The Chemistry of Pseudomonic Acid.† 18. Heterocyclic Replacement of the α,β-Unsaturated Ester: Synthesis, Molecular Modeling, and Antibacterial Activity[1]

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The electronic requirements around the C1−C3 region of pseudomonic acid analogues were investigated. Synthetic routes were developed to access a range of compounds where the α,β-unsaturated ester moiety had been replaced by a 5-membered ring heterocycle. The inhibition of isoleucyl tRNA synthetase from *Staphylococcus aureus* NCTC 6571 was determined as was the minimum inhibitory concentration (MIC) of the test compounds against that organism. Compounds possessing a region of electrostatic potential corresponding to that of the carbonyl group in the α,β-unsaturated ester, and a low-energy unoccupied molecular orbital in the region corresponding to the double bond, were found to have IC_{50} values of 0.7−5.3 ng mL^{-1}. However the MIC values of these compounds were in the range 2.0−8.0 μg mL^{-1}, reflecting their poorer penetration into the bacterial cell.

Introduction

Pseudomonic acid (**1a**), marketed by SmithKline Beecham as the topical antibacterial agent, Bactroban, is potent against Gram-positive bacteria and some Gram-negative organisms such as *Haemophilus* and *Pasteurella*.[2] The mechanism of action is the inhibition of bacterial isoleucyl tRNA synthetase,[3a,b] for which pseudomonic acid shows a much greater affinity than for the corresponding mammalian enzyme.[3b] However, *in vivo*, the ester function of **1a** is hydrolyzed to the antibacterially inactive monic acid **1b**. In our search for metabolically stable analogues, we sought to replace the α,β-unsaturated ester moiety (C1−C3) with a range of heterocycles.

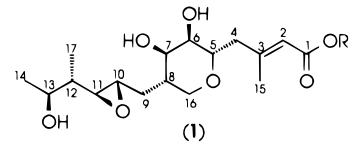
In recent years, the structure of several bacterial tRNA synthetases has been determined.[4] However, the determination of the structure of isoleucyl tRNA synthetase (IRS) has yet to be accomplished, and the precise interactions of pseudomonic acid in the active site are not yet known. We sought to build a model of some of the requirements for activity based on the structure−activity relationships of various pseudomonic acid analogues.

In this paper we report the synthesis of novel pseudomonic acid C1−C3 heterocycles, designed to test a structure-activity hypothesis developed by molecular modeling of active and inactive derivatives. The biological activity of the new compounds is reported in terms of inhibition of the IRS enzyme and whole-cell antibacterial activity.

Background

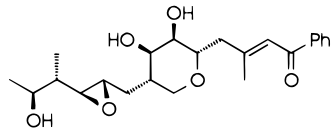
We have previously shown that the ester moiety in pseudomonic acid can be replaced by other esters, for example ethyl monate **1c**,[2] and by aryl ketones **2**[1,5] or heterocycles such as oxazole **3a**[6] or oxadiazole **3b**,[7] while retaining antibacterial activity. The 2,3-double bond is an important feature, the antibacterial activity residing solely with the *E*-geometry. Inactive compounds include the 2-phenyloxazole **3c**[7] and the *Z*-isomers (e.g. **4**).[6] Reduction of the double bond leads to reduced activity.

The knowledge that only the *E*-isomers are active, led

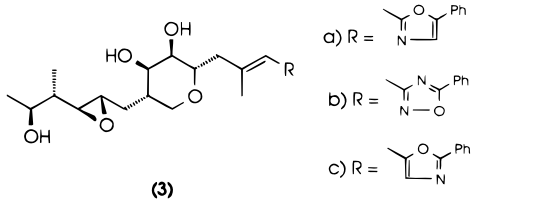


(1)

a) R = $(CH_2)_8COOH$ Pseudomonic acid A
b) R = H Monic acid A
c) R = CH_2CH_3 Ethyl Monate A



(2)



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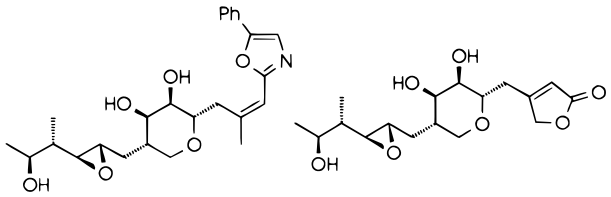

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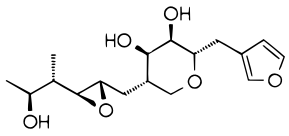

(6)

† The approved generic name for pseudomonic acid is mupirocin.
⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

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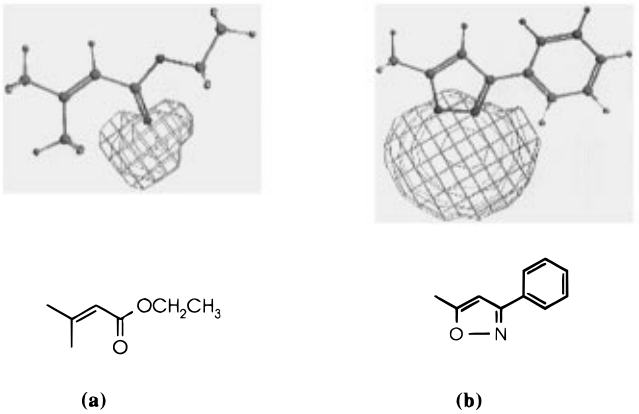


Figure 1.

us toward replacing the C1-C3 region with a cyclic system. Previous examples of this, the butenolide **5** and furan **6** showed no activity.[5] However, these lacked the alkyl or aryl substituent incorporated into the other ester bioisosteric replacements, and as such are not directly comparable.

Molecular Modeling

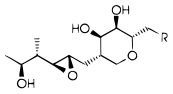
To build up a hypothesis of the requirements for activity, electronic features of the previously reported esters and replacements (**1, 2, 3a**−**c**) were examined. Of these, the electrostatic potential appeared to have the most significance. Using AM-1 potential-derived charges, electrostatic potential maps around the active ester bioisosteres (**1c, 2, 3a,b**) were calculated. The intersection of these maps, contoured at −10 kcal mol^{-1} led to a specific volume common to all active compounds, shown in Figure 1a superimposed on the ester functionality. The electrostatic potential map of the inactive **3c** at this energy level gave no intersection with this volume.

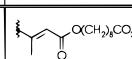
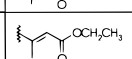
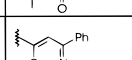
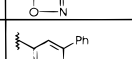
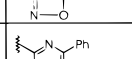
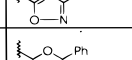
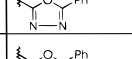
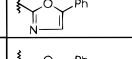
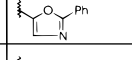
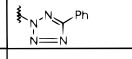
To test the hypothesis that the electrostatic potential shown in Figure 1a was a requirement for activity, the electrostatic potential of a number of 5-membered ring heterocycles (**7b, 8, 14**−**16**, **21**, **25**, and **26**) was calculated, and the intersection of the individual maps with the common volume (**1a**) was determined. The presence or absence of an intersection with this volume is given in Table 1. For example, the isoxazole corresponding to **7b** gave an electrostatic potential map at −10 kcal mol^{-1} shown in Figure 1b, the intersection of this with the common volume **1a** being equal to **1a** itself.

A second feature believed to be associated with activity in pseudomonic acid analogues is the C2−C3 double bond, which can be represented by the presence of a low-energy unoccupied molecular orbital (Figure 2a). To test the hypothesis that this was also a requirement for activity, the 5-membered ring heterocycles (**7b, 8, 14**−**16**, **21**, **25**, and **26**) were examined. For example the isoxazole corresponding to **8b** possesses a low-energy molecular orbital associated with both atoms corresponding to the double bond (Figure 2b) whereas the isoxazole **8** does not (Figure 2c). The presence or absence of these electronic features of the 5-membered ring heterocycles are given in Table 1.

The 5-membered ring heterocycles were then synthesised as described below and tested as inhibitors of isoleucyl tRNA synthetase and as antibacterial agents.

Table 1



Compound	R	Electrostatic potential	low-energy unoccupied molecular orbital	*S.aureus* NCTC 6571	
				IC_{50} (ng mL^{-1})	MIC (μg mL^{-1})
1a		✓	✓	0.85	0.13
1c		✓	✓	1.1	0.5
7b		✓	✓	5.3	4.0
8		✓	X	17% @ 8.0	>64
14		✓	✓	0.7	4.0
15		✓	✓	2.1	8.0
16		X	✓	0% @ 8.0	>64
21		✓	✓	1.9	2.0
25		✓	✓	3.6	8.0
26		✓	X	6.0	>64

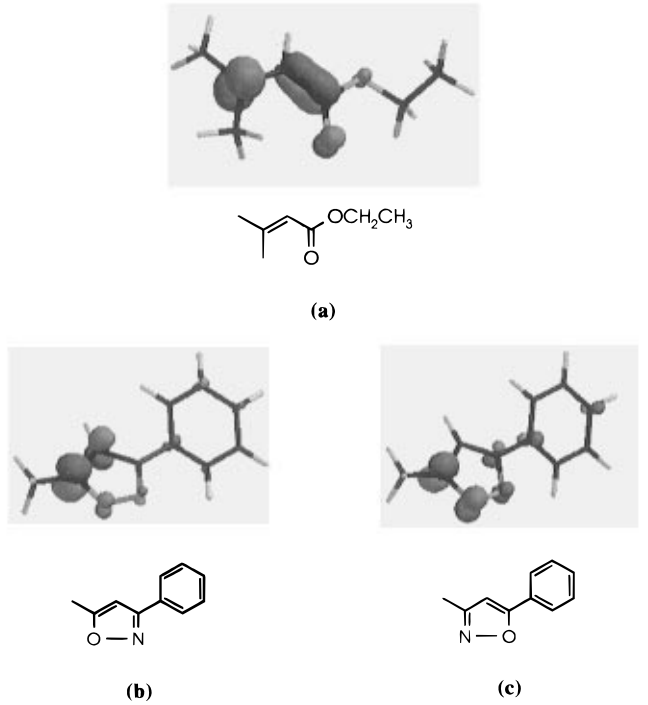
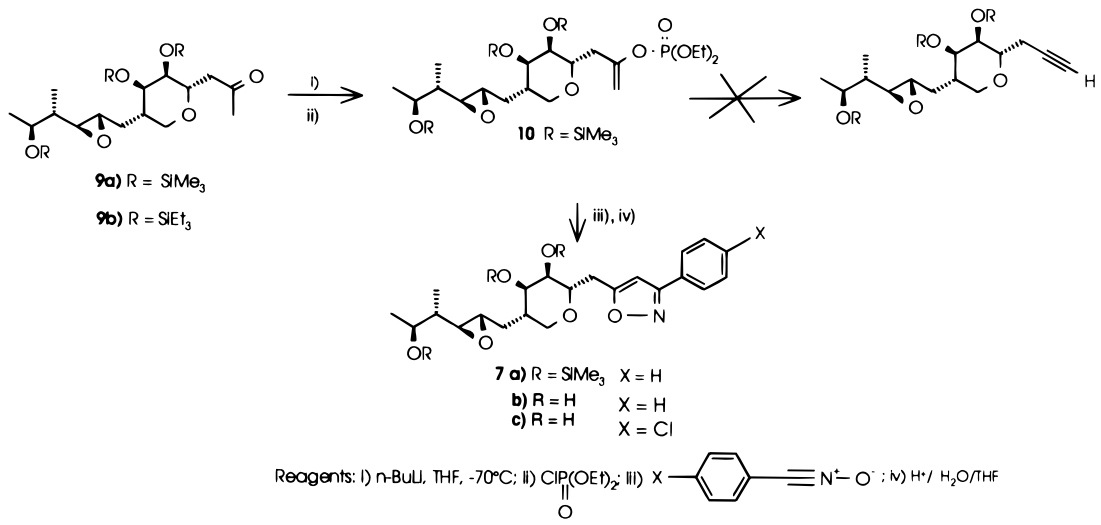


Figure 2.

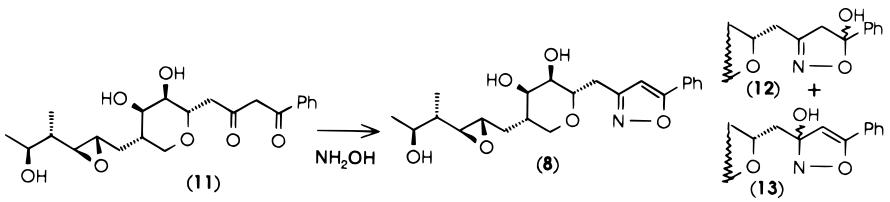
Chemistry

The first route examined for the synthesis of the 3-phenylisoxazole **7b** was a 1,3-dipolar cycloaddition of benzonitrile oxide to the corresponding acetylene (Scheme 1). The readily available ketone **9a**[4] was converted *via* the kinetic enolate to the enol phosphate **10**. However, attempted β-elimination with base gave no reaction at −70 °C, while at higher temperatures opening of the tetrahydropyran ring occurred. Enol acetates have been shown[8] to react with nitrile oxides to form isoxazolines which eliminate acetic acid on heating to form the corresponding isoxazoles. We envisaged that a similar

Scheme 1



Scheme 2



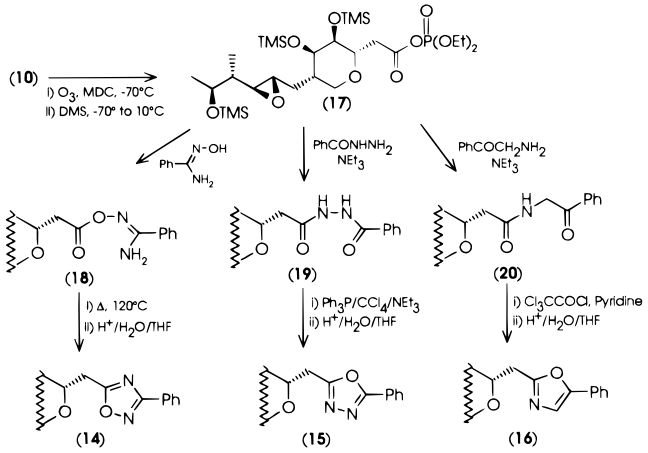
reaction might occur with the enol phosphate **10** enabling formation of the isoxazole directly. Indeed, reaction of benzonitrile oxide with the enol phosphate **10** in refluxing benzene gave a single regioisomer of isoxazole, albeit in low yield (9%). It is thought that this low yield is probably due to extensive dimerization of the nitrile oxide and also to the instability of the trimethylsilyl (TMS) protecting groups if the reaction mixture becomes slightly acidic. After cycloaddition/elimination, the TMS protecting groups were removed with mild acid. The regiochemistry of cycloaddition was determined by NOE on the corresponding 4-chloro analogue **7c**, where an NOE effect was noted between the aromatic *ortho* H and the isoxazole proton.

The synthesis of the other isoxazole regioisomer **8** was envisaged via the corresponding diketone **11**[9] (Scheme 2). Reaction of the diketone **11**[9] with hydroxylamine in ethanol gave the isoxazole **8** in low yield. This was assigned the structure by comparison with the previously assigned 3-phenylisoxazole. Also obtained were the hydroxyisoxazolines **12** and **13** in 36% and 7% yield, respectively. The outcome of the reaction of hydroxylamine with a diketone is known to depend on many features,[10] and the reaction was not optimized further. While **12** could be converted to the isoxazole **8** on acid treatment, this was accompanied by some acid-catalyzed rearrangement of the pseudomonic acid nucleus.[11]

For the synthesis of the oxadiazoles **14** and **15**, and the 2-substituted 5-phenyloxazole **16**, an activated form of the "C3-acid" was required. Although we were unable to isolate this acid directly, a direct synthesis of the mixed phosphonic anhydride was developed by ozonolysis of the enol phosphate **10**[12] (Scheme 3).

The TMS-protected mixed phosphonic anhydride **17** was not isolated but reacted with the required amidoxime, acyl hydrazide, or amino ketone in the presence of triethylamine to give the acylated derivatives **18**, **19**, and **20**, respectively. Cyclization of the acylated intermediate under standard conditions,[6a,b,13] followed by mild acid deprotection, gave the required heterocycles (**14**−**16**).

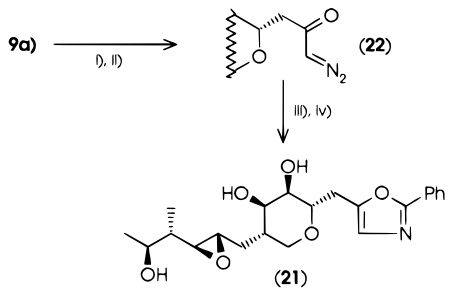
Scheme 3



Of the many reported oxazole syntheses,[14] the reaction of a carbenoid species with a nitrile appeared an amenable route to the 2-phenyloxazole (**21**). The ketone **9a** was converted *via* the kinetic enolate to the diazo ketone **22** (Scheme 4). Reaction with benzonitrile in the presence of rhodium(II) acetate gave the oxazole **21** in moderate yield.

The formation of 5-membered rings linked *via* a heteroatom required further manipulation of the pseudomonic acid skeleton (Scheme 5). Enolisation of the *tris*(triethylsilyl) ketone (**9b**)[5] under thermodynamic conditions, followed by reaction with triisopropylsilyl triflate gave the triisopropylsilyl enol ether (**23**), accompanied by the kinetic product, together with the products of epimerisation at C5. However, **23** was

Scheme 4



Reagents: I) $LiN(SiMe_3)_2$; II) Tosyl azide; III) $Rh_2(OAc)_4$, PhCN; IV) H^+/H_2O/THF

chromatographically stable, enabling its separation from the byproducts. Ozonolysis of **23** followed by reductive workup with sodium borohydride gave the alcohol **24**. This was then reacted with 5-phenyltetrazole under Mitsonobu conditions,[15] followed by fluoride deprotection to give the desired tetrazole **25**.

For the synthesis of the thiazole **26**, the ketone **9a** was converted to the bromo ketone **27** followed by reaction with thiobenzamide (Scheme 6). Dehydration of the hydroxythiazoline **28** via the mesylate gave the desired product in 29% yield.

Results and Discussion

Table 1 shows the heterocycles synthesized, together with pseudomonic acid and ethyl monate for comparison, the presence or absence of the electrostatic potential and low-energy unoccupied molecular orbitals, the inhibition of isoleucyl tRNA synthetase from *Staphylococcus aureus* NCTC 6571, and the minimum inhibitory concentration of the test compounds against that organism.

The most potent compounds (**14, 15**, and **21**) have a level of inhibition in the region of that seen with pseudomonic acid and ethyl monate and a reasonable level of whole-cell activity. All of these compounds possess both the electrostatic potential and unoccupied molecular orbital features. The isoxazole **7b**, tetrazole **25**, and thiazole **26** are slightly weaker inhibitors. Of these, the thiazole **26** lacks the unoccupied molecular orbital feature. The isoxazole **8** which also lacks the unoccupied molecular orbital feature has substantially reduced inhibition of IRS. The oxazole **16** lacking the electrostatic potential feature, does not inhibit the isolated enzyme, confirming that this is an important feature for recognition.

The electrostatic potential may correspond to a requirement for interaction with a hydrogen-bond donor in the active site. The role of the low-energy molecular orbital is less clear, but may indicate a site for charge-transfer interaction within the active site.

While the level of inhibition approaches that seen with pseudomonic acid (and is equipotent in the case of the oxadiazole **14**), none of the compounds synthesized have whole-cell activity approaching that of pseudomonic acid. It is believed that this is due to poorer penetration of these lipophilic compounds into the bacterial cell.

It is concluded that bioisosteric replacement of the α,β-unsaturated ester by aryl heterocycles has been successfully achieved by 5-membered ring heterocycles possessing electrostatic potential in the region of the carbonyl oxygen/ring nitrogen of previous C1 ester replacements. It is postulated that this may be a recognition feature by an H-bond donor in the active site.

Experimental Section

Infrared spectra were determined either in dichloromethane on a Perkin-Elmer PE 983 spectrophotometer or in KBr on a Philips PU 9706 spectrophotometer. NMR spectra were recorded on a Bruker AC-250F spectrometer. Chemical shifts are expressed in ppm (δ) relative to internal tetramethylsilane. Mass spectra were obtained on a VG ZAB mass spectrometer.

Merck Kieselgel 60 (<230 mesh ASTM) was used for column chromatography. Tetrahydrofuran (THF) was dried by distillation from calcium hydride followed by distillation from sodium benzophenone ketyl.

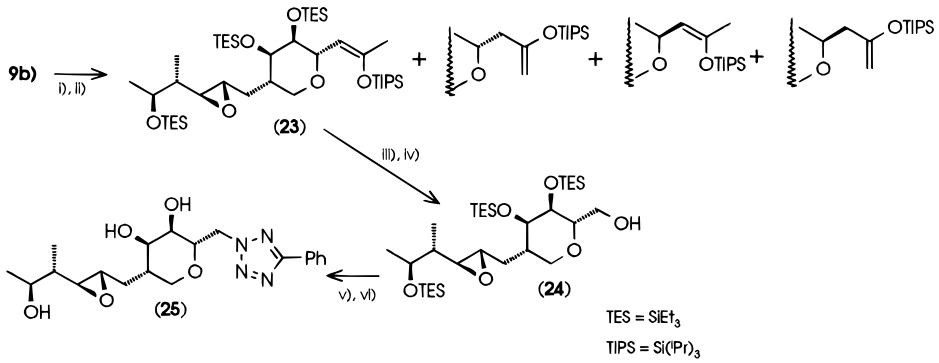
The purity of all target compounds was confirmed by HPLC, performed on a Waters Associates instrument using a C_{18} μ-Bondapak reverse-phase column with pH 4.5 0.05 M ammonium acetate buffer−methanol solutions as eluant. Detection was by UV at 240 nm and at the λ_{max} of the test compound. All target compounds were determined as >95% pure when examined by this method.

6,7,13-*O*-*Tris*(Trimethylsilyl)-1,15-bisnormon-3-yl Diethyl phosphate (10). A solution of [5(*S*)-[(2(*S*),3(*S*)-epoxy-5(*S*)-[(trimethylsilyl)oxy]-4-methylhexyl]-3(*R*),4(*R*)-*bis*[(trimethylsilyl)oxy]tetrahydropyran-2(*S*)-yl]acetone (**9a**)[4] (8.24 g, 16 mmol) in dry THF (40 mL) was added dropwise over a period of 30 min to a solution of lithium diisopropylamide (17.6 mmol) in dry THF (40 mL) at −78 °C under an argon atmosphere. After a further 30 min at −78 °C, the solution was treated with diethyl chlorophosphate (2.42 mL, 17.6 mmol). The mixture was allowed to warm to room temperature over 30 min and stirred at this temperature for a further 30 min. Saturated ammonium chloride (20 mL) was added and the mixture extracted with ethyl acetate (3 × 50 mL). The organic phases were combined, washed with brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product chromatographed on Kieselgel 60, eluting with 0−50% ethyl acetate in hexane. The product **10** was obtained as a colorless oil (6.51 g, 63%): IR (CH_2Cl_2) 3671, 1655, 1449, 1372, 1272, and 1249 cm^{-1}; ^1H NMR ($CDCl_3$) δ *inter alia* 0.91 (3H, d, J = 7.1 Hz, 17-H_3), 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 1.36 (6H, dt, J = 0.73, 6.9 Hz, CH_3), 4.16 (4H, dq, appears as five lines, J = 7.3 Hz, CH_2), and 4.77 (2H, d, J = 74.5 Hz, =CH_2); MS (EI) m/z 654 (MH^+, 1); HRMS calcd for $C_{28}H_{59}O_9PSi_3$ 654.3205, found 654.3218.

5-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-3-phenylisoxazole (7b). The enol phosphate **10** (1.3g, 2 mmol) was dissolved in dry benzene (10 mL) and treated with benzohydroximinoyl chloride[16] (343 mg, 2.2 mmol) and triethylamine (0.3 mL). The mixture was heated to reflux under an argon atmosphere for 24 h and then cooled to room temperature and the solvent evaporated under reduced pressure. The crude product was chromatographed on Kieselgel 60 eluting with 0−30% ethyl acetate in hexane to give the protected isoxazole as a colorless oil (110 mg, 9%): IR (CH_2Cl_2) 2950, 2890, 1602, and 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.10 (9H, s, $SiCH_3$), 0.17 (9H, s, $SiCH_3$), 0.18 (9H, s, $SiCH_3$), 0.90 (3H, d, J = 7.1 Hz, 17-H_3), 1.20 (3H, d, J = 6.3 Hz, 14-H_3), 2.67−2.73 (2H, m, 10-H and 11-H), 2.98 (1H, dd, J = 9.6, 15.7 Hz, 4-H), 3.15 (1H, dd, J = 2.5, 15.7 Hz, 4′-H), 6.45 (1H, s, isoxazole-H), 7.42−7.45 (3H, m, Ar-H), and 7.79−7.84 (2H, m, Ar-H); MS (EI, m/z) 625 (M^+, 1), 117 (100).

This material (100 mg, 0.16 mmol) was dissolved in a mixture of THF (3 mL) and water (0.75 mL) and treated with 5 M HCl (2 drops). After 5 min, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5 mL), and the mixture was extracted with ethyl acetate. The organic phases were combined and dried over anhydrous $MgSO_4$, the solvent was evaporated, and the crude product was chromatographed on Kieselgel 60 eluting with 5% methanol in dichloromethane to give **7b** as a white foam (56 mg, 87%): IR (KBr) 3465, 3318, 1610, and1580 cm^{-1}; UV (EtOH) λ_{max} 240.5 nm (ϵ_m 15079); ^1H

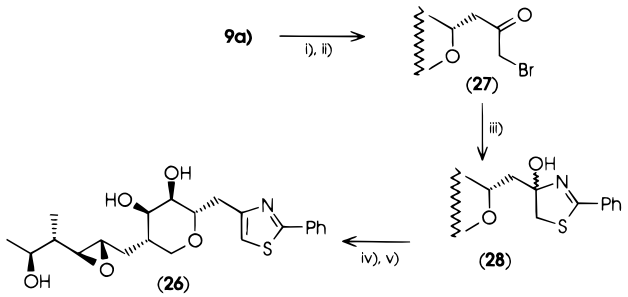
Scheme 5

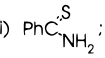


Reagents: i) LDA, -70°C; ii) TIPSOTf; iii) O_3, MDC, MeOH, -70°C; iv) $NaBH_4$, -70°C - room temp;

v) Ph_3P, DEAD, THF,  ; vi) TBAF, THF

Scheme 6



Reagents: i) TMSOTFl, ii) NBS; iii)  ; iv) NEt_3, $MeSO_2Cl$, DMAP;
v) H^+/H_2O/THF

NMR ($CDCl_3$) δ 0.92 (3H, d, J = 7.0 Hz, 17-H_3), 1.21 (3H, d, J = 6.3 Hz, 14-H_3), 2.69 (1H, dd, J = 2.0, 7.8 Hz, 11-H), 2.80 (1H, dt, J = 2.0, 5.5 Hz, 10-H), 3.01 (1H, dd, J = 8.0, 15.6 Hz, 4-H), 3.29 (1H, dd, J = 3.1, 15.6 Hz, 4′-H), 6.47 (1H, s, isoxazole-H), 7.43−7.47 (3H, m, Ar-H), 7.77−7.83 (2H, m, Ar-H);^{13}C NMR (CD_3OD) δ 12.1 (C-17), 20.2 (C-14), 30.4 and 32.9 (C-4) and (C-9), 41.7 (C-8), 43.6 (C-12), 56.7(C-10), 61.2 (C-11), 66.3 (C-16), 69.4 (C-6), 70.6 (C-7), 71.4 (C-13), 75.9 (C-5), 101.5 (C-2), 127.6 (2 × Ar), 129.9 (2 × Ar), 130.4 (Ar), 130.9 (Ar), 163.8 (C═N), and 173.2 (C−O); MS m/z 403 (M^+, 1), 188 (40), and 159 (100); found M^+ 403.2002, $C_{22}H_{29}NO_6$ requires M 403.1995.

5-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-3-(4-chlorophenyl)isoxazole (7c). The enol phosphate **10** (654 mg, 1 mmol) was reacted with chlorobenzohydroximinoyl chloride (266 mg,1.4 mmol) as described in the preparation of **7b**. After deprotection as previously described, the isoxazole **7c** was obtained as a white foam in 9% overall yield: IR (KBr) 3421, 1731, 1606, 1568, 1509, 1454, and 1430 cm^{-1}; UV (EtOH) λ_{max} 247 nm (ϵ_m 16 908); ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, J = 7.0 Hz, 17-H_3), 1.22 (3H, d, J = 6.3 Hz, 14-H_3), 2.49 (1H, dd, J = 2.1, 7.9 Hz, 11-H), 2.79 (1H, dt, J = 2.1, 6.4 Hz, 10-H), 3.00 (1H, dd, J = 8.2, 15.7 Hz, 4-H), 3.28 (1H, dd, J = 3.1, 15.7 Hz, 4′-H), 6.43 (1H, s, isoxazole-H), 7.43 and 7.73 (4H, 2 × d, Ar-H); ^{13}C NMR ($CDCl_3$) δ 12.3 (C-17), 20.3 (C-14), 30.4 and 32.9 (C-4 and C-9), 41.8 (C-8), 43.7 (C-12), 56.8 (C-10), 61.2 (C-11), 66.4 (C-16), 69.4 (C-6), 70.7 (C-7), 71.5 (C-13), 75.9 (C-5), 101.5 (C-2), 129.2, (2 × Ar), 130.2 (2 × Ar), 136.9 (Ar-Cl), 162.8 (C═N), and 173.6 (C−O); MS (EI) m/z 438 (MH^+, 10), 222 (50), 193 (95); HRMS calcd for $C_{22}H_{29}NO_6Cl$ 438.1683, found 438.1689.

3-[[5*S*-(2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl)-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-5-phenylisoxazole (8). 3(*R*),4(*R*)-Dihydroxy-2(*S*)-[4-(phenyl-2,4-dioxo-4-phenylbut-1-yl]-5(*S*)-(2(*S*),3(*S*)-epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl)tetrahydropyran (**11**)[9] (100 mg, 0.25 mmol) was dissolved in ethanol (1 mL) and treated with triethylamine (0.038 mL, 0.27 mmol) and hydroxylamine hydrochloride (19 mg, 0.27 mmol). The reaction mixture was stirred at room temperature for 3 days. Further triethylamine (0.27 mmol) and hydroxylamine hydrochloride (0.27 mmol) were added, and the mixture was stirred for a further 6 h. The solution was diluted with ethyl acetate and washed with water and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by chromatography on Keiselgel 60, eluting with 0−10% methanol in dichloromethane, to give **8** as a colorless oil (4 mg, 4%): IR (CH_2Cl_2) 3602, 3430, 1643, 1613, 1574, and 1451 cm^{-1}; UV (MeOH) λ_{max} 259 nm; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, d, J = 7.0 Hz, 17-H_3), 1.21 (3H, d, J = 6.3 Hz, 14-H_3), 2.65 (1H, dd, J = 2.0, 8.0 Hz, 11-H), 2.79 (1H, dd, J = 2.0, 5.7 Hz, 10-H), 3.00 (1H, dd, J = 6.6, 15.1 Hz, 4-H), 3.15 (1H, dd, J = 4.2, 15.1 Hz, 4′-H), 6.55 (1H, s, isoxazole-H), 7.42−7.48 (3H, m, Ar-H), and 7.76−7.80 (2H, m, Ar-H); MS (EI) m/z 403 (M^+, 2), 188 (45), 159 (100). HRMS calcd for $C_{22}H_{29}NO_6$ 403.1995, found 403.2017.

5-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-[(trimethylsilyl)oxy]-4(*S*)-methylhexyl]-3(*R*),4(*R*)-bis-[(trimethylsilyl)oxy]tetrahydropyran-2(*S*)-yl]methyl}benzamide Oxime (18). 6,7,13-*O*-Tris(trimethylsilyl)-1,15-bisnormon-3-yl diethyl phosphate (**10**) (300 mg 0.45 mmol) was dissolved in dry dichloromethane (30 mL), the mixture was cooled to −70 °C, and ozone bubbled through until a blue color was observed. Argon was bubbled through to remove excess ozone, and the solution was treated with dimethyl sulfide (0.36 mL, 0.5 mmol) and warmed to −30 °C. After 30 min the reaction mixture was treated with triethylamine (0.068 mL, 0.5 mmol) followed by benzamidoxime (68 mg, 0.5 mmol) and allowed to warm to room temperature. After 20 min the reaction mixture was washed with water and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product was chromatographed on Kieselgel 60, eluting with 10−25% ethyl acetate in hexane, to give **18** as a colorless oil (222 mg, 76%): IR (CH_2Cl_2) 3513, 3414, 3356, 1750, and 1636 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, d, J = 7.0 Hz, 17-H_3), 1.19 (3H, d, J = 6.3 Hz, 14-H_3), 2.58 (1H, dd, J = 7.7,14.4 Hz, 4-H), 2.62−2.70 (2H, m, 10-H and 11-H), 2.84 (1H, dd, J = 4.4, 14.4 Hz, 4′-H), 5.44 (2H, br s, OH and NH), 7.38−7.50 (3H, m, Ar-H), and 7.73 (2H, d, J = 6.7 Hz, Ar-H); MS (EI) m/z 639 (MH^+, 8); HRMS calcd for $C_{30}H_{55}N_2O_7Si_3$ 639.3317, found 639.3317.

5-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl)-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-3-phenyl-1,2,4-oxadiazole (14). The oxime **18** (200 mg) was dissolved in diglyme (5 mL) and heated to 120 °C for 4 h. The solution was cooled to room temperature, diluted with ethyl acetate (20 mL), and washed with water (10 mL) and brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, the solvent evaporated, and the crude product chromato-

graphed on Kieselgel 60 eluting with 10% ethyl acetate in hexane to give the protected oxadiazole as a colorless oil (89 mg, 45%): IR (CH_2Cl_2) 1595, 1571, and 1446 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (3H, d, J = 7.1 Hz, 17-H_3), 1.18 (3H, d, J = 6.3 Hz, 14-H_3), 2.65−2.68 (2H, m, 10-H and 11-H), 2.99 (1H, dd, J = 8.9, 15.1 Hz, 4-H), 3.31 (1H, dd, J = 8.9, 15.1 Hz, 4′-H), 4.04 (1H, dt, J = 3.4, 9.1 Hz, 5-H), 7.45−7.48 (3H, m, Ar-H), and 8.07−8.10 (2H, m, Ar-H); MS (EI) m/z 621 (MH^+, 1), 117 (100).

This material (67 mg) was dissolved in a mixture of THF (3 mL) and water (0.75 mL) and treated with 5 M HCl (2 drops). After 5 min, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5 mL), and the mixture was extracted with ethyl acetate. The organic phases were combined and dried over anhydrous $MgSO_4$, the solvent was evaporated, and the crude product was chromatographed on Kieselgel 60, eluting with 5% methanol in dichloromethane, to give **14** as a white solid (42 mg, 95%): IR (KBr) 3426, 1625, 1596, and 1447 cm^{-1}; UV (EtOH) λ_{max} 238 nm (ϵ_m 15 731); ^1H NMR ($CDCl_3$) δ 0.94 (3H, d, J = 7.0 Hz, 17-H_3), 1.21 (3H, d, J = 6.3 Hz, 14-H_3), 2.68 (1H, dd, J = 2.2, 8.0 Hz, 11-H), 2.80 (1H, dt, J = 2.2, 5.6 Hz, 10-H), 3.24 (1H, dd, J = 7.0, 15.5 Hz, 4-H), 3.39 (1H, dd J = 4.8, 15.5 Hz, 4′-H), 7.44 −7.50 (3H, m, Ar-H), and 8.05−8.09 (2H, m, Ar-H); ^{13}C NMR (CD_3OD) δ 10.7 (C-17), 16.7 (C-14), 29.2 and 31.3 (C-4) and (C-9), 40.2 (C-8), 42.1 (C-12), 55.2 (C-10), 59.3 (C-11), 64.9 (C-16), 67.8 (C-6), 69.1 (C-7), 69.8 (C-13), 74.0 (C-5), 126.5 (Ar), 126.7 (2xAr), 128.4 (2 × Ar), 130.7 (Ar), 167.8 (C=N), and 178.4 (C-O); MS (EI) m/z 405 (MH^+, 90), 227 (100), 119 (100); HRMS calcd for $C_{21}H_{29}N_2O_6$ 405.2026, found 405.2029.

***N′*-[2-[5(*S*)-[2(*S*),3(*S*)-Epoxy-5*S*-[(trimethylsilyl oxy]-4(*S*)-methylhexyl]-3(*R*),4(*R*)-bis[(trimethylsilyl)oxy]tetrahydropyran-2(*S*)-yl]acetylbenzoic hydrazide (19).** 6,7,13-*O*-Tristrimethylsilyl)-1,15-bisnormon-3-yl diethyl phosphate (**10**) (327 mg, 0.5 mmol) was dissolved in dry dichloromethane (30 mL), and cooled to −70 °C, and ozone was bubbled through until a blue color was observed. Argon was bubbled through to remove excess ozone, and the solution was treated with dimethyl sulfide (0.040 mL, 0.55 mmol) and warmed to −30 °C. After 30 min the reaction mixture was treated with triethylamine (0.075 mL, 0.56 mmol) followed by benzoylhydrazine (0.150 g, 0.56 mmol) and allowed to warm to room temperature. After 1 h the reaction mixture was washed with water and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product was chromatographed on Kieselgel 60, eluting with 25% ethyl acetate in hexane, to give **19** as a colorless oil (220 mg, 69%): IR (CH_2Cl_2) 3393, 3303, 1716, 1678, and 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (3H, d, J = 7.1 Hz, 17-H_3), 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 2.40 (1H, dd, J = 9.4, 15.8 Hz, 4-H), 2.68−2.88 (3H, m, 10-H, 11-H and 4′-H), 7.42−7.57 (3H, m, Ar-H), 7.80−7.84 (2H, m, Ar-H), 8.88 (1H, d, J = 6.1 Hz, N-H), and 9.49 (1-H, d, J = 6.1 Hz, N-H); MS (EI) m/z 639 (MH^+, 0.5); HRMS calcd for $C_{30}H_{55}N_2O_7Si_3$ 639.3317, found 639.3317.

5-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-2-phenyl-1,3,4-oxadiazole (15). The acyl hydrazide **19** (150 mg, 0.23 mmol) was dissolved in dry acetonitrile (1.2 mL) and treated with triethylamine (0.1 mL), tetrachloromethane (0.14 mL), and triphenylphosphine (0.18 g). The reaction mixture was stirred at room temperature for 30 min, diluted with ethyl acetate (20 mL), and washed with saturated aqueous $NaHCO_3$ (15 mL), water (10 mL), and brine (10 mL). The solution was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was dissolved in a mixture of THF (6 mL) and water (1.5 mL) and treated with 5 M HCl (4 drops). After 5 min, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5 mL), and the mixture was extracted with ethyl acetate. The organic phases were combined and dried over anhydrous $MgSO_4$, the solvent was evaporated, and the crude product was chromatographed on Kieselgel 60 eluting with 5% methanol in dichloromethane to give **15** as a white solid (63 mg, 68%): IR (KBr) 3458, 3346, 1571, and 1558 cm^{-1}; UV (EtOH) λ_{max} 251 nm (ϵ_m 18 058); ^1H NMR (CD_3OD) δ 0.95 (3H, d, J = 7.1 Hz, 17-H_3), 1.19 (3H, d, J = 6.3 Hz, 14-H_3), 2.70 (1H, dd, J = 2.3, 7.6 Hz, 11-H), 2.80 (1H, dt, J = 2.2, 5.5 Hz, 10-H), 3.12 (1H, d, J = 15.5, 8.6 Hz, 4-H), 3.41 (1H, dd, J = 15.5, 3.6 Hz, 4-H), 3.77 (1H, dq, J = 6.3, 7.4 Hz, 13-H), 4.04 (1H, dt, J = 3.6,8.8 Hz, 5-H), 7.55−7.62 (3H, m, Ar-H), and 8.02−8.06 (2H, m, Ar-H); MS (EI) m/z 404 (M^+, 5), 160 (100); HRMS calcd for $C_{21}H_{28}N_2O_6$ 404.1947, found 404.1956.

2-[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-[(trimethylsilyl)oxy]-4(*S*)-methylhexyl]-3(*R*),4(*R*)-bis[(trimethylsilyl)oxy]tetrahydropyran-2(*S*)-yl]-*N*-(2-phenyl-2-oxoethyl)acetamide (20). 6,7,13-*O*-Tris(Trimethylsilyl)-1,15-bisnormon-3-yl diethyl phosphate (10) (327 mg 0.5 mmol) was dissolved in dry dichloromethane (30 mL) and cooled to −70 °C, and ozone was bubbled through until a blue color was observed. Argon was bubbled through to remove excess ozone, and the solution was treated with dimethyl sulfide (0.40 mL, 0.55 mmol) and warmed to −30 °C. After 30 min the reaction mixture was treated with triethylamine (0.15 mL, 1.1 mmol) followed by 2-aminoacetophenone hydrochloride (102 mg, 0.6 mmol) and allowed to warm to room temperature. After 20 min the reaction mixture was washed with water and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product was chromatographed on Kieselgel 60, eluting with 25% ethyl acetate in hexane, to give 20 as a colorless oil (241 mg, 76): IR (CH_2Cl_2) 3416, 3368, 1732, and 1696 cm^{-1};^1H NMR ($CDCl_3$) δ 0.93 (3H, d, J = 7.1 Hz, 17-H_3), 1.21 (3H, d, J = 6.2 Hz, 14-H_3), 2.34 (1H, dd, J = 9.8, 15.6 Hz, 4-H), 2.66−2.75 (23H, m, 10-H, 11-H, and 4′-H),4.78 (2H, dd, CH_2NH), 7.48−7.56 (3H, m, Ar-H), and 7.98−8.02 (2H, m, Ar-H); MS (EI) m/z 639 (MH^+, 8); HRMS calcd for $C_{30}H_{55}N_2O_7Si_3$ 639.3317, found 639.3317.

2-[[5(*S*)-[2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4*S*-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-5-phenyloxazole (16). The keto amide **20** (150 mg, 0.23 mmol) was dissolved in dry dichloromethane (3 mL) and treated with pyridine (76 μL, 4 equiv) and trichloroacetyl chloride (52 μL, 2 equiv). The reaction mixture was stirred at room temperature for 30 min, diluted with dichloromethane (10 mL), washed with $NaHCO_3$, 10% citric acid, water, and brine, dried ($MgSO_4$), and evaporated. The crude product was chromatographed on Kieselgel 60 eluting with 10−20% ethyl acetate in hexane to give the protected oxazole (46 mg, 31%). This was deprotected under the conditions described for compound **18** to give the oxazole **16** as a white amorphous solid (16 mg, 55%): IR (KBr) 3464, 1760, 1629, 1558, and 1450 cm^{-1}; UV (EtOH) λ_{max} 273 (ϵ_m 17 566), 266 nm (17 782); ^1H NMR ($CDCl_3$) δ 0.95 (3H, d, J = 7.0 Hz, 17-H_3), 1.21 (3H, d, J = 6.3 Hz, 14-H_3), 2.69 (1H, dd, J = 2.2, 8.0 Hz, 11-H), 2.79 (1H, dt, J = 2.2, 5.6 Hz, 10-H), 3.22 (2H, d, J = 6.0 Hz, 4-H_2),7.25 (1H,s, oxazole-H), 7.31−7.46 (3H, m, Ar-H), and 7.51−7.64 (2H, m, Ar-H); MS (EI) m/z 403 (M^+, 2); HRMS calcd for $C_{22}H_{29}NO_6$ 403.1995, found 403.2002.

5-[[5(*S*)-(2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-2-phenyloxazole (21). A solution of 6,7,13-*O*-*tris*-(trimethylsilyl)monone (**9a**) (518 mg, 1 mmol) in dry THF (5 mL) was added dropwise to a solution of lithium bis(trimethylsilyl)-amide (1 mmol) in dry THF (3 mL) at −78 °C under an argon atmosphere. After 30 min at −78 °C the solution was treated with *p*-toluenesulfonyl azide (1.2 mmol) in dry THF (2 mL). After 30 min, the mixture was quenched with saturated ammonium chloride, allowed to warm to room temperature, and extracted with ethyl acetate. The organic phases were combined, washed with brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product chromatographed on Kieselgel 60, eluting with 10−15% ethyl acetate in hexane, to give the diazo ketone **22** as a colorless oil (107 mg, 20%): IR (CH_2Cl_2) 3417, 2959, 2898, 2107, 1640, and 1452 cm^{-1};^1H NMR ($CDCl_3$) δ 0.91 (3H, d, J = 6.9 Hz, 17-H_3), 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 2.28 (1H, d, J = 10.3, 14.6 Hz, 4-H), 2.62−2.70 (3H, m, 10-H, 11-H and 4′-H), 3.42 (1H, dd, J = 2.4, 9.1 Hz, 6-H), 3.55 (1H, d, J = 11.3 Hz, 16- H), 5.40 (1H, br s, CHN_2); MS (FAB, 3-NOBA-Na) m/z 567 (MNa^+, 45), 539 ($MNa^+ - N_2$, 30).

The diazo ketone **22** (74 mg 0.14 mmol) was dissolved in benzonitrile (2 mL), tetrakis(acetato)dirhodium(II) (7 mg) was

added, and the mixture was heated to 100 °C under an argon atmosphere for 40 min. The solution was cooled to room temperature, diluted with ethyl acetate, and washed with saturated aqueous $NaHCO_3$, water, and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. The crude product was chromatographed on Kieselgel 60 eluting with 0−10% ethyl acetate in hexane to give a colorless oil (22 mg, 25%). This was dissolved in a mixture of THF (1.5 mL) and water (0.4 mL) and treated with 5 M HCl (1 drop). After 5 min, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5 mL), and the mixture was extracted with ethyl acetate. The organic phases were combined and dried over anhydrous $MgSO_4$, the solvent was evaporated, and the crude product was chromatographed on Kieselgel 60 eluting with 5% methanol in dichloromethane to give **21** as a white solid (11 mg, 87%): IR (KBr) 3458, 1640, 1606, 1549, 1484, and1450 cm^{-1}; UV (EtOH) λ_{max} 272 nm; ^1H NMR (CD_3OD) δ 0.94 (3H, d, J = 7.1 Hz, 17-H_3), 1.19 (3H, d, J = 6.4 Hz, 14-H_3), 1.39 (1H,dq, J = 7.1, 4.9 Hz,12-H), 1.93−2.01 (1H,m, 8-H), 2.68 (1H, dd, J = 2.1, 7.9 Hz, 11-H), 2.80 (1H, dt, J = 2.1, 5.6 Hz, 10-H), 2.91 (1H, dd, J = 8.4, 15.8 Hz, 4-H), 3.24 (1H, dd, J = 2.3, 15.8 Hz, 4′-H), 3.47 (1H, dd, J = 3.1, 9.2 Hz, 6-H), 3.61 (1H, d, J = 11.3 Hz, 16-H), 3.77 (1H, dq, J = 6.4, 4.9 Hz, 13-H), 3.82−3.95 (3H, m, 5, 16′, and 7-H), 7.00 (1H, s, oxazole-H), 7.45−7.49 (3H, m, Ar-H), and 7.94−7.98 (2H, m, Ar-H); MS (EI) m/z 403 (M^+, 5), 159 (100). HRMS calcd for $C_{22}H_{29}NO_6$ 403.1995, found 403.1994.

2-[[5(*S*)-(2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-5-phenyltetrazole (25). A solution of [5(*S*)-[2(*S*),3(*S*)-epoxy-5(*S*)-[(triethylsilyl)oxy]-4-methylhexyl]-3(*R*),4(*R*)-bis[(triethylsilyl)oxy]tetrahydropyran-2(*S*)-yl]acetone (**9b**) (8.0g, 12.42 mmol) in dry tetrahydrofuran (20 mL) was added to a solution of lithium diisopropylamide (10 mmol) in dry tetrahydrofuran (20 mL) at −70 °C under an argon atmosphere. The cooling bath was removed and the mixture warmed to room temperature over a period of 15 min, and stirred for a further 30 min. Triisopropylsilyl trifluoromethanesulfonate (2.55 mL, 10 mmol) was then added, and the mixture stirred for an additional 1 h. The solvent was evaporated under reduced pressure, the residue taken up in dry pentane (10 mL), and the mixture filtered. The filtrate was evaporated under reduced pressure and the residue chromatographed on Kieselgel 60 eluting with 2-20% ethyl acetate in hexane to give the triisopropylsilyl enol ether **23** as a colorless oil (3.41 g, 43%), contaminated with 10% of the 5-epimer: ^1H NMR ($CDCl_3$) δ 0.91 (3H, d, J = 7.1 Hz, 17-H_3), 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 1.80 (3H, s, CH_3), 2.64−2.68 (2H, m, 10-H and 11-H), 3.40 (1H, dd, J = 2.3, 8.3 Hz, 6-H), 3.50 (1H, d, J = 11.4 Hz, 16-H), 3.81 (1H, appears as br s, 7-H), 3.81−3.91 (1H, m, 13-H), 3.97 (1H, d, J = 11.4 Hz, 16′-H), 4.38 (1H, d, J = 9.2 Hz, 4-H), 4.60 (1H, appears as t, J = 9.1 Hz, 5-H).

The silyl enol ether **23** (1.6 g, 2.0 mL) was dissolved in dry dichloromethane (56 mL) and methanol (32 mL) and cooled to −70 °C and ozone passed through until a blue color was observed. Argon was passed through to remove excess ozone and the solution treated with sodium borohydride (92 mg, 2.0 mmol). The mixture was stirred at −70 °C for 1 h, a further quantity of sodium borohydride (92 mg) added, and the mixture allowed to warm to room temperature. After 1 h the reaction mixture was evaporated, and the residue was dissolved in ethyl acetate (80 mL), washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was chromatographed on Kieselgel 60 eluting with 5−20% ethyl acetate in hexane to give **24** as a white foam (0.335 g, 27%); IR (CH_2Cl_2) 3582, 1459 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 2.00 (1H, *t*, J = 6.5 Hz, OH), 2.65−2.71 (2H, m, 10-H and 11-H); MS (NH_3^+ DCI) m/z 619 (MH^+, 50), 633 (MNH_4^+).

5-Phenyltetrazole (35 mg, 0.24 mmol) was dissolved in dry THF (2 mL) and treated with triphenylphosphine (63 mg, 0.24 mmol) and diethyl azodicarboxylate (38 μL, 0.24 mmol), followed by **24** (100 mg, 0.16 mmol) in dry THF (2 mL). The reaction mixture was stirred at room temperature under argon for 2 h. The solvent was evaporated and the residue chromatographed on Kieselgel 60 eluting with 5% ethyl acetate in hexane to give the protected product as a colorless oil (95 mg, 78%): ^1H NMR ($CDCl_3$) δ 1.19 (3H, d, J = 6.3 Hz, 14- Hz), 2.64−2.66 (2H, m, 10-H and 11-H), 3.54 (1H, d, J = 11.3 Hz, 16-H), 3.69 (1H, dd, J = 9.3, 2.2 Hz, 6-H), 4.33 (1H, dt, J = 9.4, 2.7 Hz, 5-H), 4.65 (1H, dd, J = 9.2, 13.6 Hz, 4-H), 4.90 (1H, dd, J = 2.8, 13.5 Hz, 4′-H), 7.45−7.53 (3H, m, Ar-H), 8.15−8.21 (2H, m, Ar-H); MS (FAB, thioglycerol) m/z 747 (MH^+, 20), 159 (100). This material (84 mg, 0.11 mmol) was dissolved in THF (4.5 mL) and treated with a solution of tetra-*n*-butylammonium fluoride (1 *M* solution in THF, 0.44 mL, 4 equiv). After the mixture was stirred for 1 h at room temperature the solvent was evaporated and the residue chromatographed on Kieselgel 60 eluting with 5% methanol in dichloromethane. The product **25** was obtained as a white foam (34 mg, 77%): IR (KBr) 3413, 1639, 1529, 1466, 1450 cm^{-1};UV (EtOH) λ_{max} 239nm (ϵ_m 16 021); ^1H NMR (CD_3OD) δ 0.92 (3H, d, J = 7.1 Hz, 17-H_3), 1.18 (3H, d, J = 6.4 Hz, 14-H_3), 1.34−1.43 (1H, m, 12-H), 1.58−1.73 (2H, m, 9-H), 1.92−1.97 (1H, m, 8-H), 2.68 (1H, dd, J = 2.2, 7.6 Hz, 11-H), 2.97 (1H, dt, J = 2.2, 5.6 Hz, 10-H), 3.55 (1H, d, J = 11.5 Hz, 16-H), 3.64 (1H, dd, J = 3.1, 5.8 Hz, 6-H), 3.72−3.78 (1H, m, 13-H), 3.86 (1H, dd, J = 2.5, 11.5 Hz, 16′-H), 3.74 (1H, appears as t, J = 3.0 Hz, 7-H), 4.7 (1H, dt, J = 2.8, 8.2 Hz, 5-H), 4.83 (1H, dd, J = 5.6, 13.8 Hz, 4-H), 4.98 (1H, dd, J = 2.8, 14.0 Hz, 4′-H), 7.43−7.53 (3H, m, Ar-H), 8.03−8.12 (2H, m, Ar-H); MS (EI) m/z 404 (M^+), 131 (100); HRMS calcd for $C_{20}H_{29}N_4O_5$ 405.2138, found 405.2142.

4-[[5(*S*)-(2(*S*),3(*S*)-Epoxy-5(*S*)-hydroxy-4(*S*)-methylhexyl]-3(*R*),4(*R*)-dihydroxytetrahydropyran-2(*S*)-yl]methyl]-2-phenylthiazole (26). A solution of 6,7,13-*O*-tris(trimethylsilyl)monone (**9a**) (540 mg, 1.04 mmol) in dry dichloromethane (10 mL) was cooled to 0 °C and treated with triethylamine (0.29 mL, 2.0 equiv) followed by (trimethylsilyl)trifluoromethanesulfonate (0.2 mL, 1.0 equiv). The mixture was stirred for 30 min. *N*-Bromosuccinimide (0.2 g, 1.1 equiv) was added and the mixture stirred at room temperature for 30 min. Triethylamine (0.29 mL, 2.0 equiv) and thiobenzamide (0.16 g, 1.1 equiv) were added, and the mixture was stirred for a further 2 h. The solvent was evaporated under reduced pressure and the residue chromatographed on Kieselgel 60, eluting with 10-20% ethyl acetate in hexane, to give the 4-hydroxythiazoline (352 mg) as a mixture of diastereomers. This was dissolved in dry dichloromethane (10 mL), cooled to 0 °C, and treated with triethylamine (0.15 mL, 2.0 equiv), methanesulfonyl chloride (0.042 mL, 1.0 equiv), and 4-(dimethylamino)pyridine (5.0 mg). The mixture was stirred at 0 °C for 1 h, the solvent evaporated under reduced pressure, and the residue chromatographed on Kieselgel 60 eluting with 5-15% ethyl acetate in hexane to give the protected product (190 mg, 29%): IR (CH_2Cl_2) 1519, 1459, 1376 cm^{-1}; ^1H NMR (CD_3OD) δ 0.91 (3H, d, J = 7.1 Hz, 17-H_3), 1.21 (3H, d, J = 6.4 Hz, 14-H_3), 2.71−2.85 (3H, m, 10, 11, and 4-H), 3.22 (1H, dd, J = 2.8, 15.0 Hz, 4′-H), 4.05 (1H, dt, J = 2.8, 9.1 Hz, 5-H), 7.22 (1H, s, thiazole-H), 7.41−7.48 (3H, m, Ar-H), 7.91−7.94 (2H, m, Ar-H); MS (EI) m/z 636 (M^+).

This material (176 mg, 0.28 mmol) was deprotected under the conditions described for **7b**. After workup, the crude product was crystallized from acetone−ether to give **26** as a white solid (68 mg, 60%): IR (KBr) 3463, 1518, 1501, 1460, 1436 cm^{-1}; UV (EtOH) λ_{max} 295 nm (ϵ_m 13 000); ^{1} H NMR (CD_3OD) δ 0.95 (3H, d, J = 7.1 Hz, 17-H_3), 1.21 (3H, d, J = 6.4 Hz, 14-H_3), 2.71 (1H, dd, J = 2.2, 7.6 Hz, 11-H), 2.81 (1H, dt, J = 2.2, 5.8 Hz, 10-H), 2.93 (1H, dd, J = 8.7, 15.1 Hz, 4-H), 3.52 (1H, dd, J = 3.1, 8.7 Hz, 16-H), 7.26 (1H, s, thiazole-H), 7.42−7.48 (3H, m, Ar-H), 7.90−7.95 (2H, m, Ar-H); ^{13}C NMR (CD_3OD) δ 12.2 (C-17), 20.2 (C-14), 33.0 (C-4), 34.6 (C-9), 41.5 (C-8), 43.7 (C-12), 56.9 (C-10), 61.3 (C-11), 66.3 (C-16), 69.9 (C-6), 70.8 (C-13), 71.6 (C-7), 77.3 (C-5), 118.4 (thiazole C-H), 127.4, 130.1 and 131.1 (Ar-CH), 134.8 (Ar-C), 158.3 (thiazole-C), 168.9 (thiazole-C); MS (EI) m/z 419 (M^+), 204 (100); HRMS calcd for $C_{22}H_{29}NO_5S$ 419.1766, found 419.1769.

Electrostatic Potential Calculations. Model fragments, containing the equivalents of the atoms shown in Figure 1, of compounds **1c, 7b, 8, 14−16**, **21**, **25**, and **26** were built using the program Chem-X[17]. Models of the ketone **2** and the

heterocyclic compounds **3a,c** were generated from the model of **1c**. The geometries of these compounds were fully optimized using the AM1 Hamiltonian within the AMPAC semiempirical molecular orbital program.[18] Electrostatic potential derived charges were calculated by the method of Ferenczy *et al.*[19]

The optimized structures of the ketone **2** and the heterocyclic compounds **3a,c** were overlaid onto the ester **1c** by a least squares fitting of the carbonyl oxygen (or equivalent nitrogen in the case of **3a,c**) and carbon atoms 1,2,3 and 15. Similarly, the heterocyclic model compounds **7b, 8, 14**−**16**, **21**, **25**, and **26** were overlaid onto **1c** by a least squares fitting the corresponding atoms of the 5-membered heterocyclic ring to the carbonyl oxygen and carbon atoms 1, 2, 3, and 15 of compound **1c**.

The common electrostatic potential map of the active compounds **1c, 2, 3a,** and **3c** was generated from the individual maps of the potentials of the region around the carbonyl oxygen (or equivalent nitrogen in the case of **3a** and **3c**). A logical AND operation on these potential maps was performed to generate the map, shown in Figure **1a**, of the electrostatic features common to these compounds. The potential maps of compounds **1c, 7b, 8, 14**−**16**, **21**, **25**, and **26** were individually AND-ed to the common potential map. Each resultant intersection map gave a specific representation of the electrostatic potential of the test molecule at −10 kcal mol^{-1} in the region identified as common to the active compounds **1c, 2, 3a**, and **3b**.

Molecular Orbital Calculations. Calculations were performed with a STO 6-31G* basis set using AM-1-optimized geometries, using the Spartan electronic structure program.[20] The orbitals shown in Figure 2 were contoured at 0.09 e/au^3.

Determination of Minimum Inhibitory Concentrations (MICs). Compounds were serially diluted in blood agar base (Oxoid) containing 5% chocolated horse blood. Organisms were grown overnight in nutrient broth media. One milliliter spots were inoculated on to the surface of the agar plates giving an inoculum of *ca.* 10^6 colony-forming units per spot. Plates were incubated for 18−24 h at 37 °C. The minimum inhibitory concentration was determined as the lowest concentration fully inhibiting bacterial growth.

Isoleucyl tRNA Synthetase (IRS) Inhibition. *Staph. aureus* strains were grown to late stationary phase in shake flasks (240 rpm) containing Nutrient Broth No. 2. For extraction of IRS, cells were harvested by centrifugation at 5000*g* and washed several times in cold phosphate-buffered saline (PBS). Bacterial synthetases were extracted by sonication in the presence of lysostaphin (150 μg/mL) in the same buffer, followed by treatment with DNase (10 μg/mL) and overnight dialysis against 20 mM Tris·HCl pH 8, containing 5 mM $MgCl_2$ and 2 mM dithiothreitol (DTT), and ultracentrifugation at 200 000*g* for 1h. All enzymes were stored at −20 °C in the presence of 30% glycerol.

IRS activity was assayed as the charging of $tRNA^{Ile}$ with ^{14}C-Ile using the method of Durekovic[21] under conditions where counts were approximately proportional to time and enzyme concentration. Assay mixtures contained 30 mM Tris, 2 mM DTT, 10 mM $MgCl_2$, 70 mM KCl, 1.56 mg/mL *E. coli* tRNA, 5 mg/ mL equine ATP, and 4.8 μM [*U*-^{14}C]-isoleucine. The concentration of each compound resulting in 50% inhibition of ^{14}C-Ile incorporation (IC_{50}) was determined after preincubation of increasing concentrations of the compound with IRS for 5 min, followed by addition of substrates and cofactors and reaction for 10 min at 37°C.

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