Pergamon

Tetrahedron Letters 39 (1998) 7313–7316

TETRAHEDRON LETTERS

Application of L-Threonine Aldolase-Catalyzed Reaction for the Preparation of a Peptidic Mimetic of RNA : A Leading Compound of Vero-toxin Inhibitors

Tsuyoshi Miura,[a] Masayuki Fujii,[b] Kazushi Shingu,[a] Ikuo Koshimizu,[a] Junko Naganoma,[a] Tetsuya Kajimoto,[a] Yoshiteru Ida*[a]

[a] *School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.*

[b] *Department of Industrial Chemistry, Faculty of Engineering, Kinki University in Kyushu, Kayanomori, Iizuka 820-0011, Japan.*

Received 9 June 1998; revised 21 July 1998; accepted 23 July 1998

Abstract: A peptidic mimetic of RNA having a guanine-adenine-guanine code as a base sequence was prepared as a leading compound of Vero-toxin inhibitors. The synthesized hexapeptide is composed from two γ-guanyl-β-hydroxy-α-L-amino acids and one γ-adenyl-β-hydroxy-α-L-amino acid, which were prepared by using L-threonine aldolase-catalyzed reaction, in addition to three glycines. On designing this peptide, the β-hydroxyl groups in the novel α-amino acids are compared to the 2'-hydroxyl groups of RNA, and glycine was chosen as the mimic of the phosphate groups in the RNA backbone.

Esherichia coli O-157 produces a fatal hemorrhagic colitis causative protein, which is named Vero-toxin.[1] Antibiotics which are commonly utilized at the clinical level do not work so effectively once the toxin has passed through the alimentary membrane and the patients start to have loose bowels. The biochemical mechanism of its toxicity expression is similar to that of ricin, a lectin from *Ricinus communis*.[2] Both proteins act as *N*-glycanase to cleave adenine from the 4323th adenosine unit of human 28s ribosomal ribonucleic acid (*r*-RNA). Since both the 3'- and 5'-sides of this adenosine are linked to guanosines through a phosphate group, RNA and its derivatives which have the guanine-adenine-guanine (G-A-G) code as a partial base sequence are expected to inhibit Vero-toxin.[3] However, ubiquitous ribonucleases can easily cleave RNA chains to small fragments of ribonucleotides, which could no longer have inhibitory activity toward Vero-toxin.

In this article, we would like to report the preparation of a peptidic mimetic of RNA (**1**) as a promising leading compound of Vero-toxin inhibitors by using L-threonine aldolase-catalyzed reaction as a key step. L-Threonine aldolase from *Candida humicola* (AKU 4586) catalyzes the aldol condensation which affords β-hydroxy-α-L-amino acids from glycine and aldehydes, as reported previously by one of the authors.[4] In our design of the peptidic RNA, the β-hydroxyl groups in the α-amino acids are compared to the intrinsic 2'-hydroxyl group in RNA, and glycine was chosen as the mimic of the phosphate groups in the RNA backbone (Figure 1). The novel peptides including **1**, which were designed in this way, could have a long metabolic half-life time due to the resistance to both natural ribonucleases and proteases. As expected, the derivatives of

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01567-6

acetaldehyde which have adenine or guanine at the α-carbon (**2**, **3**)[5] were good substrates of the enzymatic aldol condensation, to give γ-adenyl-β-hydroxy-α-L-amino acids (**4a**, **4b**) and γ-guanyl-β-hydroxy-α-L-amino acids (**5a**, **5b**).[6] The *erythro*-isomers (**4a**, **5a**) can be respectively used as mimetics of adenosine and guanosine, since both the β-carbon of **4a** (or **5a**) and the 2'-carbon in RNA have the same configuration, *i.e.*, the hydroxyl groups on both carbons orientate to the α-face in Figure 1. In order to separate the *erythro*-isomer from the *threo*-one, the isomeric mixture of **4a** and **4b** was derived to benzyl esters (**6a**, **6b**) with benzyl alcohol and *p*-toluenesulfonic acid. Furthermore, each benzyl ester (**6a**, **6b**) was derived to oxazoline (**7a**, **7b**) with methyl phenylimidate to determine the configuration of the β-carbon by means of NOE technique.[7] The isomeric mixture of **5a** and **5b** was treated in the same way to afford benzyl esters (**8a**, **8b**) and oxazolines (**9a**, **9b**).

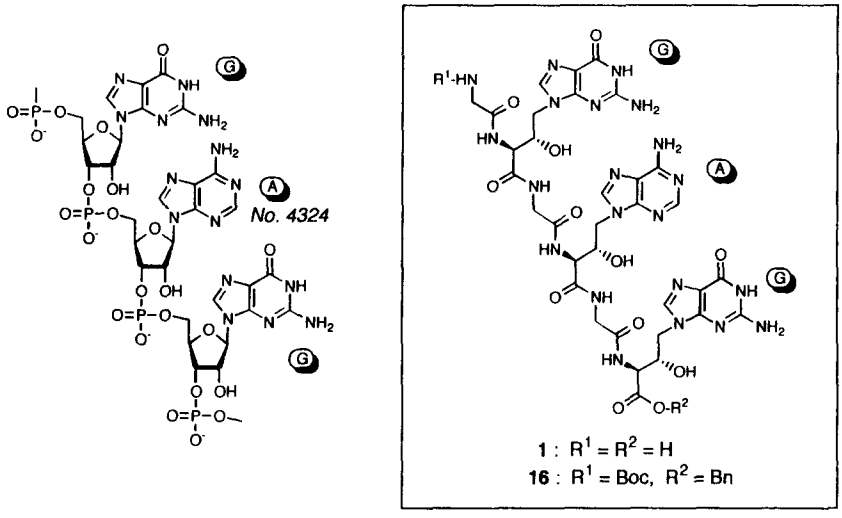
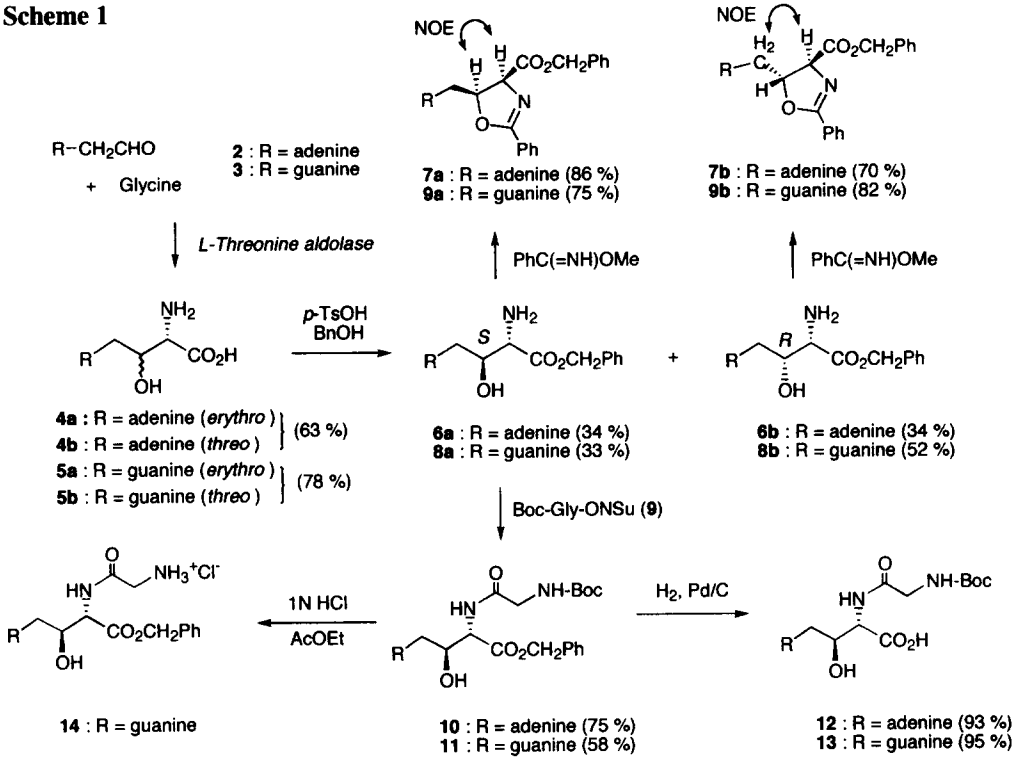


Figure 1. Structures of Vero-toxin Recognized RNA Sequence (left) and Its Peptidic Mimetic (right)

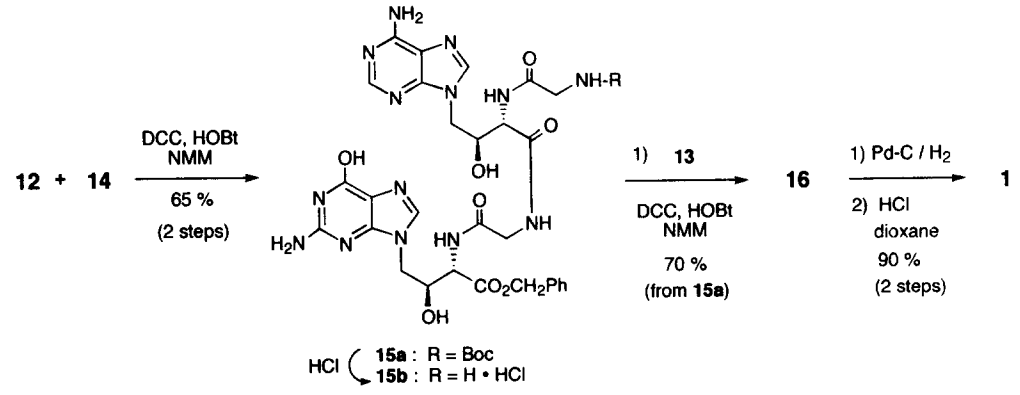
In order to synthesize our designed peptidic RNA, **6a** and **8a** were respectively coupled with *N*-hydroxylsucciminyl Boc-glycine (**9**, Boc-Gly-ONSu)[8] to provide benzyl *N*-Boc-γ-adenyl-L-*allo*-threonylglycine (**10**) and benzyl *N*-Boc-γ-guanyl-L-*allo*-threonylglycine (**11**). The benzyl groups of dipeptide **10** and **11** were removed by hydrogenation on Pd-C to afford **12**[9] and **13**[10], and the *N*-Boc group of **11** was deprotected with 1M HCl in ethyl acetate to afford **14**.[11] Compounds **12** and **14** were condensed with DCC and HOBt in the presence of *N*-methylmorpholine (NMM) in DMF[12] to give tetrapeptide **15a**. The *N*-Boc group of **15a** was deprotected with 1M HCl in dioxane to give **15b**, which was then condensed with **13** in the same way. The obtained hexapeptide **16** was deprotected with hydrogenation and following acid treatment to afford the target molecule **1**[13] (Scheme 2). The synthesized peptide **1** inhibited Vero-toxin in the μM range in the assay based on poly(U)-directed polyphenylalanine production.[14] Further derivative synthesis is now in progress and the details of an inhibitory assay of **1** toward Vero-toxin will be reported in the near future.

Our strategy for the preparation of peptidic RNA is also applicable to the synthesis of mimetics of other biologically functional RNAs.

Scheme 1



Scheme 2



Acknowledgements. We thank the Ministry of Education, Science, and Culture in Japan for a Grant-in Aid (No. 09771923). We are thankful to Drs. Yasuaki Hirai and Masazumi Miyakoshi for their useful discussion, and to Ms. Kimiko Shiobara, Dr. Mitsugu Tomioka, and Ms. Yuki Odanaka for their technical support in measuring the MS and NMR spectra.

References and Notes.

1. O'Brien, A. D.; La Veck, G. D. *Infect. Immun.*, **1983**, *40*, 675.
2. a) Endo, Y.; Tsurugi, K. *J. Biol. Chem.*, **1987**, *262*, 8128.
   b) Endo, Y.; Mitsui, K.; Mochizuki, M.; Tsurugi, K. *J. Biol. Chem.*, **1987**, *262*, 5908.
3. Chen, X.-Y.; Link, T. M.; Schraman, V. L. *J. Am. Chem. Soc.*, **1996**, *118*, 3067.
   and also see Good, L.; Nielsen, P. E. *Proc. Natl. Acad. Sci. U.S.A.*, **1998**, *95*, 2073.
4. a) Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.*, **1995**, *36*, 4081.
   b) Shibata, K.; Shingu, K.; Vassilev, V. P.; Nishide, K.; Fujita, T.; Node, M.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.*, **1996**, *37*, 2791.
5. a) Martinez, A. P.; Lee, W. W. *J. Org. Chem.*, **1965**, *30*, 317.
   b) Doel, M. T.; Jones, A. S.; Taylor, N. *Tetrahedron Lett.*, **1969**, *37*, 2285.
   c) Nollet, A. J. H.; Huting, C. M.; Pandit, U. K. *Tetrahedron*, **1969**, *25*, 5971.
6. Both β-hydroxy-α-amino acids were subjected to the reaction with D- and L-amino acid oxidases to determine the stereochemistry of the α-carbon.[4a]
7. Futagawa, S.; Inui, T.; Shibata, T. *Bull. Chem. Soc. Jpn.*, **1973**, *46*, 3308.
8. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.*, **1964**, *86*, 1839.
9. Compound **12**: ^{1}H-NMR (CD_3OD) δ 1.46 (9H, s, *t*-Bu), 3.84 (2H, s, C*H*$_2$ of glycine), 4.35 (3H, m), 4.48 (1H, dd, *J* = 14.0, 2.7 Hz, N-C*H*H), 8.19 (1H, s, adenine H-8), 8.21 (1H, s, adenine H-2). ^{13}C-NMR (CD_3OD) δ 28.7, 45.0, 48.0, 58.2, 72.0, 80.9, 119.8, 143.8, 150.9, 153.6, 157.2, 158.5, 172.5, 176.0. TOF MS: 432 ($M+Na^+$), ($C_{16}H_{23}N_7O_6$ MW, 409).
10. Compound **13**: ^{1}H-NMR (CD_3OD) δ 1.49 (9H, s, *t*-Bu), 3.80 and 3.85 (2H, each d, C*H*$_2$ of glycine), 4.17 (1H, dd, *J*= 14.6, 8.9 Hz, N-C*H*H), 4.30 (3H, m), 7.77 (1H, s, C*H* of guanine). TOF MS: 426 ($M+H^+$), ($C_{16}H_{23}N_7O_7$ MW, 425).
11. Compound **14**: ^{1}H-NMR (D_2O) δ 3.87 and 3.91 (2H, each d, *J*= 16.5 Hz, C*H*$_2$ of glycine), 4.27 (1H, dd, *J*= 13.7, 7.6 Hz, N-CH*H*), 4.38 (1H, m, C*H*-OH), 4.42 (1H, dd, *J* = 13.7, 3.7 Hz, N-C*H*H), 4.81 (1H, d, *J*= 4.3 Hz, C*H*-CO_2Bn), 5.17 and 5.20 (2H, each d, *J* = 12.5 Hz, C*H*$_2$-Ph), 7.37 (5H, m, Ph-*H*×5), 8.57 (1H, s, C*H* of guanine). ^{13}C-NMR (D_2O) δ 41.3, 48.2, 56.5, 69.0, 69.7, 109.7, 129.1, 129.6, 129.7, 135.6, 139.0, 151.1, 155.9, 156.3, 168.0, 170.5. TOF MS: 416 ($M+H^+$), ($C_{18}H_{21}N_7O_5$ MW, 415).
12. König, W.; Geiger, R. *Chem. Ber.*, **1970**, *103*, 788.
13. Compound **1**: ^{1}H-NMR (CD_3OD) δ 3.67-3.80 (6H, m, C*H*$_2$×3 of glycine), 4.18-4.37 (9H, m), 4.67-4.82 (3H, m), 8.159, 8.160, 8.162, and 8.171 (4H, each s, C*H*×4 of adenine and guanine).
14. Barbieri, L.; Valbonesi, P.; Gorini, P.; Pession, A.; Stirpe, F. *Biochem. J.*, **1996**, *319*, 507.