

1 570 625

PATENT SPECIFICATION

(11) 1 570 625

- (21) Application No. 51228/76 (22) Filed 8 Dec. 1976
 (31) Convention Application No. 16042/75
 (32) Filed 10 Dec. 1975 in
 (33) Switzerland (CH)
 (44) Complete Specification published 2 July 1980
 (51) INT CL³ C07C 103/52 A61K 31/70//C07D 309/14 493/04 498/04
 (52) Index at acceptance

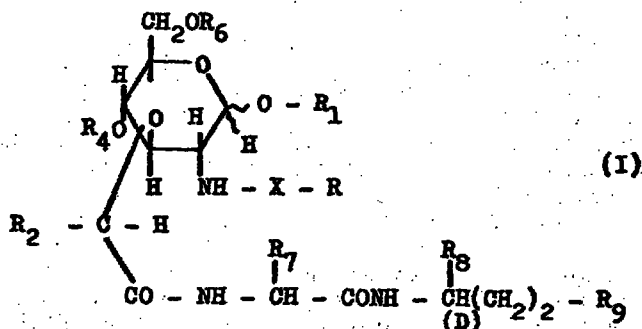
C3H 302 350 A4
 A5B 170 180 190 230 23Y 30X 30Y 38Y 390 39X H
 C2C 1377 1492 1672 167X 214 215 220 226 22Y 246 253 255 25Y
 281 28X 292 29Y 302 30Y 321 32Y 342 34Y 360 361 362
 364 366 367 368 36Y 385 450 45Y 500 50Y 510 511 51X
 535 601 610 620 623 624 628 62X 658 65X 670 672 699
 AA BC KM KP LH SD



(54) GLUCOSAMINE DERIVATIVES AND A PROCESS
 FOR THEIR MANUFACTURE

(71) We, CIBA-GEIGY AG, a body corporate organised according to the laws of Switzerland, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to new glucosamine derivatives, especially glucosamino - 3 - alkanoyl - dipeptides. The invention provides glucosamine derivatives of the general formula



and the salts thereof in which X denotes a carbonyl or sulphonyl group, R denotes an optionally substituted alkyl radical or an optionally substituted aryl radical and, if X is the carbonyl group, also denotes an alkoxy or benzyloxy radical, R₁ denotes hydrogen, alkyl or an optionally substituted benzyl radical, R₂ denotes hydrogen or lower alkyl, R₃ and R₄ denote hydrogen, alkyl or an optionally substituted benzyl or an acyl radical, R₅ denotes hydrogen, alkyl, hydroxymethyl, mercaptomethyl or phenyl, R₆ denotes an optionally esterified carboxyl group or amidised carboxyl group which is optionally substituted, and R₉ denotes an optionally esterified carboxyl group or amidised carboxyl group which is optionally substituted, with the proviso (a) that the optionally substituted alkyl radical R has more than 1 carbon atom if (1) X denotes the carbonyl group and the radical R₂ denotes methyl, or (2), if X denotes the carbonyl group, the radical R₂ represents hydrogen and R₃ and R₄ each represent a carboxyl group, and also with the proviso (b) that where R₂ and R₃ are other than hydrogen, the side-chain attached to oxygen in the 3-position of the glucosamine residue has the DLD configuration, and a process for their manufacture.

Unless specifically stated, residues, radicals or compounds modified by the term "lower" in the text which follows contain up to 7 and preferably up to 4 carbon atoms.

Alkyl is, in particular, lower alkyl, for example isopropyl, straight-chain or branched butyl, pentyl, hexyl or heptyl, which are bonded in any desired position, and above all methyl, ethyl or n-propyl.

Possible substituents of the optionally substituted alkyl group are free or functionally modified hydroxyl or mercapto groups, such as etherified or esterified hydroxyl or mercapto groups, for example lower alkoxy or lower alkyl-mercapto groups, or halogen atoms or free or functionally modified carboxyl groups, such as carbo-lower alkoxy or carbamoyl groups. The substituted alkyl radical, such as a lower alkyl radical, can carry one, two or more identical or different substituents, especially free hydroxyl groups or halogen atoms.

Aryl radicals are, in particular, monocyclic aryl radicals and also bicyclic aryl radicals especially phenyl, but also naphthyl. They can optionally be monosubstituted, disubstituted or polysubstituted, for example by lower alkyl groups, free, etherified or esterified hydroxyl, for example lower alkoxy or lower alkylenedioxy, or halogen atoms and/or trifluoromethyl groups.

If the radical X is a carbonyl group, R can also represent an alkoxy radical, especially a lower alkoxy radical, such as the methoxy or ethoxy radical, or the benzyloxy radical. The grouping —NH—X—R then forms the radical of an ester of carbamic acid.

Optionally substituted benzyl radicals are, in particular, those benzyl radicals which are optionally monosubstituted, disubstituted or polysubstituted in the aromatic nucleus, for example by lower alkyl, free, etherified or esterified hydroxyl or mercapto groups, for example lower alkoxy or lower alkylenedioxy groups, as well as lower alkylmercapto or trifluoromethyl groups and/or halogen atoms.

An optionally esterified or amidised carboxyl group is preferably, the carboxyl group itself or a carboxyl group esterified with a lower alkanol, such as methanol or ethanol, or the carbamoyl group which, on the nitrogen atom, is unsubstituted or monosubstituted or disubstituted by alkyl, especially lower alkyl, aryl, preferably phenyl, or aralkyl, such as benzyl.

The carbamoyl group can, however, also carry an alkylidene radical, such as a butylidene or pentylidene radical. The carbamoyl group R_2 can also carry a carbamoyl-methyl group on the nitrogen atom.

Acyl is, in particular, an acyl radical of an organic acid, especially of an organic carboxylic acid. Thus, acyl is in particular alkanoyl, preferably with 2—18 carbon atoms, such as acetyl or propionyl, or also aroyl, such as naphthoyl - 1 - , naphthoyl-2 and especially benzoyl, or benzoyl or naphthoyl substituted by halogen, lower alkyl, lower alkoxy, trifluoromethyl, hydroxyl or lower alkanoyloxy, as well as carbamoyl, such as unsubstituted carbamoyl, lower alkyl-carbamoyl or aryl-carbamoyl, such as methylcarbamoyl or phenyl-carbamoyl.

As a substituent of the abovementioned radicals, lower alkyl is preferably methyl or ethyl but may be also n-propyl, isopropyl or straight-chain or branched butyl.

As a substituent of the abovementioned radicals, lower alkoxy is, in particular, methoxy or ethoxy and also n-propoxy, isopropoxy, n-butoxy or isobutoxy.

As a substituent of the abovementioned radicals, lower alkylmercapto is above all methylmercapto or ethylmercapto, but also n-propyl-mercapto or isopropylmercapto.

Halogen is, for example, fluorine, chlorine or bromine.

Carbo-lower alkoxy groups which may be mentioned in particular are carbomethoxy or carboethoxy, but also carbo-n-propoxy or carboisopropoxy groups, and carbamoyl groups which may be mentioned in particular are the carbamoyl group itself.

In the abovementioned compounds, in which R_2 denotes an alkyl radical, the R_2 -acetamide radical which is linked to the oxygen atom in the 3-position of the glucosamine radical is optically active, that is to say it is in the D-form. If R_2 does not represent hydrogen, the R_2 acetamide radical is in the L-form.

Depending on the nature of their substituents, the present new compounds are neutral, acid or basic compounds. If excess acid groups are present, they form salts with bases, such as ammonium salts or salts with alkali metals or alkaline earth metals, for example sodium, potassium, calcium or magnesium. If, however, excess basic groups are present, they form acid addition salts.

Acid addition salts are, in particular, non-toxic acid addition salts which can be used pharmaceutically, such as those with inorganic acids, for example hydrochloric acid, hydrobromic acid, nitric acid, sulphuric acid or phosphoric acid, or with organic acids, such as organic carboxylic acids, for example acetic acid, propionic acid, glycollic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4 - amino - salicylic acid, 2-

phenoxybenzoic acid, 2 - acetoxy - benzoic acid, embonic acid, nicotinic acid or isonicotinic acid, or organic sulphonic acids, for example methanesulphonic acid, ethanesulphonic acid, 2 - hydroxy - ethanesulphonic acid, ethane - 1,2 - disulphonic acid, benzenesulphonic acid, p - toluene - sulphonic acid or naphthalene - 2 - sulphonic acid; and also other acid addition salts which can be used, for example, as intermediate products, for example for purifying the free compounds or in the manufacture of other salts and can also be used for characterisation, for example, those with picric acid, picrolonic acid, flavianic acid, phosphotungstic acid, phosphomolybdic acid, chloroplatinic acid, Reinecke's acid or perchloric acid.

The compounds of the present invention display valuable pharmacological properties, in particular a marked immunity-boosting action. This can be demonstrated with the aid of the test arrangement described below:

1. Boosting of the Cellular Immunity in vivo: Increase in the Delayed-type Hypersensitivity to Ovalbumin in Guinea Pigs

Pirbright guinea pigs are immunised on day 0 with 10 mg of ovalbumin in complete Freund's adjuvant by injecting 0.1 ml of an antigen-adjuvant mixture into each of the two back paws. 4 weeks later skin reactions are produced by intracutaneous injection of 100 μ g of ovalbumin in 0.1 ml of buffered physiological sodium chloride solution and quantified on the basis of the reaction volume calculated 24 hours later with the aid of the surface area of the erythema and of the increase in the thickness of the skin. The antigen-specific increase in the reaction volume which is observed after 24 hours (delayed-type reaction) is taken as a measure of cell-transmitted immunity. Ovalbumin is too weak an immunogen to induce a delayed-type reaction on its own or in a water-in-oil emulsion with incomplete Freund's adjuvant (10 parts by weight of ovalbumin solution in 0.9% by weight of NaCl mixed with 8.5 parts by weight of Bayol F and 1.5 parts by weight of Arlacel (Registered Trade Mark), A), but, for effective immunisation, has to be administered in complete adjuvant, to which Mycobacteria are added (5 mg of M butyricum, which have been destroyed and lyophilised, per 10 ml of Bayol F/Arlacel A). In order to demonstrate the immunity-boosting action of the test substances, the latter can now be mixed, in doses of 10 to 100 μ g, with the antigen/oil mixture in place of the Mycobacteria.

The glucosamine peptides according to the invention are able to imitate the effect of the Mycobacteria in the test arrangement described and quantitatively to surpass this effect.

A significant boosting of the delayed-type reactivity against ovalbumin can also be achieved when compounds of the type described are not incorporated into the antigen/oil mixture but are administered subcutaneously, in sodium chloride solution, in doses of 10 to 100 μ g per animal for several days after immunisation (for example on day 0, 1, 2, 5, 6 and 7).

This shows that compounds of the type described are able considerably to increase cellular immunity, both as a mixture with the antigen itself (adjuvant effect in the narrower sense) and when supplied at times and places which differ from those of the antigen injection (systemic immunity-boosting).

2. Boosting of the Humoral Immunity in vivo: Increase in the Production of Antibodies against Bovine Serum Albumin (BSA) in Mice

NMRI mice are immunised by intraperitoneal (i.p.) injection of 10 μ g of precipitate-free BSA on day 0. Serum samples are taken 9, 15 and 29 days later and tests are carried out, using a passive haemagglutination technique, to determine their content of anti-BSA antibodies. In the dosage used, soluble BSA is sub-immunogenic for the receiving animals, that is to say it is not able to initiate any production of antibodies or is able to initiate only a very slight production of antibodies. Additional treatment of the mice with certain immunity-boosting substances before or after the administration of the antigen leads to a rise in the antibody titre in the serum. The effect of the treatment is expressed by the score value, that is to say by the sum of the log₂ titre differences on the three days when blood is sampled, which is reached.

On intraperitoneal or subcutaneous (s.c.) administration of 100-300 mg/kg/animal on five consecutive days (day 0 to 4) after immunisation with BSA, compounds of the present invention are able significantly to increase the production of antibodies against BSA.

(13)

4

1,570,625

4

The immunity-stimulating effect of the said compounds, in contrast to that of other bacterial immunoleptic agents (for example LPS from *E. Coli*), is dependent on the antigen: injection of the new compounds results in an increase in the anti-BSA titre only in BSA-immunised mice, but not in mice which have not been immunised. It is worthy of note that the s.c. administration of the said compounds is equally as effective as the i.p. administration, that is to say the immunity-boosting action which is observed is systemic and does not depend on the stimulant having to be administered by the same route as the antigen or as a mixture with the antigen, as is the case with classical adjuvants.

The experiments illustrated show that compounds of the type described are also able specifically to increase the humoral immunity, that they improve the immunological response and that their immunity-boosting effects are based on a systemic activation of the immune system.

3. Boosting of the Humoral Immunity in vitro: T-Cell-replacement Effect in the Antibody Response of Mice Splenocytes to Sheep Erythrocytes (SE).

In many cases, lymphocytes (T-cells) emanating from the thymus are necessary for the induction of an antibody response. These cells co-operate with the precursors of antibody-forming lymphocytes (B-cells) and help them to react to stimulation by so-called T-dependent antigens by proliferation, differentiation and synthesis of antibodies. Suspensions of splenocytes from congenitally athymic nu/nu mice do not contain any functional T-cells and, for example in vitro in the presence of SE, are not able to form any anti-SE antibodies. The compounds of the present invention are, surprisingly, able functionally to replace T-cells in such cultures and to make an antibody response to SE possible. The addition of these substances to nu/nu splenocyte cultures in the presence of SE leads, within 4 days, to a considerable increase in the number of antibody-forming cells. The findings show that the said compounds are able to increase the humoral antibody formation in vitro and to compensate a defect in the T-cell system.

4. Selective Mitogenicity for B-cells: Proliferation-promoting Effect in B-lymphocyte Cultures

Suspensions of highly enriched B-lymphocytes (lymph node cells from congenitally athymic nu/nu mice) and substantially pure immature and mature T-lymphocytes (thymocytes and, respectively, cortisone-resistant thymocytes, that is to say thymocytes which persist 48 hours after a cortisone injection, from Balb/c mice) are incubated for three days in the presence of the test substances. The incorporation of H^3 -thymidine in the lymphocytes during the final 18 hours of the culture period is taken as a measure of the proliferation activity.

The compounds of the invention are mitogenic for B-lymphocytes (that is to say for the precursors of the antibody-forming cells) but not for T-lymphocytes.

They are thus able to stimulate the proliferation of lymphocytes which are involved in the humoral immune response.

5. Tolerance

Although compounds of the type described already display their boosting action in guinea pigs after, for example, an individual dose of 0.05 mg/kg administered subcutaneously, and in mice after the subcutaneous administration of 5 times 10 mg/kg, no toxic effects are observed in mice even when 5 times 300 mg/kg are administered intraperitoneally. The said substances therefore possess an excellent therapeutic range.

The compounds according to the invention are able when mixed with an antigen, to increase the immunogenicity of the latter and also, on systemic administration, to increase the immunological reactivity of the treated organism. The said substances are able to promote both the cellular and the humoral immunity and to activate the lymphocytes which are responsible for the formation of antibodies.

The new compounds can thus be used, as adjuvants mixed with vaccines, to improve the success rate of the vaccination and to improve the protection imparted by humoral antibodies and/or cellular immunity against infection by bacterial, viral or parasitic pathogens.

Finally, the compounds described are suitable, as a mixture with very diverse antigens, as adjuvants for the experimental and industrial production of antisera for

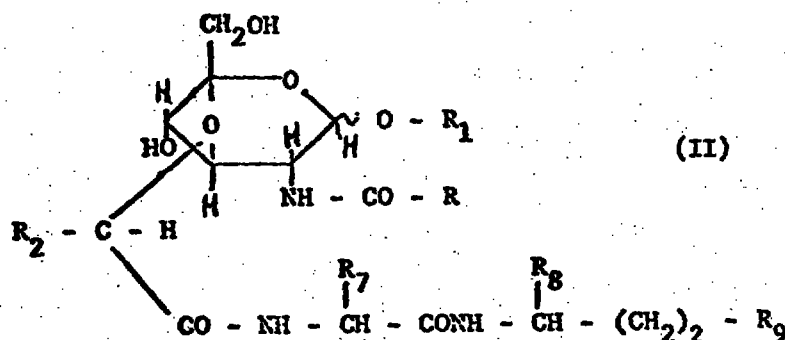
therapy and diagnosis and for inducing immunologically activated lymphocyte populations for cell transfer processes.

Moreover, the new compounds can be used, even without the addition of an antigen at the same time, to promote immune reactions which are already taking place subliminally in humans and animals. Accordingly, the compounds are especially suitable for stimulating the body's own resistance, for example in the case of chronic and acute infections or in the case of selective (antigen-specific) immunological defects, and also in the case of general (that is to say not antigen-specific) immunological defective states which are congenital or acquired, such as arise in old age, in the course of severe primary diseases and, especially after therapy with ionising rays or with hormones having an immuno-suppressive action. The said substances can thus be administered, preferably also in combination with anti-infectious antibiotics, chemotherapeutic agents or other courses of treatment, in order to counteract immunological damage. Finally, the substances described are also suitable for the general prophylaxis of infectious diseases in humans and animals.

The invention relates in particular to compounds of the formula (I) in which X denotes a carbonyl radical and R denotes a lower alkyl radical which is optionally substituted by hydroxyl or carboxyl groups or denotes a phenyl radical which is optionally substituted by lower alkyl, lower alkoxy, trifluoromethyl or halogen, and R_1 , R_2 , R_4 , R_6 , R_7 , R_8 and R_9 have the above-mentioned meaning, and their salts.

Compounds in which R_2 represents hydrogen and the other radicals possess the abovementioned meaning, and their salts are also particularly valuable.

Compounds to be singled out are, in particular, compounds of the general formula



in which R denotes lower alkyl or phenyl, R_1 denotes hydrogen or lower alkyl, R_2 denotes hydrogen or methyl, R_3 denotes hydrogen, lower alkyl or hydroxymethyl, R_4 denotes carbamoyl and R_5 denotes carboxyl, with the proviso that the lower alkyl radical R contains more than 1 carbon atom if R_2 denotes methyl, and their salts.

Compounds to be mentioned above all are those of the formula II in which R_1 denotes hydrogen, and R_2 denotes hydrogen, methyl or hydroxymethyl. Other categories of compound to be singled out are:—

1) compounds of formula I, in which R_1 and R_2 denote hydrogen, R denotes phenyl, R_3 denotes methyl, and R_4 and R_5 denote carboxyl, carbomethoxy, carbamoyl, or N - methyl - carbamoyl;

2) compounds of formula II, in which R_1 and R_2 denote hydrogen, R denotes methyl or phenyl, R_3 denotes hydroxymethyl, R_4 is carbamoyl, and R_5 is carboxyl;

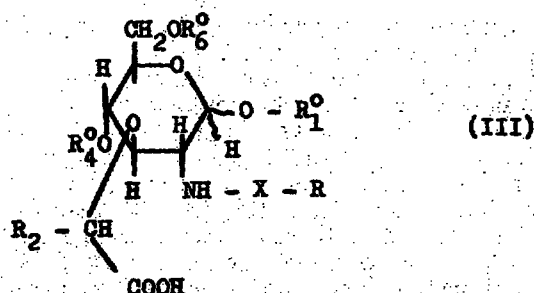
3) compounds of formula II, in which R_1 and R_2 denote hydrogen, R denotes phenyl, R_3 is methyl, R_4 is carboxyl, and R_5 denotes N - propyl - carbamoyl or N - carbamoylmethylcarbamoyl;

4) compounds of formula I, in which R_1 and R_2 represent hydrogen, R and R_3 denote methyl, and R_4 and R_5 denote carbomethoxy, carbamoyl, or N - methylcarbamoyl;

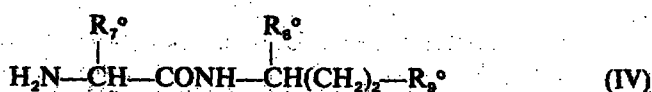
5) compounds of formula I, in which R denotes phenyl, R_1 denotes ethyl, R_2 denotes hydrogen, R_3 is methyl, R_4 is carbamoyl and R_5 is carboxyl, or R_4 and R_5 denote carbomethoxy or N - methylcarbamoyl;

6) compounds of formula I, in which R and R_3 denote methyl, R_1 is benzyl, R_2 is hydrogen, and R_4 and R_5 represent carbomethoxy or carbamoyl.

The new compounds may be obtained when a compound of the formula



in which X, R and R_2 possess the abovementioned meaning and R_1° , R_4° and R_6° represent the radicals R_1 , R_4 and R_6 respectively or represent a protective group, is condensed with a compound of the formula



in which R_7° , R_8° and R_9° possess the meaning of R_7 , R_8 and R_9 , with the proviso that carboxyl groups and, if desired, free hydroxyl groups present in these radicals are protected by protective groups and protective groups which are present are split off.

The condensation reaction is carried out, for example, by reacting the compound III, in the form of the activated carboxylic acid, with the amino compound IV or by reacting the acid III with the compound IV, in which the amino group is present in an activated form. The activated carboxyl group can be for example, an acid anhydride, preferably a mixed acid anhydride, such as an acid azide, an acid amide, such as an imidazolidine, an isoxazolidine or an activated ester. Activated esters which may be mentioned in particular are: cyanomethyl esters, carboxymethyl esters, p-nitrophenylthio esters, p-nitrophenyl esters, 2,4,5-trichlorophenyl esters, pentachlorophenyl esters, N-hydroxy-succinimide esters, N-hydroxyphthalimide esters, 8-hydroxyquinoline esters, 2-hydroxy-1,2-dihydro-1-carboethoxy-quinoline esters, N-hydroxypiperidine esters or enol esters which are obtained with N-ethyl-5-phenyl-isoxazolium 3'-sulphonate. Activated esters can also optionally be obtained with a carbodiimide with the addition of N-hydroxysuccinimide or a 1-hydroxybenzotriazole or 3-hydroxy-4-oxo-3,4-dihydro-benzo[d]-1,2,3-triazine which is unsubstituted or substituted by, for example, halogen, methyl or methoxy.

The amino group is activated by, for example, reaction with a phosphite-amide.

Amongst the methods comprising a reaction with activated esters, those with N-ethyl-5-phenyl-isoxazolium 3'-sulphonate (Woodward reagent K) or 2-ethoxy-1,2-dihydro-1-carboethoxy-quinoline or carbodiimide are to be mentioned in particular.

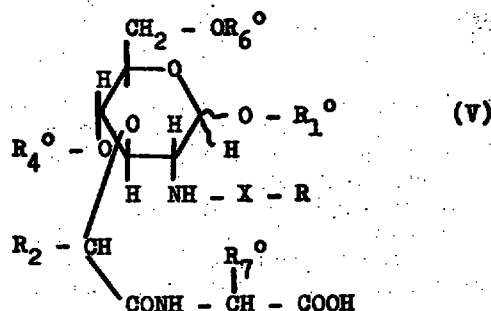
Protective groups which can be split off easily are those which are known from peptide chemistry and sugar chemistry. Protective groups which should be mentioned for carboxyl groups are, in particular, tertiary butyl, benzyl or benzhydryl and for hydroxyl groups are, in particular, acyl radicals, for example lower alkanoyl radicals, such as acetyl, aroyl radicals such as benzoyl, and, especially radicals derived from carbonic acid, such as benzyloxycarbonyl or lower alkoxy carbonyl, or alkyl, especially tert-butyl, benzyl or tetrahydropyranyl which are optionally substituted by nitro, lower alkoxy or halogen, or optionally substituted alkylidene radicals which link the oxygen atoms in the 4-position and 6-position. Such alkylidene radicals are, in particular, a lower alkylidene radical, above all the ethylidene, isopropylidene or propylidene radical, or also a benzylidene radical which is optionally substituted and preferably substituted in the p-position.

These protected groups can be split off in a conventional manner. Thus, they can be removed hydrogenolytically, for example with hydrogen in the presence of a noble metal hydrogenation catalyst, such as a palladium or platinum catalyst, or by acid hydrolysis.

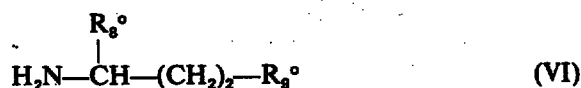
The starting materials used are known or can be manufactured in a conventional manner. Thus, compounds of the formula III can be obtained, for example, by reacting the corresponding sugar, which is unsubstituted in the 3-

position, with a halogeno - R_2 - acetic acid, in which R_2 has the abovementioned meaning, and its esters in the presence of a strong base. In these compounds, halogen is preferably bromine or especially chlorine.

Another procedure for the manufacture of the new glucosamine derivatives is to condense a compound of the formula V



in which R , R_1 , R_2 , R_4 , R_6 and R_7 have the abovementioned meaning with a compound of the formula



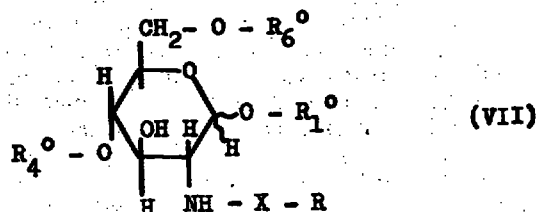
in which R_8 and R_9 have the abovementioned meaning, with the proviso that carboxyl groups and, if desired, free hydroxyl groups present in the radicals R_7 , R_8 and R_9 are protected by protective groups and to split off any protective groups which may be present.

The condensation reaction is carried out, for example, by reacting the compound V, in the form of the activated carboxylic acid, with the amino compound VI, or by reacting the acid V with the compound VI, in which the amino groups are in an activated form. The activated carboxyl group can be, for example, an acid anhydride, preferably a mixed acid anhydride, an acid amide or an activated ester. These can be, in particular, the acid anhydrides, amides or esters mentioned above. The amino group is activated, for example, by reaction with a phosphite-amide.

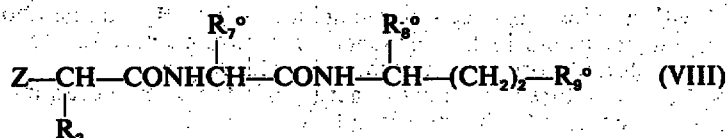
The protective groups which can be split off easily also correspond to those already mentioned above. They can be split off in a conventional manner; for example hydrogenolytically, for example with hydrogen in the presence of a noble metal hydrogenation catalyst, such as a palladium or platinum catalyst, or by acid hydrolysis.

The starting materials can be obtained in a conventional manner. Thus, it is possible, for example, to react corresponding sugars which are unsubstituted in the 3-position with a halogeno - R_2 - acetamido - acetic acid, substituted by R_7 at the alpha carbon atom or to react a compound of the formula III in the manner indicated above with a similarly substituted amino-acetic acid in which the carboxyl group is protected and to split off the protective group.

A further process method for introducing the side chain located in the 3-position of the sugar residue is to react a compound



in which X , R , R_1 , R_4 and R_6 have the abovementioned meaning and any hydroxyl groups which may be contained therein are protected by a protective group, with a compound of the formula



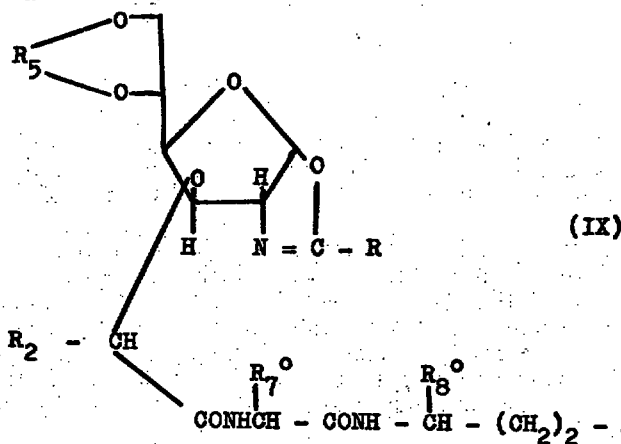
in which Z represents a reactively esterified hydroxyl group and R_7° , R_8° and R_9° have the abovementioned meaning, and to split off any protective groups which may be present.

A reactively esterified hydroxyl group is, in particular, a hydroxyl group esterified with a strong inorganic or organic acid and, especially, a hydroxyl group which is esterified with hydrogen halide acids, such as hydrochloric acid, hydrobromic acid or hydriodic acid.

The protective groups which can be split off easily correspond to those already mentioned above. They can be split off in a conventional manner, for example hydrogenolytically, for example with hydrogen in the presence of a noble metal hydrogenation catalyst, such as palladium or platinum catalyst, or by acid hydrolysis.

The starting materials used in this process variant are known.

However, the compound may also be obtained when the oxazoline ring and the dioxolane ring in a compound of the formula IX



in which R , R_2 , R_7° , R_8° , and R_9° possess the abovementioned meaning and R_5 is an alkylidene or cycloalkylidene group are split open under acid conditions with formation of the glucosamine ring and any protective groups which may be present are split off and if the radical $\text{R}-\text{CO}-$ has been removed, the $\text{X}-\text{R}$ radical is introduced into the amino group in the 2-position of the sugar molecule if this group has been liberated.

In this compound, alkylidene is, in particular, lower alkylidene, such as isopropylidene, and cycloalkylidene is preferably cyclopentylidene or cyclohexylidene.

This splitting is also carried out in a conventional manner, for example with an acid ion exchanger, especially those containing sulphonic acid groups, such as Amberlite (Registered Trade Mark) IR-120 (a styrene resin with highly acid sulpho groups) or Dowex (Registered Trade Mark) 50 (polystyrenesulphonic acids) or a strong inorganic or organic acid, such as hydrochloric acid, hydrobromic acid, sulphuric acid or a sulphonic acid, for example methanesulphonic acid, or a phenylsulphonic acid which is optionally substituted in the aromatic ring, such as p-toluenesulphonic acid, or trifluoroacetic acid. If the reaction is carried out in the presence of water a free hydroxyl group is obtained in the 1-position; if, on the other hand, the reaction is carried out in the presence of an alcohol of the formula $\text{HO}-\text{R}_1$ in which R_1 represents an optionally substituted alkyl radical, the $1-\text{O}-\text{R}_1$ compound is obtained. If one of the carboxyl groups R_7 and/or R_8 is also esterified with an alcohol, especially a lower alkanol, this can be saponified with aqueous acid, especially at elevated temperature.

However, it is also possible for the amino group in the 2-position of the sugar molecule to be liberated during this splitting. In this case, the $\text{X}-\text{R}$ radical has to be introduced subsequently. This is carried out in the customary manner by acylation or sulphonylation.

18

In the resulting compounds it is possible subsequently to split off protective groups on the peptide radical, for example by hydrogenolysis, such as, for example, with catalytically activated hydrogen, or by hydrolysis.

The starting materials used can be obtained, for example, when the R₂-acetamidopeptide radical is introduced, in one or several stages, into a corresponding oxazoline which has a free hydroxyl group in the 3-position of the sugar residue.

The resulting compounds can be converted into their salts in a conventional manner for example by reacting resulting acid compounds with alkali metal hydroxides or alkaline earth metal hydroxides or by reacting resulting basic compounds with acids.

The processes described above are carried out by conventional methods in the absence or, preferably, in the presence of diluents or solvents, if necessary with cooling or warming, under elevated pressure and/or in an inert gas atmosphere, such as a nitrogen atmosphere.

Taking into account all of the substituents present in the molecule, particularly gentle reaction conditions, such as short reaction times, the use of mild acid or basic agents in low concentration, stoichiometric ratios and the choice of suitable catalysts, solvents and temperature and/or pressure conditions, must be employed if necessary, especially in the presence of O-acyl radicals which can be hydrolysed easily.

The invention also relates to those embodiments of the process according to which a starting material is used in the form of a reactive derivative or salt. The starting materials preferably used are those which, according to the process, lead to those compounds described above as being particularly valuable.

The present invention also relates to pharmaceutical formulations which contain compounds of the formula I together with a pharmaceutical excipient. The formulations may also contain a vaccine. The pharmaceutical formulations according to the invention are those for enteral, such as oral or rectal, and parenteral administration to warm-blooded animals and which contain the pharmacological active compound on its own or together with an excipient which can be used pharmaceutically. The dosage of the active compound depends on the species of warm-blooded animal, on the age and on the individual state and on the mode of administration.

The new pharmaceutical formulations may contain from about 10% to 95% by weight, and preferably from 20% to 90% by weight, of the active compound. Pharmaceutical formulations according to the invention can, for example, be in a dosage unit form, such as dragees, tablets, capsules, suppositories or ampoules.

The pharmaceutical formulations of the present invention are manufactured in a conventional manner, for example by means of conventional mixing, granulating, dragee-making, dissolving or lyophilising processes. Apart from the types of administration mentioned, pharmaceutical formulations especially for oral use can also be obtained by combining the active compound with solid excipients, optionally granulating a resulting mixture and processing the mixture or granules, after adding suitable auxiliaries if desired or necessary, to give tablets or dragee cores.

Suitable excipients are, in particular, fillers, such as sugars, for example, lactose or sucrose; mannitol or sorbitol, cellulose formulations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders, such as starch pastes using, for example, maize starch, wheat starch, rice starch or potato starch, gelatin, tragacanth, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrating agents, such as the abovementioned starches, and also carboxymethylstarch, crosslinked polyvinylpyrrolidone, agar or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, especially, flow-regulating agents and lubricants, for example silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices, and for this purpose, inter alia, concentrated sugar solutions, which optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions in suitable organic solvents or solvent mixtures or, in order to produce coatings resistant to gastric juices, solutions of suitable cellulose formulations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, are used. Dyestuffs or pigments can be added to the tablets or dragee coatings, for example

for identification or in order to characterise different doses of the active compound.

The examples which follow illustrate the invention described above; they are not, however, intended to restrict the scope of the invention in any way. Temperatures are given in degrees centigrade, and the percentages are by weight.

Example 1

A solution of 2.6 g. of the benzyl ester of benzyl - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside in 67 ml of 60% strength acetic acid is hydrogenated under normal pressure and at room temperature, using 0.6 g of 5% strength palladium-on-charcoal as the catalyst, until the reaction has ceased (hydrogenation time about 20 minutes). The catalyst is filtered off and rinsed with a little 60% strength acetic acid and the filtrate is evaporated to dryness under a waterpump vacuum. The residue is crystallised from ethanol/ether. This gives benzyl - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside which has a melting point of 155—160° (decomposition) and an optical rotation $[\alpha]_D^{20}$ of $+105^\circ \pm 1^\circ$ (dimethyl-formamide, $c=0.58$).

The benzyl ester of benzyl - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside which is used as the starting material can be obtained as follows:

4.1 g of benzyl - 2 - amino - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxyethyl) - 2 - deoxy - α - D - glucopyranoside are dissolved in 200 ml of methanol and 3 ml of triethylamine and 0.95 ml of propionyl chloride is added dropwise at room temperature, whilst stirring. After stirring for 2 hours, the reaction mixture is evaporated to dryness and the residue is dissolved in 100 ml of water. The pH of this solution is adjusted to 4 with ice-cold 2N hydrochloric acid and the product which has separated out is filtered off, rinsed with water and dried in vacuo over sodium hydroxide. The benzyl - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxyethyl) - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside which is thus obtained can be recrystallised from methanol; melting point 257°, $[\alpha]_D^{20} = +132^\circ \pm 1^\circ$ (dimethylformamide, $c=1.086$).

1.5 g of N - ethyl - 5 - phenyl - isoxazolium 3' - sulphonate (Woodward's reagent K) are added to a solution of 3.1 g of the resulting compound in 150 ml of acetonitrile and 0.9 ml of triethylamine and the mixture is stirred at room temperature until everything has dissolved (about 60 minutes). 2.2 g of L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester - hydrochloride and 0.9 ml of triethylamine are now added and the mixture is stirred for a further 18 hours at room temperature. After distilling off the solvent under a waterpump vacuum, water is added to the residue and the insoluble matter is filtered off, washed thoroughly with water and dried. The resulting benzyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside, which has an Rf value of 0.45 on silica gel thin layer plates in the system methylene chloride/methanol, 10/1, is dissolved in 200 ml of 60% strength acetic acid and the solution is kept at 95—100°C for 1.5 hours. After cooling, the solvent is distilled off. The residue is taken up twice more in a little water and in each case is evaporated to dryness. The resulting benzyl ester of benzyl - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside is crystallised from methanol; melting point 189°, $[\alpha]_D^{20} = +100^\circ \pm 1^\circ$ (dimethylformamide, $c=1.268$).

Example 2

A solution of 4 g of the benzyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside in 80 ml of methanol is hydrogenated under normal pressure and at room temperature, using 0.4 g of 5% strength palladium-on-charcoal as the catalyst, until the reaction has ceased. The catalyst is filtered off and rinsed with a little methanol and the filtrate is evaporated to dryness under a waterpump vacuum. The residue is dissolved in 50 ml of distilled water and further hydrogenated under normal pressure and at room temperature,

using 1 g of 5% strength palladium-on-charcoal as the catalyst, until the reaction has ceased. The catalyst is filtered off and rinsed with a little water and the filtrate is evaporated to dryness. The resulting 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxyl - propyl) - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - D - glucose is dried over phosphorus pentoxide under a high vacuum; $[\alpha]_D^{20} = -10^\circ \pm 1^\circ$ (water, $c=0.930$).

The starting material can be prepared as follows:

5.3 g of N - ethyl - 5 - phenyl - isoxazolium 3'-sulphonate (Woodward's reagent K) are added to a solution of 9.5 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside in 400 ml of acetonitrile and 3 ml of triethylamine and the mixture is stirred at room temperature until a clear solution forms. 7.15 g of L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester - hydrochloride, 3 ml of triethylamine and 200 ml of acetonitrile are now added and the reaction mixture is stirred for a further 18 hours at room temperature. The benzyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside, which has crystallised out, is filtered off, washed with half-saturated sodium bicarbonate solution and water and dried; $[\alpha]_D^{20} = +81^\circ \pm 1^\circ$ (dimethylformamide, $c=0.816$).

A solution of 8 g of this compound in 400 ml of 60% strength acetic acid is kept at 80°C for 1 hour. After cooling, the solution is evaporated to dryness, a further twice 50 ml of water are added to the residue and in each case the mixture is evaporated to dryness. The resulting crystalline residue is stirred with a little water and the crystals are filtered off and dried. This gives the benzyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside which has a melting point of 200—202°, $[\alpha]_D^{20} = +77^\circ \pm 1^\circ$ (dimethylformamide, $c=0.599$).

Example 3

A solution of 0.9 g of the benzyl ester of benzyl - 2 - acetamido - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside in 40 ml of methanol is hydrogenated under normal pressure and at room temperature, using 0.2 g of 5% strength palladium-on-charcoal as the catalyst, until 22.4 ml of hydrogen have been taken up. The catalyst is filtered off and rinsed with methanol and the filtrate is evaporated to dryness. This gives benzyl - 2 - acetamido - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside, $[\alpha]_D^{20} = +33^\circ \pm 1^\circ$ (chloroform, $c=1.046$).

The starting material can be prepared as follows:

A solution of 1.4 g of stearyl chloride in 7.5 ml of methylene chloride is added dropwise in the course of 1 hour to a solution of 2.8 g of the benzyl ester of benzyl - 2 - acetamido - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl - 2 - deoxy - α - D - glucopyranoside in 30 ml of pyridine, whilst stirring and with the exclusion of moisture, and the mixture is stirred at room temperature for 48 hours. The reaction mixture is poured into ice water and extracted with methylene chloride. The organic phase is washed with ice-cold 2N hydrochloric acid and water, dried over magnesium sulphate and evaporated to dryness. The residue is purified by column chromatography on silica gel by elution with ethyl acetate.

This gives the benzyl ester of benzyl - 2 - acetamido - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $+30^\circ \pm 1^\circ$ (chloroform, $c=1.203$).

Example 4

Analogously to Example 2, benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside is subjected to a condensation reaction with L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester - hydrochloride and the protective groups are split off. This gives 2 - acetamido - 3 - O - [D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - propyl] - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

10 g of sodium hydride are added to a solution of 60 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 2 - deoxy - α - D - glucopyranoside in 600 ml of dimethylformamide and the mixture is stirred for 1.5 hours at 45°C in a nitrogen atmosphere. After cooling to 0°, 75 ml of ethyl D,L - α - bromobutyrate are added. The reaction mixture is stirred for 1 hour at room temperature and for 1 hour at 50°C and neutralised with acetic acid and the solvent is evaporated under a waterpump vacuum. The residue is partitioned between methylene chloride and water, the organic phase is washed once more with water and dried over magnesium sulphate and the solvent is evaporated. The residue, which is dried under a high vacuum, is purified by column chromatography on silica gel. Elution with methylene chloride/ethyl acetate (85/15) gives the ethyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of +113° to $\pm 1^\circ$ (chloroform, $c=0.5$), a melting point of 154°C (from methylene chloride/ether) and an Rf value of 0.21 and the ethyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (L - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of +42° $\pm 1^\circ$ (chloroform, $c=0.511$), a melting point of 240°C (from ethyl acetate) and an Rf value of 0.04.

100 ml of 1N sodium hydroxide solution are added to a solution of 38.1 g of the ethyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside in 300 ml of methanol and the mixture is kept at 60° for 1 hour. The solution is then cooled, concentrated to about 150 ml and diluted with 400 ml of ice water and 100 ml of 1N ice-cold hydrochloric acid are added. The product which has crystallised out is filtered off, washed with water and dried. This gives benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside which has a melting point of 210—213° and an $[\alpha]_D^{20}$ of +110° $\pm 1^\circ$ (dimethylformamide, $c=0.554$).

Benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (L - 1 - carboxy - propyl) - 2 - deoxy - α - D - glucopyranoside which has a melting point of 285°C and an $[\alpha]_D^{20}$ of +71° $\pm 1^\circ$ (dimethylformamide, $c=0.589$) is obtained in an analogous manner.

Example 5

3.77 g of 2 - phenyl - 4,5 - [3 - O - (D - 1 - carboxyethyl) - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline in 60 ml of acetonitrile and 15 ml of dimethylformamide are stirred together with 1.4 ml of triethylamine and 2.55 g of N - ethyl - 5 - phenyl - isoxazolium 3' - sulphonate for 1.5 hours at 0°, whereupon virtually everything goes into solution. 3.44 g of L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester - hydrochloride and a further 1.4 ml of triethylamine are then added and the mixture is stirred for 24 hours at room temperature. It is then evaporated under an oil vacuum to give a syrup and the latter is chromatographed on silica gel using a mixture of chloroform and acetone (8/2). A colourless, firm syrup which crystallised on grinding with ether is obtained. Melting point 96—99°, $[\alpha]_D^{20}=+13^\circ$ (in chloroform).

The crystalline benzyl ester is hydrogenated, using 5% strength palladium-on-charcoal, in dioxane at room temperature and under normal pressure and after evaporating in vacuo gives the parent acid as a syrup.

This is stirred in water with 10 ml of Dowex-50-H⁺ for 15 hours at room temperature. After filtering and freeze-drying, a colourless powder with a decomposition temperature of 140° is obtained. The 2 - benzoylamino - 3 - O - [- D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoyl - ethyl] - 2 - deoxy - α - β - D - glucose which is obtained contains varying amounts of water of crystallisation, depending on the drying conditions and in the above case after drying at 60° and under 0.01 mm Hg for 15 hours contains 1/3 water.

Example 6

6.0 g of 2 - phenyl - 4,5 - [3 - O - (D - 1 - carboxypropyl) - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline, 4.08 g of N - ethyl - 5 - phenyl - isoxazolium 3' - sulphonate and 2.25 ml of triethylamine are stirred in 100 ml of acetonitrile and 25 ml of dimethylformamide for 1 hour at 0—5°C,

whereupon the whole goes into solution. 5.55 g of L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester hydrochloride and a further 2.35 ml of triethylamine are then added and the mixture is stirred at room temperature for 48 hours. It is evaporated under an oil vacuum and chromatographed on silica gel in a mixture of chloroform and ethanol (9/1). 9 g of the colourless syrup which is thus obtained are hydrogenated in dioxane using 5% strength palladium-on-charcoal, the catalyst is filtered off and the filtrate is concentrated in vacuo and hydrolysed at room temperature in a mixture of 40 ml of tetrahydrofuran and 30 ml of water with 1.5 ml of trifluoroacetic acid. The water is then evaporated to dryness 4 times in vacuo and the residue is dissolved in water and lyophilised. The resulting 2 - benzoylamino - 3 - O - ([D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - propyl] - 2 - deoxy - α,β - D - glucose crystallises with 0.5 mol of water, melting point 114—152°, $[\alpha]_D^{20} = +17^\circ$ (in methanol).

Example 7

3.63 g of 2 - phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline, 3.43 g of L - alanyl - D - glutamic acid 1 - amide - γ - benzyl ester - hydrochloride, 1.21 g of N - hydroxysuccinimide, 2.16 g of dicyclohexylcarbodiimide and 1.45 ml of triethylamine are dissolved in 40 ml of dimethylformamide and the mixture is stirred for 24 hours at room temperature. It is evaporated under an oil vacuum, the residue is taken up in dichloroethane and water, the dicyclohexylurea which has precipitated out is filtered off and the organic phase is twice extracted by shaking with water and the aqueous phase is twice extracted by shaking with dichloroethane. After drying and evaporating the organic phases, a solid syrup is obtained and this is chromatographed on silica gel in a mixture of chloroform/ethanol (9/1). The resulting peptide ester, which crystallises on grinding with ether, melts at 167—168°, $[\alpha]_D^{20} = -5^\circ$ (chloroform).

4.5 g of the said ester are hydrogenated in dioxane using 5% strength palladium-on-charcoal and the catalyst is filtered off and further extracted with ethanol. The combined filtrates are evaporated and the residue is recrystallised from isopropyl alcohol. The resulting acid melts at 200—207°.

2.85 g of this acid, in a mixture of 30 ml of water and 15 ml of tetrahydrofuran are stirred with 5 ml of Dowex-50- H^+ for 15 hours at room temperature, the mixture is filtered through a hardened filter and the filtrate is evaporated to dryness in vacuo. On grinding with ether a colourless powder is obtained and this is 2 - benzoyl - amino - 3 - O - ([L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl - carbamoyl - methyl] - 2 - deoxy - α,β - D - glucose which has a melting point of 175—177° (as the hydrate).

The starting material can be prepared as follows, using a modification of the method described in Acta Chem. Scand. 18, 185 (1964):

100 g of 2 - phenyl - 4,5 - [5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline are dissolved in 1 litre of acetonitrile, with the exclusion of moisture and carbon dioxide, and 15.2 g of a 55% strength NaH/mineral oil dispersion are added in portions, whilst stirring well, and the mixture is stirred for a further one hour at room temperature. 42 ml of ethyl chloroacetate are then added dropwise at 0° and after 1.5 hours a further 42 ml are added dropwise. After 1.5 hours, a further 11.4 g of the NaH dispersion are added, the mixture is stirred for half an hour and a further 42 ml of the chloroacetate are added dropwise at 0°. After a further 2 hours, the mixture is allowed to warm to room temperature and is evaporated in vacuo—towards the end under an oil vacuum—to give a syrup. The latter is taken up in ether and extracted 3 times by shaking with water, the ether phase is dried over sodium sulphate and, after evaporating, 155 g of a brown oil are obtained. This is dissolved in 150 ml of methanol and a solution of 30 g of potassium hydroxide in 150 ml of water is added, the mixture is twice extracted with ether and the ether phase is washed once with water. The aqueous phases are freed from ether in vacuo and the pH is adjusted to 4 with 1N hydrochloric acid, using a pH meter.

The crystalline 2 - phenyl - 4,5 - [3 - O - (carboxymethyl) - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline which has precipitated out is filtered off, washed with water and dried. 107 g, that is 93% of theory, with a melting point of 186—188°, an $[\alpha]_D^{20}$ of -9° ($CHCl_3$, $c=0.9$) and an $[\alpha]_D^{20}$ of -23° ($CHCl_3$, $c=3$) are obtained.

Example 8

2.5 g of 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose (Example 8) are dissolved in a mixture of 17 ml of absolute pyridine and 4 ml of butyric anhydride. After 20 hours at room temperature and 2 hours at 50°, water is added and the mixture is concentrated in vacuo. The residue is dissolved in chloroform, the chloroform solution is extracted by shaking with 1N hydrochloric acid and water and dried over sodium sulphate and the chloroform phase is evaporated. The oily residue is extracted several times with ether and a solid amorphous mass with a melting point of 110—120° is thus obtained; this is α, β - 1,4,6 - tri - butyroyl - 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose, $R_f=0.52$ in $\text{CHCl}_3:\text{CH}_3\text{OH}=7:2$ (silica gel thin layer, Merck).

Example 9

2.3 g of 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose are dissolved in 20 ml of pyridine and 5 ml of acetic anhydride. After 3 hours at room temperature, water is added and the mixture is concentrated in vacuo. It is purified over 70 g of silica gel using $\text{CHCl}_3:\text{CH}_3\text{OH}=3:1$ and a colourless powder which has a melting point of 122—158° and an $[\alpha]_D^{20}$ of +48° (CH_3OH , $c=1.074$) is obtained; this is α, β - 1,4,6 - triacetyl - 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose, $R_f=0.54$, $\text{CHCl}_3:\text{CH}_3\text{OH}=3:2$ (silica gel thin layer, Merck).

Example 10

6.33 g of 2 - phenyl - 4,5 - (3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano) - Δ^2 - oxazoline, 5.75 g of 2 - ethoxy - N - carbethoxy - 1,2 - dihydroquinoline (EEDQ) and 9.3 ml of triethylamine are added to a solution of the trifluoroacetate of dibenzyl - L - alanyl - D - glutamate (obtained from 8.3 g of dibenzyl N - tert. - butoxycarbonyl - L - alanyl - D - glutamate and 5.1 ml of trifluoroacetic acid and 2.6 ml of dichloroethane by hydrolysing for 4 hours at 40°) in 70 ml of dichloroethane. The mixture is allowed to react at 40° for 15 hours and is diluted with chloroform and twice extracted by shaking with water and the aqueous phases are extracted once by shaking with chloroform. After drying over sodium sulphate and evaporating the chloroform solution, 19.9 g of an oil are obtained and this is purified over 400 g of Merck (Registered Trade Mark) silica gel by elution with ether and then with chloroform:acetone, 17:3. This gives pure 2 - phenyl - 4,5 - [3 - O - (1 - L - [1 - D,3 - dibenzoyloxy - carbonyl - propyl] - carbamoylethyl) - carbamoylmethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline which has a melting point of 113—116° and an $[\alpha]_D^{20}$ of -47° (CHCl_3 , $c=1.54$).

7 g of the above compound are hydrogenated in a mixture of 80 ml of tetrahydrofuran and 20 ml of water, using 1.8 g of 5% strength Pd/C, until the reaction has ceased, the catalyst is filtered off, the filtrate is evaporated in vacuo and the residue is ground with ether. This gives 4.9 g of the dicarboxylic acid as a colourless powder.

4.4 g of the above dicarboxylic acid are stirred with 11 ml of Dowex-50 W $\times$ 4 ion exchanger in a mixture of 45 ml of tetrahydrofuran and 20 ml of water for 20 hours at 40°. After filtering and clarifying with charcoal (Darco G 60), the solution is freeze-dried and colourless, amorphous 2 - benzamido - 2 - deoxy - 3 - O - [1 - L - (1 - D,3 - dicarboxy - propyl) - carbamoyl - ethyl] - carbamoylmethyl - D - glucopyranose which has an optical rotation of $[\alpha]_D^{20}=+25^\circ$ (H_2O , $c=0.997$), is obtained.

Example 11

Analogously to Example 10, 5.7 g of 2 - phenyl - 4,5[3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline are subjected to a condensation reaction with 4.9 g of L - seryl - D - glutamic acid α - amide - γ - tert. - butyl ester - hydrochloride with the addition of 2.3 ml of triethylamine and 5.2 g of 2 - ethoxy - N - carboethoxy - 1,2 - dihydroquinoline in 45 ml of dichloroethane. After 18 hours at 40° a crystalline product has formed. A further 50 ml of dichloroethane are added and the mixture is cooled in ice and filtered and

the crystals are washed with cold dichloroethane. The analytically pure, colourless crystals of 2 - phenyl - 4,5 - [3 - O - (1 - L - (1 - D - carbamoyl - 3 - tert. - butyloxy - carbonyl - propyl) - carbamoyl - 2 - hydroxyethyl) - carbamoylmethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline have a melting point of 187—188° and a $[\alpha]_D^{20}$ of +7° (CH₃OH, c=1.125).

2 g of this compound are hydrolysed with a mixture of 15 ml of methylene chloride and 5 ml of trifluoroacetic acid for 20 hours at room temperature. The mixture is evaporated under an oil vacuum, the residue is ground with ether and 2 - benzamido - 2 - deoxy - 3 - O - [1 - L - (1 - D - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - hydroxyethyl] - carbamoylmethyl - D - glucopyranose is obtained as a beige powder with a melting point of 100—115°, $[\alpha]_D^{20}$ = +23° (H₂O, c=0.886), which crystallises with 2 mols of water and 1 mol of trifluoroacetic acid. R_f = 0.28 CHCl₃:CH₃OH=1:1 (silica gel thin layer, Merck).

Example 12

Analogously to Example 10, 5.25 g of 2 - phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline and the trifluoroacetic acid salt of L - alanyl - D - glutamic acid α - n - propylamide - γ - benzyl ester—obtained from 6.2 g of N - tert. - butoxy - carbonyl - L - alanyl - D - glutamic acid α - n - propylamide - γ - benzyl ester and 4.2 ml of trifluoroacetic acid in 2.5 ml of dichloroethane after 6 hours at 40°—in 60 ml of dichloroethane are subjected to a condensation reaction, with the addition of 7.75 ml of triethylamine and 4.8 g of 2 - ethoxy - N - carboethoxy - 1,2 - dihydroquinoline. After 20 hours at 40°, the mixture is diluted with 50 ml of chloroform and twice extracted by shaking with water and the water is twice extracted by shaking with chloroform. After drying and concentrating the chloroform phase, 15 g of an oil are obtained and this is purified over 200 g of Merck silica gel by elution with ether and then with chloroform:acetone=7:3. 6.4 g of a colourless amorphous substance with an R_f of 0.35, CHCl₃:acetone=7:3 (silica gel thin layer, Merck) are obtained.

This substance is hydrogenated in 80 ml of tetrahydrofuran and 20 ml of water, using 1.8 g of 5% strength Pd/C, until the reaction has ceased, the catalyst is filtered off and the filtrate is concentrated. The acid has an R_f of 0.58, CHCl₃:CH₃OH=3:1 (silica gel thin layer, Merck). It is then stirred with 10 ml of Dowex 50-W $\times$ 4 ion exchanger, 50 ml of tetrahydrofuran and 25 ml of water for 15 hours at room temperature and for 12 hours at 40°. After filtering, clarifying the filtrate with charcoal (Darco-C-60), filtering again and freeze-drying the filtrate, colourless, amorphous 2 - benz - amido - 2 - deoxy - 3 - O - [1 - L - (1 - D - N - n - propyl - carbamoyl - 3 - carboxy - propyl) - carbamoyl - methyl - D - glucopyranose with a melting point of 65—140° and an $[\alpha]_D^{20}$ of +28° (water, c=1.03) and an R_f of 0.48, CHCl₃:CH₃OH=1:1 (silica gel thin layer, Merck) is obtained.

Example 13

7.3 g of 2 - phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline, 6.5 g of α - amino - isobutyryl - D - glutamic acid α - amide - γ - tert. - butyl ester - hydrochloride and 2.9 g of isobutyl chloroformate are dissolved in 25 ml of dimethylformamide and 50 ml of dichloroethane. A solution of 6.1 ml of triethylamine in 20 ml of dichloroethane is added dropwise to this solution at -15° to -10° in the course of 30 minutes. The mixture is then allowed to warm to room temperature and is stirred for a further 15 hours at room temperature. It is diluted with 50 ml of dichloroethane and extracted by shaking with water, twice by shaking with 0.5 N NaOH and three times by shaking with water and the aqueous phases are extracted by shaking twice with dichloroethane, the organic phases are dried and, after evaporating, 16.6 g of an oil are obtained. This is purified over Merck silica gel by elution with CHCl₃:C₂H₅OH=19:1. 9.7 g of colourless, amorphous 2 - phenyl - 4,5 - [3 - O - [1 - methyl - 1 - (1 - D - carbamoyl - 3 - tert. - butoxycarbonyl - propyl) - carbamoyl - ethyl] - 5,6 - O - isopropylidene - 3 - glucofurano] - Δ^2 - oxazoline which has an optical rotation: $[\alpha]_D^{20}$ = +6° (CHCl₃, c=1.027), a melting point of 75—89° and an R_f of 0.35, CHCl₃:C₂H₅OH=9:1 (silica gel thin layer, Merck) are obtained.

8.3 g of the above compound are allowed to stand in a mixture of 20 ml of trifluoroacetic acid, 60 ml of methylene chloride and 2 ml of water for 15 hours at

room temperature. The mixture is then evaporated in vacuo and the residue is ground with ether. The resulting pink-coloured powder is dissolved in 200 ml of water and the solution is clarified with 0.5 g of Darco-G-60 charcoal. After filtering and evaporating the filtrate, colourless, amorphous 2 - benzamido - 2 - deoxy - 3 - O - [1 - methyl - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl] - D - glucopyranose with a melting point of 110—120°, an $[\alpha]_D^{20}$ of +31° (H_2O , $c=0.88$) and an R_f of 0.52, acetone:ethanol=1:1 (silica gel thin layer, Merck), which crystallises with 0.6 mol of trifluoroacetic acid and 1.7 mols of water, is obtained.

Example 14

Analogously to Example 10, 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - ethyl - D - glucopyranoside and the trifluoroacetic acid salt of L - alanyl - D - glutamic acid α - amide - γ - tert. - butyl ester with 2 - ethoxy - N - carboethoxy - 1,2 - dihydroquinoline give the corresponding glycopeptide which has an $[\alpha]_D^{20}$ of -23° (CH_3OH , $c=1.107$) and an R_f of 0.47 ($CH_2Cl_2:CH_3OH=8:2$) and, after hydrogenation in tetrahydrofuran/water using 5% strength Pd/C, the glycopeptide gives 2 - benzamido - 2 - deoxy - 3 - O - [1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl - β - ethyl - D - glucopyranoside which has a melting point of 215—217°, an $[\alpha]_D^{20}$ of -22° (CH_3OH , $c=0.97$) and an R_f of 0.36 in $CHCl_3:CH_3OH=1:1$ (silica gel thin layer, Merck).

2 - Benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - ethyl - D - glucopyranoside which is used as the starting material is obtained as follows: 2 - Phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline is dissolved in 0.1 N HCl/ CH_3OH and the solution is left to stand for 6 hours at room temperature. It is neutralised with sodium ethylate in ethanol and evaporated to dryness and the residue is taken up in acetone. The solution is filtered through a layer of Merck silica gel, the eluate is evaporated to dryness and the residue is extracted twice with ether at room temperature. After recrystallisation from ethyl acetate, 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - ethyl - D - glucopyranoside which has a melting point of 185—188° and an $[\alpha]_D^{20}=-35^\circ$ (CH_3OH , $c=1.121$) is obtained.

9.4 g of this ester are saponified with a solution of 1.7 g of potassium hydroxide in 250 ml of ethanol and 25 ml of water for 2 hours at room temperature. The pH value is then adjusted to 3.5 with 1N hydrochloric acid and the mixture is concentrated in vacuo.

The residue is first ground with ether and is then ground with 3 times 20 ml of ice water and the mixture is filtered. Crystals of 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - ethyl - D - glucopyranoside with a melting point of 205—210° and an $[\alpha]_D^{20}$ of -40° (CH_3OH , $c=1.04$) are thus obtained.

Example 15

2 - Benzamido - 2 - deoxy - 3 - O - [(D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl] - carbamoylmethyl - D - glucose is obtained from 3 g of 2 - phenyl - 4,5 - [3 - O - [(D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl] - carbamoylmethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline by hydrolysis with 1.5 ml of trifluoroacetic acid in a mixture of 15 ml of dimethoxyethane and 15 ml of water at 40° for 3 hours. The reaction mixture is concentrated to dryness in vacuo and the residue is again extracted with ether. The residual powder is dissolved in water and the solution is treated with Darco-G-60 charcoal and filtered and the filtrate is freeze-dried. This gives a colourless, amorphous substance with a melting point of 115—155° and an $[\alpha]_D^{20}$ of +34° (water, $c=0.81$), $R_f=0.28$ $CHCl_3:CH_3OH=1:1$ (silica gel thin layer, Merck).

The starting material for this reaction is obtained as follows:

8.0 g of N - tert. - butoxycarbonyl - glycyl - D - glutamic acid α - amide - γ - benzyl ester are dissolved in a mixture of 6.3 ml of trifluoroacetic acid and 7 ml of dichloroethane and the solution is allowed to react for 2 days at room temperature and for 3 hours at 45°. 12.1 ml of triethylamine, 7.0 g of 2 - ethoxy - N - carboethoxy - 1,2 - dihydroquinoline (EEDQ) and 8.1 g of 2 - phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline and 20 ml of dimethylformamide are added to the solution, whilst cooling. After 20 hours at 40°, the mixture is concentrated under an oil vacuum and

26

the residue is partitioned between methylene chloride and water. After drying and concentrating the methylene chloride phases, a solid residue is obtained and this is extracted twice with ether and recrystallised from toluene. Yield 8.25 g; melting point 157°, $[\alpha]_D^{20} = +10^\circ$ (CHCl_3 , $c=1.48$), $R_f=0.35$ (CHCl_3 :ethanol=9:1) (silica gel thin layer, Merck).

The benzyl ester which is thus obtained is hydrogenated in 100 ml of tetrahydrofuran and 25 ml of water, using 1 g of 5% strength Pd/C, until the reaction has ceased. After filtering off the catalyst and evaporating the filtrate, the substance is chromatographed on 250 g of Merck silica gel in CHCl_3 : $\text{CH}_3\text{OH}=4:1$. 5.8 g of colourless, amorphous 2 - phenyl - 4,5 - [3 - O - ((D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl) - carbamoylmethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline which has an R_f of 0.43, CHCl_3 : $\text{CH}_3\text{OH}=3:2$ (silica gel thin layer, Merck) are obtained.

Example 16

Analogously to Example 10, 9.5 g of 2 - phenyl - 4,5 - [3 - O - carboxymethyl - 5,6 - O - isopropylidene - D - glucofurano] - Δ^2 - oxazoline are subjected to a condensation reaction with 6.25 g of L - alanyl - D - glutamic acid α,γ - diamide - hydrochloride with the addition of 3.4 ml of triethylamine and 7.95 g of 2 - ethoxy - N - carboethoxy - 1,2 - dihydroquinoline (EEDQ) in a mixture of 50 ml of dichloroethane and 150 ml of dimethylformamide. The mixture is allowed to react, whilst stirring, for 2 days at room temperature and for 4 hours at 40°. It is concentrated under an oil vacuum and the residue is extracted, first twice with ether and then twice with ice water. After drying, the product can be recrystallised from dichloroethane. This gives colourless crystals which have a melting point of 170—184°; $[\alpha]_D^{20} = +3^\circ$ (DMSO, $c=1.43$), $R_f=0.64$ CHCl_3 : $\text{CH}_3\text{OH}=3:1$ (silica gel thin layer, Merck).

6.1 g of this compound are hydrolysed with 13.5 ml of Dowex 50 ion exchanger in a mixture of 60 ml of dimethoxyethane and 60 ml of water for 15 hours at room temperature. After filtering and concentrating the filtrate, the residue is taken up in water, the mixture is clarified with Darco-G-60 charcoal and filtered and the filtrate is freeze-dried. This gives colourless, amorphous 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1,3 - dicarbamoyl - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose which has a melting point of 82—143°; $[\alpha]_D^{20} = +24^\circ$ (H_2O , $c=0.98$), $R_f=0.45$ CHCl_3 : $\text{CH}_3\text{OH}=1:1$ (silica gel thin layer, Merck). The substance crystallises with 1.23 mols of water of crystallisation.

Example 17

Analogously to Example 16, ethyl - 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - D - glucopyranoside and L - alanyl - D - glutamic acid α,γ - bis - methylamide - hydrochloride give β - ethyl - 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1,3 - bis - N - methyl - carbamoyl - propyl) - carbamoyl - ethyl] - carbamoylmethyl - D - glucopyranoside which has a melting point of 233°, an $[\alpha]_D^{20}$ of -20° (CH_3OH , $c=0.937$) and an R_f of 0.38 in CHCl_3 :ethanol, 7:3 (silica gel, thin layer plates, Merck).

Example 18

Analogously to Example 16, 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - D - glucopyranose gives 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1,3 - bis - N - methylcarbamoyl - propyl) - carbamoylethyl] - carbamoylmethyl - D - glucopyranose which has a melting point of 125—132°, and $[\alpha]_D^{20}$ of and an R_f of 0.26 in CHCl_3 :ethanol=7:3 (silica gel thin layer plates, Merck).

Example 19

Analogously to Example 16, 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - D - glucopyranose and the hydrochloride of dimethyl L - alanyl - D - glutamate give 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1,3 - bis - methoxycarbonyl - propyl) - carbamoyl - ethyl] - carbamoylmethyl - D - glucopyranose in the form of the hydrate with a melting point of 80—90°, an $[\alpha]_D^{20}$ of $+25^\circ$ (CH_3OH , $c=1.017$) and an R_f value of 0.23 in CHCl_3 :ethanol=9:1 (silica gel thin layer plates, Merck).

Example 20

Analogously to Example 16, ethyl - 2 - benzamido - 2 - deoxy - 3 - O - carboxymethyl - β - D - glucopyranoside gives ethyl - 2 - benzamido - 2 -

deoxy - 3 - O - [L - 1 - (D - 1,3 - bis - methoxycarbonyl - propyl) - carbamoyl - 3 - carboxy - propyl) - carbamoyl - methyl - D - glucopyranoside which has a melting point of 127-135°, an $[\alpha]_D^{20}$ of -17° (CH₃OH, c=1.024) and an R_f of 0.26 in ethyl acetate:acetone=2:1 (silica gel thin layer plates, Merck).

Example 21

2 - Benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - N - benzyl - carbamoyl - 3 - carboxy - propyl) - carbamoyl - methyl - D - glucopyranose is obtained analogously to Example 12.

In this case, the *p*-carboxyl group of the glutamic acid is liberated from a tert.-butyl ester by saponification with Dowex-50-H⁺ in tetrahydrofurane/water.

Example 22

2 - Benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - N - carbamoylmethyl - carbamoyl - 3 - carboxypropyl) - carbamoyl - methyl - D - glucopyranose is obtained analogously to Example 12. The starting material for the peptide part is N - tert. - butoxycarbonyl - L - alanyl - D - glutamic acid *p*-benzyl ester - α - glycine - amide.

Example 23

Analogously to Example 12, the hydrochloride of L - α - amino - valeroyl - D - glutamic acid α - amide - *p* - tert. - butyl ester gives 2 - benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylbutyl] - carbamoyl - methyl - D - glucopyranose.

Example 24

2 - Benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - propyl] - carbamoyl - methyl - D - glucopyranose is obtained analogously to Example 12.

Example 25

2 - Benzamido - 2 - deoxy - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - 2 - methylpropyl] - 1 - carbamoyl - methyl - D - glucopyranose is obtained analogously to Example 12.

Example 26

A solution of 10.7 g of the benzyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - [(D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside in 200 ml of glacial acetic acid and 100 ml of water is hydrogenated under normal pressure and at room temperature for 57 hours using 2 g of 5% strength palladium-on charcoal. The catalyst is filtered off and rinsed with water and the filtrate is evaporated. The residue is taken up in water and the mixture is filtered through 100 ml of Amberlite IR 120 (H⁺ form) ion exchanger and the filtrate is freeze-dried. 2 - Acetamido - 3 - O - [(D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoylmethyl - carbamoyl - methyl] - 2 - deoxy - D - glucose is crystallised from methanol/ethyl acetate and dried under a high vacuum. The product, which contains 1/4 mol of ethyl acetate, displays an optical rotation $[\alpha]_D^{20}=+27^\circ \pm 1^\circ$ (water, c=0.944).

The starting material used can be prepared as follows: 8 g of the benzyl ester of N - t - butoxycarbonyl - glycyl - D - isoglutamine are dissolved, at room temperature and with the exclusion of moisture, in a mixture of 18 ml of 1,2-dichloroethane and 8.4 ml of trifluoroacetic acid and the solution is left to stand for 16 hours. The reaction mixture is now diluted with 200 ml of tetrahydrofurane and, with external cooling, neutralised with triethylamine and a solution of 8.3 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxy - methyl - 2 - deoxy - α - D - glucopyranoside in 100 ml of tetrahydrofurane and 2.52 ml of triethylamine is added. After adding 5.05 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ), the mixture is stirred for 24 hours at room temperature. The product which has crystallised out is filtered off, washed with tetrahydrofurane and ether and dried. The resulting benzyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - [(D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylmethyl - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside has an optical rotation $[\alpha]_D^{20}=+66^\circ \pm 1^\circ$ (N,N - dimethylformamide, c=1.308).

28

Example 27

A solution of 4.5 g of the benzyl ester of methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside in 125 ml of 50% strength aqueous methanol is hydrogenated under normal pressure and at room temperature, using 1.0 g of 5% strength palladium-on-charcoal, until 178 ml of hydrogen has been taken up. The catalyst is filtered off and the filtrate is evaporated under a waterpump vacuum. The residue is dissolved in 50 ml of distilled water and freeze-dried. This gives methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $+49^\circ \pm 1^\circ$ (water, $c=0.939$).

The starting material can be prepared as follows:

8.1 g of the benzyl ester of N - t - butoxycarbonyl - L - alanyl - D - isoglutamine are dissolved at room temperature and with the exclusion of moisture, in a mixture of 8.1 ml of 1,2 - dichloroethane and 8.1 ml of trifluoroacetic acid and the solution is left to stand for 16 hours. The reaction mixture is diluted with 200 ml of tetrahydrofuran and, with external cooling, neutralised with triethylamine and a solution of 7.62 g of methyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 2.77 ml of triethylamine in 100 ml of tetrahydrofuran is added. After adding 5.0 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ), the reaction mixture is warmed to 40° , stirred at this temperature for 30 hours and left to stand for a further 24 hours at room temperature. The product which has precipitated out, that is to say the benzyl ester of methyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside, is filtered off, washed with tetrahydrofuran and ether and dried, $[\alpha]_D^{20}=+58^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=1.125$).

A solution of 10.5 g of the benzyl ester of methyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside in 320 ml of glacial acetic acid is diluted with 200 ml of water, whilst stirring, and the whole is stirred at $50-55^\circ\text{C}$ for 2 hours. After cooling, the solution is evaporated to dryness, a further four times 100 ml of water are added to the residue and, each time, the mixture is evaporated to dryness. This gives the benzyl ester of methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $+64^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=1.268$).

Example 28

A solution of 3.0 g of the benzyl ester of methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside in 100 ml of ethanol and 100 ml of tetrahydrofuran is hydrogenated at room temperature and under normal pressure using 0.6 g of 5% strength palladium-on-charcoal, the catalyst is filtered off and the filtrate is evaporated to dryness under a water-pump vacuum. The crystalline residue is methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $+50^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=0.921$).

The starting material can be prepared as follows:

A solution of 2.12 g of stearyl chloride in 20 ml of 1,2 - dichloroethane is added dropwise, at $0-5^\circ$, in the course of 3 hours to a solution of 3.98 g of the benzyl ester of methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside in 40 ml of absolute pyridine, whilst stirring and with the exclusion of moisture, and the mixture is left to stand for 18 hours at room temperature. The reaction mixture is diluted with chloroform, washed with water, ice-cold 2N hydrochloric acid and water, dried over magnesium sulphate and evaporated to dryness. The product, that is to say the benzyl ester of methyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl] - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside, is crystallised from ethanol/ether; $[\alpha]_D^{20}=+22^\circ \pm 1^\circ$ (chloroform, $c=1.030$).

Example 29

A solution of 6.8 g of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarbamoylpropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside in 200 ml of 50% strength aqueous methanol is hydrogenated under normal pressure at room temperature for 60 hours using 5% strength palladium-on-charcoal.

The catalyst is filtered off and the filtrate is evaporated. The residue is taken up in 50 ml of water and lyophilised. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarbamoylpropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - D - glucose, which contains 1.24 mols of water, in the form of a white powder with an $[\alpha]_D^{20}$ of $+7^\circ \pm 1^\circ$ (water, $c=0.514$).

The starting material can be prepared as follows:

5.0 g of L - alanyl - D - glutamic acid diamide - hydrochloride and 5.1 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 9.1 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside in 100 ml of N,N - dimethylformamide and 2.77 ml of triethylamine and the mixture is left to stand for 48 hours at room temperature. After distilling off the solvent, the residue is extracted thoroughly with ether and water and the product is dried and recrystallised from chloroform/methanol; $[\alpha]_D^{20}=+83^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=0.531$).

A solution of 4 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - {[L - 1 - (D - 1,3 - dicarbamoylpropyl) - carbamoylethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside in 120 ml of glacial acetic acid is diluted with 80 ml of water and stirred for 3 hours at 60° . The reaction mixture is then cooled and evaporated and a further 3 times 100 ml of water are added to the residue and the water is distilled off each time. The resulting benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarbamoylpropyl) - carbamoylethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside is recrystallised from methanol; melting point $223-225^\circ$.

Example 30

A solution of 5.7 g of the benzyl ester of benzyl - 2 - acetamido - 3 - O - {D - 1 - [(D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - methyl] - carbamoylpropyl} - 2 - deoxy - α - D - glucopyranoside in 100 ml of glacial acetic acid is hydrogenated in the presence of 5% strength palladium-on-charcoal under normal pressure and at room temperature. The catalyst is filtered off and the filtrate is evaporated. The residual 2 - acetamido - 3 - O - {D - 1 - [(D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylmethyl] - carbamoylpropyl} - 2 - deoxy - D - glucose is taken up in 50 ml of water and freeze-dried; $[\alpha]_D^{20}=+46^\circ \pm 1^\circ$ (water, $c=0.630$).

The starting material used can be prepared as follows: 5.1 g of the benzyl ester of N - t - butoxycarbonyl - glycyl - D - isoglutamine are dissolved, at room temperature and with the exclusion of moisture, in a mixture of 5.1 ml of 1,2 - dichloroethane and 5.1 ml of trifluoroacetic acid and the solution is left to stand for 16 hours. This solution is diluted with 100 ml of tetrahydrofuran and, with external cooling, neutralised with triethylamine and a solution of 6.3 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - (D - 1 - carboxypropyl) - 2 - deoxy - α - D - glucopyranoside and 1.8 ml of triethylamine in 100 ml of tetrahydrofuran and 3.2 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) is added. After 24 hours, the mixture is evaporated to dryness and the residue is partitioned between ethyl acetate and water. The organic phase is further washed with ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water and evaporated. The residual benzyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - {D - 1 - [(D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylmethyl] - carbamoylpropyl} - 2 - deoxy - α - D - glucopyranoside is crystallised from tetrahydrofuran/ether; $[\alpha]_D^{20}=+76^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=0.457$).

Mild acid hydrolysis of this product in 60% strength aqueous acetic acid leads to the benzyl ester of benzyl - 2 - acetamido - 3 - O - {D - 1 - [(D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - methyl] - carbamoylpropyl} - 2 - deoxy - α - D - glucopyranoside, which crystallises from methanol/ether; melting point $180-185^\circ$, $[\alpha]_D^{20}=+87^\circ \pm 1^\circ$ (methanol, $c=1.035$).

Example 31

4 g of the benzyl ester of N - t - butoxycarbonyl - L - alanyl - D - isoglutamine are dissolved, at room temperature and with the exclusion of moisture, in a mixture of 4 ml of trifluoroacetic acid and 4 ml of 1,2 - dichloroethane and the solution is left to stand for 16 hours. This reaction mixture is now diluted with 30 ml of 1,2 - dichloroethane and, with external cooling, neutralised with triethylamine and a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofuran is added. After adding 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydro - quinoline (EEDQ), the mixture is left to stand for 24 hours at room temperature and is evaporated to dryness. The residue is dissolved in chloroform/methanol, 9/1, and this solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water and the solvent is evaporated. The benzyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside, which is thus obtained, is recrystallised from ethanol; melting point 208–212°, $[\alpha]_D^{20} = +77^\circ \pm 1^\circ$ (N,N - dimethylformamide, c=0.546).

After splitting off the two benzyl radicals by hydrogenolysis, as described in Example 2, 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - D - glucose is obtained.

Example 32

3.8 g of the dimethyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside are dissolved in 100 ml of methanol and hydrogenated under normal pressure and at room temperature in the presence of 5% strength palladium-on-charcoal. After the absorption of hydrogen has ceased, the catalyst is filtered off and the filtrate is evaporated to dryness. The residue is taken up in 70 ml of water and freeze-dried. The resulting foam is the dimethyl ester of 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - D - glucose which has an $[\alpha]_D^{20}$ of $+23^\circ \pm 1^\circ$ (water, c=0.814).

The starting material used can be prepared as follows: 8.1 g of L - alanyl - D - glutamic acid dimethyl ester-hydrochloride and 6.95 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 12.9 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 4.0 ml of triethylamine in 100 ml of N,N - dimethylformamide and the mixture is left to stand at room temperature for 20 hours. The solvent is then evaporated, the residue is taken up in chloroform and this solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water. After drying over magnesium sulphate, the solution is evaporated to dryness. The residue is extracted with warm ethanol and the dimethyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - {[L - 1 - (D - 1,3 - dicarboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside, which has not dissolved, is filtered off and dried; $[\alpha]_D^{20} = +22^\circ \pm 1^\circ$ (chloroform, c=1.160).

15.6 g of the dimethyl ester of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - {[L - 1 - (D - 1,3 - dicarboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside are dissolved in 420 ml of glacial acetic acid and 280 ml of water and the solution is warmed to 10°C. After stirring for 4 hours at this temperature, the reaction mixture is cooled and evaporated to dryness. A further three times 100 ml of water are added to the residue and the mixture is evaporated to dryness each time. The residue is then taken up in chloroform and this solution is washed with water, dried over magnesium sulphate and evaporated to dryness. This gives the dimethyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - dicarboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside as a yellowish resin with an $[\alpha]_D^{20}$ of $+31^\circ \pm 1^\circ$ (chloroform, c=1.070).

Example 33

A solution of 6.1 g of the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - 2 - propionamido - α - D - glucopyranoside in 200 ml of 2/1 tetrahydrofuran/water is hydrogenated under normal pressure and at room

temperature in the presence of 0.6 g of 5% strength palladium-on-charcoal. After the absorption of hydrogen has ceased, the catalyst is filtered off and the filtrate is evaporated. 150 ml of water are added to the residue and the mixture is hydrogenated in the presence of 5% strength palladium-on-charcoal until no further hydrogen is taken up. The catalyst is filtered off and the filtrate is lyophilised. This gives 3 - O - β - [L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl - 2 - deoxy - 2 - propionamido - D - glucose.

The starting material used can be prepared as follows:

0.3 ml of methanesulphonic acid is added to a solution of 90 g of benzyl - 2 - acetamido - 2 - deoxy - α - D - glucopyranoside in 900 ml of N,N - dimethylformamide, whilst stirring, with the exclusion of moisture and whilst cooling with ice water. A solution of 60 ml of isopropenyl methyl ether in 240 ml of N,N - dimethyl - formamide is then added dropwise in the course of one hour and the mixture is stirred at room temperature for a further two hours and rendered alkaline with triethylamine. The solvent is distilled, the residue is taken up in ethyl acetate and this solution is washed with water, dried over magnesium sulphate and evaporated to dryness. The product, that is to say benzyl - 2 - acetamido - 2 - deoxy - 4,6 - O - isopropylidene - α - D - glucopyranoside is crystallised from ether; melting point 136—137°, $[\alpha]_D^{20} = +103^\circ \pm 1^\circ$ (chloroform, c=1.125).

52.5 g of benzyl - 2 - acetamido - 2 - deoxy - 4,6 - O - isopropylidene - α - D - glucopyranoside are dissolved in a solution of 225 g of potassium hydroxide in 750 ml of ethanol and 40 ml of distilled water and the solution is boiled under reflux for 44 hours. After cooling, the reaction mixture is concentrated to half its volume and poured onto ice. The mixture is extracted with chloroform and the organic phase is washed with water, dried over magnesium sulphate and evaporated to dryness. The residue, that is to say benzyl - 2 - amino - 2 - deoxy - 4,6 - O - isopropylidene - α - D - glucopyranoside, is crystallised from ether; melting point 145—146°C, $[\alpha]_D^{20} = +117^\circ \pm 1^\circ$ (chloroform, c=1.295).

A solution of 16.0 g of potassium bicarbonate in 192 ml of distilled water is added to a solution of 24.7 g of benzyl - 2 - amino - 2 - deoxy - 4,6 - O - isopropylidene - α - D - glucopyranoside in 192 ml of chloroform and the mixture is cooled to 0°. 8.16 g of propionyl chloride are now added dropwise in the course of 20 minutes, whilst stirring, and the mixture is stirred at this temperature for a further 50 minutes. The organic phase is now separated off, washed with water, dried over magnesium sulphate and evaporated to dryness. The product, that is to say benzyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - propionamido - α - D - glucopyranoside, is crystallised from ethyl acetate/petroleum ether; melting point 121—122°, $[\alpha]_D^{20} = +112^\circ \pm 1^\circ$ (chloroform, c=0.977).

1.25 g of pract. sodium hydride (Fluka) are added to a solution of 9.1 g of benzyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - propionamido - α - D - glucopyranoside in 90 ml of acetonitrile and the mixture is stirred for 2 hours at 40°. It is then cooled to -5 to -10°C and 4.2 ml of ethyl bromoacetate are added. After a further 20 minutes, 10 ml of ethanol are added and the reaction mixture is neutralised with glacial acetic acid and evaporated to dryness. The residue is partitioned between ether and water and the ether solution is washed with water, dried over magnesium sulphate and evaporated. The product, that is to say the ethyl ester of benzyl - 3 - O - carboxymethyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - propionamido - α - D - glucopyranoside, is crystallised from ether/petroleum ether; melting point 94—95°, $[\alpha]_D^{20} = +145^\circ \pm 1^\circ$ (chloroform, c=1.218).

22.5 ml of 1N sodium hydroxide solution are added to a solution of 6.8 g of the ethyl ester of benzyl - 3 - O - carboxymethyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - propionamido - α - D - glucopyranoside in 70 ml of methanol. After hydrolysis of the ester has ended, 7.5 ml of 1N hydrochloric acid are added and the mixture is evaporated to dryness. The product is dissolved in 50 ml of N,N - dimethylformamide and subjected to a condensation reaction with 15 mmols of L - alanine - D - isoglutamine benzyl ester - trifluoroacetate in the presence of 3.72 g of 2 - ethoxy - N - ethoxy - carbonyl - 1,2 - dihydroquinoline (EEDQ). The reaction mixture is then evaporated to dryness and the residue is taken up in chloroform. This solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water, dried over magnesium sulphate and evaporated to dryness. The product is crystallised from dilute ethanol; melting point 177—80°, $[\alpha]_D^{20} = +71^\circ \pm 1^\circ$ (chloroform, c=1.047).

15 ml of 1N hydrochloric acid are added to a solution of 8.1 g of the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 4,6 - O - isopropylidene - 2 - propionamido - α - D - glucopyranoside in 150 ml of methanol and the mixture is left to stand for one hour at room temperature. 15 ml of 1N sodium hydroxide solution are now added and the mixture is evaporated to dryness. The resulting benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - 2 - propionamido - α - D - glucopyranoside is crystallised from methanol/water and dried; melting point 208—210°, $[\alpha]_D^{20} = +75^\circ \pm 1^\circ$ (N,N - dimethylformamide, c=1.120).

Example 34

3 - O - {[L - 1 - (D - 1 - Carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 2 - caprinoyl - amido - D - glucose is obtained as a white foam, by catalytic hydrogenation of the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 2 - caprinoylamido - α - D - glucopyranoside in N,N - dimethylformamide in the presence of 5% strength palladium-on-charcoal.

The starting material used can be prepared as follows:

Analogously to the procedure described in Example 35, 24.7 g of benzyl - 2 - amino - 2 - deoxy - 4,6 - O - isopropylidene - α - D - glucopyranoside are reacted with 16.7 g of caprinoyl chloride and the reaction mixture is worked up. This gives benzyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - caprinoylamido - α - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $+81^\circ \pm 1^\circ$ (chloroform, c=1.109).

0.4 g of pract. sodium hydride is added to a solution of 3.8 g of benzyl - 2 - deoxy - 4,6 - O - isopropylidene - 2 - caprinoyl - amido - α - D - glucopyranoside in 40 ml of acetonitrile and the mixture is stirred for 2 hours at 40°. This mixture is then cooled to -10°, 1.4 ml of ethyl bromoacetate are added and the mixture is stirred for a further hour at 0°. When the mixture is worked up as described in Example 33, this gives the ethyl ester of benzyl - 3 - O - carboxymethyl - 4,6 - O - isopropylidene - 2 - caprinoylamido - α - D - glucopyranoside as a yellow-brown oil with an $[\alpha]_D^{20}$ of $+114^\circ \pm 1^\circ$ (chloroform, c=1.242).

Analogously to Example 33, the ethyl ester is hydrolysed, the product is subjected to a condensation reaction with L - alanine - D - isoglutamine benzyl ester - trifluoroacetate in the presence of EEDQ and the isopropylidene-ketal is split by mild acid hydrolysis. In this way the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 2 - caprinoylamido - α - D - glucopyranoside is obtained as a yellowish resin.

Example 35

A 5% strength solution of benzyl - 2 - acetamido - 2 - deoxy - 3 - O - {[L - 1 - (D - 1,3 - bis - methylcarbamoylpropyl) - carbamoylethyl] - carbamoylmethyl} - α - D - glucopyranoside in 1/1 distilled water/methanol is hydrogenated using 5% strength palladium-on-charcoal, the catalyst is filtered off and the filtrate is evaporated. The product, that is to say 2 - acetamido - 2 - deoxy - 3 - O - {[L - 1 - (D - 1,3 - bis - methylcarbamoylpropyl) - carbamoyl - ethyl] - carbamoylmethyl} - D - glucose is freeze-dried; $[\alpha]_D^{20} =$

The starting material can be prepared as follows:

5.6 g of L - alanyl - D - glutamic acid bis - methylamide - hydrochloride and 5.1 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline are added to a solution of 9.1 g of benzyl - 2 - acetamido - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside in 100 ml of N,N - dimethylformamide and 2.77 ml of triethylamine and the mixture is left to stand for 48 hours at room temperature. The solvent is now distilled off, water is added to the oily residue and the insoluble matter is filtered off, washed with water and dried. This product is stirred twice more with ether, filtered off and dried and is benzyl - 2 - acetamido - 4,6 - O - benzylidene - 2 - deoxy - 3 - O - {[L - 1 - (D - 1,3 - bis - methylcarbamoylpropyl) - carbamoylethyl] - carbamoyl - methyl} - α - D - glucopyranoside.

By means of mild acid hydrolysis, as described in Example 29, with 60% strength acetic acid, the benzylidene group is split off and the product, that is to say benzyl - 2 - acetamido - 2 - deoxy - 3 - O - {[L - 1 - (D - 1,3 - bis -

methylcarbamoylpropyl) - carbamoylethyl] - carbamoylmethyl] - α - D - glucopyranoside is isolated.

Example 36

A 5% strength aqueous solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - hydroxy - ethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside is hydrogenated in the presence of 5% strength palladium-on-charcoal, the mixture is filtered and the filtrate is freeze-dried. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - hydroxyethyl] - carbamoylmethyl] - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

3.26 g of L - serine - D - isoglutamine tert.-butyl ester-hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 150 ml of tetrahydrofurane and the mixture is left to stand for 20 hours at room temperature. After evaporating off the solvent, the residue is dissolved in 9/1 chloroform/methanol, the solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water and filtered and the filtrate is freed from the solvent. This gives the tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - hydroxy - ethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside.

By means of mild acid hydrolysis, as described in Example 11, the tert.-butyl ester is hydrolysed and benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - hydroxy - ethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside is obtained.

Example 37

A 5% strength solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylbutyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside in 1/1 methanol/water is hydrogenated in the presence of 5% strength palladium-on-charcoal, the mixture is filtered and the filtrate is evaporated. The residue is dissolved in distilled water and freeze-dried. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylbutyl] - carbamoylmethyl] - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

3.28 g of L - norvaline - D - isoglutamine tert.-butyl ester hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofurane and the mixture is left to stand for 24 hours at room temperature. The reaction mixture is worked up as in Example 38. This gives the tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - butyl] - carbamoyl - methyl] - 2 - deoxy - α - D - glucopyranoside.

The tert.-butyl ester is split by mild acid hydrolysis.

Example 38

A solution of 8.3 g of the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - 2 - stearoylamido - α - D - glucopyranoside in 200 ml of glacial acetic acid is hydrogenated under normal pressure and at room temperature in the presence of 5% strength palladium-on-charcoal. After working up, 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - 2 - stearoylamido - D - glucose is obtained as a white powder.

The starting material used can be prepared as follows:

0.75 g of pract. sodium hydride is added to a solution of 6.2 g of benzyl - 4,6 - O - benzylidene - 2 - deoxy - 2 - stearoylamido - α - D - glucopyranoside in 150 ml of N,N-dimethylformamide and the mixture is stirred at 40° for 1½ hours. The mixture is now cooled to -5°, 2.2 ml of ethyl bromoacetate are added and the mixture is stirred for 4 hours at room temperature. After adding 10 ml of glacial acetic acid and 400 ml of distilled water, the mixture is extracted with chloroform and the organic phase is washed with water, dried and evaporated to dryness. The

resulting ethyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - stearylido - α - D - glucopyranoside is crystallised from ethanol; melting point 151—154°, $[\alpha]_D^{20} = +94^\circ \pm 1^\circ$ (chloroform, $c=1.186$).

5 50.1 ml of 1N sodium hydroxide solution are added to a solution of 23.4 g of the ethyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - stearylido - α - D - glucopyranoside in 334 ml of methanol and 334 ml of tetrahydrofuran and the mixture is stirred for 90 minutes at 50° and evaporated. The residue is stirred in ice water and the product is filtered off, washed with water, dried and recrystallised from ethanol. Sodium benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - stearylido - α - D - glucopyranoside. $1H_2O$ melts at 225—242° (with decomposition), $[\alpha]_D^{20} = +45^\circ \pm 1^\circ$ (chloroform, $c=1.097$).

20 mmols of L - alanine - D - isoglutamine benzyl ester - trifluoroacetate and 4.95 g (20 mmols) of 2 - ethoxy - N - ethoxy - carbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 14.4 g (20 mmols) of sodium benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - stearylido - α - D - glucopyranoside monohydrate in 120 ml of tetrahydrofuran and the mixture is left to stand for 20 hours at room temperature and evaporated to dryness. The residue is dissolved in chloroform and this solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water, dried over magnesium sulphate and evaporated. The product, that is to say the benzyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - 2 - stearylido - D - glucopyranoside is recrystallised from ethanol; $[\alpha]_D^{20} = +62^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=1.148$).

Mild acid hydrolysis of this benzylidene derivative in 65% strength acetic acid leads to the benzyl ester of benzyl - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoylmethyl} - 2 - deoxy - 2 - stearylido - α - D - glucopyranoside; melting point 188—189°, $[\alpha]_D^{20} = +59^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=1.022$).

Example 39

A 5% strength aqueous solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - methylpropyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside is hydrogenated in the presence of 5% strength palladium-on-charcoal and filtered and the filtrate is lyophilised. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - methylpropyl] - carbamoylmethyl} - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

40 3.38 g of L - valine - D - isoglutamine tert.-butyl ester-hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline are added to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofuran and the mixture is left to stand for 24 hours at room temperature and worked up as described in Example 38. This gives the tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - methylpropyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside, which is hydrolysed under mild acid conditions to give benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 2 - methylpropyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside.

Example 40

55 A 5% strength solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 3 - methylbutyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside in 50% strength aqueous methanol is hydrogenated in the presence of 5% palladium-on-charcoal and the mixture is filtered and the filtrate is evaporated. The residue is dissolved in distilled water, the solution is filtered again and the filtrate is freeze-dried. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 3 - methylbutyl] - carbamoylmethyl} - 2 - deoxy - D - glucose.

The starting material used can be prepared as follows:

60 3.52 g of L - leucine - D - isoglutamine tert.-butyl ester-hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added

to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.58 ml of triethylamine in 100 ml of tetrahydrofuran and the mixture is left to stand for 24 hours at room temperature and worked up as described in Example 38. This gives the tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 3 - methylbutyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside, and mild acid hydrolysis of this leads to benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - 3 - methylbutyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside.

Example 41

A 5% strength solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylpropyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside in 50% strength aqueous methanol is hydrogenated under normal pressure and at room temperature in the presence of 5% strength palladium-on-charcoal, the catalyst is filtered off and the filtrate is evaporated. The residue is dissolved in distilled water and the solution is freeze-dried. This gives 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylpropyl] - carbamoylmethyl} - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

3.24 g of N - α - L - aminobutyryl - D - isoglutamine tert.-butyl ester-hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline are added to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofuran and after the mixture has stood for 24 hours at room temperature it is worked up as described in Example 36. The resulting tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylpropyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside is then hydrolysed under mild acid conditions to give benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylpropyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside.

Example 42

A 5% strength solution of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylphenyl - methyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside in 50% strength aqueous methanol is hydrogenated under normal pressure and at room temperature in the presence of 5% strength palladium-on-charcoal, the catalyst is filtered off and the filtrate is evaporated. The residue is dissolved in distilled water and the solution is freeze-dried and the residue is 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylphenyl - methyl] - carbamoylmethyl} - 2 - deoxy - D - glucose.

The starting material can be prepared as follows:

3.72 g of 1 - phenylglycine - D - isoglutamine tert.-butyl ester-hydrochloride and 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added to a solution of 3.7 g of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofuran and the mixture is left to stand at room temperature for 24 hours and worked up as described in Example 36. This gives the tert.-butyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - phenylmethyl] - carbamoyl - methyl} - 2 - deoxy - α - D - glucopyranoside, which is hydrolysed under mild acid conditions to give benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylphenylmethyl] - carbamoylmethyl} - 2 - deoxy - α - D - glucopyranoside.

Example 43

50 ml of distilled water are added dropwise in the course of 15 minutes to a solution of 3.5 g of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carbamoylpropyl) - carbamoyl - methyl] - carbamoyl - methyl} - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside in 50 ml of glacial acetic acid, at 50°, whilst stirring, and the mixture is stirred for 1 hour at this temperature. After

36

cooling, a further 100 ml of distilled water are added and the solvent is evaporated under a waterpump vacuum. 20 ml of water are added to the residue and the mixture is freeze-dried. This gives benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carbamoyl - propyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - β - D - glucopyranoside which has an $[\alpha]_D^{20}$ of $-44^\circ \pm 1^\circ$ (N,N - dimethylformamide, $c=0.989$).

The starting material used can be prepared as follows:

0.1 ml of methanesulphonic acid is added to a solution of 30 g of benzyl - 2 - acetamido - 2 - deoxy - β - D - glucopyranoside in 300 ml of N,N-dimethylformamide, whilst stirring and with external cooling and the exclusion of moisture. A solution of 20 ml of isopropenyl methyl ether in 60 ml of N,N-dimethylformamide is now added dropwise in the course of 1 hour and the mixture is stirred at room temperature for a further two hours and rendered alkaline with triethylamine. After evaporating off the solvent, benzyl - 2 - acetamido - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside is crystallised from ethylacetate; melting point 194° , $[\alpha]_D^{20} = -104^\circ \pm 1^\circ$ (chloroform, $c=0.850$).

4.95 g of pract. sodium hydride are added to a solution of 36.2 g of benzyl - 2 - acetamido - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside in 400 ml of acetonitrile and 100 ml of N,N-dimethylformamide and the mixture is stirred for 2 hours at 40° . This mixture is now cooled to -10°C , 17.2 ml of ethyl bromoacetate are added and the mixture is stirred for 30 minutes at 0° . 40 ml of ethanol are now added and the mixture is neutralised with glacial acetic acid and evaporated to dryness. The residue is partitioned between ether and distilled water and the ether phase is washed with water, dried over magnesium sulphate and evaporated. The product, that is to say the ethyl ester of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside is crystallised from ether; melting point $93-94^\circ$, $[\alpha]_D^{20} = -49^\circ \pm 1^\circ$ (CHCl_3 , $c=1.001$).

22.5 ml of 1N sodium hydroxide solution are added to a solution of 6.56 g of the ethyl ester of benzyl - 2 - acetamido - 3 - O - carbamoylmethyl - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside in 70 ml of methanol. After the hydrolysis of the ester has ended, 7.5 ml of 1N hydrochloric acid are added and the mixture is evaporated to dryness. The resulting sodium salt is dissolved in 50 ml of N,N-dimethylformamide and 3.7 g of L - alanyl - D - glutamic acid - diamide - hydrochloride and 3.72 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline (EEDQ) are added. After standing for 24 hours at room temperature, the mixture is evaporated to dryness, the residue is dissolved in water and the solution is extracted with methylene chloride and twice with n-butanol. The product which remains after distilling off the n-butanol is benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carbamoylpropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside.

Example 44

80 ml of distilled water are added dropwise to a solution of 5.1 g of the dimethyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carboxypropyl) - carbamoylethyl] - carbamoyl - methyl} - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside in 120 ml of glacial acetic acid, at 50° , whilst stirring, and the mixture is stirred for one hour at this temperature. After evaporating off the acetic acid, the dimethyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - β - D - glucopyranoside is obtained.

The starting material used can be prepared as follows:

6.56 g of the ethyl ester of benzyl - 2 - acetamido - 3 - O - carboxymethyl - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside are hydrolysed as described in Example 43 with sodium hydroxide solution and the reaction product is subjected to a condensation reaction with 4.24 g of L - alanyl - D - glutamic acid dimethyl ester-hydrochloride in the presence of 3.72 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline. After evaporating off the solvent, the residue is taken up in chloroform and the solution is washed with water, ice-cold 2N hydrochloric acid, water, a saturated solution of sodium bicarbonate and water, dried over magnesium sulphate and evaporated to dryness. The residue is the dimethyl ester of benzyl - 2 - acetamido - 3 - O - {[L - 1 - (D - 1,3 - bis - carboxypropyl) - carbamoylethyl] - carbamoylmethyl} - 2 - deoxy - 4,6 - O - isopropylidene - β - D - glucopyranoside.

Example 45

A 5% strength solution of the benzyl ester of benzyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoylmethylcarbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside in 2/1 tetrahydrofuran/water is hydrogenated under normal pressure and at room temperature in the presence of 5% strength palladium-on-charcoal. After about 50% of the theoretical amount of hydrogen has been taken up, the catalyst is filtered off and the filtrate is evaporated. The residue is now dissolved in distilled water and further hydrogenated. After the theoretical amount of hydrogen has been taken up, the catalyst is filtered off and the filtrate is freeze-dried. This gives 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoylmethylcarbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - D - glucose in the form of a white powder.

The starting material can be prepared as follows:

4.8 g of N - tert - butoxycarbonyl - L - alanine - D - glutamine (benzyl ester) - glycine - amide monohydrate are dissolved in a mixture of 5 ml of trifluoroacetic acid and 5 ml of 1,2 - dichloro - ethane and the solution is left to stand for 16 hours at room temperature, with the exclusion of moisture. This solution is diluted with 50 ml of tetrahydrofuran, cooled in an ice bath and neutralised with triethylamine. After adding a solution of 3.7 g of benzyl - 2 - acetamido - 3 - carboxymethyl - 2 - deoxy - α - D - glucopyranoside and 1.38 ml of triethylamine in 100 ml of tetrahydrofuran, 2.6 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline are added to the whole and the mixture is left to stand for 24 hours at room temperature and worked up as described in Example 36. This gives the benzyl ester of benzyl - 2 - acetamido - 3 - O - [[L - 1 - (D - 1 - carbamoylmethylcarbamoyl - 3 - carboxy - propyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - α - D - glucopyranoside.

Example 46

A solution of 5.2 g of the benzyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - 2 - p - tolylsulphonylamino - α - D - glucopyranoside in 120 ml of 70% strength aqueous acetic acid is hydrogenated under normal pressure and at room temperature in the presence of palladium-on-charcoal. After the theoretical amount of hydrogen has been taken up, the catalyst is filtered off and the filtrate is evaporated to dryness. The residue is dissolved in 30 ml of distilled water and the solution is freeze-dried. This gives 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoylmethyl] - 2 - deoxy - 2 - p - tolylsulphonylamino - D - glucose in the form of a white powder.

The starting material used can be prepared as follows:

A solution of 3.1 g of p-toluenesulphonyl chloride in 30 ml of methylene chloride is added dropwise, at 5°, to a solution of 6.0 g of benzyl - 2 - amino - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 3 - deoxy - α - D - glucopyranoside in 100 ml of methylene chloride and 6.3 ml of triethylamine and the mixture is left to stand for 16 hours at room temperature. This solution is then washed with water, 2N sodium hydroxide solution, water, cold 2N hydrochloric acid and water, dried over magnesium sulphate and evaporated to dryness. The residue is extracted several times with ether and dried and is benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - tolylsulphonylamino - α - D - glucopyranoside which has a melting point of 185—187°C and an $[\alpha]_D^{20}$ of +75° $\pm$ 1° (chloroform, c=0.855).

10 mmols of L - alanyl - D - isoglutamine benzyl ester - trifluoroacetate and 2.5 g of 2 - ethoxy - N - ethoxycarbonyl - 1,2 - dihydroquinoline are added to a solution of 5.7 g of benzyl - 4,6 - O - benzylidene - 3 - O - carboxymethyl - 2 - deoxy - 2 - tolylsulphonyl - amino - α - D - glucopyranoside and 1.01 g of triethylamine in 80 ml of N,N - dimethylformamide and the mixture is left to stand for 24 hours at room temperature. It is then evaporated to dryness, water is added to the residue and the insoluble matter is filtered off, rinsed with water and ether and dried in vacuo. This gives the benzyl ester of benzyl - 4,6 - O - benzylidene - 3 - O - [[L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoylethyl] - carbamoyl - methyl] - 2 - deoxy - 2 - tolylsulphonylamino - α - D - glucopyranoside in the form of a white powder.

38

Example 47

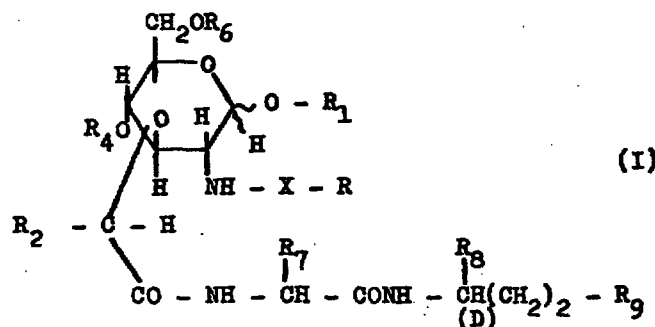
2 - (Acetaminomethylcarbonylamino) - 2 - desoxy - 3, O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoyl - methyl} - α, β - D - glucose is obtained analogously to Example 33.

Example 48

In a manner analogous to that described before 2 - trimethylacetamido - 2 - desoxy - 3, O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoylmethyl} - α, β - D - glucose is obtained as Lyophilisate.

WHAT WE CLAIM IS:—

1. A glucosamine derivative of the general formula

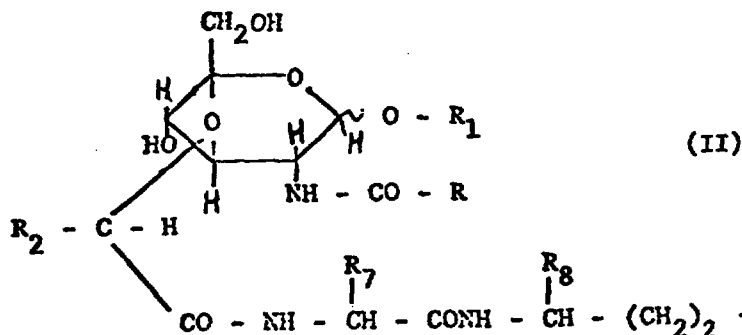


and the salts thereof, in which X denotes a carbonyl or sulphonyl group, R denotes an optionally substituted alkyl radical or an optionally substituted aryl radical and, if X is the carbonyl group, R may also denote an alkoxy or benzyloxy radical, R₁ denotes hydrogen, alkyl or an optionally substituted benzyl radical, R₂ denotes hydrogen or lower alkyl, R₃ and R₄ denote hydrogen, alkyl or an optionally substituted benzyl or an acyl radical, R₅ denotes hydrogen, alkyl, hydroxymethyl, mercaptomethyl or phenyl. R₆ denotes an optionally esterified carboxyl group or amidised carboxyl group which is optionally substituted and R₇ denotes an optionally esterified or amidised carboxyl group which is optionally substituted, with the proviso (a) that the optionally substituted alkyl radical R has more than 1 carbon atom if (1) X denotes the carbonyl group and the radical R₂ denotes methyl, or (2), if X denotes the carbonyl group, the radical R₂ represents hydrogen and R₃ and R₄ each represent a carboxyl group, and also with the proviso (b) that where R₂ and R₇ are other than hydrogen, the side-chain attached to oxygen in the 3-position of the glucosamine residue has the DLD configuration.

2. A glucosamine derivative of the formula (I), as defined in Claim 1, in which X denotes a carbonyl radical and R denotes a lower alkyl radical which is optionally substituted by hydroxyl or carboxyl groups or denotes a phenyl radical which is optionally substituted by lower alkyl, lower alkoxy, trifluoromethyl or halogen, and its salts.

3. A glucosamine derivative according to one of Claims 1 or 2, in which R₂ represents hydrogen.

4. A glucosamine derivative of the general formula





* C L M S *

29

1,570,625

29

Example 47

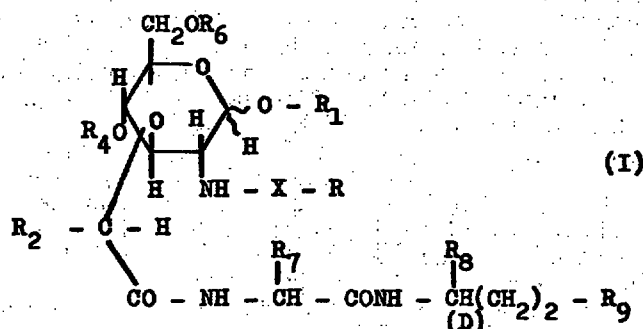
2 - (Acetaminomethylcarbonylamino) - 2 - desoxy - 3, O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoyl - methyl} - α, β - D - glucose is obtained analogously to Example 33.

Example 48

In a manner analogous to that described before 2 - trimethylacetamidio - 2 - desoxy - 3, O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoylmethyl} - α, β - D - glucose is obtained as Lyophilisate.

WHAT WE CLAIM IS:—

1. A glucosamine derivative of the general formula

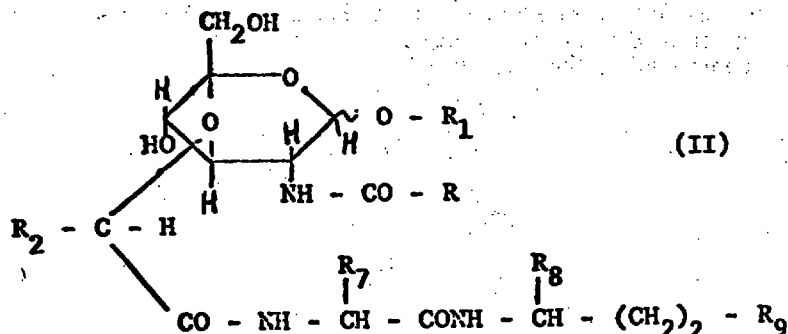


and the salts thereof, in which X denotes a carbonyl or sulphonyl group, R denotes an optionally substituted alkyl radical or an optionally substituted aryl radical and, if X is the carbonyl group, R may also denote an alkoxy or benzyloxy radical, R₁ denotes hydrogen, alkyl or an optionally substituted benzyl radical, R₂ denotes hydrogen or lower alkyl, R₄ and R₅ denote hydrogen, alkyl or an optionally substituted benzyl or an acyl radical, R₆ denotes hydrogen, alkyl, hydroxymethyl, mercaptomethyl or phenyl, R₇ denotes an optionally esterified carboxyl group or amidised carboxyl group which is optionally substituted and R₈ denotes an optionally esterified or amidised carboxyl group which is optionally substituted, with the proviso (a) that the optionally substituted alkyl radical R has more than 1 carbon atom if (1) X denotes the carbonyl group and the radical R₂ denotes methyl, or (2), if X denotes the carbonyl group, the radical R₂ represents hydrogen and R₇ and R₈ each represent a carboxyl group, and also with the proviso (b) that where R₂ and R₇ are other than hydrogen, the side-chain attached to oxygen in the 3-position of the glucosamine residue has the DLD configuration.

2. A glucosamine derivative of the formula (I), as defined in Claim 1, in which X denotes a carbonyl radical and R denotes a lower alkyl radical which is optionally substituted by hydroxyl or carboxyl groups or denotes a phenyl radical which is optionally substituted by lower alkyl, lower alkoxy, trifluoromethyl or halogen, and its salts.

3. A glucosamine derivative according to one of Claims 1 or 2, in which R₂ represents hydrogen.

4. A glucosamine derivative of the general formula





and the salts thereof, in which R denotes lower alkyl or phenyl, R₁ denotes hydrogen or lower alkyl, R₂ denotes hydrogen or methyl, R₃ denotes hydrogen, lower alkyl or hydroxymethyl, R₄ denotes carbamoyl and R₅ denotes carboxyl, with the proviso (a) that the lower alkyl radical R contains more than 1 carbon atom if R₂ denotes methyl, and (b) that where R₂ and R₃ are other than hydrogen, the side chain attached to oxygen in the 3-position of the glucosamine residue has the DLD configuration.

5. A glucosamine derivative of the formula II, according to Claim 4, in which R₁ represents hydrogen and R₃ represents hydrogen, methyl, or hydroxymethyl, and its salts.

6. Benzyl - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - 2 - propionylamino - α - D - glucopyranoside.

7. A glucosamine derivative of the formula I, according to Claim 1, in which R₁ and R₂ represent hydrogen, R denotes phenyl, R₃ denotes methyl and R₄ and R₅ represent carboxyl, carbomethoxy, carbamoyl or N - methyl - carbamoyl.

8. A glucosamine derivative of the formula II according to Claim 4, in which R₁ and R₂ represent hydrogen, R denotes methyl or phenyl, R₃ denotes hydroxymethyl, R₄ is carbamoyl and R₅ carboxyl.

9. A glucosamine derivative of the formula II, according to Claim 4, in which R₁ and R₂ represent hydrogen, R denotes phenyl, R₃ is methyl, R₄ represents carboxyl and R₅ N - propyl - carbamoyl or N - carbamoylmethylcarbamoyl.

10. A glucosamine derivative according to Claim 1, in which R₁ and R₂ represent hydrogen, R and R₃ denote methyl and R₄ and R₅ represent carbomethoxy, carbamoyl or N - methylcarbamoyl.

11. A glucosamine derivative, according to Claim 1, in which R represents phenyl, R₁ denotes ethyl, R₂ represents hydrogen, R₃ is methyl, R₄ represents carbamoyl and R₅ carboxyl or R₄ and R₅ represent carbomethoxy or N-methylcarbamoyl.

12. A glucosamine derivative, according to Claim 1, wherein R and R₃ represent methyl, R₁ is benzyl, R₂ represents hydrogen, and R₄ and R₅ represent carbomethoxy or carbamoyl.

13. Benzyl - 2 - acetamido - 3 - O - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - methyl - 2 - deoxy - 6 - O - stearoyl - α - D - glucopyranoside.

14. 2 - Acetamido - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - propyl} - 2 - deoxy - D - glucose.

15. 2 - Benzoylamino - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - carbamoyl - ethyl] - carbamoyl - ethyl} - 2 - deoxy - α,β - D - glucose.

16. 2 - Benzoylamino - 3 - O - {D - 1 - [L - 1 - (D - 1 - carbamoyl - 3 - carboxypropyl) - carbamoyl - ethyl] - carbamoyl - propyl} - 2 - deoxy - α,β - D - glucose.

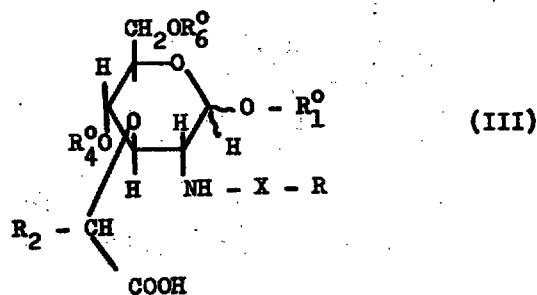
17. 2 - Benzoylamino - 3 - O - {[L - 1 - (D - 1 - carbamoyl - 3 - carboxy - propyl) - 1 - carbamoyl - ethyl] - carbamoyl - methyl} - 2 - deoxy - α,β - D - glucose.

18. A pharmaceutical formulation containing one of the compounds claimed in Claims 1 to 17 together with a pharmaceutical excipient.

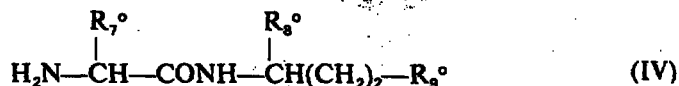
19. A pharmaceutical formulation containing one of the compounds claimed in Claims 1 to 17 together with a vaccine and, optionally, a pharmaceutical excipient.

20. Process for the manufacture of a glucosamine derivative of the general formula I as defined in claim 1, wherein:—

(a) a compound of the formula III

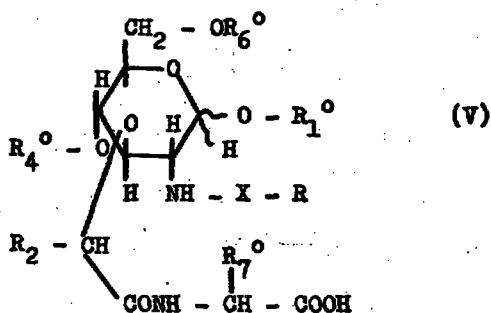


in which X, R and R₂ are as defined in claim 1, R₁^o, R₄^o, and R₆^o represent the radicals R₁, R₄, and R₆ respectively or represent a protective group, is condensed with a compound of the formula IV



5 in which R₇^o, R₈^o and R₉^o possess the meanings of R₇, R₈, and R₉ with the proviso that carboxyl groups, and if desired, free hydroxyl groups present in these radicals are protected by protective groups, and protective groups which are present are split off, or

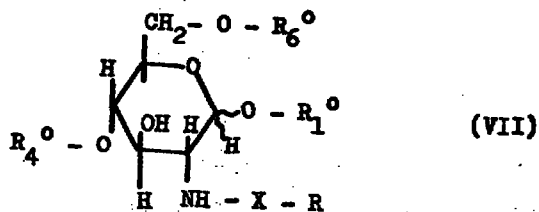
(b) a compound of the formula V



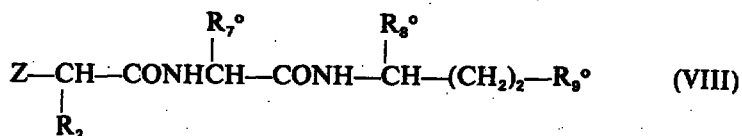
is condensed with a compound of the formula VI



and any protective groups which may be present are split off, or
(c) a compound of formula VII

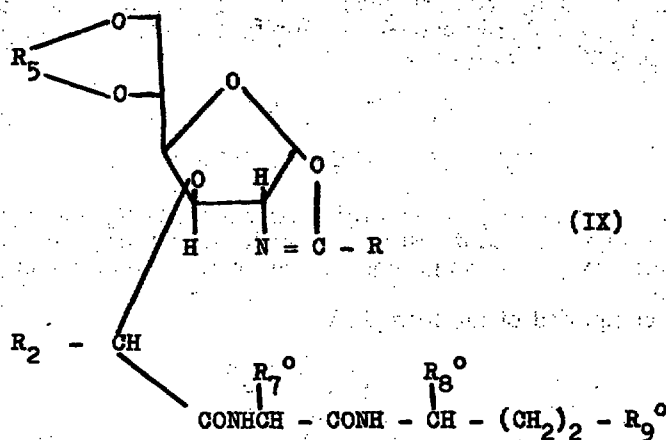


is reacted with a compound of the formula VIII



in which Z represents a reactively esterified hydroxyl group, and any protective groups which may be present are split off, or

(d) the oxazoline and dioxolane rings in a compound of formula IX



in which R_5 represents an alkylidene or cycloalkylidene radical are split open under acid conditions with formation of the glucosamine ring and any protective groups which may be present are split off, and, if the radical $R-CO-$ has been removed, introducing the $-X-R$ radical into the amino group in the 2-position of the sugar molecule, and, if desired, a compound resulting from any of the above processes is converted into a salt.

21. Process according to claim 20(a), wherein the acid of the formula III is reacted, in the form of the activated carboxylic acid, with the amino compound IV or the acid III is reacted with the compound IV in which the amino group is present in an activated form.

22. Process according to claim 20(b), wherein the acid of the formula V is reacted, in the form of the activated carboxylic acid, with the amino compound VI, or the acid V is reacted with the compound VI, in which the amino group is present in activated form.

23. Process according to claims 20—22, wherein the compounds of claims 2 to 17 are manufactured.

24. Processes for the preparation of compounds of formula I as defined in Claim 1 as defined in claims 20 to 23, with reference to examples 1 to 48.

ABEL & IMRAY,
Chartered Patent Agents,
Northumberland House,
303—306 High Holborn,
London, WC1V 7LH.