Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure–activity relationships of the truncated norzoanthamines exhibiting collagen protection toward anti-osteoporotic activity

Hironori Inoue, Kazuho Tokita, Seketsu Fukuzawa *, Kazuo Tachibana *

Department of Chemistry, School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 28 February 2014
Revised 17 April 2014
Accepted 18 April 2014
Available online xxxx

Keywords:
Alkaloid
Norzoanthamine
Osteoporosis
Collagen

ABSTRACT

The marine alkaloid norzoanthamine is a candidate drug for osteoporosis treatment. Due to its structural complexity, simplified analogues possessing similar biological activities are needed for further research. Recently, we found that the bisaminal unit, representing two-thirds of the original structure, is a bioactive equivalent. We synthesized three kinds of further truncated norzoanthamines and evaluated their collagen protection activities. No analog with collagen protection activity comparable to that of the bisaminal unit was found. Thus, we confirmed the importance of the bisaminal unit for the collagen protection activity. Furthermore, we found that the recognition tolerance of the substrate collagen is relatively large by comparing both enantiomers.

1. Introduction

The members of the zoanthamine[1] (**1**) class of alkaloids possess unique structures and biological activities.[2] Among them, norzoanthamine[3] (**2**), isolated from the colonial zoanthid *Zoanthus* sp., suppresses interleukin-6 production and increases bone weight and density in osteoporosis model mice, making it a candidate drug for osteoporosis treatment.[4,5] In the course of our search for its mode of action, we found that it accelerates collagen-hydroxyapatite composite formation, due to its collagen protection activity.[6] Marine natural products often possess curious or complicated structures, and thus they are difficult to produce by chemical synthesis for further research. Although the total synthesis of norzoanthamine has already been achieved,[7–9] the design of a simplified structure with similar biological activity represents an important and promising way to supply natural product-oriented drugs. According to the structure–activity relationship study of norzoanthamine, its seco-norzoanthamine methylester (**3**) exhibited three-fold weaker inhibition of interleukin-6 induction than that of norzoanthamine.[4] Recently, we found a truncated norzoanthamine (TZ, **4**), which includes two-thirds of the original structure and exhibits similar collagen protection activity.[10] To acquire more detailed structure–activity relationship information about norzoanthamine, we divided the bisaminal unit of TZ into three parts: pseudo-truncated norzoanthamine (p-TZ, **5**), which is a lactone-deficient TZ; northern-truncated norzoanthamine (n-TZ, **6**), which is a monoaminal unit including the lactone; and southern-truncated norzoanthamine (s-TZ, **7**), which is a monoaminal unit without the lactone (Scheme 1). Both TZ and p-TZ were previously synthesized by Kobayashi's[11,12] and Williams'[13] groups, respectively, in 1998. In this report, we synthesized more simplified norzoanthamines (p-TZ, n-TZ, and s-TZ) based on Kobayashi's scheme and discussed their structure–activity relationships.

2. Results

2.1. Synthesis of p-TZ

The optically pure (99% ee) methyl ester **9** was prepared from the commercially available 2-methylcyclohexan-1-one (**8**).[14,15] The carbonyl group of the methyl ester **9** was protected by the Noyori method, to generate the acetal **10**. The methyl ester of the acetal **10** was reduced by DIBAL-H with careful equivalent control, and the simple aldehyde fragment **11** was obtained. Using the aldehyde fragment **11** and the sulfone fragment **12**,[8] the coupling reaction was conducted to produce the hydroxyl sulfone **13**. Continuously, the hydroxyl group was oxidized by TPAP, and the sulfone group was removed by sodium mercury amalgam to afford the ketone **15**. One pot deprotection and cyclization were initially conducted. After boiling in acidic medium and drying over Na_2SO_4, the protonated molecular ion peak of the p-TZ **5** ($M+H^+$) was observed in FAB-MS. Considering the R_f value (0, CH_3Cl/MeOH = 3:1) of the obtained product, it should be the acetic acid salt, and therefore this compound could not be purified by silica gel column chromatography without a triethylamine-containing eluant. The

* Corresponding authors. Tel.: +81 3 5841 4358; fax: +81 3 5841 4357.
E-mail address: seketsuf@chem.s.u-tokyo.ac.jp (S. Fukuzawa).

http://dx.doi.org/10.1016/j.bmc.2014.04.040
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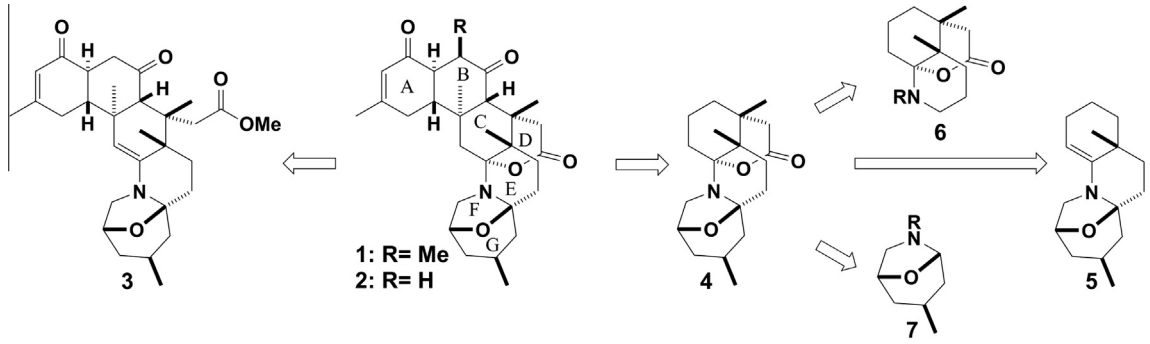


Scheme 1. Simplification of norzoanthamine.

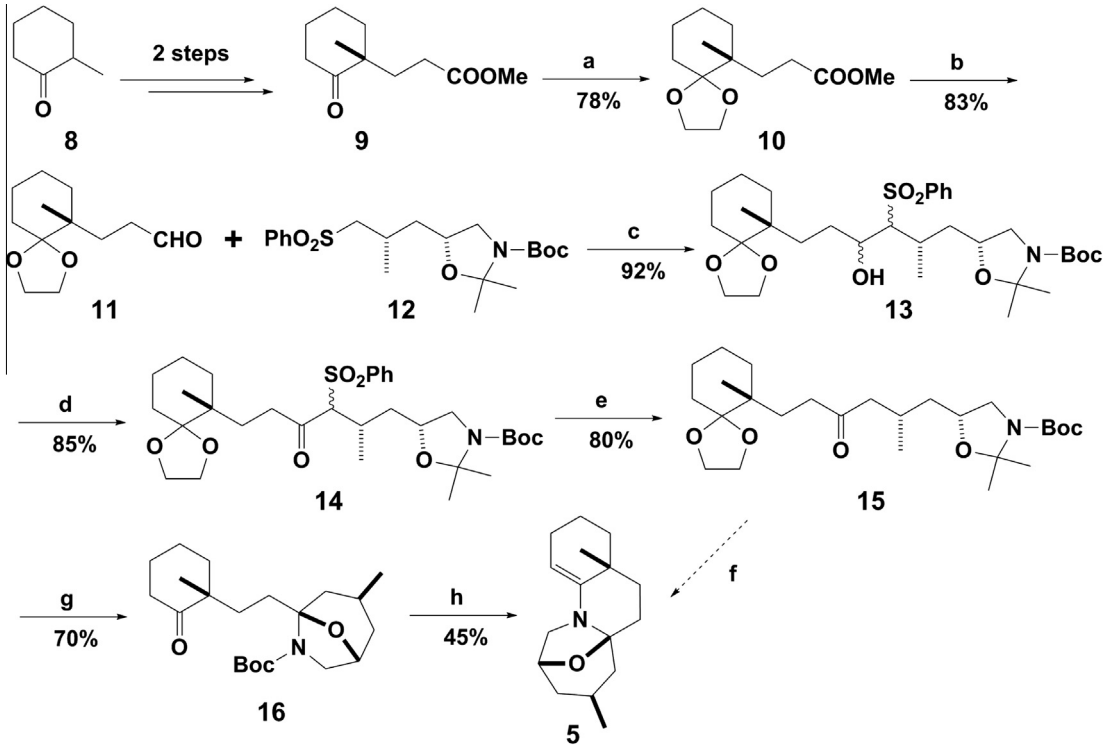
residual triethylammonium acetate was removed by activated alumina flash chromatography. However, only decomposition products were observed on TLC. Instead of using silica gel or alumina, the newly prepared product was purified by ODS column chromatography. After purification, it was quickly subjected to an NMR measurement, and the characteristic hydrogen peaks of the aminal and enamine structures were detected. However, this compound slowly degraded during the NMR measurement, and its structural elucidation was not completed. Therefore, we could not be certain whether this isolated compound was the desired one. In turn, we considered that this enamine structure is not stable under acidic conditions at high temperature, and thus used Lewis acid-promoted cyclization. First, the deprotection and the seven membered ring formation were conducted under acidic conditions with moderate heat.[9] Under these conditions, the Boc group was not deprotected. In the final step, the remaining Boc group was removed by the Lewis acid.[16] The generated secondary amine formed the enamine structure quickly, and p-TZ **5** was unambiguously obtained (Scheme 2).

2.2. Synthesis of n-TZ

The key intermediate **18** was prepared from the commercially available diketone **17** according to the Kobayashi's scheme.[11] An alkyl or an aryl substituent was introduced to the aldehyde fragment **18** by a reductive amination reaction to generate the amines **19**. The resultant secondary amino groups were then protected by Boc groups to obtain the carbamates **20**.[17] Deprotection of the TBS groups by TBAF gave the primary alcohols **21**. The obtained alcohols **21** were oxidized by TPAP to generate the aldehydes **22**, and continuously the Pinnick oxidation reaction was performed to afford the carboxylic acid **23**.[7] As the final step, an acid-mediated cyclization reaction gave n-TZ **6** (Scheme 3).[18]

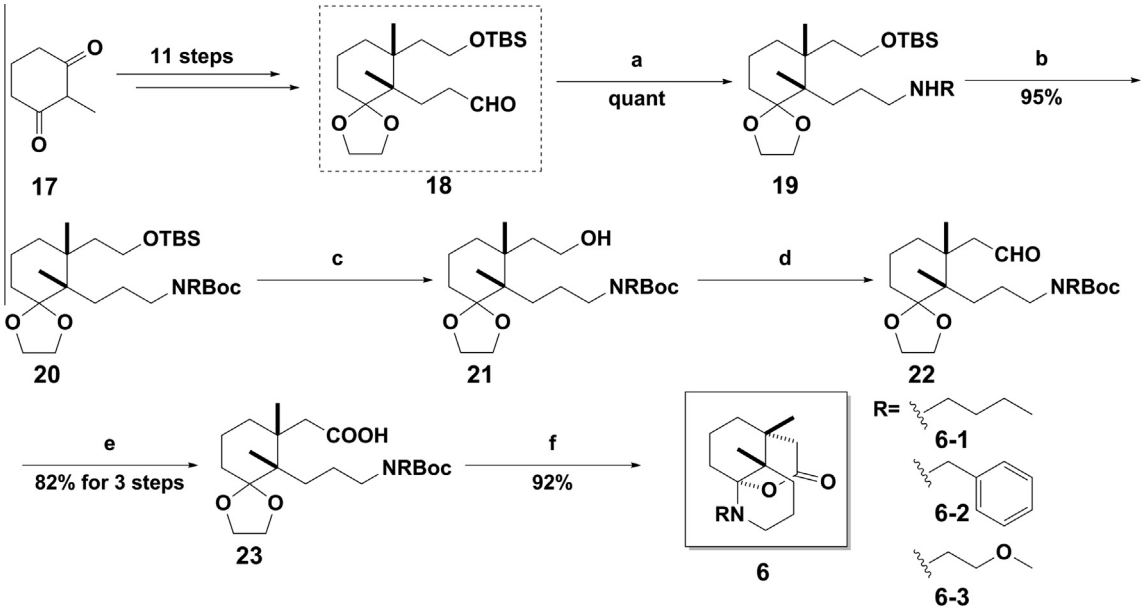
2.3. Synthesis of southern truncated norzoanthamine (s-TZ)

The diol **25** was prepared from D-glutamic acid **24** according to the Kobayashi's scheme.[11] The carbamate and hydroxyl groups of this intermediate were protected by acetonide groups, to generate



Scheme 2. Reagents and conditions: (a) $TMSO(CH_2)_2OTMS$, TMSOTf, CH_2Cl_2, −78 °C to rt; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) *n*-BuLi, THF, −78 °C; (d) TPAP, NMO, MS4A, CH_2Cl_2, rt; (e) 5% Na-Hg, Na_2HPO_4, MeOH, 0 °C to rt, 63%; (f) AcOH–H_2O (96:4), Na_2SO_4, rt to 100 °C; (g) AcOH–H_2O (96:4), rt to 60 °C; (h) TMSI, CH_3CN, 0 °C.

Please cite this article in press as: Inoue, H.; et al. *Bioorg. Med. Chem.* (2014), http://dx.doi.org/10.1016/j.bmc.2014.04.040



Scheme 3. Reagents and conditions: (a) R-NH_2, $NaBH_3CN$, CH_3CN, rt; (b) Boc_2O, Et_3N, DMAP, CH_3CN, 0 °C to rt; (c) TBAF, THF, rt; (d) TPAP, NMO, MS4A, CH_2Cl_2, rt; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH–H_2O, 0 °C to rt; (f) AcOH–H_2O (96:4), Na_2SO_4, rt to 100 °C.

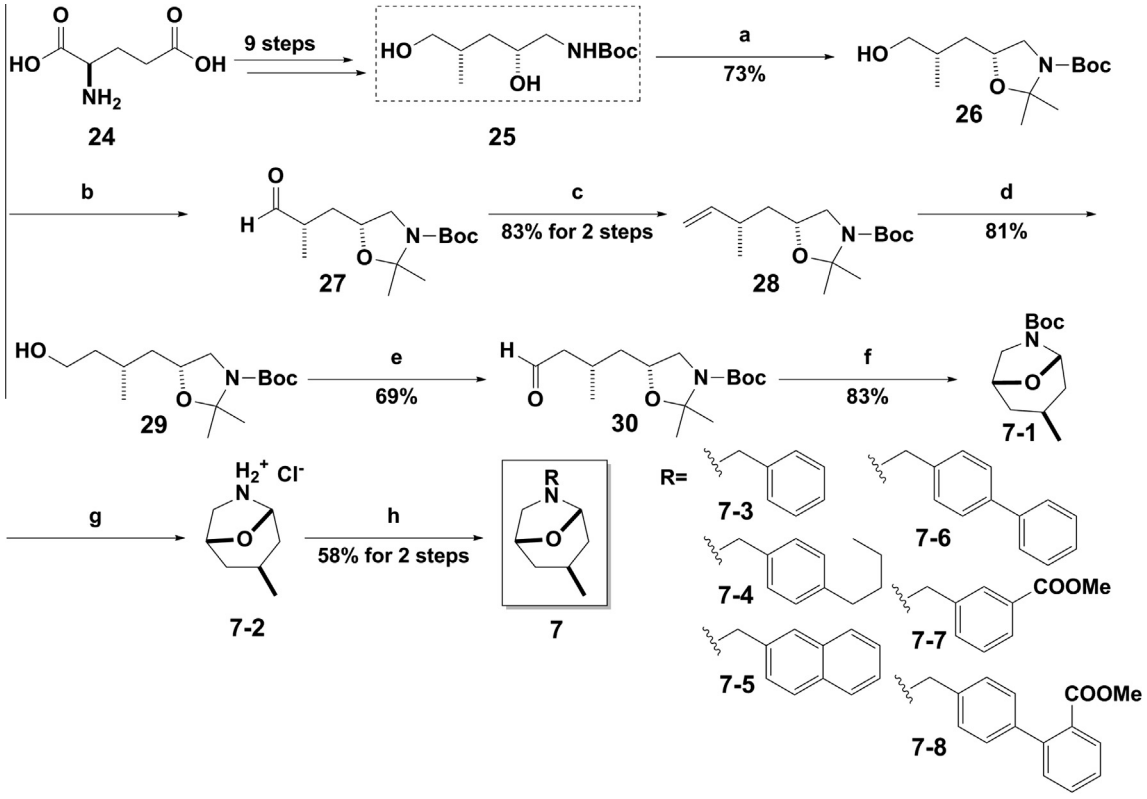
the protected aminal **26**. The aminal **26** was oxidized by SO_3·Py, and then the generated aldehyde **27** was converted to the terminal olefin **28** by a Wittig reaction. The hydroxyl group was introduced to the vinyl **28** by hydroboration. The primary alcohol **29** was oxidized to give the aldehyde **30**. Surprisingly, in comparison to the alcohol **26**, the alcohol **29** was very unstable. It occasionally decomposed during NMR measurements, and even during preservation in benzene below −20 °C. The difference between these two alcohols is only the length of the carbon skeleton. We could not find the reason why the additional C1 unit destabilizes the whole molecule. Furthermore, the aldehyde **30** was also unstable. Presumably, the moderate yield of this oxidation reaction was due to this instability. Therefore, all reactions were conducted as quickly as possible. The aldehyde **30** was cyclized under acidic conditions. When the reaction was performed with 2 N HCl, the yield was poor and a long reaction time was required. This was probably because the second cyclization reaction did not proceed quickly, and became the rate-determining step. Actually, the TLC spot of the intermediate was observed, and with time this spot disappeared and the spot of the carbamate **7**-**1** appeared. Therefore, AcOH–H_2O (96:4) was selected for the acidic conditions, and the yield was improved.[9] The Boc group of the carbamate **7**-**1** was then removed with a 4 N solution of HCl. The resultant amine **7**-**2** was too hydrophilic to purify by normal silica gel column chromatography. Therefore, the final reaction was performed without further purification. Aryl substituents were introduced to the secondary amine by an S_N2 reaction, to afford s-TZ **7** (Scheme 4).

2.4. Collagen protection assay

The collagen protecting activities of all TZ analogs are shown in Figures 1–5. After an incubation for 180 min, the residual collagen content of p-TZ **5** was about one-third of that of norzoanthamine **2** (Fig. 1). The monoaminal unit of norzoanthamine **2** including the lactone (n-TZ **6**) showed a significant decrease in collagen protection activity (Fig. 2). Although the collagen protection activity of the other monoaminal unit without the lactone (s-TZ **7**) was also weak, its degradation curve profiles were more gradual, as compared with those of n-TZ **6** (Figs. 3–5).

3. Discussion

For technical reasons, we could not examine all of the samples at once. Five samples for each collagen protection test are the maximum. To discuss the structure–activity relationships of the truncated norzoanthamines, we normalized their collagen protection activities after an incubation for 180 min. Their relative collagen contents against norzoanthamine **2** were calculated and are summarized in Table 1. The activity of TZ **4** was equal to that of norzoanthamine **2**, which was previously reported.[10] The collagen protecting activity of p-TZ **5** was weaker than those of norzoanthamine **2** and TZ **4**. The residual collagen content of p-TZ **5** was 31% of those of norzoanthamine **2** and TZ **4**, which indicated that the absence of the lactone moiety essentially decreased the activity. The lack of the hydrophobic space formed by the lactone or the planarity of the enamine might cause the reduced activity. Furthermore, we divided the bisaminal unit of the active analog TZ **4** into two monoaminals, n-TZ **6** and s-TZ **7**. Both further truncated analogs exhibited slightly different decreasing profiles. The significant decrease was observed in n-TZ **6**, and neither an alkyl nor aryl substituent on the nitrogen atom affected the activity. On the other hand, the simplest truncated analog, s-TZ **7**, showed interesting profiles. The simplest unit, the bicyclic monoaminal **7**-**2**, exhibited 31% activity as compared with the natural product **2**. The activity was increased by the addition of hydrophobic aryl groups (**7**-**3**, **4**) on the nitrogen atom. The activity was dependent on the aromatic ring size. Especially, the naphthylmethyl analog **7**-**5** showed the highest activity among the truncated norzoanthamines, except for TZ **4**. However, a biphenyl (**7**-**6**, **7**-**8**) or carbonyl (carbamate **7**-**1** and ester **7**-**7**) substituent exerted a negative effect. An ester group was also present in the seco-norzoanthamine methylester **3**, and it might cause a significant decrease in the collagen protection activity. The difference between a lactone and an ester is the structural rigidity. The enhanced fluctuation of the ester group might suppress interactions with target molecules. In addition, the enamine structure without a lactone in the seco-norzoanthamine methylester **3** is a common characteristic in p-TZ **5**. Thus, the lactone structure is considered to be the key substructure exhibiting the activity. However, n-TZ **6**, the minimum monoaminal-containing



Scheme 4. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH·H_2O, acetone, rt; (b) SO_3·Py, Et_3N, DMSO–CH_2Cl_2, 0 °C to rt; (c) $Ph_3^+CH_3Br^-$, NaHMDS, THF, 0 °C; (d) 9-BBN, $NaHCO_3$, H_2O_2, THF, 0 °C to rt; (e) TPAP, NMO, MS4A, CH_2Cl_2, rt; (f) AcOH–H_2O (96:4), 60 °C; (g) 4 N HCl, rt; (h) R-Br, Et_3N, THF, rt.

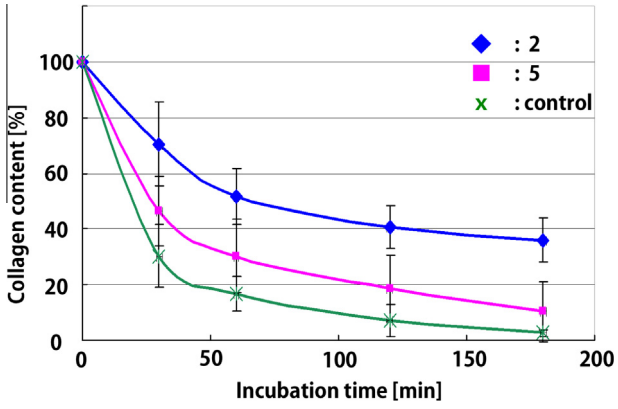


Figure 1. Collagen protection activities of p-TZ (10 μM sample concentration).

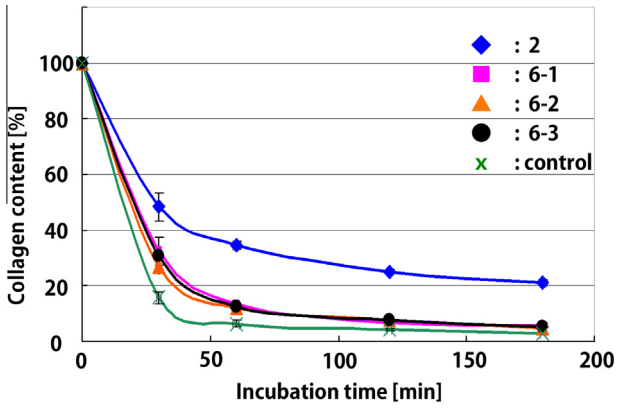


Figure 2. Collagen protection activities of n-TZs (10 μM sample concentration).

lactone, showed a significant decrease in the collagen protection activity. Comparing the collagen protection activities of both monoaminals **6** and **7**, the seven-membered bicyclic aminal s-TZ **7** is considered to be the more essential substructure than the lactone-containing bicyclic aminal n-TZ **6**. The smallest monoaminal, **7-2**, was about three-fold less active than the natural product **2** (Table 1), and its molecular weight (MW 127.2) is nearly one-third of that of the natural product **2** (MW 481.6).

We previously reported that norzoanthamine **2** functions by protecting the substrate protein, rather than competitively inhibiting the enzymatic activity. Generally, enzymes and substrate proteins are chiral molecules. To investigate the mode of action of bioactive molecules in more detail, comparing both enantiomers is effective.[19] Therefore, we synthesized the enantiomer, (−)-TZ **31**, from L-glutamic acid in the same manner as (+)-TZ **4** (Fig. 6)[11,12] and compared their collagen protection activities (Fig. 7). The activity was slightly decreased (76%, Table 1). The substrate type-I collagen is the chiral biopolymer, and is composed primarily of Gly-Pro-HyPro repeats. The fact that the enantiomer **31** showed 76% activity, as compared with the natural form **4**, implied that the recognition fidelity of the substrate collagen is relatively loose. The hydrophobic shape fitting might be the driving force for recognition.

4. Conclusion

As described above, we synthesized three kinds of further truncated norzoanthamines (p-, n-, and s-TZs), and evaluated their collagen protection activities. They possessed either the monoaminal, lactone-containing aminal (red square in Fig. 8) or the seven-membered bicyclic aminal (black square in Fig. 8). No analog with collagen protection activity comparable to the bisaminal unit (CDEFG ring in Scheme 1) **4** was found (Fig. 8). Thus, we

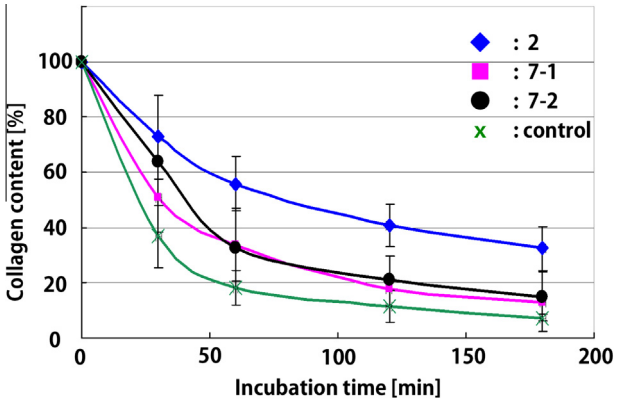


Figure 3. Collagen protection activities of s-TZs (10 μM sample concentration).

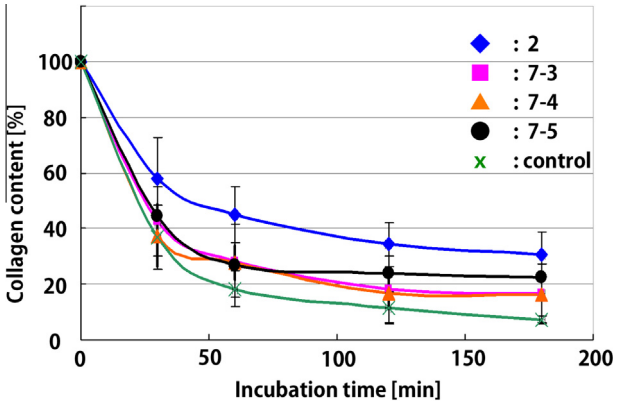


Figure 4. Collagen protection activities of s-TZs (10 μM sample concentration).

re-confirmed the importance of the bisaminal unit for exhibiting collagen protection toward an anti-osteoporotic activity. However, this investigation of further truncated analogs provided more detailed structure–activity relationship information. We found that the recognition tolerance of the substrate collagen is relatively large by comparing both enantiomers. Bottom-up design and synthesis based on our findings will enable us to provide more rationalized anti-osteoporotic drug candidates in the future.

5. Experimental section

5.1. General

^{1}H NMR spectra were recorded on a JEOL JNM-AL400 spectrometer operating at 400 MHz (^{1}H). Chemical shifts are reported in δ units and are referenced to the solvent, that is, 7.24 for $CDCl_3$. Multiplicities are indicated as: br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septet) or m (multiplet). Coupling constants (J) are reported in Hertz (Hz). Low and high resolution mass spectra were recorded on a JEOL JMS-MS700P mass spectrometer under fast atom bombardment (FAB) conditions using *m*-nitrobenzyl alcohol (NBA) as a matrix or a JEOL JMS-T100LP mass spectrometer with Direct Analysis in Real Time® unit (DART). IR spectra were recorded on a JASCO FT/IR-420 spectrometer. Optical rotations were recorded on a JASCO P-220 digital polarimeter. Analytical thin layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 plates (0.25 mm thickness). Column chromatography was performed using Kanto Chemical silica gel 60 N (40–100 mesh, spherical, neutral). Anhydrous solvents: acetone, benzene, dichloromethane (CH_2Cl_2), diethylether (Et_2O), *N*,*N*′-dimethylformamide (DMF), methanol (MeOH), pyridine and tetrahydrofuran (THF) were purchased from Kanto Chemical Co., Inc. or Aldrich, and used without

Table 1
Collagen protection activities of the truncated analogs relative to norzoanthamine **2**

Sample	Relative activity (%)
2	100
3[a]	5
4	100
5	31
6-1	17
6-2	11
6-3	15
7-1	23
7-2	31
7-3	47
7-4	45
7-5	65
7-6	20
7-7	25
7-8	25
31	76

[a] This data was not shown in this paper.[10]

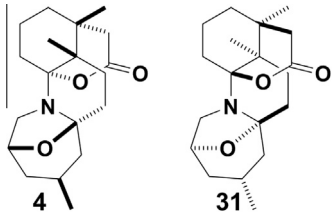


Figure 6. Structures of (+)-TZ **4** and (−)-TZ **31**.

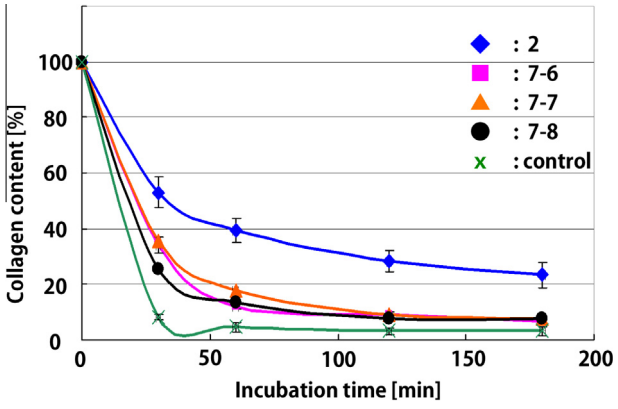


Figure 5. Collagen protection activities of s-TZs (10 μM sample concentration).

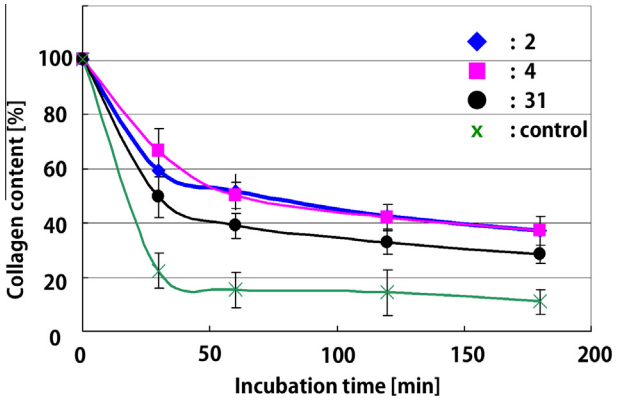


Figure 7. Collagen protection activities of TZs (10 μM sample concentration).

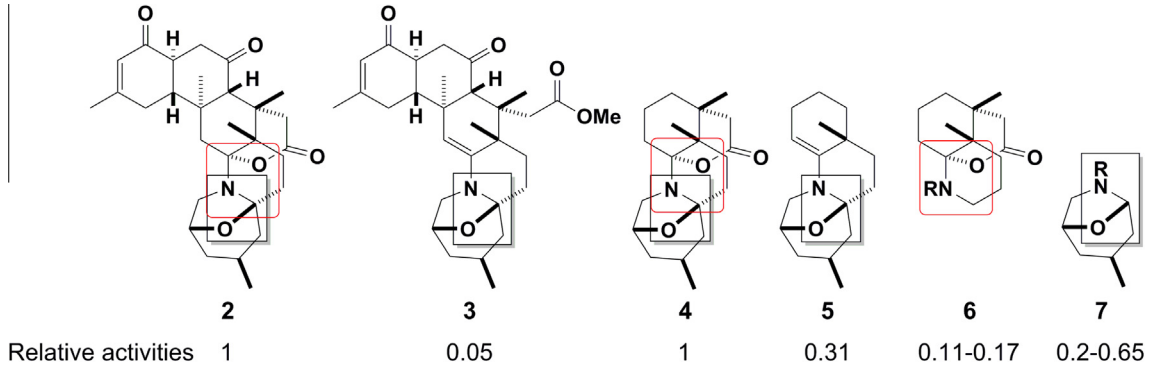


Figure 8. Structure–activity relationships of the truncated norzoanthamines.

further drying. All other reagents and solvents were purchased at highest commercial grade and used as supplied unless otherwise noted. All moisture sensitive reactions were performed under a static argon atmosphere in oven-dried glassware, otherwise noted.

5.2. Methyl (*R*)-3-(6-methyl-1,4-dioxaspiro[4.5]decan-6-yl)propanoate (10)

To a solution of the ketone **9** (302 mg, 1.52 mmol) in CH_2Cl_2 (5.3 mL) was added $TMSO(CH_2)_2OTMS$ (1.12 mL, 5.43 mmol) at room temperature. After the resulting mixture was cooled to −78 °C, to this mixture was added a solution of TMSOTf (55 μL, 0.304 mmol) and the mixture was stirred at same temperature. After 30 min, the mixture was allowed to warm to room temperature and kept at this temperature for 12 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 9:1) gave 286 mg (78% yield) of the acetal **10** as colorless oil: $[\alpha]_D^{26}$ +0.23 (*c* 0.44, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.85–3.76 (m, 4H), 3.55 (s, 3H), 2.24–2.11 (m, 2H), 1.75–1.60 (m, 2H), 1.53–1.40 (m, 4H), 1.31 (br s, 4H), 0.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 112.7, 64.9, 64.6, 51.5, 40.8, 34.7, 30.5, 30.1, 29.2, 23.5, 20.7, 19.3; IR (film, cm^{-1}) 2944, 2878, 1738, 1436, 1174, 1090; HRMS (FAB) calcd for $C_{13}H_{23}O_4$ $[(M+H)^+]$ 243.1518, found 243.1552.

5.3. (*R*)-3-(6-Methyl-1,4-dioxaspiro[4.5]decan-6-yl)propanal (11)

To a solution of the acetal **10** (270 mg, 1.11 mmol) in CH_2Cl_2 (1 mL) was added 0.99 M DIBAL-H (1.24 mL, 1.36 mmol) in hexane at −78 °C, and the mixture was stirred at that temperature for 30 min. The reaction mixture was quenched with MeOH and the mixture was allowed to warm to 0 °C. To this mixture was added a saturated aqueous solution of potassium sodium tartrate at that temperature and the mixture was stirred at room temperature overnight. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 9:1) gave 197 mg (83% yield) of the aldehyde **11** as colorless oil: $[\alpha]_D^{26}$ +30.1 (*c* 2.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.75 (s 1H), 3.95–3.86 (m, 4H), 2.45–2.30 (m, 2H), 1.82–1.66 (m, 2H), 1.61–1.51 (m, 4H), 1.41 (br s, 4H), 0.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.4, 112.7, 64.9, 64.6, 40.6, 39.2, 34.8, 30.4, 27.0, 23.5, 20.7, 19.5; IR (film, cm^{-1}) 2933, 2865, 2716, 2716, 1725, 1176, 1091; HRMS (FAB) calcd for $C_{12}H_{21}O_3$ $[(M+H)^+]$ 213.1412, found 213.1483.

5.4. *tert*-Butyl (*R*)-2,2-dimethyl-5-((*S*)-2-methyl-6-((*R*)-6-methyl-1,4-dioxaspiro[4.5]decan-6-yl)-4-oxohexyl)oxazolidine-3-carboxylate (15)

To a solution of sulfone **12** (131 mg, 0.330 mmol) in THF (3 mL) were added *t*-BuLi (0.209 mL, 0.330 mmol) at −78 °C, and then the resulting mixture was stirred at that temperature for 30 min. To this mixture was added a solution of aldehyde **11** (75.0 mg, 0.353 mL) in THF (4 mL) at −78 °C, and then the resulting mixture was stirred at that temperature for 30 min. The reaction was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 9:1) gave 197 mg (92% yield) of diastereomeric mixture of the hydroxyl sulfone **13** as yellow oil. To a solution of the diastereomeric mixture of hydroxyl sulfone **13** in CH_2Cl_2 (4 mL) was added MS4A (200 mg), NMO (114 mg, 0.973 mmol) and TPAP (1 portion) at room temperature. The resulting suspension was stirred at room temperature for 30 min. Purification by silica gel column chromatography (hexane/EtOAc = 4:1) gave 167 mg (85% yield) of diastereomeric mixture of the sulfone **14** as yellow oil. To a solution of diastereomeric mixture of the sulfone **14** in MeOH (4 mL) was added Na_2HPO_4 (1.17 g, 8.24 mmol) and 5% Na-Hg (1.17 g) at 0 °C. The resulting suspension was stirred at that temperature for 2 h. The reaction mixture was diluted with a saturated aqueous solution of NH_4Cl and EtOAc at room temperature, and the mixture was stirred at room temperature 30 min. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 5:1) gave 102 mg (80% yield) of the ketone **15** as yellow oil : $[\alpha]_D^{24}$ −11.6 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.10–4.03 (m, 1H), 3.94–3.85 (m, 4H), 3.71–3.58 (m, 1H), 3.03–2.96 (m, 1H), 2.48–2.24 (m, 4H), 2.11 (br s, 1H), 1.74–1.39 (m, 27H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.2, 152.0, 112.7, 93.0, 79.7, 71.8, 67.0, 64.9, 64.6, 51.2, 49.9, 40.6, 39.8, 38.3, 34.8, 30.4, 28.7, 28.5, 26.8, 24.7, 23.5, 20.7, 20.1, 19.4; IR (film, cm^{-1}) 2932, 2875, 1698, 1393, 1176, 1092; HRMS (FAB) calcd for $C_{26}H_{46}O_6N$ $[(M+H)^+]$ 468.3247, found 468.3353.

5.5. *tert*-Butyl (1*R*,3*S*,5*S*)-3-methyl-5-(2-((*R*)-1-methyl-2-oxocyclohexyl)ethyl)-8-oxa-6-azabicyclo[3.2.1]octane-6-carboxylate (16)

The ketone **15** (13.7 mg, 0.0293 mmol) was dissolved in AcOH–H_2O (96:4, 2 mL) at room temperature, and then the resulting mixture was stirred at 60 °C for 14 h. After the reaction mixture

was cooled to room temperature, the solution was concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 9:1) gave 7.5 mg (70% yield) of the carbamate **16** as colorless oil: $[\alpha]_D^{21}$ +86.6 (*c* 0.52, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.36–4.34 (m, 1H), 3.52–3.33 (m, 2H), 2.47–2.38 (m, 1H), 2.28–2.13 (m, 2H), 2.08–1.88 (m, 3H), 1.84–1.69 (m, 3H), 1.66–1.50 (m, 5H), 1.44 (s, 9H), 1.33–1.22 (m, 2H), 1.14 (dd, *J* = 11.9, 12.8 Hz, 1H), 1.00 (s, 3H), 0.86 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.3, 152.4, 94.0, 79.7, 72.5, 51.1, 48.0, 41.5, 39.7, 38.7, 37.7, 30.1, 29.7, 28.4, 27.6, 24.1, 22.2, 21.5, 21.0; IR (film, cm^{-1}) 2955, 2928, 1694, 1391, 1162, 1037; HRMS (FAB) calcd for $C_{21}H_{36}O_4N$ $[(M+H)^+]$ 366.2566, found 366.2625.

5.6. Pseudo-truncated norzoanthamine (p-TZ, 5)

To a solution of the carbamate **16** (7.5 mg, 0.0205 mmol) in CH_3CN (1 mL) was added TMSI (5.6 μL, 0.0409 mmol) at 0 °C, and the mixture was stirred at that temperature for 15 min. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and the mixture was stirred at that temperature for 5 min. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated under reducing pressure. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 19:1) gave 2.3 mg (45% yield) of the p-TZ **5** as yellow oil: $[\alpha]_D^{21}$ +78.6 (*c* 0.14, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.34 (t, *J* = 4.1 Hz, 1H), 4.08–4.04 (m, 1H), 2.88–2.78 (m, 2H), 2.38–2.25 (m, 2H), 2.14–2.11 (m, 2H), 2.03–1.99 (m, 3H), 1.80–1.58 (m, 3H), 1.50–1.30 (m, 5H), 1.19 (d, *J* = 6.9 Hz, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.7, 140.4, 114.4, 109.8, 71.5, 70.5, 47.0, 37.8, 36.3, 32.4, 32.1, 29.7, 25.6, 25.2, 23.7, 22.9, 18.1 (involving peaks due to imine); IR (film, cm^{-1}) 2924, 2847, 1636, 1456, ; HRMS (FAB) calcd for $C_{16}H_{26}ON$ $[(M+H)^+]$ 248.1936, found 248.1994.

5.7. General procedure of preparation of the amine (19)

To a solution of 3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propanal (**18**, 32.0 mg, 0.0832 mmol) in CH_3CN (1 mL) was added *n*-butylamine (25.0 μL, 0.253 mmol) and $NaBH_3CN$ (10.0 mg, 0.159 mmol) at room temperature, and the mixture was acidified (pH = 6) by adding AcOH, and stirred at that temperature for 12 h. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 19:1) gave 24.3 mg (58% yield) of *N*-(3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propyl)butan-1-amine (**19-1**) as colorless oil: $[\alpha]_D^{17}$ +38.7 (*c* 0.038, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.91 (ddd, *J* = 15.4, 11.0, 7.8 Hz, 2H), 3.81–3.78 (m, 2H), 3.60 (t, *J* = 7.3 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.54 (m, 2H), 1.73–1.20 (m, 16H), 0.98 (s, 3H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.87 (s, 12H), 0.03 (s, H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 114.2, 67.1, 64.9, 63.0, 60.8, 51.7, 49.7, 46.6, 40.2, 32.2, 32.0, 30.9, 28.3, 26.7, 26.1, 22.3, 20.5, 19.3, 18.4, 15.8, 14.0, −5.2; IR (film, cm^{-1}) 3309, 2927, 2874, 1253, 1078; HRMS (FAB) calcd for $C_{25}H_{52}O_3NSi$ $[(M+H)^+]$ 442.3638, found 442.3721.

5.8. *N*-Benzyl-3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propan-1-amine (19-2)

Quant as colorless oil: $[\alpha]_D^{17}$ +1.3 (*c* 0.68, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.34 (m, 2H), 7.32–7.29 (m, 3H), 4.48 (d, *J* = 6.1 Hz, 1H), 4.27 (d, *J* = 5.1 Hz, 1H), 3.94–3.83 (m, 2H), 3.81–3.75 (m, 4H), 3.60 (t, *J* = 7.3 Hz, 2H), 2.52 (t, *J* = 7.3 Hz, 2H), 2.26 (s, 2H), 2.13 (s, 2H), 1.71–1.23 (m, 13H), 0.97 (s, 3H), 0.87 (s, 9H), 0.85 (s, 3s), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 129.3, 128.4, 128.3, 126.8, 126.6, 114.2, 64.8, 63.0, 60.8, 54.0, 51.1, 47.3, 46.6, 40.2, 32.2, 31.0, 28.2, 26.8, 26.0, 22.3, 19.5, 19.3, 18.4, −5.2; IR (film, cm^{-1}) 3297, 2929, 2881, 1643, 1111, 1078; HRMS (FAB) calcd for $C_{28}H_{50}O_3NSi$ $[(M+H)^+]$ 476.3482, found 476.3580.

5.9. 3-((6*S*,7*R*)-7-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)-*N*-(2-methoxyethyl)propan-1-amine (19-3)

92% as yellow oil: $[\alpha]_D^{16}$ +7.5 (*c* 0.17, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.95–3.86 (m, 2H), 3.79 (dd, *J* = 6.1, 6.1 Hz, 2H), 3.60 (t, *J* = 7.3 Hz, 2H), 3.50 (t, *J* = 5.1 Hz, 2H), 3.34 (s, 3H), 2.80 (t, *J* = 5.2 Hz, 2H), 2.60–2.49 (m, 2H), 2.25 (br s, 1H), 2.06 (br s, 2H), 1.63–1.21 (m, 10H), 0.98 (s, 3H), 0.88 (s, 9H), 0.87 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 114.2, 71.7, 64.9, 62.9, 60.8, 59.2, 58.8, 51.7, 49.2, 46.5, 43.7, 40.2, 32.1, 30.9, 28.2, 26.6, 26.0, 22.3, 19.3, 18.4, –5.2; IR (film, cm^{-1}) 3326, 2928, 2880, 1253, 1078; HRMS (FAB) calcd for $C_{24}H_{50}O_4NSi$ $[(M+H)^+]$ 444.3431, found 444.3523.

5.10. General procedure of preparation of carbamate (20)

To a solution of the amine **19-1** (24.0 mg, 0.0543 mmol) in CH_3CN (1 mL) was added Et_3N (19.0 μL, 0.136 mmol), Boc_2O (19.0 μL, 0.0827 mmol) and DMAP (1 portion) at 0 °C, and the mixture was stirred at room temperature for 12 h. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 14:1) gave 27.5 mg (95% yield) of *tert*-butyl butyl(3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propyl) carbamate (**20-1**) as yellow oil: $[\alpha]_D^{18}$ +15.4 (*c* 0.057, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.96–3.86 (m, 2H), 3.83–3.75 (m, 2H), 3.60 (t, *J* = 7.3 Hz, 2H), 3.07 (br, 4H), 1.56–1.17 (m, 31H), 0.97 (s, 3H), 0.89 (t, *J* = 7.3 Hz, 3H), 0.87 (s, 12H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 114.2, 78.7, 64.9, 62.9, 60.7, 48.3, 46.8, 46.5, 40.2, 36.3, 32.1, 31.0, 30.4, 28.5, 27.6, 26.0, 25.5, 22.4, 20.1, 19.3, 18.4, 15.9, 13.9, –5.2; IR (film, cm^{-1}) 2929, 2876, 1693, 1251, 1078; HRMS (FAB) calcd for $C_{30}H_{60}O_5NSi$ $[(M+H)^+]$ 542.4163, found 542.4217.

5.11. *tert*-Butyl benzyl(3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propyl)carbamate (20-2)

73% as yellow oil: $[\alpha]_D^{18}$ +12.2 (*c* 0.13, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.10 (m, 5H), 4.47–4.32 (m, 2H), 3.92–3.67 (m, 4H), 3.59 (t, *J* = 7.3 Hz, 2H), 3.04–3.00 (br, 2H), 2.42 (br s, 1H), 1.59–1.23 (m, 20H), 0.94 (s, 3H), 0.87 (s, 9H), 0.83 (s, 3H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 128.4, 127.7, 127.1, 127.0, 114.2, 79.3, 64.9, 62.8, 60.7, 49.8, 48.0, 46.5, 40.2, 32.0, 32.0, 30.9, 28.5, 27.6, 26.0, 24.9, 22.4, 19.3, 18.4, 16.0, −5.2; IR (film, cm^{-1}) 2928, 2882, 1694, 1139, 1077; HRMS (FAB) calcd for $C_{33}H_{58}O_5NSi$ $[(M+H)^+]$ 576.4006, found 576.4053.

5.12. *tert*-Butyl (3-((6*S*,7*R*)-7-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propyl)(2-methoxyethyl)carbamate (20-3)

89% as colorless oil: $[\alpha]_D^{17}$ +8.0 (*c* 0.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.96–3.86 (m, 2H), 3.83–3.73 (m, 2H), 3.60 (t,

J = 7.1 Hz, 2H), 3.46 (br, 2H), 3.32 (br s, 5H), 3.11 (br, 2H), 2.31 (br, 1H), 1.53–1.16 (m, 20H), 0.97 (s, 3H), 0.87 (s, 9H), 0.86 (s, 3H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 114.2, 79.1, 71.2, 65.0, 62.9, 60.7, 58.8, 49.8, 46.7, 46.5, 40.2, 37.0, 32.1, 31.0, 28.5, 27.5, 26.0, 25.3, 22.4, 19.3, 18.4, 16.1, –5.2; IR (film, cm^{-1}) 2929, 2882, 1695, 1251, 1078; HRMS (FAB) calcd for $C_{29}H_{58}O_6NSi$ [$(M+H)^+$] 544.3955, found 544.4011.

5.13. General procedure of preparation of carboxylic acid (23)

To a solution of carbamate **20**-**1** (27.5 mg, 0.0507 mmol) in THF (1 mL) was added TBAF (1 M; 60.0 μL, 0.0600 mmol) at room temperature, and then resulting mixture was stirred at that temperature for 16 h. The reaction was quenched with water. The aqueous layer was extracted three times with EtOAc. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to afford the crude *tert*-butyl butyl(3-((6*S*,7*R*)-7-(2-hydroxyethyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-6-yl)propyl)carbamate(**21**-**1**) as yellow oil.

To a solution of the crude alcohol **21**-**1** in CH_2Cl_2 (1 mL) was added MS4A (36.0 mg), NMO (17.8 mg, 0.152 mmol) and TPAP (1 portion) at room temperature. The resulting suspension was stirred at room temperature for 10 min. Purification by silica gel column chromatography (hexane/EtOAc = 4:1) gave *tert*-butyl butyl (3-((6*S*,7*R*)-6,7-dimethyl-7-(2-oxoethyl)-1,4-dioxaspiro[4.5]decan-6-yl)propyl)carbamate (**22**-**1**) as yellow oil.

To a solution of aldehyde **22**-**1** in *t*-BuOH (1 mL) and water (0.2 mL) was added 2-methyl-2-butene (0.27 ml, 2.54 mmol) and aqueous solution of $NaClO_2$ (27.5 mg, 0.304 mmol) and NaH_2PO_4 (21.9 mg, 0.183 mmol) at 0 °C dropwise. The resulting mixture was stirred at room temperature for 3 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted three times with EtOAc. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 4:1) gave 15.8 mg (71% yield) of 2-((6*S*,7*R*)-6-(3-((*tert*-butoxycarbonyl)(butyl)amino)propyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-7-yl)acetic acid (**23**-**1**) as yellow oil: $[\alpha]_D^{18}$ +18.2 (*c* 0.042, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99–3.90 (m, 2H), 3.83–3.75 (m, 2H), 3.16–3.02 (m, 5H), 2.21 (d, J = 13.1 Hz, 1H), 1.64–1.42 (m, 21H), 1.31–1.21 (m, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.89 (t, J = 7.3 Hz, 3H); IR (film, cm^{-1}) 3127, 2929, 2873, 1697, 1152; HRMS (FAB) calcd for $C_{24}H_{44}O_6N$ [$(M+H)^+$] 442.3090, found 442.3168.

5.14. 2-((6*S*,7*R*)-6-(3-(Benzyl(*tert*-butoxycarbonyl) amino)propyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-7-yl) acetic acid (23-2)

73% Yield as yellow oil: $[\alpha]_D^{18}$ +1.7 (*c* 0.58, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.20 (m, 5H), 4.48–4.32 (m, 2H), 3.96–3.68 (m, 4H), 3.08 (br d, 3H), 2.17 (d, J = 12.9 Hz, 1H), 1.60–1.22 (m, 20H), 1.00 (s, 3H), 0.97 (s, 3H); IR (film, cm^{-1}) 3164, 2933, 2879, 1692, 1247, 1167; HRMS (FAB) calcd for $C_{27}H_{42}O_6N$ [$(M+H)^+$] 476.2934, found 476.2969.

5.15. 2-((6*S*,7*R*)-6-(3-((*tert*-Butoxycarbonyl)(2-methoxyethyl) amino)propyl)-6,7-dimethyl-1,4-dioxaspiro[4.5]decan-7-yl) acetic acid (23-3)

82% As yellow oil: $[\alpha]_D^{1.7}$ +10.2 (*c* 0.083, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00–3.92 (m, 2H), 3.84–3.74 (m, 2H), 3.46 (br s, 2H), 3.32 (br s, 5H), 3.15–3.13 (m, 3H), 2.23 (d, J = 13.3 Hz, 1H), 1.63–1.43 (m, 20H), 1.05 (s, 3H), 1.01 (s, 3H); IR (film, cm^{-1}) 3154, 2929, 2878, 1728, 1693, 1154, 1118; HRMS (FAB) calcd for $C_{23}H_{42}O_7N$ [$(M+H)^+$] 444.2883, found 444.3004.

5.16. General procedure of preparation of northern-truncated norzoanthamine (n-TZ, 6)

The carboxylic acid **23**-**1** (6.8 mg, 0.0154 mmol) was dissolved in AcOH–H_2O (96:4, 2 mL) at room temperature, and then the resulting mixture was stirred at 100 °C for 12 h. After the reaction mixture was cooled to room temperature, anhydrous Na_2SO_4 was added to the mixture. The mixture was stirred for 1 h at that temperature, and filtered and washed with MeOH. The filtrate was concentrated under reduced pressure. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 9:1) gave 2.2 mg (51% yield) of the n-TZ **6**-**1** as colorless oil : $[\alpha]_D^{23}$ –21.8 (*c* 0.022, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00–2.88 (m, 2H), 2.65 (dd, J = 11.5, 4.6 Hz, 1H), 2.52 (dd, J = 19.0, 1.7 Hz, 1H), 2.40 (ddd, J = 14.4, 7.1, 7.1 Hz, 1H), 2.22 (d, J = 19.0 Hz, 1H), 2.14–2.09 (m, 1H), 1.90–1.79 (m, 2H), 1.70–1.37 (m, 8H), 1.26–1.14 (m, 6H), 0.87 (t, J = 7.3 Hz, 3H), 0.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 100.9, 49.5, 47.1, 41.2, 38.6, 35.9, 35.2, 31.1, 29.1, 27.2, 23.1, 20.6, 20.4, 18.5, 17.8, 14.0; IR (film, cm^{-1}) 2918, 2871, 1716; HRMS (FAB) calcd for $C_{17}H_{30}O_2N$ [$(M+H)^+$] 280.2198, found 280.2266.

5.17. n-TZ 6-2

92% As colorless oil: $[\alpha]_D^{24}$ –8.54 (*c* 0.073, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.24 (m, 2H), 7.20–7.15 (m, 3H), 4.39 (d, J = 15.6 Hz, 1H), 4.31 (br s, 1H), 4.15–3.78(m, 1H), 3.35 (d, J = 15.6 Hz, 1H), 4.15–4.14 (br d, 1H), 2.88–2.81 (m, 1H), 2.62–2.59 (m, 1H), 2.52 (dd, J = 19.0, 1.7 Hz, 1H), 2.23 (d, J = 19.0 Hz, 1H), 2.24 (s, 1H), 2.12–2.03 (m, 2H), 1.88–1.76 (m, 3H), 1.62–1.41 (m, 5H), 1.29 (s, 3H), 1.19–1.12 (m, 1H), 0.84 (s 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 141.1, 128.5, 128.3, 127.3, 126.6, 104.5, 52.9, 47.6, 41.2, 38.8, 36.0, 35.1, 29.5, 27.1, 23.1, 20.5, 18.4, 17.7; IR (film, cm^{-1}) 2948, 1715, 1714, 1697; HRMS (FAB) calcd for $C_{20}H_{28}O_2N$ [$(M+H)^+$] 314.2042, found 314.2142.

5.18. n-TZ 6-3

59% as colorless oil: $[\alpha]_D^{22}$ –15.2 (*c* 0.053, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.44–3.39 (m, 2H), 3.32 (s, 3H), 3.13 (ddd, J = 15.1, 7.1, 7.1 Hz, 1H), 3.06–2.99 (m, 1H), 2.72–2.65 (m, 2H), 2.53 (dd, J = 19.0, 1.7 Hz, 1H), 2.22 (d, J = 19.0 Hz, 1H), 2.08–2.05 (m, 1H), 1.89–1.79 (m, 2H), 1.72–1.63 (m, 2H), 1.56–1.45 (m, 4H), 1.22–1.14 (m, 4H), 0.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 104.7, 71.3, 58.9, 49.5, 48.1, 41.2, 38.6, 35.9, 35.1, 29.2, 27.1, 23.1, 20.6, 18.4, 17.7; IR (film, cm^{-1}) 2947, 2874, 1716, 1116; HRMS (FAB) calcd for $C_{16}H_{28}O_3N$ [$(M+H)^+$] 282.1991, found 282.2086.

5.19. *tert*-Butyl (*R*)-5-((*S*)-3-hydroxy-2-methylpropyl)-2, 2-dimethyloxazolidine-3-carboxylate (26)

To a solution of *tert*-butyl ((2*R*,4*S*)-2,5-dihydroxy-4-methylpentyl)carbamate (**25**, 656 mg, 2.81 mmol) in dry acetone (56 mL) was added 2,2-dimethoxypropane (1.04 mL, 8.46 mmol) and *p*-TsOH·H_2O (26.7 mg, 0.140 mmol) at room temperature, and then the resulting mixture was stirred at that temperature for 4 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 3:1) gave 561 mg (73% yield) of the acetal **26** as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (ddd, J = 10.6, 6.4, 5.6 Hz, 1H), 3.68–3.60 (br d, 2H), 3.55–3.43 (m, 2H), 3.04 (br dd, 1H), 2.04 (br s, 1H), 1.83 (dt, J = 12.5, 6.3 Hz, 1H), 1.63–1.40 (m, 17H), 0.95 (d, J = 7.1 Hz, 3H); HRMS (FAB) calcd for $C_{14}H_{28}O_4N$ [$(M+H)^+$] 274.1940, found 274.2021.

5.20. *tert*-Butyl (*R*)-2,2-dimethyl-5-((*S*)-2-methylbut-3-en-1-yl)oxazolidine-3-carboxylate (28)

To a solution of the acetal **26** (146 mg, 0.534 mmol) in CH_2Cl_2 (5 mL) and DMSO (1.5 mL) was added Et_3N (0.595 mL, 4.27 mmol) and SO_3·Py (425 mg, 2.67 mmol) at 0 °C, and then the resulting mixture was stirred at room temperature for 1 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure to afford the crude aldehyde **27** as colorless oil.

To a solution of methyltriphenylphosphonium bromide (286 mg, 0.801 mmol) in THF (4 mL) was added NaHMDS (0.694 mL, 0.749 mmol) at 0 °C, and then the resulting mixture was stirred at that temperature for 1 h. The solution of the crude aldehyde **27** in THF (3 mL) was added to this reaction mixture via cannula at 0 °C, and then the resulting mixture was stirred at that temperature for 2 h. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted three times with EtOAc. Organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 19:1) gave 120 mg (83% yield) of the terminal olefin **28** as colorless oil: $[\alpha]_D^{24}$ −64.9 (*c* 0.029, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (ddd, *J* = 18.1, 10.0, 8.1 Hz, 1H), 4.99 (d, *J* = 17.1 Hz, 1H), 4.93 (d, *J* = 10.5 Hz, 1H), 4.02 (ddt, *J* = 10.4, 6.2, 5.2 Hz, 1H), 3.70–3.50 (br d, 1H), 3.01–2.90 (br dd, 1H), 2.30 (m, 1H), 1.75–1.41 (m, 15H), 1.01 (d, *J* = 6.6 Hz, 3H); IR (film, cm^{-1}) 2933, 2872, 1702, 1393; HRMS (FAB) calcd for $C_{15}H_{28}O_3N$ $[(M+H)^+]$ 270.1991, found 270.2054.

5.21. *tert*-Butyl (*R*)-5-((*R*)-4-hydroxy-2-methylbutyl)-2,2-dimethyloxazolidine-3-carboxylate (29)

To a solution of the terminal olefin **28** (120 mg, 0.445 mmol) in THF (5 mL) was added 9-BBN-H (1.07 mL, 0.535 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. To this mixture was added a saturated aqueous solution of $NaHCO_3$ (15 mL) and 30% H_2O_2 (7.5 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$. The aqueous layer was extracted three times with EtOAc. Organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 3:2) gave 104 mg (81% yield) of the alcohol **29** as colorless oil: $[\alpha]_D^{24}$ −49.2 (*c* 0.094, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (ddt, *J* = 10.2, 6.4, 5.6 Hz, 1H), 3.68 (br t, 2H), 3.62 (br dd, 1H), 2.98 (br dd, 1H), 1.73–1.36 (m, 20H), 0.95 (d, *J* = 6.3 Hz, 3H); IR (film, cm^{-1}) 3431, 2932, 2875, 1701, 1393; HRMS (FAB) calcd for $C_{15}H_{30}O_4N$ $[(M+H)^+]$ 288.2097, found 288.2190.

5.22. *tert*-Butyl (*R*)-2,2-dimethyl-5-((*S*)-2-methyl-4-oxobutyl)oxazolidine-3-carboxylate (30)

To a solution of the alcohol **29** (104 mg, 0.362 mmol) in CH_2Cl_2 (3.5 mL) was added MS4A (104 mg), NMO (127 mg, 1.08 mmol) and TPAP (1 portion) at room temperature. The resulting suspension was stirred at room temperature for 30 min. Purification by silica gel column chromatography (hexane/EtOAc = 7:1) gave 71.0 mg (69% yield) of the aldehyde **30** as colorless oil: $[\alpha]_D^{25}$ −39.6 (*c* 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 4.07 (ddd, *J* = 10.4, 6.4, 5.6 Hz, 1H), 3.71–3.58 (br dd, 1H), 3.05–2.96 (br dd, 1H), 2.47 (d, *J* = 10.7 Hz, 1H), 2.27 (d, *J* = 10.6 Hz, 1H), 2.26–2.18 (m, 1H), 1.64–1.43 (m, 17H), 1.01 (d, *J* = 6.6 Hz, 3H); IR (film, cm^{-1}) 2977, 2877, 2719, 1725, 1698, 1394; HRMS (FAB) calcd for $C_{15}H_{28}O_4N$ $[(M+H)^+]$ 286.1940, found 286.2027.

5.23. *tert*-Butyl (1*R*,3*S*,5*S*)-3-methyl-8-oxa-6-azabicyclo[3.2.1]octane-6-carboxylate (7-1)

The aldehyde **30** (38.1 mg, 0.134 mmol) was dissolved in AcOH–H_2O (96:4, 8 mL) at room temperature, and then the resulting mixture was stirred at 60 °C for 18 h. After the reaction mixture was cooled to room temperature, the solution was concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 4:1) gave 24.9 mg (83% yield) of the protected aminal **7-1** as colorless oil: $[\alpha]_D^{26}$ +50.0 (*c* 0.092, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (br d, 1H), 4.52 (m, 1H), 3.51–3.46 (br dd, 1H), 3.25–3.20 (br dd, 1H), 1.96–1.80 (m, 2H), 1.58–1.20 (m, 12H), 0.90 (d, *J* = 6.3 Hz, 3H); HRMS (FAB) calcd for $C_{12}H_{22}O_3N$ $[(M+H)^+]$ 228.1521, found 228.1610.

5.24. (1*R*,3*S*,5*S*)-3-Methyl-8-oxa-6-azabicyclo[3.2.1]octane (7-2)

The solution of the protected aminal **7-1** (8.16 mg, 0.0359 mmol) in 4 N HCl–EtOAc (1 mL) was stirred at room temperature for 30 min. The filtrate was concentrated under reduced pressure to afford the amine **7-2** as yellow oil (quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (s, 1H), 4.71 (s, 1H), 3.42 (br, 2H), 2.27–2.17 (m, 2H), 1.70–1.44 (m, 3H), 0.95 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.6, 87.81, 46.16, 37.53, 36.70, 22.49, 21.93.

5.25. General procedure of preparation of southern-truncated norzoanthamine (s-TZ, 7)

To a solution of the amine **7-2** (2.64 mg) in CH_3CN (0.5 mL) was added BnBr (20.0 μL, 0.168 mmol) and Et_3N (22.5 μL, 0.162 mmol) at room temperature. The resulting suspension was stirred at room temperature for 12 h. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl, and basidified with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted three times with EtOAc. Organic layer was washed with brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc = 9:1) gave 1.58 mg (58% yield for 2 steps) of s-TZ **7-3** as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.20 (m, 5H), 4.70 (s, 1H), 4.48 (m, 1H), 3.90 (d, *J* = 13.4 Hz, 1H), 3.76 (d, *J* = 13.4 Hz, 1H), 3.03 (d, *J* = 9.8 Hz, 1H), 2.64 (dd, *J* = 9.8, 6.6 Hz, 1H), 2.14–2.02 (m, 1H), 1.40–1.34 (m, 2H), 1.25–1.17 (m, 2H), 0.87 (d, *J* = 6.6 Hz, 3H); IR (film, cm^{-1}) 2916, 2843, 1635, 1507; HRMS (DART) calcd for $C_{14}H_{20}ON$ $[(M+H)^+]$ 218.1467, found 218.1560.

5.26. s-TZ (7-4)

72% as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.1 Hz, 2H), 4.70 (s, 1H), 4.48 (m, 1H), 3.86 (d, *J* = 13.4 Hz, 1H), 3.72 (d, *J* = 13.4 Hz, 1H), 3.03 (d, *J* = 9.8 Hz, 1H), 2.64 (dd, *J* = 9.7, 6.4 Hz, 1H), 2.57 (t, *J* = 7.8 Hz, 2H), 2.14–2.02 (m, 1H), 1.62–1.54 (m, 9H), 1.25–1.17 (m, 6H), 0.92–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.24, 128.21, 93.2, 74.6, 61.9, 57.5, 41.2, 38.7, 35.3, 33.6, 23.0, 22.4, 21.6, 13.9; IR (film, cm^{-1}) 2916, 2843, 1635, 1507; HRMS (DART) calcd for $C_{18}H_{28}ON$ $[(M+H)^+]$ 274.2093, found 274.2159.

5.27. s-TZ (7-5)

65% as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (dd, *J* = 8.2, 5.8 Hz, 4H), 7.53 (d, *J* = 7.3 Hz, 1H), 7.46–7.40 (m, 2H),

4.75 (s, 1H), 4.51 (m, 1H), 4.06 (d, J = 13.7 Hz, 1H), 3.91 (d, J = 13.4 Hz, 1H), 3.03 (d, J = 9.8 Hz, 1H), 2.71 (dd, J = 9.7, 6.4 Hz, 1H), 2.16–2.08 (m, 1H), 1.25–1.17 (m, 4H), 0.92–0.87 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 133.3, 132.5, 127.9, 127.7, 127.6, 126.9, 126.7, 125.9, 125.5, 93.3, 77.2, 62.3, 57.5, 38.7, 30.9, 23.0, 21.8; HRMS (DART) calcd for $C_{18}H_{22}ON$ [$(M+H)^+$] 268.1623, found 268.1713.

5.28. s-TZ (7-6)

77% As colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, J = 11.3, 8.5 Hz, 4H), 7.43–7.39 (m, J = 7.3 Hz, 5H), 7.46–7.40 (m, 2H), 4.75 (s, 1H), 4.51 (m, 1H), 4.06 (d, J = 13.7 Hz, 1H), 3.91 (d, J = 13.4 Hz, 1H), 3.03 (d, J = 9.8 Hz, 1H), 2.71 (dd, J = 9.7, 6.4 Hz, 1H), 2.16–2.08 (m, 1H), 1.25–1.17 (m, 4H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.0, 128.7, 127.0, 93.3, 87.7, 61.8, 58.2, 57.6, 54.3, 41.2, 38.7, 30.9, 29.7; HRMS (DART) calcd for $C_{20}H_{24}ON$ [$(M+H)^+$] 294.1780, found 294.1860.

5.29. s-TZ (7-7)

62% As colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 4.69 (s, 1H), 4.50–4.48 (m, 1H), 3.95 (d, J = 13.4 Hz, 1H), 3.90 (s, 3H), 3.78 (d, J = 13.6 Hz, 1H), 3.03 (d, J = 9.8 Hz, 1H), 2.63 (dd, J = 9.7, 6.4 Hz, 1H), 2.12–2.05 (m, 1H), 1.49–1.31 (m, 3H), 1.25–1.18 (dt, J = 6.3, 2.4 Hz, 2H), 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.1, 133.6, 130.8, 130.1, 129.0, 128.8, 94.1, 75.3, 62.4, 58.2, 56.1, 53.8, 52.8, 41.4, 39.3, 23.7, 22.4; HRMS (DART) calcd for $C_{16}H_{22}O_3N$ [$(M+H)^+$] 276.1521, found 276.1589.

5.30. s-TZ (7-8)

77% As colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 7.8 Hz, 1H), 7.50 (dt, J = 7.6, 1.5 Hz, 1H), 7.25 (m, 3H), 4.73 (s, 1H), 4.51–4.49 (m, 1H), 3.95 (d, J = 13.4 Hz, 1H), 3.80 (d, J = 13.6 Hz, 1H), 3.61 (s, 3H), 3.07 (d, J = 9.8 Hz, 1H), 2.67 (dd, J = 9.7, 6.4 Hz, 1H), 2.12–2.07 (m, 1H), 1.27–1.20 (dt, J = 6.3, 2.4 Hz, 2H), 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 139.8, 139.2, 131.2, 130.1, 129.7, 128.2, 128.0, 127.1, 96.1, 93.4, 74.6, 61.9, 57.5, 51.9, 41.1, 38.6, 23.0, 21.8; HRMS (DART) calcd for $C_{22}H_{26}O_3N$ [$(M+H)^+$] 352.1834, found 352.1894.

5.31. (−)-Tz (31)

62% Of colorless amorphous: $[\alpha]_D^{24}$ −2.4 (c 0.152, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.51–4.49 (m, 1H), 3.19 (t, J = 14.6 Hz, 1H), 3.19 (d, J = 14.4 Hz, 1H), 2.52 (dd, J = 19.0, 2.2 Hz, 1H), 2.22 (d, J = 19.0 Hz, 1H), 2.30–2.20 (m, 1H), 2.06 (dd, J = 13.2, 5.1 Hz, 1H), 1.99 (dd, J = 14.2, 4.6 Hz, 1H), 1.91–1.64 (m, 6H), 1.63–1.49 (m, 3H), 1.41 (td, J = 13.2, 2.9 Hz, 1H), 1.25–1.10 (m, 1H), 1.17 (s, 3H), 1.05 (t, J = 12.7 Hz, 1H), 0.87 (s, 3H), 0.87 (t, J = 5.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 105.6, 90.2, 74.5, 47.7, 44.8, 41.1, 39.2, 35.9, 35.5, 30.5, 30.1, 25.3, 23.6, 23.3, 22.1, 18.8, 18.6; IR (film, cm^{-1}) 2941, 1705, 1408, 1394, 1258; HRMS (DART) calcd for $C_{19}H_{30}O_3N$ [$(M+H)^+$] 320.2147, found 320.2243.

5.32. Collagen protection assay

The TZ analogs were dissolved in 1 N HCl. The resultant solution was then passed through a syringe filter to remove the insoluble impurities. After condensation, the weights of the hydrochloride salts were accurately measured. Norzoanthamine and buffer only were selected as positive and negative controls, respectively. Incidentally, the NZ preserved in our laboratory was used. Acid solubilized type I collagen from rat tail stock solution (BD Biosciences, San Jose, CA, USA) was diluted with 50 mM Tris buffer (pH 7.4), containing 100 mM NaCl and 5 mM $CaCl_2$, to make a 1.11 mg/mL solution. A 450 μL portion of the substrate collagen solution was placed in a 1.5-mL microtube. A 50 μL aliquot of each compound, in 50 mM Tris buffer (pH 7.4) containing 100 mM NaCl and 5 mM $CaCl_2$, was added, and the solution was gently mixed by vortexing. The resultant mixture was then incubated at 37 °C for 5 min. When the sample solution became cloudy, the sample preparation was performed again. The enzymatic reaction was started by adding 5 μL of 2 mg/mL collagenase (from *Clostridium histlyticum*, 1000 Mandle units/mg, EC 3. 4. 24. 3, Lot. LTK2485, Wako, Osaka, Japan) at 37 °C. After various time intervals (30, 60, 120, 180 min), an 80 μL aliquot was collected in a 1.5-mL microtube, and was stained with 1.0 mL Sirius red dye solution (Biocolor, Carrickfergus, County Antrim, UK). After gentle mixing for 30 min at room temperature, the collagen-dye complex was centrifuged at 15,000 rpm and 4 °C for 10 min. The supernatant was then drained to remove the unbound dye solution. The pellet was dissolved in 1.0 mL of 0.1 N NaOH with vigorous mixing, and then 200 μL of the mixture was transferred to a 96-well microtiter plate to measure the absorbance of the released dye at 540 nm, using a VARIOSKAN FLASH microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). All measurements were performed more than 3 times, and average values and errors were computed.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas: Creation of Biologically Functional Molecules (17035021 and 18032025), and The Global COE Program for Chemistry Innovation through Cooperation of Science and Engineering, from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and SEKISUI CHEMICAL CO., LTD.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.04.040.

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