Tritium NMR in the Analysis of Tritiated Compounds

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Abstract:

An overview is given of the possibilities of ^{3}H NMR in the characterisation of ^{3}H-labelled compounds. This technique gives information on the identity of the tritiated compounds, the position of the tritium, the distribution of the label and even the radiochemical purity of the labelled products.

Keywords: reductive dehalogenation; exchange; isotope effects

Introduction

Since the discovery of ^{3}H NMR by Tiers et al[1] the technique has developed by the pioneer-work of Elvidge and his group[2] into a powerful and essential technique in the analysis of tritiated material. At Organon it was included in 1980 as part of our analytical procedures[3] and in this article we will illustrate the possibilities of ^{3}H NMR with examples from Organon research.

Results and discussion

Quality of a ^{3}H-NMR spectrum

The amount of material that can be analysed with ^{3}H NMR is limited mainly because of radiolysis problems. Especially with deuterochloroform as solvent, we have observed isomerisation, acid catalysed exchange reactions[4] and, less seriously, protonation of amines in the NMR samples. With solvents like deuterated benzene or DMSO, this radiolysis is less serious but generally we limit the amount of material for one sample to 10 mCi (370 MBq). By addition of carrier and measurement of the ^{1}H NMR spectrum after recording of the tritium NMR any possible decomposition can be detected. With simple high-quality spectra, detection limits of 0,3 - 0,5% are possible (in such spectra the ^{13}C-satelites are visible) but in more complicated spectra the limit of detection is 5-10%.

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Applications of ^3H NMR

Usually ^3H NMR is used to determine the distribution of the tritium over the molecule and the ratio of different labelled species. In specific cases it is also possible to use ^3H NMR for the assessment of purity and identification of unknown compounds.

Scrambling of tritium during tritiation reactions is a common phenomenon so information about the labelled positions is of utmost importance. In reactions with $^3H_2$ in the presence of a catalyst these type of exchange are the result of vinylic, benzylic or allylic exchange[5]. An example is given for 2-^3H-estrone (1; Figure 1) where the reaction of 2-bromo-estrone (2) with $^3H_2$ yields as expected the label of position 2, but also at the benzylic positions 6 and 9, and even at positions 11 and 12. The latter labelling points to intermediate formation of a 9, 11-double bond on treatment with Pd/C.

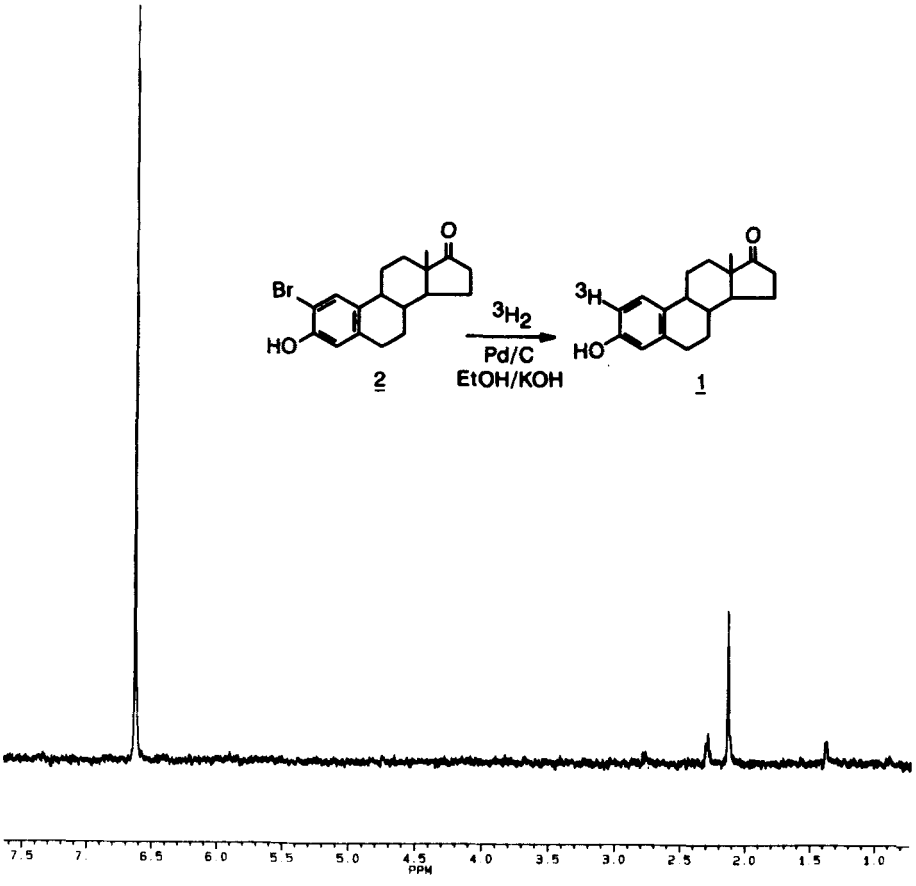

Figure 1: ^1H-decoupled ^3H-NMR spectrum (in $C^2H_3O^2H$)) of tritiated estrone (1); solvent $C^2HCl_3/C^2H_3{}^2OH$.

As shown in Figure 2 for Org 30659 (3) exchange reactions can lead to very complex labelling patterns. In this compound, prepared by exchange with $^3H_3O^+$, all enolisable positions (2α, 2β, 4, 6α, 6β and 10) have been labelled approximately to an equal extent. The label distribution is calculated from the ^3H signal integrals. However since these signals may be enhanced unequally by the different NOE effects due to the ^1H broad band decoupling the label distribution accuracy may be reduced[6].

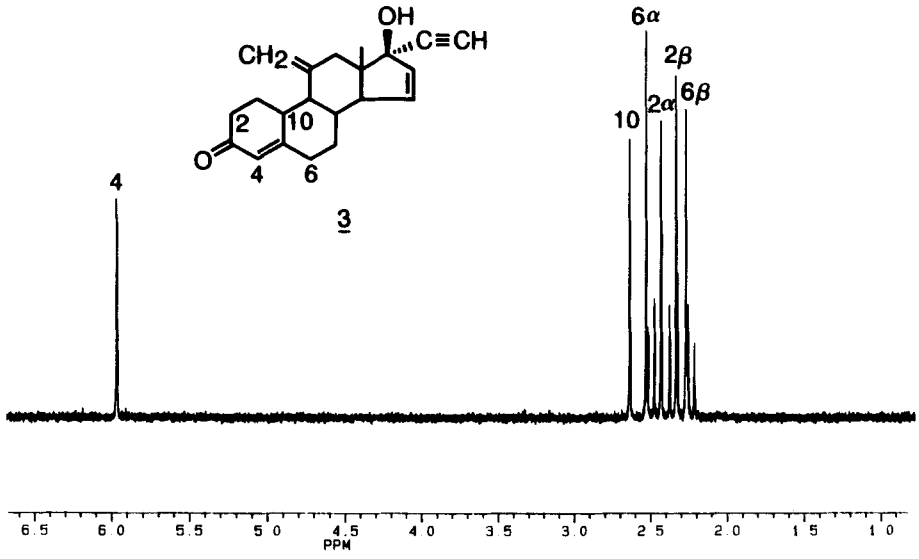

Figure 2: ^1H-decoupled ^3H-NMR spectrum of ^3H-Org 30659 (3); solvent C^2HCl_3

Information about the different labelled species present (indirect information about specific activity) can be derived in simple systems by looking at tritium-tritium couplings. In more complex systems "two-dimensional" techniques can be applied such as J-resolved ^3H-NMR spectroscopy as shown in Figure 3 for the neuropeptide Org 2766 (4).

The peptide was synthesized by reduction with $^3H_2$ of the γ-δ triple bond in the lysine side chain. The ^3H NMR spectrum obtained (Figure 3) was too complex for the desired tetra-tritiated material. ^1H-decoupled J-resolved ^3H spectroscopy as described by Williams et al[7] was applied for this material and the results are shown in Figure 4.

Since both the γ and δ tritons are diasteriotopic, and hence chemically inequivalent, four monotritiated, six ditritiated, four tritritiated and one tetratritiated species may be present with four, twelve, twelve and four ^3H signals respectively. Of these, all monotritiated-, all ditritiated, two of the four tritritiated and none of the tetratritiated species are tentatively identified. The absence of signals for two tritritiated and the tetratritiated species might be due to overlap with the other signals, even in the projection spectrum.

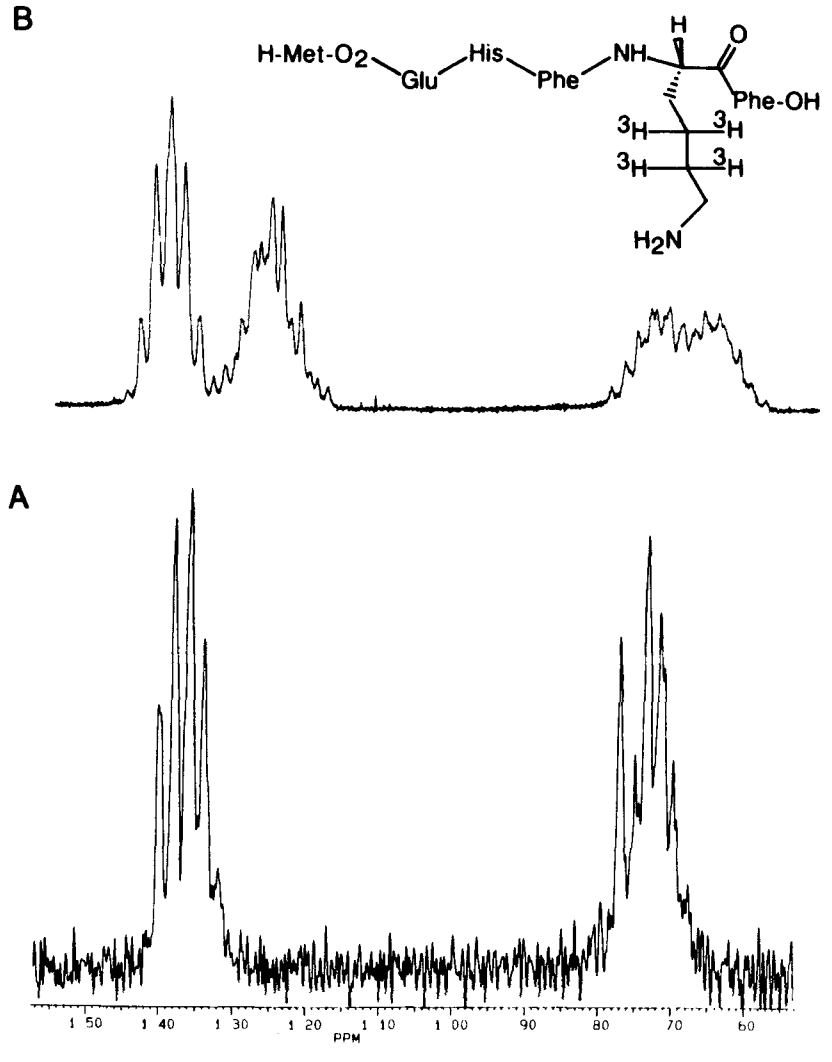

Figure 3: NMR spectra of [lysine-^{3}H] Org 2766 (4); solvent ^2H_2O; A) ^{1}H decoupled ^{3}H spectrum, B) ^{1}H spectrum.

The chemical shift differences between the di- and monotritiated species yield isotope shifts of +0.005 ppm and -0.005 ppm for the vicinally ditritiated species with 2 Hz and 7 Hz splittings respectively, and -0.014 ppm for the geminally ditritiated species (15 Hz splitting). These values are not in agreement with the vicinal (-0.01 ppm) and geminal (-0.02 ppm) isotope effects found in the literature. A tentative explanation might be found in the strong shielding of the γ-(0.32 ppm) and δ-^{3}H's (0.73 ppm) of the lysine side chain by the nearby phenyl rings of the phenylalanine units. Any slight conformational change of this lysine side chain upon tritiation will cause extra (de)-shielding and thus interfere with the normal isotope effects.

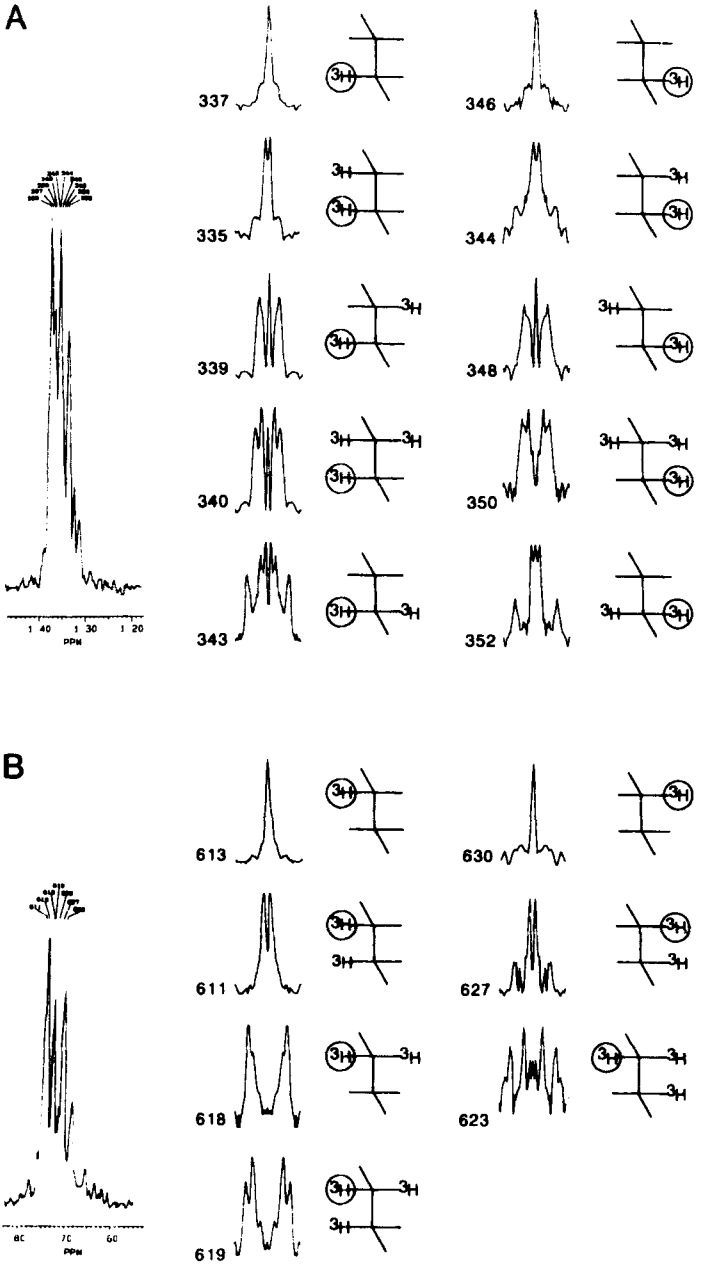

Figure 4: ^{1}H-decoupled J-resolved ^{3}H spectrum of Org 2766 (4) with cross-sections in the J-dimension.
A: δ-tritons B: γ-tritons

Application of ^3H NMR for identification of unknown products is, because of the limited amount of information present in ^3H NMR, only possible in exceptional cases when the tritium is located at or near the site of the modification[3]. An example is given in Figure 5: on an attempt to introduce an ethynyl group in the steroid (5), labelled by exchange with tritiated water,[8] an unexpected product was obtained. Based on the position of the tritium signal (and the chromatographic behaviour) the compound was identified as the 17-hydroxy compound (6) which was produced by reduction of the 17-oxosteroid with $LiALH_4$ (used for purification of the solvent and still present as a contamination).

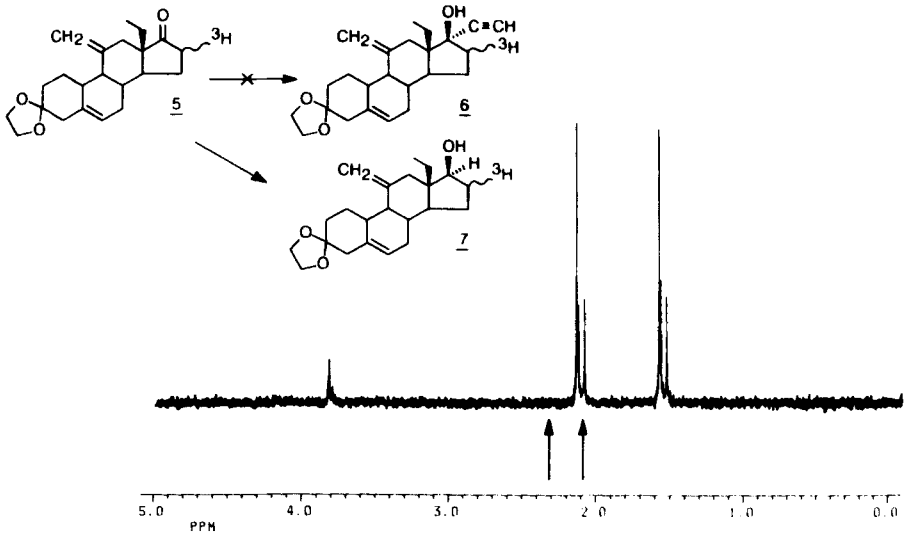

Figure 5: ^3H NMR spectrum (^1H decoupled) of 17-hydroxysteroid 7; solvent C^2HCl_3. The arrow denotes the position of the tritium for compound 6.

Although radiochemical purity generally is determined by chromatography ^3H NMR can in some cases be of help in this type of analysis. In Figures 6 and 7 two examples are given. Figure 6 gives the determination of the enantiomeric purity of [+]-[N-C^3H_3]-mianserin (8) prepared from racemic mianserin by chiral chromatography. In Figure 7 an example is given for the steroid Org 6216 (9). This product, when prepared through reoxidation with $Tl(OAc)_3$, was radiochemically pure according to all HPLC and TLC systems used. However, in the ^3H NMR spectrum signals not coïnciding with proton signals (at 2,2 and 1,8 ppm) indicated some hidden impurity. Resynthesis of the product with DDQ gave pure material according to HPLC as well as ^3H NMR (Figure 7).

Conclusions

As has been illustrated in this article ^3H NMR is a powerful and essential technique in the quality control of tritiated compounds.

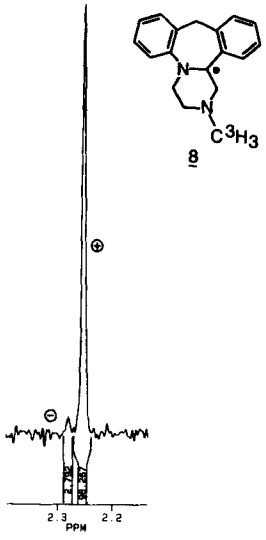

Figure 6: Part of the ^3H NMR spectrum of [N-C^3H_3]-mianserin (8) prepared by chromatography over Combriotac; solvent C^2HCl_3 containing 4% Pirkle's alcohol. enantiomeric purity: 97,5%; • denotes chiral centre.

This has been recognized by most commercial suppliers of labelled materials but strangely enough, about 60% of the papers[9] appearing on the preparation of such compounds still do not mention this technique in their analytical procedures and consequently are at risk of false-interpretations when these materials are used in biological/biochemical experiments.

Experimental part

^3H NMR spectra are recorded at a Bruker 360 operating at 384 MHz in double walled tubes essentially as described by Evans et al[2]. As internal standard tetramethylsilane was used and by multiplication with a factor of 1,06663975 this provided the standard for the tritium spectrum. The J-resolved spectra were recorded using the pulse-programme described by Williams et al[8] using 32 sub-experiments.

Of all compounds described the actual tritiation reactions using Ci-amounts of tritiated reagent were performed at Amersham Int. plc, Cardiff, U.K. while the purifications and analysis (using Waters-HPLC-systems) were done at Organon Int., Oss, The Netherlands. GC/MS measurements were performed on a Finnigan Mat TSQ70 apparatus.

^{3}H-Estrone (1)

The compound was prepared by reaction for 45 minutes at room temperature of 2-bromo-estrone (10) with tritium gas in methanol with 1% KOH in the presence of Pd/C (10%). After removal of the catalyst by filtration, neutralization and removal of labile tritium (evaporation) the material was purified by HPLC (Lichrosorb Si60) to a radiochemical purity of ≥ 96% with n-heptane / t-butyl-methylether.

Specific activity 36 Ci/mmol (1,3 TBq/mmol) as determined by HPLC combined with liquid scintillation counting.

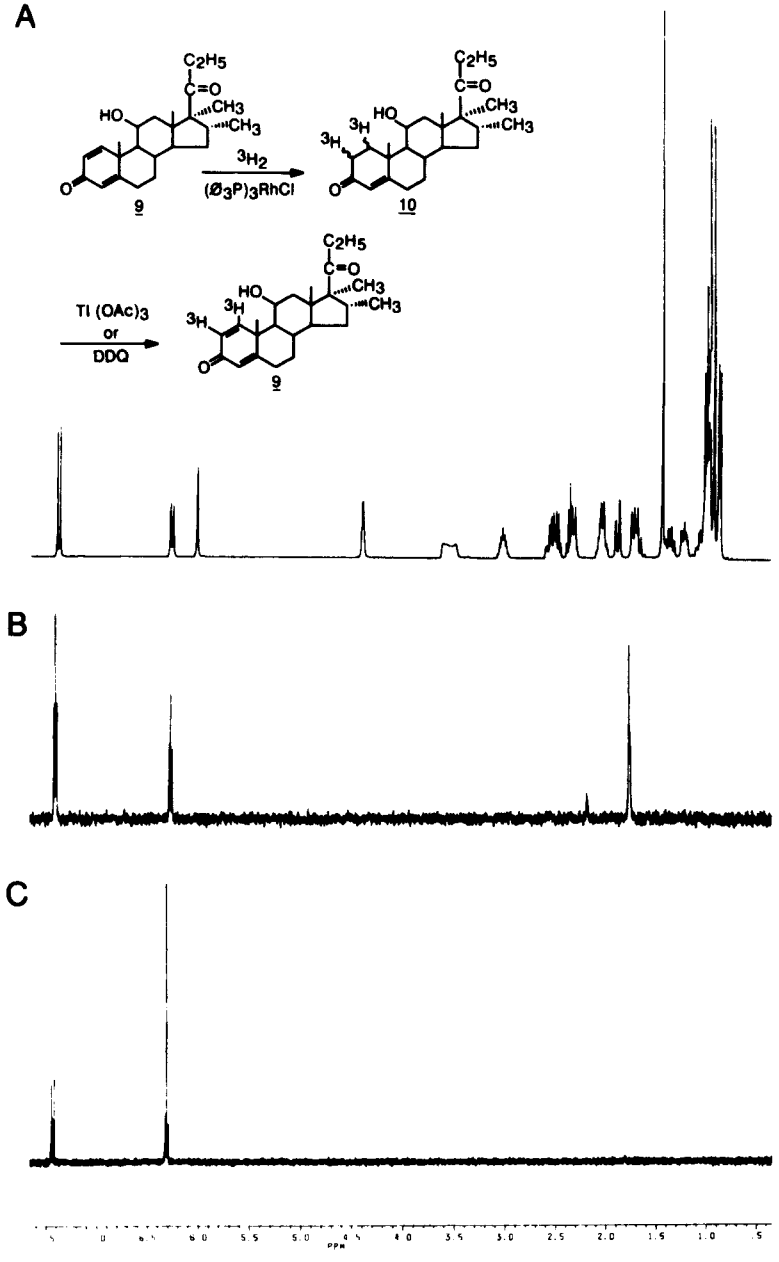

Figure 7: NMR spectra of Org 6216 (9); solvent C^2HCl_3/10% $C^2H_3O^2H$
A: ^1H NMR B: ^3H NMR (^1H-decoupled) of the product prepared with $Tl(OAc)_3$
C: ^3H NMR (^1H-decoupled) of the product prepared with DDQ.

^3H-Org 30659 (3)

The steroid was tritiated by reaction for 16 hours at 40°C in dimethyoxyethane with 25 Ci ^3H_2O and acetylchloride. After removal of volatile radioactivity the material was purified to a

radiochemical purity of > 97% HPLC (Cp spherC_{18}) with methanol/water.
Specific activity: 69 Ci/mmol (2,6 TBq/mmol) as determined by GC/MS.

Org 2766 (4)
The peptide was tritiated by reaction for 1 hour at room temperature of the lysinine-analogue in DMF/water with tritium gas in the presence of Pd/C (10%). After removal of the catalyst and labile tritium the product was purified by HPLC to a radiochemical purity of ≥ 98% (Supelcosil 18DB) with aq. ammonium acetate/acetonitrile.
Specific activity: 58 Ci/mmol (2,2 TBq/mmol) as determined by HPLC combined with liquid scintillation counting.

^{3}H-mianserin (8)
The product is prepared by reaction of excess desmethylmianserin with tritiated methyliodide in toluene for 4 days at 4°C. After removal of the excess desmethylmianserin by chromatography the product was separated into its two enantiomers by chromatography over Combriotac with methanol/acetonitrile as eluent. The final product was purified to chemical purity of > 95% by HPLC (Lichrosorb Si60) with n-hexane/2-propanol/25% aqueous ammonia.
Specific activity 83 Ci/mmol (3,1 TBq/mmol) as determined by GC/MS.

Org 6216 (9)
Org 6216 was reduced with tritium gas by reaction for 18 hours at room temperature in toluene/ethanol with tritium gas using tris(triphenylphosphine)rhodium (I) chloride as catalyst.

After removal of the labile tritium the product was purified by chromatography over silica gel (dichloromethane/acetone). The resulting 1,2-dihydro Org 6216 was oxidized by reaction for 4 hours at 120°C in toluene/acetic acid with DDQ. After filtration over aluminum oxide the product was purified to a purity of ≥ 97%. Specific activity: 28 Ci/mmol (1,0 TBq/mmol) as determined by FD-mass spectormetry.

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