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INDOLOCARBAZOLE NITROGENS LINKED BY THREE-ATOM BRIDGES: A POTENT NEW CLASS OF PKC INHIBITORS

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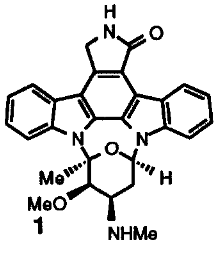
Abstract: Two different approaches to preparing a series of potent PKC inhibitors, represented by **11**, are delineated, namely, (a) reaction of indolocarbazole derivatives with appropriate 3-atom synthons followed by hydrolysis and/or hydrolysis/reduction or (b) treatment of 2-TIPS Arcyriaflavin A **10** with appropriate 3-atom synthons proceeded by N–Si bond cleavage.

Cell growth and differentiation that have gone awry are often associated with disease states such as cancer or psoriasis.[3] Over the past decade, protein kinase C (PKC),[4] a key enzyme in transmembrane signal transduction, has been implicated in these diseases as well as an array of cellular functions and inflammation.[3,5] Hence, regulation of this family of isozymes has generated considerable interest.

A variety of microbial metabolites that contain an indolocarbazole unit as the common element, interact with the catalytic domain in an ATP-competitive fashion to cause inhibition of PKC.[4] Staurosporine,[6] **1** and K-252a,[7] **2** are two examples, but many more exist.[8,9] A relationship between antagonism of PKC by this family of natural products and inhibition of tumor growth,[9,10] tumor invasion[9,11] and inflammation[3,5] has been established. The structurally simpler Arcyriarubins[12] (**3**: Arcyriarubin A) also inhibit PKC.[9] Recent investigations by Davis and co-workers have revealed that derivatives of this class, unlike Staurosporine and K-252a, are quite selective inhibitors with respect to other kinases[13].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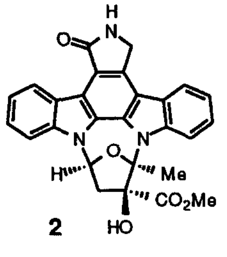
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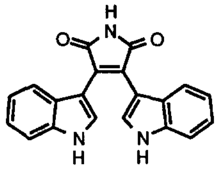
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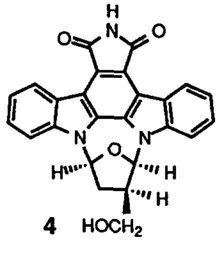
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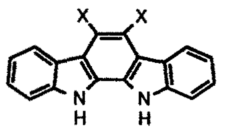
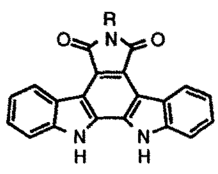
Complementary efforts from our laboratories[14] have described synthetic analogs of K-252a, modified in the carbohydrate portion, which inhibit PKC at nanomolar (**4**; IC_{50}=1.7 nM), and, in some cases, subnanomolar concentrations. Our goal was to further probe the nature of allowable changes in the carbohydrate region while maintaining the potency observed in the previous series. Specifically, we wished to define the minimum requirements of the "carbohydrate" ring and examine the effects of heteroatom incorporation into this region (Z in **11**).

We envisioned the construction of targets such as **11** from indolocarbazole[15] **5** and appropriate 5,6-substituted derivatives (**6**, **7**), or, from a protected form of Arcyriaflavin A **8**. Other work in our laboratories[14]

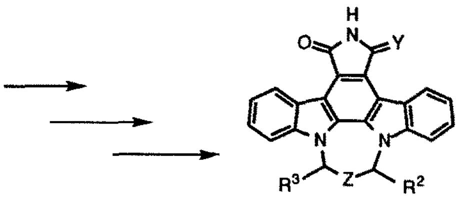


5 X=H
6 X=Br
7 X=CN

or



8 R=H
9 $R=CH_2NHR^1$
10 $R=Si(iPr_3)$

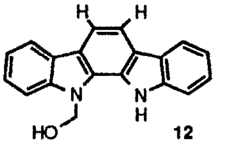


11 $Z=O, NR^1, S, SO, SO_2$
$Y=H_2, O, NH$

had revealed that both dibromo and dicyano derivatives of N,N-disubstituted indolocarbazoles were accessible, but similar reactions on the parent heterocycle **5** had not been performed.

When indolocarbazole **5** (0.17 M in DMF) was carefully treated with 2.0 equivalents of $PhNMe_3Br_3$ in DMF, the desired dibromo compound **6** was isolated in 84-88% yield. Treatment of this compound with CuCN (3.5 equiv.), a catalytic amount of NaI (10 mol% relative to CuCN) in degassed N,N-dimethyl acetamide (reflux; 4 h) led to the required dicyano derivative **7**[16,17] in 65-78% yield.

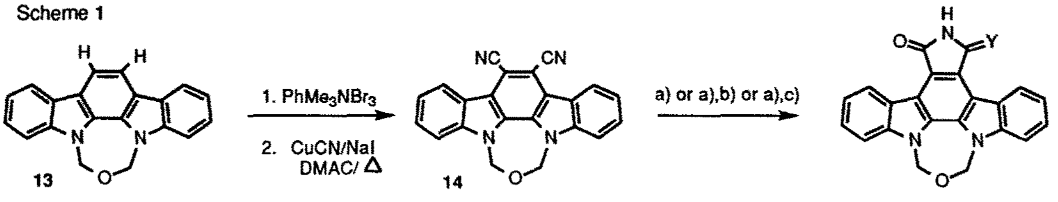
Although the dinitrile **7** could not be hydrolyzed to Arcyriaflavin A due to N-deprotonation under basic conditions, it was a convenient substrate for further modification (vide infra). Preparation of Arcyriaflavin A was efficiently accomplished by the method of Bergman.[18]



12

Installation of the three atoms linking the indolocarbazole nitrogens varied depending on the heteroatom (Z in **11**). Initial attempts to form an oxygen containing bridge focused on manipulations of the N-hydroxy methyl derivative **12** (**5**, nBu_4NOH/THF/formalin; 96% yield). Unfortunately, this easily attainable intermediate could not be parlayed into the desired ether using a variety of strategies. Further experimentation provided ether **13** by direct alkylation/cyclization of **5** (Aliquat®336/THF/50% aqueous NaOH then bis(chloromethyl)ether; 45% yield) and ultimately the desired targets **15**, **16** and **17** using our bromination/cyanation technology, followed by hydrolysis or hydrolysis/reduction.[14]

Scheme 1



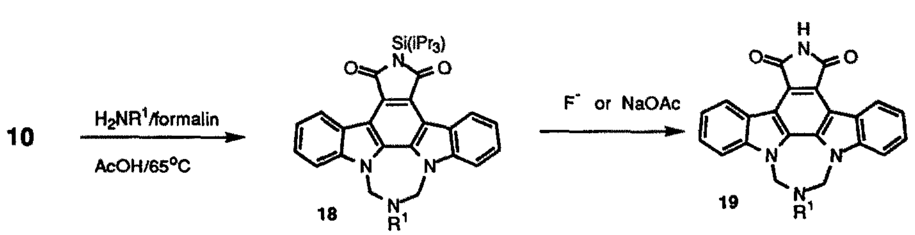
13 **14**

15 Y=NH: a) $KOH/H_2O/DMSO/100°C$
16 $Y=H_2$: a) then b) $NaCNBH_3/AcOH$
17 Y=O: a) then c) $TFA/H_2O/DMSO$

For $Z = NR^1$, an initial experiment revealed that indolocarbazole **5** underwent a Mannich reaction[19] when treated with two equivalents of formalin and one equivalent of benzylamine to give the desired cyclized product in 96% yield. We found that a wide variety of R^1 groups could be tolerated including OH and heterocycles but that use of ammonia as the amine component did not yield the prototype (Z=NH).[20]

More advanced intermediates such as **6** and **7** also underwent this process. In these examples, the reactions were much slower at room temperature than that of the parent heterocycle **5**, a consequence of substituting electron withdrawing groups Br and CN for H. Heating the reaction mixtures at 65°C allowed ring formation to occur rapidly, without a loss in efficiency. This was in marked contrast to glycosidation processes, which proceeded efficiently only in the absence of electron withdrawing substituents.[14]

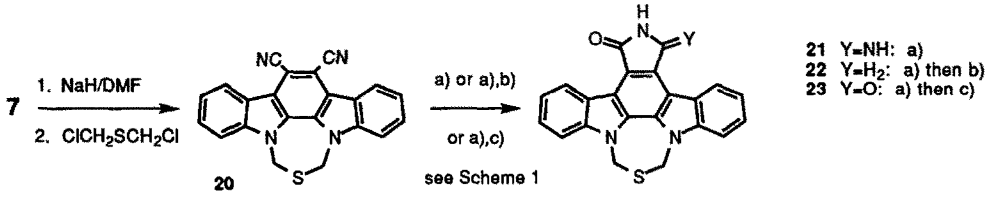
Application of these conditions to **8** led to mixtures containing not only the desired product but also competing reaction at the imide nitrogen (ie formation of **9**). This result prompted us to search for a suitable protecting group for Arcyriaflavin A, a not undaunting task, given that this compound contains three nitrogens of comparable, low nucleophilicity. After discouraging preliminary results with reagents such as SEMCl (low conversions; low yields; no detectable chemoselectivity), we found the imide nitrogen could be cleanly silylated in moderate yield ($TIPSOSO_2CF_3/iPr_2EtN$/diglyme/room temp./7 days; 20% of **10** plus unreacted starting material). Further experimentation revealed that addition of one equivalent of an N,N-disubstituted amide or N,N-dimethyl formamide to the reaction mixture greatly increased the efficiency of the process, generating the desired intermediate in 65-87% isolated yield[21].



When **10** was subjected to our Mannich protocol, cyclization products **18** were generated efficiently. Deprotection (HF·pyridine; nBu_4NF or NaOAc in $DMSO/H_2O$/65°C) gave the requisite targets **19**.

Treatment of the Mannich products derived from **7** with base (KOH/H_2O/DMSO) gave imino imides (**11**, Z=NR^1, Y=NH) and the corresponding lactams (**11**, Z=NR^1, Y=H_2) were available *via* reduction of the imino imides ($NaCNBH_3$/AcOH).

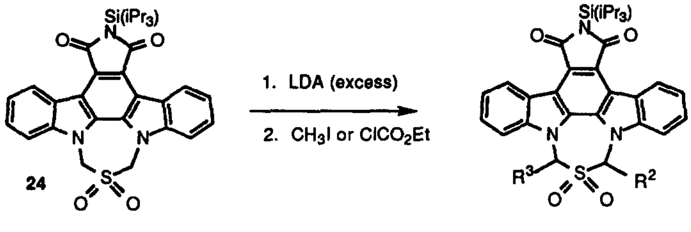
The sulfur analogs could not be produced efficiently using Mannich-type chemistry[22] (indolocarbazole/ Na_2S/formalin; <10%) but could be obtained in reasonable yields by reaction of dinitrile **7** or



2-TIPS Arcyriaflavin A **10** with NaH in an appropriate solvent (DMF; THF; 25-35% isolated yields) followed by addition of commercially available bis(chloromethyl)sulfide.

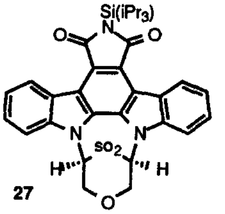
Either of these advanced intermediates could be treated as before to give substances at the sulfide oxidation level (**21**, **22** or **23**). Oxidation (mCPBA or MMPP in DMF), followed by deprotection or other functional group manipulation generated an array of sulfur containing targets (see Table 1).

The potency of compounds in the sulfone series (see Table 1, entries 13 and 14) prompted us to briefly explore substituted derivatives. Examples of formaldehyde addition, alkylation and acylation were formed by reacting N-TIPS sulfone **24** with LDA/THF at low temperature followed by addition of the appropriate electrophile. Acylation produced **26** whereas reaction with MeI generated a diastereomeric mixture of dimethylated sulfones **25**. Only the α,α'-substitution pattern was observed. The propensity for **24** to undergo dialkylation preferentially was underscored when **24** was treated with less than one equivalent of LDA followed by MeI, producing **25** plus recovered **24**. Reaction with gaseous formaldehyde gave rise to **27**, again suggesting α,α'-dialkylation to be the preferred bond-forming pathway in this system. In this case, other events must occur subsequent to formaldehyde addition (such as elimination followed by intramolecular alkoxide delivery) to form the observed oxathia analog **27**.



24

25 $R^2=R^3=CH_3$
26 $R^2=CO_2Et$, $R^3=H$



27

In vitro biological evaluation of our targets using partially purified rat brain PKC was carried out using a protocol that has been described.[23]

In general, potency was similar between imide and lactam functionalities when other groups remained constant (see Table 1, entries 6,7; 9,11). Imino imides were usually less active, in accord with previous observations.[14] Although no examples are shown in the table, alkylation of the imide or lactam nitrogen completely supresses PKC activity.[24,25]

Removal of most of the sugar-like aspects of K252a or Staurosporine does not necessarily reduce potency. In fact, most of the entries are more active than K-252a (IC_{50}=60 nM). With the exception of the trans dimethyl sulfone (entry 16), the potency of the sulfone series is comparable to or in some cases (entries 13,14 and15) greater than, Staurosporine (IC_{50}=6.0 nM)[6].

We have described the preparation of a new, wholly synthetic class of potent PKC inhibitors. All of the examples described are structurally less complex than the doubly N-glycosidated, naturally occurring compounds. This information, coupled with further *in vivo* experiments and toxicity data, will allow us to continue to evaluate selected examples for the therapeutic uses alluded to at the beginning of this article.

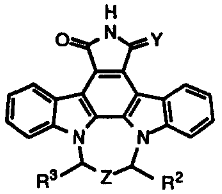


Table 1: Inhibitory Concentrations (IC_{50} nM)

Entry Number	Y	Z	R^2	R^3	PKC
1	O	O	H	H	11
2	NH	O	H	H	24
3	O	NH	H	H	145
4	O	NOH	H	H	90
5	O	NMe	H	H	86
6	O	NCH_2CH_2OH	H	H	17
7	H_2	NCH_2CH_2OH	H	H	15
8	O	$NCH(CH_2OH)_2$	H	H	10
9	O	S	H	H	14
10	NH	S	H	H	24
11	H_2	S	H	H	7.0
12	O	SO	H	H	19
13	O	SO_2	H	H	2.0
14	H_2	SO_2	H	H	5.0
15 (meso)	O	SO_2	Me	Me	2.4
16 (dl)	O	SO_2	Me	Me	120
17	O	SO_2	H	CO_2Et	20
18	O	SO_2	$—CH_2OCH_2—$		20

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References and Notes:

1. Department of Chemical Research.
2. Department of Molecular Pharmacology
3. Nagao, S.; Seishima, M.; Mori, S.; Nozawa,Y. *J. Invest. Dermatol.* **1988**, *90*, 406-408. Hegemann L; Bonnekoh B.; van Rooijen L.A.; Mahrle, G. *J. Dermatol Sci.* **1992**, *4*, 18-25. Kurtz, E. S.; Ortega-Nanos, M.; Hellyer, L.; Przekop, P.; Bailey, S. *J. Invest. Dermatol.* **1992**, *98*, 595.
4. Nishizuka, Y. *Nature* **1988**, *334*, 661-665. Nishizuka, Y. *Science* **1992**, *258*, 607-614. Nishizuka, Y. *Cancer* **1989**, *63*, 1892-1903.
5. Omari, K.; Ishii, H.; Manabe, H.; Sato, H.; Tamura, T.; Kase, H. *Drug Res.* **1988**, *38*, 809-813. Nixon, J.S. Bishop, J.; Bradshaw, D.; Davis, P. D.; Hill, C.H.; Elliott, L.H.; Kumar, H.; Lawton, G.; Lewis, E.J.; Mulqueen M.; Sedgwick, A.D.; Westmacott, D.; Wadsworth, J.; Wilkinson, S.E. *Drugs Exptl. Clin. Res.* **1991**, *17*, 389-393.

6. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiotics* **1977**, *30*, 275-282. Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S.; *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3681-3685. The absolute configuration of Staurosporine has recently been disclosed: Funato, N.; Takayanagi, H.; Konda, Y.; Toda, Y.; Harigaya, Y.; Iwai, Y.; Omura, S. *Tetrahedron Lett.* **1994**, *35*, 1251-1254.

7. Yasuzawa, T.; Iida, T.; Yoshida, M.; Hirayama, N.; Takahashi, M.; Shirahata, K.; Sano, H. *J. Antibiotics* **1986**, *39*, 1072-1078. Elliot, L.H.; Wilkinson, S.I.; Sedgwick, A.K.; Hill, C H., Lawton, G., Davis, P.D.; Nixon, J. S. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 148-154.

8. Koshino, H.; Osada, H.; Isono, K. *J. Antibiot.* **1992**, *45*, 195-198. Osada, H.; Koshino, H.; Kudo, T.; Onose, R.; Isono, K. *J. Antibiotics* **1992**, *45*, 189-194 Takahashi, I.; Saitoh, Y.; Yoshida, M.; Sano, H.; Nakano, H.; Morimoto, M.; Tamaoki, T. *J. Antibiotics* **1989**, *42*, 571-576. Tanida, S.; Takizawa, M.; Takahashi, T.; Tsubotani, S.; Harada, S. *J. Antibiotics* **1989**, *42*, 1619-1630. Takahashi, H.; Osada, H.; Uramoto, M.; Isono, K. *J. Antibiotics* **1990**, *43*, 168-173. from subphylum *urochordata*. Kinnen, R.B., Scheuer, P.J. *J. Org. Chem* **1992**, *57*, 6327-6329.

9. Gribble, G.W., Berthel, S.J *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: New York, 1993; Vol. 12, pp.365-409.

10. Tamaiki, T.; Nakano, H. *Biotechnology* **1990**, *8*, 732-735. Akinga, S ; Gomi,K.; Marimoto, M.; Tamaoki, T.; Okabe, M. *Cancer Res.* **1991**, *51*, 4888-4892.

11 O'Brian, C.A.; Ward, N.E. *J. Natl. Cancer Inst.* **1990**, *82*, 1734-1735

12. Steglich, W.; Steffan, B.; Kopanski, L., Eckhardt, G. *Angew. Chem. Int. Ed. Eng.* **1980**, *19*, 459-460.

13. Davis, P. D.; Elliott, L.H.; Harris, W.; Hill, C.H , Hurst, S.A.; Keech, E ; Kumar, M.K.H.; Lawton, G.; Nixon, J.S.; Wilkinson, S.E. *J. Med Chem.* **1992**, *35*, 994-1001. Bit, R.A.; Davis, P. D.; Elliott, L.H ; Harris, W.; Hill, C.H.; Keech, E.; Kumar, M.K.H.; Lawton, G.; Maw, A.; Nixon, J.S , Vesey, D.R.; Wadsworth, J.; Wilkinson, S.E. *J. Med Chem* **1993**, *36*, 21-29.

14. McCombie, S.W.; Bishop, R.W.; Carr, D.; Dobek, E.; Kirkup, M.P.; Kirschmeier, P.; Lin, S.; Petrin, J.; Rosinski, K.; Shankar, B.B ; Wilson, O. *BioMed. Chem. Lett.* **1993**, *3*, 1537-1542.

15. Bhide, G.V.; Tikotkar, N.L.; Tilak, B.D. *Chem. Ind.* **1957**, 363. Bloink, G.J ; Pausacker, K.H. *J. Chem. Soc.* **1950**, 1328-1331. Moldenhauer, W.; Simon, H. *Chem. Ber.* **1969**, *102*, 1198-1201

16. see reference 14 and references cited therein for examples of Ar—X → Ar—CN.

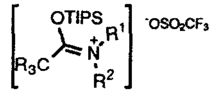
17. Isolation of the sparingly soluble 7 was best carried out by allowing it to crystallize from the reaction mixture (60-80°C). A second crop (~10% less pure) could be obtained by precipitation with 1.1 NH_4OH :H_2O and sequential washing of the solid with 1:1 NH_4OH :H_2O until no copper salts remained (ie clear washings).

18. Bergman, J.; Pelcman, B. *J. Org. Chem.* **1989**, *54*, 824-828

19. for related examples of Mannich condensations where the acidic component is a non-basic nitrogen see: Einhorn, A. *Ann. Chem.* **1905**, *343*, 207 Opitz, G.; Hellmann, H. *α-Amino-alkylierung* Verlag Chemie: Weinheim; 1960. Möhrle, H., Spillmann, P. *Tetrahedron* **1970**, *26*, 4895-4900 Katritzky, A.R.; Rachwal, S.; Hitchings, G.J. *Tetrahedron* **1991**, *47*, 2683-2732 Knapp, S.; Hale, J J.; Bastos, M.; Gibson, F.S. *Tetrahedron Lett.* **1990**, *31*, 2109-2112. Knapp, S., Hale, J.J.; Bastos, M ; Molina, A.; Chen, K.Y. *J. Org. Chem.* **1992**, *57*, 6239-6256.

20. The NH compound (Table 1, entry 3), was prepared by treating the related hydroxylamine with Ra-Ni in refluxing EtOH/THF.

21. We are exploring the generality of using this reagent as an additive in related silylations. We envisage silyl transfer occurring *via* an activated complex such as:



22. for a review of the successful use of sulfur in Mannich reactions see Tramontini, M. *Synthesis* **1973**, 757-758.

23. Bishop, W.R.; Petrin, J., Wang, L.; Ramesh, U.; Doll, R. *J. Biochem. Pharmacol.* **1990**, *40*, 2129-2135.

24. Although intermediates containing the N-TIPS group (for example, see **25**, **26** or **27**) are moderately active, we have found this to be due to partial N–Si bond cleavage under the conditions of our assay.

25. see example **3o** in: Kleinschroth, J.; Hartenstein, J.; Rudolph, C ; Schächtele, C *BioMed. Chem. Lett.* **1993**, *3*, 1959-1964.

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