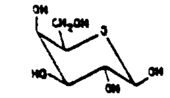
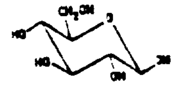
Glycoproteins with specific functions are found in a wide variety of natural sources

Glycoprotein	Source	Molecular weight	Carbohydrate content
Enzymes			
Alkaline phosphatase	Mouse liver	130,000	18%
Bromelain	Pineapple	33,000	36
Carboxypeptidase Y	Yeast	51,000	17
Hormones			
Chorionic gonadotropin	Human urine	38,000	31
Erythropoietin	Human urine	34,000	29
Lectins			
Potato		50,000	50
Soybean		120,000	6
Membrane constituents			
Glycophorin	Human erythrocytes	31,000	60
Hemagglutinin	Influenza virus	210,000	25
Rhodopsin	Bovine retina	40,000	7
Serum glycoproteins			
IgG immunoglobulin	Human serum	150,000	10
Thyroglobulin	Calf thyroid	370,000	8
Prothrombin	Human serum	72,000	8
Structural glycoproteins			
Collagen	Rat skin	300,000	0.4
Other			
Interferon	Human leukocytes	26,000	20

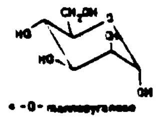
N. Sharon and H. Lis, Chem. and Eng. News, 21-44 (1981)



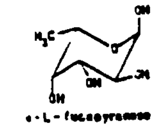
β-D-galactopyranose



β-D-glucopyranose



α-D-mannopyranose

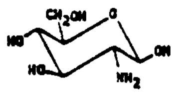


α-L-fucopyranose

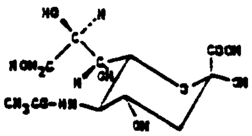
β-D-xylopyranose

β-L-arabinopyranose

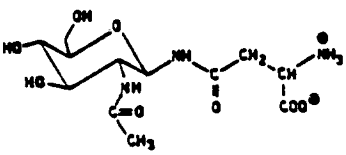
2-amino-2-deoxy-β-D-galactopyranose



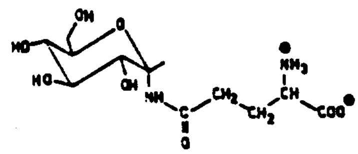
2-amino-2-deoxy-β-D-glucopyranose



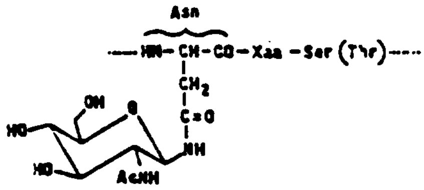
N-acetyl-neuraminic acid



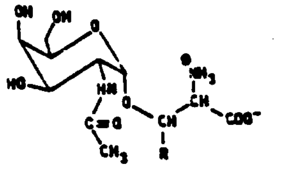
4-N-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-asparagine. Most common N-glycosidic bond present in plasmaproteins, immunoglobulins, hormones, hormone precursors, membrane glycoproteins, receptor glycoproteins, etc.



5-N-(α-D-glucopyranosyl)-L-glutamine
α-N-glucosidic bond present in a nephritogenic glycopeptide isolated from the glomerular basal membrane of rats. Glutamine has been suggested as the binding partner.

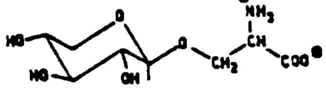


common sequence present in the connecting region of β-N-glycoproteins

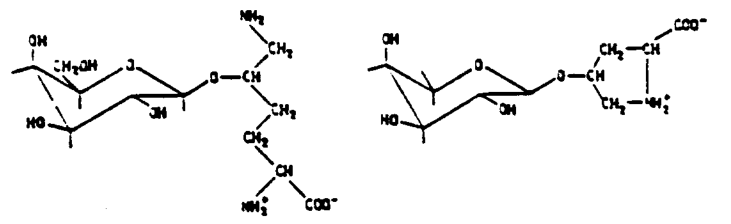


3-O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (R=H) or L-threonine ($R=CH_3$).

Characteristic of many mucous glycoproteins including the blood-group glycoproteins

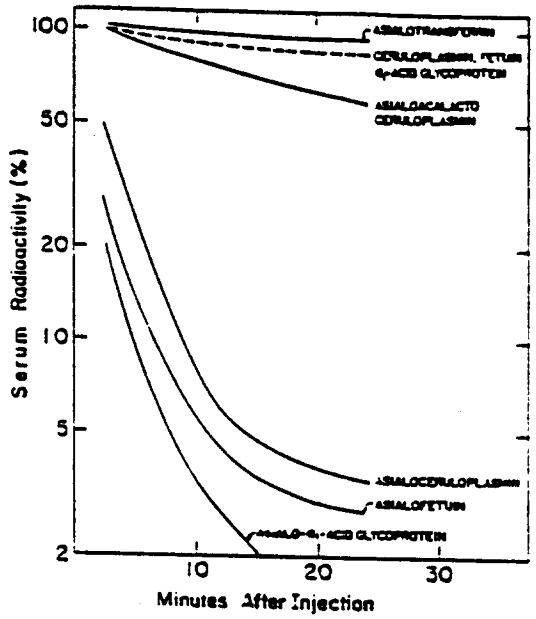


3-O-(β-D-xylopyranosyl)-L-serine
The β-O-glycosidic bond between xylose and serine is the characteristic connecting element of proteoglycans of the extracellular matrix and connective tissue.

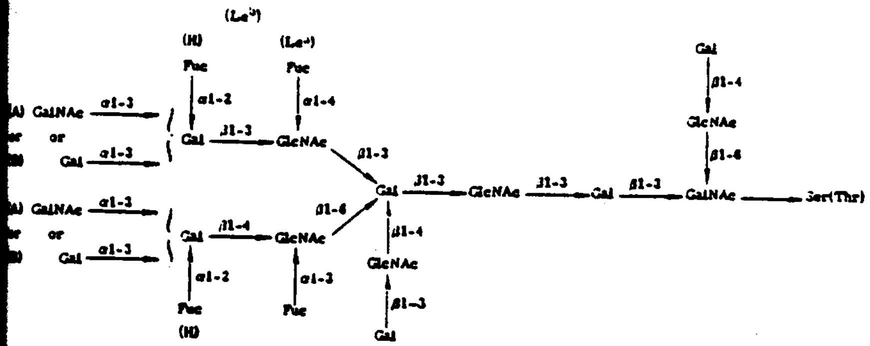


O-β-D-galattopiranosil 5-idrossi-L-lisina

O-β-D-arabinopiranosil-4-idrossi-trans-L-prolina

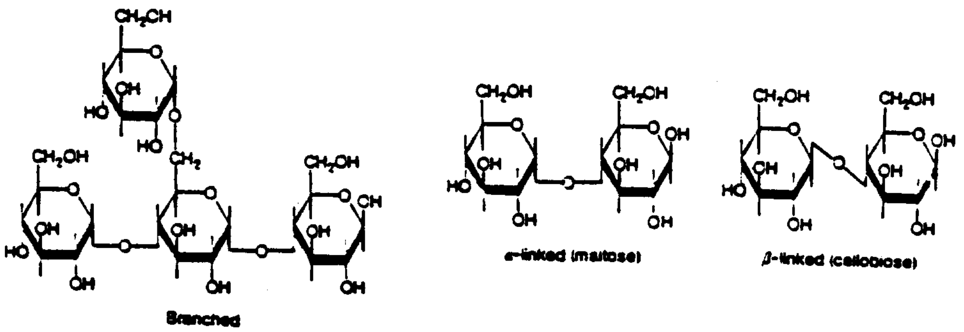


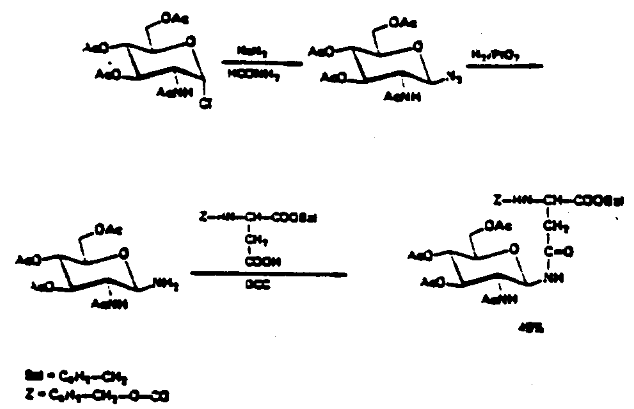
Disappearance from rabbit serum of radioactive labelled native and modified glycoproteins. [redrawn from the data of Ashwell G. and Morell A.G., Adv. Enzymol. 41, 99 (1974) by Sharon N. in "Complex Carbohydrates, Addison-Wesley Publishing Co. 1975]



Proposed composite oligosaccharide structure showing the relation of the various blood group determinants. (Modified from Lloyd and Kabat, 1968.)

Oligosaccharides have two ways of generating diversity not available to proteins or nucleic acids: α- or β-anomeric linkages and branching



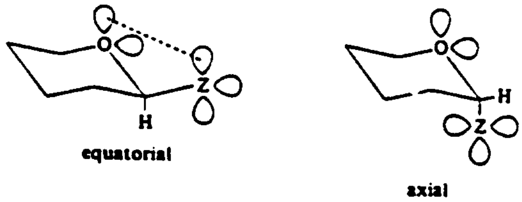


Formation of the β-N-glycosidic linkage between N-acetylglucosamine and asparagine (Redrawn from H. Kunz, Angew. Chem. Int. Ed. Engl. 25, 294 (1987)).

Oligosaccharides can have many more isomeric forms than peptides

Monomer composition	Product	Number of isomers: Peptides	Number of isomers: "Saccharides"[a]
X_2	Dimer	1	11
X_3	Trimer	1	176
XYZ	Trimer	6	1056

a Pyranose ring only. Source: Calculations by John Clamp (Biochem. Soc. Symp. 40, 3 (1974))



Interaction between ion pairs of the ring-oxigen atom and those of substituents at C-1. Z - halogen; when Z is OH, Or, or OAc there are only two ion pairs on the oxigen atom.

Neighbouring-group-assisted procedure

- β-glycosidic linkages in the D-gluco and D-galacto series
- α-glycosidic linkages in the D-manno series

In situ anomerization procedure

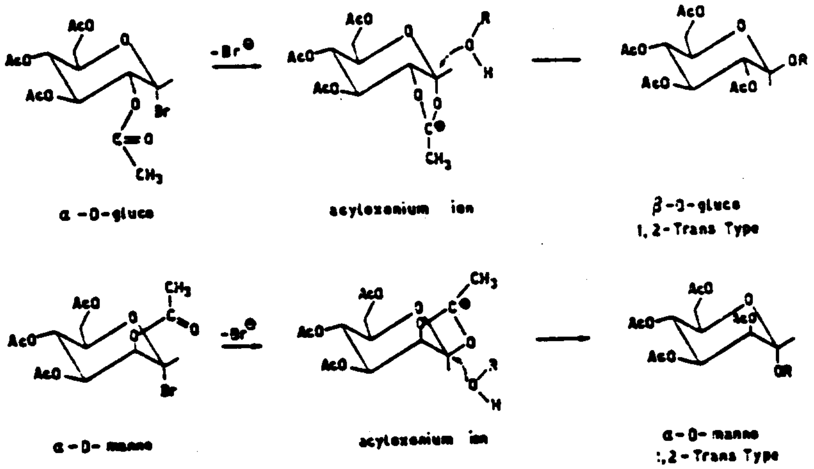
- α-glycosidic linkages in the D-gluco and D-galacto series

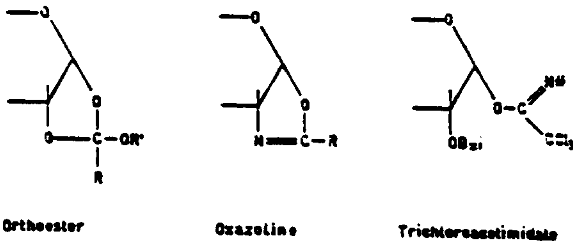
heterogeneous catalyst procedure

- β-glycosidic linkages in the D-manno series

from H. Paulsen, Chem. Soc. Review 13, 15 (1984).

Neighbouring Group Assisted Procedure

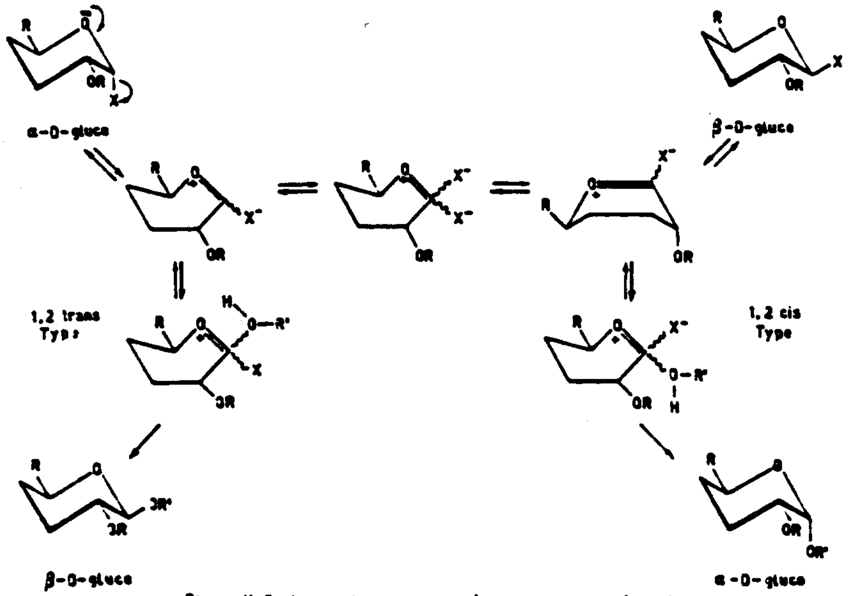




catalyst: $Hg(CN)_2$, $HgCl_2$, $AgClO_4$; Ag Triflate

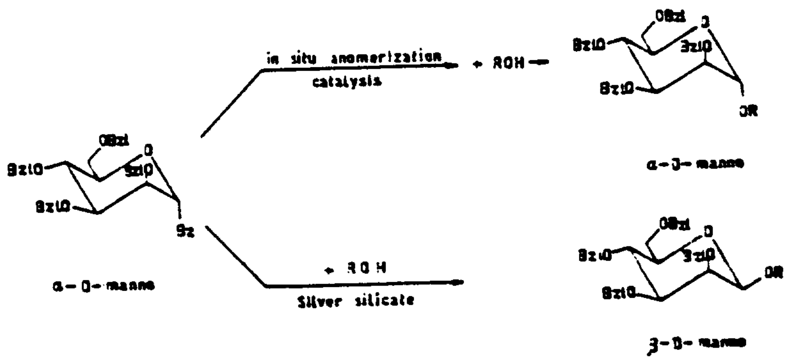
[redrawn from H. Paulsen, Chem. Soc. Review 13, 15-45 (1984)]

in situ Anomerisation Procedure



From H. Paulsen, Chem. Soc. Review 13, 15-45 (1984)

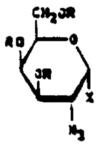
Heterogeneous Catalyst Procedure



Redrawn from H. Paulsen, Chem. Soc. Review 13, 15-45 (1984).

Reactivity parameters determining selectivity and yield in oligosaccharide syntheses

Halogen



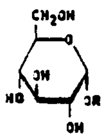
$R \sim Bzl = Bz > Ac$
$X \sim I > Br > Cl$

Catalyst

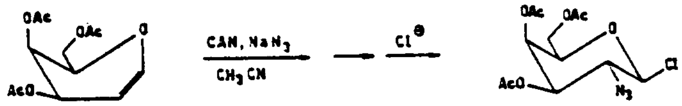
- Et_4NBr/molecular sieve (4 Å)
- $Hg(CN)_2$
- $Hg(CN)_2\ HgBr_2$
- $HgBr_2$/molecular sieve (4 Å)
- $AgClO_4$, Ag_2CO_3
- Ag triflate, Ag_2CO_3

Alcohol

$HO-Me >> HO-CH_2R > 6-OH$
$6-OH >> 3-OH > 2-OH > 4-OH$



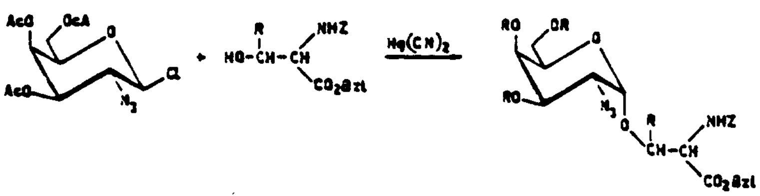
H. Paulsen, Chem. Soc. Review 13, 15-45 (1984).



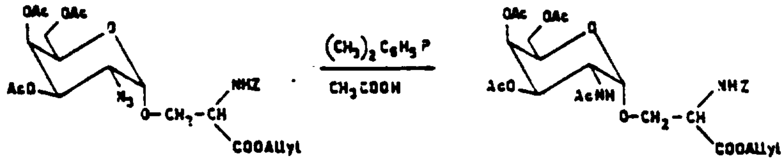
D-galactal triacetate

2-deoxy-2-azido-3,4,6-Tri-O-acetyl-β-D-galactopyranosyl chloride

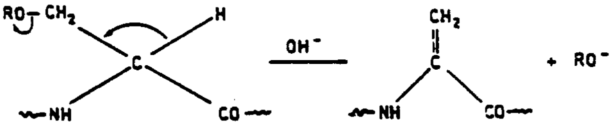
Synthesis of 2-azido-glycosyl halides by the azido nitration route; CAN = cerium ammonium nitrate.
H. Paulsen, C. Kolar, W. Steuzel, Chem. Ber. III, 2370 (1978); R.U. Lemieux, R.M. Ratcliff. Can. J. Chem. 57, 1224 (1979).



Serine (R=H); 66% - Threonine(R=CH_3); 45%. Other catalyst: $Ag_2CO_3/AgClO_3$



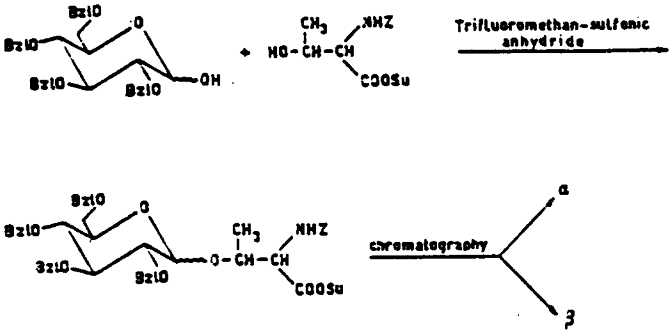
Conversion of the azido group into the acetamido group with dimethyl-phenyl-phosphane in glacial acetic acid.
H. Kunz, Proc. München-Shanghai Symp. Pept. Protein Chem. 45 (1986).



seril glicoside

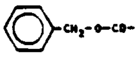
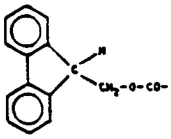
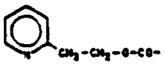
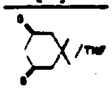
residuo 2-ammino propenoilico

β - eliminazione

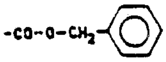


J.N. Lacombe et al. Can J. Chem. 59, 573 (1981).

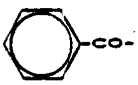
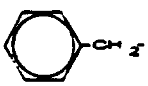
Amino-protecting groups

	Z	benzyloxycarbonyl	H_2/Pd
$(CH_3)_3C-O-CO-$	Boc	Tert-butyl-oxycarbonyl	acid labile
	Fmoc	(9-fluorenyl) methyloxycarbonyl	Base labile
$(C_6H_5)_3P^{+}-CH_2-CH_2-O-CO-$ Cl^-	Peoc	2-Triphenylphosphonioethoxy carbonyl	Base labile
	Pyoc	2-pyridylethyloxycarbonyl	1) CH_3I/CH_3CN 2) morpholine/CH_2Cl_2
$CH_2=CH-CH_2-O-CO-$	Aloc	allyloxycarbonyl	[Pd°]  /THF

Carboxy-protecting groups

	Bzl	benzyl ester	H_2/Pd
$-CO-O-(CH_3)_3$	Bu^t	tert-butyl ester	acid labile
$-CO-O-CH_2-CH_2-Br$		2-bromoethyl ester	1) NaI/acetone Zn/DMF 2) electrochemically
$-CO-O-CH_2-CH=CH_2$		allyl ester	$[(C_6H_5)_3P_3]$ RhCl 70°C, C_2H_5OH/H_2O; $[(C_6H_5)_3P]_4$Pd morpholine/THF, 20°, 30 min.; [Pd°] morpholine/THF

Sugar-hydroxyl protecting groups

CH_3-CO-	Ac	base labile
	Bz	base labile
	Bzl	H_2/Pd

Bibliografia

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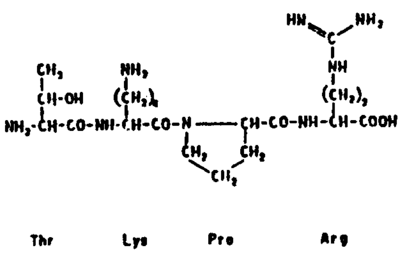
- J. Montreuil in "Adv. Carboydr. Chem. Biochem.", (R.S. Tipson and D. Horton, eds.), Vol. 37, Academic Press, New York, 1980, p. 157.
- C.P. Stowell and Y.C. Lee in "Adv. Carbohydr. Chem. Biochem.", (R.S. Tipson and D. Horton, eds.), Vol 37, Academic Press, New York, 1980, p. 225.
- N. Sharon and H. Lis in "The Proteins" (H. Neurath and R. Hill eds.) Vol. V, Academic Press, New York, 1982, p. 1.
- R. Rocchi and V. Giormani in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins", (B. Weinstein, ed.), Vol. 7, Marcel Dekker Inc., New York, 1983, p. 35.
- H. Paulsen in "Chemical Society Reviews" Vol. 13, The Royal Society of Chemistry, London, 1984, p. 15
- H. Kunz in " Angew. Chem. Int. Ed. Engl." Vol. 26, 1987, p. 294

TUFTSIN



Tuftsin's Biological Activities

- Enhancement of phagocytosis
- Stimulation of motility of granulocytes
- Increase of respiratory burst of phagocytes
- Stimulation of chemotaxis
- Influence on antibody formation
- Stimulation of immunogenic function of macrophages
- Promotion of bactericidal activity of macrophages
- Promotion of tumoricidal activity of macrophages

from J. Martinez et al., Int. J. Peptide Protein Res. 22 (1983) 119

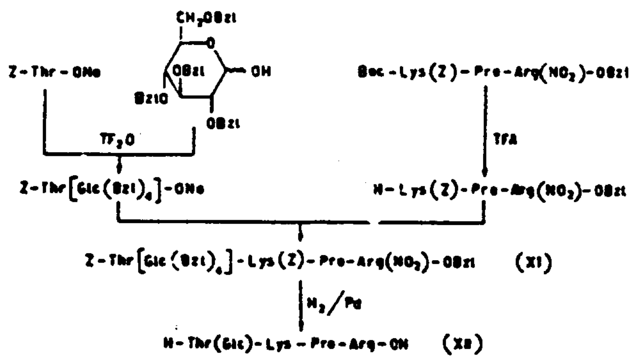


FIGURE 3
Synthesis of *O*-glucosyltuftsin.

Rocchi *et al.* *Int. J. Peptide Protein Res.* 29, 1987, 250–261

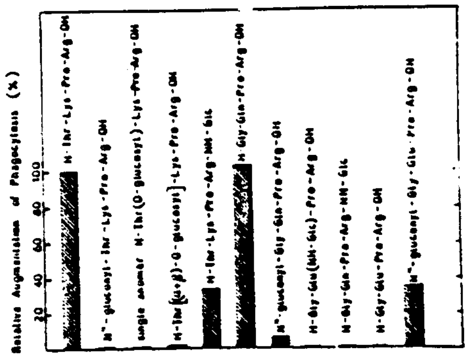


FIGURE 8

Effect of peptides on augmentation of phagocytosis. Peptides were applied to macrophages at concentration of 5 × 10⁻⁸ M. Uptake of ^{51}Cr-IgG-SRBC by the cells was measured. 100% augmentation refers to the augmentation of phagocytosis of a tuftsin concentration of 5 × 10⁻⁸ M. Zero stimulation refers to phagocytosis by macrophages without tuftsin.

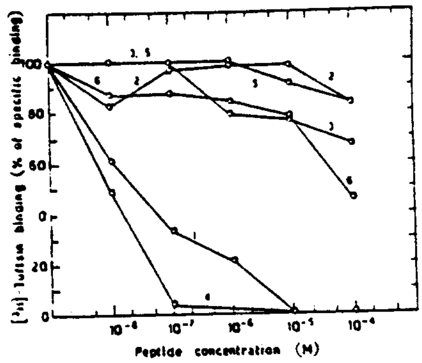


FIGURE 9

Displacement of [^{3}H]-tuftsin binding to macrophages by tuftsin, rigin and by different tuftsin analogs. Macrophages were incubated with tritiated tuftsin. The different peptides were then added at the indicated concentrations. 100% specific binding refers to the amount of [^{3}H]-tuftsin displaced by 10 uM tuftsin. 1) H-Thr-Lys-Pro-Arg-OH; 2) N^{α}-Gluconyl-Thr-Lys-Pro-Arg-OH; 3) H-Thr[O-glucosyl)-Lys-Pro-Arg-OH (single anomer); 4) H-Thr-[(α + β)-O-glucosyl]-Lys-Pro-Arg-OH; 5) H- Thr-Lys-Pro-Arg-NH-Glc; 6) H-Gly-Gln-Pro-Arg-OH.

Structure of vespulakinin 1

```
Carbohydrate(1)  Carbohydrate(2)                  bradykinin
         |        |                  _____________________________________
Thr-Ala-Thr-Thr-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
```

Carbohydrate (1): NAc-galactosamine 1 - 2, galactose 1

Carbohydrate (2): NAc-galactosamine 2 - 3, galactose 2

Vespulakinin 2 lacks the amino terminal Thr-Ala and has the same carbohydrate composition as vespulakinin 1.

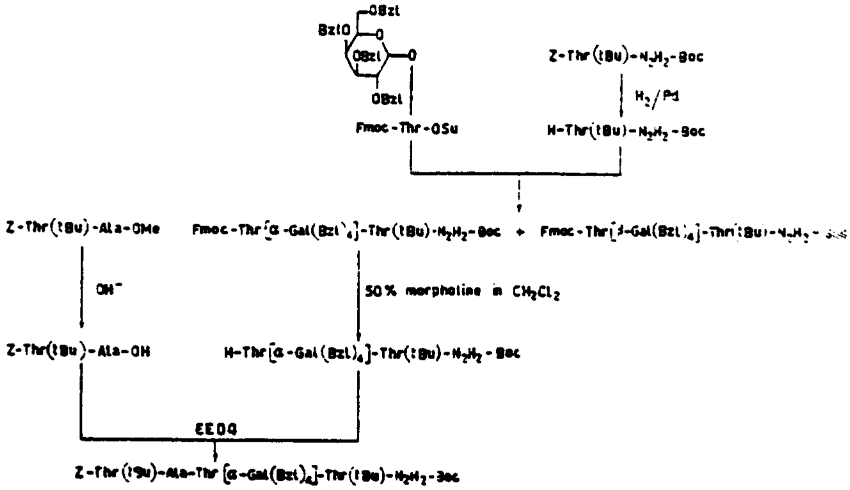
OPTICAL ROTATION OF CARBOHYDRATE-FREE VESPULAKININ 1
PREPARED BY DIFFERENT SYNTHETIC PROCEDURES

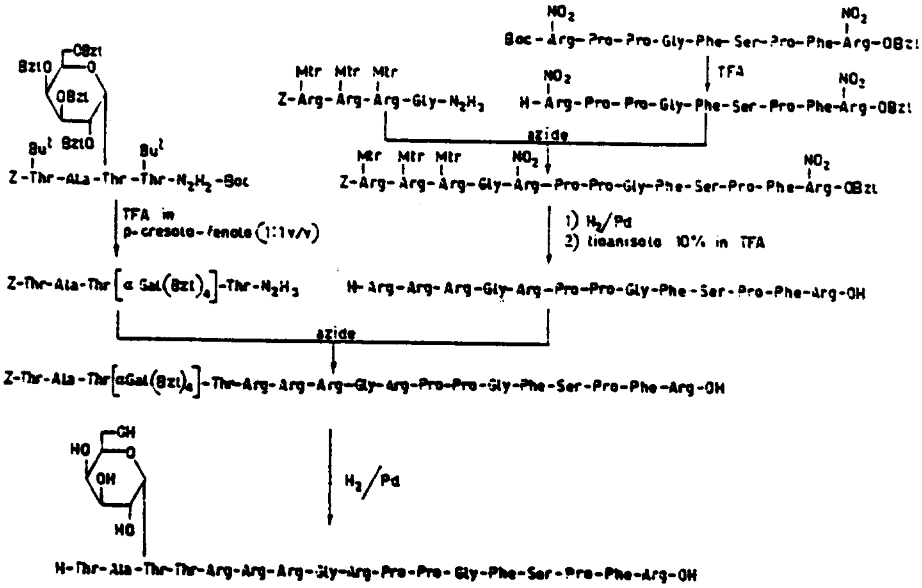
$[\alpha]_D^{25°}$ −78.6° (c 1.0, water) solid phase synthesis

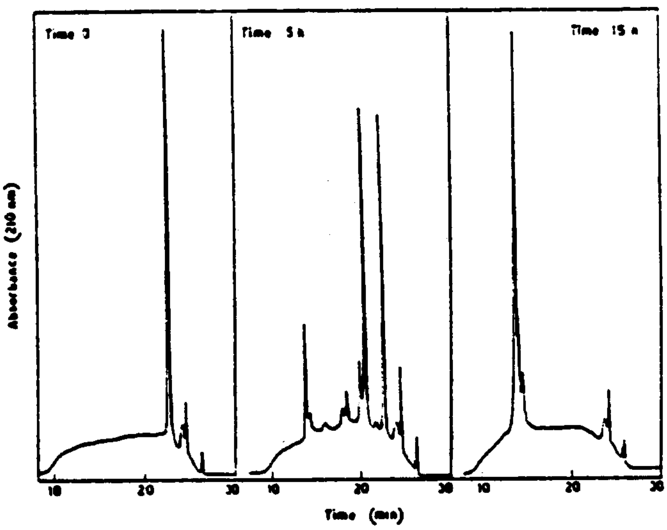
$[\alpha]_D^{25°}$ −74.5° (c 1.0, water) combination of solid phase and solution synthesis

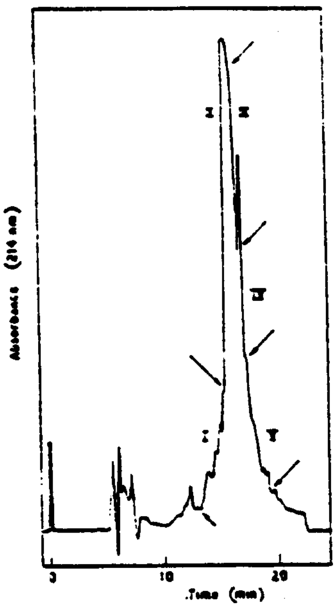
$[\alpha]_D^{25°}$ −76.5° (c 0.65, water) solution synthesis

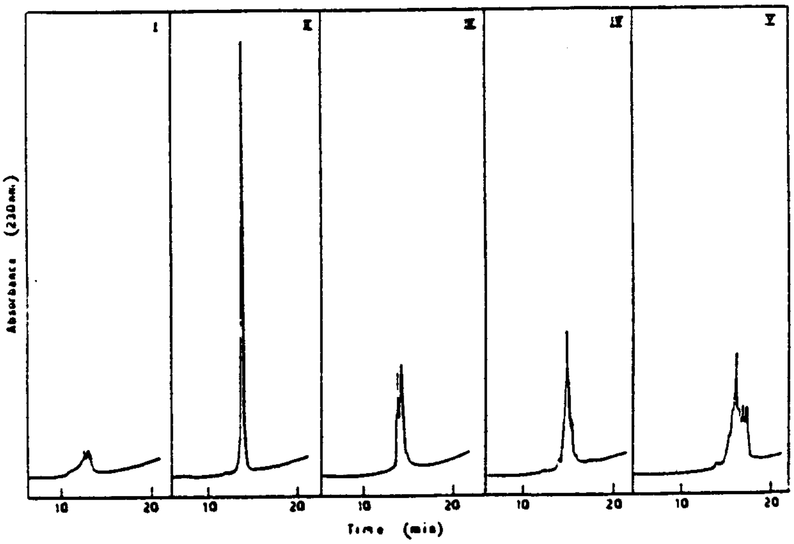
```
carbohydrate  carbohydrate                     bradykinin
      |           |                  ___________________________________
H-Thr-Ala-Thr-Thr-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
  1                                                                17
```

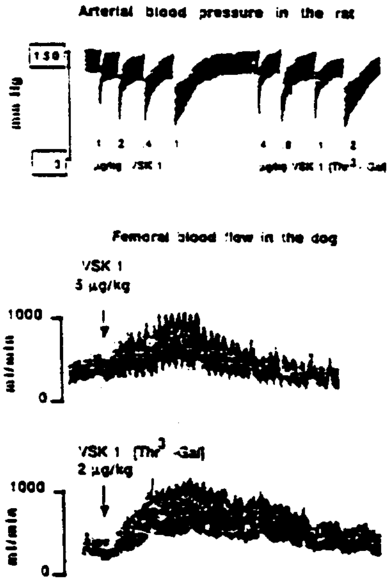

Z-Thr(tBu)-N2H2-Boc
H2/Pd
Fmoc-Thr-OSu
H-Thr(tBu)-N2H2-Boc
Z-Thr(tBu)-Ala-OMe
Fmoc-Thr[α-Gal(Bzl)4]-Thr(tBu)-N2H2-Boc + Fmoc-Thr[β-Gal(Bzl)4]-Thr(tBu)-N2H2-Boc
OH⁻
50% morpholine in CH2Cl2
Z-Thr(tBu)-Ala-OH
H-Thr[α-Gal(Bzl)4]-Thr(tBu)-N2H2-Boc
EEDQ
Z-Thr(tBu)-Ala-Thr[α-Gal(Bzl)4]-Thr(tBu)-N2H2-Boc


Boc-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OBzl
TFA
Z-Arg-Arg-Arg-Gly-N2H3
H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OBzl
azide
Z-Thr-Ala-Thr-Thr-N2H2-Boc
Z-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OBzl
TFA in p-cresolo-fenolo (1:1v/v)
1) H2/Pd
2) tioanisolo 10% in TFA
Z-Thr-Ala-Thr[α Gal(Bzl)4]-Thr-N2H3
H-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
azide
Z-Thr-Ala-Thr[αGal(Bzl)4]-Thr-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
H2/Pd
H-Thr-Ala-Thr-Thr-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

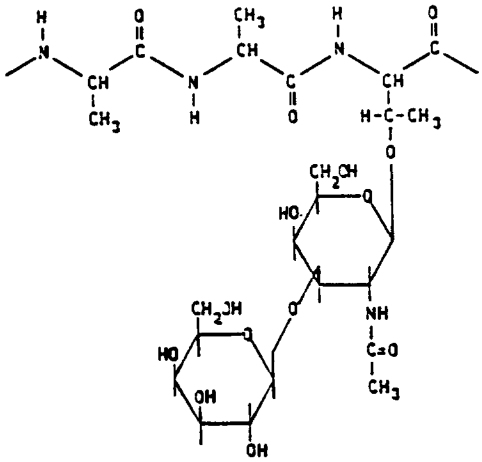

Time 0
Time 5 h
Time 15 h
Absorbance (210 nm)
Time (min)


Absorbance (214 nm)
Time (min)





At least in dogs, when injected intravenously [Thr[3]-Gal]-VTSK 1 appears to display a longer half-life than the carbohydrate-free VSK 1 as may be evaluated from the duration of the respective femoral blood flow responses.
On blood pressure of rats the potency of hypotensive activity of VSK 1 appears to be two times higher than that of glycosilated analog.



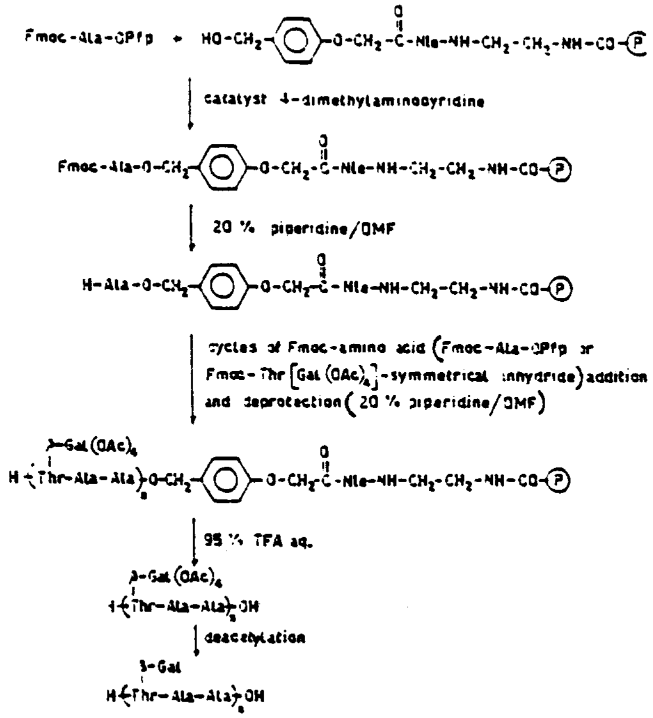
Polymer unit of the active antifreeze glycoprotein

From J.R. Vandenheede et al., J. Biol. Chem 247, 7885 (1972)

GENERAL PROPERTIES OF ANTIFREEZE GLYCOPROTEINS OF TREMATOMUS BORCHGREVINKI

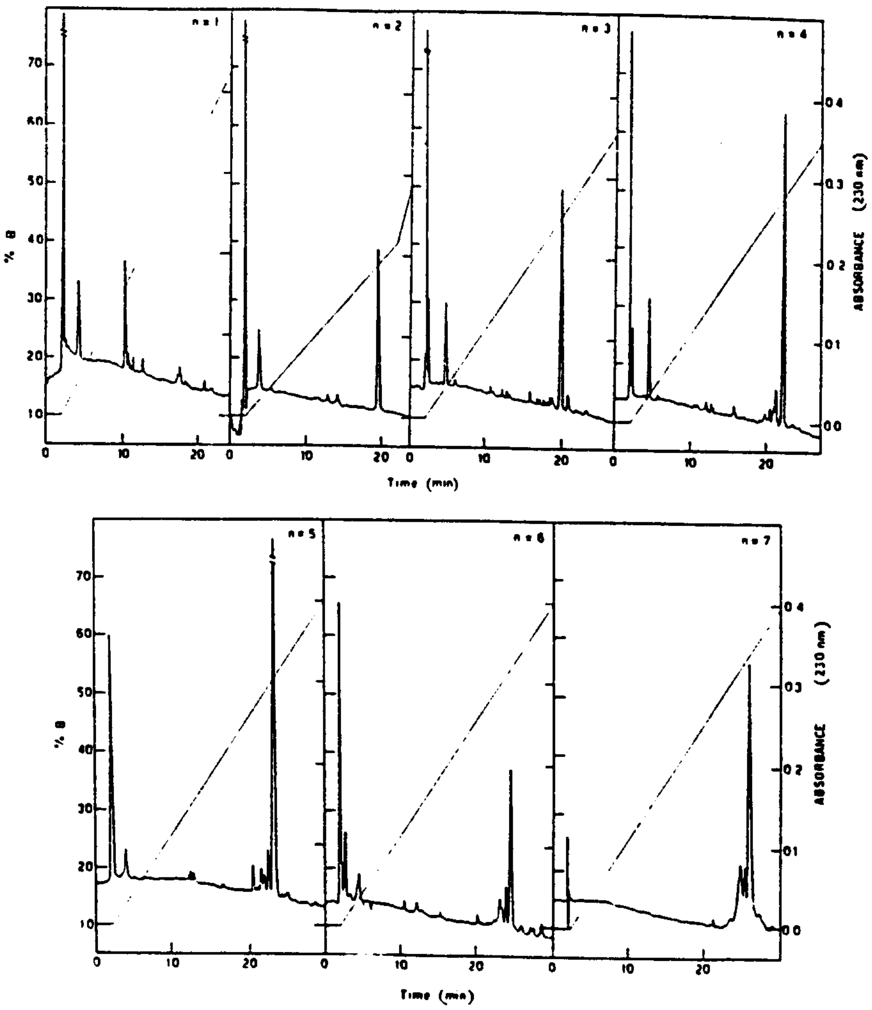
	Antifreeze glycoproteins							
	1	2	3	4	5	6	7	8
Molecular weight $\times 10^{-3}$	32	29	22	18	11	7.8	4.6	2.7
N° of triglycopeptides	50	45	35	28	17	12	7	4
Antifreeze activity	strong	strong	strong	strong	strong	none	none	none

From R.E. Feeney and Y. Yeh - Advances in Protein Chemistry vol. 32, p. 191 (1978)
Values for glycoprotein 1 are less accurate than the others, for glycoproteins 2-4 are probably correct within 5%, those for glycoproteins 6-8 are slightly more accurate.
Glycoproteins 6-8 have prolines.

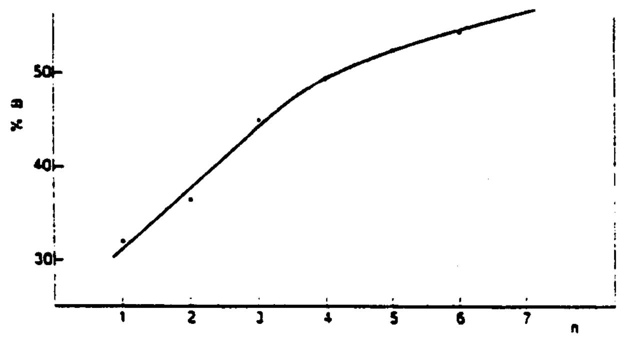


Acylation and deprotection times in the synthesis of [Thr (O-β-D-2,3,4,6-tetra-O-acetyl-galactopyranosyl)-Ala-Ala]$_7$

Amino acid	Acylation time (min)	Deprotection time (min)	Amino acid	Acylation time (min)	Deprotection time (min)
Ala 1	30	10	Ala 11	40	13
Ala 2	25	10	Thr 12	70	13
Thr 3	60	10	Ala 13	45	13
Ala 4	25	10	Ala 14	50	13
Ala 5	35	10	Thr 15	70	13
Thr 6	50	10	Ala 16	55	13
Ala 7	30	10	Ala 17	55	13
Ala 8	30	10	Thr 18	70	15
Thr 9	60		Ala 19	60	15
Thr 9 (repeated)	30	13	Ala 20	65	15
Ala 10	40	13	Thr 21	80	20



Analytical HPLC elution profiles of crude H-{Thr[β-D-Gal(OAc)$_4$]-Ala-Ala}$_n$-OH. Column Aquapore octyl RP-300 (4.6 × 220 mm). Eluant A, aqueous 0.1 % trifluoroacetic acid; B, 90 % acetonitrile in A. Flow rate 1.5 ml/min



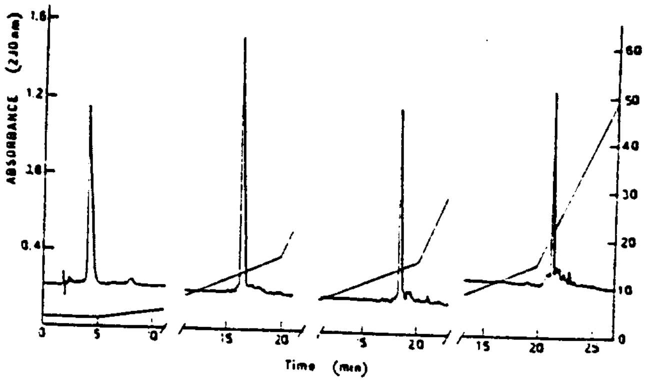
Variation in the percent of eluant B needed for eluting crude H-{Thr[β-D-Gal(OAc)$_4$]-Ala-Ala}$_n$-OH. Column Aquapore octyl RP-300 (4.6 × 220 mm). Eluant A, aqueous 0.1 % trifluoroacetic acid; B, 30 % acetonitrile in A. Flow rate 1.5 ml/min.

Alanine/Threonine ratios (r) in the crude H-{Thr[β-D-Gal(OAc)$_4$]-Ala-Ala}$_n$-Pepsyn KA

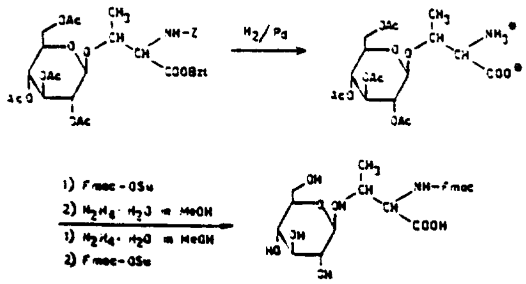
n	1	2	3	4	5	6	7
r	1.96	1.94	2.00	1.92	2.00	2.00	1.96

Amino acid analyses for synthetic
H-{Thr[β-O-Gal (OAc)$_4$]-Ala-Ala}$_n$-OH after HPLC purification.

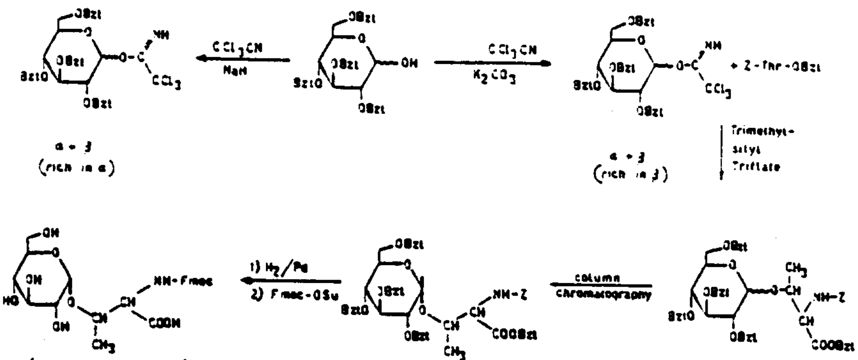
n	1	2	3	4	5	6	7
Ala	1.98	3.91	6.09	8.03	9.91	11.92	13.75
Thr	1.02	2.09	2.93	3.97	5.09	6.08	7.25



Analytical HPLC elution profiles of crude H-[Thr(β-O-Gal)-Ala-Ala]$_n$-OH. Column Aquapore octyl RP-300 (4.6 x 220 mm). Eluant A, aqueous 0.1% trifluoroacetic acid; B, 30% acetonitrile in A. Flow rate 1.5 ml/min.



Preparation of Fmoc-Thr (O-β-D-glucopyranosyl)-OH
[or Fmoc-Thr (O-β-D-galactopyranosyl)-OH]



— Fmoc-Thr(O-α-D-glucopyranosyl)-OH

α

$[\alpha]_D^{25°}$ + 35.8° (c 0.65, $CHCl_3$)*

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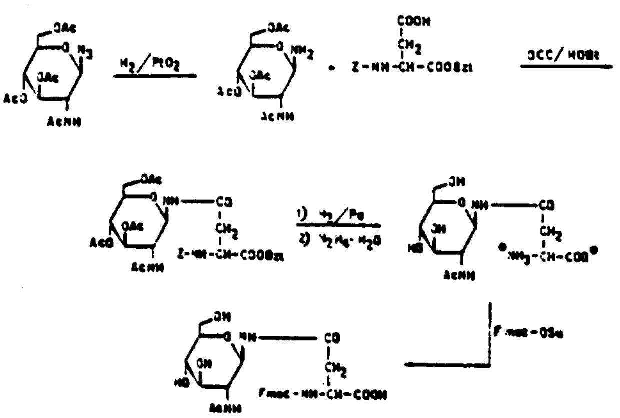
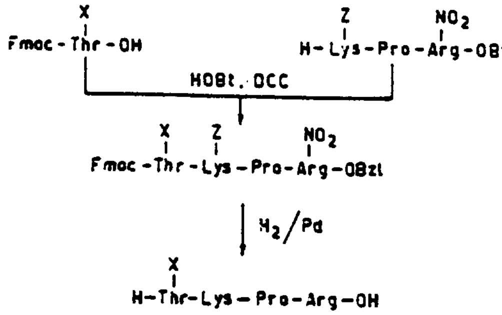
* Lemieux et al. (1981) - Can. J. Chem. 59, 473-481

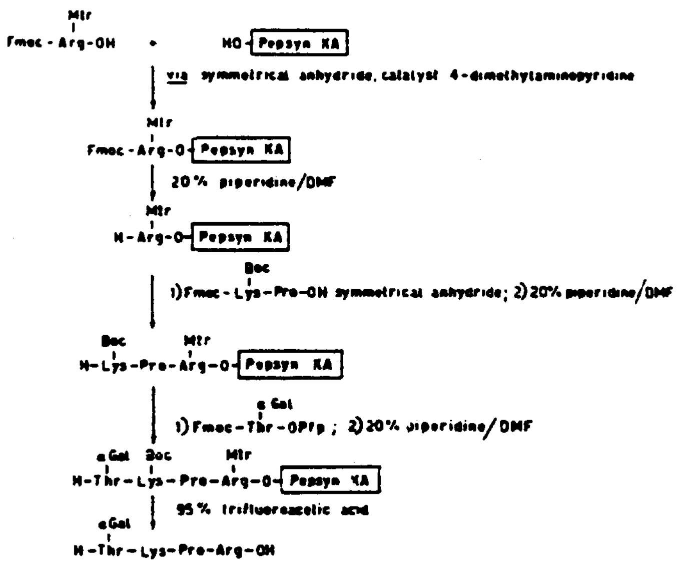
α, $[\alpha]_D^{22°}$ + 40° (c 0.4, $CHCl_3$); β, $[\alpha]_D^{22°}$ + 14.5° (c 2.53, $CHCl_3$)



4-N-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-L-asparagine



Solution synthesis of O-glycosylated tuftsins X = α-Glc, β-Glc, α-Gal.



Continuous flow solid phase synthesis of α-Gal-tuftsin on 4-hydroxymethylphenoxyacetyl-
-norleucyl derivatized polydimethyl-acrylamide kieselguhr supported resin (Pepsyn KA)

.....the separation of chemistry from biology was necessary while experimental methods and theories were being developed. Now that our science is provided with a powerful armoury of analytical and synthetic weapons chemistry can once again renew the alliance with biology, not only to the advantage of biology but also for the "glory" of chemistry.

Emil Fisher - Faraday Lecture to the Chemical Society, London, 18 October 1907