Selective Sequential Demasking of the Ester Functions of 1-Methyl-3,4,5-tris(methoxycarbonyl)pyrazole

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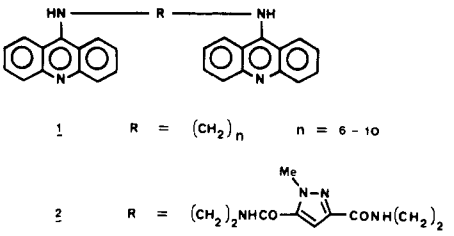
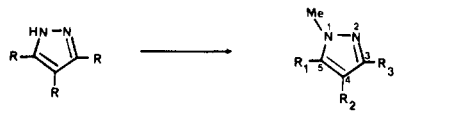
The adjacent ester functions of 1-methyl-3,4,5-tris(methoxycarbonyl)pyrazole can be selectively and sequentially demasked, providing an efficient route to heterogeneous pyrazole triamides. X-ray crystallographic determination of the structure of one of these triamides shows the order of ester hydrolysis to be 5, 4, 3.

Dimeric ligands that bind to DNA by intercalation of both ligand chromophores are of interest both as probes of DNA–ligand interactions[1] and as potential cytotoxic agents.[2] For such compounds, biological activity appears more closely related to the observed average residence times of the intercalated chromophores than to overall ligand–DNA association constants.[3,4] Thus, while diacridine derivatives linked by flexible polymethylene chains (e.g., **1**) are only marginally active, the pyrazole-linked diacridine **2** is the most cytotoxic diacridine compound yet described, with confirmed in vivo activity.[4] Such enhanced activity has been attributed to the greater rigidity of the pyrazole-containing linker chain, which results in much longer average residence times for the intercalating chromophores at their DNA binding sites.[3]

These properties, together with the high water solubility conferred by the pyrazole moiety, make it an attractive template for the elaboration of corresponding trimeric compounds that might trisintercalate into DNA. Some examples of DNA trisintercalating compounds have been reported recently,[5-7] but all employ flexible linker chains. Since maximum synthetic advantage would result from the ability to attach three different chromophores to the central template, we have explored the selective demasking of 1-methyl-3,4,5-tris(methoxycarbonyl)pyrazole (**5**). This compound is readily available via the acid-catalyzed condensation of 3-methylpentane-2,4-dione with hydrazine hydrate, followed by oxidation of the resulting trimethylpyrazole **3** and exhaustive methylation.

The three methoxycarbonyl groups of **5** exist in sufficiently different steric and electronic environments that selective, stepwise demasking seemed achievable, and this proved to be the case. Treatment of **5** with exactly 1 equiv of KOH in dry methanol at 20 °C for 3 h gave an 83% yield of a monoacid, mp 127–129 °C. Since the 3-methoxycarbonyl group of **5** is the least sterically encumbered, we tentatively assigned this monoacid structure as 1-methyl-4,5-bis(methoxycarbonyl)pyrazole-3-carboxylic acid





3, R = CH_3
4, R = COOH

5-12

	R_1	R_2	R_3
5	$COOCH_3$	$COOCH_3$	$COOCH_3$
6	$COOCH_3$	$COOCH_3$	COOH
7	COOH	$COOCH_3$	$COOCH_3$
8	CONHPh	$COOCH_3$	$COOCH_3$
9	CONHPh	COOH	$COOCH_3$
10	CONHPh	$CONH(CH_2)_2CH_3$	$COOCH_3$
11	CONHPh	$CONH(CH_2)_2CH_3$	COOH
12	CONHPh	$CONH(CH_2)_2CH_3$	$CONH(CH_2)_2OCH_3$

(**6**). However, this structure was not readily distinguished from possible isomeric structures by NMR spectrometry. In order to unequivocally determine the regiospecificity of demasking, we prepared a suitably functionalized molecule for X-ray analysis. Three different amines (aniline, *n*-propylamine, and (2-methoxyethyl)amine) were added sequentially to the pyrazole triester **5** (see below), and the structure of the resulting triamide was determined as **12** by X-ray crystallography (Figure 1).

With this information, the preparation of compound **12** can be deduced as follows. Treatment of **5** with 1 equiv of KOH in dry MeOH at 20 °C for 3 h selectively hydrolyzed the 5-methoxycarbonyl function to give the monoacid of mp 127–129 °C mentioned above and now assigned structure **7**. Addition of aniline to the corresponding acid chloride gave **8**, which was treated with 1 equiv of KOH in MeOH at reflux to selectively demask the 4-methoxycarbonyl function and give **9** in good yield. Similar addition of propylamine gave crude **10**, which was demasked in refluxing aqueous KOH to provide the acid **11** (47% yield for two steps). Final amide formation using (2-methoxyethyl)amine in the presence of 1,1′-carbonyldiimidazole gave the mixed triamide **12** in 62% yield.

These results show that selective, base-catalyzed demasking of 1-methyl-3,4,5-tris(methoxycarbonyl)pyrazole

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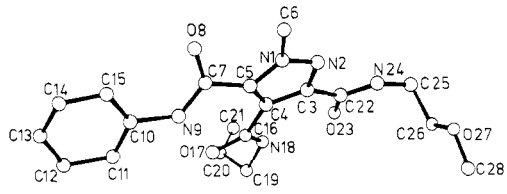

Figure 1. Molecular structure of **12**.

(**5**) is possible, but that the hydrolyses proceed in the opposite order (viz., 5, 4, 3) to that expected from the anticipated accessibilities of the three ester functions. The preparation of **12** shows that mixed triamides of 1-methylpyrazole-3,4,5-tricarboxylic acid can be efficiently obtained, and this methodology is currently being used in the synthesis of potential DNA trisintercalators.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand, under the direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer and are as read. NMR spectra were obtained on a Varian T-60 spectrometer (Me_4Si).

3,4,5-Trimethylpyrazole (3). Sodium pieces (29.9 g, 1.3 mol) were added *cautiously* to anhydrous methanol (400 mL). Pentane-2,4-dione (129 mL, 1.25 mol) was added, and the mixture was cooled to room temperature. Iodomethane (89 mL, 1.44 mol) was added dropwise over 1 h. After the mixture was refluxed for 2 h, the methanol was removed, and the residue was fractionally distilled (water pump) to yield a minor forerun of starting material (bp 62 °C) followed by 3-methylpentane-2,4-dione, bp 65.5 °C (98.7 g, 69% yield): NMR ($CDCl_3$) δ 1.25 (d, J = 7 Hz, 3-Me), 1.83 and 2.02 (2 s, 3-Me and 5-H_3 of enol form), 2.10 (s, 1-H_3 of enol form), 2.18 (s, 1-H_3), 3.82 (q, J = 7 Hz, 3-H), 16.50 (s, exchangeable on deuteration, enol OH). The enol form comprised approximately 20% of the tautomeric mixture.

The above product (78.7 g, 0.69 mol) was added dropwise to a stirred solution of hydrazine hydrate (80% solution, 45 mL, 0.718 mol), water (1.25 mL), and acetic acid (2 mL), held at 10–15 °C. The mixture was kept at 10 °C for 20 h, and the precipitated 3,4,5-trimethylpyrazole (**3**) was collected and washed with ice-water to yield 63.3 g (83%): NMR ($CDCl_3$) δ 1.85 (s, 3, CH_3), 2.15 (s, 6, CH_3), 7.10 (br s, 1, NH, exchanged on deuteration).

Pyrazole-3,4,5-tricarboxylic Acid (4). A mixture of 3,4,5-trimethylpyrazole (**3**) (63.3 g, 0.575 mol) and water (1.2 L) was heated to 70 °C in a 5-L beaker. To this rapidly stirred solution was added finely ground potassium permangamate (545 g, 3.4 mol). This was added in ca 30-g aliquots every 3–5 min as the oxidant was consumed, while the exothermic reaction was maintained around 90 °C. A slight excess of $KMnO_4$ was then added until a residual purple-pink color remained after 1 h of reflux. The mixture was filtered hot, and the solid MnO_2 was washed with boiling water. Concentrated HCl was added to the brown solution until it became clear. The mixture was cooled overnight in a refrigerator to afford 36 g (32%) of the triacid **4** as a white crystalline solid, mp 288 °C dec (lit.[8] mp 225–227 °C).

1-Methyl-3,4,5-tris(methoxycarbonyl)pyrazole (5). Pyrazole-3,4,5-tricarboxylic acid (**4**) (4.77 g, 23.85 mmol), dimethyl sulfate (13.5 mL, 6 equiv), and potassium carbonate (19.3 g, 6 equiv) in anhydrous acetone (100 mL) were stirred together at reflux for 24 h. The mixture was filtered hot, and the solid was washed with acetone. The acetone was removed and the yellow-orange residue was taken up in hot ethanol, refiltered and cooled in the refrigerator overnight to yield 3.6 g (59%) of product as white crystals: mp 93–95 °C; NMR ($CDCl_3$) δ 3.90 (s, 9, OMe), 4.20 (s, 3, NMe). Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.87; H, 4.72; N, 10.94. Found: C, 46.61; H, 4.72; N, 10.93.

1-Methyl-3,4-bis(methoxycarbonyl)pyrazole-5-carboxylic Acid (7). A mixture of (**5**) (13.0 g, 53.76 mmol), methanolic KOH (22.03 mL of a 2.44 M solution, 1 equiv), and methanol (50 mL) was stirred at room temperature for 3 h. The methanol was removed, and the white precipitate was dissolved in water (100 mL) and filtered. Dilute hydrochloric acid (54 mL of 1 N) was added, and the precipitated acid was recrystallized from hot water to give 10.2 g (83%) of crystalline material: mp 127–129 °C; NMR ($CDCl_3$, Me_2SO-d_6) δ 3.80 (s, 6, OMe), 4.20 (s, 3, NMe). Anal. Calcd for $C_9H_{10}N_2O_6 \cdot {}^1/_4H_2O$: C, 43.75; H, 4.29; N, 11.36. Found: C, 43.90; H, 4.53; N, 11.29.

1-Methyl-3,4-bis(methoxycarbonyl)-*N*-phenylpyrazole-5-carboxamide (8). A mixture of **7** (6 g, 26.3 mmol), thionyl chloride (10 mL), and DMF (1 drop) was heated to reflux for 1 h. Excess thionyl chloride was removed on a water pump followed by azeotroping with dry benzene (2 times). The oily residue was dissolved in dry acetone (50 mL), and to this solution was added (dropwise) a mixture of aniline (2.6 mL, 28.5 mmol) and triethylamine (5 mL). After the mixture was stirred for 3 min the precipitated triethylamine hydrochloride was filtered off and washed with acetone. The solvent was removed to give **8**, which was recrystallized from Et_2O–EtOH to give 6 g (80%) of product: mp 94–95 °C; NMR ($CDCl_3$) δ 3.90 (s, 6, OMe), 4.25 (s, 3, NMe), 7.00–7.75 (m, 5, Ar). Anal. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.77; H, 4.79; N, 13.25. Found: C, 56.70; H, 4.83; N, 13.48.

1-Methyl-3-(methoxycarbonyl)-5-(phenylcarbamoyl)-pyrazole-4-carboxylic Acid (9). A mixture of **8** (5.97 g, 19.7 mmol), 8.9 mL of a 2.44 M solution of methanolic KOH (1 equiv), and methanol (50 mL) was refluxed for 5 h. The solvent was removed, water (50 mL) was added, and the mixture was filtered and neutralized with 19.8 mL of 1 N HCl. The oily precipitate was extracted with ethyl acetate, dried, and concentrated to yield the amido acid **9** as fluffy white needles: mp 184 °C (EtOAc); NMR ($CDCl_3$) δ 4.12 (s, 3, OMe), 4.40 (s, 3, NMe), 7.06–7.82 (m, 5, Ar). Anal. Calcd for $C_{14}H_{13}N_3O_5$: C, 55.44; H, 4.32; N, 13.86. Found: C, 55.38; H, 4.37; N, 13.84.

1-Methyl-3-(methoxycarbonyl)-*N*-phenyl-4-(*n*-propylcarbamoyl)pyrazole-5-carboxamide (10). A mixture of **9** (155 mg, 5.11 mmol), thionyl chloride (10 drops), and 1,2-dichloroethane (5 mL) was heated to reflux for 30 min. The solvents were removed, and dichloromethane (5 mL) was added followed by *n*-propylamine (1 mL). The solution was stirred at 20 °C for 3 min; the solvents were removed, and the residue was redissolved in fresh dichloromethane, passed through a silica gel plug, and concentrated to yield **10** as a colorless oil: NMR ($CDCl_3$) δ 1.00 (t, 3, CH_3), 1.60 (q, 2, CH_2), 3.41 (t, 2, CH_2NH), 4.00 (s, 3, OMe), 4.33 (s, 3, NMe), 7.04–7.85 (m, 5, Ar).

1-Methyl-5-(phenylcarbamoyl)-4-(*n*-propylcarbamoyl)-pyrazole-3-carboxylic Acid (11). Treatment of **10** with excess KOH in boiling water for 3 min followed by filtration and acidification to pH 1 yielded a white precipitate. Recrystallisation from aqueous acetone afforded 80 mg (47% over two steps) of **11** as white needles: mp 192–193 °C; NMR ($CDCl_3$) δ 0.95 (t, 3, CH_3), 1.58 (q, 2, CH_2), 3.38 (m, 2, CH_2NH), 4.26 (s, 3, NMe) 7.06–7.84 (m, 5, Ar). Anal. Calcd for $C_{16}H_{18}N_4O_4 \cdot {}^1/_2H_2O$: C, 56.63; H, 5.64; N, 16.51. Found: C, 56.63; H, 5.71; N, 16.40.

1-Methyl-3-[(2-methoxyethyl)carbamoyl]-*N*-phenyl-4-(*n*-propylcarbamoyl)pyrazole-5-carboxamide (12). A mixture of **11** (30 mg, 0.09 mmol) and 1,1′-carbonyldiimidazole (30 mg, 2 equiv) in dry THF (5 mL) were stirred at room temperature for 1 h and then heated to reflux for 1 min. (2-Methoxyethyl)-amine (0.5 mL) was added and the mixture refluxed for 1 h. The solvents were removed; ethyl acetate was added, and the organic phase was extracted with aqueous KOH. Acidification of the aqueous phase yielded no starting material. The ethyl acetate solution was passed through a silica gel plug and concentrated to yield **12** (22 mg, 62%), which was recrystallized (vapor diffusion) as clear needles (EtOAc–hexane): mp 99–100 °C; NMR ($CDCl_3$) δ 0.95 (t, 3, CH_3), 1.58 (m, 2, CH_2), 3.35 (s, 3, OMe), 3.55 (m, 6, CH_2), 4.25 (s, 3, NMe), 7.00–7.90 (m, 5, Ar). Anal. Calcd for $C_{19}H_{25}N_5O_4$: C, 58.90; H, 6.50; N, 18.08. Found: C, 58.84; H, 6.74; N, 18.14.

X-ray Structure Determination of 12. Crystal Data: $C_{19}H_{25}N_5O_4$, M_r = 387.44, monoclinic, space group $P2_1/n$, a = 15.271 (2) Å, b = 17.277 (2) Å, c = 15.473 (3) Å, β = 99.23 (1)°, V = 4029 (4) Å^3, Z = 8, d_m = 1.27 g cm^{-3} (by flotation in petroleum ether/CCl_4), d_c = 1.277 g cm^{-3}, Mo Kα radiation of λ = 0.7107 Å, Zr filter, μ = 0.56 cm^{-1}.

Colorless, equant crystals for the X-ray diffraction study were prepared by the controlled diffusion of hexane into an ethyl

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acetate solution of the compound. The crystals were of rather poor quality and did not diffract strongly. That selected for intensity data collection was developed on {100} and {011} and measured 0.21 × 0.14 × 0.28 mm. Unit-cell constants were derived from a least-squares fit to the setting angles of 25 widely dispersed reflections on a Nonius CAD-4 diffractometer. Intensity data were collected by a variable width, variable speed $2\theta/\omega$ scan to the practical diffraction limit of $\theta = 22°$. There were no nonstatistical variations in the intensities of three standard reflections monitored throughout the data collection, and no reflection was sufficiently intense to warrant the use of attenuators. The data were corrected for Lorentz and polarization effects, but absorption corrections were not required. After averaging equivalent measurements, the data set consisted of 3320 unique reflections of which 1452 were classed as observed ($I > 2.5\sigma(I)$).[9]

Structure determination was undertaken by using direct methods. Since the crystal space group has four general equivalent positions but the unit cell contains eight molecules there are two crystallographically distinct molecules to be located (56 nonhydrogen atoms). The two independent molecules are labeled A and B. In the first E map, 49 atoms were correctly positioned, and the remainder were located from subsequent difference electron density maps. A least-squares refinement cycle in which all atoms were assigned the atomic scattering factor of carbon returned relatively low isotropic temperature factors for those atoms expected to be oxygens and nitrogens on the basis of chemical reasoning. With all atoms correctly assigned, two refinement cycles were computed. The temperature factors of some atoms at the extremities of the side chains became comparatively high, and accordingly these atoms were removed from the structure-factor calculation and a difference map computed. All atoms reappeared as single (albeit somewhat diffuse) peaks, and it was concluded that high thermal vibrations and not disorder were responsible for the high temperature factor values. The nonrigidity of packing would also be a contributory cause of the relatively peak weak diffraction by the crystals.

For the final least-squares cycles, hydrogen atoms were included in fixed calculated positions with isotropic temperature factors set approximately 10% higher than those of the atoms to which they were attached. no attempt has been made to assign anisotropic thermal parameters to any atoms as this would not be justified on the basis of the limited number of observations. Final residuals were $R = 0.103$ and $R_w = 0.110$.

(9) Programs used for unit cell determinations and initial data processing were part of the CAD-4 SDP structure determination package by B. Frenz. The direct methods structure solution and least-squares refinement were carried out with SHELX on the University of Auckland IBM 4341 computer.

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Registry No. 3, 5519-42-6; 4, 19551-66-7; 5, 20544-67-6; 6, 98688-29-0; 7, 98688-21-2; 7 (acid chloride), 98688-22-3; 8, 98688-23-4; 9, 98688-24-5; 9 (acid chloride), 98688-25-6; 10, 98688-26-7; 11, 98688-27-8; 12, 98688-28-9; $H_3CCOCH_2COCH_3$, 123-54-6; $H_3CCOCH(CH_3)COCH_3$, 815-57-6.

Supplementary Material Available: Atomic coordinates, bond lengths, and bond angles for 12 (4 pages). Ordering information is given on any current masthead page.

Structures of Diarylcarbenes and Their Effect on the Energy Separation between Singlet and Triplet States[1]

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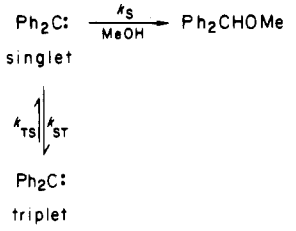
Theory indicates that expansion of the central C–C–C angle in a diarylcarbene should lead to an enhancement of its triplet–singlet energy gap. The series of carbenes diphenyl, mesitylphenyl, mesityl-*o*-tolyl, and dimesityl was used to test this hypothesis. Electron paramagnetic resonance (EPR) spectra of the triplet states of these carbenes showed that increasing ortho substitution at the aryl groups led to an expansion of the C–C–C angle. Product and kinetic studies, on reactions which reflected the singlet–triplet energy gap, showed that this expansion led to an enhanced triplet–singlet energy separation thus substantiating the theoretical prediction. The results establish a relationship between structure and the energy separation between the triplet and singlet states of simple diarylcarbenes.

Electron nuclear double resonance (ENDOR) studies[3,4] of diphenylcarbene (I) in matrices have shown that the carbene has a triplet ground state with a central C–C–C angle of 148° and a dihedral angle of 35° between the phenyl rings. Theoretical calculations[5-7] support this conclusion and further suggest[6] that the energy separation between the triplet and singlet states is very sensitive to structural distortions such as expansion of the central C–C–C angle or rotation of the phenyl rings. In fact, theory suggests[6,7] that expansion of the central C–C–C angle causes a slight destabilization of the triplet state but at the same time strongly destabilizes the singlet state in

Scheme I



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