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THE SYNTHESES OF RADIOLABELLED ORG 5222 AND ITS MAIN METABOLITE ORG 30526

Jan Vader, Frans Kaspersen, Eric Sperling, Irene Schlachter, Annie Terpstra, Peter Hilberink and Gerard Wagenaars.

N.V. Organon, Scientific Development Group, P.O. Box 20, 5340 BH, Oss, The Netherlands.

SUMMARY

The syntheses of trans-5-chloro-2,3,3a-12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (Org 5222), a potential antipsychotic compound, labelled with ^{3}H, ^{14}C and ^{36}Cl and trans-5-chloro-2,3,3a,12b-tetrahydro-1H-dibenz[2,3:6,7]-oxepino[4,5-c] pyrrole (Org 30526) labelled with ^{3}H are described. ^{3}H-labelled Org 5222 of low specific activity was prepared by a base catalyzed exchange with tritiated water of an amide precursor, ^{3}H-labelled Org 5222 with a high specific activity by a catalytic reductive dehalogenation. ^{3}H-labelled Org 30526 was prepared both by demethylation of ^{3}H-Org 5222 and by catalytic reductive iodination of 11-iodo-Org 30526. ^{14}C-labelled Org 5222 was synthesized in 6 steps using ^{14}C-sarcosine as starting material. ^{36}Cl-labelled Org 5222 was prepared by diazotation reaction in the presence of $H^{36}Cl$.

Key words: chlorine-36, electrophilic iodination, N-demethylation, tritium, ^{3}H-NMR, carbon-14 sarcosine

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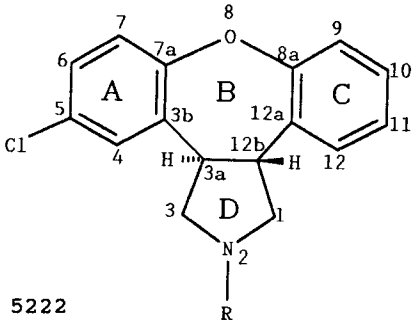
INTRODUCTION

Org 5222 (trans 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole[1], Figure 1) is a potential antipsychotic drug presently under clinical investigation. Some physico-chemical[2], metabolic[3], pharmacological[4] and neurochemical[5] properties of the compound have been published recently. Org 30526 (N-demethyl Org 5222) is one of the main metabolites of Org 5222.



1a R=CH_3 : Org 5222

1b R=H : Org 30526

Figure 1: structure of Org 5222 and Org 30526

In the course of (pre-)clinical development studies, specifically labelled compounds with ^{3}H, ^{14}C and ^{36}Cl were required. In this paper the syntheses and characterizations of these compounds are described.

RESULTS AND DISCUSSION

[5-^{36}Cl]-Org 5222

Org 5222 labelled with ^{36}Cl (β^-, 714 keV; $t_{\frac{1}{2}}$ = 3 x 10^5 years) was synthesized to be able to study the metabolic fate of the chlorine atom. The synthesis started with the deschloro-analogue of Org 5222 2 which was converted into the mononitro-compound 3 by treatment with HNO_3/H_2SO_4 in nitromethane. Reduction of the nitro group with H_3PO_3-Pd/C in refluxing methanol gave the amine 4. Diazotation of 4 with $NaNO_2/H^{36}Cl$ in the presence of powdered copper gave the aimed [5-^{36}Cl]-Org 5222 5, albeit in a low yield of 3%.

Field desorption mass spectrometry[6] indicated a specific activity of 0.54 mCi/mmol (20 MBq/mmol; 28% ^{35}Cl, 47% ^{36}Cl, 25% ^{37}Cl). This value is in agreement with the value of 0.53 mCi/mmol (20 MBq/mmol for the $H^{36}Cl$ used as starting material).

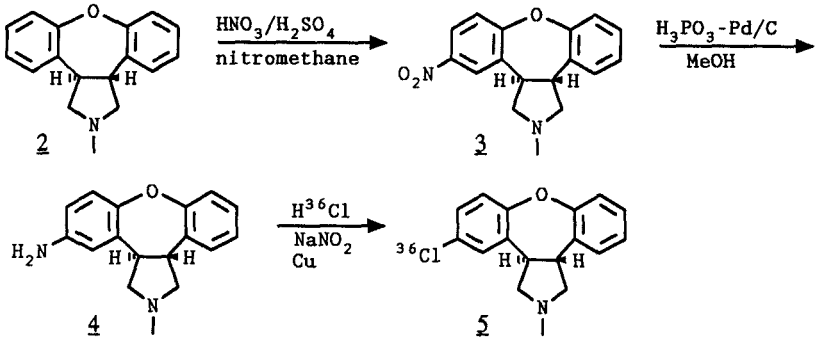


Figure 2: synthesis of [5-^{36}Cl]-Org 5222.

[3a-^{3}H]-Org 5222

[3a-^{3}H]-Org 5222 10 was synthesized from the cis amide 6 by a base catalyzed exchange with tritiated water. The resulting mixture of tritiated cis amide 7 and trans amide 8 was separated by HPLC. Unfortunately the trans amide 8 could only be reduced to the aimed trans amine 10 if the amide 8 was diluted with a thousandfold of carrier. In this way [3a-^{3}H]-Org 5222 could be synthesized, albeit only with a low specific activity of 20 mCi/mmol (740 MBq/mmol).

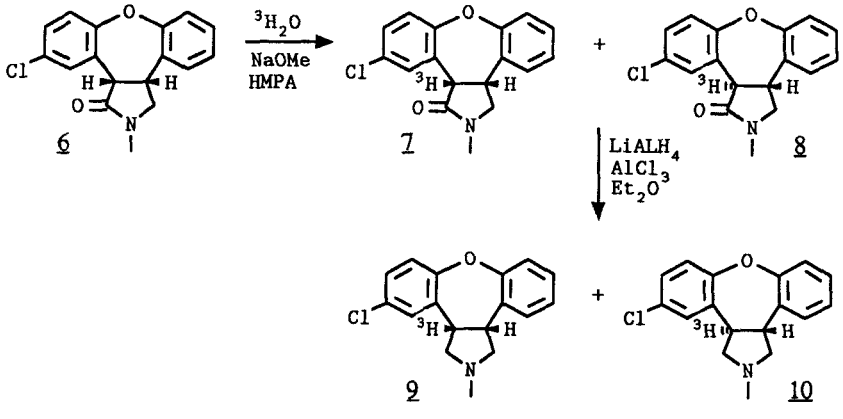
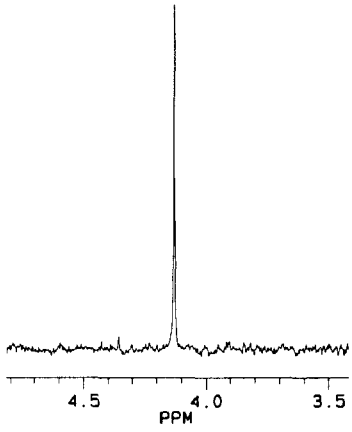
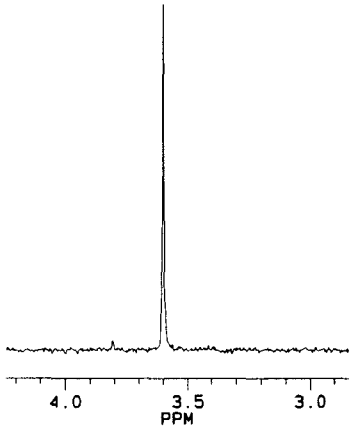


Figure 3: synthesis of [3a-^{3}H]-Org 5222.



^{3}H-NMR spectrum of amide 8 (C^2HCl_3; ^{1}H-decoupled)



^{3}H-NMR spectrum of [3a-^{3}H]-Org 5222 (C^2HCl_3; ^{1}H-decoupled)

Figure 4: ^{3}H-NMR spectra

The ^{3}H-NMR spectra of the amide 8 and the amine 10 are in agreement with labelling at position 3a of the respective trans compounds.

[11-^{3}H]-Org 5222

Aryl tritiated Org 5222 could be synthesized by a direct bromination/iodination of Org 5222 followed by a selective reductive dehalogenation. A prerequisite for a successful halogenation was the blocking of N-2, since otherwise D-ring oxidation occurred. Bromination in carbon tetrachloride gave mainly the dibromopyrrole 11 and bromination in acetic acid gave a complex mixture from which the amide 12, the imide 13 and the bromide 14 could be isolated. Bromination with bromine in 10% aqueous sulphuric acid gave the aimed bromide 14 in an acceptable yield of 45%. Although the bromide 14 may very well be a useful precursor for [^{3}H]-Org 5222, it can be anticipated that, for selectivity reasons, the iodide may be an even better precursor.

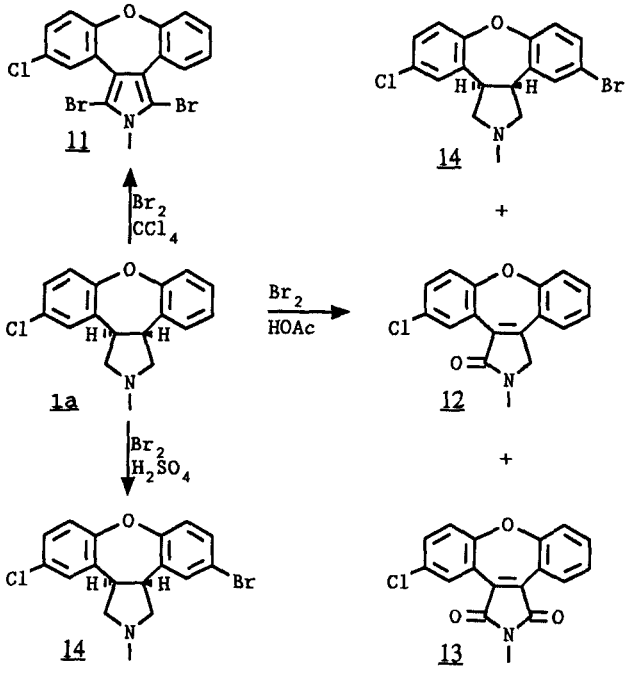


Figure 5: bromination of Org 5222

The very reactive iodination reagent N-iodosuccinicide/triflic acid, developed by Konradsson *et al.*[7] for the activation of pentenyl glycosides has been recently applied successfully in electrophilic iodinations of electron-rich[8] and electron-poor aromatic compounds[9]. By reacting with 1.0 equivalent NIS (to prevent multi-iodination) the aimed 11-iodo-Org 5222 15 was obtained in 31% yield. Subsequent reductive deiodination with $^3H_2$-Pd/C gave [11-^{3}H]-Org 5222 16 with a specific activity of 20 Ci/mmol (0.74 TBq/mmol).

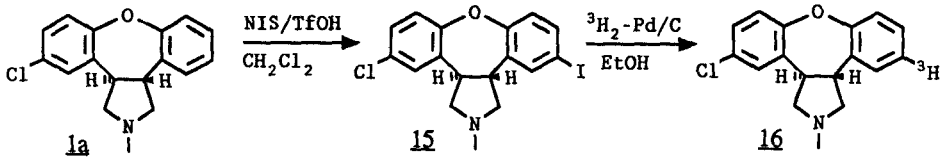


Figure 6: synthesis of [11-^{3}H]-Org 5222.

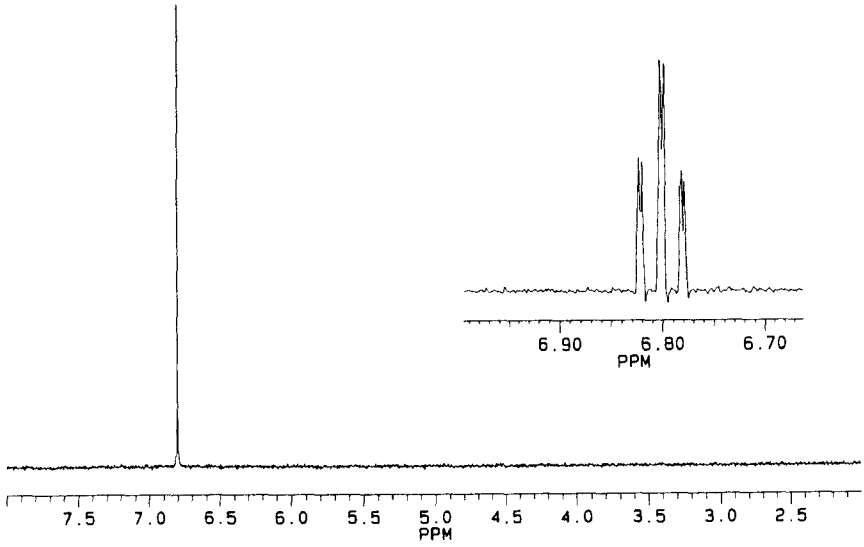


Figure 7: ^{3}H-NMR spectra of [11-^{3}H]-Org 5222 (C_6H_6; ^{1}H-decoupled and ^{1}H-coupled)

The proton decoupled ^{3}H-NMR spectrum gave a singlet at 6.80 ppm and the proton coupled spectrum gave a double triplet with coupling constants of 8 Hz and 2 Hz. These data are in agreement with labelling at position C-11.

[7,11-^{3}H]-Org 5222

Aryl tritiated Org 5222 with a higher specific activity was synthesized by the reaction sequence depicted in Figure 8. The advantage of this sequence is the control over the position of labelling since nitration deactivates the ring C sufficiently to prevent further nitration of this ring and the A ring contains only one activated position for nitration. Org 5222 1a was nitrated with nitric acid/sulphuric acid in sulpholane to give the dinitro compound 18 as the main product and a small amount of mononitro compound 17. The nitro groups in 18 were reduced with iron powder in acetic acid and the resulting diamine 19 was subsequently diazotated with sodium nitrite in hydrochloric acid in the presence of potassium iodide.

Reductive tritiation of diiodo-compound 20 with $^3H_2$-Pd/C in ethanol gave the aimed tritiated Org 5222 21. The specific activity of the material varied from 30 Ci/mmol (1.1 TBq/mmol) to 42 Ci/mmol (1.6 TBq/mmol).

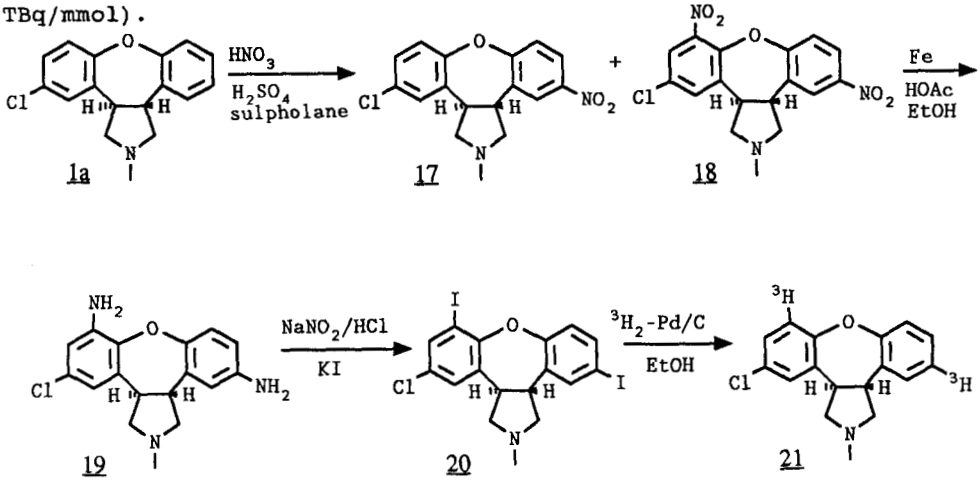


Figure 8: synthesis of [7,11-^{3}H]-Org 5222.

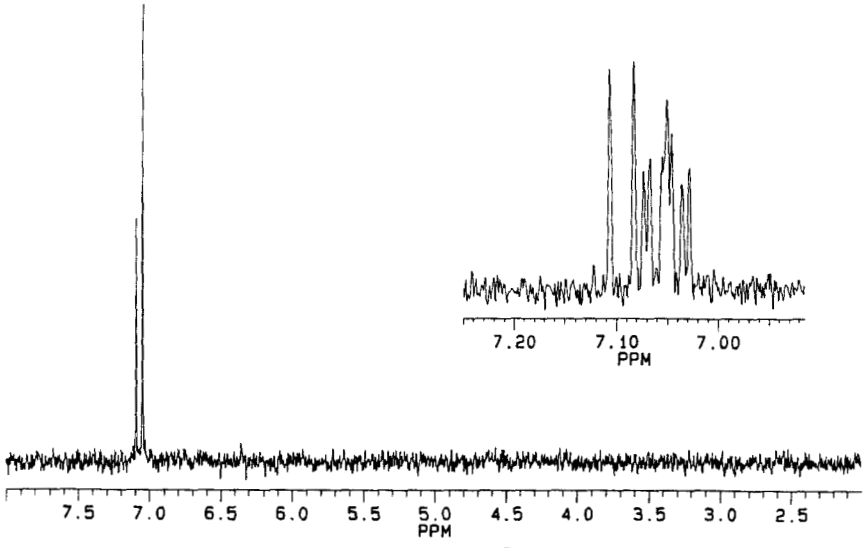


Figure 9: ^{3}H-NMR spectra of [7,11-^{3}H]-Org 5222 (C^2HCl_3; ^{1}H-decoupled and ^{1}H-coupled)

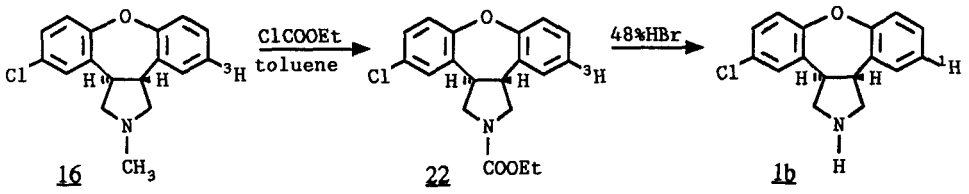
As can be seen in the proton decoupled ^{3}H-NMR spectrum the label is not equally distributed over the positions C-7 and C-11, but rather in a ratio of approximately 1/2.

The lower degree of labelling at C-7 can be explained by a relatively slow reduction of the iodo-group at C-7, during which a dilution of the tritium gas occurred as a result of exchange with the ethanol used as solvent. The positions of labelling could be deduced from the chemical shifts in combination with the splitting pattern as derived from the proton coupled ^{3}H-NMR spectrum. In contrast to the ^{3}H-NMR spectrum in figure 7, the triton at C-11 does not appear as a double triplet, but rather as a double, double doublet as result of a small difference in the two ortho couplings in $C^{2}HCl_{3}$.

^{3}H-Org 30526

The secondary amine Org 30526 is the main metabolite of Org 5222. As a result of this, the need for radiolabelled Org 30526 arose during the development of Org 5222. Since tritiated Org 5222 was available in sufficient quantities the first approach to synthesize ^{3}H-Org 30526 was based on the demethylation of ^{3}H-Org 5222. However, an initial attempt to synthesize [11-^{3}H]-Org 30526 from [11-^{3}H]-Org 5222 16 was quite unsuccessful. [11-^{3}H]-Org 5222 (specific activity : 32 mCi/mmol; 1.2 GBq/mmol) was converted into the carbamate 22 by treatment with ethyl chloroformate in refluxing toluene. This carbamate was refluxed for 2 hours in concentrated hydrobromic acid, which resulted in a rather clean chemical conversion into the secondary amine 1b as indicated by TLC in combination with UV detection. However, most of the radioactivity was lost during this reaction as tritiated water. The aimed tritiated secondary amine was obtained in only 2% radiochemical yield after purification. The specific activity of this material was only 2.6 mCi/mmol (95 MBq/mmol), indicating that > 90% of the label was lost as tritiated water.

This loss of label can be explained by the strongly acidic reaction conditions in combination with the para phenoxy substituent in the molecule.



16 22 1b

Figure 10: demethylation of ^{3}H-Org 5222

N-demethylation procedures for compounds like Org 5222 are of a more general interest. Therefore we decided to study this N-demethylation procedure in some more detail. [7,11-^{3}H]-Org 5222 21 was used as starting material for this study. The specific activity of this material was 41 Ci/mmol (1.5 TBq/mmol) and the distribution of the label 7/11 was 2/3.

This tertiary amine was converted into the carbamate 23 in 67% radiochemical yield after purification. The specific activity of the material was 41 Ci/mmol (1.5 TBq/mmol) and the degree of labelling of positions 7/11 was 2/3, so no significant loss of label had occurred during this reaction. A carbamate like 23 can be hydrolyzed to the secondary amine either under strongly basic or under strongly acidic conditions[10]. Base catalyzed hydrolysis of 23 afforded the aimed amine 24 in 54% yield after HPLC purification. The specific activity of the material was 37 Ci/mmol (1.4 TBq/mmol). This small decrease in specific activity can be explained by a dilution during the HPLC rather than by a loss of label during the reaction, as can be deduced from the unchanged ratio mono-/di-labelled material (see figure 12).

Acid catalyzed hydrolysis of the carbamate resulted in a significant loss of label. After treatment with hydrobromic acid at 100°C for 30 minutes, the carbamate was converted for approximately

50% into the amine. The amine 24 was isolated in only 17% radiochemical yield after HPLC purification. The ratio of the labelling at positions 7/11 was 3/2 indicating a preferential loss of label at position 11. Hydrobromic acid catalyzed hydrolysis of the carbamate 23 for 1 hour resulted in 70% conversion and a 18% radiochemical yield after purification. The 7-^{3}H/11-^{3}H ratio of this material was 5/1, indicating that most of the label at position 11 was lost as tritiated water during the reaction. The label at position 7 is (almost) stable during the reaction, as can be deduced from the relatively high abundance of mono-labelled material.

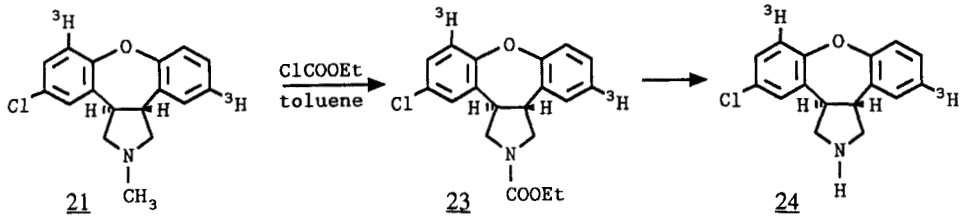
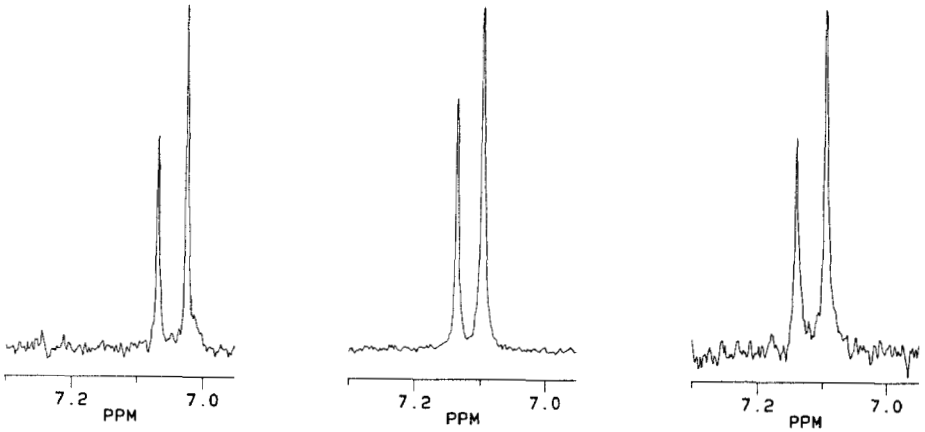


Figure 11: synthesis of [7,11-^{3}H]-Org 30526

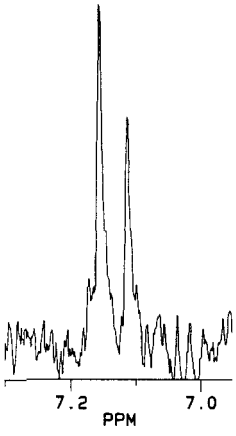


^{3}H-NMR spectrum of ^{3}H-Org 5222 21

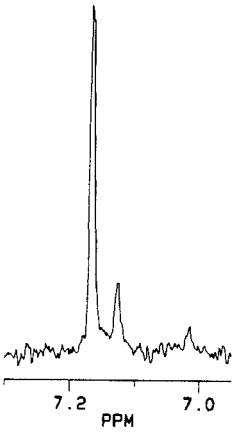
^{3}H-NMR spectrum of ^{3}H-carbamate 23

^{3}H-NMR spectrum of ^{3}H-Org 30526 24

Figure 12: ^{3}H-NMR spectra



^{3}H-NMR spectrum of ^{3}H-Org 30526 24 (acid hydrolysis, 30 min)



^{3}H-NMR spectrum of ^{3}H-Org 30526 24 (acid hydrolysis, 1 hour)

Figure 12: continued

Tritiated Org 30526 can also be synthesized by the reversed synthesis route: Org 5222 is demethylated and subsequently halogenated and reductively dehalogenated. Unlabelled Org 5222 was converted into unlabelled Org 30526 by the acid hydrolysis of the intermediate carbamate. Electrophilic iodination of Org 30526 with N-iodosuccinide/triflic acid gave the 11-iodo-Org 30526 25. Reductive tritiation of this compound using 10% palladium on charcoal as catalyst and ethanol as solvent gave the aimed [11-^{3}H]-Org 30526 26 with a specific activity of 24 Ci/mmol (880 GBq/mmol).

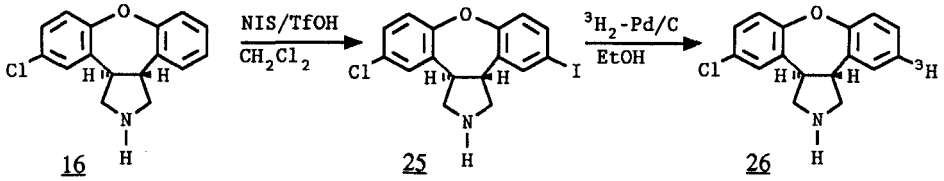


Figure 13: synthesis of [11-^{3}H]-Org 30526

Stability of ^{3}H-Org 5222 and ^{3}H-Org 30526

The free base of ^{3}H-Org 5222 could be stored for only two months in toluene at -20°C whereas the HCl salt of the material could be stored in toluene/ethanol at -20°C for more than a year with less than 5% radiolytic decomposition.

Upon storage of ^{3}H-Org 30526 in toluene/ethanol at -20°C we observed $\geq$ 5% radiolytic decomposition within two months, both when the compound was stored as a free base or as a HCl salt.

[12b-^{14}C]-Org 5222

For human metabolism studies ^{14}C-labelled Org 5222 was synthesized by a six steps synthesis as shown in Figure 14 following the same route as described by van der Burg[11].

^{14}C-sarcosine 27 was esterified with thionyl chloride in methanol and coupled with the acid chloride 30. Treatment of the amide ester 31 with potassium tert.butoxide in toluene gave the cyclic dione 32, which was subjected to further ring closure by treatment with polyphosphoric acid. Reduction of the α,ß-unsaturated amide 33 with magnesium in methanol gave a mixture of cis amide 34 and trans amide 35 which could be separated by column chromatography.

Reduction of the trans amide 35 with lithium aluminium hydride/aluminium chloride in tetrahydrofuran gave the aimed trans amine 37 and, remarkably, a small amount of cis amine 36, as a result of isomerization during the allane reduction. By using this reaction sequence ^{14}C-labelled Org 5222 was obtained in an overall yield of 2% based on the ^{14}C-sarcosine. The specific activity of the radioactive material obtained was 56 mCi/mmol (2.1 GBq/mmol) which is good agreement with the specific activity of the ^{14}C-sarcosine (53 mCi/mmol, 2.0 GBq/mmol) used as starting material.

Finally the ^{14}C-labelled Org 5222 was converted into the maleate, diluted with carrier material and recrystallized from ethanol. The specific activity of the final material was 140 µCi/mmol (52 MBq/mol).

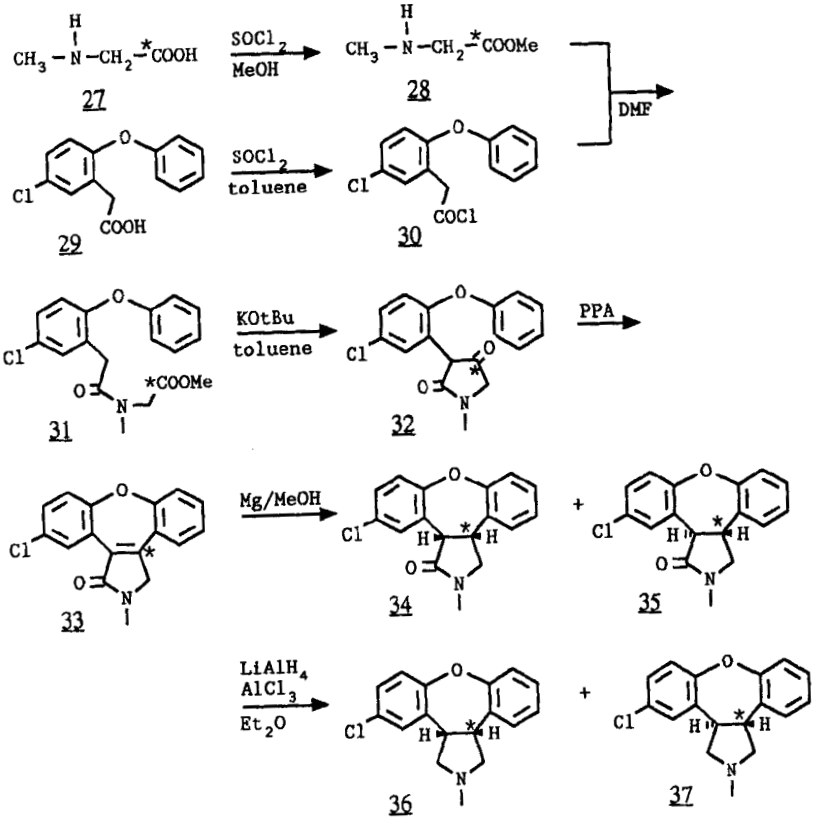
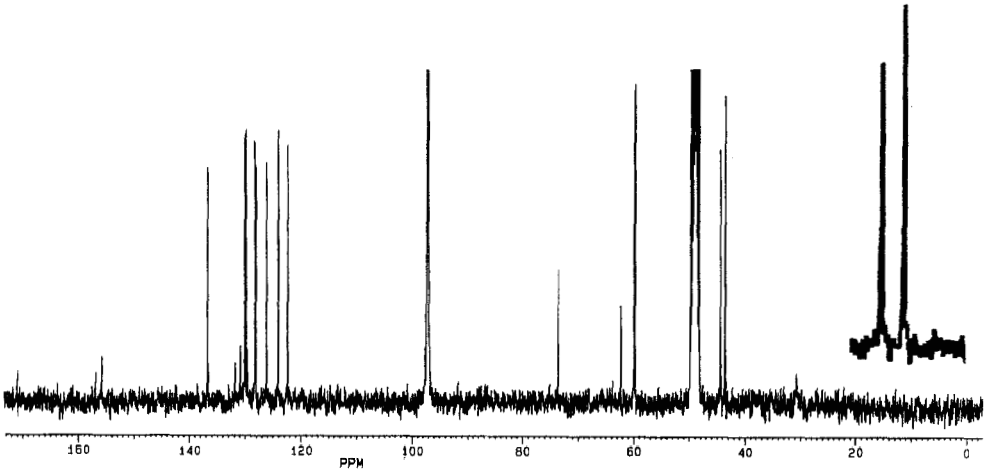
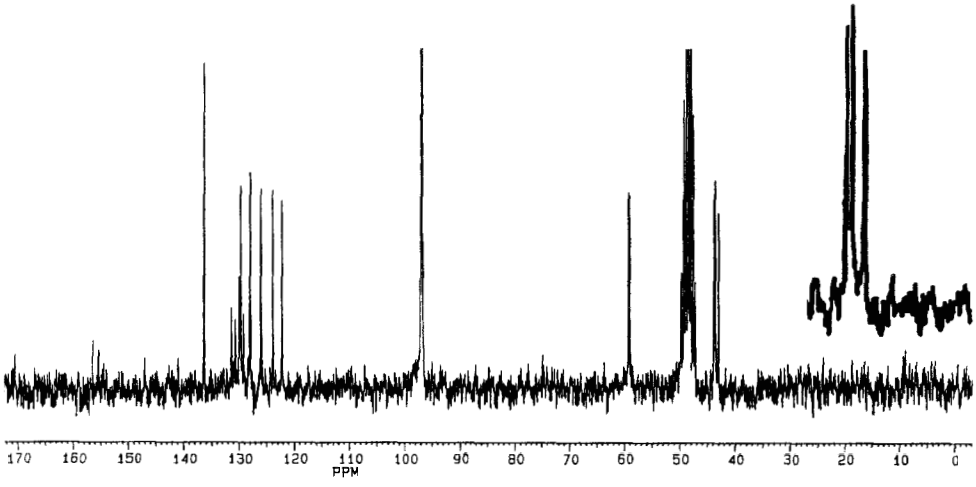


Figure 14: synthesis of [12b-^{14}C]-Org 5222

In the ^{13}C-NMR spectrum of the "carrier free" material signals of carbon tetrachloride at 97 ppm and two minor unlabelled impurities at 62 ppm and 74 ppm can be seen. The signal of C-12b at 44 ppm is absent. In the ^{13}C-NMR spectrum of the "diluted" material the signal of C-12b returns and the impurities have disappeared as a result of the extra crystallization step.



^{13}C-NMR spectrum of carrier free [12b-^{14}C]-Org 5222



^{13}C-NMR spectra of [12b-^{14}C]-Org 5222 with carrier

Figure 15: ^{13}C-NMR spectra

EXPERIMENTAL

The NMR spectra were recorded on a Bruker AC200 or AM360 spectrometer. Mass spectra were obtained from HP 5995A, Finnigan MAT 90 and Finnigan MAT TSQ 70 mass spectrometers and for the ^{36}Cl-compound from a UG 2AB-2F instrument by Dr. Lehmann, University Hospital Hamburg, Germany. Radio TLC plates were autoradiographed or scanned on a Rita 68000 linear analyzer.

HPLC purifications were done on a Waters 6000 A HPLC equipped with a Pye Unicam PU4025 UV detector and a Ramona radioactivity monitor. The reactions with $^{3}H_2$ and $^{3}H_2O$ were carried out by Amersham, Cardiff, UK. $H^{36}Cl$ was purchased from New England Nuclear, Boston, USA. ^{14}C-sarcosine was purchased from Cambridge Research Biochemicals, Billingham, UK.

Tritiated Org 30526 preparations were analyzed by TLC on silica gel with dichloromethane/methanol (8/2, v/v) and n-butanol/ acetic acid/water (4/1/1, v/v/v) as the eluents and by HPLC on μBondapak Phenyl with 0.07 M aqueous sodium dihydrogen phosphate/ acetonitrile/methanol (60/37/3, v/v/v) as mobile phase. The purities were ≥ 95%.

Radiolabelled Org 5222 preparations were analyzed by TLC on silica gel with dichloromethane/methanol (94/6, v/v), toluene/ ethanol (75/25, v/v) and/or methanol/25% ammonia (99/1, v/v) as the eluents and by HPLC on μBondapak C18 with 60% acetonitrile/ 40% 0.1 M ammonium acetate (pH=4.2) as mobile phase. The purity of the tritiated end products was ≥ 97% and the purities of the [^{36}Cl]-Org 5222 and [^{14}C]-Org 5222 were ≥ 99%.

Aqueous solutions were usually extracted three times with an appropriate organic solvent. The combined organic extracts were washed with brine, dried on sodium sulphate, filtered and evaporated to dryness under reduced pressure.

trans 2,3,3a,12b-tetrahydro-2-methyl-5-nitro-1H-dibenz[2,3:6,7] oxepino[4,5-c]pyrrole (3)

1.7 g (6.7 mmol) of 2 were dissolved in 25 ml nitromethane, and 1.3 ml of a 1:2 mixture (molar ratio) of concentrated nitric acid and concentrated sulphuric acid was added in three portions. The reaction mixture was stirred for 10 minutes, neutralized with aqueous ammonia and extracted with chloroform. Purification of the

crude product (1.6 g) on silica gel using toluene/ethanol (9/1, v/v) as the eluent gave 1.1 g (5.7 mmol) of pure 3 (55% yield). ^{1}H-NMR (C^2HCl_3): 2.56 (s, 3H), 3.1-3.4 (m, 4H), 3.5-3.8 (m, 2H), 7.1-7.3 (m, 5H), 7.98 (d, J = 3 Hz, 1H), 8.08 (dd, J_1 = 8 Hz, J_2 = 3 Hz, 1H). MS (EI) 296 (26%), 266 (2%), 57 (100%).

trans 5-amino-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (4)

300 mg (1.0 mmol) of nitro-compound 3 were dissolved in 6 ml methanol and 750 mg of phosphorous acid and 240 mg of 10% palladium on charcoal were added. The reaction mixture was refluxed for 1 hour, cooled to room temperature and filtered over hyflo. Aqueous sodium hydroxide was added and the mixture was extracted with ethyl acetate. The crude amine 4 was obtained in nearly quantitative yield (268 mg, 1.0 mmol) and used without further purification.
^{1}H-NMR (C^2HCl_3): 2.62 (s, 3H), 3.1-3.7 (m, 6H), 6.44 (d, J = 3 Hz, 1H), 6.52 (dd, J_1 = 8 Hz, J_2 = 3Hz, 1H) 7.0-7.2 (m, 4H).
MS (EI): 266 (31%), 196 (16%), 57 (100%).

[^{36}Cl]-trans 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole ([^{36}Cl]-Org 5222) (5)

35 mg of the amine 4 (0.13 mmol) were dissolved in 500 µl [^{36}Cl]-hydrochloric acid (250 µCi, 0.48 mmol) and cooled to 0°C. 10 mg (0.14 mmol) sodium nitrite in 2 ml diglyme and 10 ml dichloromethane were added and the mixture was stirred for 30 minutes at 0°C. A few milligrams of copper powder were added and the reaction mixture was stirred for 1 hour at room temperature. Volatiles were evaporated in a stream of dry nitrogen, aqueous sodium hydroxide was added and the aqueous slurry was extracted with ethyl acetate. Purification of the residue on silica gel eluting with dichloromethane/methanol (95/5, v/v) afforded pure [^{36}Cl]-Org 5222

5 (8.2 μCi, 3.3% yield). ^{1}H-NMR (C^2HCl_3): 2.65 (s, 3H), 3.15-3.40 (m, 4H), 3.6-3.8 (m, 2H), 7.05-7.25 (m, 6H).

[3a-^{3}H]-11-chloro-2,3-dihydro-2-methyl-1H-dibenz[2,3:6,7]oxepino [4,5-c]pyrrol-1-one (7 and 8)

10 mg (33 μmol) of the cis amide 6 were dissolved in 500 μl hexamethylphosphoric triamide. 25 Ci (925 GBq) tritiated water and 10 μl 0.15 M sodium methanolate in methanol were added and the mixture was stirred for 1 hour at 90°C. The reaction mixture was cooled, poured into water and extracted with toluene. Solvents were evaporated in vacuo and the residue (360 mCi, 13 GBq) was purified/separated with HPLC on μBondapak C-18 with 60% acetonitrile/40% 0.1 M ammonium acetate (pH = 4,2) as the mobile phase. 142 mCi (5.3 GBq) of pure cis amide 7 and 61 mCi (2.3 GBq) of pure trans amide 8 were obtained.
^{3}H-NMR (C^2HCl_3; ^{1}H-decoupled): 4.13 (3, 3a-^{3}H).

[3a-^{3}H]-trans 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz [2,3:6,7]oxepino[4,5-c]pyrrole (10)

16 mCi (590 MBq) of tritiated trans amide 8, and 150 mg carrier trans amide were dissolved in 2.25 ml of dry tetrahydrofuran. A mixture of 150 mg of aluminium chloride in 2.25 ml of dry ether and 108 mg of lithium aluminium hydride in 2.25 ml of dry ether were added. The reaction mixture was stirred for 1 hour at room temperature and 2.5 ml of 2 N aqueous sodium hydroxide were cautiously added. The mixture was extracted with ethyl acetate. Purification of the residue on silica gel using dichloro-methane/methanol (95/5, v/v) as the eluent afforded 4.5 mCi (170 MBq) of pure [3a-^{3}H]-Org 5222 (10).The specific activity of this material was 20 mCi/mmol (740 MBq/mmol) as determined by HPLC in combination with liquid scintillation counting; ^{3}H-NMR (C^2HCl_3; ^{1}H-decoupled); 3.60 (s, 3a-^{3}H).

trans 5-bromo-11-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (14)

19 mg (67 μmol) were dissolved in 2.5 ml of 10% aqueous sulphuric acid and 8 μl (155 μmol) bromine was added at 0°C. The reaction mixture was stirred for 1 hour at 0°C, neutralized with aqueous sodium hydroxide and extracted with ethyl acetate. Purification on aluminium oxide (type B, act. III) with toluene/ethyl acetate (95/5, v/v) afforded 11 mg (30 μmol) of pure bromide 14.

^{1}H-NMR (C^2HCl_3): 2.57 (s, 3H), 3.1-3.3 (m, 4H), 3.55-3.65 (m, 2H), 7.0-7.2 (m, 4H), 7.19 br d, J = 3Hz, 1H), 7.30 dd, J_1 = 8 Hz, J_2 = 3 Hz, 1H).

GCMS (CI-neg.): 365 (20%), 363 (24%), 288 (59%), 286 (72%), 218 (100%).

trans 5-chloro-2,3,3a,12b-tetrahydro-11-iodo-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (15)

500 mg (1.8 mmol) Org 5222 were dissolved in 10 ml of dichloromethane and 400 mg (1.8 mmol) N-iodosuccinimide and 1.6 ml (18 mmol) triflic acid were added. The reaction mixture was stirred for 1 hour at room temperature, neutralized and extracted twice with dichloromethane. Purification of the crude product (720 mg) on silica gel with dichloromethane/acetone (8/2, v/v) as the eluent gave 227 mg (0.55 mmol) of pure iodide 15.

^{1}H-NMR (C^2HCl_3): 2.64 (s, 3H), 3.1-3.4 (m, 4H), 3.6-3.7 (m, 2H), 6.95 (d, J = 8 Hz, 1H), 7.05-7.25 (m, 3H), 7.38 (d, J = 3Hz, 1H),7.52 (dd, J_1 = 8Hz, J_2 = 3 Hz, 1H). GC-MS (EI) : 413 (1.2%), 411 (3.1%), 57 (100%).

[11-^{3}H]-trans 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole ([11-^{3}H]-Org 5222, 16)

10 mg (24 μmol) of 11-iodo-Org 5222 15 were dissolved in 2 ml ethanol and 10 mg 10% palladium on charcoal were added. The mixture

was stirred under 10 Curies (370 GBq) of tritium gas for 1 hour at room temperature. The catalyst was removed by filtration and washed with ethanol. The filtrate was concentrated in vacuo and labile tritium was removed by the addition of 5 ml ethanol followed by concentration in vacuo. The residue (300 mCi, 11 GBq) was purified by HPLC as described for 7 and 8 and subsequently purified by column chromatography as described for 9.
178 mCi (6.6 GBq) of pure [11-^{3}H]-Org 5222 were obtained.
^{3}H-NMR ($C6^2H6$, ^{1}H coupled): 6.80 (dt, J_1 = 8 Hz, J_2 = 2 Hz).
The specific activity was determined by HPLC in combination with liquid scintillation counting (20 Ci/mmol, 740 GBq/mmol) and by GC-MS (19 Ci/mmol, 710 GBq/mmol).

trans 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-7,11-dinitro-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (18)

6 g (21 mmol) of Org 5222 were dissolved in 5.8 ml of sulpholane and 4 ml of a mixture of nitric acid and sulphuric acid (1/2, molar ratio) was cautiously added. The reaction mixture was stirred for 1 hour at room temperature and another 2 ml of the nitration mixture was added. After stirring for an additional hour the mixture was neutralized with aqueous ammonia and extracted with ethyl acetate. Purification on aluminium oxide (type B, act. III) afforded 0.45 g (1.4 mmol) of mono-nitro compound 17 and 3 g (8 mmol) of dinitro compound 18.
11-nitro-Org 5222 (17): ^{1}H-NMR (C^2HCl_3): 2.58 (s, 3H), 3,0-3.8 (m, 6H), 7.1-7.2 (m, 2H), 7.29 (d, J = 9 Hz, 1H), 7.99 (br d, J = 3 Hz, 1H), 8.09 (br d, J_1 = 9 Hz, J_2 = 3 Hz, 1H)
MS (EI): 330 (8%), 260 (5%), 214 (4%), 57 (100%)
7,11-dinitro-Org 5222 (18): ^{1}H-NMR (C^2HCl_3): 2.60 (s, 3H), 3.1-4.0 (m, 6H), 7.35-7.45 (m, 2H) 7.71 (d, J = 3Hz, 1H), 7.98 (br d, J = 3 Hz, 1H).
MS (EI): 375 (12%), 345 (7%), 305 (7%), 57 (100%).

trans 5,9-diamino-11-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (19)

1.0 g (2.6 mmol) of dinitro-compound 18 was dissolved in 30 ml ethanol and 5.25 ml acetic acid. 1.2 g of iron powder were added and the reaction mixture was refluxed for 3 hours.
The mixture was cooled, poured into water, the pH was adjusted to 9 with aqueous ammonia and the mixture was filtered over hyflo. Washing and extraction with chloroform gave the crude diamine, which was purified on silica gel with chloroform/methanol (7/3, v/v) as the eluent. 400 mg (1.3 mmol) of pure diamine 19 were obtained. ^{1}H-NMR (C^2HCl_3): 2.55 (s, 3H), 3.0-3.2 (m, 4H), 3.5-3.7 (m, 2H), 6.35-6.45 (m, 2H), 6.48 (br d, J = 8 Hz, 1H), 6.62 (d, J = 3Hz, 1H), 6.95 (d, J = 8 Hz, 1H).
MS (EI): 317 (2%), 316 (1%), 315 (6%), 147 (7%), 57 (100%).

trans-5-chloro-2,3,3a,12b-tetrahydro-7,11-diiodo-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5:c]pyrrole (20)

150 mg (0.42 mmol) of diamine 19 were dissolved in 3 ml of demineralized water and 0.5 ml of concentrated hydrochloric acid and the mixture was cooled to -5°C. 76 mg (1.1 mmol) of sodium nitrite in 0.5 ml water was added. The mixture was stirred for 15 minutes at 0°C and 170 mg (1.0 mmol) of potassium iodide in 0.5 ml water was added. After stirring the reaction mixture for 2 hours at room temperature aqueous ammonia was added and the mixture was extracted with ethyl acetate.
Purification of the crude product on aluminium oxide (type B act. III, with hexane/ethyl acetate (95/5, v/v) as eluent affored 67 mg (125 μmol) of pure iodide 20.
^{1}H-NMR: 2.55 (s, 3H), 3.0-3.3 (m, 4H), 3.4-3.8 (m, 2H), 7.09 (brd, J = 2 Hz, 1H) 7.24 (d, J = 8 Hz, 1H), 7.35 (br d, J = 2 Hz, 1H), 7.52 (br d, J_1 = 8 Hz, J_2 = 2 Hz, 1H) 7.65 (d, J = 2 Hz, 1H).
MS (EI): 539 (0.2%), 538 (0.1%), 537 (0.5%), 57 (100%).

[7,11-^{3}H]-trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole ([7,11-^{3}H]-Org 5222) (21)

10 mg (19 µmol) of diiodide 20 were reductively tritiated and purified in a similar way as described for [11-^{3}H]-Org 5222 16.

^{3}H-NMR (C^2HCl_3; ^{1}H coupled): 7.05 (ddd, J_1 = 2Hz, J_2 = 7 Hz, J_3 = 8 Hz), 7.09 (d, J = 7 Hz).

trans-5-chloro-2,3,3a,12b-tetrahydro-11-iodo-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (25)

252 mg (0.93 mmol) of desmethyl Org 5222 were dissolved in 15 ml of dichloromethane and 209 mg (0.93 mmol) N-iodosuccinimide and 0.8 ml (0.9 mmol) triflic acid were added. The reaction mixture was stirred for 32 hours at room temperature, neutralized and extracted with dichloromethane. The crude iodide 25 was purified on silica gel with dichloromethane/methanol (95/5, v/v) as the eluent to give 152 mg (0.38 mmol) of pure iodide 25 (41%).

^{1}H-NMR (C^2HCl_3): 3.4-3.5 (m, 2H), 3.6-3.7 (m, 2H), 3.8-3.9 (m, 2H), 6.99 (d, J = 8Hz, 1H), 7.08 (d, J = 2 Hz, 1H) 7.17 (d, J = 8 Hz, 1H), 7.24 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 1H), 7.39 (d, J = 2 Hz, 1H), 7.58 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 1H).

MS (FAB+): 397.

[11-^{3}H]-trans-5-chloro-2,3,3a,12b-tetrahydro-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole([11-^{3}H]-Org 30526, 26)

10 mg (25 µmol) of 11-iodo-Org 30526 25 were dissolved in 2 ml ethanol and 10 mg 10% palladium on charcoal were added. The reaction mixture was stirred for 6 hours under 10 Ci (370 GBq) of tritium gas at room temperature. The catalyst was removed by filtration and washed with ethanol. The filtrate was concentrated in vacuo and labile tritium was removed by the addition of 5 ml of ethanol followed by concentration in vacuo. The residue (50 mCi, 1.85 GBq) was purified by HPLC and column chromatography to give 10.1 mCi (374 MBq) of pure ^{3}H-Org 30526.

^{3}H-NMR ($C^{2}HCl_3$; ^{1}H-decoupled); 7.10 (s).
The specific activity was determined by GC-MS analysis of the trifluoroacetyl derivative (24 Ci/mmol, 890 GBq/mmol) and by HPLC in combination with liquid scintillation counting (26 Ci/mmol, 940 GBq/mmol).

[1-^{14}C]-methyl N-[2-(5-chloro-2-phenoxyphenyl)-1-oxoethyl]-N-methylglycinate (31)

To 100 mCi (3.7 GBq) of sarcosine 27 were added carefully at 0°C 3.5 ml of thionylchloride and 5 ml of methanol. The reaction mixture was stirred for 2 hours at 60-70°C. Toluene was added and the mixture was evaporated to dryness (three times), to afford the crude ester 28.
775 mg (2.9 mmol) of the acid 29 was refluxed with 2 ml of thionylchloride in 4 ml of toluene. Volatiles were evaporated in vacuo, the residue was dissolved in toluene and evaporated to dryness (three times).
The residue was dissolved in 6 ml of dry N,N-dimethylformamide and the crude ester 28 in 6 ml of N,N-dimethylformamide were added at 0°C. The reaction mixture was stirred overnight at room temperature, water was added and the mixture was extracted with ethyl acetate. The crude ester 31 (65 mCi, 2.4 GBq) was purified over aluminium oxide (type B act. III) with toluene/ ethyl acetate (9/1, v/v) and over silicagel with toluene/ethyl acetate (8/2, v/v) as the eluents. 45 mCi (1.7 GBq) of pure ester 31 were obtained (45%).

[4-^{14}C]-3-[5-chloro-2-phenoxyphenyl]-1-methyl-2,4-pyrrolidine-dione (32)

45 mCi (1.7 GBq) of the amido-ester 26 were added to 600 mg of potassium tert. butoxide in 9 ml toluene and stirred for 5 minutes at room temperature. The mixture was acidified to pH 2 with 20%

aqueous hydrochloric acid and extracted with ethyl acetate. The crude product was purified on silicagel eluting with ethyl acetate. 32 mCi (1.2 GBq) of pure pyrrolidinedione 32 was obtained (71%).

[12b-^{14}C]-11-chloro-2,3-dihydro-2-methyl-1H-dibenz[2,3:6,7] oxepino[4,5:c]-pyrrol-1-one (33)

32 mCi (1.2 GBq) of dione 32 were stirred with 1.1 g of polyphosphoric acid for 3.5 hours at 110°C. The mixture was cooled, diluted with water and extracted with dichloromethane. Purification on silicagel, with toluene/ethyl acetate (1/1, v/v) as the eluent, afforded 20 mCi (750 MBq) of pure unsaturated amide 33 (63%).

[12b-^{14}C]-trans-11-chloro-2,3-dihydro-2-methyl-1H-dibenz [2,3:6,7]oxepino[4,5-c]pyrrol-1-one (35)

20 mCi (750 MBq) of the enamide 33 were dissolved in 6 ml methanol and 100 mg magnesium turnings were added under a stream of argon. The mixture was carefully heated until the gas evolution started and stirred additionally for 1 hour at room temperature. Column chromatography on silica gel with toluene/ ethyl acetate (6/4, v/v) gave the cis amide 34 (9.8 mCi, 360 MBq) and the trans amide 35 (2.9 mCi, 110 MBq).

[12b-^{14}C]-trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (37)

120 mg of aluminium chloride in 3 ml of toluene were added to a suspension of 60 mg lithium aluminium hydride in 2 ml of dry diethylether at -78°C under nitrogen. The mixture was warmed up to 0°C and 5.4 mCi (200 MBq) of the amide 35 in 1 ml dry tetrahydrofuran were added. The reaction mixture was stirred for 30 minutes at room temperature. 400 µl of 1N aqueous sodium hydroxide were added. The mixture was stirred for 30 minutes, dried on sodium sulphate, filtered and concentrated. The residue

(4.7 mCi, 175 MBq; cis 36/trans 37 = 1/4) was chromatographed on silica gel with dichloromethane/methanol (95/5, v/v) as the eluent. 1.6 mCi (58 MBq) of pure [12b-^{14}C]-Org 5222 37 and 2.3 mCi (85 mBq) of a mixture of cis amine 36 and trans amine 37 were obtained. The pure trans amine 37 (1.6 mCi, 58 MBq) was dissolved in 50 µl of ethanol and 3.1 mg maleic acid in 25 µl of ethanol were added. Volatiles were evaporated to give [12b-^{14}C]-Org 5222 as a solid with a specific activity of 56 mCi/mmol (2.1 GBq/mmol). 450 µCi (16 MBq) of this material and 1.30 mg of Org 5222 were crystallized from ethanol. 380 µCi (14 MBq) of crystalline [12b-^{14}C]-Org 5222 were obtained with a specific activity of 140 µCi/mmol (5.2 MBq/mmol).

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