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SYNTHESIS OF A NEW FAMILY OF LIGANDS WITH BISPYRAZOLE STRUCTURE. REACTIVITY OF BISPYRAZOLYLMETHYL ETHERS

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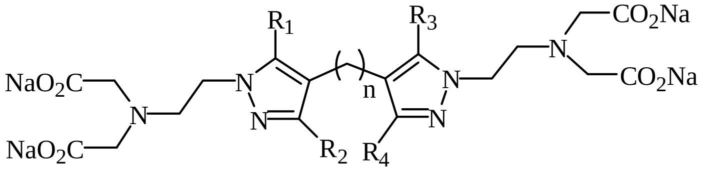
Abstract – We report the synthesis of a new family of chelating ligands containing bispyrazole structure. Some of them undergo an unusual reactivity when they react with methyl iminodiacetate. We propose a tentative mechanism that probably involves 4-chloromethylpyrazoles and the corresponding alcohols as reaction intermediates. The corresponding Gd-complexes exhibit interesting relaxivity properties being potential paramagnetic contrast agents.

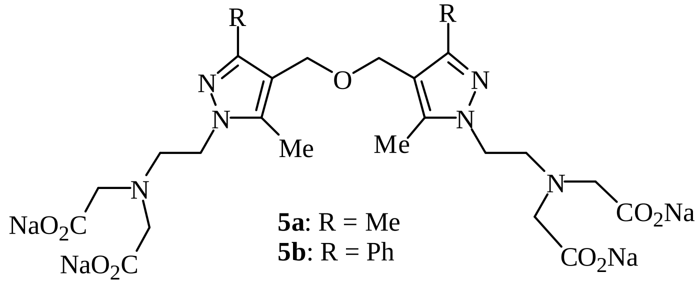
In the last decade, we have investigated heterocyclic chelating ligands with potential use as contrast agents for Magnetic Resonance Imaging (MRI). These studies cover from the designing, synthesis and characterization of pH functional probes[1] to paramagnetic contrast agents.[2] Recently, we have described a new generation of heterocyclic complexones (**1-4**) including bi or bispyrazole structures and two iminodiacetic acid units (Figure 1).[3] These ligands were able to form two intramolecular Gd(III) complexes which showed larger relaxivity values r_1 and r_2 (30 or 70 $s^{-1}mM^{-1}$), as compared with Gd-DTPA (Gd-complex of diethylentriaminepentaacetic acid; 3.8 $s^{-1}mM^{-1}$). However, these complexones present a very low affinity for Gd(III) ion.

Our investigation aims the design and synthesis of new chelating ligands with improved relaxivity and increased thermodynamic stability. On these grounds and based in our previous studies, we report here on the synthesis of a novel series of complexones having bispyrazole structure. The linkers between pyrazole rings include now oxygen atoms, which could originate more stable Gd(III) complexes (Figure 2).



1: $R_1 = R_3 = H$; $R_2 = R_4 = Ph$, n = 0
2: $R_1 = R_3 = R_2 = R_4 = Me$; n = 0
3: $R_1 = R_3 = H$; $R_2 = R_4 = C_6H_4\text{-}NO_2\text{-}p$, n = 1
4: $R_1 = R_3 = R_2 = R_4 = Me$, n = 1

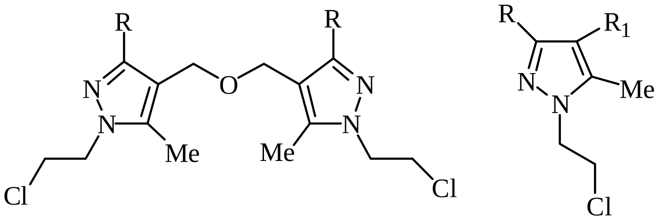
Figure 1



5a: R = Me
5b: R = Ph

Figure 2

Complexones (**5**) could be obtained from chloroethyl bispyrazoles (**6**) (Figure 3) following a similar synthetic approach previously described by Wijnberger *et al.*[4]



6a: R = Me
6b: R = Ph

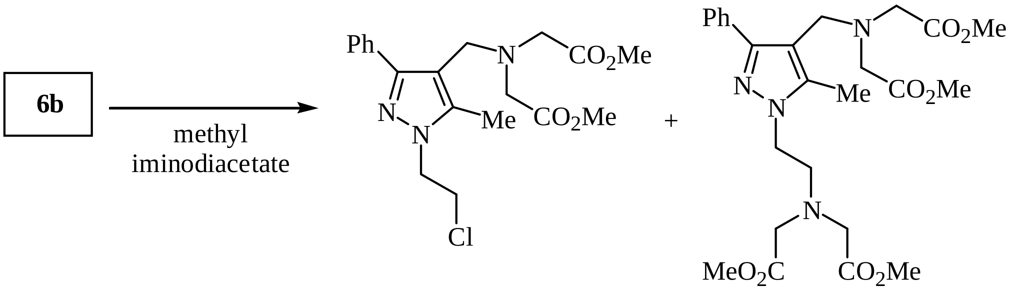
7a: R = Me, R_1 = H
7b: R = Ph, R_1 = H
8: R = Me, $R_1 = CH_2OH$

Figure 3

Condensation of pyrazoles (**7a-b**)[2] with paraformaldehyde gave different products depending on the starting substrate. When R = Me the corresponding alcohol (**8**)[3] was obtained together with traces of (**6a**). However, when R = Ph, compound (**6b**) was isolated alone. Consequently, the product obtained depended on the type of substituents at the 1-, 3- and 5- positions of the pyrazole ring.

To prepare complexone (**5b**), the chloroethyl derivative (**6b**) and methyl iminodiacetate in a ratio 1:4 was heated with at 110 °C for 4.5 h. Surprisingly, the desired compound (**5b**) was not formed. Purification of the reaction crude by column chromatography on silica gel yielded compounds (**9**) and (**10**) in 62 % and

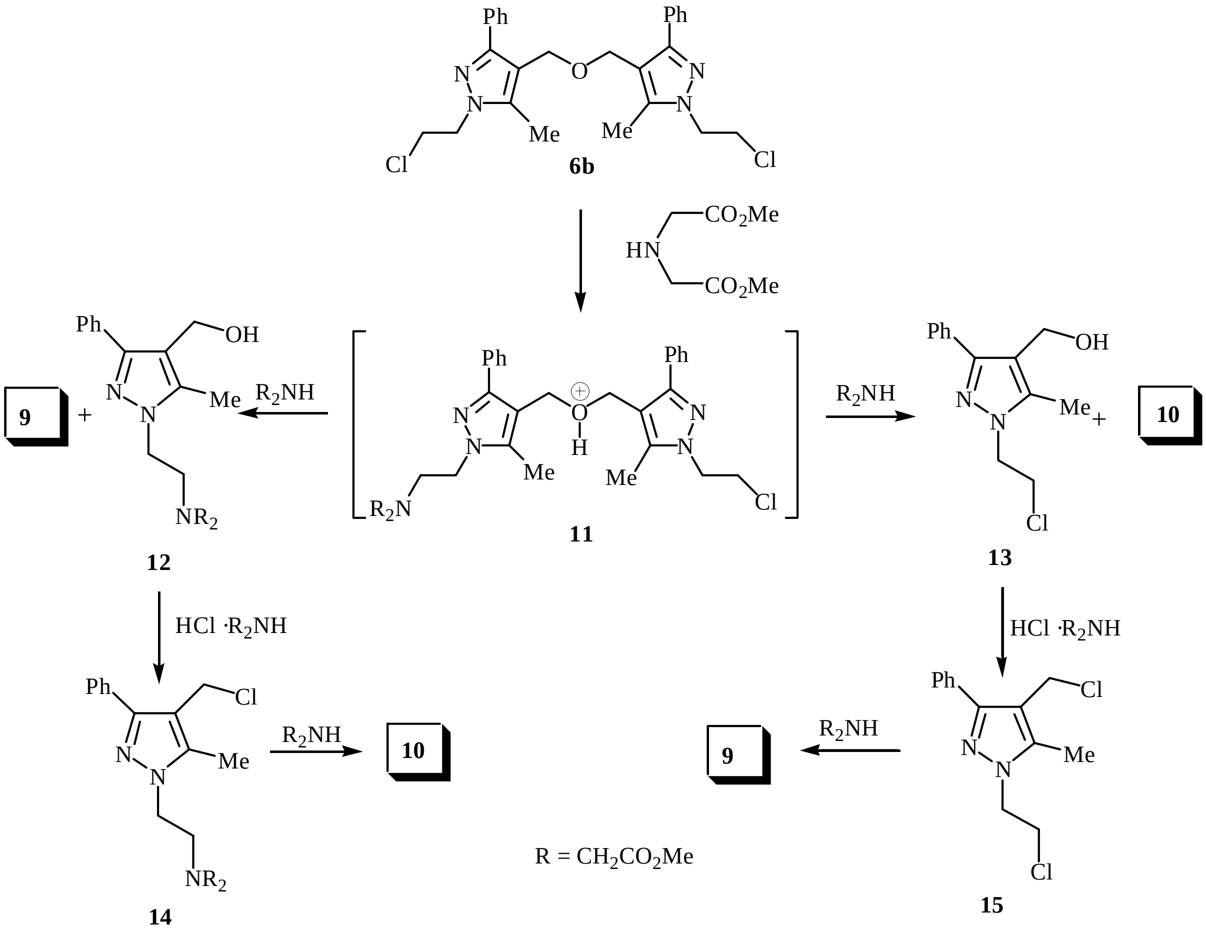
30 %, respectively (Scheme 1). Compound (**10**) was isolated exclusively when the reaction was carried out using an excess of methyl iminodiacetate (1:8).



9 (62 %) **10** (30 %)

Scheme 1

As a tentative mechanism, we propose that the formation of (**9**) and (**10**) may proceed according to Scheme 2.

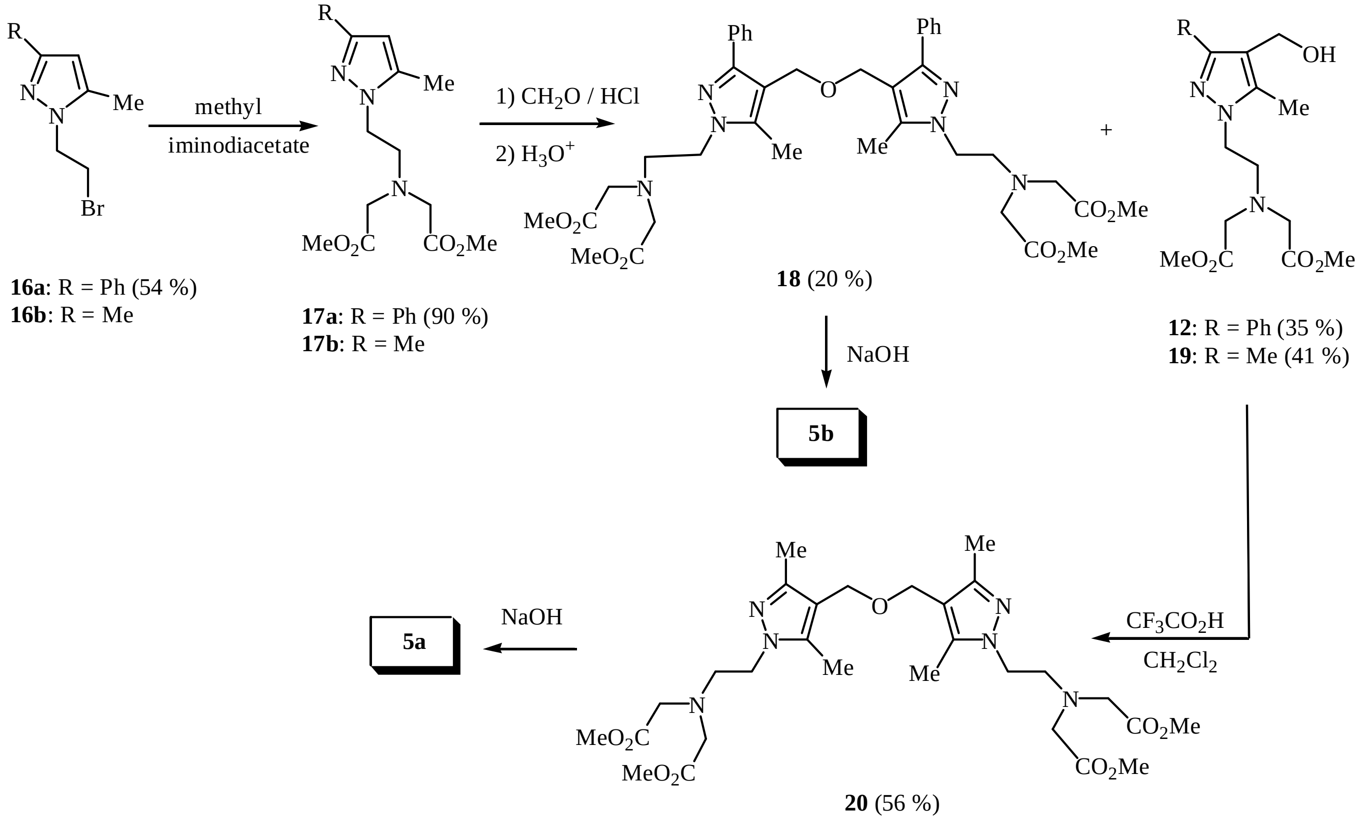


Scheme 2

Nucleophilic substitution of chloro derivative (**6b**) gives intermediate (**11**), which is able to react with methyl iminodiacetate to yield compounds (**9**) and (**10**) and most probably the corresponding alcohols (**12**) and (**13**). These alcohols readily react with HCl leading to the corresponding

4-chloromethylpyrazoles (**14**) and (**15**). Generally, 4-chloromethylpyrazoles present higher reactivity[5] than those pyrazoles containing the chloromethyl group in other positions.[6] Chloromethyl derivatives (**14**) and (**15**) readily react with methyl iminodiacetate to form products (**9**) and (**10**). The high reactivity of the 4-chloromethyl group in compound (**15**) justifies the formation of compound (**9**) as the major product. These results suggest that a catalytic amount of hydrogen chloride promoted the cleavage of 4-pyrazolylmethyl ethers generating 4-chloromethylpyrazoles, which finally reacted with methyl iminodiacetate to give products (**9**) and (**10**). Due to the high reactivity of hydroxymethyl and chloromethyl groups the proposed intermediates (**12-15**) could not be isolated.

We alternatively prepared complexones (**5**) from compounds (**16**) (Scheme 3).

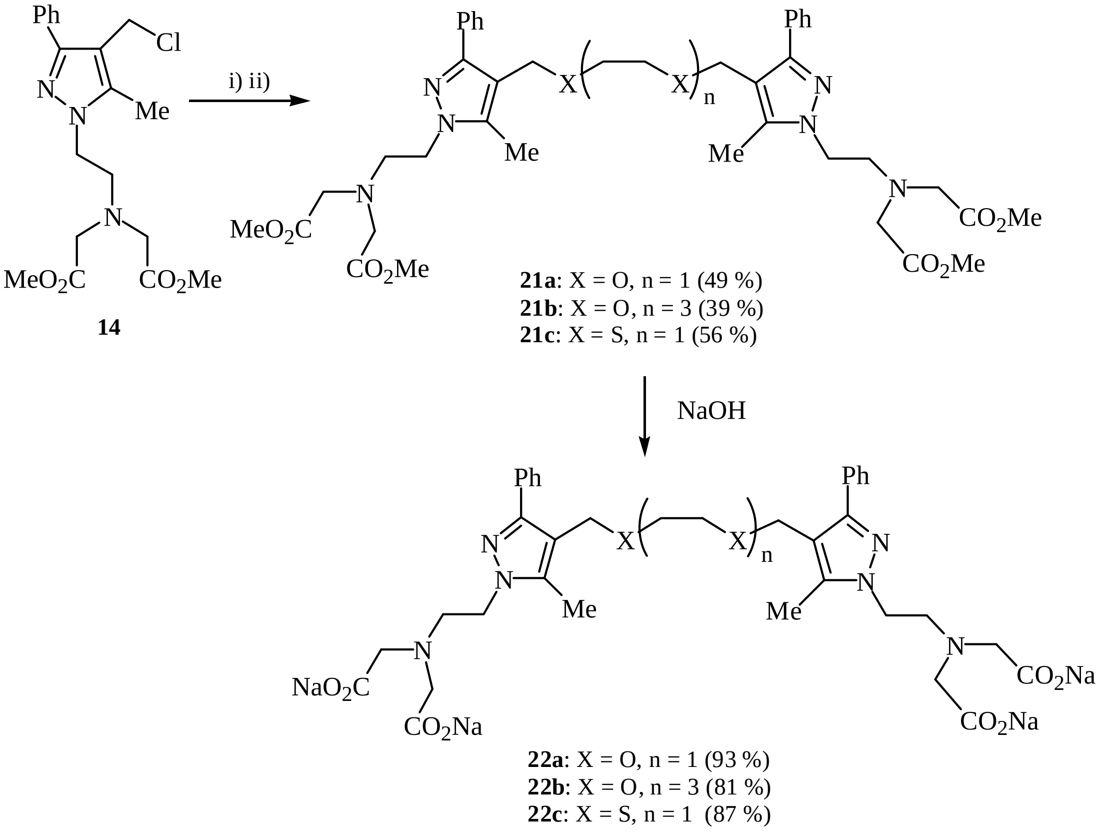


Scheme 3

Compounds (**17**) were prepared by reaction of bromoethyl derivatives (**16**) with methyl iminodiacetate. The condensation reaction of (**17a**) with paraformaldehyde yielded a mixture of ether (**18**) and alcohol (**12**), while condensation of (**17b**) under the same conditions, gave exclusively alcohol (**19**).[7] In this case, compound (**19**) could be transformed into ether (**20**) using catalytic amounts of trifluoroacetic acid in dichloromethane (stabilized with amilene) at room temperature. These conditions were described by *Miki et al.* to investigate a similar chemical behaviour of 3-hydroxymethyl pyrazolo[1,5-*a*]pyridines and related compounds.[8] Finally complexones (**5**) were obtained by basic hydrolysis of the corresponding methyl esters (**18**) and (**20**).

To confirm the suggested mechanism we performed two experiments as follow: i) reaction of ether (**18**) with methyl iminodiacetate and, ii) reaction of ether (**18**) with methyl iminodiacetate in the presence of catalytic amount of methyl iminodiacetate hydrochloride (110 ^{O}C). In the first case, we obtained the unchanged ether (**18**) while in the second, compound (**10**) was formed exclusively.[9] These results verified that the hydrogen chloride is essential for the reaction pathway.

Furthermore, complexones (**22**) were synthesized from 4-chloromethylpyrazole (**14**)[5] by reaction with ethylene glycol, triethylene glycol and thioethylene glycol, respectively to give the corresponding esters (**21**). Basic hydrolysis of the corresponding esters gave the desired compounds (**22**) (Scheme 4).



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21a: X = O, n = 1 (49 %)
21b: X = O, n = 3 (39 %)
21c: X = S, n = 1 (56 %)

22a: X = O, n = 1 (93 %)
22b: X = O, n = 3 (81 %)
22c: X = S, n = 1 (87 %)

i) NuH = ethylene glycol, triethylene glycol or thioethylene glycol; ii) NaOH.

Scheme 4.

We report a preliminary study of the gadolinium complexes of **5** and **22** as potential contrast agents for MRI. Evaluation of complexes of **5** and **22** has been carried out by measurement of their relaxivity properties in solution. Due to the low affinity constants the complexes could not be isolated. Relaxation rates of the Gd-complexes have been determined at 60 MHz using an excess of ligands to avoid the presence of free Gd(III) ion (Table 1).

Gd(III)-complexes of **5** and **22** shown slightly lower relaxivity than the Gd(III)-complexes of **1-4**, substantially higher than DTPA-Gd(III) and even than free Gd(III) ion. This circumstance is not only derived from the higher hydration number, since aqua Gd(III) exhibits much lower relaxivity. Thus, additional factors including restricted molecular rotation and/or faster water exchange, must occur which determine these improved relaxivity properties. The less rigid structure and therefore superior conformational mobility of complexones (**5**) and (**22**) could explain the lower relaxivity found in comparison with complexones (**1-4**).

Table 1. Longitudinal and transversal relaxivity (r_1, r_2; s^{-1} mM^{-1}) of aqueous solutions of Gd-complexes of complexones (**5**) and (**22**) determined at 1.5 Tesla.

Complexes[a]	$\mathbf{r_1}$[b]	$\mathbf{r_2}$[b]
Gd(III)[c]	8.3 ± 0.02	10.8 ± 0.1
5a-Gd(III)	13.8 ± 0.1	15.5 ± 0.1
5b-Gd(III)	25.9 ± 0.9	31.2 ± 0.4
22a-Gd(III)	14.5 ± 0.1	17.3 ± 0.1
22b-Gd(III)	15.9 ± 0.1	17.8 ± 0.1
22c-Gd(III)	20.5 ± 0.8	30.0 ± 0.1
DTPA-Gd(III)	4.3 ± 0.1	4.2 ± 0.1

[a] Model solutions contained 100 mM TRIS / HCl (pH~ 7, 37 ºC), 150 mM NaCl, 5 mM compounds (**5**) and (**22**) and DTPA, containing or not 0.5 mM Gd(III) in the cases of complexones (**5**) and (**22**) and 5 mM Gd(III) in the DTPA measurements. [b] Result are expressed as means ± SD of at least three independent T_1 and T_2 measurement in each sample.[c] Ref. 3

The influence of pyrazole ring in relaxivity has been observed in the Gd-complex derived from 1,2,4,5-tetrakis (pyrazol-1-yl-methyl-3-carboxylate) benzene recently reported by *Fatin-Rouge et al.*[10] This complex including four pyrazole ring shows a high relaxivity values ($r_1 = 36$ $s^{-1}mM^{-1}$ and $r_2 = 45$ $s^{-1}mM^{-1}$) and it is organized in water by self-aggregation to yield nanoparticules. As compared with those, we do not discard the possibility of that Gd-complexes of **5** and **22** undergo some type of self-association that justified the high relaxivity observed.

On the other hand, kinetic tests of **Gd-5** and **Gd-22** conducted in presence of large amount of Arsenazo III [2,2’-(1,8-dihydroxy-3,6-disulfonaphthylene-2,7-bisazo)bisbenzenearsonic acid] have revealed a too great lability of the complexes compared with DTPA and similar behavior that the complexones (**1-4**).[3]

In conclusion, in this paper we report the synthesis of new heterocyclic compounds with bispyrazole structure. Surprisingly, when the ether (**6b**) react with an excess of methyl iminodiacetate as nucleophile, undergo the cleavage of the ether group to give the corresponding tertiary amine, as single reaction product. To justify this feature we proposed a tentative mechanism that could probably involve 4-chloromethylpyrazoles as reaction intermediates. NMR studies on relaxation rate of the corresponding

Gd(III)-complexes, showed that these complexes exhibit interesting relaxivity properties. Kinetic studies revealed that the linker including now different heteroatoms do not significantly increase the paramagnetic metal chelation, as compared with the first generation of bispyrazolic complexones (**1-4**).

EXPERIMENTAL

General

Melting points were obtained on a microscope hot stage and are uncorrected. Elemental analyses were performed with Perkin-Elmer 240 apparatus. MS spectra were recorded on a GC/mass spectrometer Schimadzu QP-5000 at 70 eV. For HRMS data a VG AutoSpec instrument was used. IR spectra were determined on a Philips PU-9700 and on a Bruker vector 22 spectrophotometers. NMR spectra were recorded with a Bruker DRX-400 (400.13 MHz for ^{1}H, and 100.033 MHz for ^{13}C) and Bruker AC-200 (200.13 MHz for ^{1}H, and 50.33 MHz for ^{13}C). ^{1}H NMR and ^{13}C NMR chemical shifts (δ) in $CDCl_3$ are given from internal tetramethylsilane and ^{13}C δ in D_2O are given from external DMSO-d_6 with an accuracy of ± 0.01 for ^{1}H and ± 0.1 ppm for ^{13}C. The residual water signal in ^{1}H NMR spectra in D_2O solution was suppressed when necessary using a 1 s (low power, 0.5 watts) presaturating pulse applied with decouple. ^{1}H-^{1}H coupling constants (*J*) are accurate to ± 0.2 Hz for ^{1}H NMR espectra. TLC chromatography was performed on DC-Aulofolien/Kieselgel 60 F_{245} (Merck) and column chromatography through silica gel Merck 60 (230-400 mesh). Methyl iminodiacetate hydrochloride was basified with solid Na_2CO_3 in the minimum amount of water prior to use. D_2O (99.9 D) was purchased from Appollo Scientific (Stockport, Great Britain). The rest of the products and dry solvents were obtained from Aldrich.

1-(2-Chloroethyl)-5-methyl-3-phenylpyrazole (7b).

This compound was synthesized according to alkylation general procedure described by us for similar compounds.[2] A mixture of 3-phenyl-5-methylpyrazole (2.5 g, 0.016 mol), NaOH (1.92 g, 0.048 mol), BTBA (129 mg, 0.4 mmol), 1,2-dichloroethane (13 mL) and H_2O (5 mL) was refluxed for 24 h. Purification of the residue by column chromatography on silica gel (CH_2Cl_2 / EtOH 98:2) gave **7b** as a colorless oil (2.5 g, 71 %); mp (picrate) 149-151 °C (EtOH). Anal. Calcd for $C_{12}H_{13}N_2Cl$·picrate: C, 48.06; H, 3.59; N, 15.57. Found: C, 48.35; H, 3.66; N, 15.61. ν_{max}/cm^{-1} (film) 1555, 1450, 1315, 770, 700 ; δ_H (400 MHz; $CDCl_3$) 2.36 (3 H, s, CH_3), 3.94 (2 H, apparent t, *J* = 6.4, 6.3 Hz, CH_2Cl), 4.37 (2 H, t, *J* = 6.3 Hz, CH_2-(Azole)), 6.33 (1 H, s, H_4), 7.28 (1 H, m, aromatic), 7.39 (2 H, m, aromatics), 7.76 (2 H, d, *J* = 8.5 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 150.7, 140.2, 133.3, 128.3, 127.3, 125.3, 102.4, 49.8, 42.7, 10.9; *m/z* [EI] 220 (M^+, 62), 171 (63), 158 (100), 130 (39), 77 (48), 51 (23). Further elution with the same solvent gave 1-(2-chloroethyl)-3-methyl- 5-phenylpyrazole as a colorless oil (0.5 g, 14 %). ν_{max}/cm^{-1} (film) 1550, 1500, 1460, 1430, 770, 705 ; δ_H (400 MHz; $CDCl_3$) 2.32 (3 H, s, CH_3), 3.87 (2 H, apparent t, *J* = 6.7, 6.5 Hz, CH_2Cl), 4.34 (2 H, t, *J* = 6.6 Hz, CH_2-N(Azole)), 6.09 (1 H, s, H_4), 7.45-7.38 (5 H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 148.5, 145.3, 130.3, 128.9, 128.62, 128.57, 106.1, 50.0, 42.3, 13.4; *m/z* [EI] 220 (M^+, 21), 171 (32), 158 (100), 130 (16), 77 (13).

[2,2'-(2-Chloroethyl-5-methyl-3-phenyl-4-pyrazolylmethyl)] ether (6b).

A stream of dry hydrogen chloride was passed through a solution of **7b** (2 g, 9.1 mmol) and paraformaldehyde (332 mg) in 1,2-dichloroethane (5mL) until saturation of the solution, and the reaction mixture was refluxed for 2 h. Water (3 mL) was added and the reaction mixture was stirred until rt for 10 h. Subsequently H_2O (10 mL) was added and the water layer was made alkaline with Na_2CO_3 and extracted with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$ and evaporated *in vacuo*. Purification of the residue by column chromatography on silica gel (Hexane/ AcOEt, 6:4) gave **6b** as a white solid (1.32 g, 58 %); mp 135-137 °C (CH_2Cl_2 / Hexane). Anal. Calcd for $C_{26}H_{28}N_4OCl_2$: C, 64.60; H, 5.84; N, 11.59. Found: C, 64.52; H, 6.02; N, 11.59. ν_{max}/cm^{-1} (KBr) 1560, 1480, 1450, 1430, 1320, 1150, 1060, 700; δ_H (400 MHz; $CDCl_3$) 2.36 (6 H, s, CH_3), 3.92 (4 H, apparent t, *J* = 6.5, 6.3 Hz, CH_2Cl), 4.38 (4 H, t, *J* = 6.4 Hz, CH_2-N(Azole)), 4.45 (4 H, s, CH_2O), 7.38-7.33 (6 H, m, aromatics), 7.71 (4 H, dd, *J* = 8.3, 1.6 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 151.4, 140.0, 133.1, 128.3, 127.7, 127.6, 112.5, 61.7, 50.1, 42.6, 9.5; *m/z* [EI] 483 (M^+, 2), 262 (28), 234 (100), 185 (29), 172 (38), 104 (23), 77 (27), 63 (43).

[(2-{4-[(Bismethoxycarbonylmethylamino)methyl]-5-methyl-3-phenylpyrazol-1-yl}ethyl)methoxycarbonylmethylamino]acetic acid methyl ester (10)

A mixture of **6b** (600 mg, 1.24 mmol) and methyl iminodiacetate (850 mg, 5.28 mmol) was heated at 110 ºC for 4 h y 30 min. After cooling, the mixture was extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$ and organic solvent was evaporated *in vacuo*. Purification of the residue by column chromatography on silica gel (Hexane / AcOEt, 6:4) gave **9** as a white solid (604 mg, 62 %); mp 62-64 ºC (EtOH / H_2O). Anal. Calcd for $C_{19}H_{24}N_3O_4Cl$: C, 57.94; H, 6.14; N, 10.67. Found: C, 58.01; H, 6.11; N, 10.70. ν_{max}/cm^{-1} (KBr) 1740, 1440, 1205, 1150, 660; δ_H (400 MHz; $CDCl_3$) 2.38 (3 H, s, CH_3), 3.52 (4 H, s, CH_2N), 3.58 (6 H, s, OCH_3), 3.85 (2 H, s, C-(Azole)CH_2-N), 3.92 (2 H, apparent t, *J* = 6.4, 6.3 Hz, CH_2Cl), 4.38 (2 H, apparent t, *J* = 6.4, 6.3 Hz, CH_2-N(Azole)), 7.34-7.29 (1 H, m, aromatics), 7.39 (2 H, m, aromatics), 7.75 (2 H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 171.4, 151.7, 140.1, 133.4, 128.3, 128.1, 127.4, 111.8, 53.2, 51.1, 50.0, 46.7, 42.6, 9.6; *m/z* [EI] 334 (M^+-CO_2Me, 4), 320 (16), 233 (100). Further elution with the same solvent gave **10** as an yellow oil (386 mg, 30 %); ν_{max}/cm^{-1} (ATR) 1732, 1435, 1198, 1159, 701; δ_H (400 MHz; $CDCl_3$) 2.36 (3 H, s, CH_3), 3.19 (2 H, apparent t, *J* = 7.0, 6.5 Hz, CH_2-N), 3.50 (4 H, s, CH_2CO_2Me), 3.51 (4 H, s, CH_2CO_2Me), 3.59 (6 H, s, CO_2Me), 3.67 (6 H, s, CO_2Me), 3.82 (2 H, s, Azole-CH_2-N), 4.21 (2 H, apparent t, *J* = 7.1, 6.2 Hz, CH_2-N(Azole)), 7.31 (1 H, m, aromatic), 7.38 (2 H, m, aromatics), 7.73 (2 H, d, *J* = 8.2 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 171.6, 151.1, 139.9, 133.8, 128.4, 128.1, 127.3, 111.7, 55.5, 54.4, 53.2, 51.4, 51.2, 48.4, 46.8, 9.5; *m/z* [FAB] (Found: M^++H, 519.2457. $C_{25}H_{34}N_4O_8$ calcd 519.2455).

1-(2-Bromoethyl)-5-methyl-3-phenylpyrazole (16a).

This compound was synthesized according to general alkylation procedure described by us for the similar compounds.[2] A mixture of 3-phenyl-5-methylpyrazole (3 g, 0.019 mol), NaOH (2.28 g, 0.057 mol), BTBA (153 mg, 0.48 mmol), 1,2-dibromoethane (35.7 g, 0.19 mol) and H_2O (6 mL) was refluxed for 6.5 h. Purification of the residue by column chromatography on silica gel (CH_2Cl_2 / EtOH 98:2) gave **16a** as an yellow oil (2.7 g, 54 %); mp (picrate) 132-134 °C (EtOH). Anal. Calcd for $C_{12}H_{13}N_2Br$·picrate:C, 43.74; H, 3.26; N, 14.17. Found: C, 44.01; H, 3.32; N, 14.10. ν_{max}/cm^{-1} (film) 1550, 1445, 1375, 1305, 770, 700; δ_H (400 MHz; $CDCl_3$) 2.36 (3 H, s, CH_3), 3.77 (2 H, t, *J* = 6.7 Hz, CH_2Br), 4.27 (2 H, t, *J* = 6.7 Hz, CH_2-N(Azole)), 6.33 (1 H, s, H_4), 7.29 (1 H, m, aromatic), 7.39 (2 H, m, aromatics), 7.77 (2 H, d, *J* = 7.4 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 150.8, 139.9, 133.3, 128.4, 127.4, 125.3, 102.6, 49.8, 30.1, 11.0; *m/z* [EI] 266 (M^++2, 23), 264 (M^+, 24), 171 (36), 158 (100), 130 (24), 77 (31). Further elution with the same solvent gave 1-(2-bromoethyl)-3-methyl-5-phenylpyrazole as an yellow oil (1.01 g, 20 %); ν_{max}/cm^{-1} (film) 1550, 1495, 1450, 1295, 770, 705; δ_H (400 MHz; $CDCl_3$) 2.31 (3 H, s, CH_3), 3.68 (2 H, t, *J* = 7.0 Hz, CH_2Br), 4.39 (2 H, t, *J* = 7.0 Hz, CH_2-N(Azole)), 6.08 (1 H, s, H_4), 7.45-7.38 (5 H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 148.5, 144.9, 130.4, 128.8, 128.6, 128.5, 106.0, 49.9, 29.6, 13.4; *m/z* [EI] 266 (M^++ 2, 6), 264 (M^+, 8), 171 (19), 158 (100), 128 (18), 77 (27). Further elution, using a mixture of CH_2Cl_2 / EtOH 9:1 as eluent, gave 1,2-bis(5-methyl-3-phenylpyrazol-1-yl)ethane as a white solid (52 mg, 1 %); mp 194-196 °C (CH_2Cl_2 / Hexane). Anal. Calcd for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36. Found: C, 77.42; H, 6.49; N, 16.19. ν_{max}/cm^{-1} (ATR) 1551, 1437, 1360, 1314, 767, 687; δ_H (400 MHz; $CDCl_3$) 1.74 (6 H, s, CH_3), 4.25 (4 H, s, CH_2N), 6.19 (2 H, s, H_4), 7.30 (2 H, m, aromatics), 7.40 (4 H, m, aromatics), 7.76 (4 H, d, *J* = 7.5 Hz, aromatics);δ_C (100 MHz; $CDCl_3$) 151.0, 141.2, 133.5, 128.5, 127.5, 125.4, 102.4, 49.0, 9.8; *m/z* [EI] 342 (M^+, 7), 184 (100), 77 (38).

{[2-(5-Methyl-3-phenylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid methyl ester (17a).

A mixture of **16a** (2.1 g, 7.9 mmol) and methyl iminodiacetate (2.8 g, 17.4 mmol) was heated at 110 °C for 5 h. After cooling, the mixture was extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$ and organic solvent was evaporated *in vacuo.* Purification of the residue by column chromatography on silica gel (CH_2Cl_2) gave **17a** as an yellow oil (2.45 g, 90 %); ν_{max}/cm^{-1} (film) 1745, 1440, 1205, 735; δ_H (400 MHz; $CDCl_3$) 2.34 (3 H, s, CH_3), 3.21 (2 H, t, *J* = 6.6 Hz, CH_2-N), 3.49 (4 H, s, CH_2CO_2Me), 3.66 (6 H, s, CO_2CH_3), 4.19 (2 H, t, *J* = 6.6 Hz, CH_2-N(Azole)), 6.28 (1 H, s, H_4), 7.26 (1 H, m, aromatic), 7.35 (2 H, m, aromatics), 7.73 (2 H, d, *J* = 7.6 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 171.2, 149.9, 139.8, 133.5, 128.1, 126.9, 125.0, 102.1, 55.1, 54.1, 51.1, 48.0, 10.7; *m/z* [EI] 345 (M^+, 5), 286 (5), 187 (28), 174 (37), 158 (16), 146 (29), 128 (54), 114 (23), 77 (14), 45 (100). (Found: M$^+$, 345.1679. $C_{18}H_{23}N_3O_4$ calcd 345.1683).

{[2-(4-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-ylmethoxy-methyl}-5-methyl-3-phenylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid methyl ester (18)

Following the general procedure above, we used **17a** (1.0 g, 2.9 mmol) and paraformaldehyde (0.26 g) in 1,2-dichloroethane (3 mL). Subsequently, water (3 mL) was added and the reaction mixture was stirred at rt for 10 h. Purification of the residue by column chromatography on silica gel (Hexane/EtOH, 8:2) gave **12** as a colorless oil (308 mg, 35 %); ν_{max}/cm^{-1} (KBr) 3260, 1760, 1735, 1440, 1310, 1200, 1165, 1005, 715; δ_H (200 MHz; $CDCl_3$) 2.38 (3 H, s, CH_3), 3.19 (2 H, t, *J* = 6.5 Hz, CH_2-N), 3.47 (4 H, s, C*H*$_2$CO$_2$Me), 3.67 (6 H, s, CO_2CH_3), 4.21 (2 H, t, *J* = 6.5 Hz, CH_2-N(Azole)), 4.58 (2 H, s, C*H*$_2$OH), 7.45-7.33 (3 H, m, aromatics), 7.73 (2 H, d, *J* = 6.6 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 171.5, 150.2, 139.6, 133.4, 128.4, 127.7, 127.5, 115.2, 55.4, 54.8, 54.5, 51.5, 48.3, 9.4; *m/z* [EI] 375 (M$^+$, 6), 357 (24), 316 (14), 284 (9), 187 (48), 174 (64), 146 (49), 128 (100), 116 (23), 77 (14), 56 (28). Further elution with the same solvent gave **18** as a colorless oil (212 mg, 20 %); ν_{max}/cm^{-1} (film) 1745, 1440, 1205, 915, 735; δ_H (200 MHz; $CDCl_3$) 2.36 (6 H, s, CH_3), 3.18 (4 H, t, *J* = 6.8 Hz, CH_2-N), 3.50 (8 H, s, C*H*$_2$CO$_2$Me), 3.65 (12 H, s, CO_2CH_3), 4.21 (4 H, t, *J* = 6.6 Hz, CH_2-N(Azole)), 4.42 (4 H, s, CH_2O), 7.39-7.29 (6 H, m, aromatics), 7.72 (4 H, d, *J* = 6.0 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 171.4, 150.6, 139.8, 133.4, 128.2, 127.7, 127.3, 112.4, 61.7, 55.4, 54.3, 51.4, 48.2, 9.3; *m/z* [FAB] 733 (M$^+$+H$^+$, 13), (Found: M+,733.3572. $C_{38}H_{48}N_6O_9$ calcd 733.3561).

{[2-(4-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-3,5-dimethyl-1*H*-pyrazol-4-ylmethoxymethyl}-3,5-dimethylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid methyl ester (20)

Following the general procedure above, we used **17b**^{2} (1.19 g, 4.20 mmol), paraformaldehyde (0.38 g) in 1,2-dichloroethane (5 mL). Subsequently, water (3 mL) was added and the reaction mixture was stirred at rt for 10 h. Purification of the residue by column chromatography on silica gel (CH_2Cl_2/EtOH, 95:5) gave **19** as an yellow oil (0.58 g, 41 %); ν_{max}/cm^{-1} (film) 3350, 1745, 1435, 1210, 1005; δ_H (200 MHz; $CDCl_3$) 2.22 (3H, s, CH_3), 2.26 (3 H, s, CH_3), 3.08 (2 H, t, *J* = 6.8 Hz, CH_2-N), 3.45 (4 H, s, C*H*$_2$CO$_2$Me), 3.67 (6 H, s, OCH_3), 4.09 (2 H, t, *J* = 7 Hz, CH_2-N(Azole)), 4.43 (2 H, br s, CH_2OH); δ_C (100 MHz; $CDCl_3$) 171.6, 146.8, 138.4, 115.9, 55.5, 54.8, 54.6, 51.5, 47.9, 11.6, 9.3; *m/z* [EI] 313 (M$^+$, 4), 295 (21), 254 (27), 187 (36), 174 (55), 146 (57), 128 (100), 116 (32), 56 (44). In this reaction, traces of the ether (**20**) were isolated; *m/z* [EI] 608 (M$^+$, 4), 549 (17), 312 (45), 296 (48), 188 (32), 174 (90), 146 (60), 128 (100), 116 (40), 100 (22), 59 (25). To a solution of alcohol (**19**) (200 mg, 0.64 mmol) in CH_2Cl_2 (3 mL, stabilized with amilene) was added CF_3CO_2H (3 drops) and the reaction mixture was refluxed for 4.5 h. After cooling was added H_2O (5 mL) and the water layer was made alkaline with Na_2CO_3 and extracted with

CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$ and evaporated *in vacuo*. Purification of the residue by column chromatography on silica gel (CH_2Cl_2/EtOH, 98:2) gave **20** as an yellow oil (109 mg, 56 %); ν_{max}/cm^{-1} (film) 1741, 1436, 1204, 1047; δ_H (400 MHz; $CDCl_3$) 2.16 (6 H, s, CH_3), 2.21 (6 H, s, CH_3), 3.07 (4 H, t, J = 7 Hz, CH_2N), 3.47 (8 H, s, CH_2CO_2Me), 3.65 (12 H, s, OCH_3), 4.06 (4 H, t, J = 7 Hz, CH_2-N(Azole)), 4.22 (4 H, s, CH_2O); δ_C (100 MHz; $CDCl_3$) 171.2, 146.9, 138.2, 112.8, 61.2, 55.2, 54.2, 51.2, 47.6, 11.3, 9.1; *m/z* [EI] 608 (M^+, 4), 549 (17), 312 (45), 296 (48), 188 (32), 174 (90), 146 (60), 128 (100), 116 (40), 100 (22), 59 (25). (Found: M+, 608.3188. $C_{28}H_{44}N_6O_9$ calcd 608.3170).

{[2-(4-{2-[2-(2-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-yl-methoxy}ethoxy)ethoxy]ethoxymethyl}-5-methyl-3-phenylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid methyl ester (21b)

To a solution of **14**[5] (780 mg, 1.98 mmol) in dichloroethane (1.5 mL) was added triethylene glycol (149 mg, 0.99 mmol) and the reaction mixture was heated at 80 °C for 2 h. After cooling was added H_2O (5 mL) and the water layer was made alkaline with Na_2CO_3 and extracted with CH_2Cl_2 (stabilized with amilene). The combined organic extracts were dried with $MgSO_4$ and evaporated *in vacuo*. Purification of the residue by column chromatography on silica gel (CH_2Cl_2/EtOH, 98:2) gave **21b** as an yellow oil (294 mg, 39 %); ν_{max}/cm^{-1} (ATR) 1743, 1478, 1202, 777, 702; δ_H (400 MHz; $CDCl_3$) 2.34 (6 H, s, CH_3), 3.19 (4 H, t, J = 6.9 Hz, CH_2-N), 3.51 (8 H, s, CH_2CO_2Me), 3.64 (12 H, br s, CH_2O), 3.67 (12 H, CO_2Me), 4.20 (4 H, apparent t, J = 7.1, 6.4 Hz, CH_2-N(Azole)), 4.39 (4 H, s, Azole-CH_2-O), 7.25-7.38 (6 H, m, aromatics), 7.70 (4 H, d, J = 8 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 171.4, 150.8, 139.7, 133.5, 128.2, 127.9, 127.3, 112.4, 70.45, 70.42, 68.6, 63.0, 55.4, 54.3, 51.4, 48.2, 9.5; *m/z* [FAB]. (Found: M+ 865.4346. $C_{44}H_{61}N_6O_{12}$, calcd 865.4347).

Basic hydrolysis. General procedure.

A mixture of corresponding ester (1 equivalent) and (0.6 %; H_2O MQ), NaOH (4 equivalents) was stirred at room temperature until consumption of the starting material was detected by TLC. Subsequently, the reaction mixture was washed with CH_2Cl_2 and the water was removed *in vacuo*.

({2-[4-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-3,5-dimethyl-1*H*-pyrazol-4-ylmethoxy}methyl-3,5-dimethylpyrazol-1-yl]ethyl}methoxycarbonylmethylamino)acetic acid sodium salt (5a)

According to the general procedure we used **20** (290 mg, 0.48 mmol) and NaOH (76 mg, 1.9 mmol). The mixture was stirred at rt for 20 h to give **5a** (261 mg, 86 %) as a white solid. ν_{max}/cm^{-1} (ATR) 3431, 1603, 1435, 1410, 762; δ_H (400 MHz; D_2O) 2.06 (6 H, s, CH_3), 2.17 (6 H, s, CH_3), 2.86 (4 H, apparent t, J = 7.8, 7.3 Hz, CH_2-N), 3.17 (8 H, s, CH_2CO_2Na), 4.08 (4 H, apparent t, J = 7.8, 7.6 Hz, CH_2-N(Azole)), 4.29 (4 H, s, CH_2O); δ_C (100 MHz; D_2O) 179.0, 148.2, 140.5, 112.5, 60.1, 58.3, 53.3, 46.2, 10.0, 8.3.

{[2-(4-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-ylmethoxymethyl}-5-methyl-3-phenylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid sodium salt (5b)

According to the general procedure we used **18** (200 mg, 0.27 mmol) and NaOH (44 mg, 1.1 mmol). The mixture was stirred at rt for 54 h to give **5b** (188 mg, 90 %) as a white solid. ν_{max}/cm^{-1} (ATR) 3420, 1595, 1410; δ_H (400 MHz; D_2O) 2.24 (6 H, s, CH_3), 2.92 (4 H, t, J = 7.7 Hz, CH_2-N), 3.20 (8 H, CH_2CO_2Na), 4.18 (4 H, t, J = 7.7 Hz, CH_2-N(Azole)), 4.36 (4 H, s, CH_2O), 7.34-7.35 (6 H, m, aromatics), 7.43 (4 H, m, aromatics); δ_C (100 MHz; D_2O) 178.3, 149.8, 140.7, 131.3, 127.7, 127.3, 126.8, 111.2, 59.4, 57.7, 52.6, 46.0, 27.7.

({2-[4-(2-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-yl-methoxy}ethoxymethyl)-5-methyl-3-phenylpyrazol-1-yl]ethyl}methoxycarbonylmethylamino)acetic acid sodium salt (22a)

According to the general procedure we used the corresponding ester (**21a**)[5] (52 mg, 0.067 mmol) and NaOH (11 mg, 0.27 mmol). The mixture was heated at 60 °C for 24 h to give **22a** (50 mg, 93%) as a white solid. ν_{max}/cm^{-1} (ATR) 3282, 1572, 1407, 1119, 712; δ_H (400 MHz; D_2O) 2.26 (6 H, s, CH_3), 2.97 (4 H, apparent t, J = 7.7, 7.0 Hz, CH_2N), 3.22 (8 H, s, CH_2CO_2Na), 3.64 (4 H, CH_2O), 4.20 (4 H, apparent t, J = 7.6, 7.4 Hz, CH_2-N(Azole)), 4.31 (4 H, s, Azole-CH_2O), 7.19 (4 H, m, aromatics), 7.25 (2 H, m, aromatics), 7.49 (4 H, m, aromatics) ; δ_C (100 MHz; D_2O) 177.5, 150.0, 140.6, 131.2, 127.6, 127.3, 127.0, 111.5, 67.7, 61.4, 57.6, 52.7, 45.9, 7.8.

{[2-(4-{2-[2-(2-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-yl-methoxy}ethoxy)ethoxy]ethoxymethyl}-5-methyl-3-phenylpyrazol-1-yl)ethyl]methoxycarbonylmethylamino}acetic acid sodium salt (22b)

According to the general procedure we used the corresponding ester (**21b**) (219 mg, 0.25 mmol) and NaOH (40 mg, 1 mmol). The mixture was stirred at rt for 24 h to give **22b** (181 mg, 81 %) as a white solid. ν_{max}/cm^{-1} (ATR) 3443, 1598, 1333; δ_H (400 MHz; D_2O) 2.26 (6 H, s, CH_3), 2.95 (4 H, apparent t, J = 7.6, 6.7 Hz, CH_2N), 3.21 (8 H, s, CH_2CO_2Na), 3.52 (4 H, s, CH_2O), 3.56 (8 H, br s, CH_2O), 4.18 (4 H, apparent t, J = 7.5, 7.4 Hz, CH_2-N(Azole)), 4.31 (4 H, s, Azole-CH_2O), 7.40-7.32 (6 H, m, aromatics), 7.52 (4 H, m, aromatics); δ_C (100 MHz; D_2O) 178.9, 150.7, 141.5, 132.2, 128.7, 128.3, 127.6, 112.2, 69.8, 69.5, 68.2, 62.3, 58.5, 53.5, 46.8, 8.7.

({2-[4-(2-{1-[2-(Bismethoxycarbonylmethylamino)ethyl]-5-methyl-3-phenyl-1*H*-pyrazol-4-ylmethylsulfanyl}ethylsulfanylmethyl)-5-methyl-3-phenylpyrazol-1-yl]ethyl}methoxycarbonylmethylamino)-acetic acid sodium salt (22c)

According to the general procedure we used the corresponding ester (**21c**)[5] (196 mg, 0.24 mmol) and NaOH (39 mg, 0.97 mmol). The mixture was heated at 60 °C for 24 h to give **22c** (178 mg, 87 %) as a white solid. ν_{max}/cm^{-1} (ATR) 3442, 1597, 1405; δ_H (400 MHz; D_2O) 2.22 (6 H, s, CH_3), 2.32 (4 H, s, CH_2S), 2.92 (4 H, apparent t, J = 7.8, 7.2 Hz, CH_2N), 3.19 (8 H, s, CH_2CO_2Na), 3.49 (4 H, s, Azole-CH_2S), 4.17 (4 H, apparent t, J = 7.6, 7.4 Hz, CH_2-N(Azole)), 7.39-7.30 (6 H, m, aromatics), 7.46 (4 H, m, aromatics); δ_C (100 MHz; D_2O) 178.4, 149.1, 139.4, 131.6, 128.1, 127.6, 127.3, 111.5, 57.9, 53.0, 46.2, 30.3, 23.6, 8.2.

Determination of relaxivity. ^{1}H NMR relaxation times T_1 and T_2 of aqueous solutions of Gadolinium complexes of **5** and **22** were measured to determined relaxivity r_1 and r_2. All measurements were made using a Bruker Minispec NMR spectrometer operating at 60 MHz. Before each measurement the spectrometer was tuned and calibrated. T_1 values were determined by the inversion-recovery method (d1-180^0 (ph1)-τ-90^0(ph1)-aq) and T_2 values were determined by the Carr-Purcell-Maiboom-Gill sequence (d1-90^o(ph1)-[τ-180^o(ph2)-τ]$_n$-aq), using, in both cases, not less than 3 different τ values. Three different measurements of T_1 or T_2 were performed in every sample. Typically, 5 mM compounds (**5-22**) and DTPA were dissolved in 100 mM Tris/HCl, 150 mM NaCl containing or not 0.5 mM Gd(III) in the cases of complexones (**5-22**) and 5 mM Gd(III) in the DTPA measurements and the final pH was adjusted with HCl and NaOH solutions. These pH values were direct read as measured on a Crison micropH 2002 equipped with a glass electrode at 20 °C. The glass electrode was calibrated by measuring the electromotive force of standar buffers at pH 4.000 and 7.020. The paramagnetic contribution was measured using the corresponding complex in same conditions. Relaxivities $r_{1(2)}$ were calculated according to the expression:

$$r_{1(2)} = \Delta(1 / T_{1(2)}) / [\mathrm{Gd(III)}]$$

where, for every complexone, Δ is the difference in relaxation rates ($1 / T_{1(2)}$) of the water protons in the presence and absence of Gd(III), and [Gd(III)] the molar concentration of Gd(III).

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