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# Manipulation of Transmembrane Transport by Synthetic K + Ionophore Depsipeptides and Its Implications in Glucose-Stimulated Insulin Secretion in $\beta$ -Cells

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José García-Calvo, Tomás Torroba, Virginia Brañas-Fresnillo, Germán Perdomo, Irene Cózar-Castellano, et al.. Manipulation of Transmembrane Transport by Synthetic K + Ionophore Depsipeptides and Its Implications in Glucose-Stimulated Insulin Secretion in  $\beta$ -Cells. Chemistry - A European Journal, 2019, 25 (39), pp.9287-9294. 10.1002/chem.201901372 . hal-03831759

**HAL Id: hal-03831759**

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# CHEMISTRY

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### Accepted Article

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This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

**To be cited as:** *Chem. Eur. J.* 10.1002/chem.201901372

**Link to VoR:** <http://dx.doi.org/10.1002/chem.201901372>

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# Manipulation of transmembrane transport by synthetic K<sup>+</sup> ionophore depsipeptides and its implications in glucose-stimulated insulin secretion in $\beta$ -cells

José García-Calvo,<sup>[a]</sup> Tomás Torroba\*<sup>[a]</sup>, Virginia Brañas-Fresnillo,<sup>[b]</sup> Germán Perdomo,<sup>[b]</sup> Irene Cózar-Castellano,\*<sup>[c]</sup> Yu-Hao Li,<sup>[d]</sup> Yves-Marie Legrand,<sup>[d]</sup> and Mihail Barboiu\*<sup>[d]</sup>

This paper is dedicated to the memory of the late Dr. Stefano Marcaccini

**Abstract:** Cyclic depsipeptide Cereulide toxin is a very well known potassium electrogenic ionophore particularly sensitive to pancreatic beta cells. The mechanistic details of specific activity are unknown. Here, we describe a series of synthetic substituted cereulide potassium ionophores that cause an impressive selective activation of glucose-induced insulin secretion in a constitutive manner in rat insulinoma INS1E cells. Our study demonstrates that a different electroneutral K<sup>+</sup> transport mechanism by the anionic mutant depsipeptides when compared with classical electrogenic Cereulides can have an important impact of pharmacological value on glucose-stimulated insulin secretion.

## Introduction

Natural cyclic depsipeptides valinomycin and cereulide are well known K<sup>+</sup> ionophores.<sup>[1]</sup> While valinomycin is a biosynthetic product currently used as antibiotic,<sup>[2]</sup> cereulide is an emetic toxin produced by specific strains of *Bacillus cereus*,<sup>[3]</sup> which is a common cause of several diseases.<sup>[4]</sup> Because *B. cereus* is a ubiquitous agent in nature,<sup>[5]</sup> its spores can be found as a natural contaminant in several food products,<sup>[6]</sup> so the presence of cereulide at low concentrations is an undetected risk. It is currently assumed that cereulide disrupts the transmembrane potential in mitochondria of eukaryotic cells, leading to their degeneration.<sup>[4]</sup> The higher toxicity (about 15 times more toxic<sup>[7]</sup>) of cereulide [D-O-Leu-D-Ala-L-O-Val-L-Val]<sub>3</sub> relative to valinomycin [D-O-Hyi-D-Val-L-O-Lac-L-Val]<sub>3</sub> is claimed to be due

to a higher affinity<sup>[8]</sup> for K<sup>+</sup> at physiological plasma concentration. Whereas ingestion of high doses of cereulide causes acute emetic toxicity, very low doses of cereulide also exert toxicity,<sup>[9]</sup> being pancreatic beta-cells (insulin-producing cells) particularly sensitive.<sup>[10]</sup> Chronic or repeated low dose exposure to cereulide could impair the mitochondrial activity of the beta-cells, thereby leading to hampered insulin secretion, thus contributing to the incidence of type 2 diabetes. It has been previously shown in different study models of beta-cells, INS1E, MIN6 and rodent islets cells, that natural cereulide at lower concentrations (0.25-0.5 ng/mL) inhibits glucose-induced insulin secretion,<sup>[11]</sup> whereas at higher concentrations (1-5 ng/mL) induces cell death.<sup>[10-12]</sup> Since the natural cereulide includes minor variants of the main structure,<sup>[13]</sup> the so-called isocereulides,<sup>[14-15]</sup> the activity of cereulide in live beta cells is therefore a still unsolved matter. With all these considerations in mind, we have considered herein novel a different unexplored mechanistic approach by using a series of four novel synthesized cyclic mutant depsipeptides and their natural cereulide counterpart as specific ionophores for which the K<sup>+</sup> binding and transport through bilayer membrane and the glucose stimulated insulin secretion of beta cells can be systematically correlated with an important view to their pharmacological value: the anionic mutant exhibit a different mechanism of transport from its neutral counterpart. Interestingly, this anionic mutant trigger constitutive insulin secretion from beta cells, yet the neutral mutants elicited reduction of the glucose-triggered insulin release from beta cells.

## Results and Discussion

First, we performed the design and synthesis of novel depsipeptides, based on a modification of natural cereulide, that are selective K<sup>+</sup> membrane ionophores and present activity on live beta-cells (Scheme 1). The design of mutant cereulides can be done by modifying aminoacids as well as hydroxyacids in the cyclic structure. Any modification should be designed by preserving the 3D structure of the natural cereulide, preserving the internal H-bonds otherwise the cation binding properties should be lost. We selected for the modification the only disordered component observed in the X-ray single-crystal structure, thus, the (S)-2-hydroxy-3-methylbutanoic acid fragment. Small changes in this part of the macrocycle should have the least modification of the whole structure due to the fact that there are a few accommodations of the lateral group in the crystal without perturbing the H-bonds. The position of the carboxylic ester

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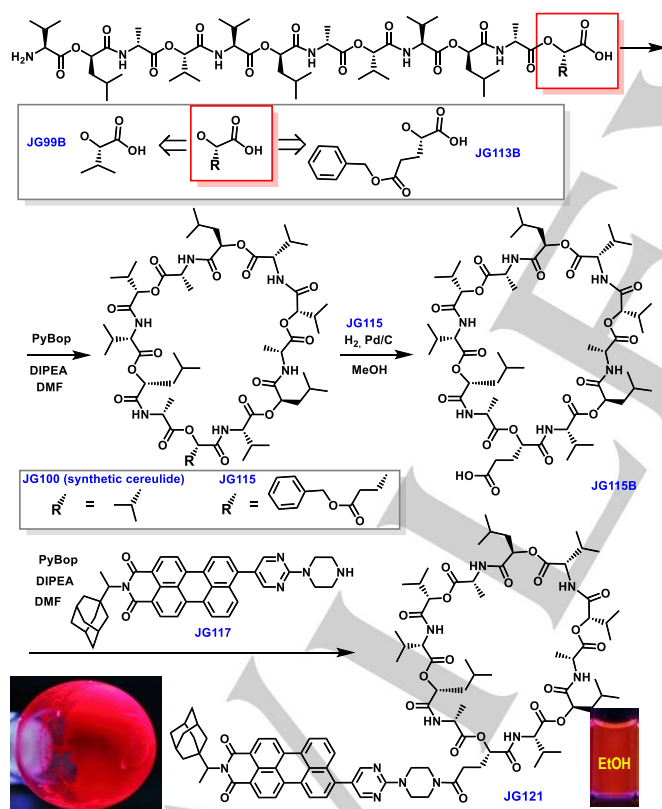
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anchoring group, suitable for further modifications is another important issue. A carboxylic group too close to the depsipeptide backbone should compete with the closest carbonyl group for the H-bonding, therefore disrupting the 3D structure of the natural cereulide with unknown consequences for  $K^+$  affinity. Therefore, we selected to place the carboxylic group at a distance sufficiently far to prevent competition for H-bonds. Our approach consisted in the substitution of the first (*S*)-2-hydroxy-3-methylbutanoic acid in a classical sequence<sup>[3]</sup> by (*S*)-5-(benzyloxy)-2-hydroxy-5-oxopentanoic acid and performing the synthesis of a new depsipeptide **JG113B**, which is then submitted to cyclization,<sup>[16-17]</sup> to give **JG115**, and debenzoylation under hydrogenation, to give **JG115B**, which bears a free carboxylic acid on a lateral chain. In parallel, synthetic sCereulide **JG100** was obtained by cyclization of **JG99B**. For live imaging experiments we selected as fluorescent tag a 9-[2-(piperazin-1-yl)pyrimidin-5-yl]perylene-3,4-monoimide **JG117** (Scheme 1), obtained from Suzuki reaction of a 9-bromoperylene-3,4-monoimide<sup>[18]</sup> and a 2-[(*N*-Boc-piperazin-1-yl)pyrimidin-5-yl]boronic ester, used for extended conjugation and activation of fluorogenic probes,<sup>[19]</sup> followed by acid deprotection of the amine group. Amide formation between the depsipeptide **JG115B** and the fluorescent tag **JG117** gave the fluorescent depsipeptide **JG121** (Scheme 1).



**Scheme 1.** Synthetic pathway to modified depsipeptides. Insets: Fluorescence of **JG121** in ethanol solution (right) and in the solid state (left).

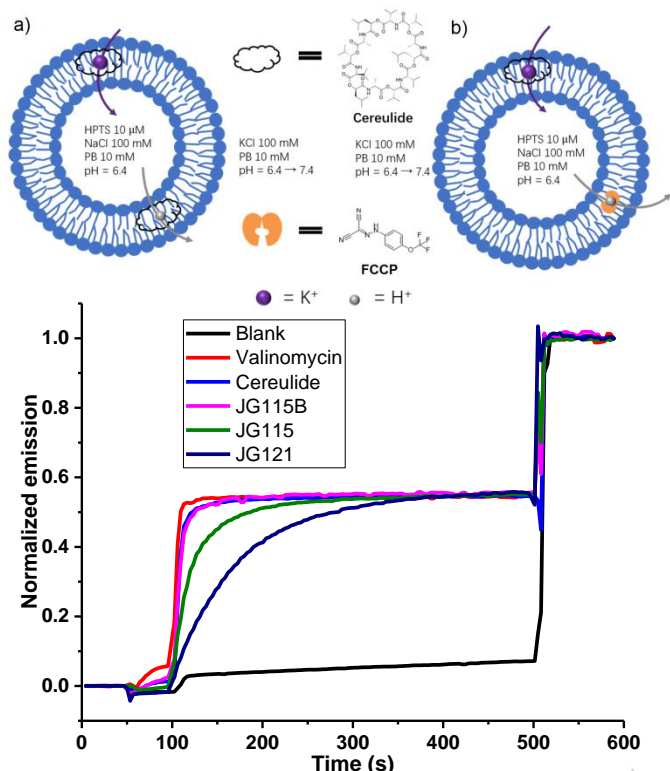
Compound **JG121**, a bright red fluorescent solid, is soluble in common solvents except in pure water and hexane (similarly to the natural cereulide); it showed an orange fluorescence in

ethanol  $\lambda_{max} = 592$  nm,  $\Phi_{JG121}(EtOH) = 0.43 \pm 0.02$ ; its lifetime,  $\tau_{JG121}(EtOH) = 2.91$  ns, was similar to other perylenemonoimide dyes<sup>[20]</sup> and the fluorescence did not change in the presence of  $K^+$ . The compound was solvatochromic in fluorescent emission, from yellow-green,  $\lambda_{max} = 557$  nm in cyclohexane, to red,  $\lambda_{max} = 610$  nm in DMSO ( $\lambda_{exc} = 500$  nm). These structures comprise selected examples bearing different steric hindrances and indeed altered electrostatics, but also providing the first example of a fluorescent cereulide derivative, albeit the increased steric hindrance for transport studies, allow intracellular imaging and localization of a cereulide derivative.

The affinities for  $K^+$  of commercial valinomycin, sCereulide **JG100** and modified depsipeptides **JG115**, **JG115B**, **JG121** synthesized in this work, have been evaluated by determining the equilibrium constants in ethanolic solutions in relation to a known fluorogenic probe<sup>[3]</sup> (See the Supporting Information Section). With this method, the equilibrium binding constants, given in order of affinity are:  $\log K(\mathbf{JG100} \text{ EtOH}) = 5.99 \pm 0.02$ ,  $\log K(\text{valinomycin EtOH}) = 5.97 \pm 0.02$ ,  $\log K(\mathbf{JG115B} \text{ EtOH}) = 5.97 \pm 0.01$ ,  $\log K(\mathbf{JG115} \text{ EtOH}) = 4.59 \pm 0.01$  and  $\log K(\mathbf{JG121} \text{ EtOH}) \ll \log K(\mathbf{JG115} \text{ EtOH})$ , therefore the affinity for  $K^+$  in the cases of sCereulide, valinomycin and **JG115B** was very similar, the affinity in the case of **JG115** was more than one order lower than previous values and the method only confirmed that the affinity for  $K^+$  in the case of **JG121** was much lower than the previous one, giving a dramatic tendency of decreasing affinity for  $K^+$  with the increase in the bulkiness of the lateral group. While small changes in the structure affect the  $K^+$  transport in natural ionophores valinomycin or cereulide,<sup>[8]</sup> the manipulation of  $K^+$  transport in cells by changes in the structure opens a new route from toxins to drugs that we have evaluated by  $K^+$  transport experiments through bilayer membranes.<sup>[21]</sup>

The  $K^+$  transport fluorescent assays were performed by using the Fast Filter method, in order to read quasi instantaneously the emission at 510 nm under alternate excitation at 403 and 460 nm.<sup>[22]</sup> The experiments were performed in unilamellar vesicles (LUV) prepared from a mixture of lipids. Inside the LUV there was an aqueous solution of 10 mM sodium phosphate, pH 6.4, and 100 mM NaCl. Outside the LUV there was an aqueous solution (1.85 mL) of 10 mM sodium phosphate, pH 6.4, and 100 mM KCl. Upon initiating the experiment, 0.02 mL of 1 mM active compound in DMSO was added to the measurement cell containing HPTS-loaded LUV (HPTS: 8-hydroxypyrene-1,3,6-trisulfonic acid) after 50 s. Then, 29  $\mu$ L of 0.5 M aqueous NaOH were added after 100 s, bringing external pH to 7.4. Upon addition of active compound and pH increase outside the LUV, fluxes of cations and  $H^+$  across the bilayer modified the ratio of protonated and non-protonated HPTS, which was followed by fluorescence.<sup>[23]</sup> Maximal changes in dye emission were obtained at the end of each experiment by lysis of the liposomes with detergent (0.04 mL of 5% aqueous Triton x100). **JG115**, **JG115B**, **JG100** (sCereulide) and **JG121** were tested and compared to the reference compound, valinomycin (Figure 1).

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**Figure 1.** Schematic representation of the transport experiments through bilayer membranes in the presence of depsipeptides ionophores a) without or b) with FCCP proton transporter. c) Intensity ratio ( $I_{460}/I_{403}$ ) over time of solutions containing HPTS-loaded LUV by the DMSO (blank), valinomycin (for reference), **JG115**, **JG115B**, **JG100** or **JG121** addition (@ 50 sec.) followed by base addition (@ 100 sec.) and finally Triton for lysis (@ 500 sec.). All spectra were normalized (max/initial emission).

The HPTS transport assays (Figure 1a) showed very close transport rates for valinomycin, sCereulide-**JG100** and **JG115B**, and it is strongly slowed down for both **JG115** and **JG121** containing bulky substituents on the macrocycle of the ionophore (Figure 1c). The rate of transport follows the sequence of the determined  $K^+$  binding constants. The transport mechanism is most probably reminiscent to a carrier- $K^+$  translocation, for which the diffusion through the membrane is highly dependent on the size of the molecule related to grafted substituents.

The Hill analysis<sup>[24]</sup> (Table 1) indicated that the most active carrier is the reference sCereulide-**JG100** ( $EC_{50}=0.85 \mu\text{M}$ ), which is almost 1.5 times more active than bulkier substituted ionophores **JG115** and **JG115B**. Surprisingly **JG121** has very low transport activity because of the bulky fluorescent tag, which presumably hinders the translocation of the macrocycle within the bilayer membrane. The Hill coefficients are mostly corresponding to one molecule of ionophore, binding the  $K^+$  with a lower degree of cooperativity, confirming the carrier mechanism proposed for the transport. The transport rates are controlled via membrane potential (i.e. electrogenic valinomycin, **JG100**, **JG121** and **JG115**) or pH gradient (i.e. electroneutral **JG115B**- $K^+$ ).<sup>[25][26]</sup> Then,

once removing the rate-limiting step which is  $H^+$  transport by adding FCCP, a specific proton transporter (FCCP: carbonyl cyanide *p*-(trifluoromethoxy)hydrazine),<sup>[27][28]</sup> (Figure 1b), the  $K^+$  transport activities are remarkably increasing by 2-3 orders of magnitude for electrogenic **JG100**- $K^+$  or **JG121**- $K^+$  and **JG115**- $K^+$  carriers (Table 1). Despite a minimal structural modification, a lower activity is observed for the electroneutral carrier **JG115B**- $K^+$  which is seven times less active and probably less membrane permeable than electrogenic **JG100**- $K^+$ , in presence of FCCP. Moreover, the pseudo-first order initial transport rates  $k_{K^+}=0.0048 \text{ s}^{-1}$  and  $k_{Na^+}=0.0006 \text{ s}^{-1}$  of **JG115B** are reminiscent with a selectivity for  $K^+$   $S_{K^+/Na^+} = 8$  in the absence of FCCP, which is strongly increasing toward  $S_{K^+/Na^+} = 129$  ( $k_{K^+}=0.3352 \text{ s}^{-1}$  and  $k_{Na^+}=0.0026 \text{ s}^{-1}$ ) in the presence of FCCP.

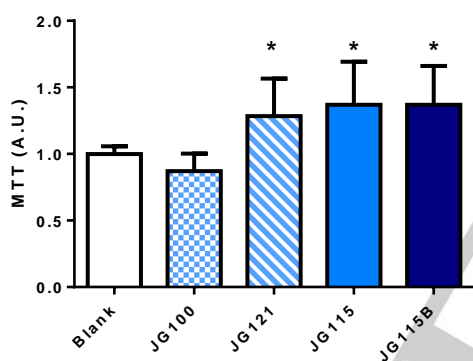
**Table 1.** Hill analysis results of  $K^+$  transport in the presence of **JG100**, **JG115**, **JG115B**, or **JG121** with or without FCCP,  $EC_{50}$  values expressed as  $\mu\text{M}$  needed to obtain 50% ion transport activity and  $n$  is Hill coefficient.

	HTPS assay without FCCP		HTPS assay with FCCP	
	$EC_{50}$ (300)/ $\mu\text{M}$	$n$	$EC_{50}$ (300)/ $\mu\text{M}$	$n$
<b>JG100</b>	0.85	1.22	0.00147	1.24
<b>JG115</b>	1.28	1.05	0.03177	1.17
<b>JG115B</b>	1.18	2.06	0.01069	1.32
<b>JG121</b>	N. D.*	N. D.*	0.01082	1.29

These results demonstrate that the neutral sCereulide-**JG100** and its derivatives **JG115** and **JG121** showed clear evidence for electrogenic transport of  $K^+$  similar to valinomycin, which means, for these carriers, transport rate follows as  $K^+ > H^+$ . This is most probably producing the loss of  $K^+$  gradient inducing a strong membrane depolarization and other effects on cellular functions. Lower activity based on better  $K^+/Na^+$  selectivity is realized by electroneutral **JG115B**- $K^+$  carrier, facilitating binding of the cations and producing less cell membrane depolarization and dysfunctions by preserving better the transmembrane potential. These results may be of direct relevance and have direct applications in biomedicine,<sup>[29]</sup> on the way to control processes related to  $K^+$  equilibria in metabolic disorders such as diabetes mellitus.<sup>[30]</sup> Therefore, the use of the ionophores to modulate transport in live beta cells will open a new avenue for the creation of useful new drugs. For the study, rat insulinoma (an insulin-producing neuroendocrine tumor arising from beta cells of the rat pancreas) INS1E cells<sup>[31]</sup> were used for the glucose stimulated insulin secretion (GSIS) assays. For the experiments, INS1E cells were seeded on cell culture 24-well plates at a density of 250,000 cells per well for 72 hours. 24 hours before GSIS 0.25 ng/mL of sCereulide or its derivatives were added to the cell culture. The day of the assay cells were preincubated for 2 hours with glucose-free medium. Then, they were washed twice and incubated for 30 min at 37 °C in glucose-free HEPES balanced salt solution (HBSS). Next, cells were washed once

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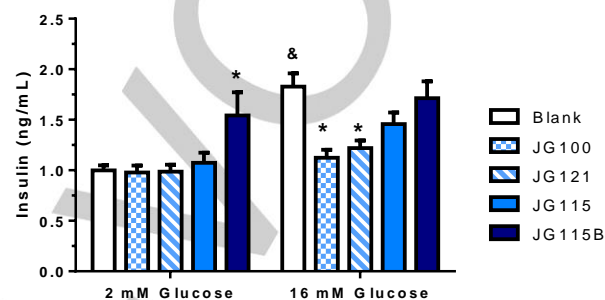
with glucose-free HBSS and then insulin secretion was stimulated by using static incubation for 30 min period in 1 ml of the same buffer containing 2 or 16 mM glucose respectively. Secretion samples were measured by Rat Insulin ELISA and statistical analysis of data was performed. Distribution of variables was checked using the Kolmogorov-Smirnov test. Comparisons between groups were performed by ANOVA followed by Tukey's Multiple Comparison Test. Differences were considered significant at  $p < 0.05$ . The effect of sCereulide and its derivatives on beta-cell viability was studied by the MTT assay method, which measures cell metabolic activity (MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide).<sup>[32]</sup> sCereulide **JG100** (0.25 ng/mL) did not produce any effect on cell viability in INS1E cells, but interestingly, **JG121**, **JG115** and **JG115B** increased by ~30% cell viability as measured by MTT (Figure 2). These results indicated that none of the novel synthetic cereulides induced cytotoxicity when used at a concentration of 0.25 ng/mL. Thus, this was an optimal concentration to study a potential effect of this family of products on beta-cell function.



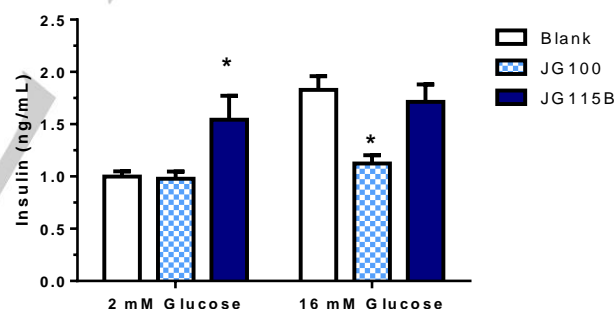
**Figure 2.** Beta-cell viability after treatment with sCereulide or the new depsipeptides. Cell viability was measured in INS1E cells by MTT 24 h after treatment with sCereulide **JG100** or **JG121**, **JG115**, and **JG115B**, using a final concentration of 0.25 ng/mL. N=6. \* $p < 0.05$  versus Blank (control/vehicle).

Pancreatic beta-cell function is intended to synthesize and to secrete insulin in response to glucose overload. Therefore, monitoring the effect of cereulide ionophores on beta-cell function, was performed by measuring insulin secretion in response to glucose challenge in INS1E cells that have been treated with vehicle (Blank/control) or any of the cereulide related molecules (**JG121**, **JG115** and **JG115B**). The vehicle used to resuspend cereulide related molecules is 0.0001% DMSO in cell culture medium. Control INS1E cells secreted 80% more insulin when treated with 16 mM glucose (compared to 2 mM); this positive effect on insulin secretion was abrogated when beta-cells were treated with sCereulide-**JG100** or **JG121**. Unexpectedly, **JG115B** induced a constitutive secretion of insulin, independently of glucose concentration (Figure 3). Figure 4 shows more specifically the opposite effects of **JG100** and **JG115B**.

Meanwhile **JG100** inhibited insulin secretion after a glucose challenge; **JG115B** stimulated insulin secretion under low glucose (2 mM) concentrations when insulin secretion should be off. Because **JG115B** increases the insulin secretion over the blank experiment when there is low amount of glucose, this result indicates that modifications in cereulide structure are able to alter beta-cell function. More specifically, these results point to the change of the (S)-2-hydroxy-3-methylbutanoic acid by (S)-2-hydroxypentanedioic acid as the responsible for the changes in beta-cell functionality.



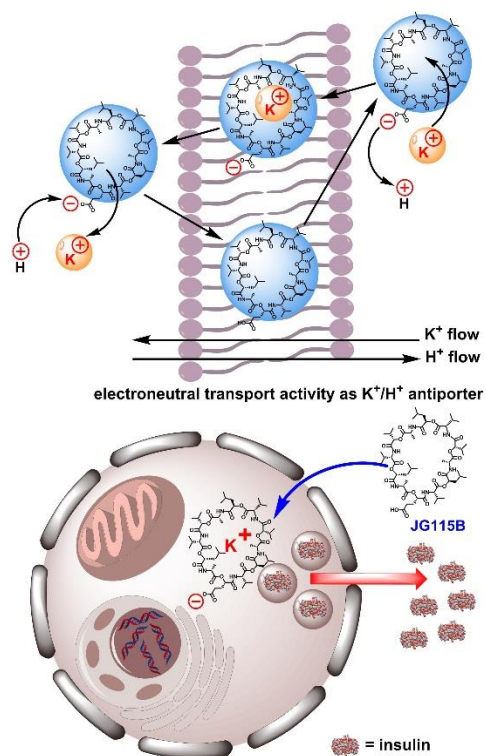
**Figure 3.** Beta-cell function after treatment with cereulide or its derivatives. Beta-cell function was measured by glucose stimulated insulin secretion 24 h after treatment with **JG100** or **JG121**, **JG115**, and **JG115B** (0.25 ng/mL). \* $p < 0.05$  versus Blank; & $p < 0.05$  versus same condition-2mM glucose.



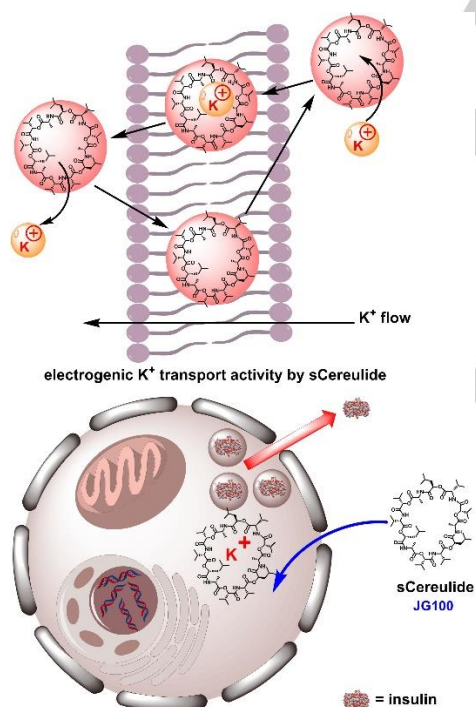
**Figure 4.** Comparison of Blank, **JG100** and **JG115B**. N = 5-6 in triplicates. \* $p < 0.05$  versus Blank

The mechanism by which **JG115B** induces insulin secretion, as well as **JG115**-**JG121**, is illustrated in Figures 5-6. At physiological pH it is expected that the carboxylic acid group of **JG115B** exists in its anionic form, therefore the active species **JG115B**<sup>-</sup>·K<sup>+</sup> is a neutral zwitterionic complex that acts in electroneutral transport activity as monovalent cation-proton antiporter.<sup>[33][34]</sup> The rest of compounds have no acidic groups therefore the complexes **JG100**·K<sup>+</sup> or **JG121**·K<sup>+</sup> and **JG115**·K<sup>+</sup> are positively charged species with electrogenic K<sup>+</sup> transport activity across lipid bilayers.

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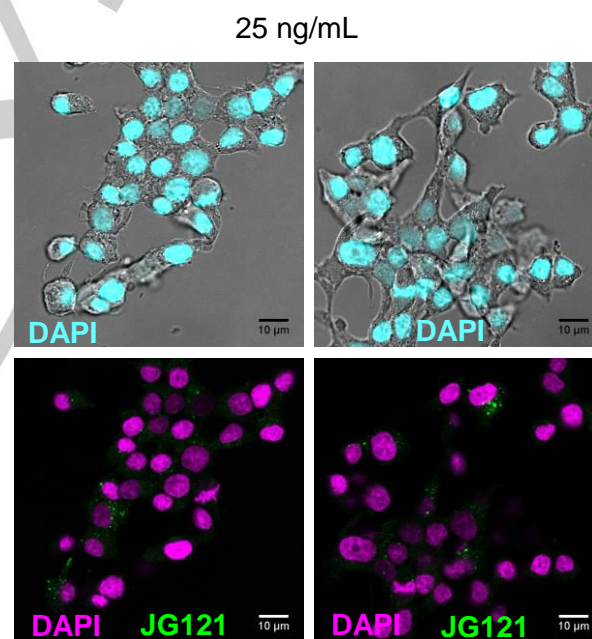
**Figure 5.** Potassium cation flow and opposite proton flow by electroneutral transport activity and the consequent glucose-stimulated insulin secretion by the potassium cation-proton antiporter action of **JG115B**.



**Figure 6.** Potassium cation flow by electrogenic transport activity and the consequent impaired glucose-stimulated insulin secretion by the potassium cation transport action of **sCereulide JG100**.

The different mechanism of transport activity,<sup>[35][36]</sup> triggers a divergent physiological response in the insulin secretion mechanism.<sup>[37][38]</sup> Previous studies have related the effect of cereulide-induced GSIS inhibition to impaired mitochondrial production of ATP, strongly depending on membrane depolarization caused by specific carriers like **sCereulide**.<sup>[10][12][39]</sup>

We know that **JG115B** acts in an opposite way and with lower  $K^+$  activity than **sCereulide**, working as a selective potassium cation-proton antiporter<sup>[40]</sup> with lower membrane depolarization and an activator of mitochondrial function, leading to increased ATP production. In support of this line of thinking, we have observed that **JG115B** induced cellular metabolic activity, as assessed by MTT. An increased cellular metabolic activity closes the channels and consequently induces membrane depolarization, which stimulates electrical activity triggering insulin release. Consequently, because ATP-dependent  $K^+$  channels are targets for the treatment of type 2 diabetes, modified cereulides represent a new avenue for developing new drugs for the treatment of this disease.



**Figure 7.** Confocal microscopy projection images of probe **JG121** 25 ng/mL inside INS1E cells. Nuclei are stained with Hoechst dye (blue channel).

With the fluorescently tagged cereulide **JG121** we also performed cellular localization studies in INS1E cells, in order to confirm that the cyclic depsipeptides are cell penetrating compounds, an important feature when studying biological events mediated by  $K^+$  ion interactions.<sup>[41-43]</sup> The INS1E cells, cultured under standard conditions, were incubated with 2.5 and 25 ng/mL of the probe from an initial solution in DMSO. The nucleus of fixed cells was stained with DAPI (4',6'-diamino-2-fenilindol). All confocal cell

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images were pseudo-colored (Figure 7). At 2.5 ng/mL the fluorescence was faint but clearly distinguished from the DAPI stain (See the Supporting Information). On the other hand, at 25 ng/mL the fluorescence of **JG121** was clearly seen inside the cells (Figure 7). Therefore the fluorescent probe **JG121** can be considered as a useful tool for the visualization of modified cereulide in live INS1E cells.

## Conclusions

In conclusion, we have shown for the first time that synthetic cereulide at 0.25 ng/mL mimics the deleterious effect of natural cereulide on insulin secretion in beta-cells and the changes in the backbone composition of the synthetic cereulide altered the biological properties of this molecule. Thus, substitution of one hydroxyacid, the (S)-2-hydroxy-3-methylbutanoic acid by (S)-2-hydroxypentanedioic acid led to a modified cyclic depsipeptide **JG115B**, related to cereulide, that worked as a K<sup>+</sup> cation transporter with a different electroneutral mechanism and caused glucose-induced insulin secretion in a constitutive manner in rat insulinoma INS1E cells. This constitutes an example of the potential of formal toxins to develop new synthetic drugs from controlled modification of structure and mechanism of action in cyclic depsipeptides, and an advance in the understanding and control of bilayer membrane transport and their physiological activity in live beta-cells.

## Experimental Section

Detailed synthetic procedures for the remaining compounds, complete characterization of all materials and full details about the instrumentation and methods, as well as additional experimental data can be found in the Supporting Information.

**Synthesis of JG115B:** *N,N*-Dicyclohexylcarbodiimide (DCC) (127 mg, 0.615 mmol) was added to a stirred solution of 5-benzyl 1-(*tert*-butyl) (S)-2-hydroxypentanedioate **JG110** (171 mg, 0.58 mmol), Boc-(L-Val-D-O-Leu-D-Ala-L-O-Val-L-Val-D-O-Leu-D-Ala-L-O-Val-L-Val-D-O-Leu-D-Ala)-OH **JG112** (680 mg, 0.58 mmol), and 4-(dimethylamino)pyridine (DMAP) (14.2 mg, 0.12 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) at 0°C. The resulting suspension was warmed until room temperature and stirred for 2 h. Then the suspension was filtered and concentrated on CH<sub>2</sub>Cl<sub>2</sub>. Purification by flash column chromatography (ethyl acetate [EtOAc]/heptane, from 1:8 to 1:4) afforded the protected acyclic modified cereulide derivative **JG113** (713 mg, 85%) as a white solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.34 (d, 1H, *J* = 6.3 Hz, NH), 8.09 (d, 1H, *J* = 6.0 Hz, NH), 7.88-7.93 (m, 4H, NH), 7.32 (m, 5H, C<sub>Ar</sub>H), 5.31 (m, 2H, NH + CH), 5.17 (m, 1H, CH), 5.09 (s, 2H, CH<sub>2</sub>), 5.05-4.92 (m, 3H, CH), 4.74 (dd, 4H, *J* = 8.4, *J* = 4.5 Hz, 4xCH), 4.61-4.49 (m, 2H, 2xCH), 4.42 (m, 1H, CH), 4.29 (m, 2H, CH), 4.07-3.93 (m, 1H, CH), 3.82-3.77 (m, 1H, 0.5xCH<sub>2</sub>), 2.48 (t, 1H, *J* = 7.6 Hz, 0.5xCH<sub>2</sub>), 2.32 (m, 3H, 3xCH), 2.11 (t, 1H, *J* = 8.4 Hz, 0.5xCH<sub>2</sub>), 1.76-1.57 (m, 11H, 5xCH + 3xCH<sub>2</sub>), 1.47-1.33 (m, 28H, CH + 9xCH<sub>3</sub>), 1.02-0.83 (m, 48H, 16xCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 172.6 (C=O), 172.4 (C=O), 171.5 (C=O), 171.1 (C=O), 170.8 (C=O), 170.6 (C=O), 170.5 (C=O), 170.4 (C=O), 170.3 (C=O), 170.0 (C=O), 169.6 (C=O), 168.4 (C=O), 156.6 (C=O), 135.9 (C<sub>Ar</sub>), 128.4 (C<sub>Ar</sub>H), 128.1 (C<sub>Ar</sub>H), 128.1 (C<sub>Ar</sub>H), 82.0 (C<sub>q</sub>), 80.8 (C<sub>q</sub>), 72.5 (CH), 66.3 (CH<sub>2</sub>), 60.3 (CH), 49.6 (CH), 49.2 (CH), 47.9 (CH), 41.2 (CH<sub>2</sub>), 33.9 (CH<sub>2</sub>), 30.2 (CH), 29.8 (CH), 28.2 (CH<sub>3</sub>), 27.8 (CH<sub>3</sub>), 26.0 (CH<sub>2</sub>), 24.3 (CH), 24.2

(CH), 23.3 (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>), 23.0 (CH<sub>3</sub>), 21.1 (CH<sub>3</sub>), 21.0 (CH<sub>3</sub>), 20.7 (CH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 19.3 (CH<sub>3</sub>), 19.1 (CH<sub>3</sub>), 18.9 (CH<sub>3</sub>), 16.5 (CH<sub>3</sub>), 16.2 (CH<sub>3</sub>), 14.1 (CH<sub>3</sub>). HRMS (MALDI+, DCTB) *m/z* calcd. for C<sub>73</sub>H<sub>118</sub>N<sub>6</sub>NaO<sub>23</sub>: 1469.8141 (M+Na<sup>+</sup>); found: 1469.8330. Then, HCl(g) was bubbled into a solution of **JG113** (540 mg, 0.37 mmol) in EtOAc (12 mL). The resulting mixture was stirred for 4 hours and then concentrated *in vacuo*, to afford **JG113B** (490 mg, 99%) as a white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 8.46 (m, 2H, NH), 7.32 (m, 5H, C<sub>Ar</sub>H), 5.12 (m, 4H, 2xCH + CH<sub>2</sub>), 4.87 (m, 2H, 2xCH), 4.49 (m, 2H, 2xCH), 4.38 (m, 1H, CH), 4.12 (m, 2H, 2xCH), 3.46 (m, 1H, 0.5xCH<sub>2</sub>), 3.27 (m, 1H, 0.5xCH<sub>2</sub>), 2.49 (t, 1H, *J* = 7.7 Hz, 0.5xCH<sub>2</sub>), 2.22 (m, 3H, CH + CH<sub>2</sub>), 1.85-1.58 (m, 15H, 5xCH + 5xCH<sub>2</sub>), 1.42 (m, 9H, 3xCH<sub>3</sub>), 1.08-0.88 (m, 48H, 16xCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 173.7 (C=O), 172.3 (C=O), 171.9 (C=O), 171.5 (C=O), 171.4 (C=O), 171.3 (C=O), 171.1 (C=O), 170.9 (C=O), 170.8 (C=O), 170.7 (C=O), 170.6 (C=O), 170.4 (C=O), 168.4 (C=O), 136.1 (C<sub>Ar</sub>), 128.2 (C<sub>Ar</sub>H), 127.9 (C<sub>Ar</sub>H), 78.5 (CH), 73.9 (CH), 72.4 (CH), 71.7 (CH), 66.0 (CH<sub>2</sub>), 58.1 (CH), 49.3 (CH), 43.9 (CH<sub>2</sub>), 40.5 (CH<sub>2</sub>), 32.9 (CH<sub>2</sub>), 30.4 (CH), 30.2 (CH), 29.5 (CH), 29.3 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 24.2 (CH), 22.3 (CH<sub>3</sub>), 20.3 (CH<sub>3</sub>), 20.1 (CH<sub>3</sub>), 19.6 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 18.0 (CH<sub>3</sub>), 17.9 (CH<sub>3</sub>), 17.7 (CH<sub>3</sub>), 17.2 (CH<sub>3</sub>), 17.0 (CH<sub>3</sub>), 16.6 (CH<sub>3</sub>), 16.4 (CH<sub>3</sub>), 16.3 (CH<sub>3</sub>), 16.0 (CH<sub>3</sub>). HRMS (MALDI+, DCTB) *m/z* calcd. for C<sub>64</sub>H<sub>102</sub>N<sub>6</sub>O<sub>21</sub>: 1291.7171 (M+H<sup>+</sup>); found: 1291.7493. Then, DIPEA (0.35 mL, 1.062 mmol) was added to dry DMF (350 mL) at room temperature, followed by PyBop (203 mg, 0.39 mmol). Then, a solution of the deprotected acyclic modified cereulide derivative **JG113B** (0.48 g, 0.36 mmol) in DMF (40 mL) was slowly added (2-3 hours) to the previous solution and afterwards the mixture was stirred for 16 h. Then the solvent was evaporated *in vacuo*. Purification of the residue by flash column chromatography (EtOAc/heptane, 1:3; R<sub>f</sub>, 0.28) afforded the benzyl protected modified cereulide **JG115** (155 mg, 40%) as a white solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.17 (m, 1H, NH), 7.99 (m, 2H, *J* = 6.6 Hz, NH), 7.92 (m, 2H, *J* = 6.3 Hz, NH), 7.70 (m, 2H, 2xNH), 7.61 (m, 2H, *J* = 5.3 Hz, 2xNH), 7.32 (m, 5H, C<sub>Ar</sub>H), 5.31 (m, 1H, CH), 5.23 (m, 1H, CH), 5.08 (s, 2H, CH<sub>2</sub>), 5.04 (s, 1H, CH), 4.94 (d, 1H, *J* = 3.0 Hz, CH), 4.87 (d, 1H, *J* = 3.5 Hz, CH), 4.49 (t, 1H, *J* = 7.4 Hz, CH), 4.34 (t, 1H, *J* = 6.8 Hz, CH), 4.23-4.13 (m, 2H, 2xCH), 3.99-3.79 (m, 3H, 2xCH), 2.39-2.27 (m, 9H, 3xCH + 3xCH<sub>2</sub>), 1.72 (s, 9H, 3xCH + 3xCH<sub>2</sub>), 1.41 (m, 9H, 3xCH<sub>3</sub>), 1.06-0.90 (m, 48H, 16xCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 172.6 (C=O), 172.3 (C=O), 171.9 (C=O), 171.7 (C=O), 171.6 (C=O), 170.8 (C=O), 170.7 (C=O), 170.5 (C=O), 170.4 (C=O), 169.5 (C=O), 135.9 (C<sub>Ar</sub>), 128.5 (C<sub>Ar</sub>H), 128.4 (C<sub>Ar</sub>H), 128.3 (C<sub>Ar</sub>H), 128.2 (C<sub>Ar</sub>H), 128.1 (C<sub>Ar</sub>H), 79.0 (CH), 78.0 (CH), 73.8 (CH), 73.4 (CH), 72.9 (CH), 72.6 (CH), 66.3 (CH<sub>2</sub>), 60.3 (CH), 60.1 (CH), 58.6 (CH), 49.2 (CH), 41.0 (CH<sub>2</sub>), 40.6 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 40.3 (CH<sub>2</sub>), 30.5 (CH), 30.2 (CH), 29.7 (CH), 29.2 (CH<sub>2</sub>), 28.7 (CH), 28.5 (CH), 28.4 (CH), 25.8 (CH<sub>2</sub>), 24.5 (CH), 24.3 (CH), 23.4 (CH<sub>3</sub>), 23.3 (CH<sub>3</sub>), 23.0 (CH<sub>3</sub>), 21.8 (CH<sub>3</sub>), 21.1 (CH<sub>3</sub>), 21.0 (CH<sub>3</sub>), 19.8 (CH<sub>3</sub>), 19.6 (CH<sub>3</sub>), 19.5 (CH<sub>3</sub>), 19.2 (CH<sub>3</sub>), 19.1 (CH<sub>3</sub>), 18.6 (CH<sub>3</sub>), 16.9 (CH<sub>3</sub>), 16.6 (CH<sub>3</sub>), 15.9 (CH<sub>3</sub>), 15.7 (CH<sub>3</sub>), 15.6 (CH<sub>3</sub>). HRMS (MALDI+, DCTB) *m/z* calcd. for C<sub>64</sub>H<sub>100</sub>N<sub>6</sub>NaO<sub>20</sub>: 1295.6885; found: 1295.7059 (M+Na<sup>+</sup>); *m/z* calcd. for C<sub>64</sub>H<sub>100</sub>KNaO<sub>20</sub>: 1311.6624 (M+K<sup>+</sup>); found 1311.6959. Then, palladium on carbon (10% wt/wt Pd, 0.5 g) was added to a stirred solution of benzyl protected modified cereulide **JG115** (120 mg, 0.09 mmol) in MeOH (10 mL) at room temperature. The resulting mixture was placed under a hydrogen atmosphere and stirred vigorously for 1 hour. Next, the mixture was filtered over celite and concentrated *in vacuo* to obtain the carboxylic acid modified cereulide **JG115B** (98 mg, 88%) as a white solid, which was used without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.33-8.17 (m, 1H, NH), 7.98 (m, 3H, 3xNH), 7.72 (m, 1H, *J* = 6.3 Hz, NH), 5.18 (m, 3H, 3xCH), 5.06 (m, 1H, CH), 4.90 (dd, 2H, *J* = 8.8 Hz, *J* = 3.2 Hz, 2xCH), 4.65-4.48 (m, 1H, CH), 4.35-4.21 (m, 2H, 2xCH), 4.14-4.06 (m, 1H, CH), 3.93 (m, 2H, 2xCH), 2.30-2.17 (m, 3H, CH + CH<sub>2</sub>), 1.69 (s, 9H, 3xCH + 3xCH<sub>2</sub>), 1.43 (m, 9H, 3xCH<sub>3</sub>), 1.05-0.86 (m, 48H, 16xCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 172.3 (C=O), 171.7 (C=O), 79.0 (CH), 73.9 (CH), 73.3 (CH), 72.9 (CH), 72.8 (CH), 60.3 (CH), 49.3 (CH), 40.6 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 30.4 (CH), 30.2 (CH), 28.6 (CH), 28.4 (CH), 26.3 (CH<sub>2</sub>), 24.4 (CH), 24.3 (CH), 23.3 (CH<sub>3</sub>), 23.3 (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>), 21.6 (CH<sub>2</sub>), 21.0 (CH<sub>3</sub>), 19.7 (CH<sub>3</sub>),



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19.6 (CH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 19.1 (CH<sub>3</sub>), 18.6 (CH<sub>3</sub>), 16.8 (CH<sub>3</sub>), 16.5 (CH<sub>3</sub>), 15.5 (CH<sub>3</sub>). HRMS (MALDI, DCTB) calcd. for C<sub>57</sub>H<sub>94</sub>N<sub>6</sub>NaO<sub>20</sub>: 1205.6415; found: 1205.6589 (M+Na<sup>+</sup>); m/z calcd. for C<sub>57</sub>H<sub>94</sub>KN<sub>6</sub>O<sub>20</sub>: 1221.6154 (M+K<sup>+</sup>); found: 1221.6452.

**Synthesis of JG121:** The following compounds were dissolved in a mixture of Tol:BuOH:H<sub>2</sub>O (8:2:0.6 ml) in a 100 mL Schlenk, provided with magnetic stirrer, cooler and N<sub>2</sub> atmosphere: *N*-(1-(1-Adamantyl)ethyl)-1,3-dioxo-perylene-8-bromo-3,4-dicarboxylmonoimide **JG73** (150 mg, 0.27 mmol), (2-(4-*N*-Boc-piperazin-1-yl)pyrimidin-5-yl)boronic acid pinacol ester (113.2 mg, 0.29 mmol), Na<sub>2</sub>CO<sub>3</sub> (143 mg, 1.35 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (15 mg, 0.014 mmol). The resulting mixture was heated under reflux for 24 hours. After that, 100 mL of water were added and the mixture was extracted with DCM (3x75 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated. The product was purified by column chromatography (SiO<sub>2</sub>, DCM:MeOH 4%, v/v), affording **JG116** as a purple solid, 149 mg, 74% yield; m.p.: 318–320 °C (decomposition). IR (KBr, cm<sup>-1</sup>): 2955 (C<sub>Ar</sub>-H), 2920 (C<sub>Ar</sub>-H), 2852 (C-H), 1734 (C=O), 1647 (C=O), 1597 (C<sub>Ar</sub>=C<sub>Ar</sub>), 1461 (C-O), 1381, 1248, 1168 (-CH<sub>3</sub>), 1134 (C-N), 1362, 1350, 1305, 1073, 997, 947 (fingerprint). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm): 8.52 (s, 2H, 2x C<sub>Ar</sub>H=N), 8.34 (m, 2H, 2x H<sub>Ar</sub>), 8.19–8.15 (m, 2H, 2x H<sub>Ar</sub>), 8.06 (d, 2H, J = 8.3 Hz, 2x H<sub>Ar</sub>), 7.86 (d, 1H, J = 8.4 Hz, H<sub>Ar</sub>), 7.47 (t, 1H, J = 7.5 Hz, H<sub>Ar</sub>), 7.40 (d, 1H, J = 7.8 Hz, H<sub>Ar</sub>), 5.08 (q, 1H, J = 7.3 Hz, NCHCH<sub>3</sub>), 3.95 (m, 4H, CH<sub>2</sub>), 3.59 (m, 4H, 2x CH<sub>2</sub>), 1.97 (s, 3H, 3x CH), 1.85–1.61 (m, 15H, 6x CH<sub>2</sub>+CH<sub>3</sub>), 1.52 (s, 9H, 3x CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm): 165.5 (N-C=N), 164.8 (C=O), 161.1 (O-C=O), 158.3 (C<sub>Pyrr</sub>H), 155.0 (C<sub>Ar</sub>), 136.4 (C<sub>Ar</sub>), 136.3 (C<sub>Ar</sub>), 136.3 (C<sub>Ar</sub>), 132.3 (C<sub>Ar</sub>), 131.3 (C<sub>Ar</sub>H), 131.0 (C<sub>Ar</sub>H), 129.5 (C<sub>Ar</sub>), 128.8 (C<sub>Ar</sub>), 128.4 (C<sub>Ar</sub>), 128.0 (C<sub>Ar</sub>H), 127.2 (C<sub>Ar</sub>H), 126.2 (C<sub>Ar</sub>), 123.6 (C<sub>Ar</sub>H), 123.1 (C<sub>Ar</sub>H), 122.0 (C<sub>Ar</sub>H), 121.0 (C<sub>Ar</sub>), 120.2 (C<sub>Pyrr</sub>), 119.9 (C<sub>Ar</sub>H), 80.3 (C(CH<sub>3</sub>)<sub>3</sub>), 58.1 (NCHCH<sub>3</sub>), 43.9 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 38.1 (C<sub>q</sub>), 37.1 (CH<sub>2</sub>), 28.9 (CH), 28.6 (C(CH<sub>3</sub>)<sub>3</sub>), 13.4 (CH<sub>3</sub>). HRMS (MALDI+, DCTB) m/z (%): calcd. for C<sub>47</sub>H<sub>47</sub>N<sub>5</sub>O<sub>4</sub>: 745.3623 (M<sup>+</sup>); found: 745.4080. UV-Vis (CHCl<sub>3</sub>), λ nm (log ε): 511 (4.6). Then, the *boc*-protected product, **JG116**, (150 mg, 0.20 mmol) was dissolved in DCM (20 mL). Then, trifluoroacetic acid (5 mL, ρ = 1.489 g/mL, 65.3 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 30 minutes. Thereafter, 50 mL of water and a 5% NaOH basic solution were added until pH=10. Then, an extraction was carried out with DCM:water (4x50 mL, DCM). The combined organic extracts were dried using Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent evaporated under reduced pressure to get the product **JG117**, purple-red solid, 123 mg, 95% yield; m.p.: 247–250 °C. IR (KBr, cm<sup>-1</sup>): 3433 (N-H), 2956 (C<sub>Ar</sub>-H), 2923 (C<sub>Ar</sub>-H), 2854 (C-H), 1738 (C=O), 1692 (C=O), 1651 (C=O), 1594, 1573, 1450 (C-O), 1352, 1261, 1037, 955, 813, 753. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm): 8.52 (m, 4H, C<sub>Ar</sub>H), 8.40–8.31 (m, 4H, 4x C<sub>Ar</sub>H), 7.93 (d, J = 8.5 Hz, 1H, C<sub>Ar</sub>H), 7.58 (t, J = 7.7 Hz, 1H, C<sub>Ar</sub>H), 7.49 (d, J = 7.7 Hz, 1H, C<sub>Ar</sub>H), 5.09 (q, J = 7.3 Hz, 1H, NCHCH<sub>3</sub>), 4.03 (m, 4H, CH<sub>2</sub>), 3.11 (m, 4H, CH<sub>2</sub>), 2.51 (s, 1H, NH), 1.97 (s, 3H, 3x CH), 1.83–1.63 (m, 15H, 6x CH<sub>2</sub>+CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm): 165.5 (N-C=N), 164.8 (C=O), 158.2 (C<sub>Pyrr</sub>H), 136.7 (C<sub>Ar</sub>), 132.7 (C<sub>Ar</sub>), 131.9 (C<sub>Ar</sub>H), 131.1 (C<sub>Ar</sub>H), 129.6 (C<sub>Ar</sub>), 128.5 (C<sub>Ar</sub>), 128.0 (C<sub>Ar</sub>H), 127.2 (C<sub>Ar</sub>H), 126.6 (C<sub>Ar</sub>), 125.0 (C<sub>Ar</sub>H), 123.7 (C<sub>Ar</sub>H), 123.2 (C<sub>Ar</sub>H), 122.1 (C<sub>Ar</sub>), 122.0 (C<sub>Ar</sub>), 121.2 (C<sub>Ar</sub>), 120.3 (C<sub>Ar</sub>H), 120.1 (C<sub>Ar</sub>H), 58.0 (NCHCH<sub>3</sub>), 45.3 (CH<sub>2</sub>), 44.0 (CH<sub>2</sub>), 40.2 (CH<sub>2</sub>), 38.0 (C<sub>q</sub>), 37 (CH<sub>2</sub>), 28.8 (CH), 13.2 (CH<sub>3</sub>). MS (MALDI+, DIT) m/z (%): 646.3213 (M<sup>+</sup>, 100), 647.3271 (M<sup>+</sup>+1, 47.8), 648.3383 (M<sup>+</sup>+2, 11.6). HRMS (MALDI): calcd. for C<sub>42</sub>H<sub>39</sub>N<sub>5</sub>O<sub>2</sub>: 645.3104; found: 646.3213 (M+H<sup>+</sup>). UV-Vis (CHCl<sub>3</sub>), λ nm (log ε): 509 (4.5). Then, DIPEA (12.3 μl, 0.037 mmol) was added dropwise to a stirred solution of the perylene derivative **JG117** (12 mg, 0.019 mmol) in dry DMF (10 mL) at room temperature, followed by addition to a solution of carboxylic acid modified cereulide **JG115B** (22 mg, 0.019 mmol) in DMF (5 mL). Finally, PyBop (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) (11 mg, 0.02 mmol) was added, and the mixture was stirred for 2 h. Then, the solution was concentrated *in vacuo* and extracted with CH<sub>2</sub>Cl<sub>2</sub>:H<sub>2</sub>O (3x50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and

concentrated *in vacuo*. Purification by flash column chromatography (SiO<sub>2</sub>, EtOAc/hexane, 2:3 v/v) afforded the fluorescent perylenemonoimide modified cereulide **JG121** (25.2 mg, 75 %) as a red solid. IR (KBr, cm<sup>-1</sup>) 3432, 2958, 2923, 2853, 1738 (C=O), 1651 (C=O), 1592, 1452, 1351, 1289, 1261, 1198, 1313, 1181, 1100, 1045, 954. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.58 (m, 2H, C<sub>Ar</sub>H), 8.52–8.41 (m, 5H, C<sub>Ar</sub>H + NH), 8.33 (dd, 2H, J = 4.5 Hz, J = 2.9 Hz, C<sub>Ar</sub>H), 8.27 (d, 1H, J = 4.6 Hz, NH), 8.13–8.04 (m, 3H, C<sub>Ar</sub>H + NH), 7.98 (d, 1H, J = 8.4 Hz, NH), 7.63 (t, J = 8.0 Hz, 1H, C<sub>Ar</sub>H), 7.53 (d, 1H, J = 7.8 Hz, C<sub>Ar</sub>H), 5.11 (q, 1H, J = 7.2 Hz, CH), 5.01 (dd, 1H, J = 9.4 Hz, J = 4.1 Hz, CH), 4.85–4.76 (m, 5H, 5x CH), 4.24 (dd, 3H, J = 7.4 Hz, J = 4.7 Hz, 3x CH), 4.06–3.75 (m, 8H, 2x CH + 3x CH<sub>2</sub>), 3.72–3.64 (m, 2H, CH<sub>2</sub>), 2.68 (dd, 1H, J = 43.3 Hz, J = 10.5 Hz, 0.5x CH<sub>2</sub>), 2.28 (d, 6H, J = 5.1 Hz, 2x CH + 2x CH<sub>2</sub>), 1.98 (s, 3H, 3x CH), 1.87–1.69 (m, 12H, 6x CH<sub>2</sub>), 1.62 (dd, J = 13.6 Hz, J = 6.2 Hz, 14H, 7x CH<sub>2</sub>), 1.55–1.48 (m, 9H, 3x CH<sub>3</sub>), 1.14 (d, J = 6.0 Hz, 9H, 3x CH<sub>3</sub>), 1.06–0.88 (m, 39H, 13x CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 175.6 (C=O), 175.5 (C=O), 175.4 (C=O), 175.3 (C=O), 175.2 (C=O), 172.7 (C=O), 172.6 (C=O), 172.2 (C=O), 172.1 (C=O), 171.0 (C=O), 165.7 (C=N), 165.0 (C=O), 161.0 (C=O), 158.3 (C<sub>Pyrr</sub>H), 137.0 (C<sub>Ar</sub>), 132.9 (C<sub>Ar</sub>), 132.1 (C<sub>Ar</sub>H), 131.4 (C<sub>Ar</sub>H), 129.9 (C<sub>Ar</sub>), 128.7 (C<sub>Ar</sub>), 128.2 (C<sub>Ar</sub>H), 127.4 (C<sub>Ar</sub>H), 126.6 (C<sub>Ar</sub>), 123.9 (C<sub>Ar</sub>H), 122.1 (C<sub>Ar</sub>), 120.5 (C<sub>Ar</sub>H), 120.3 (C<sub>Ar</sub>H), 79.8 (CH), 79.7 (CH), 75.1 (CH), 73.8 (CH), 62.0 (CH), 61.9 (CH), 58.2 (CH), 50.4 (CH), 48.6 (CH<sub>2</sub>), 48.5 (CH<sub>2</sub>), 41.0 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 38.2 (C<sub>q</sub>), 37.1 (CH<sub>2</sub>), 30.3 (CH), 30.2 (CH), 29.8 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 28.9 (CH), 28.8 (CH), 28.7 (CH), 26.3 (CH<sub>2</sub>), 26.2 (CH<sub>2</sub>), 24.5 (CH), 23.3 (CH<sub>3</sub>), 21.3 (CH<sub>3</sub>), 21.2 (CH<sub>3</sub>), 20.5 (CH<sub>3</sub>), 20.4 (CH<sub>3</sub>), 20.3 (CH<sub>3</sub>), 19.5 (CH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 18.7 (CH<sub>3</sub>), 16.8 (CH<sub>3</sub>), 16.7 (CH<sub>3</sub>), 15.1 (CH<sub>3</sub>), 15.0 (CH<sub>3</sub>), 13.3 (CH<sub>3</sub>). HRMS (MALDI+, DIT) m/z calcd. for C<sub>99</sub>H<sub>131</sub>KN<sub>11</sub>O<sub>21</sub>: 1848.9153 (M+K<sup>+</sup>); found: 1848.9389.

## Acknowledgements

We gratefully acknowledge financial support from the Ministerio de Economía y Competitividad, Spain (Projects CTQ2015-71353-R, SAF2016-77871-C2-1-R and SAF2016-77871-C2-2-R), Junta de Castilla y León, Consejería de Educación y Cultura y Fondo Social Europeo (Project BU263P18) and the EFSD European Research Programme on New Targets for Type 2 Diabetes supported by an educational research grant from MSD to ICC and GP (EFSD-2017). This work was also supported in part by 1000 Talent Plan, WQ20144400255 of SAFEA, China. Y.-H. L. wishes to thank the Innovation and Talent Introduction Base of Photoelectronic and Functional Molecular Solid Material (Grant No. 90002-18011002) from Sun Yat-sen University and the China Scholarship Council (No. 21606380054) for financial support.

**Keywords:** Fluorescent probes • Cereulide • Depsipeptides • Ionophore • Insulin • Beta-cells

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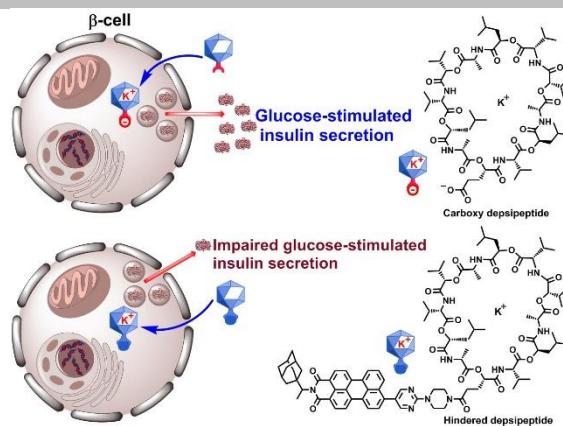
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Substitution of one hydroxyacid in the structure of an electrogenic  $K^+$  ionophore, cereulide, led to a modified cyclic depsipeptide that worked as a  $K^+$  ionophore with a different electroneutral transport mechanism and caused glucose-induced insulin secretion in a constitutive manner in rat insulinoma INS1E cells.



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**Manipulation of transmembrane transport by synthetic  $K^+$  ionophore depsipeptides and its implications in glucose-stimulated insulin secretion in  $\beta$ -cells**