Chapter 4

Strategies Towards the Synthesis of Staurosporine Indolocarbazole Alkaloid and Its Analogues

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Additional information is available at the end of the chapter

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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 anticancer 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 topoisomerase* (target for cancer chemotherapy), whereas those with two *N*-glycosidic bonds, e.g., staurosporine (**1**), are mainly *protein kinase C (PKC) inhibitors* [14].

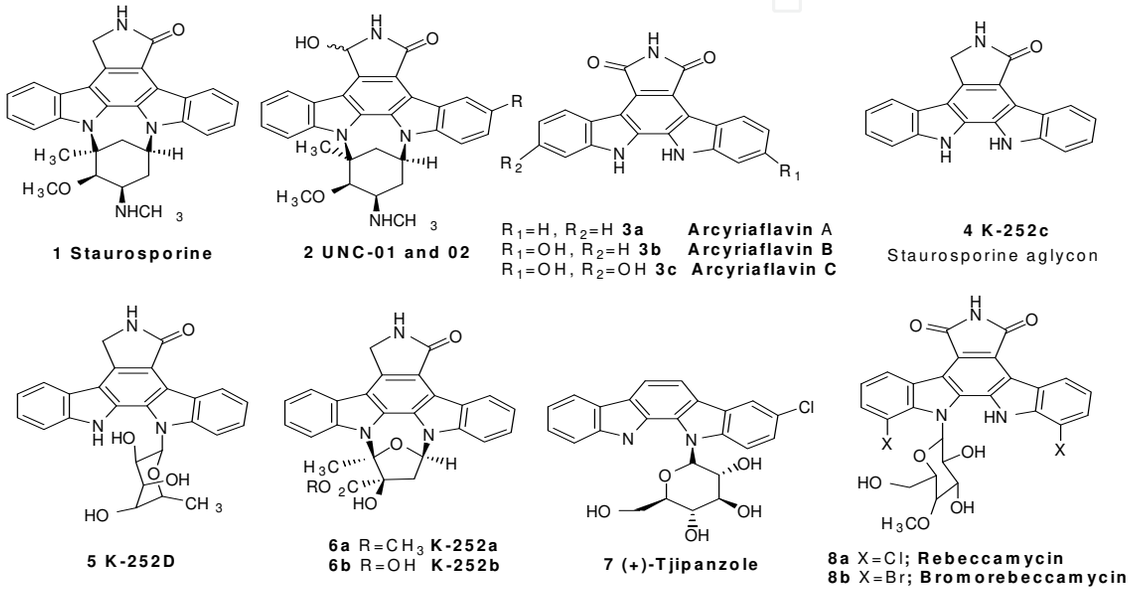

Figure 1. Well-known indolocarbazole alkaloids

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-

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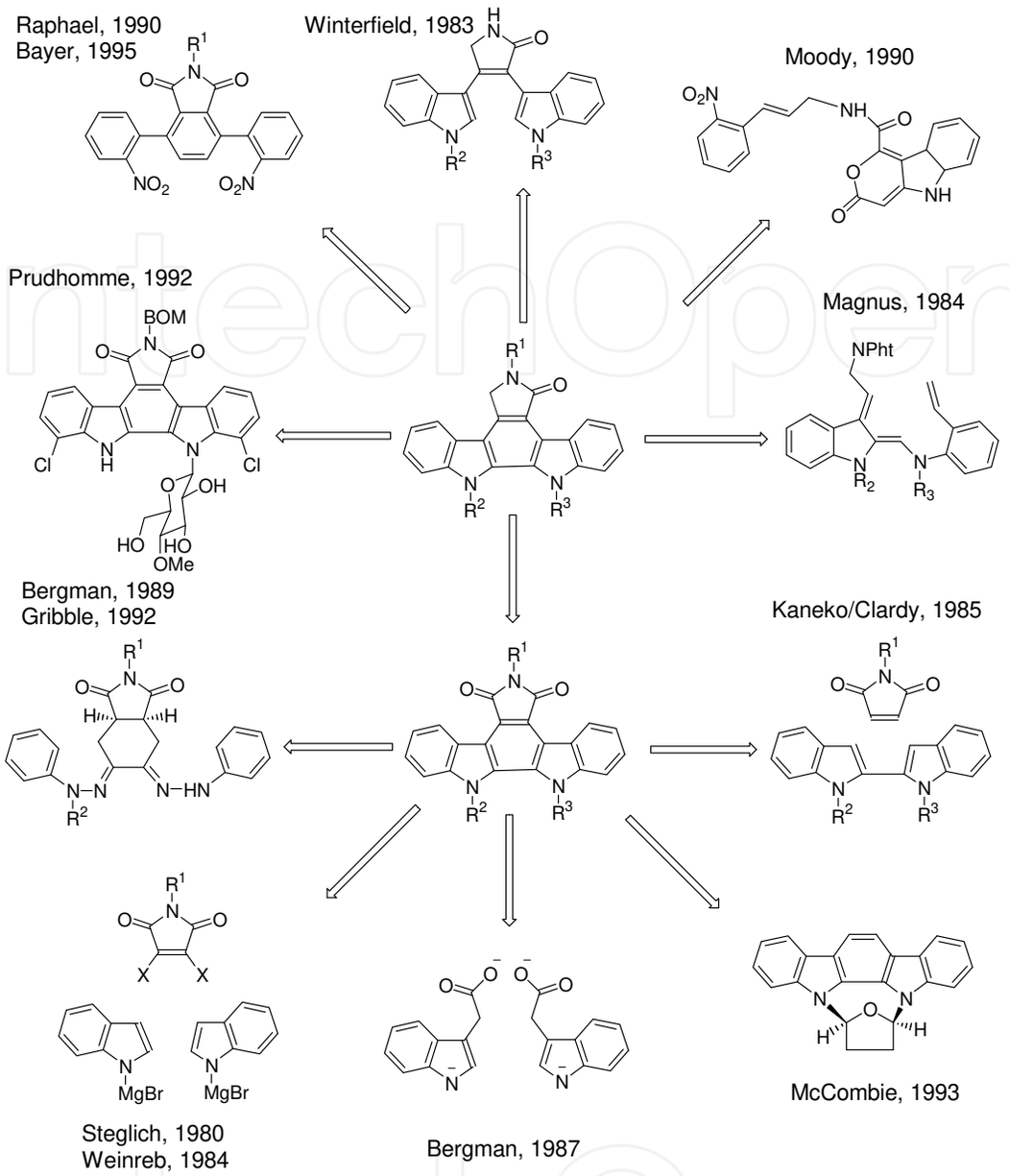

Figure 2. Approaches to indolocarbazole alkaloids

terization 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 (R_1 =R_2 =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.

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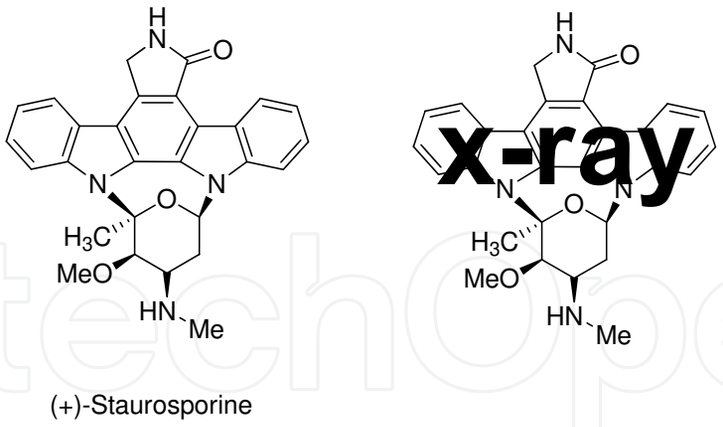


Figure 3. Single crystal X-ray analysis of staurosporine

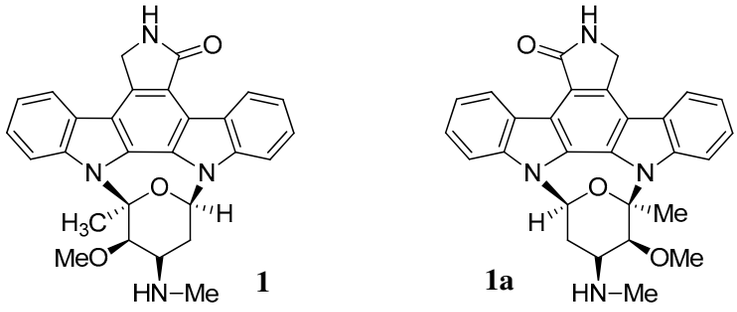
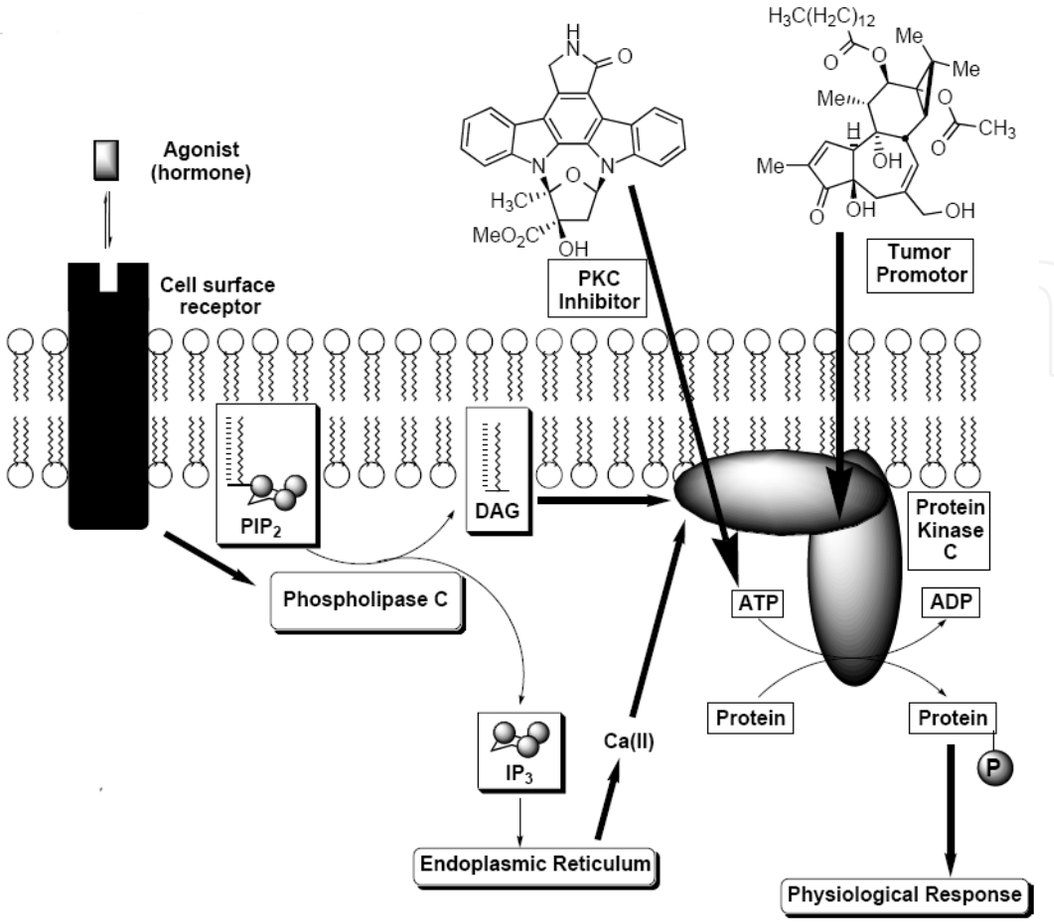


Figure 4. Structures of staurosporine (**1**) and *ent*-staurosporine (**1a**).

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.



(Adapted from: B. M. Stolz, PhD Thesis, Yale University 1997)

Figure 5. Mechanism of PKC inhibitors

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

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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).

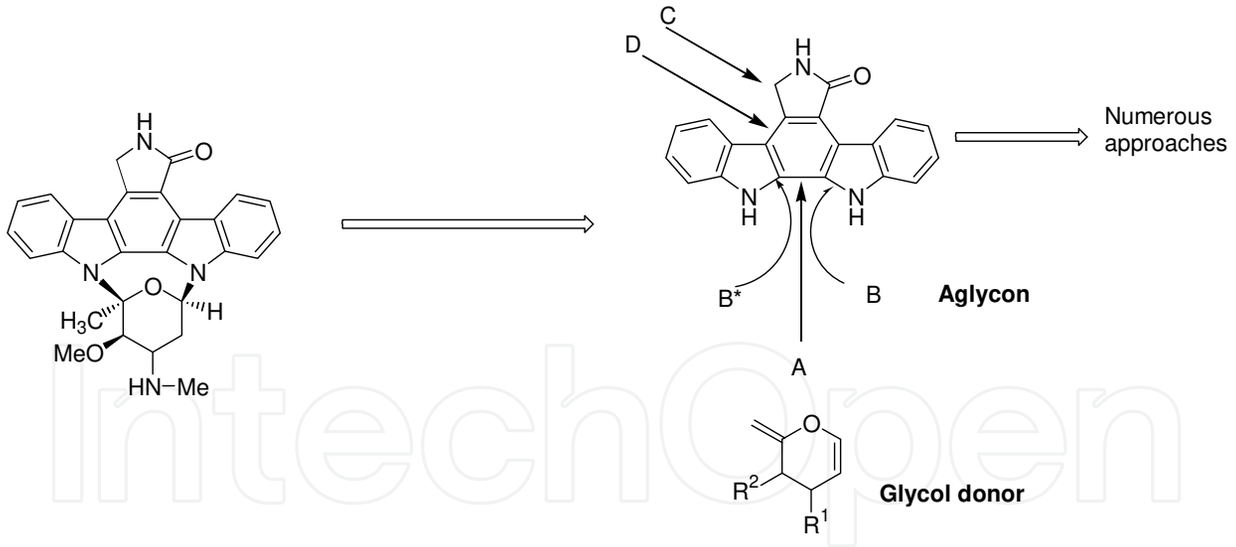


Figure 6. Retrosynthetic analysis of staurosporine (**1**).

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 diazo-lactam initiated cycloaromatization (D).

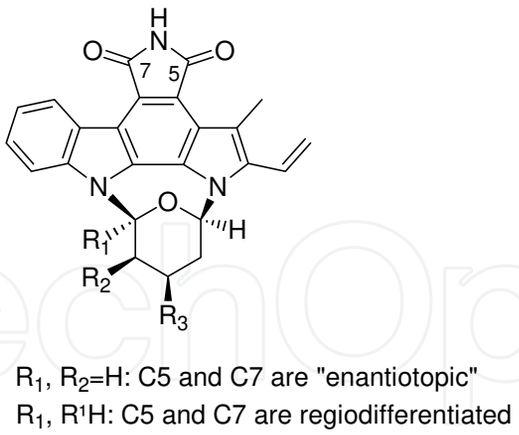
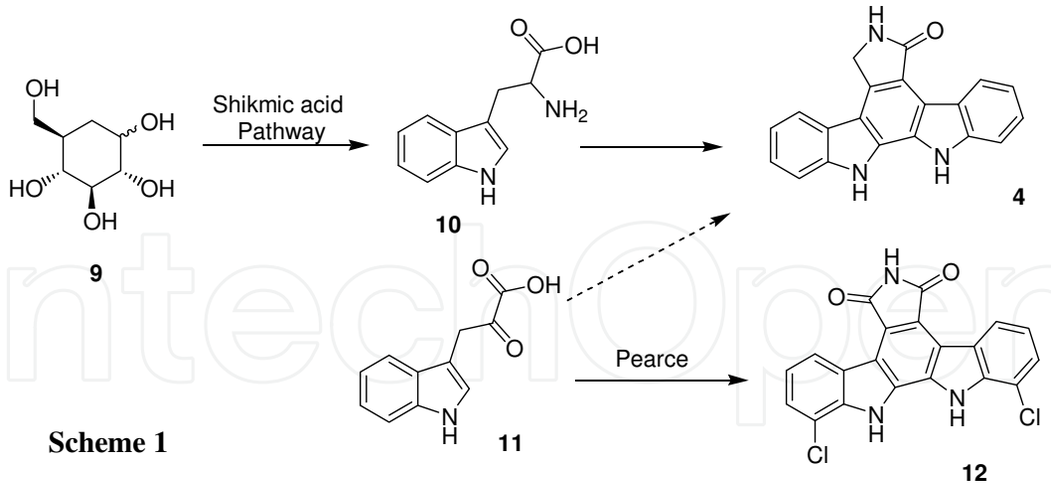


Figure 7. Effect of substitution on differentiation of C5 and C7.

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].



Scheme 1

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, ie., direct precursor to staurosporine biosynthesis [37].

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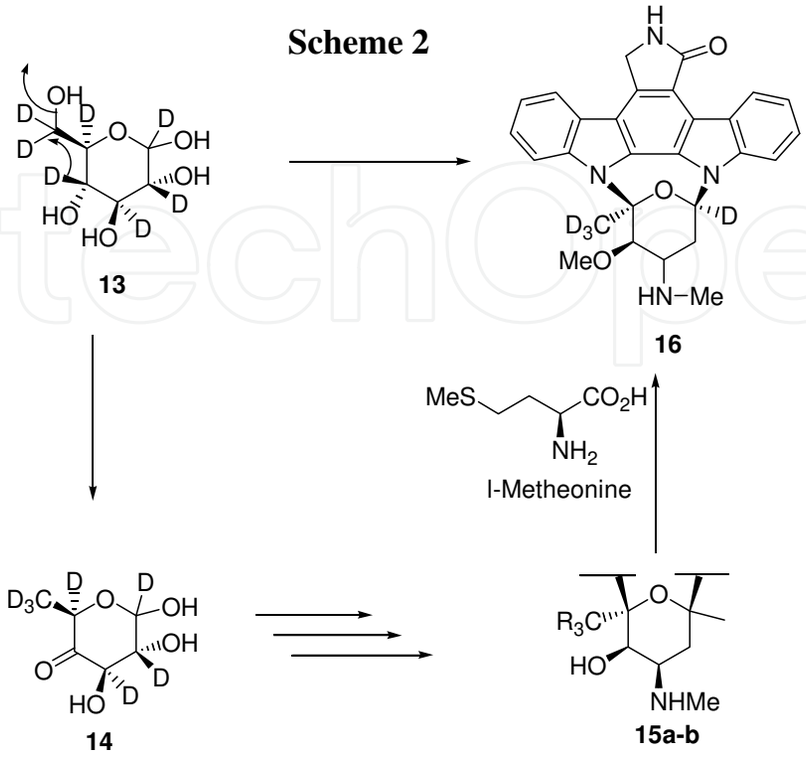

2.2.3. About this pathway

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-ristosamine. 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].

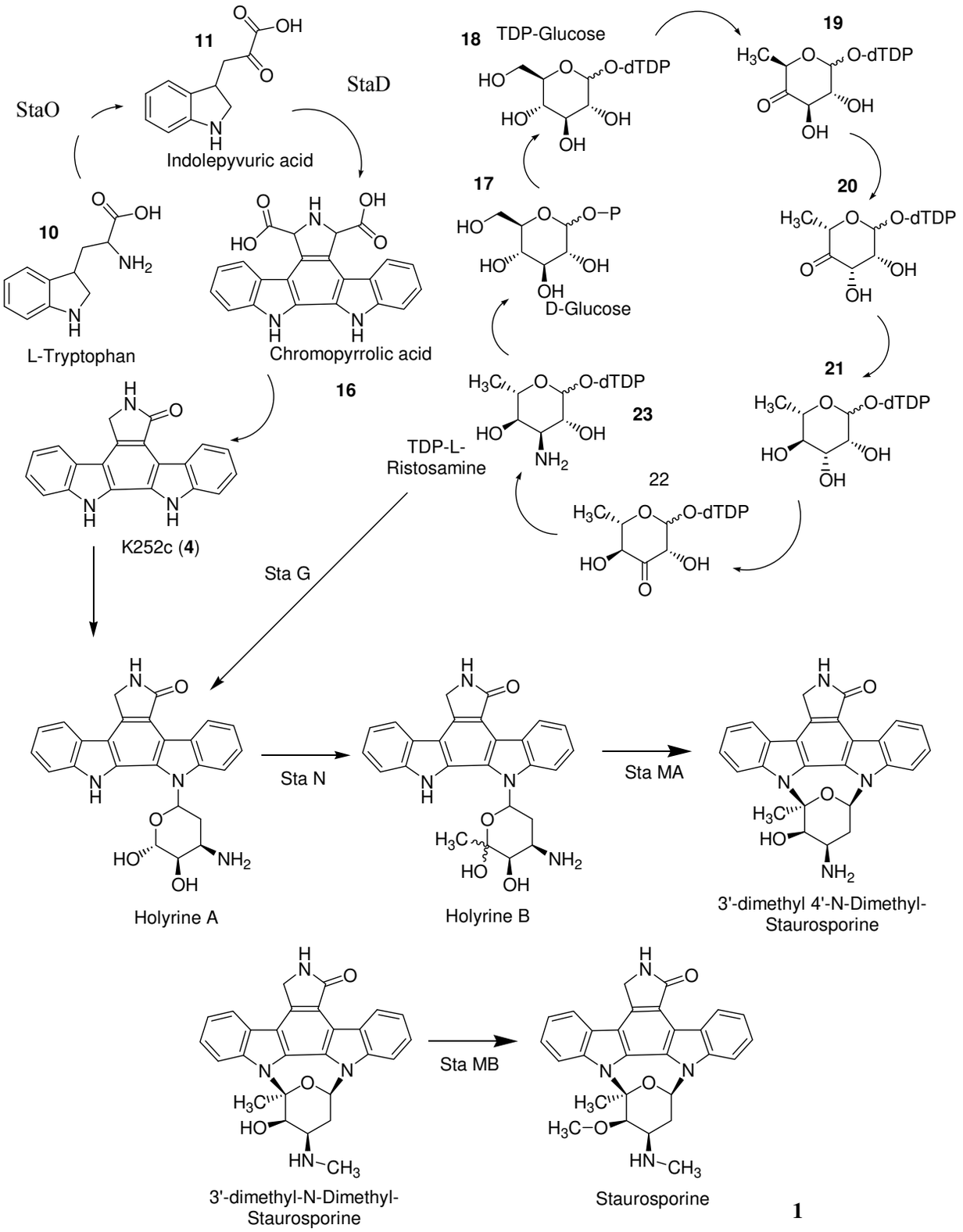

Figure 8. Biosynthetic pathways of Staurosporine in Stryptomyces sp. TPA0274. The genes associated in synthetic steps are shown (dTDP-deoxy-thymidine-5-diphosphate).

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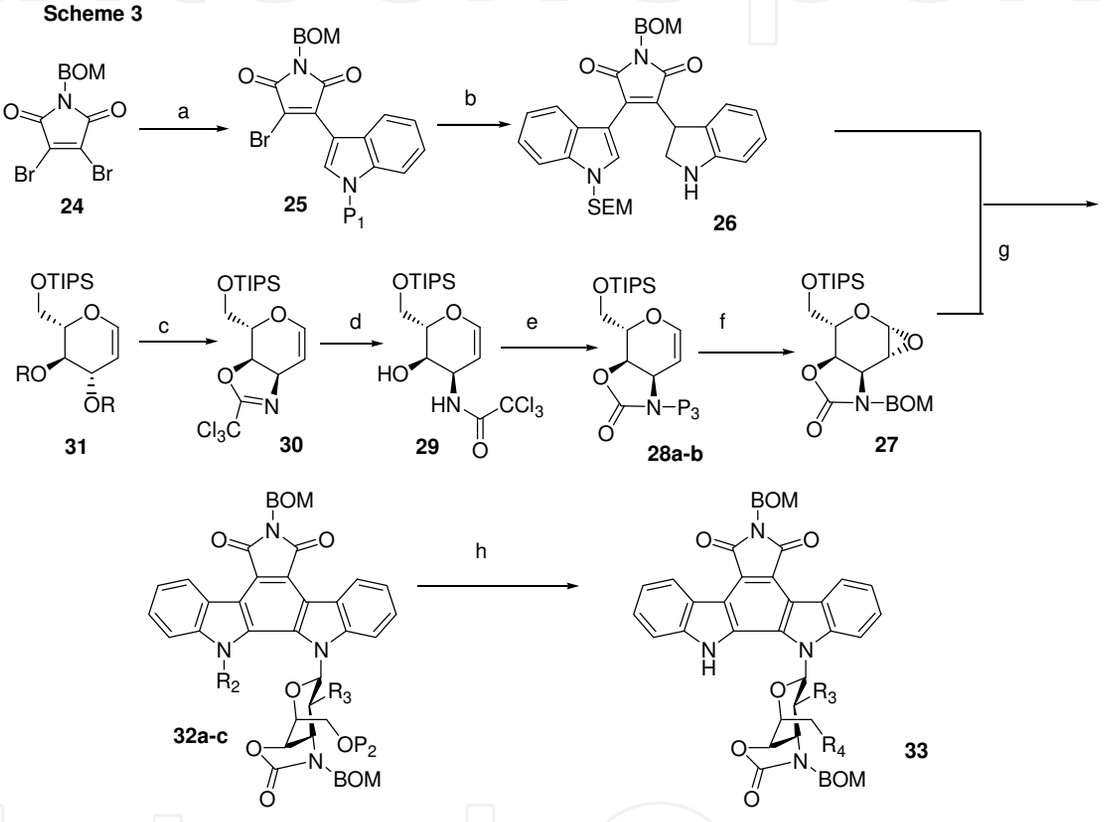
2.3. First total synthesis of staurosporine and ent-staurosporine (Danishefsky et al., 1995)

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



(a) (i) Indole Grignard, PhH 0 ^{o}C to rt, overnight, 82%(P_1 = H). (ii) NaH, THF, room temp., then SMECl, 91% (P_1= SEM). (b) Indole Grignard, PhH 0^{o}C to rt, overnight, 75%. (c) NaH, CH_2Cl_2, 0^{o}C then Cl_3CCN,0^{o}C to rt,(R= $CNHCCl_3$ then BF3.OEt2 -78^{o}C, 78%. (d) cat. TsOH,H_2O, pyr, 80^{o}C, 80%. (e) (i) NaH, CH2Cl2, 0^{o}C to rt, 92% of 27 (P_2 = TIPS, P_3=H)(ii) NaH, DMF, then BOMCl, 40 ^{o}C, 65%((P_2 = TIPS, P_3=BOM) and 22% of 27 (iii) TBAF, THF, 0 ^{o}C 95%, (P_2 = H, P_3=BOM) (iv) NaH, DMF, 0^{o}C to rt then PMBCl, 0^{o}C to rt 92% of 28 (P_2 = PMB, P_3=BOM) (f) Dimethyldioxirane, CH_2Cl_2, 0 ^{o}C 100%, of α–epoxide β-epoxide. (g) (i) 21, NaH THF, rt then 11 and 12, rt to reflux 47%of 30 (P_2 = PMB, R_2=SEM R_3 =OH) (ii) Thiophosgene, DMAP, Pyr, CH_2Cl_2 reflux then C_6F_5OH reflux, 79% (P_2 = PMB, R_2=SEM R_3 =$OCSOC_6H_5$ (iii) n-Bu_3SnH, AIBN, PhH reflux, 74% 31 (P_2 = PMB, R_2=SEM R_3 =H) (iv) DDQ CH_2Cl_2 H_2O 0 ^{o}C to rt 97% (P_2 = H, R_2=SEM R_3 =H) (v) TBAF, THF reflux, 91% of 32 (P_2 = H, R_2=H R_3 =H) (h) (i) hυ, cat. I2, air, PhH, rt 73% of 16 (R_4 =OH). (ii) I_2, PPh_3, imidazole, CH_2Cl_2, 0 ^{o}C to rt 84% (R^4 =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_2' 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 carbanolamide linkage but this portion was smoothly accomplished by using benzeneselenol. By performing two steps on **42**, Danishefsky et al. obtained a 1:l 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*.

Monosaccaharide synthesis

Danishefsky et al. assumed the upcoming C_3' methoxy and C_4' methylamino vestiges would be existing in an oxazolidinone ring. Protecting the nitrogen with a benzyloxymethyl group, C_1'-*p*-methoxybenzyl ether would protect a primary alcohol that could be utilized in designing the exo-glycal essential for intramolecular indole glycosylation (Scheme 5).

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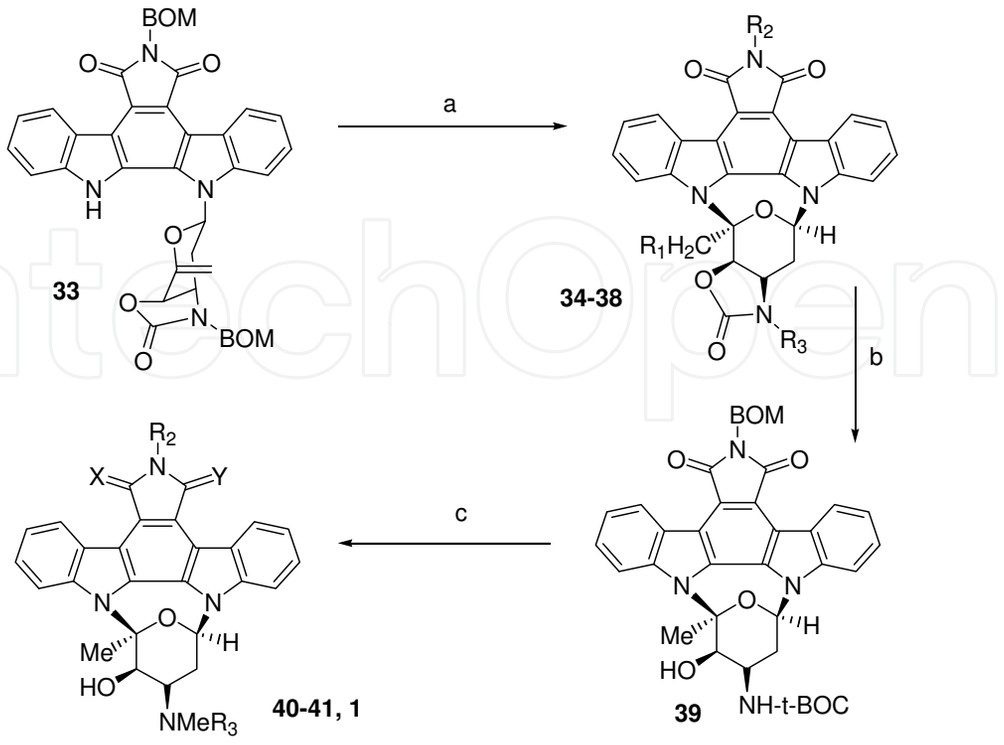
Scheme 4



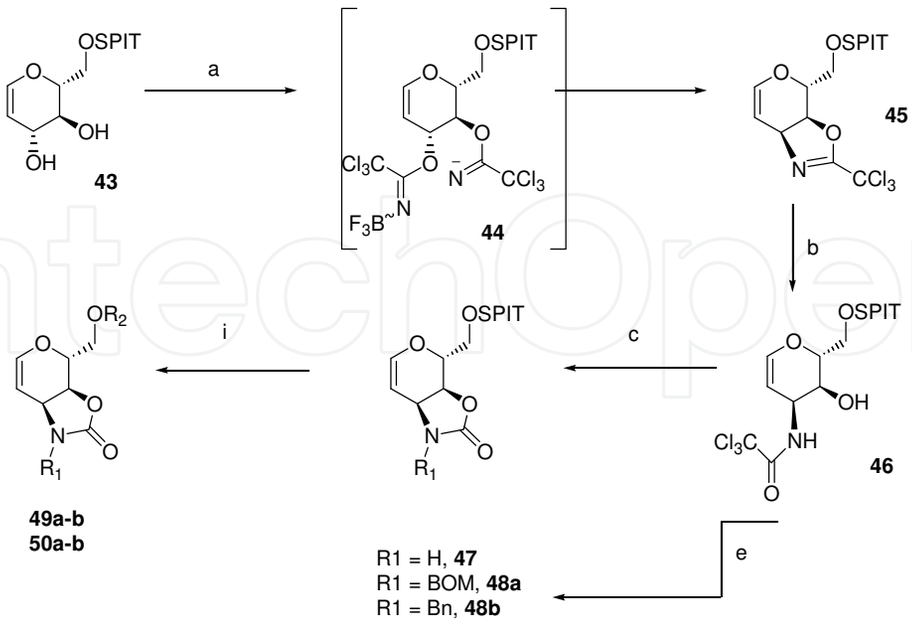
16 (R_4 =I), THF, DBU, roomtemperature, 89% of 7. (a) (i) t-BoOK, I_2, THF, MeOH, room tempeature, 65% of 18 (R_1= I, R_2, R_3 = BOM) and 15% of recovered 17. (ii) n-Bu3 SnH, SnH, AIBN, PhH reflux 99% of 19 (R_1= H, R_2, R_3 = BOM) (iii) H2, Pd, (OH)2, EtOAc, MeOH, room temp. then NaOMe, MeOH, 90% of 20(R_1= R_2=R_3 = H) (iv) $(BOC)_2O$, THF, cat. DMAP, room temp. 81% of 21 (R_1= = H, R_2, R_3 = BOC). (v) NaH, DMF, MeOH, room temperature, then BOMCl, 82% of 22 (R_1=HI, R_2= BOM R_3 = BOC). (b) Cs_2CO_3, MeOH, room temp. 93%. (c) (i) NaH, $(CH_3)_2$ SO_4, THF, DMF, room temp. 86% of 24. (X, Y=O R_2=BOM R_3 = BOC). (ii) H_2 $Pd(OH)_2$, EtOAc, MeOH, room temp. then NaOMe in MeOH 84% (X, Y=O R_2=H R_3 = BOC) (iii)TFA, CH2Cl2, room temp. 97% of 25 (X, Y=O R_2,R_3 = H) (iv) NaBH4, EtOH, room temp. work up (X, Y= O, OH R_2, R_3 =H), then PhSeHcat. TsOH, CH_2Cl_2 room temp. 39% of (X=H2 Y=O R_2, R_3 =H) 39% of 26 (X=O, Y=H2 R_2, R_3 = H), and 5% of 25.

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.

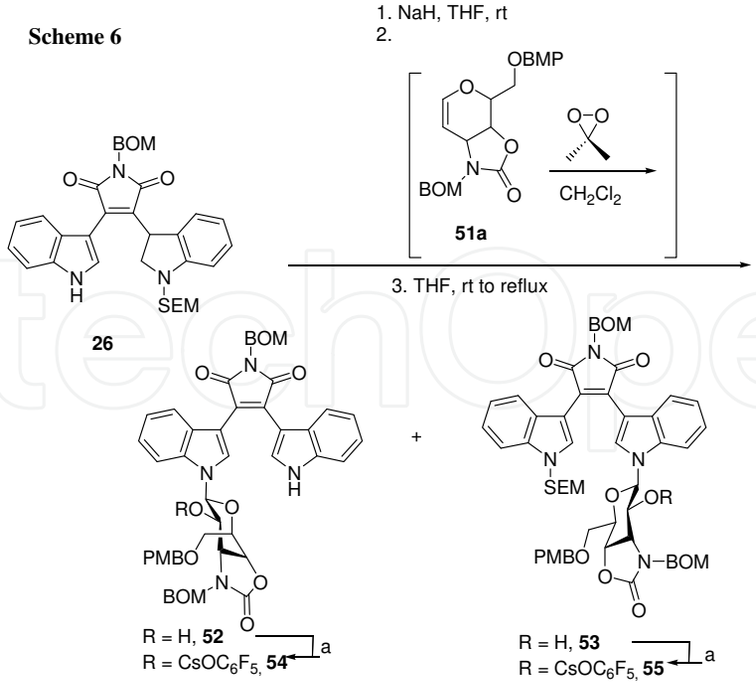
Oxazolidinone glycal **50a** and its derived epoxide proved to be effective as functional versions of target glycals. Danishefsky et al. next focused on the first glycosidic bond (Scheme 6), for which bis-indolyl maleimides were effective glycosyl acceptors for 1,2-anhydrosugar donors. Thus, the sodium 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



a) NaH, CH_2Cl_2 0 °C then Cl_3CCN, 0 °C to rt then BF_3. OEt_2 -78°C to 78% (b) TsOH H_2O, pyr, 80°C, 80% (c) NaH, CH_2Cl_2 0 °C to rt, 92% (d) NaH, TBAI, DMF then BMOCl, 40 °C 65% and 22% recovered 48. (e) NaH, TBAI, DMF, then BnBr, 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



(a) Thiophosgene, DMAP, Pyr, CH_2Cl_2 reflux then C_6F_5OH, reflux, 79%

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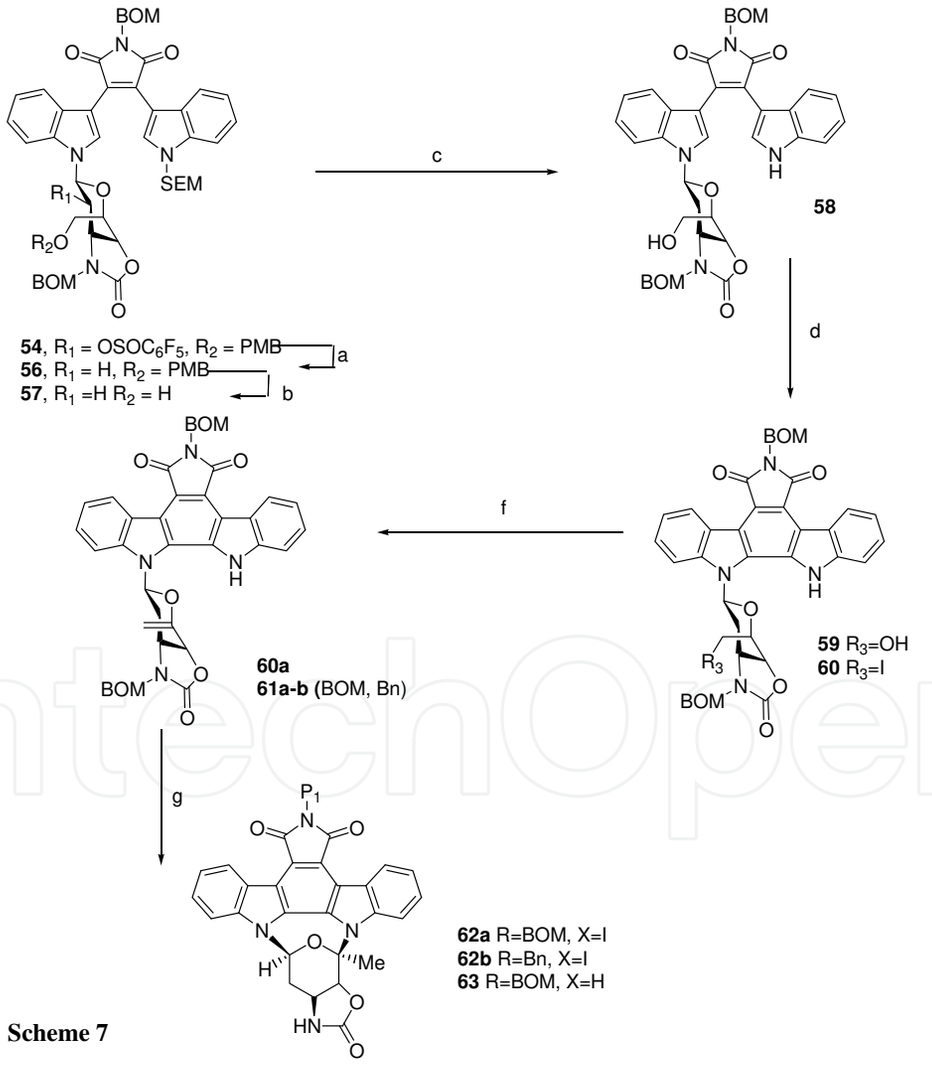
Alteration of the functional group was essential to construct the second glycosidic bond. It was performed by deoxygenating the newly created alcohol at C5′, deprotecting the indole moiety, establishing 2,2′ indolic bond, and finally formation of exo-glycal (Scheme 7).

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 nucleophillic nitrogen attacks the activated exo-glycal.

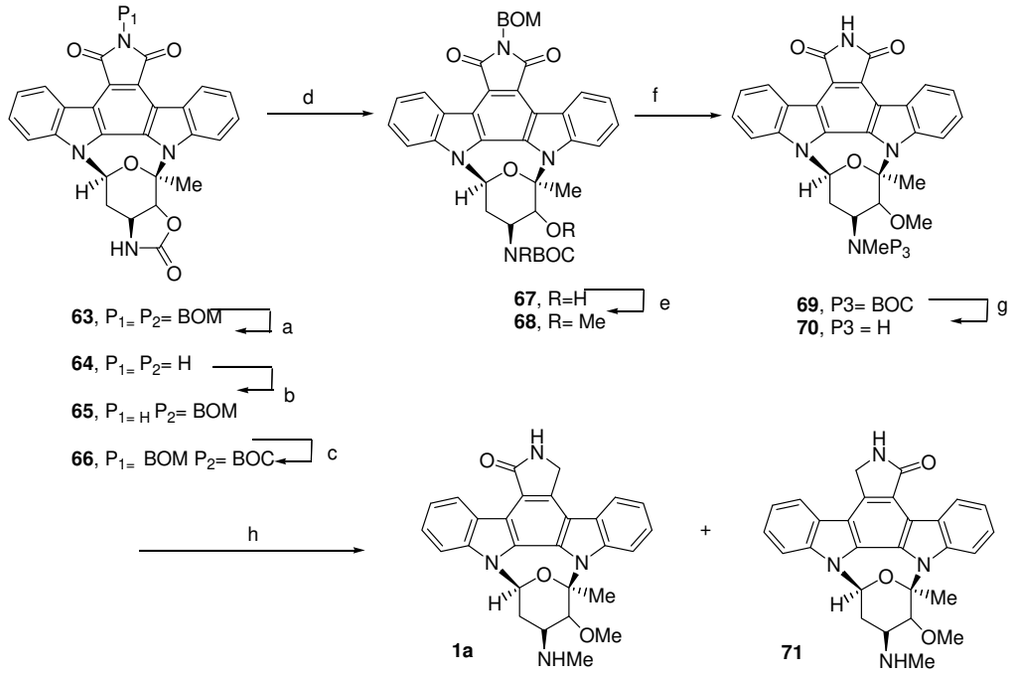


Scheme 7

(a) n-Bu_3SnH. AIBN ,PhH, reflux, 74%. (b) DDQ, CH_2Cl_2, H_2O, 0 °C to rt, 97% (c) TBAF, THF, reflux, 91%. (d) hυ, cat. I_2, air, PhH, rt, 73% (e) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C to rt, 84% (f) DBU, THF, 0 °C, 89%; (h) KOtBu, I_2, THF:MeOH, rt.

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.



Scheme 8

(a) H_2, $Pd(OH)_2$, EtOAc, MeOH, rt, then NaOMe in MeOH, 92% (b) BOC_2O, THF, cat. DMAP, rt, 81% (c) NaH, DMF, rt, then BOMCl, 82% (d) Cs_2CO_3, MeOH, rt, 93% (e) NaH, $CH_3)_2SO_4$, THF, DMF, rt, 86% (f) H_2, $Pd(OH)_2$, EtOAc, MeOH, rt, then NaOMe in MeOH, 84% (g) TFA, CH_2Cl_2, rt, 97%, (h) $NaBH_4$, EtOH, rt, workup, then PhSeHcat. TsOH, CH_2Cl_2, rt 39% of 2, 39% of 89 and 15%of 88

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-

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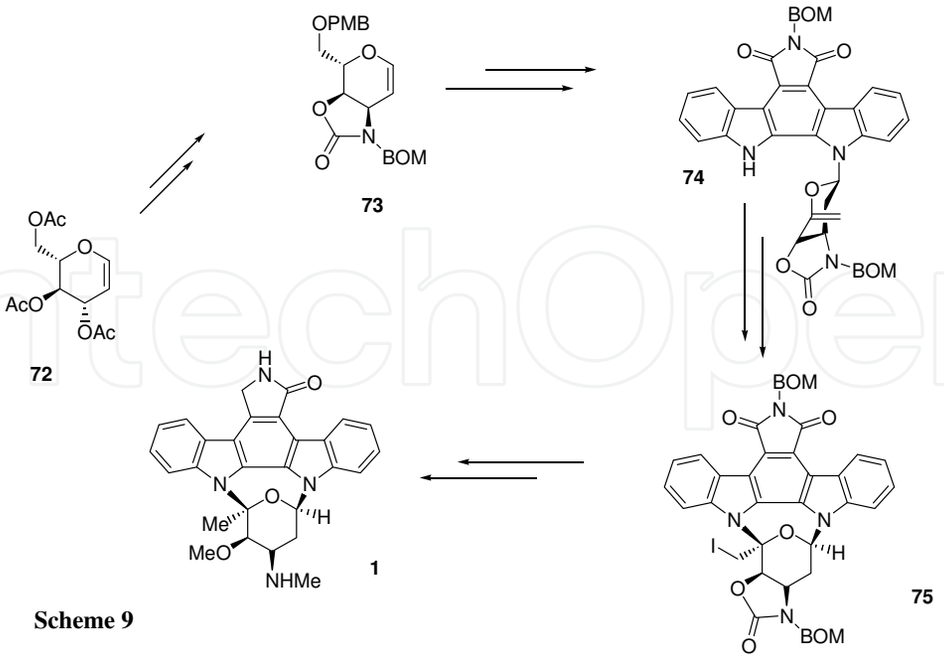

Scheme 9

carbazole alkaloids can also be affected by a different mechanism than inhibition of PKC, i.e. inhibition of topoisomerase I (Table 2).

Compound	PKC inhibition: IC_{50} (μM)		Cytotoxicity	
			HL-60	833K
Staurosporine (**1**)	α	0.02	0.014	0.0065
	β_1	< 0.005		
	β_2	< 0.005		
ent-Staurosporine (**2**)	α	0.29	3.84	0.312
	β_1	0.19		
	β_2	0.15		
isostaurosporine (**94**)	α	0.98	2.39	0.55
	β_1	0.08		
	β_2	0.05		
ent-isostaurosporine (89)	α	0.03	6.55	0.272
	β_1	0.02		
	β_2	0.01		
imide(95)(*ent*-82)	α	1.25	1.68	0.72
	β_1	0.25		
	β_2	0.10		
imide **82**	α	0.24	593	454
	β_1	0.02		
	β_2	0.03		

Cytotoxicities are given as IC_{50}'s in μM units

Table 1. PKC Inhibition and in Vitro Cytotoxicity

Compound	topo I inhibition	
	DNA cleavage	inhibition of supercoiled DNA relaxation
Staurosporine (**1**)	++	+
ent-Staurosporine (**2**)	++	+
isostaurosporine (**94**)	++	+
ent-isostaurosporine (89)	+++	+
imide(95)(*ent*-82)	++	++++
imide **82**	+	+
camptothecin	++++	++++

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

Table 2. Topo I Inhibition

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 steroselective 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**).

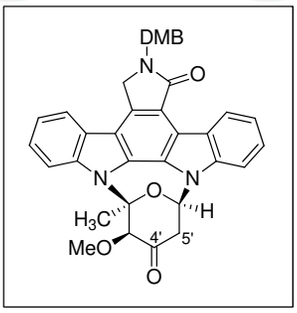

Figure 9. Key intermediate **76**

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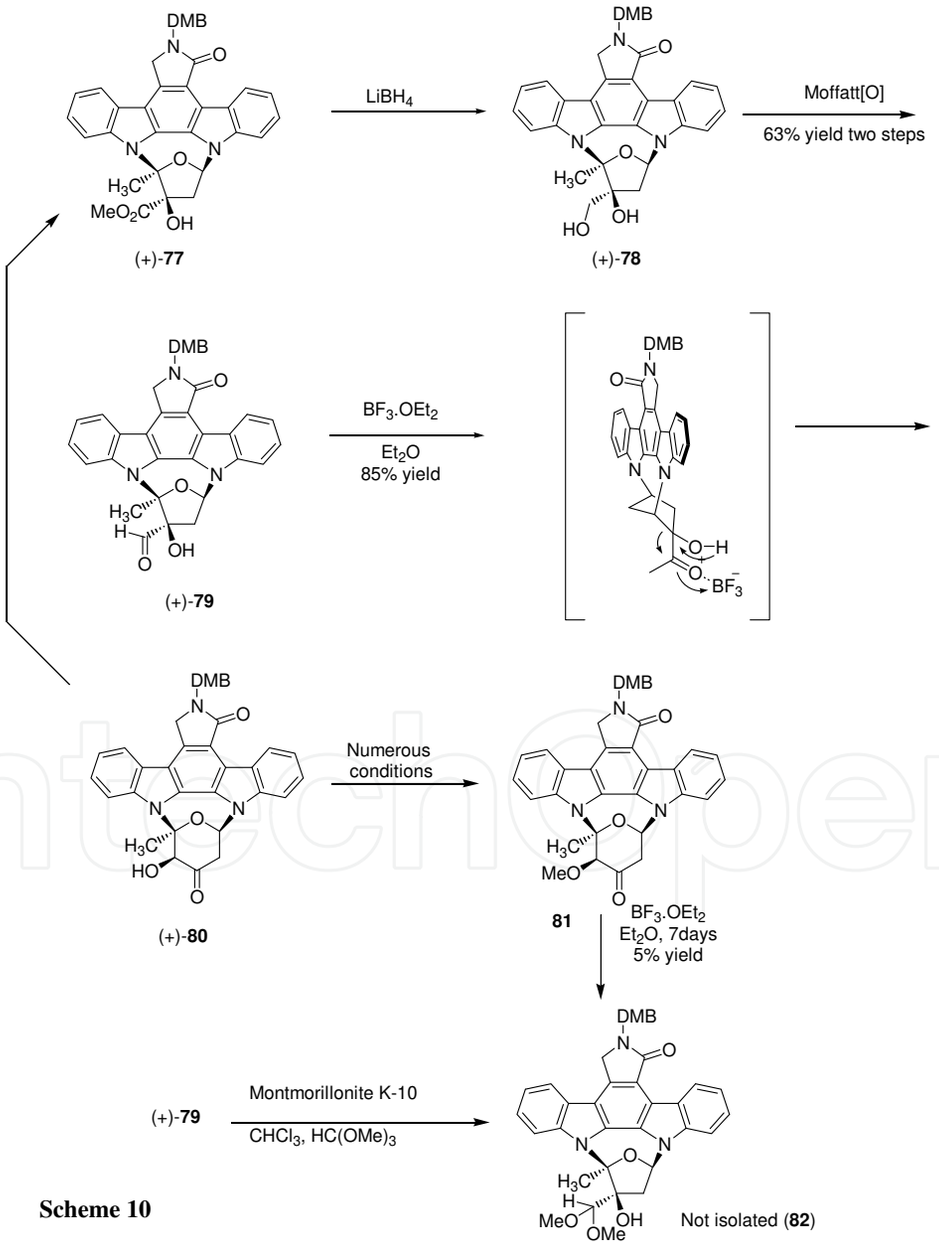
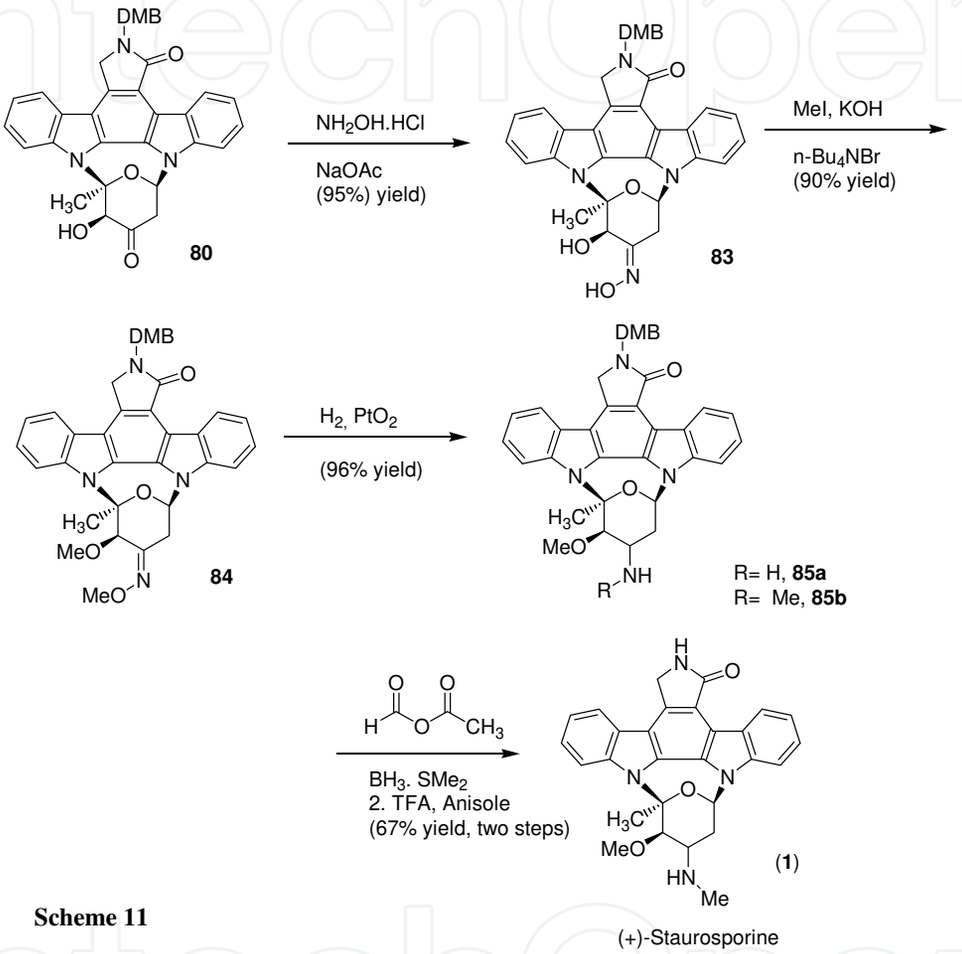
The inspiration for developing this approach derived from Wood's recognition that ketone **76** might be accessed from aldehyde **79** *via* a Tiffaneu-Demyanov-like ring expansion (Schemes 10 and 11). In designing this ring expansion approach, Wood et al. addressed the issues of regio- and stereochemical 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 $NH_2OH.HCl$ 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*-Bu_4NBr in THF) occurred cleanly to afford (-)-**84** and set the stage for a stereoselective reduction (H_2/PtO_2) 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

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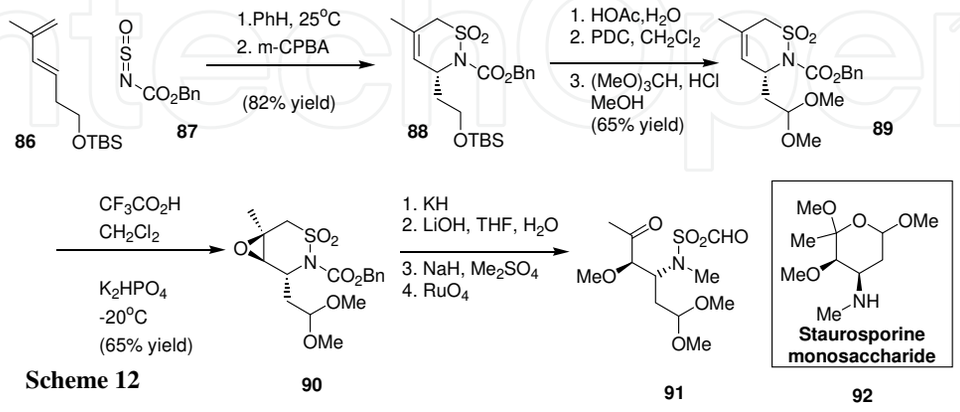
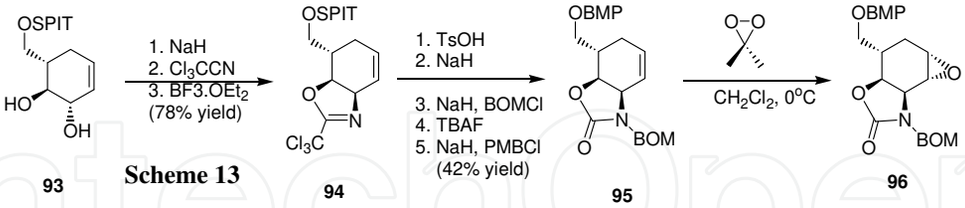
sulfinylcarbamate (**87**) resulted in a mixture of diastereomeric sulfoxides which after oxidation yielded the corresponding sultam (**88**) and then converted to acetal **99.** Subjecting to diastereoselectively epoxidition of olefin **89** using trifluoroperacetic acid afforded **90**. Hydrolytic-reductive opening of epoxide **90** followed by olefin cleavage resulted in keto-acetal **91**, a critical synthon for the staurosporine carbohydrate (**92**).



Scheme 12

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).

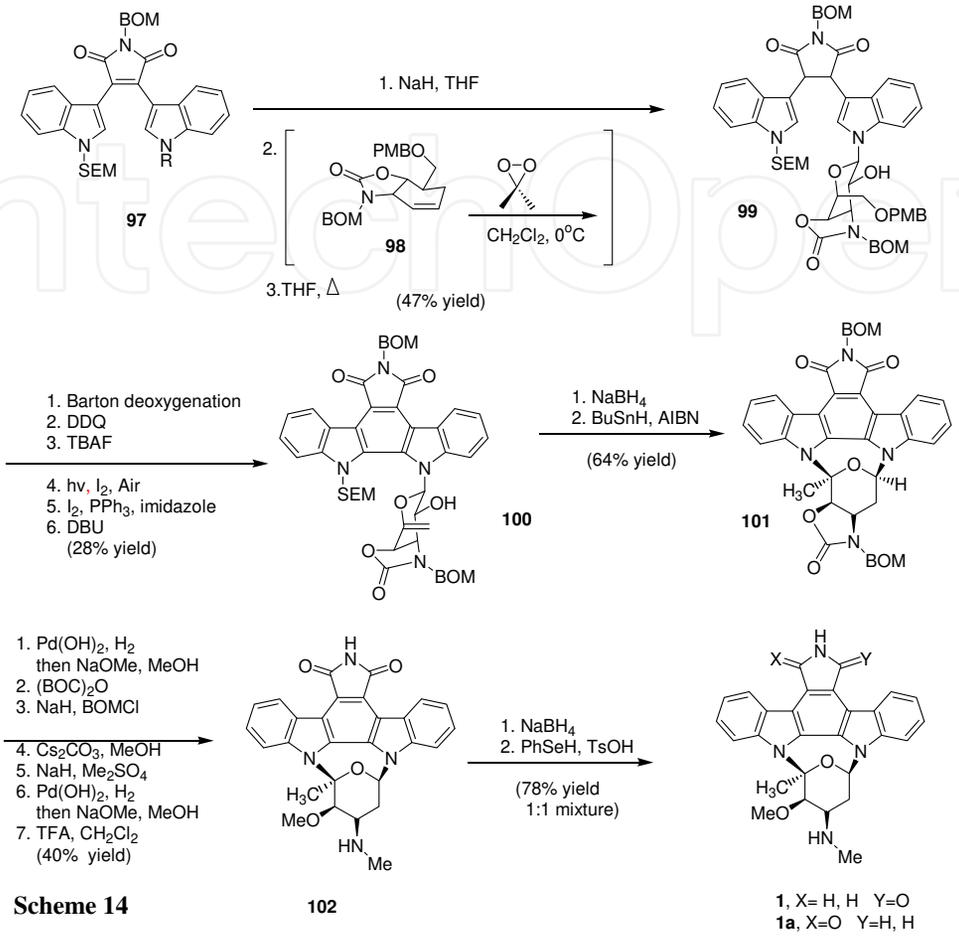


Scheme 13

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**.



Scheme 14

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.

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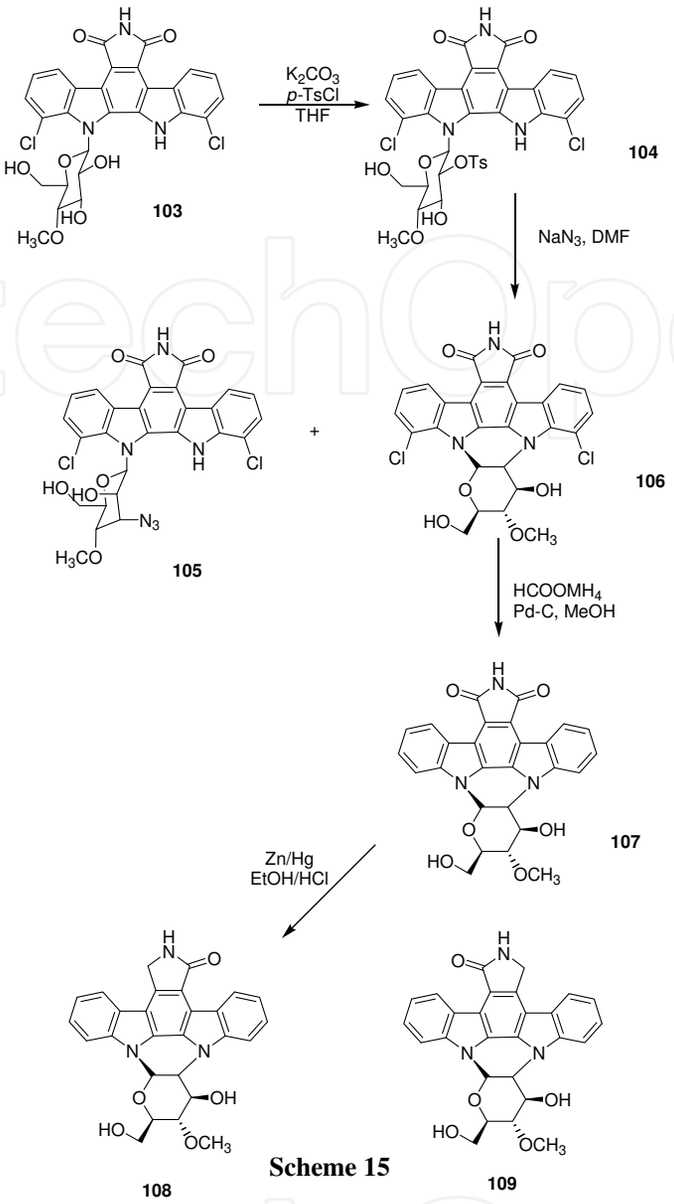
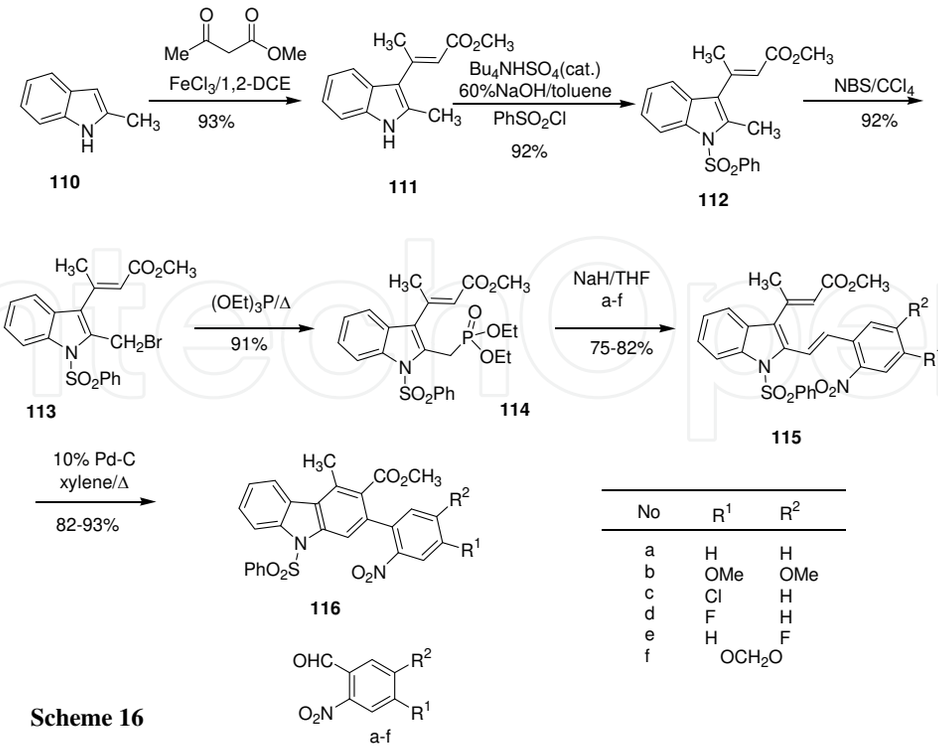


Scheme 15

Triphenylphospite-mediated nitrene insertion of 2-nitroarylcarbazole was performed at a moderate temperature using anhydrous $ZnBr_2$ as catalyst. In addition, an alternative synthetic protocol for preparing ICZs involving concurrent electrocyclization followed by nitrene insertion was adopted as in Scheme 17 by Mohankrishna et al. [50].

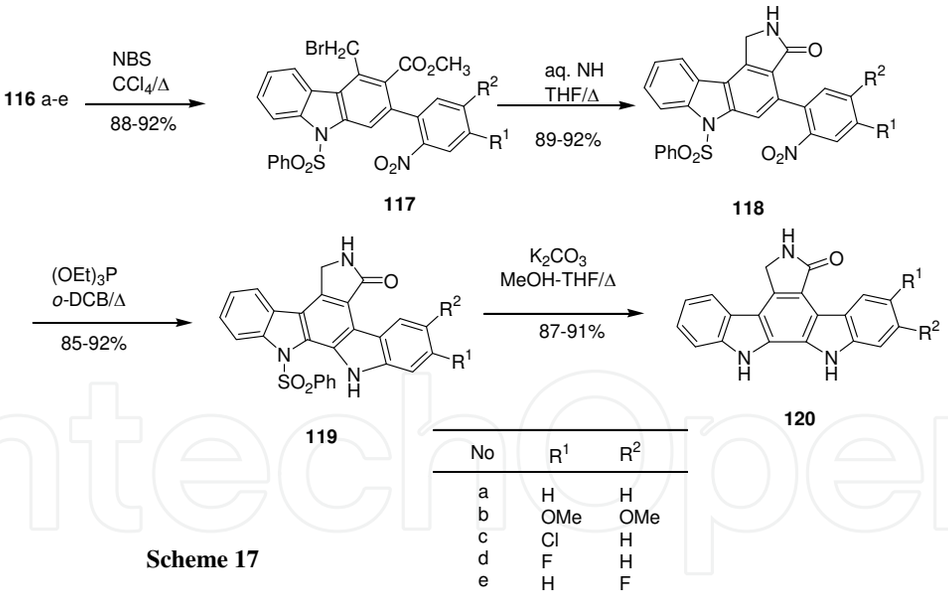
3.3.4. Synthesis of pyrrolidin-2-ones and staurosporine aglycon (K-252c) by intermolecular Michael reaction

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].



No	R^1	R^2
a	H	H
b	OMe	OMe
c	Cl	H
d	F	H
e	H	F
f	OCH_2O	

Scheme 16



No	R^1	R^2
a	H	H
b	OMe	OMe
c	Cl	H
d	F	H
e	H	F

Scheme 17

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.

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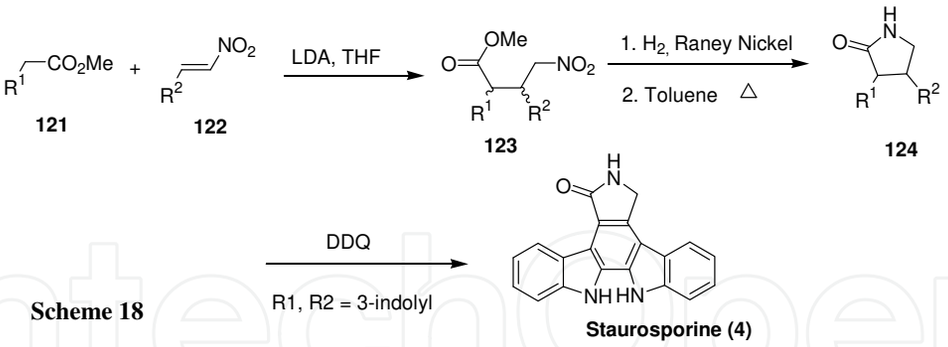


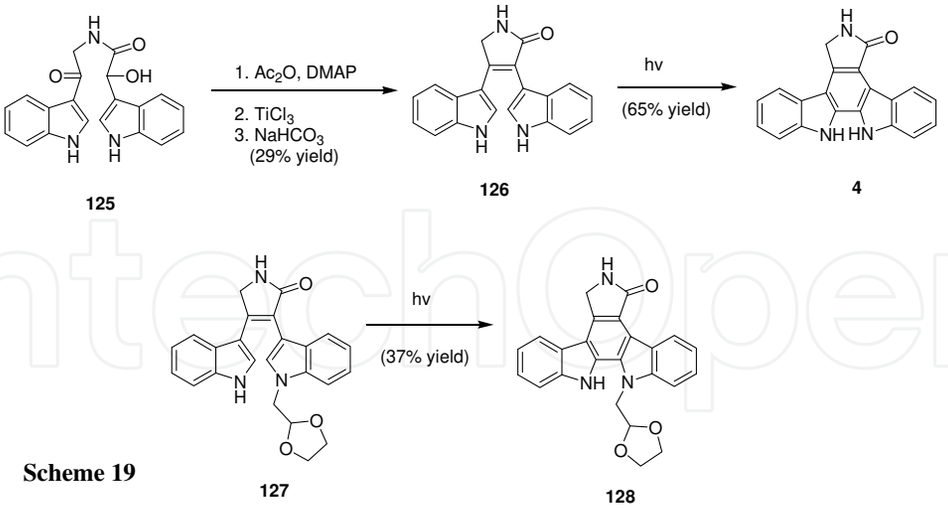
Scheme 18

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).

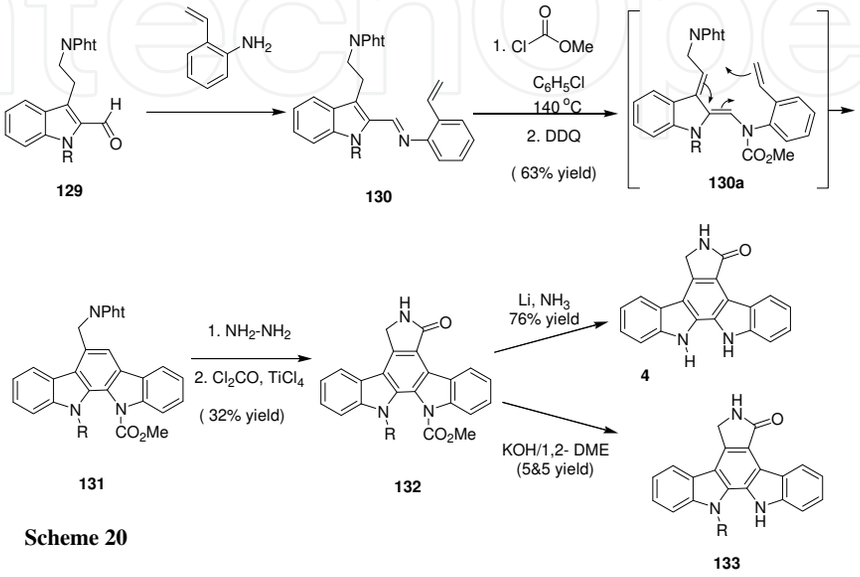


Scheme 19

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.



Scheme 20

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 nitrene 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). Nitrene formation by deoxygenation using triethylphosphire produced K252c (**4**, staurosporinone) [57-58].

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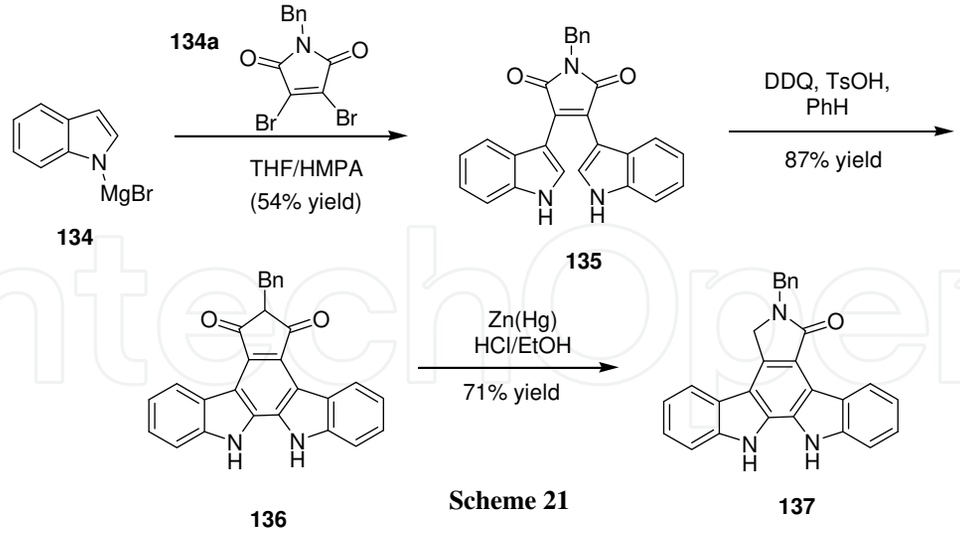
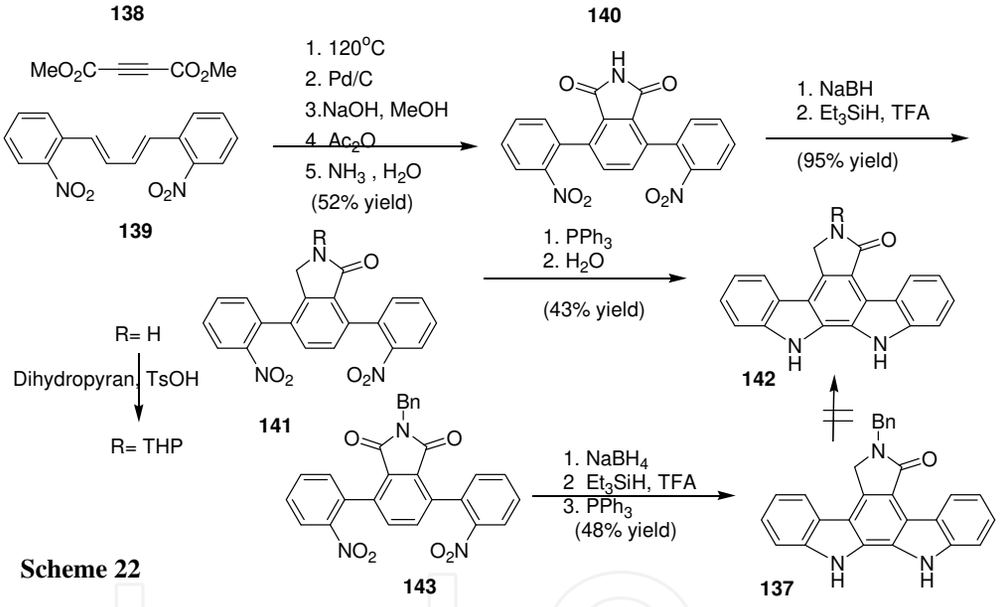


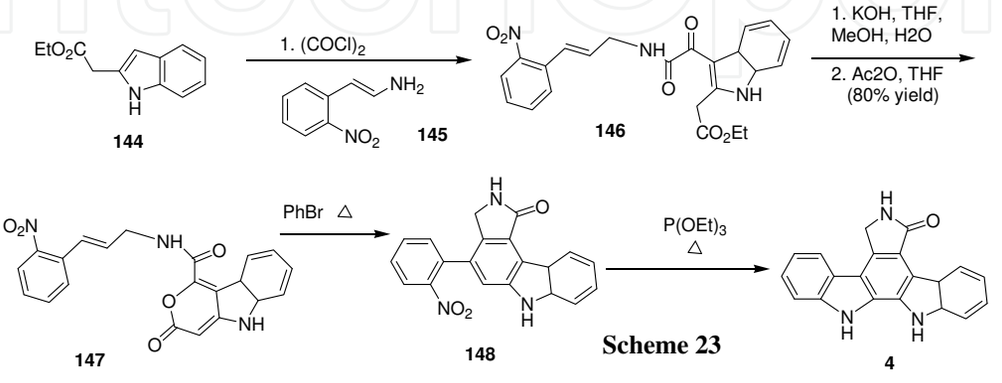
134 135 136 137

Scheme 21



138 139 140 141 142 143 137

Scheme 22



144 145 146 147 148 4

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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References

[1] Shewach DS. Introduction to cancer chemotherapeutics. Chem. Rev. 2009; 109: 2859-2661.

[2] Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J. Ethnopharmacol. 2005; 100: 72-79.

[3] Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. Chem. Rev. 2009; 109: 3012-3043.

[4] Sreedhar AS, Csermely P. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: A comprehensive review. Pharmacol. Ther. 2004; 101: 227-257.

[5] Pechan PM. Heat shock proteins and cell proliferation. FEBS Lett. 1991; 280: 1-4.

[6] McCubrey JA, Steelman LS, Abrams SL, Lee JT, Chang F, Bertrand FE, Navolanic P M, Terrian DM, Franklin RA, D'Assoro, AB, Salisbury JL, Mazzarino MC, Stivala F, Libra M. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. Adv. Enzyme Regul. 2006; 46: 249-279.

[7] Lloyd DG, Golfis G, Knox AJ, Fayne D, Meegan MJ, Oprea TI. Oncology exploration: charting cancer medicinal chemistry space. Drug Discovery Today. 2006; 11: 149-159.

[8] Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discovery 2005; 4: 206-220.

[9] Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. 2003; 66: 1022-1037.

[10] Newman DJ. Natural Products as leads to potential drugs: An old process or the new hope for drug discovery?. J. Med. Chem. 2008; 51: 2589-2599.

[11] Akinaga S, Sugiyama K, Akiyama T. UCN-01 (7-hydroxystaurosporine) and other indolocarbazole compounds: a new generation of anti-cancer agents for the new century?. Anti-Cancer Drug Design. 2000; 15: 43-52.

[12] Prudhomme M. Rebeccamycin analogues as anti-cancer agents. Eur. J. Med. Chem. 2003; 38: 123-140.

[13] Mucke HA. CEP-1347 (Cephalon). IDrugs. 2003; 6: 377-383.

[14] Marminon C, Anizon F, Moreau P, Le´once S, Pierre A, Pfeiffer B, Renard P, Prudhomme M. Syntheses and antiproliferative activities of new rebeccamycin derivatives with the sugar unit linked to both indole nitrogens. J. Med. Chem. 2002; 45: 1330-1339.

[15] Knolker H-J, Reddy KR. Isolation and synthesis of biologically active carbazole alkaloids. Chem. Rev. 2002; 102: 4303-4427.

[16] Omura S, Iwai Y, Hirano A, Nakagawa A, Awaya J, Tsuchia H, Takakashi Y, Masuma R. A new alkaloid am-2282 of streptomyces origin taxonomy, fermentation, isolation and preliminary characterization. J. Antibiotics. 1977; 30: 275-282.

[17] Furusaki A, Hoshiba N, Matsumoto T, Hirano A, Iwai Y, Omura S. X-ray crystal structure of staurosporine: a new alkaloid from a streptomyces strain. J. Chem. Soc. Chem. Commun. 1978; 800-801.

[18] Furusaki A, Hoshiba N, Matsumoto T, Hirano A, Iwai Y, Omura S. The crystal and molecular structure of staurosporine, a new alkaloid from a *streptomyces* strain. Bull. Chem. Soc. Jpn. 1982; 5: 3681-3685.

[19] Gribble G, Berthel S. Studies in Natural Products Chemistry; Elsevier Science Publishers: New York, 1993; 12: 365-409.

[20] Sapi J, Massiot G. *The Alkaloids*; Academic Press: New York, 1995; 47: 173-226.

[21] Funato N, Takayanagi H, Konda Y, Toda Y, Hariyage Y, Iwai Y, Omura S. Absolute configuration of staurosporine by X-ray analysis. Tetrahedron Lett. 1994; 35: 1251-1254.

[22] Gani OA, Engh RA. Protein kinase inhibition of clinically important staurosporine analogues. Nat. Prod. Rep. 2010; 27(4): 489-498.

[23] Alessi DR. The protein kinase C inhibitors RO 31 8220 and GF 109203X are equally potent inhibitors of MAPKAP kinase -1b (Rsk-2) and p70 S6 kinase. FEBS Lett. 1997; 402: 121-123.

[24] Tamoki T, Nomoto H, Takahishi I, Kato Y, Morimoto M, Tomita F. Staurosporine, a potent inhibitor of phospholipid Ca±2 dependent protein kinase. Biochem. Biophys. Res. Commun. 1986; 135: 397-402.

[25] Prudhomme M. Biological targets of antitumor indolocarbazoles bearing a sugar moiety. Curr. Med. Chem.2004; 4: 509-521.

[26] Omura S, Iwai Y, Hirano A, Japan Kokai 78 73, 501; *Chem. Abstr.* 1978; 89: 178086b; idem, Ger. Offen. 2,745,326; *Chem. Abstr.* 1978;, 89: 58348y.

[27] Oka S, Kodama M, Takada H, Tomizuka N, Suzuki H, Agric G. Staurosporine, a potent platelet aggregation inhibitor from a *streptomyces* species. Biol. Chem. 1986; 50: 2723-2727.

[28] McAlpine JB, Karwoski JP, Jackson M, Mullaly MM, Hochlowski JE, Premachandran U, Burres NS. MLR-52, (4'-demethylamino4'5'-dihydroxystaurosporine), a new inhibitor of protein kinase c with immunosuppressive activity. J. Antibiotics 1994; 47: 281-288.

[29] Sato W, Yusa K, Naito M, Tsuruo T. Staurosporine, a potent inhibitor of C-kinase, enhances drug accumulation in multidrug-resistant cells. Biochem. Biophys. Res. Commun. 1990; 173: 1252-1257.

[30] Wakusawa S, Inoko K, Miyamoto K, Kajita S, Hasegawa K, Koyama M. Staurosporine derivatives reverse multidrug resistance without correlation with their protein kinase inhibitory activities. J. Antibiotics 1993; 46: 353-355.

[31] For a review, see: Bergman, J. Studies in Natural Products Chemistry; Elsevier Science Publishers: New York, 1988; 1: 3-30.

[32] Wood JL, Stoltz, B. M.; Dietrich, H.-J. Total Synthesis of (+)- and (-)-K252a. J. Am. Chem. Soc. 1995; 117: 10413-10414.

[33] Meksuriyen D, Cordell GA. Biosynthesis of Staurosporine, 1. 1H- and 13C-NMR assignments. J. Nat. Prod. 1988; 51: 884-892.

[34] Meksuriyen D, Cordell GA. Biosynthesis of staurosporine, 2. Incorporation of tryptophan. J. Nat. Prod. 1988; 51: 893-899.

[35] Pearce CP, Doyle TW, Forenza S, Lam KS, Schroeder DR. The biosynthetic origins of rebeccamycin. J. Nat. Prod. 1988; 51: 937-940.

[36] Yang S-W, Cordell GA. Biosynthesis of staurosporine: Incorporation of glucose. J. Nat. Prod. 1996; 59: 828-833.

[37] Hoehn P, Ghisalba O, Moerker T, Peter HH. "3'-Demethoxy-3'-hydroxystaurosporine, a novel staurosporine analogue produced by a blocked mutant." J. Antibiot. (Tokyo) 1995; 48(4): 300-305.

[38] Weidner S, Kittelmann M, Goeke K, Ghisalba O, Zahner H. 3'-Demethoxy-3'-hydroxystaurosporine-O-methyltransferase from *Streptomyces longisporoflavus* catalyzing the last step in the biosynthesis of staurosporine. J. Antibiot. (Tokyo) 1998; 51(7): 679-682.

[39] Howard-Jones AR, Walsh CT. Staurosporine and rebeccamycin aglycones are assembled by the oxidative action of StaP, StaC, and RebC on chromopyrrolic acid. J. Am. Chem. Soc. 2006; 128 (37): 12289-12298.

[40] Link JT, Raghavan S, Danishefsky SJ. First total synthesis of staurosporine and *ent*-Staurosporine. J. Am. Chem. Soc. 1995; 117: 552-553.

[41] Link JT, Gallant M, Danishefsky SJ. The first synthesis of a fully functionalized core structure of staurosporine: sequential indolyl glycosidation by endo and exo glycols. J. Am. Chem. Soc. 1993; 115: 3782-3783.

[42] Winterfield E. In Heterocycles in Bio-Organic Chemistry; Bergmann, J., Ed.; The Royal Society of Chemistry: London, 1991; 18-27.

[43] Joyse RP, Gainor JA, Weinreb SM. Synthesis of the aromatic and monosaccharide moieties of staurosporine J. Org. Chem. 1987; 52: 1177-1185.

[44] McCombie SW, Bishop RW, Carr D, Dobek E, Kirkup MP, Kirschmeir P, Lin SI, Petrin J, Rosinski K, Shankar BB, Wilson O. Indolocarbazoles. 1. Total synthesis and protein kinase inhibiting characteristics of compounds related to K-252c. Bioorg. Med. Chem. Lett. 1993; 3:1537-1542.

[45] Link JT, Raghavan S, Gallant M, Danishefsk SJ, Chou TC, Ballas LM. Staurosporine and *ent*-Staurosporine: The first total syntheses, prospects for a regioselective approach, and activity profiles J. Am. Chem. Soc. 1996; 118, 2825-2842.

[46] Wood JL, Stolz BM, Goodman SN, Onwuene K. Design and implementation of an effective synthetic approach to pyranosylated indolocarbazoles: Total synthesis of (+)-RK286c, (+)-MLR-52, (+)-Staurosporine, and (-)-TAN-1030a. J. Am. Chem. Soc. 1997; 119: 9652-9661.

[47] Weinreb SM, Garigipati RS, Gainor JA. Natural product synthesis *via* cycloadditions with *N*-sulfinyl dienophiles. Heterocycles. 1984: 21; 309-324.

[48] Gallant M, Link JT, Danishefsky SJ. A Stereoselective synthesis of indole-8-*N*-glycosides: An application to the synthesis of Rebeccamycin J. Org. Chem. 1993; 58: 343-349.

[49] Anizon F, Moreau P, Sancelme M, Voldoire A, Prudhomme M, Ollier M, SeveÁre D, Riou J-F, Bailly C, Fabbro D, Meyer T, Aubertin AM. Syntheses, biochemical and biological evaluation of staurosporine analogues from the microbial metabolite rebeccamycin. Bioorg. Med. Chem. 1998; 6: 1597-1604.

[50] Rajeshwaran GG, Mohanakrishnan AK. Synthetic studies on indolocarbazoles: Total synthesis of staurosporine aglycon. Org. Lett. 2011; 13 (6): 1418-1421.

[51] Mahboobi S, Eibler E, Koller M, Kumar KCS, Popp A. Synthesis of pyrrolidin-2-ones and of staurosporine aglycon (K-252*c*) by intermolecular Michael reaction. J. Org. Chem. 1999; 64: 4697- 4704.

[52] Burkhard S, Winterfeld E. Reactions with indole derivatives, XLVIII. A simple synthesis of the staurosporine aglycon. Heterocycles 1983; 20: 469-476.

[53] Bruning J, Hache T, Winterfeld E. The First regioselective synthesis of a monosubstituted staurosporine precursor. Synthesis. 1994; 25-27.

[54] [Magnus PD, Sear NL. Indole-2,3-quinodimethanes : Synthesis of selectively protected derivatives of the fused dimeric indole alkaloid staurosporinone. Tetrahedron. 1984; 40: 2795-2797.

[55] Hughes I, Raphael RA. Synthesis of arcyriaflavin B. Tetrahedron Lett. 1983; 24: 1441-1444.

[56] Hughes I, Nolan WP, Raphael RA. Synthesis of the indolo[2,3-*a*]carbazole natural products staurosporinone and arcyriaflavin B. J. Chem. Soc., Perkin Trans. 1. 1990; 2475-2480.

[57] [57] Moody CJ, Rahimtoola KF. Synthesis of the staurosporine aglycone. J. Chem. Soc., Chem. Commun. 1990; 1667-1668.

[58] Moody CJ, Rahimtoola KF, Porter B, Ross BC. Synthesis of the staurosporine aglycon. J. Org. Chem. 1992; 2105-2114.

[59] For a comprehensive review on the therapeutic potential of PKC inhibitors, see: Bradshaw, D.; Hill, C. H.; Nixon, J. S.; Wilkinson, S. E. Agents and Actions. 1993; 38: 135-147.