

## Previews

### Nature's Protection Racket

(2S)-4-amino-2-hydroxybutyrate (AHBA) is a side chain that is important for the antibiotic activities of aminoglycosides. The elucidation of the biosynthetic pathway to AHBA, by Spencer et al. in this issue of *Chemistry & Biology* [1], reveals several surprises and will facilitate biosynthetic engineering of new improved aminoglycoside antibiotics.

The use of protecting groups to mask functional groups within a molecule, and thereby facilitate regioselective transformations, has been one of the cornerstones of organic synthesis [2]. Nowhere has this been more evident than in the total synthesis of natural products, which are typically rich in diverse, and often delicate, functionality [3]. However, the use of protecting groups is inefficient, adding extra steps to a synthetic pathway. As a result, many of today's synthetic chemists strive to develop new reactions that are sufficiently mild and selective to obviate the need for protecting groups. In this respect, the chemist can only aspire to achieve the kind of selectivity and efficiency afforded by enzymes. After all, evidence suggests that nature has been biosynthesizing natural products, without the need for protecting groups, for over a billion years. In spite of this, there are rare examples when even nature resorts to using protecting groups to facilitate a subsequent enzymatic transformation, particularly when a substrate may otherwise undergo spontaneous nonenzymatic cyclization. For example, in lysine biosynthesis (Figure 1A), the hydrated open-chain form of the precursor L-tetrahydrodipicolinate (L-THDP, 1) is acylated to give *N*-acyl- $\alpha$ -amino- $\epsilon$ -ketopimelate 2 [4, 5]. The *N*-acyl-blocking group, which is succinate in *E. coli* or acetyl in some other prokaryotes, frees the  $\epsilon$ -keto group, facilitating a subsequent transamination to give *N*-acyl diaminopimelate (DAP) 3. Hydrolysis of the acyl-protecting group followed by epimerization and decarboxylation gives lysine. Similarly in the biosynthesis of arginine in *E. coli*, glutamate is *N*-acetylated before the  $\gamma$ -carboxyl group is phosphorylated and reduced to *N*-acetylglutamate- $\gamma$ -semialdehyde. Transamination of the aldehyde followed by hydrolysis of the acetyl-protecting group reveals ornithine, which is transformed to arginine via the urea cycle [6]. In the absence of the *N*-acetyl-protecting group, glutamate- $\gamma$ -semialdehyde spontaneously cyclizes and is reduced, by pyrroline-5-carboxylate reductase, to give proline.

In addition to amino acid metabolism, recent studies on the catabolism of amines have revealed other examples of natural protective-group chemistry. For instance (Figure 1B), putrescine 4 is degraded in *E. coli* by first protecting one of the amino groups with  $\gamma$ -glutamate 5. This allows oxidation of the other amino group to the aldehyde 6, which would spontaneously cyclize in the absence of the protecting group. Further oxidation to the acid 7 and hydrolysis of the glutamyl group gives

$\gamma$ -aminobutyrate (GABA) [7]. Finally, in the degradation of isopropylamine to L-alanine by *Pseudomonas* species, the amine is first protected with a  $\gamma$ -glutamyl group before hydroxylation and subsequent hydrolysis of the protecting group [8].

In this issue of *Chemistry & Biology*, Spencer et al. [1] have elucidated the biosynthetic pathway to the AHBA side chain of the aminoglycoside butirosin (Figure 1C). Intriguingly, this pathway provides another example in which nature has apparently evolved a protective-group strategy. In AHBA biosynthesis, the phosphopantetheine group of BtrI, an acyl-carrier protein (ACP), is  $\gamma$ -L-glutamylated by an ATP-dependent ligase, BtrJ. Activation of the  $\gamma$ -carboxylate occurs through the formation of an acyl-phosphate intermediate with release of ADP, which is distinct from the activation step in the transfer of amino acids to peptidyl-carrier proteins (PCPs), which occurs via aminoacyl adenylate intermediates. Decarboxylation of  $\gamma$ -L-Glu-S-BtrI 8 gives GABA-S-BtrI 9, which surprisingly undergoes  $\gamma$ -L-glutamylated, in a reaction that is also catalyzed by BtrJ. Subsequent  $\alpha$ -hydroxylation of 10 gives  $\gamma$ -L-Glu-AHBA-S-BtrI 11. Presumably, the dual function enzyme BtrJ catalyzes the second acylation to protect the  $\gamma$ -amino group of GABA-S-BtrI or AHBA-S-BtrI from cyclizing onto the activated thioester, which would result in the loss of GABA or AHBA from the ACP. Although the mode of transfer of AHBA to the aminoglycoside core was not determined, it was proposed that the enzymes BtrG or BtrH catalyze the acyl-transfer with hydrolysis of the  $\gamma$ -glutamyl group occurring in the last step to give butirosin. Therefore, the  $\gamma$ -glutamyl group might also serve to protect the producing organism from the antibiotic effects of butirosin [1].

The pathway to AHBA also provides additional evidence of the important role that carrier proteins play in secondary metabolism. In addition to the well-characterized role of ACPs and PCPs in the biosynthesis of fatty acids, polyketides, and nonribosomal peptides, it has become apparent that phosphopantetheinylated carrier proteins play a wide role in the biosynthesis of other classes of natural products. For example, PCPs and adenylation-PCP didomains are used to activate and tether proline, tyrosine, and histidine in the biosynthesis of undecylprodigiosin [9], novobiocin [10], and nikkomycin [11], respectively. As with the biosynthesis of AHBA, the carrier proteins in these examples serve to sequester proteinogenic amino acids, thereby diverting a fraction of the available primary metabolic pool to secondary metabolism [12]. Any subsequent intermediates that are generated by downstream tailoring enzymes remain tethered to the carrier protein and are therefore unable to interfere with existing primary metabolic pathways. This could include the aberrant incorporation of nonproteinogenic amino acids into proteins during the course of biological evolution [13]. The carrier protein can further serve to channel and protect any unstable intermediates, so-called "hot potatoes" [14]. Also, given that the carrier protein is a covalent extension of the substrate, protein-protein interactions

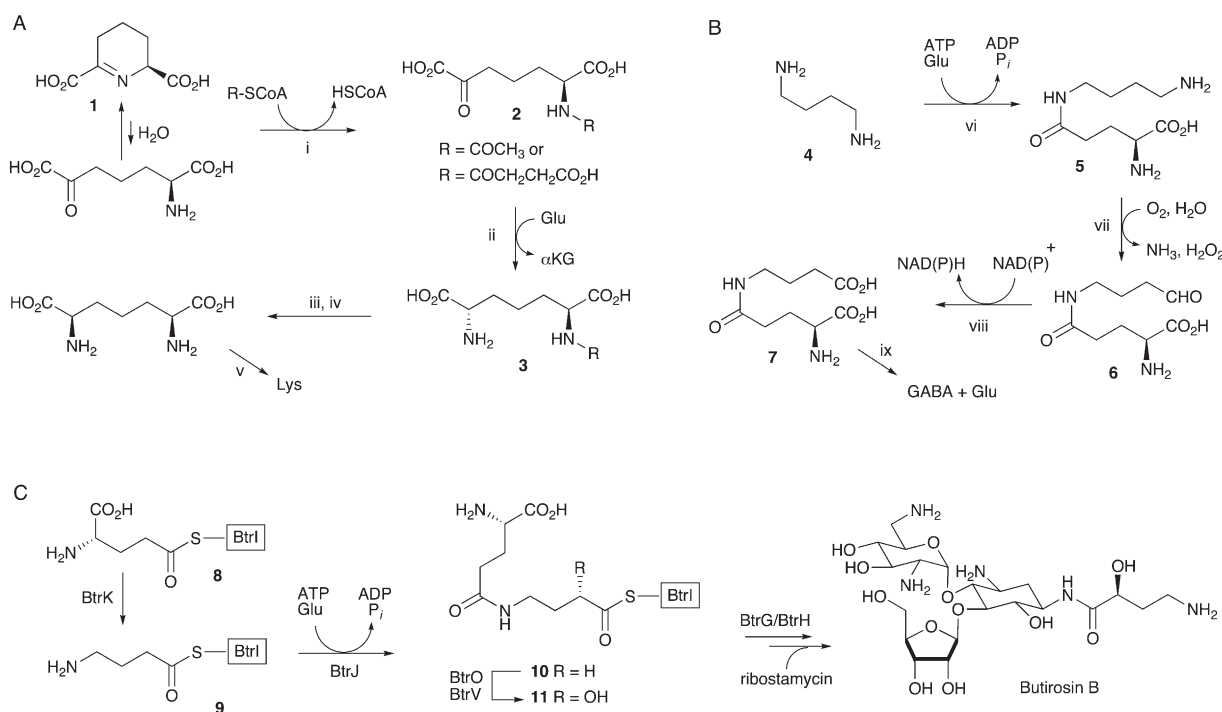


Figure 1. Examples of Protective-Group Chemistry Employed by Nature

(A) Biosynthesis of L-lysine in bacteria [4, 5]: (i) THDP *N*-Acyltransferase; (ii) *N*-Acyl-DAP aminotransferase; (iii) *N*-Acyl-DAP deacylase; (iv) DAP epimerase; and (v) DAP decarboxylase.

(B) Putrescine degradation in *E. coli* [7]: (vi) YcjK,  $\gamma$ -glutamyl-putrescine synthetase; (vii) OrdL,  $\gamma$ -glutamyl-putrescine oxidase; (viii) AldH,  $\gamma$ -glutamyl-aminobutyraldehyde dehydrogenase; and (ix) YcjL,  $\gamma$ -glutamyl-GABA hydrolase.

(C) Biosynthesis of the AHBA side chain of butirosin: see this issue of *Chemistry & Biology* [1].

undoubtedly serve to modulate the activities of downstream tailoring enzymes. Finally, the electron-withdrawing thioester bond between intermediates and the phosphopantetheine group of the carrier protein not only activates the carboxyl group of substrate for subsequent cyclization [10], reduction [12], hydrolysis [11], or in the case of AHBA, amino acylation [1] but also stabilizes  $\alpha$ -carbanions facilitating  $\alpha,\beta$ -oxidations [1, 12]. Indeed, the general utility of phosphopantetheinylated carrier proteins extends beyond metabolism in prokaryotes and lower eukaryotes and has recently been demonstrated in higher eukaryotes. For example, a PCP-like domain within  $\alpha$ -amino adipate-semialdehyde dehydrogenase is implicated in the degradation of lysine in humans [15]. Similarly, in *Drosophila*, a PCP-containing enzyme (Ebony) has been shown to activate  $\beta$ -alanine for a subsequent acylation of histamine [16].

The emergence of bacterial resistance to naturally occurring aminoglycoside antibiotics has fueled the search for new analogs [17]. The AHBA side chain is a useful pharmacophore in the design of new analogs because it affords butirosin and semisynthetic aminoglycosides increased protection against resistance enzymes, which chemically modify this class of antibiotics [17]. Despite this, the total synthesis and even the semisynthesis of aminoglycoside analogs is complicated by the need to protect and differentiate the multiple reactive-amino and hydroxyl groups. The development of methods that enable engineered biosynthesis

of new aminoglycoside analogs is, thus, extremely important. Although the enzyme that transfers AHBA to ribostamycin in the biosynthesis of butirosin has yet to be identified, the findings of Spencer et al. [1] make it highly likely that AHBA will be transferred from the carrier protein BtrI, remarkably with a  $\gamma$ -glutamyl *N*-protecting group still intact. This discovery should spark future research toward the engineered biosynthesis of novel aminoglycoside antibiotics incorporating AHBA or related acyl side chains.

#### Jason Micklefield

School of Chemistry  
The University of Manchester  
Sackville Street, PO Box 88  
Manchester, M60 1QD  
United Kingdom

#### Selective Reading

- Li, Y., Llewellyn, N.M., Giri, R., Huang, F., and Spencer, J.B. (2005). *Chem. Biol.* 12, this issue, 665–675.
- Green, T.W., and Wuts, P.G.M. (1999). *Protective Groups in Organic Synthesis*, Third Edition (New York: Wiley).
- Nicolaou, K.C., and Snyder, S.A. (2004). *Proc. Natl. Acad. Sci. USA* 101, 11929–11936.
- Cox, R.J. (1996). *Nat. Prod. Rep.* 13, 29–43.
- Beaman, T.W., Blanchard, J.S., and Roderick, S.L. (1998). *Biochemistry* 37, 10363–10369.
- Caldovic, L., and Tuchman, M. (2003). *Biochem. J.* 372, 279–290.

7. Kurihara, S., Oda, S., Kato, K., Kim, H.G., Koyanagi, T., Kumagai, H., and Suzuki, H. (2005). *J. Biol. Chem.* **280**, 4602–4608.
8. de Azevedo Wäsch, S.I., van der Ploeg, J.R., Maire, T., Lebreton, A., Kiener, A., and Leisinger, T. (2002). *Appl. Environ. Microbiol.* **68**, 2368–2375.
9. Thomas, M.G., Burkart, M.D., and Walsh, C.T. (2002). *Chem. Biol.* **9**, 171–184.
10. Chen, H., and Walsh, C.T. (2001). *Chem. Biol.* **8**, 301–312.
11. Chen, H., Hubbard, B.K., O'Connor, S.E., and Walsh, C.T. (2002). *Chem. Biol.* **9**, 103–112.
12. Chen, H., Thomas, M.G., O'Connor, S.E., Hubbard, B.K., Burkart, M.D., and Walsh, C.T. (2001). *Biochemistry* **40**, 11651–11659.
13. Tze-Fei Wong, J. (1983). *Proc. Natl. Acad. Sci. USA* **80**, 6303–6306.
14. Perham, R.N. (2000). *Annu. Rev. Biochem.* **69**, 961–1004.
15. Praphanphoj, V., Sacksteder, K.A., Gould, S.J., Thomas, G.H., and Geraghty, M.T. (2001). *Mol. Genet. Metab.* **72**, 336–342.
16. Richardt, A., Kemme, T., Wagner, S., Schwarzer, D., Marahiel, M.A., and Hovemann, B.T. (2003). *J. Biol. Chem.* **278**, 41160–41166.
17. Haddad, J., Vakulenko, S., and Mobashery, S. (1999). *J. Am. Chem. Soc.* **121**, 11922–11923.