



(11) (21) (C) **2,028,094**
(86) 1990/02/16
(87) 1990/09/02
(45) 2000/11/21

(72) Norberg, Thomas, SE

(72) Kallin, Elisabeth, SE

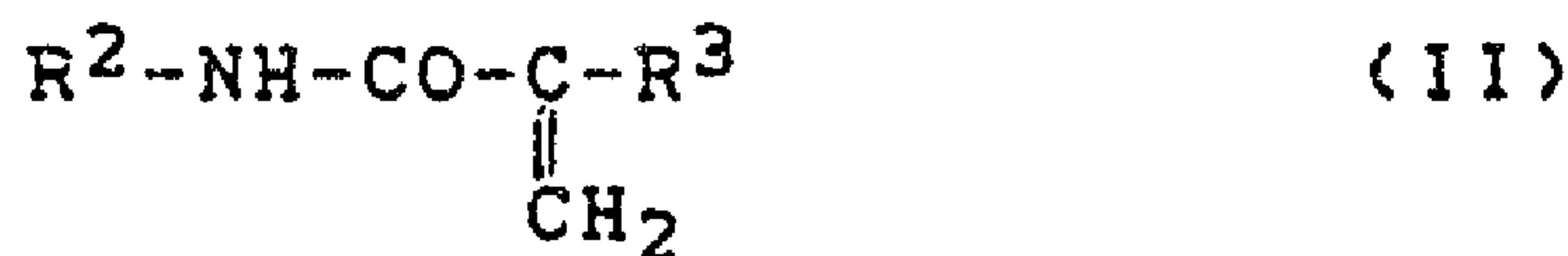
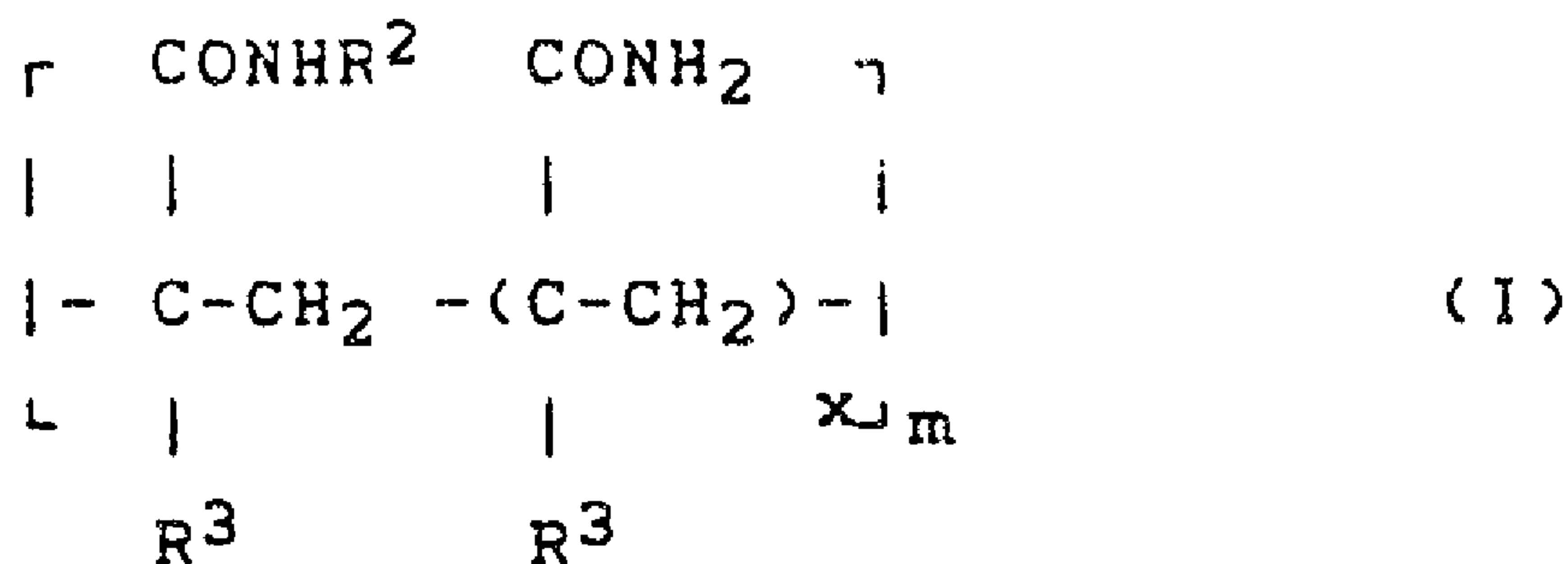
(73) PROCUR Aktiebolag, SE

(51) Int.Cl.⁶ C07H 5/06, C08F 224/00

(30) 1989/03/01 (8900605-0) SE

(54) **COPOLYMERES CARBOHYDRATEACRYL- ET
METHACRYLIQUES; PROCEDE DE FABRICATION**

(54) **CARBOHYDRATEACRYL- AND METHACRYLCOPOLYMERS
AND THEIR MANUFACTURE**



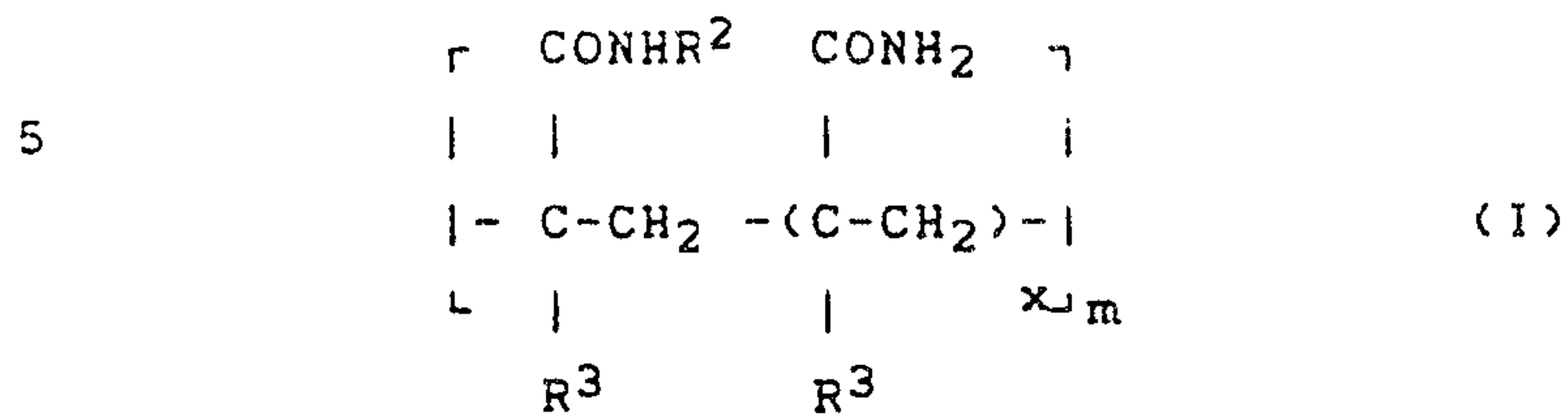
(57) L'invention concerne un copolymère de glycosylamine N-acylé ainsi qu'un amide ayant la formule générale (I), dans laquelle R² représente un résidu de sucre de réduction; R³ représente H ou CH₃; x représente un nombre entier compris entre 0 et environ 20; et m est tel que la masse moléculaire du copolymère est comprise entre environ 5 kDa et 2000 kDa; une glycosylamine N-acryloyle ou méthacryloyle ayant la formula (II), dans laquelle R² représente un résidu de sucre de réduction; et R³ représente H ou CH₃. L'invention concerne en outre des procédés de préparation du composé.

(57) A copolymer of an N-acylated glycosylamine and an amide having the general formula: (see formula I) wherein R² is a reducing sugar residue; R³ is H or CH₃; x is an integer from 0 to about 20; and m is such that the molecular weight of the copolymer is from about 5 kDa to about 2000 kDa; an N-acryloyl- or methacryloyl-glycosylamine having the formula: (see formula II) wherein R² is a reducing sugar residue; and R³ is H or CH₃; and processes for its preparation.



ABSTRACT

A copolymer of an N-acylated glycosylamine and an amide having the general formula:



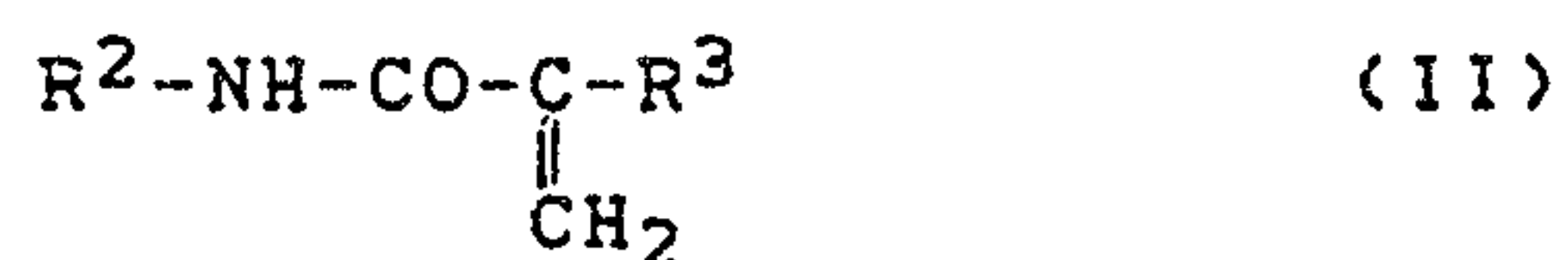
10 wherein R^2 is a reducing sugar residue;

R^3 is H or CH_3 ;

x is an integer from 0 to about 20; and

m is such that the molecular weight of the copolymer is from about 5 kDa to about 2000 kDa;

15 an N-acryloyl- or methacryloyl-glycosylamine having the formula:



wherein R^2 is a reducing sugar residue; and

20 R^3 is H or CH_3 ; and

processes for its preparation.

Carbohydrateacryl- and methacrylcopolymers and their
manufacture.

5 The present invention relates to copolymers of glycosyl
amines and amides, to N-acryloyl- or methacryloyl-glycosylamines
and to processes for their preparation.

BACKGROUND OF THE INVENTION

10 Carbohydrate structures, exposed on the surface of cells
or occurring in soluble form in body fluids, are important in
many biological recognition processes. To investigate such pro-
cesses, low molecular weight oligosaccharides are sometimes not
satisfactory. Therefore, a great deal of effort has been devoted
to the development of techniques (ref.1 to 4)) for attaching
15 oligosaccharides to larger molecules such as proteins, to give
high molecular weight, multivalent conjugates. The obtained
"neoglycoproteins" can be used as immunizing antigens to produce
carbohydrate-directed antibodies, or as antigens in immunoassays
to detect such antibodies.

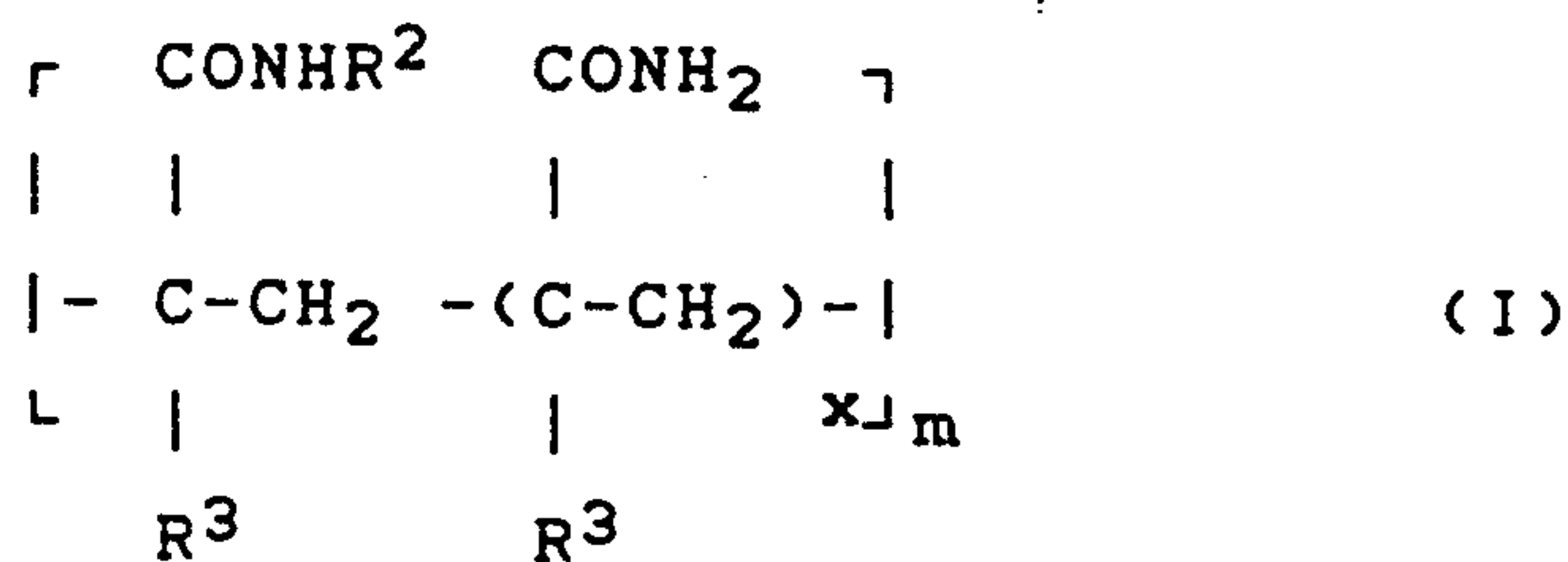
20 However, for many applications, it is problematic to use
protein conjugates. For example, in immunoassays using carbohyd-
rate antigens, the presence of protein epitopes is highly unde-
sirable. An alternative to proteins in these cases are water-
-soluble, weakly immunoreactive polymers of the polyacrylamide
25 type. Several reports have recently appeared describing the pre-
paration of oligosaccharide-acrylamide copolymers, both linear
(ref.5 to 14) and crosslinked (ref.15 to 17). The general
strategy for preparation of these conjugates has been to attach
an olefinic group to a carbohydrate, and then copolymerize this
30 derivative with acrylamide. The olefinic group has been
introduced into the carbohydrate molecule either as an allyl
glycoside at an early stage in a synthetic scheme (ref.5-7, 9-12
and 15), by acryloylation of an amino function of a mono- or
oligosaccharide derivative (ref.8, 11, 13, 14, 17), or by other
35 methods (ref. 16). These known techniques are, however, subject
to drawback in that they cannot be directly applied to reducing
di- or oligosaccharide reactants, since the reaction conditions
necessary result in cleavage of interglycosidic linkages.

Few reports have appeared to date (ref.14,18) on the attachment of an olefinic group onto a *reducing* oligosaccharide. Reducing oligosaccharides of great complexity and structural variety can be isolated from natural sources such as milk (ref.19), urine (ref.20), and faeces (ref.20), and also from chemical or enzymatic hydrolyzates of glycoproteins (ref.21), glycolipids (ref.22,23), or lipopolysaccharides (ref.24).

SUMMARY OF THE INVENTION

The present invention is directed to new techniques for the attachment of an olefinic group to the anomeric position of reducing saccharides. Generally, the invention is based on conversion of the saccharides used into the corresponding β -glycosyl amines which are then N-acryloylated or N-methacryloylated. The N-acryloyl- or N-methacryloylglycosylamines formed are then copolymerized with acrylamide or methacrylamide to form high molecular weight, linear polymers. These new polymers are useful in for example applications involving coating antigens in ELISA assays.

According to a first aspect the invention thus provides for a copolymer of glycosyl amine and an amide, said copolymer having the general formula:



wherein R^2 is a reducing sugar residue;

R^3 is H or CH_3 ;

x is an integer from 0 to about 20; and

m is such that the molecular weight of the copolymer is from about 5 kDa to about 2000 kDa.

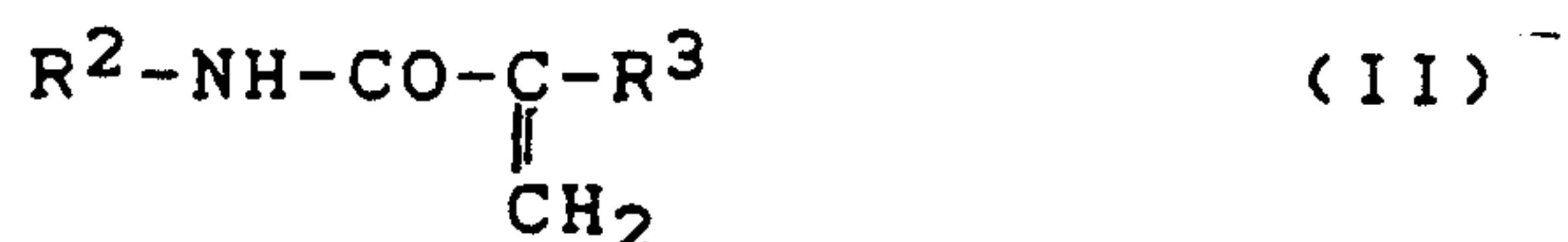
It is preferred that R^2 in formula I above is a mono-, di- or oligo-saccharide residue. Particularly preferred are saccharides having 1 to 10 monosaccharide units, especially 1 to 6 units.

Although the invention is not to be construed to be limited thereto, examples of saccharides of which R^2 denotes the residue are: lactose, lacto-N-tetraose, lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-difucohexaose I, 2'-fucosyllactose, 3'-siallyllactose, A-tetrasaccharide, cellobiose.

In formula I above x is preferably from 1 to about 15, and m is preferably such that the molecular weight of the copolymer is from about 10 to about 500 kDa. It is preferred that R^3 of formula I is hydrogen corresponding to using acrylamide as one repeating entity in the copolymer I.

In the above formula I symbol m is defined by functional statement on molecular weight of the copolymer. Generally, the molecular weight range 5 to 2000 kDa as given above corresponds to an approximate range for symbol m of from about 5 to about 3000, such as from about 50 to about 750. The fact that these ranges are of an approximative nature is, of course, due to the fact that the other variables of formula I, i.e. R^2 , R^3 and x , result in varying molecular weights for the entity within the main bracket of the formula.

According to another aspect the invention provides for new N-acryloyl- or methacryloyl-glycosylamines having the formula:

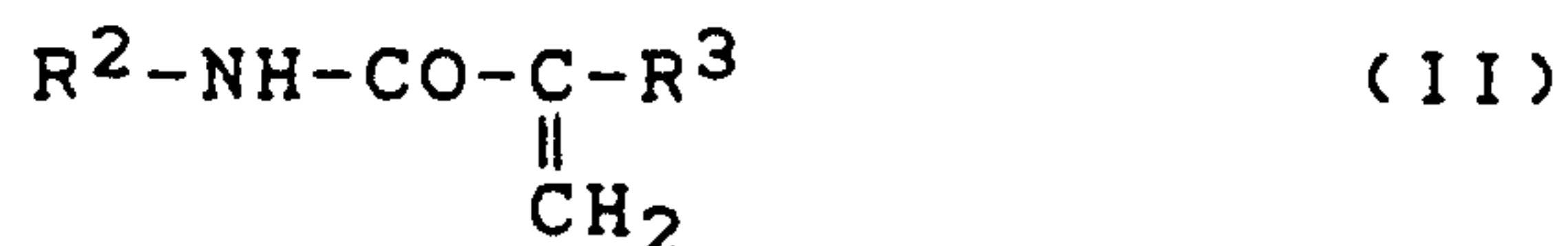


wherein R^2 is a reducing sugar residue; and

R^3 is H or CH_3 .

In this formula II R^2 and R^3 have the meanings defined in relation to general formula I above.

According to a further aspect the invention provides for a process for preparing an N-acryloyl- or methacryloyl-glycosylamine having the formula:



wherein R^2 is a reducing sugar residue; and

R^3 is H or CH_3 ,

comprising the steps:

- a) reacting a reducing sugar in solution and ammonium hydrogen carbonate to form an N-acryloyl glycosylamine;
- b) reacting the glycosylamine obtained in step a) with a reactive derivative of acrylic or methacrylic acid; and

c) recovering the resulting N-acryloyl glycosylamine.

It is preferred to use a halide or anhydride in step b) above, and especially preferred is the use of acryloyl chloride in said step.

5 According to yet another aspect the invention provides for a process for preparing copolymers having the general formula I as defined above, said process comprising the steps:

a) polymerizing in solution an N-acryloyl- or N-methacryloyl glycosylamine and acrylamide or methacrylamide; and

10 b) recovering the resulting copolymer.

In this process the amide is preferably used in stoichiometric excess, and the use of acrylamide in step a) is particularly preferred.

As previously indicated the copolymers of the present
15 invention are useful in a variety of applications, of which some are exemplified below.

As a first example of practical use of the present invention there may be mentioned the copolymer based on the N-acryloyl or -methacryloyl glycosylamine of lacto-N-tetraose (β -
20 D-Galp-(1->3)- β -D-GlcNAcp-(1->3)- β -D-Galp-(1->4)-D-Glc), and acrylamide, said copolymer being further described below in the exemplifying section of the specification. This copolymer is useful in its capacity of acting as a bacterial receptor enabling diagnosing and inhibiting bacterial adherence of for
25 example pneumococci as disclosed in published European patent application 0126043, published November 21, 1984.

Another example of practical application of the invention is the use of the corresponding copolymer based on the so called A-tetrasaccharide (α -D-GalNAcp-(1->3)- [α -L-Fucp-(1->2)]- β -D-
30 Galp-(1->4)- β -D-Glc) in that the copolymer shows excellent binding to anti-A and is thus useful in blood-testing using for example copolymer-coating in ELISA-assays.

A third example of practical application relates to the corresponding copolymer based on lacto-N-fucopentaose (α -L-Fucp-(1->2)- β -D-Galp-(1->3)- β -D-GlcNAcp-(1->3)- β -D-Galp-(1->4)-D-Glc), said copolymer being useful in the control of uterine implantation of embryos in mammals. This application is in accordance with published European patent application No. 0298 064, published January 4, 1989. The usefulness of the copolymer based on this carbohydrate is emphasized by the fact that an amplified effect in the control of uterine implantation is obtained due to the multivalent character of the copolymer.

The present invention will now be further described by non-limiting examples with reference to the appended drawings, wherein:

Fig. 1 illustrates the process steps involved in the manufacture of the copolymer;

Fig. 2 illustrates NMR specter on reactions between lactose and ammoniumhydrogen carbonate.

Fig. 3 illustrates three different intermediates in such manufacturing process; and

Fig. 4 is a diagram showing the average molecular weight of the copolymers versus gel filtration elution volume.

EXAMPLES

General procedures

Pure oligosaccharides, isolated from human milk (ref.19), urine (ref.20) or other sources were treated with aqueous ammonium bicarbonate, essentially as previously described (ref. 26), to give the corresponding glycosylamines (Fig. 1). The yields were as reported in Table 1. In all cases where oligosaccharides terminating with 4-linked glucose were used, β -pyranosidic glycosylamines were the main product. Less than 5% of the bis- β -glycosylamine (ref.27,28) was detected (NMR, H-1 at

δ 4.32). No α -anomeric product signals were detected in any of the NMR spectra.

For closer inspection of the reaction path leading to the glycosylamines, the reaction between lactose and ammonium bicarbonate was performed in D_2O and monitored by ^1H NMR spectroscopy. It was shown (Fig. 2) that after 24 h at room temperature, about 50% of the lactose had been converted. After 5 days, more than 95% of the starting material had disappeared. However, only minor amounts of the glycosylamine 1 (H-1 at δ 4.12, H-2 at δ 3.20) were detected. Instead, signals from another major product (H-1 at δ 4.70, H-2 at δ 3.38) were present (see Fig. 3). This product was assumed to be the N-glycosylcarbamate 2. Signals

from another, unidentified minor product (H-1 at δ 6.29, H-2 at δ 4.71) were also present. That 2 was indeed present in the reaction mixture was also indicated by a FAB-MS spectrum of the mixture, where peaks corresponding to the acid of 2 (m/z 386) and 2 (5 m/z 403) were detected. After processing of the reaction mixture by evaporation, adsorption to a cation exchange column, elution with methanolic ammonia and evaporation, the ^1H NMR spectrum of a D_2O solution showed presence of pure 1 (H-1 at δ 4.12), the yield was 82%. Therefore, any N-glycosylcarbamate (2) present in the reaction mixture must have decomposed into the glycosylamine (1) during processing, an expected reaction for this type of derivative (ref.29). The importance of 2 as an intermediate was indicated by the fact, that reaction of lactose with concentrated aqueous solutions of ammonium acetate, ammonium formate, or ammonium chloride gave less than 10% conversion (15 TLC evidence) after 5 days at room temperature.

The influence of pH on the stability of glycosylamine solutions was also investigated. Lactose glycosylamine (1) was dissolved in phosphate buffers of different pH and the solutions (20 were monitored polarimetrically. Solutions in the pH range 8.0-10.0 were found to undergo little change during several days at room temperature. This was also confirmed by the observation, that the ^1H NMR spectrum of 1 in D_2O (10 mg/mL, pH 7.7) did not change during this period. Lowering the pH increased the rate of (25 decomposition of 1. At pH 5, for example, 1 was completely converted to lactose in less than 1 hour. However, at low pH (0.5 M aqueous HCl) 1 was again stable. These results are in good agreement with those reported for similar glycosylamines (ref.30). However, we also noted a lower stability of glycosylamines in (30 borate buffers. At pH 10.0 in 0.1 M borate buffer, lactose glycosylamine was converted to lactose in less than 5 min (optical rotation and TLC evidence). The reason for this needs to be investigated.

On basis of the above stability investigations, it was (35 concluded that N-acylation of the glycosylamines in hydroxylic solvents should be possible, provided that the acylation reaction is fast and selective for amino groups, and that the pH during the reaction can be kept above 8. Indeed, several acylating agents were successfully used, and this communication re-

ports on the results with acryloyl chloride. Treatment of the glycosylamines in Table 2 with acryloyl chloride in aqueous methanol using sodium carbonate as buffer gave satisfactory yields of the corresponding N-acryloyl glycosylamines (fig. 1, Table 2). The N-acryloyl glycosylamines were, as predicted (ref.30), much more stable towards hydrolysis than the glycosylamines. However, the presence of the acryloyl group introduced a marked tendency to self-polymerization (ref. 13,14), therefore addition of small amounts of a radical inhibitor to the solutions of these compounds was necessary during some operations.

Radical copolymerization of the oligosaccharide N-acryloyl glycosylamines with acrylamide in aqueous solution using ammonium persulfate/tetramethylethylenediamine (TEMED) as initiator system (ref.5) gave linear polymers (fig.1, Table 3). The carbohydrate contents of the polymers were determined by integration of appropriate signals in the ^1H NMR spectra, and also independently by the anthrone-sulfuric acid colorimetric method (ref.31). The yield of carbohydrate incorporated into the polymer varied from 48 to 82%. The molar ratio oligosaccharide groups/ $\text{CH}-\text{CH}_2$ groups in the polymer agreed well with the corresponding ratio in the pre-polymerization mixture (Table 3). This is in contrast to what is the case (ref.6,9,12) when allyl glycosides are copolymerized with acrylamide. Here lower yields are obtained and much lower oligosaccharide/ $\text{CH}-\text{CH}_2$ ratios are found in the polymer than in the reaction mixture, since the reactivity of allyl glycosides in radical reactions is lower than that of acrylamide. Obviously, N-acryloyl sugar derivatives have a higher reactivity (ref.11) in this respect.

The average molecular weights of the polymers (usually in the range of 100-500 kDa) were determined from the gel filtration elution volume using dextran standards for calibration (fig. 4). The molecular weights so determined agreed well with those determined by ultrafiltration through filters with different pore sizes.

In order to find optimal conditions for obtaining a high molecular weight polymer, several sets of experiments were performed with the N-acryloyl derivative of lactose. It was found that, as expected (ref.32-34), the molecular weight increased with decreasing concentration of ammonium persulfate and increa-

sing concentration of monomers. However, too high a concentration of monomer resulted in an insoluble product. The reaction temperature is expected (ref.32-33) to affect polymer molecular weight, but the effect was found to be moderate when varying
5 between 0° and 20°C. We also found little effect of pH (5 and 9) of the reaction mixture on the molecular weight distribution of the polymer, and there was no detectable change when the acrylamide to sugar monomer ratio was changed from 10:1 to 2:1. Factors that were found to be important for a good result were the
10 purity of the monomers and an oxygen-free reaction mixture (ref.7). Factors that were not investigated but are known (ref.32-34) to affect polymer molecular weight are initiator type and presence of chain transfer agents such as salts or alcohols.

15 The obtained copolymers exhibited strong specific binding to antibodies against the carbohydrate portion when used as coating antigens in ELISA assays. Thus, as reported before (ref.6,11), carbohydrate-acrylamide copolymers are alternatives to glycolipid or glycoprotein antigens in immunological assays.
20 Other biological properties of carbohydrate-acrylamide copolymers, such as the ability to inhibit or promote various biological processes are being investigated in this laboratory.

Degassed distilled water was used. All reactions except the preparation of glycosylamines were performed under nitrogen.
25 Concentrations were performed at <30°C (bath). Optical rotations were recorded at 21°C with a Perkin-Elmer 241* polarimeter. NMR spectra were recorded at 27°C in D₂O with a Bruker AM 500 instrument, using acetone methyl signals (δ_H 2.225 and δ_C 23.2) as internal standards. The FAB-MS spectra were recorded with a VG
30 ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 keV. The samples were dissolved in thioglycerol and the positive ions were extracted and accelerated over a potential of 10 kV. Thin layer chromatography was performed on silica gel 60 F₂₅₄* (Merck, Darmstadt, FRG) using
35 4:3:3:2 ethyl acetate:acetic acid:methanol:water as eluant. The spots were visualized by charring with 5% sulfuric acid. Acrylamide (enzyme grade, Eastman Kodak Co, Rochester, NY, USA) was used without further purification. Bond-Elut C-18* and SCX* cartridges and Sepralyte C18* silica gel were from Analytichem Inter-
*Trade-mark

national (Harbor City, USA). Bio-Gel P2* (Bio-Rad, Richmond, USA) and Fractogel TSK HW55(F)* (Merck, Darmstadt, FRG) columns were packed and eluted with water. Dextran standards were from Pharmacosmos* (Viby, Denmark). Ultrafiltration equipment (Omega cells) were from Filtron AB* (Bjärred, Sweden). The tetrahydrofuran (Riedel-de Haen, FRG) used contained 250 mg/L of 2,6-di-tert-butyl-4-methylphenol as stabilizing agent.

EXAMPLE 1

10 Preparation of Glycosylamines:

Solid ammonium bicarbonate was added until saturation to a solution of oligosaccharide (50 mg) in water (2.5 mL). The mixture was stirred in an open vessel at room temperature for 3-7 days. Ammonium bicarbonate was added at intervals, saturation was assured by always keeping a portion of solid salt present in the mixture. When TLC indicated no more conversion, the mixture was diluted with water (5 mL) and concentrated to half the original volume. The residue was diluted to 20 mL with water and concentrated to 5 mL. This process was repeated once, then the residue was diluted to 10 mL and lyophilized. The crude product was purified by dissolving in water (1 mL) and passing the solution through a cation exchange resin (Bond-Elut SCX, H⁺-form, 0.5 g cartridge). After washing the resin with water, the glycosylamine was eluted with 2 M ammonia in 1:1 methanol-water (2.5 mL). The eluate was concentrated to 1 mL and then lyophilized.

4-O-(β-D-Galactopyranosyl)-β-D-glucopyranosylamine (1).

Treatment of lactose (50 mg) as described above gave 1 (41 mg, 82%), $[\alpha]_D +37^\circ$ (c 1.0 water), litt. (ref.35) $[\alpha]_D +38.5$ (water).

NMR data: ¹³C, δ61.1 (C-6), 61.9 (C-6'), 69.4 (C-4'), 71.8 (C-2'), 73.4 (C-3'), 74.8 (C-2), 76.0 (C-3), 76.2 (C-5'), 76.5 (C-5), 79.5 (C-4), 85.7 (C-1), 103.7 (C-1'); ¹H, δ3.20 (dd, J_{1,2} 8.7, J_{2,3} 9.4 Hz, H-2), 3.54 (dd, J_{1',2'} 7.8, J_{2',3'} 9.9 Hz, H-2'), 3.55 (ddd, J_{4,5} 9.6, J_{5,6a} 5.0, J_{5,6b} 2.3 Hz, H-5), 3.62 (dd, J_{2,3} 9.4, J_{3,4} 8.7 Hz, H-3), 3.64 (dd, J_{3,4} 8.7, J_{4,5} 9.6 Hz, H-4), 3.66 (dd, J_{2',3'} 9.9, J_{3',4'} 3.4 Hz, H-3'), 3.72 (ddd, J_{4',5'} 1.1, J_{5',6'a} 3.8, J_{5',6'b} 8.1 Hz, H-5'), 3.75 (dd, J_{5',6'a} 3.8, J_{6a,6b} 11.6 Hz, H-6'a), 3.78 (dd, J_{5,6a} 5.0, J_{6a,6b}

*Trade-mark

12.1 Hz, H6a), 3.79 (dd, $J_{5',6'b}$ 8.1, $J_{6'a,6'b}$ 11.6 Hz, H-6'b), 3.92 (dd, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.1 Hz, H-4'), 3.94 (dd, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 12.1 Hz, H-6b), 4.11 (d, $J_{1,2}$ 8.7 Hz, H-1), 4.45 (d, $J_{1',2'}$ 7.8 Hz, H-1').

5 Anal. Calcd. for $C_{12}H_{23}NO_{10} \times H_2O$: C, 40.1; H, 7.0; N, 3.9. Found: C, 40.3; H, 6.8; N, 3.8. A FAB-MS spectrum showed an M+1 ion at m/z 342.

EXAMPLE 2

N-Acryloylation of Glycosylamines

10 Sodium carbonate (100 mg) and methanol (1.0 mL) was added to a solution of the glycosylamine (0.14 mmol) in water (1.0 mL). The mixture was stirred at 0°C while acryloyl chloride (60 μ L, 0.74 mmol) in tetrahydrofuran (0.5 mL) was added during 5 min. After 10 min, the solution was diluted with water (3 mL) and concentrated to 2 mL. The solution was again diluted with water (2 mL), 200 μ L of 0.5% 2,6-di-tert-butyl-4-methylphenol in tetrahydrofuran (inhibitor solution) was added, and the solution was concentrated to 1-2 mL. This solution was applied onto a C-18 silica gel column (2.0 x 5.0 cm), packed in water. Elution with water gave salts, unreacted glycosylamine, and reducing sugar in the first fractions, and the desired product in the later fractions. In some cases, elution of the product was preferably speeded up by adding methanol to the eluant. The fractions containing product were pooled, mixed with a few drops of inhibitor solution, and concentrated to 2 mL. This solution was purified by gel filtration on a Bio-Gel P2 column. Appropriate fractions were pooled and lyophilized.

N-Acryloyl-4-O-(β -D-galactopyranosyl)- β -D-glucopyranosylamine

30 (3):

Treatment of 1 (50 mg) with acryloyl chloride (60 mL) as described above gave 3 (51 mg, 88%), $[\alpha]_D -7^\circ$ (c 0.5, water). NMR data: ^{13}C , δ 60.7 (C-6), 61.9 (C-6'), 69.4 (C-4'), 71.8 (C-2'), 72.3 (C-2), 73.3 (C-3'), 75.9 (C-3), 76.2 (C-5'), 77.3 (C-5), 78.6 (C-4), 80.1 (C-1), 103.7 (C-1'), 130.20, 130.25 (CH=CH₂), 170.2 (C=O); ^1H , δ 3.49 (dd, $J_{1,2}$ 9.2, $J_{2,3}$ 9.2 Hz, H-2), 3.56 (dd, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.9 Hz, H-2'), 3.67 (dd, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.4 Hz, H-3'), 3.70 (m, H-5), 3.72 (m, H-3), 3.73 (m, H-4), 3.75 (m, H-5'), 3.77 (dd, $J_{5',6'b}$ 3.8, $J_{6'a,6'b}$ 11.6

Hz, H-6'b), 3.80 (dd, $J_{5',6'a}$ 8.2, $J_{6'a,6'b}$ 11.6 Hz, H-6'a),
 3.82 (dd, $J_{5,6b}$ 4.4 $J_{6a,6b}$ 12.3 Hz, H-6b), 3.93 (dd, $J_{3',4'}$ 3.4,
 $J_{4',5'}$ 1.6 Hz, H-4'), 3.94 (dd, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12.3 Hz, H-
 -6a), 4.46 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 5.08 (d, $J_{1,2}$ 9.2 Hz, H-1),
 5 5.87 (dd, J 3.7 and 7.9 Hz, CH=CH₂), 6.31 (m, CH=CH₂).

Anal. Calcd. for C₁₅H₂₅NO₁₁: C, 45.6; H, 6.4; N, 3.5.
 Found: C, 41.1; H, 6.1; N, 3.6.

EXAMPLE 3

10 Copolymerization of N-Acryloylglycosylamines with Acrylamide:

A solution of the N-acryloylglycosylamine (52 μ mol) and
 acrylamide (210 μ mol, 15 mg) in distilled water (400 μ L) was
 deaerated by flushing with nitrogen for 20 min. The solution was
 then stirred at 0°C and N,N,N',N'-tetramethylethylenediamine (2
 15 μ L) and ammonium persulfate (1 mg) were added. The mixture was
 slowly stirred at 0°C for 2 h, and then at room temperature o-
 vernight. The viscous solution was diluted with water (1 mL) and
 purified by gel filtration on Fractogel HW 55(F). Fractions con-
 taining polymer were pooled and lyophilized.

20

Copolymer of N-Acryloyl-4-O-(β -D-galactopyranosyl)- β -D-glucopy- ranosylamine and acrylamide:

Treatment of 3 (20 mg) with acrylamide (7.2 mg, 2 eq) as
 described above gave copolymer (18 mg, 54% calculated from 3),
 25 $[\alpha]_D +7^\circ$ (c 0.1, water). Analysis of the material by ¹H NMR
 spectroscopy (D₂O, 50°C) showed presence of approximately 1 lac-
 tose unit per 4.6 CHCH₂ units (theoretical value: 1/3). The mo-
 lecular weight distribution of the copolymer, as determined by
 gel filtration, was 50-1000 kDa, centered around 300 kDa.

REFERENCES

1. E. Kallin, H. Lönn, and T. Norberg, Glycoconjugate J., 3, 311-319 (1986).
- 5 2. C.P. Stowell and Y.C. Lee, Adv. Carbohydr. Chem. Biochem., 37, 225-281.
- 10 3. J.D. Aplin and J.C. Wriston, Jr, C.R.C.Crit.Rev.Biochem., 259-306 (1981).
4. Y.C. Lee and R.T. Lee in The Glycoconjugates, Vol IV; M.I. Horowitz, Ed.; Academic press: New York, 1982, p 57-83.
- 15 5. V. Horejsi, P. Smolek, and J. Kocourek, Biochem. Biophys. Acta, 538, 293-298 (1978).
- 20 6. A. Ya. Chernyak, K.V. Antonov, N.K. Kochetkov, L.N. Padyukov, and N.V. Tsvetkova, Carbohydr. Res., 141, 199-212 (1985).
7. A. Ya. Chernyak, A.B. Levinsky, B.A. Dmitriev, and N.K. Kochetkov, Carbohydr. Res., 128, 269-282 (1984).
- 25 8. A. Ya. Khorlin and N.V. Bovin, Bioorg. Chem., 11, 671-673 (1985).
9. P. Kosma, J. Gass, G. Schulz, R. Christian, and F.M. Unger, Carbohydr. Res., 167, 39-54 (1987).
- 30 10. P. Kosma, G. Schulz, and F.M. Unger, Carbohydr. Res., 180, 19-28 (1988).
- 35 11. R. Roy and F. Tropper, Glycoconjugate J., 5, 203-206 (1988).
12. R. Roy, C.A. Laferrière, A. Gamian, H.J. Jennings, J. Carbohydr. Chem., 6, 161-165 (1987).

13. R.L. Whistler, H.P. Panzer, and H.J. Roberts, *J. Org. Chem.*,
26, 1583-1588 (1961).
- 5 14. J. Klein and D. Herzog, *Macromol, Chem.*, 188, 1217-1233
(1987).
15. V. Horejsi and J. Kocourek, *Biochem. Biophys. Acta*, 297,
346-351 (1973).
- 10 16. R.T. Lee, S. Cascio, and Y.C. Lee, *Anal. Biochem.*, 95,
260-269 (1979).
- 15 17. P.H. Weigel, R.L. Schnaar, S. Roseman, and Y.C. Lee in
Methods in Enzymology, Vol 83, V. Ginsburg, Ed.; Academic
press: Orlando, 1982, p 294-299.
18. K. Kobayashi, H. Sumitomo, and Y. Ina, *Polymer J.*, 15,
667-671 (1983).
- 20 19. A. Kobata in *Methods in Enzymology*, Vol 28, V. Ginsburg,
Ed.; Academic Press: Orlando, 1973, p 262-271.
20. A Lundblad and A. Chester in *The Molecular Immunology of
Complex Carbohydrates*, A. Wu, Ed.; Plenum Press: New York,
25 1988.
21. A. Kobata In *Biology of Carbohydrates*, Vol 2; V. Ginsburg
and P.W. Robbins, Eds.; John Wiley: New York, 1984, p 87-
-161.
- 30 22. H. Wiegandt and H. Bücking, *Eur. J. Biochem.*, 15, 287-292.
(1970).
23. B. Nilsson and D. Zopf, *Arch. Biochem. Biophys.*, 222,
35 628-648 (1983).
24. S.B. Svensson and A.A. Lindberg, *J. Immunol.*, 120,
1750-1757 (1978).

25. E. Kallin, H. Lönn, and T. Norberg, *Glycoconjugate J.*, 5, 145-150 (1988).
26. L.M. Likhoshesterov, O.S. Novikova, V.A. Derevitskaja, and N. K. Kochetkov, *Carbohydr. Res.*, 146, C1-C5 (1986).
27. B. Paul and W. Korytnyk, *Carbohydr. Res.*, 67, 457-468 (1978).
28. H.S. Isbell and H.L. Frush, *J.Org. Chem.*, 23, 1309-1319 (1958).
29. R. Howe in *Rodd's Chemistry of Carbon compounds*, Vol 1C; S. Coffey, Ed.; Elsevier: Amsterdam, 1965, p 290-291.
30. H. Paulsen and K.-W. Pflughaupt in *The Carbohydrates*, Vol 1B, W. Pigman and D. Horton, Eds.; Academic Press: New York, 1980, p 881-927.
31. J.E. Hodge and B.T. Hofreiter in *Methods in Carbohydrate Chemistry*, vol 1; R.L. Whistler and M.L. Wolfrom, Eds.; Academic Press: New York, 1962, p 380-394.
32. W.M. Thomas and D.W. Wang in *Encyclopedia of Polymer Science and Engineering*, Vol 1, Second Ed.,: Wiley: New York, 1985, p 169-211.
33. S. Sandler and W. Karo, *Polymer Synthesis*; Vol 1; Academic Press: New York, 1974, p 343-365.
34. Y. Yamazaki and H. Maeda, *Agric. Biol. Chem.*, 45, 2091-2103 (1981).
35. F. Micheel, R. Frier, E. Plate, and A. Hiller, *Chem. Ber.*, 85, 1092-1101 (1952).

Table 1: Conversion of reducing oligosaccharides to the corresponding glycosylamines

Oligosaccharide	Glycosylamine, yield (%)
5	
Lactose	82
Lacto-N-tetraose ^a	82
Lacto-N-fucopentaose I ^b	81
10 Lacto-N-fucopentaose II ^c	78
Lacto-N-difucohexaose I ^d	88
2'-Fucosyllactose	83
3'-Siallylactose	74
A-tetrasaccharide ^e	88
15 Cellobiose	72

a. β -D-Galp-(1→3)- β -D-GlcNAcp-(1→3)- β -D-Galp-(1→4)-D-Glc

b. α -L-Fucp-(1→2)- β -D-Galp-(1→3)- β -D-GlcNAcp-(1→3)- β -D-Galp-(1→4)-D-Glc

20

c. β -D-Galp-(1→3)-[α -L-Fucp-(1→4)]- β -D-GlcNAcp-(1→3)- β -D-Galp-(1→4)-D-Glc

d. α -L-Fucp-(1→2)- β -D-Galp-(1→3)-[α -L-Fucp-(1→4)]- β -D-GlcNAcp-(1→3)- β -D-Galp-(1→4)-D-Glc

e. α -D-GalNAcp-(1→3)-[α -L-Fucp-(1→2)]- β -D-Galp-(1→4)- β -D-Glc

(Nomenclature according to IUPAC-recommendations)

25

Table 2: Acryloylation of glycosylamines

Glycosylamine	N-acryloylglycosylamine (%)
30	
Lactose	88
Lacto-N-tetraose	74
Lacto-N-fucopentaose I	53*
Lacto-N-fucopentaose II	61*
2'-Fucosyllactose	92
35	
A-tetrasaccharide	65*

* Yield not optimized

Table 3: Synthesis of copolymers

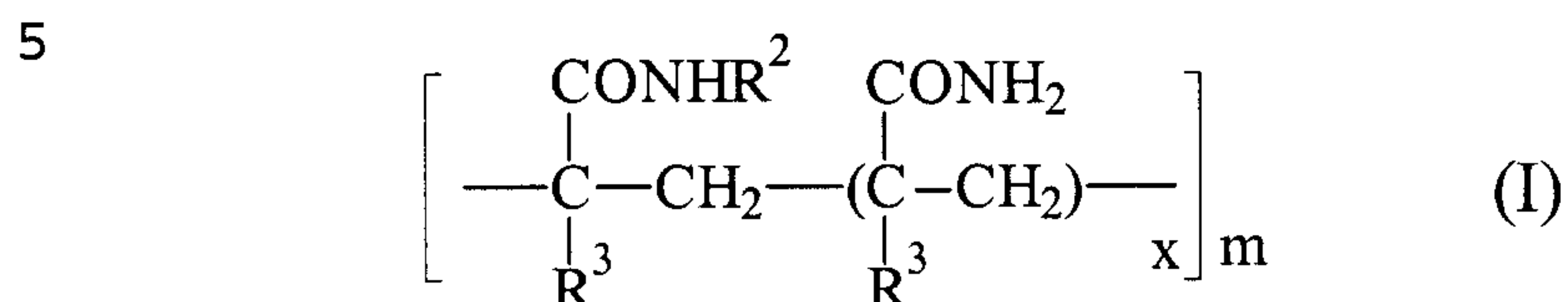
5	Oligo- saccharide	Yield of copolymer*	Charged ratio**	Polymer ratio**	[α]D
10	Lactose	54	1:3	1: 4,6	+6
	Lacto-N-tetraose	82	1:4	1: 5	-1
	Lacto-N-fucopentaose I	59	1:4	1: 6	-11
	A-tetrasaccharide	48	1:6	1:14	+18
	2'-Fucosyllactose	65	1:6	1: 9	-30

* Yield calculated from starting N-acryloylglycosylamine

** Ratio = ratio N-acryloylglycosylamine/CHCH₂-unit

CLAIMS:

1. A copolymer of an N-acylated glycosylamine and an amide characterized by having the general formula:



wherein R^2 is a reducing sugar residue;

10 R^3 is H or CH_3 ;

x is an integer from 0 to about 20; and

m is such that the molecular weight of the copolymer is from about 5 kDa to about 2000 kDa.

15 2. A copolymer according to claim 1, wherein R^2 is a mono, di- or oligo-saccharide residue.

3. A copolymer according to claim 2, wherein R^2 is the residue of a saccharide having 1 to 10 monosaccharide units.

20

4. A copolymer according to claim 2, wherein R^2 is the residue of a saccharide having 1 to 6 monosaccharide units.

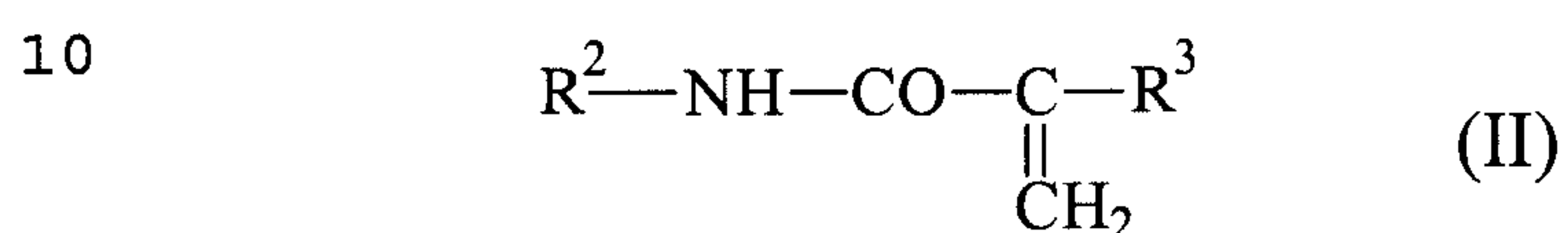
5. A copolymer according to claim 3 or 4, wherein R^2 is
25 the residue of a saccharide selected from: lactose, lacto-N-tetraose, lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-difucohexaose I, 2'-fucosyllactose, 3'-siallyllactose, A-tetra-saccharide, cellobiose.

30 6. A copolymer according to any one of claims 1 to 5 wherein x is from 1 to about 15.

7. A copolymer according to any one of claims 1 to 6, wherein m is such that the molecular weight of the copolymer is from about 10 to about 500 KDa.

5 8. A copolymer according to any one of claims 1 to 7, wherein R³ is H.

9. An N-acryloyl- or methacryloyl-glycosylamine having the formula:



wherein R² is a reducing sugar residue; and
R³ is H or CH₃.

15

10. An N-acryloyl- or methacryloyl-glycosylamine according to claim 9, wherein R² is a mono-, di- or oligo-saccharide residue.

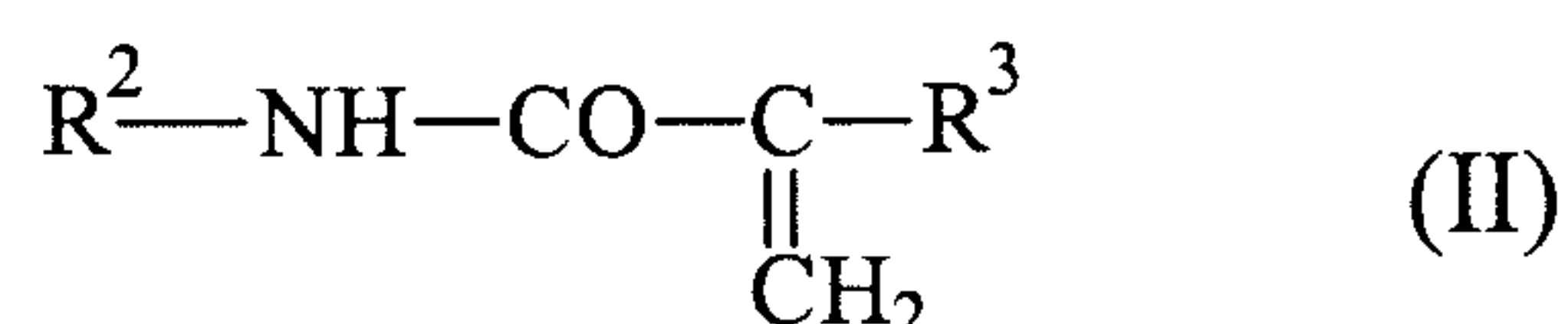
20 11. An N-acryloyl- or methacryloyl-glycosylamine according to claim 10, wherein R² is the residue of a saccharide having 1 to 10 monosaccharide units.

12. An N-acryloyl- or methacryloyl-glycosylamine according to claim 10, wherein R² is the residue of a saccharide having 1 to 6 monosaccharide units.

13. An N-acryloyl- or methacryloyl-glycosylamine according to claim 11 or 12, wherein R² is the residue of a saccharide selected from: lactose, lacto-N-tetraose, lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-difucohexaose 1, 2'-fucosyllactose, 3'-sialyllactose, A-tetrasaccharide, cellobiose.

14. An N-acryloyl- or methacryloyl-glycosylamine according to any one of claims 9 to 13, wherein R^3 is H.

15. A process for preparing an N-acryloyl- or methacryloyl-glycosylamine having the formula:



wherein R^2 is a reducing sugar residue; and

10 R^3 is H or CH_3 ,

comprising the steps:

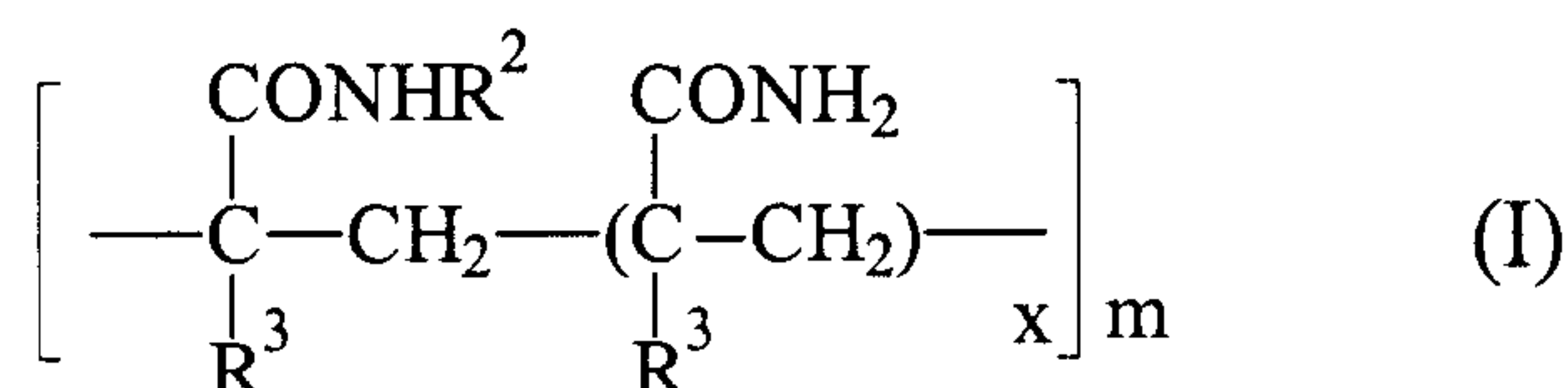
- a) reacting a reducing sugar in solution and ammonium hydrogen carbonate to form an N-acryloyl glycosylamine;
- b) reacting the glycosylamine obtained in step a) with a
15 reactive derivative of acrylic or methacrylic acid; and
- c) recovering the resulting N-acryloyl glycosylamine.

16. A process according to claim 15, wherein an acryloyl or methacryloyl halide or anhydride is used in step b).

20

17. A process according to claim 16, wherein acryloyl chloride is used in step b).

18. A process for preparing a copolymer of a glycosylamine
25 and an amide characterized by having the general formula:



30 wherein R^2 is a reducing sugar residue;

R^3 is H or CH_3 ;

x is an integer from 0 to about 20; and

m is such that the molecular weight of the copolymer is from about 5 kDa to about 2000 kDa, comprising the steps:

- a) polymerizing in solution an N-acryloyl- or N-methacryloyl glycosylamine and acrylamide or methacrylamide; and
- 5 b) recovering the resulting copolymer.

19. A process according to claim 18, wherein the acrylamide or methacrylamide is used in stoichiometric excess.

10 20. A process according to claim 18 or 19, wherein acrylamide is used in step a).

FETHERSTONHAUGH & CO.
OTTAWA, CANADA

PATENT AGENTS

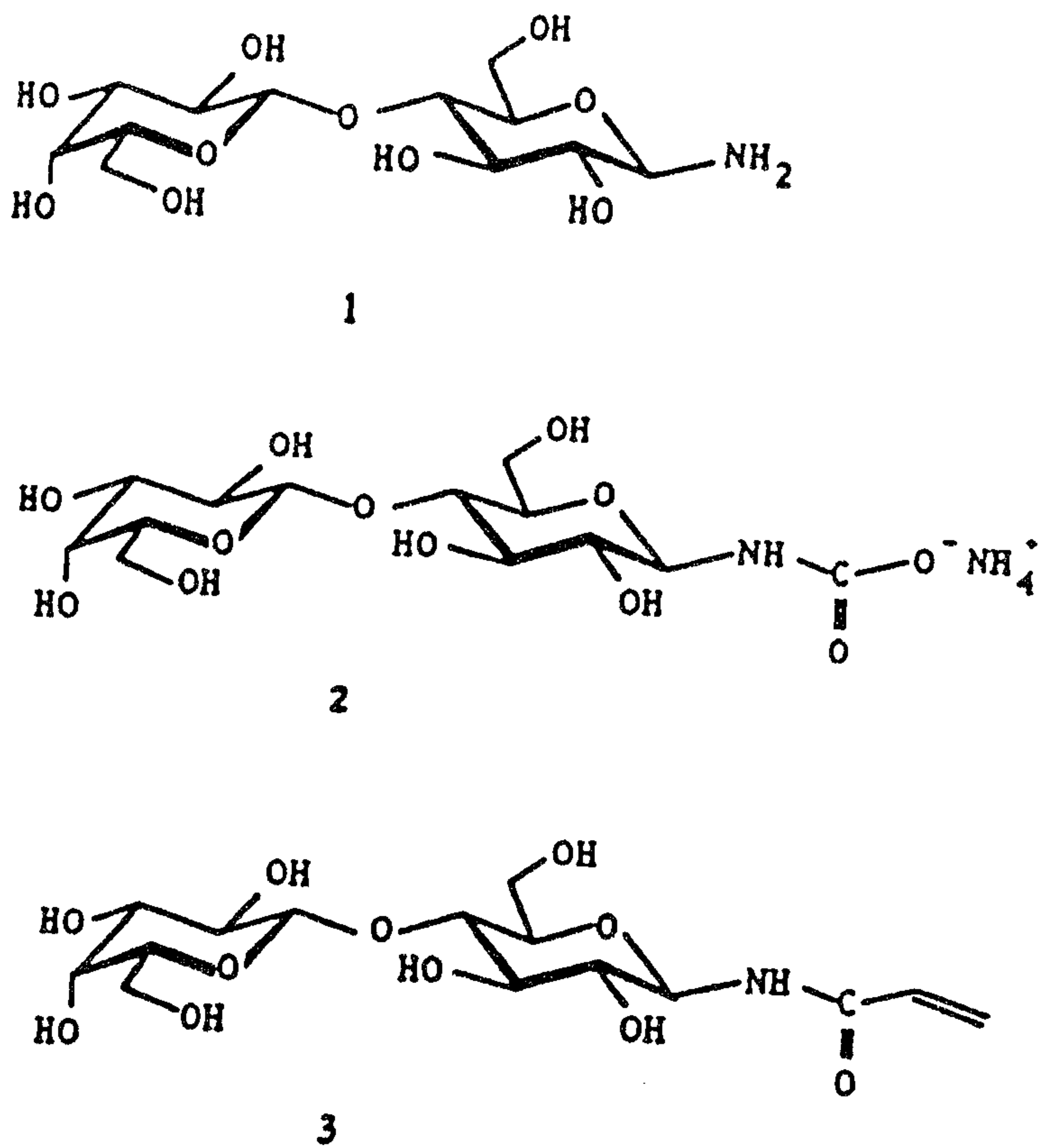


FIG. 1

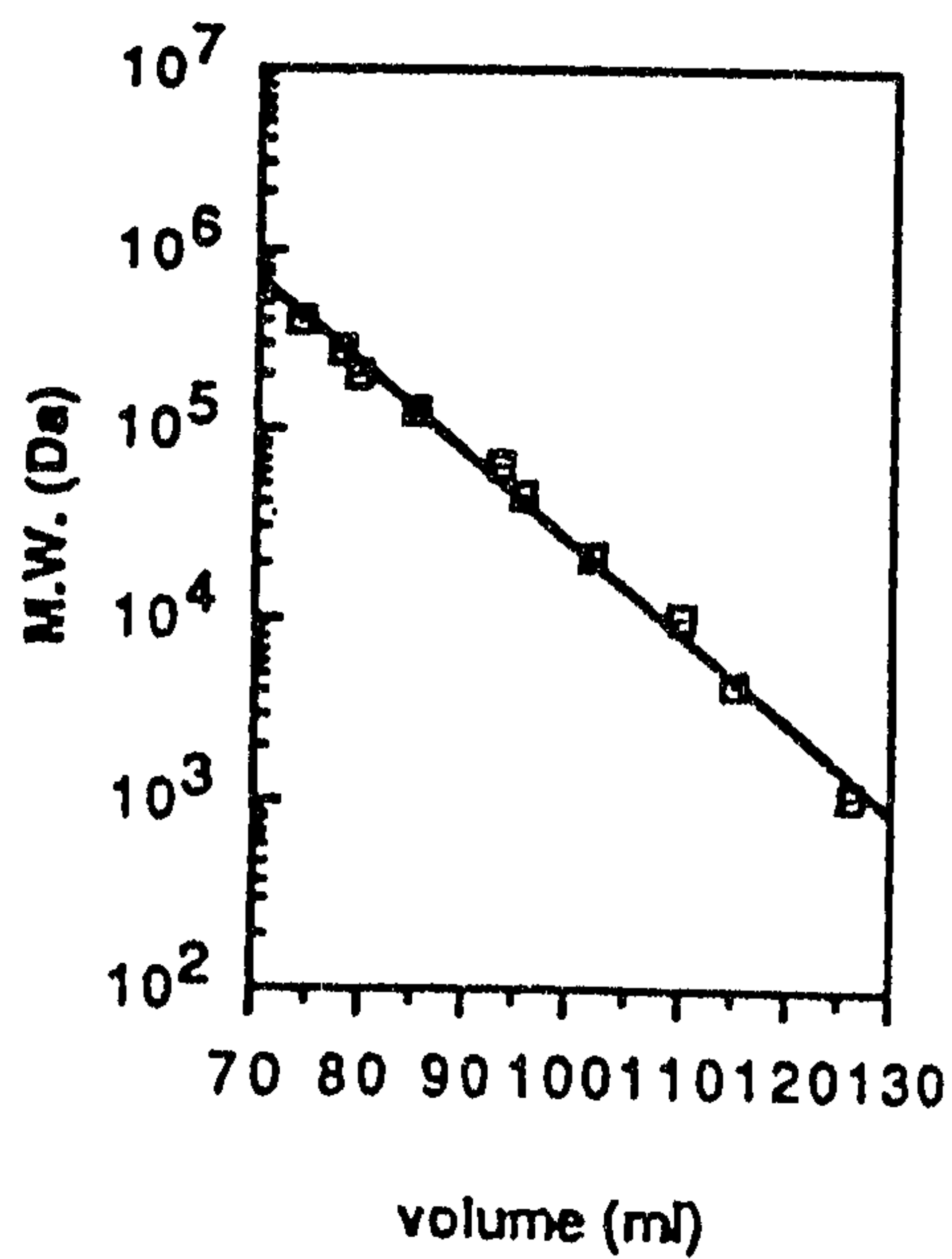


FIG. 2

Patent Agents
Fetherstonhaugh & Co.

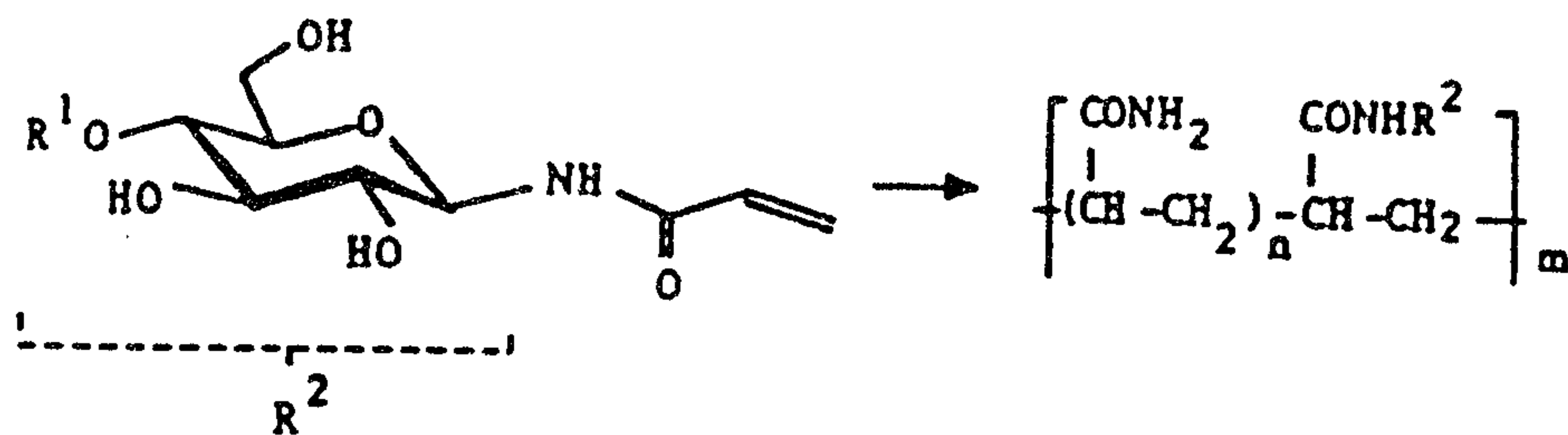
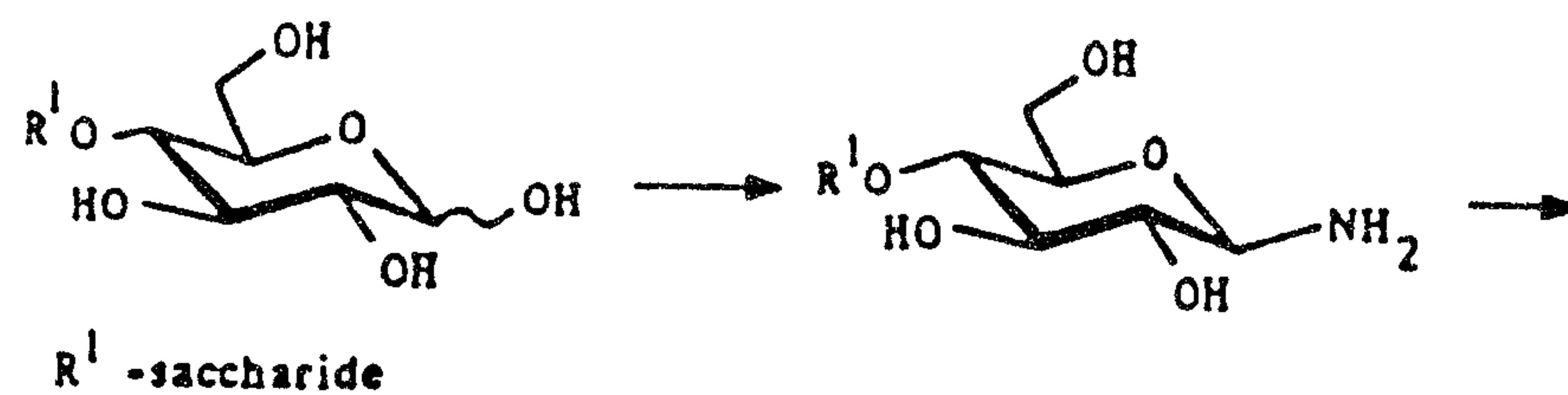


FIG. 3

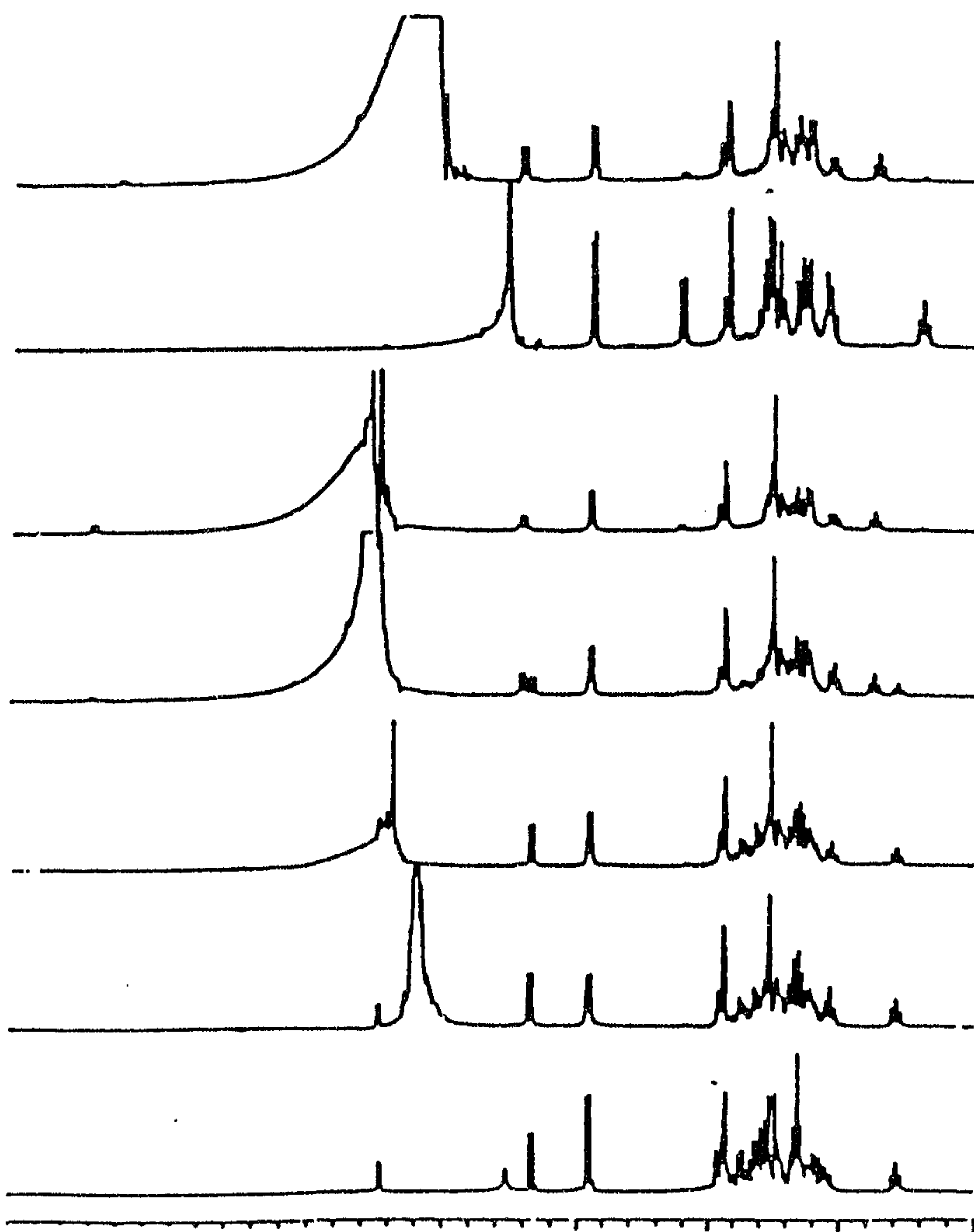


FIG. 4

Patent Agents
Fetherstonhaugh & Co.

