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Effective 2,6-substitution of piperidine nitroxyl radical by carbonyl compound

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ABSTRACT

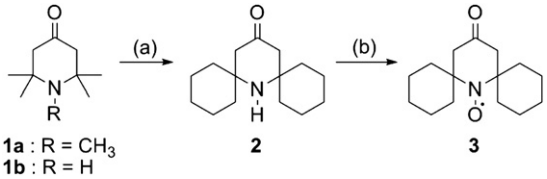
Nitroxyl radicals (nitroxides) with unpaired electron are widely used as antioxidants, contrast agents, and spin probes. Although piperidine nitroxyl radicals have many applications, these are mainly tetramethylpiperidine compounds, and only a few reports consider the substitution of N–O surround as a reaction site, such as 2,2,6,6-tetrasubstituted piperidine nitroxyl radicals. Our results revealed that the 2,6-position of the 2,2,6,6-tetramethylpiperidin-4-one compound was substituted by cyclohexyl groups to produce 2,2,6,6-tetrasubstituted piperidin-4-one derivatives under mild reaction conditions. An interesting result was obtained by using ^{15}N-labeled NH_4Cl instead of $^{14}NH_4Cl$: it gave ^{15}N-labeled 2,2,6,6-tetrasubstituted piperidin-4-one-1-oxyls with a high ^{15}N content. In conclusion, the new method for the synthesis of nitroxyl radicals readily yields 2,2,6,6-tetrasubstituted piperidin-4-one under mild conditions.

1. Introduction

Nitroxyl radicals (nitroxides) with unpaired electron are widely used as antioxidants,[1–3] contrast agents,[4,5] spin probes,[6–8] spin labels,[9,10] hindered amine light stabilizers,[11] nitroxide-mediated radical polymerization agents,[12,13] and radical batteries.[14,15] In particular, the most commonly used nitroxyl radicals, tempol, are starting to be used in human applications. For example, the results of a phase I study have shown that radiation-induced alopecia can be prevented by the topical application of tempol, which is a typical piperidine nitroxyl radical, to the scalp.[16] Such piperidine nitroxyl radicals are usually formed by the reaction of 4-oxo-2,2,6,6-tetramethylpiperidine, which is known as triacetonamine, because it is synthesized from three molecules of acetone and ammonium chloride.[17] Although piperidine nitroxyl radicals have wide applications, there are mainly tetramethylpiperidine compounds and only a few reports considering the substitution of N–O surround as reaction site, such as 2,2,6,6-tetrasubstituted piperidine nitroxyl radicals. Miura et al. successfully synthesized 7-aza-15-oxodispiro[5.1.5.3]hexadec-7-yloxyl using 2,2,4,6,6-pentamethyltetrahydropyrimidine (acetonine) as the intermediate.[12] Wetter et al. synthesized 2,2,6,6-tetraethylpiperidin-4-one-1-oxyl with bisphosphonate as the starting material.[13] Recently, we also reported that 2,2,6,6-tetraethylpiperidin-4-one-1-oxyl was synthesized from acetonine and tetrahydro-4*H*-thiopyran-4-one. This compound has the characteristic of ascorbic acid resistance,[18] and therefore, differs from commonly used piperidine type nitroxyl radicals. These results suggest that the development of a 2,6-substituted compound would potentially have a new scope and application. Therefore, we attempted to develop an effective method of synthesis for a series of 2,6-substituted piperidine nitroxyl radicals.

2. Results and discussion

First, we focused on the steric properties of the existing compound 2,2,6,6-tetramethylpiperidin-4-one-1-oxyl. It has a twist-boat conformation[19] and a bulky tetramethyl group substituted at the 2,6-position. Hence, we expected that the carbonyl group of the 2,2,6,6-tetramethylpiperidin-4-one compound (**1b**), as a precursor of 2,2,6,6-tetramethylpiperidin-4-one-1-oxyl, would be enolized easily. Our results revealed that the four methyl groups at the 2,6-position in the commercially available compound **1a** were substituted by cyclohexyl groups to produce 2,6-substituted piperidin-4-one derivative **2** under mild reaction conditions (yield 34%, Scheme 1). Subsequent oxidation with hydrogen peroxide in



Scheme 1. Synthetic route for 7-aza-15-oxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl. Reagents and conditions: (a) cyclohexanone, NH_4Cl, DMSO, 60 °C, 5 h, 34% from **1a**, 16% from **1b**; (b) H_2O_2, $Na_2WO_4 \cdot 2H_2O$, EtOH, rt, 24 h, 83%.

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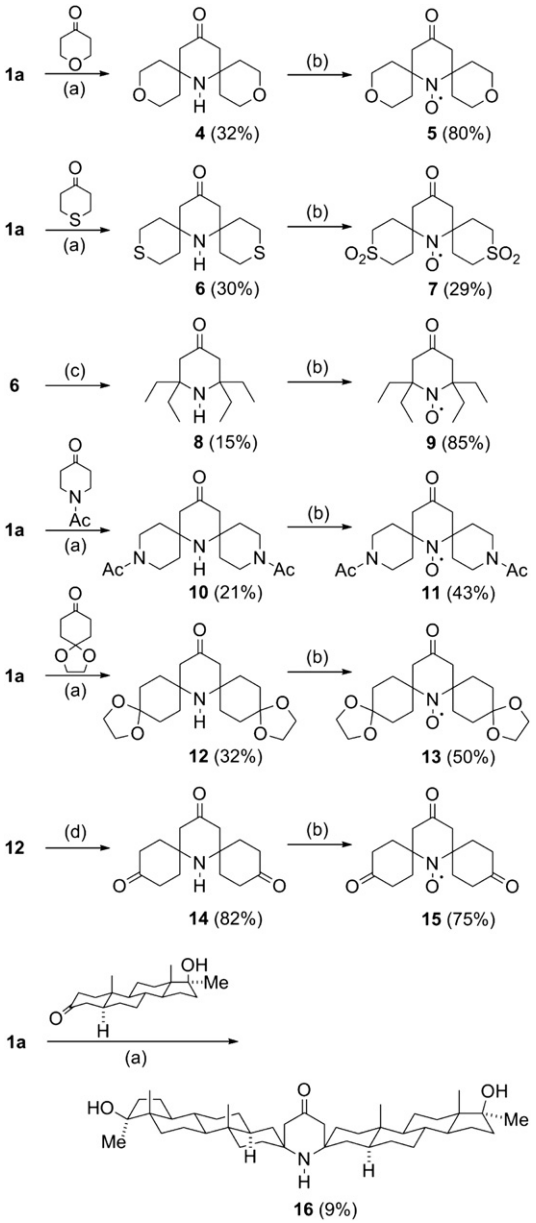
the presence of sodium tungstate gave the nitroxyl radical **3**. As an alternative method, we added the strong base, Triton B, to the reaction mixture of **1a**, and the yield was increased to 48%. We assumed that the carbonyl group was more easily enolized by the Triton B and the aldol reaction was more advanced. However, we did not try other base reagents, so further investigation was needed to clarify the effect of the base compound on the reaction yield. The four methyl groups in 2,2,6,6-tetramethylpiperidin-4-one (**1b**) was also substituted by carbonyl compounds to afford 2,2,6,6-tetrasubstituted piperidin-4-one derivatives. This reaction was induced using the same method as that used to produce **1a** and cyclohexanone; however, the reaction time was 15–20 h, and the yield was low (16%). This reaction also required the four methyl substituents of piperidine, because when piperidin-4-one was used as a substrate, it was unable to react with cyclohexanone under the same conditions (data not shown). Instead of cyclohexanone, a substrate containing ketone was used to produce the corresponding 2,2,6,6-tetrasubstituted piperidin-4-one (Scheme 2; compounds **8** and **14** were synthesized from compounds **6** and **12**, respectively). Tetrahydro-4*H*-pyran-4-one, tetrahydro-4*H*-thiopyran-4-one, and 1-acetyl-piperidin-4-one gave compounds **4**, **6**, and **10**, respectively. However, there was not much difference in the yield according to the type of heteroatom on the ring. Compound **6** was oxidized with H_2O_2 and the sulfide was also oxidized to sulfone to afford compound **7**. Moreover, the desulfurization of compound **6** with Raney-Ni afforded compound **8**. Spiro compounds such as 4-(ethylenedioxy) cyclohexanone also afforded a moderate yield of 2,2,6,6-tetrasubstituted piperidin-4-one derivatives **13**. In addition, the ketal hydrolysis of compound **12** afforded compound **14**. Further, the use of steroidal ketone produced compound **16**, and this structure was confirmed by X-ray crystallographic analysis (Fig. 1).



Scheme 2. Synthetic route for 2,2,6,6-tetrasubstituted piperidine nitroxyl radicals, reagents and conditions: (a) carbonyl compound, NH_4Cl, DMSO, 60 °C, 5 h; (b) H_2O_2, $Na_2WO_4{\cdot}2H_2O$, EtOH, rt, 24 h; (c) Raney-Ni, EtOH, 60 °C, 2 h; (d) HCl, AcOH, 70 °C, 5 h.

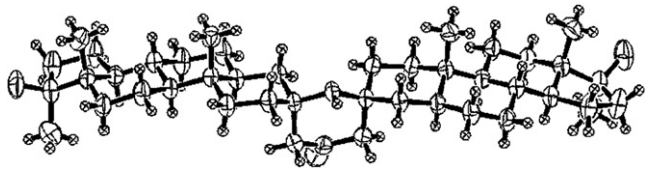
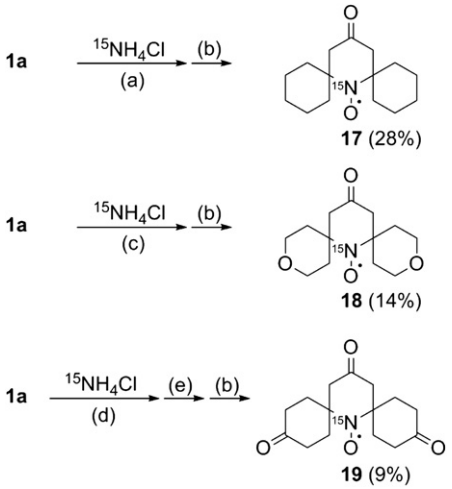
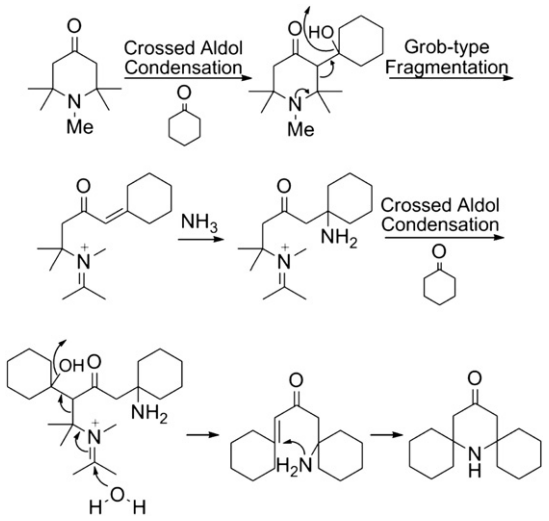


Figure 1. Molecular structure of compound **16** with thermal ellipsoids at the 50% probability level.

An interesting result was obtained by using ^{15}N-labeled NH_4Cl instead of $^{14}NH_4Cl$; this gave ^{15}N-labeled 2,2,6,6-tetrasubstituted piperidin-4-one-1-oxyls **17**, **18**, and **19** with a high ^{15}N content (>98%, Scheme 3). These results suggested that the external NH_4X compound was the source of nitrogen during the reaction. The basicity of the starting compound **1a** or **1b** was greater than that of ammonia, so the HX of NH_4X was quenched by the compound **1a** or **1b** and the ammonia was produced. From the results, we inferred that the reaction mechanism consists of the following six steps in one reaction mixture (Scheme 4); 2,2,6,6-tetramethylpiperidine reacts with carbonyl compound via crossed aldol condensation. The steric hindrance between the carbonyl compound and the *N*-methyl-substituted piperidine leads to Grob-type fragmentation when an ammonium substrate is added to the reaction mixture. After a crossed aldol condensation reaction, acetone and ammonia species such as methylamine are eliminated. Finally, the ring is restructured. The yield of each six reaction steps would be more than 80%, based on the calculation from the final product yield. However, this is a tentative mechanism, and an in-depth study should be conducted to obtain detailed information on the actual reaction mechanism.



Scheme 3. Synthetic route for ^{15}N-labeled nitroxyl radicals. Reagents and conditions: (a) cyclohexanone, $^{15}NH_4Cl$, DMSO, 60 °C, 5 h; (b) H_2O_2, $Na_2WO_4{\cdot}2H_2O$, EtOH, rt, 24 h; (c) tetrahydro-4*H*-pyran-4-one, $^{15}NH_4Cl$, DMSO, 60 °C, 5 h; (d) 1,4-cyclohexandione monoethylene acetal, $^{15}NH_4Cl$, DMSO, 60 °C, 5 h; (e) HCl, AcOH, 70°C, 5 h.



Scheme 4. Tentative mechanism for 2,6-substitution of piperidin-4-one.

3. Conclusion

In conclusion, the new method for the synthesis of nitroxyl radicals readily yields 2,2,6,6-tetrasubstituted piperidin-4-one under mild conditions. ^{15}N-labeled nitroxyl radicals can be synthesized by using ^{15}N-labeled NH_4Cl as an external source of nitrogen. The electron spin resonance spectral characteristics of the nitrogen nuclei of nitroxyl radicals containing ^{15}N nuclei (I=1/2) are different from those of the ^{14}N-substituted molecule (I=1). A reduction in the spectral multiplicity of the ^{15}N analogue leads to an increase in its sensitivity when it is subjected to a magnetic field.[20,21] Different types of 2,6-substituted nitroxyl radicals can be synthesized by the new method. The new nitroxyl radicals could be used as antioxidants, contrast agents, and nitroxide-mediated radical polymerization agents.

4. Experimental section

4.1. General

All commercially available materials were used as received. TLC: silica gel *60* plates (*Merck*) detected with iodine vapor. Column chromatography: Wakogel® C-200 (75–150 μm, *Wako Pure Chemical Industries, Ltd., Osaka, Japan*). Melting points were measured with the Yanaco micro melting point apparatus MP-500P (*Yanaco, Kyoto, Japan*). Mass spectra were measured on a JMS-600H (*JEOL, Akishima, Japan*). IR spectra were recorded by using the attenuated total reflection (ATR) method on an FT/IR-4200 (*JASCO, Japan*). ^{1}H NMR spectra were recorded on a Unity INOVA 400 (400 MHz, *Varian*) or INOVA 500 plus (500 MHz, *Varian*) using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are given from TMS (0 ppm) and coupling constants are expressed in hertz (Hz). ^{13}C NMR spectra were recorded on an ECA300 (75 MHz, *JEOL, Akishima, Japan*). Elemental analyses were performed using the center of elemental analysis with a CHN CORDER MT-6 (*Yanaco, Kyoto, Japan*). ESR spectra were recorded on a JES-RE1X (*JEOL, Akishima, Japan*), and nitroxides were dissolved in 10 mM of phosphate buffer (pH 7.4).

4.2. 7-Azadispiro[5.1.5.3]hexadecan-15-one (2) (general procedure for 2,2,6,6-tetrasubstituted piperidin-4-one)

NH_4Cl (3.21 g, 60 mmol) was added portionwise to a stirred solution of 1,2,2,6,6-pentamethylpiperidin-4-one (**1a**) (1.69 g, 10 mmol) or 2,2,6,6-tetramethylpiperidin-4-one (**1b**) (1.55 g, 10 mmol) and cyclohexanone (2.94 g, 30 mmol) in dimethyl sulfoxide (15 mL) at room temperature. The mixture was then heated for 5 h at 60 °C. It was diluted with H_2O (40 mL), acidified with 7% aq HCl (10 mL), and extracted with ether (×3) to remove a neutral fraction. The reaction mixture was adjusted to pH 9 using 10% aq K_2CO_3 and then extracted with AcOEt (×4). The AcOEt extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was separated by column chromatography with hexane/AcOEt (85:15) to afford 7-azadispiro[5.1.5.3]hexadecan-15-one (**2**) (799 mg, 34%; 376 mg, 16% when **1b** was used as the starting compound) as a white solid after recrystallization. As an alternative, Triton B (2 ml) was added to a stirred solution of **1a** (1.69 g, 10 mmol), cyclohexanone (2.94 g, 30 mmol), and NH_4Cl (2.68 g, 50 mmol) in dimethyl sulfoxide (15 mL), and stirred for 2.5 h at 50 °C. The reaction mixture was worked up in the same manner and gave compound **2** (48% yield); mp: 103 °C (hexane/AcOEt, lit.[12] mp: 101–103 °C,); MS (FAB$^+$): 236.3 (M^++1); ν_{max}/cm^{-1}: 1689 (C=O); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 1.36–1.46 (8H, m), 1.47–1.56 (8H, m), 1.68–1.71 (4H, m), 2.31 (4H, s); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm): 22.53 (CH_2), 25.87 (CH_2), 40.96 (CH_2), 52.47 (C), 57.02 (CH_2), 211.57 (C=O). Found: C, 76.51; H, 10.68; N, 5.93%. Calcd for $C_{15}H_{25}NO$: C, 76.55; H, 10.71; N, 5.95%.

4.3. 7-Aza-15-oxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (3) (general procedure for oxidation of 2,2,6,6-tetrasubstituted piperidin-4-one)

Compound **2** (235 mg, 0.99 mmol) and $Na_2WO_4 \cdot 2H_2O$ (50 mg, 0.15 mmol) were added in ethanol (10 mL); H_2O_2 (30%, 2 mL) was slowly added to the solution, which was stirred for 24 h at room temperature. After stirring, the solution was saturated with K_2CO_3 and extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and evaporated. The residue was recrystallized from hexane to give a pale yellow scale-like crystal (83% yield, 208 mg); mp: 114.2 °C (lit.[12] mp 114–116 °C); MS (FAB$^+$): 250.3 (M^+); ν_{max}/cm^{-1}: 1717 (C=O). Found: C, 71.86; H, 9.62; N, 5.57%. Calcd for $C_{15}H_{24}NO_2$: C, 71.96; H, 9.66; N, 5.59%; A_N=1.407 mT.

4.4. 7-Aza-3,11-dioxadispiro[5.1.5.3]hexadecan-15-one (4)

Tetrahydro-4*H*-pyran-4-one (3.00 g, 30 mmol) was used in place of cyclohexanone according to the method for compound **2**. The product was separated by column chromatography with $CHCl_3$ to afford a white prismatic crystal; yield: 32%; mp: 167 °C (AcOEt); MS (FAB$^+$): 240.1 (M^++1); ν_{max}/cm^{-1}: 1692 (C=O); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 1.64 (8H, t, J=5.6 Hz), 2.40 (4H, s), 3.54–3.60 (4H, m), 3.81–3.87 (4H, m); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm):40.61 (CH_2), 52.21 (C), 54.96 (CH_2), 63.85 (CH_2), 209.34 (C=O). Found: C, 65.17; H, 8.85; N, 5.86%. Calcd for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85%.

4.5. 7-Aza-3,11-dioxa-15-oxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (5)

Compound **4** (130 mg, 0.54 mmol) was oxidized according to the method for compound **3**. The crude product was separated by column chromatography with $CHCl_3$ and recrystallized from AcOEt to afford compound **5** (110 mg, 80%) as an orange crystal; mp: 149.5 °C (AcOEt); MS (FAB$^+$): 255.2 (M^++1); ν_{max}/cm^{-1}: 1717 (C=O). Found: C, 61.23; H, 7.97; N, 5.40%. Calcd for $C_{13}H_{20}NO_4$: C, 61.40; H, 7.93; N, 5.51%; A_N=1.364 mT.

4.6. 7-Aza-3,11-dithiadispiro[5.1.5.3]hexadecan-15-one (6)

Tetrahydro-4*H*-thiopyran-4-one (3.49 g, 30 mmol) was used in place of cyclohexanone according to the method for compound **2**. The product was separated by column chromatography with hexane/AcOEt (1:1) and recrystallized from AcOEt to afford compound **6** as a dark scale-like crystal; yield: 30%; mp: 155–157 °C (AcOEt); MS (FAB$^+$): 272.2 (M^++1); ν_{max}/cm^{-1}: 1697 (C=O); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 0.8 (1H, br s), 1.75–1.90 (8H, m), 2.27 (4H, s), 2.43–2.50 (4H, m), 2.88–2.95 (4H, m); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm): 24.26 (CH_2), 41.66 (CH_2), 53.22 (C), 55.94 (CH_2), 209.24 (C=O). Found: C, 57.54; H, 7.78; N, 5.11%. Calcd for $C_{13}H_{21}NOS_2$: C, 57.52; H, 7.80; N, 5.16%.

4.7. 7-Aza-3,11-dithia-3,3,11,11,15-pentaoxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (7)

Compound **6** (210 mg, 0.78 mmol) and $Na_2WO_4\cdot 2H_2O$ (118 mg, 0.36 mmol) were added in methanol (3 mL); H_2O_2 (30%, 1 mL) was slowly added to the solution, which was stirred for 48 h at room temperature. After stirring, the solution was saturated with K_2CO_3 and extracted with $CHCl_3$/MeOH (3:1). The extracts were dried over anhydrous sodium sulfate and evaporated. The residue was recrystallized from H_2O at a 29% yield (80 mg) to afford compound **7** as a pale yellow crystal; mp: 212.5–214.2 °C; MS (FAB$^+$): 351.0 (M^++1); ν_{max}/cm^{-1}: 1712 (C=O), 1287 (SO_2), 1122 (SO_2). Found: C, 44.38; H, 5.79; N, 3.93%. Calcd for $C_{13}H_{21}NO_6S_2$: C, 44.56; H, 5.75; N, 4.00%; A_N=1.295 mT.

4.8. 2,2,6,6-Tetraethylpiperidin-4-one (8)

A suspension of freshly prepared Raney-Ni in ethanol (8 mL) was added to a well-stirred solution of compound **6** (489 mg, 1.8 mmol) in ethanol (15 mL), and the mixture was stirred at 60 °C. After 2 h, the mixture was filtered through a pad of Celite® and the filtrates were concentrated in vacuo. The residue was separated by column chromatography with $CHCl_3$ to give an orange oil **8** at a 15% yield (57 mg); MS (FAB$^+$): 212.2 (M^++1); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 0.8–0.9 (12H, m), 1.35–1.55 (8H, m), 2.27 (4H, s). Found: C, 73.81; H, 11.99; N, 6.25%. Calcd for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63%. The analytical data are in agreement with literature values.[13]

4.9. 2,2,6,6-Tetraethylpiperidin-4-one-1-oxyl (9)

Compound **8** was oxidized according to the method for compound **3**. The analytical data are in agreement with literature values.[13]

4.10. 3,11-Diacetyl-3,7,11-triazadispiro[5.1.5.3]hexadecan-15-one (10)

NH_4Cl (3.21 g, 6 equiv) was added portionwise to a stirred solution of 1,2,2,6,6-pentamethylpiperidin-4-one (**1a**) (0.845 g, 5 mmol) and 1-acetyl-piperidin-4-one (2.12 g, 15 mmol) in dimethyl sulfoxide (12 mL) at room temperature. The mixture was then heated for 8 h at 60 °C. It was diluted with H_2O (40 mL), acidified with 7% aq HCl (10 mL), and extracted with ether (×3) to remove a neutral fraction. The reaction mixture was adjusted to pH 9 using 10% aq K_2CO_3 and then extracted with $CHCl_3$ (×4). The $CHCl_3$ extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was separated by column chromatography with $CHCl_3$/MeOH (95:5) and recrystallized from hexane/AcOEt to afford compound **10** (328 mg, 21%) as a pale yellow solid; mp: 164.2 °C; MS (FAB$^+$): 322.3 (M^++1); ν_{max}/cm^{-1}: 1687 (C=O), 1634 (N–*Ac*); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 1.58–1.66 (8H, m), 2.08 (6H, s), 2.38 (4H, s), 3.37–3.71(8H, m); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm): 21.48 (CH_3), 37.59 (CH_2), 39.96 (CH_2), 42.62 (CH_2), 52.41 (C), 55.94 (CH_2), 168.93 (C=O), 208.71 (C=O); HRMS (FAB$^+$): found: 322.2104. Calcd for $C_{17}H_{28}N_3O_3$ ([M+H]$^+$): 322.2131.

4.11. 3,11-Diacetyl-15-oxo-3,7,11-triazadispiro[5.1.5.3]hexadec-7-yl-7-oxyl (11)

Compound **10** was oxidized according to the method for compound **3**. The product was separated by column chromatography with $CHCl_3$/MeOH (95:5) and recrystallized from hexane/AcOEt to afford compound **11** as a yellow solid; mp: 162–163 °C (hexane/AcOEt); MS (FAB$^+$): 337.3 (M^++1); ν_{max}/cm^{-1}: 1720 (C=O), 1637 (–*N*–*CO*–CH_3); HRMS (FAB$^+$): found: 337.1996. Calcd for $C_{17}H_{27}N_3O_4$ ([M+H]$^+$): 337.2002.; A_N=1.359 mT.

4.12. 1,4,14,17-Tetraoxa-9-azatetraspiro[4.2.1.2.4.2.3.2]tetracosan-21-one (12)

1,4-Cyclohexanedione monoethylene acetal was used in place of cyclohexanone according to the method for compound **2**. The product was separated by column chromatography with $CHCl_3$ and recrystallized from hexane/AcOEt to afford compound **12** as a white powder; yield: 32%; mp: 190.6 °C (hexane/AcOEt); MS (FAB$^+$): 352.4 (M^++1). ν_{max}/cm^{-1}: 1699 (C=O), 1083 (–O–CH_2–CH_2–O–); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 1.56–1.74 (12H, m), 1.85–1.92 (4H, m), 2.32 (4H, s), 3.93(8H, br s); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm):31.14 (CH_2), 37.86 (CH_2), 52.84 (C), 56.38 (CH_2), 64.41 (CH_2), 64.43 (CH_2), 108.42 (C), 210.59 (C–O). Found: C, 64.95; H, 8.30; N, 4.05%. Calcd for $C_{19}H_{29}NO_5$: C, 64.93; H, 8.32; N, 3.99%.

4.13. 1,4,14,17-Tetraoxa-9-aza-21-oxotetraspiro[4.2.1.2.4.2.3.2]tetracos-9-yl-9-oxyl (13)

Compound **12** was oxidized according to the method for compound **3**. The product was separated by column chromatography with $CHCl_3$ and recrystallized from hexane/AcOEt to afford compound **13** as an orange needle; yield: 50%; mp: 183.2–184.2 °C (hexane/AcOEt); MS (FAB$^+$): 367.3 (M^++1); ν_{max}/cm^{-1}: 1713 (C=O). Found: C, 62.18; H, 7.38; N, 3.88%. Calcd for $C_{19}H_{28}NO_6$: C, 62.28; H, 7.70; N, 3.82%; A_N=1.374 mT.

4.14. 7-Aza-3,11,15-trioxodispiro[5.1.5.3]hexadecane (14)

Ten percent aq HCl (0.2 mL) was dropped portionwise into a well-stirred solution of compound **12** (536 mg, 1.53 mmol) in H_2O (2 mL) and acetic acid (5 mL) and the mixture was heated at 70 °C. The reaction mixture was diluted with H_2O and adjusted to pH 7, then extracted with AcOEt. The extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was separated by column chromatography (hexane/AcOEt, 1:4) to afford compound **14** after recrystallization using hexane and AcOEt at an 82% yield (330 mg) as a white powder; mp: 99.7 °C; MS (FAB$^+$): 264.2 (M^++1); ν_{max}/cm^{-1}: 1705 (C=O); ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 1.24 (1H, br s), 1.87–2.05 (8H, m), 2.29–2.67 (8H, m), 2.49 (4H, s); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm): 37.25 (CH_2), 39.15 (CH_2), 52.56 (C), 56.72 (CH_2), 208.61 (C=O), 209.93 (C=O). Found: C, 68.39; H, 8.08; N, 5.34%. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32%.

4.15. 7-Aza-3,11,15-trioxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (15)

Compound **14** (215 mg, 0.82 mmol) was oxidized according to the method for compound **3**. The product was separated by column chromatography with $CHCl_3$ and recrystallized from hexane/AcOEt to afford compound **15** (170 mg, 75%) as a yellow solid; mp: 161.5 °C

(hexane/AcOEt); MS (FAB$^+$): 279.3 (M^++1); ν_{max}/cm^{-1}: 1708 (C=O). Found: C, 64.71; H, 7.25; N, 5.06%. Calcd for $C_{15}H_{20}NO_4$: C, 64.73; H, 7.24; N, 5.03%; A_N=1.351 mT.

4.16. Bis(17′β-hydroxy-17′α-methyl-5′α-androstan-3′-spiro)-2,6-piperidin-4-one (16)

17′β-Hydroxy-17′α-androstan-3-one (2.25 g, 7.39 mmol) was used in place of cyclohexanone according to the method for compound **2** and compound **16** (210 mg, 0.32 mmol, 9%) was obtained as a white solid. This compound was identified with X-ray crystallographic analysis; mp: 292.7 °C (toluene); MS (FAB$^+$): 648.0 (M^+); ν_{max}/cm^{-1}: 1705 (C=O), 2920 (OH, br); ^{1}H NMR (500 MHz; $CDCl_3$) δ (ppm): 0.60–0.64 (2H, m), 0.81 (6H, s), 0.83 (6H, s), 0.86–0.88 (2H, m), 0.99–1.05 (2H, m), 1.15–1.28 (14H, m), 1.21 (6H, s), 1.32–1.34 (2H, m), 1.40–1.48 (7H, m), 1.54–1.60 (7H, m), 1.65–1.81 (7H, m), 2.41 (4H, s); ^{13}C NMR (75 MHz; $CDCl_3$) δ (ppm): 12.07, 14.07, 20.76, 23.32, 25.92, 28.65, 31.74, 35.25, 35.85, 36.46, 37.13, 39.08, 42.54, 44.01, 45.65, 50.26, 50.75, 54.53, 57.81, 81.80, 211.71. CCDC No. 761583.

4.17. 7-[^{15}N]-Aza-15-oxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (17)

$^{15}NH_4Cl$ was used in place of NH_4Cl according to the general procedure for compound **2** to afford 7-[^{15}N]-azadispiro[5.1.5.3]hexadecan-15-one as a white solid (34% yield). The product was oxidized according to compound **3** to give the title compound as a pale yellow scale-like crystal (83% yield); mp: 115.4–117.4 °C (hexane); MS (FAB$^+$): 251.3 (M^+); ν_{max}/cm^{-1}: 1717 (C=O). Found: C, 71.67; H, 9.79; N, 5.67%. Calcd for $C_{15}H_{24}{}^{15}NO_2$: C, 71.68; H, 9.62; N, 5.57%; A_{15N}=1.974 mT.

4.18. 7-[^{15}N]-Aza-3,11-dioxa-15-oxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (18)

$^{15}NH_4Cl$ and tetrahydro-4*H*-pyran-4-one were used in place of NH_4Cl and cyclohexanone, respectively, according to the general procedure for compound **2**, to afford 7-[^{15}N]-aza-3,11-dioxadispiro[5.1.5.3]hexadecane-15-one as a white prismatic crystal (32% yield). The product was oxidized according to the method for compound **3** to afford the title compound as an orange crystal (45% yield); mp: 171.7–172.0 °C (AcOEt); MS (FAB$^+$): 256.23 (M^++1); ν_{max}/cm^{-1}: 1719 (C=O). Found: C, 61.18; H, 7.90; N, 5.52%. Calcd for $C_{13}H_{20}{}^{15}NO_4$: C, 61.16; H, 7.90; N, 5.48%; A_{15N}=1.912 mT.

4.19. 7-[^{15}N]-Aza-3,11,15-trioxodispiro[5.1.5.3]hexadec-7-yl-7-oxyl (19)

$^{15}NH_4Cl$ and 1,4-cyclohexanedione monoethylene acetal were used in place of NH_4Cl and cyclohexanone, respectively, according to the general procedure for compound **2** to afford 9-[^{15}N]-aza-1,4,14,17-tetraoxatetraspiro[4.2.1.2.4.2.3.2]tetracosan-21-one as a white powder (33% yield). The ketal group of the product was hydrolyzed according to the method for compound **14** to afford 7-[^{15}N]-aza-3,11,15-trioxodispiro[5.1.5.3]hexadecane as a white powder (74% yield). The product was oxidized according to the method for compound **3** to afford the title compound as a yellow solid (38% yield); mp: 165.8 °C (hexane/AcOEt); MS (FAB$^+$): 280.22 (M^++1); ν_{max}/cm^{-1}: 1707 (C=O). Found: C, 64.47; H, 7.22; N, 5.01%. Calcd for $C_{15}H_{20}{}^{15}NO_4$: C, 64.50; H, 7.22; N, 5.01%; A_{15N}=1.888 mT.

Supplementary data

Crystallographic data for compound **16** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 761583. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336 033 or e-mail: deposit@ccdc.cam.ac.uk).

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