Engineering bisabolene synthase for novel activities

Bisabolene is a sesquiterpene found to have properties important for a biofuel in hydrogenated form (bisabolane). Bisabolene synthase produces bisabolene from farnesyl pyrophosphate bisaboyl cation. Initial work has been focused on redesigning the enzyme active site (Figure 2) to produce novel activities and novel terpenes. So far 75 enzyme mutants have been designed and produced using site-directed mutagenesis and screened for novel activities. Screening is carried out using gas chromatography, mass spectrometry and any novel terpenes will be characterised using 2D NMR. The malachite green assay is being adapted from the work of Vardakou to screen the mutants for pyrophosphate cleavage. This assay utilises an inorganic pyrophosphatase, converting pyrophosphate into two inorganic phosphate ions, which consequently form the malachite green complex (Figure 3). Pyrophosphate cleavage is the first step in the reaction mechanism, therefore this assay can be used to test both active and inactive mutants. Testing the inactive mutants can tell us whether the cleavage of pyrophosphate is the inhibited step inactivating the enzyme. From these 75 mutants potential catalytically important residues have been identified along with inactive mutants and mutations increasing the native product profile up to double the wild-type production level.

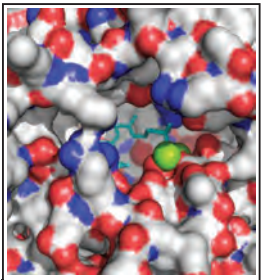

Figure 2: crystal structure of the active site of *Abies grandis* bisabolene synthase (3SAE) with farnesyl thio-pyrophosphate (cyan) and catalytically important magnesium ions (green) bound.

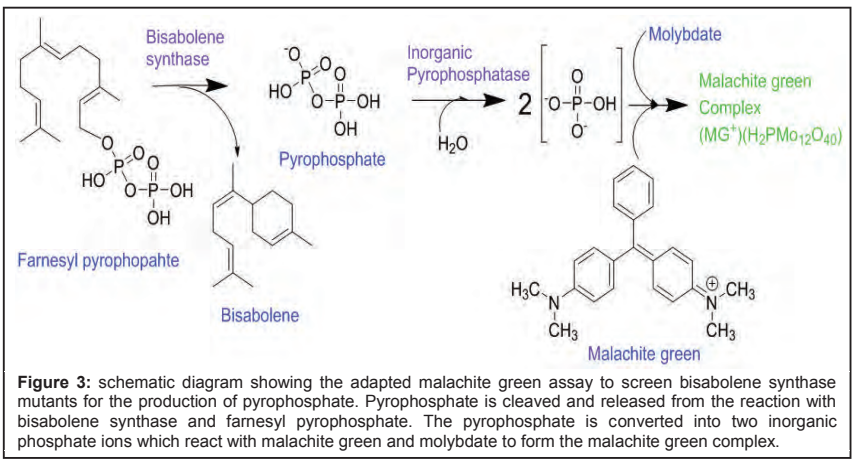

Figure 3: schematic diagram showing the adapted malachite green assay to screen bisabolene synthase mutants for the production of pyrophosphate. Pyrophosphate is cleaved and released from the reaction with bisabolene synthase and farnesyl pyrophosphate. The pyrophosphate is converted into two inorganic phosphate ions which react with malachite green and molybdate to form the malachite green complex.

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Economical and scalable synthesis of 6-amino-2-cyanobenzothiazole

Jacob Hauser, Hester Beard, Stuart Warriner and Robin Bon

Introduction

2-Cyanobenzothiazoles (CBTs) are useful building blocks for both luciferin derivatives for bioluminescent imaging (BLI) and handles for fast (k ~ 10 $M^{-1}s^{-1}$) biorthogonal ligations (Figure 1). A particularly versatile CBT is 6-amino-2-cyanobenzothiazole (ACBT, **9**), which has an amine handle for straight-forward derivatisation. Previously reported routes to ACBT are low-yielding, difficult to scale up, and/or use costly and highly toxic (combinations of) reagents. We developed a safe, economical and scalable synthesis of ACBT.

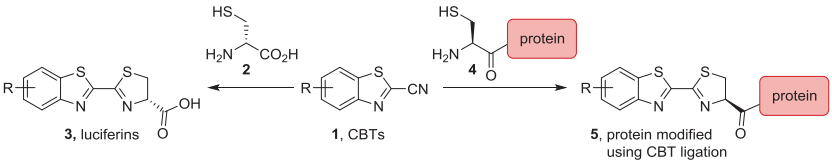

Figure 1: functionalised CBTs **1** can be used for the synthesis of luciferins **3** and for bio-orthogonal ligations such as the site-specific labelling/immobilisation of proteins **4**.

Results

After variation of reagents, catalyst, solvent and reaction temperature, we found that the 2-cyano group of ACBT can be installed under mild conditions through the DABCO-catalysed cyanation of 2-chloro-6-nitro-benzothiazole **7** (Figure 2). Any unreacted cyanide in the reaction mixture was safely quenched by the addition of an $FeCl_3$ solution, and calorimetric analysis showed that the rate and energetic profile of the cyanation reaction could be controlled by the slow addition of a dilute aqueous NaCN solution to the reaction mixture, preventing potential thermal runaway upon scale-up. We also developed an improved procedure for the reduction of 2-cyano-6-nitro-benzothiazole **8** using iron powder. Our route allowed the safe synthesis of ACBT on multi-gram scale and in high purity. In addition, the sole use of filtrations and crystallisations for purification of all intermediates and products, in combination with the endothermic nature of the controlled cyanation procedure, will enable straight-forward further scale up if required.

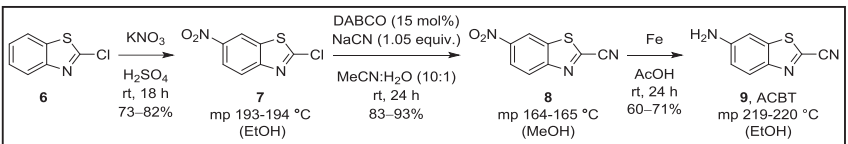

Figure 2: optimised procedure for ACBT synthesis.

Publications

Hauser J.R., Beard H.A., Bayana M.E., Jolley K.E., Warriner S.L. & Bon R.S. (2016) Economical and scalable synthesis of 6-amino-2-cyanobenzothiazole. *Beilstein J. Org. Chem.* **12**:2019-2025.

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Collaborators

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