

Bicyclic acidic amino acids as mimetics of Glu or Asp

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Introduction

Many hormones are peptides, for instance insulin, glucagon, antidiuretic hormone (ADH), thyrotropin-releasing hormone (TRH), and growth hormone. They often have a fast clearance due to enzymatic degradation. One approach to avoid this is to incorporate unnatural amino acids in the sequence.

Glucagon is an important hormone involved in carbohydrate metabolism. It is produced by the pancreas and released when the blood glucose level is low. This causes the liver to convert stored glycogen into glucose and release it into the bloodstream. In case of severe hypoglycemia (low blood glucose) when the victim is unconscious or for other reasons cannot take glucose orally, an injectable form of glucagon is a vital first aid. The dose for an adult is typically 1 milligram, and the glucagon is given by intramuscular, intravenous or subcutaneous injection, and quickly raises blood glucose levels.

Glucagon is known to be metabolic labile between position 2 and 3 as well as between position 17 and 18. By placing an unnatural amino acid between these positions it may be possible to suppress both types of enzymatic cleavage and thereby to increase metabolic stability.

In order to test the effect of incorporating unnatural amino acids into the sequence of glucagon on the activity, unnatural amino acid **1**, which has a negative moiety and resembles both Glu (**2**) and Asp (**3**) (Fig. 1), was prepared and substituted for an Asp in glucagon, and the resulting glucagon analogue tested on the human glucagon receptor.

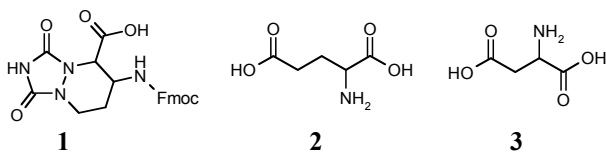


Fig. 1. Target amino acid (**1**), Glu (**2**), and Asp (**3**).

Results and Discussion

Fmoc-protected bicyclic amino acid **1** was prepared in 5 steps from commercially available [1,2,4]triazolidine-3,5-dione in 63% overall yield. The key steps in the synthesis are Diels-Alder cycloaddition to form the bicyclic system [1] and, after rearrangement of the formed double bond to a conjugated α - β unsaturated system [2], a Michael type conjugate addition of ammonia giving the corresponding amino acid ester. After hydrolysis and Fmoc-protection the amino acid (**1**) is ready

for peptide synthesis using standard peptide coupling conditions. Chemistry was developed to build unnatural amino acid **1** into the sequence of glucagon. A glucagon analogue (**11**, Fig. 2) in which the Asp9 residue is substituted with unnatural amino acid **1** was successfully prepared as a diastereomeric mixture in good yield (0.53% overall) and high purity. Comparison of the UV response after Fmoc deprotections shows that coupling of the unnatural amino acid **1** proceeded with similar efficiency as the natural amino acids in the sequence. Furthermore, glucagon analogues **12a** and **12b** (Fig. 2) in which the Asp15 residue is substituted with unnatural amino acid **1** was prepared and the diastereomers partly separated (**12a** containing mainly one diastereomer and **12b** mainly the other).

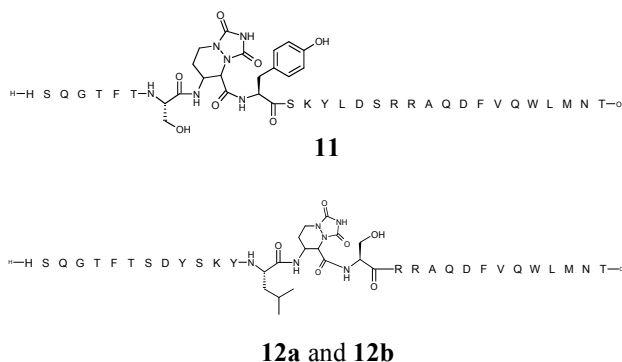


Fig. 2. Glucagon analogues containing unnatural amino acid **1**.

Glucagon analogues **11**, **12a**, and **12b** induced cAMP production on BHK cells, but displayed very low potency compared to the natural hormone, glucagon, on the human glucagon receptor. The EC₅₀ of **12a** and **12b** were 399 nM and 134 nM respectively, however, the exact EC₅₀ of **11** could not be determined due to problems by obtaining a full dose response curve.

Acknowledgments

János Tibor Kodra is acknowledged for inspiration and useful suggestions. Furthermore, Novo Nordisk A/S and the Danish Ministry of Science, Technology and Innovation are acknowledged for financial support.

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