Carbohydrate Research, 253 (1994) 167–183
Elsevier Science B.V., Amsterdam

Synthesis of 5-aminopentyl 4,6-*O*-[(*R*)-1-carboxyethylidene]-β-D-galactopyranoside and its use as a ligand for the affinity chromatography of human serum amyloid P protein

Thomas Ziegler *, Elisabeth Eckhardt, Jochen Strayle and Helmut Herzog

Institut für Organische Chemie und Isotopenforschung, Universität Stuttgart, Pfaffenwaldring 55, 70569 Stuttgart (Germany)

(Received June 1st, 1993; accepted August 9th, 1993)

ABSTRACT

A series of 2,3-di-*O*-benzoyl-D-galactopyranosides, α-allyl (**5**), α-benzyl (**6**), β-ethyl-1-thio (**7**), β-phenyl-1-thio (**8**), and α-methyl (**9**), were prepared from the corresponding 4,6-*O*-benzylidene derivatives and were acetalated in acetonitrile with methyl pyruvate, to give diastereoselectively the 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-D-galactopyranosides **10–16**. The latter were converted into the 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-D-galactopyranosyl α- and β-trichloroacetimidates **19** and **20**, α- and β-fluorides **21** and **22**, the α-bromide **23**, and the α-chloride **24**, respectively. These donors, including the phenyl 1-thiogalactoside **14**, reacted with 5-[(benzyloxycarbonyl)amino]pentanol to give the corresponding protected β-D-galactoside **27**, deblocking of which afforded the title compound **1**. Binding of **1** to epoxypropyl-modified acrylamide beads gave an affinity adsorbent that was used to isolate serum amyloid P protein from human serum.

INTRODUCTION

The α_1-glycoprotein amyloid P (AP) is a unique plasma constituent found in amyloid fibrils and at concentrations of 30–45 mg/L in human serum[1]. It is built up of 10 subunits with a molecular mass of 23 000 daltons each, is continuously produced in the liver of all vertebrates, and belongs to the pentraxin family (C-reactive protein, hamster female protein). Although the explicit physiological function of serum amyloid P (SAP) protein remains enigmatic, several interesting features of this plasma component are known. For example, the concentration of SAP is increased in arthritis, malignancy, and at the end of pregnancy, and decreased in liver diseases[1]. Recently, it has been shown that AP is also a constituent of senile plaques in Alzheimer's disease[2]. Most obvious, however, is

* Corresponding author.

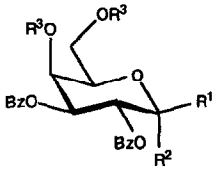
SSDI 0008-6215(93)E0293-A

the ability of SAP to exhibit a Ca-dependent self-aggregation[3] and binding to several other proteins such as C_4-binding protein and fibronectin[4,5]. Furthermore, SAP has been referred to as a lectin since it also binds to heparin, heparan and dermatan sulfate[6,7], and agarose. The latter binding ability of SAP was discovered to be a consequence of the presence of pyruvic acid acetals as trace contaminants in agarose. Thus, Hind et al. showed that SAP binds specifically to 4,6-*O*-(1-carboxyethylidene)-β-D-galactose residues in a Ca-dependent fashion[8–10]. This unique feature of SAP has recently been adapted by Urbányi and Medzihradszky to a "rapid method to isolate serum amyloid P component from human plasma" using agarose as the affinity material[11]. However, since the specific binding of SAP to agarose strongly depends on the content of pyruvic acid acetals[8], the efficiency of that affinity chromatography is dramatically influenced by the "quality" of the agarose that is used. Therefore, we describe here the convenient preparation and application for SAP isolation of a polyacrylamide-based chromatography material modified with the title pyruvated galactosyl ligand.

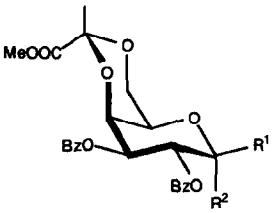
RESULTS AND DISCUSSION

Synthesis of 5-aminopentyl 4,6-O-[(R)-1-carboxyethylidene]-β-D-galactopyranoside (**1**). — For the preparation of affinity adsorbents, it is generally recommended to couple low molecular weight ligands, like monosaccharides, via a "spacer arm" to a suitable matrix in order to avoid low steric availability of the ligand[12]. We therefore constructed a 5-aminopentyl glycoside for that purpose. Similar aminoalkyl glycosides have been used previously by others[13–15] as superior ligands for affinity adsorbents and by us[16–18] for the synthesis of glycoconjugates containing pyruvated D-glucose residues. For the synthesis of the ligand 5-aminopentyl 4,6-*O*-[(*R*)-1-carboxyethylidene]-β-D-galactopyranoside (**1**), an efficient procedure for 1,2-*trans*-selective coupling of a suitable pyruvate acetal-containing galactosyl donor with 5-[(benzyloxycarbonyl)amino]pentanol[19] was needed. Thus, starting from the monosaccharide diols **5–9**, we first prepared a series of 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-D-galactopyranosides (**10–16**) that were potential precursors for these donors. Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (**5**), benzyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (**6**), and ethyl 2,3-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**7**) were obtained from the corresponding 4,6-*O*-benzylidene derivatives **2** and **3** and ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[20], respectively, by acid hydrolysis as previously described[21,22] for compounds **8** and **9**.

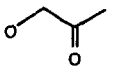
Table I summarizes the results of the boron trifluoride-catalyzed acetalation of diols **5–9** with methyl pyruvate. A significant improvement of the previously described diastereoselective preparation of 4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-D-galactosides from either 4,6-bis(trimethylsilyl) ethers[21], 4,6-(1,1,3,3,-tetraisopropyl-1,3-disiloxane-1,3-diyl) ethers[23], or monosaccharide diols in dichloromethane[24] could now be achieved with acetonitrile as the solvent. In general,

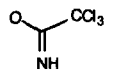


	R1	R2	R3
2	H	OAllyl	PhCH
3	H	OBn	PhCH
4	OBn	H	PhCH
5	H	OAllyl	H
6	H	OBn	H
7	SEt	H	H
8	SPh	H	H
9	H	OMe	H



	R1	R2
10	H	OAllyl
11	H	OBn
12	SEt	H
13	H	SEt
14	SPh	H
15	H	SPh
16	H	OMe
17	H	X1
18	H, OH	
19	H	X2
20	X2	H
21	H	F
22	F	H
23	H	Br
24	H	Cl
25	X3	H
26	EtO	H

X1 =


X2 =


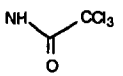
X3 =


TABLE I

Synthesis of 4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-D-galactopyranosides **10–16** from monosaccharides diols **5–9** and methyl pyruvate at room temperature in dichloromethane and acetonitrile

Starting material	Dichloromethane			Acetonitrile		
	Time (h)	Product	Yield (%)	Time (h)	Product	Yield (%)
5	20	**10**	47	3	**10**	70
6	24	**11**	[a]	2	**11**	63
7	12	**12**	[b]	2	**12**	34
					13	17
8	24	**14**	28 [c]	2.5	**14**	91
		15	30			
9	24	**16**	58 [c]	2.5	**16**	82

[a] Inseparable mixture. [b] Complete decomposition of **12**. [c] Taken from ref 24.

reaction times at room temperature were shorter and the yields of the pyruvated galactosides **10–16** were higher for acetalations in acetonitrile than in dichloromethane (Table I). Furthermore, decomposition of the starting material or the product (i.e., for conversion **6** → **11** and **7** → **12**) or anomerisation (i.e., for conversion **8** → **14, 15,**) was prevented in acetonitrile, and TLC of the crude mixture revealed a surprisingly clean reaction of the diols **5–9**. However, the ethyl 1-thio-β-D-galactoside **7**, which decomposed in dichloromethane, afforded an anomeric mixture of the pyruvated galactosides **12** and **13** in acetonitrile. In all cases, solely the diastereomers having *R* configuration at the pyruvate acetal centre were formed as could be concluded from ^{13}C NMR spectra[21,25]. Preliminary results also showed that this "direct" pyruvation of monosaccharide diols in acetonitrile was also applicable for the convenient diastereoselective synthesis of 4,6-*O*-[(*S*)-1-methoxycarbonylethylidene] derivatives in the D-*gluco* and D-*manno* series.

For the preparation of suitable pyruvated galactosyl donors, the allyl galactoside **10** was treated with a catalytic amount of palladium dichloride in degassed aqueous acetic acid with strict exclusion of air to afford an anomeric mixture of the 1-*O*-unprotected derivative **18** (91%, α : β 6 : 1). When the reaction was performed without exclusion of air, **18** (43%) was accompanied by the oxidation product[26] **17** (33%). Alternatively, **18** was obtained hydrogenolytically from benzyl galactoside **11** in 96% yield. Treatment of alcohol **18** with trichloroacetonitrile and potassium carbonate then gave the imidates **19** and **20**, respectively. Stopping the reaction after complete disappearance of the starting material (4 days, room temperature) afforded the α-imidate **19** (66%) and the β-imidate **20** (20%), whereas after prolonged reaction time (14 days) only **19** (81%) was obtained. Next, the phenyl 1-thiogalactosides **14** and **15** were converted into the fluorides **21** and **22**, using Nicolaou's method[27] [diethylaminosulfur trifluoride (DAST)–NBS]. Surprisingly, we found that the reaction occurred with inversion at the anomeric center. Thus, the phenyl 1-thio-β-D-galactoside **13** afforded a 65 : 35 mixture of the α-fluoride **21** and the β-fluoride **22** isolated in 68% overall yield, whereas the corresponding α anomer **15** gave exclusively **22** (85%). A similar inversion at the anomeric center was recently found for the conversion of ethyl 1-thioglycosides into fluorides by dimethyl(methylthio)sulfonium tetrafluoroborate[28]. Similarly, the β-thiogalactoside **14** was converted with bromine into the bromide **23** (53%). The chloride **24** was obtained in 74% yield from methyl galactoside **16** upon treatment with dichloromethyl methyl ether[29] as previously performed[21] with the corresponding *R*/*S*-mixture of **16**.

The pyruvated galactosyl donors **13**, **19**, and **21–24** thus prepared were subsequently treated, under appropriate activation, with 5-[(benzyloxycarbonyl)amino]-pentanol, and Table II summarizes the results. From all the donors tested here, the trichloroacetimidate **19** gave the highest yields of the desired aminopentyl galactoside **27**. However, when the glycosylation was performed in acetonitrile as the solvent, an excess of imidate **19** has to be used in order to bring the reaction to

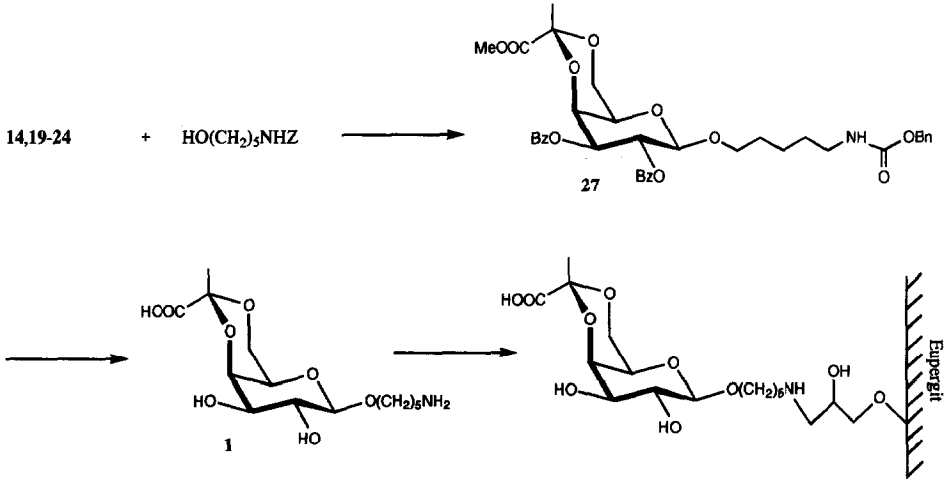
TABLE II

Reaction of pyruvated galactosyl donors **14** and **19–24** with 5-[(benzyloxycarbonyl)amino]pentanol, to give compound **27**

Donor	**14**	**19**	**19**	**21**	**22**	**23**	**24**
Promoter	NIS	Me_3SiOTf [a]		BF_3	BF_3	AgOTf	AgOTf
Yield of **27** (%)	44 [b]	quant [c]	98 [d]	83	62 [e]	71	74

[a] Tf = trifluoromethanesulfonyl. [b] Compound **13** (53%) was reisolated. [c] In acetonitrile, **25** (22%) was obtained in addition to **27**. [d] In dichloromethane. [e] Compound **26** (10%) was obtained in addition to **27**.

completion (TLC). This was because acid-catalyzed rearrangement[30] of the imidate occurred, affording the trichloroacetamido β-D-galactopyranoside **25** (22%) as a byproduct. Although the galactoside **27** was isolated in practically quantitative yield, purification of the latter was somewhat tedious because the contaminating trichloroacetamide could not be removed by a single chromatography run. In dichloromethane, the conversion **19** → **27** proceeded more cleanly and only traces of the byproduct **25** could be detected in TLC. All other donors gave significantly lower yields. The reaction of the phenyl 1-thiogalactoside **14** could not be brought to completion. Only the glycosylation with the α-fluoride **21**, according to Kunz's method[31], gave **27** in an acceptable 83% yield. From the more reactive β-fluoride **22**, the galactoside **27** (62%) was accompanied by the corresponding ethyl galactoside **26** (10%) formed by reaction of the donor with the diethyl ether content of the promoter (BF_3 · etherate). Finally, **27** was deblocked by first removing the benzoyl groups (Zemplén) followed by saponification of the methyl ester of the pyruvate acetal and subsequent hydrogenolysis of the amino protective group, to give the target ligand **1** in 97% overall yield.



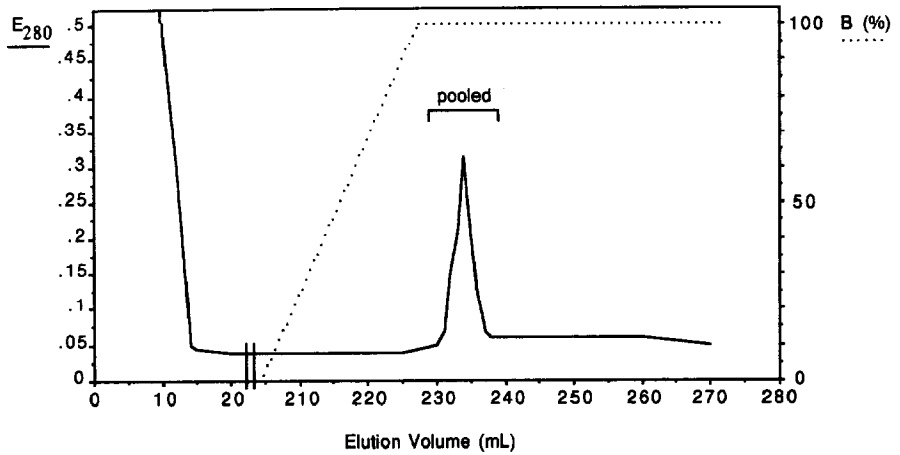


Fig. 1. Elution diagram of human serum amyloid protein P (SAP). Application of human serum with buffer *A* (0.1 M Tris·HCl, 0.1 M $CaCl_2$, 0.15 M NaCl, pH 7.8). Gradient elution (dotted line) with buffer *B* (0.1 M Tris·HCl, 0.15 M NaCl, 4 mM EDTA, pH 7.8).

Preparation of the affinity adsorbent and isolation of SAP from human. — As carrier material for the ligand **1**, we chose epoxypropyl-modified polyacrylamide beads (Eupergit®). The coupling was performed simply by agitating a suspension of the carrier in a buffered solution of **1** at room temperature until the monitored concentration of **1** remained unchanged. In a typical run using 5 g of dry carrier material, the initial concentration of **1**, determined by the phenol–sulfuric acid method[32], decreased during 5 days from 4.5 to 3.8 mM. Comparing the amount of ligand that disappeared from the solution with that which was determined as being bound to the carrier, a content of 7.7–13.5 μmol of **1** per gram of carrier was thus achieved.

For the isolation of SAP, human serum containing calcium chloride was passed over a column packed with the affinity adsorbent. After exhaustive washing of the column, calcium was removed by gradient elution with an EDTA-containing buffer. This abolished the calcium-dependent binding of SAP to the carrier-bound ligand **1** and eluted the protein. Fig. 1 shows the elution diagram. The fractions containing SAP were pooled and dialyzed against phosphate buffer without further chromatographic purification — an additional step that was previously necessary when agarose was used as affinity material[11]. Thus, starting from 45 mL of human serum a total amount of 0.6 mg of SAP was obtained. The protein was homogeneous and identical to an authentic sample according to SDS-PAGE (Fig. 2). It showed a molecular mass of 23 000 daltons in the denaturated form and ca. 230 000 daltons (not shown here) in the native form, as was previously determined[1,11].

In summary, the convenient preparation and application of an SAP-specific affinity adsorbent provides an efficient and rapid method for the isolation of pure SAP which will now be studied in detail concerning its specific binding to pyruvated sugars.

EXPERIMENTAL

For general methods, see ref 33. SAP isolation was performed using a Pharmacia FPLC-system 500 with 2 pumps P 500 and controller LCC 501 plus. SDS-PAGE was performed on Pharmacia gradient gels 10–15, using a Phast-system with Development unit. Gels were stained with Coomassie Brilliant Blue and scanned with a Desaga CD 60 densitometer. Compounds **8** and **9** were prepared as previously described[21]. Eupergit C® was purchased from Röhm & Haas, and an authentic sample of SAP from Sigma.

Allyl 2,3-di-O-benzoyl-4,6-O-benzylidene-α-D-galactopyranoside (**2**).—Benzoyl chloride (8.3 mL, 71.4 mmol) was added to a solution of allyl 4,6-*O*-benzylidene-α-D-galactopyranoside[34] (2.75 g, 8.9 mmol) in pyridine (10 mL) and the mixture was stirred at room temperature for 5 h. A small amount of water was added, in order to hydrolyze the excess of benzoyl chloride, and the mixture was poured into water and extracted with CH_2Cl_2. The organic layers were subsequently washed with aq HCl and aq $NaHCO_3$, and concentrated. Crystallization of the residue from EtOH afforded **2** (4.08 g, 89%); mp 153–155°C; $[\alpha]_D$ + 176.0° (*c* 1.7, $CHCl_3$); NMR data δ_H 5.93–5.73 (m, 3 H, H-2,3, C*H*=CH_2), 5.57 (s, 1 H, PhC*H*), 5.52 (d, 1 H, $J_{1,2}$ 1.5

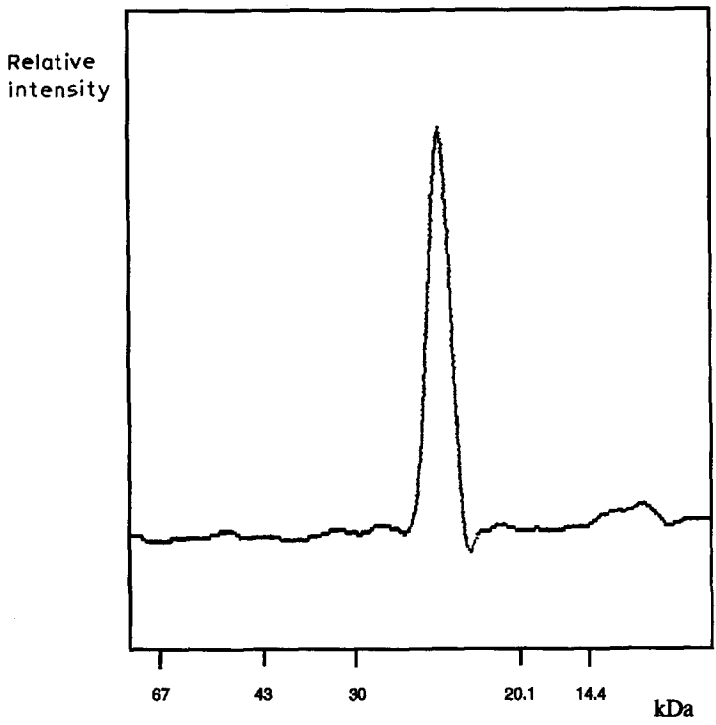


Fig. 2. Densitogram of the SDS-PAGE of the isolated SAP. Marker proteins: bovine serum albumin (67 kDa), chicken ovalbumin (43 kDa), carbonic anhydrase from bovine erythrocytes (30 kDa), soya bean trypsin inhibitor (20.1 kDa), cow milk α-lactalbumin (14.4 kDa).

Hz, H-1), 4.67 (bd, 1 H, H-4), 4.34 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ −12.5 Hz, H-6a), 4.31–3.46 (m, 4 H, H-5, 6b, CH=CH_2); δ_C 100.7 (PhCH), 96.3 (C-1), 74.3 (C-4), 69.4, 68.7 (C-2,3), 69.2, 68.8 (C-6, CH_2CH=CH_2), 62.5 (C-5). Anal. Calcd for $C_{30}H_{28}O_8$: C, 69.76; H, 5.46. Found: C, 69.66; H, 5.37.

Benzyl 2,3-di-O-benzoyl-4,6-O-benzylidene-α-D-galactopyranoside (**3**) *and benzyl 2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside* (**4**).—A suspension of D-galactose (10 g, 55.5 mmol) in benzyl alcohol (80 mL) containing 2% of HCl was stirred at 80°C until a clear solution was obtained (1 h). The mixture was stirred at 100°C for 3 h, cooled to room temperature, neutralized by addition of $BaCO_3$, and filtered through a layer of Celite. Diethyl ether (250 mL) and hexane (250 mL) were added to the filtrate, and the precipitate was collected by decantation and dissolved in water (50 mL). The aq solution was washed with hexane and concentrated. The oily residue (9.0 g) was mixed with benzaldehyde (25 mL) and $ZnCl_2$ (10 g), and the resulting mixture was vigorously stirred at room temperature for 4 h. Water (100 mL) and hexane (300 mL) were added, and the resulting solid was collected by filtration and washed repeatedly with water and hexane, to give crude benzyl 4,6-*O*-benzylidene-D-galactopyranoside (5.68 g, 29%), as a 1.6:1 $\alpha:\beta$ mixture; Significant NMR data: δ_H 5.12 (bs, 1.6 H, H-1α), 4.32 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1β).

Benzoyl chloride (10 mL, 86 mmol) was added to a solution of the above crude benzylidene derivative (5.6 g, 15.8 mmol) in pyridine (50 mL) and the mixture was stirred at room temperature for 3 h. Workup as described for **2** and chromatography gave, first, **3** (4.82 g, 54%), as a colorless oil; $[\alpha]_D$ + 141.3° (*c* 0.8, $CHCl_3$); NMR data: δ_H 5.85 (dd, 1 H, $J_{1,2}$ 2.9, $J_{2,3}$ 10.7 Hz, H-2), 5.80 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 5.56 (s, 1 H, PhC*H*), 5.46 (d, 1 H, H-1), 4.79, 4.63 (2 d, 2 H, *J* −12.3 Hz, PhCH_2), 4.65 (bd 1 H, H-4), 4.29 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ −12.5 Hz, H-6a), 4.08 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.92 (bs, 1 H, H-5); δ_C 100.7 (PhCH), 96.5 (C-1), 74.2, 69.4. 68.7 (C-2,3,4), 70.1 (PhCH_2), 69.1 (C-6), 62.7 (C-5). Anal. Calcd for $C_{34}H_{30}O_8$: C, 72.07; H, 5.34. Found: C, 72.24; H, 5.40.

Eluted next was **4** (3.21 g, 36%); mp 172–173°C (from EtOH) (lit.[35] mp 177–178°C).

Allyl 2,3-di-O-benzoyl-α-D-galactopyranoside (**5**).—A suspension of **2** (0.90 g, 1.7 mmol) in AcOH (80 mL) and water (10 mL) was stirred at 80°C until a clear solution was obtained (1.5 h). The solution was cooled to room temperature, concentrated, and coevaporated with toluene. Chromatography of the residue afforded **5** (0.55 g, 76%) as a colorless foam; $[\alpha]_D$ + 177.1° (*c* 0.9, $CHCl_3$); NMR data: δ_H 5.93–5.68 (m, 3 H, H-2,3, C*H*=CH_2), 5.32 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.48 (bd, 1 H, H-4), 4.28–4.06 (m, 2 H, H-6a,6b); δ_C 95.9 (C-1), 71.1, 69.8, 69.2, 68.8 (C-2,3,4,5), 63.2 (C-6). Anal. Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.71; H, 5.74.

Benzyl 2,3-di-O-benzoyl-α-D-galactopyranoside (**6**).—A suspension of **3** (2.24 g, 4.25 mmol) in AcOH (80 mL) and water (10 mL) was stirred at 75°C until a clear solution was obtained (1 h). Workup as described for **5** and chromatography

afforded **6** (1.8 g, 89%) as a colorless foam; $[\alpha]_D$ + 181.8° (*c* 1.4, $CHCl_3$); NMR data: δ_H 5.73 (bd, 2 H, H-2,3), 5,37 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.77, 4.58 (2 d, 2 H, *J* −12.3 Hz, PhCH_2), 4.48 (bs, 1 H, H-4), 4.10 (bt, 1 H, $J_{5,6}$ 4.6 Hz, H-5), 4.00–3.66 (m, 2 H, H-6a,6b); δ_C 96.0 (C-1), 71.1, 69.8, 69.4, 68.8 (C-2,3,4,5), 69.9 (PhCH_2), 63.2 (C-6). Anal. Calcd for $C_{27}H_{26}O_8$: C, 67.77; H, 5.48. Found: C, 67.66; H, 5.44.

*Ethyl 2,3-di-*O*-benzoyl-1-thio-β-*D*-galactopyranoside* (**7**).—A suspension of ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[20] (0.91 g, 1.75 mmol) in AcOH (35 mL) and water (12 mL) was stirred at 90°C until a clear solution was obtained (5 h). Workup as described for **5** and chromatography afforded **7** (0.6 g, 79%); mp 158°C; $[\alpha]_D$ −89.9° (*c* 0.9, $CHCl_3$); NMR data: δ_H 5.84 (t, 1 H, $J_{1,2}$ 9.9, $J_{2,3}$ 9.9 Hz, H-2), 5.35 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.73 (d, 1 H, H-1), 4.45 (bt, 1 H, H-4), 4.05–3.89 (m, 2 H, H-6a,6b), 3.81 (bt, 1 H, H-5), 2.90–2.60 (m, 2 H, SCH_2), 1.27 (t, 3 H, *J* 7.4 Hz, CH_3); δ_C 84.0 (C-1), 78.1, 75.4, 68.6, 67.9 (C-2,3,4,5), 62.9 (C-6), 24.1 (SCH_2), 14.9 (CH_3). Anal. Calcd for $C_{22}H_{24}O_7S$: C, 61.10; H, 5.59; S, 7.41. Found: C, 61.09; H, 5.62; S, 7.19.

*2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-*D*-galactopyranosides* (**10–16**).—*General procedure.* $BF_3 \cdot$ etherate (2.0 mol equiv) was added under Ar at room temperature to a solution of the appropriate galactoside-diol **5–9** (1.0 mol equiv) and methyl pyruvate (2.0 mol equiv) in (*a*) CH_2Cl_2 or (*b*) MeCN. The solution was stirred at room temperature until TLC showed complete consumption of the starting material. The mixture was poured into aq $NaHCO_3$ (reactions in MeCN were first diluted with CH_2Cl_2), the organic layer was separated, and the aq layer was extracted twice with CH_2Cl_2. Concentration of the combined organic layers and chromatography afforded **10–16**.

*Allyl 2,3-di-*O*-benzoyl-4,6-*O*[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galacto-pyranoside* (**10**).—(*a*) According to the general procedure, **5** (1.49 g, 4.5 mmol), methyl pyruvate (0.9 g, 9.1 mmol), and $BF_3 \cdot$ etherate (1.14 mL, 9.1 mmol) in CH_2Cl_2 (5 mL) afforded, after 20 h, **10** (1.01 g, 47%); $[\alpha]_D$ +160.7° (*c* 1.5, $CHCl_3$); NMR data: δ_H 5.91–5.78 (m, 1 H, C*H*=CH_2), 5.72 (dd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.7 Hz, H-2), 5.65 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 5.38 (d, 1 H, H-1), 5.35–5.11 (m, 2 H, CH=CH_2), 4.60 (dd, 1 H, $J_{4,5}$ 0.8 Hz, H-4), 4.34–4.01 (m, 2 H, CH_2CH=CH_2), 4.07 (bd, 2 H, H-6a,6b), 3.84 (bd, 1 H, H-5), 3.67 (s, 3 H, $COOCH_3$), 1.61 (s, 3 H, CH_3); δ_C 98.7 (*C*–$COOCH_3$), 96.2 (C-1), 69.8, 69.3, 68.4 (C-2,3,4), 68.9 (*C*H_2CH=CH_2), 65.3 (C-6), 61.7 (C-5), 52.4 ($COOCH_3$), 25.8 (CH_3). Anal. Calcd for $C_{27}H_{28}O_{10}$: C, 63.28; H, 5.51. Found: C, 63.15; H, 5.55.

(*b*) According to the general procedure, **5** (1.84 g, 4.3 mmol), methyl pyruvate 0.87 g, 8.6 mmol), and $BF_3 \cdot$ etherate (1.08 mL, 8.6 mmol) in MeCN (5 mL) afforded, after 2 h, **10** (1.53 g, 70%).

*Benzyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galac-topyranoside* (**11**).—(*a*) According to the general procedure, **6** (0.48 g, 1.0 mmol), methyl pyruvate (0.2 g, 2.0 mmol), and $BF_3 \cdot$ etherate (0.25 mL, 2.0 mmol) in CH_2Cl_2 (1 mL) afforded, after 24 h, an inseparable mixture (TLC).

(*b*) According to the general procedure, **6** (1.45 g, 3.0 mmol), methyl pyruvate

(0.73 g, 6.0 mmol), and $BF_3 \cdot$ etherate (0.74 mL, 6.0 mmol) in MeCN (12 mL) afforded, after 2 h, **11** (1.06 g, 63%); $[\alpha]_D$ + 145.6° (*c* 0.7, $CHCl_3$); NMR data: δ_H 5.76 (dd, 2 H, $J_{1,2}$ 3.3, $J_{2,3}$ 10.7 Hz, H-2), 5.67 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.42 (d, 1 H, H-1), 4.75, 4.61 (2 d, 2 H, *J* −12.3 Hz, PhCH_2), 5.59 (bd, 1 H, H-4), 4.02 (bd, 2 H, H-6a,6b), 3.79 (bs, 1 H, H-5), 3.66 (s, 3 H, $COOCH_3$), 1.60 (s, 3 H, CH_3); δ_C 98.7 (*C*–$COOCH_3$), 96.4 (C-1), 70.2 (Ph*C*H_2), 69.7, 69.3, 68.3 (C-2,3,4), 65.2 (C-6), 61.7 (C-5), 52.4 ($COOCH_3$), 25.8 (CH_3). Anal. Calcd for $C_{31}H_{30}O_{10}$: C, 66.19; H, 5.38. Found: C, 66.26; H, 5.25.

*Ethyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-β-*D*-galactopyranoside* (**12**) *and ethyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-α-*D*-galactopyranoside* (**13**).—(*a*) According to the general procedure, **7** (0.43 g, 1.0 mmol), methyl pyruvate (0.2 g, 2.0 mmol), and $BF_3 \cdot$ etherate (0.25 mL, 2.0 mmol) in CH_2Cl_2 (1 mL) afforded, after 12 h, complete decomposition of the starting material without any formation of **12** (TLC).

(*b*) According to the general procedure, **7** (0.59 g, 1.4 mmol), methyl pyruvate (0.28 g, 2.8 mmol), and $BF_3 \cdot$ etherate (0.34 mL, 2.8 mmol) in MeCN (3 mL) afforded, after 2 h, first **13** (0.12 g, 17%); $[\alpha]_D$ + 146.5° (*c* 1.5, $CHCl_3$); NMR data: δ_H 5.98–5.09 (m, 2 H, $J_{2,3}$ 9.6 Hz, H-1,2), 5.50 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 4.60 (bd, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 4.19 (bs, 1 H, $J_{5,6a}$ 1.6, $J_{5,6b}$ 1.5 Hz, H-5), 4.12 (dd, 1 H, $J_{6a,6b}$ −13.1 Hz, H-6a), 4.04 (dd, 1 H, H-6b), 3.68 (s, 3 H, $COOCH_3$), 2.62–2.56 (m, 2 H, SC$H_2$$CH_3$), 1.62 (s, 3 H, CH_3), 1.25 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3); δ_C 98.7 (*C*–$COOCH_3$), 82.6 (C-1), 69.8, 69.5, 67.9 (C-2,3,4), 65.3 (C-6), 61.7 (C-5), 52.4 ($COOCH_3$), 25.7 (CH_3), 24.3 (S$CH_2$$CH_3$), 14.7 ($SCH_2CH_3$). MS for $C_{26}H_{28}O_9S$: *m/z* 517 (MH^+).

Eluted next was **12** (0.24 g, 34%); $[\alpha]_D$ + 69.7° (*c* 1.4, $CHCl_3$); NMR data: δ_H 5.91 (t, 1 H, $J_{1,2}$ 9.9, $J_{2,3}$ 9.9 Hz, H-2), 5.24 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.69 (d, 1 H, H-1), 4.57 (bd, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 4.16 (dd, 1 H, $J_{5,6a}$ 1.4, $J_{6a,6b}$ −12.9 Hz, H-6a), 4.03 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 3.66 (s, 3 H, $COOCH_3$), 3.61 (bs, 1 H, H-5), 2.91–2.71 (m, 2 H, SC$H_2$$CH_3$), 1.59 (s, 3 H, CH_3), 1.30 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3); δ_C 98.6 (*C*–$COOCH_3$), 82.9 (C-1), 73.7 (C-4), 69.2, 69.0 (C-2,3), 67.0 (C-5), 65.3 (C-6), 52.4 ($COOCH_3$), 25.6 (CH_3), 23.2 (S$CH_2$$CH_3$), 14.8 ($SCH_2CH_3$). Anal. Calcd for $C_{26}H_{28}O_9S$: C, 60.45; H, 5.46; S, 6.21. Found: C, 60.53; H, 5.48; S, 5.59.

*Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-β-*D*-galactopyranoside* (**14**) *and phenyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-α-*D*-galactopyranoside* (**15**).—(*a*) According to ref 24, **8** afforded, after 24 h in CH_2Cl_2, **14** (28%) and **15** (30%).

(*b*) According to the general procedure, **8** (1.0 g, 2.1 mmol), methyl pyruvate (0.42 g, 4.2 mmol), and $BF_3 \cdot$ etherate (0.52 mL, 4.2 mmol) in MeCN (2 mL) afforded, after 2.5 h, **14** (1.07 g, 91%); identical in all respects to the previously obtained compound[24].

*Methyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galactopyranoside* (**16**).—(*a*) According to ref 24, **9** afforded, after 24 h in CH_2Cl_2, **16** (58%).

(*b*) According to the general procedure, **9** (5.61 g, 13.9 mmol), methyl pyruvate (2.84 g, 27.9 mmol), and $BF_3 \cdot$ etherate (3.5 mL, 27.9 mmol) in MeCN (12 mL) afforded, after 2.5 h, **16** (5.53 g, 82%), identical in all respects to the previously obtained compound[24].

2-Oxopropyl 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonylethylidene]-α-D-galactopyranoside (**17**) *and 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonylethylidene]-D-galactopyranose* (**18**).—(*a*) A solution of **10** (1.54 g, 3.0 mmol) and a catalytic amount of $PdCl_2$ (40 mg) in carefully degassed aq AcOH (90%, 40 mL) was stirred at 60°C while N_2 was bubbled through the solution. When TLC showed complete conversion of the starting material into a slower moving product (6 h), the solution was cooled to room temperature and concentrated. Chromatography of the residue afforded **18** (1.29 g, 91%) as a 6:1 α/β mixture; NMR data (significant peaks): δ_H (Me_2SO-d_6) 5.60 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2α), 5.47 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1α), 5.00 (t, 1 H, $J_{1,2} = J_{2,3} = 6.8$ Hz, H-2β). Anal. Calcd for $C_{24}H_{24}O_{10}$: C, 61.02; H, 5.12. Found: C, 60.82; H, 5.24.

(*b*) Treatment of **10** (0.94 g, 1.8 mmol) and a catalytic amount of $PdCl_2$ (50 mg) in aq AcOH (90%, 40 mL) as described above, but without the protective N_2 atmosphere, showed the formation of two slower moving products in TLC. Chromatography after workup as described above afforded, first, **17** (0.32 g, 33%); $[\alpha]_D$ +146° (*c* 1.0, $CHCl_3$); NMR data: δ_H 5.77 (dd, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 10.8 Hz, H-2), 5.68 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.39 (d, 1 H, H-1), 4.62 (bd, 1 H, $J_{4,5} < 1.0$ Hz, H-4), 4.21, 4.22 (2 s, 2 H, OCH_2), 4.06, 4.04 (2 s, 2 H, H-6a,6b), 3.95 (s, 1 H, H-5), 3.68 (s, 3 H, $COOCH_3$), 2.11 (s, 3 H, $O{=}CCH_3$), 1.61 (s, 3 H, CH_3); δ_C 204.8 ($O{=}CCH_3$), 98.7 (C–$COOCH_3$), 97.7 (C-1), 73.2 (OCH_2), 69.6, 68.9, 68.2 (C-2,3,4), 65.2 (C-6), 62.0 (C-5), 52.5 ($COOCH_3$), 26.5 ($O{=}CCH_3$), 25.7 (CH_3). Anal. Calcd for $C_{27}H_{28}O_{11}$: C, 61.36; H, 5.34. Found: C, 61.11; H, 5.47.

Eluted next was **18** (0.37 g, 43%).

(*c*) A suspension of **11** (1.0 g, 1.78 mmol) and a catalytic amount of Pd–C (10%, 100 mg) in AcOH (50 mL) was treated at room temperature with H_2 at atmospheric pressure until TLC showed complete conversion of the starting material (3 days). The mixture was filtered through a layer of Celite and concentrated. Chromatography of the residue afforded **18** (0.81 g, 96%).

2,3-Di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonylethylidene]-α-D-galactopyranosyl trichloroacetimidate (**19**) *and 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonylethylidene]-β-D-galactopyranosyl trichloroacetimidate* (**20**).—(*a*) A suspension of **18** (0.88 g, 1.9 mmol), trichloroacetonitrile (0.5 mL), and K_2CO_3 (0.43 g, 3.1 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 4 days. The mixture was centrifuged and the supernatant solution was concentrated. Chromatography of the residue afforded, first, **19** (0.75 g, 66%); $[\alpha]_D$ +123.5° (*c* 1.0, $CHCl_3$); NMR data: δ_H 8.59 (s, 1 H, NH), 6.83 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.01 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 5.71 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 4.74 (bd, 1 H, $J_{4,5} < 1.0$ Hz, H-4), 4.15 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ −13.3 Hz, H-6a), 4.05 (dd, 1 H, $J_{5,6b}$ 1.6 Hz, H-6b), 4.04 (bs, 1 H, H-5), 3.68 (s, 3 H, $COOCH_3$), 1.63 (s, 3 H, CH_3); δ_C 160.6 (C=NH), 94.5

(C-1), 90.9 (CCl_3), 69.1, 67.1 (2 C, 1 C, C-2,3,4), 64.9 (C-6), 64.1 (C-5), 52.5 ($COOCH_3$), 25.7 (CH_3). Anal. Calcd for $C_{26}H_{24}Cl_3NO_{10}$: C, 50.63; H, 3.92; N, 2.27; Cl, 17.24. Found: C, 50.48; H, 3.87; N, 2.19; Cl, 17.52.

Eluted next was **20** (0.23 g, 20%); mp 157–158°C (acetone–hexane); $[\alpha]_D$ +96.2° (*c* 1.0, $CHCl_3$); NMR data: δ_H 6.13–6.00 (m, 2 H, H-1,2), 5.28 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 3.6 Hz, H-3), 4.62 (bs, 1 H, $J_{4,5}$ < 1.0 Hz, H-4), 4.21 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ −13.1 Hz, H-6a), 4.08 (dd, 1 H, $J_{5,6b}$ 1.9 Hz, H-6b), 3.76 (bs, 1 H, H-5), 3.67 (s, 3 H, $COOCH_3$), 1.63 (s, 3 H, CH_3); δ_C 161.6 (C=NH), 98.8 (*C*–$COOCH_3$), 96.4 (C-1), 90.3 (CCl_3), 72.6 (C-4), 68.7, 67.9 (C-2,3), 66.6 (C-5), 64.7 (C-6), 52.5 ($COOCH_3$), 25.6 (CH_3). Anal. Calcd for $C_{26}H_{24}Cl_3NO_{10}$: C, 50.63; H, 3.92; N, 2.27; Cl, 17.24. Found: C, 50.86; H, 4.00; N, 2.33; Cl, 17.25.

(*b*) Treatment of **18** (1.4 g, 3.0 mmol) with trichloroacetonitrile (5 mL) and K_2CO_3 (5 g, 36.2 mmol) in CH_2Cl_2 (25 mL) for 14 days at room temperature, as described above, afforded **19** (1.47 g, 81%).

*2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galactopyranosyl fluoride* (**21**) *and 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-β-*D*-galactopyranosyl fluoride* (**22**).—(*a*) DAST (270 μL, 2.2 mmol) was added under Ar at −15°C to a solution of **14** (0.8 g, 1.4 mmol) in CH_2Cl_2 (9 mL) followed by NBS (0.33 g, 1.9 mmol). The orange mixture was stirred at −15°C until TLC showed complete consumption of the starting material (3.5 h). The mixture was diluted with CH_2Cl_2, washed with aq $NaHCO_3$, and concentrated. Chromatography of the residue afforded, first, **21** (0.3 g, 45%); $[\alpha]_D$ +147.5° (*c* 1.4, $CHCl_3$); NMR data: δ_H 6.04 (dd, 1 H, $J_{1,2}$ 2.6, $J_{1,F}$ 54 Hz, H-1), 5.80 (dq, 1 H, $J_{2,3}$ 10.7, $J_{2,F}$ 23.6 Hz, H-2), 5.62 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 4.69 (bd, 1 H, H-4), 4.17–4.02 (m, 2 H, H-6a,6b), 4.03 (bs, 1 H, H-5), 3.69 (s, 3 H, $COOCH_3$), 1.62 (s, 3 H, CH_3); δ_C 169.8 ($COOCH_3$), 105.4 (d, $J_{1,F}$ 227 Hz, C-1), 98.9 (*C*–$COOCH_3$), 69.1, 68.5 (C-3,4), 67.7 (d, $J_{2,F}$ 23.9 Hz, H-2), 64.7 (C-6), 63.8 (C-5), 52.6 ($COOCH_3$), 25.6 (CH_3). Anal. Calcd for $C_{24}H_{23}FO_9$: C, 60.76; H, 4.89. Found: C, 60.86; H, 4.90.

Eluted next was **22** (0.15 g, 23%); $[\alpha]_D$ +90.9° (*c* 1.2, $CHCl_3$); NMR data: δ_H 5.93 (dq, 1 H, $J_{1,2}$ 7.2, $J_{2,3}$ 10.5, $J_{2,F}$ 12 Hz, H-2), 5.48 (dd, 1 H, $J_{1,F}$ 53 Hz, H-1), 5.23 (dq, 1 H, $J_{3,4}$ 3.5, $J_{3,F}$ < 1 Hz, H-3), 4.60 (bt, 1 H, H-4), 4.23–4.05 (m, 2 H, H-6a,6b), 3.70 (bs, 1 H, H-5), 3.68 (s, 3 H, $COOCH_3$), 1.63 (s, 3 H, CH_3); δ_C 169.9 ($COOCH_3$), 107.4 (d, $J_{1,F}$ 218 Hz, C-1), 98.9 (*C*–$COOCH_3$), 71.8 (d, $J_{3,F}$ 11 Hz, C-3), 69.1 (d, $J_{2,F}$ 24 Hz, C-2), 68.9 (C-4), 66.1 (C-5), 64.6 (C-6), 52.5 ($COOCH_3$), 25.5 (CH_3). Anal. Calcd for $C_{24}H_{23}FO_9$: C, 60.76; H, 4.89. Found: C, 60.57; H, 4.98.

(*b*) Treatment of **15** (0.96 g, 1.7 mmol), DAST (315 μL, 2.6 mmol), and NBS (0.39 g, 2.2 mmol) in CH_2Cl_2 (22 mL) for 15 h at −15°C, as described above, afforded **22** (0.69 g, 85%).

*2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galactopyranosyl bromide* (**23**).—Bromide (0.64 g, 4.0 mmol) was added at room temperature to a solution of **14** (2.0 g, 3.5 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred until TLC showed complete conversion of the starting material into a single faster

moving product (2 h). The mixture was washed with aq $NaHCO_3$ and aq $Na_2S_2O_5$, and concentrated. Chromatography of the residue afforded **23** (1.0 g, 53%); $[\alpha]_D$ +195.8° (*c* 1.3, $CHCl_3$); NMR data: δ_H 6.92 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 5.71–5.70 (m, 2 H, H-2,3), 4.69 (bd, 1 H, $J_{3,4}$ 1.6 Hz, H-4), 4.10 (bs, 3 H, H-5,6a,6b), 3.69 (s, 3 H, $COOCH_3$), 1.61 (s, 3 H, CH_3); δ_C 98.8 (*C*–$COOCH_3$), 89.8 (C-1), 69.8 (C-4), 68.7 (C-3), 67.7 (C-2), 66.2 (C-5), 64.6 (C-6), 52.6 ($COOCH_3$), 25.6 (CH_3). MS for $C_{24}H_{23}BrO_9$: *m/z* 535, 537 (1:1, MH^+).

*2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-*D*-galactopyranosyl chloride* (**24**).—A suspension of methyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-α-D-galactopyranoside (**16**; 1.2 g, 2.5 mmol), dichloromethyl methyl ether (6 mL), and a catalytic amount of freshly molten $ZnCl_2$ (20 mg) in $CHCl_3$ (20 mL) was heated at 65°C until TLC showed complete conversion of the starting material into a faster moving product (11 h). The mixture was concentrated, coevaporated with toluene, and chromatographed to afford **24** (1.01 g, 83%), identical in all respects to the previously described compound[21].

*2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-*N*-trichloroacetyl-β-D-galactopyranosylamine* (**25**), *ethyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-β-D-galactopyranoside* (**26**), *and 5-[(benzyloxycarbonyl)amino]pentyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-β-D-galactopyranoside* (**27**).—(*a*) A solution of *N*-iodosuccinimide (NIS) (0.31 g, 1.4 mmol) and trifluoromethanesulfonic acid (12 μL, 0.14 mmol) in 1:1 CH_2Cl_2–ether (15 mL) was added at −40°C to a suspension of **14** (0.68 g, 1.2 mmol), 5-[(benzyloxycarbonyl)amino] pentanol[19] (0.33 g, 1.4 mmol), and 4A molecular sieves (0.5 g) in CH_2Cl_2 (15 mL), and the resulting red mixture was stirred at −40°C. After 12 h, when TLC showed no reaction, additional trifluoromethanesulfonic acid (10 μL) was added at 0°C and the mixture was stirred at room temperature for 2 weeks. The solution was diluted with CH_2Cl_2, filtered, washed with aq $NaHCO_3$, and concentrated. Chromatography of the residue afforded, first, starting material **14** (0.36 g, 53% reisolated).

Eluted next was **27**; $[\alpha]_D$ +45.9° (*c* 0.8, $CHCl_3$); NMR data: δ_H 5.79 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.4 Hz, H-2), 5.19 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.07 (s, 2 H, PhCH_2), 4.64 (d, 1 H, H-1), 4.61–4.57 (m, 1 H, OCH_2), 4.52 (bd, 1 H, H-4), 4.14 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ −12.8 Hz, H-6a), 4.06 (dd, 1 H, $J_{5,6b}$ 1.8 Hz, H-6b), 3.97 (dt, 1 H, *J* 5.9, −11.8 Hz, OCH_2), 3.65 (s, 3 H, $COOCH_3$), 3.53–3.44 (m, 2 H, H-5, $NHCH_2$), 2.92 (bq, 1 H, $NHCH_2$), 1.59 (s, 3 H, CH_3), 1.55–1.42 (m, 2 H, CH_2), 1.39–1.17 (m, 4 H, CH_2); δ_C 101.3 (C-1), 98.9 (*C*–$COOCH_3$), 72.8 (C-2), 69.6 (PhCH_2), 69.1 (2 C, C-3,4), 66.5 (OCH_2), 65.7 (C-5), 65.1 (C-6), 52.4 ($COOCH_3$), 40.9 ($NHCH_2$), 29.5, 28.9, 23.1 (3 CH_2), 25.6 (CH_3). Anal. Calcd for $C_{37}H_{41}NO_{12}$: C, 64.25; H, 5.97; N, 2.02. Found: C, 63.78; H, 5.93; N, 1.98.

(*b*) Trimethylsilyl trifluoromethanesulfonate (10 μL, 0.05 mmol) was added at 0°C to a solution of 5-[(benzyloxycarbonyl)amino]pentanol (0.12 g, 0.5 mmol) in MeCN (2 mL). To that mixture was added a solution of **19** (0.5 g, 0.81 mmol) in MeCN (5 mL) at 0°C during 0.5 h. The mixture was neutralized by addition of

pyridine (0.5 mL), diluted with CH_2Cl_2, washed with aq $NaHCO_3$, and concentrated. Chromatography of the residue afforded, first, **25** (0.11 g, 22%); $[\alpha]_D$ +105.4° (*c* 1.1, $CHCl_3$); NMR data: δ_H 5.81 (bt, 1 H, $J_{1,2}$ 9.1, $J_{2,3}$ 9.9 Hz, H-2), 5.45 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 5.35 (t, 1 H, $J_{1,NH}$ 9.1 Hz, H-1), 4.63 (bd, 1 H, H-4), 4.17 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ −13.1 Hz, H-6a), 4.05 (dd, 1 H, $J_{5,6b}$ 1.8 Hz, H-6b), 3.76 (bd, 1 H, H-5), 3.69 (s, 3 H, $COOCH_3$), 1.63 (s, 3 H, CH_3); δ_C 98.9 (C–$COOCH_3$), 91.6 (CCl_3), 80.4 (C-1), 72.0, 69.1, 68.5, 67.6 (C-2,3,4,5), 65.0 (C-6), 52.6 ($COOCH_3$), 25.6 (CH_3). Anal. Calcd for $C_{26}H_{24}Cl_3NO_{10}$: C, 50.63; H, 3.92; N, 2.27. Found: C, 50.31; H, 4.06; N, 2.14.

Eluted next was crude **27** that was rechromatographed and stored in a vacuum oven for 24 h, to give 0.35 g of pure material (100%).

(*c*) Treatment of a solution of 5-[(benzyloxycarbonyl)amino]pentanol (0.12 g, 0.5 mmol) and trimethylsilyl trifluoromethanesulfonate (10 μL, 0.05 mmol) in CH_2Cl_2 (2 mL) with a solution of **19** (0.5 g, 0.81 mmol) in CH_2Cl_2 (5 mL), as described in (*b*), afforded **27** (0.34 g, 98%).

(*d*) $BF_3 \cdot$ etherate (262 μL, 2.1 mmol) was added at room temperature to a solution of **21** (0.25 g, 0.53 mmol), 5-[(benzyloxycarbonyl)amino]pentanol (0.14 g, 0.6 mmol), and Et_3N (105 μL, 0.75 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred for 1 h, washed with aq $NaHCO_3$, and concentrated. Chromatography of the residue afforded **27** (0.22 g, 83%).

(*e*) Treatment of **22** (0.5 g, 1.05 mmol), 5-[(benzyloxycarbonyl)amino]pentanol (0.28 g, 1.2 mmol), Et_3N (209 μL, 1.5 mmol), and $BF_3 \cdot$ etherate (526 μL, 4.2 mmol) in CH_2Cl_2 (20 mL) for 1 h at room temperature and workup as described above afforded, first, **26** (50 mg, 10%); $[\alpha]_D$ +77.7° (*c* 0.3, $CHCl_3$); NMR data: δ_H 5.80 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.4 Hz, H-2), 5.20 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 4.69 (d, 1 H, H-1), 4.53 (bd, 1 H, H-4), 4.16 (dd, 1 H, $J_{5,6a}$ 1.6, $J_{6a,6b}$ −12.8 Hz, H-6a), 4.07 (dd, 1 H. $J_{5,6b}$ 1.9 Hz, H-6b), 3.68–3.55 (m, 1 H, H-5), 3.99–3.55 (m, 2 H, OCH_2), 3.66 (s, 3 H, $COOCH_3$), 1.60 (s, 3 H, CH_3), 1.15 (t, 3 H, J 7.1 Hz, CH_2CH_3); δ_C 170.2 ($COOCH_3$), 100.9 (C-1), 98.8 (C–$COOCH_3$), 72.9 (C-4), 69.0, 68.9 (C-2,3), 65.7 (C-5), 65.3, 65.1 (OCH_2, C-6), 52.4 ($COOCH_3$), 25.6 (CH_3), 15.0 (CH_2CH_3). MS for $C_{26}H_{28}O_{10}$: m/z 441 ($M^+ - COOCH_3$).

Eluted next was **27** (0.45 g, 62%).

(*f*) Silver trifluoromethanesulfonate (385.4 mg, 1.5 mmol) was added at room temperature to a solution of **23** (535.4 mg, 1.0 mmol), 5-[(benzyloxycarbonyl)amino]pentanol (356.0 mg, 1.5 mmol), and 2,4,6-trimethylpyridine (84.8 mg, 0.7 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 15 min, diluted with CH_2Cl_2, filtered, and washed with aq $NaHCO_3$ and aq $Na_2S_2O_5$. Concentration and chromatography of the residue afforded **27** (490 mg, 71%).

(*g*) A solution of **24** (1.53 g, 3.12 mmol) and 2,4,6-trimethylpyridine (303 mg, 2.5 mmol) in CH_2Cl_2 (12 mL) was added under Ar at 0°C to a suspension of 5-[(benzyloxycarbonyl)amino]pentanol (0.81 g, 3.4 mmol), silver trifluoromethanesulfonate (2.39 g, 9.36 mmol), and 3A molecular sieves (0.5 g) in CH_2Cl_2 (8 mL), and the mixture was stirred until TLC showed complete consumption of the

starting materials (35 min). Workup as described under (*f*) and chromatography afforded **27** (1.6 g, 74%).

5-Aminopentyl 4,6-O-(R)-1-carboxyethylidene-β-D-galactopyranoside (**1**).—A solution of **27** (396.2 mg, 0.57 mmol) and a catalytic amount of NaOMe in MeOH (5 mL) was stirred at 50°C for 5 h and cooled to room temperature. The solution was neutralized by addition of Lewatit ion-exchange resin (H^+), filtered, concentrated, and eluted from silica gel with 10:1 CH_2Cl_2–MeOH. Carbohydrate-containing fractions were pooled and concentrated, and the oily residue was dissolved in 0.1 M NaOH (10 mL) and MeOH (5 mL). After 12 h at room temperature, the solution was again neutralized with Lewatit resin (H^+) and filtered. A catalytic amount of Pd–C (10%, 50 mg) was added and the suspension was treated at atmospheric pressure with H_2 for 5 h. Filtration through a layer of Celite, concentration, and chromatography of the residue on Bio-Gel P2 with water afforded, after lyophilization of the appropriate fractions, **1** (186.3 mg, 97%); $[\alpha]_D$ −25.1° (*c* 0.8, H_2O); NMR data: δ_C 179.0 (COOH), 105.1 (C-1), 103.6 (*C*–COOH), 74.6 (C-2), 73.8 (C-3), 73.3 (OCH_2), 72.7 (C-4), 68.9 (C-5), 67.7 (C-6), 42.3 ($NHCH_2$), 31.0, 29.5, 28.1 (3 CH_2), 25.0 (CH_3). FABMS: *m/z* 336 (MH^+), 358 ($M + Na^+$).

Binding of **1** *to Eupergit*.—Eupergit C® (5 g) was repeatedly suspended in water (25 mL) and filtered, and finally washed with aq Na_2CO_3 (buffer *A*, 0.3 M, pH 11). The wet material was suspended in buffer *A* (100 mL) containing **1** (4.5 mM) and the mixture was shaken at room temperature. Aliquots (50 μL) were withdrawn and the carbohydrate content of each sample was determined by the phenol–H_2SO_4 method[32]. After 5 days, a constant concentration of 3.8 mM of **1** was achieved. The solid material was collected by filtration, washed with 0.3 M buffer *A* (3 × 300 mL), water (600 mL), 0.1 M buffer *A* (3 × 100 mL, pH 8) containing NaCl (0.5 M), and 0.1 M NaOAc (3 × 100 mL, pH 4) containing NaCl (0.5 M). The material was suspended in buffer *B* (1 M ethanolamine–HCl, pH 8, 50 mL) and shook at room temperature for 16 h. After that period, the material was collected again by filtration, and washed with water (3 × 100 mL) and 25 mM NaCl (3 × 100 mL). A sample of the adsorbent was lyophilized and the carbohydrate content was determined[32] to be 13.5 μmol/g.

Isolation of serum amyloid P protein (SAP).—A suspension of the aforementioned adsorbent in water (10 mL) was packed into a column (Pharmacia, length 10 cm, i.d. 10 cm) which was equilibrated with buffer *A* (0.1 M Tris · HCl, 0.1 M $CaCl_2$, 0.15 M NaCl, pH 7.8). Human serum (45 mL) was mixed with buffer *A* (50 mL), delipidated by ultracentrifugation at 40 000*g* for 40 min at 4°C, and filtered through a 0.45-μm membrane. The filtrate was applied to the column at a flow rate of 1 mL/min. The column was washed with buffer *A* until no more protein could be detected (see elution diagram Fig. 1). The bound SAP was liberated from the column by gradient elution with buffer *B* (0.1 M Tris · HCl, 0.15 M NaCl, 4 mM EDTA, pH 7.8) at a flow rate of 0.5 mL/min and collecting 1-mL fractions. The SAP-containing fractions were pooled, dialyzed against phosphate buffer (10

mM, pH 7.5) with an Amicon cell, and finally concentrated to a total volume of 2 mL. Protein determination according to the Pierce BCA protein assay[36] showed a total amount of SAP of 600 μg. SDS-PAGE revealed the isolated protein to be homogeneous (Fig. 2) and having a molecular mass of 23000 daltons for the subunit and 230000 daltons in the native form, and to be identical to an authentic sample of SAP.

ACKNOWLEDGMENTS

We thank Professor Dr. Dr. h.c. F. Effenberger for helpful discussions and for providing the laboratory facilities. We also thank Dr. med. L. Gabriel for a gift of human serum, and Dr. P. Fischer and J. Rebell for performing the NMR spectra. This work was financially supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1 M. Skinner and A.S. Cohen, *Methods Enzymol.*, 163 (1988) 523–536.
2 R.N. Kalaria, *Adv. Behav. Biol.*, 38 (1990) 87–90.
3 R.H. Painter, I. De Escallon, A. Massey, L. Pinteric, and S.B. Stern, *Ann. N.Y. Acad. Sci.*, 389 (1982) 199–215.
4 F.C. De Beer, M.L. Baltz, S. Holford, A. Feinstein, and M.B. Pepys, *J. Exp. Med.*, 154 (1981) 1134–1149.
5 A. Pollak, H. Coradello, U. Latzka, A. Lischka, and G. Lubec, *Klin. Wochenschr.*, 94 (1982) 291–293.
6 H. Hamazaki, *J. Biol. Chem.*, 262 (1987) 1456–1460.
7 E.C. Williams, B.J. Huppert, and S. Asakura, *J. Lab. Clin. Med.*, 120 (1992) 159–167.
8 C.R.K. Hind, P.M. Collins, D. Renn, R.B. Cook, D. Caspi, M.L. Baltz, and M.B. Pepys, *J. Exp. Med.*, 159 (1984) 1058–1069.
9 C.R.K. Hind, P.M. Collins, and M.B. Pepys, *Biochim. Biophys. Acta*, 802 (1984) 148–150.
10 C.R.K. Hind, P.M. Collins, M.L. Baltz, and M.B. Pepys, *Biochem. J.*, 225 (1985) 107–111.
11 Z. Urbányi and D. Medzihradszky, *J. Chromatogr.*, 578 (1992) 130–133.
12 J. Carlsson, J.-C. Janson, and M. Sparrman, in J.-C. Janson, and L. Rydén (Eds.), *Protein Purification*, VCH, Weinheim, 1989, pp 275–329.
13 R. Barker, C.K. Chiang, I.P. Trayer, and R.L. Hill, *Methods Enzymol.*, 34 (1974) 317–328.
14 C.K. Chiang, M. McAndrew, and R. Barker, *Carbohdyr. Res.*, 70 (1979) 93–102.
15 J. Vernon, S. Roseman, and Y.C. Lee, *Carbohydr. Res.*, 82 (1980) 59–69.
16 T. Ziegler, *Angew. Chem.*, 104 (1992) 1369–1371; *Angew. Chem. Int. Ed. Engl.*, 31 (1992) 1358–1360.
17 T. Ziegler and E. Eckhardt, *Tetrahedron Lett.*, 33 (1992) 6615–6618.
18 T. Ziegler, E. Eckhardt, and V. Birault, *J. Org. Chem.*, 58 (1993) 1090–1099.
19 T. Suami, Jpn. Kokai Tokkyo Koho Jp 58 49, 395 (1983); *Chem. Abstr.*, 99 (1983) P 212875h.
20 A.F. Bochkov, V.M. Dashunin, and N.K. Kochetkov, *Izv. Akad. Nauk SSSR*, 3 (1975) 632–638.
21 T. Ziegler, E. Eckhardt, and G. Herold, *Liebigs Ann. Chem.*, (1992) 441–451.
22 E.J. Reist, R.R. Spencer, D.F. Calkins, B.R. Baker, and L. Goodman, *J. Org. Chem.*, 30 (1985) 2312–2317.
23 T. Ziegler, E. Eckhardt, K. Neumann, and V. Birault, *Synthesis*, (1992) 1013–1017.
24 T. Ziegler, E. Eckhardt, and G. Herold, *Tetrahedron Lett.*, 31 (1992) 4413–4416.
25 P.A.J. Gorin, M. Mazurek, H.S. Duarte, M. Iacomini, and J.H. Duarte, *Carbohydr. Res.*, 100 (1982) 1–15.
26 J. Tsuji, *Synthesis*, (1984) 369–384.
27 R.E. Dolle and K.C. Nicolaou, *J. Am. Chem. Soc.*, 107 (1985) 1695–1698.
28 L. Blomberg and T. Norberg, *J. Carbohydr. Chem.*, 11 (1992) 751–760.

29 H. Groß, I. Farkas, and R. Bognár, *Z. Chem.*, 18 (1978) 201–210.
30 M.G. Hoffmann and R.R. Schmidt, *Liebigs Ann. Chem.*, (1985) 2403–2419.
31 H. Kunz and W. Sager, *Helv. Chim. Acta*, 68 (1985) 283–287.
32 M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
33 T. Ziegler, *Carbohydr. Res.*, preceding paper.
34 R. Gigg and C.D. Warren, *J. Chem. Soc.*, (1968) 1903–1911.
35 G.J.F. Chittenden and J.G. Buchanan, *Carbohydr. Res.*, 11 (1969) 379–385.
36 P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D.C. Klenk, *Anal. Biochem.*, 150 (1985) 76–85.