(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 199747483 B2**
(10) Patent No. **728398**

(54) Title
Acylated cyclodextrin derivatives

(51)[7] International Patent Classification(s)
C08B 037/16 **A61K 047/48**

(21) Application No: **199747483**

(22) Application Date: **1997.10.06**

(87) WIPO No: **WO98/20044**

(30) Priority Data

(31) Number	(32) Date	(33) Country
08/740778	**1996.11.01**	**US**

(43) Publication Date : **1998.05.29**
(43) Publication Journal Date : **1998.07.23**
(44) Accepted Journal Date : **2001.01.11**

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OPI DATE 29/05/98 APPLN. ID 47483/97
AOJP DATE 23/07/98 PCT NUMBER PCT/US97/18105
AU9747483

CT)

(51) International Patent Classification 6 :
C08B 37/16, A61K 47/48

A1

(11) International Publication Number: WO 98/20044

(43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/US97/18105

(22) International Filing Date: 6 October 1997 (06.10.97)

(30) Priority Data:
08/740,778 1 November 1996 (01.11.96) US

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(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, 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, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ACYLATED CYCLODEXTRIN DERIVATIVES

(57) Abstract

A cyclodextrin derivative, wherein at least 60 percent of the free hydroxy groups of said cyclodextrin are acylated with acyl groups where at least one of said acyl groups comprise a free carboxylic group.

ACYLATED CYCLODEXTRIN DERIVATIVES

Background of the Invention

In 1904, Schardinger first characterized cyclodextrins as cyclic oligosaccharides. The α, β, and γ-cyclodextrins, which consist of six, seven, and eight glucose units, respectively, are the most common natural cyclodextrins.

Cyclodextrins have been used as inclusion complexes by complexing with a guest compound or molecule as a host compound or molecule. Such inclusion complexes have been used to mask the bitter taste or unpleasant odor of a guest compound, to solubilize a hardly soluble guest compound, to enlarge the stability of a guest compound against heat, light, or air, to stabilize emulsions, or as a sustained release preparation using a hydrophobic alkylated cyclodextrin. See U.S. Patent No. 4,869.904. However, no one has attempted to make carboxyacylated cyclodextrins, or used such cyclodextrin derivatives to form ionic sustained release compositions.

Summary of the Invention

In one aspect, the present invention features a cyclodextrin derivative, wherein at least 60 percent of the free hydroxy groups of said cyclodextrin are acylated with acyl groups where at least one of said acyl groups comprises a free carboxylic group;

said cyclodextrin is α-cyclodextrin, β-cyclodextrin, or γ-cyclodextrin; and

said acyl groups are selected from COE_1, where E_1 is selected from the group consisting of C_{2-32} carboxy alkyl, C_{3-32} carboxy alkenyl, C_{7-37} carboxyaryl, C_{8-38} carboxyaryl alkyl, and C_{9-39} carboxyaryl alkenyl and COE_2, where E_2 is selected from the group consisting of C_{1-30} alkyl, C_{2-30} alkenyl, C_{6-36} aryl, C_{7-37} arylalkyl, and C_{8-38} aryl alkenyl, wherein at least one of said acyl group is COE_1.

In a preferred embodiment, between 10 and 80 (e.g., between 30 and 80) percent of the free hydroxy groups of the cyclodexrin are acylated with COE_1 and between 10 and 80 (e.g., between 15 and 60) percent of the cyclodextrin are acylated with COE_2. In still a further preferred embodiment, E_1 and C_{2-10} carboxy alkyl (e.g., COE_1 is $CO(CH_2)_nCOOH$ (where n = 2-3)) and E_2 is C_{1-10} alkyl (e.g., COE_2 is $CO(CH_2)_nCH_3$ (where n = 0-5)).

In another aspect, the invention features a copolymer comprising the cyclodextrin derivative described above, wherein the cyclodextrin derivative comprises at least one free hydroxy group which is acylated with a polyester comprising hydroxy acid monomers. In one embodiment, the copolymer has an average molecular weight of between 500 - 40,000 daltons (e.g., 500 - 10,000). In a further embodiment, the polyester comprises hydroxy acid monomers selected from the group consisting of lactic acid, glycolic acid, hydroxy caproic acid, or any optically active isomer thereof. Such polyesters can be manufactured by reacting said cyclodextrin derivative with lactide, glycolide, caprolactone, p-dioxanone, trimethyl carbonate, or any optically active isomer thereof.

In still another aspect, the invention features a composition comprising the cyclodextrin derivative described above and a drug, the drug comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of the drug present in the composition is ionically bonded to the cyclodextrin derivative. In one embodiment, the composition comprises between 1 and 30 (e.g., between 10 and 20) percent, by weight, of the drug. In a further embodiment, the drug is a polypeptide. In still a further embodiment, the polypeptide comprises between 4 and 200 amino acids

(e.g., between 4 and 50 amino acids). Examples of the polypeptide include somatostatin, bombesin, calcitonin, amylin, parathyroid hormone, parathyroid hormone related protein, gastrin releasing peptide, luteinizing hormone releasing hormone, growth hormone, growth hormone releasing factor, interferons, erythropoietin, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal peptide, thyrotropin releasing hormone, corticotropin releasing hormone, Acetyl-Ser-Asp-Lys-Pro, arginine vasopressin, angiotensin, and any fragments, agonists, or antagonists thereof.

In yet another aspect, the invention features a composition comprising the copolymer described above and a drug, the drug comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of the drug present in the composition is ionically bonded to the cyclodextrin derivative. In one embodiment, the composition comprises between 1 and 30 (e.g., between 10 and 20) percent, by weight, of the drug. In a further embodiment, the drug is a polypeptide.

As used herein, "lower alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, sec-butyl, and the like. "Lower alkenyl" groups include those branched and straight chain aliphatic hydrocarbon groups having one or several double bonds. Examples of alkenyl groups include vinyl, allyl, isopropenyl, butenyl, pentenyl, hexenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, isoprenyl, and the like. All alkyl, alkenyl, and alkynyl groups are noncyclic.

As used herein, "aryl" is intended to include any stable monocyclic, bicyclic, or tricyclic carbon ring(s) of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of aryl groups include phenyl, naphthyl, anthracenyl, biphenyl, tetrahydronaphthyl, indanyl, phenanthreynl, and the like. The term "carboxy" is meant to include the recited chemical group (e.g., alkyl, alkenyl, aryl, arylalkyl, arylalkenyl) substituted with 1 to 3 carboxy groups.

Other features and advantages of the present invention will be apparent from the detailed description of the invention, and from the claims.

Throughout this specification and the claims, the words "comprise", "comprises" and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

Description of the Invention

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Example 1: Preparation of Acylated β-Cyclodextrin (ACD)

Cyclodextrin (Amaizo, American Maize Products Corp. Hammond, IN) was dried at 90°C under reduced pressure (0.1 mm Hg) to a constant weight. Dried cyclodextrin (CD) was then transferred to a reaction flask equipped for stirring. After purging with argon, the CD was heated at 50°C for 30 min. at 0.1 mm Hg,

cooled to 25°C, and repurged with dry argon. Calculated amount of the acylating reagent, as a liquid anhydride (except in ADC No. 1 where the anhydrides were dissolved in 15 ml of acetic acid), containing a catalytic amount (i.e., 1 percent by weight) of p-toluene sulfonic acid (except in ACD No. 1 and ACD No. 2 where 1 percent H_2SO_4 was used instead of p-toluene sulfonic acid) was transferred to the reaction flask, mixed under a dry argon atmosphere, and then heated. The amount of CD and acylating agents used as well as the heating scheme is described in Table I. At the conclusion of the reaction, the resulting mixture was allowed to cool slightly and then poured on to a vigorously stirring ice-water mixture. The resulting precipitate was filtered, rinsed several times with cold water, and air dried. The product was then isolated and dried until constant weight under vacuum, first at 25°C and then 50°C. The product was characterized for equivalent weight, as reported in Table I, by measuring titratable carboxylic acid functionally using benzyl alcohol solution of the product and potassium hydroxide in benzyl alcohol with bromophenol red as an indicator.

TABLE I. Preparation and Properties of Acylated β-Cyclodextrins (ACD)

ACD No.	REACTANTS[1]	HEATING SCHEME Temp (°C)/ Time (min.)	EQUIVALENT WEIGHT (Da)
1	51.0g CD, 55.0g A, 28.7g S	95/180	1104
2	25.5g CD, 28.1g A, 14.4g S	95/135	874
3	12.8g CD, 9.69g A, 10.8g G	95/120	561
4	12.8g CD, 18.0g P, 8.20g G	95/15; 100/30; and 105/30	829
5	12.7g CD, 12.2g P, 10.7g G	90/60	657
6	12.7g CD, 10.1g P, 13.9g G	70/20	524
7	12.7g CD, 10.1g P, 13.9g G	60/10 and 65/30	511
8	12.7g CD, 12.3g B, 13.9g G	60/10 and 65/45	574
9	12.7g CD, 8.90g B, 20.5g G	65/10 and 60/50	401
10	12.7g CD, 5.31g B, 20.4g G	65/5 and 60/50	346

[1] CD is β-Cyclodextrin, A is Acetic Anhydride, P is Propionic Anhydride, B is Butyric Anhydride, S is Succinic Anhydride, and G is Glutaric Anhydride

Example 2: Grafting Acylated Cyclodextrin (G-ACD) with Lactones

Predetermined amounts of the above acylated cyclodextrin derivative (ACD), lactone or mixture of lactones, and a catalytic amount (i.e., <0.2 percent by weight) of stannous octoate were transferred to a dry polymerization flask equipped for stirring, under an inert dry atmosphere. The amount of ACD and lactones used are described in Table II. The mixture was then heated under vacuum at about 45°C for 30 min., cooled to room temperature, and then purged with dry argon. The reactants were then heated while stirring as described in Table II. At the conclusion of the reaction, the temperature was lowered to abut 110°C, and vacuum was applied for 0.5 to 1 hr. to remove distillable volatiles.

The polymerization flask was cooled to room temperature and purged with argon. The grafted copolymer product (G-ACD) was isolated, dissolved in acetone, and then precipitated in ice water. The precipitate was filtered and air dried. The resulting powder was further dried under vacuum to a constant weight. The product was characterized for equivalent weight, as reported in Table II, by measuring titratable carboxylic acid functionality.

TABLE II. Preparation of Lactone-Grafted ACD (G-ACD)

G-ACD No.	REACTANTS[1]	HEATING SCHEME Temp (°C)/ Time (Hr.)	EQUIVALENT WEIGHT (Da)
1	2.50 ACD No. 4, 7.90g L, 2.10g G	150/6	2060
2	2.50 ACD No. 3, 7.90g L, 2.10g G	150/7	1055
3	4.00 ACD No. 7, 6.32g L, 1.68g G	150/6.5	1100
4	2.67 ACD No. 7, 6.32g L, 1.68g G	150/10.5	1148
5	3.30 ACD No. 7, 6.27g CL, 0.34g G	150/10	1072
6	2.66 ACD No. 8, 6.32g L, 1.68g G	150/7	945
7	5.34 ACD No. 9, 12.65g L, 3.37gG	150/3.5	681

[1] G is glycolide, L is D,L-Lactide, and CL is ϵ-Caprolactone

Example 3: Preparation of Polypeptide Composition Containing of Acylated β-Cyclodextrin (U-CON)

Predetermined amount of the above acylated β-cyclodextrin (ACD) was dissolved in a minimum volume of acetone (from 5-15 weight/volume percent) and filtered through a micro-syringe with 0.45 μm porous filter. The filtrate was cooled and a 1N sodium hydroxide aqueous solution was added to neutralize carboxylic groups in the

ACD. A concentrated, cold solution (3-15 weight/volume percent) of the acetate salts of the polypeptides Lanreotide™ (D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2; Kinerton Ltd., Dublin, Ireland) or Decapeptyl™ (pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2; Kinerton Ltd.) was added to acetone solution of the neutralized ACD dropwise with stirring. The product was left at 25°C for 0.5-1 hr. and then precipitated in a stirring ice-water bath. The resulting precipitate was filtered, rinsed thoroughly with water, and air dried. The product was then isolated and dried under vacuum to constant weight at room temperature. The weight of the dried composition was determined, and the composition was characterized for percent content of peptide, as measured by elemental analysis of the percent of nitrogen present in the composition (Quantitative Technologies, Inc., Whitehouse, NJ), as reported in Table III. The particulate product was reduced in size by grinding to achieve an average particle size of about 100 μ before storage under reduced pressure.

TABLE III. Preparation and Properties of Polypeptide/ Acylated β-Cyclodextrin Conjugates (U-CON)

U-CON No.	REACTANTS[1]	PERCENT NITROGEN	PERCENT PEPTIDE
1	0.11g ACD No. 1, 0.01g L	0.54	3.84
2	0.10g ACD No. 2, 0.02g L	1.40	9.96
3	0.035 ACD No. 4, 0.016g L	1.42	10.11
4	0.08g ACD No. 3, 0.028g L	2.47	17.58
5	0.074g ACD No. 5, 0.028g L	2.72	19.36
6	0.819g ACD No. 5, 0.215g L	2.18	15.50
7	0.811g ACD No. 5, 0.212g L	2.00	14.20
8	0.803g ACD No. 4, 20.199g L	2.42	17.20
9	3.00g ACD No. 5, 0.502g L	2.20	15.60
10	2.005g ACD No. 4, 0.500g D	2.49	17.70
11	0.201g ACD No. 7, 0.051g D	2.19	12.10
12	0.402g ACD No. 6, 0.131g D	3.75	20.72
13	0.??? ACD No. 8, 0.202g D	3.68	20.33
14	2.004g ACD No. 8, 0.671g L	1.45	10.32
15	0.601g ACD No. 9, 0.202g D	3.34	18.45
16	1.2015g ACD No. 10, 0.401g D	4.45	24.58
17	2.055g ACD No. 9, 0.671g L	2.57	18.29

[1] L is Lanreotide™ and D is Decapeptyl™

Example 4: Preparation of Polypeptide Conjugates of Lactone-Grafted Acylated β-Cyclodextrin (G-CON)

Predetermined amount of the grafted ACD (G-ACD) was converted to a peptide composition following the same procedure used in preparing the U-CON's in Example 3. Reaction composition and characterization data of the different G-CON's are reported in Table IV.

TABLE IV. Preparation and Properties of Polypeptide/ Lactone-Grafted ACD Conjugates (G-CON)

G-CON No.	REACTANTS[1]	PERCENT NITROGEN	PERCENT PEPTIDE
1	0.079g G-ACD No. 1, 0.027g L	0.62	4.41
2	0.077g G-ACD No. 2, 0.028g L	1.00	7.12
3	1.00g G-ACD No. 3, 0.252g L	0.96	6.80
4	1.99g G-ACD No. 4, 0.502g L	0.74	5.30
6	0.202g G-ACD No. 4, 0.051g D	0.53	2.92
7	2.003g G-ACD No. 5, 0.5019g L	1.16	8.26
8	1.206g G-ACD No. 6, 0.4015g D	1.98	10.93
9	1.2035g G-ACD No. 7, 0.4036g D	3.56	19.67
10	2.0078g G-ACD No. 6, 0.6706g L	1.69	12.03

[1] L is Lanreotide™ and D is Decapeptyl™

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the claims.

What is claimed is:

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A cyclodextrin derivative, wherein at least 60 percent of the free hydroxy groups of said cyclodextrin are acylated with acyl groups where at least one of said acyl groups comprises a free carboxylic group;

said cyclodextrin is α-cyclodextrin, β-cyclodextrin, or γ-cyclodextrin; and

said acyl groups are selected from COE_1, where E_1 is selected from the group consisting of C_{2-32} carboxy alkyl, C_{3-32} carboxy alkenyl, C_{7-37} carboxyaryl, C_{8-38} carboxyaryl alkyl, and C_{9-39} carboxyaryl alkenyl and COE_2, where E_2 is selected from the group consisting of C_{1-30} alkyl, C_{2-30} alkenyl, C_{6-36} aryl, C_{7-37} arylalkyl, and C_{8-38} aryl alkenyl, wherein at least one of said acyl group is COE_1.

2. A cyclodextrin derivative of claim 1, wherein between 10 and 80 percent of said free hydroxy groups of said cyclodextrin are acylated with COE_1, and between 10 and 80 percent of said cyclodextrin are acylated with COE_2.

3. A cyclodextrin derivative of claim 2, wherein COE_1, is $CO(CH_2)_nCOOH$ (where n = 2-3) and COE_2 is $CO(CH_2)_nCH_3$ (where n = 0-5) and said cyclodextrin is β-cyclodextrin.

4. A copolymer comprising said cyclodextrin derivative of claim 1, wherein said cyclodextrin derivative comprises at least one free hydroxy group which is acylated with a polyester comprising hydroxy acid monomers.

5. A copolymer of claim 4, wherein said copolymer has an average molecular weight of between 500 - 40,000 daltons.

6. A copolymer of claim 5, wherein said polyester comprises hydroxy acid monomers selected from the group consisting of lactic acid, glycolic acid, hydroxy caproic acid, or any optically active isomer thereof.

7. A copolymer comprising said cyclodextrin derivative of claim 3, wherein said cyclodextrin derivative comprises at least one free hydroxy group which is acylated with a polyester comprising hydroxy acids.

8. A copolymer of claim 7, wherein said polyester comprises hydroxy acid monomers selected from the group consisting of lactic acid, glycolic acid, hydroxy caproic acid, or any optically active isomer thereof and said copolymer has an average molecular weight of between 500 - 40,000 daltons.

9. A composition comprising said cyclodextrin derivative of claim 1 and a drug, said drug comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of said drug present in said composition is ionically bonded to said cyclodextrin derivative.

10. A composition of claim 9, wherein said composition comprises between 1 and 30 percent, by weight, of said drug.

11. A composition of claim 10, wherein said drug is a polypeptide.

12. A composition of claim 11, wherein said polypeptide comprises between 4 and 50 amino acids.

13. A composition of claim 12, wherein said polypeptide is somatostatin, LHRH, calcitonin, or an analog thereof.

14. A composition comprising said copolymer of claim 4 and a drug, said drug comprising at least one effective ionogenic amine, wherein at least 50 percent, by weight, of said drug present in said composition is ionically bonded to said cyclodextrin derivative.

15. A composition of claim 14, wherein said composition comprises between 1 and 30 percent, by weight, of said drug.

16. A composition of claim 15, wherein said drug is a polypeptide.

17. A composition of claim 16, wherein said polypeptide comprises between 4 and 50 amino acids.

18. A composition of claim 17, wherein said polypeptide is somatostatin, LHRH, calcitonin, or an analog thereof.

19. A composition according to claim 13, wherein said somatostatin analog is D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2.

20. A composition according to claim 13, wherein said LHRH analog is pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

21. A composition according to claim 18, wherein said somatostatin analog is D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cyc]-Thr-NH_2.

22. A composition according to claim 18, wherein said LHRH analog is pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

Dated this 17th day of October 2000

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