An Economical Synthesis Route in the Ocean for the Marine Natural Product, Bryostatin

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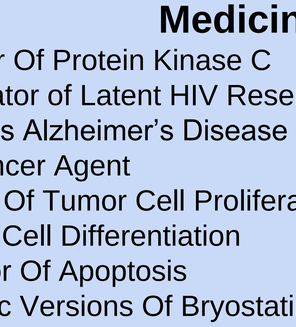
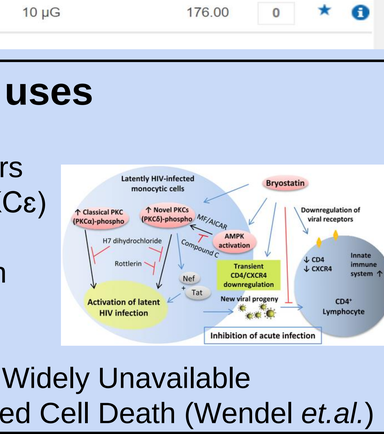
Abstract:
Bryostatin is a marine natural product found in minute amounts in nature. Cost 17 million USD/gram, this molecule targets latent HIV reservoirs, has potential for slowing the progression of Alzheimer's, and demonstrates strong anti-cancer properties. We have developed an economical synthesis of bryostatin through the creation of a bioreactor that cultivates a symbiotic bacterium which lives on the bryosome B. Nerintina. This bioreactor is comprised of inexpensive organic materials providing essential nutrients which promotes the growth of the bacterium. Our bioreactor was deployed off the Gulf Coast for a short period to attract our symbiotic bacterium. This bioreactor was returned to the lab where it was monitored weekly for 2 months with sample extraction every other day. Each sample was labeled and stored in cold storage to prevent the degradation of bryostatin. After our collection period, we ran each sample through a UV Spectrometer to determine the possible presence of Bryostatin. Promising samples were, then, ran through a liquid chromatography mass spectrometer to determine the different types of Bryostatin present

History/Facts

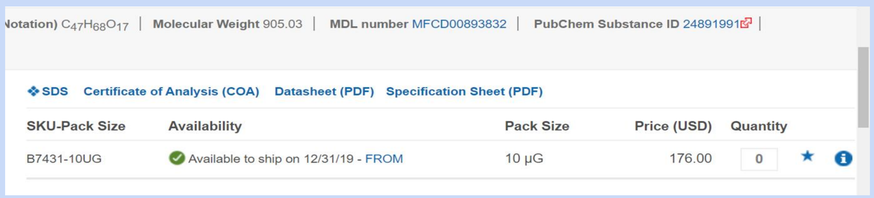
- Jack Rudloe, a marine collector based in Panacea, Florida, sent marine invertebrate specimens to the NCI for testing at the request of Dr. Jonathan Hartwell
- *Bugula neritina* showed promising in testing against PS-100 Leukemia
- Dr. George R. Pettit (Arizona State University) first isolated the active compound and named it **Bryostatin**
- *Bugula* colony is ~3-5 inches across and has an outer coating of Calcium Carbonate in addition to other calcium compounds
- Bryostatin is present in *Bugula* at 10^{-7} % by mass
- A picture of *Bugula neritina* has been provided
- In 1991, 14 tons of *Bugula* produced only 18 grams of Bryostatin





Synthesis/ Pricing

- Lab synthesis of Bryostatin is an economically infeasible process
- Bryostatin 1, 2, 3, 7, 9, and 16 have lb synthesis steps
  - Number of steps ranges from 29 (Byro. 1, Keck) to 88 (Bryo. 3, Keio University)
- Most recent synthesis of Bryo. 1 was completed in 2017 (Keck)
- Bryo. 8 was synthesized in 2018 by Song, et. al.
- Current Bryo-1 Price: 176 (USD) per 10 ug
- Price Of Top Alzheimer Drug Razadyne: 344 (USD) per 60 tablets of 4 mg apiece
- Comparison: For 1 Month of Treatment a patient would pay 4.2 million (USD) for a Bryo-1 prescription.



Medicinal uses

- Activator Of Protein Kinase C
- Reactivator of Latent HIV Reservoirs
- Reverses Alzheimer's Disease (PKCε)
- Anti-Cancer Agent
- Inhibitor Of Tumor Cell Proliferation
- Induces Cell Differentiation
- Promotor Of Apoptosis
- Synthetic Versions Of Bryostatin-1 Widely Unavailable
- Reduces Chikungunya-Virus Induced Cell Death (Wendel *et.al.*)

References

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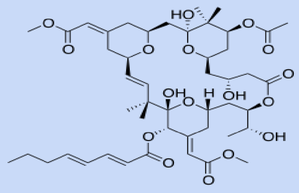
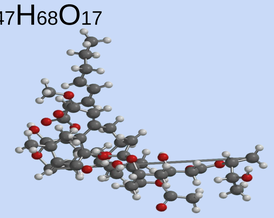
- Wu R.; Chen, H.; Chang, N.; Xu, Y.; Jiao, J.; Zhang, H. Unlocking the Drug Potential of the Bryostatin Family: Recent Advances in Product Synthesis and Biomedical Applications. *Chem. Eur. J.* **2019**,25,1-31.
- Additional References can be sent as requested

Bryostatin 1

Molecular Formula: $C_{47}H_{68}O_{17}$



2D Structure



3D Structure

Bioactivity Properties -------
- GPCR Ligand - 3.06
- Ion Channel Modulator - 3.53
- Kinase Inhibitor - 3.58
- Nuclear Receptor Ligand - 3.29
- Protease Inhibitor - 2.46
- Enzyme Inhibitor - 2.94

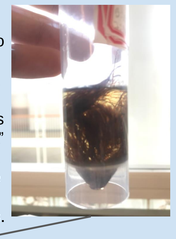
Physical Properties -------
- mi LogP 5.38
- TPSA 240.14
- # atoms 64
- Molar Weight 905.04

Explanation
- These calculations show different properties
- The biological properties show how the drug interacts with the different binding sites with the body.
- The physical properties provide the base on how the drug needs to be administered due to the different physical properties, solubility, ect.

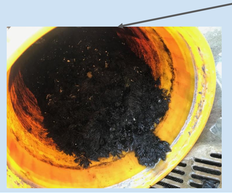
Our Method

The Bioreactor first begins with inexpensive materials combined together on the site at which we choose to deploy it. The cheap materials provide the media that attracts the bacteria we are targeting.

The Bioreactor is then transported to the lab to allow the biofilm to grow. Over the weeks, The bioreactor is monitored with samples "Like the one to the left" are taken to be tested. Different indicators are used to determine presence of bryostatins.



After the Bioreactor is assembled it is deployed out in the water for a certain amount of time to allow the bacteria to migrate.

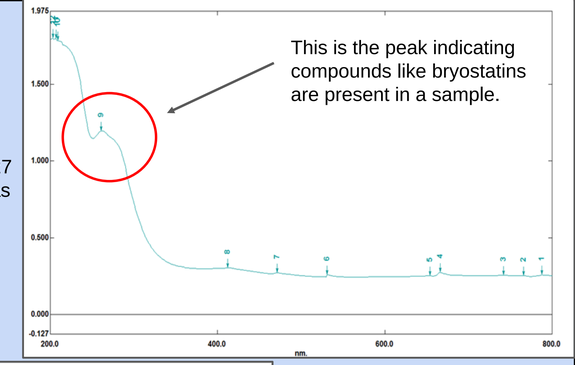


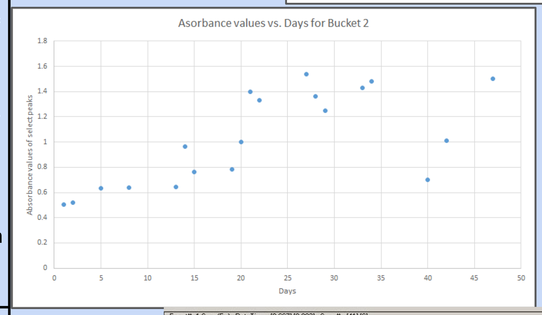
Once the concentration of Bryostatins is highest, the bioreactor is set to dry and be prepped for extraction

Conclusion

Results conclude that our method is viable for the production of the marine natural product bryostatin. Bioreactor tests show that bryostatins are naturally synthesized during the two months of cultivation. With further trials, we can optimize our method and increase the yield of the desired product. Eventually, our method can be scaled to commercial levels. This scaling can allow a more economic source of bryostatins for the market and further allowing research in the many field that could not be done due to high cost.

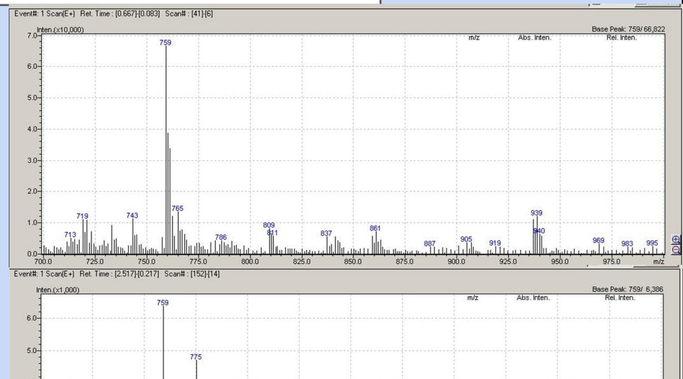
This graph is Ultraviolet Spectra of a sample taken 27 days after the bioreactor was taken from the ocean.





This graph is used to determine which samples are best to use for liquid chromatography mass spectrometry. Also graphs like these help determine peak times for extraction.

This is a Liquid chromatography Mass spectrometry (LCMS). LCMS shows different masses of fragments present. These fragments can then be used to determine which samples are good for NMR. Charts like this help determine from the L



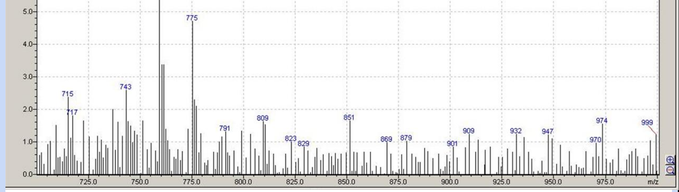
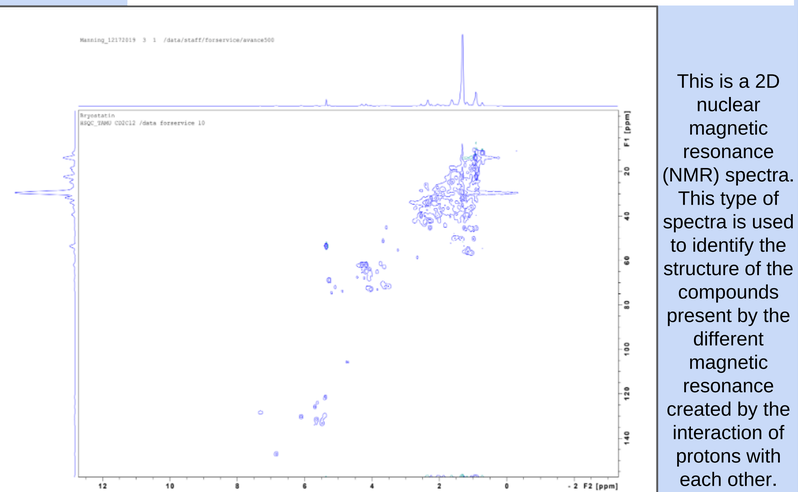


TABLE I The monoisotopic masses of bryostatin 1–18, the side chain masses and the empirical formulas of the chemical species

Bryo. #	*Monoisotopic mass*	*M. M.+ (Na+): 22.9892*	*M. M.± (H2): 2.0156*	*Group R1 monoisotopic mass (attached)*	*Group R2 monoisotopic mass (attached)*	*Empirical formula*
1	904.4456	927.4348	902.4300 906.4613	59.0133: CH_3COO	139.0759: $CH_3(CH_2)_2(CH_3)_2COO$	$C_{45}H_{68}O_{17}$
2	862.4350	885.4243	860.4194 864.4507	139.0759: OH	139.0759: $CH_3(CH_2)_2(CH_3)COO$	$C_{42}H_{66}O_{16}$
3	888.4143	911.4035	886.3987 890.4300	59.0133: CH_3COO	139.0759: $CH_3(CH_2)_2(CH_3)COO$	$C_{44}H_{64}O_{17}$
4	894.4613	917.4505	892.4456 896.4769	101.0602: $(CH_3)_2CHCH_2COO$	87.0446: $CH_3(CH_2)_2COO$	$C_{44}H_{70}O_{17}$
5	866.4300	889.4192	864.4143 868.4456	101.0602: $(CH_3)_2CHCH_2COO$	59.0133: CH_3COO	$C_{42}H_{66}O_{17}$
6	852.4143	875.4035	850.3987 854.4300	87.0446: $CH_3(CH_2)_2COO$	59.0133: CH_3COO	$C_{41}H_{64}O_{17}$
7	824.3830	847.3722	822.3674 826.3987	59.0133: CH_3COO	59.0133: CH_3COO	$C_{39}H_{60}O_{17}$
8	880.4456	903.4348	878.4300 882.4613	87.0446: $CH_3(CH_2)_2COO$	87.0446: $CH_3(CH_2)_2COO$	$C_{45}H_{68}O_{17}$
9	852.4143	875.4035	850.3987 854.4300	87.0446: $CH_3(CH_2)_2COO$	59.0133: CH_3COO	$C_{43}H_{66}O_{17}$
10	808.4245	831.4137	806.4088 810.4401	101.0602: $(CH_3)_2CCOO$	1.0078: H	$C_{42}H_{64}O_{15}$
11	766.3775	789.3667	764.3619 768.3932	59.0133: CH_3COO	1.0078: H	$C_{39}H_{58}O_{15}$
12	932.4769	955.4661	930.4613 934.4926	87.0446: $CH_3(CH_2)_2COO$	139.0759: $CH_3(CH_2)_2(CH_3)COO$	$C_{48}H_{70}O_{17}$
13	794.4088	817.3980	792.3932 796.4245	87.0446: $CH_3(CH_2)_2COO$	1.0078: H	$C_{43}H_{62}O_{15}$
14	824.4194	847.4086	822.4037 826.4350	101.0602: $(CH_3)_2CCOO$	17.0027: OH	$C_{43}H_{62}O_{16}$
15	920.4405	943.4297	918.4249 922.4562	59.0133: CH_3COO	155.0708: $CH_3CH_2CHOH(CCH_3)_4$-COO	$C_{47}H_{68}O_{18}$
16	790.4139	813.4031	788.3983 792.4296	101.0602: $(CH_3)_2CCOO$	1.0078: H	$C_{42}H_{62}O_{14}$
17	790.4139	813.4031	788.3983 792.4296	101.0602: $(CH_3)_2CCOO$	1.0078: H	$C_{42}H_{62}O_{14}$
18	808.4245	831.4137	806.4088 810.4401	101.0602: $(CH_3)_2CCOO$	1.0078: H	$C_{42}H_{64}O_{15}$



This is a 2D nuclear magnetic resonance (NMR) spectra. This type of spectra is used to identify the structure of the compounds present by the different magnetic resonance created by the interaction of protons with each other.