Synthesis, Optimization, and Biological Evaluation of Corrinated Conjugates of the GLP-1R Agonist Exendin-4

Ian C. Tinsley,# Tito Borner,# MacKenzie L. Swanson, Oleg G. Chepurny, Sarah A. Doebley, Varun Kamat, Ian R. Sweet, George G. Holz, Matthew R. Hayes, Bart C. De Jonghe, and Robert P. Doyle*

ABSTRACT: Corrination is the conjugation of a corrin ring containing molecule, such as vitamin B$_{12}$ (B12) or B12 biosynthetic precursor dicyanocobinamide (Cbi), to small molecules, peptides, or proteins with the goal of modifying pharmacology. Recently, a corrinated GLP-1R agonist (GLP-1RA) exendin-4 (Ex4) has been shown in vivo to have reduced penetration into the central nervous system relative to Ex4 alone, producing a glucoregulatory GLP-1RA devoid of anorexia and emesis. The study herein was designed to optimize the lead conjugate for GLP-1R agonism and binding. Two specific conjugation sites were introduced in Ex4, while also utilizing various linkers, so that it was possible to identify Cbi conjugates of Ex4 that exhibit improved binding and agonist activity at the GLP-1R. An optimized conjugate (22), comparable with Ex4, was successfully screened and subsequently assayed for insulin secretion in rat islets and in vivo in shrews for glucoregulatory and emetic behavior, relative to Ex4.

INTRODUCTION

The dramatic rise of type 2 diabetes (T2D) and obesity as comorbidities has driven a concomitant rise in new pharmaceutical interventions to treat such disorders, either together or individually.1–7 One extremely successful family of pharmaceuticals in this field has been that of glucagon-like peptide-1 receptor agonists (GLP-1RAs),8–13 such as exenatide (i.e., Exendin-4; Ex4), liraglutide,14–17 and semaglutide.16–18 Exenatide and liraglutide confer glucoregulation in tandem with a body weight reduction of 5–6% over ~1 year,16,17 while semaglutide can produce a weight loss of 10–12% over the same time period.16,17 The hypogagic effects of all GLP-1RAs are however accompanied by nausea and vomiting (emesis) in upward of 25% of patients,19–25 the result of penetrance and direct action of the GLP-1RA in the central nervous system (CNS), particularly in the nucleus tractus solitarius and area postrema of the hind-brain.26–29 Thus, the development of a GLP-1RA that does not access the CNS would be expected then to mitigate the side effects of nausea and emesis and reduce the hypogagic effects. The reasons for removing the side effects are obvious, but less clear is the fact that there is a current unmet clinical need to broaden pharmacopoeia for certain subpopulations of T2D patients, including lean individuals, those in a state of cachexia (wasting of body tissues/chronic weight loss), or those who must avoid weight loss from other life-threatening diseases such as chronic obstructive pulmonary disease, cystic fibrosis, cancer, and HIV, among others.30–34 Thus, we set out to create GLP-1RAs with reduced brain penetrance but with retained, comparable pharmacodynamic profiles on pancreatic GLP-1R populations (Figure 1).35–39 Recently, we have taken the approach of utilizing components of the vitamin B$_{12}$ (B12)
dietary uptake pathway including that of the corrin ring containing B12 themselves as well as B12 precursors such as dicyanocobinamide (Cbi) in a process we have coined "corrination." The benefits of corrination over other conjugation modes lie in the ability to specifically target select corrin binding proteins such as intrinsic factor, transcobalamin, or haptocorrin for use in targeting or to affect pharmacokinetics, modify solubility, and alter drug localization, all without having to reverse the process to allow for maintained drug function (pending suitable design). Our recent report explored the conjugation of Cbi with Ex4, to produce Cbi-Ex4 (1), and its effects were tested in vivo in the musk shrew (Suncus murinus), a mammal capable of emesis in response to GLP-1R targeting therapeutics. Our data showed that Cbi-Ex4 enhanced the glucoregulatory response following an intraperitoneal glucose tolerance test (IPGTT), without producing hypophagia, anorexia, body weight loss, and, more importantly, without emesis, all characteristics of classical Ex4-based therapies. We hypothesized that these results are due to reduced drug penetration into the CNS (see Figure 1). This proof-of-concept corrinated Ex4 (1) was, however, notably less potent an agonist for the GLP-1R than Ex4 alone (200 vs 30 pM, respectively, in the same GLP-1R FRET assay). With that in mind, we set out to optimize 1 in terms of receptor binding and agonism for the purposes of future translation. As such,
we needed to synthesize and characterize a new library of Cbi-Ex4 conjugates. Of note here is that there is a dearth of synthetic cobinamide chemistry in the literature, mostly focusing on the novel cobinamides to treat hydrogen sulfide\textsuperscript{5,46} or cyanide poisoning\textsuperscript{47–54} and even less so in terms of conjugation chemistry (an excellent exception being that from the Gryko group using Cbi-PNA).\textsuperscript{55} hence, we wanted to produce Cbi-linkers that would be readily amenable to conjugation to Ex4 but indeed any targeted peptide moving forward. Here, we report a set of 16 new constructs (12–27) conjugated between Cbi and Ex4 at two positions (K12 and K40) by various linkers with a set of diverse chemical properties including hydrophobicity, amphiphilicity, and rigidity. The successful design and synthesis lead to the production of a new series of corrinated Ex4 compounds with comparable agonism and binding to unconjugated Ex4. One of these conjugates (22) displayed equipotency and binding at the GLP-1R in vitro and was subsequently selected for ex vivo screening for insulin secretion in rat islets and in vivo in shrews for glucoregulation, food intake, body weight, and emesis, compared to Ex4 and 1.

**RESULTS AND DISCUSSION**

**Design.** Our initial report on the effects of corrination on Ex4 included evidence for glucoregulation without emesis nor a reduction in food intake.\textsuperscript{59} In these subsequent studies, we have looked to optimize 1 with a focus on agonism and binding at the GLP-1R while also expanding to the little explored chemistry pertaining to the synthesis of Cbi conjugates. As such, the structure–activity data presented in these studies integrates synthesis, receptor agonism, receptor binding, ex vivo insulin secretion, in vivo glucoregulation, emetic response, and food intake to explore the role of the peptide conjugate site (Scheme 1) and/or the role of a specific set of spacers (linkers) as shown in Figure 2. In terms of the linkers, there are three subsets that were chosen to sample space across particular structural features, namely, steric, hydrophobic, and amphiphilic. The Ex4 conjugate site was restricted to K12 and a K40 residue added to the C-terminus of Ex4 (Ex40) since both sites were proven to be amenable to modification with minimal loss of function in our hands.\textsuperscript{35–39} and in prior literature.\textsuperscript{56–58}

**Chemistry.** The Cbi linker compounds (4–11) and Ex4 conjugates of such (12–27) amounted to a library of 32 compounds. The experimental details are shown in Scheme 1, with linkers (Figure 2) indicated as R-groups. Initially, Cbi was produced in-house via microwave-assisted reaction of B12 with sodium cyanide in EtOH as previously described.\textsuperscript{59} The purple dicyanocobinamide (CN\textsubscript{2}Cbi) produced was then activated with an excess of 1,1′-carbonyl-di-(1,2,4-trizole) (CDT) in dry N-methyl-2-pyrrolidone (NMP) and heating at 40 °C under argon for 10 min or until complete dissolution was observed. Upon dissolution, a specific linker (Figure 2; 3A–3H) was added in large excess (20×) relative to the activated CN\textsubscript{2}Cbi along with triethylamine (TEA), and the reaction was allowed to proceed for 1 h, again at 40 °C under argon with stirring. Initially, it was observed via RP-HPLC tracking on a C18 column (data not shown) that the reaction proceeded slowly, so in subsequent experiments, a second equivalent of linker (20×) and TEA were added at the 1 h time point and the reaction was allowed to proceed, as before, for an additional hour. Based on the additional step, yields for all Cbi-linkers were in the 30–50% range with facile purification to produce the Cbi-Linkers (4–11) with purities at, or in excess of, 97% (as tracked by RP-HPLC; see the Supporting Information). It should also be noted, and such is indicated in the color scheme used in Scheme 1 for the corrin rings, that a color change was observed upon formation of the Cbi-linkers, going from purple to orange upon purification of the Cbi-linkers in water. This color change was previously reported by Zhou and Zeldner\textsuperscript{60} and assigned to the formation of isomers, namely, α-cyano-β-aqua- and α-aqua-β-cyano-Cbi (as indicated in Scheme 1). This suggestion of isomer formation was supported by the HPLC traces of the linkers, which clearly showed the presence of both isomers (subsequently confirmed by nuclear magnetic resonance (NMR); Figure S7). Given the fact that such isomers were not expected to negatively affect the subsequent chemistry or biology, isomers were combined prior to peptide coupling and were characterized via NMR, electrospray ionization-mass spectrometry (ESI-MS), and electronic absorption spectroscopy (EAS).

Coupling of 4–11 with K12-azido- or K40-azido-modified Ex4 (noted throughout here simply as Ex4 or Ex40) to produce conjugates 12–27 was achieved via copper(I)-mediated alkyne-azide cycloaddition over 16 h at room temperature in a 4:1 dimethylformamide (DMF)/H\textsubscript{2}O solvent system in the presence of the tertiary amine tris((1-benzyl-4-triazolyl)methyl)amine (TBTA) to stabilize copper(I).\textsuperscript{51} Yields obtained were quantitative based on the starting peptide, and all conjugates were produced to at least 97%.

**Figure 3.** Effect of Cbi conjugation on the secondary structure of Ex4 or Ex40. CD spectra were collected with a sample concentration of 40 μM at pH 7 between 200 and 250 nm. % helicity was measured at 222 nm.
Conjugation of Cbi to Ex4 or Ex40 peptides maintains agonism at the GLP-1R. Nonlinear regression analysis was performed with GraphPad Prism 8. All compounds were assayed at least as triplicate independent runs. Data are shown as mean ± SEM.

### Structural Studies Using Circular Dichroism (CD)

All conjugates were investigated for the effects of corrination on helicity at a concentration of 40 μM in water at pH 7.0 (Figure 3). Immediately, it was evident that there was minimal variation in percent helicity whereupon the Cbi was conjugated at the Ex4 C-terminal K40 (20–27; % helicity ranged from ~20 to 36%; Ex40 control 33.1%; Table 1). There was however a noticeable variation in the percent helicity for the conjugates (12–19) whereupon Cbi was conjugated to the K12 within the Ex4 helix (% helicity range ~20–46%; Ex4 control 24.3; Table 1). These results are consistent with the fact that conjugation at the C-terminus renders less consequence to the linker type or spacer distance between Cbi and the peptide, given that this region is away from the helical region of Ex4 and does not interfere with the role of this region with receptor binding/agonism. The variation noted upon conjugation at the K12 residue renders the linker critical with the highest % helicity noted as 41% with the amphiphilic PEG spacer 3F (Figure 2, Table 1) and the lowest percent helicity noted with the small alkyl spacers 3B–3D (Figure 2, Table 1). No overall correlation between the structure and agonism or binding was observed upon data fitting (not shown).

### Biological Evaluation

To determine how the conjugation site of the peptide affects its function and the role linker choice plays in the attachment of the peptide to Cbi, we sought to screen 12–27 utilizing in vitro assays to determine potency and binding at the GLP-1R.

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**Table 1. EC<sub>50</sub> and IC<sub>50</sub> Values with Hill Slopes and % Helicity of Cbi Conjugates 12–27**

|     | EC<sub>50</sub> (pM)<sup>a</sup> | EC<sub>50</sub> Hill slope<sup>b</sup> | % helicity<sup>c</sup> | IC<sub>50</sub> (nM)<sup>d</sup> | IC<sub>50</sub> Hill slope<sup>e</sup> |
|-----|-------------------------------|---------------------------------|---------------------|----------------|---------------------|
| Ex4 | 12.5 ± 3.7                     | 1.1970                          | 24.3                | 5.98 ± 0.94    | −1.3010             |
| 12  | 75.4 ± 4.1                     | 0.9110                          | 39.2                | 24.6 ± 6.3     | −1.1170             |
| 13  | 105.6 ± 4.1                    | 1.0100                          | 26.9                | 135 ± 122.5    | −0.5661             |
| 14  | 33.2 ± 4.4                     | 1.4170                          | 29.0                | 79.5 ± 28.8    | −0.7858             |
| 15  | 38.9 ± 15.6                    | 1.0470                          | 20.5                | 82.7 ± 15.4    | −1.6990             |
| 16  | 34.1 ± 9.7                     | 0.9976                          | 24.4                | 36.5 ± 16.0    | −1.1770             |
| 17  | 36.5 ± 7.6                     | 1.0980                          | 41.6                | 45.9 ± 16.3    | −1.1740             |
| 18  | 40.5 ± 17.1                    | 1.3030                          | 33.1                | 34.3 ± 11.9    | −1.1530             |
| 19  | 31.2 ± 16.9                    | 1.1130                          | 45.6                | 11.6 ± 2.4     | −1.1270             |
| Ex40| 10.4 ± 3.9                     | 1.1160                          | 33.1                | 7.34 ± 1.37    | −0.9107             |
| 20  | 13.4 ± 4.2                     | 1.1890                          | 28.1                | 26.2 ± 6.3     | −1.1350             |
| 21  | 37.8 ± 11.5                    | 1.1830                          | 29.8                | 21.4 ± 8.0     | −0.8940             |
| 22  | 20.7 ± 8.3                     | 1.2090                          | 36.6                | 11.9 ± 2.5     | −1.3970             |
| 23  | 10.7 ± 6.0                     | 1.0120                          | 31.0                | 27.2 ± 6.3     | −1.1780             |
| 24  | 16.4 ± 5.1                     | 1.1370                          | 33.1                | 15.1 ± 4.1     | −1.2540             |
| 25  | 26.0 ± 14.1                    | 0.9817                          | 28.9                | 18.5 ± 4.6     | −1.0840             |
| 26  | 27.9 ± 4.2                     | 0.9957                          | 34.4                | 26.4 ± 8.0     | −1.2370             |
| 27  | 23.1 ± 7.1                     | 1.2130                          | 29.3                | 24.6 ± 6.3     | −1.2790             |

<sup>a</sup>Data represents EC<sub>50</sub> obtained using nonlinear regression analysis of data from highest FRET values obtained for each data point. Experiments were performed as three independent runs. 
<sup>b</sup>Data represents the Hill slope obtained using nonlinear regression analysis of data from highest FRET values obtained for each data point. Experiments were performed as three independent runs. 
<sup>c</sup>Data represents mean residue ellipticity [θ]<sub>222</sub> determined from the CD spectra of a 40 μM solution of peptide in H<sub>2</sub>O at RT pH 7.0. Average [θ]<sub>222</sub> values utilized to calculate percent helicity were obtained by performing the experiment in triplicate. Percent helicity was calculated using 100 × ([θ]<sub>222</sub>/max[θ]<sub>222</sub>). max[θ]<sub>222</sub> = −40,000 [1 − (2.5/n)], where n is the residue number. Experiments were performed as three independent runs. 
<sup>d</sup>Data represents IC<sub>50</sub> values obtained from competitive binding assays against red fluorescent GLP-1 using nonlinear regression analysis from highest values obtained for each data point. Experiments were performed as two independent runs. 
<sup>e</sup>Hill slopes were obtained using nonlinear regression analysis from highest values obtained for each data point. Results are expressed as mean ± SEM.
In Vitro Functional Agonism (EC$_{50}$) at Human GLP-1R. Our previously published Cbi-Ex4 construct 1 resulted in agonism at the GLP-1R with an EC$_{50}$ of 200 pM. The design of the newly constructed conjugates, 12−27, aimed to increase the potency of the original construct to that comparable of unconjugated Ex4 (<30 pM). 12−27 were assessed utilizing in vitro screening in HEK C20 cells stably expressing the human GLP-1R and cAMP FRET reporter H188 produced in-house (see Methods). To determine if the azido modification to lysine at position K12 (Ex4) or the addition of this residue to position 40 (Ex40) had any effect on agonism at GLP-1R, they were also screened for agonism to compare with native Ex4 (nEx4; no azido modifications), resulting in EC$_{50}$ values of 20 pM for nEx4, 12.5 ± 3.7 pM for Ex4, and 10.4 ± 3.9 pM for Ex40 (Table 1). All newly synthesized compounds were functional at the GLP-1R with improved potency in all cases (EC$_{50}$ range 13.4 ± 4.2 to 105.6 ± 4.1 pM) to that of our previously reported construct 1 (200 pM; Table 1 and Figure 4). In general, the Ex4 conjugates 12−20 were inferior to the Ex40 conjugates (20−27) and displayed greater variance, with EC$_{50}$ values ranging from 31 to 106 pM for 12−19 compared to 13 to 38 pM for 20−27. As with the variance observed in the folded state for 12−19, it is likely that proximity and/or interactions of the Cbi moiety to the helix interferes with receptor binding and/or interactions necessary for agonism. Conjugations performed at the C-terminal end of the peptide would be expected to place the corrin ring away from such residues as we have shown previously with B12 conjugates of the neuropeptide PYY$_{3−36}$. One Cbi-Ex4 conjugate however (19), which bridged Ex4 at the K12 residue with Cbi through one of the hydrophobic, rigid linkers (3H), was shown to be equipotent to Ex4 with an EC$_{50}$ value of 31.2 ± 16.9 pM and an IC$_{50}$ of 11.6 ± 2.4 nM (compared to 5.98 ± 0.94 nM for Ex4 alone; Table 1). All Ex40-based conjugates (20−27) were shown to be equipotent to the unconjugated peptide. Such results are consistent with the fact that addition of, and conjugation to, the K40 residue is optimal when conjugating Cbi to Ex4.

In Vitro Competitive Binding (IC$_{50}$) at Human GLP-1R. As shown in Table 1, the presence of Cbi influences the ability of the conjugate to agonize the GLP-1R compared to that of Ex4 or Ex40. To further investigate how conjugation of Cbi affects Ex4 or Ex40, a series of competitive binding assays against GLP-1red (a red fluorescent analog of GLP-1) were conducted. nEx4 was utilized as a reference competitor with published sensitivity to GLP-1RAs and the same serum B12 binding proteins (IF and Haptocorrin) as that found in humans and thus was considered an ideal model for the in vivo studies. We observed that shrews treated with 1, 22, or nEx4 display similar improvements in glucose handling following glucose load compared to vehicle controls (Figure 6A). Posthoc analyses showed that all three compounds significantly suppressed blood glucose (BG) at 20 and at 40 min after glucose administration versus vehicle treatment (all p < 0.0001). Remarkably, BG values at 20 min post glucose injection in animals receiving 22 were significantly lower than those in animals treated with 1, denoting an improved glucose tolerance/superior pharmacological efficacy (p < 0.01). Additionally, the variation in plasma glucose concentrations, represented as area under the curves (0−60’ and 0−120’, respectively) for 22, did not differ from nEx4 and were significantly lower than controls (Figure 6B, C, all p < 0.05).

We then analyzed the effects of 1, 22, and Ex4 on food intake and body weight at a single, proof-of-concept, dose, in
line with previous reports. Ex4 administration produced anorexia in shrews at all measured time points (Figure 6D), while 1 had no impact on feeding. 22 treatment suppressed food intake similar to Ex4 at 6, 24, and 48 h. The anorectic effect of Ex4 treatment was paralleled by a significant reduction in body weight at 24 h ($p < 0.05$), which was not observed following treatment with 1 or 22 (Figure 6E).

To test whether 22 treatment retained the non-emetic properties of the lead conjugate 1, indicative of an altered pharmacodynamic profile, despite its comparable potency and GLP-1R binding affinity compared to 1, and comparable to nEx4, we compared the emetogenic properties of 1, 22, and native Ex4 in shrews. Ex4 induced profound emesis in the majority of the shrews tested (Figure 6F). Indeed, 80% of the animals exhibited emesis upon administration of native Ex4 within minutes after injection (29 ± 16 min). In line with a
previous report, treatment with 1 did not cause emesis in any of the shrews tested. Importantly, the number of single emetic episodes occurring in the 120 min window after drug administration was also significantly reduced after 22 administration compared to Ex4 and did not differ from vehicle- or 1-treated groups. Only two animals that received 22 experienced emesis with an average latency of 70 ± 29 min. The greater emetic effect observed for 22 over 1 is likely a consequence of the greater binding and agonism of 22 at the GLP-1R, an effect that is likely to be mitigated, without loss of glucoregulation, by use of lower doses of 22 (on-going work). The difference in the emetogenic profiles of the corrinated constructs versus native Ex4 is further emphasized by the graphical visualization of emetic intensity, recurrence, and latency for each individual animal across time (Figure 6G).

■ CONCLUSIONS

Neuroendocrine associated pharmacological side effects such as nausea and emesis are often downplayed or dismissed, left in the shadow of the overriding target, be it glucoregulation, weight loss, etc. Indeed, the weight loss gleaned from GLP-1RAs, for example, has proven to be a beneficial "side effect" of T2D treatment due to the high comorbidity of T2D with obesity. In many cases, however, such as lean T2D patients, or patients with comorbidities where nutritional status is critical (cystic fibrosis, cancer-cachexia, sarcopenia, chronic obstructive pulmonary disease, etc.), removal of the CNS-associated side effects would be greatly beneficial. Indeed, GLP-1RAs have proven to be life-altering, and the ability to expand their use, or increase patient compliance when using, should not be understated. Herein, we conceived 16 new Cbi-Ex4/Ex40 conjugates with the aim of designing a construct with equipotent binding and agonism of the GLP-1R to that of Ex4 with a view of maintaining activity at peripheral GLP-1R populations and mitigating such activity in CNS populations. We were able to successfully design several constructs with comparable binding and agonism at GLP-1R as Ex4, with one conjugate, 22 (IC_{50} 11.9 ± 2.5 nM, EC_{50} 20.7 ± 8.3 pM), translated into ex vivo and in vivo studies. 22 increased glucose secretion compared to vehicle controls in a glucose-dependent insulin secretion assay in rat islets. In an in vivo IPGTT, 22 provided glucoregulation comparable to Ex4. Critically, 22 showed a near absence of emesis and mild body weight lowering actions compared to profound emesis and body weight loss observed for NE4x.

While corrination is poorly explored, especially as it pertains to applications that seek to prevent CNS penetration while maintaining peripheral activity of a target drug, it shows considerable promise as a platform technology, moving beyond GLP-1RAs. An additional highlight of Cbi is its ability to applications that seek to prevent CNS penetration while maintaining peripheral activity of a target drug, it shows considerable promise as a platform technology, moving beyond GLP-1RAs. An additional highlight of Cbi is its ability to applications that seek to prevent CNS penetration while maintaining peripheral activity of a target drug, it shows considerable promise as a platform technology, moving beyond GLP-1RAs. An additional highlight of Cbi is its ability to

■ EXPERIMENTAL SECTION

General Considerations. All commercial reagents and anhydrous solvents purchased were used without further purification. All reactions containing air- and/or moisture-sensitive reactants were performed under argon. Compounds were purified using a Shimadzu Prominance HPLC using a C18 column (Eclipse XDB-C18 5 μm, 4.6 × 150 mm). All compounds had purities at, or in excess of, 97% (as tracked by RP-HPLC; see the Supporting Information). Products were analyzed for mass using a Shimadzu LCMS-8040. ^1H NMR and ^15C NMR spectra were acquired on a Bruker NMR spectrometer (Avance 400 MHz) in D_{2}O at a D of 3 for all compounds. EAS was collected on a Varian Cary 60Bio UV–visible spectrophotometer in a 1 mL quartz cuvette with baseline correction. CD spectra were recorded with a Jasco J-715 circular dichroism spectropolarimeter in 40 μM H_{2}O in a 0.1 cm quartz cuvette. Data was analyzed and fit using GraphPad Prism 8.0. We established a new clone of H188 cells designated here as HEK293-GLP-1R-H188-C20. This clone was obtained by G418 antibiotic resistance selection after cotransfection of cells with the human GLP-1R and the cAMP FRET reporter H188. Cells were grown in a Memmert Incubator I (Schwabach, Germany) at 37 °C gassed with 5% CO_{2} at ~95% humidity. FRET assays were conducted utilizing a Molecular Devices FlexStation 3 Multi-Mode Microplate Reader. Data was analyzed utilizing Molecular Devices SoftMax Pro v.5.4.6. Linear regression analyses were performed utilizing GraphPad Prism 8.

Materials. The following were purchased from Sigma-Aldrich: acetanilide (Cat # 14851), diethyl ether (Cat # 673811), ethanol (Cat # 459844), ethyl acetate (Cat # 319902), isopropyl alcohol (Cat # 34863), methanol (Cat # 34885), n-methyl-2-pyrrolidone (Cat # 328634), triethylamine (Cat # 808352), vitamin B_{12} (Cat # V2876), sodium cyanide (Cat # 205222), 1,1′-carbonyl-di-(1,2,4-trizole) (Cat # 21863), propargylamine (Cat # P36900), 1-amino-3-butyne (Cat # 715190), 4-pentyne-1-amine (Cat # 779490), hex-5-yne-1-amine (Cat # COM497S12576), bovine serum albumin (Cat # A7030), Dulbecco’s modified Eagle medium (Cat # D6429), fetal bovine serum (Cat # 12303C), G-418 disulfate salt solution (Cat # G8168), and HEPES (Cat # H0877). The following were purchased from ThermoFisher Scientific: penicillin-streptomycin (Cat # 15140122) and trypsin–EDTA (Cat # 25200072). The following were purchased from Fisher Scientific: calcium chloride dihydrate (Cat # BP510), magnesium chloride hexahydrate (Cat # BP214), potassium chloride (Cat # BP366), and sodium chloride (Cat # BP358–1). The following were purchased from BroadPharm: propargyl-PEG2-amine (Cat # BP22519) and propargyl-PEG4-amine (Cat # BP22520). The following were purchased from Enamine: (4-ethylphenyl)methanamine (Cat # EN300–233648) and (3-ethylphenyl)methanamine (Cat # EN300–248722). Krebs-Ringer Bicarbonate (KRB) buffer was prepared as described previously. d-Glucose (Cat # G 5767) and nEx4 (Cat # E7144) were purchased from Sigma-Aldrich. Ex4 K12-azido and Ex4 K40-azido were produced by Genscript (Piscataway, New Jersey, United States). The rat insulin radioimmunoassay (RIA) kit was purchased from Millipore Sigma, Burlington, MA (Cat no. RI-13 K). The human GLP-1R plasmid was provided by M. Reinhoff (Tufts University School of Medicine to author G.G.H.). The H188 plasmid was provided by K. Jalink, Netherlands Cancer Institute to authors G.G.H. and R.P.D.

Methods. Synthesis of Dicyanocobinamide (Cbi) (2). The synthesis of dicyanocobinamide (Cbi) was performed according to previously reported methods. Briefly, to a 10 mL microwave reaction vessel containing a magnetic stir bar were added B12 (cyanocobalamin) (303.8 mg, 0.225 mmol) (see Figures S1–S6), sodium cyanide (NaCN) (36.9 mg, 0.7096 mmol), and 5 mL of iPrOH, and the vessel was sealed. The reaction was heated to 120 °C for 10 min. Following completion of the microwave reaction, the remaining solution was transferred and diluted using isopropyl alcohol (iPrOH). The reaction was purified utilizing a normal-phase silica column. The reaction was eluted using 100% methanol (MeOH). The product eluted as a purple product. The isolated product was precipitated utilizing diethyl ether (Et_{2}O) and allowed to dry producing a solid, purple product, 2 (see Figure 2), in 80% yield (187.4 mg, 0.1795 mmol). Solubility was measured to be 400 mg/mL in distilled water. 2 was purified by RP-HPLC as described for method A1, to 98%; t_{R} = 2.67 min; ESI-MS expected m/z = 1042, observed m/z = [M–CN]^{+} 1016. H NMR (400 MHz, D_{2}O): δ 5.90 (s, 1H), 3.95–3.87 (m, 1H), 3.85 (d, J = 8.4 Hz, 1H), 3.75 (d, J = 10.4 Hz, 1H), 3.41 (m, 1H), 3.36 (s, 3H), 3.30 (m, 2H), 3.25 (d, J = 4.7 Hz, 1H), 3.17 (dd, J = 6.8, 6.9 Hz, 1H), 2.92–2.86 (m, 3485

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method A1 to produce di was redissolved in a minimal amount of MeOH (50 mL) and centrifuged (5 min, 4000 rpm, RT). The crude solid was added and stirred for 1 h after which point a second equivalent of CDT (19.9 mg, 0.0191 mmol) with CDT (51.1 mg, 0.311 mmol) and stirring the solution for 1 h at which time 3E (25.1 µL, 25.4 mg, 0.177 mmol) and TEA (50 µL) were added and stirred for 1 h after which a second equivalent amount of 3E and TEA were added and allowed to stir for 1 h to give the target compound, which was purified using RP-HPLC method A1 to produce 8 as an orange solid to 98% purity. Yield 35% (6.0 mg, 0.0055 mmol). The product obtained was in the form of two different isomers with the aquo-group located on the alpha and beta positions (α-cyano-β-aqua- and α-aqua-β-cyano-). EAS Ext Coeff ff = 22,919 M-1 cm-1. See Figures S62–S66.

Cbi-PEG4-Alkyn (9). 9 was prepared according to the general procedure described above, combining 2 (15.5 mg, 0.0149 mmol) with CDT (51.3 mg, 0.313 mmol) and stirring the solution for 1 h at which time 3F (18.8 µL, 19.5 mg, 0.0843 mmol) and TEA (50 µL) were added and stirred for 1 h after which a second equivalent amount of 3F and TEA were added and allowed to stir for 1 h to give the target compound, which was purified using RP-HPLC method A1 to produce 9 as an orange solid to 97% purity. Yield 32% (6.0 mg, 0.0055 mmol). The product obtained was in the form of two different isomers with the aquo-group located on the alpha and beta positions (α-cyano-β-aqua- and α-aqua-β-cyano-). EAS Ext Coeff ff = 19,439 M-1 cm-1. See Figures S73–S77.

Cbi-4EPMA-Alkyn (10). 10 was prepared according to the general procedure described above, combining 2 (14.9 mg, 0.0143 mmol) with CDT (50.1 mg, 0.305 mmol) and stirring the solution for 1 h at which time 3G (23.8 mg, 0.181 mmol) and TEA (50 µL) were added and stirred for 1 h after which a second equivalent amount of 3G and TEA were added and allowed to stir for 1 h to give the target compound, which was purified using RP-HPLC method A1 to produce 10 as an orange solid to 97% purity. Yield 10% (1.5 mg, 0.001 mmol). The product obtained was in the form of two different isomers with the aquo-group located on the alpha and beta positions (α-cyano-β-aqua- and α-aqua-β-cyano-). EAS Ext Coeff ff = 15,886 M-1 cm-1. See Figures S84–S88.

Cbi-3EMPA-Alkyn (11). 11 was prepared according to the general procedure described above, combining 2 (15.7 mg, 0.0151 mmol) with CDT (51.1 mg, 0.311 mmol) and stirring the solution for 1 h at
which time 3H (21.0 µL, 21.2 mg, 0.162 mmol) and TEA (50 µL) were added and stirred for 1 h after which a second equivalent amount of 3H and TEA were added and allowed to stir for 1 h to give the target compound, which was purified using RP-HPLC method A1 to produce 11 as an orange solid to 97% purity. Yield 14% (2.6 mg, 0.002 mmol). The product obtained was in the form of two different isomers with the aqua-group located on the alpha and beta positions (α-cyano-β-aqua- and α-cyano-β-aqua-). t½: 12.6 and 13.0 min; ESI-MS-expected m/z = 1191, observed m/z = [M+H]⁺: 1172, [M−H₂O + H⁺]⁻: 587. H NMR (400 MHz, D₂O): δ 6.69 (1H, s, Ar-H, α-aqua isomer) 6.42 (1H, s, Ar-H, α-aqua isomer). EAS Ext Coeff = 15,669 M⁻¹ cm⁻¹. See Figures S59–S99.

General Procedure for Copper-Mediated Alkyne-Acylo. Zycle-Addition. To a 5 mL round bottom flask containing a stir bar, copper(I) iodide (Cul) (3.3 mg, 0.017 mmol) and TBTA (7.0 mg, 0.013 mmol) were added and stirred to 1 mL of 4:1 DMF/H₂O. The reaction mixture was allowed to stir at room temperature until a color change occurred (clear to yellowish brown) (~15 min). The solution was treated with azido-exendin-4/40 (2.0 mg, 0.004 mmol) (see Figures S12–S17) and the corresponding Cbi-alkyne (4–11) (4.8 mg, 0.004 mmol) and allowed to stir overnight.

Cbi-Pro-Ex4 (12). 12 was prepared according to the general procedure described above; combining 4 (1.1 mg, 0.001 mmol) with Cul (4.0 mg, 0.021 mmol), TBTA (7.5 mg, 0.014 mmol), and Ex4 (3.1 mg 0.007 mmol) gave the target compound, which was purified using RP-HPLC method A2 to produce 12 as a red solid to 98% purity, t½: 11.7 min; ESI-MS-expected m/z = 5327, observed m/z = [M−H₂O + 2H⁺]²⁻: 1775, [M−H₂O + CH₂OH]⁻: 1364, [M−H₂O + 3H⁺]⁺: 1328. See Figures S32–S35.

Cbi-But-Ex4 (13). 13 was prepared according to the general procedure described above; combining 5 (5.7 mg, 0.005 mmol) with Cul (4.2 mg, 0.022 mmol), TBTA (7.5 mg, 0.014 mmol), and Ex4 (4.2 mg 0.0010 mmol) gave the target compound, which was purified using RP-HPLC method A2 to produce 13 as a red solid to 98% purity, t½: 12.0 min; ESI-MS-expected m/z = 5341, observed m/z = [M−H₂O + 2H⁺]²⁻: 1775, [M−H₂O + 3H⁺]⁺: 1364, [M−H₂O + 4H⁺]³⁺: 1322. See Figures S34–S36.

Cbi-Pent-Ex4 (14). 14 was prepared according to the general procedure described above; combining 6 (4.9 mg, 0.0043 mmol) with Cul (3.6 mg, 0.019 mmol), TBTA (6.2 mg, 0.012 mmol), and Ex4 (3.9 mg 0.0009 mmol) gave the target compound, which was purified using RP-HPLC method A2 to produce 14 as a red solid to 98% purity, t½: 12.0 min; ESI-MS-expected m/z = 5355, observed m/z = [M−H₂O + 2H⁺]²⁻: 1780, [M−H₂O + 3H⁺]⁺: 1335. See Figures S45–S47.

Cbi-Hex-Ex4 (15). 15 was prepared according to the general procedure described above; combining 7 (9.7 mg, 0.0084 mmol) with Cul (3.5 mg, 0.018 mmol), TBTA (6.6 mg, 0.012 mmol), and Ex4 (5.3 mg 0.0013 mmol) gave the target compound, which was purified using RP-HPLC method A3 to produce 15 as a red solid to 98% purity, t½: 12.0 min; ESI-MS-expected m/z = 5369, observed m/z = [M−H₂O + 2H⁺]²⁻: 1784, [M−H₂O + 3H⁺]⁺: 1339. See Figures S56–S58.

Cbi-PEG2-Ex4 (16). 16 was prepared according to the general procedure described above; combining 8 (2.0 mg, 0.0017 mmol) with Cul (3.6 mg, 0.019 mmol), TBTA (7.1 mg, 0.013 mmol), and Ex4 (3.0 mg 0.0007 mmol) gave the target compound. t½: 12.0 min; ESI-MS-expected m/z = 5341, observed m/z = [M−H₂O + 2H⁺]²⁻: 1800, [M−H₂O + 3H⁺]⁺: 1350. See Figures S67–S69.

Cbi-PEG4-Ex4 (17). 17 was prepared according to the general procedure described above; combining 9 (3.0 mg, 0.0023 mmol) with Cul (3.4 mg, 0.018 mmol), TBTA (7.3 mg, 0.014 mmol), and Ex4 (4.1 mg 0.0010 mmol) gave the target compound. t½: 12.3 min; ESI-MS-expected m/z = 5504, observed m/z = [M−H₂O + 2H⁺ + CH₂CN]⁻: 1870, [M−H₂O + 3H⁺ + CH₂OH]⁻: 1404, [M−H₂O + 4H⁺]⁺: 1098. See Figures S79–S80.
with Cul (3.0 mg, 0.016 mmol), TBTA (6.7 mg, 0.013 mmol), and Ex40 (2.3 mg 0.0005 mmol) gave the target compound, which was purified using RP-HPLC method A2 to produce 26 as a red solid to 98% purity. \( \text{Ex40 (2.0 mg 0.0005 mmol)} \) gave the target compound, which was purified using RP-HPLC method A2 to produce 27 as a red solid to 97% purity. Ex40 (3.4 mg, 0.018 mmol), TBTA (7.0 mg, 0.013 mmol), and CuI (3.4 mg, 0.018 mmol), TBTA (7.0 mg, 0.013 mmol), and CuI (3.4 mg, 0.018 mmol) gave the target compound, which was determined through an increase in the 485/535 nm FRET ratio, indicative of an increase in the cAMP level through binding to the GLP-1R. To each well at 5 \( \mu \)L of standard extracellular saline with 11 mM glucose and 0.1% BSA at 37 °C, gassed with 5% CO2 for 60 min. Subsequently, islets were placed into 96-well plates containing desired amounts of glucose and agents as indicated (Figure S5) and incubated for an additional 60 min. At the end of this period, the supernatant was assayed for insulin by RIA.

**In Vivo Study Design in Shrews.** Adult male shrews \( (n = 32, \text{Suncus murinus}) \) weighing \( \geq 60 \) g were bred at the University of Pennsylvania. These animals generated in our lab were originally derived from a colony maintained at the University of Pittsburgh Cancer Institute (a Taiwanese strain derived from stock supplied by the Chinese University of Hong Kong). Shrews were single-housed in plastic cages (37.3 × 23.4 × 14 cm, Inniove), fed \( \text{ad libitum} \) with a mixture of feline (75%, Laboratory Feline Diet 5003, Lab Diet) and ferret food (25%, High Density Ferret Diet SL14, Lab Diet), and had \( \text{ad libitum} \) access to tap water except where noted. All animals were housed under a 12/12 h light/dark cycle in a temperature- and humidity-controlled environment. Shrews were habituated to single housing in their home cages and injected IP at least 1 week prior to experimentation. All experimental injections in shrews were separated by at least 72 h. Behavioral experiments were conducted in a within-subjects, Latin square design.

**Effects of Ex4, 1, and 22 on Energy Balance in Shrews.** We first evaluated in shrews the effects on food intake and body weight of doses of nEx4, 1, and 22. Food intake was evaluated using our custom-made feedometers, consisting of a standard plexiglass rodent housing cage (29 × 19 × 12.7 cm) with mounted food hoppers resting on a plexiglass cup (to account for spillage). Shrews had \( \text{ad libitum} \) access to powdered food through a circular (3 cm diameter) hole in the cage. Food was removed 1 h prior to the dark onset. Shortly before the dark onset, shrews \( (n = 10) \) received IP injection of Ex4 (5 nmol/kg), 1 (5 nmol/kg), 22 (5 nmol/kg), or vehicle (1 mL/100 g BW sterile saline). Food intake was manually measured at 6, 24, and 48 h post injection. BW was measured at 0, 24, and 48 h. Treatments occurred in a within-subject, counter-balanced design and were at least 3 days apart.

**Effects of Ex4, 1, and 22 on Glucosemetabolism Following an IPGTT in Shrews.** The protocol for performing an IPGTT in shrews was similar to that previously described.\(^{22,24}\) Brieﬂy, 3 h before the dark onset, shrews \( (n = 12) \) were food- and water-deprived. Two hours intraperitoneal baseline BG levels were determined by a small drop of tail blood and measured using a standard glucometer. nEx4 (5 nmol/kg), 1 (5 nmol/kg), and 22 (5 nmol/kg), or vehicle (1 mL/100 g BW sterile saline) were then administered IP. BG was measured 30 min later \( (t = 0 \text{ min}) \), and then each shrew received an IP bolus of glucose \( (2 \text{ g/kg}) \). Subsequent BG readings were taken at 20, 40, 60, and 120 min after glucose injection. After the final BG reading, food and water were returned. IPGTT studies were carried out in a within-subject, counter-balanced design and separated by at least 72 h.

**Effects of Ex4, 1, and 22 on Emesis in Shrews.** Shrews \( (n = 10) \) were habituated to IP injections and to clear plastic observation chambers \( (23.5 × 15.25 × 17.8 \text{ cm}) \) for four consecutive days prior to experimentation. The animals were injected IP with nEx4, 1, 22 (all at 5 nmol/kg), or vehicle, placed in their respective emetic cages, and then video-recorded (Vixia HF-R62, Canon) for 20 min. All recordings started within 2 min after drug administration. After 120 min, the animals were returned to their cages. Treatments were separated by 72 h. Analysis of emetic episodes was performed by an observer blinded to treatment groups. Emetic episodes were characterized by strong rhythmic abdominal contractions associated with either oral expulsion from the gastrointestinal tract (vomiting) or without the passage of materials (retching). Latency to the first emetic episode, the total number of emetic episodes, and the number of emetic episodes per minute were recorded.
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00185.

RP-HPLC purity traces, electrospray ionization mass spectra, $^1$H and $^{13}$C nuclear magnetic resonance spectra, electronic absorption spectra, and in vitro dose response curves (PDF)

AUTHOR INFORMATION

Corresponding Author
Robert P. Doyle — Department of Chemistry, Syracuse University, Syracuse, New York 13244, United States; Department of Medicine, State University of New York, Upstate Medical University, Syracuse, New York 13210, United States; orcid.org/0000-0001-6786-5656; Email: rpdoyle@syr.edu

Authors
Ian C. Tinsley — Department of Chemistry, Syracuse University, Syracuse, New York 13244, United States
Tito Borner — Department of Biobehavioral Health Sciences, University of Pennsylvania, School of Nursing, Philadelphia, Pennsylvania 19104, United States
MacKenzie L. Swanson — Department of Chemistry, Syracuse University, Syracuse, New York 13244, United States
Oleg G. Chepurny — Department of Medicine, State University of New York, Upstate Medical University, Syracuse, New York 13210, United States
Sarah A. Doebble — Department of Biobehavioral Health Sciences, University of Pennsylvania, School of Nursing, Philadelphia, Pennsylvania 19104, United States
Varun Kamat — Department of Medicine, University of Washington, Medicine Diabetes Institute, Seattle, Washington 98109, United States
Ian R. Sweet — Department of Medicine, University of Washington, Medicine Diabetes Institute, Seattle, Washington 98109, United States
George G. Holz — Department of Medicine, State University of New York, Upstate Medical University, Syracuse, New York 13210, United States
Matthew R. Hayes — Department of Psychiatry, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania 19104, United States
Bart C. De Jonghe — Department of Biobehavioral Health Sciences, University of Pennsylvania, School of Nursing, Philadelphia, Pennsylvania 19104, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c00185

Author Contributions
$^*$I.C.T. and T.B. contributed equally to the work.

Author Contributions
The project was conceived by R.P.D. All syntheses, purification, and chemical characterizations (NMR/CD/ESMS) were performed by I.C.T. and M.L.S. All EC$_{50}$ measurements were conducted by O.G.C. and I.C.T. Stably transfected HEK-hGLP-1R-H188 C20-cells were generated by O.G.C. Binding experiments were conducted by Euroscreent Fast (Gosselies, Belgium) using compounds synthesized by I.C.T. and M.L.S. Insulin secretion in islets was performed by I.R.S. and V.K. All in vivo work was designed by M.R.H., B.C.D.J., and T.B. and was conducted by T.B. and S.A.D. The manuscript was written mainly by I.C.T. and R.P.D., with contributions from all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare the following competing financial interest(s): RPD is the named inventor of a patent associated with this work, which is assigned to Syracuse University. R.P.D., I.C.T., T.B., B.C.D.J., and M.R.H. are owners of Cantius Therapeutics LLC (Lansdale, Pennsylvania, United States), which played no role in this work. MRH receives research support from Eli Lilly & Co. and Boehringer Ingelheim for projects unrelated to the current manuscript.

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ABBREVIATIONS
3EPMA, (3-ethylphenyl)methanamine; 4EPMA, (4-ethylphenyl)methanamine; AUC, area under the curve; BG, blood glucose; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; Cbi, dicyanobonamide; CD, circular dichroism; CDT, 1,1’-carbonyl-di-(1,2,4-triazole); CNS, central nervous system; DMEM, Dulbecco’s modified Eagle medium; DMF, dimethylformamide; EAS, electronic absorption spectra; EC$_{50}$, half-maximal effective concentration; EPAC, exchange protein directly activated by cAMP; Ex4, exendin-4 with K12 azido modification; Ex40, exendin-4 with an azido-lysin added as residue 40; FBS, fetal bovine serum; FRET, Forster resonance energy transfer; GLP-1R, glucagon like peptide-1 receptor; GPCR, G-protein coupled receptor; GSIS, glucose-stimulated insulin secretion; HEM, human embryonic kidney cells; IC$_{50}$, half-maximal inhibitory concentration; IPGTt, intraperitoneal glucose tolerance test; ISR, insulin secretion rate; nEx4, native Exendin-4; NMP, 1-ethyl-2-pyrrolidinone; NMR, nuclear magnetic resonance; PNS, peripheral nervous system; RIA, radioimmunoassay; RP-HPLC, reversed-phase high-performance liquid chromatography; SD, Sprague–Dawley rat; SEM, standard error of the mean; SES, standard extracellular saline; T2D, type 2 diabetes mellitus; BTA, tris(benzyltriazolylmethyl)amine; TEA, triethylamine; t$_{50}$, retention time

REFERENCES
(1) Chen, L.; Magliano, D. J.; Zimet, P. Z. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. Nat. Rev. Endocrinol. 2012, 8, 228–236.
(2) Flegal, K. M.; Carroll, M. D.; Ogden, C. L.; Curtin, L. R. Prevalence and trends in obesity among US adults, 1999–2008. JAMA 2010, 303, 235–241.
(3) Sherwin, R.; Jastreboff, A. M. Year in diabetes 2012: The diabetes tsunami. J. Clin. Endocrinol. Metab. 2012, 97, 4293–4301.
(4) Franks, P. W.; McCarthy, M. I. Exposing the exposures responsible for type 2 diabetes and obesity. Science 2016, 354, 69–73.

(5) Upadhyay, J.; Polyzos, S. A.; Perakakis, N.; Thakkar, B.; Paschosu, S. A.; Katsiki, N.; Underwood, P.; Park, K. H.; Seufert, J.; Kang, E. S.; Sternthal, E.; Karagiannis, A.; Mantzoros, C. S. Pharmacotherapy of type 2 diabetes: An update. Metabolism 2018, 78, 13–42.

(6) Sadry, S. A.; Drucker, D. J. Emerging combinatorial hormone therapies for the treatment of obesity and T2DM. Nat. Rev. Endocrinol. 2013, 9, 425–433.

(7) Drucker, D. J.; Sherman, S. I.; Bergenstal, R. M.; Buse, J. B. The safety of incretin-based therapies—review of the scientific evidence. J. Clin. Endocrinol. Metab. 2011, 96, 2027–2031.

(8) Hayes, M. R.; Mietlicki-Baase, E. G.; Kanoski, S. E.; De Jonghe, B. C. Incretins and amylin: neuroendocrine communication between the gut, pancreas, and brain in control of food intake and blood glucose. Annu. Rev. Nutr. 2014, 34, 237–260.

(9) Holst, J. J. The physiology of glucagon-like peptide 1. Physiol. Rev. 2007, 87, 1409–1439.

(10) Baggio, L. L.; Drucker, D. J. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007, 132, 2131–2157.

(11) Lovshin, J. A.; Schmidt, H. D. GLP-1 influences food and drug reward. Curr. Opin. Behav. Sci. 2016, 9, 66–70.

(12) Kanoski, S. E.; Fortin, S. M.; Arnold, M.; Grill, H. J.; Hayes, M. R. Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exenatide-4. Endocrinology 2011, 152, 3103–3112.

(13) Sisley, S.; Gutierrez-Aguilar, R.; Scott, M.; D’Alessio, D. A.; Sandoval, D. A.; Seeley, R. J. Neuronal GLP1R mediates liraglutide’s anorectic but not glucose-lowering effect. J. Clin. Invest. 2014, 124, 2456–2463.

(14) Kofoed, J.; Bielsen, P. F.; Huusfeldt, P. O.; Johansen, N. L.; Madsen, K.; Pederson, F. Z.; Thøgersen, H.; Wilken, M.; Agerö, H. Potent Derivatives of glucagon-like peptide 1 with pharmacokinetic properties suitable for once daily administration. J. Med. Chem. 2000, 43, 1664–1669.

(15) Madsen, K.; Knudsen, L. B.; Agersøe, H.; Nielsen, P. F.; Thøgersen, H.; Wilken, M.; Johansen, N. L. Structure-activity and protraction relationship of long-acting glucagon-like peptide-1 derivative: importance of fatty acid length, polarity, and bulkiness. J. Med. Chem. 2007, 50, 6126–6132.

(16) Knudsen, L. B.; Lau, J. The discovery and development of liraglutide and semaglutide. Front. Endocrinol. 2019, 10, 155.

(17) O’Niel, P. M.; Birkenfeld, A. L.; McGowan, B.; Mosenzon, O.; Pederson, S. D.; Wharton, S.; Carson, C. G.; Jepsen, C. H.; Kabisch, M.; Wilding, J. P. H. Efficacy and safety of semaglutide compared with dulaglutide versus sitagliptin after 2 years in metformin-treated patients with type 2 diabetes (AWARD-5): a randomized, phase III study. Diabetes, Obes. Metab. 2015, 17, 849–858.

(18) Wang, T.; Gou, Z.; Wang, F.; Ma, M.; Zhai, S. D. Comparison of GLP-1 analogues versus sitagliptin in the management of type 2 diabetes: systematic review and meta-analysis of head-to-head studies. PLoS One 2014, 9, No. e103798.

(19) Kanoski, S. E.; Fortin, S. M.; Arnold, M.; Grill, H. J.; Hayes, M. R. Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exenatide-4. Endocrinology 2011, 152, 3103–3112.
(38) Henry, K. E.; Elfers, C. T.; Burke, R. M.; Chepurny, O. G.; Holz, G. G.; Blevins, J. E.; Roth, C. L.; Doyle, R. P. Vitamin B12 conjugation of peptide-YY(3-36) decreases food intake compared to native peptide-YY(3-36) upon subcutaneous administration in male rats. Endocrinology 2015, 156, 1739–1749.

(39) Borner, T.; Workinger, J. L.; Tinsley, I. C.; Fortin, S. M.; Stein, L. M.; Chepurny, O. G.; Holz, G. G.; Wierzeba, A. J.; Gryko, D.; Nexo, E.; Shaulson, E. D.; Bameza, A.; Rodriguez Da Silva, V. A.; De Jonghe, B. C.; Hayes, M. R.; Doyle, R. P. Correlation of a GLP-1 receptor agonist for glycemic control without emesis. Cell Rep. 2020, 31, 107768.

(40) Workinger, J. L.; Kuda-Wedagedara, A. N. W.; Julin, M. M.; White, J. M.; Nexo, E.; Viola, N. T.; Doyle, R. P. Systemically administered plant recombiant holino-intrinsic factor targets the liver and is not affected by endogenous B12 levels. Sci. Rep. 2019, 9, 12269.

(41) De Jonghe, B. C.; Lawler, M. P.; Horn, C. C.; Tordoff, M. G. Pica as an adaptive response: kaolin consumption helps rats recover from chemotherapy-induced illness. Physiol. Behav. 2009, 97, 87–90.

(42) Ueno, S.; Matsuaki, S.; Saito, H. Suncus murinus: a new experimental model in emesis research. Life Sci. 1987, 41, 513–518.

(43) Chan, S. W.; Lin, G.; Yew, D. T. W.; Yeung, C. K.; Rudd, J. A. Separation of emetic and anorexic responses of exendin-4, a GLP-1 receptor agonist in Suncus murinus (house musk shrew). Neuropharmacology 2013, 70, 141–147.

(44) Chan, S. W.; Lin, G.; Yew, D. T. W.; Rudd, J. A. A physiological role of glucagon-like peptide-1 receptors in the central nervous system of Suncus murinus (house musk shrew). Eur. J. Pharmacol. 2011, 668, 340–346.

(45) Anantharam, P.; Whitley, E. M.; Mahama, B.; Kim, D. S.; Sarkar, S.; Santana, C.; Chan, A.; Kanthasamy, A. G.; Kanthasamy, A.; Boss, G. R.; Rumbelha, W. Cobinamide is effective for treatment of hydrogen sulhide-induced neurological sequelae in a mouse model. Anna. N.Y. Acad. Sci. 2017, 1408, 61–78.

(46) Hendry-Hofer, T. B.; Ng, P. C.; McGrath, A. M.; Mukai, D.; Brenner, M.; Mahon, S.; Maddy, J. K.; Boss, G. R.; Bebarta, V. S. Intramuscular aminotetrazole cobinamide as a treatment for inhaled hydrogen sulfide poisoning in a large swine model. Ann. N.Y. Acad. Sci. 2020, 1479, 159–167.

(47) Ma, J.; Dasgupta, P. K.; Zelder, F. H.; Boss, G. R. Cobinamide chemistries for photometric cyanide determination. A merging zone liquid core waveguide cyanide analyzer using cyanoaquacobinamide. Anal. Chim. Acta 2012, 736, 78–84.

(48) Männel-Croise, C.; Probst, B.; Zelder, F. A straightforward method for the colorimetric detection of endogenous biological cyanide. Anal. Chem. 2009, 81, 9493–9498.

(49) Brenner, M.; Mahon, S. B.; Lee, J.; Kim, J.; Mukai, D.; Goodman, S.; Kreuter, K. A.; Ahdout, R.; Mohammad, O.; Sharma, V. S.; Blackledge, W.; Boss, G. R. Comparison of cobinamide to vitamin B12 analog cobinamide is an effective antidote for oral cyanide poisoning. J. Med. Toxicol. 2016, 12, 370–379.

(50) Greenawald, L. A.; Snyder, J. L.; Fry, N. L.; Sailor, M. J.; Boss, G. R.; Finklea, H. O.; Bell, S. Development of a cobinamide-based end-of-service-life indicator for detection of hydrogen cyanide gas. Sens. Actuators B Chem. 2015, 221, 379–385.

(51) Wierzb, A. J.; Maximiou, K.; Wincenciuk, A.; Równicki, M.; Wojciechowska, M.; Nexo, E.; Trylska, J.; Gryko, D. Does a conjugation site affect transport of vitamin B12-peptide nucleic acid conjugates into bacterial cells. Chem. – Eur. J. 2018, 24, 18772–18778.

(52) Evers, A.; Haack, T.; Lorenz, M.; Bossart, M.; Elvert, R.; Henkel, B.; Stengelin, S.; Kurz, M.; Glien, M.; Dudda, A.; Lorenz, K.; Kaderiet, D.; Wagner, M. Design of novel exendin-based dual glucagon-like peptide 1 (GLP-1) glucagon receptor agonists. J. Med. Chem. 2017, 60, 4293–4303.

(53) Dai, S.; Liu, S.; Li; C.; Zhou, Z.; Wu, Z. Site-selective modification of exendin 4 with variable molecular weight dextrans by oxime-ligation chemistry for improving type 2 diabetic treatment. Carbohydr. Polym. 2020, 249, 116864.

(54) Lee, J. G.; Ryu, J. H.; Kim, S. M.; Park, M. Y.; Kim, S. H.; Shin, Y. G.; Sohn, J. W.; Kim, H. H.; Park, Z. Y.; Seong, J. Y.; Kim, J. I. Replacement of the C-terminal Trp-cage of exendin-4 with a fatty acid improves therapeutic utility. Biochim. Pharmacol. 2018, 151, 59–68.

(55) Ő Proinsias, K.; Karczewski, M.; Zieleniewska, A.; Gryko, D. Micro-wave-assisted cobinamide synthesis. J. Org. Chem. 2014, 79, 7752–7757.

(56) Zhou, K.; Zelder, F. Identification of diastereomeric cyanoaque cobinamides with a backbone-modified vitamin B12 derivative and with 2H NMR spectroscopy. Eur. J. Inorg. Chem. 2011, 53–57.

(57) Berg, R.; Straub, B. F. Advancements in the mechanistic understanding of the copper-catalyzed azide-alkyne cycloaddition. Beilstein J. Org. Chem. 2013, 9, 2715–2750.

(58) Tribaduiza, E. C.; Chen, C.; Beinborn, M. A Small molecule ligand of the glucagon-like peptide 1 receptor targets its amino-terminal hormone binding domain. J. Biol. Chem. 2001, 276, 37787–37793.

(59) Klarenbeek, J.; Goedhart, J.; van Batenburg, A.; Groenewald, D.; Jalink, K. Fourth generation EPAC-based FRET sensors for cAMP feature exceptional brightness, photostability and dynamic range: characterization of dedicated sensors for FLIM, for ratiometry and with high affinity. PLoS One 2015, 10, e0122513.

(60) Khod ihr, L.; McCowen, K. C.; Blackburn, G. L. Obesity and its comorbid conditions. Clin. Cornerstone. 1999, 2, 17–31.

(61) Mroz, P. A.; Perez-Tilve, D.; Mayer, J. P.; DiMarchi, R. D. Sterechemical inversion as a route to improved biophysical properties of therapeutic peptides exemplified by glucagon. Commun. Chem. 2019, 2, 2.

(62) Chabenne, J. R.; Mroz, P. A.; Mayer, J. P.; DiMarchi, R. D. Structural refinement of glucagon for therapeutic use. J. Med. Chem. 2020, 63, 3447–3460.

(63) Chabenne, J. R.; DiMarchi, M. A.; Gelfanov, V. M.; DiMarchi, R. D. Optimization of the native glucagon sequence for medicinal purposes. J. Diabetes Sci. Technol. 2010, 4, 1322–1331.

(64) Chen, W.; Lisowski, M.; Khalil, G.; Sweet, I. R.; Shen, A. Q. Microencapsulated 3-Dimensional sensor for the measurement of oxygen in single isolated pancreatic islets. PLoS One 2012, 7, No. e33070.

(65) Milikin, B. T.; Chepurny, O. G.; Doyle, R. P.; Holz, G. G. FRET reporter assays for cAMP and calcium in a 96-well format using genetically encoded biosensors expressed in living cells. Bio-Protoc. 2020, 10, No. e3641.

(66) Sweet, I. R.; Cook, D. L.; DeJulio, E.; Wallen, A. R.; Khalil, G.; Chabenne, J. R.; DiMarchi, M. A. Replacement of the C-terminal Trp-cage of exendin-4 with a fatty acid improves therapeutic utility. Biochim. Biophys. Acta. 2013, 1831, 778–787.

(67) Matsumoto, S.; Shibata, S.; Kirchhof, N.; Hiraoka, K.; Sageshima, J.; Zhang, X. W.; Gilmore, T.; Ansite, J.; Zhang, H. J.; Sutherland, D. E. R.; Hering, B. J. Immediate reversal of diabetes using genetically encoded biosensors expressed in living cells. BioProtoc. 2020, 10, No. e3641.
primates following intraportal transplantation of porcine islets purified on a new histidine-lactoioniate-iodixanol gradient. Transplantation. 1999, 67, S220.

(72) Jung, S.-R.; Reed, B. J.; Sweet, I. R. A highly energetic process couples calcium influx through L-type calcium channels to insulin secretion in pancreatic beta-cells. Am. J. Physiol. 2009, 297, E717–E727.