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Stereoselective Syntheses of 4-Oxa Diaminopimelic Acid and Its Protected Derivatives via Aziridine Ring Opening

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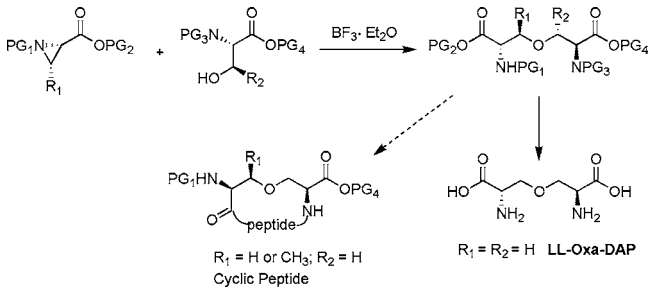
ABSTRACT



Regio- and stereoselective aziridine ring opening with oxygen nucleophiles derived from serine and threonine provides a route to stereochemically pure 4-oxa-2,6-diaminopimelic acid (oxa-DAP) and its methyl-substituted derivatives. Oxa-DAP is a substrate of DAP epimerase, a key enzyme for biosynthesis of L-lysine and formation of peptidoglycan precursors. Orthogonally protected analogues of lanthionine and β-methyllanthionine wherein oxygen replaces sulfur were prepared that could be used for solid-supported peptide synthesis to make oxa derivatives of lantibiotics.

Bacterial enzymes on the biosynthetic route to L-lysine also generate key precursors for the peptidoglycan cell wall layer.[1] *meso*-Diaminopimelic acid (*meso*-DAP) (**1b**) occurs in the peptidoglycan of virtually all Gram-negative bacteria, whereas its metabolic product, L-lysine, is used analogously in many Gram-positive organisms.[2] The enzymes on the pathway provide an opportunity for development of new antibiotics with low mammalian toxicity because mammals do not make DAP and require L-lysine in their diet.[1] One of them, DAP epimerase, catalyzes the reversible conversion of LL-DAP (**1a**) to *meso*-DAP (**1b**). Its unusual mechanism does not rely on metals or cofactors but rather utilizes two cysteine residues as a thiol−thiolate pair to deprotonate the α-carbon of the substrate and reprotonate from the opposite side (Figure 1).[2c,d] DAP epimerase exhibits very strict substrate specificity. *meso*-Lanthionine (**3a**), which has a sulfur atom instead of

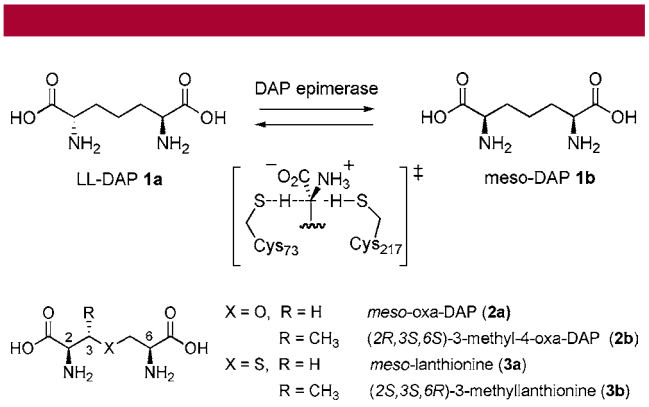


Figure 1. Interconversion of LL-DAP and *meso*-DAP by DAP epimerase, and structures of oxa-DAP, lanthionine, and β-methyllanthionine.

(1) For recent reviews, see: (a) Vederas, J. C. *Can. J. Chem.* **2006**, *84*, 1197−1207 and references cited therein. (b) Cox, R. J.; Sutherland, A.; Vederas, J. C. *Bioorg. Med. Chem.* **2000**, *8*, 843−872.

(2) (a) van Heijenoort, J. *Nat. Prod. Rep.* **2001**, *18*, 503−519. (b) Wiseman, J. S.; Nichols, J. S. *J. Biol. Chem.* **1984**, *259*, 8907−8914. (c) Diaper, C. M.; Sutherland, A.; Pillai, B.; James, M. N. G.; Semchuk, P.; Blanchard, J. S.; Vederas, J. C. *Org. Biomol. Chem.* **2005**, *3*, 4402−4411. (d) Pillai, B.; Cherney, M. M.; Diaper, C. M.; Sutherland, A.; Blanchard, J. S.; Vederas, J. C.; James, M. N. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8668−8673.

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a CH_2 group at the 4-position, is not accepted by the enzyme.[3] Significant differences in bond lengths and bond angles apparently cannot be accommodated in the very tight active site of this epimerase. However, it seemed that replacement of the CH_2 at the 4-position in DAP with an oxygen atom could generate a substrate or inhibitor as the predicted bond lengths (1.43 vs 1.54 Å) and bond angles (109°28′ vs 111°43′) of C−C−C and C−O−C bonds are more comparable.[4] In this context, we decided to develop a stereoselective method for the synthesis of ether-bridged bisamino acids as there were no general methods for the synthesis of such compounds.[5] Ideally, development of such methodology would give access not only to oxa-DAP but also to orthogonally protected analogues. The latter could be used in peptide synthesis to mimic lanthionine and β-methyllanthionine (Figure 1, **3a** and **3b**) in lantibiotics, such as nisin and lacticin 3147.[6] These potent antimicrobial agents are effective at nanomolar concentrations against a range of Gram-positive bacteria, including organisms resistant to conventional antibiotics.

Our synthetic strategy involves ring opening of serine- and threonine-derived aziridines bearing an electron-withdrawing group on the nitrogen by the hydroxyl side chain of suitably protected amino acids in the presence of a Lewis acid catalyst (Figure 2).

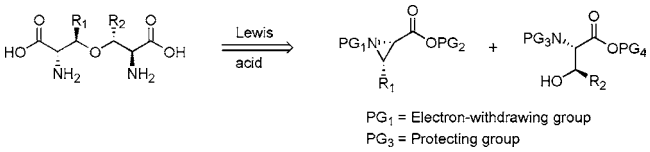


Figure 2. Aziridine ring opening with oxygen nucleophiles.

The chemistry of aziridine ring opening with stoichiometric quantities of a variety of nucleophiles in the presence of Lewis acids is well-documented in the literature.[7] However, due to poor nucleophilicity of the hydroxyl group, examples[8] of ring openings with oxygen nucleophiles are usually limited to solvolysis reactions in the corresponding alcohol as solvent. In order to use serine and threonine as nucleophiles in lower concentration, the reactivity of the hydroxyl needs to be enhanced. This could possibly be accomplished via disruption of the hydrogen bond between the oxygen lone pair and hydrogen of monoprotected neighboring amine.[9] Bisprotection of the amino group could lead to favorable reversal of hydrogen bonding to increase the electron density on the oxygen, thereby improving its nucleophilicity (Figure 3). The phthalimido (Pht) group was chosen for diprotection

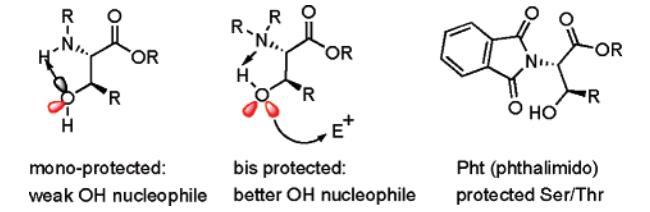
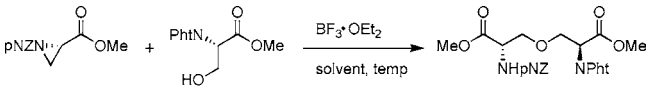


Figure 3. H-Bonding patterns and effects on nucleophilicity of hydroxyls of protected serine or threonine derivatives.

of the amino group because of the ease of preparation and removal.[10] To activate the aziridine for regioselective ring opening at the β-position, the electron-withdrawing *p*-nitrobenzyloxycarbonyl group (pNZ) was selected for nitrogen protection.[8a]

The ring opening of pNZ-aziridinocarboxylate ester **4a** with Pht-Ser-OMe **5a** (5.0 equiv) and $BF_3{\cdot}OEt_2$ (0.1 equiv) affords the desired product **6a**. Although the reaction is sluggish and requires 2 days for completion, the yield (56%) is comparable to those using oxygen nucleophiles as solvent (Table 1, entry 1).[8] Encouraged by this positive result, the reaction was examined with respect to the solvent, temperature, and reagent stoichiometries (Table 1). Although

Table 1. Optimization of Aziridine Ring Opening Conditions



4a **5a** **6a**

entry	nu/E^+/$BF_3{\cdot}OEt_2$	solvent	temp	time	% yield
1	5/1/0.1	$CHCl_3$	rt	2 days	57
2	2/1/0.2	$CHCl_3$	rt	4 days	60
3	2/1/0.2	toluene	110 °C	5 h	70
4	2/1/0.5	toluene	110 °C	1.5 h	72

(3) Lam, L. K. P.; Arnold, L. D.; Kalantar, T. H.; Kelland, J. G.; Lane-Bell, P. M.; Palcic, M. M.; Pickard, M. P.; Vederas, J. C. *J. Biol. Chem.* **1988**, *263*, 11814−11819.

(4) (a) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans.* **1987**, *2*, S1−S19. (b) Blukis, V.; Kasai, P. H.; Myers, R. J. *J. Chem. Phys.* **1963**, *38*, 2753−2760.

(5) For synthesis of a mixture of oxa-DAP isomers, see: Zahn, H.; Dietrich, R.; Gerstner, W. *Chem. Ber.* **1955**, *88*, 1734−1746.

(6) For selected reviews, see: (a) Chatterjee, C.; Paul, M.; Xie, L.; van der Donk, W. A. *Chem. Rev.* **2005**, *105*, 633−684. (b) McAuliffe, O.; Ross, R. P.; Hill, C. *FEMS Microbiol. Rev.* **2001**, *25*, 285−308. (c) Sahl, H. G.; Bierbaum, G. *Annu. Rev. Microbiol.* **1998**, *52*, 41−79.

(7) For reviews, see: (a) Hu, X. E. *Tetrahedron* **2004**, *60*, 2701−2743 and references cited therein. (b) Tanner, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 599−619. (c) Zhou, P.; Chen, B.; Davis, F. A. *Aziridines and Epoxides in Organic Synthesis*; Yudin, A. K., Ed.; Wiley-VCH: Weinheim, Germany, 2006; p 89.

(8) (a) McKeever, B.; Pattenden, G. *Tetrahedron* **2003**, *59*, 2071−2712. (b) Nakajima, K.; Neya, M.; Yamada, S.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3049−3050. (c) BhanuPrasad, B. A.; Sekar, G.; Singh, V. K. *Tetrahedron Lett.* **2000**, *41*, 4677−4679. (d) Ho, M.; Wang, M.; Pham, D. T. *Tetrahedron Lett.* **1991**, *32*, 1283−1286.

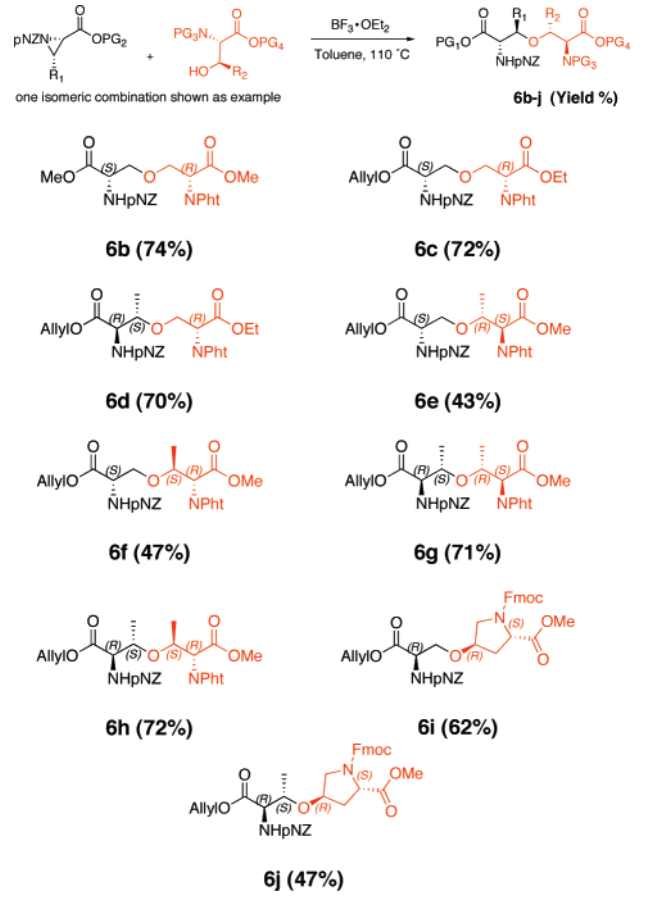
(9) Enhanced nucleophilicity of the hydroxyl groups via intramolecular H-bonding has been offered as an explanation for glycosidation of serine and threonine. (a) Szabo, L.; Li, Y.; Polt, R. *Tetrahedron Lett.* **1991**, *5*, 585−588. (b) Polt, R.; Jennifer, T. L.; Li, Y.; Hurby, V. *J. Am. Chem. Soc.* **1992**, *114*, 10249−10258.

(10) Bose, A. K. *Organic Syntheses*; Wiley & Sons: New York, 1973; Collect. Vol. V, pp 973−974.

reaction yields are not improved dramatically, optimization shows that using 0.5 equiv of $BF_3{\cdot}OEt_2$ catalyst in refluxing toluene (Table 1, entry 4) shortens the reaction time significantly from 4 days to 1.5 h and affords the best yield.[11]

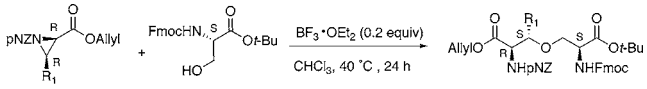
The scope of the reaction could be explored using several primary and secondary hydroxyl side chain containing amino acids as the nucleophiles with pNZ-protected, β-substituted, and β-unsubstituted aziridines. In all cases, the reaction proceeds smoothly with the optimized conditions to give a single isomer of the oxygen-bridged bisamino acids in moderate to good yields (Scheme 1).[12]

Scheme 1. Preparation of Bisamino Acid Ether Derivatives



Interestingly, the *sec*-*sec* dimethyl ethers **6g** and **6h** could be formed stereochemically pure and in good yields. Reactions leading to *sec*-*sec* ethers are often reported to undergo stereocenter scrambling.[13] Ring opening is also successful with a sterically demanding cyclic secondary alcohol as the nucleophile to afford **6i** and **6j** in good yields. As derivatives **6b** and **6f** are protected oxygen mimics of lanthionine and β-methyllanthionine isomers found in lantibiotics,[6] it seemed desirable to investigate orthogonal protecting groups that are suitable for Fmoc solid phase peptide synthesis. These could then be used for the preparation of analogues of lantibiotics.[14] Hence, the reaction was examined using pNZ[15] and allyl protection for the aziridine component and with Fmoc and *t*-butyl protecting groups on the serine nucleophile. All four protecting groups can be manipulated selectively on solid support. However, all attempts to use Fmoc-Ser-O*t*Bu as nucleophile under the optimized conditions (toluene, 110 °C) led to a complex mixture of products, presumably due to the instability of the *t*-Bu group. Fortunately, milder conditions using 0.2 equiv of $BF_3{\cdot}OEt_2$ in ethanol-free $CHCl_3$ at 40 °C could be employed. Using these conditions, the orthogonally protected oxygen analogues **7b** and **7c** of lanthionine and β-methyllanthionine, respectively, can be obtained in modest yields. These compounds are suitable for direct incorporation onto solid support after removal of *t*-Bu group (Table 2). The lower yields can be attributed to the

Table 2. Synthesis of Orthogonally Protected Oxygen Analogs of Lanthionine and β-Methyllanthionine



entry	R_1	product	% yield
1	H	(2*S*,6*S*)-**7a**	36
2	H	(2*R*,6*S*)-**7b**	37
3	CH_3	(2*R*,3*S*,6*S*)-**7c**	40

decreased nucleophilicity of the oxygen lone pairs resulting from unfavorable H-bonding as described in Figure 3, as well as decreased temperature. Nonetheless, the yields in these cases are still preparatively useful.

In order to verify the stereochemical integrity of the aziridine ring opening, ^{13}C NMR spectra of a mixture of stereochemically pure **7a** and **7b** were examined. Appearance of individual peaks for each diastereomer at different parts per million values (Figure 4) clearly illustrate the stereochemical integrity of the ring opening.

Having successfully obtained protected LL- and *meso*-oxa-DAP derivatives **6a** and **6b**, we attempted global removal of the protecting groups using 6 N HCl. Unfortunately, this provides only partially deprotected product with the pNZ group still attached, probably due to the acidic stability of pNZ.[16] Simple hydrogenolytic deprotection of the remaining

(11) A side product was isolated in 6% yield resulting from the attack of OH on the pNZ to form a benzyl ether. For such a side reaction, see: Osterkamp, F.; Wehlan, H.; Koert, U.; Wiesner, M.; Raddatz, P.; Goodman, S. *Tetrahedron* **1999**, *55*, 10713−10734.

(12) Intramolecular aziridine rearrangement with formation of oxazoline (5%) was observed during the synthesis of **6e**. For rearrangement of acylaziridines to oxazolines using Lewis acids, see: (a) Ferraris, D.; Druey, W. J., III; Cox, C.; Lectka, T. *J. Org. Chem.* **1998**, *63*, 4568−4569. (b) Tomasini, C.; Vecchione, A. *Org. Lett.* **1999**, *1*, 2153−2156.

(13) Kuethe, J. T.; Marcoux, J. F.; Wong, A.; Wu, J.; Hillier, M. C.; Dormer, P. G.; Davies, I. W.; Hughes, D. L. *J. Org. Chem.* **2006**, *71*, 7378−7390.

(14) Pattabiraman, V. R.; Stymiest, J. L.; Derksen, D. J.; Martin, N. I.; Vederas, J. C. *Org. Lett.* **2007**, *9*, 699−702.

(15) Isidro-Llobet, A.; Guasch-Camell, J.; Alvarez, M.; Albericio, F. *Eur. J. Org. Chem.* **2005**, *14*, 3031−3039.

(16) Shields, J. E.; Carpenter, F. H. *J. Am. Chem. Soc.* **1961**, *83*, 3066−3070.

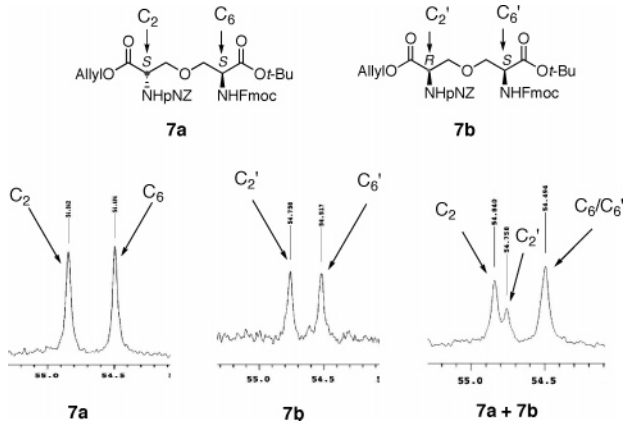
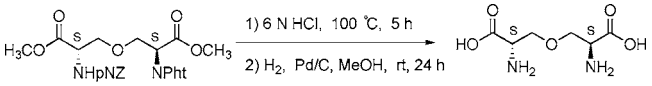


Figure 4. Portions of the 125 MHz ^{13}C NMR spectra of diastereomers **7a**, **7b**, and a mixture of **7a** and **7b** at 27 °C in $CDCl_3$.

pNZ gives the completely deprotected LL- (**8a**) and *meso*-oxa-DAP (**2a**) isomers required for evaluation with DAP epimerase (Scheme 2).

Scheme 2. Deprotection to Obtain LL-Oxa-DAP



6a
6b 2*S*, 6*R* - isomer

8a (38%)[a]
2a 2*S*, 6*R* - isomer (41%)

[a] Isolated yields over two steps after cationic ion exchange column.

The enzymatic studies with LL-oxa-DAP (**8a**) employed DAP epimerase from *Haemophilus influenzae*.[1,2c,d] The enzymatic reactions were done in deuterated solvents in order to monitor the exchange of α-hydrogen with a deuterium by the epimerase using NMR and mass spectrometry.[17] Incubation of LL-oxa-DAP (**8a**) with DAP epimerase in deuterated Tris buffer results in change of the integral for the α-hydrogen in the ^1H NMR spectrum due to incorporation of deuterium at the α-position. This demonstrates that DAP epimerase accepts LL-oxa-DAP (**8a**) as a substrate. Initial velocity for the enzymatic reaction could be obtained by monitoring the change in integral values for the α-proton of **8a** and **1a** in separate reactions as a function of time. LL-Oxa-DAP (**8a**) exhibits an initial velocity that is 26% of that of the natural substrate LL-DAP (**1a**) (Figure 5). Although

(17) (a) Campbell, I. D. *Fresenius Z. Anal. Chem.* **1986**, *324*, 437−441. (b) Petersen, B. O.; Krah, M.; Duss, J.; Thomsen, K. K. *Eur. J. Biochem.* **2000**, *267*, 361−369.

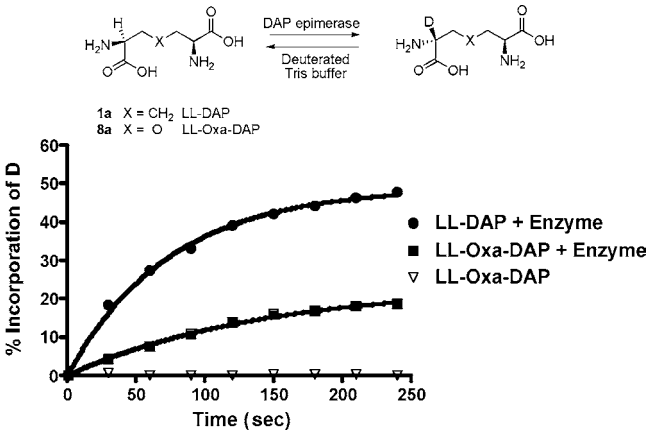


Figure 5. Time course of epimerization of LL-DAP (**1a**) and LL-oxa-DAP (**8a**) catalyzed by DAP epimerase. As both *meso*- and LL-isomers are substrates/products and there is an isotope effect for removal of α-hydrogen, the conversion to deuterated product slows as the reaction proceeds.

DAP epimerase accommodates the substitution of oxygen atom for a methylene group at the 4-position in its natural substrate, the presence of a polar oxygen atom appears to disrupt the tight hydrophobic interactions in the closed enzyme active site.[2d]

In conclusion, a facile, regio- and stereoselective procedure to prepare ether-linked bisamino acids via aziridine ring opening with oxygen nucleophiles has been developed. LL-Oxa-DAP (**8a**), obtained via the new methodology, was identified as an effective substrate for DAP epimerase, in contrast to lanthionine (the 4-thia analogue of DAP). Furthermore, analogues of lanthionine and *β*-methyllanthionine with orthogonal protecting groups amenable for solid phase peptide synthesis have been prepared which could be readily incorporated as building blocks into cyclic peptides and lantibiotics.

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Supporting Information Available: Experimental procedures and spectral data for all compounds synthesized and DAP epimerase assay. This material is available free of charge via the Internet http://pubs.acs.org.

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