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Construction of a kinase inhibitor library via parallel synthesis

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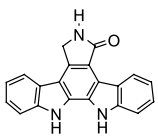
Abstract—A modified core structure of indolo[2,3-*a*]carbazole template has been immobilized. EtMgBr was identified as a substrate compatible base providing solid-phase dianion formation. Depending upon the nature of the electrophile, the core structure has been selectively functionalized to generate a library of kinase inhibitors.

Indolo[2,3-*a*]carbazole (**1**) and the structurally related bisindolylmaleimide (**2**) constitute core structures of several natural[1] and natural product-like compounds.[2] Some of them are well-known protein kinase inhibitors,[3] a property which affords these molecules therapeutic potential in a wide variety of diseases including cancer.[4] However, lack of specificity[5] of these molecules among kinases has remained a liability. With the emergence of natural product based combinatorial synthesis in recent years,[6] we envisioned that parallel synthesis could be a viable approach to generate potent and specific kinase inhibitors from this class of molecules. Logical structural diversification could generate compounds with enhanced kinase specificity presumably via additional interaction with non-conserved regions proximal to the ATP site of kinases.[7]



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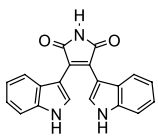

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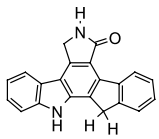

3

Our starting template was the indenopyrrolocarbazole **3**, where one of the indole nitrogens of indolo[2,3-*a*]carbazole (**1**) has been replaced with a methylene.[8] Herein, we report the immobilization of **3** and its elaboration via carbon–carbon and/or carbon–nitrogen bond forming chemistries at the indene and indole NH positions, respectively. Our objective was to generate an array of potential kinase inhibitors related to this scaffold.

The lactam nitrogen in **3** represents a suitable resin attachment point. After some experimentation, Rink acid resin[9] was found to afford efficient immobilization of **3**. We have devised conditions under which the lactam nitrogen can be selectively tethered without complicating reactions at the indole nitrogen. Thus, the reaction of Rink acid resin (**4**, ~0.5–6 mmol/gm loading) with **3** (2–3 equivalent) in a solvent mixture of benzene:dimethylformamide (DMF) or preferably using benzene:*N*-methylpyrrolidinone (NMP), along with an equivalent of *p*-TsOH at reflux temperature gave the immobilized product resin **5** (Scheme 1). The advantages of the use of Rink acid resin are that: (a) it can be removed with 1% trifluoroacetic acid (TFA) in dichloromethane. Facile resin detachment allows monitoring of the reaction progress[10] by mass spectrometry or by TLC/HPLC; (b) it also offers an advantage over a corresponding non resin-lactam protecting group like 4,4′-dimethoxydiphenylmethyl group for **3** using solution chemistry. A major side product (**7b**, Scheme 1) arises in solution phase chemistry during the deprotection step, due to the migration of the departing carbocation (generated from the 4,4′-dimethoxy diphenylmethyl group) to an activated indole aromatic position. However, in resin bound reactions, side product (**7a**), if formed, would remain attached to the resin.

Treatment of the resin **5** with a number of bases under various reaction conditions and subsequent treatment with an electrophile resulted in complex mixtures of products.[11] After considerable experimentation, it was found that EtMgBr serves as a suitable base for **5** (Scheme 1) to generate a dianion which unlike other bases tried, reacted with a variety of electrophiles to provide the desired product(s). Upon treatment with an

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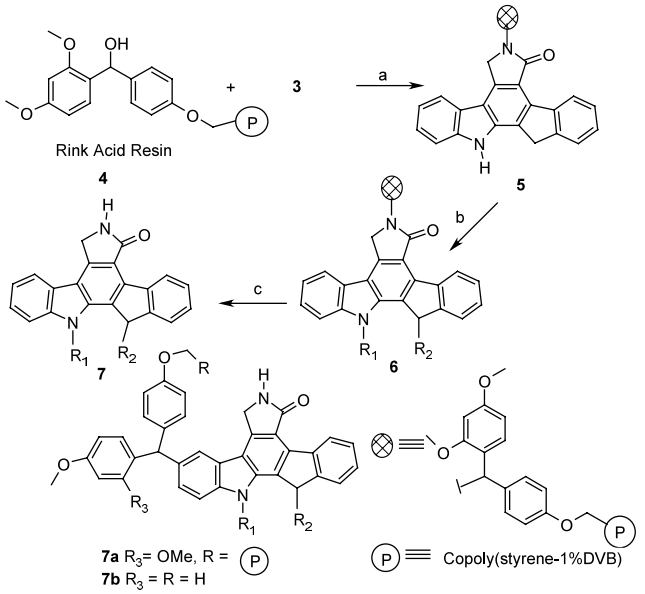


Scheme 1. *Reagents and conditions*: (a) TsOH (1 equiv.), benzene–NMP, reflux; (b) (i) EtMgBr (>10 equiv.), THF, 45 min, (ii) HMPA (HMPA:THF, 1:1), Halo-electrophile (>15 equiv.), 3 h at rt and at 70°C, overnight, (iii) quenching with dil. NH_4Cl solution, resin washing; (c) 1% TFA in dichloromethane, rt, 1 h.

excess of EtMgBr, a suspension of **5** turns red in color, thereby serving as an indicator of dianion formation, and this coloration disappears upon the addition of an electrophile. These reactions are generally clean, the only major impurities following release from resin, are the starting material (**3**) and in some instances an ethyl transfer product (**7**, R_1=H, R_2=ethyl), which arises due to transfer of the ethyl group from EtMgBr to the indene position of **5**, presumably via a free radical mechanism. The dianion of **5** must be generated under an inert atmosphere.

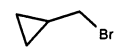
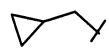
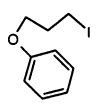
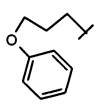
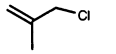
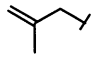
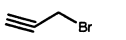
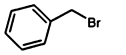
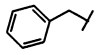
Table 1 records the reaction of the dianion of **5** with different halides (only representative examples shown). Use of hexamethylphosphoramide (HMPA) was found to be required for efficient alkylation. Under low HMPA concentrations (THF:HMPA, 4:1) and at room temperature, the dianion generated from **5** upon reaction with an alkyl halide provided a mixture of monoalkylated (alkylation at *N* or *C*); bis-alkylated (*N*,*C*- and/or *C*,*C*-bis-alkylated) products along with unreacted starting material. After some optimization, it was found that by increasing the amount of HMPA in the reaction medium and by elevating the reaction temperature (70°C), it was possible to obtain predominant formation of *N*,*C*-bis-alkylated compounds. A variety of alkyl, allyl, propargyl, and benzyl halides have been used as electrophiles. The products were obtained in good to excellent chemical yields (60–99%) with a purity range of 70–90%, based upon the HPLC analysis.

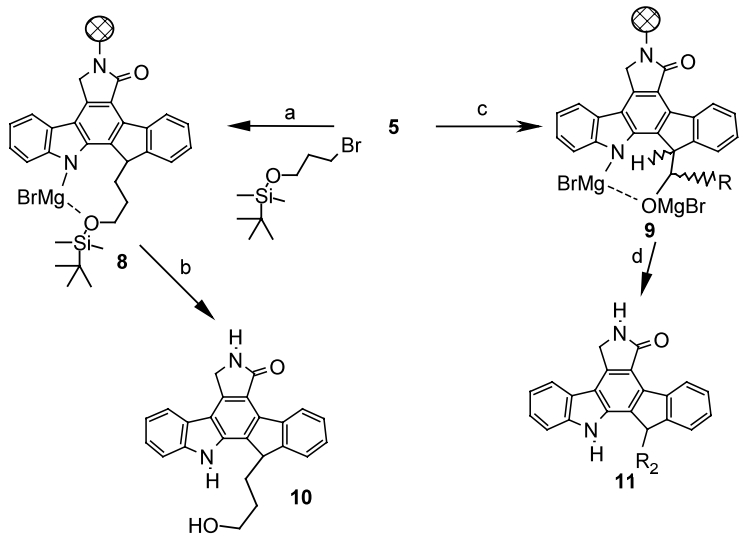
An exception was observed in the case of 3-bromo-1-*t*-butyldimethylsilyloxypropane (Scheme 2) which resulted in only monoalkylation at the indene position. The origin of such substrate dependent monoalkylation is not clear. We postulate that magnesium mediated chelate (**8**) formation,[12] as shown in Scheme 2, prevents alkylation at the indole nitrogen in this example, resulting in formation of monoalkylation product **10**.

Support for this postulation comes from the reactions of electrophiles such as aldehydes (Table 2, only representative examples with different electrophiles shown) with the dianion **5** which resulted in the monoalkylated product **11** presumably via such chelation[13] (intermediate **9**), as shown in Scheme 2. Unlike halo-electrophiles, fairly clean reactions were observed with aldehydes proceeding at room temperature and under low HMPA conditions (THF:HMPA, 4:1). Even though both of the added reagents (EtMgBr and aldehyde) present in the medium react vigorously with each other, the outcome of the solid-phase carbanion reactions were not effected as (a) the aldehyde/ketone were added last and (b) these reagents were added in higher molar equivalents than EtMgBr.

The products (**11**, Table 2), obtained following cleavage from the resin, were found to consist of a mixture of diastereomers of varying ratios and in these cases (acetaldehyde versus propargyl aldehyde, Table 2 entries 1 and 2) reversal of facial selectivities was also observed.[14] Ketones like ethyl pyruvate and chloroacetone also reacted in analogous fashion to provide the tertiary alcohols as diastereomeric mixtures (Table 2, entries 3 and 4). The presence of ester group and 1,2-halo alcohol products (entries 3 and 4, Table 2) attest to the mildness of these reaction conditions. Similarly, propylene oxide and trimethylene oxide also reacted smoothly to afford the desired products (Table 2, entries 5 and 6). In the case of the epoxide, a mixture of diastereomers with some facial bias was also observed.

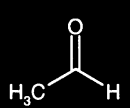
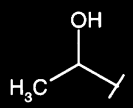
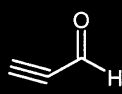
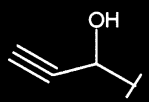
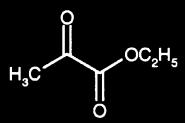
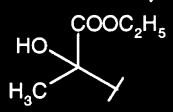
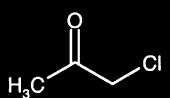
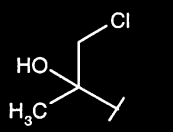
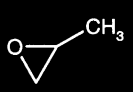
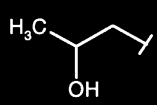
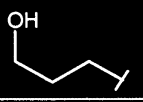
Table 1. Reactions of alkyl, aryl, allyl and propargyl halides with the dianion of **5**

Entry	Electrophile	Product **7** R_1 & R_2	Yield[a] %	Purity[b]	MS[c]
1			99	77	419
2			90	81	579
3			96	80	419
4			99	85	387
5			87	87	490



Scheme 2. *Reagents and conditions*: (a) (i) EtMgBr (>10 equiv.), THF, 45 min, (ii) HMPA (HMPA:THF, 4:1), 3-bromo-1-*t*-butyldimethylsilyloxypropane (>15 equiv.), 3 h at rt and at 70°C, overnight; (b) 1% TFA in dichloromethane, rt, overnight; (c) (i) EtMgBr (>10 equiv.), THF, 45 min, (ii) HMPA (HMPA:THF, 1:4), aldehyde (>15 equiv.), rt, 3 h–overnight; (d) (i) quenching with dil. NH_4Cl solution, resin washing, (ii) 1% TFA in dichloromethane, rt, 1 h.

Table 2. Reactions of aldehydes and other electrophiles[a] with ethylmagnesium bromide mediated anions of **5**

Entry	Electrophile	Product **11** R_2	Purity[b]	%Yield[c]	M+H (amu)	Diastereomeric Ratio[d]
1			>95	61	355	74:26
2			86	63	365	17:83
3[e]			85	56	427	77:23
4			>85	70	403,405	~55:45
5			85	98	369	~20:80
6[f]			>90	65	369	-

[a] Representative examples are shown above. [b] Yields were determined on the basis of initial resin loading. [c] Purity was determined from reverse phase HPLC. [d] Diastereomeric ratios and sense of facial selectivities were estimated and correlated from ^{1}H-NMR and HPLC. Also see ref. 13. [e] This reaction was carried out at –40°C. [f] The product from this reaction was identical to the product (**10**) obtained from alkylation reaction of **5** with 3-iodo-1-*t*-butyldimethylsilyloxypropane (Scheme 2).

The origin of facial selectivities observed in cases of product mixture **11** derived from aldehydes, unsymmetrical ketones and epoxide is difficult to explain. Possibly stereoselective product formation is due to epimerization of the indene hydrogen of the initial adducts to form the thermodynamically more stable isomer. However, other possibilities to rationalize facial selectivities cannot be ruled out. By using these solid-phase synthetic protocols, we have synthesized an array of compounds with diverse molecular architecture attached to the indenopyrrolocarbazole scaffold **3** to form a chemical library of kinase inhibitors.[15] The kinase inhibition data for those compounds would be published in due course.

In conclusion, we have demonstrated that the scaffold **3** can be immobilized on rink acid resin with a facile removal technique. Complete new structural segments can be incorporated at indene and/or indole nitrogen positions via EtMgBr-mediated anion chemistry in the solid-phase affording a library of potential kinase inhibitors.

Acknowledgements

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7. Such additional binding possibilities have been postulated: recent modeling has shown the presence of a hydrophobic pocket adjacent to the ATP binding site in protein kinase A (PKA) active site, and as suggested by authors, could be exploited by inhibitors from various chemical classes for additional binding affinity and selectivity. Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S. E.; Plé, P. A.; Lohmann, J.-J. M.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Lambert-van der Brempt, C. *J. Med. Chem.* **1999**, *42*, 5369.
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9. (a) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787; (b) The Rink acid resin was purchased from Novabiochem.
10. A small amount of resin can be taken out from the reaction mixture and can be filtered through a glass pipet containing a cotton plug. After washing and discarding the solvent, the resin can be further washed with a small amount of 1% TFA in dichloromethane, the washings can be analyzed by mass spectroscopy or by TLC/HPLC.
11. The initial reactions were tried on a resin bound mixture of **3** and the lactam regioisomer (5-oxo) compounds. Bases surveyed: NaH, KH, BuLi, LDA, Li/Na/K-hexamethyldisilazides, K-O*t*Bu, etc. Alkylation reactions gave complex mixture of products with these bases.
12. Molecular scaffold-imposed proximity of the coordinating groups might be partially responsible for such chelation.
13. Our initial thinking was that the aldehydes and ketones might be forming hemi-aminal/ketal type linkages with the indole nitrogen, which would fragment upon acidic resin cleavage, providing monosubstituted product mixture **11**. However, extremely mild resin cleavage condition, and no bis substituted products with epoxide and oxetane, respectively, favors our proposed chelation mechanism.
14. Diastereomeric ratios were determined with ^{1}H NMR and HPLC. The relative stereochemistries of the diastereomers have not been proved.
15. Spectroscopic and chromatographic data for representative compounds: Compound **7** ($R_1=R_2=CH_2CCH$): ^{1}H NMR (DMSO-d_6): δ 9.46 (d, $J=7$ Hz, 1H), 8.67 (s, 1H, NH), 8.03 (d, $J=8$ Hz, 1H), 7.81 (d, $J=7$ Hz, 1H), 7.61 (t, $J=8$ Hz, 1H), 7.36–7.45 (m, 2H), 7.25–7.23 (m, 1H), 7.18–7.14 (m, 1H), 5.72 (dd, $J=2$, 15 Hz, 1H), 5.28 (dd, $J=1$, 15 Hz, 1H), 4.95 (s, 2H), 4.93 (m, 1H), 3.35 (m, 1H), 2.86 (dd, $J=7$, 15 Hz, 1H), 2.54 (t, $J=2$ Hz, 1H), 2.51 (s, 1H). MS: 387 (M+H). HPLC (Zorbax Rx C8, analytical column, CH_3CN–H_2O 10–100% (containing 0.1% TFA) over 40 min at 1.2 mL/min) retention time = 28.4 min. IR (neat): 2120, 2240 cm^{-1} acetylenic st.
Compound **11** (R_2 = MeCH(OH)-): ^{1}H NMR (DMSO-d_6): δ 11.75 (s, 0.25H), 10.97 (s, 0.75H), 9.36 (d, $J=7.55$ Hz, 1H), 8.52 (s, 1H), 7.97–7.21 (series of m, 7H), 6.15 (d, $J=2.35$ Hz, 0.75H), 5.20 (d, $J=2.35$ Hz, 0.25H), 4.9–4.79 (overlapping m and s, 3H), 4.52 (d, $J=4.39$ Hz, 0.25H), 4.45 (d, $J=2.92$ Hz, 0.75 Hz), 0.48 (d, $J=6.14$ Hz, 2.25H), 0.29 (d, $J=6.08$ Hz, 0.75H). MS: 355 (M+H), HPLC (Zorbax Rx C8, analytical column, CH_3CN–H_2O 10–100% (containing 0.1% TFA) over 40 min at 1.2 mL/min) retention times = 21.97 min (26%) and 25.81 min (74%), respectively.