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Synthesis of a Morphine-6-Glucuronide Hapten, N-(4-Aminobutyl)normorphine-6-Glucuronide, and Related Haptens

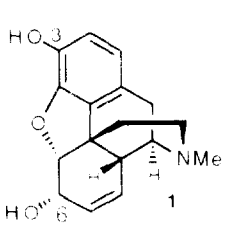
Richard T. Brown*[a], Neil E. Carter[a], Keith W. Lumbard[b] and Feodor Scheinmann[b]

[a] *Department of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, UK.*

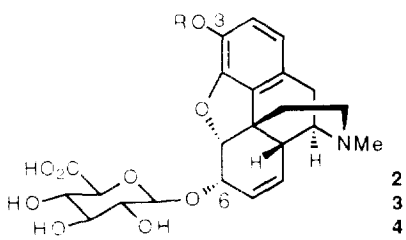
[b] *Salford Ultrafine Chemicals and Research Ltd., Synergy House, Guidhall Close, Manchester Science Park, Manchester M15 6SY, UK*

Abstract For use as a hapten in the radioimmunoassay of morphine-6-glucuronide **2** (M6G), N-(4-aminobutyl)normorphine-6-glucuronide **12** has been prepared, together with related haptens, using the imidate coupling method developed for synthesis of M6G; a rebuttal of recent critical comments on our synthesis of M6G is included

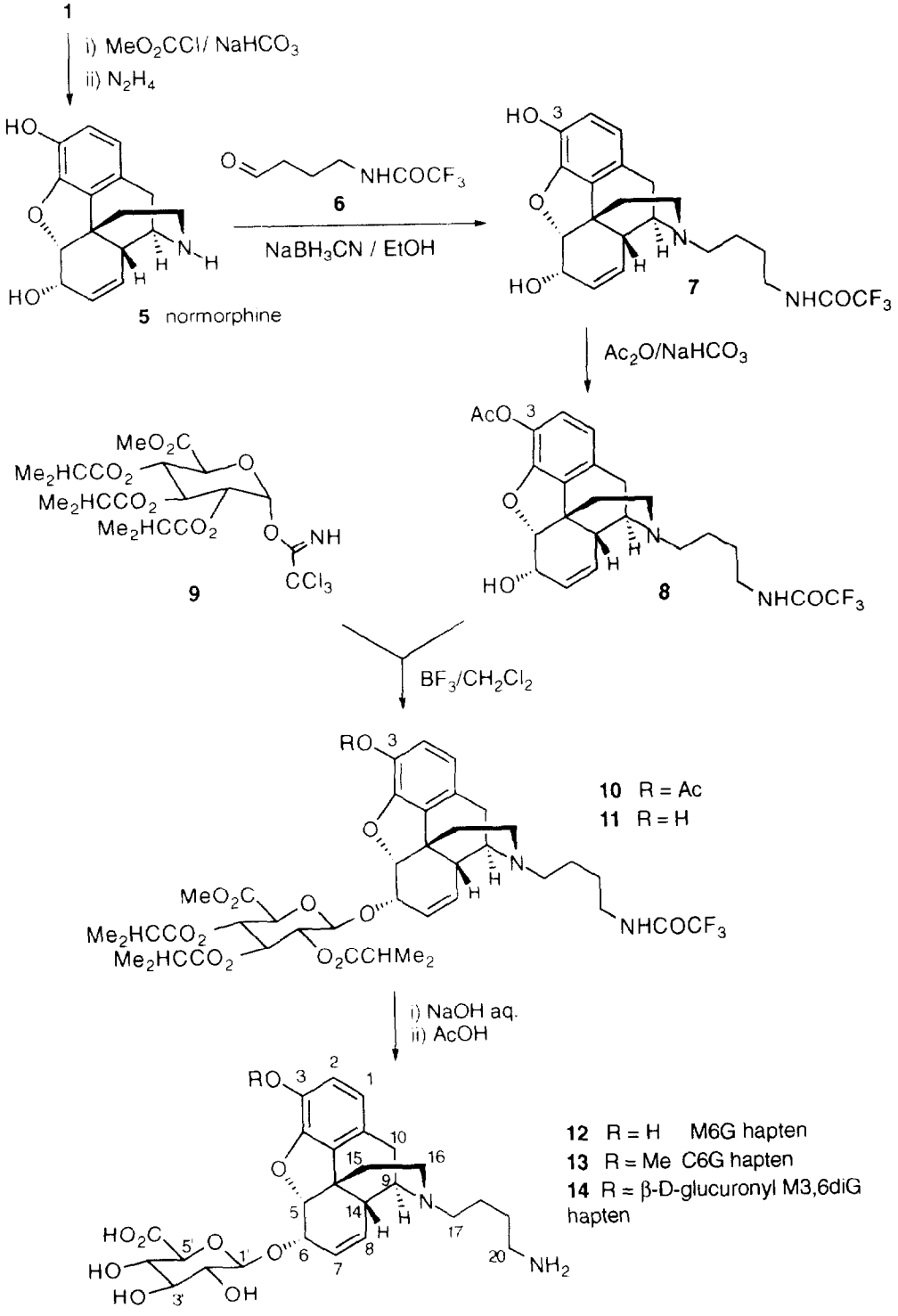
Since it was established that morphine-6-β-D-glucuronide **2** (M6G) is not only a metabolite of morphine **1** but also has greater analgesic activity[1,2], substantial amounts have been required for clinical trials and evaluation. In our hands, coupling of the sugar moiety to morphine by the Koenigs-Knorr reaction, *i.e.* heating methyl 2,3,4-triacetyl-1-α-bromoglucuronate and 3-acetylmorphine in benzene with Ag_2CO_3, as originally used by Yoshimura *et al.*[3] and subsequently by others[4], was adequate for gram quantities of M6G, but gave very variable yields ranging from zero to *ca.* 70%. The rather unreliable yield and the heterogeneous nature of the reaction rendered it unsuitable for scaling up to the kilogram level, and a further serious drawback was the use of heavy metal ions, which must not be present even in trace amounts in potential medicines. Hence we developed homogeneous procedures[5] avoiding these problems where we use acid catalysed coupling of glucuronate ester derivatives with 3-acylmorphines to give only the β-glucuronide routinely in yields of 50-69%. Alkaline hydrolysis and subsequent precipitation by neutralisation with [*not* crystallisation from[6]] acetic acid affords in *ca.* 90% yield, routinely on a 25 gram scale, pure M6G m.p. 249-250°(dec.) which we have supplied to various research laboratories. Likewise, pure morphine-3,6-diglucuronide **3** (M3,6diG) has been prepared from morphine in 35-50% overall yield, and codeine-6-glucuronide **4** (C6G) from codeine in 70% yield[7]. These results are contrary to statements made about both our procedure and Yoshimura's by Sainsbury in a recent publication[6], where the proffered alternative is effectively only a lower-yielding (45%) variant of the Koenigs-Knorr coupling, little different from previous versions.



1



2 R = H
3 R = β-D-glucuronyl
4 R = Me


1
i) MeO2CCl/ NaHCO3
ii) N2H4
5 normorphine
6
NaBH3CN / EtOH
7
Ac2O/NaHCO3
8
9
BF3/CH2Cl2
10 R = Ac
11 R = H
i) NaOH aq.
ii) AcOH
12 R = H M6G hapten
13 R = Me C6G hapten
14 R = β-D-glucuronyl M3,6diG hapten

As a result of the clinical interest in M6G there was a requirement for a hapten to raise a specific antiserum to be used in its radioimmunoassay. To this end we have devised a synthesis of N-(4-aminobutyl)-normorphine-6-glucuronide **12**, modelled on our synthesis of M6G using the imidate coupling method[8]. Thus, normorphine **5** was made in 71% yield from morphine by heating with methyl chloroformate and $NaHCO_3$ under reflux, followed by heating with aqueous hydrazine[9]. 4,4-Diethoxybutanamine was reacted with ethyl trifluoroacetate in presence of triethylamine for two days at room temperature to give 4,4-diethoxy-1-trifluoroacetamidobutane, which was hydrolysed overnight with aqueous acetic acid to afford 4-trifluoroacetamidobutanal **6** in 48% overall yield[10]. Reductive coupling of the aldehyde and normorphine with $NaCNBH_3$ in ethanol then led to the morphine hapten amide **7** $[\alpha]_D$ -94° (c 1.1 CH_2Cl_2) (69% yield after chromatography on Sephadex LH20), characterised *inter alia* by a molecular ion at *m/z* 438.1766 ($C_{22}H_{25}N_2O_4F_3$), a trifluoroacetamide C=O i.r. band at 1754 cm^{-1}, and additional signals for the butanamide side chain at δ 3.15 (t, H_2-20), 2.4 (t, H_2-17) and 1.4 (m, H_2-18/H_2-19) in the ^1H n.m.r. spectrum[11]. Careful treatment with $NaHCO_3$ and acetic anhydride to avoid cleavage of the trifluoroacetamide selectively formed the 3-acetate **8** (71% yield; M^+ 480.1872 = $C_{24}H_{27}N_2O_5F_3$).

By ring-opening D-glucurono-6,3-lactone with sodium methoxide, followed by acylation with isobutyryl chloride in pyridine, the 1β anomer of methyl 1,2,3,4-tetra-O-isobutyryl-D-glucopyranuroate was prepared in 49% yield, m.p.127°C after recrystallisation from petrol. Selective hydrolysis of the C-1 isobutyrate ester in CH_2Cl_2 with ammonia gave the corresponding hemiacetal (73% yield), which on treatment with CCl_3CN and anhydrous Na_2CO_3 afforded ethyl 2,3,4-tri-O-isobutyryl-1-α-trichloroacetimidoyl-D-glucopyranuroate **9**, m.p. 81°C from isopropanol (85% yield)[5]. The imidate was a single α-anomer, as indicated by the H-1 n.m.r. doublet at δ 6.66 with an axial-equatorial coupling of 3.7 Hz.

The 3-acetylmorphine hapten amide **8** was then reacted (6 mmolar scale) in presence of BF_3 with an excess of imidate **9**, the function of the relatively hindered isobutyrate esters being to minimise competitive 6-transacylation whilst retaining β-stereoselectivity and ease of removal. Subsequent chromatography on silica in CH_2Cl_2/MeOH unfortunately resulted in partial 3-deacetylation to give largely mixtures of the expected product **10** and the corresponding phenol **11** (*ca.* 40% combined yield from **8**), but from one fraction the pure phenol was isolated (610 mg, 11% yield) and identified by spectra characteristic of both sugar ester and morphine moieties: M^+ 840.3748 = $C_{41}H_{55}N_2O_{13}F_3$; λ_{max} 281 nm shifting to 297 nm with alkali; ν_{max} 3348 (OH), 1752 (C=O) cm^{-1}; ^1H n.m.r. (CD_3OD): δ 6.69 (d *J* 8, H-2), 6.51 (d *J* 8, H-1), 5.61(t *J* 10, H-3'), 5.50 (bd *J* 9, H-8), 4.42 (d *J* 8, H-5'), 3.47 (s, CO_2Me), 1.23-1.02 (3 x 6H d, $COCHMe_2$). Finally, alkaline hydrolysis of **11** or the product mixture and neutralisation with acid afforded in *ca.* 85% yield the desired hapten **12**, N-(4-aminobutyl)normorphine-6-β-D-glucuronide, as an amorphous powder: (FAB) M+1 519.2359 = $C_{26}H_{35}N_2O_9$; ^1H n.m.r. (300 MHz, D_2O): δ 6.67 (d *J* 8, H-2), 6.56 (d *J* 8, H-1), 5.74(bd *J* 10, H-8), 5.30 (d *J* 10, H-7), 5.12 (d *J* 6, H-5), 4.67 (d *J* 8, H-1'), 4.48 (bs, H-6), 4.02 (bs, H-9), 3.71 (d *J* 8, H-5'), 3.52 (dt *J* 8, H-4',3'), 3.36 (t *J* 8, H-2'), 3.2-2.6 (9H m, H_2-10, 16, 17, 20, H-14), 2.20 (bt *J* 12, H-15_{ax}), 1.90 (bd *J* 12, H-15_{eq}), 1.73 (4H bs, H_2-18, 19).

In subsequent work by Chapman *et al.* the hapten **12** was coupled to bovine thyroglobulin to afford a conjugate which raised a specific antiserum for M6G in a rabbit. This antiserum had negligible cross-reactivity with morphine, M3G and related compounds, and was used to develop a simple, sensitive, rapid and effective radioimmunoassay for M6G in human plasma[12].

The morphine hapten amide **7** also provided access to a series of related opiate haptens. Thus, methylation with diazomethane gave the corresponding codeine hapten (FAB M+1 453.1991 = $C_{42}H_{57}N_2O_{13}F_3$) which on coupling with the imidate **9** afforded, in 47% yield after chromatography, the precursor of C6G hapten **13** (FAB M+1 853.3735 = $C_{42}H_{56}N_2O_{13}F_3$). Again, direct coupling of **7** with **9** led to the precursor of M3,6diG hapten **14** in 50% yield (FAB M+1 1239.5290 = $C_{60}H_{82}N_2O_{22}F_3$). Catalytic hydrogenation of all products gave near-quantitative yields of the corresponding 7,8-dihydro derivatives. All of these compounds have potential as haptens for development of a specific radioimmunoassay of the corresponding opiate as with **12**.

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Dedication To Emeritus Professor Hans Suschitzky on the occasion of his 80th birthday.

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11. **7** ^{1}H n.m.r. (500 MHz, CD_3OD): δ 6.36 (d *J* 7, H-2), 6.25 (d *J* 7, H-1), 5.43(bd *J* 10, H-8), 5.15 (dt *J* 10, 3, H-7), 4.62 (d *J* 7, H-5), 4.09 (bs, H-6), 3.32 (q *J* 3, H-9), 3.14 (t *J* 7, H_2-20), 2.78 (d *J* 18, H-10_{eq}), 2.52 (dd *J* 13, 6, H-15_{ax}), 2.47 (t *J* 3, H-14), 2.39 (t *J* 7, H_2-17), 2.23 (dd *J* 13, 3, H-15_{eq}), 2.17 (dd *J* 18, 7, H-10_{ax}), 1.89 (td *J* 13, 6, H-16_{ax}), 1.60 (bd *J* 13, H-16_{eq}), 1.45-1.37 (m, H_2-18, 19).
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