Bioorganic & Medicinal Chemistry Letters, Vol.2, No.6, pp. 583-588, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

SYNTHESIS OF A POTENTIAL INHIBITOR OF UDP-GLUCURONOSYLTRANSFERASE

D. Noort[a,b], N.C.R. van Straten[a], G.J.P.H. Boons[a], G.A. van der Marel[a], X. Bossuyt[c], N. Blanckaert[c], G.J. Mulder[b], J.H. van Boom[a]

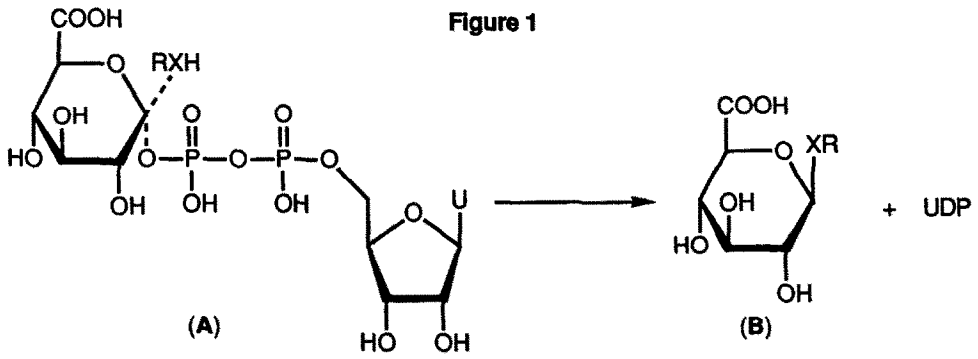
[a]Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden; [b]Division of Toxicology, Center for Bio-Pharmaceutical Sciences, P.O. Box 9503, 2300 RA Leiden, The Netherlands; [c]Laboratory of Biological Chemistry, University of Leuven, B-3000 Leuven, Belgium

(*Received* 3 *March* 1992)

Abstract: A convenient synthesis of phosphonomethyl 1-*O*-(2,2,2-triphenyl)ethyl-α-D-*gluco*-2-heptulopyranosiduronate (**2**) is presented. The target compound proved to be an inhibitor of UDP-glucuronosyltransferase *in vitro*.

Glucuronidation of hydroxyl, thiol, amino or carboxylate functions in xenobiotics and endogenous compounds [e.g. RXH; X=O, S, NH or C(O)O] is a major detoxification pathway catalyzed by UDP-glucuronosyltransferases (UDPGT)[1]. It has been proposed[2] that the glucuronidation reaction proceeds *via* transition-state **A** (see Figure 1) resulting from the attack of an aglycon RXH on the anomeric centre of

Figure 1

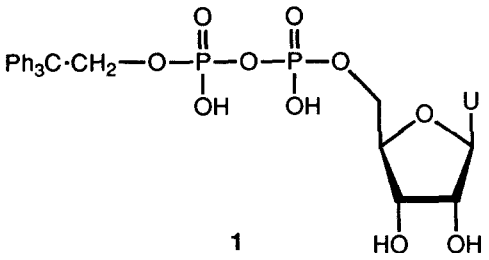


the sugar nucleotide UDP-glucuronic acid. Collapse of the transition state (TS) will yield uridine 5'-diphosphate (UDP) and the ß-glucuronide **B**, which is readily excreted due to the hydrophilicity of the glucuronic acid moiety.

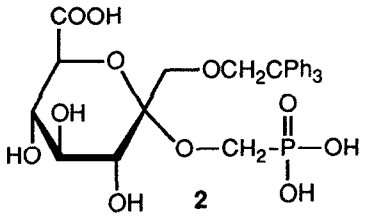
Glucuronidation also plays a pivotal role in drug metabolism and it may thus be anticipated that UDPGT inhibition could improve the therapeutic efficiency of a drug. For example, the anti-Human

Immunodeficiency Virus drug 3'-azido-3'-deoxythymidine (AZT) is rapidly metabolized *via* glucuronidation and excreted as its glucuronide[3].

Recent studies in our laboratories revealed that the TS-analogue 2,2,2-triphenylethyl-UDP (**1**), which contains both a uridine and an aglycon moiety, was not only inhibitory towards UDPGT activity in the microsomal fraction from rat liver[2], but also in an intact cellular system *in vitro* (i.e. isolated rat hepatocytes)[4].



1

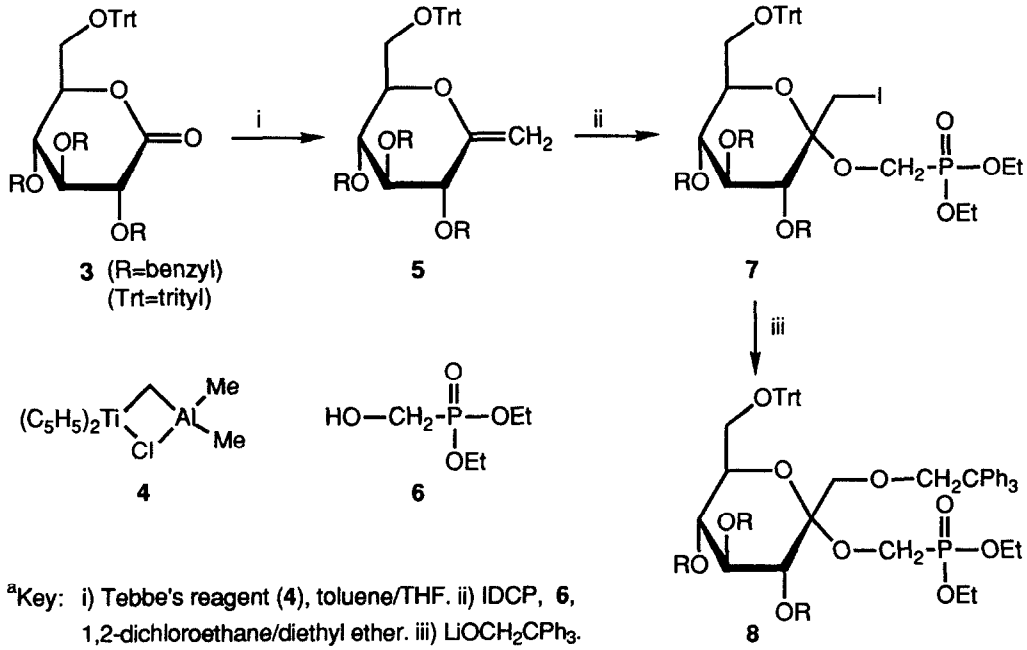


2

We here report the synthesis of phosphonomethyl 1-*O*-(2,2,2-triphenyl)ethyl-α-D-*gluco*-2-heptulopyranosiduronate (**2**) which exerts a distinct inhibitory effect on UDPGT activity *in vitro*.

The design and synthesis route to the new TS-analogue **2** is based on the following heuristic considerations. Thus, in view of the strong inhibitory effect of **1** on UDPGT activity, it would be reasonable to incorporate the triphenylethyl (TPE) moiety. Further, replacement of the UDP unit by a more stable

Scheme 1[a]

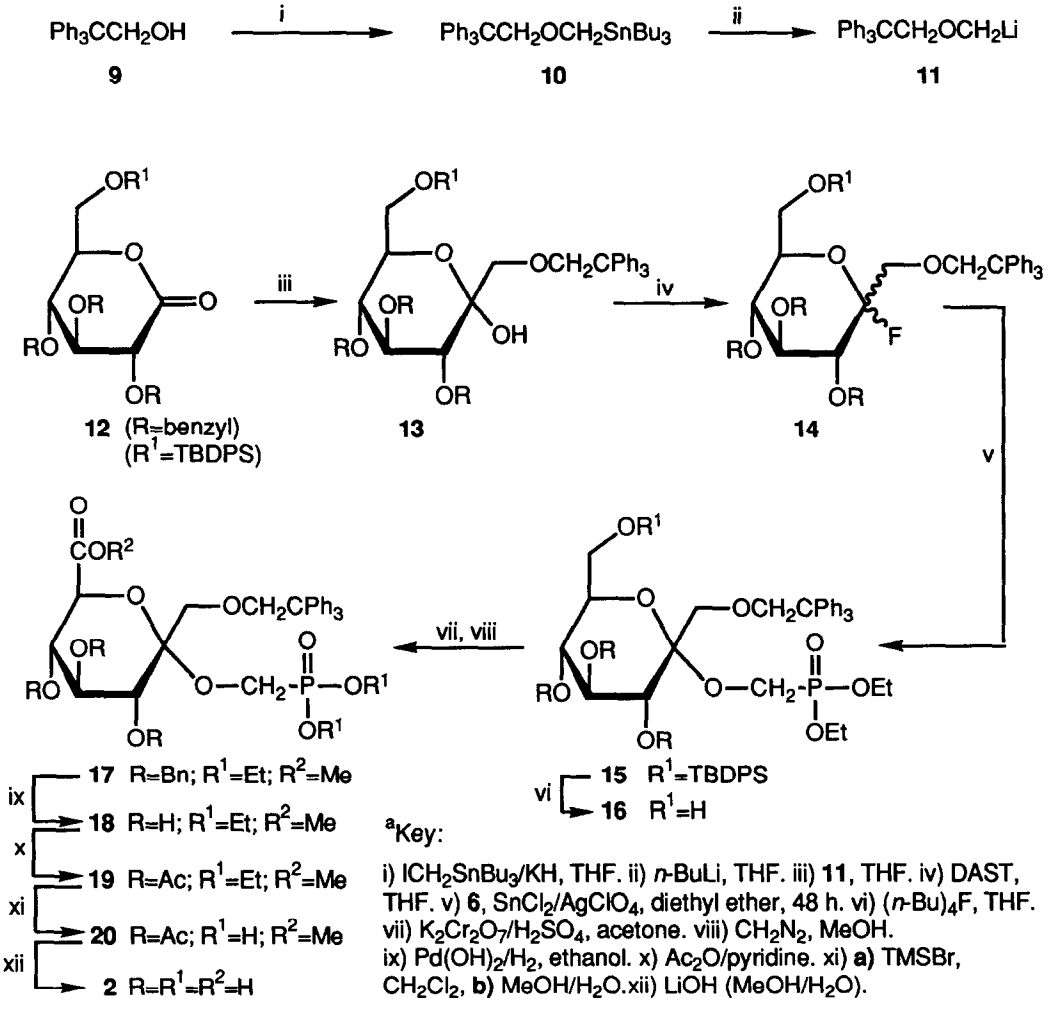


[a]Key: i) Tebbe's reagent (**4**), toluene/THF. ii) IDCP, **6**, 1,2-dichloroethane/diethyl ether. iii) $LiOCH_2CPh_3$.

phosphonomethylene[5] function will shorten the synthetic route. In addition, the recently reported[6] stereospecific iodonium ion promoted reaction of exocyclic glycals (e.g. **5**) with alcohols resulting in 1-iodoheptulosides enables the construction of the requisite configuration at the anomeric centre.

A first attempt to assemble target compound **2** is outlined in Scheme 1 and commences with the introduction of the phosphonomethylene function. Thus, iodonium *sym*-dicollidine perchlorate[7] (IDCP) assisted condensation of diethyl(hydroxymethyl)phosphonate[8] (**6**) with 2,6-anhydro-3,4,5-tri-*O*-benzyl-1-deoxy-7-*O*-trityl-D-*gluco*-hept-1-enitol (**5**), prepared by methylenation of readily accessible lactone **3**[9] with Tebbe's reagent[10] (**4**), gave the expected α-ketoside[11] **7**[12] (m.p. 135°C; α_D^{20} +54.4°) in 84% yield. Unfortunately, direct replacement of the iodine atom in **7** by lithium 2,2,2-triphenylethoxide, giving fully protected **8**, was abortive[13].

Scheme 2[a]



[a]Key:

i) ICH_2SnBu_3/KH, THF. ii) *n*-BuLi, THF. iii) **11**, THF. iv) DAST, THF. v) **6**, $SnCl_2/AgClO_4$, diethyl ether, 48 h. vi) $(n\text{-}Bu)_4F$, THF. vii) $K_2Cr_2O_7/H_2SO_4$, acetone. viii) CH_2N_2, MeOH. ix) $Pd(OH)_2/H_2$, ethanol. x) Ac_2O/pyridine. xi) **a)** TMSBr, CH_2Cl_2, **b)** $MeOH/H_2O$. xii) LiOH ($MeOH/H_2O$).

An alternative pathway to compound **2** is presented in Scheme 2. A key element of this pathway is the introduction, at an early stage of the synthesis, of the TPE unit *via* the organolithium reagent **11**. The latter was readily accessible by tin/lithium exchange[14] of the corresponding tributylstannane derivative **10** which, in turn, was prepared by the reaction of **9** with iodomethyl tributylstannane according to Seyferth[15]. Addition of the properly protected D-gluconolactone **12**[16] to **11**, generated *in situ* by quenching **10** with *n*-butyllithium, led to the exclusive formation of the anomerically pure[11] 1-*O*-(2,2,2-triphenyl)ethyl-α-D-*gluco*-heptulopyranose **13** in 86% yield (α_D^{20} +40.1°).

The phosphonate function was now introduced by the following two-step procedure. Treatment of **13** with diethylaminosulfur trifluoride (DAST)[17] furnished the stable ketoglycosyl fluoride **14** (α/ß mixture) in a quantitative yield. Glycosylation of diethyl(hydroxymethyl)phosphonate (**6**) by the ketopyranosyl fluoride **14** in the presence of the promoter $SnCl_2/AgClO_4$[18] gave, after purification, the fully protected α-linked[11] phosphonomethyl derivative **15** (α_D^{20} +34.2°) in 52% yield.

At this stage, the silyl protecting group of HO-7 in **15** was removed and the resulting hydroxyl was converted into the required carboxylate methyl ester. Thus, removal of the *tert*-butyldiphenylsilyl group with fluoride ions afforded homogeneous **16** (yield 92%; α_D^{20} +29.3°). Oxidation of **16** could be realized most effectively[19] with Jones reagent[20] to give, after methylation of the carboxylate group with diazomethane, fully protected **17** (overall yield 60%; α_D^{20} +48.7°).

Complete deblocking of **17** to give the target compound **2** was executed as follows. Hydrogenolysis of the benzyl groups followed by acetylation of **18** yielded homogeneous **19**. Hydrolysis of the phosphonate diethyl ester by trimethylsilyl bromide (TMSBr)[21] and subsequent addition of $MeOH/H_2O$ afforded **20**. Finally, deesterifation of **20** resulted, after purification and conversion (Dowex 50 W, Na^+-form) into the trisodium salt, in the isolation of homogeneous **2** [α_D^{20} +59.3° (c 1, H_2O)].

Preliminary biological studies indicated that the TS-analogue **2**, in a 20-fold excess with respect to UDP-glucuronic acid, acts as an inhibitor of 4-methylumbelliferone (78% inhibition) and bilirubin (41% inhibition) glucuronidation in a rat liver microsomal fraction. A detailed study on the inhibitory effect of **2** will be published in due course.

Acknowledgement. This work was supported by a grant from the Foundation for Medical Research MEDIGON.

References and Notes

1. Mulder, G.J.; Coughtrie, M.W.H.; Burchell, B. *Conjugation Reactions in Drug Metabolism. An Integrated Approach*; Mulder G.J., Ed.; Taylor and Francis: London, pp 51-106, 1990.
2. Noort, D.; Coughtrie, M.W.H.; Burchell, B.; Van der Marel, G.A.; Van Boom, J.H.; Van der Gen, A.; Mulder, G.J. *Eur. J. Biochem.* **1990**, *188*, 309.
3. Resetar, A.; Spector, T. *Biochem. Pharmacol.* **1989**, *38*, 1389.
4. Noort, D.; Meijer, E.A.; Visser, T.J.; Meerman, J.H.N.; Van der Marel, G.A.; Van Boom, J.H.; Mulder, G.J. *Molec. Pharmac.* **1991**, *40*, 316.

5. Holy, A. *Nucleosides and Nucleotides* **1987**, *6*, 147.
6. Noort, D.; Veeneman, G.H.; Boons, G.J.P.H.; Van der Marel, G.A.; Mulder, G.J.; Van Boom, J.H. *Synlett*, **1990**, 205.
   Recently, similar iodonium-ion mediated glycosylation reactions were reported by others. See for instance: Haudrechy, A.; Sinaÿ, P. *Tetrahedron Lett.* **1990**, *31*, 5765. Thiem, J.; Kleeberg, M. *Carbohydrate Res.* **1990**, *205*, 333.
7. Lemieux, R.U.; Morgan, A.R. *Can. J. Chem.* **1965**, *43*, 2190.
8. Kluge, A.F. *Org. Synth.* **1986**, *64*, 80.
9. Lactone **3** was prepared by regioselective tritylation (trityl chloride/pyridine) of 2,3,4-tri-*O*-benzyl-α/ß-D-glucopyranose followed by Swern oxidation.
10. Cannizzo, L.F.; Grubbs, R.H. *J. Org. Chem.* **1985**, *50*, 2386.
11. The α-configuration of compounds **7**, **13** and **15** was *inter alia* assigned on the basis of the characteristic chemical shift (3.9-4.1 ppm) and pattern (dd, $J_{3,4}\approx J_{4,5}$=9.0-9.5 Hz) of the proton at the C-4 position. See also: Nicotra, F.; Panza, L.; Russo, G. *Tetrahedron Lett.* **1991**, *32*, 4035.
12. Satisfactory elemental analytical data were obtained for compounds **2**, **5**, **7**, **10**, **12**, **13**, **14**, **15**, **16**, **17** and **19**. Optical rotations were measured in $CHCl_3$ (c 1) unless stated otherwise.
    Relevant ^1H NMR data (300 MHz; δ in ppm) of compounds **2**, **5**, **7**, **13**, **17** and **19**:
    2: δ 4.9-4.5 (AB, 2H, -$C\underline{H}_2CPh_3$; J=9.7 Hz); 3.95-3.75 (AB, 2H, H_1+$H_{1'}$; J=11.2 Hz), 3.55-3.35 (m, 2H, $C\underline{H}_2P$). **5**: δ 6.95 (m, 2H, =$C\underline{H}_2$). **7**: δ 4.1 (m, 4H, 2x $C\underline{H}_2CH_3$), 3.9 (dd, 1 H, H_4); 3.8-3.7 (m, 2H, $C\underline{H}_2P$), 3.6-3.4 (AB, 2H, $C\underline{H}_2I$; J=10.9 Hz), 1.2 (t, 6H, 2x $C\underline{H}_3$). **13**: δ 4.5 (AB, 2H, $C\underline{H}_2CPh_3$), 4.0 (dd, 1H, H_4), 3.5 (AB, 2H, H_1+$H_{1'}$; J=11 Hz). 1.0 (s, 9H, 3x $C\underline{H}_3$-TBDPS). **15**: δ 4.1 (m, 4H, 2x $C\underline{H}_2CH_3$), 3.95 (dd, 1H, H_4), 3.9-3.6 (AB, 2H, H_1+$H_{1'}$; J=11.0 Hz), 1.2 (m, 6H, 2x $CH_2C\underline{H}_3$). **17**: 3.32 (s, 3H, $OC\underline{H}_3$). **19**: δ 4.6-4.3 (AB, 2H, $C\underline{H}_2CPh_3$; J=9.6 Hz), 4.1-4.2 (m, 4H, $OC\underline{H}_2CH_3$), 3.7 (s, 1H, $OC\underline{H}_3$), 3.7-3.2 (m, 4H, H_1+$H_{1'}$, $C\underline{H}_2P$), 2.0-1.8 (3x s, 9H, 3x $C\underline{H}_3$, acetyl), 1.2 (m, 6H, 2x $CH_2C\underline{H}_3$).
    Relevant ^{13}C {^1H} NMR data (50.1 MHz; δ in ppm) of compounds **2**, **5**, **7**, **13**, **14**, **15** and **17**:
    2: δ 177.5 ($\underline{C}$=O), 146.2 (C_q, $C\underline{Ph}_3$), 101.0 (d, C_2; $J_{C\text{-}O\text{-}C\text{-}P}$=10.0 Hz), 79.3 ($\underline{C}H_2CPh_3$), 70.2 ($C_1$), 59.8-57.9 ($\underline{C}H_2P$ + $\underline{C}Ph_3$). **5**: δ 156.5 (C_2), 94.4 (C_1). **7**: δ 100.4 (d, C_2; $J_{C\text{-}O\text{-}C\text{-}P}$=10.3 Hz), 62.5 (m, $\underline{C}H_2CH_3$ + C_7), 56.8-53.4 (d, $\underline{C}H_2P$; $J_{C\text{-}P}$=172 Hz), 16.1 (d, $\underline{C}H_3$; $J_{P\text{-}O\text{-}C\text{-}C}$=6.0 Hz). **13**: δ 145.5 (C_q, $C\underline{Ph}_3$), 97.6 (C_2), 79.4 ($\underline{C}H_2CPh_3$), 72.1 (C_1), 57.6 (C_q, $\underline{C}Ph_3$). **14**: δ 115.5-111.0 (d, C_2; $J_{C\text{-}P}$=226 Hz). **15**: δ 101.6 (d, C_2; $J_{C\text{-}O\text{-}C\text{-}P}$=10.3 Hz), 71.0 ($C_1$), 63.5-62.4 (m, $\underline{C}H_2CH_3$ + C_7), 57.4-53.9 (d, $\underline{C}H_2P$, $J_{C\text{-}P}$=173 Hz), 16.4 (d, $CH_2\underline{C}H_3$; $J_{P\text{-}O\text{-}C\text{-}C}$=5.9 Hz). **17**: δ 169.5 ($\underline{C}$=O), 102.4 (d, C_2; $J_{C\text{-}O\text{-}C\text{-}P}$=10.3 Hz), 69.8 ($C_1$), 52.3 ($O\underline{C}H_3$).
    Relevant ^{31}P NMR data (δ in ppm) of compounds **2**, **7**, **15**, **16** and **17**:
    2: δ 18.1; **7**: δ 20.5; **15**: δ 20.4; **16**: δ 20.4; **17**: δ 20.9.
13. It was also established that nucleophilic substitution could not be effected, in contrast with expectation, by cesium acetate in HMPA. See ref. 6 (Haudrechy *et al.*).
14. Still, W.C. *J. Am. Chem. Soc.* **1978**, *100*, 1481.
15. Seyferth, D.; Andrews, S.B. *J. Organomet. Chem.* **1971**, *30*, 151.
16. Lactone **12** was prepared by regioselective silylation (*tert*-butyldiphenylsilyl chloride/pyridine) of 2,3,4-

tri-*O*-benzyl-α/ß-D-glucopyranose followed by Swern oxidation.

17. Rosenbrook, Wm., Jr.; Riley, D.A.; Lartey, P.A. *Tetrahedron Lett.* **1985**, *26*, 3.
18. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.
19. Attempts to convert **16** in the carboxylate methyl ester derivative **17** *via* the two-step method of McDonald *et al.* (i.e. Swern oxidation of HO-7 and subsequent NIS/MeOH mediated conversion of the resulting aldehyde function) were not successful. See: McDonald, C.; Holcomb, H.; Kennedy, K.; Kirkpatrick, E.; Leathers, T.; Vanemon, P. *J. Org. Chem.* **1989**, *54*, 1213.
20. Fügedi, P. *J. Carbohydr. Chem.* **1987**, *6*, 377.
21. McKenna, C.E.; Higa, M.T.; Cheung, N.H.; McKenna, M.C. *Tetrahedron Lett.* **1977**, 155.