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| **Preprint Title** | Unexpected one-pot formation of 1*H*-6a,8a-epiminotricyclopenta[a,c,e][8]annulene system from cyclopentanone, ammonia and dimethyl fumarate. Synthesis of highly strained polycyclic nitroxide and EPR study |
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**Unexpected one-pot formation of 1*H*-6a,8a-epiminotricyclopenta[a,c,e][8]annulene system from cyclopentanone, ammonia and dimethyl fumarate. Synthesis of highly strained polycyclic nitroxide and EPR study.**

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**Abstract.** The unexpected formation of a highly strained polycyclic amine was found in a one-pot synthesis from cyclopentanone, dimethylfumarate and ammonium acetate. This multistep reaction includes 1,3-dipolar cycloaddition of dimethyl fumarate to the cyclic azomethine ylide formed *in situ* from cyclopentanone and ammonia. The polycyclic amine product was easily converted into a sterically-shielded polycyclic nitroxide. The EPR spectra and spin relaxation behavior of the nitroxide was studied in solution. The spin relaxation seems well suited for use as a biological spin label and are comparable with those of cyclic nitroxides with two spirocyclic moieties adjacent to N-O• group.

## Introduction

Domino reaction have attracted much attention as an approach for synthesis of complex molecules in a few steps[1]. The utility of multicomponent reactions involving amines, actvated olefines and carbonyl compounds for synthesis of heterocyclic compounds have been repeatedly demonstrated.[2,3] We recently used the domino reaction of aminoacid, ketone and dimethyl fumarate for one-pot synthesis of a substituted pyrrolidine, which then was converted into reduction-resistant pyrrolidine nitroxide.[4] Here we report the unexpected formation of a highly-strained polycyclic amine from cyclopentanone, dimethylfumarate and ammonium acetate. This multistep reaction obviously includes 1,3-dipolar cycloaddition of dimethyl fumarate with cyclic azomethine ylide formed *in situ* from cyclopentanone and ammonia. The polycyclic amine product was then converted to sterically-shielded polycyclic nitroxide.

Sterically-hindered nitroxides have high chemical stability [5] and can be used as spin labels to study biopolymers in cells[6]. Introduction of spiro-cyclic moieties has a smaller effect on the reduction rates of nitroxides than does the introduction of linear alkyl substituents, however, spiro-cyclic nitroxides may have much longer spin relaxation time at 70–150 K which make them attractive agents for spin labeling[7,8,9]. Sterically-hindered nitroxides can be used as spin labels for measurements at room temperature[10]. In this paper we examined the properties of this new nitroxide, in particular, the electron spin relaxation at different temperatures in water/glycerol solution.

## Results and discussion

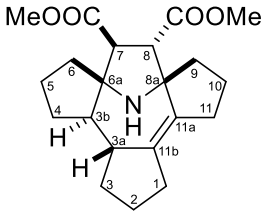
### Synthesis

*Polycyclic Amine*

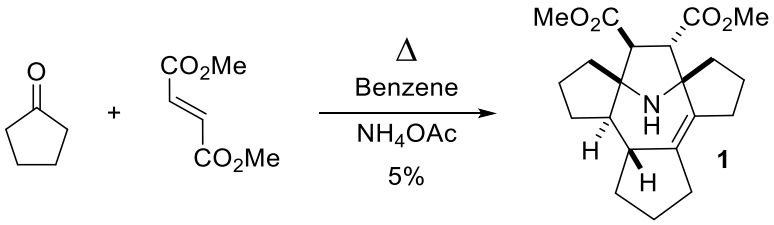
A mixture cyclopentanone, dimethyl fumarate and ammonium acetate was refluxed in benzene in a Dean-Stark apparatus. The reaction mixture underwent a strong tarring and after standard extraction with acid solution, the main product **1** was isolated with a small yield (ca. 5%). The $^{1}$H NMR spectra of **1** show signals of two ester groups, multiple signals of methylene and methyne protons with the overall intensity of 22 protons. $^{13}$C NMR spectrum showed signals from 21 carbon atoms, including an isolated, fully-substituted C=C moiety at 133.5 and 137.5 ppm, methoxy groups at 51.5 and 51.6 ppm and carboxylate groups at 172.7 and 175.3 ppm. There are also four signals of CH groups at 45.9, 55.1, 59.9 and 60.4 ppm. The two downfield CH groups belong to isolated spin systems in the $^{1}$H NMR spectra at 3.19 and 3.44 ppm with hfs

constants 3 Hz. In the HMBC spectrum the later protons show cross peaks with carboxylate carbons and with nodal carbons at 76.08 and 76.12 ppm. The above data imply presence of a 2,2,5,5-tetrasubstituted-3,4-bis(methoxycarbonyl)pyrrolidine ring. The $^{1}$H signal at 3.19 ppm shows a cross-peak with an atom of a fully-substituted C=C moiety, while another low-field signal of a CH group at 3.44 ppm interacts with a CH-group carbon at 55.1 ppm. The hydrogen of the later group shows cross-peaks with the carbon of $^{3a}$CH group at 45.9 ppm and with the carbon of $^{11b}$C=C moiety at 137.5 ppm. These interactions clearly indicate 9-azabicyclo[4.2.1]non-2-ene system in structure of **1**. The remaining assignments were made on the basis of HSQC, COSY $^{1}$H-$^{1}$H and $^{1}$H-$^{13}$C MNR spectra (see Table 1). The structure of the compound was finally confirmed with X-ray analysis and element analyses data. Possible mechanism of **1** formation is represented in scheme 2.

**Table 1.** Assignment of the signals in NMR spectra of **1** on the basis of HSQC, COSY $^{1}$H-$^{1}$H and $^{1}$H-$^{13}$C spectra.




| | $^{13}$C (ppm) | $^{1}$H (ppm) | | $^{13}$C (ppm) | $^{1}$H (ppm) |
|---|---|---|---|---|---|
| $^{10}CH_2$ | 21.0 | 1.71;2.00 | $^{3a}$CH | 45.9 | 2.45 |
| $^{2}CH_2$ | 24.1 | 1.43;1.68 | $CH_3$ | 51.5; 51.6 | 3.57; 3.66 |
| $^{5}CH_2$ | 25.3 | 1.51;1.63 | $^{3b}$CH | 55.1 | 2.39 |
| $^{11}CH_2$ | 30.1 | 1.93;2.24 | $^{7}$CH | 59.9 | 3.44 |
| $^{1}CH_2$ | 32.4 | 2.01;2.15 | $^{8}$CH | 60.4 | 3.19 |
| $^{4}CH_2$ | 33.9 | 1.41;1.94 | $^{6a}$C, $^{8a}$C | 76.08; 76.12 | - |
| $^{3}CH_2$ | 34.4 | 1.11;1.87 | $^{11a}$C | 133.5 | |
| $^{6}CH_2$ | 36.4 | 1.45;1.63 | $^{11b}$C | 137.5 | |
| $^{9}CH_2$ | 40.9 | 1.96;2.01 | $CO_2$ | 172.7; 175.3 | |




**Scheme 1.** Synthesis of compound 1.

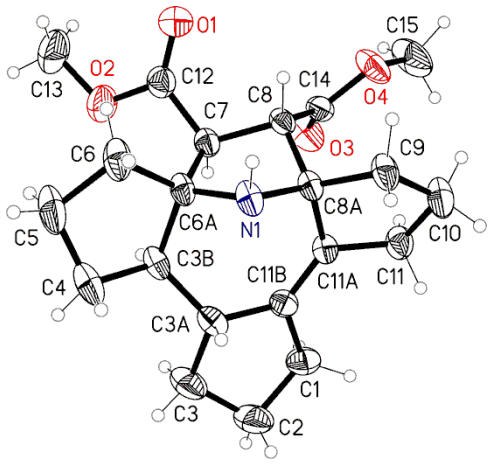
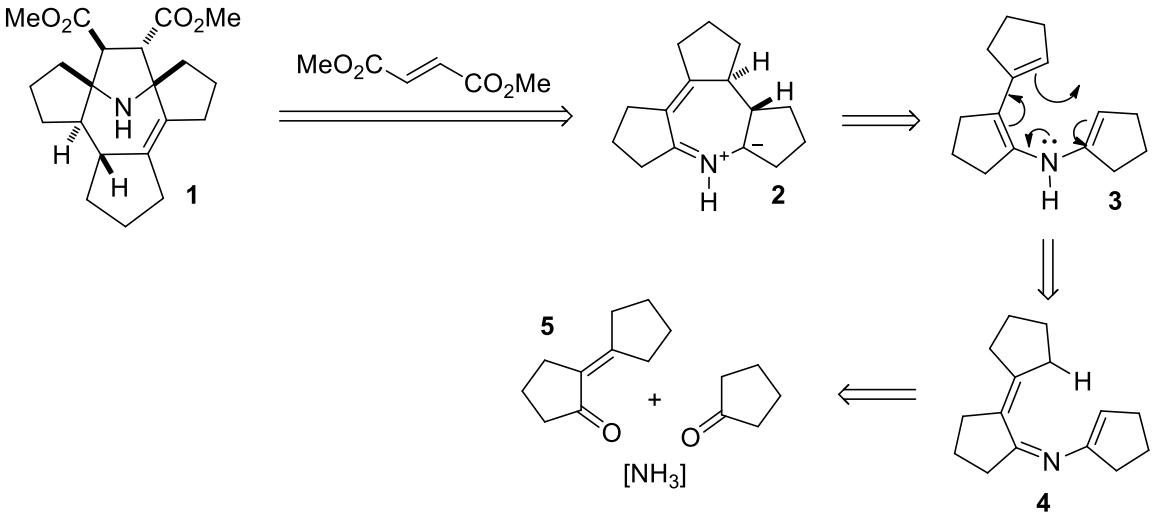



**Fig. 1.** X-ray structure of compound 1.




**Scheme 2.** Possible mechanism of **1** formation.

It is well-known that cyclopentanone is prone to self-condensation. In the presence of ammonia these reactions may lead to heterocycle formation[11]. Presumably, prototropic shift in enamine-imine intermediate **4** is followed by electrocyclization to cyclic azomethine ylide, which then reacts with dimethyl fumarate in a 1,3-dipolar cycloaddition. The suggested mechanism accounts for the trans-position of the methyne hydrogens in the azepine ring: electrocyclization proceeds via a conrotatory mechanism due to the antisymmetry of the HOMO (Fig. 2).

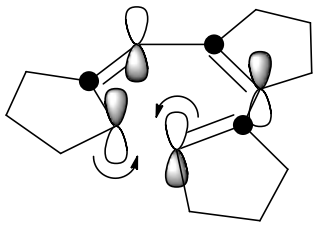


**Fig. 2.** The possible mechanism for trans-position of methyne hydrogens in the azepine ring: electrocyclization proceeds via a conrotatory mechanism due to the antisymmetry of the HOMO

Selective formation of a single diastereomer in the 1,3-dipolar cycloaddition reaction is likely to result from the secondary interaction of orbitals of the π-systems of the dipole and dipolarophyl (Fig. 3).

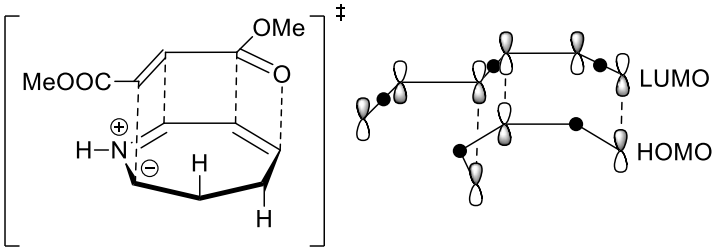
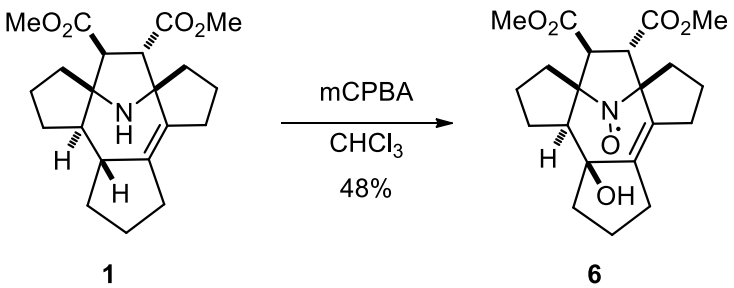



**Figure 3.** Selective formation of a single diastereomer in the 1,3-dipolar cycloaddition reaction.

*Nitroxide*

Oxidation of **1** with m-CPBA afforded the nitroxide **6** with 48% yield (Scheme 3). It is noteworthy that oxidation of the amino group is accompanied with the stereospecific hydroxylation at position 4 of the 2,3,4,7-tetrahydroazepine ring. The structure assignment was performed on the basis of single-crystal X-ray analysis (Fig. 4). A possible mechanism of this hydroxylation is shown in scheme 4. Oxidation of amines with peracids is known to proceed via oxoammonium cation formation.[12] Close proximity of this reactive group to the allyl hydrogen results in hydride abstraction with carbocation **9** formation and subsequent cyclization to bicyclic alkoxyamine **10**. The resulting isoxazolidine ring is then opened with m-CPBA in the normal way, retaining the configuration of the asymmetric center at $^{3a}$C-OH.[13]




**1** **6**

**Scheme 3.** Synthesis of nitroxide **6**.

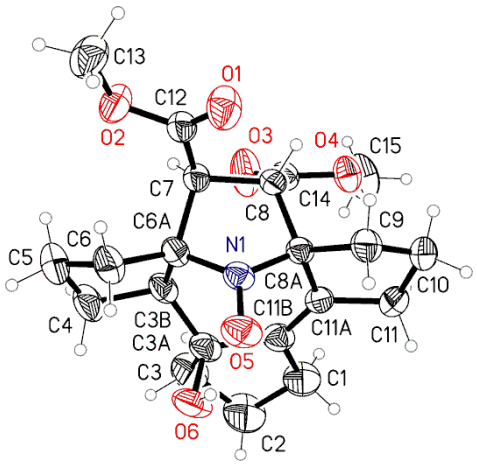
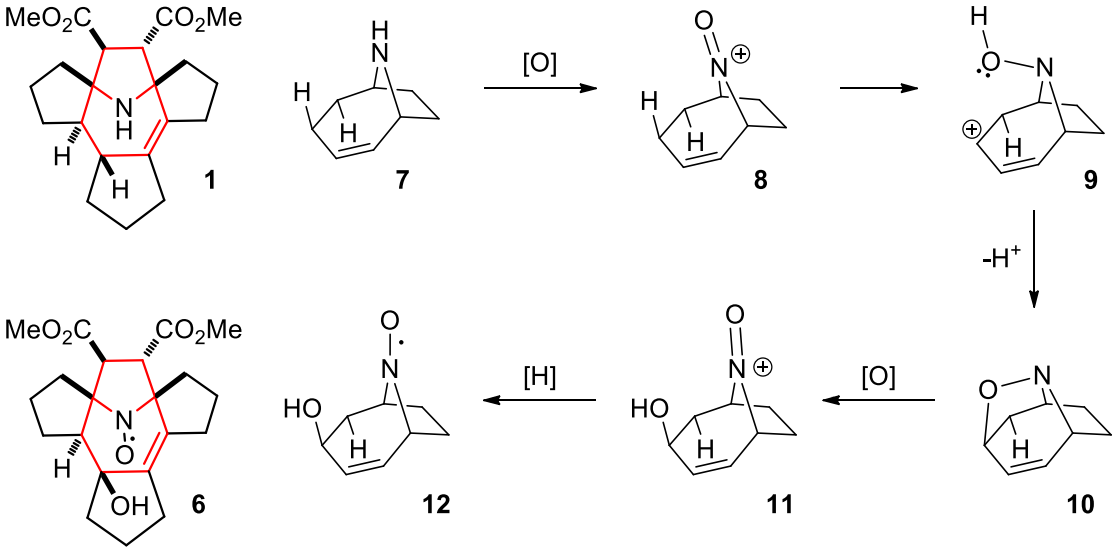



**Figure 4.** X-ray structure of compund **6**.




**Scheme 4.** Proposed mechanism of nitroxide **6** synthesis.

Steric strain in molecules **1** and **6** is characterized by elongation of bonds C6A-C7 1.579(2), C8-C8A 1.573(2) Å in **1** and C3B-C6A 1.580(2), C8-C8A 1.573(2) Å in **6**. Conformation of the azepane ring in **1** is close to a distorted boat with a kink at C3A-N1 line, while in **6** the conformation of this ring is close to distorted half-chair. Presumably, this difference is due to formation of an intramolecular hydrogen bond O6-H…O5 (H…O 1.95(2) Å, O-H…O 154(2)º) in molecule **6**. In the crystal of **1** the amino group participates in intermolecular hydrogen bonding N1-H…O3 (H…O 2.39(2) Å, N-H…O 167(1)º) with chains of the molecules formation along the axis *a*.

**EPR measurements.**

Figure 4 demonstrates the X-band CW EPR spectra of nitroxide **6** in water/glycerol solution at 180 K and at room temperature and corresponding simulations (red) with the parameters listed in the Figure 4 caption.

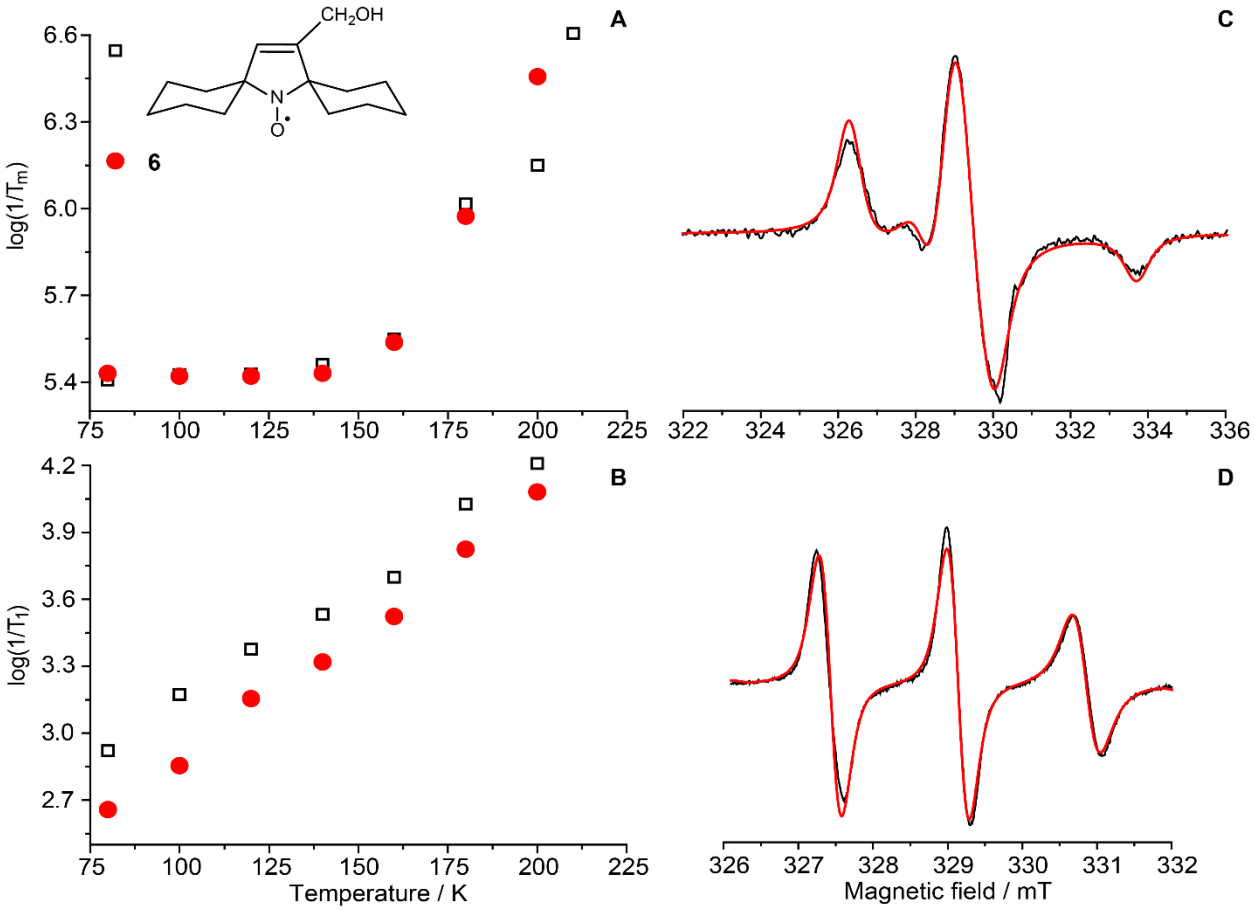



**Fig.4. A-B.** Temperature dependence of electron spin relaxation times of nitroxides in water-glycerol at X-band frequency. The solid red dots are for nitroxide **6**; the open black squares are for spirocyclohexa-substituted nitroxide. **C-D.** CW EPR spectra of nitroxide **6** at 180 K (**C**) and at room temperature (**D**) in water-glycerol. The parameters of HFI, g-factors and correlation time are A(N) =[0.71; 0.71; 3.71] mT, g = [2.0087; 2.0055; 2.0019], $t_{corr}$ = 0.53 ns (at 298 K).

The electron spin relaxation of nitroxides with different bulky substituents has been studied in water-glycerol solution at low temperatures in numerous papers.[9,14,15,16] Previously we investigated electron spin relaxation of a series of nitroxides with different bulky substituents in water solution and in trehalose.[10] Because **6** is a new class of hindered nitroxide, we investigated its electron spin relaxation properties in water-glycerol solution which is the usual solvent for biomolecular distance measurements by PELDOR/DEER. If rotational motions of the radical are prevented, the primary relaxation mechanisms are (i) modulation of $^{14}$N hyperfine interaction (HFI) anisotropy of the NO group by librational motion, and (ii) modulation of the HFI with

other nuclei of the radical by rotation of the groups containing those nuclei (e.g. rotation of methyl groups). The temperature dependence of spin relaxation reveals the relevant mechanism. For comparison, the same relaxation for spirocyclohexa-substituted nitroxide[10] is shown on the same Figure 4. The shapes of $T_m$ vs. $T$ dependence are generally similar compared to the trends observed in frozen solutions. The 2,5-tetramethyl-substituted pyrrolidine and piperidine nitroxides have a local maximum in their phase relaxation ($1/T_m$) at $T$>100 K as thermally-activated rotation of methyl groups becomes rapid; at $T$>140 K this rotation becomes fast enough to average the HFI anisotropy, leading to some decrease in phase relaxation rates; finally, at $T$>220 K librational motion of the NO group governs phase relaxation and its rate continues to increase with the temperature in soft matter.[17,18] Consequently, the common tetramethyl substituted nitroxides are characterized by a local bell-shaped maximum in their phase relaxation at $T$>100 K, that is absent in more hindered nitroxides. In contrast to the 2,5,-tetramethyl-substituted nitroxides, no bell-like shape is observed for the phase relaxation of the nitroxides with two spirocyclohexane moieties adjacent to N-O• group [8,9,10] as well as for nitroxide **6**. Temperature dependence of $1/T_m$ is similar for both radicals and generally similar to that obtained in frozen solutions with a difference about 15 % in region of temperatures 180-170 K.

**Conclusions.**

Unexpected formation of highly strained polycyclic amine from cyclopentanone, dimethylfumarate and ammonium acetate was found, and it it was converted to sterically shielded polycyclic nitroxide. The temperature dependence of electron spin relaxation in water-glycerol solution is found to be very similar to that of nitroxides with two spirocyclohexane moieties adjacent to N-O• group studied previously despite the very different structures of the nitroxide molecules.

**Experimental**

$^1$H NMR spectra were recorded at 400 or 600 MHz, and $^{13}$C NMR spectra were recorded at 100 or 150 MHz. 1H and 13C chemical shifts (δ) were internally referenced to the residual solvent peak. IR spectra were acquired on FT-IR spectrometer in KBr and are reported in wave numbers ($cm^{-1}$). Reactions were monitored by TLC carried out using UV light 254 nm, 1 % aqueous permanganate and Dragendorff reagent as visualizing agents. Column chromatography was performed on silica gel 60 (70−230 mesh). X-ray diffraction data were obtained with a Bruker KAPPA APEX II diffractometer using ϕ, ω scans with Mo-Kα radiation (λ = 0.71073 Å) and a graphite monochromator. CCDC 1947797 (for **1**) and 1947798 (for **6**) contain the

supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre. www.ccdc.cam.ac.uk.

**Synthesis of and dimethyl 2,3,3a,3b,4,5,6,7,8,9,10,11-dodecahydro-1H-6a,8a-epiminotricyclopenta [a,c,e][8]annulene-7,8-dicarboxylate (1):**

A mixture of ammonium acetate (616 mg, 8 mmol), cyclopentanone (1.3 ml, 15 mmol), dimethylfumarate (576 mg, 4 mmol) and benzene (10 ml) was placed into Dean-Stark apparatus and stirred under reflux for 48 h. The solvent was distilled off in vacuum and the residue was dissolved in ethyl acetate. Organic layer was washed with 5% sodium hydro carbonate solution and then extracted with 5% sulfuric acid. Acidic extracts were basified with $Na_2CO_3$ and extracted with ethyl acetate and the solution was extracted with 5% sulfuric acid. Acidic extracts were basified with $Na_2CO_3$ and extracted with ethyl acetate. The extract was dried with $Na_2CO_3$ and the solvent was distilled off in vacuum to give dark oil, which was purified using column chromatography on silica gel (hexane/ethyl acetate – 4/1). **1**: 5%, colorless crystals. m.p. 146.8-148.4. IR (KBr): 3298 (N-H), 1734 1718 (C=O). $^1$H NMR (400 MHz; $CDCl_3$, δ): 1.08-1.16 (m, 1H), 1.35-1.48 (m, 3H), 1.49-1.57 (m, 1H), 1.60-1.69 (m, 3H), 1.70-1.76 (m, 1H), 1.84-1.90 (m,1H), 1.91-1.98 (m, 2H), 1.99-2.08 (m, 3H), 2.14-2.20 (m, 1H), 2.20-2.29 (m, 1H), 2.36-2.41 (m, 1H), 2.42-2.49 (m, 1H), 2.58-2.80 (br, 1H), 3.19 (d $J_d$=3.1 Hz, 1H), 3.44 (d $J_d$= 3.1 Hz, 1H), 3.57 (s, 3H), 3.66 (s, 3H). $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$, δ): 21.0, 24.0, 25.3, 30.0, 32.3, 33.8, 34.3, 36.3, 40.8, 45.9, 51.4, 51.5, 55.0, 59.8, 60.4, 76.0, 76.1, 133.5, 137.4, 172.7, 175.3. Anal. Calcd for $C_{21}H_{29}NO_4$: C, 70.17; H, 8.13; N, 3.90. Found: C, 69.84; H, 7.89; N, 3.96. X-ray: triclinic system, *P*-1, *a* 6.2467(4), *b* 9.2039(8), *c* 16.7575(15) Å, α 98.858(4), β 94.176(3), γ 95.362(3)º, *V* 944.07(13) Å$^3$, *Z* 2, $2\theta_{max}$ 27.271°, 4168 independent reflections ($R_{int}$ 0.0429), $R_1$ 0.0602, $wR_2$ 0.1696, S 1.030 [for 3234 $I > 2\sigma(I)$].

**Synthesis of 3a-hydroxy-7,8-bis(methoxycarbonyl)-2,3,3a,3b,4,5,6,7,8,9,10,11-dodecahydro-1H-6a,8a-epiminotricyclopenta[a,c,e][8]annulene N-oxyl (6):**

A 70% m-CPBA solution (200 mg, 0.9 mmol) was added to the solution of amine **1** (108 mg, 0.3 mmol) in chloroform (2 lm) and stirred at room temperature for 30 min. The reaction mixture was washed 3 times with a 5% solution of sodium bicarbonate. The extract was dried with $Na_2CO_3$ and the solvent was distilled off in vacuum to give a red oil, which was purified using column chromatography on silica gel (hexane/ethyl acetate – 4/1). 48%, red crystals. IR(KBr) 3438 (O-H) 1730 (C=O). X-ray: triclinic system, *P*-1, *a* 8.2240(5), *b* 10.9423(6), *c*

11.4515(8) Å, α 83.440(3), β 75.611(3), γ 83.119(2)º, $V$ 987.17(11) Å$^3$, $Z$ 2, $2\theta_{max}$ 27.292°, 4388 independent reflections ($R_{int}$ 0.0375), $R_1$ 0.0439, $wR_2$ 0.1215, S 1.011 [for 3451 $I > 2\sigma(I)$].

**EPR measurements**

Continuous wave (CW) EPR measurements were carried out using commercial X-band spectrometer Bruker Elexsys E540. The CW EPR spectra were simulated using EasySpin[19]. Pulse EPR experiments were carried out using commercial X/Q-band Bruker Elexsys E580 spectrometer equipped with an Oxford flow helium cryostat and temperature control system. ER 4118X-MD5W resonator was used for X-band measurements. $T_m$ was measured using a two-pulse electron spin echo (ESE) sequence; $T_1$ was measured using inversion-recovery technique with inversion π-pulse and detecting two-pulse ESE sequence. Unless indicated otherwise, the π-pulse lengths were 20 ns.

**Acknowledgements**

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