# SYNTHESIS AND ANTIBACTERIAL EVALUATION OF *N*-ALKYL VANCOMYCINS†

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Over eighty *N*-alkyl vancomycins were synthesized by reductive alkylation of vancomycin with the appropriate aldehydes. The *N*-alkyl vancomycins exhibit greater antibacterial activity than the corresponding *N*-acyl vancomycins and the parent antibiotic. Some of these semisynthetic vancomycins are five times more active than vancomycin. The *N*-alkyl vancomycins also show longer elimination half-lives in rats than vancomycin.

For the past 30 years, vancomycin has been the drug of choice to treat severe Gram-positive infections caused by methicillin-resistant *Staphylococcus aureus*. Recent reports have described glycopeptides with acylamido side chains on a sugar residue,[2~6] and some of these compounds, *e.g.* teicoplanin, have been claimed to have more favorable antibacterial and pharmacokinetic properties than vancomycin.[7] Synthesis of several *N*-acyl vancomycins and structure activity relationship studies revealed no substantial advantage of these *N*-acyl vancomycins over the parent antibiotic.[8]

As an extension to the structure-activity relationship (SAR) of the *N*-acyl vancomycin research, we undertook the synthesis and evaluation of *N*-alkyl vancomycins.

## Chemistry

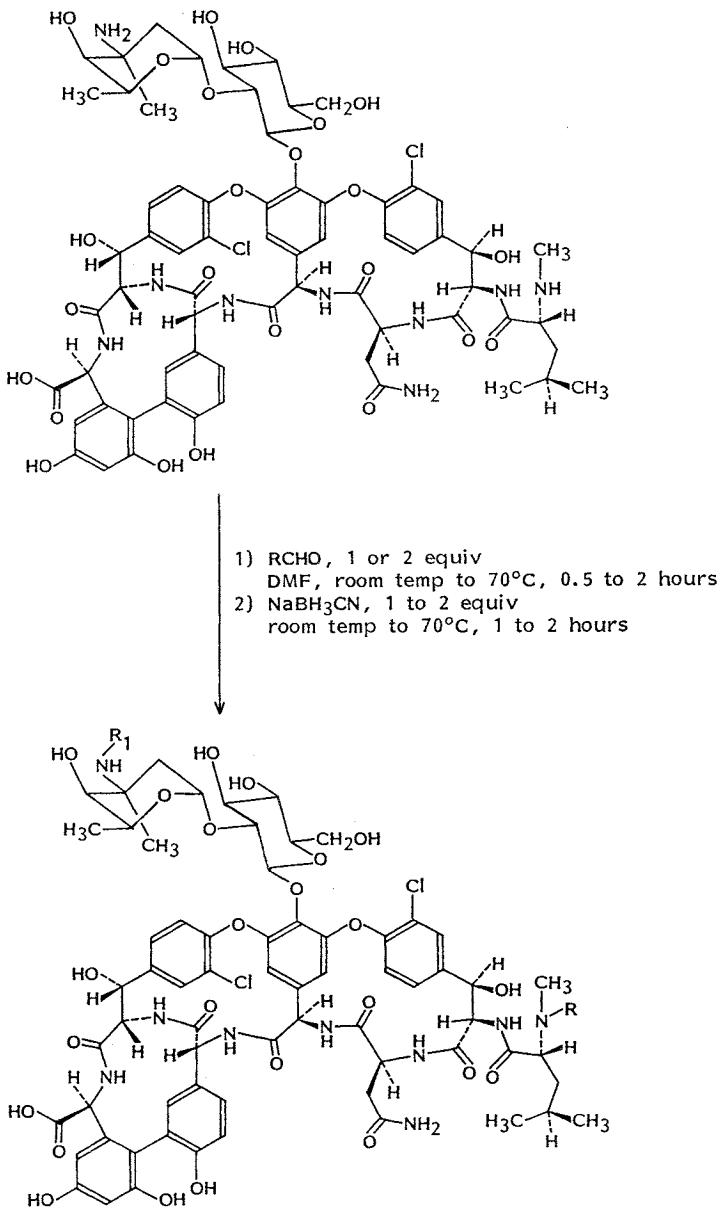
The *N*-alkyl vancomycins were obtained by reductive alkylation of vancomycin with the appropriate aldehydes. This reaction could yield two mono-*N*-alkyl derivatives substituted at the amino groups of vancosamine sugar and *N*-methyl leucine residue, respectively, and one di-*N*-alkyl vancomycin. The reaction products were monitored by analytical HPLC. Not all reactions produced all the three possible products. However, if all three *N*-alkyl vancomycins were available in a series, the retention times of the *N*-alkyl vancomycins were diagnostic of the site of alkylation as previously observed for the *N*-acyl derivatives.[8] The mono-*N*-alkyl derivative substituted on vancosamine eluted first, then the second mono-*N*-alkyl vancomycin functionalized on *N*-methyl leucine, and finally the di-*N*-alkyl vancomycin (see Table 1).

The ratio of the three *N*-alkyl vancomycins from the reductive alkylation reaction depended on the reaction conditions which could be adjusted to yield the desired *N*-alkyl derivative as the major product. The reaction mixture was purified by preparative HPLC.

The structures of the *N*-alkyl vancomycins were confirmed by fast atom bombardment mass spectrometry (FAB-MS). The molecular ion indicates if the derivatives is a mono- or di-*N*-alkyl vancomycin. The fragmentation pattern of the mono-*N*-alkyl derivative clearly establishes the site of substitution between the two alternative amino groups in vancomycin. Thus, the mono-*N*-alkyl vancomycin alkylated on the *N*-methyl leucine affords the disaccharide, vancosaminyl-*O*-glucose and van-

† See ref 1.

Fig. 1. Synthesis of *N*-alkyl vancomycins.



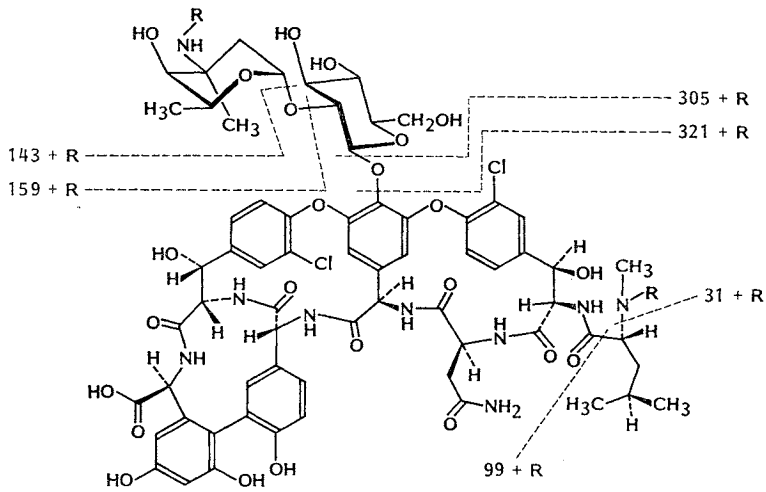

Two mono-*N*-alkyl and one di-*N*-alkyl vancomycins

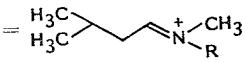
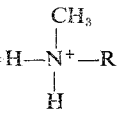
cosamine fragments, whereas the devancosamine vancomycin, aglucovancomycin, and *N*-methyl leucine fragments show increased mass corresponding to the alkyl residue. The mono-*N*-alkyl vancomycin substituted on vancosamine gives vancosaminyl-*O*-glucose and vancosamine fragments containing the additional mass due to alkylation on the amino group of vancosamine.

## SAR of *N*-Alkyl Vancomycins

The first derivatives prepared were the *N*-decyl vancomycins with an aliphatic straight chain

Fig. 2. FAB-MS fragmentation pattern of *N*-alkyl vancomycins.




| R on vancosamine | R on leucine |
|---|---|
| M+H=Vancomycin+R+H=1,447+R+H | M+H=Vancomycin+R+H=1,447+R+H |
| 1,305=Devancosamine vancomycin+H | 1,305+R=Devancosamine vancomycin+R+H |
| 1,143=Aglucovancomycin+H | 1,143+R=Aglucovancomycin+R+H |
| 143+R=Vancosamine+R | |
| 159+R=Vancosamine+R+oxygen | 99+R=  |
| 305+R=Vancosamine+glucose+R | |
| | 31+R=  |

similar to the naturally occurring *N*-acylamido glycopeptides.[2~6] A comparison of the antibacterial activities of the *N*-decyl vancomycins with the corresponding *N*-decanoyl vancomycins shows that the $C_{10}$ alkyl analogs are more active than the corresponding alkanoyl series. Furthermore, the mono-*N*-decyl vancomycin **5** is more active *in vitro* than the parent vancomycin, equivalent to vancomycin *in vivo*, and shows longer elimination half-life in rats. Encouraged by this result, we undertook an extensive SAR of *N*-alkyl vancomycins, and over eighty derivatives were prepared and evaluated.

A series of the two mono-*N*-alkyl and one di-*N*-alkyl derivatives belonging to nine aliphatic and five aromatic *N*-alkyl vancomycins were prepared and their antibacterial activity compared. As in the SAR of the *N*-acyl vancomycin series,[8] the general trend is that the *N*-alkyl derivatives substituted on vancosamine are more active than those substituted on *N*-methylleucine, and both mono-substituted vancomycins are more active than the corresponding di-*N*-alkyl vancomycins.

Having established that the mono-*N*-alkyl vancomycins substituted on vancosamine are the most active of the three derivatives, the reaction conditions for compounds described on Table 3 were adjusted so that the most active mono-*N*-alkyl derivative was the major product of the reaction. The other product for the ten series of compounds in Table 3 were either the other mono-*N*-alkylated derivative or the di-*N*-alkyl compound.

The *in vitro* and *in vivo* antibacterial activity of all thirty-eight mono-*N*-alkyl vancomycins substituted on vancosamine were determined. The pharmacokinetics of the most active derivatives and

Table 1. Comparison of *N*-acyl and *N*-alkyl vancomycins.

| | Compound | FAB-MS[a] (*m/z*) | $R_t$[b] | MIC ($\mu$g/ml)[c] | | | | | | | | | | $ED_{50}$ (mg/kg×2, sc) | | | Serum (iv, rat) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S.A.1 | S.A.2 | S.A.3 | S.A.4 | S.E.1 | S.E.2 | S.Py. | S.Pn. | S.D.1 | S.D.2 | S.A.1 | S.Py. | S.Pn. | $T_{1/2}$ (hours) | Peak conc (mg/ml) |
| 1 | Vancomycin | 1,448 | 6.66 | 0.5 | 0.5 | 1 | 1 | 2 | 1 | 0.5 | 0.5 | 1 | 2 | 1.8 | 0.8 | 0.9 | 0.75 | 160 |
| 2 | R=H, $R_1$=*n*-$C_9H_{19}CO$ | 1,602 | 11.63 | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 1 | 0.5 | 2 | 5 | 2.9 | 1.4 | — | — |
| 3 | R=*n*-$C_9H_{19}CO$, $R_1$=H | 1,602 | 12.64 | 0.5 | 0.5 | 0.5 | 1 | 4 | 2 | 4 | 4 | 2 | 4 | 10 | 6.8 | 7.2 | — | — |
| 4 | R=$R_1$=*n*-$C_9H_{19}CO$ | 1,755 | 16.55 | 2 | 4 | 4 | 8 | 32 | 8 | 4 | 16 | 8 | 8 | — | — | — | — | — |
| 5 | R=H, $R_1$=*n*-$C_{10}H_{21}$ | 1,588 | 11.14 | 0.13 | 0.13 | 0.13 | 0.13 | 0.25 | 0.13 | 0.06 | 0.13 | 0.25 | 0.25 | 1.8 | 0.65 | 0.68 | 3.4 | 203 |
| 6 | R=*n*-$C_{10}H_{21}$, $R_1$=H | 1,588 | 12.05 | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.5 | 0.5 | 1 | 5 | 4.6 | 3.6 | — | — |
| 7 | R=$R_1$=*n*-$C_{10}H_{21}$ | 1,728 | 14.76 | 2 | 4 | 4 | 4 | 16 | 4 | 2 | 4 | 4 | 4 | — | — | — | — | — |

[a] Column shows molecular ion as $M^+$ or $M^+ + 1$.

[b] HPLC: Waters $\mu$Bondapak $C_{18}$ column, UV detection at 254 nm, gradient; acetonitrile - water, 0.2% triethylamine buffer solvent systems in the following gradients.

| System | Gradient |
|---|---|
| A | 5% $CH_3CN$ → 80% $CH_3CN$ |
| B | 10% $CH_3CN$ → 60% $CH_3CN$ |

$R_t$: Retention time in minutes.

[c] MICs determined by standard agar dilution method.

S.A. 1: Benzylpenicillin-sensitive *Staphylococcus aureus* X 1.1, S.A. 2: benzylpenicillin-resistant *S. aureus* V41, S.A. 3: methicillin-resistant *S. aureus* X400, S.A. 4: methicillin-resistant *S. aureus* S13E, S.E. 1: macrolide-resistant *Staphylococcus epidermidis* 270, S.E. 2: penicillin, methicillin and macrolide-sensitive *S. epidermidis* 222, S.Py.: *Streptococcus pyogenes* C203, S.Pn.: *Streptococcus pneumoniae* Park, S.D. 1: *Streptococcus faecium* X66, S.D. 2: *Streptococcus faecalis* 2041.

Table 2. *In vitro* antibacterial activity of *N*-alkyl vancomycins.

| | Compound | MIC ($\mu$g/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S.A. 1 | S.A. 2 | S.A. 3 | S.A. 4 | S.E. 1 | S.E. 2 | S.Py. | S.Pn. | S.D. 1 | S.D. 2 |
| **8** | R=H, $R_1$=*n*-$C_3H_7$ | 1 | 1 | 1 | 1 | 4 | 1 | 0.5 | 0.5 | 1 | 1 |
| **9** | R=*n*-$C_3H_7$, $R_1$=H | 1 | 2 | 2 | 2 | 4 | 2 | 1 | 1 | 2 | 8 |
| **10** | R=$R_1$=*n*-$C_3H_7$ | 2 | 2 | 2 | 2 | 4 | 4 | 2 | 1 | 2 | 8 |
| **11** | R=H, $R_1$=*n*-$C_4H_9$ | 1 | 1 | 1 | 1 | 4 | 2 | 1 | 0.5 | 2 | 4 |
| **12** | R=*n*-$C_4H_9$, $R_1$=H | 1 | 2 | 2 | 2 | 4 | 2 | 1 | 0.5 | 2 | 4 |
| **13** | R=$R_1$=*n*-$C_4H_9$ | 0.5 | 1 | 1 | 1 | 2 | 1 | 0.5 | 0.5 | 1 | 2 |
| **14** | R=H, $R_1$=*n*-$C_7H_{15}$ | 0.25 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.125 | 0.5 | 1 |
| **15** | R=*n*-$C_7H_{15}$, $R_1$=H | 1 | 1 | 1 | 1 | 4 | 2 | 1 | 0.5 | 1 | 4 |
| **16** | R=$R_1$=*n*-$C_7H_{15}$ | 0.5 | 1 | 1 | 1 | 2 | 1 | 0.5 | 0.5 | 1 | 1 |
| **17** | R=H, $R_1$=*n*-$C_8H_{17}$ | 0.25 | 0.25 | 0.25 | 0.25 | 2 | 0.5 | 0.125 | 0.125 | 0.25 | 0.5 |
| **18** | R=*n*-$C_8H_{17}$, $R_1$=H | 0.5 | 0.5 | 1 | 1 | 2 | 1 | 0.5 | 1 | 1 | 2 |
| **19** | R=$R_1$=*n*-$C_8H_{17}$ | 1 | 1 | 1 | 1 | 4 | 2 | 0.5 | 1 | 1 | 2 |
| **5** | R=H, $R_1$=*n*-$C_{10}H_{21}$ | 0.125 | 0.125 | 0.25 | 0.125 | 0.25 | 0.25 | 0.125 | 0.125 | 0.25 | 0.25 |
| **6** | R=*n*-$C_{10}H_{21}$, $R_1$=H | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.5 | 0.5 | 1 |
| **7** | R=$R_1$=*n*-$C_{10}H_{21}$ | 2 | 4 | 4 | 4 | 16 | 4 | 2 | 4 | 4 | 4 |
| **20** | R=H, $R_1$=*n*-$C_5H_{10}OH$ | 1 | 1 | 2 | 1 | 4 | 2 | 1 | 1 | 2 | 8 |
| **21** | R=*n*-$C_5H_{10}OH$, $R_1$=H | 2 | 2 | 2 | 2 | 8 | 2 | 2 | 2 | 2 | 8 |
| **22** | R=$R_1$=*n*-$C_5H_{10}OH$ | 2 | 2 | 2 | 2 | 8 | 8 | 2 | 2 | 2 | 8 |
| **23** | R=H, $R_1$=$C_2H_5OC_3H_6$ | 1 | 1 | 2 | 1 | 4 | 2 | 0.5 | 0.5 | 1 | 4 |
| **24** | R=$C_2H_5OC_3H_6$, $R_1$=H | 2 | 2 | 8 | 2 | 8 | 8 | 2 | 2 | 2 | 8 |
| **25** | R=$R_1$=$C_2H_5OC_3H_6$ | 2 | 8 | 8 | 2 | 32 | 8 | 2 | 2 | 8 | 32 |
| **26** | R=H, $R_1$=$(CH_2)_2CH(CH_3)_2$ | 0.5 | 1 | 1 | 1 | 4 | 1 | 0.5 | 0.25 | 0.5 | 4 |
| **27** | R=$(CH_2)_2CH(CH_3)_2$, $R_1$=H | 1 | 1 | 2 | 1 | 4 | 2 | 0.5 | 0.5 | 1 | 4 |
| **28** | R=$R_1$=$(CH_2)_2CH(CH_3)_2$ | 1 | 2 | 2 | 1 | 4 | 2 | 1 | 1 | 1 | 4 |
| **29** | R=H, $R_1$=$C_6H_{11}CH_2$ | 0.5 | 0.5 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 2 |
| **30** | R=$C_6H_{11}CH_2$, R=H | 2 | 2 | 2 | 2 | 8 | 4 | 2 | 2 | 2 | 8 |
| **31** | R=$R_1$=$C_6H_{11}CH_2$ | 2 | 2 | 2 | 2 | 8 | 2 | 1 | 1 | 2 | 4 |
| **32** | R=H, $R_1$=$C_6H_5CH_2$ | 0.06 | 0.125 | 0.125 | 0.125 | 0.5 | 0.125 | 0.06 | 0.015 | 0.125 | 0.5 |
| **33** | R=$C_6H_5CH_2$, R=H | 8 | 8 | 16 | 8 | 32 | 16 | 4 | 4 | 8 | 64 |
| **34** | R=$R_1$=$C_6H_5CH_2$ | 8 | 8 | 8 | 8 | 32 | 8 | 4 | 1 | 8 | 32 |
| **35** | R=H, $R_1$=$C_6H_5(CH_2)_2$ | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.25 | 0.5 | 2 |
| **36** | R=$C_6H_5(CH_2)_2$, $R_1$=H | 2 | 2 | 2 | 2 | 8 | 4 | 2 | 1 | 2 | 8 |
| **37** | R=$R_1$=$C_6H_5(CH_2)_2$ | 2 | 2 | 2 | 2 | 8 | 2 | 1 | 0.25 | 2 | 4 |
| **38** | R=H, $R_1$=$C_6H_5(CH_2)_3$ | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 0.5 | 0.25 | 0.25 | 0.5 | 2 |
| **39** | R=$C_6H_5(CH_2)_3$, $R_1$=H | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.5 | 0.5 | 0.5 | 4 |
| **40** | R=$R_1$=$C_6H_5(CH_2)_3$ | 0.5 | 0.5 | 0.5 | 0.5 | 4 | 1 | 0.125 | 0.125 | 0.5 | 2 |
| **41** | R=H, $R_1$=*p*-$HOC_6H_4CH_2$ | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 0.06 | 0.5 | 2 |
| **42** | R=*p*-$HOC_6H_4CH_2$, $R_1$=H | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 0.5 | 2 | 8 |
| **43** | R=$R_1$=*p*-$HOC_6H_4CH_2$ | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 0.125 | 1 | 4 |
| **44** | R=H, $R_1$=*p*-$CH_3OC_6H_4CH_2$ | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 1 | 0.25 | 0.25 | 0.5 | 2 |
| **45** | R=*p*-$CH_3OC_6H_4CH_2$, $R_1$=H | 2 | 2 | 2 | 2 | 8 | 4 | 1 | 1 | 2 | 8 |
| **46** | R=$R_1$=*p*-$CH_3OC_6H_4CH_2$ | 2 | 2 | 4 | 2 | 8 | 4 | 2 | 2 | 2 | 8 |

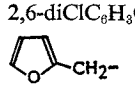
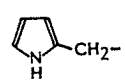
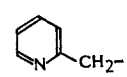
Abbreviations: See Table 1.

Table 3. *In vitro* antibacterial activity of *N*-alkyl vancomycins.

| | Compound | MIC ($\mu$g/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S.A. 1 | S.A. 2 | S.A. 3 | S.A. 4 | S.E. 1 | S.E. 2 | S.Py. | S.Pn. | S.D. 1 | S.D. 2 |
| **47** | R=$C_2H_5$, $R_1$=H | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 0.5 | 2 | 4 |
| **48** | R=$R_1$=$C_2H_5$ | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 4 |
| **49** | R=H, $R_1$=*n*-$C_5H_{11}$ | 0.5 | 1 | 1 | 1 | 2 | 1 | 0.5 | 0.125 | 1 | 2 |
| **50** | R=*n*-$C_5H_{11}$, $R_1$=H | 2 | 2 | 2 | 2 | 4 | 2 | 1 | 0.5 | 2 | 8 |
| **51** | R=H, $R_1$=*n*-$C_6H_{13}$ | 0.5 | 1 | 1 | 0.5 | 2 | 1 | 0.5 | 0.125 | 1 | 1 |
| **52** | R=*n*-$C_6H_{13}$, $R_1$=H | 1 | 1 | 2 | 2 | 4 | 2 | 1 | 0.5 | 2 | 4 |
| **53** | R=H, $R_1$=*n*-$C_9H_{19}$ | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.125 | 0.25 | 0.25 | 0.5 |
| **54** | R=$R_1$=*n*-$C_9H_{19}$ | 1 | 1 | 1 | 2 | 4 | 2 | 1 | 2 | 1 | 2 |
| **55** | R=H, $R_1$=$(C_2H_5)_2CHCH_2$ | 0.5 | 0.5 | 1 | 1 | 4 | 1 | 0.25 | 0.25 | 1 | 4 |
| **56** | R=$(C_2H_5)_2CHCH_2$, $R_1$=H | 1 | 2 | 2 | 2 | 4 | 2 | 1 | 1 | 2 | 8 |
| **57** | R=H, $R_1$=$CH_3S(CH_2)_3$ | 0.5 | 1 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 4 |
| **58** | R=$CH_3S(CH_2)_3$, $R_1$=H | 2 | 8 | 8 | 2 | 8 | 8 | 2 | 2 | 8 | 32 |
| **59** | R=H, $R_1$=$(C_6H_5)_2CHCH_2$ | 0.25 | 0.25 | 0.5 | 0.5 | 2 | 0.5 | 0.25 | 0.06 | 0.25 | 1 |
| **60** | R=$(C_6H_5)_2CHCH_2$, $R_1$=H | 4 | 4 | 4 | 4 | 8 | 8 | 4 | 2 | 4 | 8 |
| **61** | R=H, $R_1$=*p*-$CH_3SC_6H_4CH_2$ | 0.25 | 0.25 | 0.5 | 0.25 | 1 | 0.5 | 0.25 | 0.125 | 0.25 | 1 |
| **62** | R=*p*-$CH_3SC_6H_4CH_2$, $R_1$=H | 2 | 2 | 2 | 2 | 8 | 2 | 2 | 2 | 2 | 8 |
| **63** | R=H, $R_1$=*p*-$C_4H_9C_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.06 | 0.125 | 0.125 | 0.125 | 0.125 | 0.06 | 0.25 |
| **64** | R=$R_1$=*p*-$C_4H_9C_6H_4CH_2$ | 4 | 4 | 4 | 4 | 8 | 4 | 2 | 2 | 2 | 4 |
| **65** | R=H, $R_1$=*p*-$C_4H_9OC_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.125 | 0.25 | 0.125 | 0.06 | 0.06 | 0.06 | 0.25 |
| **66** | R=$R_1$=*p*-$C_4H_9OC_6H_4CH_2$ | 4 | 4 | 4 | 4 | 8 | 4 | 2 | 8 | 4 | 8 |

Abbreviations: See Table 1.

Table 4. *In vivo* antibacterial activity and pharmacokinetics of mono-*N*-alkyl vancomycins.

| Compound (R=H, $R_1$=as shown) | | MIC ($\mu$g/ml) | | | $ED_{50}$ (mg/kg×2, sc) | | | Serum (iv, rat) | |
|---|---|---|---|---|---|---|---|---|---|
| | | S.A.1 | S.Py. | S.Pn. | S.A.1 | S.Py. | S.Pn. | $T_{1/2}$ (hours) | 5 minutes conc (mg/ml) |
| **11** | $n$-$C_4H_9$ | 1 | 1 | 0.5 | 6.3 | 1.9 | 1.3 | 0.5 | 45 |
| **51** | $n$-$C_6H_{13}$ | 0.5 | 0.5 | 0.125 | 3.6 | 1.1 | 0.9 | 0.68 | 109 |
| **17** | $n$-$C_8H_{17}$ | 0.25 | 0.125 | 0.125 | 3.4 | 0.9 | 0.4 | 2.1 | 256 |
| **5** | $n$-$C_{10}H_{21}$ | 0.13 | 0.06 | 0.13 | 1.8 | 0.65 | 0.68 | 3.4 | 203 |
| **67** | $n$-$C_{12}H_{25}$ | 0.25 | 0.125 | 0.25 | 5.7 | 0.3 | 0.4 | 2.9 | 254 |
| **55** | $(C_2H_5)_2CHCH_2$ | 0.5 | 0.25 | 0.25 | 1.8 | 1.1 | 1.7 | 1.7 | 95 |
| **29** | $C_6H_{11}CH_2$ | 0.5 | 0.5 | 0.25 | 1.8 | 1.4 | 0.9 | — | — |
| **20** | $(CH_2)_5OH$ | 1 | 1 | 1 | 5.2 | 5.8 | 1.8 | — | — |
| **23** | $C_2H_5O(CH_2)_3$ | 1 | 0.5 | 0.5 | 6.2 | 4.3 | 2.0 | — | — |
| **57** | $CH_3S(CH_2)_3$ | 0.5 | 0.5 | 0.25 | 3.7 | 1.4 | 1.5 | 0.68 | 97 |
| **32** | $C_6H_5CH_2$ | 0.06 | 0.06 | 0.015 | 0.8 | 1.0 | 0.9 | 1.84 | 89 |
| **59** | $(C_6H_5)_2CHCH_2$ | 0.25 | 0.25 | 0.06 | 0.6 | 1.3 | 0.8 | 1.49 | 116 |
| **35** | $C_6H_5(CH_2)_2$ | 0.5 | 0.5 | 0.25 | 1.0 | 3.1 | 1.0 | — | — |
| **38** | $C_6H_5(CH_2)_3$ | 0.5 | 0.25 | 0.25 | 1.1 | 1.6 | 1.2 | 1.74 | 79 |
| **68** | $C_6H_5CH(CH_3)CH_2$ | 0.5 | 0.03 | 0.125 | 1.8 | 0.8 | 0.9 | — | — |
| **69** | $p$-$(CH_3)_2CHC_6H_4CH_2$ | 0.25 | 0.25 | 0.25 | 1.1 | 0.9 | 0.9 | 1.6 | 86 |
| **63** | $p$-$C_4H_9C_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.7 | 0.4 | 0.8 | 5.4 | 156 |
| **70** | $p$-$C_5H_{11}C_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.6 | 0.8 | 0.6 | — | — |
| **71** | $p$-$C_8H_{17}C_6H_4CH_2$ | 0.5 | 0.25 | 0.25 | 0.19 | 0.62 | 0.23 | — | — |
| **44** | $p$-$CH_3OC_6H_4CH_2$ | 0.5 | 0.25 | 0.25 | 1.1 | 0.6 | 2.3 | — | — |
| **65** | $p$-$C_4H_9OC_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.7 | 0.5 | 0.6 | 2.4 | 205 |
| **72** | $p$-$C_5H_{11}OC_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.6 | 0.9 | 0.7 | — | — |
| **73** | $p$-$C_8H_{17}OC_6H_4CH_2$ | 0.25 | 0.25 | 0.25 | 0.2 | 0.2 | 0.2 | — | — |
| **61** | $p$-$CH_3SC_6H_4CH_2$ | 0.25 | 0.25 | 0.125 | 1.1 | 2.1 | 0.9 | — | — |
| **74** | $p$-$CNC_6H_4CH_2$ | 0.5 | 0.25 | 0.125 | 1.8 | 2.5 | 1.2 | 0.68 | 112 |
| **41** | $p$-$HOC_6H_4CH_2$ | 0.5 | 0.5 | 0.06 | 0.9 | 1.1 | 1.2 | 0.67 | 145 |
| **75** | $p$-$(CH_3)_2NC_6H_4CH_2$ | 0.5 | 0.25 | 0.5 | 1.3 | 3.1 | 1.9 | 2.0 | 119 |
| **76** | $p$-$(C_2H_5)NC_6H_4CH_2$ | 0.25 | 0.25 | 0.125 | 1.1 | 0.7 | 1.9 | 5.8 | 110 |
| **77** | $p$-$CH_3CONHC_6H_4CH_2$ | 0.5 | 0.5 | 0.25 | 1.7 | 4.9 | 3.7 | — | — |
| **78** | $p$-$BrC_6H_4CH_2$ | 0.25 | 0.25 | 0.03 | 1.3 | 0.8 | 0.4 | 1.7 | 136 |
| **79** | $m$-$BrC_6H_4CH_2$ | 0.5 | 0.25 | 0.125 | 0.9 | 1.2 | 0.7 | 1.6 | 120 |
| **80** | $o$-$BrC_6H_4CH_2$ | 1 | 0.5 | 0.25 | 1.4 | 1.0 | 1.3 | — | — |
| **81** | $p$-$ClC_6H_4CH_2$ | 0.5 | 0.25 | 0.03 | 0.8 | 1.8 | 0.7 | 2.0 | 188 |
| **82** | 2,6-di$ClC_6H_3CH_2$ | 0.5 | 0.5 | 0.5 | 2.5 | 1.8 | 3.1 | — | — |
| **83** |  | 1 | 1 | 0.5 | 1.3 | 1.9 | 3.3 | — | — |
| **84** |  | 1 | 0.5 | 0.25 | 1.4 | 1.8 | 2.5 | — | — |
| **85** |  | 1 | 0.5 | 0.25 | 1.9 | 6.8 | 3.1 | 0.43 | 117 |

Abbreviations: See Table 1.

representative structural types were also undertaken. In the aliphatic straight chain series, increasing the chain length enhances activity and the optimum chain length seems to be the $C_{10}$ analog. Branching the aliphatic side chains, or substituting with oxygen or sulfur (compounds **20**, **23**, **29**, **55** and **57**) does not seem to increase activity. The benzyl derivative **32**, and the benzyl derivatives substituted at the 4-position with an aliphatic side chain, especially compounds **63**, **65**, **70** and **76**, are more active

Table 5. MIC[a] and MBC[a] for *Streptococcus* sp. (Group D).

| Compound | *Streptococcus* sp. (Group D) strain No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 238 | | Guze | | Mitis | | Shrigley | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Vancomycin | 2 | >128 | 0.25 | >128 | 0.25 | >128 | 1.0 | >128 |
| **5** | 0.03 | 64 | 0.008 | 64 | 0.008 | >128 | 0.008 | >128 |

[a] μg/ml.

Table 6. Rat endocarditis using *Streptococcus faecium* X66.

| Compound | Dosage (mg/kg×28) | 5th Day[a] | | 9th Day[a] | | 16th Day[a] | |
|---|---|---|---|---|---|---|---|
| | | % Cured | % With $10^4$ drop | % Cured | % With $10^4$ drop | % Cured | % With $10^4$ drop |
| Vancomycin | 20 | 33 | 33 | 100 | 100 | 100 | 100 |
| **32** | 20 | 100 | 100 | 66 | 100 | 100 | 100 |

[a] Days after initiation of treatment.

than the aliphatic series in the *in vivo* models. The most active compounds are the octylbenzyl (**71**) and octyloxybenzyl (**73**), derivatives. Substitution at the *para*-position of the benzyl moiety with hetero atoms like oxygen, sulfur, nitrogen or halogens does not seem to enhance activity. The heterocyclic analogs **83**, **84** and **85** also have no advantage over the benzyl analog **32**. In general, these mono-*N*-alkyl vancomycins have more favorable pharmacokinetics than the parent vancomycin. Butyl and butyloxy derivatives, **63** and **65**, have elimination half-lives of 5.4 and 2.4 hours in rats, respectively.

Table 7. Rat descending pyelonephritis with *Streptococcus faecalis* Guze.

| Compound | Dosage (mg/kg×12) | % Cured | % With $10^4$ log drop |
|---|---|---|---|
| Vancomycin | 10 | 80 | 80 |
| **63** | 10 | 100 | 100 |
| **65** | 10 | 90 | 90 |
| **32** | 10 | 60 | 70 |
| Vancomycin | 2 | 10 | 50 |
| **63** | 2 | 70 | 100 |
| **65** | 2 | 40 | 80 |
| **32** | 2 | 10 | 70 |

Since several of the *N*-alkyl vancomycins were more active than the parent antibiotic, further evaluations of representative members of the *N*-alkyl vancomycin series were undertaken. Comparison between the MIC and MBC of the mono-*N*-decyl vancomycin **5** and vancomycin for four strains of Group D *Streptococcus* sp. showed that both compounds were bacteriostatic. The mono-*N*-benzyl vancomycin **32** and the parent vancomycin were examined in the rat endocarditis model using *Streptococcus faecium* X66. Both compounds had comparable activity in this test.

Finally, the mono-*N*-benzyl (**32**), mono-*N*-butylbenzyl (**63**), and mono-*N*-butyloxybenzyl (**65**) derivatives were evaluated in the descending pyelonephritis rat model with *Streptococcus faecalis* Guze. The order of activity in the above test of the *N*-alkyl vancomycins were: Mono-*N*-butylbenzyl (**63**) >mono-*N*-butyloxybenzyl (**65**) >mono-*N*-benzyl (**32**)=vancomycin.

Several compounds in the *N*-alkyl vancomycin series were more active than vancomycin. Octylbenzyl (**71**), octyloxybenzyl (**73**), butylbenzyl (**63**), butyloxybenzyl (**65**) and benzyl (**32**) derivatives had the best activity and are up to five times more active than vancomycin.

## Experimental

### General Procedure for the Preparation of *N*-Alkyl Vancomycins

The desired aldehyde was added to a solution of vancomycin base in DMF. The solution was stirred and a slight excess of sodium cyanoborohydride was added to the intermediate SCHIFF's base formed. After completion of the reaction, the mixture of products was separated by chromatography. A shorter reaction time and an equimolar amount of the aldehyde favored the formation of mono-*N*-alkyl vancomycins. Longer reaction time and an excess of aldehyde gave the di-*N*-alkyl derivative as the major product.

Example: Vancomycin base (5 g, 3.5 mmol) was dissolved in DMF and *n*-decyl aldehyde (0.7 ml, 3.72 mmol) was added. The reaction mixture was stirred for 2 hours in a 70°C oil bath.

Sodium cyanoborohydride (275 mg, 4.4 mmol) was added to the solution containing the SCHIFF's base formed *in situ*. The reaction mixture was stirred for 2 hours in the 70°C oil bath, then cooled to room temperature and the DMF evaporated to dryness. The reaction mixture was purified by reverse-phase HPLC using a Waters Prep Pak/500 column and an acetonitrile - water gradient. The eluates were monitored by analytical HPLC using UV detection at 280 nm. Appropriate fractions were pooled and 794 mg of mono-*N*-decyl vancomycin **5** was obtained. The identity of the product was confirmed by FAB-MS.

Chromatography: The conditions used for the analytical and preparative HPLC were described earlier.[8]

FAB-MS: FAB-MS spectra were determined using a VGZ AB-3F mass spectrometer. Samples were dispersed in thioglycerol and introduced into the spectrometer on a cooled FAB target.

### Antibacterial Activity *In Vitro*

The MICs for the aerobic bacteria strains were determined in an agar dilution assay. Mueller-Hinton agar containing 1% supplement C (Difco Laboratories, Detroit, Michigan) was used. The dilutions of the antibiotics were made in water and mixed with the melted agar prior to pouring the plates. The various bacteria were inoculated onto the medicated plates using a Cotlara replicator at an inoculum of $10^4$ cfu/spot. The plates were then incubated for 20~24 hours at 35°C. End points were read to discrete colonies.

### Antibacterial Activity *In Vivo*

The therapeutic efficacy of the vancomycin derivatives were determined in standard mouse protection tests. An experimental systemic infection was produced using ICR random sex mice (Harland Laboratories, Cumberland, Indiana), by intraperitoneal inoculation of a suitable diluted broth culture of the infecting organism. The test compounds (*N*-alkyl vancomycins) were administered subcutaneously at 1 and 5 hours post-infection. Five 2-fold dilutions of each antibacterial agent was tested and there were eight mice for each dose level. All the mice were observed for a period of 7 days, after which the effective dose ($ED_{50}$) was calculated by the method of REED and MUENCH.[9] Under the conditions of the test, all infected and untreated mice died within 48 hours.

### MIC and MBC Determination

Determination of the broth MIC was carried out in Mueller-Hinton broth supplemented with 50 mg $Ca^{2+}$ and 25 mg $Mg^{2+}$ per liter in autotiter plates. To determine the MBC (99.99 killing) of the antibiotic, subcultures onto Mueller-Hinton agar without antibiotics were made using a 10-$\mu$l loopful from those dilutions on the MIC plates which failed to show macroscopic turbidity. These plates were read after 72 hours of incubation.

### Production and Treatment of Endocarditis

Groups of 8~10 female Sprague Dawley rats weighing 200~220 g were anesthetized and the right carotid artery was exposed. A silastic catheter was inserted *via* the right carotid artery through the aortic valve into the left ventricle of the heart and secured in place with a silk ligature. Forty eight hours after catheterization, the rats were injected *via* the tail vein with 0.5 ml of *S. faecium* X66 culture containing $5\times10^8$ organisms per ml. Subcutaneous therapy with the test antibiotics began

15 minutes prior to infection and at 12-hour intervals for 14 days, a total of 28 treatments. At the 5th, 9th day of treatment, and also at the conclusion of therapy the hearts were removed, homogenized, diluted and plated on Trypticase soy agar. The plates were incubated 48 hours prior to counting the colonies. The results are expressed as a cure if the animal had no growth at the lowest dilution of the heart homogenate ($10^{-2}$). A positive effect was recorded where the treated animal had a colony count of at least $10^4$ less than the average colony count of the infected untreated control animals.

Production and Treatment of Descending Rat Pyelonephritis

Groups of 8~10 female Sprague Dawley rats weighing 190~220 g were anesthetized and the left ureter was exposed and occluded for 20 minutes. The rats were then injected with 0.5 ml of the test organism *via* the femoral vein. The occlusion was then removed. Antimicrobial therapy commenced 4 to 5 hours postinfection and continued two times a day for a total of 12 doses. Four hours after the last treatment the rats were sacrificed, the left kidney removed, homogenized, diluted and plated for colony counts in Trypticase soy agar. The plates were incubated for 48 hours prior to counting. The therapeutic results were expressed as: The percentage of rats cured, meaning the percentage of rats with a colony cell count per kidney of less than $10^2$ per g of kidney tissue and the percentage of rats with at least a $10^4$-reduction in bacterial titer compared with the average of the infected control rat's kidneys. Infected control rats were treated with 0.125% carboxymethylcellulose only.

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