Glycosylated Derivatives of Benzophenone, Benzhydrol, and Benzhydril as Potential Venous Antithrombotic Agents

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A series of glycosylated derivatives of benzophenone, benzhydrol, and benzhydril has been synthesized and evaluated for potential activity as venous antithrombotic agents. Studies on structure–activity relationships revealed that compounds having an electron-withdrawing group in the benzhydril or benzhydrol moiety, and specifically those having the β-D-xylopyranosyl structure in the sugar moiety, were good antithrombotic agents in a rat model of venous thrombosis.

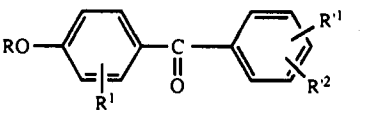
Introduction

Heparin, a glycosaminoglycan component of a parent proteoglycan, is a highly sulfated polydisperse polysaccharide that has been used for over half a century as an anticoagulant and antithrombotic agent.[1] Heparin exhibits the undesirable side effect of hemorrhagic complications after intravenous injection.[1a,2] Thus, although heparin enjoys widespread clinical use, it has been cited as the drug most responsible for death in otherwise healthy patients.[3]

The relatively recent introduction of low-molecular-weight (LMW) fractions of heparin obtained by various means (fractionation, depolymerization) has provided therapeutic agents offering a supposedly decreased risk of hemorrhage.[4] Fractions having a molecular weight of less than 5000 retain their ability to bind to antithrombin III (AT III) and to inhibit activated factor X (Xa), but the antithrombin activity is greatly decreased.[5] Furthermore, the therapeutic potential of these compounds is improved because of their more favorable bioavailability, even after subcutaneous administration.[6] Dermatan sulfate (DS), a heterogeneous glycosaminoglycan that is also a component of a parent proteoglycan, had been shown experimentally to be antithrombotic, even though it is devoid of AT III-binding activity, the compound inhibiting thrombin via heparin co-factor II (HC II).[7] Thus, although this compound elicits a somewhat less potent antithrombotic effect, the risk of hemorrhage is virtually absent.[8] However DS has to be obtained by tedious extraction and is only effective when administrated parenterally.[9] Although DS is absorbed intact, its bioavailability is low, and therapeutically active blood levels are rarely obtained.[10]

This paper describes for the first time the venous antithrombotic effect of a chemically defined entities following intraperitoneal, oral, and intravenous administration in the rat.

Chemistry: see Scheme I. As a part of broader study on venous antithrombotic agents, a number of novel glycosylated benzophenone derivatives having the formula I were selected for general screening. Various methods for producing glycosides in a highly stereoselective manner have been reported.[11] In the present work, the classical Koenigs–Knorr reaction[11] employing Ag_2O as activator



R = glycosyl

R^1, R'^1, R'^2 = H, alkyl, halo, NO_2, CF_3, CN, OMe

was the most commonly used (method B). In some cases, NaH was selected as the base for the glycosidation reaction (method A). The 1,2-cis glycosides **11** and **12** were prepared from the per-*O*-acetylglycopyranose together with a Lewis acid such as $SnCl_4$ as activator for the glycosidation[12] (method C).

The target benzhydrol (V) and benzhydril glycosides (VI) were prepared from the appropriate protected glycosyl precursors and benzophenone derivatives (I).[13] The benzophenone phenols were obtained by condensation of the Grignard reagent of the appropriate substituted anisoles with an aromatic acyl chloride[14] (except for the nitro derivative of benzophenone), followed by demethylation in acid medium of the resultant methoxy ether. The 4′-nitro-3-methoxybenzophenone was obtained by the reaction of 4-fluoro-1-nitrobenzene and a substituted morpholine.[15] The latter was synthesized from 3-methoxybenzaldehyde, morpholine, and potassium cyanide in acid medium.[15] The target benzophenone phenols were obtained in excellent yields, and their physical constants are summarized in Table I.

The methods of synthesis and data for the glycosylated products are summarized in Table II.

Two routes were chosen to obtain the benzhydril glycosides (VI). First, the benzophenone β-D-xylopyranosides (II) were reduced with sodium borohydride in trifluoroacetic acid[16] with subsequent deacylation by the Zemplén method,[17] affording compounds VI in good to excellent yield. Alternatively, the β-D-xylopyranosides (IV) were deacylated and then reduced to the benzhydrols (V) with sodium borohydride in methanol to give the latter as mixtures of epimers without induction of reduction in excellent yield.[18] The benzhydrils (VI) were then obtained by reduction of the benzhydrols (V) in the manner used for reduction of the benzophenones to the benzhydril.

Pharmacology Evaluation

1. **Technique.** The technique employed involves partial stasis in the presence of slight endothelium

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Scheme I

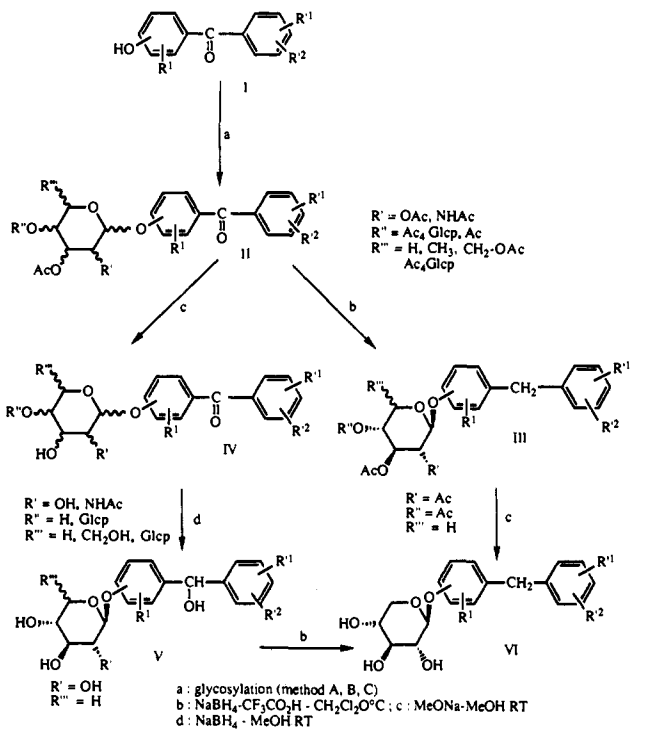
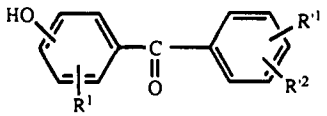


Table I



position of OH	R'1	R'2	R1	mp, °C
4	2'-CH_3	H	3-CH_3	142
3	4'-NO_2	H	H	117
3	2'-CH_3	H	H	112
2	2'-CH_3	H	5-CH_3	95

alteration, as previously described by Millet et al.[19] Male Wistar rats (IFFA CREDO, France) weighing 250 g are anaesthetized with urethane (10 mL/kg body weight of a 12% urethane solution by intraperitoneal injection). A midline laparotomy is performed, and abdominal organs are displayed on saline-impregnated gauze. The inferior vena cava is cleared from surrounding tissues from the left renal vein to the iliac vein. A partial ligature just beneath the left renal vein is made with a cotton thread enclosing the vein together with a G 26 3/8-in. gauge needle. A Schwartz clamp is placed just above the iliac bifurcation to delimit a venous bag.

Saline is flushed into this bag at a constant rate of 10 mL/min during 15 s at a point just above the clamp. Thereafter, the perfusion needle is removed and the hole sealed with a cyanoacrylate adhesive, after which the needle and the clamp are removed. Organs are replaced, and the body temperature of the rats was maintained at a temperature close to 37 °C. Fifteen minutes after the partial venous flow has been restored, two Schwartz clamps are placed, one at the level of the structure and the other at the iliac bifurcation. The vein segment is split longitudinally, and the thrombus is removed and dried for 24 h at 55 °C before weighing.

The activity of these compounds is given as percentage reduction of thrombus formation by weight.

2. Treatment. The compounds were administered intraperitoneally to rats with (carboxymethyl) cellulose as vehicle at doses of 100 mg/kg in a volume of 5 mL/kg for the substances of which the lethal dose exceeded 800 mg/kg (in mice). Otherwise antithrombotic activity was assessed at $^1/_{10}$ of the LD_{50}.

The heparin used as reference compound was monitored by iv route at various time intervals before thrombogenic challenge with factor Xa, a dose-related antithrombotic effect, this being significant from the dose 0.12 mg/kg and producing an ED_{50} (dose inhibiting the effect of factor Xa by 50%) of 0.15 mg/kg.

Results and Discussion

As may be seen from Table III, significant antithrombotic activity, although admittedly low by the intraperitoneal route, was displayed solely by the D-xylopyranosides having the β configuration at the anomeric carbon atom. Other sugar structures and configurations were inactive. The results shown in Table III prompted the synthesis of a number of β-D-xylopyranosides of benzophenone derivatives for systematic evaluation of structure–activity relationships. The antithrombotic activity of these compounds is summarized in Table IV.

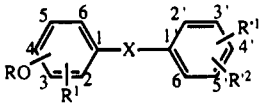
The data of Table IV showed that compound **70**, having the β-D-xylopyranosyl group at the meta position of 4'-nitrobenzophenone, was more active than the para-substituted analog (compound **55**). The analog of **70** lacking the 4'-nitro group (compound **74**) was about as active as **70**. No clear structure–activity trends toward antithrombotic activity could be discerned from compounds **60** and **68** having an electron-donating group at C-4' and compounds **70** and **56** where the 4'-substituent is electron-withdrawing.

Some of the benzophenone derivatives in Table IV were selected for reduction of the carbonyl group to the corresponding benzhydrol and/or benzhydril compounds. The antithrombotic activities of these products are summarized in Table V. Surprisingly the reduction of the para-glycosylated 4'-nitrobenzophenone **55** (itself inactive as a venous antithrombotic compound) afforded the highly active benzhydrol **79** and the benzhydril **86**. These two products are the leading candidates for effective antithrombotic agents in the rat model. The compounds **79** and **86** have been selected for evaluation of their oral antithrombotic activity in the rat model.[19] The technique employed was based on that of Wessler as described in the rabbit.[20] Oral administration of these two compounds was 4 h before injection of the thrombogenic stimulus (Xa) and reduced thrombus weight in a dose-related manner with an ED_{50} of 48 mg/kg of body weight for **79** and an ED_{50} of 24 mg/kg of body weight for **86**.

Up to now, heparin has not yet been revealed to be active by oral administration. Compound **79**, being more soluble in the poly(ethylene glycol) 400, was chosen for comparison with heparin by intravenous route. The data are summarized in Table VI.

Conclusion and Perspective in the Future. We have reported for the first time the synthesis of the β-D-xylopyranoside and demonstrated a venous antithrombotic activity following oral administration (**79**, **86**). The biological activity of the separated epimers of **79** (mixture 50/50) is quite the same as the parent compound (unpublished results). We believe that β-D-xylopyranose was the pharmacophore for the antithrombotic activity. How-

Table II



no.	position	R	R'1	R'2	R1	mp, °C	formula	anal.	X	$[\alpha]^{20}_D$, deg	method	procedure	yield, %
1	4	Ac4-β-Glcp	4'-NO2	H	H	214	$C_{27}H_{27}NO_{13}$	CHN	C=O	-88 (c 0.5, $CHCl_3$)	B		37
2	4	Ac4-β-Glcp	4'-Cl	H	H	212	$C_{27}H_{27}ClO_{11}$	CH	C=O	-20.5 (c 0.5, $CHCl_3$)	B		71
3	4	Ac4-β-Glcp	H	H	H	170	$C_{27}H_{28}O_{11}$	CH	C=O	-21.2 (c 0.5, $CHCl_3$)	B		81
4	4	Ac4-β-Galp	4'-NO2	H	H	148	$C_{27}H_{27}NO_{13}$	CHN	C=O	+3.2 (c 0.5, $CHCl_3$)	B		61
5	4	Ac4-β-Galp	4'-Cl	H	H	140	$C_{27}H_{27}ClO_{11}$	CH	C=O	+5.6 (c 0.5, $CHCl_3$)	B		45
6	4	Ac3-β-GlcNAcp	4'-NO2	H	H	238	$C_{27}H_{28}N_2O_{12}$	CHN	C=O	-15.3 (c 0.5, $CHCl_3$)	A		46
7	4	Ac3-β-GlcNAcp	2'-Cl	H	H	196	$C_{27}H_{28}NClO_{10}$	CHN	C=O	-10.2 (c 0.5, $CHCl_3$)	A		60
8	4	Ac3-β-GlcNAcp	4'-CF3	H	H	200	$C_{28}H_{28}NF_3O_{10}$	CHN	C=O	-14.8 (c 0.5, $CHCl_3$)	A		56
9	4	Ac3-β-GlcNAcp	2'-CH3	H	3-CH3	175	$C_{29}H_{23}NO_{10}$	CHN	C=O	-16.9 (c 0.5, $CHCl_3$)	A		51
10	4	Ac4-α-Manp	4'-NO2	H	H	70 foam	$C_{27}H_{27}NO_{13}$	CHN	C=O	+78 (c 0.5, $CHCl_3$)	B		29
11	4	Ac4-β-Manp	4'-NO2	H	H	120	$C_{27}H_{27}NO_{13}$	CHN	C=O	-73.3 (c 0.5, $CHCl_3$)	C		50
12	4	Ac3-α-Xylp	4'-NO2	H	H	foam	direct desacetylated		C=O	+130 (c 0.5, $CHCl_3$)	C		26
13	4	Ac3-β-Xylp	4'-NO2	H	H	149	$C_{24}H_{23}NO_{11}$	CHN	C=O	-33 (c 1, $Cl(CH_2)_2Cl$)	B		65
14	4	Ac3-β-Xylp	4'-CN	H	H	150	$C_{25}H_{23}NO_9$	CHN	C=O	-29 (c 0.5, $CHCl_3$)	B		55
15	4	Ac3-β-Xylp	4'-Cl	H	H	148	$C_{24}H_{23}ClO_9$	CH	C=O	-44 (c 1, $CHCl_3$)	B		72
16	4	Ac3-β-Xylp	2'-CH3	H	3-CH3	102	$C_{26}H_{28}O_9$	CH	C=O	-33 (c 0.5, MeOH)	B		50
17	4	Ac3-β-Xylp	4'-CF3	H	H	134	$C_{25}H_{23}F_3O_9$	CH	C=O	NA	B		28
18	4	Ac3-β-Xylp	4'-OCH3	H	H	144	$C_{25}H_{26}O_{10}$	CH	C=O	-21 (c 0.5, MeOH)	B		20
19	4	Ac3-β-Xylp	H	H	H	132	$C_{24}H_{24}O_9$	CH	C=O	-44 (c 0.5, $CHCl_3$)	B		30
20	4	Ac7-β-Mal[c]	4'-NO2	H	H	191	$C_{39}H_{43}NO_{21}$	CHN	C=O	+34 (c 0.5, $CHCl_3$)	B		48
21	4	Ac7-β-Lac[d]	4'-NO2	H	H	100	$C_{39}H_{43}NO_{21}$	CHN	C=O	-26 (c 0.5, $CHCl_3$)	B		40
22	4	Ac3-β-GlcpA[b]	4'-NO2	H	H	NA	$C_{26}H_{25}NO_{13}$	CHN	C=O	NA	B		56
23	4	Ac3-α-Rhap	4'-NO2	H	H	foam	$C_{25}H_{25}NO_{11}$	CHN	C=O	-85 (c 0.5, $CHCl_3$)	B		43
24	4	Ac3-β-Xylp	3'-NO2	H	H	145	$C_{24}H_{23}NO_{11}$	CHN	C=O	-26 (c 0.5, MeOH)	B		75
25	4	Ac3-β-Xylp	2'-Cl	4'-Cl	H	142	$C_{25}H_{22}Cl_2O_9$	CH	C=O	-20 (c 0.5, MeOH)	B		85
26	4	Ac3-β-Xylp	4'-CH3	H	H	138	$C_{25}H_{26}O_9$	CH	C=O	-22 (c 0.5, MeOH)	B		60
27	4	Ac3-β-Xylp	2'-Cl	H	H	165	$C_{24}H_{23}ClO_9$	CH	C=O	-24 (c 0.5, MeOH)	B		59
28	3	Ac3-β-Xylp	4'-NO2	H	H	144	$C_{24}H_{23}NO_{11}$	CHN	C=O	NA	B		73
29	3	Ac3-β-Xylp	4'-Cl	H	H	126	$C_{24}H_{23}ClO_9$	CH	C=O	-26 (c 0.5, MeOH)	B		53
30	3	Ac3-β-Xylp	2'-CH3	H	H	90	$C_{25}H_{26}O_9$	CH	C=O	-40 (c 0.5, $CHCl_3$)	B		71
31	3	Ac3-β-Xylp	4'-CH3	H	H	94	$C_{25}H_{26}O_9$	CH	C=O	-45 (c 0.5, $CHCl_3$)	B		80
32	3	Ac3-β-Xylp	H	H	H	148	$C_{24}H_{24}O_9$	CH	C=O	-28 (c 0.5, MeOH)	B		72
33	2	Ac3-β-Xylp	2'-CH3	H	5'-CH3	NA	$C_{26}H_{28}O_9$	CH	C=O	NA	B		
34	4	Ac3-β-Xylp	4'-OCH3	H	H	80	$C_{25}H_{28}O_9$	CH	CH_2	-21 (c 0.5, EtOAc)		A	62
35	4	Ac3-β-Xylp	H	H	H	112	$C_{24}H_{26}O_8$	CH	CH_2	-27 (c 1, EtOAc)		A	42
36	4	Ac3-β-Xylp	3'-NO2	H	H	105	$C_{24}H_{25}NO_{10}$	CHN	CH_2	-25 (c 0.5, EtOAC)		A	43
37	4	Ac3-β-Xylp	4'-NO2	H	H	foam	$C_{24}H_{25}NO_{10}$	CHN	CH_2	-33 (c 0.7, $CHCl_3$)		A	62
38	4	Ac3-β-Xylp	2'-Cl	H	H	85	$C_{24}H_{25}ClO_8$	CH	CH_2	-21 (c 0.5, $CHCl_3$)		A	43
39	4	Ac3-β-Xylp	4'-Cl	H	H	101	$C_{24}H_{25}ClO_8$	CH	CH_2	-19 (c 0.5, AcOEt)		A	39
40	4	Ac3-β-Xylp	2'-Cl	4'-Cl	H	99	$C_{24}H_{24}Cl_2O_8$	CH	CH_2	-17 (c 0.5, EtOAC)		A	65
41	3	Ac3-β-Xylp	4'-Cl	H	H	99	$C_{24}H_{25}ClO_8$	CH	CH_2	-43 (c 0.5, $CHCl_3$)		A	100
42	3	Ac3-β-Xylp	H	H	H	110	$C_{24}H_{26}O_8$	CH	CH_2	-46 (c 0.5, $CHCl_3$)		A	100

[a] All sugars are of the D configuration, except for rhamnose, which is L. [b] As methyl ester. [c] Hepta-*O*-acetyl-β-maltosyl. [d] Hepta-*O*-acetyl-β-lactosyl. NA: not available. The $J_{1,2}$ values in ^{1}H-NMR spectra for the component sugar were all in range 7–9 Hz, except for compounds 11 and 12 (2–4 Hz).[24]

ever, the dose used was high. Our target in the future is thus active compounds at low doses by changing the heteroatom of D-xyloside series. Of course, the mechanism of action will be investigated in our laboratory.

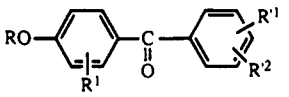
Experimental Section

Melting points were determined on a Kofler melting-point apparatus. ^{1}H-NMR spectra were recorded for $CDCl_3$ or Me_2SO-d_6 solutions with a Brucker WD 80 FT instrument. Optical rotations were recorded with a Perkin-Elmer Model 241 or a Jobin Yvon digital-readout polarimeter. Satisfactory elemental analyses for C, H, N were obtained for all new compounds.

Method A. 4-(4'-Nitrobenzoyl)phenyl 2-Acetamido-3,4,5-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7). To a solution of 5 g (20.5 mmol) of (4'-nitrobenzoyl)phenol in DMF (25 mL) and dichloromethane (25 mL) was added 0.54 g (22 mmol) of NaH under a nitrogen atmosphere. The mixture was stirred for 2 h at room temperature, and then 8.3 g (23.25 mmol) of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[21] was added portionwise and the final solution was stirred for 3 h at 40 °C. The mixture was poured into ice–water, and then the aqueous solution was extracted with ethyl acetate. The organic layer was washed with 1 M NaOH and with water until the pH was neutral and dried over $MgSO_4$. Ethyl acetate was removed by evaporation under diminished pressure. The residual oil was treated with ethyl ether and the resultant precipitate recrystallized from ethyl acetate to yield compound 7 (6.9 g, 46%): mp 238 °C; $[\alpha]^{20}_D$ -15.3° (c 0.5, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6) δ 1.79 (s, 3 H, NHAc), 1.95 (s, 3 H, OAc), 2.00 (s, 6 H, OAc) 4.17 (m, 4 H), 4.98 (m, 1 H), 5.22 (dd, J = 9.5 Hz, 1 H) 5.58 (d, $J_{1,2}$ = 8.35 Hz, 1 H, H-1), 7.2 (d, J = 8.4 Hz, 2 H, Ar), 7.8 (d, 2 H Ar), 7.93 (d, 2 H, Ar), 8.10 (d, J = 9.6 Hz, 1 H, NH), 8.40 (d, 2 H, Ar).

Method B. [4-(4-Nitrobenzoyl)phenyl] 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (14). A mixture of (4-nitrobenzoyl)phenol (2.45 g, 10 mmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[22] (3.4 g, 10 mmol) in anhydrous CH_3CN (200 mL) was stirred with 2.4 g (10.3 mmol) of freshly prepared Ag_2O for 30 min at ambient temperature. Ethyl acetate (500 mL) was added, and the solid salt was removed by filtration. The organic layer was washed with 1 M NaOH and water until the pH was neutral and then dried over $MgSO_4$. After evaporation under diminished pressure, the residual oil was crystallized from ethyl ether and

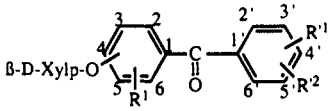
Table III



no.	R[a]	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	LD_0	activity,[d] %
43	β-Glcp	4'-NO_2	H	H	196	$C_{19}H_{19}NO_9 \cdot H_2O$	CHN	−54 (c 0.5, MeOH)	>800	0
44	β-Glcp	4'-Cl	H	H	155	$C_{19}H_{19}ClO_7 \cdot {}^1/_2H_2O$	CHCl	−57 (c 0.5, MeOH)	>800	0
45	β-Glcp	H	H	H	163	$C_{19}H_{20}O_7 \cdot {}^1/_2H_2O$	CH	−55 (c 0.5, MeOH)	>800	25 (NS)
46	β-Galp	4'-NO_2	H	H	220	$C_{19}H_{19}NO_9$	CHN	−39 (c 0.6, Py)	>800	0
47	β-Galp	4'-Cl	H	H	202	$C_{19}H_{19}ClO_7 \cdot {}^1/_2H_2O$	CHCl	−43 (c 0.5, MeOH)	>800	0
48	β-GlcpNAc	4'-NO_2	H	H	206	$C_{21}H_{22}N_2O_5 \cdot H_2O$	CHN	+12.5 (c 0.6, MeOH)	>800	0
49	β-GlcpNAc	2'-Cl	H	H	226	$C_{21}H_{22}NO_7$	CHNCl	+20 (c 0.4, MeOH)	>800	20 (NS)
50	β-GlcpNAc	4'-CF_3	H	H	238	$C_{22}H_{22}F_3NO_7 \cdot {}^1/_2H_2O$	CHNF	+20 (c 0.4, MeOH)	>800	19 (NS)
51	β-GlcpNAc	2'-CH_3	H	3-CH_3	238	$C_{23}H_{27}NO_7$	CHN	+4 (c 0.5 MeOH)	>800	12 (NS)
52	α-Manp	4'-NO_2	H	H	206	$C_{19}H_{19}NO_9$	CHN	+120 (c 0.5, MeOH)	>800	0
53	β-Manp	4'-NO_2	H	H	120	$C_{19}H_{19}NO_9$	CHN	−58 (c 0.5, MeOH)	>800	0
54	α-Xylp	4'-NO_2	H	H	172	$C_{18}H_{17}NO_8$	CHN	+163 (c 0.5, MeOH)	600[e]	38 (NS)
55	β-Xylp	4'-NO_2	H	H	200	$C_{18}H_{17}NO_8$	CHN	−27 (c 0.5, MeOH)	>800	0
56	β-Xylp	4'-CN	H	H	206	$C_{19}H_{17}NO_6$	CHN	−29 (c 0.3, MeOH)	ND	0
57	β-Xylp	4'-Cl	H	H	174	$C_{18}H_{17}ClO_6$	CH	−27 (c 0.1, MeOH)	>800	37
58	β-Xylp	2'-CH_3	H	3CH3	135	$C_{20}H_{22}O_6$	CH	−25 (c 0.5, MeOH)	ND	62
59	β-Xylp	4'-CF_3	H	H	160	$C_{19}H_{17}F_3O_6$	CH	−20 (c 0.6, MeOH)	>800	0
60	β-Xylp	4'-CH_3O	H	H	180	$C_{19}H_{20}O_7$	CH	−26 (c 0.6, MeOH)	>800	44
61	β-Xylp	H	H	H	140	$C_{18}H_{18}O_6$	CH	−20 (c 0.7, EtOAc)	750[e]	77
62	β-Mal[c]	4'-NO_2	H	H	158	$C_{25}H_{29}NO_{14}$	CHN	+64 (c 0.5, MeOH)	>800	17 (NS)
63	β-Lac	4'-NO_2	H	H	208	$C_{25}H_{29}NO_{14}$	CHN	−21 (c 0.5, Pyr)	>800	0
64	β-GlcpA	4'-NO_2	H	H	132	$C_{19}H_{17}NO_{10}$	CHN	−68 (c 0.5, MeOH)	>800	20 (NS)
65	α-Rhap	4'-NO_2	H	H	110	$C_{19}H_{19}NO_8$	CHN	−123 (c 0.5, MeOH)	>800	0

[a] All sugars are of the D configuration, except for rhamnose, which is L. [b] Lac = lactose. [c] Mal = maltose. [d] Antithrombotic activity at 100 mg/kg for compounds where the nonlethal dose (LD_0) >800 mg, otherwise antithrombotic activity was assessed at $^1/_{10}$ of LD_{50}; activity is given by intraperitoneal route as percentage of reduction of thrombus formation by weight, and 5–10 rats are used by lot of tests. The injection of the stimulus was 4 h after administration of the tested compound. [e] LD_{50}, ND = not determined, NS = not significant.

Table IV



no.	position of β-D-Xylp	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	LD_0	activity,[a] %
66	4	3'-NO_2	H	H	164	$C_{18}H_{17}NO_8$	CHN	−30 (c 0.2, MeOH)	>800	39
67	4	2'-Cl	4'-Cl	H	172	$C_{18}H_{16}Cl_2O_6 \cdot {}^1/_2H_2O$	CH	−22 (c 0.7, MeOH)	>800	18 (NS)
68	4	4'-CH_3	H	H	162	$C_{19}H_{20}O_6 \cdot H_2O$	CH	−27 (c 0.6, MeOH)	>800	0
69	4	2'-Cl	H	H	90	$C_{18}H_{17}ClO_6 \cdot H_2O$	CH	−21 (c 0.7, MeOH)	>800	76
70	3	4'-NO_2	H	H	108	$C_{18}H_{17}NO_8$	CHN	−30 (c 0.7, MeOH)	>800	61
71	3	4'-Cl	H	H	80	$C_{18}H_{17}ClO_6 \cdot {}^1/_2H_2O$	CHCl	−26 (c 0.5, MeOH)	220[b]	0
72	3	2'-CH_3	H	H	168	$C_{19}H_{20}O_6$	CH	−33 (c 0.8, MeOH)	>800	0
73	3	4'-Me	H	H	154	$C_{19}H_{20}O_6$	CH	−29 (c 0.8, MeOH)	>800	0
74	3	H	H	H	140	$C_{18}H_{18}O_6$	CH	−30 (c 0.5, MeOH)	>800	60
75	2	2'-Me	H	5-CH_3		$C_{18}H_{18}O_6$	CH	NA	>800	0

[a] See Table I. [b] LD_{50}. NA = not available.

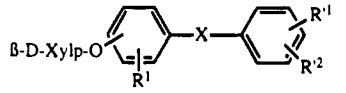
recrystallized from MeOH to yield 6.5 g (65%) of 14: mp 149 °C; $[\alpha]^{20}_D$ −33° (c 1, 1,2-dichloroethane); ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 9 H, OAc), 3.90 (m, 2H, H-5), 5.16 (m 3 H, H-2,3,4), 5.70 (d, $J_{1,2}$ = 6.5 Hz 1 H, H-1), 7,19 (d, J = 8.0 Hz, 2 H, Ar), 7.80 (d, J = 8 Hz, 2 H, Ar), 7.90 (d, J = 8 Hz, 2 H, Ar), 8.4 (d, J = 8 Hz, 2 H, Ar).

Method C. 4-(4-Nitrobenzoyl)phenyl 2,3,4-Tri-*O*-acetyl-α-D-xylopyranoside (13). To a solution of 10 g (31.4 mmol) of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose[23] and 10 g (41 mmol) of a (4-nitrobenzoyl)phenol solution in CH_2Cl_2 (100 mL) was added $SnCl_4$ (8 mL, 68 mmol) under a nitrogen atmosphere. The mixture was stirred for 4 h at 60 °C and then poured onto ice. The residue was extracted with CH_2Cl_2, washed with a solution of $NaHCO_3$ and water until the pH was neutral, and then dried over $MgSO_4$. Evaporation under diminished pressure gave an oil that was chromatographed on a column of silica gel (solvent 8:1 $CHCl_3$–EtOAc). The solvent was removed to afford 13 as an oil yield 6 g (26%): $[\alpha]^{20}_D$ +130° (c 0.5, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 3 H, OAc), 2.04 (s, 6 H, OAc), 3.70 (m, 2 H), 5.14 (m, 2 H), 5.55 (t, 1 H), 6.03 (d, $J_{1,2}$ = 3.5 Hz, 1 H, H-1), 7.30 (d, J = 8 Hz, 2 H, Ar), 7.80 (d, J = 8 Hz, 2 H Ar), 7.90 (d, J = 8 Hz, 2 H, Ar), 8.4 (d, J = 8 Hz, 2 H, Ar).

Reduction of Benzophenone to Benzhydril (Procedure A). 4-(4-Nitrobenzyl)phenyl 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside (38). To a mixture of 2.45 g (5 mmol) of 14 in CF_3CO_2H (15 mL) and CH_2Cl_2 (20 mL) was added portionwise at 0 °C 1.17 g (30 mmol) of $NaBH_4$. The mixture was stirred at room temperature for 8 h until the starting material disappeared (solvent 4:1 toluene–EtOAc). The residue was hydrolyzed on ice, and the acid was neutralized with a solution of $NaHCO_3$. The aqueous phase was then extracted with EtOAc and the extract washed with water until it was neutral. Evaporation under diminished pressure gave an oil that was chromatographed on a column of silica gel with 9:1 toluene–EtOAc to yield 5.5 g (100%) of 38 as an oil: $[\alpha]^{20}_D$ −33° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.07 (3 H, OAc), 2.08 (3 H, OAc), 2.14 (3 H, OAc), 3.70 (dd, J = 2.6 Hz, J = 13 Hz, 1 H, H-5), 4.02 (s, 2 H), 4.12 (dd, J = 4 Hz, J = 13 Hz, 1 H, H-5'), 5.07 (d, $J_{1,2}$ = 7 Hz, 1 H, H-1), 5.34 (m, 3 H), 6.94 (d, J = 9 Hz, 2 H, Ar), 7.11 (d, J = 9 Hz, 2 H, Ar, NaH), 7.3 (d, J = 8 Hz, 2 H, Ar), 8.15 (d, J = 8 Hz, 2 H, Ar).

Reduction of Benzophenone to Benzhydrol (Procedure B). 4-(4-Chloro-α-hydroxybenzyl)phenyl β-D-Xylopyranoside (81). To a suspension of 5 g (13.7 mmol) of 57 in methanol (120 mL) was added $NaBH_4$ (0.55 g, 14 mmol) portionwise. The

Table V



no.	position of β-D-Xylp	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	X	LD_0	activity,[a] %
76	4	2'-Cl	4'-Cl	H	152	$C_{18}H_{18}Cl_2O_6$	CH	−17 (c 0.5, MeOH)	CHOH	350[b]	37
77	4	H	H	H	190	$C_{18}H_{20}O_6$	CH	−26 (c 0.5, MeOH)	CHOH	>800	37
78	4	3'-NO_2	H	H	125	$C_{18}H_{19}NO_6$	CHN	−15 (c 0.5, MeOH)	CHOH	600[b]	63
79	4	4'-NO_2	H	H	142	$C_{18}H_{19}NO_6$	CHN	−17 (c 0.5, MeOH)	CHOH	>800	80
80	4	2'-Cl	H	H	210	$C_{18}H_{19}NO_6$	CH	−23 (c 0.5, MeOH)	CHOH	>800	51
81	4	4'-Cl	H	H	182	$C_{18}H_{19}NO_6$	CHCl	−22 (c 0.5, MeOH)	CHOH	>800	39
82	4	2'-CH_3	H	3-CH_3	110	$C_{20}H_{24}O_6$	CH	−9.5 (c 0.4, MeOH)	CHOH	450[b]	14 (NS)[c]
83	4	4'-OCH_3	H	H	153	$C_{19}H_{22}O_6$	CH	−28 (c 0.5, MeOH)	CH_2	>800	43[c]
84	4	H	H	H	160	$C_{18}H_{20}O_6$	CH	−28 (c 0.5, MeOH)	CH_2	>800	12 (NS)[c]
85	4	3'-NO_2	H	H	139	$C_{18}H_{19}NO_7$	CHN	−24 (c 0.5, MeOH)	CH_2	>800	32 (NS)[c]
86	4	4'-NO_2	H	H	166	$C_{18}H_{19}NO_7$	CHN	−20 (c 0.5, MeOH)	CH_2	650	80[c]
87	4	2'-Cl	H	H	183	$C_{18}H_{19}ClO_5$	CHCl	−27 (c 0.5, MeOH)	CH_2	>800	0[c]
88	4	4'-Cl	H	H	142	$C_{18}H_{19}ClO_5$	CH	−25 (c 0.5, MeOH)	CH_2	>800	22[c]
89	4	2'-Cl	4'-Cl	H	158	$C_{18}H_{18}Cl_2O_5$	CH	−26 (c 0.5, MeOH)	CH_2	640[b]	25 (NS)[c]
90	3	4'-Cl	H	H	84	$C_{18}H_{19}ClO_6 \cdot {}^1/_2H_2O$	CH	−37 (c 0.5, MeOH)	CHOH	>800	0
91	3	H	H	H	168	$C_{18}H_{20}O_6$	CH	−2 (c 0.5, MeOH)	CHOH	>800	19 (NS)[c]
92	3	4'-Cl	H	H	136	$C_{18}H_{19}ClO_5$	CH	−35 (c 0.5, MeOH)	CH_2	500[b]	46[c]
93	3	H	H	H	140	$C_{18}H_{20}O_5$	CH	−38 (c 0.5, MeOH)	CH_2	200[b]	39 (NS)[c]

[a] See Table I. [b] LD_{50}. [c] The injection of the stimulus was 4 h after administration of the tested compound otherwise the injection of the thrombogenic agent was 2 h later. NS = not significant.

Table VI

	79[b]	heparin[c]
ED_{50},[a] mg/kg iv	17.8 (14.5–21.1)	0.15 (0.12–0.17)

[a] Calculated by linear regression with their corresponding 95% confidence limits (in parentheses). [b] 79 was administered by iv route before injection of thrombogenic agent (Wessler Xa). [c] Obtained from Terhormon, Novara, Italy.

mixture was stirred for 1 h at room temperature and the solution then deionized by adding Amberlite IR-120 (H^+) cation-exchange resin until the pH became neutral. The diastereoisomeric mixture thus formed was chromatographed, after evaporation of the solvent, on a column of silica gel (3:1 toluene–MeOH) to yield 4.33 g (87%) of 81 as a mixture of epimers: mp 182 °C; $[\alpha]^{20}_D$ −22° (c 0.5, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 3.3 (m, 4 H), 3.7 (m, 1 H), 4.8 (d, $J_{1,2}$ = 7.04 Hz, 1 H, H-1), 5.0 (broad, 1 H), 5.3 (broad, 1 H), 5.6 (d, J = 3 Hz, 1 H), 5.9 (d, J = 3 Hz, 1 H), 6.9 (d, J = 9.5 Hz, 2 H, Ar), 7.26 (d, J = 9.5 Hz, 2 H, Ar), 7.35 (s, 4 H, Ar).

Zemplén Deacylation. 4-(4-Chlorobenzoyl)phenyl β-D-Xylopyranoside (57). A solution of 16 (3.4 g, 7 mmol) in dry methanol (50 mL) was stirred with a 3 M solution of methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was deionized by addition of Amberlite IR-120 (H^+) cation-exchange resin, the resin filtered off, and the filtrate taken to dryness. The residue was recrystallized from methanol to yield 2.3 g (88%) of 57: $[\alpha]^{20}_D$ −27° (c 0.1, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 3.34 (m, 1 H), 3.8 (m, 1 H), 5.1 (m, 3 H), 5.4 (d, $J_{1,2}$ = 4.8 Hz, 1 H, H-1), 7.17 (d, J = 8 Hz, 2 H, Ar), 7.52–7.84 (m, 6 H, Ar).

α-(3-Methoxyphenyl)-4-morpholinoacetonitrile. To a mixture of 3-methoxybenzaldehyde (3 g, 22 mmol), p-toluenesulfonic acid (4 g, 27 mmol), and morpholine (3.8 g, 44 mmol) was added potassium cyanide (1.43 g, 22 mmol). The mixture was stirred for 3 h under reflux. After neutralization with a concentrated solution of $NaHCO_3$ and extraction with ethyl acetate, the organic layer was washed with water until the pH was neutral. Ethyl acetate was removed by evaporation under diminished pressure, and the solid residue was recrystallized from isopropyl ether to yield 4.12 g (81%), mp 40 °C.

3-Methoxy-4'-nitrobenzophenone. The preceding compound (4.12 g, 18 mmol) was added portionwise to a suspension of sodium hydride (0.53 g, 18 mmol) and N,N-dimethylformamide (40 mL) at 0 °C. After 30 min at this temperature, a solution of 1-fluoro-4-nitrobenzene 2.5 g (18 mmol) in 20 mL of N,N-dimethylformamide was added slowly. The mixture was stirred at room temperature until the starting materials had disappeared. After hydrolysis on ice, the precipitate was removed by filtration and washed with water until the pH was neutral. The crude, wet material was stirred under reflux for 1 h in 40 mL of 70% aqueous acetic acid. From this hydrolyzed product the residue was removed by filtration and washed with water until the pH was neutral to give 3.2 g (70%) of the final compound. This pure compound was used directly for the next step.

4'-Nitro-3-hydroxybenzophenone. The preceding compound (3 g, 11.6 mmol) was stirred under reflux for 4 h in 5 mL of acetic acid and 6 mL of 62% hydrogen bromide. The acid medium was hydrolyzed with cold water, the residue removed by filtration, and the product recrystallized from isopropyl ether to give 2.03 g (70%) of the product, mp 116 °C.

General Method for Synthesis of Benzophenone from Grignard Reagent: Preparation of 2'-Methyl-3-methoxybenzophenone. Under an atmosphere of nitrogen 9.35 g (50 mmol) of 3-bromoanisole dissolved in 5 mL of anhydrous THF was added slowly to a suspension of 1.8 g (75 mmol) of magnesium in 5 mL of THF. The reflux was maintained during the addition of 3-bromoanisole until the magnesium had disappeared. This Grignard reagent was added slowly to a solution of 2-methylbenzoyl chloride (7.22 g, 50 mmol) in 10 mL of THF at −20 °C. The reaction was stirred overnight at room temperature and then hydrolyzed with a cooled solution of M HCl. The aqueous solution was extracted with ethyl ether. The organic layer was washed with water until the pH was neutral, dried over $MgSO_4$, and evaporated under diminished pressure to yield 1.22 g (86%) of this crude compound that was used directly for the next step.

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