Synthesis, Physical Properties, and Cytotoxicity of Nitroxyl – Aziridine Hybrid

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Nitroxyl – aziridine hybrid, a candidate for a magnetic resonance imaging (MRI) probe and an anticancer drug, was synthesized by aziridine formation reaction of nitroxyl-introduced aldehyde and guanidinium ylide in good diastereoselectivity. The relative configuration at aziridine C(2) – C(3) bond of the major diastereoisomer was determined to be *cis* by X-ray crystallographic analysis. Application of chiral guanidinium ylide resulted in the formation of the corresponding optically active aziridine in 84% ee. Reversible one-electron redox potential and quantitative spin yield of the hybrid were observed in cyclic voltammogram and electron spin resonance, respectively. However, cytotoxicity of the hybrid against cancer cell lines used was not observed.

Introduction. – Nitroxyl compounds such as TEMPO (=2,2,6,6-tetramethylpiperidin-1-yloxyl; **1**) and PROXYL (=2,2,5,5-tetramethylpyrrolidin-1-yloxyl; **2**) (*Fig. 1,a*) are well-known stable radical species and widely used for synthetic and medicinal purpose, *e.g.*, as spin labels [1], spin traps [2], oxidizing catalysts/reagents [3], antioxidants [4], and magnetic resonance imaging (MRI) contrast agents [5]. Because selective accumulation of **1** in hamster and mice melanotic melanomas [6] was observed, design, synthesis, and cytotoxicities were examined for the hybrids of nitroxyl and antimelanomic drugs such as nitrosourea, so-called spin-labeled *N*-ethylnitrosoureas (SLENU) [7][1]). Recent successful application of SLENU as noninvasive real-time MRI probes based on high permeability of nitroxyl compounds for blood – brain barrier [9] implies the importance of the series of these compounds. On the other hand, aziridines play an important role in cytotoxicity of anticancer drugs such as thiotepa [10] and mitomycins [11]. Thus, a nitroxyl – aziridine hybrid is a very interesting candidate for not only a spin-labeled compound but also an anti-cancer drug. We have developed a new synthetic method for 3-aryl (or 3-alkenyl)-aziridine-2-carboxylates **3** from aromatic (or olefinic) aldehydes and guanidinium salt **4** in the presence of a base

[1]) For an example of synthesis of spin-labeled analogs of antitumor podophyllotoxin glycoside, see [8].

such as NaH and *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG) [12]. Optically active **3** can be effectively obtained, when chiral guanidinium salt **5** instead of achiral **4** is used (*Fig. 1*,*b*). We herein report synthesis, physical properties, and cytotoxicity of nitroxyl–aziridine hybrid.

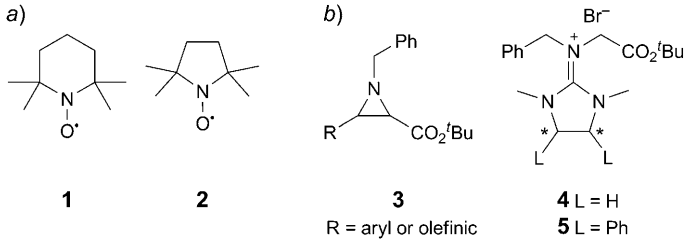
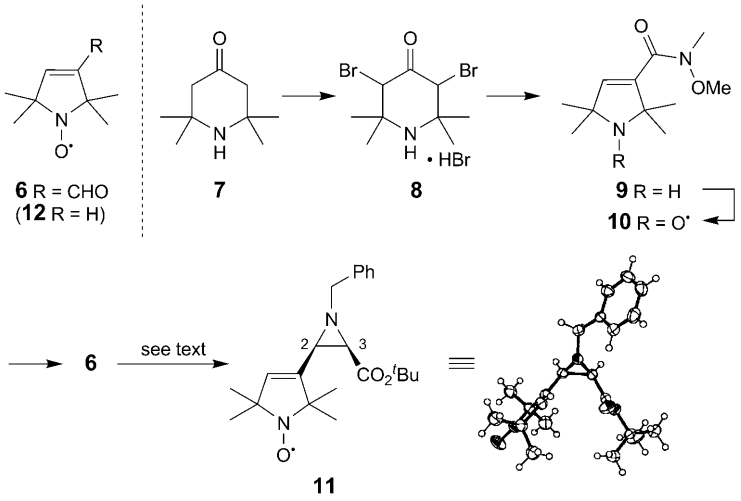


Fig. 1. *Structures of* a) *stable nitroxyls* **1** *and* **2**, b) *aziridine* **3** *and guanidinium salts* **4** *and* **5**

Results and Discussion. – 3-Formyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxyl (**6**) was selected as a nitroxyl-transferring olefinic aldehyde, and it was synthesized according to the procedure reported in [13] (*Scheme*): treatment of commercially available 2,2,6,6-tetramethylpiperidin-4-one (**7**) with Br_2 in AcOH gave dibromo derivative **8** as a mixture of *cis*- and *trans*-diastereoisomers. *Favorskii*-type reaction of **8** in the presence of *N*,*O*-dimethylhydroxylamine gave the *Weinreb* amide **9**. After oxidation of the secondary amine part of **9** with H_2O_2 in the presence of tungsten (W) catalyst to nitroxyl **10**, treatment of which with diisobutylaluminum hydride (DIBAL) afforded the key olefinic aldehyde **6**. Reaction of **6** and guanidinium salt **4** in the presence of a base gave the desired aziridine (±)-**11**. Yield and diastereoselectivity

Scheme. *Synthesis of Nitroxyl-Aziridine Hybrid* **11**



of (±)-**11** were dependent on the base and the solvent used: NaH in DMF gave 80% yield and an 8:1 ratio of the diastereoisomers; TMG in THF led to 33% yield and a 3.5:1 ratio. A more polar diastereoisomer was obtained as major isomer in each case. The relative configuration at the C(2)–C(3) bond of the major aziridine was determined to be *cis* by X-ray crystallographic analysis[2]), as expected from the results of other olefinic aldehydes such as cyclohexenecarbaldehyde [12c] (*Scheme*). Application of chiral guanidinium salt (*S*,*S*)-**5** gave the corresponding optically active (−)-*cis*-**11** in 84% ee with chemical yield and diastereoselectivity similar to those with achiral **4** when NaH was used as a base. The absolute configuration of (−)-*cis*-**11** was tentatively determined as (2*R*,3*R*) based on former results with benzaldehyde [12a].

Cyclic voltammograms (CV) of the nitroxyl–aziridine hybrid (±)-*cis*-**11** showed one-electron redox process for the nitroxyl/*N*-oxoammonium couple similar to those of other nitroxyls **6** and **10** (*Fig. 2*,*a*). The peak separation ($\Delta E_p = 0.065 - 0.070$ V) estimated from the oxidation (E_{pa}) and the reduction (E_{pc}) peak currents confirmed the reversible one-electron redox process of these nitroxyls[3])[4]). The durability of the nitroxyl (±)-*cis*-**11** against electrochemical redox cycling was also tested. The resulting eight-time repetitive voltammograms displayed reproducible patterns (*Fig. 2*,*b*), indicating a good durable nature of the redox couple. Electron spin resonance (ESR) spectrum for the nitroxyl (±)-*cis*-**11** in benzene solution at room temperature exhibited triplet resonance in $|\Delta m_s| = 1$ region (*Fig. 3*). The *g* value of 2.0059 and nitrogen hyperfine splitting ($a_N = 1.4$ mT) of (±)-*cis*-**11** are typical of nitroxyl

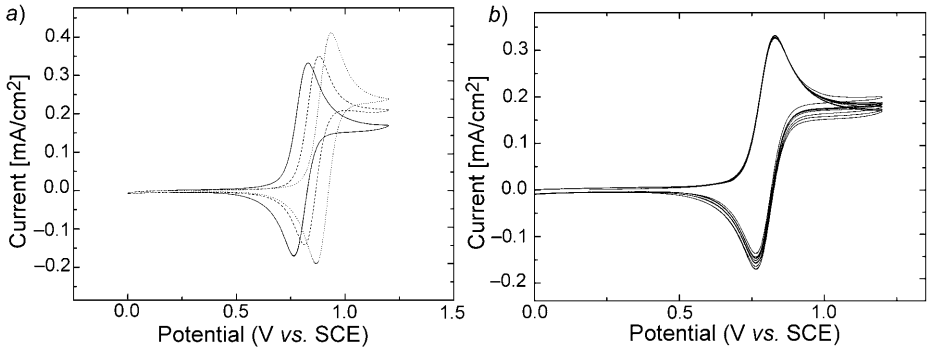


Fig. 2. a) *CV of* **6** (dot), **10** (dash), *and* (±)-*cis*-**11** (line) *measured in MeCN at a Pt disk electrode*; b) *redox cycling of* (±)-*cis*-**11**

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[2]) CCDC-720905 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

[3]) E_{pa} and E_{pc} values (V *vs.* SCE) were estimated as 0.940 and 0.870 for **6**, 0.879 and 0.814 for **10**, and 0.830 and 0.763 for (±)-*cis*-**11**, respectively. Calculated $E_{1/2}$ ($=(E_{pa}+E_{pc})/2$) and ΔE_p ($=E_{pa}-E_{pc}$) values were 0.905 and 0.070 for **6**, 0.847 and 0.065 for **10**, and 0.797 and 0.067 for (±)-*cis*-**11**, respectively.

[4]) For recent example of experimental and theoretical studies of the redox potentials of cyclic nitroxyls, see [14].

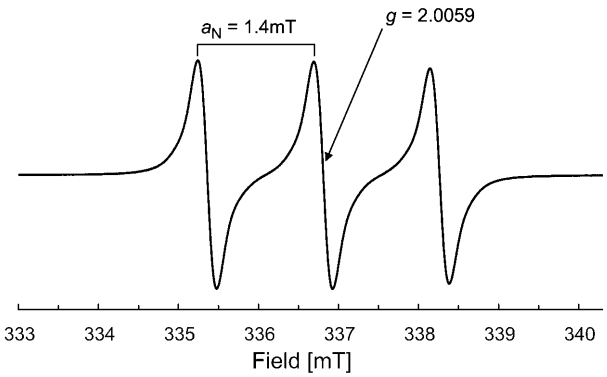

Fig. 3. *ESR* (X-Band, 9.4560 GHz) *Spectrum of (±)*-cis-**11** *in benzene* (0.1 mM) *at room temperature*

radical[5]). The spin yield for (±)-*cis*-**11** was estimated to be 98.2% by integration of the ESR signal. The quantitative spin yield means the radical is robust even at room temperature and in air.

Cytotoxicities of the nitroxyl-aziridine hybrid (±)-*cis*-**11**, as well as of aldehyde **6** and amide **10** were examined; however, none of these compounds exhibited the growth-inhibitory effect against cancer cell lines used (MCF-7, HeLa, and Caki-1).

Conclusions. – Nitroxyl–aziridine hybrid **11** was synthesized *via* aziridine formation reaction of nitroxyl-introduced olefinic aldehyde **6** and guanidinium salts **4** and **5**. The relative configuration of the major diastereoisomer of aziridine **11** was determined to be *cis*. Existence of stable radical in this hybrid was confirmed with CV and ESR; however, no inhibitory effect for cancer cell proliferation was observed. Trials for application of the hybrid as MRI probe and as chiral catalyst of enantioselective oxidation reaction are now in progress[6]).

Experimental Part

General. Anh. DMF and THF were used as purchased from *Kanto Chemical* and *Wako Chemical*, resp. Column chromatography (CC): *Kanto Chemical* silica gel *60 spherical*. TLC: *Merck Art 5715 DC-Fertigplatten Kieselgel 60 F_{254}*. M.p.: *Yanagimoto MPSI* melting point apparatus; uncorrected. IR Spectra: *Attenuated Total Reflectance* (ATR) system on a *JASCO FT/IR-300E* spectrophotometer, $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR (400 MHz) Spectra: in $CDCl_3$ on a *JEOL JNM-ECP-400*; chemical shifts in δ [ppm] rel. to Me_4Si as internal reference; coupling constants *J* in Hz. MS: *JEOL JNM MS-GCMATE* for EI-MS, and *JEOL JNM HX-110* for HR-FAB-MS, resp.

CV of nitroxyls **6**, **10**, and (±)-*cis*-**11** (2 mM) was carried out in MeCN (*Kanto Chemical*, spectroscopic grade) soln. containing 0.1M tetrabutylammonium perchlorate (*Tokyo Kasei*, >98%) under N_2 using a *BAS model ALS 750A* electrochemical analyzer. A *BAS* working Pt disk electrode (area 0.021 cm^2) and a *BAS* counter Pt plate were immersed in the main compartment of a two-compartment

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[5]) The *g* value of 2.00594 and nitrogen hyperfine splitting ($a_N = 1.45$ mT) of **12** (*Scheme 1*) was reported in [15].

[6]) For examples of the application of chiral nitroxyl derivatives to asymmetric oxidation of racemic alcohols and amines, see [16].

cell. In the auxiliary compartment, which is separated from the main one by a sintered glass frit, was immersed a KCl agar bridge connected to a *TOA* reference saturated calomel electrode (SCE). CW X-Band ESR spectrum for (±)-*cis*-**11** in benzene in 5-mm o.d. ESR quartz tubes was acquired on a *JEOL JES-TE200* spectrometer with 100-kHz field modulation. Spectra were obtained using single mode cavity, with the oscillating magnetic field perpendicular (TE102) to the swept magnetic field. The *g* value was referenced using MnO dispersed in MnO_2. Spin concentration of each sample was determined both by careful integration of the ESR signal standardized with that of 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-yloxyl (TEMPOL).

*(3-{1-Benzyl-3-[(*tert*-butoxy)carbonyl]aziridin-2-yl}-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yl)oxidanyl* (**11**). Under Ar, DMF (1.5 ml) was added to a mixture of NaH (60%, 75 mg, 1.87 mmol), **6** (150 mg, 0.89 mmol), and **4** (461 mg, 1.16 mmol) at −20°, and the whole mixture was stirred at the same temp. for 5 min. MeCN (40 ml) and SiO_2 (12 g) were added, and the whole mixture was stirred at r.t. for 12 h, then filtered off. The precipitate was washed with MeCN. The filtrate and the washings were combined and evaporated *in vacuo*. The residue was purified by CC (SiO_2; hexane/AcOEt 10:1) to give (±)-*cis*-**11** (235 mg, 71%) and (±)-*trans*-**11** (39 mg, 9%).

Data of (±)-cis-**11**. Yellow needles (recryst. from hexane/CH_2Cl_2). M.p. 110–111°. R_f (hexane/AcOEt 2:1) 0.49. IR: 1729. ^{1}H-NMR 1.40 (br. *s*, 9 H); 2.27 (br. *s*, 1 H); 3.74 (br. *s*, 1 H); 4.00 (br. *s*, 1 H); 7.22–7.41 (*m*, 5 H); signals derived from H-atoms on the dihydro-tetramethylpyrrole part and of C(2) of the aziridine part were not detected due to paramagnetism of *cis*- and *trans*-**11**. EI-MS: 371 (15, M^+), 341 (19), 285 (31), 270 (62), 242 (76), 91 (100). Anal. calc. for $C_{22}H_{31}N_2O_3$: C 71.13, H 8.41, N 7.54; found: C 71.11, H 8.62, N 7.55.

Data of (±)-trans-**11**. Colorless oil. R_f (hexane/AcOEt 2:1) 0.60. IR: 1735. ^{1}H-NMR 1.41 (br. *s*, 9 H); 2.05 (br. *s*, 1 H); 4.18 (br. *s*, 2 H); 6.90–7.60 (*m*, 5 H). HR-FAB-MS 371.2348 ($[M+H]^+$, $C_{22}H_{31}N_2O_3^+$; calc. 371.2335).

*(3-{(2*R*,3*R*)-1-Benzyl-3-[(*tert*-butoxy)carbonyl]aziridin-2-yl}-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yl)oxidanyl* ((−)-*cis*-**11**). Under Ar, DMF (0.3 ml) was added to a mixture of NaH (60%, 7 mg, 0.18 mmol), **6** (22 mg, 0.13 mmol), and (*S*,*S*)-**5** (81 mg, 0.15 mmol) at −20°, and the whole mixture was stirred at the same temp. for 1 h and at r.t. for 15 min. MeCN (4 ml) and SiO_2 (0.4 g) were added, and the whole mixture was stirred at r.t. for 24 h, then filtered off. The precipitate was washed with MeCN. The filtrate and the washings were combined and evaporated *in vacuo*. The residue was purified by CC (SiO_2; hexane/AcOEt 3:1) to give (−)-*cis*-**11** (38 mg, 78%) and *trans*-**11** (3 mg, 7%).

Data of (−)-*cis*-**11**. Yellow oil. $[\alpha]_D^{23} = -35.9$ ($c = 0.7$, $CHCl_3$). Chiral HPLC: (*DAICEL CHIRALCEL OD-H*; hexane/^{i}PrOH 100:1; flow rate, 1.0 ml/min; detection, 254 nm): 12.3 (92%) and 15.1 (8%) min.

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