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Novel Glucagon Receptor Antagonists with Improved Selectivity over the Glucose-Dependent Insulinotropic Polypeptide Receptor

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Optimization of a new series of small molecule human glucagon receptor (hGluR) antagonists is described. In the process of optimizing glucagon receptor antagonists, we counter-screened against the closely related human gastric inhibitory polypeptide receptor (hGIPR), and through structure activity analysis, we obtained compounds with low nanomolar affinities toward the hGluR, which were selective against the hGIPR and the human glucagon-like peptide-1 receptor (hGLP-1R). In the best cases, we obtained a >50 fold selectivity for the hGluR over the hGIPR and a >1000 fold selectivity over the hGLP-1R. A potent and selective glucagon receptor antagonist was demonstrated to inhibit glucagon-induced glycogenolysis in primary rat hepatocytes as well as to lower glucagon-induced hyperglycemia in Sprague−Dawley rats. Furthermore, the compound was shown to lower blood glucose in the ob/ob mouse after oral dosing.

Introduction

Blood glucose levels are maintained via a tightly controlled balance between insulin and a number of counter-regulatory hormones. Glucagon is a 29 amino acid peptide secreted from the α-cells in response to low plasma levels of glucose. Glucagon is a timely and potent activator of hepatic glucose production, whereas the role of insulin in the liver is to inhibit hepatic glucose production and induce hepatic glucose utilization and storage. Inappropriately, high glucose production from the liver is believed to be an important contributor to the development of hyperglycemia in type 2 diabetes. This is presumably a result of hepatic insulin resistance in combination with lack of suppression of glucagon secretion from the α-cells in response to elevated glucose. In type 2 diabetes, the glucagon level is elevated relative to the blood glucose and insulin levels. Therefore, new therapeutic agents, capable of blocking the effect of glucagon on hepatic glucose production, has attracted attention for treatment of hyperglycemia in type 2 diabetes.[1–6]

The receptor for the gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors belong to the same family of G-protein coupled seven-transmembrane domain receptors as the glucagon receptor and share structural similarities. The primary role of GIP in healthy subjects is to stimulate glucose-dependent insulin secretion.[7] In addition, GIP has been shown to have an inhibitory effect on β-cell apoptosis[8] and has been suggested to play a role in lipid metabolism.[9] GIP receptor knockout mice have been demonstrated to be protected from high-fat diet-induced obesity.[10]

In humans with type 2 diabetes, the insulinotropic effect of GIP is reduced,[11] but bolus administration of GIP to patients with type 2 diabetes has been shown to stimulate insulin secretion.[12] In a recent study, a long-acting GIP analogue has been shown to decrease nonfasting blood glucose, improve glucose tolerance, and enhance insulin secretion in ob/ob mice.[13] The role of GIP in type 2 diabetes is however still an issue of debate.[14] Nevertheless, GIP does stimulate first phase insulin secretion in type 2 diabetes and thus it would most likely not be a desirable property of an antidiabetic agent to inhibit the GIP receptor.

GLP-1 is an important incretin and is the basis for two new emerging drug classes for the treatment of type-2 diabetes, the GLP-1 analogues and the dipeptidyl peptidase IV (DPP-IV) inhibitors. Thus, glucagon receptor antagonists must be selective against the GLP-1 receptor.

A novel type of orally bioavailable small-molecule glucagon receptor antagonist, based on β-alanine urea derivatives, was recently published.[15] We have found that this series of compounds was capable of binding to and antagonize the GIP receptor function. Other series reported by Merck have demonstrated selectivity of antagonism of the hGluR over the hGIPR.[16,17]

We have addressed this selectivity issue of antagonism of the hGluR versus the hGIPR and have optimized the previously published series of small-molecule hGluR antagonists containing a β-alanine motif to obtain improved binding selectivity over the hGLP-1R and particularly the hGIPR. This was accomplished by changing the β-alanine motif to an isoserine (α-hydroxy-β-alanine) motif.

Chemistry

In general, the hGluR antagonists were synthesized as outlined in Scheme 1. The 4-formyl benzoic acid methyl ester was reacted with various amines in a reductive amination to give the secondary amine intermediates **1**. For the reaction with 4-*tert*-butyl-cyclohexyl amine the pure trans-4-[(4-*tert*-butyl-cyclohexylaminomethyl]benzoic acid methyl ester of which was obtained as described previously.[15] The secondary amines **1** were reacted with various phenyl isocyanates and subsequently treated with NaOH to give the benzoic acid ureas derivatives

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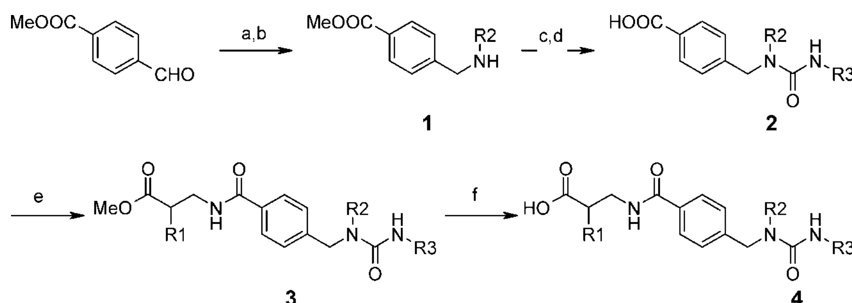
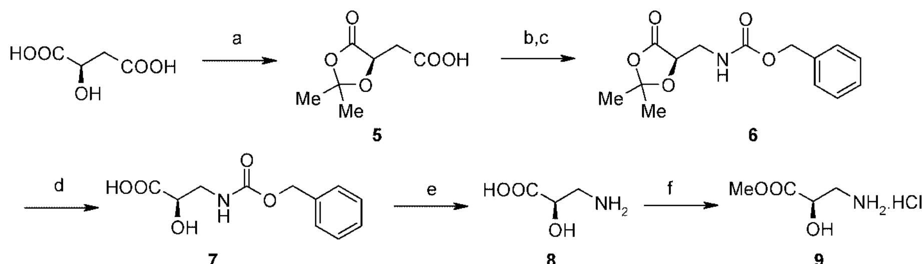
Scheme 1. General Reaction Scheme for Synthesis of Glucagon Antagonists[a]



[a] Reagent and conditions: (a) R2-NH_2, methanol, 60 min, reflux; (b) $NaBH_3CN$, acetic acid, methanol, 60 min, rt; (c) R3-NCO, dichloromethane, 16 h, rt; (d) 4 N NaOH in ethanol (1:3), 16 h, rt; (e) EDAC/HOBt, DMF, 30 min, then $MeOOCCH(R1)CH_2NH_2 \cdot HCl$, diisopropylethylamine, 16 h, rt; (f) 2 N NaOH in ethanol (1:8), 1 h, rt.

Scheme 2. Synthesis of (*R*)-Isoserine[a]



[a] Reagent and conditions: (a) 2,2-dimethoxypropane, toluene, reflux, 2 h (81%); (b) DPPA, triethylamine, toluene, 85 °C; (c) benzyl alcohol, toluene, 85 °C, 17 h, (40%); (d) 1 N HCl in acetonitrile (1:1), 3 h, 40°C (87%); (e) H_2/Pd−C, 17 h, rt, (92%); (f) thionylchloride, methanol, −30°C, (99%).

2, which could be transformed into a range of test compounds using standard chemistries. Initially the racemic mixture of commercially available (*R*,*S*)-isoserine protected as ethyl ester was coupled to the benzoic acid derivatives **2** to give **3**, which was either hydrolyzed to the test compound **4** and tested as a racemic mixture or **3** was separated by chiral HPLC into the pure (*R*) and (*S*) forms of the ethyl ester were converted into the carboxylic acids by saponification to give the (*R*) and (*S*) forms of the hGluR antagonists, respectively (Scheme 1).

For larger scale preparation, a suitable synthesis for the enantiomers of isoserine was necessary. Enantioselective synthesis of (*S*)-isoserine as well as enzymatic methods have been described earlier[18,19] and also the conversion of L-malic acid into (*S*)-isoserine have previously been described.[20] We modified a procedure from Burger et al.[20] for the synthesis the both enantiomers of isoserine from commercially available malic acid. Because both enantiomers of malic acid are available with an eantiomeric purity above 95%, the method could be used for the synthesis of both (*R*)- and (*S*)-isoserine. The conversion of D-malic acid to (*R*)-isoserine and the corresponding methyl ester hydrochloride is outlined in Scheme 2.

D-Malic acid was converted into 2-[(4*R*)-2,2-dimethyl-5-oxo-1,3-dioxolana-4-yl]acetic acid **5** by refluxing with an excess of 2,2-dimethoxypropane in toluene and isolated in a reasonably pure form by simple evaporation. The Curtius rearrangement of **5** was carried out with diphenylphosphoryl azide (DPPA) and triethylamine in toluene. The intermediate isocyanate was not isolated but trapped with benzyl alcohol to give the benzyloxycarbonylamino intermediate **6**. Crude **6** was treated with 1 N HCl in acetonitrile to give crystalline benzyloxycarbonyl protected (*R*)-isoserine **7**, which was purified by recrystallization from toluene. The pure (*R*)-isoserine **8** was obtained by removal of the benzyloxycarbonyl with hydrogenolysis (10% Pd/C, H_2, ethanol). The pure (*R*)-isoserine was protected as the methyl ester using $SOCl_2$ in methanol.

Biological Methods

The biological methods are described in detail in the Experimental Section.

Receptor Assays. Receptor assays were carried out using plasma membranes purified from BHK[a] cells expressing either the cloned human glucagon GIP or GLP-1 receptor. The hGluR binding assay was done as previously published.[21] Binding assays for the cloned human GLP-1 and GIP receptors were done similarly using BHK cell lines expressing the cloned human GIP and GLP-1 receptor, and the assay for the isolated rat glucagon receptor followed same protocol using rat liver membrane preparations.

Primary Hepatocyte Assays. As a model system for hepatic glucose production, rat hepatocytes were isolated and cultured as described earlier.[22] The hepatocytes were loaded with glycogen, and the effect of the glucagon antagonists on glucagon-induced glycogenolysis was examined.

Pharmacokinetics. The compounds were dosed iv and po to fasted male Sprague−Dawley rats weighing ∼200 g. The compounds were dissolved in 5% ethanol, 20% propyleneglycol, 10% hydroxypropyl-β-cyclodextrin, and phosphate buffer pH 7.5−8.0. Postdose ethylenediaminetetraacetic acid (EDTA) blood samples were collected by heart puncture under carbon dioxide anesthesia at time 5, 15, 30, 60, 120, 240, and 360 min for the iv dosing and at time 15, 30, 60, 90, 120, 240, and 360 min for the po dosing. Plasma samples were prepared by centrifugation (3000*g*, 10 min) and stored at 20 °C pending analysis. Plasma samples were analyzed by high turbulence liquid chromatography (HTLC) combined with tandem mass spectrometry (MS/MS). The PK analysis was performed using noncompartmental methods.

[a] Abbreviations: BHK, baby-hamster kidney; iv, intravenous; po, oral; sc, subcutaneous; PK, pharmacokinetics; $t_{1/2}$, half-life; Fpo, oral bioavailability; SAR, structure activity relationship.

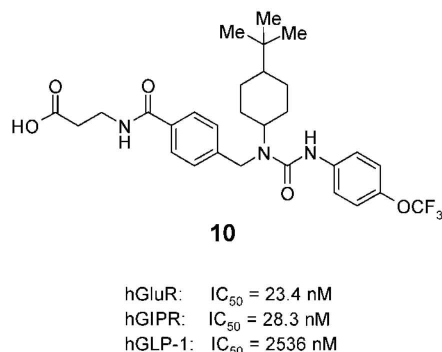


Figure 1

Glucagon Challenged Rat. To demonstrate acute in vivo efficacy, the effect of the compounds on glucagon-stimulated hyperglycemia was studied in rats. Nonfasted male Sprague–Dawley rats (200 g) were maintained in the anaesthetized state during the test by sc administration of a 1:1 mixture of Hypnorm (fentanyl, 0.05 mg/mL, and fluanizone, 2.5 mg/mL, Janssen Pharma Ltd., Copenhagen, Denmark) and Dormicum (Midazolam, 1.25 mg/mL, Roche, Basel, Switzerland). Test compounds were administered intravenously. A catheter was inserted in a jugular vein for administration of compounds. Approximately 60 min after initiation of anesthesia, test compounds (0, 3, and 10 mg/kg) and glucagon (3 μg/kg) were administered with 5 min interval, respectively. Samples for determination of blood glucose concentrations were taken from the tail tip 25 and 5 min prior to administration of the compound to represent average basal values and again 10 min after administration of glucagon (time for peak response of glucagon). The results were expressed as δ values calculated as the value obtained 10 min after glucagon administration minus the average of the two basal values. This method was adapted from the method previously published[21]

Acute Effect on Blood Glucose in ob/ob Mice. Male ob/ob mice (11–13 weeks of age) used for the studies were selected from a larger group of mice having the highest blood glucose (BG) levels. Selected mice were randomized into treatment groups having matching BG levels. Compounds (0 and 100 mg/kg) were administered orally by gavage in the morning, and blood samples (5 μL) for determination of BG were obtained approximately $^1/2$ h predose and at 2, 4, 6, and 24 h postdose. The mice had no access to food the first 6 h after dosing and subsequently had free access to food. Food and water intake were measured.

Results and Discussion

We have recently described a new type of orally available glucagon receptor antagonists based on β-alanine derivatives.[15] The β-alanine hGluR antagonist **10** (Figure 1) was selective over the hGLP-1R but displayed equally high binding affinity for the hGIPR as for the hGluR.

In our search for selective hGluR antagonists, we found that the β-alanine moiety was very sensitive toward receptor affinity as reported earlier[15] as well as selectivity. The β-alanine urea derivative **11** could be made more selective when the β-alanine was replaced with (*R*,*S*)-isoserine **12** (Table 1). We reported previously that the cyclehexenyl-3,5 dichlorophenyl urea β-alanine derivative **13** lowered blood glucose in a murine type 2 diabetes model.[15] Estimated from the IC_{50} values **13** has a 5-fold greater affinity for the hGluR (IC_{50} = 12 nM) than for the hGIPR (IC_{50} = 64 nM). The racemic (*R*,*S*)-isoserine **14** displayed a similar affinity toward the hGluR (IC_{50} = 14 nM) but showed a reduced affinity toward the hGIPR (IC_{50} = 701 nM) corresponding to a 54-fold selectivity. It was concluded that by introducing a hydroxy group next to the carboxylic acid moiety in the β-alanine of our hGluR antagonist would be neutral toward the hGluR receptor binding affinity while it would impair the binding to the hGIPR. Separation of **14** into the two enantiomers revealed an affinity difference of the two enatiomers and subsequent independent synthesis of the *R*-isomer and *S*-isomer showed that the *R*-isomer had the highest IC_{50} (**15** 3.9 nM and **16** 51 nM). The *R*-isomer also had improved selectivity, IC_{50} for the GIP receptor were 359 and 445 nM for **15** and **16**, respectively, equivalent to approximate 10-fold increased selectivity.

Methylation of the hydroxyl group **20** seriously diminished the receptor affinity toward the hGluR (IC_{50} = 693 nM). The isoserine derivatives **15** and **18** also showed low affinity for the hGLP-1R. In the case of **15**, the hGluR/hGLP-1R ratio was 4300-fold compared to the β-alanine derivative **13** (hGluR/hGLP-1-R = 400-fold). These data suggest that the introduction of the isoserine improves the selectivity toward the GLP-1 and GIP receptors.

Focused libraries were synthesized, and it was revealed that the SAR was similar to what was observed in the β-alanine series.[15] The compounds **21**–**36** in Table 2 showed that the combination of R2 being a bulky lipophilic moiety with the combination of R3 being a phenyl group with electron withdrawing or lipophilic substituents gave fairly potent glucagon receptor antagonists. The best substituents for R2 were cyclohexylphenyl and cyclohexenylpheny and the best for R3 were 3,5-di-CF_3, 3,5-dichloro, and 3,4-CF_2-O-CF_2-O. In contrast to R3 being an unsubstituted phenyl group (**27**), the potency dropped 10-fold compared to when a Cl was added in the metaposition of R3 (**28**).

In all cases, the receptor binding affinities for the hGluR were always higher than the binding affinities for the hGIPR, although in the case of **29**, the difference was only 2-fold.

The best selectivity was seen for **36**, where the hGluR/hGIPR ratio was 94-fold.

Pharmacokinetic Studies in Rats. The most potent isoserine compounds that showed hGluR selectivity above 10-fold over the hGIPR were tested for pharmacokinetic properties. The plasma half-lives and oral availability in rats were tested, and several compounds showed oral bioavailability. Compound **15** showed an oral bioavailability of >30% and a $t_{1/2}$ (iv) of 53 min and compound **22** had an acceptable profile with an oral bioavailability of >20% and $t_{1/2}$ (iv) = 72 min. In comparison the β-alanine **13** has been reported to have a F_{po} = 69% and $t_{1/2}$ = 82 min in rats,[15] and it was our general observation that the introduction of the hydroxyl group seemed to reduced the oral bioavailability as well as the plasma half-life of this series of compounds (Table 3).

Cultured Primary Hepatocyte Studies. To examine a biological end-point of glucagon receptor inhibition in a cellular system, the effects of the isoserine compound **15** and the β-alanine compound **13** on glucagon-stimulated glucose production from primary rat hepatocytes in culture were measured and compared. As can be seen in Figure 2, inhibition of glucagon-stimulated glycogenolysis was observed with both compounds with IC_{50} values of approximately 820 ± 250 nM for **13** and 200 ± 40 nM for **15**. These values are considerably higher than those obtained in the binding assays for the rat receptor (IC_{50}: ratGlucR, **13** = 36 nM, **15** = 43 nM), but this difference may be due to the fact that these compounds bind to albumin and the hepatocyte culture medium contains 0.1% HSA (human

Table 1. Glucagon/GIP Receptor Potency Dependence on Stereochemistry

Compound	R1	Config.	R2	R3	hGluR binding affinity IC_{50} (nM)	hGIPR binding affinity IC_{50} (nM)	hGLP-1R binding affinity IC_{50} (nM)
11	H	-	Me Me Me	OCF_3	36	38	10773
12	OH	*R/S*	Me Me Me	OCF_3	55	315	n.d.
13	H	-		Cl Cl	12	64	4995
14	OH	*R/S*		Cl Cl	14	701	n.d.
15	OH	*R*		Cl Cl	3.9	359	17045
16	OH	*S*		Cl Cl	51	445	9323
17	H	*trans*	Me Me Me	CF_3 CF_3	16	93	n.d.
18	OH	*R, trans*	Me Me Me	CF_3 CF_3	8	87	7731
19	OH	*S, trans*	Me Me Me	CF_3 CF_3	82	373	4879
20	OMe	*R*		Cl Cl	693	3020	21767

serum albumin), as well as the fact that primary hepatocytes are metabolic active. There was no observed effect of these compounds at concentration up to 25 μM on basal or adrenaline-stimulated glycogenolysis, indicating that the observed effect is mediated by specific inhibition of the hepatic glucagon receptor. In addition, both **15** and **13** increased lactate production, indicating an increased flux through the glycolysis. These hepatocyte studies most importantly demonstrate that the glucagon receptor antagonists have the expected biological effect.

Pharmacodynamic Studies. To examine the effects in vivo, compound **15** was tested in a glucagon-challenged rat model. The compound dose-dependently inhibited the glucagon-stimulated rise in blood glucose (Figure 3). The minimum effective iv dose for **15** was 3 mg/kg.

Acute Studies in ob/ob Mice. The leptin deficient, obese model of type 2 diabetes, the ob/ob mouse, is characterized by hyperglycemia and hyperinsulinemia. In an oral single-dose study with no access to food for 6 h, **15** reduced the observed hyperglycemia (Figure 4).

Receptor Selectivity and GIP Antagonism. To directly test the effect of these compounds on GIP stimulated insulin secretion, studies using the isolated perfused rat pancreas were carried out.[23] The results presented in Figure 5 demonstrate that

Table 2. SAR Table of the Isoserine Glucagon Antagonists

Compound	Config.	R2	R3	hGluR binding affinity IC_{50} (nM)	hGIPR binding affinity IC_{50} (nM)	Compound	Config.	R2	R3	hGluR binding affinity IC_{50} (nM)	hGIPR binding affinity IC_{50} (nM)
21	*trans*	Me, Me, Me	Me, Me	27	220	**29**	-		SCF_3	6	12
22	-		Br	8	216	**30**	-		CF_3, CN	9	198
23	-		CF_3, Br	7	380	**31**	-		Me, Me, Me	18	74
24	-		CF_3, OMe	14	279	**32**	-		Cl, Cl	4	93
25	-		CF_3, OMe	9	145	**33**	-		F, F, O, O, F, F	6	83
26	*trans*	Me, Me, Me	CF_3, Me	10	98	**34**	-		CF_3, F	13	277
27	-			166	1392	**35**	-		SMe	9	411
28	-		Cl	14	406	**36**	-		SO_2CF_3, OCF_3	5	497

10, which is a potent GluR antagonist as well as potent GIPR antagonist (IC_{50}: ratGlucR = 23 nM, ratGIP = 42 nM, ratGlucR/ratGIPR = 1.7-fold), was able to inhibit GIP stimulated insulin secretion from the isolated perfused rat pancreas (Figure 5a). However in comparison **15** (IC_{50}: ratGlucR = 43 nM, ratGIP = 501, ratGlucR/ratGIPR = 12-fold) and **13** (IC_{50}: ratGlucR = 36 nM, ratGIPR = 216, ratglucR/ratGIPR = 6-fold), which are just as potent glucagon antagonists but with reduced affinity for the GIP receptor, did not inhibit GIP stimulated insulin secretion, even at a 10-fold higher concentration than used for **10** (Figure 5).

Conclusion

GIP is an incretin hormone that stimulates first-phase insulin secretion. GIP receptor inhibition is not a desirable extra effect for a glucagon receptor antagonist. We have previously reported a β-alanine series of orally bioavailable glucagon receptor antagonist,[15] and this series has now been optimized to obtain a better selectivity especially over the GIP receptor with the same high affinity for the glucagon receptor. **15** was orally bioavailable, and furthermore, we found that substituting the β-alanine with an isoserine on the compounds from a previous series[15] in many cases lead to decreased GIP receptor affinity. Glucagon receptor antagonists containing isoserines have been

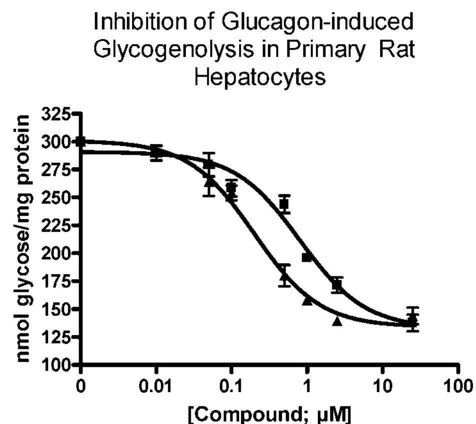


Figure 2. Inhibition of glucagon-stimulated glycogenolysis in cultured rat hepatocytes. For glycogenolysis experiments, glycogen-filled hepatocytes were stimulated with 0.5 nM glucagon and increasing concentrations of **15** (▲) or **13** (■) as indicated, and the glucose production was measured in the medium after 60 min of stimulation.

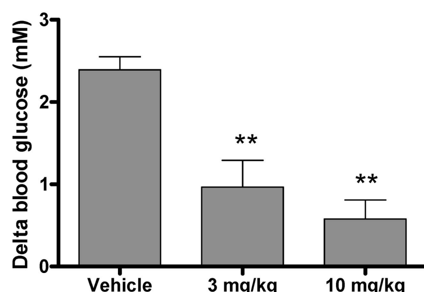


Figure 3. Effect of **15** in glucagon challenged rats. Anesthetized animals were given an intravenous dose of the compounds as shown 5 min prior to a 3 μg/kg glucagon load. The blood glucose values for each individual rat are given. ** = $p \leq 0.001$ *** = $p \leq 0.0005$.

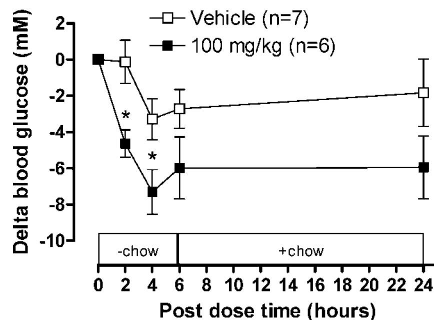


Figure 4. The acute effects of a single oral dose of **15** in ob/ob mice. These animals had no access to food during the first 6 h following dosing with 100 mg/kg. Blood glucose is presented as the change from baseline.

reported previously;[24,25] however, no details regarding SAR or selectivity toward hGIPR have been reported previously. We found in our series the (*R*)-form was the more potent of the two enantiomers. The SAR of the (*R*)-isoserine ureas was similar to what was observed with the previous reported β-alanine urea series where optimal binding was obtained with electron withdrawing groups on the distal aromatic group in combination with cyclohexyl or cyclohexenyl on the proximal aromatic group. Up to 100-fold selectivity for glucagon receptor over the GIP receptor was obtained in one case (**38**), and in all cases, the introduction of a hydroxyl group on the β-alanine moiety gave compounds that showed selectivity for the glucagon receptor. The most promising compound, **15**, which was a potent glucagon receptor antagonist with a 54-fold selectivity over the GIP receptor, inhibited glucagon-induced glycogenolysis primary rat hepatocytes and inhibited glucagon-induced hyperglycemia in Sprague−Dawley rats; in addition, **15** acutely decreased hyperglycemia in ob/ob mice when dosed orally. The physiological advantage of selective glucagon receptor antagonism was demonstrated by the observation that compound **15** is able to inhibit glucagon stimulated rise in blood glucose (Figure 3) but does not inhibit GIP stimulated insulin secretion from the rat pancreas (Figure 5). An approximately 10-fold reduction in GIP receptor affinity resulted in minimal effect on insulin secretion.

Experimental Section

General Synthesis. ^{1}H NMR spectra were recorded in deuterated solvents at 200, 300, or 400 MHz (DRX 200, DRX 300, and AMX2 400 from Bruker Instruments, respectively). Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane (δ = 0 ppm). Elemental analyses were performed by the microanalytical laboratories at Novo Nordisk A/S, Denmark, and University of Southern Denmark. Column chromatography was performed on silica gel 60 (40−63 μm). Melting points were determined in open capillary tubes on a Büchi 535 apparatus and are uncorrected. Chemicals and solvents used were commercially available and were used without further purification. Yields refer to pure materials and are not optimized. The preparation methods are illustrated by single representative experimental procedures.

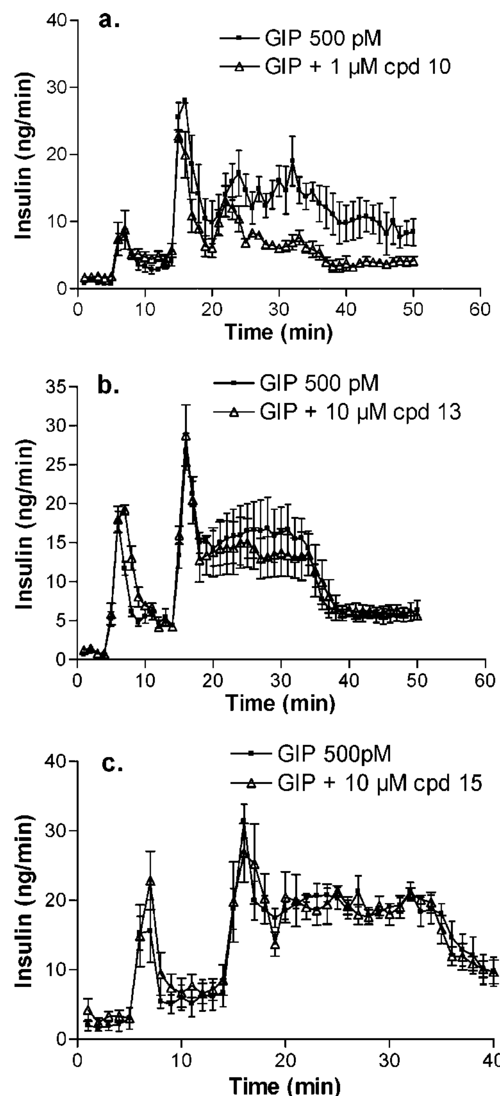


Figure 5. Isolated rat pancreas were perfused with 80 mg/dL glucose during the period 1−4 min and switched to 160 mg/dL glucose during the period 5−50 min. GIP (500pM) was infused during 15−34 min to stimulate insulin secretion, and compound **10** (a), **13** (b), and **15** (c) were infused during the period 5−50 min (n = 4 for each group).

Table 3. PK Properties of Compound **15**, **18**, **22**, **23**, **24**, **25**, **35**, and **36** in Rats[a]

compound	$t_{1/2}$ (min, iv)	F_{po} (%)
15	53	32
18	41	11
22	72	21
23	46	14
24	53	7
35	43	8
36	55	10

[a] Rats were dosed 4 mg/kg of compound po and 2mg/kg iv.

(*RS*)-Isoserine Ethyl Ester Hydrochloride. Dry ethanol (40 mL) was cooled on an ice bath, and thionyl chloride (4 mL) was added dropwise, maintaining the temperature below 5 °C. To this cold

solution was added (*RS*)-isoserine (2.5 g, 23.79 mmol), and stirring was continued until a homogeneous solution was obtained. The ice bath was removed and stirring was continued for 17 h at room temperature. The solution was concentrated in vacuo to afford 4.0 g (100%) of (*RS*)-isoserine ethyl ester hydrochloride as an oil. ^{1}H NMR (DMSO-d_6): δ 1.22 (t, 3H), 3.00 (dm, 2H), 4.15 (q, 2H), 4.40 (dd, 1H), 6.30 (br s, 1H), 8.32 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 14.7 (q), 42.4 (t), 61.7 (t), 67.7 (d), 171 (s).

(*R*)-Isoserine Hydrochloride. To a suspension of D-(+)-malic acid (15.0 g, 0.1119 mol) in dry toluene (150 mL) was added 2,2-dimethoxypropane (50 mL, 0.392 mol). The mixture was refluxed at 100 °C for 2 h and evaporated in vacuo. The residue was dissolved in diethyl ether (150 mL) and subjected to flash column chromatography using diethyl ether as eluent (200 mL). The pure fractions were pooled and evaporated in vacuo, and the residue was stirred in *n*-hexane. The precipitate was collected, washed with *n*-hexane, and dried to afford 15.7 g (81%) of (*R*)-(2,2-dimethyl-5-oxo-[1,3]dioxolan-4-yl)acetic acid (**5**) as a solid. ^{1}H NMR (acetone-d_6): δ 1.57 (ds, 6H), 2.85 (m, 2H), 4.80 (dd, 1H), 11.0 (br s, 1H). ^{13}C NMR (acetone-d_6): δ 25.9 (q), 26.8 (q), 36.2 (t), 71.5 (d), 111.2 (s), 170.7 (s), 172.7 (s). Anal. calcd for $C_7H_{10}O_5$: C, 48.28; H, 5.79. Found: C, 48.31; H. 6.09. mp 113−114 °C.

A mixture of (*R*)-(2,2-dimethyl-5-oxo-[1,3]dioxolan-4-yl)acetic acid as a solid (**5**) (10.0 g, 57.41 mmol), triethylamine (10 mL, 68.89 mmol), and diphenylphosphoryl azide (14 mL, 63.15 mmol) in dry toluene (100 mL) was heated and stirred at 85 °C. When the gas evolution had ceased, stirring was continued for an additional hour. Dry benzyl alcohol (6.3 mL, 63.15 mmol) was added, and heating was continued for 17 h. After evaporation in vacuo, the residue was partitioned between dichloromethane, water, and brine. The aqueous phase was further extracted twice with dichloromethane. The combined organic phases were washed twice with saturated sodium hydrogen carbonate. After drying (magnesium sulfate), filtration, and concentration in vacuo of the organic phase, the residue was subjected to flash column chromatography with dichloromethane as eluent. This afforded 6.4 g (40%) of (*R*)-(2,2-dimethyl-5-oxo-[1,3]dioxolan-4-ylmethyl)carbamic acid benzyl ester (**6**) as an oil. ^{1}H NMR (Acetone-d_6): δ 1.56 (s, 6H), 3.61 (m, 2H), 4.64 (dd, 1H), 5.08 (dd, 2H), 6.51 (br s, 1H), 7.29−7.38 (m, 5H).

To a solution of (*R*)-(2,2-dimethyl-5-oxo-[1,3]dioxolan-4-yl-methyl)carbamic acid benzyl ester (**6**) (6.0 g, 25.08 mmol) in acetonitrile (100 mL) was added hydrochloric acid (1 N, 100 mL). The mixture was stirred for 3 h at 40 °C and concentrated in vacuo to half the original volume. The solid was collected by filtration and washed with water. The crude product was stirred for 5 min in acetone (100 mL) and filtered. Toluene was added to the clear and colorless filtrate, and the whole was concentrated in vacuo until a precipitate was obtained. The precipitate was collected by filtration and dried to afford 4.45 g (87%) of (*R*)-3-benzyloxycarbonylamino-2-hydroxypropionic acid (**7**). ^{1}H NMR (acetone-d_6): δ 3.45 (ddd, 1H), 3.58 (ddd, 1H), 4.29 (dd, 1H), 5.08 (s, 2H), 6.41 (br s, 1H), 7.29−7.38 (m, 5H). ^{13}C NMR (acetone-d_6): δ 45.1 (t), 66.4 (t), 70.4 (d), 128.3 (d), 128.9 (d), 138.0 (d), 157.2 (s), 173.8 (s). Anal. calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.85. Found: C, 55.35; H, 5.72; N, 5.82. mp 131−132 °C.

(*R*)-3-Benzyloxycarbonylamino-2-hydroxypropionic acid (**7**) (4.4 g, 18.39 mmol) was dissolved in absolute ethanol (150 mL). Under a nitrogen atmosphere palladium on activated carbon (10%, 0.5 g) was added, and the mixture was hydrogenated at 1 atm for 17 h. The catalyst was filtered off and washed with water. The combined filtrate and washings were concentrated to about 20 mL by evaporation in vacuo. A precipitate was obtained by dropwise addition of methanol (100 mL). The precipitate was filtered off, washed with methanol, and dried to afford 1.78 g (92%) of (*R*)-isoserine (**8**) as a solid. ^{1}H NMR (D_2O): δ 3.07 (dd, 1H), 3.30 (dd, 1H), 4.19 (dd, 1H). ^{13}C NMR (D_2O): δ 43.0 (t), 68.9 (d), 177.5 (s). Anal. calcd for $C_3H_7NO_3$: C, 34.29; H, 6.71; N 13.33. Found: C, 34.35; H, 6.83; N, 13.19. mp 200−201 °C. $[\alpha]_D = +33.05$ (*c* = 1, H_2O at 20 °C)

(*R*)-Isoserine Ethyl Ester Hydrochloride. The compound was prepared in analogy with the method outlined above for (*RS*)-isoserine ethyl ester hydrochloride.

(*R*)-Isoserine Methyl Ester Hydrochloride. To methanol (2000 mL) thionyl chloride (209 mL) was added dropwise while the temperature was maintained below −10 °C. After the addition of the thionyl chloride, the mixture was stirred while the temperature was kept below −30 °C. To this cold solution was added (*R*)-isoserine (120 g, 1.14 mol) while temperature was kept below −30 °C. When all the (*R*)-isoserine had dissolved, the cooling was removed and the mixture was stirred overnight at room temperature. The solvent was removed in vacuo followed by coevaporation with toluene. The remaining crystals were washed with toluene (750 mL) for 30 min, filtered, and washed with *n*-hexane (300 mL). The crystals dried in vacuo to yield 176 g (99%) of (*R*)-isoserine methyl ester hydrochloride. Anal. calcd for $C_4H_9NO_3$: C, 30.88; H, 6.48; N, 9.00. Found: C, 30.98; H, 6.60; N, 8.94. mp 113−114 °C. $[\alpha]_D = +20.75$ (*c* = 1, H_2O at 20 °C).

(*S*)-Isoserine Ethyl Ester Hydrochloride. The compound was prepared in analogy from (*S*)-isoserine, which was prepared from L-(−)-malic acid similar to that described above.

Test compounds were in general prepare as illustrated with the following representative example.

(*R*)-3-{4-[1-(4-Cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)ureidomethyl]benzoylamino}-2-hydroxypropionic acid (25). 4-Formylbenzoic acid methyl ester (6.65 g, 40.5 mmol) was dissolved in hot methanol (175 mL). To this mixture, 4-cyclohexylaniline (7.1 g, 40.5 mmol) was added. To the resulting suspension, more methanol (75 mL) was added and the mixture was heated at reflux for 1 h. After cooling to 0 °C, the mixture was filtered and the solid was washed with ice-cold methanol and dried in vacuo at 40 °C for 16 h to afford 10.95 g of 4-[(4-cyclohexylphenylimino)methyl]benzoic acid methyl ester. This compound (10.93 g, 34 mmol) was suspended in methanol (200 mL) and glacial acetic acid (27 mL) was added followed by sodium cyano borohydride (1.9 g, 30 mmol) in small portions. The mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was dissolved in DCM (200 mL) and washed with 5% aqueous sodium carbonate (5 × 80 mL), dried (magnesium sulfate), and concentrated in vacuo. The residue was added ethyl acetate (100 mL) and *n*-heptane (200 mL) and concentrated in vacuo to half the original volume. The solid was filtered, washed with *n*-heptane, and dried in vacuo at 40 °C for 16 h to afford 9.52 g (87%) of 4-((4-cyclohexylphenylamino)methyl)benzoic acid methyl ester (**1**). ^{1}H NMR (DMSO-d_6): δ 1.2−1.4 (5H, m), 1.65 (5H, m), 2.30 (1H, t), 3.84 (3H, s), 4.30 (2H, d), 6.18 (1H, t), 6.50 (2H, d), 6.87 (2H, d), 7.49 (2H, d), 7.92 (2H, d);

4-[1-(Cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)ureidomethyl]benzoic Acid (2). 5-Methoxy-3-(trifluoromethyl)-aniline (2.0 g, 10.5 mmol) was dissolved in ethyl acetate (10 mL) and dry HCl in ethyl acetate (15 mL) was added and the solvent was removed in vacuo. The solid was coevaporated with toluene (3 × 15 mL). Toluene (75 mL) and diphosgene (13 mL) were added, and the reaction mixture was refluxed under a nitrogen atmosphere for 2.5 h. Excess diphosgene was removed in vacuo, and the clear oil was coevaporated with toluene. The residue was dissolved in DCM (75 mL), and 4-((4-cyclohexylphenylamino)-methyl)benzoic acid methyl ester (2.3 g, 7.1 mmol) was added. The reaction mixture was stirred overnight at room temperature, the solvent was removed in vacuo, and the residual oil was purified by column chromatography on silica gel, eluting with a mixture of heptane and ethyl acetate (7:3) to obtain 3 g (78%) of 4-[1-(cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)ureidomethyl]benzoic acid methyl ester as an oil. ^{1}H NMR (DMSO-d_6): δ 1.22 (broad, 1H), 1.37 (broad, 4H), 1.7 (broad, 1H), 1.79 (broad, 4H), 3.77 (s, 3H), 3.83 (s, 3H), 4.98 (s, 2H), 6.81 (s, 1H), 7.18 (d, 2H), 7.23 (d, 2H), 7.42 (m, 3H), 7.51 (s, 1H), 7.90 (d, 2H), 8.53 (s, 1H), 10.01 (s, 1H).

4-[1-(Cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)-ureidomethyl]benzoic acid methyl ester (3.0 g) was dissolved in absolute ethanol (50 mL), sodium hydroxide (4 N, 15 mL) was

added, and the reaction mixture was stirred at room temperature for 16 h. The organic solvent was removed in vacuo, and additional water (50 mL) was added, pH was adjusted with hydrochloric acid (4 N) to acidic reaction, and then ethyl acetate (200 mL) was added. The organic phase was washed with water (5 × 50 mL), dried (magnesium sulfate), filtered, and evaporated in vacuo. The residue was recrystallized from acetonitrile (25 mL) to afford 1.83 g (63%) of 4-[1-(cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)-ureidomethyl]benzoic acid (**2**) as crystals. ^{1}H NMR (DMSO-d_6): δ 1.22 (m, 1H), 1.37 (m, 4H), 1.70 (m, 1H), 1.79 (m, 4H), 3.77 (s, 3H), 4.95 (s, 2H), 6.81 (s, 1H), 7.18 (d, 2H), 7.23 (d, 2H), 7.40 (d, 2H), 7.42 (s, 1H), 7.51 (s, 1H), 7.89 (d, 2H), 8.55 (s, 1H), 12.90 (s, 1H). Anal. calcd for $C_{29}H_{29}F_3N_2O_4$: C, 66.15; H, 5.55; N, 5.32. Found: C, 66.65; H, 5.70; N, 5.33. mp 148−150 °C.

4-[1-(Cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)-ureidomethyl]benzoic acid (**2**) (420 mg, 0.8 mmol) was dissolved in DMF (10 mL), and then 1-hydroxybenzotriazole (160 mg, 1.2 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethyl-carbodiimide hydrochloride (230 mg, 1.2 mmol) were added. The reaction mixture was allowed to stand for 30 min, then (*R*)-isoserine ethyl ester (260 mg, 1.2 mmol) and diisopropylethylamine (210 μL, 1.2 mmol) dissolved in DMF (5 mL) were added and the reaction mixture was stirred at room temperature for 16 h. Water (50 mL) and ethyl acetate (100 mL) were added and the organic phase was washed with water (5 × 50 mL), dried (magnesium sulfate), filtered, and evaporated in vacuo. The residue was purified by column chromatography on silica gel, eluting with a mixture of heptane and ethyl acetate (1:3) to afford 510 mg (99%) of (*R*)-3-{4-[1-(4-cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl)ureidomethyl]benzoylamino}-2-hydroxypropionic acid ethyl ester (**3**) as an amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.12 (t, 3H), 1.22 (m, 1H), 1.37 (m, 4H), 1.70 (m, 1H), 1.79 (broad, 4H), 3.41 (m, 1H), 3.52 (m, 1H), 3.77 (s, 3H), 4.06 (q, 2H), 4.21 (q, 1H), 4.95 (s, 2H), 5.68 (d, 1H), 6.81 (s, 1H), 7.18 (d, 2H), 7.23 (d, 2H), 7.33 (d, 2H), 7.42 (s, 1H), 7.51 (s, 1H), 7.77 (d, 2H), 8.47 (t, 1H), 8.53 (s, 1H). (*R*)-3-{4-[1-(4-Cyclohexylphenyl)-3-(3-methoxy-5-trifluoromethylphenyl) ureidomethyl]benzoylamino}-2-hydroxypropionic acid ethyl ester (**3**) (510 mg, 0.8 mmol) was dissolved in ethanol (15 mL) and sodium hydroxide (2 N, 2 mL) was added. The reaction mixture was stirred at room temperature for 60 min. Then ethanol was removed in vacuo, water (50 mL) was added, and pH was adjusted with 4 N hydrochloric acid to acidic reaction. Filtration and washing with water (5 × 5 mL) and drying in vacuo afforded 460 mg (94%) of **25** as a crystalline solid. ^{1}H NMR (DMSO-d_6): δ 1.22 (m, 1H), 1.37 (m, 4H), 1.70 (m, 1H), 1.79 (broad, 4H), 3.37 (m, 1H), 3.51 (m, 1H), 3.77 (s, 3H), 4.09 (t, 1H), 4.95 (s, 2H), 6.80 (s, 1H), 7.18 (d, 2H), 7.23 (d, 2H), 7.33 (d, 2H), 7.42 (s, 1H), 7.51 (s, 1H), 7.77 (d, 2H), 8.47 (t, 1H), 8.53 (s, 1H). Anal. $C_{30}H_{31}N_3O_5$1.25 H_2O: C, H, N. mp 117−121 °C.

(*S*)-3-{4-[1-(4-Cyclohex-1-enylphenyl)-3-(3,5-dichlorophenyl)-ureidomethyl]benzoylamino}-2-hydroxypropionic acid (16). A 750 mg racemic mixture of (*RS*)-3-{4-[1-(4-cyclohex-1-enylphenyl)-3-(3,5-dichlorophenyl)ureidomethyl]benzoylamino}-2-hydroxypropionic acid ethyl ester was eluted on a OD PREP-HPLC (50 mm × 500 mm) from Daicell with absolute ethanol/heptane 4:96 with a flow at 120 mL/min. The fastest eluting fraction was isolated and solvent removed in vacuo. The (*S*)-3-{4-[1-(4-Cyclohex-1-enylphenyl)-3-(3,5-dichlorophenyl)ureidomethyl]benzoylamino}-2-hydroxypropionic acid ethyl ester was hydrolyzed with NaOH similar to what has been described for the procedure above. Anal. calcd for $C_{30}H_{29}Cl_2N_3O_5 \cdot {}^3/_4H_2O$: C, 60.46; H, 5.16; N, 7.05. Found: C, 60.75; H, 5.54; N, 6.78.

(*R*)-3-{4-[1-(4-Cyclohexen-1-ylphenyl)-3-(3-methanesulfonyl-4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}-2-hydroxypropionic acid (36). To a solution of methyl iodide (59.0 g, 0.41 mol) in DMF (150 mL) was added potassium carbonate (23.0 g, 0.16 mol). 2-(Trifluoromethoxy)thiophenol (16.0 g, 0.08 mol) was added in portions during 30 min. The reaction mixture was then stirred vigorously overnight. Water (250 mL) was added. The reaction mixture was extracted with ethyl acetate (2 × 150 mL). The combined organic phases were washed with a 50% saturated aqueous solution of sodium chloride (4 × 100 mL), dried (magnesium sulfate), and concentrated in vacuo to give 15.0 g (90%) of 1-methylsulfanyl-2-trifluoromethoxybenzene.

1-Methylsulfanyl-2-trifluoromethoxybenzene (15.0 g, 72 mmol) was dissolved in dichloromethane (200 mL) and *m*-chloroperoxybenzoic acid (39.0 g, 216 mmol) was added in small portions during 30 min. The reaction mixture was then allowed to stand overnight. Dichloromethane (200 mL) was added, followed by slow addition of sodium hydroxide (2 N, 200 mL). The organic phase was separated and washed with sodium hydroxide (2 N, 3 × 150 mL), dried (magnesium sulfate), and concentrated in vacuo to give 15.8 g (91%) of 1-methylsulfonyl-2-trifluoromethoxybenzene ^{1}H NMR ($CDCl_3$): δ 8.11 (d, 1H), 7.71 (t, 1H), 7.48 (m, 2H) 3.23(s 1H). Anal. calcd for $C_8H_7F_3O_3S$: C, 40.0; H, 2.94. Found: C, 40.20; H, 2.90. mp 148−150 °C.

1-Methylsulfonyl-2-trifluoromethoxybenzene (15.7 g, 65 mmol) was dissolved in concentrated sulfuric acid (27 mL), and the solution was heated to 40 °C. Nitric acid (100%, 27 mL) was added dropwise over 45 min. The reaction mixture was allowed to stand overnight at 60 °C, cooled, and then poured on crushed ice (300 mL). The precipitated product was isolated by filtration, washed with water (10 × 50 mL), and dried (magnesium sulfate), affording 17.5 g (94%) of 3-methylsulfonyl-4-trifluoromethoxynitrobenzene. ^{1}H NMR (DMSO-d_6): δ 8.69 (d, 1H), 8.64 (d, 1H), 7.95 (d, 1H) 3.45 (s 3H). Anal. calcd for $C_8H_6F_3NO_5S$: C, 33.69; H, 2.12; N, 4.91. Found: C, 33.90; H, 2.07; 4.91. mp 102−104 °C.

3-Methylsulfonyl-4-trifluoromethoxynitrobenzene (17.5 g, 61 mmol) was dissolved in methanol (400 mL), followed by addition of palladium on carbon (10%, 50% water, 3.2 g). The reaction mixture was hydrogenated for 17 h at 1 atm of hydrogen, filtered, and concentrated in vacuo to give 14.3 g (92%) of 3-methylsulfonyl-4-trifluoromethoxyaniline. ^{1}H NMR (DMSO-d_6): δ 7.26 (d, 1H), 7.14 (d, 1H), 6.85 (dd, 1H) 5.89 (s, 2H) 3.21(s, 3H). Anal. calcd for $C_8H_8F_3NO_3S$: C, 37.65; H, 3.16; N, 5.49. Found: C, 37.65; H, 3.14; N, 5.45. mp 106−109 °C.

To 3-methylsulfonyl-4-trifluoromethoxyaniline (2.0 mmol, 500 mg) dissolved in ethyl acetate (6 mL) was added 3 N hydrochloric acid in ethyl acetate (5 mL) followed by concentration in vacuo. The residue was treated with toluene (3 × 5 mL) and each time concentrated in vacuo. To the residue was added toluene (10 mL) and diphosgene (6 mmol, 0.73 mL) under a nitrogen atmosphere, and the suspension was gently refluxed for 2 h. Additional diphosgene (6 mmol, 0.73 mL) was added, and refluxing was continued overnight. The reaction mixture was concentrated in vacuo to afford 3-methylsulfonyl-4-trifluoromethoxyphenyl isocyanate, which was used directly in the subsequent step to make **36** according to the method described for compound **25**.

3-Amino-2(*R*)-methoxypropionic Acid. To an ice-cooled solution of methanol (250 mL) was added acetyl chloride (12.5 mL), and the solution was stirred for 1 h at 0 °C. (*R*)-Malic acid (20.0 g) was added, and the solution was stirred for 16 h at room temperature. Solvent was removed by evaporation in vacuo, leaving a quantitative yield of (*R*)-2-hydroxysuccinic acid dimethyl ester as an oil. ^{1}H NMR ($CDCl_3$): δ 4.52 (dd, 1H), 3.80 (s, 3H), 3.71 (s, 3H), 3.55 (bs, 1H), 2.88 (dd, 1H), 2.80 (dd, 1H).

The above (*R*)-2-hydroxysuccinic acid dimethyl ester was redissolved in methyl iodide (100 mL), freshly prepared silver oxide (30.2 g) was added, and the mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with acetonitrile (200 mL) and filtered through celite to remove silver salts and excess silver hydroxide. The filtrate was taken to dryness to leave (*R*)-2-methoxysuccinic acid dimethyl ester as an oil (23.2 g, 88%). ^{1}H NMR ($CDCl_3$): δ 4.20 (dd, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.48 (s, 3H), 2.80 (dd, 2H).

The above (*R*)-2-methoxysuccinic acid dimethyl ester was suspended in 2 N aqueous hydrochloric acid and heated to reflux for 30 min to give a clear solution. Upon evaporation of solvent in vacuo, a quantitative yield of 2(*R*)-methoxysuccinic acid was obtained as oil. The oil was redissolved in acetic anhydride (120 mL) and heated to 110 °C for 2 h. Solvent was removed by rotary evaporation to leave oil. Ice-cooled methanol (150 mL) was added,

and the mixture was stirred for 3 h at 0 °C followed by 16 h at room temperature. The solvent was removed to leave of (*R*)-2-methoxysuccinic acid 1-methyl ester (5.0 g, 30.8 mmol, 24%). ^{1}H NMR ($CDCl_3$): δ 10.30 (bs, 1H), 4.19 (dd, 1H), 3.80 (s, 3H), 3.50 (s, 3H), 2.86 (dd, 1H), 2.78 (dd, 1H).

This was dissolved in thionyl chloride (16 mL) and heated to reflux for 2 h. Thionyl chloride was removed in vacuo, followed by coevaporation with acetonitrile. The neat acid chloride was dissolved in toluene (50 mL). Trimethylsilylazide (5.0 mL, 38.2 mmol) was added, and the mixture was heated to 100 °C overnight. Then *tert*-butanol (30 mL) was added, and heating was continued for an additional 16 h. The reaction mixture was cooled, and insoluble material was removed by filtration. The organic phase was washed with water (100 mL), saturated sodium hydrogen carbonate solution (100 mL), 10% citric acid solution (100 mL), water (100 mL), and saturated sodium chloride solution (100 mL), then dried over anhydrous sodium sulfate. The solvent was removed in vacuo. The residual oil was further purified by column chromatography using 20% ethyl acetate/heptane as eluent. Pure fractions (TLC plates were stained with ammonium molybdate/cerium sulfate/sulfuric acid) were pooled and solvent removed in vacuo to afford. 3-*tert*-butoxycarbonylamino-2(*R*)-methoxypropionic acid methyl ester (600 mg, 9%). ^{1}H NMR ($CDCl_3$): δ 6.93 (t, 1H), 3.83 (t, 1H), 3.64 (s, 3H), 3.25 (s, 3H), 3.18 (dd, 2H), 1.36 (s, 9H).

3-*tert*-Butoxycarbonylamino-2(*R*)-methoxypropionic acid methyl ester (500 mg, 2 mmol) was dissolved in 10% TFA in DCM (20 mL), and the reaction mixture was stirred at 30 min at ambient temperature. The solvent was removed in vacuo, and the residue coevaporated twice from 30 mL of 1 N hydrochloric acid in ether to afford 320 mg (88%) of 3-amino-2(*R*)-methoxypropionic acid methyl ester hydrochloride. ^{1}H NMR ($CDCl_3$): δ 8.25 (s, 3H), 4.21 (dd, 1H), 3.71 (s, 3H), 3.40 (s, 3H), 3.15 (m, 1H), 2.98 (m, 1H). 3-Amino-2(*R*)-methoxypropionic acid was used in the subsequent step to make **20** according to the method described for compound **25**.

Isolated Perfused Rat Pancreas. Surgery was performed on anesthetized rats (sodium pentobarbital) in order to isolate the pancreas as previously described.[26,27] A perfusate, consisting of a modified Krebs−Ringer bicarbonate buffer containing 3% dextran and 0.2% bovine serum albumin (Fraction V, RIA grade, Sigma), gassed with 95% O_2/5% CO_2 to achieve pH 7.4, was perfused at a rate of 4 mL/min into the abdominal aorta. The portal venous outflow was collected at 1 min intervals. Following a 10 min equilibration period, 80 mg/dL glucose was infused from periods 1−4 min, after which the perfusate was switched to 160 mg/dL glucose from 5 to 50 min ± the compounds to be tested. During the period from 15 to 34 min, 500 pM GIP was introduced through a sidearm infusion. Insulin levels in the collected perfusate was measured by radioimmunoassay as previously described.[28]

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Supporting Information Available: Characterization data for for compounds **11**−**36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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