

Discovery and biosynthetic pathway analysis of cyclopentane- β -lactone globilactone A



戈惠明

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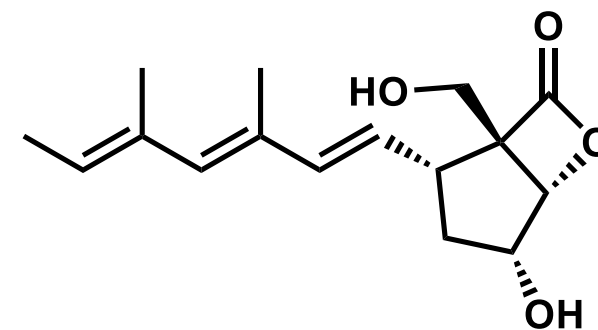
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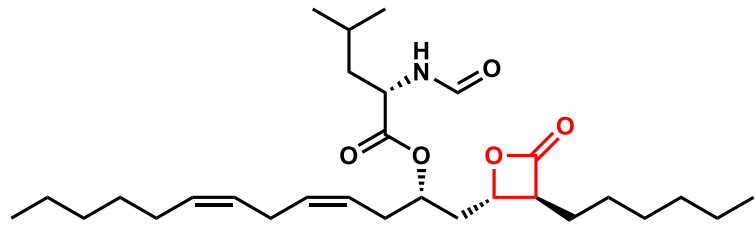
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Yongle Luo

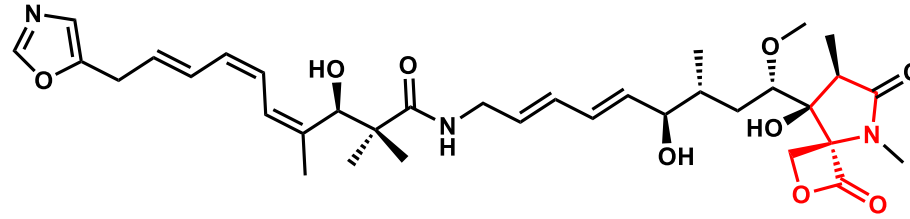
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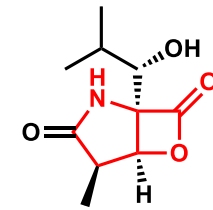
1.1 β -lactone natural products



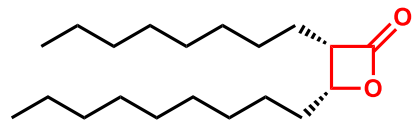
Lipstatin



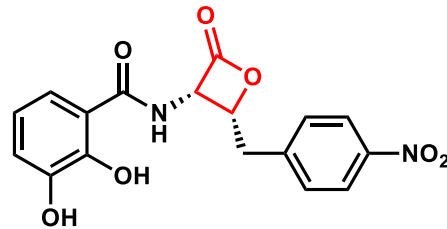
Oxazolomycin



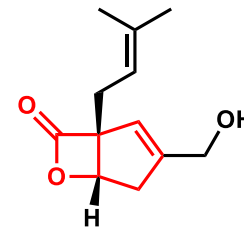
Omuralide



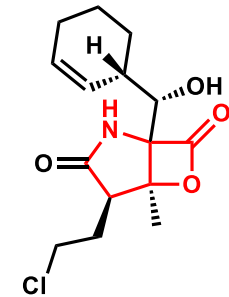
cis-3-Octyl-4-nonyloxetan-2-one



Obafluorin

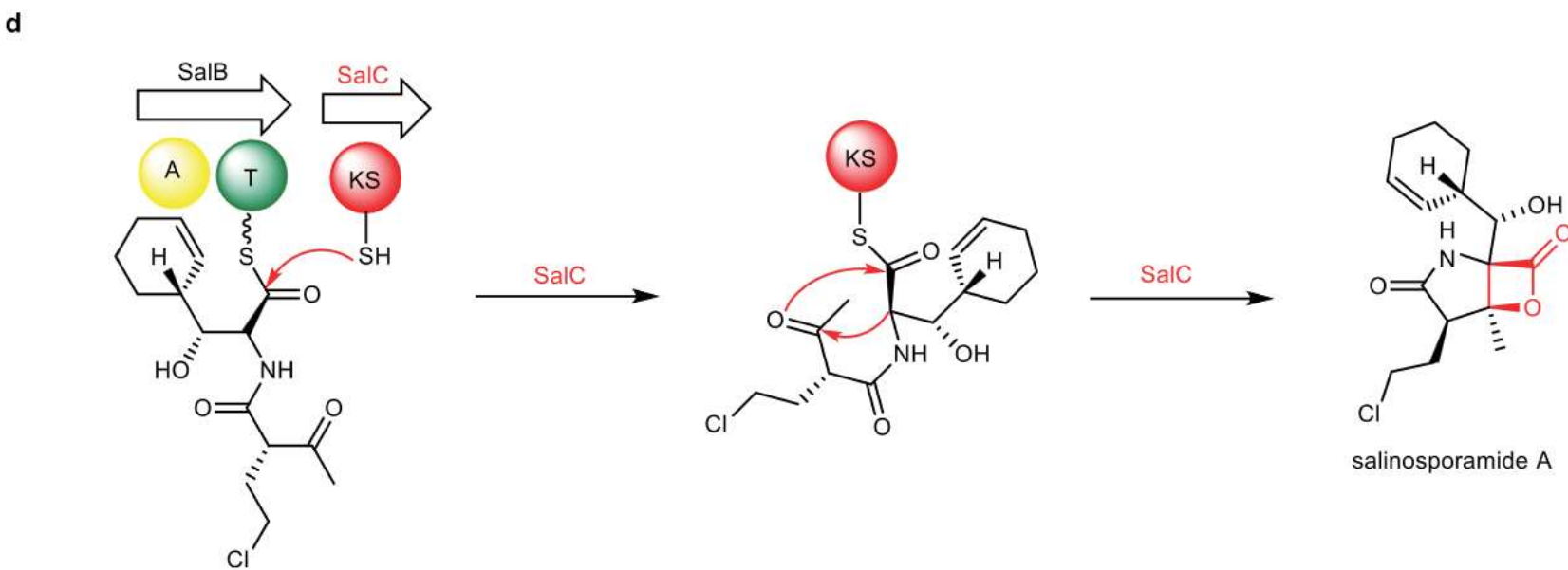
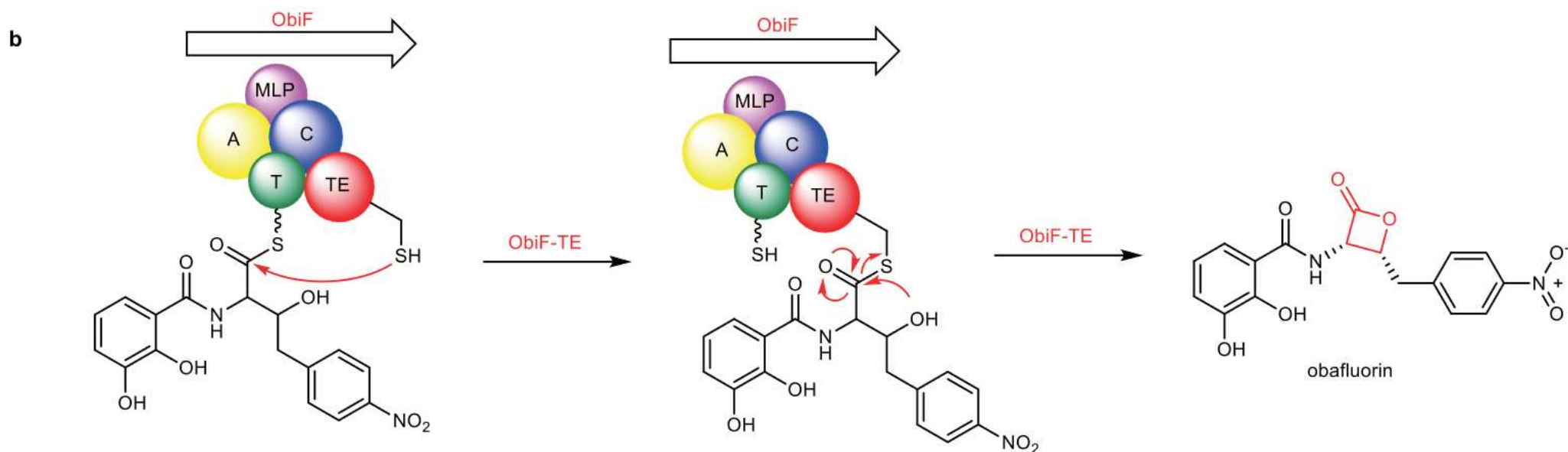


Vibralactone



Salinosporamide A

1.2 Enzymatic mechanisms of known β -lactone ring biosynthesis



2.1 Discovery of Globilactones

Identify a putative PKS and NRPS-like hybrid BGC from *S. globisporus* NPDC060234



Construct a cosmid library of *S. globisporus* NPDC060234 genomic DNA



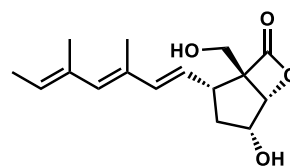
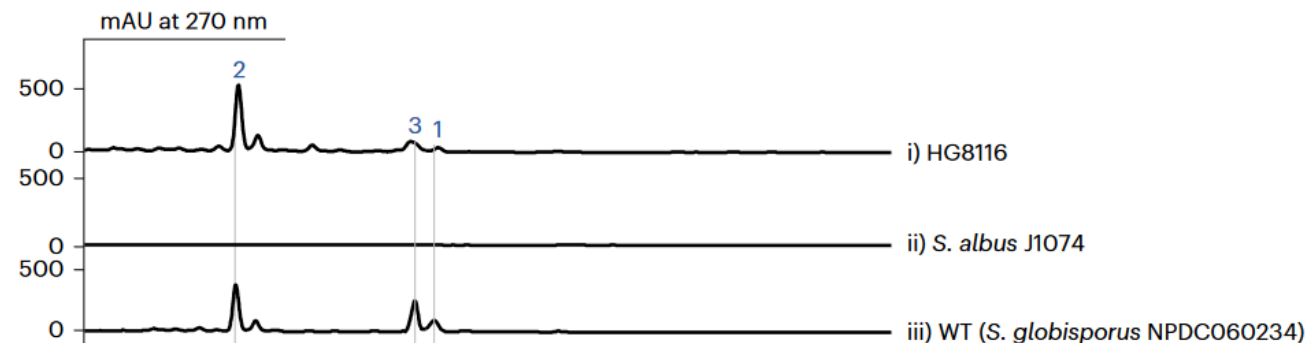
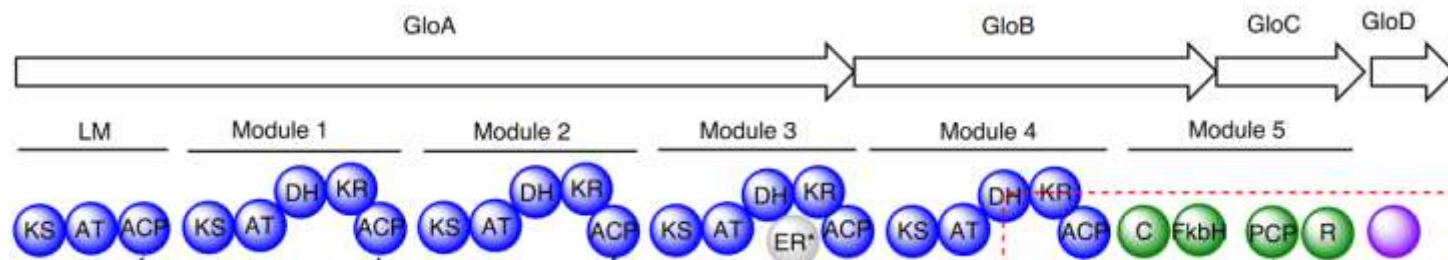
Find one positive cosmid **pHG8116** from ~2000 clones



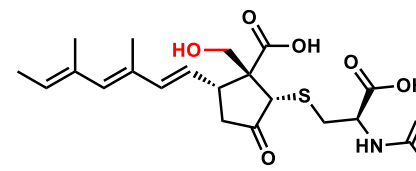
Heterologous expression of pHG8116 cosmid in *S. albus* J1074 host



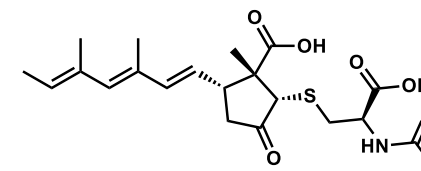
LC-MS analysis



1
Globilactone A

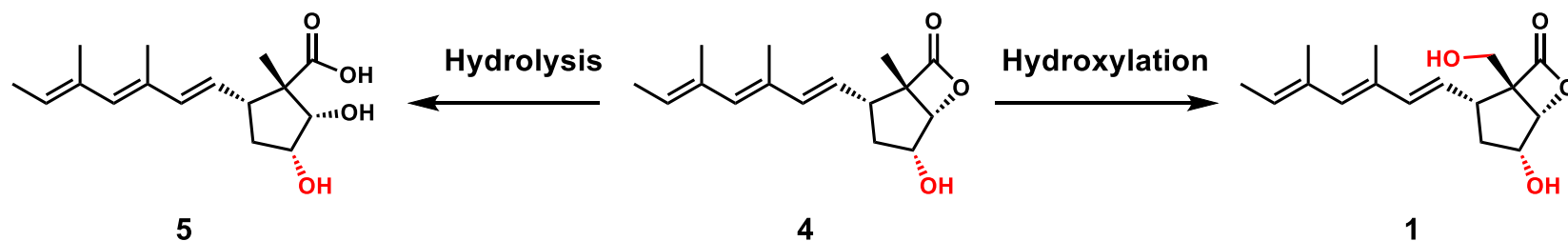
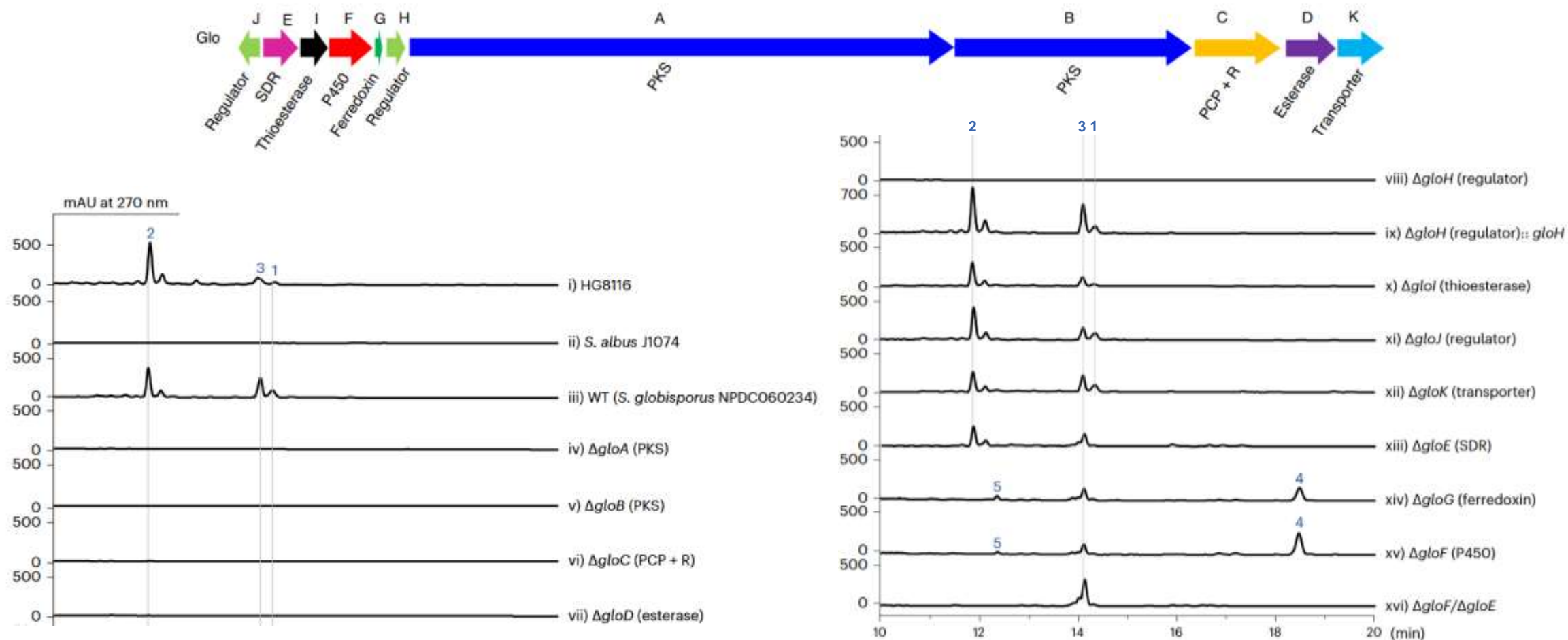


2
Globilactone B

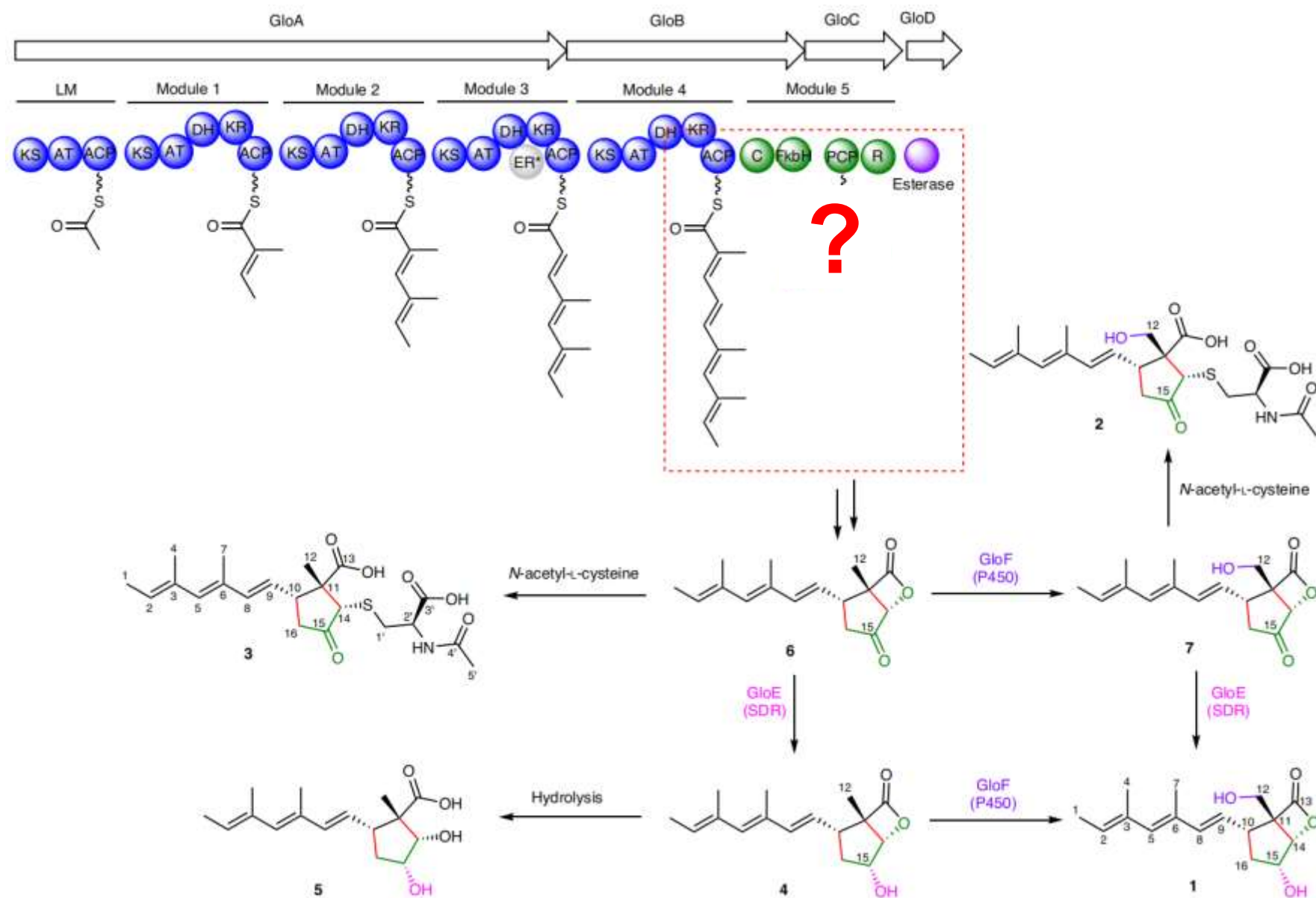


3
Globilactone C

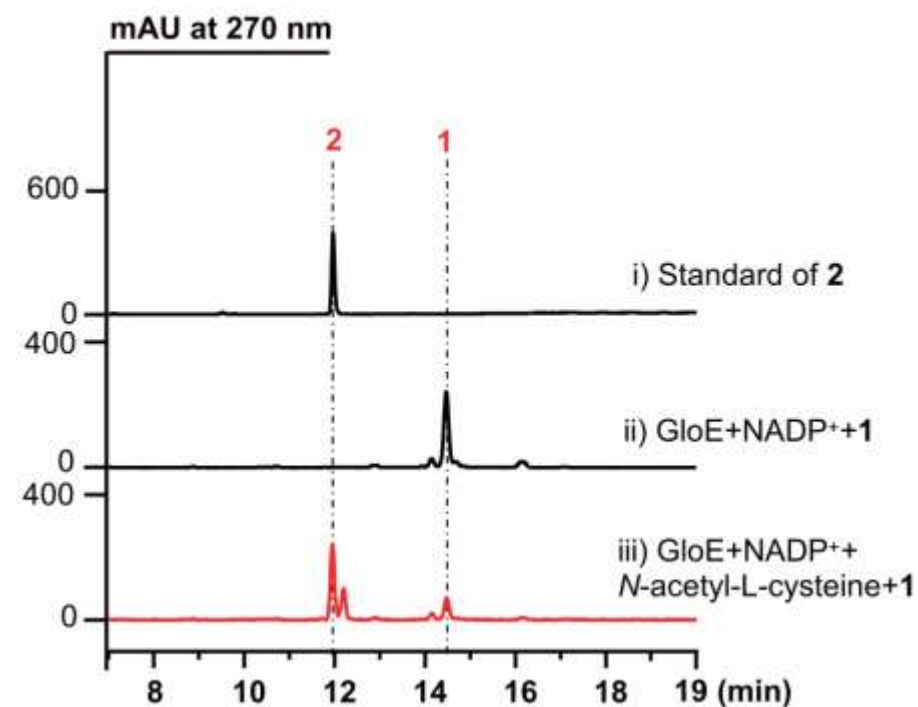
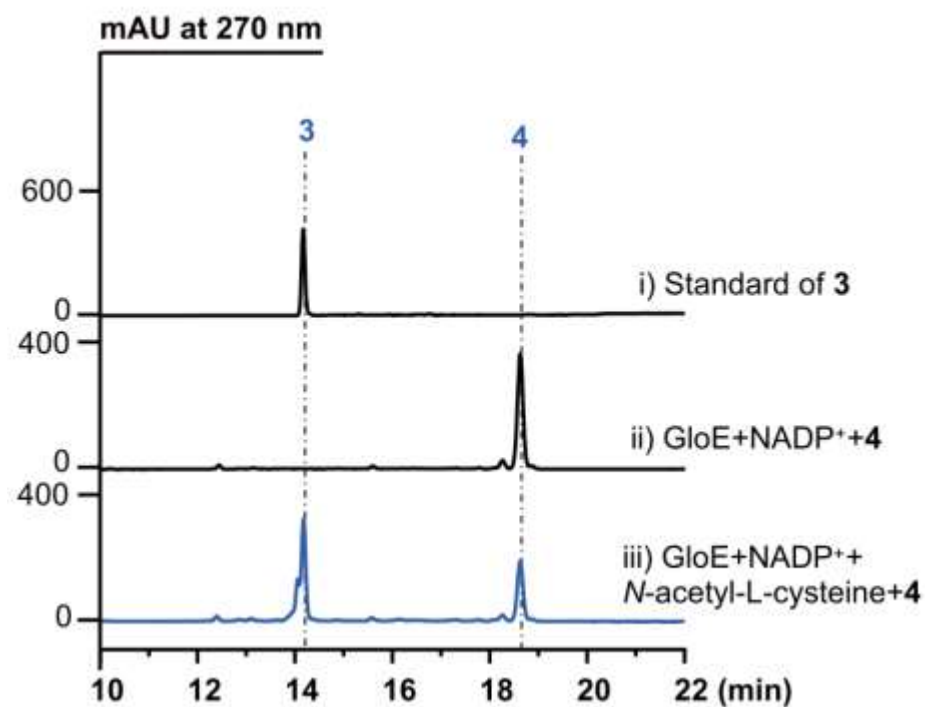
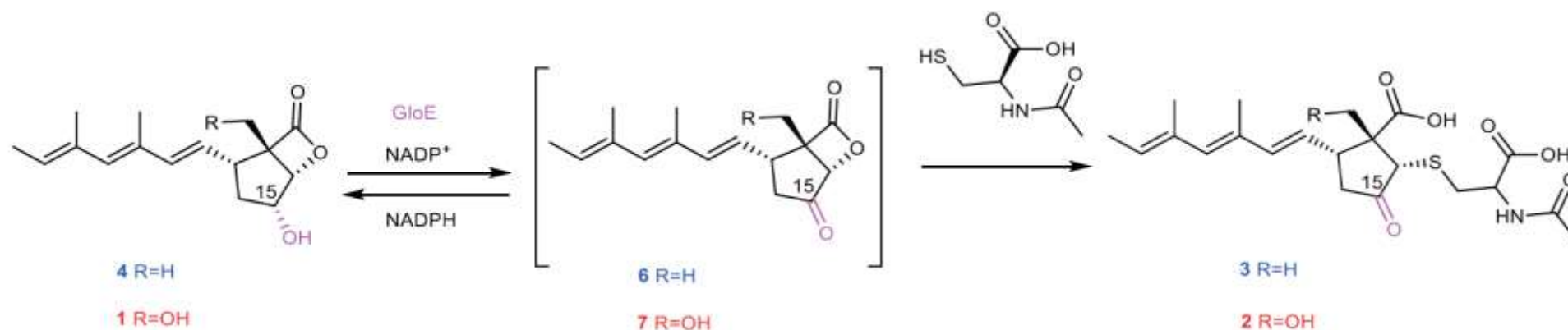
2.2 Functional characterization of the *glo* biosynthetic cluster



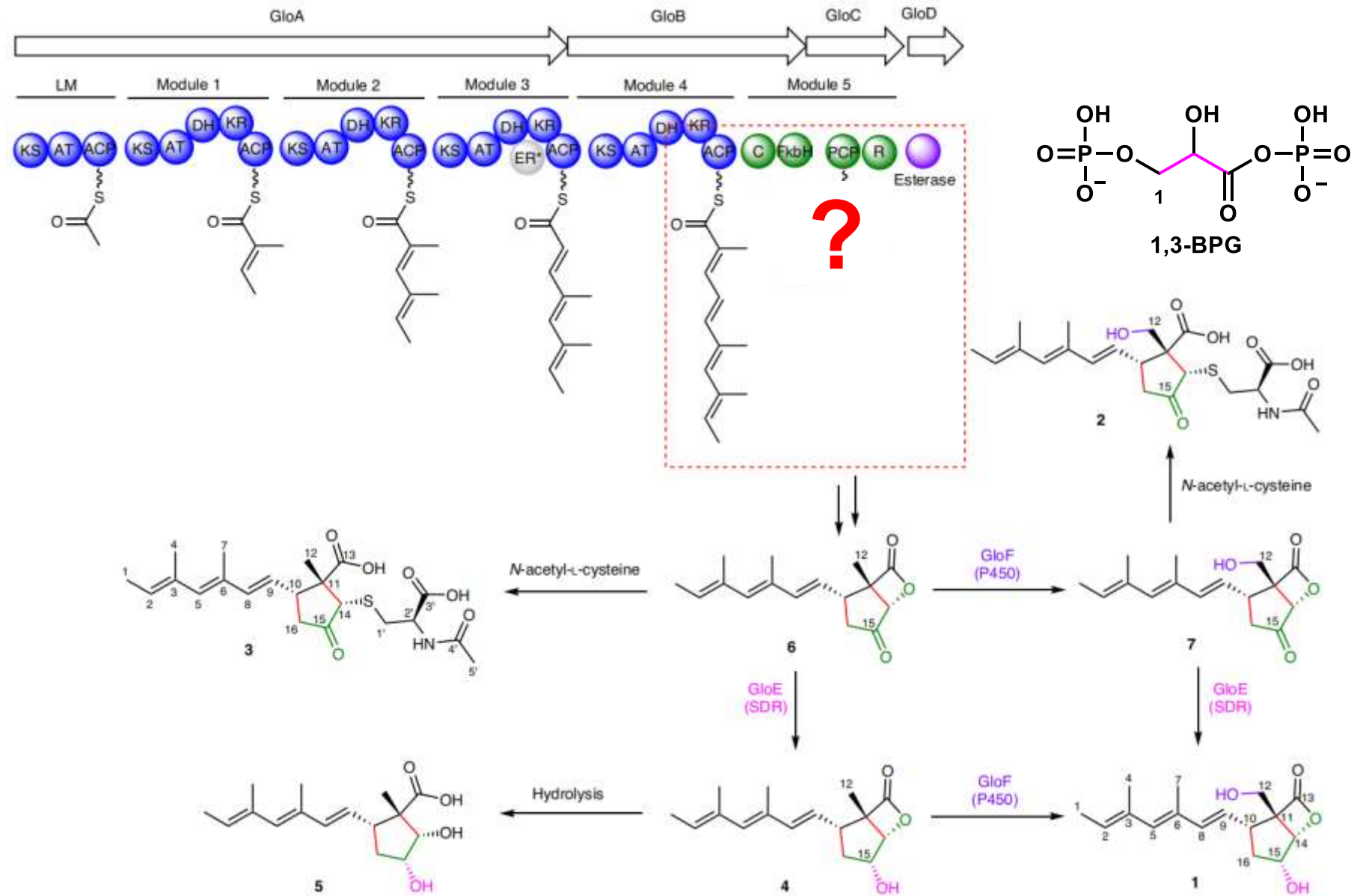
2.3 Proposed biosynthetic pathway of compound 1-3



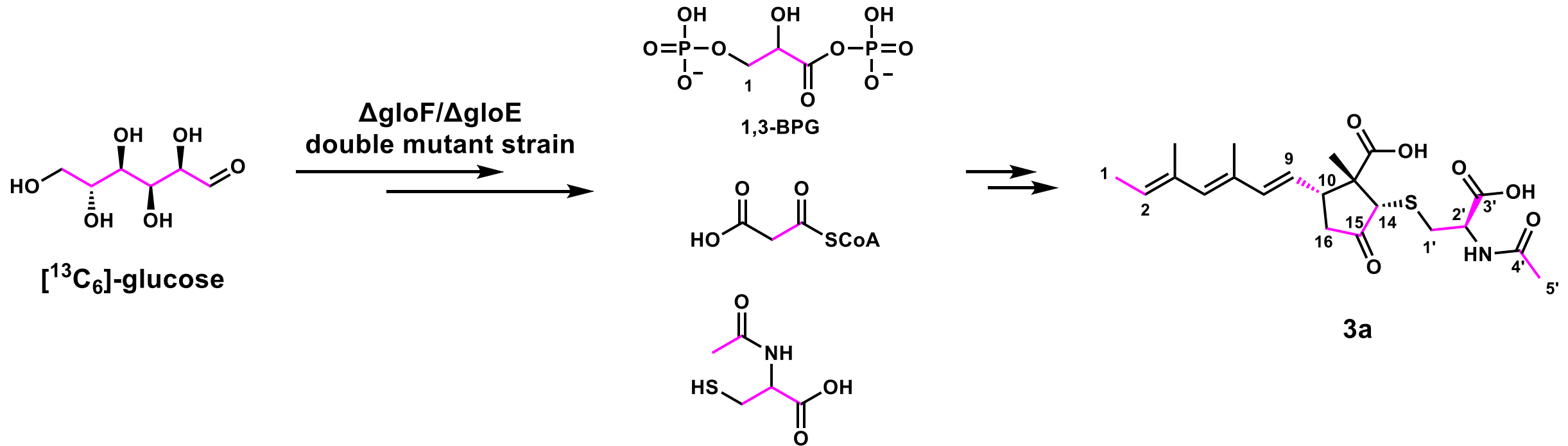
2.4 Biochemical characterization of GloE (SDR)



The biosynthetic origin of C14-C16?



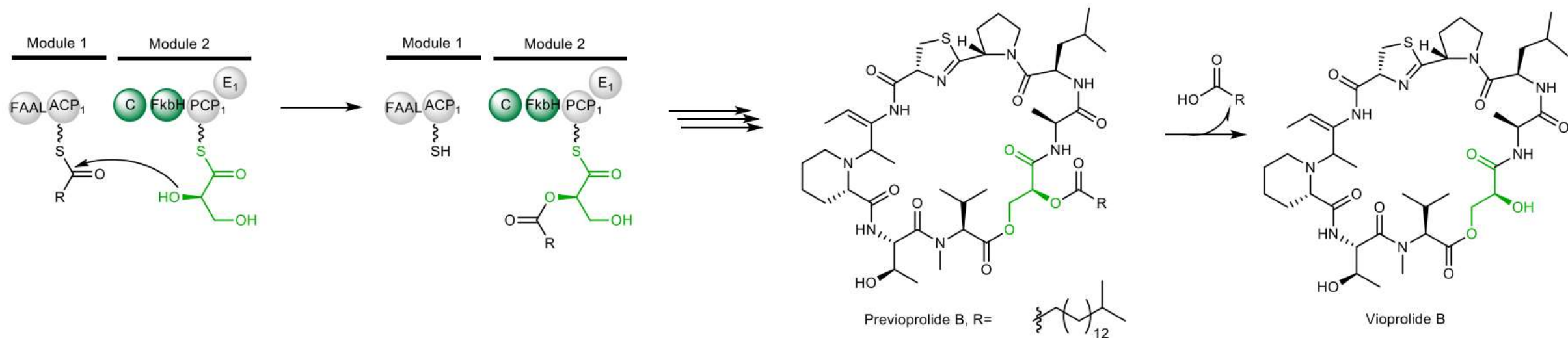
3.1 [$^{13}\text{C}_6$] glucose feeding experiment reveals the biosynthetic origin



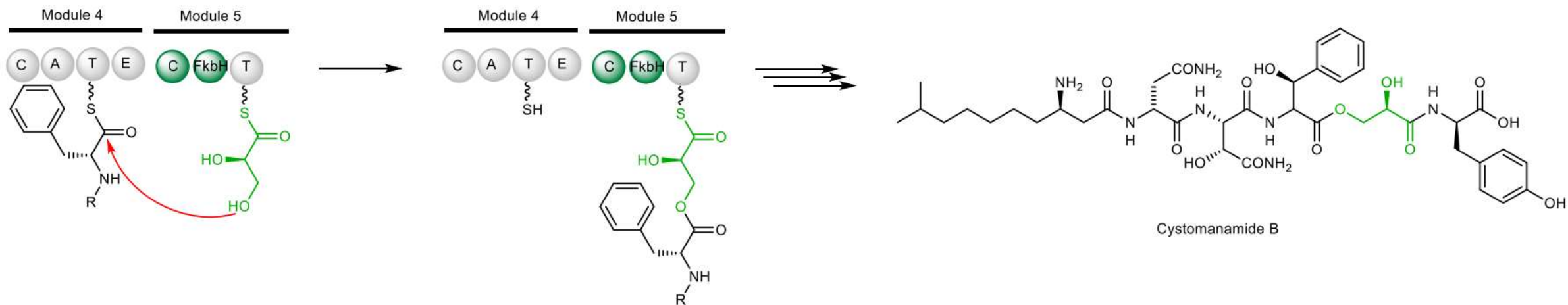
C14–C15–C16 three-carbon unit in globilactones is most likely originated from 1,3-BPG

Background: FkbH diverts 1,3-BPG into PKS or NRPS assembly line

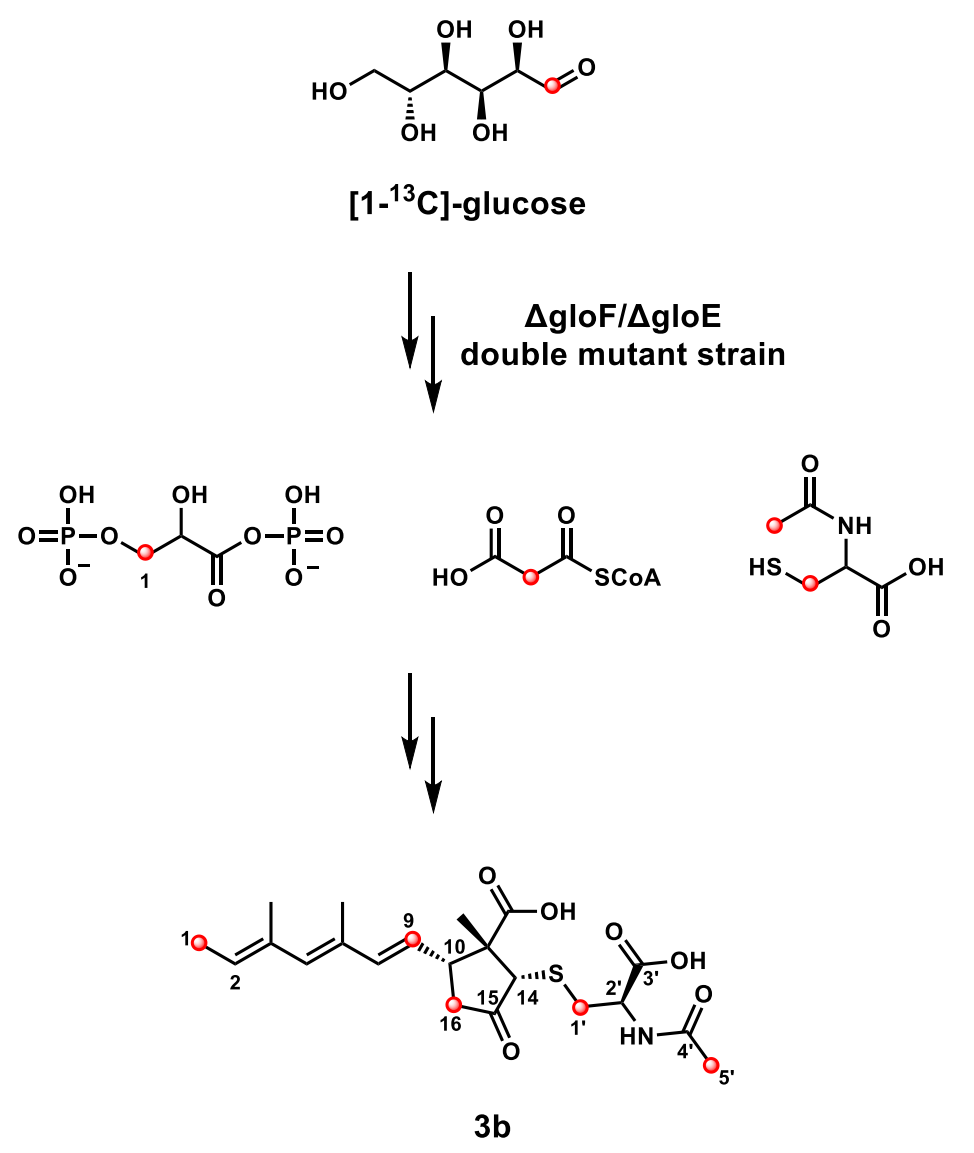
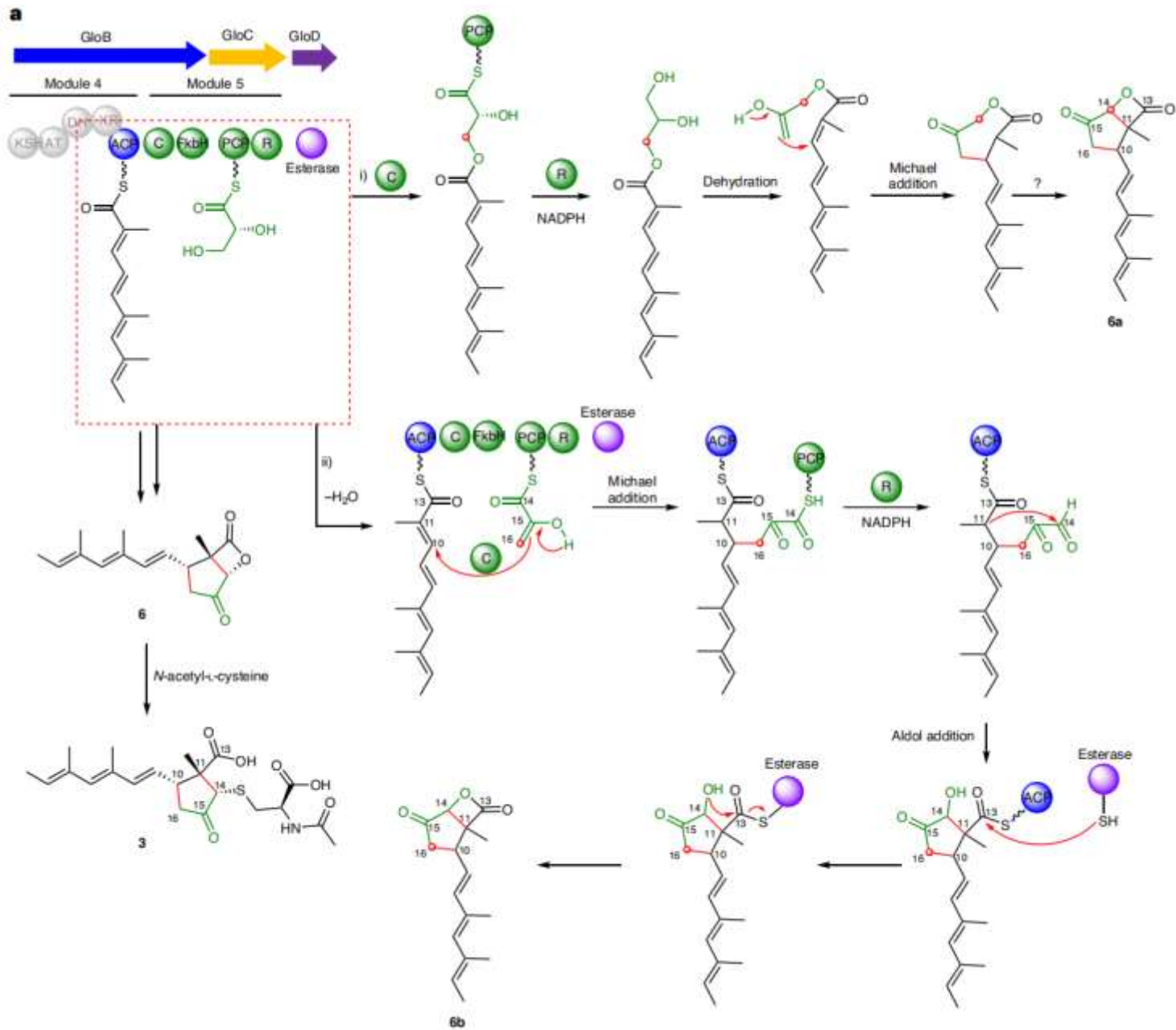
A



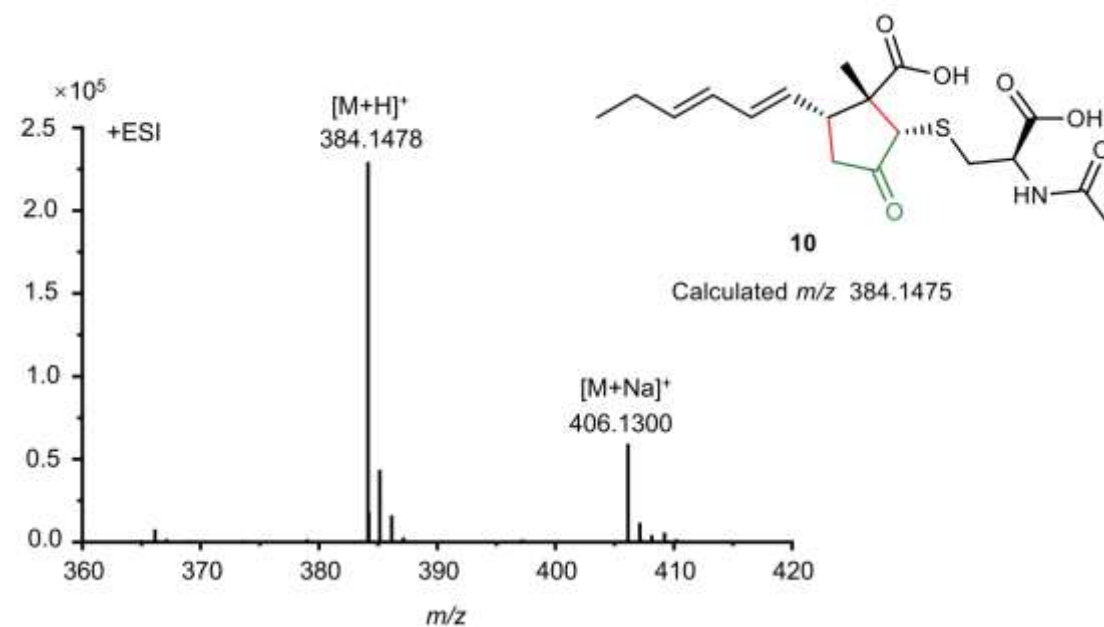
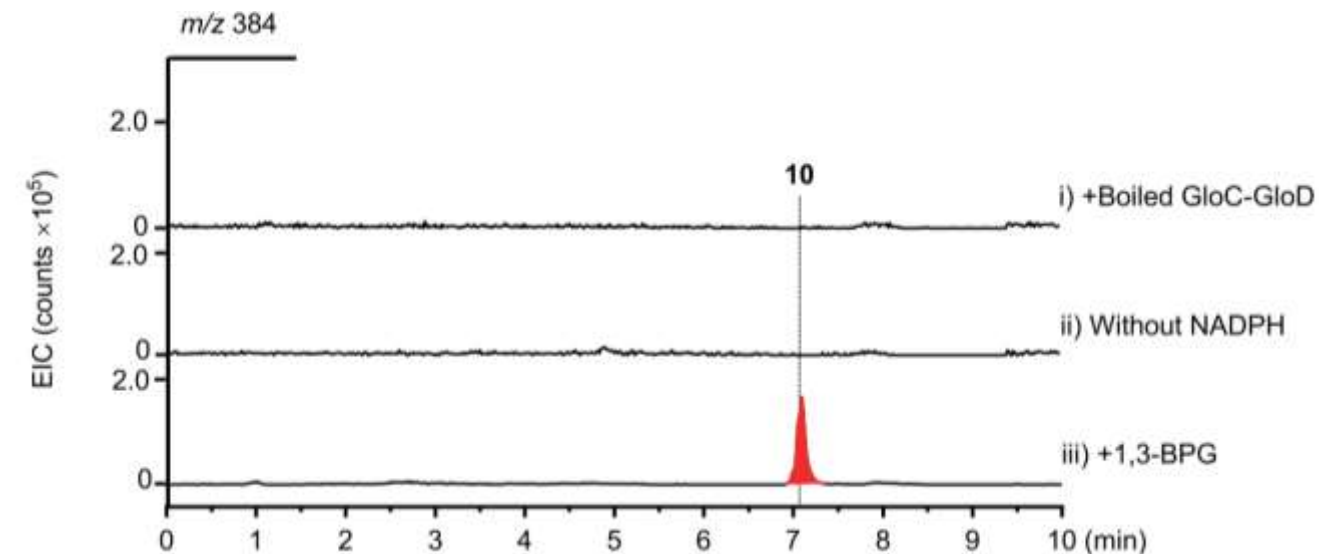
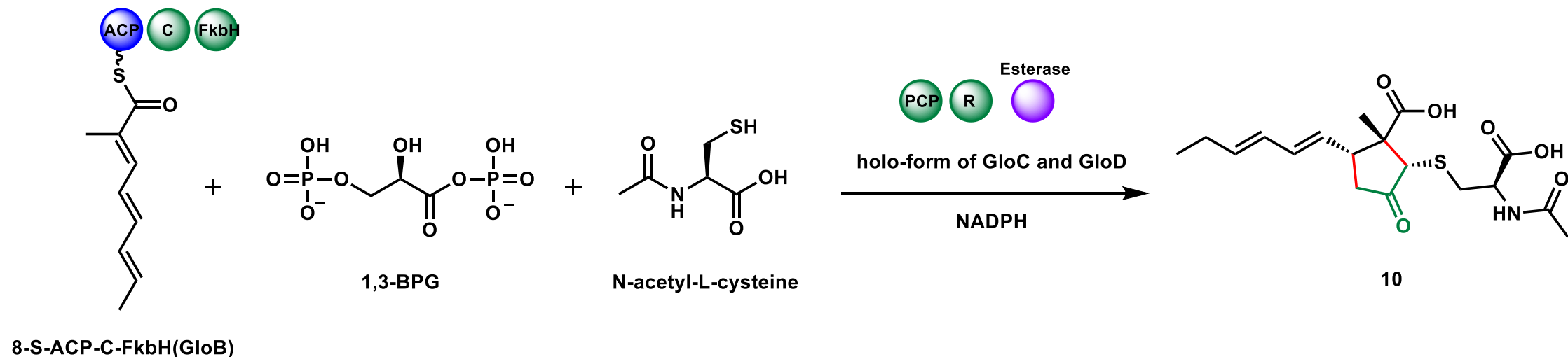
B



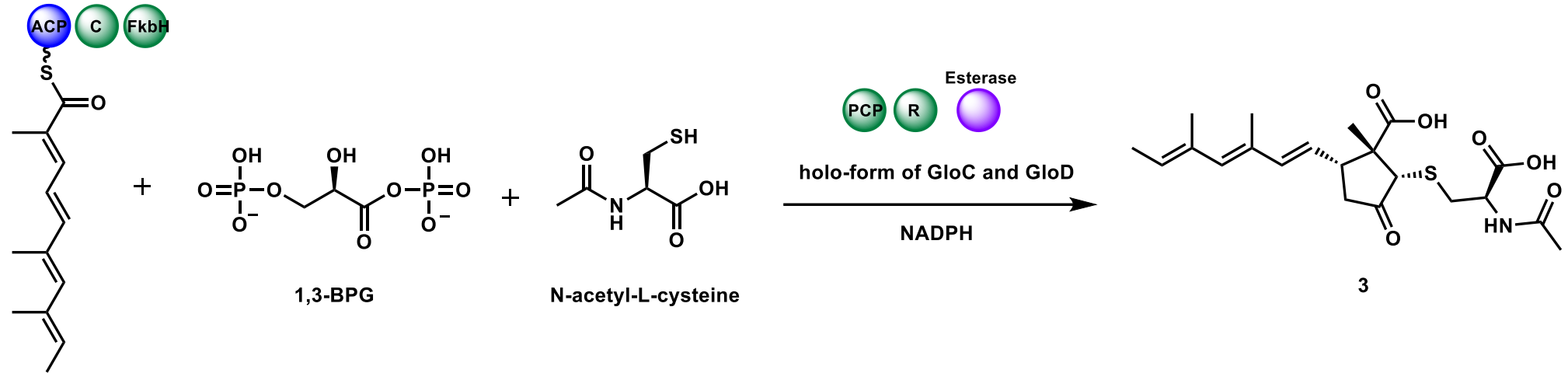
3.2 [1-¹³C] glucose feeding experiment reveals route ii more reasonable



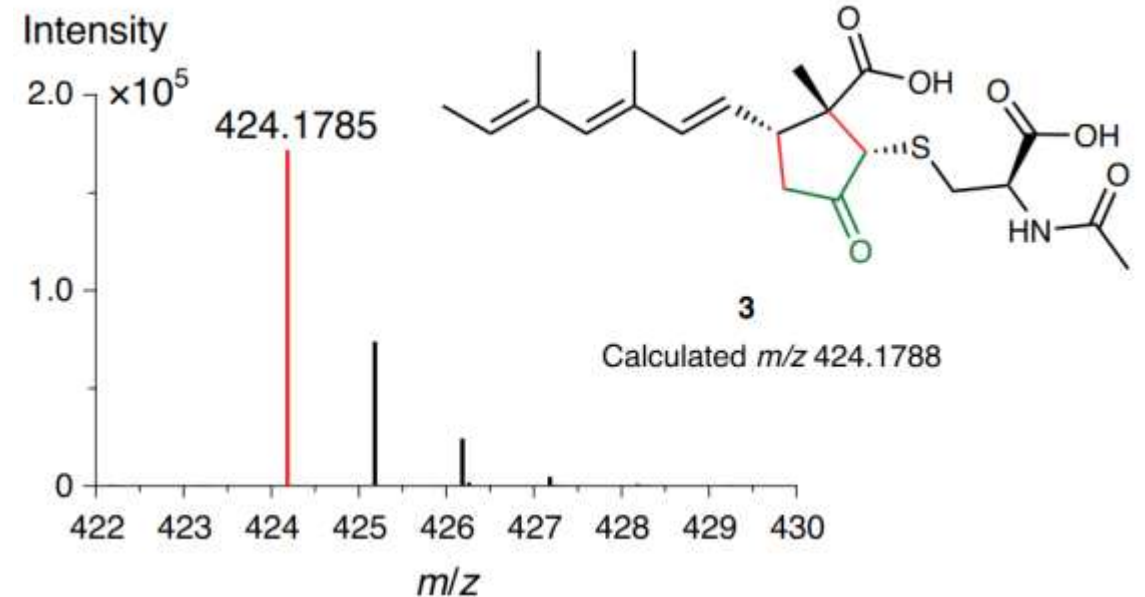
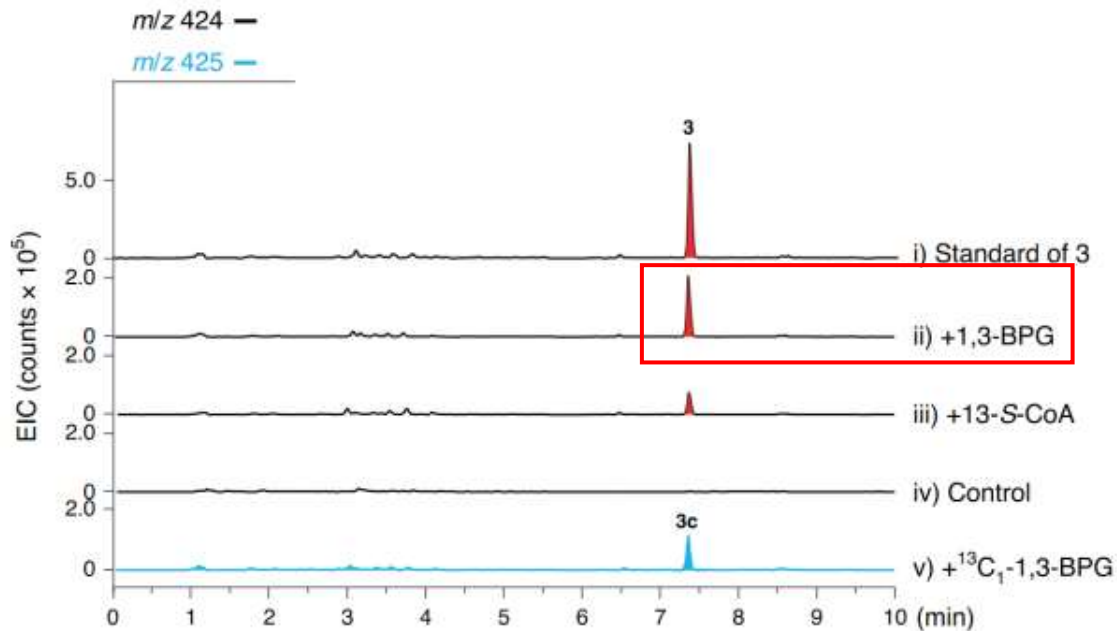
4.1 In vitro reconstitution of globilactone C



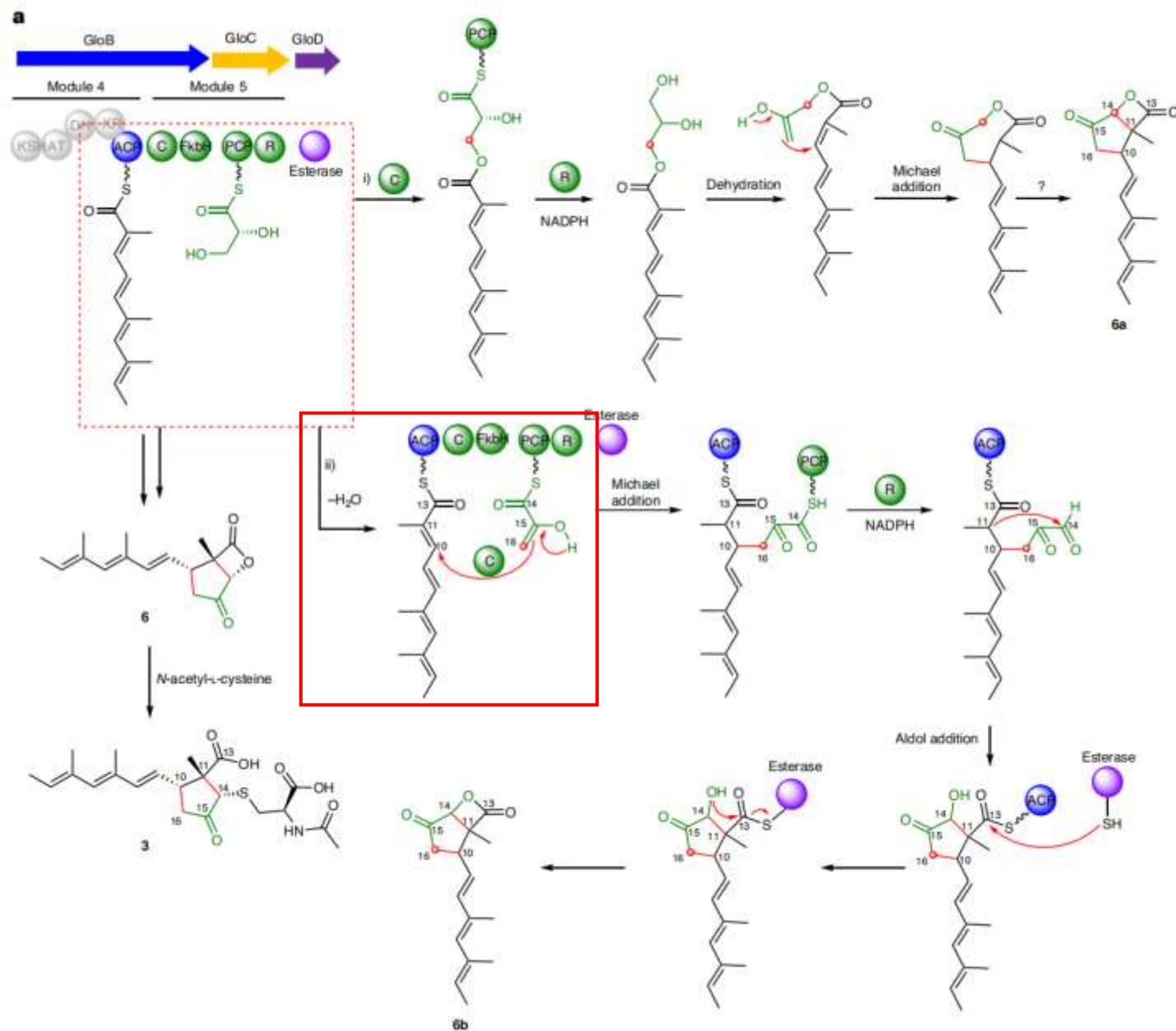
4.1 In vitro reconstitution of globilactone C



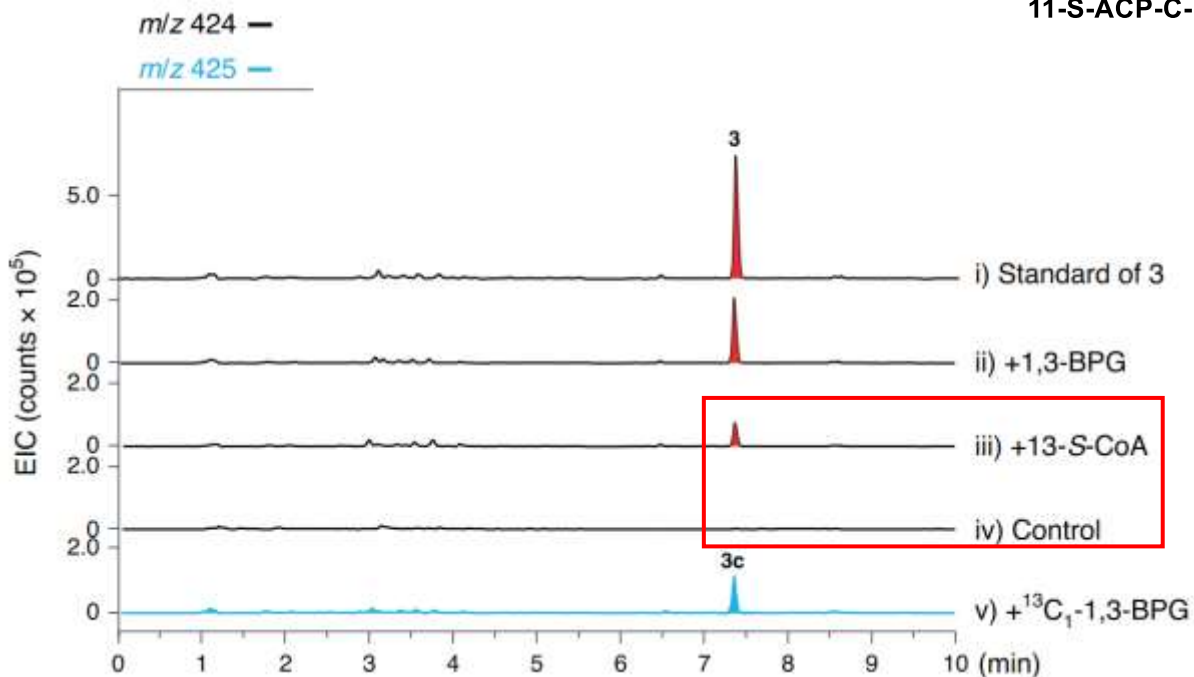
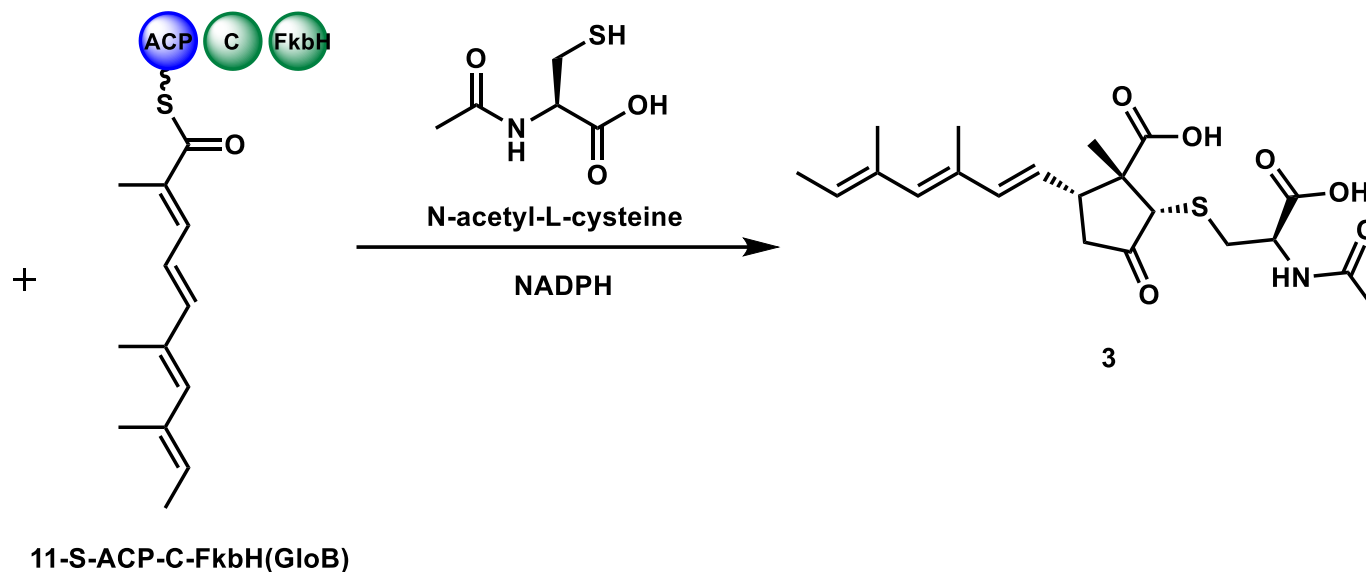
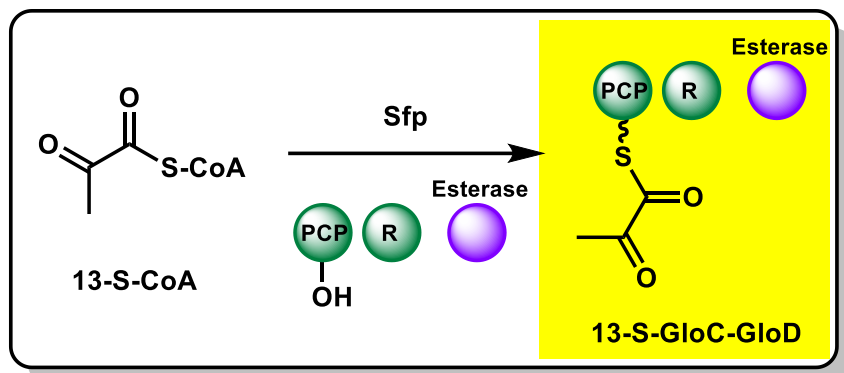
11-S-ACP-C-FkbH(GloB)



β -elimination step in route ii

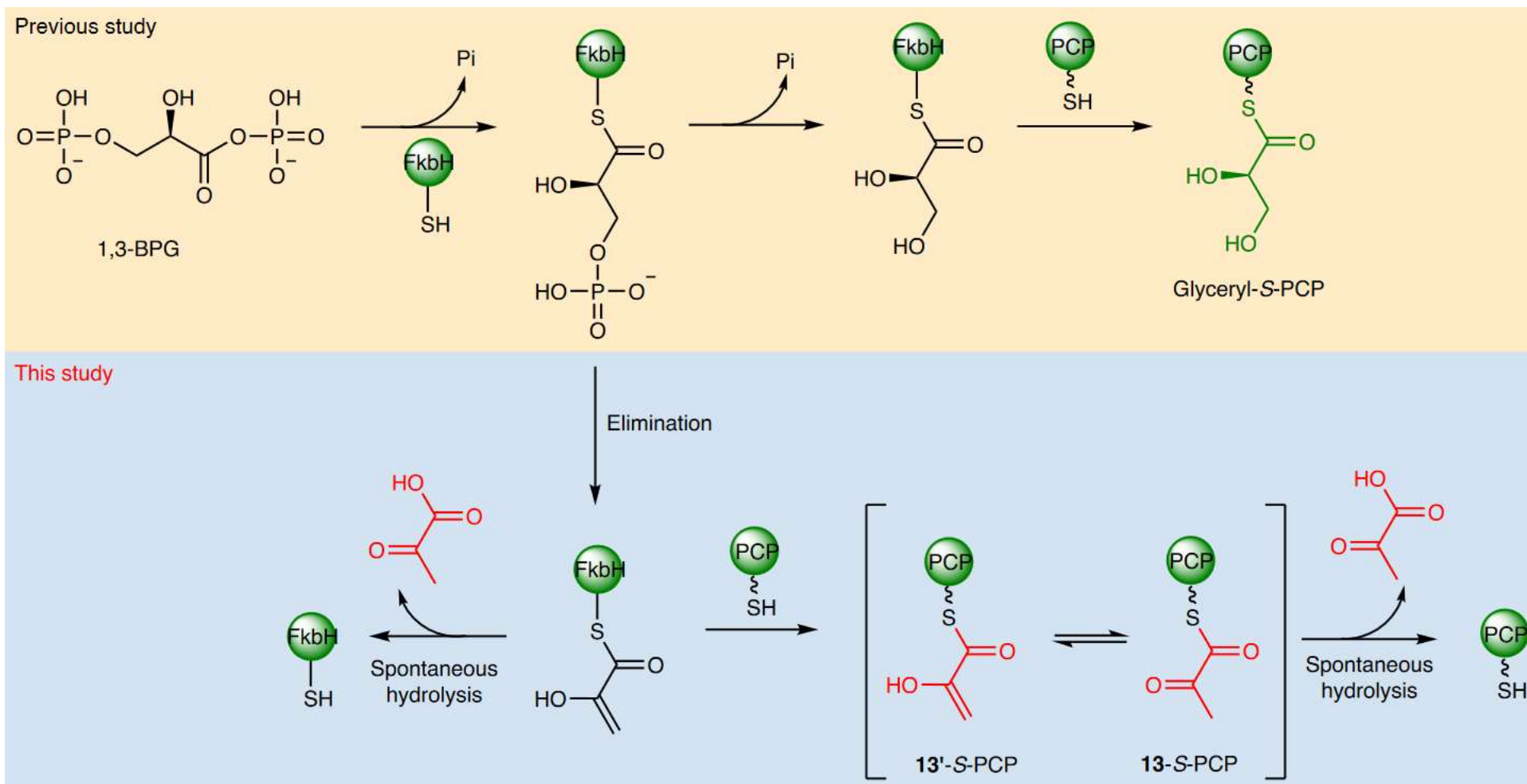


4.2 FkbH domain catalyzed dehydration to form pyruvoyl-S-PCP

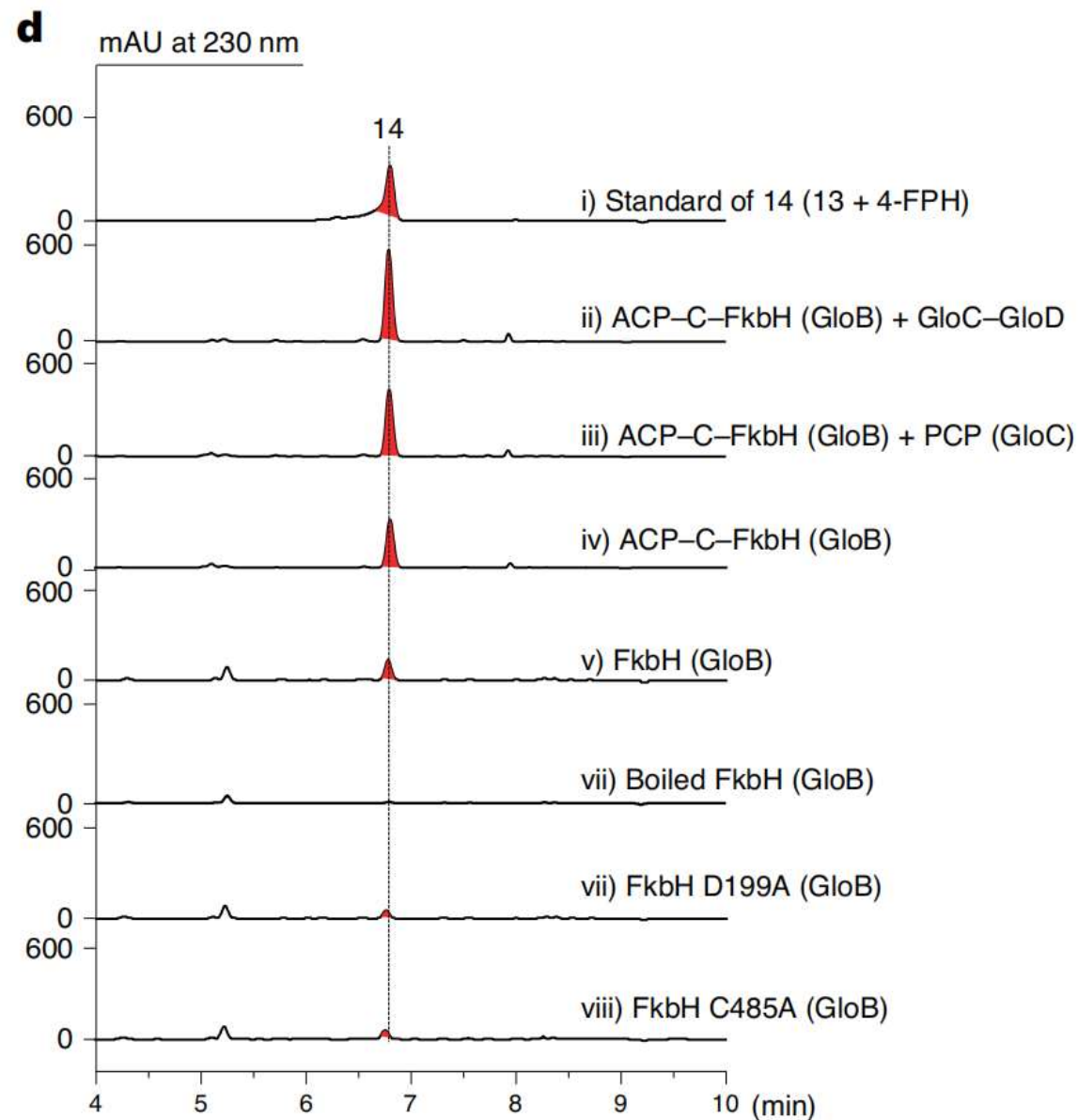
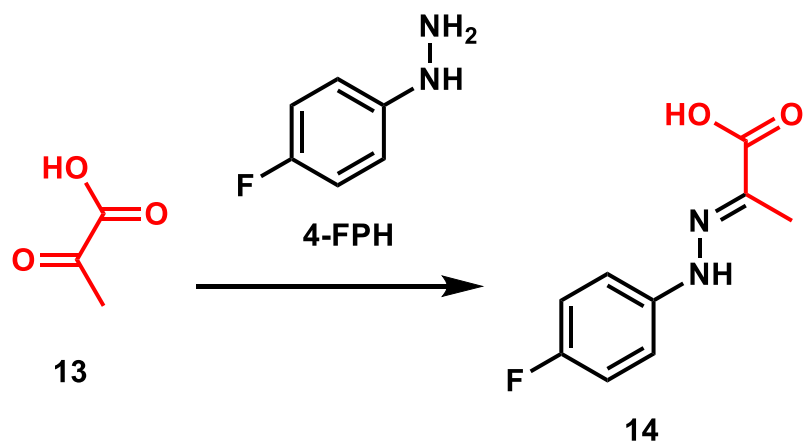


β -elimination step is indeed involved in the biosynthesis of globilactone biosynthesis

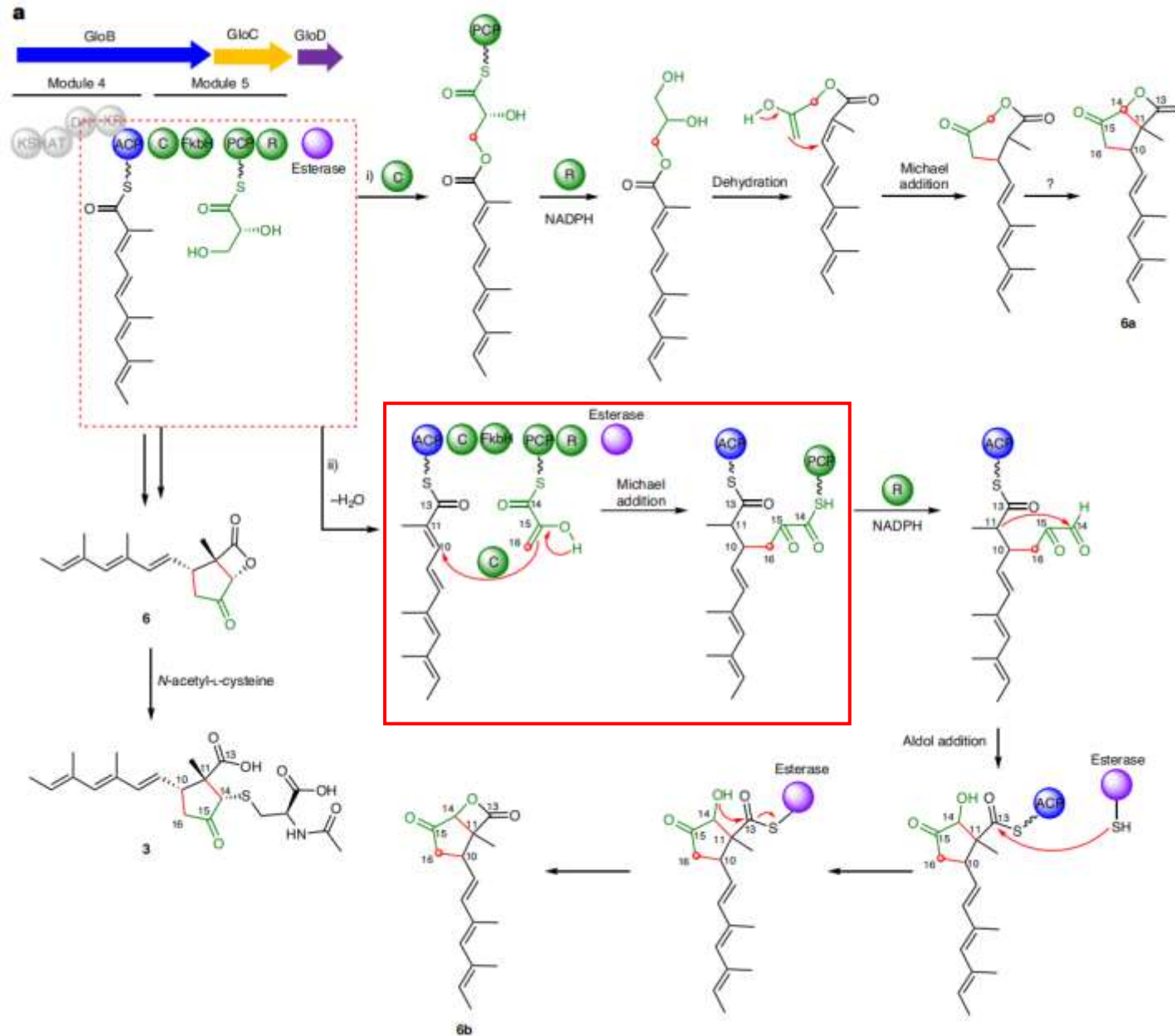
4.2 Proposed mechanism of 13-S-PCP formation catalyzed by FkbH



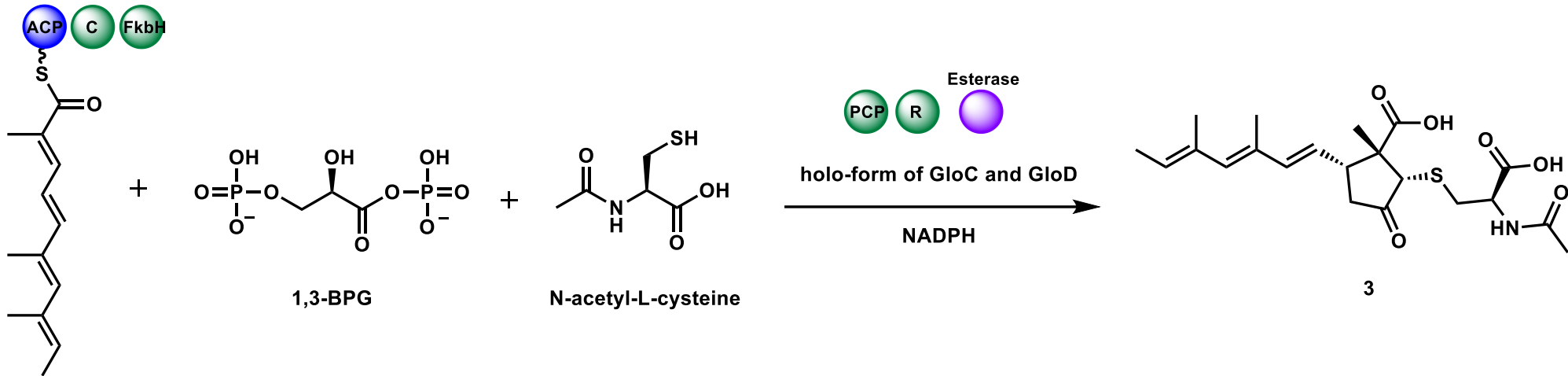
4.3 FkbH domain catalyzed dehydration to form pyruvoyl-S-PCP



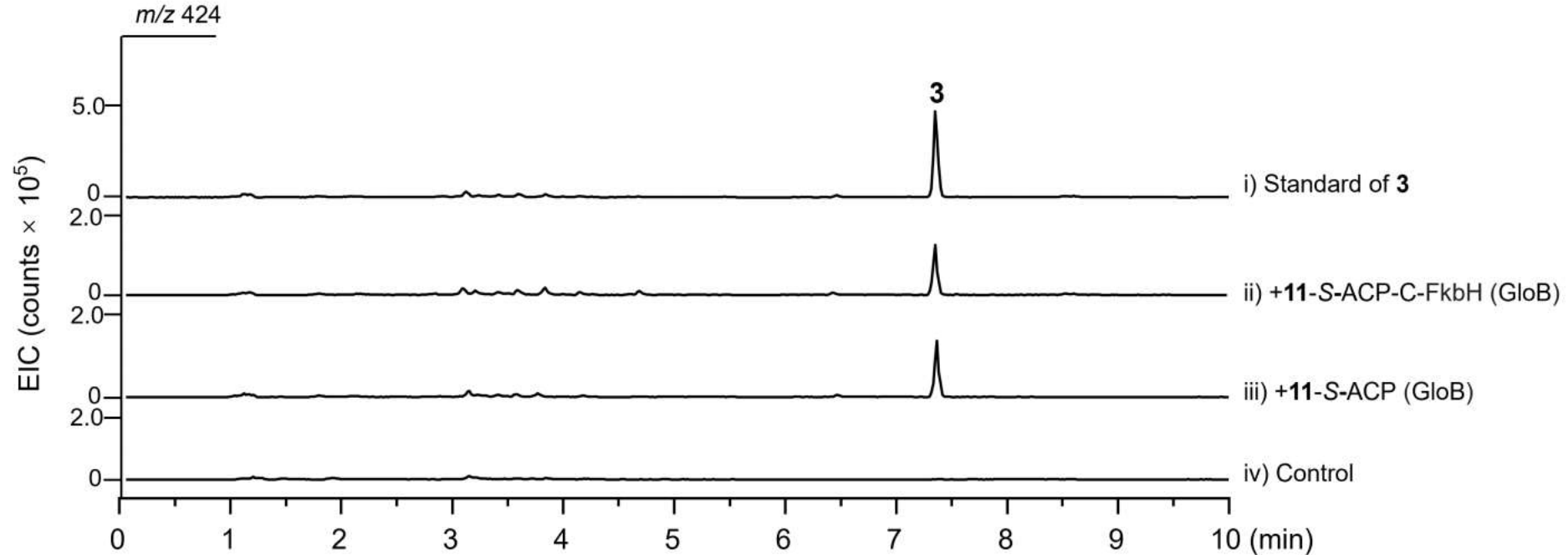
Michael additional step in route ii



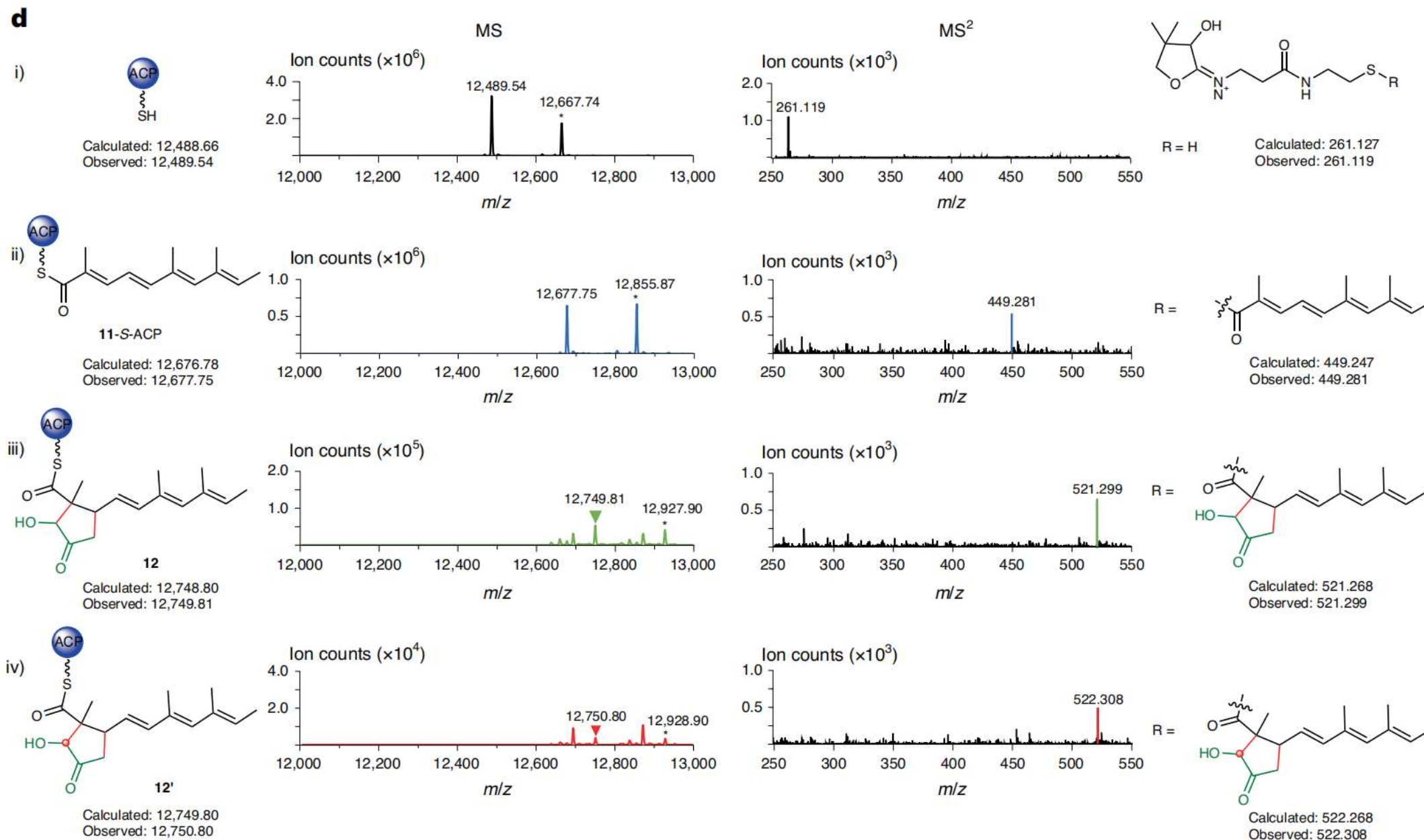
4.3 C domain (GloB) catalyzes the Michael-type addition



11-S-ACP-C-FkbH(GloB)

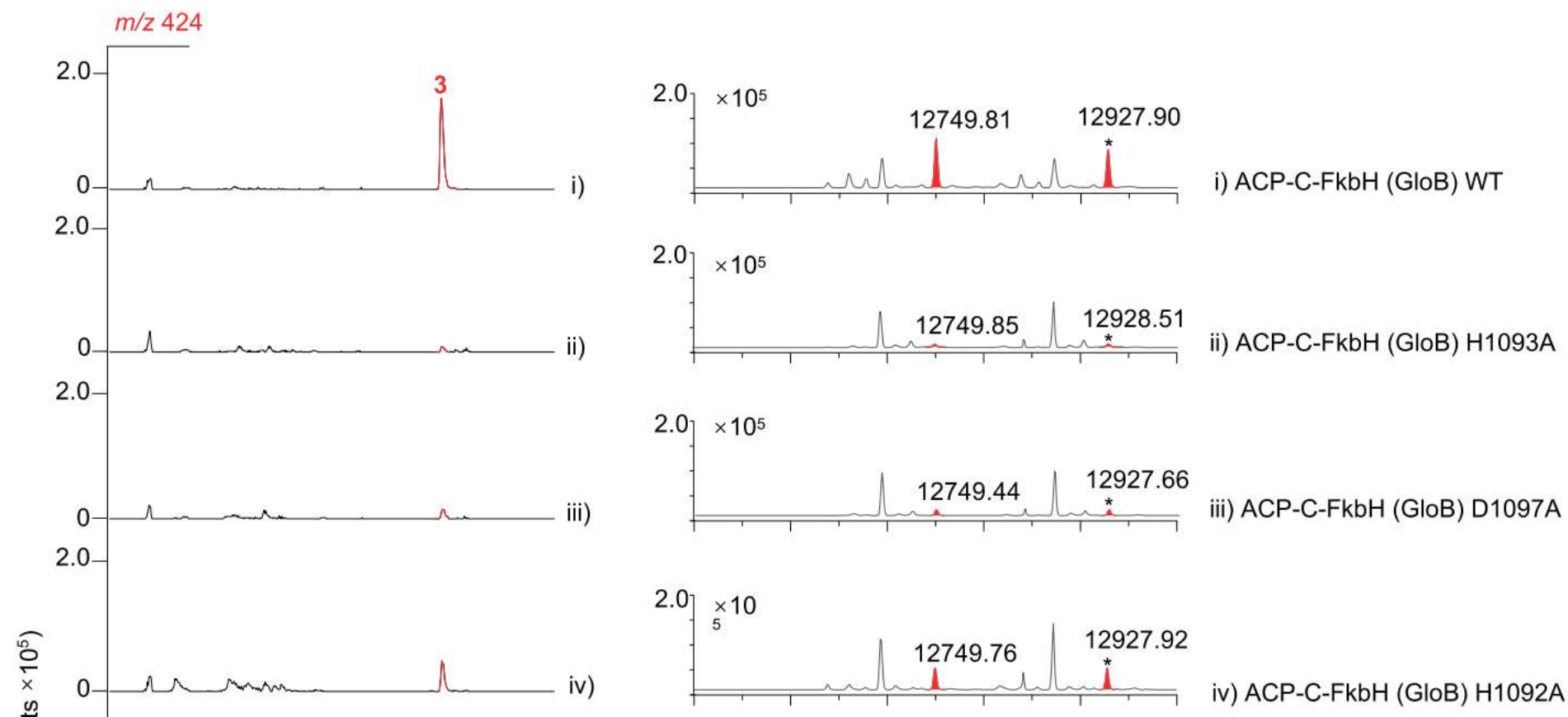


4.3 C domain (GloB) catalyzes the Michael-type addition

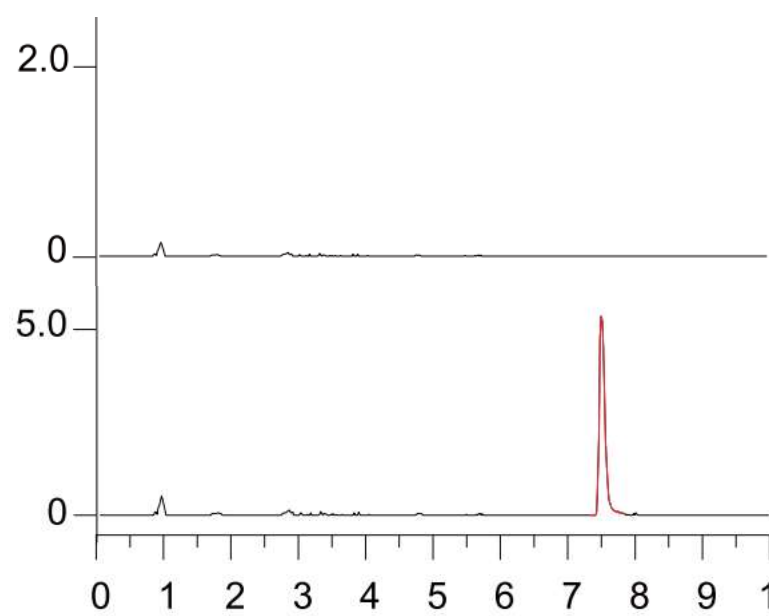
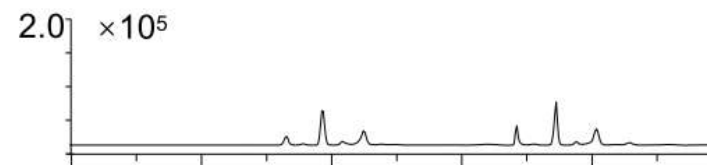
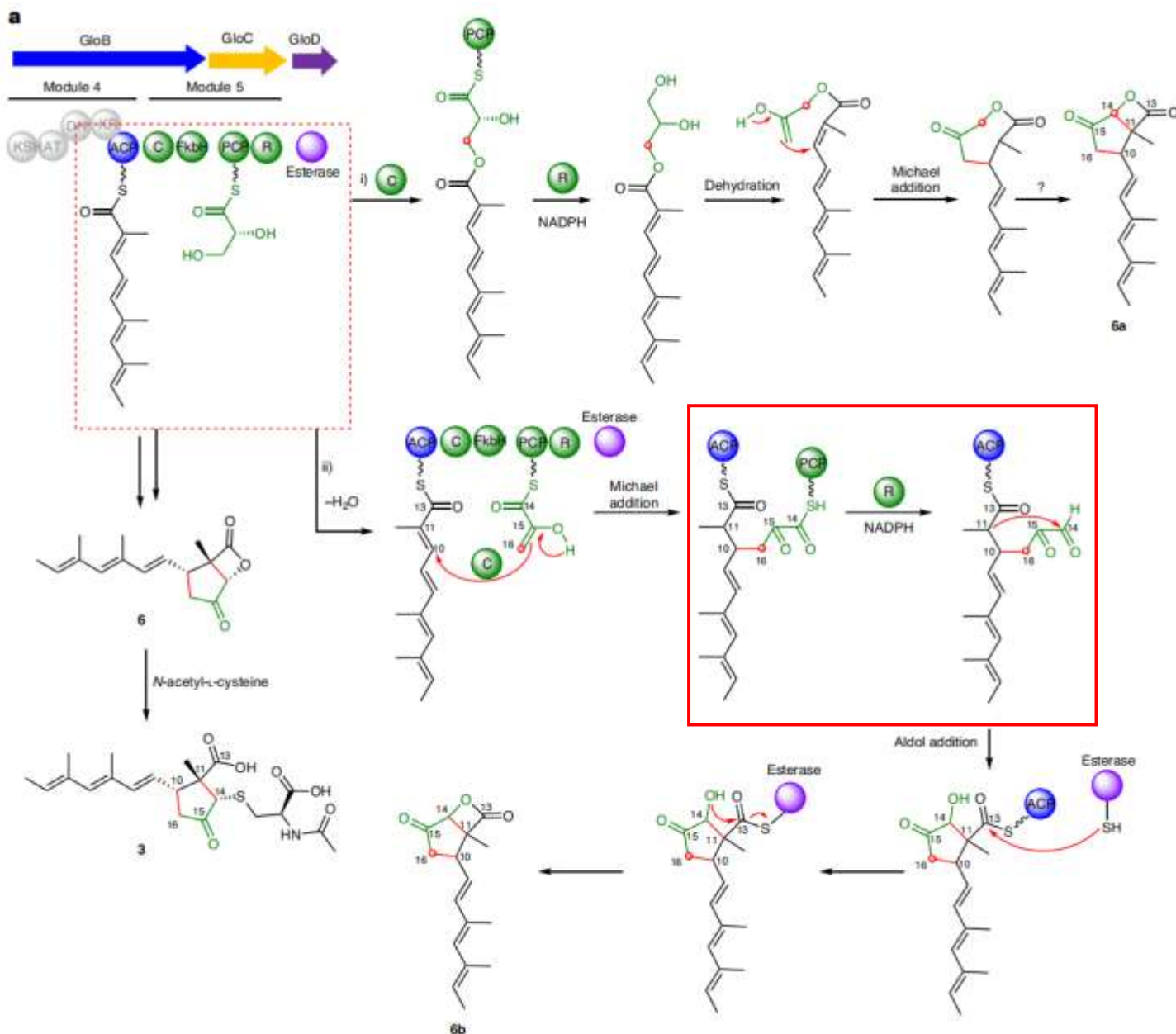


4.3 C domain (GloB) catalyzes the Michael-type addition

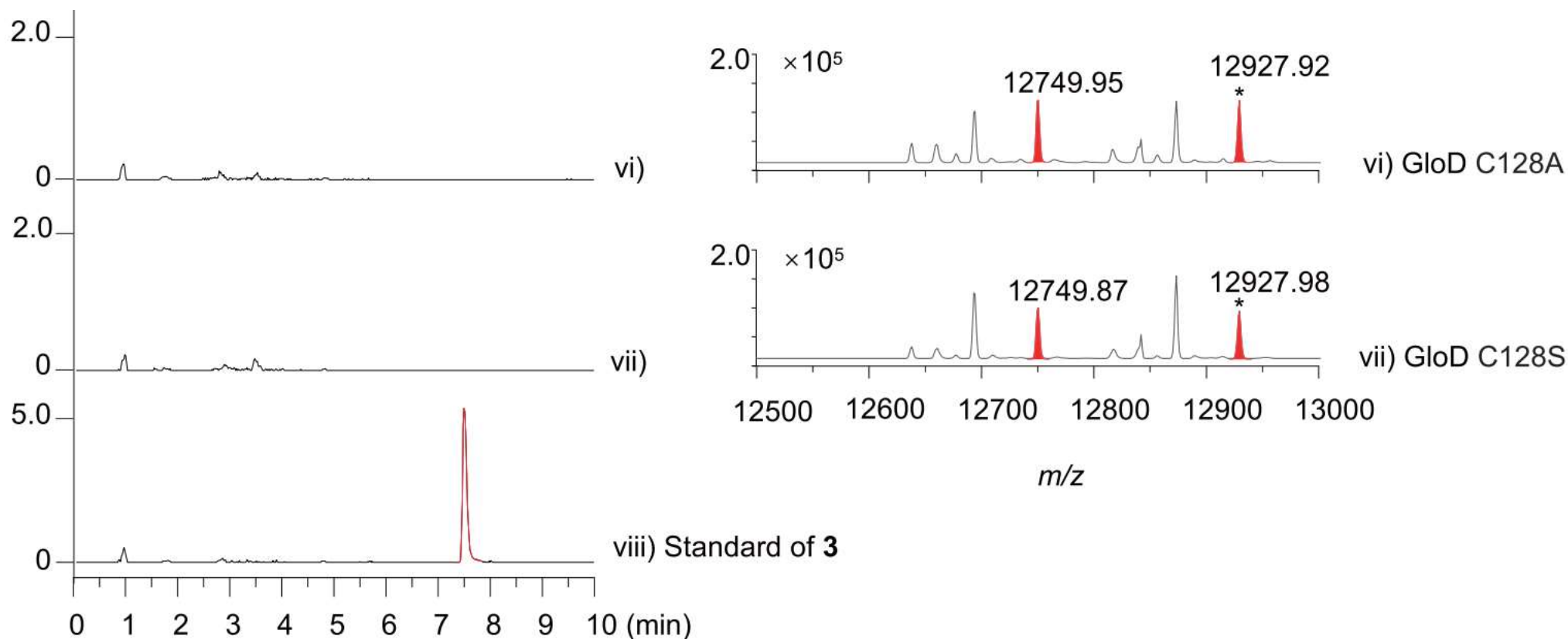
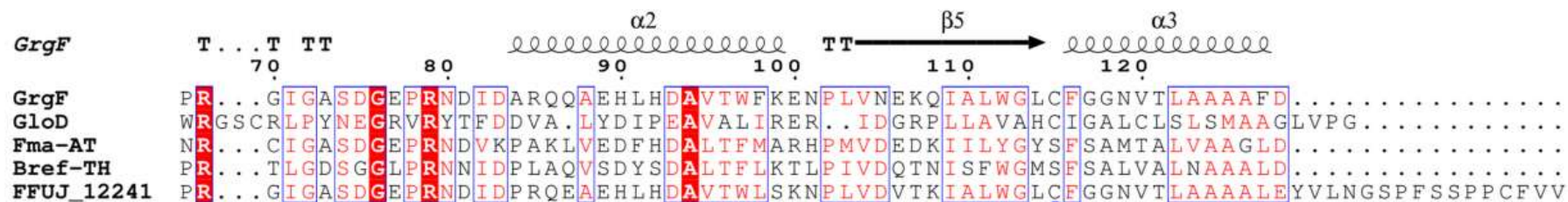
	60	70	80	90	100	110	120																																																																									
GloB-C	E	V	R	R	A	L	D	G	E	L	L	R	W	S	P	V	G	E	D	A	...	D	V	D	Q	R	L	R	E	L	M	E	P	P	F	D	L	A	A	A	.	P	L	W	R	F	E	L	L	A	F	P	S	G	E	Q	V	L	V	F	G	A	H	H	A	V	S	D	L	A	S	L	M	L	V	A				
AntC-C	E	V	C	A	A	G	S	V	R	P	V	L	Q	V	V	D	C	P	E	...	E	E	V	E	A	H	V	A	G	A	L	R	H	A	F	D	L	T	R	D	L	P	L	W	A	G	.	L	Y	G	T	G	A	S	R	T	L	V	L	V	.	L	H	H	S	A	A	D	G	W	S	L	R	P	L	A				
CesA-C	I	V	I	N	H	G	Q	L	K	I	H	Q	V	D	I	S	Q	Y	D	E	S	E	Q	I	K	V	L	E	K	I	L	R	E	D	R	Q	R	P	F	Q	L	D	E	Y	.	P	L	I	R	V	A	L	V	K	K	N	E	Y	E	Y	E	L	I	W	S	F	H	H	I	S	I	D	G	W	S	I	F	L	V	L
CyrD-C	V	V	L	K	Q	V	N	V	P	W	N	T	L	D	W	R	E	L	S	S	N	D	Q	Q	Q	L	K	Q	L	L	Q	T	O	R	E	Q	G	F	N	L	S	Q	A	.	P	L	M	R	C	T	L	V	R	L	G	E	D	N	Y	K	F	I	W	S	H	H	H	I	L	M	D	G	W	C	L	S	I	I	F	
DidB-C	E	V	L	P	A	P	A	P	A	E	A	L	P	V	V	Q	I	A	P	A	E	D	A	D	A	A	L	V	A	A	G	L	A	L	G	H	E	S	F	D	L	A	V	G	.	P	L	A	R	F	R	L	F	R	C	A	P	D	L	H	G	L	G	V	V	L	H	H	I	V	A	D	G	W	S	A	G	L	C	L
DidE-C	I	V	V	P	E	.	R	R	G	V	L	E	I	R	A	A	A	E	P	...	S	S	L	Q	A	L	A	E	A	E	A	R	K	A	F	D	L	T	R	D	I	P	F	R	A	A	.	L	V	P	V	G	P	E	R	H	L	L	L	I	T	M	H	H	I	A	S	D	G	W	S	M	G	V	F	L				
HctE-C	Q	V	D	R	H	Q	E	V	D	F	L	Q	V	D	A	S	T	W	S	K	...	E	E	L	N	Q	R	V	V	E	A	H	K	H	P	F	N	L	E	K	E	.	S	G	M	R	V	R	W	F	V	R	S	F	Q	E	H	I	L	L	L	T	I	H	H	I	A	C	D	G	W	S	L	D	I	I	F			
KtzE-C	L	I	M	A	R	A	E	L	P	W	R	E	V	R	L	A	D	D	G	...	A	F	Q	R	F	L	A	D	D	R	D	E	G	F	D	L	A	R	P	.	P	L	V	R	M	T	L	V	H	I	G	Q	T	R	A	E	L	V	L	S	A	H	H	A	L	F	D	G	W	S	E	P	L	I	A					
VioA-C	H	V	R	P	A	A	R	M	P	L	P	L	S	D	I	S	G	L	P	A	S	R	R	G	E	E	A	R	L	F	Q	E	E	A	R	R	P	L	D	V	E	R	G	R	.	P	L	L	R	A	R	L	I	Q	L	D	S	R	E	H	Q	L	L	L	T	V	H	H	L	V	A	D	G	L	S	L	E	I	L	V



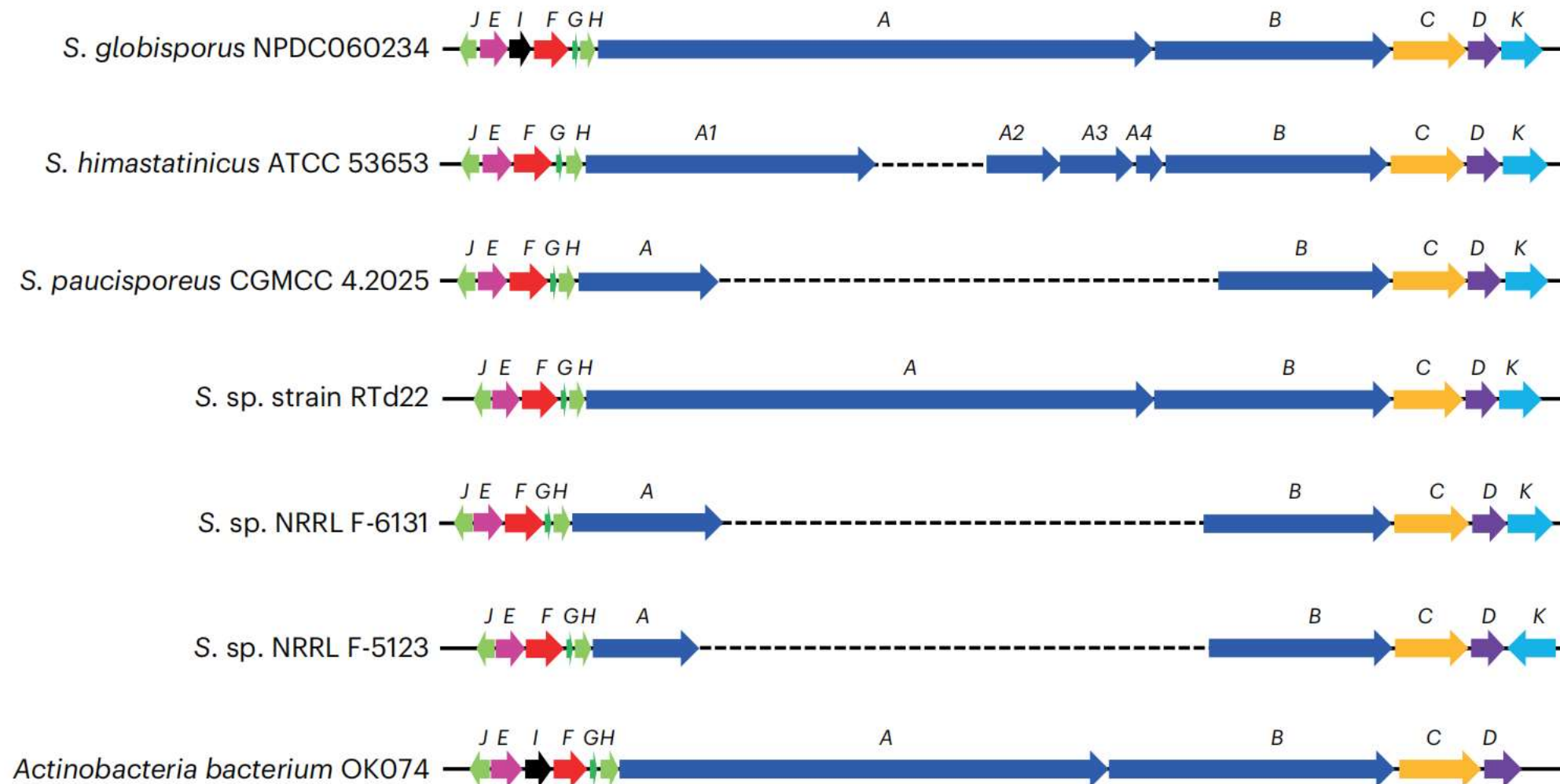
R domain catalyzes the reduction of thioester bond on PCP



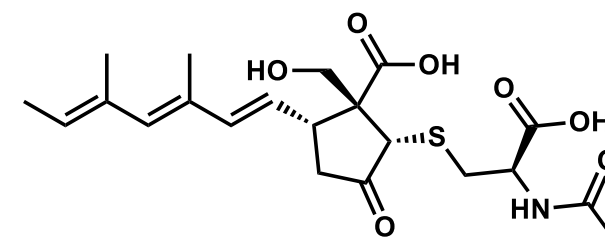
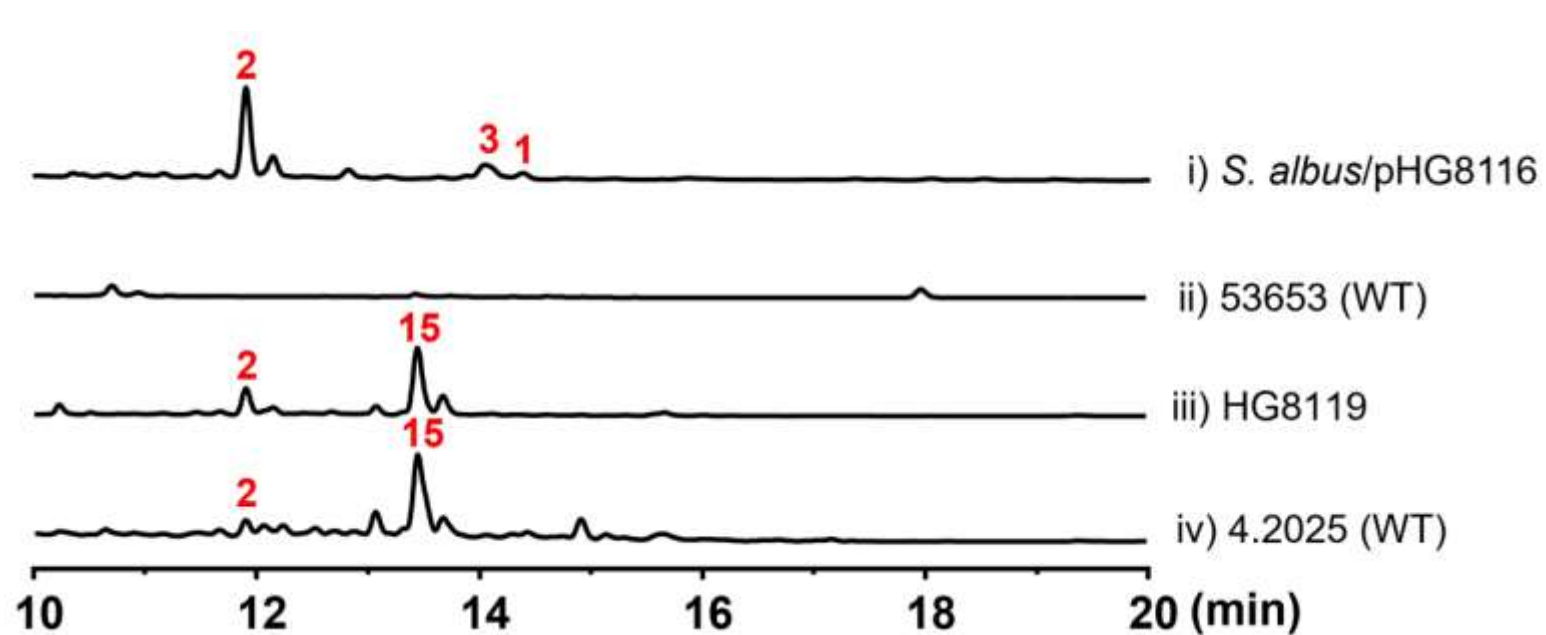
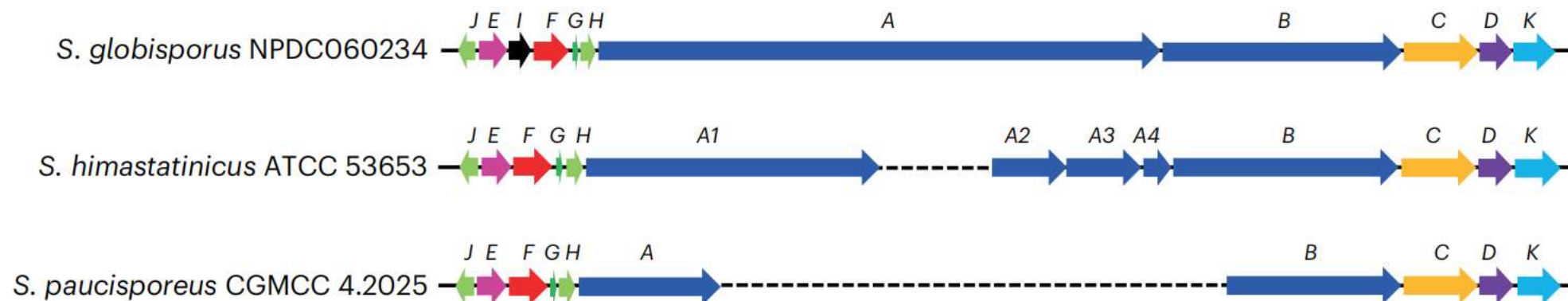
4.4 GloD is responsible for β -lactone ring formation



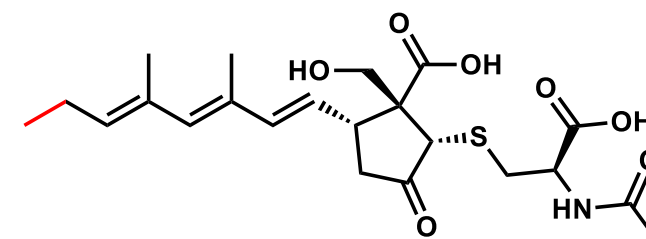
5 Genome mining of homologous glo clusters



5 Genome mining of homologous glo clusters

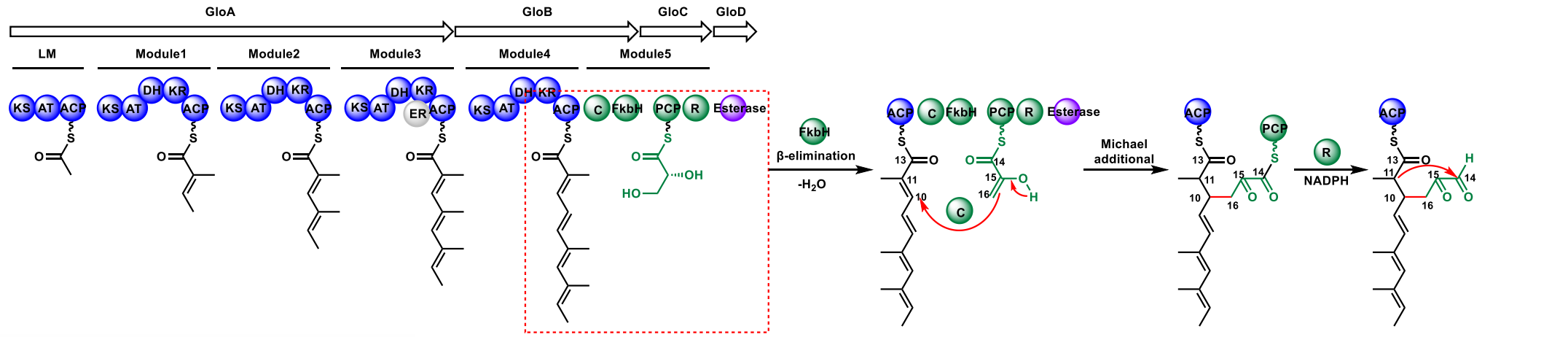


2
Globilactone B



15

Summary

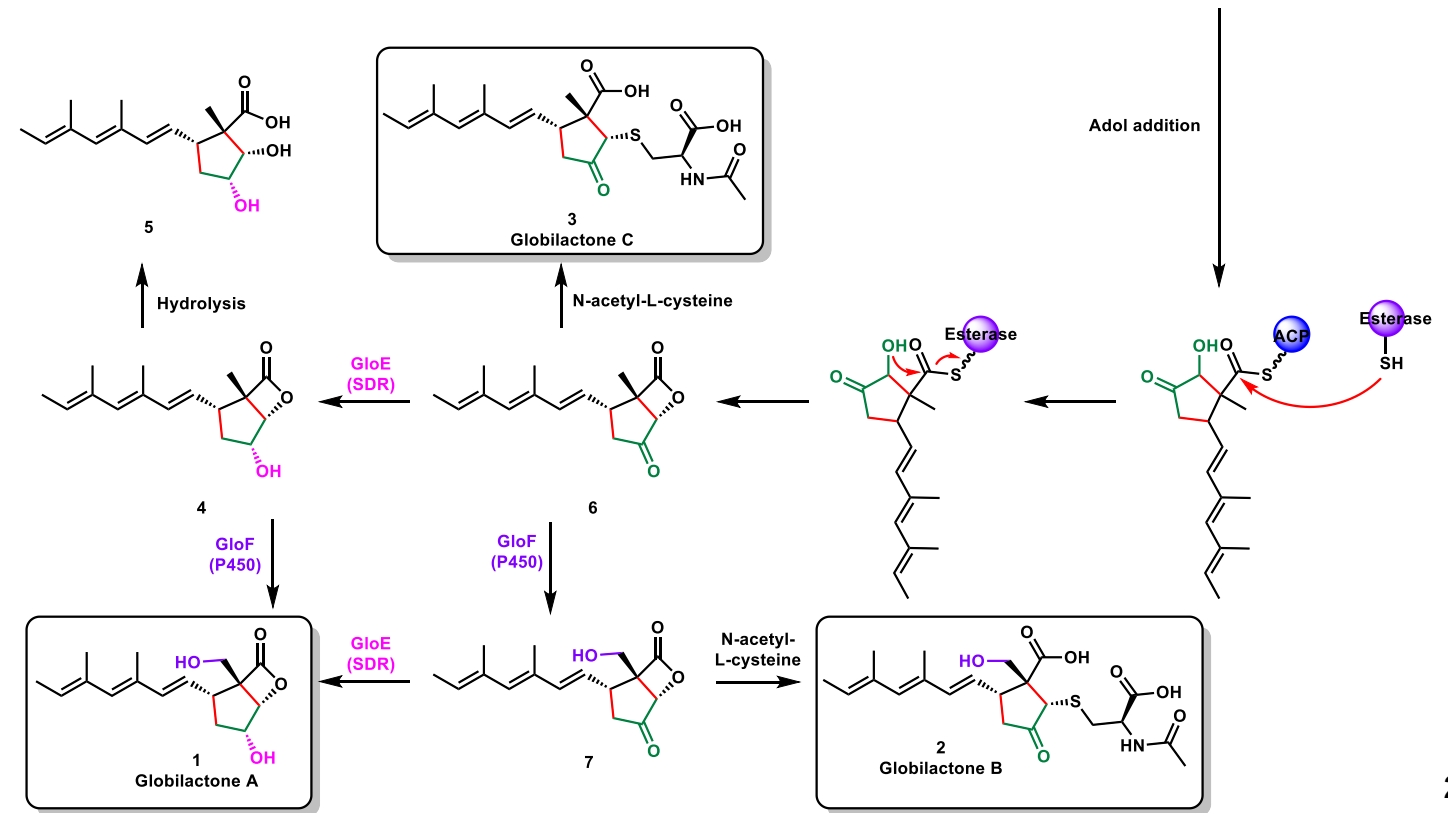


Genome mining identified a unique C-FkbH-PCP-R module

Heterologous expression, isolation and structure elucidation

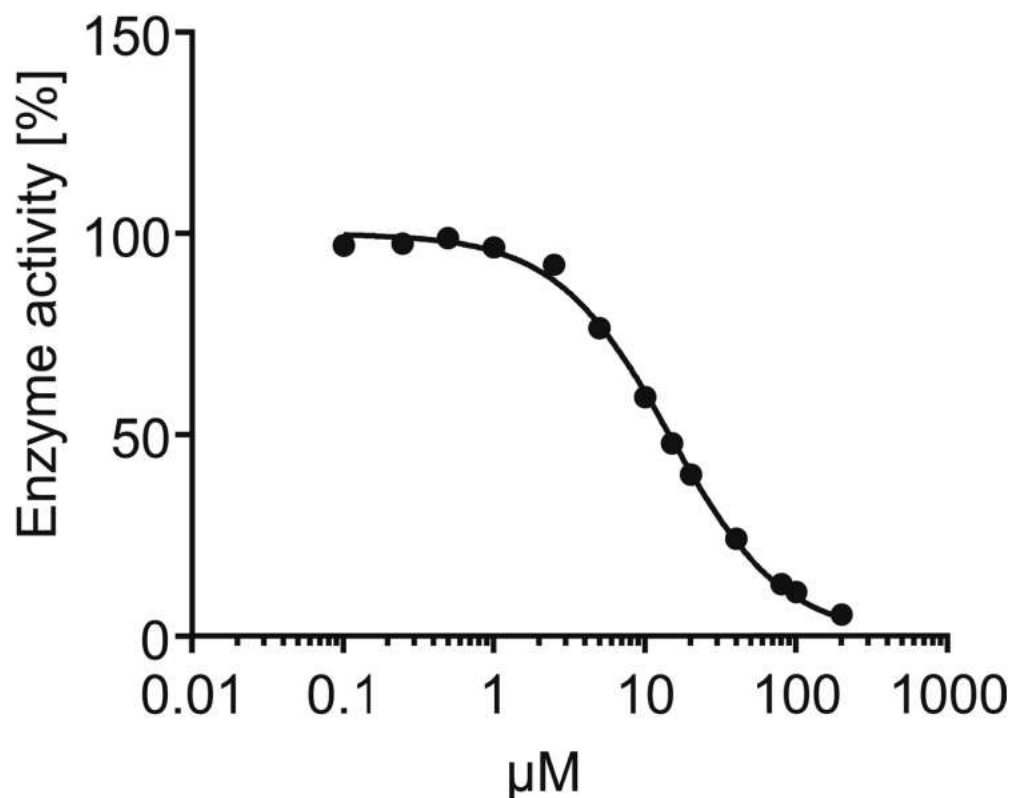
Discovered a β -lactone globilactone

Analyzed the biosynthetic pathway analysis of globilactone



Thanks!

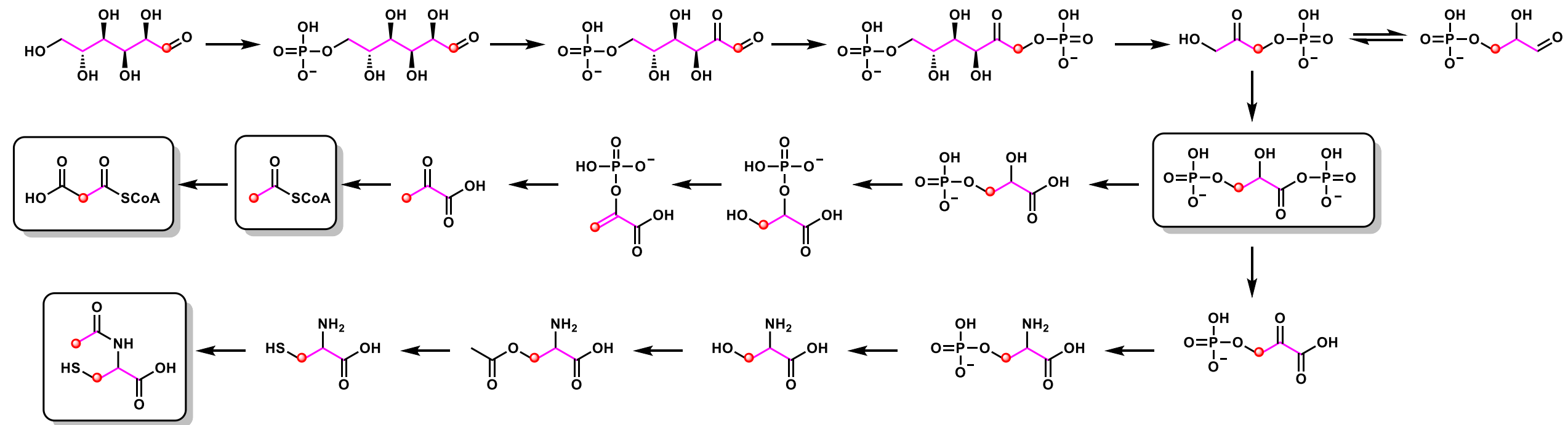
Biological activities



Compounds 1–3 showed no significant activity against bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus* and *Klebsiella pneumoniae*, as well as against lung cancer cells (A549) at a dose of 100 μM

**Inhibitory activity against the class A β-lactamase of 1.
An IC₅₀ value against class A β-lactamase of 1 was 15.05 μM.**

From glucose to 1,3-BPG



INADEQUATE (150 MHz, CD₃OD) spectrum of 3a with feeding of [U-¹³C₆] glucose

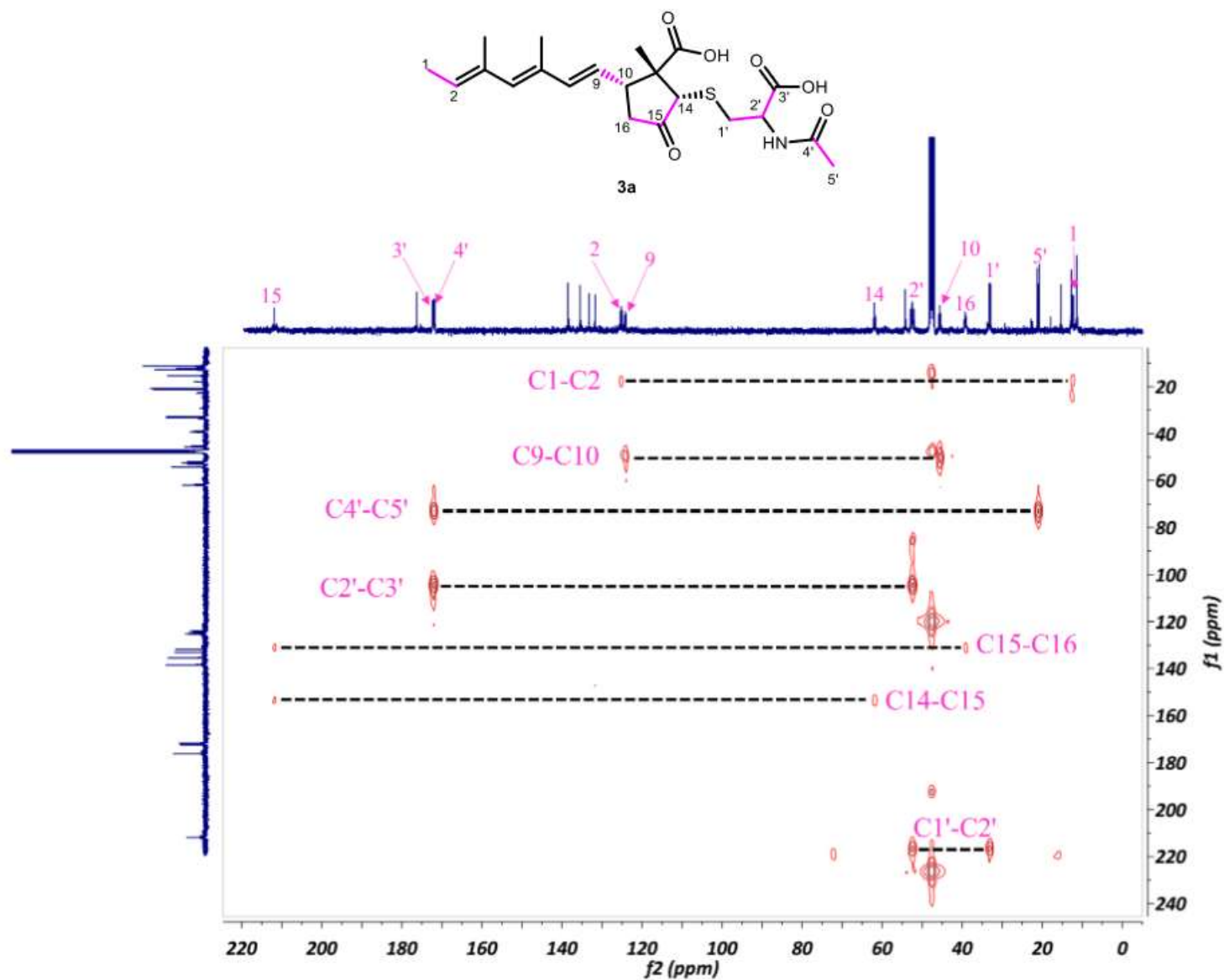


Figure S60. INADEQUATE (150 MHz, CD₃OD) spectrum of 3a with feeding of [U-¹³C₆] glucose.

Overlap DEPT NMR (100 MHz, CD₃OD) spectrum of 3b (green) and 3 (red)

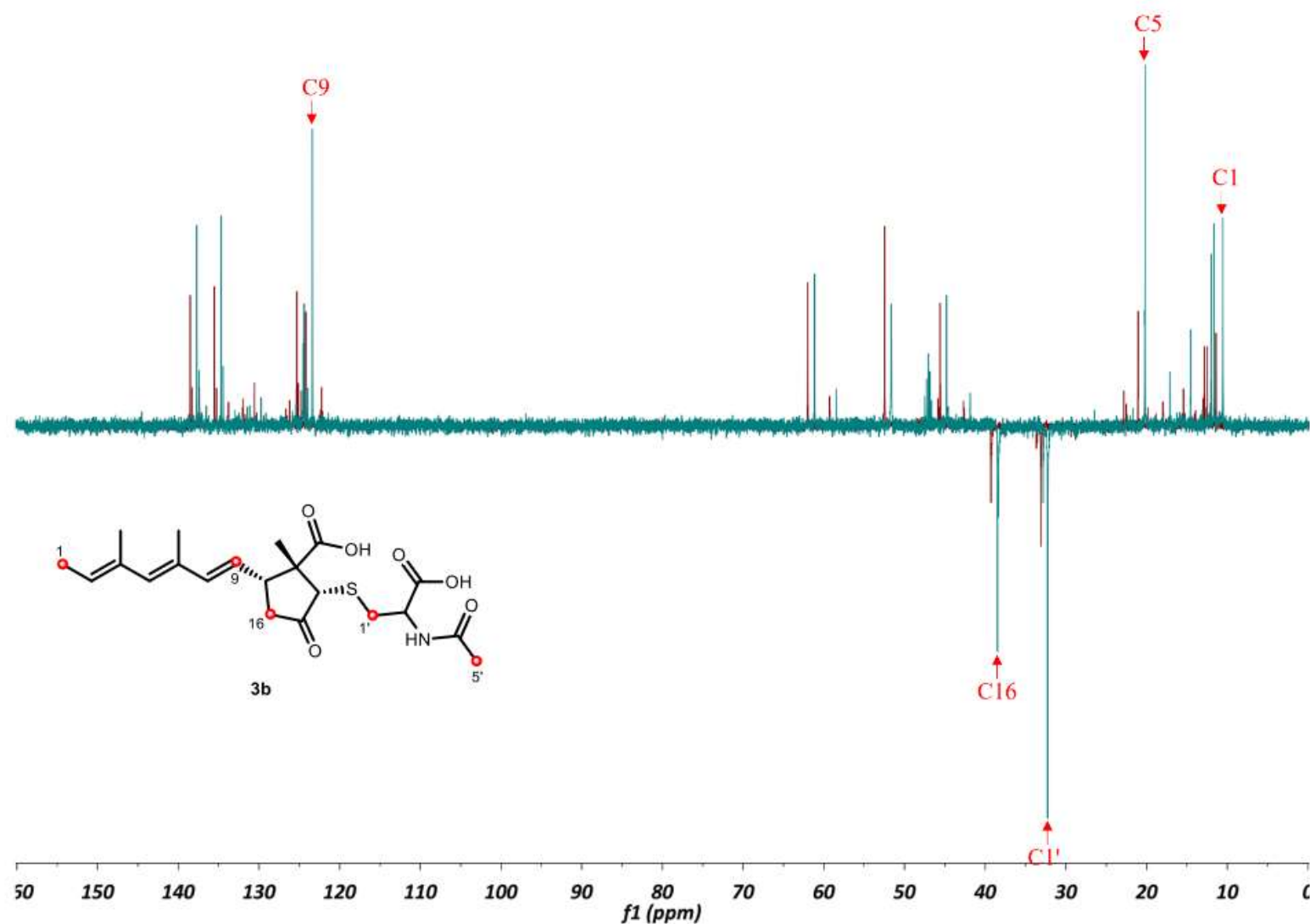


Figure S64. Overlap DEPT NMR (100 MHz, CD₃OD) spectrum of **3b** (green) and **3** (red). Triangles represent enriched carbon signals.

From glucose to 1,3-BPG

