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Chapter 4

Strategies Towards the Synthesis of Staurosporine Indolocarbazole Alkaloid and Its Analogues

B. Purna Chandra Rao, Osvaldo N. Oliveira Jr. and Ravi Varala

Abstract

In this Chapter we revisit the main strategies used for years in synthesizing staurosporine indolocarbazole alkaloid and its analogues, which are promising compounds for treating cancer. In addition to describing the details of the synthesis strategies, including the key challenges that had to be faced, we offer a historical perspective of the development in the field.

Keywords: Indolocarbazole, alkaloids, cancer, synthesis, sugar moiety, glycosylation

1. Introduction

1.1. Aims and significance

Cancer is one of the most serious threats against human health [1], which has motivated extensive research into a plethora of chemotherapeutic agents [2-3]. The need for new anti-cancer drugs arises not only from the limitations of current drugs, but also from the development of drug resistance [4-6]. Several strategies exist for designing such novel drugs, for which the essential criterion is the selection of a suitable starting point from the vast chemical space [7]. Natural products, in this context, are privileged structures [8] and biologically prevalidated leads, for they contain molecules that probably evolved to exert highly specialized functions. About 74% of anticancer compounds originate from natural products or from natural product-derived products [9]. The variety of structures in products is key for new therapeutics [10].

The indolocarbazole family of natural products (hereafter referred to as ICZ’s) was discovered in 1977 in actinomycetes, bacteria commonly found in soil, and is now investigated by medicinal chemists especially due to its antitumor and neuroprotective properties [11-13].
Figure 1 illustrates that ICZs are a structurally diverse family of natural products. The four types of aglycons include: A) the parent indolo[2,3-a]carbazole nucleus, such as that found in tjipanazole F2 (7); B) an imide, as in rebeccamycin (8) and arcyriaflavin D (3); C) hydroxy lactams, as in the UNC compounds (2); and D) simple lactams, found in 1, 4, 5 and 6. In all of these aglycon types, substitution with halides, ethers, phenols, has been done at various positions on the aromatic heterocycle. The compounds possessing the pyrroloindolocarbazole system with one $N$-glycosidic bond, such as rebeccamycin (8), act by inhibiting DNA topoiso‐merase (target for cancer chemotherapy), whereas those with two $N$-glycosidic bonds, e.g., staurosporine (1), are mainly protein kinase C (PKC) inhibitors [14].

We can also further divide ICZs based on the pattern of attachment of aglycon to the sugar moiety into four sub-patterns, viz.: A) ICZs having no sugar moiety, such as 3, 4; B) ICZs possessing one indole $N$-glycosidic linkages, such as 5, 7 and 8; C) ICZs with pyranose fused ring with two indole $N$-glycosidic linkages (e.g., 1, 2); and, D) ICZs with furanose fused ring with two indole $N$-glycosidic linkages (e.g., 6). The synthetically most challenging subgroups of indolocarbazoles are the cyclofuranosylated [e.g., K252a (6a)] and cyclopyranosylated [e.g., staurosporine (1)] congeners.

Knolker and Reddy reviewed the synthesis and biological activity of carbazole alkaloids, depicted in Figure 2, where different synthetic strategies for indolocarbazole alkaloids were discussed [15].

1.2. Motivation for the chapter

Potent drugs against cancer normally have to fulfill a number of requirements in terms of its toxicity to tumor cells and solubility for efficient delivery. This requires a full-fledged charac-
A characterization of drug candidates, including possible synthetic strategies. In this Chapter we concentrate on indolocarbazoles such as staurosporine, the most potent PKC inhibitors isolated to date, which probably act by occupying the ATP binding site and preventing protein phosphorylation. There is hence the need of synthetic routes to prepare indolocarbazole derivatives that are selective toward specific malfunctioning kinases associated with a disease. Furthermore, clinically useful compounds should have enhanced solubility in water, as compared to the poorly soluble ICZs. Since most indolocarbazoles with potent biological activities have substituents on the benzene portion of the core, enhanced solubility has been attempted with at least three approaches. The first is to introduce a hydrophilic group on the
imide nitrogen, e.g. the N-bis(hydroxymethyl)methylamino group. The second possibility consists in elongating the carbohydrate side chain. The third approach is to replace the uncharged sugar residue of ICZ with a positively charged amino-carbohydrate. Many of the recent synthetic approaches toward indolo[2,3-]carbazole glycosides separately address the syntheses of the sugar and heterocyclic portions, leaving glycosylation as the consummate step. One of the major difficulties associated with the synthesis of biologically-active ICZ alkaloids, such as Staurosporine, is the regiocontrol required for the glycosylation step. Left undiscriminated to the last, the attachment of a chiral sugar moiety to a specific indolic nitrogen indolocarbazole moiety (R₁ = R₂ = H) occurs nonselectively, thus producing regioisomers.

Well-known examples of pharmaceutically important glycosylated natural products include macrolide antibiotics, aromatic polyketides, glycopeptides, indolocarbazoles, aminoglycosides, and cardiac glycosides. The sugar moieties are often essential for the biological activity in such natural products. Thus, altering the structures and/or substitution patterns of sugar appendages on aglycone moieties, a process known as glycodiversification, could potentially generate glyco-conjugates with enhanced biological activity. Therefore, glycodiversification may ultimately lead to new antibiotics against drug-resistant infectious bacteria, improved cytotoxic agents for treating cancer, or potent chemicals for combating other ailments.

1.3. Definition of the problem

The indolocarbazole acceptor is generally a weaker nucleophile than the bis(indoly1)-maleimide or indole acceptor, which limits application of established glycosylation methodologies to the indolocarbazole aglycones.

1.4. History of staurosporine

1.4.1. Isolation

Omura et al reported in 1977 a new alkaloid, isolated from *Streptomyces staurosporeus* during a search for new alkaloids in actinomycetes, found to possess potent hypotensive properties in addition to broad spectrum antifungal activity [16]. It was originally named as AM-2282 (1), whose structure solved by single crystal X-ray analysis contained an indolocarbazole subunit with the two indole nitrogens bridged by glycosyl linkages (see Figure 3) [17-18]. AM-2282 was then renamed staurosporine (1), and became the first of over 50 compounds to be characterized in this family of alkaloids possessing the indolo[2,3-a]carbazole subunit [19-20].

Structure 1a, the enantiomer of the natural product, was originally assigned to staurosporine, and not until recently has the assignment of the absolute configuration of staurosporine been revised to that shown in structure 1 (Figure 4) [21].

This isolation of staurosporine sparked research into related natural and synthetic compounds, particularly for treating cancer with nanomolar inhibition of protein kinases (PKC) [22]. Many staurosporine analogues are in phase III clinical trials to treat cancer and about ten such PKC inhibitors have been approved for use in clinical level.
1.4.2. The importance of protein kinase C inhibitors

Protein kinase C (PKC) is a family comprised of at least eight serine/threonine specific kinases that are approximately 77 kD in size. The importance of PKC in regulating signal transduction pathways and ultimately cellular response has been well-established [59]. Activation of PKC occurs through a series of events that begins with specific binding of an extracellular agonist to a cell surface receptor. This binding results in activation of phospholipase C which then cleaves inositol triphosphate (IP3) from phosphatidylinositol-4,5-biphosphate (PIP2) and leaves behind a molecule of 1,2-diacylglycerol (DAG) in the membrane. Phosphorylation ultimately results in cellular responses by modifying the function of rate-limiting enzymes and regulatory proteins implicated in metabolic pathways.

As already mentioned, indolocarbazoles such as K252a and staurosporine are the most powerful PKC inhibitors isolated to date. This mode of PKC binding, illustrated in Figure 5, unfortunately results in a relatively non-selective inhibition of several kinases. The preparation of indolocarbazole derivatives possessing selectivity toward specific malfunctioning kinases associated with a disease state would be a solution; thus, an efficient and general synthetic route to the indolocarbazoles is desirable.
1.4.3. Pharmacology of staurosporine and its analogues

The recent literature on staurosporine analogues has provided valuable inputs into their biochemical pharmacology and generated discussion on the suitability of protein kinase C as potential target for anticancer drugs. The following conclusions are particularly pertinent with respect to pharmacological mechanisms [23]:

1. staurosporine analogues such as UCN-01 and CGP 41251 are inhibitors not only of PKC, but of a ‘cocktail’ of kinases;

2. the composition of this cocktail and expression of its constituent kinases in a given neoplasm determine the nature and extent of pharmacological efficacy; and

3. slight alterations in molecular structure dramatically alter individual components of this cocktail.

Indolocarbazoles are all biologically active and display such properties as antimicrobial, antifungal, and antitumor activity, in addition to acting as hypotensive or platelet aggregation

(Adapted from: B. M. Stolz, PhD Thesis, Yale University 1997)
agents [24-27]. Three representative examples of this class are staurosporine (1), rebeccamycin (8), and K-252a (6) (see Figure 1). Rebeccamycin (8) causes topoisomerase I-mediated DNA cleavage and is presently in late-stage clinical trials as an anticancer agent. Additionally, staurosporine (1) and K-252a (6a) are potential antitumor agents acting as potent inhibitors of protein kinase C (PKC). Staurosporine has also been reported to possess immunosuppressive activity and to reverse multidrug resistance [28-30]. It is because of its nanomolar inhibition of PKC, however, that staurosporine has attained its current acclaim.

2. Synthesis of staurosporine and its analogues

2.1. Introduction

Staurosporine can be divided into two distinct parts: the “northern” indolocarbazole aglycon and the “southern” carbohydrate portion of the molecule, as shown in Figure 6. One can envision that by so dissecting the molecule, a convergent synthetic approach would be possible in which a lactam-protected derivative of aglycon could be coupled with a bis-glycal derivative (no commitment is made as to the functional nature of $R_1$ or $R_2$).

![Figure 6. Retrosynthetic analysis of staurosporine (1).](http://dx.doi.org/10.5772/63832)

From Figure 7 one may infer that aglycon 2 is itself a natural product, commonly referred to as staurosporinone or K-252c. Because it constitutes a major unit of many indolocarbazole natural products, several approaches to its synthesis have been developed [31-32]. Classified by the last covalent bond(s) formed, these approaches include cycloaromatization (A), double nitrene C-H insertion (B, B’), nitrene C-H insertion (B’), maleimide reduction (C), and diazolactam initiated cycloaromatization (D).
2.2. Biosynthetic pathway of staurosporine

2.2.1. Biogenesis of the indolocarbazole nucleus

Cordell and Pearce independently reported the first indolocarbazole biosynthesis in 1988 [33-35], both identifying aglycon units of ICZs (1 and 8 (Figure 1)), to be derived from two intact tryptophan units. Tryptophan (10) was in fact utilized in the aglycon biosynthesis, produced by Streptomyces staurosporeus from D-glucose (9), probably via the shikimic acid pathway (Scheme 1) [36].

2.2.2. Biosynthesis of indolocarbazole carbohydrates

The carbohydrate precursor to staurosporine has been shown to be D-glucose and the N- and O-methyl groups are derived from L-methionine as shown in Scheme 2. Hoehn reported the isolation of 15b by cofermentation and bioconversion studies and found that O-methylation is the last step, i.e., direct precursor to staurosporine biosynthesis [37].
2.2.3. About this pathway

The first enzyme identified in staurosporine biosynthesis was the one catalyzing the very last step (3'-'O-demethyl-staurosporine methyltransferase). A *Streptomyces longisporoflavus* mutant defective in this enzyme was reported in 1995 [37], while the enzyme was identified in 1998 [38]. The complete staurosporine biosynthetic gene cluster was cloned from *Streptomyces sp.* L-amino acid oxidase staO initiates synthesis by converting L-tryptophan to the imine form of indole-3-pyruvate (2-imino-3-(indol-3-yl)propanoate). StaD (staD) then catalyzes coupling of two IPA imines to yield chromopyrrolate. Formation of the indolocarbazole core of staurosporine is catalyzed by two enzymes: staP converts chromopyrrolate into three indolocarbazole compounds, K-252c, 7-hydroxy-K252c and arcyriaflavin A, by intramolecular C-C bond formation and oxidative decarboxylation, while StaC is required to ensure that the main product is K-252c.

The next step is glycosylation, which is catalyzed by two enzymes. K252c N-glycosyltransferase (staG) catalyzes N-glycosidic bond formation between N-13 and C-6' of the nucleotide sugar dTDP-L-ristsamine. Cytochrome P450 StaN (staN) then catalyzes an additional C-N bond formation between N-12 and C-5'. These two enzymes convert K-252c to 3'-O-demethyl-4'-N-demethyl-staurosporine via the intermediates holyrine A and holyrine B. The final steps in the pathway are two methylation reactions. staMA catalyzes N-methylation of 3'-O-demethyl-4'-N-demethyl-staurosporine and staMB catalyzes O-methylation, which results in staurosporine (*Figure 8*) [39].
Figure 8. Biosynthetic pathways of Staurosporine in Stryptomyces sp. TPA0274. The genes associated in synthetic steps are shown (dTDP=deoxy-thymidine-5-diphosphate).
2.3. First total synthesis of staurosporine and ent-staurosporine (Danishefsky et al., 1995)

It was not until 1995 that the first total synthesis of staurosporine (1) was reported by Danishefsky et al. [40]. A central challenge in total synthesis by previous groups was that of constructing the two glycosidic bonds to weakly nucleophilic indolic nitrogens [41-44]. Danishefsky observed oxazolidinone glycal 27b to function as the glycosyl donor and bis(indolyl)maleimide 26 to function as the aglycon acceptor (Scheme 3). Aglycon 26 was synthesized from benzyloxymethyl (BOM) dibromomaleimide 24 in the modular fashion shown.

![Scheme 3](image-url)

(a) (i) Indole Grignard, PhH 0°C to rt, overnight, 82%(P1 = H). (ii) NaH, THF, room temp., then SMECl, 91%(P1 = SEM). (b) Indole Grignard, PhH 0°C to rt, overnight, 75%. (c) NaH, CH2Cl2, 0°C then Cl3CCN,0°C to rt,(R= C3N=CCl3 then BF3.OEt2 -78°C, 78%. (d) cat. TsOH,H2O, Pyr, 80°C, 80%. (e) (i) NaH, CH2Cl2, 0°C to rt, 92% of 27 (P2= TIPS, P3=SEM) (ii) NaH, DMF, then BOMCl, 0°C to rt, 92% of 27 (P2= TIPS, P3=BOC) and 22% of 27 (iii) TBAF, THF, 0°C 95%, (P2 = H, P3=BOC) (iv) NaH, DMF, 0°C to rt then PMBCl, 0°C to rt 92% of 28 (P2 = PMB, P3=BOC) (i) Dimethyldioxirane, CH2Cl2, 0°C 100%, of α-epoxide β-epoxide. (g) (i) 21, NaH THF, rt then 11 and 12, rt to reflux 47%(30 (P2 = PMB, R2=SEM R3=OH) (ii) Thiophosgene, DMAP, Pyr, CH2Cl2 reflux then C6F5OH reflux, 79%(P2 = PMB, R2=SEM R3=OCSOC6H5 (iii) n-Bu3SnH, AIBN, PhH, reflux, 74% 31 (P2 = PMB, R2=SEM R3=H) (iv) DDQ CH2Cl2, H2O 0°C to rt 97%(P2 = H, R2=SEM R3=H) (v) TBAF, THF reflux, 91% of 32 (P2 = H, R2=H R3=H) (vi) (i) h2, cat. 12, air, PhH, rt 73% of 16 (R4=OH). (ii) I2, PPh3, imidazole, CH2Cl2, 0°C to rt 84%(R4=I)

Triisopropylsilyl-L-glucal 31 (TIPS-L-glucal) was converted to its bis- (trichloroacetimidate) and then to oxazoline 30 by an apparent vinylogous Schmidt glycosylation. The oxazolidinone, fashioned from derivative 29, was protected as its BOM derivative 27. The TIPS protecting
group was cleaved, and a p-methoxybenzyl ether (PMB) was installed. Accordingly, glycal 28b was oxidized with 2,2-dimethyldioxirane. The mixture of epoxides (27) was treated with the sodium salt of 26 to furnish indole glycoside 32 with 47% yield.

Compound 32a was subjected to Barton deoxygenation to remove C$_{3}$' hydroxyl, affording 32b. Seco-system 32c was obtained by further deprotection of C$_{6}$' PMB and the indolic SEM groups. Photolytic oxidative cyclization resulted in compound 33 (Scheme 3). The exo-glycal, which was essential for intramolecular glycosylation, was performed using iodination strategy of 33 followed by elimination. Treatment of 34 with potassium tert-butoxide and iodine eventually resulted 35. Thereafter, reacting with tri-n-butyltin hydride and deprotecting the BOM groups, compound 37 was available. For compound 38, a BOC group was introduced particularly on the oxazolidinone ring to facilitate disconnection of oxazolidinone. The BOC group would protect against dimethylation of the amine during the opening reaction. To safeguard the imide ring during sequential modifications, which would generate N-methyl and O-methyl functions, compound 38 was converted into 39. Treatment of 39 with cesium carbonate in methanol led to 40. Next, the O-methyl and single N-methyl groups were incorporated to yield 41, which on further deprotection afforded 7-oxostaurosporine (42) (Scheme 4). 7-Oxo compound 42 was transformed into staurosporine.

A methodology was developed to convert the 7-oxo compound 42 to staurosporine itself. It started with a reduction with sodium borohydride (In Scheme 4, 40-42). It was not that easy to deoxygenate the carbamoyl linkage but this portion was smoothly accomplished by using benzeneselenol. By performing two steps on 42, Danishefsky et al. obtained a 1:1 mixture of isostaurosporine (43) and staurosporine (1) [40]. After separation, homogeneous fully synthetic staurosporine (1) was isolated. The total synthesis of staurosporine (1) was thus completed.

2.4. Staurosporine and ent-staurosporine: The first total syntheses, prospects for a regioselective approach, and activity profiles (Danishefsky et al., 1996)

The total syntheses of staurosporine and ent-staurosporine was achieved again by Danishefsky et al, by constructing both the glycosidic bonds from glycal precursors [45]. The first glycosidic bond was originated from direct epoxidation of endo-glycal to give 1,2-anhydro sugar, which was later made to react with indole anion through intermolecular coupling. They used the strategy of intramolecular iodo glycosylation for the second bond using an exo-glycal [45].

The authors dealt with the problem of indole glycosylation, functional group management in the pyranose ring, and regiochemical harmonization in the course of the first total synthesis of staurosporine (1) detailed herein. It is an electrophilically induced cyclization of the second indolic nitrogen onto a novel exo-glycal to establish the staurosporine core skeleton.

Monosaccharide synthesis

Danishefsky et al. assumed the upcoming C$_{3}$' methoxy and C$_{3}$' methlamino vestiges would be existing in an oxazolidinone ring. Protecting the nitrogen with a benzyloxymethyl group, C$_{3}$' -p-methoxybenzyl ether would protect a primary alcohol that could be utilized in designing the exo-glycal essential for intramolecular indole glycosylation (Scheme 5).
Consistent with the discussion above, they formulated the donor to be a glucal of the type \(50a\). This sequence shown in Scheme 5 provided oxazolidinone glycal \(50a\) which proved to be an effective glycosyl donor subjecting to proper activation. It was noted that oxazolidinone would provide stereochemical guidance in activating the endo-glycal en route to the first indole glycosylation.

**Glycosylation and Elaboration.**

Oxazolidinone glycal \(50a\) and its derived epoxide proved to be effective as functional versions of target glycals. Danishefsky et al. next focused on the first glycosidic bond (Scheme 6), for which bis-indolyl maleimides were effective glycosyl acceptors for 1,2-anhydrosugar donors. Thus, the sodium anion of bis-indolyl maleimide \(26\) was synthesized and treated with a solution of 1,2-anhydrosugars prepared from epoxidation of glycal \(50a\) using 3,3-dimethyl-dioxirane. A mixture of expected indole glycoside \(52\) (47% yield) and indole glycoside \(53\) (10% yield) was obtained upon heating the reaction.
Scheme 5

43

\[ \text{OSPIT} \text{a} \rightarrow \text{OSPIT} \]

44

\[ \text{OSPIT} \text{b} \rightarrow \text{OSPIT} \]

45

49a-b

50a-b

R = H, 47
R = BOM, 48a
R = Br, 48b

a) NaH, CH₂Cl₂ 0°C then Cl₂CCN, 0°C to rt then BF₃·OEt₂, -78°C to 78% (b) TsOH H₂O, pyr, 80°C, 80% (c) NaH, CH₂Cl₂ 0°C to rt, 92% (d) NaH, TBAI, DMF then BMOCl, 40°C 65% and 22% recovered 48. (e) NaH, TBAI, DMF, then Br₂Br, 0°C to rt, 94% (f) TBAF, THF, 0°C, 95% (g) NaH, DMF, 0°C to rt then PMBCl, 0°C to rt, 92%

Scheme 6

1. NaH, THF, rt
2. \[ \text{OSPIT} \text{a} \rightarrow \text{OSPIT} \]
3. THF, rt to reflux

51a

52

53

R = H, 52
R = CsOC₂F₅, 54

(a) Thiophosgene, DMAP, Pyr, CH₂Cl₂ reflux then C₆F₅OH, reflux, 79%
Alteration of the functional group was essential to construct the second glycosidic bond. It was performed by deoxygenating the newly created alcohol at C5’, deprotecting the indole moiety, establishing 2,2’ indolic bond, and finally formation of exo-glycal (Scheme 7).

**The Key Cyclization**

Early screening of the reaction of indolocarbazole glycoside 61 with an array of electrophiles failed to establish conditions to perform cyclization and lead to the fully functionalized core of staurosporine (1). Indolocarbazole glycoside 61 should have its activated exo-glycal and thereby undergo a conformational change so that cyclization would be made possible. The sterically demanding aglycon must be in an axial conformation rather than the preferred equatorial conformation. Cyclization to 62 thus resulted as the nucleophilic nitrogen attacks the activated exo-glycal.
Completion of the Synthesis

To complete the total synthesis of ent-staurosporine (2), a two-step deprotection strategy (hydrogenation followed by aminal hydrolysis) delivered 64 from 63 in high yield (Scheme 8). Danishefsky et al. preferred to clarify the monosaccharide domain prior to reducing the maleimide function [45]. The most efficient method involved reduction of the imide group with sodium borohydride to provide a 1:1:1:1 mixture of hydroxy lactams. Further reduction to ent-staurosporine (1a) and ent-isostaurosporine (71) was then successfully finished using phenylselenol and p-TSA. Compounds 1a and 71 were each isolated in a homogeneous state from the 1:1 mixture generated from this two-step sequence.

Upon successfully completing the chemistry in the ent-series, the strategy towards total synthesis of staurosporine (1) was evident (Scheme 9). Initially, tri-O-acetyl-L-glucal 72 was transformed into the corresponding oxazolidinone 73. Compound 74 resulted from coupling to the aglycon, deoxygenation, photocyclization, and finally by exposing exo-glycal. Thereafter, performing the crucial cyclization step yielded 75. Opening the oxazolidinone, methylation, deprotection, and reduction furnished staurosporine (1) and isostaurosporine (1a).

Danishefsky et al. evaluated ent-staurosporine (1a), ent-isostaurosporine (71), a related imide 64, and their corresponding enantiomers for their in vitro antitumor activity, their capacity to inhibit PKC (Table 1), and their ability to inhibit topoisomerase I. The cytotoxicity of indolo-
Carbazole alkaloids can also be affected by a different mechanism than inhibition of PKC, i.e. inhibition of topoisomerase I (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>PKC inhibition:</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (µM)</td>
<td>HL-60</td>
</tr>
<tr>
<td>Staurosporine (1)</td>
<td>α 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ &lt; 0.005</td>
<td></td>
</tr>
<tr>
<td>ent-Staurosporine (2)</td>
<td>α 0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂ 0.15</td>
<td></td>
</tr>
<tr>
<td>isostaurosporine (94)</td>
<td>α 0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂ 0.05</td>
<td></td>
</tr>
<tr>
<td>ent-isostaurosporine (89)</td>
<td>α 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂ 0.01</td>
<td></td>
</tr>
<tr>
<td>imide(95) (ent-82)</td>
<td>α 1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ 0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂ 0.10</td>
<td></td>
</tr>
<tr>
<td>imide 82</td>
<td>α 0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁ 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂ 0.03</td>
<td></td>
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</tbody>
</table>

Cytotoxities are given as IC₅₀'s in µM units

Table 1. PKC Inhibition and in Vitro Cytotoxicity
Table 2. Topo I Inhibition

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA cleavage inhibition</th>
<th>DNA relaxation inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staurosporine (1)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ent-Staurosporine (2)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>isostaurosporine (94)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ent-isostaurosporine (89)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>imide(95)(ent-82)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>imide 82</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>camptothecin</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Relative potencies are compared with camptothecin (++++) at 100µM

2.5. Wood and Stolz’s synthesis of staurosporine

A total synthesis of the natural product (+)-staurosporine has been achieved [46] along with other ICZs. The synthetic strategy involved stereoselective ring expansion of a furanosylated indolocarbazole [(+)-79] to a pyranosylated congener [(+)-80] that serves a common intermediate in the production of 1 and other desired ICZs.

2.5.1. Retrosynthetic analysis: The development of a ring expansion approach to the pyranosylated indolocarbazoles

Wood and Stolz began by considering approaches that involved ring expansion of a furanosylated intermediate. Noting the striking structural homology of 1 and other related ICZs, they envisioned a strategy that would allow access to these congeners via a common intermediate. Specifically, R-methoxy ketone 76 was viewed as ideal since the stereogenic centers common in place and flexibility for stereocontrolled functionalization at C(4’) and C(5’) is maintained. Thus, reductive amination would produce staurosporine (1).

Figure 9. Key intermediate 76
The inspiration for developing this approach derived from Wood's recognition that ketone 76 might be accessed from aldehyde 79 via a Tiffaneu-Demyanov-like ring expansion (Schemes 10 and 11). In designing this ring expansion approach, Wood et al. addressed the issues of regio- and stereochemical outcome and the known propensity of similar systems to undergo skeletal rearrangement (i.e., 77 to 78, Scheme 10). From Scheme 3, it could be envisioned that the planned rearrangement occurs with migration of either bond $a$ or bond $b$ of aldehyde 79, to produce regioisomeric hydroxy ketones 80 or 81, respectively. Thus, Wood et al. [46] assumed bond $a$ would migrate to the si face of the aldehyde, producing a product (80) that possesses both the regio- and stereochemistry needed for further progressive steps towards staurosporine.

Scheme 10
2.5.2. Completion of staurosporine

Next, Wood and Stolz [46] treated (+)-80 with NH₂OH.HCl to produce corresponding oxime (-)-83 in 95% yield. In contrast to ketone (+)-80, bis-methylation of (-)-83 under phase transfer conditions (MeI, KOH, and n-Bu₄NBr in THF) occurred cleanly to afford (-)-84 and set the stage for a stereoselective reduction (H₂/PtO₂) that furnished amine (+)-85a. Mono-methylation and deprotection then afforded (+)-staurosporine (1) in 67% yield (two steps, Scheme 11).

![Scheme 11]

3. The synthesis of carbohydrates for indolocarbazole synthesis

Only a few methodologies have been developed for synthesizing complex carbohydrate intermediates for use in the total synthesis of indolocarbazole alkaloids such as staurosporine (1). Some of those strategies are summarized in the succeeding sections:

3.1. Synthesis of staurosporine monosaccharide (Weinreb et al.)

Weinreb published the synthesis of aminohexose fragment of staurosporine via an N-sulfinyl Diels-Alder [4+2] cycloaddition [43,47]. From Scheme 12, cycloaddition of diene 86 and benzyl...
sulfinylcarbamate (87) resulted in a mixture of diastereomeric sulfoxides which after oxidation yielded the corresponding sultam (88) and then converted to acetal 99. Subjecting to diastereoselectively epoxidation of olefin 89 using trifluoroperacetic acid afforded 90. Hydrolytic-reductive opening of epoxide 90 followed by olefin cleavage resulted in keto-acetal 91, a critical synthon for the staurosporine carbohydrate (92).

3.2. Staurosporine glycal precursor (Danishefsky et al).

Danishefsky exploited glycal epoxide 93 as the glycosyl donor in his first total synthesis of staurosporine [41,48]. Glycal 94, a derivative of L-glucal, was transformed into its corresponding oxazoline 95 by a modified Schmidt reaction. Conversion to oxazolidinone proceeded under standard conditions, and finally treatment with Murry’s reagent provided the glycal epoxide (96, Scheme 13).

3.3. Methods describing the combination of carbohydrate and indolocarbazole

3.3.1. The Danishefsky synthesis of (+)- and (-)-staurosporine

Danishefsky formulated a strategy to staurosporine [41], in which epoxidation of glycal (-)-98 with maleimide 97 resulted in one of the indole N-glycosidic linkages to form 99. Treatment of olefin 99 using Barton deoxygenation, iodine and t-BuOK followed by radical dehalogenation provided the pyranosylated indolocarbazole 101 with 64% yield. Deprotection and methylation followed as shown in Scheme 14 (i.e., 101 → 102), after which reduction of imide 102 led to a 1:1 mixture of 1 and 1a.
3.3.2. Syntheses, biochemical and biological evaluation of staurosporine analogues from the microbial metabolite rebeccamycin

To synthesize staurosporine analogues from rebeccamycin, different structural variations were exploited by Prudhomme et al., including coupling of the sugar moiety to the second indole nitrogen, dechlorination and then reduction of imide to amide [49].

The synthesized compounds 105-109 in Scheme 15 were tested for their ability to bind to DNA and inhibit topoisomerase I and protein kinase C [49]. The cytotoxicity of dechlorinated imide analogue 108 correlates well with its DNA binding and anti-topoisomerase I activities.

3.3.3. Synthetic studies on indolocarbazoles: Total synthesis of staurosporine aglycon

Mohankrishnan et al. synthesized staurosporine aglycon and its analogues with 28-36% overall yield, using 2-methylindole (110) as synthetic precursor [50]. The key steps for the synthesis of indolocarbazole alkaloids involved electrocyclization and nitrene insertion reactions as depicted in Schemes 16 and 17.
Triphenylphosphite-mediated nitrone insertion of 2-nitroarylcarbazole was performed at a moderate temperature using anhydrous ZnBr₂ as catalyst. In addition, an alternative synthetic protocol for preparing ICZs involving concurrent electrocyclization followed by nitrone insertion was adopted as in Scheme 17 by Mohankrishna et al. [50].

3.3.4. Synthesis of pyrrolidin-2-ones and staurosporine aglycon (K-252c) by intermolecular Michael reaction

3,4-Disubstituted pyrrolidin-2-ones, a group of compounds with interesting biological properties, are related to staurosporinone. The most important property is inhibition of protein kinase C (PKC), so that this antiproliferative agent can interfere with the cell cycle. The synthetic strategy permits preparation of said compounds using an intermolecular Michael addition, starting from nitroethene derivatives and substituted acetate Michael donors [51].
Enantioselective syntheses can also be carried out using chiral auxiliaries in this strategy. Reduction of the nitro group using raney nickel and subsequent lactamization, the desired lactam precursor of staurosporine, which is essential for the biological activity, is obtained according to Scheme 18. The easiest and shortest (in contrast to the published routes of staurosporinone) synthetic strategy of staurosporinone within three steps with good to moderate yields is obtained.
3.4. Syntheses of the indolo[2,3-a]carbazole nucleus

Synthetic strategies for preparing the indolo[2,3-a]carbazole nucleus have been already summarized in Figure 2 based on the key bond formations, type of structure synthesized (aglycon), and research group. In the following section some of the methodologies are described briefly.

3.4.1. Winterfeld’s strategy to synthesis of staurosporinone

In 1983, Winterfeld published the first synthesis of K252c as shown in Scheme 19 [52-53]. The synthesis of lactam 126 was successfully achieved by intramolecular aldol reaction of ketoamide 125 and then followed by titanium-mediated deoxygenation. Oxidative Photocyclization of 126 resulted in indolocarbazole 4 (staurosporinone).

3.4.2. Magnus’ approach

Magnus published a synthetic methodology to selectively protect staurosporinones, just after Winterfeld’s report [54]. Intramolecular Diels-Alder cycloaddition of indole-2,3-quinidomethane 130a was the crucial step in his synthetic strategy (see Scheme 22). Imine 130 was
prepared from condensation of tryptamine derivative 129 and 2-aminostyrene and then subjecting to acylation yielded indole-2,3-quinidomethane 130a (in situ) and initiated an intramolecular Diels-Alder reaction. Oxidative work-up with DDQ resulted in indolocarbazole 131. Deprotecting phthalimide group on 131 followed by acylation gave bis-protected staurosporinone 132. Interestingly, the indoles could be selectively deprotected (e.g., 132→4 or 132→133, Scheme 20) to facilitate regioselective introduction of a sugar portion.

3.4.3. The Weinreb approach

Weinreb exploited a synthetic strategy for the synthesis of bis indolyl maleimides to furnish maleimide 135 from indole-Grignard 134 and imide 134a [43]. DDQ mediated oxidative cyclization of 135 resulted N-benzyl imide 136. To complete the synthesis, Clemmenson reduction was performed for desymmetrizing 136, to produce the corresponding lactam 137 (Scheme 21).

3.4.4. Raphael’s approach

Raphael staurosporinone synthesis based on intermolecular Diels-Alder methodology and nitrone insertion chemistry is depicted in Scheme 22 [55-56]. Reaction of numerous dienophiles with diene 139 following dehydrogenation afforded triaryl products such as 140a and b. In an initial attempt, 140b was reduced and cyclized in good yield to afford lactam 137, a compound previously prepared by Weinreb and Bergman [43].

3.4.5. The Moody approach

Moody utilized the pyranoindolone 147 to regulate intramolecular Diels-Alder reaction with subsequent aromatization to carbazole 148 (Scheme 23). Nitrone formation by deoxygenation using triethylphosphire produced K252c (4, staurosporinone) [57-58].
Scheme 21

Scheme 22

Scheme 23
4. Conclusion

In this book chapter, a brief introduction to biologically active indolocarbazole alkaloids was presented, with emphasis on the isolation and synthetic pathways of powerful protein kinase inhibitors such as Staurosporine indolocarbazole alkaloid and its analogues. Glycosylation on indolic moiety and concerns were discussed apart from the synthesis of staurosporinone aglycon and sugar portion. We do hope that this book chapter will be a valuable addition to the chemists dealing with indolocarbazole alkaloids from pharmaceutical industry and synthetic organic point of view.

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