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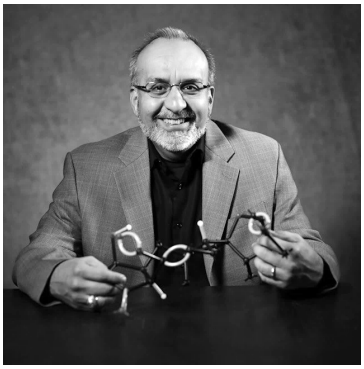
FROM THE WEST SIDE OF SAN ANTONIO TO PHARMACOPHORE-DIRECTED RETROSYNTHESIS

Daniel Romo
Department of Chemistry & Biochemistry, Baylor University, Waco, TX 76798
Email: Daniel_Romo@baylor.edu



Daniel Romo is Schotts Professor of Chemistry at Baylor University and co-Director of the Baylor Synthesis and Drug-Lead Discovery Laboratory. He began his independent research career at Texas A&M University (as a proud Aggie '86) and rose through the ranks to Professor in 1993-2003. In 2014, he became the Gradipore Chair at Texas A&M before moving to Baylor in Fall 2015. The chemistry and biology of bioactive natural products is at the heart of Romo's research interests and this interest drives all of his group's research projects described in >175 manuscripts to date.

The field of natural products is an exciting and enduring inter-disciplinary area for discoveries in basic cell biology and human health. Natural products are unique and often structurally complex molecules that have evolved to interact in highly specific ways with cellular receptors found in various organisms, and due to protein homology, also receptors found in humans.

My Journey.–I am very humbled and grateful to be named the 2024 Distinguished Texas Scientist by the Texas Academy of Science. I am especially humbled as I think about all the people who made this journey possible. Beginning with my God, who as I look back at the innate passion I had for learning and exploring His world, I still to this day am bewildered by why He instilled this in me. Certainly, God placed many people in my history and along my path that inspired, encouraged, and supported me, which leads to my story. Without question, I was inspired by stories about my great grandparents who left San Luis Potosi, Mexico as Pancho Villa was terrorizing store owners such as them, and seeing first-hand how hard my father and mother worked to provide for their family. This passion to provide a better life for their family, including my grandparents (on my mom's side) who lived with us, led my father, with only a 5th grade education, and my mother to start their own business, Romo Sales. My father was an institutional supplier of dry goods to local restaurants in San Antonio

My grandparents, Jose Guevara (a.k.a. 'apa') and Reyes Estalla Guevara (a.k.a. 'granmo') with my mother (age 2) Minerva Guevara Romo (left); family business "Romo Sales" started on the West side of San Antonio with our first delivery truck (Mom and Sister pictured) (right).

where I was born and raised. This business provided well for our family and taught me, my brother, and my sister the value of hard work since my brother and I were required to partake in the family business daily by restocking my father's truck after school so he could be ready to go out and sell his merchandise the following day. I must mention the inspiration provided by my grandmother (a.k.a. 'granmo' a.k.a. Reyes Guevara) who took care of two rascals while my parents both worked outside the home during our young lives. 'Granmo' continued to do this, including cooking and cleaning, despite being paralyzed in half of her body following a stroke when I was ~5 years old. I must also acknowledge a cadre of fantastic teachers in my formative years, including: Mr. Eisenberg, Clever, and Moenkhe and Mrs. Roher and Gulick through my elementary and middle school years. I also had some fantastic high school teachers at Thomas Jefferson HS such as my science teachers: Mr. Miles (who showed me the art of growing crystals and my mom's patience in the messes I made in the kitchen continuing my crystal growth education!), Mrs. Thompson, Mr. Karpenski (these latter two teachers allowed me to develop an Independent Study course

Team Rapamycin Celebration Dinner (1993) with Stephanie Meyer, DR, Laura Romo, Donna Johnson, Stuart Schreiber (left to right; inset: Dr. Tetsuo Miwa who had returned to Japan).

in my senior year as I ran out of science classes to take), Mr. Serna (physics), Ms. Tondre (computer science); Mr. Weiss (photography) and Mr. Salas (drafting). These fantastic teachers inspired me with the passion they had for teaching their respective subjects and serving as role models for the next generation. Mrs. Mendez, my high school counselor, helped and encouraged me immensely with college and scholarship applications enabling me to attend the greatest university, Texas A&M University in 1982 (unbiased, Gig'em), free of charge thanks primarily to the Baumberger Foundation of San Antonio and several other scholarships that she helped me obtain. It was at Texas A&M that I had the privilege to take Organic Chemistry I and II with Prof. Larry Klein who explained things so well, including beautiful board work, that I fell in love with the subject. His teaching inspired this undergrad chemistry major to not attend dental school as originally planned, but rather devote his career to the pursuit of organic synthesis. In my sophomore year, I realized that I wanted to do exactly what Prof. Klein was doing including laboratory research and I received the first taste of organic synthesis, and natural product synthesis in particular, in his lab. I later carried out Senior Thesis research with Prof. Kenn Harding (Chemistry Dept) and Jeffrey Pommerville (Biology Dept) at Texas A&M where I had my first taste of the chemistry/biology interface through synthesizing and then testing novel derivatives of the water mold sex pheromone, sirenin (Pommerville et al. 1988). This experience still impacts my research interests to this day, as I learned

first-hand how small molecules that I synthesized, purified, and characterized could have a direct impact on living organisms as I peered through a microscope to observed how these sperm-like male gametes of a water mold, *Alloymces macrogynus*, responded to a concentration gradient of the compounds I had synthesized. I obtained my Ph.D. at Colorado State University in 1991 under the supervision of a highly supportive mentor, Prof. Albert Meyers, in synthetic methodology (Meyers & Romo 1989; Romo et al. 1990; Romo & Meyers 1992; Romo et al. 1992). Most importantly this is also where I met my wonderful wife of 37 years now, Laura Larson. We were blessed with our first son Matthew as I was writing my dissertation. We then moved to Cambridge, MA so I could pursue post-doctoral studies at Harvard with Prof. Stuart Schreiber. My wife secured a position in the Mass Spectrometry Lab (thanks to John Porco!) and so we both worked in the Chemistry Dept while my son attended Harvard Law School *Day Care*. After leading a team at Harvard in the synthesis of rapamycin (Romo et al. 1993), I began my independent research career at Texas A&M University (yes, full circle!) as an Assistant Professor in 1993. I rose through the ranks, with another four sons making their appearance along the way: Zachary (an Aggie), Nathan (a Baylor Bear), Jedidiah (an Aggie), and much later 'adopted' son, Bryan. I ultimately was named the Gradipore Professor of Chemistry in 2014 before moving to Baylor in 2015. As I reflect on this journey, I am extremely humbled and feel extremely blessed to know that my path was clearly directed by my Lord and Savior, Jesus Christ as I can see no other way for this kid from the West side of San Antonio to become a synthetic organic chemist and currently the Schotts Professor of Chemistry at Baylor University.

The Field of Organic Synthesis and Natural Products.–Organic synthesis is a field which enables chemists to synthesize any molecule we can dream up but, in my case, molecules isolated from various organisms (i.e., natural products). These often highly complex molecules are biosynthesized by a variety of organisms including bacterial, fungi, sea animals like sponges and coral (or bacteria living within them), and plants. The reason that these molecules have useful medicinal properties is often ascribed to an organism's desire to protect

itself through a type of chemical warfare. Going deeper, what is amazing is that because these molecules are assembled using proteins to biosynthesize them and the fact that they are designed to interact with a microbial enemy's proteins in order to kill them, it is not surprising that these molecules also have interactions with proteins in our bodies given that we have many related proteins to those found in various organisms, most notably bacteria. In fact, >50% of all currently used medicines are natural products themselves or are natural product derivatives such as aspirin and penicillin.

"…and their fruit will be for food and their leaves for healing."
Ezekiel 47:12 (NASB)

From a Love of Puzzles to Organic Synthesis.–My mom once told me that from an early age I loved puzzles and this innate interest must have led me to the pursuit of synthetic organic chemistry and natural product synthesis. This interest continues to this day given that a structurally interesting natural product is very much like a puzzle that drives practitioners of organic synthesis to figure out how to reproduce it in the laboratory. Furthermore, natural products are critical tools for unravelling the inner workings of cells, which are puzzles in themselves, and ones that can be deciphered through the use of natural products as cellular probes. We navigate our study of natural products through implementing the scientific method and synthetic chemists know better than most that only through much failure does one find success. I was surprised to learn, only many years after becoming a scientist, that the scientific method is actually contained in the Bible in succinct form. While Paul may not have had scientific truths but rather spiritual truths in mind when he wrote this, this is in fact the way I approach my faith and my science.

"….examine everything; hold firmly to that which is good…" 1 Thessalonians 5:21 (NASB)

Synthetic organic chemists develop plans and then build molecules from simple, commercially available starting materials by constructing each required bond including carbon-carbon, carbon-oxygen, and

carbon-nitrogen bonds in an orchestrated and methodical manner to ultimately deliver a targeted molecule. More often than not, our initial (or even secondary or tertiary!) plans do not work so a high level of resilience and tenacity is required in synthetic organic chemistry as we typically must 'go back to the drawing board' several times until a solution is found. One analogy is that we are engineers and construction workers at the molecular level dreaming up ways to construct complex sky scrapers by first developing plans and then using the 'rules' of organic chemistry developed over the past ~200 years to attempt to synthesize these molecules in the laboratory. This field began with Fred Wohler's synthesis of urea in 1828 and the complexity of molecules that have been achieved in this field is inspiring with many great synthetic organic chemists such as Profs. Robert Woodward and E. J. Corey and many others showing us what is possible.

Retrosynthetic Analysis.–A powerful and unified strategy utilized to realize elegant and concise solutions to the total synthesis of complex natural products is retrosynthetic analysis. First articulated by E. J. Corey in The Logic of Chemical Synthesis (Corey & Cheng 1989), retrosynthetic analysis has become a cornerstone to modern synthetic endeavors allowing for the deconvolution of complex natural products into increasingly simplified intermediates and eventually commercially available starting materials. The increased capabilities realized through retrosynthesis fostered the generation of ideas which encompass an "ideal synthesis", a term used by Hendrickson (Truax & Romo 2020) which has evolved to include: concise and convergent strategies (step economy) (Wender et al. 2006), decreasing reactant waste throughout a synthesis (atom economy) (Trost 1991), and eliminating unnecessary redox manipulations redox economy) (Newhouse et al. 2009). The concept of an ideal synthesis is one that all chemists aspire to achieve; however, the completion of a complex natural product is not always considered simultaneously with collection of information regarding functionality required for a natural product's biological effects. This two-fold goal can become quite challenging, requiring a balance between synthetic efficiency (Hendrickson 1975; Trost 1991; 1995; 2002) and gained structure activity relationship (SAR) data. This is best articulated in Wender's function-oriented synthesis (FOS) strategy

(Wender et al. 2004; 2006; Wender 2014). Given the high percentage of drugs that were inspired by natural product scaffolds, or are natural products themselves (Newman & Cragg 2016), the continued harvesting of the vast information content available from natural products and their cellular receptors is essential.

Natural Products: A God-given Playground for Synthetic Chemists.–My students and I have collaborated over the past ~30 years to successfully develop synthetic strategies for the total synthesis of natural products and, in several instances, we have utilized the established synthetic routes to prepare tool compounds that enable unraveling of the molecular details of the bioactivity of the natural product in a variety of cell types. As a tribute to the wonderful students that I have been blessed to work with over the years (42 Ph.D students, 9 M.S. students, 37 post-doctoral fellows, >160 undergraduates), I show the collection of natural products that my students have synthesized over the years (Figure 1, name of primary contributor(s) shown and date first synthesized) and I would not be receiving this award were it not for their hard work and diligent efforts of my students.

In some cases, in collaboration with some fantastic biological collaborators over the years including Prof. Jun O. Liu (Johns Hopkins), Prof. Stephan Sieber (Tech. U. of Munich, Germany), Prof. Jerry Pelletier/Prof. Bushan Nagar (McGill U., Canada), Jeffrey Smith (Burnham Med.) and many others, we have been able to gain a molecular-level understanding of how these molecules exert their bioactivity inside cells. Two examples are depicted in Figure 2. Our collaborative work with Prof. Liu's team revealed that pateamine A is a potent inhibitor of the RNA helicase eIF4A bound to RNA (Figure 2a). This protein unwinds RNA in preparation for conversion to protein by the ribosome at the initiation phase of protein synthesis. An X-ray crystal structure of the ternary complex was secured leading to our first view of this interfacial inhibitor. Ultimately, this leads to apoptosis, programmed cell death, and novel derivatives of this protein translation inhibitor are showing great promise as lead compounds, in particular as a combination therapy for pancreatic cancer in mouse model studies. Our studies in this area with Prof. Susan Bates (Columbia Medical) will

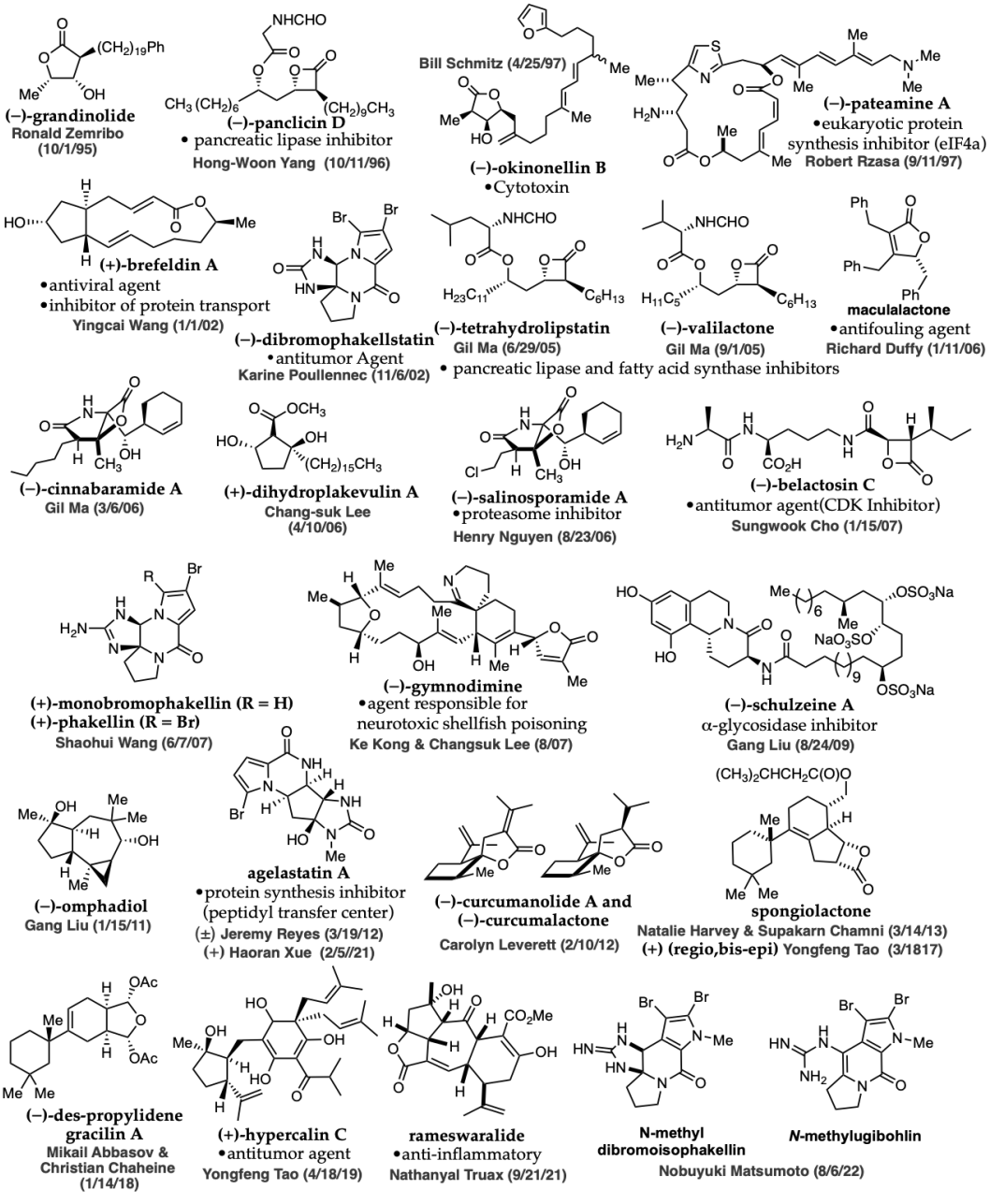


Figure 1. Selected natural products and derivatives synthesized by the Romo Group from 1993-2022.

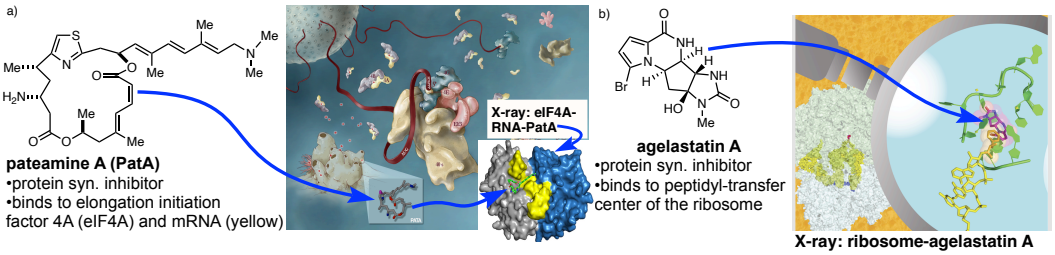


Figure 2. (a) Molecular level details of the mode of action of pateamine A (PatA). We determined with our collaborators that PatA is a protein translation inhibitor forming a ternary complex with eIF4A and leading to inhibition of protein synthesis, at the initiation phase, leading to stress granule formation and apoptosis. (b) Molecular level details of the mode of action of agelastatin A found to also be a translation inhibitor, however in this case at the elongation phase of protein synthesis through binding to the peptidyl-transfer center of the ribosome.

soon be transitioning to pre-clinical studies to ideally address this very aggressive type of cancer. In another collaborative project at the chemistry-biology interface, again with the Liu group but, in this case, X-ray crystallography conducted by the Yusupov group confirmed that agelastatin A is also a protein translation inhibitor but this time at the elongation phase of protein synthesis (Figure 2b). Agelastatin A binds to the peptidyl transfer center of the ribosome and importantly this natural product is blood-brain barrier penentrable and thus holds promise for glioblastoma therapy.

Pharmacophore-Directed Retrosynthesis (PDR).–In one major area of the Romo Research Group, natural products are selected for chemical synthesis based on compelling and potent biological activity in addition to challenging synthetic hurdles that must be overcome to access these molecules. Recently, we have started to approach our total synthesis efforts quite differently than in the first ~23 years of my independent research program. While we have always been driven by the synthesis of biologically active natural products toward gaining a molecular level understanding of their biological effects, our approach rarely strayed from classic total synthesis ideology, particularly with regard to traditional retrosynthetic analysis. We recently gained perspective for the shortcomings of this approach in hindsight due to our extensive and continued studies of the immunosuppressive and antitumor marine natural product, pateamine A (PatA, 1a, Figure 3) (Rzasa et al. 1998),

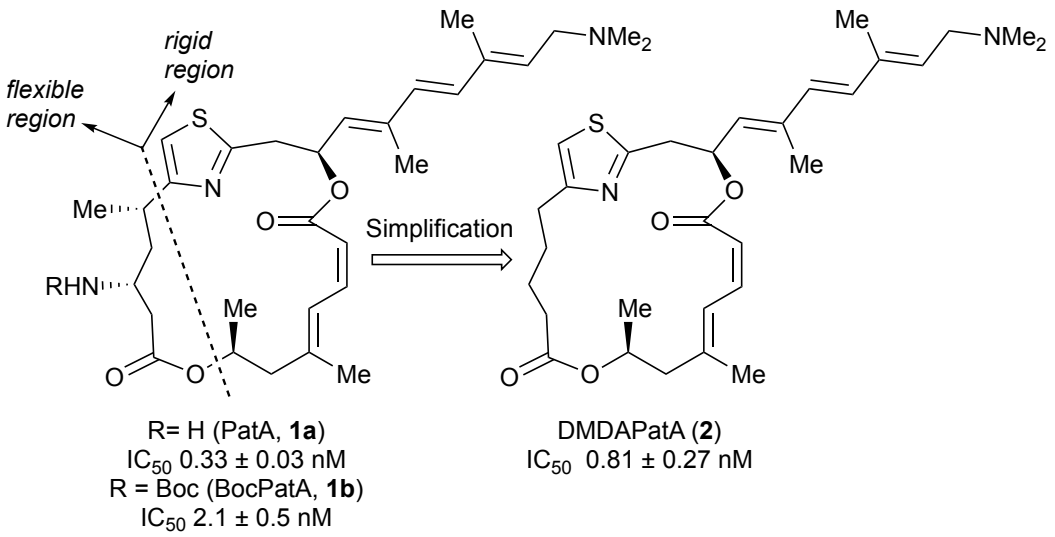


Figure 3. Pateamine A served as inspiration for development of pharmacophore-directed retrosynthesis. (IC_{50} values for the interleukin-2 reporter gene assay using the same population of Jurkat cells.)

a natural product first isolated by Munro and Blunt from a sponge off the New Zealand shores (Northcote et al. 1991). We were initially drawn to PatA due to its novel structure, reported immunosuppressive activity, yet unknown mechanism of action. PatA was one of our group's earliest success stories in delivering a reasonably efficient total synthesis, performing preliminary minimal SAR and ultimately determining its mechanism of action through synthesis of a biotin conjugate that enabled affinity chromatography. Through a fruitful collaboration with the Liu Group (Johns Hopkins) using our biotin conjugate, they determined that PatA exerts its potent antiproliferative activity by binding to elongation initiation factor-4A (eIF4A) resulting in inhibition of cap-dependent eukaryotic protein translation. Formation of the PatA-eIF4A complex stalls the translation initiation complex on mRNA *in vitro* leading to protein synthesis inhibition, stress granule formation, and ultimately apoptosis (Low et al. 2005; 2007a, b; 2005; 2014; Dang et al. 2006; 2009). However, we did not anticipate that our journey with PatA almost 25 years later would lead to the strategy that we now approach all our natural products with, namely pharmacophore-directed retrosynthesis (PDR). We completed a total synthesis of PatA in 1998 (Rzasa et al. 1998) and later that year we reported an improved synthetic route which in collaboration with

the Liu group provided our first SAR data for PatA and related analogues (Romo et al. 1998). Subsequently, we published the design, synthesis, and bioactivity of several additional PatA analogues, most notably DMDAPatA (2), which we proposed possessed the required pharmacophoric elements for PatA's bioactivity reminiscent and certainly inspired by Wender's FOS ideas. During our initial bioactivity studies of PatA and derivatives, we found that Boc-PatA (1b) had only a ~6-fold decrease (0.3 vs 2.1 nM) in the IL-2 assay, which we were employing at the time to pursue the reported immunosuppressive activity, relative to the natural product. This led us to hypothesize that PatA consisted of a less conformationally constrained region (red, 'scaffolding domain', Figure 3) which could tolerate alteration, while modification of the more conformationally rigid region ('binding domain', blue) resulted in significantly diminished bioactivity. Thus, a convergent route to des-methyl, des-amino PatA (DMDAPatA) devoid of two of four total stereocenters was developed building on our established synthesis of PatA through application of Danishefsky's Diverted Total Synthesis (DTS). Bioactivity studies subsequently revealed that DMDAPatA actually displayed similar bioactivity to the natural product (Romo et al. 2004). Our studies of PatA and its analogues continue to this day including initial animal studies with Eisai for various cancers (Kuznetsov et al. 2009) and most recently, pancreatic cancer with the Bates group at Columbia Medical (Safari et al. 2024), the initial finding of DMDAPatA's comparable activity to PatA with a greatly simplified structure led to an intriguing retrospective question. *Could the design and synthesis of DMDAPatA have been conceived earlier and thus synthesized en route to pateamine A rather than many years later?* This strategy, which we have termed 'pharmacophore-directed retro-synthesis (PDR),' builds on concepts first introduced by Wender to bring function to the forefront of any target-oriented synthesis project. Our continued interest in total synthesis of bioactive natural products, particularly those with unknown biological receptors, led us to conceive of PDR. To restate a question posited in our first disclosure of the concept of PDR: *Can the total synthesis of natural products, in particular with limited SAR or unknown or unconfirmed cellular targets, be more closely aligned to proposed biological activity during the retrosynthetic planning stages?*

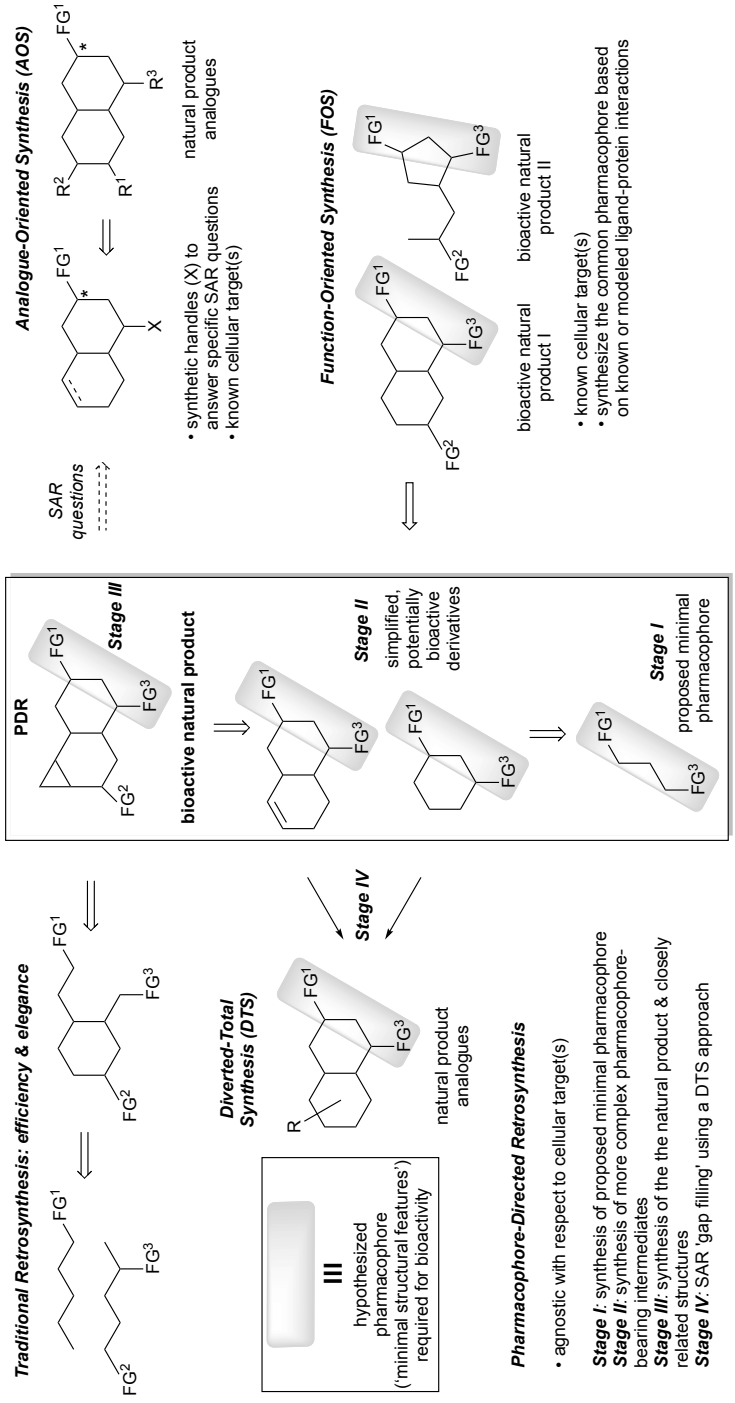


Figure 4. Overview of various synthetic strategies bring biological function to the forefront including those employed to utilize bioactive natural products as starting points for biological studies building on ideas of FOS with comparison to PDR. The latter enables gathering of SAR information en route to a given natural product via target-oriented synthesis

(Abbasov et al. 2019). A total syn-thesis following PDR principles would allow the gathering of valuable SAR information during the course of a total synthesis since multiple intermediates possessing the putative pharmacophore would be accessed *en route* to the natural product. This ultimately increases the potential of identifying simplified equipotent versions much earlier in a total synthesis effort.

We termed the strategy pharmacophore-directed retrosynthesis (PDR) to emphasize the *importance of considering the pharmacophore* or "pharmacophoric" elements of a natural product at the retrosynthetic planning stage, thus allowing the design of a total synthesis wherein the putative pharmacophore is synthesized first followed by more elaborate derivatives with increasing structural complexity *en route* to the natural product. PDR aims to utilize Wender's notion of bringing function to the forefront of synthesis (FOS) while employing the logic of retrosynthesis to target simplified derivatives bearing the proposed pharmacophore (Wender & Zercher 1991). This approach has the potential to reveal simplified analogues much earlier in a total synthesis effort with the important caveat that for many natural products, but certainly not all (Towle et al. 2001; Posner & O'Neill 2004; Wender et al. 2004), the entire structure may indeed be required to obtain comparable bioactivity to the natural product (Scheme 1). However, application of PDR enables one to determine this requirement prior to completion of the natural product which in some cases may be available in quantities useful for control experiments in concurrent biological studies.

PDR was developed to be applied to natural products in which only minimal information is known regarding the structural features required for bioactivity and limited or no information regarding the natural product's putative cellular target(s). To apply PDR to a given natural product, one first develops a hypothesis for the 'pharmacophore' or 'minimal structural requirements for bioactivity' for a particular natural product or class of natural products. It is important to note that within PDR, the term 'pharmacophore' is loosely used compared to what a medicinal chemist might use as a definition taking into account all hydrogen-bonding, hydrophobic interaction, π–π interactions, etc.

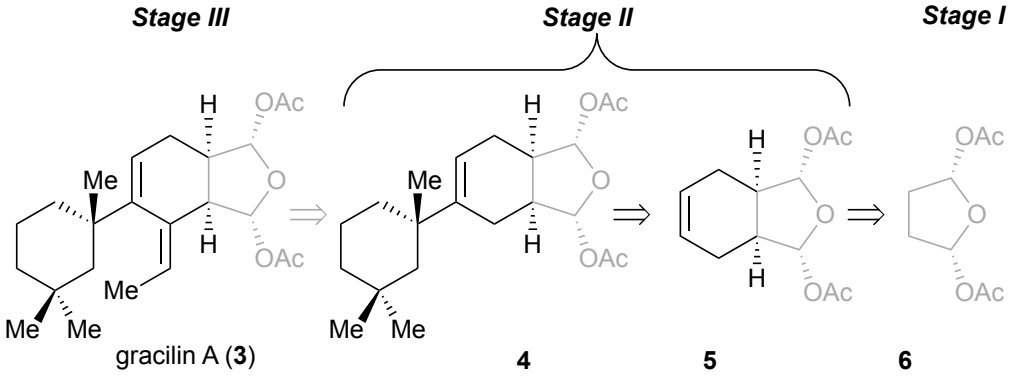
Thus, application of PDR does not require prior identification of the cellular target nor docking experiments to reveal the actual pharmacophore; however, computational methods could be used to ascertain the major conformation and exposed hydrogen-bonding donor/acceptors of the natural product. Furthermore, the absence of cellular target identity precludes the use of both structure and ligand-based modelling to aid in actual pharmacophore determination as in FOS. Thus, to apply PDR, the development of a hypothesis for a 'pharmacophore' is required and can be informed by several considerations: i) overall structural and conformational analysis of the natural product with chemical intuition, ii) existing SAR data of natural product congeners or biosynthetic precursors often provided by isolation chemists, iii) the bioactivity of structurally related natural products, and in the easiest application iv) the presence of reactive functionality which may covalently modify cellular targets (e.g. beta-lactones, epoxides, aldehydes).

With a proposed pharmacophore in hand, a retrosynthesis is designed to access the hypothesized pharmacophore early in the total synthesis effort, and subsequent elaboration to more complex pharmacophore-containing intermediates enables gathering of SAR information at a much earlier stage in a total synthesis effort (Scheme 2). The forward synthesis can proceed in stages. Stage I seeks to synthesize the simplest form of the proposed pharmacophore. Ideally, the target from stage I will be utilized as a synthon to access more complex derivatives in Stage II, however this may not always be practical, especially in cases where the proposed pharmacophore is reactive or unstable. Finally, natural product derivatives synthesized in Stage II can be utilized as intermediates for the synthesis of the natural product and closely related derivatives (Stage III). The derivatives resulting from Stages I – III provide valuable SAR information regarding the essential structural features of the natural product required for bioactivity. Inevitably this will lead to further questions regarding the motifs and moieties present on the synthetic derivatives and natural product. Fortunately, as previously mentioned, the PDR approach towards synthesis provides multiple advanced intermediates which can be utilized for a final stage (Stage IV) of SAR 'gap fill'

utilizing Danishefsky's DTS without the need to develop a new synthetic strategy to allow for derivatization. The stages not only help to provide a near-term target, but they also help facilitate concurrent synthetic and biological studies.

PDR Applied to the Gracilins.–PDR prioritizes the collection of SAR information for natural products; therefore, while the economies of synthesis are still of concern, it holds lower priority, as displayed in the primarily linear routes required to utilize PDR for full benefit. To date, we have applied PDR to two natural products that indeed led to the identification of simplified natural product derivatives possessing in some cases more potent activity compared to the natural product, providing initial proof of concept for the utility of PDR.

The gracilins, isolated from *Spongionella gracilis* (Leirós et al. 2014; 2015; Sanchez et al. 2016), are a family of natural products, many of which contain a unique bis-acetoxy furanose (Scheme 1, red), wherein gracilin A was reported to possess both neuroprotective and immunosuppressive activity (Rateb et al. 2009). Given that only minimal information regarding SAR and no prior synthetic work was available for these natural products, we targeted gracilin A (3) for application of PDR to interrogate these two bioactivities in a collaborative effort with the Botana group (Universidad de Santiago de Compastella) who had reported potential interactions of these sponge isolates, particularly gracillin A, with the cyclophilins.



Scheme 1. Various stages of PDR applied to gracilin A.

As described above, the first step of applying PDR to a target molecule is the development of a hypothesized pharmacophore. In the case of gracilin A and related congeners what readily stood was the common bis-acetoxy furanose moiety (Figure 5a) which can be seen as a masked 1,4-dialdehyde that upon hydrolysis could engage protein targets through Schiff base formation and further condensation, Paal-Knorr pyrrole synthesis (Kornienko & La Clair 2017). In the case of macfarlandin E (11, Figure 5b), the Overman group had demonstrated that a simplified *t*-butyl derivative 12, possessing a similar masked 1,4-dialdehyde engaged lysine in a Paal-Knorr condensation under simulated physiological conditions (Schnermann et al. 2010). Furthermore, computational studies provided evidence that the bis-acetoxy motif of gracilin A (1) and a structurally related natural product aplysulphurin-1 (not shown) binds divalent cations including Ca^{2+}, which might be related to their bioactivity. Thus, these lines of evidence led to our hypothesis that the pharmacophore of gracilin A is the bis-acetoxy furanose which became our initial synthetic target for stage I. We therefore developed a retrosynthetic analysis that would intercept multiple intermediates bearing the hypothesized bis-acetoxy furanose pharmacophore (red). Specifically, simple tetrahydrofuran 6 (Stage I), the simplified bicycle 5 (Stage II) and tricycle 6 (Stage III, Scheme 1) were initially targeted for synthesis through application of PDR.

In Stage I, oxidation and reduction of furan gave the minimal pharmacophore as a mixture of diastereomers 6/14 (Scheme 2) which were assayed together. In practice, the reactivity of the bis-acetoxy furanose did not allow for maintenance of this moiety throughout the sequence, rather it was introduced in just a few steps from various intermediates. The core of the gracilins was available in optically active form employing our recently disclosed Diels-Alder lactonization organocascade to afford the bicyclic TBS enol ether 15. This key intermediate provided access to the simple bicyclic core 17 through diol 16, and also the tricyclic derivative 20 via lactone 19. The tricyclic derivative 21, obtained as a separable mixture of diastereomers and regioisomers, possessed all functionality found in gracilin A (3) with the exception of the exocyclic alkylidene. Given the availability of gra-

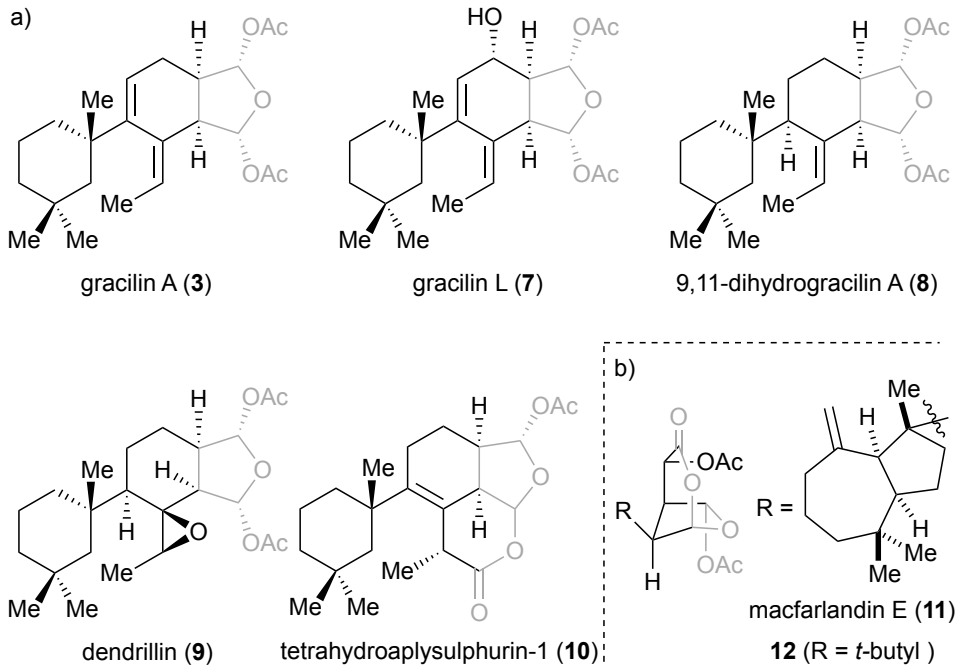
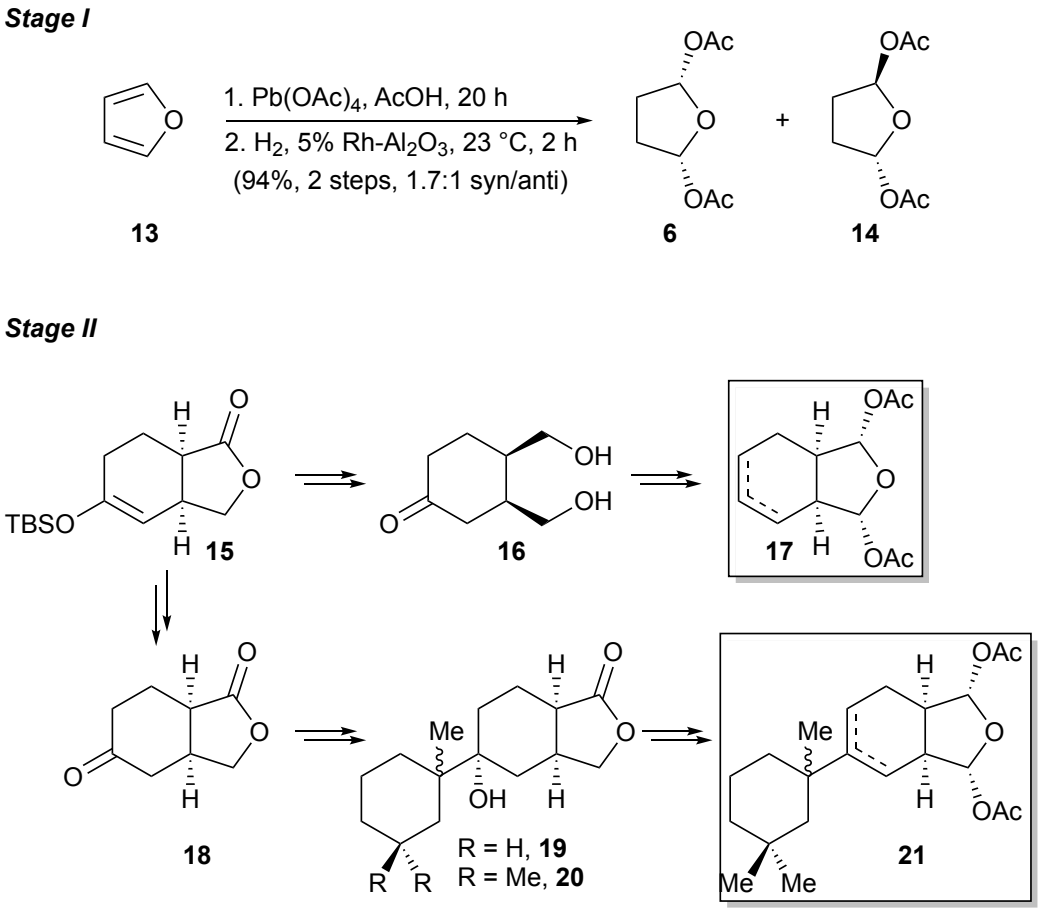


Figure 5. (a) Members of the spongiane family of diterpene natural products including gracilin A (3). (b) Macfarlandin E (11) and *t*-butyl derivative 12 bearing a related masked 1,4-ketoaldehyde and shown to undergo a Paal-Knorr pyrrole synthesis with lysine in simulated physiological conditions.

cilin A (3), its synthesis was not required as a comparator for bio-logical studies, precluding the need to complete Stage III in this case.

A compelling case for application of PDR to natural products came from the fact that a simplified derivative of gracilin A displayed nanomolar activity toward cyclophilin A as determined by surface plasmon resonance (SPR) (K_D 5.34 ± 1.68 nM) measurements which was ~500X more potent than gracillin A (Figure 6). Our studies further revealed the necessity of the cyclohexyl moiety since highly simplified bis-acetoxy furanoses 6/14 were unsurprisingly inactive while an interesting interplay between the C10-quaternary carbon stereochemistry and the alkene regiochemistry was revealed by careful HPLC separation of all four diastereomers of tricyclic derivatives 22 that were not initially optimized for diastereoselectivity nor regioselectivity.



Scheme 2. An overview of route used to complete stage I and II of the gracilin PDR route, showing intermediates both used for testing and as starting points for stage IV DTS.

As expected, a number of new questions were generated upon initial synthesis of gracilin A derivatives through application of PDR (Abbasov et al. 2014). To expand the SAR profile through 'gap-filling' and to specifically answer questions regarding selectivity for immunosuppressive (Abbasov 2019) vs. neuro-protective activity of the gracilin derivatives through interaction with either CypD versus CypA, we made use of DTS with intermediates previously synthesized. This enabled a more comprehensive view of structural features that led to the greatest selectivity between CypD and CypA and therefore selective neuroprotection or immunosuppression.

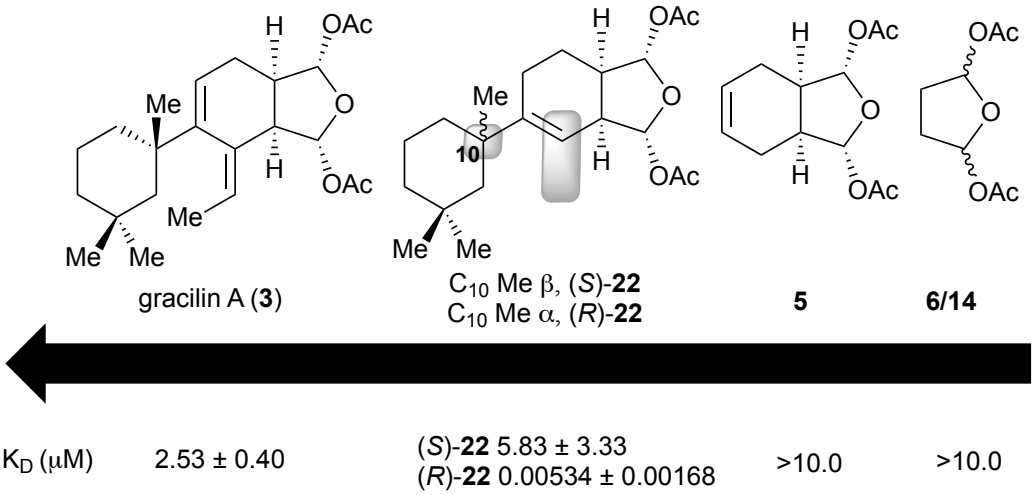


Figure 6. Selected bioactivity data resulting from PDR applied to the gracilins highlighting the importance of C_{10} stereochemistry and the superfluous exocyclic alkene.

Closing Thoughts.–I will end this recounting of my God-guided scientific journey with its unusual beginnings and surprising roads by again paying homage to the many wonderful co-workers that I have been blessed to work with throughout the years, my students who have done research in my laboratory. Furthermore, because of my formative undergraduate days in the Klein and Harding research laboratories, I was inspired to provide a forum for undergraduates to experience research including organic synthesis and perhaps, like me, decide to pursue a career in this exciting field. I have had many undergraduates who worked in my lab but in Fall 2010, I initiated the Texas A&M Undergraduate MiniPharma Research Program, which is a team-based semi-autonomous group of undergraduates working in the areas of molecule modeling, synthesis, and biological assays. In 2016, after I moved to Baylor, I restarted this research program now known as the Baylor Undergraduate MiniPharma Program (http://sites.baylor.edu /minipharma/)

We now have several examples where PDR has assisted in finding useful compounds beyond the natural product we were originally pursuing. I anticipate further discoveries through use of PDR leading to simpler versions of natural products that could become useful cellular probes or even drug leads for human ailments. In the area of translational research, we are beginning to push our basic discoveries

at the chemistry-biology towards drug-lead development through efforts of the Baylor Synthesis and Drug-Lead Discovery Laboratory established with Prof. John Wood when I moved my research group to Baylor University. This is the next venture in my journey where we hope to advance some of our basic discoveries that shed light on deleterious cellular events to alleviate various human ailments including cancer and most recently hypoxic ischemic brain injury in infants.

"Trust in the Lord with all your heart and lean not on your own understanding, in all your ways acknowledge Him and He will make your paths straight." Prov. 3:5-6

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