Pyrimido[5,4-*c*]pyrrolo[2,1-*a*]isoquinoline: a new potential DNA-interactive ring system

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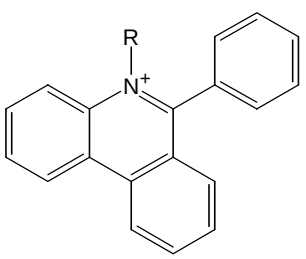
Dedicated to Professor Domenico Spinelli on the occasion of his 70th birthday
(received 29 Nov 02; accepted 14 Jan 03; published on the web 22 Jan 03)

Abstract
The acid catalyzed decomposition of the azide **9** failed to give the title compounds, which were however obtained by a Pschorr-type cyclization on reactive 1-(6-aminopyrimidin-5-yl)-pyrroles of type **13**. Derivatives of type **14** and **15** were fully characterized by NMR data. Theoretical calculations demonstrated that the new compounds possess properties suitable for DNA-intercalation.

Keywords: TAP, TFMSA-catalyzed decomposition of azides, Pschorr-type cyclization, pyrimido[5,4-*c*]pyrrolo[2,1-*a*]-isoquinoline

Introduction

Polycyclic heterocycles having a planar structure can be effective pharmacophore units of drugs endowed with anti-tumor activity because they can intercalate into double-stranded DNA. Phenanthridine- and acridine- derivatives of type **1** and **2** are well known compounds possessing such a property whose principal driving forces are stacking- and charge-transfer- interactions, as well as hydrogen bonding and electrostatic forces.[1] In particular, it has been demonstrated that the biological activity of acridine derivatives of type **2** (R=$NHSO_2Me$) correlates with their DNA association constants, the more active compounds being those that bind more tightly to DNA.[2]



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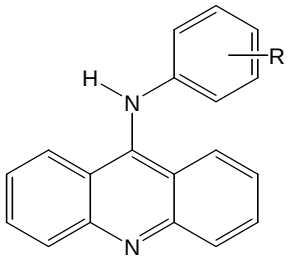

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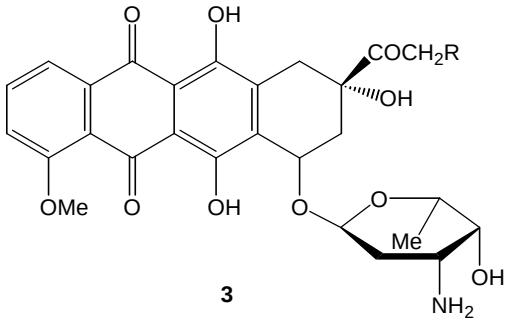

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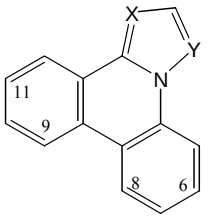
The ethidium derivatives **1** (R=Et) and anthracycline antitumor antibiotics **3** also bind tightly to DNA and show a strong specificity for guanine (G) and cytosine (C) residues.[3] Doxorubicin (**3**, R=OH), in particular, interacts with the 2-amino group of guanine.[4] In addition, compounds of types **2** and **3** target the topoisomerase II.[5]

For a long time, we have been interested in studying heterocycles annelated with either pyrrole or indole rings as potential DNA-interactive agents. In particular several azolo-phenanthridine derivatives of type **4** [pyrazolo (X=CR, Y=N); triazolo (X=Y=N); pyrrolo (X=Y=CR)] have been prepared [6–10] and some of them showed anti-proliferative activity in *in vitro* tests.[11] Moreover, some pyrrolo[1,2-*f*]phenanthridines, and in particular compound **5**, showed unique properties—being able not only to reduce the HIV-induced cytopathogenicity, but also to stimulate the growth of the same MT cells at lower concentrations.[12] Structure–activity relationship (SAR) studies in the class of pyrrolo[1,2-*f*]phenanthridines have shown that the presence of amino or methoxy groups on the 6- and/or 11- positions of the polycondensed system is relevant for the appearance of the biological activity.[11,12]



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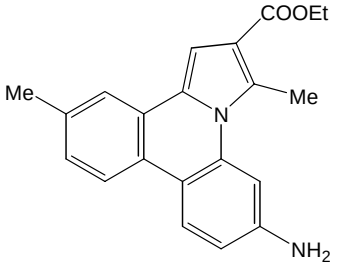

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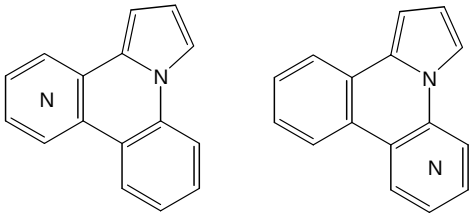
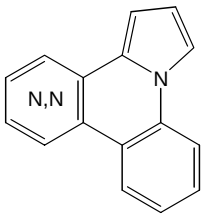
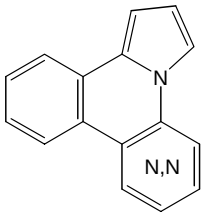
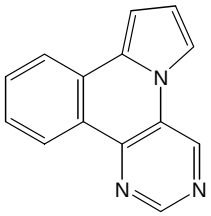


DAP





TAP



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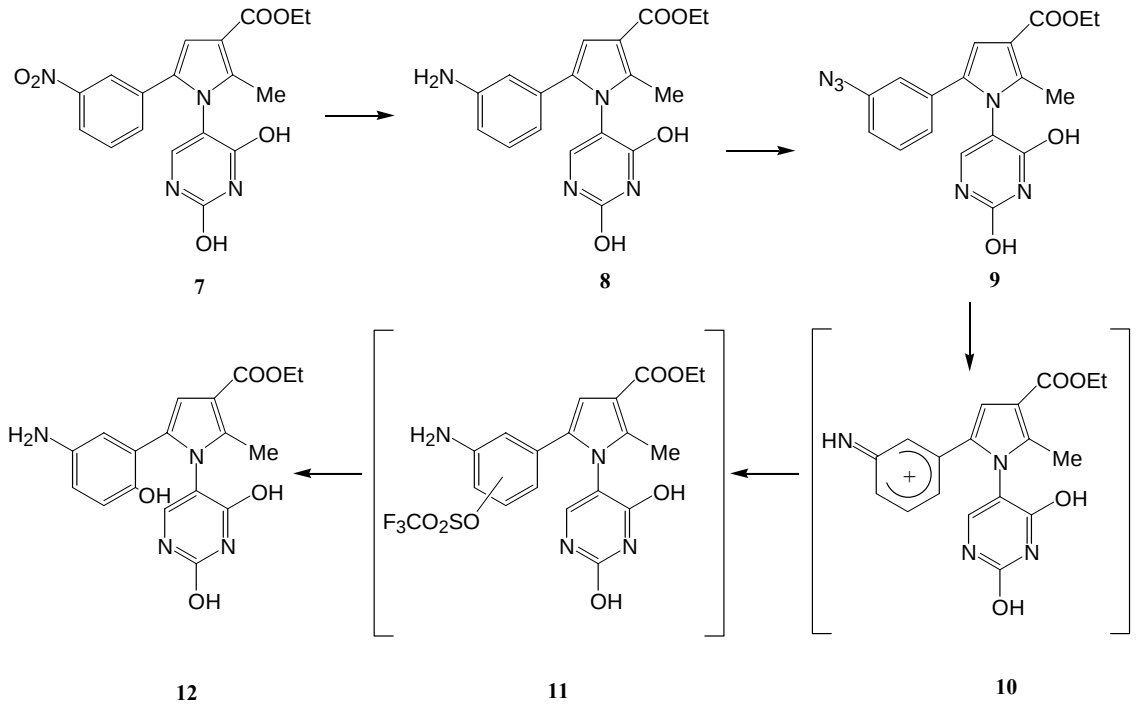
On the basis of these results, it was interesting to explore further this type of planar heterocycles by making isosteric modifications of the phenanthridine moiety. The introduction of one or two nitrogens could lead to several classes of hitherto unknown heterocyclic systems, annelated with the pyrrole ring, such as **D**i**A**za**P**henanthrenes (**DAP**) or **T**ri**A**za-**P**henanthrenes (**TAP).** In this paper, we report our approach to the new ring system TAP, namely pyrimido[5,4-*c*]pyrrolo[2,1-*a*]isoquinolines, of type **6**.

Results and Discussion

The synthetic approach to TAP could involve either the acid-catalyzed decomposition of (3-azidoaryl- or heteroaryl-)-pyrroles, or the Pschorr-type cyclization of (2-aminoaryl/heteroaryl)-pyrroles, according to the procedures already employed successfully for the synthesis of the pyrrolo[1,2-*f*]phenanthridines. These methods can also allow the functionalization of the phenanthrene rings with electron-donating groups suitable for the interaction with DNA.[2]

For this purpose we first utilized the method involving the acid catalyzed decomposition of 1-heteroaryl-2-(3-azidophenyl)-pyrroles. Ethyl 1-(2,4-dihydroxypyrimidin-5-yl)-2-methyl-5-(3-nitro-phenyl)-pyrrole-3-carboxylate (**7**) was prepared from a suitable 1,4-diketone and 5-aminouracil according to the procedure recently reported by us.[13] Reduction of the nitro group with hydrogen and Pd on charcoal afforded the amino derivative **8** in good yield. This last was diazotized with sodium nitrite in hydrochloric acid, then treated with sodium azide to give the corresponding 2-(3-azidophenyl)-pyrrole **9** (Scheme 1).

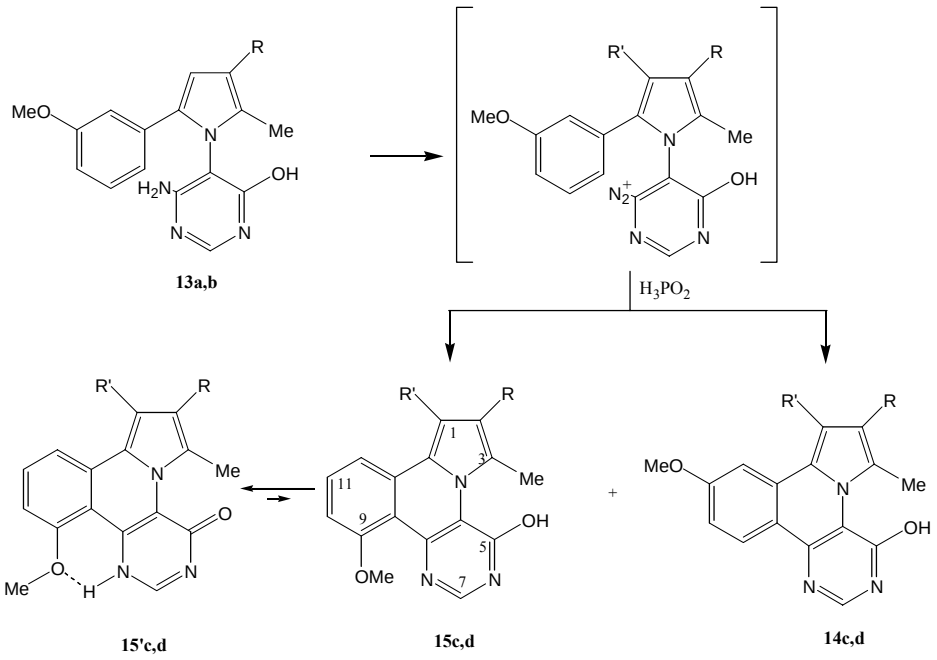


Scheme 1

The azide, dissolved in trifluoroacetic acid, was decomposed with an equivalent of trifluoromethane-sulfonic acid (TFMSA), from 0°C to room temperature. From the complex reaction mixture it was possible to isolate only the hydroxyphenyl derivative **12**, and no traces of a polycyclic product of type **6** could be isolated. In this acid catalyzed decomposition of the azide, the intermediate cation of type **10** is involved. Such an intermediate, being a π-carbocation, needs a sufficiently high electron density on the attacking substrates under the reaction conditions. We have already demonstrated that a phenyl group is not sufficient to bring about the cyclization reaction, whereas increasing yields of cyclized product were obtained by introducing an activated aryl or an electron rich pyrrole moiety.[14] Therefore we thought that the presence of the two hydroxyl groups could activate the pyrimidine moiety sufficiently to bring about the cyclization reaction. However the extensive protonation of the substrate reduced the amount of the reactive intermediate **10**. Therefore compound **12**, the only material isolable from the reaction mixture, was formed by competing intermolecular nucleophilic reactions with triflate, leading to the intermediate **11**, or by reacting directly with water, as observed in our previous reports.[9,10]

A different approach to the title compounds of type **6**, involving a Pschorr- type cyclization of 1-(6-aminopyrimidin-5-yl)-pyrroles of type **13**, was then undertaken. The amino derivatives **13a,b** were prepared in good yields from 1-(3-methoxyphenacyl)-1,4-pentanediones and 4,5-diamino-6-hydroxypyrimidine.[13]

The compounds **13a,b** were then diazotized in sulfuric acid with a large excess of sodium nitrite, followed by treatment with hypophosphorous acid (Scheme 2).



Scheme 2. a, R=H; **b**, R=Ac; **c**, R=H, R'=NO_2; **d**, R=Ac, R'=H.

From the complex reaction mixture, after column chromatography, it was possible to isolate two isomeric compounds identified as the 11- and 9-methoxy- substituted derivatives of types **14** and **15** respectively.

When amine **13a** was the starting material, because of the presence of a large excess of nitrite, nitrosation of the pyrrole β- position during the diazotization reaction, followed by oxidation, was observed. Such a behavior is not unusual with pyrrole substrates.[15] Since both the β- carbons were prone to substitution, the position of the nitro group, *ortho* to the phenyl group, was assigned by analogy with literature reports, taking into account the driving and/or activating effects of the substituents already present in the pyrrole moiety,[16] and on the basis of theoretical consideration of the ^{13}C- NMR carbon shifts (see below).

Diazotization of the amine **13a** afforded the cyclized products, **14c** and **15c,** only when the reaction was carried out in the presence of methanol, whereas in the case of amine **13b** the formation of the isomers **14d** and **15d** can be modulated by slight variation of the experimental conditions. When methanol is added to the reaction mixture to increase the poor solubility of the starting material, the diazotization reaction is faster and the less hindered isomer (11-methoxy, **14d**) is formed. In the absence of methanol the diazotization is slow and has to be carried out at 30°C; under these conditions the 9-methoxy isomer **15d** is formed preferentially. This product, although it is more constrained, once formed can be stabilized by intramolecular hydrogen bonding in the tautomeric form **15′**, which was also the only form present in solution, as evidenced by NMR spectral data.

The structures of derivatives **14c,d** and **15c,d** were assigned on the basis of the spectral data, in particular ^{1}H- and ^{13}C- NMR. From the proton NMR data it is possible to identify the two isomers **14** and **15**, deriving from cyclization on the ortho- and *para*- positions of the phenyl ring, by considering the different coupling patterns of the aromatic protons of the isoquinoline moiety. The position of the nitro group in derivatives of type **c** was determined on the basis of the chemical shift of the proton at C-2, which was found as a singlet at 6.87 and 6.51 respectively, and of the proton on C-12, shifted 0.3–0.5 ppm downfield by the nitro group, compared to the other derivatives of type **d**.

To assign all the resonances in the ^{13}C- NMR we evaluated the correlation between experimental and calculated chemical shifts. In particular, the structures of all the isomers **14** and **15** were fully optimized,[17] *in vacuo* and in $CDCl_3$, by SCF calculation with the semi-empirical PM3 method, which gave a prediction of the ^{13}C- NMR chemical shifts by the neural-net technique.[18] Table 1 reports the experimental and calculated values for the new TAP derivatives. Generally, we found a good linear correlation between the two sets of values, with $r^2 = 0.91–0.95$ in the case of derivatives **c** and **d** respectively. For the 9-methoxy- substituted isomers it was possible to establish the predominance of tautomer **15′**, in which C-5 was deshielded by up to 27 ppm compared with the chemical shift of the corresponding carbon atom in the 11-methoxy derivatives.

Table 1. ^{13}C- NMR data (δ_C, ppm) for pyrimido[5,4-*c*]pyrrolo[2,1-*a*]isoquinoline derivatives

	14c		14d		15'c		15'd	
	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
C-1	129.34	123.97	113.58	123.57	113.94	118.19	121.68	121.20
C-2	120.75	128.08	123.25	104.34	118.39	126.83	117.49	110.60
C-3	162.59	156.67	130.89	152.57	160.02	149.47	174.35	158.19
C-4a	129.36	125.13	128.80	124.27	119.49	118.54	136.85	131.85
C-5	185.77	162.98	159.97	162.90	192.13	170.37	187.27	168.98
C-7	129.52	160.74	129.86	159.73	130.26	160.88	123.54	131.69
C-8a	162.05	152.52	131.20	154.38	170.27	158.13	159.96	157.44
C-8b	121.97	118.96	128.84	126.66	127.84	122.93	117.49	115.34
C-9	116.64	125.98	116.27	127.33	159.68	147.48	159.96	157.70
C-10	114.28	115.66	109.10	108.39	111.05	113.47	113.53	118.24
C-11	162.61	158.06	157.94	157.69	123.59	129.83	129.83	160.11
C-12	102.51	112.56	105.40	105.95	97.89	111.59	121.65	119.62
C-12a	137.02	126.90	130.86	136.45	137.03	130.83	136.85	130.88
C-12b	171.49	160.49	151.52	156.30	170.28	158.44	159.60	151.59
CH_3	27.27	17.86	14.53	14.76	12.17	17.29	13.37	14.06
COCH_3			29.14	27.87			30.13	25.22
OCH_3	55.44	38.48	55.33	38.00	55.40	34.53	55.47	40.97
CO			194.06	211.17			191.35	212.61

To investigate the potential ability of derivatives of the new ring system, pyrimido[5,4-*c*]pyrrolo[2,1-*a*]isoquinoline, to interact with DNA, we utilized the structures optimized *in vacuo* to calculate the values of some molecular descriptors[17] [accessible surface area (ASA), ionization potential, LUMO and HOMO orbital energies]. Table 2 reports our findings on derivatives of type **14** and **15**, together with those of amsacrine [AMSA, **2**, (*R*=2-methoxy- 4-methanesulfonamido-)], and doxorubicin **3** (DOXO), chosen as reference compounds.

Table 2. Molecular descriptors for TAP and reference drugs

Compounds	ASA (Å^3)	Ionization Potential[a]	LUMO[a]	HOMO[a]
AMSA	396.95	8.486	-1.163	-8.486
DOXO	525.40	9.079	-1.566	-9.079
14c	303.33	9.247	-1.643	-9.247
15c	300.44	9.114	-1.563	-9.114
15'c	297.25	9.168	-1.638	-9.168
14d	322.85	8.809	-1.268	-8.809
15d	326.85	8.662	-1.326	-8.662
15'd	320.42	8.588	-1.303	-8.588

[a] eV

All the new derivatives have LUMO and HOMO energies in the range calculated for the known intercalators, and also all the other parameters are of the same order of magnitude as the active compounds. Therefore, it can be assumed that the new ring system can constitute a probable pharmacophore for potential DNA- interactive compounds.

In conclusion, in this paper we report the synthesis of the new ring system, pyrimido[5,4-*c*]pyrrolo[2,1-*a*]isoquinoline, through a Pschorr- type cyclization of suitable 1-(6-aminopyrimidin-5-yl)-pyrroles. The derivatives of this polycyclic heterocycle, TAP, that can be considered an isostere of the known intercalators belonging to the phenanthridine class, have shown to be potential DNA- interactive compounds in preliminary molecular modeling studies.

Experimental Section

General Procedures. All melting points were taken on a Buchi–Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H- and ^{13}C- NMR spectra were measured at 200- and 50.3 MHz respectively in $(CD_3)_2SO$ solution, unless otherwise specified, using a Bruker AC-E Series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230–400 Mesh ASTM or with Biotage FLASH40i chromatography module (prepacked cartridge system). For all new compounds, analyses were within ±0.4% of theoretical values.

Ethyl 5-(3-aminophenyl)-1-(2,4-dihydroxypyrimidin-5-yl)-2-methylpyrrole-3-carboxylate (8). A solution of **7** (2.4 mmol) in ethanol was reduced overnight with hydrogen over 10% Pd on charcoal in a Parr apparatus at 50 psi at room temperature. Removal of the catalyst and evaporation of the solvent under reduced pressure gave a solid that was collected and washed with dichloromethane. Yield 81%, white powder, m.p. 105°C: IR ν; 3673 (OH), 3562 (OH), 3353 and 3230 (NH_2) 1662 (CO) cm^{-1}: ^{1}H NMR δ; 1.27 (3H, t, J= 6.8 Hz, CH_3), 2.30 (3H, s, CH_3), 4.20 (2H, q, J= 6.8 Hz, CH_2), 5.14 (2H, bs, NH_2), 5.75 (1H, s, pyrrole H-4), 6.29 (1H, dt, J=7.8, 2.0 Hz, phenyl H-4) 6.43 (1H, s, pyrimidine H-6), 6.48 (1H, dt, J=7.8, 2.0 Hz, phenyl H-6), 6.95 (1H, t, J=7.8 Hz, phenyl H-5), 7.69 (1H, d, J= 2.0 Hz, phenyl H-2), 11.26 (1H, pyrimidine OH), 11.51 (1H, pyrimidine OH). ^{13}C NMR δ; 11.4 (q), 14.4 (q), 58.9 (t), 108.4 (d), 111.6 (s), 111.8 (s), 112.8 (d), 113.7 (d), 115.3 (d), 128.7 (d), 132.6 (s), 135.2 (s), 138.5 (s), 142.5 (d), 148.6 (s), 150.7 (s), 161.2 (s), 164.4 (s). (Found: C, 61.19; H, 5.12; N, 15.86%. $C_{18}H_{18}N_4O_4$ requires C, 61.01; H, 5.12; N, 15.81%).

Ethyl 5-(3-azidophenyl)-1-(2,4-dihydroxypyrimidin-5-yl)-2-methylpyrrole-3-carboxylate (9). To a suspension of **8** (2.4 mmol) in HCl (37%, 8.4 mmol) and water (2.5 ml), $NaNO_2$ (2.4 mmol) in water (3 ml) was added at 0–5°C. After 30 minutes sodium azide (4.8 mmol) in water (3 ml) was added dropwise; the reaction mixture was stirred for 6h, from 0°C to room temperature. The precipitate was filtered off and dried in the desiccator under vacuum. Yield 74%, yellow–orange powder, m.p. 185°C: IR ν; 3463 (OH), 3379 (OH), 2102 (N_3) 1686

(CO) cm^{-1}: ^1H NMR δ; 1.28 (3H, t, J=7.3 Hz, CH_3), 2.33 (3H, s, CH_3), 4.22 (2H, q, J= 7.3 Hz, CH_2), 6.65 (1H, s, pyrrole H-4), 6.93 (1H, s, pyrimidine H-6), 6.99 (1H, d, J= 1.5 Hz, phenyl H-2), 7.06 (1H, dt, J=7.3, 1.5 Hz, phenyl H-6), 7.39 (1H, t, J=7.3 Hz, phenyl H-5), 7.84 (1H, dt, J=7.3, 1.5 Hz, phenyl H-4), 11.40 (1H, pyrimidine OH), 11.50 (1H, pyrimidine OH): ^{13}C NMR δ; 11.5 (q), 14.4 (q), 59.0 (t), 109.5 (d), 111.4 (s), 112.1 (s), 117.8 (d), 117.9 (d), 124.4 (d), 130.1 (d), 133.2 (s), 133.6 (s), 139.4 (s), 139.5 (s), 143.1 (d), 150.6 (s), 161.3 (s), 164.2 (s). (Found: C, 56.67; H, 4.23; N, 22.03. $C_{18}H_{16}N_6O_4$ requires C, 56.84; H, 4.24; N, 22.03%).

Decomposition of ethyl 5-(3-azidophenyl)-1-(2,4-dihydroxypyrimidin-5-yl)-2methylpyrrole-3 -carboxylate (9). To a solution of azido derivative **9** (1.5 mmol) in trifluoroacetic acid (2 ml), trifluoromethanesulfonic acid (TFMSA) (0.13 ml, 1.45 mmol) was added dropwise at 0°C. The reaction mixture was stirred for 6 days at room temperature. After neutralization with Na_2CO_3, the solution was extracted with ethyl acetate; the combined organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography using dichloromethane/methanol 95/5 as eluent. The only product isolated was ethyl 5-(3-amino-6-hydroxyphenyl)-1-(2,4-dihydroxypyrimidin-5-yl)-2-methylpyrrole-3-carboxylate (**12**). Yield 25%, brown powder, m.p. >290°C: IR ν; 3560–3243 (OH and NH_2) 1687 (CO) cm^{-1}: ^1H NMR δ; 1.27 (3H, t, J= 7.5 Hz, CH_3), 2.34 (3H, s, CH_3), 4.21 (2H, q, J= 7.5 Hz, CH_2), 5.57 (2H, bs, NH_2), 6.34 (1H, s, pyrrole H-4), 6.51 (1H, s, pyrimidine H-6), 6.57 (1H, dd, J=8.5, 3.2 Hz, phenyl H-4), 7.06 (1H, d, J=8.5 Hz, phenyl H-5), 7.69 (1H, d, J=3.2 Hz, phenyl H-2): ^{13}C NMR δ; 11.5 (q), 14.3 (q), 59.0 (t), 111.0 (s), 111.2 (d), 112.0 (s), 114.1 (d), 115.9 (d), 122.0 (d), 125.9 (s), 127.6 (s), 136.9 (s), 139.0 (s), 141.7 (d), 148.8 (s), 150.5 (s), 160.7 (s), 164.2 (s). (Found; C, 58.57; H, 4.92; N, 15.18. $C_{18}H_{18}N_4O_5$ requires C, 58.37; H, 4.90; N 15.13%).

Substituted 5-hydroxy-3-methyl-1-nitropyrimido[5,4-*c*]pyrrole[2,1-*a*]isoquinolines 14c, 15, (15')c. To a stirred solution of **13a** (3.4 mmol) in H_2SO_4 (50%, 17 mmol) and methanol (5 ml), a solution of $NaNO_2$ (10.2 mmol) in water (4 ml) was added dropwise at room temperature. After 30 min the reaction mixture was treated with hypophosphorous acid (50%, 7.4 ml) and stirred at 4°C overnight. The solution was extracted with dichloromethane; the combined organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography using cyclohexane/ethyl acetate 9/1 as eluent.

The first compound eluted was **15(15')c** (yield 20%), yellow oil: IR ν; 3510 (broad OH), 1434 cm^{-1} (NO_2): ^1H NMR ($CDCl_3$) δ; 2.53 (3H, s, CH_3), 3.87 (3H, s, CH_3), 6.87 (1H, s, H-2), 7.18 (1H, dd, J=7.5, 2.7 Hz, H-10), 7.38 (1H, s, H-7), 7.37–7.42 (1H, m, H-11), 7.91 (1H, dd, J=7.5, 2.7 Hz, H-12). (Found: C, 59.33; H, 3.73; N, 17.45. $C_{16}H_{12}N_4O_4$ requires C, 59.33; H, 3.73; N, 17.45%). Further elution gave an inseparable mixture (1:3) of derivatives **15(15')c** and **14c** (yield 25%). For **14c**: ^1H NMR ($CDCl_3$) δ 2.70 (3H, s, CH_3), 3.87 (3H, s, CH_3), 6.51 (1H, s, H-2), 7.15–7.20 (1H, m, H-10), 7.37 (1H, s, H-7), 7.32–7.38 (1H, m, H-9), 7.78 (1H, dd, J=3.2, 1.7 Hz, H-12).

2-Acetyl-5-hydroxy-3-methyl-11-methoxypyrimido[5,4-*c*]pyrrole[2,1-*a*]isoquinoline (14d). To a stirred solution of **13b** (2.9 mmol) in H_2SO_4 (50%, 14.8 mmol) and methanol (7 ml), a solution of $NaNO_2$ (8.9 mmol) in water (4 ml) was added dropwise at room temperature. After

30 min the reaction mixture was treated with hypophosphorous acid and stirred at 4°C overnight. The solution was extracted with dichloromethane; the combined organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography using cyclohexane/ethyl acetate 9/1 as eluent. The first compound eluted was **15(15')d** (yield <2%).

Further elution gave **14d (**yield 23%), yellow oil: IR ν; 3418 (OH), 1708 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ; 1.25 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.85 (3H, s, CH_3), 6.84 (1H, s, H-1), 7.18 (1H, dd, J=7.8, 1.5 Hz, H-10), 7.25 (1H, d, J=1.5 Hz, H-12), 7.26 (1H, s, H-7), 7.29 (1H, d, J=7.8 Hz, H-9). (Found; C, 66.41; H, 4.72; N, 13.10. $C_{18}H_{15}N_3O_3$ requires C, 67.28; H, 4.71; N, 13.10%).

2-Acetyl-5-hydroxy-3-methyl-9-methoxypyrimido[5,4-*c*]pyrrole[2,1-*a*]isoquinoline [15(15')d]. To a suspension of **13b** (2.9 mmol) in H_2SO_4 (50%, 14.8 mmol) $NaNO_2$ (20.7 mmol) was added in small portions; the mixture was stirred for 2 days at 30°C. Hypophosphorous acid (50%, 6.4 ml) was added and the mixture was stirred overnight at room temperature. After neutralization with $NaHCO_3$, the solution was extracted with ethyl acetate; the combined organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography using cyclohexane/ethyl acetate 8/2 as eluent to give **15(15')d** (yield 24%), as an uncrystallizable yellow oil: IR ν; 3346 (OH), 1680 (CO) cm^{-1}: ^1H NMR ($CDCl_3$) δ; 2.31 (3H, s, CH_3), 2.76 (3H, s, CH_3), 3.87 (3H, s, CH_3), 7.22 (1H, dd, J=8.3, 2.5 Hz, H-10), 7.41 (1H, t, J=8.3 Hz, H-11), 7.52 (1H, s, H-1), 7.50–7.60 (2H, m, H-12 and H-7). (Found; C, 66.82; H, 4.68; N, 13.35. $C_{18}H_{15}N_3O_3$ requires C, 67.28; H, 4.71; N, 13.08%).

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