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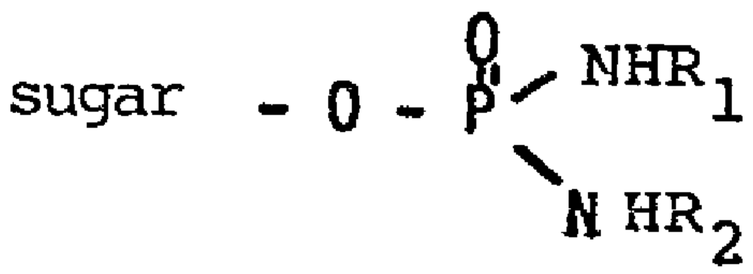
(54) **CONJUGATS DE SACCHARIDES INHIBITEURS DES TUMEURS**

(54) **TUMOUR-INHIBITING SACCHARIDE CONJUGATES**



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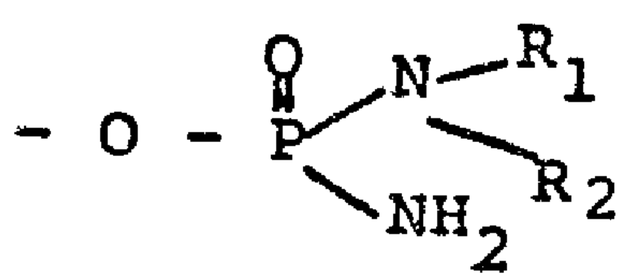
and sugar



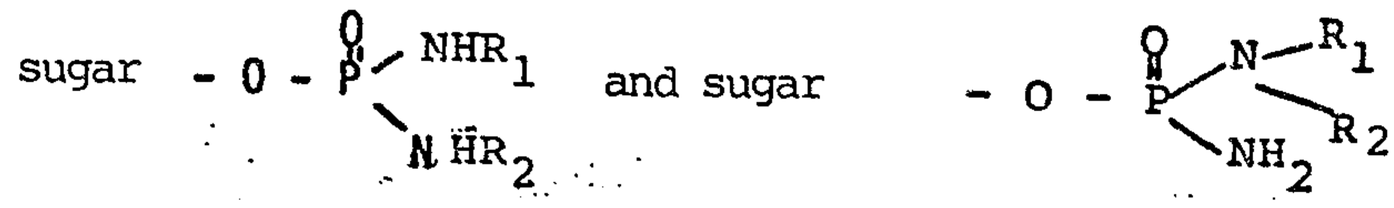
1a

(57) The invention relates to a method for the preparation of glyco-conjugates of phosphorus amides with the general formula (see formulas 1 and 1a) where the connection of the sugar with the phosphorus amide-lost residue, and the ifosfamide-lost residue, resp., occurs preferably in the 1-position, and where R_1 and R_2, which can be the same or different, denote hydrogen, lower C_1-C_4 alkyl or C_1-C_6 haloalkyl and where as sugar there can be present mono-, di-, or polysaccharides in all existing isomeric and enantiomeric forms, wherein in a known way protected bromium sugars are conjugated with the respective phosphorus compounds, and freed of the protective residues, and to the use of said compounds as anti-tumour drugs.

Tumour-inhibiting Saccharide Conjugates

ABSTRACT

The invention relates to a method for the preparation of glyco-conjugates of phosphorus amides with the general formula



1

1a

where the connection of the sugar with the phosphorus amide-lost residue, and the ifosfamide-lost residue, resp., occurs preferably in the 1-position, and where R_1 and R_2, which can be the same or different, denote hydrogen, lower C_1-C_4 alkyl or C_1-C_6 haloalkyl and where as sugar there can be present mono-, di-, or polysaccharides in all existing isomeric and enantiomeric forms, wherein in a known way protected bromium sugars are conjugated with the respective phosphorus compounds, and freed of the protective residues, and to the use of said compounds as anti-tumour drugs.

In the Federal Republic of Germany, mortality due to cancer ranks second, following cardiovascular diseases, in mortality statistics. Besides surgery and radiation, anti-neoplastic chemotherapy has nowadays become an established cancer therapy.

In spite of excellent surgical technique, improved radiation therapy, and many newly developed chemotherapeutic agents, in the last years it was not possible to improve the heilungsrate of malignant tumours, although very good results have been achieved with single kinds of tumours, as f.i. Hodgkin lymphoma (morbus Hodgkin).

There is, therefore, still a need to render possible fundamental improvements in chemotherapy, based on steadily increasing knowledge on the biochemistry of the tumour cell.

The main object in developing of new anti-neoplastic chemotherapeutic agents is to improve the selectivity, and thus to decrease undesired side effects.

Although meanwhile many biochemical differences between the tumour cell and the normal cell are known, these differences are not too significant.

Many of the agents used at present therefore already has a certain selectivity, and thereby an useful therapeutic index, but there is still a long way to go to obtain absolute selectivity.

One possibility to reach that goal is the use of "pro-drugs", i.e. drugs which are activated in a particular way at the site or inside the target cell, or which are detoxified with particular efficiency by non-target cells. Following another approach one tries to direct the drug to the site of or into the target cell or at least to enrich it there ("drug targeting").

Many concepts of drug targeting are based on a specific binding of a drug to the target cell or on different uptake mechanisms of

non-target and target cell. Also quantitative differences can be utilized in this respect.

By using the hybridoma technique (Köhler and Milstein, 1975, Nature 256:495) it is f.i. possible to produce specific monoclonal antibodies (MAB's) and with their help to recognize tumour-associated antigens (TAA's).

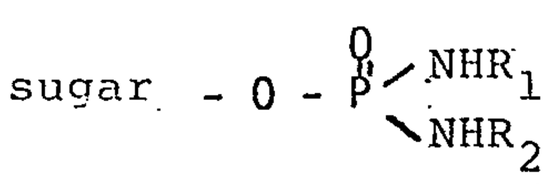
The glycoside esters to be prepared can be obtained by known methods, in particular by the further modified Koenigs-Knorr reaction or the imidate method.

A summary for such Methods, and of stereo-selective glycosylation, for which at present, depending on the stereochemistry of the linkage desired, there are three basic methods available, is given in particular in Paulsen, 1984, Chem. Soc. Rev. 13: 15.

It is known, though, that not every linkage desired can be prepared stereoselectively, despite the many glycosylation methods available. Every glycosyl transfer presents as a unique problem, and there are often no universal reaction conditions (Schmidt, 1986, Angew. Chem. 98: 213).

Therefore, the present inventions relates to glycoconjugates of certain, effective anti-tumour agents to be used as anti-neoplastic agents largely preserving the activity of those agents, but strongly deminishing their toxicity.

As typical examples, conjugates corresponding to the following general formula have been prepared:



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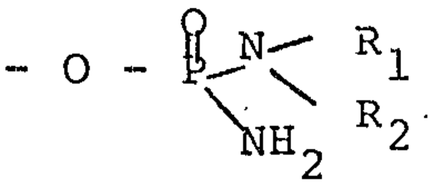


1a

where linkage of the sugar to the phosphoric acid amide-lost residue preferably occurs in the 1-position. Galactose, mannose, glucose, mannan, galactan, glucan, and branched-chain sugars, particularily in position 3 and 6, are to be named as most important sugars, while, however, basically all sugars can be employed.

R_1 and R_2 can be the same or different, and represent as follows: hydrogen, lower C_1-C_4 alkyl, C_1-C_6 halogenoalkyl, preferably C_1-C_4 halogenoalkyls, and in particular C_2 halogenoalkyl.

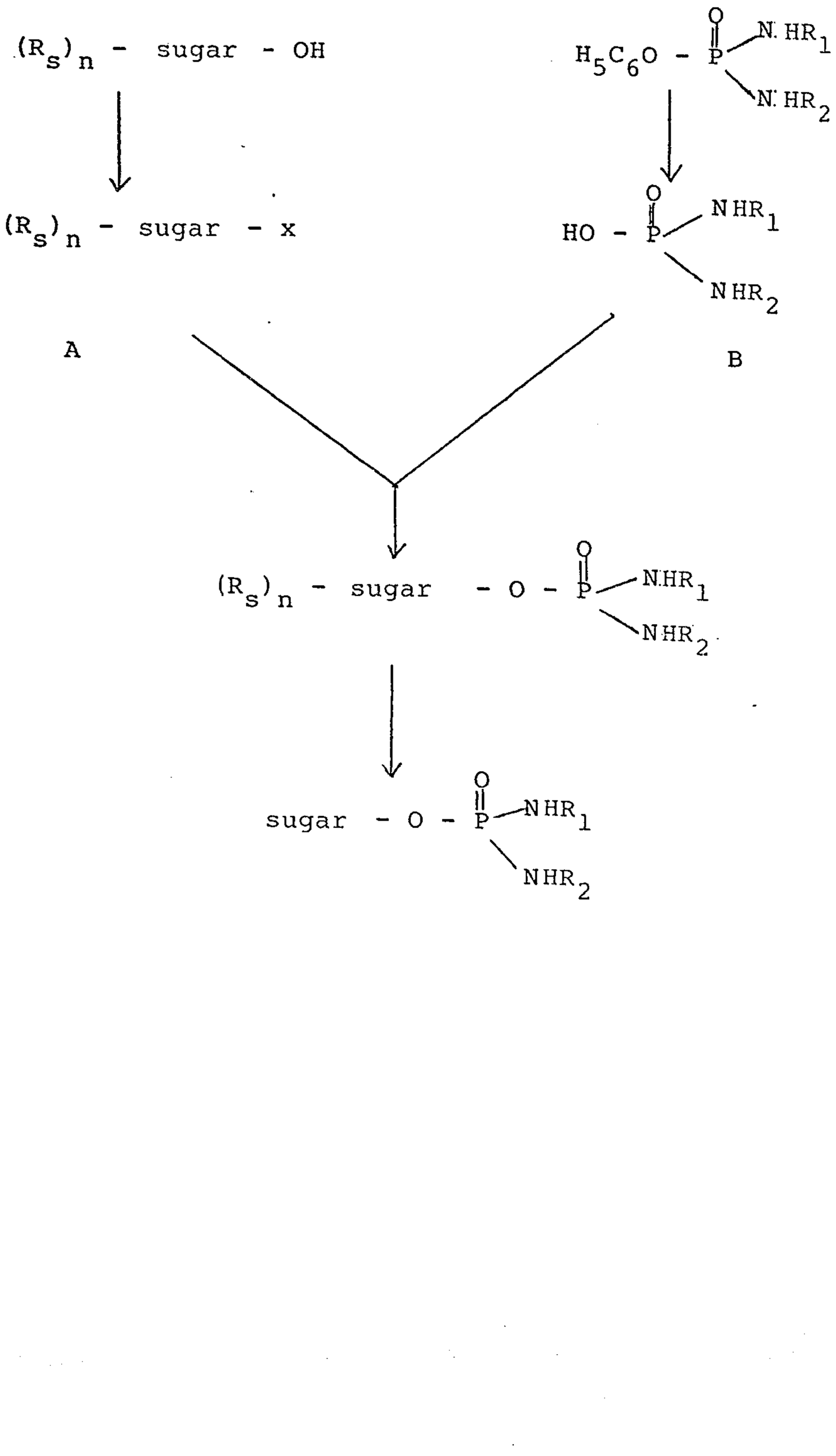
All protective groups customary with OH-groups can be employed as protective groups, f.i. benzyl, acetyl, trityl groups, and they are split off, too, in a known way, enzymatically, by hydrogenolysis, under acidic or alkaline conditions.

Generally, the glycosylically linked phosphamide mustard and ifosfamide mustard, desired and used according to the present invention, can be prepared as follows:

The units to be linked, the phosphorus compounds can be prepared according to the protocol by Lorenz and Wießler, 1985, Arch. Pharm. 318: 577.

Each of these two compounds can be obtained with protected brominated saccharides according to the protocol illustrated by the example of compound 28 β,glucose-β IPM, and the disaccharide compound 50 and 51, according to the accompanying figures 1 and 2, resp..

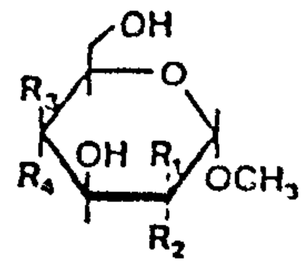
A protected sugar A is reacted with a phosphoric acid compound B by allowing both to react in a solvent, preferably a polar solvent, f.i. acetonitrile, CH_2Cl_2, or toluene, at a temperature ranging from 20 °C to 120 °C for a period from 1 h to 48 h, and with the addition of an auxiliary base, f.i. Et_3N, $Et(i\text{-}Prop)_2N$. The general protocol of the method can be depicted by the following schematic formula:



The reaction with ifosfamide occurred in a corresponding way.
R_s = a protective group, f.i. benzyl,
X = a reactive group, f.i. bromine.

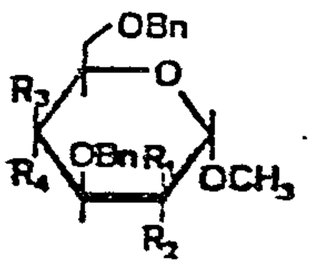
Starting from the 1-O-methyl pyranosides of glucose, galactose, and mannose, the corresponding 2,3,4,6-tetra-O-benzyl α-D-glycopyranosylbromides according to fig. 1 can be prepared.

Fig. 1. Synthesis if the benzylated bromoglycoses



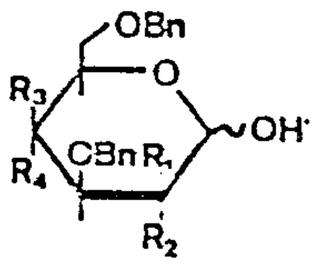
R_1	R_2	R_3	R_4	
H	OH	H	OH	Methyl-α-D-Glc
H	OH	OH	H	Methyl-α-D-Gal
OH	H	H	OH	Methyl-α-D-Man

BnCl
Base



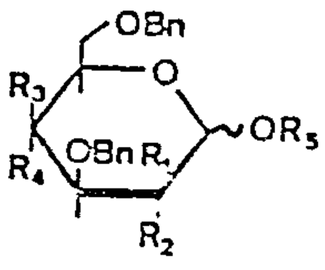
R_1	R_2	R_3	R_4	
H	OBn	H	OBn	10
H	OBn	OBn	H	11
OBn	H	H	OBn	12

H^+



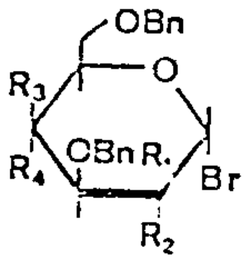
R_1	R_2	R_3	R_4	
H	OBn	H	OBn	13
H	OBn	OBn	H	14
OBn	H	H	OBn	15

p-NO_2BzCl
o.
Ph—N=C=O



R_1	R_2	R_3	R_4	R_5	
H	OBn	H	OBn	CO-Ph-NO_2	16
H	OBn	OBn	H	CO-NH-Ph	17
OBn	H	H	OBn	CO-Ph-NO_2	18

HBr



R_1	R_2	R_3	R_4	
H	OBn	H	OBn	19
H	OBn	OBn	H	20
OBn	H	H	OBn	21

The benzylation occurs in a known way, f.i. in dioxane with benzyl chloride in the presence of KOH for methyl α-D-glucopyranosid, and -galactopyranoside, resp., while methyl α-D-mannopyranoside is reacted, f.i., in benzyl chloride/NaH. The benzylated methyl glycosides 10, 11, and 12 are subsequently hydrolysed, f.i. with H_2SO_4/HOAc to give 13, and with HCl/HOAc to give 14 and 15, resp.. The compound 13 and the isomeric mannose 15 then can be derivatized to give the corresponding 2,3,4,6-tetra-O-benzyl 1-O-p-nitrobenzoyl D-glycopyranoses 16 and 18, both of which can be conveniently purified by recrystallisation. As this is not as easily possible for the p-nitrobenzoate of 2,3,4,6-tetra-O-benzylgalactose, in this case the derivatisation with phenyl isocyanate in pyrimidine to give 2,3,4,6-tetra-O-benzyl 1-O-(N-phenylcarbamoyl) D-galactopyranose 17, which can be crystallized, is preferred (Kronzer and Schuerch, 1974, Carboh. Res. 33: 273).

The benzyl-protected α-bromohalogenoses 19, 20, and 21, formed, f.i., by treatment with HBr in CH_2Cl_2, and after usual processing, namely filtering off p-nitrosobenzoic acid, and aniliniumbromide, resp., and removal of excess HBr, are suitably used directly and without any further purification in the subsequent glycosylation reactions.

The glycosyl donors protected by benzyl groups now do not have a substituent at C-2 which could exert an influence directing the glycosylation. The reaction of the brominated sugars provided with protective benzyl groups with phosphamide and isfosfamide, resp., occurred in dichloromethane/triethylamine. The yield of the reaction of 16, 17, and 18 to 22, 23, and 24, as well as the ratio of the anomeres and the eluent in the column chromatography is given in table 1. The separation as achieved by column chromatography.

Table 1

	Yield (%)	α:β	EE:PE
22	68	56:44	60:40
23	65	50:50	60:40
24	47	55:45	80:20

In the HPLC analysis the strong dependence of retention times on concentration as well as the significant tailing of the conjugates has to be noted. Ascertainment of structure and stereochemistry was done by ^{1}H and ^{13}C NMR spectroscopy.

For the preparation of larger amounts a technique was employed wherein by stereoselective synthesis one anomere is formed preferentially, thus making it possible to dispense with HPLC purification.

According to Schmidt, 1986, Angew. Chem. 98: 213, benzyl-protected O-glycosyl trichloroacetimidates can be employed for stereoselective syntheses. Synthesis of the was performed according to methods described in the literature, starting from the tetra-O-benzylglycoses 13, 14, and 15.

Using potassium carbonate as base, the β-imidate 25β is obtained in a cinetically controlled reaction, with NaH a fast anomerization giving the thermodynamically more stable 25α is achieved. In an analogous manner, the galactosyl imidate 26α is obtained from 14, the mannosyl imidate 27α from 15. Compared to the bromine-activated benzyl glycosides these imidates have the

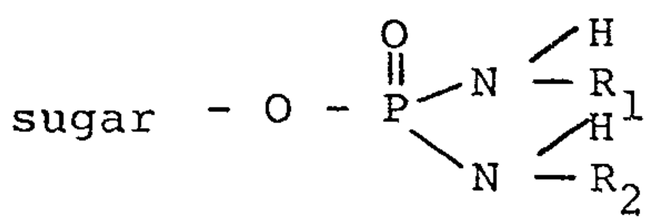
advantage of greater stability; the can readily be recovered and stored.

Thus the imidates were reacted with IMP 4b under different reaction conditions. F.i. dichloromethane or acetonitrile were used as solvents, and BF_3, diethylether or HCl in dichloromethane as catalysts. The reaction occurred faster in CH_3CN than in CH_2Cl_2. The mannosyl imidate 27α reacted selectively with 4b to give mannoside 24α.

The protected glycosides can be deprotected (freed of their protective groups) f.i. by catalytic hydrogenation with Pd/activated charcoal at room temperature. The course of the hydrogenation can be monitored with thin-layer chromatography. The detection can be done with methanolic sulfuric acid, however, detection with 4-(p-nitrobenzyl) pyridine reagent (NBP) is more sensitive.

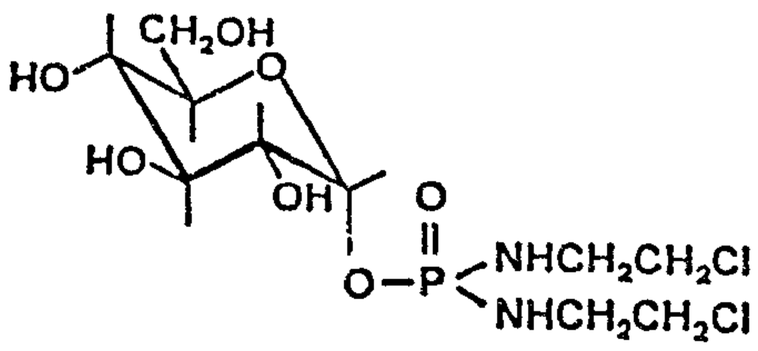
After the reaction was completed the catalyst was removed by filtration, the filtrate was concentrated by rotation evaporation, and dried under high-vacuum. When using the pure anomeric glycosides 22, 23, and 24, the corresponding glycosides 28, 29, and 30 were obtained as pure anomeres, too, (fig. 2).

The corresponding phosphamide conjugates, as well as the conjugates of the derivatives according to the formula:



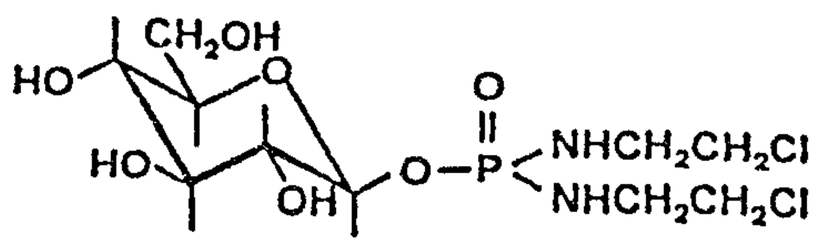
can be obtained in a corresponding manner.

Fig. 2: 6 de-protected diastereomeric glycosyl-IPM conjugates



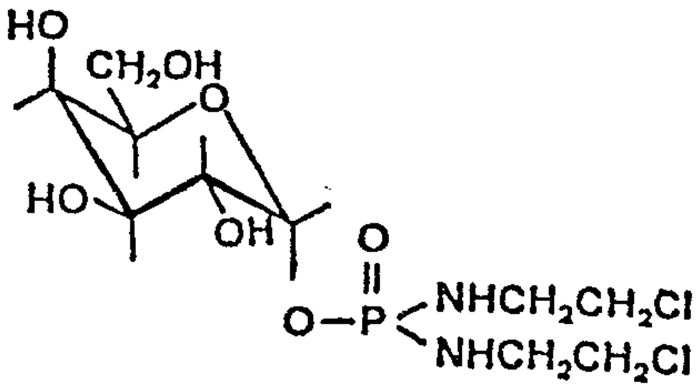
28α

Glc-α-IPM



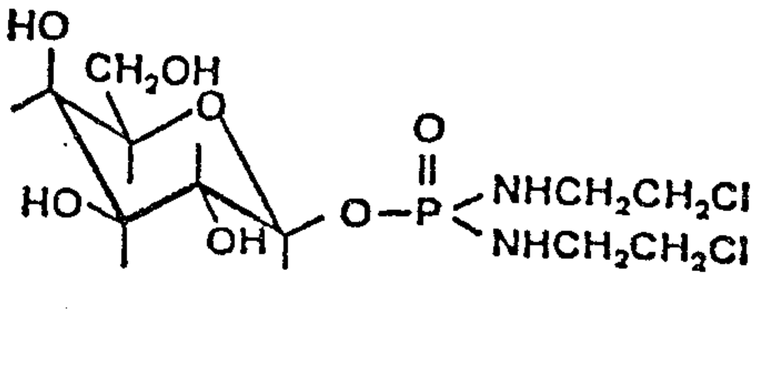
28β

Glc-β-IPM



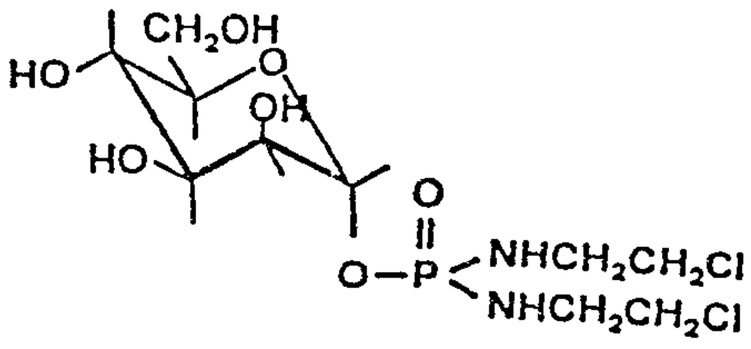
29α

Gal-α-IPM



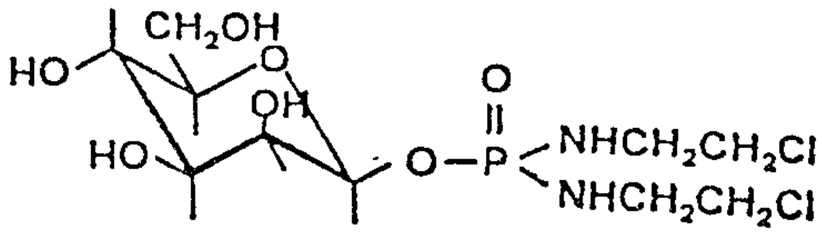
29β

Gal-β-IPM



30α

Man-α-IPM



30β

Man-β-IPM

Purification of the products forming as clear, colourless, highly viscous oils is not necessary. Should side-products be formed during hydrogenation, anyway, these can be removed by short column chromatography on silica (acetonitrile/methanol mixtures).

HPLC analysis permits to ascertain anomere ratios in the case of glucoside 28 and mannoside 30.

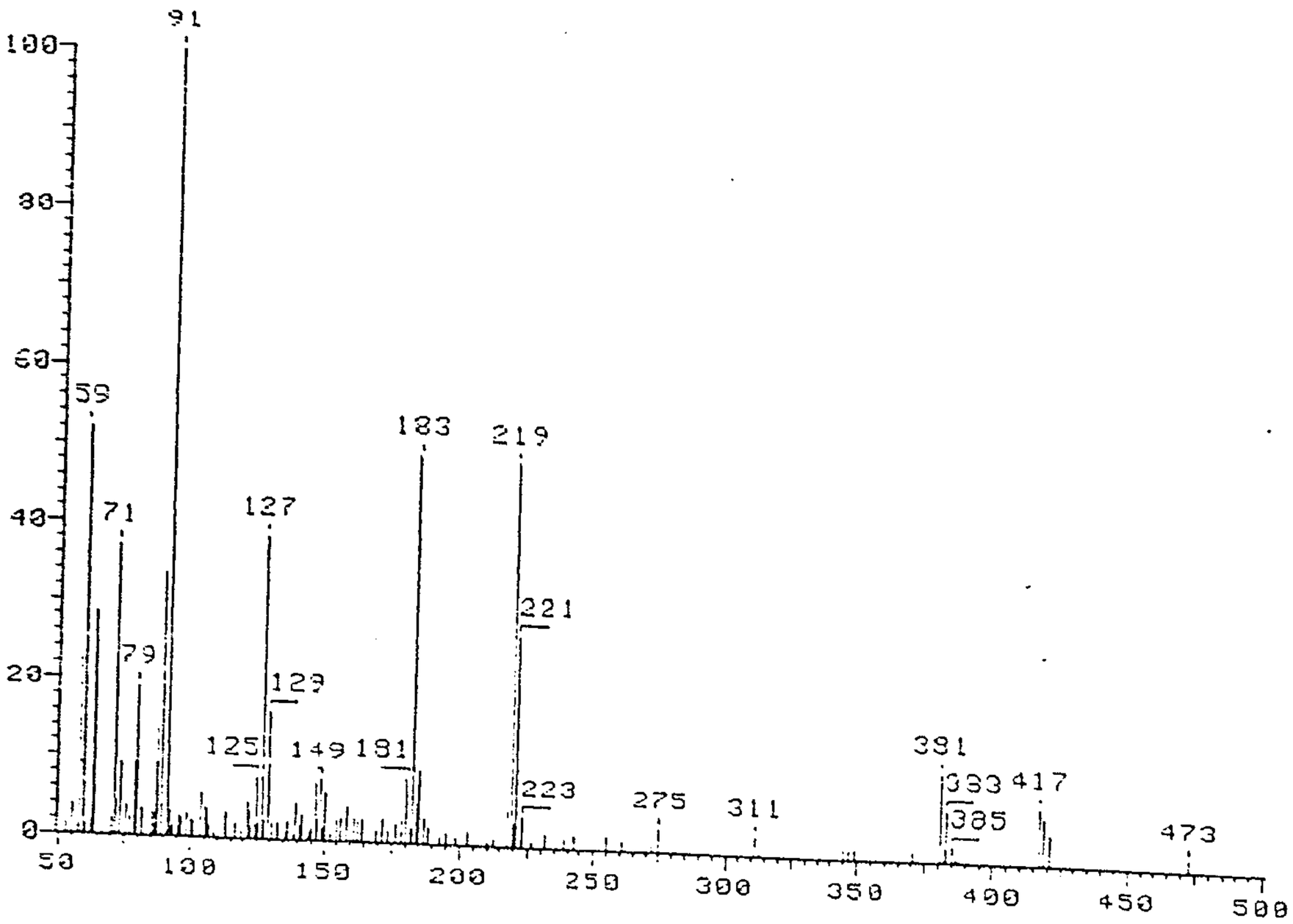
Table 2: Reactions of the trichloroacetimidates with IPM

starting compound	solvent	reaction condition	product	yield	$\alpha:\beta$
25α	CH_2Cl_2	RT, 1 d	22	-	
		RF, 6 h		56%	1:6
	CH_3CN	RT, 1 d		low	
		RF, 6 h		42%	1:20
25β	CH_2Cl_2	RF, 4h		51%	2:3
	CH_3CN	RF, 6h		45%	1:1.1
26α	CH_2Cl_2	RT, 4 h	23	-	
		RF, 8 h		48%	2:5
	CH_3CN	RF, 6h		42%	1:3
27α	CH_2Cl_2	RT, 4 h	24	-	
		RF, 4 h		48%	
	CH_3CN	RF, 6h		47%	only α

RT = room temperature

The identity of the compounds could be ascertained by FAB-MS and ^{1}H-NMR spectroscopy. Moreover, the configuration of the sugar residues was confirmed by enzymatic reactions. As an example, the FAB spectrum (negative) of Glc-β-IPM is given in fig. 3.

Fig. 3



Using glycerol as a matrix, negative as well as positive FAB spectra gave significant information. From all 6 compounds signals of the negative molecule-peak ions $(M-H)^-$, and the positive molecule-peak ions $(M+H)^+$ could be seen. In each case there occurred 3 peaks in a characteristical ratio, a phenomenon caused by the fact that the molecule contains 2 chlorine atoms, and that chlorine naturally occurs not as one pure isotope but as ^{35}Cl and ^{37}Cl in a ratio of 3:1, resulting in a ration of the isotope peaks of appr. 9:6:1. For the ions $(M+H)^+$ the expected values for m/e = 383, 385, 387, for $(M-H)^-$ the corresponding values m/e = 381, 383, and 385 are found. A characteristical fragment ion, namely that of the alkylating aglycone IPM was definitely shown with the signals m/e = 221, 223, 225 for $(IPM+H)^+$, and m/e = 219, 221, and 223 for$(IPM-H)^-$; here, too, the typical distribution of the isotope peaks is found. In fig. 4, the two triplets of the ions $(M-H)^-$ and $(IPM-H)^-$ of Glc-β-IPM 28β are clearly visible. The signals m/e = 91 and 183 are derived from the glycerol matrix used.

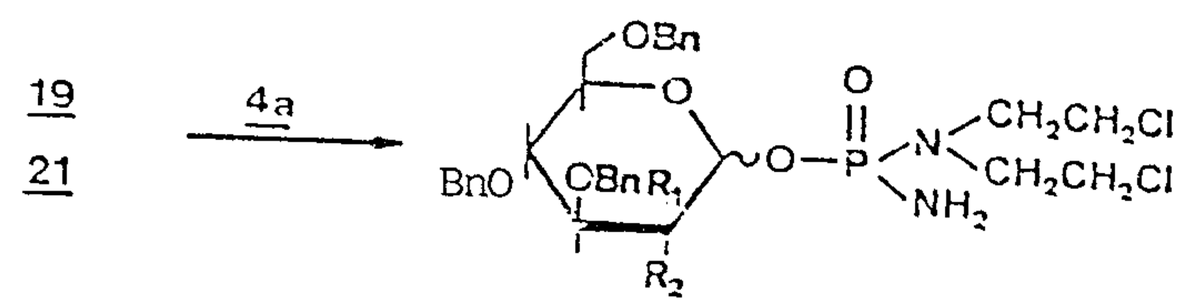
The attribution of the configuration at the anomeric centre, as for the protected starting compounds, was possible with ^1H-NMR by the typical couplings of the proton H-1 and its chemical shift; table 3 summarizes these parameters.

The PM 4a, isomeric with IPM, reacted with 2,3,4,6-tetra-O-benzyl α-D-glucopyranosid 19 and the respective mannosyl donor 21 in dichloromethane/triethylamine to give 2,3,4,6-tetra-O-benzyl D-glucopyranosyl N,N-bis-(2-chloroethyl) phosphoric acid diamide 31, and the 2-epimeric 32, resp., (fig. 4).

Table 3

Chemical shifts and coupling of the anomeric proton of the de-protected monosaccharide-IPM conjugate

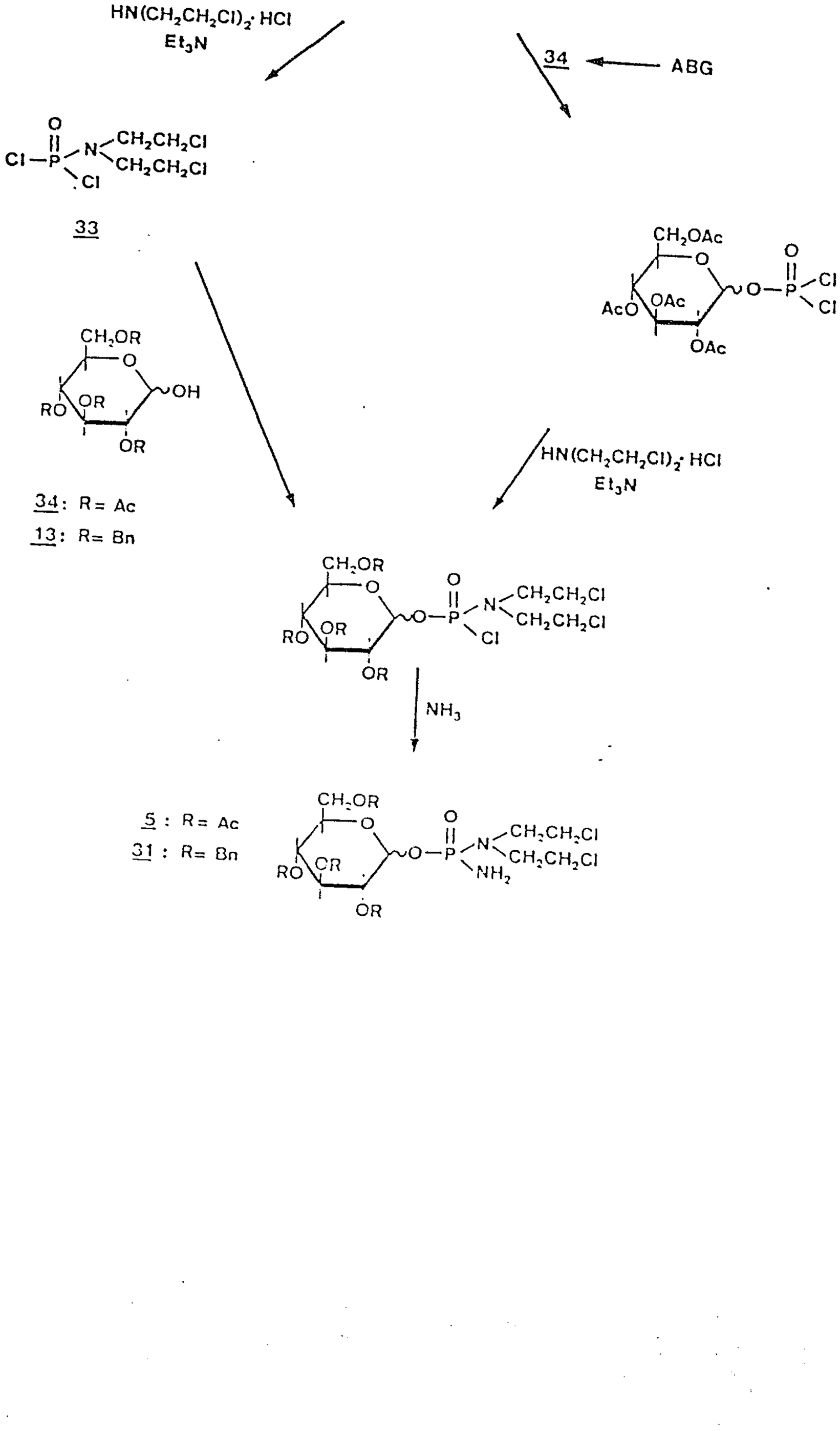
	δ(H-1)	$J_{1,2}$	$J_{1,P}$
<u>28α</u>	5.605	3.4	7.8
<u>28β</u>	5.004	8.0	8.0
<u>29α</u>	5.642	3.1	7.8
<u>29β</u>	4.950	8.0	8.0
<u>30α</u>	5.564	2.0	8.0
<u>30β</u>	5.282	1.1	8.6



	R_1	R_2
<u>31</u>	H	OBn
<u>32</u>	OBn	H

Fig. 4: Glycosylation of PM <u>4a</u>

Still other ways of synthesis are possible for the preparation of the glycosyl-PM conjugates 5 or 31, as is shown in fig. 5.



In an analogous way (examples 1 and 2) via the hepta-O-benzylglycoses 43 and 44

	R_1	R_2
43	OBn	H
44	H	OBn

the disaccharide-IPM conjugates 50 and 51 were obtained.

	R_1	R_2
50	OH	H
51	H	OH

Table 4 shows the ^{1}H-NMR data of the de-protected disaccharide conjugates.

Table 4

	δ(H-1)	$J_{1,2}$	$J_{1,P}$	δ(H-1')	$J_{1',2'}$
50α	5.623	3.6	7.8	4.478	8.0
50β	5.049	8.0	8.0	4.473	8.0
51α	5.622	3.6	7.8	4.537	7.9
51β	5.044	8.0	8.0	4.532	8.0

Here, too, the identity of all four diastereomeric compounds was ascertained by 2D-NMR-COSY. Fig. 6a/6b show as representative examples the 2D spectra of the de-protected cellobiose-IPM conjugate 51α and 51β.

Fig. 6a: 500 MHz ^{1}H-^{1}H-2D-NMR spectrum of the cellobiose conjugate 51α

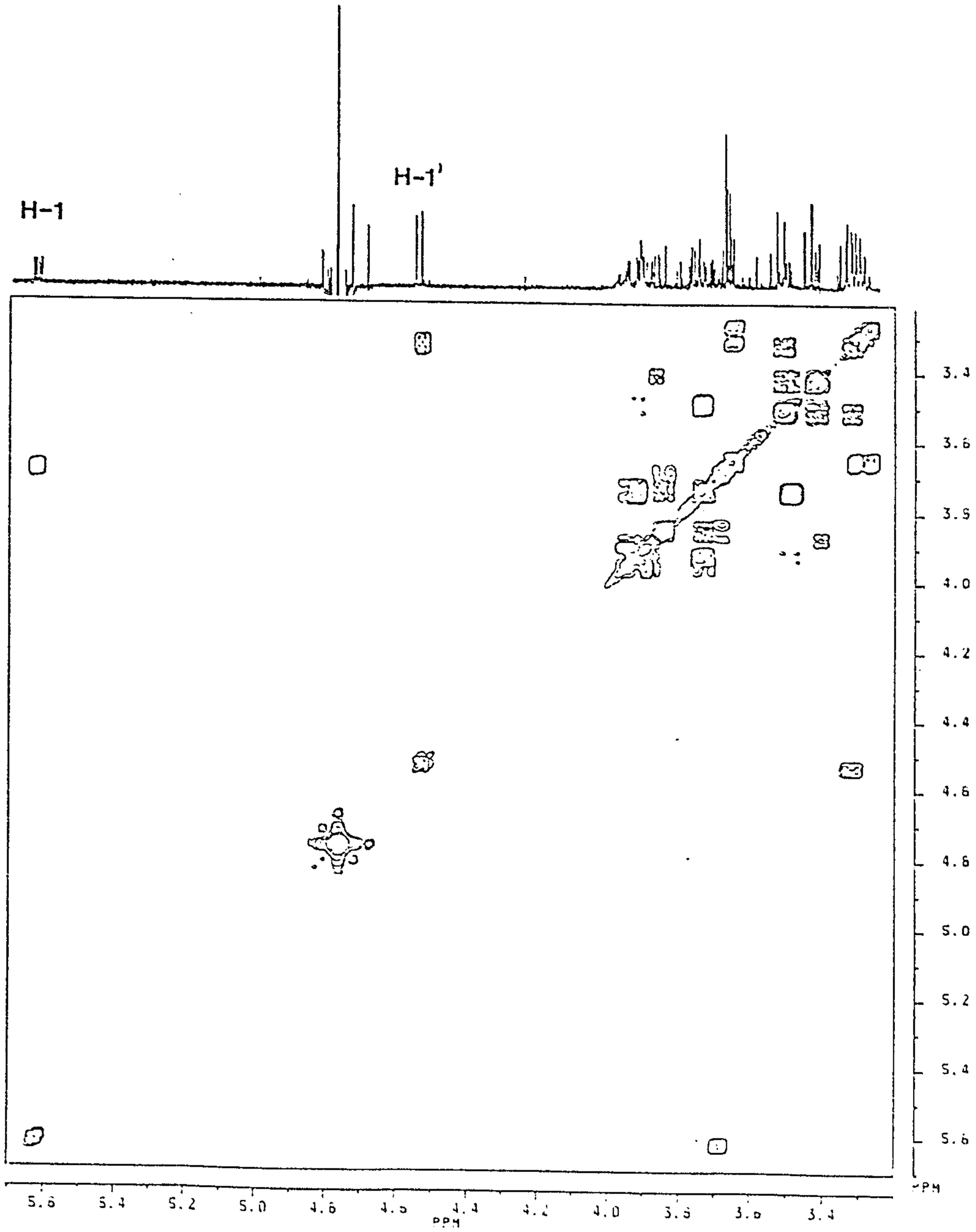
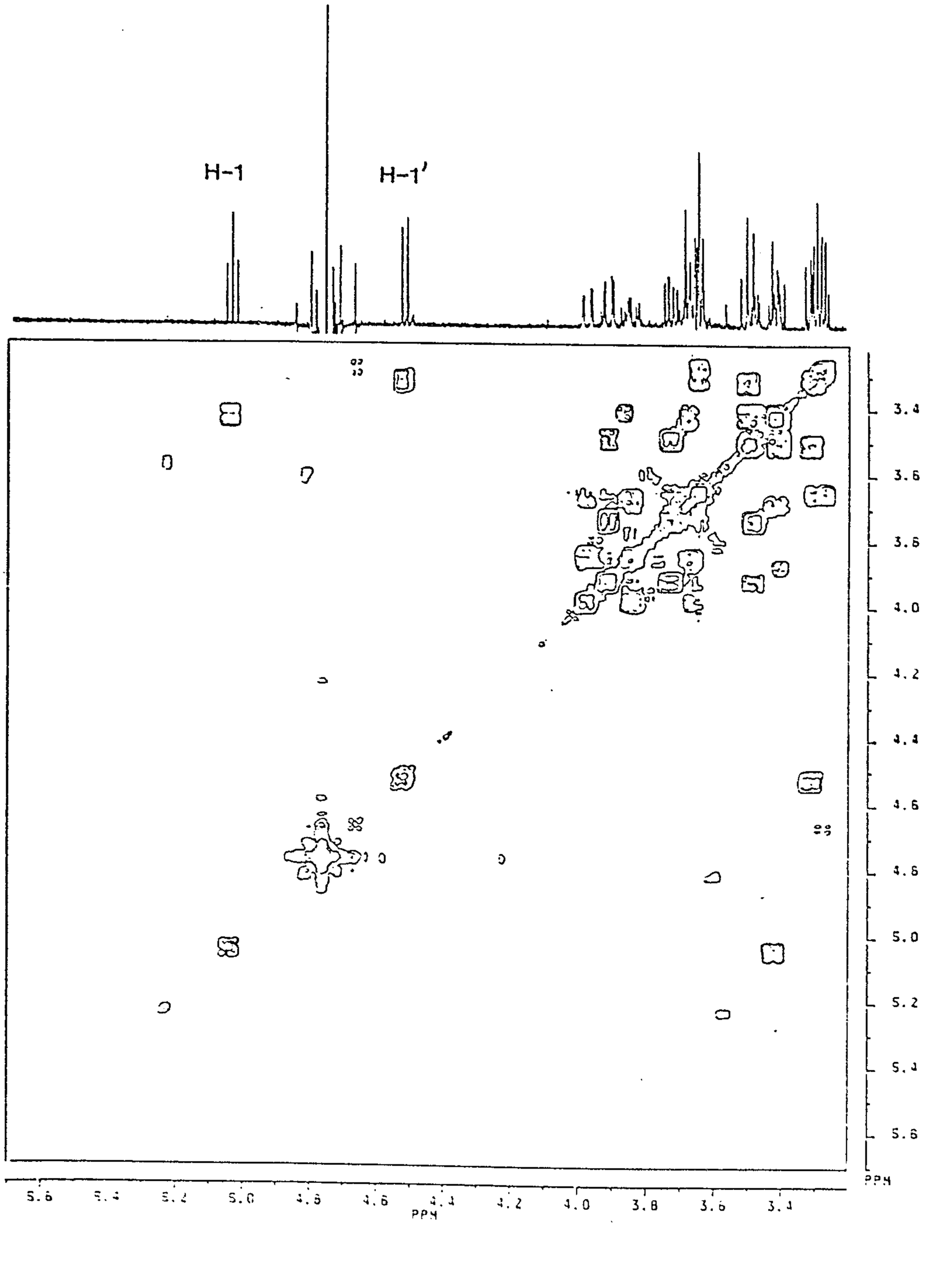


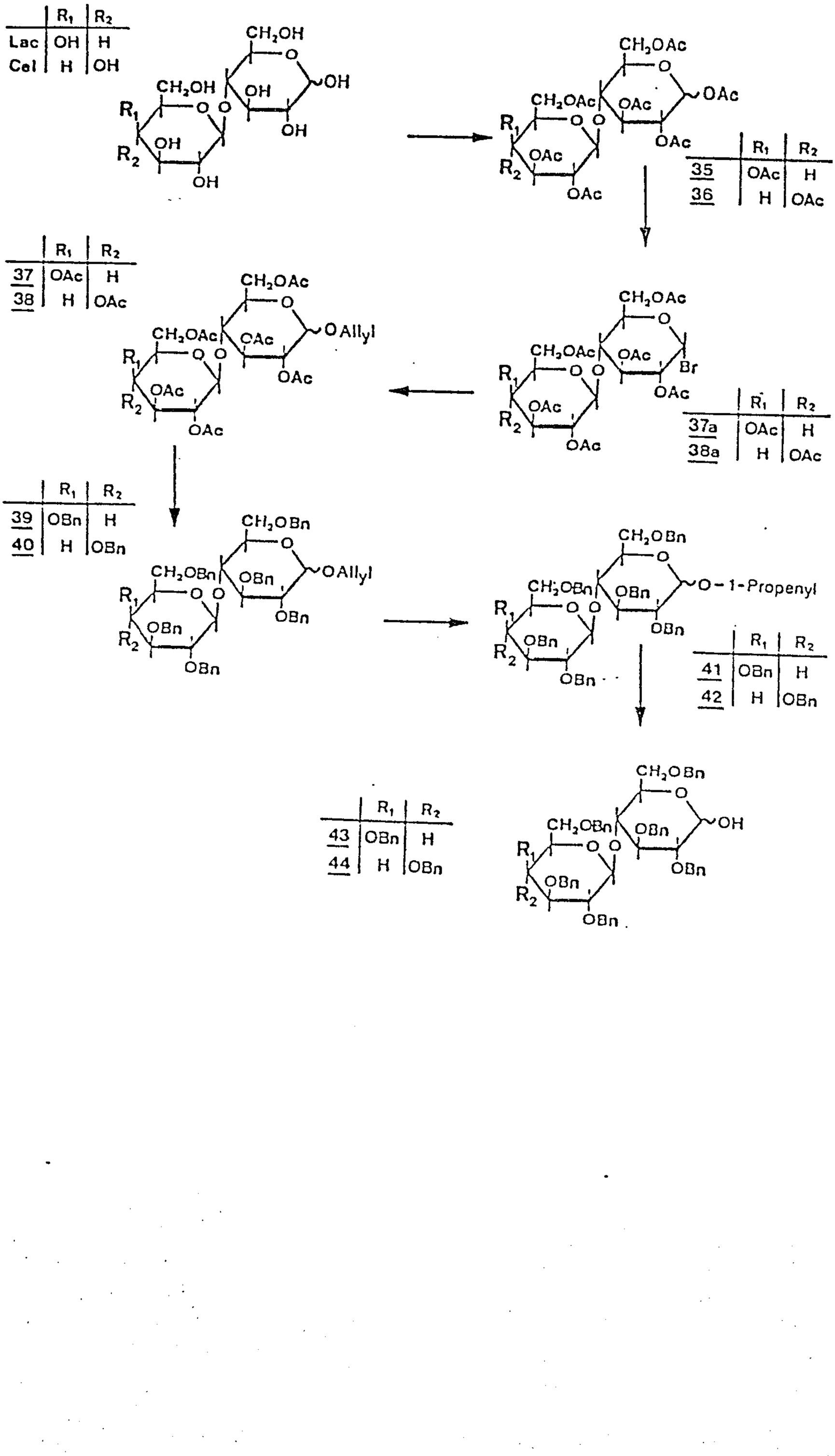
Fig. 6b: 500 MHz ^1H-^1H-2D-NMR spectrum of the cellobiose conjugate 51β



Also in this case the structure of the sugar residue, i.e. lactose and cellobiose, resp., was confirmed by enzymatic reactions.

In the construction of disaccharide-coupled IPM conjugates the use naturally occurring disaccharides, such as lactose or cellobiose, as the starting material, as opposed to constructing the conjugate from mono-sugar, has the advantage that the glucosidic bond between the sugars is already there, and thereby one step of stereoselective synthesis can be saved. Thus, the synthesis protocols described above are also used for the preparation of disaccharide-coupled IPM conjugates. First, a benzyl-protected disaccharide was necessary, the 1-O-position of which could be activated either with hydrogen bromide or with trichloroacetonitrile. In this way, based on the method of S. Koto (Koto et al., 1982, Nihon-Kagakkai: Nihon-Kagaku-Kaishi (J. Chem. Soc. Jpn.) 10: 1651), the disaccharide components 2,3,6,-2',3',4',6'-hepta-O-benzyl lactose and the isomeric cellobiose derivative 44 were synthesized (fig. 7).

Fig. 7


Lac OH H
Cel H OH
35 OAc H
36 H OAc
37 OAc H
38 H OAc
37a OAc H
38a H OAc
39 OBn H
40 H OBn
41 OBn H
42 H OBn
43 OBn H
44 H OBn
Allyl
1-Propenyl
Br

On incubation of the glycosides in HEPES or water with corresponding glycosidases a rapid cleavage of the glycosidic bond could be observed.

The conjugates Lac-IPM 50 and Cel-IPM 51, proved stable at room temperature in methanolic solution, just as the monosaccharide conjugates 28 - 30 (TLC analysis). Because each in 50 and 51 two glycosidic bonds are present, showing the same (51β) or a different configuration (f.i. 50α), along with single glucosidases enzyme mixtures were used to check the enzymatic release of IPM. The enzymatic cleavage of the pure anomeric conjugates 50 and 51 was monitored by TLC; intact conjugates or the cleavage products 28α, 28β or IPM were detected with NBP.

All conjugates were rapidly cleaved by suitable glycosidases and can release f.i. the metabolite of the general formula 1, and 1a, resp..

In the case of the disaccaride conjugates 50 and 51α cleavage of two different glycosidic bonds was required for the release of IPM. Only 52β, showing two bonds with the same configuration, could be completely cleaved by a single enzyme, β-glucosidase.

This was confirmed by measuring the biological efficiency, namely the cytotoxic activity in vitro by studies done on a murine retrothelial sarcoma, and on rat mammary tumour cell lines, and on Eb/Esb cell lines.

In vivo studies were done with the P388 leukemia in the mouse and the rat 1C32 tumour. Results of the in vitro and in vivo studies are found in fig. 8 to 13.

Fig. 8: Proliferation of the RES culture and long-term incubation with 28α (a) and 28β (b).

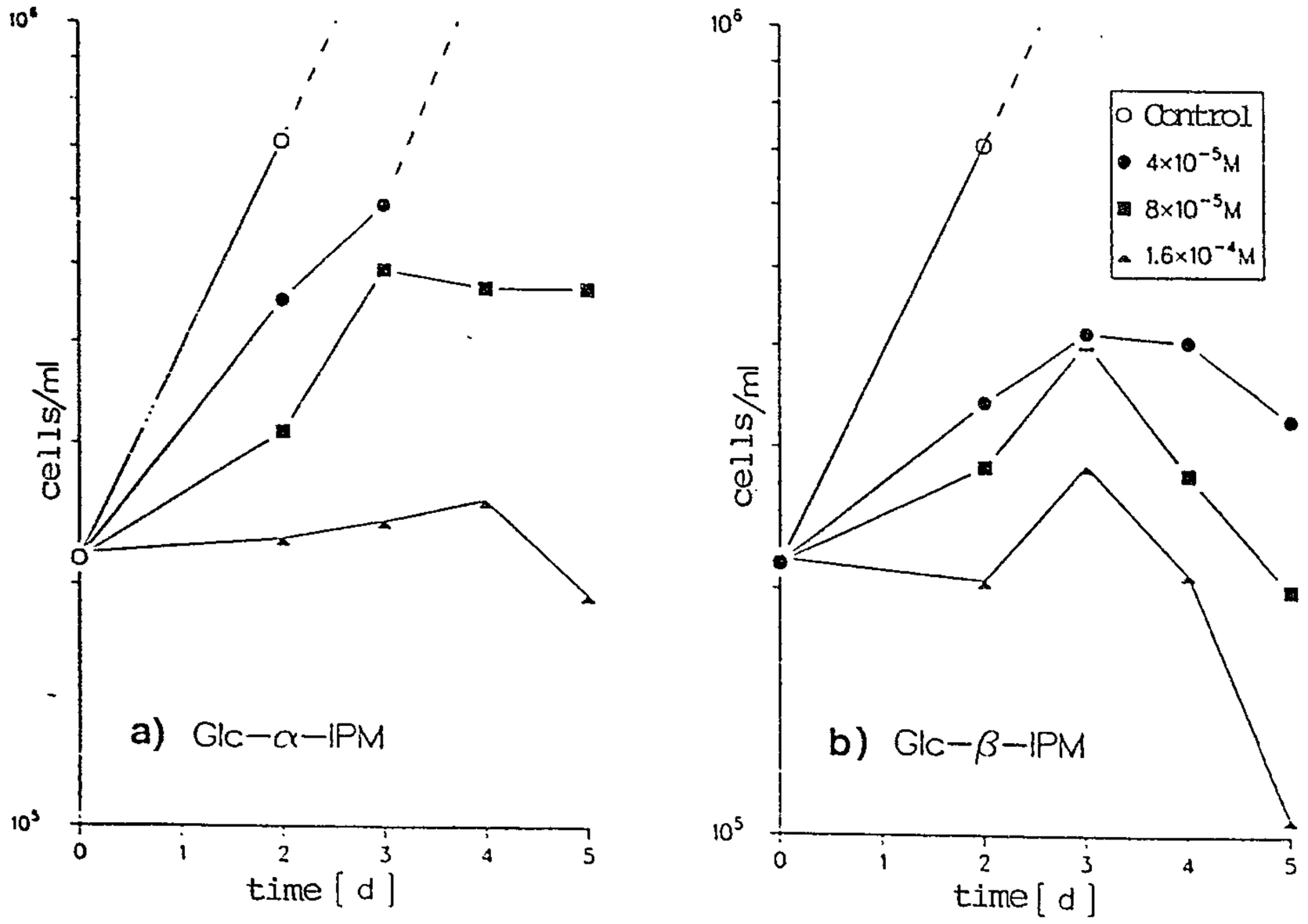
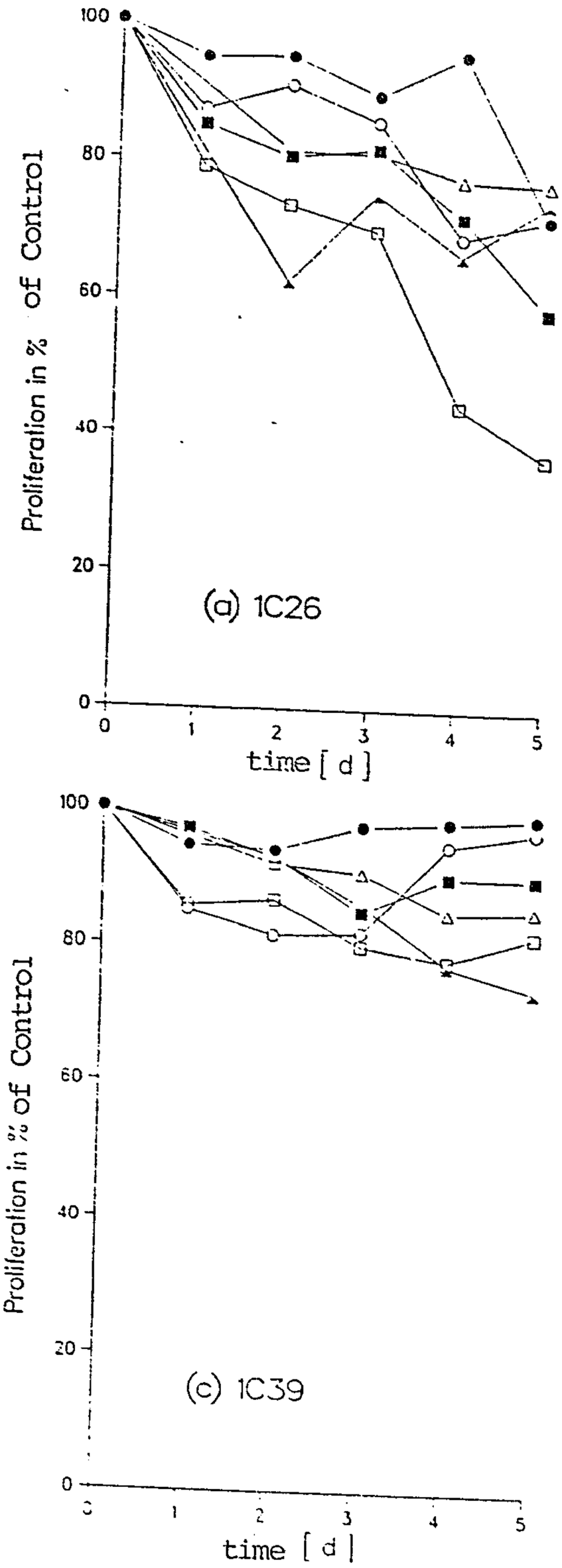
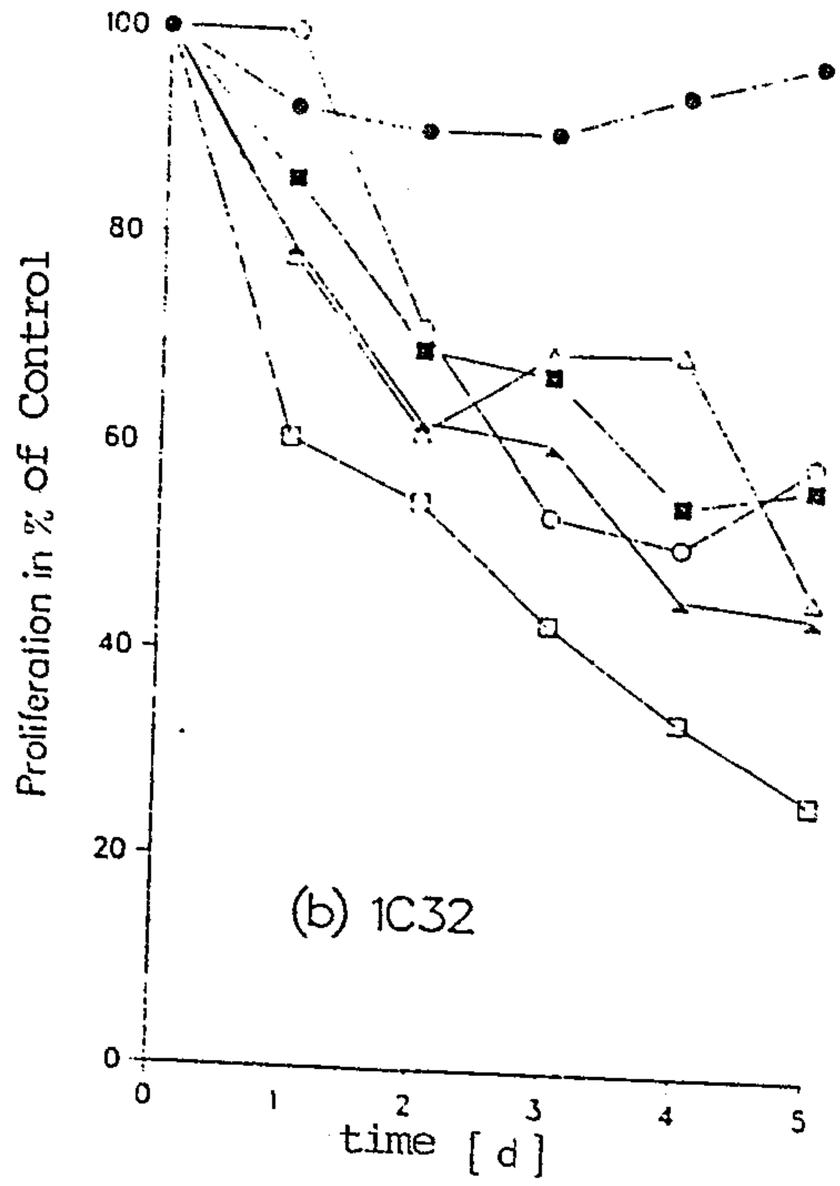


Fig. 9: Proliferation of mammary tumour cell-lines 1C26 (a), 1C32 (b), and 1C39 (c) after incubation with $8 \cdot 10^{-5}$ M monosaccharide-Imp conjugates 28 - 30 for 2 h





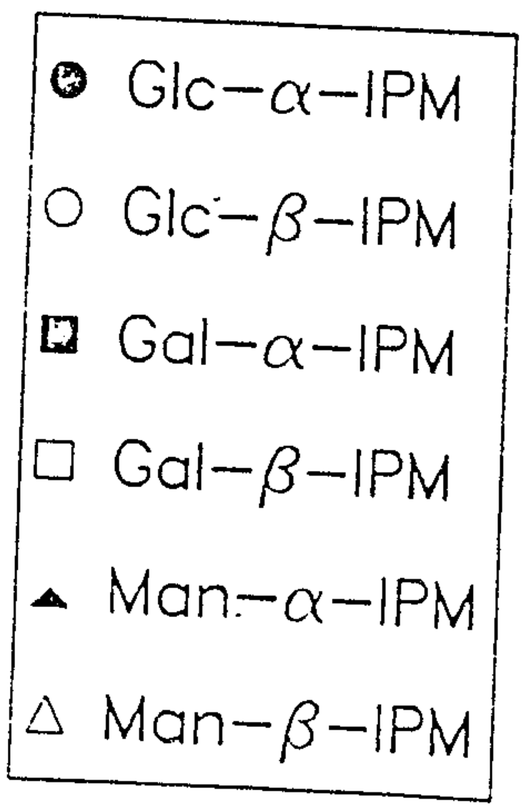
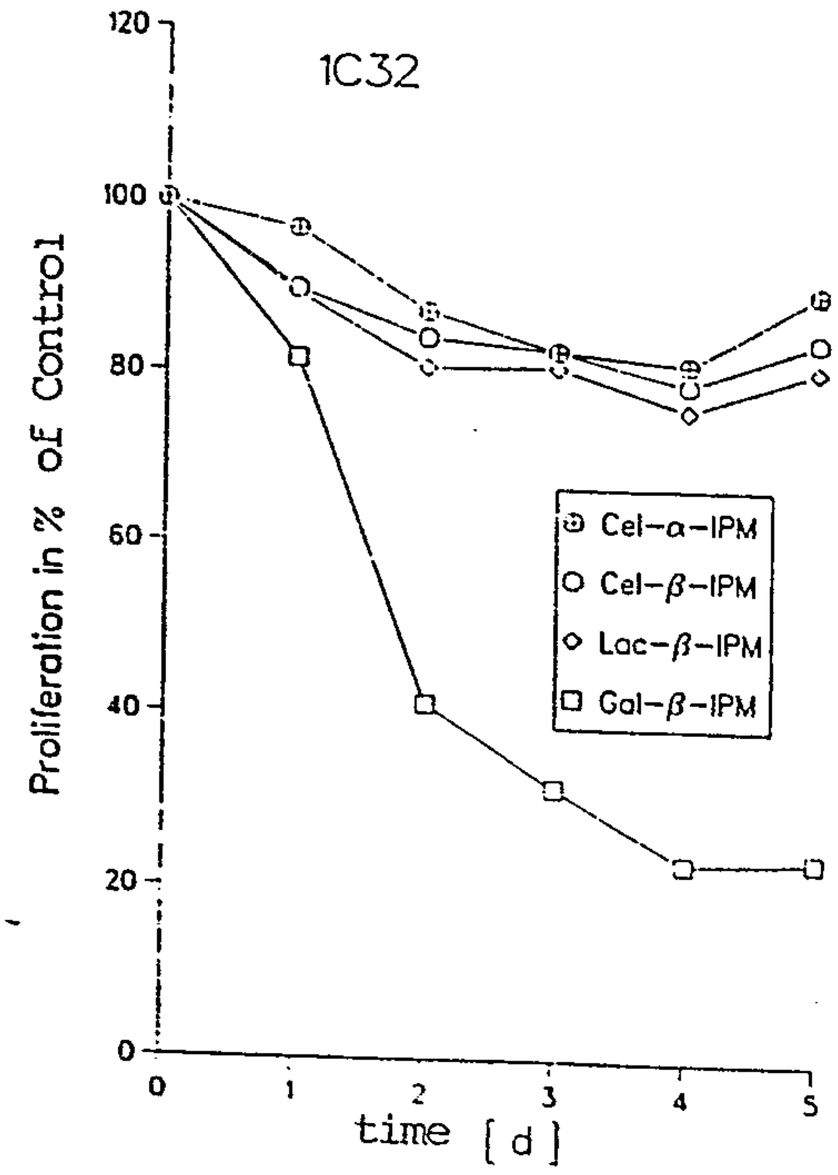


Fig. 10: Proliferation of mammary tumour cell-line 1C32 after incubation ($8 \cdot 10^{-5}$ M) with the disaccharide conjugates 50 and 51 for 2 h



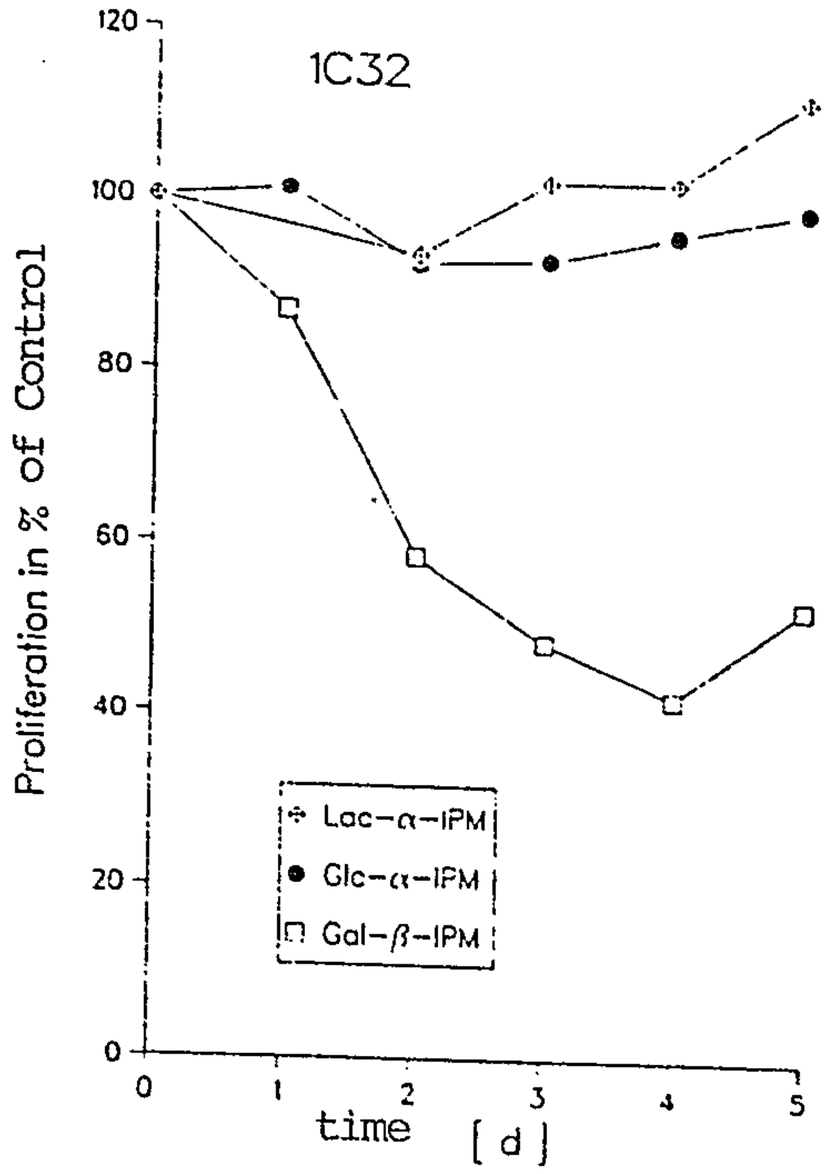
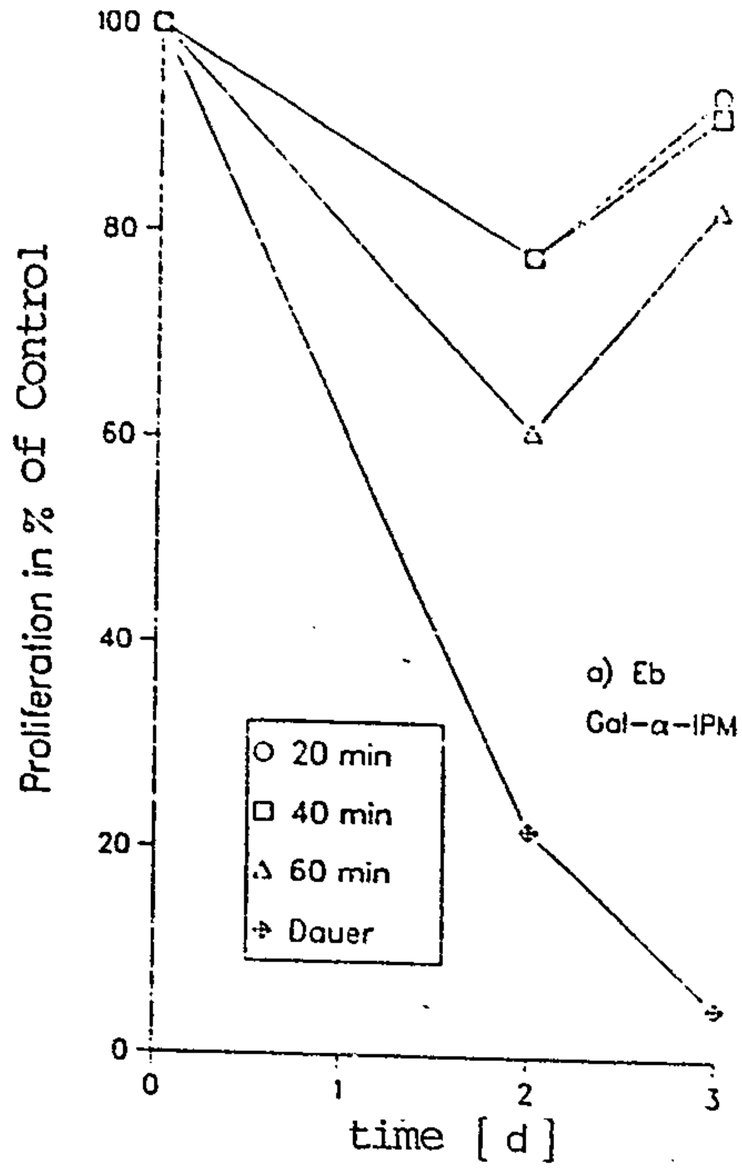
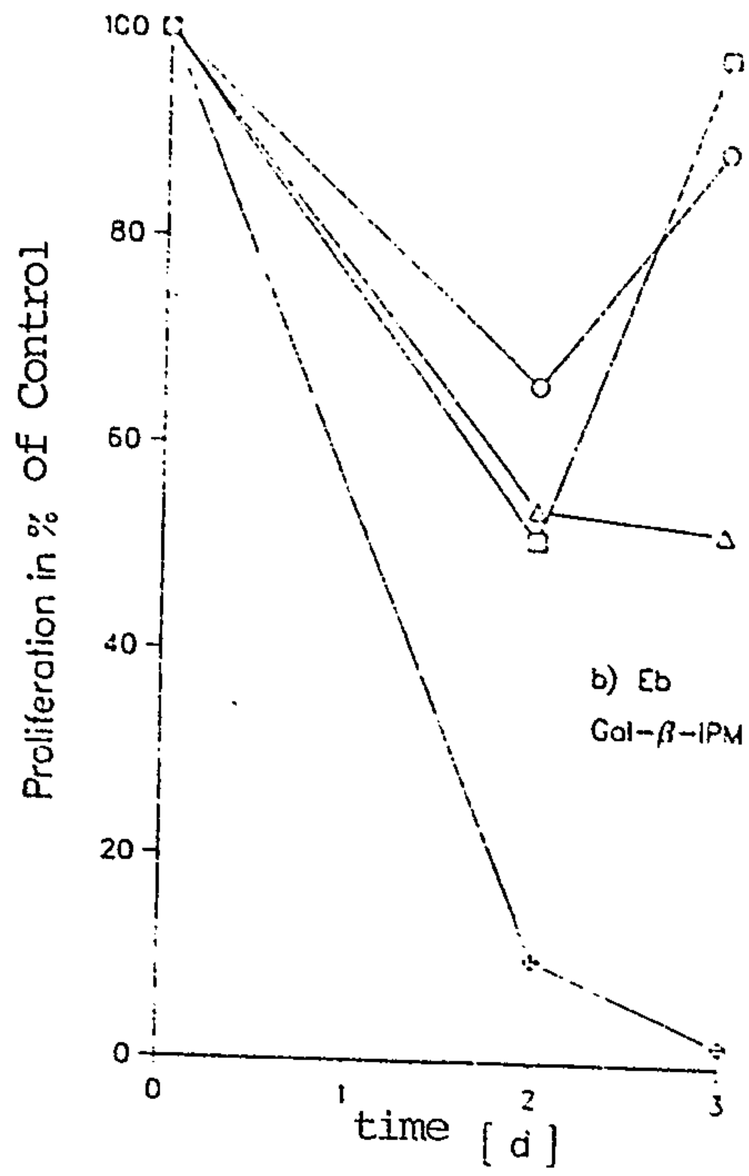
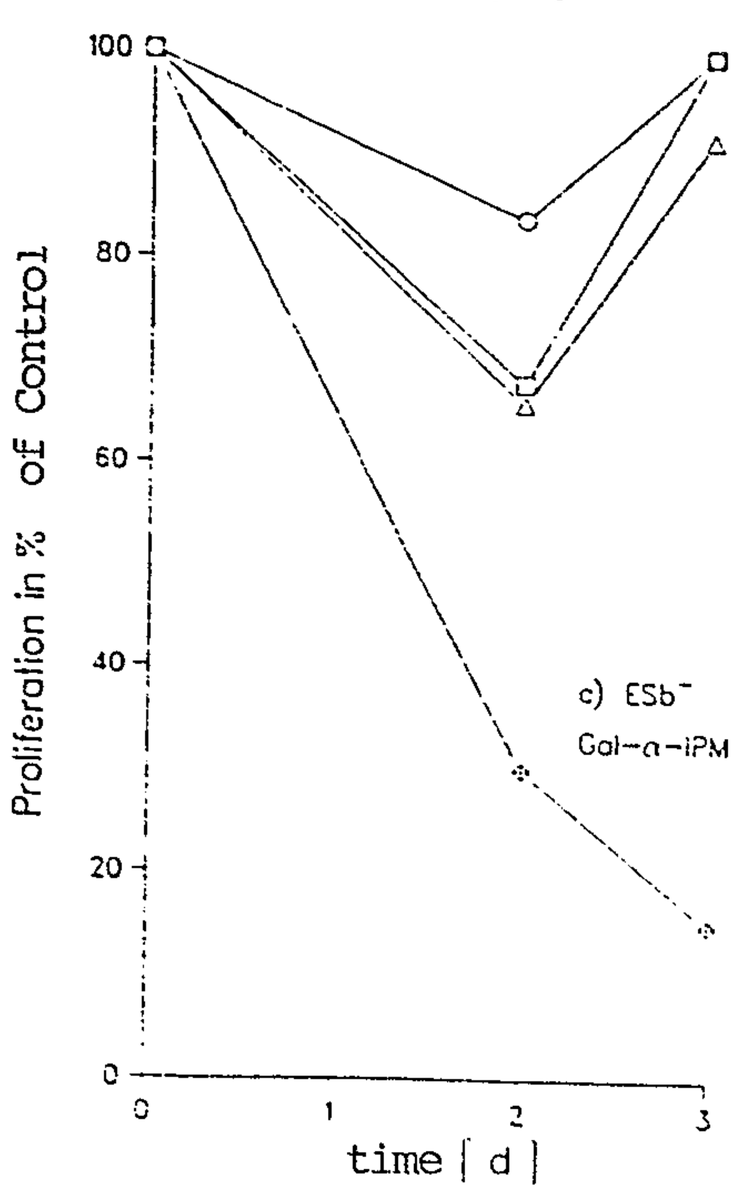


Fig. 11: Dependence of proliferation of Eb (a and b), and ESb- (c and d) on incubation time; incubation was performed with Gal-α-IPM (a and c), and Gal-β-IPM (b and d), resp., at $8 \cdot 10^{-5}$ M each.







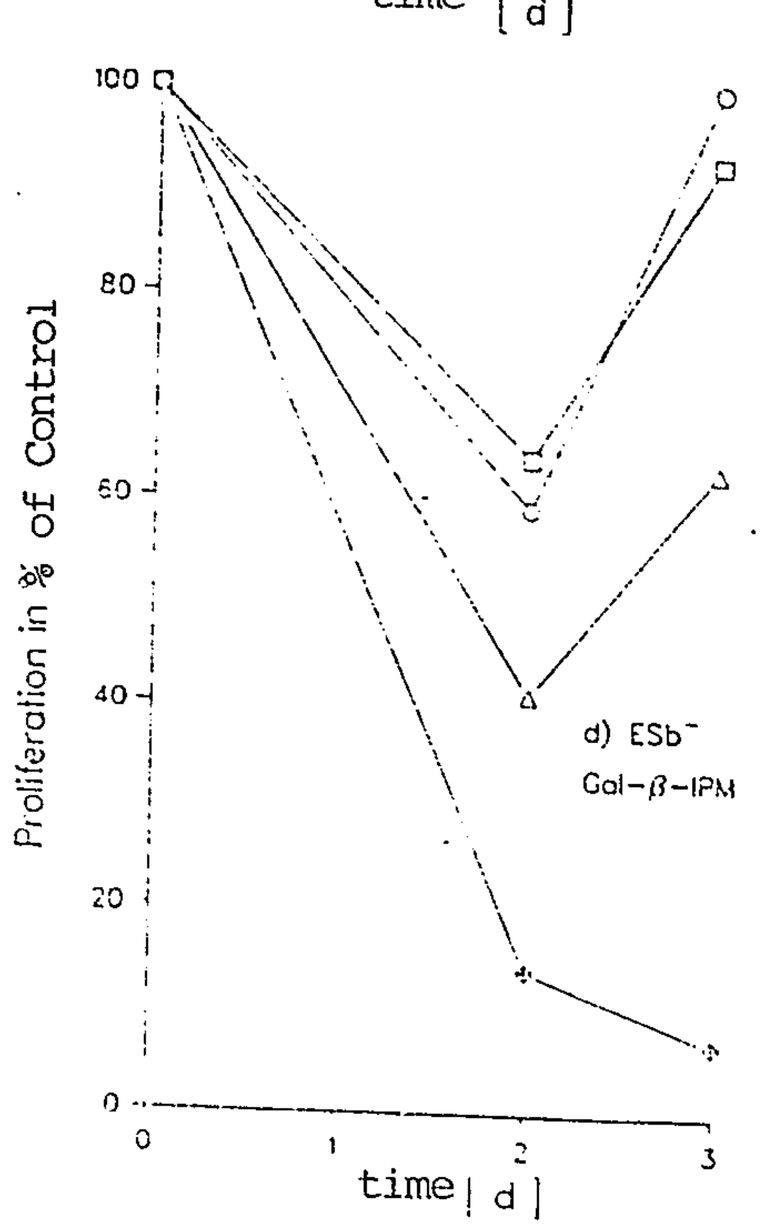


Fig. 12: Survival times of female (a) and male (b) mice treated with Glc-β-IPM; protocol: 5 × 1 o.a. dose an days 1-5; (result of an NCI-study).

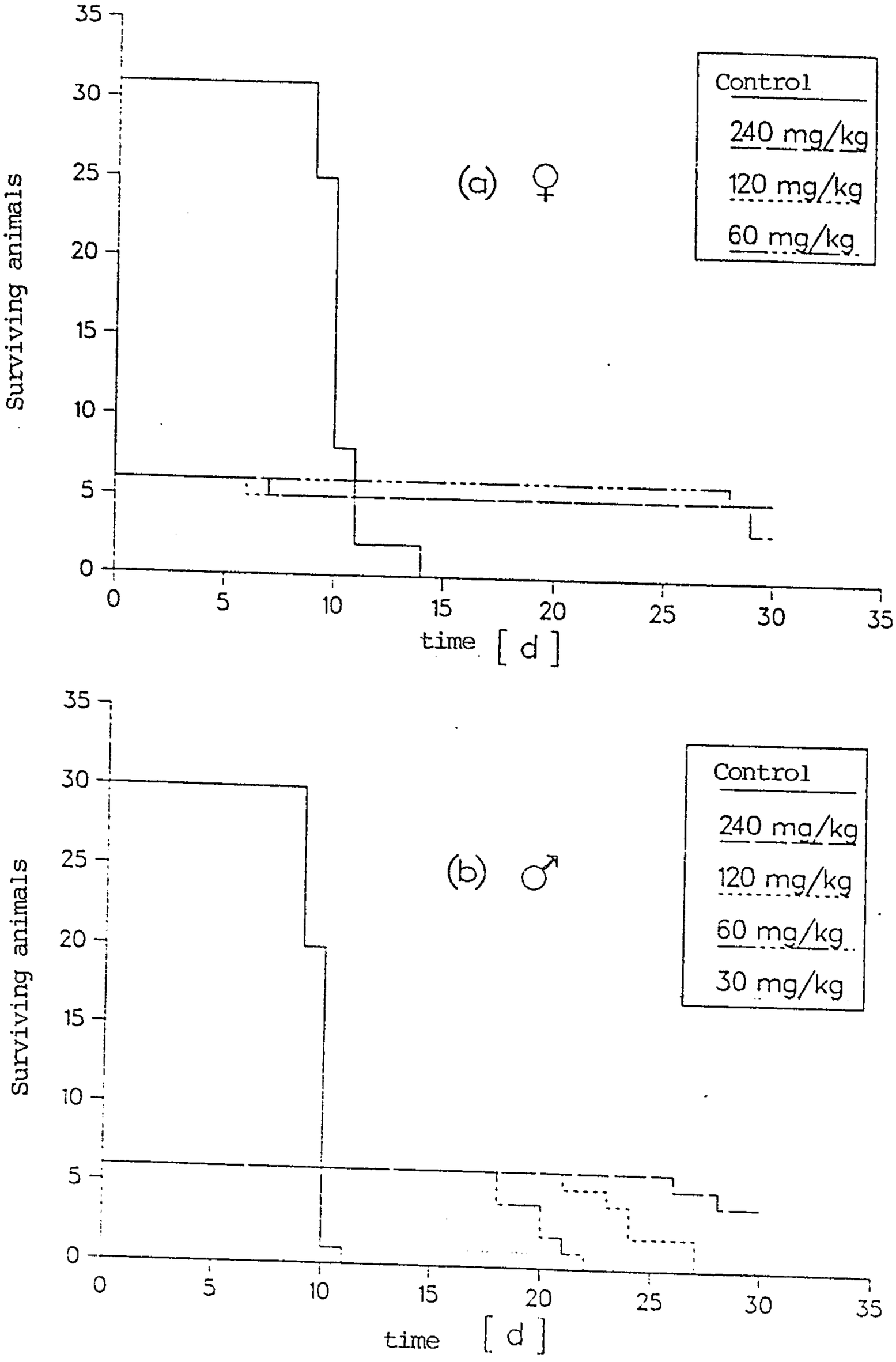
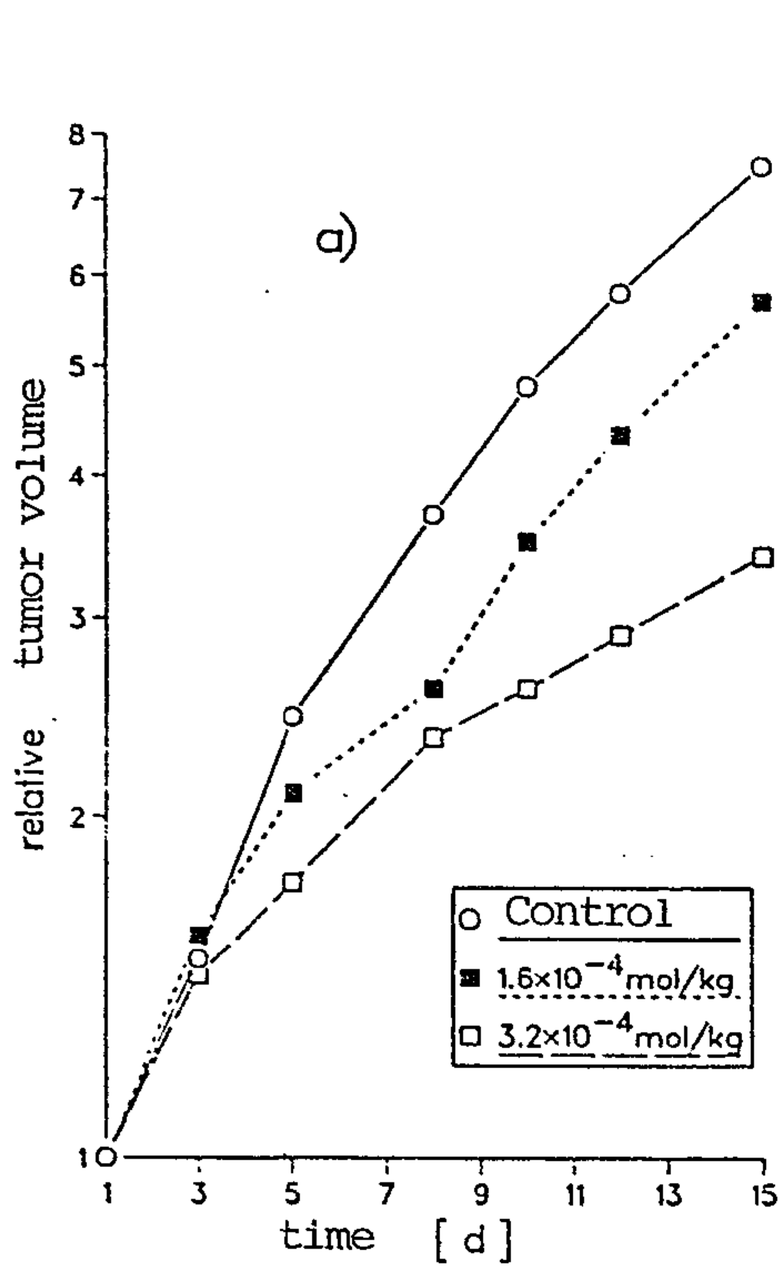
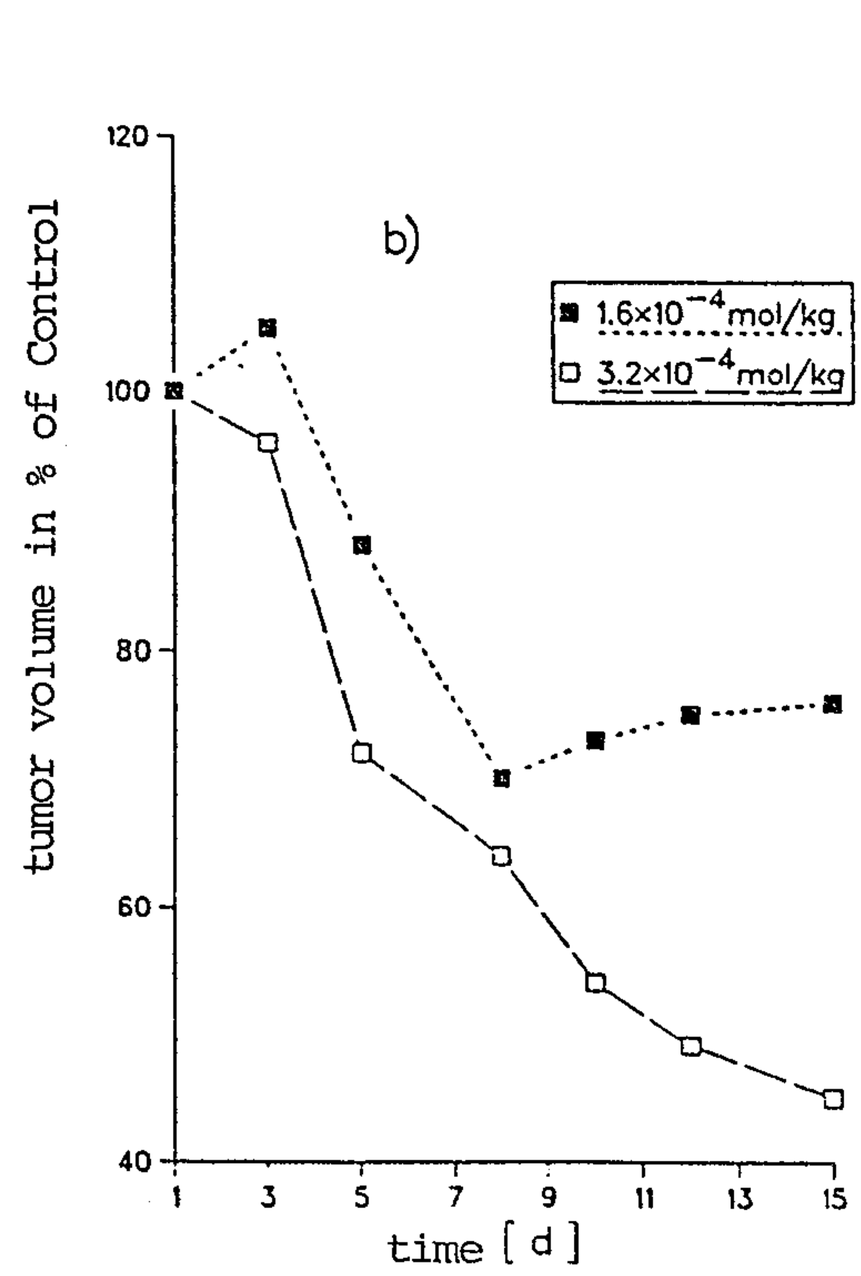


Fig. 13: Growth of the transplanted tumour 1C32 under therapy with Gal-IPM (5 × 1 dose); a) increase of tumour volume, b) tumour volume in % of control





Determination of toxicity showed that upon application of 100 and 1000 mg/kg Glc-β-IPM i.p. to male CD_2F_1 mice no acute toxicity could be observed. Autopsy of the animals after 28 days gave no findings. Also the BD6 rats with transplanted 1C32 tumour, treated with Gal-IPM (5-122.5 and 5-245 mg/kg, resp.) did not show any toxicity. The result of autopsy (among others liver, kidneys, spleen, brain, bone marrow) on day 15 after the onset of treatment gave no finding.

Summarizing, it can be stated that in vitro studies on a retrothelial sarcoma demonstrate the cytotoxicity of Aglyca-PM and -IPM, as well as the monosaccharide-IPM conjugate according to the general formula 1 and 1a, resp., and of the disaccharide conjugates acc. to the general formula 1 and 1a, resp.. In in vitro studies certain gradations become evident with the mammary tumour cell-lines 1C29, 1C32, and 1C39, because Gal-β-IPM is always the most effective agent. In vivo studies, too, showed a good effectiveness in the P388 model and with the solid 1C32 mammary tumour, and without acute toxicity. As also bone-marrow toxicity, studied in Glc-β-IPM as an example, is very low, an important requirement in the development of new anti-neoplastically active chemotherapeutic agents is fulfilled.

The following examples illustrate in detail the preparation of various, exemplary compounds.

Example 1

General protocol for the preparation of the benzyl-protected glycosyl-phosphoric acid diamides

Protocol A: Activation with hydrogenbromide

4.35 mmol of p-nitrobenzylglycose, protected in a known manner, (f.i. 3.0 g of 16), and of the N-phenyl carbamoyl derivative 17, resp., after drying over P_2O_5 were dissolved in 10 ml absol. CH_2Cl_2 (three-necked flask). At -20 °C 30 ml of a CH_2Cl_2 solution saturated with HBr were slowly injected under dry nitrogen. After a few seconds p-nitrobenzoic acid precipitates. After heating to r.t. (room temperature) (within 30 min.) stirring was continued for 1 h at r.t., and thereafter the reaction mixture was filtered with suction through a inverting frit into a second three-necked flask. Following evaporation of CH_2Cl_2 (stirring with a magnetic stirrer, water jet vacuum, waterbath, r.t.), twice 5 ml diethyl ether were added and each time evaporated as described above to remove remaining HBr. The resulting yellow to orange-coloured oil was now dissolved in 20 ml CH_2Cl_2, and 1.0 g 4a or 4b or 54 (4.6 mmoles), and 0.85 ml triethylamine (6 mmoles) were added. After stirring for 3 d at r.t. the reaction mixture was filtered, washed twice with little water, and the organic phase was dried over Na_2SO_4 and rotation-evaporated. Subsequently, column chromatography of the mostly yellow, highly viscous oils was performed, and the respective anomere was found enriched in early, and late fractions, resp.. In all cases, perfect purity of anomeres could be achieved by preparative HPLC, sometimes also by crystallisation.

All working steps were performed under dry nitrogen, using dried solvents, and in the dark.

Protocol B: Reaction of the phosphoric acid diamides with glycosylimidates

1.0 mmole glycosylimidate (f.i. 25) was dissolved in 20 ml acetonitrile. After the addition of 1.0 mmole phosphoric acid diamide there was stirred for 6 h under reflux (in the dark). Following filtration and rotation-evaporation chromatography on silica was performed in a known manner.

Protocol C: Cleavage of protective benzyl groups

0.1 mmole of the benzyl-protected monosaccharide derivatives 22, 23, 24, or 55, and the disaccharide conjugates 48 or 49 were dissolved in 15 ml MeOH. Following addition of appr. 5 mg Pd/activated charcoal (oxidized form, containing 10 % Pd) per 0.1 mequ benzyl groups to be cleaved off (that is f.i. 20 mg catalyst per 0.1 mmole 22, 35 mg per 0.1 mmole 48) hydrogenation was performed at r.t. until the cleavage of all protective groups could be demonstrated by TLC analysis (after 1 - 4 h). If hydrogenation was terminated immediately, the de-protected product was obtained pure after filtration and rotation-evaporation of the solution. With unnecessarily long reaction time the de-protected glycoside decomposed, and IPM 4b formed. In this case, the desired product could be recovered after TLC chromatography (silica, CH_3CN:MeOH = 70:30).

TLC:

Silica, CH_3CN:MeOH = 30:70, R_f (28α) = 0.58, R_f (4b) = 0.18;

Silica, CH_3CN:MeOH:H_2O = 75:20:5

R_f-Values:

0.44 (28α),	0.48 (28β)
0.43 (29α),	0.45 (29β)
0.43 (30α),	0.41 (30β)
0.29 (50 and 51),	0.13 (4b)

For detection plates were sprayed with NBP reagent (2.5 %, in acetone), heated to 120 °C for 10 min., and, after cooling to r.t., sprayed with 0.5 M NaOH. The IPM conjugates were coloured deep-blue, while IPM itself was light blue.

Phosphoric acid amide mustard and ifosfamide mustard were prepared according to protocols known from the literature.

Monosaccharide-PM/IPM conjugates

(analogous to 6)

Batch: 0.55 g 4a (2.5 mmoles) (N,N-di-(2-chloroethyl) phosphoric acid diamide)
1.03 g ABG (2.5 mmoles)

Yield: 30 mg as yellow, clear oil (2.2 % of expected), mixture of diastereomeres (A+B, see NMR spectra)

Analysis:

		C	H	N
$C_{18}H_{29}Cl_2N_2O_{11}P$	exp.	39.24	5.30	5.08
(551.3)	obs.	39.53	5.38	4.94

with D_2O, $-NH_2$), 3.35–3.70 (m, 8H, 2× $-CH_2CH_2Cl$), 3.82 (m, 1H, H–5) 4.148 (dd, H–6a(A), $J_{5,6a}$=5.0 Hz, $J_{6a,6b}$=12.5 Hz), 4.208 (dd, H–6a(B), $J_{5,6a}$=4.9 Hz, $J_{6a,6b}$=12.5 Hz), 4.244 (dd, H–6b(A), $J_{5,6b}$=2.1 Hz, $J_{6a,6b}$=12.5 Hz), 4.288 (dd, H–6b(B), $J_{5,6b}$=2.1 Hz, $J_{6a,6b}$=12.5 Hz). 5.02–5.12 (m, 2H), 5.21 and 5.23 (2t, together 1H, (B+A), J=9.5 Hz), 5.298 and 5.316 (2t, together 1H, H–1(A) und H–1(B), $J_{1,2}$=$J_{1,P}$=7.8 Hz).

MS: m/e = 331 (M-PM)

2,3,4,6-Tetra-O-acetyl β-D-glucopyranosyl N,N'-di-(2-chloroethyl)-phosphoric acid diamide 6.

To a mixture of 1.1 g 4b (5 mmoles) and 2.05 g acetobromoglucose (5 mmoles) in 50 ml dry acetone 0.5 g triethylamine (5 mmoles) were added dropwise. The reaction mixture was stirred for 48 h at r.t. in the dark. After filtration and rotation-evaporation the residue was dispersed in CH_2Cl_2, washed with little 0.1 M HCl, saturated $NaHCO_3$ solution, and water, dried over Na_2SO_4, and chromatographed on silica (acetone:n-hexane = 40:60). 80 mg 6 were obtained as a clear, colourless oil which crystallized after months at 4 °C.

M.p.: 91 °C

TLC: silica, acetone:n-hexane = 1:1, R_f = 0.13

Analysis:

		C	H	N	Cl
$C_{18}H_{29}Cl_2N_2O_{11}P$	exp.	39.24	5.30	5.08	12.87
(551.3)	obs.	39.42	5.40	4.80	12.58

Penta-o-pivaloyl β-D-glucose 7

(acc. to Kund and Harreus, 1982, Liebig's Ann. Chem. 41)

Batch: 29 g D-glucose (0.16 moles)
121.2 pivaloyl chloride (1.0 mole)
200 ml chloroform, 120 ml pyridine

Yield: 66 g 7 (69 % of theory)

M.p.: 154 °C (Lit.: 156 - 158 °C)

Analysis:

		C	H
$C_{31}H_{52}O_{11}$	exp.	61.98	8.72
(600.8)	obs.	62.16	8.79

2,3,4,6-Tetra-O-pivaloyl α-D-glucopyranosyl bromide 8

(acc. to Kund and Harreus, 1982, Liebig's Ann. Chem. 41)

Batch: 12 g 7 (20 mmoles)
20 ml HBr in glacial acetic acid (33 %)
20 ml CH_2Cl_2

Yield: 6.4 g (55.5 % of theory)

M.p.: 142 °C

Analysis:

		C	H
$C_{26}H_{43}BrO_9$ (579.5)	exp.	53.89	7.48
	obs.	54.24	7.94

$J_{2,3}$=9.5 Hz), 5.21 and 5.64 (2t, 2H, J=9.5 Hz, H-4 and H-3), 6.62 (d, 1H, H-1, $J_{1,2}$=4.2 Hz).

2,3,4,6-Tetra-O-pivaloyl β-D-glucopyranosyl N,N'-di-(2-(chloroethyl) phosphoric acid diamide 9

To a mixture of 0.38 g 4b (1.725 mmoles) and 1.0 g 8 (1.725 mmoles) in 50 ml acetone 0.5 g Ag_2CO_3 (1.8 mmoles) were added at r.t.. After stirring for 24 h (in the dark) at r.t., the reaction mixture was filtered, concentrated, and chromatographed over silica (acetone:n-hexane = 25:75). 160 mg 9 (12.9 % of theory) were obtained as a clear, colourless oil which crystallized after months at +4 °C.

M.p.: 94 °C

TLC: silica, acetone:n-hexane = 1:1, R_f = 0.43

Analysis:

		C	H	N	Cl
$C_{30}H_{54}Cl_2N_2O_9P$ (719.54)	exp.	50.08	7.42	3.89	9.86
	obs.	51.35	7.98	3.24	9.36

Methyl 2,3,4,6-tetra-O-benzyl α-D-glucopyranoside 10

(acc. to Methods in Carbohydrate Chemistry, 1972)

Batch: 50 g methyl α-D-glucopyranoside (257 mmoles)
250 g KOH (powdered)
150 ml dioxane
318 ml benzylchloride (2.76 moles)

Yield: 116 g 10 (81.3 % of theory) as highly-viscous, yellow, clear oil

Analysis:

		C	H
$C_{35}H_{38}O_6$ (554.68)	exp.	75.79	6.91
	obs.	76.19	6.93

Methyl 2,3,4,6-tetra-O-benzyl α-D-galactopyranoside 11

(analogous to 10)

Batch: 40 g methyl α-D-galactopyranoside (206 mmoles)
200 g KOH (powdered)
200 ml dioxane
280 ml benzylchloride

Yield: 102 g 11 (89.3 % of theory) as yellow, clear oil

Analysis:

		C	H
$C_{35}H_{38}O_6$ (554.68)	exp.	75.79	6.91
	obs.	76.07	6.67

Methyl 2,3,4,6-tetra-O-benzyl α-D-mannopyranoside 12

(acc. to Koto et al., 1976)

Batch: 18 g methyl α-D-mannopyranoside (92.7 mmoles)
81 g NaH suspension (20 %)
450 ml benzylchloride

Raw yield: After the washing process 2 phases form. After removing the upper, colourless phase (white oil), 52 g yellow, slightly turbid oil were obtained which could be reacted to 15 without further purification.
Part of the oil was chromatographed (silica, EE:PE = 40:60): yellow, clear oil

- ^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.31 (s, 3H, -OMe), 3.65-4.15 (m, 6H, sugar protons), 4.43-5.10 (m, 9H, H-1 und 4×-C$\underline{H}_2$-Ph), 7.1-7.45 (m, 20H, 4×-Ph).

2,3,4,6-Tetra-O-benzyl α-D-glucopyranose 13

(acc. to Methods in Carbohydrate Chemistry, 1982)

Batch: 115 g 10 (207 mmoles)

Yield: 54 g 13 (48.3 % of theory)

M.p.: 152 °C (recrystallized from methanol)

Analysis:

		C	H
$C_{34}H_{36}O_6$ (540.66)	exp.	75.53	6.71
	obs.	75.69	6.91

2,3,4,6-Tetra-O-benzyl α-D-galactopyranose 14
(acc. to Kronzer and Schuerch, 1974, Carboh. Res. 33: 273)

Batch: 30 g 11 (54 mmoles)
500 ml acetic acid (80 %)
150 ml 1 N HCl

Yield: 28.6 g 14 (98 % of theory), yellow, clear oil

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.47 (d, 1H, exchangeable with D_2O, -OH), 3.5-4.3 (m, 6H, sugar protons), 4.35-5.05 (m, 8H, 4×-$C\underline{H}_2$-Ph), 5.28 (dd, 1H, H-1, $J_{1,2}$=3.2 Hz), 7.1-7.5 (m, 20H, 4×-Ph).

2,3,4,6-Tetra-O-benzyl D-mannopyranose 15

50 g of the mixture 12 (appr. 90 mmoles, still contains some white oil) were dissolved in 800 ml glacial acetic acid and heated to 80 - 85 °C. 120 ml 2 N HCl were added dropwise within 60 min.; after further 90 min. 200 ml water were added, the reaction mixture was cooled to r.t., and subsequently extracted with 3 200 ml toluene. The organic phase was washed with sat. $NaHCO_3$ solution and water, dried over Na_2SO_4, and concentrated by rotation-evaporation. The resulting brown syrup was chromatographed (silica, EE:EP = 30:70) and 32.2 g 15 (68.3 % of theory) were obtained as a yellow oil.

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.5-4.3 (m, 7H, 1H exchangeable with D_2O, -OH and 6 sugar protons), 4.35-5.05 (m, 8H, 4×-$C\underline{H}_2$-Ph), 5.22 (d, 1H, H-1, $J_{1,2}$~2 Hz), 7.05-7.4 (m, 20H, 4×-Ph).

2,3,4,6-Tetra-O-benzyl 1-O-p-nitrobenzoyl D-glucopyranoside 16

(acc. to Methods in Carbohydrate Chemistry, 1972)

Batch: 15.5 g 13 (28.7 mmoles)
6 g p-nitrobenzoyl chloride (32.3 mmoles)
3.75 ml pyridine

Yield: 15.6 g 16 (78.8 % of theory) as white powder

M.p.: 93 - 98 °C (from ethanol)

Recrystallization from diisopropylether gave 16α as needles.

M.p.: 122°C

Analysis:		C	H	N
$C_{41}H_{39}NO_9$	exp.	71.39	5.70	2.03
(689.76)	obs.	71.37	5.67	2.04

From the mother liquor 16β crystallized, m.p. 98 °C

2,3,4,6-Tetra-O-benzyl 1-O-(N-phenylcarbamoyl) D-galactopyranose 17

(acc. to Kronzer and Schuerch, 1974, Carboh. Res. 33: 273)

Batch: 18.7 g 14 (34.6 mmoles)
50 ml pyridine
5.4 ml phenylisocyanate

Yield: 8.0 g 17 (35 % of theory), (α:β = 35:65)

M.p.: 120 - 122 °C (from ethanol)

Analysis:

		C	H	N
$C_{41}H_{41}NO_7$	exp.	74.64	6.26	2.12
(659.78)	obs.	74.21	5.97	2.36

From the mother liquor 17α crystallizes, m.p. 143 °C;

2,3,4,6-Tetra-O-benzyl 1-O-p-nitrobenzoyl α-D-mannopyranoside 18

(acc. to Koto et al., 1976, Bull. Chem. Soc. Jpn. 49: 2639)

Batch: 9.8 g 15 (18.1 mmoles)
4.9 g p-nitrobenzoyl chloride
50 ml pyridine

Yield: after flash chromatography (silica 60, EE:PE = 25:75) 7.7 g 18 (61.7 % of theory) crystallized from diisopropyl ether

M.p.: 105 °C

Analysis:

		C	H	N
$C_{41}H_{39}NO_9$	exp.	71.39	5.70	2.03
(689.76)	obs.	71.46	5.58	1.90

2,3,4,6-Tetra-O-benzyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 22

1. acc. to protocol A

Batch: 3.0 g 16 (4.35 mmoles)

Yield: after column chromatography (silica, EE:PE = 60:40) 2.2 g 22 (68 % of theory), mixture of anomeres: α:β = 5:4 (^{1}H-NMR).

TLC: Silica, EE:PE = 60:40, R_f = 0.28, R_f (α) > R_f (β)

HPLC (anal.): Silica, EE:hexane = 75:25, flow: 1.0 ml/min.
R_t (α) = 12.53', R_t (β) = 14.04';

HPLC (prep.): Silica, EE:hexane:MeOH = 58:42:0.5, flow: 10.0 ml/min
R_t (α) = 45', R_t (β) = 55';

Analysis:

		C	H	N	Cl	
$C_{38}H_{45}Cl_2N_2O_7P$ (743.60)	exp.	61.38	6.10	3.77	9.54	
	obs.	61.15	6.22	3.78	9.61	22α
	obs.	60.82	6.29	3.61	9.37	22β

2. acc. to protocol B

Batch: 4.5 g 25α (6.75 mmoles)
1.45 g 4b (6.57 mmoles)

Yield: after column chromatography (see above): 2.06 g 22 (42.2 % of theory), anomeric ratio α:β appr. 1:20 (acc. to ^{1}H-NMR and HPLC)

Batch: 50 mg 25β (0.073 mmoles)
16.1 mg 4b (0.073 mmoles)

TLC shows product with R_f = 0.28 and little tetrabenzyl glucose 13, as well as some starting material 25β, anomeric ratio α:β = 1:1.1 (HPLC)

2,3,4,6-Tetra-O-benzyl D-galactopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 23

1. acc. to protocol A

Batch: 2.6 g 17 (3.94 mmoles)
0.9 g 4b (4.07 mmoles)

Yield: after column chromatography (silica, EE:PE = 60:40): 1.9 g 23 (65 % of theory), mixture of anomeres α:β = 1:1 (^{1}H-NMR)

TLC: Silica, EE:PE = 60:40, R_f = 0.27, R_f (α) > R_f (β)

HPLC (anal.): Silica, EE:hexane = 75:25, flow: 1.0 ml/min:
R_t (α) = 14.26', R_t (β) = 18.03';

HPLC (prep.): Silica, EE:hexane:MeOH = 58:42:0.5,
R_t (α) = 51 ', R_t (β) =62';

Analysis:

		C	H	N	Cl	
$C_{38}H_{45}Cl_2N_2O_7P$	exp.	61.38	6.10	3.77	9.54	
(743.60)	obs.	61.26	6.19	3.76	9.76	23α
	obs.	61.16	6.26	3.76	9:66	23β

2. acc. to protocol B

Batch: 685 mg 26α (1.0 mmole)
221 mg 4b (1.0 mmole)

Yield: in the filtered reaction mixture the ratio of anomeres α:β was 55:45 (HPLC). After TLC (see above) 260 mg 23 (38 % of theory) were obtained.

2,3,4,6-Tetra-O-benzyl D-mannopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 24

1. acc. to protocol A

Batch: 2.3 g 18 (3.33 mmoles)
0.75 g 4b (3.39 mmoles)

Yield: after column chromatography (silica, EE:PE = 80:20) 1.16 g 24 (47 % of theory), anomeric mixture: α:β = 55:45 (^{1}H-NMR)

TLC: Silica, EE:PE = 60:40, R_f (α) = 0.23, R_f (β) = 0.19

HPLC (anal.): Silica, EE:hexane = 75:25, flow: 1.0 ml/min.
R_t (α) = 13.05', R_t (β) = 21.61';

HPLC (prep.): Silica, EE:hexane:MeOH = 64:36:0.5, flow: 10 ml/min., R_t (α) = 45', R_t (β) = 70';

Analysis:

		C	H	N	
$C_{38}H_{45}Cl_2N_2O_7P$ (743.60)	exp.	61.38	6.10	3.77	
	obs.	60.98	6.32	3.52	24α
	obs.	60.98	6.19	3.29	24β

2. acc. to protocol B

Batch: 3.2 g 27α (4.67 mmoles)
1.04 g 4b (4.70 mmoles)

Yield: TLC showed almost quantitative reaction to 24α with R_f = 0.23. In the filtered reaction mixture only the α-anomere is found (HPLC). After column chromatography 1.6 g 24α (45 % of theory) were obtained.

O-(2,3,4,6-Tetra-O-benzyl α-D-glucopyranosyl) trichloroacetimidate 25α

(acc. to Schmidt and Stumpp, 1983, Liebig's Ann. Chem., 1249)

Batch: 9.2 g 13 (17 mmoles)
7.7 ml trichloroacetonitrile
680 mg NaH

Yield: 10.9 g 25α (93.6 % of theory), colourless, clear oil.

TLC: Silica, PE:e = 1:1, R_f = 0.44

^{1}H-NMR: 90 MHz, $CDCl_3$.
δ = 3.6–5.1 (m, 14H), 6.51 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 6.95–7.55 (m, 20H, 4× -Ph), 8.55 (s, 1H, -NH-).

O--(2,3,4,6-Tetra-O-benzyl β-D-glucopyranosyl) trichloroacetimidate 25β

(acc. to Schmidt et al., 1984, Liebig's Ann. Chem., 680)

Batch: 1.3 g 13 (2.4 mmoles)
1.25 g K_2CO_3 (dried)
1.25 ml trichloroacetonitrile

Yield: after gel filtration on silica: slightly yellowish oil (1.6 g, 97 % of theory, α:β = 1:5); after TLC (silica, E:PE = 2:3) pure 25β was obtained as a colourless oil (50 mg, 30 % of theory)

TLC: Silica, PE:E = 1:1, R_f = 0.37

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.55–3.90 (m, 6H), 4.40–5.05 (m, 8H), 5.82 (d, 1H, H–1), 7.1–7.5 (m, 20H, 4× –Ph), 8.70 (s, 1H, –NH–).

O-(2,3,4,6-Tetra-O-benzyl D-galactopyranosyl) trochloroacetimidate 26

(acc. to Schmidt et al., 1984, Liebig's Ann. Chem., 1343)

Batch: 1.5 g 14 (2.77 mmoles)
1.4 ml trichloroacetonitrile
80 mg NaH

Yield: after gel filtration on silica an anomeric ratio of α:β of 4:1 was determined

(^{1}H-NMR: 90 MHz, $CDCl_3$, Nr. H14208, δ = 5.72 (d, 0.2H, H–1 (β), $J_{1,2}$=8.0 Hz), 6.52 (d, 0.8 H, H–1 (α), $J_{1,2}$=3.7 Hz), 8.51 (s, 0.8H, –NH– (α) exchangeable with D_2O), 8.60 (s, 0.2H, –NH– (β), exchangeable with D_2O).

After column chromatography (silica, E:PE = 1:1) 2 fractions were obtained:
F1: 1130 mg 26α (59.5 % of theory) as colourless oil,
^{1}H-NMR: 500 MHz, $CDCl_3$, Nr. H14112
F2: 320 mg 26 (16.8 % of theory) as yellowish oil (α:β = 2:1)

^{1}H-NMR: 500 MHz, $CDCl_3$, Nr. H14113

TLC: Silica, PE:E = 1:1, R_f (α) = 0.43, R_f (β) = 0.34

O-(2,3,4,6-Tetra-O-benzyl α-D-mannopyranosyl) trichloroacetimidate 27α

(acc. to Schmidt et al., 1984, Liebig's Ann. Chem., 1343)

Batch: 4.5 g 15 (8.27 mmoles)
4 ml trichloroacetonitrile
45 mg NaH

Yield: after column chromatography: 4.45 g 27α (65 % of theory) as colourless oil

TLC: Silica, E:PE = 3:2, R_f = 0.51

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.65–5.0 (m, 14H), 6.33 (d, 1H, H-1, $J_{1,2}$~1.5 Hz), 7.0–7.5 (m, 20H, 4× -Ph), 8.52 (s, 1H, -NH-).

α-D-Glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 28α

Hydrogenation of 22α acc. to protocol C

^{1}H-NMR: 500 MHz, D_2O, Nr. 13943
δ = 3.25-3.32 (m, 4H, 2× $-CH_2-$), 3.488 (t, 1H, J=9.5 Hz), 3.61-3.67 (m, 5H mit 2×$-CH_2$), 3.719 (t, 1H, J-9.5 Hz), 3.75-3.90 (m, 3H), 5.605 (dd, 1H, H-1, $J_{1,2}$=3.4 Hz, $J_{1,P}$=7.8 Hz).

FAB-MS: Nr. MSN 13608

positiv:	m/e =	383, 385, 387	$(M+H)^+$
		221, 223, 225	$(IPM+H)^+$

β-D-Glucopyranosyl N,N'-die-(2-chloroethyl) phosphoric acid diamide 28β

Hydrogenation of 22β acc. to protocol C

Analysis:

		C	H	N
$C_{10}H_{21}Cl_2N_2O_7P$	ep.	31.35	5.53	7.31
(383.10)	obs.	31.03	5.04	6.82

α-D-Galactopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 29α

Hydrogenation of 23α acc. to protocol C

^{1}H-NMR: 500 MHz, D_2O, Nr. 13945
δ = 3.26-3.32 (m, 4H, 2× $-CH_2-$), 3.635-3.665 (m, 4H, 2 $-CH_2-$), 3.74-4.05 (m, 5H), 4.098 (m, 1H), 5.642 (dd, 1H, H-1, $J_{1,2}$=3.1 Hz, $J_{1,P}$=7.8 Hz).

FAB-MS: Nr. MSN 13612

positiv	m/e =	383	$(M+H)^+$
		221, 223, 225	$(IPM+H)^+$
negativ	m/e =	381, 383, 385	$(M-H)^-$
		219, 221, 223	$(IPM-H)^-$

β-D-Galactopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 29β

Hydrogenation of 23β acc. to protocol C

^1H-NMR: 500 MHz, D_2O, Nr. 13946
δ = 3.26-3.32 (m, 4H, 2× $-CH_2-$), 3.605 (d, 1H), 3.63-3.67 (m, 4H, 2× $-CH_2-$), 3.692 (dd, 1H, J=3.5 und J=10.0 Hz), 3.70-3.95 (m, 4H), 4.950 (t, 1H, H-1, $J_{1,2}=J_{1,P}=8.0$ Hz).

FAB-MS: Nr. MSN 13613

negativ	m/e =	381, 383, 385	$(M-H)^-$
		219, 221, 223	$(IPM-H)^-$

α-D-Mannopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 30α

Hydrogenation of 24α acc. to protocol C

^1H-NMR 500 MHz, D_2O, Nr. 13947

δ = 3.26-3.32 (m, 4H, 2× $-CH_2-$), 3.5-4.0 (m, 9H mit 4H bei 3.63-3.67, 2× $-CH_2-$), 4.018 (dd, 1H), 5.564 (dd, 1H, H-1, $J_{1,2}=2.0$ Hz, $J_{1,P}=8.0$ Hz).

FAB-MS: Nr. 13614
negativ m/e = 381, 383, 385 $(M-H)^-$
219, 221, 223 $(IPM-H)^-$

β-D-Mannopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 30β

Hydrogenation of 24β acc. to protocol C

^1H-NMR: 500 MHz, D_2O, Nr. 13948
δ = 3.26-3.33 (m, 4H, 2× $-CH_2-$), 3.45 (m, 1H, H-5), 3.604 (t, 1H, H-4, $J_{3,4}=J_{4,5}=9.8$ Hz), 3.63-3.67 (m, 4H, 2× $-CH_2-$), 3.703 (dd, 1H, H-3, $J_{2,3}=3.2$ Hz, $J_{3,4}=9.8$ Hz), 3.754 (dd, 1H, H-6a, $J_{5,6a}=6.2$ Hz, $J_{6a,6b}\sim12.5$ Hz), 3.931 (dd, 1H, H-6b, $J_{5,6b}=2.1$ Hz, $J_{6a,6b}\sim12.5$ Hz), 4.052 (dd, 1H, H-2, $J_{1,2}\sim1.1$ Hz, $J_{2,3}=3.2$ Hz), 5.282 (dd, 1H, H-1, $J_{1,2}\sim1.1$ Hz, $J_{1,P}=8,6$ Hz).

FAB-MS: Nr. 13615
negativ m/e = 381, 383, 385 $(M-H)^-$
219, 221, 223 $(IPM-H)^-$

Di-(2-chloroethyl) phosphoric acid diamide dichloride 33

(acc. to Friedman and Seligman, 1954, J. Am. Chem. Soc. 76: 655)

Batch: 130 ml $POCl_3$ (1.4 mole)
50 g Bis-(2-chloroethyl) amine hydrochloride

Yield: 53 g 33 as white crystals

M.p.: 54 °C (from acetone/PE)

Analysis:

		C	N	N	Cl
$C_4H_3Cl_4NOP$ (258.9)	exp.	18.56	3.11	5.41	54.77
	obs.	18.67	3.13	5.35	54.90

Example 2

Disaccharide-IPM conjugates

Octa-O-acetyl lactose 35

A mixture of 100 g lactose (147 mmoles), 400 ml acetanhydride, and 25 g water-free sodium acetate was stirred at 120 - 135 °C for 60 min..After cooling it was poured on ice-water and extracted with CH_2Cl_2. The organic phase was washed neutral with sat. $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated by rotation-evaporation. Crystallization from ethanol yielded 153 g 35 (80 % of theory).

M.p.: 79 - 92 °C

TLC: Silica, Toluene:2-butanone = 10:4, R_f = 0.43

Analysis:

		C	H
$C_{28}H_{38}O_{19}$ (678.6)	exp.	49.56	5.64
	obs.	49.37	5.80

Allyl-4-O-(2,3,4,6-tetra-O-acetyl β-D-galactopyranosyl) 2,3,6-tri-O-acetyl D-glucopyranoside 37

(acc. to Koto et al., 1982, J. Chem. Soc. Jpn. 10: 1651)

34 g (50 mmoles) 35 were dissolved in 60 ml $CHCl_3$. At 0 °C 20.6 ml acetylbromide (276 mmoles) and 4.56 ml H_2O were added. After stirring for 2.5 h at r.t. the yellow, clear solution was concentrated by rotation-evaporation, and a yellow foam (hepta-O-acetyl α-D-lactosylbromide) was obtained with:

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 1.95-2.20 (m, 21H, 7× -OAc), 3.7-5.65 (m, 13H, sugar protons), 6.51 (d, 1H, H-1, $J_{1,2}$=4 Hz).

The foam was dissolved in 400 ml allyl alcohol at 35 °C. After addition of 30 g silver carbonate the mixture was stirred for 1 d at r.t. (in the dark, followed by filtration and concentration by rotation-evaporation. The residue was dispersed in ether and, after another filtration and concentration step chromatographed on silic 60 (toluene:2-butanone = 10:1 -> 10:3). 18.2 g 37 (53.8 % of theory) were obtained as a clear oil.

TLC: Silica, toluene:2-butanone = 10:4 (10:1), R_f = 0.47 (0.08)

Analysis:

		C	H
$C_{29}H_{40}O_{18}$	exp.	51.48	5.96
(676.62)	obs.	51.44	5.92

Allyl-4-O-(2,3,4,6-tetra-O-acetyl β-D-glucopyranosyl) 2,3,6-tri-O-acetyl D-glucopyranoside 38

(analogous to 37)

Batch: 29 g α-D-cellobiose octaacetate (42.6 mmoles) (Ega-Chemie)
17.6 ml acetylbromide
3.9 ml H_2O

Intermediate product: hepta-O-acetyl α-D-cellobiosylbromide

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 6.51 (d, 1H, H-1, $J_{1,2}$=4 Hz)

Yield: After column chromatography (silica, EE:PE) and crystallisation from diisopropyl ether 16.5 g 38 (57 % of theory) were obtained.

M.p.: 179 °C

TLC: Silica, toluene:2-butanone = 10:4, R_f = 0.49;

Analysis:

		C	H
$C_{29}H_{40}O_{18}$ (679.62)	exp.	51.48	5.96
	obs.	51.45	6.07

Allyl-4-O-(2,3,4,6-tetra-O-benzyl β-D-galactopyranosyl) 2,3,6-tri-O-benzyl D-glucopyranoside 39

(acc. to Koto et al., 1982, J. Chem. Soc. Jpn. 10: 1651=

Batch: 19.5 g 37 (28.8 mmoles)
800 ml benzylchloride
105 g KOH, powdered

Yield: after column chromatography (silica, toluene:2-butanone = 100:1 -> 10:1, and crystallization from EE/hexane 21.3 g 39 (73 % of theory) were obtained as needles.

TLC: Silica, toluene:2-butanone = 10:1, R_f = 0.50

M.p.: 73 °C

Analysis:

		C	H
$C_{64}H_{68}O_{11}$ (1013.24)	exp.	75.87	6.76
	obs.	75.66	6.63

Allyl 4-O-(2,3,4,6-tetra-O-benzyl β-D-glucopyranosyl) 2,3,6-tri-O-benzyl D-glucopyranoside 40

(analogous to 39)

Batch: 2.65 g 38 (3.92 mmoles)
100 ml benzylchloride
14.3 g KOH, powdered

Yield: after column chromatography and crystallisation from diisopropyl ether/hexane 2.82 g 40 (71 % of theory) were obtained.

TLC: Silica, toluene:2-butanone = 10:1, R_f = 0.50

M.p.: 102 °C

Analysis:

		C	H
$C_{64}H_{68}O_{11}$ (1013.24)	exp.	75.87	6.76
	obs.	76.17	6.57

4-O-(2,3,4,6-Tetra-O-benzyl β-D-galactopyranosyl) 2,3,4-tri-O-benzyl D-glucopyranose 43

A) Isomerization with t-BuOK to give 1-propenyl ether 41

A mixture of 4.9 g 39 (4.84 mmoles) and 1.3 g t-BuOK in 30 ml DMSO was stirred for 2 h at 110 °C under nitrogen. DMSO was

removed by rotation-evaporation, the residue was dissolved in a mixture of ether/water. The ether phase was isolated, the water phase was reextracted twice with ether. The combined ether phases were dried over Na_2SO_4 and concentrated by rotation-concentration. 4.02 g 41 (4.13 mmoles) were obtained as a brown oil (raw yield: 85 %).

TLC: Silica, toluene:2-butanone = 10:1, R_f = 0.62

B) Hydrolysis of the 1-propenyl ether 41 with $HgCl_2$ to give 43

(acc. to Gigg and Warren, 1968, J. Chem. Soc. (C), 1903)

To a mixture of 4.02 g 41 (4.13 mmoles) and 1130 mg HgO in 10 ml acetone/water (10:1) 1150 mg $HgCl_2$ in 10 ml acetone/water (10:1) were added dropwise over 5 min.. After stirring for 1 h at r.t. the reaction mixture was filtered through Celite, concentrated by rotation-evaporation, and dispersed in ether. The ether phase was washed with 10 ml of a half-saturated KJ solution, and with water. After drying over Na_2SO_4 and rotation-evaporation it was chromatographed over silica (toluene:2-butanone = 100:1 -> 10:5). 43 was obtained as a yellow oil which crystallized from ether/PE: 2.6 g (55 % of theory, based on 39, anomeric mixture, α:β appr. 2:1 after ^{13}C-NMR).

M.p.: 103 °C

TLC: Silica, toluene:2-butanone = 10:1, R_f = 0.22

Analysis:

		C	H
$C_{61}H_{64}O_{11}$	exp.	75.29	6.63
(973.17)	obs.	74.79	6.65

4-O-(2,3,4,6-Tetra-O-benzyl β-D-glucopyranosyl) 2,3,4-tri-O-benzyl D-glucopyranose 44

1. analogous to 43: Isomerization with t-BuOK to give the 1-propenyl ether 42 and subsequent hydrolysis with $HgCl_2$

Yield: 47.7 % of theory (after column chromatography)

2. Isomerization with Tris(triphenylphosphin) rhodium chloride ($RhCl(PPh_3)_3$) and subsequent hydrolysis with 1 N HCl

(acc. to Corey and Suggs, 1973, J. Org. Chem. 38: 3224)

125 mg 40 (0.123 mmoles) were boiled for 3 h in 30 ml EtOH/water with 2 mg diazabicyclo{2.2.2} octane (0.027 mmoles) and 12 mg $RhCl(PPh_3)_3$ (0.009 mmoles). Then 6 ml 1 N HCl were added and boiled for another 2 h . After cooling $NaHCO_3$ solution was added and extracted with ether. The organic phase was washed with water, dried over night over Na_2SO_4, and rotation-evaporated. After column chromatography (silica, toluene:2-butanone = 100:1 -> 100:5) 109 mg 44 (91 % of theory, based on 40, anomeric mixture, α:β appr. 3:1 after ^{13}C-NMR) were obtained as a colourless oil.

TLC: Silica, toluene:2-butanone = 10:1, R_f = 0.22

^1H-NMR: 90 MHz, $CDCl_3$
δ = 3.05 and 3.25 (2d, 1H, -OH, (α und β), exchangeable with D_2O), 3.3-5.2 (m, 28H), 7.1-7.5 (m, 35H, 7× -Ph).

^{13}C-NMR: Nr. C14897, $CDCl_3$
δ = 91.38 (s, C-1α), 97.42 (s, C-1β), 102.68 (s, C-1').

FAB-MS: Nr. 13689 (pos., Glycerin, DMF/HCl)
m/e = 973 $(M+H)^+$

4-O-(2,3,4,6-Tetra-O-benzyl β-D-galactopyranosyl 2,3,6-tri-O-benzyl 1-O-p-nitrobenzoyl D-glucopyranose 45

To 480 mg 43 (0.49 mmoles) in 20 ml CH_2Cl_2 2 ml of a solution of 130 mg p-nitrobenzoylchloride and 0.3 ml pyridine in CH_2Cl_2 were dropwise at r.t.. After stirring for 20 h at r.t. almost no starting material 43 was present in TLC (silica, toluene:2-butanone = 10:1), but two products with R_f = 0.42 and 0.49. After washing with 0.5 N HCl, 1 N $NaHCO_3$, and water, and drying over Na_2SO_4 a highly viscous oil was obtained. From ethanol 435 mg 45 (79 % of theory, anomeric mixture, α:β = 3:7 after ^{1}H-NMR) crystallized.

M.p.: 112 °C

Analysis:

		C	H	N
$C_{68}H_{67}O_{14}N$ (1122.28)	exp.	72.77	6.02	1.25
	obs.	73.05	6.25	1.11

4-O-(2,3,4,6-Tetra-O-benzyl β-D-glucopyranosyl 2,3,6-tri-O-benzyl 1-O-p-nitrobenzoyl D-glucopyranose 46

(analogous to 45)

Batch:
520 mg 44 (0.534 mmoles)
150 mg p-nitrobenzoyl chloride
0.4 ml pyridine

Yield: After 20 h TLC (silica, toluene:2-butanone = 10:1) showed two products with R_f = 0.43 and 0.49, and a little starting material with R_f = 0.22. After column chromatography (silica, toluene:2-butanone = 20:1) 320 mg 46 were obtained as oil (53.4 % of theory). From diisopropyl ether 46α crystallized.

M.p.: 169 °C

Analysis:

		C	H	N
$C_{68}H_{67}O_{14}N$ (1122.28)	exp.	72.77	6.02	1.25
	obs.	72.70	6.01	1.07

O-[4-O-(2,3,4,6-Tetra-O-benzyl β-D-glucopyranosyl 2,4,6-tri-O-benzyl α-D-glucopyranosyl] trichloroacetimidate 47

(analogous to 25α)

Batch: 130 mg 44 (0.133 mmoles)
60 µl trichloroacetonitrile
5.3 mg NaH

Yield: after column chromatography (silica, E:PE = 2:3) 120 mg 47 (80 % of theory) were obtained as a clear, colourless oil.

TLC: silica, E:PE = 3:2, R_f = 0.49

^{1}H-NMR: 90 MHz, $CDCl_3$
δ = 3.3–5.2 (m, 27H), 6.44 (d, 1H, H-1, $J_{1,2}$=4 Hz), 7.1–7.4 (m, 35H, 7× -Ph), 8.57 (s, 1H, -NH-, exchangeable with D_2O).

$C_{63}H_{64}Cl_3NO_{11}$ (1117.56)

4-O-(2,3,4,6-Tetra-O-benzyl β-D-galactopyranosyl) 2,3,6-tri-O-benzyl D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 48

acc. to protocol A

Batch: 100 mg 45 (0.089 mmoles)
21 mg 4b (0.095 mmoles)

Yield: after column chromatography (silica, EE:PE = 40:60 -> 70:30) 2 fractions were obtained:
F1: 7 mg 48, β >> α (6.7 % of theory)
F2: 40 mg 48, α >> β (38.2 % of theory)

TLC: silica, EE/PE = 80:20, R_f (α) = 0.37, R_f (β) = 0.42;

$C_{65}H_{73}Cl_2N_2O_{12}P$ (1176.18)

^{1}H-NMR: Nr. H15189, 90MHz, $CDCl_3$, $\alpha >> \beta$
Nr. H15326, 90MHz, $CDCl_3$, $\beta >> \alpha$

HPLC (anal.): Silica, EE:hexane:MeOH = 72:28:0.7, flow: 1.0 ml/min.;
R_t (α) = 10.32', R_t (β) = 8.73'.

HPLC (prep.): Silica, EE:hexane:MeOH = 64:36:0.5, flow: 10.0 ml/min.;
R_t (α) = 35', R_t (β) = 27';

4-O-(2,3,4,6-Tetra-O-benzyl β-D-glucopyranosyl) 2,3,6-tri-O-benzyl D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 49

acc. to protocol B

Batch: 40 mg 47 (0.036 mmoles)
9 mg 4b (0.041 mmoles)

Yield: after column chromatography (silica, EE:PA = 60:40) and prep. HPLC 2 fractions were obtained (each a clear, colourless oil):
F1: 18 mg 49β (42.5 % of theory)
F2: 7 mg 49α (10.9% of theory)

TLC: Silica, EE/PE = 80:20, R_f (α) = 0.35, R_f (β) = 0.42

$C_{65}H_{73}Cl_2N_2O_{12}P$ (1176.18)

HPLC (anal.): Silica, EE:hexane:MeOH = 72:28:1, flow: 1.0 ml/min.
R_t (α) = 7.03', R_t (β) = 5.89';

HPLC (prep.): Silica, EE:hexane:MeOH = 64:36:0.5, flow: 10.0 ml/min.
R_t (α) = 35', R_t (β) = 25';

4-O-(β-D-galactopyranosyl) α-D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 50α

Hydrogenation of 48α acc. to protocol C

$C_{16}H_{31}Cl_2N_2O_{12}P$ (541.31)

^{1}H-NMR: 500 MHz, D_2O, Nr. H15701
δ = 3.27-3.34 (m, 4H, 2× $-CH_2-$), 3.563 (dd, 1H, H-2', $J_{1',2'}$=8.0 Hz, $J_{2',3'}$=9.8 Hz), 3.65-4.0 (m, 15H with 4H at 3.65-3.68, 2× $-CH_2-$), 4.478 (d, 1H, H-1', $J_{1',2'}$=8.0 Hz), 5.623 (dd, 1H, H-1, $J_{1,2}$=3.6 Hz, $J_{1,P}$=7.8 Hz).

FAB-MS: Nr. MSN 14075

positiv	m/e = 221, 223, 225	$(IPM+H)^+$
	545, 547, 549	$(M+H)^+$

4-O-(β-D-Galactopyranosyl) β-D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 50β

Hydrogenation of 48β acc. to protocol C

$C_{16}H_{31}Cl_2N_2O_{12}P$ (541.31)

^1H-NMR: 500 MHz, D_2O, ^1H-^1H-2D-COSY, Nr. 15834
δ = 3.27-3.34 (m, 4H, 2× $-CH_2-$), 3.43 (dd, 1H, H-2, $J_{1,2}$=8.0 Hz), 3.558 (dd, 1H, H-2', $J_{1',2'}$=8.O Hz, $J_{2',3'}$=10.0 Hz), 3.65-3.68 (m, 4H, 2× $-CH_2-$), 3.68-3.90 (m, 8H), 3.938 (dd, 1H, J=3.4 and J-1.0 Hz), 3.989 (dd, 1H, J=1.9 and J=12.6 Hz), 4.473 (d, 1H, H-1', $J_{1',2'}$=8.0 Hz), 5.049 (t, 1H, H-1, $J_{1,2}=J_{1,P}$=8.0 Hz).

FAB-MS: Nr. MSN 14076

positiv	m/e =	221, 223, 225	$(IPM+H)^+$
		545, 547, 549	$(M+H)^+$

4-O-(β-D-glucopyranosyl) α-D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 51α

Hydrogenation of 49α acc. to protocol C

$C_{16}H_{31}Cl_2N_2O_{12}P$ (541.31)

^1H-NMR: 500 MHz, D_2O, ^1H-^1H-2D-COSY, Nr. 15856
δ = 3.27-3.32 (m, 4H, 2× $-CH_{1,2}-$), 3.333 (dd, 1H, H-2', $J_{1',2'}$=7.9 Hz, $J_{2',3'}$=9.4 Hz), 3.40-3.45 (m, 2H), 3.47-3.55 (m, 2H), 3.64-3.68 (m, 4H, 2× $-CH_2-$), 3.69 (dd, 1H, H-2), 3.70-3.99 (m, 6H), 4.537 (d, 1H, H-1', $J_{1',2'}$=7.9 Hz), 5.622 (dd, 1H, H-1, $J_{1,2}$=3.6 Hz, $J_{1,P}$=7.8 Hz).

FAB-MS: Nr. MSN 14077

positiv	m/e =	221, 223, 225	$(IPM+H)^+$
		545, 547	$(M+H)^+$

4-O-(β-D-Glucopyranosyl) β-D-glucopyranosyl N,N'-di-(2-chloroethyl) phosphoric acid diamide 51β

Hydrogenation of 49β acc. to protocol C

$C_{16}H_{31}Cl_2N_2O_{12}P$ (541.31)

^{1}H-NMR: 500 MHz, D_2O, ^{1}H-^{1}H-2D-COSY, Nr. 15857
δ = 3.27-3.32 (m, 4H, 2× $-CH_{1,2}-$), 3.33 (dd, 1H, H-2', $J_{1',2'}$=8.0 Hz, $J_{2',3'}$~10 Hz), 3.428 (dd, 1H, H-2, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.0 Hz), 3.44-3.54 (m, 3H), 3.64-3.68 (m, 4H, 2× $-CH_2-$), 3.69-3.72 (m, 3H), 3.745 (dd, 1H, H-6'a, $J_{5',6'a}$=5.8 Hz, $J_{6'a,6'b}$=12.3 Hz), 3.855 (m, 1H, H-6b), 3.928 (dd, 1H, H-6'b, $J_{5',6'b}$=2.1 Hz, $J_{6'a,6'b}$~12.3 Hz), 3.990 (dd, 1H, H-6a, $J_{5,6a}$=2.0 Hz, $J_{6a,6b}$=12.2 Hz), 4.532 (d, 1H, H-1', $J_{1',2'}$=8.0 Hz), 5.044 (t, 1H, H-1, $J_{1,2}$=$J_{1,P}$=8.0 Hz).

FAB-MS: Nr. MSN 14078

positiv	m/e =	221, 223, 225	$(IPM+H)^+$
		545, 547, 549	$(M+H)^+$

Example 3

Maltotriose is peracetylated (using sodium acetate/acetanhydride). The product has R_f 0,48, $CHCl_3$/ethylacetate 1:1 on silica gel). From the product the 1-bromide is prepared using HBr/glacial acetic acid at 0°C (product: R_f = 0,58, same conditions as above). From this product the 1-alkyl-maltotrioside is prepared using allyl alcohol/Ag_2CO_3, product: R_f = 0,60, same conditions as above). From this product alkyl-2,3,6,2',3',6',2",3",4",6" deca-O-benzyl-maltotrioside is prepared using benzyl chloride/KOH at 120°C (product: R_f = 0,51, toluene/ethyl acetate 10:1 using silica gel). After isomerization to the enol ether the latter is saponified using 1N HCl to get the 1-OH compound (product: R_f = 0,17, toluene/ethyl acetate 10:1, using silica gel). From this product the trichloroacetimidate is prepared by reaction with NaH and trichloroacetonitrile (product: $R_{f\alpha}$ =0,48, same conditions as above). From this product the glykoconjugate is prepared in acetonitrile using ifosfamide mustard under reflux (product: R_f = 0,24, ethyl acetate/hexan 6:4, using silica gel). By hydrogenation with 10 % Pd/activated carbon in CH_3OH at ambient temperature the benzylic groups are split off (product:R_f =0,22, $CHCl_3$/methanol 1:1 using silica gel).

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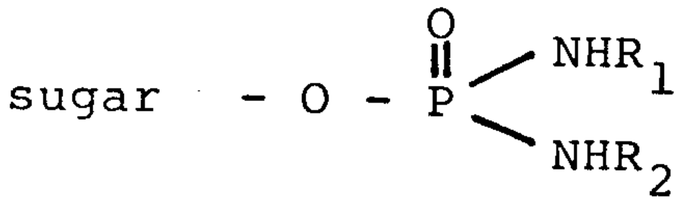
CLAIMS

1. Glycoconjugates of phosphorus amide mustard or ifosfamide mustard with the formula:

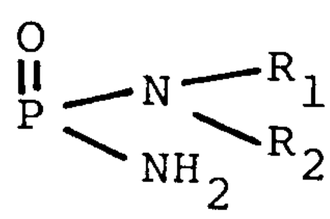
α-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
β-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
α-D-galactopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
α-D-mannopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
β-D-mannopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
4-O-(β-D-galactopyranosyl) α-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
4-O-(β-D-galactopyranosyl) N,N'-di(2-chloroethyl) phosphoric acid diamide,
4-O-(β-D-galactopyranosyl) β-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
4-O-(β-glucopyranosyl) α-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide,
4-O-(β-glucopyranosyl) β-D-glucopyranosyl N,N'-di(2-chloroethyl) phosphoric acid diamide.

2. Method for the preparation of glycoconjugates of phosphoric acid amide mustard and ifosfamide mustard with the general formula:



1

and



1a

where the sugar is linked to the phosphorus amide-lost residue or the ifosfamide-lost residue and where R_1 and R_2, which can be the same or different, denote hydrogen, lower C_1 - C_4 alkyl or C_1 - C_6 halogenoalkyl and where as sugar there can be present mono-, di-, or polysaccharides in all existing isomeric and enantiomeric forms, characterized by conjugating protected brominated sugars with the respective phosphorus compounds, and removing the protective residues.

3. The method of claim 2, wherein the sugar is linked to the phosphorus amide-lost residue and the infosfamide-lost residue in the 1-position.

4. The method of claim 2 or 3, wherein said C_1 - C_6 halogenoalkyl is C_1 - C_4 halogenoalkyl.

5. The method of claim 4, wherein said C_1 - C_4 halogenoalkyl is C_2-halogenoalkyl.

6. Use of the compounds according to any one of claims 1 to 5 as antitumour agents.

7. Use of the compounds according to claim 6 against breast carcinoma, Morbus Hodgkin, or tumours in the gastro-intestinal tract.

FIG. 1

Synthesis by the example of 28ß

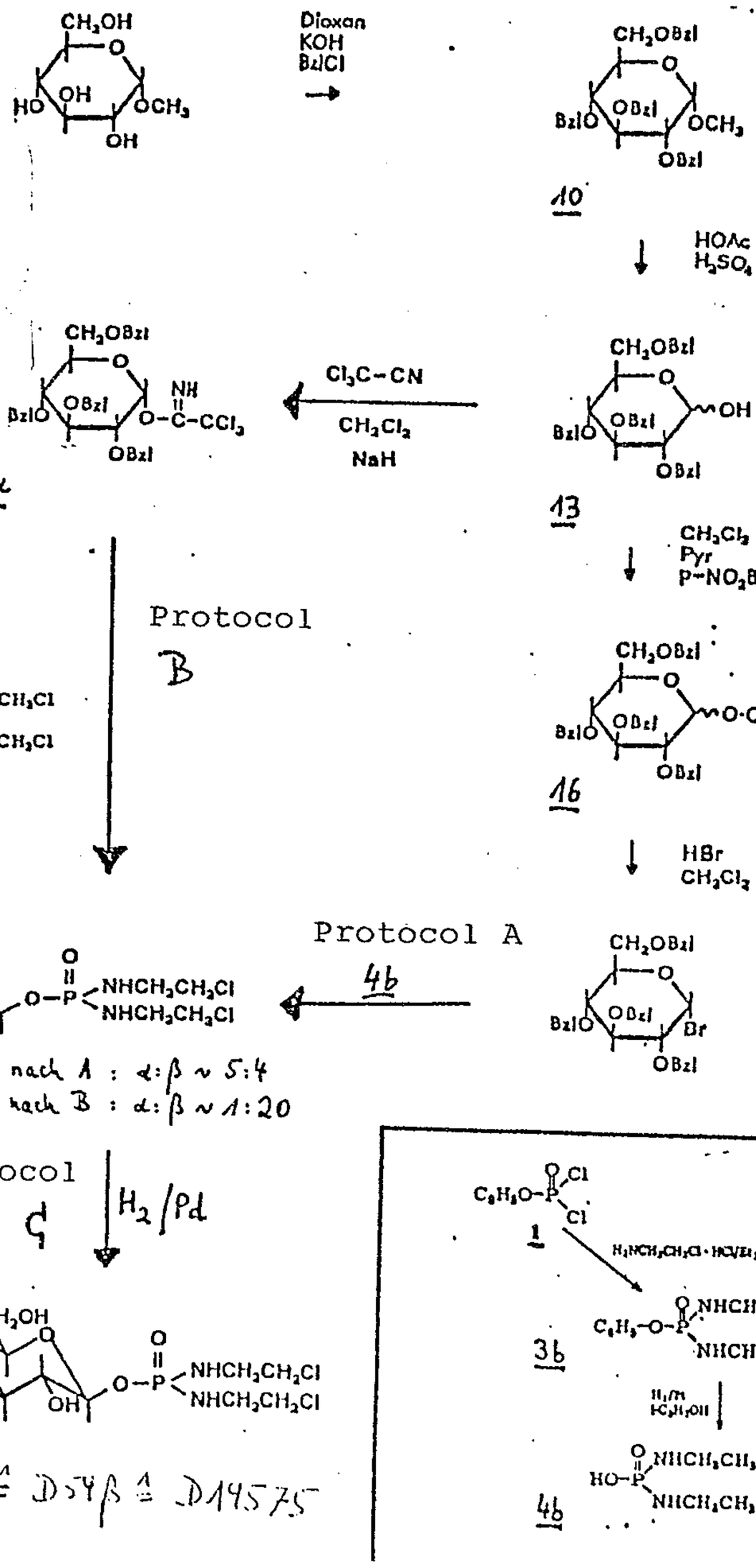


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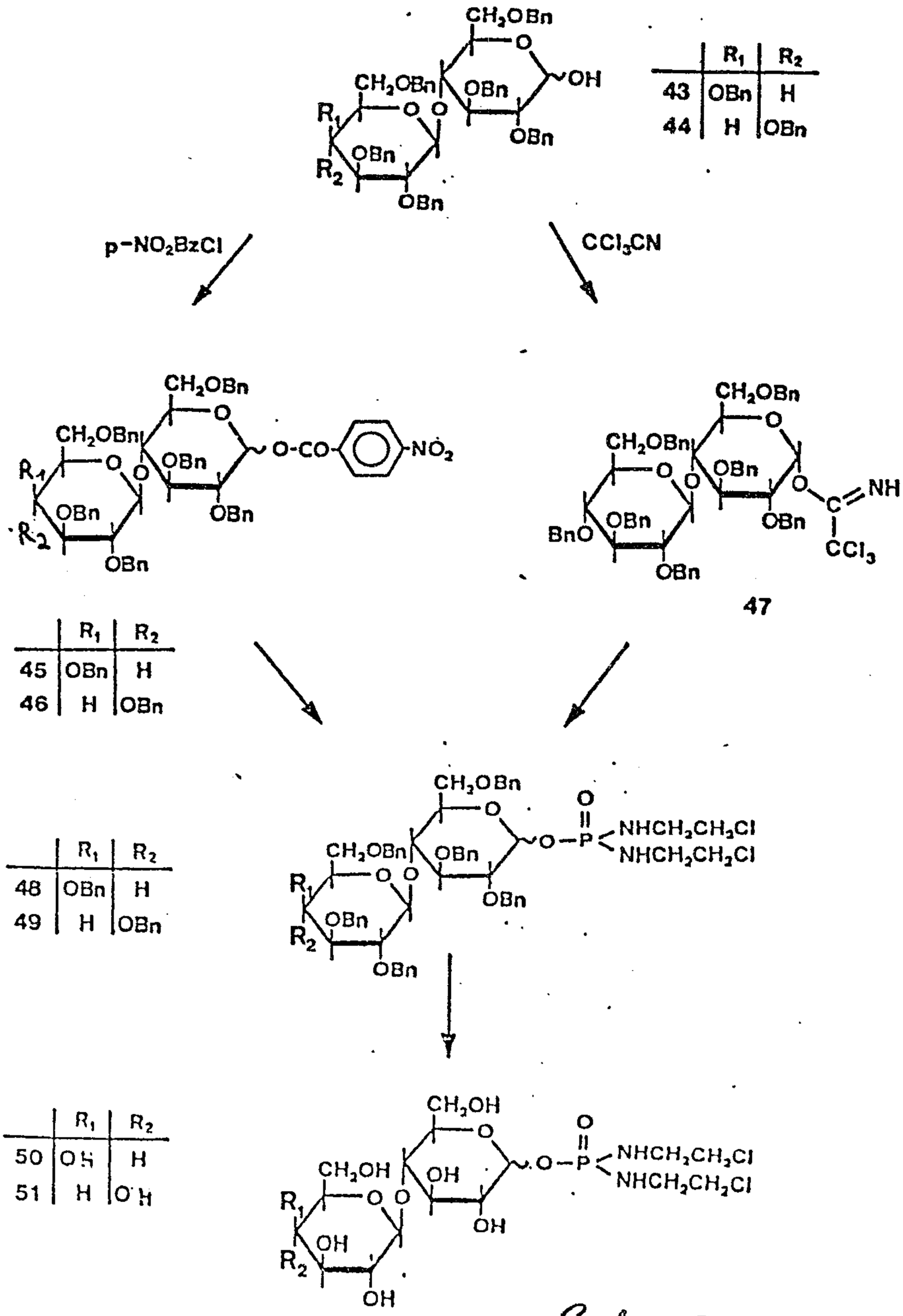
Fig. 2
Synthesis of the disaccharide-IPM conjugates 50 and 51 starting from the hepta-O-benzyl glycoses 43 and 44



	R_1	R_2
43	OBn	H
44	H	OBn

	R_1	R_2
45	OBn	H
46	H	OBn

	R_1	R_2
48	OBn	H
49	H	OBn

	R_1	R_2
50	OH	H
51	H	OH

47

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