SYNTHESIS OF LOSOXANTRONE METABOLITES ISOLATED FROM HUMAN URINE

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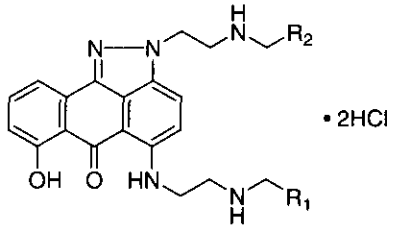
Abstract - The first regiospecific synthesis of the monoacid metabolites (**2**) and (**3**) of losoxantrone is reported. In addition, synthesis of the diacid metabolite (**4**) *via* two different routes is also described. The chemical structures of these metabolites as well as their intermediates have been fully characterized.

Losoxantrone (DuP 941, biantrazole, (**1**)) is an antitumor agent in clinical studies for the treatment of breast cancer.[1]

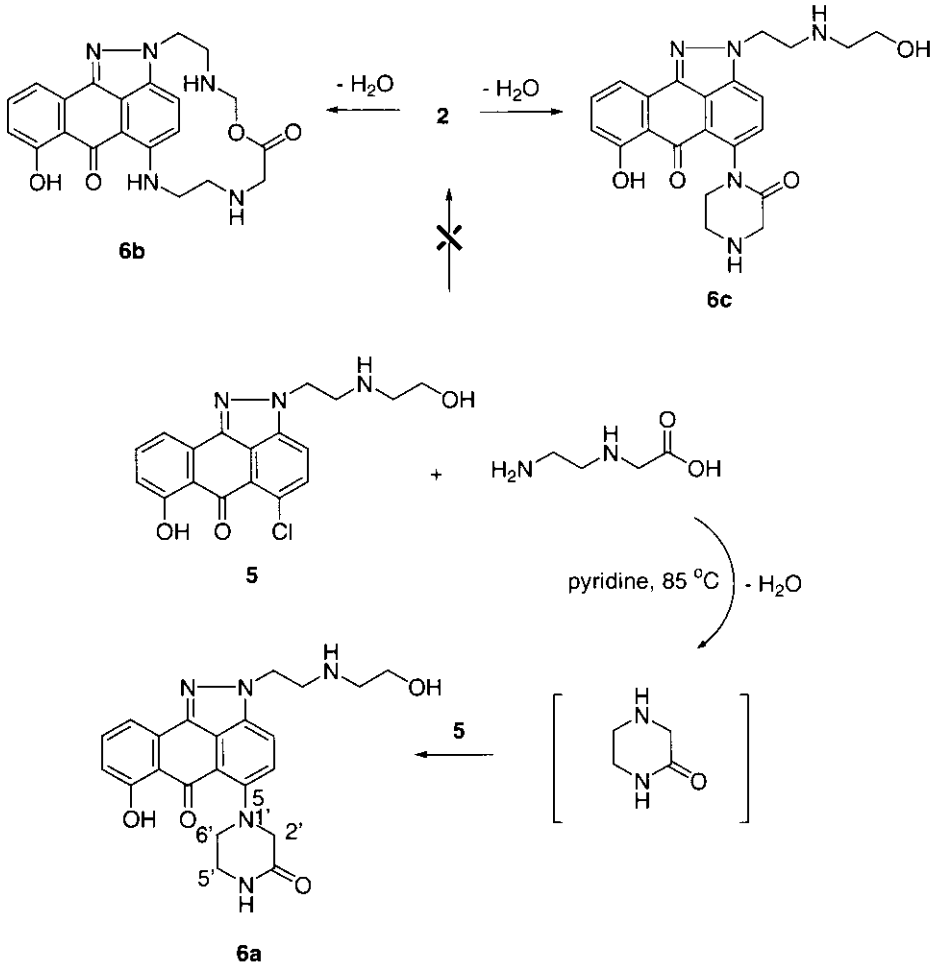


1 (losoxantrone, DuP 941, biantrazole), $R_1 = R_2 = CH_2OH$
2, $R_1 = CO_2H$, $R_2 = CH_2OH$
3, $R_1 = CH_2OH$, $R_2 = CO_2H$
4, $R_1 = R_2 = CO_2H$

Its metabolites detected by HPLC-MS from human urine have been reported.[2] The report, solely based on MS spectral data, suggested the presence of a monocarboxylic acid (**2** or **3**) and a dicarboxylic acid (**4**), derived from oxidation of hydroxyethylaminoethyl side chains. Later, a more sensitive HPLC trace implicated the presence of both regioisomeric monocarboxylic acids (**2** and **3**); however, no structural proof was presented.[3] To unambiguously determine the

presence of these metabolites, we desired to synthesize authentic standards. In addition, the question whether these metabolites possess anticancer activity as well as toxicity could then be answered. In this paper, we present the first synthesis of the monoacids (**2**) and (**3**), and the diacid (**4**).

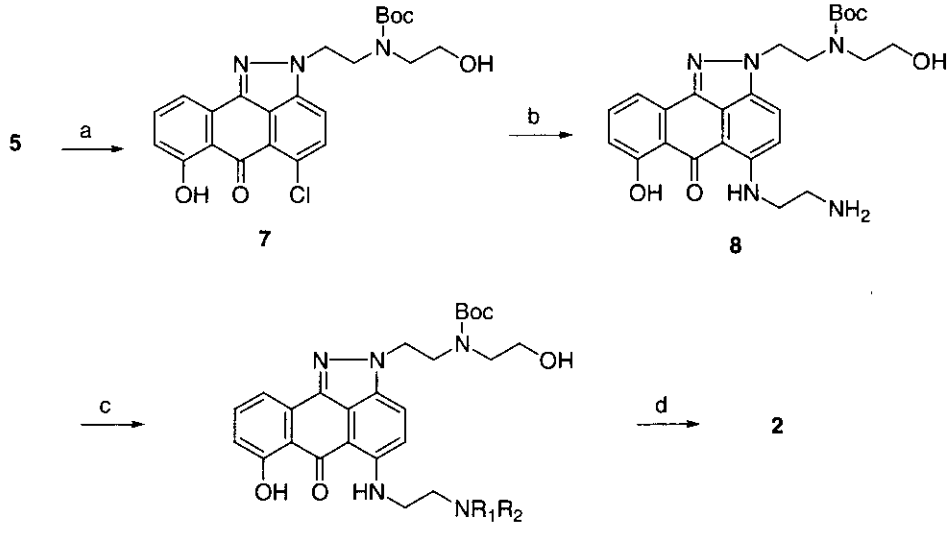
We envisioned that **2** could be derived in one-step from chloro displacement of **5**[4] with *N*-(2-aminoethyl)glycine.



To our surprise and disappointment, reaction of **5** with *N*-(2-aminoethyl)glycine in pyridine at 85 °C gave a product, which initially we thought was **2**. But its base peak at 422 (M - H) was 18 a.m.u. less than expected. The most likely explanation for the low mass is that the carboxylic group is involved in some form of internal cyclization with concomitant loss of water. One possible isomer would be the formation of the lactone with the terminal hydroxyl to give **6b**.

This structure can be ruled out by the detection of the terminal hydroxyl in the proton NMR spectrum. Assignment of the hydroxyl was confirmed by its correlation to the adjacent methylene in the COSY spectrum. Two other possible isomers are the lactams (**6a**) and (**6c**). Only **6a** can be confirmed as the unexpected product by NMR. The COSY spectrum shows that only one of the methylene protons (i.e., the 5' methylene) in the lactam is coupled to the secondary amide proton and the HMBC (Heteronuclear Multiple Bond Correlation) spectrum shows that the other two lactam methylene protons (i.e., the 2' and 6' methylenes) are long range coupled to the aromatic carbon at the 5 position. As the HMBC experiment was optimized for the observation of 3-bond-carbon-proton couplings, these observations confirm **6a** as the unexpected product. The formation of **6a** was rationalized by formation of piperazin-2-one *via* dehydration of *N*-(2-aminoethyl)glycine, which then reacted with **5** to give **6a** in 34% yield.

Scheme 1[a]



9a: R_1 = H, R_2 = CH_2CO_2Bu-*t*
9b: R_1 = R_2 = CH_2CO_2Bu-*t*

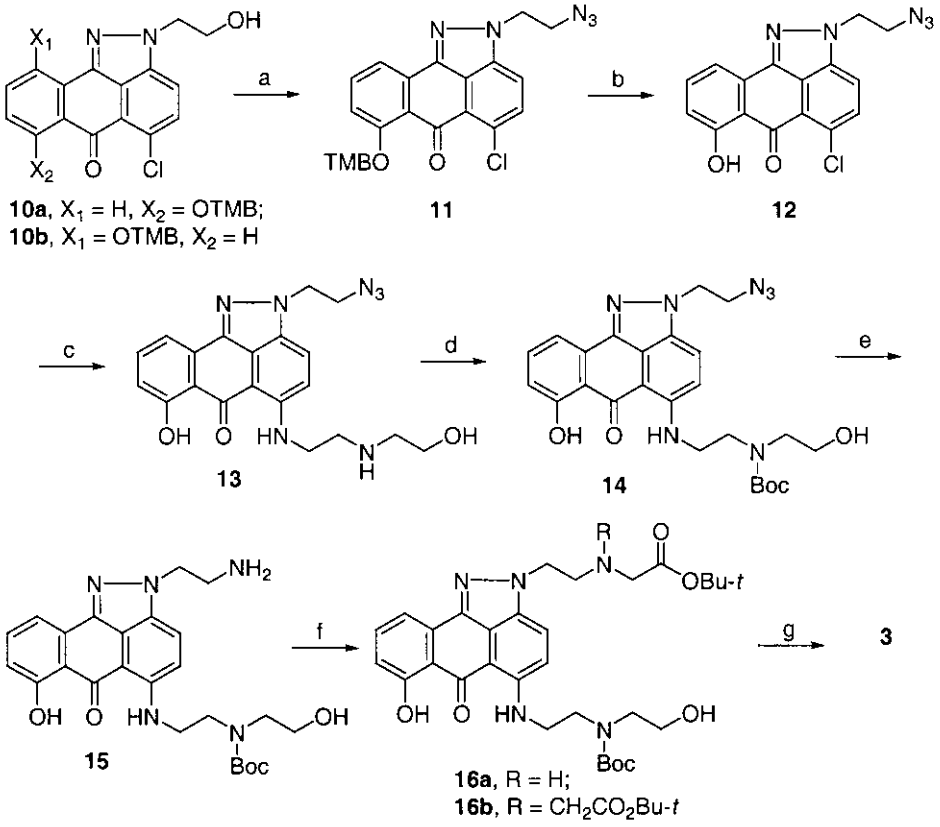
[a] Reagents and conditions: (a) Boc_2O, Et_3N, DMF, rt, 2 h, 87%;
(b) ethylenediamine, pyridine, 75-90 °C, 6 h, 50%;
(c) $BrCH_2CO_2Bu$-*t*, KI, Et_3N, THF, rt, 4 days, 62% (**9a**), 2.3% (**9b**),
27% (**8**); (d) 4 N HCl, dioxane, rt, overnight, then 53 °C, 1 h, 100%.

We then decided to install the oxidized side chain in **2** in a stepwise manner as depicted in Scheme 1. After protection of the amino group **5** as its Boc derivative (**7**) (87% yield),

displacement of chlorine with ethylenediamine afforded **8** in 50% yield. Subsequent *N*-alkylation with *tert*-butyl bromoacetate produced a mixture of *mono*- and *di*-alkylated products (**9a**) and (**9b**), and starting material (**8**). The desired compound (**9a**) was isolated in 62% yield after flash chromatography. Hydrolysis of the *tert*-butyl ester and simultaneous removal of the Boc protecting group were effected by 4 N HCl in dioxane to yield **2** quantitatively.

Synthesis of metabolite (**3**) was accomplished in seven steps from the mixture (**10a/10b**)[4] as shown in Scheme 2.

Scheme 2[a]



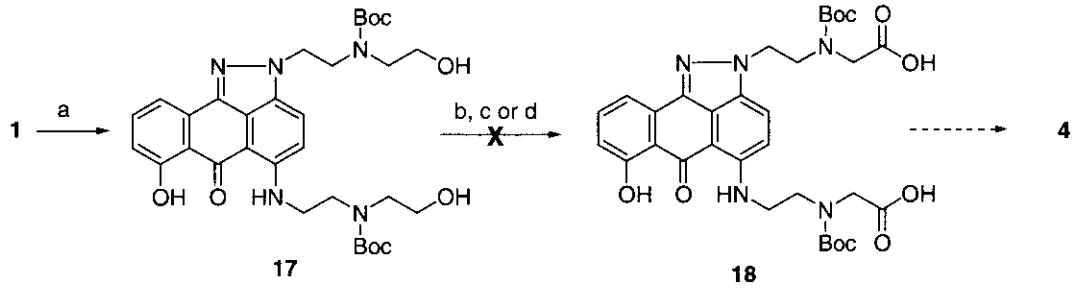
10a, X_1 = H, X_2 = OTMB;
10b, X_1 = OTMB, X_2 = H

[a] Reagents and conditions: (a) $(PhO)_2P(O)N_3$, DBU, toluene, rt, 0.5 h, 84 °C, 7 h, 75-80 °C, overnight, 58%; (b) 4 N HCl, CH_2Cl_2, 0 °C to rt, overnight, 67%; (c) $H_2N(CH_2)_2NH(CH_2)_2OH$, pyridine, 85-90 °C, 6 h, 66%; (d) Boc_2O, CH_2Cl_2, rt, overnight, 92%; (e) PPh_3, THF, H_2O, 58-64 °C, 3 h, 90%; (f) $BrCH_2CO_2Bu$-*t*, Et_3N, THF, rt, 28 h, 70% (**16a**), 5% (**16b**), 10% (**15**); g) 4 N HCl, dioxane, rt, 2 days, 93%.

Treatment of (**10a/10b**) (4:1 ratio) with diphenylphosphoryl azide[5] gave the azide (**11**) in 58% yield. The *O*-trimethylbenzyl protecting group in **11** was readily hydrolyzed by aqueous hydrochloric acid, furnishing **12** in 67% yield. Nucleophilic chloro-displacement with 2-(2-aminoethylamino)ethanol produced **13** in 66% yield. After protection of the amine as its Boc derivative (**14**), the azide was reduced with PPh_3 in aqueous THF to give **15** in 90% yield. The remaining steps in the synthesis were similar to those of **2** as described above. That is, *N*-alkylation produced a mixture of **16a** (70% yield), **16b** (5% yield) and **15** (10%), and acid hydrolysis of **16a** furnished **3** in 93% yield.

Our initial strategy for synthesis of the diacid (**4**) was to protect the secondary amine moieties in **1** as their Boc derivatives to give **17**, followed by hydroxyl oxidation to **18,** and then removal of the Boc protecting groups by acid hydrolysis (Scheme 3).

Scheme 3[a]



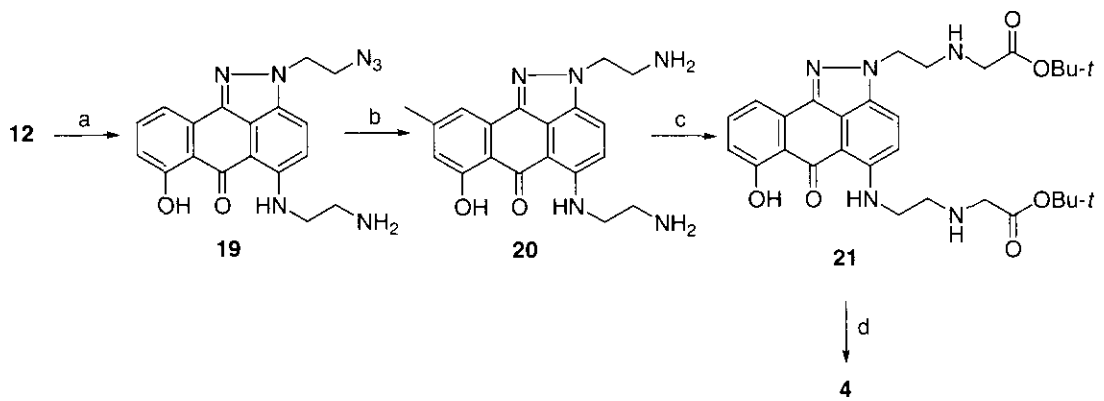
[a] Reagents and conditions: (a) Boc_2O, Et_3N, DMF, rt, 3 h, 96%; (b) $RuCl_3$, $NaIO_4$, aqueous MeCN, rt, overnight; (c) CrO_3, aqueous H_2SO_4, acetone, rt, overnight; (d) pyridium dichromate, DMF, rt, overnight.

Reaction of **1** with Boc_2O using standard protocol furnished **17** in 96% yield. Unfortunately, oxidation of **17** with $RuCl_3/NaIO_4$[6] in aqueous acetonitrile gave no reaction and only starting material was recovered. When **17** was treated with chromic trioxide[7] in aqueous sulfuric acid or pyridinium dichromate[8] in DMF, both reactions resulted in complex mixtures.

We then pursued the route depicted in Scheme 4. Reaction of the azido chloride (**12**) with ethylenediamine in pyridine at 83 °C gave **19** in 80% yield. The azide in **19** was reduced with

PPh_3 in aqueous THF at 65 °C to obtain the amine (**20**) in 73% yield. Subsequent alkylation with 1 equivalent of *tert*-butyl bromoacetate in the presence of Et_3N in DMF gave a mixture of products from which the desired **21** was isolated in 9.4% yield after tedious flash chromatography. Treatment of **21** with 4 N HCl in dioxane at room temperature furnished **4** in 66% yield.

Scheme 4[a]

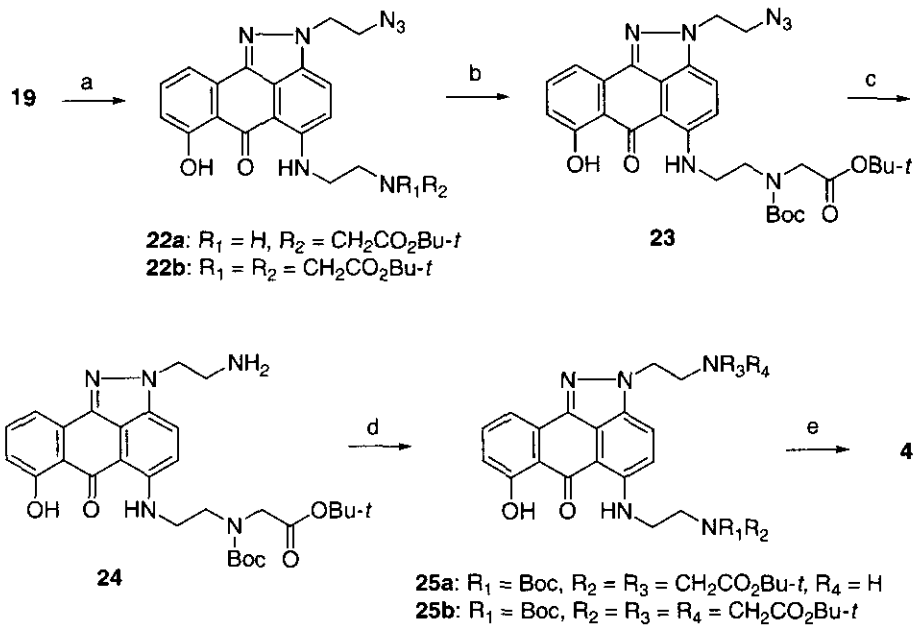


[a] Reagents and conditions: (a) ethylenediamine, pyridine, 85 °C, 4.5 h, 80%; (b) PPh_3, THF, H_2O, 65 °C, 2 h, 73%; (c) $BrCH_2CO_2Bu$-*t*, Et_3N, DMF, rt, 3 h, 9.4%; (d) 4 N HCl, dioxane, rt, 4 h, 66%.

Due to the poor yield of **21**, an alternate synthesis of **4** was developed as shown in Scheme 5. *N*-Alkylation of azide (**19**) with *tert*-butyl bromoacetate in the presence of diisopropylethylamine in *N,N*-dimethylacetamide (DMAC) at room temperature overnight produced **22a** (50% yield), **22b** (22% yield) and recovered starting material **19** (11% yield). With **22a** in hand, the remaining 4-step sequence toward **4** was similar to the synthesis of **3**. Thus, Boc protection of the amine gave **23** in 94% yield, and the azide was reduced to the amine (**24**) in 54% yield. Subsequent alkylation and chromatography led to isolation of **25a** (63% yield), **25b** (15% yield) and **24** (11% yield). Acid hydrolysis of **25a** produced **4** in 84% yield.

In summary, we have achieved the regiospecific synthesis of the monoacid metabolites (**2**) and (**3**) of losoxantrone. In addition, we have also completed the synthesis of the diacid metabolite (**4**) *via* two different routes. The chemical structures of these metabolites as well as their intermediates have been fully characterized.

Scheme 5[a]



[a] Reagents and conditions: (a) $BrCH_2CO_2Bu$-*t*, $(i\text{-}Pr)_2NEt$, DMAC, rt, overnight, 50% (**22a**), 22% (**22b**); (b) Boc_2O, CH_2Cl_2, rt, overnight, 94%; (c) PPh_3, THF, H_2O, 58-64 °C, 3 h, 54%; (d) $BrCH_2CO_2Bu$-*t*, $(i\text{-}Pr)_2NEt$, DMAC, rt, overnight, 63% (**25a**), 15% (**25b**), 11% (**24**); (e) 4 N HCl, rt, overnight, then 50 °C, 1 h, 84%.

EXPERIMENTAL

All reactions were carried out with continuous stirring under an atmosphere of dry nitrogen. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on either Varian VXRS-300 or VXRS-400 spectrometers using tetramethylsilane as an internal standard. Melting points are uncorrected. TLC was performed on E. Merck 15719 silica gel 60 (230-400 mesh). Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ.

7-Hydroxy-2-[2-[(2-hydroxyethyl)*amino*]ethyl]-5-(3-oxo-1-piperazinyl)anthra[1,9-*cd*]-pyrazol-6(2*H*)-one (6a) A mixture of **5** (0.36 g, 0.9 mmol), *N*-(2-aminoethyl)glycine (1.0 g, 8.5 mmol) in 9 mL of pyridine was heated at 80 °C for 20 h. After cooling, the solvent and excess reagent in the solution were evaporated. Flash chromatography (silica gel, 10% MeOH/CH_2Cl_2 with 0-1% NH_4OH) gave 0.13 g (34% yield). **6a**: mp 168-170 °C; IR (KBr) 3396, 3304, 3286, 3200, 3092, 2926, 2848, 1680, 1662, 1640, 1612, 1580, 1494, 1448, 1404, 1388, 1378, 1360,

1332, 1284, 1254, 1204, 1182, 1160, 1030, 994, 858, 824, 790, 778 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.02 (s, 1H), 8.13 (s, 2H), 8.12 (d, 1H, *J* = 9.0 Hz), 7.43 (m, 1H, *J* = 7.5, 6.8 Hz), 7.61 (m, 1H, *J* = 7.5, 2.1 Hz), 7.41 (d, 1H, *J* = 9.0 Hz), 6.93 (m, 1H, *J* = 6.8, 2.1 Hz), 4.63 (t, 2H, *J* = 6.1 Hz), 4.46 (br s, 1H), 3.97 (s, 2H), 3.61 (m, 2H), 3.52 (m, 2H), 3.39 (t, 2H, *J* = 5.6 Hz), 3.09 (t, 2H, *J* = 6.1 Hz), 2.61 (t, 2H, *J* = 5.6 Hz); HRMS calcd for $C_{22}H_{24}N_5O_4$ 422.1828, found 422.1821. Anal. Calcd for $C_{22}H_{25}N_5O_4$•0.5 H_2O: C, 61.39; H, 5.62, N, 16.27. Found: C, 61.58; H, 5.45; N, 16.56.

1,1-Dimethylethyl [2-(5-chloro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-2(6*H*)-yl]ethyl] (2-hydroxyethyl)carbamate (7) To a suspension of **5** (1.0 g, 2.8 mmol) and triethylamine (0.28 g, 2.8 mmol) in 12 mL of DMF was added a solution of di-*tert*-butyl dicarbonate (0.61 g, 2.8 mmol) in 2 mL of DMF. After stirring at rt for 2 h, the solution was poured into ice water. The aqueous mixture was extracted with ethyl acetate. The combined extracts were washed with water, and dried (Na_2SO_4) and the solvent evaporated. Flash chromatography twice (silica gel 35 g, 0-2% MeOH/CH_2Cl_2; silica gel 25 g, 0-2% MeOH/CH_2Cl_2) furnished 1.11 g (87% yield). **7**: mp 78-82 °C; IR (KBr) 3434, 1692, 1638, 1612, 1596, 1482, 1452, 1220, 1178, 1158, 830, 784 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.30, 13.26 (2s, 1H), 7.66-7.51 (m, 4H), 7.03-7.00 (m, 1H), 4.74-4.68 (m, 2H), 4.06-3.60 (m, 5H), 3.29 (br, 1H), 3.16 (br, 1H), 1.22, 0.99 (2s, 9H); MS *m/z* 458.2 (M + H^+); HRMS calcd for $C_{23}H_{25}N_3O_5Cl$ 458.1483, found 458.1490. Anal. Calcd for $C_{23}H_{24}N_3O_5Cl$: C, 60.33; H, 5.28; N, 9.18; Cl, 7.74. Found: C, 60.38; H, 5.18; N, 9.20; Cl, 7.73.

1,1-Dimethyl ethyl[2-[5-[(2-aminoethyl)amino]-7-hydroxy-6-oxoanthra[1,9-*cd*] pyrazol-2(6*H*)-yl]ethyl](2-hydroxyethyl)carbamate (8) A solution of **7** (0.21 g, 0.46 mmol) and ethylenediamine (0.28 g, 4.7 mmol) in 1 mL of pyridine was heated at 75-90 °C (oil bath temperature) for 6 h. After evaporation, the residue was purified by flash chromatography (silica gel, 25 g, 0-5% MeOH/CH_2Cl_2) to give 0.11 g (50% yield). **8**: mp 78-88 °C; IR (KBr) 3348, 3290, 1690, 1660, 1604, 1572 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.90 (br, 1H), 8.87 (s, 1H), 7.60-7.30 (m, 3H), 6.94 (d, 1H, *J* = 7.7 Hz), 6.76 (d, 1H, *J* = 8.8 Hz), 4.66-4.60 (m, 2H), 3.80-3.68 (m, 4H), 3.51-3.45 (m, 2H), 3.27-3.10 (m, 4H), 1.21, 0.94 (2 s, 9H); MS *m/z* 482.2 (M + H^+); HRMS calcd for $C_{25}H_{32}N_5O_5$ 482.2403, found 482.2395. Anal. Calcd for $C_{25}H_{31}N_5O_5$: C, 62.36; H, 6.49; N, 14.54. Found: C, 62.00; H, 6.44; N, 14.38.

1,1-Dimethylethyl *N*-[2-[[2-[2-[[(1,1-dimethylethoxy)carbonyl](2-hydroxyethyl)amino]-ethyl]-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-5-yl]amino]ethyl]glycine (9a) A mixture of **8** (0.30 g, 0.62 mmol), potassium iodide (30 mg, 1.8 mmol), triethylamine (130 mg,

1.28 mmol) and *tert*-butyl bromoacetate (122 mg, 0.62 mmol) in 20 mL of THF was stirred at rt for 4 days. The solvent in the mixture was evaporated and the resulting residue was partitioned between water and dichloromethane. The aqueous layer was further extracted with CH_2Cl_2. The combined extracts were washed with water, and dried (Na_2SO_4) and the solvent evaporated. Flash chromatography furnished 0.23 g (62% yield) of **9a**, 0.01 g (2.3% yield) of **9b** and 0.08 g (27% yield) of **8**. **9a**: mp 59-66 °C; IR (KBr) 3426, 3285, 1732, 1694, 1660, 1604, 1572, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.98, 13.92 (2 s, 1H), 8.92 (br, 1H), 7.65-7.43 (m, 3H), 6.97-6.87 (m, 2H), 4.71, 4.65 (2 br, 2H), 3.81-3.70 (m, 4H), 3.57-3.52 (m, 2H), 3.41 (s, 2H), 3.34-3.01 (m, 4H), 1.79 (br, 1H), 1.48 (s, 9H), 1.20, 0.93 (2 s, 9H); MS *m/z* 596 (M + H^+); HRMS calcd for $C_{31}H_{42}N_5O_7$ 596.3084, found 596.3094. Anal. Calcd for $C_{31}H_{41}N_5O_7$: C, 62.51; H, 6.94; N, 11.76. Found: C, 62.34; H, 6.99; N, 11.60. **9b:** ^1H NMR ($CDCl_3$) δ 14.06, 13.99 (2 s, 1H), 8.96 (br, 1H), 7.66 (d, 1H, *J* = 7.3 Hz), 7.54 (t, 2H, *J* = 8.2 Hz), 7.02-6.95 (m, 2H), 4.70 (m, 2H), 3.82-3.59 (m, 6H), 3.55 (s, 4H), 3.28-3.13 (m, 4H), 1.48 (s, 18H), 1.21, 0.95 (2 s, 9H); MS *m/z* 732 (M + Na^+).

***N*-[2-[[2,6-Dihydro-7-hydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]-6-oxoanthra[1,9-*cd*]-pyrazol-5-yl]amino]ethyl]glycine Dihydrochloride (2)** The material was prepared in quantitative yield from **9a** following a procedure identical to that described for **4** (Method A). **2**: mp 244.5-248.5 °C (decomp); IR (KBr) 3510, 3390, 3298, 2816, 2742, 2406, 1754, 1660, 1616, 1604, 1576, 1564, 780 cm^{-1}; ^1H NMR (DMSO-d_6) δ 14.12 (s, 1H), 9.39 (br, 2H), 9.29 (br, 2H), 8.97 (m, 1H), 8.24 (d, 1H, *J* = 9 Hz), 7.72-7.64 (m, 2H), 7.42 (d, 1H, *J* = 9.5 Hz), 7.00-6.92 (m, 1H), 5.27 (br, 1H), 5.02-4.95 (m, 1H), 3.96 (s, 4H), 3.72-3.50 (m, 4H), 3.24 (m, 2H), 3.08 (m, 2H); MS *m/z* 440 (M + H^+); HRMS calcd for $C_{22}H_{26}N_5O_5$ 440.1934, found 440.1933. Anal. Calcd for $C_{22}H_{27}N_5O_5Cl_2 \cdot H_2O$: C, 49.82; H, 5.51; N, 13.20; Cl, 13.37. Found: C, 49.91; H, 5.15; N, 13.21; Cl, 13.48.

2-(2-Azidoethyl)-5-chloro-7-[(2,4,6-trimethylphenyl)methoxy]anthra[1,9-*cd*]pyrazol-6(2*H*)-one (11) To the solution of an isomeric mixture of **10a/10b** (4:1 ratio) (5.0 g, 11.2 mmol) and diphenylphosphoryl azide (3.70 g, 13.4 mmol) in 50 mL of toluene was added a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (2.05 g, 13.4 mmol) in toluene (10 mL) under nitrogen with cooling in an ice bath. After stirring at rt for 0.5 h, the reaction mixture was heated at 75-80 °C overnight. The product in the reaction mixture was collected on a filter and washed with toluene and water to give 2.05 g of the crude product. The toluene in the filtrate was evaporated, and the residue was treated with CH_2Cl_2 and MeOH (1:2.5) and placed in a

refrigerator to yield an additional 0.92 g of product. The crude products were combined and purified by flash chromatography (silica gel 50 g, 100% CH_2Cl_2) to give 2.44 g (58% yield). **11**: mp 201-202 °C; IR (KBr) 3420, 2116, 1656, 1606, 1590, 1558, 1480, 1454, 1316, 1256, 1206, 1004, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.84 (dd, 1H, *J* = 7, 1 Hz), 7.58 (t, 1H, *J* = 8 Hz), 7.52 (d, 2H, *J* = 3 Hz), 7.14 (dd, 1H, *J* = 8, 1 Hz), 6.90 (s, 2H), 5.23 (s, 2H), 4.57 (t, 2H, *J* = 6 Hz), 3.89 (t, 2H, *J* = 6 Hz), 2.43 (s, 6H), 2.29 (s, 3H); MS *m/z* 472(M + H^+); HRMS calcd for $C_{26}H_{23}N_5O_2Cl$, 472.1540, found 472.1530. The analytical sample was obtained by dissolving 50 mg of **11** in 5 mL of CH_2Cl_2, which was then triturated with 15 mL of MeOH. Anal. Calcd for $C_{26}H_{22}N_5O_2Cl$: C, 66.17; H, 4.70; N, 14.84; Cl, 7.51. Found: C, 65.97; H, 4.60; N, 14.81; Cl, 7.50.

2-(2-Azidoethyl)-5-chloro-7-hydroxyanthra[1,9-*cd*]pyrazol-6(2*H*)-one (12) An ice-cold suspension of **11** (1.0 g, 2.1 mmol) and 4 N HCl (5 mL) in 15 mL of dichloromethane was stirred at ambient temperature overnight. The solvent and excess reagent in the mixture were evaporated. The residue was treated with 5 mL of MeOH and 20 mL of Et_2O, and kept in a refrigerator for 0.5 h. The resulting precipitates were collected on a filter, and purified by flash chromatography (silica gel 35 g, 100% CH_2Cl_2) to give 0.48 g (67% yield). **12**: mp 164-165 °C; IR (KBr) 3416, 2134, 2104, 1636, 1608, 1594, 1570, 1452, 1218, 784 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.29 (s, 1H), 7.68-7.55 (m, 4H), 7.03 (dd, 1H, *J* = 7, 1 Hz), 4.61 (t, 2H, *J* = 5.5 Hz), 3.92 (t, 2H, *J* = 5.5 Hz); MS *m/z* 340 (M + H^+); HRMS calcd for $C_{16}H_{11}N_5O_2Cl$ 340.0601, found 340.0589. Anal. Calcd for $C_{16}H_{10}N_5O_2Cl$: C, 56.57; H, 2.97; N, 20.61; Cl, 10.44. Found: C, 56.39; H, 2.76; N, 20.69; Cl, 10.16.

2-(2-Azidoethyl)-7-hydroxy-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthra[1,9-*cd*]-pyrazol-6(2*H*)-one (13) A mixture of **12** (0.34 g, 1 mmol) and 2-(2-aminoethylamino)ethanol (1.04 g, 10 mmol) in 2 mL of pyridine was heated at 85-90 °C (oil bath temperature) for 6 h. After cooling to rt, the mixture was treated with 3 mL of isopropanol, and then kept in a refrigerator overnight. The precipitates were collected on a filter and purified by flash chromatography (silica gel 25 g, 0-10% MeOH/CH_2Cl_2) to give 0.27 g (66% yield). **13**: mp 126-127.5 °C; IR (KBr) 3286, 2108, 1660, 1574, 1554 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.88 (s, 1H), 9.05 (br, 1H), 7.71 (dd, 1H, *J* = 6.5, 1 Hz), 7.59-7.53 (m, 2H), 6.98-6.92 (m, 2H), 4.59 (t, 2H, *J* = 5.5 Hz), 3.89 (t, 2H, *J* = 5.5 Hz), 3.73 (t, 2H, *J* = 5 Hz), 3.60 (q, 2H, *J* = 6 Hz), 3.09 (t, 2H, *J* = 6 Hz), 2.92 (t, 2H, *J* = 5 Hz); MS *m/z* 408 (M + H^+); HRMS calcd for $C_{20}H_{22}N_7O_3$ 408.1784, found 408.1788. Anal. Calcd for $C_{20}H_{21}N_7O_3$: C, 58.96; H, 5.20; N, 24.07. Found: C, 58.68; H, 5.12;

N, 23.87.

1,1-Dimethylethyl [2-[[2-(2-azidoethyl)-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-5-yl]amino]ethyl](2-hydroxyethyl)carbamate (14) A solution of **13** (0.2 g, 0.49 mmol) and di-*tert*-butyl dicarbonate (0.11 g , 0.49 mmol) in 20 mL of dichloromethane was stirred at rt overnight. The solvent in the solution was evaporated. Flash chromatography (silica gel 20 g, 0-2% MeOH/CH_2Cl_2) gave 0.23 g (92%). **14**: mp 60-63 °C; IR (KBr) 3432, 3280, 2104, 1690, 1660, 1604, 1572 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.89 (br, 1H), 8.89 (br, 1H), 7.73-7.54 (m, 3H), 6.99, 6.97 (2 s, 2H), 4.60 (t, 2H, *J* = 5.5 Hz), 3.89 (t, 2H, *J* = 5.5 Hz), 3.80-3.62 (m, 6H), 3.47-3.41 (m, 2H), 1.49 (s, 9H); MS *m/z* 508 (M + H^+); HRMS calcd for $C_{25}H_{30}N_7O_5$ 508.2308, found 508.2327. Anal. Calcd for $C_{25}H_{29}N_7O_5$: C, 59.16; H, 5.76; N, 19.32. Found: C, 58.92; H, 5.73; N, 19.13.

1,1-Dimethylethyl [2-[[2-(2-aminoethyl)-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]-pyrazol-5-yl]amino]ethyl](2-hydroxyethyl)carbamate (15) The material was prepared in 90% yield from **14** following a procedure identical to that described for **24**. **15:** mp 120-122 °C; IR (KBr) 3360, 3300, 1690, 1662, 1604, 1572 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.90 (br, 1H), 8.83-8.79 (m, 1H), 7.70, 7.68 (2s, 1H), 7.58-7.51 (m, 2H), 6.96, 6.93 (2 br s, 2H), 4.50 (t, 2H, *J* = 5.5 Hz), 3.79-3.78 (m, 2H), 3.63 (br, 4H), 3.49-3.44 (m, 2H), 3.30 (t, 2H, *J* = 5.5 Hz), 1.49 (s, 9H); MS *m/z* 482 (M + H^+); HRMS calcd for $C_{25}H_{32}N_5O_5$ 482.2403, found 482.2409. Anal. Calcd for $C_{25}H_{31}N_5O_5 \cdot 0.5H_2O$: C, 61.15; H, 6.58; N, 14.26. Found: C, 61.43; H, 6.29; N, 14.14.

1,1-Dimethylethyl *N*-[2-[5-[[2-[[(1,1-dimethylethoxy)carbonyl](2-hydroxyethyl)amino]-ethyl]amino]-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-2(6*H*)-yl]ethyl]glycine (16a) A suspension of **15** (0.40 g, 0.83 mmol), potassium iodide (41 mg, 0.25 mmol), triethylamine (171 mg, 1.69 mmol) and *tert*-butyl bromoacetate (164 mg, 0.84 mmol) in 40 mL of THF was stirred at rt for 28 h. The solvent in the reaction mixture was evaporated, and the remaining residue was partitioned between dichloromethane and water. The aqueous layer was further extracted with dichloromethane. The combined extracts were washed with water, dried (Na_2SO_4), and the solvent concentrated. Flash chromatography (silica gel 40 g, 0-50% MeOH/CH_2Cl_2) furnished 0.35 g (70% yield) of **16a**, 0.03 g (5% yield) of **16b**, and 0.04 g (10% yield) of **15**. **16a**: mp 132-134 °C; IR (KBr) 3460, 3334, 3300, 1734, 1692, 1658, 1598, 1572, 1232, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.91 (br, 1H), 8.85 (br, 1H), 7.73, 7.71 (2 s, 1H), 7.60-7.52 (m, 2H), 6.96, 6.94 (2 br s, 2H), 4.57 (t, 2H, *J* = 5.5 Hz), 3.78 (br, 2H), 3.65 (br, 4H), 3.45 (br, 2H), 3.30 (s, 2H), 3.21 (t, 2H, *J* = 5.5 Hz), 1.49 (s, 9H), 1.41 (s, 9H); MS *m/z* 440 (M + H^+); HRMS calcd

for $C_{31}H_{42}N_5O_7$ 596.3084, found 596.3068. Anal. Calcd for $C_{31}H_{41}N_5O_7$: C, 62.51; H, 6.94; N, 11.76. Found: C, 62.20; H, 6.89; N, 11.62. **16b:** ^{1}H NMR ($CDCl_3$) δ 13.97 (br, 1H), 8.89 (br, 1H), 7.82 (d, 1H, *J* = 9.2 Hz), 7.73 (d, 1H, *J* = 7.2 Hz), 7.53 (t, 1H, *J* = 7.9 Hz), 6.96 (m, 2H), 4.65 (t, 2H, *J* = 6.2 Hz), 3.78 (m, 2H), 3.74-3.54 (m, 4H), 3.46 (m, 2H), 3.35 (s, 4H), 3.28 (t, 2H, *J* = 6.4 Hz), 1.49, 1.43 (2 s, 27H); MS *m/z* 732 (M + Na^+)

***N*-[2-[7-Hydroxy-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-6-oxoanthra[1,9-cd]-pyrazol-2(6*H*)-yl]ethyl]glycine Dihydrochloride (3)** A mixture of **16a** (100 mg, 0.17 mmol) in 4 N HCl in dioxane (10 mL) was stirred at rt for 2 days. The solid was collected on a filter and washed with ether to give 80 mg (93% yield). **3**: mp 223-225 °C; IR (KBr) 3430, 3342, 3168, 2968, 2794, 2542, 1730, 1658, 1602, 1568, 1220, 828 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 14.08 (s, 1H), 9.58 (br, 1H), 9.13 (br, 2H), 8.94 (t, 1H, *J* = 6.5 Hz), 8.21 (d, 1H, *J* = 9 Hz), 7.70-7.63 (m, 2H), 7.47 (d, 1H, *J* = 9 Hz), 6.97-6.92 (m, 1H), 5.30 (br, 1H), 4.99 (t, 2H, *J* = 6 Hz), 3.98-3.92 (m, 4H), 3.69 (t, 2H, *J* = 5 Hz), 3.59-3.54 (m, 2H), 3.22 (br, 2H), 3.06 (br, 2H); MS *m/z* 440 (M + H^+); HRMS calcd for $C_{22}H_{26}N_5O_5$ 440.1934, found 440.1932. Anal. Calcd for $C_{22}H_{27}N_5O_5Cl_2$: C, 51.57; H, 5.31; N, 13.67; Cl, 13.84. Found: C, 51.65; H, 5.32; N, 13.60; Cl, 13.82.

1,1-Dimethylethyl [2-[5-[[2-[[(1,1-dimethylethoxy)carbonyl](2-hydroxyethyl)amino] ethyl]-amino]-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-2-yl]ethyl](2- hydroxyethyl)-carbamate (17) A suspension of **1** (0.1 g, 0.2 mmol) and triethylamine (41 mg, 0.4 mmol) in 5 mL of DMF was stirred for a few minutes to become a clear solution. To the solution was added di-*tert*-butyl dicarbonate (0.13 g, 0.6 mmol), stirred at rt for 3 h and then poured into ice water. The aqueous solution was extracted with EtOAc (3 x 40 mL). The combined extracts were washed with water (2 x 40 mL), and dried ($MgSO_4$) and the solvent concentrated to give 0.12 g (96% yield) as an orange solid. **17**: mp 75-77 °C. IR (KBr) 3435, 2975, 2933, 1693, 1661, 1604, 1572, 1478, 1456, 1410, 1367, 1352, 1342, 1322, 1281, 1265, 1248, 1224, 1174, 1146, 1055, 783 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.90 (br, 1H), 8.86 (m, 1H), 7.66 (d, 1H, *J* = 7.7 Hz), 7.57 (d, 1H, *J* = 7.7 Hz), 7.28 (m, 1H), 6.97 (d, 2H, *J* = 8.1 Hz), 4.66 (m, 2H), 3.77-3.62 (m, 10 H), 3.44 (br, 2H), 3.26-3.15 (m, 2H), 1.50 (s, 9H), 1.20, 0.98 (s, br, 9H); MS *m/z* 626 (m + H^+).

5-[(2-Aminoethyl)amino]-2-(2-azidoethyl)-7-hydroxyanthra[1,9-*cd*]pyrazol-6(2*H*)-one (19) A mixture of **12** (1.40 g, 4.1 mmol) and ethylenediamine (2.48 g, 41.3 mmol) in 8.5 mL of pyridine was heated at 83 °C for 4.5 h. After cooling, to the solution was added 13 mL of *i*-

PrOH and placed in a refrigerator overnight. The precipitated red solid was collected on a filter and washed with cold *i*-PrOH. A 2nd crop was obtained from the filtrate. The crude products were combined and purified by flash chromatography (silica gel 35 g, 0-4% MeOH/CH_2Cl_2) to give 1.19 g (80% yield). **19**: mp 164.5-166 °C; IR (KBr) 3382, 3304, 2919, 2859, 2100, 1662, 1598, 1573, 1546, 1454, 1408, 1337, 1275, 1259, 1214, 1178, 1155, 1120, 828, 805, 784, 718, 634 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.94 (s, 1H), 8.97 (t, 1H, *J* = 5 Hz), 7.72 (dd, 1H, *J* = 6.5, 1 Hz), 7.61-7.53 (m, 2H), 7.00-6.91 (m, 2H), 4.60 (t, 2H, *J* = 5.5 Hz), 3.89 (t, 2H, *J* = 5.5 Hz), 3.54 (q, 2H, *J* = 6 Hz), 3.12 (t, 2H, *J* = 6 Hz); MS *m/z* 364.1 ($M + H^+$). Anal. Calcd for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.72; N, 26.98. Found: C, 59.39; H, 4.56; N, 26.91.

2-(2-Aminoethyl)-5-[(2-aminoethyl)amino]-7-hydroxyanthra[1,9-*cd*]pyrazol-6(2*H*)-one (20) A solution of **19** (0.1 g, 0.28 mmol) and triphenylphosphine (0.072 g, 0.28 mmol) in 10 mL of tetrahydrofuran and 0.1 mL of water was heated at 65 °C for 2 h. Additional 7.2 mg of triphenylphosphine was added. The solution was continued to heat at 65 C for I h and then cooled to rt overnight. The reaction mixture was added ether. The solid was collected on a filter, washed with ether to give 69 mg (73% yield) of bright reddish orange solid. **20**: mp 158-163 °C. IR (KBr) 3368, 3054, 2933, 2866, 1661, 1604, 1571, 1513, 1458, 1409, 1340, 1266, 1226, 1182, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.90 (br, 1H), 8.96 (t, 1H, *J* = 5.5 Hz), 7.71 (dd, 1H, *J* = 7.6, 1.1 Hz), 7.56 (m, 2H), 6.96 (dd, 1H, *J* = 8.1, 1.1 Hz), 6.90 (d, 1H, *J* = 9.1 Hz), 4.51 (t, 2H, *J* = 5.7 Hz), 3.53 (q, 2H, *J* = 5.9 Hz), 3.31 (t, 2H, *J* = 5.7 Hz), 3.12 (t, 2H, *J* = 6.1 Hz); MS *m/z* 338 ($M + H^+$).

1,1-Dimethylethyl [[2-[5-[[2-[[2-(1,1-dimethylethoxy)-2-oxoethyl]amino]ethyl]amino]-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-2-yl]ethyl]amino]acetate (21) A solution of **20** (54 mg, 0.16 mmol), *tert*-butyl bromoacetate (62 mg, 0.32 mmol) and triethylamine (32 mg, 0.32 mmol) in 4 mL of DMF was stirred at rt for 3 h. The solution was poured into water and then extracted with EtOAc (3 x 40 mL). The combined extracts were washed with water (1 x 40 mL), and dried (MgSO4) and the solvent concentrated to give 40 mg of crude product. Flash chromatography (silica gel, 0-5% MeOH/CH_2Cl_2) gave 8.5 mg (9.4%) of an orange solid. **21**: ^1H NMR ($CDCl_3$) δ 13.99 (s, 1H), 8.97 (m, 1H), 7.75 (dd, 1H, *J* = 7.7, 1.1 Hz), 7.66 (d, 1H, *J* = 9.1 Hz), 7.56 (t, 1H, *J* = 7.9 Hz), 6.98 (m, 1H), 4.59 (t, 2H, *J* = 6.1 Hz), 3.59 (q, 2H, *J* = 6.0 Hz), 3.41 (s, 2H), 3.29 (s, 2H), 3.22 (t, 2H, *J* = 6.1 Hz), 3.04 (t, 2H, *J* = 6.2 Hz), 1.48 (s, 9H), 1.41 (s, 9H); MS *m/z* 566.4 ($M + H^+$); HRMS calcd for $C_{30}H_{40}N_5O_6$ 566.2979, found 566.2983.

1,1-Dimethylethyl *N*-[2-[[2-(2-azidoethyl)-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]-

pyrazol-5-yl]amino]ethyl]glycine (22a) To an ice-cold solution of **19** (0.36 g, 1.0 mmol) in 10 mL of *N,N*-dimethylacetamide (DMAC) was added *tert*-butyl bromoacetate (0.20 g, 1.0 mmol) and a solution of diisopropylethylamine (0.13 g, 1.0 mmol) in 2.5 mL of DMAC. After stirring at ambient temperature overnight, the solution was poured into water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined extracts were washed with water (2 x 25 mL), and dried ($MgSO_4$) and the solvent evaporated. Flash chromatography (silica gel, 0.2-5% MeOH/CH_2Cl_2 with 0-0.1% NH_4OH) gave 0.24 g (50% yield) of **22a**, 0.13 g (22% yield) of **22b** and 0.04 g (11% yield) of **19**. **22a**: mp 125-126 °C; IR (KBr) 3302, 2132, 2100, 1728, 1662, 1604 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.94 (s, 1H), 8.89 (m, 1H), 7.70 (d, 1H, *J* = 7.7 Hz), 7.57-7.53 (m, 2H), 6.97 (d, 1H, *J* = 8.1 Hz), 6.90 (d, 1H, *J* = 9.2 Hz), 4.58 (t, 2H, *J* = 5.1 Hz), 3.87 (t, 2H, *J* = 5.1 Hz), 3.55 (m, 2H), 3.41 (s, 2H), 3.03 (t, 2H, *J* = 5.7 Hz), 1.48 (s, 9H); MS *m/z* 478 ($M + H^+$); HRMS calcd for $C_{24}H_{28}N_7O_4$ 478.2203, found 478.2206. Anal. Calcd for $C_{24}H_{27}N_7O_4$: C, 60.37; H, 5.70; N, 20.53. Found: C, 60.30; H, 5.57; N, 20.51. **22b:** mp 86-87 °C; IR (KBr) 2978, 2102, 1737, 1660, 1602, 1573, 1455, 1409, 1393, 1368, 1339, 1281, 1263, 1218, 1152, 784 cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.04 (s, 1H), 8.95 (m, 1H), 7.72 (dd, 1H, *J* = 7.6, 1.1 Hz), 7.63 (d, 1H, *J* = 9.1 Hz), 7.56 (d, 1H, *J* = 8.1 Hz), 7.03 (d, 1H, *J* = 9.5 Hz), 6.98 (dd, 1H, *J* = 8.1, 1.1 Hz), 4.60 (t, 2H, *J* = 5.5 Hz), 3.89 (t, 2H, *J* = 5.5 Hz), 3.61 (q, 2H, *J* = 6.2 Hz), 3.55 (s, 4H), 3.15 (t, 2H, *J* = 6.6 Hz), 1.48 (s, 18H); MS *m/z* 592 ($M + H^+$).

1,1-Dimethylethyl *N*-[2-[[2-(2-azidoethyl)-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]-pyrazol-5-yl]amino]ethyl]-*N*-[(1,1-dimethylethoxy)carbonyl]glycine (23) A solution of **22a** (0.34 g, 0.70 mmol) and di-*tert*-butyl dicarbonate (0.19 g, 0.87 mmol) in 50 mL of dichloromethane was stirred at rt overnight. The solution was washed with water (2 x 30 mL), and dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (silica gel 20 g, 0-0.3% MeOH/CH_2Cl_2) afforded 0.38 g (94% yield). **23**: mp 55 °C started to shrink, at 62 °C turned red; IR (KBr) 3440, 2104, 1744, 1700, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.00, 13.91 (2 s, 1H), 8.94 (m, 1H), 7.75 (d, 1H, *J* = 7.7 Hz), 7.69-7.55 (m, 2H), 7.16 (d, 1H, *J* = 7.7 Hz), 7.69-7.55 (m, 2H), 7.16 (d, 1H, *J* = 9.2 Hz), 7.06-6.97 (m, 1H), 4.62 (t, 2H, *J* = 5.5 Hz), 3.90-3.83 (m, 4H), 3.74-3.61 (m, 4H), 1.58, 1.51 and 1.47 (3 s, 18H); MS *m/z* 595 ($M + NH_4^+$), 578 ($M + H^+$, 100); HRMS calcd for $C_{29}H_{36}N_7O_6$ 578.2727, found 578.2722. Anal. Calcd for $C_{29}H_{35}N_7O_6$: C, 60.30; H, 6.11; N, 16.97. Found: C, 60.21; H, 6.15; N, 16.93.

1,1-Dimethylethyl *N*-[2-[[2-(2-aminoethyl)-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9- *cd*]-pyrazol-5-yl]amino]ethyl]-*N*-[(1,1-dimethylethoxy)carbonyl]glycine (24) A solution of **23**

(0.33 g, 0.57 mmol) and triphenylphosphine (0.18 g, 0.69 mmol) in 25 mL of THF and 0.3 mL of water was stirred at 58-64 °C for 3 h. The solvents in the solution were evaporated to dryness. Flash chromatography three times (silica gel 30 g, 0-3% MeOH/CH_2Cl_2 with 0.1% NH_4OH; silica gel 20 g, 0-2% MeOH/CH_2Cl_2 with 0.05% NH_4OH; silica gel 5 g, 0-1% MeOH/CH_2Cl_2 with 0.05% NH_4OH) furnished 0.17 g (54% yield). **24**: mp 67 °C (shrink), 75 °C (turned from orange to red), 85 °C (completely melted). IR (KBr) 3434, 1744, 1700, 1660, 1604 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.98 (br, 1H), 8.90 (m, 1H), 7.71 (d, 1H, *J* = 7.7 Hz), 7.60 (d, 1H, *J* = 9.5 Hz), 7.54 (t, 1H, *J* = 7.9 Hz), 7.25 (d, 1H, *J* = 9.5 Hz), 6.95 (d, 1H, *J* = 9.5 Hz), 4.51 (t, 2H, *J* = 5.7 Hz), 3.92 and 3.84 (2 s, 2H), 3.71-3.58 (m, 4H), 3.31 (t, 2H, *J* = 5.7 Hz), 1.51 and 1.47 (2 s, 18H); MS *m/z* 552 (M + H^+); HRMS calcd for $C_{29}H_{38}N_5O_6$ 552.2822, found 552.2833. Anal. Calcd for $C_{29}H_{37}N_5O_6$•0.5 H_2O: C, 62.13; H, 6.83; N, 12.49. Found: C, 62.30; H, 6.62; N, 12.21.

1,1-Dimethylethyl *N*-[(1,1-dimethylethoxy)carbonyl]-*N*-[2-[[2-[2-[[2-(1,1-dimethylethoxy)-2-oxoethyl]amino]ethyl]-2,6-dihydro-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-5- yl]amino]-ethyl]glycine (25a) The material was prepared from **24** following a procedure identical to that described for **22a.** 46.4 mg (63% yield) of **25a**, 13 mg (15% yield) of **25b** and 7.2 mg (11% yield) of **24**. **25a:** mp 59-65 °C; IR(KBr) 3434, 1736, 1702, 1660, 1604 cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.02, 13.96 (2 s, 1H), 9.0-8.85 (m, 1H), 7.75 (d, 1H, *J* = 7.3 Hz), 7.69 (d, 1H, *J* = 9.1 Hz), 7.57 (m, 1H), 7.12 (d, 1H, *J* = 9.1 Hz), 6.98 (m, 1H), 4.60 (t, 2H, *J* = 5.9 Hz), 3.93, 3.84 (2 s, 2H), 3.72 (m, 2H), 3.61 (t, 2H, *J* = 6.4 Hz), 3.30 (s, 2H), 3.22 (t, 2H, *J* = 5.8 Hz), 1.51, 1.47 (2 s, 18H), 1.41 (s, 9H); MS *m/z* 666 (M + H^+); HRMS calcd for $C_{35}H_{38}N_5O_8$ 666.3503, found 666.3493. Anal. Calcd for $C_{35}H_{37}N_5O_8$•0.5 H_2O: C, 62.30; H, 7.17; N, 10.38. Found: C, 62.45; H, 7.06; N, 10.22. **25b** (orange solid)**:** ^1H NMR ($CDCl_3$) δ 14.04, 13.99 (2 s, 1H), 8.94 (br, 1H), 7.86 (d, 1H, *J* = 9.2 Hz), 7.74 (d, 1H, *J* = 7.7 Hz), 7.56 (m, 1H), 7.11 (d, 1H, *J* = 9.2 Hz), 6.98 (m, 1H), 4.67 (t, 2H, *J* = 6.6 Hz), 3.93, 3.84 (2 s, 2H), 3.71 (m, 2H), 3.61 (t, 2H, *J* = 6.1 Hz), 3.37, 3.35 (2 s, 4H), 3.29 (t, 2H, *J* = 6.6 Hz), 1.47, 1.44 (2 s, 36H); MS *m/z* 802 (M + Na^+).

***N*-[2-[5-[[2-[(Carboxymethyl)amino]ethyl]amino]-7-hydroxy-6-oxoanthra[1,9-*cd*]pyrazol-2(6*H*)-yl]ethyl]glycine Dihydrochloride (4) Method A: from 25a.** A mixture of **25a** (16 mg, 0.024 mmol) in 1 mL of 4 N HCl was stirred at rt overnight. The mixture was added another 1 mL of 4 N HCl, warmed at 50 °C (oil bath temperature) for 1 h, and then at rt for 2 h. The solvent in the mixture was evaporated to dryness. The residue was triturated with ether and filtered to give 10.7 mg (84% yield) as an orange solid. **4**: mp 232-233 °C (decomp); IR (KBr)

1736, 1659 cm $^{-1}$; ^{1}H NMR (DMSO-d_6) δ 14.11 (s, 1H), 8.98 (t, 1H, *J* = 6.4 Hz), 8.19 (d, 1H, *J* = 9.3 Hz), 7.68 (m, 2H), 7.40 (d, 1H, *J* = 9.2 Hz), 6.96 (m, 1H), 4.96 (t, 2H, *J* = 5.9 Hz), 3.93 (m, 2H), 3.91 (s, 2H), 3.88 (s, 2H), 3.55 (t, 2H, *J* = 6.0 Hz), 3.23 (t, 2H, *J* = 6.1 Hz); MS *m/z* 454 (M + H^+); HRMS calcd for $C_{22}H_{24}N_5O_6$ 454.1727, found 454.1711. Anal. Calcd for $C_{22}H_{25}N_5O_6$ Cl_2•0.9 H_2O: C, 48.70; H, 4.98; N, 12.91; Cl, 13.07. Found: C, 48.98; H, 5.19; N, 12.82; Cl, 12.83.

Method B: from 21. A mixture of **21** in 4 N HCl in dioxane (1 mL) was stirred at rt for 4 h. The solid was collected on a filter, washed with ether to give 3.5 mg (66% yield) as an orange solid.

4: MS *m/z* 454 (M + H^+); HRMS calcd for $C_{22}H_{24}N_5O_6$ 454.17267, found 454.1720.

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