

## CARBON-14 AND TRITIUM LABELED GUANADREL SULFATE

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### SUMMARY

Carbon-14 and tritium labeled (1,4-dioxaspiro[4.5]dec-2-ylmethyl)guanidine sulfate, or guanadrel sulfate\* (1) were synthesized. The carbon-14 label was incorporated into the dioxolane ring portion of guanadrel sulfate. The tritium labels were introduced into the cyclohexane ring portion of the molecule, at the beta and gamma positions with respect to the ketal linkage. The ketal resulting from the reaction of the original 2-cyclohexen-1-one with 3-phthalimido-1,2-propanedial contained a double bond in the  $\beta$ - $\gamma$  position rather than the  $\alpha$ - $\beta$  position of the original 2-cyclohexen-1-one. This double bond shift provided a convenient method of introducing tritium into the 3- and 4-positions of cyclohexanone. Proof of the double bond position is discussed.

Key Words: Carbon-14, tritium, guanadrel, synthesis.

### INTRODUCTION

Guanadrel sulfate (Hylorel<sup>®</sup>) (4) is an antihypertensive (2-7) drug of demonstrated clinical utility. We prepared radioactive forms of this drug for

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\*Guanadrel sulfate is the USAN name for (1,4-dioxaspiro[4.5]dec-2-ylmethyl)guanidine sulfate.

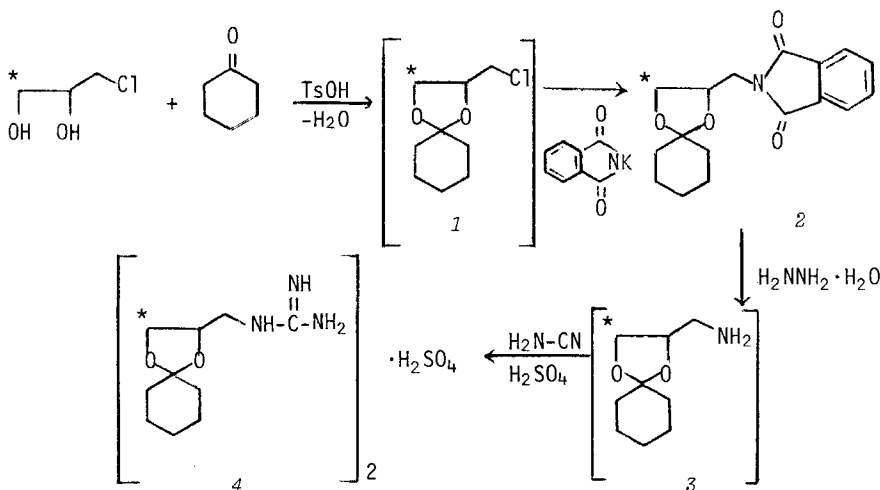
studying its metabolic transformations in test animals and man. Because the ketal function in guanadrel readily undergoes acid catalyzed hydrolysis (8), we labeled the propanediol portion of the molecule with carbon-14, and the cyclohexanone portion with tritium, so that the metabolic fate of both fragments of the molecule could be investigated, should hydrolysis occur *in vivo*.

#### DISCUSSION

##### Carbon-14-Labeled Guanadrel

The synthesis of carbon-14 labeled guanadrel sulfate is outlined in Scheme 1. Ketalization of cyclohexanone with 3-chloro-1,2-[1-<sup>14</sup>C]propanediol produced 2-chloromethyl-1,4-dioxaspiro[4.5]decane (1). Treatment of 1 with potassium phthalimide led to 2-phthalimidomethyl-1,4-dioxaspiro[4.5]decane (2). The phthalimide group of 2 was removed with hydrazine hydrate, and the resulting amine 3 was added to cyanamide in the presence of dilute sulfuric acid to afford carbon-14 labeled guanadrel sulfate (4).

Scheme 1. Synthesis of Carbon-14 Labeled Guanadrel



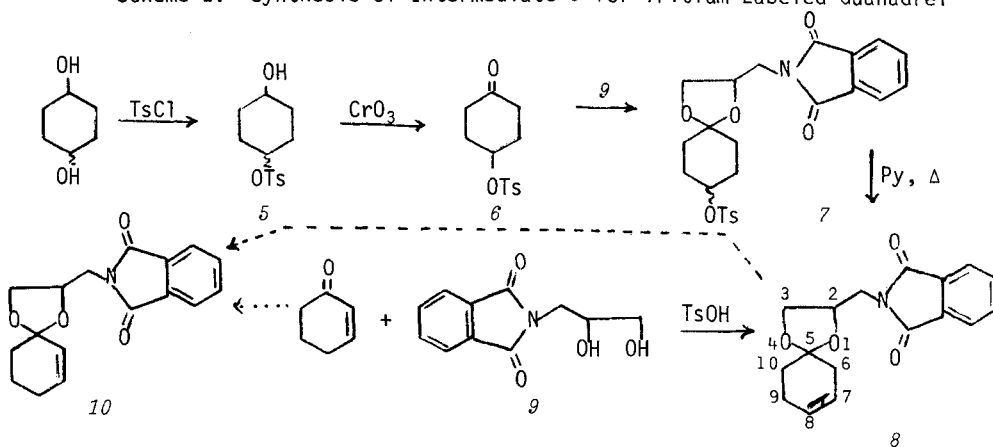
\* denotes carbon-14 label

##### Tritium Labeled Guanadrel

Guanadrel labeled with tritium in the cyclohexyl ring could be readily

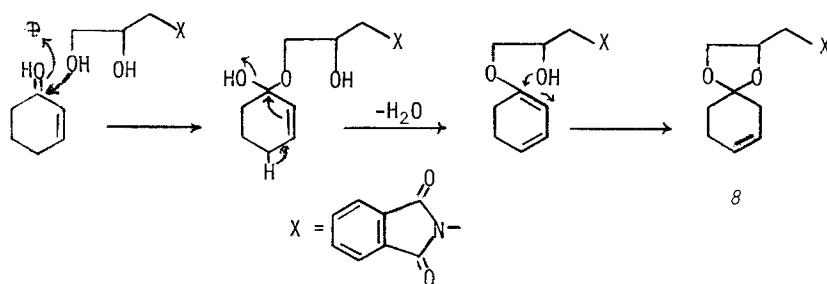
synthesized according to Scheme 1 by using tritium labeled cyclohexanone as the starting material. Because guanadrel readily undergoes hydrolysis to give a diol and cyclohexanone, we wished to introduce tritium at one or both of the 3- and 4-positions of cyclohexanone, so that the label would not be easily removed from the labeled cyclohexanone arising from guanadrel upon hydrolysis. Tritium incorporated in the 2-position of cyclohexanone would be easily lost through enolization of the ketone function. To avoid handling volatile radioactive material such as tritiated cyclohexanone, we chose to delay introducing tritium until a crystalline intermediate was attained in the synthesis. Therefore we prepared 2-phthalimido-1,4-dioxaspiro[4.5]dec-7-ene (*8*) which contains a double bond,  $\beta$ - $\gamma$  to the ketal function, to serve as the site for incorporating tritium by means of catalytic reduction with tritium gas. Scheme 2 outlines the preparation of compound *8* from 1,4-dihydroxy cyclohexane, which was tosylated (compound *5*) and oxidized to give 4-tosyloxy-cyclohexanone (*6*). Ketalization of *6* with 3-phthalimido-1,2-propanediol (*9*), obtained by treating 3-chloro-1,2-propanediol with potassium phthalimide, afforded 2-phthalimidomethyl-8-tosyloxy-1,4-dioxaspiro[4.5]decane (*7*). The elements of *p*-toluenesulfonic acid were eliminated from *7* to give the desired compound *8*.

Scheme 2. Synthesis of Intermediate *8* for Tritium Labeled Guanadrel



Although the proton magnetic resonance (pmr) spectrum of the unsaturated ketal was compatible with the assigned structure **8**, the isomeric structure **10**, could not be unequivocally ruled out for lack of models. The position of the double bond in **8** was important because we wished to avoid incorporating tritium into the cyclohexane ring position adjacent to the ketal carbon in guanadrel, as would occur if the double bond positioned as shown in structure **10** was reduced with tritium gas. We therefore attempted to prepare compound **10** for comparison with our compound **8**. Ketalization of 2-cyclohexen-1-one with the diol **9** surprisingly gave a ketal identical to **8** with respect to melting point, thin-layer chromatography, and pmr spectrum. In order to produce the same 1,4-dioxaspiro[4.5]decene from 2-cyclohexen-1-one and **3**, or from **7**, a double bond shift must occur either during the ketalization of 2-cyclohexen-1-one (solid arrows, Scheme 2), or after the elimination of *p*-toluenesulfonic acid from **7**, to produce **10** (broken arrows, Scheme 2). The latter alternative appeared unlikely under the mildly basic reaction conditions used (refluxing pyridine). A double bond shift during the formation of a ketal from an  $\alpha,\beta$ -unsaturated ketone, on the other hand, can be rationalized as shown in Scheme 3, and an analogy can be found in the steroid field, such as the

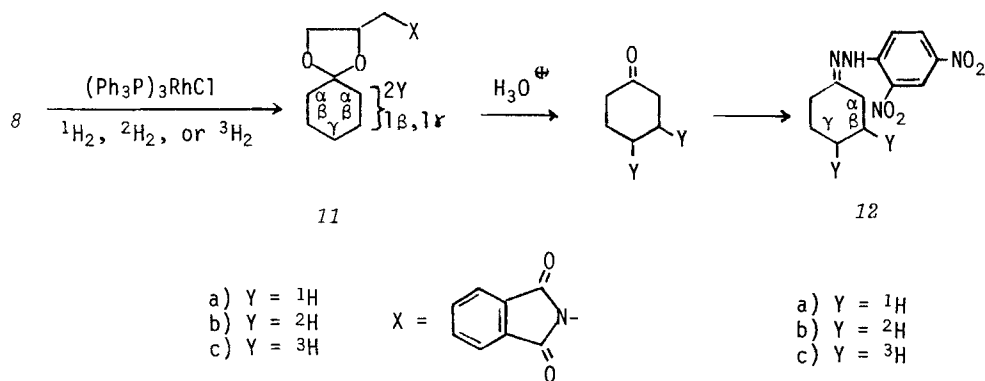
Scheme 3. Ketalization of 2-Cyclohexen-1-one



ketalization of  $\Delta^4$ -3-ketosteroids (**9-13**) to give  $\Delta^5$ -3-ketals. To prove the position of the double bond as shown in structure **8**, we reduced both samples of compound **8** with deuterium gas in the presence of *trans*-(triphenylphosphine)-rhodium chloride\* (Scheme 4). The resulting compound **11b** was hydrolyzed

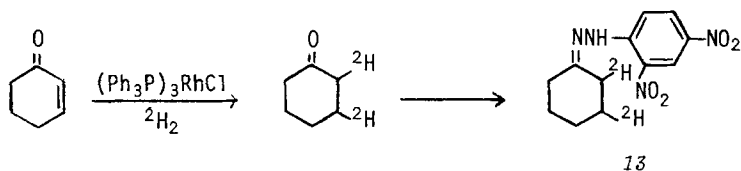
\*Attempts to reduce **8** in the presence of heterogeneous catalysts such as platinum oxide, platinum and palladium supported on charcoal were unsuccessful, presumably because of the non-planar spiro structure of **8**.

to deuterium labeled cyclohexanone which was converted to the 2,4-dinitrophenylhydrazone (2,4-DNP) *12b*. The pmr spectra of both samples of *12b* showed that the ratio of protons at the  $\alpha$  position (2.53  $\delta$ ) to those at the  $\beta$  and  $\gamma$  positions (1.80  $\delta$ ) was 1 to 1 (4 at  $\alpha$ , and 4 at  $\beta$  and  $\gamma$ )\*. Reduction of *8* with hydrogen gas followed by hydrolysis to cyclohexanone and conversion of the ketone to its 2,4-DNP afforded compound *12a*. The  $\alpha$  to  $\beta$  and  $\gamma$  proton ratio of *12a* was found to be 4:6, as expected. We also prepared the 2,4-DNP *13* of a sample of [2,3- $^2\text{H}$ ]-cyclohexan-1-one obtained by reducing 2-cyclohexen-1-one with deuterium gas

Scheme 4. Reduction of *8* and Conversion to 2,4-DNP

(Scheme 5), and the  $\alpha$  to  $\beta$  and  $\gamma$  proton ratio in *13* proved to be 3:5. These results proved that the double bond in *8* was positioned  $\beta$ ,  $\gamma$  to the ketal

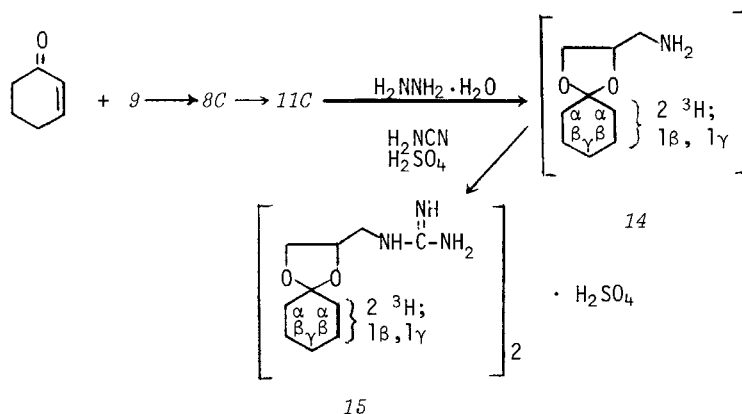
Scheme 5. Reduction of 2-Cyclohexenone and Conversion to 2,4-DNP



\*The pmr spectra were obtained in  $\text{CDCl}_3$  containing tetramethylsilane (TMS) as a reference for chemical shifts. The nitrogen proton (11.20  $\delta$ ) and aromatic protons (7.83 - 9.16  $\delta$ ) served as standards for quantifying the protons at the  $\alpha$ ,  $\beta$ , and  $\gamma$  positions.

function, and that during the reaction of 2-cyclohexen-1-one with the diol *9*, the  $\alpha, \beta$  double bond in the starting ketone had shifted into the  $\beta, \gamma$  position in the ketal product. This shift provided a convenient method of preparing the intermediate *8* for the synthesis of tritiated guanadrel (*15*) with the labels located  $\beta$  and  $\gamma$  to the ketal linkage (Scheme 6). Since the compound *8* is

Scheme 6. Synthesis of Tritium Labeled Guanadrel Sulfate



readily reducible with deuterium or tritium, and the reduction product *11* easily undergoes hydrolysis to give cyclohexanone, *8* should be a useful intermediate for preparing labeled cyclohexanone with no labels in the alpha position where they are susceptible to removal through enolization.

To confirm the expectation that tritium labeled guanadrel (*15*) obtained according to Scheme 6 would contain no potentially labile tritium after hydrolysis of *15* to tritiated cyclohexanone, we hydrolyzed a sample of the tritiated ketal *11C* (obtained from 2-cyclohexen-1-one and the diol *9* followed by reduction with tritium gas), and subjected the resulting tritiated cyclohexanone to base treatment prior to converting it to the 2,4-DNP *12C*. In a separate experiment, we established by means of mass spectroscopy that under the same basic conditions the protons at the positions alpha to the ketone

function were readily exchangeable with deuterium from deuterium oxide. The molar specific activities of 12C and its precursor 11C were identical, which indicated absence of any exchangeable tritium in the cyclohexanone moiety of 11C, and validated the assignment of the tritium label positions as shown in structure 11C. Tritium labeled guanadrel, when prepared from 11C, would therefore also contain no potentially exchangeable tritium. The conversion of 11C to tritiated guanadrel (15) paralleled the two final steps in the synthesis of carbon-14 labeled guanadrel, and is shown in Scheme 6. The phthaloyl group was removed from 11C and addition of the resulting amine 14 to cyanamide in the presence of dilute sulfuric acid afforded the product 15.

#### EXPERIMENTAL SECTION

Radioactivity determinations were carried out with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer. The external standard method was used, with Diotol (Burdick-Jackson) as the scintillation solvent. Thin-layer chromatography (tlc) analyses were done on 2.5 x 10 cm or 5 x 20 cm glass plates precoated with a 250  $\mu$ m layer of silica gel GF (Analtech). Developed zones were visually detected under ultraviolet light (254 nm) or by exposure to iodine vapor. Radioactive zones were detected with a Vanguard Model 880 Autoscanner equipped with a Model 885 Glass Plate Scanner. The pmr spectra were obtained with a Varian Model A-60A spectrometer. Infrared (ir) spectra were obtained with a Digilab Model FTS-14D spectrophotometer and ultraviolet (uv) spectra with a Cary Model 15 spectrometer. Mass spectra were obtained with an LKB Type 9000 mass spectrometer equipped with direct inlet probe. Melting points were determined in capillary tubes and are uncorrected. Microanalyses were obtained for the indicated elements, and the results were all within  $\pm 0.4\%$  of the theoretical values.

#### 2-Phthalimidomethyl-1,4-dioxo[3-<sup>14</sup>C]spiro[4.5]decane (2)

A mixture of 9.16 mg of 3-chloro-1,2-[1-<sup>14</sup>C]propanediol\* (nominally 12.6

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\*Supplied by the former Mallinckrodt Labeled Compounds, now California Bio-nuclear Corporation, Sun Valley, CA 91352, U.S.A.

mCi, 15.2 mCi/mmol), 122 mg of 3-chloro-1,2-propanediol (1.93 mmol total, Eastman Organic Chemicals), 20 mg of *p*-toluenesulfonic acid monohydrate (Aldrich, 99%), and 393 mg of cyclohexanone (4.0 mmol, Aldrich) in 20 ml of toluene was refluxed for 4 hours under N<sub>2</sub> under a Dean-Stark water trap. The solution was filtered to remove traces of tarry materials and concentrated under reduced pressure. The residue was dissolved in 20 ml of dimethylformamide (DMF) and 740 mg of potassium phthalimide (4.0 mmol, Aldrich) was added. The mixture was heated with stirring at 185°C under N<sub>2</sub> overnight, cooled, and diluted with ether. Insoluble materials were removed by filtration, and the filtrate was washed with water and brine, and dried over magnesium sulfate. After evaporation of ether, the residue was triturated with acetone to remove traces of insoluble materials. The acetone solution was concentrated and the residue was crystallized from acetone-water to give 435 mg of **2** (75% yield), m.p. 94-3°C, [lit. m.p. 91-2°C(1)]; specific activity 15.7 μCi/mg or 4.74 mCi/mmol single component by tlc (19:1 v/v dichloromethane: methanol, R<sub>f</sub> 0.74; 3:1 v/v cyclohexane: ethyl acetate, R<sub>f</sub> 0.31); ir (Nujol mull) ν<sub>max</sub> cm<sup>-1</sup>: 1765, 1710 (C=O), 1160, 1115, 1030, 1010, 940, 920 (C-O and C-N); uv (in hexane) λ<sub>max</sub> nm (ε): 283 (1,350), 289 (1,550), 298 (1,400), λ<sub>SH</sub> 220 (14,950), 239 (8,450); pmr (in CDCl<sub>3</sub>, TMS) δ: 7.8 (4H, aromatic H), 4.6 (1H, O-C-H), 3.5-4.2 (4H, CH<sub>2</sub> not in cyclohexane ring), 1.2-1.8 (10H, CH<sub>2</sub> in cyclohexane ring).

(1,4-Dioxa[3-<sup>14</sup>C]spiro[4.5]dec-2-ylmethyl)guanidine Sulfate (4)

A solution of 298 mg of **2** (1.0 mmol) and 100 mg of hydrazine hydrate (2.0 mmol, MCB Reagents) in 5 ml of absolute ethanol was refluxed under N<sub>2</sub> for 2 hours. The mixture was cooled and diluted with 15 ml of ether. The precipitates were filtered and washed with ether. The combined filtrate and washing were concentrated to give a partially crystalline residue which was triturated with *n*-pentane and filtered. The *n*-pentane solution was evaporated under N<sub>2</sub> to give 169 mg of the amine **3**\* (~100% yield) as an oil (tlc, 10:9:1

\*Because of the sensitivity of the amine **3** to air, the reaction and workup mixtures containing **3** were handled at all times under a nitrogen tent.



v/v methanol: water: ammonium hydroxide,  $R_f$  0.74). A mixture of this oil (1.0 mmol), 0.5 ml of water, 2.0 ml of 0.2 N  $H_2SO_4$  (0.4 mmol), and 126 mg of purified\* cyanamide (3.0 mmol) was heated at 85°C (oil bath) with stirring under  $N_2$  for 48 hours, at which time another 21 mg of cyanamide (0.5 mmol) was added and the reaction was allowed to proceed for another 24 hours. The mixture was filtered and the filtrate was evaporated to dryness under vacuum. The solid residues were dissolved in 5 ml of methanol, the solution was heated to reflux, and 20 ml of absolute ethanol was added. The mixture was distilled while the volume was maintained at 20-30 ml by the periodic addition of a total of 50 ml of absolute ethanol, followed by 25 ml of a 3:1 v/v mixture of absolute ethanol: i-propanol. The volume was finally reduced to 5 ml and the mixture was stirred at room temperature overnight to yield 182 mg of **4** (70%), m.p. 240-241°C (dec.); specific activity 17.9  $\mu Ci/mg$  or 9.39 mCi/mmol; tlc showed presence of a single component (10.9:1 v/v methanol: water: 14.8 N ammonium hydroxide,  $R_f$  0.29; 39:1 v/v methanol: 14.8 N ammonium hydroxide,  $R_f$  0.12) identical to a standard sample of guanadrel sulfate; anal. - C, H, N.

#### 1,4-Cyclohexanediol Monotosylate (5)

*p*-Toluenesulfonyl chloride (36.9 g, 0.194 mol, Aldrich) was added in portions to a stirred solution of 25 g of 1,4-cyclohexanediol (0.215 mol, Eastman Organic Chemicals) in 100 ml of dry pyridine. The mixture was stirred at room temperature for 18 hours and poured into 300 ml of ice water. The mixture, containing much of the less soluble 1,4-cyclohexanediol ditosylate as crystalline precipitates (tlc, 9:1 dichloromethane: methanol,  $R_f$  0.79), was filtered and the filtrate was extracted with dichloromethane. The extracts were washed with dilute HCl, water, and brine in that order, and dried over magnesium sulfate. The solution was concentrated and the residual oil was chromatographed on a 100 x 3.8 cm (I.D.) column of silica gel (500 g, 70-230

\*Commercial cyanamide (Aldrich) was triturated with ether, filtered, and the filtrate concentrated to give pure cyanamide.

mesh, EM Laboratories) eluted with 9:1 v/v dichloromethane: methanol. Fractions of the eluate containing the monotosylate were combined, concentrated, and the residue was crystallized from ether-hexane to give 31.5 g of a mixture of the *cis* and *trans*-isomers of  $\delta$ , m.p. 77-106°C [lit. m.p. 94-95°C (14), 97.2-97.8°C (15)]; tlc of this material showed presence of a single component (9:1 v/v dichloromethane:methanol,  $R_f$  0.50), uv (in methanol)  $\lambda_{\max} \text{ nm } (\epsilon)$ : 225 (13,100), 256 (479), 262 (600), 267 (540), 272 (489); ir (in Nujol mull)  $\nu_{\max} \text{ cm}^{-1}$ : 3590, 3560 (OH), 1600, 1495 (C=C), 1345, 1190, 1175, 1125, 930 (-SO<sub>2</sub>-O-), 1095, 1075, 1030, 970 (C-O), 870, 810 (C-H); anal. - C,H,S. No attempt was made to separate the isomers since the mixture was to be oxidized to give a single product ( $\theta$ ) in the next step.

#### 4-Tosyloxycyclohexanone ( $\theta$ )

Chromium trioxide (44.4 g, 0.444 mol, MCB Reagents) was added cautiously in portions with stirring to 700 ml of cold (0°) pyridine. The mixture was stirred for 15 minutes and 15.0 g of  $\delta$  (0.055 mol) in 100 ml of pyridine was added in one portion. The mixture was stirred at room temperature overnight, diluted with 300 ml of ether, and filtered through a pad of celite. The filtrate was concentrated and the residue triturated with 300 ml of ether. The ether solution was washed with 4 x 100 ml of water, 2 x 100 ml of 1N HCl, 2 x 100 ml of H<sub>2</sub>O and 100 ml of brine in that order, and dried over MgSO<sub>4</sub>. Removal of solvent afforded 11.77 g of the ketone  $\theta$  (79.7% yield) after recrystallization from benzene-hexane, m.p. 95.5-96.5°C, chromatographically homogeneous (tlc, 19:1 v/v dichloromethane: methanol,  $R_f$  0.77, 7:3 v/v hexane: acetone,  $R_f$  0.33); uv (in ethanol)  $\lambda_{\max} \text{ nm } (\epsilon)$ : 225 (12,800), 256 (488), 262 (614), 267 (563), 273 (510); pmr (in CDCl<sub>3</sub>, TMS) $\delta$ : 7.8, 7.35 (4H aromatic H), 4.9 (1H, O-C<sup>1</sup>-H), 2.5 (7H, CH<sub>3</sub>, O=C<sup>1</sup>-CH<sub>2</sub>), 2.1 (4H, O-C<sup>1</sup>-CH<sub>2</sub>); anal. -C,H,S.

#### 3-Phthalimido-1,2-propanediol ( $\vartheta$ )

A mixture of 18.54 g of potassium phthalimide (100 mmol), 11.00 g of 3-chloro-1,2-propanediol (100 mmol) and 25 ml of DMF was gently refluxed with stirring under N<sub>2</sub> for 6 hours. The cooled mixture was filtered and the solids were washed with acetone. The combined filtrate and washing were concentrated

and the residual oil was boiled with benzene. The hot solution was decanted to separate traces of insoluble oil. The solution on cooling afforded 12.0 g of  $\vartheta$  (54% yield), m.p. 114-6°C; [lit. m.p. 112-3°C (16)]; homogeneous by tlc (9:1 v/v dichloromethane: methanol,  $R_f$  0.27); uv (in ethanol)  $\lambda_{\max}$  nm ( $\epsilon$ ): 219 (40,050), 294 (1,850),  $\lambda_{\text{sh}}$ : 232 (13,350), 241 (9,500), 297 (1,800); pmr (in  $\text{CDCl}_3$ , TMS) $\delta$ : 7.75 (4H, aromatic H), 4.75 (d, 1H, CH-OH), 4.45 (t, 1H, CH<sub>2</sub>-OH), 3.5-4.0 (m, 5H, CH<sub>2</sub>, CH); anal. - C, H, N.

2-Phthalimidomethyl-8-tosyloxy-1,4-dioxaspiro[4.5]decane ( $\zeta$ )

A mixture of 4.95 g of  $\vartheta$  (22.4 mmol), 6.00 g of  $\epsilon$  (22.3 mmol), and 50 mg of *p*-toluenesulfonic acid in 200 ml of benzene was refluxed with stirring under N<sub>2</sub> under a Dean-Stark water trap for 3.5 hours. The solvent was removed at reduced pressure and the residue was chromatographed on a 500 g, 1 m x 3.8 cm (I.D.) column of silica gel eluted with 19:1 v/v dichloromethane: methanol. After a forerun of 150 ml, the eluate was collected in 20 ml fractions. From the pooled fractions 36-50, there was obtained 7.92 g of  $\zeta$  (75% yield), m.p. 136-143°C\*, chromatographically homogeneous by tlc (19:1 v/v dichloromethane: methanol,  $R_f$  0.71); uv (in ethanol)  $\lambda_{\max}$  nm ( $\epsilon$ ): 219 (52,600), 261 (1,250), 265 (1,350), 273 (1,450), 293 (1,900),  $\lambda_{\text{sh}}$ : 240 (9,300), 256 (1,150), 297 (1,850); ir (Nujol mull)  $\nu_{\max}$  cm<sup>-1</sup> 1770, 1715 (C = O), 1615, 1600, 1495 (C = C), 1395, 1355, 1190, 1170, 1125 (-SO<sub>2</sub>-O), 1085, 1015, 915, 910 (C-O, C-N), 720, 715, 685 (C-H); pmr (in  $\text{CDCl}_3$ , TMS) $\delta$ : 7.8-7.4 (8H, aromatic H), 4.6 (1H, O-C-H of cyclohexane ring), 4.4 (1H, O-C-H of dioxolane ring), 3.6-4.2 (4H, CH<sub>2</sub> not in cyclohexane ring), 2.5 (3H, CH<sub>3</sub>), 1.4-2.0 (8H, CH<sub>2</sub> in cyclohexane ring); anal. -C,H,N.

2-Phthalimido-1,4-dioxaspiro[4.5]dec-7-ene ( $\theta$ )

A) Elimination of *p*-Toluenesulfonic Acid from  $\zeta$

A solution of 10.70 g of  $\zeta$  (22.6 mmol) in 100 ml of pyridine was refluxed under N<sub>2</sub> overnight. The solvent was removed at reduced pressure and the residue was chromatographed on silica gel with 3:1 v/v cyclohexane: ethyl acetate to give 1.43 g of  $\theta$  (21.2% yield) after recrystallization

\*Melting point was not improved by recrystallization from benzene-pentane. The wide range was probably due to diastereoisomeric mixture since two chiral centers are present in  $\zeta$  (C-2 and C-8).

pentane, m.p. 74-5°C; chromatographically homogeneous by tlc (3:1, v/v cyclohexane: ethyl acetate,  $R_f$  0.30); uv (in ethanol)  $\lambda_{\max}$  nm ( $\epsilon$ ): 219 (41,200), 293 (1,950),  $\lambda_{sh}$ : 233 (12,050), 239 (8,950), 298 (1,850); ir (Nujol mull)  $\nu_{\max}$  cm<sup>-1</sup>: 1770, 1710 (C=O), 1610 (C=C), 1430, 1395, 1115, 1060, 1035, 1030 (C-O, C-N), 725, 715 (C-H); pmr (in CDCl<sub>3</sub>, TMS)  $\delta$ : 7.8 (4H, aromatic H), 5.6 (2H, C=C-H), 4.55 (1H, O-C-H), 4.1 (2H, O-CH<sub>2</sub>), 3.8 (2H, N-CH<sub>2</sub>), 1.8-2.4 (6H, CH<sub>2</sub> in cyclohexene ring); anal. -C,H,N.

#### B) Ketalization of 2-Cyclohexen-1-one with 9

A mixture of 1.086 g of 2-cyclohexen-1-one (11.3 mmol, Eastman Organic Chemicals), 2.500 g of 9 (11.3 mmol), 40 mg of *p*-toluenesulfonic acid and 100 ml of benzene was refluxed under N<sub>2</sub> under a Dean-Stark water trap overnight. The mixture was concentrated at reduced pressure and the residue was chromatographed on a column of 80 g of silica gel eluted with 3:1 v/v cyclohexane: ethyl acetate. The crude product was recrystallized from pentane to give 2.52 g (74.6% yield) of 8, which was identical (tlc, m.p., mixed m.p.) to the sample of 8 prepared from 7 as described above.

Assignment of the double bond in 8 to the 7,8- and/or 8,9-positions rather than the 6,7-position (structure 10) was based on pmr data (see below) of samples of deuterium labeled cyclohexanone 2,4-DNP, 13 and 12, derived respectively from [2,3-<sup>2</sup>H]cyclohexan-1-one and intermediate 8 which had been prepared with either method A or method B. See also the "Discussion and Results" section for details of this study.

#### Reduction of 8 to 11

Reduction of 8 was carried out separately with hydrogen, deuterium, and tritium\* gases to give 11a, 11b, and 11c, respectively. A solution of 300 mg of 8 (1.00 mmol) and 150 mg of (Ph<sub>3</sub>P)<sub>3</sub>RhCl (Aldrich) in 35 ml of benzene was stirred at room temperature under the appropriate gas until the theoretical amount of gas had been absorbed (2-3 hours). The mixture was diluted with 100 ml of ether and refluxed for 30 minutes. The mixture was poured onto a dry column of 30 g of 30-60 mesh Florisil (Floridin Co.),

\*Reduction with tritium gas was carried out by New England Nuclear Corp., Boston, Mass., U.S.A., on a sample of 8 prepared from 2-cyclohexen-1-one and the diol 9 (Method B).

and the column was eluted with 200 ml of ether. The eluate was concentrated and the residue was crystallized from a mixture of 3 ml of acetone and 15 ml of water or from *i*-propanol (2-3 ml per g). The procedure typically afforded 295 mg of *11* (97% yield), 93-4°C, tlc: 3:1 v/v cyclohexane: ethyl acetate,  $R_f$  0.31, same as *2*.

Crude tritium labeled 2-phthalimido-1,4-dioxaspiro[4.5]decane (*11a*), nominally 18 mg, 28 mCi/mg, was mixed with 2.00 g of non-labeled material (*11a*) and recrystallized from 4 ml of hot *i*-propanol to give 1.90 g of *11c*, m.p. 93-94°C; sp. act. 222  $\mu$ Ci/mg, or 66.9 mCi/mmol; radiochemically pure by tlc (3:1 v/v cyclohexane: ethyl acetate,  $R_f$  0.31, same as *2*

#### Alternative Preparations of *11a*

2-Phthalimido-1,4-dioxaspiro[4.5]decane (*11a*) was also prepared in 64% yield from 3-chloro-1,2-propanediol, cyclohexanone (Eastman Organic Chemicals), and potassium phthalimide (see procedure for the preparation of *2*). In a third method, *11a* was obtained in  $\sim$  90% yield by the condensation of cyclohexanone with IX in a manner similar to the preparation of *8* from *9* (Method B).

#### Conversion of *11* to Cyclohexanone 2,4-Dinitrophenylhydrazone (*12*)

The hydrolysis of *11* and conversion of the resulting cyclohexanone to *12* were carried out in one step. 2,4-Dinitrophenylhydrazine (500 mg, 2.52 mmol, Eastman Organic Chemicals) was dissolved in 6 ml of conc.  $H_2SO_4$  and the solution was diluted with 3 ml of water and 10 ml of ethanol. This solution was added with swirling to a solution of 300 mg of *11* (1.0 mmol) in 5 ml of ethanol. The resulting crystalline precipitates were filtered, washed with ethanol and dried, 264 mg (94% yield), m.p. 160-161°C [lit. m.p. 160°, 162° (17)], readily recrystallized from hot EtOH; pmr (CDCl<sub>3</sub>, TMS) $\delta$  : for *12a*, 11.20 (s, broad, 1H, N-H), 7.83-9.16 (m, 3H, aromatic H), 2.53 (s, broad, 4H,  $\alpha$ -H), 1.80 (s, broad, 6H,  $\beta$ - and  $\gamma$ -H); for *12b* obtained from *11b* prepared by either method A or method B, 11.20 (1H), 7.83-9.16 (3H), 2.53 (4H), 1.80 (4H).

To prepare *12c*, 301 mg (1.00 mmol) of *11c*, sp. act. 23.4  $\mu$ Ci/mg or 7.06

mCi/mmol, in 2 ml of methanol was stirred with 1 ml of 1N HCl at room temperature for 30 minutes. To the solution, pH  $\sim$  1, was added 253 mg (4.5 mmol) of KOH pellets. The mixture was stirred for 1 hour. To the solution, pH  $\sim$  14, was added with stirring 10 ml of 2,4-dinitrophenylhydrazine solution (2.0 g in 10 ml of conc. H<sub>2</sub>SO<sub>4</sub>, 15 ml of water, and 50 ml of ethanol). The resulting precipitates were recrystallized from 35 ml of hot ethanol to give 228 mg of *12c* (82% yield), sp. act. 25.2  $\mu$ Ci/mg or 7.00 mCi/mmol, m.p. 160-161°C, single component by tlc (dichloromethane, R<sub>f</sub> 0.58).

[2,3-<sup>2</sup>H]Cyclohexan-1-one 2,4-Dinitrophenylhydrazone 13

A solution of 192 mg of 2-cyclohexen-1-one (2.0 mmol) in 30 ml of benzene containing 50 mg of (Ph<sub>3</sub>P)<sub>3</sub>RhCl was stirred under deuterium gas for 2.5 hours, refluxed with 100 ml of ether and passed through a dry column of 30 g of 30-60 mesh Florisil followed by 200 ml of ether. Removal of ether and treatment of the residue in ethanol with 2,4-dinitrophenylhydrazine gave *13*, recrystallized from hot ethanol, m.p. 160-161°C; pmr (CDCl<sub>3</sub>, TMS) $\delta$ : 11.20 (s, broad, 1H, N-H), 7.83-9.16 (m, 3H, aromatic H), 2.53 (s, broad, 3H,  $\alpha$ -H), 1.80 (s, broad, 5H,  $\beta$ -, and  $\gamma$ -H).

[1,4-Dioxa[7,8-<sup>3</sup>H]spiro[4.5]dec-2-ylmethyl)guanadine Sulfate (15)

The procedure for preparing carbon-14 labeled guanadrel sulfate (*4*) from *2* described above was followed. From 422 mg of *11c* (sp. act. 66.9 mCi/mmol), tritium labeled guanadrel sulfate (*15*) was obtained in 70% yield, m.p. 240-241°C; sp. act. 254  $\mu$ Ci/mg or 66.7 mCi/mmol; chromatographically homogeneous by tlc (10:9:1 methanol: water: 14.8N ammonium hydroxide, R<sub>f</sub> 0.29) and identical to a standard sample of guanadrel sulfate; anal. -C,H,N.

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