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Muraymycins, Novel Peptidoglycan Biosynthesis Inhibitors: Semisynthesis and SAR of Their Derivatives

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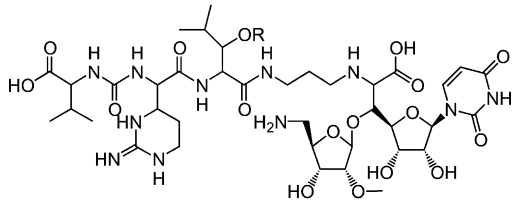
Abstract—Sixteen muraymycin derivatives **2**–**17** were synthesized based on selective reactions of the primary and secondary amino groups of muraymycin C1 (**1**) with isocyanates and aldehydes. Disubstituted derivatives **3**–**9** demonstrated no activity against either MraY or MurG at $\leq 100\,\mu g/mL$ whereas mono substituted derivatives **11**–**17** demonstrated good inhibitory activity, well correlated with the lipophilicity of the substituent introduced. In particular, the activity of derivatives **13** and **14** was comparable to that of muraymycin C1 in this assay.

Since peptidoglycan is an essential bacterial cell wall polymer, peptidoglycan biosynthesis provides a unique and selective target for antibiotic action.[1] MraY, which is located in the membrane, is one of the enzymes required for peptidoglycan biosynthesis. Therefore, inhibition of MraY will lead to loss of cell shape and integrity followed by bacterial death.[2,3] In previous publications,[4,5] muraymycins A1 and A3, which inhibited MraY, were reported. These new antibiotics demonstrated excellent activity against MraY, good in vitro activity against Gram-positive bacteria and good in vivo activity (ED_{50} ~1–2 mg/kg in mice) but moderate therapeutic index of ~4 as measured by the ratio of LD_{50} to ED_{50}. However, muraymycin C1 (**1**) with good enzyme inhibitory activity and no apparent toxicity did not demonstrate good antimicrobial activity. We speculated that the lipophilic 12-guanidino or 12-hydroxyguanidino lauroyl group at the hydroxyl group of the hydroxyleucyl moiety may be responsible for transporting the active structure to the target enzyme in the membrane, and that the 12-guanidino and 12-hydroxyguanidino moieties may also be responsible for the toxicity. Since muraymycin C1 was readily available,[4] we undertook the semisynthetic modification of muraymycin C1 in order to improve the activity and the therapeutic index. Instead of modifying the hydroxyl group of the muramycin C1 hydroxyleucyl moiety, we decided to introduce lipophilic groups onto the primary amino group of the amino ribose moiety and/or the secondary amino group at the 15 position of Muraymycin C1.[6] Here we report the synthesis and antimicrobial activities of urea, hydantoin and *N*-alkyl derivatives of muramycin C1.



Muraymycin A1: R = $COC_{11}H_{22}N(OH)C(NH_2)$=NH
Muraymycin A3: R = $COC_{11}H_{22}NHC(NH_2)$=NH
Muraymycin C1 (**1**): R = H

Muraymycin derivatives **2**–**7** were synthesized based on selective reactions of the primary and secondary amino groups of Muraymycin C1 (**1**) with isocyanates. Muraymycin C1 also reacted with aldehydes followed by treatment with either sodium cyanoborohydride in methanol or sodium triacetoxyborohydride[7] in DMF to give alkylated derivatives **8**–**9**. With one equivalent of isocyanates, or aldehydes, the major products were derived from the primary amino group. When the primary amino group was protected with 2,4-pentanedione, the products **11**–**17** from the secondary amino group were obtained. Thus, reaction of **1** (50 μmol) with

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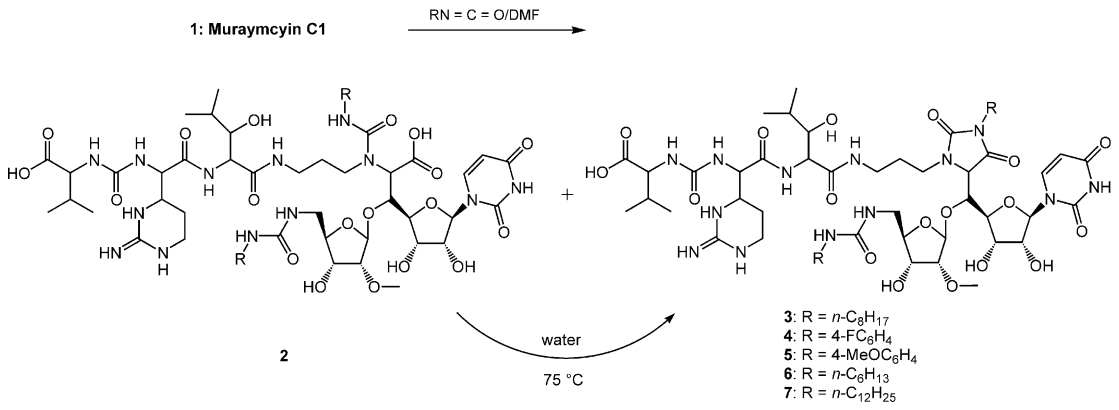
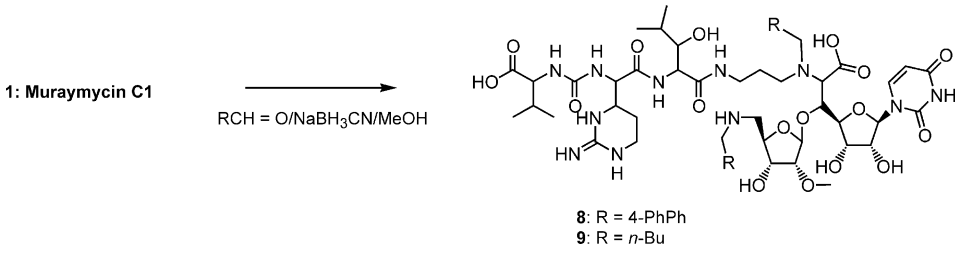
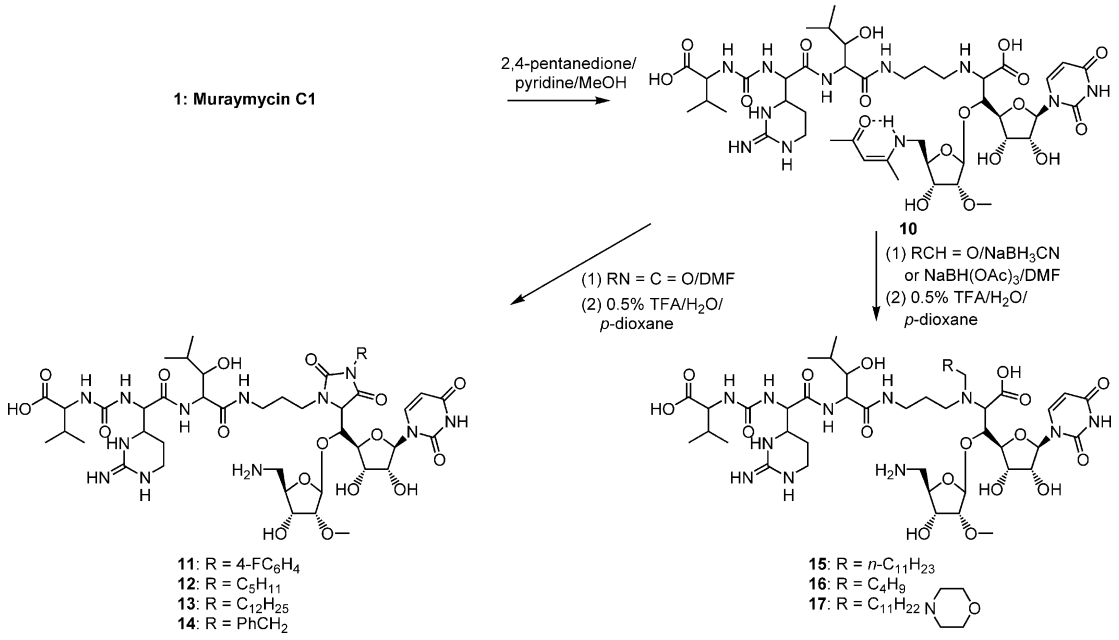


Scheme 1.



Scheme 2.



Scheme 3.

n-octylisocyanate (130 μmol) in 4.0 mL of DMF (rt, 20 h) gave a mixture of **2** (M_r = 1255.9) and **3** (M_r = 1237.9), which were isolated together by preparative HPLC.[8] After heating the mixture in 5 mL of water (70 °C, 45 min), 27 mg (44%) of **3** (M_r = 1237.9) was obtained (Scheme 1).[9] Similarly, derivatives **4**–**7** were synthesized. Reaction of **1** (21 μmol) with biphenylcarboxaldehyde (42 μmol) in 0.4 mL of methanol, followed by reduction with sodium cyanoborohydride (21 μmol) gave 4.0 mg (15%) of **8** as a white solid (Scheme 2). In the same manner, **9** was prepared. Reaction of **1** (50 μmol) with 2,4-pentanedione (200 μL) in 2.5 mL methanol containing pyridine (200 μL) (rt, overnight), followed by removal of the volatile materials under reduced pressure, gave 5-amino-pent-3-en-2-one derivative **10**. Reaction of **10** (50 μmol) with 4-fluorophenylisocyanate (95 μmol), followed by treatment with 0.5% TFA in a 1:1 mixture of water and *p*-dioxane (rt, 2 h) gave 24 mg (44% overall yield from **1**) of **11** as a colorless solid (Scheme 3).[10] Similarly, derivatives **12**–**14** were prepared. Reaction of **10** with aldedydes, followed by reduction with either sodium cyanoborohydride or

sodium triacetoxyborohydride[7] in DMF and deprotection with 0.5% TFA in a mixture of *p*-dioxane and water, gave mono alkylated derivatives **15**–**17** (Scheme 3). Derivatives **2**–**9** and **11**–**17** were isolated by preparative HPLC and were characterized by H^1 NMR and MS spectroscopic analyses.

The MurG biochemical assay utilized *Staphylococcus epidermides* membranes to catalyze the late steps in cell wall biosynthesis including MraY, the phospho-MurNAc pentapeptide translocase and MurG, the UDP-*N*-acetylglucosaminyl transferase. The following procedure was adapted from the method described by Mengin-Lecreaulx et al.[11] In this procedure, the formation of Lipid II was assessed using radiolabeled UDP-*N*-acetylglucosamine.

S. epidermides membranes (50 μg), compound, UDP-MurNAc pentapeptide (400 μM), and 3.2 μM nCi [^{14}C]-UDP-*N*-acetylglucosamine were incubated at room temperature for 30 min. The reaction was terminated by boiling in a water bath for 1 min. Samples (2 μL) of each reaction were analyzed by TLC. The samples were spotted onto K6 silica plates and chromatographed in Isobutyric Acid: 1 M NH_4OH (5:3). The plates were exposed to film and the inhibition of lipid II formation could be monitored by comparing the sample area to the control area.

Sixteen muraymycin derivatives **2**–**17**[12] were synthesized based on selective reactions of the primary and secondary amino groups of muraymycin C1 (**1**) with isocyanates and aldehydes. When the primary amino group of the amino ribose moiety was protected with 2,4-pentanedione, the product from the secondary amino group at the 15 position of Muraymycin C1 was obtained. As was evident from Table 1, disubstituted derivatives **3**–**9** all demonstrated no inhibitory activity at $\leq$100 μg/mL against either MraY or MurG. However, compounds **11**–**17** derived from the secondary amino group all demonstrated good inhibitory activity. Among hydantoin derivatives **11**–**14**, the activity was well correlated with the lipophilicity of the substituent introduced. In particular, derivatives **13** with $C_{12}H_{25}$ and **14** with $PhCH_2$ demonstrated good level of inhibition at 6.25 μg/mL which was comparable to that of muraymycin C1 (**1**) in the assay. The same observation could be applied to the tertiary amino derivatives **15**–**17**. Of these, derivative **15** with *n*-$C_{11}H_{23}$ demonstrated better level of inhibition at 50 μg/mL.

Table 1. The inhibitory activity of muraymycin derivatives against either MraY or MurG

Compd	100 μg/mL	50 μg/mL	25 μg/mL	6.25 μg/mL
1	+[a]	+	+	+
2	−	−	−	−
3	−	−	−	−
4	−	−	−	−
5	−	−	−	−
6	−	−	−	−
7	−	−	−	−
8	−	−	−	−
9	−	−	−	−
10	−	−	−	−
11	+	+	+	−
12	+	−	−	−
13	+	+	+	+
14	+	+	+	+
15	+	+	−	−
16	+	−	−	−
17	+	−	−	−

[a]'+' indicates reduced production of lipid II relative to the control (judged by intensity) on the film, thus indicating inhibition of either MraY or MurG. Since Muraymycins A1 and A3 inhibited the production of labeled lipid I and lipid II due to inhibition of MraY, its closely related derivatives might be considered inhibitors of MraY.[5]

Acknowledgements

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References and Notes

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6. The chemical name of derivative **9**: 16-[(*R*)-{(2*S*,3*S*,4*R*,5*R*)-5-[2,4-dioxo-3,4-dihydro-1(2*H*)-pyrimidinyl]-3,4-dihydroxytetrahydro-2-furanyl}({(3*R*,4*S*,5*R*)-4-hydroxy-3-methoxy-5-[(pentylamino)methyl]tetrahydro-2-furanyl}oxy)methyl]-9-(1-hydroxy-2-methylpropyl)-6-(2-iminohexahydro-4-pyrimidinyl)-2-isopropyl-4,7,10-trioxo-15-pentyl-3,5,8,11,15-pentaazaheptadecane-1,17-dioic acid.
7. Abdel-Magid, A. F.; Maryanoff, C. A.; Carson, K. G. *Tetrahedron Lett.* **1990**, *31*, 5595.
8. HPLC conditions: column: Prodigy ODS 4.6×150 mm mobile phase: gradient, A = 0.02% TFA/water; B = 0.02% TFA/acetonitrile flow rate: 1.0 mL/min detection: 215 nm, MSD retention time: 5.3 min.
9. Preparation of **3**: To a suspension of 47.3 mg (50 μmol) of **1** (λ_{max} nm in water = 259) in 4.0 mL of *N*,*N*-dimethylformamide was added 20.2 mg (130 μmol) of *n*-octyl isocyanate at room temperature. The reaction mixture was stirred for 20 h. The reaction was monitored by MS and checked by LC/MS. The reaction mixture was concentrated to about 1 mL, and the fractions with molecular weights of 1237.9 and 1255.9 were isolated together by preparative HPLC using Prodigy ODS column. After heating the mixture in 5 mL of water at 70 °C for 45 min, 27 mg (43.6%) of **3** (M_r = 1237.9) was obtained. Molecular formula: $C_{56}H_{95}N_{13}O_{18}$; M_r: positive ion electrospray MS m/z = 1238.85 $(M+H)^+$ and 619.86 $(M+2H)^{2+}$; ultraviolet absorption spectrum: λ_{max} nm (water) = 262.
10. Preparation of **11**: To a solution of 47.3 mg (50 μmol) of **1** (λ_{max} nm in water = 259) in 2.5 mL of methanol was added 200 μL of pyridine and 200 μL of 2,4-pentanedione at room

temperature. The reaction mixture was stirred overnight [the reaction was monitored by mass spectroscopy (MS)]. After the reaction was complete, the volatile materials were removed under reduced pressure. *N*,*N*-dimethylformamide (DMF) (3 mL) was added to the residue, followed by addition of 13.0 mg (95 μmol) of 4-fluorophenyl isocyanate. The reaction mixture was stirred at room temperature for 50 h (the reaction was monitored by MS). The volatile materials were removed under reduced pressure to afford a residue to which was added 5 mL of 0.5% trifluoroacetic acid (TFA) in a 1:1 mixture of water and *p*-dioxane. The mixture was stirred at room temperature for 2 h. The reaction was monitored by MS and the desired product is identified by liquid chromatography/mass spectrum (LC/MS). After concentration of volatiles to about one mL, 24 mg (44% yield) of **11** was obtained as a colorless solid using preparative HPLC (Prodigy ODS column). Molecular formula: $C_{45}H_{65}FN_{12}O_{17}$; M_r: positive ion electrospray MS $m/z = 1065.2$ $(M+H)^+$ and 533.2 $(M+2H)^2$; ultraviolet absorption spectrum: λ_{max} nm (water) $= 261$.

11. Mengin-Lecreaulx, D.; Texier, L.; Rousseau, M.; van Heijenoort, J. *J. Bacteriol*. **1991**, *173*, 4625.

12. The content of this manuscript came from the poster, paper #: 1160 presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy Chicago, IL. 16–19 December 2001.