From Deep Sea Sponge To Pilot Plant

Adventures in natural product synthesis

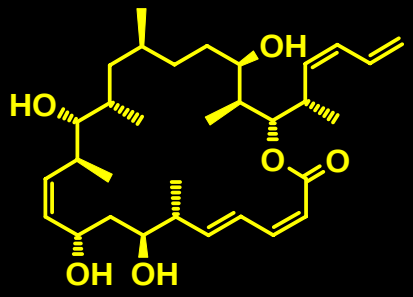
S. J. Mickel, Novartis

Discussion points

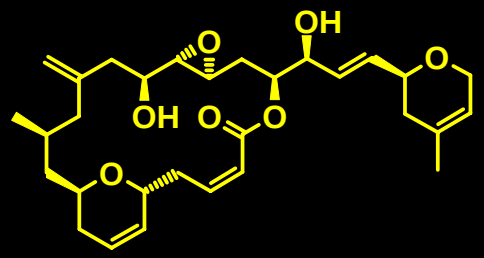
- Background
- Comments to Disco Structure
- Route selection
- Execution
- Statistics
- Outlook

Discodermolide: A Novel Microtubule Stabilizing Agent

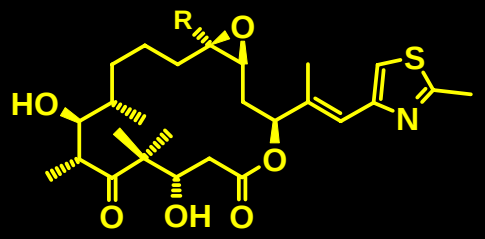
Marine natural product patented by HBOI (1990)
Licensed to Novartis in 1998



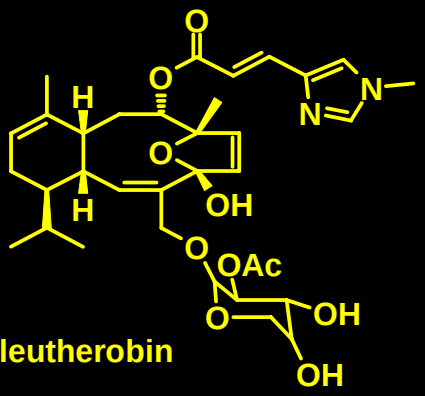
dictyostatin



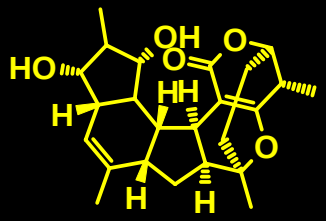
lailimalide



R = H: epothilone A
R = Me: epothilone B



eleutherobin



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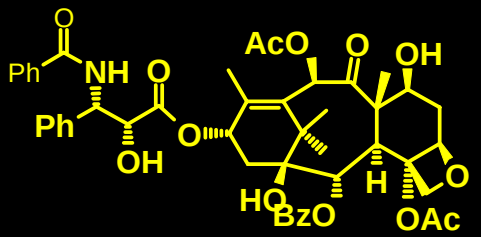
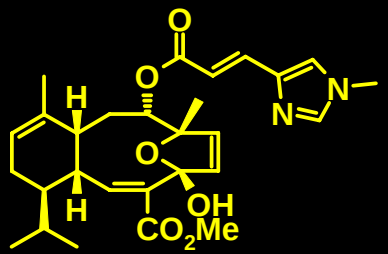
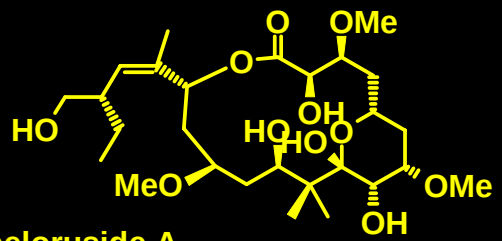


Taxol



sarcodictyin



peloruside A

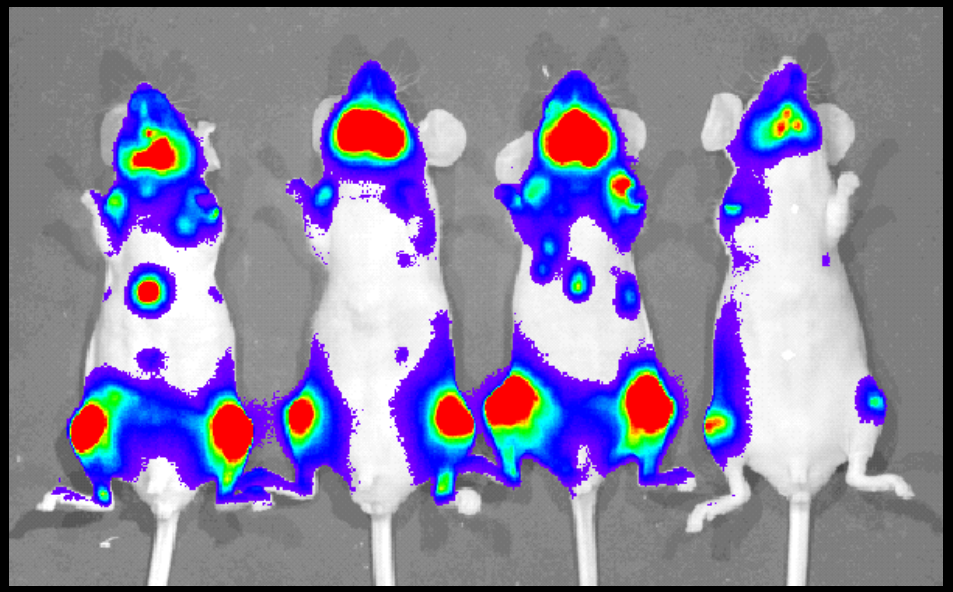
Discodermolide: A Novel Microtubule Stabilizing Agent

Mechanism of action: stabilization of microtubules, mitotic arrest → apoptosis (clinically validated)

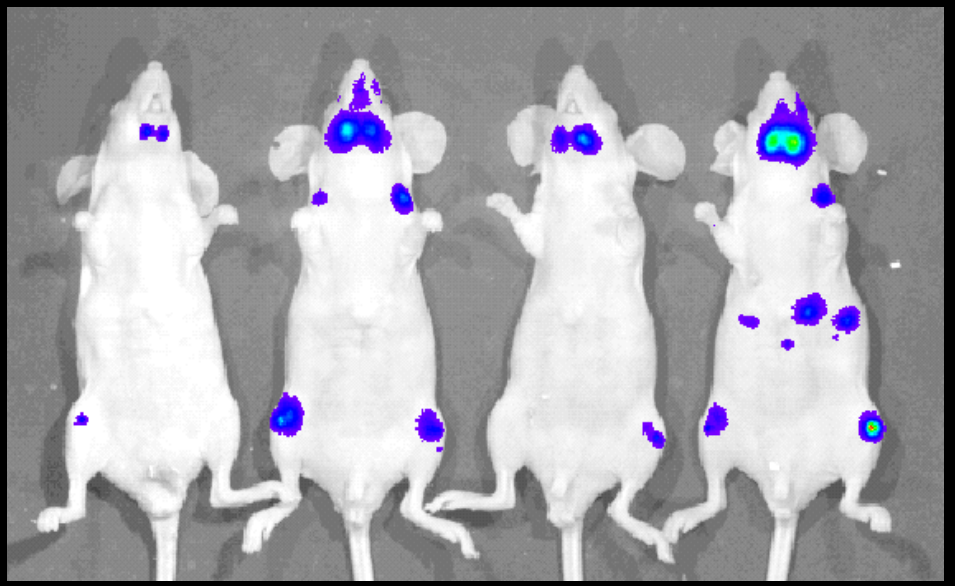
Potent inhibitor of paclitaxel (PTX) – resistant cell lines;
Active *in vivo* vs. PTX-resistant tumors
Phase I August 2002 (advanced solid tumors)

Bone Metastasis Imaging Model

PC-3M2AC6 luciferized human prostate carcinoma

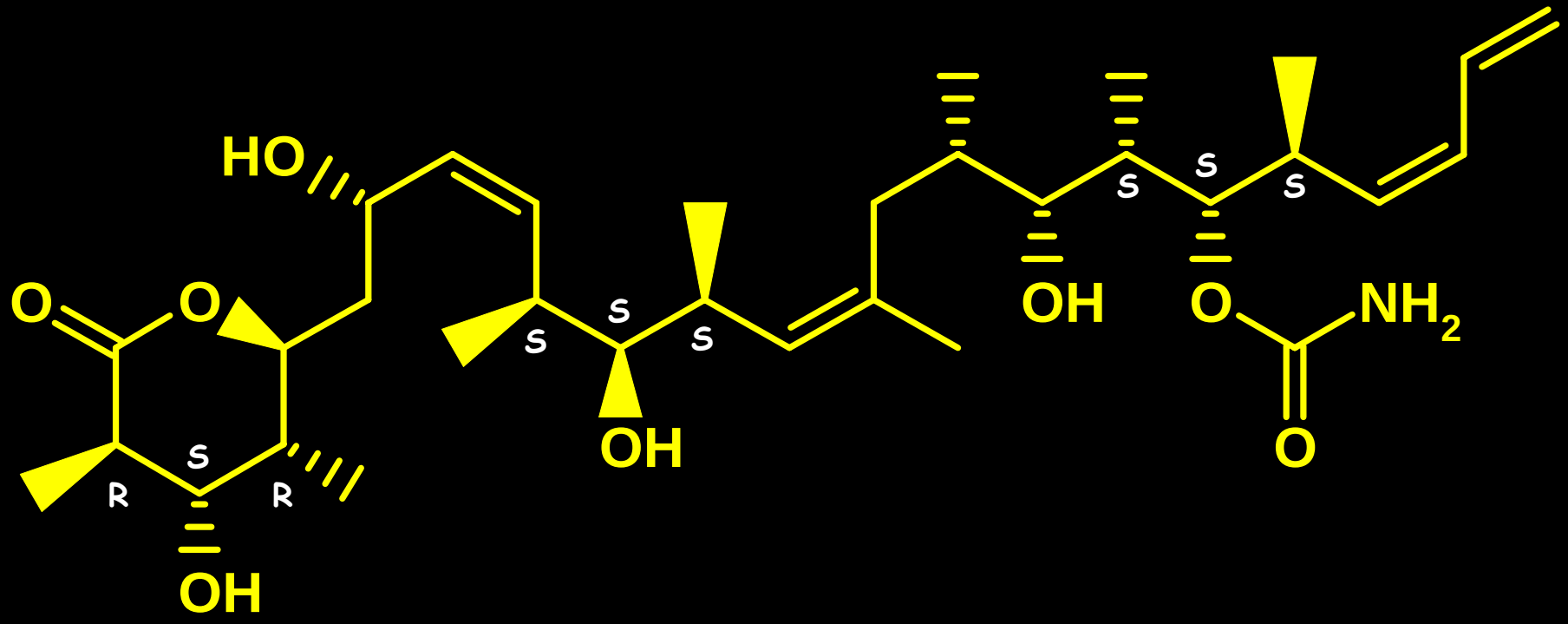


VEHICLE TREATED MICE



15 mg/kg XAA296 (ONE DOSE)

Discodermolide Structure and retro synthesis


HO
O
O
S
R
R
OH
S
S
S
OH
S
S
S
OH
O
NH2
O

Route Selection

Selected route must be capable of delivering

100 - 1000g

amounts of discodermolide

Evaluation of Published Syntheses (as of ca. 2000)

Smith
Myles
Schreiber
Marshall
Paterson

Evans
Golec
Heathcock
Masamune

- Overall yield
- Crystalline intermediates
- Chromatography
- Problematic reactions
- Changes in Oxidation state
- Organometallic reagents
- Stereochemistry
- Final crystallisation

Evaluation of Published Syntheses (as of ca. 2000)

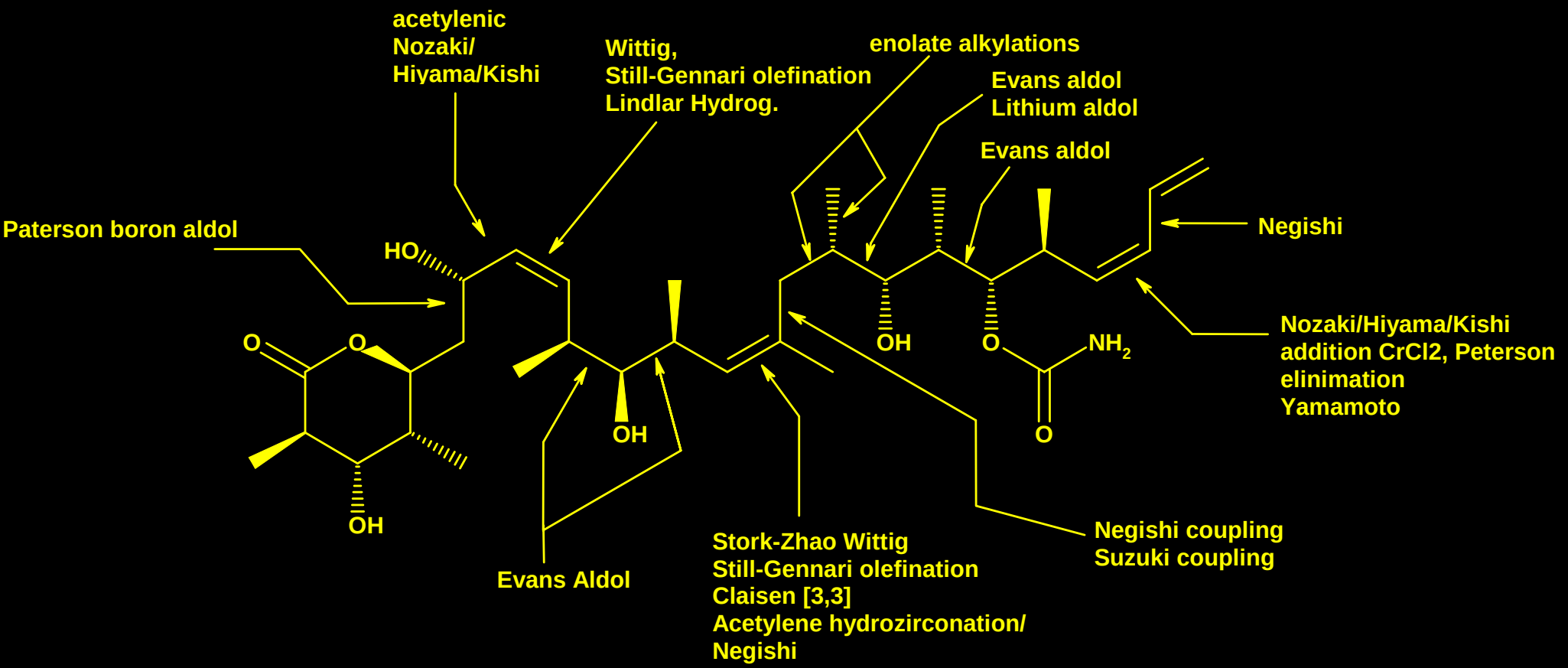
Smith → High pressure reaction

Myles → Not stereoselective, MOMCl

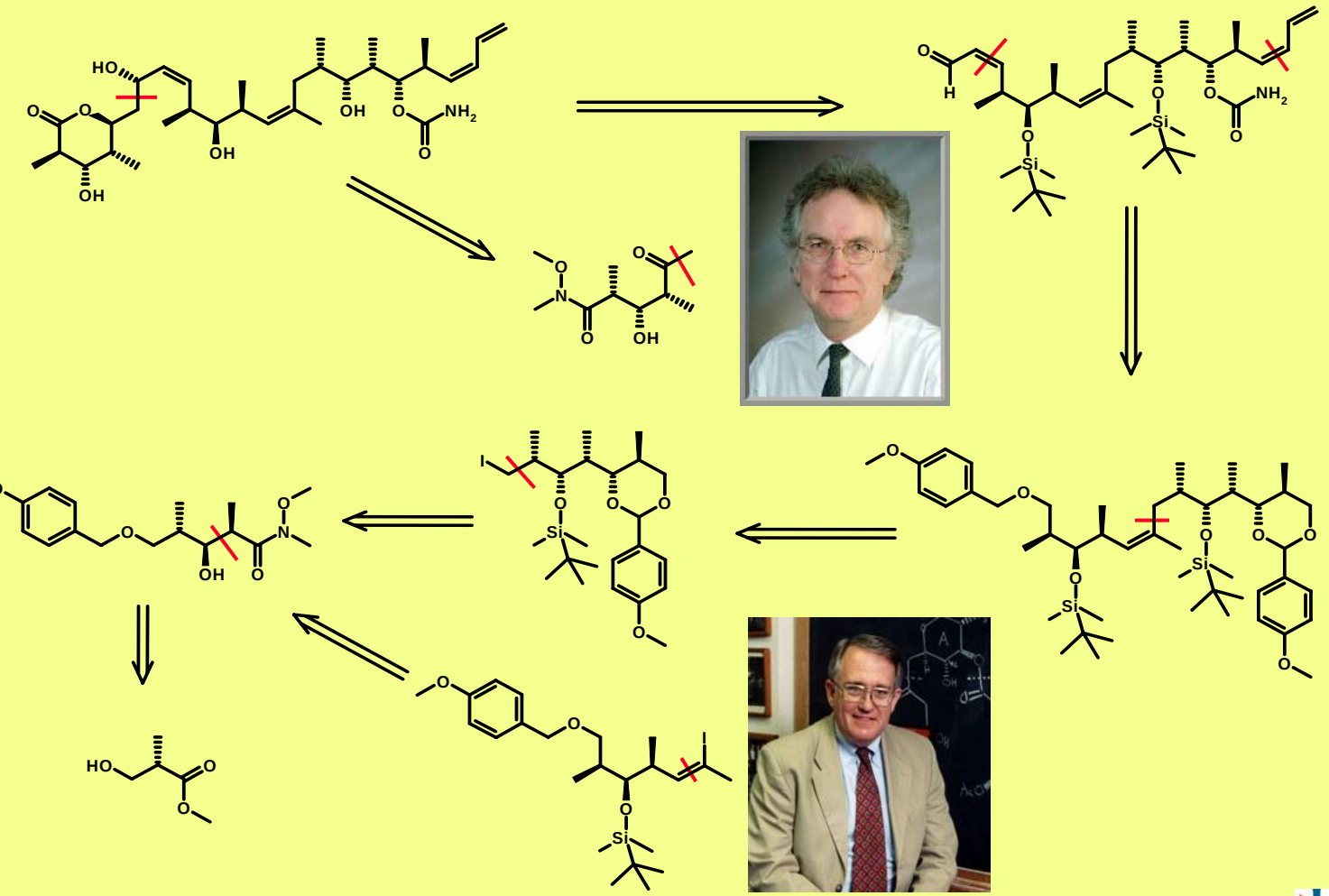
Schreiber → O_3, PhSTMS, $HgCl_2$

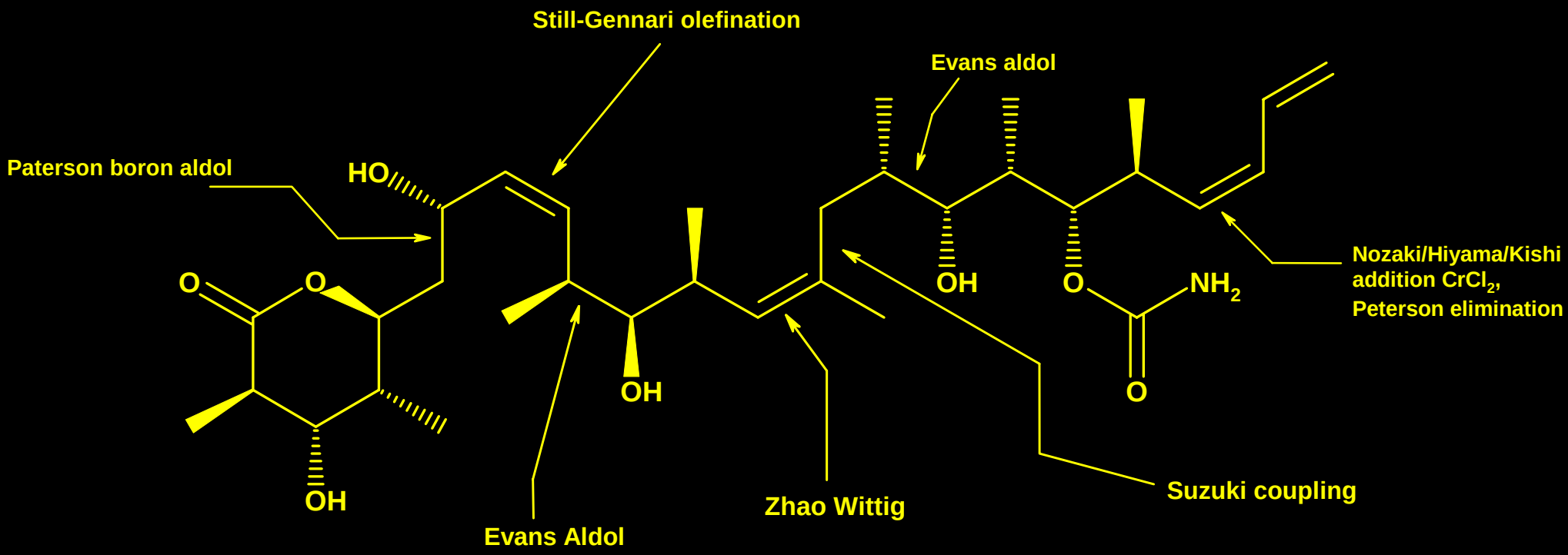
Marshall → Low M.wt.acetylenes, Sn chemistry

Paterson → Selenium chemistry, B chemistry

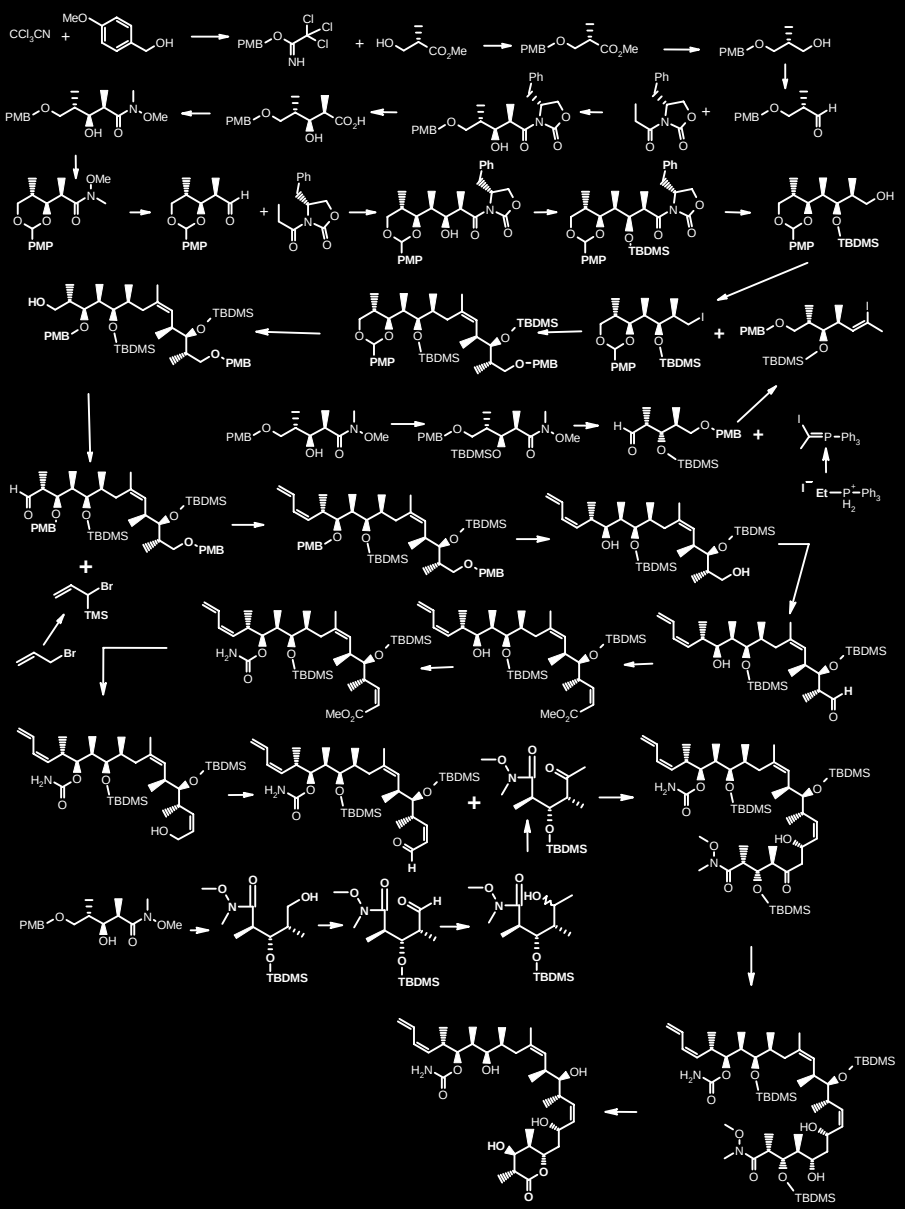

acetylenic
Nozaki/
Hiyama/Kishi
Wittig,
Still-Gennari olefination
Lindlar Hydrog.
enolate alkylations
Evans aldol
Lithium aldol
Evans aldol
Paterson boron aldol
Negishi
HO
O
O
OH
OH
OH
O
NH2
O
Nozaki/Hiyama/Kishi
addition CrCl2, Peterson
elinimation
Yamamoto
Evans Aldol
Stork-Zhao Wittig
Still-Gennari olefination
Claisen [3,3]
Acetylene hydrozirconation/
Negishi
Negishi coupling
Suzuki coupling

Retrosynthetic Analysis
(Novartis-Smith-Paterson- Hybrid)




Still-Gennari olefination
Evans aldol
Paterson boron aldol
HO
O
O
OH
OH
OH
O
NH2
O
Nozaki/Hiyama/Kishi
addition CrCl2,
Peterson elimination
Evans Aldol
Zhao Wittig
Suzuki coupling

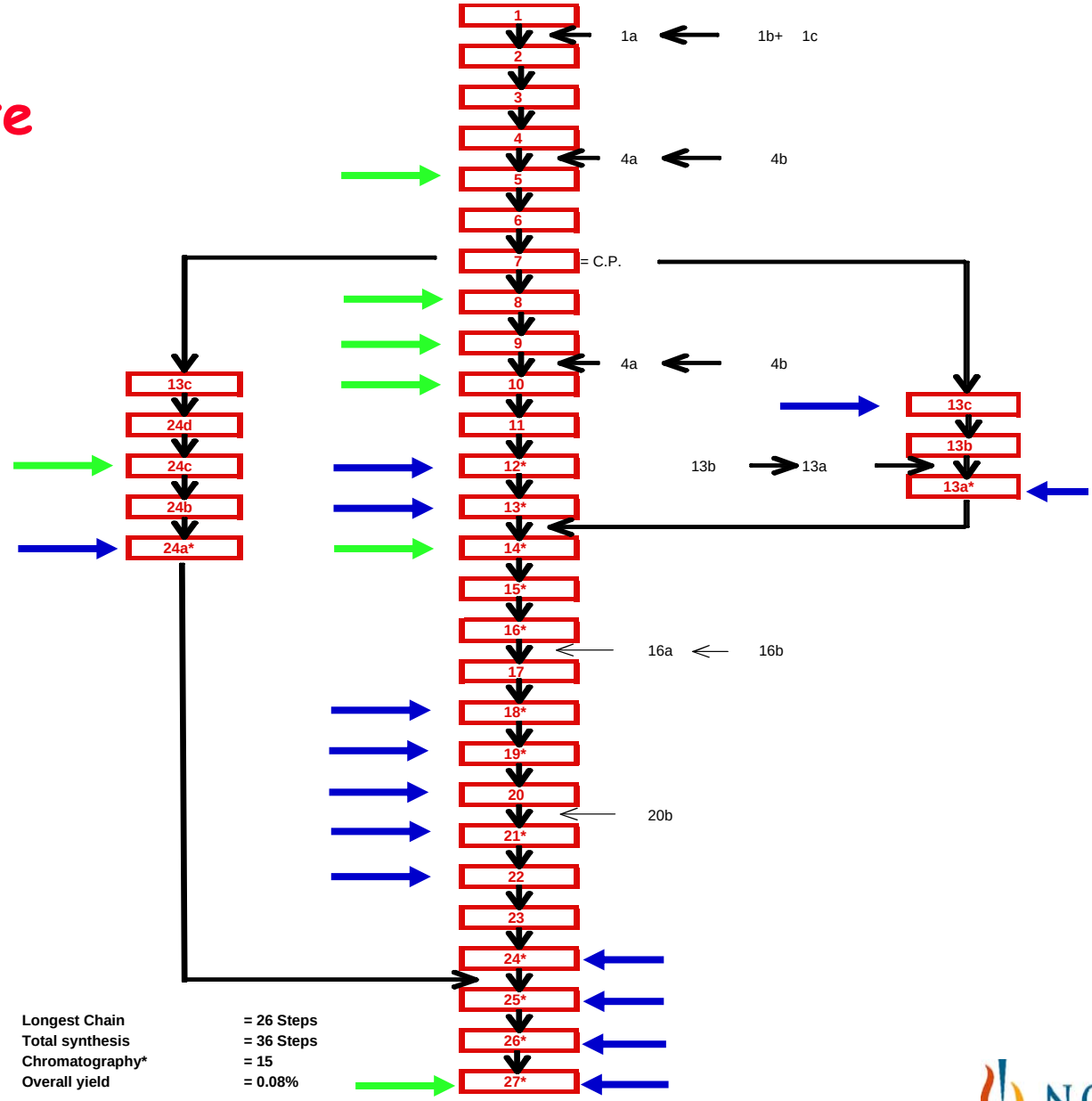
Chosen route



Synthetic route Schematic

= chromatography

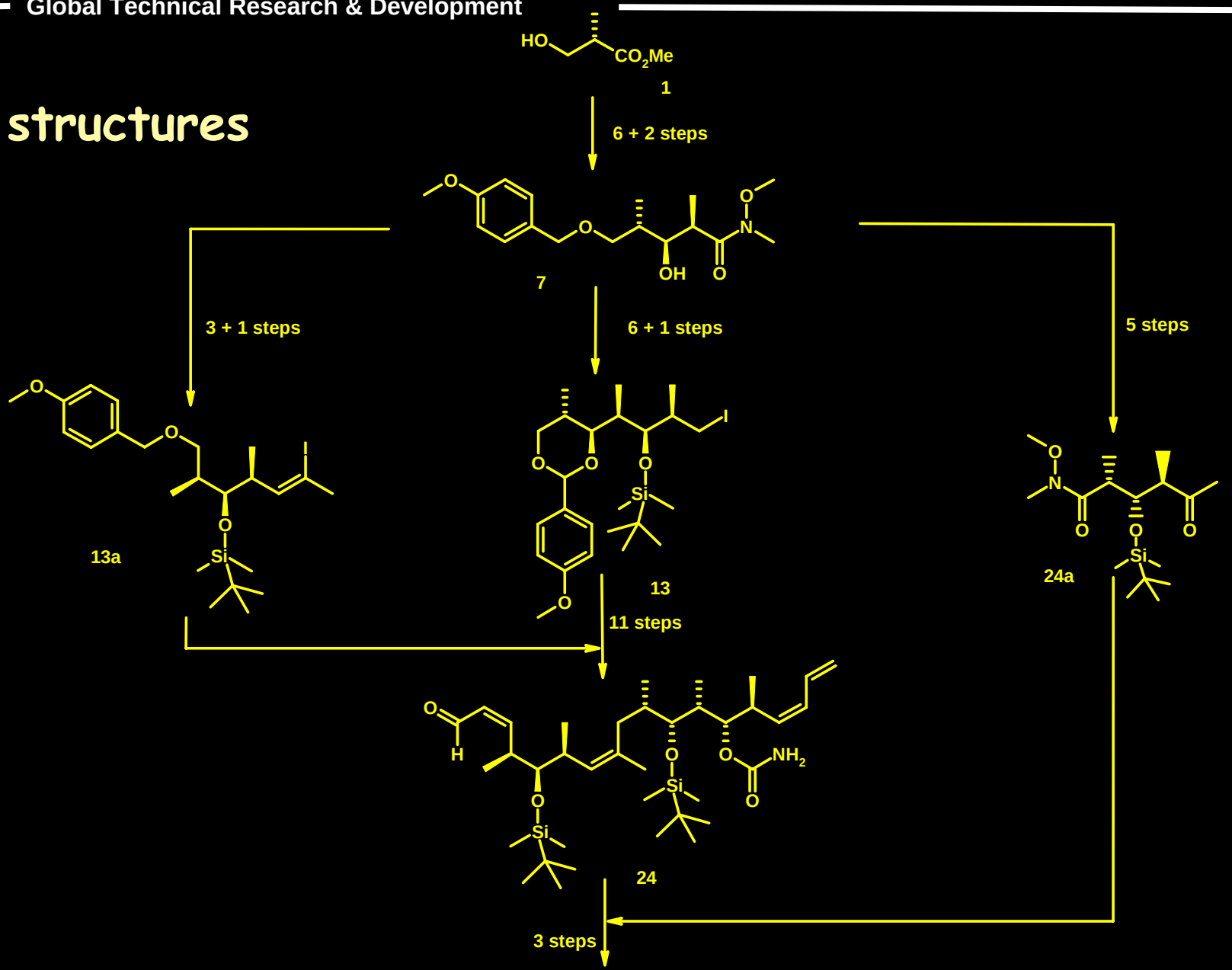
= crystalline

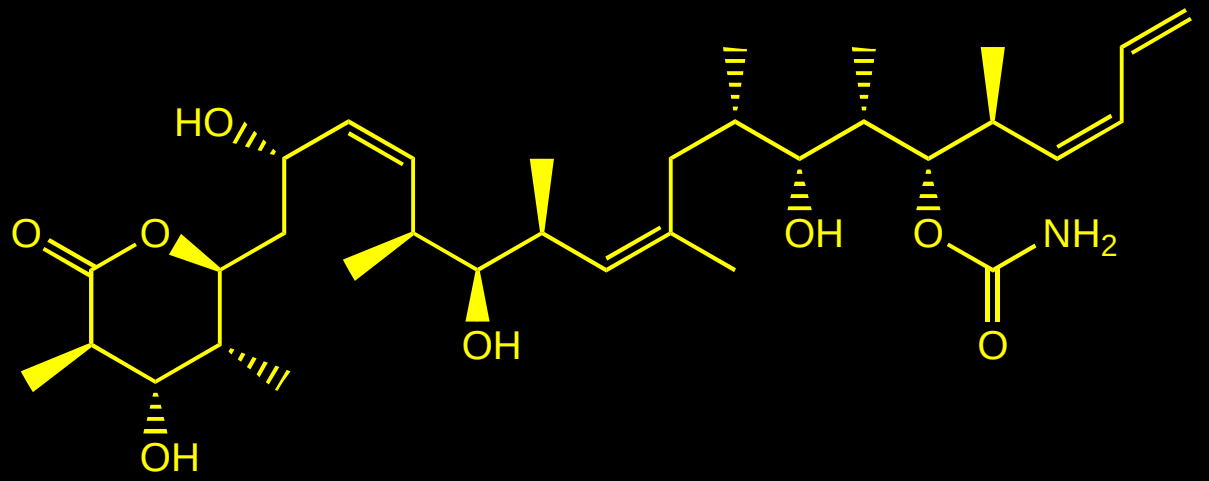


Longest Chain	= 26 Steps
Total synthesis	= 36 Steps
Chromatography*	= 15
Overall yield	= 0.08%

NOVARTIS

Key structures




HO
O
O
OH
OH
OH
O
NH2
O

Execution

2 – 3

2 3

Scale, 55 Kg 2 = 230 Mol/reaction 4 times

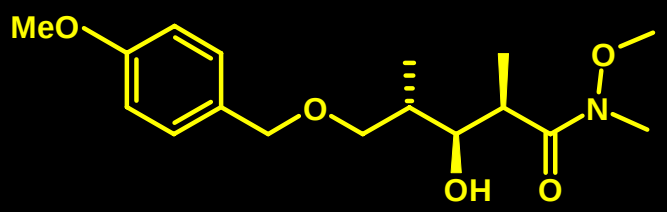
$LiAlH_4$ yield 85%

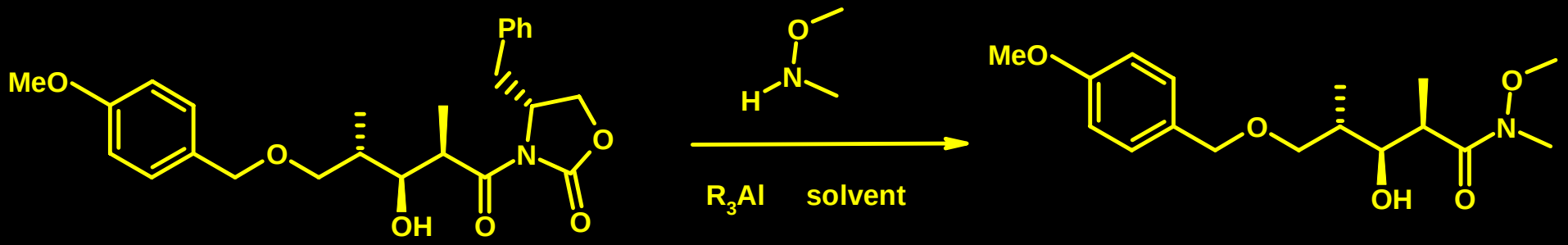
Work up, very slow filtration >24 hours

$LiBH_4$ 86% yield

Work up, quench HOAc, extraction, <3 hours

Common Precursor

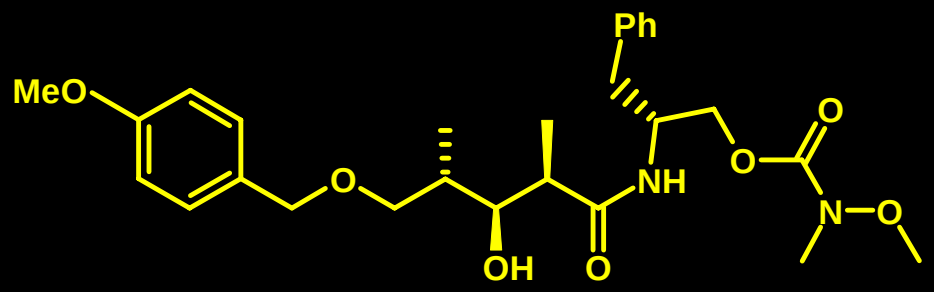




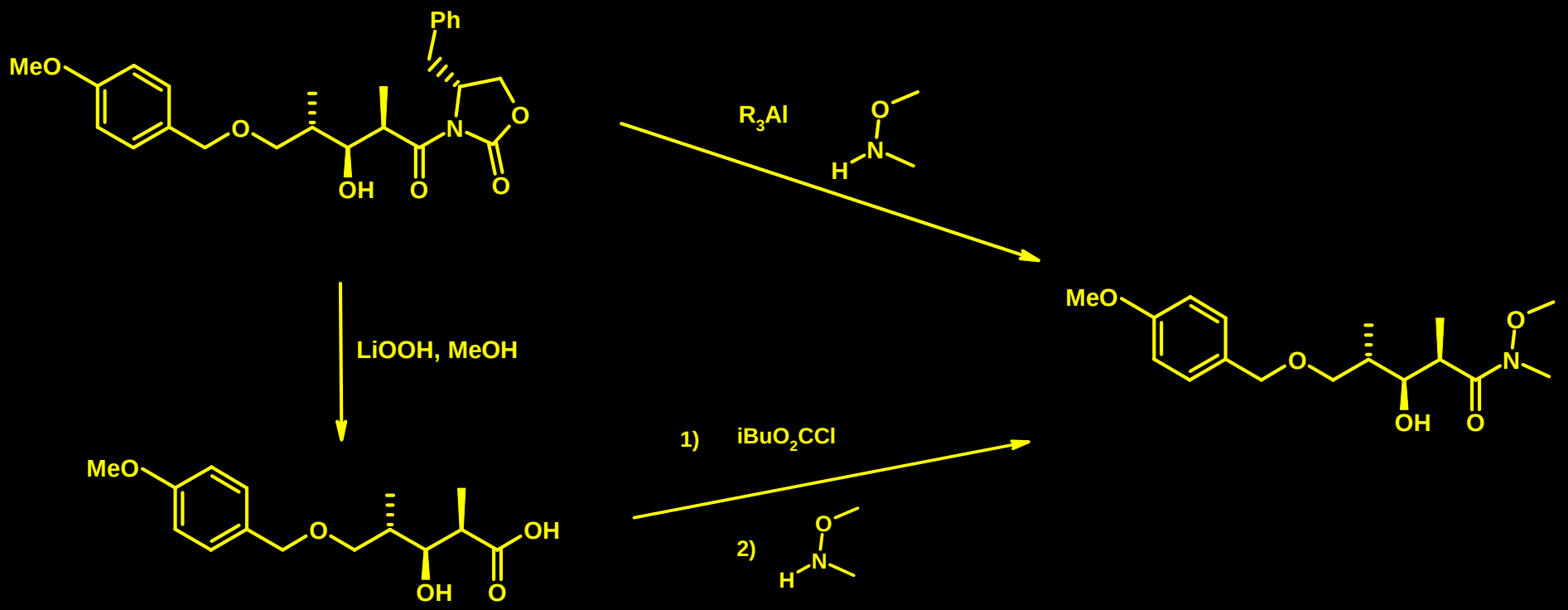
Common Precursor Synthesis

1 Thermal problems

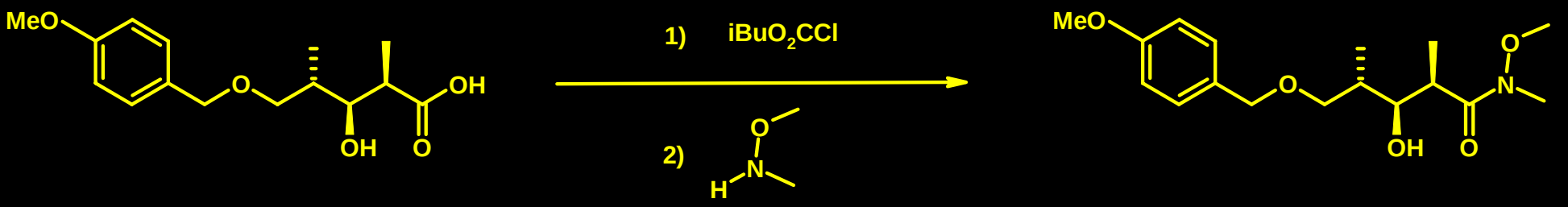
2 Side product



Common Precursor

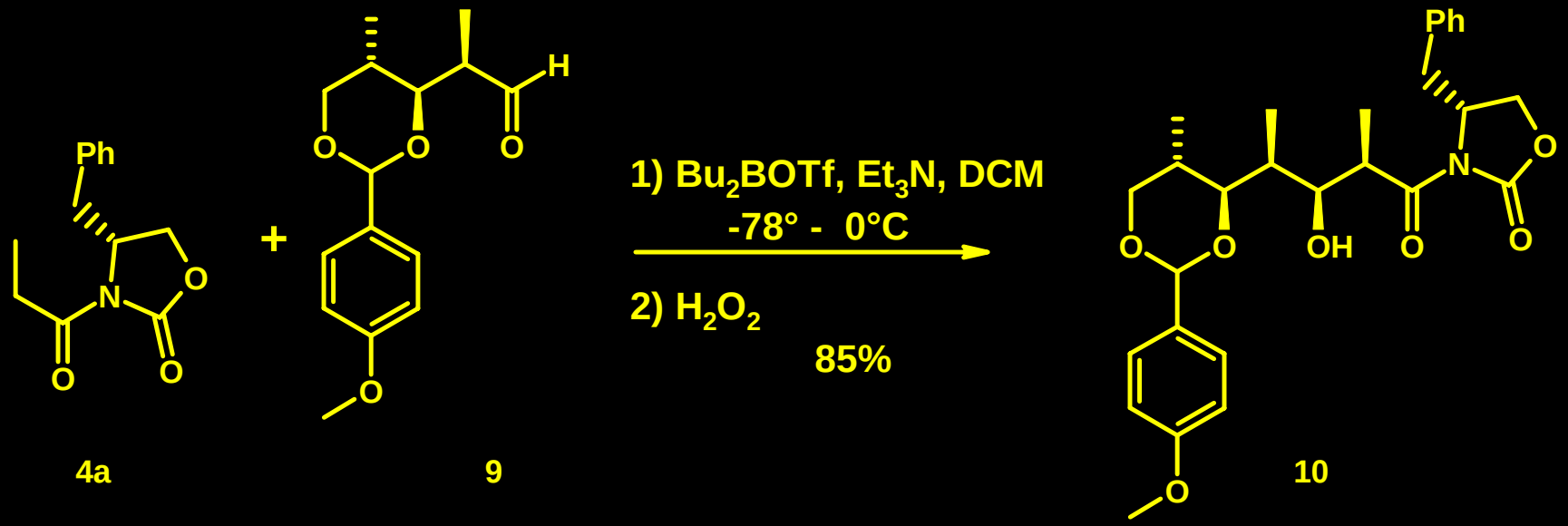


Common Precursor



Campaign	yield	Absolute quantities
1 (6g)	30%	3.5Kg
2 (60g)	35%	35.0Kg
3 (500g)	61%	110.0Kg

9 - 10

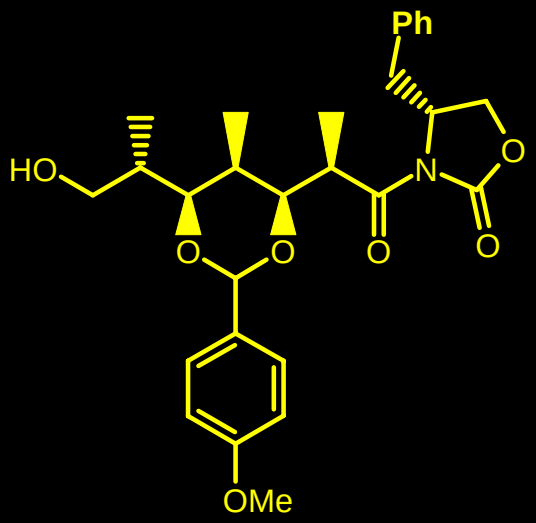


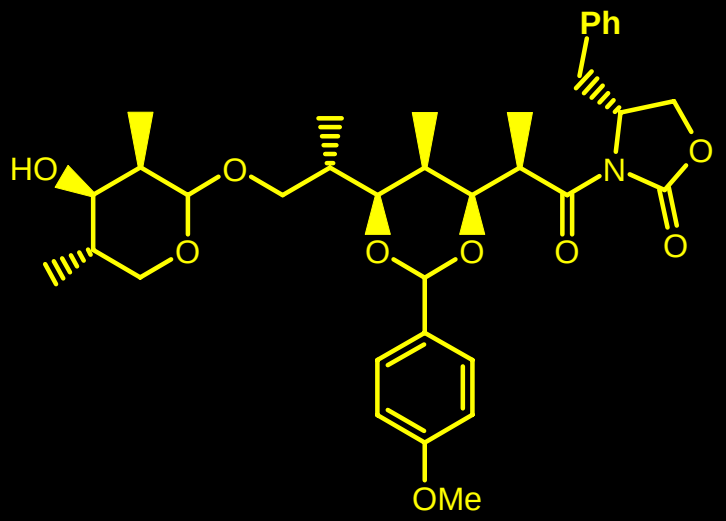
4a 9 10

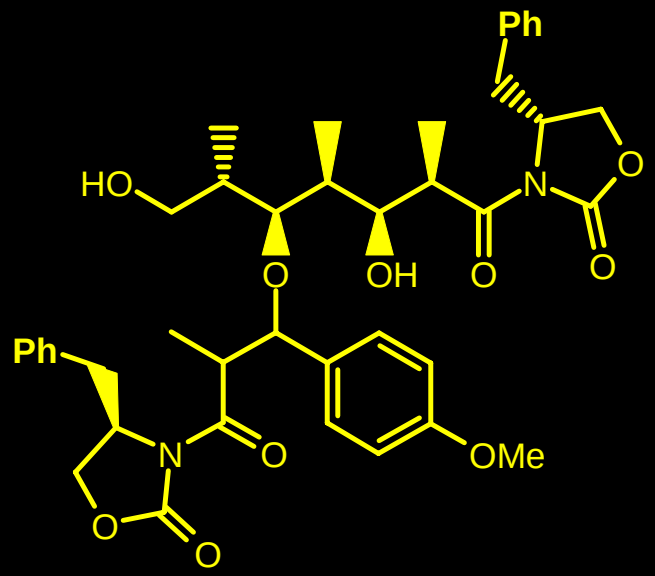
1st Campaign, 1.40 Kgs 9 = 5.3Mol/ reaction, 4 reactions

2nd Campaign, 14.4 Kgs 9 = 54.5Mol/reaction, 3 reactions

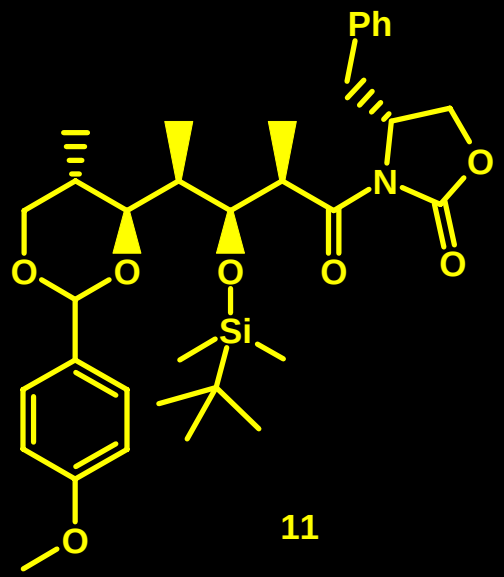
Isolated side products







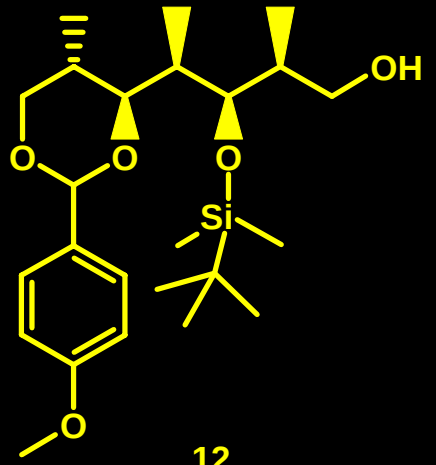
11 - 12



11

$LiBH_4$, EtOH, THF

60%



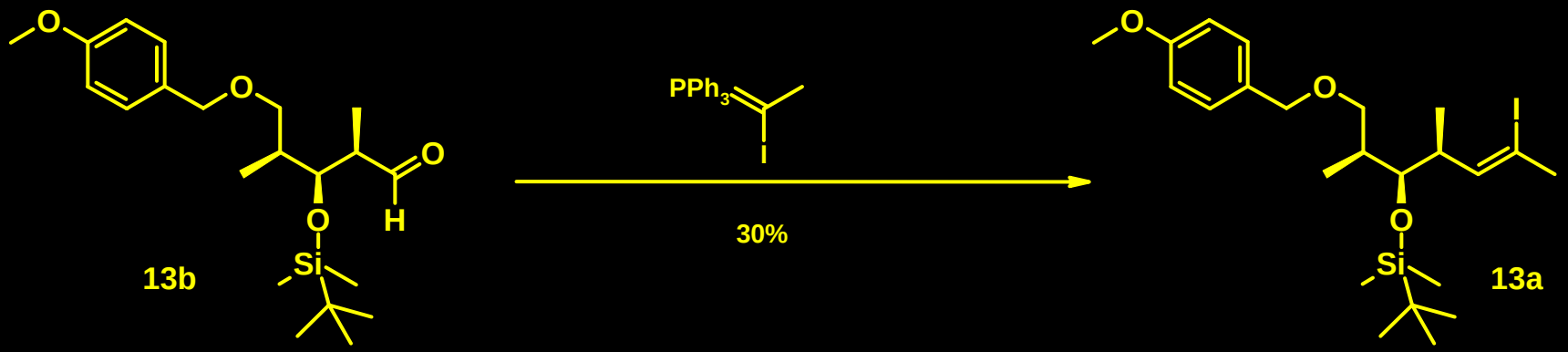
12

1st Campaign, 5.0Kgs 11 = 8.2 Mol, 3 reactions

2nd Campaign, 31.7Kgs 11 = 52 Mol, 2 reactions

Chromatography required

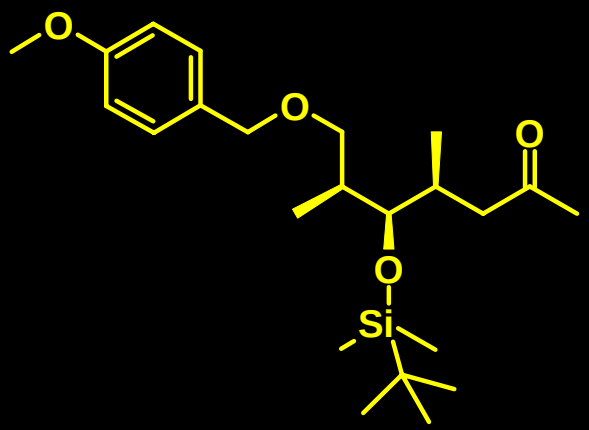
13a



1st Campaign, 2.5 Kgs 13b = 6.6 Mol, 4 reactions

2nd Campaign, 28 Kgs 13b, = 74 Mol, 10 reactions

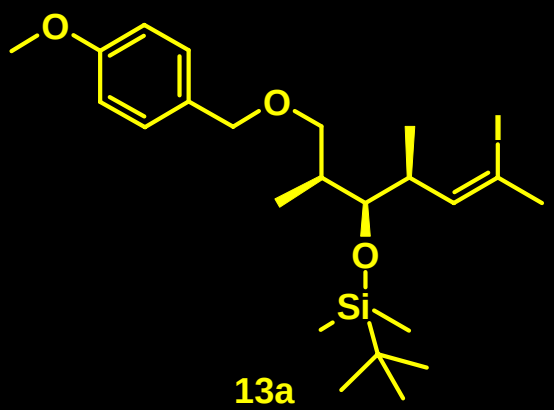
13a side products



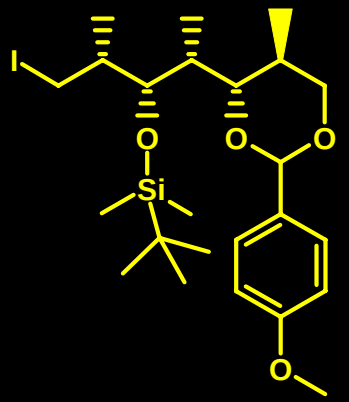
13a side products

Campaign	Yield	Absolute quantities
1 (6g)	22%	0.5Kg
2 (60g)	20-30%	3.4Kg
3 (500g)	30.5%	11.8Kg

14 Suzuki Coupling



13a

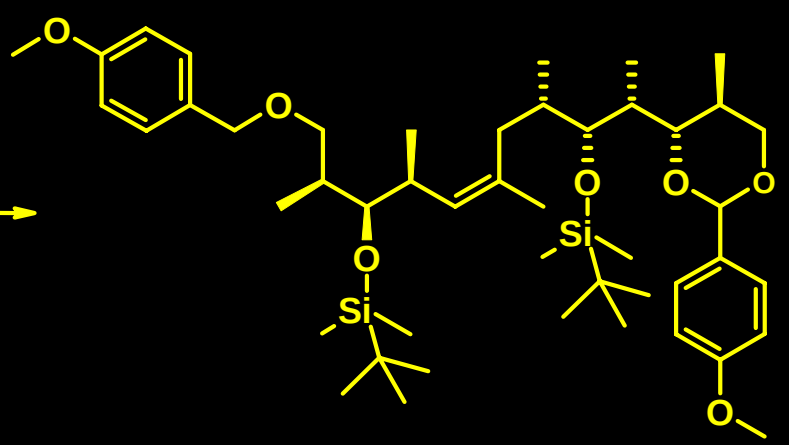


13

t-BuLi, 9-MeO-BBN

Pd catalyst
DMF, $CsCO_3$

70%



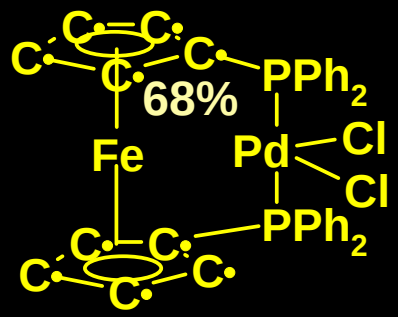
14

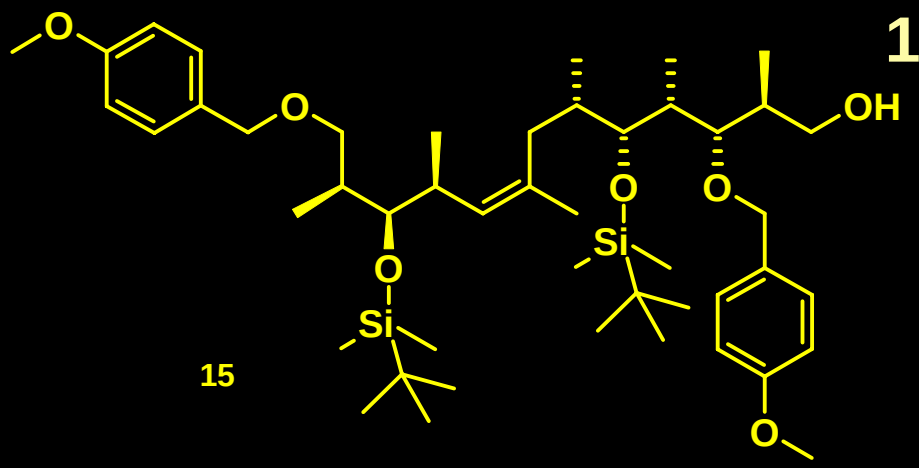
2nd Campaign 11.8Kgs 13a = 23mol, 3 reactions

14 Suzuki Coupling

- Very complex process
- Large excess of 13 necessary
- Product isolated from RM by crystallisation
- 70% yield
- Catalyst structure

Campaign	Overall yield	Absolute quantities
1 (6g)	50%	0.15Kg
2 (60g)	53%	3.7Kg
3 (500g)	68%	11.4Kg



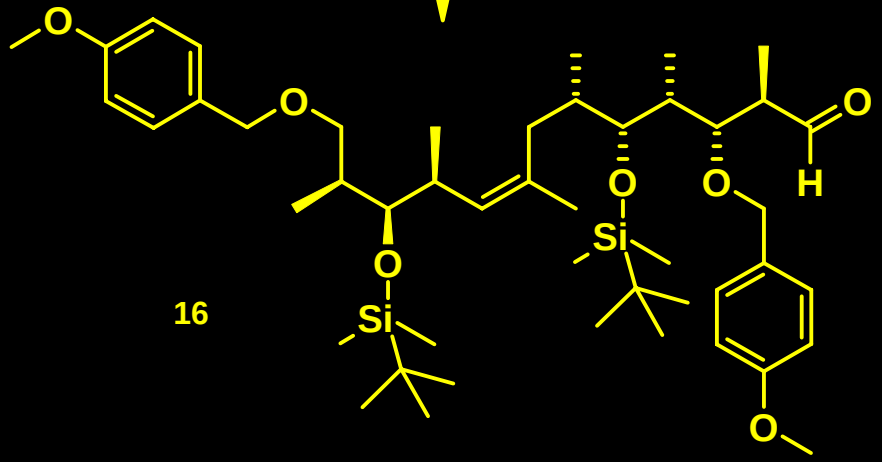


15

SO_3, Pyridine

Et_3N, DMSO

92%

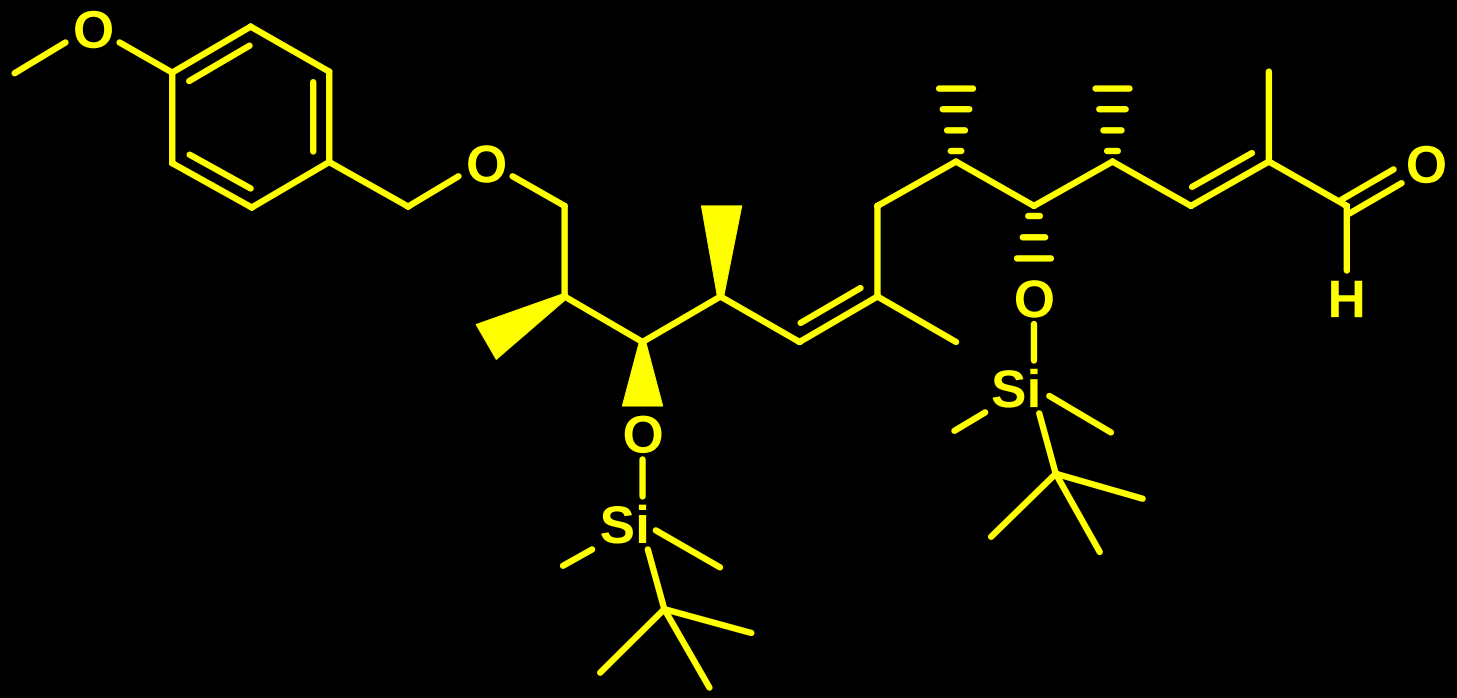


16

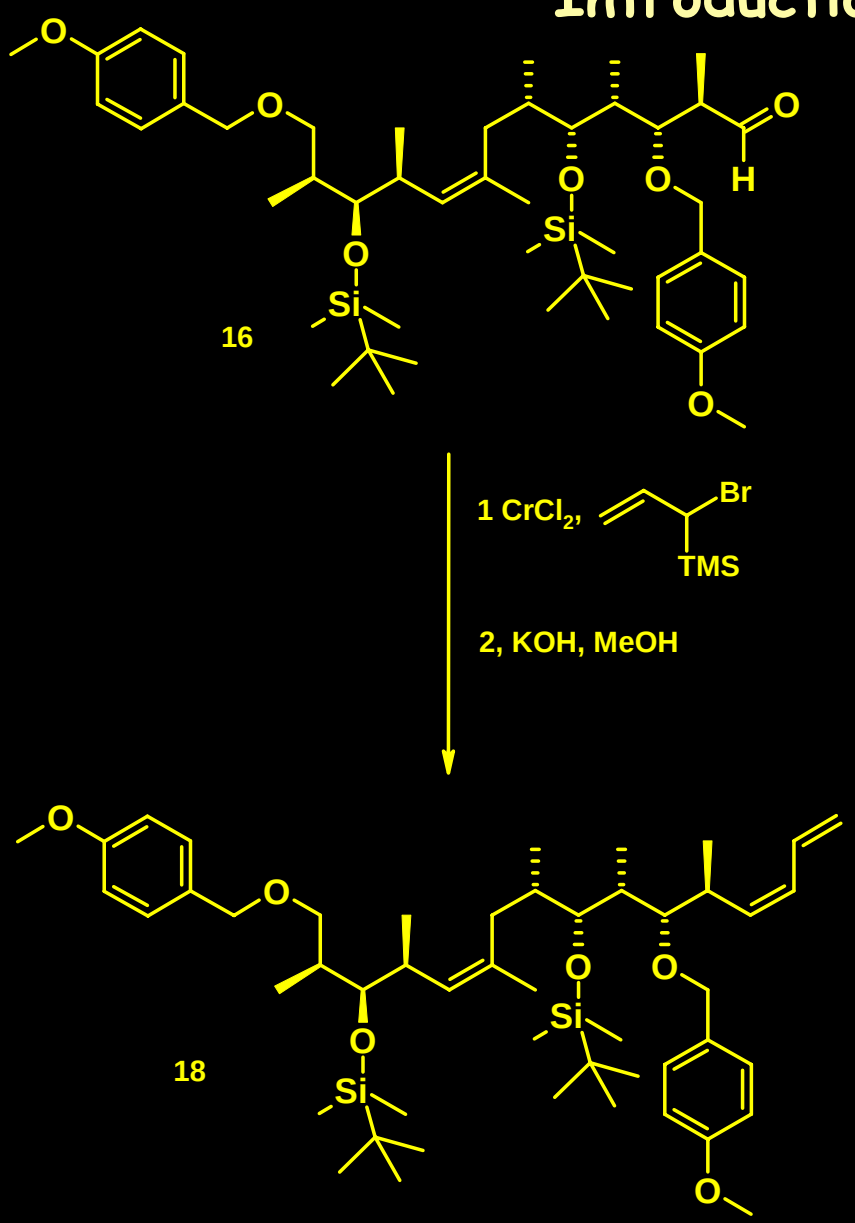
1st campaign
A15 1.0x
Et_3N 4.2x
SO_3-Py 3.1x
Bisulphate
70% yield 17% side product

2nd campaign
A15 1.0x
Et_3N 3.4x
SO_3-Py 2.5x
Bicarbonate
92% yield 2% side product
1.7Kgs 15, = 2.0Mol, twice
Planned 9 Kgs 15, = 11Mol, 3 reactions

16 Side product



Introduction of *cis*-diene unit



- Scale
- 1st Campaign 1.6 kg, 1.88 mol, twice
- 2nd Campaign 11.6kg, 14.4 mol,
- three times

- Requires 4.5-5.0 x $CrCl_2$ = 3Kg

- Two step process

1st Campaign complete after 2 hours RT

2nd Campaign

1st batch no reaction after 2 hours RT!

+ 20% $CrCl_2$, complete after 18Hr RT!

Before 2nd batch Lab test OK

2nd batch as 1st batch – NO REACTION

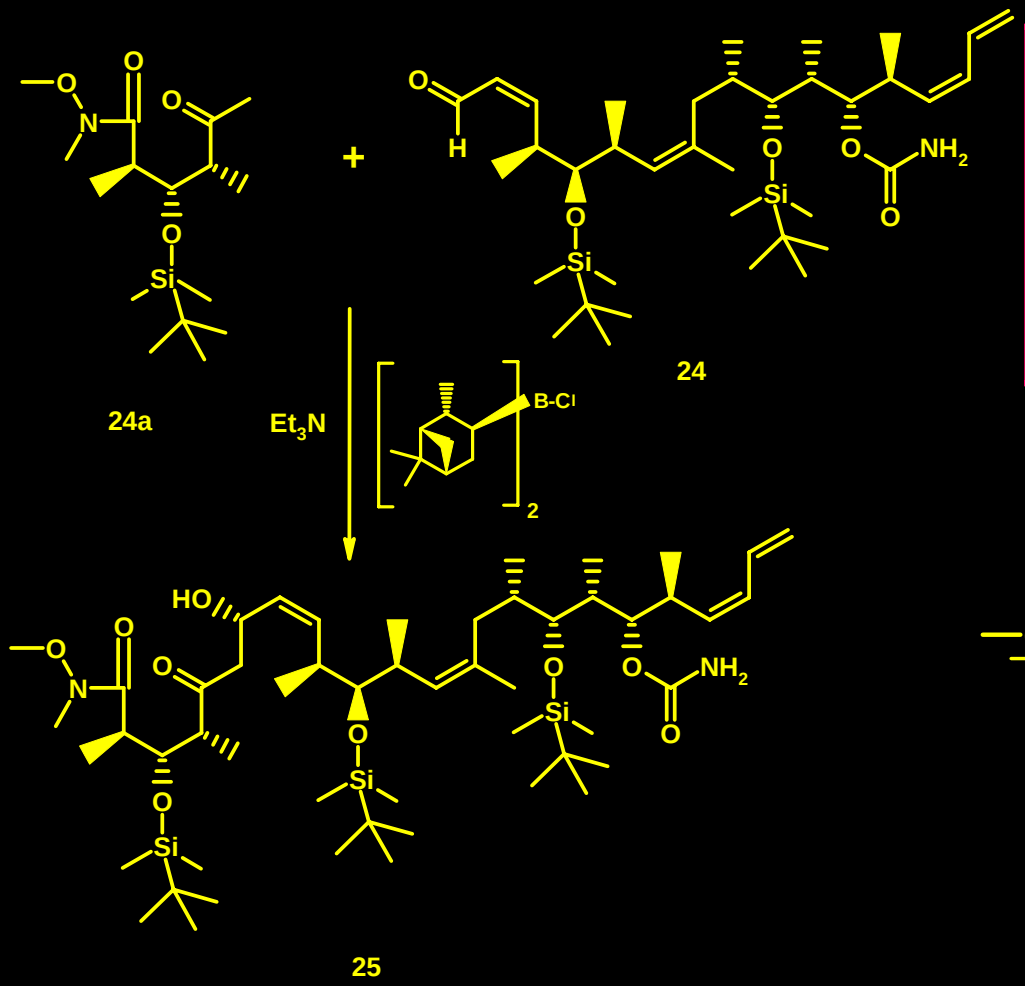
RM aerated! Complete after 18Hrs RT!

3rd batch as 2nd batch – NO REACTION

Heated to 40°C, complete within 2 Hrs!

Campaign	Overall yield	Absolute quantities
1 (6g)	80%	0.3Kg
2 (60g)	82%	2.7Kg
3 (500g)	65%	7.3Kg

25



Test campaign (2 x ~20g 24)

Yield 30-60%

Solid (+)-DIP-Cl

Peroxide work-up

Chromatography

Initial 2nd campaign 2x 50 gram 24

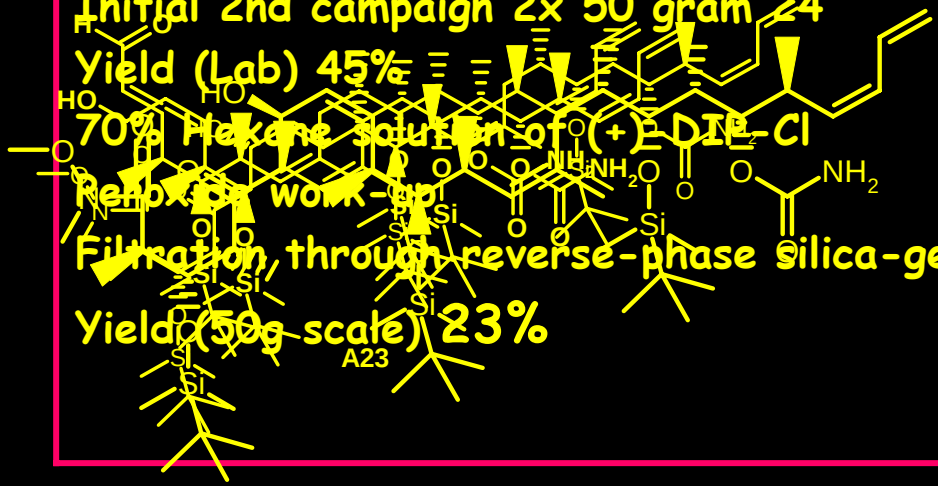
Yield (Lab) 45%

70% Hexane solution of (+)-DIP-Cl

Peroxide work-up

Filtration through reverse-phase silica-gel

Yield (50g scale) 23%

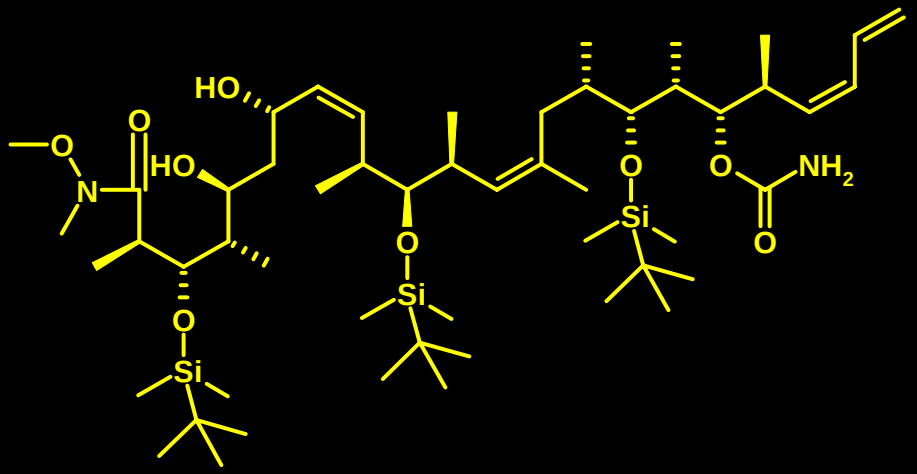


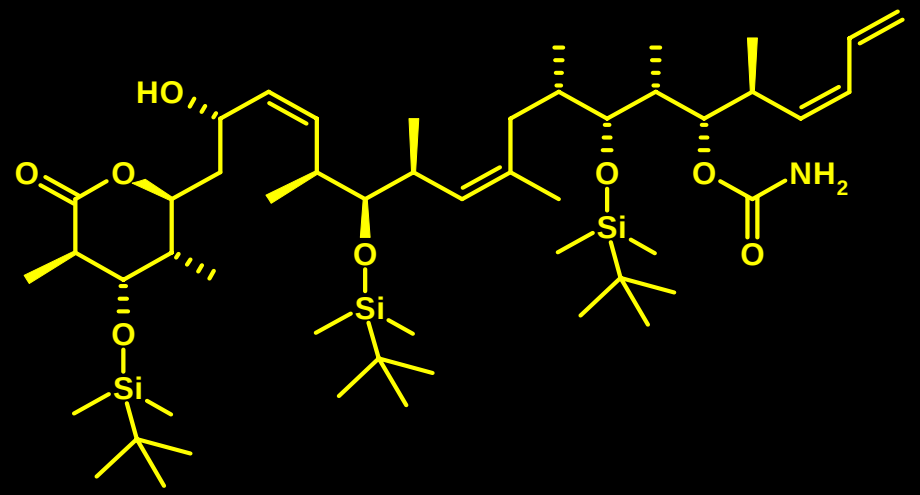
Aldol chromatography

- 2 reactions combined = 700g
- Diluted 368kg MeCN/TBME/H_2O 85/15/10
- Apply to 20kg RP-18 silica-gel (column 120x30cm)
  - Elute 1060kg MeCN/TBME/H_2O 85/15/10
  - Then 150kg MeCN/TBME 1/1
  - Collect 20kg fractions
  - Combine product fractions
  - Evaporate to 10% of original volume
  - Extract TBME, evaporate

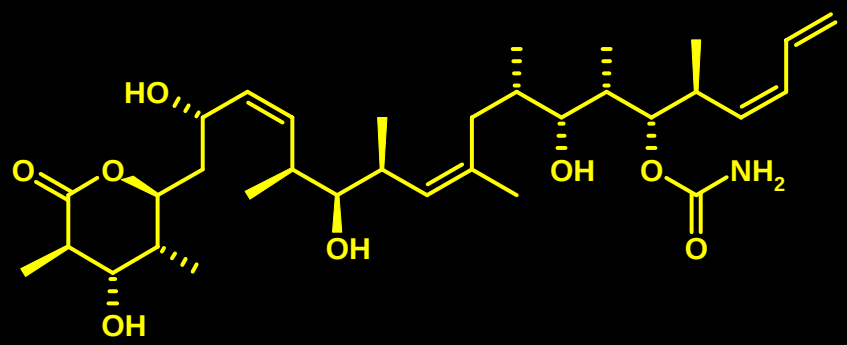
- **Obtain 150g pure aldol product epimer free**

27/28





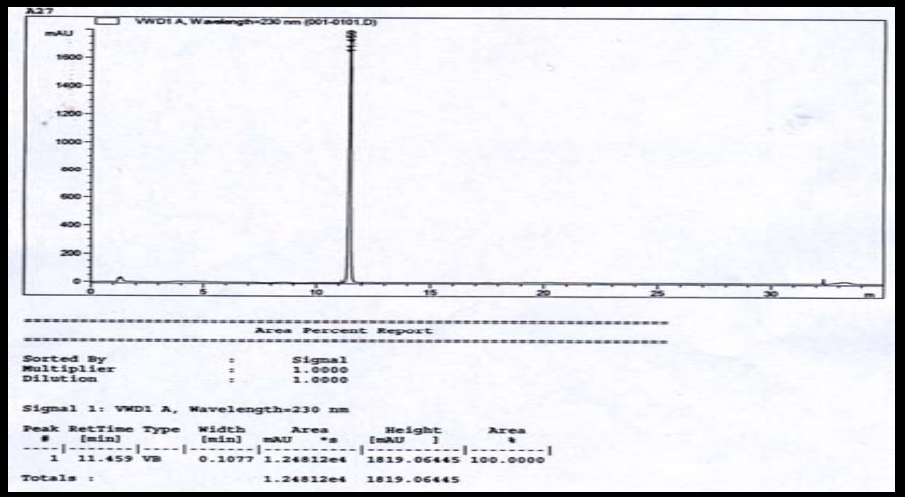
MeOH/HCl



1st Campaign, 75g 26, = 77mMol/ reaction, 3 reactions; 50% yield

2nd Campaign, 1Kg 26, = 1 Mol planned, 3 reactions

HPLCs of Synthetic Disco

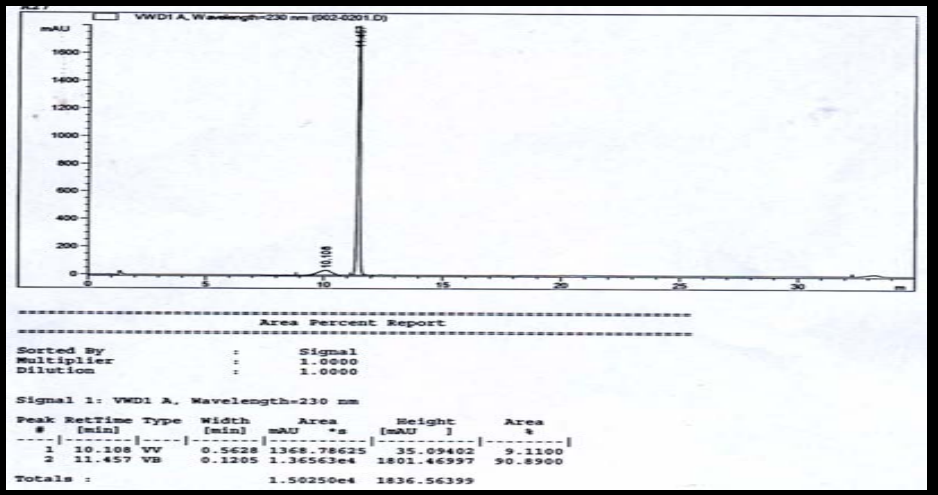


Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: VWD1 A, Wavelength=230 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	11.459	VB	0.1077	1.24812e4	1819.06445	100.0000
Totals :				1.24812e4	1819.06445	

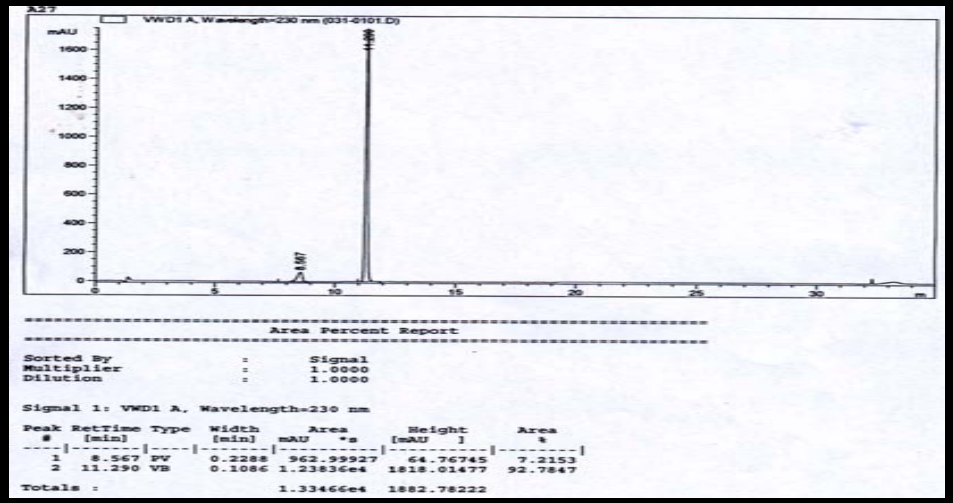


Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: VWD1 A, Wavelength=230 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	10.108	VV	0.5628	1368.78625	35.09402	9.1100
2	11.457	VB	0.1205	1.36563e4	1801.46997	90.8900
Totals :				1.50250e4	1836.56399	

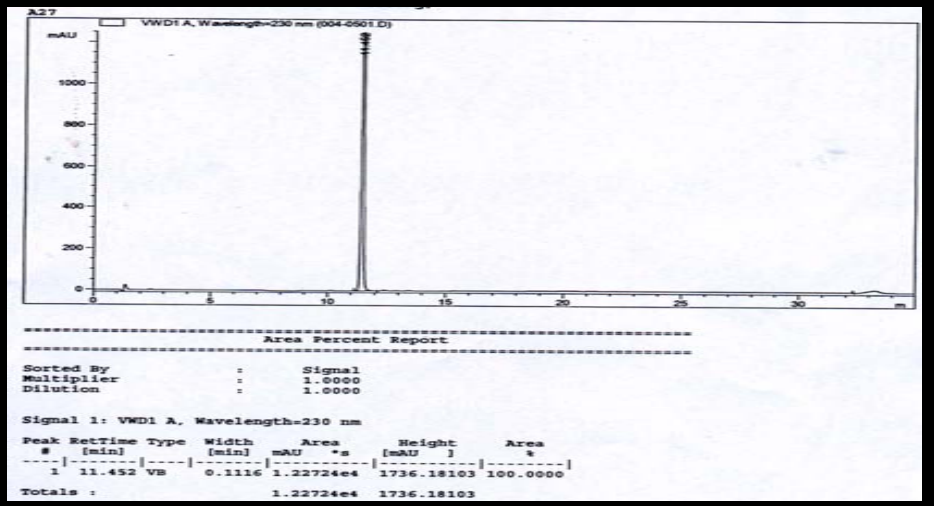


Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: VWD1 A, Wavelength=230 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.567	PV	0.2288	962.99927	64.76745	7.2153
2	11.290	VB	0.1086	1.23836e4	1818.01477	92.7847
Totals :				1.33466e4	1882.78222	



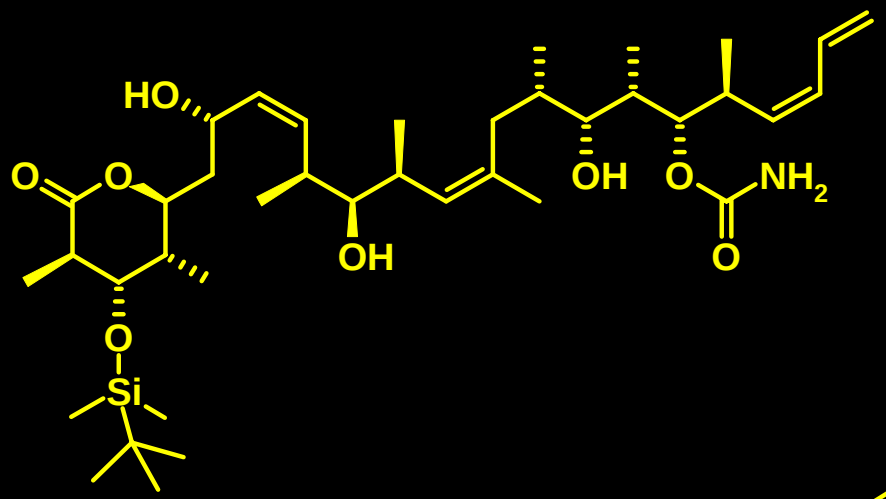
Area Percent Report

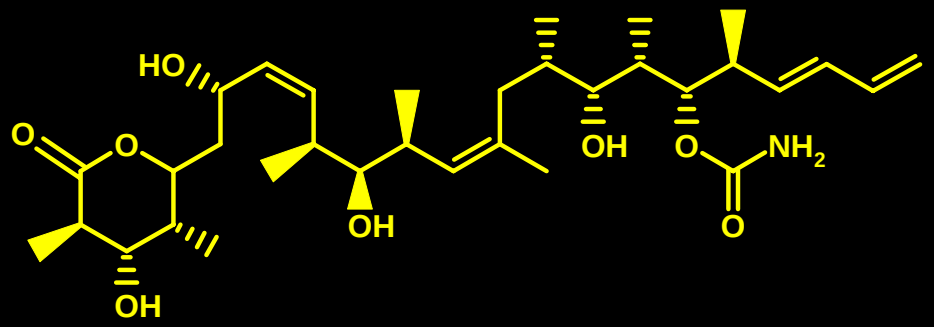
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

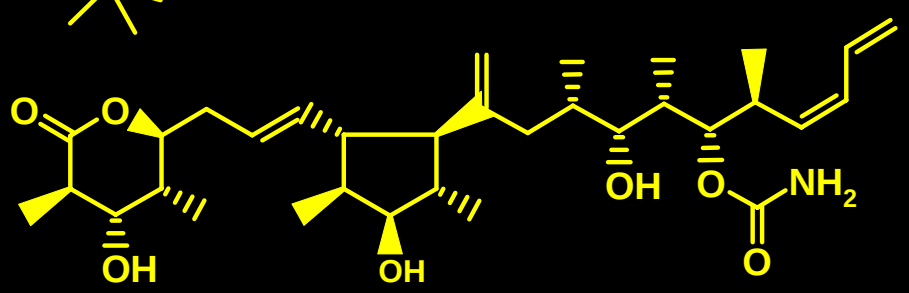
Signal 1: VWD1 A, Wavelength=230 nm

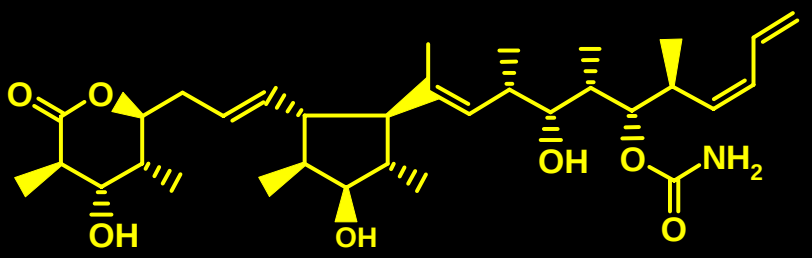
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	11.452	VB	0.1116	1.22724e4	1736.18103	100.0000
Totals :				1.22724e4	1736.18103	

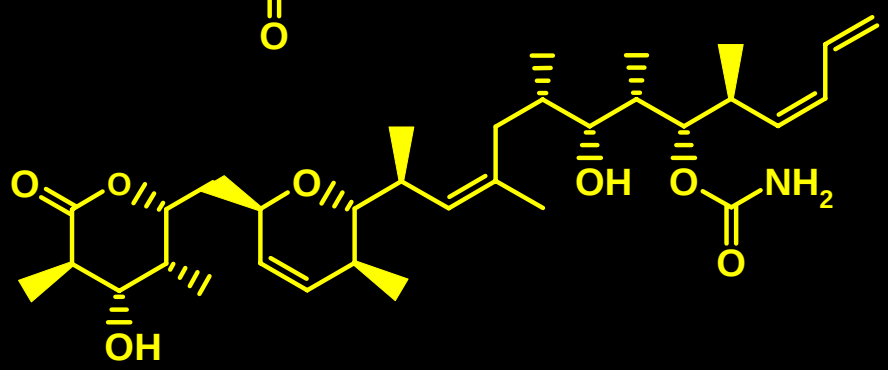
Cleavage side products



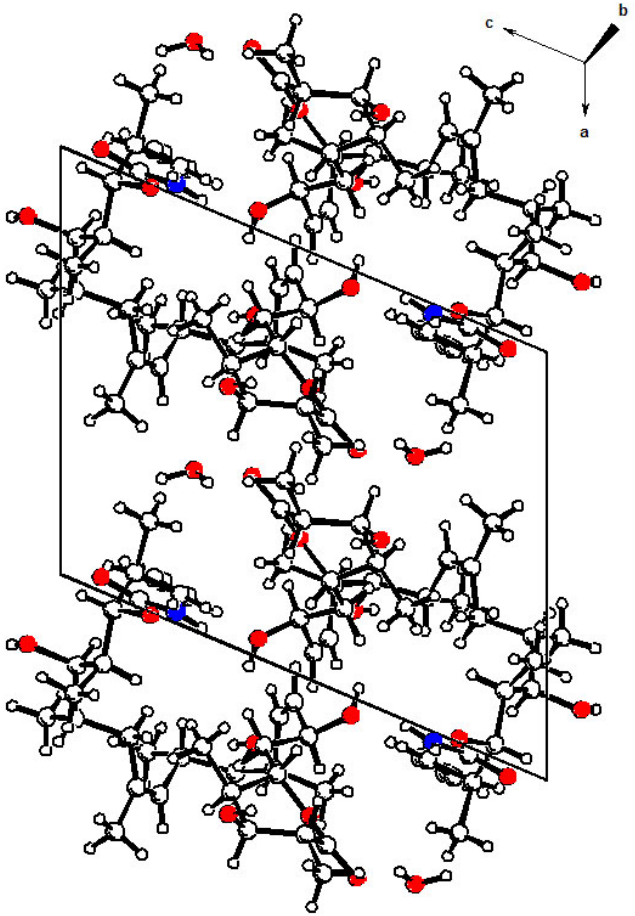


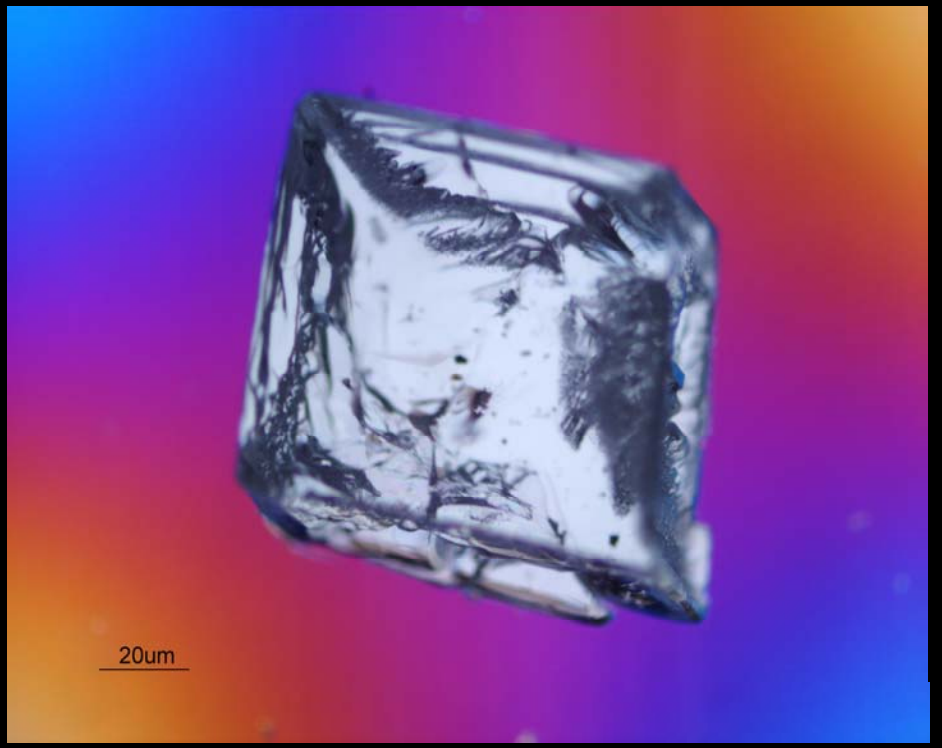




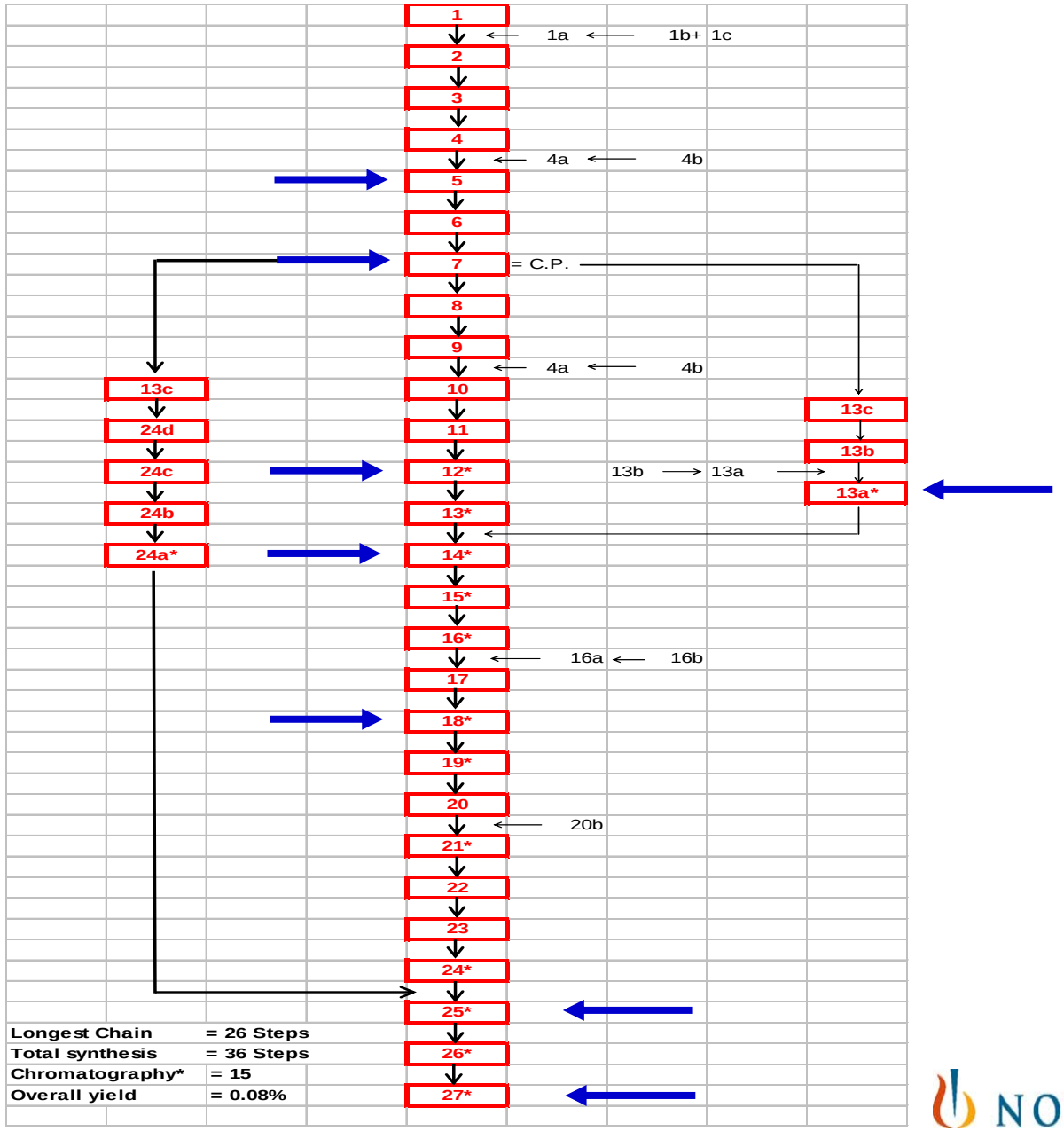


NVP-XAA296-NXA monohydrate





Problem steps



NOVARTIS

Costs and Future

Disco from the phase 1 campaign cost

Lots of SFr/Kg

Future:

find and develop an alternative synthesis which is simpler, shorter, and cheaper with the aim of producing up to 25Kgs per annum