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Integration of Optimized Substituent Patterns to Produce Highly Potent 4-Aryl-pyridine Glucagon Receptor Antagonists

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Abstract—Optimized substituent patterns in 4-aryl-pyridine glucagon receptor antagonists were merged to produce highly potent derivatives containing both a 3-[(1*R*)-hydroxyethyl] and a 2′-hydroxy group. Due to restricted rotation of the phenyl–pyridine bond, these analogues exist as four isomers. A diastereoselective methylcopper reaction was developed to facilitate the synthesis, and single isomers were isolated with activities in the range $IC_{50} = 10$–25 nM.

The incidence of type 2 diabetes is expected to significantly increase by 2010.[1] As no current therapies prevent the disease's progression, many research groups are focusing their efforts toward the identification of novel approaches.[2] One such strategy targets modulation of the glucagon receptor. The mechanism of action of glucagon and its role in the regulation of blood glucose levels have been known for quite some time.[3] However, only during the last decade have studies shown that potent peptide antagonists significantly decrease blood glucose levels in diabetic animal models.[4] More recently, it was demonstrated that a small molecule could act as an antagonist, triggering a strong interest in the research community.[5]

From a high throughput screening assay, we discovered compound **1** (Scheme 1), which exhibited modest activity against the human glucagon receptor (binding affinity, $IC_{50} = 7$ μM). Efforts to optimize compound **1** led to the discovery of more potent analogues, such as **2**[6] (R = 1-propyl, $IC_{50} = 0.11$ μM) and **3**[7] (*R* = 1-pentyl, $IC_{50} = 0.19$ μM). Thereafter, our goal was to merge the key pharmacophoric groups of these two series into a hybrid structure of type **4,** striving for an additive effect in potency. The two main moieties contributing to high binding affinities with the glucagon receptor are the 3-[(1*R*)-hydroxyethyl] group of structure **2** and the 2′-hydroxyl group of structure **3**. In this article, we present the preparation and SAR analysis of such compounds (**4**), as well as the development of a diastereoselective methylcopper approach to address challenges encountered in the synthesis of **4**.

Compounds of type **4** have four possible isomers by virtue of their two chiral elements; the stereocenter of the 3-(1-hydroxyethyl) group, and the axis of chirality resulting from restricted rotation about the phenyl–pyridine bond.[8] We decided to synthesize compound **4** first in an achiral fashion and separate the mixture of isomers by chiral chromatography for biological evaluation.[9] *In situ* formation of the magnesium complex of diethyl acetone-1,3-dicarboxylate followed by condensation with isobutyryl chloride provided the 2,6-diisopropyl pyrone **6**, by a one-pot process[7] (Scheme 2). Treatment of pyrone **6** with ammonia gave the 4-hydroxypyridine, which was methylated to the 4-methoxypyridine **7**. Treatment of compound **7** with Grignard reagent **8** afforded phenylpyridine **9** in good yield.[7] Selective reduction of one of the ester groups of compound **9** with Red-Al®, followed by a PCC oxidation and

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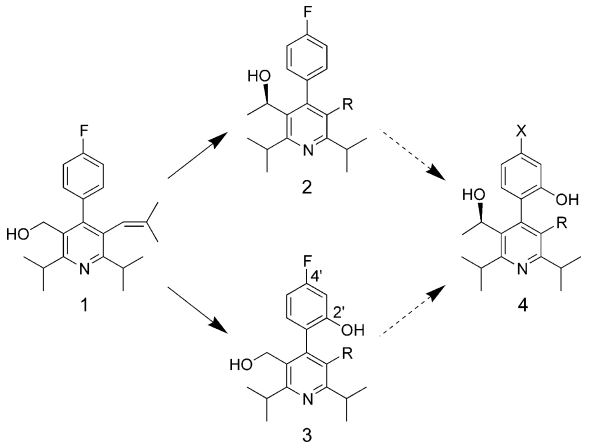
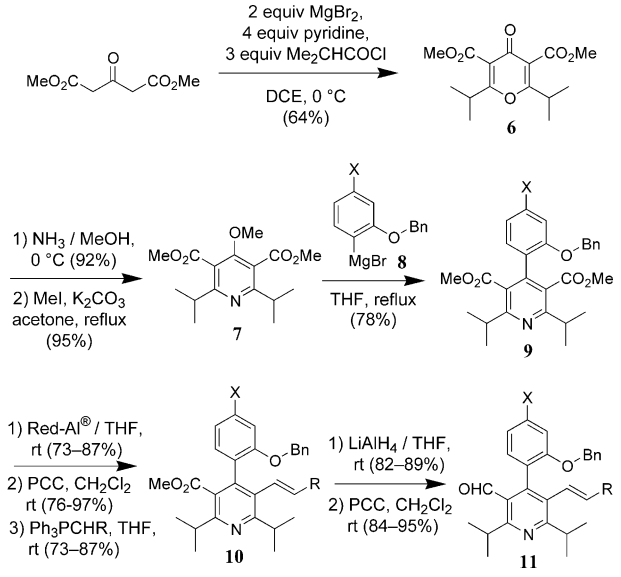


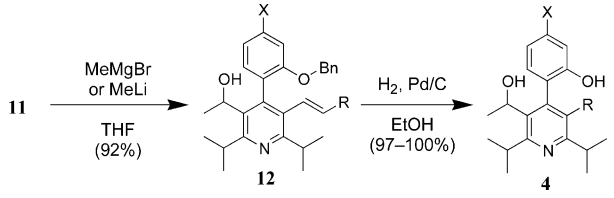
Scheme 1. Optimization summary of a class of 4-aryl-pyridine glucagon antagonists (R = ethyl to pentyl, X = H or F).



Scheme 2. Synthesis of intermediate **11**.

Wittig reaction afforded alkene **10**. Standard $LiAlH_4$ reduction of the ester group of compound **10**, followed by PCC oxidation gave the desired aldehyde precursor **11**. Compound **11** was obtained as a racemic mixture, since the benzyloxy group at the 2′ position introduces an element of chirality of the biaryl axis (vide supra).

Intermediate **11** was then treated with methyllithium or methylmagnesium bromide to provide the hydroxyethyl compound **12** (Scheme 3). Hydrogenation of **12** served to saturate the olefin moiety as well as to remove the benzyl protecting group, affording the desired analogue **4**.



Scheme 3. Synthesis of analogues **4**.

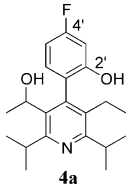
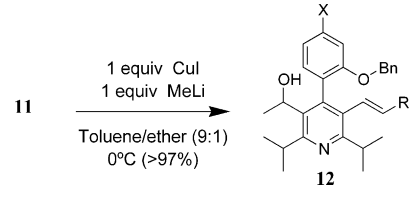


Figure 1. Potent glucagon antagonist analogue of type 4.

Compound **4a** (X = F and R = C_2H_5, Fig. 1) was one of the first examples of type **4** synthesized and was obtained as a ca. 1:2 diastereomeric mixture. The diastereomers were separated through standard silica gel chromatography (10% ethyl acetate in hexanes), and the faster-eluting diastereomer exhibited high affinity to the human glucagon receptor (IC_{50} = 20 nM), while the slower-eluting diastereomer had marginal activity (IC_{50} = 2000 nM). Using chiral chromatography,[10] the former (more active) diastereomer was resolved, to provide only one potent enantiomer (IC_{50} = 16 nM vs 5000 nM for the two enantiomers). This highly potent analogue demonstrated that combining the key pharmacophoric groups of compounds **3** and **4** resulted in an additive effect in potency. Thus we focused our efforts toward a detailed SAR evaluation of this new sub-class of analogues. However, their synthesis via Scheme 3, which produced the more potent diastereomer as the minor product, was inefficient. The development of a diastereoselective and scalable synthesis became a priority.

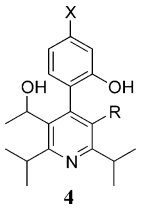
During our search for a diastereoselective synthesis, we discovered that aldehyde **11** gave encouraging results when treated with alkylcopper reagents using procedures developed by Sato and coworkers.[11] After a series of optimization experiments, we found that the best results were obtained when the methylcopper reagent was generated by using a 1:1 ratio of methyllithium and copper iodide in a mixture of toluene and ether (9:1) at 0 °C (Scheme 4).[12] Using these conditions, a nearly quantitative yield of product was obtained, with a diastereomeric ratio of >90:10 in favor of the desired stereoisomer. This represented a substantial synthesis improvement, as the original method (Scheme 3) provided predominantly the less potent diastereomer. The diastereomers were separated by standard chromatography, and the racemic mixture of the desired isomer was carried until the end of the synthesis, where they were easily separated by chiral chromatography.[10]

With an improved synthesis in hand, we then focused our efforts on a detailed SAR investigation of analogues of type **4,** and a summary of their activities is listed in Table 1. The activities (IC_{50}) of diastereomers **D1** (first-eluting



Scheme 4. Diastereoselective synthesis of intermediates **12**.

Table 1. Glucagon antagonist activities of analogues **4**



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Compd	X	R	D1 IC_{50} (nM)	D2 IC_{50} (nM)	E1 of D1 IC_{50} (nM)	E2 of D1 IC_{50} (nM)
4a	F	Et	20	2000	5000	16
4b	H	Pr	40	1100	190	25
4c	F	Pr	18	1000	8000	10
4d	F	Bu	28	1000	—	—
4e	F	Pent	30	2200	8000	12

D1, first diastereomer to elute (as mixture of E1 and E2); D2, second diastereomer to elute; E1, first enantiomer to elute; E2, Second enantiomer to elute.

diastereomer) ranged from 18 to 40 nM and were up to 100-fold more potent than diastereomers **D2** (second-eluting diastereomer).[13] The second-eluting enantiomer (**E2**) of diastereomer **D1** was the more potent enantiomer, exhibiting slightly stronger activities as compared to their parent diastereomers **D1**, while the first-eluting enantiomers (**E1**) were considerably less active. As previously observed,[14] the fluoro substituent at the 4′ position confers an approximately 2-fold increase in potency (compare diastereomers **D1** and enantiomers **E2** of **4b** and **4c**). The most potent analogue, **E2** of **4c**, exhibited an IC_{50} value of 10 nM, establishing it as one of the most active glucagon antagonists reported to date. This compound was also evaluated for antagonistic activity according to a published procedure.[15] In that assay, the potent isomer of analogue **4c** (**E2** of **D1**) exhibited functional activity (cAMP production $IC_{50} = 6.5$ nM) comparable to that measured in the receptor binding affinity assay.

We also sought to determine the absolute configuration of the most active isomers **E2** (Table 1). Assuming that the hydroxyethyl group retains the *R* configuration established for compound **2**,[16] X-ray crystal structure analysis[17] allowed us to assign the absolute configuration of compound **13** (Fig. 2), in which both hydroxy groups are *syn* to each other and appear to form an internal hydrogen bond.

In summary, we have discovered new highly potent glucagon antagonists by integrating the key pharmacophoric groups of compounds **2** and **3**. Isomer **E2** of **D1** of compound **4c** (Table 1) exhibited the greatest potency with an IC_{50} of 10 nM. Also, a diastereoselective alkylation was developed to improve the synthetic accessibility to this new series.

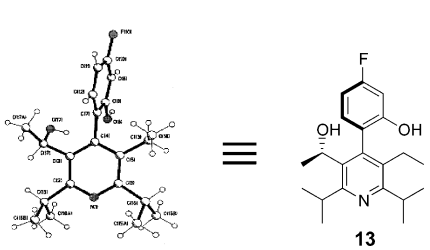

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Figure 2. X-ray crystal structure (XP plot) of the potent glucagon antagonist **13** (**4a, E2** of **D1** in Table 1).

Acknowledgements

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References and Notes

1. Amos, A. F.; McCarty, D. J.; Zimmet, P. *Diabetic Med.* **1997**, *14*, S7.
2. (a) Zhang, B. B.; Moller, D. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 461. (b) Cobb, J.; Dukes, I. *Annu. Rep. Med. Chem.* **1998**, *33*, 213.
3. (a) Dohlman, H. G.; Thorner, J.; Caron, M. G.; Lefkowitz, R. J. *Annu. Rev. Biochem.* **1991**, *60*, 653. (b) Christophe, J. *Biochim. Biophys. Acta* **1995**, *1241*, 45. (c) Unger, R. H.; Orci, L. *N. Engl. J. Med.* **1981**, 1518. (d) Gerich, J. E.; Lorenzi, M.; Bier, D.; Schneider, V.; Tsalikian, E.; Karam, J.; Forsham, P. H. *New Engl. J. Med.* **1975**, *292*, 985. (e) Unger, R. H. *Metabolism* **1978**, *27*, 1691.
4. (a) Unson, C. G.; Gurzenda, E. M.; Merrifield, R. B. *Peptides* **1989**, *10*, 1171. (b) Salzig, T. M.; Unson, C.; Merrifield, B. *Diabetes* **1996**, *45*, 220.
5. (a) Livingston, J. N.; Schoen, W. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 189. (b) Connell, R. D. *Exp. Opin. Ther. Pat* **1999**, *9*, 701. (c) Cascieri, M. A.; Koch, G. E.; Ber, E.; Sadowski, S. J.; Louizides, D.; De Laszlo, S. E.; Hacker, C.; Hagmann, W. K.; MacCoss, M.; Chicchi, G. G.; Vicario, P. P. *J. Biol. Chem.* **1999**, *274*, 8694. (d) Madsen, P.; Knudsen, L. B.; Wiberg, F. C.; Carr, R. D. *J. Med. Chem.* **1998**, *41*, 5150.
6. (a) Ladouceur, G. H.; Cook, J. H.; Doherty, E. M.; Schoen, W. R.; MacDougall, M. L.; Livingston, J. N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 461. (b) Schoen, W. R.; Ladouceur, G. H.; Cook, J. H.; Lease, T. G.; Wolanin, D. J.; Kramss, R. H.; Hertzog, D. L.; Osterhout, M. H. US Patent 6,218,431, 2001. *Chem. Abstr.* **2001**, *134*, 311111.
7. Smith, R. A.; Hertzog, D. L.; Osterhout, M. H.; Ladouceur, G. H.; Korpusik, M.; Bobko, M. A.; Jones, H.; Phelan, K.; Romero, R. H.; Hundertmark, T.; MacDougall, M. L.; Livingston, J. N.; Schoen, W. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1303.
8. Lloyd-Williams, P.; Giralt, E. *Chem. Soc. Rev.* **2001**, *30*, 145.
9. All data reported herein reflect purified and characterized (^{1}H NMR, MS) samples. The human glucagon receptor binding assay was carried out as described previously.[6a]
10. Racemates were resolved by using a Waters Preparative LC 2000HPLC system with a chiral column (BRB-9668A, 6×50 cm ID). The mobile phase consisted of 99% hexanes and 1% of a solution of 1:99 acetic acid/ethanol (150 mL/min). As an example, for the racemate (**D1**) of compound **4c**, **E1** was collected between 17 and 23 min and E2 was collected between 23 and 32 min.
11. Sato, F.; Kobayashi, Y.; Takahashi, O.; Chiba, T.; Takeda, Y.; Kusakabe, M. *J. Chem. Soc. Comm.* **1985**, *1636*.
12. (a) An example of a typical procedure: To CuI (5.69 g, 29.9 mmol) in toluene (85 mL) was slowly added a solution of 1.4 M of methyllithium in diethyl ether (21 mL, 29.4 mmol) at

0 °C. The mixture was stirred for 15 min before the addition of aldehyde **11** (5.5 mmol) in toluene (8 mL). During the addition of aldehyde **11**, the temperature was carefully monitored to ensure that it was maintained around 0 °C. The reaction mixture was stirred for an additional 30 min at 0 °C, then quenched with a saturated solution of NH_4OH (250 mL). After an additional 30 min, the mixture was further diluted with a saturated solution of NH_4Cl (300 mL) and extracted with ether (3×150 mL). The combined organic layers were dried (MgSO4) and concentrated to give a colorless oil. The oil was filtered through a pad of silica gel (5:1 hexanes/EtOAc) and the filtrate concentrated to give the product as a colorless oil (29.6 mmol, 99%).

(b) A mechanistic rationale for the different stereoselectivity observed for the methylcopper reagent, as compared to that observed for methyllithium, is complicated by the lack of a clear understanding of the structures of these reagents in solution. Indeed, we have found that the stereoselectivity is influenced by the reagent as well as the solvent. Coordination of the reagent to the benzyloxy group may play a role in this process, but we have insufficient data to be able to propose an interpretation for these findings.

13. Standard errors for the binding data were $\leq 35\%$.
14. Table 2 in ref 6a.
15. Selected compounds were also tested in a functional cell assay, and all were determined to be competitive antagonists. IC_{50} values for cAMP production are as follows: for **4a**-E2-D1, 8 nM, **4b**-E2-D1, 12 nM, **4c**-E2-D1, 6.5 nM, **4e**-E2-D1, 11 nM. The procedure for the functional assay has been described previously.[6a]
16. The secondary alcohol of compound **2** was assigned the *R* configuration by X-ray crystal structure analysis of its Mosher ester derivative.[6a]
17. Computations and molecular graphics were performed using SHELXTL-5 on a HP-Vectra 5/90 XU PC. The structure was solved by direct methods. All remaining non-hydrogen atoms were found by successive electron density map calculations. H-atoms could also be detected, but were constructed in geometrically meaningful positions. Anisotropic displacement parameters were applied to all but the H-atoms for which an isotropic displacement factor (1.5 times Uequiv of the corresponding atom for CH_3 hydrogens, 1.2 times Uequiv of the corresponding atom for all other H-atoms in the structure) is calculated.