

METHODS OF MAKING INCRETIN ANALOGS

The disclosure relates generally to biology, chemistry and medicine, and more particularly it relates to methods of synthesizing, via hybrid liquid solid phase synthesis (HLSPS), an incretin analog having activity at one or more of the glucose-dependent insulintropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and glucagon (GCG) receptors.

Over the past several decades, the prevalence of diabetes has continued to rise. Type 2 diabetes (T2DM) is the most common form of diabetes, accounting for about 90% of all diabetes. T2DM is characterized by high blood glucose levels caused by insulin resistance. The current standard of care for T2DM includes dieting and exercising, as well as treating with oral glucose-lowering therapeutics and/or injectable glucose-lowering therapeutics, including incretin-based therapies, such as GLP-1 receptor agonists, GIP/GLP-1 dual receptor agonists and even GIP/GLP-1/GCG (GGG) tri-receptor agonists.

Intl. Patent Application Publication Nos. WO 2019/125938 and 2019/125929 generally describe incretin analogs that act as GGG tri-receptor agonists and a method of synthesizing the same via standard solid phase peptide synthesis. *See also*, Intl. Patent Application Publication Nos. WO 2014/049610, 2015/067716, 2016/198624, 2017/116204, 2017/153575 and 2018/100135. Likewise, Intl. Patent Application Publication Nos. WO 2013/164483 and 2016/111971 describe compounds stated to have GLP-1 and GIP activity. Moreover, Intl. Patent Application Publication No. WO 2020/023386 describes peptides having GIP and GLP1 receptor agonist activity.

There is a need, however, for alternative methods of making such incretin analogs and intermediates thereof to enable pharmaceutically elegant production with commercially desired purity. Likewise, there is a need for efficient methods and stable intermediates to provide incretin analogs efficiently, with fewer purification steps.

To address this need, the disclosure describes methods of making incretin analogs via HLSPS or native chemical ligation (NCL), where such methods use from two to four intermediate compounds to make the incretin analog.

Summary

In one aspect of the present disclosure, there is provided a method of making an incretin analog of SEQ ID NO:6, the method comprising the step of:

coupling, via hybrid liquid solid phase synthesis, four intermediate compounds selected from the groups consisting of:

- 5 a. SEQ ID NOS:7, 8, 9 and 10,
 b. SEQ ID NOS:7, 11, 12, and 10, and
 c. SEQ ID NOS:7, 13, 14 and 10.

In another aspect of the present disclosure, there is provided a method of making an incretin analog of SEQ ID NO:6, the method comprising the step of:

10 coupling, via hybrid liquid solid phase synthesis, three intermediate compounds selected from the groups consisting of:

- a. SEQ ID NOS:7, 13 and 15,
 b. SEQ ID NO S:16, 17 and 10,
 c. SEQ ID NOS:18, 12 and 10, and
15 d. SEQ ID NOS:7, 45 and 10.

In another aspect of the present disclosure, there is provided a method of making an incretin analog of SEQ ID NO:6, the method comprising the step of:

coupling, via hybrid liquid solid phase synthesis, two intermediate compounds selected from the groups consisting of:

- 20 a. SEQ ID NOS:15 and 19 and
 b. SEQ ID NOS:18 and 20.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:7 or a pharmaceutically acceptable salt thereof.

25 In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:8 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

30 SEQ ID NO:9 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:10 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:11 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:12 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:13 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising

SEQ ID NO:14 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:15, or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:16 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:17 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:18 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:19 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:20 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:21 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:22 or a pharmaceutically acceptable salt

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:23 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:24 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:25 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:26 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:27 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:28 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:38 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:39 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:40 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising Boc-Y(Aib)EGT(α MeF(2F))TSD(4Pal)SI(α MeL)L (SEQ ID NO:30), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising:

SEQ ID NO:31 or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising Q(Aib)EFI(D-Glu)(α MeY)LIEG (SEQ ID NO:32), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising GPSSGAPPPS (SEQ ID NO:33), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising Boc-Y(Aib)EGT(α MeF(2F))TS (SEQ ID NO:34), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising Q(Aib)EFI(D-Glu)(α MeY)LIEGGPSSGAPPPS-NH₂ (SEQ ID NO:35), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising EFI(D-Glu)(α MeY)LIEGGPSSGAPPPS-NH₂ (SEQ ID NO:36), or a pharmaceutically acceptable salt thereof.

In another aspect of the present disclosure, there is provided an intermediate compound comprising CQ(Aib)EFI(D-Glu)(α MeY)LIEGGPSSGAPPPS-NH₂ (SEQ ID NO:37), or a pharmaceutically acceptable salt thereof.

_____ In another aspect of the present disclosure, there is provided an intermediate
compound selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID
NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID
NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID
5 NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID
NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID
NO:61, SEQ ID NO:62, or a pharmaceutically acceptable salt thereof.

_____ In another aspect of the present disclosure, there is provided a method of making
10 an incretin analog of SEQ ID NO:29, the method comprising the step of:
_____ coupling, via hybrid liquid solid phase synthesis, intermediate compounds
selected from the groups consisting of:
_____ a. SEQ ID NOS:7, 62, 42 and 31,
_____ b. SEQ ID NOS:43, and 44.

15

Description

In a first embodiment, the incretin analog can include an amino acid sequence of: