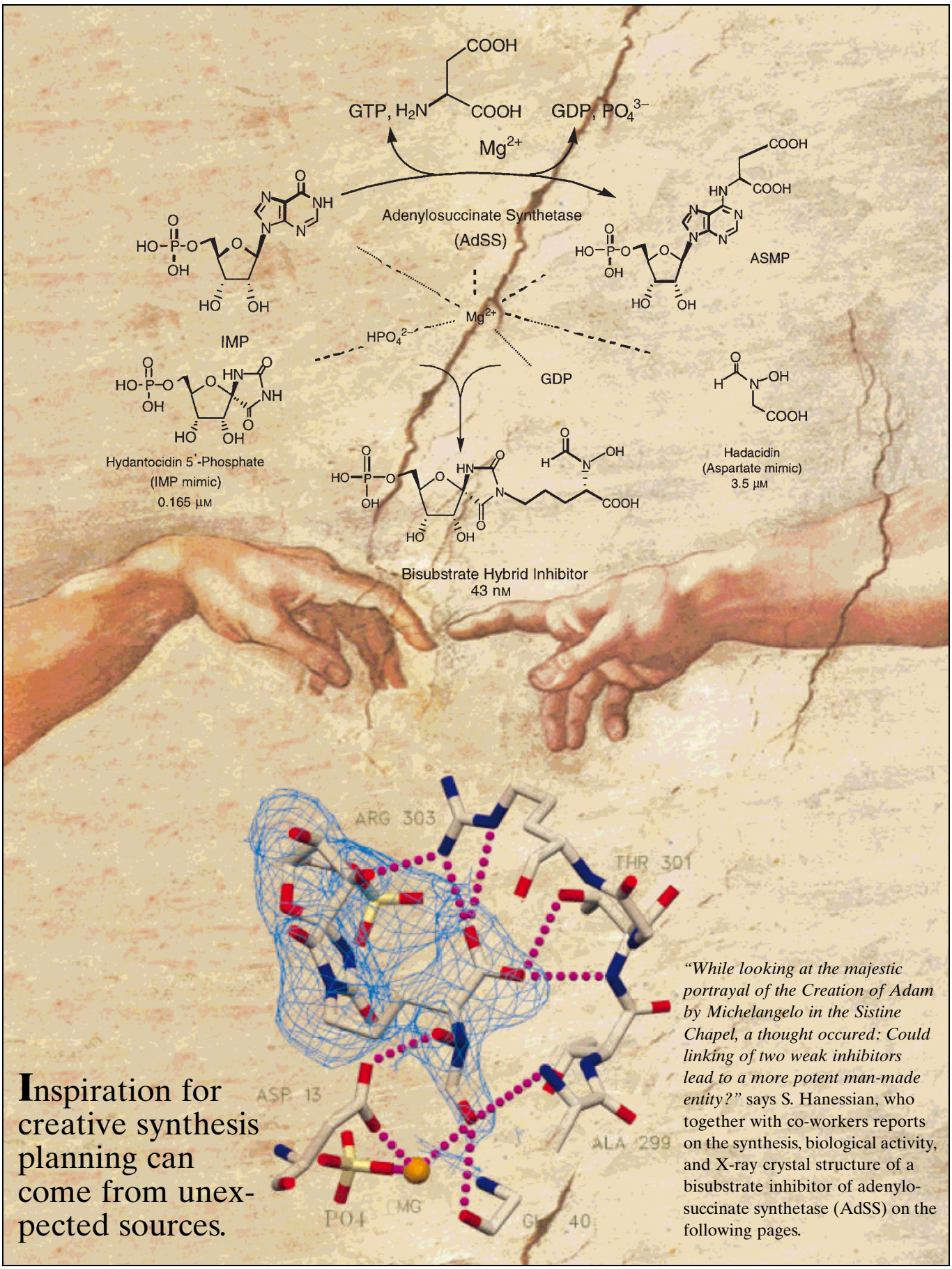

Inspiration for creative synthesis planning can come from unexpected sources.

"While looking at the majestic portrayal of the Creation of Adam by Michelangelo in the Sistine Chapel, a thought occured: Could linking of two weak inhibitors lead to a more potent man-made entity?" says S. Hanessian, who together with co-workers reports on the synthesis, biological activity, and X-ray crystal structure of a bisubstrate inhibitor of adenylosuccinate synthetase (AdSS) on the following pages.

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An Enzyme-Bound Bisubstrate Hybrid Inhibitor of Adenylosuccinate Synthetase**

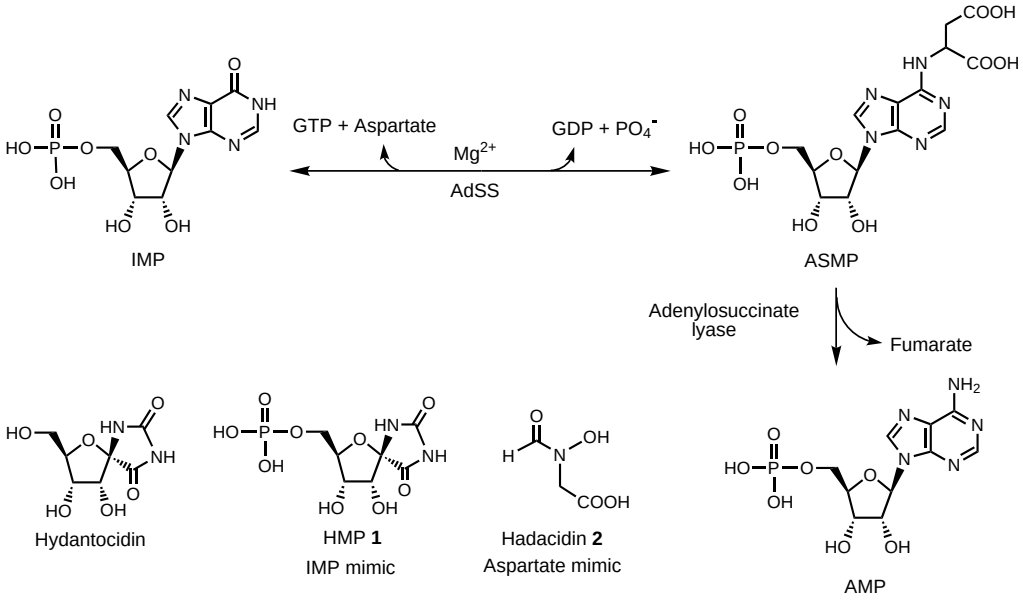
Stephen Hanessian,* Pu-Ping Lu, Jean-Yves Sancéau, Philippe Chemla, Keigo Gohda, Raymonde Fonne-Pfister, Lars Prade, and Sandra W. Cowan-Jacob

Nature has been a generous provider of a plethora of compounds displaying fascinating structural and functional stereochemical diversity and biological activity. Such compounds are also endowed with intriguing biological activity affecting a wide cross-section of physiological functions in the animal and plant world. Nowhere is this more evident than in the area of drug discovery and the development of therapeutically important medicines.[1] The great advances in physical methods of structure elucidation such as X-ray crystallography and NMR analysis of proteins have provided the synthetic chemist with powerful tools to creatively design inhibitor molecules on a "rational basis".[2] Thus, a crystal structure of an organic molecule nestled in the active site of a given enzyme[3] offers a powerful visual image of critical interactions, which can be used to optimize new structures through chemical modifications. While great efforts have been directed at the exploitation of such approaches in medicinal chemistry, relatively little information is available in the area of plant enzymology with respect to "designed" inhibitors.[4]

The enzyme adenylosuccinate synthetase (AdSS) is involved in the first step in the conversion of inosine monophosphate (IMP) into adenosine monophosphate (AMP, Scheme 1).[5] The process is mediated by GTP, magnesium ions, and aspartate, which lead to the aspartylated nucleotide adenylosuccinate monophosphate (ASMP) with release of GDP and phosphate. The enzyme adenylosuccinate lyase then completes the transformation with the production of AMP and release of fumarate.

Hydantocidin, a unique spirocyclic hydantoin riboside, isolated from *Streptomyces hygroscopicus*,[6] inhibits AdSS from *E. coli* as well as from the plant. As a pro-herbicide, it is first converted into the 5′-phosphate **1** (hydantocidin monophosphate, HMP; see Scheme 1), and as such, it is believed to mimic IMP or AMP.[7] Convincing evidence comes from the structure of the cocrystal HMP and AdSS, where the inhibitor molecule has replaced IMP in the active site.[7c]

An unrelated natural product, hadacidin (*N*-formyl-*N*-hydroxy glycine, **2**; see Scheme 1), which also possesses herbicidal activity, exerts its activity on AdSS as a competitive inhibitor of aspartic acid, thus affecting the enzyme at a different site.[8] An example of simultaneously capturing and visualizing two different inhibitors with a common enzyme is provided by the crystal structure of AdSS harboring HMP and hadacidin.[9] These two crystallographic studies presented a unique opportunity to probe new aspects of inhibitor design for AdSS.[7c, 9]



Scheme 1. Biosynthesis of AMP and structures of natural inhibitors of adenylosuccinate synthetase (AdSS).

Considering the relative positions of HMP, hadacidin, Mg^{2+}, and phosphate (Scheme 2), it occurred to us that the covalent attachment of HMP and hadacidin by a judicious choice of a linker unit would lead to an HMP–hadacidin hybrid with improved binding, hence a more potent herbicidal activity. Molecular modeling and simulated docking experiments revealed that a bisubstrate hybrid with a C_3 methylene bridge ending with an (2*S*)-*N*-formyl-*N*-hydroxy amino acid such as **3** would be a preferred hybrid compared to the 2*R* isomer **4**, or to the C_2-linked variants. We report herein on the validation of this hypothesis through synthesis and X-ray crystal structure analysis in an unequivocal way.

The camphorsultam derivative **5**[10] was treated with allyl bromide in the presence of powdered zinc in a mixture of aqueous ammonium chloride and THF to produce the (*S*)-

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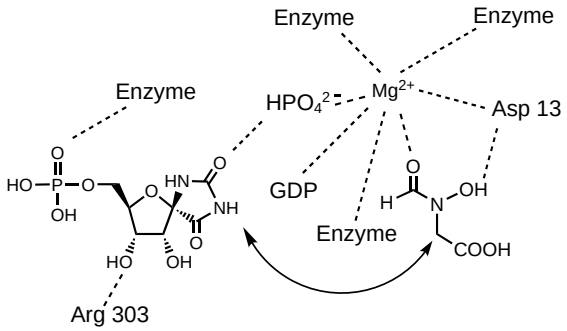
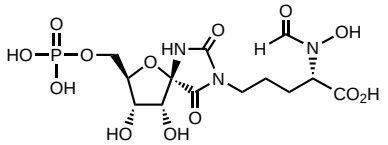
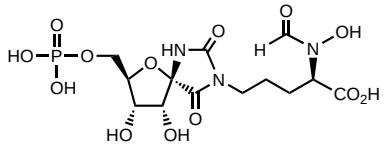


Scheme 2. Interactions of hydantocidin phosphate and hadacidin with AdSS. Structures of bisubstrate hybrids **3** and **4**.



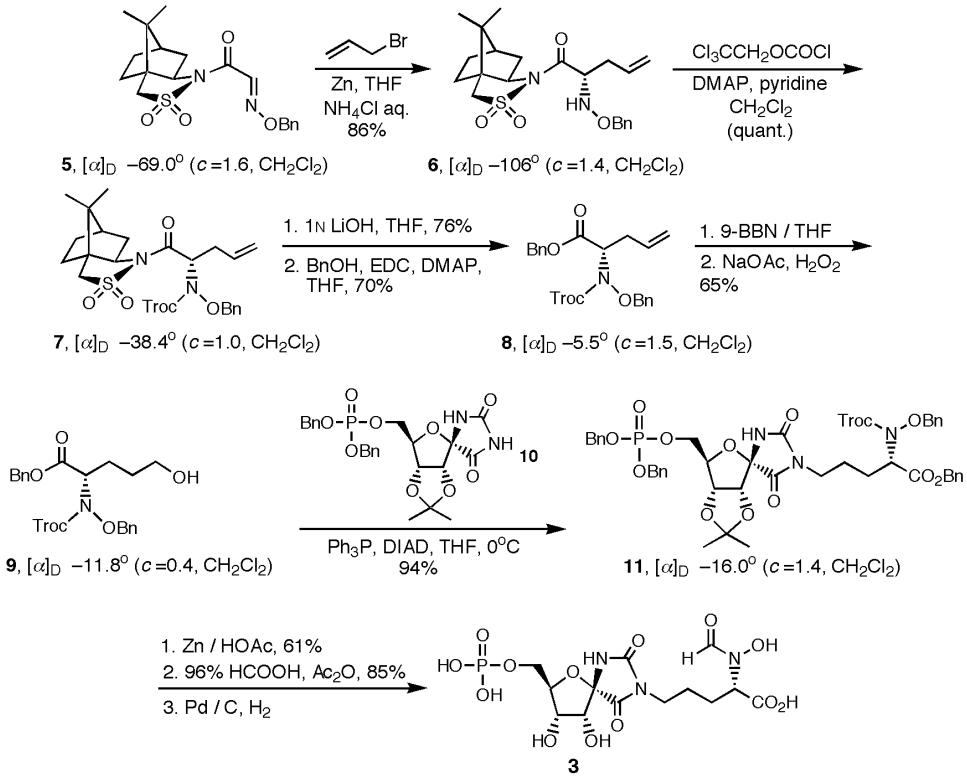


allyl glycine derivative **6** in excellent yield and with high diastereoselectivity (Scheme 3). Protection of the amine group as the *N*-trichlorethoxy-carbonyl derivative **7**, cleavage of the chiral amide motif, and reesterification afforded the benzyl ester **8** in good overall yield. Hydroboration proceeded uneventfully to give the primary alcohol **9**, which was coupled to the HMP dibenzyl derivative **10** under the conditions of Mitsunobu reaction[11] to afford the coupled product **11** in excellent yield. Deprotection of the *N*-Troc group followed by N-formylation with concomitant hydrolysis of the acetal function afforded an O-benzyl-protected precursor of the target compound. Debenzylation under catalytic hydrogenolysis by careful monitoring gave the hybrid molecule **3** as an amorphous powder. The analogous (2*R*)-amino acid analogue **4** was similarly prepared starting with the other enantiomer of the sultam.

Enzyme inhibition studies proved to be most gratifying: (2*S*)-hybrid **3** inhibited AdSS from *E. coli* and wheat at concentrations of 0.043 and 0.200 μM, respectively, which was much lower than observed for the 2*R* isomer **4** and the natural substrates **1** and **2** (Table 1).[12] Thus, linking two relatively weakly active inhibitors to produce a hybrid molecule such as **3** enhanced the enzymatic inhibitory activity significantly compared to the individual natural substrates.

Table 1. Inhibitory activity of HMP, hadacidin, and bisubstrate hybrids on AdSS from *E. coli* and wheat.

Inhibitor	IC_{50} [μM]	
	E. coli AdSS	wheat AdSS
HMP (**1**)	0.675	1.35
hadacidin (**2**)	3.5	12
3	0.043	0.200
4	0.665	8.93



Scheme 3. Synthesis of hybrid **3**. 9-BBN = 9-borabicyclo[3.3.1]nonane, Bn = benzyl, DIAD = diisopropyl azodicarboxylate, DMAP = dimethylaminopyridine, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, Troc = trichlorethoxycarbonyl.

Structural validation of the biological results was secured from X-ray crystallographic data of AdSS complexed with the 2*S* and 2*R* hybrids individually at a resolution of 2.0 and 2.2 Å, respectively.[13] The left side of Figure 1 depicts the orientation of the 2*S* hybrid **3**, which occupies the sites originally harbored by IMP and aspartate. Magnesium and phosphate are seen at their expected positions, as well as interactions with amino acids. The C_3 methylene chain in **3** adopts a conformation which presumably allows for maximum binding of the HMP and hadacidin counterparts to AdSS.[14] Curiously the 2*R* hybrid **4** also occupies the same relative positions with subtle differences at the amino acid end and in the conformation of the C_3 methylene chain[14] (right side of Figure 3).

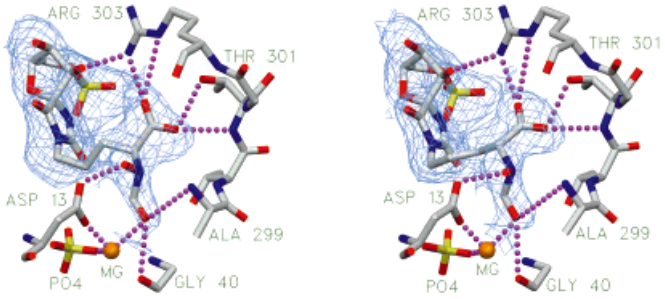


Figure 1. X-ray crystal structures of the complexes formed between AdSS and **3** (left) and **4** (right) showing the electron density at a cutoff of 1 σ. Hydrogen bonds with amino acids in AdSS are indicated. Note the different conformations of the C_3 methylene chain in **3** and **4**.[13]

The C_2-linked hybrid—readily available from the common intermediate **8** by oxidative cleavage and reduction, then Mitsunobu coupling and deprotection—was also more active than either HMP or hadacidin, but not as active as the C_3-linked hybrid **3**.[15] It is possible that in this case, it is the enzyme that must undergo a conformational change to adapt to the inhibitor, rather than the reverse as in the case of **3**.

There are very few examples of successfully linking two entities to achieve a hybrid-type structure that improves enzyme inhibitory activity.[16] Recently, great strides have been made in this direction, by the ingenious application of structure–activity relationships (SAR) determined from NMR spectroscopy for the discovery of high-affinity ligands.[17] To the best of our knowledge the design and synthesis of a bisubstrate inhibitor such as **3** is unprecedented in the herbicide field.

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[1] For excellent coverage, see *Burger's Medicinal Chemistry and Drug Discovery*, *Vol. 1–5* (Ed.: M. W. Wolff), Wiley, New York, **1997**.
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[12] For enzyme assays, see ref. [7c].
[13] For details of the X-ray structure determination and analysis, see the Supporting Information.
[14] The dihedral angles for the C_3 methylene chain in **3** and **4** respectively are summarized in Table 2.

Table 2. Dihedral angles [°] in **3** and **4**.

Angle	**3**	**4**
N(hydantoin)-C1-C2-C3	66.4	–51.9
C1-C2-C3-Cα(glycine)	–166	–178
C2-C3-Cα-CO_2H	167	–150
C2-C3-Cα-NH_2	–72.8	89.4

[15] For the *R* and *S* hybrids with a C_2 chain, the IC_{50} for AdSS was 0.24 μM: S. Hanessian, P.-P. Lu, J.-Y. Sancéau, P. Chemla, K. Gohda, R. Fonne-Pfister, L. Prade, S. W. Cowan-Jacob, unpublished results.
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