Lessons from the Synthetic Chemist Nature

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Lessons from the Synthetic Chemist *Nature*

Gerrit Jürjens, Andreas Kirschning* and David A. Candito

This conceptual review examines the ideal multistep synthesis from the perspective of nature. We suggest that besides step- and redox economies, one other key to efficiency is steady state processing with intermediates that are immediately transformed to the next intermediate when formed. We discuss four of nature’s strategies (multicatalysis, domino reactions, iteration and compartmentation) that commonly proceed via short-lived intermediates and show that these strategies are also part of the chemist’s portfolio. We particularly focus on compartmentation which in nature is found microscopically within cells (organelles) and between cells and on a molecular level on multiprotein scaffolds (e.g. in polyketide synthases) and demonstrate how compartmentation is manifested in modern multistep flow synthesis.
1. Introduction

What is a perfect multistep synthesis? This is a key question which has received much attention recently, despite the fact that as early as 1975 Hendrickson had already provided a definition for an "ideal synthesis". According to him it is one which: "...creates a complex molecule...in a sequence of only construction reactions involving no intermediary refunctionalisations, and leading directly to the target, not only its skeleton but also its correctly placed functionality." 1

Many years later this general statement was complemented with ideas such as, atom, step, and redox-economy that serve as standards by which the efficiency of chemical reactions and syntheses can be measured. 2 However, some of these concepts are restricted to individual chemical transformations and the underlying mechanisms to achieve selectivity. The Baran group has discussed and provided criteria that allow for comparison and even quantification of multistep synthesis. They cited three specific criteria: a) overall yield, b) step count and c) percent ideality. 3 The authors also acknowledge that the % ideality should be used with caution and is more of a simple tool than a true overarching measure of efficiency. Today, these straightforward concepts are generally accepted within the synthetic community as they reflect an important step toward striving for improved efficiency.

In several aspects, nature can be seen as an ideal synthetic chemist. Understanding the biosynthesis of a natural product is often a productive starting point for the design of highly efficient, biomimetic total syntheses. 4 Still, chemists have yet to achieve the same exquisite levels of selectivity, particularly, chemoselectivity, in the design of multistep processes. However, analysis of biosynthetic pathways reveals that atom-, redox- and step economies are not always well fulfilled in nature although from the synthetic chemist’s point of view biosyntheses of complex natural products are regarded to be highly efficient. This may sound like a contradiction.

Herein, we discuss four fundamental strategies that are shared in both synthetic chemistry and nature, in so doing, we wish to shed light on this seeming contradiction. Based on these strategies, it is interesting to see the interplay between the chemist and nature, where the chemist can be seen to borrow from nature and in certain cases even improve on nature’s design. The hope of this highlight is to discuss these fundamental strategies in a broad context taking examples from biosynthesis and the state of the art in organic synthesis and presenting them side by side. To clarify the scope of this review it is not about biomimetic synthesis and we have deliberately not chosen biomimetic chemical syntheses as this has been extensively reviewed elsewhere. 5

In this unique setting we hope to inspire the synthetic chemist to think more on natural systems for inspiration. Too often does the chemist draw a line between themselves and biologists, we hope to encourage a blurring of the lines. 6

2. Step- and Redox-economies in Nature

In order to discuss the issue of step-economy in nature let us first take a look at erythronolide A (1a). It is composed of a macrolactone ring that is biosynthesised by a polyketide synthase type 1 (PKS). Starting from propionyl-CoA transformations (additional four steps have to be added, if one starts counting from acetyl-CoA, additional six steps if transacylation steps from AT to ACP on the PKS are included, and additional seven steps if the activation and loading of the starter and elongation units are included) occur in a linear fashion to yield 6-deoxyerythronolide B (1b). After this, five additional biosynthetic tailoring steps fashion...creates a complex

The biosynthesis of the pseudomonic acids, secondary metabolites originally isolated from Pseudomonas fluorescens NCIMB 10586, is an illustrative showcase for poor step- and redox-economy in nature (Scheme 2). A tetrahydropyran ring is a central structural element of the pseudomonic acids and starting from the advanced biosynthetic intermediate 3 it is formed by a six step sequence that includes two oxidations, two reductions an epoxide ring-opening and a dehydration to yield pyran 4. A chemist would likely suggest a single step alcohol addition onto the olefinic double bond in an anti-Markovnikov fashion. Despite these facts pseudomonic acids are produced by fermentation and are used under the name mupirocin against staphylococci and non-enteric streptococci. One may speculate why many biosyntheses are linear and rather long, occasionally with poor redox-economy. From a chemist's perspective the repertoire of reactions and building blocks nature can rely on is rather small. Additionally, it has been hesistant to develop new biosynthetic pathways. nature rather sticks to established multistep processes. Often iteration and the addition of "decorating" or "tailoring" steps lead to ever longer linear biosyntheses. From an evolutionary point of view long biosyntheses...
originates from genes that were incorporated into biosynthetic gene clusters and became finally operative, when the new metabolite provided improved survival for the producing organism.\(^{14}\)

![Scheme 2. Biosynthesis of the pyran ring in pseudomonic acids.](image)

Unlike most chemical syntheses, biosyntheses are not batch but steady state processes in which intermediates are directly processed as they are formed. We believe that this fact is important to approach the topic of a perfect multistep synthesis from nature’s perspective. Therefore, we shall begin with a “Gedanken-experiment” of a conventional multistep batch synthesis in order to be able to mirror it with nature’s multistep processes before we discuss the four synthetic strategies.

3. The flaw of Multistep Batch Synthesis is its Batch Character

Synthetic chemists commonly conduct multistep processes under batch conditions in which intermediates are formed in equimolar amounts and if necessary need to be isolated and/or further purified. Practically, batch mode synthesis is time consuming and uneconomical and here step, step and redox-economies do matter.

![Figure 1. The concept of multistep batch synthesis exemplified in a “Gedankenexperiment” (amounts of materials in case of quantitative transformations).](image)

In this context, the patented synthesis of Olanzapine (7) is illustrative.\(^{15}\) Olanzapine is commercialised under the name ZYPREXA\(^{16}\) and was among the top 20 best selling drugs worldwide in 2011. ZYPREXA\(^{16}\) is an atypical neurolepticum and is used for the treatment of bipolar disorders and schizophrenia.\(^{16}\) It is known to exert antagonistic activity towards the dopamine receptor type 4 (\(D_4\)-receptor) and the serotonine receptor type 2 (57HT\(_2\)-receptor).\(^{17}\) The patent\(^{15}\) describes a four step batch protocol starting from \(1\)-fluoro-\(2\)-nitrobenzene (5) and \(2\)-amino-\(5\)-methylthiophene-3-carbonitrile (6). Assuming the preparation of 1 kg (3.2 mol) of 7 with quantitative yield for each step (Figure 1). This would require about 0.5 kg of starting materials 5 and 6, and more than 0.8 kg of three intermediates are formed that need to be isolated. As a consequence multistep reaction sequences should ideally avoid work-up and isolation of intermediates.

![Figure 2. A simplistic view on the bioproduction of erythromycin A (2).](image)

At first glance the bioproduction of complex natural products such as erythromycin A (2) may look like a true batch protocol (figure 2). D-Glucose is the starting material, Saccharopolyspora erythrea acts as the “catalytic” system and the fermenter resembles the flask or the reactor. Harvesting the bioreactor, yields the antibiotic 2, still being used in the clinic.

The gross simplification depicted in figure 2 treats Saccharopolyspora erythrea as a black box or a batch reactor. Using the tools of molecular biology and biochemistry we can pry into these multiscatalytic biosystems. In fact, the biosynthesis of erythromycin A (2) is a highly orchestrated multistep and rather linear steady state process, catalyzed by enzymes, many being part of a catalytic megacomplex, the polyketide synthase (PKS, vide infra). In contrast to the multistep batch process depicted in figure 1, these catalytic architectures circumvent the stoichiometric formation of intermediates.

![Figure 3. Enzyme-catalyzed multistep biosynthesis (A\(^1\)–A\(^3\)= substrates, building blocks, B= product of biosynthesis/ natural product; E\(^1\)–E\(^3\)= reactive intermediates, E\(^4\)–E\(^5\)= enzymes, E\(^6\)= enzyme that catalyzes a multistep process via highly reactive intermediates F\(^1\)–F\(^5\)).](image)

Primary metabolism “feeds” the biosynthetic pathways of secondary metabolite production and is organised in a complex network of enzymatic transformations and routes. A biosynthetic intermediate is often generated from different sources and the intermediate can be the starting point for different biosynthetic routes as exemplified in figure 3. In most cases, building blocks A are activated carboxylic acids such as acetyl- or propionyl-CoA, aminoacyl phosphates or alkyl diphosphates. Often tailoring transformations such as acylations, alkylations or glycosylations implement further “decoration” into the backbone (e. g. E\(^6\)=B, figure 3). Intermediates I are difficult to detect or to track, so that first insights into biosynthetic pathways came from the isolation of biosynthetic end product B, while manifestation of intermediates I was necessary to provide a detailed description of the pathways.\(^{19}\) These biosyntheses either rely on a cocktail of individual enzymes (e.g. in terpene biosynthesis)\(^{20}\) or are based on megaenzyme complexes such as the polyketide synthases that are e.g. part of erythromycin (2) biosynthesis, or non-ribosomal peptide synthases (NRPS). In both cases the templating property of PKS and NRPS guarantee spatial separation or compartmentation of each step (see also chapter 4.4).

Now that we have provided an overview of secondary metabolite biosynthesis, with an emphasis on polyketides biosynthesis, we will go on to discuss four strategies that nature has developed in order to allow for efficient multistep synthesis with seeming disregard to the chemists notion of step count and atom economy. Typically, the strategies are based on steady state processes and are a)
4. Nature’s Synthetic Strategies are Based on Steady State Processes

4.1 Strategy 1: Continuous processing by multicatalysis

As previously discussed, nature relies on linear steady state processes, in which individual enzymes are responsible for single transformations. The early steps of terpene biosynthesis are a typical example for a multicatalytic biosynthetic pathway (Scheme 3). Two distinct biosynthetic pathways, namely the mevalonate and the deoxyxylulose route, provide nature’s isoprene analogues isopentenyl pyrophosphate [IPP (8)] and dimethylallyl pyrophosphate [DMAPP (9)]. The former pathway utilises six enzymes to reach IPP starting from SCoA activated acetic acid, while the latter route requires seven enzymes starting from pyruvate (12) and glyceraldehyde-3-phosphate (13). Then, an iterative process provides geranyl pyrophosphate [GPP (10)], farnesyl pyrophosphate [FPP (11)], geranyl geranyl pyrophosphate and squelene. Each of these advanced intermediate building blocks represents one of the terpene classes (e.g. mono-, di-, sesqui-, tri- and tetraterpenes).

Scheme 3. Overview on the biosynthesis of acyclic terpene pyrophosphates, a typical example of a biological multicatalytic system [IPP= isopentenyl pyrophosphate (8); DMAPP= dimethylallyl pyrophosphate (9); GPP= geranyl pyrophosphate (10); FPP= farnesyl pyrophosphate (11) (enzymes involved: a. isopentyl-diphosphate delta-isomerase; b. farnesyl diphosphate synthase; c. geranylgeranyl diphosphate synthase; d. farnesyl-diphosphate farnesyltransferase].

Figure 4. Multicatalytic systems in biosynthesis and chemical synthesis. Multicatalytic reactions and sequences have also emerged in organic synthesis. These systems could be purely based on biocatalysts and synthetic catalysts or on biocatalyst / synthetic catalyst combinations. Within the category of synthetic catalysts further subdivisions can be made based upon whether the catalyst is an organocatalyst or based on inorganic/organometallic catalysts (Figure 4). The combination of multiple catalysts has several benefits such as reduced waste, reduced overall time to realise multistep syntheses, the ability to control unstable intermediates and the ability to generate molecular complexity from simple precursors. However, certain points must be taken into account, such as the compatibility of starting materials/intermediates and catalysts with each other and with the reaction conditions (temperature, solvent, pressure etc.). Additionally, the relative rates of the individual processes, and the selectivity of the individual catalysts must be considered, such that reactions occur in a definite sequence on precisely the intermediate which one intends. Despite many parameters needing to be controlled, researchers have begun to uncover reactions and strategies which already led to several impressive examples. An exciting new field in the arena of multicatalysis is the use of several metal catalysts that operate orthogonally in one pot and provide highly complex molecular architectures from simple precursors. Jeong and co-workers reported one of the first examples of a multicatalytic palladium/rhodium system with multiple C-C bonds being formed (Scheme 4). Initially, a palladium-catalyzed allylation between malonyl derivative 14 and allyl acetate 15 takes place to furnish intermediate 17. This intermediate enters a second catalytic cycle where rhodium triggers a Pauson-Khand type reaction (PKR). Via intermediates 18 and 19 the bicyclic ring system 16 is generated in excellent yield. The authors found that the chosen rhodium precatalyst is critical to the success of the multicatalytic cascade. This example demonstrates the multitude of opportunities chemists have compared to nature when developing multistep sequences, as there are no analogous catalytic reactions known in nature.

Scheme 4. Multicatalytic Pd-Rh-promoted allylic alkylation-PKR (for purposes of clarity, only key intermediates are depicted). Several examples of cooperative utilisation of transition metal catalysts, organocatalysts and even enzymes in one-pot have appeared in the literature. Jørgensen et al. showed that proline-derivative 22 and a π-Lewis acid based on gold or copper, can affect the reaction of propargyl malononitrile 21 with α,β-unsaturated aldehydes 20, to furnish highly enantioenriched cyclopentene carbaldehydes 23 (Scheme 5). Initially, the organocatalyst is responsible for iminium formation and directs highly selective 1,4-addition (intermediates 24 and 25). Then, the π-Lewis acid takes over (intermediate 26) disposing the alkyne toward intramolecular 5-exo-dig cyclisation to provide chiral cyclopentene carbaldehydes (intermediates 27 and 28). From control experiments it became evident that during transformation of 26 to 27 the two catalysts operate cooperatively.
As early as 2002 a multicyclic system was reported that utilizes an enzyme, an organocatalyst and a metal catalyst in water as the solvent in one-pot (Scheme 6). The conversion of methyl β-D-galactoside (29) to methyl 4-deoxy-6-aldehyde-β-D-galactoside (30) was initiated by the action of D-galactose oxidase that affects the oxidation at C-6. The resulting aldehyde (intermediate 31), in equilibrium with its hydrate, was warmed up and L-proline promoted dehydration to form the corresponding enal (intermediate 32). Finally, hydrogenation over palladium on charcoal gave the deoxygenated hexoside in excellent yield. This process proceeded without purification or isolation of intermediates, only reagents are added and conditions are altered. Remarkably, this process does not require protection of hydroxyl groups and it demonstrates the power of multicyclicity in multistep processes.

4.2 Strategy 2: Domino reactions

Domino or cascade reactions have emerged as an important tool for the organic chemist and over the past 20 years there has been a great deal of research in this area and a wealth of new processes have become available. As defined by Tietze, a domino reaction is a process whereby a starting material passes through two or more intermediates on its way to the final product, in a sequential manner, where functionality generated in the first step allows for further transformations to take place. The whole process occurs under the same reaction conditions and commonly all components are present from the very beginning (figure 5).

Nature also relies on this concept and in this context terpene cyclases are excellent examples. These enzymes have the ability to control the reactivity of intermediate carbocations of a domino sequence that results in the large number of known oligocyclic terpenes. During cyclisation the ring sizes and the configuration of stereogenic centers formed are dictated by the defined folding of the linear pyrophosphate precursor and governed by stereoelectronic effects in the enzyme active site, where “special” conformations can be stabilised. Commonly, the domino sequence is initiated by formation of a 3,3-disubstituted allyl cation which reacts intramolecularly with one of the trisubstituted alkene moieties of the substrate. A new carbocation is formed which commonly has three options: a) internal or external trapping of a nucleophile, b) deprotonation and formation of an alkene or c) Wagner-Meerwein rearrangement and formation of a new carbocation (figure 5).

Figure 5. Principal concept of domino reactions and comparison to terpene cyclases. (A= substrate of terpene cyclases, B= cyclisation product, 1-1= reactive intermediates, Cy= terpene cyclase).

The taxadiene synthase that operates in the biosynthesis of the yew tree (Taxus brevifolia) metabolite paclitaxel (38, TAXOL®) is an illustrative example. Paclitaxel inhibits microtubule depolymerisation and commercial is one of the most successful anti-cancer compounds. This diterperne is based on a 6-8-6 tricyclic carbon backbone which is formed from geranyl-geranyl pyrophosphate (33) in an enzyme-catalyzed domino reaction (Scheme 7). The cascade proceeds through several cationic intermediates I-III formed either by π-solvolyse, elimination or H-shifts. A series of tailouring oxidations and esterifications provide paclitaxel via baccatin III (34). Within this biosynthesis, another remarkable domino sequence occurs during the tailouring phase of the taxane backbone to form the oxetane ring as depicted in Scheme 8.
Cationic intermediates also drive iminium biosyntheses of alkaloids. Anionic species govern cascade sequences in polyether and ladder polyether biosyntheses as studied in detail for monensin. Radical cascade reactions are not very widespread in nature. Often these sequences are short and represent rearrangements commonly initiated by S-adenosyl methionine or by vitamin B_{12}.

**Scheme 8.** Proposed domino sequence in the formation of the oxetane ring in paclitaxel (35).

In a similar fashion chemists have utilised this concept of creating reactive intermediates based on carbocations, carbanions and C-centered radicals to build up several carbocyclic systems in one step. An impressive example was disclosed by the Kilburn group, in which SmI_{2} is used to reductively initiate a radical cascade of cyclisation-ring expansion-cyclisation via proposed intermediates starting from cyclopropyl ketone 36. The use of HMPA is supposed to influence the respective size of the coordinating samarium, thus stabilizing either intermediate I or II and hence controlling the relative stereochemistry of the product 37 and 38, respectively.

**Scheme 9.** SmI_{2} promoted cyclisation-ring expansion-cyclisation cascade by Kilburn et al.35

Although this domino reaction differs from terpene cyclase-promoted biosyntheses in that radicals instead of carbocations are the highly reactive intermediates, the underlying principles of domino reactions are alike. In the case of terpene cyclases the resulting stereochemistry is controlled by the asymmetric environment provided by the biocatalyst which exerts a defined conformation of the substrate. In the Kilburn example the starting material prefolds to a preferred conformer in solution, which can further be adjusted by choice of additional ligands on the samarium species. This concept of creating a reactive intermediate which initiates a second very different reaction is rather common in transition metal catalysis. Recently, Tietze et al. published an example of a palladium catalyzed carbopalladation-C-H activation sequence (Scheme 10). The reaction starts with Pd insertion into the carbon halogen bond followed by a 6-exo-dig cyclisation through carbometalation of the triple bond to furnish intermediate 44. The close proximity of the aryl group to the metal center initiates a C-H insertion of the palladium, resulting in the formation of the product 43 after reductive elimination.

**Scheme 10.** A combined Heck/C-H activation.36

**4.3 Strategy 3: Iterative reactions**

Iteration of steps as was discussed for the acyclic terpene pyrophosphates in Scheme 3, is a third concept how nature simplifies biosynthetic processes.

**Scheme 11.** Dihydromonacolin (46), a precursor of the hypolipidemic drug lovastatin 47 and the iterative biosynthesis of 46 (KS= ketosynthase, AT= acyl transferase, DH= dehydratase, MT= methyl transferase, KR=...
The catalytic domains are organised in the one multifunctional (LovB) and one monofunctional protein (LovC) that are responsible for
the biosynthesis of 46. The individual enzymatic units within the
domains are iteratively used up to eight times before release from
the PKS. The final product 46 is formed after a bioprocess
consisting of seven iterations that only differ in the number of
enzymatic units operating and the length of the carbon backbone of
the ACP-bound intermediates. As a result only seven different
enzymes are required to perform 21 reactions. At first glance, the
iterative process is not revealed in the structure of 46. But the
structural complexity is further increased because of an intervening
intramolecular Diels-Alder cycloaddition, which most likely
being terminated due to the steric congestion present in the product.
Structural parameters such as bulkiness lead to termination of the
iterations are not fully understood yet, it is discussed that folding of
the substrate in the enzyme pocket is crucial and once a certain
chain length is reached the iterative process stops. Noteworthy, the
biosynthesis nicely demonstrates the high reactivity of intermediate
48 – a lack of the cyclase results in spontaneous cyclisation to other
products such as 51 and 52.

Iterative sequences have also been developed by chemists. This
strategy provides complexity in a modular fashion from simple
precursors. In analogy to iteration by fungal PKS or type II PKS,
the Yamamoto group found that persiseryl enol ethers 53 can be utilised
for iterative aldol processes with aldehydes to yield aldol products
54 with high syn-selectivity (Scheme 13).

Although a new aldehyde is formed, the second aldol reaction is
retarded due to the large size of the persiseryl group. Additives such as
Phl facilitated oligoaldol processes. For example one triple aldol
reaction provided polyketide-type triol 57 from silyl enol ether 56
(R= H) and 3-nitro propanol 55 (Scheme 14). It was postulated that
organoisodienes react with (Me₃Si)₂SiNTf₂ to furnish a cationic,
sterically less congested complex of the type R²-Si(SiMe₃)₃⁺ NTF₂.
The iterative process was successfully applied in the synthesis of
the polymethoxy-1-alkene 61 that was, among other metabolites,
isolated from the polytoxin producing blue-green algae Tolypothrix
conglutinate. Here, this aldol reaction proceeds three times before
being terminated due to the steric congestion present in the product.
1-Iodo-2-phenylacetylene serves as a mediator. The fact that
structural parameters such as bulkiness lead to termination of the
iterative cycle also likely play a role in type 2 PKS biosynthesis as
discussed above.

Scheme 13. Iterative aldol reactions and proposed transition state for
syn-product.

Scheme 14. Iterative triple aldol reaction and in the total synthesis of the
polymethoxy-1-alkene rac-52.
The group of Martin Burke developed a powerful methodology based upon iterative cross-coupling reactions of bifunctional MIDA-boronates (Scheme 15). In principal, the polarity of elements n or i can be flexibly chosen such that selective cross-coupling occurs in a stereoretentive sense to give the adduct n+i, deprotection and activation of the boronate then takes place after which time additional steps may invert the polarity of the substrate. Possible options are the conversion of the vinyl boronate into a vinyl halide or the formation of pinacol boronates. This cycle can be repeated several times to access fragments in stereochemically pure form bearing appropriate functionality for further reactions. The different principal options of this new methodology becomes evident when studying the total syntheses of the polyenes synechoxanthin (67) (Scheme 16).45

The synthesis of synechoxanthin (67) started from aryl iodide 62 by Suzuki-Miyaura cross coupling with mixed boronate 63. The resulting vinyl MIDA boronate moiety was transformed into the corresponding iodide 65 so that the same elongation process with building block 63 could be repeated again. The resulting polyenyl MIDA-borate was activated as boronic ester 66 for the dimerisation step with dihalo alkene 64. In a remarkable recent paper the Burke group demonstrated that 12 different MIDA building blocks are in principal sufficient to iteratively prepare about 75% of all known polynye natural products.46

4.4 Strategy 4: Spatial compartmentation of individual steps – from polyketide biosynthesis to flow chemistry

The fourth strategy of nature’s multistep biosynthesis is spatial compartmentation which can be discussed from two perspectives, a microscopic and a molecular view. Microscopic compartmentation refers to cell organelles which have individual metabolic functions and which are characterised by different chemical conditions with respect to enzyme compositions and pH. Building blocks and metabolites are transferred between cell organelles or even between different cell types as found in plants by active transport systems or passively by e.g. osmosis. In that context organelles are chemical reactors which form a network with active mass transport among them. Microscopic compartmentation has been studied in plants to some degree.21 For example, in Catharanthus roseus both intra- and intercellular compartmentation play an important role in the biosynthesis of different types of terpenoid indole alkaloid skeletons such as vindoline 72, which is a precursor of the commercially important anticancer agents vinblastine and vincristine (Scheme 17).58

The early terpenoid precursors such as loganin 68 from the mevalonate terpenoid pathway are made in internal phloem parenchyma, the other important precursor tryptamine 70 in the epidermis. The last step of the terpenoid precursor secologanin 69 occurs in epidermis cells. Strictosidine synthase is present in the vacuoles and catalyzes the condensation of tryptamine 70 and secologandin 69 to yield the intermediate strictosidine 71. Finally, vindoline 72 is formed in other specialised cells, the ideoblasts and laticifers so that an intercellular transport of strictosidine must take place and the biosynthesis is compartmented. In essence, the flux through a pathway is controlled by transport from the site of production of a precursor to the site of the next enzyme.

The idea of compartmentation and passive transport is also found in synthetic chemistry at the interphase of two phases which can actively be promoted by phase transfer catalysts. E.g., this concept has been exploited for increasing the life time of free radicals using micelles as reactor compartments.22

Spatial separation can also be realised on a nanoscopic scale, where template structures based on e.g. multiple protein complexes are able to separate biosynthetic intermediates from each other. Such protein complexes are specifically folded bringing various catalytic domains in close proximity thereby allowing bound biosynthetic intermediates to be processed by transfer to the next catalytic domain (Figure 6).
As mentioned above, erythromycin A (2) is a classical example of a complex antibiotic produced by *Saccharopolyspora erythraea*. Its type 1 polyketide synthase (PKS) is able to carry out 20 steps required to form the complex carbon backbone of 6-deoxythronolide (1) the advanced precursor of erythromycin A (2) (Schemes 1 and 21). This type 1 polyketide synthase represents an example of molecular compartmentation. This megaenzyme complex is based on three large proteins that are composed of several enzymes and which can span several so-called PKS modules. Altogether, the erythromycin PKS is organised in seven modules (one starting and six extender modules) (Scheme 18).

In each module acyl transferases (AT) choose a starting building block (propionyl-CoA) or six extender units (methylmalonyl-CoA), respectively, and transfer them to the individual acyl carrier proteins (ACP) that play a central role for the following transformations. The ACP-bound intermediates are handed over from one catalytic site to the next and from one module to the next. A set of enzymatic domains (e.g. KS, KR, DH, ER), each module is responsible for C2-homologation and functional group manipulations (keto → alcohol → alkene → alkane).

This modular character and the permanent attachment of intermediates to the PKS lead to spatial separation of each chemical transformation. As the PKS operates continuously under steady state conditions all intermediates are only present in minute amounts (in theory, one per protein).
Conceptually, there is a counterpart in synthetic chemistry. Multistep flow synthesis using linearly assembled flow devices resembles such a continuous compartmented process.\(^5\) Indeed, continuous flow processes are the best technical option to achieve spatial separation of individual transformations and the individual reactor may be regarded as an individual PKS module (Scheme 21 and Figure 9). In continuously operated multistep flow synthesis the burden of work-up is minimised and isolation protocols ideally take place after the last step. Furthermore, it is possible to set up flow systems in which two short linear synthetic flow sequences converge for allowing production of structurally complex products from two fragments. Technically, this concept is well established. E.g. one technical way to produce acrylic acid proceeds as a truly multistep continuous process. It starts with the steamcracking process of crude oil followed by a two step catalytic oxidation of propene via acrolein to acrylic acid. It is estimated that 5-10\(^6\) tons of acrylic acid are produced worldwide much of it in continuously.\(^5\)

![Figure 8. Double-helical model for modular polyketide synthases (PKs). The ACP is situated in the center to allow interaction with neighbouring domains AT, KS, KR and KS.](image)

![Scheme 19. McQuade’s continuous three-step flow process of ibuprofen (75).](image)

A second illustrative example, the continuous multistep flow synthesis of olanzapine (Zyprexa, 82), was recently reported by our own laboratories (Scheme 20).\(^5\) A continuous three-step synthesis of thieno[1,5]-benzodiazepine 80 started from aryl iodide 78 and thiophene 79 which were coupled via a Buchwald-Hartwig amination. The resulting intermediate 83 first had to be passed through an in-line extractor for removing base-derived ammonium salts then through a pad of silica that trapped palladium black before hydrogenation of the nitro group occurred in the second reactor. The aniline intermediate 84 that left this reactor was collected in a glass vessel where remaining hydrogen gas was liberated. At this point a stream of HCl in MeOH was added and the solution was injected into the tubular reactor that was heated inductively. As a result thieno[1,5]-benzodiazepine 80 was collected after 30 hours of continuous operation in 88% yield without the need of chromatographic purification. Finally, the synthesis of olanzapine 82 was achieved in 83% yield after coupling with piperazine 81 using an inductively heated fixed bed reactor containing a solid-phase bound titanium Lewis acid.

![Scheme 20. The continuous multistep flow process of olanzapine (Zyprexa) (82).](image)
A most remarkable work was published earlier this year by Ley and coworkers. During the total synthesis of the methyl ester of spirangien A (90, Scheme 21) a polyketide-based natural product isolated from myxobacteria Sorangium cellulosum So ce90 several polyketide fragments and hence the western part were synthesised under continuous multistep flow conditions without isolation of intermediates.

Strategically, intermediate 85 was transformed into the key building block 86. It contains a stereotriade that is found at C16-C18 and C26-C28, respectively, of spirangien A (89). Consequently, alkene 86 was transformed into the two advanced fragments 87 and 88. These were merged convergently by nucleophilic addition of the alkyln lithium derivative derived from spirangien A (89). At this point the authors switched from an overall flow process to the batch mode. The synthesis contains a lot of technical details and synthetic subtleties, so that only some key features of individual reactions and reaction sequences can be highlighted which demonstrate the state of the art of modern flow synthesis (Scheme 22).

Utilizing the technically simple device of a tube-in-tube reactor suitable for continuous reactions with gases, the Ley group conducted an asymmetric hydrogenation using Pfaltz’s catalyst (R,R)-91 with alkene 85, which yielded hydrogenation product 92 in excellent yield and with good selectivity (Scheme 22). The catalyst’s special feature is associated with the fact that it can be applied for the hydrogenation of non functionalised alkenes without the need of additional polar coordinating sites. In the following, the protecting group was switched to an acetonide and all reagents and byproducts were removed from product 92 by pumping the reaction mixture through a cartridge of polymer supported benzylamine and basic alumina oxide. Finally, the resulting ester 93 was selectively reduced to yield the corresponding aldehyde which was followed by the experimentally challenging Roush crotylation with Z-but-2-enylboronate 94 to furnish fragment 86. It is noteworthy that aluminium containing byproducts were removed from the stream using a fixed bed material composed of a polyalcohol immobilised on the polymer IRA-743. It is now possible to perform the Roush crotylation directly by addition of the boronate to the flow system. Control of stoichiometry was achieved by measuring the concentration of the resulting aldehyde using a FlowIR cell. Data processing, allowed automatic adjustment of the flow rate of the pump that is responsible for the addition of boronate 94.

It has to be stressed, that in several aspects this total synthesis is not biomimetic, although continuous processes and compartmentation were exploited. Ley’s synthesis does not follow the linear logic of the polyketide synthase but has the flexibility to dissect the spirangien A backbone into three major fragments which are merged in a convergent fashion, instead.

5. Outlook

This conceptual review has examined the ideal multistep synthesis by analyzing nature. This article is not about biomimetic natural product synthesis but we rather suggest that the key to efficiency is steady state processing with intermediates that are immediately transformed to the next intermediate when formed until the target secondary metabolite is formed. We discussed four strategies (multicatalysis, domino reactions, iteration and compartmentation) that commonly proceed via short-lived intermediates. Nature, unlike chemists, uses these four strategies flexibly, almost combinatorially like a juggler which is still a huge challenge for synthetic chemists. In essence, we believe that atom, step and redox-economy can only cover some major aspects of defining the ideal synthesis.

The flexible use of these and additional strategies should increase the efficiency of processes by minimizing work-up protocols of intermediates so that isolation of the final product is the only purification issue. We believe that harnessing and enhancing these strategies should not only be a priority for academia but also for industry. We envision that a combination of these methods may one day change ‘traditional’ synthetic chemistry and open up rapid access to a world of natural product-like compounds with astonishing complexity with only one or two purification steps (Scheme 23). We are aware that it is still a long way to go to develop such processes, but we believe we should not shy from such an opportunity to access a larger part of the chemical space in a more economical fashion.

55 VBPR = variable back pressure regulator; OP = QuadraPure; BZA = benzyl amine BAr = tetrakis[3,5-bis(trifluoromethyl)phenyl]borate.

Scheme 21. Ley’s total synthesis of the methyl ester of Spirangien A (90).
processes are conducted. With the multitude of reactions discovered since Wöhler’s first preparation of urea in 1828, synthetic chemists have the potential to develop more powerful multistep processes than nature, if only we can manage to put them into use effectively.

5. Notes and References

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7 We do not discuss the important class of alkaloids, because they are not biosynthesised through a common biosynthetic pathway.
12 The fermentation yield is based on the average dilution of 0.07 h⁻¹ in a cyclic fed batch with the specific production rate of 0.75 mg g⁻¹ h⁻¹ by addition of 6 g L⁻¹ glucose medium and a constant dilution of 2 g L⁻¹ biomass on the one hand and on a chemostat cultivation with a dilution rate of 0.07 h⁻¹ using otherwise the same parameters and a specific production rate of 0.31 mg g⁻¹ h⁻¹ as reported in reference 11. It must be noted that exact fermentation conditions and yields of the continuously operated industrial process are not available.
14 Convergency is not unknown in biosynthesis as exemplified for the biosynthesis of erythromycin A (2). The rare deoxysugars L-mycarose (O-methylation to yield L-cladinose is a tailoring step) and D-desosamine are attached to the aglycon at a late stage and both are biosynthesised in seven steps from D-glucose.
19 In the past two decades molecular biology and genetic engineering has contributed tremendously in deciphering biosynthetic pathways.
23 For simplification, other topics such as diversification from a common advanced intermediate (see T. Newhouse, P. S. Baran, R. W. Hoffmann, Chem. Soc. Rev. 2009, 38, 3010-3021 and R. D. Firn, C. G. Jones, Nat. Prod. Rep. 2003, 20, 382-391) or multicomponent reactions (MCR) are skipped. In fact, the latter is hardly found in nature.
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<td>16</td>
<td>This is a borderline case, because it could also fit the category of domino reactions.</td>
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<td>11</td>
<td>In cell biology the term compartmentation comprises organelles in such as mitochondria, lysosomes, the endoplasmic reticulum, the cell nucleus etc. Here we use this term to refer to distinct catalytic domains of megaenzyme complexes such as the PKS. There are true uses of such organelles in biosynthesis – a) morphine b) p450 operate in peroxisomes etc.</td>
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Graphical abstract

Nature’s strategy of performing ideal multistep (bio)synthesis are based on multicatalysis, domino reactions, iteration and compartmentation. These are discussed and compared with chemical synthesis in this conceptual review.