ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 325 (2000) 72–80

CARBOHYDRATE RESEARCH

Note

Synthesis of antioxidative and anti-inflammatory drugs glucoconjugates

Rainer K. Uhrig [a,b,*], Martin A. Picard [a,b], Konrad Beyreuther [a], Manfred Wiessler [b]

[a] *Zentrum für Molekularbiologie Heidelberg (ZMBH), Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany*
[b] *Molekulare Toxikologie (C0300), Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*

Received 11 June 1999; revised 22 November 1999; accepted 23 November 1999

Abstract

Glucoconjugates of (±)-ibuprofen, (±)-α-tocopherol (vitamin E), gentisic acid, gallic acid, 2,6-bis(*tert*-butyl)-4-thiophenol, and *N*-acetyl-L-cysteine were prepared with the objective of increasing the bioavailability of such antioxidant and anti-inflammatory drugs. The *O*-glucosides were synthesized using benzylated α-D-glucopyranosyl trichloracetimidate as glycosyl donor. For the synthesis of the *S*-glucosides, the glycosyl donor 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose provided higher yields than the corresponding O-acetylated imidate.

Keywords: Glycosylation; Antioxidants; Anti-inflammatory drugs; Glucose transporter; Blood–brain barrier; Cancer; Alzheimer's disease

1. Introduction

In recent years, evidence has accumulated suggesting that oxidative stress and inflammation are a common feature of several chronic and degenerative diseases [1]. Brain disorders, such as Alzheimer's disease, Parkinson's disease and stroke, might be involved due to the brain's high vulnerability to oxidative stress [2]. For cancer, epidemiological studies have shown that nutrients containing vitamins and other natural antioxidants (e.g., vitamin E, gallic acid derivatives) might lower the risk [3]. Non-steroidal anti-inflammatory drugs have been reported to be beneficial for arthritis [4]. Whole-body autoradiography studies in rats with Glufosfamid, the β-D-glucoside of the chemotherapeutic agent Ifosfamide mustard (IPM), provides evidence for a selective enrichment of the glucoside in the brain. This enrichment was not observed in the case of the β-L-glucoside of IPM, which indicates that specialized glucose transporters may be responsible for this effect [5,6]. Moreover, uptake measurements of the glucosides of IPM using oocytes of *Xenopus laevis* as an in vitro expression system for mammalian glucose transporters show that this selective uptake of the β-D-glucoside is a characteristic feature of a sodium-coupled glucose transporter called SGLT-3 (= SAAT-1) [7]. The concept that glucoconjugates are transported by cellular glucose transporters has also been reported for other glucoconjugates [8–11]. The expres-

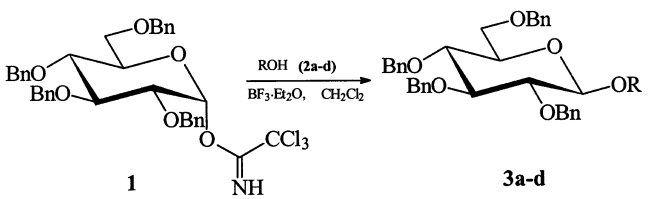
* Corresponding author.
E-mail address: r.uhrig@dkfz-heidelberg.de (R.K. Uhrig)

0008-6215/00/$ - see front matter
PII: S0008-6215(99)00311-0

sion of the high-affinity Na^+-D-glucose transporter SGLT-1 in human brain was demonstrated by Poppe et al. [12]. We present here novel synthetic routes to the glucosides of the antioxidants or anti-inflammatory drugs gallic acid, 2,6-bis(*tert*-butyl)-4-thiophenol, *N*-acetyl-L-cysteine ethyl ester, (±)-ibuprofen and an improved synthesis for glucosides of (±)-α-tocopherol and gentisic acid, which may help to overcome the problem of restricted bioavailability of such drugs.

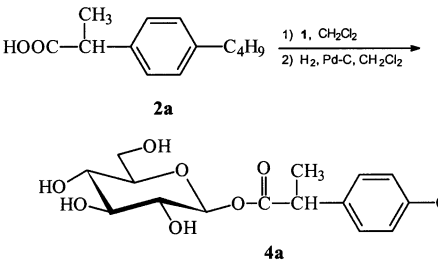
2. Results and discussion

The synthesis of the *O*-glucosides was performed in dry dichloromethane and under an atmosphere of nitrogen using the benzylated trichloroacetimidate (**1**) as glycosyl donor [13]. For the *O*-acyl glucoside, the reaction was performed at room temperature without any catalyst [14]. The synthesis of the *O*-aryl glucosides was performed using boron trifluoride diethyl etherate as catalyst at −40 °C. Deprotection of the sugar moiety was achieved by hydrogenation with palladium on charcoal. Coupling of aglycones containing more than one potential glycosylation site required additional protection and deprotection steps, which are described in the respective sections.



1 **3a-d** **4a-d**

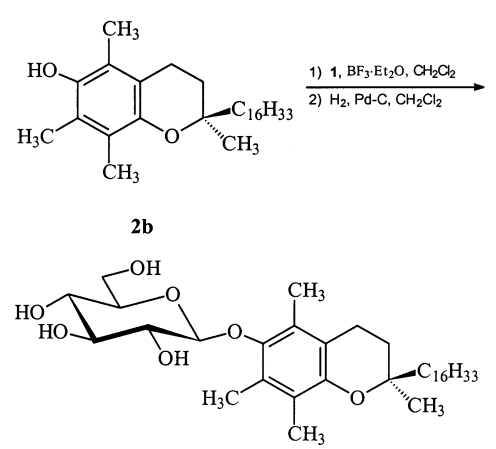
The 1-*O*-acyl glucoside **4a** of (±)-ibuprofen (**2a**) was prepared by the autocatalytic reaction of **2a** with **1** yielding 67% of the protected glucoside. Deprotection was performed by hydrogenation with palladium on charcoal in dichloromethane–acetone yielding **4a** (76%), which, however, showed a poor stability and decomposed slowly even at −20 °C.



2a **4a**

The synthesis of glucosides of α-tocopherol (**2b**) has been described by three other groups using various acetylated sugar analogues as glycosyl donors [15–17]. Up to now, only the use of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**5**) as glycosyl donor, as reported by Lahmann and Thiem [17], gave good yields. These authors obtained a 67% yield by using a six-fold excess of α-tocopherol. With equimolar amounts of the reagents, the yield was diminished to about 40%. Deprotection of the acetylated glucoside was nearly quantitative [17].

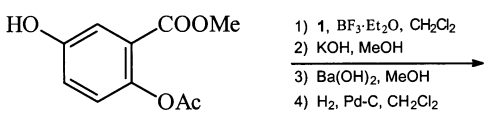
Performing the glycosylation reaction of **2b** (1 equivalent) with **1** (1.2 equivalents), we achieved only moderate yields of the benzylated glucoside of **2b** (30–40%). Due to the very similar physical properties of this derivative and the aglycone itself, the glucoside could only be purified by crystallization from ethanol, thereby resulting in substantial loss. Deprotection by hydrogenolysis with palladium on charcoal gave yields between 70 and 80%. The severe loss of the protected glucoside during the purification procedure could be avoided by direct hydrogenation of the mixture resulting from the glycosylation step,



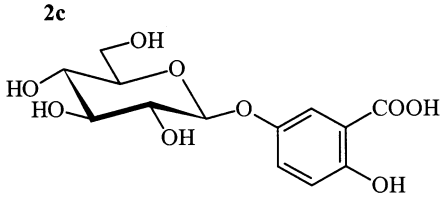
2b **4b**

followed by column chromatography. Thus, the overall yield in **4b** could be increased to 67%, a result similar to the synthesis of Lahmann and Thiem [17]. The advantage of our approach is that only equimolar amounts of the reactants are needed.

The only described synthesis of the β-D-glucoside of gentisic acid (**4c**) used a Königs–Knorr reaction of **2c** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (15%) [18,19]. The glycosylation of **2c** with **1** yielded 91% of the glucoside. Deacetylation with barium hydroxide in methanol, followed by ester saponification with a mixture of potassium and barium hydroxide in methanol, gave a partly deprotected glucoside (55%). Hydrogenolysis with palladium on charcoal in dichloromethane led to the fully deprotected glucoside **4c** (80%).



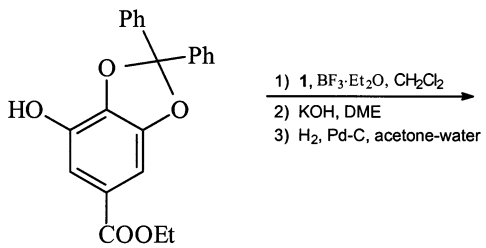
2c



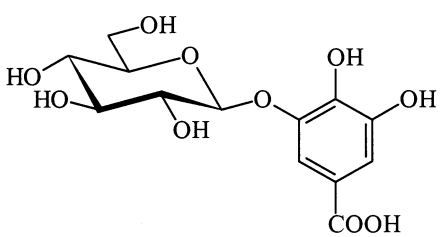
4c

Until now only *O*-acyl glucosides of gallic acid ('α- and β-glucogallin') were obtained by chemical synthesis [20–22]. The only routes to *O*-aryl glucosides of gallic acid are enzymatic synthesis or by direct isolation from plants [23–28]. Gallic acid possesses four reactive groups, three of which have to be protected to achieve a regioselective glycosylation. Gallic acid ethyl ester is commercially available and protection of the C-3,4 hydroxyl groups could be performed by reaction with dichlorodiphenylmethane in pyridine, yielding **2d** (61%). The major advantage of this protective group is that it can be removed by hydrogenation [29].

The glycosylation was performed with **1** as described above, yielding 60% of **3d**. However, the subsequent hydrogenation was more difficult than expected and the diphenylmethylene protective group was not cleaved by the usual catalyst (10% Pd–C). The cleavage required a more reactive catalyst such as 20% palladium on charcoal. Since we found no method of cleaving the ethyl ester after deprotection of the glucoside, we changed the order of reaction steps by cleaving the ethyl ester before hydrogenation. The ester cleavage was performed by using potassium hydroxide in 1,2-dimethoxyethane (89%), followed by hydrogenolysis with 20% palladium on charcoal in acetone containing 10% water (94%).

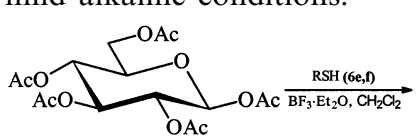


2d

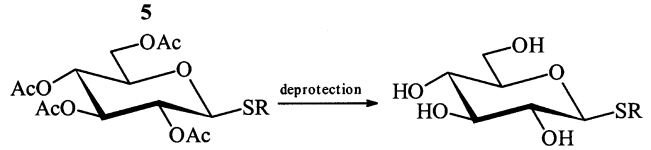


4d

For the synthesis of thioglucosides **8e,f** 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**5**) was used as glycosyl donor. The reaction was performed in dry dichloromethane at room temperature using boron trifluoride diethyl etherate as catalyst and a five-fold excess of **5**. Deprotection could be performed under mild alkaline conditions.



5

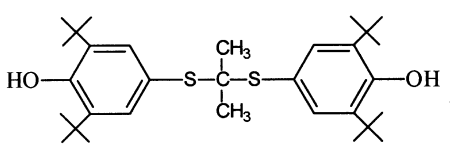


7e,f **8e,f**

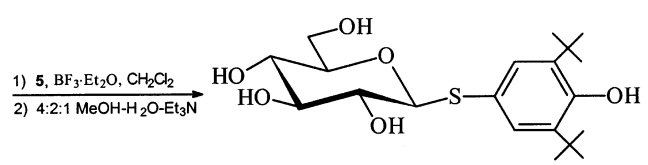
For the synthesis of the β-D-glucoside of 2,6-bis(*tert*-butyl)-4-thiophenol (**8e**), direct glycosylation of the highly sensitive 2,6-bis-(*tert*-butyl)-4-thiophenol gave only poor yields

of **8e** (~ 10–20%) using **5** as glycosyl donor. However, this yield could be increased by up to 74% by in situ generation of the highly reactive educt using its acetonide, the synthetic antioxidant probucol (4,4′-isopropylidenedithio)bis[2,6-di-*tert*-butylphenol] (**6e**) as precursor. Deprotection under mild alkaline conditions (4:2:1 MeOH–water–Et_3N) yielded 74% of the unprotected glucoside **8e**.

For the synthesis of cysteine glucosides two methods have been described so far. Monsigny et al. [30] used a 3-iodo-L-serine derivative as glycosyl acceptor in a reaction with an acetylated glycosyl isothiouronium salt. Although they achieved a very good yield (72%) for the thioglycosylation step, a great deal of synthetic work was required for the preparation of the adducts. Interestingly, Baran and Drabarek [31] successfully synthesized a cysteine glucoside via a Königs–Knorr reaction using silver salts as catalyst and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide as glycosyl donor in a 50% yield.

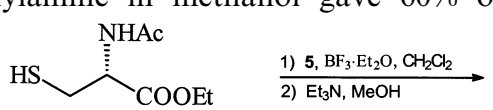


6e

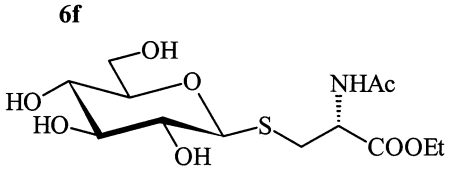


8e

For the regioselective glycosylation of *N*-acetyl-L-cysteine, the carboxy group was protected as its ethyl ester (46%). The glycosylation step with **5** yielded 45% of the acetylated glucoside. Deprotection with 10% triethylamine in methanol gave 60% of the deprotected glucoside **8f**, with however partial racemization at the cysteine moiety.



6f



8f

3. Experimental

General methods[1]. — Thin-layer chromatography (TLC) was performed on precoated plates of silica gel (Polygram Sil G/UV_{254}, Machery & Nagel), detection by UV absorption and/or spraying with 0.1% vanillin in 50% H_2SO_4 followed by heating at 160 °C. Column chromatography was performed on silica gel (Kieselgel 60, 63–200 mesh, Machery & Nagel). Ratios of solvent mixtures for chromatography are specified in terms of volume. NMR spectra were recorded at 250 MHz (^1H) or 63 MHz (^{13}C) on a Bruker AM 250 with Me_4Si (δ 0) as internal standard by 'Zentrale Spektroskopie, DKFZ', Heidelberg. Mass spectrometry was performed on a Finnigan MAT TSQ 7000 by 'Zentrale Spektroskopie, DKFZ', Heidelberg. Elemental analysis were performed with a Carlo Erba Elemental Analyzer CHNS EA 1108 by 'Mikroanalytische Abteilung, Max-Planck-Institut für Medizinische Forschung', Heidelberg.

General procedure for the synthesis of O-*acyl glucosides (GP 1).* — A solution of the acyl compound and the benzylated α-D-glucopyranosyl trichloracetimidate (**1**) was reacted at room temperature (rt) until no further reaction was detectable (TLC). The mixture was washed with 0.05 M NaOH and brine. After drying over Na_2SO_4, the solvents were removed under reduced pressure. The products were purified by column chromatography or crystallization.

General procedure for the synthesis of O-*aryl glucosides (GP 2).* — A solution of $BF_3{\cdot}Et_2O$ in dry CH_2Cl_2 was slowly added to a solution of the aromatic compound and benzylated α-D-glucopyranosyl trichloracetimidate (**1**) at −40 °C under nitrogen atmosphere. The mixture was stirred until no further reaction was detectable (TLC). The excess of $BF_3{\cdot}Et_2O$ was decomposed with $NaHCO_3$. After dilution with CH_2Cl_2, the mixture was washed

[1] Note of the Editor: The normally required polarimetric data for chiral compounds were not available for this manuscript.

with water, dried over Na_2SO_4 and the solvents were removed under reduced pressure. The raw product was purified by column chromatography or crystallization.

General procedure for the synthesis of the S-*Glucosides (GP 3).*—A solution of $BF_3{\cdot}Et_2O$ in dry CH_2Cl_2 was slowly added to a solution of the aromatic compound and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**5**) at rt under nitrogen atmosphere. The mixture was stirred overnight and stopped by decomposition of the catalyst over $NaHCO_3$. After dilution with CH_2Cl_2, the mixture was washed with water, dried with Na_2SO_4 and the solvents were removed under reduced pressure. The raw product was purified by column chromatography or crystallization.

General procedure for the deprotection of benzyl groups (GP 4).—A slurry of palladium on charcoal was added to the solution of the protected glucoside under a nitrogen atmosphere. The atmosphere was changed from nitrogen to hydrogen and the reaction was continued until no further reaction was detectable. After filtration the solvents were removed under reduced pressure and the product was purified by column chromatography or crystallization.

*1-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-(±)-ibuprofen* (**3a**).—A solution of compound **2a** (2.2 g, 11 mmol) in dry CH_2Cl_2 (100 mL) was reacted with a solution of **1** (8.6 g, 12.0 mmol) in dry CH_2Cl_2 (25 mL) for 4 h according to GP 1. After purification by column chromatography (9:1 hexane–EtOAc), **3a** (5.37 g, 7.37 mmol, 67%) was obtained as an oil; ^{1}H NMR ($CDCl_3$) (two sets of signals, diastereomers): δ 7.15 (m, 24 H, 4 $CH_2C_6H_5$, H-2′, H-3′, H-5′, H-6′), 5.60 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.45 (m, 15 H, 4 $CH_2C_6H_5$, H-2, H-3, H-4, H-5, H-6a, H-6b, ROOC–CHCH$_3$–R′), 2.35 (d, 2 H, CH_2CH(CH$_3$)$_2$), 1.75 (m, 1 H, CH$_2$CH(CH$_3$)$_2$), 1.50 (d, 3 H, ROOC–CHCH$_3$–R′), 0.85 (m, 6 H, CH$_2$CH(CH$_3$)$_2$); ^{13}C NMR ($CDCl_3$) (two sets of signals, diastereomers): δ 173.06 (COOR), 140.76, 140.62 (C-4′), 138.44, 138.08, 137.97, 137.88, 137.20, 136.73 (C-1′, 4 $CH_2C_6H_5$), 129.49, 129.28 (C-2′, C-6′), 128.34, 128.06, 127.93, 127.85, 127.83, 127.76, 127.66, 127.59, 127.56, 127.53, 127.46, 127.30, 127.26 (C-3′, C-5′, 4 $CH_2C_6H_5$), 94.73, 94.42 (C-1), 84.74, 84.59 (C-2), 80.92, 80.76 (C-3), 77.29, 77.16 (C-4), 75.79, 75.48 (C-5), 75.60, 75.55, 74.92, 74.75, 74.35, 73.49 (4 $CH_2C_6H_5$), 68.10 (C-6), 45.20 (ROOC–CHCH$_3$–R′), 44.98, 44.93 (CH_2CH(CH$_3$)$_2$), 30.06, 29.98 (CH$_2$CH-(CH$_3$)$_2$), 22.34, 22.28 (CH$_2$CH(CH$_3$)$_2$)), 18.49, 18.44 (ROOC–CHCH$_3$–R′); ESIMS: $C_{47}H_{52}O_7$ (728.92), m/z 751.3 (60), $[M+Na]^+$.

1-(β-D-Glucopyranosyloxy)-(±)-ibuprofen (**4a**).—A solution of compound **3a** (212 mg, 0.29 mmol) in CH_2Cl_2 (20 mL) and acetone (5 mL) was reacted under a hydrogen atmosphere in the presence of 20% palladium on charcoal (205 mg) according to GP 4. After purification by column chromatography (4:1 Et_2O–acetone), a colourless film of **4a** (81 mg, 0.22 mmol, 76%) was obtained; ^{1}H NMR (CD_3OD) (two sets of signals, diastereomers): δ 7.20 (m, 2 H, H-2′, H-6′), 7.11 (d, 2 H, H-3′, H-5′), 5.50 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.75 (m, 2 H, H-6a, H-6b), 3.60 (m, 1 H, ROOC–CHCH$_3$–R′), 3.30 (m, 4 H, H-2, H-3, H-4, H-5), 2.42 (d, 2 H, CH_2CH(CH$_3$)$_2$), 1.80 (m, 1 H, CH$_2$CH(CH$_3$)$_2$), 1.52 (m, 3 H, ROOC–CHCH$_3$–R′), 0.89 (d, 6 H, CH_2CH(CH$_3$)$_2$); ^{13}C NMR (CD_3OD) (two sets of signals, diastereomers): δ 175.22, 175.15 (COOR), 141.72 (C-4′), 138.86, 238.79 (C-1′), 130.35, 130.29 (C-2′, C-6′), 128.44 (C-3′, C-5′), 96.05, 95.99 (C-1), 78.86 (C-5), 78.11, 78.04 (C-2), 73.98, 73.92 (C-3), 70.99, 70.92 (C-4), 62.28, 62.19 (C-6), 46.27, 46.21 (ROOC–CHCH$_3$–R′), 46.03 (CH_2CH(CH$_3$)$_2$), 31.40 (CH$_2$CH-(CH$_3$)$_2$)), 22.71 (CH$_2$CH(CH$_3$)$_2$)), 19.43, 19.20 (ROOC–CHCH$_3$–R′); Anal. Calcd for $C_{19}H_{28}O_7$ (368.42): C, 61.94; H, 7.7. Found: C, 61.21; H, 7.81.

6-(β-D-Glucopyranosyloxy)-(±)-α-tocopherol (**4b**).—Compound **2b** (2.15 g, 5 mmol) and **1** (3.97 g, 6 mmol) were reacted in the presence of $BF_3{\cdot}Et_2O$ (120 ml, 1 mmol) in dry CH_2Cl_2 (110 mL) for 3 h according to GP 1. Following flash chromatography (9:1 hexane–EtOAc) the mixture of **2b** and **3b** (3.57 g) in EtOAc (370 mL) was hydrogenated for 4 h in the presence of 10% palladium on charcoal (3.7 g) according to GP 4. After purification by column chromatography (20:1 EtOAc–MeOH), white crystals of **4b** (2 g, 3.37 mmol, 67%) formed spontaneously; mp 143–145 °C; ^{1}H NMR ($CDCl_3$): δ 5.22 (bs, 1 H, OH), 4.72

(bs, 1 H, OH), 4.61 (bs, 1 H, OH), 4.57 (dd, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.74 (m, 5 H, H-2, H-3, $J_{4,5}$ 9.5 Hz, H-4, H-6a, H-6b), 3.18 (m, 1 H, H-5), 2.76 (bs, 1 H, OH), 2.47 (m, 2 H, H-3′), 2.08 (m, 9 H, 5′-CH_3, 7′-CH_3, 8′-CH_3), 1.73 (m, 2 H, H-4′), 1.31 (m, 24 H, 2′-CH_3, $C_{16}H_{33}$), 0.86 (m, 12 H, $C_{16}H_{33}$); ^{13}C NMR ($CDCl_3$): δ 148.41 (C-6′), 145.4 (C-1a′), 128.36 (C-8′), 126.61 (C-7′), 122.78 (C-4a′), 117.37 (C-5′), 104.30 (C-1), 69.74, 74.31, 74.81, 75.15 (C-2′, C-2, C-3, C-4, C-5), 61.76 (C-6), 40.74, 39.37, 37.29, 37.44, 37.58, 37.67 ($C_{16}H_{33}$), 32.79 (2′-CH_3), 31.13 (C-3′), 27.97, 24.47, 24.80, 23.46, 23.70, 22.63, 22.72, 20.69, 21.08, 19.69, 19.75 (C-7′, $C_{16}H_{33}$), 11.81, 12.92, 13.77 (5′-CH_3, 7′-CH_3, 8′-CH_3); Anal. Calcd for $C_{35}H_6O_7$ (592.85): C, 70.91; H, 10.2. Found: C, 70.64; H, 10.34.

Methyl 2-acetoxy-5-(2,3,4,6-tetra-O-*benzyl-β*-D-*glucopyranosyloxy)benzoate* (**3c**).—To compound **2c** (1.7 g, 8.08 mmol), prepared as described elsewhere [18,19], $BF_3{\cdot}Et_2O$ (300 ml, 2.5 mmol) in dry CH_2Cl_2 (80 mL) and **1** (6.6 g, 5 mmol) were added according to a slight modification of GP 2. The imidate **1** (6.6 g, 5 mmol) was added to the mixture of **2c** (1.7 g, 8.08 mmol) in three portions (3.3 g (2.5 mmol) at 0 h and 1.65 g (1.25 mmol) at 1.5 and 3 h, respectively). Extraction was performed with EtOAc instead of CH_2Cl_2. After purification by column chromatography (5:1 hexane–EtOAc), **3c** (5.4 g, 7.37 mmol, 91%) was obtained. Recrystallization from MeOH gave white crystals; mp 79–80 °C; ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 1 H, H-6′), 7.25 (m, 21 H, 4 $CH_2C_6H_5$, H-4′), 7.01 (d, 1 H, H-3′), 4.75 (m, 9 H, 4 $CH_2C_6H_5$, H-1), 3.81 (s, 3 H, OCH_3), 3.65 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.31 (s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 169.90 (COOR), 164.39 ($COCH_3$), 154.71 (C-3′), 145.71 (C-6′), 138.44, 138.1, 138.03, 138.01, 128.39, 128.33, 128.17, 127.89, 127.84, 127.78, 127.71, 127.65, 127.60 (4 $CH_2C_6H_5$), 124.73 (C-4′), 123.77 (C-1′), 122.35 (C-2′), 120.01 (C-5′), 101.90 (C-1), 84.61, 81.91, 77.55, 75.21 (C-2, C-3, C-4, C-5), 75.74, 75.11, 75.01, 73.50 (4 $CH_2C_6H_5$), 68.66 (C-6), 52.19 (OCH_3), 20.91 ($COCH_3$); Anal. Calcd for $C_{44}H_{44}O_{10}$ (732.82): C, 72.12; H, 6.05. Found: C, 72.51; H, 5.96.

2-Hydroxy-5-(2,3,4,6-tetra-O-*benzyl-β*-D-*glucopyranosyloxy)benzoate* (**3c′**).—A suspension of **3c** (1.0 g, 1.36 mmol) in MeOH (100 mL) was mixed with a saturated $Ba(OH)_2$ solution (30 mL). Subsequently, EtOH, isopropanol and *tert*-butanol (each 15 mL) were added and the mixture was stirred for 25 h at rt. The mixture was neutralized with 1 M HCl and diluted in Et_2O. Following drying over Na_2SO_4, the organic solvents were removed under reduced pressure. The raw product and KOH powder (1 g) were suspended in a saturated solution of $Ba(OH)_2$ (30 mL), 1,2-dimethoxyethane (100 mL) and water (70 mL) and reacted for 5 days at rt. Repeating the work-up procedure of the first step resulted in spontaneous crystallization of colourless needles of **3c′** (550 mg, 0.81 mmol, 60%); mp 137–138 °C; ^{1}H NMR ($CDCl_3$): δ 10.20 (bs, 1 H, OH), 7.61 (d, 1 H, H-6′), 7.3 (m, 21 H, 4 $CH_2C_6H_5$, $J_{4'/6'}$ 3.0 Hz, H-4′), 6.91 (d, 1 H, $J_{3'/4'}$ 9.1 Hz, H-3′), 4.75 (m, 9 H, 4 $CH_2C_6H_5$, H-1), 3.7 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b); ^{13}C NMR ($CDCl_3$): δ 173.00 (COOH), 158.02 (C-3′), 149.60 (C-6′), 138.41, 138.06, 137.94, 137.82, 128.39, 128.33, 128.18, 127.91, 127.87, 127.79, 127.67, 127.28 (4 $CH_2C_6H_5$), 118.58 (C-4′), 118.16 (C-1′), 112.89 (C-2′), 111.27 (C-5′), 102.56 (C-1), 84.60, 81.99, 77.66, 76.48 (C-2, C-3, C-4, C-5), 75.76, 75.14, 75.00, 73.48 (4 $CH_2C_6H_5$), 68.75 (C-6); ES-IMS: $C_{41}H_{40}O_9$ (676.76), m/z 699.8 (80), $[M+Na]^+$.

2-Hydroxy-5-(β-D-*glucopyranosyloxy)benzoate* (**4c**).—Compound **3c′** (40 mg, 0.062 mmol) was reacted with 20% palladium on charcoal (50 mg) in acetone (20 mL) and water (2 mL) for 4 h according to GP 4. Without further purification, white crystals of **4c** (20 mg, 55.8 mmol, 80%) formed spontaneously out of the aqueous solution; mp 98–99 °C; ^{1}H NMR (D_2O): δ 7.55 (d, 1 H, H-6′), 7.30 (dd, 1 H, H-4′), 6.95 (d, 1 H, H-3′), 5.00 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 3.95 (dd, 1 H, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 12.4 Hz H-6a), 3.25 (dd, 1 H, H-6b, $J_{5,6b}$ 5.6 Hz), 3.10 (m, 4 H, H-2, H-3, H-4, H-5); ^{13}C NMR (D_2O): δ 176.67 (COOH), 160.45 (C-5′), 153.94 (C-2′), 130.23 (C-4′), 122.70 (C-6′), 122.45 (C-3′), 118.22 (C-1′), 106.02 (C-1), 80.80 (C-5), 80.30 (C-2), 77.65 (C-3), 74.18 (C-4), 65.27 (C-6); Anal. Calcd for $C_{13}H_{16}O_9{\cdot}2H_2O$ (352.29): C, 44.32; H, 5.72. Found: C, 44.56; H, 5.96.

Ethyl 3,4-(diphenylmethylendioxy)-5-hydroxybenzoate (**2d**).—Ethyl 3,4,5-trihydroxybenzoate (396 mg, 2 mmol) was dissolved in acetone (0.9 mL) and pyridine (0.16 mL). Then a solution of diphenyldichloromethane (0.38 mL, 2 mmol) in acetone (0.45 mL) was added slowly and the mixture was stirred for 16 h. The reaction was stopped by carefully adding a solution of NaOH (0.16 g) in water (0.45 mL) and stirring for a further 2 h. The oily phase was separated and extracted with Et_2O. The aqueous phase was acidified with concd HCl (pH 2–3) and also extracted with Et_2O. The combined organic solutions were washed with 10% $NaHCO_3$ and water and dried over Na_2SO_4. Solvents were removed under reduced pressure and after column chromatography (9:1 petroleum ether–EtOAc) white crystals of **2d** (440 mg, 1.21 mmol, 61%) formed spontaneously; mp 148–150 °C; ^{1}H NMR ($CDCl_3$): δ 7.37, 7.56 (m, 12 H, H-4, H-6, C(C_6H_5)$_2$), 5.67 (s, OH), 4.32 (q, 2 H, J 7.12 Hz, OCH_2CH$_3$), 1.34 (t, 3 H, OCH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 166.23 (COOR), 148.28 (*C*(C_6H_5)$_2$), 138.03, 138.93, 139.46 (C-3, C-4, C-5), 129.37, 128.31, 126.3 (C(*C*$_6$H$_5$)$_2$), 124.65 (C-1), 103.29, 113.99 (C-2, C-6), 61.12 (O*C*H$_2$CH$_3$), 14.25 (OCH$_3$*C*H$_3$); Anal. Calcd for $C_{22}H_{18}O_5$ (362.38): C, 72.92; H, 5.01. Found: C, 73.08; H, 5.11.

Ethyl 3,4-(diphenylmethylendioxy)-5-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyloxy)benzoate (**3d**).—Compound **2d** (362 mg, 1 mmol) and **1** (793 mg, 1.2 mmol) were reacted in the presence of $BF_3{\cdot}Et_2O$ (120 ml, 1 mmol) in neat CH_2Cl_2 (25 mL) for 3 h according to GP 1. After crystallization from EtOH, white crystals of **3d** (530 g, 0.599 mmol, 60%) formed spontaneously; mp 106–109 °C; ^{1}H NMR ($CDCl_3$): δ 7.26, 7.52 (m, 32 H, H-2′, H-6′, C(C_6H_5)$_2$, 4 CH$_2$C$_6$H$_5$), 5.15 (m, 2 H, $J_{1,2}$ 6.5 Hz, H-1, H-2), 4.97 (d, 1 H, H-3), 4.82 (3 H, CH_2C$_6$H$_5$), 4.53 (m, 3 H, H-4, H-6a, H-6b), 4.28 (q, 2 H, J 7.1 Hz, OCH_2CH$_3$), 3.68 (m, 6 H, H-5, CH_2C$_6$H$_5$), 1.30 (t, 3 H, OCH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 165.65 (COOR), 148.57 (*C*(C_6H_5)$_2$), 138.09, 138.16, 138.55, 139.44, 139.51, 139.81, 140.09 (C-3′, C-4′, C-5′, C(C_6H_5)$_2$), 118.7, 124.81, 126.3, 126.35, 127.52, 127.61, 127.65, 127.76, 127.83, 127.92, 128.28, 128.33, 128.37, 129.28, 129.34 (C-1′, C(C_6H_5)$_2$, 4 CH$_2$*C*$_6$H$_5$), 102.28, 105.07, 114.81 (C-1, C-2′, C-6′), 75.25, 77.38, 81.81, 84.47 (C-2, C-3, C-4, C-5), 73.48, 74.98, 75.04, 75.72 (4 CH_2C$_6$H$_5$), 68.29 (C-6), 60.9 (O*C*H$_2$CH$_3$), 14.27 (OCH$_2$*C*H$_3$); Anal. Calcd for $C_{56}H_{52}O_{10}$ (885.02): C, 76.00; H, 5.92. Found: C, 75.82; H, 6.09.

3,4-(Diphenylmethylendioxy)-5-(2,3,4,6-tetra-O-benzyl)-β-D-glucopyranosyloxy)benzoic acid (**3d′**).—Compound **3d** (4.5 g, 5 mmol) and KOH powder (3 g) were reacted in 1,2-dimethoxyethane (175 mL). After 20 h the reaction was quenched with water (90 mL), neutralized with 1 M HCl (pH 6–7) and extracted with Et_2O. The organic solution was washed with a saturated solution of $NaHCO_3$ and water and dried over Na_2SO_4. After removing the solvents under reduced pressure **3d′** (3.8 g, 4.45 mmol, 89%) was obtained as a white powder; mp 59–61 °C; ^{1}H NMR ($CDCl_3$): δ 7.26, 7.54 (m, 32 H, H-2′, H-6′, C(C_6H_5)$_2$, 4 CH$_2$C$_6$H$_5$), 5.14 (2 H, $J_{1,2}$ 7 Hz, H-1, $J_{3,4}$ 10.9 Hz, H-3), 4.97 (1 H, $J_{2,3}$ 10.9, H-2), 4.83 (3 H, CH_2C$_6$H$_5$), 4.52 (m, 3 H, $J_{4,5}$ 11 Hz, H-4, $J_{6a,6b}$ 12.2 Hz, H-6a, H-6b), 3.68 (m, 6 H, H-5, CH_2C$_6$H$_5$); ^{13}C NMR ($CDCl_3$): δ 171.00 (COOH), 148.57 (*C*(C_6H_5)$_2$), 138.04, 138.13, 138.55, 139.34, 139.4, 140.21, 140. 66 (C-3′, C-4′, C-5′, C(*C*$_6$H$_5$)$_2$), 118.96, 123.43, 126.32, 126.36, 127.56, 127.63, 127.68, 127.74, 127.83, 127.9, 128.29, 128.35, 129.36, 129.42 (C-1′, C(C_6H_5)$_2$, 4 CH$_2$*C*$_6$H$_5$), 102.26, 105.61, 115.56 (C-1, C-2′, C-6′), 75.23, 77.42, 81.80, 84.47 (C-2, C-3, C-4, C-5), 73.50, 75.01, 75.75 (*C*H$_2$C$_6$H$_5$), 68.32 (C-6); Anal. Calcd for $C_{54}H_{48}O_{10}$ (856.96): C, 75.96; H, 5.65. Found: C, 75.95; H, 5.80.

5-(β-D-Glucopyranosyloxy)benzoic acid (**4d**).—Compound **3d′** (54 mg, 0.061 mmol) was reacted for 4 h in the presence of 20% palladium on charcoal (20 mg) in acetone (10 mL) and water (1 mL) according to GP 4. Compound **4d** (20 mg, 0.057 mmol, 94%) was obtained as a white powder without further purification; mp 143–145 °C; ^{1}H NMR (CD_3OD): δ 7.36, 7.18 (2 s, 2 H, H-2′, H-6′), 4.76 (CD_3OD, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 3.69, 3.84 (m, 2 H, H-6a, H-6b), 3.49 (m, 4 H, H-2, H-3, H-4, H-5); ^{13}C NMR (CD_3OD): δ 169.89 (COOH), 141.61, 146.70, 146.78 (C-3′, C-4′, C-5′), 122.28 (C-1′), 112.16, 113.57 (C-2′, C-

6′), 104.32 (C-1(b)), 101.48 (C-1(a)), 73.36, 74.61, 74.78 (C-2, C-3, C-4, C-5 (a)), 71.06, 74.86, 77.6, 78.23 (C-2, C-3, C-4, C-5 (b)), 62.18 (C-6). Anal. Calcd for $C_{13}H_{16}O_{10}\cdot H_2O$ (350.28): C, 44.58; H, 5.14. Found: C, 44.36; H, 5.14.

*4-[2,6-Bis(*tert-*butyl)-phenyl] 2,3,4,6-tetra*-O-*acetyl-1-thio-β*-D-*glucopyranoside* (**7e**).—Compound **5** (390 mg, 1 mmol) was reacted for 16 h with probucol (103.4 mg, 0.2 mmol) in the presence of $BF_3\cdot Et_2O$ (26 μL, 0.2 mmol) in CH_2Cl_2 (5 mL) according to GP 3. After purification by column chromatography (4:1 petroleum ether–EtOAc), white crystals of **7e** (170 mg, 0.29 mmol, 74%) formed spontaneously; mp 142–144 °C, ^{1}H NMR ($CDCl_3$): δ 7.30 (s, 2 H, H-3′, H-5′), 5.35 (s, 1 H, OH), 5.2 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.02 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.87 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.57 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.31 (dd, 1 H, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.12 (dd, 1 H, H-6b), 3.72 (ddd, 1 H, $J_{5,6a}$ 4.5 Hz, $J_{5,6b}$ 2.2 Hz, H-5), 2.10, 2.07, 2.00, 1.99 (4 s, 12 H, 4 COCH_3), 1.44 (s, 18 H, 2 C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$): δ 169.06, 169.38, 170.20, 170.63 (*C*OCH$_3$), 154.87 (C-1′), 136.48 (C-2′, C-6′), 132.00 (C-3′, C-5′), 119.48 (C-4′), 85.89 (C-1), 67.99, 69.81, 74.30, 75.88 (C-2, C-3, C-4, C-5), 62.21 (C-6), 34.33 (2 *C*(CH$_3$)$_3$), 30.14 (2 CH(*C*H$_3$)$_3$), 20.54, 20.58, 20.75, 20.82 (CO*C*H$_3$); Anal. Calcd for $C_{28}H_{40}O_{10}S\cdot 0.5H_2O$ (577.69): C, 58.22; H, 7.15; S, 5.54. Found: C, 58.41; H, 7.04; S, 5.64.

*4-[2,6-Bis(*tert-*butyl)-phenyl] 1-thio-β*-D-*glucopyranoside* (**8e**).—Compound **7e** (4.2 g, 7.4 mmol) was reacted for 6 h in a solution of MeOH (200 mL), water (100 mL) and Et_3N (50 mL). After removing the solvents under reduced pressure and column chromatography, white–yellowish crystals of **8e** (2.2 g, 5.4 mmol, 74%) formed spontaneously; mp 81–83 °C; ^{1}H NMR (CD_3OD): δ 7.41 (s, 2 H, H-3′, H-5′), 4.80 (s, 1 H, OH), 4.40 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 3.86 (dd, 1 H, H-6b), 3.67 (m, 1 H, $J_{6a,6b}$ 12 Hz, H-6a), 3.30 (m, 3 H, H-2, H-3, H-4), 3.16 (m, 1 H, $J_{5,6a}$ 5.4 Hz, $J_{5,6b}$ 1.8 Hz, H-5), 1.41 (s, 18 H, 2 C(CH_3)$_3$); ^{13}C NMR (CD_3OD): δ 155.73 (C-1′), 139.82 (C-2′, C-6′), 131.48 (C-3′, C-5′), 123.67 (C-4′), 90.31 (C-1), 71.55, 73.62, 79.72, 81.95 (C-2, C-3, C-4, C-5), 63.15 (C-6), 35.68 (2 *C*(CH$_3$)$_3$)), 30.77 (2 C(*C*H$_3$)$_3$); Anal. Calcd for $C_{20}H_{32}O_6S\cdot 0.5H_2O$ (409.53): C, 58.68; H, 8.07; S, 7.82. Found: C, 58.62; H, 7.94; S, 7.87.

N-*Acetyl*-L-*cysteine ethyl ester* (**6f**).—To a solution of *N*-acetyl-L-cysteine (10 g, 61.5 mmol) in EtOH (300 mL), $SOCl_2$ (5 mL, 67.5 mmol) was added dropwise under a nitrogen atmosphere, avoiding temperatures higher than 30 °C. The reaction was stopped after 3 h by adding water (200 mL). After extraction with EtOAc, drying over Na_2SO_4 and removing the solvents under reduced pressure **6f** (5.4 g, 28.3 mmol, 46%) was obtained as an oil; ^{1}H NMR ($CDCl_3$): δ 6.42 (bs, 1 H, NH), 4.87 (dt, 1 H, $J_{2,3}$ 4.1 Hz, H-2), 4.26 (q, 2 H, *J* 7.2 Hz, OCH_2CH$_3$), 3.03 (m, 2 H, $J_{3,SH}$ 8.9 Hz, H-3), 2.08 (s, 3 H, COCH_3), 1.33 (m, 4 H, SH, OCH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 169.84, 170.09 (COOR, *C*OCH$_3$), 62 (O*C*H$_2$CH$_3$), 53.53 (C-2), 26.86 (C-3), 23.11 (*C*OCH$_3$), 14.17 (OCH$_2$*C*H$_3$); Anal. Calcd for $C_7H_{13}NO_3S$ (191.25): C, 43.96; H, 6.85; S, 7.32. Found: C, 43.66; H, 6.88; S, 7.25.

N-*Acetyl-3-(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)*-L-*cysteine ethyl ester* (**7f**).—Compound **5** (10.9 g, 28 mmol) was reacted for 3 h with **6f** (5.4 g, 28 mmol) in the presence of $BF_3\cdot Et_2O$ (6.9 ml, 56 mmol) in CH_2Cl_2 (40 mL) according to GP 3. After purification by column chromatography (EtOAc) white crystals of **7f** (6.56 g, 12.6 mmol, 45%) formed spontaneously; mp 75–78 °C, ^{1}H NMR ($CDCl_3$): δ 6.47 (bs, 1 H, NH), 5.23 (dd, 1 H, $J_{3,4}$ 9.4 Hz, 3-H), 5.07, 4.98 (2 dd, 2 H, $J_{2,3}$ 9.3, H-2, $J_{4,5}$ 10 Hz, H-4), 4.79 (m, 1 H, H-2′), 4.54 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.21 (m, 4 H, H-3′, *J* 7.2 Hz, OCH_2CH$_3$), 3.72 (ddd, 1 H, $J_{5,6a}$ 6 Hz, $J_{5,6b}$ 4.6 Hz, H-5), 3.23 (dd, 1 H, H-6b), 3.05 (dd, 1 H, $J_{6a,6b}$ 14 Hz, H-6a), 2.10 (s, 3 H, (NH)COCH_3), 2.07, 2.05, 2.03, 2.01 (4 s, 12 H, 4 (O)COCH_3), 1.29 (m, 3 H, OCH$_2$CH_3); ^{13}C NMR: δ 169.31, 169.38, 169.82, 169.97, 170.32, 170.53 (COOR, 5 *C*OCH$_3$), 83.25 (C-1), 68.06, 69.78, 73.53, 76.11 (C-2, C-3, C-4, C-5), 61.83, 61.85 (C-6, O*C*H$_2$CH$_3$), 51.89 (C-2′), 31.68 (C-3′), 22.86 ((NH)CO*C*H$_3$)), 20.47, 20.49, 20.63 (4 (O)CO*C*H$_3$), 14.1 (OCH$_2$*C*H$_3$); Anal. Calcd for $C_{21}H_{31}NO_{12}S$ (521.54): C, 48.36; H, 5.99; N, 2.69; S, 6.15. Found: C, 48.45; H, 6.08; N, 2.83; S, 6.27.

N-*Acetyl-3-(β-*D*-glucopyranosyl)-*L*-cysteine ethyl ester* (**8f**).—Compound **7g** (156.3 mg, 0.3 mmol) was reacted in a solution of 10% Et_3N in neat MeOH (24 mL) under a nitrogen atmosphere. After 16 h the solvents were removed under reduced pressure. After column chromatography (6:1 petroleum ether–EtOAc) a colourless oil of **8f** (67 mg, 0.18 mmol, 60%) was obtained; ^{1}H NMR (CD_3OD) (two sets of signals, diastereomers): δ 5.32 (bs, 1 H, NH), 4.65 (m, 1 H, H-2′), 4.37 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.13 (m, 2 H, J 7.2 Hz, OCH_2CH_3), 3.86 (d, 2 H, $J_{3,4}$ 10.6 Hz, H-3, $J_{4,5}$ 10 Hz, H-4), 3.67 (m, 2 H, $J_{5,6a}$ 8.1 Hz, H-5), 3.44 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.08 (m, 4 H, $J_{6a,6b}$ 13.8 Hz, H-6a, H-6b, 3), 1.99 (s, 3 H, $COCH_3$), 1.26 (m, 3 H, $(O)CH_2CH_3$); ^{13}C NMR (CD_3OD) (two sets of signals, diastereomers): δ 172.17, 173.36 (COOR, $COCH_3$), 88.85, 86.79 (C-1), 71.39, 71.65, 73.1, 74.25, 74.51, 75.6, 79.57, 82.26 (C-2, C-3, C-4, C-5), 62.81 (OCH_2CH_3), 62.66 (C-6), 54.42 (C-2′), 33.77, 31.99 (C-3′), 22.4, 20.85 ($COCH_3$), 14.45 (1 C, OCH_2CH_3); Anal. Calcd for $C_{21}H_{31}NO_{12}S$ (371.41): C, 42.04; H, 6.78; N, 3.77; S, 8.63. Found: C, 42.48; H, 6.41; N, 3.57; S, 8.12.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft (DFG) for support through SFB 317.

References

[1] B. Halliwell, *Ann. Int. Med.*, 107 (1987) 525–545.
[2] R.J. Reiter, *FASEB J.*, 9 (1985) 526–533.
[3] E. Negri, C. La-Vecchia, S. Franceschi, B. D'Avanzo, F. Parazzini, *Int. J. Cancer*, 48 (1991) 350–354.
[4] L.J. Marnett, *Cancer Res.*, 52 (1992) 5575–5589.
[5] J. Pohl, B. Bertram, P. Hilgard, M.R. Nowrousian, J. Stüben, M. Wießler, *Cancer Chemother. Pharmacol.*, 35 (1995) 364–370.
[6] M. Wießler, K. Beyreuther, German Patent application 195 24 515.6-43 (1994); *Chem. Abstr.*, 126 (1997) 131743.
[7] M. Veyhl, K. Wagner, C. Volk, V. Gorboulev, K. Baumgarten, W.-M. Weber, M. Schaper, B. Bertram, M. Wießler, H. Koepsell, *Proc. Natl. Acad. Sci. USA*, 95 (1998) 2914–2919.
[8] M.P. Lostao, et al., *J. Membr. Biol.*, 142 (1994) 161–170.
[9] T. Mizuma, K. Ohta, M. Hayashi, S. Awazu, *Biochem. Pharmacol.*, 43 (1992) 2037–2039.
[10] T. Mizuma, K. Ohta, S. Awazu, *Biochim. Biophys. Acta*, 1200 (1994) 117–122.
[11] T. Matsuoka, T. Nishizaki, G.E. Kisby, *J. Neurochem.*, 70 (1998) 772–777.
[12] R. Poppe, U. Karbach, S. Gambaryan, H. Wiesinger, M. Lutzenburg, M. Kraemer, O.W. Witte, H. Koepsell, *J. Neurochem.*, 69 (1997) 84–94.
[13] G. Grundler, R.R. Schmidt, *Justus Liebigs Ann. Chem.*, 11 (1984) 1826–1847.
[14] R.R. Schmidt, J. Michel, *J. Carbohydr. Chem.*, 4 (1985) 141–169.
[15] T. Shoji, K. Mizuraya, Dainippon Ink and Chemicals, Inc. (1992) Jpn., Kokai Tokkyo Koho JP 04 05,298 [92 05,298]; *Chem. Abstr.*, 116 (1992) 255969d.
[16] Sunstar, Inc. (1985) Jpn., Kokai Tokkyo Koho JP 60 56,994 [85 56,994]; *Chem. Abstr.*, 104 (1986) 34290a.
[17] M. Lahmann, J. Thiem, *Carbohydr. Res.*, 299 (1997) 23–31.
[18] G. Wagner, *Arch. Pharm.*, 291 (1958) 278.
[19] S. Fujii, H. Aoki, M. Komoto, K. Munakata, *Agric. Biol. Chem.*, 35 (1971) 1526–1534.
[20] E. Fischer, M. Bergmann, *Dtsch. Chem. Ber.*, 51 (1918) 1760–1809.
[21] O.T. Schmidt, J. Herok, *Justus Liebigs Ann. Chem.*, 587 (1954) 63–66.
[22] O.T. Schmidt, H. Schmadel, *Justus Liebigs Ann. Chem.*, 649 (1961) 149–157.
[23] G.G. Gross, *FEBS Lett.*, 148 (1982) 67–70.
[24] G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.*, 31 (1983) 1652–1658.
[25] Y. Kashiwada, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.*, 34 (1986) 3237–3243.
[26] S. Klick, K. Herrmann, *J. Chromatogr.*, 441 (1988) 335–345.
[27] M. Yamanaka, K. Shimomura, K. Sasaki, K. Yoshiohira, K. Ishimaru, *Phytochemistry*, 40 (1995) 1151–1154.
[28] N. Tanaka, K. Shimomuraand, K. Ishimaru, *Phytochemistry*, 40 (1995) 1149–1150.
[29] W. Bradley, R. Robinson, G. Schwarzenbach, *J. Chem. Soc.*, 793 (1930).
[30] M.L.P. Monsigny, D. Delay, M. Vaculik, *Carbohydr. Res.*, 59 (1977) 589–593.
[31] E. Baran, S. Drabarek, *Pol. J. Chem.*, 52 (1978) 941–946.