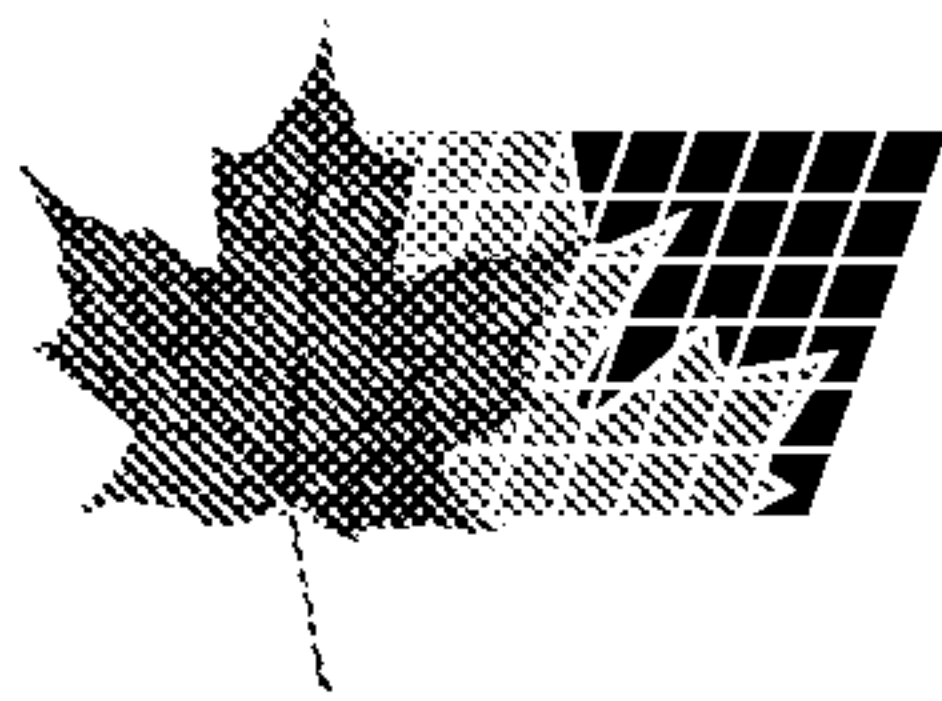




(72) BERGERON, RAYMOND J., JR., US

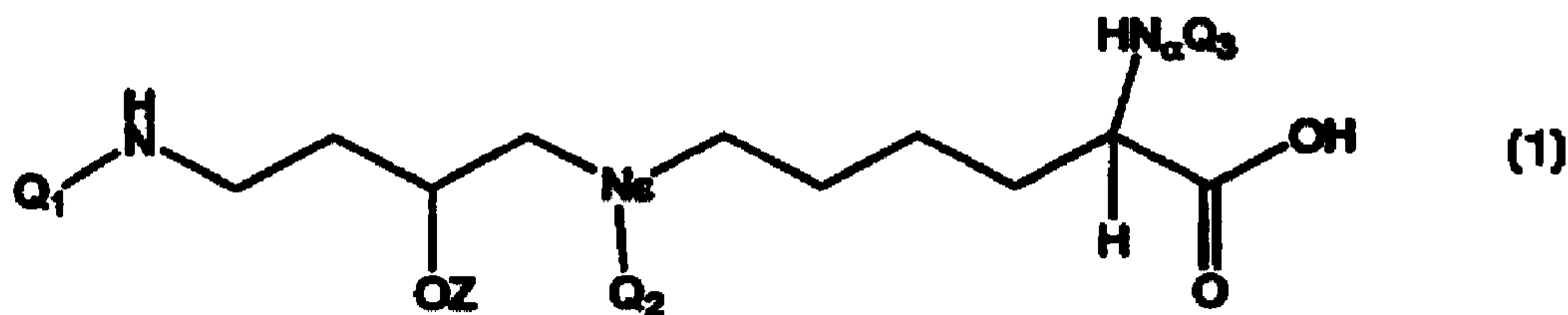
(71) UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC., US

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(54) **PEPTIDES CONTENANT HYPUSINE**

(54) **HYPUSINE PEPTIDES**



(57) L'invention concerne de nouveaux peptides synthétiques de la formule (1) obtenus suivant un procédé qui met en oeuvre le réactif d'hypusine. Dans ladite formule, Q₁, Q₂ et Q₃ peuvent être identiques ou différents et sont des groupes de protection amines, à condition que Q₃ soit orthogonal relativement à Q₁ et Q₂; et Z est un groupe de protection hydroxy. L'invention concerne également des procédés améliorés de synthèse peptidique mettant en oeuvre ledit réactif d'hypusine pour préparer de nouveaux peptides contenant hypusine.

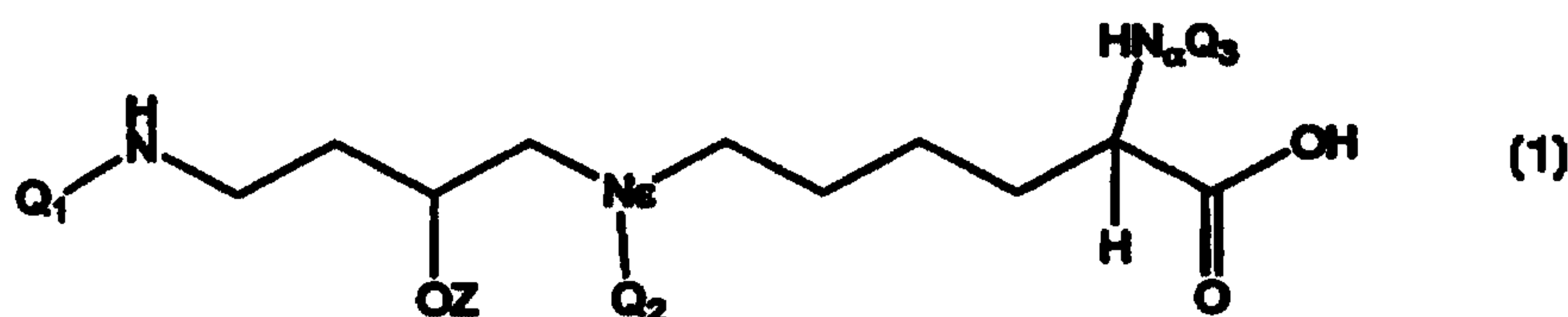
(57) The invention relates to novel peptides synthesized according to a method utilizing the hypusine reagent of formula (1) wherein Q₁, Q₂, and Q₃ may be the same or different and are amino protective groups, provided that Q₃ is orthogonal to Q₁ and Q₂; and Z is a hydroxy protective group, as well as improved methods of peptide synthesis wherein the above-described hypusine reagent is employed to prepare novel hypusine-containing peptides.

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(21) International Application Number: PCT/US98/17221 (22) International Filing Date: 19 August 1998 (19.08.98) (30) Priority Data: 08/975,656 21 November 1997 (21.11.97) US (71) Applicant: UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC. [US/US]; University of Florida, 109 Grinter Hall, P.O. Box 115500, Gainesville, FL 32611-5500 (US). (72) Inventor: BERGERON, Raymond, J., Jr.; 6220 Northwest 56th Lane, Gainesville, FL 32653 (US). (74) Agent: BARAN, Alexandra, J.; Cooley Godward LLP, 5 Palo Alto Square, 3000 El Camino Real, Palo Alto, CA 94306 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>

(54) Title: HYPUSINE PEPTIDES



(57) Abstract

The invention relates to novel peptides synthesized according to a method utilizing the hypusine reagent of formula (1) wherein Q₁, Q₂, and Q₃ may be the same or different and are amino protective groups, provided that Q₃ is orthogonal to Q₁ and Q₂; and Z is a hydroxy protective group, as well as improved methods of peptide synthesis wherein the above-described hypusine reagent is employed to prepare novel hypusine-containing peptides.

HYPUSINE PEPTIDES

Field of the Invention

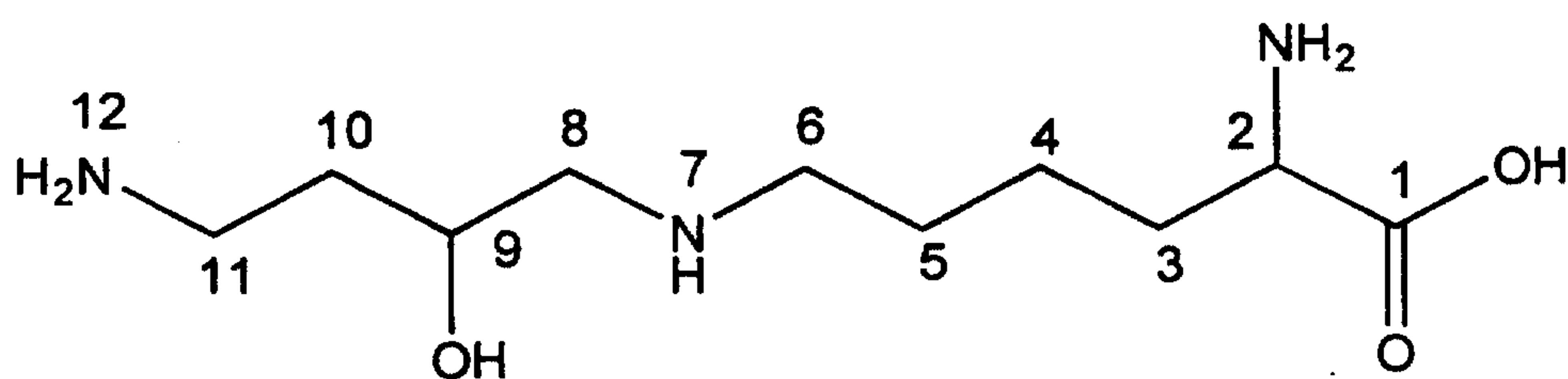
The present invention relates to novel hypusine-containing peptides.

5

BACKGROUND OF THE INVENTION

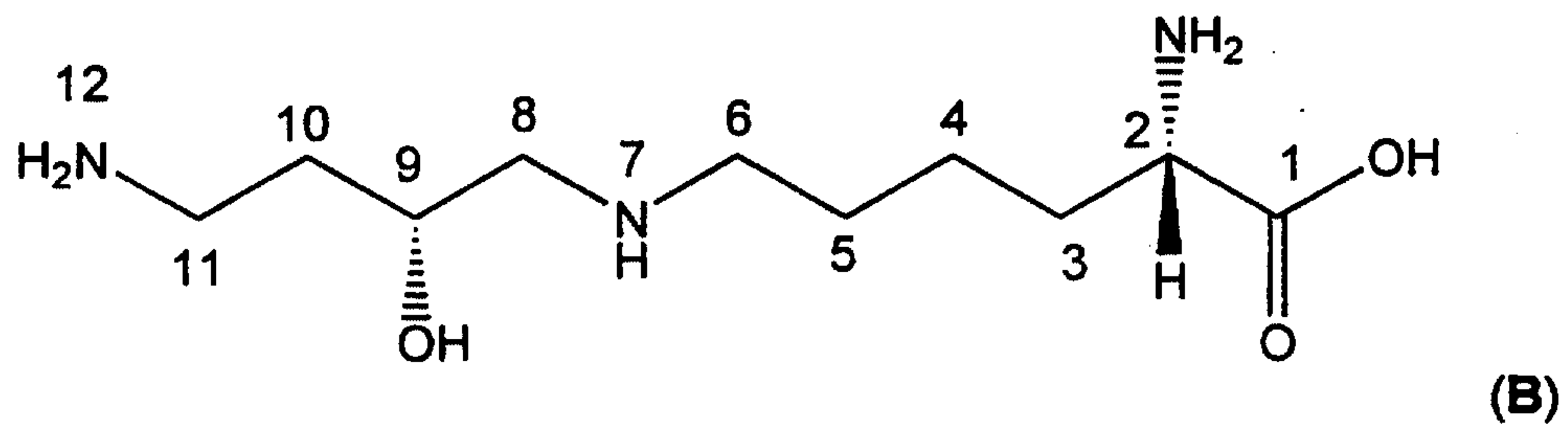
Description of the Prior Art

Hypusine [N_ϵ - (4-amino-2-hydroxybutyl) lysine], or [(2S, 9R)-2, 11-diamino-9-hydroxy-7-azaundecanoic acid], an unusual naturally occurring amino acid, having
10 the structure:



(A)

was first isolated from bovine brain extracts by Shiba et al. in 1971 [Biochim. Biophys. Acta., Vol. 244, pages 523-531 (1971)]. The molecule has two chiral centers at positions 2 and 9, each of which can be classified R or S by the Cahn-Ingold-Prelog method. The (2S, 9R) diastereomer (B), formed as a post-translational modification
15



of lysine, has been shown to occur on a precursor protein of the eukaryotic initiation factor 5A (formerly called eIF-4D) [Cooper et al, Proc. Natl. Acad. Sci. U.S.A., Vol. 80, pages 1854-1857 (1983); and Safer, Eur. J. Biochem., Vol. 186, pages 1-3 (1989)]. This initiation factor 5A is unique in that it is the only known cellular protein that contains the amino acid hypusine (Hpu). In the mid-1970's, eIF-5A was shown to stimulate ribosomal subunit joining and to enhance 80 S-bound Met-t-RNA_i reactivity with puromycin [Anderson et al, FEBS Lett., Vol. 76, pages 1-10 (1977); and Kemper et al, J. Biol. Chem., Vol. 251, pages 5551-5557 (1976)]. Later, in 1983, Cooper et al, supra, suggested that a hypusine-modified protein serves as an important initiation factor in all growing eukaryotic cells. In 1986, Park et al [J. Biol. Chem., Vol. 261, pages 14515-14519 (1986)] isolated the eIF-5A protein from human red blood cells and elucidated the amino acid sequence surrounding the single hypusine residue, as Thr-Gly-Hpu-His-Gly-His-Ala-Lys. Furthermore, and most interesting because of the potential application to the control of HIV replication [Bevec et al., J. Proc. Natl. Acad. Sci. U.S.A., Vol. 91, pages 10829-10833 (1994); and Ruhl et al, J. Cell Biol., Vol. 123, pages 1309-1320 (1994)], the synthesis of eIF-5A analogues are of great therapeutic significance.

Since hypusine is specific to eIF-5A, antibodies derived from hypusine-containing peptides could be used to quantitate the levels of eIF-5A directly and with

high specificity. Interest in developing an antibody assay of eIF-5A to investigate the physiological role of this important initiation factor prompted total synthesis of hypusine and its (2S, 9R)-diastereomer [Bergeron et al., J. Org. Chem., Vol. 58, pages 6804-6806 (1993)]. The key step in the synthesis involved the N_ε-alkylation
5 of N_ε-benzyl-N_α-carbobenzyloxy-(L)-lysine benzyl ester with (R)- or (S)-epichlorohydrin to give the respective (2S, 9R)- and (2S, 9S)-chlorohydrins. Subsequent displacement of the respective chlorides by cyanide ion provided the protected hypusine skeletons. The final step, hydrogenation over PtO₂ in AcOH, followed by neutralization and re-acidification, yielded the respective (2S, 9S)- and
10 (2S, 9R)-hypusine dihydrochlorides. A comparison of the reported hypusine optical rotation with that of the synthetic (2S, 9R)-hypusine **B** confirmed the stereochemical integrity of both chiral centers throughout the synthesis.

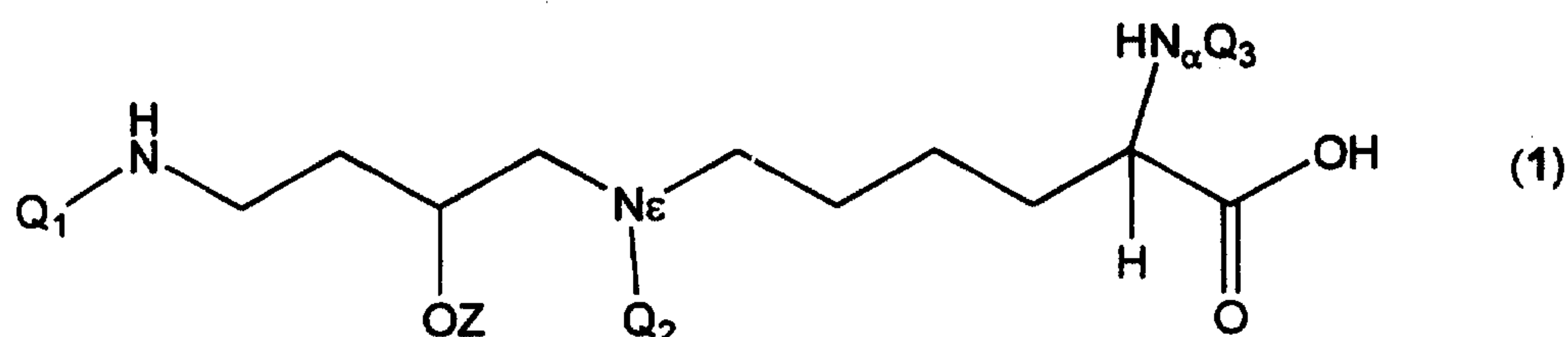
Synthetic methodology for accessing hypusine itself exists and it was desirable to have a selectively-protected hypusine reagent which could be used to
15 incorporate this unusual amino acid into selected peptides. Copending application Serial No. 08/962, 300, filed October 31, 1997 entitled "Hypusine Reagent for Peptide Synthesis", the entire contents and disclosure of which are incorporated herein by reference, describes a selectively protected hypusine reagent useful for incorporating hypusine into peptides, as well as methods for preparation of the
20 hypusine reagent.

It is an object of the present invention to provide novel hypusine-containing peptides, as well as methods for their synthesis utilizing the above-described hypusine reagent.

SUMMARY OF THE INVENTION

The above and other objects are realized by the present invention, one embodiment of which relates to novel peptides containing the hypusine moiety.

More specifically, the present invention relates to novel hypusine-containing peptides synthesized utilizing the hypusine reagent:



wherein: Q_1 , Q_2 , and Q_3 may be the same or different and are amino protective groups, provided that Q_3 is orthogonal to Q_1 and Q_2 ; and
Z is a hydroxy protective group.

A further embodiment of the invention relates to compounds of structure (2)



which may be synthesized using hypusine reagent (1), wherein Hpu is the hypusine amino acid residue, S and T are each independently peptide residues from zero to about 12 amino acids in length. Compounds of the invention find utility in the study of biochemical processes involving hypusine.

Another embodiment of the invention concerns improved methods of peptide synthesis wherein the above-described hypusine reagent is employed to prepare novel hypusine-containing peptides.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 and 2 depict example reaction schemes for preparing peptides of the invention. Figures 1 and 2 correspond to the chemistry described in Examples 1 through 8.

DETAILED DESCRIPTION OF THE INVENTION

10

The novel peptides (2) of the present invention comprise any synthetic peptide that incorporates within its structure the hypusine moiety, which is synthesized according to a method involving the use of the above-described hypusine reagent (1).

15

In compounds of structure (2), S and T are peptide residues from zero to about 12 amino acids in length, and preferably, are peptide residues from zero to about six amino acids in length. Most preferably, S and T are peptides residues from zero to about three amino acids in length S and T may vary independently in length and in composition of amino acid residues. The terminal amino acid residue of T may be hydroxylated. Non-limiting examples of peptides of the invention are:

20

L-Ser-L-Thr-L-Ser-L-Lys-L-Thr-Gly-Hpu-L-His-Gly-L-His-L-Ala-L-Lys,
L-Cys-L-Thr-Gly-Hpu-L-His-Gly,
L-Cys-L-Thr-Gly-Hpu-L-His-Gly-OH,
Hpu-L-His-Gly,
L-Thr-Gly-Hpu-L-His-Gly,

L-Lys-L-Thr-Gly-Hpu-L-His-Gly,

wherein the Hpu linkage is the (2S, 9R)-diastereomer thereof.

Compounds of the invention find utility in the study of biochemical processes involving hypusine, such as in the study of transport mechanisms for eIF5A.

5 The peptides of the invention may be prepared employing conventional steps of peptide synthesis except that the above-described hypusine reagent (1) is employed to incorporate the hypusine moiety into the peptide chain. Conventional peptide synthesis steps are disclosed, for example, in Moroder et al., "Hormonal Receptors in Digestive Tract Physiology," G. Rosselin et al., eds., Elsevier/North-
10 Holland Biomedical Press, Amsterdam, pages 129-135 (1979); and Moroder et al., Z. Physiol. Chem., Vol. 360, pages 787-790 (1979).

The synthesis of peptides is generally carried out through the condensation of the carboxyl group of an amino acid, and the amino group of another amino acid, to form a peptide bond. A sequence can be constructed by repeating the condensation
15 of individual amino acid residues in stepwise elongation or, in some cases, by condensation between two pre-formed peptide fragments (fragment condensation). In such condensations, the amino and carboxy groups that are not to participate in the reaction must be blocked with protecting groups which should be readily introduced, be stable to the condensation reactions and be removed selectively from
20 the completed peptide. If a peptide involves amino acids with side chains that may react during condensation, the problem of protection becomes increasingly difficult. A great range of reactive groups and side chains (amino, carboxy, thiol, hydroxy and the like) must be adequately blocked. Their blocking must be stable to unmasking of the α -amino or α -carboxy block for stepwise condensation and must be readily
25 removed at the final stage, leaving the completed peptide moiety intact.

Several methods are known wherein peptides are synthesized in vitro. The principal methodology used for peptide synthesis involves variations of the solid-phase methodology developed by Merrifield et al. See, for example, Erickson et al., "The Proteins," third edition, Vol. 2, Chapter 3, Academic Press, New York (1976).

5 Solid phase peptide synthesis involves attachment of a first amino acid to a solid support, such as a resin, followed by sequential addition of subsequent amino acids which results in assembly of the peptide chain on the solid support.

Peptides can also be synthesized by related methods involving coupling peptide fragments to solid supports as discussed by Erickson et al., supra, pages
10 268-269. This technique involves the synthesis of small peptide segments containing a few amino acids, which segments are then coupled to each other using fragment condensation techniques to form larger peptides. Fragment condensation techniques can be combined with standard solid phase techniques wherein small peptides are attached to resins followed by sequential attachment of single amino
15 acids or other peptide segments. Alternatively, sequential attachment of small peptides to single resin-bound amino acids can also be accomplished. The combination of the two approaches provides flexibility to synthetic schemes.

Upon completion of a particular synthesis, the synthesized peptide is then removed from the resin, usually by chemical means such as treatment with
20 hydrofluoric acid (HF). The chemical treatment also removes various amino acid and peptide protecting groups, such as CBZ, t-BOC or tosyl, which mask the reactivity of amino acid functional groups during synthesis.

In most peptide syntheses, the initial attachment to the resin involves the C-terminal amino acid of the peptide to be synthesized, which amino acid is covalently
25 attached to the resin through an ester or amide linkage involving its α -carboxyl

group. Synthesis then proceeds from the C- to the N-terminal. N-terminal to C-terminal peptide synthesis is less frequently used because the chemistry is more difficult and unwanted side reactions are more common.

The first amino acid may be covalently attached to the resin, in some cases, through its functional side chain. Initial attachment of an amino acid to the resin by means of the side chain functional group allows the possibility of bi-directional synthesis starting with the attached amino acid. Bi-directional synthesis cannot be performed if the initial amino acid is attached through the α -COOH or α -NH₂ group. Side chain functional groups which have been used for attachment to resins include the sulfhydryl group of cysteine, the imidazole group of histidine, the δ -amino group of ornithine, the ϵ -amino group of lysine and the γ -carboxyl group of glutamic acid. A review of the chemistry of solid phase peptide synthesis, including attachment of amino acids to resins via the α -COOH, α -NH and functional side chain groups, is found in Erickson et al., supra.

By using the insoluble resin support, it is possible to isolate the product of each coupling reaction simply by filtering the resin and washing it free of by-products and excess starting materials. In fact, the synthetic processes are so simplified and the time required for one cycle is so shortened that in recent years, it has become quite common to use automated peptide synthesizers. [See, for example, Barany et al., "The Peptides," Vol. 2, Academic Press, Inc., New York (1979), pages 1-284; or Kemp-Vellaccio, "Organic Chemistry," pages 1030-1032 (1980).]

Although the hypusine reagent described herein may be employed to access any hypusine-containing peptide, the method of the invention will be illustrated with reference to the following syntheses. It will be understood that any conventional peptide synthesis may be modified to prepare a hypusine-containing peptide by

simply utilizing the herein described hypusine reagent at any convenient stage thereof.

While the hypusine reagent allows for the assembly of a variety of hypusine-containing peptides, the hypusine-containing pentapeptide found in eIF-5A capped at its N-terminus with L-Cys, i.e., L-Cys-Thr-Gly-Hpu-His-Gly is a typical target peptide. The L-Cys, which is not contained in the natural peptide, was fixed to the sequence with the idea of being able to covalently link the peptide via a disulfide bond to a larger protein, in order to ultimately generate antibodies. The solution synthesis, a convergent approach, was carried out by elaborating from the C- to the N-terminus, as shown in Fig. 1, and involved the coupling of three appropriately-protected pieces: Cys-Thr-Gly, Hpu and His-Gly.

The carboxyamidomethyl (CAM) ester developed by Martinez et al. [Tetrahedron, Vol. 41, pages 739-743 (1985); and Tetrahedron Lett., Vol. 47, pages 5219-5222 (1983)] was employed as a carboxyl protecting group in generating the Cys-Thr-Gly fragment. This protecting group is orthogonal to the BOC, CBZ and FMOC groups. Thus, the synthesis of the masked Cys-Thr-Gly fragment 15 began with the N-BOC-Gly-CAM ester [Martinez et al., Tetrahedron, supra]. Removal of the BOC group with trifluoroacetic acid (TFA) gave the amine salt (60%) which was immediately coupled with N-FMOC-(O-t-butyl)-L-threonine to give the N-FMOC-(O-t-butyl)-L-Thr-Gly-CAM ester 13 in 85% yield. Treatment of 13 with 10% diethylamine (DEA) in DMF followed by coupling with N, S-di-CBZ-L-cysteine with BOP and DIEA afforded the N, S-di-CBZ-L-Cys-(O-t-butyl)-L-Thr-Gly-CAM ester 14 in 50% yield. Removal of the CAM ester with aqueous Na₂CO₃ followed by acidification with aqueous citric acid generated the N, S-di-CBZ-L-Cys-(O-t-butyl)-L-Thr-Gly acid 15 in 77% yield.

The design of the His-Gly fragment **17** was predicated on obtaining efficient coupling between the N $_{\alpha}$ His group and reagent **1**. Previous work by Yamashiro et al. [J. Am. Chem. Soc., Vol. 94, pages 2855-2859 (1972)] demonstrated that t-butyl-carbonylation of the imidazole side chain prior to the condensation step substantially
5 increased coupling yields between systems containing His residues and N-BOC groups. For this reason, the His-Gly fragment **17** was assembled in two steps. First, the condensation of glycine t-butyl ester and N $_{\alpha}$ -CBZ-L-His with diphenyl-phosphorylazide (DPPA) [Shioiri et al., J. Am. Chem. Soc., Vol. 94, No. 17, pages 6203-6205 (1972)] afforded N $_{\alpha}$ -CBZ-L-His-Gly t-butyl ester **16** (72%). In the second
10 step, the His side chain was masked by the treatment with di-t-butyl dicarbonate in THF to give the N $_{\alpha}$ -CBZ-N $_{im}$ -BOC-L-His-Gly t-butyl ester **17** in 78% yield.

As shown in Fig. 1, the final hexapeptide **12** was constructed stepwise from the three aforementioned fragments, i.e., **1**, **15** and **18**. Hydrogenolysis of the N $_{\alpha}$ -CBZ group of **17** provided the amine HCl salt (74%) which was condensed with
15 hypusine reagent **1** to give the di-CBZ-THP protected Hpu-His-Gly tripeptide **18** in 85% yield. The amine generated by treatment of the N $_{\alpha}$ -FMOC in **18** with 4-aminomethyl-piperidine (68%) [Beyermann et al., J. Org. Chem., Vol. 55, pages 721-728 (1990)] was acylated by the tripeptide acid **15** and BOP to give the masked conjugate **19** in 81% yield. The final deprotection of **19** with HBr/acetic acid in TFA
20 and a "cocktail" of scavengers (phenol, pentamethylbenzene, triisopropylsilane, 1,2-ethanedithiol) at room temperature developed by Wang et al. [Int. J. Peptide. Res., Vol. 40, pages 344-349 (1992)] gave the Cys-Thr-Gly-Hpu-His-Gly as its tetrakis-trifluoroacetic acid salt **12** in 22% yield. The peptide was fully assigned by DOCOSY, TOCSY and HMOC NMR.

Polymer bounded synthesis of the hexapeptide **12** was performed on a HMP-resin [Wang, *supra*] using an Applied Biosystems 432A Synthesizer. The Cys and His residues of the hexapeptide were initially protected with trityl groups while the Thr was protected as its t-butyl ether. Attempts to release the free hexapeptide by
5 refluxing in TFA/phenol or with HBr/acetic acid in TFA with phenol and pentamethylbenzene, respectively, did not succeed. Use of the more labile 4-methoxytrityl and 4-methyltrityl groups to protect the Cys and His derivatives, respectively, produced the protected hexapeptide **20** (Fig. 2). Final deprotection of **20** was then achieved with HBr/acetic acid in TFA using a "cocktail" of four cation scavengers. This method
10 generated very few side products and provided **12** after purification by HPLC in 24% yield. All analytical data were consistent with hexapeptide **12** * 4 TFA previously prepared by the solution-phase method.

In summary, the hypusine reagent described has been demonstrated to be a highly useful synthon in accessing the eIF-5A pentapeptide sequence. While the
15 yields are generally excellent for these kinds of systems, the most notable feature is the flexibility that this methodology offers in synthesizing related eIF-5A mimics.

EXAMPLE 1

FMOC-Thr(O-t-butyl)-Gly Carboxyamido

20 methyl (CAM) Ester (14)

N_α-BOC-Gly CAM ester [Martinez et al., *Tetrahedron*, *supra*] (1.16 g, 5.0 mmol) was dissolved in TFA (10 ml) and stirred 10 minutes at 0° C. The amine TFA salt was precipitated with diethyl ether (130 ml), filtered and dried (0.75 g, 60%). The amine salt (0.75 g) was then dissolved in a dry DMF (10 ml) solution containing
25 FMOC-Thr (O-t-butyl)-OH (1.20 g, 3.0 mmol) and BOP (1.33 g, 3.0 mmol). The

solution was cooled to 0° C, and diisopropylethylamine (DIEA, 1.15 ml, 6.6 mmol) was added dropwise. The solution was warmed to room temperature and stirred overnight. The volatiles were removed in vacuo, the resulting oil was dissolved in ethyl acetate (200 ml) and washed with 1 M citric acid, water, 5% aqueous NaHCO₃ solution and water. The organic layer was dried over MgSO₄ and concentrated. Flash chromatography (90% ethyl acetate/hexane) gave **14** as a colorless solid (1.30 g, 85%). ¹H NMR (CDCl₃) δ 7.77(d, 2H, J = 7.5 Hz), 7.65 (m, 1H), 7.60 (d, 2H, J = 7.5 Hz), 7.45- 7.27 (m, 4H), 6.60 (s br, 1H), 5.87 (d br, 1H), 5.54 (s-br, 1H), 4.67 (m, 2H) , 4.51 – 4.35 (m, 2H), 4.28 – 4.12 (m, 4H), 4.05 (m, 1H), 1.29 (s, 9H), 1.08 (d, 3H, J = 6.4 Hz). Anal. calcd. for C₂₇H₃₃N₃O₇: C 63.39; H 6.50, N 8.21. Found: C 63.14; H 6.60, N 8.10. [α]_D²² -0.8° (c = 1.00, CH₃OH).

EXAMPLE 2

CBZ-Cys (CBZ) -Thr (O-t-butyl) -Gly

Carboxyamidomethyl Ester (15)

A solution of **14** (0.46 g, 0.90 mmol) was dissolved in a solution of diethylamine (DEA, 0.8 ml) in dry DMF (8 ml). After stirring overnight at room temperature, the volatiles were removed in vacuo and the residue dissolved in 15 ml dry DMF. N, S-di-CBZ-L-Cys (0.35 g, 0.90 mmol) and BOP (0.40 g, 0.90 mmol) were added and the turbid solution cooled to 0° C. DIEA (0.25 g, 1.89 mmol) was added, and the solution was stirred at room temperature for 2 days. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate (100 ml) and washed with 10% citric acid, water, 5% aqueous NaHCO₃ solution and water. The organic layer was dried over MgSO₄ and concentrated in vacuo. The resulting oil was purified by flash column chromatography (80% ethyl acetate/10% hexane/10%

CHCl₃ to obtain **15** (0.29 g, 50%). mp 95 – 97°C. ¹H NMR (CDCl₃) δ 7.70 (m, 1H), 7.28 (s, 10H), 6.78 (s, 1H), 6.00 (s, 1H), 5.50 (s, 1H), 5.23 (s, 2H), 5.17 (s, 2H), 4.62 (m, 2H), 4.41 (m, 1H), 4.30 (m, 1H), 4.25 (m, 1H), 4.15 (m, 1H), 3.94 (m, 1H), 3.30 (m, 2H), 1.65 (s, 1H), 1.20 (s, 9H), 1.07 (d, 3H). Anal. calcd. for C₃₁H₄₀N₄O₁₀S: C 56.35; H 6.10; N 8.48. Found: C 56.62; H 6.14, N 8.38. [α]²⁵_D – 14° (c = 1.10, CH₃OH).

EXAMPLE 3

CBZ-Cys(CBZ)-Thr(O-t-butyl)-Gly-OH (16)

To a solution of **15** (0.90 g, 1.36 mmol) dissolved in DMF (14 ml) was added aqueous Na₂CO₃ (2.72 mmol, 8 ml) at room temperature. The solution warmed upon addition and water was added until the solution was clear. After 5 minutes, the pH was adjusted to 6 with citric acid (0.5 N, 9 ml). The reaction mixture was concentrated in vacuo; the residue was dissolved in ethyl acetate and washed with 0.5 N citric acid. The organic layer was dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography using LH-20 lipophilic Sephadex (50 g; eluting with 1% EtOH/toluene) to give **16** as a white solid (0.63 g, 77%). mp 124 – 125°C; ¹H NMR (CD₃OD) δ 7.39 – 7.25 (m, 10H), 5.24 (s, 2H), 5.12 (s, 2H), 4.46 (dd, 1H, J = 8.6, 5.0 Hz), 4.33 (d, 1H, J = 3.0 Hz), 4.16 (m, 1H), 3.92 (m, 2H), 3.45 (dd, 1H, J = 14.3, 5.0 Hz), 3.16 (dd, 1H, J = 14.3, 8.6 Hz), 1.20 (s, 9H), 1.09 (d, 3H, J = 6.4 Hz). Anal. calcd. for C₂₉H₃₇N₃O₉S: C 57.70; H 6.18; N 6.96. Found: C 57.76; H 6.24; N 7.02. [α]²²_D +8.6° (c = 0.50, CHCl₃).

EXAMPLE 4**CBZ-His-Gly-O-tert-Butyl (17)**

Glycine t-butyl ester hydrochloride (2.20 g, 13.0 mmol) and N $_{\alpha}$ -CBZ-L-His (3.44g, 11.9 mmol) were dissolved in dry DMF (40 ml). The solution was cooled to 0°C under an N₂ atmosphere, and diphenylphosphoryl azide (DPPA, 3.58 g, 13.0 mmol) was added dropwise over 5 minutes. The clear solution was stirred for 10 minutes and turned turbid upon addition of triethylamine (2.63 g, 26 mmol). The solution was stirred at room temperature overnight. The solution was concentrated and the residue purified by flash chromatography (8% EtOH/CHCl₃) to give **17** as a white solid (3.45 g, 72%). mp 64 - 66°C. ¹H NMR (CD₃OD) δ 7.56 (d, 1H, J = 1.1 Hz), 7.30 (m, 5H), 6.88 (s, 1H), 5.04 (m, 2H), 4.42 (m, 1H), 3.80 (m, 2H), 3.12 (m, 1H), 2.91 (m, 1H), 1.46 (s, 9H). Anal. calcd. for C₂₀H₂₆N₄O₅: C 59.69; H 6.51; N 13.92. Found: C 59.43; H 6.46; N 13.81.

EXAMPLE 5**CBZ-His(BOC)-Gly-O-tert-Butyl (18)**

A solution of di-t-butyl dicarbonate (1.33 g, 6.0 mmol) in 3 ml THF was added dropwise at 0°C to a solution of **17** (2.0 g, 5.0 mmol) in 15 ml THF. The reaction mixture was stirred overnight at room temperature and concentrated in vacuo. The residue was purified by flash chromatography (60% ethyl acetate/hexane) to give **18** (1.96 g, 78%). ¹H NMR (CDCl₃) δ 8.00 (s, 1H), 7.30 (m, 6H), 6.60 (m, 1H), 5.10 (s, 2H), 4.58 (m, 1H), 3.85 (m, 2H), 3.03 (m, 2H), 2.38 (s, 1H), 1.60 (s, 9H), 1.40 (s, 9H). Anal. calcd. for C₂₀H₂₆N₄O₅: C 59.75; H 6.82; N 11.15. Found: C 59.69; H

6.87; N 11.09. $[\alpha]^{22}_D -4.0^\circ$ (c = 1.00, CH₃OH).

EXAMPLE 6

Fmoc-Hpu(N7,N12-di-CBZ, O9-THP)–

His(BOC)-Gly-O-tert-Butyl (19)

5 To a solution of **18** (185 mg, 0.37 mmol) and 1 N HCl (370 μ l) in ethanol (15 ml) was added 10% Pd–C (18 mg), and the reaction mixture was hydrogenated for 2.5 hours at room temperature. The reaction mixture was filtered through Celite, concentrated in vacuo and purified by flash chromatography (CHCl₃/EtOH = 9:1) to give H-His-(BOC)-Gly-O-t-Bu hydrochloride (101 mg, 68%). A portion of this
10 ammonium salt (69 mg, 0.17 mmol) and hypusine reagent **1** (134 mg, 0.17 mmol) were dissolved in 12 ml dry DMF. The solution was cooled to 0° C and BOP (87 mg, 0.20 mmol) was added and stirred for 20 minutes. DIEA (47.5 mg, 0.37 mmol) was added dropwise at 0° C and the solution warmed to room temperature and stirred overnight. The volatiles were removed under reduced pressure, and the residue was
15 dissolved in ethyl acetate and washed with 5% aqueous NaHCO₃ solution and water. The organic layer was dried over MgSO₄ and concentrated in vacuo and the residue purified by flash chromatography (4% EtOH/CHCl₃) to give **19** as a colorless oil (165 mg, 85%). ¹H NMR (600 MHz) (CD₃OD) δ 7.86 (m, 1H), 7.70 (d, 2H, J = 7.5 Hz), 7.58 (m, 2H), 7.31 – 7.11 (m, 15H), 5.00 (m, 2H), 4.92 (s, 2H), 4.60 (m, 1H), 4.50 –
20 4.32 (m, 1H), 4.30 – 4.26 (m, 2H), 4.08 (t, 1H, J = 6.8 Hz), 3.90 (m, 1H), 3.86 – 3.52 (m, 5H), 3.38 – 2.76 (m, 8H), 1.70 – 1.12 (m, 14H), 1.40 (s, 9H), 1.28 (s, 9H). HRMS m/z calcd. for C₆₃H₈₀N₇O₁₄ 1158.5763; found 1158.5739. $[\alpha]^{26}_D -1.5^\circ$ (C = 1.00, CHCl₃).

EXAMPLE 7**CBZ-Cys(CBZ)-Thr(O-t-Bu)-Gly-Hpu(N7, N12-di-CBZ,****O9-THP)-His(BOC)-Gly-O-t-Bu (20)**

4-(Aminomethyl)-piperidine (1.0 ml) was added to **19** (156 mg, 0.14 mmol) dissolved in CHCl₃ (10 ml). The clear solution was stirred for 2 hours at room temperature. An additional portion of 4-(aminomethyl)-piperidine (1.0 ml) was added and stirring was continued another 40 minutes. The reaction mixture was taken up in 50 ml CHCl₃ and extracted three times with phosphate buffer (pH = 5.5, 75 ml each). The organic layer was dried over Na₂SO₄, concentrated and purified by flash chromatography on silica (10% MeOH/CHCl₃) to give H-Hpu (N7, N12-di-CBZ, O9-THP) – His(BOC)-Gly-O-t-Bu as a colorless oil (88 mg, 68%). A portion of the colorless oil (10 mg, 11 μmol) and **16** (7 mg, 11 μmol) in dry DMF (2 ml) was cooled to 0°C. BOP (6 mg, 12 μmol) was added and stirred for 30 minutes. Diisopropylethylamine (4.5 μl, 26 μmol) was added dropwise and the solution was warmed to room temperature and stirred overnight. The reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (15 ml) and extracted with 5% aqueous NaHCO₃ solution (10 ml) and water (10 ml). The organic layer was concentrated under reduced pressure and the residue purified by flash chromatography (10% EtOH/CHCl₃; R_f = 0.35) to give **20** as a colorless oil (13 mg, 81%). ¹H NMR (600 MHz) (CD₃OD) δ 7.99 (m, 1H), 7.28 – 7.14 (m, 21H), 5.17 – 5.07 (m, 2H), 5.06 – 4.88 (m, 6H), 4.58 (dd, 1H, J = 9.0, 4.2 Hz), 4.51 – 4.32 (m, 2H), 4.26 (m, 1H), 4.14 (m, 1H), 4.10 (m, 1H), 4.08 – 3.98 (m, 2H), 3.86 – 3.58 (m, 6H), 3.40 – 2.94 (m, 8H), 2.88 (m, 1H), 1.70 – 0.94 (m, 17H), 1.48 (s, 9H), 1.34 (s, 9H),

1.08 (s, 9H). HRMS m/z calcd. for $C_{77}H_{105}N_{10}O_{20}S$ 1521.7227; found 1521.736.

$[\alpha]^{26}_D -1.2^\circ$ (c = 1.00, $CHCl_3$).

EXAMPLE 8

Cys-Thr-Gly-Hpu-His-Gly, Trifluoroacetic Acid Salt (12)

5

Method (a)

A solution of **20** (13 mg, 7.2 μ mol) and phenol (5 mg, 50 μ mol) was heated to reflux for 90 minutes in degassed TFA (5.0 ml) under an argon atmosphere. The reaction mixture was concentrated in vacuo and the residue applied to a C-18 plug (Supelco; water/acetonitrile = 88/22 + 0.1% TFA). Further purification was performed by preparative HPLC (solvent systems A, aqueous 0.1% TFA; and B, 0.1% FA in CH_3CN ; linear gradient of 0-20% B in 50 minutes; flow rate 4.0 ml/minute; detection at 214 nm; retention time = 8.4 minutes) using a C-18 reverse phase column (Dynamax 300 A C_{16}) to give **12** as a colorless oil (2 mg, 24%). 1H NMR (D_2O) (600 MHz) δ 8.69 (d, 1H, J = 1.2 Hz), 7.38 (s, 1H), 4.80 (m, 1H), 4.49 (d, 1H, J = 4.9 Hz), 4.41 (t, 1H, J = 5.6 Hz), 4.36 (dd, 1H, J = 8.5, 6.0 Hz), 4.29 (m, 1H), 4.11 (m, 1H), 4.09 – 4.00 (m, 4H), 3.37 (dd, 1H, J = 15.5, 7.2 Hz), 3.29 – 3.06 (m, 9H), 1.99 (m, 1H), 1.86 (m, 2H), 1.76 (m, 3H), 1.45 (m, 2H), 1.32 (d, 3H, J = 6.4 Hz). MS (MALDI-Tof) m/z calcd. for $C_{27}H_{48}N_{10}O_9S$ 688.33 (M^+), found 688.96.

20

Method (b)

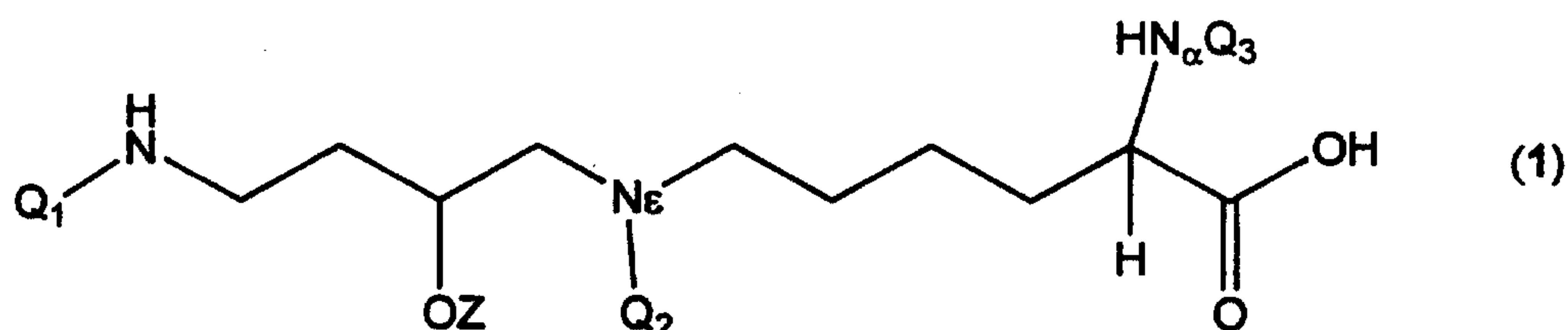
The polymer-bound peptide **21** was synthesized using an Applied Biosystems 432A Synthesizer. Amino acid analysis for **21**: Gly 2.09, His 1.03, Thr 0.88. An aliquot of **21** (49 mg, 19.3 μ mol), phenol (250 mg) and pentamethylbenzene (250 mg) were dissolved in degassed TFA (5.0 ml) at 0°C. Saturated HBr in acetic acid

25

solution (0.2 ml), triisopropylsilane (0.1 ml) and 1, 2-ethanedithiol (0.1 ml) were added under an argon atmosphere. The solution was stirred at room temperature for 1 hour and concentrated under reduced pressure. The residue was dissolved in 10% acetic acid (10 ml) and extracted with methyl tert-butyl ether (3 x 25 ml). The aqueous layer was concentrated in vacuo and the residue was purified on a preparative HPLC as in method (a) above using a C-18 reverse phase column (Dynamax 300 A C₁₈) to give **12** as a colorless oil (5.2 mg, 24%). ¹H NMR and MS analytical data were identical to those for **12** prepared by method (a) above. $[\alpha]_D^{26} - 16.7^\circ$ (c = 0.30, H₂O). Amino acid analysis: Gly 1.94, His 1.02, Thr 1.04.

I claim:

1. A peptide containing a hypusine moiety, wherein said peptide is synthesized according to a method of preparing peptides comprising the use of a hypusine reagent having the formula:



wherein: Q_1 and Q_2 may be the same or different and are amino protective groups;

Q_3 is an amino protective group which is orthogonal to Q_1 and Q_2 ; and

Z is a hydroxy protective group.

10 2. A peptide of claim 1 wherein said hypusine moiety is the (2S, 9R), (2R, 9S), (2S, 9S) or the (2R, 9R) diastereomer.

3. A peptide of claim 1 comprising the hexapeptide:

L-CYS-L-THR-GLY-HPU-L-HIS-GLY.

15

4. A peptide of claim 3 wherein said -HPU- linkage is the (2S, 9R), (2R, 9S), (2S, 9S) or the (2R, 9R) diastereomer thereof.

5. A peptide of claim 1 comprising the tripeptide:

HPU-L-HIS-GLY.

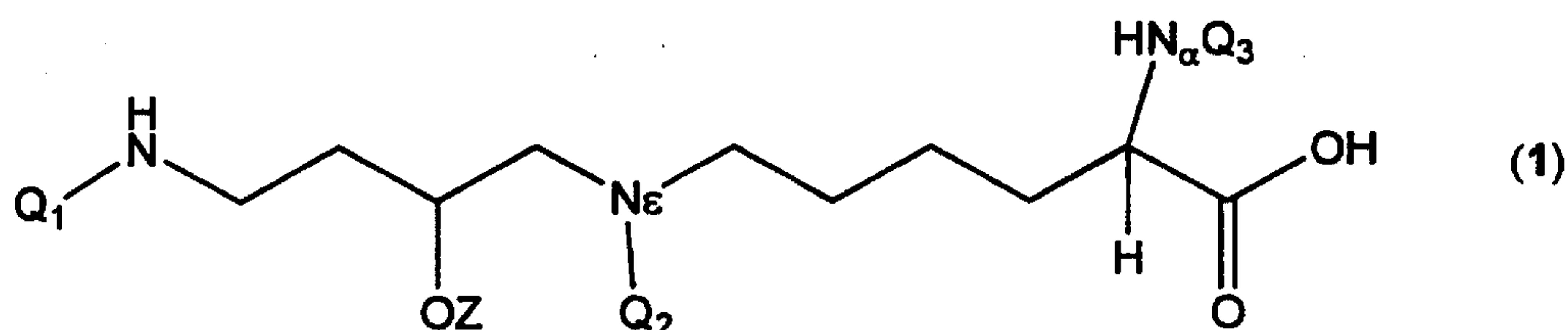
6. A peptide of claim 5 wherein said –HPU– linkage is the (2S, 9R), (2R, 9S), (2S, 9S) or the (2R, 9R) diastereomer thereof.

5 7. A peptide of claim 1 comprising the pentapeptide:

L-THR-GLY-HPU-L-HIS-GLY.

8. A pentapeptide of claim 9 wherein said –HPU– linkage is the (2S, 9R), (2R, 9S), (2S, 9S) or the (2R, 9R) diastereomer thereof.

10 9. In a method of preparing peptides, the improvement comprising synthesizing a peptide containing a hypusine moiety using a hypusine reagent having the formula:



wherein: Q_1 and Q_2 may be the same or different and are amino protective groups;

15 Q_3 is an amino protective group which is orthogonal to Q_1 and Q_2 ; and

Z is a hydroxy protective group.

10. A method according to claim 9 wherein said method of preparing peptides comprises synthesis of a peptide chain on an insoluble support.

20

11. A peptide of formula:



wherein Hpu is a hypusine amino acid residue, S and T are peptide residues each independently comprising from zero to about 12 amino acids.

5 12. A peptide according to claim 11, wherein said peptide is L-Cys-L-Thr-Gly-hypusine-L-His-Gly.

 13. A peptide according to claim 11, wherein said peptide is L-Ser-L-Thr-L-Ser-L-Lys-L-Thr-Gly-hypusine-L-His-Gly-L-His-L-Ala-L-Lys.

10

 14. A peptide according to claim 11, wherein said peptide is hypusine-L-His-Gly.

 15. A peptide according to claim 11, wherein said peptide is L-Thr-Gly-hypusine-L-His-Gly.

15

 16. A peptide according to claim 11, wherein said peptide is L-Cys-L-Thr-Gly-Hpu-L-His-Gly-OH.

20 17. A peptide according to claim 11, wherein said peptide is L-Lys-L-Thr-Gly-hypusine-L-His-Gly.

 18. A peptide of formula (2) synthesized by utilizing the reagent of claim 1.

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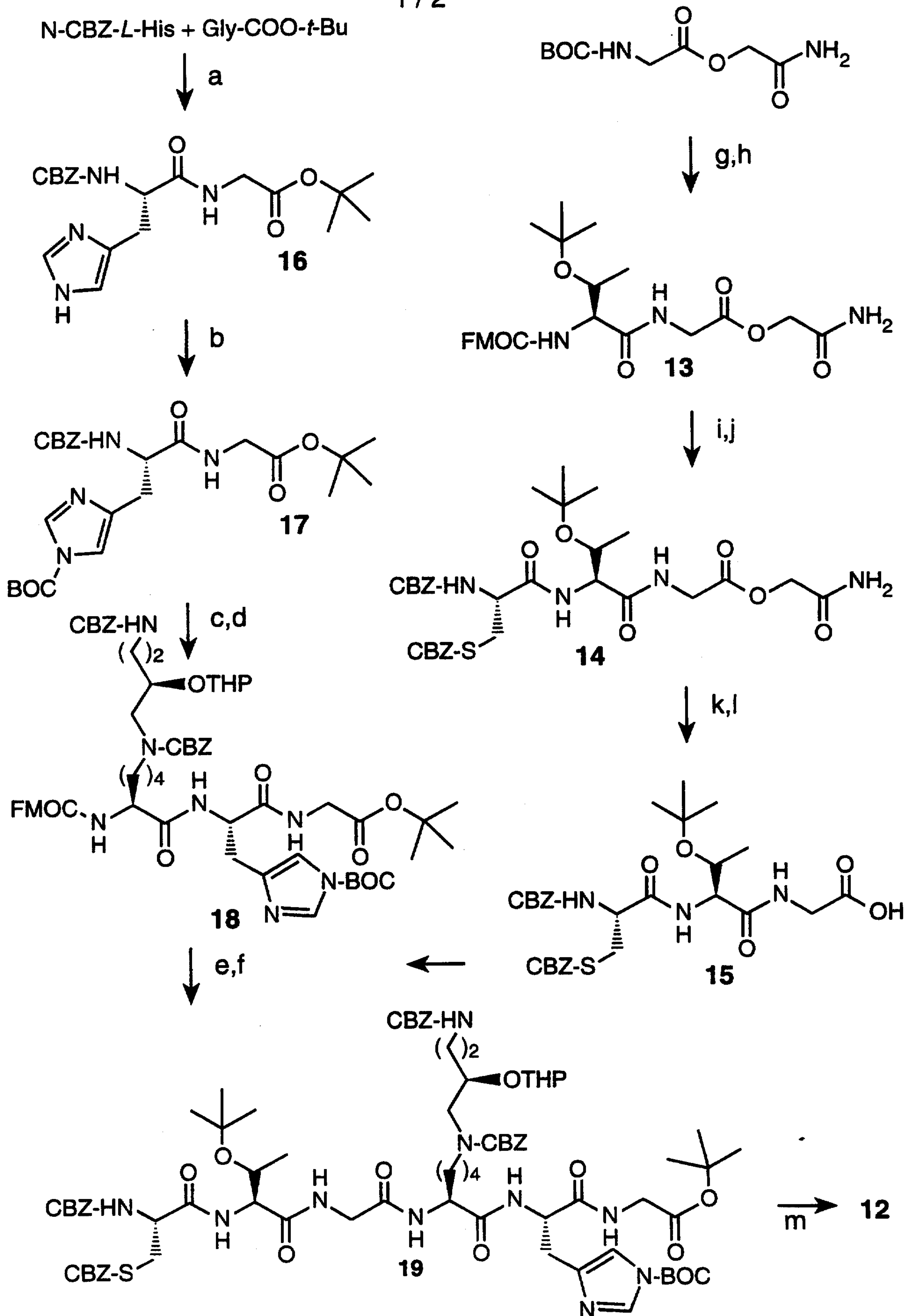


FIG. 1
SUBSTITUTE SHEET (RULE 26)

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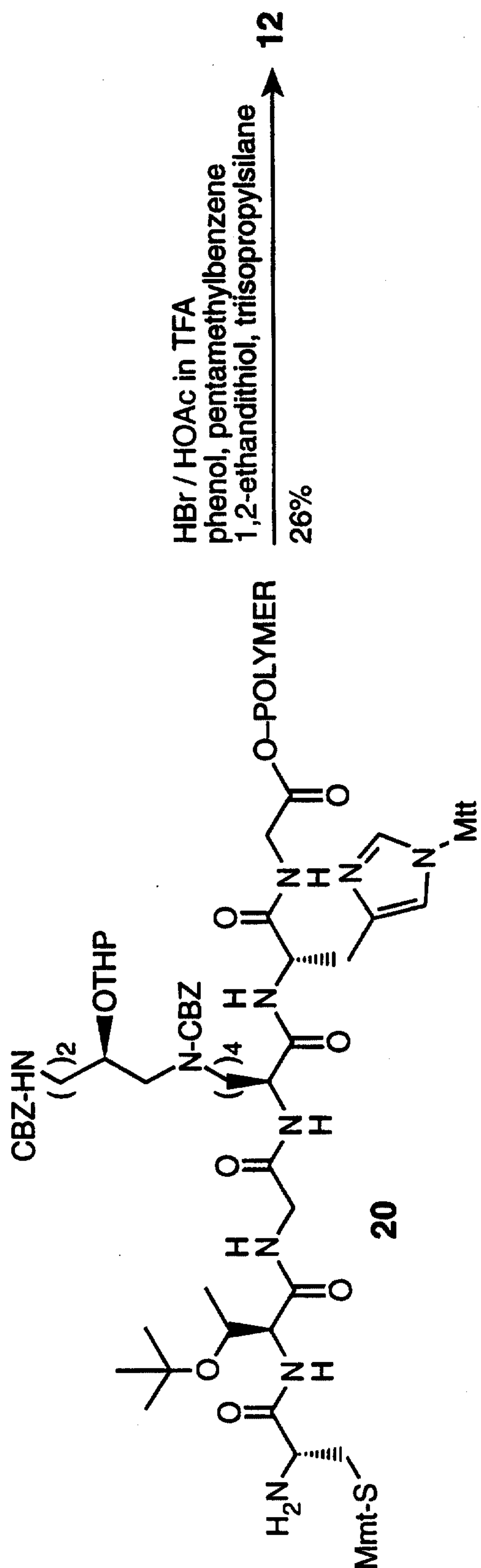


FIG.-2