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Synthesis of Bisindolylmaleimide Macrocycles

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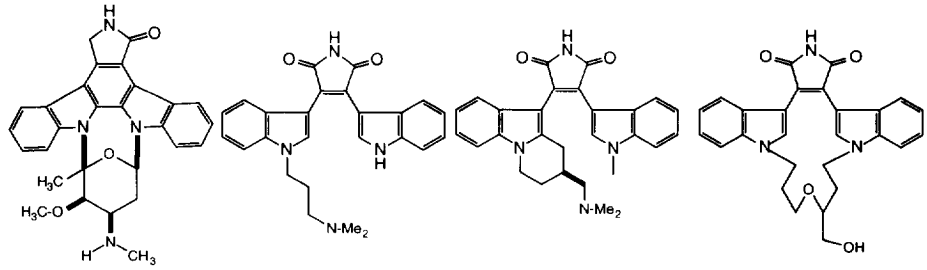
Abstract: The synthesis of a novel class of N-N'-macrocyclic bisindolylmaleimides is reported. The key step involves a remarkably efficient intramolecular cyclization reaction. The method was further developed to provide an efficient synthesis of this type of macrocycle through an intermolecular alkylation with subsequent intramolecular cyclization.

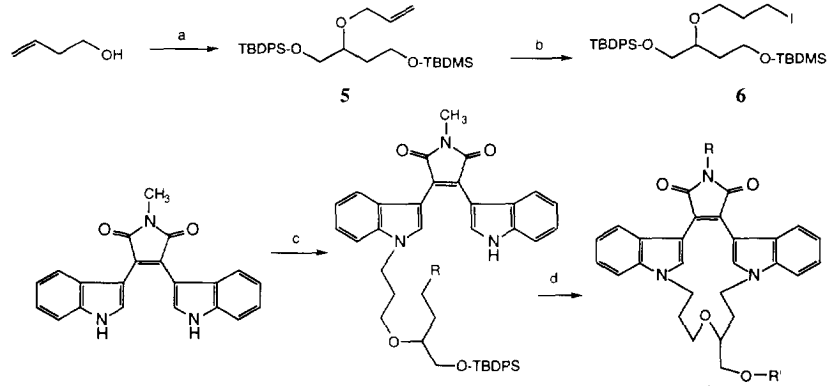
The remarkable potency that Staurosporine (**1**) has demonstrated as a protein kinase inhibitor has attracted considerable attention in both the synthetic and medicinal chemistry community.[1] Staurosporine has a limited selectivity *in vitro* for both ATP dependent kinase and the individual PKC isozymes. Therapeutically, an antagonist which posses both kinase selectivity for protein kinase C (PKC), in addition to PKC isozyme selectivity are potentially useful pharmacological agents.[2] The (bis)indolylmaleimide GF109203X (**2**) has been recognized as a PKC kinase selective agent.[3] Both **2** and conformational restricted analogs of **2**, *visa vie* Ro 32-0432 (**3**), are potent PKC kinase selective as well as PKC isozyme selective antagonists.[4] We would like to disclose our synthesis of a new structural type of (bis)indolylmaleimide which are N-N' bridged (bis)indolylmaleimide macrocycles, *visa vie* LY 320283 (**4**). These structural types of compounds are both kinase selective and PKC isozyme selective antagonists. The key step of our synthesis of these compounds involves a remarkably efficient macrocyclization reaction.



Staurosporine:1 **GF109203X: 2** **Ro 32-0432: 3** **LY 317644: 4**

We first needed to establish that this type of macrocycle could be efficiently formed through an intramolecular alkylation of the indole nitrogen. A convergent approach to the macrocycle was used in which a N-N' bridging component was synthesized and then alkylated in a stepwise sequence with 2,3-(bis)indolyl-N-methylmaleimide.[5] This method is illustrated for a 15-atom N-N' bridged macrocycle.

The selectively protected N-N' bridging component was prepared from 3-butenol (Scheme I). Protection of the primary hydroxyl as the TBDMS ether, oxidative 1,2-dihydroxylation of the alkene, selective protection of the primary hydroxyl as the TBDPS ether, and allylation the secondary alcohol using allyl trichloroacetimidate[6] gave alkene **5** in >90% yield from 3-butenol. Alkene **5** was useful as an intermediate leading to either the 15 atom bridged macrocycle or through oxidative degradation (*vide infra*) to the 14 atom bridged macrocycle. Oxidative hydroboration of alkene **5** followed by a Finklestein conversion produced differentially protected iodide **6** in 74% yield.

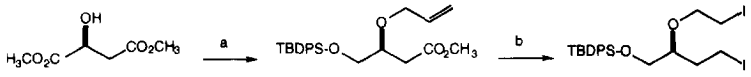


Scheme I: a. i. TBDMS-Cl, imidazole, CH_2Cl_2. ii. OsO_4, NMMO, acetone/water (9:1). iii. TBDPS-Cl, imidazole, CH_2Cl_2. iv. CH_2Cl_2, C_6H_{12}, $H_2C{=}CHCH_2OCNHCCl_3$. 91% overall yield, 0.25 mole scale. b. i. 9-BBN, THF, 0 °C/NaOH, H_2O_2. ii. Ms-Cl, Et_3N, Et_2O, 0 °C -> RT. iii. NaI, acetone, 0.3% $NaHCO_3$. 74% overall yield, 50 mmol scale. c i. 2 equivalents (bis)indolyl-N-methylmaleimide, **6**, DMF, Cs_2CO_3 (4 eq). ii. MeOH, 1% /mol TsOH (cat), 0 °C. iii. Ms-Cl, Et_3N, Et_2O, 0 °C -> RT. iv. NaI, acetone, 0.3% $NaHCO_3$. 39% overall yield, 50 mmol scale. d. i. DMF, Cs_2CO_3 (4 eq), 80 hr syringe pump addition (>95% yield). ii. 5N NaOH, EtOH 12 hr then 50 °C 3 hr, acid work-up. iii. HMDS (10 eq), MeOH (5 eq), DMF. 75% overall yield, 50 mmol scale.

Alkylation of 2,3-(bis)indolyl-N-methyl-maleimide **7** (2 equivalents) with iodide **6** gave the mono-alkylated product **8** in 59% yield, and the bis alkylated compound in 12% yield.[7] Selective removal of the TBDMS- group in **8**, followed by Finklestein conversion to the iodide gave **9** in 74% yield. Iodide **9** underwent remarkably clean macrocylization to produce (bis)indolyl-N-methylmaleimide macrocycle **10** in >95% isolated yield on slow addition to a DMF slurry of cesium carbonate.[8] The (bis)indolyl-N-methylmaleimide macrocycle **10** was hydrolyzed under basic conditions with loss of the TBDPS- group forming the anhydride during acidic work-up and subsequent conversion to the N-N' bridged-2,3-(bis)indolyl-N-H-maleimide **4** by HMDS/MeOH mediated maleimide formation in 77% yield.[9] We have found these cyclization conditions to be general for 13, 14, and 15 atom (bis)indolyl-N-methylmaleimide macrocycles.[10]

This class of compounds has also been prepared enantiomerically pure using an optically active four carbon atom precursor (Scheme II). Selective reduction[11] of dimethyl (R) or (S) malate ester gave the 1,2 diol. Protection of the primary alcohol followed by allylation of the secondary alcohol produced ester **11** in 45% yield. Alkene **11** could be modified for a stepwise alkylation of **7**, however we were also interested in the preparation of the dihalo compound **12**. Reduction of ester **11** to the

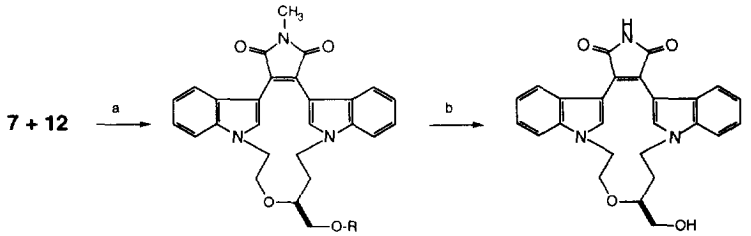
alcohol, followed by ozonolysis of the olefin with reductive work-up produced the diol which was converted to the diiodo compound **12** by a double Finklestein procedure in 64% overall yield.



11 **12**

Scheme II: a. i. BMS-$NaBH_4$, THF 0 °C. ii. TBDPS-Cl, imidazole, CH_2Cl_2. iii. CH_2Cl_2, C_6H_{12}, $H_2C{=}CHCH_2OCNHCCl_3$. 45% overall yield, 0.25 mole scale. b. i. DIBAL-H, THF, -78 -> -20 °C. ii. O_3, MeOH, -78 °C then $NaBH_4$ work-up. iii. Ms-Cl, Et_3N, Et_2O, 0 °C -> RT. iv. NaI, acetone, 0.3% $NaHCO_3$. 64% overall yield, 100 mmol scale.

Having demonstrated efficient intramolecular closure of the macrocycle, we then focused on combining the intermolecular and intramolecular alkylation steps. This method is illustrated for a 14-atom N-N' bridged macrocycle (Scheme III). Reaction of **7** and **12** under high dilution to a 65 °C DMF slurry of cesium carbonate (4 equivalents) gave the (bis)indolyl-N-methylmaleimide macrocyclization product as a mixture of alcohol **13** (42%) and silyl ether **14** (36%) in 78% overall yield. Partial loss of the TBDPS- protecting group during the reaction was inconsequential for our purpose since it is removed in the next synthetic step. Base hydrolysis followed by acidic work up and subsequent conversion to the N-H-maleimide produced (bis)indolyl-NH-maleimide macrocycle **15** in 63% overall yield. The (bis)alkylation reaction also appears to be a general method for the synthesis of 13, 14, and 15 atom (bis)indolyl-N-methylmaleimide macrocycles.



13 R = TBDPS
14 R = H

LY 326449: 15

Scheme III: a. **7** and **12** added together by syringe pump (72 hr), DMF, Cs_2CO_3 (4 eq), 60 °C. 78% combined yield of **13** and **14**, 100 mmol scale. b. i. **13** and **14**, 5N NaOH, EtOH 12 hr then 50 °C 3 hr, acid work-up. ii. HMDS (10 eq), MeOH (5 eq), DMF. 63% overall yield, 100 mmol scale.

The macrocycles prepared using this methodology are PKC kinase selective antagonists. The PKA/PKC IC_{50} ratio as a measure of kinase selectivity for **4** and **15** was >10,000. This class of compounds is also PKC isozyme selective. The PKCα/PKCβII IC_{50} ratio as a measure of isozyme selectivity for **4** and **15** was 25 and 20 against the human cloned isozymes. The IC_{50} value against PKCβII was 270 nM (± 24 nM, n=3 SD) for **4** and 32 nM (± 2 nM, n=2) for **15**. The IC_{50} values of **4** and **15** against rat brain PKC, which contains at least four isozymes (α, βI, βII, γ,), was 6.4 μM (± 0.4 μM, n=2) and 0.55 μM (± 0.17 μM, n=2)respectively. We will report the biological activity and an extensive structure activity relationship study of this new macrocycle class of isozyme and kinase selective PKC inhibitors in due course.[12]

Acknowledgment: We would like to thank the Physical Chemistry Department at Lilly Research Laboratories (LRL) for collecting analytical data and the LRL custom synthesis laboratory personnel Elizabeth Aaron and Gib Staten for the preparation of some intermediates.

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6. Wessel, H.-P.; Iversen, T.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2247. Clibe, L. A.; Overman, L. E. *Org. Syn.* **1978**, *58*, 4. Investigation of a large number of basic alkylation conditions and reagents did not give a satisfactory result.

7. All compounds gave appropriate physical spectra data [IR, NMR (^{1}H and ^{13}C), LRMS(FD) and HRMS(EI) or elemental analysis and α_D where appropriate], the chiral compounds were also confirmed by conversion to the Mosher ester and subsequent ^{19}F-NMR analysis, selected spectral data is given:
4: ^{1}H-NMR(d_6-DMSO): 2.1 (m, 4H), 2.4 (m, 2H), 3.28 (br m, 2H), 3.4 (m, 1H), 4.25 (m, 4H), 4.5 (t, J=6 Hz, 1H), 7.0-7.9 (m, 10H), 11.0 (s, 1H); ^{13}C-NMR(d6-DMSO): 20.9, 28.9, 30.3, 30.9, 34.3, 40.2, 41.6, 42.4, 62.4, 65.9, 78.1, 104.0, 104.1, 110.0 110.1, 119.6, 119.7, 121.4, 121.8, 24.8, 126.5, 126.6, 127.9, 131.5, 131.6, 131.7, 135.8, 135.9, 139.1, 151.4, 172.2; combustion analysis (theory): C 71.05 (71.19), H 5.43 (5.53), N 9.02 (9.23), O 14.12 (14.05); IR:(KBr): 2948.6, 1720.7 (vs), 1545.2, 1468.0, 1391.8, 1327.2, 1188.3, 1049.4, 744.6 (vs); MP:>280 °C.
15: ^{1}H NMR (d_6-DMSO): 1.6 (m, 1H); 2.05 (m, 1H); 3.4 (m, 1H); 3.46 (m, 1H); 3.6 (m, 1H); 3.85 (m, 1H); 4.1-4.4 (m, 5H); 4.65 (t, 1H); 7.07 (t, 2H); 7.15 (t, 2H); 7.4 (s, 1H); 7.42 (d, 1H); 7.48 (s, 1H); 7.5 (d, 1H); 7.75 (d, 1H); 7.8 (d, 1H); 10.9 (s, 1H). MS: MW = 441.50; observed 441 (FD, $CHCl_3$). IR: 3446, 2931, 1703, 1470, 1288, 743 cm^{-1}. $[a]_D$ -11.26 (c 1.0, MeOH) at 25° C.

8. The use of Cs_2CO_3 in DMF to effect cyclization in dithiacycloalkanes is known: Meier, H.; Dai, Y. *Tetrahedron Lett.* **1993**, *34*, 5277. We experienced remarkably lower yields of cyclized product using other bases or solvents.

9. Davis, P. D.; Bit, R. A. *Tetrahedron Lett.* **1990**, *31*, 5201.

10. We have noticed that ring closure is more facile in the 15 atom macrocycle, the order of ease of closure observed was 15>14>13. In a different 15 atom system similar to **4**, using slow reverse addition conditions, cyclization occurred at room temperature in >90% yield while the 13 atom homologue required a 100 °C reaction temperature and cyclized in 60% yield.

11. Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T; Nomizu, S; Moriwake, T.*Chem. Lett.* **1984**, 1389. In our hands about 10% of the 1,3 reduction product was also obtained, and was separated by chromatography after protection of the primary alcohol as the TBDPS- ether.

12. The assay method will be reported in detail : Jirousek,M. R.; Gillig, J. R.; Heath, W. F.; Johnston, C. M.; McDonald III, J. H. Neel, D. A.; Rito, C. J.; Stramm, L. E.; Melikian-Badalian, A.; Baevsky, M.; Ballas, L.M.; Hall, S. E.; Faul, M. M. Winneroski, L. "LY333531: A Potent and Isozyme Selective Inhibitor of Protein Kinase C. Cellular, Kinase, PKC Isozyme Activity." Manuscript in preperation. Briefly: PKC Enzyme Assay. PKC enzymes = alpha, beta I, beta II, gamma, delta, epsilon, eta and zeta. Assay components in a total volume of 250 µL are as follows: Vesicles consisting of 120 µg/mL phosphatidylserine (Avanti Polar Lipids) and sufficient diacylglycerol (Avanti Polar Lipids) to activate the enzyme to maximum activity in 20 mM HEPES buffer (Sigma, St. Louis, Missouri), pH 7.5, 940 µM calcium chloride (Sigma, St. Louis, Missouri) for assaying the alpha, beta-1, beta-2 and gamma enzyme only, 1 mM EGTA for all the enzymes, 10 mM magnesium chloride (Sigma, St. Louis, Missouri) and 30 µM (gamma-32P) ATP (DuPont). For all the enzymes either histone type HL (Worthington) or myelin basic protein is used as substrate. The assay is started by addition of protein kinase C enzyme incubated at 30°C for 10 minutes and stopped by adding 0.5 mL of cold trichloroacetic acid (Amresco) followed by 100 µL of 1 mg/mL bovine serum albumin (Sigma, St. Louis, Missouri). The precipitate is collected by vacuum filtration on glass fiber filters employing a TOMTEC™ filtration system and quantified by counting in a beta scintillation counter.

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