Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2013, **23**, 76–77
Mendeleev Communications

Synthesis of 2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine – bridged nitric oxide synthase inhibitor with enhanced lipophilicity

Olga N. Zefirova,*[a,b] Evgeniya D. Plotnikova,[a] Evgeniya V. Nurieva,[a] Danil I. Peregud,[c] Mikhail V. Onufriev[d] and Natalia V. Gulyaeva[d]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 0290; e-mail: olgaz@org.chem.msu.ru*
[b] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*
[c] *National Research Center on Addictions, 119002 Moscow, Russian Federation. E-mail: peregud_d@yahoo.com*
[d] *Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, 117485 Moscow, Russian Federation. E-mail: nata_gul@pisem.net*

DOI: 10.1016/j.mencom.2013.03.006

2-Thia-4-azabicyclo[3.3.1]non-3-en-3-amine was synthesized as a lipophilic analogue of NO-synthase inhibitor 2-amino-5,6-dihydro-4*H*-1,3-thiazine and found to be a potent inhibitor of inducible NO-synthase *in vitro*. The crystal structure of the key intermediate 2-thia-4-azabicyclo[3.3.1]nonane-3-thione (obtained by cyclization of *trans*-3-bromocyclohexylamine with carbon disulfide) was determined by X-ray analysis.

The problem of low lipophilicity of the lead compound often takes place in drug design, being usually solved by introduction of the additional substituents to the molecule. Recently,[1,2] we suggested a new means to enhance lipophilicity of non-aromatic monocyclic compounds by their 'structural insertion' into the bridged or cage system. As an example of this approach we initiated the elaboration of the bridged analogues of 2-amino-5,6-dihydro-4*H*-1,3-thiazine **1** (Figure 1), inducible nitric oxide synthase (NOS) inhibitor[3] possessing good antihypotensive and radio-protective activity, but low elimination half-life because of the low lipophilicity (see ref. 4 and citations therein).

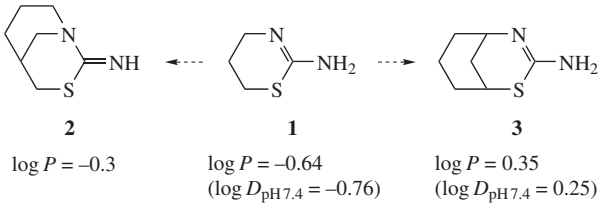


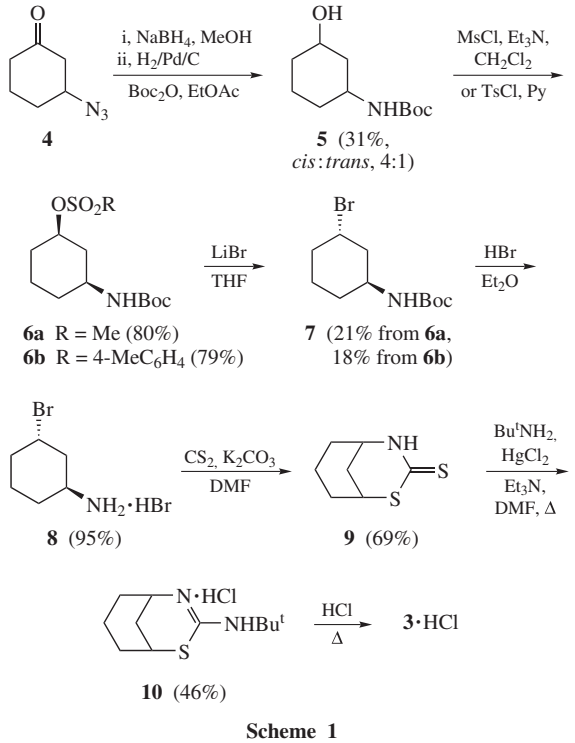
Figure 1 Lipophilic analogues **2** and **3** of the lead-compound 2-amino-5,6-dihydro-4*H*-1,3-thiazine **1**. The values of log *P* and log *D* were calculated using ACD/Labs program.

Earlier we synthesized 3-thia-1-azabicyclo[3.3.1]nonane-2-imine **2**, which was found to be not active as NOS inhibitor in spite of the predictions made by computer modeling.[2] The purpose of the present work was to synthesize and to test 2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine **3**, an analogue of **1** designed by attaching an additional cycle to the positions at C^4 and C^6. According to the calculations, compound **3** should be noticeably more lipophilic than the parent molecule **1** (Figure 1).

First we tried to prepare bridged thiazine **3** by reaction of 3-bromocyclohexene with thiourea *via* intramolecular cyclization of the intermediate 1-(cyclohex-2-en-1-yl)thiourea. However, even under the vigorous conditions (reflux in isopropyl alcohol) the nucleophilic substitution was not achieved and only the starting reactants were recovered. Thus, another synthetic route was chosen, namely, cyclization of *trans*-3-bromocyclohexylamine with carbon bisulfide followed by thiocarbonyl modification (similar approach was used for the synthesis of bridged structure **2**[2]).

Since the only operationally difficult method of 3-bromocyclohexylamine synthesis is mentioned in the literature[5] (and no characteristics of the compound are presented), we developed an original one (Scheme 1). A starting compound, 3-azidocyclohexanone **4**, was synthesized from cyclohexenone according to described procedure.[6] Azido ketone **4** was reduced with sodium borohydride to the corresponding alcohol, its azido group was then subjected to palladium-catalyzed hydrogenation[7] and simultaneous protection of the formed amino group gave product **5** as a racemic mixture of *cis*- and *trans*-isomers in a 4:1 ratio (as determined from ^{1}H NMR spectrum; the signals were ascribed on the basis of spectral data of structurally close compounds[8]).

It should be mentioned, that we also synthesized compound **5** from 1,3-cyclohexanedione *via* 3-aminocyclohexenone with its



Scheme 1

following reduction to the corresponding amino alcohol[8] and protection of amino group.† However, this method was more complicated operationally.

An attempt of the direct replacement of hydroxy group in **5** with bromine substituent using standard treatment with CBr_4 in the presence of triphenylphosphine[9] led to the elimination products only. Mesylation and tosylation of hydroxy group of compound **5** allowed us to isolate *cis*-isomers of compounds **6a**,**b** and to carry out the nucleophilic substitution in these compounds using lithium bromide in aprotic solvents. However, elimination was predominating and the maximal yield of amino bromide **7** (21%) has been achieved after 15 h reflux of mesylate **6a** and LiBr in THF. In the ^{1}H NMR spectrum of compound **7** only resonances of *trans*-isomer were observed. The following deprotection of amino group in **7** gave *trans*-3-bromocyclohexylamine **8** (as hydrobromide).

The reaction of the obtained compound **8** with carbon disulfide[2] afforded 2-thia-4-azabicyclo[3.3.1]nonane-3-thione **9** in a good yield.‡ Since this compound represents a novel type of bridged heterocycles we performed its X-ray analysis, which unambiguously proved the structure of thione **9** (Figure 2).§

As shown in Figure 2 the cycle with two heteroatoms in molecule of **9** is strongly flattened due to the presence of sp^2-hybridized carbon atom and a substituent at C^3 of the bridged structure and adopts a conformation of distorted envelope (for calculated puckering parameters see Online Supplementary Materials).

Reaction of thione **9** with *tert*-butylamine in the presence of mercury(II) chloride[10] led to the replacement of thiocarbonyl by *tert*-butylamino group and afforded bridged product **10** (see Scheme 1). ^{1}H NMR spectrum of **10** is characterized by the singlet of nine protons of *tert*-butyl group at 1.49 ppm and two multiplets at 9.83 ppm and 10.78 ppm, corresponding to the protons at exo- and endocyclic nitrogen atoms. The resonance of the C^3 carbon atom in ^{13}C NMR spectrum is observed at 166.6 ppm (198 ppm in **9**) and proves the formation of isothiourea fragment.

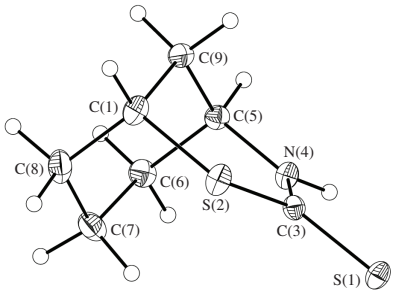


Figure 2 The general view of 2-thia-4-azabicyclo[3.3.1]nonane-3-thione **9** in representation of atoms by thermal ellipsoids (p = 50%).

Removal of the *tert*-butyl substituent in **10** as described[10] provided the target 2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine **3** as a hydrochloride. Two resonances (broad singlet at 8.0–9.0 ppm and singlet at 10.77 ppm with relative integrated intensity ~2 and 1) corresponding to the NH_2^+ and NH groups are present in ^{1}H NMR spectrum of **3**.¶ The data of elemental analysis and mass spectrometry (m/z 156) also prove the structure of thiazine **3**.

The synthesized bridged compound **3** (as racemic mixture) was tested *in vitro* for the inhibiting activity to inducible isoform of nitric oxide synthase (*i*NOS, isolated from lipopolysaccharide-stimulated mouse macrophages, Cayman Chemical, USA, 22.43 units per mg, 4.97 mg cm^{-3}, 111.5 units per ml, catalog number 60862). The activity of *i*NOS was determined by radiometric method from the rate of [^{3}H]-L-citrulline accumulation in the NOS-catalyzed oxidation of NOS substrate [^{3}H]-L-arginine (0.37 Ci mmol^{-1}) as reported.[2] The binding results revealed that at 1 μM an inhibition percent (*i.e.*, the difference between the activities of the samples without inhibitors and those containing inhibitors expressed as a percent of the activity of samples without inhibitors) for the lead compound **1** is 77%, and for thiazine **3** – 76%. Thus, their *in vitro* activity is nearly equal and the synthesized 2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine **3** is an effective *i*NOS inhibitor with enhanced lipophilicity. Antihypotensive activity of this compound in the experiments *in vivo* is under study.

This work was supported by the Russian Foundation for Basic Research (project no. 12-03-00720) and Russian Academy of Sciences. Part of the equipment for the work was purchased at the expense of M.V. Lomonosov Moscow State University Program of Development. Authors are grateful to Dr. K. A. Lyssenko for the X-ray crystallography experiment.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2013.03.006.

References

1 O. N. Zefirova, I. S. Raguzin, V. V. Gogol, E. V. Nurieva and M. S. Belenikin, *Mendeleev Commun.*, 2011, **21**, 242.
2 O. N. Zefirova, E. V. Nurieva, V. I. Chupakhin, I. S. Semenova, D. I. Peregud, M. V. Onufriev and N. V. Gulyaeva, *Mendeleev Commun.*, 2010, **20**, 323.
3 S. K. Shah, S. K. Grant, M. Maccoss, K. Shankaran, H. Qi and R. Guthikonda, *International Patent* WO 96/14842, 23.05.1996.
4 T. P. Trofimova, O. N. Zefirova, A. A. Mandrugin, V. M. Fedoseev, D. I. Peregud, M. V. Onufriev, N. V. Gulyaeva and S. Ya. Proskuryakov, *Vestn. Mosk. Univ., Ser. Khim.*, 2008, **49**, 328 (*Moscow University Chem. Bull.*, 2008, **63**, 274).
5 J. v. Braun, W. Haensel and F. Zobel, *Liebigs Ann. Chem.*, 1928, **462**, 283.
6 S.-G. Kim and T.-H. Park, *Synth. Commun.*, 2007, **37**, 1027.
7 S. B. Boga, J. S. Aidhen and I. S. Aidhen, *Molecules*, 2005, **10**, 871.
8 G. Bartoli, C. Cimarelli and G. Palmieri, *J. Chem. Soc., Perkin Trans. 1*, 1994, 537.
9 S. G. Newman, C. S. Bryan, D. Perez and M. Lautens, *Synthesis*, 2011, 342.
10 L. Gauzy, Y. Le Merrer, J.-C. Depezay, D. Damour-Barbalat and S. Mignani, *Tetrahedron Lett.*, 1999, **40**, 3705.

Received: 22nd November 2012; Com. 12/4023

† For synthetic details and characteristics of all the compounds, see Online Supplementary Materials.

‡ Attempts to synthesize the target bridged compound **3** *via* interaction of amino bromide **8** with sodium thiocyanate or thiourea were unsuccessful.

§ *Crystal data for* **9**. Crystals of **9** ($C_7H_{11}NS_2$, M = 173.29, from $CHCl_3$) are monoclinic, space group $P2_1/c$, at 100(2) K: a = 9.9364(8), b = = 7.3175(6) and c = 11.2579(9) Å, β = 105.6639(16)°, V = 788.16(11) Å^3, Z = 4, d_{calc} = 1.460 g cm^{-3}, μ(MoKα) = 0.594 cm^{-1}. Intensities of 6436 reflections were measured with Bruker APEX-II CCD [λ(MoKa) = = 0.71073 Å, 2θ <58°] and 2083 independent reflections were used in the further refinement. The structure was solved by direct method and refined by the full-matrix least-squares technique against F^2 in the anisotropic–isotropic approximation. The refinement converged to wR_2 = 0.0808 and GOF = 0.985 for all independent reflections [R^1 = 0.0308 was calculated against F for 1932 observed reflections with $I > 2\sigma(I)$]. All calculations were performed using SHELXTL PLUS 5.1.

CCDC 911410 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif. For details, see 'Notice to Authors', *Mendeleev Commun.*, Issue 1, 2013.

¶ Spectral data indicate the presence of a single tautomer of compound **3** and do not allow assigning it to the specific form. In Figure 1 we give the most expected biologically active tautomer.