(1.00 g, 4.4 mmol) in super-dry THF (3 ml) and methyl iodide (4.4 mmol) in THF (3 ml). The reaction mixture was stirred for a further 40 min at −78°, then allowed to warm to room temperature before being quenched with a saturated solution of NH_4Cl. The solvent was then evaporated to leave crude chloroethanesulphonamide **13**, which was purified as before to give 0.714 g (67%) of product which was identified spectroscopically.

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The synthesis of glucuronides derived from the antidepressant drugs mianserin and Org 3770

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Abstract. The synthesis of glucuronides derived from mianserin and its 6-aza analogue (Org 3770) is described. Several methods were investigated. The most successful approach was the coupling of 1,2,3,4,10,14b-hexahydro-8-hydroxy-2-(trifluoroacetyl)dibenzo[*c*,*f*]pyrazino[1,2-*a*]azepine or its 6-aza analogue with methyl [trichloroethanimidoyl 2,3,4-tris-*O*-(phenylmethyl)-α-D-glucopyranosid]uronate catalyzed by BF_3. Fully protected glycosides were obtained as diastereomeric β/α mixtures. After deprotection the glucuronides of 2-demethylmianserin and 2-demethyl-Org 3770 were synthesized. The corresponding *N*(2)-methyl analogues were obtained by reductive methylation. The synthetic β-glucuronides were identical with the isolated metabolites of mianserin and Org 3770.

Introduction

It is now well established that β-D-glucuronides are formed as metabolites of many xenobiotics[1]. These metabolites are generated in a reaction of phenols, alcohols and carboxylic acids with uridine-5′-diphospho-α-D-glucuronic acid mediated by β-glucuronyl transferases. Studies[2,3] with antidepressants such as mianserin **1a** and Org 3770 **1b** revealed that large amounts of β-glucuronides are formed during their metabolism.

The metabolic reactions leading to these products are shown in Fig. 1. In phase-I metabolism, compounds **1a,b** are hydroxylated at position 8 and partially demethylated; in phase-II metabolism, β-*O*-glucuronidation occurs (see formation **17a,b** and **18a,b** in Fig. 1).

In order to study the biological and pharmacological properties of these glucuronides reasonable amounts should be available.

Isolation of glucuronides from urine[3] of animals to which the antidepressants were administered or *in-vitro* biochemical[4] synthesis only provides small amounts. However, an appropriate organic synthesis would give enough material. Up to

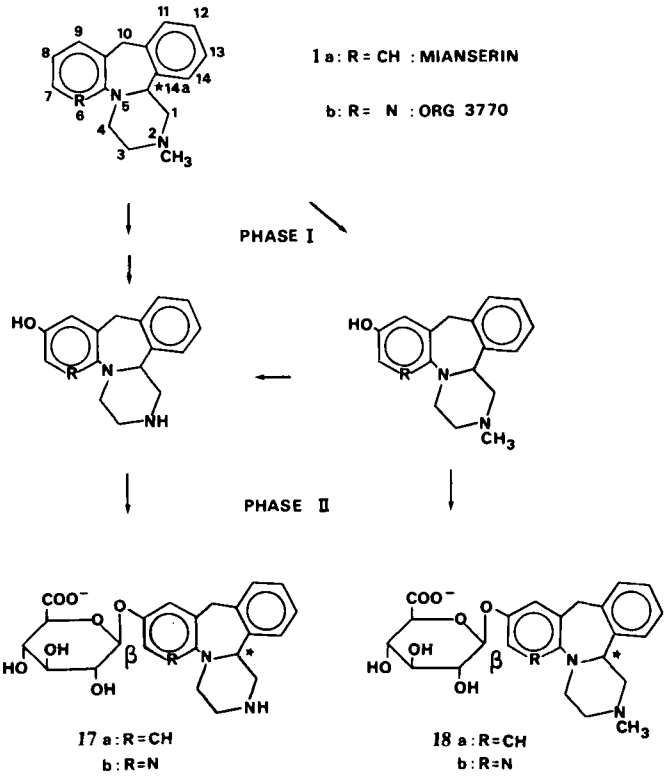


Fig. 1. Part of the metabolism of mianserin and Org 3770.

[1] See e.g. *B. Testa* and *P. Jenner*, Drug Metabolism, Chemical and Biochemical Aspects, Marcel Dekker Inc., New York 1976.
[2] *G. D. de Jongh, H. M. van den Wildenberg, H. Nieuwenhuyse* and *F. van der Veen*, Drug Metab. Dispos. **9**, 48 (1981).
[3] *L. P. C. Delbressine, M. Moonen* and *F. M. Kaspersen* (to be published).
[4] For a typical example see *B. K. Wilson* and *J. A. Thompson*, Drug Metab. Dispos. **12**, 161 (1984).

now several synthetic methods have been described, the application of which will be discussed for the synthesis of the above-mentioned glucuronides (*i.e.* **17a,b**; **18a,b**).

Results and discussion

Synthesis of aglycons **5a,b**

The synthesis of β-glucuronides **17a,b and 18a,b** requires a coupling reaction between a suitably protected D-glucuronic acid and 8-hydroxy derivatives of mianserin and Org 3770 respectively (**3a,b**). The aglycons **3a,b** were readily prepared in a three-step procedure starting from the parent compounds **1a,b** (see Fig. 2). Bromination of **1a,b** in acetic acid afforded **2a,b** in 80% yield. The 8-bromo compounds **2a,b** were converted into the corresponding 8-hydroxy derivatives **3a,b** by a method described by *Buck–Köbrich*[5] (yield 30–45%).

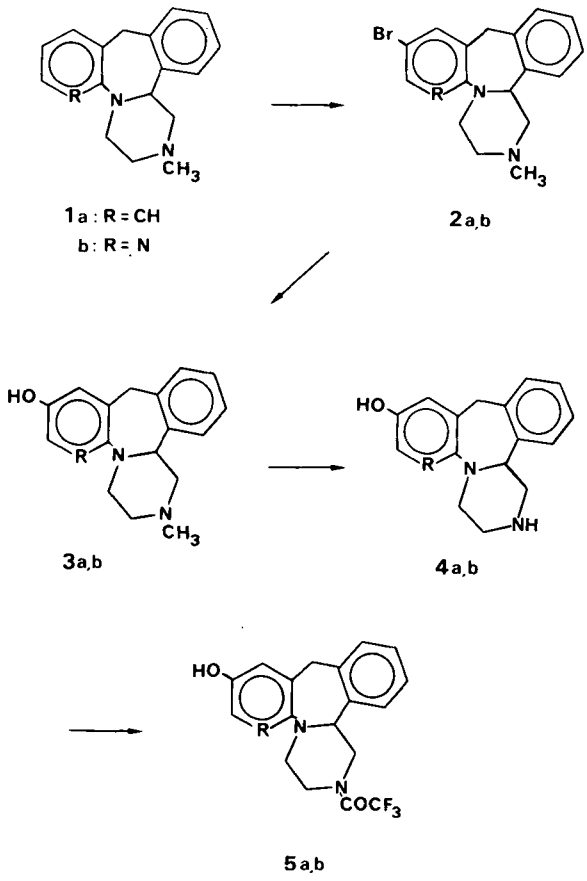


Fig. 2

Preliminary coupling reactions of aglycons **3a,b** with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-1-α-D-glucopyranuronate **7** failed completely. This is probably caused by the presence of the nucleophilic N(2) nitrogen atom[6].
In order to circumvent interference by the N(2) function, the *N*-methyl derivatives **3a,b** were converted into the corresponding N-(trifluoroacetyl) compounds **5a,b**. Thus, demethylation of **3a,b** was achieved by ethyl chloroformate/hydrogen bromide treatment to give **4a,b** in yields of about 60%. Compounds **4a,b** were quantitatively converted into **5a,b** by reaction with trifluoroacetic anhydride.

Glucuronide formation

The fully protected glucopyranuronate **7** has been used frequently as glycon in the synthesis of β-D-glucuronides[7]; it is easily prepared in a three-step synthesis starting from glucuronolactone **6**[8] (Fig. 3).

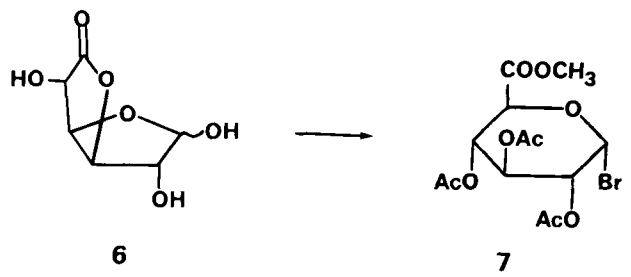


Fig. 3

Several methods for the coupling of **7** with aglycons **5a** and/or **5b** were investigated. The classical *Koenigs–Knorr* reaction, using silver carbonate in toluene at 110°C under nitrogen, resulted in oxidation of the aglycon (**5b**) without glycoside formation. We assume that this side-reaction is facilitated due to the presence of an oxidation-sensitive *p*-hydroxyaniline moiety in the aglycon and the high temperature required. Since it has been shown recently[9] that silver silicate (on alumina or silica) is a more reactive promoter for coupling reactions than silver carbonate, we turned our attention to the use of the former reagent. Coupling of glycon **7** with **5a,b** in the presence of silver silicate at room temperature indeed led to the formation of glucuronide derivatives. Unfortunately, however, 1H-NMR revealed the formation of *ortho* esters **8** (see Fig. 4; H1 at 5.85 ppm) instead of glycosides. The formation of *ortho* esters is a well-known undesired side-reaction; *Vlahov* and *Snatzke*[10] have introduced a pivaloyl-protected analogue of **7** (*i.e.* **9**) to suppress this reaction. However, coupling of **9** with **5a,b** in the presence of silver silicate did not result in the formation of coupling product.

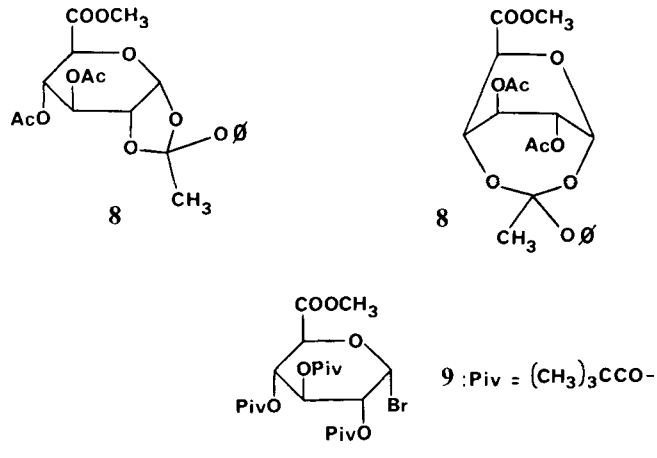


Fig. 4. ∅ *represents the tetracyclic moiety.*

[5] *P. Buck* and *G. Köbrich*, Tetrahedron Lett. 1563 (1967).
[6] Similar problems were encountered by *J. E. Oatis* et al. (*J. E. Oatis, J. P. Baker, J. R. McCarthy* and *D. R. Knapp*, J. Med. Chem. **26**, 1687 (1983)) in the coupling reaction of **7** with propranolol, a compound which also contains a nucleophilic nitrogen function.
[7] *D. Keglevic* in Advances in Carbohydrate Chemistry and Biochemistry, Vol. **36**, 57. Ed. *R. S. Tipson* and *D. Horton*, Academic Press, New York 1979 and references cited therein.
[8] *G. N. Bolleback, J. W. Long, D. G. Benjamin* and *J. A. Lindquist*, J. Am. Chem. Soc. **77**, 3310 (1955).
[9a] *H. Paulsen, W. Kutscher* and *O. Lockhoff*, Chem. Ber. **114**, 3233 (1981);
[b] *H. Paulsen and W. Kutscher*, Liebigs Ann. Chem. 557 (1983).
[10] *J. Vlahov* and *G. Snatzke*, Liebigs Ann. Chem. 570 (1983).

Conrow and *Bernstein*[11] reported a substantial improvement in yields of aryl steroid glucuronides when cadmium carbonate was applied instead of silver carbonate. Coupling of **5a** with **7** in toluene at 110° with cadmium carbonate afforded a complex mixture of glucuronides in an yield of 15%. 1H-NMR indicated the formation[12] of β/α *O*-glycosides, *ortho* esters and *C*-glucuronides.

A completely different coupling method was introduced by *Berrang* et al.[13] who used a lithium phenoxide as nucleophile in a reaction with glycopyranosyl bromide **7**. Application of this method with the lithium salt of **5b** on **7** failed and only decomposition of **7** was observed. Also the coupling of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate with aglycon **5b** in the presence of trimethylsilyl trifluoromethanesulfonate[14] failed.

Recently, *Schmidt*[15] et al. developed a new coupling reaction in carbohydrate chemistry. In this procedure a trichloroacetimidate function is used as leaving group at the anomeric centre. The advantages in glucuronide synthesis are the following: Benzyl ethers instead of acyl esters can be used as glycon-protecting groups and therefore no *ortho* esters can be formed. Furthermore the reaction is performed at low temperature and catalyzed by *Lewis* acids, so that no oxidative side-reactions are expected. *Schmidt* et al.[16] already applied this reaction for the synthesis of *O*-glucuronides starting from methyl [trichloroethanimidoyl 2,3,4-tris-*O*-(phenylmethyl)--α-D-glucopyranosid]uronate **14**. Compound **14** was easily prepared by trichloroacetonitrile treatment of **13** with sodium hydride as a base. Carbohydrate derivative **13** was prepared as shown in Fig. 5.

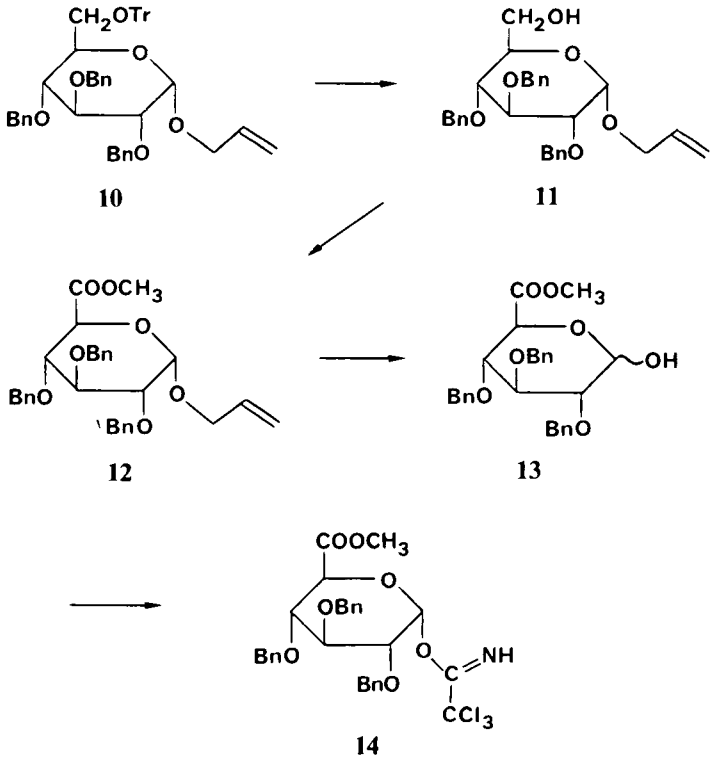


Fig. 5

In our approach of the synthesis of **13** the easily removable 1-*O*-allyl group was used as protective group in carbohydrate derivatives **10–12** instead of the earlier applied 1-*O*-methyl group[17]. Reaction of α-imidate **14** with **5a** or **5b** in dichloromethane with boron trifluoride etherate as a catalyst gave the glucuronides **15a** and **15b**, respectively (see Fig. 6), in high yields (70–80%). It is worthwhile mentioning that the reactivities of aglycon **5a** and pyridine analogue **5b** differ considerably.

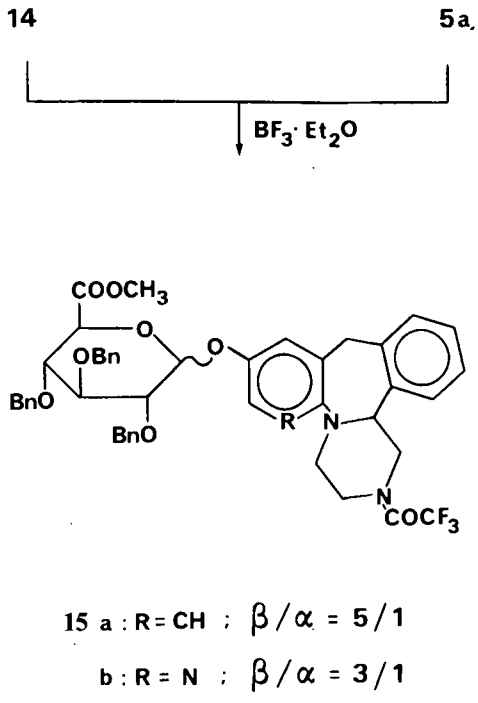
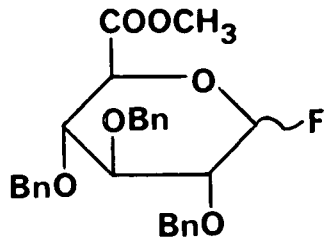


Fig. 6

Thus, the mianserin derived aglycon **5a** already couples at −20°C (reaction time 1–2 h), whereas the Org 3770-derived aglycon **5b** reacted at room temperature (reaction time 8 h), resulting in a somewhat lower β/α ratio of **15b** relative to **15a**. Fortunately, the β/α isomers of **15b** could be separated by chromatography on silica gel. In addition, whereas **5a** could be coupled with **14** in the presence of a catalytic amount of boron trifluoride (0.11 eq.), aglycon **5b** required more than one equivalent of the catalyst (1.1 eq.).



16

A side-reaction in the latter coupling was the formation of 1-fluoro carbohydrate derivative **16**. We propose that this product is formed by reaction of **14** with hydrogen fluoride present in boron trifluoride etherate[18]. This side-reaction

[11] *R. B. Conrow* and *S. Bernstein*, J. Org. Chem. **36**, 863 (1971).

[12] A similar mixture has been found earlier by *W. E. Dick* (Carbohydr. Res. **70**, 313 (1979)) in the Cd-carbonate-catalyzed reaction with phenol.

[13] *B. Berrang, C. E. Twine, G. L. Hennessee* and *F. I. Carroll*, Synth. Commun. **5**, 231 (1975).

[14a] *T. Ogawa, K. Beppu and S. Nakabay*, Carbohydr. Res. **93**, C 6 (1981).

[b] *B. Fischer, A. Nudelman, M. Ruse, J. Herrig, H. E. Gottlieb* and *E. Keinan*, J. Org. Chem. **49**, 4988 (1984).

[15] *R. R. Schmidt* and *M. Reichrath*, Angew. Chem. Int. Ed. Engl. **18**, 466 (1979).

[16] *R. R. Schmidt* and *G., Grundler*, Synthesis 885 (1981).

[17] *R. R. Schmidt* and *E. Rücker*, Tetrahedron Lett. 1421 (1980).

[18] With freshly distilled BF_3 etherate the amount of fluorosugar formed is less than with technical BF_3 etherate. At this moment we are studying the formation of fluorosugars via trichloroacetimidates derivatives.

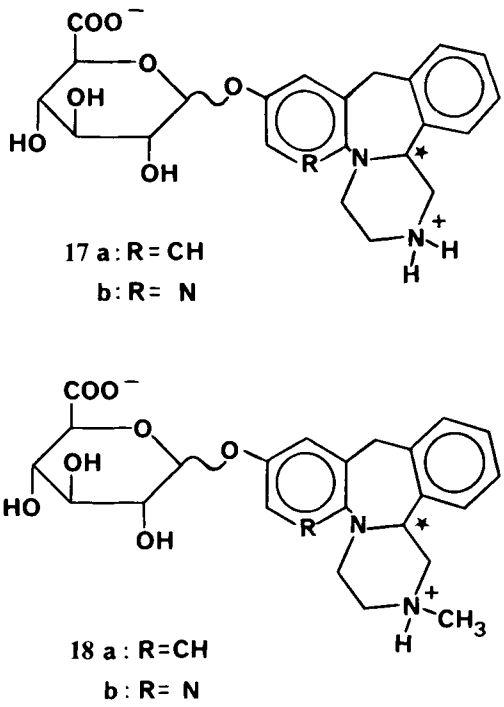


Fig. 7

provides an interesting synthesis of 1-fluoro carbohydrates, which are currently in the picture as building blocks[19] for the synthesis of oligosaccharides as well as *N*- or *C*-glucosides.

Deprotection and N-*methylation*

The fully protected glucuronides **15a,b** were deprotected in a two-step procedure. Firstly, the benzyl groups were removed by hydrogenolysis (Pd/C); secondly the methyl ester and trifluoroacetimidoyl function were hydrolyzed simultaneously by sodium hydroxide treatment in aqueous methanol to afford demethyl glucuronides **17a,b** (Fig. 7) in virtually quantitative yields.

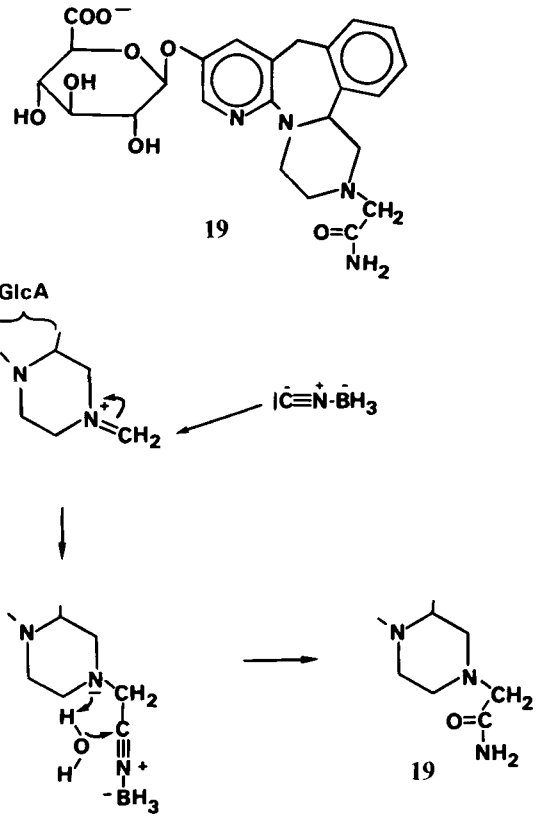


Fig. 8

[19a] *S. Hashimoto, M. Hayashi* and *R. Noyori*, Tetrahedron Lett. 1379 (1984);
[b] *K. C. Nicolaou, R. E. Dolle, A. Chucholowski* and *J. L. Randall*, Chem. Commun. 1153 (1984);
[c] *K. C. Nicolaou, A. Chucholowski, R. E. Dolle* and *J. L. Randall*, Chem. Commun. 1155 (1984);
[d] *K. C. Nicolaou, R. E. Dolle, D. P. Papahatjis* and *J. L. Randall*, J. Am. Chem. Soc. **106**, 4189 (1984).

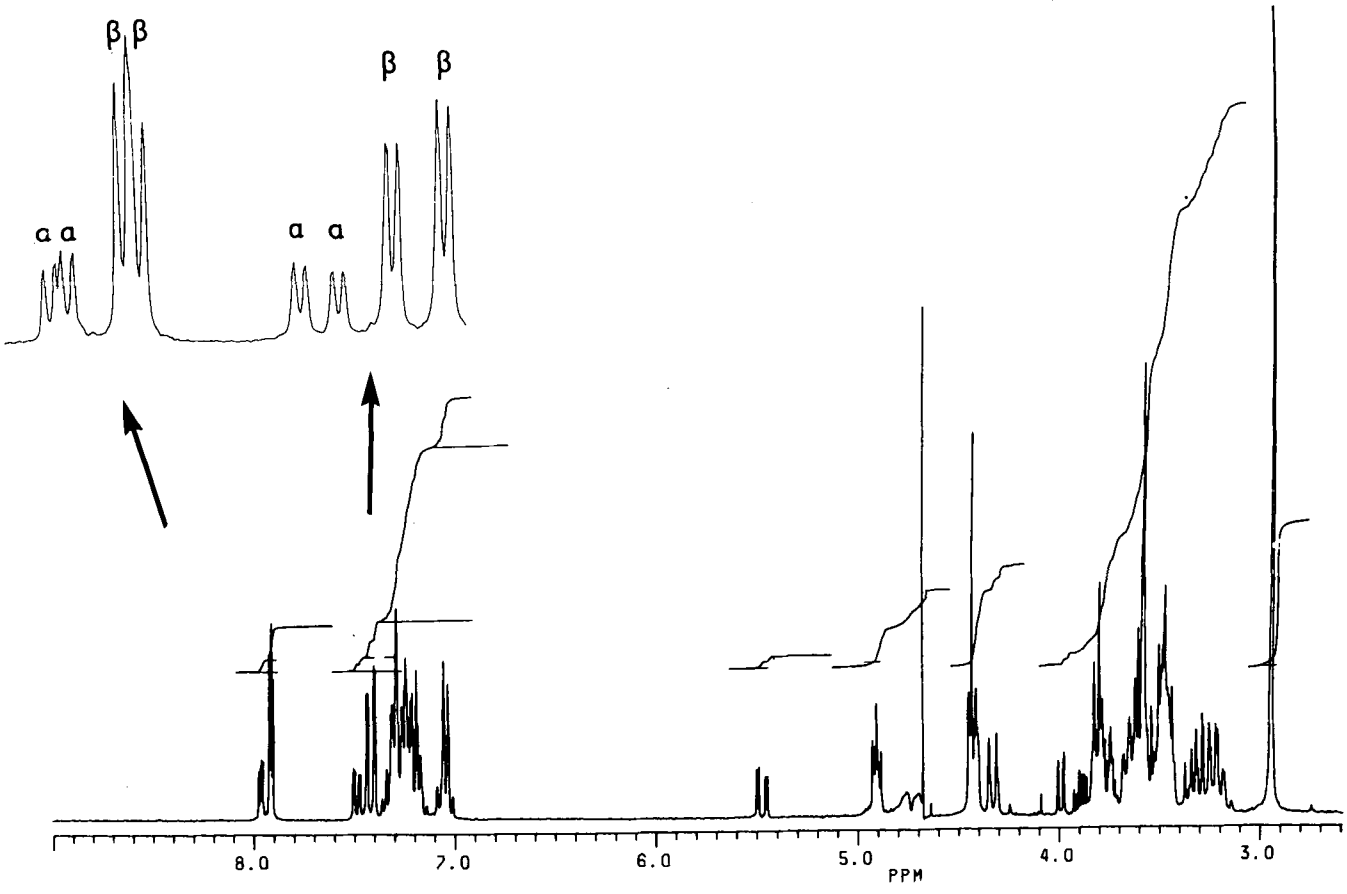


Fig. 9. ^{1}*H-NMR spectrum in* D_2O *of the diastereomeric* α *and* β *glucuronides of 8-hydroxy-Org 3770* (**18b**).

Finally, reductive methylation was performed by treating **17a,b** with formaldehyde and sodium cyanoborohydride in aqueous methanol (pH 7–8) to give, after purification by HPLC, the *N*-methyl glucuronides **18a,b** in about 60% yield. In this reaction a side-product was isolated which was identified as **19** by ^{1}H-NMR, IR and FAB-mass spectrometry. The proposed mechanism for the formation of **19** is depicted in Fig. 8. Thus, instead of hydride addition, the intermediate iminium ion may also be trapped by cyanide to give the corresponding cyanomethyl function, hydrolyzation of which is facilitated by boron-*Lewis* acids to afford amino-carbonyl-methyl derivative **19**. When the reaction was performed using short reaction times the intermediate nitriles **20a,b** were isolated as side-products.

Analysis of glucuronides **17a,b** *and* **18a,b**

Since mianserin and Org 3770 have an asymmetric centre at position 14b two pairs of diastereomeric β/α glucuronides of **15a,b**, **17a,b** and **18a,b** respectively were formed. ^{1}H-NMR analysis of the β/α *N*-methyl glucuronides **18a,b** clearly showed that apart from preference of β over α glycoside formation, no diastereoselective coupling reaction had occurred (see Fig. 9). Fortunately, the complex mixture of diastereomeric β/α glucuronides of **18a,b** could be separated by HPLC, which also shows that the diastereomers were formed in equimolar amounts (see Fig. 10a). However, the separation of the β/α mixtures of the glucuronides at the protected stage by chromatography on silica gel was more convenient as mentioned previously. In the case of **17b** one of the β-diastereomers could be isolated in pure form by recrystallisation of the mixture from methanol.

The synthesized demethyl β-glucuronides **17a,b** and the *N*-methyl glucuronides **18a,b** turned out to be identical with the isolated metabolites. Thus, ^{1}H-NMR and HPLC (*e.g.* see Fig. 10) analyses as well as FAB-mass spectrometry (*e.g.* see Fig. 11) of synthetic **17a,b**, **18a,b** and the corresponding metabolites, corroborated their equality and structure. However, it should be noted that biotransformation of **1a,b** shows a (species-dependent) enantioselectivity resulting in the formation of an excess of one of the diastereomeric β-glucuronides (see Fig. 10, upper curve).

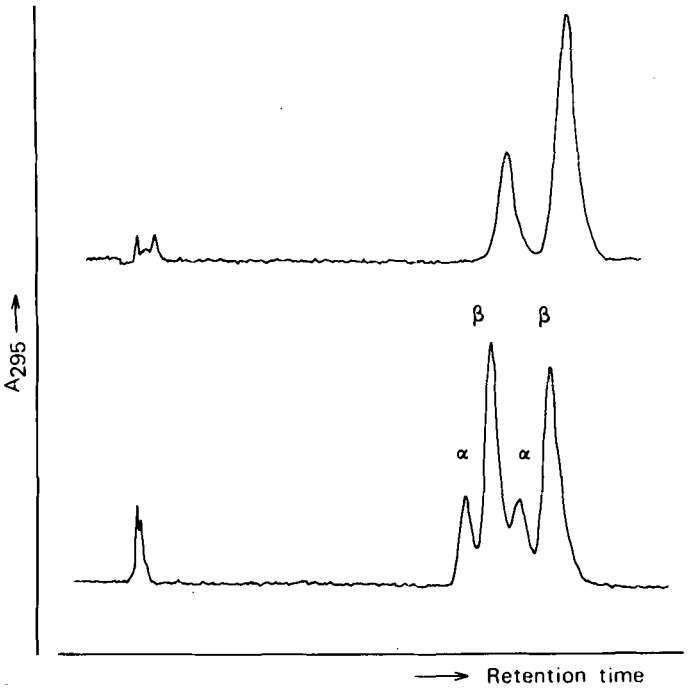


Fig. 10. HPLC pattern of the glucuronides of 8-hydroxy-Org 3770 (**18b**).
Lower curve: synthetic glucuronides.
Upper curve: natural glucuronides isolated from the urine of a female dog.
Column: Nova Pak C_{18} with aq. 0.01 M ammonium phosphate pH 8.0/acetonitrile (1/10 v/v) as mobile phase.

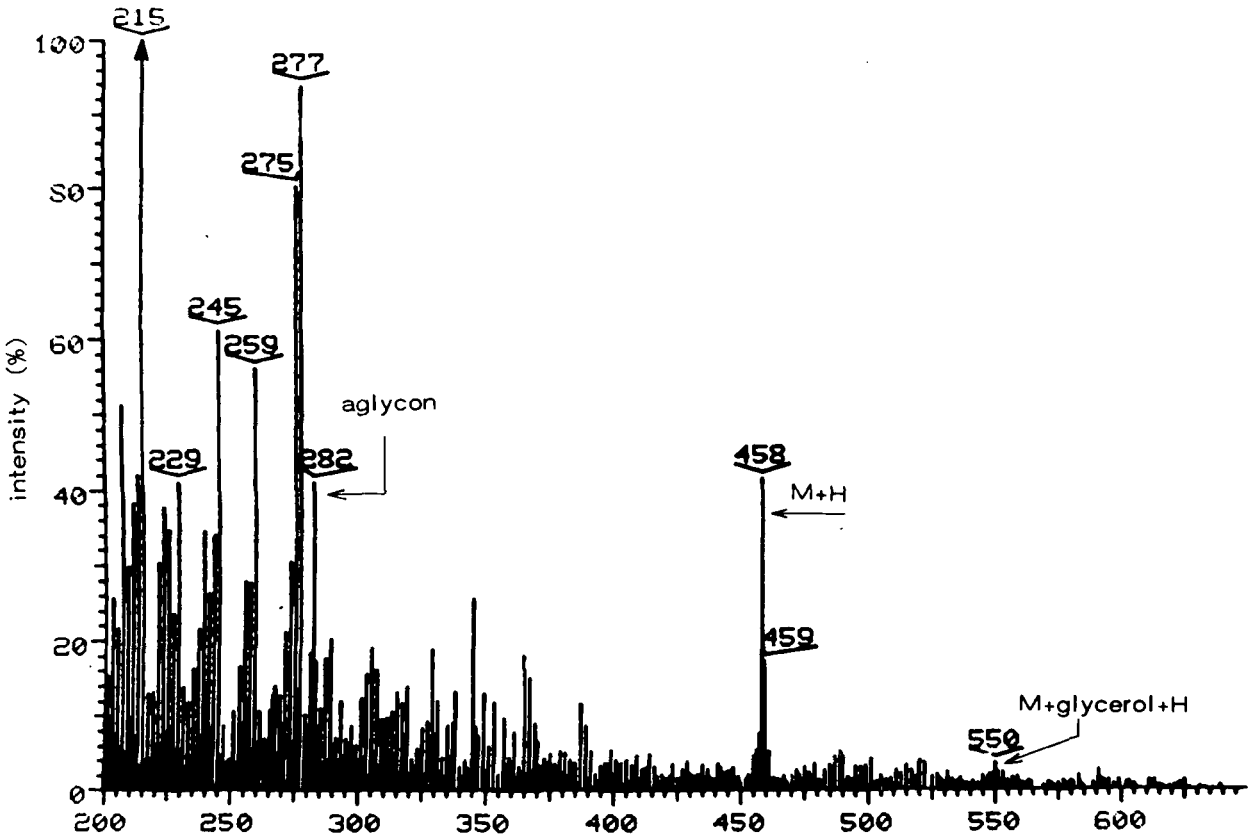


Fig. 11. FAB mass spectrum (glycerol background subtracted) of the synthetic glucuronides of 8-hydroxy-Org 3770 (**18b**).

Experimental

Mass spectra were determined using a Varian MAT 311-A spectrometer in the EI mode or the positive FAB option (M-Scan using Xe and glycerol as matrix). NMR spectra were recorded on a Bruker AM 360 FTNMR spectrometer. Chemical shifts are given in ppm (δ) relative to TMS as internal standard. IR spectra were obtained on a Perkin Elmer 580 IR spectrophotometer or a Digilab FTS-15/90 spectrometer. HPLC experiments were performed on a Waters HPLC model 6000 A using as detector a Schoefel fluorometer (excitation at 295 nm) or an Pye Unicam LC 3 UV-detector (measurement at 254 nm).

1,2,3,4,10,14b-Hexahydro-8-bromo-2-methyldibenzo[c,f]pyrazino-[1,2-α]azepine (8-bromomianserin) (**2a**)

Mianserin (**1a**; 10 g, 37.8 mmol) was dissolved in acetic acid (125 ml) and bromine (4 ml) was slowly added over a period of 30 min. After 4 h stirring at room temperature the precipitate was filtered off and washed with acetic acid and diethyl ether. The precipitate was suspended in diluted aq. ammonia and the mixture was extracted with chloroform (3 × 100 ml). After drying on sodium sulphate, the chloroform was evaporated yielding bromomianserin **2a** (8 g, 62% yield). An analytically pure sample was obtained by conversion of the bromomianserin into its maleate salt and recrystallisation from ethanol yielding colourless crystals; m.p. 136°–137°C. ^{1}H-NMR ($CDCl_3$): δ 6.85 (doublet *J* 8 Hz, H6); 6.90–7.40 (m, H7, H9 – H14). Mass spectrum (EI): *m/z* 342/344 (100%, M^+); *m/z* 271/273 [70%, $(M - NC_4H_9)^+$].

1,2,3,4,10,14b-Hexahydro-8-bromo-2-methyl-pyrazino[2,1-a]pyrido-[2,3-c][2]benzazepine (8-bromo-Org 3770) (**2b**)

The bromination was carried out as described for **2a**. An analytically pure sample was obtained by chromatography over silica gel (methanol/acetone 9/1 v/v) and recrystallisation from *n*-hexane; m.p. 135°–137°C. ^{1}H-NMR ($CDCl_3$): δ 8.25 (d, *J* 6 Hz); 7.48 (d, *J* 6 Hz, H7); 7.15–7.55 (m, H11 – H14). Mass spectrum (EI): *m/z* 343/345 (10% M^+); *m/z* 273/275 [100%, $(M-C_4H_8N)^+$].

1,2,3,4,10,14b-Hexahydro-2-methyldibenzo[c,f]pyrazino[1,2-a]-azepin-8-ol (8-hydroxymianserin) (**3a**)

A solution of the bromo compound (**2a**; 1g g, 2.9 mmol) in THF (35 ml dried and freshly distilled over $LiAlH_4$) was added slowly at −60°C to a solution of butyllithium (3.5 ml; 1.6 M in hexane, Merck) in THF (12 ml). After stirring for 45 min at −60°C, the reaction mixture was cooled to −196°C and nitrobenzene (3.5 ml) was added. The mixture (yellow) was allowed to warm up to −60°C and turned bluish. After stirring at −60°C for 3½ h. the mixture was warmed to room temperature and 10% aq. H_2SO_4 was added (65 ml). After extraction with diethyl ether (2 × 50 ml) the pH of the aqueous layer was adjusted to 10 with aq. ammonia and the mixture was extracted with chloroform (3 × 15 ml). After drying on sodium sulphate the $CHCl_3$ extracts were chromatographed over alumina (with chloroform/methanol 30/1) yielding pure 8-hydroxymianserin (**3a**) (0.24 g; yield 30%); m.p. 216°–218°C (as maleate). ^{1}H-NMR ($CDCl_3$): δ 6.62 ppm (d, *J* 8 Hz, H7); 6.65 ppm (d, *J* 8 Hz); 6.85 (d, *J* 8 Hz, H6); 6.90–7.15 (m, H11 – H14). Mass spectrum (EI): *m/z* 280 (100%, M^+); *m/z* 209 [58%, $(M-NC_4H_9)^+$].

1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]-pyrido[2,3-c]-[2]benzazepin-8-ol (8-hydroxy-Org 3770) (**3b**)

The hydroxylation of 8-bromo-Org 3770 was done in the same way as the hydroxylation of mianserin. The product was purified by chromatography over alumina with toluene/ethyl acetate (1/1, v/v) as mobile phase; yield 45%; m.p. 256°C. ^{1}H-NMR ($CDCl_3$): δ 7.70 d, *J* 1.5 Hz; H7); 6.90 (d, *J* 1.5 Hz; H9); 2.33 (s, N – CH_3). Mass spectrum (EI): *m/z* 281 (20%, M^+); *m/z* 211 [100%, $(M - NC_4H_8)^+$].

1,2,3,4,10,14b-Hexahydrodibenzo[c,f]pyrazino[1,2-a]azepin-8-ol (8-hydroxy-2-demethylmianserin) (**4a**)

To a solution of ethyl chloroformate (1.65 ml) in toluene (10 ml) a solution of 8-hydroxymianserin (**3a**) (1.5 g in 10 ml toluene; 5.3 mmol) was added slowly. After the addition the mixture was refluxed for 2 h. Toluene (10 ml) was added and the mixture was extracted with aq. HCl (10%; 2 × 20 ml) and washed with water (2 × 20 ml). The toluene layers were dried and evaporated to dryness. The residue was dissolved in 48% aq. HBr (15 ml) and this mixture was refluxed under nitrogen for 5 h. After adjustment of the pH to 7–8 with solid $NaHCO_3$ the reaction mixture was extracted with chloroform containing 5% methanol. This extract was chromatographed over silica gel with methanol/acetone (95/5 v/v) yielding pure 8-hydroxy-2-demethylmianserin **4a** (75% after correction for the recovery of the starting material from the HCl extracts); m.p. >245°C (decomposition). ^{1}H-NMR (CD_3OD): δ 6.60 (m, H7 and H9); 6.85 (d, H6); 6.90–7.15 (m, H11–H14). Mass spectrum (EI): *m/z* 266 (100%, M^+); *m/z* 224 [75%, $(M-NC_2H_4)^+$]; *m/z* 209 [60%, $(M - NC_3H_7)^+$].

1,2,3,4,10,14b-Hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benza-zepin-8-ol (8-hydroxy-2-demethyl-Org 3770) (**4b**)

This product was prepared as described for **4a**. It was purified by chromatography over silica gel with methanol/acetone (9/1, v/v); yield 70%; m.p. 300°C (as diHCl-salt). ^{1}H-NMR ($CDCl_3/CD_3OD$): δ 7.60 (d, *J* 1.6 Hz, H7) and 6.90 (d, *J* 1.6 Hz, H9). Mass spectrum (EI): *m/z* 267 (20% M^+); *m/z* 225 [45%, $(M - NC_2H_4)^+$]; *m/z* 211 [100%, $(M - NC_3H_6)^+$].

1,2,3,10,14b-Hexahydro-2-(trifluoroacetyl)dibenzo[c,f]pyrazino-[1,2-a]azepin-8-ol [N-(trifluoroacetyl)-2-demethyl-8-OH-mianserin] (**5a**)

To trifluoracetic anhydride (7 ml) demethyl-8-OH-mianserin (**4a**, 700 mg; 2.63 mmol) was added in portions of 25 mg over a period of 30 min. The reaction mixture was poured into an aq. solution of $NaHCO_3$ (10%, 100 ml) and the mixture was extracted with chloroform containing 10% methanol (2 × 25 ml). After drying on sodium sulphate and removal of the solvent, pure **5a** was obtained as a slightly yellow solid; yield 760 mg (80%). IR (KBr): 1670 cm^{-1} (broad, amide bond). ^{1}H-NMR ($CDCl_3$): δ 6.80–7.50 (m, aromatic protons). Mass spectrum (EI): *m/z* 362 (100% M^+); *m/z* 265 [45% $(M-COCF_3^+)$].

1,2,3,4,10,14b-Hexahydro-2-(trifluoracetyl)pyrazino[2,1-a]pyrido-[2,3-c][2]benzazepin-8-ol [N-(trifluoracetyl)demethyl-8-OH-Org 3770] (**5b**)

The compound (slightly yellow crystals) was prepared with an identical procedure as used for the synthesis of **5a**. Yield 85%. IR (KBr): 1670 cm^{-1} (broad, amide band). ^{1}H-NMR ($CDCl_3/CD_3OD$); δ 7.80 (d, *J* 3.5 Hz, H9); 7.05 (d, *J* 3.5 Hz, H7). Mass spectrum (EI): *m/z* 363 (100%, M^+); *m/z* 265 [50%, $(M-COCF_3^+)$.]

Methyl 2,3,4-tris-O-(phenylmethyl)-α-D-glucopyranuronate (**13**)

The syntheses of compounds **10** and **11** have been described by *Gent* et al.[21] starting from allyl α-D-glucopyranoside. Compound **11** (9.5 g, 19.4 mmol) was dissolved in acetone and cooled (−10°C). To this mixture was added dropwise a solution of chromium(VI) oxide (5.0 g, 33 mmol) in diluted sulphuric acid (3.5 M, 20 ml). After the addition was complete the reaction mixture was stirred at room temperature. After 3 h the solution was poured on ice and extracted with dichloromethane, dried ($MgSO_4$) and then concentrated *in vacuo*. The residue was treated with excess diazomethane in methylene chloride. After 15 min the reaction was stopped by adding acetic acid. Concentration *in vacuo* afforded crude methyl [allyl 2,3,4-tris-*O*-(phenylmethyl)-α-D-glucopyranosid]uronate, **12**, which was purified by chromatography over silica gel with *n*-hexane/ethyl acetate (9/1, v/v) yielding pure **12** (7.65 g; yield 77%). Compound **12** (7.65 g, 14.8 mmol) was dissolved in a mixture of acetic acid (30 ml); water (1.5 ml), NaOAc (3.1 g) and $PdCl_2$ (3.1 g, 17.5 mmol) were added and the mixture was stirred under nitrogen at room temperature for 2 days. The mixture was dissolved in dichloromethane (250 ml) and washed successively with water, aqueous $NaHCO_3$ (10%) and water, dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography of the residue on silica gel (dichloromethane/acetone 98/2 v/v) afforded pure 13 (4.8 g, yield 69%). $[\alpha]_D^{20}$ +25.8 (*c* 1 in $CHCl_3$). M.p. 110°–112°C. ^{1}H-NMR ($CDCl_3$): δ 5.20 (d, anomeric proton); 3.70 (s, CH_3 group).

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[20] *J. V. Paukstellis* in *A. G. Cook*, Enamines, pp. 169–209, Marcell Dekker Inc., New York 1969.

[21] *P. A. Gent* and *R. Gigg*, J. Chem. Soc. Perkin Trans. I, 1835 (1974).

Methyl [1,2,3,4,10,14b-hexahydro-2-(trifluoracetyl)dibenzo-[c,f]pyrazino[1,2-a]azepin-8-yl 2,3,4-tris-O-(phenylmethyl)-D-gluco-pyranosid]uronate (**15a**)

The protected glucuronic acid derivative **13** (956 mg, 2 mmol) was converted into the imidate **14** as described by *Schmidt*[16]. Aglycon **5a** (361 mg, 1 mmol) and glycon **14** (746 mg, 1.2 mmol) were dissolved in dry dichloromethane (8 ml). To this solution was added freshly distilled boron trifluoride etherate (0.1 mmol). The reaction mixture was stirred under nitrogen for 2 h at −25°C after which TLC revealed the reaction to be complete. After work-up and purification by column chromatography **15a** was obtained as a diastereomeric α/β mixture 575 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 7.30 (s, phenyl groups); 5.25 and 5.35 (anomeric β-protons); 3.68 (s, $COOCH_3$).

Methyl [1,2,3,4,10,14b-hexahydro-2-(trifluoroacetyl)pyrazino-[2,1-a]pyrido[2,3-c][2]benzazepin-8-yl 2,3,4-tris-O-(phenylmethyl)--D-glucopyranosid]uronate (**15b**)

Compound **14** (1.12 g; 8 mmol) and aglycon **5b** (548 mg, 1.5 mmol) were dissolved in dry dichloromethane (25 ml). To this solution was added freshly distilled boron trifluoride etherate (2 mmol). The reaction mixture was stirred under nitrogen for 8 h at 20°C. The organic layer was washed with aqueous $NaHCO_3$ (10%) and water, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was chromatographed on a column of silica gel with dichloromethane/acetone (98/2 v/v) to give **15b** (1.0 g; 81%, β/α mixture) together with **5b** (30 mg) and the fluoro derivative **16** (58 mg; 6.7%; β/α mixture).
The β/α mixture of **15b** (80 mg) was further purified by silica gel chromatography to afford pure β-anomer of **15b** (58 mg oil) and pure α-anomer of **15b** (19 mg oil).

Methyl 1-fluoro-1-deoxy-2,3,4-tris-O-(phenylmethyl)-D-glucopyranuronate (**16**)

^{1}H-NMR ($CDCl_3$): δ 5.26 and 5.40 [H1(β), J_{HF} 73 Hz and J_{HH}: 6.6 Hz]; 5.44 and 5.58 [H1(α), J_{HF} 53 Hz, J_{HH} 3 Hz].

Protected glucuronides (**15b**)

^{1}H-NMR ($CDCl_3$): δ 7.97 (m, diastereomeric H7 protons); 7.25 (s, benzylgroup); 5.22 and 5.18 (anomeric α-protons); 3.68 (s, $COOCH_3$).

Deprotection

Compound **15b**-β (50 mg, 0.06 mmol) was dissolved in a mixture of methanol (8 ml) and acetic acid (0.2 ml) and hydrogenolyzed in the presence of palladium on carbon (10%, 50 mg). The mixture was shaken for 5 h at room temperature after which TLC analysis showed the reaction to be complete. The catalyst was filtered off and washed with methanol (50 ml). The solvent was evaporated under reduced pressure to afford the debenzylated derivative in a quantitative yield (33 mg)[23]. The residue was dissolved in methanol (3.5 ml), 1 N aq. NaOH (0.3 ml) was added and the mixture was stirred for $1\frac{1}{2}$ h at room temperature. The pH was adjusted to 7 with acetic acid and the mixture was evaporated to dryness. The product was desalted by chromatography over Sephadex LH20 using methanol as mobile phase to give crude demethyl glucuronide **17b** (25 mg). The corresponding mianserin analogue was deprotected in an identical way.

Reductive methylation

The crude demethylglucuronide **17b** (25 mg) was dissolved in a minimal amount of methanol/water (1/1 v/v) and the pH was adjusted to 7 with solid $NaHCO_3$. Formaldehyde (40%, 0.05 ml in water) and $NaBH_3CN$ (20 mg) were added and the mixture was stirred for 1 h at room temperature. After the reaction excess $NaBH_3CN$ was destroyed with acetic acid (stirring for 30 min) and the pH was readjusted to 7 with solid $NaHCO_3$. After evaporation of the solvent the mixture was desalted by chromatography over Sephadex LH20 using methanol as eluent.
Purification was performed by chromatography over silica gel with dichloromethane/methanol/water (70/35/10 v/v) as mobile phase. Separation of the glucuronides was done by HPLC on Spherisorb 5 ODS C_{18} with 0.1 M aq. ammonium acetate pH 4.0/methanol (gradient from 10% methanol to 90% methanol) or NovaPak C_{18} with 0.01 M aq. ammonium phosphate pH 6.0/acetonitrile (96/4 v/v) for **18a** and 0.1 M aq. ammonium acetate pH 8.0/acetonitrile (gradient from 10% acetonitrile to 14% acetonitrile) for **18b**. In all systems the flow was 2 ml/min.

[1,2,3,4,10,14b-Hexahydrodibenzo[c,f]pyrazine[1,2-a]azepin-8--ylium D-glycopyranosid]uronate (glucuronides of 8-hydroxydemethyl-mianserin) (**17a**)

^{1}H-NMR ($CDCl_3$): δ 6.95–7.25 (m, aromatic protons); 5.05 (d, anomeric α-protons); 4.98 (d, anomeric β-protons). Mass spectrum (FAB): *m/z* 443 [(M + H)$^+$, vw].

[1,2,3,4,10,14b-Hexahydropyrazino[2,1-a]-pyrido[2,3-c][2]benza-zepin-8-ylium D-glycopyranosid]uronate (glucuronides of 8-hydroxy--demethyl-Org 3770 (**17b**)

^{1}H-NMR (D_2O): δ 7.95; 7.94; 7.90; 7.87 (doublets diastereomeric H9 protons); 7.49; 7.48; 7.42 and 7.41 (doublets, diastereomeric H7 protons); 6.90–7.35 (m, H11 – H14 protons); 5.49 and 5.05 (doublets, anomeric α-protons); 4.90 and 4.95 (double doublets, anomeric β-protons). Mass spectrum (FAB): *m/z* 444 [(M + H)$^+$w].

[1,2,3,4,10,14b-Hexahydro-2-methyldibenzo[c,f]pyrazino[1,2-a]-azepin-8-ylium glycopyranosid]uronate (glucuronides of 8-hydroxy-mianserin) (**18a**)

^{1}H-NMR (D_2O): δ 6.90–7.35 ppm (m, diastereomeric aromatic protons); 5.52 and 5.50 (doublets anomeric α-protons); 5.00 and 4.49 (doublets, anomeric β-protons); 2.93 (s, N – CH_3). Mass spectrum (FAB): *m/z* 457 [(M + H)$^+$]; *m/z* 281 [(aglycon + H)$^+$].

[1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c]-[2]benzazepin-8-ylium D-glycopyranosid]uronate (glucuronides of 8-hydroxy-Org 3770 (**18b**)

^{1}H NMR, see Fig. 9. Mass spectrum, see Fig. 11.

[1,2,3,4,10,14b-Hexahydro-2-[(aminocarbonyl)methyl]pyrazino-[2,1-a]pyrido[2,3-c][2]benzazepin-8-ylium D-β-glycopyranosid]uronate (**19b**)

IR (KBr): 1670 cm^{-1} (amide); 1610 cm^{-1} (COO^-). ^{1}H-NMR (CD_3OD): δ 7.76 (d, H7); 7.27 (d, H9); 7.02–7.17 (m, H11 – H14); 4.76 (m, anomeric β-protons); 3.40 (s, N – CH_2 – CO). Mass spectrum (FAB): *m/z* 501 [(M + H)$^+$]; *m/z* 325 [(aglycon + H)$^+$].

[1,2,3,4,10,14b-Hexahydro-2-(cyanomethyl)dibenzo[c,f]pyrazino-[1,2-a]azepin-8-ylium D-glycopyranosid]uronate (**20a**)

IR (KBr): 1600 cm^{-1} (broad COO^-). ^{1}H-NMR (CD_3OD): δ 6.90–7.50 (m, aromatic protons); 5.40 (m, anomeric α-protons); 4.90 (m, aromatic β-protons); 3.72 (s, CH_2 – CN). Mass spectrum (FAB): *m/z* 504 [(M + Na)$^+$]; *m/z* 482 [(M + H)$^+$].

[1,2,3,4,10,14b-Hexahydro-2-(cyanomethyl)pyrazino[2,1-a]pyrido-[2,3-c][2]benzapin-8-ylium D-glycopyranosid]uronate

IR (KBr): 1600 cm^{-1} (broad, COO^-). ^{1}H-NMR (CD_3OD): δ 7.98; 7.97; 7.96 and 7.95 (doublets; diastereomeric H7 protons); 7.42; 7.39; 7.38; 7.37 (doublets, diastereomeric H9 protons); 5.40 (anomeric α-protons); 4.80 (anomeric β-protons); 3.65 (s, CH_2 – CN). Mass spectrum (FAB): *m/z* 505 [(M + Na)$^+$]; *m/z* 483 [(M + H$^+$)]; *m/z* 329 [(aglycon + Na)$^+$]; *m/z* 307 [(aglycon + H)$^+$].

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[22] *T. Ogawa, S. Nakabayashi* and *T. Kitajima*, Carbohydr. Res. **114**, 225 (1983).

[23] In case of the mianserin glucuronides they could be separated at this stage on HPLC on NovaPak C_{18} with 0.1 M aq. ammonium acetate/acetonitrile (74/26 v/v).