

SIMPLE METHODS FOR THE LABELLING OF N-METHYL AMINES USING ISOTOPICALLY LABELLED METHYL IODIDE.

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Summary

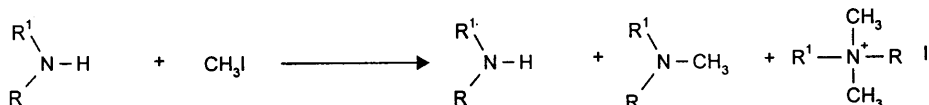
Methods for the synthesis of isotopically labelled N-methyl amines using the target amine and isotopically labelled methyl iodide in a quaternisation/dequaternisation strategy are described. The method has been further refined to allow the synthesis of such amines without any isotopic dilution.

Key Words: Amines, N-Methyl, Isotopic Labelling, Quaternisation, Demethylation.

Introduction

The commercial availability of several isotopically labelled versions of methyl iodide makes isotopic labelling *via* direct methylation of suitable nucleophilic substrates an attractive strategy. For many oxygen, sulphur and non-basic nitrogen nucleophiles this is often a quite straightforward process. However, with amine nucleophiles, direct alkylation usually leads to mixtures of products, with formation of the quaternary ammonium salt as a major product (Scheme 1).

Scheme 1



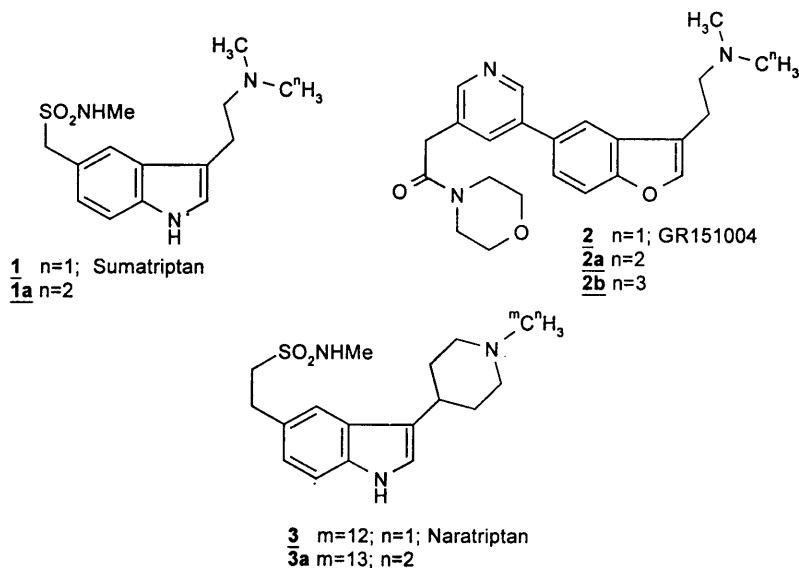
Furthermore, the above method requires the corresponding N-desmethyl amine as the labelling substrate. Such analogues are not always readily available. Strategies to

address these problems were outlined in a poster presented at the 6th International Symposium of The International Isotope Society (1). The studies described here were directed towards the labelling of compounds that have been prepared as part of a programme to discover novel 5HT receptor agonists, potentially useful in the treatment of migraine.

Discussion and Results

Sumatriptan **1** is a selective 5HT₁ agonist, which has proved to be an effective new treatment for migraine (2). In the course of the search for improved analogues, other potential drug candidates have been synthesised, including compounds **2** & **3**.

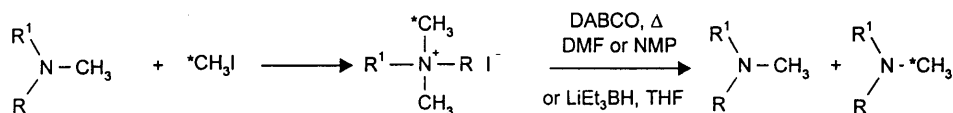
Scheme 2



These compounds contain an N-methylamino function, and therefore potentially could be labelled *via* direct alkylation with isotopically labelled methyl iodide using the strategy outlined in Scheme 3. The method involves quaternisation of the target tertiary amine with isotopically labelled methyl iodide (normally a high-yield reaction taking place under mild conditions) followed by demethylation of the resultant quarternary salt. This may be effected by heating with diazabicyclooctane (DABCO) in dimethylformamide (DMF) or N-methylpyrrolidinone (NMP) at 120-150°C as described by Ho (3a); or by heating with lithium triethylborohydride in THF as reported by Cooke and Parlman (4). Although the

chemical yields from this sequence are often good (ca 65%), there is an inevitable loss of isotopic label that is inherent in the method.

Scheme 3



(* isotopically labelled methyl group: R, R¹ = alkyl, cyclo alkyl, aryl etc, but NOT benzyl or allyl)

The demethylation step leads to the production of a mixture of isotopic and non-isotopic N-methyl tertiary amines in the statistically predicted ratio. Thus whilst trimethylammonium salts retain two thirds of the isotopic label, the corresponding dimethylammonium salts produce amines in which only half the isotopic methyl groups are retained. Nevertheless, this strategy represents a very simple and rapid means of labelling the N-methyl group. The reaction sequence is short (two steps) and simple to carry out, and there is no need for the synthesis of new analogues to use as the labelling substrate since the labelling target also serves as the non-isotopic starting material. Labelled versions of compounds **1** - **3** that have been synthesised using this strategy are tabulated below. (Table 1).

Table 1

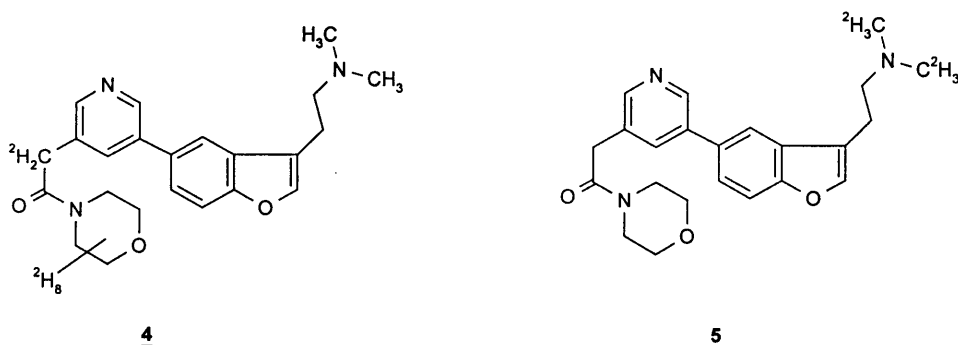
Compound	Label Source	Reaction Conditions (yield)	Isotopic Abundance /Specific Activity
1a	C ² H ₃ I	(i) C ² H ₃ I (1.6 mol.), MeOH/DCM, 20°, 16h, (80%) (ii) DABCO (16 mol), DMF, 130°, 5h, (78%).	67% C ² H ₃ (NMR)
2a	C ² H ₃ I	(i) C ² H ₃ I (1.0 mol.), DCM, 20°, 24h, (84%) (ii) DABCO (5 mol), NMP, 200°, 40 min, (49%).	67% C ² H ₃ (NMR)
3a	¹³ C ² H ₃ I	(i) ¹³ C ² H ₃ I (1.24 mol), acetone, 20°, 18h, (95%). (ii) LiEt ₃ BH, THF, reflux, 21h, (82%).	50% ¹³ C ² H ₃ (NMR)
2b	C ³ H ₃ I	(i) C ³ H ₃ I (1.4 mol), toluene/DCM, 20°, 24h, (48%) (ii) DABCO (12.5 mol), NMP, 200°, 4h, (5%)	39Ci/mmol (81Ci/mmol) [†]

[†] Following isotopic enrichment *via* preparative HPLC (see below).

As predicted, the deuterated sumatriptan **1a** and GR151004 **2a**, retained two thirds of the isotopic label, whereas naratriptan **3a** retained only half of the label in the final product (estimated from the intensity of the N-methyl proton peak in the ¹H NMR spectra).

The tritiated compound **2b** showed initial specific activity considerably lower than that of the methyl iodide used in the synthesis (*ca* 80Ci/mmol). This again reflects the statistical loss of label in the dequaternisation step, possibly exacerbated by residual unreacted starting amine in the quaternary salt used in the dequaternisation step. However, it proved possible to enrich these products to essentially the same isotopic abundance as the starting methyl iodide by exploiting an unusual, but not unknown (5-7), separation of labelled and non-labelled amines on normal-phase HPLC. This separation seems to be confined to compounds in which the isotopic label is adjacent to a *basic* nitrogen centre. A perdeuterated version **4** of GR151004, in which all eight hydrogens on the *amidic* morpholine ring are replaced by deuterium (8), showed no separation from unlabelled drug. In contrast, the trideuterated **2a**, and hexadeuterated **5** analogues (9), showed small, but significant, retention time differences, not only from the unlabelled analogue **2**, but also from each other (Table 2). These observations are in line with previously documented observations (6,7,10) regarding the increased basicity of isotopically labelled amines.

Scheme 4



These differences, also seen with the tritium labelled analogues, were exploited at Amersham International (11) to enrich the specific activity of the labelled GR151004 by preparative HPLC.

In principle, preparative HPLC could also be used to enhance the isotopic content of the deuterium labelled analogues. However, these materials are often prepared for use as internal standards in mass-spectrometric assays, where it is important for assay sensitivity that no extraneous unlabelled material is introduced, and also significant weights (mg as opposed to μg) of standards are required to support major assay programmes. This

mitigated against the use of preparative HPLC, and led to the development of alternative strategies to produce isotopically pure compounds.

Table 2

Compound	Retention Time (system 1)	Retention Time (system 2)
<u>2</u> [² H ₀]GR151004	5.94min	5.59min
<u>2a</u> [² H ₃]GR151004	-	6.05min
<u>6</u> [² H ₆]GR151004	6.89min	6.64min
<u>5</u> [² H ₁₀]GR151004	5.91min	-
<u>1</u> [² H ₀]GR43175	5.67min	-
<u>1a</u> [² H ₃]GR43175	6.28min	-

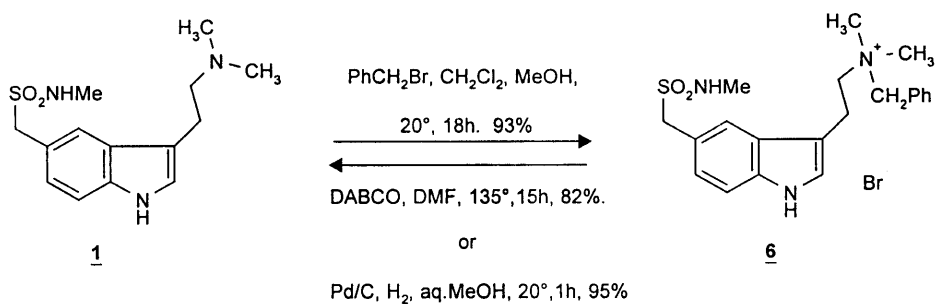
HPLC run on Capital 3 μ SiO₂ column (15x0.46cm) with a flow rate of 1ml/min.

Eluent 1:- Ethyl acetate-methanol-triethylamine; 700:300:1

Eluent 2:- Tetrahydrofuran-methanol-triethylamine; 700:300:1

Using sumatriptan 1, it was demonstrated that the N-benzyl group could be removed *selectively* from the quaternary salt 6 either by treatment with DABCO as above, or, more conveniently, by catalytic hydrogenation (Scheme 5). (This removal of the benzyl substituent by DABCO contrasts with what is seen with other alkyl or aryl groups, and is a consequence of the ease of nucleophilic substitution at benzylic centres).

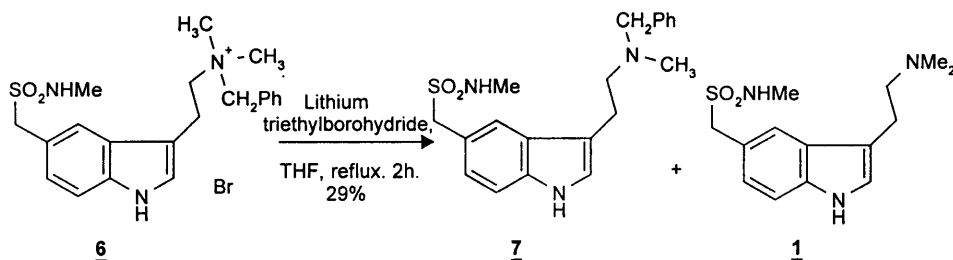
Scheme 5



Thus, if an N-methyl labelled version of the benzyl quaternary salt **6** were obtained, then dequaternisation could be effected to give a tertiary amine retaining all the isotopically labelled N-methyl group.

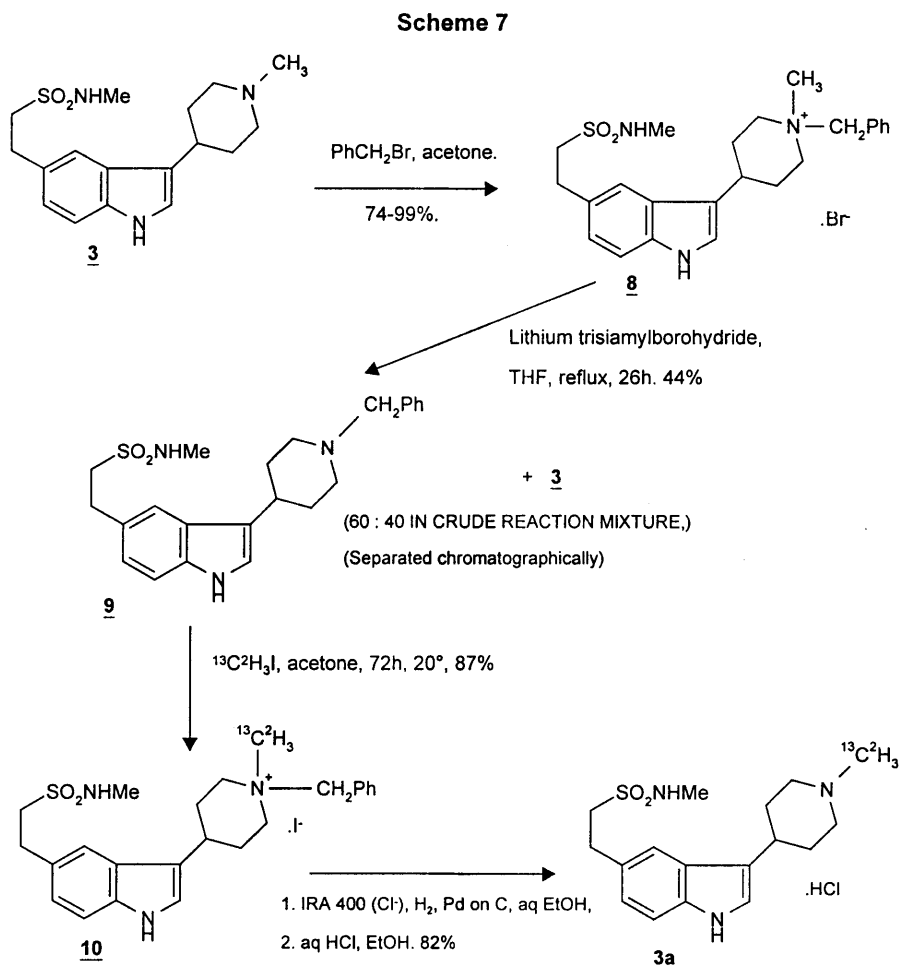
This strategy required an N-benzylated tertiary amine, such as **7**, for use as the quaternisation substrate. Whilst such an analogue could be prepared by a *de-novo* synthesis, this would negate many of the advantages of the simple quaternisation-dequaternisation strategy outlined earlier. A solution to this problem was indicated by work from Cooke and Parlman (4), who reported that the dequaternisation of methyltrialkylammonium salts (including benzyltrimethylammonium iodide) with lithium triethylborohydride lead to selective **demethylation** to the corresponding tertiary amine. This is in contrast to dealkylations using other nucleophiles such as DABCO or phenylthiolate anion, which lead predominantly to **debenzylation**. These dealkylations proceed *via* nucleophilic attack at one of the quaternary alkyl carbons, with the tertiary amine being displaced as the leaving group (3a, 3b). On electronic grounds, attack at the benzylic carbon would be favoured over attack at a methyl carbon, thereby leading predominantly to debenzylation. Presumably the enhanced steric requirements of the bulky trialkylborohydride nucleophiles forces the reaction towards attack at the less hindered, although electronically disfavoured methyl carbon. (Loss of the indolylalkyl substituent is also possible, however, no evidence for this was seen).

Scheme 6



Preliminary experiments using triethylborohydride with the quaternary salt **6** gave a mixture from which the desired N-benzyl tertiary amine **7** was isolated chromatographically. TLC data suggested that the other major product was the corresponding debenzylated amine **1** (Scheme 6). Thus although this reaction did not lead to the exclusive formation of the N-benzylated amine **7**, it gave a mixture containing synthetically significant proportions of the requisite product.

This synthesis of N-benzyl amines from the corresponding N-methyl analogues was then applied to the synthesis of labelled naratriptan **3a**:-

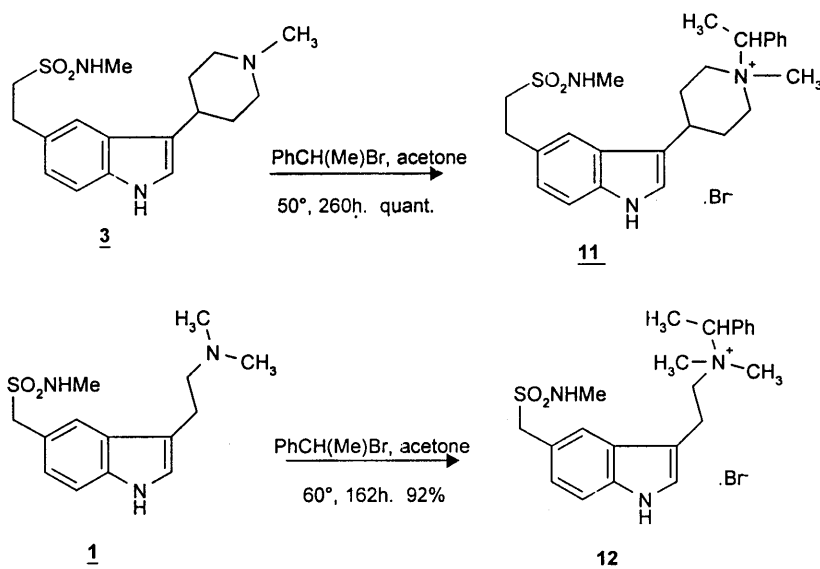


Reaction of naratriptan **3** with benzyl bromide, followed by dealkylation of the resultant quaternary salt **8** with trialkylborohydride reagents produced mixtures of the N-methyl and N-benzyl amines **3** and **9** respectively. In line with the findings of Newkome et al (12), it was found that the use of the sterically hindered lithium triisiamylborohydride favoured the requisite demethylation pathway and gave a mixture of N-benzyl and N-methyl amines in a ratio of 6:4 (HPLC estimation). The pure N-benzyl amine **9** was isolated chromatographically from this mixture in 44% yield. Quaternisation of **9** with trideutero [^{13}C]-methyl iodide, followed by hydrogenative debenzylation in the presence of IRA400 chloride ion exchange resin, produced labelled naratriptan **3a** in 71% yield from labelled

methyl iodide (Scheme 7). Hydrogenative debenzoylation of iodide salts was carried out in the presence of the ion exchange resin to suppress poisoning of the catalyst by iodide ion, this would not be necessary if the counter ion was bromide or chloride.

The enhancement of the demethylation reaction due to steric factors was then investigated in more detail, using quaternary salts obtained from naratriptan **3** or sumatriptan **1**. In addition to examining the effect exerted by the alkyl groups attached to the hydride reagent, the effect of increased steric demand from the benzyl group attached to the quaternary nitrogen centre was also assessed by studying the quaternary salts obtained by reaction of these amines with α -methyl benzylbromide (Scheme 8):-

Scheme 8



The results from these experiments are reported in Table 3. The experiments were carried out as follows:- The quaternary salts **6** & **12** derived from sumatriptan, or the corresponding naratriptan derivatives **8** & **11** (5-50mg) were treated with an excess (> 5 equiv.) of the hydride reagent (A, B or C) in tetrahydrofuran (THF) at 60° for the times shown in the table. These reactions were then quenched by the addition of methanol, and the ratios of starting material and dealkylation products [**1** plus **7** or **13**] or [**3** plus **9** or **14**]; (Scheme 9)] in the crude reaction mixture determined by HPLC.

Scheme 9

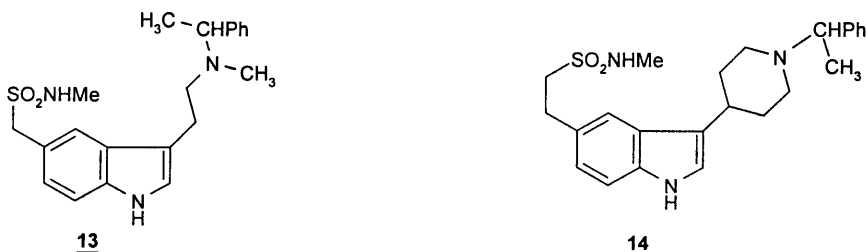


Table 3

Expt. No.	Substrate	Hydride reagent	Reaction Time (h)	Product Profile	Proportion of demethylation in dealkylated product
1	<u>8</u>	A	4	<u>9</u> 17%; <u>3</u> 79%; <u>8</u> 3%	18%
2	<u>8</u>	B	4	<u>9</u> 34%; <u>3</u> 65%; <u>8</u> 0%	34%
3	<u>8</u>	C	4	<u>9</u> 29%; <u>3</u> 21%; <u>8</u> 49%	58%
4	<u>11</u>	A	22	<u>14</u> 72%; <u>3</u> 28%; <u>11</u> 0%	72%
5	<u>11</u>	B	22	<u>14</u> 95%; <u>3</u> 5%; <u>11</u> 0%	95%
6	<u>11</u>	C	22	<u>14</u> 91%; <u>3</u> 4%; <u>11</u> 5%	96%
6a	<u>11</u>	C	92	<u>14</u> 97%; <u>3</u> 3%; <u>11</u> 0%	97%
7	<u>6</u>	A	18	<u>7</u> 30%; <u>1</u> 70%; <u>6</u> 0%	30%
8	<u>6</u>	B	18	<u>7</u> 46%; <u>1</u> 52%; <u>6</u> 2%	47%
9	<u>6</u>	C	18	<u>7</u> 59%; <u>1</u> 38%; <u>6</u> 3%	61%
10	<u>12</u>	A	18	<u>13</u> 86%; <u>1</u> 14%; <u>12</u> 0%	86%
11	<u>12</u>	B	18	<u>13</u> 97%; <u>1</u> 3%; <u>12</u> 0%	97%

Reagents:-

A = Lithium triethylborohydride; B = Lithium tri-*sec*-butylborohydride; C = Lithium trisiamylborohydride.

It should be noted that although the proportions in the table are reported as area/area ratios of the UV response at 225nm, measurement of the relative UV response factors at this wavelength for naratriptan 2, the quaternary salt 8 and the N-benzyl analogue 9 showed a total spread of <5% for all three components. It was therefore concluded that the UV area ratio measurement gives a sufficiently accurate reflection of the composition of the reaction mixture for valid conclusions to be drawn about the effects of the structural changes to substrates and reducing agents.

The above experiments clearly show that, although increasing the steric bulk of either the hydride reducing agent ($C > A$), or the benzyl substituent [**11** or **12** > **8** or **6**], both result in increased selectivity towards removal of the methyl group, the effect from changes to the benzyl substituent is much the more significant. Dealkylations on **11** or **12** with the moderately hindered lithium trisecbutylborohydride, resulted in >95% demethylation (expts. 5 & 11), whereas the maximum level of demethylation achieved on the unsubstituted benzyl analogues **8** and **6** was only ca 60% (expts. 3 & 9), even when the highly hindered lithium trisiamylborohydride was used.

Reaction of amine **13** with [$^{13}\text{C}^2\text{H}_3$] methyl iodide (2 equivs; THF; 20°; 350h) gave a quantitative yield of a labelled analogue of quaternary salt **12**. This was then debenzylated (24h), under hydrogenation conditions similar to those used in the synthesis of labelled naratriptan **3a**, to give a 96% yield of labelled sumatriptan. Thus the use of α -methylbenzyl bromide in this labelling strategy results in the most efficient formation of a requisite benzylamino labelling substrate, and thence the labelled target molecule. However, this efficiency is gained at the expense of longer reaction times, particularly in the quaternisation steps.

The greater influence of steric crowding on the benzylic carbon is a consequence of the likely reaction mechanism. The $\text{S}_{\text{N}}2$ attack by the trialkylborohydride almost certainly involves approach by the hydrido-atom towards the alkyl carbon, followed by expulsion of the tertiary amine and concomitant formation of the alkane and trialkylborane (Scheme 10).

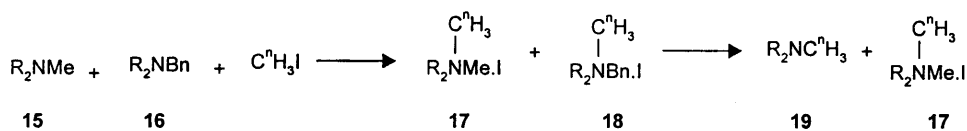
Scheme 10



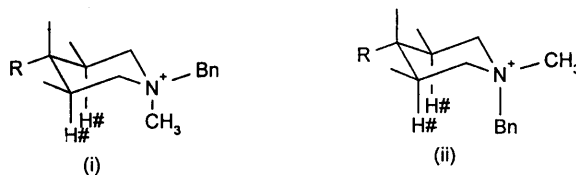
Thus substituents on the benzylic carbon are **directly** attached to an atom that is a reaction centre, and would therefore be expected to have a greater steric impact than modifications to the substituents "R" attached to the boron atom, which are more remote from the centre of reaction.

Although in the above work, the benzyl/methyl amine mixtures were purified to prevent isotopic dilution of the final product with unlabelled methyl amine, this may not prove to be necessary. Provided that the mixture of benzylic **16** and methyl amines **15** is **totally** converted into the corresponding quaternary salts **17** and **18**, the hydrogenative dealkylation step will result only in dequaternisation of the **benzylic** quaternary salt **18**, leaving the bismethylated quaternary product **17** unchanged (Scheme 11). The resultant mixture, containing only the requisite labelled tertiary amine **19** plus the bismethylated quaternary salt **17**, should present a less challenging separation problem than that posed by the mixtures of methyl and benzylic amines. However, for this strategy to produce isotopically pure material, it is essential that the quaternisation step is driven to completion, which could prove somewhat profligate in the use of labelled methyl iodide.

Scheme 11

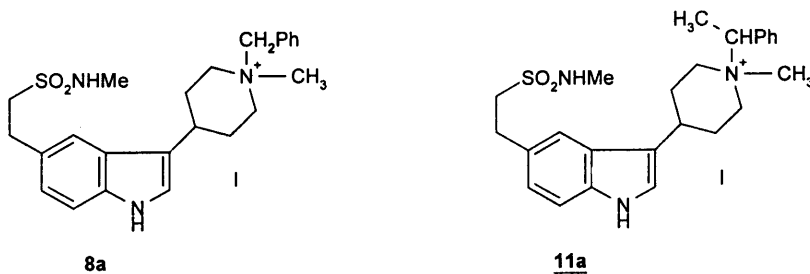


Finally, interesting stereochemical effects have been observed in the above quaternisation/dequaternisation reactions on the cyclic amine substrate **3**. Quaternisation of **3** with either benzyl bromide or α -methylbenzyl bromide results in a product displaying two quite distinct signals for the N-benzylic and N-methyl protons. [4.70 & 4.76 δ and 2.98 & 3.08 δ respectively in compound **8**; 4.90 & 5.35 δ and 2.96 & 3.04 δ respectively in compound **11**]. These two sets of signals were attributed to the two geometric isomers (i) and (ii), where the quaternary N-methyl substituent lies in a 1-4 *cis* (i) or 1-4 *trans* (ii) relationship to the bulky indolic substituent (R). The accuracy of this assignment was confirmed by nOe experiments which demonstrated the proximity of the axial N-alkyl substituent protons to the corresponding *trans* 3-axial ring protons (H#).



The quaternary salts **8** and **11**, which were produced by reacting the N-methyl amine **3** with the appropriate benzylic bromides, were found to contain approximately equal proportions of the two isomers (i) and (ii). (Ratio (i):(ii) is 4:6 by ¹H NMR estimation). However, the corresponding salts **8a** and **11a**, produced by treating the benzylic amines **9** and **14** with methyl iodide contained a much higher proportion of the isomer (i) (ca 9:1) in which the methyl group lies *cis* to the indolic group. This probably reflects the influence of the more sterically demanding benzylic group in amines **9** or **14**, leading to a preponderance of the conformer in which the benzylic group also occupies the equatorial orientation favoured by the bulky indolic substituent. This inequality in the conformer distribution is reflected in a higher proportion of axial attack in the quaternisation step.

Scheme 12



A dequaternisation experiment, similar to those described in Table 3, was then carried out on **8a** using lithium trisiamylborohydride (reagent "C" in the table). This resulted in a demethylation to debenzylation ratio of 7:3 compared with that of 6:4 observed with **8** (Expt.3; Table 3). Assuming that in each substrate, the *cis* methyl substituent occupies an axial orientation, thereby allowing the bulky indolic group to occupy the sterically favoured equatorial position, the above result may indicate that, in addition to favouring removal of the less bulky methyl group, there may also be a preference towards removal of the axial substituent in the dequaternisation step. However, the change in the ratio is probably too small to allow firm conclusions to be drawn.

Conclusion

Methods have been developed for isotopically labelling N-methyl substituents on tertiary amines. These methods rely on simple quaternisation/dequaternisation procedures starting from the unlabelled version of the target molecules. High isotopic abundance may

be achieved either *via* an HPLC separation of labelled and non-labelled species, or *via* a benzylic analogue, also prepared by quaternisation/dequaternisation methods. Conditions for optimising the formation of these benzylic intermediates have been established.

Experimental

General Methods: ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity 400MHz spectrometer. High resolution mass spectrometry (MS) was performed using a VG Autospec spectrometer.

Synthesis of quaternary salts:-

A solution of the tertiary amine in acetone, was treated with the relevant alkyl halide (iodomethane or benzyl bromide; 1-3 equivalents) and the mixture allowed to stand at ambient temperature (ca. 20°) for 1-3 days, during which time the product precipitated out of solution and was collected by filtration. [For reactions run at very high dilution, (<0.001M), the reaction mixture was heavily diluted with diethyl ether at the end of the reaction to induce precipitation of the product] Washing the precipitated product with diethyl ether to remove unreacted starting material gave material of sufficient purity for further use, in yields of typically 80-100%.

For quaternisations using α -methylbenzyl bromide the reactions were performed in similar fashion, but heated at reflux for 2.5 to 12 days. The products from these reactions were then isolated by evaporation of the acetone followed by trituration of the residual gum in ether.

Using the above methods the following quaternary salts were obtained:-

Benzyl-dimethyl-[2-(5-methylsulfamoylmethyl-1H-indol-3-yl)-ethyl]-ammonium bromide 6

δ_{H} (400MHz, DMSO- d_6) 11.11 (1H, br s, indole NH), 7.66-7.52 (6H, m, phenyl and indole 4H), 7.40 (1H, d, indole 7H), 7.33 (1H, d, indole 2H), 7.16 (1H, d of d, indole 6H), 6.86 (1H, q, SO_2NHCH_3), 4.72 (2H, s, PhCH_2N), 4.37 (2H, s, $\text{CH}_2\text{SO}_2\text{NHCH}_3$), 3.60 (2H, m, $\text{CH}_2\text{CH}_2\text{N}^*$), 3.31 (2H, m, $\text{CH}_2\text{CH}_2\text{N}^*$), 3.14 (6H, s, $^*\text{N}(\text{CH}_3)_2$), 2.56 (3H, d, SO_2NHCH_3).

1-Benzyl-1-methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-piperidinium bromide 8

δ_{H} (400MHz, DMSO- d_6) 11.03 and 10.86 (1H, s, indole NH), 7.64-7.44 (6H, m, indole 4H and phenyl), 7.35 and 7.20 (1H, d, indole 2H), 7.32 and 7.29 (1H, d, indole 7H), 7.04-6.92 (2H, m, indole 6H and SO_2NHCH_3), 4.74 and 4.68 (2H, s, NCH_2Ph), 3.67-3.12 (7H, m, piperidine CH and NCH_2 x2, and $\text{SO}_2\text{CH}_2\text{CH}_2$), 3.08 and 2.98 (3H, s, $^*\text{NCH}_3$), 3.03 (2H, m, $\text{SO}_2\text{CH}_2\text{CH}_2$), 2.61 (3H, d, SO_2NHCH_3), 2.46-2.00 (4H, m, piperidine CH_2 x2); (Found: m/z [Electrospray +ve] 426.221127 [M $^+$]. $\text{C}_{24}\text{H}_{32}\text{N}_3\text{O}_2\text{S}$ requires 426.221524)

1-Benzyl-1-methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-piperidinium iodide 8a

δ_{H} (400MHz, DMSO- d_6) 10.9 (1H, br s, indole NH), 7.68-7.55 (5H, m, phenyl), 7.51 (1H, s, indole 4H), 7.33 (1H, d, indole 7H), 7.24 (1H, d, indole 2H), 7.03 (1H, d, indole 6H), 6.99 (1H, q, SO₂NHCH₃), 4.70 (2H, s, CH₂Ph), 3.70-3.40 (4H, m, piperidine NCH₂ x2), 3.3 (2H, m, SO₂CH₂CH₂), 3.12 (3H, s, *NCH₃), 3.10-2.98 (3H, m, piperidine CH and SO₂CH₂CH₂), 2.65 (3H, d, SO₂NHCH₃), 2.24-2.04 (4H, m, piperidine CH₂ x2); (Found: m/z [Electrospray +ve] 426.221575 [M*]. C₂₄H₃₂N₃O₂S requires 426.221524)

1-Benzyl-1-[¹³C-2H3]methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-piperidinium iodide 10

δ_{H} (400MHz, DMSO- d_6) 10.84 (1H, s, indole NH), 7.64-7.52 (5H, m, phenyl), 7.47 (1H, s, indole 4H), 7.29 (1H, d, indole 7H), 7.20 (1H, d, indole 2H), 6.99 (1H, d of d, indole 6H), 6.94 (1H, q, SO₂NHCH₃), 4.66 (2H, d, NCH₂Ph), 3.66-3.40 (4H, m, piperidine NCH₂ x2), 3.28 (2H, m, SO₂CH₂CH₂), 3.10-2.96 (3H, m, piperidine CH and SO₂CH₂CH₂), 2.61 (3H, d, SO₂NHCH₃), 2.20-2.00 (4H, m, piperidine CH₂ x2); δ_{C} (100.6MHz, DMSO- d_6) 44.3 (*N¹³CH₃ enhanced); m/z (Thermospray) 430 (M*, 28%) 340 ([M-C₇H₇]*, 100%); (Found: C, 51.5; H, 5.8; N, 7.3%. C₂₃¹³C₁H₂₉²H₃N₃O₂SI requires: C, 51.7; H, 5.8; N, 7.5%).

1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-1-(1-phenyl-ethyl)-piperidinium bromide 11

δ_{H} (400MHz, DMSO- d_6) 10.94 and 10.85 (1H, s, indole NH), 7.70-7.45 (6H, m, indole 4H and phenyl), 7.38 and 7.20 (1H, d, indole 2H), 7.32 and 7.29 (1H, d, indole 7H), 7.04-6.92 (2H, m, indole 6H and SO₂NHCH₃), 5.34 and 4.90 (1H, q, PhCHCH₃), 4.00-1.80 (9H, m's, piperidine CH and CH₂ x4), 3.30 (2H, m, SO₂CH₂CH₂), 3.04 and 2.94 (3H, s, *NCH₃), 3.02 (2H, m, SO₂CH₂CH₂), 2.61 (3H, d, SO₂NHCH₃), 1.80 and 1.70 (3H, d, PhCHCH₃); (Found: m/z [Electrospray +ve] 440.237183 [M*]. C₂₅H₃₄N₃O₂S requires 440.237174)

1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-1-(1-phenyl-ethyl)-piperidinium iodide 11a

δ_{H} (400MHz, DMSO- d_6) 10.85 (1H, s, indole NH), 7.74-7.48 (5H, m, phenyl), 7.46 (1H, s, indole 4H), 7.29 (1H, d, indole 7H), 7.20 (1H, d, indole 2H), 6.99 (1H, d, indole 6H), 6.94 (1H, q, SO₂NHCH₃), 4.90 (1H, q, PhCHCH₃), 3.74-3.56 (2H, m, axial piperidine NCH₂ x2), 3.48 (1H, m, piperidine CH) 3.28 (2H, m, SO₂CH₂CH₂), 3.14-2.96 (7H, m, equatorial piperidine NCH₂ x2, *NCH₃ and SO₂CH₂CH₂), 2.62 (3H, d, SO₂NHCH₃), 2.24-1.90 (4H, m, piperidine CH₂ x2), 1.80 (3H, d, PhCHCH₃); (Found: m/z [Electrospray +ve] 440.235561 [M*]. C₂₅H₃₄N₃O₂S requires 440.237174)

Dimethyl-[2-(5-methylsulfamoylmethyl-1H-indol-3-yl)-ethyl]-(1-phenyl-ethyl)-ammonium bromide 12

δ_{H} (400MHz, CD₃OD) 7.66-7.51 (6H, m, phenyl and indole 4H), 7.39 (1H, d, indole 7H), 7.25 (1H, s, indole 2H), 7.20 (1H, d of d, indole 6H), 4.94 (1H, q, PhCHCH₃), 4.41 (2H, s, CH₂SO₂NHCH₃), 3.70-3.30 (4H, m, CH₂CH₂N*), 3.18 (3H, s, *NCH₃) 3.10 (3H, s, *NCH₃), 2.63 (3H, s, SO₂NHCH₃), 1.86

(3H, d, PhCHCH₃); (Found: m/z [Electrospray +ve] 400.207697 [M⁺]. C₂₂H₃₀N₃O₂S requires 400.205874)

N-Methyl-C-(3-{2-[methyl-(1-phenyl-ethyl)-amino]-ethyl}-1H-indol-5-yl)-methanesulfonamide **13**

Quaternary salt **12** (0.453g, 0.943mmol) was suspended in anhydrous THF (50ml) to which lithium trisecbutylborohydride (L-Selectride; 1M solution in THF, 4.71ml, 4.71mmol) was added. The mixture was refluxed under nitrogen for two hours then quenched by the addition of methanol (ca. 1.5ml; caution, hydrogen evolved). The mixture was evaporated and partitioned between ethyl acetate (50ml) and 2N hydrochloric acid (50ml). The layers were separated and the organic phase further extracted with 2N hydrochloric acid (2 x 50ml). To the combined aqueous layers ethyl acetate (25ml) was added and the solution made basic by the addition of solid potassium carbonate. The aqueous layer was further extracted with ethyl acetate (5 x 25ml), the combined organic fractions dried and evaporated to yield *the title compound* (0.309g, 0.80mmol, 70%) as a yellow solid. δ_H (400MHz, CD₃OD) 7.40-7.25 (7H, m, phenyl and indole 4H, 7H), 7.13 (1H, d of d, indole 6H), 7.00 (1H, s, indole 2H), 4.33 (2H, s, CH₂SO₂NHCH₃), 3.74 (1H, q, PhCHCH₃), 3.02-2.60 (4H, m, CH₂CH₂N), 2.57 (3H, s, SO₂NHCH₃), 2.41 (3H, s, NCH₃), 1.45 (3H, d, PhCHCH₃); (Found: m/z [Electrospray +ve] 386.190106 [MH⁺]. C₂₁H₂₈N₃O₂S requires 386.190224).

2-{3-[1-(1-Phenyl-ethyl)-piperidin-4-yl]-1H-indol-5-yl}-ethanesulfonic acid methylamide **14**

After reaction for 70h at reflux, the quaternary salt **11** similarly gave *the title compound 14*

δ_H (400MHz, DMSO-d₆) 10.68 (1H, s, indole NH), 7.47 (1H, s, indole 4H), 7.37-7.20 (6H, m, phenyl and indole 7H), 7.05 (1H, d, indole 2H), 6.94 (1H, d of d, indole 6H), 6.91 (1H, q, SO₂NHCH₃), 3.50 (1H, q, PhCHCH₃), 3.26 (2H, m, SO₂CH₂CH₂), 2.98 (2H, m, SO₂CH₂CH₂), 3.06 and 2.85 (2H, m, axial piperidine NCH₂ x2), 2.66 (1H, m, piperidine CH), 2.60 (3H, d, SO₂NHCH₃), 2.15-1.53 (6H, m, equatorial piperidine NCH₂ x2 and piperidine CH₂ x2), 1.34 (3H, d, PhCHCH₃); (Found: m/z [Electrospray +ve] 426.221843 [MH⁺]. C₂₄H₃₁N₃O₂S requires 426.221524)

2-{3-(1-Benzyl-piperidin-4-yl)-1H-indol-5-yl}-ethanesulfonic acid methylamide **9**

Quaternary salt **8** (3.01g; 5.94mmol) was refluxed with a tetrahydrofuran solution of lithium trisiamylborohydride (LS-Selectride; 33ml 1M solution; 33mmol). After work-up similar to the above, the crude product was purified by column chromatography over silica (150g), eluting with a mixture of dichloromethane - ethanol - 0.88 ammonia (20:8:1). Evaporation of appropriate fractions gave *the title compound* (1.17g; 2.84mmol; 48% yield) as a pale brown solid. δ_H (400MHz, DMSO-d₆) 10.70 (1H, s, indole NH), 7.43 (1H, s, indole 4H), 7.36-7.30 (6H, m, phenyl and indole 7H), 7.06 (1H, d, indole 2H), 6.95 (1H, d, indole 6H), 6.92 (1H, q, SO₂NHCH₃), 3.51 (2H, s, PhCH₂N), 3.27 (2H, m, SO₂CH₂CH₂), 3.00 (2H, m, SO₂CH₂CH₂), 2.90 (2H, m, axial piperidine NCH₂ x2), 2.74 (1H, m, piperidine CH), 2.61 (3H, d, SO₂NHCH₃), 2.11 (2H, m, equatorial piperidine NCH₂ x2), 1.92 (2H,

m, axial piperidine CH_2 x2), 1.65 (2H, m, equatorial piperidine CH_2 x2); (Found: m/z [Electrospray +ve] 412.206193 $[\text{MH}^+]$. $\text{C}_{23}\text{H}_{29}\text{N}_3\text{O}_2\text{S}$ requires 412.205874).

C-{3-[2-(Benzyl-methyl-amino)-ethyl]-1H-indol-5-yl]-N-methyl-methanesulfonamide **7**

Quaternary salt **6** (0.253g; 0.542mmol) in tetrahydrofuran (5ml) was treated with a solution of lithium triethylborohydride (Super Hydride; 3ml; 1M solution in tetrahydrofuran; 3mmol) and the mixture heated at reflux for 2h. After the usual work-up the crude product was purified by column chromatography over silica (15g), eluting with dichloromethane-ethanol-0.88 ammonia (100:8:1). Evaporation of appropriate fractions gave *the title compound* (0.058g; 0.156mmol; 29% yield) as a cream solid. δ_{H} (400MHz, CDCl_3) 8.06 (1H, broad, indole NH), 7.48 (1H, s, indole 4H), 7.34 (1H, d, indole 7H), 7.34-7.22 (5H, m, phenyl), 7.20 (1H, d of d, indole 6H), 7.06 (1H, d, indole 2H), 4.33 (2H, s, $\text{CH}_2\text{SO}_2\text{NHCH}_3$), 3.89 (1H, q, SO_2NHCH_3), 3.60 (2H, s, PhCH_2N), 2.98 (2H, m, $\text{CH}_2\text{CH}_2\text{N}$), 2.73 (2H, m, $\text{CH}_2\text{CH}_2\text{N}$), 2.64 (3H, d, SO_2NHCH_3), 2.34 (3H, s, NCH_3).

2-[3-(1-[^{13}C -2H3]Methyl-piperidin-4-yl)-1H-indol-5-yl]-ethanesulfonic acid methylamide hydrochloride **3a**

The quaternary salt **10** (1.3048g; 2.34mmol) was dissolved in ethanol-water (2:1 v/v; 150ml). Amberlite resin (IR400, chloride form; 6g) was added followed by 10% palladium on carbon catalyst (0.275g). The mixture was stirred at 20° under an atmosphere of hydrogen for 3h, during which time 78ml of hydrogen was taken up. The reaction mixture was diluted with water (75ml), filtered, and the product isolated by solvent evaporation. The resulting pale yellow foam was partitioned between aqueous potassium carbonate solution and ethyl acetate, the layers separated, and the aqueous phase extracted with two further aliquots of ethyl acetate. The organic extracts were combined, washed with saturated brine, then dried over sodium sulphate. Evaporation of the dried extracts gave labelled naratriptan base as a white foam (0.735g; 2.16mmol; 92% yield).

This base was converted into its hydrochloride salt by dissolution in hot (ca 75°) ethanol (8ml) followed by addition of 2N hydrochloric acid (2ml). The requisite salt crystallised out on cooling, and was recovered by filtration. The product was dried *in vacuo* at 70° for 24h to give *the title compound* as a white solid (0.7205g; 1.916mmol; 82% yield). δ_{H} (400MHz, DMSO-d_6) 10.88 (1H, s, indole NH), 10.61 (1H, s, NH^+), 7.58 (1H, s, indole 4H), 7.29 (1H, d, indole 7H), 7.11 (1H, s, indole 2H), 7.02-6.94 (2H, m, indole 6H and SO_2NHCH_3), 3.46 (2H, m, axial piperidine NCH_2 x2), 3.30 (2H, m, $\text{SO}_2\text{CH}_2\text{CH}_2$), 3.18-2.94 (5H, m, $\text{SO}_2\text{CH}_2\text{CH}_2$, equatorial piperidine NCH_2 x2 and piperidine CH); 2.62 (3H, d, SO_2NHCH_3), 2.16-2.02 (4H, m, piperidine CH_2 x2); m/z (Thermospray) 340/342 (MH^+ , 100%); (Found: C, 53.9; H, 6.7; N, 10.95; S, 8.6%. $\text{C}_{16}^{13}\text{C}_1\text{H}_{22}^2\text{H}_3\text{N}_3\text{O}_2\text{S.HCl}$ requires: C, 54.3; H, 7.0; N, 11.2; S, 8.5%).

Acknowledgements

The authors thank Mr Peter Moore and Miss Zoe Heaton for NMR data.

References

1. Fellows I., Carr RM., deBoeck N., Montgomery S., Waterhouse I. and Sutherland DR. – *Synthesis and Applications of Isotopically Labelled Compounds 1997* (Ed Heys JR. and Mellilo DG), John Wiley & Sons Ltd., 1998, 507.
2. North PC., Oxford AW., Feniuk W., Humphrey PPA. and Connor HE – *Medicinal Chemistry: Principles and Practice* (Ed. King FD.), Royal Society of Chemistry, 1994, 265.
3. (a) Ho TL. – *Synthesis* **702**, (1972)
(b) Ho TL. – *Synth. Comm.* **3**: 99 (1973)
4. Cooke MP. and Parlman RM. – *J. Org. Chem.* **40**: 531 (1975)
5. Filer CN., Fazio R. and Ahern DG. – *J. Org. Chem.* **46**: 3344 (1981)
6. Heys JR. – *J. Chrom.* **407**: 37 (1987)
7. Gerardy BM., Poupaert JH., Vandervorst D., Dumont P., Declerq J-P., Van Meerssche M. and Portoghesi PS. – *J. Lab. Comp. Radiopharm.* **22**: 5 (1985)
8. Synthesised by amide formation with the corresponding acid and deuterated morpholine, in the presence of diphenylphosphoryl azide, followed by exchange of the alpha protons in deuterio-methanol with NaOD catalysis.
9. Prepared by permethylation of the corresponding primary amine with trideuteromethyl iodide. Followed by demethylation of the resultant quaternary salt with DABCO in NMP.
10. Van Der Linde W. and Robertson RE. – *J. Amer. Chem. Soc.* **86**: 4505 (1964)
11. Chapelle MR. – *Personal Communication*. The collaboration with Amersham Life Science is gratefully acknowledged.
12. Newkome GR., Majestic VK. and Sauer JD. – *Org. Prep. Proced. Int.* **12**: 345 (1980)