Bioorganic & Medicinal Chemistry Letters 19 (2009) 2103–2106

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of novel neamine derivatives effectively targeting to RNA

Yanli Xu, Hongwei Jin, Zhenjun Yang, Liangren Zhang, Qi Wang, Manning Li, Lihe Zhang *

State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, PR China

ARTICLE INFO

Article history:
Received 26 December 2008
Revised 18 February 2009
Accepted 9 March 2009
Available online 13 March 2009

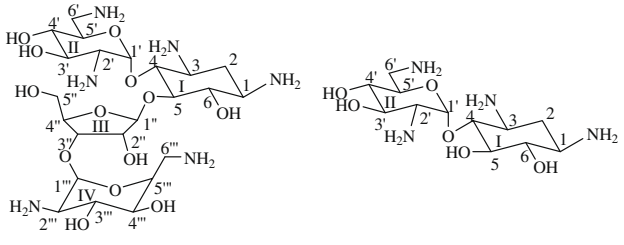
Keywords:
Neamine derivative
TAR RNA
16S RNA
Molecular modeling

ABSTRACT

Three new derivatives of neamine, **3** (NE), **6** (NEA) and **9** (NEL), were synthesized by connecting arginine or lysine to 5-hydroxyl group of neamine using ethylenediamine as a linker. The binding affinities of these derivatives to A site of 16S RNA and TAR RNA indicate that the modification on 5-hydroxyl of neamine by amino acid can enhance the binding affinity of neamine. Compound **9** (NEL) shows some antibacterial activities. These results demonstrate that modification on 5-hydroxyl group of neamine may provide a promising way for the development of potential candidates effectively targeting to RNAs.

RNA has become an attractive target for drug design due to its structural complexity and diversity and the important roles played in the process of life.[1,2] The recognition between mRNA and tRNA is an important step in the process of protein synthesis, and in bacteria the recognition occurs at the A site decoding region of 16S rRNA.[3] Neomycin B (Fig. 1), an effective aminoglycoside antibiotic, can bind potentially to the A site of 16S RNA and induce miscoding or inhibition of the bacterial protein synthesis, thus leading to bacterial cell death in the end.[4] Besides binding to 16S RNA, neomycin B can also potentially target to some other RNAs including HIV-1 trans-activation response element (TAR) RNA.[5] The interaction between TAR RNA and Tat protein has been known the key step in the transcription of HIV-1 genome by activating and stabilizing the synthesis of HIV-1 mRNA.[6] Neomycin B can prevent the binding of Tat protein to TAR RNA thus inhibiting the replication of HIV-1 virus.[7] Unfortunately, neomycin B can not be directly used in clinic mainly because of its nonspecific electrostatic interaction to RNA in a large part which causes the high toxicity.[8] In addition, it is relatively unstable and prone to be modified by aminoglycoside modifying enzymes (AME) which can lead to drug resistance.[9]

Neamine (Fig. 1), a simplified neomycin B mimic, keeps the same targeting site as neomycin B and indicates the lower toxicity. However, compared with neomycin B, it shows only poor antibiotic activity and can not also be clinically used.[10] It appears a promising way to keep the minimal structural motif and optimize the structure of neamine. Many efforts have been made to prepare new neamine derivatives potentially targeting to RNAs, among which amino acid modified aminoglycosides has been proved to be an effective strategy.[11,12] Arginine-rich peptide region of HIV Tat and Rev proteins is the important binding site in the recognition of the target RNAs.[13,14] Many aminoglycoside-arginine conjugates (AACs) and arginine peptide-aminoglycoside conjugates (APACs) have been successfully synthesized, including arginine conjugates of neomycin B, paromomycin, gentamicin and kanamycin A.[15–26] These AACs and APACs all display a high binding affinities to HIV virus RNAs and show potent antiviral activities. Lysine-rich protein is another one of the HIV RNA binding proteins.[13] The mono-lysine neamine conjugate and hexa-lysine neomycin conjugate also both showed higher bioactivities compared with their mother compound neamine and neomycin, respectively.[27,28,17] Up to now, all of the reported aminoglycoside conjugates were designed connecting neamine with arginine, arginine peptide or lysine directly to one of amino groups on the aminosugar moiety. Here, we reported the synthesis of neamine derivatives, **3** (NE), **6** (NEA) and **9** (NEL),

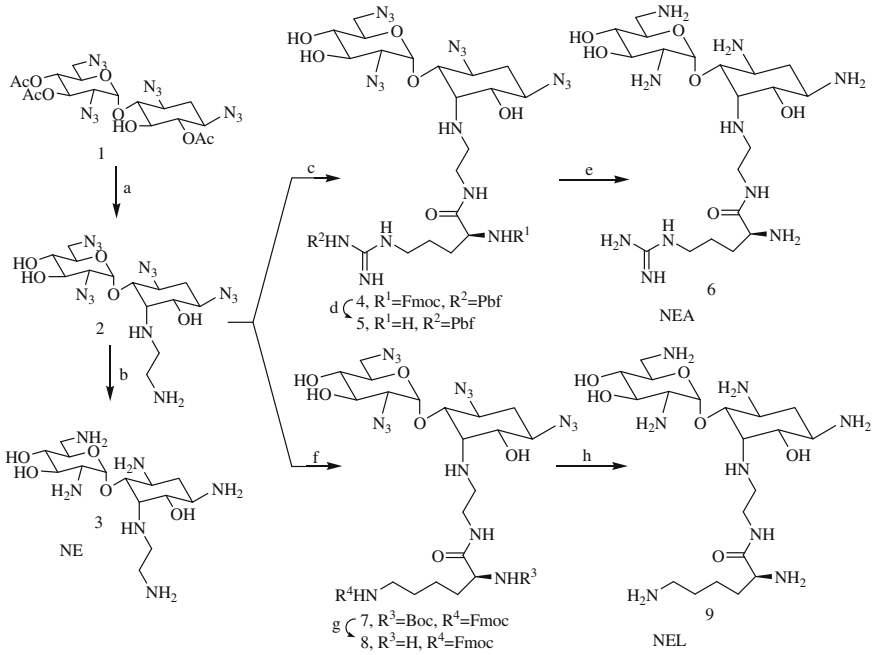


Neomycin B Neamine

Figure 1. Structures of neomycin B and neamine.

* Corresponding author. Tel.: +86 10 82801700; fax: +86 10 82802724.
E-mail address: zdszlh@bjmu.edu.cn (L. Zhang).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.021



Scheme 1. Synthesis of neamine derivatives **3** (NE), **6** (NEA) and **9** (NEL). Reagents and conditions: (a) (i) Tf_2O, Py, CH_2Cl_2; (ii) $H_2N(CH_2)_2NH_2$, rt; (b) H_2S, $Py/Et_3N/H_2O$ (4/3/2), rt; (c) Fmoc-Arg(Pbf)-OH, HOBt, DCC, DMF, 0 °C to rt; (d) Et_2NH/DMF (1:9), rt; (e) (i) $CF_3COOH/H_2O/PhSCH_3$ (94/3/3); (ii) H_2S, $Py/Et_3N/H_2O$ (4/3/2), rt; (f) Boc-Lys(Fmoc)-OH, HOBt, DCC, DMF, 0 °C to rt; (g) CF_3COOH/CH_2Cl_2 (1:4), rt; (h) (i) Et_2NH/DMF (1:9); (ii) H_2S, $Py/Et_3N/H_2O$ (4/3/2), rt.

in which amino acid modified on the 5-hydroxyl of neamine (Scheme 1). The amino groups on neamine play an important role in their binding to the target RNAs. Keeping the amino group free in the neamine molecule and modification of other positions of neamine may lead the derivatives contributing a various interaction to RNA. In this report, compounds **3** (NE), **6** (NEA) and **9** (NEL) show the interesting binding properties to the A site of 16S RNA and TAR RNA and the binding data were discussed by molecular modeling. In addition, compound **9** (NEL) exhibits some antibacterial activities against *Staphylococcus* aureus (MIC = 25 μM) and *Escherichia coli* (MIC = 50 μM).

Compound **1** and **2** were obtained by the published procedures.[29,30] As shown in Scheme 1, the reduction of all of four azido-groups on compound **2** can directly afford the desired product **3** (NE) in 83% yield. For the synthesis of compounds **6** (NEA) and **9** (NEL), Fmoc-Arg(Pbf)-OH or Boc-Lys(Fmoc)-OH was condensed with compound **2** in the presence of HOBt/DCC in DMF at room temperature to obtain the intermediate **4** or **7** in 79% or 74% yield, respectively. After removal of the protected groups on arginine/lysine moiety, compound **5** and **8** were obtained in 85% and 76% yields, respectively. As shown in Scheme 1, Fmoc group on arginine α-amino group and lysine ε-amino group can be removed using Et_2NH/DMF (1:9) and the deprotections of Pbf group on arginine δ-guanidino group and the Boc group on lysine α-amino group can be completed using $CF_3COOH/H_2O/PhSCH_3$ (94/3/3) and CF_3COOH/CH_2Cl_2 (1:4), respectively. Compounds **6** (NEA) and **9** (NEL) were obtained in 64% and 71% yield, respectively, by the reduction of intermediate **5** and **8** by H_2S in a mixture of pyridine and triethylamine. All the new neamine derivatives **3**, **6** and **9** were purified by reversed phase chromatography on C18 column (1‰ CF_3COOH in H_2O) and obtained as the appropriate trifluoroacetic salts in the end. The intermediates and final products were characterized by ^1H NMR, ^{13}C NMR, HRMS and optical rotations.

The binding properties of three neamine derivatives **3**, **6** and **9** to the A site of 16S RNA (Fig. 2) were evaluated by SPR (Surface Plasmon Resonance) using Biacore 3000 instrument, the dissociation constants (K_D value in μM) were calculated from the slope

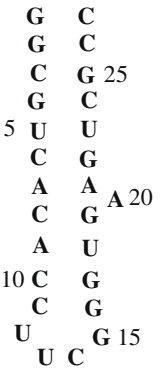


Figure 2. Structure of A site of 16S RNA.

of the *Scatchand* plot.[31,32] The SPR results for neamine and its three derivatives **3**, **6** and **9** binding to the A site of 16S RNA are summarized in Table 1. The K_D value of neamine was determined as 19 μM. After modification of an ethylenediamine at 5-hydroxyl of neamine, compound **3** shows 7 μM of K_D value which is 2.7 times potential in comparison with neamine. Interestingly, compounds **6** and **9** further increase their potential in binding to the A site of 16S RNA and pose 2 μM and 1 μM of K_D values which indicate 3.5 and 7 times potential in comparison with derivative **3**, respectively.

The binding properties of neamine and its derivatives **3**, **6** and **9** to TAR RNA (Fig. 3) were also determined by SPR.[31,32] The results

Table 1
The K_D values of neamine and its derivatives **3**, **6** and **9** binding to A site of 16S RNA

Compd	16S RNA (μM)
Neamine	19
3 (NE)	7
6 (NEA)	2
9 (NEL)	1

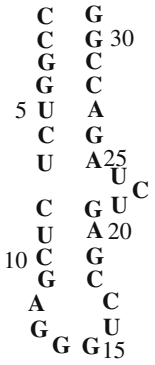


Figure 3. Structure of HIV-1 TAR RNA.

are summarized in Table 2. The K_D value of neamine is 24 μM. Three neamine derivatives **3**, **6** and **9** show more potential binding activities to TAR RNA in comparison with neamine, the K_D value is 14 μM, 3 μM and 2 μM, respectively.

The SPR data indicate that the ethylenediamine modified neamine **3** (NE) could increase the binding affinity to RNA in comparison with neamine, ethylenediamine in NE plays not only a linker but a functional group for the binding to RNA. Based on the structure of compound **3**, an arginine or lysine was conjugated to the terminal amino group of ethylenediamine and two novel neamine derivatives **6** and **9** were synthesized and showed the higher binding affinities to RNA compared with compound **3**. The K_D data of compounds **6** and **9** indicate more interactions between these compounds with RNA in existence. It is unexpected that the lysine conjugate **9** shows the stronger binding activity (Table 1 and 2) compared with the arginine conjugate **6**. To obtain more information about the interactions between the neamine derivatives **6** and **9** and A site of 16S RNA, molecular modeling study was performed.[33] The structure of A site of 16S RNA fragment was extracted from the protein databank (PDB code 1PBR) and the software AUTODOCK 3.0 was used for the molecular docking calculation of three neamine derivatives. By computer calculation, the final configuration (Fig. 4a, b, and c) with the lowest docking energy was obtained and used for the analysis.

Table 2
The K_D values of neamine and its derivatives **3**, **6** and **9** binding to TAR RNA

Compd	TAR RNA (μM)
Neamine	24
3 (NE)	14
6 (NEA)	3
9 (NEL)	2

The molecular docking study suggests that except the hydrogen bonds and electrostatic interactions which are formed between the neamine moiety of compound **3** and 16S RNA, the NH_2 group on

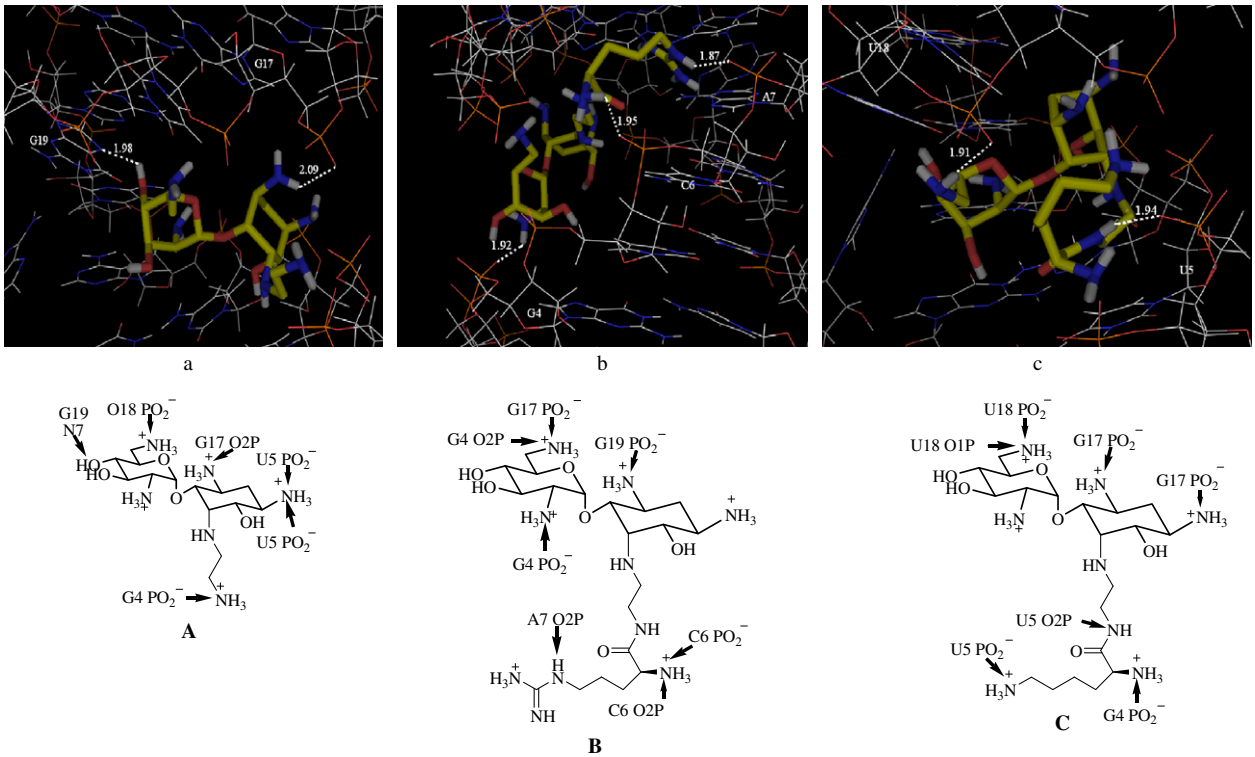


Figure. 4. Molecular modeling for the interactions between compounds **3** (NE) (a, A), **6** (NEA) (b, B), **9** (NEL) (c, C) and A site of 16S RNA. a, b and c show the hydrogen bonds (dotted line) only and A, B and C indicate the interactions including all the hydrogen bonds and electrostatic interactions.

Table 3
The MIC values (μM/L) of neamine and its three derivatives **3**, **6** and **9**

Compd	*E. coli*	*S. aureus*	*P. aeruginos*
Neamine	50	50	>100
3 (NE)	100	>100	50
6 (NEA)	100	>100	>100
9 (NEL)	50	25	>100

ethylenediamine side chain of compound **3** can also form an electrostatic interaction with PO_2^- of G4 on the A site (Fig. 4a and A). After conjugation of an arginine or lysine to compound **3**, more interactions including hydrogen bonds and electrostatic interactions are observed between **6** or **9** and the A site of 16S RNA (Fig. 4b, B, c, and C). Comparing with the molecular modeling of compound **6** or **9**, it shows clearly that the arginine residue in compound **6** contributes only one NH of δ-guanidino group and one α-NH_2 group for the interaction with RNA and the ethylenediamine moiety loses its interaction with RNA. However, in the case of compound **9**, the ω-NH_2 and α-NH_2 of lysine residue and the NH of ethylenediamine part contribute significantly for the interaction with the A site of 16S RNA. These results are in agreement with the experimental data and could explain the difference of binding affinity between compound **6** and **9**.

The antibacterial activities of neamine and its three neamine derivatives against *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *P. aeruginosa (Pseudomonas aeruginosa)* ATCC 27853 were determined, respectively,[34] the results are summarized in Table 3. Compound **9** shows some activities against *S. aureus* (MIC = 25 μM) and *E. coli* (MIC = 50 μM). This result exhibits that compound **9** could be used as a lead for the further structural optimization.

In summary, three new neamine derivatives **3**, **6** and **9** were synthesized by modifying 5-hydroxyl of neamine. Their binding affinities to 16S and TAR RNA indicate that the modification to 5-hydroxyl of neamine by amino acid can enhance the binding affinity of neamine. Compound **9** (NEL) shows more potential of binding affinity compared with its arginine derivative and exhibits some antibacterial activities against *S. aureus* (MIC = 25 μM) and *E. coli* (MIC = 50 μM). These results demonstrate that lysine modifying at 5-position of neamine may provide a promising way for the development of potential candidates effectively targeting to RNAs.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (20332010) and The Ministry of Science and Technology of China (2004cb518904).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.021.

References and notes

1. Sucheck, S. J.; Wong, C. H. *Curr. Opin. Chem. Biol.* **2000**, *4*, 678.
2. Ecker, D. J.; Griffey, R. H. *Drug Discovery Today* **1999**, *4*, 420.
3. Ramakrishna, V.; Moore, P. B. *Curr. Opin. Struct. Biol.* **2001**, *11*, 144.
4. Fourmy, D. I.; Recht, M.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347.
5. Mei, H. Y.; Cui, M.; Heldsinger, A.; Heldsinger, A.; Lemrow, S. M.; Loo, J. A.; Sannes-Lowery, K. A.; Sharmeen, L.; Czarnik, A. W. *Biochemistry* **1998**, *37*, 14204.
6. Gait, M. J.; Karn, J. *Trends Biochem. Sci.* **1993**, *18*, 255.
7. Mei, H. Y.; Galan, A. A.; Halim, N. S.; Mack, D. P.; Moreland, D. W.; Sanders, K. B.; Truong, H. N.; Czarnikt, A. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2755.
8. Koeda, T.; Umemura, K.; Yokoda, M.. In *Aminoglycoside Antibiotics*; Umezawa, H., Hooper, I. R., Eds.; Springer: Berlin, New York, 1982; Vol. 62, p 293.
9. Tanaka, N. *Arch. Pharm. Res.* **1983**, *6*, 93.
10. Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S. C.; Wong, C. H. *J. Am. Chem. Soc.* **1999**, *121*, 6527.
11. Silva, G. S.; Carvalho, I. *Curr. Med. Chem.* **2007**, *14*, 1101.
12. Zhou, J.; Wang, G. N.; Zhang, L. H.; Ye, X. S. *Med. Res. Rev.* **2007**, *27*, 279.
13. Calnan, B. J.; Tidor, B.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Science* **1991**, *252*, 1167.
14. Weeks, K. M.; Ampe, C.; Schultz, S. C.; Steitz, T. A.; Crothers, D. M. *Science* **1990**, *249*, 1281.
15. Litovchick, A.; Evdokimov, A. G.; Lapidot, A. *Biochemistry* **2000**, *39*, 2838.
16. Lapidot, A.; Vijayabaskar, V.; Litovchick, A.; Yu, J. G.; James, T. L. *FEBS Lett.* **2004**, *577*, 415.
17. Kawamoto, S. A.; Sudhahar, C. G.; Hatfield, C. L.; Sun, J.; Behrman, E. J.; Gopalan, V. *Nucleic Acids Res.* **2008**, *36*, 697.
18. Lapidot, A.; Peled, A.; Berchanski, A.; Pal, B.; Kollet, O.; Lapidot, T.; Borkow, G. *Biochim. Biophys. Acta (BBA)* **2008**, *1780*, 914.
19. Borkow, G.; Lara, H. H.; Lapidot, A. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1047.
20. Borkow, G.; Vijayabaskar, V.; Lara, H. H.; Kalinkovich, A.; Lapidot, A. *Antiviral Res.* **2003**, *60*, 181.
21. Eubank, T. D.; Biswas, R.; Jovanovic, M.; Litovchick, A.; Lapidot, A.; Gopalan, V. *FEBS Lett.* **2002**, *511*, 107.
22. Hamasaki, K.; Ueno, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 591.
23. Cabrera, C.; Gutierrez, A.; Barretina, J.; Blanco, J.; Litovchick, A.; Lapidot, A.; Clotet, B.; Este, J. A. *Antiviral Res.* **2002**, *53*, 1.
24. Litovchick, A.; Evdokimov, A. G.; Lapidot, A. *FEBS Lett.* **1999**, *445*, 73.
25. Lapidot, A.; Vijayabaskar, V.; Litovchick, A.; Yu, J. H.; James, T. L. *FEBS Lett.* **2004**, *577*, 415.
26. Hegde, R.; Borkow, G.; Berchanski, A.; Lapidot, A. *FEBS J.* **2007**, *274*, 6523.
27. Watanable, K.; Katou, T.; Lkezawa, Y.; Yajima, S.; Shionnoya, H.; Akagi, T.; Hamasaki, K. *Nucleic Acids Symp. Ser.* **2007**, *51*, 209.
28. Yajima, S.; Shionoya, H.; Akagi, T.; Hamasaki, K. *Bioorg. Med. Chem.* **2006**, *14*, 2799.
29. Wu, B. G.; Yang, J.; He, Y.; Swayze, E. E. *Org. Lett.* **2002**, *4*, 3455.
30. Cai, L.; Li, Q.; Ren, B.; Yang, Z. J.; Zhang, L. R.; Zhang, L. H. *Tetrahedron* **2007**, *63*, 8135.
31. Davis, T. M.; Wilson, W. D. *Methods Enzymol.* **2001**, *340*, 22.
32. Hendrix, M.; Priestley, Z. S.; Joyce, G. F.; Wong, C. H. *J. Am.Chem. Soc.* **1997**, *119*, 3641.
33. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Hucy, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
34. Jorgensen, J. H.; Turnidge, J. D.; Washington, J. A. In *Manual of Clinical Microbiology*; Murray, P. R., Baron, E. J. M., Pfaller, A., Tenover, F. C., Yolken, R. H., Eds., 7th Ed.; ASM: Washington, D.C., 1999; p 1526.