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

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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

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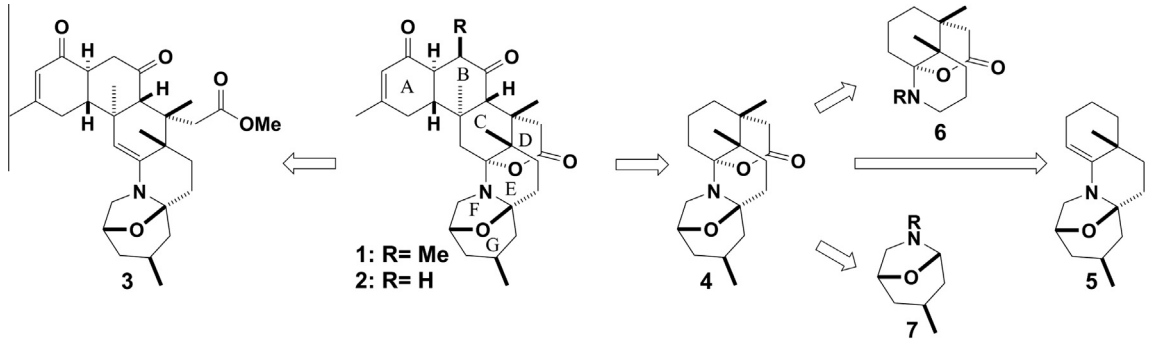


Scheme 1. Simplification of norzoanthamine.

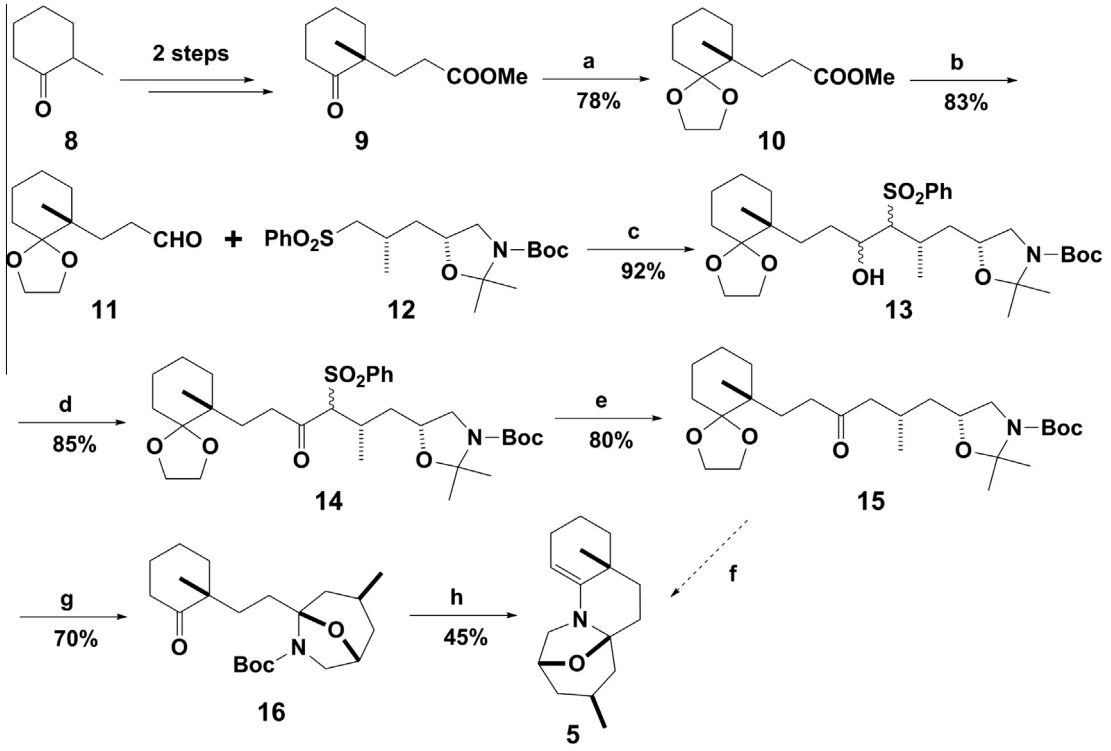
residual triethylammonium acetate was removed by activated alumina flash chromatography. However, only decomposition products were observed on TLC. Instead of using silica gel or alumina, the newly prepared product was purified by ODS column chromatography. After purification, it was quickly subjected 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.

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