Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1451–1454

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibitors of the Bacterial Cell Wall Biosynthesis Enzyme MurC

Folkert Reck,[a,*] Stephen Marmor,[b] Stewart Fisher[b] and Mark A. Wuonola[a]

[a]*Chemistry, Infection Discovery, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA*
[b]*Biochemistry, Infection Discovery, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA*

Received 12 March 2001; accepted 6 April 2001

Abstract—A series of phosphinate transition-state analogues of the L-alanine adding enzyme (MurC) of bacterial peptidoglycan biosynthesis was prepared and tested as inhibitors of the *Escherichia coli* enzyme. Compound **4** was identified as a potent inhibitor of MurC from *Escherichia coli* with an IC_{50} of 49 nM.

Rapidly developing bacterial resistance to existing drugs has become the major problem in antibacterial therapy and necessitates continuous research for novel antibacterials. Especially, agents directed against novel bacterial targets are expected to have the potential for low initial bacterial resistance. The biosynthesis of peptidoglycan as a target in bacterial cell wall synthesis is validated by several important classes of antibiotics. One key enzyme, the ligase MurC, has been shown to be essential in *Escherichia coli*.[1] MurC catalyzes the conversion of UDP-MurNAc to UDP-MurNAc-Ala in the assembly of the disaccharide-peptide unit required for peptidoglycan biosynthesis (Fig. 1).

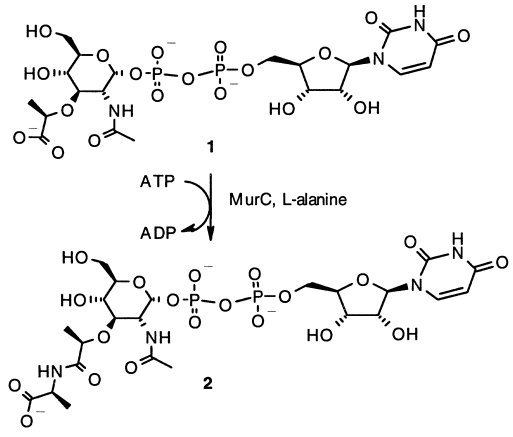


Figure 1. The reaction catalyzed by MurC.

We were interested in the design and synthesis of transition state analogues of MurC. The reaction pathway catalyzed by MurC involves the acyl phosphate intermediate **3**[2,3] (Fig. 2).

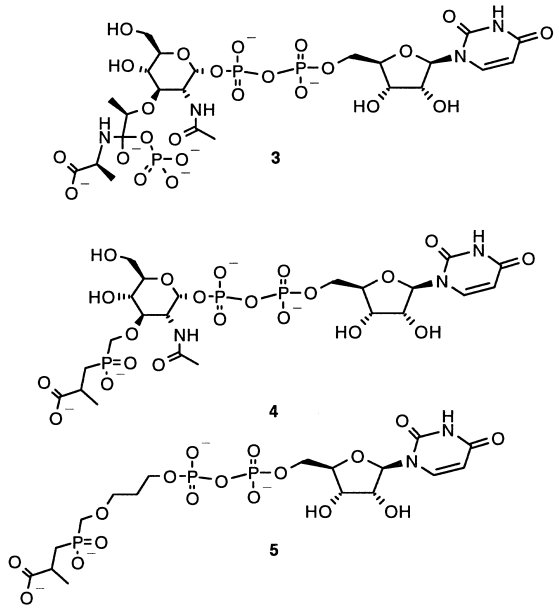


Figure 2. MurC, acyl phosphate intermediate **3** and transition state analogues **4** and **5**.

For several ligases that involve acyl phosphate intermediates, such as MurD,[4,5] MurE,[6] and D-Ala-D-Ala ligase,[7] potent phosphinate inhibitors have been developed. Phosphinates mimic the tetrahedral reaction center of the transition state. In the case of D-Ala-D-Ala-ligase, phosphinates have been shown to undergo phos-

*Corresponding author. Fax: +1-781-839-4500; e-mail: folkert.reck@astrazeneca.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00251-7

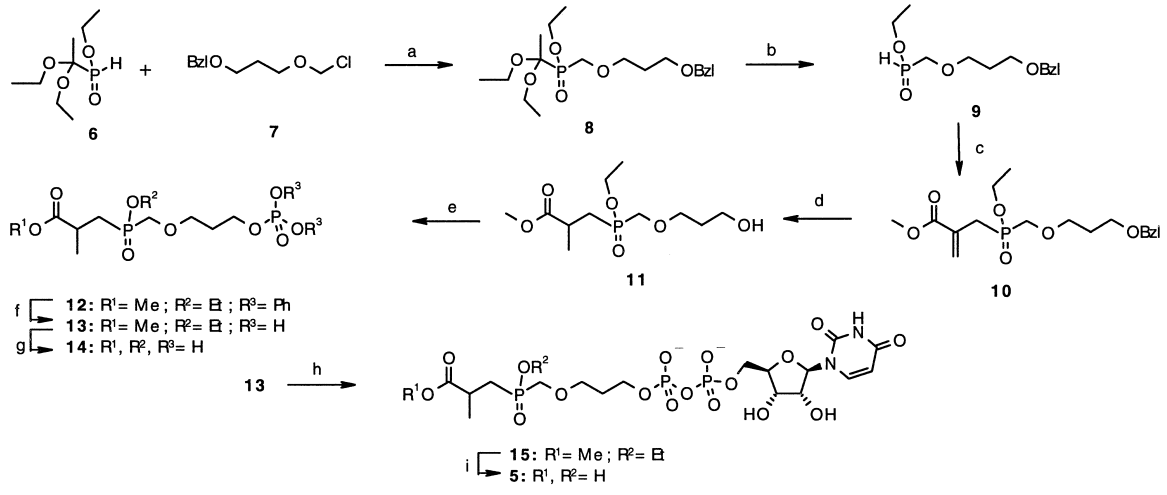
phorylation in the active site by the co-substrate ATP. Therefore, we expected that phosphinate analogues of the product of the MurC reaction, such as compounds **4** and **5** might produce tight binding inhibitors of MurC. In the design of **4** we did not include the methyl group of the lactic acid moiety, and chose not to define the stereochemistry at the L-alanyl-derived moiety, in order to simplify the synthesis considerably. For additional simplification, in **5** the hexose is substituted by a 1,3-propanediol spacer.

Here, we report on the synthesis and biological evaluation of compounds **4** and **5** along with compounds derived from their synthetic precursors.

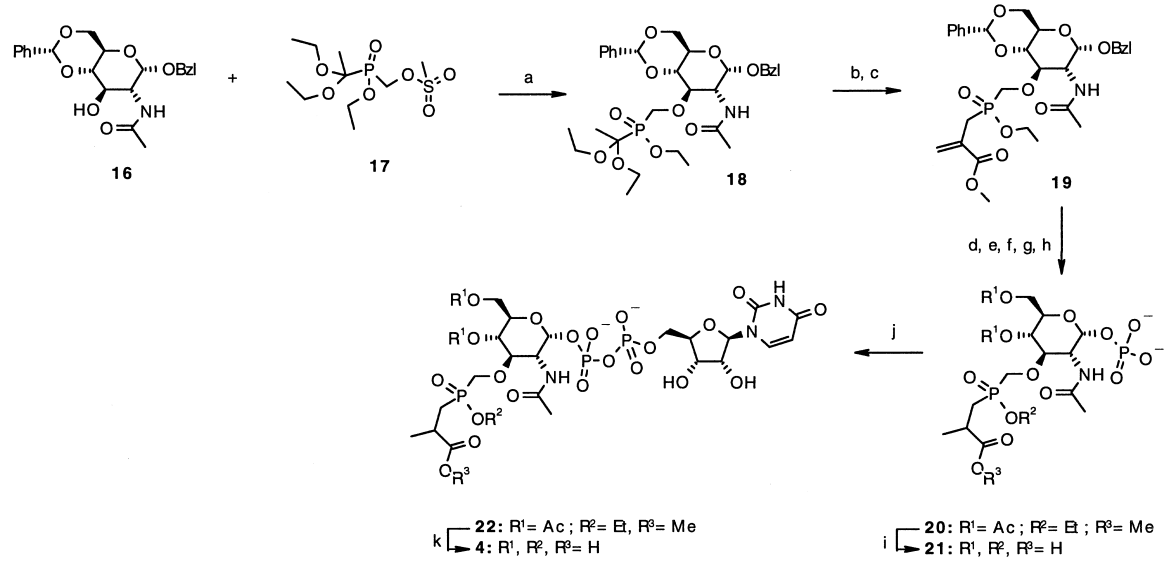
Compounds **4** and **5** include an ether linkage in α-position to the phosphinate moiety, a structural feature that is difficult to establish, and has little literature precedent for complex molecules. Other known phosphinate transition state analogues of ligases have at this position a nitrogen instead of an oxygen. Formation of this ether linkage was perceived as the major challenge in the synthesis of **4** and **5**.

Synthesis of **5** is outlined in Scheme 1. The starting material **6** was prepared from phosphinic acid according to Baylis.[8] Compound **6** has the advantage that it allows selective deprotection under mild conditions for stepwise assembly of complex phosphinates. The chloromethyl ether **7** was obtained in quantitative yield by treating commercially available monobenzylated 1,3-propanediol with formaldehyde/HCl. Arbuzov reaction of **6** and **7** proceeded to **8** under mild silylating conditions[9] in 29% yield. Side reactions arose from partial loss of the ketal and ester protection groups on the phosphinate. An alternative route via alkylation of the corresponding primary alcohol with mesylate **17** was explored, but proved to be less compatible with the protecting groups on the phosphinic acid moiety. Selective removal of the ketal protecting group in **8** was achieved under mild conditions with $CHCl_3$/TMSCl[8] to give **9** in 97% yield. Arbuzov reaction with methyl bromomethyl acrylate under silylating conditions[10] afforded **10** in 83% yield. Concomitant hydrogenation of the double bond and benzylether in **10** proceeded smoothly to the alcohol **11** which was phosphorylated with diphenyl chlorophosphate/DMAP to give **12** in 94% yield. Deprotection of **12** by hydrogenation over PtO_2 to **13**, followed by saponification gave phosphate **14** in nearly quantitative yield. Coupling of **13** with UMP-morpholidate/tetrazole in pyridine afforded the UDP-containing derivative **15** in 27% yield. Finally, saponification of **15** gave the diastereomeric mixture of **5** in 88% yield.

For the synthesis of **4** (Scheme 2) the major problem was again formation of the ether linkage alpha to the phosphinate. Commercially available **16** was chosen as a suitable and convenient starting material. Alkylation of **16** with 1.6 equiv of mesylate **17** (the latter was prepared from the corresponding alcohol[11] with mesylchloride/DIPA in 73% yield) and sodium hydride as base gave **18** as a mixture of two diastereomers in 10% yield. The low yield is the result of decomposition of the mesylate under reaction conditions; most of **16** could be recovered during workup. Removal of the ketal protecting group in **18** was effected with $CHCl_3$/TMSCl. In our experience, deprotection of the ketal of the Baylis protecting group requires at least a trace of water, and as a consequence the benzylidene protecting group in **18** was partially intermittently lost during ketal removal in **18**. However, after ketal removal the benzylidene protection could be reestablished in good yield by azeotropic distillation with toluene. The resulting crude intermediate was then subjected to Arbuzov reaction with methyl bromomethyl acrylate under silylating conditions[10] to afford **19** in 49% overall yield. In **19**, saturation of the double bond and removal of the benzylidene and benzyl groups was effected by hydrogenation over Pd/C. The crude product was then peracetylated with acetic anhydride/pyridine. Treatment with aminoethanol in THF selectively removed the anomeric acetate to give the free anomeric intermediate



Scheme 1. Reagents: (a) 2,6-lutidine (4 equiv), BSA (1 equiv)/CH_3CN, 90 °C, 1 h, 29%; (b) TMSCl (1.1 equiv), H_2O (0.5 equiv)/$CHCl_3$, rt, 5 h, 97%; (c) TEA (2.2 equiv), TMSCl (2 equiv), then methyl (2-bromomethyl) acrylate (1.1 equiv), 0 °C–rt, 2 h, 83%; (d) Pd/C, H_2/MeOH, 15 min, 96%; (e) $PClO(OPh)_2$ (1.4 equiv), DMAP (1.6 equiv)/CH_2Cl_2, 0 °C, 15 min, 94%; (f) PtO_2, H_2/MeOH/AcOH, 2 h, 99%; (g) LiOH (2.3 M, aq), rt, 2 h, 99%; (h) UMP-morpholidate (2 equiv), 1*H*-tetrazole (4 equiv)/pyridine, rt, 27%; (i) LiOH (2.3 M, aq), rt, 2 h, 88%.



Scheme 2. Reagents: (a) NaH/DMF, 0 °C–rt, 18 h, 10%; (b) TMSCl (1.1 equiv), H_2O (0.5 equiv)/$CHCl_3$, rt, 1 h; (c) TMSCl (4 equiv), TEA (4.5 equiv), 0 °C, 30 min, then methyl (2-bromomethyl) acrylate (1.2 equiv), 5 min, 49% (b-c); (d) Pd/C, H_2/MeOH, HOAc (10:1), rt, 1 day; (e) Ac_2O/pyridine, rt, 16 h; (f) aminoethanol (2 equiv)/THF, rt, 4 h, 50% (d-f); (g) *n*-BuLi (1 equiv)/THF, −78 °C, 2 min, then $PClO(OPh)_2$ (1.3 equiv), 1 h; (h) PtO_2, H_2/THF, −78 °C–rt, 16 h, 96% (g-h); (i) NaOH (0.5 M, aq), rt, 1.5 h, 80%; (j) **20**, UMP-morpholidate (2.5 equiv)/pyridine, rt, 9 days, 37%; (k) NaOH (0.5 M, aq), 1.5 h, 79%.

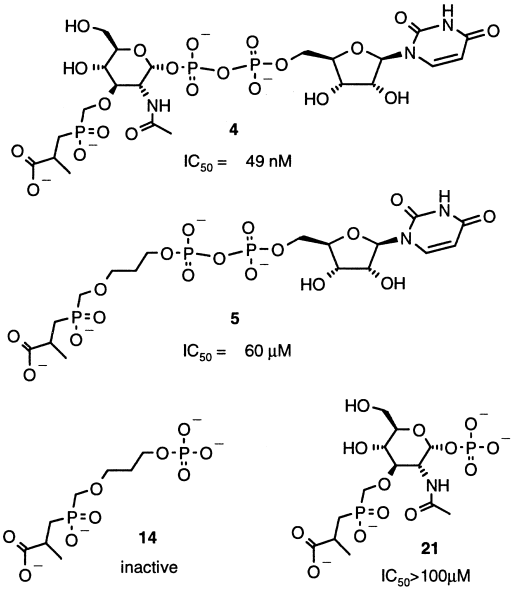


Figure 3. Inhibition of MurC from *E. coli* by compounds **4**, **5**, **14**, and **21**.

as a complex diastereomeric mixture (eight theoretical diastereomers) in 50% yield. Stereoselective formation of the α-phosphate was achieved according to the protocol by Inage[12] with butyllithium and diphenylchlorophosphate and after deprotection of the unstable intermediate by hydrogenation over PtO_2 the unprotected phosphate **20** was isolated as a mixture of two major diastereomers in 96% yield. Saponification of **20** afforded the unprotected anomeric phosphate **21** as a 1:1 diastereomeric mixture in 80% yield. Coupling of **20** with UMP-morpholidate in pyridine proceeded in 37% yield to **22**. Finally, saponification of **22** gave **4** in 79% yield as a 1:1 mixture of the two diastereomers.

Compounds **4**, **5**, **14**, and **21** were tested for inhibition of MurC from *E. coli* using 37 nM enzyme, 200 µM ATP, 120 µM L-alanine and 75 µM UDP-MurNAc. The readout was detection of the product phosphate by the malachite green assay.[13] Results are summarized in Figure 3. The phosphinate **4** exhibited an IC_{50} of 49 nM and is thus the most potent inhibitor of MurC reported. The results indicate that substitution of the des-methylmuramic acid in **4** with a flexible 1,3-propanediol spacer decreases potency by over three orders of magnitude. Also, the UDP moiety is necessary for potent inhibition, since the terminal phosphates **14** and **21** are inactive.

References and Notes

1. Bocquel, M. T.; Fairley, M.; Mengin-Lecreulx, D.; Parquet, C.; Realo, E.; Salah Bey, K.; Taburet, Y.; Harnois, M.; van Heijenoort, J. 97th General Meeting American Society for Mircobiology. Abs. *Genetics and Molecular Biology* **1997**, *H-2*, 285.
2. Falk, P. J.; Ervin, K. M.; Volk, K. S.; Ho, H. T. *Biochemistry* **1996**, *35*, 1417.
3. Bouhss, A.; Dementin, S.; van Heijenoort, J.; Parquet, C.; Blanot, D. *FEBS Lett*. **1999**, *453*, 15.
4. Tanner, M. E.; Vaganay, S.; Vanheijenoort, J.; Blanot, D. *J. Org. Chem*. **1996**, *61*, 1756.
5. Gegnas, L. D.; Waddell, S. T.; Chabin, R. M.; Reddy, S.; Wong, K. K. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1643.
6. Zeng, B.; Wong, K. K.; Pompliano, D. L.; Reddy, S.; Tanner, M. E. *J. Org. Chem*. **1998**, *63*, 10081.
7. Parsons, W. H.; Patchett, A. A.; Bull, H. G.; Schoen, W. R.; Taub, D.; Davidson, J.; Combs, P. L.; Springer, J. P.; Gade-

busch, H.; Weissberger, B.; Valiant, M. E.; Mellin, T. N.; Busch, R. D. *J. Med. Chem*. **1988**, *31*, 1772.
8. Baylis, E. K. *Tetrahedron Lett*. **1995**, *36*, 9385.
9. Thottathil, J. K.; Przybyla, C. A.; Moniot, J. L. *Tetrahedron Lett*. **1984**, *25*, 4737.
10. Thottathil, J. K.; Ryono, D. E.; Przybyla, C. A.; Moniot, J. L.; Neubeck, R. *Tetrahedron Lett*. **1984**, *25*, 4741.
11. Froestl, W.; Mickel, S. J.; Hall, R. G.; von Sprecher, G.; Strub, D.; Baumann, P. A.; Brugger, F.; Gentsch, C.; Jaekel, J.; Olpe, H.-R.; Rihs, G.; Vassout, A.; Waldmeier, P. C.; Bittiger, H. *J. Med. Chem*. **1995**, *38*, 3297.
12. Inage, M.; Chaki, H.; Kusumoto, S.; Shiba, T. *Chem. Lett*. **1982**, 1281.
13. Lanzetta, P. A.; Alvarez, L. J.; Reinach, P. S.; Candia, O. A. *Anal. Biochem*. **1979**, *100*, 95.