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Platinum(II) complexes with steroidal esters of L-methionine and L-histidine: Synthesis, characterization and cytotoxic activity

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Abstract—Twelve steroidal platinum(II) complexes were synthesized by reaction of potassium tetrachloroplatinate with steroidal esters of L-methionine and L-histidine. The steroidal esters coordinated as bidentate ligands via S and N donor atoms of L-methionine and via two N donor atoms of L-histidine. Cholesterol, cholestanol, diosgenine, pregnenolone, dehydroepiandrosterone, testosterone, estrone, and estradiol were used as the steroidal compounds. The esters and complexes prepared were characterized by infrared, mass, and ^{1}H NMR spectroscopy and elemental analysis. Platinum complexes were tested for in vitro cytotoxicity against several cancer cell lines: T-lymphoblastic leukemia CEM, breast carcinoma MCF-7, lung carcinoma A-549, multiple myeloma RPMI 8226, and one normal cell line human fibroblast BJ.

1. Introduction

Since the discovery of cisplatin[1,2] as an efficient chemotherapy drug, many platinum complexes with significant anti-tumor activities have been prepared.[3–9] Despite of their high activity, many such products, including cisplatin, have various side effects that limit their use.[10–13] Due to this fact, many research teams try to prepare platinum compounds with a high activity and simultaneously with low or no toxicity. Sulfur containing L-methionine or L-cystein play an important role in metabolism of platinum based anticancer drugs.[14] Borch and Pleasants describe that the high toxicity of these drugs is caused by interaction of platinum with methionyl or cysteinyl residues in proteins.[15] Moreover, L-methionine is used as an inhibitor of nephrotoxicity of cisplatin forming the complex [Pt(L-Met)$_2$].[16] These facts prepossess us to study the cytotoxic activity of new platinum(II) complexes with L-methionine. We include here also platinum(II) complexes with L-histidine. Although there are no significant studies about interaction of platinum with L-histidyl residues in enzymes, L-histidine is one of the most important metal binding sites in biological systems.[17,18] Several metal L-histidine complexes were reported to reduce the cisplatin induced nephrotoxicity and gastrointestinal toxicity.[19] Steroidal units have been chosen with respect to possible translocation into the nucleus of the mammary tumor cells by the steroidal hormone receptor system, especially in breast cancer cells.[20–22] Many steroidal platinum complexes have been prepared up to now.[23–31]

Herein we report the synthesis of platinum(II) complexes coordinated via L-methionine or L-histidine esters with steroids such as cholesterol (**1**), 5α-cholestan-3β-ol (cholestanol, **2**), diosgenin (**3**), pregnenolone (**4**), estrone (**5**), testosterone (**6**), dehydroepiandrosterone (DHEA, **7**), and estradiol (**8**). Furthermore, several such steroids display significant anti-tumor activity (e.g., estrogens or androgens)[32–34] which could increase the cytotoxic activity of prepared compounds. In addition, these complexes were investigated to establish how the steroidal units affect the cytotoxic activity against several tumor cell lines, T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF-7, lung carcinoma cell line A-549, multiple myeloma cell line RPMI 8226, and one normal cell line human fibroblast BJ (Fig. 1).

Keywords: Platinum(II) complexes; Steroid; L-Methionine; L-Histidine; Cytotoxicity; NMR spectroscopy.
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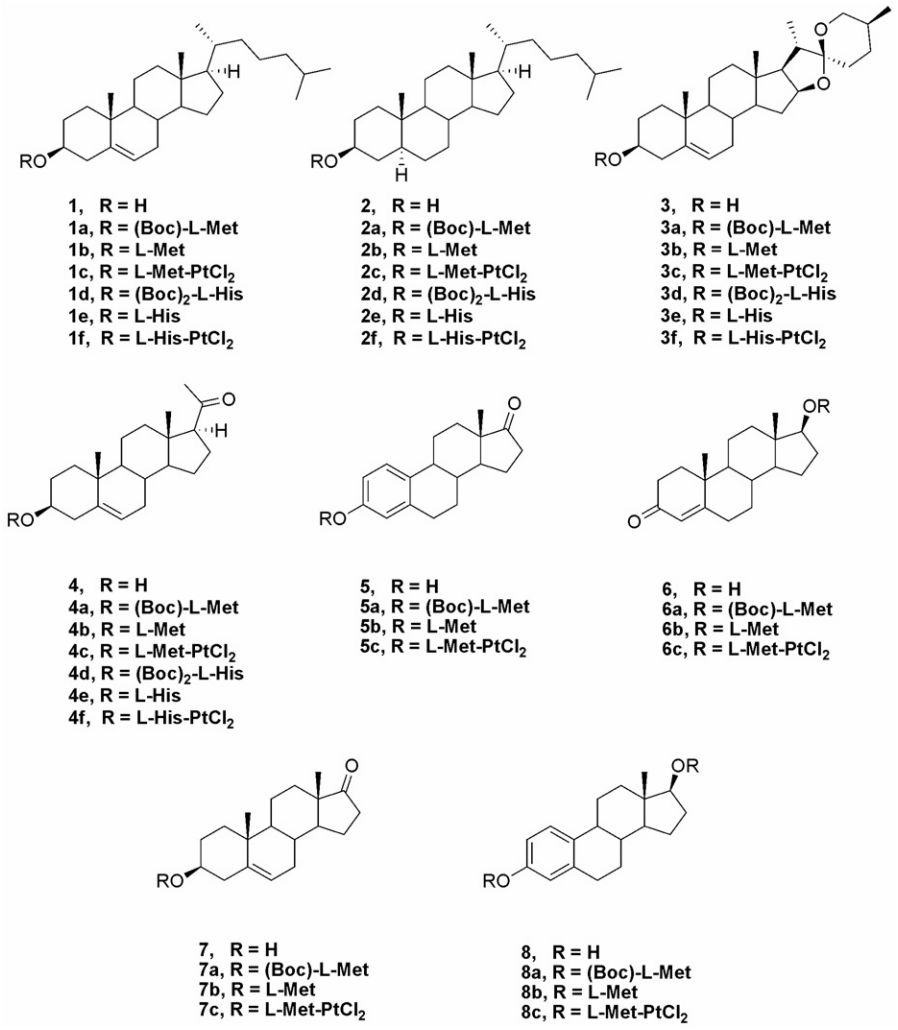


Figure 1. List of prepared compounds.

2. Results and discussion

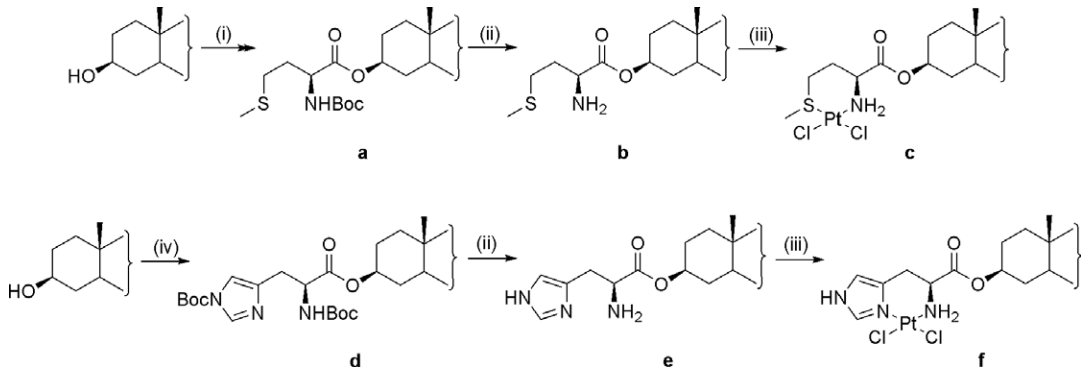
2.1. Synthesis of platinum complexes

Twelve platinum(II) complexes (**1c**–**8c**, **1f**–**4f**) were synthesized by the reaction of corresponding steroidal esters of L-methionine (**1b**–**8b**) or L-histidine (**1e**–**4e**) with potassium tetrachloroplatinate in aqueous DMF. All esters were prepared by esterification using DCC[35] with corresponding N-protected amino acid and steroid, followed by deprotection of amino group(s) with trifluoroacetic acid (Scheme 1). Due to very low stability of L-histidine ester with estrone (**5**), testosterone (**6**), dehydroepiandrosterone (**7**), and estradiol (**8**), no platinum complexes of these steroids were prepared.

2.2. Spectroscopic studies

In contrast to free ligands, IR spectra of the platinum complexes showed significant shift of the NH_2 band to lower frequencies for about 120–150 cm^{-1} (e.g., 3384 cm^{-1} for **4b** and 3269 cm^{-1} for **4c**, 3382 cm^{-1} for **2e** and 3208 cm^{-1} for **2f**). All complexes showed a strong band of ester group around 1730 cm^{-1}. The difference between free ligand and complex is undistinguished, only about 1–7 cm^{-1} (e.g., 1727 cm^{-1} for **2e** and 1730 cm^{-1} for **2f**, 1724 cm^{-1} for **2b** and 1730 cm^{-1} for **2c**). C–O stretching vibrations (around 1230 cm^{-1}) are also very strong, but without any significant change toward free ligands. Several platinum complexes showed very broad band of water (~3436 cm^{-1}), which corresponds with data obtained from elemental analysis. If there is no previous band, then it is possible to see NH stretching vibration (~3435 cm^{-1}) of L-histidine complexes (**2f** and **4f**). Furthermore, the two weak absorption close to 320 cm^{-1} indicate a *cis* metal–chlorine configuration. Very weak C=C vibrations were not observed in several complexes. Selected IR frequencies are given in Table 1.

Conjugates of diosgenine with L-methionine and L-histidine (**3b**, **3e**) and their complexes with platinum (**3c** and **3f**) were chosen for detailed analysis of their ^{1}H and ^{13}C NMR spectra in $CDCl_3$. For the structural assignment of hydrogen and carbon signals the combination of homonuclear and heteronuclear 2D NMR spectra (H,H-COSY, H,C-HSQC, and H,C-HMBC) were used. Proton and carbon-13 chemical shifts are summarized in Table 2.



Scheme 1. General scheme of synthesis of esters and platinum complexes. Reagents: (i) Boc-L-Met, DCC/benzene; (ii) TFA/CH_2Cl_2; (iii) $K_2[PtCl_4]$/DMF, H_2O; (iv) $(Boc)_2$-L-His, DCC/benzene.

Table 1. Selected IR frequencies (cm^{-1}) of platinum complexes **1c**–**8c** and **1f**–**4f** in KBr

Compound	ν(NH, H_2O)	ν(PtN–H)	ν(C=O)	ν(C=C)	ν(C–O)	ν(Pt–N)	ν(Pt–Cl)
1c	3437	3257	1731	n	1231	541	315 320
1f	3435	3210	1732	n	1233	543	318 322
2c	3437	3260	1730	—	1229	542	315 320
2f	3434 (NH imidazole)	3208	1730	—	1233	545	318 322
3c	—	3256	1730	1671	1234	542	317 321
3f	3437	3210	1731	1671	1232	n	318 321
4c	—	3257	1733 1699	n	1233	542	317 321
4f	3435 (NH imidazole)	3210	1730 1698	n	1232	541	318 322
5c	—	3256	1757 1734	1608, 1493 (arom.)	1205	540	317 320
6c	—	3256	1736 1664	1612	1232	540	317 320
7c	3426	3258	1737	1669	1220	542	317 321
8c	3429	3259	1737 1708	1611, 1495 (arom.)	1212,1227	n	317 320

n, not observed.

Already ^1H and namely ^{13}C NMR spectra of conjugates **3b** and **3e** showed line broadening of some signals, especially those methionyl and histidyl residue, that could be detected only indirectly (from ^1H,^{13}C-HSQC spectra) and/or not detected at all (carbonyl carbon atom of ester group). Still more pronounced line-broadening effects were observed for complexes with platinum **3c** and **3f**. In case of L-methionine complex **3c** the doubling of certain signals in the vicinity of platinum was observed in ^1H and ^{13}C NMR spectra. This doubling can be explained by formation of a new chiral center at sulfur atom of methionyl residue during complexation as it can be seen from Table 2. Complexation influences only ^1H and ^{13}C chemical shifts of methionyl residue whereas atoms of steroid ring A were not influenced by that.

2.3. Cytotoxicity studies

Synthesized platinum(II) complexes **1c**–**8c** and **1f**–**4f** were tested for cytotoxic activity in the following tumor cell lines: T-lymphoblastic leukemia CEM, epithelial breast carcinoma MCF-7, epithelial lung carcinoma A-549, and lymphoblast-like multiple myeloma RPMI 8226. Normal cells of human fibroblast BJ were used as a control for toxicity. The TCS_{50} values of cisplatin are also included for comparison. The TCS_{50} value of compound **4c** demonstrates similar activity in case of A-549 cell line in comparison to cisplatin. However, the toxicity of **4c** to BJ is almost one order less as cisplatin toxicity. This result may inspire the next studies. Furthermore, all prepared platinum complexes failed to demonstrate any significant activity against MCF-7 cell line. On the other hand, five complexes showed sig-

Table 2. ^{1}H and ^{13}C chemical shifts of compounds **3b**, **3c**, **3e**, and **3f** in $CDCl_3$

Proton	3b	3c	3e	3f	Carbon	3b	3c	3e	3f
1α (ax)	1.14	1.10	1.11	1.10	1	36.85	36.78	36.63	36.58
1β (eq)	1.88	1.87	1.86	1.87	2	27.61	27.48	27.45	27.43
2α (eq)	1.86	1.90	1.83	1.86	3	75.13	76.62; 76.55	75.73	76.56
2β (ax)	1.61	1.66	1.59	1.63	4	37.96	37.77	37.77	37.69
3	4.66	4.68	4.63	4.63	5	139.27	139.10	139.08	138.95
4α (eq)	~2.33	~2.34	~2.31	2.25	6	122.71	122.86	122.85	123.00
4β (ax)	~2.33	~2.34	~2.31	2.25	7	32.01	32.00	32.01	31.94
6	5.38	5.37	5.36	5.36	8	31.34	31.32	31.32	31.21
7α (ax)	1.55	1.55	1.56	1.54	9	49.88	49.83	49.88	49.78
7β (eq)	2.01	1.99	2.00	1.99	10	36.68	36.64	36.79	36.72
8 (ax)	~1.63	~1.62	1.63	1.63	11	20.79	20.78	20.79	20.72
9 (ax)	0.97	0.96	0.96	0.96	12	39.68	39.65	39.68	39.58
11α (eq)	~1.50	~1.50	~1.50	~1.50	13	40.23	40.22	40.23	40.16
11β (ax)	~1.50	~1.50	~1.50	~1.50	14	56.39	56.38	56.42	56.31
12α (ax)	1.18	1.18	1.18	1.18	15	31.81	31.80	31.81	31.67
12β (eq)	1.74	1.74	1.74	1.74	16	80.77	80.74	80.74	80.74
14 (ax)	1.11	1.11	1.11	1.11	17	62.02	62.03	62.07	61.89
15α	1.99	1.99	1.99	1.98	18	16.27	16.27	16.27	16.19
15β	1.29	1.29	1.29	1.29	19	19.31	19.31	19.25	19.23
16α	4.41	4.41	4.41	4.41	20	41.58	41.56	41.57	41.50
17	1.78	1.77	1.77	1.78	21	14.52	14.52	14.53	14.40
18	0.79	0.78	0.79	0.79	22	109.28	109.25	109.25	109.33
19	1.04	1.03	1.03	1.02	23	31.35	31.34	31.35	31.23
20	1.87	1.87	1.87	1.88	24	28.77	28.76	28.78	28.64
21	0.97	0.97	0.97	0.97	25	30.27	30.25	30.26	30.15
23	1.65	~1.63	~1.63	~1.63	26	66.82	66.80	66.81	66.77
23′	1.60	~1.63	~1.63	~1.63	27	17.13	17.12	17.13	17.03
24	1.62	1.64	1.62	1.62	C=O	n	169.29; 169.21	n	168.95
24′	1.46	1.46	1.46	1.45	Cα	53.18	52.96; 54.07	53.75	52.04
25	1.63	1.63	1.63	1.63	Cβ	32.97	33.51	28.78	33.72
26	3.47	3.47	3.49	3.48	Cγ	30.25	29.02; 31.87	134.67	136.72
26′	3.38	3.37	3.37	3.37	SCH_3	15.31	23.34; 22.02	—	—
27	0.79	0.79	0.79	0.79	Cδ	—	—	117.88	115.03
Hα	3.69	3.97; 4.06	3.94	3.97	Cε	—	—	n	132.26
Hβ	2.09	~3.06	3.18	3.44					
Hβ′	1.91	~3.06	2.99	3.22					
Hγ	~2.64	2.64; 2.15	—	—					
Hγ′	~2.64	3.34; 2.59	—	—					
SCH_3	2.12	2.71; 2.64	—	—					
Hδ	—	—	7.77	8.24					
Hε	—	—	6.90	7.01					

n, not determined.

Table 3. TCS_{50} (μmol/L) values for complexes **1c**–**8c** and **1f**–**4f** obtained from the Calcein AM assays with the tested cancer and normal cell lines; means ± SD obtained from three independent experiments performed in triplicate

Compound	Cell lines (TCS_{50}, μmol/L)				
	CEM	MCF-7	RPMI 8226	A-549	BJ
1c	>50	>50	>50	>50	>50
1f	>50	>50	>50	>50	>50
2c	>50	>50	>50	>50	>50
2f	49.5 ± 0.4	>50	>50	>50	>50
3c	21.5 ± 4.2	44.6 ± 2.6	18.8 ± 2.0	>50	>50
3f	41.8 ± 3.3	>50	46.2 ± 0.5	>50	>50
4c	18.1 ± 3.1	>50	32.2 ± 4.1	43.1 ± 5.6	>50
4f	22.5 ± 1.8	>50	37.4 ± 5.1	>50	>50
5c	14.5 ± 1.2	>50	20.3 ± 2.8	>50	>50
6c	19.1 ± 0.5	>50	46.4 ± 2.9	>50	>50
7c	33.1	>50	>50	>50	>50
8c	45.1	>50	47.1	>50	>50
Cisplatin	1.6 ± 0.5	9.2 ± 1.4	2.4 ± 0.6	42.4 ± 0.4	5.1 ± 0.2

nificant activity against CEM cell lines, especially complex **5c** with IC_{50} = 14.5 μmol/L. Remaining four complexes **3c**, **4c**, **4f**, and **6c** showed cytotoxic activity in the range of 18–23 μmol/L. Significant activity was

also found in case of RPMI 8226 cell line, although the activity is not as high as for CEM cell line. Complex **5c** demonstrates the highest activity (IC_{50} ca. 20 μmol/L) against RPMI 8226 cell line. In contrast to cisplatin (IC_{50} = 5 μmol/L) no synthesized new platinum complex demonstrates cytotoxic activity against normal human cells as is shown on human fibroblast cells BJ. This fact is very interesting and inspiring, too. TCS_{50} values are summarized in Table 3.

3. Conclusion

Within the worldwide research in the field of anti-tumor agents, number of platinum(II) complexes were studied. Our study demonstrates that synthesis of new platinum(II) complexes of steroidal esters with L-methionine and L-histidine can result in compounds with significant cytotoxic activity against tumor cell lines of different histogenetic origin, mainly of lymphoblast-like tumors. Furthermore, we discovered that these complexes are not toxic against human fibroblast BJ, chosen as an example of normal cells. The synthesized complexes, except complexes with cholesterol and cholestanol units, are also highly polar compounds (e.g., they are very soluble in polar solvents, e.g., alcohols), which is very useful in biological screening, as it is possible to eliminate DMSO as part of solubility system, or in in vivo screenings, where using of polar solvents is necessary. The next advantage of steroidal platinum complexes is also possible using of many modified steroidal alcohol of all kind to increase cytotoxic activity and simultaneously decrease the toxicity. Hence, this kind of complexes represents interesting class of compounds for further studies.

4. Experimental

4.1. Materials and methods

The melting points were determined on a Hund H 600 apparatus (Helmut Hund, Germany). The elemental analyses (C, H, N) were carried out on a Perkin-Elmer 2400 II elemental analyzer. Optical rotations were measured on Autopol IV polarimeter (Rudolf Research Analytical, Flanders, USA) at 25 °C in chloroform (unless otherwise stated) and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g. Infrared spectra were recorded on a Bruker IFS 55 spectrometer in chloroform or in KBr in case of platinum complexes. Wave numbers are given in cm^{-1}. ^{1}H NMR spectra were taken in $CDCl_3$ on a Bruker AVANCE-400 (at 400 MHz) instrument with tetramethylsilane as an internal reference, unless otherwise stated. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. All values were obtained by first-order analysis. Compounds **3b**, **3c**, **3e**, and **3f** were measured on Bruker AVANCE-600 (^{1}H at 600.13 MHz and ^{13}C at 150.9 MHz frequency) in $CDCl_3$. Mass spectra (FAB) were obtained with a ZAB-EG spectrometer at 70 eV. Samples prepared were identified by their melting points, by thin-layer chromatography (TLC) on silica gel G (ICN Biochemicals; detection by spraying with sulfuric acid and heating), and by their IR and ^{1}H NMR spectra. For column chromatography, neutral silica gel (60 μm) was used (Fluka).

All steroids used in this work were purchased from Steraloids, Inc. Potassium tetrachloroplatinate, Boc-L-methionine, $(Boc)_2$-L-histidine dicyclohexylamime salt, DCC, and trifluoroacetic acid were purchased from Sigma–Aldrich. $(Boc)_2$-L-histidine was prepared from its dicyclohexylamine salt. The salt was dissolved in ethyl acetate and the solution was extracted with saturated aqueous solution of citric acid.

4.2. Synthesis

4.2.1. General procedure for preparation of *N*-(*tert*-butoxycarbonyl)-L-methioninate. DCC (140 mg, 0.68 mmol) and DMAP (2 mg, 0.016 mmol) were added to a solution of steroidal alcohol (200 mg) and Boc-L-methionine (130 mg, 0.52 mmol) in anhydrous benzene (10 mL). The mixture was stirred vigorously and after completion (12 h) the white precipitate was filtered off. Solvent was removed from the filtrate under reduced pressure and the resulting crude oil product was purified by flash chromatography on silica using EtOAc/petroleum ether (ratio mentioned in each experiment).

4.2.1.1. Cholest-5-en-3β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (1a). Starting with cholesterol (**1**) (200 mg, 0.52 mmol), chromatography (1:15) and evaporation of solvents gave colorless oil product of **1a** (262 mg, 82%), $[\alpha]_D$ −14 (*c* 0.33). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1732, 1710, 1500, 1368, 1165. ^{1}H NMR (400 MHz): δ 0.68 (3H, s), 0.86, 0.87 (both 3H, d, J = 6.8 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.02 (3H, s), 1.45 (9H, s), 2.11 (3H, s), 2.31–2.33 (2H, m), 2.50–2.61 (2H, m), 4.35 (1H, q, J = 4.5 Hz), 4.67 (1H, m, ΣJ = 32.1 Hz), 5.12 (1H, br d, J = 7.5 Hz), 5.38 (1H, d, J = 5.2 Hz). MS *m*/*z* (rel intensity): 640 ([M+Na]$^+$, 37), 369 (100). Anal. Calcd for $C_{37}H_{63}NO_4S$: C, 71.91; H, 10.28; N, 2.27. Found: C, 71.99; H, 10.20; N, 2.21%.

4.2.1.2. 5α-Cholestan-3β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (2a). Starting with cholestanol (**2**) (200 mg, 0.52 mmol), chromatography (1:15) and evaporation of solvents gave colorless oil product of **2a** (271 mg, 85%), $[\alpha]_D$ +29 (*c* 0.27). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1729, 1710, 1500, 1368, 1167. ^{1}H NMR (400 MHz): δ 0.65, 0.82 (both 3H, s), 0.859, 0.864 (both 3H, d, J = 6.8 Hz), 0.90 (3H, d, J = 6.4 Hz), 1.45 (9H, s), 2.10 (3H, s), 2.50–2.55 (2H, m), 4.34 (1H, q, J = 5.6 Hz), 4.75 (1H, m, ΣJ = 32.4 Hz), 5.12 (1H, br d, J = 7.6). MS *m*/*z* (rel intensity): 642 ([M+Na]$^+$, 44), 371 (100). Anal. Calcd for $C_{37}H_{65}NO_4S$: C, 71.68; H, 10.57; N, 2.26. Found: C, 71.73; H, 10.55; N, 2.22%.

4.2.1.3. Spirost-5-en-3β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (3a). Starting with diosgenin (**3**) (200 mg, 0.48 mmol), chromatography (1:10) and evaporation of solvents gave amorphous powder of **3a** (271 mg, 87%), mp 86–88 °C; $[\alpha]_D$ −64 (*c* 0.26). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1730, 1710, 1500, 1368, 1167, 981. ^{1}H NMR (400 MHz): δ 0.79 (3H, s), 0.79 (3H, d, J = 6.6 Hz), 0.97 (3H, d, J = 7.2 Hz), 1.04 (3H, s), 1.45 (9H, s),

2.11 (3H, s), 2.33 (2H, br d, J = 8.0 Hz), 2.51–2.56 (2H, m), 3.38 (1H, t, J = 11.2 Hz), 3.47 (1H, ddd, J = 10.8, 4.4, 1.6 Hz), 4.36 (1H, q, J = 7.2 Hz), 4.41 (1H, m, ΣJ = 22.4 Hz), 4.66 (1H, m, ΣJ = 32.4 Hz), 5.12 (1H, br d, J = 8.0 Hz), 5.38 (1H, d, J = 4.0 Hz). MS m/z (rel intensity): 642 ($[M+H]^+$, 37), 397 (100). Anal. Calcd for $C_{37}H_{59}NO_6S$: C, 68.80; H, 9.21; N, 2.17. Found: C, 68.85; H, 9.15; N, 2.14%.

4.2.1.4. 20-Oxo-pregn-5-en-3β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (4a). Starting with pregnenolone (**4**) (200 mg, 0.63 mmol), chromatography (1:7) and evaporation of solvents gave amorphous powder of **4a** (277 mg, 80%), mp 45–47 °C; $[\alpha]_D$ −15 (c 0.31). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1731, 1704, 1500, 1368, 1167. ^1H NMR (400 MHz): δ 0.64, 1.03 (both 3H, s), 1.45 (9H, s), 2.11, 2.13 (both 3H, s), 2.33 (2H, br d, J = 7.2 Hz), 2.56–2.54 (2H, m), 4.36 (1H, q, J = 4.8 Hz), 4.67 (1H, m, ΣJ = 32.0 Hz), 5.12 (1H, br d, J = 7.6 Hz), 5.38 (1H, d, J = 4.8 Hz). MS m/z (rel intensity): 548 ($[M+H]^+$, 34), 299 (100). Anal. Calcd for $C_{31}H_{49}NO_5S$: C, 67.97; H, 9.02; N, 2.56. Found: C, 67.96; H, 9.05; N, 2.55%.

4.2.1.5. 17-Oxo-estra-1,3,5(10)-trien-3-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (5a). Starting with estrone (**5**) (200 mg, 0.74 mmol), chromatography (1:7) and evaporation of solvents gave amorphous powder of **5a** (272 mg, 74%), mp 52–56 °C; $[\alpha]_D$ +65 (c 0.32). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1756, 1733, 1712, 1609, 1585, 1501, 1493, 1369, 1159. ^1H NMR (400 MHz): δ 0.91 (3H, s), 1.46 (9H, s), 2.14 (3H, s), 2.51 (1H, dd, J = 18.8, J' = 8.8 Hz), 2.64 (2H, t, J = 7.2 Hz), 2.89–2.92 (2H, m), 4.64 (1H, q, J = 4.4 Hz), 5.19 (1H, d, J = 8.0 Hz), 6.82 (1H, d, J = 2.4 Hz), 6.87 (1H, dd, J = 8.4, J' = 2.4 Hz), 7.29 (1H, d, J = 8.4 Hz). MS m/z (rel intensity): 524 ($[M+Na]^+$, 100), 269 (45). Anal. Calcd for $C_{28}H_{39}NO_5S$: C, 67.03; H, 7.84; N, 2.79. Found: C, 66.98; H, 7.91; N, 2.76%.

4.2.1.6. 3-Oxo-androst-4-en-17β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (6a). Starting with testosterone (**6**) (200 mg, 0.69 mmol), chromatography (1:7) and evaporation of solvents gave amorphous powder of **6a** (299 mg, 83%), mp 87–90 °C; $[\alpha]_D$ +71 (c 0.36). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1732, 1711, 1663, 1616, 1500, 1369, 1165. ^1H NMR (400 MHz): δ 0.85, 1.19 (both 3H, s), 1.44 (9H, s), 2.10 (3H, s), 4.41 (1H, q, J = 5.2 Hz), 4.64 (1H, dd, J = 8.8, J' = 8.4 Hz), 5.12 (1H, d, J = 8.4 Hz), 5.73 (1H, s). MS m/z (rel intensity): 542 ($[M+Na]^+$, 100), 271 (31). Anal. Calcd for $C_{29}H_{45}NO_5S$: C, 67.02; H, 8.73; N, 2.69. Found: C, 67.08; H, 8.70; N, 2.69%.

4.2.1.7. 17-Oxo-androst-5-en-3β-yl *N*-(*tert*-butoxycarbonyl)-L-methioninate (7a). Starting with dehydroepiandrosterone (**7**) (200 mg, 0.69 mmol), chromatography (1:7) and evaporation of solvents gave amorphous powder of **7a** (316 mg, 88%), mp 114–116 °C; $[\alpha]_D$ +19 (c 0.27). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1733, 1711, 1669, 1500, 1368, 1165. ^1H NMR (400 MHz): δ 0.88, 1.05 (both 3H, s), 1.44 (9H, s), 2.11 (3H, s), 2.34 (2H, br d, J = 7.6 Hz), 2.46 (1H, dd, J = 19.2 Hz, J' = 8.4 Hz), 2.51–2.56 (2H, m), 4.36 (1H, m), 4.67 (1H, m, ΣJ = 32.4 Hz), 5.12 (1H, br d, J = 8.4 Hz), 5.41 (1H, d, J = 4.4 Hz). MS m/z (rel intensity): 520 ($[M+H]^+$, 91), 271 (100). Anal. Calcd for $C_{29}H_{45}NO_5S$: C, 67.02; H, 8.73; N, 2.69. Found: C, 66.97; H, 8.76; N, 2.65%.

4.2.1.8. Estra-1,3,5(10)-trien-3,17β-diyl bis[*N*-(*tert*-butoxycarbonyl)-L-methioninate] (8a). Starting with estradiol (**8**) (200 mg, 0.73 mmol), chromatography (1:7) and evaporation of solvents gave amorphous powder of **8a** (426 mg, 79%), mp 59–61 °C; $[\alpha]_D$ +24 (c 0.26). IR $\nu(CHCl_3)$ cm^{-1}: 3437, 1754, 1711, 1495, 1369, 1161. ^1H NMR (400 MHz): δ 0.84 (3H, s), 1.45, 1.46 (both 9H, s), 2.11, 2.14 (both 3H, s), 2.53–2.58 (2H, m), 2.64 (2H, t, J = 7.6 Hz), 2.85–2.88 (2H, m), 4.43 (1H, bdd, J = 12.4 Hz, J' = 6.8 Hz), 4.63 (1H, bdd, J = 13.0 Hz, J' = 8.0 Hz), 4.75 (1H, t, J = 8.4 Hz), 5.13 (1H, br d, J = 8.0 Hz), 5.19 (1H, br d, J = 7.8 Hz), 6.8 (1H, d, J = 2.4 Hz), 6.85 (1H, dd, J = 8.4 Hz, J' = 2.4 Hz), 7.28 (1H, d, J = 8.4 Hz). MS m/z (rel intensity): 757 ($[M+Na]^+$, 100), 255 (77). Anal. Calcd for $C_{38}H_{58}N_2O_8S_2$: C, 62.10; H, 7.95; N, 3.81. Found: C, 62.02; H, 8.00; N, 3.78%.

4.2.2. General procedure for preparation of N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate. DCC (140 mg, 0.68 mmol) and DMAP (2 mg, 0.016 mmol) were added to a solution of steroidal alcohol (200 mg) and $(Boc)_2$-L-histidin (190 mg, 0.54 mmol) in anhydrous benzene (10 mL). The mixture was stirred vigorously and after completion (15 h) the white precipitate was filtered off. Solvent was removed from the filtrate under reduced pressure and the resulting crude oil product was purified by flash chromatography on silica using EtOAc/petroleum ether (ratio mentioned in each experiment).

4.2.2.1. Cholest-5-en-3β-yl N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate (1d). Starting with cholesterol (**1**) (200 mg, 0.52 mmol), chromatography (1:10) and evaporation of solvents gave colorless oil product of **1d** (289 mg, 77%), mp 144–145 °C; $[\alpha]_D$ −17 (c 0.33). IR $\nu(CHCl_3)cm^{-1}$: 3437, 1754, 1709, 1491, 1392, 1255, 1156, 1012. ^1H NMR (400 MHz): δ 0.68 (3H, s), 0.86 (6H, br d, J = 6.8 Hz), 0.91 (3H, d, J = 6.4 Hz), 1.01 (3H, s), 1.43, 1.60 (both 9H, s), 1.91–2.04 (2H, m), 2.26–2.31 (2H, m), 3.03 (2H, d, J = 4.7 Hz), 4.51 (1H, m), 4.63 (1H, m, ΣJ = 31.9 Hz), 5.35 (1H, d, J = 4.0 Hz), 5.66 (1H, d, J = 8.4 Hz), 7.13, 7.99 (both 1H, s). MS m/z (rel intensity): 724 ($[M+H]^+$, 64), 369 (100). Anal. Calcd for $C_{43}H_{69}N_3O_6$: C, 71.33; H, 9.61; N, 5.80. Found: C, 71.35; H, 9.57; N, 5.77%.

4.2.2.2. 5α-Cholestan-3β-yl N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate (2d). Starting with cholestanol (**2**) (200 mg, 0.52 mmol), chromatography (1:10) and evaporation of solvents gave colorless product of **2d** (295 mg, 79%), mp 111–113 °C; $[\alpha]_D$ +14 (c 0.29). IR $\nu(CHCl_3)$ cm^{-1}: 3436, 1754, 1708, 1492, 1392, 1255, 1155, 1012. ^1H NMR (400 MHz): δ 0.64, 0.81 (both 3H, s), 0.859, 0.863 (both 3H, br d, J = 6.8 Hz), 0.90 (3H, d, J = 6.4 Hz), 1.43, 1.60 (both 9H, s), 1.92–1.97 (2H, m), 3.02 (2H, d, J = 5.2 Hz), 4.50 (1H, m,

ΣJ = 18.4 Hz), 4.72 (1H, m, ΣJ = 32.4 Hz), 5.67 (1H, d, J = 8.4 Hz), 7.12, 7.98 (both 1H, s). MS *m*/*z* (rel intensity): 726 ($[M+H]^+$, 87), 371 (100). Anal. Calcd for $C_{43}H_{71}N_3O_6$: C, 71.13; H, 9.86; N, 5.79. Found: C, 71.18; H, 9.85; N, 5.78%.

4.2.2.3. Spirost-5-en-3β-yl N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate (3d). Starting with diosgenin (**3**) (200 mg, 0.48 mmol), chromatography (1:8) and evaporation of solvents gave colorless product of **3d** (301 mg, 83%), mp 173–175 °C; $[\alpha]_D$ −62 (*c* 0.33). IR ν($CHCl_3$) cm^{-1}: 3436, 1754, 1709, 1490, 1391, 1255, 1155, 1012. ^1H NMR (400 MHz): δ 0.787 (3H, s), 0.790, 0.97 (both 3H, d, J = 6.8 Hz), 1.03 (3H, s), 1.43, 1.60 (both 9H, s), 2.29 (2H, d, J = 7.2 Hz), 3.03 (2H, d, J = 4.0 Hz), 3.37 (1H, t, J = 10.4 Hz), 3.47 (1H, m), 4.41 (1H, q, J = 7.6 Hz), 4.51, 4.63 (both 1H, m), 5.36 (1H, br s), 5.66 (1H, d, J = 8.4 Hz), 7.13, 7.99 (both 1H, s). MS *m*/*z* (rel intensity): 752 ($[M+H]^+$, 100), 397 (53). Anal. Calcd for $C_{43}H_{65}N_3O_8$: C, 68.68; H, 8.71; N, 5.59. Found: C, 68.73; H, 8.70; N, 5.52%.

4.2.2.4. 20-Oxo-pregn-5-en-3β-yl N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate (4d). Starting with pregnenolone (**4**) (200 mg, 0.63 mmol), chromatography (1:5) and evaporation of solvents gave amorphous powder of **4d** (314 mg, 76%), mp 154–155 °C; $[\alpha]_D$ +8 (*c* 0.28). IR ν($CHCl_3$) cm^{-1}: 3436, 1754, 1703, 1492, 1391, 1255, 1155, 1012. ^1H NMR (400 MHz): δ 0.75, 1.13 (both 3H, s), 1.56, 1.72 (both 9H, s), 2.25 (3H, s), 2.42 (2H, d, J = 7.6 Hz), 2.66 (1H, t, J = 8.8 Hz), 3.16 (2H, d, J = 4.8 Hz), 4.64 (1H, m), 4.76 (1H, m, ΣJ = 27.6 Hz), 5.48 (1H, d, J = 4.0 Hz), 5.79 (1H, d, J = 8.8 Hz), 7.26, 8.12 (both 1H, s). MS *m*/*z* (rel intensity): 654 ($[M+H]^+$, 29), 315 (100). Anal. Calcd for $C_{37}H_{55}N_3O_7$: C, 67.97; H, 8.48; N, 6.43. Found: C, 67.91; H, 8.56; N, 6.39%.

4.2.3. General procedure for deprotection of *N*-(*tert*-butoxycarbonyl)-L-methioninate. Trifluoroacetic acid (0.7 mL, 9.4 mmol) was added to a solution of Boc-L-methionine ester (200 mg) in dichloromethane (7 mL). The mixture was stirred 1 h and after completion it was neutralized by pyridine (2 mL). The solvents were evaporated under reduced pressure and the residue was diluted in ethyl acetate, washed with water, dried over magnesium sulfate, and the solvent was evaporated under reduced pressure.

4.2.3.1. Cholest-5-en-3β-yl L-methioninate (1b). Starting with ester **1a** (200 mg, 0.32 mmol), the reaction according to general procedure afforded oil product of **1b** (153 mg, 91%), $[\alpha]_D$ −24 (*c* 0.30). IR ν($CHCl_3$) cm^{-1}: 3383, 1725, 1670, 1440, 1381, 1191. ^1H NMR (400 MHz): δ 0.59, 0.843 (both 3H, s), 0.85 (6H, d, J = 6.8 Hz), 0.94 (3H, J = 6.6 Hz), 2.11 (3H, s), 2.31 (1H, dd, J = 12.0 Hz, J' = 2.3 Hz), 2.41 (1H, ddd, J = 12.8 Hz, J' = 5.0 Hz, J'' = 2.1 Hz), 2.45 (2H, t, J = 7.5 Hz), 3.35 (1H, dd, J = 8.5 Hz, J' = 4.6 Hz), 4.79 (1H, m, ΣJ = 32.7 Hz), 5.27 (1H, m). MS *m*/*z* (rel intensity): 518 ($[M+H]^+$, 17), 369 (100). Anal. Calcd for $C_{32}H_{55}NO_2S$: C, 74.22; H, 10.71; N, 2.70. Found: C, 74.16; H, 10.75; N, 2.69%.

4.2.3.2. 5α-Cholestan-3β-yl L-methioninate (2b). Starting with ester **2a** (200 mg, 0.32 mmol), the reaction according to general procedure afforded colorless product of **2b** (156 mg, 90%), mp 85–87 °C; $[\alpha]_D$ +14 (*c* 0.32). IR ν($CHCl_3$) cm^{-1}: 3384, 1724, 1446, 1383, 1190. ^1H NMR (400 MHz): δ 0.65, 0.82 (both 3H, s), 0:860, 0.865 (both 3H, d, J = 6.8 Hz), 0:90 (3H, d, J = 6.4 Hz), 2.62 (2H, t, J = 8.0 Hz), 3.52 (1H, dd, J = 8.0 Hz, J' = 4.8 Hz), 4.73 (1H, m, ΣJ = 32.4 Hz). MS *m*/*z* (rel intensity): 520 ($[M+H]^+$, 100), 371 (62). Anal. Calcd for $C_{32}H_{57}NO_2S$: C, 73.93; H, 11.05; N, 2.69. Found: C, 73.89; H, 11.11; N, 2.66%.

4.2.3.3. Spirost-5-en-3β-yl L-methioninate (3b). Starting with ester **3a** (200 mg, 0.31 mmol), the reaction according to general procedure afforded colorless product of **3b** (157 mg, 93%), mp 112–116 °C; $[\alpha]_D$ −73 (*c* 0.32). IR ν($CHCl_3$) cm^{-1}: 3384, 1726, 1680, 1455, 1379, 1188, 1049, 981, 898. ^1H NMR (400 MHz): δ 0.785 (3H, d, J = 6.4 Hz), 0.786 (3H, s), 0.97 (3H, d, J = 6.8 Hz), 1.04, 2.12 (both 3H, s), 2.32 (2H, d, J = 7.6 Hz), 2.63 (2H, t, J = 7.6 Hz), 3.37 (1H, t, J = 10.8 Hz), 3.47 (1H, dd, J = 11.0 Hz, J' = 4.5 Hz), 3.55 (1H, dd, J = 8.0 Hz, J' = 4.5 Hz), 4.41 (1H, q, J = 7.5 Hz), 4.65 (1H, m, ΣJ = 32.8 Hz), 5.38 (1H, br d, J = 5.6 Hz). MS *m*/*z* (rel intensity): 546 ($[M+H]^+$, 100), 397 (73). Anal. Calcd for $C_{32}H_{51}NO_4S$: C, 70.42; H, 9.42; N, 2.57. Found: C, 70.37; H, 9.49; N, 2.54%.

4.2.3.4. 20-Oxo-pregn-5-en-3β-yl L-methioninate (4b). Starting with ester **4a** (200 mg, 0.37 mmol), the reaction according to general procedure afforded oil product of **4b** (148 mg, 91%), $[\alpha]_D$ −34 (*c* 0.32). IR ν($CHCl_3$) cm^{-1}: 3384, 1726, 1701, 1439, 1380, 1190. ^1H NMR (400 MHz): δ 0.64, 1.03, 2.12, 2.13 (all 3H, s), 2.33 (2H, br d, J = 7.6 Hz), 2.54 (1H, t, J = 8.8 Hz), 2.63 (2H, t, J = 6.8 Hz), 3.55 (1H, dd, J = 8.0 Hz, J' = 4.8 Hz), 4.66 (1H, m, ΣJ = 32.4 Hz), 5.39 (1H, br d, J = 4.8 Hz). MS *m*/*z* (rel intensity): 448 ($[M+H]^+$, 100), 299 (31). Anal. Calcd for $C_{26}H_{41}NO_3S$: C, 69.76; H, 9.23; N, 3.13. Found: C, 69.68; H, 9.28; N, 3.14%.

4.2.3.5. 17-Oxo-estra-1,3,5(10)-trien-3-yl L-methioninate (5b). Starting with ester **5a** (200 mg, 0.40 mmol), the reaction according to general procedure afforded oil product of **5b** (139 mg, 87%), $[\alpha]_D$ +77 (*c* 0.25). IR ν($CHCl_3$) cm^{-1}: 3388, 1756, 1734, 1610, 1584, 1494, 1159. ^1H NMR (400 MHz): δ 1.03, 2.27 (both 3H, s), 2.53 (1H, m), 2.63 (1H, dd, J = 19.2 Hz, J' = 9.2 Hz), 2.86 (2H, t, J = 8.4 Hz), 3.02–3.05 (2H, m), 4.00 (1H, dd, J = 7.6 Hz, J' = 5.2 Hz), 6.94 (1H, d, J = 2.4 Hz), 6.99 (1H, dd, J = 8.8 Hz, J' = 2.4 Hz), 7.42 (1H, d, J = 8.6 Hz). MS *m*/*z* (rel intensity): 402 ($[M+H]^+$, 73), 269 (100). Anal. Calcd for $C_{23}H_{31}NO_3S$: C, 68.79; H, 7.78; N, 3.49. Found: C, 68.71; H, 7.83; N, 3.41%.

4.2.3.6. 3-Oxo-androst-4-en-17β-yl L-methioninate (6b). Starting with ester **6a** (200 mg, 0.39 mmol), the reaction according to general procedure afforded oil product of **6b** (143 mg, 89%), $[\alpha]_D$ +39 (*c* 0.32). IR ν($CHCl_3$) cm^{-1}: 3384, 1732, 1666, 1615, 1439, 1232, 1197. ^1H NMR (400 MHz): δ 0.85, 1.19, 2.11 (all 3H, s), 3.92 (1H, bm), 4.67 (1H, t, J = 8.4 Hz), 5.73 (1H, s).

MS *m/z* (rel intensity): 420 ([M+H]$^+$, 100), 271 (18). Anal. Calcd for $C_{24}H_{37}NO_3S$: C, 68.69; H, 8.89; N, 3.34. Found: C, 68.66; H, 8.93; N, 3.29%.

4.2.3.7. 17-Oxo-androst-5-en-3β-yl L-methioninate (7b). Starting with ester **7a** (200 mg, 0.39 mmol), the reaction according to general procedure afforded oil product of **7b** (140 mg, 87%), $[\alpha]_D$ +40 (*c* 0.25). IR $\nu(CHCl_3)$ cm^{-1}: 3385, 1731, 1438, 1190. ^{1}H NMR (400 MHz): δ 0.89, 1.05, 2.12 (all 3H, s). 2.33–2.35 (2H, m), 2.46 (1H, dd, J = 18.4 Hz, J' = 8.8 Hz), 2.62 (2H, t, J = 7.2 Hz), 3.55 (1H, m), 4.65 (1H, m, ΣJ = 32.4 Hz), 5.41 (1H, br d, J = 4.4 Hz). MS *m/z* (rel intensity): 420 ([M+H]$^+$, 100), 271 (68). Anal. Calcd for $C_{24}H_{37}NO_3S$: C, 68.69; H, 8.89; N, 3.34. Found: C, 68.64; H, 8.95; N, 3.32%.

4.2.3.8. Estra-1,3,5(10)-trien-3,17β-diyl bis(L-methioninate) (8b). Starting with diester **8a** (200 mg, 0.27 mmol), the reaction according to general procedure afforded oil product of **8b** (118 mg, 81%), $[\alpha]_D$ +21 (*c* 0.25). IR $\nu(CHCl_3)$ cm^{-1}: 3375, 1769, 1727, 1584, 1502, 1191. ^{1}H NMR (400 MHz): δ 0.84, 2.12, 2.14 (all 3H, s), 2.65 (1H, t, J = 6.8 Hz), 2.71–2.75 (2H, m), 2.85–2.89 (2H, m), 3.60 (1H, dd, J = 8.0 Hz, J' = 4.8 Hz), 3.83 (1H, dd, J = 8.0 Hz, J' = 4.8 Hz), 4.75 (1H, t, J = 8.0 Hz), 6.79 (1H, d, J = 2.4 Hz), 6.84 (1H, dd, J = 8.4 Hz, J' = 2.4 Hz), 7.28 (1H, d, J = 8.4 Hz). MS *m/z* (rel intensity): 535 ([M+H]$^+$, 100), 255 (86). Anal. Calcd for $C_{28}H_{42}N_2O_4S_2$: C, 62.89; H, 7.92; N, 5.24. Found: C, 62.85; H, 8.00; N, 5.19%.

4.2.4. General procedure for deprotection of N^{α},N^{τ}-bis(*tert*-butoxycarbonyl)-L-histidinate. Trifluoroacetic acid (1.0 mL, 13.5 mmol) was added to a solution of $(Boc)_2$-L-histidine ester (200 mg) in dichloromethane (7 mL). The mixture was stirred 1 h and after completion it was neutralized by ammonium solution in chloroform (2 mL). The solvents were evaporated under reduced pressure and the residue was diluted in ethyl acetate, washed with water, dried over magnesium sulfate, and the solvent was evaporated under reduced pressure.

4.2.4.1. Cholest-5-en-3β-yl L-histidinate (1e). Starting with ester **1d** (200 mg, 0.28 mmol), the reaction according to general procedure afforded colorless product of **1e** (122 mg, 84%), mp 83–86 °C; $[\alpha]_D$ −30 (*c* 0.20). IR $\nu(CHCl_3)$ cm^{-1}: 3465, 3382, 1730, 1565, 1468, 1205, 1134. ^{1}H NMR (400 MHz): δ 0.68 (3H, s), 0.915 (6H, d, J = 6.6 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.01 (3H, s), 2.25–2.35 (2H, m), 2.95 (1H, dd, J = 16.0 Hz, J' = 7.2 Hz), 3.16 (1H, dm, J = 16.0 Hz), 3.89 (1H, br s), 4.64 (1H, m, ΣJ = 32.1 Hz), 5.38 (1H, d J = 4.8 Hz), 6.90, 7.73 (both 1H, s). MS *m/z* (rel intensity): 524 ([M+H]$^+$, 100), 369 (68). Anal. Calcd for $C_{33}H_{53}N_3O_2$: C, 75.67; H, 10.20; N, 8.02. Found: C, 75.64; H, 10.25; N, 8.05%.

4.2.4.2. 5α-Cholestan-3β-yl L-histidinate (2e). Starting with ester **2d** (200 mg, 0.28 mmol), the reaction according to general procedure afforded colorless product of **2e** (125 mg, 86%), mp 170–172 °C; $[\alpha]_D$ +5 (*c* 0.27). IR $\nu(CHCl_3)$ cm^{-1}: 3464, 3382, 1727, 1563, 1468, 1198, 1133. ^{1}H NMR (400 MHz): δ 0.65, 0.82 (both 3H, s), 0.860, 0.865 (both 3H, d, J = 6.8 Hz), 0.90 (3H, d, J = 6.4 Hz), 1.93–1.95 (2H, m), 2.83 (1H, dd, J = 15.2 Hz, J' = 8.4 Hz), 3.07 (1H, dd, J = 14.8 Hz, J' = 3.6 Hz), 3.71 (1H, br s), 4.73 (1H, m, ΣJ = 32.0 Hz), 6.85, 7.56 (both 1H, s). MS *m/z* (rel intensity): 526 ([M+H]$^+$, 100), 371 (55). Anal. Calcd for $C_{33}H_{55}N_3O_2$: C, 75.38; H, 10.54; N, 7.99. Found: C, 75.30; H, 10.61; N, 8.02%.

4.2.4.3. Spirost-5-en-3β-yl L-histidinate (3e). Starting with ester **3d** (200 mg, 0.27 mmol), the reaction according to general procedure afforded colorless product of **3e** (122 mg, 83%), mp 120–123 °C; $[\alpha]_D$ −90 (*c* 0.31). IR $\nu(CHCl_3)$ cm^{-1}: 3468, 3382, 1732, 1453, 1200, 1050, 980, 899. ^{1}H NMR (400 MHz): δ 0.788 (3H, s), 0.790 (3H, d, J = 6.4 Hz), 0.97 (3H, d, J = 7.2 Hz), 1.03 (3H, s), 2.25–2.35 (2H, m), 2.99 (1H, br s), 3.17 (1H, d, J = 12.4 Hz), 3.37 (1H, t, J = 11.2 Hz), 3.47 (1H, m, ΣJ = 12.8 Hz), 3.92 (1H, br s), 4.41 (1H, dt, J = 8.4 Hz, J' = 6.8 Hz), 4.63 (1H, m, ΣJ = 32.8 Hz), 5.37 (1H, d, J = 4.4 Hz), 6.91, 7.77 (both 1H, s). MS *m/z* (rel intensity): 552 ([M+H]$^+$, 100), 397 (23). Anal. Calcd for $C_{33}H_{49}N_3O_4$: C, 71.83; H, 8.95; N, 7.62. Found: C, 71.75; H, 9.01; N, 8.91%.

4.2.4.4. 20-Oxo-pregn-5-en-3β-yl L-histidinate (4e). Starting with ester **4d** (200 mg, 0.31 mmol), the reaction according to general procedure afforded colorless product of **4e** (117 mg, 84%), mp 85–87 °C; $[\alpha]_D$ +9 (*c* 0.43). IR $\nu(CHCl_3)$ cm^{-1}: 3463, 3379, 1728, 1698, 1358, 1193. ^{1}H NMR (400 MHz): δ 0.63, 1.01, 2.12 (all 3H, s), 2.53 (1H, t, J = 8.4 Hz), 3.03 (1H, br s), 3.20 (1H, m), 3.96 (1H, br s), 4.64 (1H, m, ΣJ = 32.8 Hz), 5.37 (1H, d, J = 4.4 Hz), 6.93, 7.85 (both 1H, s). MS *m/z* (rel intensity): 454 ([M+H]$^+$, 100), 299 (18). Anal. Calcd for $C_{27}H_{39}N_3O_3$: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.40; H, 8.73; N, 9.22%.

4.2.5. General procedure for platination. Solution of potassium tetrachloroplatinate (90 mg, 0.22 mmol) in DMF (2 mL) and distilled water (2 mL) was added to a solution of steroidal ester of L-methionine or L-histidine (0.24 mmol) in DMF (2 mL). The resulting mixture was stirred in the dark for 3 days. Then, a drop of DMSO was added to destroy the excess of $K_2[PtCl_4]$ and the stirring was continued for 1 h. The solvent was evaporated and the residue was stirred vigorously in a saturated aqueous potassium chloride solution (5 mL) for 20 min. The resulting suspension was filtered, washed with water, and dried in a desiccator over phosphorus pentoxide for 1 day. Analytical data of complexes **1c**–**8c** and **1f**–**4f** are given in Table 4. Optical rotations of complexes **1c** and **2c** were not determined due to very low solubility in most organic solvents.

4.3. In vitro studies

4.3.1. Cell cultures. All cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). The screening cell lines (T-lymphoblastic leukemia cell line CEM; breast carcinoma cell line MCF-7, lung carcinoma cell line A-549, multiple myeloma

Table 4. Analytical data for complexes **1c**–**8c** and **1f**–**4f**

Compound	Formula	Anal. Calcd/Found (%)			Yield (%)	Mp (°C) (decomp.)	Optical rotation (in DMF)
		C	H	N			
1c	$C_{32}H_{55}Cl_2NO_2PtS{\cdot}0.5H_2O$	48.48	7.12	1.77	85	(>258)	n
		48.40	7.19	1.66			
1f	$C_{33}H_{53}Cl_2N_3O_2Pt{\cdot}1.5H_2O$	49.43	6.83	5.24	79	(>292)	−12 (*c* 0.16)
		49.27	6.90	5.04			
2c	$C_{32}H_{57}Cl_2NO_2PtS{\cdot}0.5H_2O$	48.35	7.35	1.76	87	(>245)	n
		48.39	7.43	1.77			
2f	$C_{33}H_{55}Cl_2N_3O_2Pt$	50.06	7.00	5.31	77	(>280)	+34 (*c* 0.33)
		50.14	7.19	5.18			
3c	$C_{32}H_{51}Cl_2NO_4PtS$	47.34	6.33	1.73	88	(>255)	−28 (*c* 0.29)
		47.51	6.36	1.73			
3f	$C_{33}H_{49}Cl_2N_3O_4Pt{\cdot}2H_2O$	46.42	6.26	4.92	78	(>243)	−32 (*c* 0.32)
		46.34	6.23	4.79			
4c	$C_{26}H_{41}Cl_2NO_3PtS$	43.76	5.79	1.96	84	134–136	+26 (*c* 0.25)
		43.62	5.87	1.88		(>185)	
4f	$C_{27}H_{39}Cl_2N_3O_3Pt$	45.07	5.46	5.84	80	195–198	+26 (*c* 0.26)
		44.92	5.50	5.79		(>217)	
5c	$C_{23}H_{31}Cl_2NO_3PtS$	41.38	4.68	2.10	80	152–155	+62 (*c* 0.33)
		41.29	4.72	1.99		(>193)	
6c	$C_{24}H_{37}Cl_2NO_3PtS$	42.04	5.44	2.04	85	(>224)	+24 (*c* 0.28)
		41:90	5.51	2:00			
7c	$C_{24}H_{37}Cl_2NO_3PtS{\cdot}0.5H_2O$	41.50	5.51	2.02	87	(>240)	+6 (*c* 0.27)
		41.55	5.44	2.10			
8c	$C_{28}H_{42}Cl_4N_2O_4Pt_2S_2{\cdot}2H_2O$	31.00	4.09	2.58	75	(>235)	+12 (*c* 0.37)
		31.11	3.99	2.69			

n, not determined.

cell line RPMI 8226, and human fibroblast BJ) were cultured in DMEM medium (Gibco BRL) supplemented with 10% fetal calf serum, 4 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, at 37 °C in a fully humidified atmosphere containing 5% CO_2.

4.3.2. Cytotoxicity assay. The cell suspension of approximate density of 1.25×10^5 cells/mL was redistributed into 96-well microtiter plates and after 3 h of stabilization the tested platinum derivates were added in different concentrations. Platinum derivates were dissolved in dimethylsulfoxide (DMSO) before addition to cultures. Control cultures were treated with DMSO alone. The final concentration of DMSO in the reaction mixture never exceeded 0.6%. Fourfold dilutions of the intended test concentration were added at time zero in 20 μmL aliquots to the microtiter plate wells. Usually, each tested compound was evaluated at six fourfold dilutions. In routine testing, the highest well concentration was 50 μmol/L (but it can be the matter of change dependent on the agent). After 72 h of cultivation, the cells were incubated with Calcein AM solution (Molecular Probes) for 1 h. Fluorescence of viable cells was quantified with Fluoroscan Ascent (Microsystems). The tumor cell survival (TCS) was calculated using the following equation: $TCS = (OD_{drug\ exposed\ well}/\text{mean}\ OD_{control\ wells}) \times 100\%$. The TCS_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from the obtained dose–response curves. Cisplatin was used as a positive control.

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