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THE SYNTHESIS OF ORG 3770 LABELLED WITH ^{3}H, ^{13}C AND ^{14}C

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SUMMARY

The syntheses of 1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]pyrido [2,3-c][2]benzazepine (Org 3770) labelled with ^{3}H (and ^{2}H), ^{13}C and ^{14}C are described. Tritiated Org 3770 was prepared either by exchange under alkaline conditions with tritiated water or catalytic reductive dehalogenation of a chloro analogue with $^{3}H_2$. ^{13}C-labelled material was obtained in a seven-step synthesis starting from ^{13}C-labelled benzene whereas ^{14}C-Org 3770 was prepared in a three-step synthesis starting with $^{14}CO_2$. All labelled compounds were analyzed by TLC, HPLC, MS and NMR.

Key words: Antidepressant, catalytic dechlorination, NMR, mass spectrometry.

INTRODUCTION

Org 3770 (1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c] [2]benzazepine; (Figure 1) is a potential antidepressant drug under clinical development[1]. For metabolic studies in animal and man and for the determination of the bioavailability, the compound labelled with ^{3}H, ^{14}C and ^{13}C was needed. The synthesis and analyses of these compounds together with those of deuterated material are described in this article.

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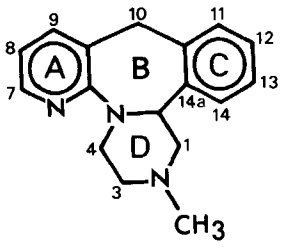

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Figure 1

RESULTS

^{3}H-Org 3770

Previous experiments with a related compound mianserin[2] indicated that the benzylic hydrogens at position 10 can be exchanged with deuterium under strong alkaline conditions and such an exchange seemed to be the most direct approach to tritiated Org 3770. Reaction of 1 with tritiated water in hexamethylphosphoric triamide in the presence of NaOH gave [10-^{3}H]-Org 3770 with a specific activity of 1 Ci/mmol (37 GBq/mmol, 25% of the theoretically possible incorporation). As illustrated by the ratio of the tritium signals in Figure 2 exchange at the equatorial position is favoured by a factor 5 over exchange at the axial position similar to that in mianserin. The rate of exchange had increased by a factor 10 in comparison with mianserin in agreement with the presence of an electronegative nitrogen in ring A. Although the labelled compound was stable in plasma, loss of label from the parent drug was observed in vivo; thus for metabolic studies this compound could not be used.

As an alternative [12-^{3}H]-Org 3770 1b was prepared by reductive dechlorination of 12-chloro-Org 3770 2 with $^{3}H_{2}$ over Pd/C (Figure 3). By addition of 1% KOH the reductive dechlorination proceeded smoothly (complete reaction within 2 hours) while reasonably high specific activities were obtained for two batches prepared by this method: 11,9 Ci/mmol (440 GBq/mmol) and 18,1 Ci/mmol (670 GBq/mmol) (as measured by FD mass spectrometry).

The tritium NMR spectrum was in agreement with the position of the ^{3}H at C_{12}: a singlet at 6,88 ppm (in $C^{2}H\,Cl_{3}$) in the ^{1}H decoupled spectrum and a double triplet (J=1,9 Hz and J=7 Hz) in the ^{1}H coupled spectrum.

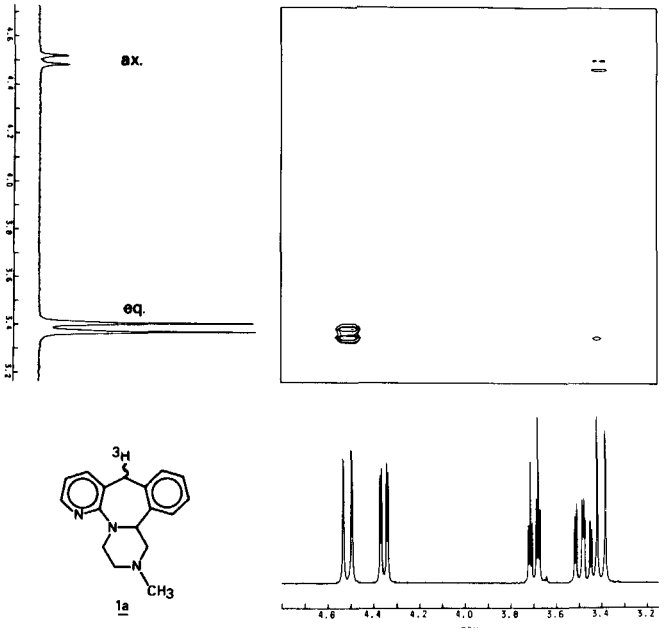

Figure 2: ^{1}H-^{3}H correlation spectrum for [10-^{3}H]-Org 3770; abscissa ^{1}H-spectrum, ordinate ^{3}H-spectrum. Solvent $C^{2}HCl_3$.

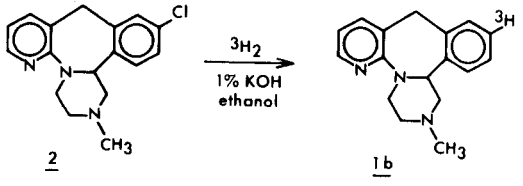

Figure 3:

With deuterated reagents using the same reaction conditions we obtained [10,10-$^{2}H_2$]- Org 3770 with and [12-^{2}H]-Org 3770 both with isotopic abundances of >98%.

^{13}C-Org 3770

Since ^{13}C-Org 3770 was intended for assessment of the bioavailability by a GC/MS method, it should contain at least four ^{13}C-atoms to avoid overlap between the molecular clusters of the unlabelled Org 3770 and that of ^{13}C-Org 3770. Because uniformly labelled $^{13}C_6$-benzene is relatively cheap, this material was selected as starting material. It was converted into $^{13}C_6$-Org 3770 1c in a yield of 7% (mean for two batches) as shown in Figure 4.

$^{13}C_6$-Benzene was converted in a 50% yield into α-bromo-acetophenone 3 by reaction with acetic anhydride and $AlCl_3$ followed by bromination with Br_2. Amine 4, the other synthon for $^{13}C_6$-Org 3770 was prepared by an elaborate reaction sequence starting with 2-chloronicotinonitrile (5) since more direct approaches (e.g. reaction of ethyl-2-amino-pyridine-3-carboxylate with acetic acid derivatives or ethyl-2-chloro-pyridine-3-carboxylate with glycine derivatives) failed. 2-Chloro-3-cyano-pyridine 5 was coupled to ethylglycinate 6 to give 7 in 80% yield. By reaction with methylamine (at low temperature to avoid attack at the cyano-group) the amide 8 was formed in 86% yield and reduction of the cyano-group using Raney Nickel and sodium hypophosphite[3] gave in quantitative yield the

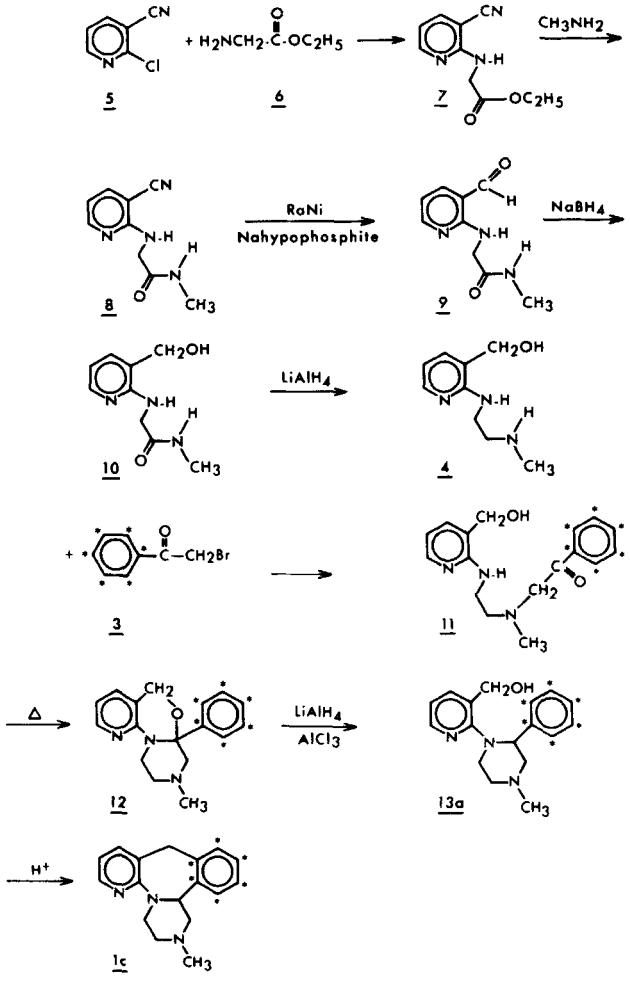

Figure 4: Synthesis of [$^{13}C_6$]-Org 3770.

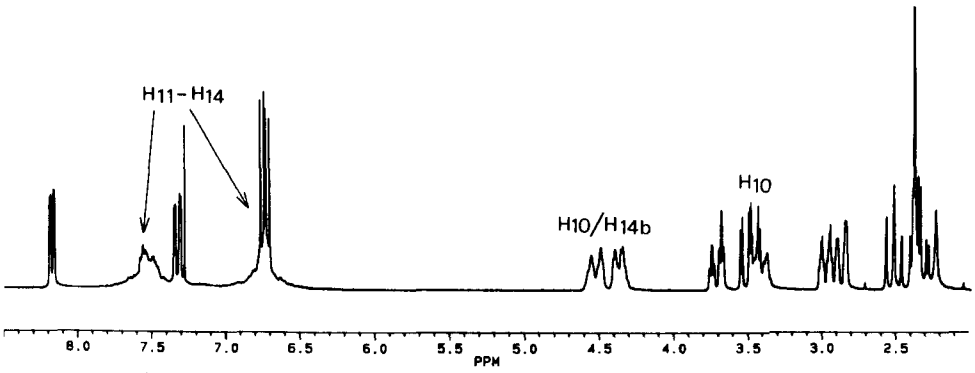

Figure 5: ^{1}H NMR spectrum of $^{13}C_6$-Org 3770; solvent C^2HCl_3

aldehyde 9. The reduction of 9 to 4 (yield 60%) was done in two steps because of solubility problems. First the aldehyde function was reduced with $NaBH_4$, then the amide with $LiAlH_4$.

Ring closure of 11 by heating yielded 12 in 30%; reduction of 12 with $LiAlH_4/AlCl_3$ at room temperature (at higher temperatures over-reduction to the corresponding 3-methylpyridine occurred) gave 13a in 90% yield. Reaction with sulphuric acid and purification of the resulting product by chromatography and crystallization gave pure ^{13}C-Org 3770 (1c) in 50% yield. The isotopic abundance as measured by MS (m/z = 271; 99,2% enrichment for one batch and 96,7% enrichment for the other batch) was in agreement with the starting material used. The ^{13}C-NMR spectrum was complicated by second order effects and could not be interpreted but the large-range ^{1}H-^{13}C-couplings in the ^{1}H-NMR spectrum (with H_{10ax}, H_{10eq} and H_{14b}, Figure 5) confirmed the labelling of the C-ring with ^{13}C.

^{14}C-Org 3770

Since Org 3770 labelled with ^{14}C was intended for metabolic studies in man we selected position 10 for labelling. This position had proved to be stable in metabolic studies in animals while one ^{14}C atom would give sufficiently high specific activity for this type of study.

The synthesis of ^{14}C-Org 3770 1d is shown in Figure 6.

The nitropyridine 14 (prepared by coupling of 2-chloro-3-nitropyridine with 1-methyl-3-phenylpiperazine) was reduced with H_2 on Pd/C to the amine 15 (yield 90%). The bromoderivative 16 was prepared through the intermediate diazonium

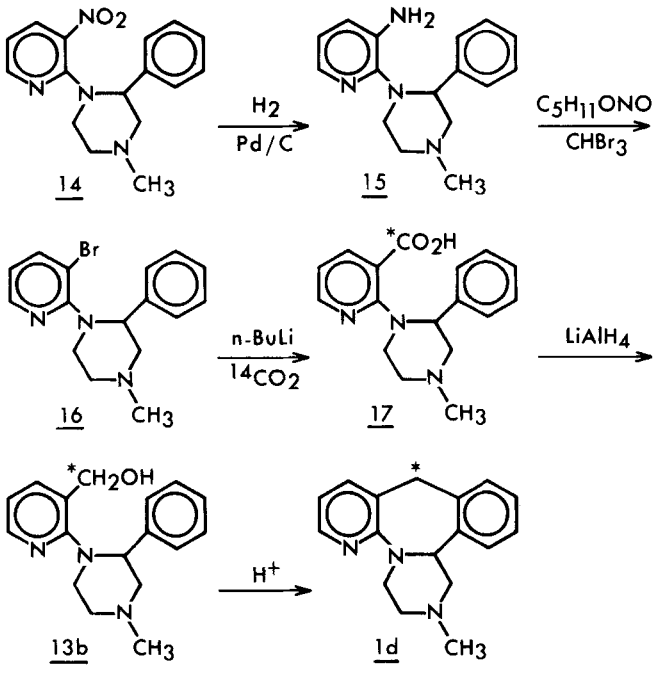

Figure 6: Synthesis of ^{14}C-Org 3770

salt; of all the methods used (HBr/Br_2 with $NaNO_2$, CuBr/HBr with $NaNO_2$ or $CuBr_2$ and $NaNO_2$) the best yield (20%) was obtained with bromoform and pentyl nitrite[4]. Radioactivity was introduced by reaction with n-BuLi and $^{14}CO_2$ (100 mCi; 3,7 GBq). Next to acid 17 (yield 51%; 22,3 mCi) we isolated butylcarboxylic acid (Figure 7, 18; 13 mCi), the ketone 19 (6 mCi, formed by reaction of the lithium salt of 16 with 18 or by reaction of 17 with butyl lithium) and the oxidation product 20 (30% yield).

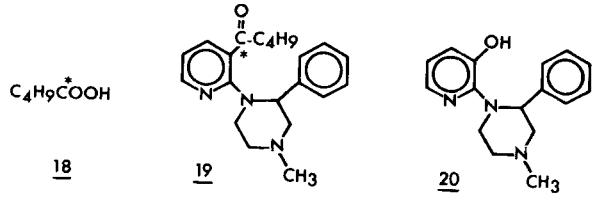

Figure 7: Side-products formed in the synthesis of ^{14}C-Org 3770.

Using unlabelled material we also tested the possibilities of preparing 17 through reaction of the diazonium salt of 15 with cyanide. However, this was not very successful since generation of the diazonium salt using $NaNO_2$/HCl or $NaNO_2$/HBF_4 followed by reaction with CuCN gave only 2% cyanide 21 while the main products were either the chloride 22 or the fluoride 23 next to the ring closure products 24 (resulting from electrophilic ring closure) and 25 (formed via attack of cyanide at the nitrogen of the diazonium group followed by a t-amino effect type ring closure[6]) (Figure 8).

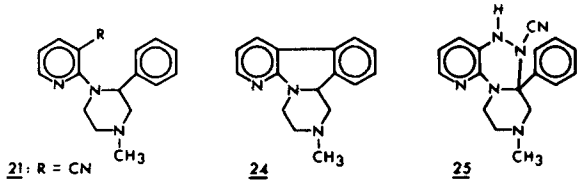

Figure 8:

The acid 17 was converted into the alcohol 13b by reaction with $LiAlH_4$. Due to solubility problems no complete conversion was obtained and next to 18% 13b 25% starting material was recovered. Ring closure with sulphuric acid gave pure [10-^{14}C]-Org 3770 in a 62% yield. The compound had a radiochemical and chemical purity of $\geq$ 99% as determined by TLC and NMR. The position of the ^{14}C at C_{10} was confirmed by ^{13}C NMR spectroscopy since the signal of C_{10} in the labelled compound was absent (see Figure 9) while in the ^{1}H NMR spectrum a slight upfield shift of the protons at C_{10} was observed[7]. The specific activity was determined by EI-mass spectrometry as 58,4 mCi/mmol (2,1 GBq/mmol) in agreement with the specific activity of the $^{14}CO_2$ used in the synthesis.

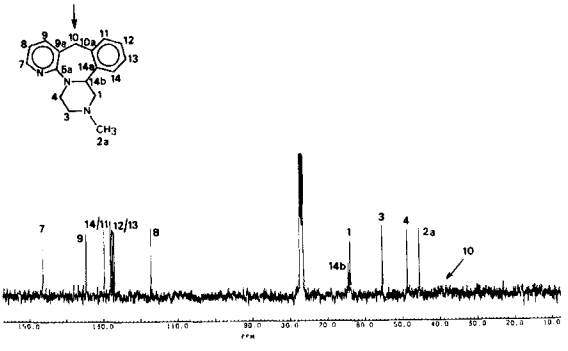

Figure 9: ^{13}C NMR spectrum of [10-^{14}C]-Org 3770; solvent C^2HCl_3.

Experimental

The NMR spectra were recorded on a Bruker AC200 or AM360 spectrometer. The IR spectra were obtained at a Digilab FTS15/90 and the UV spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS spectrometer. Mass spectra were measured on an HP 5995A, Finnigan MAT 90 or a Finnigan MAT TSQ 70 mass spectrometer and for the tritiated compounds on a VG ZAB-ZF instrument by Dr. W. Lehmann, University Hospital, Hamburg, F.R.G. The radioactivity on TLC plates was measured by autoradiography or on a Berthold LB 282/283 linear analyzer. HPLC purifications were done on a Waters 6000A HPLC equipped with a Pye Unicam UV detector (PU4025) at 254 nm and a Berthold radioactivity monitor type LB 503. Melting points were measured on a Büchi 535 apparatus (2 ^{0}/min). The reactions with $^{3}H_{2}$ were carried out at Amersham International plc., Cardiff, U.K. and the reaction with $^{14}CO_{2}$ was performed at ICI, Billingham, U.K.

[10-^{3}H]-Org 3770

Org 3770 (10 mg) was dissolved in a mixture of 2 ml hexamethylphosphoric triamide and 0,5 ml of a solution of sodium methoxide in methanol (2,5 M) and tritiated water, obtained by oxidation of 25 Ci (92,5 GBq) tritium gas (isotopic abundance 80%) were added. The reaction mixture was stirred at 80 ^{0}C for 2 hours and poured into water. The resulting mixture was extracted with dichloromethane (2x5 ml). The organic layer was dried over sodium sulphate and after evaporation of the solvent, the residue was purified by HPLC on a μBondapak C_{18} column with 0,05 M tetramethylammonium phosphate (pH=3)/acetonitrile (3:7, v/v) as mobile phase. The fractions containing [^{3}H]-Org 3770 were collected and the acetonitrile was removed in vacuo. The resulting solution was brought on a Seppak C_{18} column (prewashed with methanol and water) and the column was washed with water (10 ml). Pure [^{3}H]-Org 3770 was obtained by eluting the column with methanol (5 ml).

[12-^{3}H]-Org 3770

10 mg of 12-chloro-1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]-pyrido[2,3c][2]benzazepine (2) (supplied by the Organic Chemical R&D Labs, Organon Int.) were dissolved in 2 ml of ethanol containing 1% (m/v) KOH, 10 mg of Pd/C (10%) were added and the reaction mixture was stirred for 2 hours at room temperature under 10 Curies (370 GBq) of tritium gas. The catalyst was removed by filtration and washed with 5 ml of ethanol. The filtrate was concentrated in vacuo and labile tritium was removed by dissolving the residue in ethanol followed by lyophilization (twice). The residue was purified by HPLC as described for [10-^{3}H]-Org 3770. Yields: 148 mCi (5,5 GBq) and 267 mCi (9,8 GBq).

Analytical procedures

The tritiated products had radiochemical purities of $\geq$ 97% both by TLC (silica gel with butanol-1/acetic acid/water (4:1:1, v/v) dichloromethane/methanol/aq. ammonia (24%) (89:10:1, v/v), toluene/ethanol (8:2, v/v) or chloroform/methanol (9:1, v/v) and HPLC on μBondapak C_{18} with 0,05 M aq. tetramethylammonium phosphate (pH=3)/acetonitrile (8:2, v/v) as mobile phase.

[^{13}C]-Org 3770

ethyl 2[3-cyanopyridin-2-yl)amino]acetate 7

11 g of ethyl glycinate. HCl 6 were dissolved in 200 ml of DMSO and 4,5 g of Na_2CO_3 were added. After stirring for 10 min the suspension was filtered over Hyflo. To the filtrate 10 g of 2-chloro-3-cyano-pyridine 5 and 10 g of KF were added and this mixture was heated for 3 h at 120 °C and another 15 h at room temperature. The solution was poured into 400 ml of water and extracted twice with 200 ml of dichloromethane. The organic layers were washed with 100 ml of saturated NaCl-solution, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by chromatography over silica gel (elution with dichloromethane/acetone, 97:3, v/v). Yield: 12 g (75%) of 7 as colourless oil.
^1H NMR (C^2HCl_3): 4,25 ppm (glycine H's); 6,68; 7,71 and 8,28 (pyridine H's).
MS (EI): m/z 205 (11%, $M^{+\cdot}$); m/z = 132 (100%, M-.$COOC_2H_5$]$^+$).

2-[3-cyanopyridin-2-yl)amino]-N-methylacetamide 8

12 g of 7 were dissolved in 100 ml of toluene, cooled to 0 °C and 10 ml of methylamine were added. The resulting solution was left for 50 h at 4 °C and the crystals were isolated by filtration. The filtrate was treated again with methylamine and processed in the same way yielding 10 g (85%) of 8 as colourless crystals; m.p. 150,5-151,7 °C.
^1H NMR (C^2HCl_3): 2,87 ppm (N-CH_3); 4,18 ppm (glycine H's); 6,72, 7,71 and 8,80 ppm (pyridine-H's).
IR (DRIFT/KBr): 2210 cm^{-1} (CN); 1673 cm^{-1} (amide).

2-[(3-formylpyridin-2-yl)amino]-N-methylacetamide 9

6 g of the nitrile 8 were dissolved in 180 ml of a mixture of water/acetic acid/pyridine (1:1:2 v/v) and 12 g of sodium hypophosphite and 2,1 g of Raney Nickel were added. After stirring for 2 h at 20 °C another 2 g of Raney Nickel were added and the mixure was stirred for another 2 h. The suspension was poured into 150 ml of H_2O and the pH was adjusted to 10 with concentrated aq. NH_4OH (about 75 ml). The solution was saturated with NaCl and extracted three times with 200 ml of ethyl acetate. The organic layers were washed with saturated aqueous NaCl-solution, dried over Na_2SO_4 and evaporated to dryness, yielding 6 g of the aldehyde 10 (98%) as an oil.
^1H NMR: 2,82 ppm (N-CH_3); 4,25 ppm (glycine H's); 6,80, 7,82 and 8,35 ppm (pyridine H's); 9,89 ppm (aldehyde).
MS (FAB pos/glycerol): m/z = 286 (17% M + H + glycerol]$^+$); m/z = 194 (100% M+H]$^+$).

2-[(3-hydroxymethylpyridin-2-yl)amino]-N-methylacetamide 10

6 g of aldehyde 9 were dissolved in 200 l of methanol and about 1 g of $NaBH_4$ was carefully added in small portions. After stirring for 2 h at 20 °C the excess of $NaBH_4$ was destroyed with 1,5 ml of acetic acid and the crude product was

evaporated to dryness and purified by chromatography over silica gel (elution with dichloromethane/methanol 9:1 v/v). Yield 4,2 g (70%) of 10 as colourless crystals; m.p. 150,5–152,0 °C.
^{1}H NMR (C^2HCl_3): 2,78 ppm (N-CH_3); 4,11 ppm (glycine); 4,65 ppm (CH_2OH); 6,61, 7,29 and 8,08 ppm (pyridine H's).
MS (EI): m/z = 195 (16%, $M^{+\cdot}$), m/z = 137 (65%, M-$C_2H_4NO^{1+}$); m/z = 119 (39%, M-$C_2H_6NO_2^{1+}$), m/z = 92 (100%, $C_6H_6N^+$).

2-[2-(methylamino)ethylamino]-3-pyridinemethanol 4

4,2 g of 10 were dissolved in 600 ml of dry dioxane and 2 g of $LiAlH_4$ were carefully added under nitrogen. After refluxing for 1 h another 2 g of $LiAlH_4$ were added and the reaction mixture was refluxed for another 2 h. The reaction mixture was cooled in an ice bath and 8 ml of H_2O were carefully added; the suspension was filtered over hyflo, the filtrate dried over Na_2SO_4 and evaporated to dryness by chromatography over alumina B (elution with dichloromethane/-methanol, 9:1, v/v). 2,4 g (62%) of 4 were obtained as an oil.
IR (CCl_4): 3610 and 3390 cm^{-1} (OH and NH); 1600 cm^{-1} (pyridine); 1515 cm^{-1} (NH).
^{1}H NMR (C^2HCl_3/C^2H_3OH): 2,43 ppm (N-CH_3); 2,84 and 3,57 ppm (C_2H_4); 4,52 ppm (CH_2OH); 6,55, 7,31 and 7,97 ppm (pyridine H's).
^{13}C NMR (C^2HCl_3/C^2H_3OH): 35,6 ppm N-CH_3; 40,3, 50,8 and 62,4 ppm (CH_2's); 112,4, 120,2, 136,3, 136,5, 146,6 and 157,4 ppm (pyridine).

[^{13}C]-α-bromoacetophenone 3

2,5 g of [$^{13}C_6$]-benzene 17 (MSD isotopes, 99,4% atom and 99,2% ^{13}C) were dissolved in 15 ml of CS_2 and 8,8 g of powdered dry $AlCl_3$ were added and the suspension was gently refluxed, 2,7 g of freshly distilled acetic anhydride were slowy added (vigorous reaction) and the mixture was refluxed for 1 h. The CS_2 was distilled off and the residue was cooled to 30 °C. The remaining red liquid was carefully added to 50 ml of ice water to which a few drops of concentrated HCl were added. The mixture was saturated with NaCl and extracted three times with 20 ml of diethyl ether. The organic layers were washed with 10 ml of 10% NaOH and twice with 10 ml of a saturated NaCl-solution. The combined layers were dried over Na_2SO_4 and the diethyl ether was distilled off yielding 2,4 g (63%) of ^{13}C-acetophenone (as colourless liquid).
^{1}H NMR (C^2HCl_3): 2,61 ppm (CH_3); 6,95–8,50 ppm (aromatic H's).
^{13}C NMR (C^2HCl_3): clusters around 127 ppm, 132 ppm and 136 ppm.
2,3 g acetophenone were dissolved in 20 ml of anhydrous diethyl ether, cooled to 0 °C and 3,75 g of bromine were added. After gentle warming to room temperature the mixture discoloured spontaneously from brown to yellow. The reaction mixture was poured into 100 ml of ice water and extracted twice with 25 ml of diethyl ether. After drying over Na_2SO_4 it was evaporated to dryness and the residue was purified by chromatography over a Lobar Fertigsäule B (elution with toluene/-hexane 60:40, v/v), yielding 3,1 g (80%) of 3 as colourless crystals; 48,0–49,8 °C.

^{1}H NMR: 4,46 ppm (CH_2); 6,90-8,60 ppm (aromatic H's).
^{13}C NMR: 30,2 ppm (CH_2); clusters around 129 and 134 ppm (aromatic C-atoms).
MS (EI): m/z = 204 and 206 (~5% $M^{+\cdot}$); m/z = 111 (100%, M-$CH_2Br^{]+}$).

[^{13}C]-2-[2-[3-(hydroxymethyl)-2-pyridinylamino]ethylamino]-1-phenylethanone 11
2,4 g of amine 4 were dissolved in 15 ml of dry dioxane and 1,4 g of triethylamine were added. 3,1 g of 3 in 10 ml dioxane were added slowly and the mixture was stirred for 1 h at 20 °C followed by 1 h at 0 °C. The precipitated triethylamine. HBr was filtered off and washed with dioxane and the filtrate was evaporated (T < 30 °C) to dryness. The residue was dissolved in H_2O and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated (T < 30 °C) to dryness. Yield: 4,5 g (97%) of 11 as a brown oil.

[^{13}C]-6a,7,8,9-tetrahydro-8-methyl-6a-phenyl-5H,10H-pyrazino[2,1-b]pyrido[2,3-d][1,3]-oxazine 12
4,5 g of 11 were heated on an oil-bath at 120 °C for 3 h. The reaction product was purified by chromatography over silica gel (elution with dichloromethane/-acetone, 80:20, v/v). Yield: 1,3 g (31%) of 11 as pink crystals; m.p. 141,5-145,0 °C.
^{1}H NMR (C^2HCl_3): 2,29 ppm (N-CH_3); 4,21 and 4,52 ppm (H_6's); 4,75 ppm (H_2); 6,70-7,05 ppm and 7,55-7,95 ppm (phenyl H's); 6,56, 6,96 and 8,12 ppm (pyridine H's).
^{13}C NMR (C^2HCl_3): clusters at 128 and 139 ppm.
MS (EI): m/z = 287 (12% $M^{+\cdot}$); m/z = 217 (95%, M-$C_4H_8N^{]+}$); m/z = 111 (98%, $^{13}C_6{}^{12}CH_5O^+$).

[^{13}C]-2-(4-methyl-2-phenyl-1-piperazinyl)-3-pyridinemeethanol 13a
A solution of 800 mg of powdered dry $AlCl_3$ in 15 ml of anhydrous diethyl ether was added at 20 °C to a suspension of 400 mg of $LiAlH_4$ in 15 ml of anhydrous diethyl ether. This mixture was cooled to -80 °C and 1,3 g of 12 in 20 ml of dry THF were added dropwise and the mixture was stirred for 1 h at 20 °C. The mixture was cooled to -10 °C and 6 ml of 1,5 N NaOH were slowly added. The suspension was filtered over hyflo and washed with diethyl ether. The filtrate was dried on sodium sulphate and evaporated to dryness yielding 1,2 g (77%) of 13a as colourless crystals; m.p. 116°,5-117°,8 C.
^{1}H NMR (C^2HCl_3): 2,37 ppm (N-CH_3); 4,62 and 4,86 ppm (CH_2OH); 4,60-4,80 ppm (piperazine H_2); 6,50-7,00 ppm and 7,35-7,80 ppm (phenyl H's) 6,87, 7,38 and 8,15 ppm (pyridine H's).
MS (EI): m/z = 289 (9% $M^{+\cdot}$); m/z = 219 (34%, M-$C_4H_8N^{]+}$); m/z = 201 (32%, M-$C_4H_{10}NO^{]+}$); m/z = 165 (100%, M-$C_6H_8N_2O^{]+\cdot}$).

[^{13}C]-Org 3770 1c
1,2 g of 13a were cooled to -40 °C and 2,5 ml of concentrated H_2SO_4 were added and the mixture was stirred for 2 h at 60 °C. After cooling down about 30 ml of water were added and after cooling to -10 °C the pH of the solution was adjusted

to 10 by adding aq. ammonia (24%). The product was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to dryness to yield 950 mg (85%) of crude 1c. The crude 1c was purified by chromatography over Alox B (eluted with hexane/ethyl acetate 7:3, v/v) to yield 830 mg. For the final purification the product was treated twice with 100 mg of charcoal in n-hexane (containing 1% of methanol) followed by crystallization from methanol/water (1:1, v/v) yielding 600 mg (53%) Org 3770 as colourless crystals, m.p. 123,8–125,8 °C. No impurities were detectable either on TLC, HPLC or GC.

^{13}C-NMR (C^2HCl_3): clusters from 126 ppm to 131 ppm (C_{11}–C_{14}) and 136 ppm–139 ppm (C_{10a} and C_{14a}).

IR-spectrum (DRIFT/KBr): 1585 cm^{-1}, 1565 cm^{-1}, 1460 cm^{-1} and 1440 cm^{-1}.

UV-spectrum (ethanol): λ_{max} at 293,4 nm, E (1%, 1 cm) = 215

[^{14}C]-Org 3770

4-methyl-1-(3-nitro-2-pyridinyl)-2-phenylpiperazine 14

8,7 g of 1-methyl-3-phenylpiperazine and 7,7 g of 2-chloro-3-nitro-pyridine were dissolved in 175 ml of dry DMF: 8,7 g of dry KF were added and the solution was stirred for 15 h at 140 °C. After cooling to 20 °C the reaction mixture was poured into 1 l of H_2O and extracted 4 times with 150 ml of dichloromethane. The organic layers were washed with 500 ml of H_2O and after drying over Na_2SO_4, evaporated to dryness. The residue was purified by chromatography over Alox B (elution with hexane/ethyl acetate 75:25, v/v) followed by chromatography over silica gel (elution with toluene–ethanol 95:5, v/v) to yield 11 g (70%) of 14.

2-(4-methyl-2-phenyl-1-piperazinyl)-3-pyridin-amine 15

11 g of 14 were hydrogenated in 250 ml of ethanol at 20 °C and 275 kPa using 2 g of 10% palladium on charcoal as catalyst. After 1 h the reaction product was filtered over hyflo and the filtrate evaporated to dryness to yield 9 g (90%) of 15 as colourless crystals.

1-(3-bromo-2-pyridinyl)-4-methyl-2-phenylpiperazine 16

9 g of 15 dissolved in 50 ml of bromoform, were slowly added at 80 °C to a solution of 7 ml pentyl nitrite in 50 ml of bromoform and the solution was stirred for 1 h at 100 °C. The reddish-brown solution was evaporated to dryness and purified by chromatography over Alox B (elution with hexane/ethyl acetate, 8:2, v/v) and silica gel (elution with toluene/ethanol, 9:1, v/v). Yield: 2 g (18%) of 16. For a final purification the product was crystallized from diethyl ether to yield 1 g of pure bromide 16 as slightly pink-coloured crystals; m.p. 106,0–107,5 °C.

IR (DRIFT/KBr): 1572 cm^{-1} (pyridine); 753 and 750 cm^{-1} (monosubstituted benzene).

NMR (C^2HCl_3): 6,70,; 7,75 and 8,05 ppm (pyridine-H's); 7,0–7,4 ppm (phenyl-H's); 4,72 ppm (H_2); 2,35 ppm ($N-CH_3$).

MS (EI): m/z = 331 and 333 (7%; $M^{+\cdot}$); m/z = 261 and 263 (20%; $M-C_4H_8N^{]+}$); m/z = 185 and 187 (18%; $C_6H_6N_2Br^{]+}$); m/z = 159 (100%; $C_{11}H_{13}N^{]+}$).

[carboxyl-^{14}C]-2-(4-methyl-2-phenyl-1-piperazinyl)-3-pyridine-carboxylic acid 17

0,8 Ml of n-butyllithium (15% solution in n-hexane; 1,5 equivalents) were added under stirring and in a nitrogen atmosphere to a solution of the bromo-compound 16 (250 mg) in 5 ml of diethyl ether (sodium dried) at -60 °C. The mixture was stirred at -60 °C for another 2 h and subsequently carbonated at -60 °C with $^{14}CO_2$ (spec. act. 58 mCi·mmol^{-1}; 2,1 GBq·mmol^{-1}). After 30 min stirring at room temperature, 5 ml of distilled water was added. The reaction mixture was extracted with ethyl acetate (2x5 ml) and the extract (44 mCi; 1,6 GBq) was also evaporated to dryness.
From the ethyl acetate extract 2,4 mCi (89 MBq) of pure acid 17 was isolated after chromatography over silica gel (elution with dichloromethane/methanol, 95:5, v/v). The aqueous residue was also purified by chromatography over silica gel (elution with dichloromethane/methanol, 9:1, v/v) yielding 19,9 mCi (736 MBq) of acid 17.

[^{14}C]-2-(4-methyl-2-phenyl-1-piperazinyl)-3-pyridinemethanol 13b

To a suspension of 150 mg of $LiAlH_4$ in 3 ml of dry THF, 22,3 mCi (825 MBq) of acid 17 in 2 ml of THF were added and the mixture was stirred for 3 h at 60 °C. After cooling to 20 °C about 1,5 ml of H_2O was added and the suspension was filtered over hyflo and washed with diethyl ether. The filtrate was dried over Na_2SO_4 and evaporated to dryness and the residue was purified over silica gel (elution with dichloromethane/methanol, 9:1, v/v).
Yield 4 mCi (148 MBq; 18%) of 13b.
^{1}H NMR (C^2HCl_3): 6,90, 7,38 and 8,14 ppm (pyridine-H's); 7,0-7,3 ppm (phenyl-H's); 4,60 and 4,83 ppm (CH_2OH); 4,71 ppm (H_2); 2,40 ppm ($N-CH_3$).

[10-^{14}C]-Org 3770 1d

4 mCi (148 MBq) of alcohol 13b were cooled to -40 °C and 0,15 ml of concentrated sulphuric acid were added under nitrogen atmosphere and the solution was stirred for 2 h at 60 °C. After cooling to -30 °C 1 ml of H_2O was added and the pH of the solution was adjusted to 10 with concentrated aq. NH_4OH. The product was extracted with ethyl acetate, dried over Na_2SO_4, and evaporated to dryness. The crude 1d was purified by chromotography over silica gel (elution with dichloromethane/-methanol 95:5, v/v) to yield 2,45 mCi (90,7 MBq; 61%) of pure [10-^{14}C]-Org 3770. The radiochemical purity was $\geq$ 99% as determined by TLC on silica gel with dichloromethane/methanol (9:1, v/v) and toluene/ethanol (8:2, v/v).
^{1}H NMR (C^2HCl_3): 2,40 ppm ($N-CH_3$); 2,38 ppm (H_{3ax}); 2,50 ppm (H_{1ex}); 2,85 ppm (H_{1eq}); 2,97 ppm (H_{3eq}); 3,41 ppm (H_{10eq}); 3,51 ppm (H_{4ax}); 3,71 ppm (H_{4eq}); 4,40 ppm (H_{14b}); 4,52 ppm (H_{10ax}); 6,73 ppm (H_8); 7,10-7,20 ppm ($H_{11}-H_{14}$); 7,31 ppm (H_9); 8,14 ppm (H_7).

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