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"COMPUESTOS NOVEDOSOS QUE TIENEN UNA
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TRANSPORTADOR DE GLUCOSA
DEPENDIENTE DEL SODIO PRESENTE EN EL
INTESTINO O RIÑÓN"
MITSUBISHI TANABE PHARMA
CORPORATION

RESPUESTA A REQUERIMIENTO ARTICULO 46

HUMBERTO RUBIO CAMACHO, mayor de edad, domiciliado en Bogotá, D.C., identificado con la cédula de ciudadanía número 17'192.062 de Bogotá, abogado graduado portador de la Tarjeta Profesional No. 50.007 expedida por el Consejo Superior de la Judicatura, actuando en carácter de Apoderado de **MITSUBISHI TANABE PHARMA CORPORATION**, domiciliada en 2-10, Dosho-machi 3-chome, Chuo-ku, Osaka-shi, Osaka 541-8505, Japón; encontrándome dentro de la debida oportunidad procesal para dar respuesta al auto proferido por su Despacho, mediante Oficio No. 9925 y Concepto Técnico No 964, notificado por fijación en lista de 28 de Agosto de 2009, me permito manifestar y anexar:

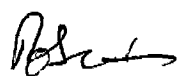
I.- Manifiesto y Anexo:

Para dar cumplimiento con el artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina, estamos anexando las publicaciones completas conforme a lo solicitado por el examinador de los siguientes documentos:

- MANIS M O; BRASELTON W E.,
DRUG METABOLISM AND DISPOSITION, 19860101 WILLIAMS AND WILKINS, BALTIMORE MD,
US - ISSN 0090-9556, Vol: 14, Nr:2, Page(s): 166 - 174
- LIN P. ET AL
SYNTHESIS, 20030101 GEORG THIEME VERLAG, STUTTGART, DE - ISSN
0039-7881, Page(s): 255-261

Conforme a lo anterior hemos atendido el requerimiento y exigencia del examinador.

Atentamente,


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METABOLISM OF 4,4'-METHYLENEBIS(2-CHLOROANILINE) BY CANINE LIVER AND KIDNEY SLICES

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ABSTRACT:

4,4'-Methylenebis(2-chloroaniline) (MBOCA) metabolism in canine liver and kidney slices was investigated using HPLC to separate the metabolites. Liver slices metabolized 5–10% of the ¹⁴C-MBOCA in 60 min and produced seven metabolites resolved by HPLC. The major metabolite, representing approximately 80% of the metabolism, was 2-amino-5-[(4-amino-3-chlorophenyl)methyl]-3-chlorophenyl hydrogen sulfate, previously identified as the major urinary metabolite in dogs. An MBOCA-glucoside was identified by mild acid hydrolysis, which released MBOCA and glucose. An O-glucuronide was characterized as labile to β-glucuronidase, stable to arylsulfatase, and mild acid. It was formed in increased amounts when 2,6-dichloro-4-nitrophenol (DCNP) was added to the incubation. Two other glucuronide metabolites were labile to mild acid and β-glucuronidase, stable to arylsulfatase, and were formed in decreased amounts in the presence of D-(+)-galactosamine (D-gal) and p-nitrophenyl sulfate (PNPS). Renal cortical slices metabolized 3–5% of the

¹⁴C-MBOCA in 90 min, producing six metabolites. Based on retention time and lability to hydrolysis, three of these, the MBOCA-glucoside, a glucuronide, and 2-amino-5-[(4-amino-3-chlorophenyl)methyl]-3-chlorophenyl hydrogen sulfate were also found as kidney metabolites. One additional sulfur-containing metabolite was labile to mild acid and arylsulfatase. The major kidney metabolite represented 25–40% of the metabolism and was unaffected by mild acid, β-glucuronidase, arylsulfatase, DCNP, and D-gal. Covalent binding in liver slices was 20–27 pmol/mg of wet weight/60 min and in kidney was 9–13 pmol/mg of wet weight/90 min. Binding was not altered in either tissue by D-gal, PNPS, or low concentrations of DCNP. Renal medullary slice incubations produced no ¹⁴C-MBOCA metabolites observed by HPLC with UV absorbance or radioactivity monitoring. Tissue covalent binding was 1.2 pmol/mg/90 min and was unchanged by the addition of aspirin or indomethacin, but doubled with 1 mM arachadonic acid.

MBOCA² is a commercially important curing agent used in the production of diisocyanate polymers. Human exposure has occurred as a result of environmental contamination and with industrial use. MBOCA is an arylamine, a class of compounds which has been associated with human bladder carcinogenesis. It is a carcinogen in mice (1) and rats (1–4) and causes bladder cancer in dogs (5), a test animal for arylamine-induced bladder cancer.

Metabolism is believed necessary for arylamines to exert their carcinogenic effect. Arylamine bladder carcinogens may be activated by hepatic metabolism to hydroxylamines, conjugated and transported to the urine as N-hydroxy-N-glucuronides (6). These are acid-labile forms which can release the hydroxylamine, a proximate carcinogen, in the mildly acidic urine (6–8). Metabolism by the urothelium has resulted in the mutagenic activation of 2AF, BZ, 4AB, and 2NA (9, 10) and in nucleic acid binding

of BZ (11). 2AAF was metabolized to ring- and N-hydroxylated products and to 2AF by rat and human bladder culture (12).

Renal metabolism of arylamines is also of interest due to the physiological proximity of the kidney to the bladder. Studies have focused on the medulla and cooxidation of arylamines by PES. BZ, 2AF, 4AB, and 2-amino-4(5-nitro-2-furyl)thiazole have been cooxidized by PES from ram seminal vesicle or rabbit renal medulla (11, 13–15). PES-mediated DNA (16), RNA (14–16), and macromolecular binding (13) as well as mutagenesis (17) have been demonstrated using microsomal incubations. Furthermore, BZ was cooxidized by renal medullary slices to macromolecular binding species (18).

In contrast to those studies, renal cortical metabolism studies have had limited success. 2AAF and 2AF were activated to bacterial mutagens (19–22), and N-hydroxylation has been observed for 2AAF (23). However, BZ was not metabolized by rabbit cortical microsomes (13) and 2NA was not metabolized by rat cortical tissue slices (24). Furthermore, production of conjugated metabolites capable of being excreted in the urine has not been reported.

The metabolism of MBOCA has not been extensively studied. It is rapidly metabolized in rats and dogs and is excreted in urine and bile. In rats, 69–73% of the dose was recovered in feces and 22–29% in urine in 48 hr (25), only 1–2% of which was the parent compound. In the dog, 46% of the dose was recovered in the urine in 24 hr, only 0.54% of which was the parent compound (26). Seventy-five per cent of the excreted MBOCA was as the *ortho*-hydroxy sulfate metabolite (27). Biliary excretion represented 32% of the dose in the dog. Neither rat nor dog excreted the parent compound in the bile (25, 26).

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² Abbreviations used are: MBOCA, 4,4'-methylenebis(2-chloroaniline); 4AB, 4-aminobiphenyl; 2NA, 2-naphthylamine; 2AF, 2-aminofluorene; 2AAF, 2-acetylaminofluorene; BZ, benzidine; DCNP, 2,6-dichloro-4-nitrophenol; D-gal, D-(+)-galactosamine; PNPS, p-nitrophenyl sulfate; PES, prostaglandin endoperoxide synthase; PIC A, tetrabutylammonium phosphate; [B-¹⁴C]MBOCA, 4,4'-[¹⁴C]methylenebis(2-chloroaniline) (bridge labeled); [U-¹⁴C]MBOCA, 4,4'-methylenebis(2-chloro[¹⁴C]aniline (uniformly ring labeled); ACN, acetonitrile; LSS, liquid scintillation spectrometry.

The present study was undertaken to evaluate the potential of the liver and kidney for metabolism of MBOCA to reactive and conjugated products. The dog was used due to its sensitivity to arylamine-induced bladder cancer.

Materials and Methods

Animals. Healthy adult male mongrel dogs, weighing 11–19 kg, were used. They had free access to food and water prior to the experiments and were used within 3 days of receipt. Dogs less than about 1.5 years old did not produce the same metabolic profile in *in vitro* incubations and were excluded from the study.

Chemicals. Glucuronic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI), and aqueous counting solution was obtained from Amersham Corp. (Arlington Heights, IL), DCNP from Alpha Products (Danvers, MA). $\text{Na}_2^{35}\text{SO}_4$, 43 Ci/mg, 220 mCi/ml, was from ICN Pharmaceuticals (Irvine, CA), and ^{14}C -toluene (4×10^5 dpm/ml) was from New England Nuclear (Boston, MA). β -Glucuronidase-free arylsulfatase, β -glucuronidase, D-gal, PNPS, and deoxycholic acid were from Sigma Chemical Co. (St. Louis, MO), Soluene 350 and Demilume were from United Technologies Packard (Downers Grove, IL), and the ion pair reagent PIC A was from Waters Associates (Milford, MA). Solvents were HPLC, UV, spectrometric grade and were purchased from Burdick and Jackson (Muskegon, MI). Water was purified by passing distilled water through a 4-bowl Milli-Q (Millipore Corp., Bedford, MA) water purification system containing a 0.25- μm filter.

[^{14}C]MBOCA (58 mCi/mmol) and [^{14}C]MBOCA (10.9 mCi/mmol) from Amersham Corp. were provided by the Michigan Toxic Substances Control Commission through the Office of Radiation, Chemical and Ecological Safety, Michigan State University. They were purified by HPLC on a C_{18} μ Bondapak column (Waters Associates) using ACN/ H_2O (47:53, v/v) prior to use. Radiochemical purity was judged to be greater than 99% by quantitative GC/MS and LSS. MBOCA was a gift from Daniel E. Williams (Division of Environmental Epidemiology, Michigan Department of Health) and was purified before use by HPLC as above.

Metabolism *in vitro*. Dogs were killed by injection of pentobarbital (80 mg/kg). Liver samples were excised, cut into 1-inch pieces, rinsed in cold saline, and placed in ice-cold phosphate-buffered medium (slice buffer) containing 96.7 mM NaCl, 7.4 mM sodium phosphate, 40 mM KCl, and 0.74 mM CaCl_2 at pH 7.4 (28). Kidneys were excised, divided into four pieces, rinsed in saline, and placed in cold slice buffer. Medullary tissue was dissected away from corticomedullary tissue and stored separately. All experiments were initiated within 3 hr.

The slices were prepared by hand using a slice block and razor blade, and were thin enough to curl when picked up with a forceps. A total of 200 ± 10 mg of liver or 400 ± 20 mg of kidney tissue was incubated in 4 ml of slice buffer. [^{14}C]MBOCA, 100 nmol/ml (9.09×10^{-4} μCi /nmol), was added in 20 μl of dimethylsulfoxide. Incubations were performed in a Dubnoff shaking water bath at 90 cycles/min and 37°C under 100% O_2 for 60 min (liver) or 90 min (kidney). In experiments to define the effect of substrate concentration, MBOCA was added at 0.005–0.1 $\mu\text{mol}/\text{ml}$, while radioactivity was added in a constant amount (2×10^5 dpm/ml). [^{14}C]MBOCA was used for the 0.005- $\mu\text{mol}/\text{ml}$ incubation due to its higher specific activity. In experiments to define the effect of incubation time, incubations were performed for 0–90 min (liver) or 0–120 min (kidney). Incubations were stopped by addition of 4 ml of cold ACN and were placed in ice for 15 min. Controls were inactivated with ACN prior to adding [^{14}C]MBOCA or [^{14}C]MBOCA.

Metabolite Separation and Quantitation. Slices were homogenized in the incubation media with a Wheaton-Teflon-glass homogenizer. One ml of 0.05 M PIC A was added to the homogenate and the mixture was extracted three times with 3 ml of dichloromethane. The three extracts were pooled, dried over anhydrous sodium sulfate, evaporated under N_2 , resuspended in 150 μl of ACN/ H_2O (1:1), and filtered through 0.2- μm regenerated cellulose filters (BAS, The Anspec Co., Ann Arbor, MI). Glass fiber prefilters were sometimes used. Samples were kept at –20°C until used. Recovery of radioactivity was 90–100% in the extract and

75–85% in the filtrate. Calculations were based on total dpm in the incubation mixture.

The HPLC system consisted of an M6000 and an M45 pump, a model 720 system controller, a U6K injector, and a model 440 detector at 254 nm (Waters Associates). The metabolites were separated on a 5- μm C_{18} Novapak radial compression column (Waters Associates) with a 35-min linear gradient of 23–42.5% ACN, 2.5 mM PIC A at 3 ml/min or a 25-min 23–33% ACN gradient in 2.5 mM PIC A followed by a 20-min 33–42.5% gradient, both at 2.3 ml/min. Metabolite peaks were observed by UV absorbance at 254 nm. The peaks were quantified from fractions collected through 60 min and counted by LSS using a Packard B4600 refrigerated scintillation spectrometer and predetermined quench curve. Fractions with less than 3 times background cpm were not considered for further analysis. Recovery from the column was 100%.

Binding. Macromolecular covalent binding was determined on the extracted homogenates. Homogenates were brought to 10% trichloroacetic acid, precipitated on ice for 15 min, and centrifuged, and the supernatants were discarded. The precipitates were extracted six times with methanol/ether (3:1) and four times with ethyl acetate. They were allowed to dry, moistened with 0.3 ml of 5% deoxycholic acid, kept overnight, and solubilized with 2–3 ml of Soluene 350 at 50°C. Fifteen ml of Demilume were added and the samples were counted. Quench was corrected with an internal standard (^{14}C -toluene). Binding was expressed as pmol/mg of wet weight of tissue. In a control experiment background dpm levels were reached with four extractions of methanol/ether followed by three extractions of ethyl acetate.

Conjugated Metabolites. Formation of metabolites conjugated with sulfate was evaluated by the addition of reported inhibitors or enhancers of sulfation (29, 30), or a specific label for sulfur. In the first instance, slices were incubated with 100 nmol/ml of cold MBOCA and 2 mM $\text{Na}_2^{35}\text{SO}_4$ (7.4×10^5 dpm) (liver) or 2 mM $\text{Na}_2^{35}\text{SO}_4$ (8×10^5 dpm) (kidney). Incubation extracts were analyzed by HPLC-LSS and 1-min or peak fractions were collected across the gradient. The quench curve for ^{14}C was used to determine dpm. In the second and third instances, liver or kidney slices were preincubated 10 min with either 0–2 mM PNPS or 0–10 μM DCNP prior to adding [^{14}C]MBOCA in order to enhance or inhibit sulfate conjugate formation. Metabolism was evaluated by HPLC-LSS determination of metabolites and by measurement of covalent binding in tissue slices.

Sulfate conjugate formation was also evaluated with arylsulfatase. Dichloromethane incubation extracts were pooled and stored at –20°C until use. The extracts were divided, solvent was evaporated under N_2 , and the extracts were resuspended in 2 ml of 0.2 M Tris-HCl, pH 7.1. The samples were mixed with 4–8 units of arylsulfatase and all tubes were incubated at 37°C for 4 hr in a shaking water bath. Samples and controls were extracted following the addition of 0.2 ml of 0.05 M PIC A and analyzed by HPLC-LSS.

Formation of glucuronide conjugates was evaluated by addition of D-gal, an inhibitor of glucuronidation (30, 31). Slices were preincubated 10 min with 0–15 mM D-gal prior to the addition of [^{14}C]MBOCA. Metabolism was evaluated by HPLC-LSS and measurement of binding as above.

Glucuronide formation was also evaluated by post-incubation hydrolysis experiments. Dichloromethane extracts were pooled and stored, then divided and dried as described above. The extracts were resuspended in 2 ml of 0.075 M phosphate buffer, pH 7.0, and these were used for three experiments. For mild acid hydrolysis, the samples were treated with 50 or 100 μl of 1 M citric acid (final pH 3.5–4.7) and were incubated for 60 min at 37°C as before. The samples were neutralized with 0.01 N NaOH. Enzymatic hydrolysis was performed with 500–1000 units of β -glucuronidase and incubation for 4 hr at 37°C. Thermal hydrolysis was tested by incubating the samples at 37°C for 24 hr and comparing to refrigerated controls. Samples and controls for these three experiments were extracted, prepared, and analyzed by HPLC-LSS following the addition of 0.2 ml of 0.05 M PIC A.

Structure of the Metabolite at 9.5 min. This metabolite was isolated from *in vitro* incubations for further analysis. The dichloromethane extracts from three incubations were pooled, dried, and resuspended in

5 ml of H₂O. This was applied to a C₁₈ Sep Pak column (Waters Associates) previously activated with ACN and equilibrated with H₂O. The column was rinsed with 10 ml of H₂O and the metabolites eluted with 10 ml of ACN/H₂O (1:1). The ACN was evaporated under N₂ and the sample was filtered before HPLC on a 5- μ m C₁₈ radial compression column (Waters Associates). To isolate the metabolite, a 40-min hyperbolic (No. 8) gradient, 15–60% ACN, 50 mM ammonium acetate, pH 7.45, at 2.5 ml/min was used. The column eluant was evaporated to dryness, resuspended in ACN, and stored at 4°C.

HPLC-LSS analysis of the metabolite was performed before and after hydrolysis. Seventy-five nmol of the isolated conjugate were dissolved in H₂O and maintained at room temperature for 48 hr. This resulted in complete disappearance of the peak at 9.5 min and appearance of MBOCA as determined by HPLC-LSS.

The metabolite was also analyzed by GC/MS. The conjugate was too unstable to derivatize and run directly, but the carbohydrate released on hydrolysis was suitable for derivatization and GC/MS. Sixty nmol were derivatized with trimethylsilylimidazole/*N,O*-bis(trimethylsilyl)trifluoroacetamide/pyridine (1:1:3) for 20 min at 100°C. The reagents were evaporated under N₂ and the sample was resuspended in dichloromethane. Mannose, galactose, glucose, and glucuronic acid were also derivatized and used as reference standards. The derivatives were analyzed by GC/MS on a Finnigan 3200 instrument with a Riber SADR data system. Chromatography was carried out on two separate columns, 1% OV17 and 1% OV1, both on gas chrom Q, using 30 ml/min of He and a temperature program of 140–260°C at 12°C/min beginning 0.5 min after injection. Mass spectra were obtained at 70 eV.

Renal Medullary Metabolism. Metabolism was evaluated through binding studies using 400 mg of tissue and a 90-min incubation under 100% O₂. Studies included substrate concentration dependence, addition of arachadonic acid (0.2 or 1 mM), and addition of the PES inhibitors indomethacin (0.28 mM) and aspirin (1 mM). The studies were done under yellow light. Arachadonic acid was diluted in ethanol and stored in the dark, under N₂, at –20°C. Its purity was tested prior to use by thin layer chromatography. LHP-K plates were used and 99:1 ethyl acetate/acetic acid was the solvent system. Iodine vapors were used for visualization of arachadonic acid at *R_f* = 0.67.

Statistical Methods. Differences from control values were determined at *p* < 0.05 by one-way analysis of variance. The least significant differences test was used for individual comparisons where analysis of variance indicated that differences existed.

Results

Liver. Liver slice incubations produced a major radioactive metabolite with a retention time of 25 min and six additional metabolites with retention times of 9.5, 10.1, 11.5, 12.2, 13.5, and 23.5 min (fig. 1). These were consistent from one animal to the next except for the peak at 13.5 min which was sometimes missing and could not be further characterized. The production of metabolites increased in linear fashion with time for 90 min. At 60 min, 5–10% of the MBOCA was metabolized, approximately 80% of which was the major metabolite.

Incubation of the extracted metabolite fraction with β -glucuronidase decreased the metabolites eluting at 10.1, 11.5, and 12.2 min and hydrolysis at pH 3.5–4.7 decreased the peaks at 9.5, 10.1, and 12.2 min (fig. 2). Incubation in the presence of 3 mM D-gal, an inhibitor of glucuronidation (30, 31), resulted in a decrease in formation of the peaks at 9.5, 10.1, and 12.2 min (table 1). Incubation in the presence of DCNP, a sulfation inhibitor which can increase the glucuronidation of phenols (29), caused a 4.5-fold increase in formation of the peak at 11.5 min (table 1). These results suggested that the metabolites at 9.5, 10.1, 11.5, and 12.2 min were glucuronides.

The metabolite at 9.5 min was identified as a glucoside. The compound was shown by HPLC-LSS analysis to decompose to

MBOCA on standing in water at room temperature. The sugar moiety released was identified as glucose by GC/MS. The mass spectrum was the same as the glucose reference (fig. 3) and the GC retention time and pattern of α and β anomers was identical (fig. 4).

During incubations with Na₂³⁵SO₄, two MBOCA metabolites, the peaks at 23.5 and 25.5 min, were labeled by ³⁵S (fig. 5). Two other radioactive peaks were shown to be artifactual. The peak at 23.5 min was present only in trace quantities and was not characterized further by other experiments with D-gal, DCNP, or PNPS. The metabolite at 25.5 min corresponded to the previously identified major canine urinary metabolite, the MBOCA-*ortho*-hydroxy sulfate (27). Both had identical retention times on HPLC and were labile in the presence of arylsulfatase and stable in the presence of β -glucuronidase (fig. 2 and ref. 27).

Macromolecular covalent binding increased linearly with substrate concentrations of 0.005–0.1 μ mol/ml and with time for 0–90 min under conditions of oxidative metabolism. Binding was 19.9 $\pm$ 1.8 pmol/mg of wet weight in control incubations for PNPS and D-gal. It was neither increased nor decreased by the addition of up to 2 mM PNPS (18.7 $\pm$ 2.3 pmol/mg) or 15 mM D-gal (23.0 $\pm$ 4.7 pmol/mg), although these compounds significantly altered the production of the MBOCA glycoside and glucuronide metabolites (table 1). Binding was 30 pmol/mg in control incubations for DCNP. This decreased significantly only at 10 and 100 μ M DCNP, where production of all metabolites except that at 11.5 min, was decreased. These studies indicated that metabolism was required for binding but that neither the glucoside nor the glucuronide conjugates measured by HPLC played a major role in tissue binding.

Kidney Cortex. Renal cortical slice incubations produced a major radioactive metabolite with a retention time of 15.5 min and five additional metabolites with retention times of 9.5, 12.2, 21.5, and 23.5, and 25.5 min (fig. 6). The production of metabolites increased in a linear fashion with time to 120 min. At 90 min, 3–5% of the ¹⁴C-MBOCA was metabolized, with the major metabolite representing from 25 to 40% of the total metabolites. The structure of this metabolite was not elucidated by the methods used. It was not sensitive to mild acid, β -glucuronidase, or arylsulfatase hydrolysis, was not labeled by ³⁵S, and was unaffected by incubation with D-gal or DCNP. It was decreased following incubation with PNPS (table 2).

The metabolites which eluted at 9.5, 12.2, and 25.5 min appeared the same as the corresponding liver metabolites. The MBOCA-glucoside at 9.5 min was labile in mild acid but stable to β -glucuronidase and arylsulfatase hydrolysis (fig. 7). Its production was inhibited by D-gal (table 2). A difference from liver was noted in its response to incubation with PNPS, where a decrease was observed in liver but an increase was seen in kidney (tables 1 and 2). A glucuronide eluting at 12.2 min was labile with mild acid and β -glucuronidase but stable to arylsulfatase hydrolysis (fig. 7). The *ortho*-hydroxy-sulfate at 25.5 min was stable to mild acid and β -glucuronidase but labile to arylsulfatase (fig. 7). It was labeled by ³⁵S in both organs (figs. 5 and 8).

Two other metabolites were labeled with ³⁵S (fig. 8). The metabolite at 21.5 min was produced in small quantities and could not be characterized further. The metabolite at 23.5 min was sensitive to arylsulfatase and mild acid hydrolysis. The peaks at 5 and 30 min were artifactual.

Macromolecular binding increased linearly with substrate concentrations of 0.005–0.1 μ mol/ml under conditions for oxidative metabolism. Binding levels in control incubations were 13.1 $\pm$

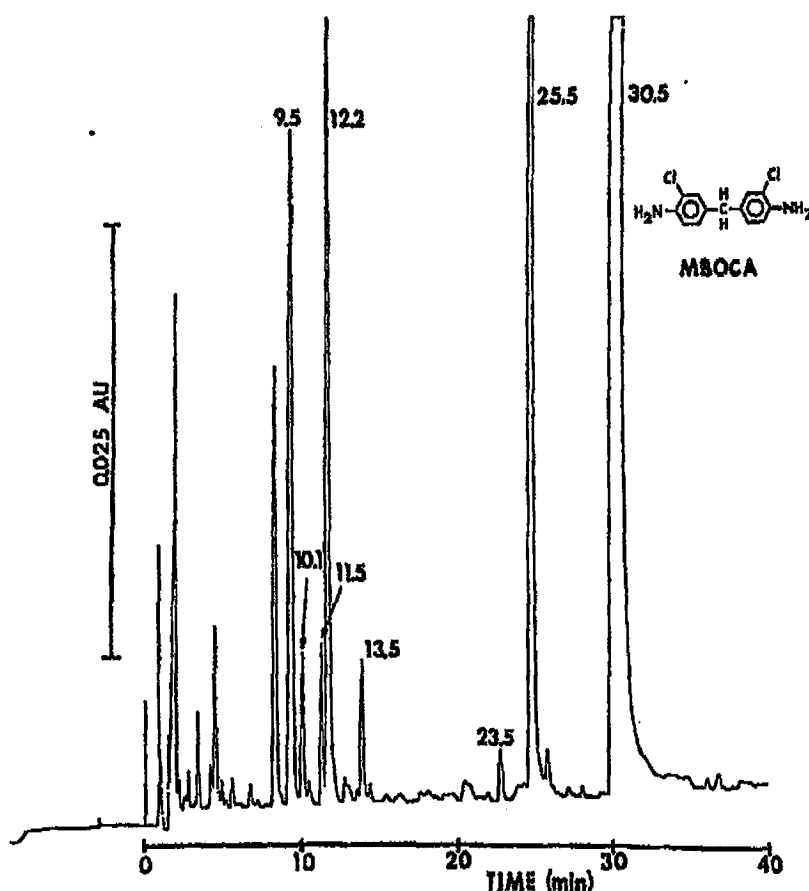


FIG. 1. HPLC chromatogram of metabolites from a hepatic tissue slice incubation.

Metabolite peaks observed by UV absorbance at 254 nm are distinguished by retention time. A 35-min linear gradient from 23–42.5% ACN, in 2.5 mM PIC A, at 3 ml/min was used on a 5- μ m radial compression Nova-pak column (Waters Associates).

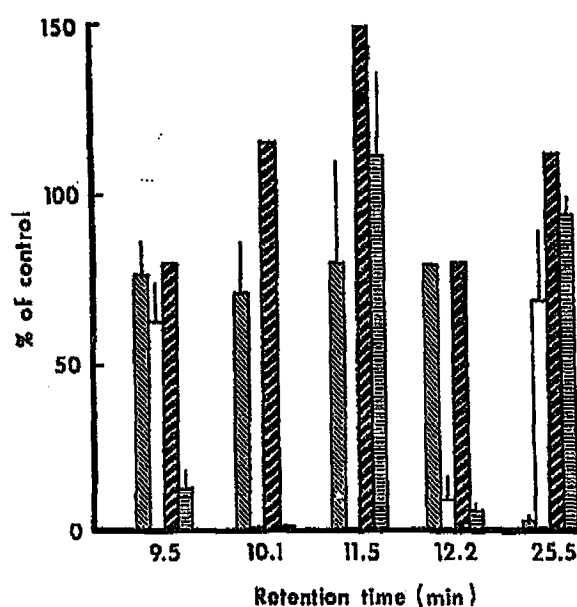


FIG. 2. Post-incubation hydrolysis of metabolites produced by hepatic tissue slices.

■, arylsulfatase (4–8 units), □, β -glucuronidase (500–1000 units); ▨, heat 37°C, 24 hr; ▩, citric acid, pH 3.5–4.7. $N = 3$ except for heat where $N = 2$. Standard error bars are shown.

0.84 (PNPS), 9.1 ± 0.3 (D-gal), and 13.4 ± 1.9 (DCNP) pmol/mg of wet weight/90 min. These values did not change significantly in the presence of up to 2 mM PNPS or 15 mM D-gal but decreased at 10 μ M and 100 μ M DCNP to 8.3 ± 2.4 and 6.1 ± 0.3 pmol/mg of wet weight/90 min. This indicated that metab-

olism was necessary for binding to occur and that the metabolites at 9.5, 12.2, and 15.5 min did not play a major role in binding.

Renal Medulla. Renal medullary slice incubations produced no metabolites identifiable by HPLC-LSS. Tissue binding was concentration dependent with 0.005–0.1 μ mol/ml of ^{14} C-MBOCA. It was unchanged by 1 mM aspirin, 0.28 mM indomethacin, or 0.2 mM arachadonic acid but doubled with 1 mM arachadonic acid to 2.21 ± 0.71 pmol/mg of wet weight/90 min ($N = 3$).

Discussion

Incubations of [^{14} C]MBOCA with canine liver and kidney slices produced metabolites characterized as glucoside, glucuronide, and sulfate conjugates. Of the seven metabolites from liver and six from kidney incubations, three were produced by both organs. These were an MBOCA-glucoside, a glucuronide, and the MBOCA-*ortho*-hydroxy sulfate previously identified as the major canine urinary metabolite (27).

2-Amino-5-[(4-amino-3-chlorophenyl)methyl]-3-chlorophenyl hydrogen sulfate, an MBOCA *ortho*-hydroxy sulfate, represented 80% of the metabolism in liver slices and was 75% of the MBOCA excreted in urine (27). The *ortho*-hydroxy sulfate is the major canine urinary metabolite of the carcinogenic arylamines BZ, 2NA and 4AB (32, 33) and the noncarcinogenic 1-naphthylamine (33). It has been considered a detoxication product because the BZ, 2NA, and 2AF metabolites were negative in the mouse bladder implantation model (34–36).

Other evidence suggests that some *ortho*-hydroxy metabolites may be toxic or carcinogenic. Two conjugates, 3-hydroxy-4-aminobiphenyl sulfate and 2-amino-1-naphthyl glucosiduronate,

TABLE I

Liver slice metabolite production

Liver metabolites were measured by HPLC-LSS and are presented in nmol/200 mg/60 min. Values are means of three determinations with standard errors in parentheses.

Retention Time min	D-(+)-Galactosamine		2,6-Dichloro-4-nitrophenol ^a		p-Nitrophenyl Sulfate	
	Control	3.0 mM	Control	0.1 μ M	Control	0.5 mM
9.5	3.07 (0.98)	0.83 (0.25) ^b	3.80	3.18	2.45 (0.16)	1.55 (0.30) ^b
10.1	1.17 (0.18)	0.44 (0.11) ^b	1.61	1.60	0.96 (0.12)	0.56 (0.08) ^b
11.5	0.95 (0.38)	0.57 (0.18)	0.20	0.95	1.08 (0.33)	0.55 (0.27)
12.2	2.96 (0.22)	0.74 (0.15) ^b	5.46	4.87	2.62 (0.54)	0.76 (0.22) ^b
23.5	0.58 (0.18)	0.63 ^c	1.62	1.37	0.43 (0.07)	0.59 (0.18)
25.5	14.83 (1.30)	12.09 (2.16)	29.66	28.01	13.00 (0.02)	17.10 (3.41)

^a N = 2.

^b Significantly different from control, $p < 0.05$.

^c N = 1.

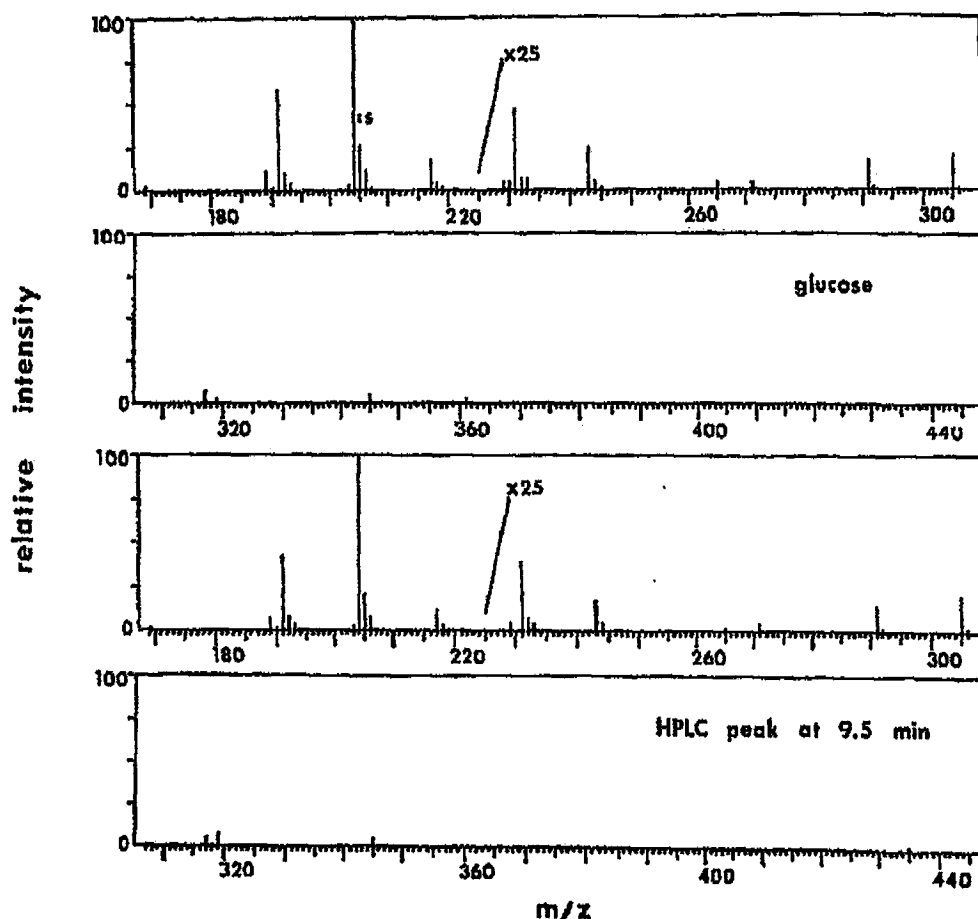


FIG. 3. Electron impact mass spectra of the trimethylsilyl-derivatized carbohydrate moiety from the 9.5-min HPLC peak and a glucose standard.

The derivative was analyzed by GC/MS on a Finnigan 3200 spectrometer at 70 eV. Chromatography was carried out on 1% OV17 using 30 ml/min of helium and a temperature program of 140–260°C at 12°C/min beginning 0.5 min after injection.

and the orthoaminophenols 1-amino-2-naphthol and 2-amino-1-naphthol produced tumors in the mouse bladder implantation model (35). In addition, *o*-aminophenol was cytotoxic *in vitro* and more genotoxic than *N*-phenylhydroxylamine as indicated by sister chromatid exchange (37). Previous studies demonstrated that the MBOCA-*ortho*-hydroxy sulfate bound to DNA and protein upon hydrolysis with arylsulfatase (27). Thus, although conjugation with sulfate may be a detoxication mechanism, the *ortho*-hydroxy arylamine metabolite may be reactive.

An MBOCA-glucoside was produced by both liver and kidney. It was not sensitive to β -glucuronidase or arylsulfatase but hy-

drololyzed in mild acid or water at room temperature, releasing glucose and MBOCA. It may have been an *N*-glucoside. The *N*-glucoside of a secondary amine, 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole, was a major canine biliary and urinary metabolite (38) and it was also resistant to β -glucuronidase hydrolysis and labile under acidic conditions. In general, *N*-conjugates of arylamines have been identified as *N*-glucuronides (33, 39–41). Formation of 2NA-*N*-glucoside was observed in rat liver and kidney slice incubations containing 0.2% glucose (42). Further studies demonstrated that it was an artifact, produced in incubation media in the absence of slices and not in incubations in

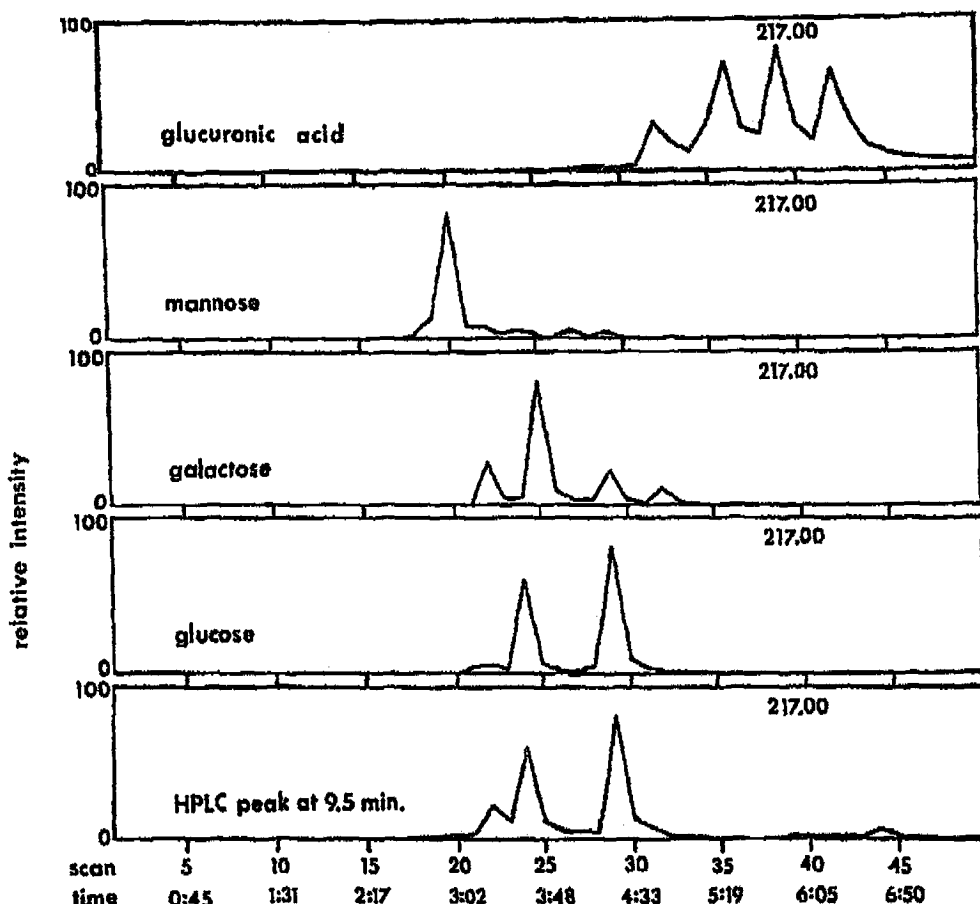


FIG. 4. Selected ion chromatogram of the trimethylsilyl-derivatized carbohydrate moiety from the 9.5-min HPLC peak and glucuronic acid, mannose, galactose, and glucose standards.

Chromatography was carried out on 1% OV17 using 30 ml/min of helium and a temperature program of 140–260°C at 12°C/min beginning 0.5 min after injection. The ion current chromatogram of the fragment at m/z 217 was plotted.

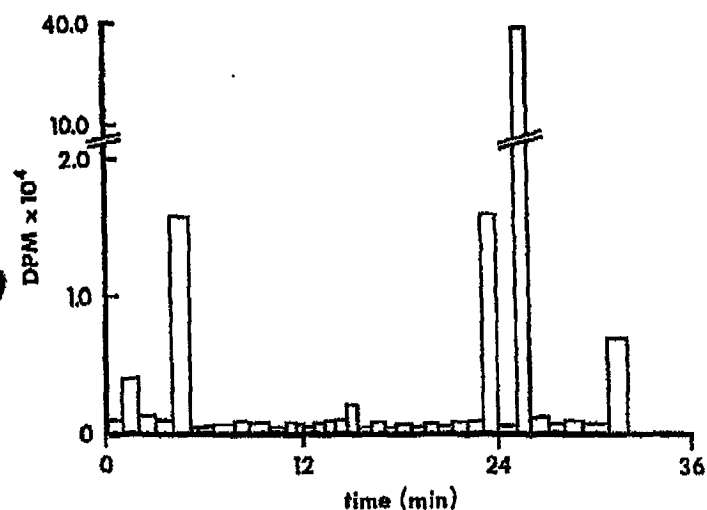


FIG. 5. Labeling of hepatic metabolites with $\text{Na}_2^{35}\text{SO}_4$.

Plot of the LSS analysis of 1-min or peak fraction collections during a 35-min linear HPLC gradient from 23–42.5% ACN, 2.5 mM PIC A at 3 ml/min. A 5- μm radial compression Nova-pak column (Waters Associates) was used.

the absence of added glucose. In the current experiments, the MBOCA-glucoside is unlikely to have occurred spontaneously for three reasons. First, the media did not contain glucose. Second, the metabolite was not produced in the absence of liver slices even in media with 0.2% glucose added. Third, in the presence of PNPS, the MBOCA-glucoside varied in opposite

directions in liver and kidney. Glucosidation can supplement glucuronidation when the aglycon load is high (43). The UDP-glucosyltransferases are normal constituents of both liver and kidney.

Liver slice incubations produced an *O*-glucuronide. It was labile to β -glucuronidase, stable to mild acid hydrolysis, and increased up to 500% in the presence of DCNP, a compound known to enhance the glucuronidation of phenols while inhibiting sulfation (29). In our experiments inhibition of sulfation was not observed, perhaps due to the relative proportion of metabolites. A small decrease in the *ortho*-hydroxy sulfate may have gone undetected and still have produced a large increase in the minor metabolite. The increase in the MBOCA-*O*-glucuronide did not occur with a concurrent increase in tissue binding, indicating that it was a detoxication product.

Liver and kidney slices produced two glucuronide conjugates that were labile to both mild acid and β -glucuronidase. These are characteristics of arylamine *N*-glucuronides and *N*-hydroxy-*N*-glucuronides (7, 8, 44, 45). Formation of both metabolites was decreased in the presence of D-gal which inhibits *O*-glucuronide formation (30) but which has not been used previously to inhibit formation of either of the *N*-glucuronide conjugates. The effect is not unexpected, however, because D-gal traps uridine nucleotides as UDP-D-gal, a compound that inhibits UDP-glucose dehydrogenase.

Extra-bladder metabolism of arylamines to acid label *N*-conjugates may play a role in bladder carcinogenesis. The parent compound may be concentrated in the bladder following hy-

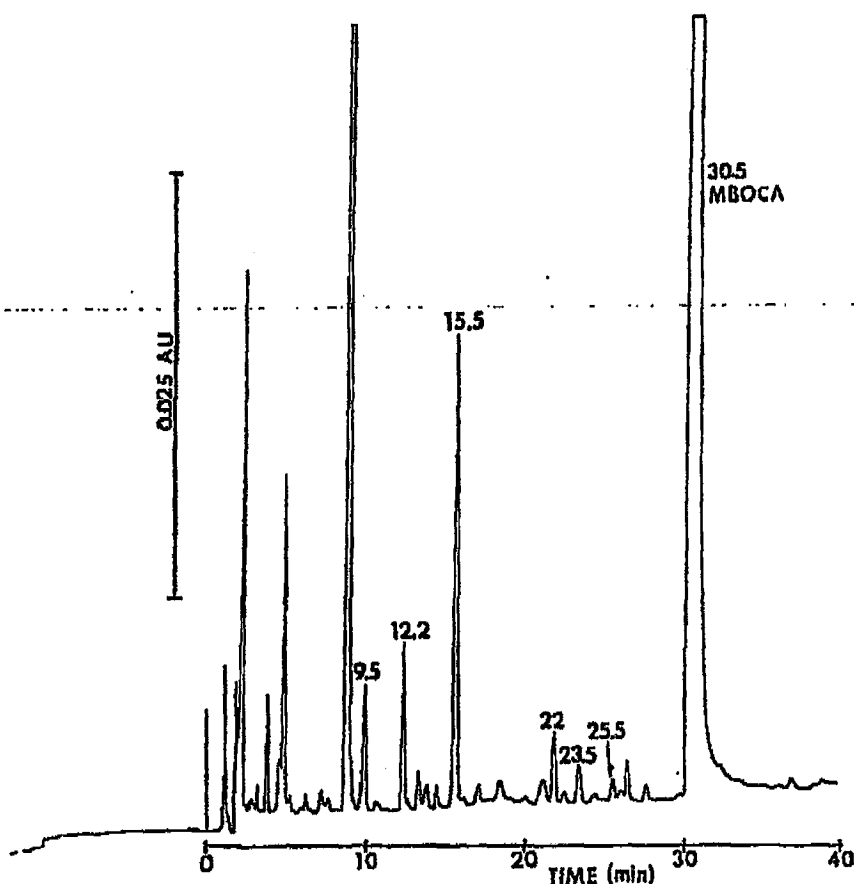


FIG. 6. HPLC chromatogram of metabolites from renal cortical tissue slice incubation.

Metabolite peaks are identified by retention time. A 35-min linear gradient from 23–42.5% ACN, 2.5 mM PIC A at 3 ml/min was used on a 5- μ m radial compression Nova-pak column (Waters Associates). UV absorbance was monitored at 254 nm.

TABLE 2

Kidney slice metabolite production

Kidney metabolites were measured by HPLC-LSS and are presented in nmol/400 mg/90 min. They are the means of three determinations, with standard error in parentheses, except where noted.

Retention Time <i>min</i>	D-(+)-Galactosamine		2,6-Dichloro-4-nitrophenol ^a		<i>p</i> -Nitrophenyl Sulfate	
	Control	0.6 mM	Control	0.1 μ M	Control	0.5 mM
9.5	0.74 (0.13)	0.11 (0.18) ^a	0.86 (0.25)	0.75 (0.07)	0.87 (0.07)	1.40 (0.25) ^a
12.2	1.62 (0.39)	1.26 (0.48)	2.69 (0.60)	2.40 (0.40)	1.72 (0.74)	2.28 (0.48)
15.5	3.27 (0.39)	3.05 (0.63)	4.84 (1.08)	5.38 (1.70)	3.26 (0.86)	1.26 (0.27) ^a
21.5	1.08 (0.23)	0.98 (0.18)	1.29 (0.23)	0.99 (0.26)	1.16 (0.24)	1.59 (0.49)
23.5	0.55 (0.03)	0.70 (0.19)	0.69 (0.12)	0.68 (0.17)	1.16 (0.53)	0.50 (0.05)
25.5	1.05 (0.56)	1.62 (1.12)	0.65 (0.13)	0.43 (0.11)	0.98 (0.40)	0.98 ^b

^a Significantly different from control, $p < 0.05$.

^b $N = 2$.

drolysis of the conjugate in acidic urine. The arylamine may then be absorbed by the urothelium and metabolically activated. Poupko *et al.* (46) showed that canine and rat urothelial cytochrome P₄₅₀ enzymes metabolized 4AB to the hydroxylamine and that the rate per nmol of cytochrome P₄₅₀ was equal in liver and bladder. BZ and 2NA have been activated to protein and nucleic acid binding species by dog bladder microsomal preparations (47). Both have also been *N*-acetylated by rat and human bladder organ cultures (48). Swaminathan *et al.* (49) demonstrated activation of 2-amino-4-(5-nitro-2-furyl)thiazole to protein and nucleic acid binding forms in rat bladder culture. In addition, bovine urothelial cells have activated 2AAF, 4AB, 2NA, and BZ to bacterial mutagens in V79 cells (9, 10).

In the present study, renal cortical incubations produced con-

jugated and reactive metabolites of MBOCA. Glucoside, glucuronide, and sulfate conjugates were identified. Previous attempts to demonstrate renal cortical metabolism have had limited success. Mutagenic activation of 2AF and 2AAF by S9 or microsomes has been reported for mice (19, 20) and rat (21) kidney. Ring- and *N*-hydroxylation of 2AAF has been observed in mice (23). However, no metabolism of 2NA was observed in rat kidney slices even when deacetylation and acetylation of 2NA metabolites was observed (24). Zenser *et al.* (13) reported a lack of BZ metabolism by either liver or renal cortex in rabbits. The present study provides evidence that canine kidney cortex has the potential to metabolize arylamines to hydroxylated and conjugated products which may be excreted in the urine.

Acknowledgments. The skillful technical assistance of Ms.

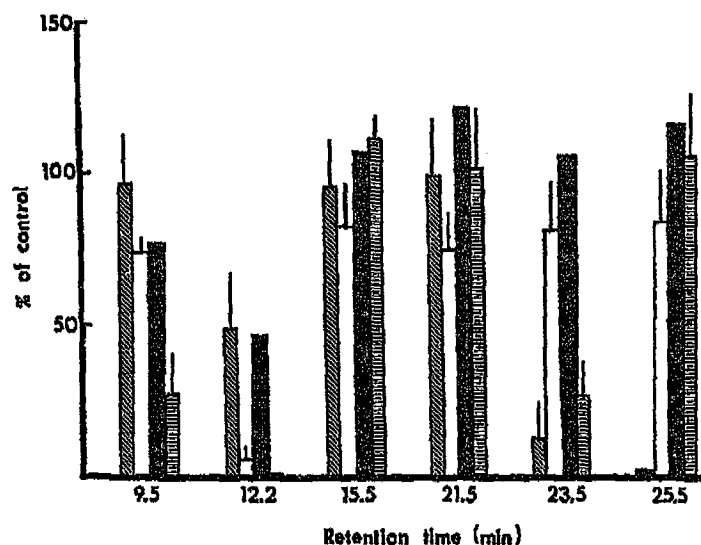


FIG. 7. Post-incubation hydrolysis of the metabolites produced by renal cortical tissue slices.

■, arylsulfatase (4-8 units). □, β -glucuronidase (500-1000 units); ■, heat 37°C, 24 hr; ▨, citric acid, pH 3.5-4.6. $N = 3$ except for heat where $N = 2$. Standard error bars are shown.

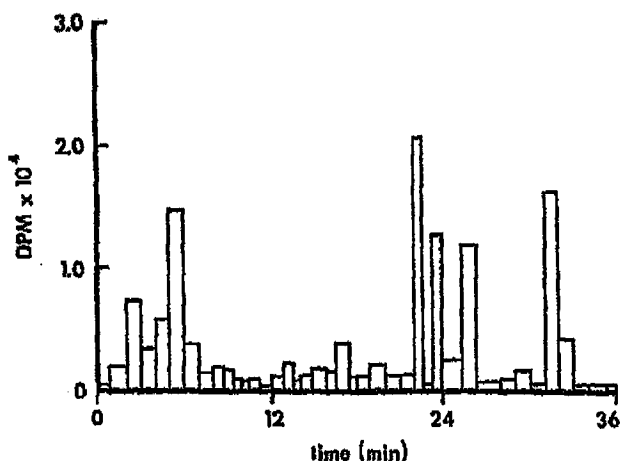


FIG. 8. Labeling of renal cortical metabolites with $\text{Na}_2^{35}\text{SO}_4$.

HPLC analysis of metabolites isolated following the incubation of cortical slices with MBOCA in the presence of $\text{Na}_2^{35}\text{SO}_4$. A plot of the LSS analysis of 1-min or peak fractions collected during a 35-min linear HPLC gradient from 23-42.5% ACN, 2.5 mM PIC A at 3 ml/min is shown. Compounds were chromatographed on a 5- μm radial compression Nova-pak column (Waters Associates).

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Syntheses of Guanidinoglycosides with the Inventive use of Mitsunobu Conditions and 1,8-Diazabicyclo[5.4.0]undec-7-ene

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Abstract: A series of novel guanidinoglycosides was successfully synthesized. This was accomplished with the use of Mitsunobu conditions as a strategy to convert the glycopyranose anomeric hydroxy group to give the corresponding substituted masked guanidines in high yields. Subsequent deprotection and coupling with Fmoc protected β -amino acid, afforded a series of N,N' -substituted-methylisothioureas. Cleavage of Fmoc followed by concomitant cyclization was achieved with a catalytic amount of DBU to give the guanidinoglycosides.

Key words: guanidine, glycosides, Mitsunobu reaction, coupling, cyclization

The significance of the guanidine moiety's role in many biologically active compounds cannot be disputed. Discovered in 1944, Streptomycin contains two essential guanidine groups at C-1 and C-3 and is an important member of the antibiotic family. It is active against gram-negative microbial infections and has beneficial effects in the treatment of infections caused by intracellular pathogens.² Over the years, numerous streptomycin analogs have been prepared and analogs without both guanidino groups were found to be inactive.³ Cotner and Smith performed extensive work on phosphotyrosine binding by ammonium and guanidinium-modified cyclodextrins for the inhibition of inappropriate mitogenic signaling.⁴ Meanwhile, Reitz et al. prepared a series of carbohydrate biguanidines as potential hypoglycemic agents and succeeded in achieving activities equivalent to those of Phenformin and Metformin.⁵ More recently, Goodman and co-workers synthesized a new class of guanidinylation reagents: N,N' -diBoc- and N,N' -diCBz- N'' -triflylguanidine,⁶ which efficiently guanidylate aryl-, alkyl-amines and amino acids to the corresponding guanidines in quantitative yields. The authors also used the reagents to convert aminoglycosides to their corresponding guanidinoglycosides, and demonstrated that these were able to inhibit replication of the HIV virus with good activities.⁷

Recently, our group reported the synthesis of novel guanidinoglycosides⁸ via aza-Wittig type reaction of iminophosphoranes.⁹ A series of β -glycosyl isothiocyanates was used as starting materials for the formation of glycosyl carbodiimides, which in turn underwent addition and spontaneous cyclization with β -amino acid methyl esters

in the presence of Et_3N to yield the desired products. However, with the former synthetic route, we were restricted to the forming of guanidinoglycosides that were di-substituted at the N-1 position. As such, we embarked on the quest of designing an alternative route to enable the synthesis of guanidinoglycosides that are mono-substituted at the N-3 position (the previously N-1 position). In this paper, we report the successful syntheses of these guanidinoglycosides with the inventive use of the Mitsunobu reaction and DBU in guanidine formation.

Classical Mitsunobu reactions entail the activation of an alcohol entity with triphenylphosphine and diethyl azodicarboxylate so as to allow the 'attack' of a nucleophile that results in an $\text{S}_{\text{N}}2$ -like reaction.¹⁰ Applying the theory, we envisaged that, with a powerful guanidinylation reagent, the anomeric hydroxy group of a 2,3,4,6-*O*-acetylated glycopyranose should undergo Mitsunobu reaction to yield the guanidinoglycosides. A search through the literature revealed that diurethane-protected thiopseudourea, diurethane- and triurethane-protected guanidines are excellent guanidinylation reagents, which react with primary or secondary alcohols under the Mitsunobu conditions to yield the (masked) guanidines.^{6b,11}

We have chosen 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea as the guanidinylation reagent for two reasons: it is commercially available and is relatively cheap (US\$39.10/5 g). As expected, the Mitsunobu reaction with 2,3,4,6-*O*-acetylated galacto- and gluco-pyranose proceeded with ease to yield 85 and 68% of 1a and 1b, respectively (Scheme 1). ¹H NMR spectra indicated that both purified glycosyl substituted thiopseudoureas 1a and 1b contained five ($\alpha_1:\alpha_2:\beta_1:\beta_2:\beta_3 = 1:1:0.5:0.9:0.5$) and four products ($\alpha_1:\alpha_2:\beta_1:\beta_2 = 1:0.5:1:1.5$), respectively. The α and β anomers were assigned based on the $J_{1,2}$ coupling constants. We suspected that these products were mixtures of rotomers since HRMS gave only the desired molecular peak. Boc-Deprotection with TFA (80%) gave 2a (59%, α only) and 2b (75%, $\alpha:\beta = 19:1$). The reaction was completed within 15 min at 0 °C. Since only the desired deBoc product was obtained from compound 1, it indicates that the initial mixtures were indeed the rotomers.¹²

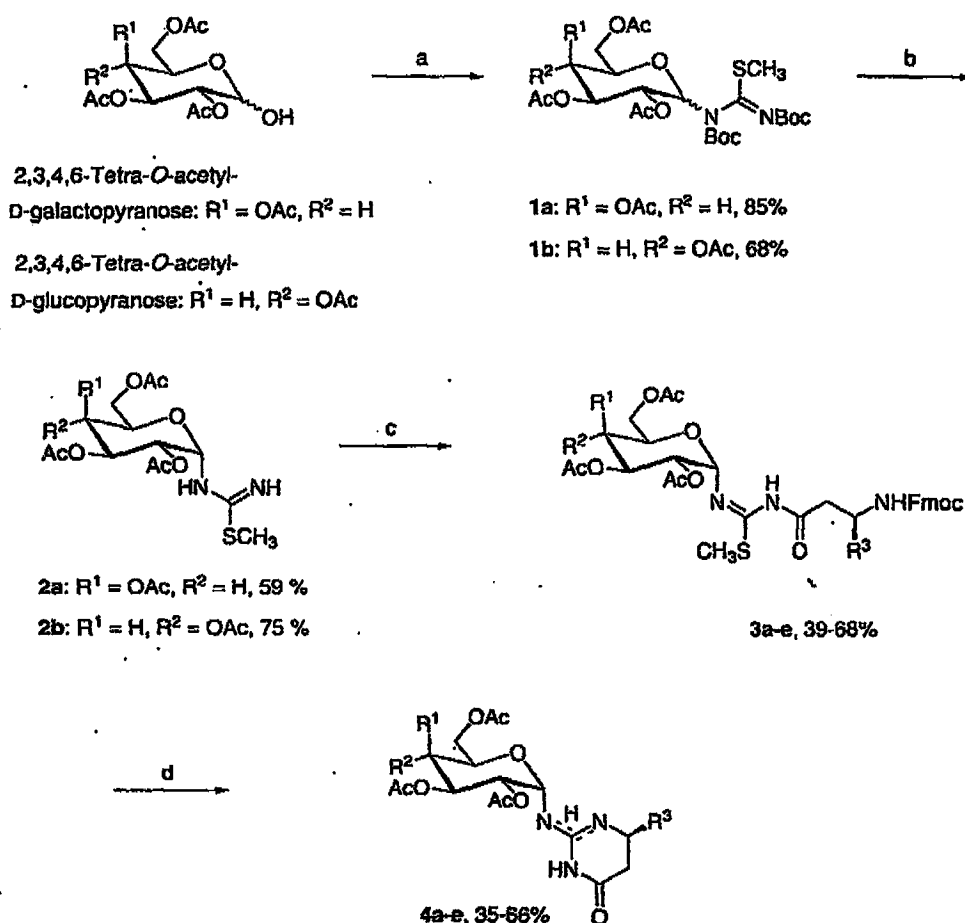
Standard coupling conditions were employed during the next step in the syntheses of disubstituted masked guanidines 3a-e (Table 1). It is important to note that the reaction, though it proceeded smoothly, did not reach completion. The progress of the reaction was monitored

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Scheme 1 Reagents and Conditions: (a) *N,N'*-BocNHC(SMe)NBoc, Ph₃P, DEAD, THF, 0 °C to r.t., 18 h; (b) TFA, CH₂Cl₂, anisole, 15 min, 0 °C; (c) Fmoc-protected β-amino acid, HOBt, DIC, DMA, *i*-Pr₂NEt, CH₂Cl₂, 0 °C to r.t., 24 h; (d) DBU, THF, r.t., 1 h.

by TLC and MS for 48 hours and observations indicated that the reaction typically reached a plateau after 24 hours. The pure isolated yields of 3a–e were in the range of 39–68% (Table 1). The coupling reactions of α-2a,b and Fmoc protected β-amino acids gave only the α-disubstituted masked guanidines 3a–e.

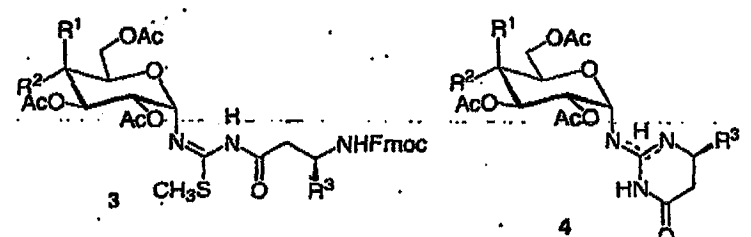
The next step was to unmask the guanidine by cleaving the Fmoc protecting group and cyclizing the free amine to yield the guanidinoglycoside. The classical way of cleaving Fmoc employs the use of large excess of secondary amine, such as piperidine in DMF. However, subsequent experiments have been carried out to show the superiority of DBU over other common reagents with Fmoc cleavages.¹³ Although DBU is commonly employed as a base for cyclization reactions,¹⁴ none was found using DBU as the reagent for the in situ cleavage of Fmoc and cyclization to form the guanidine moiety. We predicted that the use of DBU should not only cleave the Fmoc protecting group but also cause the in situ intramolecular cyclization to guanidinoglycoside 4. Using a catalytic amount of DBU (0.7% equiv), 3 was successfully converted to the desired guanidinoglycoside 4 (35–66%) within an hour. In some cases (4b–c,e), hydrolysis of the guanidine moiety at the anomeric position was observed to yield the 2,3,4,6-

O-acetylated glycopyranoses (17, 15 and 4%, respectively).

While performing HRMS, compounds 2–4 sometimes gave peaks that corresponded to (M + 45)⁺. For example, with 2a, on top of the desired molecular peak (HRMS calcd for C₁₆H₂₃N₂O₉S: 421.1281; found: 421.1282), an extra peak at 465.1176 (corresponding to C₁₇H₂₃N₂O₁₁S) was also observed. The extra peak was suspected to be a result of CO₂ trapped in the basic molecule. It was removed by bubbling argon gas through the sample solution prior to HRMS analysis in neutral conditions. The ¹³C NMR spectra of compounds 2–4 also gave an extra carbon peak at δ = 158–159. The peak disappeared when fresh samples were prepared.

We have also isolated two cyclized products (4c-I, 4c-II and 4d-I, 4d-II) from 4c and 4d, respectively. The more polar products 4-II show very broad signals in their ¹H NMR spectra and yet strong (M + H)⁺ HRMS signals. On the other hand, the less polar compounds 4-I gave a very strong (M + H + CO₂)⁺ HRMS signal. We suspected that products 4-I are actually the trapped CO₂ form of products 4-II. An experiment was thus carried out by stirring 4c-II with dry ice in THF. Compound 4c-II was found to slowly convert to 4c-I as monitored by TLC and ¹H NMR.

Table 1 Isolated Yields of Methylisothiourcas 3 and Guanidinoglycosides 4



	R ¹	R ²	R ³	Isolated Yields (%)	
				3	4
a	OAc	H	CH ₂ (4-CH ₃ C ₆ H ₄)	65	45
b	OAc	H	H	56	35
c	H	OAc	CH ₂ (4-CH ₃ C ₆ H ₄)	68	65
d	H	OAc	CH ₂ CH ₂ Ph	67	56
e	H	OAc	CH ₂ (4-NO ₂ C ₆ H ₄)	39	66

The chemical structures of compounds 3 and 4 were carefully studied using ¹H, ¹³C and 2D NMR spectroscopy. For example, the successful conversion of 3b to 4b was confirmed by the disappearance of the SCH₃ group (δ = 14.93 and 174.07) as well as the appearance of the guanidine group (δ = 161.24) from their ¹³C NMR spectra. Furthermore, a ¹H-¹³C HMBC experiment on 4b also showed a correlation between the anomeric proton and the guanidine carbon (C-2) but not the carbonyl carbon (C-4), thus indicating that the amide coupling occurred at the less hindered amine (Figure 1).

Similarly, the ¹H-¹³C HMBC correlation between the amide proton and carbonyl carbon confirmed the imino position of 3d. However, unlike our previously reported N-1 disubstituted guanidinoglycosides,⁸ we were unable to determine the location of the imino-group on the guanidine moiety of guanidinoglycosides 4 using ¹H-¹⁵N 2D NMR experiments.

In this report, we have described a synthetic route that not only complements our previous work but also provided another convenient and efficient way to synthesize novel 2-glycosylamino-dihydropyrimidinone for future biologi-

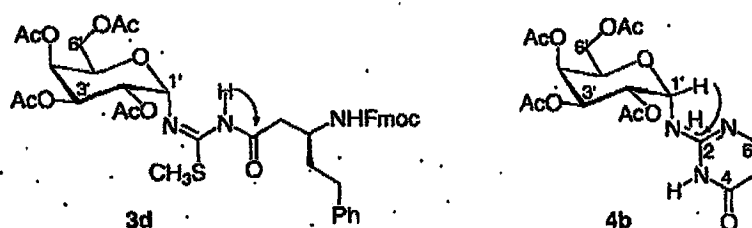
cal evaluation. More importantly, we have described two novel uses of Mitsunobu conditions and DBU, both of which are efficient and simple in application.

THF was distilled from sodium benzophenone ketyl prior to use. CH₂Cl₂ was distilled from CaH₂. Commercially available chemicals were used without further purification. Analytical TLC was carried out on precoated glass plates (Merck silica gel 60, F₂₅₄). Column chromatography was performed with silica gel (420–630 mesh) from Merck. All the NMR spectra were recorded with a Bruker Avance DMX 400 MHz instrument in CDCl₃ solutions and were calibrated using TMS as an internal standard. HRMS were determined using a Marina Biospectrometry workstation via ESI⁺.

Additional abbreviations: *N*-hydroxybenzotriazole (HOBt), *N,N*-dimethylacetamide (DMA), and 1,3-diisopropylcarbodiimide (DIC).

N-(2,3,4,6-Tetra-*O*-acetyl-D-galactopyranosyl)-*N,N'*-bis(*tert*-butoxycarbonyl)methylisothiourca (1a)

A mixture of Ph₃P (450 mg, 1.72 mmol) and 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (498 mg, 1.72 mmol) were dissolved in freshly distilled THF under argon. The mixture was cooled to below 0 °C with an ice/salt-bath, stirred for 10 min before DEAD (271.7 μL, 1.72 mmol) was added dropwise. After another

Figure 1 ¹H-¹³C HMBC correlations of methylisothiourca 3d and guanidinoglycoside 4b.

10 min, a solution of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose (500 mg, 1.43 mmol) in distilled THF was transferred to it via a delivery needle. The entire mixture was warmed up to r.t., stirred overnight and concentrated. Triphenylphosphine oxide was removed by precipitating the crude mixture in Et₂O. The filtrate was concentrated and purified using flash chromatography (33% EtOAc in hexane) to give pure 1a (750 mg, 85%, $\alpha_1:\alpha_2:\beta_1:\beta_2 = 1:1:0.5:0.9:0.5$) as a yellow, viscous syrup. Further separation of 1a into 1a-I and 1a-II was achieved by developing the preparative TLC 3 times (25% EtOAc in hexane).

From 1a-I ($\alpha_1, \alpha_2, \beta_1$)

α_1 Anomer

¹H NMR: $\delta = 6.34$ (d, 1 H, $J = 3.5$ Hz, H-1), 5.48 (dd, 1 H, $J = 3.0, 1.0$ Hz, H-4), 5.44–5.43 (overlapped with β_1 -H-4 and α_2 -H-3, 1 H, H-3), 5.28 (dd, 1 H, $J = 10.9, 3.4$ Hz, H-2), 4.38 (t, 1 H, $J = 6.7$ Hz, H-5), 4.11–4.01 (m, 2 H, H-6a, H-6b), 2.40 (s, 3 H, SCH₃), 2.11, 2.00, 1.98, 1.96 (4 s, 12 H, OAc), 1.47, 1.45 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 174.79, 170.35, 170.27, 170.17, 170.10, 159.07, 150.35, 91.50, 68.50, 67.50, 67.42, 66.79, 61.12, 27.91, 20.72, 14.80$.

α_2 Anomer

¹H NMR: $\delta = 6.18$ (d, 1 H, $J = 3.4$ Hz, H-1), 5.47 (dd, $J = 3.0, 1.3$ Hz, H-4), 5.44–5.43 (overlapped with α_1 -H-3 and β_1 -H-4, 1 H, H-3), 5.34 (dd, 1 H, $J = 11.0, 3.1$ Hz, H-2), 4.35 (t, 1 H, $J = 6.6$ Hz, H-5), 4.11–4.01 (m, 2 H, H-6a, H-6b), 2.40 (s, 3 H, SCH₃), 2.11, 2.00, 1.99, 1.95 (4 s, 12 H, OAc), 1.47, 1.45 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 170.77, 170.33, 170.09, 169.95, 153.36, 93.10, 68.81, 67.36, 67.18, 66.43, 61.12, 27.91, 20.72, 14.09$.

β_1 Anomer

¹H NMR: $\delta = 5.85$ (d, 1 H, $J = 6.2$ Hz, H-1), 5.80 (dd, 1 H, $J = 9.7, 3.5$ Hz, H-3), 5.44–5.43 (overlapped with α_1 -H-3 and α_2 -H-3, 1 H, H-4), 5.42 (dd, 1 H, $J = 9.6, 6.2$ Hz, H-2), 4.55 (dt, 1 H, $J = 6.4, 1.9$ Hz, H-5), 4.11–4.01 (m, 2 H, H-6a, H-6b), 2.38 (s, 3 H, SCH₃), 2.07, 2.02, 1.99, 1.95 (4 s, 12 H, OAc), 1.46 (s, 18 H, Boc).

¹³C NMR: $\delta = 170.39, 170.10, 169.96, 169.65, 161.79, 157.34, 151.97, 80.86, 71.20, 67.88, 67.09, 66.41, 61.63, 27.91, 20.72, 15.88$.

HRMS: m/z calcd for C₂₆H₄₁N₂O₁₃S (M + H)⁺: 621.2328; found: 621.2323.

From 1a-II (β_2, β_3)

β_2 Anomer

¹H NMR: $\delta = 5.66$ (d, 1 H, $J = 8.3$ Hz, H-1), 5.42–5.37 (m, 2 H, H-2, H-4), 5.06 (dd, 1 H, $J = 10.4, 3.4$ Hz, H-3), 4.13–4.10 (m, 2 H, H-6a, H-6b), 4.03 (t, 1 H, $J = 6.5$ Hz, H-5), 2.36 (s, 3 H, SCH₃), 2.09, 1.98, 1.97, 1.94 (4 s, 12 H, OAc), 1.44, 1.43 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 175.19, 170.30, 170.14, 170.03, 169.19, 158.90, 150.24, 94.06, 71.54, 71.01, 67.07, 66.78, 61.07, 27.94, 20.64, 20.59, 20.53, 15.58$.

β_3 Anomer

¹H NMR: $\delta = 5.92$ (t, 1 H, $J = 9.7$ Hz, H-2), 5.42–5.37 (overlapped with β_2 protons, 1 H, H-4), 4.99 (dd, 1 H, $J = 10.0, 3.4$ Hz, H-3), 4.94 (br d, 1 H, $J = 9.5$ Hz, H-1), 4.13–4.10 (m, 2 H, H-6a, H-6b), 3.94 (t, 1 H, $J = 6.6$ Hz, H-5), 2.33 (s, 3 H, SCH₃), 2.07, 2.00, 1.97, 1.92 (4 s, 12 H, OAc), 1.47 (s, 18 H, Boc).

¹³C NMR: $\delta = 170.35, 170.19, 170.07, 169.48, 160.63, 157.24, 150.45, 85.64, 72.88, 72.35, 67.74, 66.08, 61.16, 27.91, 20.97, 20.71$ (carbons overlapped with β_2), 14.71.

HRMS: m/z calcd for C₂₆H₄₁N₂O₁₃S (M + H)⁺: 621.2328; found: 621.2327.

N-(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl)-*N*'-bis(*tert*-butoxycarbonyl)methylisothiourea (1b)

Compound 1b (602 mg, 68%, $\alpha_1:\alpha_2:\beta_1:\beta_2 = 1:0.5:1:1.5$) was prepared as 1a from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (500 mg, 1.43 mmol).

α_1 Anomer

¹H NMR: $\delta = 6.29$ (d, 1 H, $J = 3.7$ Hz, H-1), 5.54 (t, 1 H, $J = 9.9$ Hz, H-3), 5.10 (t, 1 H, $J = 9.9$ Hz, H-4), 5.03 (dd, 1 H, $J = 9.7, 3.8$ Hz, H-2), 4.22 (dd, 1 H, $J = 12.4, 4.5$ Hz, H-6a), 4.18–4.12 (m, 1 H, H-5), 4.02 (dd, 1 H, $J = 12.5, 2.1$ Hz, H-6b), 2.39 (s, 3 H, SCH₃), 2.03, 1.97 (9), 1.97 (7), 1.96 (4 s, 12 H, OAc), 1.44, 1.43 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 174.87, 170.57, 170.12, 169.92, 169.47, 161.91, 90.81, 69.80, 69.46, 68.18, 67.81, 61.29, 27.85, 20.65, 20.54, 14.76$.

α_2 Anomer

¹H NMR: $\delta = 6.11$ (d, 1 H, $J = 3.6$ Hz, H-1), 5.45 (t, 1 H, $J = 10.0$ Hz, H-3), 5.09 (dd, 1 H, $J = 9.5, 4.0$ Hz, H-2), 5.05–5.00 (overlapped with other anomers, 1 H, H-4), 4.25–4.00 (overlapped with other anomers, H-5, H-6a, H-6b), 2.34 (s, 3 H, SCH₃), 2.03, 1.98 (3), 1.98 (0), 1.97 (4 s, 12 H, OAc), 1.45, 1.44 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 170.46, 169.91, 169.37, 169.32, 160.02, 153.13, 92.39, 69.75, 69.60, 69.14, 67.61, 61.23, 27.82, 20.70, 20.53, 14.03$.

β_1 Anomer

¹H NMR: $\delta = 5.75$ (t, 1 H, $J = 7.7$ Hz, H-3), 5.70 (d, 1 H, $J = 6.2$ Hz, H-1), 5.24 (dd, 1 H, $J = 7.8, 6.2$ Hz, H-2), 5.00 (dd, 1 H, $J = 9.5, 7.3$ Hz, H-4), 4.41 (ddd, 1 H, $J = 9.4, 4.0, 2.9$ Hz, H-5), 4.20 (dd, 1 H, $J = 12.2, 4.2$ Hz, H-6a), 4.07 (dd, 1 H, $J = 12.3, 2.6$ Hz, H-6b), 2.34 (s, 3 H, SCH₃), 2.01, 2.00, 1.97, 1.96 (4 s, 12 H, OAc), 1.44, 1.43 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 170.64, 169.66, 169.54, 169.48, 157.27, 151.86, 80.81, 71.55, 71.13, 68.62, 68.18, 61.95, 27.85, 20.70, 20.65, 15.78$.

β_2 Anomer

¹H NMR: $\delta = 5.70$ (t, 1 H, $J = 9.4$ Hz, H-3), 5.13 (t, 1 H, $J = 9.4$ Hz, H-2), 5.03 (t, 1 H, $J = 9.8$ Hz, H-4), 5.03 (d, 1 H, $J = 9.5$ Hz, H-1), 4.15 (dd, 1 H, $J = 12.4, 4.8$ Hz, H-6a), 4.09 (dd, 1 H, $J = 12.3, 2.4$ Hz, H-6b), 3.65 (ddd, 1 H, $J = 9.9, 4.6, 2.5$ Hz, H-5), 2.33 (s, 3 H, SCH₃), 2.01, 1.98, 1.96, 1.93 (4 s, 12 H, OAc), 1.45, 1.42 (2 s, 18 H, Boc).

¹³C NMR: $\delta = 170.50, 170.14, 169.40, 169.34, 157.09, 150.42, 84.75, 74.08, 73.92, 68.20, 67.81, 61.83, 27.89, 20.66, 20.54, 15.62$.

HRMS: m/z calcd for C₂₆H₄₁N₂O₁₃S (M + H)⁺: 621.2328; found: 621.2327.

N-(2,3,4,6-Tetra-*O*-acetyl- α -D-galactopyranosyl)methylisothiourea (2a)

To 1a (560 mg, 0.90 mmol) in distilled CH₂Cl₂ (1 mL) at 0 °C was added anisole (70 μ L, 0.64 mmol) and TFA (7 mL, 90 mmol). The solution was stirred for 15 min at 0 °C. The solvent was removed in vacuo, and the residue co-evaporated twice with hexane and then redissolved in MeOH. Powdered NaHCO₃ was added portionwise to neutralize the solution. The solution was filtered, concentrated and purified on a column (silica gel; 60% EtOAc in hexane) to afford pure 2a.

Yield: 223 mg (59%); colorless syrup.

¹H NMR: $\delta = 6.39$ (d, 1 H, $J = 3.7$ Hz, H-1), 5.52 (d, 1 H, $J = 1.3$ Hz, H-4), 5.50 (dd, 1 H, $J = 13.5, 3.2$ Hz, H-3), 5.31 (dd, 1 H, $J = 10.3, 3.7$ Hz, H-2), 4.45 (t, 1 H, $J = 6.7$ Hz, H-5), 4.12 (dd, 1 H, $J = 11.3, 7.2$ Hz, H-6a), 4.07 (dd, 1 H, $J = 11.2, 6.6$ Hz, H-6b), 2.49 (s, 3 H, SCH₃), 2.15, 2.04, 2.02, 1.99 (4 s, 12 H, OAc).

¹³C NMR: $\delta = 175.60, 170.38, 170.21, 170.05, 91.01, 68.31, 67.63, 67.51, 66.89, 61.20, 20.75, 20.67, 13.68$.

HRMS: calcd for $C_{16}H_{25}N_2O_9S$ ($M + H$)⁺: 421.1281; found: 421.1282.

***N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)methylisothiourea (2b)**

Compound 2b was prepared the same way as 2a from 1b (560 mg, 0.90 mmol).

Yield: 284 mg (75%); α : β = 19:1; colorless syrup. The α - and β -anomers were separated and characterized individually.

α Anomer

¹H NMR: δ = 6.34 (d, 1 H, J = 3.7 Hz, H-1), 5.62 (t, 1 H, J = 9.9 Hz, H-3), 5.15 (t, 1 H, J = 9.8 Hz, H-4), 5.07 (dd, 1 H, J = 10.3, 3.8 Hz, H-2), 4.28 (dd, 1 H, J = 12.3, 3.7 Hz, H-6a), 4.25–4.21 (m, 1 H, H-5), 4.07 (dd, 1 H, J = 12.3, 1.9, H-6b), 2.50 (s, 3 H, SCH₃), 2.08, 2.02, 2.01 (9), 2.01 (4 s, 12 H, OAc).

¹³C NMR: δ = 175.75, 170.73, 170.17, 170.10, 169.58, 90.36, 70.62, 69.61, 69.26, 68.00, 61.44, 20.70, 20.65, 20.62, 13.71.

HRMS: m/z calcd for $C_{16}H_{25}N_2O_9S$ ($M + H$)⁺: 421.1281; found: 421.1282.

β Anomer

¹H NMR: δ = 5.29 (t, 1 H, J = 9.4 Hz, H-3), 5.08 (t, 1 H, J = 9.7 Hz, H-4), 5.02–4.99 (m, 2 H, H-1, H-2), 4.24 (br d, 1 H, J = 11.8 Hz, H-6a), 4.13 (br d, 1 H, J = 12.4, H-6b), 3.81 (ddd, 1 H, J = 10.1, 4.5, 2.5 Hz, H-5), 2.34 (s, 3 H, SCH₃), 2.07, 2.04, 2.03, 2.01 (4 s, 12 H, OAc).

¹³C NMR: δ = 170.68, 170.19, 169.50, 162.80, 83.20, 73.24, 72.97, 71.34, 68.31, 61.95, 20.74, 20.69, 20.62, 20.59, 13.0.

HRMS: m/z calcd for $C_{16}H_{25}N_2O_9S$ ($M + H$)⁺: 421.1281; found: 421.1282.

Compounds 3; General Procedures

To a solution of Fmoc-protected β -amino acid (1.1 equiv) and HOBT (1.1 equiv) in DMA (0.6 mL) and CH₂Cl₂ (5 mL) was added DIC (1.1 equiv). It was stirred for 10 min before being added to an ice-cooled solution of 2 (1 equiv) and *i*-Pr₂NEt (1.1 equiv) in CH₂Cl₂ (5 mL). The resulting solution was allowed to stir at r.t. for 24 h. The mixture was concentrated and by-product urea was removed by precipitation using hexane. Purification of the residue on preparative TLC (50% EtOAc in hexane) afforded pure 3.

***(S)*-*N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-galactopyranosyl)-*N'*-[2-(9-fluorenylmethylcarbonyl)amino-3-(4-methylphenyl)propylcarbonyl]methylisothiourea (3a)**

Compound 3a was prepared from 2a (64 mg, 0.15 mmol) and Fmoc-(*S*)-3-amino-4-(4-methylphenyl)butyric acid (70 mg, 0.17 mmol).

Yield: 80 mg (65%); white foam.

¹H NMR: δ = 12.12 (s, 1 H, NHCO), 7.69 (d, 2 H, J = 7.5 Hz, Fmoc), 7.49–7.47 (m, 2 H, Fmoc), 7.33 (t, 2 H, J = 7.4 Hz, Fmoc), 7.23 (t, 2 H, J = 7.4 Hz, Fmoc), 7.09–6.92 (m, 4 H, C₆H₄), 6.31 (br s, 1 H, H-1), 5.46 (br s, 1 H, H-4), 5.41 (br d, 1 H, J = 11.1 Hz, H-3), 5.26 (dd, 1 H, J = 10.7, 3.2 Hz, H-2), 5.22 (d, 1 H, J = 10.2 Hz, FmocNH), 4.35 (t, 1 H, J = 6.4 Hz, H-5), 4.35–4.24 (m, 2 H, OCH₂CH), 4.20–4.08 (m, 2 H, NCH, OCH₂CH), 4.08–3.98 (m, 2 H, H-6a, H-6b), 2.94–2.75 (m, 2 H, CH₂C₆H₄), 2.65–2.45 (m, 2 H, CH₂CO), 2.39 (s, 3 H, SCH₃), 2.24 (s, 3 H, CH₃C₆H₄), 2.09, 1.96, 1.94 (3 s, 12 H, OAc).

¹³C NMR: δ = 174.04, 170.31, 170.11, 169.65, 155.60, 143.82, 141.27, 136.53, 133.90, 129.43, 129.04, 127.64, 127.00, 125.04, 119.93, 91.64, 68.55, 67.39, 67.33, 66.66, 61.02, 49.39, 47.18, 39.95, 39.44, 21.01, 20.68, 20.63, 14.93.

HRMS: m/z calcd for $C_{42}H_{48}N_3O_{12}S$ ($M + H$)⁺: 818.2959; found: 818.2960.

***(S)*-*N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-galactopyranosyl)-*N'*-[2-(9-fluorenylmethylcarbonyl)aminoethylcarbonyl]methylisothiourea (3b)**

Compound 3b was prepared from 2a (95 mg, 0.23 mmol) and Fmoc- β -alanine (77 mg, 0.25 mmol).

Yield: 90 mg (56%); white foam.

¹H NMR: δ = 12.20 (br s, 1 H, NHCO), 7.69 (d, 2 H, J = 7.5 Hz, Fmoc), 7.51 (d, 2 H, J = 7.3 Hz, Fmoc), 7.33 (t, 2 H, J = 7.5 Hz, Fmoc), 7.24 (t, 2 H, J = 7.3 Hz, Fmoc), 6.32 (d, 1 H, J = 3.1 Hz, H-1), 5.46 (br s, 1 H, H-4), 5.45–5.35 (m, 1 H, H-3), 5.29–5.23 (m, 2 H, NHFmoc, H-2), 4.33 (d, 2 H, J = 6.8 Hz, OCH₂CH), 4.38–4.32 (hidden, 1 H, H-5), 4.18–4.10 (m, 1 H, OCH₂CH), 4.08–4.01 (m, 2 H, H-6), 3.48–3.41 (m, 2 H, NCH₂), 2.40 (br s, 2 H, CH₂CO), 2.09 (s, 3 H, SCH₃), 1.97, 1.95, 1.94 (3 s, 12 H, OAc).

¹³C NMR: δ = 174.07, 170.32, 170.13, 156.29, 143.81, 141.28, 127.68, 127.01, 125.01, 119.96, 91.73, 68.59, 67.38, 67.32, 66.71, 61.01, 47.18, 36.97, 35.89, 20.64, 14.93.

HRMS: m/z calcd for $C_{34}H_{40}N_3O_{12}S$ ($M + H$)⁺: 714.2332; found: 714.2333.

***(S)*-*N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-*N'*-[2-(*N*-fluorenylmethylcarbonyl)amino-3-(4-methylphenyl)propylcarbonyl]methylisothiourea (3c)**

Compound 3c was prepared from 2b (100 mg, 0.24 mmol) and Fmoc-(*S*)-3-amino-4-(4-methylphenyl)butyric acid (110 mg, 0.26 mmol).

Yield: 133 mg (68%); white foam.

¹H NMR: δ = 12.12 (br s, 1 H, NH), 7.69 (d, 2 H, J = 7.6 Hz, Fmoc), 7.49–7.47 (m, 2 H, Fmoc), 7.33 (t, 2 H, J = 7.4 Hz, Fmoc), 7.24 (t, 2 H, J = 7.4 Hz, Fmoc), 7.06–6.98 (m, 4 H, C₆H₄), 6.27 (d, 1 H, J = 2.6 Hz, H-1), 5.54 (t, 1 H, J = 9.8 Hz, H-3), 5.20 (d, 1 H, J = 8.1 Hz, NH), 5.10 (t, 1 H, J = 9.9 Hz, H-4), 5.03 (dd, 1 H, J = 10.3, 3.5 Hz, H-2), 4.29 (br s, 2 H, OCH₂CH), 4.22 (dd, 1 H, J = 12.6, 3.5 Hz, H-6a), 4.13–4.12 (m, 3 H, H-5, NCH, OCH₂CH), 4.01 (br d, 1 H, J = 13.4 Hz, H-6b), 2.94–2.75 (m, 2 H, CH₂C₆H₄), 2.65–2.46 (m, 2 H, CH₂CO), 2.41 (s, 3 H, SCH₃), 2.24 (s, 3 H, C₆H₄CH₃), 2.01, 1.98, 1.97, 1.96 (4 s, 12 H, OAc).

¹³C NMR: δ = 174.26, 170.62, 170.19, 169.95, 169.67, 169.47, 159.00, 155.62, 143.84, 141.29, 136.56, 133.91, 129.45, 129.05, 127.66, 127.02, 125.06, 119.94, 91.00, 69.82, 69.57, 69.41, 67.83, 66.65, 61.29, 49.41, 47.20, 40.00, 39.45, 21.02, 20.68, 20.58, 14.98.

HRMS: m/z calcd for $C_{42}H_{48}N_3O_{12}S$ ($M + H$)⁺: 818.2960; found: 818.2947.

***(S)*-*N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-*N'*-[2-(9-fluorenylmethylcarbonyl)amino-4-phenylbutylcarbonyl]methylisothiourea (3d)**

Compound 3d was prepared from 2b (130 mg, 0.31 mmol) and Fmoc-(*S*)-3-amino-5-phenylpentanoic acid (142 mg, 0.34 mmol).

Yield: 169 mg (67%); white foam.

¹H NMR: δ = 12.10 (s, 1 H, NHCO), 7.66 (d, 2 H, J = 7.4 Hz, Fmoc), 7.50 (d, 2 H, J = 7.0 Hz, Fmoc), 7.29 (dd, 2 H, J = 10.9, 7.2 Hz, Ph), 7.21 (t, 2 H, J = 7.3 Hz, Fmoc), 7.18 (t, 2 H, J = 7.0 Hz, Fmoc), 7.10–7.04 (m, 3 H, Ph), 6.26 (d, 1 H, J = 3.1 Hz, H-1), 5.53 (t, 1 H, J = 9.9 Hz, H-3), 5.28 (d, 1 H, J = 9.6 Hz, FmocNH), 5.09 (t, 1 H, J = 9.9 Hz, H-4), 5.01 (dd, 1 H, J = 10.0, 3.4 Hz, H-2), 4.33 (d, 2 H, J = 6.2 Hz, OCH₂CH), 4.19 (dd, 1 H, J = 12.7, 3.5 Hz, H-6a), 4.13–4.11 (m, 2 H, OCH₂CH, H-5), 3.98 (br d, 1 H, J = 11.1 Hz, H-6b), 3.94–3.91 (m, 1 H, NHCH), 2.65–2.50 (m, 4 H, CH₂CO).

CH_2Ph), 2.35 (s, 3 H, SCH_3), 1.98, 1.95, 1.94, 1.93 (4 s, 12 H, OAc), 1.92–1.75 (m, 2 H, $\text{CH}_2\text{CH}_2\text{Ph}$).

^{13}C NMR: δ = 174.02, 170.48, 170.05, 169.83, 169.41, 169.34, 155.68, 143.74, 143.65, 141.16, 140.76, 128.35, 128.20, 127.54, 126.89, 125.96, 124.89, 124.77, 119.82, 90.84, 69.69, 69.42, 69.27, 67.69, 66.38, 61.16, 47.85, 47.11, 41.57, 35.61, 32.35, 20.54, 20.45, 14.80.

HRMS: m/z calcd for $\text{C}_{42}\text{H}_{48}\text{N}_3\text{O}_{12}\text{S}$ ($M + \text{H}$) $^+$: 818.2959; found: 818.2951.

(*S*)-*N*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-*N'*-(2-(9-fluorenylmethylcarbonyl)amino-3-(4-nitrophenyl)propylcarbonyl)methylisothiourea (3e)

Compound 3e was prepared from 2b (130 mg, 0.31 mmol) and Fmoc-(*S*)-3-amino-4-(4-nitrophenyl)-butyric acid (152 mg, 0.34 mmol).

Yield: 102 mg (39%); white foam.

^1H NMR: δ = 12.19 (s, 1 H, NHCO), 8.07 (d, 2 H, J = 7.6 Hz, C_6H_4), 7.69 (d, 2 H, J = 7.5 Hz, Fmoc), 7.45 (br s, 2 H, Fmoc), 7.33 (t, 2 H, J = 7.5 Hz, Fmoc), 7.31–7.23 (m, 4 H, Fmoc, C_6H_4), 6.29 (br s, 1 H, H-1), 5.54 (t, 1 H, J = 9.4 Hz, H-3), 5.20 (br s, 1 H, Fmoc-NH), 5.11 (t, 1 H, J = 9.9 Hz, H-4), 5.03 (br d, 1 H, J = 7.2 Hz, H-2), 4.33 (br s, 2 H, OCH_2CH), 4.23 (dd, 1 H, J = 12.5, 3.2 Hz, H-6a), 4.15–4.12 (m, 3 H, H-5, CHC_6H_4 , OCH_2CH), 4.02 (br d, 1 H, J = 12.1 Hz, H-6b), 3.07–2.90 (m, 2 H, $\text{CH}_2\text{C}_6\text{H}_4$), 2.72–2.55 (m, 2 H, CH_2CO), 2.42 (s, 3 H, SCH_3), 2.02, 1.98, 1.97 (3 s, 12 H, OAc).

^{13}C NMR: δ = 174.11, 170.62, 170.20, 169.94, 169.45, 169.19, 155.50, 146.95, 145.06, 143.63, 141.33, 130.00, 127.75, 127.02, 124.89, 123.88, 120.01, 91.06, 69.77, 69.60, 69.42, 67.78, 66.49, 61.23, 48.99, 47.14, 40.24, 39.50, 20.67, 20.57, 15.00.

HRMS: m/z calcd for $\text{C}_{41}\text{H}_{45}\text{N}_4\text{O}_{12}\text{S}$ ($M + \text{H}$) $^+$: 849.2653; found: 849.2659.

Compounds 4; General Procedures

To 3 (1 equiv) in freshly distilled THF (0.5 mL) was added DBU (0.7% equiv). The mixture was stirred at r.t. for 1 h. The mixture was concentrated and purified on preparative TLC (33% EtOAc in hexane) to afford pure 4.

(*S*)-2-(2',3',4',6'-Tetra-*O*-acetyl- α -D-galactopyranosyl)amino-6-(4-methylbenzyl)-5,6-dihydro-4-pyrimidinone (4a)

Compound 4a was prepared from 3a (20 mg, 0.025 mmol).

Yield: 6 mg (45%); white foam.

^1H NMR: δ = 7.10 (d, 2 H, J = 7.8 Hz, C_6H_4), 7.00 (d, 2 H, J = 8.0 Hz, C_6H_4), 6.23 (d, 1 H, J = 3.7 Hz, H-1'), 5.42 (dd, 1 H, J = 3.2, 1.1 Hz, H-4'), 5.38 (dd, 1 H, J = 10.9, 3.3 Hz, H-3'), 5.24 (dd, 1 H, J = 10.8, 3.7 Hz, H-2'), 4.37 (t, 1 H, J = 6.8 Hz, H-5'), 4.02 (d, 2 H, J = 6.6 Hz, H-6a', H-6b'), 3.92–3.86 (m, 1 H, H-6), 2.86 (dd, 1 H, J = 13.8, 6.1 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.78 (dd, 1 H, J = 13.8, 7.7 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.70 (dd, 1 H, J = 16.8, 5.1 Hz, H-5a), 2.52 (dd, 1 H, J = 16.8, 9.6 Hz, H-5b), 2.27 (s, 3 H, $\text{C}_6\text{H}_4\text{CH}_3$), 2.08, 1.98, 1.94, 1.91 (4 s, 12 H, OAc).

^{13}C NMR: δ = 170.36, 170.27, 170.18, 169.79, 167.27, 161.79, 137.49, 131.54, 129.89, 128.99, 90.76, 68.25, 67.63, 67.37, 66.76, 61.13, 50.16, 40.47, 35.78, 21.07, 20.78, 20.68, 20.63.

HRMS: m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_{10}$ ($M + \text{H}$) $^+$: 548.2245; found: 548.2422.

(*S*)-2-(2',3',4',6'-Tetra-*O*-acetyl- α -D-galactopyranosyl)amino-5,6-dihydro-4-pyrimidinone (4b)

Compound 4b was prepared from 3b (50 mg, 0.07 mmol).

Yield: 11 mg (35%); colorless foam.

^1H NMR: δ = 6.22 (d, 1 H, J = 3.5 Hz, H-1'), 5.40 (dd, 1 H, J = 3.2, 1.3 Hz, H-4'), 5.36 (dd, 1 H, J = 10.9, 3.3 Hz, H-3'), 5.22 (dd, 1 H, J = 10.9, 3.5 Hz, H-2'), 4.63 (t, 1 H, J = 6.7 Hz, H-5'), 4.05 (d, 2 H, J = 6.7 Hz, H-6'a, H-6'b), 3.69 (ddd, 1 H, J = 13.2, 10.5, 5.4 Hz, H-6a), 3.58 (dt, 1 H, J = 13.1, 6.4 Hz, H-6b), 2.75 (dt, 1 H, J = 16.9, 5.4 Hz, H-5a), 2.66 (ddd, J = 16.9, 10.4, 6.5 Hz, H-5b), 2.09, 1.98, 1.97, 1.90, (4 s, 12 H, OAc).

^{13}C NMR: δ = 170.49, 170.25, 169.86, 168.62, 161.92, 90.70, 68.19, 68.11, 66.93, 61.36, 37.04, 30.42, 20.82, 20.75, 20.67, 20.63.

HRMS: m/z calcd for $\text{C}_{18}\text{H}_{26}\text{N}_3\text{O}_{10}$ ($M + \text{H}$) $^+$: 444.1618; found: 444.1614.

(*S*)-2-(2',3',4',6'-Tetra-*O*- α -acetyl- α -D-glucopyranosyl)amino-6-(4-methylbenzyl)-5,6-dihydro-4-pyrimidinone (4c)

Compound 4c-I (8.4 mg, 31%, colorless foam) and 4c-II (9 mg, 34%, colorless foam) were prepared from 3c (40 mg, 0.05 mmol).

Compound 4c-I

^1H NMR: δ = 7.15 (d, 2 H, J = 7.8 Hz, C_6H_4), 7.06 (d, 2 H, J = 8.0 Hz, C_6H_4), 6.26 (d, 1 H, J = 3.8 Hz, H-1'), 5.56 (t, 1 H, J = 9.9 Hz, H-3'), 5.14 (t, 1 H, J = 9.9 Hz, H-4'), 5.07 (dd, 1 H, J = 10.3, 3.8 Hz, H-2'), 4.26 (dd, 1 H, J = 12.5, 3.5 Hz, H-6a'), 4.18 (dt, 1 H, J = 10.3, 2.7 Hz, H-5'), 4.05 (dd, 1 H, J = 12.5, 2.1 Hz, H-6b'), 3.97–3.91 (m, 1 H, H-6), 2.92 (dd, 1 H, J = 13.8, 6.1 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.83 (dd, 1 H, J = 13.8, 7.8 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.76 (dd, 1 H, J = 16.8, 5.1 Hz, H-5a), 2.57 (dd, 1 H, J = 16.8, 9.6 Hz, H-5b), 2.33 (s, 3 H, $\text{C}_6\text{H}_4\text{CH}_3$), 2.07, 2.03, 2.00, 1.99 (4 s, 12 H, OAc).

^{13}C NMR: δ = 170.70, 170.03, 170.00, 169.59, 167.16, 161.59, 137.54, 131.47, 129.90, 128.98, 90.12, 69.96, 69.46, 69.24, 67.87, 61.35, 50.17, 40.46, 35.79, 21.07, 20.71, 20.67, 20.57.

HRMS: m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_{10}$ ($M + \text{H}$) $^+$: 548.2246; found: 548.2243.

Compound 4c-II

^1H NMR: δ = 7.09 (d, 2 H, J = 7.5 Hz, C_6H_4), 6.97 (d, 2 H, J = 7.8 Hz, C_6H_4), 5.61 (br s, 1 H, NH), 5.44 (br s, 1 H, H-3'), 5.23 (br s, 1 H, H-1'), 4.94 (t, 1 H, J = 9.7 Hz, H-4'), 4.87 (br d, 1 H, J = 6.5 Hz, H-2'), 4.00 (br d, 1 H, J = 10.7 Hz, H-6a'), 3.82–3.72 (br m, 1 H, H-5'), 3.75–3.65 (br m, 1 H, H-6), 3.38 (br d, 1 H, J = 11.8 Hz, H-6b'), 2.82 (br d, 1 H, J = 11.1 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.65 (br d, 1 H, J = 15.6 Hz, H-5a), 2.63–2.57 (m, 1 H, $\text{CH}_2\text{C}_6\text{H}_4$), 2.40 (dd, 1 H, J = 16.7, 9.5 Hz, H-5b), 2.25 (s, 3 H, $\text{C}_6\text{H}_4\text{CH}_3$), 2.02, 1.99, 1.95, 1.93 (4 s, 12 H, OAc).

^{13}C NMR: δ = 170.35, 170.13, 169.98, 169.60, 137.29, 132.60, 129.83, 128.88, 79.91, 70.65, 70.09, 68.38, 67.48, 61.86, 50.85, 41.04, 36.85, 20.97, 20.84, 20.68, 20.59.

HRMS: m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_{10}$ ($M + \text{H}$) $^+$: 548.2246; found: 548.2247.

(*S*)-2-(2',3',4',6'-Tetra-*O*-acetyl- α -D-glucopyranosyl)amino-6-ethylphenyl-5,6-dihydro-4-pyrimidinone (4d)

Compound 4d-I (11.2 mg, 34%, white foam) and 4d-II (7.2 mg, 22%, white foam) were prepared from 3d (50 mg, 0.06 mmol).

Compound 4d-I

^1H NMR: δ = 7.25 (t, 2 H, J = 7.3 Hz, Ph), 7.18 (t, 1 H, J = 7.4 Hz, Ph), 7.11 (d, 2 H, J = 7.0 Hz, Ph), 6.23 (d, 1 H, J = 3.8 Hz, H-1'), 5.52 (t, 1 H, J = 9.9 Hz, H-3'), 5.10 (t, 1 H, J = 9.8 Hz, H-4'), 5.02 (dd, 1 H, J = 10.3, 3.8 Hz, H-2'), 4.23 (dd, 1 H, J = 12.3, 3.5 Hz, H-6a'), 4.21–4.17 (m, 1 H, H-5'), 4.02 (dd, 1 H, J = 12.2, 1.7 Hz, H-6b'), 3.68–3.61 (m, 1 H, H-6), 2.75 (dd, 1 H, J = 16.8, 5.4 Hz, H-5a), 2.70–2.63 (m, 2 H, CH_2Ph), 2.50 (dd, 1 H, J = 16.8, 8.4 Hz, H-5b), 2.02, 1.97, 1.95, 1.94 (4 s, 12 H, OAc) 1.97–1.82 (m, 2 H, $\text{CH}_2\text{CH}_2\text{Ph}$).

^{13}C NMR: δ = 170.71, 170.01, 169.92, 169.56, 167.32, 161.92, 139.19, 128.86, 128.19, 126.71, 90.18, 69.93, 69.47, 69.23, 67.86, 61.37, 47.78, 35.93, 35.83, 31.29, 20.73, 20.66, 20.58.

HRMS: m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_{10}$ ($M + H$) $^+$: 548.2245; found: 548.2248.

Compound 4d-II

^1H NMR: δ = 7.24 (t, 2 H, J = 7.3 Hz, Ph), 7.16 (t, 1 H, J = 7.3 Hz, Ph), 7.10 (d, 2 H, J = 7.3 Hz, Ph), 5.68 (br s, 1 H, NH), 5.48 (t, 1 H, J = 9.8 Hz, H-3'), 5.22 (d, 1 H, J = 4.3 Hz, H-1'), 4.98 (t, 1 H, J = 9.7 Hz, H-4'), 4.89 (dd, 1 H, J = 10.3, 4.0 Hz, H-2'), 4.08–4.03 (m, 2 H, H-6a', H-6b'), 3.94–3.91 (m, 1 H, H-5'), 3.56–3.50 (m, 1 H, H-6), 2.66–2.62 (m, 3 H, CH_2Ph , H-5a), 2.38 (dd, 1 H, J = 16.3, 7.6 Hz, H-5b), 2.11, 1.96, 1.95 (3 s, 12 H, OAc), 1.88–1.79 (m, 2 H, $\text{CH}_2\text{CH}_2\text{Ph}$).

^{13}C NMR: δ = 170.43, 170.19, 169.99, 169.68, 139.82, 128.78, 128.21, 126.57, 79.46, 70.47, 70.13, 68.72, 67.70, 62.33, 48.46, 36.43, 36.28, 31.55, 20.83, 20.69, 20.64.

HRMS: m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_{10}$ ($M + H$) $^+$: 548.2246; found: 548.2245.

(S)-2-(2',3',4',6'-Tetra-O-acetyl- α -D-glucopyranosyl)amino-6-(4-nitrobenzyl)-5,6-dihydro-4-pyrimidinone (4e)

Compound 4e was prepared from 3e (20 mg, 0.024 mmol).

Yield: 9 mg (66%); white foam.

^1H NMR: δ = 8.18 (d, 2 H, J = 8.6 Hz, C_6H_4), 7.23 (d, 2 H, J = 8.6 Hz, C_6H_4), 6.19 (d, 1 H, J = 3.7 Hz, H-1'), 5.51 (t, 1 H, J = 9.9 Hz, H-3'), 5.09 (t, 1 H, J = 9.9 Hz, H-4'), 5.01 (dd, 1 H, J = 10.3, 3.8 Hz, H-2'), 4.20 (dd, 1 H, J = 12.5, 3.4 Hz, H-6a'), 4.16–4.11 (m, 1 H, H-5'), 4.00 (dd, 1 H, J = 12.6, 2.1 Hz, H-6b'), 4.01–3.95 (m, 1 H, H-6), 3.00 (d, 2 H, J = 6.9 Hz, $\text{CH}_2\text{C}_6\text{H}_4$), 2.76 (dd, 1 H, J = 16.7, 5.3 Hz, H-5a), 2.54 (dd, 1 H, J = 16.7, 8.6 Hz, H-5b), 2.01, 1.97, 1.95, 1.94 (4 s, 12 H, OAc).

^{13}C NMR: δ = 170.67, 169.97, 169.92, 169.51, 166.35, 157.65, 147.60, 142.13, 130.13, 124.42, 90.34, 69.87, 69.44, 69.34, 67.82, 61.31, 49.63, 40.59, 35.57, 20.71, 20.65, 20.58.

HRMS: m/z calcd for $\text{C}_{25}\text{H}_{31}\text{N}_4\text{O}_{12}$ ($M + H$) $^+$: 579.1939; found: 579.1934.

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(54) C-GLYCOSIDES AND PREPARATION OF THEREOF AS ANTIDIABETIC AGENTS

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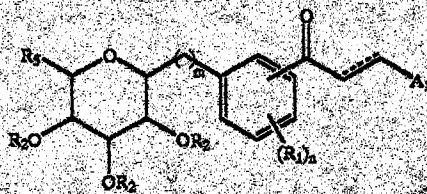
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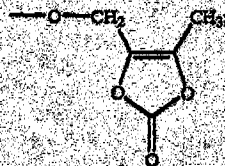
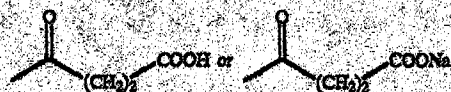
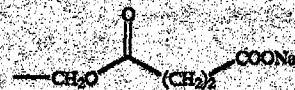
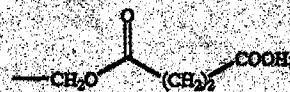
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(57) ABSTRACT

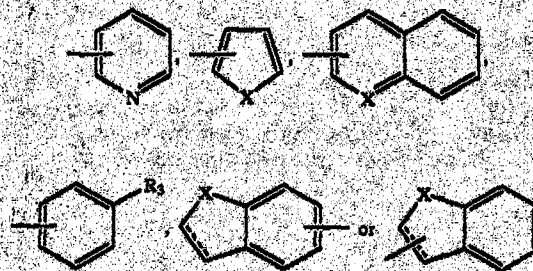
C-glycoside derivatives are disclosed, which are represented by the following formula (I) and its pharmaceutical acceptable salt, and which are useful for the treatment and/or prevention of diabetes and hypoglycemia.



wherein: with the provisos that

R₁ is H, OH, lower alkyl, O-lower alkyl orR₂ is H, —COO-lower alkyl,R₃ is —CH₂OH, —CH₂OCO₂-lower alkyl,—CH₂OSO₃H, —COOH or —COONa;

wherein: A is



(with the provisos that X is oxygen atom, nitrogen atom or sulfur atom R₃ is lower alkyl when is 1, R₃ is including —OH, —O-lower alkyl, . . . means saturated or unsaturated carbon bond;

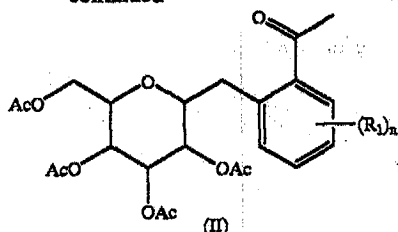
m is 0 or 1;

n is 0, 1 or 2;

above mentioned-lower alkyl means C₁-C₃;

or a pharmaceutical acceptable salt.

-continued



[0061] As pharmaceutical acceptable salts of a compound of general formula (I), sodium salt, potassium salt and inorganic base are mentioned.

[0062] In case of A₁ contains pyridine base, salts of inorganic and organic acids are mentioned. As the salt of inorganic acid, hydrochloride and sulfate are mentioned. As the salt of organic acid, acetate, succinate and fumarate are mentioned.

[0063] A compound of general formula (I) can be used itself or formulated to pharmaceutical product such as powder, granule, tablet and capsule by known pharmaceutical technology and can be orally administrable. It can be also administered not orally such as direct administration to rectal and in the form of injection. An effective dosage of the compound is from 10 to 1000 mg once to several times a day for adults, through it may be adjusted depending an age, a body weight and symptoms.

[0064] PHARMACOLOGICAL EXPERIMENT

[0065] The pharmacological test of urinary glucose excretion in rat is described that follow.

[0066] Measurement of Urinary Glucose Excretion in Rats

[0067] 20 mg of test compounds were dissolved in 10 mL saline containing 20% dimethylsulfoxide.

[0068] Test compound (10 mg/5 mL/kg, i.p.) was administered twice an 8 hr interval to male SD rats (6 weeks old, 3 animals/group). An equal volume of vehicle was given to the control group. Urine was collected for 24 hr after first administration in metabolic cages.

[0069] The urine was centrifuged and urine volume was measured. Glucose concentration (mg/dl) was assayed using Glucose CII test Wako (Wako Pure Chemicals, Japan). The amounts of glucose excretion during 24 hr was calculated from the following equation.

$$[0070] \text{ Glucose secretion (mg/24hr)} = (A \times B) / 100$$

[0071] (A; the urine volume, B; the glucose concentration)

[0072] The result are shown in Table 8.

TABLE 8

Compound	Dose (mg/kg)	Urinary glucose excretion (mg/24 hr)
1	10	4.1
3	10	241
4	10	289

TABLE 8-continued

Compound	Dose (mg/kg)	Urinary glucose excretion (mg/24 hr)
5	10	228
6	10	89
22	10	124

EXAMPLE

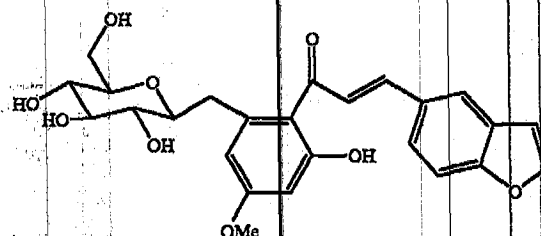
[0073] The following Example are provided only for the purpose of the preparation of the compound and not restrict the disclosed invention. The following compound 1-31 and 42-58 correspond to that of table 1-7.

Example 1

3-(Benzo[b]furan-5-yl)-1-(2'-β-D-glucopyranosylmethyl-6'-hydroxy-4'-methoxy)acrylophenone

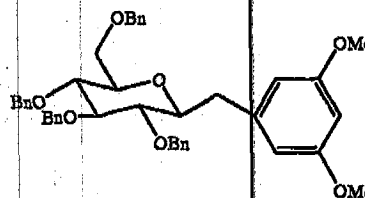
[0074]

(Compound 1)



[0075] (a) 1-(3,5-Dimethoxyphenylmethyl)-1-deoxy-2,3,4,6-tetra-O-benzyl-β-D-glucopyranose

(Compound 33)



[0076] To 2,6-Anhydro-3,4,5,7-tetra-O-benzyl-1-deoxy-D-glucohept-1-enitol (2.0 g, XII), obtained from tetra benzylgluconolactone (XI) by Tebbe reaction, was added 9-BBN (0.5M in THF) at room temperature. After refluxing for 7 hr, the reaction mixture was cooled to room temperature and 3M K₃PO₄ (3.3 mL) was added. This was followed by the addition of 1,3-dimethoxyiodobenzene (1.2 g), and PdCl₂(dppf) (160 mg). Resulting solution was stirred at room temperature for 3 hr. The reaction mixture was poured into Et₂O (30 mL) and washed with brine. Organic layer was dried over Na₂SO₄. Filtration and obtained residue was followed by chromatography on silicagel (10:1 n-hexane/EtOAc) to give the compound 33 in 92% yield.



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(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

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A61P 43/00, A61P 3/10, C07C 39/15, C07C 69/18,
C07C 69/16, C07C 69/78, C07C 43/23, C07C 323/18

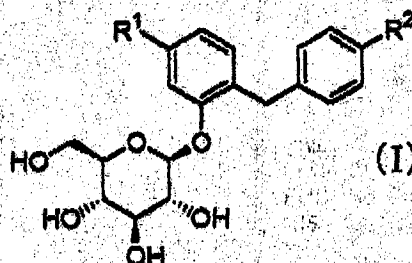
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(54) Titre : DERIVES GLUCOPYRANOSYLOXYBENZYL BENZENE, PREPARATIONS MEDICINALES LES CONTENANT
ET INTERMEDIAIRES POUR LA PREPARATION DESDITS DERIVES

(54) Title: GLUCOPYRANOSYLOXYBENZYL BENZENE DERIVATIVES, MEDICINAL COMPOSITIONS CONTAINING
THE SAME AND INTERMEDIATES FOR THE PREPARATION OF THE DERIVATIVES



(57) Abrégé/Abstract:

Glucopyranosyloxybenzylbenzene derivatives of the general formula (I) and salts thereof, which exhibit excellent human SGLT2 inhibiting activity and are useful as preventive or therapeutic drugs for diabetes, obesity and so on; and intermediates for the preparation of the derivatives wherein R¹ is hydrogen or hydroxylated lower alkyl; R² is lower alkyl, lower alkoxy, lower alkylthio, hydroxylated lower alkyl, hydroxylated lower alkoxy, hydroxylated lower alkylthio, or the like.

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado
HUMBERTO RUBIO CAMACHO

ASUNTO	Radicación	06-12953
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	
	Folios	6

07265

Patente de Invención



Patente de Modelo de Utilidad



Vía Nacional



Vía PCT (fase nacional)



Cap. I



Cap. II



No. Sol. Internacional

PCT/JP2004/011311

Fecha de Presentación Internal.

30-07-2004

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:
inicialmente)

Folios: 13 a 70

(como

se

radicó

Reivindicaciones:
inicialmente)

Folios 71 a 87

(como

se

radicó

Prioridad:

Fecha: 01-08-2003; país y número: 60/491,523.

2. Objeto de la Invención

Titulo de la invención: "COMPUESTOS NOVEDOSOS QUE TIENEN UNA ACTIVIDAD INHIBIDORA CONTRA EL TRANSPORTADOR DE GLUCOSA DEPENDIENTE DEL SODIO PRESENTE EN EL INTESTINO O EL RIÑON"

Problema Técnico Planteado: la necesidad de eliminar el exceso de glucosa directamente en la orina, con el fin de normalizar la concentración de glucosa en la sangre.

Solución: Derivados glucosilados inhibidores de SGLT.

IPC: A61P3/10; A61K31/7034

3. De la solicitud de Patente

a. Reivindicaciones (artículo 30 D 486)

las reivindicaciones 1, 2, 3, 4, 5, 7, 8, 9, 12 carecen de concisión debido a que se presenta un número demasiado alto de alternativas posibles. Se recomienda al solicitante limite el alcance de las siguientes expresiones: "anillo monocíclico", "heterocíclico no saturado", "sustituido opcionalmente", "anillo heterocíclico fusionado no saturado", "un grupo alquilo inferior", "grupo alcanoilo inferior", "alcoxicarbonilo inferior", "alcoxi", "hidroxialquilo", "haloalquilo", "haloalcoxi", "alcoxialcoxi", "alquenilo", "alquinilo", "cicloalquenilo", "cicloalquiloxi", "arilo", "ariloxi", "arilalcoxi", "dialquilamino", "alcanoilamino", "alcoxicarbonilamino", "dialquilcarbamoilo", "alcanoilo", "alquilsulfonilamino", "arilsulfonilamino", "alquiltio", "alquilsulfinilo", "alquilsulfonilo", "arilsulfonilo", "alquiltio", "dialquilsulfamoilo".

Las reivindicaciones 1-16 reclaman un profármaco de los compuestos descritos anteriormente. Sin embargo la descripción carece de información que permita establecer la estructura del profármaco, la forma como puede obtenerse el mismo y su utilidad para resolver el problema técnico objeto de la presente solicitud.

Las reivindicaciones mencionadas no cumplen con el requisito de concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486.y/o art.22 Tratado PCT.

4. Unidad de invención (artículo 25 D 486)

Analizar y argumentar la falta de unidad de invención haciendo énfasis en los diferentes conceptos inventivos que se presentan y las razones por las cuáles no existe una relación entre ellos.

Concluir indicando que no cumplen con el requisito de unidad de invención.

5. Excepción a la patentabilidad (artículo 20 D486 literal d))

Las reivindicaciones 18 y 19 reclaman el método para el tratamiento de la diabetes. Estas reivindicaciones no son patentables.

6. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de la solicitud prioritaria 01-08-2003

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

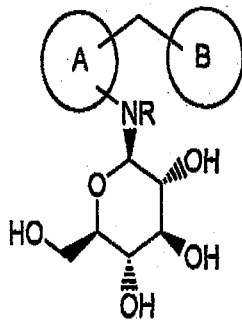
143
142

Nº	Documento	Título	Fecha de publicación*
D1	The american society for pharmacology and experimental therapeutics. Vol 14, No. 2	METABOLISM OF 4,4'-METHYLENEBIS(2-CHLOROANILINE) BY CANINE LIVER AND KIDNEY SLICES	1986
D2	US2001/00441674	C-GLYCOSIDES AND PREPARATION OF THEREOF AS ANTIDIABETIC AGENTS	15-11-2001
D3	CA2402609	GLUCOPYRANOSYLOXYBENZYL BENZENE DERIVATIVES, MEDICINAL COMPOSITIONS CONTAINING THE SAME AND INTERMEDIATES FOR THE PREPARATION OF THE DERIVATIVES	20-09-2001

7. Análisis de Patentabilidad (Art 14 D486)

Novedad (Art. 16 D 486)

La reivindicación 1 reclama un derivado de la fórmula (I):



El documento D1 divulga el compuesto N-[4-[(4-amino-3-chlorophenyl)methyl]-2-clorofenil]-beta-D-glucopiranosilamina MBOCA-glucosido (resumen). Este compuesto se encuentra comprendido dentro de la materia reclamada por D1. En consecuencia la reivindicación 1 no cumple con el requisito de novedad.

en esta solicitud?

Nivel Inventivo (Art18 D486)

El estado de la Técnica más cercano es D2 que divulga derivados glucosidicos útiles para el tratamiento de la diabetes (resumen)

La solicitud se diferencia de D2 esencialmente en la presencia del grupo anilina que une la glucopiranososa con el anillo A

El Efecto Técnico que logra la diferencia no se puede establecer, debido a que la solicitud carece de información que evidencie la utilidad de los compuestos para resolver el problema técnico, o una eventual ventaja de los mismos. Sin embargo D2 enseña un estudio de actividad farmacológica (pág. 16 párrafo 64), en donde se demuestra que la mayoría de los compuestos ensayados incrementan la eliminación de glucosa a través de la orina.

El Problema Técnico Objetivo de esta solicitud consiste en posibles inhibidores de SGLT alternativos y análogos a los divulgados en el estado de la técnica.

D3 describe derivados glucosídicos en los cuales la glucopiranososa se encuentra unida al anillo aromático mediante el oxígeno, evidenciando que a pesar de la sustitución de la cadena carbonada de los compuestos divulgados por D2 por un elemento como el oxígeno, se conserva la actividad inhibidora sobre SGLT. En consecuencia es evidente que el experto en la materia busque análogos de glucopiranososa realizando modificaciones a la estructura en D2 como las sugeridas por D3. Así, los compuestos y composiciones de las reivindicaciones 1 a 17 carecen de nivel inventivo

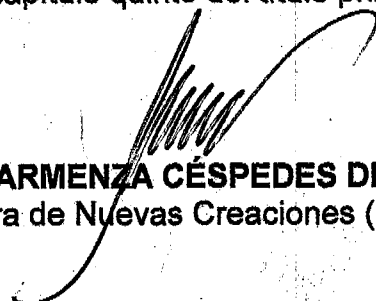
8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud se encuentran afectados así:

Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	The american society for pharmacology and experimental therapeutics. Vol 14, No. 2	1	Novedad
D2	US2001/00441674	1 a 17	Nivel Inventivo
D3	CA2402609	1 a 17	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única Nº 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Directora de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 04 JUN. 2010

CONCEPTO TÉCNICO No.1852	EXAMINADOR TÉCNICO Código No. 202073
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