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INDOLOCARBAZOLES. 3. SYNTHESIS OF NOVEL AZA ANALOGS OF STAUROSPORINE AND K 252a AS PKC INHIBITORS.

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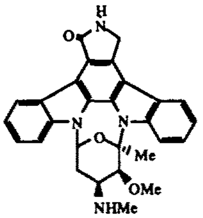
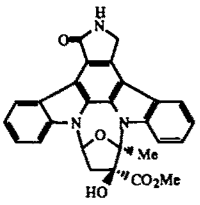
Abstract: Indolocarbazole **4** and arcyriaflavin A **19** reacted under basic conditions with 1-benzyl-2,6-bis (benzotriazolyl)-piperidine to give **5** and **20**. As an extension of this methodology other related bis benzotriazole derivatives were synthesized and coupled with **19** to obtain a variety of aza derivatives **3**. N-debenzylation of these compounds gave novel PKC inhibitors.

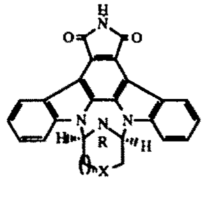
The vital and complex role played by the protein kinase C (PKC) isozymes in cellular processes continues to focus interest on inhibitors of these kinases, and their potential therapeutic applications.[1] The diverse family of indolocarbazole natural products[2] has provided staurosporine **1** and K-252a **2** as potent inhibitors of PKC; our own studies related to these compounds have resulted in a convenient route to highly potent analogs of **2**,[3] and uncovered an unusual skeletal rearrangement in the carbohydrate portion.[4] In this paper, we describe the synthesis and properties of hitherto unknown aza analogs **3** of the natural products.



1



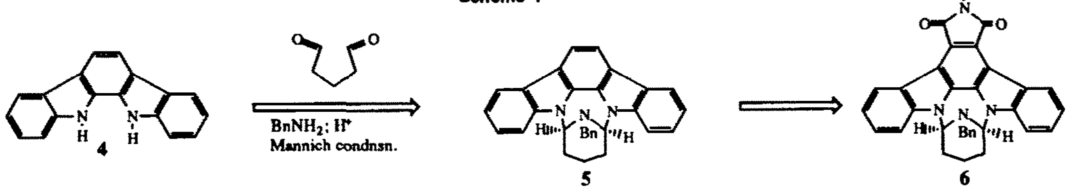
2



Aza Derivatives
3

Our earlier studies [3] encouraged us to use the more reactive parent indolocarbazole[5] **4** as the substrate for N-functionalization (Scheme 1), but initial attempts to effect Mannich condensation[6] between **4**, $PhCH_2NH_2$, and glutaraldehyde to give **5** led only to complex mixtures. **5** remained a target however, since we could implement our recent protocol for imide ring introduction to prepare **6**. As an alternative we sought milder, preferably nucleophilic conditions to accomplish the desired transformation.

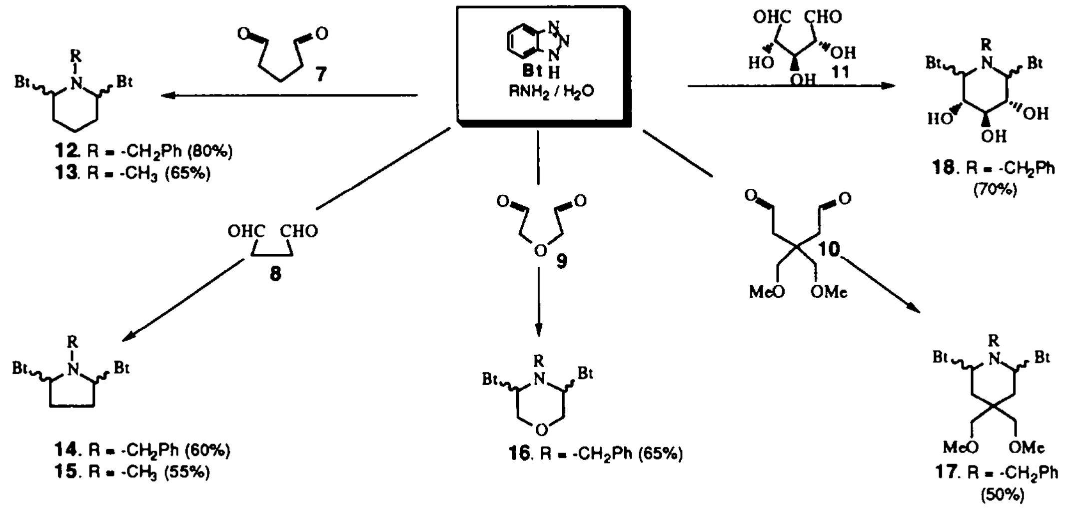
Scheme 1



Katritzky's 2,6 bis-(benzotriazolyl) piperidine **12**[7,8] attracted our attention as a potential aza glycosidating agent. Accordingly, treatment of an aqueous mixture of glutaraldehyde **7** and 2 equivalents of benzotriazole with benzyl amine furnished **12**. Efficient coupling ensued when preformed dianion of indolocarbazole **4** (**4**; NaH; THF) was treated with one equivalent of **12**, to provide **5** (70 % yield).[13]

Encouraged by this result, a variety of other bis-benzotriazolyl derivatives **13**, **14**, **15**, **16**, **17**, and **18** were synthesized starting from readily accessible corresponding dialdehydes,[9] including a highly substituted sugar derived dialdehyde **11**.[10] The benzotriazole chemistry is quite general and yields are good to modest as shown in Scheme 2.[11]

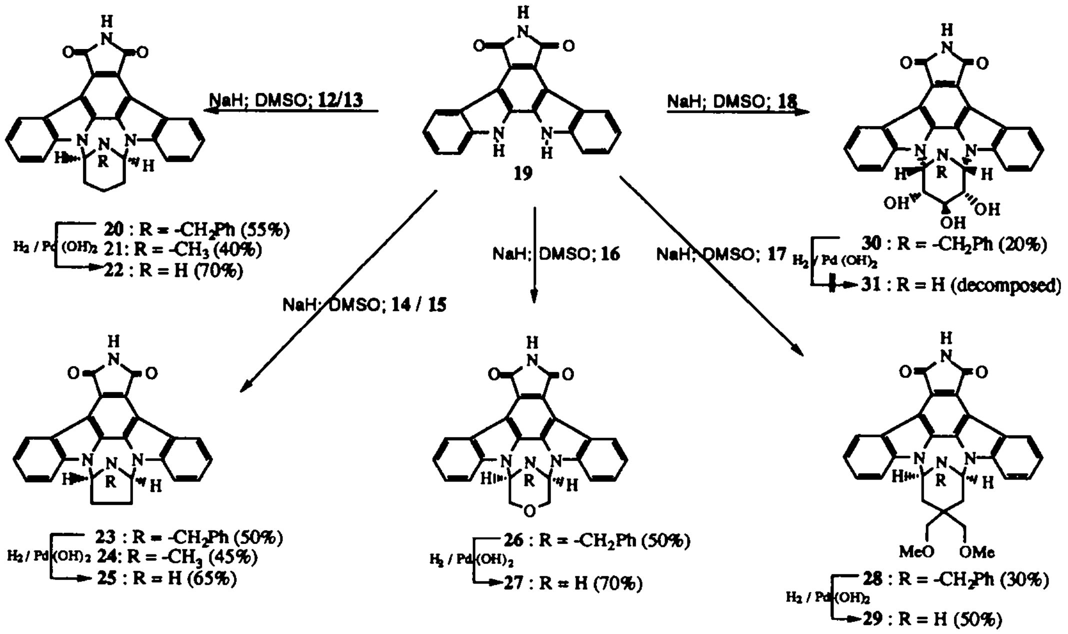
Scheme 2



With an efficient protocol for construction of bottom portion of the molecule in hand, we turned our attention to putting in place the cyclic imide which is a requirement for biological activity. We found, however, that although **5** could be dibrominated, attempted CuCN displacement [3] led to decomposition. Since the crucial C-N bond forming processes which led to **5** involved the nitrogen anion of **4**, we examined the possibility of using the trianion derived from arcyriaflavin A **19** which is readily prepared by the Bergman method.[12] The tri anion (NaH / DMSO) was treated with bis(benzotriazolyl) derivative **12** (two equivalents) to obtain the desired coupled product **20**; however, in lower yield (55%) vs. the dianion of **4**.

Other bis-(benzotriazolyl) derivatives **13**, **14**, **15**, **16**, **17**, and **18** also underwent coupling with the trianion of **19** to provide products **21**, **23**, **24**, **26**, **28**, and **30** respectively.[13] The yields were modest ranging from 55 to 20%, decreasing with increasing substitution on the bottom ring, as shown in Scheme 3. However, the easy availability of the starting materials, and the simplicity of the method renders this route to be a reasonable and practical one. The N-benzyl products required debenzylation for bioactivity. Debenzylation was carried out over Pearlman's catalyst under atmospheric hydrogen.

Scheme 3



In Vitro Biological Evaluation:

These compounds were evaluated for their inhibition of 1-oleoyl-2-acetylglycerol activated PKC (a mixture of isozymes).[14] The results are summarized in Table 1. None of them were as active as staurosporine **1** (IC_{50} = 8 nM), however, some were comparable or superior to K252a **2** (IC_{50} = 60 nM). Significant selectivity was not observed when assayed against pure PKC isozymes and other serine-threonine kinases.[15] Further work is ongoing in an effort to achieve selectivity against these kinases and will be reported in due course.

Table 1

Compound	PKC IC_{50}	Compound	PKC IC_{50}	Compound	PKC IC_{50}
19	550 nM	22	28 nM	27	70 nM
20,23,26,28, 30	~5,000 nM	24	50 nM	29	1200 nM
21	250 nM	25	230 nM	31	---

Acknowledgments: We would like to thank Dr. Robert W. Bishop for the biological evaluation and acknowledge the Physical-Analytical Department staff members for spectra and analysis.

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9. Synthesis of dialdehydes: Succinaldehyde **8** was synthesized by 0.6 N HCl hydrolysis of 2,5-dimethoxy tetrahydrofuran. The resulting aqueous solution was used after neutralizing with sodium bicarbonate. Dialdehyde **9** was synthesized by $NaIO_4$ oxidation of 1,4-anhydroerythritol (Aldrich). Dialdehyde **10** was obtained from ozonolysis of the corresponding bis allyl derivative which itself was procured by $LiAH_4$ reduction of diethyl diallylmalonate (Aldrich) followed by methylation.
10. Dialdehyde **11** was synthesized from 1,2-O-isopropylidene-D-glucofuranose according to our recently reported procedure: Shankar, B. B.; Kirkup, M. P.; McCombie, S. W.; Ganguly, A. K. *Tetrahedron Lett.* **1993**, *34*, 7171.
11. ^{1}H NMR spectra were complicated as these compounds were obtained as cis and trans mixtures and regioisomeric with respect to 1,2 nitrogens of benzotriazole. Most of them were crystalline and gave accurate C, H, N, elemental analysis, with the exception of **12** and **15** which were gummy and were used in the crude form. The geometric nature of the bis-benzotriazolyl derivatives is inconsequential since the nucleophilic displacement possesses SN_1 character and proceeds via an iminium intermediate.
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13. All new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, MS. **Selected Spectroscopic Data**: Compound **5**: CI^+/CH_4 MS m/z = 476 $(M+1)^+$; ^{1}H-NMR (200 MHz, CDCl3) d 1.46 (2H, m), 2.26 (4H, m), 3.84 (2H, s), 5.84 (2H, t, J = 2 Hz), 7.06-7.5 (11H, m), 7.94 (2H, s), 8.2 (2H, d, J = 8 Hz). Compound **20**: CI^+/CH_4 MS m/z = 497 $(M+1)^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) d 1.19 (1H, m), 1.49 (1H, m), 2.2 (4H, m), 3.85 (2H, s), 6.1 (2H, br.s), 7.05-7.72 (11H, m), 9.11 (2H, d, J = 8Hz), 11.1 (1H, s). Compound **23**: CI^+/CH_4 MS m/z = 483 $(M+1)^+$; ^{1}H-NMR (200 MHz, DMSO-d_6) d 1.8 (2 X 1H, ABX system, m), 2.7 (2 X 1H, ABX system, m), 3.76 (2H, s), 6.37 (2H, br.s), 7.05-7.8 11H, m), 9.08 (2H, d, J = 7.8 Hz), 11.12 (1H, s). Compound **26**: CI^+/CH_4 MS m/z = 499 $(M+1)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) d 4.02 (4H, t, J = 8 Hz), 3.87 (2H, s), 5.98 (2H, s), 7.1-7.75 (11H, m), 9.11 (2H, d, J = 8 Hz), 11.1 (1H, s). Compound **28**: FAB-MS m/z = 585 $(M+1)^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) d 2.0 (2H, s), 2.1 (2H, s), 2.2 (2H, ABX system, d, J = 11Hz), 2.36 (2H, ABX system, dd, J = 11,4 Hz), 3.25 (3H, s), 3.33 (3H, s), 3.82 (2H, s), 6.12 (2H, br. m), 7.02 (2H, m), 7.26 (3H, m), 7.42 (2H, t, J = 7 Hz), 7.58 (2H, t, J = 7 Hz), 7.66 (2H, d, J = 8 Hz), 9.09 (2H, J = 8 Hz), 11.1 (1H,s). Compound **30**: FAB-MS m/z = 545 $(M+1)^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) d 3.22 (1H, m), 3.75 (2H, s), 4.04 (2H, m), 4.88 (1H, d, J = 4 Hz, D_2O exch.), 5.38 (2H, d, J = 4 Hz, D_2O exch.), 6.08 (2H, d, J = 4 Hz), 7.04 (2H, m), 7.26 (3H, m), 7.34 (2H, t, J = 7 Hz), 7.5 (2H, t, J = 7 Hz), 7.68 (2H, d, J = 8 Hz), 9.08 (2H, d, J = 8 Hz), 11.05 (1H, s).
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