*Chemistry of Heterocyclic Compounds, Vol. 46, No. 5, 2010*

# NEW METHOD FOR THE SYNTHESIS OF DIFFICULTLY AVAILABLE STERICALLY HINDERED TRITIUM-LABELED PYRIDINIUM DERIVATIVES

**N. E. Shchepina[1]*, V. V. Avrorin[2], G. A. Badun[3], V. M. Fedoseev[3], and S. B. Lewis[4]**

*Direct phenylation of the nitrogen atom in pyridines was noted in a study of the ion-molecule reactions of free nucleogenic phenyl cations generated by β-decay of tritium in labeled benzene. A one-step synthesis of unreported biologically active tritium-labeled N-phenyl-2,6-lutidinium and 2,4,6-collidinium salts. Steric factors, which significantly reduce the yield in the case of sym-collidine, play a major role in the direct phenylation of nitrogen.*

**Keywords**: nucleogenic phenyl cations. N-phenyl-2,6-lutidinium and N-phenyl-2,4,6-collidinium salts, tritium, direct phenylation of pyridine nitrogen atom.

The discovery of gene therapy has opened new and astounding possibilities in medicine, permitting a revolutionary approach to the treatment of disease on the cellular level. When a cell mechanism is weakened due to a defective gene, a functional gene contained in a suitable vector may be introduced directly into the injured cell, tissue, or organ. After internalization, DNA is transferred to the nucleus, where it combines with the "host" gene. The genetic code is read and is finally transformed into a protein needed for eliminating the cell imbalance [1-5]. Despite apparent simplicity, the success of this new treatment approach largely depends on the development of efficient transport processes. Viral transfer agents are presently the most efficient system for the transmission and transfection of foreign DNA into a living cell. However, such transfer agents unfortunately have significant side effects such as immunogenicity, the limited size of DNA, which can be introduced into a virus, mutagenicity, difficulties in preparation and storage, and, in some cases, high toxicity [6]. A safer alternative to viral transport is the use of cationic lipids [7-9], including pyridinium cationic lipids, which display low cytotoxicity [10-13]. Studies have shown that the efficiency of introduction in this case is directly related to the possibility of redistribution of the positive charge of the cation head, which is achieved by charge

* To whom correspondence should be addressed, e-mail: neschepina@mail.ru.

[1]Natural Sciences Institute, Perm State University, Perm 614990, Russia.
[2]St. Petersburg State University, St. Petersburg 199034, Russia; e-mail: radiochem@yandex.ru.
[3]M. V. Lomonosov Moscow State University, Moscow 119991, Russia; e-mail: badun@radio.chem.msu.ru.
[4]James Madison University, Harrisonburg, VA, USA; e-mail: lewissb@jmu.edu.

Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 5, pp. 692-698, May, 2010. Original article submitted November 30, 2009.

delocalization within the heterocyclic ring. Greatest promise is found for methylpyridinium derivatives, specifically, 2,4,6-trimethylpyridinium salts and also their 2,4,6-triphenylpyridinium analogs used as efficient membrane markers [14, 15]. Kourai et al. [16] have also shown that the presence of electron-donor methyl groups in the heterocyclic ring of pyridinium derivatives markedly enhances their antibacterial activity. A comparison of N-alkyl- and N-arylpyridinium salts has revealed that N-phenylpyridinium salts specifically have the greatest aromaticity and, thus, more efficient gene transport [17-19].

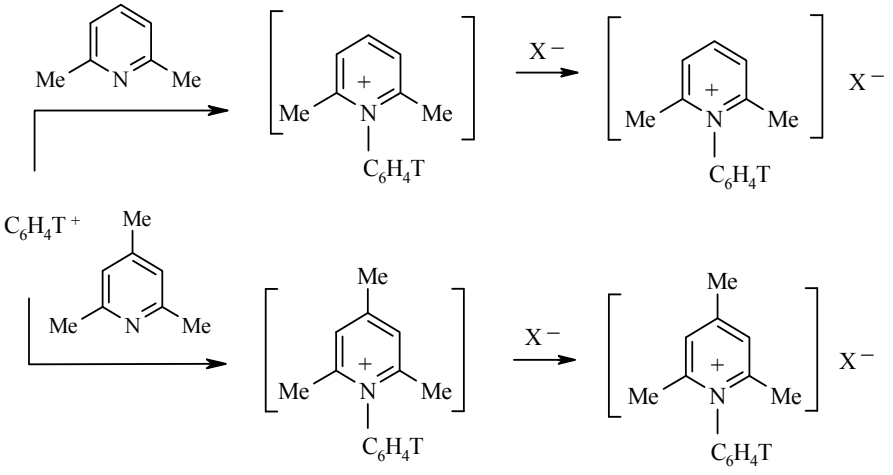
However, the synthesis of N-phenylpyridinium salts has encountered significant difficulties since the direct phenylation of the pyridine nitrogen atom has proved impossible [20, 21]. The only convenient reaction for the preparation of N-phenylpyridinium salts is the reaction of the corresponding pyrillium derivatives with aromatic amines, in the given case, with aniline [22, 23]. We should note that this reaction can be carried out only for collidine derivatives [24]. For disubstituted pyridines, this reaction is possible only when the molecule has phenyl substituents in the heterocyclic ring [25]. The study of the biological processes and mechanism of gene therapy requires the use of fine instruments and, hence, the labeled atom method is extremely promising [26-30]. We have previously shown the feasibility of the direct phenylation of the nitrogen atom in pyridine and methylpyridines (picolines) using nucleogenic phenyl cations [31, 32].

In the present work, we studied the direct phenylation of the nitrogen atom in polymethylpyridines, namely, 2,6-dimethylpyridine and 2,4,6-trimethylpyridine, and developed a simple method for prepared previously unreported tritium-labeled N-phenyl-2,6-lutidinium and N-phenyl-2,4,6-collidinium salts, which have been difficult to obtain.

Free tritium-labeled phenyl cations were generated by spontaneous tritium β-decay within doubly-tritium-labeled benzene according to the following scheme:

$$C_6H_4T_2 \xrightarrow{\beta^-} C_6H_4T^+ + He$$

Quaternary pyridinium cations are formed in the reaction of nucleogenic phenyl cations with the unshared electron pair of the nitrogen atom. The stabilization of these pyridinium cations by a suitable anion leads to tritium-labeled N-phenyllutidinium and N-phenylcollidinium salts:




The yields of the tetrafluoroborates obtained are given in Table 1. For comparison, the yields of the onium salts in the case of unsubstituted and methyl-substituted pyridines are also given.

The data in Table 1 show that shielding of the pyridine atom by the methyl substituents leads to a reduction in the radiochemical yield of quaternary salt. The facility of electrophilic attack at the nitrogen atom

depends predominantly on two major factors: 1) Nucleophilicity of the nitrogen atom and 2) Steric hindrance. The basicity (p$K_a$ value) is somewhat enhanced in going from pyridine to picolines and then to lutidine and collidine [33-36]. This, in turn, may lead to an increase in the yields of the products of phenylation of the nitrogen atom. However, at the same time, the electron-donor methyl substituents in the pyridine molecule stabilize azaarene ions and, thereby, facilitate concurrent electrophilic substitution in the heterocyclic ring [37-42].

Steric factors probably play the major role in the addition of free phenyl cations to the nitrogen atom as seen from a comparison of the yields of N-phenyl-substituted pyridinium and picolinium salts and then lutidinium and collidinium salts (see Table 1). The lowest yields of quaternary onium derivative are found for 2,4,6-collidine, where the steric hindrance of the 2-$CH_3$ and 6-$CH_3$ groups has the greatest effect.

TABLE 1. Yields of Quaternary Pyridinium Derivatives

| Onium salt | Yield, % |
|---|---|
| 2,6-Dimethyl-N-phenylpyridinium (2,6-lutidinium) tetrafluoroborate | 29±2 |
| 2,4,6-Trimethyl-N-phenylpyridinium (2,4,6-collidinium) tetrafluoroborate | 20±1 |
| 2-Methy-N-phenylpyridinium (2-picolinium) tetrafluoroborate [32] | 35±3 |
| 3-Methyl-N-phenylpyridinium (3-picolinium) tetrafluoroborate [32] | 36±2 |
| 4-Methyl-N-phenylpyridinium (4-picolinium) tetrafluoroborate [32] | 25±2 |
| N-Phenylpyridinium tetrafluoroborate [31] | 62±4 |

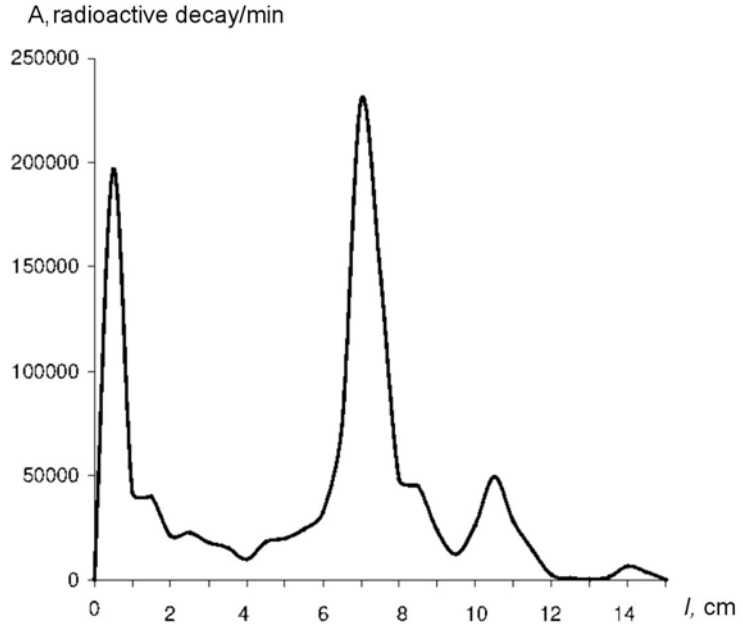



Fig. 1. Radioactivity distribution on the chromatographic plate in the analysis of labeled products formed in the radiochemical synthesis in the 2,4-lutidine/$C_6H_4T_2$/KI system.

Thus, a study of the ion-molecule reactions of nucleogenic phenyl cations with polymethylpyridines showed the direct phenylation of the nitrogen atom. This reaction features the one-step synthesis of previously unreported tritium-labeled N-phenyl-substituted 2,6-lutidinium and 2,4,6-collidinium salts, which are important materials for the detailed investigation of the mechanisms of antibacterial activity and transport processes in gene therapy.

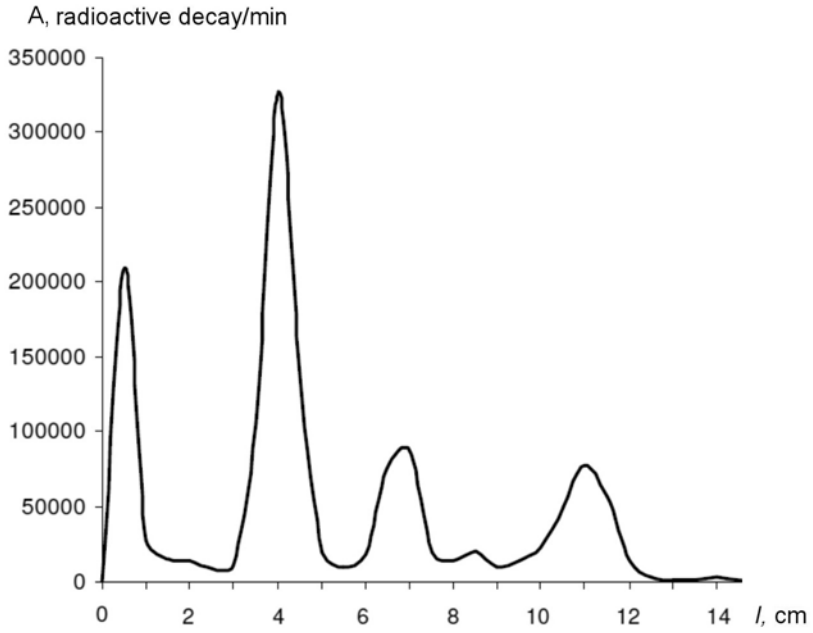



Fig. 2. Radioactivity distribution on the chromatographic plate in the analysis of labeled products formed in the radiochemical synthesis in the 2,4,6-collidine/$C_6H_4T_2$/KI system.

## EXPERIMENTAL

Commercially available 2,6-lutidine and 2,4,6-collidine were used as the substrates for the nuclear chemical synthesis. Inactive N-phenyl-4,6-diphenyl-2-picolinium iodide used as the reference and support was synthesized by reported methods [22-25] from the corresponding pyrillium salts, mp 243-244°C (mp 243-245°C [43]).

**Doubly-tritium-labeled Benzene,** used as the source of nucleogenic phenyl cations, was obtained from *p*-dibromobenzene and gaseous tritium by the catalytic replacement of halogen by tritium [32]. The volumetric specific activity of the solution obtained in hexane was 4 Ci/cm$^3$. The mass concentration of benzene in the solution was $7.1 \cdot 10^{-2}$ mmol/cm$^3$. The specific activity was 56.3 Ci/mmol, which corresponds to the multiplicity of the label virtually equal to 2. The chemical purity of benzene obtained was not less than 99%.

**Nuclear Chemical Synthesis (General Method)**. The ion-molecule reactions were carried out in sealed ampules containing tritiated benzene (source of phenyl cations) and the substrates, namely, 2,6-dimethylpyridine and 2,4,6-trimethylpyridine in ~1:1000 mol ratio (1 μl hexane solution of $C_6H_4T_2$ and 6.4 μl 2,6-lutidine or 7.3 μl 2,4,6-collidine) on crystals of the stabilizing salt (potassium tetrafluoroborate or potassium iodide). The ampules with the reaction mixture were maintained for not less than one month to accumulate the reaction products in amounts sufficient for reliable determination. Unreacted benzene was distilled off. The phenyl-substituted tritium-labeled lutidinium or collidinium derivative synthesized was then subjected to chromatographic separation and identified by preparative chromatography on Reverse Phase C18 silica gel glass plates with a fluorescent indicator. Acetonitrile was the eluent. Segments of the chromatogram (0.5 cm) were read in a dioxane scintillator and their radioactivity was measured using a Rack-beta scintillation counter produced in the USA.

Typical radiograms are given in Figs. 1 and 2.

This work was carried out with the support of the Russian Basic Research Fund, Grant 07-03-00881.

**REFERENCES**


1. N. S. Templeton and D. D. Lasic (editors), *Gene Therapy*. *Therapeutic Mechanisms and Strategies*, Marcel Dekker, New York (2000).
2. N. R. Lemoine and D. N. Cooper, in: D. N. Cooper, S. E. Humphries, and T. Strachan (editors), *Human Molecular Genetics*, BIOS Sci. Publ., Oxford, UK (1996).
3. K. R. Smith, *Arch. Med. Res.*, **34**, 247 (2003).
4. K. R. Smith, *J. Biotechnol*, **99**, 1 (2002).
5. T. Friedmann, *Nat. Med.*, **2**, 144 (1996).
6. A. Mountain, *Trends Biotechnol.*, **18**, 119 (2000).
7. X. Guo and F. C. Szoka, *Acc. Chem. Res.*, **36**, 335 (2003).
8. M. A. Ilies, W. A. Seitz, and A. T. Balaban, *Curr. Pharm. Des.*, **8**, 2441 (2002).
9. M. A. Ilies and A. T. Balaban, *Expert Opin. Ther. Pat.*, **11**, 1729 (2001).
10. I. Van der Woude, A. Wagenaar, A. A. Meekel, M. V. ter Beest, M. H. Ruiters, J. B. Engberts, and D. Hoekstra, *Proc. Nat. Acad. Sci. USA*, **94**, 1160 (1997).
11. A. A. P. Meekel, A. Wagenaar, J. Smisterova, J. E. Kroeze, P. Haadsma, B. Bosgraaf, M. C. A. Stuart, A. Brisson, M. H. J. Ruiters, D. Hoekstra, and J. B. F. N. Engberts, *Eur. J. Org. Chem.*, 665 (2000).
12. A. Roosjen, J. Smisterova, C. Driessen, J. T. Anders, A. Wagenaar, D. Hoekstra, R. Hulst, and J. B. F. N. Engberts, *Eur. J. Org. Chem.*, 1271 (2002).
13. M. A. Ilies, W. A. Seitz, M. T. Caproiu, M. Wentz, R. E. Garfield, and A. T. Balaban, *Eur. J. Org. Chem.*, 2645 (2003).
14. V. Menchise, G. De Simone, V. Alterio, A. Di Fiore, C. Pedone, A. Scozzafava, and C. T. Supuran, *J. Med. Chem.*, **48**, 5721 (2005).
15. M. A. Ilies, B. H. Johnston, F. Makori, A. Miller, W. A. Seitz, E. B. Thompson, and A. T. Balaban, *Arch. Biochem. Biophys.*, **435**, 271 (2005).
16. H. Kourai, H. Takechi, T. Horie, K. Takeichi, and I. Shibasaki, *Bokin Bobai*, **13**, No. 6, 245 (1985).
17. A. T. Balaban, A. Dinculescu, J. Elguero, and R. Faure, *Magn. Reson. Chem.*, **23**, 553 (1985).
18. S. R. Salman, A. H. Hassan, and M. A. R. Khyat, *Bull. Chem. Soc. Jpn.*, **61**, 2271 (1988).
19. A. T. Balaban, W. A. Seitz, A. C. Ilies, E. B. Thompson, R. E. Garfield, B. H. Johnson, A. L. Miller, and M. J. Wentz, US Pat. 2005196863; http://www.freepatentsonline.com/7456197.pdf.
20. K. H. Pausacker, *Aust. J. Chem.*, **11**, 200 (1958).
21. F. Brody and P. R. Ruby, in: E. Klingsberg (editor), *Pyridine and Its Derivatives*, vol. 114, Interscience, New York (1960), pt. 1, p. 289.
22. K. Dimroth, *Angew. Chem.*, **72**, 331 (1960).
23. C. Toma and A. T. Balaban, *Tetrahedron Suppl.*, **22**, No. 7, 9 (1966).
24. A. Camerman, L. H. Jensen, and A. T. Balaban, *Acta Crystallogr.*, **B25**, 2623 (1969).
25. E. A. Zvezdina, M. P. Zhdanova, I. I. Nechayuk, I. A. Barchan, Yu. N. Simkina, and T. A. Buchnaya, *Khim.-farm. Zh.*, **20**, 1328 (1986).
26. B. W. Fox, *Int. J. Radiat. Biol.*, **13**, 504 (1968).
27. R. B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, Elsevier Academic, Amsterdam (2004).
28. M. Saljoughian, *Synthesis*, **13**, 1781 (2002).
29. G. V. Sidorov and N. F. Myasoedov, *Usp. Khim.*, **68**, 254 (1999).
30. P. Shevchenko, I. Yu. Nechaev, and N. F. Myasoedov, *Tritium-Labeled Lipophilic Compounds* [in Russian], Nauka, Moscow (2003).
31. N. E. Shchepina, V. D. Nefedov, M. A. Toropova, V. V. Avrorin, and D. S. Gembitskii, *Radiokhimiya*, **41**, 523 (1999).

32. N. E. Shchepina, V. V. Avrorin, G. A. Badun, V. M. Fedoseev, S. E. Ukhanov, and S. B. Lewis, *Radiokhimiya*, **49**, 551 (2007).
33. J. Joule and G. Mills, in: M. A. Yurovskaya (editor), *Chemistry of Heterocyclic Compounds* [Russian translation], Mir, Moscow (2004).
34. I-Jen Chen and A. D. MacKerell, Jr., *Theor. Chem. Acta*, **103**, 483 (2000).
35. M. Makowski, R. Sadowski, D. Augustin-Nowacka, and L. Chmurzynski, *J. Phys. Chem. A*, **105**, 6743 (2001).
36. A. Augustin-Nowacka, M. Makowski, and L. Chmurzynski, *J. Chem. Thermodyn.*, **34**, 391 (2002).
37. A. R. Katritzky and C. D. Johnson, *Angew. Chem. Int. Ed. Engl.*, **6**, 608 (1967).
38. A. R. Katritzky and B. J. Ridgewell, *J. Chem. Soc.*, 3753 (1963).
39. R. A. Abromowitch and J. G. Saha, *Adv. Heterocycl. Chem.*, **6**, 229 (1966).
40. C. D. Johnson, A. R. Katritzky, B. J. Ridgewell, and M. Viney, *J. Chem. Soc. (B)*, 1204 (1967).
41. A. R. Katritzky and R. Taylor, *Adv. Heterocycl. Chem.*, **47**, 1 (1990).
42. A. R. Katritzky and W. O. Fan, *Heterocycles*, **34**, 2179 (1992).
43. I. G. S. Brooker, D. S. Daniel, and R. C. Taber, US Pat. 3639127; http://www.freepatentsonline.com/3639127.pdf.