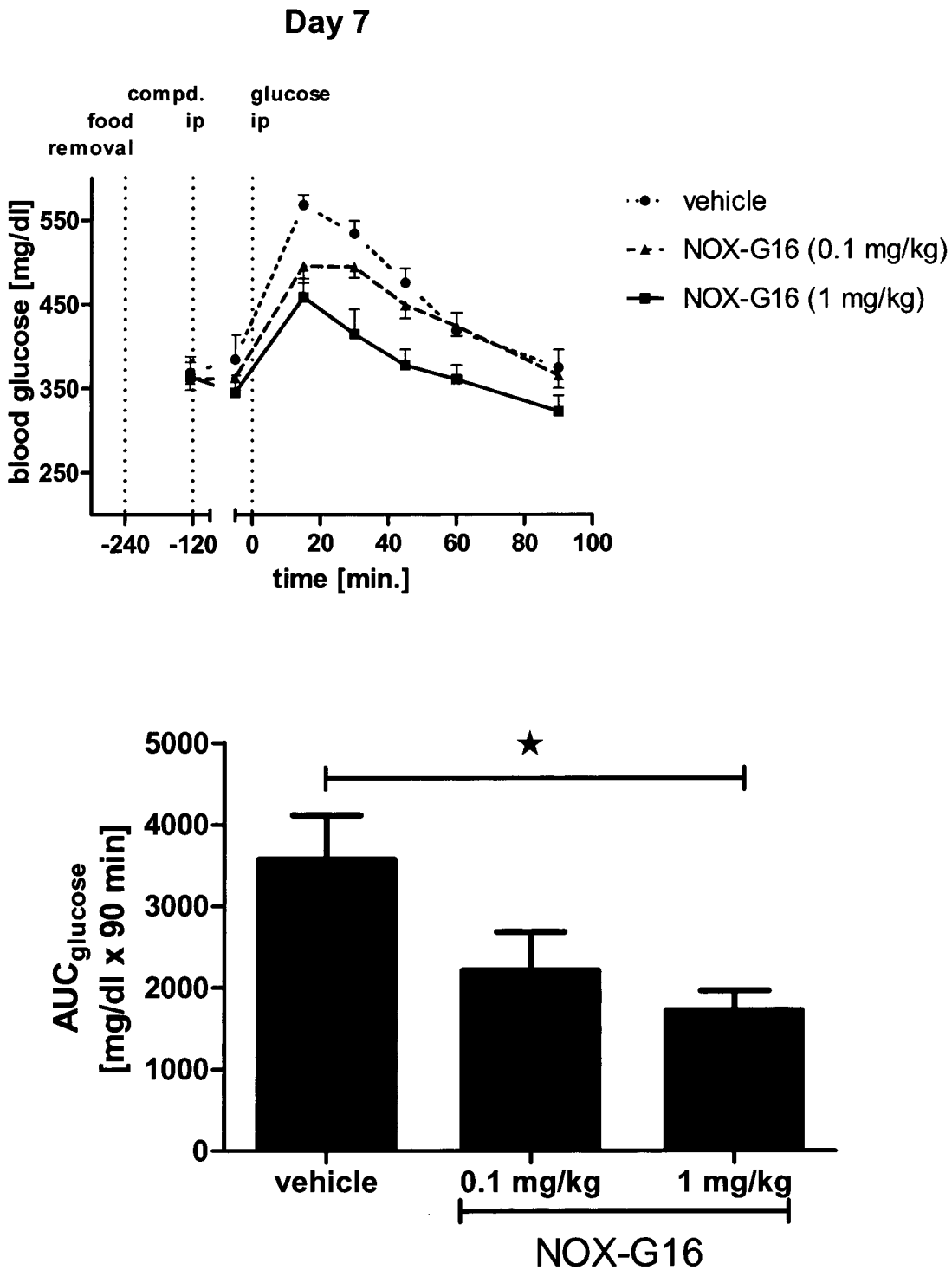

Day 7
compd. ip
glucose ip
food removal
blood glucose [mg/dl]
550
450
350
250
-240
-120
0
20
40
60
80
100
time [min.]
vehicle
NOX-G16 (0.1 mg/kg)
NOX-G16 (1 mg/kg)
AUC glucose [mg/dl x 90 min]
5000
4000
3000
2000
1000
0
★
vehicle
0.1 mg/kg
1 mg/kg
NOX-G16

Fig.27C

Day 9

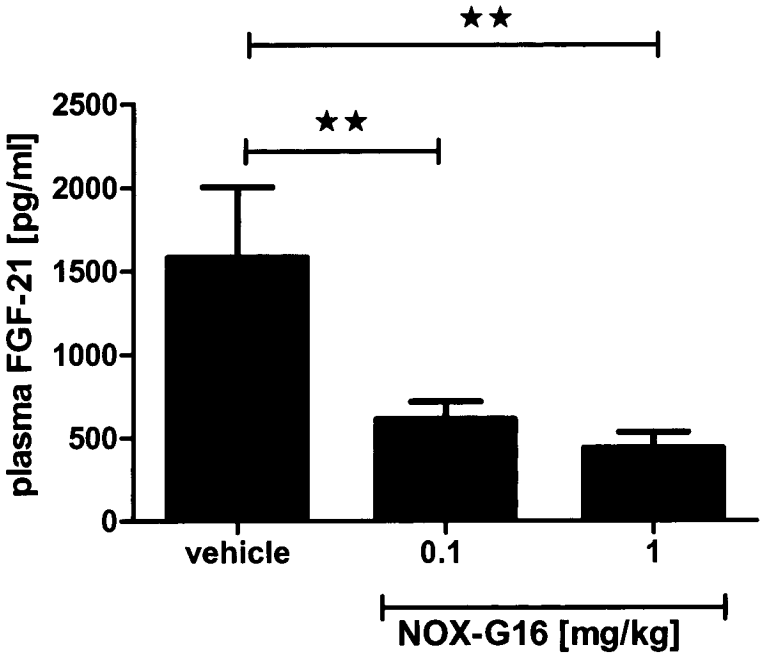

★★
★★
2500
2000
1500
1000
500
0
plasma FGF-21 [pg/ml]
vehicle
0.1
1
NOX-G16 [mg/kg]

Fig.28

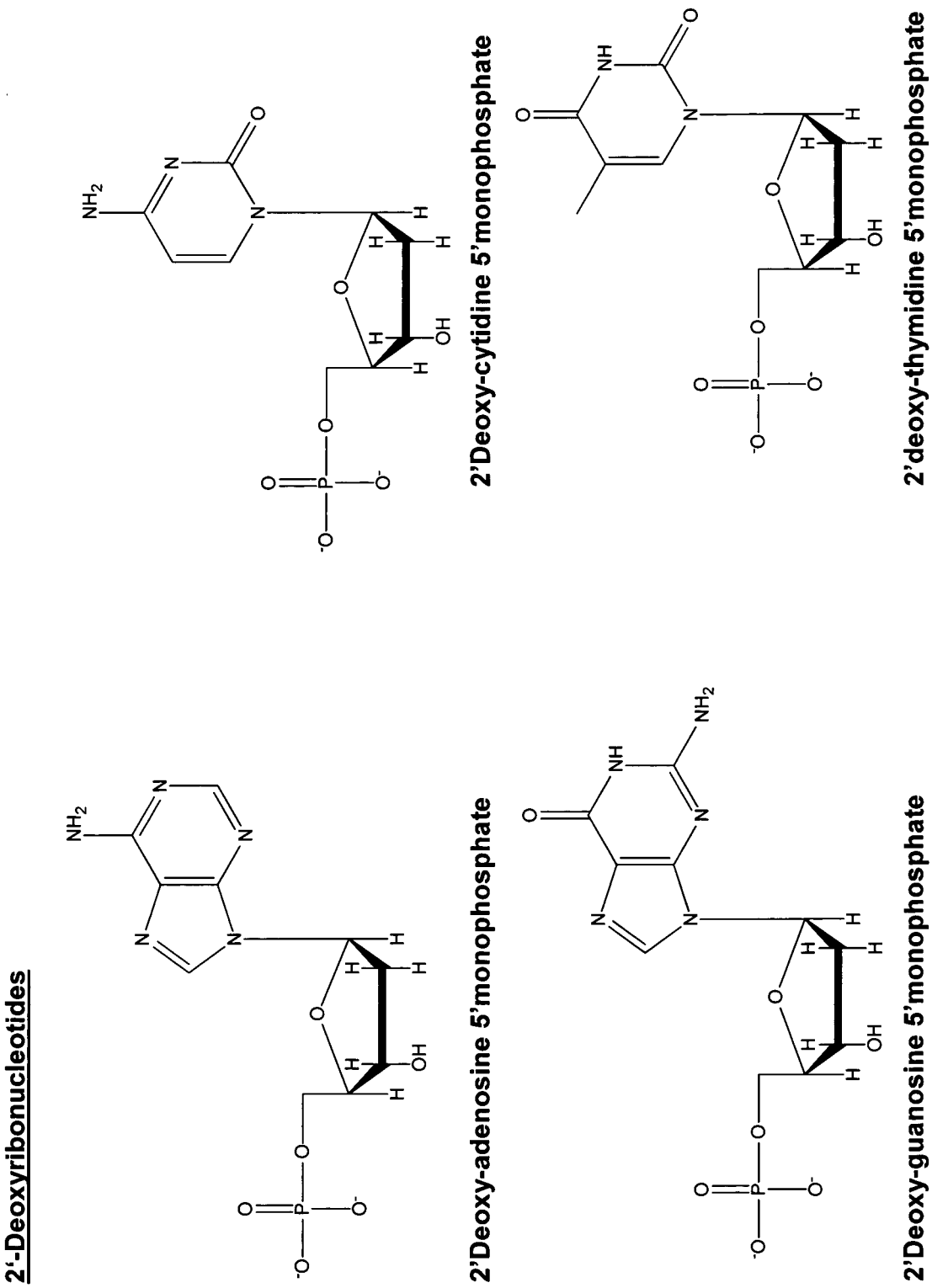

2'-Deoxyribonucleotides
2'Deoxy-cytidine 5'monophosphate
2'deoxy-thymidine 5'monophosphate
2'Deoxy-adenosine 5'monophosphate
2'Deoxy-guanosine 5'monophosphate

Fig. 29

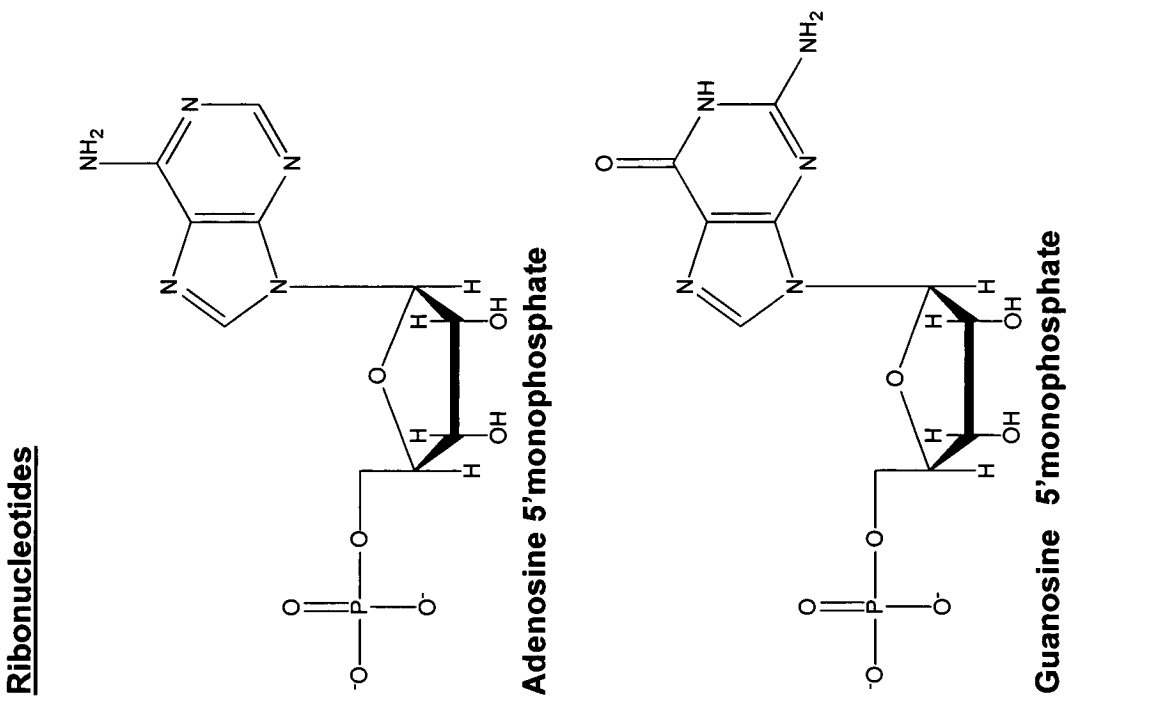
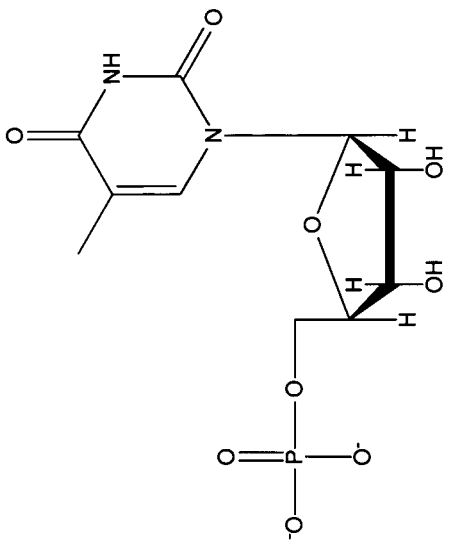

Ribonucleotides
Adenosine 5'monophosphate
Guanosine 5'monophosphate
Cytidine 5'monophosphate
Uridine 5'monophosphate

Fig. 30 A

Ribonucleotides



Thymidine 5'monophosphate (5-Methyl-uridine 5'monophosphate)

Fig. 30B