	Experiment title: BAG	**Experiment number**: LS-2087
Beamline: ID29	**Date of experiment**: from: 18th July to: 19th July	**Date of report**: Feb 18th
Shifts:	**Local contact(s)**:	*Received at ESRF:*

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Report:

EvaD

Chloroeremomycin (Figure 1) is an important member of the vancomycin family of glycopeptide antibiotics. All members of which consist of an identical crosslinked heptapeptide scaffold to which a number of sugar moieties are attached, common to all vancomycin derivatives is the monosaccharide glucose, which is attached to the oxygen of the phenyl side-chain of residue 4. Additionally, chloroeremomycin also contains the 4-epi isomer of vancosamine (3-amino-2,3,6-trideoxy-3-C-methyl-L-arabino-hexopyranose), which is bound to the glucose 2'O as well as the β-OH-Tyr-6 of the glycopeptide[i]. This sugar differentiates chloroeremomycin from other vancomycin derivatives and it contributes to its antibacterial efficacy as it is thought to promote - together with the glucose substituent - the antibiotic dimerisation, which is important in the mode of action of this group of compounds. These antibiotics are made by fermentation of bacteria not chemical synthesis. Thus

improvments in the efficacy of vancomycin will have to come from engineering of enzymes to produce novel antibiotics.

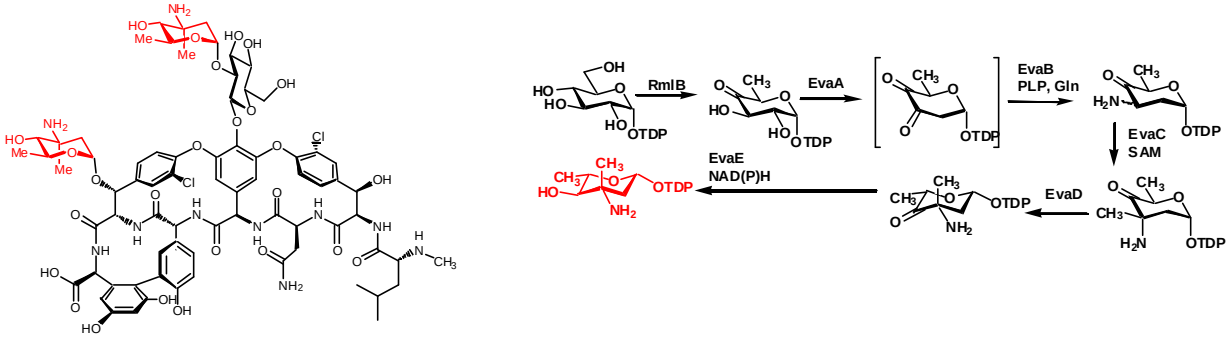

Figure 1 (left) Chloreomomycin, in red is shown epivancosamine. Figure 2 (right) The biosynthetic pathway of dTDP-epivancosamine.

We have collected a data set on EvaD, the fourth step in the pathway to 1.5Å and a data set on a substrate analogue complex to 1.4Å. We have determined the structure of the enzyme. Only part of the substrate is bound in the crystal, the rest is either disordered (not recognised) or has decomposed in solution. The data in hand do help establish the mechanism of the enzyme. The paper describing the data collection has been submitted to Acta CrystD. The native set collected is detailed below.

Data collection on Eva D

Values in Parenthesis refer to the highest resolution shell

	Data obtained at the ESRF
Wavelength (Å)	0.933
Resolution (Å)	37.27-1.50
Space Group	$P2_12_12$
Unit-cell Parameters (Å, º)	a = 98.57, b = 72.00, c = 57.10, $\alpha = \beta = \gamma = 90$
V_m (two molecules per asymmetric unit) ($Å^3$ Da^{-1})	2.3
Percentage solvent	42.6
Total measurements	292,414
Unique reflections	65,259
I/σ	7.3 (2.6)
Average redundancy	4.5 (4.1)
Data completeness (%)	100 (100)
R_{merge} † (%)	5.6 (26.3)

† R_{merge} = ΣΣ I(h)j – (I(h)) / ΣΣ I(h)j where I(h) is the measured diffraction intensity and the summation includes all observations

SSB

We used the beamline to collect data on crystal of single stranded DNA binding protein. This has a novel structure and due to poor quality (SeMEt all merohedral twinned) crystals we attempted to collected data at a wavelength of 1.8Å on native crystals to solve by location of the anomalous sulfur substructure. We collected 720° of data to 2.4Å, R-merge 9%, multiplicity 20. However, SOLVE, SHAKENBAKE, SHELX and RANTAN have all failed to located the sulfur atoms.