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"N-ACETYLNEURAMINIC ACID DERIVATIVES AND PROCESS FOR THE
PREPARATION THEREOF"

5 This invention relates to new N-acetylneuraminic acid derivatives, and relates in particular to new N-acetylneuraminic acid derivatives having immunological activity and to a process for the preparation thereof.

10 It is known that N-acetylneuraminic acid exists in animals and on the cell surface of some bacteria as a sialo-complex (glycoproteins, glycolipids, oligosaccharides, polysaccharides). Recently, it has been recognised that this compound is important medically and pharmaceutically in conditioning the functioning of the nervous system, differentiation of cells and immunity, treatment of inflammation, cancer, viral infection and as a hormone
15 receptor. This compound has been observed to be a specific active molecule localized on the cell surface.

However, the roles of this compound in the sialo-complex are still a matter of conjecture.

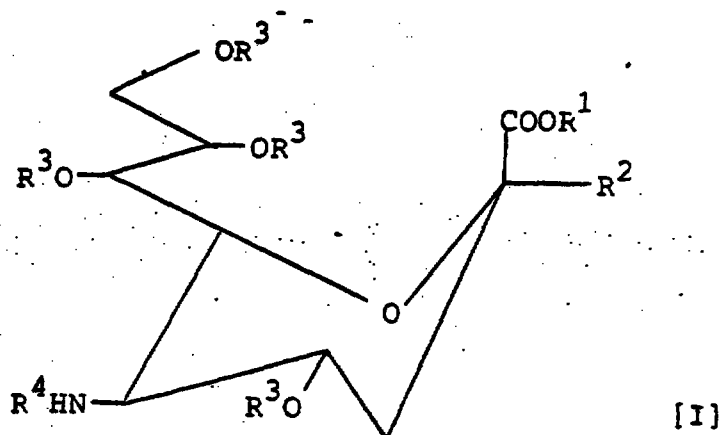
20 A number of organic chemists who engage in the investigation of natural substances have studied this compound and they have reported various kinds of simple derivatives thereof [see, for example, J. Biol. Chem. 244, 1306 (1969); Z. Physiol. Chem., 352, 1715 (1971)]. However, no derivative having a conspicuous physiological
25 activity has yet been observed.

The recently developed multilateral treatments for such diseases as every kind of cancer inclusive of malignant tumours of the hematogenic organs and collagenosis diseases,

have shown that an apothanasia effect is, in fact, attained. Since, an immoderate use of adrenocortical hormones and immunosuppressors is almost inevitable with such treatments, the problems of side-effects as well as the reduction or decrease in so-called immunity capacity are matters of grave concern.

Therefore, there is a strong demand for safer and more effective medicines for use in the treatment of many diseases and which are not accompanied by serious side-effects.

According to one aspect of this invention, there are provided N-acetylneuraminic acid derivatives represented by the following general formula (I):



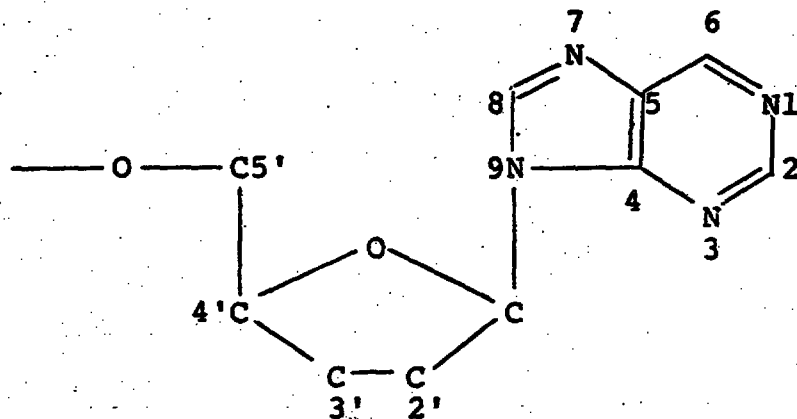
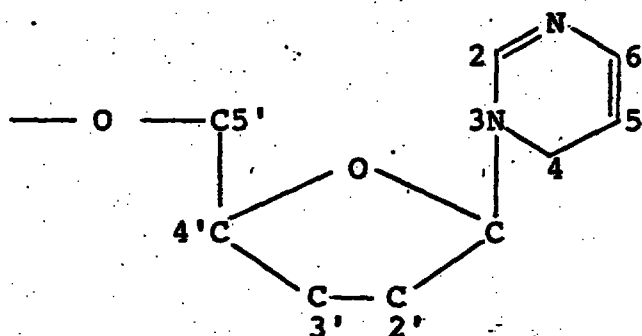
wherein R^1 represents a lower alkyl, or a lower alkyl-substituted or non-substituted aralkyl or aryl group, R^2 stands for a residue of a nucleoside (as hereinafter defined) or a residue of a glucose (as hereinafter defined), and the groups R^3 represent H or an acetyl group independently and R^4 represents an acetyl group.

It has in fact been found that by chemically modifying N-acetylneuraminic acid with a sialic acid which is an inherent component of the living body, N-acetylneuraminic acid derivatives having a high immunological activity without entraining any serious side-effects could be produced and that the above-mentioned disadvantages could be eliminated.

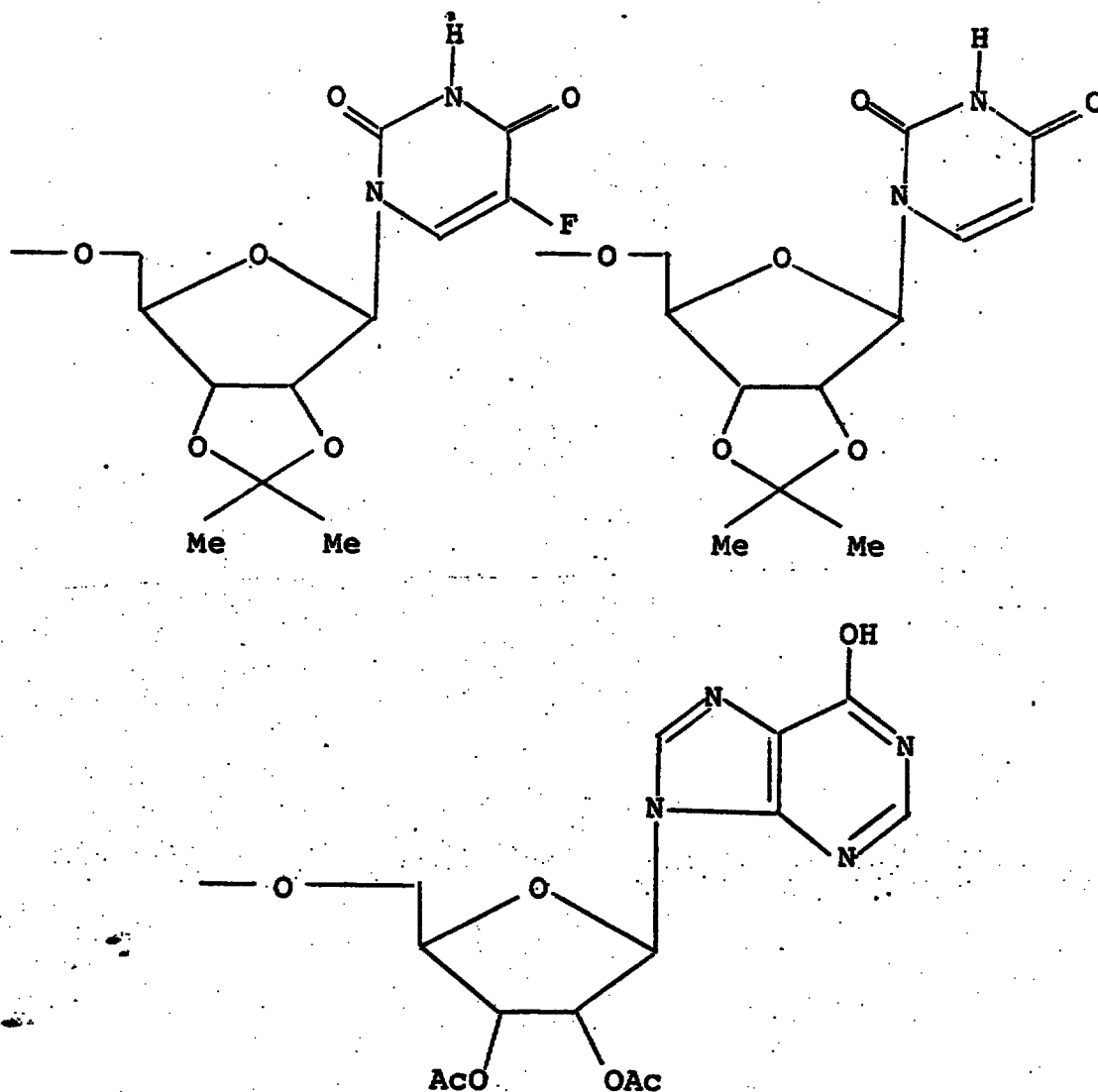
According to the present invention, the term "lower alkyl" means C₁₋₄ alkyl, preferably methyl or ethyl.

5 The term "nucleoside residue" used herein means a residue in which a saccharide and a purine or a pyrimidine base are bonded through a glycoside bond, and these residues may include a substituent or substituents and/or rings fused to the characteristic rings thereof. The basic backbones of these residues are represented by the following structural formulae:

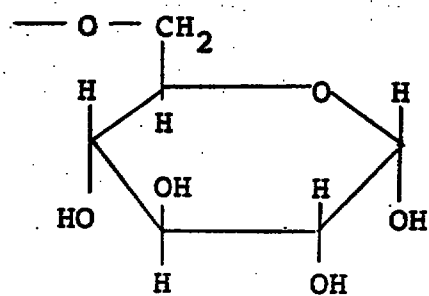
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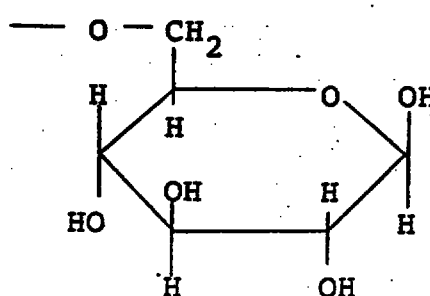
The following residues, for example, may be used:



The term "glucose residue" herein used denotes both D- and L-residues; the D-glucose residue is preferably used. The following residues are the typical ones:

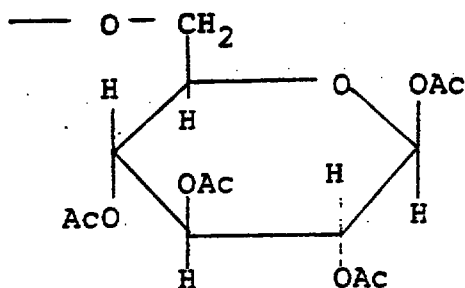


α -D-glucopyranosyl

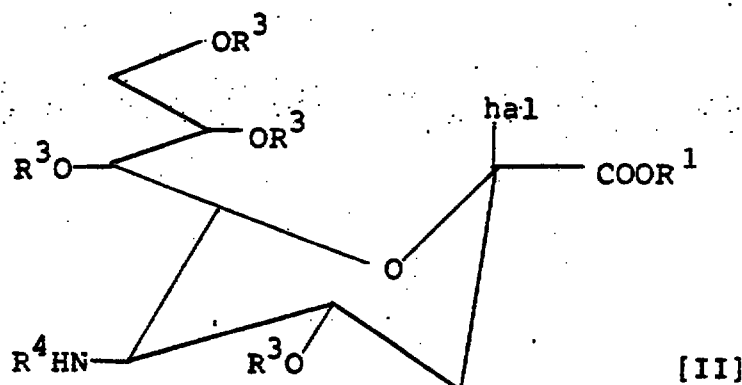


β -D-glucopyranosyl

The term "glucose residue" also includes the residues in which part of or all of the hydroxy groups are esterified with for example acetic acid:



- 5 According to a second aspect of the invention, there is provided a process for the preparation of the N-acetyl-neuraminic acid derivatives of this invention which comprises subjecting a compound of the formula (II):



- 10 wherein R^1 stands for a lower alkyl or a lower alkyl substituted or non-substituted aralkyl or aryl group, R^3 and R^4 are all acetyl and hal represents a
15 halogen atom, and a nucleoside or a glucose as hereinbefore defined to Koenigs-Knorr reaction, and then if desired partially or completely deacetylating the resulting product.

- Use may be made in the Koenigs-Knorr reaction of for example Ag_2CO_3 , Ag_2O , $Hg(CN)_2$, $HgBr_2$, $AgClO_4$ as a catalyst. In a preferred embodiment, use is made of a molecular sieve
20 such as molecular sieve 3A or 4A together with a catalyst so as effectively to remove hydrogen halides generated

during the reaction, to enhance the reaction rate and to improve the yield of the reaction product.

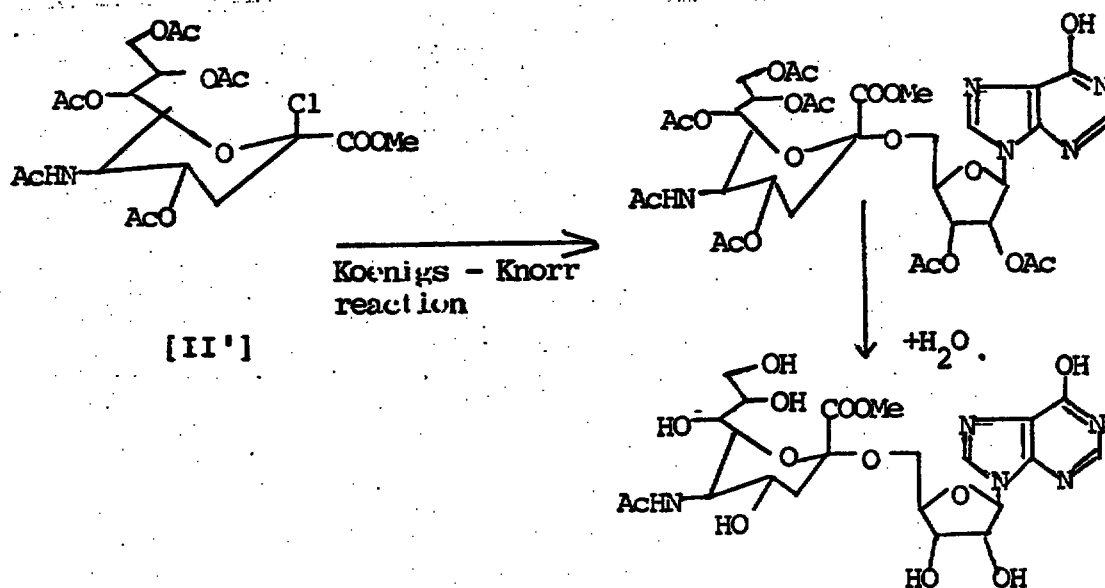
Although there is no critical range of reaction temperatures, the reaction is usually to be carried out at room temperature both from the economical point of view and because of the simplicity of such operation.

The reaction time is in a range of from 30 minutes to 24 hours.

Acetonitrile or nitromethane is preferably used as reaction medium in carrying out the process of the present invention.

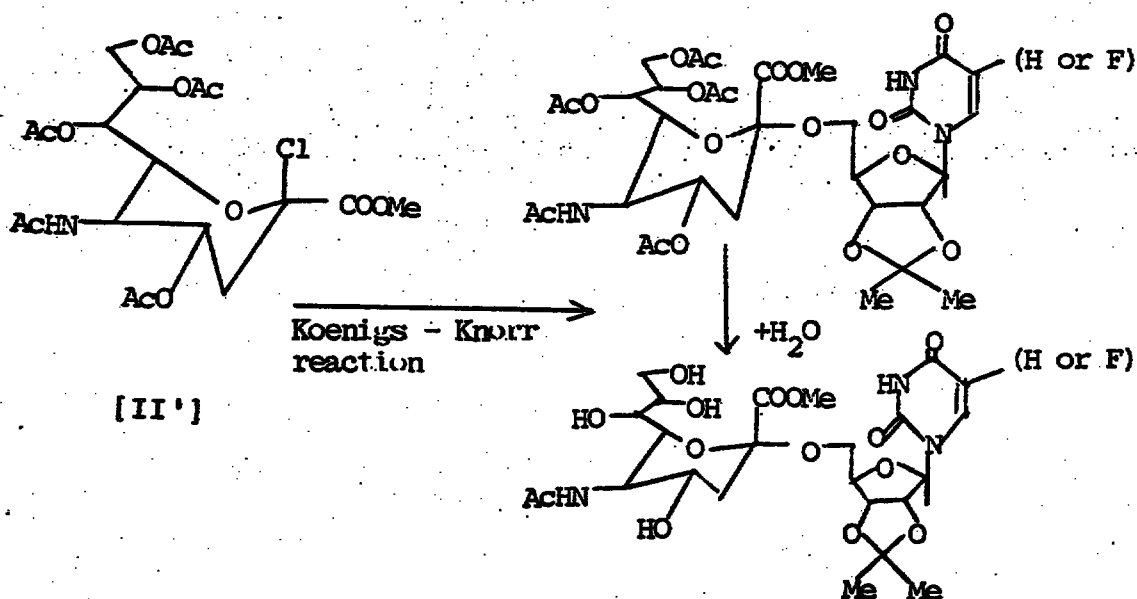
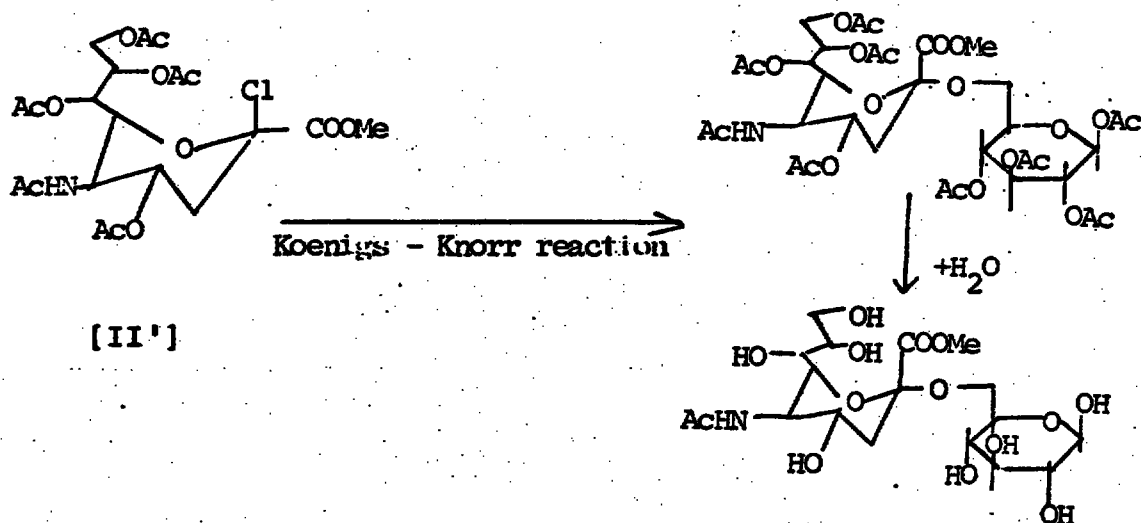
The deacetylation reaction may be carried out by agitating the product having acetyl group(s), obtained after the Koenigs-Knorr reaction, at a temperature of -20 to 0°C for about 20 minutes in a reaction medium such as methanol, reaction being carried out in the presence of an alkali metal alcoholate. The reaction solution is neutralised, for example with Dowex 50 x 8 (H⁺) and the reaction product is recovered using a conventional procedure. (Dowex is a Registered Trade Mark).

Preferred examples of the process for preparing new N-acetylneuraminic acid derivatives are hereinafter illustrated by reaction schemes.



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Starting materials of the formula [II] used in the process of the present invention are known substances and may be prepared for example according to the process disclosed in the paper of Richard Kuhn et al. Chem. Ber., 99, 611 (1966), which process comprises three steps i.e., esterifying N-acetylneuraminic acid to obtain β -D-N-acetylneuramate, acetylating the esterified product to get

4,7,8,9-tetra-O-acetyl- β -D-N-acetylneuraminate and finally halogenating the resultant acetylated product.

Alternatively, the compound [II] may also be prepared by a new process developed by the present inventors, which
5 comprises esterifying N-acetylneuraminic acid and then simultaneously acetylating and halogenating the resultant esterified N-acetylneuraminic acid with acetyl halide. The compound [II] may be obtained in higher yield than in the method of R.Kuhn et al. This process is also excellent from the points of
10 view of both safety and economy and is utilised preferably in a preferred preliminary step to the process of this invention.

The compounds [II] may be prepared in overall yield of about 90% by esterifying N-acetylneuraminic acid and then
15 reacting the resultant product in an acetyl halide solution at ambient temperature (there is no limitation on the reaction temperature) for several hours to achieve simultaneous acetylation and halogenation.

R.Kuhn et al discloses in the aforesaid paper a halide (chloride) of a N-acetylneuraminic acid derivative
20 together with a molecular formula. However, the melting point thereof is not disclosed and it is not believed that the chloride has been prepared.

The compounds [I] of the present invention have an impressive activity for conditioning immunity.

25 The immunological functions may be determined by the use of the following procedures:

(i) Effect on the activation of murine splenic lymphocytes with Con A:

Suppressor T cells are non-specifically activated by
30 Concanavalin A (Con A). An investigation of the effect of N-acetylneuraminic acid derivatives of the invention on such a reaction system was therefore carried out. The investigation was carried out according to the following procedure. Firstly, Con A and examples of the compounds
35 [I] of the invention were added to splenic lymphocytes (SPC) obtained from BALB/C mice to prepare samples. These samples were cultured on a microplate at 37°C for 20 hours

in a 5% CO₂ atmosphere, then tritium labelled thymidine was added to the samples and the samples were cultured at 37°C for another 18 hours. The SPC was then collected and the amount of ³H-thymidine incorporated into the SPC was determined by using a scintillation counter.

As a result, it was observed that the incorporation of ³H-thymidine into SPC was accelerated and enhanced and that the activation of T cells by Con A was also enhanced by the presence of compounds [I] of the present invention (products of Examples 9 and 10 hereinafter). Results of tests carried out using typical compounds are shown in Table 1.

Table 1
Effect of neuraminic acid derivatives on the DNA Synthesis of Con A-Stimulated Lymphocytes

	Derivative	Concentration (M)	Stimulation Index of [⁶⁻³ H] Thymidine Incorporation a)	
			With Con A	Without Con A
	None		1.00	1.00
	Compound II'	10 ⁻⁴	1.10	0.93
20	Product of Ex. 9	10 ⁻⁵	1.57	1.26
	Product of Ex. 10	10 ⁻⁴	1.81	1.24
	FIU	10 ⁻⁵	0.02	0.47
	DAI	10 ⁻⁴	1.07	1.24
	Compound II' + FIU	10 ⁻⁵	0.02	0.30
25	Compound II' + DAI	10 ⁻⁴	1.02	1.33

FIU: 5-fluoro-2',3'-isopropylidene uridine

DAI: 2',3'-di-O-acetylinosine

a) Results of typical experiments.

As shown in Table I, Con A-induced increase of DNA synthesis of lymphocytes was significantly enhanced by the compounds of Ex. 9 (10⁻⁵ M) and Ex. 10 (10⁻⁴ M). FIU, a component of the product of Ex. 9 and DAI, that of the

product of Ex. 10, did not induce any increase in DNA synthesis in Con A-stimulated lymphocytes. Almost complete inhibition of $[6^{-3}\text{H}]$ thymidine incorporation was observed in a FIU (10^{-5} M) -treated culture. Compound II', a component common to the products of Ex. 9 and Ex. 10, seemed to induce an increase of DNA synthesis, but the rate of increase was not significant. Furthermore, the mixtures of compound [II'] (10^{-5} M) and FIU (10^{-5} M), and of compound [II'] (10^{-4} M) and DAI (10^{-4} M) exerted the same effects as those of FIU or DAI alone on the DNA synthesis of Con A-stimulated lymphocytes. The products of Ex. 9 and Ex. 10 enhanced DNA synthesis of lymphocytes even when cultured without Con A (Table I).

(ii) Effect of murine splenic lymphocytes on immunoglobulin synthesis

Investigation of the effect of N-acetylneuraminic acid derivatives having T cell activation effect on immunoglobulin synthesis was carried out by measuring the number of plaque forming cells (PFC). To do this, SPC was cultured together with sheep red blood cells (SRBC) and each of the compounds [I] of the invention and comparative substance to be set out in Table 2 at 37°C for 5 days. Then, SRBC and a complement were further added to the sensitized SPC. The resultant sample was cultured in a Cunningham chamber at 37°C for 3 to 12 hours and then the number of PFC was determined.

It was found that the number of PFC was reduced and the cell viability was identical to the control (free of the compound [I]). This means that the suppressive effect on immunoglobulin synthesis is enhanced by the compounds of formula [I].

Table 2

Effect of disaccharide nucleosides and their molecular constituents on the primary PFC response to SRBC in vitro

5	<u>Treatment</u>	<u>Concentration (M)</u>	<u>PFC/2 x 10⁶ cultured lymphocytes a)</u>
	None	-	57 ± 4
	FIU	10 ⁻⁵	11 ± 3
	DAI	10 ⁻⁴	28 ± 6
	Compound II'	10 ⁻⁴	43 ± 11
10	Product of Ex.9	10 ⁻⁵	16 ± 2
	Product of Ex.10	10 ⁻⁴	25 ± 17

a) Mean ± S.E. of triplicate cultures.

From the results shown in Table 2, it can be seen that the products of Examples 9 and 10 and their starting materials induced a decrease in PFC response to SRBC.

Con A-stimulated lymphocytes exhibited suppressor activity on the primary PFC response to SRBC. This induction of suppressor cells by Con A was significantly enhanced by the presence of the product of Ex. 9 (10⁻⁵ m) and the product of Ex. 10 (10⁻⁴ M) during the incubation with Con A. Furthermore, SPC precultured with the products of Ex. 9 and Ex. 10 in the absence of Con A also exhibited the suppressor activity. On the other hand, FIU, DAI and compound [II'] did not induce the suppressor activity of the cells (Table III).

The induction of suppressor cells by the products of Examples 9 and 10 no longer occurred with the pre-treatment of lymphocytes with anti Thy-1 antiserum plus complement. These results suggest that these disaccharide nucleosides can induce suppressor T cells.

Table 3

Effect of the treatment of Con A-stimulated and non-stimulated cells with disaccharide nucleosides on the suppressor activity of the cells

5	Treatment ^{a)}	PFC/2 x 10 ⁶ cultured lymphocytes ^{b)}		
		Exp. 1	Exp. 2	Exp. 3
	None (Control)	103 ± 17	111 ± 4	130 ± 16
	FIU (10 ⁻⁵ M)	---	---	108 ± 10
10	DAI (10 ⁻⁴ M)	---	---	108 ± 8
	Compound II' (10 ⁻⁴ M)	---	---	145 ± 24
	Product of Ex. 9 (10 ⁻⁴ M)	83 ± 16	54 ± 2	72 ± 5
	Product of Ex. 10 (10 ⁻⁴ M)	67 ± 9	34 ± 7	89 ± 17
	Con A (2 g/ml)	68 ± 3	65 ± 8	---
15	[Ex. 9] + Con A	51 ± 6	39 ± 13	---
	[Ex.10] + Con A	29 ± 9	27 ± 8	---
20	a) 2 x 10 ⁵ lymphocytes were incubated with disaccharide nucleosides and their molecular constituents in the presence or absence of Con A at 37°C for 45 hr, and then the cells were co-cultured with normal fresh spleen cells (2 x 10 ⁶ cells) plus SRBC (1 x 10 ⁶ cells).			

b) Mean ± S.E. of triplicate cultures.

Hitherto, it has been recognised that the function of suppressor T cell is reduced in the case of autoimmune disease such as collagen disease. Hence, N-acetylneuraminic acid derivatives according to the present invention having an activation effect on suppressor T cell, may be expected to have clinical effectiveness as an immunity control agent.

The following non-limiting Examples illustrate this invention:

EXAMPLE 1

Preparation of methyl 2-chloro-4,7,8,9-tetra-O-acetyl-β-D-N-acetylneuraminate

N-acetylneuraminic acid (1g) was dissolved in methanol (100 ml) and 2 g of Dowex 50 x 8 (H⁺) were added

to the solution obtained. The mixture was agitated at room temperature for 2 hours, then filtered and the filtrate was evaporated to dryness. The resultant residue was re-crystallised from methanol-ether and 1 g of methyl β -D-N-acetylneuramate was obtained as colourless acicular crystals (yield 95%).

Physical properties:

m.p. 175 - 178°C

$[\alpha]_D^{20}$ -28°C (c=1, H₂O)

Elemental Analysis: C₁₂H₂₁NO₉

calculated: C 44.58; H 6.55; N 4.33

found : C 44.62; H 6.57; N 4.23

To 1 g of methyl β -D-N-acetylneuramate thus obtained were added 10 ml of acetylchloride and the mixture was stirred at ambient temperature for 5 hours. The excess amount of acetylchloride was then distilled off under reduced pressure, benzene was added to the solution and the solvent was again distilled off to obtain 1.5 g (yield 95%) of the title compound as a colourless amorphous substance. The crude substance was recrystallised from benzene-ether-petroleumether mixed solvent and 1.26 g (yield 80%) of the title compound was obtained as colourless acicular crystals.

Physical properties:

m.p. 116 - 118°C

$[\alpha]_D^{20}$ -68°C (c=1, chloroform)

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm⁻¹) 1735, 1654, 1532

¹H NMR (CDCl₃) δ_H (TMS)

1.92(3H, s), 1.99 - 2.10 (OAcx4),

2.87(1H, dd, J=5.0 and 12.0 Hz),

3.91(3H, s)

EXAMPLE 2

Preparation of 2',3'-di-O-acetyl-5'-
O-(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-
O-acetyl-1-methoxycarbonyl-D-glycero- α -
D-galacto-octopyranosyl) inosine

5 550 mg of 2',3'-di-O-acetylinosine, 150 mg of $\text{Hg}(\text{CN})_2$,
300 mg of HgBr_2 and 500 mg of molecular sieve (4A) were
added to 30 ml of acetonitrile, followed by 510 mg of
methyl 2-chloro-4,7,8,9-tetra-O-acetyl- β -D-N-acetylneur-
10 aminate (compound [II']). The mixture was then agitated
at ambient temperature for 48 hours. The resultant solution
was filtered and the filtrate was evaporated to dryness.
50 ml of ethyl acetate were added to the residue and the
solution formed was washed two times with 30% by weight
15 aqueous potassium iodide solution to remove $\text{Hg}(\text{CN})_2$ and
 HgBr_2 . The organic phase was then dried over Glauber's
salt and the solvent was distilled off. Purification was
effected by subjecting the crude product to alumina
column chromatography and eluting the benzene-ethylacetate.
20 The title compound was obtained in an amount of 430 mg
(yield 52%) as a colourless powder.

Physical properties:

$[\alpha]_D^{25} -16^\circ\text{C}$ (c=1, methanol)

Elemental Analysis: $\text{C}_{34}\text{H}_{43}\text{N}_5\text{O}_{19}$

25 calculated: C 49.46; H 5.25; N 8.48

found : C 49.15; H 5.41; N 8.11

Mass Spectroscopy (FD) m/z

825 (M^+)

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}) 3300, 1740, 1660, 1530

30 ^1H NMR (CDCl_3) δ_{H} (TMS)

1.88 - 2.20 (OAcx7), 2.76 (1H, dd, J=13.0 and
4.5Hz), 3.78 (3H, s), 5.95 (1H, d, J=2.2Hz),
8.20 (1H, s), 8.44 (1H, s)

EXAMPLE 3

Preparation of 2',3'-isopropylidene-5'-O-(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-O-acetyl-1-methoxycarbonyl-D-glycero- α -D-galacto-octopyranosyl) uridine

50 ml of acetonitrile were added to a mixture of 1 g of 2',3'-isopropylideneuridine, 150 mg of $\text{Hg}(\text{CN})_2$ and 300 mg of HgBr_2 , followed by 1 g of molecular sieve (4A).

The mixture obtained was reacted with 510 mg of compound [II'] under agitation at ambient temperature for 24 hours. The solution obtained was filtered and the filtrate was evaporated to dryness at 40°C under reduced pressure to remove the solvent. 100 ml of ethylacetate were added to the residue, and the solution obtained was washed with 30% by weight aqueous potassium iodide solution to remove $\text{Hg}(\text{CN})_2$ and HgBr_2 . The ethylacetate solution was dried over Glauber's salt, the solvent was removed and oily material was obtained. Substances soluble in ether were removed from the oily material, and after the addition of 10 ml of chloroform non-soluble material was removed. By the addition of ether to the chloroform solution, precipitates were formed and filtered off and dried. In this way, 300 mg (yield 40%) of a colourless powder of the title compound were obtained.

Physical properties

$[\alpha]_D^{22} -2.1^\circ$ (c=1, methanol)

Elemental Analysis: $\text{C}_{32}\text{H}_{43}\text{N}_3\text{O}_{18}$ MW=757.70

calculated: C 50.73; H 5.72; N 5.55

found : C 50.56; H 5.90; N 5.22

Mass Spectroscopy: m/z 757 (M^+), 742 (M^+-15)
714 (M^+-43), 698 (M^+-59)

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 1735, 1678, 1530

^1H NMR (CDCl_3) δ_{H} (TMS)

1.48 (s, 3H), 1.70 (s, 3H), 1.88 (s, 3H), 2.00 -
2.20 (OAcx4), 2.60 (dd, 1H, J=4.0 and 13.0Hz)
3.80 (s, 3H), 5.68 (d, 1H, J=7.0Hz), 7.60 (d, 1H,
J=7.0Hz)

5

EXAMPLE 4

Preparation of 2',3'-isopropylidene-5'-
O-(4-N-acetyl-2,4-dideoxy-1-methoxy-
carbonyl-D-glycero- α -D-galacto-octapyranosyl)
uridine:

10

The compound obtained in Example 3 was dissolved in
10 ml methanol to form a solution which was reacted with a
solution prepared by dissolving 100 mg of potassium metal
in 10 ml of methanol and the resultant solution was agi-
tated at 0°C for 20 minutes. The resulting solution was
15 neutralised with Dowex 50 x 8 (H⁺) at -20°C, filtered,
concentrated and evaporated to dryness. The resulting .
residue was dissolved in 5 ml of methanol and dioxane was
further added to form a precipitate which was filtered
off. In this way 230 mg (yield 60%) of a colourless
20 powder of the title compound were obtained.

Physical properties:

$[\alpha]_D^{20}$ -9.5° (c=1, H₂O)

Elemental Analysis: C₂₄H₃₅N₃O₁₄, MW=589,55

calculated: C 48.89; H 5.98; N 7.13

25

found : C 48.25; H 6.03; N 7.05

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm⁻¹): 3300, 1735, 1645, 1530

¹H NMR (D₂O) δ_H (DSS)

1.55 (s, 3H), 1.66 (s, 3H), 2.30 (s, 3H), 3.4 (s,
3H), 5.96 (d, 1H, J=8.5Hz), 5.99 (d, 1H,
30 J=1.2Hz), 7.90 (d, 1H, J=8.5Hz)

EXAMPLE 5

Preparation of methyl 1',2',3',4'-tetra-O-acetyl- β -D-glucopyranosyl-(6' $\rightarrow$ 2)-4,7,8,9-tetra-O-acetyl- α -D-N-acetylneuramate

5 1.5 g of 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose was dissolved in 50 ml of acetonitrile and 1 g of molecular sieve (4A), 150 mg of $\text{Hg}(\text{CN})_2$ and 300 mg of HgBr_2 were added to the solution, followed by 1 g of compound [II']. The resulting solution was stirred at ambient temperature
10 for 12 hours and then filtered. The filtrate was distilled at 40°C under reduced pressure to remove solvent and evaporated to dryness. 100 ml of ethylacetate were added to the residue obtained, and the solution thus formed was washed with 30% by weight aqueous potassium
15 iodide solution to remove $\text{Hg}(\text{CN})_2$ and HgBr_2 . The resulting ethylacetate solution was dried over Glauber's salt and then the solvent was distilled off. The residual powder was subjected to silica gel column chromatography and eluted by means of a mixed benzene-ethylacetate solvent to purify
20 the product. In this way 650 mg (yield 40%) of a colourless powder of the title compound were obtained.

Physical properties:

$[\alpha]_D^{24} +3.2^\circ$ (c=1, methanol)

Elemental Analysis: $\text{C}_{34}\text{H}_{47}\text{NO}_{22}$, MW=821.74

25 calculated: C 49.70; H 5.77; N 1.71

found : C 49.02; H 5.52; N 1.40

Mass Spectroscopy: m/z

821 (M^+), 762 (M^+-59)

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 1735, 1655, 1530

30 ^1H NMR (CDCl_3) δ_{H} (TMS)

1.89 (s, 3H), 1.99 - 2.12 (OAcx8), 3.74 (s, 3H)

EXAMPLE 6

Preparation of methyl D-glucopyranosyl-
(6'→2)-α-D-N-acetylneuraminate

500 Mg of compound obtained in Example 5 were dissolved in 10 ml of methanol to form a solution to which a solution of 100 mg of potassium metal in 10 ml methanol was added. The mixed solution was then stirred at 0°C for 20 minutes and then was neutralised with Dowex 50 x 8 (H⁺), filtered, concentrated and evaporated to dryness. The resulting residue was dissolved in 5 ml of ethanol and ether was added to form a precipitate. The precipitate was filtered off to yield 220 mg (yield 75%) of a colourless powder of the title compound.

Physical properties:

[α]_D²⁰ +25° (c=1, H₂O)

Elemental Analysis: C₁₈H₃₁NO₁₄, MW=485.44

calculated: C 44.54; H 6.44; N 2.89

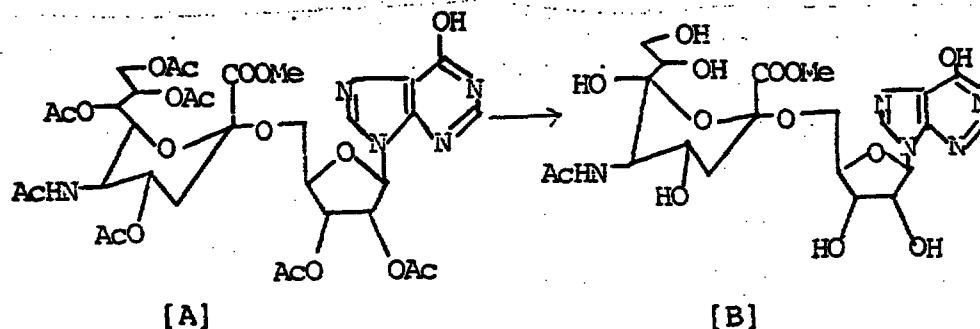
found : C 44.15; H 6.42; N 2.52

I.R. ν_{max}^{KBr} (cm⁻¹): 3300, 1735, 1645, 1530

¹H NMR (D₂O) δ_H (DSS): 2.05 (s, 3H), 3.85 (s, 3H)

EXAMPLE 7

Preparation of 5'-O-(4-N-acetyl-2,4-dideoxy-1-methoxycarbonyl-D-glycero-α-D-galacto-octopyranosyl)-inosine



Compound [A] (300 mg) was dissolved in 10 ml of methanol, to form a solution to which a solution of 80 mg of potassium metal in 10 ml of methanol was added. The resulting solution was stirred at 0°C for 20 minutes, after which it was neutralised by the addition of Dowex 50 x 8 (H⁺) at -20°C. The neutralised solution was filtered, concentrated and evaporated to dryness. The residue thus obtained was dissolved in 1 ml of water and ethanol was added to the solution to enable a precipitate of the title compound to form. As a result, 150 mg (yield 71%) of the title compound [B] were obtained as a colourless powder.

Physical properties:

$[\alpha]_D^{20} -22^\circ$ (c=1, H₂O)

Elemental Analysis: C₂₂H₃₁N₅O₁₃, MW=573

calculated: C 46.07; H 5.45; N 12.21

found : C 45.65; H 5.61; N 12.18

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm⁻¹): 3300, 1735, 1655, 1530

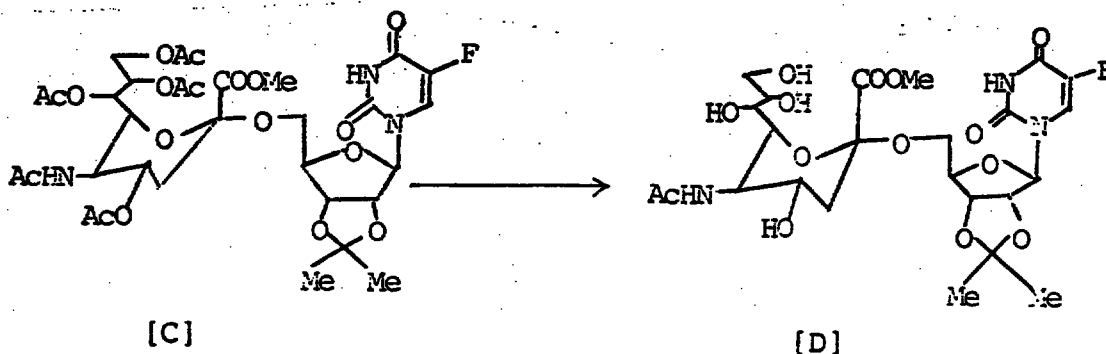
¹H NMR (D₂O) δ_H (DSS):

2.05(s, 3H), 3.85(s, 3H)

8.15(s, 1H), 8.62(s, 1H)

EXAMPLE 8

Preparation of 5-fluoro-2',3'-isopropylidene-5'-O-(4-N-acetyl-2,4-dideoxy-1-methoxycarbonyl-D-glycero- α -D-galactooctopyranosyl) uridine



A solution obtained by dissolving 500 mg of compound [C] in 10 ml of methanol was reacted with a solution of 100 mg of potassium metal in 10 ml of methanol, reaction being effected at 0°C for 20 minutes with stirring. Dowex 50 x 8 (H⁺) was added to the solution at -20°C to neutralise it and the solution was filtered, concentrated and evaporated to dryness. The resulting residue was then dissolved in 5 ml of methanol and dioxane was added to precipitate the title compound. This was separated off from the solvent and the compound [D] was obtained in an amount of 180 mg (yield 48%) as a colourless powder.

Physical properties:

$[\alpha]_D^{20} -1.1^\circ$ (c=1, methanol)

Elemental Analysis: C₂₄H₃₄N₃O₁₄F, MW=607

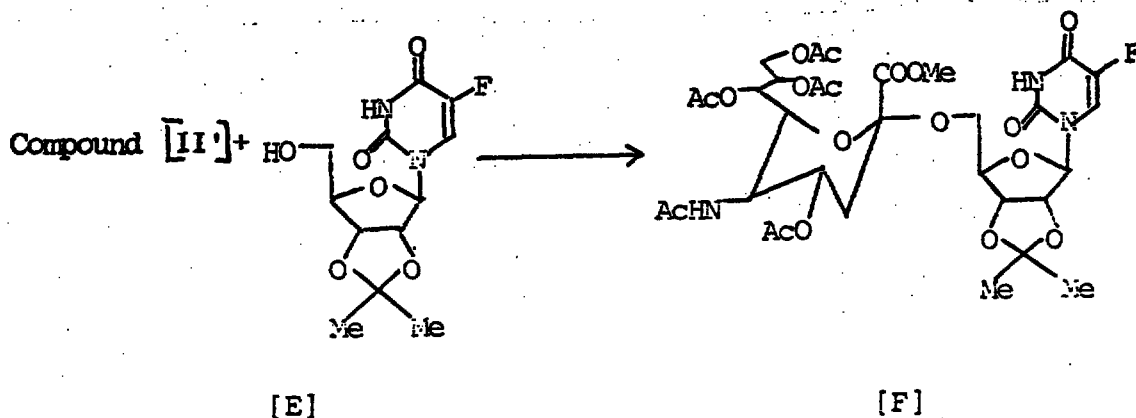
calculated: C 47.45; H 5.60; N 6.92

found : C 47.92; H 5.45; N 6.21

I.R. $\nu_{\text{max}}^{\text{KBr}}$ (cm⁻¹): 3300, 1735, 1640, 1535

EXAMPLE 9

Preparation of 5-fluoro-2',3'-isopropylidene-5'-O-(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-O-acetyl-1-methoxycarbonyl-D-glycero- α -D-galacto-octopyranosyl)-uridine



Method (i)

500 mg of 5-fluoro-2',3'-isopropylideneuridine [E], 150 mg of $\text{Hg}(\text{CN})_2$ and 300 mg of HgBr_2 were dissolved in 30 ml of CH_3CN , and then 1 g of a molecular sieve powder (3A) was added. After 1 hour agitation, 510 mg of the compound [II] were introduced and the suspension obtained was stirred at ambient temperature over 24 hours. The resulting product was filtered and the solvent of the filtrate was distilled off at 40°C under reduced pressure until a dry residue resulted. The residue obtained was dissolved in 100 ml of ethylacetate and washed with the aid of 30% by weight aqueous potassium iodide solution to remove $\text{Hg}(\text{CN})_2$ and HgBr_2 . The ethylacetate phase was dried over Glauber's salt and the solvent was distilled off to obtain an oily substance. Ether soluble substances were removed from the oil, and 10 ml of chloroform were added to enable chloroform insoluble materials to be removed. Ether was added to the chloroform solution to enable the title compound to precipitate out from the chloroform solution. The precipitate was purified by subjecting it to alumina column chromatography and eluting with ethylacetate-ethanol mixture. Thus, 62 mg (yield 8%) of the compound [F] were obtained as a colourless powder.

Physical properties:

$[\alpha]_D^{25} -1.4^\circ$ (c=1, methanol)

Elemental Analysis: $\text{C}_{32}\text{H}_{42}\text{N}_3\text{O}_{18}\text{F}$

calculated: C 49.55; H 5.42; N 5.45

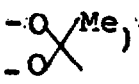
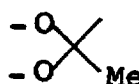
found : C 49.24; H 5.80; N 5.12

Mass Spectroscopy m/z:

775 (M^+), 760 ($\text{M}^+ - \text{Me}$), 732 ($\text{M}^+ - \text{COOMe}$), 716 ($\text{M}^+ - \text{COOMe}$)

I.R. KBr_{max} (cm^{-1}): 1735, 1680, 1530

^1H NMR (CDCl_3) δ_{H} (TMS):

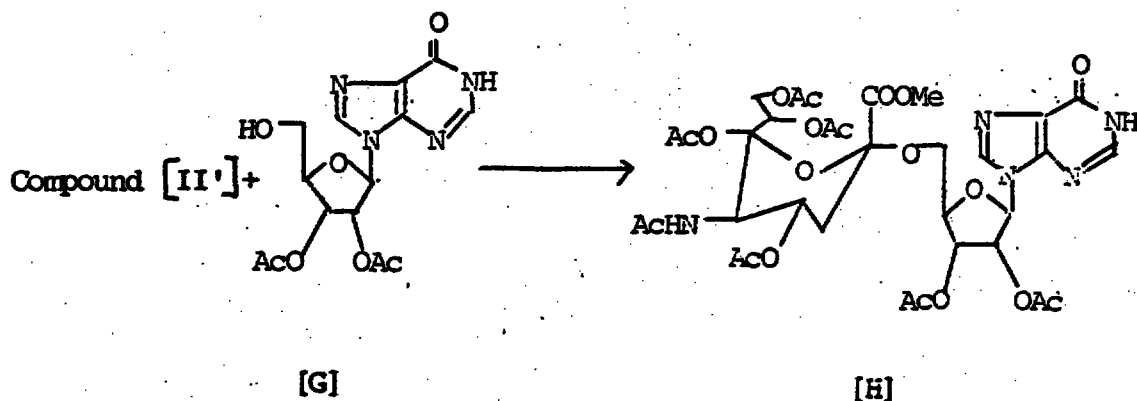
1.48 (3H, s, , 1.74 (3H, s, ,
1.89 - 2.20 (15H, all s, -NHAc and -OAc),
2.63 (1H, dd, J=4.0 and 14.0Hz, 3"-H eq.)
3.80 (3H, s, methylester), 5.95 (1H, d, J=2.0Hz,
5 1'-H), 7.62 (1H, d, J=7Hz, 6- H)

Method (ii)

The compound [E] (500 mg) was dissolved in 10 ml of
CH₃NO₂, then 350 mg of AgClO₄ and 200 mg of powdered
molecular sieve (4A) were added and the mixture obtained
10 was stirred for 30 minutes. A solution of 510 mg of the
compound [II'] in 5 ml of CH₃NO₂ was then added to the
mixture and the resulting solution was stirred at ambient
temperature for 2 hours. The resulting solution was
filtered and 50 ml of ethylacetate were added to the
15 filtrate followed by 20 ml of saturated sodium chloride
solution. The CH₃NO₂-ethylacetate solution was dried over
Glauber's salt and, after filtration of the solution, the
solvent was distilled off. The residue was extracted with
either to remove ether-soluble substances and then ex-
20 tracted with chloroform to remove chloroform-insoluble
materials. Ether was added to the resulting chloroform
solution to enable the title compound to precipitate. The
precipitate was purified by subjecting it to alumina column
chromatography and followed by elution with ethylacetate-
25 ethanol mixed solvent. 150 Mg (yield 20%) of the compound
[F] were obtained as a colourless powder.

EXAMPLE 10

Preparation of 2',3'-di-O-acetyl-5'-O-
(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-O-
30 acetyl-1-methoxycarbonyl-D-glycero- α -D-
galacto-octopyranosyl) inosine



550 Mg of the compound [G], 150 mg of $\text{Hg}(\text{CN})_2$, 300 mg of HgBr_2 and 500 mg of powdered molecular sieve (4A) were added to 30 ml of CH_3CN and the resulting mixture was agitated for 1 hour. 510 Mg of the compound [II'] were then introduced into the mixture and the resulting solution was stirred at ambient temperature for 48 hours. The reaction solution was then filtered, and the filtrate was concentrated and evaporated to dryness. The residue obtained was dissolved in 50 ml of ethylacetate and the solution was washed twice with the aid of 30% by weight aqueous potassium iodide solution to remove $\text{Hg}(\text{CN})_2$ and HgBr_2 . Then, the ethylacetate solution was dried over Glauber's salt and the solution was distilled to remove solvent. The oily material resulting from the distillation was subjected to alumina column chromatography using benzene-ethylacetate-ethanol to purify the product, and thus 210 mg (yield 25%) of the title compound were obtained as a colourless powder.

Physical properties:

$$[\alpha]_D^{25} -16^\circ \quad (c=1, \text{methanol})$$

Elemental Analysis: $\text{C}_{34}\text{H}_{43}\text{N}_5\text{O}_{19}$

calculated: C 49.46; H 5.25; N 8.48

found : C 49.15; H 5.41; N 8.11

Mass Spectroscopy (FD): m/z 825 (M^+)

I.R. ν_{max} KBr (cm^{-1}): 3300, 1740, 1660, 1530

^1H NMR (CDCl_3) δ_{H} (TMS):

1.88 - 2.20 (21H, all s, NHAc and OAcx6)

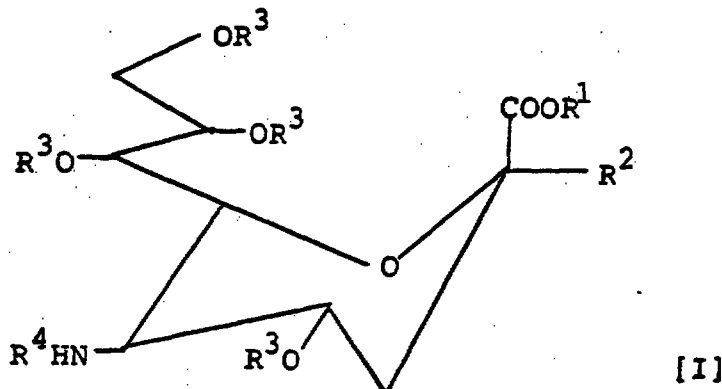
2.76 (1H, dd, $J=13.0$ and 4.5Hz , 3-Heq)

5 3.78 (3H, s, methylester), 5.95 (1H, s, $J=2.2\text{Hz}$,

1'-H), 8.20 (1H, s, 2-H), 8.44 (1H, s, 6-H)

Claims:

1. N-acetylneuraminic acid derivatives of the general formula [I]:



wherein R^1 represents a lower alkyl or a lower alkyl-substituted or non-substituted aralkyl or aryl group, R^2 stands for a nucleoside residue as hereinbefore defined or a glucose residue as hereinbefore defined, ^{the} groups R^3 represent a hydrogen atom or an acetyl group independently and R^4 represents an acetyl group.

2. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 5-fluoro-2',3'-isopropylideneuridine residue and each R^3 and R^4 is an acetyl group.

3. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 5-fluoro-2',3'-isopropylideneuridine residue, each R^3 is a hydrogen atom and R^4 is an acetyl group.

4. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 2',3'-di-O-acetylinosine residue, and each R^3 and R^4 is an acetyl group.

5. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is an inosine residue, each R^3 is a hydrogen atom and R^4 is an acetyl group.

6. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a



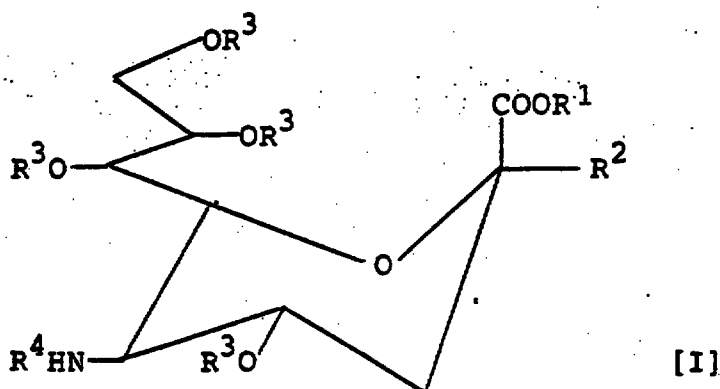
β -D-glucopyranosyl residue, each R^3 is a hydrogen atom and R^4 is an acetyl group.

7. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 1,2,3,4-tetra-O-acetyl- β -D-glucopyranosyl residue and each R^3 and R^4 is an acetyl group.

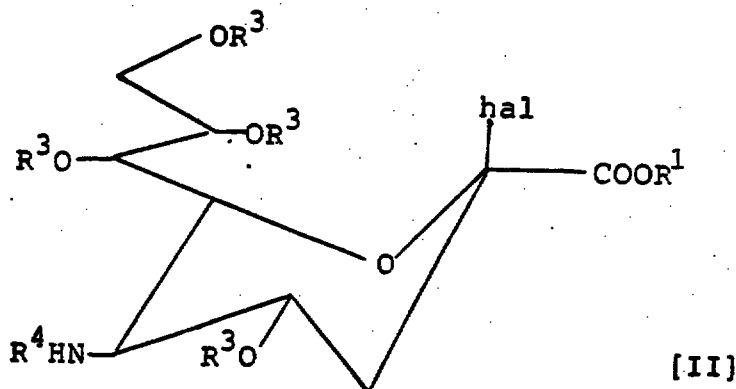
8. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 2',3'-isopropylideneuridine residue, each R^3 is a hydrogen atom and R^4 is an acetyl group.

9. An N-acetylneuraminic acid derivative as set forth in claim 1, wherein R^1 is a methyl group, R^2 is a 2',3'-isopropylideneuridine residue and each R^3 and R^4 is an acetyl group.

10. A process for preparing an N-acetylneuraminic acid derivative of the following general formula [I] :

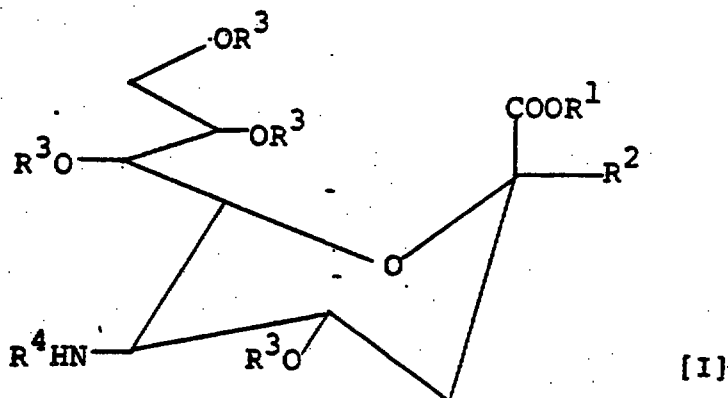


wherein R^1 , R^2 , R^3 and R^4 have the meanings given in claim 1, which comprises subjecting a compound of the general formula [II]:

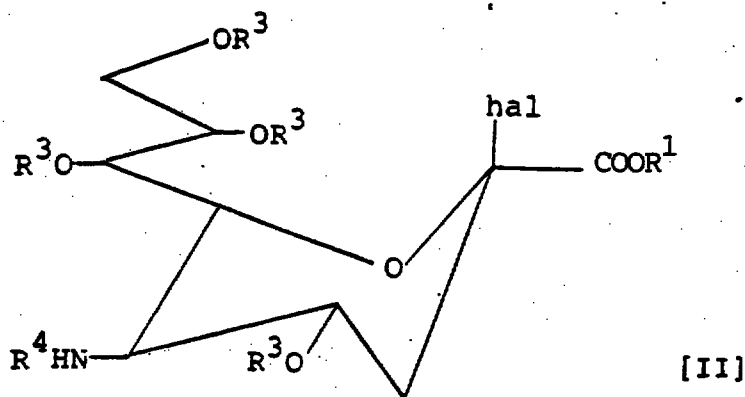


wherein R^3 and R^4 are all acetyl, R^1 has the aforesaid meaning and hal represents a halogen atom, and a nucleoside as hereinbefore defined or a glucose as hereinbefore defined to Koenigs-Knorr reaction, and optionally partially or completely deacetylating the resultant product.

11. A process for preparing an N-acetylneuraminic acid derivative of the general formula [I]:



wherein R^1 , R^2 , R^3 and R^4 have the meanings given in claim 1, which comprises esterifying N-acetylneuraminic acid, then simultaneously acetylating and halogenating the resulting product to form a compound of the general formula [II]:



wherein R^1 has the aforesaid meaning hal represents a halogen atom and R^3 and R^4 are all acetyl, then subjecting said compound [II] and a nucleoside as hereinbefore defined or a glucose as hereinbefore defined to Koenigs-Knorr reaction and optionally partially or completely deacetylating the resultant product.

12. A process as claimed in claim 11, wherein the simultaneous acetylation and halogenation is carried out by reacting the esterification product of N-acetylneuraminic acid with an acetyl halide.

13. A process as claimed in claim 12, wherein the acetyl halide is acetyl chloride.

14. A process as claimed in any one of claims 10 to 13, wherein R^1 of the compound II is a methyl group.

15. A process as claimed in any one of claims 10 to 14, wherein said nucleoside or glucose is selected from 5-fluoro-2',3'-isopropylidenuridine, 2',3'-isopropylideneuridine, 2',3'-di-O-acetylinosine and 1,2,3,4-tetra-O-acetyl- β -D-glucopyranose.

16. A process for preparing an N-acetylneuraminic acid derivative of the general formula I set out in claim 1, substantially as described in any one of the foregoing Examples 2 to 8, Example 9 (Method (i) or (ii)) or Example 10, optionally in association with Example 1.

17. An N-acetylneuraminic acid derivative, whenever produced by the process claimed in any one of claims 10 to 16.

