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Synthesis and DNA cleavage evaluation of epoxypiperidine derivatives bearing a dehydroamino acid unit

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ABSTRACT

Epoxypiperidine derivatives bearing a dehydroamino acid unit were designed and synthesized as novel DNA alkylating agents based on the structure of azinomycins. A relaxation assay of plasmid DNA revealed that the epoxypiperidine derivative **3** has a DNA cleavage activity. Based on the studies, it would appear that the electron density of the amino group of epoxypiperidines plays a critically important role in the DNA cleavage.

1. Introduction

DNA alkylating agents comprise an extremely important class of clinical therapeutics in the treatment of cancer, a large number of which have been discovered from natural products and their synthetic analogs.[1] Azinomycins A and B, isolated from *Streptomyces* sp. as antitumor antibiotics in 1986, present a new structural class of dual DNA alkylating (interstrand cross-linking) agents (Fig. 1).[2] As is obvious from the structure, the aziridine and oxirane rings are known to be responsible for the alkylation of a guanosine and/or adenosine residue of DNA duplex.[3]

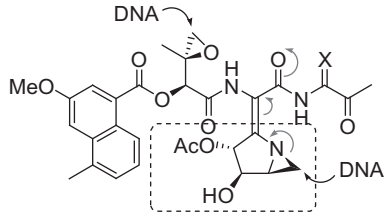

Figure 1. Structures of azinomycins A and B.

We are interested in azinomycins because of their remarkable biological activities and peculiar structure, the highly unstable 4-hydroxy-1-azabicyclo[3.1.0]hexane ring system.[4] We previously designed and synthesized 3,4-epoxypiperidine derivatives as novel DNA alkylating agents based on a hypothetical interconversion of the 3,4-epoxypiperidine and 4-hydroxy-1-azabicyclo[3.1.0]hexane (Fig. 2).[5] As was anticipated, 3,4-epoxypiperidines **1** and **2** showed DNA cleavage activity at concentrations of 100 and 10 μM, respectively, in a relaxation assay of plasmid DNA. Our studies further revealed that both the amino group and epoxide are important for DNA cleavage. Although the mechanism of action is yet to be

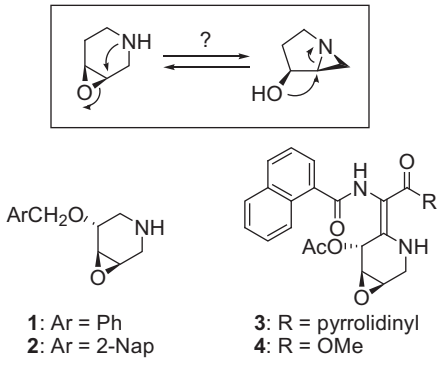

Figure 2. Design of epoxypiperidine derivatives **1**–**4** based on the hypothetical interconversion of the 3,4-epoxypiperidine and 4-hydroxy-1-azabicyclo[3.1.0]hexane.

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known, 3,4-epoxypiperidine might be a stable precursor of 4-hydroxy-1-azabicyclo[3.1.0]hexane. As part of our continuous effort to study the structure–activity relationship of the epoxypiperidines, we report herein, the synthesis and evaluation of the 3,4-epoxypiperidines bearing a dehydroamino acid unit.

From the structure of azinomycins, it is interesting to decipher how the attachment of the unique dehydroamino acid moiety affects the activity of the epoxypiperidines. It is expected that, in azinomycins, conjugation of the aziridine ring with the unsaturated amide moiety would enhance the aziridine-ring cleavage by a DNA nucleophile as indicated by red arrows in Figure 1.[6] We first sought to address this curiosity by designing compounds **3** and **4** (Fig. 2). As the naphthyl group (Nap) of **2** and azinomycin appears to be also an important component probably acting as an interaction site with DNA,[5a] we introduced it into the dehydroamino acid unit. The synthetic strategy of **3** and **4** is shown in Figure 3. We planned to synthesize the compounds **3** and **4** through ring-opening reactions of azlactone **5** by using pyrrolidine and MeOH, respectively. The azlactone **5** would be obtained by a coupling reaction of 1-naphtyl azlactone **6** and thioimidate **7**.

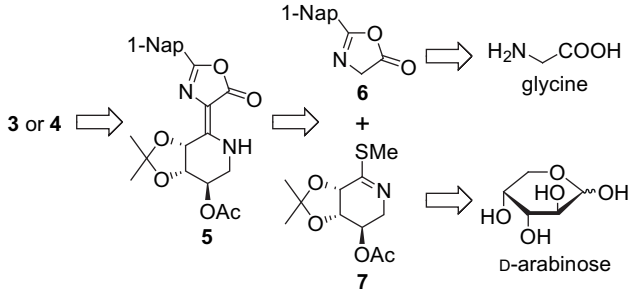
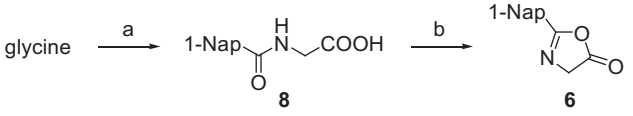


Figure 3. Retrosynthetic analysis for the 3,4-epoxypiperidine derivatives **3** and **4**.

2. Results and discussion

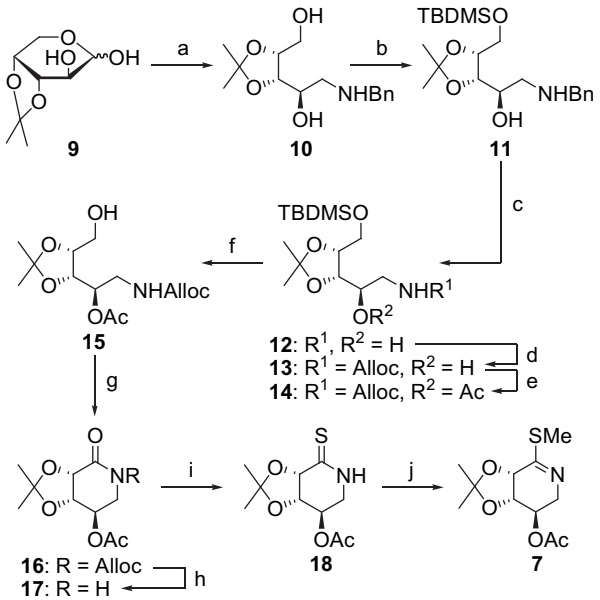
Synthesis of the compounds, **3** and **4**, was achieved as follows. 1-Naphthyl azlactone **6** was readily prepared from glycine in 88% via an intramolecular condensation of **8** by treatment with DCC in DMF (Scheme 1).



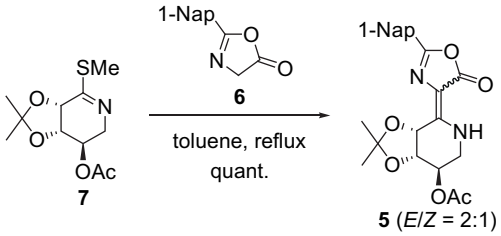
Scheme 1. Preparation of the 1-naphthyl azlactone **6**. Reagents and conditions: (a) 1-naphthoyl chloride, NaOH aq, dioxane, rt; (b) DCC, DMF, 50 °C, 88% (two steps).

The thioimidate **7** was synthesized as shown in Scheme 2. The starting material **9** was prepared from D-arabinose using a known procedure.[7] Reductive amination of **9** employing a catalytic amount of Pt_2O gave amine **10** in 63%. The amine **10** was converted to alcohol **15** in five steps, with an overall yield of 86%. PCC oxidation of **15** directly provided the desired lactam **16**. After removal of Alloc group, thiolactam **18** was obtained by heating with Lawesson's reagent.[8] Methylation of **18** using MeOTf afforded the desired thioimidate **7** exclusively without production of *N*-methyl thiolactam.

The coupling reaction of azlactones and thioimidates was reported by Terashima's group in 2003.[9] Following their method, compounds **6** and **7** were reacted in toluene under reflux to afford the coupling product **5** in quantitative yield (Scheme 3). The product **5** was obtained as an equilibrium mixture of geometrical isomers (*E*/*Z*=2:1). The existence of hydrogen bonding between the amine and the carbonyl group might suggest the favorable formation of the *E* isomer (Fig. 4). The isomers could be differentiated by ^{1}H NMR ($CDCl_3$). That is to say, the H-3 resonance of *Z*-**5** appears at lower field (δ 5.99 ppm) than that of *E*-**5** (δ 5.72 ppm) due to a magnetic deshielding effect by the carbonyl group on the azlactone ring.



Scheme 2. Synthesis of the thioimidate **7**. Reagents and conditions: (a) $BnNH_2$, MeOH, 50 °C, then H_2, PtO_2, MeOH, 50 °C, 63%; (b) TBDMSCl, imidazole, DMF, 0 °C, 86%; (c) 20 wt.% $Pd(OH)_2$/C, HCO_2NH_4, EtOH, reflux; (d) AllocCl, Na_2CO_3, MeOH, H_2O, 0 °C; (e) Ac_2O, Py, 0 °C; (f) TBAF, AcOH, THF, 0 °C to rt, quant. (four steps); (g) PCC, MS3 Å, CH_2Cl_2, rt; (h) $Pd(PPh_3)_4$, sodium 2-methylhexanoate, CH_2Cl_2, rt, 68% (two steps); (i) Lawesson's reagent, toluene, 65 °C, 84%; (j) MeOTf, CH_2Cl_2, 0 °C, 97%.



Scheme 3. Coupling reaction of the 1-naphthyl azlactone **6** and thioimidate **7**.

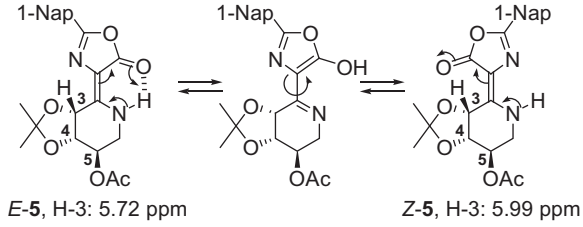
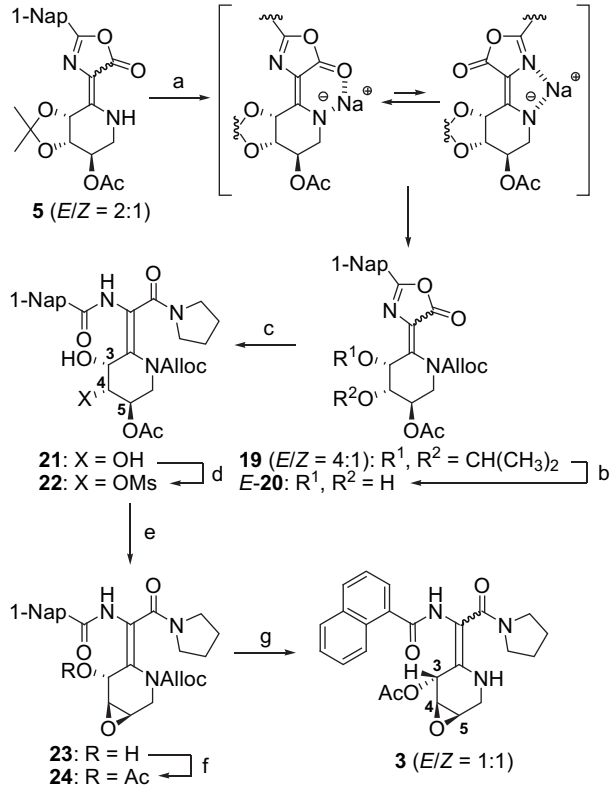


Figure 4. Equilibrium of the isomers *E*-**5** and *Z*-**5**.

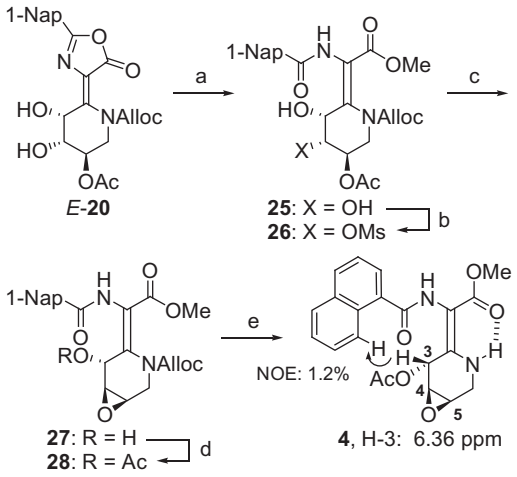
Prior to transformation of the azlactone ring to the dehydroamino acid system, the piperidine amino group of **5** was protected by an Alloc group to yield **19**. The preferential formation of the *E* isomer (*E*/*Z* ratio of **19** was 4:1 based on ^{1}H NMR) may be attributed to the higher stability of the *E* isomer anion having an O–Na^+ chelation structure. We add that the ratio of *E*/*Z* isomers did not differ after *N*-Alloc protection. The *E*/*Z* isomers were separated by column chromatography after removal of the acetonide group by BCl_3, to give the desired *E*-**20** in 42% yield. Treatment of *E*-**20** with pyrrolidine successfully provided the desired dehydroamino acid **21**. The regioselective mesylation of **21** took place under typical mesylation conditions to give **22** as a single compound (72%, two steps). The regiochemistry of **22** could not be determined spectroscopically at this stage, but was confirmed after derivatizing to

24. The compound **22** was converted into epoxypiperidine **23** by using K_2CO_3 in MeOH. Acetylation of **23** gave **24**, ^{1}H NMR of which showed the acetoxymethine hydrogen at 5.69 ppm. This fact strongly supports that the mesylation of **21** exclusively proceeded at the C-4 hydroxyl group. Finally, removal of the Alloc group of **24** afforded the desired epoxypiperidine **3** as a 1:1 equilibrium mixture of *E*/*Z* isomers. The isomeric mixture was then used for DNA cleavage assay without further separation (Scheme 4).



Scheme 4. Preparation of the pyrrolidine amide **3**. Reagents and conditions: (a) AllocCl, NaH, THF, 0 °C, 67%, (recovery of SM, 25%, *E*/*Z*=2:1); (b) BCl_3, CH_2Cl_2, −78 °C, 42%, (*Z*-**20**, 14%; recovery of SM, 42%, *E*/*Z*=4:1); (c) pyrrolidine, toluene, 0 °C; (d) MsCl, Py, 0 °C, 72% (two steps); (e) K_2CO_3, MeOH, 0 °C to 10 °C, 58%; (f) Ac_2O, Py, rt; (g) Pd$(PPh_3)_4$, HCO_2NH_4, CH_2Cl_2, 0 °C, 82% (two steps).

The methyl ester derivative **4** was similarly prepared from diol *E*-**20** (Scheme 5). In contrast to the pyrrolidine amide **24**, isomerization of the double bond was not observed when the Alloc group of **28** was removed at the final step, and the methyl ester **4** was obtained as a single isomer. ^{1}H NMR study of **4** revealed that the H-3 has NOE correlation with the aromatic hydrogen. Therefore, the methyl ester **4** has an *E*-configuration. The *E* isomer is considered to be stable due to an intramolecular hydrogen bonding between the amino group and methyl ester.



Scheme 5. Preparation of the methyl ester **4**. Reagents and conditions: (a) MeOH, TEA, −20 °C; (b) MsCl, Py, 0 °C, 82% (two steps); (c) K_2CO_3, MeOH, 0 °C to rt, 44%; (d) Ac_2O, Py, rt; (e) Pd$(PPh_3)_4$, HCO_2NH_4, CH_2Cl_2, 0 °C, 74% (two steps).

The epoxypiperidines **3** and **4** were then evaluated by a relaxation assay of supercoiled plasmid DNA as in the previous report.[5] Supercoiled plasmid DNA (form I) metamorphoses into an open circular DNA (form II, relaxed DNA) upon DNA damage, and this metamorphosis can be analyzed by agarose gel electrophoresis. Each epoxypiperidine was incubated with supercoiled plasmid DNA at 37 °C for 24 h prior to analysis. The results of the DNA cleavage assay are shown in Figure 5.

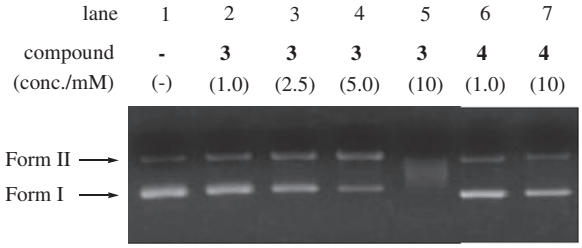


Figure 5. DNA cleavage activity for the epoxypiperidines **3** and **4**. Form I: supercoiled pBR 322 DNA; Form II: relaxed DNA.

As can be seen from the gel, the DNA form I was clearly converted into form II at 5.0 mM concentration of **3** (lane 4). Increasing the concentration of **3** to 10 mM led to the disappearance of both bands (lane **5**), presumably due to critical cleavage of DNA resulting to fragmentation. On the other hand, no significant activity was observed for epoxypiperidine **4** up to 10 mM concentration.

The compound **3** was evaluated as a mixture of geometrical isomers. However, we point out that the stereochemistry about the double bond would not be a factor of the activity, as the double bond could easily isomerize via imino–enol intermediate **29** (Fig. 6). On comparing the activity of **3** with **4**, amide **3** showed DNA cleavage, but ester **4** did not. This observation could be explained by a difference of electron-withdrawing property between an amide and an ester. The electron density on the piperidine–nitrogen is lowered by the conjugated carbonyl group as shown by black arrows on the structure for **3** or **4** in Figure 6.[10] As an ester group is more electron-withdrawing than an amide group, it is thought that, in the case of ester **4**, formation of the aziridine ring by intramolecular nucleophilic attack

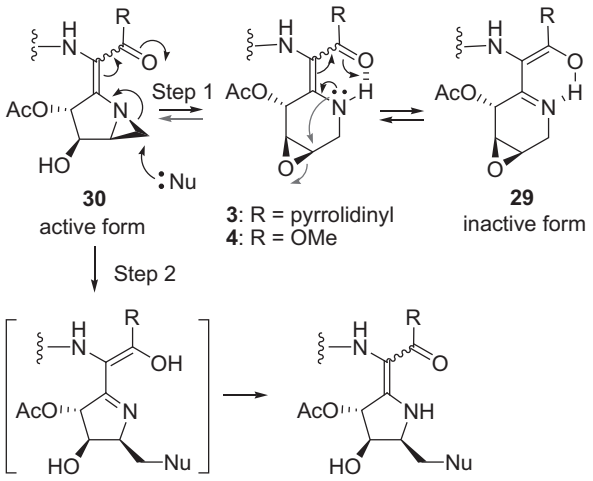


Figure 6. Possible transformations of the epoxypiperidine derivatives **3** and **4**.

shown by red arrows is hindered. That is to say that ester **4** can transform to an inactive form **29**, but cannot transform to an active form **30**, while amide **3** can transform to both forms, **29** and **30**.[12]

Meanwhile, on comparing the activity of **3** with a simple epoxypiperidine derivative **2**, **3** unexpectedly showed lower activity (5.0 mM) than did **2** (10 μM).[5a] This fact could be attributed to a similar reason with the amide group slightly decreasing the nucleophilicity of the proximal piperidine–nitrogen to prevent the formation of the aziridine (step 1) rather than activation of the aziridine (step 2). Simultaneously, this suggests that the step 1 might be a rate-determining step. It might be also considered that the affinity of **3** with DNA is lower than that of **2** due to the unfavorably arranged naphthyl group of **3**. Although the mechanistic issues still remain to be solved, it would appear, based on our present results, that the electron density and/or nucleophilicity of the piperidine–nitrogen is critical for DNA cleavage.[11]

3. Conclusion

In summary, we have demonstrated the design, synthesis, and evaluation of epoxypiperidine derivatives bearing the dehydroamino acid unit of azinomycins. The newly-designed epoxypiperidines **3** and **4** were successfully prepared in 19 synthetic steps via coupling of 1-naphthyl azlactone **6** and thioimidate **7**. Relaxation assay of supercoiled plasmid DNA revealed that epoxypiperidine **3** has a DNA cleavage activity at a concentration of 5.0 mM. Although epoxypiperidine **3** was not as active as the simple epoxypiperidines **1** or **2**, it should be noted that the activity is dependent on a variety of factors. In fact, we recently reported that a proximal aromatic ring to the epoxypiperidine is a highly important unit for the DNA cleavage activity.[5b] Altogether, these results should guide us in our future design of advanced epoxypiperidine analogs. Further studies on the mechanism and structure–activity relationship of epoxypiperidines are ongoing.

4. Experimental

4.1. General

All chemicals from commercial sources were used without purification unless otherwise stated. All reactions were performed under an atmosphere of nitrogen unless noted otherwise. Reactions were monitored by thin-layer chromatography (TLC) plates that were visualized using UV light and *p*-anisaldehyde or phosphomolybdic acid staining, followed by heating. Column chromatography was carried out with BW-127ZH, FL-100D, FL-100B, PSQ-100B, BW-300, FL-60D silica gel purchased from Fuji Silysia. IR spectra were obtained from KBr plates using a JASCO FT-IR-4200 instrument. NMR spectra, including ^{1}H, ^{13}C, and NOE experiments, were recorded on a JEOL EX-270, JEOL AL-300 or JEOL LA-500 spectrometer. Proton and Carbon chemical shifts were referenced to residual solvent peaks or tetramethylsilane (0.00 ppm). High-resolution mass spectra were measured on a JEOL JMS-600 or JMS-700 mass spectrometer.

*4.1.1. N-(1-Naphthoyl)glycine (**8**).* A solution of 1-naphthoyl chloride (30 mL, 200 mmol) in dioxane (100 mL) was added to a solution of glycine (15 g, 200 mmol) in 1.0 M NaOH aq (200 mL). The mixture was stirred at room temperature for 30 min, and then washed with AcOEt. The aqueous layer was acidified by an addition of 5.0 M HCl aq. A precipitate was collected by filtration and dried under vacuum to afford the product **8** (37 g) as a white powder. Compound **8**: Mp 132–134 °C. IR ν_{max} (KBr): 3050, 1729, 1650, 1536, 1400, 1213 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 4.17 (2H, s), 7.48–7.58 (3H, m), 7.68 (1H, dd, J=1, 7.5 Hz), 7.88–7.92 (1H, m), 7.98 (1H, d, J=7.5 Hz), 8.32–8.35 (1H, m).

*4.1.2. 2-(1-Naphthyl)-4H-oxazol-5-one (**6**).* A mixture of **8** (33 g) and *N*,*N*-dicyclohexyl carbodiimide (38 g, 180 mmol) in DMF (300 mL) was stirred at 50 °C for 6 h. The mixture was then diluted with Et_2O, and washed with water and brine. After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/hexane=1:1) to afford the product **6** (29 g, 88%, two steps) as a yellow powder. Compound **6**: Mp 109–112 °C. IR ν_{max} (KBr): 3059, 2945, 1816, 1650, 1574, 1509, 1320 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 4.59 (2H, s), 7.25–7.69 (3H, m), 7.92 (1H, d, J=8 Hz), 8.05 (1H, d, J=8 Hz), 8.17 (1H, d, J=7 Hz), 9.25 (1H, d, J=9 Hz). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: 55.5, 121.4, 124.4, 125.7, 126.4, 128.0, 128.6, 130.0, 130.5, 133.5, 133.6, 163.0, 175.4. MS (FAB) m/z: 212 ($M+H^+$). HRMS Calcd for $C_{13}H_{10}NO_2$: 212.0712. Found: 212.0717.

*4.1.3. 3,4-O-Isopropylidene-β-D-ribofuranose (**9**)[7].* The compound **9** was prepared from D-arabinose according to a known procedure.[7] 2-Methoxypropene (190 mL, 2.0 mol), Drierite® (20 g) and *p*-toluenesulfonic acid monohydrate (350 mg, 2.0 mmol) were added to a solution of D-arabinose (150 g, 1.0 mol) in anhydrous DMF (700 mL) at 0 °C, and the mixture was stirred at 5 °C for 8 h. Sodium carbonate (100 g) was added to the mixture, and the resulting mixture was stirred at 0 °C for 1 h and then filtrated. The filtrate was poured onto water at 0 °C, and the aqueous solution was washed with CH_2Cl_2. The organic layer was then extracted with water, and the combined aqueous solution was concentrated under reduced pressure. The crude product was purified by recrystallization (toluene) to afford the product **9** (146 g, 77%) as a white solid. Compound **9**: Mp 86–87 °C (toluene) [lit.[7] mp 82–84 °C]. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.37 (3H, s), 1.52 (3H, s), 3.80–3.88 (2H, m), 4.11–4.32 (3H, m), 4.56 (3/10H, d, J=7 Hz), 5.19 (7/10H, d, J=4 Hz).

*4.1.4. (2R,3S,4R)-5-Benzylamino-2,3-isopropylidenedioxypentane-1,4-diol (**10**).* Benzylamine (95 mL, 0.87 mol) was added to a solution of **9** (150 g, 0.79 mol) in MeOH (2.0 L), and the mixture was stirred at 50 °C for 6 h. To a suspension of PtO_2 (8.0 g) in MeOH (1.0 L) was added the mixture, and the resulting mixture was further stirred at 50 °C for 19 h under hydrogen atmosphere. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by recrystallization (AcOEt/hexane=1:3) to afford the product **10** (140 g, 63%) as colorless crystals. Compound **10**: Mp 63 °C (AcOEt/hexane). $[\alpha]_D^{25}$ +2.25 (*c* 0.65, $CHCl_3$). IR ν_{max} (KBr): 3320, 2982, 1650, 1539, 1059 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.36 (3H, s), 1.50 (3H, s), 2.77 (1H, ABX, J_{AX}=5 Hz, J_{AB}=12 Hz), 2.88 (1H, ABX, J_{AX}=7 Hz, J_{AB}=12 Hz), 3.72 (1H, ABX, J_{AX}=4 Hz, J_{AB}=12 Hz), 3.76–3.87 (2H, m), 3.82 (2H, s), 4.07 (1H, dd, J=3, 7 Hz), 4.20 (1H, ddd, J=3, 4, 5 Hz), 7.21–7.38 (5H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: 25.2, 27.2, 52.3, 53.6, 60.8, 67.3, 77.3, 78.2, 108.2, 127.1, 128.0, 128.4, 139.4. MS (EI) m/z: 281 (M^+, 6), 120 (100), 91 (100). HRMS Calcd for $C_{15}H_{23}NO_4$: 281.1627. Found: 281.1644.

*4.1.5. (2R,3S,4R)-5-N-Benzylamino-1-tert-butyldimethylsiloxy-2,3-isopropylidenedioxypentan-4-ol (**11**).* Imidazole (17 g, 240 mmol) and *tert*-butyldimethylsilyl chloride (24 g, 160 mmol) were added to a solution of **10** (45 g, 16 mmol) in anhydrous DMF (150 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. Water was added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH=10:1) to afford the product **11** (54 g, 86%) as a colorless oil. Compound **11**: $[\alpha]_D^{21}$ −8.7 (*c* 0.95, $CHCl_3$). IR ν_{max} (KBr): 3478, 2930, 1459, 1375, 1253, 1216, 1089 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.07

(6H, s), 0.89 (9H, s), 1.34 (3H, s), 1.47 (3H, s), 2.76 (1H, ABX, J_{AX}=5 Hz, J_{AB}=12 Hz), 2.83 (1H, ABX, J_{AX}=7 Hz, J_{AB}=12 Hz), 3.74 (1H, ABX, J_{AX}=4 Hz, J_{AB}=11 Hz), 3.80 (2H, s), 3.90 (1H, m), 3.92 (1H, ABX, J_{AX}=7 Hz, J_{AB}=11 Hz), 4.08 (1H, dd, J=3, 7 Hz), 4.13 (1H, ddd, J=3, 4, 7 Hz), 7.18–7.33 (5H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: −5.4, 18.3, 25.2, 25.9, 27.2, 52.4, 53.8, 61.8, 67.6, 77.2, 78.3, 108.0, 126.7, 127.9, 128.2, 140.1. MS (EI) m/z: 395 (M^+, 12), 380 (25), 338 (77), 120 (100), 91 (80). HRMS Calcd for $C_{21}H_{37}NO_4Si$: 395.2492. Found: 395.2493.

*4.1.6. (2R,3S,4R)-5-Amino-1-tert-butyldimethylsiloxy-2,3-isopropylidenedioxypentan-4-ol (**12**).* To a solution of **11** (54 g, 140 mol) in EtOH (500 mL) was added 20 wt.% $Pd(OH)_2/C$ (20 g) and ammonium formate (90 g, 1.4 mol), and the mixture was refluxed for 15 min. After filtration, the filtrate was concentrated under reduced pressure to afford the compound **12** (42 g) as a colorless oil. Compound **12**: $[\alpha]_D^{21}$ −11.0 (c 1.05, $CHCl_3$). IR ν_{max} (KBr): 3370, 2933, 1254, 1093 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.10 (6H, s), 0.90 (9H, s), 1.36 (3H, s), 1.48 (3H, s), 2.74 (2H, br s), 2.86–3.00 (2H, m), 3.74 (1H, ABX, J_{AX}=8 Hz, J_{AB}=11 Hz), 3.83 (1H, m), 3.94 (1H, ABX, J_{AX}=7 Hz, J_{AB}=11 Hz), 4.10 (1H, dd, J=2, 6 Hz), 4.16 (1H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: −5.5, 18.2, 25.0, 25.7, 27.1, 44.9, 61.6, 68.9, 77.2, 77.9, 107.9. MS (FAB) m/z: 306 ($M+H^+$). HRMS Calcd for $C_{14}H_{32}NO_4Si$: 306.2101. Found: 306.2126.

*4.1.7. (2R,3S,4R)-5-N-Allyloxycarbonylamino-1-tert-butyldimethylsiloxy-2,3-isopropylidenedioxypentan-4-ol (**13**).* To a solution of **12** (42 g) in MeOH (150 mL) was added water (600 mL), sodium carbonate (25 g, 240 mmol), and allyl chloroformate (20 mL, 190 mmol) at 0 °C, and the mixture was stirred for 30 min. Saturated $NaHCO_3$ was added to the mixture, and the resulting mixture was stirred at room temperature for 10 min, and then extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under vacuum to afford the product **13** (54 g) as a colorless oil. Compound **13**: $[\alpha]_D^{21}$ +3.69 (c 0.95, $CHCl_3$). IR ν_{max} (KBr): 3737, 2933, 1717, 1533, 1253, 1096 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.10 (6H, s), 0.90 (9H, s), 1.35 (3H, s), 1.48 (3H, s), 3.29 (1H, ABXY, J_{AX}=4 Hz, J_{AY}=8 Hz, J_{AB}=13 Hz), 3.47 (1H, m), 3.76 (1H, ABX, J_{AX}=3 Hz, J_{AB}=11 Hz), 3.92 (1H, m), 3.94 (1H, ABX, J_{AX}=7 Hz, J_{AB}=11 Hz), 4.07 (1H, dd, J=3, 6 Hz), 4.16 (1H, ddd, J=4, 6, 6 Hz), 4.56 (2H, d, J=5 Hz), 5.19 (1H, dd, J=2, 10 Hz), 5.29 (1H, dd, J=2, 18 Hz), 5.92 (1H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: −5.4, 18.3, 25.1, 25.8, 27.1, 44.6, 61.3, 65.4, 67.8, 76.9, 77.6, 108.2, 117.3, 132.8, 156.3. MS (FAB) m/z: 390 ($M+H^+$). HRMS Calcd for $C_{18}H_{36}NO_6Si$: 390.2312. Found: 390.2311.

*4.1.8. (2R,3S,4R)-4-Acetoxy-5-N-allyloxycarbonylamino-1-tert-butyldimethylsiloxy-2,3-isopropylidenedioxypentane (**14**).* To a solution of **13** (54 g) in anhydrous pyridine (70 mL) was added 4-(dimethylamino)pyridine (0.98 g, 8.0 mmol) and acetic anhydride (18 mL, 190 mol) at 0 °C, and the mixture was stirred for 2 h. Saturated $NaHCO_3$ was added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under vacuum to afford the product **14** (59 g) as a colorless oil. Compound **14**: $[\alpha]_D^{22}$ −23.5 (c 0.99, $CHCl_3$). IR ν_{max} (KBr): 3344, 2932, 1734, 1522, 1373, 1251, 1086 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.07 (6H, s), 0.89 (9H, s), 1.35 (3H, s), 1.45 (3H, s), 2.09 (3H, s), 3.46 (1H, ABX, J_{AX}=5 Hz, J_{AB}=12 Hz), 3.52 (1H, ABX, J_{AX}=3 Hz, J_{AB}=12 Hz), 3.69 (2H, d, J=5 Hz), 4.16–4.28 (2H, m), 4.56 (2H, d, J=5 Hz), 5.16 (1H, m), 5.20 (1H, dd, J=1, 11 Hz), 5.30 (1H, dd, J=1, 17 Hz), 5.91 (1H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: −5.4, 18.3, 21.1, 25.5, 25.8, 27.2, 42.9, 61.5, 65.5, 69.7, 77.0, 77.2, 108.7, 117.4, 132.7, 156.1, 170.0. MS (FAB) m/z: 454 ($M+Na^+$). HRMS Calcd for $C_{20}H_{38}NO_7Si$: 432.2418. Found: 432.2412.

*4.1.9. (2R,3S,4R)-4-Acetoxy-5-N-allyloxycarbonylamino-2,3-isopropylidenedioxypentan-1-ol (**15**).* To a solution of **14** (59 g) in THF (300 mL) was added acetic acid (46 mL, 0.80 mol) and TBAF solution (1.0 M in THF, 180 mL, 180 mmol) at 0 °C, and the mixture was stirred at room temperature for 24 h. The mixture was diluted with AcOEt, and washed with saturated $NaHCO_3$, water, and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=1:0 to 75:1) to afford the product **15** (43 g, quant., four steps) as a colorless oil. Compound **15**: $[\alpha]_D^{22}$ +7.10 (c 0.87, $CHCl_3$). IR ν_{max} (KBr): 3341, 2986, 1730, 1538, 1375, 1243, 1048 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.36 (3H, s), 1.47 (3H, s), 2.10 (3H, s), 3.36 (1H, ddd, J=6, 6, 15 Hz), 3.56 (1H, ABXY, J_{AX}=6 Hz, J_{AY}=6 Hz, J_{AB}=15 Hz), 3.72 (1H, ABXY, J_{AX}=4 Hz, J_{AY}=6 Hz, J_{AB}=15 Hz), 3.69 (1H, ABX, J_{AX}=6 Hz, J_{AB}=12 Hz), 3.74 (1H, ABX, J_{AX}=5 Hz, J_{AB}=12 Hz), 4.20–4.36 (2H, m), 4.56 (2H, d, J=5 Hz), 5.15 (1H, dd, J=6, 9 Hz), 5.21 (1H, dd, J=1, 10 Hz), 5.30 (1H, dd, J=1, 17 Hz), 5.91 (1H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: 21.2, 25.4, 27.2, 42.8, 60.8, 65.7, 69.9, 76.0, 77.2, 108.8, 117.6, 132.5, 156.4, 170.3. MS (FAB) m/z: 318 ($M+H^+$). HRMS Calcd for $C_{14}H_{24}NO_7$: 318.1553. Found: 318.1557.

*4.1.10. (3S,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-3,4-isopropylidenedioxypiperidin-2-one (**16**).* Pyridinium chlorochromate (92 g, 0.42 mol) and molecular sieves 3 Å (45 g) were added to a solution of **15** (45 g, 140 mmol) in CH_2Cl_2 (2.5 L), and the mixture was stirred at room temperature for 30 h. Water was added to the mixture, and the resulting mixture was filtrated. The filtrate was extracted with Et_2O, and the combined organic layer was washed with water and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure to afford the product **16** (41 g) as a colorless oil. Compound **16**: $[\alpha]_D^{22}$ +0.26 (c 0.97, $CHCl_3$). IR ν_{max} (KBr): 2937, 1784, 1729, 1458, 1375, 1227, 1055 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.37 (3H, s), 1.52 (3H, s), 2.06 (3H, s), 3.87 (1H, ABX, J_{AX}=2 Hz, J_{AB}=14 Hz), 4.27 (1H, ABXY, J_{AX}=2 Hz, J_{AY}=4 Hz, J_{AB}=14 Hz), 4.44 (1H, ddd, J=2, 2, 7 Hz), 4.65 (1H, d, J=7 Hz), 4.76 (2H, d, J=6 Hz), 5.02 (1H, m), 5.29 (1H, dd, J=2, 11 Hz), 5.44 (1H, dd, J=2, 17 Hz), 5.96 (1H, m). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: 20.7, 24.1, 26.0, 43.1, 67.8, 67.9, 75.1, 76.1, 111.1, 118.7, 130.8, 152.9, 165.9, 169.1. MS (FAB) m/z: 314 ($M+H^+$). HRMS Calcd for $C_{14}H_{20}NO_7$: 314.1240. Found: 314.1247.

*4.1.11. (3S,4S,5R)-5-Acetoxy-3,4-isopropylidenedioxypiperidin-2-one (**17**).* Tetrakis(triphenylphosphine) palladium (0) (1.6 g, 1.4 mmol), triphenylphosphine (14 g, 55 mmol), and sodium 2-methylhexanoate (96 g, 0.63 mol) were added to a solution of **16** (41 g) in anhydrous CH_2Cl_2 (1.5 L), and the mixture was stirred at room temperature for 1 h. After dilution with AcOEt, the resulting mixture was washed with water and brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=1:0 to 50:1) to afford the product **17** (22 g, 68%, two steps) as a white solid. Compound **17**: Mp 99–100 °C. $[\alpha]_D^{25}$ −62.3 (c 0.56, $CHCl_3$). IR ν_{max} (KBr): 3228, 2988, 1746, 1685, 1375, 1232, 1058 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, s), 1.45 (3H, s), 2.05 (3H, s), 3.29 (1H, ABXY, J_{AX}=2 Hz, J_{AY}=4 Hz, J_{AB}=13 Hz), 3.71 (1H, AB, J=13 Hz), 4.39 (1H, dd, J=5, 7 Hz), 4.48 (1H, d, J=7 Hz), 5.05 (1H, m), 7.84 (1H, br s). ^{13}C NMR (75.45 MHz, $CDCl_3$) δ_C: 20.8, 24.6, 26.6, 40.4, 67.9, 72.9, 74.1, 110.7, 169.4, 169.7. MS (EI) m/z: 230 (M^+, 100), 214 (77), 172 (68), 169 (59), 130 (49), 112 (48), 84 (31). HRMS Calcd for $C_{10}H_{16}NO_5$: 230.1028. Found: 230.1028.

*4.1.12. (3S,4S,5R)-5-Acetoxy-3,4-isopropylidenedioxypiperidine-2-thione (**18**).* Lawesson's reagent (42 g, 100 mmol) was added to a solution of **17** (22 g, 90 mmol) in toluene (500 mL), and the mixture was stirred at 65 °C for 30 min. After dilution with AcOEt, the mixture was washed with saturated $NaHCO_3$ and brine and then dried over Na_2SO_4. The solvent was removed under reduced

pressure, and the residue was purified by recrystallization (AcOEt/hexane=1:1) to afford the product **18** (20 g, 84%) as colorless crystals. Compound **18**: Mp 164 °C (AcOEt/*n*-hexane). $[\alpha]_D^{26}$ −161.8 (*c* 0.56, $CHCl_3$). IR ν_{max} (KBr): 3269, 2987, 1748, 1544, 1375, 1232, 1139, 1053 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.41 (3H, s), 1.50 (3H, s), 2.10 (3H, s), 3.44 (1H, ABXY, J_{AX}=2 Hz, J_{AY}=3 Hz, J_{AB}=14 Hz), 3.69 (1H, ABXY, J_{AX}=2 Hz, J_{AY}=2 Hz, J_{AB}=13 Hz), 4.39 (1H, ddd, *J*=2, 3, 7 Hz), 4.95 (1H, d, *J*=7 Hz), 5.09 (1H, d, *J*=3 Hz). ^{13}C NMR (75.45 MHz, $CDCl_3$) δ_C: 20.9, 24.1, 26.3, 42.8, 67.5, 73.2, 78.0, 110.4, 169.7, 197.8. MS (EI) *m*/*z*: 245 (M^+, 100), 230 (32), 188 (23), 128 (57), 100 (41). Anal. Calcd for $C_{10}H_{15}NO_4S$: C, 48.96; H, 6.16; N, 5.71; S, 13.07. Found: C, 48.97; H, 6.05; N, 5.67; S, 12.96.

*4.1.13. (3S,4S,5R)-5-Acetoxy-3,4-isopropylidenedioxy-2-methylthio-3,4,5,6-tetrahydropyridine (**7**).* Methyl trifluoromethanesulfonate (8.9 mL, 80 mmol) was added to a solution of **18** (13 g, 53 mmol) in anhydrous CH_2Cl_2 (500 mL) at 0 °C, and the mixture was stirred for 2 h at the same temperature. After an addition of saturated $NaHCO_3$, the mixture was extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under vacuum to afford the product **7** (13 g, 97%) as a white solid. Compound **7**: Mp 35–37 °C. $[\alpha]_D^{25}$ −36.6 (*c* 0.21, $CHCl_3$). IR ν_{max} (KBr): 2930, 1747, 1627, 1373, 1234, 1163, 1058 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.40 (3H, s), 1.45 (3H, s), 2.08 (3H, s), 2.33 (3H, s), 3.72 (1H, ABX, J_{AX}=5 Hz, J_{AB}=16 Hz), 3.86 (1H, ABX, J_{AX}=3 Hz, J_{AB}=16 Hz), 4.35 (1H, dd, *J*=4, 6 Hz), 4.43 (1H, d, *J*=6 Hz), 5.02 (1H, m). ^{13}C NMR (75.45 MHz, $CDCl_3$) δ_C: 12.0, 21.0, 25.4, 26.8, 49.3, 68.5, 72.1, 73.4, 110.8, 166.9, 169.9. MS (EI) *m*/*z*: 259 (M^+, 39), 199 (86), 186 (82), 142 (100), 126 (73). HRMS Calcd for $C_{11}H_{17}NO_4S$: 259.0878. Found: 259.0911.

*4.1.14. 4-[(3R,4S,5R)-5-Acetoxy-3,4-isopropylidenedioxypiperidin-2-ylidene]-2-(1-naphtyl)-4H-oxazol-5-one (**5**).* The compound **6** (25 g, 54 mmol) was added to a solution of **7** (3.5 g, 14 mmol) in anhydrous toluene (40 mL), and the mixture was stirred at 100 °C for 36 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=1:6 to 1:4) to afford the product **5** (5.0 g, quant., *E*/*Z*=2:1) as a yellow powder. Compound **5**: Mp 86–88 °C. IR ν_{max} (KBr): 3308, 2987, 2927, 1745, 1712, 1634, 1590, 1509, 1342, 1212, 1160, 1063 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.46 (3H, s), 1.48 (3H, s), 2.00 (3H, s), 3.57 (1H, m), 3.80 (1H, m), 4.37 (1/3H, ddd, *J*=2, 2, 7 Hz), 4.45 (2/3H, ddd, *J*=2, 2, 7 Hz), 5.02 (1H, s), 5.72 (2/3H, d, *J*=7 Hz), 5.99 (1/3H, dd, *J*=2, 7 Hz), 7.42–7.63 (3H, m), 7.80–7.90 (2H, m), 8.08 (1H, m), 9.28 (1H, d, *J*=6 Hz). MS (FAB) *m*/*z*: 445 ($M+Na^+$). HRMS Calcd for $C_{23}H_{22}N_2O_6Na$: 445.1376. Found: 445.1377.

*4.1.15. 4-[(3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-3,4-isopropylidenedioxypiperidin-2-ylidene]-2-(1-naphtyl)-4H-oxazol-5-one (**19**).* Sodium hydride (60% in oil, 610 mg, 15 mmol) was added to a solution of **5** (3.2 g, 7.6 mmol) in anhydrous THF (70 mL) at 0 °C, and the mixture was stirred for 30 min at the same temperature. After an addition of allyl chloroformate (1.5 mL, 11 mmol), the resulting mixture was further stirred at room temperature for 15 h. Saturated $NaHCO_3$ was added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=1:3) to afford the product **19** (2.5 g, 67%, *E*/*Z*=4:1) as a yellow powder. The starting **5** was recovered in 25% (0.9 g, *E*/*Z*=2:1). Compound **19**: Mp 65–66 °C. IR ν_{max} (KBr): 3023, 2987, 1790, 1751, 1720, 1652, 1512, 1369, 1218, 1056 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.48 (3/5H, s), 1.51 (12/5H, s), 1.56 (3/5H, s), 1.58 (12/5H, s), 1.96 (3H, s), 3.91 (4/5H, AB, *J*=14 Hz), (1/4H, ABX, J_{AX}=2 Hz, J_{AB}=14 Hz), 4.15–4.28 (1H, m), 4.44–4.53 (1H, m), 4.56–4.82 (2H, m), 4.92–5.45 (3H, m), 5.69–6.03 (1H, m), 5.96 (4/5H, d, *J*=7 Hz), 6.24 (1/5H, d, *J*=7 Hz), 7.53–7.52 (3H, m), 7.93 (1H, d, *J*=8 Hz), 8.06 (1H, d, *J*=8 Hz), 8.29 (1H, d, *J*=8 Hz), 9.41 (1H, d, *J*=8 Hz). MS (FAB) *m*/*z*: 507 ($M+H^+$). HRMS Calcd for $C_{27}H_{27}N_2O_8$: 507.1767. Found: 507.1753.

*4.1.16. 4-[(3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-3,4-dihydroxypiperidin-2-ylidene]-2-(1-naphtyl)-4H-oxazol-5-one (**20**).* Boron trichloride solution (1.0 M in hexane, 20 mL, 20 mmol) was added dropwise over 10 min to a solution of **19** (1.0 g, 2.0 mmol) in anhydrous CH_2Cl_2 (100 mL) at −78 °C. After stirring for 30 min at −78 °C, saturated $NaHCO_3$ solution was added to the reaction mixture, which was gradually warmed up to room temperature. The resulting mixture was extracted with AcOEt, and the combined organic layer was washed with water and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH=50:1) to afford the product *E*-**20** (390 mg, 42%) as a yellow powder and *Z*-**20** (131 mg, 14%) as a yellow powder. The starting **19** was recovered in 42% (420 mg, *E*/*Z*=4:1). Compound *E*-**20**: Mp 66–68 °C. IR ν_{max} (KBr): 3412, 2939, 1796, 1728, 1659, 1565, 1512, 1315, 1231, 1144, 1055 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 2.12 (3H, s), 3.28 (1H, s), 3.75–4.40 (3H, m), 4.57–4.80 (2H, m), 5.08–5.35 (4H, m), 5.92 (1H, m), 7.52–7.73 (3H, m), 7.95 (1H, d, *J*=8 Hz), 8.09 (1H, d, *J*=8 Hz), 8.25 (1H, dd, *J*=1, 7 Hz), 8.99 (1/2H, d, *J*=9 Hz), 9.06 (1/2H, d, *J*=8 Hz). MS (FAB) *m*/*z*: 467 ($M+H^+$). HRMS Calcd for $C_{24}H_{23}N_2O_8$: 467.1454. Found: 467.1443. Compound *Z*-**20**: Mp 71–73 °C. IR ν_{max} (KBr): 3425, 2930, 1790, 1727, 1659, 1567, 1512, 1374, 1234, 1141, 1060 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 2.14 (3H, s), 3.12 (1H, d, *J*=6 Hz), 3.58 (1H, ABX, J_{AX}=7 Hz, J_{AB}=13 Hz), 3.98 (1H, ddd, *J*=4, 7, 7 Hz), 4.35 (1H, ABX, J_{AX}=4 Hz, J_{AB}=13 Hz), 4.65 (1H, m), 4.99 (1H, dd, *J*=1, 10 Hz), 5.09 (1H, ddd, *J*=1, 3, 17 Hz), 5.32 (1H, ddd, *J*=5, 7, 7 Hz), 5.56 (1H, dd, *J*=3, 3 Hz), 7.58 (2H, dd, *J*=8, 15 Hz), 7.66 (1H, ddd, *J*=1, 8, 16 Hz), 7.93 (1H, dd, *J*=1, 8 Hz), 8.08 (1H, d, *J*=8 Hz), 8.28 (1H, dd, *J*=1, 7 Hz), 9.31 (1H, d, *J*=8 Hz). HRMS Calcd for $C_{24}H_{23}N_2O_8$: 467.1454. Found: 467.1445.

*4.1.17. (3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-2-[(E)-1-(1-pyrrolidinyl)carbonyl-1-(1-naphthoyl)amino]methylidene-3,4-dihydroxypiperidine (**21**).* Pyrrolidine (73 μL, 0.88 mmol) was added to a solution of *E*-**20** (400 mg, 0.88 mmol) in anhydrous THF (10 mL) at −20 °C, and the mixture was stirred for 15 min at the same temperature. The solvent was removed under reduced pressure to afford the product **21** (450 mg) as a colorless amorphous solid. Compound **58b**: Mp 218–220 °C. IR ν_{max} (KBr): 3379, 2977, 2881, 1742, 1715, 1651, 1619, 1453, 1399, 1238, 1051 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.87 (4H, br s), 2.06 (3H, s), 3.33 (4H, br s), 3.68 (1H, br s), 3.87 (1H, br s), 4.23 (1H, br s), 4.49 (2H, s), 4.82 (1H, br s), 5.09 (1H, br s), 5.15 (1H, d, *J*=11 Hz), 5.25 (1H, d, *J*=17 Hz), 5.82 (1H, br s), 7.35–7.59 (3H, m), 7.85 (2H, d, *J*=8 Hz), 7.93 (1H, d, *J*=8 Hz), 8.40 (1H, d, *J*=7 Hz). HRMS Calcd for $C_{28}H_{32}N_3O_8$: 538.2189. Found: 538.2195.

*4.1.18. (3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-3-hydroxy-2-[(E)-1-(1-pyrrolidinyl)carbonyl-1-(1-naphthoyl)amino]methylidene-4-methanesulfonyloxypiperidine (**22**).* Methanesulfonyl chloride (0.12 mL, 1.7 mmol) was added to a solution of **21** (450 mg) in anhydrous pyridine (10 mL) at 0 °C, and the mixture was stirred at the same temperature for 2 h. The mixture was then added $NaHCO_3$ and extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt) to afford the product **22** (380 mg, 72%, two steps) as a white powder. Compound **22**: Mp 111–119 °C. $[\alpha]_D^{22}$ +125.2 (*c* 0.30, $CHCl_3$). IR ν_{max} (KBr): 3248, 2976, 2877, 1747, 1715, 1666, 1611, 1512, 1452, 1396, 1359, 1291, 1229, 1177, 1139, 1099, 1056 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.87 (4H, br s), 2.04 (3H, s), 3.12 (3H, s), 3.15–3.57 (4H, m), 3.79 (1H, br s), 4.06 (2H, br s), 4.49 (1H, br s), 5.13–5.26 (3H, br s), 5.32 (1H, br s), 5.70 (1H, br

s), 7.35–7.67 (3H, m), 7.75 (1H, d, *J*=7 Hz), 7.83 (1H, d, *J*=8 Hz), 7.90 (1H, d, *J*=8 Hz), 8.33 (1H, d, *J*=8 Hz), 9.54 (1H, br s). HRMS Calcd for $C_{29}H_{34}N_3O_{10}S$: 616.1965. Found: 616.1978.

*4.1.19. (3R,4R,5R)-N-Allyloxycarbonyl-3-hydroxy-2-[(E)-1-(1-pyrrolidinyl)carbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**23**).* Potassium carbonate (110 mg, 0.80 mmol) was added to a solution of **22** (240 mg, 0.40 mmol) in MeOH (4.0 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and then at 10 °C for 4 h. After an addition of AcOEt, the mixture was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt) to afford the product **23** (110 mg, 58%) as a white powder. Compound **23**: Mp 89–95 °C. $[\alpha]_D^{23}$ +211.7 (*c* 0.27, $CHCl_3$). IR ν_{max} (KBr): 3267, 2980, 2877, 1712, 1668, 1649, 1615, 1512, 1449, 1397, 1337, 1289, 1259, 1223, 1133, 1065, 1045 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.82 (4H, br s), 2.71 (1H, br s), 3.18–3.53 (5H, m), 3.53–3.93 (2H, m), 4.24–4.46 (1H, m), 4.46–4.77 (2H, m), 5.12–5.36 (3H, m), 5.84 (1H, m), 7.45–7.62 (3H, m), 7.86 (1H, dd, *J*=2, 7 Hz), 7.93 (1H, d, *J*=8 Hz), 7.96 (1H, d, *J*=8 Hz), 8.51 (1H, d, *J*=9 Hz), 9.59 (1H, br s). HRMS Calcd for $C_{26}H_{28}N_3O_6$: 478.1978. Found: 478.1975.

*4.1.20. (3R,4R,5R)-3-Acetoxy-N-allyloxycarbonyl-2-[(E)-1-(1-pyrrolidinyl)carbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**24**).* Acetic anhydride (48 μL, 0.50 mmol) was added to a solution of **23** (80 mg, 0.17 mmol) in anhydrous pyridine (1.7 mL), and the mixture was stirred for 2 h. The mixture was added saturated $NaHCO_3$ at 0 °C, and extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the product **24** (86 mg) as a white powder. Compound **24**: Mp 85–91 °C. $[\alpha]_D^{23}$ +300.3 (*c* 0.14, $CHCl_3$). IR ν_{max} (KBr): 3302, 2974, 2878, 1712, 1678, 1642, 1511, 1486, 1436, 1396, 1337, 1256, 1132, 1057 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.91 (4H, br s), 2.07 (3H, s), 2.71 (1H, br s), 3.32–3.72 (5H, m), 3.72–4.00 (2H, m), 4.36–4.68 (3H, m), 5.22 (1H, dd, *J*=1, 10 Hz), 5.30 (1H, ddd, *J*=1, 3, 17 Hz), 5.69 (1H, s), 5.93 (1H, m), 7.47–7.63 (3H, m), 7.87 (1H, dd, *J*=2, 7 Hz), 7.89 (1H, d, *J*=7 Hz), 7.98 (1H, d, *J*=8 Hz), 8.56 (1H, d, *J*=7 Hz), 9.51 (1H, br s). HRMS Calcd for $C_{28}H_{30}N_3O_7$: 520.2084. Found: 520.2101.

*4.1.21. (3R,4R,5R)-3-Acetoxy-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**3**).* Tetrakis(triphenylphosphine) palladium (0) (20 mg, 17 μmol) and ammonium formate (210 mg, 3.4 mmol) were added to a solution of **24** (86 mg) in anhydrous CH_2Cl_2 (1.7 mL) at 0 °C, and the mixture was stirred at the same temperature for 1.5 h. After an addition of AcOEt, the mixture was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=3:1) to afford the product **3** (60 mg, 82%, two steps, *E*/*Z*=1:1) as a white foam. Compound **3**: IR ν_{max} (KBr): 3289, 3009, 2950, 1745, 1664, 1612, 1511, 1481, 1280, 1220, 1023 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.85 (2H, m) 1.93 (2H, m), 2.03 (3/2H, s), 2.08 (3/2H, s), 3.22 (1/2H, d, *J*=3 Hz), 3.24 (1/2H, d, *J*=4 Hz), 3.30 (1/2H, d, *J*=4 Hz), 3.33 (1/2H, d, *J*=5 Hz), 3.38–3.53 (3H, m), 3.73 (1/2H, m), 3.82 (1/2H, m), 3.99 (1/2H, AB, *J*=19 Hz), 4.01 (1/2H, AB, *J*=18 Hz), 4.22 (1/2H, AB, *J*=19 Hz), 4.30 (1/2H, AB, *J*=21 Hz), 5.46 (1H, s), 5.59 (1/2H, d, *J*=8 Hz), 5.67 (1/2H, d, *J*=8 Hz), 7.36–7.53 (3H, m), 7.66 (1/2H, d, *J*=7 Hz), 7.68 (1/2H, d, *J*=8 Hz), 7.79 (1H, d, *J*=8 Hz), 7.86 (1H, d, *J*=9 Hz), 8.33 (1H, d, *J*=8 Hz). HRMS Calcd for $C_{24}H_{26}N_3O_5$: 436.1874. Found: 536.1860.

*4.1.22. (3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-3,4-dihydroxypiperidine (**25**).* Triethylamine (0.32 mL, 2.3 mmol) was added to a solution of *E*-**20** (220 mg, 0.46 mmol) in MeOH (4.6 mL) at −20 °C, and the mixture was stirred for 10 min at the same temperature. After an addition of AcOEt, the mixture was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the product **25** (230 mg) as a white powder. Compound **25**: Mp 79–85 °C. IR ν_{max} (KBr): 3294, 2951, 1736, 1512, 1493, 1406, 1316, 1238, 1053 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 2.13 (3H, s), 2.84 (1H, br s), 3.63–3.84 (2H, m), 3.79 (3H, s), 4.50–4.76 (3H, m), 4.82 (1H, d, *J*=4 Hz), 5.12–5.40 (3H, m), 5.86 (1H, m), 7.46–7.70 (3H, m), 7.84 (1H, d, *J*=7 Hz), 7.92 (1H, d, *J*=8 Hz), 8.02 (1H, d, *J*=8 Hz), 8.38 (1H, d, *J*=6 Hz). HRMS Calcd for $C_{25}H_{27}N_2O_9$: 499.1717. Found: 499.1725.

*4.1.23. (3R,4S,5R)-5-Acetoxy-N-allyloxycarbonyl-3-hydroxy-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-4-methanesulfonyloxypiperidine (**26**).* Methanesulfonyl chloride (47 μL, 0.69 mmol) was added to a solution of **25** (230 mg) in anhydrous pyridine (4.5 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt, and the combined organic layers were washed with water and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=3:2) to afford the product **26** (220 mg, 82%, two steps) as a white powder. Compound **26**: Mp 95–99 °C. $[\alpha]_D^{24}$ +150.5 (*c* 0.57, $CHCl_3$). IR ν_{max} (KBr): 3307, 3022, 2951, 1731, 1666, 1512, 1491, 1437, 1402, 1362, 1318, 1221, 1177, 1141, 1094, 1050 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 2.11 (3H, s), 2.91 (1H, br s), 3.09 (3H, s), 3.55–3.89 (4H, m), 4.37 (1H, br s), 4.57 (2H, br s), 4.78 (1H, d, *J*=7 Hz), 5.09 (1H, br s), 5.20 (1H, d, *J*=9 Hz), 5.31 (1H, dd, *J*=1, 17 Hz), 5.50 (1H, br s), 5.85 (1H, m), 7.35–7.70 (3H, m), 7.80 (1H, d, *J*=7 Hz), 7.89 (1H, d, *J*=7 Hz), 7.99 (1H, d, *J*=8 Hz), 8.36 (1H, d, *J*=8 Hz). HRMS Calcd for $C_{26}H_{29}N_2O_{11}S$: 577.1492. Found: 577.1501.

*4.1.24. (3R,4R,5R)-N-Allyloxycarbonyl-3-hydroxy-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**27**).* Potassium carbonate (78 mg, 0.57 mmol) was added to a solution of **26** (160 mg, 0.29 mmol) in MeOH (3.0 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and then at 10 °C for 4 h. After an addition of AcOEt, the mixture was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=1:1) to afford the product **27** (55 mg, 44%) as a white powder. Compound **27**: Mp 76–82 °C. $[\alpha]_D^{26}$ +341.7 (*c* 0.45, $CHCl_3$). IR ν_{max} (KBr): 3297, 3013, 2951, 1728, 1666, 1512, 1493, 1436, 1403, 1333, 1272, 1213, 1131, 1038 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 3.43–3.58 (2H, m), 3.77 (3H, s), 3.93 (1H, AB, *J*=15 Hz), 4.39 (1H, AB, *J*=14 Hz), 4.58 (2H, m), 4.99 (1H, s), 5.13 (1H, s), 5.17 (1H, d, *J*=11 Hz), 5.32 (1H, d, *J*=17 Hz), 5.84 (1H, m), 7.42–7.67 (3H, m), 7.85 (1H, d, *J*=7 Hz), 7.92 (1H, d, *J*=8 Hz), 8.02 (1H, d, *J*=8 Hz), 8.39 (1H, d, *J*=8 Hz). HRMS Calcd for $C_{23}H_{23}N_2O_7$: 439.1505. Found: 439.1502.

*4.1.25. (3R,4R,5R)-3-Acetoxy-N-allyloxycarbonyl-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**28**).* Acetic anhydride (31 μL, 0.21 mmol) was added to a solution of **27** (45 mg, 0.10 mmol) in anhydrous pyridine (1.0 mL), and the mixture was stirred for 4 h. The mixture was added saturated $NaHCO_3$ at 0 °C, and extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the product **28** (47 mg) as a white powder. Compound **28**: Mp 67–73 °C. $[\alpha]_D^{26}$ +276.1 (*c* 0.21, $CHCl_3$). IR ν_{max} (KBr): 3319, 3016, 2945, 1735, 1718, 1674, 1510, 1483, 1435, 1401, 1373, 1337, 1280, 1253, 1230, 1057 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ: 2.07 (3H, s), 3.36 (1H, m), 3.49 (1H, dd, *J*=4, 8 Hz), 3.65–4.00 (1H, m), 3.80 (3H, s), 4.33–4.66

(3H, m), 5.17–5.38 (2H, m), 5.66 (1H, d, J=2 Hz), 5.84 (1H, m), 7.38–7.67 (3H, m), 7.87 (1H, dd, J=2, 7 Hz), 7.90 (1H, dd, J=1, 8 Hz), 7.98 (1H, d, J=8 Hz), 8.58 (1H, dd, J=1, 8 Hz). ^{13}C NMR (67.80 MHz, $CDCl_3$) δ_C: 20.5, 44.2, 50.8, 51.8, 52.6, 66.7, 67.1, 117.8, 118.1, 124.5, 125.6, 126.0, 126.4, 127.4, 128.1, 130.4, 131.4, 131.5, 131.8, 131.9, 133.7, 153.8, 163.0, 167.4, 171.5. HRMS Calcd for $C_{25}H_{25}N_2O_8$: 481.1611. Found: 481.1630.

*4.1.26. (3R,4R,5R)-3-Acetoxy-2-[(E)-1-methoxycarbonyl-1-(1-naphthoyl)amino]methylidene-4,5-epoxypiperidine (**4**).* Tetrakis(triphenylphosphine) palladium (0) (6.0 mg, 5.2 μmol) and ammonium formate (120 mg, 2.0 mmol) were added to a solution of **28** (47 mg) in anhydrous CH_2Cl_2 (1.0 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. After an addition of AcOEt, the mixture was washed with water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (AcOEt/hexane=3:2) to afford the product **4** (29 mg, 74%, two steps) as a white powder. Compound **4**: Mp 93–95 °C. $[\alpha]_D^{24}$ −16.1 (*c* 0.14, $CHCl_3$). IR ν_{max} (KBr): 3289, 3008, 2949, 1745, 1664, 1612, 1511, 1481, 1437, 1369, 1279, 1220, 1070, 1023 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.15 (3H, s), 3.50 (2H, m), 3.74 (3H, s), 3.86 (2H, s), 6.36 (1H, s), 6.76 (1H, br s), 7.47–7.58 (3H, m), 7.75 (1H, d, J=8 Hz), 7.86 (1H, dd, J=1, 8 Hz), 7.92 (1H, d, J=9 Hz), 8.37 (1H, d, J=8 Hz), 8.90 (1H, br s). HRMS Calcd for $C_{21}H_{21}N_2O_6$: 397.1400. Found: 397.1408.

4.2. Examination of relaxation assay of supercoiled plasmid DNA[5]

To a solution of supercoiled pBR 322 DNA (0.15 μg) in pH 7.0 TE buffer (9 μL) was added a DMSO solution of compound **3** or **4** (1 μL, 10–100 mM), and the mixture was incubated for 24 h at 37 °C. Under the concentrations examined, precipitation was not observed. The resulting DNA analysis was conducted using electrophoresis (tris-acetate–EDTA buffer, ethidium bromide 1.3 μM solution) on 0.7% native agarose gel at 7.4 v/cm for 30 min.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.08.027.

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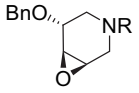
10. Our previous studies revealed that a free piperidine-amino group plays an important role in DNA cleavage.[5a] Compound **1** was shown to be more active than **31**, while **32** did not exhibit any activity up to 100 μM. We have examined the DNA binding of **2** with DNA, but could not obtain clear data probably due to nonspecific and multi-site binding of **2** with DNA. In addition, NMR analysis did not show any structural change of **1** or **2** by an addition of DNA.



1: R = H > **31**: R = Me > **32**: R = CO_2Et (inactive)

11. We also mention that both *N*-Alloc epoxypiperidines **24** and **28** do not show any activity up to 10 mM.
12. The NMR analysis of **3** or **4** shows only one form, and we could observe neither **29** nor **30**. Therefore the ratio of these compounds could not be calculated. However, based on the fact that the double bond of dehydroamino acid derivatives isomerizes via an imino–enol structure like **29** as shown in Figure 6, the equilibrium between **3** (or **4**) and **29** should exist. In addition, the formation of **30** was supported by our previous studies, which revealed that a free piperidine-amino group (electron-density and/or nucleophilicity of piperidine-amino group) plays an important role in DNA cleavage.[5,10,11]