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Synthesis of new heteroaryldi(diindolyl)methanes: Colorimetric detection of DNA by di(diindolylmethyl)carbazoles

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Abstract. We have synthesized di(diindolylmethyl)carbazoles and di(diindolylmethyl)pyrroles by the reaction of substituted indoles with the corresponding carbazole and pyrroledicarboxaldehydes by employing a new catalyst $PPh_3.CF_3SO_3H$. We have also demonstrated the utility of di(diindolylmethyl) carbazole derivatives for the colourimetric and fluorometric detection of DNA.

Keywords. Di(diindolylmethyl)carbazoles; di(diindolylmethyl)pyrroles, triindolylmethane; UV-Vis titrations; fluorometric titrations.

1. Introduction

Biosensor technologies that focus on the direct detection of nucleic acids are currently an area of interest as they play a major role in clinical, forensic, and pharmaceutical applications.[1,2] The molecular probes that cause an increase in both absorbance and emission intensity by association with the host biomacromolecules (e.g. DNA, RNA, and proteins) are very useful photoluminescent markers in genomics and proteomics.[3] These simple and straightforward spectroscopic methods are especially advantageous because small organic dyes absorb and emit at wavelengths that do not interfere with the absorption of the DNA bases ($\lambda_{max} \sim 260$ nm). Indeed, spectrophotometric and spectrofluorimetric titrations are direct methodologies that indicate the association of a specific dye with DNA.[4] The design of new small molecular organic fluorophores is not a simple task.[5]

Carbazole[6], indole[7] and pyrrole[8] fragments are featured widely in a variety of pharmacologically and biologically active compounds. *Bis*(indolyl) alkanes and their derivatives constitute an important group of bioactive metabolites of terrestrial and marine origin[9] and hence there is a great deal of synthetic interest of these compounds. Although the extensive work have done on the simple alkyl and aryl diindolylmethanes[10], the reports on the synthesis of heteroaryl diindolylmethanes are very rare. In particular there were no reports on the synthesis of pyrrole diindolylmethanes to the best of our knowledge. Earlier attempt to the synthesis of diindolyl(pyrrolyl) methane by the reaction of pyrrole-2-carboxaldehyde with indole in presence of M.K-10 clay catalyst leads to triindolylmethane instead of diindolylmethane.[11]

2. Experimental

2.1 *Materials and reagents*

All the products obtained were purified by column chromatography using neutral silica gel (100–200 mesh). Hexane was used as a co-eluent. ^1H, ^{13}C and ^{31}P NMR were recorded in 400 MHz spectrometer. The chemical shifts are reported in ppm downfield to TMS ($\delta = 0$) for ^1H NMR and relative to the central DMSO resonance ($\delta = 40{\cdot}15$ ppm) for ^{13}C NMR. Mass spectral data was obtained from LC-MS. IR spectra were recorded on a FT–IR spectrometer using KBr pellets. Elemental analysis was carried out in CHN analyzer. Melting points are uncorrected. CT-DNA was purchased from Aldrich and it was sonicated to dissolve completely in 5 mM Tris, 50 mM NaCl, pH = 7·1. Di(diindolylmehyl)carbazoles were dissolved in DMSO and used for DNA binding studies.

2.2 *Typical procedure for the preparation of* ***3a***

A mixture of 9-methyl-3,6-carbazoledicarboxaldehyde (0·25 g, 1·0 mmol), indole (0·48 g, 4·10 mmol) and

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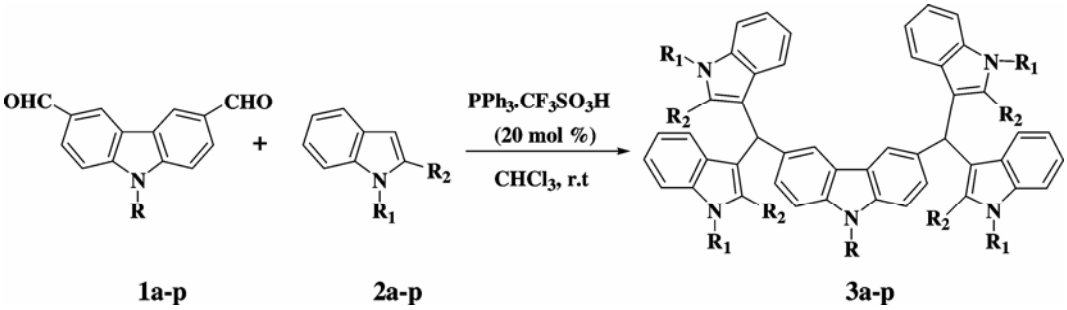


Scheme 1. Synthesis of di(diindolylmethyl)carbazoles.

Table 1. Synthesis of di(diindolylmethyl)carbazoles.

Entry	R	R_1	R_2	Product	Time (h)	Yield (%)[a]
a	CH_3	H	H	**3a**	1	75
b	C_2H_5	H	H	**3b**	1	71
c	*n*-C_4H_9	H	H	**3c**	1	77
d	CH_2Ph	H	H	**3d**	1	70
e	CH_3	CH_3	H	**3e**	1	78
f	C_2H_5	CH_3	H	**3f**	1	80
g	*n*-C_4H_9	CH_3	H	**3g**	1	81
h	CH_2Ph	CH_3	H	**3h**	1	79
i	CH_3	H	CH_3	**3i**	3	76
j	C_2H_5	H	CH_3	**3j**	3	71
k	*n*-C_4H_9	H	CH_3	**3k**	3	73
l	CH_2Ph	H	CH_3	**3l**	3	69
m	CH_3	H	Ph	**3m**	12	64
n	C_2H_5	H	Ph	**3n**	12	61
o	*n*-C_4H_9	H	Ph	**3o**	12	60
p	CH_2Ph	H	Ph	**3p**	12	62

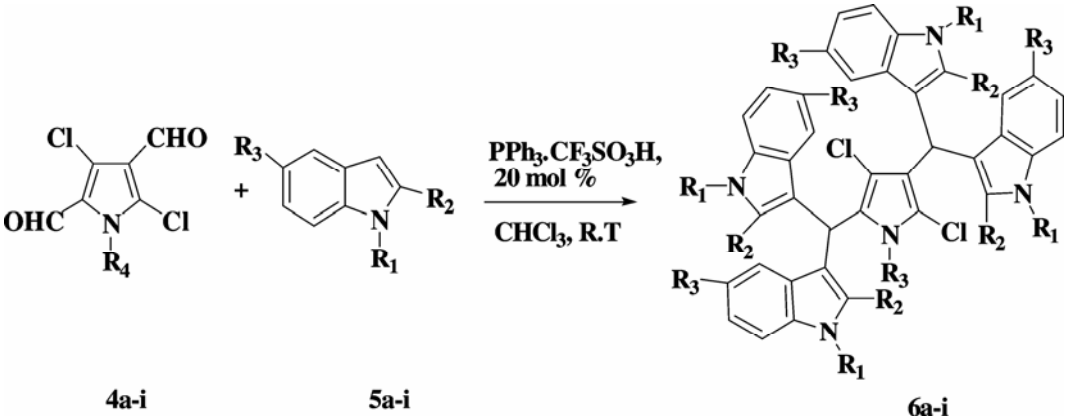
[a]Yields are reported after column purification of the compounds

TPP triflate (20 mol%) in chloroform (15 mL) was stirred at room temperature for one hour. After completion of the reaction (TLC), the solvent was evaporated under reduced pressure and the crude material was subjected to column chromatography (hexane: ethyl acetate = 85 : 15) to afford the pure product in 75% yield. m.p. 115–116°C; IR (KBr): 3404, 3043, 1607, 1485, 1413, 1228, 1083, 1158, 845, 790, 787, 683 cm^{-1}; ^1H NMR (400 MHz, TMS, DMSO-d_6) δ: 3·64 (3H, *s*, CH_3), 5·97 (2H, *s*), 6·78–6·92 (8H, *m*), 6·99–7·05 (4H, *m*), 7·27–7·34 (8H, *m*) 7·48 (4H, *s*), 8·06 (2H, *s*), 10·56 (4H, *s*, NH); ^{13}C NMR (100 MHz, TMS, DMSO-d_6) δ: 37·5, 65·4 (aliphatic C), 108·9, 111·9, 118·7, 119·5, 119·8, 121·3, 122·7, 124·3, 126·8, 127·3, 128·6, 135·7, 137·2, 138·9 (aromatic C); LC-MS: $m/z = 668$ ($M–H^+$), negative mode; Anal. Calcd. for $C_{47}H_{35}N_5$; C, 84·28; H, 5·27; N, 10·46% found: C, 84·41; H, 5·35; N, 10·22%.

3. Results and discussion

We report here the synthesis of di(diindolylmethyl)carbazole and pyrrole derivatives catalysed by a new catalyst $PPh_3.CF_3SO_3H$ (20 mol%). Di(diindolylmethyl)carbazoles **3a–3p** were synthesized in good yields by the reaction of 9-alkylcarbazole-3,6-dicarboxaldehydes **1a–1p** with various indole derivatives in the presence of a catalytic amount (20 mol%) of $PPh_3.CF_3SO_3H$ in $CHCl_3$ at room temperature (scheme 1). The results are summarized in table 1, clearly indicating the scope and generality of the reaction. The more reaction time was observed in the case of 2-phenylindole derivatives. This may be due to the steric hindrance and the electron withdrawing nature of the phenyl ring which renders the nucleophilicity of the indole.

As shown in scheme 2, di(diindolylmethyl)pyrrole derivatives were formed by treating with 3,5-



Scheme 2. Synthesis of di(diindolylmethyl)pyrroles.

Table 2. Synthesis of di(diindolylmethyl)pyrroles.

Entry	R_1	R_2	R_3	R_4	Product	Time	Yield (%)[b]
a	H	H	H	H	**6a**	2	74
b	CH_3	H	H	H	**6b**	2	72
c	H	CH_3	H	H	**6c**	3	70
d	H	Ph	H	H	**6d**	12	59
e	H	Ph	H	CH_3	**6e**	12	56
f	H	(4-Br)Ph	H	H	**6f**	12	61
g	H	(4-OMe)Ph	H	H	**6g**	12	58
h	CH_2Ph	H	H	H	**6h**	2	59
i	H	H	Br	H	**6i**	2	63

[b]Yields are reported after column purification of the compounds

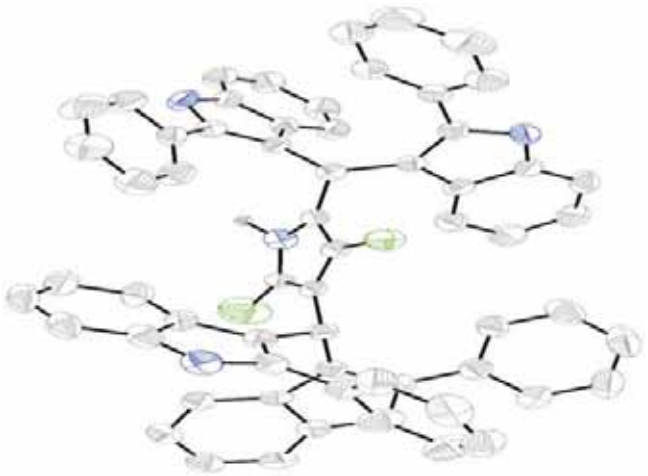


Figure 1. ORTEP diagram of **6e**. Hydrogen atoms are omitted for clarity.

dichloro-2,4-pyrroledicarboxaldehyde with various indole derivatives. The results are summarized in table 2. The formation of triindolylmethane (~20%) was observed with indole only. There was no formation of the corresponding triindolylmethane with other indole derivatives. The formation of triindolylmethane may be considered to proceed through the successive formation of the expected diindolylmethane and indoleninium species. The structure of **6e** was also confirmed further by the single crystal X-ray analysis (figure 1).[12]

The interaction of the compound **3d** in DMSO (40 μM), was investigated by titrating with CT-DNA in Tris buffer. As shown in figure 2, on addition of 7 μM of DNA, the absorption band centered at 489 nm is disappeared and a new band at higher energy was observed at 447 nm. The binding constant was deduced to be $1{\cdot}4 \times 10^5$ M^{-1} using nonlinear least-squares treatment of UV/Vis titrations. As can be expected from UV/vis data (figure 2), colour change occurs from orange to pale yellow colour of the dye **3d** by the addition of 10 μM of DNA (figure 3). Further addition of DNA (100 μM) caused the colour change of the solution from pale yellow to light reddish brown (figure 3) while in the UV-Visible spectrum (figure 2), the band at 447 nm dis-

appears and a new band appeares at 491 nm. This optical response may be due to the non-covalent interactions between DNA and di(diindolylmethyl) carbazole moiety. We have also carried out ^{1}H and

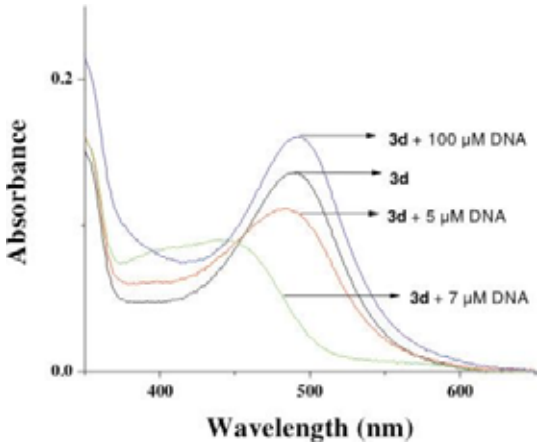


Figure 2. Family of UV-Vis spectra taken in the course of the titration of DMSO solution of **3d** (40 μM) with CT-DNA in Tris buffer.

Figure 3. Colour changes upon the addition of CT-DNA to the DMSO solution of **3d** (50 μM) from left to right: (**a**) **3d**, (**b**) **3d** + 10 μM of DNA, (**c**) **3d** + 100 μM of DNA.

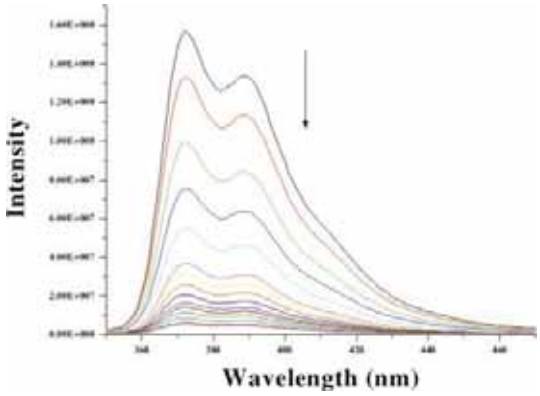


Figure 4. Fluoroscent titration curves for **3d** upon incremental additions of CT-DNA. DNA concentrations: 0, 2.5, 5, 7, 9.5, 15, 20, 25, 30, 40, 50 μM.

^{31}P NMR titrations of **3i** with DNA. All the protons of di(diindolylmethyl)carbazole moiety were moving to the upfield which indicates that the absence of hydrogen bonding and the presence of non-covalent interactions. In ^{31}P the singlet was splitted into four new peaks (see supplementary information).

Other di(diindolylmethyl)carbazole derivatives were also exhibiting similar behaviour like **3d**. On the addition of DNA aliquots to **3i** (40 μM), the peak at 514 nm disappears and a new peak at 407 nm appears in the UV-Vis spectrum which results the colour change of the dye from pink to pale yellow (see supplementary information). Further addition of DNA (100 μM), the peak at 407 nm disappears and a new peak at 512 nm evolved (see supplementary information). Again the colour changes from pale yellow to intensive pink colour (see supplementary information). The colour of **3p** changes from violet to pale yellow corresponding to the disappearance of the peak at 551 nm and the appearance of a new peak at 440 nm upon the addition of DNA aliquots. Again the colour changes from pale yellow to violet after addition of DNA (100 μM) and the peak at 440 nm disappeared and a new peak at 548 nm appeared (see supplementary information).

But di[di(1-methylindolylmethyl)]carbazole derivatives did not give any colour change after the addition of excess of DNA also. Absorbance of the peak at 498 nm decreased but there was no new peak was observed (see supplementary information). This clearly indicates that the NH protons were playing a major role for the optical response of the other di(diindolylmethyl)carbazoles.

All di(diindolylmethyl)carbazole derivatives are fluorescent. The fluorescent intensity of all derivatives was going to quench after the addition of DNA. As shown in figure 4, the intensity of characteristic emission maximum at 372 nm and 389 nm gradually decreases upon the incremental addition of DNA upon exciting at 280 nm. Di(diindolylmethyl) pyrroles could not give any colour change even after adding the excess of DNA also.

3. Conclusions

In conclusion, we have synthesized di(diindolylmethyl)carbazoles and pyrroles by employing a new catalyst $PPh_3.CF_3SO_3H$ and also demonstrated the utility of the di(diindolylmethyl)carbazole derivatives for the colourimetric and fluorometric detection of DNA.

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12. The CCDC deposition number of compound 6e is 653448; formula: $C_{71}H_{61}Cl_2N_5O_4$; unit cell parameters: a 16.6198(10), b 21.5543(13), c 17.8697(10), β 110.6790(10), space group P2(1)/n