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SYNTHESIS AND INHIBITORY EFFECT OF A TRISUBSTRATE TRANSITION STATE ANALOGUE FOR UDP GLUCURONOSYLTRANSFERASES

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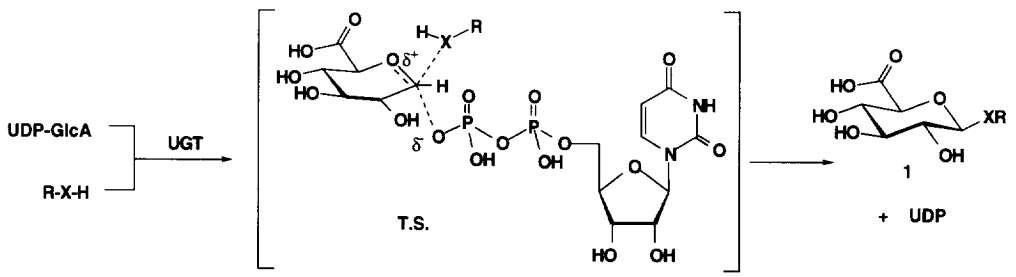
Abstract: Trisubstrate UGT transition state analogue **2** is readily accessible by nucleophilic ring-opening of 1,2-anhydroglucose precursor **5** with diethylmalonate anion followed by reduction of the ethyl ester moieties (**6**→**7**). Subsequent C_6 oxidation (**8**→**9**), NIS/*cat.* TfOH-mediated introduction of the androsterylmethylene unit (**12**→**15**) and phosphitylation with 5'-uridine phosphoramidite **16** furnished, after oxidation and deprotection, target derivative **2**, the two individual diastereomers of which (**2a** and **2b**) were separated by HPLC. Trisubstrate analogues **2a,b** show a different inhibition pattern for several UGT isoforms, indicating isoenzyme selectivity. Moreover, $C_{7'}$-epimers **2a** and **2b** exert a different inhibitory effect on UGT2B15.

Glucuronidation of xenobiotics and endogenous compounds is a major metabolic detoxification pathway[1] catalyzed by UDP glucuronosyltransferases (UGT's), a family of membrane-bound (iso)enzymes present in mammalian liver cells. The glucuronidation is presumed to proceed *via* the transition state[2] (T.S.) depicted in Figure 1 and comprises transfer of a glucuronosyl residue from α-linked uridine-5'-diphosphate glucuronic acid (UDP-GlcA) to an aglycon (RXH). The resulting β-glucuronide (GlcA-XR, **1**) is readily excreted due to the

Figure 1



hydrophilicity of the glucuronic acid unit. Enzymatic glucuronidation also plays a pivotal role in the biotransformation of drugs.[3] Consequently, the design[4] of isoenzyme-selective inhibitors[5] of UGT, which may improve the therapeutic efficiency of a drug without affecting the detoxification of endogenous substrates, is a worthwile goal.

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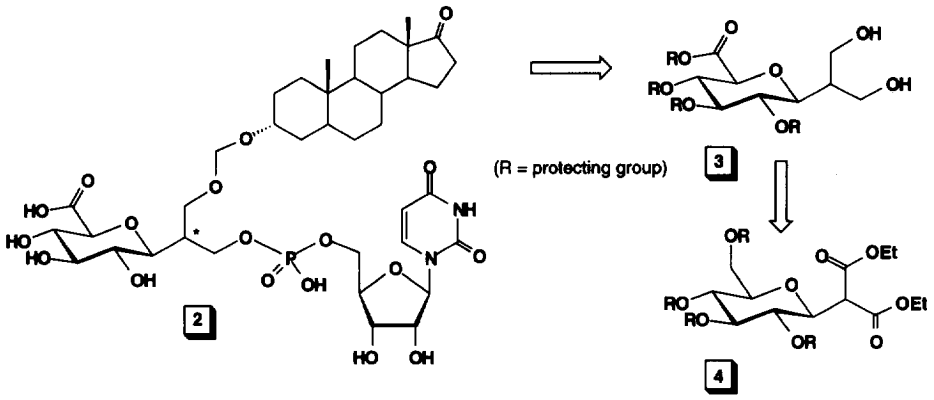
As part of an ongoing program directed towards the design of potential UGT inhibitors[6-8], we here report the assembly of UGT trisubstrate analogue **2** (see Figure 2), containing a methylene acetal-linked androsterone unit, as well as its inhibitory activity and selectivity towards several UGT isoenzymes.

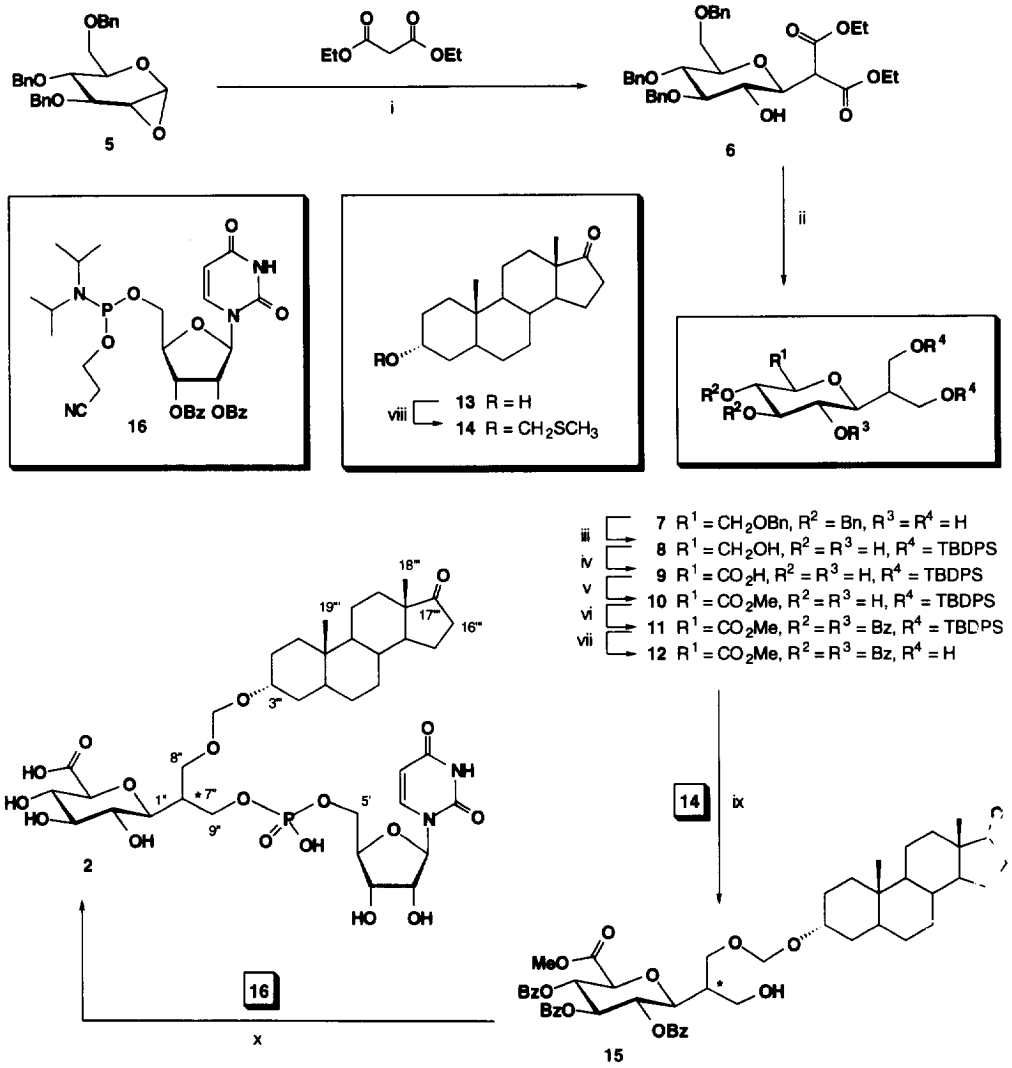
Figure 2



Retrosynthetic analysis reveals that target compound **2** is accessible starting from a suitably protected 2-glucuronosyl-1,3-propanediol derivative (**3**), which in turn can be prepared from the corresponding 2-glucosylmalonate (**4**). It was reasoned that *C*-glucoside **4** can be synthesized by ring-opening of a 1,2-anhydro glucose precursor with malonyl anion.[9] Reaction of known 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose[10] (**5**, Scheme 1) with the anion of diethylmalonate did not lead to the desired *C*-glucoside **6**, but merely resulted in hydrolysis of the 1,2-epoxide into the corresponding 1,2-diol. However, $ZnCl_2$-mediated condensation of **5** with diethylmalonate anion[11] gave the 2-glucosyl malonate **6** in a yield of 76%, reduction of which afforded the triol **7**. Silylation of the primary hydroxyl functions in **7** with TBDPSCl, followed by hydrogenolysis of the benzyl protective groups over Pd/C led to the tetraol **8**. The C_6 in **8** was now oxidized using 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) and sodium hypochlorite (NaOCl) under phase-transfer conditions[12] to give carboxylate **9**. Subsequent esterification of the crude acid **9** with diazomethane furnished methyl ester **10** in 62% overall yield from **8**. Benzoylation of **10** and desilylation of the fully protected *C*-glucuronide **11** with tetra-*n*-butylammonium fluoride (TBAF) in the presence of pyridine-HCl gave the key intermediate diol **12**. The requisite androsterone moiety was now introduced by iodonium ion-mediated reaction of **12** with methylthiomethyl-androsterone **14**. Thus, reaction[13] of commercially available androsterone (**13**) with AcOH/Ac_2O/DMSO afforded the corresponding methylthiomethyl derivative **14** in 84% yield. *N*-Iodosuccinimide (NIS)/*cat.* triflic acid (TfOH)-assisted condensation[14] of diol **12** with methylthiomethyl ether **14** led to methylene acetal-linked derivative **15** as a mixture of diastereoisomers (7R:7S = 1:1) in 69% yield. Finally, phosphitylation of the primary alcohol in **15** with the uridine-5'-phosphoramidite **16**[15] under the agency of 1*H*-tetrazole followed by *t*-BuOOH-mediated oxidation of the intermediate phosphite triester, and then removal of the base-labile 2-cyanoethyl and benzoyl protective groups, gave the phosphodiester derivative **2**. Purification of the crude phosphate by HW-40 gel filtration gave homogeneous target compound **2**, the individual $C_{7''}$-epimers of which (**2a** and **2b**) were separated by reverse-phase HPLC.

The identity of trisubstrate analogues **2a** and **2b** was unambiguously corroborated by mass spectrometry and ^{31}P, ^{1}H and ^{13}C NMR-spectroscopy.[16]

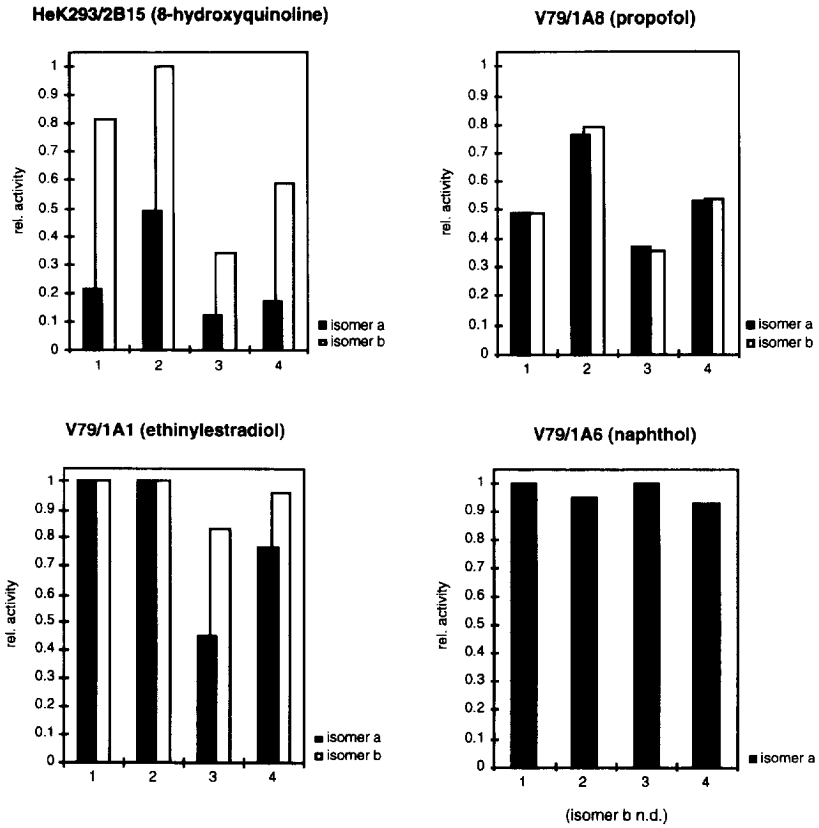
Scheme 1



Key: (i) NaH, $ZnCl_2$, THF, 30 min, 76%; (ii) $LiAlH_4$, Et_2O, reflux, 25 min, 81%; (iii) a. TBDPSCl, DMAP, pyr, 3 h; b. H_2 (3 atm.), Pd/C, *t*-BuOH/AcOH/H_2O (20:1:2, v/v/v), 12 h, 70%; (iv) TEMPO, NaOCl, KBr, $NaHCO_3$, NaCl, $(n\text{-}Bu)_4NCl$, CH_2Cl_2/H_2O (1:1, v/v), 30 min; (v) CH_2N_2, CH_2Cl_2/Et_2O, 5 min (4:1, v/v), 62% (2 steps); (vi) BzCl, pyr, 3 h, 91%; (vii) TBAF, pyr-HCl, THF, 12 h, 74%; (viii) AcOH/Ac_2O/DMSO (1:4:5, v/v/v), 30 °C, 12 h, 84%; (ix) NIS, *cat.* TfOH, 1,2-dichloroethane/THF (3:1, v/v), 10 min, 69%; (x) a. 1*H*-tetrazole, CH_2Cl_2/CH_3CN (1:2, v/v), 30 min; b. *t*-BuOOH (80% in *t*-BuOO*t*-Bu), 10 min; c. LiOH, H_2O/MeOH (1:3, v/v), 1 h, 76%.

The inhibitory effect of the two individual diastereoisomers **2a** and **2b** on the UGT activity was investigated using several cloned and expressed UGT isoforms[17] and their appropriate model substrates: V79/1A8 (propofol), HeK293/2B15 (8-hydroxyquinoline), V79/1A1 (ethinylestradiol) and V79/1A6 (naphthol). The relative extent[18] of glucuronidation of the four substrates in the presence of **2** is summarized in Figure 3.

Figure 3



Key: lane 1: [S] = 50 μM, [**2**] = 100 μM; lane 2: [S] = 250 μM, [**2**] = 100 μM; lane 3: [S] = 50 μM, [**2**] = 500 μM; lane 4: [S] = 250 μM, [**2**] = 500 μM

The most pronounced inhibitory activity is observed for UGT2B15, the isoenzyme that plays an important role in steroid glucuronidation and the only family 2 isoform used in this study. In this respect, it is of interest to note that isomer **2a** proves to be a better inhibitor than its $C_{7''}$-epimer **2b**, which may be attributed to a different spatial alignment of the androsterone and uridine moieties in the respective isomers. On the other hand, both isomers of trisubstrate analogue **2** exert a comparable inhibitory effect on UGT1A8. The degree of UGT1A1 inhibition is considerably lower than that detected for UGT2B15 and UGT1A8. At higher inhibitor

concentration (500 μM) the two isomers again show a different inhibitory effect. Finally, transition state analogue **2a** showed no inhibition of UGT1A6.

In conclusion, this paper describes an efficient and flexible synthetic route towards the first trisubstrate transition state analogue for UGT. The assembly of inhibitor **2** is based on a novel procedure for the preparation of *C*-glycosides from 1,2-anhydro sugars (*i.e.* **5→6**), regioselective TEMPO-mediated oxidation (*i.e.* **8→9**) and iodonium-ion-mediated introduction of the required androsterylmethylene moiety (*i.e.* **12→15**). Trisubstrate analogue **2** shows a different inhibition pattern for several UGT isoforms, indicating isoenzyme selectivity. The most distinct inhibitory effect is observed for UGT2B15, an isoenzyme which plays a pivotal role in steroid glucuronidation. The remarkable difference in UGT2B15-inhibitory activity between diastereomers **2a** and **2b** indicates that the degree of inhibition is influenced by the relative orientation of the aglycon, uridine and glucuronic acid moieties. Inhibitors **2a,b** are quite water-soluble, which implies that their uptake by intact cells, as is required for UGT inhibition *in vivo*, will be relatively slow. However, trisubstrate analogues **2a,b** present promising lead compounds for the future development of more lipid-soluble *in vivo* active UGT inhibitors.

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11. In a similar way, $ZnCl_2$-mediated reaction of diethylmalonate anion with 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-galactopyranose provided the respective β-*C*-galactoside (74% yield). It was also established that *C*-glucoside **6** undergoes a stereoselective cyclization to the corresponding (2*R*)-3-ethyl-2-(3',4',6'-tri-*O*-benzyl-β-D-glucopyranosyl)-1,2'-malonyl lactone upon prolonged treatment with NaH (3 eq., 12 h, 76% from **5**). Finally, transformation of *C*-glucoside **6** with DMSO/Ac_2O and subsequent reduction of the intermediate 2'-ulose with $NaBH_4$ afforded the corresponding 2-(β-D-mannosyl)-malonate (79% yield).
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15. Phosphoramidite **16** was prepared by phosphitylation of commerically available 2',3'-di-*O*-benzoyl-uridine with 2-cyanoethyl-*N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine (DIPEA, CH_2Cl_2, 1 h, 94%).
16. Representative spectral data for diastereomer **2a**: ^{31}P NMR (D_2O): δ 0.77. ^1H NMR (D_2O): δ 7.97 (d, 1H, H_6, $J_{5,6}$ = 8.1 Hz), 5.96 (d, 1H, $H_{1'}$, $J_{1',2'}$ = 3.5 Hz), 5.94 (d, 1H, H_5), 4.75 (d, 1H, OCH_2O, $J_{H,H}$ = 7.5 Hz), 4.73 (d, 1H, OCH_2O), 4.32 (t, 1H, $H_{2'}$, $J_{2',3'}$ = 3.5 Hz), 4.31 (t, 1H, $H_{3'}$, $J_{3',4'}$ = 3.6 Hz), 4.24 (m, 1H, $H_{4'}$), 4.17 (ddd, 1H, $H_{5'A}$, $J_{4',5'A}$ = 2.4 Hz, $J_{5'A,5'B}$ = 11.9 Hz, $J_{5'A,P}$ = 4.2 Hz), 4.09 (ddd, 1H, $H_{5'B}$, $J_{4',5'B}$ = 2.9 Hz, $J_{5'B,P}$ = 4.9 Hz), 4.01 (m, 2H, $H_{9''A}/H_{9''B}$), 3.89 (m, 1H, $H_{3''}$), 3.81 (dd, 1H, $H_{8''A}$, $J_{7'',8''A}$ = 4.9 Hz, $J_{8''A,8''B}$ = 10.4 Hz), 3.66 (dd, 1H, $H_{8''B}$, $J_{7'',8''B}$ = 7.3 Hz), 3.60 (d, 1H, $H_{5''}$, $J_{4'',5''}$ = 8.9 Hz), 3.56 (dd, 1H, $H_{1''}$, $J_{1'',2''}$ = 10.4 Hz, $J_{1'',7''}$ = 1.2 Hz), 3.50 (dd, 1H, $H_{3''}$, $J_{2'',3''}$ = 8.2 Hz, $J_{3'',4''}$ = 9.6 Hz), 3.47 (dd, 1H, $H_{2''}$), 3.44 (dd, 1H, $H_{4''}$), 2.49 (ddd, $H_{16'''A}$, $J_{15'''A,16'''A}$ = 0.9 Hz, $J_{15'''B,16'''A}$ = 8.6 Hz, $J_{16'''A,16'''B}$ = 19.8 Hz), 2.44 (m, 1H, $H_{7''}$), 2.15 (ddd, $H_{16'''B}$, $J_{15'''A,16'''B}$ = 8.0 Hz, $J_{15'''B,16'''B}$ = 2.0 Hz), 1.96-0.78 (m, 20H, 2 x $H_{1'''}$/2 x $H_{2'''}$/2 x $H_{4'''}/H_{5'''}$/2 x $H_{6'''}$/2 x $H_{7'''}/H_{8'''}/H_{9'''}$/2 x $H_{11'''}$/2 x $H_{12'''}/H_{14'''}$/2 x $H_{15'''}$), 0.88 (s, 3H, 3 x $H_{18'''}$), 0.80 (s, 3H, 3 x $H_{19'''}$). ^{13}C {^1H} NMR* (D_2O): δ 218.1 ($C_{17''}$), 177.4 ($C_{6''}$), 156.5 (C_6), 154.4 (C_2), 152.0 (C_4), 103.2 (C_5), 92.9 (OCH_2O), 89.2 ($C_{1'}$), 84.0 ($C_{4'}$, $J_{4',P}$ = 8.4 Hz), 78.2 ($C_{2''}$), 76.3 ($C_{1''}$), 74.8 ($C_{2'}$), 73.2 ($C_{3'''}$), 72.9 ($C_{4''}$), 72.0 ($C_{3''}$), 71.1 ($C_{5''}$), 70.5 ($C_{3'}$), 66.0 ($C_{8''}$), 65.4 ($C_{5'}$, $J_{5',P}$ = 4.0 Hz), 64.9 ($C_{9''}$, $J_{9'',P}$ = 4.0 Hz), 54.9 ($C_{9'''}$), 51.7 ($C_{14'''}$), 49.4 ($C_{13'''}$), 41.5 ($C_{7''}$, $J_{7'',P}$ = 8.0 Hz), 40.4 ($C_{5'''}$), 36.9, 36.2 ($C_{10'''}/C_{16'''}$), 35.4 ($C_{8'''}$), 33.2, 33.1, 31.8, 31.0, 28.7, 26.6, 22.2, 20.4 ($C_{1'''}/C_{2'''}/C_{4'''}/C_{6'''}/C_{7'''}/C_{11'''}/C_{12'''}/C_{15'''}$), 14.0 ($C_{18'''}$), 11.5 ($C_{19'''}$). MS (ESI): m/z = 860 $(M\text{-}H)^-$.
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18. The UGT activity in the presence of **2a** or **2b** was related to a control experiment carried out in the absence of inhibitor. The control activities (nmol/min/mg) at substrate concentrations of 250 μM for the four UGT isoforms are: HeK293/2B15 = 0.194; V79/1A6 = 0.235; V79/1A1 = 0.168; V79/1A6 = 1.27. The assay conditions were: 100 mM tris/maleate (pH = 7.4), 5 mM $MgCl_2$ and substrate concentrations from 25 μM to 500 μM. The inhibitor was dissolved in DMSO and a control curve with 5 μL of DMSO per assay was carried out concurrently with the inhibition study. The assays were started by addition of 10 μL of 20 mM UDP-GlcA (containing 0.1 μCi of [^{14}C]-UDP-GlcA per 10 μL). Incubations were run for 40 min and then terminated by the addition of 100 μL of methanol (-20 °C). Assays were left on ice until the protein could be removed by centrifugation at 1000 g for 5 min. The supernatant (150 μL) was directly injected onto HPLC, comprising a binary gradient of 0-86% acetonitrile in 0.05 M ammonium acetate developed over 13 min on a spherisorb 5ODS2 column. [^{14}C]-Labelled UDP-GlcA and glucuronide detection was executed by a heterogeneous radiochemical method using a 500 μL flow cell packed with silanized cerium-activated glass as scintillant. Two independent experiments were carried out in each assay.

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