Thermal Atropisomerism of Aglucovancomycin Derivatives: Preparation of (*M*,*M*,*M*)- and (*P*,*M*,*M*)-Aglucovancomycins

Dale L. Boger,* Susumu Miyazaki, Olivier Loiseleur, Richard T. Beresis, Steven L. Castle, Jason H. Wu, and Qing Jin

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received June 2, 1998

Abstract: The degradation of vancomycin to a series of aglucovancomycin derivatives containing modifications in key functional groups and a study of their thermal atropisomerism are detailed. In all of the cases, selective isomerism of the DE ring system atropisomers was observed under conditions where the CD and AB stereochemistries were unaffected. Competitive retro aldol ring cleavage of the CD and DE ring systems (CD > DE) was observed but could be minimized by the choice of solvent and thermal conditions (DE ring system) or precluded by alcohol protection (CD ring system). Similarly, competitive main chain succinimide formation through the loss of ammonia from the Asn residue could be minimized by the choice of thermal conditions or prevented by carboxamide protection. Resynthesis of natural aglucovancomycin, (*M*,*M*,*M*)-**2**, and its unnatural DE atropisomer (*P*,*M*,*M*)-**2** from **6** are described. The comparative antimicrobial activity of the key derivatives and their unnatural DE ring system *P*-diastereomers are disclosed.

Introduction

Vancomycin (**1**, Figure 1) is the prototypical member of a class of clinically important glycopeptide antibiotics[1−4] used for the treatment of methicillin-resistant *Staphylococcus aureus* and against enterococci and bacterial infections in patients allergic to β-lactam antibiotics.[5] The structural complexity of vancomycin, the interest in defining its structural features responsible for inhibition of cell wall biosynthesis in sensitive bacteria,[6] and the emergence of clinical resistance[7] have renewed interest in **1** and related agents.

The inherent control of the vancomycin CD and DE ring system atropisomer stereochemistries remains one of the most significant challenges yet to be addressed in efforts directed at its examination or total synthesis.[8,9] In recent studies, we defined conditions under which the isolated CD and DE ring system atropisomers may be equilibrated, and this has allowed the unnatural atropisomers to be thermally equilibrated,[10,11] chromatographically reisolated, and recycled to provide the desired natural stereochemistry. Significantly, the DE atropisomer equilibration in the isolated ring system occurred much more rapidly (10−20 min, 130 °C) than that in the stand alone CD ring system (>10 h, 130 °C) and indicated that it may be possible to preferentially equilibrate the vancomycin DE versus CD atropisomers within the intact CDE ring system. We recently implemented this preferential DE equilibration in a synthesis of a vancomycin CDE ring system in a manner that indirectly addresses the control of the atropisomer stereochemistry.[12,13]

Herein, we report studies of the thermal atropisomerism of a series of vancomycin aglycons that define its scope, limitations, and potential competitive reactions. These studies define conditions that permit a selective DE versus CD atropisomerism and have provided an appropriately protected vancomycin aglycon which not only minimizes competitive thermal reactions but also may serve as a relay intermediate in the total synthesis of the natural product itself. Thus, thermal atropisomerism of **6** provided a 1:1 mixture of (*M*,*M*,*M*)-**6** and

(1) McCormick, M. H.; Stark, W. M.; Pittenger, G. E.; Pittenger, R. C.; McGuire, G. M. *Antibiot. Annu.* **1955**−**1956**, 606.

(2) (a) Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915. (b) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580. (c) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature* (*London*) **1978**, *271*, 223.

(3) Williams, D. H.; Kalman, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 2768.

(4) Nagarajan, R. *J. Antibiot.* **1993**, *46*, 1181. Cooper, R. D. G.; Thompson, R. C. *Ann. Rep. Med. Chem.* **1996**, *31*, 131. Malabarba, A.; Nicas, T. I.; Thompson, R. C. *Med. Res. Rev.* **1997**, *17*, 69.

(5) Weidemann, B.; Grimm, H. In *Antibiotics in Laboratory Medicine*; Lorian, V.; Ed.; Williams and Wilkins: Baltimore, 1996; pp 900−1168.

(6) Williams, D. H.; Searle, M. S.; Westwell, M. S.; Mackay, J. P.; Groves, P.; Beauregard, D. A. *Chemtracts*: *Org. Chem.* **1994**, *7*, 133. Try, A. C.; Sharman, G. J.; Dancer, R. J.; Bardsley, B.; Entress, R. M. H.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2911.

(7) Walsh, C. T.; Fisher, S. L.; Park, I.-S.; Prahalad, M.; Wu, Z. *Chem. Biol.* **1996**, *3*, 21.

(8) Rao, A. V. R.; Gurjar, M. K.; Reddy, K. L.; Rao, A. S. *Chem. Rev.* **1995**, *95*, 2135. Evans, D. A.; DeVries, K. M. In *Glycopeptide Antibiotics*; Nagarajan, R., Ed.; Marcel Dekker: New York; 1994; pp 63−104.

(9) For recent disclosures: (a) Evans, D. A.; Barrow, J. C.; Watson, P. S.; Ratz, A. M.; Dinsmore, C. J.; Evrard, D. A.; DeVries, K. M.; Ellman, J. A.; Rychnovsky, S. D.; Lacour, J. *J. Am. Chem. Soc.* **1997**, *119*, 3419. (b) Konishi, H.; Okuno, T.; Nishiyama, S.; Yamamura, S.; Koyasu, K.; Terada, Y. *Tetrahedron Lett.* **1996**, *37*, 8791. (c) Pearson, A. J.; Chelliah, M. V. *J. Org. Chem.* **1998**, *63*, 3087. (d) Rao, A. V. R.; Gurjar, M. K.; Lakshmipathi, P.; Reddy, M. M.; Nagarajan, M.; Pal, S.; Sarma, B. V. N. B. S.; Tripathy, N. K. *Tetrahedron Lett.* **1997**, *38*, 7433. (e) Bois-Choussy, M.; Vergne, C.; Neuville, L.; Beugelmans, R.; Zhu, J. *Tetrahedron Lett.* **1997**, *38*, 5795. (f) Nicolaou, K. C.; Boddy, C. N. C.; Natarajan, S.; Yue, T.-Y.; Li, H.; Braese, S.; Ramanjulu, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 3421.

(10) Boger, D. L.; Borzilleri, R. M.; Nukui, S.; Beresis, R. T. *J. Org. Chem.* **1997**, *62*, 4721. Boger, D. L.; Borzilleri, R. M.; Nukui, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3091.

(11) Boger, D. L.; Castle, S. L.; Miyazaki, S.; Wu, J. H.; Beresis, R. T.; Loiseleur, O. *J. Org. Chem.*, in press.

(12) Boger, D. L.; Loiseleur, O.; Castle, S. L.; Beresis, R. T.; Wu, J. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3199.

(13) Boger, D. L.; Beresis, R. T.; Loiseleur, O.; Wu, J. H.; Castle, S. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 721.

S0002-7863(98)01928-3 CCC: $15.00
Published on Web 08/22/1998

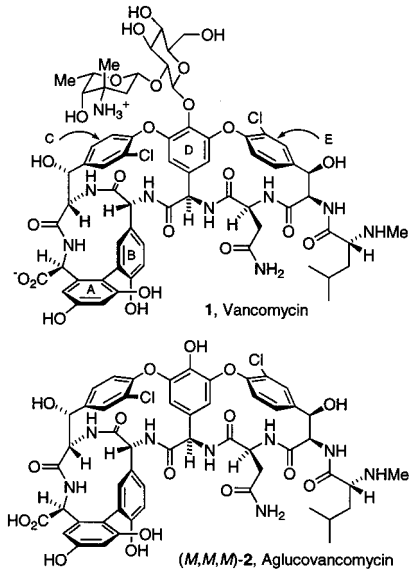
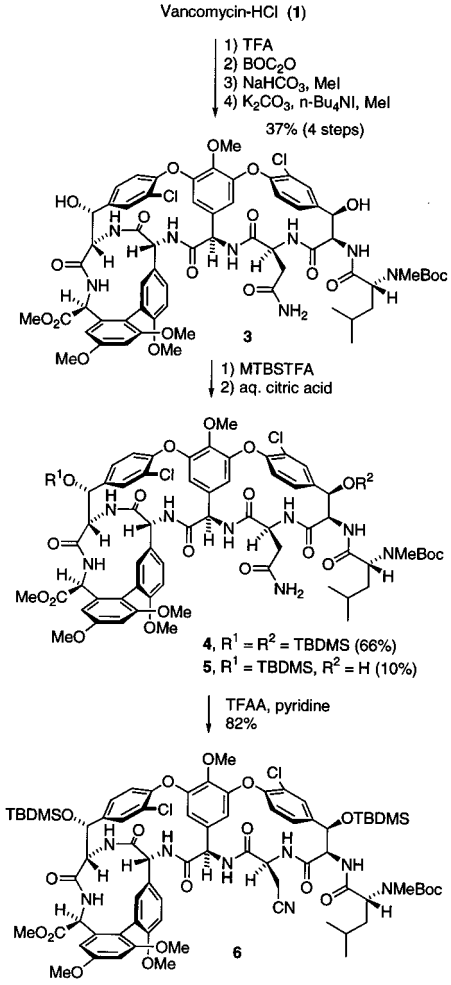


Figure 1.

(*P*,*M*,*M*)-**6** and their conversions to the natural vancomycin aglycon **2** [(*M*,*M*,*M*)-**2**] and (*P*,*M*,*M*)-**2**, respectively, are described. The former efforts constitute the final stages of an anticipated total synthesis of the vancomycin aglycon, and the latter have provided the corresponding key analogue possessing the unnatural DE ring system (*P*)-stereochemistry.

Preparation of Substrates. Degradation of Vancomycin. The degradation of vancomycin to a series of aglucovancomycin derivatives is summarized in Scheme 1. Removal of the disaccharide to provide aglucovancomycin **2** was accomplished by TFA treatment[14,15] (50 °C, 7.5 h), and shorter reaction periods provided lower overall conversions to **3**. Although aglucovancomycin (**2**) could be purified by semipreparative reverse phase HPLC (C18, 2.5 × 10 cm, $CH_3CN-0.07\%$ TFA/H_2O, 17:83, 10 mL/min, $R_t = 18$ min), it proved more convenient to carry the crude material forward without purification. *N*-BOC formation (BOC_2O, $NaHCO_3$, dioxane$-H_2O$, 25 °C, 2 h) followed by methyl ester formation (CH_3I, $NaHCO_3$, DMF, 25 °C, 2 h) and final exhaustive methyl ether protection of the phenols (CH_3I, K_2CO_3, DMF, 25 °C, 6 h) provided **3** in 37% overall yield for the four steps. The intermediate *N*-BOC aglucovancomycin proved too polar to easily purify by normal phase chromatography and selective diazomethane esterification, or a single step exhaustive esterification and methyl ether formation were not as effective as the two-step alkylative procedure, and efforts to reverse the esterification ($TMSCHN_2$) and *N*-BOC protection proved unsuccessful. The most convenient procedure entailed conducting the four steps on crude material with a final purification of the readily handled **3**. O-Silylation of the C_3^2 and C_3^6 alcohols enlisting $CF_3CONMeTBDMS$[13] (50 equiv, CH_3CN, 50 °C, 9.5 h) provided **4** (66%) and a small amount of the C_3^6 mono-TBDMS ether **5** (10%). Shorter reaction periods (4.5 h) at lower reaction temperatures (40 °C) provided a near 1:1 mixture of the readily separable **4** (54%) and **5** (44%). A third intermediate involving presumed silylation of the C_4^3 carboxamide was also observed but was cleaved with an aqueous citric acid workup. The selective monosilylation was established to occur with protection of the C_3^6 alcohol by 1D and 2D $^1H-^1H$ NMR and the alternative C_3^2 mono-TBDMS ether was not detected. Clean dehydration of the Asn carboxamide to provide

Scheme 1



the nitrile **6** (82%) was accomplished by treatment of **4** with TFAA-pyridine in CH_2Cl_2 (0 °C, 10 min).[16]

Thermal Atropisomerism Studies. The now classic studies leading to the structure elucidation of vancomycin which include initial chemical degradation studies revealing the amino acid composition and unusual amino sugar vancosamine,[17] high-field NMR studies that yielded further information on the unusual amino acid structures, their connectivity linkages and stereochemical relationships,[3] and the X-ray structure of CDP-I,[2c] a degradation product produced by acidic thermal treatment (pH 4.2, 70−80 °C, 40 h), provided the basis for the first proposed structure.[2c] This was shortly thereafter revised first to the correct (*M*) versus (*P*) DE ring system atropisomer on the basis of further NMR studies[2b] and finally to **1** with a revision for backbone incorporation of an Asn versus *iso*-Asn residue.[2a] This latter revision included chemical studies that documented the intermediates in the conversion of vancomycin to its degradation product CDP-I by way of an intermediate backbone succinimide (Scheme 2). No DE ring-opened intermediates reflecting cleavage of the backbone amide[2a] linking the central phenylglycine and Asn residues or retro aldol[2b] at the C_3^2 site were detected. Selective ring-expansion hydrolysis of the succinimide with release of ring strain was suggested to account for the exclusive formation of the CDP-I isomers, and the subsequent atropisomerism between the two CDP-I isomers was shown to

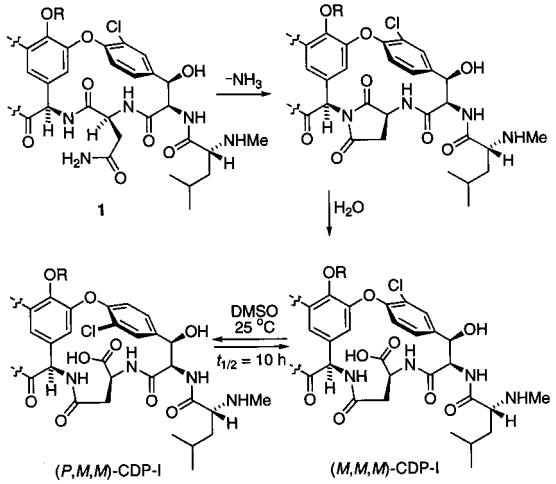
(14) Nagarajan, R.; Schabel, A. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1306.

(15) For the use of HCl, see: Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18.

(16) Both DCC-py and EDCI-py provided only the recovered starting material under similar reaction conditions.

(17) Smith, G. A.; Smith, K. A.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2108 and references cited therein.

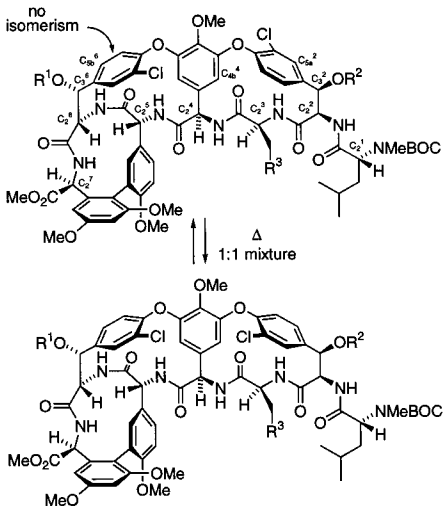
Scheme 2



occur readily ($t_{1/2}$ = 10 h, DMSO, 25 °C, 7:3 *P*/*M*),[2a] Scheme 2. Atropisomerism of the DE ring system was observed only within the ring-expanded CDP-I isomers and not within the 16-membered DE ring system of vancomycin. To date, the assumption has been that the barrier to isomerism of either the CD or DE ring system within vancomycin is too large to permit their observation.[2] Analogous to these efforts, our attempts to thermally equilibrate vancomycin (**1**) or aglucovancomycin (**2**) in aprotic polar (DMSO or DMF, 130 °C, 2.5 h) or nonpolar solvents (*o*-$Cl_2C_6H_4$, 130 °C, 2.5 h) led to extensive or partial degradation, respectively, from which no information on the atropisomerism could be established. Consequently, we examined a number of thermally less sensitive aglycon derivatives which permitted their conventional handling (SiO_2, organic solvents) and allowed us to address the impact of the critical substituents.

In the conduct of the studies, two significant competitive reactions were observed at the temperatures required for atropisomerism, and only isomerism of the DE, not the CD, ring system was observed.[18] This latter observation is consistent with expectations based on our prior studies of preferential DE versus CD atropisomerism with the isolated ring systems.[10−13] The two main competitive reactions were established to be retro aldol ring cleavage preferentially of the CD versus DE ring system which we will refer to as more polar products in the following discussion and main chain succinimide formation (less polar products). Protection of the C_3^6 alcohol eliminated the most competitive retro aldol ring cleavage reaction (more polar products) isolating its most susceptible occurrence to the CD ring system, and dehydration of the Asn carboxamide to a nitrile prevented competitive succinimide formation (less polar products). That the most competitive retro aldol reaction is confined to the CD ring system was established with the observation that it is only detected with **3** and not **5** upon modest thermal treatment in nonpolar solvents (*o*-$Cl_2C_6H_4$, ≤140 °C). Notably, **5** still possesses a C_3^2 free alcohol and the potential for retro aldol DE ring cleavage, and this was not observed under the thermal conditions most successful for atropisomerism (*o*-$Cl_2C_6H_4$, 130−140 °C) but was observed in more polar solvents (DMF, 140 °C). An identical preferential sensitivity of the CD versus DE ring system to retro aldol ring cleavage has been

(18) Although the AB ring system is capable of atropisomerism under the thermal conditions examined, only the natural (*M*)-atropisomer was detected, reflecting its thermodynamic preference, see: Evans, D. A.; Dinsmore, C. J.; Ratz, A. M.; Evrard, D. A.; Barrow, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 3417.



4, R^1 = TBDMS R^2 = TBDMS R^3 = $CONH_2$
5, R^1 = TBDMS R^2 = H R^3 = $CONH_2$
6, R^1 = TBDMS R^2 = TBDMS R^3 = CN

for **6** (*o*-$Cl_2C_6H_4$): E_a = 23.6 kcal/mol, $\Delta H^{\ddagger}$ = 22.9 kcal/mol, $\Delta S^{\ddagger}$ = –6.2 eu, $\Delta G^{\ddagger}$(130 °C) = 25.4 kcal/mol

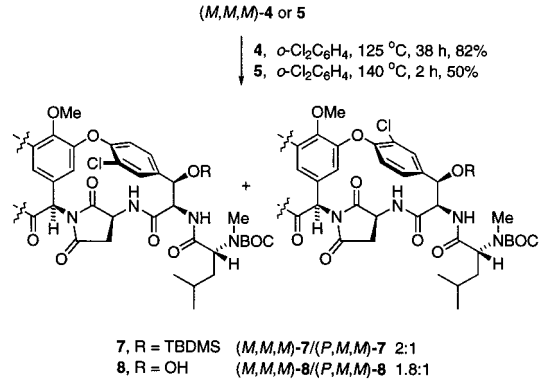
Compd	Conditions	k (h^{-1})	$t_{1/2}$ (h)
4	110 °C, *o*-$Cl_2C_6H_4$	0.019	14.3
4	130 °C, *o*-$Cl_2C_6H_4$	0.17	1.6
5	110 °C, *o*-$Cl_2C_6H_4$	0.003	94
6	110 °C, *o*-$Cl_2C_6H_4$	0.033	8.24
6	130 °C, *o*-$Cl_2C_6H_4$	0.11	2.78
6	140 °C, *o*-$Cl_2C_6H_4$	0.35	0.82

Figure 2.

observed in our studies of the isolated vancomycin CD, DE, and CDE ring systems.[10−13] Thus, C_3^6 but not C_3^2 alcohol protection is required for clean thermal atropisomerism.

The most successful and the most extensively investigated substrate was **6**. For all of the substrates including **6**, the thermal atropisomerism appeared cleaner in a nonpolar (*o*-$Cl_2C_6H_4$) versus polar (DMF, DMSO) aprotic solvent although the effect of other solvents was not surveyed. Selective and rapid DE isomerism occurred at 110−140 °C (*o*-$Cl_2C_6H_4$), and only traces of competitive byproducts were observed (Figure 2). Given the past assumption that interconversion of the vancomycin atropisomers is not possible, the relative ease with which the DE ring system isomerizes is especially notable ($t_{1/2}$ = 2.8 h, 130 °C). This atropisomerism is substantially faster than the observed DE atropisomerism within the isolated CDE ring system we have examined[12,13] ($t_{1/2}$ = 20.4 h, 130 °C) and comparable to that observed within the isolated DE ring system itself ($t_{1/2}$ < 1 h, 130 °C).[10,11] Even at 110 °C, a reasonable rate of isomerization ($t_{1/2}$ = 8.2 h) is observed. Preparative isomerism at 130 °C (5 h, 80% recovery) and 140 °C (1.5 h, 70% recovery) provided a separable 1:1 mixture of natural (*M*,*M*,*M*)-**6** and (*P*,*M*,*M*)-**6** possessing the unnatural DE atropisomer stereochemistry. The isomerism at 130 °C appeared better than that at either the more vigorous conditions of 140 °C or the slower conditions at 110 °C and was enlisted for our preparative studies. The assignment of the atropisomer structure and stereochemistry followed from resubjection of (*P*,*M*,*M*)-**6** to the thermal conditions with reequilibration to the 1:1 mixture of atropisomers and 2D $^1H-^1H$ NMR (CD_3OD, 313 K, 600 MHz). The latter revealed that the characteristic nOe cross-peaks of the CD ring system (*M*)-stereochemistry were unperturbed [C_{5a}^6/C_2^6 (m) and C_{5a}^6/C_3^6 (s)], while those of the DE ring system were now diagnostic of the unnatural

Scheme 3

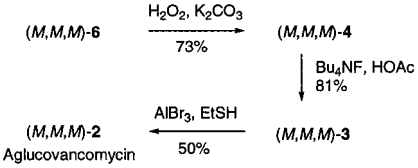


(*P*)-stereochemistry [C_{5b}^2/C_3^2 (s) and C_{6b}^2/C_{4b}^4 (m)] rather than the natural (*M*)-stereochemistry [C_{5a}^2/C_3^2 (m), C_{5a}^2/C_2^2 (w), and C_{6b}^2/C_{4b}^4 (w)].

In contrast, **4** incorporating the free Asn carboxamide and both the C_3^2 and C_3^6 alcohols protected as TBDMS ethers exhibited a more complex atropisomerism complicated by competitive succinimide formation. The competitive succinimide formation in *o*-$Cl_2C_6H_4$ was minimal at the lower temperature of 110 °C (10 h, 80% **4** and 5% **7**) and it became more prominent at 130 °C (1.8 h, 74% **4** and 14% **7**). The two atropisomers of the succinimide **7** were preparatively isolated in superb yield by extending the thermal treatment (125 °C, 38 h, 82%) to provide a 2:1 mixture of (*M*)/(*P*) DE atropisomers (Scheme 3). Although complicated by the competitive succinimide formation, the rate of atropisomerism by the carboxamide **4** appears to be slightly faster than that of the nitrile **6**, and analogous observations have been made with the isolated DE ring system.[11] The identity of the DE (*P*)-atropisomer was established by 2D $^1H-^1H$ NMR, and the succinimide atropisomers were easily distinguishable by additional changes in the characteristic Asn C*H*$_2$CONH$_2$ resonances. Resubjecting **7** to thermal atropisomerism revealed that the succinimide isomerizes much more slowly than the parent compound **4** with little or no isomerization observed at 130 °C and only very slow isomerization at 140 °C (*o*-$Cl_2C_6H_4$). At these temperatures and reaction times, competitive decomposition was observed, and only an approximate atropisomerism rate was established ($k = 0.07$ h^{-1}, 140 °C). This slow rate of interconversion of the succinimide atropisomers established that unnatural (*P*,*M*,*M*)-**7** is most likely derived from a sequence involving first atropisomerism of **4** and subsequent succinimide formation and is consistent with the lower temperature studies where atropisomerism is kinetically faster than competitive succinimide formation.

Similarly, **5** underwent an analogous atropisomerism of the DE ring system with no evidence of CD isomerism. However, the atropisomerism was complicated by competitive succinimide formation in *o*-$Cl_2C_6H_4$ analogous to observations made with **4**, and under these conditions and up to temperatures of 140 °C (2 h), no DE retro aldol ring cleavage was observed. Only at 110 °C where succinimide formation is slow could an approximate rate of DE atropisomerism be established. At the higher temperatures, competitive succinimide formation precluded accurate assessments of the isomerization rates (Scheme 3). Interestingly and analogous to observations made with **3**, competitive succinimide formation appears to be slowed by C_3^2 OTBDMS protection with **4**, and it was observed more prominently with both **5** and **3**. Conducting analogous studies in DMF (140 °C) led to the observation of a slow competitive retro aldol cleavage of the DE ring system.[19]

Scheme 4



Finally, initial studies conducted with **3** revealed that atropisomerism in polar solvents including DMF (140 °C, 3 h, 40% or 110 °C, 17 h, 41%) provided extensive conversion to the polar products including the CD retro aldol ring cleavage product[20] (45−50%). At the lower temperature of 110 °C (DMF), only one prominent retro aldol product arising from cleavage of the CD ring system was observed. At 140 °C, two retro aldol products were detected which most likely reflect both CD and DE ring cleavage with the former appearing in less than 30 min and the latter arising more slowly (2 h).[20] The competitive retro aldol reactions representing more polar products were minimized in *o*-$Cl_2C_6H_4$ (6−10%), and isomerization at 110 °C (17 h, 60%) and 140 °C (3 h, 63%) under conditions that also minimize succinimide formation provided respectable recoveries of a 1:1 mixture of **3** atropisomers. Under these conditions, 20% (110 °C) and 25% (140 °C) of the succinimide atropisomers were also isolated. As in observations made with **5** versus **4**, the competitive generation of the succinimide appears more significant with **3**, suggesting the C_2^3 OTBDMS protection found in **4** may either slow its formation or facilitate atropisomerism. Thus, useful interconversions are possible even with **3**, although those of **6** are much cleaner and easier to predictably control.

Conversion of (*M*,*M*,*M*)-6 to Aglucovancomycin. The preparation of natural aglucovancomycin from (*M*,*M*,*M*)-**6** was also examined in efforts to address two key issues associated with a projected vancomycin aglycon total synthesis. The first was whether conditions could be developed that would permit hydration or hydrolysis of an Asn residue nitrile. The second and far more significant issue was whether conditions could be developed for aryl methyl ether cleavage. Thus, treatment of (*M*,*M*,*M*)-**6** with $K_2CO_3-H_2O_2$ (8 equiv/40 equiv) under modified Katritzky conditions[21] (DMSO−H_2O, 25 °C, 3.5 h) cleanly provided the carboxamide **4** (73%) without cleavage of the TBMDS ethers or C-terminal methyl ester (Scheme 4). If the reaction times were extended, competitive TBDMS ether cleavage was observed. Deprotection of the TBDMS ethers (60 equiv of Bu_4NF, 60 equiv of HOAc, THF, 25 °C, 29 h, 81%) followed by treatment of **3** with $AlBr_3$ (40 equiv, EtSH, 25 °C, 5 h) provided (*M*,*M*,*M*)-**2** (50%). Significantly, the final conversion of **3** to **2** involves the cleavage of the four methyl ethers and the methyl ester and *N*-BOC deprotection. The sample of (*M*,*M*,*M*)-**2** obtained by this sequence was identical in all respects with authentic aglucovancomycin (**2**) derived from the degradation of **1** (^1H NMR, 1:1 comixture ^1H NMR, HPLC R_t and 1:1 comixture HPLC).

Conversion of (*P*,*M*,*M*)-6 to (*P*,*M*,*M*)-2. The Vancomycin Aglycon with the Unnatural DE Atropisomer Stereochemistry. Analogous to the efforts described above, (*P*,*M*,*M*)-**6** obtained by preparative thermal atropisomerism of (*M*,*M*,*M*)-**6**

(19) The product aldehyde exhibited a characteristic ^1H NMR signal at δ 9.85 (DMF-d_7) and appeared at the following rates: 0.5 h (≤5%), 1.5 h (≤10%), 4 h (ca. 50%). Extensive conversion to both polar and nonpolar products provided a complex mixture.

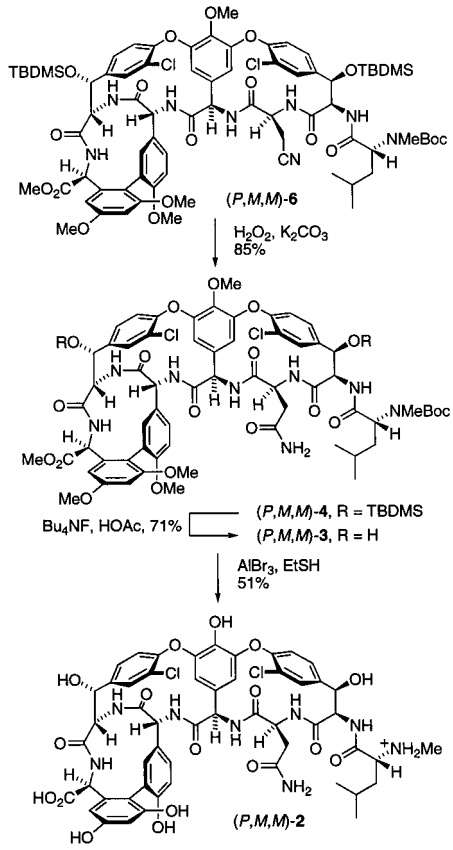
(20) The diagnostic -CHO signals in the ^1H NMR (CD_3OD, 313 K, 600 MHz) were observed at δ 9.87 (CD retro aldol) and δ 9.85 (DE retro aldol).

(21) Katritzky, A. R.; Pilarski, B.; Urogdi, L. *Synthesis* **1989**, 949.

Table 1. Antimicrobial Activity, MIC (μg/mL)

compd	*Staphylococcus aureus* (ATCC 25923)	*Enterococcus faecium* (ATCC 35667)	*Enterococcus faecium* (vancomycin-resistant)
vancomycin (**1**)	1.25	2.5	250
(*M*,*M*,*M*)-**2**	0.7	2.8	180
(*P*,*M*,*M*)-**2**	14	28	>1800
(*M*,*M*,*M*)-**3**	13	110	>1700
(*P*,*M*,*M*)-**3**	>44	>44	>1400
(*M*,*M*,*M*)-**4**	>120	>120	>1900
(*P*,*M*,*M*)-**4**	>56	>56	>1800
(*M*,*M*,*M*)-**6**	>130	>130	>2100
(*P*,*M*,*M*)-**6**	>90	>90	>1400
(*M*,*M*,*M*)-**9**	0.7	1.4	45

Scheme 5

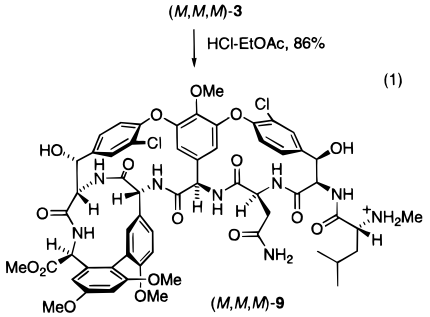


was converted to (*P*,*M*,*M*)-**2**, aglucovancomycin possessing the unnatural DE atropisomer stereochemistry. Thus, conversion of the nitrile to the corresponding carboxamide under the modified Katritzky conditions of $K_2CO_3-H_2O_2$ (85%) followed by the cleavage of the TBDMS ethers ($Bu_4NF-HOAc$, 71%) and final exhaustive deprotection of the four methyl ethers, methyl ester, and *N*-BOC group ($AlBr_3$, EtSH, 51%) provided (*P*,*M*,*M*)-**2** (Scheme 5).

Antimicrobial Activity. The antimicrobial activities of aglucovancomycin (**2**) and its unnatural (*P*,*M*,*M*)-atropisomer were examined along with those of a number of the semisynthetic derivatives and vancomycin itself against two vancomycin-sensitive and one vancomycin-resistant bacterial strains (Table 1). Aglucovancomycin (**2**) was found to be essentially equipotent with vancomycin (**1**) against the three bacterial strains examined, consistent with prior observations.[4] Unnatural (*P*,*M*,*M*)-**2** was found to be 10× less potent than natural (*M*,*M*,*M*)-**2** and showed no enhanced selectivity against the vancomycin-resistant *Enterococcus faecium*. This substantially diminished activity of the unnatural DE atropisomer is an interesting and important contrast to the properties of monodechlorovancomycin[22] which lacks the DE aryl chloride or the related orienticins which exhibit essentially equipotent activity unaffected by the lack of a DE aryl chloride. Thus, while the presence of the chloride in the natural (*M*)-atropisomer does not appear to significantly potentiate the antimicrobial properties,[22] its presence in the unnatural (*P*)-atropisomer substantially diminishes the antimicrobial potency.

In addition, (*M*,*M*,*M*)-**3** exhibited surprisingly potent activity despite N-acylation of the terminal *N*-methyl-Leu residue. Although it was 10−20× less active than **2**, the residual activity is significant, given its critical role in binding *N*-acyl-D-Ala-D-Ala, and similar observations have been implicated in prior studies.[4] Analogous to the observations made with **2**, (*M*,*M*,*M*)-**3** possessing the natural DE atropisomer stereochemistry was more potent than (*P*,*M*,*M*)-**3**.

Significantly, permethylation of the four phenols with (*M*,*M*,*M*)-**9**, which was obtained by *N*-BOC deprotection of (*M*,*M*,*M*)-**3** (HCl-EtOAc, 86%, eq 1), provided an aglucovancomycin



(1)

derivative that was at least as active if not slightly more potent than **1** or **2** and slightly more effective against the vancomycin-resistant bacteria. Such derivatives of the natural products have not, to our knowledge, been disclosed or explored and may prove to be an important class to pursue. Notably, the C-terminal carboxylate of **9** is functionalized as a methyl ester, and this derivatization site constitutes one of the more successful used for modulating physiochemical and transport/absorption properties without negatively impacting in vitro antimicrobial activity.[4] Thus, the assessment of the methyl ester with **9** (and **3**, **4**, and **6**) versus the use of the free carboxylic acid would not be expected to alter the in vitro antimicrobial properties against the three bacterial strains examined.

(22) The removal of the vancomycin C_{6a}^2-Cl provides an antibiotic that exhibits 70% of the antimicrobial activity of vancomycin and binds representative di- and tripeptides slightly less effectively than vancomycin, see: Harris, C. M.; Kannan, R.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc*. **1985**, *107*, 6652. In addition, the C_{6a}^2-Cl of the related antibiotic eremomycin fits into a pocket at the antibiotic dimerization interface and its removal reduces K_{dim} by a factor of 80, see: Mackay, J. P.; Gerhard, U.; Beauregard, D. A.; Maplestone, R. A.; Williams, D. H. *J. Am. Chem. Soc*. **1994**, *116*, 4573.

Experimental Section

Degradation of Vancomycin. (*M,M,M*)-3. Vancomycin·HCl (1.03 g, 0.71 mmol) was treated with CF_3CO_2H (25 mL), and the resulting solution was stirred at 50 °C for 7.5 h. The mixture was concentrated in vacuo, and the residue was triturated with EtOAc−hexane (1:1, 100 mL). The resulting precipitate was collected by filtration, washed with EtOAc (50 mL), and dried under vacuum to afford aglucovancomycin (**2**) as a crude residue. A sample of crude **2** (200 mg) prepared as described above was purified by semipreparative reverse phase HPLC (C18, 2.5 × 10 cm, CH_3CN−0.07% TFA/H_2O, 17:83, 10 mL/min, R_t = 18 min) to afford pure aglucovancomycin (**2**, 88 mg) as a white film.

A solution of the crude aglucovancomycin (**2**, from 1.03 g **1**) in dioxane−H_2O (2:1, 15 mL) was treated sequentially with $NaHCO_3$ (198 mg, 2.36 mmol) and BOC_2O (388 mg, 1.78 mmol) at 0 °C. The reaction mixture was slowly warmed to 25 °C and was stirred for 2 h. The reaction mixture was cooled to 0 °C, quenched by the addition of HOAc (0.135 mL, 2.36 mmol), concentrated in vacuo, and the residue was triturated with EtOAc−hexane (1:1, 50 mL). The resulting precipitate was collected by filtration, washed with EtOAc−hexane (1:1, 50 mL), and dried under vacuum to afford *N*-BOC-aglucovancomycin as a crude residue.

A solution of crude *N*-BOC-aglucovancomycin in DMF (10 mL) was treated sequentially with $NaHCO_3$ (198 mg, 2.36 mmol) and CH_3I (0.244 mL, 3.93 mmol) at 0 °C under Ar. The reaction mixture was slowly warmed to 25 °C and stirred for 2 h. The reaction mixture was cooled to 0 °C and quenched by the addition of H_2O (200 mL) followed by 10% aqueous HCl (5 mL). The resulting precipitate was collected by filtration, washed with H_2O (20 mL), and dried under vacuum to afford *N*-BOC-aglucovancomycin methyl ester as a crude residue.

A solution of crude *N*-BOC-aglucovancomycin methyl ester in DMF (10 mL) was treated sequentially with K_2CO_3 (342 mg, 2.47 mmol), *n*-Bu_4NI (114 mg, 0.31 mmol), and CH_3I (0.386 mL, 6.2 mmol). The reaction mixture was slowly warmed to 25 °C and stirred. After 3.5 h, additional K_2CO_3 (171 mg, 1.23 mmol) and CH_3I (0.386 mL, 6.2 mmol) were added. The reaction mixture was stirred for 2.5 h, quenched by the addition of 1% aqueous HCl (70 mL) at 0 °C, and extracted with EtOAc (2 × 140 mL). The combined organic layers were washed with H_2O (30 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 2.5 × 20 cm, 5−7% CH_3OH−$CHCl_3$ gradient elution) afforded (*M,M,M*)-**3**[23] (340 mg, 929 mg theoretical, 37%) as a white solid.

(*M,M,M*)-4. A solution of (*M,M,M*)-**3** (189 mg, 144 μmol) in anhydrous CH_3CN (1.9 mL) was treated with $CF_3CONMeTBDMS$ (1.7 mL, 7.2 mmol) under Ar, and the resulting mixture was stirred at 50 °C for 9.5 h. The reaction mixture was poured into EtOAc−20% aqueous citric acid (3:1, 40 mL) and stirred at 25 °C for 12 h. The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 40 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (15 mL) and saturated aqueous NaCl (15 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 2.5 × 20 cm, 50−85% EtOAc−hexane and then 10% CH_3OH−$CHCl_3$ gradient elution) afforded (*M,M,M*)-**4**[23] as a white film (147 mg, 222 mg theoretical, 66%) and (*M,M,M*)-**5**[23] as a white film (20.1 mg, 206 mg theoretical, 10%).

(*M,M,M*)-6. A solution of (*M,M,M*)-**4** (137 mg, 88.6 μmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C was treated sequentially with pyridine (42.9 μL, 530 μmol) and TFAA (50.0 μL, 354 μmol). The resulting mixture was stirred at 0 °C for 10 min, diluted with EtOAc (20 mL), and quenched by the addition of 1% aqueous HCl (2 mL). The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with H_2O (5 mL) and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 1.5 × 15 cm, 50−60% EtOAc−hexane gradient elution) afforded (*M,M,M*)-**6**[23] (111 mg, 135 mg theoretical, 82%) as a white film.

Thermal Atropisomerism Studies. General Procedure. Thermal Interconversion of the Atropisomers (*M,M,M*)-6 and (*P,M,M*)-6. A solution of (*M,M,M*)-**6** (2 mg, 1.3 μmol) in *o*-dichlorobenzene (0.1 mL) saturated with Ar was warmed at 130 °C for 5.5 h. Periodically, *o*-dichlorobenzene was evaporated under a flow of N_2 and replaced with CD_3OD for the determination of the ratio of (*M,M,M*)-**6** and (*P,M,M*)-**6** by ^1H NMR (313 K, 600 MHz), Table 2. Removal of CD_3OD and its replacement with *o*-dichlorobenzene allowed further heating of (*M,M,M*)-**6** and its emerging isomer (*P,M,M*)-**6** with the unnatural DE atropisomer stereochemistry. Upon completion of the kinetic investigation, evaporation of *o*-dichlorobenzene and PTLC (SiO_2, 3% CH_3OH−$CHCl_3$) afforded the recovery of the starting material (*M,M,M*)-**6**[23] (1.0 mg, 2 mg theoretical, 49%) and (*P,M,M*)-**6**[23] (0.7 mg, 2 mg theoretical, 36%) as white films.

Analogous experiments enlisting (*M,M,M*)-**6** were conducted at 110 °C and 140 °C in *o*-dichlorobenzene (Table 2).

Table 2

compd	conditions	*t* (h)	(*M,M,M*)/(*P,M,M*)
6	*o*-$C_6H_4Cl_2$, 110 °C	0.62	96:4
		1.62	91:9
		3.77	86:14
		7.77	75:25
		13.77	69:31
6	*o*-$C_6H_4Cl_2$, 130 °C	0.50	97:3
		1.00	92:8
		1.50	88:12
		2.50	79:21
		3.50	69:31
		4.50	62:38
		5.50	58:42
6	*o*-$C_6H_4Cl_2$, 140 °C	0.28	90:10
		0.53	83:17
		0.87	72:28
		1.20	65:35
		1.53	50:50
4	*o*-$C_6H_4Cl_2$, 110 °C	1.00	94:6
		2.00	93:7
		5.00	87:13
		9.00	82:15
4	*o*-$C_6H_4Cl_2$, 130 °C	0.67	88:12
		1.33	78:22
		2.50	64:36
		2.98	60:40

Thermal Interconversion of the Atropisomers (*M,M,M*)-4 and (*P,M,M*)-4. This was conducted following the general procedure. The atropisomerism of **4** incorporating the free Asn carboxamide residue exhibited a more complex atropisomerism, resulting from a competitive succinimide formation. Investigated temperatures and heating time frames were selected where the amount of succinimide formation remains minimal (Table 2).

Formation of Succinimides (*M,M,M*)-7 and (*P,M,M*)-7. A solution of (*M,M,M*)-**4** (5 mg, 3.2 μmol) in *o*-dichlorobenzene (0.3 mL) saturated with Ar was warmed at 125 °C for 38 h. Evaporation of the solvent and PTLC (SiO_2, 3% CH_3OH−$CHCl_3$) afforded (*M,M,M*)-**7**[23] (2.7 mg, 4.9 mg theoretical, 55%) and (*P,M,M*)-**7**[23] (1.3 mg, 4.9 mg theoretical, 27%) as white solids.

Thermal Interconversion of the Atropisomers (*M,M,M*)-5 and (*P,M,M*)-5. This was conducted following the general procedure. The atropisomerism of **5** in *o*-dichlorobenzene was complicated by competitive succinimide formation analogous to the observation made with **4**. Only at 110 °C where succinimide formation is slow could an approximate rate of DE atropisomerism be established.

Formation of Succinimides (*M,M,M*)-8 and (*P,M,M*)-8. A solution of (*M,M,M*)-**5** (2.5 mg, 1.8 μmol) in *o*-dichlorobenzene (0.2 mL) saturated with Ar was warmed at 140 °C for 2 h. Evaporation of the solvent and PTLC (SiO_2, 2% CH_3OH−$CHCl_3$) afforded (*M,M,M*)-**8**[23] (0.8 mg, 2.5 mg theoretical, 33%) and (*P,M,M*)-**8**[23] (0.4 mg, 2.59 mg theoretical, 17%) as white solids.

Thermal Interconversion of the Atropisomers (*M,M,M*)-3 and (*P,M,M*)-3. The atropisomerism of **3** in *o*-dichlorobenzene was complicated by competitive formation of succinimide and other unidentified compounds which precluded assessment of the isomerization rates.

(23) Full characterization data and diagnostic and strong 2D ^1H−^1H nOe's are provided in the Supporting Information.

In DMF, upon heating at 110−140 °C, rapid formation of a compound more polar than (*M*,*M*,*M*)-**3** was observed and shown to arise from retro aldol CD ring cleavage.

Retro Aldol CD Ring-Cleaved 3. A solution of (*M*,*M*,*M*)-**3** (5.0 mg, 3.8 μmol) in DMF (0.2 mL) saturated with Ar was warmed at 140 °C for 0.5 h. Evaporation of the solvent and PTLC (SiO_2, 5% CH_3OH−$CHCl_3$) afforded the retro aldol CD ring-cleaved product[23] (0.8 mg, 2.5 mg theoretical, 33%) as a white solid.

Conversion of (*M*,*M*,*M*)-6 to Aglucovancomycin [(*M*,*M*,*M*)-2] and (*P*,*M*,*M*)-6 to (*P*,*M*,*M*)-2. (*P*,*M*,*M*)-6. A solution of (*M*,*M*,*M*)-**6** (39.9 mg, 2.62 μmol) in *o*-dichlorobenzene (3 mL) was stirred at 120 °C for 16.5 h. The reaction mixture was concentrated in vacuo. PTLC (SiO_2, 40% EtOAc−$CHCl_3$) afforded the recovery of starting material (*M*,*M*,*M*)-**6** (17.5 mg, 39.9 mg theoretical, 44%) and (*P*,*M*,*M*)-**6**[23] (15.4 mg, 39.9 mg theoretical, 39%) as a white film.

Preparation of 4 from 6. A solution of (*M*,*M*,*M*)-**6** (3.5 mg, 2.3 μmol) in DMSO (0.6 mL) at 25 °C was treated sequentially with H_2O_2 (10 μL, 98 μmol) and 10% aqueous K_2CO_3 (25.0 μL, 20 μmol). The resulting mixture was stirred at 25 °C for 3.5 h, diluted with EtOAc (15 mL), and quenched by the addition of 0.1% aqueous HCl (2 mL). The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with H_2O (10 mL) and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. PTLC (SiO_2, 6% CH_3OH−$CHCl_3$) afforded (*M*,*M*,*M*)-**4**[23] (2.6 mg, 3.5 mg theoretical, 73%) as a white film.

By using the same procedure, we obtained (*P*,*M*,*M*)-**4**[23] (2.5 mg, 2.9 mg theoretical, 85%) as a white film from (*P*,*M*,*M*)-**6** (2.9 mg, 1.9 μmol).

Preparation of 3 from 4. A solution of (*M*,*M*,*M*)-**4** (1.6 mg, 1.04 μmol) in THF (50 μL) was treated with *n*-Bu_4NF−HOAc (1:1, 1 M solution in THF, 31 μL, 31 μmol) at 25 °C, and the resulting mixture was stirred at 25 °C. After 11 h, additional *n*-Bu_4NF−HOAc (1:1, 1 M solution in THF, 31 μL, 31 μmol) was added. The reaction mixture was stirred for 18 h, quenched by the addition of H_2O (2 mL) at 0 °C, and extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with H_2O (2 × 15 mL) and saturated aqueous NaCl (20 mL), dried (Na_2SO_4), and concentrated in vacuo. PTLC (SiO_2, 9% CH_3OH−$CHCl_3$) afforded (*M*,*M*,*M*)-**3**[23] (1.1 mg, 1.4 mg theoretical, 81%) as a white film.

By using the same procedure, we obtained (*P*,*M*,*M*)-**3**[23] (3.4 mg, 4.8 mg theoretical, 71%) as a white film from (*P*,*M*,*M*)-**4** (5.6 mg, 3.6 μmol).

Preparation of Aglucovancomycin 2 from 3. A vial charged with (*M*,*M*,*M*)-**3** (5.0 mg, 3.8 μmol) was treated with $AlBr_3$ (38.1 mg, 0.143 mmol) in EtSH (0.2 mL) under Ar. The resulting mixture was stirred at 25 °C for 5 h, diluted with $CHCl_3$ (0.5 mL), cooled to 0 °C, and quenched by the addition of CH_3OH (0.1 mL). The solvent was removed by a stream of N_2. The crude mixture was purified by PTLC (SiO_2, 55% CH_3OH−EtOAc) and semipreparative reverse phase HPLC (CH_3CN−0.07% TFA/H_2O, 17:83, 10 mL/min, R_t = 18 min) to afford (*M*,*M*,*M*)-**2**[23] (2.4 mg, 4.8 mg theoretical, 50%) as a white film.

By using the same procedure, we obtained (*P*,*M*,*M*)-**2**[23] (2.0 mg, 3.9 mg theoretical, 51%) as a white film from (*P*,*M*,*M*)-**3** (4.1 mg, 3.1 μmol).

(*M*,*M*,*M*)-9. A solution of (*M*,*M*,*M*)-**3** (30.3 mg, 23.1 μmol) in anhydrous dioxane (1 mL) was treated with 4 N HCl−EtOAc (2 mL) under Ar, and the resulting mixture was stirred at 25 °C for 0.5 h. The reaction mixture was concentrated in vacuo, and the residue was triturated with EtOAc (5 mL) to give (*M*,*M*,*M*)-**9**[23] (24.9 mg, 28.8 mg theoretical, 86%) as a white solid.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA41101), the Skaggs Institute for Chemical Biology, the award of a sabbatical leave for S.M. (Japan Tobacco), the award of a Swiss National Foundation postdoctoral fellowship and a Ciba-Geigy Jubiläums Stiftung (O.L.), the award of a National Institutes of Health postdoctoral fellowship (R.T.B., CA71102), and the award of a NSF predoctoral fellowship (S.L.C., GER-9253922).

Supporting Information Available: Full characterization of (*M*,*M*,*M*)- and (*P*,*M*,*M*)-**2**−**8**, (*M*,*M*,*M*)-**9**, and diagnostic or strong 2D ^1H−^1H nOe's are provided (7 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA981928I