Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 12 pp 2187–2192, 1996

PII: S0968-0896(96)00222-2
0968-0896/96 $15.00+0.00

Synthesis and Biological Evaluation of 2-Amino-2-deoxy- and 6-Amino-6-deoxy-cyclomaltoheptaose Polysulfates as Synergists for Angiogenesis Inhibition

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Abstract—2-Amino-2-deoxy-cyclomaltoheptaose was prepared from β-cyclodextrin perbenzoate [heptakis(2,3,6-tri-*O*-benzoyl)cyclomaltoheptaose] by a series of reactions including selective de-*O*-benzoylation at C-2 of one of the perbenzoylated D-glucopyranosyl moieties, oxidation to the 2-ulose derivative, oxime formation, and reduction to the 2-amino-2-deoxy-D-glucose moiety. This compound and 6-amino-6-deoxycyclomaltoheptaose accessible from β-cyclodextrin through the known procedure were sulfated to give polysulfated aminocyclomaltoheptaose derivatives (**3**, **5**). Employing β-cyclodextrin polysulfate as a reference compound, the synergistic effects of **3** and **5** for cortexolone on angiogenesis inhibitory activity were examined by rabbit-corneal micropocket assay system. In contrast to the significant anti-angiogenesis activity of the β-cyclodextrin polysulfate–cortexolone pair, neither **3** nor **5** showed any cooperative activity with cortexolone in the inhibition of basic FGF-induced angiogenesis.

Introduction

In 1989, Folkman et al.[1] reported that β-cyclodextrin tetradecasulfate (cyclomaltoheptaose tetradecasulfate, **1**) administered together with a steroid inhibits angiogenesis 100- to 1000-fold more effective than heparin, known as a synergist of certain steroids for anti-angiogenesis activity in the chicken embrio bioassay.[2] Furthermore, they showed that **1** also augmented the anti-angiogenic effect of cortexolone, one of the angiostatic steroids, against corneal neovascularization in rabbits. In contrast to the heterogeneous polysaccharide, heparin, polysulfated cyclodextrins are comparatively simple synthetic molecules and are expected to have an increased reproducibility in the enhancement of angiostatic steroids. As in the case for heparin, the synergistic effects of **1** were considered to depend upon its polysulfated structure and additionally upon ability of inclusion complex formation with steroids as a guest compound. Since heparin-binding cell growth factors are known strongly to induce angiogenesis,[3] such a reversed activity of heparin as a synergist of angiostatic cortexolone seems interesting. These facts prompted us to examine whether amino-deoxy-cycloglycan polysulfates with the same ring size as β-cyclodextrin show similar cooperative activities with cortexolone, considering the structure of such cycloglycan derivatives more closely resemble a glycosaminoglycans like heparin.

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Key words: sulfated β-cyclodextrin, amino-deoxy-β-cyclodextrin, angiogenesis inhibition, synergists, basic fibroblast growth factor.

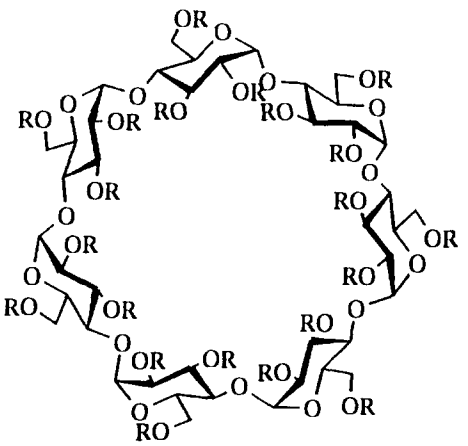
In this paper, we firstly describe a new process for the preparation of a new D-glucosamine-containing cycloglycan, i.e., 2-amino-2-deoxy-cyclomaltoheptaose (**2**)[4] and its sulfation to the heptadecasulfate derivative **3** (Fig. 1). In addition, the syntheses of reference compound, cyclomaltoheptaose heptadecasulfate (**4**), as well as 6-amino-6-deoxy-cyclomaltoheptaose octadecasulfate (**5**) as an isomer of **3**, using almost the same reaction conditions for sulfation as for **2** are described. Secondly, we describe the evaluation of their biological activity, comparing **3** and its isomer **5** with the reference polysulfate **4** as synergists for the anti-angiogenic effects ascribable to cortexolone.

Results and Discussion

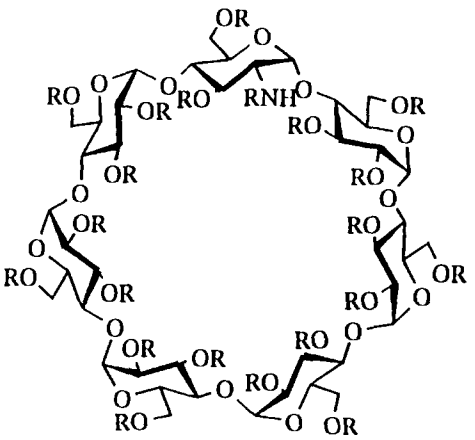
We already reported a novel synthesis[4] of **2** by insertion of a D-glucosamine moiety into the α-cyclodextrin skeleton, which involved a series of reactions such as ring opening of the starting cycloglycan chain, glycosidation with a D-glucosamine precursor, and recyclization through intramolecular glycosidation. The present paper discloses a simpler approach to **2**, employing heptakis(2,3,6-tri-*O*-benzoyl)cyclomaltoheptaose[5] (β-cyclodextrin perbenzoate, **6**) as the starting material. The key step was selective removal of one of the benzoyl groups at C-2 of the D-glucopyranosyl moieties constituting **6**. This was achieved by treatment of **6** in pyridine with 5 molar equivalents of hydrazine hydrate at room temperature.[6] After 25 h, the expected cyclo{→4)-hexakis[*O*-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl)-(1→4)]-*O*-(3,6-di-*O*-benzoyl-α-D-glucopyranosyl)-(1→} (**7**) was obtained in 24% yield, along with unchanged **6** (56%) by repeating column chroma-

tography on silica gel. In the ^{1}H NMR spectrum of **7** in DMSO-d_6 at 100 °C, a one-proton multiplet appeared at significant upper magnetic field (δ 3.59–3.60) and this signal became doublet of doublets with coupling constants of 3.4 and 9.8 Hz after addition of D_2O, suggesting that **7** had a free hydroxyl group at the 2-position. It is noteworthy that a regioselective reaction of monosaccharide[6] was applicable to the heptasaccharide system. Furthermore, Swern oxidation[7] of **7** with dimethylsulfoxide and trifluoroacetic anhydride at low temperature gave the crude mono-2-oxo derivative. Without further purification, the ketone was immediately converted into the oxime in pyridine at 70–80 °C and then *O*-benzoylated. Chromatographic purification on silica gel afforded the expected cycloglycan derivative **8** in nearly 60% overall yield, which contained the 3,6-di-*O*-benzoyl-2-benzoyloxyimino-2-deoxy-α-D-*arabino*-hexopyranosyl moiety as a constituent. Stereoselective reduction of the benzoyloxyimino group to an amino group was effected by hydroboration[8] of **8** with a large excess of borane in tetrahydrofuran and was followed by complete removal of benzoates. The resulting ninhydrin-positive product was purified through two successive column chromatography, using columns packed with active charcoal and then with CM-Sephadex (NH_4^+ form), giving 2-amino-2-deoxy-cyclomaltoheptaose (**2**) in almost 60% yield (Scheme 1). This product was identical in all aspects with an authentic sample,[4] confirming the D-*gluco* configuration of the newly constructed amino-sugar constituent.

Sulfation of **2**, as well as its known positional isomer 6-amino-6-deoxy-cyclomaltoheptaose[9] was carried out in a similar way as for the sulfation of cyclomaltoheptaose (β-cyclodextrin), using sulfur trioxide trimethylamine complex in *N,N*-dimethylformamide as the reagent. The reaction conditions employed were almost the same as those of reported by Folkman et al.,[1] and average degrees of sulfation for **3**–**5** determined by elemental analyses were 17, 17 and 18, respectively. However, Pitha et al.[10] reported that sulfation of β-cyclodextrin gave a mixture of several derivatives with nearly symmetrical distributions of degree of sulfation that was determined by fast-atom bombardment (FAB) mass spectrometry in negative mode.



1 and 4: R = SO_3Na or H



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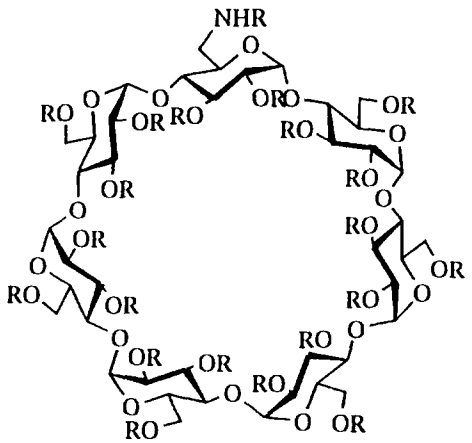


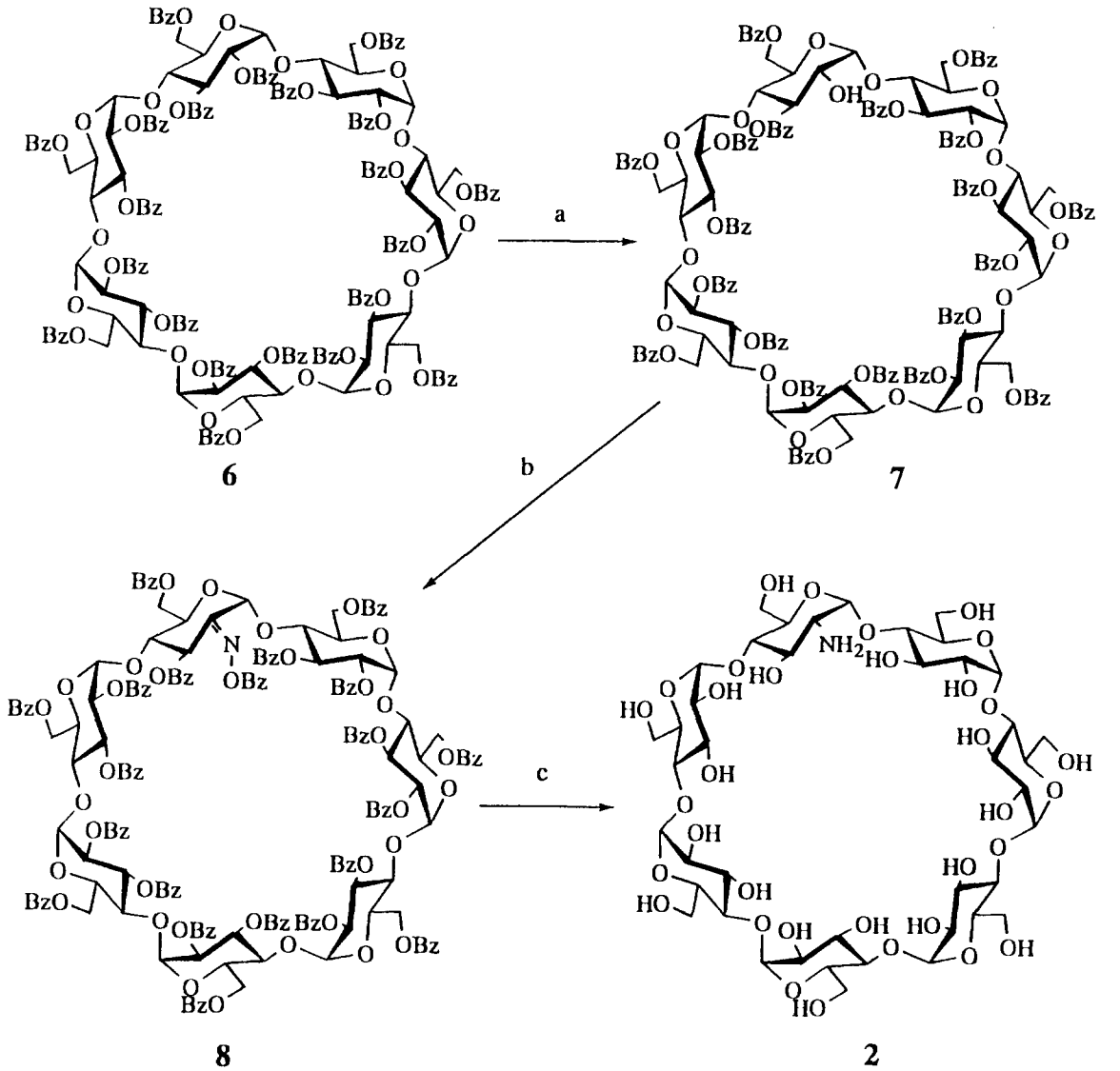
5: R = SO_3Na or H

Figure 1. Structure of polysulfated cycloglycans. The average degrees of sulfation of **3**, **4**, and **5** were 17, 17, and 18, respectively.

Indeed, the FAB mass spectrum of **3** showed three molecular ion peaks of 2744, 2846, and 2948 amu corresponding to degree of substitution from 17 to 19. Since the hydroxyl group at 3-position of β-cyclodextrin is less reactive than those at 2- or 6-positions in several reactions,[11] it is assumed that some of the hydroxyl group at 3-positions are unsubstituted. Furthermore, the fact that treatment of **3** with sodium nitrite in aqueous acetic acid did not affect its FAB mass spectrum suggested that the amino group located at C-2 position was substitited by a sulfate group.

Effects of **3** and **5** as a synergist of cortexolone on angiogenesis inhibitory activity were evaluated in comparison with the effect of the reference compound **4** by rabbit-corneal micropocket assay system, according to J. Folkman[1] with some modifications. The assay was based upon the following scheme: (1) angiogenesis was artificially induced by administration of basic Fibroblast growth factor (b-FGF),[12] (2) the induced angiogenesis was suppressed to some extent by administration of cortexolone, (3) the cooperative effects by administration of **3**–**5** together with cortexolone were observed. Each of b-FGF, cortexolone, and sulfated cycloheptaoses (**3**, **4**, or **5**) were separately incorporated into sustained-release polymer pellets of ethylene–vinyl acetate copolymer.[13] Contents of those reagents held in each pellet were adjusted closely identical those employed by Folkman et al.[1] in a previous similar experiment (see Experimental Section). Several combinations of these pellets such as b-FGF alone, b-FGF + one of the cycloglycan polysulfates, b-FGF + cortexolone and b-FGF + cortexolone + one of the cycloglycan polysulfates were implanted into a pocket made in rabbit corneas by surgery.[14] Anti-angiogenesis activity was judged by stereomicroscopy by measuring neovascular length.

Angiogenesis could be observed at the most position of upper half limbs 3 days after implantation of b-FGF



Scheme 1. Synthesis of 2-amino-2-deoxy-β-cyclodextrin (**2**). Reagents: (a) $H_2NNH_2 \cdot H_2O$, pyridine; (b) (i) $(CF_3CO)_2O$, DMSO, Et_3N, CH_2Cl_2, (ii) $H_2NOH \cdot HCl$, pyridine; (iii) BzCl, pyridine; (c) (i) BH_3, THF, (ii) NaOMe, MeOH, (iii) NaOH, H_2O.

Table 1. Influence of polysulfated cycloglycans on angiogenesis induced by b-FGF

		Vascular length (mm)							
		Days post operation							
		3	6	9	13	17	20	24	28
b-FGF	Mean	0.75	1.97	2.55	3.20	3.20	3.40	3.49	3.38
	SD	0.08	0.26	0.32	0.69	0.49	0.59	0.53	0.38
b-FGF + **4**	Mean	0.97	2.00	2.47	2.94	2.94	3.18	3.18	3.38
	SD	0.24	0.18	0.34	0.48	0.33	0.45	0.32	0.34
b-FGF + **3**	Mean	0.82	2.28	2.65	3.08	3.03	3.16	3.52	3.16
	SD	0.13	0.24	0.26	0.30	0.28	0.26	0.27	0.66
b-FGF + **5**	Mean	0.70	1.96	2.40	2.67	2.38	2.81	2.64	2.62
	SD	0.09	0.19	0.47	0.70	0.46	0.44	0.52	0.54

Cortex = Cortexolone. [a]$p < 0.001$ (compared with b-FGF treatment). [b]$p < 0.01$ (compared with b-FGF treatment). [c]$p < 0.05$ (compared with b-FGF treatment). [d]$p < 0.001$ (compared with b-FGF treatment). [e]$p < 0.01$ (compared with b-FGF + Cortexolone treatment). [f]$p < 0.05$ (compared with b-FGF + Cortexolone treatment).

pellets. The vessel length grew 2 mm 6 days after the implantation and more than 3 mm 13 days after the implantation, whereas neovascular length retained less than 0.5 mm in sham-operated eyes. Implantation of neither **3**, **4**, nor **5** with b-FGF exerted significant influence on the effect of b-FGF, suggesting a poor potency of such polysulfated cycloglycans alone to control neovascularization stimulated by b-FGF (Table 1).

On the contrary, implantation of a cortexolone pellet caused significant suppression of FGF-induced angiogenesis five and more days after the surgery for implantations. When mean maximal vessel lengths were measured, the vessel growth decreased to about 60% of the value in the eyes treated with b-FGF alone. In the case of administration of **4** together with cortexolone, the vessel growth further dropped to ca. 40% more than 13 days after implantation. In contrast to **4**, monoaminocycloglycan polysulfates **3** and **5** did not show any synergism with cortexolone but seemed to have slight tendency to supress the effect of cortexolone. (Table 2, Fig. 2).

Table 2. Effects of polysulfated cycloglycans as a synergist of angiostatic cortexolone

		Vascular length (mm)					
		Days of operation					
		5	8	13	20	24	29
b-FGF	Mean	1.33	1.91	2.29	2.54	2.70	2.92
	SD	0.10	0.31	0.42	0.67	0.86	0.89
b-FGF + cortex	Mean	0.85[a]	1.03[b]	1.32	1.85	1.56	1.82
	SD	0.30	0.64	0.97	1.38	1.49	1.62
b-FGF + cortex + **4**	Mean	0.97	1.25[b]	1.07[c]	1.02[c]	1.00[c]	1.05[c]
	SD	0.06	0.41	0.72	1.25	1.09	1.07
b-FGF + cortex + **3**	Mean	1.29[d]	1.80[f]	1.78	1.83	1.90	2.00
	SD	0.16	0.41	0.62	0.82	0.97	0.98
b-FGF + cortex + **5**	Mean	1.25[e]	1.81[f]	2.03	2.19	2.19	2.25
	SD	0.10	0.49	0.98	1.15	1.21	1.21

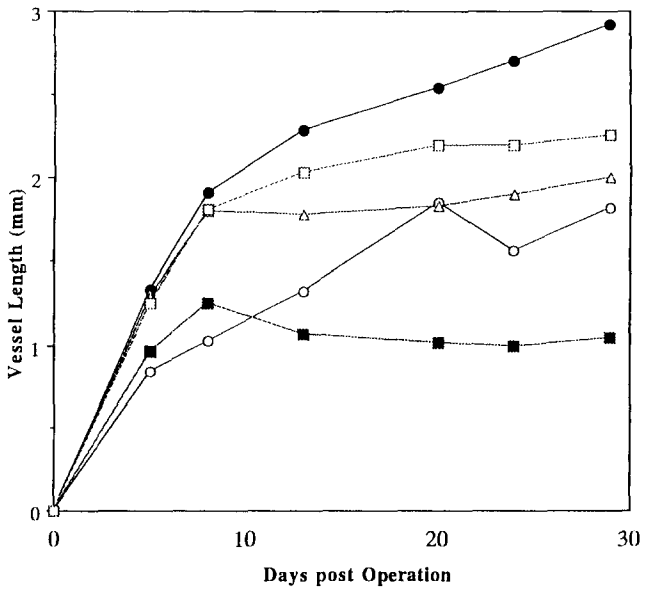


Figure 2. Effects of polysulfated cycloglycans as a synergist of angiostatic cortexolone. Approximate changes of vessel length are drawn on the basis of the mean values in Table 2. Solid circle: b-FGF; open circle: b-FGF + cortexolone; solid square: b-FGF + cortexolone + **4**; open square: b-FGF + cortexolone + **5**; open triangle: b-FGF + cortexolone + **3**.

The results that **4** and cortexolone, administered together, inhibited neovascularization induced by b-FGF in a synergistic manner were essentially the same as Folkman's experiments[1] regarding the cooperative effect of **1** and cortexolone on angiogenesis stimulated by endotoxin. This means that employment of the different stimulators for angiogenesis and such a small difference with respect to the extent of sulfation degree between **4** (D.S. = 17) and **1** (D.S. = 14) have no critical influence on the experimental data. On the contrary, substitution of only one sulfonyloxy group in cycloglycan polysulfate with a sulfonylamino group brings about a serious effect, resulting in complete loss of synergistic character regardless of the substitution position. Since the fitting of cortexolone into the hydrophobic cavities of **3**, **4**, and **5** would be almost the same because of their equal ring sizes, such a critical difference of the synergistic potency between **4** and **3**, **5** suggests the existence of some unknown but essential role of sulfonylamino groups in biological fenomena like angiogenesis.

Experimental

Chemistry

General methods. Optical rotations were determined with a Jasco DIP-370 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz or 500 MHz with Jeol JNM-GX 400 or Jeol JNM-GX 500 spectrometers for solution in DMSO-d_6, using tetramethylsilane as the internal standard unless otherwise noted. Fast-atom bombardment (FAB) mass spectrum was recorded with a Jeol JMA-HX110 spectrometer in the negative mode using triethanolamine as a liquid matrix. Reactions were monitored by TLC on a precoated plate of silica

gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Silica gel 60 (70–230 mesh; E. Merck) or activated charcoal (63–297 μm, Wako Pure Chemical Industries, Ltd, Osaka, Japan) were used for column chromatographic purification. Medium pressure column chromatography was carried out using Lobar column Lichroprep Silica gel 60 (E. Merck). Solvent extracts were dried over anhydrous sodium sulfate unless otherwise specified, and solutions were concentrated under diminished pressure below 40 °C. Analytical samples were dried at 60–65 °C over phosphorus pentoxide for 5–6 h in vacuo. β-Cyclodextrin was purchased from Nihon Shokuhin Kako Co., Ltd and dried under reduced pressure at 90–100 °C for 8 h prior to use.

Cyclo{→4)-hexakis[*O*-(2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl)-(1→4)]-*O*-(3,6-di-*O*-benzoyl-α-D-glucopyranosyl)-(1→} (**7**). To a solution of heptakis-(2,3,6-tri-*O*-benzoyl)cyclomaltoheptaose[5] (**6**; 59.77 g, 18 mmol) in pyridine (650 mL) was added hydrazine hydrate (4.5 mL, 90 mmol) and the mixture was stirred at room temperature for 25 h. Acetone (50 mL) was added to the mixture to quench the reaction and the solvent was evap under red. pres. The residual syrup was applied on a column of silica gel, and eluted with toluene:EtOAc (15:1 v/v) to give 33.64 g (56%) of unchanged **6** and crude product. Further purification of the product by medium pressure chromatography with toluene:EtOAc (9:1 v/v) of the product gave 13.78 g (24%) of **7**: $[\alpha]_D^{15}+9.7°$ (*c* 0.29, $CHCl_3$). ^1H NMR (DMSO-d_6, 100 °C): δ 3.59–3.60 (m, 1H, H-2^1), 3.97 (d, $J=6.71$ Hz, 1H, OH), 4.11 (t, $J=9.16$ Hz, 1H, H-4), 4.30 (t, $J=9.16$ Hz, 1H, H-4), 5.03 (d, $J=3.97$ Hz, 1H, H-1^1), 5.04–5.11 (m, 5H, 5×H-2), 5.14 (dd, $J=3.66$ and 10.38 Hz, 1H, H-2), 5.46–5.49 (m, 2H, H-1 and 3^1), 5.53 (d, $J=3.97$ Hz, 1H, H-1), 5.56 (d, $J=3.35$ Hz, 1H, H-1), 5.59 (d, $J=3.97$ Hz, 1H, H-1), 5.82–5.86 (m, 4H, 4×H-3), 5.89 (t, $J=10.38$ Hz, 1H, H-3), 5.97 (t, $J=10.07$ Hz, 1H, H-3). After addition of D_2O, the peak of δ 3.59–3.60 became doublet of doublets with *J* values of 3.4 and 9.8 Hz. $C_{182}H_{150}O_{55}$ (3217.15), calcd: C, 67.95; H, 4.70; found: C, 67.72; H 4.60.

Cyclo{→4)-hexakis[*O*-(2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl)-(1→4)]-*O*-(3,6-di-*O*-benzoyl-2-benzoyloxyimino-2-deoxy-α-D-arabino-hexopyranoyl)-(1→} (**8**). To a stirring mixture of DMSO (4.5 mL, 54 mmol) in CH_2Cl_2 (30 mL) at −78 °C under argon atmosphere was added dropwise trifluoroacetic anhydride (3.8 mL, 27 mmol), and the mixture was stirred at the same temperature for 45 min. To the resulting suspension at −78 °C was added dropwise a soln of **7** (4.3 g, 1.4 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred at −78 °C for 1 h. After addition of Et_3N (7.5 mL, 54 mmol), the mixture was warmed to room temperature, stirred for 1 h, and partitioned between H_2O and $CHCl_3$. The organic layer was successively washed with 2 M HCl, aq satd $NaHCO_3$, and brine, dried, and concd to dryness, giving 5.4 g of a pale yellow syrup; TLC analysis with toluene:EtOAc (4:1 v/v) showed two major spots (R_f 0.64 and 0.49). A soln of the resulting syrup and hydroxyamine hydrochloride (1.35 g, 19 mmol) was stirred at 70–80 °C for 3.5 h, poured into water, and extracted with $CHCl_3$. The organic layer was successively washed with ice cold 2 M HCl, aq $NaHCO_3$, and brine, dried, and evapd, giving 4.5 g of syrup; TLC with toluene:EtOAc (4:1 v/v) revealed a major spot (R_f 0.49). To an ice cold solution of the residual syrup in pyridine (80 mL) was added benzoyl chloride (1 mL, 8.6 mmol). The mixture was stirred at room temperature for 1 day, poured into ice water, extracted with $CHCl_3$. The organic layer was successively washed with 2 M HCl, H_2O, aq $NaHCO_3$, and brine, dried, and evapd to dryness. The residual syrup was applied onto a column of silica gel and eluted with toluene:EtOAc (15:1 v/v) to give a product, which was further purified by medium pressure column chromatography with toluene:EtOAc (8:1 v/v), giving 2.24 g (59%) of **8**: $[\alpha]_D^{15}+16.1°$ (*c* 0.36; $CHCl_3$). ^1H NMR (DMSO-d_6, 100 °C): δ 5.06–5.13 (m, 3H, 3×H-2), 5.19 (dd, $J=9.46$ and 3.97 Hz, 1H, H-2), 5.29 (dd, $J=10.07$ and 3.35 Hz, 1H, H-2), 5.56–5.57 (m, 4H, 4×H-1), 5.61 (d, $J=3.35$ Hz, 1H, H-1), 5.65 (d, $J=3.36$ Hz, 1H, H-1), 5.80 (t, $J=8.7$ Hz, 1H, H-3), 5.94 (t, $J=9.01$ Hz, 1H, H-3), 5.96 (t, $J=9.16$ Hz, 1H, H-3), 6.14 (d, $J=9.15$ Hz, 1H, H-3^1), 6.19 (*s*, 1H, H-1^1). $C_{189}H_{153}O_{56}N$ (3334.25), calcd: C, 68.08; H, 4.63; N 0.42; found: C, 67.98; H, 4.63; N, 0.39.

2-Amino-2-deoxycyclomaltoheptaose (**2**). Compound **8** (2.24 g, 0.67 mmol) was dissolved in 1 M borane THF soln (24 mL) and the soln was stirred under an argon atmosphere at room temperature for 2 days. MeOH (20 mL) was carefully added to the ice-cold soln to decompose the excess reagent and the solvent was evapd under red. pres. The residue was dissolved in 0.01 M methanolic NaOMe (50 mL). The soln was stirred at room temperature for 1 day, poured into water (100 mL), concd to half of its volume, and partitioned between CH_2Cl_2 and H_2O. The aq layer was washed with CH_2Cl_2 several times, and concd to dryness. The residue was dissolved in 4 M aq NaOH (35 mL) and heated at 95 °C for 3 days, neutralized with 1 M HCl, and filtered through Celite pad. The filtrate was concd, applied onto a column of active charcoal (30×200 mm), and successively eluted with H_2O, EtOH:H_2O (6:4 v/v), and 2-propanol:H_2O (7:3 v/v). The ninhydrin positive fractions were concentrated, filtered through a Millipore filter (0.5 μm), and subjected to ion-exchange chromatography with CM-Sephadex C-25 (NH_4^+ form) with 0.02 M NH_4OH to give 0.45 g (59%) of **2**: $[\alpha]_D^{15}+140°$ (*c* 0.25, H_2O), ref. 4 $[\alpha]_D^{23}+146°$ (*c* 0.18, H_2O). ^1H NMR (D_2O; TMS as the external standard) δ 2.76 (dd, $J=3.36$ and 10.38 Hz, 1H, H-2^1), 3.48 (pseudo-t, $J=$ca. 9 Hz, 7H, 7×H-4), 3.55 (dd, $J=3.4$ and 9.8 Hz, 6H, 6×H-2), 3.86–3.82 (m, 21 H, 7×H-5,6a,6b), 3.86 (pseudo-t, $J=$ca. 9.5 Hz, 6H, 6×H-3), 4.90 (d, 1H, $J=3.66$ Hz, H-1^1), 4.97 (*pseudo-d*, $J=$ca. 3.7 Hz, 6H, 6×H-1); ^1H NMR (D_2O containing 1 drop of 20% DCl in D_2O; TMS as the external standard) δ 3.37 (dd, $J=3.66$ and 10.99 Hz, 1H, H-2^1), 4.07 (t, $J=9.77$ Hz, 1H, H-3^1),

4.98–5.00 (m, 6H, 6 × H-1), 5.22 (d, J = 3.4 Hz, 1H, H-1^1).

Cyclomaltoheptaose polysulfate (4). A soln of β-cyclodextrin (100 mg, 0.09 mmol) and sulfur trioxide trimethylamine complex (1.03 g, 7.4 mmol) in DMF (8 mL) was stirred at 60–70 °C for 24 h, filtered, and washed with small amount of DMF. The collected precipitate was dissolved in 10% aq NaOAc (5 mL), poured into EtOH (100 mL), and filtered to give an amorphous powder. Chromatographic purification of the product on Sephadex G-25 with H_2O as the eluant and subsequently on SP Sephadex C-25 (Na^+ form) with H_2O followed by lyophilization gave 0.122 g (54%) of hygroscopic powdery **4**: $[\alpha]_D^{22}$ + 73° (*c* 0.25, H_2O); R_f 0.14 (1-propanol:pyridine:AcOH:H_2O, 15:10:3:15, v/v/v/v). $C_{42}H_{53}O_{86}S_{17}Na_{17} \cdot 2H_2O$ (2905.74), calcd: C, 17.36; H, 1.98; S, 18.76; found: C, 17.43; H, 2.28; S, 18.45.

2-Amino-2-deoxycyclomaltoheptaose polysulfate (3). Compound **7** (44 mg, 0.039 mmol) was treated with $SO_3 \cdot NMe_3$ (0.46 g, 3.5 mmol) in a similar way as described above, giving 130 mg (56%) of hygroscopic powdery **3**: $[\alpha]_D^{22}$ + 70° (*c* 0.15, H_2O); R_f 0.40 (1-propanol:pyridine:AcOH:H_2O 15:10:3:17, v/v/v/v); FAB-MS: 2744, 2846, 2948. $C_{42}H_{54}O_{85}NS_{17}Na_{17} \cdot 2H_2O$ (2904.76), calcd: C, 17.37; H, 2.01; N, 0.48; S, 18.77; found: C, 17.57; H, 2.11; N, 0.58; S, 18.79.

6-Amino-6-deoxycyclomaltoheptaose polysulfate (5). 6-Amino-6-deoxycyclomaltoheptaose[9] (102 mg, 0.09 mmol) was treated with $SO_3 \cdot NMe_3$ (1.0 g, 7.2 mmol) in a similar way as described above, giving 130 mg (56%) of hygroscopic powdery **5**: $[\alpha]_D^{22}$ + 67° (*c* 0.21, H_2O); R_f 0.30 (1-propanol:pyridine:AcOH:H_2O, 15:10:3:17, v/v/v/v). $C_{42}H_{53}O_{88}NS_{18}Na_{18} \cdot 2H_2O$ (3006.80), calcd: C, 16.78; H, 1.91; N, 0.47; S, 19.19; found: C, 17.10; H, 2.01; N, 0.42; S, 19.15.

Biological evaluation

Male New Zealand white rabbits, weighing 2.3–3.4 kg, were used for corneal micropocket assay experiments.

Preparation of pellets. Pellets of ethylene–vinyl acetate copolymer (33 wt% vinyl acetate, Aldrich) were rinsed with sterile phosphate buffered saline, dried, and dissolved in 1,2-dichloroethane (10% w/v). Each of b-FGF (California Biotechnology Inc.), cortexolone, and three kinds of cycloglycan polysulfates were separately mixed with the above polymer solution. Each mixture was dried in a vacuum desiccator to remove 1,2-dichloroethane and the resulted polymer matrixes trapping b-FGF or other reagents were cut into the implantation pellets (1 mm × 1 mm × 1 mm). Each pellets contained b-FGF (250 ng), cortexolone (30 μg), or one of the cycloglycan polysulfates (20 μg).

Implantation technique. An oblong small pocket was fashioned within the corneal stroma by surgical operation. Various combinations of pellets (2–3 pieces) were deposited in the bottom of each pockets, which sealed spontaneously.

Observation of eyes. Neovascular lengths in corneal implanted were measured with the aid of a stereomicroscope (Olympus, OMK-1).

(Received in Japan 25 May 1996; accepted 21 August 1996)

Acknowledgment

The authors wish to thank Ms M. Yoshida and her collaborators of RIKEN for elemental analyses.

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