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Preparation and NMR Spectroscopic Studies of the Glucuronides of Irbesartan

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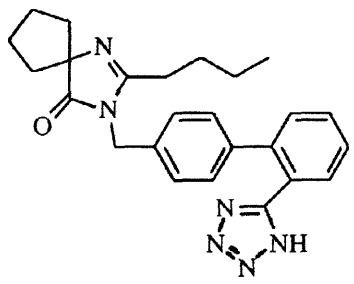
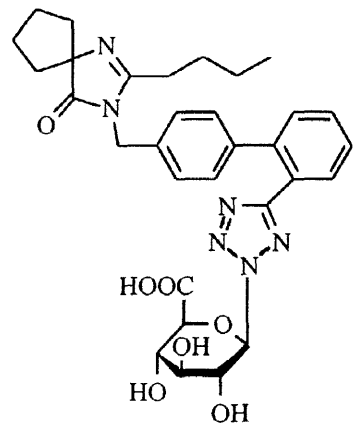
Abstract: The preparation of N1- and N2- glucuronides of Irbesartan (SR47436) is described. The regiochemistry of the products was established from NMR spectroscopic studies, and the N2-glucuronide was identical to material obtained from natural sources.

Keywords: angiotensin II antagonist; tetrazole glucuronide; nÖe enhancement; $^{13}C/^{1}H$ correlation.

Irbesartan (SR47436, BMS 186295) **1** is a highly potent and specific AT1 angiotensin II antagonist, recently introduced to the market.[1] The 5-(biphenyl-2-yl)tetrazole sub-unit is common to a number of angiotensin II inhibitors,[2] and glucuronidation of the tetrazole is a major metabolic pathway in all the cases reported.[3-5] NMR spectroscopic experiments have indicated that N2- of the tetrazole is the predominant site of glucuronidation in one case,[4] consistent with the general course of the reaction of tetrazoles with electrophiles.[6] In the case of **1**, the N-2 glucuronide **2** has been identified as a metabolite and has been prepared by incubation of **1** with D-glucuronic acid in microsomal fractions.[5] However, the quantities available by this route were insufficient to enable definitive assignment of the regiochemistry or detailed examination of its pharmacological profile, and so a synthesis was necessary.



1



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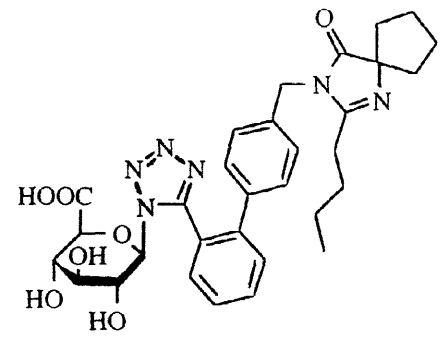

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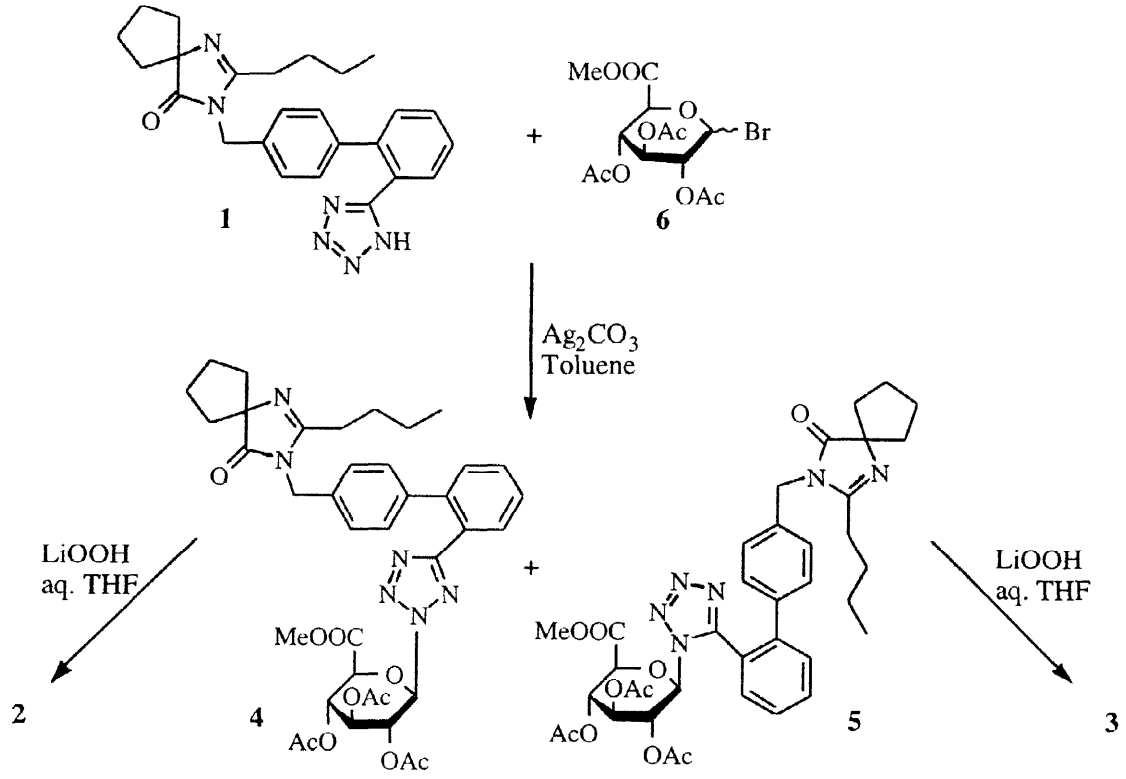

Scheme 1. Synthesis of Glucuronides **2** and **3**.

Glucuronides **2** and **3** were synthesised in two steps. Protected glucuronides **4** and **5** were prepared by coupling of **1** with the bromo-sugar **6**,[7] and were separated by chromatography. Treatment of **4** with excess aqueous base at room temperature failed to deprotect the material completely whilst, on heating, the sugar unit was removed at a rate greater than that of deprotection, returning **1**. Effective deprotection was, however, achieved using lithium hydroperoxide in aqueous THF.[8] Glucuronide **2**, prepared by the latter method, was identical to material prepared by incubation with macaque liver homogenates,[5] as determined by HPLC and NMR spectroscopy. Deprotection was also effected by sodium methoxide in tetrahydrofuran (the methyl ester presumably being cleaved during aqueous work-up), but this was accompanied by isomerisation, so that either **4** or **5** gave rise to mixtures of **2** and **3**. Comparable cationotropic processes have been observed with triazoles.[9]

Structures **2-5** were assigned unambiguously following NMR spectroscopic studies of **4** and **5**, the ^{1}H and ^{13}C chemical shifts being recorded in Tables 1 and 2 respectively. Carbon 5 (*21* in Figure 1) of the tetrazole ring can be assigned with certainty by examination of the ^{3}J carbon/proton correlation (HMBC) spectrum in which coupling to the proton *ortho-* to the tetrazole is observed. Thus, the resonances of carbon *21* in **2** and **4** appear at 166.5 ppm and 166.1 ppm respectively, while those in **5** and **3** occur at 155.5 ppm and 156.5 ppm respectively. The latter are comparable to the chemical shift of 155 ppm observed in the spectrum of **1**. These chemical shift values are also in the same order as those reported previously for C-5 of 2- and 1-methyltetrazoles respectively.[10] In the ^{3}J HMBC spectrum of **5**, C-*21* is coupled to the anomeric proton (*n*). Since this can occur in this isomer only (and indeed, no such coupling is observed in the HMBC spectrum of **3**), this constitutes an unequivocal assignment of the structures of **4** and **5**, and therefore of **2** and **3**.

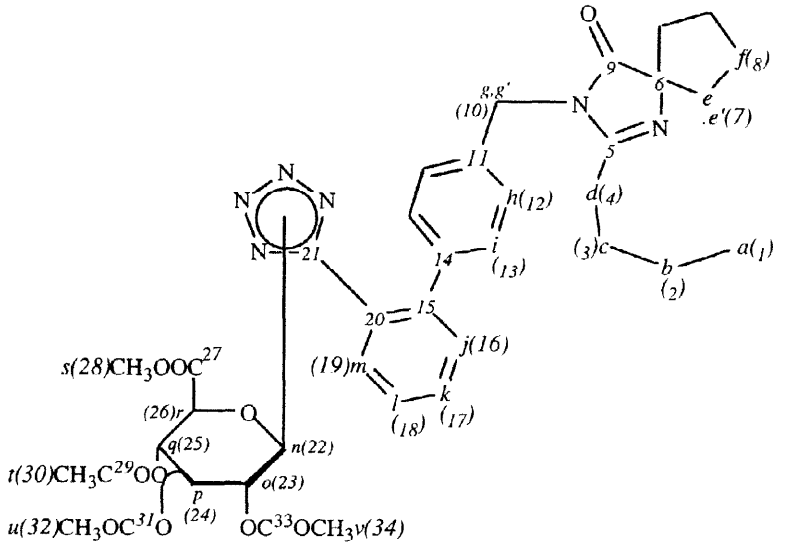

Figure 1. Schematic representation of positions in **1** - **5**

Table 1. ^{1}H NMR data for Protected Glucuronides **4** and **5** (in $CDCl_3$).

Proton	**4** δ_H/PPM	multiplicity (*J*/Hz)	^{1}H correlation (from COSY)	**5** δ_H/PPM	multiplicity (*J*/Hz)	^{1}H correlation (from COSY)
a	0.85	t (7.3)	*b*	0.83	t (7.4)	*b*
b	1.31	sextet (7.6)	*a,c*	1.28	sextet (7.4)	*a, c*
c	1.56	quintet (7.4)	*b,d*	1.55	quintet (8.4)	*b, d*
d	2.32	t (7.7)	*c*	2.34	t (7.7)	*c*
e,	1.80	m (2H)	*e',f*	-	-	-
e',f	1.85-2.05	m (6H)	*e*	1.80-2.05	m (e,e',f)	-
g	4.67	s	-	4.52, 4.66	2d (16.2; *g,g'*)	*g,g'*
h	7.06	d (8.4)	*i*	7.05	d (8.4)	*i*
i	7.13	d (8.4)	*h*	7.12	d (8.4)	*h*
j	7.39	dd (7.6, 1.1)	*k*	7.56	ddd (7.9, 1.3, 0.6)	*k*
k	7.52	dt (7.4, 1.4)	*j*	7.69	ddd (7.9, 7.4, 1.5)	*j*
l	7.45	dt (7.4, 1.5)	*m*	7.53	ddd (7.4, 7.3, 1.3)	*m*
m	7.81	dd (7.6, 1.1)	*l*	7.45	ddd, (7.7, 1.4, 0.5)	*l*
n	6.02	d (9.3)	*o*	5.18(0)	d (9.2)	*o*
o	5.76	t (9.2)	*n,p*	5.65	t (9.0)	*n*
p	5.43	t (9.2)	*o,q*	5.18(2)	t (9.5)	-
q	5.37	t (9.6)	*p,r*	5.23	t (9.5)	*r*
r	4.29	d (9.6)	*q*	3.77	d (9.6)	*q*
s	3.73	s	-	3.74	s	
t	2.04/2.03	s	-	1.99/1.98	s	-
u	2.03/2.04	s	-	1.98/1.99	s	-
v	1.80	s	-	1.74	s	-

Table 2. ^{13}C NMR Data for **1** and protected glucuronides **4** and **5**.

Carbon	**1** (CD_3OD)	**4** ($CDCl_3$)			**5** ($CDCl_3$)		
	δ_C/PPM	δ_C/PPM	HMQC	HMBC	δ_C/PPM	HMQC	HMBC
1	13.6	13.7	*a*	*b,c*	13.6	*a*	*b,c*
2	21.5	22.3	*b*	*a,c,d*	22.3	*b*	*a,c*
3	26.6	27.8	*c*	*a,b,d*	27.8	*c*	*a,b,d*
4	27.5	28.8	*d*	*b,c*	28.5	*d*	*b,c*
5	161.1	161.7	-	*c,d,g*	ca. 164[b]	-	*c,d,g*
6	75.8	76.4	-	-	76.7	-	-
7	36.8	37.4	*e,e'*	*f*	37.4	*e,e'*	-
8	25.4	26.1	*f*	*e,e'*	26.0	*f*	*e,e'*
9	185.7	186.7	-	*g*	186.7	-	*g,g'*
10	42.2	43.4	g	h	43.3	*g,g'*	*h*
11	136.3	135.4	-	*g,i*	136.8	-	*g,g'i*
12	126.3	126.5	*h*	*g,h,i*	127.5	*h*	*g,g',h,i*
13	129.2	129.7	*i*	*h,i*	129.3	*i*	*h*
14	141.0	140.2	-	*h,j*	138.0	-	*h,j*
15	138.3	141.5	-	*i,k,m*	141.6	-	*i,k,m*
16	130.5	130.9	*j*	*obscured*	130.9	*j/m*	*k/l*
17	130.1	130.4	*k*	*obscured*	132.2	*k*	*m*
18	131.1	127.7	*l*	*j*	128.2	*l*	*j*
19	127.8	130.7	*m*	*obscured*	130.9	*j,m*	*k/l*
20	123.4	125.3	-	*j,l*	121.4	-	*j,k,l*
21	155.0	166.1	-	*m*	155.5	-	*j,m,n*
22		86.4	*n*	*o,r*	82.3	*n*	*o,r*
23		69.7	*o*	*n*	70.0	*o*	*v*
24		72.2	*p*	*n,o,q,r*	72.0	*p*	*o,q,r,u*
25		68.6	*q*	*p,r*	68.4	*q*	*t*
26		74.8	*r*	*n,q*	74.9	*r*	*n,p,q*
27		165.9	-	*q,r,s*	165.6	-	*q,s*
28	-	53.1	*s*	-	53.3	*s*	-
29	-	169.2	-	*q,t*	169.0	-	*q,t*
30[a]	-	20.5	*t/u*	-	20.5	*t/u*	-
31	-	170.0	-	*p,u*	170.1	-	*p,u*
32[a]	-	20.4	*u/t*	-	20.4	*u/t*	-
33	-	168.1	-	*o,v*	167.9	-	*o,v*
34		20.1	*v*	-	20.1	*v*	-

a. assignments of signals due to *30* and *32* may be reversed. b. chemical shift taken from HMBC spectrum

Upon pre-irradiation at the resonance frequency of the anomeric proton in the ^{1}H NMR spectra of **4** and **5**, nÖe enhancements were observed for the signals of protons *p* and *r*. Both protons are on the same face of the pyran ring and the nÖe experiments demonstrate that both species are β-glucuronides. Also, in the spectrum of **5**, signals of protons *m* and *i* on the biphenyl unit were enhanced in the nuclear Överhauser experiment. This is consistent with the observations cited by Stearns and co-workers as evidence in the assignment of N-2 regiochemistry for the natural glucuronide of DuP 753.[4] Molecular mechanics minimisation studies[11] indicate that the anomeric proton in **5** may approach within 1.01 Å of proton *m*, whilst a minimum distance of 4.4 Å may be attained in **4**. The observed nÖe enhancements provide strong support for the regiochemistry assigned, but definitive evidence is provided by the HMBC experiment. The benzylic protons (*g*) which, in the spectrum of **4**, are observed as a singlet, give rise to an AB quartet in the spectrum of **5**. This proton non-equivalence may be ascribed to steric congestion in **5**. In the ^{1}H NMR spectrum of **5**, three resonances occur between 5.10 and 5.25 ppm. In order to confirm the multiplicities (and assignments) of these signals, double irradiation experiments were carried out involving irradiation of signals at 3.77 and 5.65 ppm. When the former was decoupled, the signal at 5.23 ppm reduced to a doublet, while decoupling of the latter reduced the signals at 5.180 and 5.182 ppm to a singlet and a doublet respectively. This gives added confidence to the assignments in Table 2.

Experimental

Analyses were carried out by CHN Analysis Ltd., South Wigston, Leicester. Melting points were recorded using a Büchi 510 capillary melting point apparatus. Infrared spectra were recorded using a Nicolet 510 FTIR spectrometer. Mass spectra were recorded using a VG Autospec magnetic sector instrument at the University of York. NMR spectra were recorded using Jeol GSX-270 and Lambda-400 spectrometers.

Protected Glucuronides 4 and 5. A suspension of **1** (3.394 g, 8 mmol) and silver carbonate (1.399 g, 5 mmol) in toluene (40 ml) containing methyl tri-*O*-acetyl-2-bromo-β-D-glucopyranuronate (3.004 g)[7] was stirred at 100°C with protection from light for 18 h. The mixture was filtered through celite, the filter pad was washed with a little ethyl acetate, and the combined filtrates were concentrated under reduced pressure. Column chromatography of the residue on silica gel, eluting with 2:1 ethyl acetate - hexane, gave **4** (3.515 g, 59%) as a white solid, M.P. 89.5-90°C. ν_{max} 2959, 1760, 1723, 1634 cm^{-1}. MS(CI+) 745 ([MH]$^+$), 717 ([MH-CO]$^+$); HRMS Calc. for $C_{38}H_{45}N_6O_{10}$ 745.3197; Found: 745.3201. followed by **5** (1.776 g, 30%) as a white foam, MS(CI+) 745 ([MH]$^+$), 717 ([MH-CO]$^+$); HRMS Calc. for $C_{38}H_{45}N_6O_{10}$ 745.3197; Found: 745.3196 and **1** (0.244 g, 7%).

N-2 Glucuronide 2. Hydrogen peroxide (30%, 10.4 ml) was added to a stirred suspension of lithium hydroxide (3.86 g) in water (40 ml), forming a solution within 3-4 minutes. The mixture was stirred for a further 10 min, then added to a stirred solution of **4** (3.515 g, 4.8 mmol) in THF (130 ml). A precipitate formed within 15 min, and the mixture was quenched by addition of sodium thiosulfate after 75 min, the reaction being complete as judged by t.l.c. after 45 min. The mixture was acidified to pH 2.5 with 1M hydrochloric acid and crude product was extracted three times with ethyl acetate. The combined extracts were dried (Na_2SO_4) and solvent was removed under reduced pressure to leave chromatographically pure glucuronide **2** (2.978 g, 100%) which was recrystallised from ethanol to give white crystals, dec. 187-190°C (without melting). Found: C, 59.09; H, 6.22; N, 12.74. $C_{31}H_{36}N_6O_7.1.5H_2O$ requires C, 58.94; H, 6.22; N, 12.74 %. ν_{max} 3397, 2959, 1772, 1725, 1630 cm^{-1}. δ_H (CD_3OD) 0.88 (t, *a*), 1.32 (m, *b*), 1.51 (m, *c*), 1.85

(2H, m, *e'*), 1.95 (6H, m, *e,f*), 2.43 (t, *d*), 3.61 (dd, *p*), 3.69 (dd, *q*), 4.07 (d, *r*), 4.12 (dd, *o*), 4.77 (s, *g*), 5.86 (d, *n*), 7.10 (d, *h*), 7.16 (d, *i*), 7.46 (d, *j*), 7.53 (dd, *l*), 7.61 (dd, *k*), 7.84 (d, *m*). δ_C (CD_3OD) 14.0 (*1*), 20.9 (*2*), 23.2 (*8*), 27.0 (*3*), 28.5 (*4*), 38.4 (*7*), 44.3 (*10*), 72.7 (*23*), 73.0 (*25*), 77.1 (*26*), 77.9 (*24*), 78.9 (*6*), 90.7 (*22*), 127.2 (*20*), 127.6 (*12*), 128.85 (*19*), 131.0 (*13*), 131.6 (*18*), 131.85 (*16 & 17*), 136.55 (*11*), 141.65 (*15*), 143.0 (*14*), *ca.* 162 (*5*), 166.55 (*21*), 175.6 (*27*), 187.2 (*9*). MS(FAB+) 627 (13%, $[MNa]^+$), 605 (50%, $[MH]^+$), 154 (100%), 137 (73%), 136 (62%). HRMS Calc. for $C_{31}H_{36}N_6O_7$ 605.2724; Found: 605.2721.

N-1 Glucuronide 3. Hydrolysis of **5** in the manner described above gave **3** (100%) as a gum. MS(FAB+) 605 (5%, $[MH]^+$), 154 (100%), 137 (70%), 136 (70%). δ_H (CD_3OD) 0.88 (t, *a*), 1.33 (m, *b*), 1.54 (m, *c*), 1.80 (2H, m, *e'*), 1.95 (6H, m, *e,f*), 2.42 (t, *d*), 4.24 (dd, *q*), *ca.* 4.3 (*p*), 4.45 (d, *r*), 4.76 (s, *g*), 5.87 (dd, *o*), 6.23 (d, *n*), 7.12 (d, *h*), 7.18 (d, *i*), 7.48 (2d, *j,m*), 7.53 (dd, *l*), 7.60 (dd, *k*). δ_C (CD_3OD) 14.0 (*1*), 23.2 (*2*), 24.2 (*3*), 27.0 (*8*), 28.75 (*4*), 38.4 (*7*), 44.25 (*10*), 70.6 (*23*), 72.0 (*25*), 76.3 (*26*), 77.5 (*6 & 24*), 89.95 (*22*), 127.15 (*20*), 127.6 (*12*), 128.8 (*19*), 131.0 (*13*), 131.55 (*18*), 131.65 (*17*), 131.9 (*16*), 136.8 (*11*), 141.6 (*15*), 142.95 (*14*), 156.5 (*21*), 174.7 (*27*), 187.95 (*9*); no resonance observed for C*5*. HRMS Calc. for $C_{31}H_{36}N_6O_7$ 605.2724; Found: 605.2731.

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References

1. Bernhart, C., Perreaut, P.M., Ferrari, B.P., Muneaux, Y.A., Assens, J.-L.A., Clément, J., Haudricourt, F., Muneaux, C.F., Taillades, J.E., Vignal, M.-A., Gougat, J., Guiraudou, P.R., Lacour, C.A., Roccon., A., Cazaubon, C.F., Brelière, J.-C., Le Fur, G., and Nisato, D., *J. Med. Chem.*, **1993**, *36*, 3371.
2. Wexler, R.R., Greenlee,W.J., Irvin, J.D., Goldberg, M.R., Prendergast, K., Smith, R.D., and Timmermans, P.B.M.W.M., *J. Med. Chem.*, **1996**, *39*, 625.
3. e.g. Bowers, G.D., Eddershaw, P.J., Hughes, S.Y., Manchee, G.R., and Oxford, J., *Rapid Commun. Mass Spec.*, **1994**, *8*, 217; Colletti, A.E., and Krieter, P.A., *Drug Metab. Dispos.*, **1994**, *22*, 183; Nohara, A., Kuriki, H., Ishiguro, T., Saijo, T., Ukawa, K., Maki, Y., and Sanno, Y., *J. Med. Chem.*, **1979**, *22*, 290; Ellingboe, J.W., Collini, M.D., Quagliato, D., Chen, J., Antane, M., Schmid, J., Hartupee, D., White, V., Park, C.H, Tanikella, T., and Bagli, J.F., *J. Med. Chem.*, **1998**, *41*, 4251.
4. Stearns, R.A., Doss, G.A., Miller, R.R., and Chiu, S.-H.L., *Drug Metab. Distrib.*, **1991**, *19*, 1160.
5. Perrier, L., Bourrié, M., Marti, E., Tronquet, C., Massé, D., Berger, Y., Magdalou, J., and Fabre, G., *J. Pharmacol. Exp. Ther.*, **1994**, *271*, 91.
6. Kolbobskii, G.I., and Ostrovskii, V.A., *Russ. Chem. Revs.*, **1994**, *63*, 797; Butler, R.N., in Katritzky, A.R., and Rees, C.W., "Comprehensive Heterocyclic Chemistry" (Pergamon, 1984), vol. 5, p. 791.
7. Bollenback, G.N., Long, J.W., Benjamin, D.G., Lindquist, J.A., *J. Am. Chem. Soc.*, **1955**, *77*, 3310.
8. Evans, D.A., Britton, T.C., and Ellman, J.A., *Tetrahedron Lett.*, **1987**, *28*, 6141.
9. Katritzky, A.R., Darabantu, M., Aslan, D.C, and Oniciu, D.C., *J. Org. Chem.*, **1998**, *63*, 4323.
10. Elguero, J., Marzin, C., and Roberts, J.D., *J. Org. Chem.*, **1974**, *39*, 357.
11. Calculations were carried out using the Amber forcefield in Macromodel.™