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Reactions of per-*O*-Benzoyl-β-D-Gal*f* Isothiocyanate, a Chiral Resolving Agent

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Abstract: Tetra-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate (**1**) was readily prepared (90% yield) from per-*O*-benzoyl-D-galactofuranose, *via* the glycosyl bromide. Reaction of **1** with alcohols, amines and amino acids afforded a variety of glycosylthiourethanes and glycosylthioureides. The isothiocyanate **1** showed to be useful as a chiral resolving agent. The resolution of racemic primary and secondary amines and aminoalcohols was readily accomplished, in analytical and preparative scale, by condensation of such compounds with **1**. © 1997 Elsevier Science Ltd.

INTRODUCTION

Glycosyl isothiocyanates are versatile intermediates in synthesis as they can be transformed into glycosyl heterocycles, nucleosides and a variety of biologically important *N*-glycosyl derivatives.[1,2] Particularly, glycosylthioureas are interesting because of their antifungal and antimicrobial properties.[3] Also, glycosylthioureido sugars are analogues of phosphatosugars in which the phosphate has been replaced by the non-ionic, isosteric thiourea group.[4,5] The reaction of isothiocyanates with oligovalent, multibranched amines to yield oligoantennary, thiourea-bridged glycoconjugates has been recently reported.[6]

Besides their use in the synthesis of asymmetric molecules of biological interest, the isothiocyanate group has been employed for chiral derivatization. For example, the terpene isothiocyanates synthesized by Nambara *et al*[7] showed to be useful for the resolution of racemic amines by high perfomance liquid chromatography (HPLC). Per-*O*-acyl-D-glycopyranosyl isothiocyanates having *gluco*[8-11] and *arabino*[12] configurations have been employed for the resolution of amino compounds. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate has also been applied to the resolution of chiral oxiranes[13] and thiols.[14] Recently,[15] *(R,R)*- and *(S,S)-trans* diaminocyclohexane-based isothiocyanates were synthesized and employed for the HPLC-resolution of amines and amino alcohols.

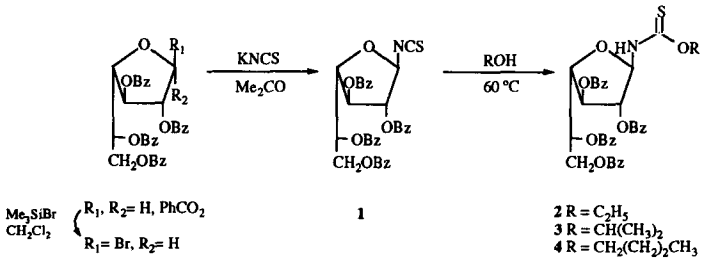
In this paper we report the synthesis of 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate (**1**). The preparative scale reactions of **1** with alcohols, primary and secondary amines and amino alcohols, to afford the corresponding thiourethanes and thioureides was studied. The condensation of **1** with the amino group of aminoacids was also performed. The products are analogs of the linkage unit of *N*-linked glycoproteins.[16] Furthermore, we have proved the usefulness of **1** as a highly selective and stable chiral

derivatizing reagent for the preparative and analytical resolution of primary and secondary amino compounds (amines, amino alcohols and amino acids).

RESULTS AND DISCUSSION

We have just reported[17] a facile and high yielding procedure for the synthesis of per-*O*-acyl-D-glycofuranosyl isothiocyanates from acylated glycofuranosyl chlorides. Now we found that 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate (**1**) can be also obtained from the per-*O*-benzoylgalactofuranose *via* the glycosyl bromide. This intermediate was easily obtained by reaction of the former with bromotrimethylsilane. The crude galactofuranosyl bromide undergoes nucleophilic substitution by the thiocyanate, under smooth conditions, to give the glycosyl isothiocyanate **1** in 90% yield. The reaction was regiospecific (no thiocyanate was formed) and diastereoselective, in favor of the β anomer. This result agrees with our recent report[17] that glycofuranosyl chlorides yield exclusively the isothiocyanate under the conditions (acetone, 25 °C, 2 h) which led mainly to thiocyanates in the case of the glycopyranosyl halides.[18]

Derivatization of **1** with nucleophilic reagents would provide a variety of interesting products. Therefore we studied addition of alcohols, amines and amino acids to the isothiocyanate group of **1**. Compound **1**, reacts rapidly with EtOH to give the corresponding thiourethane **2** (92% yield). Longer reaction times were required for completion when **1** is poorly soluble in the alcohol, as in the case of *n*-butanol (acetonitrile was employed as co-solvent to increase the solubility) or when secondary alcohols were used. For example, the addition of 2-propanol to **1** was completed after 16 h of reaction whereas *tert*-butanol is practically unreactive under the same conditions.



In contrast, addition of primary or secondary amines to the NCS group of **1** took place at room temperature in just 1-2 h. Thus, treatment of **1** with phenacylamine for 2 h yielded the corresponding thiourea **5**, in 91% yield. The ^1H and ^{13}C NMR spectra of **5** confirmed the structure proposed and excluded the presence of possible imidazolic or aminothiazolic derivatives, which could result from the cyclodehydration of *N*-phenacylthioureas.[19] Thus, the ^1H NMR spectrum of **5** showed the presence of the CH_2 phenacyl group at 5.05 ppm and resonances at 52.3 (CH)$_2$, 183.2 (C=S) and 193.4 ppm (C=O) were observed in the ^{13}C NMR spectrum. Fuentes *et al*[4] have also reported that a *N*-glycopyranosyl-*N'*-phenacylthiourea derivative analogue of **5** does not undergo cyclization to the glycosyl heterocycle.

The addition of diethylamine to **1** led almost quantitatively, after 2 h of reaction, to the thiourea derivative **6**. The ^1H NMR spectrum of **6** showed two complex multiplets for the CH_2 groups of ethylamine (δ 3.71 and 3.56) as they are differentiated because of the chiral enviroment. Compound **1** reacted rapid and

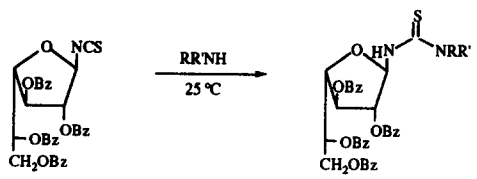
regioselectively with the amino group of amino alcohols to give the corresponding thioureides. For example, reaction of **1** with (2*S*)-1-amino-2-propanol led quantitatively to the galactofuranosyl thiourea **7**, which was isolated crystalline in 89% yield. The ^{13}C NMR spectrum of **7** showed, among other signals, the resonances for the thiocarbonyl (183.3 ppm) and anomeric (87.7 ppm) carbons and the signals of the amino alcohol moiety: 64.5 (*C*HOH), 51.3 (NH*C*H_2) and 21.0 ppm (*C*H_3).

Table 1. ^1H NMR (200MHz) Chemical Shifts (ppm) of Compounds **1-11**

Comp	d (ppm),*J* (Hz)						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6,6' ($J_{5',6}$)($J_{6,6'}$)	*O*- or *N*'- substituent
1[a]	5.59 (<1)	5.72 (<1)	5.75 (4.2)	4.86 (4.2)	6.10 (3.4)	4.80; 4.72 (6.5)(11.9)	
2[b]	6.46 (4.4)	5.82 (3.3)	5.60 (3.6)	4.85	5.93	4.74 - 4.61	Et: 4.45(c), 1.30(t)
3[b]	6.45 (4.4)	5.81 (3.3)	5.60 (3.8)	4.85	5.91	4.72	*i*Pr: 5.48(m), 1.70(m), 1,42(m)
4[b]	6.46 (4.4)	5.84 (3.3)	5.60 (3.8)	4.86	5.93	4.69*	*n*Bu: 4,41(t), 1.65(m), 1.38(m), 0.90(t)
5[a]	6.23 (2.3)	5.82 (3.3)	5.75 (4.5)	4.87*	6.13	4.87*	5.05(t, NCH_2, J_{HNH}: 3.7)
6[a]	6.98 (4.2)	5.94 (<1)	5.73 (1.3)	4.43 (9.0)	6.03 (3.2)	4.97; 4.67 (6.1) (12.4)	Et: 3.71(m), 3.56(m); 1.15(t), $J_{NH,H1}$=8.6
6[b]	6.80 (5.3)	5.96	5.82*	4.54	5.82* (2.7)	4.82; 4.66 (6.5) (12.3)	Et: 3.77(m), 3.51(m); 0.98(t), $J_{NH,H1}$=8.8
7[a]	6.12 (2.6)	5.72 (2.5)	5.79 (4.3)	4.76*	6.10	4.86; 4.76*	4.01(m), 3.39(m), 1.18(d)
7[b]	6.64	5.57	5.66	4.72*	5.95	4.72*	3.81(m), 3.55(m), 1.06(d)
8[a]	6.08 (2.06)	5.66 (2.5)	5.72 (4.3)	4.74*	6.09	4.84; 4.74*	4.03(m), 3.36(m), 1.20(d)
8[b]	6.65	5.57	5.66	4.75*	5.93	4.75*	3.78(m), 3.60(m), 1.07(d)
9[a]	6.10 (2.6)	5.59 (2.4)	5.62 (4.3)	4.70*	5.98	4.70*	gli: 4.25 (CH_2)
10[a]	6.0 (2.3)	5.61 (2.1)	5.74 (4.5)	4.81*	6.07	4.81*	ala: 5.00(c), 1.50(d)
11[a]	6.04 (2.3)	5.66 (2.3)	5.75 (4.5)	4.81*	6.08	4.81*	leu: 5.10(t), 1.73, 1.27, 0.95(d)

[a] Cl_3CD, [b] DMSO-d_6, *center of a complex multiplet.

Some chiral isothiocyanate derivatives have been successfully employed for the conversion of racemic amino acids,[10-12] amines and amino alcohols[15] into enantiomeric derivatives, which can be separated by high-



1

5 R = CH_2COPh R' = H
6 R = R' = Et
7 R = (*S*)$CH_2CH(OH)CH_3$ R' = H
8 R = (*R*)$CH_2CH(OH)CH_3$ R' = H
9 R = CH_2CO_2Et R' = H
10 R = $CH(CH_3)CO_2Et$ R' = H
11 R = $CH(CH_2CHMe_2)CO_2Me$ R' = H

performance liquid chromatography (HPLC). In order to examine the utility of the thiocyanate derivative **1** as a chiral resolving agent, we performed the reaction of **1** with racemic 1-amino-2-propanol.

The condensation was conducted in acetonitrile, and in order to avoid kinetic resolution (one enantioner reacts faster than the other) an excess of **1** was employed to force the reaction to completion. The reaction mixture showed by TLC two spots of almost equal intensity (R_F 0.46 and 0.50). The lower moving component had identical mobility as compound **7**. The separation of the diastereoisomers was successfully accomplished by column chromatography, which afforded **7** and the *N'*[(R)2-hydroxypropyl] thiourea derivative **8**, in 36% and 45% yield respectively. This experiment proved the utility of **1** as a chiral resolving agent even in preparative scale. The reagent is also interesting for analytical purposes, as the diastereomeric thioureas **7** and **8** were clearly separated by TLC. The original mixture of **7** and **8** was also subjected to RP-HPLC analysis. This technique has been employed to resolve enantiomeric amino compounds, mainly by the indirect method *via* covalent diastereomer formation using chiral isothiocyanates.[10-15] Furthermore, the thiourea group is sensitive to UV detection[7] and **1** would show increased sensitivity, compared with the acetyl glycopyranosyl isothiocyanates,[8-12] owing to the higher molar absorptivity of the benzoates. The mixture of **7** and **8** was readily resolved by RP-HPLC in a Spherisorb ODS column, using 4:1 methanol-water as solvent. The retention times were 11.1 (for **7**) and 8.9 min (for **8**) being the separation factor (α) 1.25.

In order to extend this methodology to other amino compounds, the reaction of **1** with D,L-alaninol ((±)-2-amino-1-propanol) was conducted. The mixture was resolved as two well separated peaks (α = 1.17) having retention times of 9.8 (*R*-isomer) and 8,4 min (*S*-isomer). Reaction of **1** with the ethyl ester derivative of amino acids such as glycine, L-alanine and L-leucine gave the corresponding thioureides,which showed by HPLC well defined peaks having retention times of 9.2, 11.5 and 15.2 min, respectively. The aminoacid could be recovered from the thioureide without racemization by using 99% trifluoroacetic acid.[20]

Glycosyl isothiocyanates have been used in the synthesis of *N*-glycopeptides[16,21,22] and other types of glycoconjugates.[23-25] Therefore, condensation between **1** and amino acids was performed in preparative scale. Amino acids were previously derivatized as their alkyl esters, and then condensed with **1** to afford the thioureides **9-11** in 80-90% yield. The structures of compounds **9-11** were confirmed by NMR spectroscopy. For example, the ^1H NMR spectrum of **9** showed the methylene group of the glycine moiety as a double doublet at 4.25 ppm. This signal collapsed into a singlet upon addition of D_2O. The quartet and triplet for the OEt group appeared at 4.03 and 1.10 ppm, respectively.

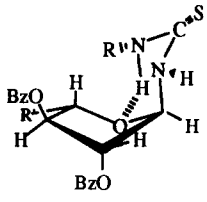
Table 2. ^{13}C NMR (25.3 MHz) Chemical Shifts (ppm) of Compounds **1-11**

Comp.	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C=S*	*O or N'-substituent*
1[a]	89.4	82.4	76.9	84.1	70.1	63.3	142.7	
2[a]	88.8	80.0	78.8	81.9	71.6	63.5	191.7	Et: 67.1, 14.1
3[b]	89.0	80.2	78.5	80.4	71.9	63.6	190.9	*i*Pr: 73.7, 21.6
4[b]	89.0	80.2	78.4	80.3	71.8	63.5	190.9	*n*Bu: 70.8, 30.1, 18.6, 13.6
5[a]	88.4	80.9	78.3	81.4	71.0	63.3	183.3	Ph*C*H2*C*O: 193.4, 52.3
6[b]	85.6	76.2	75.5	77.4	71.3	63.5	179.5	Et: 44.8, 12.4.
7[b]	87.7	79.6	78.3	80.2	71.8	63.5	183.3	*(S)*CH2*C*HOHCH3: 64.5, 51.3, 21.0
8[a]	87.6	79.6	78.3	80.1	71.8	63.5	183.8	*(R)*CH2*C*HOHCH3: 64.5, 51.4, 21.1
9[a]	88.4	80.8	78.2	81.5	71.5	63.4	183.8	*C*H2: 61.6
10[a]	88.3	80.7	78.1	81.5	70.9	63.3	183.2	*C*H3CHCO2: 61.7, 18.3
11[a]	88.3	81.1	77.9	81.9	70.9	63.4	183.8	leu: 56.7, 41.4, 25.O, 22.7, 22.4

[a] Recorded in Cl3CD. [b] Recorded in DMSO-*d*6.

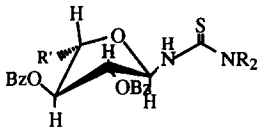
Comparison of the spectral data for the products (Table1) led us to the observation that the coupling constants of the thioureides (except those of **6**) are quite different from those of the thiourethanes. Application of the pseudorotational analysis procedure, based on the dependence of $^3J_{H,H}$ on pseudorotation parameters indicated that the glycosyl thioureides populate mainly the $E_0 \rightleftharpoons {}^1T_0 \rightleftharpoons {}^1E$ segment of the pseudorotation itinerary, whereas the thiourethanes (**2-4**) and **6** are shifted conformationally towards the opposite side of the cycle. Particularly, the large value for $J_{1,2}$ and the small values for $J_{3,4}$ in the thiourea derivative **6** suggests that the compound populates almost exclusively the ${}^0E \rightleftharpoons {}^0T_1 \rightleftharpoons E_1$ region. Searching for the reason of this rather surprising conformational behavior for structurally similar compounds, we observed that the thiourethanes **2-4** and the thiourea **6** had no H atom on the O or N', whereas all the other derivatives had H covalent bonded to N'. This fact suggested that a H-bonding could be involved in the stabilization of the thioureides. The existence of a NH···O-ring hydrogen bond in thioureido sugars was recently demonstrated[5] by rotational barrier calculations, temperature-induced NH shifts and other NMR data. Similarly, we suggest stabilization of H-N' containing thioureides in the ${}^1T_0$ pseudorotamer region due to the formation of N'-H···O-ring hydrogen bond (**12**), which generates a stable *trans*-fused bicyclic system. The absence of H at N' would preclude the possibility of conformational stabilization by hydrogen bonding, and a conformation such as **13** would be favored, due to steric preference of the bulky substituent at C-1 for a *quasy*-equatorial orientation.

Furthermore, the thiourethanes **2-4** and the thioureide **6** showed $J_{1,2}$ values larger than 4 Hz, against the empirical rule[25] which establishes this value as the upper limit for a *trans* relationship for H-1 and H-2. As we have earlier suggested,[26] such a rule should be applied with caution for the anomeric assignment of furanosides having other than an oxygen substituent on C-1.



$E_0 \rightleftharpoons {}^1T_0 \rightleftharpoons {}^1E$

12



$E_0 \rightleftharpoons {}^1T_0 \rightleftharpoons {}^1E$

13

In conclusion, we proved that the reaction of **1** with aminoacids is a potential model for the synthesis of neoglycoconjugates and a reagent for the resolution of chiral amines and aminoacids.

EXPERIMENTAL SECTION

Melting points were determined with a Thomas-Hoover apparatus. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. NMR Spectra were recorded with a Bruker AC 200 spectrometer. Column chromatography was performed on silica gel 60 (Merck). Thin layer chromatography (TLC) was carried out on precoated aluminium plates of silica gel 60 F_{254} (Merck), using mixtures of toluene-EtOAc as solvent having the following composition: 9:1 (solvent a), 4:1(solvent b), 1:1 (solvent c). The spots were visualized by exposure to UV light and by spraying the plates with 10% (v/v) H_2SO_4 in EtOH, followed by heating. 1,2,3,5,6-Penta-O-benzoyl-D-galactofuranose (**1a**) was prepared[27] by benzoylation of D-galactose at 100 °C.

2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate (1). To a solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (2.1 g, 3.0 mmol) in anhydrous CH_2Cl_2 (15 mL), bromotrimethylsilane (4 mL, 30.3 mmol) was added, with external cooling (0 °C). The mixture was stirred for 24 h at room temperature, when TLC examination showed a single spot (R_F 0.32, solvent a), less polar than the starting material (R_F 0.52). The solution was concentrated *in vacuo*, and then toluene was added in order to remove the excess of reagent. Crude 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl bromide was dissolved in anhydrous acetone (10 mL) and potassium thiocyanate (0.87 g, 9.0 mmol) was added. After 2 h of stirring at room temperature, TLC examination showed a single spot (R_F 0.74). The mixture was filtered and the filtrate concentrated to a syrup which crystallized upon addition of EtOH. Rapid recrystallization from the same solvent afforded compound **1** (1.72 g, 90 %), mp 138-140 °C (lit.[17] 138-140 °C), $[\alpha]_D$ -98° (*c* 0.9, $CHCl_3$).

Addition of alcohols to 1

Compound **1** (0.35 g, 0.5 mmol) was dissolved in ethanol (10 mL), isopropanol (10 mL) or in a 1:1 mixture of n-BuOH:MeCN (10 mL) and the solution was heated at 60°C until all the starting material **1** was converted into a product of lower chromatographic mobility. The reaction times were: 2 h for EtOH and 16 h for i-PrOH and *n*-BuOH. After evaporation of the solvent the syrupy thiouretanes **2-4** were respectively obtained. Analytically pure samples were obtained by purification by column chromatography (40:1, toluene-EtOAc).

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*O*-ethyl thiourethane (2).** Yield 0.35 g (92%), R_F 0.56 (solvent a), $[\alpha]_D$ +2° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{37}H_{32}O_{10}NS$: C, 65.00; H, 4.86; N, 2.05; S, 4.69. Found: C, 64.91; H, 4.69; N, 2.20; S, 4.88.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*O*-isopropyl thiourethane (3).** Yield 0.35 g (91 %), R_F 0.73 (solvent a), $[\alpha]_D$ +11° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{38}H_{34}O_{10}NS$: C, 65.51; H, 4.92; N, 2.01; S, 4.60. Found: C, 65.36; H, 4.80; N, 1.93; S, 4.42.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*O*-*n*-butyl thiourethane (4).** Yield 0.34 g (87%), R_F 0.7 (solvent a), $[\alpha]_D$ +10° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{39}O_{10}H_{36}NS$: C, 65.90, H, 5.11, N, 1.97; S, 4.51. Found: C, 65.62, H, 5.39, N, 2.05; S, 4.36.

Addition of amines to 1.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-phenacyl thiourea (5).** To a solution of **1** (0.20 g, 0.31 mmol) in dry 3:1 acetone-toluene (5 mL), phenacylamine hydrochloride (0.06 g, 0.35 mmol) and EDPA (0.038 mL, 0.42 mmol) were added. The resulting solution was kept at room temperature for 1 h, and then concentrated. The residue was purified by column chromatography (49:1 toluene-EtAcO) to give compound **5** (0.22 g, 91%), $[\alpha]_D$ -18° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{43}H_{36}O_{10}N_2S$: C, 66.83; H, 4.70; N, 3.62; S, 4.15. Found: C, 66.51; H, 4.72; N, 3.61; S, 4.44.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N',N'*-diethyl thiourea (6).** To a solution of **1** (0.20 g, 0.31 mmol) in dry toluene (5 mL), diethylamine (0.054 mL, 0.52 mmol) was added and the mixture was stirred for 2 h at room temperature. The solvent was evaporated and the residue crystallized from ethanol. Recrystallized from the same solvent compound **6** (0.18 g, 81 %) gave mp 145-146°C, $[\alpha]_D$ -13° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{39}H_{38}O_9N_2S$: C, 65.90; H, 5.39; N, 3.94; S, 4.51. Found: C, 65.80; H, 5.57; N, 3.94; S, 4.72.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-2(*S*)-hydroxypropyl thiourea (7).** To a solution of **1** (0.20 mg, 0.31 mmol) in CH_3CN (6mL), (*S*)-1-amino-2-propanol (0.04 mL, 0.77 mmol) was added. The mixture was stirred at 0 °C for 2 h, and then concentrated. The residue was chromatographed with toluene-EtOAc, and from the fractions having R_F 0.46 (solvent c) crystalline **7** (0.20 g, 89 %) was obtained. After recrystallization from isopropylether, **7** gave mp 84-85 °C, $[\alpha]_D$ -11° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{38}O_{10}H_{36}N_2S$: C, 64.03; H, 5.09; N, 3.93; S, 4.50. Found: C, 63.91; H, 4.96; N, 4.01; S, 4.71.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-2(*S*)-hydroxypropyl thiourea (7) and *N*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-2(*R*)-hydroxypropyl thiourea (8).** Racemic 1-amino-2-propanol (0.04 mL, 0.77 mmol) was added to a solution of **1** (0.20g, 0.31 mmol) in CH_3CN (6 mL). The reaction was conducted as indicated above, and the resulting syrup showed by TLC two spots of equal intensity (R_F 0.46 and 0.50, solvent c). Separation by column chromatography (toluene-EtOAc) afforded the faster moving compound, which was characterized as **8** (0.10 g, 45 %). After crystallization from isopropylether, **8** gave m.p. 95-96 °C, $[\alpha]_D$ +29° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{38}O_{10}H_{36}N_2S$: C, 64.03; H, 5.09; N, 3.93; S, 4.50. Found: C, 63.87; H, 4.89; N, 3.97; S, 4.70.

From the next chromatographic fraction compound **7** was isolated (0.08 g, 36 %), and showed the same properties indicated above.

Addition of aminoacids to 1

Compound **1** (0.7 g, 1.1 mmol) and the amino acid ester hydrochloride (1.3 mmol) were dissolved in dry toluene (20 mL) containing EDPA (1.5 mmol), and the solution was stirred at room temperature for 3 h. The mixture was diluted with toluene (50 mL), washed with water, dried ($MgSO_4$), and the solvent evaporated. The thioureides **9-11** were purified by column chromatography employing toluene and increasing proportions of EtOAc as solvent.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-carboxyethylmethylene thiourea (9).** Yield 0.76 g (86%), R_F 0.33 (solvent b), $[\alpha]_D$ -31° (*c* 1, $CHCl_3$), Anal. Calcd for $C_{39}H_{36}O_{11}N_2S$: C, 63.23; H, 4.90; N, 3.78; S, 4.33. Found: C, 63.29; H, 4.89; N, 3.70; S, 4.20.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-2(*S*)-carboxyethyl(methyl)methyne thiourea (10).** Yield 0.75 g (91%), R_F 0.42 (solvent b), $[\alpha]_D$ -6° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{40}O_{11}H_{38}N_2S$: C, 63.65; H, 5.07; N, 3.71; S, 4.25. Found: C, 63.92; H, 5.15; N, 3.90; S, 4.33.

***N*-(2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl)-*N'*-2(*S*)carboxymethyl(isobutyl)methyne thiourea (11).** Yield 0.69 (80%), R_F 0.55 (solvent b), $[\alpha]_D$ -20° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{42}O_{11}H_{42}N_2S$: C, 64.42 H, 5.41; N, 3.58; S, 4.09. Found: C, 64.19 H, 5.31; N, 3.50; S, 4.29.

Recovering of the aminoacid from the corresponding thioureide **10** (0.037g, 0.05 mmol) was accomplished by treatment with 99% trifluoroacetic acid (2.0 mL) overnight, at room temperature. HPTLC analysis was performed with methanol-water-acetonitrile (1:1:4) as solvent, using a chiralplate.[20] Only one spot of R_F 0.46 coincident with L-alanine and well differentiated from D-alanine (R_F 0.42) was detected with the ninhydrine reagent.

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REFERENCES

1. Mukerjee, A.K.; Share, R.A. *Chem. Rev.* **1991**, *91*, 1-24
2. Witczak, Z.J. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 91-145
3. Jastalska, D.; Dzierzynska, J. *Acta Pol. Pharm.* **1969**, *26*, 125-198.
4. Fuentes, J.; Moreda, W.; Ortiz,C.; Robina, I.; Welsh, C. *Tetrahedron* **1992**, *48*, 6413-6424.
5. García Fernández, J.M.; Ortíz Mellet, C.; Díaz Pérez, V.M.; Jimenez Blanco, J.L.; Fuentes, J. *Tetrahedron* **1996**, *52*, 12947-12970.
6. Lindhorst,T.K.; Kieburg, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953-1956.
7. Nambara, T.; Ikegawa, S.; Hasegawa, M.; Goto, J. *Anal. Chim. Acta* **1978**, *101*, 111-116.
8. Nimura, N.; Ogura, H.; Kinochita, T. *J. Chromatogr.* **1980**, *202*, 375-379
9. Nishi, H.; Fujimura, N.; Yamaguchi, H.; Fukuyama, T. *J. Chromatogr.* **1991**, *539* , 71-81
10. Tian, Z.; Hrinyo-Pavlina, T.; Roeske, R.W. *J. Chromatogr.* **1991**, *541*, 297-302.
11. Lobell, M.; Schneider, M.P. *J. Chromatogr.* **1993**, *633*, 287-294.
12. Kinoshita, T.; Kasahara, Y.; Nimura, N. *J. Chromatogr.* **1981**, *210*, 77-81.
13. Gal, J. *J. Chromatogr.* **1985**, *331*, 349-357.
14. Ito, S.; Ota, A.; Yakamoto, K.; Kawashima, Y. *J. Chromatogr.* **1992**, *626*, 187
15. Kleidernigg, O.P.; Poseh, K.; Lindner, W. *J. Chromatogr.* **1996**, *729*, 33-42.
16. Gunther, W. and Kunz, H. *Agew. Chem. Int. Ed. Engl.* **1990**, *102*, 1068-1069.
17. Marino, C.; Varela, O.; Lederkremer, R.M. de *Carbohydr. Res.* in press.
18. Pakulski, Z.; Pierozynsky, D; Zamojski, A. *Tetrahedron* **1994**, *50*, 2975-2992
19. Fuentes Mota, J.; Pradera, A.; Ortíz Mellet, M.A.; García Fernández, J.M. *Carbohydr. Res.* **1986**, *153*, 318-324.
20. Borrás, F.; Offord, R.E. *Nature* **1970**, *227*, 716-718.
21. Garg, H.G.; Jeanloz, R.W. *Adv. Carbohydr. Chem. Biochem.* **1985**, *43*, 135-201.
22. García Fernández, J.M.; Ortiz Mellet, C.; Jiménez Blanco, J.L.; Fuentes, J. *J. Org. Chem.* **1994**, *59*, 5565-5572.
23. Pradera, M.A.; Molina, J.L.; Fuentes, J. *Tetrahedron* **1995**, *51*, 923-954.
24. Reitz, A.B.; Tuman, R.W.; Marchone, C.S.; Jordan, A.D. Jr; Browden, C.R.; Maryanof, B.J. *Med. Chem.* **1989**, *32*, 2110-2116.
25. Bundle, D.R.; Lemieux, R.D. *Method. Carbohydr. Chem.* **1976**, *7*, 79
26. Zunzain, P.A.; Varela, O. *J. Chem. Research (S)* **1995**, 486-487.
27. Marino, C.; Varela, O.; Lederkremer, R.M. *Carbohydr. Res.* **1989**, *190* , 65-76.

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