In This Issue

New Enzymes for New Alkaloids

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Madagascar periwinkle (*Catharanthus roseus*) produces several pharmaceutically important terpene indole alkaloids. Metabolic engineering of the pathway that makes these compounds is a promising strategy to generate novel molecules and potential new drug candidates. However, reengineering efforts in this alkaloid system are limited by the narrow substrate scope of the enzyme at the entry point of the pathway. Bernhardt et al. report a strategy for rapid identification of enzyme variants that can be used to expand the scope of alkaloid biosynthesis in one of Nature's most prolific small-molecule factories.

Inflammation: Glycodendrimers to the Rescue

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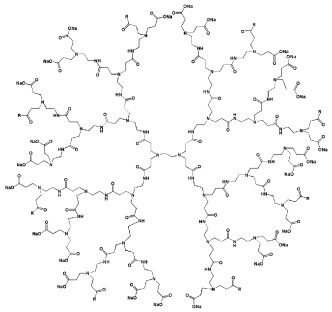
Synthetic, amine-functionalized heparin oligosaccharides can be attached to hyperbranched macromolecules such as dendrimers to create novel heparin conjugates for applications in glycobiology. As De Paz et al. report, the multivalent presentation of heparin epitopes on the surface of dendrimers drastically amplifies the binding affinity to heparin-binding proteins such as growth factors. Blocking interactions between heparan sulfate and different protein families such as selectins and chemokines that play a key role in inflammatory processes may be another area of dendrimer application. New anti-inflammatory drugs with minimal side effects may be based on synthetic heparin dendrimers.



Stereocontrol of PKS Achieved by KR domains

PAGE 898

Polyketide synthases control the chiralities of multiple substituents during the synthesis of complex molecules like erythromycin. The complicated architecture of these megasynthases has traditionally hindered efforts to determine how stereocontrol is achieved. Current structural and functional data of isolated ketoreductase domains presented by Keatinge-Clay reveal that they are responsible for setting nearly all substituent chiralities. By comparing the structures of ketoreductases that operate on the same substrate to yield different products, the mechanisms by which ketoreductases set unique combinations of stereochemistries were elucidated. From this analysis, a protocol was developed to predict the structure of a polyketide based on the sequence of its synthase.

Designing a MHC I Single Chain Trimer

PAGE 909

Due to their preassembled nature, MHC I single chain trimer (SCT) proteins bypass conventional antigen processing, express efficiently at the cell surface, and potently stimulate CD8 T cells in vivo. These properties afford us a unique opportunity to broaden the production of antigen-specific DNA vaccines to include the induction of subdominant immune responses. Mitaksov et al. present a novel approach for generating soluble covalently linked pMHC reagents that is based on coupling of peptide and class I HC using covalent disulfide bond trapping. The produced disulfide-trap SCT and soluble pMHC reagents are potential diagnostic tools and modulators of T cell responses to pathogens and tumors.

Organelle Specific ROS Production "On Demand"

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Reactive oxygen species (ROS) cause cellular damage and are implicated in human illness ranging from cancer to Alzheimer's disease. While the effects of ROS have been studied in depth within living systems, little is known about how the subcellular origin of ROS impacts the cellular response. In order to develop a clearer picture, a set of chemical tools were engineered that allowed oxidative stress to be studied in specific organelles. By delivering oxidant-bearing peptide conjugates specifically to mitochondria or nuclei, oxidative stress was generated site-specifically. It was found that the specific organelle targeted by oxidative stress influences significantly the viability of the cell and the nature of the cell's stress response. These comparative studies, enabled by the development of organelle-specific oxidants, are the first to examine cellular responses to site-specific oxidative stress.

Mechanism of Pikromycin PKS Product Partioning

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Polyketide natural products are renowned for their architecturally complex chemical structures and diverse bioactivity. Accordingly, there is considerable interest in generating novel natural product molecules through manipulation and engineering of metabolic pathways for polyketide biosynthesis. The pikromycin polyketide synthase (PKS) is unprecedented in its ability to generate both 12- and 14-membered ring macrolactone products. Kittendorf et al. investigated possible mechanisms responsible for this unusual product partitioning. The results of this comprehensive in vitro study demonstrate that the docking domain-mediated protein-protein interaction between the final two monomodules of the pikromcyin PKS is a requirement for assembly of both macrolactone products.

Engineering Polyketide Synthase: Structure Paves the Way

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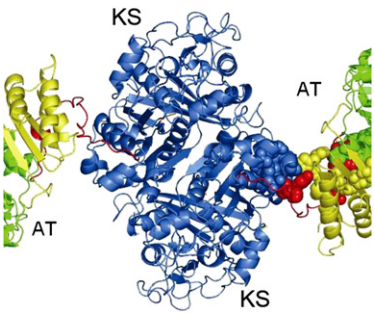
Tang et al. report the 2.6-Å X-ray crystal structure of a 190-kDa homodimeric fragment from module 3 of the 6-deoxyerthronolide B synthase. Its ketosynthase domain is covalently bound to the inhibitor cerulenin. The overall structure reveals two well-organized catalytic domains and two interdomain linker regions. In conjunction with other mechanistic results, the authors propose a model for the interaction of the catalytic domains with the acyl carrier protein (ACP) domain. These results pave the way for structure-based engineering of polyketide synthases.



Mutasynthetic Approach Leads to Natural Product Diversification

PAGE 955

The study by Anderle et al. reports improved mutasynthetic approaches for the production of aminocoumarin antibiotics. The versatility of mutasynthesis was significantly improved by metabolic engineering of the producer strains using heterologous genes that code for enzymes with suitable substrate specificities, as well as by two-stage feeding procedures in which two different mutant strains are used in succession. These methods offer the potential to significantly increase the structural diversity of natural products obtainable by mutasynthesis experiments in drug discovery programs.

