Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2018, **28**, 64–65

Mendeleev Communications

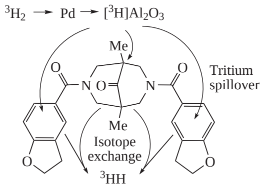
Synthesis of tritium-labeled PAM-43

Igor Yu. Nagaev,*[a] Konstantin V. Shevchenko,[a] Valeriy P. Shevchenko,*[a] Nicolay F. Myasoedov,[a] Vladimir V. Grigoriev,[b] Mstislav I. Lavrov,[c] Ekaterina V. Bondarenko[d] and Elena E. Kalashnikova[d]

[a] *Institute of Molecular Genetics, Russian Academy of Sciences, 123182 Moscow, Russian Federation. Fax: +7 499 196 0221; e-mail: nagaev@img.ras.ru*
[b] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 496 524 9508*
[c] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 932 8846*
[d] *Cyclomemorin Ltd., 249030 Obninsk, Kaluga Region, Russian Federation. Fax: +7 484 396 5879*

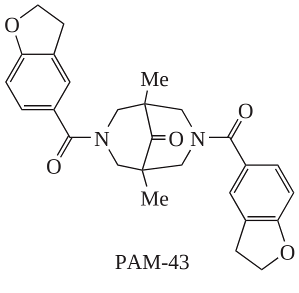
DOI: 10.1016/j.mencom.2018.01.021

The deuterium- and tritium-labeled PAM-43 was obtained in 23% yield and with a specific radioactivity of 132 Ci mmol^{-1}. As determined by mass spectrometry, a PAM-43 molecule contained 80% of deuterium in 2,3-dihydrobenzofuran fragments, while the central fragment contained 20% of deuterium.



Dementia is the deterioration of intellectual and other mental processes due to organic brain disease; 46.8 million people worldwide suffer from Alzheimer's disease, and this number will increase to 74.7 million until 2030.[1] In Russia, the market segment of anti-dementia drugs is limited by anticholinesterase drugs (donepezil, rivastigmine and galantamine), an antagonist of NMDA receptors (memantine) and a Ginkgo biloba extract. However, these drugs do not have a positive effect on the long-term prognosis and exert a number of side effects. Recently, new approaches have been developed to the use of modified compounds with neuroprotective activity.[2,3]

Cyclomemorin (Obninsk, Russia) proposed the development and market launch of a new drug with a stimulating effect on integrative brain functions, facilitating learning, improving memory and possessing a neuroprotective action to treat dementia and, possibly, Alzheimer's disease. The active compound of this drug is PAM-43.



PAM-43

The preclinical tests of this compound include pharmacokinetic and pharmacodynamic studies that require radiolabeled PAM-43. The aim of this work was to synthesize tritium-labeled PAM-43 with a specific radioactivity of at least 100 Ci mmol^{-1}.[†]

At the first stage, it was necessary to choose a better method between liquid-phase or solid-phase ones, or isotope exchange with tritiated water.[5–8] The liquid-phase methods and isotopic exchange with tritiated water turned out to be ineffective. When a solid-phase method is used, the support and catalyst types are of great importance.[9–12]

The use of an additional carrier with an organic compound, which is then mixed with a catalyst, makes it possible to carry out isotope exchange at higher temperatures. Therefore, a screening was conducted for the effect of isotope exchange between PAM-43 and tritium gas using Al_2O_3, zeolite, graphene, $CaCO_3$, SiO_2 and nanodiamonds. The reaction was carried out at 160 °C for 10 min.

The amount of hydrogen isotopes in PAM-43 molecules, the yield and the formation of by-products are strongly affected by the carrier. Depending on the carrier applied, the label contents of the samples differed by a factor of more than 4, and the yields of PAM-43, by factors of 2–3. The best results were obtained with Al_2O_3 and zeolite. However, in the latter case, a more complex mixture of products was formed (Figure 1).

The catalyst was 5% Rh/Al_2O_3 or 5% Pd/Al_2O_3, and the reaction was carried out at 160–200 °C for 10 min. The yield varied

---

[†] The labeling was performed using deuterium and tritium–protium mixtures according to a published procedure.[4] Mass-spectrometric data were obtained on an LCQ Advantage MAX instrument with electrospray ionization, direct injection of a sample solution with a concentration of 10 μg ml^{-1} in 0.1% acetic acid and further fragmentation of the molecular peak in the analyzer by ion collision at 35 eV. The chromatographic data were processed using a Chrom@Spec chromatography data station (ZAO Ampersand, Russia). Radioactivity was measured on a scintillation counter with a tritium detection efficiency of 30% in a dioxane scintillator. HPLC analysis was carried out on a Milichrom-A02 chromatograph (detection wavelengths, 200–240 nm) using a ProntoSIL-120-5-C18 AQ DB-2003 column (2 × 75 mm; particle size, 5 μm), in a gradient of methanol in a buffer solution (0.2 M $LiClO_4$ + 0.005 M $HClO_4$, pH 2.24) for 12 min at 35 °C; the eluent flow rate was 0.2 ml min^{-1}. The retention times of PAM-43 in the methanol gradients from 20 to 100 and from 40 to 100% were 7.87 and 5.29 min, respectively. When using a Reprosil pur C18aq column, 4 × 150 mm, the methanol–water system (1:1), and a flow rate of 1 ml min^{-1}, the retention time of PAM-43 was 4.27 min. With a Reprosil pur C18aq column, 8 × 250 mm, methanol–water–acetic acid system (50 : 50 : 0.5), and a flow rate of 2 ml min^{-1}, the retention time of PAM-43 was 11.34 min.

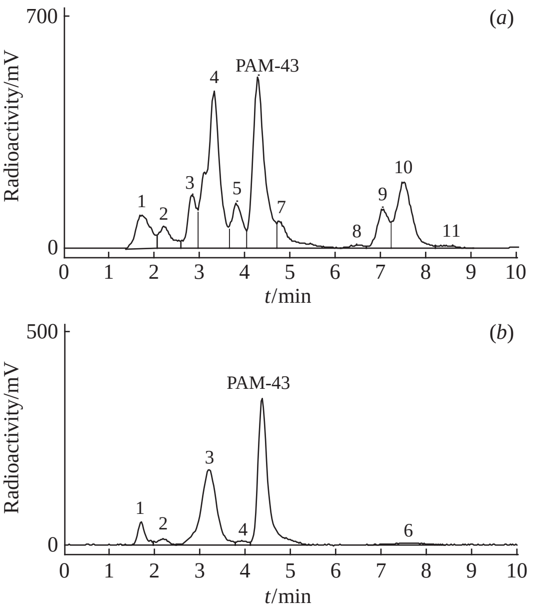


Figure 1 Distribution of radioactivity in HPLC of PAM-43 using (*a*) zeolite and (*b*) Al_2O_3 carriers (catalyst : carrier : PAM-43 ratio, 2 : 30 : 1).

from 80 to 10% or from 90 to 20% within the temperature range in the case of 5% Rh/Al_2O_3 or 5% Pd/Al_2O_3, respectively.

In experiments with 100% tritium, a solution of PAM-43 (6 mg) in methanol (0.5 ml) was mixed with Al_2O_3 (180 mg). The solvent was removed on a rotary evaporator and the residue was lyophilized. An aliquot portion of the dry mixture (62 mg) was mixed with 5% Pd/Al_2O_3 (4 mg) and transferred to a reaction ampoule. Then, the vial was evacuated to 0.1 Pa, filled with a tritium gas to a pressure of 400 hPa, and kept at 150 °C for 10 min. An excess of tritium was removed by evacuation. The catalyst material was extracted with methanol (5 × 2 ml) and separated by filtration. Labile tritium was removed several times by a dissolution of the substance in methanol (5 × 2 ml) and evaporation of the latter. After preparative HPLC (Figure 2),† the yield of PAM-43 was 32%, and the molar radioactivity was 52 Ci $mmol^{-1}$.

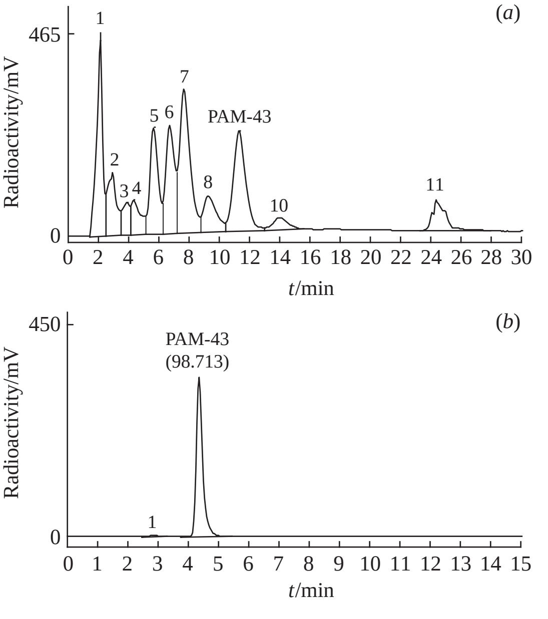


Figure 2 Preparative separation of reaction products in the synthesis of labeled PAM-43: (*a*) product isolation, (*b*) HPLC after purification.

When an additional carrier was not used, the reaction had to be performed at lower temperatures. At 120, 140 and 160 °C (10 min), the fractions of PAM-43 molecules containing no deuterium atoms were 15, 6 and 1%, respectively, while the yield of PAM-43 in this temperature range decreased from 48 to 14%. When working with a tritium–protium mixture, the specific radioactivity of PAM-43 was recalculated to 100% tritium. With this technique, the specific radioactivity of PAM-43 varied from 90 to 165 Ci $mmol^{-1}$ in a temperature range from 120 to 160 °C. The specific radioactivity increased by 70% or only 10% as the reaction temperature was raised from 120 to 140 °C, or from 145 to 165 °C, respectively. As a result of the study, the reaction at 150 °C was found optimal for the incorporation of tritium into PAM-43.

In this procedure, a solution of PAM-43 (5 mg) in methanol (0.3 ml) was mixed with 5% Pd/Al_2O_3 (100 mg). Methanol was removed on a rotary evaporator, the residue was lyophilized. An aliquot of the dry mixture (63 mg) was transferred to the reaction ampoule. The reaction was then carried out at 150 °C for 10 min followed by the treatment described above. The yield of PAM-43 was 23%, and its specific radioactivity was 132 Ci $mmol^{-1}$.

Based on the structure of PAM-43, one could expect that the bulk of the tritium label occurred in the aromatic fragments of this compound. Indeed, when 5% Pd/Al_2O_3 and a catalyst-to-substance ratio of 20 : 1 were used, the deuterium amount incorporated into 2,3-dihydrobenzofuran fragments was four times greater than that in the central fragment. The resulted tritium labeled PAM-43 with a specific radioactivity of 132 Ci $mmol^{-1}$ is suitable for subsequent studies and for determining its distribution in the body, primarily, in the brain.

This work was supported in part by the Skolkovo Foundation (agreement no. G51/16 of 2016) and the Russian Science Foundation (grant no. 16-14-00077).

References

1 M. Prince, A. Wimo, M. Guerchet, G-C. Ali, Yu-T. Wu and M. Prina, *World Alzheimer Report 2015: The Global Impact of Dementia*, Alzheimer’s Disease International (ADI), London, 2015.

2 S. A. Kozin, V. A. Mitkevich and A. A. Makarov, *Mendeleev Commun.*, 2016, **26**, 269.

3 T. A. Gudasheva, *Russ. Chem. Bull. Int. Ed.*, 2015, **64**, 2012 (*Izv. Akad. Nauk, Ser. Khim.*, 2015, 2012).

4 J. Scheigetz, C. Berthelette, C. Li and R. J. Zamboni, *J. Label. Compd. Radiopharm.*, 2004, **47**, 881.

5 V. P. Shevchenko, I. Yu. Nagaev and N. F. Myasoedov, *Mechennye tritiem lipofil’nye soedineniya (Tritium-labeled Lipophilic Compounds)*, ed. V. M. Fedoseev, Nauka, Moscow, 2003 (in Russian).

6 C. N. Filer, *J. Label. Compd. Radiopharm.*, 2010, **53**, 739.

7 S. A. de Keczer, T. S. Lane, T. Voronin and M. R. Masjedizadeh, *J. Label. Compd. Radiopharm.*, 2005, **48**, 1013.

8 C. N. Filer, *J. Label. Compd. Radiopharm.*, 2017, **60**, 96.

9 V. P. Shevchenko, I. Yu. Nagaev, V. V. Bezuglov, N. F. Myasoedov and A. B. Susan, *J. Label. Compd. Radiopharm.*, 2007, **50**, 448.

10 R. Voges, J. R. Heys and T. Moenius, *Preparation of Compounds Labeled with Tritium and Carbon-14*, Wiley, 2009.

11 E. A. Evans, *Tritium and Its Compounds*, 2^{nd} edn., Butterworths, London, 1974.

12 V. P. Shevchenko, I. Yu. Nagaev, N. F. Myasoedov, *J. Label. Compd. Radiopharm.*, 2010, **53**, 693.

Received: 2nd May 2017; Com. 17/5239