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# Studies toward the Synthesis of 4-(2-R-ethyl)amino-2,2,5,5-tetramethyl-3-imidazoline 1-Oxyls. Nucleophilic Substitution of Bromide in the *N*-Alkyl Chain of the 1,2,4-Oxadiazol-2-one Precursor

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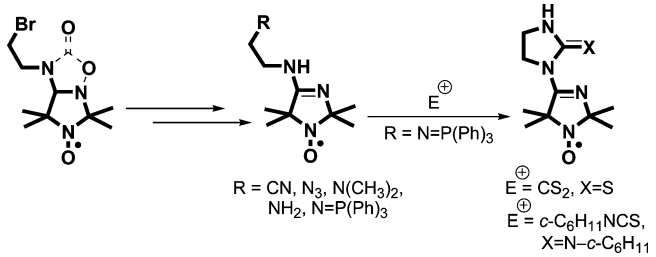


A synthetic approach to access the new nitroxides of the amidine type exhibiting pH-dependent EPR spectra through substitution of a halide in the *exo*-*N*-halogenoalkyl chain of 1-(2-bromoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one is reported. In this approach, an oxycarbonyl moiety of the oxadiazolone heterocycle plays the role of a "protecting group" for the amidine functionality. A nucleophilic cleavage of the oxadiazolone heterocycle under mild nonbasic conditions, applicable to substrates bearing substituents vulnerable to attack by strong basic nucleophiles, is elaborated. The approach allows for the new amidine nitroxides bearing various functional groups (e.g., such as CN, $N_3$, $NH_2$, COOEt) to be synthesized. A series of nitroxides obtained through the Staudinger/intermolecular aza-Wittig reaction of the azido derivative is also described. The nitroxides synthesized here were found to have pH-dependent two-component EPR spectra indicative of a slow, on the EPR time scale, $R^{\bullet} \leftrightarrows R^{\bullet}H^{+}$ chemical exchange, and p$K_a$ values ranging from 2.8 to 12.5 units. The guanidine derivatives synthesized in this work show the highest p$K_a$ values (p$K_a$ = 10.2 and 12.5, respectively) ever reported for the nitroxide pH-probes of a "basic type".

The ability of biological tissue to actively maintain constant concentration of hydrogen ions is of fundamental importance in living organisms: it plays a critical role in sustaining the metabolic function of proteins and other macromolecules.[1] Proton concentration, defined as pH, has been proven to change significantly during myocardial ischemia,[2] chronic heart failure,[3] tumors,[4] and inflammation.[5] Therefore, a real-time pH assessment is of considerable relevance for detecting pathophysiological conditions. Since first reports in the early nineteen eighties[6] significant progress in EPR-based *noninvasive* methods for pH monitoring has been made.[7] The method is based on

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(1) *Regulation of tissue pH in plants and animals*: *a reappraisal of current techniques*; Egginton, S.:, Taylor, E. W., Raven, J. A., Eds.; Cambridge University Press: Cambridge, UK, 1999.

(2) Bond, J. M.; Chacon, E.; Herman, B.; Lemasters, J. J. *Am. J. Physiol. Cell Physiol.* **1993**, *265*, C129−C137.

(3) Scott, A. C.; Wensel, R.; Davos, C. H.; Georgiadou, P.; Kemp, M.; Hooper, J.; Coats, A. J. S.; Piepoli, M. F. *Circulation* **2003**, *107*, 300−306.

(4) Raghunand, N.; Gillies, R. J. *Drug Resist. Update* **2000**, *3*, 39−47.

(5) Issberner, U.; Reeh, P. W.; Steen, K. H. *Neurosci. Lett*. **1996**, *208*, 191−194.

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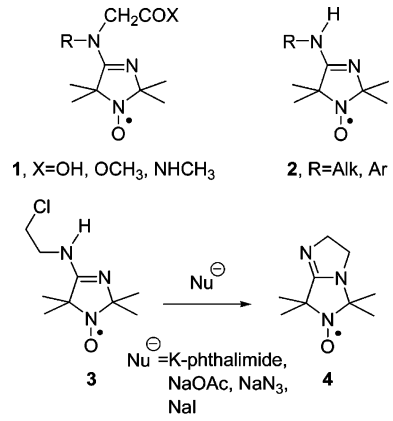
dependence of magnetic parameters of the EPR spectra (i.e., isotropic nitrogen hyperfine coupling constant, $a_N$, and *g*-factor) of some stable nitroxides on the reversible protonation of functional groups adjacent to the N−O moiety.[6a,8] Owing to recent developments of low-[9] and high-frequency EPR spectroscopy[10] and EPR-based techniques such as EPR imaging (EPRI),[11] longitudinally detected ESR (LODESR),[12] and proton electron double-resonance imaging (PEDRI),[13] this method has grown into a powerful tool with many biophysical and biomedical applications.[7,9,11h,14,15] However, the potential of this method is considered to be far beyond its current state. For the further progress of the method, a series of new nitroxides with pH-dependent EPR spectra and specific task-oriented properties needs to be designed and synthesized.[9c] Recently, we described an approach to synthesize new imidazoline nitroxides **1** (Scheme 1) having a protonatable *N*′,*N*′-disubstituted amidine functionality.[16] Various functional groups could be introduced into the nitroxide molecule within this approach, thus allowing one to manipulate the properties of the spin probe to a greater extent. Over the last two decades the *N*′-monosubstituted 4-alkyl(aryl)-amino-2,2,5,5-tetramethyl-3-imidazoline 1-oxyls **2** were among the most widely used EPR pH-probes in biophysical studies

**SCHEME 1**




because they are easy to synthesize and have p$K_a$ values matching the physiological range of pH.[7] Amidines **2** could be easily obtained through 1,3-dipolar cycloaddition of isocyanates to the aldonitrone 2,2,5,5-tetramethyl-3-imidazoline 1-oxyl followed by the alkaline cleavage of the oxadiazolone heterocycle formed.[17] The only obvious shortcoming of this synthesis is that the structure of the alkyl(aryl) substituent in the *exo*-*N*-alkyl chain in most cases is strictly predetermined by the structure of the isocyanate used. Until now, chemical transformations did not allow for much diversity with respect to the structure of the alkyl(aryl) group.[17] We believe that introducing functional groups into the *exo*-*N*-alkyl chain of 4-alkylamino-2,2,5,5-tetramethyl-3-imidazoline 1-oxyls would provide additional ways to tune the properties of pH spin probes. We consider the substitution of halide in 4-(2-halogenoethyl)-amino-2,2,5,5-tetramethyl-3-imidazoline 1-oxyl with nucleophiles to be the most attractive way to modify the *exo*-*N*-alkyl chain. Unfortunately, all the previous attempts to realize this approach have failed. Specifically, under conditions of the nucleophilic substitution of the chloride in 4-(2-chloroethyl)-amino-2,2,5,5-tetramethyl-3-imidazoline 1-oxyl **3** with NaI, $NaN_3$, NaOAc, and potassium phthalimide the only product isolated was the bicyclic amidine 2,3,4,5,6,7-hexahydro-6-oxyl-5,5,7,7-tetramethyl-7*H*-imidazo[1,5-*a*]imidazole **4** (Scheme 1).[17c] This product obviously results from the intramolecular nucleophilic attack of the lone electron pair of the *endo*-cyclic nitrogen atom of the amidine group on the *exo*-*N*-chloroethyl fragment.

Here we report the synthetic approach to substitute a halide in the *exo*-*N*-halogenoalkyl chain in a way to overcome the undesired reaction of intramolecular alkylation. We also report on properties and chemical transformations of the resulting compounds that allowed us to access new nitroxides exhibiting pH-dependent EPR spectra. The key step in the synthesis is the nucleophilic substitution of the bromide in the cycloadduct 1-(2-bromoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one **5**. In our approach an oxycarbonyl moiety of the oxadiazolone heterocycle plays the role of a "protecting group" for the amidine functionality.

## Results and Discussion

The oxadiazolone derivative **5a** was obtained in a good yield through 1,3-dipolar cycloaddition of the commercially available

(6) (a) Khramtsov, V. V.; Weiner, L. M.; Grigor'ev, I. A.; Volodarsky, L. B. *Chem. Phys. Lett.* **1982**, *91* (1), 69−72. (b) Khramtsov, V. V.; Weiner, L. M.; Eremenko, S. I.; Belchenko, O. I.; Schastnev, P. V.; Grigor'ev, I. A.; Reznikov, V. A. *J. Magn. Reson.* **1985**, *61*, 397−408. (c) Khramtsov, V. V.; Weiner, L. M. *Russ. Chem. Rev.* **1988**, *57* (9), 824−839.

(7) Khramtsov, V. V.; Volodarsky, L. B. In *Biological Magnetic Resonance*; Berliner, L. J., Ed.; Plenum Press: New York, 1998; Vol. 14, pp. 109−180.

(8) Keana, J. F. W.; Acarregui, M. J.; Boyle, S. L. M. *J. Am. Chem. Soc.* **1982**, *104*, 827−830.

(9) (a) Halpern, H. J.; Spencer, D. P.; van Polen, J.; Bowman, M. K.; Nelson, A. C.; Dowey, E. M.; Teicher, B. A. *Rev. Sci. Instrum.* **1989**, *60*, 1040−1050. (b) Berliner, L. J.; Koscielniak, J. Low field EPR spectrometers: L-band. In *EPR Imaging and In-Vivo ESR*; Eaton, G., Eaton, S., Ohno, K., Eds.; CRC Press: Boca Raton, FL, 1991; pp 65−72. (c) Foster, M. A.; Grigor'ev, I. A.; Lurie, D. J.; Khramtsov, V. V.; McCallum, S.; Panagiotelis, I.; Hutchison, J. M.S.; Koptioug, A.; Nicholson, I. *Magn. Reson. Med.* **2003**, *49*, 558−567. (d) Khramtsov, V. V.; Grigor'ev, I. A.; Lurie, D. J.; Foster, M. A.; Zweier, J. L.; Kuppusamy, P. *Spectroscopy* **2004**, *18*, 213−225. (e) Potapenko, D. I.; Foster, M. A.; Lurie, D. J.; Kirilyuk, I. A.; Hutchison, J. M. S.; Grigor'ev, I. A.; Bagryanskaya, E. G.; Khramtsov, V. V. *J. Magn. Reson.* **2006**, *182* (1), 1−11.

(10) Very High Frequency (VHF) ESR/EPR. In *Biological Magnetic Resonance*; Grinberg, O. Y., Berliner, L. J., Eds.; Kluwer Academic/Plenum Publishers: New York, 2004; Vol. 22.

(11) (a) Berliner, L. J.; Fujii, H. *Science* **1985**, *227*, 517−519. (b) Kuppusamy, P.; Li, H.; Ilangovan, G.; Cardounel, A. J.; Zweier, J. L.; Yamada, K.; Krishna, M. C.; Mitchell, J. B. *Cancer Res.* **2002**, *62*, 307−312. (c) Fujii, H.; Berliner, L. J. *Magn. Reson. Med.* **1999**, *42*, 691−694. (d) Khan, N.; Swartz, H. *Mol. Cell Biochem.* **2002**, *234*−235, 341−357. (e) Sotgiu, A.; Mäder, K.; Placidi, G.; Colacicchi, S.; Ursini, C. L.; Alecci, M. *Phys. Med. Biol.* **1998**, *43*, 1921−1930. (f) Gallo, P.; Colacicchi, S.; Ferrari, M.; Gualtieri, G.; Sotgiu, A. *Cardioscience* **1991**, *2*, 221−224. (g) Lurie, D. J. *Br. J. Radiol.* **2001**, *74*, 782−784. (h) Khramtsov, V. V.; Grigor'ev, I. A.; Foster, M. A.; Lurie, D. J.; Nicholson, I. *Cell. Mol. Biol.* **2000**, *46*, 1361−1374.

(12) Nicholson, I.; Robb, F. J.; McCallum, S. J.; Koptioug, A.; Lurie, D. J. *Phys. Med. Biol.* **1998**, *43*, 1851−1855.

(13) (a) Lurie, D. J.; Li, H.; Petryakov, S.; Zweier, J. L. *Magn. Reson. Med.* **2002**, *47*, 181−186. (b) Lurie, D. J.; Foster, M. A.; Yeung, D.; Hutchison, J. M. *Phys. Med. Biol.* **1998**, *43*, 1877−1886.

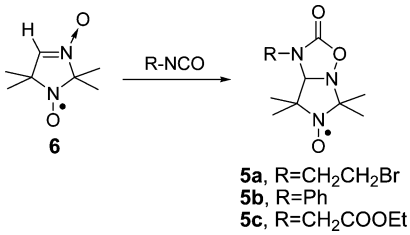
(14) (a) Khramtsov, V. V.; Marsh, D.; Weiner, L. M.; Reznikov, V. A. *Biochim. Biophys. Acta* **1992**, *1104*, 317−324. (b) Gallez, B.; Mäder, K.; Swartz, H. *Magn. Reson. Med.* **1996**, *36*, 694−697.

(15) (a) Smirnov, A. I.; Ruuge, A.; Reznikov, V. A.; Voinov, M. A.; Grigor'ev, I. A. *J. Am. Chem. Soc.* **2004**, *126*, 8872−8873. (b) Möbius, K.; Savitsky, A.; Wegener, C.; Plato, M.; Fuchs, M.; Schnegg, A.; Dubinskii, A. A.; Grishin, Y. A.; Grigor'ev, I. A.; Kühn, M.; Duché, D.; Zimmermann, H.; Steinhoff, H.-J. *Magn. Reson. Chem.* **2005**, *43*, S4−S19.

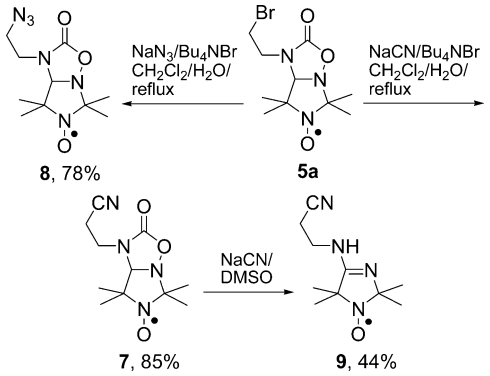
(16) Voinov, M. A.; Polienko, J. F.; Schanding, T.; Bobko, A. A.; Khramtsov, V. V.; Gatilov, Y. V.; Rybalova, T. V.; Smirnov, A. I.; Grigor'ev, I. A. *J. Org. Chem.* **2005**, *70*, 9702−9711.

(17) (a) Berezina, T. A.; Martin, V. V.; Volodarsky, L. B.; Khramtsov, V. V.; Weiner, L. M. *Bioorg. Khim.* **1990**, *16*, 262−269. (b) Balakirev, M.; Khramtsov, V. V.; Berezina, T. A.; Martin, V. V.; Volodarsky, L. B. *Synthesis* **1992**, *12*, 1223−1225. (c) Berezina, T. A.; Reznikov, V. A.; Volodarsky, L. B. *Tetrahedron* **1993**, *49* (46), 10693−10704.

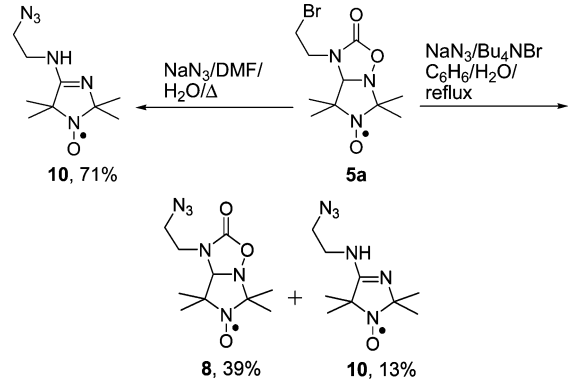
**SCHEME 2**




**SCHEME 3**




**SCHEME 4**




2-bromoethyl isocyanate to the aldonitrone 2,2,5,5-tetramethyl-3-imidazoline 1-oxyl **6** (Scheme 2). The bromoethyl derivative **5a** was chosen as a starting compound in the synthesis for the reason that bromide is a better leaving group than chloride.

Thus, under the phase-transfer catalysis conditions ($CH_2Cl_2$/$H_2O$/$Bu_4NBr$, reflux) the nucleophilic substitution of the bromide in the cycloadduct **5a** with the cyanide ion gave 1-(2-cyanoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*]-[1,2,4]oxadiazol-2-one **7** in 85% yield (Scheme 3). Under the same conditions the substitution of the bromide with azide ion afforded 1-(2-azidoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one **8** in 78% yield (Scheme 3).

We found that the composition of the reaction products in substitution of the bromide with the azide ion varies depending on conditions used. Thus, the substitution under the phase-transfer conditions specified above gave solely azidoethyl derivative **8**, while using the high-boiling benzene instead of dichloromethane ($C_6H_6$/$H_2O$/$Bu_4NBr$, reflux) resulted in the formation of the mixture of products **8** and **10** in approximately 4:1 molar ratio (Scheme 4). When aqueous DMF or DMSO (containing 10% of $H_2O$) was used as a solvent for the nucleophilic substitution and the temperature was increased to 110 °C the only product of the reaction was the amidine **10**. Compound **10** is identical with that obtained in the customary manner by hydrolytic cleavage of the oxadiazolone **8** under alkaline conditions ($CH_3ONa$/$CH_3OH$ or NaOH/$CH_3OH$). In all cases a 2-fold molar excess of $NaN_3$ was used. No product **4** resulting from the intramolecular alkylation was detected in the reaction mixture. This observation provides evidence that the nucleophilic substitution rate prevails over the oxadiazolone heterocycle cleavage rate. For convenience, the experimental conditions are summarized in Table S1 (Supporting Information).

In principle, the cleavage of the oxadiazolone heterocycle might occur under both thermolysis conditions[18] and as a result of attack by the nucleophile on the carbonyl carbon atom,[17c,19] though the arguments in favor of nucleophilic pathway presented seem to be quite questionable.

When compound **8** and reference compound **5b** (Scheme 2) were heated in DMF or DMSO at 110 °C without a nucleophile present, amidines **10** and **2** (R = Ph),[17c] respectively, were obtained in good yields.

As we found, the reaction of **5a** with 1 equiv of $NaN_3$ in aqueous DMSO at 55 °C affords in 4 h the azido cycloadduct **8** as the sole product. However, when 2 equiv of $NaN_3$ was applied at the same conditions, the mixture of **8** and **10** in 1:1.5 molar ratio was obtained in 4 h (reaction was interrupted at this point). For more discussion and for a complete set of conditions used for the oxadiazolone heterocycle cleavage, see the Supporting Information.

We believe that the attack of a nucleophile on the carbonyl carbon atom of the oxadiazolone heterocycle is the main reaction pathway under mild conditions, when reaction temperature does not exceed 50−55 °C. At more harsh conditions (100−110 °C) the thermolytic pathway of the heterocycle cleavage most likely operates along with the nucleophilic cleavage. At elevated temperatures and in the absence of the nucleophile the thermolytic pathway seems to become a dominating one. Plausible schemes for 1,2,4-oxadiazol-2-one conversion into an amidine under both nucleophilic and thermolytic conditions are shown in Schemes 5 and 6, respectively.

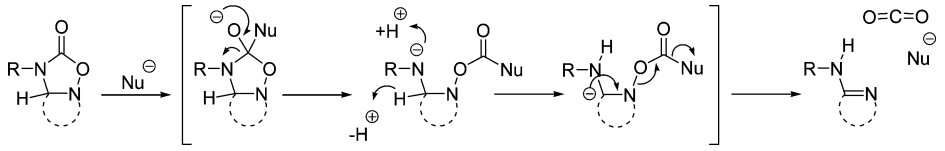
Thermal fragmentation of the oxadiazolone heterocycle is presumably triggered by heterolysis of the weak N−O bond (Scheme 6, Path A). In Path B, the transition state of the fragmentation would take advantage of the stabilization provided by the aromatic transition state. Both pathways seem to be thermodynamically highly favorable, because they produce stable $CO_2$ and a compound with a C═N double bond.

The nucleophilic cleavage of the oxadiazolone heterocycle under mild conditions, which we have discovered, would be quite applicable to substrates bearing substituents vulnerable to attack by strong basic nucleophiles like $CH_3ONa$ or NaOH. To examine this hypothesis, the ester cycloadduct **5c** was reacted with 1 equiv of $NaN_3$ in DMSO containing 10% of $H_2O$ at 55 °C. As a result, the ester amidine **11** was obtained in 74% yield (Scheme 7; see the Supporting Information for more details). The same reaction with KBr was interrupted after 75 h; the ester amidine **11** was isolated in 38% yield, and 27% of

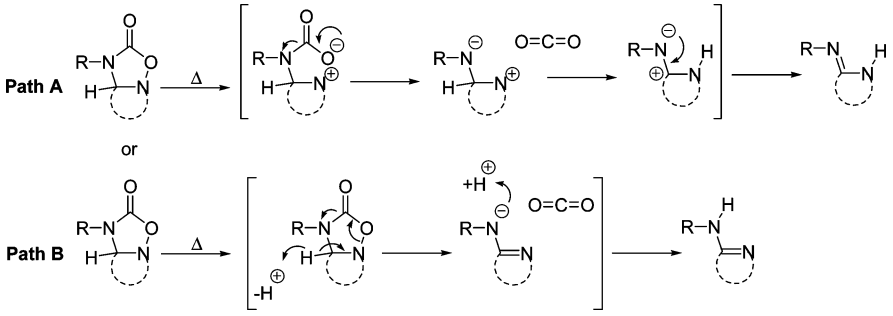
(18) (a) Black, D. St. C.; Crozier, R. F.; Davis, V. C. *Synthesis* **1975**, *4*, 205−221. (b) Seidl, H.; Huisgen, R.; Grashey, R. *Chem. Ber.* **1969**, *102*, 926−930.

(19) (a) Goerdeler, J.; Schimpf, R. *Chem. Ber.* **1973**, *106*, 1496−1500. (b) Wilkerson, C. J.; Green, F. D. *J. Org. Chem.* **1975**, *40*, 3112−3118. (c) Hisano, T.; Yoshikawa, S.; Muraoka, K. *Chem. Pharm. Bull.* **1974**, *22*, 1611−1616.

**SCHEME 5**



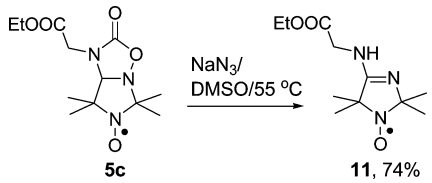
**SCHEME 6**




unreacted cycloadduct **5c** was also recovered. This observation explains, at least, why no detectable amount of azide amidine **10** was formed during the bromide substitution in **5a** with $NaN_3$ at 55 °C: the bromide anion released in the course of the reaction seems to provide, in this condition, very little assistance in the oxadiazolone heterocycle cleavage. To have the heterocycle cleaved, one more equivalent of more reactive $NaN_3$ must be added. As we found, the outcome of the thermolysis is not affected by the presence of water in DMF. It is likely that the nucleophilicity of water is too low to affect this reaction noticeably (see the Experimental Section and Table S2, Supporting Information, for further details).

The Staudinger procedure seems to be the most effective way to modify compounds containing an azido group thus far developed. Typically, this reaction proceeds under mild conditions, almost quantitatively, without noticeable formation of any side products.[20] The iminophosphorane formed is a rather reactive intermediate and can be rapidly hydrolyzed to the primary amine[21] or reacted with almost any kind of electrophilic reagent.[20,21b,c] Carbodiimide[22] and isothiocyanate[23,24] derivatives

**SCHEME 7**




**5c** **11**, 74%

are of general use for modification of proteins and chemically modified nucleic acids. In this work we used the Staudinger/intermolecular aza-Wittig reaction sequence to approach the carbodiimido and isothiocyanato derivatives starting from amidine nitroxide **10**; such derivatives would be valuable pH-sensitive spin labels for biophysical applications.

When the ether solution of azido derivative **10** was treated with P(*n*-Bu)$_3$ under argon, a vigorous nitrogen evolution was observed. The reaction was over within 40 min (as determined by TLC). $PPh_3$ and $PEt_3$ were also used in this reaction. For $PPh_3$, refluxing in THF was needed. Because of the high reactivity, the intermediate iminophosphorane **12** was not isolated as an individual compound and characterized. Further reaction of **12a**,**b** with carbon disulfide afforded a crystalline solid. While elemental analysis of this solid was consistent with isothiocyanato derivative **13** (Scheme 8), the IR spectrum did not show absorptions in the region $\nu$ 2040−2220 cm$^{-1}$, characteristic of the stretching vibrations of the heterocumulene −N=C=S group.

The crystal structure of the product was resolved by a single-crystal X-ray diffraction analysis (Figure 1) that allowed us to describe it as 2′,2′,5′,5′-tetramethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-thione **14**—the product of intramolecular cyclization of the isothiocyanato group on the *exo*-cyclic nitrogen atom of the amidino group (Scheme 8).

Reaction of the iminophosphorane **12b**,**c** with cyclohexyl isothiocyanate performed in a similar manner yielded a yellow crystalline solid with elemental analysis consistent with the structure of carbodiimido derivative **15** (Scheme 8). No absorptions near $\nu$ 2130 cm$^{-1}$ characteristic of the −N=C=N− group were observed in the IR spectrum of the compound, but rather intense bands characteristic of C=N stretching vibration were revealed at $\nu$ 1596 and 1661 cm$^{-1}$. Similar to the reaction with carbon disulfide, we assigned the resulting compound the structure of guanidine derivative cyclohexyl(2′,2′,5′,5′-tetram-

(20) (a) Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635−646. (b) Staudinger, H.; Hauser, E. *Helv. Chim. Acta* **1921**, *4*, 861−886. (c) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287* (5460), 2007−2010. (d) Köhn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106−3116.

(21) (a) Wallace, K. J.; Hanes, R.; Anslyn, E.; Morey, J.; Kilway, K. V.; Siegel, J. *Synthesis* **2005**, *12*, 2080−2083. (b) Gololobov, Y. G.; Zhmurova, I. N.; Kasukhin, L. F. *Tetrahedron* **1981**, 37, 437−472. (c) Gololobov, Y. G.; Kazukhin, L. F. *Tetrahedron* **1992**, *48* (8), 1353−1406.

(22) (a) Beechey, R. B.; Roberton, A. M.; Holloway, C. T.; Knight, I. G. *Biochemistry* **1967**, *6*, 3867−3879. (b) Musser, S. M.; Larsen, R. W.; Chan, S. I. *Biophys. J.* **1993**, *65*, 2348−2359. (c) Hassinen, I. E.; Vuokila, P. T. *Biochim. Biophys. Acta* **1993**, *1144* (2), 107−124. (d) Kumarev, V. P.; Knorre, D. G. *Dokl. Akad. Nauk S.S.S.R.* **1970**, *193* (1), 103−105. (e) Azzi, A.; Bragadin, M. A.; Tamburro, A. M.; Santato, M. *J. Biol. Chem.* **1973**, *248*, 5520−5526. (f) Girvin, M. E.; Fillingame, R. H. *Biochemistry* **1995**, *34*, 1635−1645.

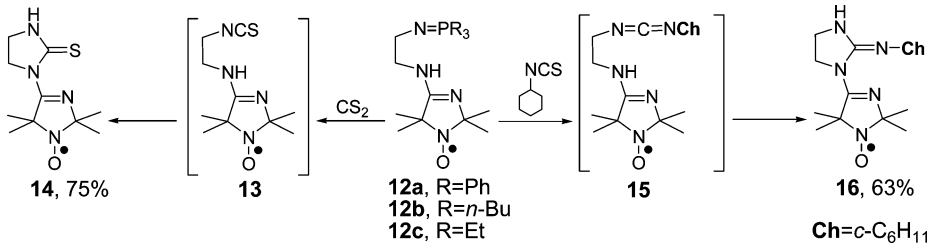
(23) (a) Tonomura, Y.; Watanabe, S.; Morales, M. *Biochemistry* **1969**, *8*, 2171−2176. (b) Coock, R.; Morales, M. *Biochemistry* **1969**, *8*, 3188−3194. (c) Schmidt, P. G.; Bertanowicz, M. S.; Rich, D. H. *Biochemistry* **1973**, *21*, 1830−1835. (d) Maeda, D. Y.; Berman, F.; Murray, T. F.; Aldrich, J. V. *J. Med. Chem.* **2000**, *43*, 5044−5049.

(24) Proudnikov, D.; Mirzabekov, A. *Nucleic Acids Res.* **1996**, *24* (22), 4535−4532.

(25) (a) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, *18*, 734−736. (b) Baldwin, J. E.; Cutting, J.; DuPont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. *J. Chem. Soc., Chem. Commun.* **1976**, *18*, 736−738.

(26) Voinov, M. A.; Martin, V. V.; Volodarskii, L. B. *Russ. Chem. Bull.* **1992**, *41* (11), 2091−2095.

**SCHEME 8**



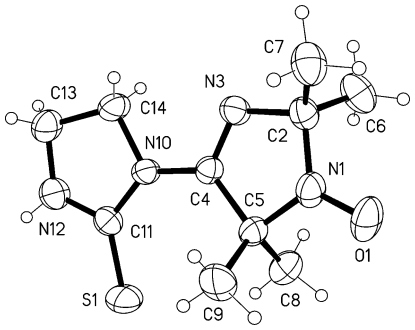




**FIGURE 1.** ORTEP presentation of **14**. Thermal ellipsoids are drawn at the 50% probability level. Selected bond distances (Å) and angles (deg): N(3)−C(4) 1.2789(19), C(4)−N(10) 1.3894(18), S(1)−C(11) 1.6606(16), N(10)−C(11) 1.3861(19), C(11)−N(12) 1.331(2), N(3)−C(4)−N(10)−C(14) 171.44(15), C(4)−N(10)−C(11)−S(1) −3.2(3).

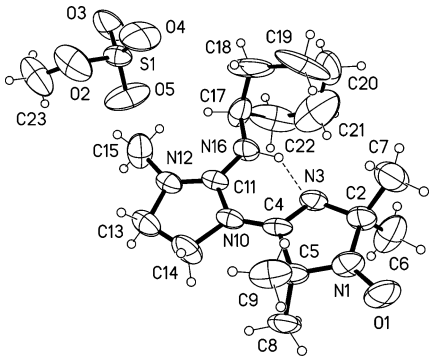
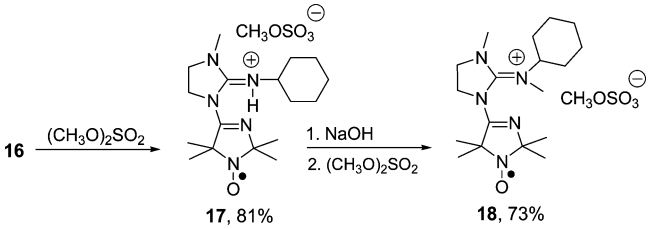



**FIGURE 2.** ORTEP presentation of **17**. Thermal ellipsoids are drawn at the 50% probability level. Selected bond distances (Å) and angles (deg): N(3)−C(4) 1.286(4), C(4)−N(10) 1.367(4), N(10)−C(11) 1.388-(4), C(11)−N(16) 1.303(4), N(16)−C(17) 1.475(4), N(3)−C(4)−N(10)−C(11) −5.6(11), C(4)−N(10)−C(11)−N(16) 1.8(10).

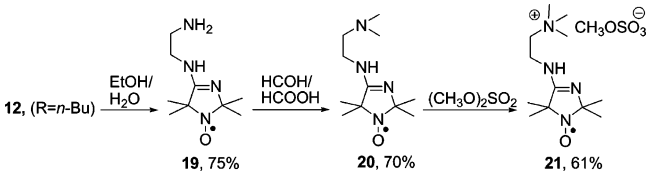
ethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-ylidene)amine **16**. The assignment was confirmed by X-ray diffraction analysis (Figure 2). For this purpose, the more readily crystallizable *N*-methyl guanidinium methyl sulfate **17** was prepared (Scheme 9). Further alkylation of **17** allowed us to obtain the guanidinium salt **18**. The regiochemical assignment of the position of a new methyl group in **18** was made by analogy to the literature data.[27,28]

To the author's best knowledge, the nitroxides bearing guanidine moiety have never been reported before. The literature data indicate that the guanidinium group is the most popular binding site motif in nature and in synthetic receptors for oxoanions[29] and peptides[30] due to its exceptional binding

**SCHEME 9**




**SCHEME 10**




capacity. From the prospects of molecular recognition chemistry, the guanidine **16** and corresponding guanidinium derivatives **17** and **18** could be of interest as nitroxide receptors for EPR protein and DNA/RNA studies.

The iminophosphorane **12b** was readily hydrolyzed by aqueous ethanol to give amino derivative **19** in 75% yield (Scheme 10). Reductive alkylation of **19** by an Eschweiler−Clarke reaction afforded dimethylamino derivative **20** in a good yield. A reduced reactivity of the amidino group in **20** toward alkylating reagents[16,26] enabled us to selectively alkylate the amino group with dimethyl sulfate and afforded a quaternary ammonium salt **21** in 61% yield (Scheme 10).

The X-band (9.5 GHz) EPR spectra of the amidine nitroxides synthesized were studied as a function of pH. All new nitroxides, with the exception of guanidinium salt **18**, were found to show a reversible effect of pH on EPR spectra. Irreversible character of the titration curve for **18** is likely to arise from hydrolysis of the guanidine group in a strong alkaline medium.[31] Figure 3 shows a series of EPR spectra of the amino derivative **19** in aqueous buffers with pH ranging from 1.52 to 6.37 units. The

(27) Xie, H.; Zhang, S.; Duan, H. *Tetrahedron Lett.* **2004**, *45*, 2013−2015.

(28) Gao,Y.; Arritt, S. W.; Twamley, B.; Shreeve, J. M. *Inorg. Chem.* **2005**, *44*, 1704−1712.

(29) (a) Mehrota, M. M.; Sternbach, D. D.; Rodriguez, M.; Charifson, P.; Berman, J. *Bioorg. Med. Chem. Lett.* **1996**, *6* (16), 1941−1946. (b) Schug, K. A.; Lindner, W. *Chem. Rev.* **2005**, *105*, 67−113. (c) Orner, P.; Hamilton, A. D. *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *41*, 141−147. (d) Houk, R. J. T.; Tobey, S. L.; Anslyn, E. V. *Top. Curr. Chem.* **2005**, *255*, 199−229. (e) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 841−864.

(30) (a) Salvatella, X.; Peczuh, M. W.; Gairi, M.; Jain, R. K.; Sanchez-Quesada, J.; Mendoza, J.; Hamilton, A. D.; Giralt, E. *Chem. Commun.* **2000**, *15*, 1399−1400. (b) Sanchez-Quesada, J.; Seel, C.; Prados, P.; Mendoza, J. *J. Am. Chem. Soc.* **1996**, *118*, 277−278. (c) Peczuh, M. W.; Hamilton, A. D.; Sanchez-Quesada, J.; Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328. (d) Haack, M. W.; Peczuh, X.; Salvatella, J.; Sanchez-Quesada, J.; Mendoza, J.; Hamilton, A. D.; Giralt, E. *J. Am. Chem. Soc.* **1999**, *121*, 11813−11820.

(31) (a) Bell, J. *J. Chem. Soc.* **1926**, 1213−1219. (b) Warner, R. C. *J. Biol. Chem.* **1942**, *142*, 705−723. (c) Eloranta, J. *Suomen Kem.* **1961**, *34*, 107−110. (d) Homer, R. B.; Alwis, K. W. *J. Chem. Soc., Perkin II* **1976**, *7*, 781−784.

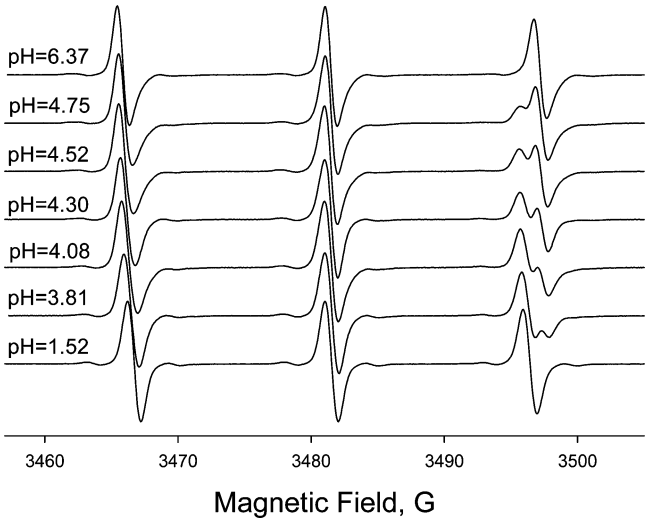
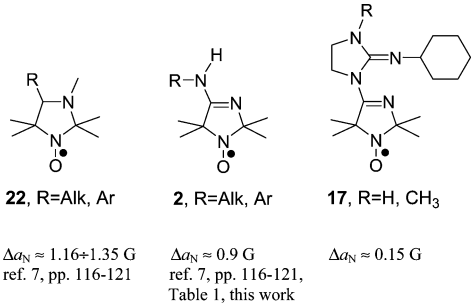



**FIGURE 3.** Representative room temperature 9.5 GHz (X-band) EPR spectra of the amino nitroxide **19** taken in 1.5 mM buffer solutions at various pH values (concentration of nitroxide ca. 0.5 mM).

**SCHEME 11**




two-component EPR spectra shown in Figure 3 are indicative of a slow, on the EPR time scale, $R^{\bullet} \leftrightarrows R^{\bullet}H^{+}$ chemical exchange[6b] and were observed for all nitroxides studied in this work. Nitroxides **9**−**11** and **19**−**21** showed spectra with a partially resolved high-field nitrogen hyperfine component similar to that shown in Figure 3.

For nitroxides **14**, **16**, and **17** even at pH close to p$K_a$ where one would expect the existence of both protonated and non-protonated nitroxide species, only broadening of the high-field nitrogen hyperfine component was observed. For **14** (p$K_a$ = 2.82), this broadening is apparently attributed to the rate of proton exchange being in an intermediate regime on the EPR time scale (cf. refs 6b and 7). Line broadening of the high-field component for nitroxides **16** and **17** is due to a small difference between hyperfine splitting of protonated ($a_N R^{\bullet}H^{+}$) and non-protonated ($a_N R^{\bullet}$) forms of the nitroxide. For these nitroxides the difference between $a_N R^{\bullet}H^{+}$ and $a_N R^{\bullet}$ was found to be only $\Delta a_N \approx 0.15$ G, which is about one-sixth of that for nitroxides **9**−**11** and **19**−**21** ($\Delta a_N \approx 0.9$ G, see Table 1 and refs 7 and 16). The most likely reason for such a small $\Delta a_N$ is that for protonated forms of nitroxides **16** and **17** the positive charge is delocalized over the guanidinium moiety of the molecule. This effect noticeably diminishes the effective charge at the N-3 atom of the imidazoline heterocycle. As a result, the nitrogen hyperfine coupling constant of the nitroxide is only slightly affected by the protonation. To illustrate this relationship it would be appropriate to mention here that $\Delta a_N$ gradually decreases in the order **22** > **2** > **17** (Scheme 11).[7] Moreover, for nitroxide **17** (p$K_a$ = 12.5), the rate of proton exchange does not satisfy the condition of slow exchange (3 < p$K_a$ < 11, ref 6b). Thus, the width of the high-field hyperfine component of **17** is most likely affected by both above-mentioned effects.

**TABLE 1. Magnetic Parameters ($a_N R^{\bullet}H^{+}$ and $a_N R^{\bullet}$), $\Delta a_N$, and p$K_a$ Values for Nitroxides 9−11, 14, 16, 17, and 19−21**

| | $a_N$, G | | | |
|---|---|---|---|---|
| no. | $R^{\bullet}H^{+}$ | $R^{\bullet}$ | $\Delta a_N$, G | p$K_a$ |
| **9** | 14.87 ± 0.03 | 15.73 ± 0.02 | 0.86 ± 0.036 | 5.06 ± 0.02 |
| **10** | 14.85 ± 0.01 | 15.74 ± 0.01 | 0.89 ± 0.014 | 5.47 ± 0.02 |
| **11** | 14.97 ± 0.01 | 15.87 ± 0.01 | 0.90 ± 0.014 | 4.76 ± 0.03 |
| **14** | 14.81 ± 0.05 | 15.53 ± 0.03 | 0.72 ± 0.058 | 2.82 ± 0.05 |
| **16** | 15.45 ± 0.01 | 15.60 ± 0.01 | 0.15 ± 0.014 | 10.2 ± 0.10 |
| **17** | 15.44 ± 0.06 | 15.61 ± 0.05 | 0.17 ± 0.078 | 12.5 ± 0.10 |
| **19** | 14.80 ± 0.01 | 15.61 ± 0.02 | 0.81 ± 0.022 | 4.11 ± 0.02 |
| **20** | 14.83 ± 0.01 | 15.70 ± 0.02 | 0.87 ± 0.022 | 3.73 ± 0.02 |
| **21** | 14.69 ± 0.01 | 15.63 ± 0.02 | 0.94 ± 0.022 | 3.80 ± 0.02 |

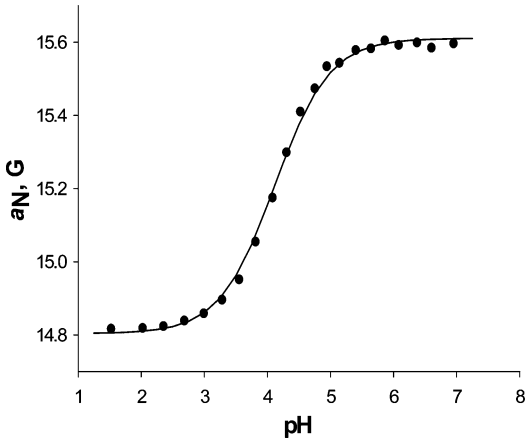



**FIGURE 4.** Experimentally measured splitting between low-field and central nitrogen hyperfine components of the nitroxide **19** as a function of pH. Spectra were taken at room temperature in 1.5 mM buffer solutions. The corresponding least-squares Henderson−Hasselbalch titration curve is shown as a solid line.

Though the EPR spectra of nitroxides described in this work do not satisfy fast exchange conditions at X-band (9.5 GHz), the experimentally observed splitting between the low-field and the central nitrogen hyperfine components still could be taken as an approximation of the weighted average of the isotropic nitrogen hyperfine coupling constants.[7,16] Figure 4 shows experimentally measured splitting between these two components of the first-derivative experimental EPR spectra, $a_N$, for the nitroxide **19**, calibrated as a function of pH. Magnetic parameters of the amidine nitroxides synthesized in this work ($a_N R^{\bullet}H^{+}$, $a_N R^{\bullet}$, and $\Delta a_N$) and corresponding observed p$K_a$ values are summarized in Table 1.

It is worthwhile to note here that guanidine derivatives **16** and **17** synthesized in this work show the highest p$K_a$ values (p$K_a$ = 10.2 and 12.5, respectively) ever reported for nitroxide probes of a "basic type" (cf. ref 7). Recently, lipophilic pH-sensitive nitroxide probes of an "acidic type" with p$K_a$ values in the range of 9−12 pH units have been described.[32]

## Conclusion

We have synthesized a series of new nitroxides using the approach based on the nucleophilic substitution of bromide in

(32) Reznikov, V. A.; Skuridin, N. G.; Khromovskikh, E. L.; Khramtsov, V. V. *Russ. Chem. Bull., Int. Ed.* **2003**, *52* (9), 2052−2056.

the *exo*-*N*-bromoalkyl chain of the cycloadduct, 1-(2-bromoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]-oxadiazol-2-one. In this approach an oxycarbonyl moiety of the oxadiazolone heterocycle plays the role of "protecting group" for the amidine functionality. The approach we report here allows one to overcome the undesired intramolecular alkylation of the amidino group with *N*-halogenoalkyl moiety that the researchers have encountered earlier. A nucleophilic cleavage of the oxadiazolone heterocycle under mild nonbasic conditions is elaborated and its applicability to substrates bearing substituents vulnerable to attack by strong basic nucleophiles (such as $CH_3ONa$ or NaOH) is demonstrated. Within this approach, new nitroxides bearing various functional groups (e.g., CN, $N_3$, $NH_2$, and COOEt) were synthesized. A series of nitroxides obtained through Staudinger/intermolecular aza-Wittig reaction of the azido derivative is also described. The nitroxides synthesized here were shown to have pH-dependent two-component EPR spectra indicative of a slow, on the EPR time scale, $R^{\bullet} \leftrightarrows R^{\bullet}H^{+}$ chemical exchange, and p$K_a$ values ranging from 2.8 to 12.5 units. The guanidine nitroxides synthesized in this work show the highest p$K_a$ values (p$K_a$ = 10.2 and 12.5, respectively) ever reported for nitroxide pH-probes of a "basic type". The synthetic approach to access nitroxides with pH-dependent EPR spectra described here would facilitate further progress in the application of the noninvasive EPR-based method for pH monitoring in biophysical and biomedical studies.

## Experimental Section

**Synthesis of 1-(2-R)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one 5 (General Procedure).** Commercially available isocyanate (15 mmol) was added to the solution of the aldonitrone **6** (10 mmol) in dry $CH_2Cl_2$ (10 mL). The reaction mixture was allowed to stay at room temperature for 1−5 days. The progress of reaction was monitored by chromatography on TLC plates ($SiO_2$, $CHCl_3$/MeOH, 100:3). After the reaction was completed the solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). **1-(2-Bromoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one 5a** was obtained as orange crystals (3.08 g, 9.98 mmol, 99%): mp 111−112 °C dec (EtOAc/hexane, 1:3); IR (KBr, $cm^{-1}$) 1744 (C═O). Anal. Calcd for $C_{10}H_{17}BrN_3O_3$: C, 39.10; H, 5.58; N, 13.67. Found: C, 38.99; H, 5.50; N, 13.40. **1-(2-Ethoxy-2-oxoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one 5c** was obtained as orange crystals (2.43 g, 8.50 mmol, 85%): mp 91−92 °C dec (EtOAc/hexane, 1:2); IR (KBr, $cm^{-1}$) 1771 (C═O), 1749 (COOEt). Anal. Calcd for $C_{12}H_{20}N_3O_5$: C, 50.35; H, 6.99; N, 14.69. Found: C, 50.61; H, 7.15; N, 14.61.

**1-(2-Cyanoethyl)-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo-[1,5-*b*][1,2,4]oxadiazol-2-one 7. Method A:** A solution of NaCN (0.2 g, 4.1 mmol) in $H_2O$ (3 mL) and $Bu_4NBr$ (0.05 g, 0.15 mmol) were added to the solution of **5a** (0.5 g, 1.63 mmol) in $CH_2Cl_2$ (15 mL). The resulting mixture was refluxed under vigorous stirring for 15 h. The progress of reaction was monitored by chromatography on TLC plates ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). After the reaction was completed, the organic layer was separated, and the water phase was extracted with $CH_2Cl_2$ (2 × 5 mL). Combined organic extracts were dried over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). The product obtained after evaporation of solvent solidified to give **7** as yellow crystals (0.35 g, 1.38 mmol, 85%): mp 161−162 °C dec (EtOAc/hexane, 3:2); IR (KBr, $cm^{-1}$) 2251 (CN), 1758 (C═O). Anal. Calcd for $C_{11}H_{17}N_4O_3$: C, 52.17; H, 6.77; N, 22.11. Found: C, 52.42; H, 6.60; N, 21.94.

**Method B:** NaCN (0.03 g, 0.65 mmol) was added to the solution of **5a** (0.2 g, 0.65 mmol) in DMSO (7 mL). The resulting mixture was heated for 1 h in an oil bath at temperatures not exceeding 55 °C. After the reaction was completed, the mixture was diluted with $H_2O$ and extracted with $CHCl_3$ (3 × 5 mL). Combined organic extracts were washed with brine (8 × 5 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). Yield: 0.14 g (0.56 mmol, 85%).

**1-(2-Azidoethyl)-6-oxyl-5,5,7,7-tetramethyl-tetrahydroimidazo-[1,5-*b*][1,2,4]oxadiazol-2-one 8. Method A:** A solution of $NaN_3$ (0.13 g, 2 mmol) in $H_2O$ (3 mL) and $Bu_4NBr$ (0.05 g, 0.15 mmol) were added to the solution of **5a** (0.31 g, 1 mmol) in $CH_2Cl_2$ (5 mL). The resulting mixture was refluxed under vigorous stirring for 4 h. The progress of the reaction was monitored by chromatography on TLC plates ($SiO_2$, $CH_2Cl_2$/EtOAc, 9:1). After the reaction was completed, the organic layer was separated, and the water phase was extracted with $CH_2Cl_2$ (2 × 5 mL). Organic extracts were combined and dried over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). The product solidified after evaporation of solvent (0.21 g, 0.78 mmol, 78%).

**Method B:** $NaN_3$ (0.21 g, 3.2 mmol) was added to the solution of **5a** (1.01 g, 3.3 mmol) in the mixture of DMSO or DMF (20 mL) and $H_2O$ (2 mL). The resulting mixture was heated in an oil bath at temperatures not exceeding 55 °C. The progress of the reaction was monitored by chromatography on TLC plates ($SiO_2$, hexane/THF, 10:1). After the reaction was over the mixture was diluted with $H_2O$ and extracted with $CHCl_3$ (5 × 5 mL). Combined organic extract was washed with brine (8 × 5 mL) and dried over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CH_2Cl_2$/EtOAc, 5:1). The product solidified after evaporation of solvent to give **8** as yellow crystals (DMSO: 0.67 g, 2.5 mmol, 76%; DMF: 0.71 g, 2.6 mmol, 79%): mp 93−95 °C dec (hexane/EtOAc, 5:1); IR (KBr, $cm^{-1}$) 2100 ($N_3$), 1757 (C═O). Anal. Calcd for $C_{10}H_{17}N_6O_3$: C, 44.61; H, 6.36; N, 31.20. Found: C, 44.63; H, 6.38; N, 30.67.

**Method C:** $NaN_3$ (0.86 g, 13.2 mmol) was added to the solution of **5a** (2.02 g, 6.6 mmol) in a mixture of DMSO (20 mL) and $H_2O$ (2 mL). The resulting mixture was heated in an oil bath at the temperature range of 50−56 °C. The progress of reaction was monitored by chromatography on TLC plates ($SiO_2$, hexane/THF, 10:1) and interrupted after 4 h. A workup procedure was performed similar to that described in Method A. Yield of **8**: 0.5 g, 1.86 mmol, 28%. Yield of **10**: 0.65 g, 2.89 mmol, 44%.

**Method D:** A solution of $NaN_3$ (0.21 g, 3.25 mmol) in $H_2O$ (1.5 mL) was added to a mixture of benzene (5 mL), **5a** (0.5 g, 1.63 mmol), and $Bu_4NBr$ (0.052 g, 0,16 mmol). The resulting mixture was refluxed for 4 h. A workup procedure was performed similar to that described in Method A. Yield of **8**: 0.17 g, 0.63 mmol, 39%. Yield of **10**: 0.04 g, 0.18 mmol, 11%.

**4-[(2-Cyanoethyl)amino]-1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazole 9.** NaCN (0.02 g, 0.4 mmol) was added to a solution of **7** (0.1 g, 0.4 mmol) in DMSO (5 mL). The resulting mixture was heated for 8 h in an oil bath at temperatures not exceeding 55 °C. The reaction mixture was diluted with $H_2O$ and extracted with $CHCl_3$ (3 × 5 mL). Combined organic extracts were washed with brine (8 × 5 mL), an organic layer was dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. The residue solidified after evaporation of solvent to give **9** as canary-yellow crystals (0.036 g, 0.17 mmol, 44%): mp 209−211 °C dec (hexane/EtOAc, 2:5); IR (KBr, $cm^{-1}$) 3352, 1547 (N−H), 2251 (CN), 1626 (C═N). Anal. Calcd for $C_{10}H_{17}N_4O$: C, 57.42; H, 8.13; N, 26.79. Found: C, 57.56; H, 8.21; N, 26.70.

**4-[(2-Azidoethyl)amino]-1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazole 10. Method A:** A solution of $NaN_3$ (0.42 g, 6.6 mmol) in $H_2O$ (2 mL) was added to a solution of **5a** (1.01 g, 3.3 mmol) in DMF or DMSO (20 mL). The resulting mixture was heated in an oil bath at 110 °C for 5 h. The progress of the reaction was monitored by chromatography on TLC plates ($SiO_2$, $CHCl_3$/

methanol, 15:1). After the reaction was over the mixture was diluted with $H_2O$ (20 mL) and extracted with $CHCl_3$ (5 × 5 mL). Combined organic extracts were washed with brine (4 × 5 mL) and dried over $MgSO_4$, and the residue obtained after removal of solvent under reduced pressure was separated by column chromatography ($SiO_2$, $CHCl_3$). The product solidified after evaporation of solvent to give **10** as a yellow crystals (in DMF: 0.53 g, 2.35 mmol, 72%; in DMSO: 0.52 g, 2.3 mmol, 70%): mp 86−88 °C dec (hexane/EtOAc, 5:1); IR (KBr, $cm^{-1}$) 3363, 1545 (N−H), 2105 ($N_3$), 1627 (C═N). Anal. Calcd for $C_9H_{17}N_6O$: C, 48.00; H, 7.64; N, 37.29. Found: C, 47.54; H, 7.69; N, 37.21.

**Method B:** Compound **8** (0.27 g,1 mmol) was dissolved in 3 mL of 1 N $CH_3ONa/CH_3OH$ and the solution was allowed to stay at room temperature for 3 h, solvent was removed under reduced pressure, then the residue was dissolved in 15 mL of $H_2O$ and extracted with $CHCl_3$. The organic solution was extracted with 3% HCl, and the organic layer was discarded; the aqueous solution was neutralized with $Na_2CO_3$ and extracted with $CHCl_3$. Further workup was performed similar to Method A. Yield: 0.2 g, 0.92 mmol, 92%.

**Method C:** A solution of **8** (0.37 mmol) in 5 mL of dry DMF or DMSO was heated at 110 °C. The reaction times were as follows: DMF, 5.5 h; DMSO, 12 h. The reaction mixture was diluted with brine (5 mL) and extracted with EtOAc (5 × 5 mL). Organic extract was thoroughly washed with brine (8 × 5 mL) and dried over $MgSO_4$, solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CHCl_3$). Yield in DMF: 71%. Yield in DMSO: 68%.

**Cleavage of the Reference Oxadiazolone 5b under Nucleophilic Conditions.** $NaN_3$ (0.12 g, 1.9 mmol) was added to a solution of **5b** (0.44 g, 1.9 mmol) in a mixture of DMSO (20 mL) and $H_2O$ (2 mL). The resulting mixture was heated at 55 °C for 65 h. Further workup was performed similar to that specified for **9**. Analytical data for **2**, R = Ph, are in a good agreement with those published in the literature. The IR spectrum is identical with that of the reference compound **2**, R = Ph, synthesized through the literature procedure.[17c] **2**, R = Ph, was obtained as a yellow crystalline compound in 95% yield.

**Thermolysis of 1-Phenyl-6-oxyl-5,5,7,7-tetramethyltetrahydroimidazo[1,5-*b*][1,2,4]oxadiazol-2-one 5b.** A solution of **5b** (0.37 mmol) in 5 mL of dry DMF or DMSO was heated at 110 °C. The reaction times were as follows: DMF, 64 h; DMSO, 89 h. The reaction mixture was diluted with brine (5 mL) and extracted with EtOAc (5 × 5 mL). Organic extract was thoroughly washed with brine (8 × 5 mL) and dried over $MgSO_4$, solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CHCl_3$) to give **2** (R = Ph). Yield in DMF: 60%. Yield in DMSO: 62%.

**(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazol-4-ylamino)acetic Acid Ethyl Ester 11. Method A:** $NaN_3$ (0.18 g, 2.8 mmol) was added to a solution of **5c** (0.8 g, 2.8 mmol) in DMSO (20 mL). The resulting mixture was heated in an oil bath at temperatures not exceeding 55 °C for 14 h. The course of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/methanol, 15:1). The mixture was diluted with $H_2O$ and extracted with $CHCl_3$ (3 × 5 mL). Combined organic extracts were washed with brine (7 × 5 mL), an organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was separated by column chromatography ($SiO_2$, $CHCl_3$). The residue, obtained after evaporation of solvent, solidified to give **11** as orange crystals (0.5 g, 2.1 mmol, 74%): mp 93−95 °C dec (hexane/EtOAc, 3:1); IR (KBr, $cm^{-1}$) 3331, 1565 (N−H), 1753 (COOEt), 1635 (C═N). Anal. Calcd for $C_{11}H_{20}N_3O_3$: C, 54.56; H, 8.26; N, 17.36. Found: C, 54.68; H, 8.28; N, 17.15.

**Method B:** A solution of $NaN_3$ (0.011 g, 0.175 mmol) was added to a solution of **5c** (0.05 g, 0.175 mmol) in 2 mL of dry DMF. Water (0.075 mL) was added to facilitate the solubility of $NaN_3$ in DMF. The resulting mixture was heated in an oil bath at 110 °C for 2 h. The course of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/EtOAc, 2:1). Further workup was performed similar to Method A. Yield: 0.036 g, 0.149 mmol, 86%.

**Method C:** A solution of **5c** (0.05 g, 0.175 mmol) in 2 mL of dry DMSO was heated in an oil bath at 110 °C for 17 h. The course of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/EtOAc, 2:1). Further workup was performed similar to Method A. Yield: 0.032 g, 0.132 mmol, 75%.

**Method D:** A solution of **5c** (0.05 g, 0.175 mmol) in 2 mL of DMF (freshly distilled over $P_2O_5$) was heated in an oil bath at 110 °C for 31 h. The course of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/EtOAc, 2:1). Further workup was performed similar to Method A. Yield: 0.012 g, 0.049 mmol, 28%.

**Method E:** A solution of **5c** (0.05 g, 0.175 mmol) in 2 mL of DMF containing 5% v/v $H_2O$ was heated in an oil bath at 110 °C for 31 h. The course of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/EtOAc, 2:1). Further workup was performed similar to Method A. Yield: 0.009 g, 0.037 mmol, 22%.

**Method F:** A solution of KBr (0.021 g, 0.175 mmol) was added to a solution of **5c** (0.05 g, 0.175 mmol) in 2 mL of dry DMF. Water (0.075 mL) was added to facilitate the solubility of KBr in DMF. The resulting mixture was heated in an oil bath at 110 °C for 2 h. The progress of the reaction was monitored by TLC ($SiO_2$, $CHCl_3$/EtOAc, 2:1). Further workup was performed similar to Method A. Yield: 0.033 g, 0.137 mmol, 80%.

**2′,2′,5′,5′-Tetramethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-thione 14. Method A:** A solution of azide **10** (0.35 g, 1.56 mmol) in dry THF (10 mL) was placed into a flask that was flushed with argon, and P(*n*-Bu)$_3$ (0.4 mL, 1.59 mmol) was added under argon upon stirring. After the vigorous $N_2$ evolution had ceased, the reaction mixture was stirred for an additional 30 min at room temperature and then heated to 50 °C for 20 min. The reaction mixture was allowed to cool to the ambient temperature and $CS_2$ (3 mL, 50.75 mmol) was added dropwise. The resulting mixture was allowed to stay under stirring for 12 h, and the residue, obtained after evaporation of solvent, was separated by chromatography ($SiO_2$, $CHCl_3$).

**Method B:** $PPh_3$ (0.44 g, 1.7 mmol) was added under argon to the stirred solution of **10** (0.25 g, 1.1 mmol) in dry THF (3 mL). The reaction mixture was refluxed under stirring for 90 min, and $CS_2$ (3 mL, 50.75 mmol) was added dropwise. The resulting mixture was refluxed for 2 h, the residue, obtained after evaporation of solvent, was separated by chromatography (Kieselgel, $CH_2Cl_2/CH_3CN$, 3:2) to give **14** as canary-colored crystals (0.28 g, 1.16 mmol, 75%, method A; 0.08 g, 0.32 mmol, 35%, method B): mp 140−141 °C dec (hexane/EtOAc, 1:2); IR (KBr, $cm^{-1}$) 3295, 1511 (NH), 1580 (C═N). Anal. Calcd for $C_{10}H_{17}N_4OS$: C, 49.79; H, 7.10; N, 23.21. Found: C, 49.84; H, 7.04; N, 23.01.

**Cyclohexyl(2′,2′,5′,5′-tetramethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-ylidene)amine 16. Method A:** P(*n*-Bu)$_3$ (0.4 mL, 1.59 mmol) was added under argon to the stirred solution of **10** (0.35 g, 1.56 mmol) in dry THF (10 mL). After the vigorous $N_2$ evolution had ceased, the reaction mixture was stirred for an additional 30 min at room temperature and for 20 min at 50 °C. The reaction mixture was allowed to cool to ambient temperature and cyclohexyl isothiocyanate (0.3 mL, 2.03 mmol) was added dropwise. The resulting mixture was allowed to stay under stirring at ambient temperature for 12 h. The residue, obtained after evaporation of solvent, was separated by column chromatography ($Al_2O_3$, $CH_3CN$).

**Method B:** A 1 M solution of $PEt_3$ (1.36 mL, 1.36 mmol) was added under argon to the stirred solution of **10** (0.33 g, 1.33 mmol) in dry THF (5 mL). After the vigorous $N_2$ evolution had ceased, the reaction mixture was stirred for an additional 40 min at room temperature and for 20 min at 40 °C. The reaction mixture was allowed to cool to ambient temperature and cyclohexyl isothiocyanate (0.24 g, 1.73 mmol) was added to the resulting mixture dropwise. The reaction mixture was allowed to stay under stirring at ambient temperature for 12 h. The residue, obtained after evaporation of solvent, was separated by column chromatography

($Al_2O_3$, $CH_3CN$) to give **16** as a pale yellow crystals (0.3 g, 0.98 mmol, 63%, method A; 0.23 g, 0.75 mmol, 52%, method B): mp 140−142 °C dec (hexane/EtOAc, 4:1); IR (KBr, $cm^{-1}$) 3256, 1535 (NH), 1596 (C═N amidine), 1661 (C═N guanidine). Anal. Calcd for $C_{16}H_{28}N_5O \cdot 0.25H_2O$: C, 62.03; H, 9.21; N, 22.62. Found: C, 61.58; H, 9.20; N, 22.49.

**Cyclohexyl(3,2′,2′,5′,5′-pentamethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-ylidene)ammonium Methyl Sulfate 17.** $(CH_3)_2SO_4$ (0.05 mL, 0.53 mmol) was added to a solution of **16** (0.11 g, 0.35 mmol) in dry ether (5 mL), and the mixture was allowed to stay at ambient temperature for 8 h. The yellow crystals of **17** formed (0.12 g, 0.28 mmol, 81%) were collected on a filter: mp 165−168 °C dec (EtOAc); IR (KBr, $cm^{-1}$) 1604 (C═N amidine), 1679 (C═N guanidine), 3550, 3486 ($NH^+$). Anal. Calcd for $C_{18}H_{34}N_5O_5S \cdot H_2O$: C, 48.00; H, 8.00; N, 15.56. Found: C, 48.43; H, 7.93; N, 15.61.

**Cyclohexyl(3,2′,2′,5′,5′-pentamethyl-1′-oxyl-4,5,2′,5′-tetrahydro-3*H*,1′*H*-[1,4′]biimidazolyl-2-ylidene)methylammonium Methyl Sulfate 18.** A solution of **17** (0.16 g, 0.37 mmol) in water (10 mL) was basified (NaOH) to pH 12 and extracted with ether (3 × 5 mL), then the organic extract was dried over $Na_2SO_4$. The drying agent was filtered off and $(CH_3)_2SO_4$ (0.05 mL, 0.56 mmol) was added to the ether solution. The reaction mixture was allowed to stay at ambient temperature for 8 h. The ether layer was discarded, and the oily residue formed on the bottom of the flask was treated with dry hexane to give **18** as yellow crystals (0.12 g, 0.27 mmol, 73%): mp 157−159 °C dec (hexan/EtOAc, 1:3); IR (KBr, $cm^{-1}$) 1602 (C═N amidine), 1689 (C═N guanidine). Anal. Calcd for $C_{19}H_{36}N_5O_5S \cdot H_2O$: C, 49.13; H, 8.19; N, 15.09. Found: C, 49.00; H, 7.87; N, 15.67.

**4-(2-Aminoethylamino)-1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazole 19.** $PBu_3$ (0.96 mL, 3.5 mmol) was added under argon to a stirred solution of **10** (0.5 g, 2.25 mmol) in dry THF (10 mL). After the vigorous $N_2$ evolution had ceased, the reaction mixture was stirred for an additional 30 min. The solvent was removed under reduced pressure, 20 mL of the aqueous $C_2H_5OH$ solution (1:1) was added to the residue, and the reaction mixture was allowed to stay under stirring at ambient temperature for 6 h. $C_2H_5OH$ was distilled off under reduced pressure, the water layer was acidified with 3% HCl to pH 4 and extracted with $CHCl_3$ (5 × 5 mL), and the organic layer was discarded. The water layer was neutralized ($NaHCO_3$) and extracted with $CHCl_3$ (5 × 5 mL); the organic layer was discarded again. The water layer was basified (NaOH) to pH 12 and extracted with $CHCl_3$ (7 × 5 mL), then the organic layer was dried over $K_2CO_3$. Solvent was removed under reduced pressure to give **19** as yellow crystals (0.33 g, 1.67 mmol, 75%): mp 66−68 °C dec (hexane/EtOAc, 1:1); IR (KBr, $cm^{-1}$) 3365 ($NH_2$), 1626 (C═N), 1549 (NH). Anal. Calcd for $C_9H_{19}N_4O \cdot 0.75H_2O$: C, 50.82; H, 9.65; N, 26.35. Found: C, 51.18; H, 9.39; N, 26.09.

**4-[(2-Dimethylaminoethyl)amino]-1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazole 20.** Formic acid (90%, 12.5 mmol) was added portionwise to an ice-cooled solution of amine **19** (0.5 g, 2.5 mmol) in a 35% solution of formaldehyde (5.5 mmol). The resulting mixture was heated in an oil bath at 60 °C for 30 min, diluted with water, basified (NaOH) to pH 12, and extracted with ether (7 × 5 mL). An organic extract was dried over $K_2CO_3$, the solvent was removed under reduced pressure, and **20** was obtained as yellow crystals (0.4 g, 1.8 mmol, 70%): mp 89−90 °C dec (hexane/EtOAc, 1:1); IR (KBr, $cm^{-1}$) 3325 ($N(CH_3)_2$), 1627 (C═N), 1548 (NH). Anal. Calcd for $C_{11}H_{23}N_4O \cdot {}^1/_6H_2O$: C, 57.39; H, 10.14; N, 24.35. Found: C, 57.37; H, 9.94; N, 24.10. Mass spectrum (M+): calcd for $C_{11}H_{23}N_4O$ 227.18718, found 227.18862.

**[2-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-imidazol-4-ylamino)ethyl]trimethylammonium Methyl Sulfate 21.** $(CH_3O)_2SO_2$ (0.034 mL, 0.33 mmol) was added to a solution of **20** (0.075 g, 0.33 mmol) in dry ether (5 mL) and the resulting mixture was allowed to stay at ambient temperature for a 6 h. The residue, obtained after evaporation of solvent, solidified after triturating with an ether/$CH_3CN$ mixture (20:1) to give **21** as pale yellow crystals (0.071 g, 0.2 mmol, 61%): mp 115−117 °C dec (ether/$CH_3CN$, 2:1); IR (KBr, $cm^{-1}$) 3353 (NH), 1628 (C═N), 1543 (NH). Anal. Calcd for $C_{13}H_{29}N_4O_5S \cdot 0.25H_2O$: C, 43.63; H, 8.25; N, 15.66. Found: C, 43.53; H, 8.24; N, 15.39.

**X-Band EPR Titration of the Nitroxides.** Nitroxides were titrated in a buffer solution (10 mM citric acid, 10 mM sodium phosphate, and 10 mM sodium borate). The pH value of nitroxide solutions was adjusted with HCl or NaOH solutions. The pH measurements were performed with a digital pH meter with accuracy ±0.05 pH unit. Hyperfine coupling constants, $a_N$, were measured as a distance between low-field and central nitrogen hyperfine components of the EPR spectra.

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**Supporting Information Available:** Experimental conditions for the nucleophilic substitution of bromide in **5a** and details of the thermolytic and nucleophilic cleavage of the oxadiazolone heterocycle; crystallographic data in CIF format, details of X-ray data collection and structure determination, and crystal data and experimental parameters for the crystal structure analyses of **14** and **17**; discussion of the results of crystallographic analyses; general experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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