Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3345–3350

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Muraymycins, Novel Peptidoglycan Biosynthesis Inhibitors: Synthesis and SAR of Their Analogues†

Ayako Yamashita,* Emily Norton, Peter J. Petersen, Beth A. Rasmussen, Guy Singh, Youjin Yang, Tarek S. Mansour and Douglas M. Ho‡

Chemical Sciences and Infectious Diseases, Wyeth Research, Pearl River, NY 10965, USA

Received 24 December 2002; accepted 8 May 2003

Abstract—A series of Muraymycin analogues was synthesized. These analogues showed excellent antimicrobial activity against gram-positive organisms. These analogues also showed excellent inhibitory activity against the target peptidoglycan biosynthesis enzyme MraY, the cell membrane associated transglycosylase responsible for the formation of Lipid II.

Over the past years, a large number of naturally derived semi-synthetic or synthetic antibiotics in various classes such as glycopeptides, tetracyclines and macrolides, have improved the quality of these compounds for the treatment of infections caused by a broad range of bacteria. However, despite the historical success of this strategy in combating bacterial infections, relatively few antibacterial agents of microbial origin are currently being pursued in the clinic. This is primarily attributable to the considerable difficulty in identifying structurally novel antibiotics with potent and selective antimicrobial properties.[1] On the other hand, as a consequence to the indiscriminate use of antibiotics, the emergence of multi-drug resistant pathogens has resulted in an epidemic situation. This problem, therefore, necessitated the search for new antibacterial agents by employing different strategies, such as relying on advanced screening technologies, understanding structural basis for the design of new generation of antibacterial compounds,[2] or unraveling the understanding of resistance mechanisms[3] to current antibacterial agents in the hope that a new generation of antimicrobial agents emerges.

This paper outlines our approach to new antibacterial agents, based on expanding the structure activity relationship of muraymycins[4,5] (**1a**,**b** and **2a**,**b**) via the development of synthetic routes toward the total synthesis of muraymycins, a novel class of peptidoglycan biosynthesis inhibitors,[6] with potent activity against gram-positive organisms with minimum inhibitory concentration (MIC) of 1–8 μg/mL. The new target analogues differ from naturally occurring muraymycins by replacement of the cyclic arginine amino acid with arginine and by the removal of the 5′-amino ribose sugar moiety **3** as well as the removal of the lipophilic side chain X (Fig. 1). During this process, we found that the truncated muraymycins (**19**, Scheme 4) were as active as the natural products against gram positive organisms.

A synthetic route was developed to allow for rapid introduction of various amino acids and linkers in the acyclic portion of muraymycins. The first amino acid moiety (glycine) was planned to be introduced by aldol reaction of an uridyl-5′ aldehyde with a suitable glycine derivative. Toward this end, aldehyde **4**[7] was synthesized from D-uridine. Aldol reaction between **4** and an anion of dibenzylglycine *tert*-butyl ester,[8] generated by treatment with LDA in THF at −78 °C, afforded a mixture of four diastereomers, which were separated by chromatography (Scheme 1). The two major components (**5** and **6**) were deprotected by catalytic hydrogenation, to form their free amines (**7** and **8**). The absolute stereochemistry of the newly created chiral centers at positions 5 and 6 in **7** and **8** was determined by the X-ray crystallographic analyses of their

†A part of this work was presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, USA. Yamashita, A.; Norton, E.; Labthavikul, P.; Petersen, P. J.; Rasmussen, B. A.; Singh, G.; Yang, Y.; Mansour, T. S. Abstract No. 1161.

*Corresponding author. Fax: +1-845-602-5561; e-mail: yamashita@wyeth.com

‡Current address: Director, Small Molecule X-ray Facility, Department of Chemistry, Princeton University, Princeton, NJ 08544, USA.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00671-1

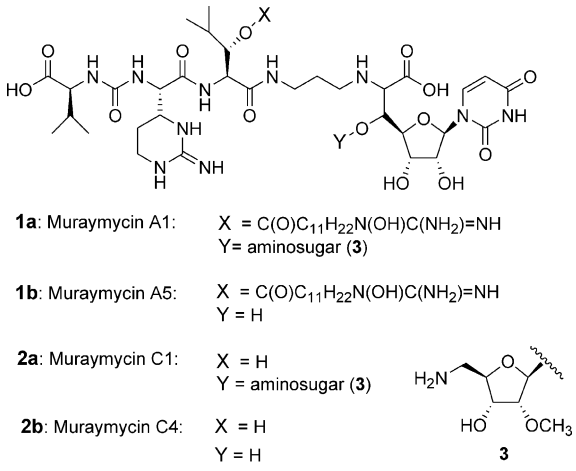
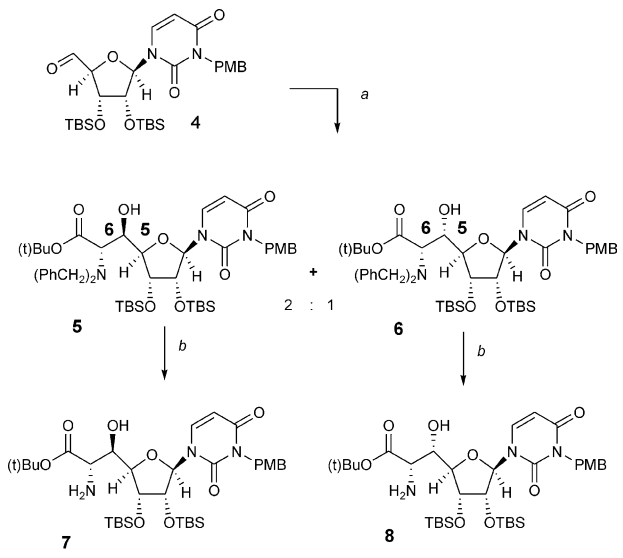


Figure 1.

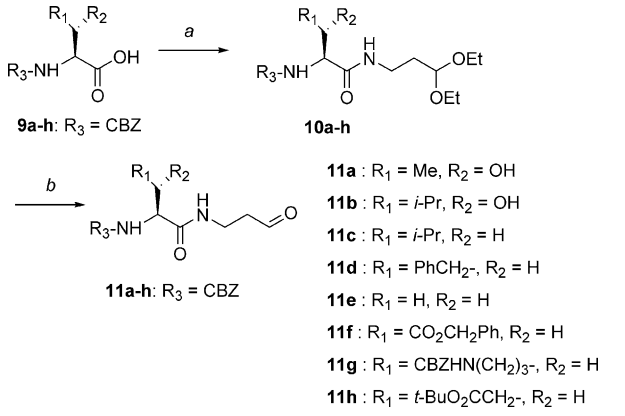


Scheme 1. (a) $(PhCH_2)_2NCH_2CO_2Bu(t)$, LDA, THF, −78 °C, 2 h, then −30 °C, 15 h; (b) H_2, 10% Pd/C, MeOH.

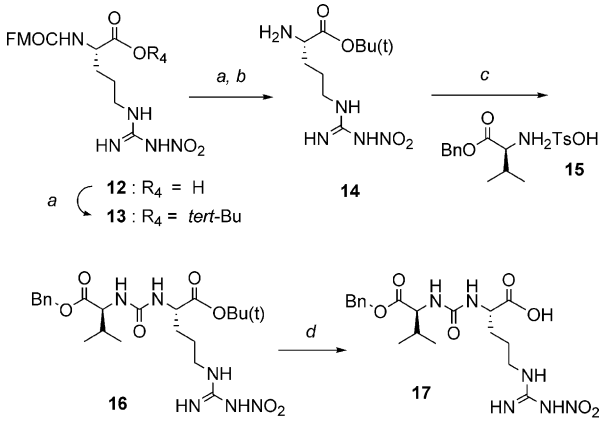
para-nitrophenyl urea derivatives, to be (**7**: 5*R*,6*S*) and (**8**: 5*S*,6*S*).[9]

To illustrate the methodology towards the assembly of muraymycin analogues, compounds **7** and **8** were independently used for further transformations as outlined in Schemes 2–4. The requisite linker aldehyde **11a–h** was prepared in two steps from the protected amino acid **9a–h** (R_3 = CBZ), which was coupled with aminopropane diethyl acetal to give an amide **10a–h**, followed by acid treatment to form **11a–h** (Scheme 2). The urea moiety **17** was prepared in four steps from the FMOC protected arginine **12** by the following processes: (a) esterification, **13**, (b) removal of the FMOC group, **14**, (c) urea formation, **16**, with valine benzyl ester (**15**), and (d) regeneration of the acid (Scheme 3).

Reductive amination of **7** with **11a** using $NaBH(OAc)_3$ resulted in the expected formation of the uridyl-dipeptide **18a**. This reaction proved to be applicable to various L-leucine and (2*S*,3*S*)-hydroxyleucine[10] derivatives (**11b** and **11c**) exemplified by three combinations of R_1



Scheme 2. (a) $H_2N(CH_2)_2CH(OEt)_2$, HOBT, EDC, DIPA, THF; (b) HCl, THF.



Scheme 3. (a) Isobutylene, *c*-H_2SO_4, CH_2Cl_2; (b) piperidine, rt, 1 h; (c) **15**, triphosgene, DIPA, rt; (d) TFA, CH_2Cl_2, 0 °C.

and R_2 substituents of **11**. Further progress of **18a** was achieved by removal of the CBZ group by catalytic hydrogenation, to give the free amine **19a**. Coupling between **17** and **18a** under the conditions using hydroxybenzotriazole (HOBT) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) allowed for the construction of the fully protected final target molecule **20a** in good isolated yield (Scheme 4).[11] In a similar fashion, **20b** and **20c** were isolated. The (5*S*,6*S*) diastereomer **8** was also successfully converted to the corresponding threonine intermediate **21a**, then to the unprotected amine **22a**. This result suggests that the stereochemical difference between **7** and **8** at the C5 position has no outcome in influencing the reductive amination and deprotection steps under the reported experimental conditions.

The antibacterial activity, as measured by the minimal inhibitory concentration (MIC, μg/mL), of **18a** and **19a** as well as **21a** and **22a** was evaluated in order to examine the effect of the protecting group (CBZ) on the terminal amines (Table 1). Table 1 also illustrates the stereochemical effect between **19a** and **22a** at the C5 position with regards to their antimicrobial activity. Table 1 illustrates that both **19a** and **22a** were active in the soluble peptidoglycan assay (SPG) with better activity obtained for **22a** (IC_{50} 11 μg/mL). This suggests

a stereochemical preference for 5*S*. However, the MIC values for **22a** did not track with its SPG activity. Compound **19a** showed better MIC activity (4–8 μg/mL) than **22a** (8–>128 μg/mL) against various gram-positive organisms.

Based on the above antibacterial data, a variety of analogues **19a–h**[12] were prepared by coupling between **7** and **11a–h**, and were also evaluated for their antibacterial activities (Table 2). A consistent trend was observed in the analogues carrying PMB, TBS and *tert*-butyl protecting groups, in that the terminal unprotected amine forms possess varied antimicrobial activities (MICs 4–>128 μg/mL) against gram-positive organisms, although their CBZ forms somewhat lost their activities. Remarkably, this trend extends to the aspartate and lysine analogues (**19f** and **19g**).

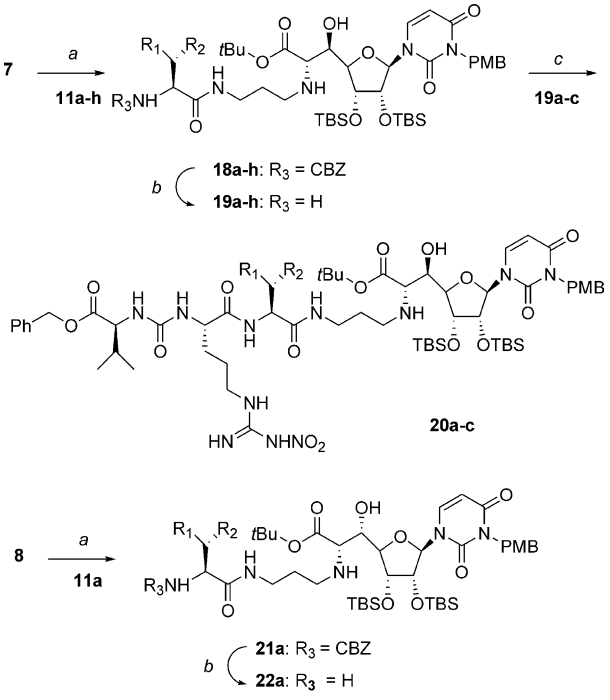
The role of various protecting groups in **18** and **20** with respect to their antimicrobial activities (Table 2) was studied by employing a stepwise deprotection strategy. This approach was applied to leucine containing analogue **18c** (Scheme 5). Upon treatment of **18c** with nBu$_4$NF, both TBS groups were removed to afford the corresponding diol **23c**. The CBZ group in **23c** was readily removed by catalytic hydrogenation, to form the free amine **24c**. Hydrolysis of the *tert*-butyl ester in **23c**, followed by removal of the CBZ group, provided a prototype of uracil protected derivative **26c** via **25c** for antimicrobial evaluation.

Alternatively, the PMB group of the uracil ring in **18c** was removed by treatment with ceric ammonium nitrate (CAN), to form the unprotected uracil **27c**. Catalytic hydrogenation of **27c** cleaved the CBZ group, to form

Table 1. Comparison of in vitro activity for two diastereomers

Compd	**18a**	**19a**	**21a**	**22a**	**1a**
IC_{50} SPG (μg/mL)	32	32	10.7	10.7	—
	Minimal inhibitory concentration (MIC) (μg/mL)				
Gram-negative (10)	>128	>128	64–128	>128	8–32
Candida albicans GC 4531	128	>128	32	128	—
Staphylococcus aureus GC 4536	>128	4	128	128	44
Staphylococcus aureus GC 1131	>128	8	128	128	4
Staphylococcus aureus GC 2216	>128	4	128	128	8
CNS[a] GC 4538	>128	4	128	128	2
CNS GC 4538	>128	4	128	128	1
CNS GC 4547	>128	8	128	8	8
Enterococcus faecalis GC 842	128	4	64	>128	4
Enterococcus faecalis GC 2242	>128	8	64	128	32
Enterococcus faecalis GC 4555	>128	4	64	128	16

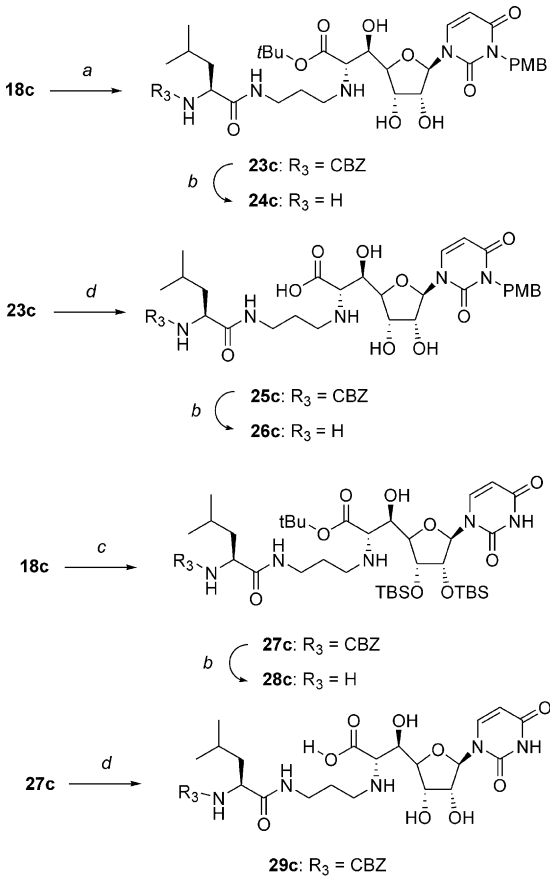
[a]CNS, coagulase-negative staphylococcus.



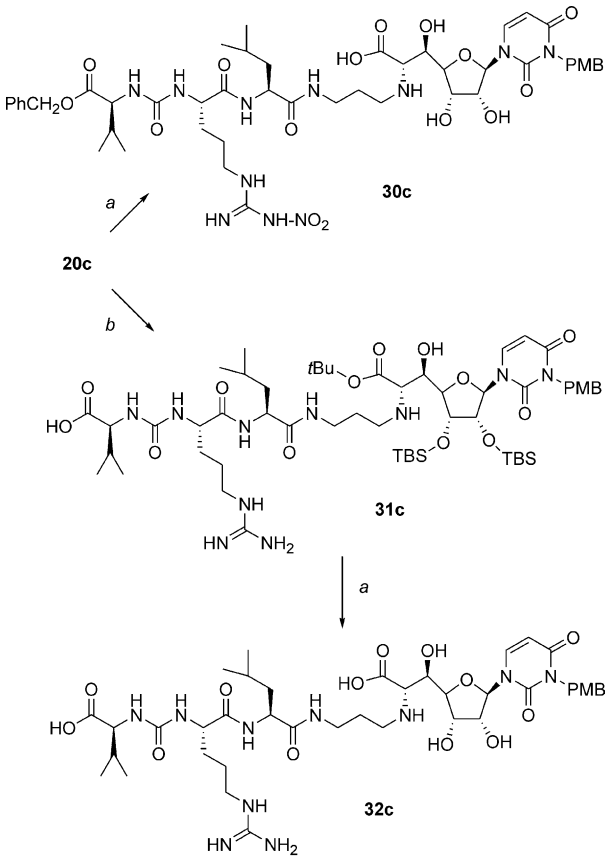
Scheme 4. (a) **11a–h**, NaBH(OAc)$_3$, AcOH, THF; (b) H_2, 10% Pd/C, MeOH; (c) **17**, HOBT, EDC, THF.

Table 2. In vitro activity of muraymycin analogues

Compd	Gram-negatives (10)	*Staphylococci* (6)	*Enterococci* (3)
18b	>128	>128	128
19b	>128	4–16	4–8
18c	>128	>128	>128
19c	>128	4–64	4–16
18d	>128	128	128
19d	>128	>128	16–>128
18e	>128	>128	128
19e	64–>128	4–8	4–8
18f	>128	>128	>128
19f	128–>128	16–>128	4–32
18g	>128	>128	>128
19g	128–>128	4–16	8
18h	>128	>128	>128
19h	>128	>128	>128
20a	64–>128	128	64–128
20b	>128	>128	128–>128
20c	>128	>128	>128
23c	128–>128	128–>128	128
24c	128–>128	>128	128–>128
25c	128–>128	>128	128
26c	128–>128	>128	128
27c	128–>128	>128	64–>128
28c	128–>128	1–2	2
29c	128–>128	>128	128–>128
30c	128–>128	>128	128–>128
31c	128–>128	>128	128–>128
32c	128–>128	>128	128–>128



Scheme 5. (a) nBu$_4$NF, THF; (b) H_2, 10% Pd/C, MeOH; (c) CAN, MeCN, 65 °C; (d) TFA, CH_2Cl_2.



Scheme 6. (a) TFA, CH_2Cl_2; (b) H_2, 10% Pd/C, MeOH, cat amount AcOH.

Table 3. In vitro activity of muraymycin analogues

Organism	Compd			
	19b	**19e**	**19g**	**28c**
	Minimal inhibitory concentration (MIC) (μg/mL)			
Escherichia coli GC 4559	>128	>128	>128	>128
Escherichia coli GC 4560	>128	8	8	32
Escherichia coli GC 3226	>128	>128	128	>128
Serratia marcescens GC 4077	>128	>128	128	128
Morganella morganii GC 4531	>128	64	128	128
Klebsiella pneumoniae GC 4534	>128	128	>128	128
Enterobacter cloacae GC 3783	>128	>128	>128	128
Escherichia coli GC 2214	>128	>128	128	128
Pseudomonas aeruginosa GC 2214	>128	>128	>128	>128
Pseudomonas aeruginosa GC 4532	>128	>128	>128	128
Candida albicans GC 3066	>128	>128	>128	32
Staphylococcu aureus GC 4536	16	4	8	1
Staphylococcu aureus GC 1131	4	4	4	1
Staphylococcu aureus GC 2216	4	8	8	2
CNS GC 4538	4	4	8	1
CNS GC 4538	4	8	8	2
CNS GC 4547	16	4	16	2
Enterococcu faecalis GC 842	8	4	8	2
Enterococcu faecalis GC 2242	4	4	8	2
Enterococcu faecalis GC 4555	4	8	8	2

the free N-terminal analogue **28c**. Treatment of **27c** with TFA not only cleaved both TBS groups, but also hydrolyzed the *tert*-butyl ester, to give **29c**. The process of deprotection of **20c** followed the condition described above. Catalytic hydrogenation cleaved both the benzyl and the NO_2 groups, while treatment with TFA cleaved the *tert*-butyl ester and the two TBS groups (Scheme 6).

Table 2 also demonstrates the SAR studies using the leucine analogue **18c**. When the TBS groups are removed, the antibacterial activity was lost. However, in

Table 4. The inhibitory activity of muraymycin analogues against MraY or MurG

Compd	100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL	6.25 μg/mL
2a	+[a]	+	+	+	+
19a	+	−	−	−	−
19b	+	+	−	−	−
19c	+	+	+	−	−
19d	−	−	−	−	−
19e	+	+	−	−	−
19f	+	+	−	−	−
19g	+	+	−	−	−
19h	−	−	−	−	−
22a	+	+	−	−	−
28c	+	+	+	+	−
32c	−	−	−	−	−

[a] + indicates reduced production of lipid II relative to the control (judged by intensity) on the film, thus indicating inhibition of either MraY of MurG. Since muraymycin A1 inhibited the production of labeled lipid I and II due to inhibition of MraY, its closely related analogues might be considered inhibitors of MraY.

the unique case of **28c** in which the PMB group in the uracil ring was removed, the MIC value is restored upon removal of the CBZ group. This result was consistent with earlier observations.

More extensive in vitro activities (MICs) of selected compounds (**19b**, **19e**, **19g**, and **28c**) are shown in Table 3. Compound **28c** demonstrated good antimicrobial activity against various gram-positive organisms.

The inhibition of lipid II formation by muraymycin analogues is summarized in Table 4. With the exception of **19d** and **19h**, most of the compounds of this type inhibited lipid II formation. This was most significant for **19c**, where the inhibition was observed as low as 25 μg/mL. These results corresponded well to the in vitro antimicrobial tests, that **19a**,**b**,**c**,**e**,**g** inhibited lipid II formation also showed moderately good antibacterial activity against gram-positive isolates (MIC 4–16 μg/mL). Compounds **19d** and **19h** did not inhibit lipid II formation, and showed poor antibacterial activities (MIC 16–>128 μg/mL). These results indicated that the substitution at R_1 in **19** has significant impact on the inhibitory activity of target and bacteria. Overall, compound **28c** was the most potent one tested. It inhibited lipid II formation at concentrations as low as 12.5 μg/mL, although **28c** has the same substitutions as compound **19c** with *iso*-propyl at R_1 and hydrogen at R_2.

In conclusion, we have demonstrated the establishment of a versatile methodology towards the synthesis of various muraymycin analogues. The SAR in this series establishes useful trends. Importantly, we have shown that smaller fragments of the natural products provide an excellent avenue for further development of potent antimicrobial agents.

Method for In Vitro Antibiotics Evaluation

The in vitro activities of the antibiotics were determined by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[13] Muller-Hinton II broth (MHBII: BBL Cockeysville, MD, USA) was the medium employed in the testing procedures. Microtiter plates containing serial dilutions of each antimicrobial agent were incubated with each organism to yield the appropriate density (10^5 CFU/mL) in a 100-μL final volume. The plates were incubated for 18–22 h at 35 °C in ambient air. The minimum inhibitory concentration (MIC) for all isolates was defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye.

Determination of Lipid II Formation and Soluble Peptidoglycan

The biological assay determining lipid II formation utilizes *S. epidermides* membrane to catalyze the late steps in cell wall biosynthesis including those performed by MraY, the MurNAc pentapeptide translocase, and MurG, the UDP-*N*-acetylglucosamyl transferase. According to the reported method (*J. Bacteriol.* **1991**, *173*, 4625–4636), the formation of lipid II is assessed using radiolabeled UDP-*N*-acetylglucosamine, *S. epidermides* membranes, compound, UDP-MurNAc pentapeptide, and [^{14}C]-UDP-*N*-acetylglucosamine. The reaction was incubated at room temperature for 30 min and was terminated by boiling in a water bath for 1 min. The samples of each reactions were analyzed by separation using TLC. The plates were exposed to film, and inhibition of lipid II formation was monitored by comparing the area of the experimental sample to the area of the control. The soluble peptidoglycan was determined using a 96-well format to quantitate the production of radioactive labeled peptidoglycan by *S. epidermides*. The method was modified from that of Boothby et al. (1971).

Acknowledgements

Authors thank analytical support provided by Discovery Analytical Chemistry Department, Wyeth Research, Pearl River, NY, and Princeton, NJ, USA.

References and Notes

1. Singh, M. P.; Greenstein, M. *Curr. Opin. Drug Discov. Develop.* **2000**, *3*, 167.
2. Brodersen, D. E.; Clemons, W. M., Jr.; Carter, A. P.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Cell* **2000**, *103*, 1143.
3. Healy, V. L.; Lessard, I. A. D.; Roper, D. I.; Knox, J. R.; Walsh, C. T. *Chem. Biol.* **2000**, *7*, R109.
4. Isolation and structure determination of muraymycin core skeleton: McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Lenoy, E.; Petersen, P. P.; Siegel, M. M.; Sign, G.; Williamson, R. T. *J. Am. Chem. Soc.* **2002**, *124*, 10260.
5. Biological activities of Muraymycins and derivatives were reported: (a) Rasmussen, B. A.; McDonald, L. A.; Petersen, P. P.; Rothstein, D.; Singh, G. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chcago, IL; Abstr #1162. (b) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.;

Lenoy, E.; Petersen, P. P.; Siegel, M. M.; Singh, G. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chcago, IL; Abstr #1159. (c) Lin, Y.-I.; Li, Z.; Francisco, G. D.; McDonald, L. A.; Davis, R. A.; Singh, G.; Yang, Y.; Mansour, T. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2341. (d) Singh, G.; Yang, Y.; Rasmussen, B. A.; Petersen, P. P.; McDonald, L. A.; Yamashita, A.; Lin, Y.-I.; Norton, E.; Francisco, G. D.; Li, Z.; Barbieri, L. R., 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chcago, IL; Abstr #1163.

6. (a) Isono, K.; Uramoto, M.; Kusakabe, H.; Kimura, K.; Izaki, K.; Nelson, C. C.; McCloskey, J. A. *J. Antibiot.* **1985**, *38*, 1617. (b) Kimura, K.; Ikeda, Y.; Kagami, S.; Yoshihara, M. *J. Antibiot.* **1998**, *51*, 1099.

7. Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4697.

8. Banifi, L.; Cardani, S.; Potenza, D.; Scolastico, C. *Tetrahedron* **1987**, *43*, 2317.

9. **Preparation of 7 and 8, and their *para*-nitrophenyl urea derivatives:** To a cooled (−78 °C) solution of *tert*-butyl 2-(dibenzylamino)propanoate (4.75 g, 15.23 mmol) in anhydrous THF (60 mL) was added LDA (7.6 mL, 15.23 mmol, Aldrich) dropwise under argon. After strring for 1 h at this condition, a solution of **4** (4.5 g, 7.62 mmol) in THF (10 mL) was added via syringe. The resulting mixture was stirred at −78 °C for 2 h, then at −35 °C for 15 h, and quenched by addition of water. The aqueous layer was extracted with EtOAc, and the combined extracts were washed with sat. brine solution, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed (flash column, silica gel, *n*-hexane/CH_2Cl_2/MeOH/ether = 50/50/1/1), to give **6** (990 mg, 14%) and **5** (2.14 g, 31%). Further elution gave a mixture of two other diastereomers (710 mg, 10%) and the recovered **4** (1.6 g, 36%). Compounds **5** and **6** were separately hydrogenated using 10% Pd/C in MeOH, to give **7** and **8**, respectively. A solution of **7** and *para*-nitrophenylisocyanate (1.2 mol equiv) in THF was stirred at room temperature under nitrogen for 24 h. The solution was concentrated in vacuo, and the residue was chromatographed (flash column, silica gel, EtOAc/hexenes) to give the corresponding urea. Crystals suitable for X-ray diffraction were obtained from methanol. The *para*-nitrophenyl urea of **8** was prepared in a similar manner, and crystals for X-ray were obtained from acetonitrile. The crystallographic data for the *para*-nitrophenyl urea derivatives of **7** and **8** have been deposited with the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EW, UK, as supplementary publication nos CCDC 199820 and 199821, respectively.

10. (2*S*,3*S*)-3-Hydroxyleucine was prepared by using the literature procedure: Panek, J. S.; Masse, C. E. *J. Org. Chem.* **1998**, *63*, 2382.

11. Typical reaction conditions for formation of **18**, **19**, and **20** are as follows: **Preparation of 18a**: A mixture of **7** (230 mg, 0.319 mmol), **11a** (108 mg, 0.35 mmol), $NaBH(OAc)_3$ (135 mg, 0.64 mmol) and AcOH (3 drops) in anhydrous THF (5 mL) was stirred at room temperature under nitrogen for 4 h, and concentrated in vacuo. The residue was chromatographed (silica gel, 5% MeOH in CH_2Cl_2), to give **18a** (174 mg, 54%) as a pale-yellow solid. **Preparation of 19a**: A mixture of **18a** (146 mg, 0.144 mmol) was hydrogenated using 10% Pd/C (30 mg) in MeOH (3 mL) at room temperature under atomospheric pressure. The catalyst was removed by filtration and the filtrate was concentrated in vacuo, to give **19a** as a white amorphous solid (123 mg, 97%). **Preparation of 20a**: A solution of **17** (53 mg, 0.117 mmol), HOBT (16 mg, 0.117 mmol) and EDC (22 mg, 0.117 mmol) in anhydrous THF (3 mL) was stirred at room temperature for 30 min. To this mixture were added a solution of **19a** (98 mg, 0.111 mmol) in anhydrous THF (2 mL) and Hunig base (4 drops), and the resulting mixture was stirred at room temperature under nitrogen for 16 h. The reaction mixture was partitioned between EtOAc and H_2O. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed (satd brine), dried (Na_2SO_4), and concentrated in vacuo. The product was purified by chromatography (silica gel, 7% MeOH/CH_2Cl_2), to give **20a** as a white solid (84 mg, 58%).

12. Definition of R_1 and R_2 for **a**–**h** in compounds **18a**–**h**, **19a**–**h**, **20a**–**c**, **21a**, **22a**, and **23c**–**32c** is same as those in **11a**–**h** (Scheme 2).

13. NCCLS4. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standards: M7-A5, Vol. 20. National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2000.