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Semi-Synthetic Glycopeptide Antibacterials

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Abstract—Studies leading to the discovery of TD-6424 and their relevance to other hydrophobically-substituted glycopeptides are reviewed along with a brief comparison of properties for related agents currently undergoing clinical evaluation.

The glycopeptide antibiotic vancomycin has received increasing use with the emergence and spread of multi-drug-resistant Gram-positive bacterial pathogens.[1–5] While vancomycin-resistant enterococci have become an important problem, staphylococcal infections are both more common and more virulent. Thus, a bigger challenge may ultimately result from vancomycin-tolerant staphylococci and strains with intermediate susceptibility or frank resistance to vancomycin.[6–14]

Following the work of Nagarajan, Nicas, Allen, and colleagues to improve the activity of vancomycin, other groups have pursued a semi-synthetic approach to the discovery of glycopeptide antibacterials active against resistant bacteria.[15–23] For some analogues, physical, pharmacokinetic and safety properties were also different than for the parent glycopeptides.[21,23] While progress has been made in identifying lead candidates, structure–activity relationships (SARs) appear to be complex.[16,24] Studies of the mechanism of action (MOA) for these agents in relation to both their bactericidal activity and spectrum have focused on self-association driven by substrate affinity, and more recently on direct inhibition of the transglycosylase reaction involved in polymerization of the cell wall.

However, no one hypothesis can completely account for the antibacterial properties, and distinct and multiple mechanisms may be present among members of this class. The urgent need for additional agents active against resistant pathogens and the intriguing potential of multiple mechanisms of action prompted us to prepare our own glycopeptide antibacterials. Here we review the clinical candidate derived from this work, TD-6424 (Fig. 1), briefly discuss observations of SARs and MOA made during discovery of this agent (Fig. 2), and compare the properties of TD-6424 with reports on other glycopeptide antibacterials (Table 1).

Hydrophobically-substituted derivatives of vancomycin were prepared by regioselective reductive alkylation of the vancosamine nitrogen.[21,25–27] The decyl-aminoethyl vancomycin analogue THRX-689909 was further substituted at the resorcinol position via Mannich reaction conditions using formalin and the appropriate amine to yield TD-6424.[21] TD-6424 exhibted in vitro antibacterial activity against both *Staphylococcus aureus*, and vancomycin-resistant enterococci (Table 1). Leading up to the discovery of TD-6424 a number of analogues had been prepared with increasing size for the hydrophobic substituent. The antibacterial properties of these *N*-alkylated vancomycin analogues were dependent on the length of the hydrophobic substituent as well as on the bacterial species (Fig. 2). Increasing size of the hydrophobic-subtituent group resulted in a slight initial increase in activity followed by reduction in activity against methicillin-resistant *S. aureus*,[28] while against the VanA enterococci activity increased with increasing substituent size until a size corresponding to 13 methylene equivalents was achieved. No further improvement in activity was observed with larger hydrophobic substituents. We noted the similarity of our findings to those reported by the Lilly group.[24,28,29]

In addition to the differences between the antibacterial activities against *S. aureus* and enterococci observed

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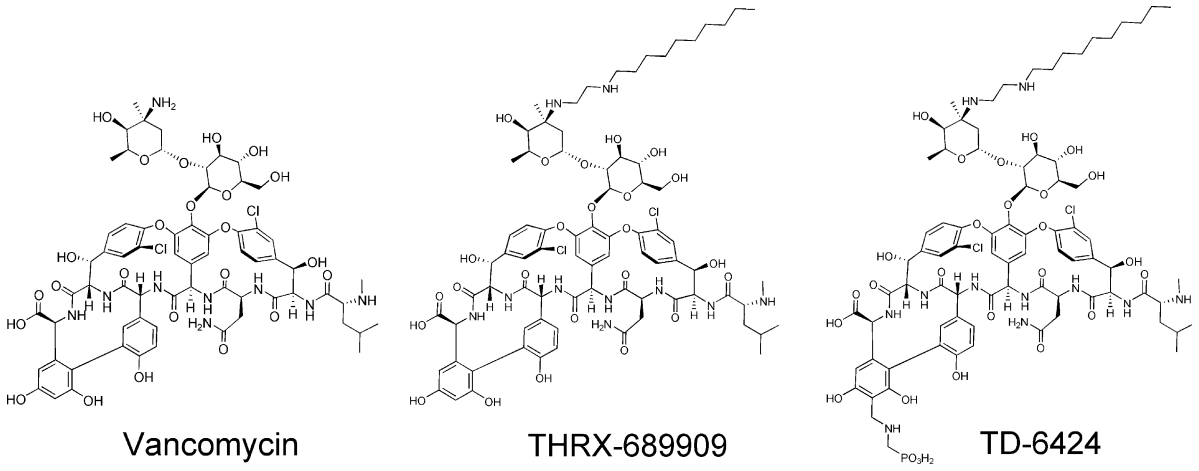

Figure 1. Structures of TD-6424, THRX-689909 and vancomycin.

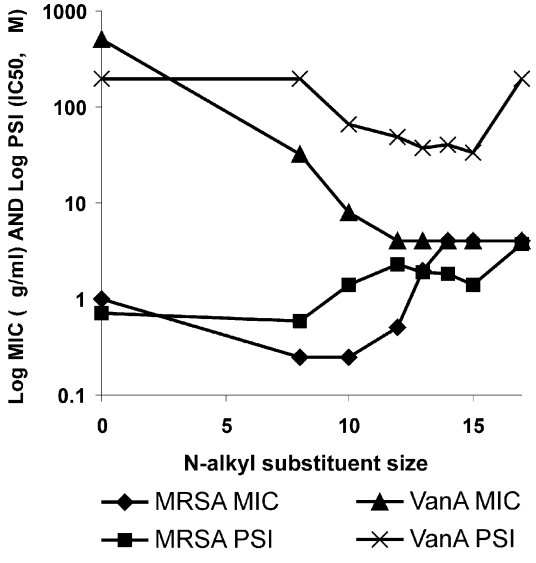

Figure 2. Structure–activity relationships of *N*-alkylated vancomycin derivatives. MRSA, *S. aureus* ATCC 33591; VanA, *E. faecalis* MGH-01; MIC, minimal inhibitory concentration (μg/mL); and PSI, IC_{50} (μM) for peptidoglycan synthesis inhibition.

with this glycopeptide series, we noted a poor association between antibacterial activity and ability of the molecules to inhibit peptidoglycan synthesis. Vancomycin exerts its antibacterial activity through inhibition of peptidoglycan synthesis via binding of the lipid II substrate intermediate.[30] This may affect both the transglycosylation and transpeptidation reactions involved in cell wall polymerization.[30] The activity is believed to be impacted by self-association, or formation of non-covalent vancomycin dimers, with correspondingly enhanced substrate affinity.[31–34] If hydrophobic substitution of the glycopeptide antibiotic were to result in enhanced self-association then it might be expected that both inhibition of peptidoglycan synthesis and antibacterial activity would be enhanced.[35–37] We performed a variety of studies to evaluate a role for enhanced substrate binding but could not support this hypothesis. Our findings suggested further complexity and a second mechanism of action with TD-6424.

Although other semi-synthetic glycopeptide antibacterials may directly interact with the transglycosylase enzyme and directly inhibit cell wall synthesis,[19,20] in our glycopeptide series MIC was not associated with transglycosylase inhibition when using the same *Escherichia coli* model as previously used by others.[20] For example, IC_{50} for the transglycosylase reaction decreased from 11.6 to 0.01 μg/mL for the series of glycopeptide analogues having hydrophobic susbtituents of sizes between six and fifteen methylene equivalents, while MICs against methicillin-resistant *S. aureus* were 1.6 μg/mL for all with the exception of THRX 689909 MIC of 0.4 μg/mL. Instead, studies with analogues leading to the discovery of TD-6424 suggested disruption of membrane function.[28,29,38] Further examination revealed that both fatty acid and subsequent phospholipid syntheses were inhibited in staphylococci, streptococci, and enterococci by our substituted vancomycin derivatives.[28,29] TD-6424 IC_{50}s for [^{14}C]-glycerol incorporations into lysylphosphatidylglycerol were 23 μg/mL for *S. aureus* ATCC

Table 1. Antibacterial activity[a,c] of glycopeptides against important gram-positive pathogens

		TD-6424	Oritavancin	Dalbavancin	Vancomycin
MRSA	MIC_{90}[a]	1	1	0.25	4
VRSA	MIC[a]	2	0.5	NA[b]	>128
S. pneumoniae	MIC_{90}	0.008	0.008	0.06	0.5
Eneterococcus spp., VanA	MIC_{90}	8	4	128	>128

[a]MIC, minimal inhibitory concentration (μg/mL) determined by NCCLS methods, MIC_{90}, MIC for 90% of strains assayed (N = 100–200).
[b]NA, data not available.
[c]References for susceptibility data.[14,24,29,39–43,45–50,56]

33591, 15 μg/mL for *Streptococcus agalactiae* KPB-01, and 10 μg/mL for VanA *Enterococcus faecalis* MGH-01. Neither vancomycin, teicoplanin, nor penicillin G inhibited phospholipid synthesis in these bacteria. Clearly TD-6424 acts through multiple mechanisms, some distinct from that of vancomycin. The varying effects of substituents on *S. aureus* and enterococci suggest that different mechanisms may predominate in one bacterial species versus another.

TD-6424 and related analogues exhibited enhanced in-vitro bactericidal activity including concentration-dependent bactericidal action superior to that of vancomycin, and longer post-antibiotic effect. Although their in vivo efficacy was also superior, some of these molecules were less soluble than vancomycin and showed nephrotoxic potential in animal models.[21] For example, BUN values for mice treated with 50 mg/kg THRX-689909 were 5-fold greater than values from vehicle-treated mice. Urinary clearance was reduced and liver and kidney deposition were greater than observed with vancomycin.[21] In an attempt to improve the clearance and distribution, a second substituent group at the carboxy or resorcinol positions was introduced.[21] Some of the compounds derived from this series did exhibit reduced nephrotoxic potential, showing tissue distribution profiles similar to vancomycin, with rapid clearance in the urine. TD-6424 was among this group of compounds.

In addition to improved tissue distribution and clearance, TD-6424 C_{max} and C_{min} were 45 and 5 μg/mL following a 5-mg/kg dose in human volunteers.[39,51,52] TD-6424 has an 7 h half-life in man which is consistent with once daily dosing.[29,51,52] While it is known that the introduction of a hydrophobic appendage to a glycopeptide causes an increase in half life, other hydrophobically substituted glycopeptides like oritavancin, derived from chloroeremomycin, and dalbavancin have half-lives of approximately 1 week.[24,53,54] The shorter half-life of TD-6424 may be due to its vancomycin core structure.

TD-6424 and oritavancin are broadly active against Gram-positive pathogens (Table 1).[11,24,29,39–43] Both agents are active in vitro and in vivo against drug-resistant staphylococci including MRSA and the recently isolated fully vancomycin-resistant *S. aureus*.[44] TD-6424 and oritavancin are also active against VanA enterococci in contrast to both dalbavancin and vancomycin.[45] Finally, TD-6424 and oritavancin are both more active than other compounds against *Streptococcus pneumoniae* including penicillin-resistant isolates.[29,39,40,46–49]

TD-6424 exhibits a longer post-antibiotic effect than vancomycin, or literature values reported for dalbavancin.[29,40,50] and is uniformly active in both immuno-suppressed and -competent animal models of disease.[29,39] Serum bactericidal titers against MRSA determined with patient samples obtained during the Phase I clinical studies of TD-6424 were 256–>512 and 8–32 at peak and trough, respectively.[29,39] Corresponding values reported for dalbavancin were 16–64 at peak concentrations and 4 at trough concentrations.[55] Human Phase II efficacy studies with TD-6424 are ongoing. Phase III studies with oritavancin have recently been completed and dalbavancin Phase III studies are underway.

In conclusion, TD-6424 has the potential to be an important tool for the treatment of serious bacterial infections with the possibility of clinically important improvements over vancomycin. While much progress has been made in identification of potentially useful semi-synthetic glycopeptides, the complex MOA and SARs for these compounds suggests that each agent may have unique and distinctive properties.

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References and Notes

1. Witte, W. J. *Antimicrob. Chemother.* **1999**, *44*, 1.
2. Moellering, R. C., Jr. *Clin. Infect. Dis.* **1998**, *27*, S135.
3. Chambers, H. F. *Clin. Microbiol. Rev.* **1997**, *10*, 781.
4. Malabarba, A.; Nicas, T.; Ciabatti, R. *Eur. J. Med. Chem.* **1997**, *32*, 459.
5. Ziglam, H. M.; Finch, R. G. *Clin. Microb. Infect.* **2001**, *7*, S53.
6. Smith, T. L.; Pearson, M. L.; Wilcox, K. R.; Cruz, C.; Lancaster, M. V.; Robinson-Dunn, B.; Tenover, F. C.; Zervos, M. J.; Band, J. D.; White, E.; Jarvis, W. R. *N. Engl. J. Med.* **1999**, *340*, 493.
7. Hiramatsu, K.; Aritaka, N.; Hanaki, H.; Kawasaki Hosoda, Y.; Hori, S.; Fukuchi, Y.; Kobayashi, I. *Lancet* **1997**, *350*, 1670.
8. CDC. *MMWR* **2002**, *51*, 565.
9. CDC. *MMWR* **2002**, *51*, 902.
10. CDC. *JAMA* **2002**, *288*, 2116.
11. Chang, S.; Sievert, D. M.; Hageman, J. C.; Boulton, M. L.; Tenover, F. C.; Downes, F. P.; Shah, S.; Rudrik, J. T.; Pupp, G. R.; Brown, W. J.; Cardo, D.; Fridkin, S. K. *N. Engl. J. Med.* **2003**, *348*, 1342.
12. Leclerq, R.; Derlot, E.; Duval, J.; Courvalin, P. *N. Engl. J. Med.* **1988**, *319*, 157.
13. Uttley, A. H. C.; Collins, C. H.; Naidoo, J.; George, R. C. *Lancet* **1988**, *1988*, 1 57.
14. Bozdogan, B.; Chaitram, J.; Appelbaum, P. C.; Whitener, C.; Browne, F. A.; Tenover, F. C. *Abstracts of Papers*, 103rd Amer. Soc. for Microbiology Annual Meet., May 18–22, 2003; Amer. Soc. for Microbiology: Washington, DC, 2003; A-075.
15. Nagarajan, R. *J. Antibiot.* **1993**, *46*, 1181.
16. Nagarajan, R. In *Glycopeptide Antibiotics*; Nagarajan, R., Ed.; Marcel Dekker: New York, 1994; p 195.
17. Nicas, T. I.; Allen, N. E. In *Glycopeptide Antibiotics*; Nagarajan, R., Ed.; Marcel Dekker: New York, 1994; p 219.
18. Nicas, T. I.; Mullen, D. L.; Flokowitsch, J. E.; Preston, D. A.; Snyder, N. J.; Zweifel, M. J.; Wilkie, S. C.; Rodriguez, M. J.; Thompson, R. C.; Cooper, R. D. *Antimicrob. Agents Chemother.* **1996**, *40*, 2194.

19. Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507.
20. Chen, L.; Walker, D.; Sun, B.; Hu, Y.; Walker, S.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5658.
21. Leadbetter, M. R.; Linsell, M.; Fatheree, P.; Trapp, S.; Lam, B.; Nodwell, M.; Shaw, J.; Pace, J.; Judice, K. *Abstracts of Papers*, 42nd ICAAC, San Diego, CA, Sept. 27–30, 2002; Am. Soc. Microbiol.: Washington, DC, 2002; F-367.
22. Blizzard, T. A.; Kim, R. M.; Morgan, J. D., II; Chang, J.; Kohler, J.; Kilburn, R.; Chapman, K.; Hammond, M. L. *Biorg. Med. Chem. Lett.* **2002**, *12*, 849.
23. Yoshida, O.; Yasukata, T.; Sumino, Y.; Munekage, T.; Narukawa, Y.; Nishitani, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3027.
24. Allen, N. E.; Nicas, T. I. *FEMS Microbiol. Rev.* **2002**, *26*, 511.
25. Rodriguez, M. J.; Snyder, N. J.; Zweifel, M. J.; Wilkie, S. C.; Stack, D. R.; Cooper, R. D. G.; Nicas, T. I.; Mullen, D. L.; Butler, T. F.; Thompson, R. C. *J. Antibiotics* **1998**, *51*, 560.
26. Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L.; Felty-Duckworth, A. M. *J. Antibiotics* **1989**, *42*, 63.
27. Pavlov, A. Y.; Lazhko, E. I.; Preobrazhenskaya, M. N. *J. Antibiotics* **1997**, *50*, 509.
28. Debabov, D., Pace, J.; Kaniga, K.; Nodwell, M.; Farrington, L.; Campbell, B.; Karr, D.; Leadbetter, M.: Linsell, M.; Wu, T.; Krause, K.; Johnston, D.; Lane, C.; Quast, K.; Bazzini, B.; Phi, B.; Schmidt, D.; White, L.; Higgins, D.; Christensen, B.; Judice, K. *Abstracts of Papers*, 42nd ICAAC, San Diego, CA, Sept. 27–30, 2002. Am. Soc. Microbiol.: Washington, DC, 2002; F-364.
29. Pace, J. L. *Abstracts of Papers*, 42nd ICAAC, San Diego, CA, Sept. 27–30, 2002; Am. Soc. Microbiol.: Washington, DC, 2002; p 614.
30. Bugg, T. D. H.; Wright, G. D.; Dutka-Malen, S. S.; Arthur, M.; Courvalin, P.; Walsh, C. T. *Biochemistry* **1991**, *30*, 10408.
31. Nieto, M.; Perkins, R.; Reynolds, P. E. *Biochem. J.* **1972**, *126*, 139.
32. Gerhard, U.; Mackay, J. P.; Maplestone, R. A.; Williams, D. H. *J. Am. Chem. Soc.* **1993**, *115*, 232.
33. Sharman, G. J.; Williams, D. H. *Chem. Commun.* **1997**, *7*, 723.
34. Rao, J.; Lahiri, J.; Issacs, L.; Weis, R. M.; Whitesides, G. M. *Science* **1998**, *280*, 708.
35. Beauregard, D. A.; Williams, D. H.; Gwynn, M. N.; Knowles, D. J. *Antimicrob. Agents Chemother.* **1995**, *39*, 781.
36. Goldstein, B. P.; Rosina, R.; Parenti, F. In *Glycopeptide Antibiotics*; Nagarajan, R., Ed.; Marcel Dekker: New York, 1994; p 273.
37. Allen, N. E.; leTourneau, D. L.; Hobbs, J. N., Jr.; Thompson, R. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 2344.
38. Pace, J.; Quast, K.; Chen, Q.; Linsell, M.; Krause, K.; Farrington, L.; Nodwell, M.; Leadbetter, M.; Schmidt, D.; Mabery, E.; Soriano, E.; Fatheree, P.; Bazzini, B.; Bao, J.; Gao, D.; Higgins, D.; Griffin, J.; Karr, D.; Christensen, B.; Judice, K. *Abstracts of Papers*, 12th ECCMID, Milan, Italy, April 24–27, 2002; ESCMID: Basel, Switzerland, 2002; O134.
39. Pace, J.; Judice, K.; Hegde, S.; Leadbetter, M.; Linsell, M.; Kaniga, K.; Reyes, N.; Farrington, L.; Debabov, D.; Nodwell, M.; Christensen, B. *Abstracts of Papers*, 10th International Symposium Staphylococci and Staphylococcal Infection, Tsukuba, Japan, Oct. 16–19, 2002; Japanese Symposium on Staphylococci and Staphylococcal Infections: Tokyo, Japan, 2002; 265-01.
40. King, A.; Phillips, I.; Farrington, L.; Pace, J.; Kaniga, K. *Abstracts of Papers*, 13th ECCMID, Glasgow, Scotland, May 10–13, 2003; ESCMID: Basel, Switzerland, 2003; p 799.
41. Jones, R. N.; Barrett, M. S.; Erwin, M. E. *Antimicrob. Agents Chemother.* **1996**, *41*, 488.
42. Karlowsky, J. A.; Loutit, J.; Porter, S. B.; Blosser-Middleton, R. S.; Jones, M. E.; Thornsberry, C.; Sahm, D. *Abstract of Papers*, 13th ECCMID, Glasgow, Scotland, May 10–13, 2003. ESCMID: Basel, Switzerland, 2003; p 801.
43. Reynolds, P. *Abstracts of Papers*, 37th ICAAC, Toronto, CN, Sept. 28–Oct. 1, 1997; Am. Soc. Microbiol.: Washington, DC, 1997; C-165.
44. Weigel, L. M., McDougal, L. K.; Clark, N.; Killgore, G.; Tenover, F. C.; Appelbaum, P. C.; Bozdogan, B. *Abstracts of Papers*, 103rd Am. Soc. for Microbiology Annual Meet., May 18–22, 2003; Am. Soc. for Microbiology: Washington, DC, 2003; A-111.
45. Jones, R. N.; Biedenbach, D. J.; Johnson, D. M.; Pfaller, M. A. *Abstracts of Papers*, 42nd ICAAC, San Diego, CA, Sept. 27–30, 2002; Am. Soc. Microbiol.: Washington, DC, 2002; p 2276.
46. Patel, R.; Rouse, M. S.; Piper, K. E.; Cockerill, F. R.; Steckelberg, J. M. *Diagn. Microbiol. Infect. Dis.* **1998**, *30*, 89.
47. Coyle, E. A.; Rybak, M. J. *Antimicrob. Agents Chemother.* **2001**, *45*, 706.
48. Garcia-Garrote, F.; Cercenado, E.; Alcala, L.; Bouza, E. *Antimicrob. Agents Chemother.* **1998**, *42*, 2452.
49. Zeckel, M. L. D.; Preston, D. A.; Allen, B. S. *Antimicrob. Agents Chemother.* **2000**, *44*, 1370.
50. Gomez, M.; Hackbarth, C. J.; Lopez, S.; Trias, J.; White, R. 101st Am. Soc. Microbiol. Gen. Meet., Orlando, FL, May 20–24, 2001; Am. Soc. Microbiol.: Washington, DC, 2001; A-4.
51. Barriere, S.; Shaw, J. P.; Seroogy, J.; Kaniga, K.; Pace, J.; Judice, K.; Mant, T. *Abstracts of Papers*, 13th ECCMID, Glasgow, Scotland, May 10–13, 2003; ESCMID: Basel, Switzerland, 2003; P1214.
52. Barriere, S.; Genter, F.; Spencer, E.; Kitt, M.; Hoelscher, D.; Morganroth, J. *Abstracts of Papers*, 13th ECCMID, Glasgow, Scotland, May 10–13, 2003; ESCMID: Basel, Switzerland, 2003; O144.
53. Leighton, A., White, R.; Chaudhari, U.; Van Saders, C.; Baylor, M.; Perry, M.; Henkel, T.; Kelly, E.; Campbell, K. C. M. *Abstracts of Papers*, 41st ICAAC, Chicago, IL Dec. 7–11, 2001; Am. Soc. Microbiol.: Washington, DC, 2001; 2192.
54. Dowell, J. A.; Gottlieb, A. B.; Van Saders, C.; Dorr, M. B.; Leighton, A.; Cavaleri, M.; Guanci, M.; Colombo, L. 2002. *Abstracts of Papers*, 42nd ICAAC, San Diego, CA, Sept. 27–30, 2002; Am. Soc. Microbiol.: Washington, DC, 2001; p 1386.
55. White, R. J.; Brown, G. L.; Cavellero, M.; Romano, G. *Abstracts of Papers*, 40th ICAAC, Toronto, Canada, Sept. 17–20, 2000; Am. Soc. Microbiol.: Washington, DC, 2000; p 2196.
56. Candiani, G.; Abbondi, M.; Borogonovi, M.; Romano, G.; Parenti, F. J. *Antimicrob. Chemother.* **1999**, *44*, 179.