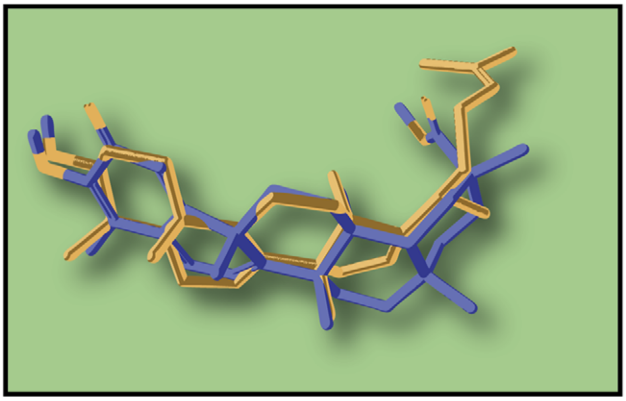
In This Issue

Biosynthesis of an RNA Polymerase Inhibitor

PAGE 1031

Streptolydigin is a potent inhibitor of bacterial RNA polymerase with limited cross-resistance with other inhibitors such as rifampicin, thus making streptolydigin a good candidate for the development of antibacterial derivatives with improved antibiotic activity. Since streptolydigin has been also described as potential antitumor agent, the opportunity for developing derivatives with enhanced antitumor activity seems promising. The characterization of the streptolydigin gene cluster from *Streptomyces lydicus,* reported now by Olano et al., has shown its tetramic acid moiety being synthesized by a hybrid type I PKS-NRPS system, and the use of combinatorial biosynthesis has allowed the generation of several novel glycosylated derivatives with antibacterial activity.

OMG, MGL! They Found What?



PAGE 1045

Monoacylglycerol lipase (MGL) is the primary enzyme responsible for the deactivation of the endocannabinoid 2-arachidonoyl-sn-glycerol (2-AG). Previous studies in rodent models suggest that blockade of MGL produces antinociception. All currently available MGL inhibitors exert their effects by forming covalent bonds with the enzyme and may therefore carry potential clinical disadvantages through this irreversible mode of action. In the present study, King and colleagues screened a commercial chemical library to identify two bioisosteric terpenoids that inhibit MGL through a rapidly reversible mechanism and may thus serve as starting points for the further development of MGL as a therapeutic target.

Blood-Feeding Ticks Meet Proteomics

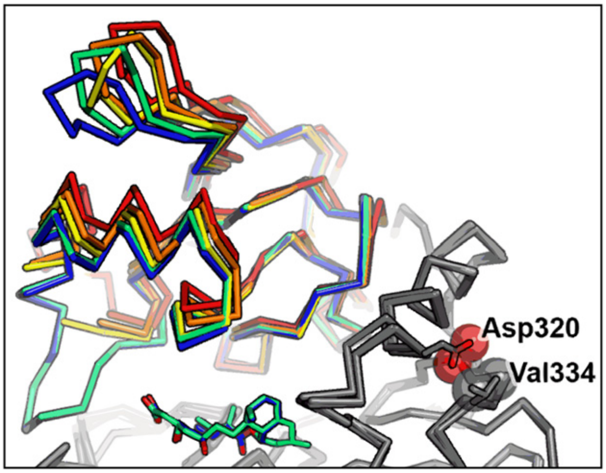
PAGE 1053

Hemoglobin digestion is an essential process for blood-feeding parasites. We deconvoluted the hemoglobinolytic cascade in the tick *Ixodes ricinus*, a vector of Lyme disease and tick-borne encephalitis. Horn et al. demonstrate the role of a network of digestive peptidases through imaging with specific activity-based probes and activity profiling. Selective inhibitors were applied to dissect the roles of the individual peptidases and determine the peptidase-specific cleavage map of the hemoglobin molecule. Because of their central function in nutrition of the parasite, the identified enzymes are potential targets to developing novel antitick vaccines that limit parasite survival and disease transmission.

When LovD Turns to Serious Synthesis

PAGE 1064

Simvastatin is the active pharmaceutical ingredient of the blockbuster cholesterol-lowering drug Zocor. In this work, Gao et al. employed directed evolution to engineer the acyltransferase LovD to be an efficient simvastatin synthase. Improved mutants were obtained through directed evolution, and the best mutant displaying a ~11-fold increase in whole cell biosynthesis of simvastatin acid was compared to the parent LovD. In addition, seven crystal structures of LovD (parent, mutant, and cocrystal with ligands) were determined. This collection of structures enhanced our understanding of the catalytic mechanism of LovD and afforded insights into how beneficial mutations improved the catalytic properties of LovD. The successful redesign of LovD into a simvastatin synthase demonstrates the vast potential of utilizing enzymes involved in secondary metabolism as useful biocatalysts.



Chemistry & Biology

In This Issue

Diels-Alder and DNA Barcodes

PAGE 1075

DNA-encoded chemical libraries are promising tools for the discovery of binders towards protein targets of pharmaceutical relevance. DNA-encoded small molecules can be enriched in affinity-based selections and their unique DNA ‘‘barcode’’ allows the amplification and identification by high-throughput sequencing. In the approach described by Buller et al., ligands were selected out of 4000 DNA-encoded compounds generated by Diels-Alder cycloadditions against trypsin, human serum albumin, the anti-apoptotic Bcl-xL protein, and tumor necrosis factor (TNF). Sequence enrichment profiles corresponding to the ‘‘barcoded’’ library members were validated by affinity measurements of single compounds, revealing a novel class of TNF binders, which completely inhibited TNF-mediated killing of L-M fibroblasts in vitro. The approach might provide an alternative to conventional small molecule high-throughput screening as it lays a foundation for manipulating large DNA-encoded chemical libraries.

Ribosome under Attack

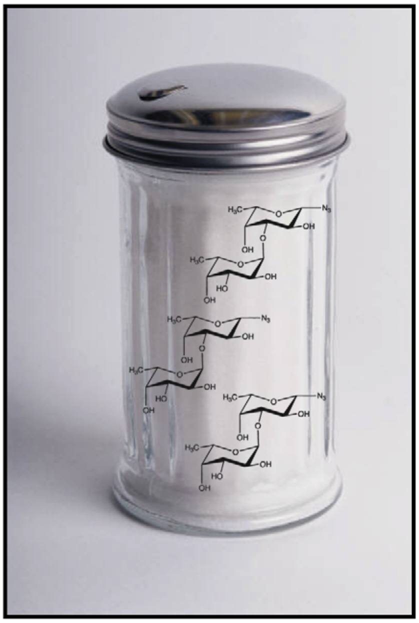
PAGE 1087

The thiopeptide antibiotics thiostrepton (ThS) and nosiheptide (NoS) target the ribosome and inhibit translation factor function, whereas GE2270A/T binds to elongation factor EF-Tu and prevents ternary complex formation. Using several in vitro translational machinery assays to screen a library of thiopeptide antibiotic precursor compounds, Starosta et al. identified four families of compounds that are either themselves inhibitory or relieve the inhibitory effects of ThS, NoS, or GE2270T. Some of these precursors represent distinct compounds with respect to their ability to bind to ribosomes, demonstrating the potential of such assays for identifying lead compounds that might be missed using conventional inhibitory screening protocols.

La Dolce Vita: Removing a Roadblock in Glycosynthase Startegy

PAGE 1097

Carbohydrate synthesis, not regulated by universally conserved codes, is significantly more complex, conceptually and practically, than nucleic acids and protein synthesis. Cellular polysaccharides are endowed with breathtaking structural variety and the diverse stereochemistry of the monosaccharide building blocks. The advent of glycosynthases, glycoside hydrolases mutants able to synthesize sugars, could, in principle, overcome some problems surrounding chemical synthesis strategies, if these engineered enzymes are applied to obtain polysaccharides of desired structure and composition. However, only β-glycosynthases could be reliably produced so far. Cobucci-Ponzano et al. now show an approach to prepare two α-fucosynthases that efficiently produce fucosylated oligosaccharides of biotechnological interest. The strategy relies on the use of glycoside azide derivatives. The methodology is of general applicability, thus expanding the glycosynthetic approach to the entire class of α-glycosidases.



Amination of Polyketide Starter Unit

PAGE 1109

Analyses of the gene cluster for the biosynthesis of a cytotoxic macrolactam, elaborated on in this issue by Jørgensen et al., provides exciting insights into the general questions surrounding biosynthesis of natural products. The authors propose involvement of two distinct PKS systems, one functioning together with NRPS-related enzymes. The synthesis of the polyketide starter and the manner in which it is aminated represents a previously unobserved mechanism for generation of an acyl amino acid starter unit. Understanding of the biosynthetic pathway, access to the genes, and the demonstrated possibility to manipulate them open new possibilities for modification of the macrolactam molecule and generation of novel analogs with improved pharmacological properties.