

## **Ureidopeptide GLP-1 analogues with prolonged activity *in vivo* via signal bias and altered receptor trafficking†**

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Electronic Supplementary Information

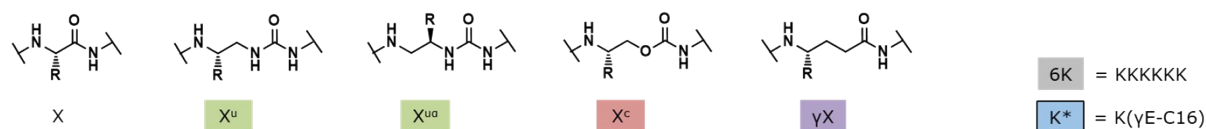
## Additional Results

### Screening of different urea residues with various side chains

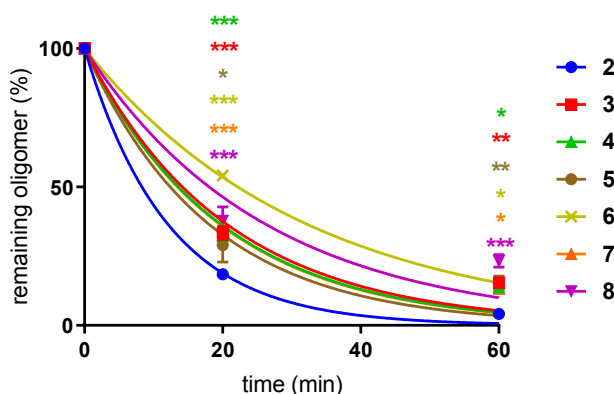
**Table S1:** Ureido screening of GLP-1 at position 2.

	Sequence										Compound	EC <sub>50</sub> (nM) <sup>a</sup>	SE (nM) <sup>b</sup>	Potency (%) <sup>c</sup>																															
	1	5	10	15	20	25	30	35	40																																				
<b>1</b>	H	H	A	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	OH	GLP-1	0.10 <sup>d</sup>	0.01 <sup>e</sup>	100									
<b>2</b>	H	H	A	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1-NH <sub>2</sub>	0.19	0.06	53									
<b>3</b>	H	H	A <sup>u</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[A <sup>u</sup> ] <sup>2</sup>	0.14	0.05	70									
<b>4</b>	H	H	A <sup>v</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[A <sup>v</sup> ] <sup>2</sup>	1.4	0.3	7									
<b>5</b>	H	H	A <sup>c</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[A <sup>c</sup> ] <sup>2</sup>	0.8	0.3	13									
<b>6</b>	H	H	A <sup>aa</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[A <sup>aa</sup> ] <sup>2</sup>	3.4	0.9	3									
<b>7</b>	H	H	A <sup>g</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[A <sup>g</sup> ] <sup>2</sup>	0.8	0.2	13									
<b>8</b>	H	H	A <sup>v</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[V <sup>v</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S1</b>	H	H	A <sup>s</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[S <sup>s</sup> ] <sup>2</sup>	1.3	0.4	8									
<b>S2</b>	H	H	A <sup>r</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[R <sup>r</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S3</b>	H	H	A <sup>c</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[C <sup>c</sup> ] <sup>2</sup>	65	39	0									
<b>S4</b>	H	H	A <sup>q</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[Q <sup>q</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S5</b>	H	H	A <sup>e</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[E <sup>e</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S6</b>	H	H	A <sup>i</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[I <sup>i</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S7</b>	H	H	A <sup>l</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[L <sup>l</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S8</b>	H	H	A <sup>k</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[K <sup>k</sup> ] <sup>2</sup>	>10000	N/A	<0.01									
<b>S9</b>	H	H	A <sup>f</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[F <sup>f</sup> ] <sup>2</sup>	0.5	0.1	21									
<b>S10</b>	H	H	A <sup>t</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[T <sup>t</sup> ] <sup>2</sup>	3.3	1.0	3									
<b>S11</b>	H	H	A <sup>w</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[W <sup>w</sup> ] <sup>2</sup>	2.2	0.6	5									
<b>S12</b>	H	H	A <sup>g</sup>	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R	G	NH <sub>2</sub>	GLP-1[G <sup>g</sup> ] <sup>2</sup>	0.24	0.06	42									
<b>9</b>	H	H	G	E	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	NH <sub>2</sub>	Exenatide	0.19	0.08	53	
<b>10</b>	H	H	G	E	G	T	F	T	S	D	L	S	K	Q	L	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	NH <sub>2</sub>	Ex4 <sup>L</sup>	0.28	0.12	36	
<b>11</b>	H	H	A <sup>u</sup>	E	G	T	F	T	S	D	L	S	K	Q	L	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	NH <sub>2</sub>	Ex4 <sup>L</sup> [A <sup>u</sup> ] <sup>2</sup>	0.08	0.05	125	
<b>12</b>	H	H	G	E	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	6K	NH <sub>2</sub>	Lixisenatide	0.26	0.11	38
<b>13</b>	H	H	G	E	G	T	F	T	S	D	L	S	K	Q	L	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	6K	NH <sub>2</sub>	Lixi <sup>L</sup>	0.06	0.03	164
<b>14</b>	H	H	A <sup>u</sup>	E	G	T	F	T	S	D	L	S	K	Q	L	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S	6K	NH <sub>2</sub>	Lixi <sup>L</sup> [A <sup>u</sup> ] <sup>2</sup>	0.09	0.03	118
<b>15</b>	H	H	A	E	G	T	F	T	S	D	V	S	S	L	E	G	Q	A	A	K*	E	F	I	A	W	L	V	R	G	R	G	OH	Liraglutide	58	20	0.2									

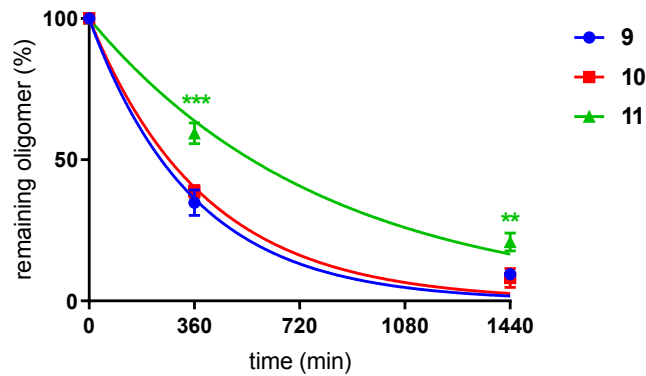
<sup>a</sup> GLP-1R potency (EC<sub>50</sub>). <sup>b</sup> SE on the EC<sub>50</sub>. <sup>c</sup> Percentage of potency compare to GLP-1-NH<sub>2</sub> (**2**). <sup>d</sup> Mean value of 22 experiments. <sup>e</sup> SEM on the EC<sub>50</sub>. Unless otherwise stated, the data are EC<sub>50</sub> values ± SE obtained by non-linear regression on points concentration-response curves performed in duplicates (n=2). GLP-1R: GLP-1 receptor; EC<sub>50</sub>: half maximal effective concentration; SE: standard error; SEM: standard error of the mean.



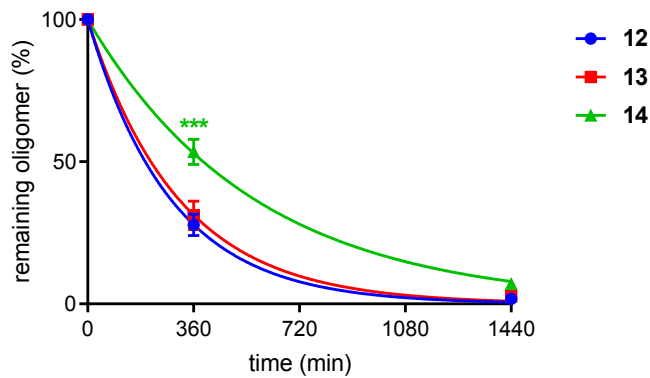
### In vitro Mouse plasma stability studies



**Figure S1:** Mouse plasma degradation assay (Method MP1). Stability of selected GLP-1 analogues in mouse plasma. Data are mean  $\pm$  SEM, n = 3. Statistic by two-way anova and Bonferroni post-test: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, comparing peptide 2 to oligomers.

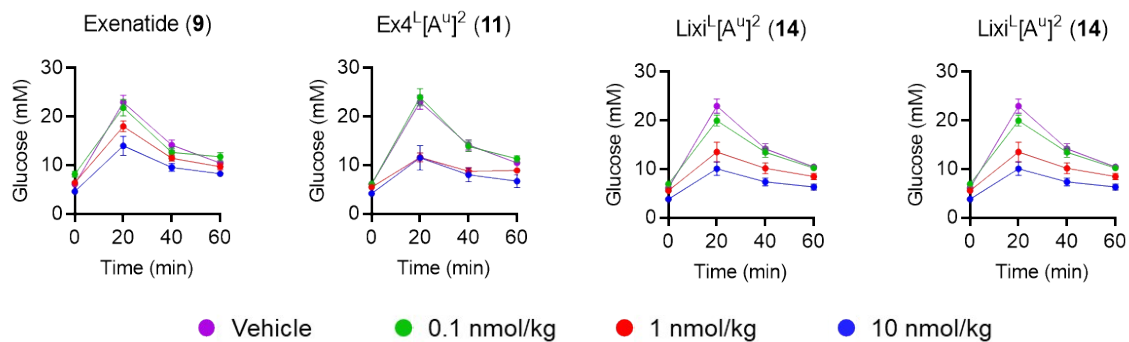


**Figure S2:** Mouse plasma degradation assay (Method MP2). Stability of selected exenatide analogues in mouse plasma. Data are mean  $\pm$  SEM, n = 3. Statistic by two-way anova and Bonferroni post-test: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, comparing peptide 9 to oligomers.



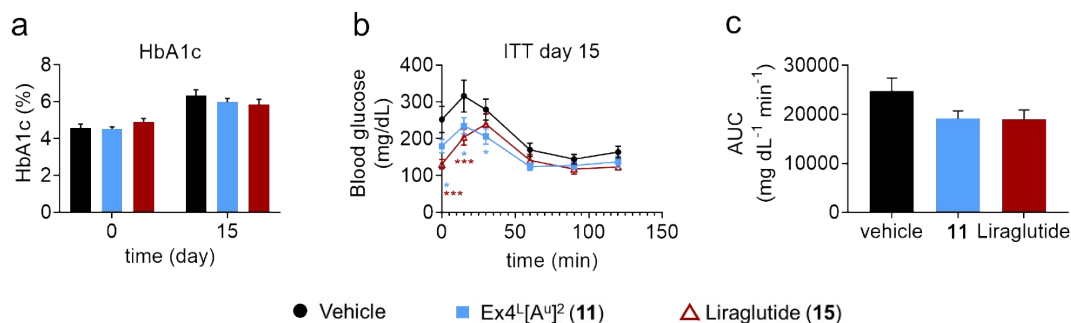
**Figure S3:** Mouse plasma degradation assay (Method MP3). Stability of selected lixisenatide analogues in mouse plasma. Data are mean  $\pm$  SEM, n = 3. Statistic by two-way anova and Bonferroni post-test: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, comparing peptide 12 to oligomers.

### Dose-response study in healthy mice



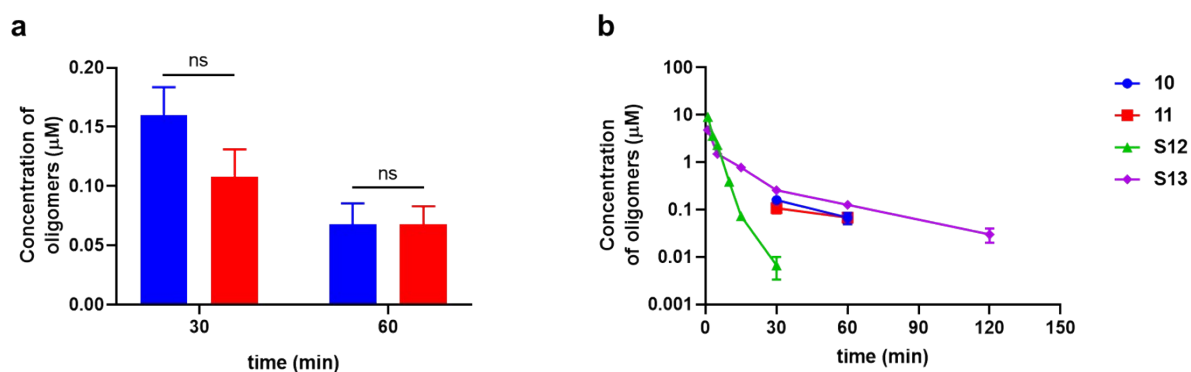
**Figure S4 :** Dose-response relationships of exenatide (9), Lixi<sup>L</sup> (13) and their putative ureidopeptides Ex4<sup>L</sup>[A<sup>U</sup>]<sup>2</sup> (11) and Lixi<sup>L</sup>[A<sup>U</sup>]<sup>2</sup> (14) in healthy mice (C57BL/6J, male, 25-30 g). IPGTT (2 mmol/kg glucose) performed 6 hours after administration of indicated agonist dose in lean C57Bl/6 mice (n=8). Dosage and formulation: 0.01 to 10 nmol·kg<sup>-1</sup> in 100  $\mu$ L saline *i.p.*. Fasted 4 h. Data are mean  $\pm$  SEM.

## Study in db/db mice treated over 15 days



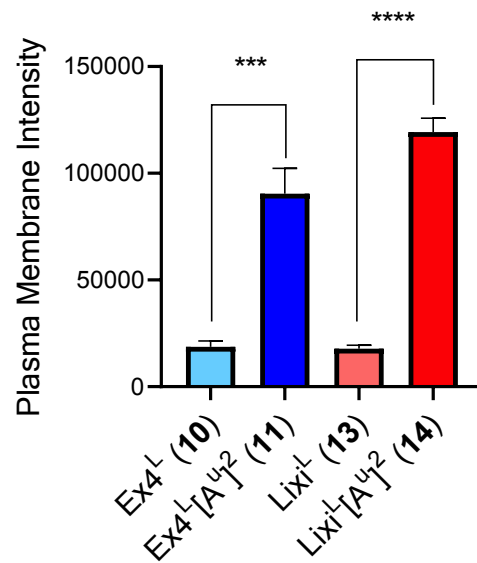
**Figure S5** : Comparative pharmacodynamic of Ex4<sup>L</sup>[A<sup>u</sup>]<sup>2</sup> (**11**), Lixi<sup>L</sup>[A<sup>u</sup>]<sup>2</sup> (**14**) and liraglutide (**15**) in db/db mice treated over 15 days. Dosage: 100 µg kg<sup>-1</sup> (25 nmol kg<sup>-1</sup>) *s.c.* once a day. Formulation: 20 µg mL<sup>-1</sup> in PBS 1×. (a) HbA1c level at the beginning (day 0) and at the end (day 15) of the treatment. (b) ITT after 6 h of the dosing at day 15: trace and AUC. ITT: 2 U/kg of insulin *i.p.* at T0. Data are mean ± SEM (n=10). Statistic by two-way anova and Bonferroni post-test: comparing vehicle to oligomers; one way anova with Dunnett's multiple comparison test: comparing vehicle to oligomers. ITT: Insulin tolerance test; AUC: Area under the curve; *s.c.*: subcutaneous; *i.p.*: intra peritoneal.

## PK Studies



**Figure S6** : Pharmacodynamic of Ex4<sup>L</sup> (**10**) and Ex4<sup>L</sup>[A<sup>u</sup>]<sup>2</sup> (**11**), GLP-1-G<sup>2</sup>-NH<sub>2</sub> (**S12**)<sup>[1]</sup> and GLP-1-G<sup>2</sup>[A<sup>u</sup>A<sup>u</sup>]<sup>28-31</sup> (**S13**)<sup>[1]</sup> in healthy mice. Mice: male C57BL/6J; dose: 2 mg k<sup>-1</sup>; formulation: 1 mg mL<sup>-1</sup> in PBS 1X; route: *i.v.* Data are mean ± SEM (n=4 for **10** and **11**, n=3 for **S13** and **S14**). Statistic by two-way anova and Bonferroni post-test: ns = p>0.05 (non-significant). *i.v.* intra venous. The PK data of **S12** and **S13** were taken from our previously reported work.<sup>[1]</sup>

### SNAP-GLP-1R internalization quantification



**Figure S7** : Quantification of SNAP-GLP-1R internalisation from Figure 6b data: Plasma membrane intensities quantified from area under the curve (AUC) of baseline-subtracted full-width at half-maximum peaks of membrane intensity profiles for the different conditions measured. 3 profiles analysed per image of a total of 2-3 images per experiment, n=3 experiments. \*\*\*p<0.001 and \*\*\*\*p<0.0001, one-way ANOVA with Sidak's multiple comparison test. Note that increased plasma membrane intensity corresponds to decreased internalisation propensity.

## Methods

### **Statistical analysis.**

GraphPad Prism 7.0 and 8.0 were used for all analyses. Replicate measurements were taken from different samples. Statistical analyses (two-way t-test, two-way ANOVA and Bonferroni post-test, one-way ANOVA with Dunnett's multiple comparison test, two-way ANOVA with Tukey's test, one-way ANOVA with Sidak's multiple comparison test) were applied when indicated. P values lower than 0.05 were considered significant.

### **Synthesis of oligomers 2-14 and S1-S12**

#### **General Procedure P1**

Oligomers **2-14** and **S1-S12** were synthesized using the following general procedure as previously described:<sup>[1]</sup>

Sieber resin ( $\approx$  160mg, loading 0.62mmol/g) was swelled in DMF (3 mL) for 30 min. All steps were performed under microwave irradiation. The synthesis were conducted with microwave irradiation using the Liberty Blue™ microwave peptide synthesizer from CEM.

#### A1: Fmoc deprotection

The *N*-Fmoc protecting group was removed with 20% piperidine in DMF (3 ml) with the standard liberty blue methods.<sup>[2]</sup>

#### A2: Coupling of Fmoc-amino acid

*N*-Fmoc- $\alpha$  amino acid (5 eq. relative to the resin loading) were coupled with PyBOP or DIC/Oxyma (5 eq. relative to the resin loading) and DIEA (10 eq. relative to the resin loading) as coupling reagent using the standard liberty blue methods.<sup>[2]</sup>

#### A3: Coupling of activated N<sub>3</sub>-building bloc

Each activated monomer (3 eq. relative to the resin loading) was coupled twice using DIEA (10 eq. relative to the resin loading) under microwave irradiation (70°C, 50W, 20 min) in DMF (4 mL).

#### A4: Reduction of azide group

The reduction of the azido group was performed twice in a mixture of 1,4-dioxane/H<sub>2</sub>O (7:3 v/v) (5 mL) with a 1M PMe<sub>3</sub> solution in THF (10 eq. relative to the resin loading) under microwave irradiation (50°C, 50W, 30 min).

#### A5: Cleavage from the resin

After completion of the synthesis, the resin was transferred into a syringe with a frit, and washed three times with DMF, three times with CH<sub>2</sub>Cl<sub>2</sub> and three times with Et<sub>2</sub>O. Cleavage from the resin was performed using 95% TFA with 2.5% triisopropylsilane and 2.5% water (3 mL). After 2h the resin was filtered and discarded. Diethyl ether was added to precipitate the oligomer and the solid was triturated and filtrated.

#### Purification and characterization:

Analytical RP-HPLC analyses were performed on a Dionex U3000SD using a Macherey-Nagel Nucleodur C18ec column (4 x 100 mm, 3  $\mu$ m) at a flow rate of 1 mL/min with UV detection at 200 nm. The mobile phase was composed of 0.1% (v/v) TFA-H<sub>2</sub>O (Solvent A) and 0.1% (v/v) TFA-CH<sub>3</sub>CN (solvent B).

Semi preparative purification of all compound was performed on a Dionex U3000SD using a Macherey-Nagel Nucleodur C18ec column (10 x 250 mm, 5  $\mu$ m) at a flow rate of 4 mL/min with UV detection at 200 nm. The mobile phase was composed of 0.1% (v/v) TFA-H<sub>2</sub>O (Solvent A) and 0.1% (v/v) TFA-CH<sub>3</sub>CN (solvent B).

LC-MS analyses were carried out on a UHPLC (Agilent 1290 Infinity) coupled to a ESI -MS Tof (Agilent 6230 ESI).

Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on an Agilent 6560 DTIMS-Q-TOF spectrometer (Agilent Technologies, Santa Clara, CA), with the dual-ESI source operated in positive ion mode.

## Synthesis of oligomers 15

### Procedure P2

*N*-Fmoc-Lys(Boc)-OH at position 20 was replaced by *N*-Fmoc-Lys(Alloc)-OH and *N*-Fmoc-His(Trt)-OH at position 1 was replaced by *N*-Boc-His(Boc)-OH.

The oligomer was synthesized using procedure **P1**. Then the resin was transferred in a 10 mL syringe, 5 mL of DCM was added and the Alloc group was removed using Pd(Ph<sub>3</sub>)<sub>4</sub> (30 mg, 0.25 equiv relative to the resin loading) and phenylsilane (135  $\mu$ L, 1.1 equiv relative to the resin loading) at room temperature for 45 min. After filtration and washes with DMF (2x) and DCM (3x), DCM (5 mL), *N*-Fmoc-Glu-*OtBu* (222 mg, 5 equiv relative to the resin loading), PyBop (260 mg, 5 equiv relative to the resin loading), and DIEA (93  $\mu$ L, 5 equiv relative to the resin loading) were loaded on the resin and it was shaken for 2 hours at room temperature. Fmoc group was removed with piperidine in DMF (20%), 2 times 20 min. The resin was washed with DMF (2x) and DCM (3x), then palmitoyl chloride (93  $\mu$ L), and DIEA (260  $\mu$ L) were loaded on the resin and it was shaken for 2 hours at room temperature. The cleavage, the purification and the characterization were the same as procedure **P1**.

## Cell lines and culture

- PathHunter CHO-GLP-1R- $\beta$ arr2 cells (DiscoverX) were grown in the manufacturer's proprietary medium 6. INS-1 832/3 cells (a kind gift from Prof Christopher Newgard, Duke University, USA) were grown in RPMI supplemented with 11 mM D-glucose, supplemented with 10% FBS, 10 mM HEPES, 2 mM L-glutamine, 1 mM pyruvate,  $\mu$ M  $\beta$ -mercaptoethanol and 1% penicillin/streptomycin.
- Stable SNAP-GLP-1R-expressing INS-1 832/3 cells were generated from INS-1 832/3 cells where endogenous GLP-1R was deleted by CRISPR/Cas9 (a gift from Dr. Jacqui Naylor, MedImmune)<sup>[5]</sup> by transfection of pSNAP-GLP-1R (Cisbio) and G418 (1 mg/ml) selection, and cultured as above in the presence of G418.
- HEK-293T cells (Multispan inc., lot#.DC1267-062017) were suspended in cell culture media (DMEM 1X + GlutaMAX (Gibco 31966-021)) complemented with FBS 10% (Sigma Aldrich F7524), Pen/Strep 1% (Sigma Aldrich P4333).
- $\beta$ TC6 cells were suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4) and 500  $\mu$ M IBMX.

## Cyclic AMP measurements

### *In vitro* pharmacology: Affinity (IC<sub>50</sub>)

The affinity of oligomers at the mouse GLP-1 receptor endogenously expressed in  $\beta$ TC6 cells was determined in a radioligand binding assay (performed by Cerep S.A., catalog 2015, ref. 0228) as previously described.<sup>[4]</sup> Cell membrane homogenates (20  $\mu$ g protein) are incubated for 120 min at 37°C with 0.025 nM [<sup>125</sup>I]GLP-1(7-36) in the absence or presence of the test compound in a buffer containing 50 mM Hepes-NaOH (pH 7.4), 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1 mM EDTA, 0.025% bacitracin and 1% BSA. Nonspecific binding is determined in the presence of 1  $\mu$ M GLP-1(7-36). Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with an ice-cold buffer containing 50 mM Tris-HCl and 500 mM NaCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is exenatide (**9**), which is tested in each experiment at several concentrations to obtain a competition curve from which its IC<sub>50</sub> is calculated using Graphpad Prism.

### *In vitro* pharmacology on HEK-293T cells (EC<sub>50</sub>: Method C1)

The agonist activity of compounds at the human GLP-1 receptor exogenously expressed in HEK-293T cells (Multispan inc., lot#.DC1267-062017) was determined by measuring their effects on cAMP production using the HTRF detection method (performed by UREKA SARL). The cells were distributed in 384-well microplates at a density of 1.0x10<sup>4</sup> cells/well. Stock solutions of the test compounds or the reference agonist were prepared at a concentration of 1 mM in DMSO. Then, compounds to be tested were diluted in cell culture media and transferred to the plate containing the cells to reach final assay concentrations in the range 10<sup>-8</sup> to 10<sup>-15</sup> M. The cells were incubated for 10 min at 5% CO<sub>2</sub> at 37°C. The reaction was then stopped by addition of fluorescence donor (anti-cAMP antibody labeled with europium cryptate) and the fluorescence acceptor (D2-labeled cAMP) mixed in lysis buffer (cAMP Gs-Dynamic kit, Cisbio). After 120 min at room temperature, a microplate reader (F500 Tecan) was used to measure the fluorescence transfer at  $\lambda_{ex}$  = 337 nm and  $\lambda_{em}$  = 620 nm and 665 nm. The ratio of the signal measured at 665 nm on signal measured at 620 nm is used to determine the cAMP concentration. The results are given as a percent of the control response to 10 nM Forskolin. The standard reference agonist is GLP-1-G2-NH<sub>2</sub> (peptide 1), which is tested in each experiment at several concentrations to generate a concentration response curve from which its EC<sub>50</sub> value is calculated using GraphPad Prism.

### *In vitro* pharmacology on immortalised beta cells (EC<sub>50</sub>: Method C2)

The agonist activity of compounds at the mouse GLP-1 receptor endogenously expressed in  $\beta$ TC6 cells was determined by measuring their effects on cAMP production using the HTRF detection method (performed by Cerep S.A., catalog 2015, ref. 2181) as previously described.<sup>[1,3]</sup> The cells were distributed in microplates at a density of 1.5x10<sup>4</sup> cells/well and incubated for 10 min at room temperature in the presence of HBSS (basal control), the test compound or the reference agonist. Following incubation, the cells were lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at  $\lambda_{ex}$ = 337 nm and  $\lambda_{em}$ = 620 nm and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the



signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 10 nM GLP-1(7-37). The standard reference agonist is GLP-1(7-37), which is tested in each experiment at several concentrations to generate a concentration-response curve from which its EC<sub>50</sub> value and SE are calculated using GraphPad Prism.

*In vitro* pharmacology on immortalised CHO-GLP-1R cells (EC<sub>50</sub>: Method C3)

PathHunter CHO-GLP-1R cells were stimulated for 30 min at 37°C with indicated concentration of agonist in serum free medium without phosphodiesterase inhibitors. The reaction was terminated by addition of cAMP detection reagents (cAMP Dynamic 2, Cisbio) and read by HTRF.

### **β-arrestin-2 recruitment assay**

PathHunter CHO-GLP-1R cells were stimulated for 30 min at 37°C with indicated concentration of agonist in serum free medium. The reaction was terminated by addition of PathHunter detection reagents (DiscoverX) and luminescent signal recorded.

### **Calculation of signal bias**

Biased signalling was quantified using a modified form of the operational model.<sup>[6]</sup> LogR values were calculated for each ligand in each assay, and ΔLogR were determined by subtracting from the corresponding values for URK-062. Bias was then determined as ΔΔLogR by subtracting ΔLogR for cAMP from β-arrestin-2. As all ligands were run in parallel in both pathways, calculations were done on a per-assay basis<sup>3</sup>. Bias was compared statistically by determining the difference between LogR for cAMP and β-arrestin-2 for each ligand and performing a randomised-block 1-way ANOVA on this value.

### **Insulin secretion *in vitro***

*In vitro* insulin secretion was performed as previously described.<sup>[7]</sup> INS-1 832/3 cells were primed at 3 mM glucose overnight before the assay. After washing, cells were added in suspension to the normal growth medium at 11 mM glucose, with or without agonist. In 96 well plates. After overnight incubation, a sample of supernatant was removed and analysed for insulin by HTRF (Insulin Hi Range, Cisbio).

### **Surface SNAP-GLP-1R labelling and internalization**

SNAP-GLP-1R-expressing INS-1 832/3 cells were labeled at 37°C with 1 μM of SNAP-Surface 549 fluorescent probe (New England Biolabs) in full media prior to being stimulated with 10 nM ligands for 30 minutes, fixed in 4% paraformaldehyde, mounted in Prolong Diamond antifade reagent with 4,6-diamidino-2-phenylindole (Life Technologies), and imaged with a Zeiss LSM-780 inverted confocal laser-scanning microscope in a 63x/1.4 numerical aperture oil-immersion objective from the Facility for Imaging by Light Microscopy (FILM) at Imperial College London, and analyzed in Fiji.

### **Mouse plasma stability (Method MP1)**

Stock solutions of the oligomers were prepared at a concentration of 250  $\mu\text{M}$  in mQ water. The oligomer was then diluted 1/50 with a solution of plasma/PBS pH 7.4 (1/1) to afford a final concentration of 5 $\mu\text{M}$  and incubated at 37°C. (4 $\mu\text{L}$  of the stock solution was diluted with 196  $\mu\text{L}$  of plasma/PBS, pH 7.4, 1/1) Each compound was also incubated in the absence of plasma (196  $\mu\text{L}$  of H<sub>2</sub>O/PBS, pH 7.4, 1/1). At the indicated time (20min and 60min) an aliquot of 70  $\mu\text{L}$  was removed from each experimental reaction and pipetting into 175  $\mu\text{L}$  of Acetonitrile at 0°C to quench the reaction. (t = 0 min was determined using the reaction without plasma). The samples were frozen at -80°C before analysis. The frozen sample were defrosted, stirred with a vortex 5 min and finally centrifuged 5 min at 16°C. The supernatant was analyzed by LC-MS. The time course of oligomer degradation was determined by integrating the area of the peak in the extracted ion chromatogram.

### **Mouse plasma stability (Method MP2)**

Stock solutions of the oligomers were prepared at a concentration of 250  $\mu\text{M}$  in DMSO. The oligomer was then diluted 1/50 with a solution of plasma/PBS pH 7.4 (1/1) to afford a final concentration of 5 $\mu\text{M}$  and incubated at 37°C. (4 $\mu\text{L}$  of the stock solution was diluted with 196  $\mu\text{L}$  of plasma/PBS, pH 7.4, 1/1) Each compound was also incubated in the absence of plasma (196  $\mu\text{L}$  of H<sub>2</sub>O/PBS, pH 7.4, 1/1). At the indicated time (20min and 60min) an aliquot of 70  $\mu\text{L}$  was removed from each experimental reaction and pipetting into 175  $\mu\text{L}$  of Acetonitrile at 0°C to quench the reaction. (t = 0 min was determined using the reaction without plasma). The samples were frozen at -80°C before analysis. The frozen sample were defrosted, stirred with a vortex 5 min and finally centrifuged 5 min at 16°C. The supernatant was analyzed by LC-MS. The time course of oligomer degradation was determined by integrating the area of the peak in the extracted ion chromatogram.

### **Mouse plasma stability (Method MP3)**

Stock solutions of the oligomers were prepared at a concentration of 250  $\mu\text{M}$  in DMSO. The oligomer was then diluted 1/50 with a solution of plasma/PBS pH 7.4 (1/1) to afford a final concentration of 5 $\mu\text{M}$  and incubated at 37°C. (4 $\mu\text{L}$  of the stock solution was diluted with 196  $\mu\text{L}$  of plasma/PBS, pH 7.4, 1/1) Each compound was also incubated in the absence of plasma (196  $\mu\text{L}$  of H<sub>2</sub>O/PBS, pH 7.4, 1/1). At the indicated time (20min and 60min) an aliquot of 70  $\mu\text{L}$  was removed from each experimental reaction and pipetting into 10  $\mu\text{L}$  of HCL 12N solution. The remaining mixture was then pipetting into 165  $\mu\text{L}$  of Acetonitrile at 0°C to quench the reaction. (t = 0 min was determined using the reaction without plasma). The samples were frozen at -80°C before analysis. The frozen sample were defrosted, stirred with a vortex 5 min and finally centrifuged 5 min at 16°C. The supernatant was analyzed by LC-MS. The time course of oligomer degradation was determined by integrating the area of the peak in the extracted ion chromatogram.

### **Animals.**

For the pharmacodynamics studies performed by Physiogenex S.A.S., mice were housed in ventilated and enriched housing cages (310 x 125 x 127 mm<sup>3</sup>) throughout the experimental phase. The mice were housed in groups of 3 animals during the study, on a normal 12 hours light cycle (at 8:00 pm lights off), 22  $\pm$  2 °C and 50  $\pm$  10 % relative humidity. A standard chow diet (RM1 (E) 801492, SDS) and tap water were provided ad libitum. All animal protocols done by Physiogenex S.A.S were reviewed and approved by the local (Comité régional d'éthique de Midi-Pyrénées) and national (Ministère de l'Enseignement

Supérieur et de la Recherche) ethics committees (protocol number 05049-06). For the dose-response study, animal procedures were approved by British Home Office under the UK Animal (Scientific Procedures) Act 1986 (Project Licence 70/7596). Lean male C57Bl/6J mice (6-8 weeks of age, body weight 25-30 g, obtained from Charles River) were maintained at 21-23°C and light-dark cycles (12:12h schedule, lights on at 07:00). *Ad libitum* access to water and normal chow (RM1, Special Diet Services) was provided unless otherwise stated. Mice were housed in groups of five. For the pharmacokinetics studies performed by TechMedILL service (PCBIS platform, CNRS UMS3286), mice were housed in polycarbonate cages (PCT2L12SHT, Allentown) enriched with play tunnels throughout the experimental phase. The mice were housed in groups of 9 animals during the study, under controlled environment ( $22 \pm 1$  °C) with a relative humidity ( $50 \pm 10$  %) and a normal 12 hours light cycle (at 8:00 pm lights off). A standard chow diet (A04, SAFE, France) and tap water were provided *ad libitum*. All animal protocols done by TechMedILL service were reviewed and approved by the agriculture ministry regulating animal research in France (Ethics regional committee for animal experimentation Strasbourg, APAFIS 1341#2015080309399690).

#### **IPGTT experiments in healthy mice (IPGTT 3 h and 6 h).**

After the acclimation period of 5 days, 20-25 g male C57Bl/6J mice 8 weeks old (Charles River laboratories) were randomized into groups (6 mice per group) according to their body weight. The mice were acutely treated with vehicle or 1 µg per mouse ( $10 \text{ nmol kg}^{-1}$ ) of oligomers via *i.v.* injections (formulation: 4 µg mL<sup>-1</sup> in PBS 1×) IPGTTs (glucose 2 g kg<sup>-1</sup> *i.p.*) were performed after 3 or 6 hours of dosing with a fasting period of 6 hours. Blood glucose was measured before the administration of glucose and after at 30, 60, 90 and 120 min.

#### **Fasted Blood Glucose and IPGTT experiments in healthy mice (IPGTT 9 h).**

After the acclimation period of 5 days, 20-25 g male C57Bl/6J mice 8 weeks old (Charles River laboratories) were randomized into groups (6 mice per group) according to their body weight. The mice were acutely treated with vehicle or 1 µg per mouse ( $10 \text{ nmol kg}^{-1}$ ) of oligomers via *i.v.* injections (formulation: 4 µg mL<sup>-1</sup> in PBS 1×). The mice were fasted and blood glucose was measure before dosing (T0) and after 2, 4, 6, 8 and 9 hours. An IPGTT (glucose 2 g kg<sup>-1</sup> *i.p.*) was performed after 9 hours of dosing with a fasting period of 9 hours. Blood glucose was measured before the administration of glucose and after 30, 60 and 90 min.

#### **Fed Blood Glucose experiments in healthy mice (30 h).**

After the acclimation period of 5 days, 20-25 g male C57Bl/6J mice 8 weeks old (Charles River laboratories) were randomized into groups (6 mice per group) according to their body weight. The mice were acutely treated with vehicle or 1 µg per mouse ( $10 \text{ nmol kg}^{-1}$ ) of oligomers via *i.v.* injections (formulation: 4 µg mL<sup>-1</sup> in PBS 1×) Fed blood glucose was measure before dosing (T0) and after 3, 6, 18, 24, 27 and 30 hours. An IPGTT (glucose 2 g kg<sup>-1</sup> *i.p.*) was performed after 9 hours of dosing with a fasting period of 9 hours. Blood glucose was measured before the administration of glucose and after 30, 60 and 90 min.

#### **Dose-response study in healthy mice.**

Mice were lightly fasted (4 hours) before IP administration of varying doses of each agonist or vehicle (100  $\mu$ L saline). After a further 6 hours, blood glucose was measured (GlucoRx) from the tail followed by IP administration of 2 g/kg 20% glucose. Blood glucose was monitored at 20, 40 and 60 minute time-points. To derive the ED<sub>50</sub> (dose required to give a 50% maximal response), 3-parameter fitting was performed on the area-under-curve for each set of data.

### **Study in db/db mice (15 days).**

After the acclimation period of 5 days, male db/db mice 8 weeks old (Charles River laboratories) were randomized into groups (n=10) according to their body weight, HOMA-IR and HbA1C measured after an overnight fast and 8 non diabetic mice were excluded. Mice were chronically treated during 15 days via s.c. route (once daily) at around 8AM with the vehicle or with 100  $\mu$ g kg<sup>-1</sup> (25 nmol kg<sup>-1</sup>) of the oligomers. Formulation: 20  $\mu$ g mL<sup>-1</sup> in PBS 1 $\times$ . On the 7<sup>th</sup> day of treatment, fed blood glucose was measured at 1, 2, 3, 6 and 24 h after dosing. On the 12<sup>th</sup> day of treatment, mice were fasted 6 hours and an OGTT (glucose 1 g kg<sup>-1</sup> p.o.) was performed 6 hours (2 PM) after the dosing. Blood glucose was measure 30 and 0 min before the glucose injection and after 15, 30, 60, 90, and 120 min. Plasma insulin was measure 30 min before the glucose injection and 15 min after. On the 15<sup>th</sup> day of treatment, mice were fasted 6 hours and an ITT (insulin 2 U kg<sup>-1</sup> i.p.) was performed 6 hours (2 PM) after the dosing. Blood glucose was measure before insulin injection and after 15, 30, 60, 90, and 120 min. Blood samples were collected for measuring HbA1c just before the start of the treatments and at the end of the treatment period.

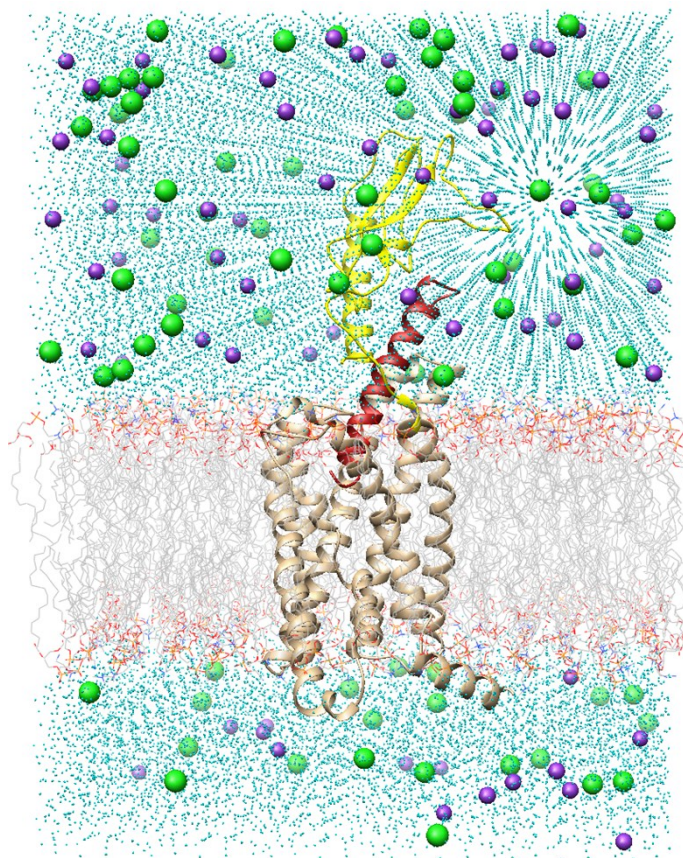
### **Pharmacokinetics.**

Fifteen mice (male C57BL/6J mice (Janvier Labs, France) 9 weeks old, 20-25 g) were treated with GLP-1 analogues via *i.v.* injections (2 mg kg<sup>-1</sup>) formulated at 1 mg mL<sup>-1</sup> in PBS 1 $\times$ . After different time points mice were sacrificed and blood sample were collected. The plasma was separated by centrifugation and the samples were frozen at -80°C before analysis. A volume of 400  $\mu$ L of each sample of plasma was mixed with 1 ml of acetonitrile to precipitate the protein and extract the compound. The sample were then vortexed and centrifuged (15 000  $\times$  g, 5 min, 16 °C) to sediment the precipitated protein. The supernatant was analysed by LC-MS/MS using a UHPLC coupled to LC-MS 8030 Shimadzu triple quadrupole.

### **Molecular dynamics simulation of GLP-1R in complex with ureidopeptide 3**

A three dimensional model of human GLP-1 receptor (GLP-1R) was obtained from the cryo-EM structure of rabbit GLP1-R in complex with human GLP-1 (PDB ID 5VAI)<sup>[8]</sup>. Human GLP-1R which shares 92% sequence identity with the rabbit template was directly obtained by mutating corresponding residues in SYBYL X2.1.1 (Cetera Inc, Princeton, NJ). GLP-1 bound peptide was then modified to ureido peptide 3 by substituting Ala<sup>u</sup> for Ala2. Dihedral angle values for main chain atoms of Ala<sup>u</sup>2 were manually assigned to that of a right-handed 2.5 helix, in agreement with our previous work on the NMR structure elucidation of an ureido heptamer in methanol<sup>[9]</sup>. Hydrogen atoms were added with AMBER16 (University of California, San Francisco). Previously developed AMBER parameters were used to model ureido alanine<sup>[9]</sup>. The CHARMM-GUI interface<sup>[10]</sup> was used to orient the receptor-peptide complex in a 114 \* 113 \*136 Å hydrated phospholipid bilayer (60 cholesterol + 120 POPC +

120 POPE) at a 0.150 M NaCl concentration (75 Na<sup>+</sup> and 79 Cl<sup>-</sup>) and containing 27,692 TIP3P water molecules (Figure S8).



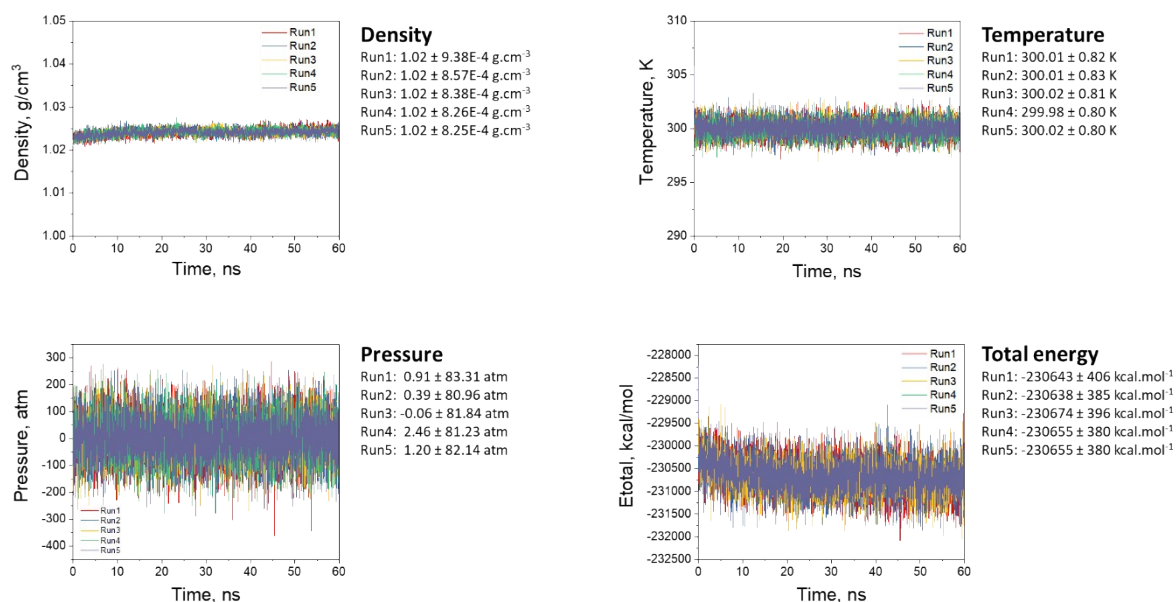
**Figure S8:** Starting orientation of human GLP-1R (transmembrane domain, tan ribbons; extracellular domain, yellow ribbons) bound to ureido peptide 3 (fire brick ribbons), in a phospholipid bilayer (cpk-colored lines) hydrated by a 10 Å shell of water molecules (cyan dots) at 0.150 M NaCl concentration (Na<sup>+</sup>, purple spheres; Cl<sup>-</sup>, green spheres)

The full system containing 125,694 atoms was first refined in AMBER16 using the ffSB14 force field<sup>[11]</sup> and the lipid14 parameter set.<sup>[12]</sup> A harmonic positional restraint of 20 kcal.mol<sup>-1</sup>Å<sup>-2</sup> was first set on GLP-1R and peptide 3 for 10,000 steps of steepest descent followed by 20,000 steps of conjugate gradient minimization. A non-bonded cut off value of 10 Å was used to compute non-bonded interactions with the particle-mesh Ewald (PME) to handle long-range electrostatic interactions with periodic boundaries. A second minimization step similar to the first one was performed while reducing the positional restraints to a value of 7 kcal.mol<sup>-1</sup>Å<sup>-2</sup> for the receptor-peptide atoms, excepted for the His1-Ala<sup>42</sup> N-terminal part of the ureido peptide for which the restraint was looser (1.5 kcal.mol<sup>-1</sup>Å<sup>-2</sup>). A last fully unrestrained minimization protocol (10,000 steps of steepest-descent + 20,000 steps of conjugate gradient) was then applied to the full system.

All atoms were then submitted to a molecular dynamics (MD) protocol consisting of 10 iterative equilibrium steps of 25 fs each using a time step of 1 fs, a Langevin thermostat and a collision frequency of 1 ps<sup>-1</sup>. In the first three steps, a constant volume was applied and the temperature gradually increased from 100 to 300K while restraining differently the protein-peptide complex and the ions (restraint of 10 kcal.mol<sup>-1</sup>Å<sup>-2</sup>) from the phospholipid bilayer and water molecules (restraint of 2.5 kcal.mol<sup>-1</sup>Å<sup>-2</sup>). In the next four steps, a constant pressure of 1 atm was applied with a Berendsen barostat, a isotropic position scaling, and a pressure relaxation time of 2.0 ps. The SHAKE algorithm

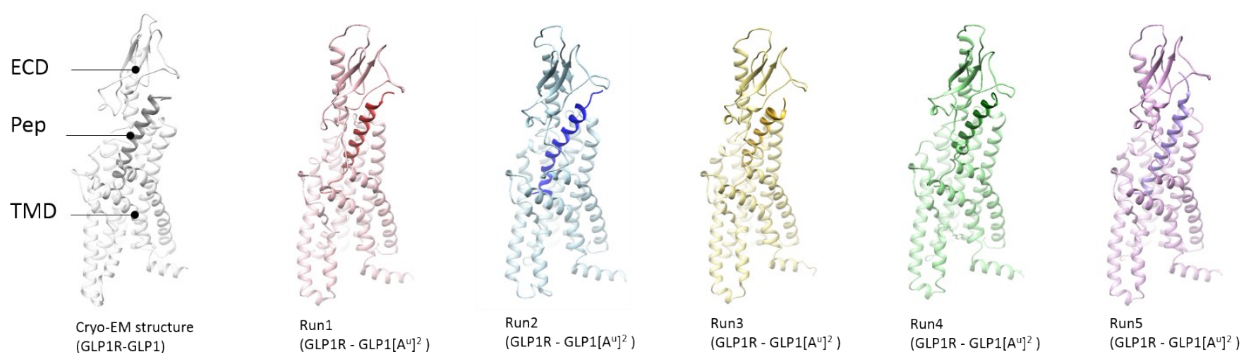
was applied to constrain bonds involving hydrogen atoms and harmonic positional restraints were gradually decreased from 5 to 0.5 kcal.mol<sup>-1</sup>Å<sup>-2</sup> (protein + peptide), and from 2.5 to 0.1 kcal.mol<sup>-1</sup>Å<sup>-2</sup> for the hydrated phospholipid bilayer. In the last three equilibrium steps of NPT dynamics, the phospholipid bilayer was free to move, while the restraints on the protein-peptide complex were decreased from 1 to 0.5 kcal.mol<sup>-1</sup>Å<sup>-2</sup> and lastly removed. The system was next simulated for three final steps of 2 ns NPT dynamics each, where the time step was set to 2 fs and the skinnb parameter set to a value of 5 Å. Last, 5 independent production NPT dynamics trajectories of 60 ns each were run to sample the conformational space of the receptor-peptide complex in a hydrated phospholipid bilayer. All simulations were run on a NVIDIA Tesla K80 GPU accelerator using the CUDA version of AMBER PMEMD module.

Recording statistics along the entire trajectory for density, temperature, pressure and total energy shows that all simulations were well equilibrated (Figure S9).



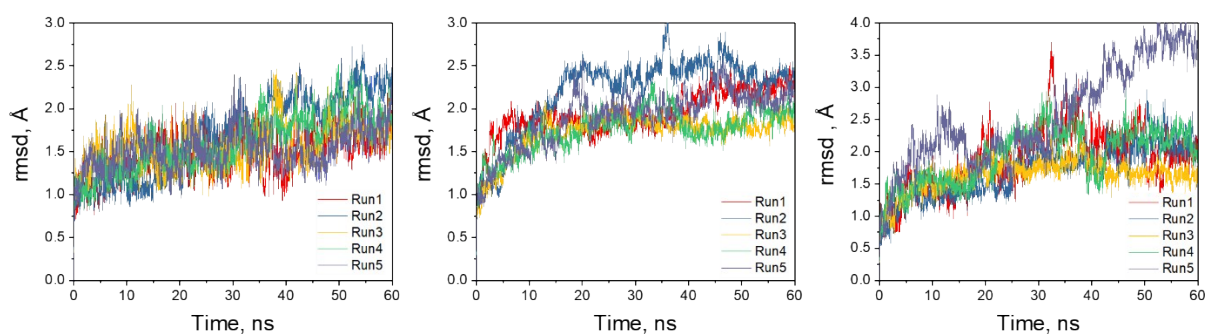
**Figure S9:** Time course and average value for density, temperature, pressure and total energy along the 60 ns production molecular dynamics trajectories of GLP1-R in complex with the ureido peptide 3 in a fully hydrated phospholipid bilayer.

Atomic coordinates were saved every 20 ps, therefore producing a set of 3,000 snapshots for each trajectory out of which the last 10 ns (50-60 ns simulation time) were kept for producing a time-averaged structure (Figure S10).



**Figure S10:** Time-average structure of GLP-1R in complex with ureido peptide 3 (GLP1[A<sup>u</sup>]2) for 5 independent molecular dynamics simulations. Receptor and peptide are displayed by ribbons. The relative position of the peptide with respect to the extracellular (ECD) and transmembrane domain (TMD) of GLP-1R is indicated for the GLP-1R/GLP1 cryo-EM structure.

Root-mean square deviations (rmsd) of main chain atomic coordinates to that of the starting conformation shows that neither the GLP-1R nor the peptide main chain atoms encounter major conformational changes (rmsd < 2.0 Å), excepted for the fifth run for which the ureido peptide is drifting away from its initial positions, mostly at its C-terminal end (Figure S11). Altogether, the first four trajectories are remarkably stable considering the large size of the simulated system (125,964 atoms).



**Figure S11:** rms deviations of main chain atomic coordinates for the extracellular domain (ECD: Thr24-Pro137), the transmembrane domain (TMD: Glu138-Arg421) and the ureido peptide 3 (His1-Gly31).

Receptor-peptide non-bonded interactions were computed with the in-house developed IChem toolkit<sup>[13]</sup> and converted into a receptor-ligand interaction fingerprint<sup>[14]</sup> for ease of comparisons of MD structures with respect to the GLP-1R/GLP-1 cryo-EM template (Figure S12)

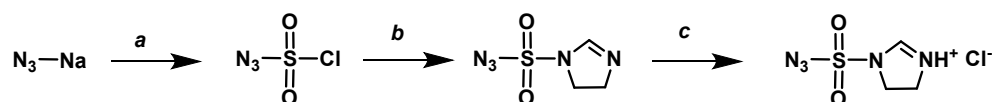




## Synthesis of Azido building block and Fmoc- $\gamma$ -amino acids

The azido building blocks for the Ala<sup>u</sup>, Ala<sup>u $\alpha$</sup> , Ser<sup>u</sup>, Glu<sup>u</sup>, Leu<sup>u</sup>, Lys<sup>u</sup>, Phe<sup>u</sup>, Trp<sup>u</sup>, Val<sup>u</sup>, Ile<sup>u</sup> residues were synthesized as previously reported.<sup>[1,15–18]</sup>

### 1-(azidosulfonyl)-4,5-dihydro-1H-imidazol-3-ium chloride



**a:** SO<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>CN, 0°C, N<sub>2</sub>; **b:** Imidazole, CH<sub>3</sub>CN, 0°C; **c:** HCl (2M) in Et<sub>2</sub>O, 0°C

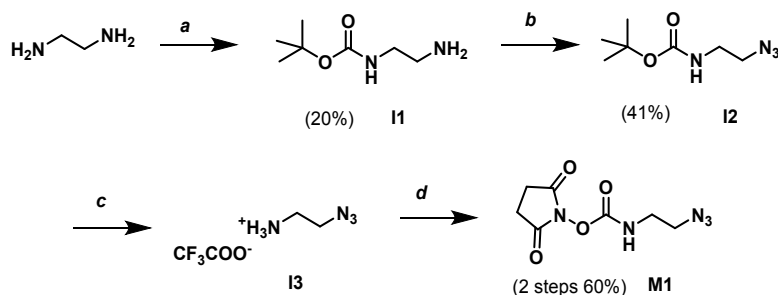
Figure S13 : Synthesis of Imidazolium salt

**a:** Sodium azide (14.0 g, 215 mmol) was suspended in CH<sub>3</sub>CN (150 mL) at 0 °C under N<sub>2</sub> atmosphere. Sulfonyl chloride (17.4 mL, 215 mmol) was added dropwise over 10 min. The mixture was stirred overnight from 0°C to room temperature.

**b:** The mixture was cooled down to 0°C, and imidazole (27.9 g, 409 mmol) was added. The mixture was stirred for 2.5 h at 0 °C. EtOAc (100ml) and H<sub>2</sub>O was added. The organic layer was washed with H<sub>2</sub>O and NaHCO<sub>3</sub> (sat), dried over MgSO<sub>4</sub> and concentrated to remove half of the solvent.

**c:** The mixture was cooled to 0 °C and the compound was precipitated upon addition of HCl (2M) in Et<sub>2</sub>O. The salt was filtered, washed with Et<sub>2</sub>O and dried under vacuum (26 g, 125 mmol, 58%).

### Monomer M1; 2,5-dioxopyrrolidin-1-yl (2-azidoethyl)carbamate (N<sub>3</sub>-G<sup>u</sup>)



**a:** Boc<sub>2</sub>O, DCM, 0°C; **b:** K<sub>2</sub>CO<sub>3</sub>, CuSO<sub>4</sub>·5H<sub>2</sub>O, Imidazolium salt, CH<sub>3</sub>CN/H<sub>2</sub>O; **c:** TFA; **d:** DIEA, DSC, DCM, 0°C

Figure S14 : Synthesis of monomer M1

**a:** Ethylenediamine (6.00 g, 99.8 mmol) was dissolved in anhydrous DCM (100 mL) under N<sub>2</sub> atmosphere at 0 °C. Di-tert-butyl dicarbonate (6.53 g, 29.9 mmol) dissolved in DCM (20ml) was added dropwise over 1 h. The mixture was stirred overnight. Organic phase was washed with brine, dried over MgSO<sub>4</sub> and concentrated.

**b:** I1 (3.1 g, 19.3 mmol) was dissolved in CH<sub>3</sub>CN/H<sub>2</sub>O (50:50, 150 ml). Potassium carbonate (4.01 g, 29.0 mmol), Copper(II) sulfate pentahydrate (48.0 mg, 193  $\mu$ mol) and imidazolium salt (4.87 g, 23.2 mmol) were added. The mixture was stirred 1.5 h at RT. EtOAc was added and the layers were separated. The aqueous layer was extracted with EtOAc. Organic layers were combined and washed with KHSO<sub>4</sub>, brine, dried over MgSO<sub>4</sub> and concentrated. The compound was purified by flash chromatography on silica gel (liquid loading). Eluent Cyclo/EtOAc 90:10 and then 80:20.

**c:** **12** (1.5 g, 12.3 mmol) was dissolved in Trifluoroacetic acid (30 mL) at 0 °C and stirred 2 h. TFA was evaporated and then coevaporated with cyclohexane. The compound was dissolved in H<sub>2</sub>O and lyophilized.

**d:** N,N'-Disuccinimidyl carbonate (1.99 g, 7.8 mmol) was dissolved in anhydrous DCM (20 mL) and cooled to 0°C. TFA salt (**13**) (1.3 g, 6.5 mmol) and N,N-Diisopropylethylamine (2.15 mL, 12.3 mmol) dissolved in anhydrous DCM (100 mL) were added and the mixture was stirred for 3 h at room temperature. The organic phase was washed with KHSO<sub>4</sub> (1M), brine, dried over MgSO<sub>4</sub> and concentrated. The compound was triturated in hexane to afford the monomer **M1** as a white powder (1.1 g, 4.8 mmol, 4.8% yield).

**Melting point** (*M.p.*) 82.2 °C; **<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 300 MHz) δ: 6.00 (s, 1H), 3.51 (m, 4H), 2.87 (s, 4H); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ: 169.95, 151.57, 50.41, 41.18, 25.48; **HRMS** (*m/z*) calcd for C<sub>7</sub>H<sub>9</sub>N<sub>5</sub>O<sub>4</sub>Na<sup>+</sup> [M + Na]<sup>+</sup> 250.0547, found 250.0542.

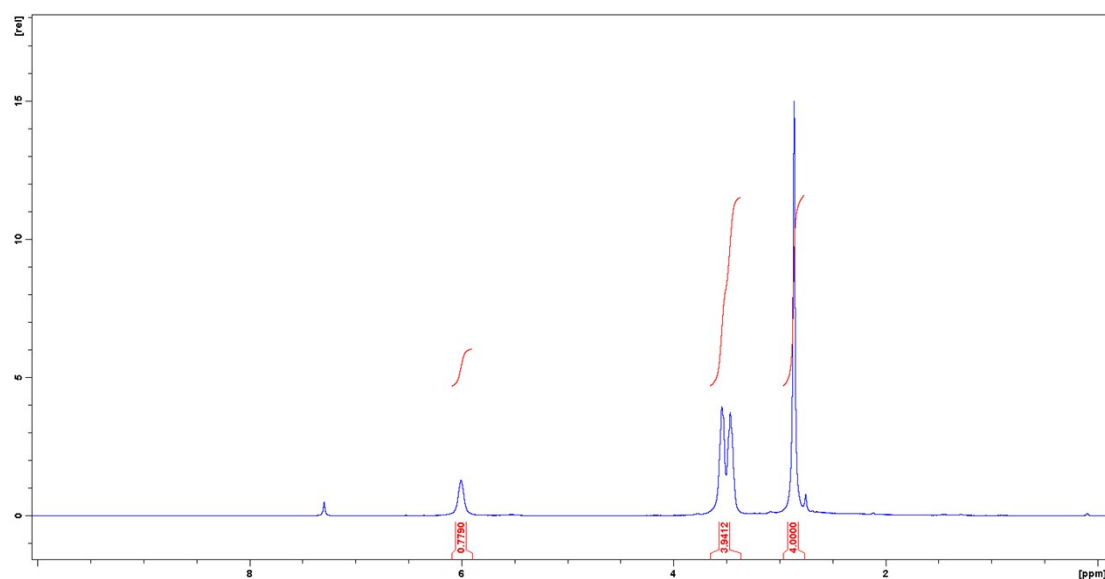


Figure S15 : <sup>1</sup>H NMR of monomer **M1** in CDCl<sub>3</sub> (300 MHz)

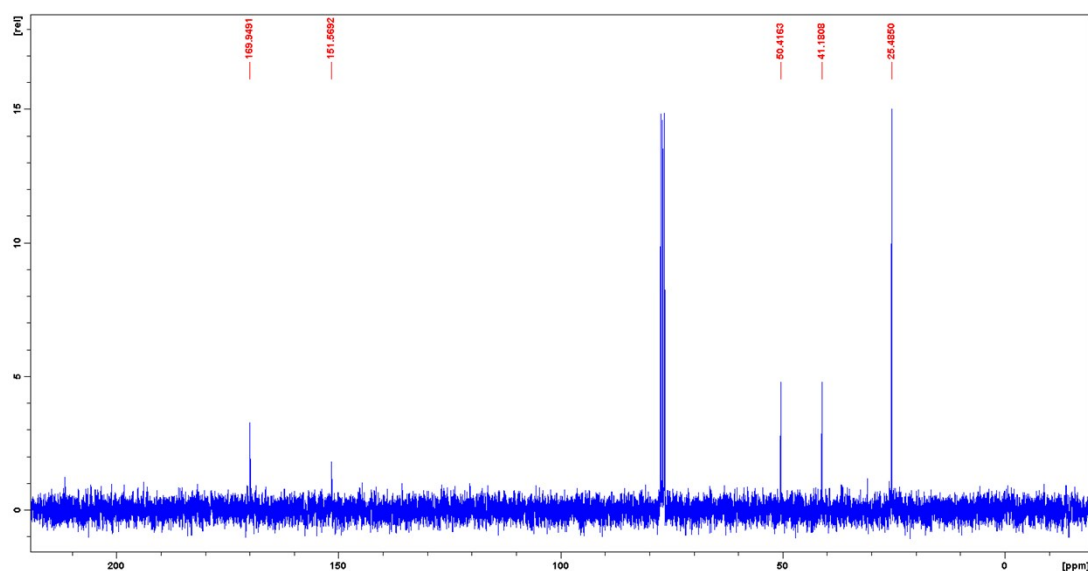
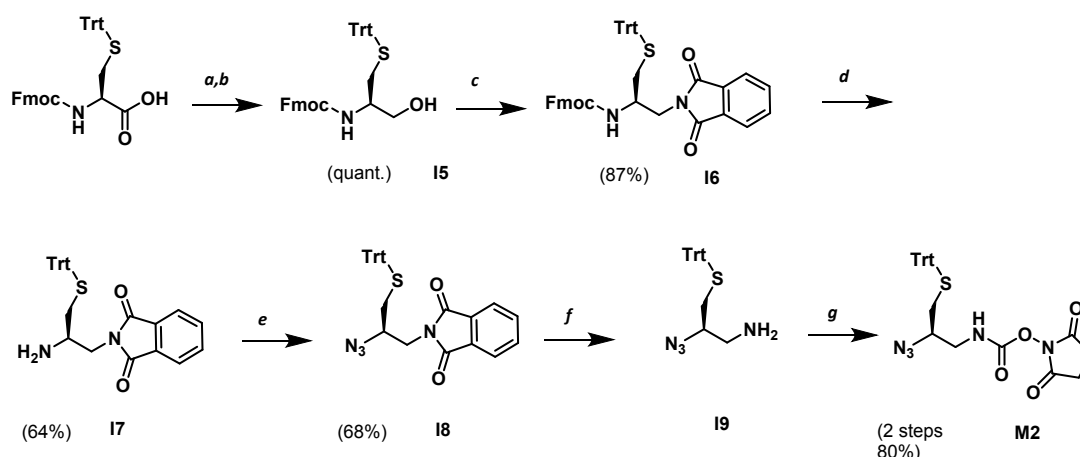


Figure S16 : <sup>13</sup>C NMR of monomer **M1** in CDCl<sub>3</sub> (75 MHz)

**Monomer M2; 2,5-dioxopyrrolidin-1-yl (S)-(2-azido-3-(tritylthio)propyl)carbamate; (N<sub>3</sub>-C<sup>u</sup>)**



**a:** *i*BuOCOCI, NMM, THF, -10 °C; **b:** NaBH<sub>4</sub>, THF, H<sub>2</sub>O, 0 °C; **c:** PPh<sub>3</sub>, DIAD, Phtalimide, THF, 0 °C; **d:** piperidine, dioxane; **e:** K<sub>2</sub>CO<sub>3</sub>, CuSO<sub>4</sub>·5H<sub>2</sub>O, Imidazolium salt, CH<sub>3</sub>CN/H<sub>2</sub>O; **f:** N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, 70 °C; **g:** DSC, EtOAc, 0 °C

**Figure S17 : Synthesis of monomer M2**

**a:** Fmoc-L-Cys(Trt)-OH (5.00 g, 8.5 mmol) was dissolved in dry THF (in 500 mL double neck flask) at (-10 °C, ice + brine) under N<sub>2</sub> atmosphere. 4-Methymorpholine (1.0 mL, 9.4 mmol) was added. Isobutyl chloroformate (1.2 mL, 9.0 mmol) was added dropwise via an addition funnel. The mixture was stirred 45 min at -10 °C.

The white precipitate was filtered and washed with THF.

**b:** Sodium borohydride (600 mg, 17.1 mmol) was dissolved in 5 ml of H<sub>2</sub>O and the previous filtrate was added dropwise at 0 °C.

The mixture was stirred for 3 h at RT.

THF was evaporated and the compound was solubilized in EtOAc, washed with KHSO<sub>4</sub> (1M), NaHCO<sub>3</sub> (sat), brine, dried over MgSO<sub>4</sub> and concentrated.

**c:** I5 (10.59 g, 8.5 mmol) was dissolved in anhydrous THF (50 ml) at 0 °C and triphenylphosphine (2.68 g, 10.2 mmol) and Phtalimide (1.5 g, 10.2 mmol) were added. DIAD (2 ml, 10.2 mmol) was added dropwise at 0 °C and the mixture was stirred for 48h at RT.

The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. Eluent Cyclohexane/EtOAc 90:10 and then 80:20.

**d:** I6 (5.1 g, 7.4 mmol) was dissolved in dioxane and piperidine (1.1 ml, 11.1 mmol) was added. The mixture was stirred overnight at room temperature.

The solvent was evaporated and the compound was purified by flash chromatography on silica gel. Eluent DCM/MeOH; gradient 98:2 to 90:10.

**e:** I7 (2.27 g, 4.7 mmol) was dissolved in CH<sub>3</sub>CN/H<sub>2</sub>O (50:50, 100 ml). Potassium carbonate (0.9 g, 7.2 mmol), Copper(II) sulfate pentahydrate (12.0 mg, 50 μmol) and imidazolium salt (1.19 g, 5.7 mmol) were added. The mixture was stirred 1.5 h at RT. EtOAc was added and the layers were separated. The aqueous layer was extracted with EtOAc. The organic layers were combined and washed with KHSO<sub>4</sub> and brine, dried over MgSO<sub>4</sub> and concentrated. The compound was purified by flash chromatography on silica gel (liquid loading). Eluent Cyclo/EtOAc 90:10 and then 80:20.

**f:** I8 (1.62 g, 3.2 mmol) was dissolved in MeOH (15 ml) with DCM (4 ml). Hydrazine monohydrated (470 μL, 9.6 mmol) was added and the mixture was stirred at 70 °C for 3 h. The white precipitate was filtrated and washed with EtOAc. The MeOH was evaporated. The compound was dissolved in EtOAc, washed with water and brine and then dried over MgSO<sub>4</sub>. The compound was used as is in the next step without evaporation.

**g:** N,N'-Disuccinimidyl carbonate (0.82 g, 3.2 mmol) was suspended in EtOAc (20 mL) at 0 °C. **19** (3.2 mmol) previously dissolved in EtOAc (150 mL) was added dropwise at 0 °C and the mixture was stirred for 2 h at room temperature.

Half of the solvent was evaporated. The organic layer was washed with KHSO<sub>4</sub> (1M), and brine, dried over MgSO<sub>4</sub> and concentrated. The mixture was triturated in hexane to afford the monomer **M2** as a white powder (1.32g, 2.56 mmol, 30% yield).

**Melting point (M.p.)** 65.2 °C; **<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 300 MHz) δ: 7.55-7.21 (m, 15H), 5.43 (m, 1H), 3.35-3.24 (m, 1H), 3.06-2.95 (m, 1H), 2.86 (m, 1H), 2.84 (s, 4H), 2.57-2.43 (m, 2H); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ: 169.69, 151.38, 144.24, 129.58, 128.19, 127.04, 67.56, 60.87, 44.13, 33.52, 25.47; **HRMS** (*m/z*) calcd for C<sub>27</sub>H<sub>29</sub>N<sub>6</sub>O<sub>4</sub>S<sup>+</sup> [M + NH<sub>4</sub>]<sup>+</sup> 533.1966, found 533.1947

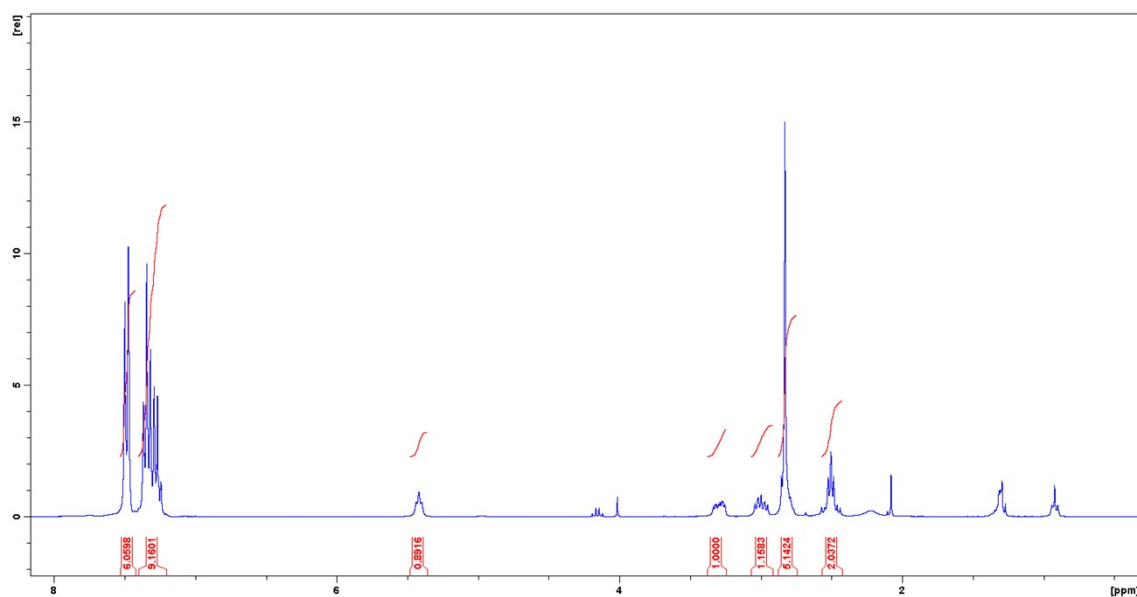


Figure S18 : <sup>1</sup>H NMR of monomer **M2** in CDCl<sub>3</sub> (300 MHz)

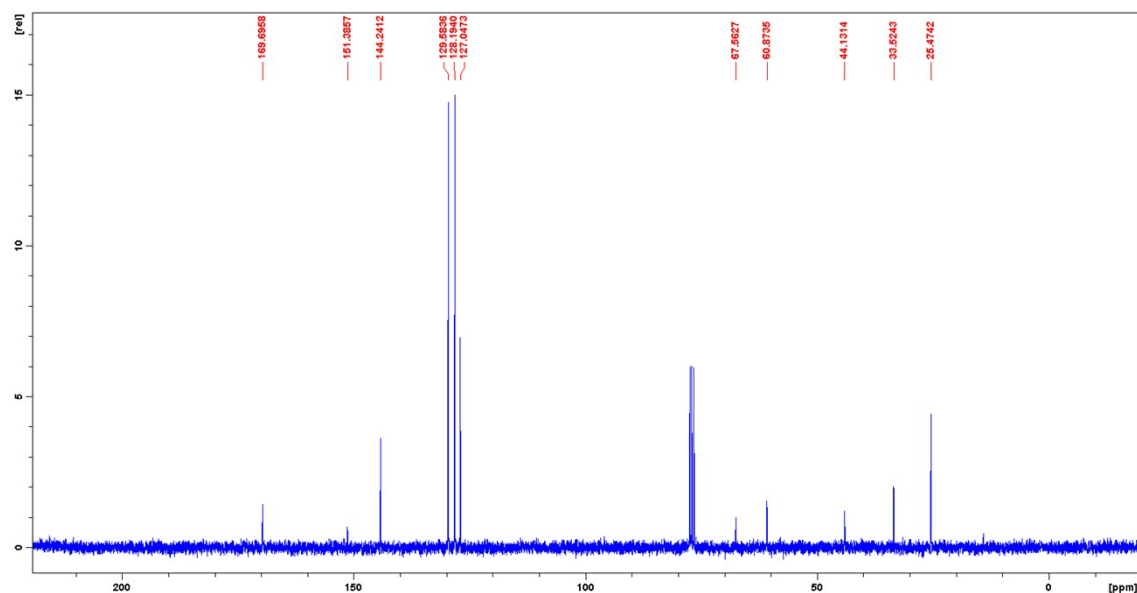
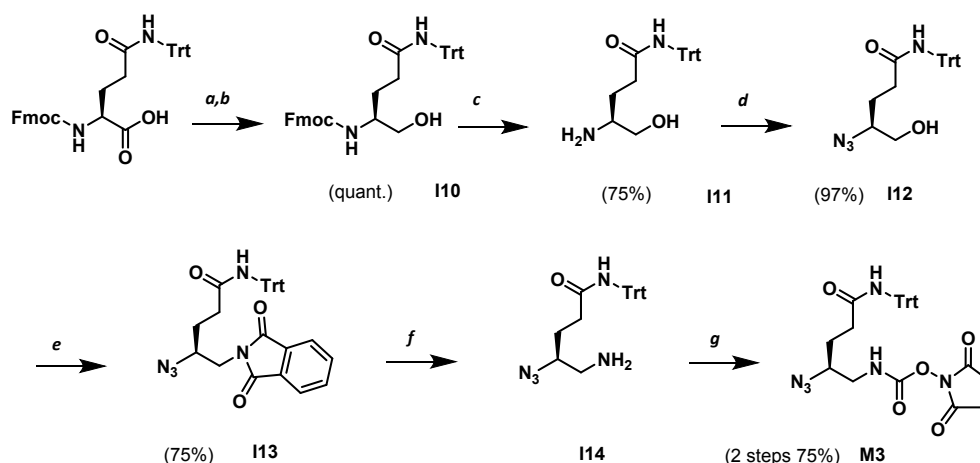


Figure S19 : <sup>13</sup>C NMR of monomer **M2** in CDCl<sub>3</sub> (75 MHz)

**Monomer M3; 2,5-dioxypyrrolidin-1-yl (R)-(2-azido-5-oxo-5-(tritylamino)pentyl)carbamate; (N<sub>3</sub>-Q<sup>u</sup>)**



**a:** *i*BuOCOC<sub>l</sub>, NMM, THF, -10°C; **b:** NaBH<sub>4</sub>, THF, H<sub>2</sub>O, 0°C; **c:** piperidine, dioxane; **d:** K<sub>2</sub>CO<sub>3</sub>, CuSO<sub>4</sub>·5H<sub>2</sub>O, Imidazolium salt, CH<sub>3</sub>CN/H<sub>2</sub>O; **e:** PPh<sub>3</sub>, DIAD, Phtalimide, THF, 0°C; **f:** N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, 70°C; **g:** DSC, EtOAc, 0°C

**Figure S20: Synthesis of monomer M3**

**a:** Fmoc-L-Gln(Trt)-OH (12.6 g, 20.6 mmol) was dissolved in dry THF (in 500 mL double neck flask) at (-10°C, ice + salt bath) under N<sub>2</sub>. 4-Methymorpholine (2.5 mL, 22.7 mmol) was added. Isobutyl chloroformate (2.8 mL, 21.6 mmol) was added dropwise via an addition funnel. The mixture was stirred 45 min at -10 °C.

The white precipitate was filtered and washed with THF.

**b:** Sodium borohydride (1.56 g, 41.3 mmol) was dissolved in 5 ml of H<sub>2</sub>O and the previous filtrate was added dropwise at 0°C.

The mixture was stirred 3 h at RT.

THF was evaporated and the compound was solubilized in EtOAc and washed with KHSO<sub>4</sub> (1M), NaHCO<sub>3</sub> (sat), brine, dried over MgSO<sub>4</sub> and concentrated.

**c:** **I10** (14.4 g, 24.2 mmol) was dissolved in dioxane (80ml) and piperidine (20 ml) was added. The mixture was stirred 2 h at room temperature. The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. Eluent DCM/MeOH; gradient 100:0 to 80:20.

**d:** **I11** (5.80 g, 15.5 mmol) was dissolved in CH<sub>3</sub>CN/H<sub>2</sub>O (50:50, 100 ml) and MeOH (100 ml). Potassium carbonate (2.14 g, 15.49 mmol), Copper(II) sulfate pentahydrate (40.0 mg, 150 μmol) and imidazolium salt (3.90 g, 18.6 mmol) were added. The mixture was stirred for 2 h at RT. MeOH was evaporated. EtOAc was added and the phase were separated. Aqueous phase was extracted with EtOAc. Organic phases were combined and washed with KHSO<sub>4</sub> and brine, dried with MgSO<sub>4</sub> and concentrated.

**e:** **I12** (6.00 g, 14.98 mmol) was dissolved in anhydrous THF (50 ml) at 0 °C and triphenylphosphine (4.72 g, 17.98 mmol) and Phtalimide (2.65 g, 17.98 mmol) were added. DIAD (3.56 ml, 17.98 mmol) was added dropwise at 0 °C and the mixture was stirred 2 h at RT.

The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. (liquid loading). Eluent Cyclohexane/EtOAc. Gradient 90:10 to 60:40.

**f:** **I13** (6.00 g, 11.3 mmol) was dissolved in MeOH (150 ml). Hydrazine monohydrated (1.65 mL, 33.9 mmol) was added and the mixture was stirred at 70 °C for 3 h. MeOH was evaporated. The compound was dissolved in EtOAc and washed with water and brine, dried with MgSO<sub>4</sub>. The compound was used as is in the next step without evaporation.

**g:** N,N'-Disuccinimidyl carbonate (5.42 g, 21.1 mmol) was suspended in EtOAc (20 mL) at 0 °C. **I14** (21.1 mmol) previously dissolved in EtOAc (150 mL) was added dropwise at 0 °C and the mixture was stirred 2 h at room temperature.

The solvent was half evaporated. The organic phase was washed with  $\text{KHSO}_4$  (1M), brine, dried over  $\text{MgSO}_4$  and concentrated. The mixture was triturated in hexane to afford the monomer **M3** as a white powder (18.5g, 15.8 mmol, 77% yield).

**Melting point** (*M.p.*) 68.7 °C;  **$^1\text{H NMR}$**  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.37-7.20 (m, 15H), 6.87 (s, 1H), 6.19 (m, 1H), 3.53 (m, 1H), 3.27-3.10 (m, 2H), 2.76 (s, 4H), 2.46 (m, 2H), 1.93-1.76 (m, 2H);  **$^{13}\text{C NMR}$**  (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 170.99, 169.91, 151.88, 144.44, 128.63, 128.03, 127.13, 70.68, 60.63, 44.45, 32.45, 25.44; **HRMS** (*m/z*) calcd for  $\text{C}_{29}\text{H}_{29}\text{N}_6\text{O}_5^+$  [ $\text{M} + \text{H}$ ] $^+$  541.2194, found 541.2208.

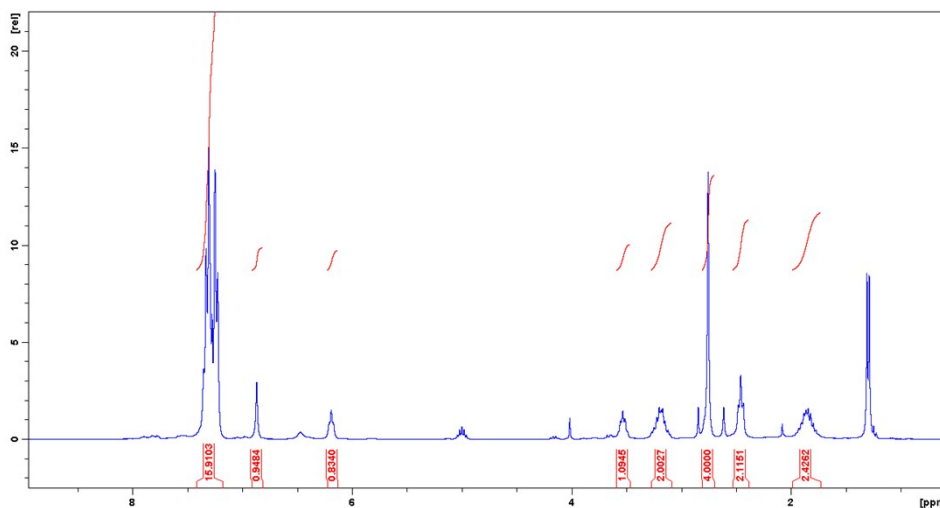


Figure S21 :  $^1\text{H NMR}$  of monomer **M3** in  $\text{CDCl}_3$  (300 MHz)

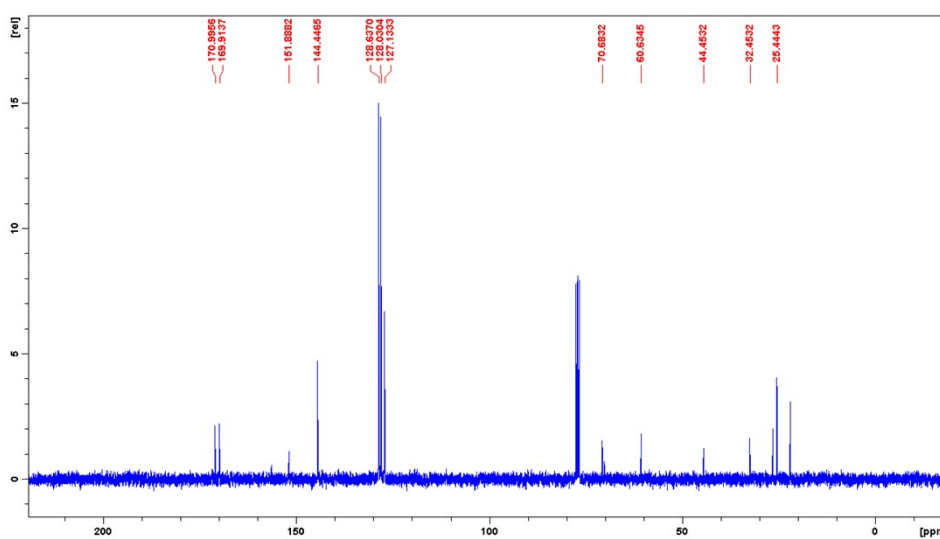
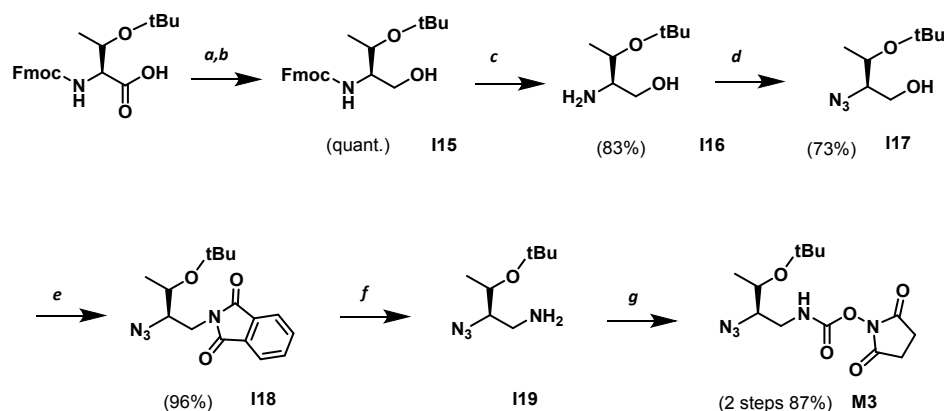


Figure S22 :  $^{13}\text{C NMR}$  of monomer **M3** in  $\text{CDCl}_3$  (75 MHz)

**Monomer M4; 2,5-dioxypyrrolidin-1-yl ((2S,3R)-2-azido-3-(tert-butoxy)butyl)carbamate; (N<sub>3</sub>-T<sup>u</sup>)**



**a:**  $i\text{BuOCOCl}$ , NMM, THF,  $-10^\circ\text{C}$ ; **b:**  $\text{NaBH}_4$ , THF,  $\text{H}_2\text{O}$ ,  $0^\circ\text{C}$ ; **c:** piperidine, dioxane; **d:**  $\text{K}_2\text{CO}_3$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Imidazolium salt,  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ; **e:**  $\text{PPh}_3$ , DIAD, Phtalimide, THF,  $0^\circ\text{C}$ ; **f:**  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , MeOH,  $70^\circ\text{C}$ ; **g:** DSC, EtOAc,  $0^\circ\text{C}$

**Figure S23 : Synthesis of monomer M4**

**a:** Fmoc-L-Thr(tBu)-OH (10.0 g, 25.2 mmol) was dissolved in dry THF (100 ml) at  $-10^\circ\text{C}$ , (ice + salt bath) under  $\text{N}_2$  atmosphere. 4-Methymorpholine (3.0 mL, 27.7 mmol) was added. Isobutyl chloroformate (3.4 mL, 21.4 mmol) was added dropwise via an addition funnel. The mixture was stirred 45 min at  $-10^\circ\text{C}$ . The white precipitate was filtered and washed with THF.

**b:** Sodium borohydride (1.9 g, 50.3 mmol) was dissolved in 5 ml of  $\text{H}_2\text{O}$  and the previous filtrate was added dropwise at  $0^\circ\text{C}$ . The mixture was stirred 3h at RT. THF was evaporated. The compound was solubilized in EtOAc and washed with  $\text{KHSO}_4$  (1M),  $\text{NaHCO}_3$  (sat) and brine (sat), dried with  $\text{MgSO}_4$  and concentrated.

**c:** **I15** (9.5 g, 24.8 mmol) was dissolved in dioxane (80ml) and piperidine (20 ml) was added. The mixture was stirred 2h at room temperature. The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. Eluent Cyclohexane/ EtOAc; gradient 50:50 to 0:100. And EtOAc/MeOH, 90:10.

**d:** **I16** (3.3 g, 20.5 mmol) was dissolved in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (50:50, 100 ml). Potassium carbonate (6.29 g, 45.5 mmol), Copper(II) sulfate pentahydrate (51.0 mg, 200  $\mu\text{mol}$ ) and imidazolium salt (5.13 g, 24.6 mmol) were added. The mixture was stirred 2 h at RT. EtOAc was added and the phase were separated. Aqueous phase was extracted with EtOAc. Organic phases were combined and washed with  $\text{KHSO}_4$  and brine, dried over  $\text{MgSO}_4$  and concentrated. The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. Eluent Cyclohexane/ EtOAc, 90:10.

**e:** **I17** (2.80 g, 15.0 mmol) was dissolved in anhydrous THF (50 ml) at  $0^\circ\text{C}$  and triphenylphosphine (4.71 g, 17.9 mmol) and Phtalimide (2.64 g, 17.9 mmol) were added. DIAD (3.5 ml, 17.9 mmol) was added dropwise at  $0^\circ\text{C}$  and the mixture was stirred 2 h at RT. The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. (liquid loading). Eluent Cyclohexane/EtOAc. Gradient 90:10.

**f:** **I18** (4.56 g, 14.4 mmol) was dissolved in MeOH (50 ml). Hydrazine monohydrated (2.0 mL, 43.2 mmol) was added and the mixture was stirred at  $70^\circ\text{C}$  for 2 h. The white precipitate was filtrated and washed with EtOAc. MeOH was evaporated. The compound was dissolved in EtOAc and washed with water and brine, dried with  $\text{MgSO}_4$ . The compound was used as is in the next step without evaporation.

**g:** N,N'-Disuccinimidyl carbonate (3.69 g, 14.4 mmol) was suspended in EtOAc (20 mL) at  $0^\circ\text{C}$ . **I19** (14.4 mmol) previously dissolved in EtOAc (150 mL) was added dropwise at  $0^\circ\text{C}$  and the mixture was stirred 2 h at room temperature.

The solvent was half evaporated. The organic phase was washed with  $\text{KHSO}_4$  (1M), brine, dried over  $\text{MgSO}_4$  and concentrated. The mixture was triturated in hexane to afford the monomer **M4** as a white powder (4.1 g, 12.5 mmol, 50% yield).

**Melting point** (*M.p.*) 65.3 °C;  **$^1\text{H NMR}$**  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 6.07 (m, 1H), 3.78 (m, 1H), 3.52-3.42 (m, 2H), 3.27 (m, 1H), 2.85 (s, 4H), 1.25 (s, 9H), 1.21 (d,  $J = 6.18$  Hz, 3H);  **$^{13}\text{C NMR}$**  (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 169.92, 151.57, 74.93, 68.52, 65.47, 42.21, 28.29, 25.48, 19.37; **HRMS** ( $m/z$ ) calcd for  $\text{C}_{13}\text{H}_{21}\text{N}_5\text{O}_5\text{Na}^+$  [ $\text{M} + \text{Na}$ ] $^+$  350.1435, found 350.1438.

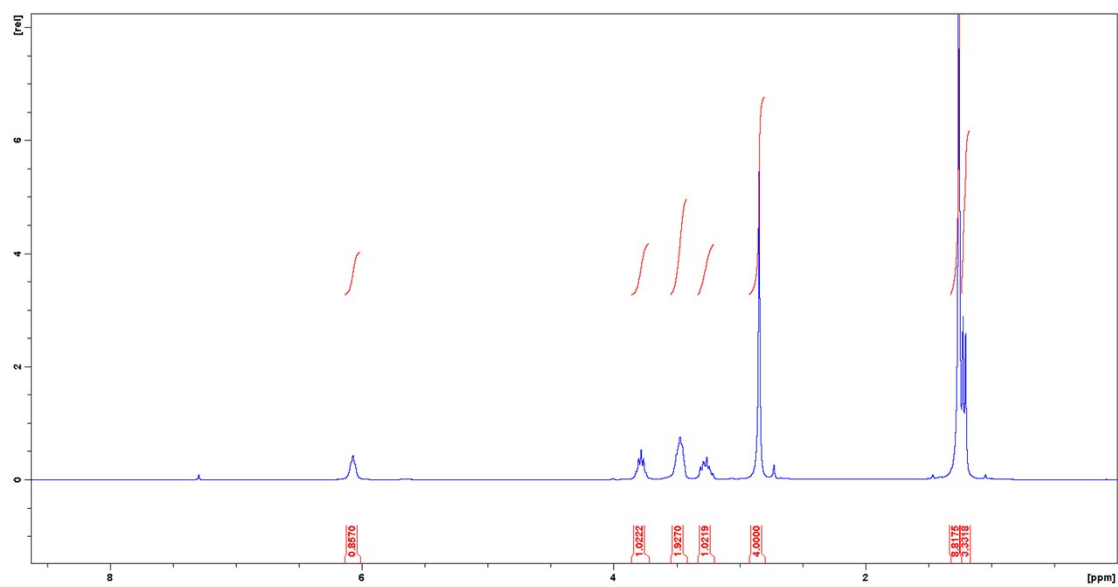


Figure S24 :  $^1\text{H NMR}$  of monomer **M4** in  $\text{CDCl}_3$  (300 MHz)

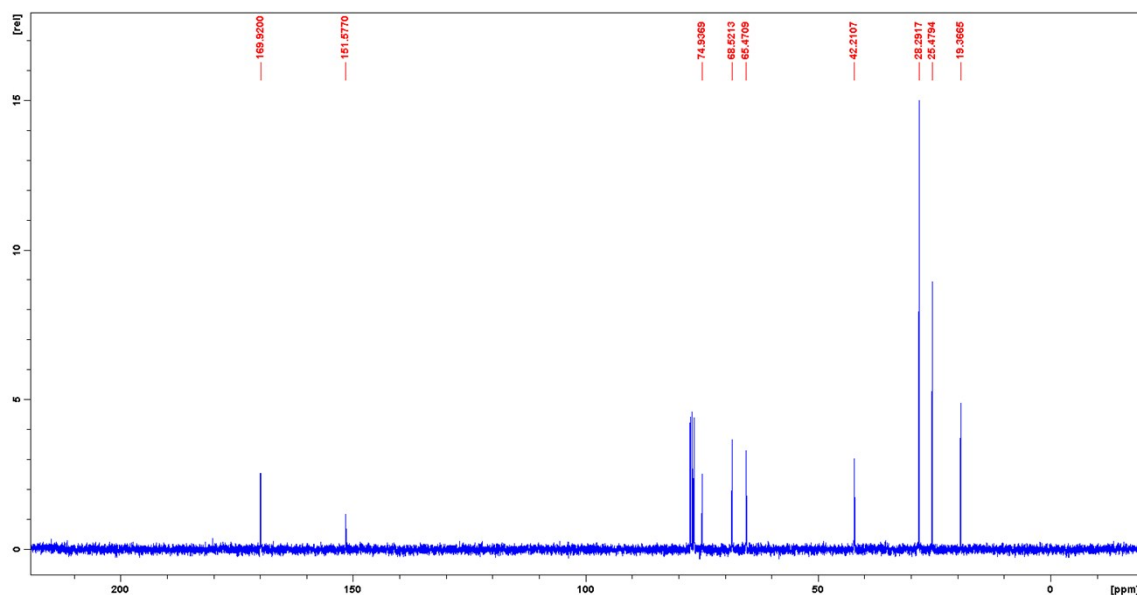
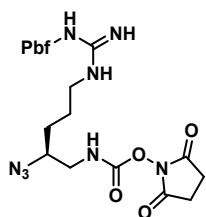


Figure S25 :  $^{13}\text{C NMR}$  of monomer **M4** in  $\text{CDCl}_3$  (75 MHz)



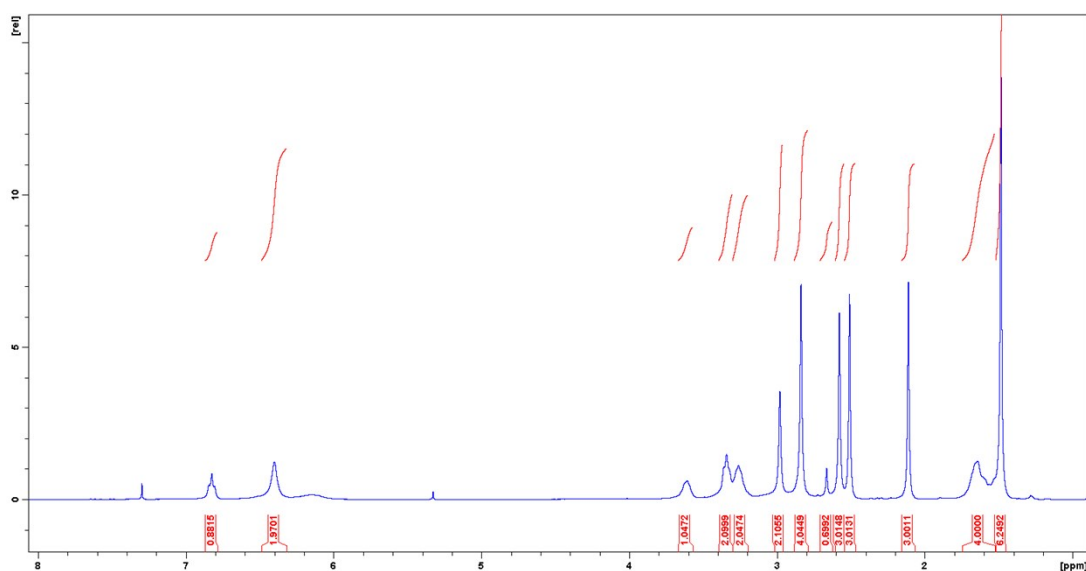
**Monomer M5; 2,5-dioxopyrrolidin-1-yl (R)-(2-azido-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentyl)carbamate; (N<sub>3</sub>-R<sup>u</sup>)**



**M5**

Monomer M5 was synthesized by NovAliX.

**Melting point (M.p.)** 81.4 °C; **<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 300 MHz) δ:6.83 (m, 1H), 6.45-6.35 (m, 2H), 3.66-3.56 (m, 1H), 3.39-3.30 (m, 2H), 3.30-3.19 (m, 2H), 2.98 (s, 2H), 2.84 (s, 4H), 2.66 (s, 1H), 2.58 (s, 3H), 2.51 (s, 3H), 2.11 (s, 3H), 1.73-1.53 (m, 4H), 1.48 (s, 6H); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ:170.6; 158.96; 156.22; 152.26; 138.43; 132.37; 124.86; 117.68; 86.61; 61.08; 44.85; 43.18; 28.61; 28.45; 25.55; 25.34; 19.30; 17.97; 12.49; **HRMS** (*m/z*) calcd for C<sub>24</sub>H<sub>35</sub>N<sub>8</sub>O<sub>7</sub>S<sup>+</sup> [M + H]<sup>+</sup> 579.2344, found 579.2341.



**Figure S26 : <sup>1</sup>H NMR of monomer M5 in CDCl<sub>3</sub> (300 MHz)**

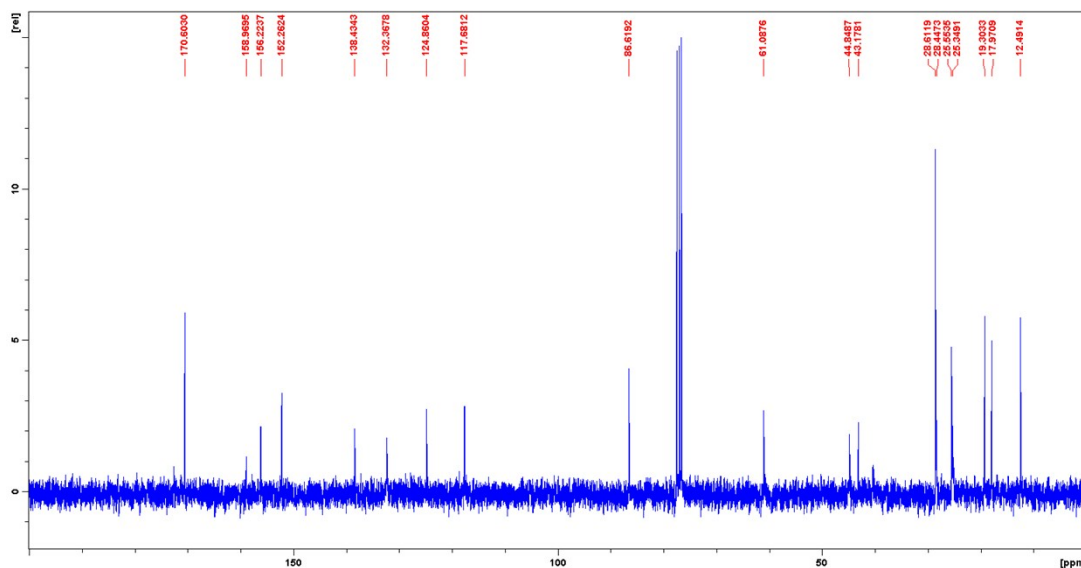
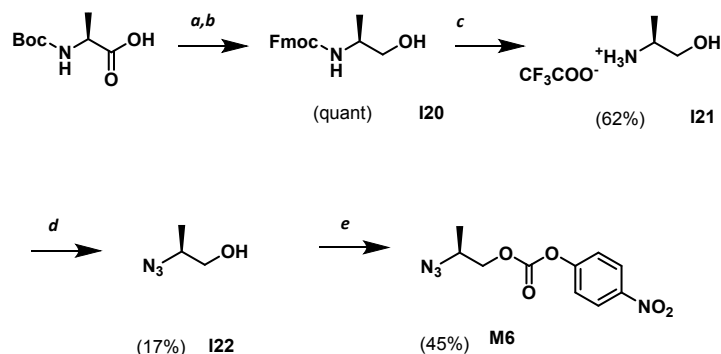


Figure S27 :  $^{13}\text{C}$  NMR of monomer **M5** in  $\text{CDCl}_3$  (75 MHz)

### Monomer **M6**; (S)-2-azidopropyl (4-nitrophenyl) carbonate; ( $\text{N}_3\text{-A}^c$ )



**a:**  $i\text{BuOCCl}$ , NMM, THF,  $-10^\circ\text{C}$ ; **b:**  $\text{NaBH}_4$ , THF,  $\text{H}_2\text{O}$ ,  $0^\circ\text{C}$ ; **c:** TFA; **d:**  $\text{K}_2\text{CO}_3$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Imidazolium salt,  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ; **e:** Pyridine, 4-nitrophenylchloroformate, DCM,  $\text{N}_2$ ,  $0^\circ\text{C}$ ;

Figure S28 : Synthesis of monomer **M6**

**a:** Boc-L-Ala-OH (10.0 g, 52.9 mmol) was dissolved in dry THF (100 ml) at  $-10^\circ\text{C}$ , (ice + salt bath) under  $\text{N}_2$ . 4-Methymorpholine (6.4 mL, 58.1 mmol) was added. Isobutyl chloroformate (7.2 mL, 55.5 mmol) was added dropwise via an addition funnel. The mixture was stirred 45 min at  $-10^\circ\text{C}$ . The white precipitate was filtered and washed with THF.

**b:** Sodium borohydride (4.0 g, 105.7 mmol) was dissolved in 8 ml of  $\text{H}_2\text{O}$  and the previous filtrate was added dropwise at  $0^\circ\text{C}$ . The mixture was stirred overnight at room temperature. THF was evaporated. The compound was solubilized in EtOAc and washed with  $\text{KHSO}_4$  (1M),  $\text{NaHCO}_3$  (sat) and brine (sat), dried over  $\text{MgSO}_4$  and concentrated.

**c:** **I20** (9.2 g, 52.9 mmol) was dissolved in TFA (40 ml). The mixture was stirred 3h at room temperature. TFA was evaporated and coevaporated with cyclohexane. The compound was dissolved in  $\text{Et}_2\text{O}$  and extracted with  $\text{H}_2\text{O}$ . The aqueous phase was lyophilized to afford **I21**.

**d:** **I21** (6.2 g, 32.7 mmol) was dissolved in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (50:50, 100 ml). Potassium carbonate (10.01 g, 72.4 mmol), Copper(II) sulfate pentahydrate (82.0 mg, 330  $\mu\text{mol}$ ) and imidazolium salt (8.2 g, 39.2 mmol) were added. The mixture was stirred 1.5 h at room temperature. EtOAc was added and the phase were separated. Aqueous phase was extracted with EtOAc. Organic phases were combined and washed with  $\text{KHSO}_4$  and brine. The solvent was evaporated and the compound was purified by flash column chromatography on silica gel. Eluent Cyclohexane/ EtOAc, 90:10.

**e: 122** (0.57 g, 5.6 mmol), was dissolved in DCM under N<sub>2</sub>. Pyridine (0.5 ml, 6.2 mmol) was added and the reaction mixture was cooled to 0°C. 4-nitrophenylchloroformate (2.27 g, 11.3 mmol) dissolved in DCM (5ml) was added dropwise and the reaction mixture was stirred 5h. The organic phase was washed with NaHCO<sub>3</sub> (1M), dried over MgSO<sub>4</sub> and concentrated. The compound was purified by flash column chromatography on silica gel. Eluent Cyclohexane/ EtOAc, 90:10. Monomer **M6** (0.67 g, 2.5 mmol, 5% yield).

**Melting point** (*M.p.*) 35.3 °C; **<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 300 MHz) δ: 8.35-8.29 (m, 2H), 7.46-7.39 (m, 2H), 4.33 (dd, 1H, J= 10.89Hz, J = 4.26 Hz), 4.24 (dd, 1H, J= 11.45Hz, J= 7.01 Hz), 3.96-3.87 (m, 1H) 1.37 (d, 3H, J= 6.75 Hz) ; **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ: 155.35, 152.26, 145.55, 125.38, 121.79, 71.42, 55.59, 15.81; **HRMS** (*m/z*) calcd for C<sub>10</sub>H<sub>11</sub>N<sub>4</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup> 267.0724, found 267.0725.

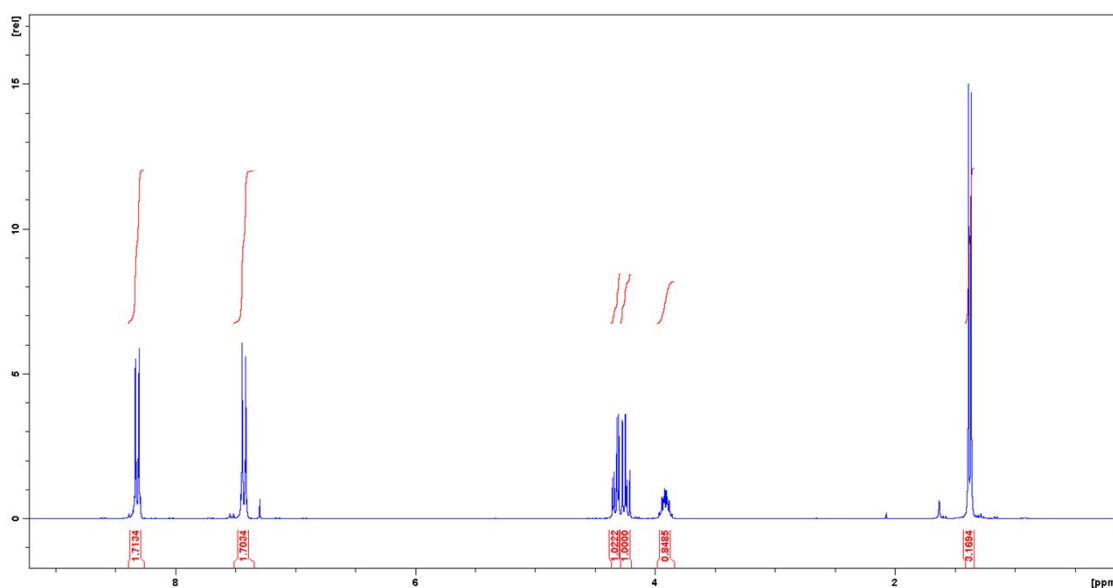


Figure S29 : <sup>1</sup>H NMR of monomer **M6** in CDCl<sub>3</sub> (300 MHz)

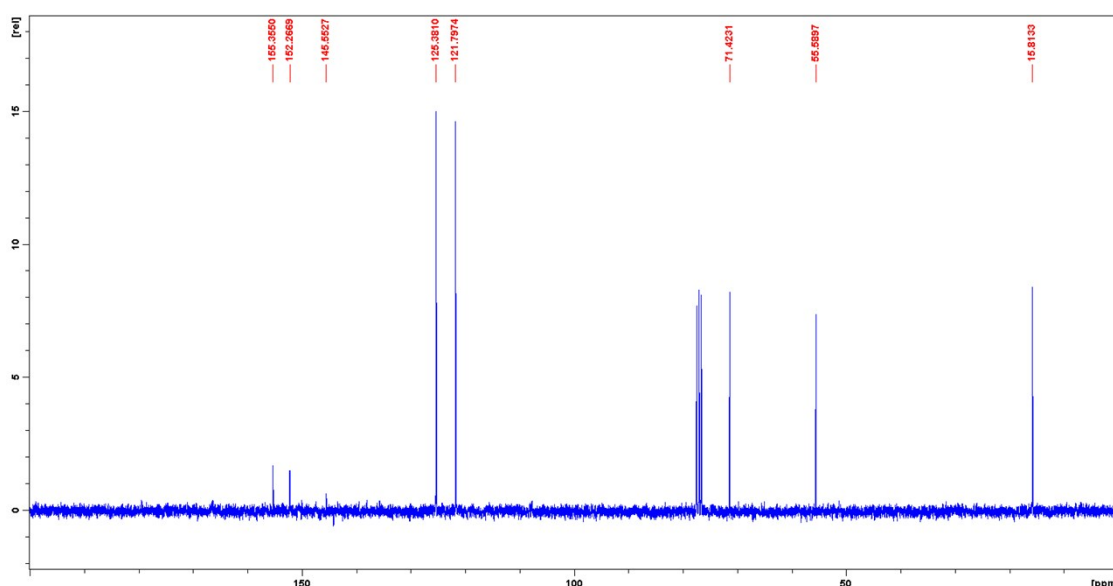
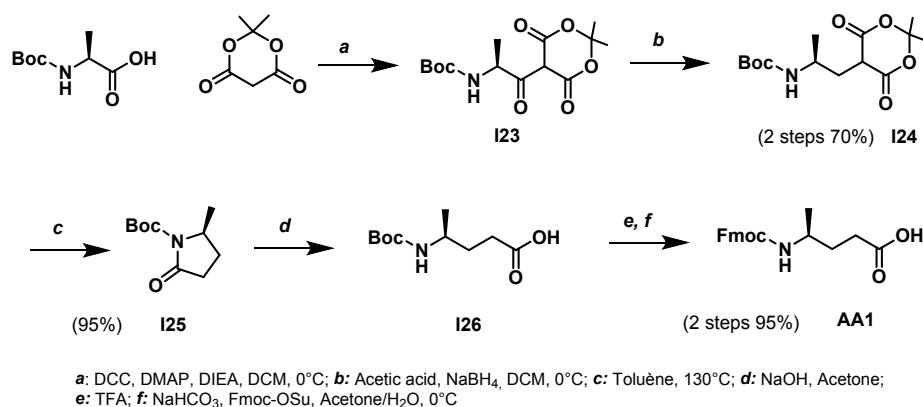


Figure S30 : <sup>13</sup>C NMR of monomer **M5** in CDCl<sub>3</sub> (75 MHz)

**$\gamma$ -Amino acid AA1; (S)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)pentanoic acid; (Fmoc- $\gamma$ A)**



**Figure S31** : Synthesis of Fmoc- $\gamma$ -amino acid **AA1**

**a:** Meldrum's acid (9.14 g, 63.4 mmol) was dissolved in DCM (200 ml). Boc-L-Ala-OH (10.0 g, 52.9 mmol), DMAP (0.59 g, 5.28 mmol) and DIEA (13.93 ml, 81.9 mmol) were added at 0 °C. DCC (13.8 g, 63.4 mmol) dissolved in DCM (60 ml) was added dropwise to the previous mixture. The reaction mixture was stirred over night at 0 °C. The white solid was filtered and discarded. The organic phase was washed with KHSO<sub>4</sub> (1M) and brine (sat), dried with MgSO<sub>4</sub> and used as a solution in the next step **b:** The previous solution of **I23** was cooled to 0°C and Acetic acid (30.2 ml, 528.7 mmol) was added. NaBH<sub>4</sub> (4.99 g, 132.2 mmol) was added portionwise and the mixture was stirred at room temperature overnight. Water was added. The organic phase was then washed with water and brine (sat), dried with MgSO<sub>4</sub> and concentrated. **I24** was recrystallized from DCM/Hexane.

**c:** **I24** (8.6 g, 27.3 mmol) was dissolved in toluene (200 ml) and stirred at 130°C for 4h. After completion, the toluene was evaporated.

**d:** **I25** (5.43g, 27.3 mmol) was dissolved in Acetone (40 ml) and NaOH (1M) (50 ml) was added, the mixture was stirred 1h at room temperature. After completion, acetone was evaporated. The aqueous phase was washed with DCM and then acidified with HCl (6N) ( $\approx$ 15 ml). The compound was extracted twice with DCM. Organic phases were combined, washed with brine, dried over MgSO<sub>4</sub> and concentrated.

**e, f:** **I26** (5.92 g, 27.2 mmol) was dissolved in TFA (40 ml) at 0 °C and stirred 40 min at room temperature. TFA was then evaporated and coevaporated with cyclohexane. The TFA mixture was dissolved in H<sub>2</sub>O/Acetone (160 ml, 50:50) and cooled to 0°C. NaHCO<sub>3</sub> (2.29 g, 27.2 mmol) was added portionwise until pH 9. Fmoc-OSu (10.0 g, 27.3 mmol) was added and the mixture was stirred overnight at room temperature. Acetone was evaporated. The aqueous phase was washed with Et<sub>2</sub>O and then acidified with HCl (6N). The compound was extracted three times with DCM. Organic phases were combined, washed with brine, dried over MgSO<sub>4</sub> and concentrated. **AA1** was recrystallized from DCM/Hexane with a yield of 95%. (8.8 g, 26 mmol, 35% yield).

**Melting point (M.p.)** 124.5 °C; **<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 300 MHz)  $\delta$ : 7.79 (d,  $J$ = 7.44 Hz, 2H), 7.62 (d,  $J$ = 10.07 Hz, 2H), 7.46-7.29 (m, 4H), 5.75 (m, 1H), 4.73 (d,  $J$ = 8.46 Hz, 1H), 4.52-4.37 (m, 2H), 4.27-4.20 (m, 1H), 3.86-3.73 (m, 1H), 2.45-2.31; (m, 2H), 1.90-1.59 (m, 2H), 1.19 (d,  $J$ = 6.29 Hz, 3H) **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 168.61, 143.95, 142.45, 141.33, 128.24, 127.46, 125.24, 120.19, 72.96, 47.34, 46.37, 31.86, 30.80, 24.57; **MS** ( $m/z$ ) calcd for C<sub>20</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup> [M + H]<sup>+</sup> 340.1543, found 340.2445.

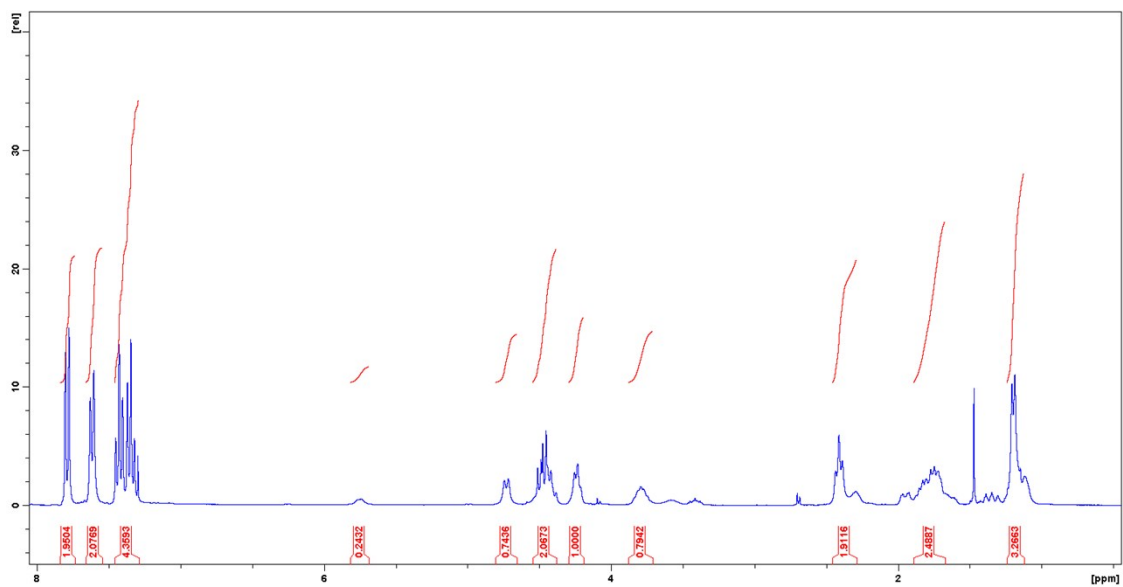


Figure S32 :  $^1\text{H}$  NMR of  $\gamma$ -amino acid AA1 in  $\text{CDCl}_3$  (300 MHz)

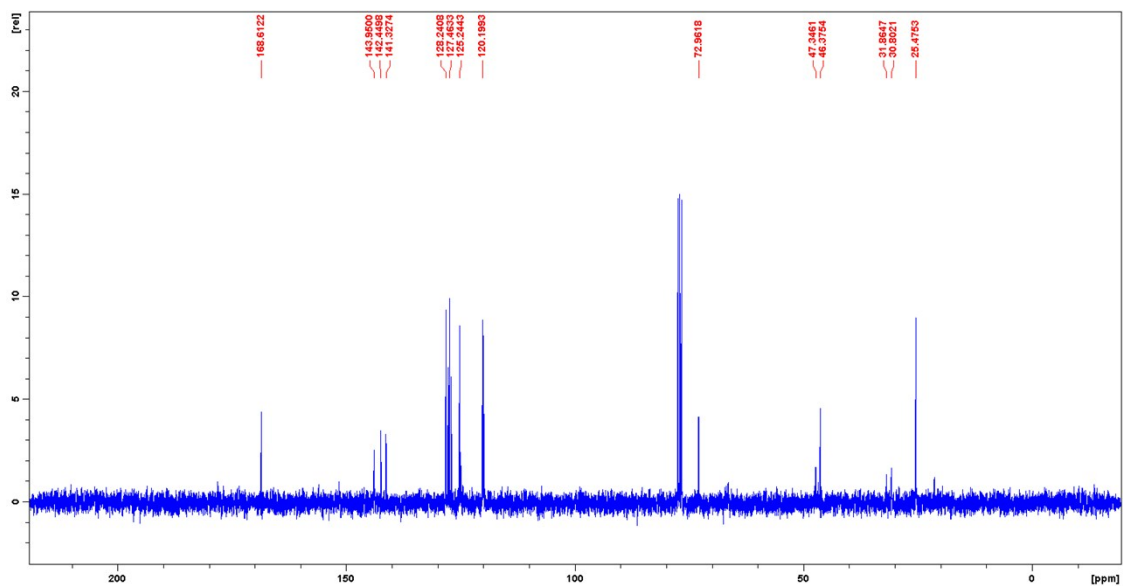
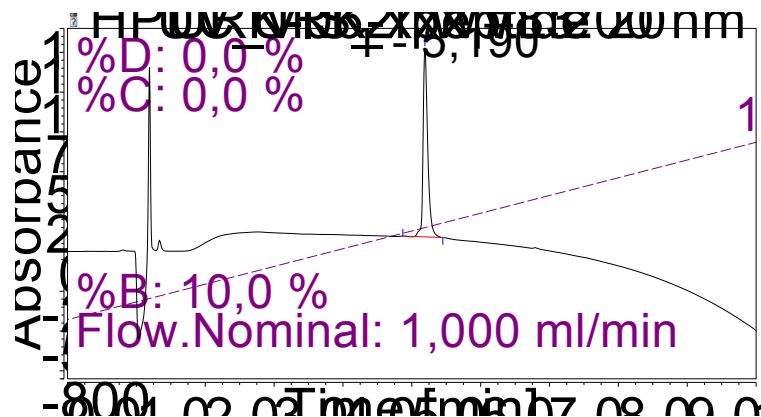


Figure S33 :  $^{13}\text{C}$  NMR of  $\gamma$ -amino acid AA1 in  $\text{CDCl}_3$  (75 MHz).

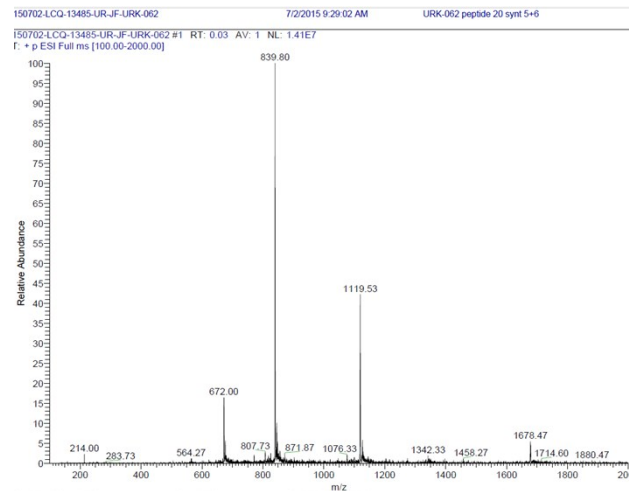
## Characterization of oligomers 2-15 and S1-S12 (analysis and biological activity)

### H-HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (2, URK-062)

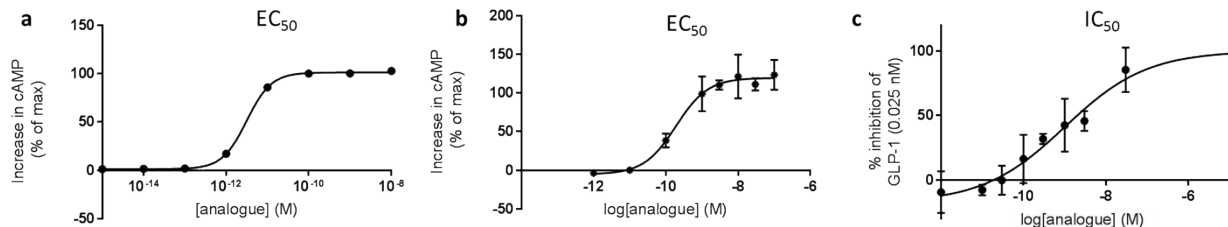
Peptide **2** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **2** was purified by semi-preparative HPLC. 40 mg was obtained (yield 12 %). **HPLC**:  $R_t = 5.19$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** ( $m/z$  3354.73): 672.00 [M+5H]<sup>5+</sup>, 839.80 [M+4H]<sup>4+</sup>, 1119.53 [M+3H]<sup>3+</sup>



**Figure S34**: HPLC profile of peptide **2** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S35** : electrospray ionization spectrum of peptide **2** performed on a DTIMS-Q-TOF spectrometer, with the dual-ESI source operated in positive ion mode.



**Figure S36** : (a-b) Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **2** in cells expressing the GLP-1R (a) Method C1 (b) Method C2. (c) Concentration-inhibition curve of **2** in a radioligand ([<sup>125</sup>I]GLP-1(7-36)) binding assay on cell membrane homogenates for affinity (IC<sub>50</sub>) to GLP-1R determination.

### H-HA<sup>6</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (3, URK-286)

Oligomer **3** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **3** was purified by semi-preparative HPLC. 4.6 mg was obtained (yield 1.3 %). **HPLC**:  $R_t = 5.22$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** ( $m/z$  3383.78): 677.60 [M+5H]<sup>5+</sup>, 847.20 [M+4H]<sup>4+</sup>, 1129.00 [M+3H]<sup>3+</sup>

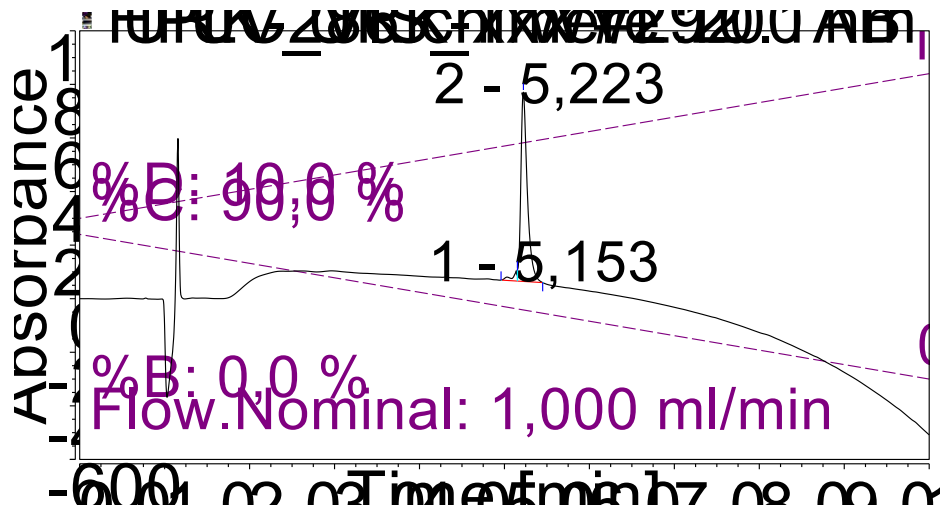


Figure S37 : HPLC profile of oligomer **3** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

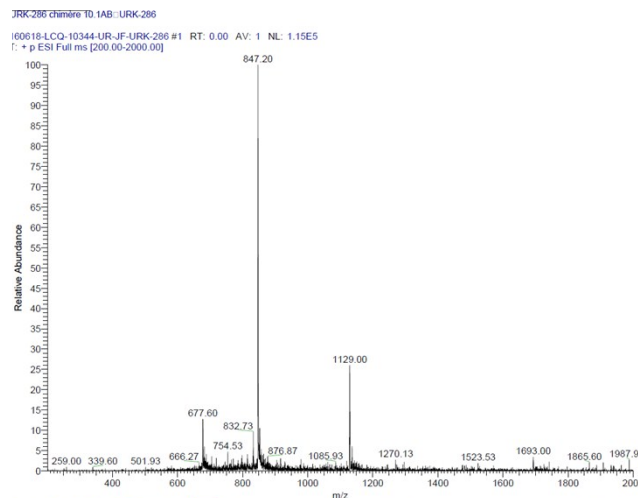


Figure S38 : electrospray ionization spectrum of peptide **3** performed on a DTIMS-Q-TOF spectrometer, with the dual-ESI source operated in positive ion mode.

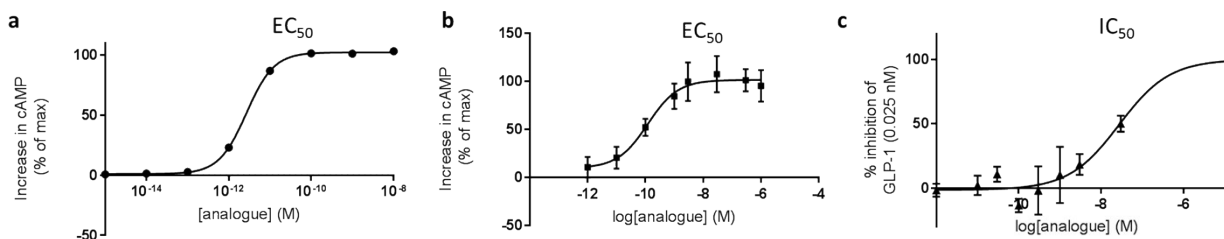
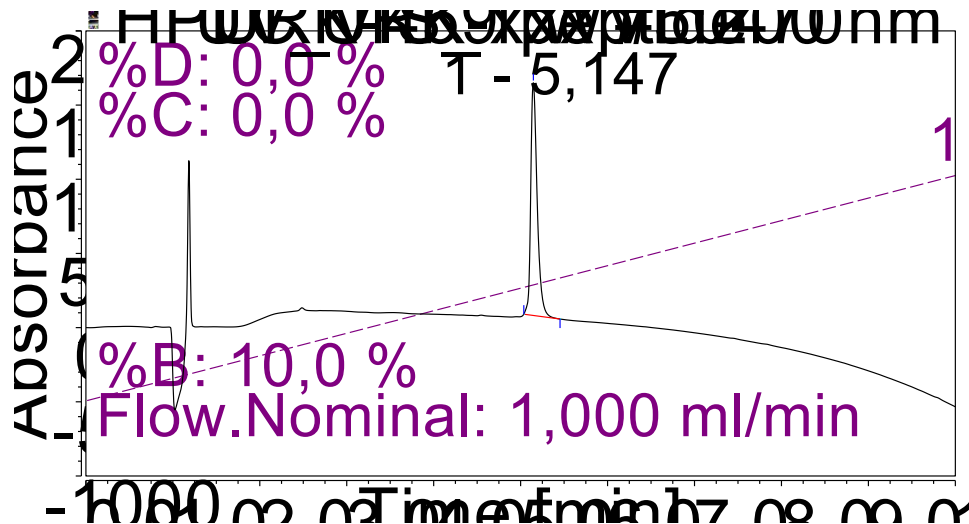


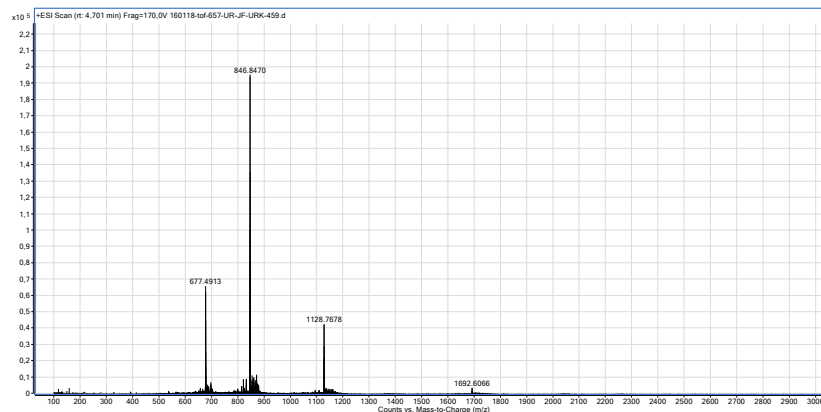
Figure S39 : (a-b) Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **3** in cells expressing the GLP-1R (a) Method C1 (b) Method C2. (c) Concentration-inhibition curve of **3** in a radioligand ([<sup>125</sup>I]GLP-1(7-36)) binding assay on cell membrane homogenates for affinity (IC<sub>50</sub>) to GLP-1R determination.

### H-HyAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (**4**, URK-459)

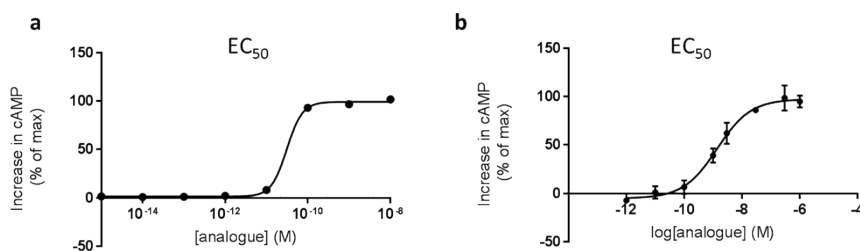
Peptide **4** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **4** was purified by semi-preparative HPLC. 2.5 mg was obtained (yield 0.8 %). **HPLC**: R<sub>t</sub> = 5.15 min (10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3382.79): 677.49 [M+5H]<sup>5+</sup>, 846.85 [M+4H]<sup>4+</sup>, 1128.77 [M+3H]<sup>3+</sup>, 1692.61 [M+2H]<sup>2+</sup>



**Figure S40** : HPLC profile of oligomer **4** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S41** : LC-MS spectrum of oligomer **4** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

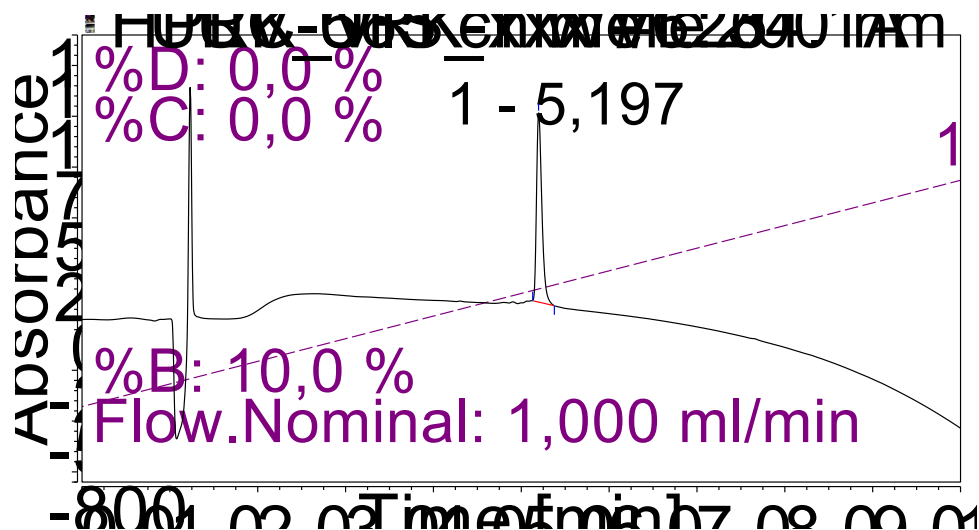


**Figure S42** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **4** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

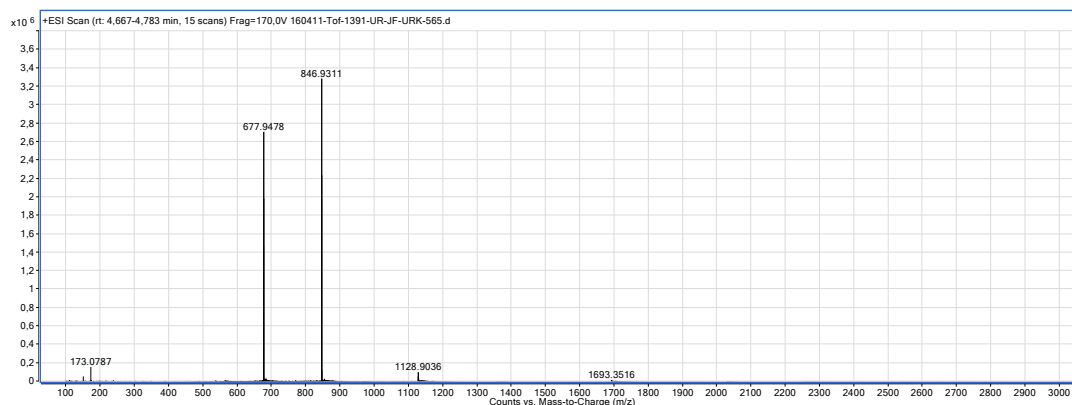


### H-HA<sup>c</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (5, URK-565)

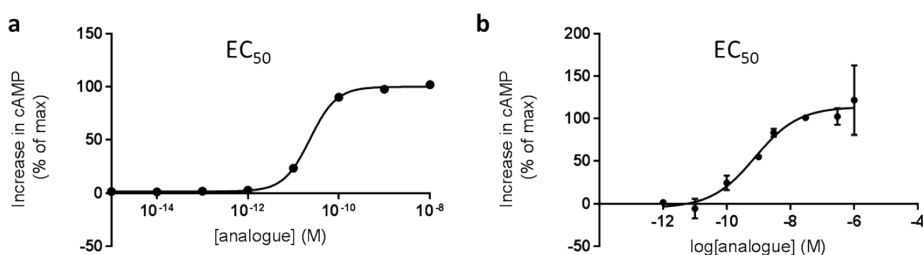
Oligomer **5** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **5** was purified by semi-preparative HPLC. 2.75 mg was obtained (yield 0.8 %). **HPLC**:  $R_t = 5.19$  min (10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3384.76): 677.95 [M+5H]<sup>5+</sup>, 846.93 [M+4H]<sup>4+</sup>, 1128.90 [M+3H]<sup>3+</sup>, 1693.35 [M+2H]<sup>2+</sup>



**Figure S43** : HPLC profile of oligomer **5** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



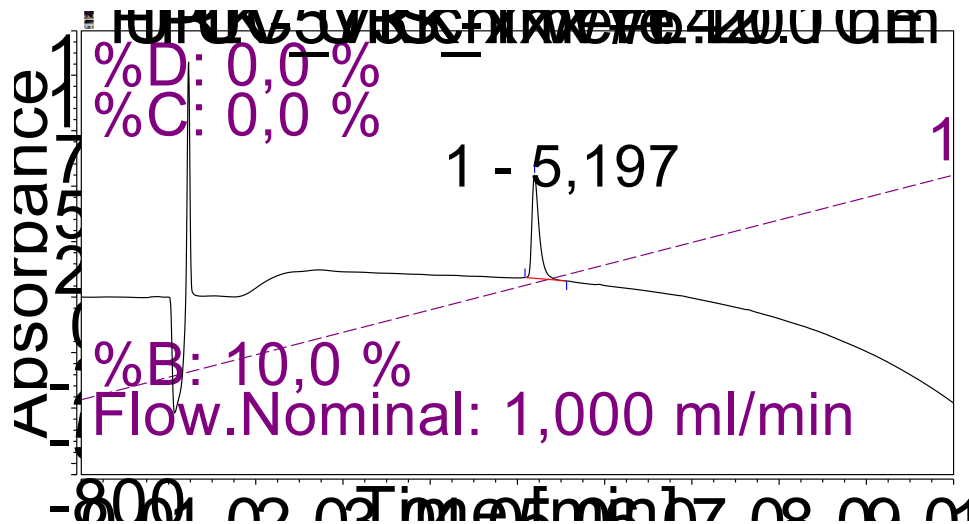
**Figure S44** : LC-MS spectrum of oligomer **5** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



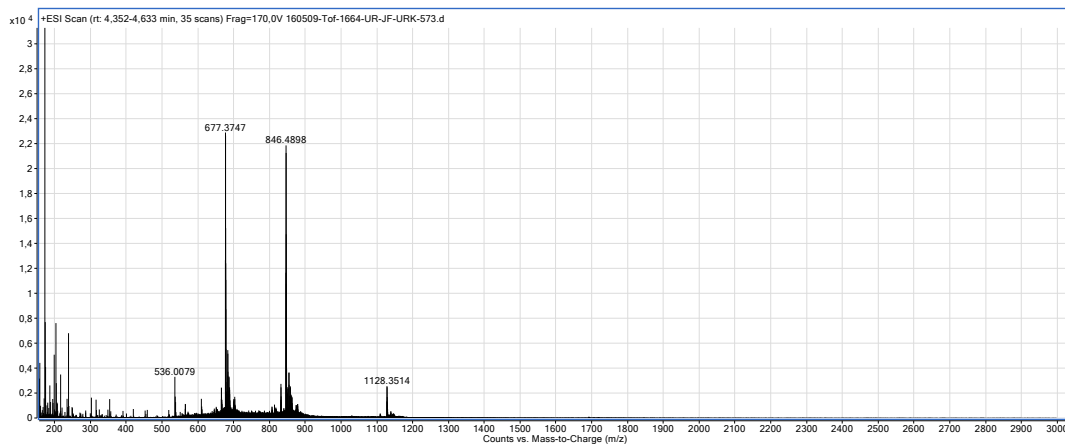
**Figure S45** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **5** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

**H-HA<sup>u</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (6, URK-573)**

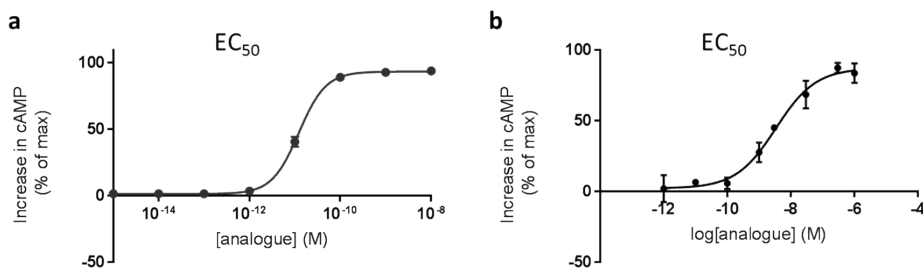
Oligomer **6** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **6** was purified by semi-preparative HPLC. 1.2 mg was obtained (yield 0.3 %). **HPLC**:  $R_t = 5.20$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3383.78): 677.37 [M+5H]<sup>5+</sup>, 846.49 [M+4H]<sup>4+</sup>, 1128.35 [M+3H]<sup>3+</sup>



**Figure S46** : HPLC profile of oligomer **6** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S47** : LC-MS spectrum of oligomer **6** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S48** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **6** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

### H-HG<sup>u</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (7, URK-521)

Oligomer **7** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **7** was purified by semi-preparative HPLC. 2.4 mg was obtained (yield 0.7 %). **HPLC**:  $R_t = 5.19$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3369.75): 674.94 [M+5H]<sup>5+</sup>, 843.42 [M+4H]<sup>4+</sup>, 1123.89 [M+3H]<sup>3+</sup>

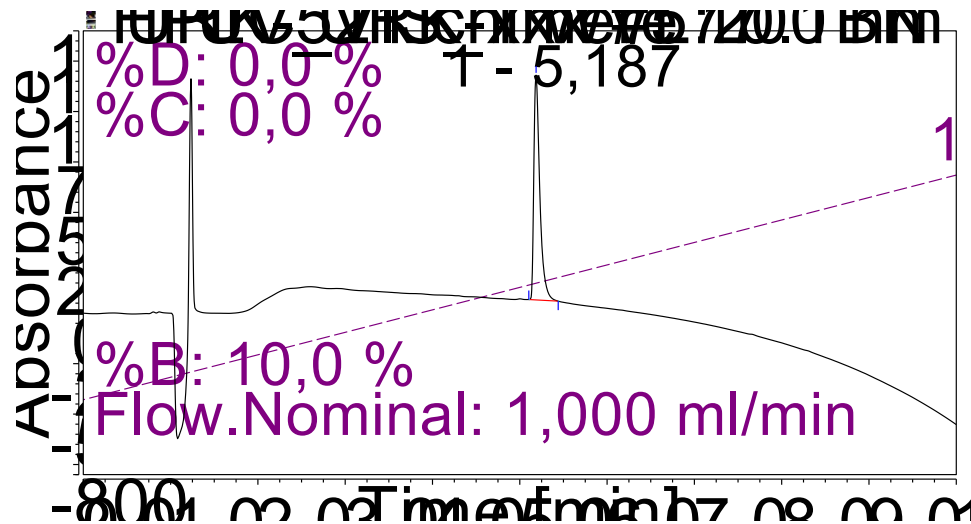


Figure S49 : HPLC profile of oligomer **7** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

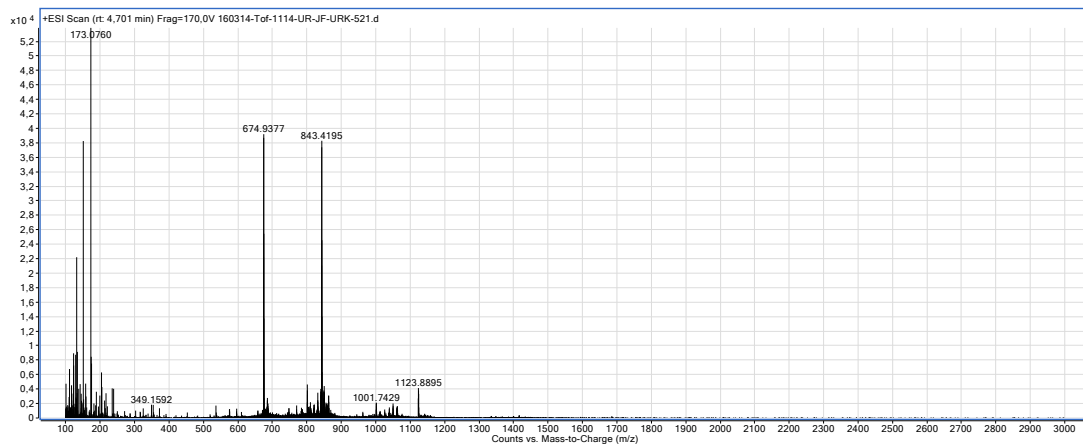


Figure S50 : LC-MS spectrum of oligomer **7** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

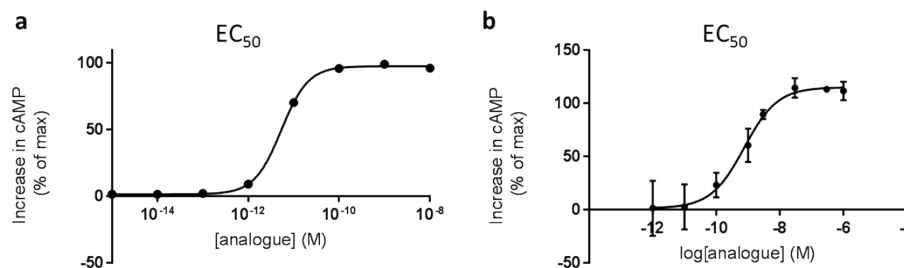
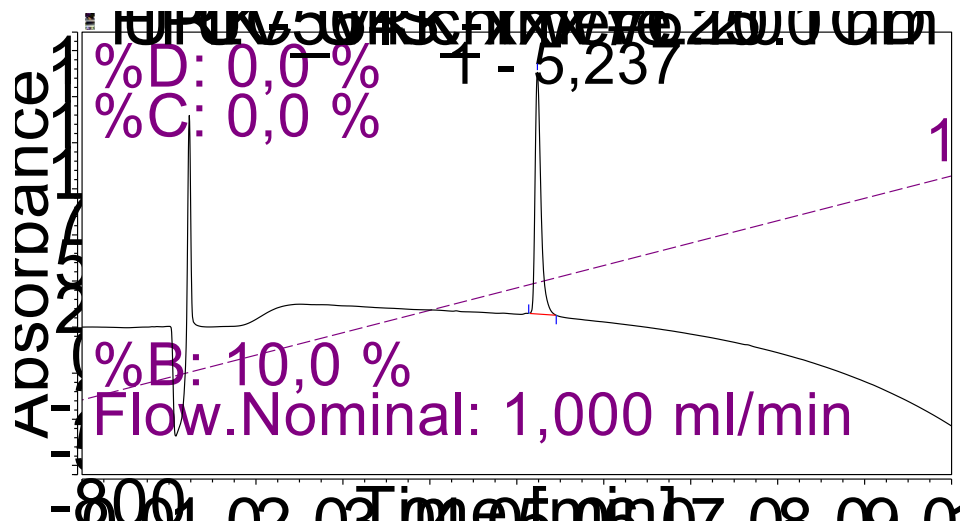


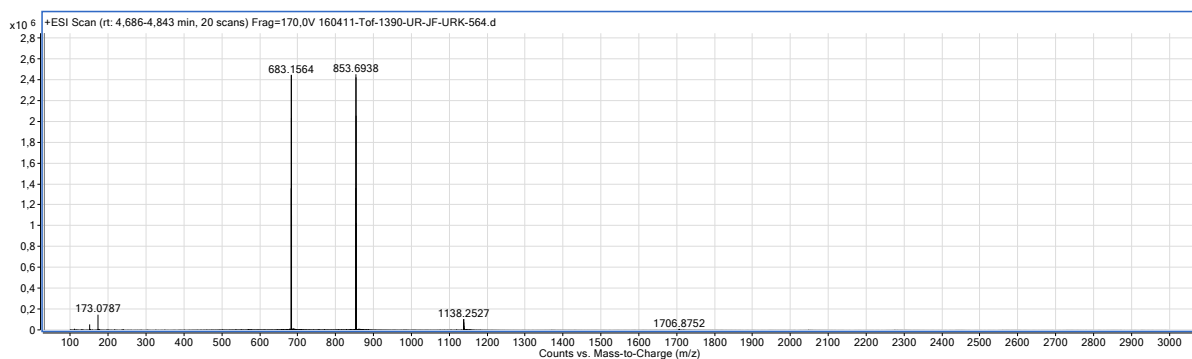
Figure S51 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **7** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

**H-HV<sup>a</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (8, URK-564)**

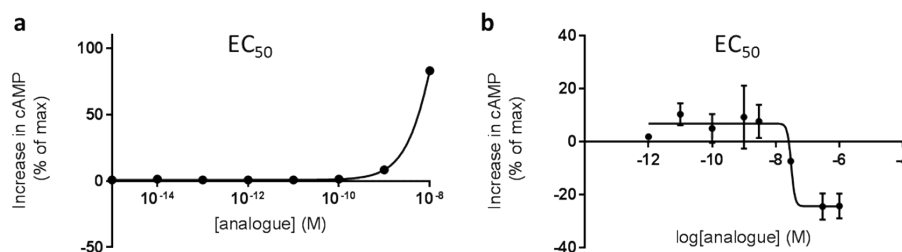
Oligomer **8** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **8** was purified by semi-preparative HPLC. 2.7 mg was obtained (yield 0.8 %). **HPLC**:  $R_t = 5.23$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3411.83): 683.15 [M+5H]<sup>5+</sup>, 853.69 [M+4H]<sup>4+</sup>, 1138.25 [M+3H]<sup>3+</sup>, 1706.87 [M+2H]<sup>2+</sup>



**Figure S52** : HPLC profile of oligomer **8** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S53** : LC-MS spectrum of oligomer **8** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S54** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **8** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

### H-HAEGTFTSDLSKQMEEEAVKLFIEWLKNNGPSSGAPPPS-NH<sub>2</sub> (9, URK-075)

Peptide **9** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **9** was purified by semi-preparative HPLC. 23 mg was obtained (yield 5.5 %). **HPLC**: R<sub>t</sub>= 5.19 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** (*m/z* 4186.63): 842.88 [M+5H]<sup>5+</sup>, 1047.65 [M+4H]<sup>4+</sup>, 1396.23 [M+3H]<sup>3+</sup>

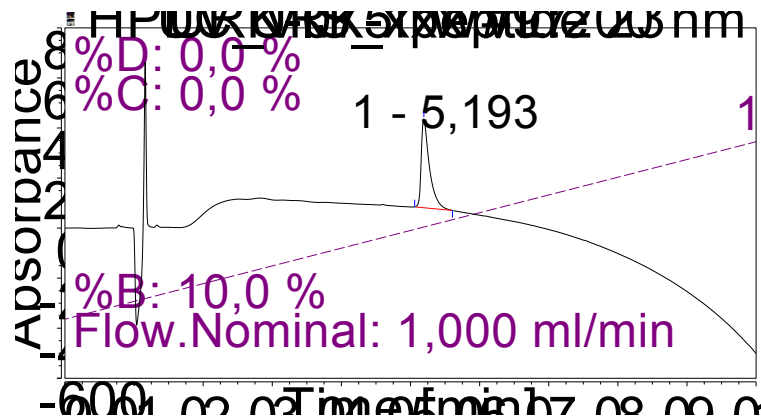


Figure S55 : HPLC profile of peptide **9** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

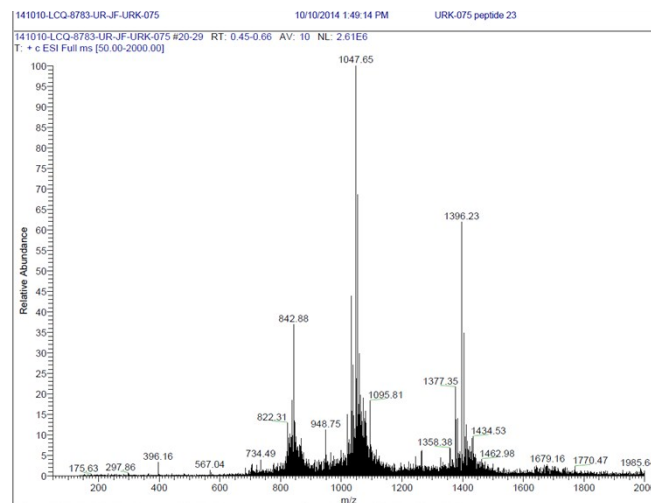


Figure S56 : electrospray ionization spectrum of peptide **9** performed on a DTIMS-Q-TOF spectrometer, with the dual-ESI source operated in positive ion mode.

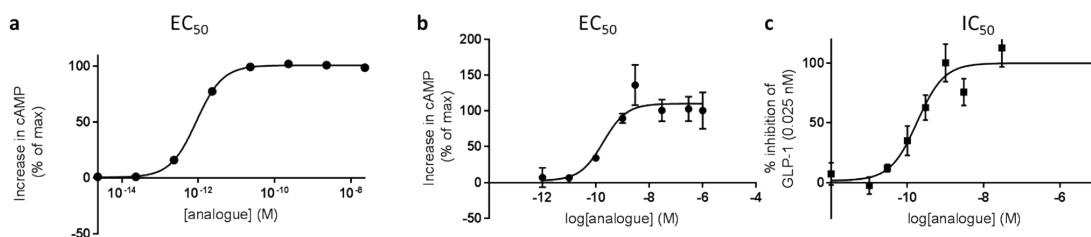
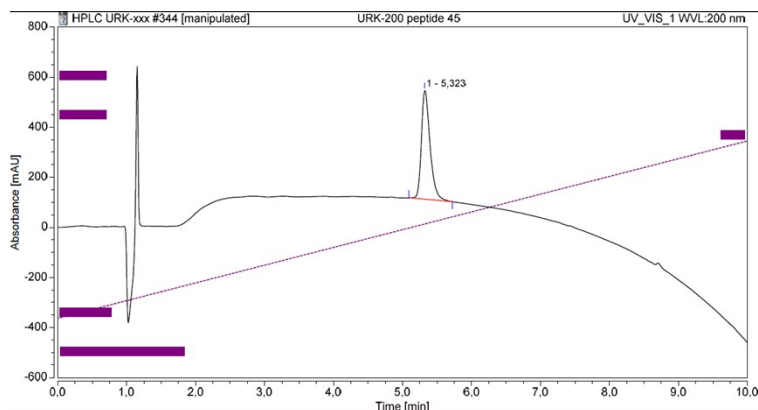


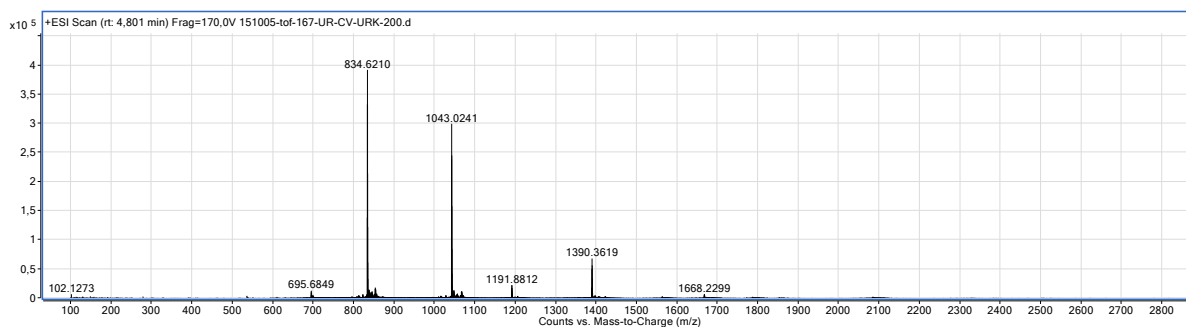
Figure S57 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **9** in cells expressing the GLP-1R (a) Method C1 (b) Method C2. (c) Concentration-inhibition curve of **9** in a radioligand ([<sup>125</sup>I]GLP-1(7-36)) binding assay on cell membrane homogenates for affinity (IC<sub>50</sub>) to GLP-1R determination.

## H-HAEGTFTSDLSKQLEEEAVKLFIEWLKNNGPSSGAPPPS-NH<sub>2</sub> (**10**, URK-200)

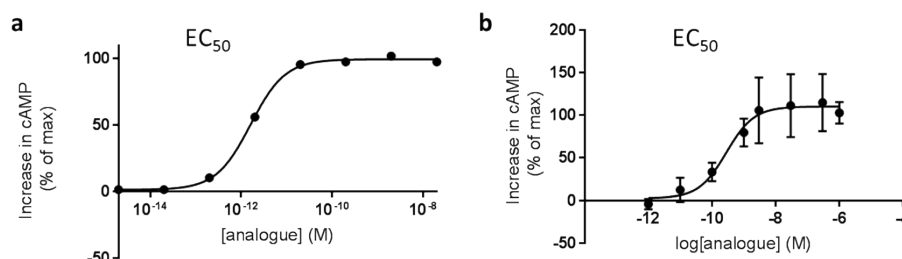
Peptide **10** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **10** was purified by semi-preparative HPLC. 11.8 mg was obtained (yield 2.8 %). **HPLC**:  $R_t = 5.32$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** ( $m/z$  4168.60): 834.62 [M+5H]<sup>5+</sup>, 1043.02 [M+4H]<sup>4+</sup>, 1390.36 [M+3H]<sup>3+</sup>



**Figure S58** : HPLC profile of peptide **10** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S59** : LC-MS spectrum of peptide **10** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S60** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **10** in cells expressing the GLP-1R (a) Method C1 (b) Method C2 (c) Concentration-inhibition curve of **10** in a radioligand ([<sup>125</sup>I]GLP-1(7-36)) binding assay on cell membrane homogenates for affinity (IC<sub>50</sub>) to GLP-1R determination.

## H-HA<sup>6</sup>EGTFTSDLKQLLEEEAVKLFIEWLKNNGPSSGAPPPS-NH<sub>2</sub> (**11**, URK-434)

Oligomer **11** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **11** was purified by semi-preparative HPLC. 2.9 mg was obtained (yield 0.7 %). **HPLC**:  $R_t = 5.28$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  4211.67): 843.24 [M+4H]<sup>4+</sup>, 1053.80 [M+3H]<sup>3+</sup>, 1404.73 [M+2H]<sup>2+</sup>

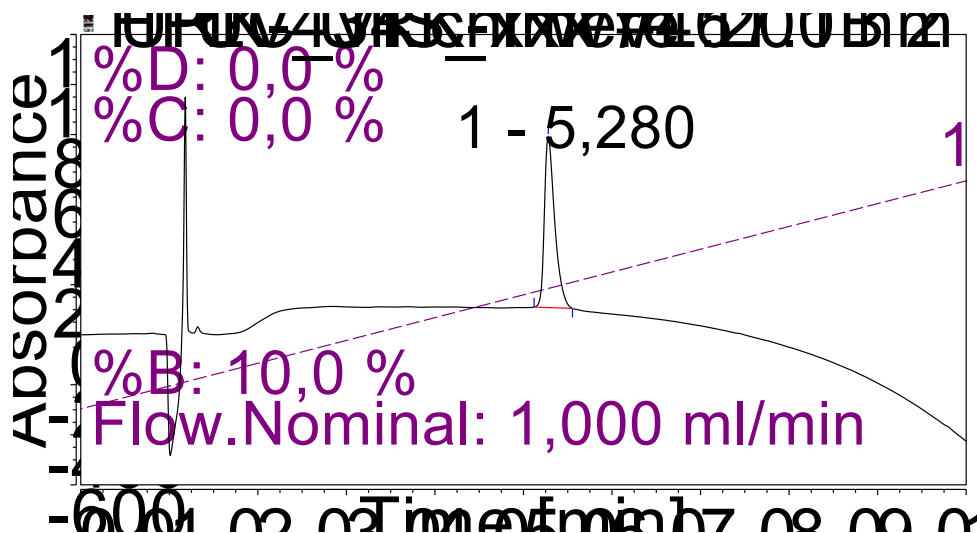


Figure S61 : HPLC profile of oligomer **11** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

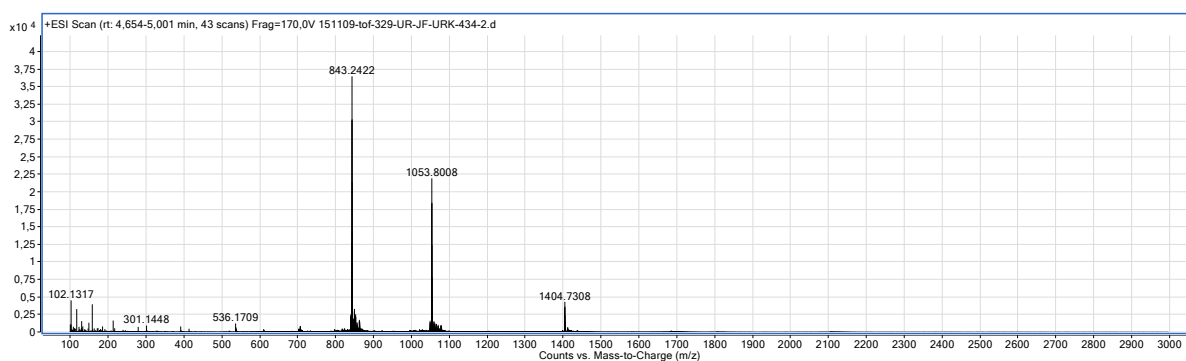


Figure S62 : LC-MS spectrum of oligomer **11** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

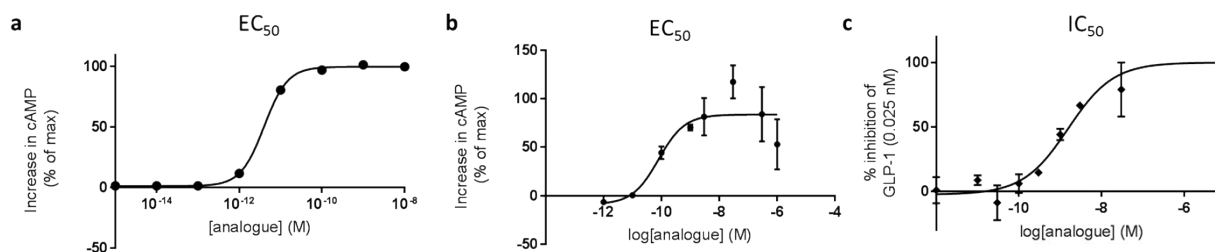
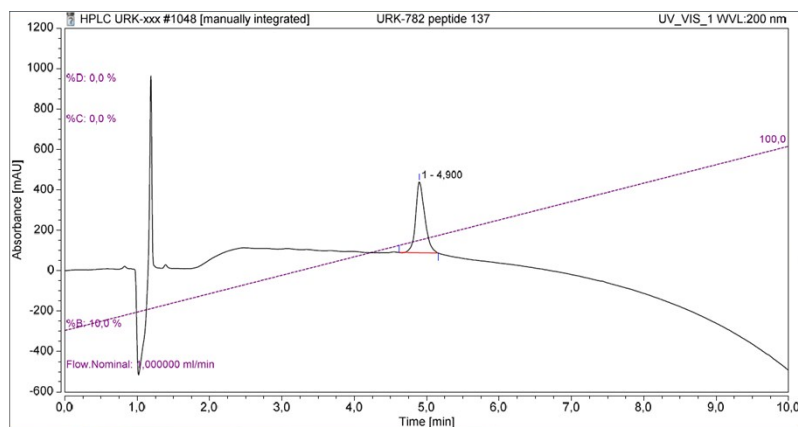


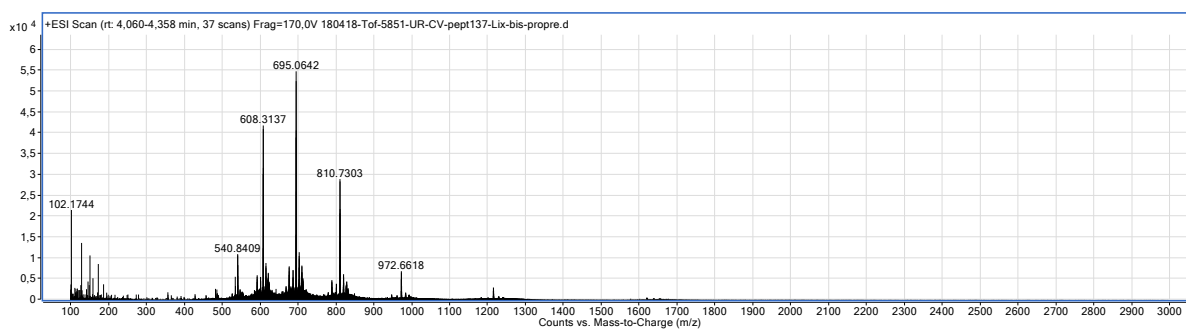
Figure S63 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **11** in cells expressing the GLP-1R (a) Method C1 (b) Method C2. (c) Concentration-inhibition curve of **11** in a radioligand ([<sup>125</sup>I]GLP-1(7-36)) binding assay on cell membrane homogenates for affinity (IC<sub>50</sub>) to GLP-1R determination.

## H-HAEGTFTSDLSKQMEEEAVKLFIEWLKNNGPSSGAPPSKSKKKK-NH<sub>2</sub> (**12**, URK-782)

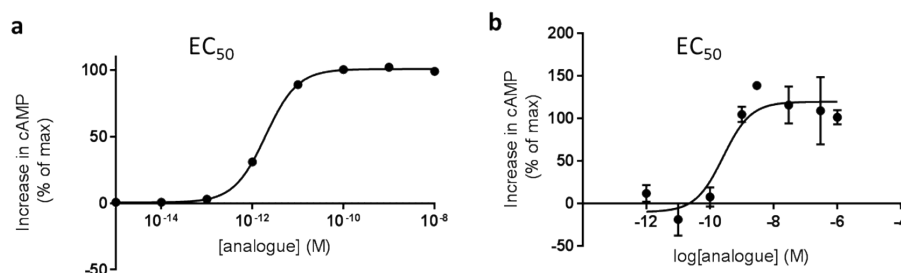
Peptide **12** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **12** was purified by semi-preparative HPLC. 1.1 mg was obtained (yield 0.62%). **HPLC**:  $R_t = 4.90$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** ( $m/z$  4858.56): 540.84 [M+9H]<sup>9+</sup>, 608.31 [M+8H]<sup>8+</sup>, 695.06 [M+7H]<sup>7+</sup>, 810.73 [M+6H]<sup>6+</sup>, 972.66 [M+5H]<sup>5+</sup>



**Figure S64** : HPLC profile of oligomer **12** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S65** : LC-MS spectrum of oligomer **12** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

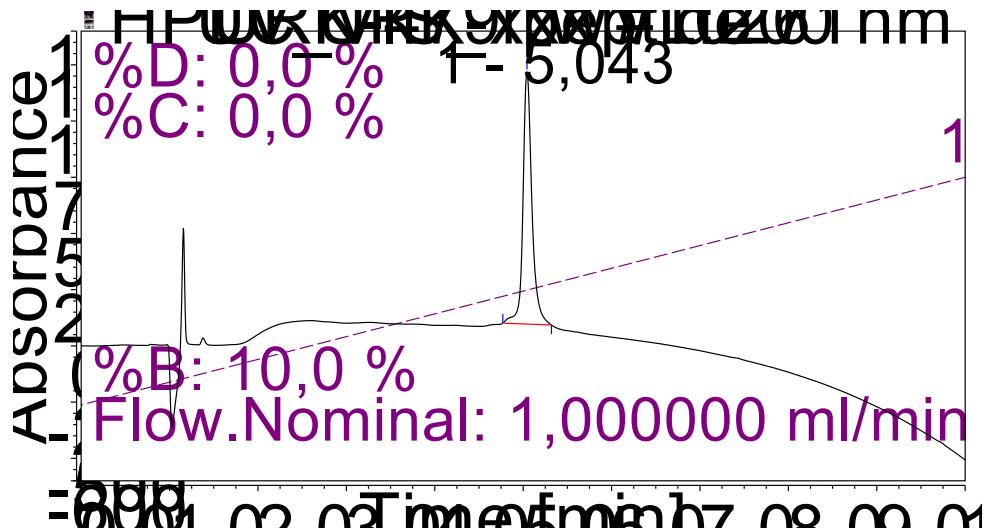


**Figure S66** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **12** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

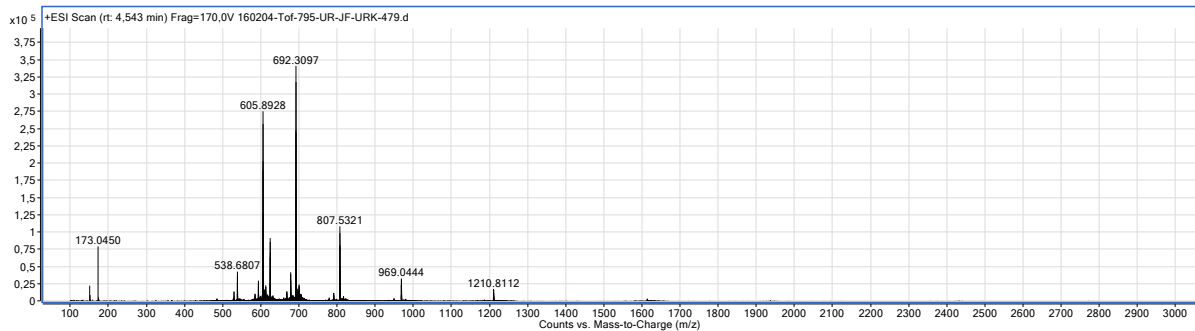


### H-HAEGTFTSDLSKQLEEEAVKLFIEWLKNNGPSSGAPPSKSKKKKK-NH<sub>2</sub> (**13**, URK-479)

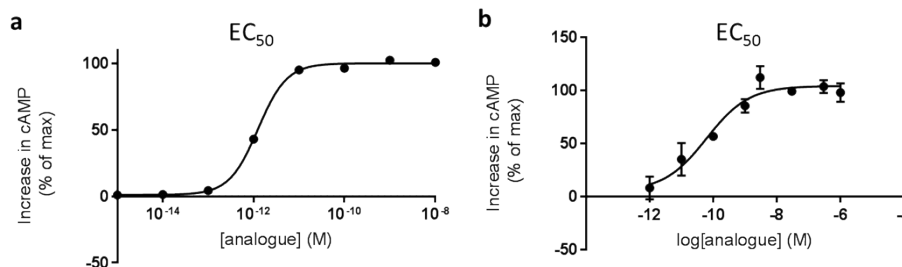
Peptide **13** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **13** was purified by semi-preparative HPLC. 2.97 mg was obtained (yield 0.6 %). **HPLC**:  $R_t$  = 5.04 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **ESI-MS** ( $m/z$  4840.53): 538.68 [M+9H]<sup>9+</sup>, 605.89 [M+8H]<sup>8+</sup>, 692.31 [M+7H]<sup>7+</sup>, 807.53 [M+6H]<sup>6+</sup>, 969.04 [M+5H]<sup>5+</sup>, 1210.81 [M+4H]<sup>4+</sup>



**Figure S67** : HPLC profile of oligomer **13** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



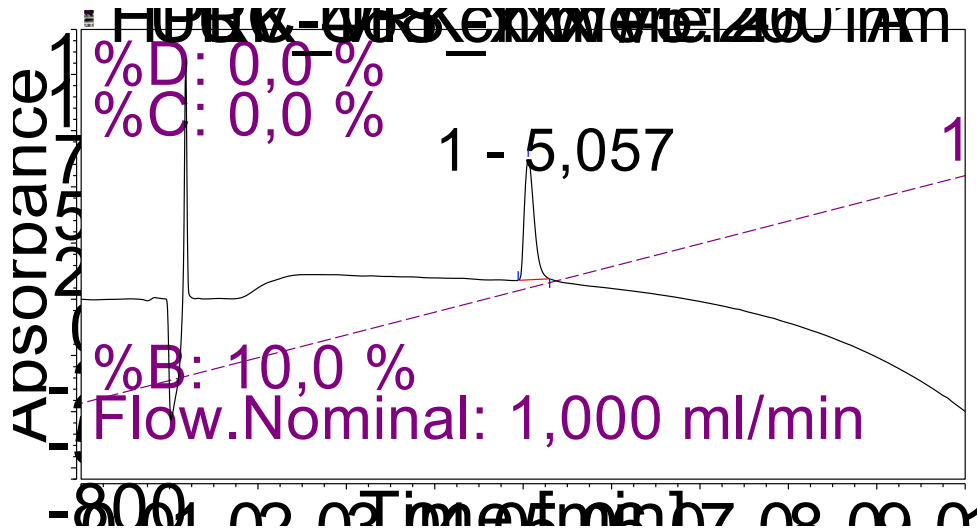
**Figure S68** : LC-MS spectrum of oligomer **13** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



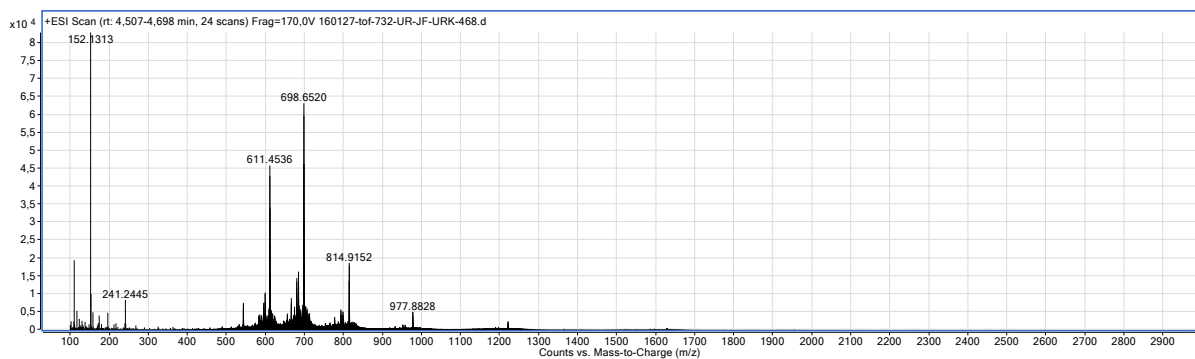
**Figure S69** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **13** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

**H-HA<sup>6</sup>EGTFTSDLKQLLEEEAVKLFIEWLKNNGPSSGAPPSKKKKKK-NH<sub>2</sub> (14, URK-468)**

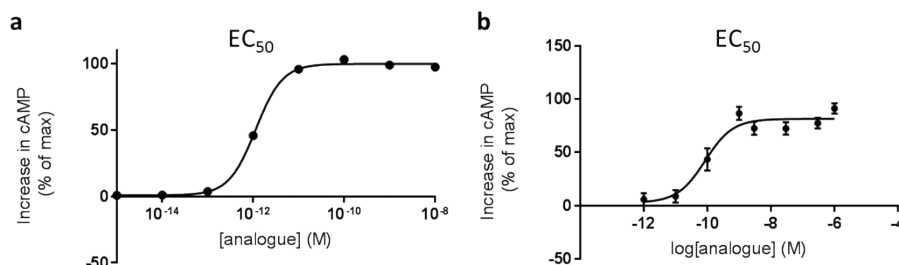
Oligomer **14** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **14** was purified by semi-preparative HPLC. 3.4 mg was obtained (yield 0.7 %). **HPLC**:  $R_t = 5.06$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  4883.6): 611.45 [M+8H]<sup>8+</sup>, 698.65 [M+7H]<sup>7+</sup>, 814.91 [M+6H]<sup>6+</sup>, 977.88 [M+5H]<sup>5+</sup>



**Figure S70** : HPLC profile of oligomer **14** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



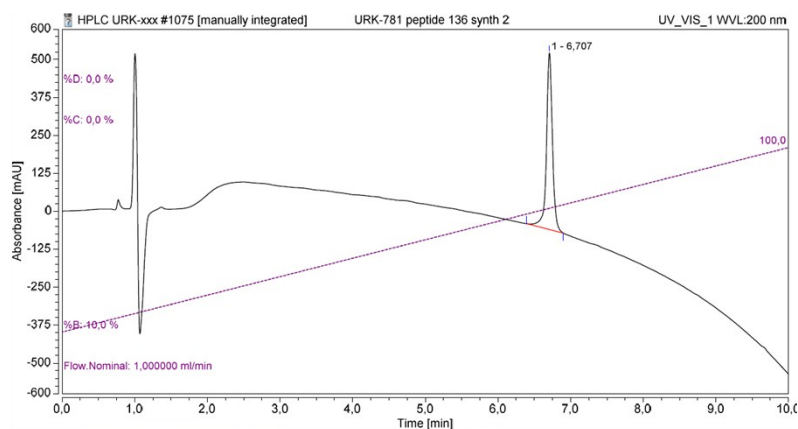
**Figure S71** : LC-MS spectrum of oligomer **14** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



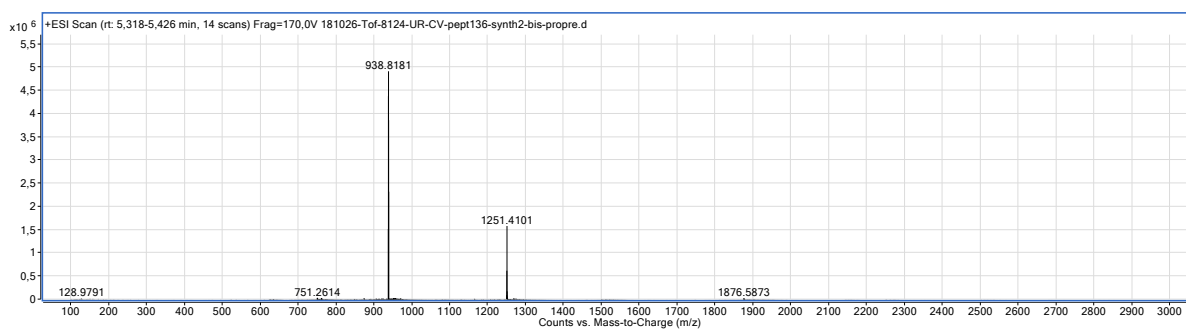
**Figure S72** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **14** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

## H- HAEGTFTSDVSSYLEGQAAK\*EFIAWLVRGRG-NH<sub>2</sub> (15, URK-781)

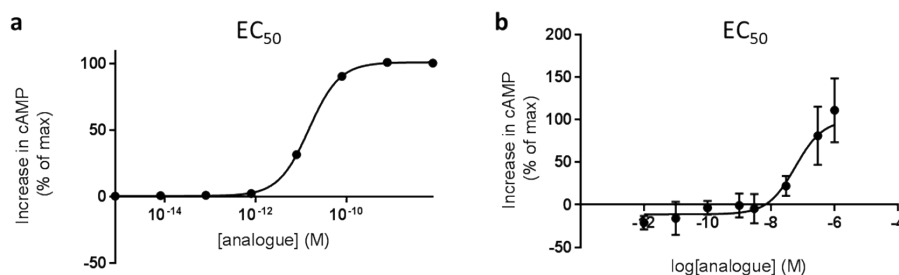
Oligomer **15** has been synthesized using the general procedure **P2** starting from sieber resin (160 mg, 0.1 mmol). The final product **15** was purified by semi-preparative HPLC. 0.77 mg was obtained (yield 0.2 %). **HPLC**:  $R_t = 6.70$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3751.26): 938.82 [M+4H]<sup>4+</sup>, 1251.41 [M+3H]<sup>3+</sup>, 1876.58 [M+2H]<sup>2+</sup>



**Figure S73** : HPLC profile of oligomer **15** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



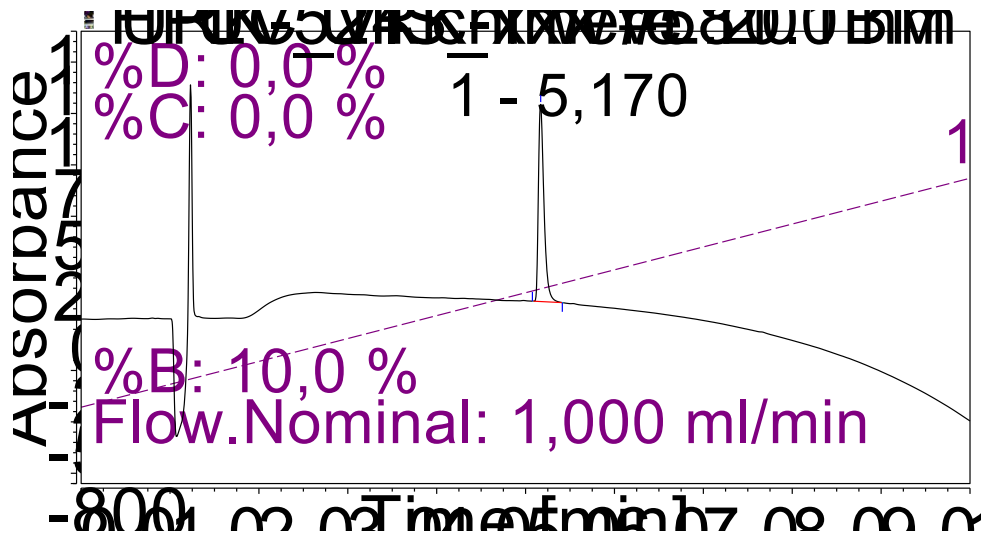
**Figure S74** : LC-MS spectrum of oligomer **15** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



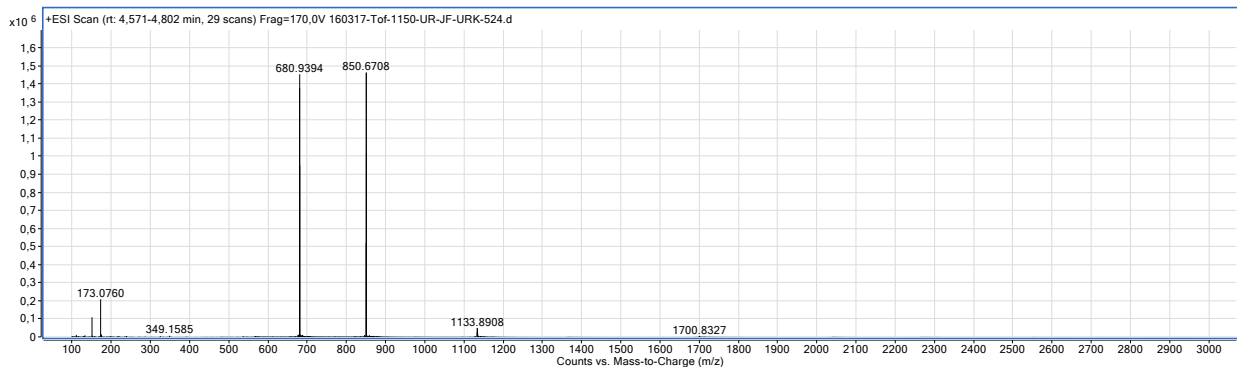
**Figure S75** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **15** in cells expressing the GLP-1R (a) Method C1 (b) Method C2.

### H-HS<sup>4</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (**S1**, URK-524)

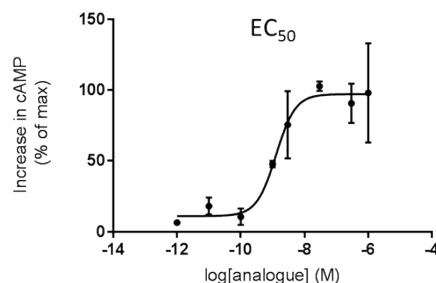
Oligomer **S1** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S1** was purified by semi-preparative HPLC. 1.8 mg was obtained (yield 0.5 %). **HPLC**:  $R_t = 5.17$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3397.77): 674.94 [M+5H]<sup>5+</sup>, 843.42 [M+4H]<sup>4+</sup>, 1123.89 [M+3H]<sup>3+</sup>



**Figure S76** : HPLC profile of oligomer **S1** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



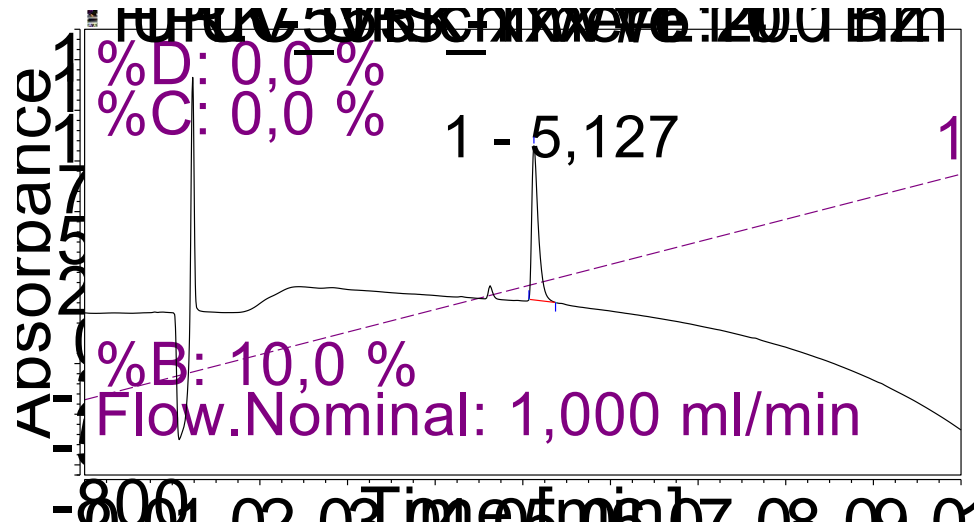
**Figure S77** : LC-MS spectrum of oligomer **S1** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



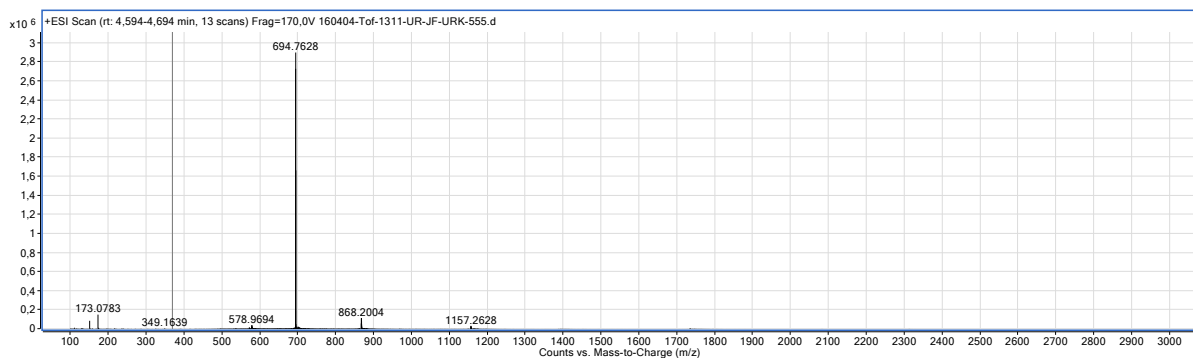
**Figure S78** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S1** in cells expressing the GLP-1R (Method C2).

### H-HR<sup>U</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S2, URK-555)

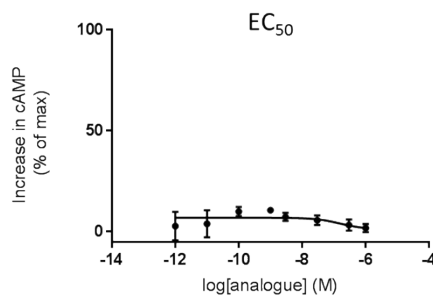
Oligomer **S2** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S2** was purified by semi-preparative HPLC. 2.2 mg was obtained (yield 0.6 %). **HPLC**:  $R_t = 5.13$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3468.89): 578.97 [M+6H]<sup>6+</sup>, 694.76 [M+5H]<sup>5+</sup>, 868.20 [M+4H]<sup>4+</sup>, 1157.26 [M+3H]<sup>3+</sup>



**Figure S79** : HPLC profile of oligomer **S2** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



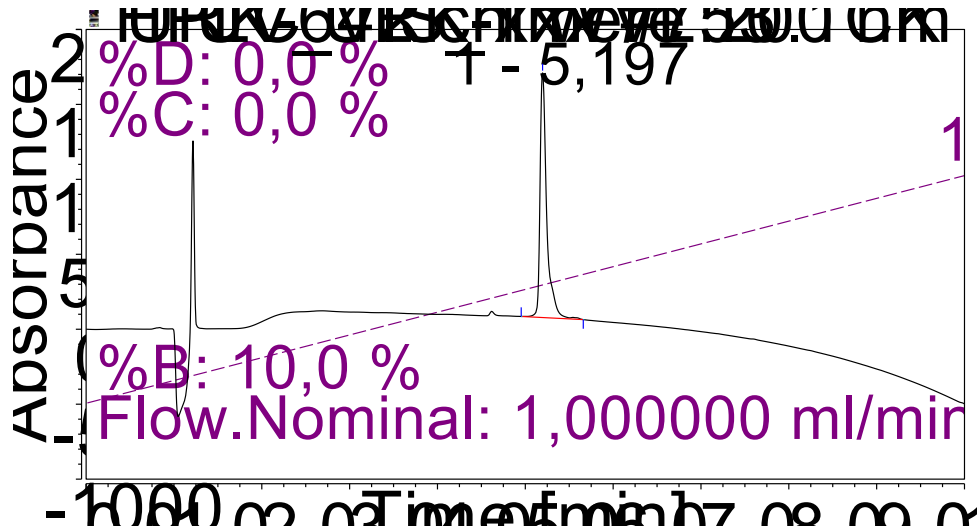
**Figure S80** : LC-MS spectrum of oligomer **S2** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



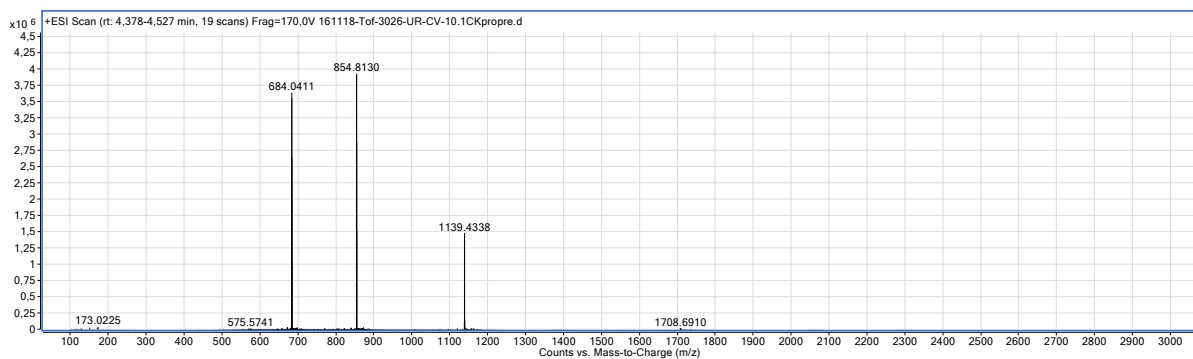
**Figure S81** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S2** in cells expressing the GLP-1R (Method C2).

**H-HC<sup>e</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S3, URK-642)**

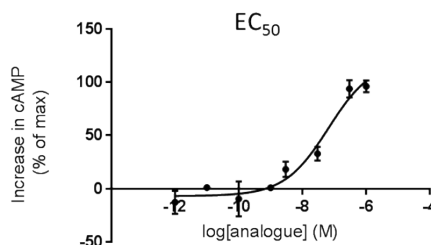
Oligomer **S3** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S3** was purified by semi-preparative HPLC. 3.3 mg was obtained (yield 0.96 %). **HPLC**: R<sub>t</sub>= 5.19 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3415.84): 684.04 [M+5H]<sup>5+</sup>, 854.81 [M+4H]<sup>4+</sup>, 1139.43 [M+3H]<sup>3+</sup>, 1708.69 [M+2H]<sup>2+</sup>



**Figure S82** : HPLC profile of oligomer **S3** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S83** : LC-MS spectrum of oligomer **S3** performed on UHPLC coupled to an ESI-MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S84** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S3** in cells expressing the GLP-1R (Method C2).

### H-HQ<sup>e</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S4, URK-548)

Oligomer **S4** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S4** was purified by semi-preparative HPLC. 2.8 mg was obtained (yield 0.8 %). **HPLC**: R<sub>t</sub>= 5.16 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3440.83): 688.95 [M+5H]<sup>5+</sup>, 860.94 [M+4H]<sup>4+</sup>, 1147.91 [M+3H]<sup>3+</sup>, 1721.36 [M+2H]<sup>2+</sup>

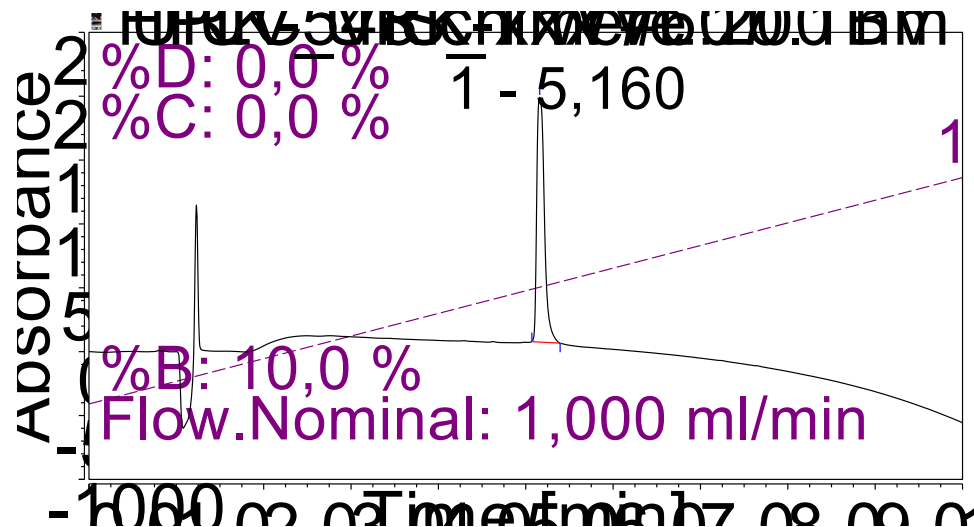


Figure S85 : HPLC profile of oligomer **S4** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

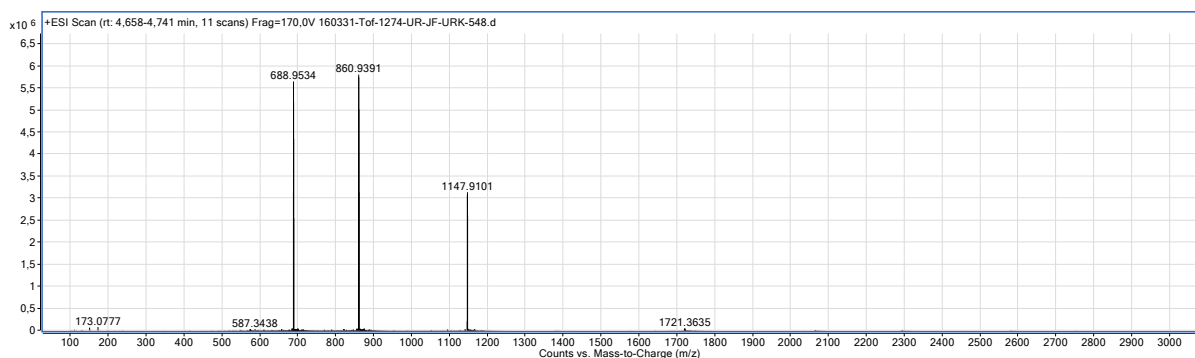


Figure S86 : LC-MS spectrum of oligomer **S4** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

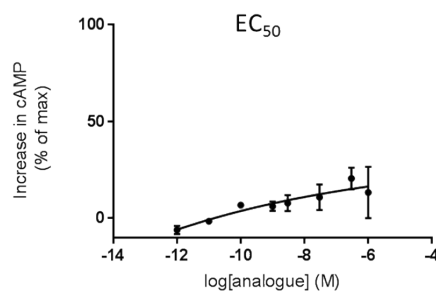
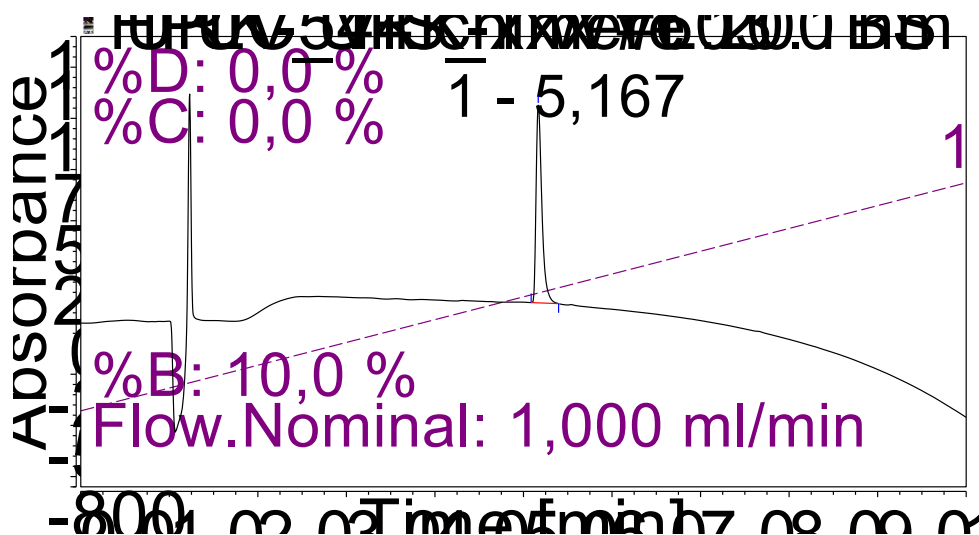


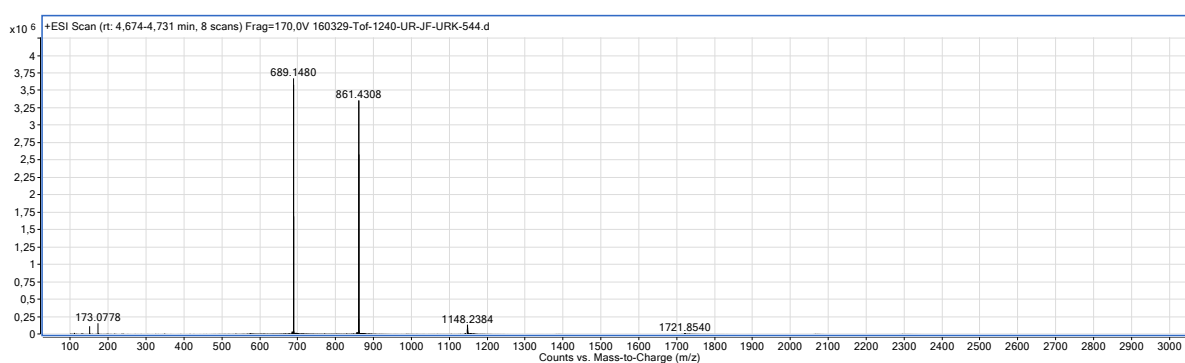
Figure S87 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S4** in cells expressing the GLP-1R (Method C2).

**H-HE<sup>e</sup>EGTFTSDVSSYLEGQAQAEKFIWLVKGRG-NH<sub>2</sub> (S5, URK-544)**

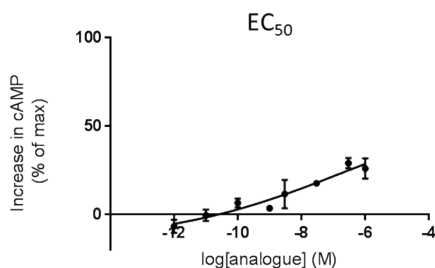
Oligomer **S5** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S5** was purified by semi-preparative HPLC. 2.8 mg was obtained (yield 0.8 %). **HPLC**:  $R_t = 5.16$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3441.81): 689.15 [M+5H]<sup>5+</sup>, 861.43 [M+4H]<sup>4+</sup>, 1148.24 [M+3H]<sup>3+</sup>, 1721.85 [M+2H]<sup>2+</sup>



**Figure S88** : HPLC profile of oligomer **S5** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S89** : LC-MS spectrum of oligomer **S5** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S90** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S5** in cells expressing the GLP-1R (Method C2).



## H-HI<sup>14</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S6, URK-562)

Oligomer **S6** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S6** was purified by semi-preparative HPLC. 2.2 mg was obtained (yield 0.6 %). **HPLC**:  $R_t = 5.27$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3425.86): 685.96 [M+5H]<sup>5+</sup>, 857.19 [M+4H]<sup>4+</sup>, 1142.92 [M+3H]<sup>3+</sup>, 1713.88 [M+2H]<sup>2+</sup>

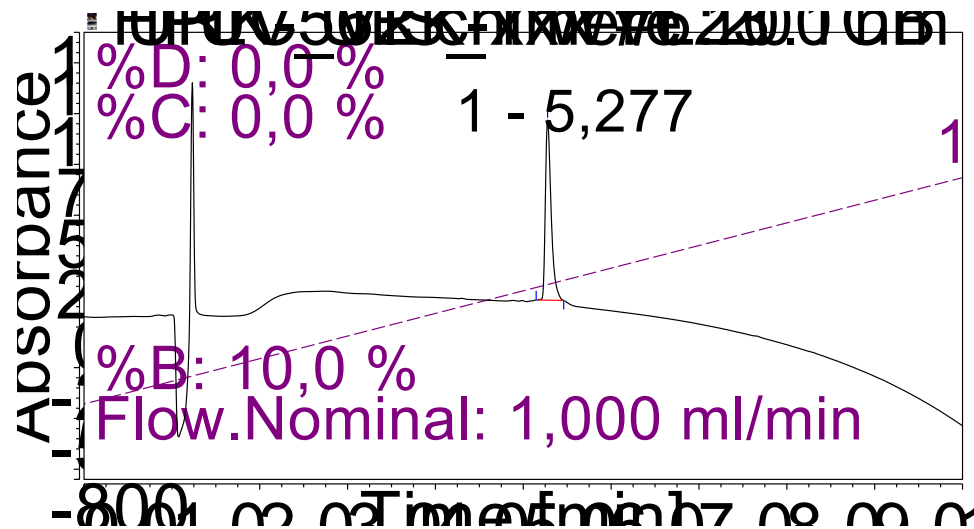


Figure S91 : HPLC profile of oligomer **S6** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

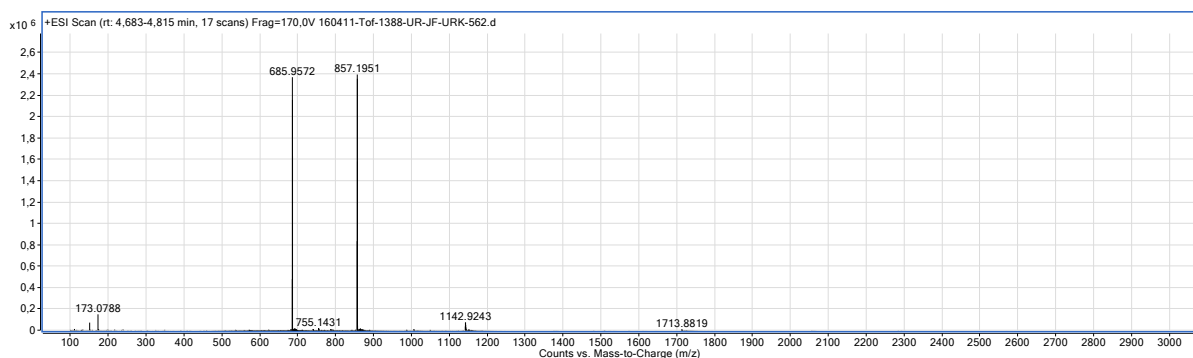


Figure S92 : LC-MS spectrum of oligomer **S6** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

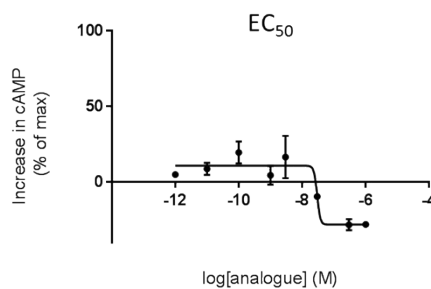


Figure S93 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S6** in cells expressing the GLP-1R (Method C2).

## H-HL<sup>E</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S7, URK-551)

Oligomer **S7** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S7** was purified by semi-preparative HPLC. 2.1 mg was obtained (yield 0.6 %). **HPLC**:  $R_t = 5.29$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3425.86): 686.15 [M+5H]<sup>5+</sup>, 857.44 [M+4H]<sup>4+</sup>, 1142.92 [M+3H]<sup>3+</sup>

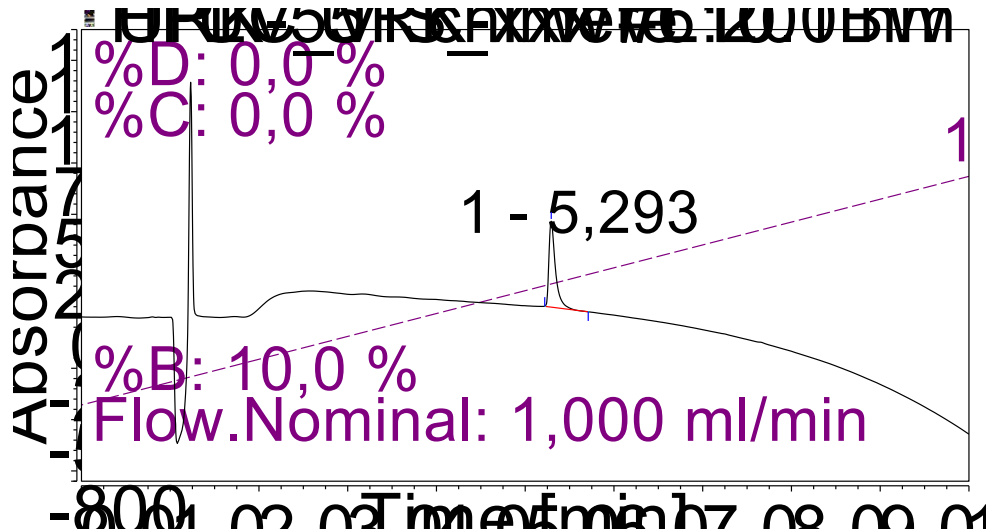


Figure S94 : HPLC profile of oligomer **S7** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

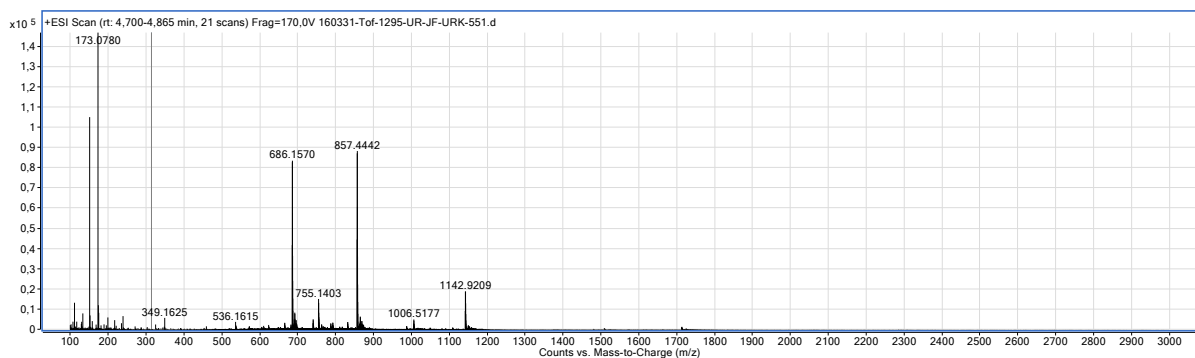


Figure S95 : LC-MS spectrum of oligomer **S7** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

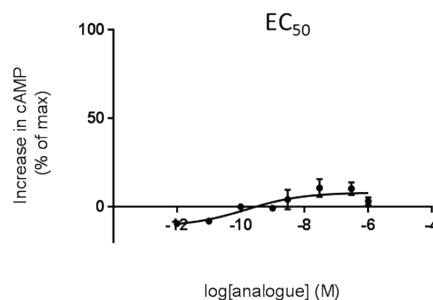
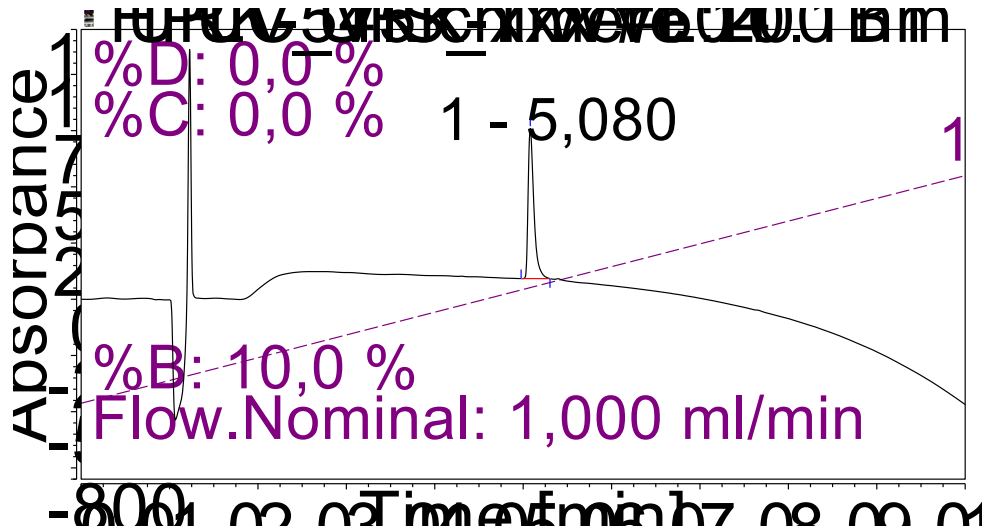


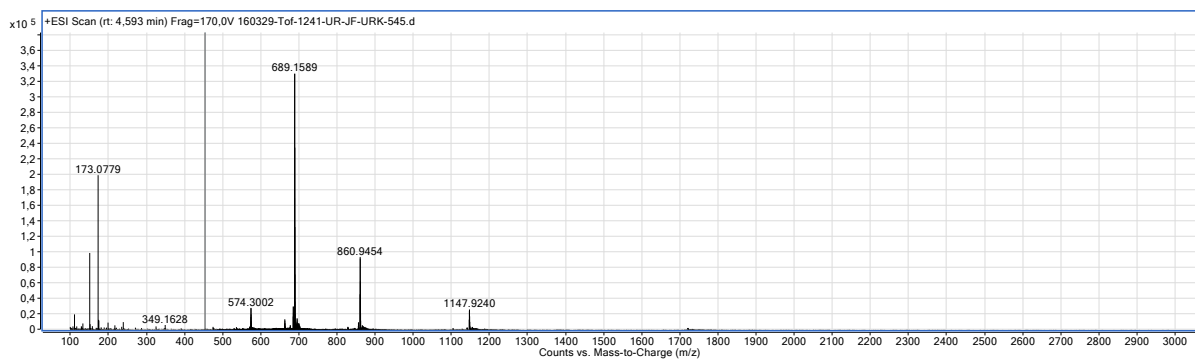
Figure S96 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S7** in cells expressing the GLP-1R (Method C2).

**H-HK<sup>4</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S8, URK-545)**

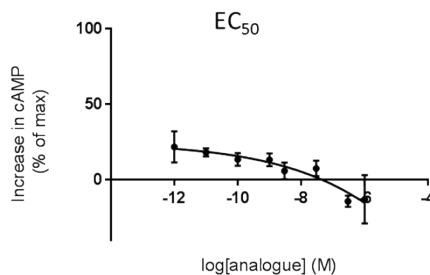
Oligomer **S8** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S8** was purified by semi-preparative HPLC. 3.1 mg was obtained (yield 0.9 %). **HPLC**: R<sub>t</sub>= 5.08 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3440.87): 574.30 [M+6H]<sup>6+</sup>, 686.16 [M+5H]<sup>5+</sup>, 860.94 [M+4H]<sup>4+</sup>, 1147.92 [M+3H]<sup>3+</sup>



**Figure S97** : HPLC profile of oligomer **S8** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S98** : LC-MS spectrum of oligomer **S8** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S99** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S8** in cells expressing the GLP-1R (Method C2).

### H-HF<sup>4</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S9, URK-546)

Oligomer **S9** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S9** was purified by semi-preparative HPLC. 2.6 mg was obtained (yield 0.8 %). **HPLC**:  $R_t = 5.08$  min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** ( $m/z$  3459.87): 577.63 [M+6H]<sup>6+</sup>, 692.75 [M+5H]<sup>5+</sup>, 865.68 [M+4H]<sup>4+</sup>, 1154.25 [M+3H]<sup>3+</sup>, 1730.87 [M+2H]<sup>2+</sup>

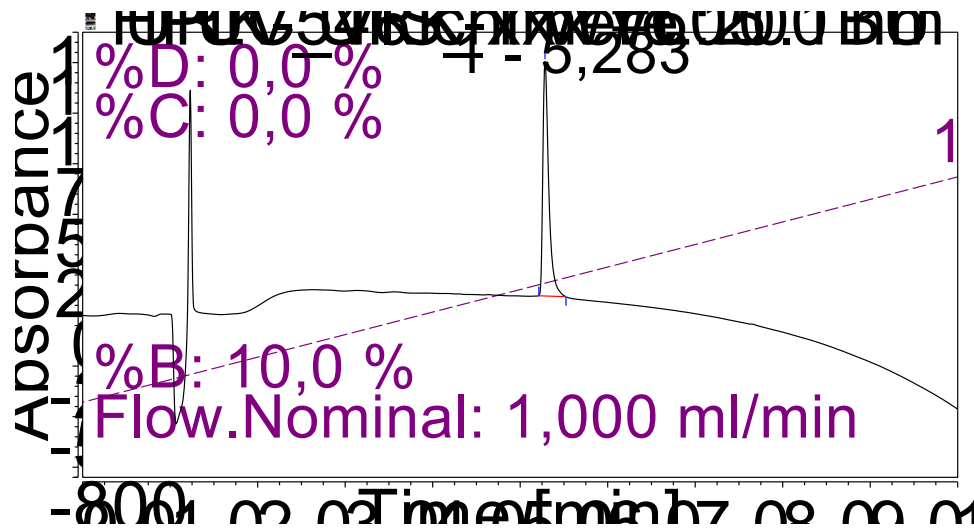


Figure S100 : HPLC profile of oligomer **S9** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

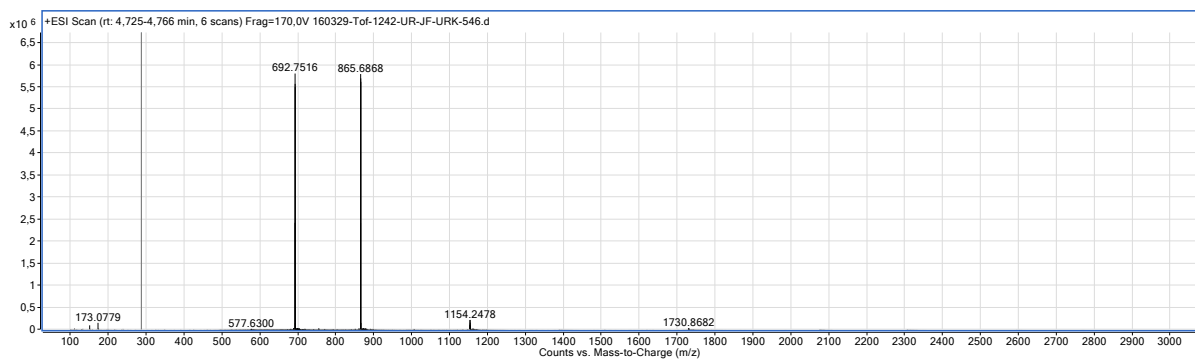


Figure S101 : LC-MS spectrum of oligomer **S9** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

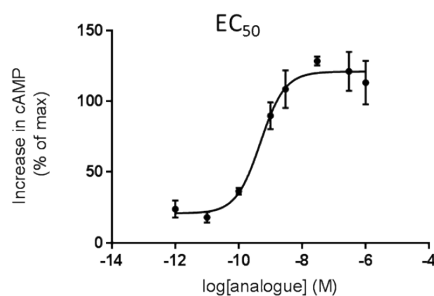
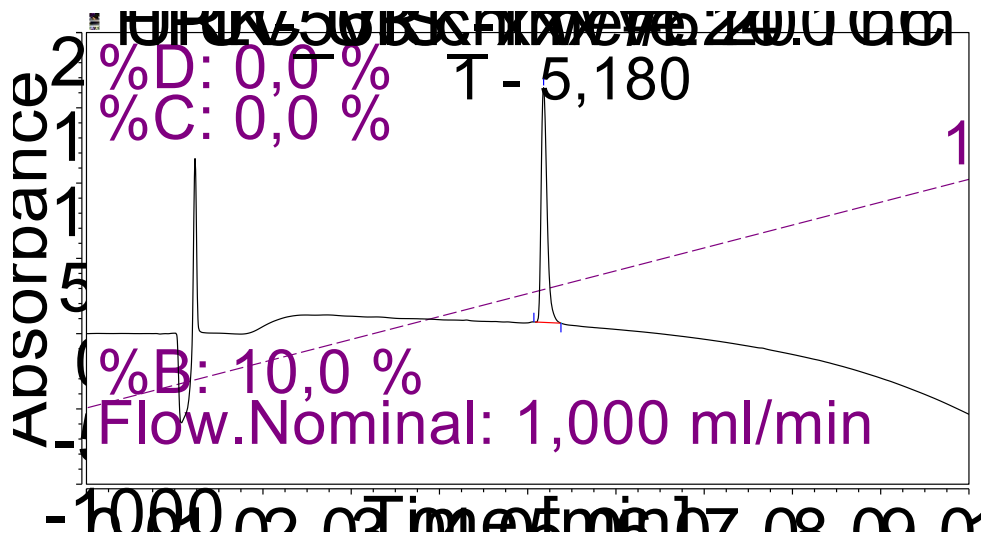


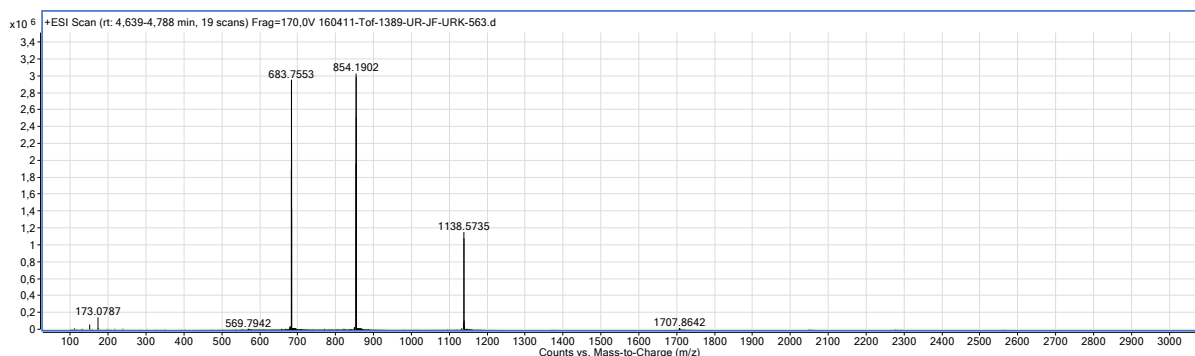
Figure S102 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **9** in cells expressing the GLP-1R (Method C2).

**H-HT<sup>6</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (S10, URK-563)**

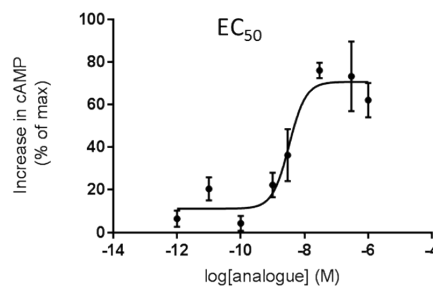
Oligomer **S10** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S10** was purified by semi-preparative HPLC. 2.1 mg was obtained (yield 0.6 %). **HPLC**: R<sub>t</sub>= 5.18 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3413.8): 569.79 [M+6H]<sup>6+</sup>, 683.75 [M+5H]<sup>5+</sup>, 854.19 [M+4H]<sup>4+</sup>, 1138.57 [M+3H]<sup>3+</sup>, 1707.86 [M+2H]<sup>2+</sup>



**Figure S103** : HPLC profile of oligomer **S10** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).



**Figure S104** : LC-MS spectrum of oligomer **S10** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).



**Figure S105** : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S10** in cells expressing the GLP-1R (Method C2).

## H-HW<sup>u</sup>EGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (**S11**, URK-554)

Oligomer **S11** has been synthesized using the general procedure **P1** starting from sieber resin (160 mg, 0.1 mmol). The final product **S11** was purified by semi-preparative HPLC. 2.2 mg was obtained (yield 0.6 %). **HPLC**: R<sub>t</sub>= 5.34 min (10-100% CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18); **LC-MS** (*m/z* 3498.91): 700.75 [M+5H]<sup>5+</sup>, 875.69 [M+4H]<sup>4+</sup>, 1167.25 [M+3H]<sup>3+</sup>

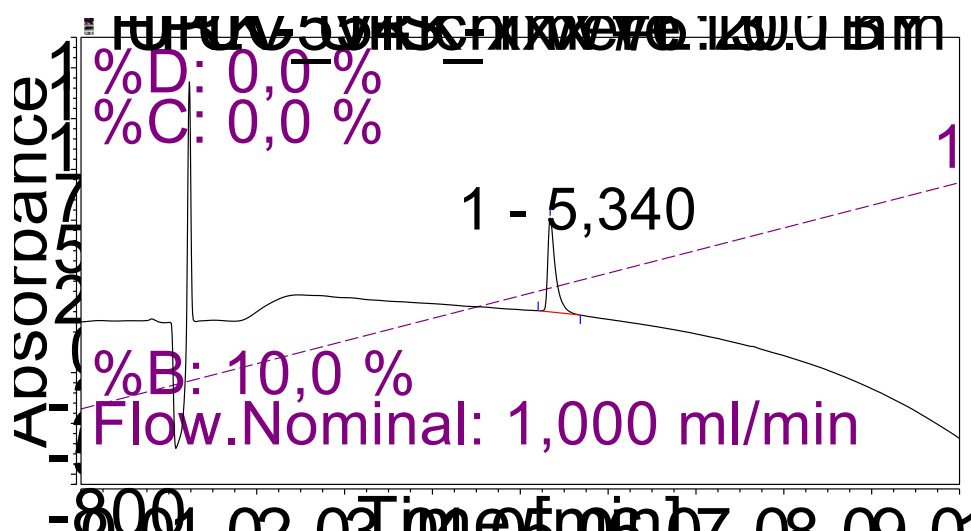


Figure S106 : HPLC profile of oligomer **S11** (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % TFA in H<sub>2</sub>O 0.1% TFA, 10 min, C18).

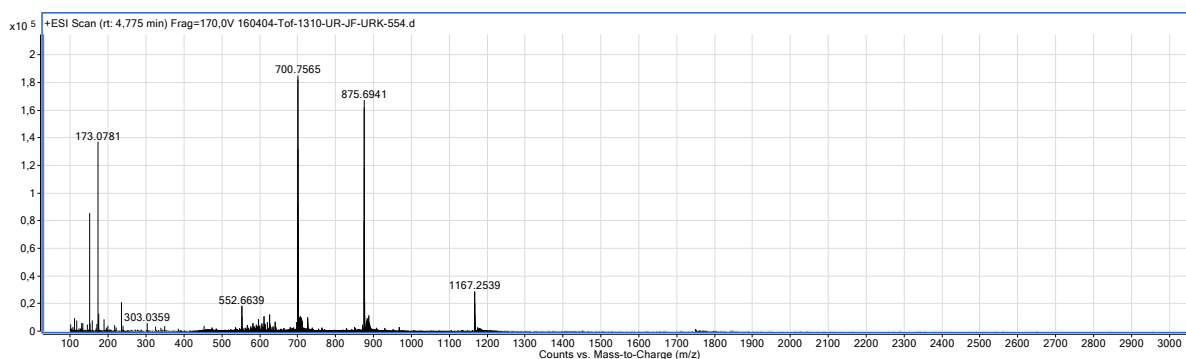


Figure S107 : LC-MS spectrum of oligomer **S11** performed on UHPLC coupled to an ESI -MS ToF with the dual-ESI source operated in positive ion mode. (Gradient: 10-100%; CH<sub>3</sub>CN 0.1 % Formic acid in H<sub>2</sub>O 0.1% formic acid, 6 min, C18).

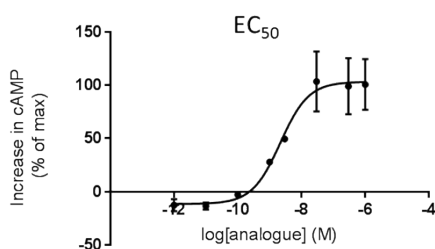


Figure S108 : Concentration-response curve (receptor-mediated cAMP produced) for EC<sub>50</sub> determination of **S11** in cells expressing the GLP-1R (Method C2).

## H-HGEGTFTSDVSSYLEGQAAKEFIAWLVKGRG-NH<sub>2</sub> (**S12**)

The synthesis of peptide **S12** has already been described<sup>[1]</sup>.

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