PAVOL JOZEF ŠAFÁRIK UNIVERSITY IN KOŠICE
Faculty of Science

Supplementary material

In vitro selective inhibitory effect of silver(I) aminoacidates against bacteria and intestinal cell lines and elucidation of mechanism action by means of DNA binding properties, DNA cleavage and cell cycle arrest

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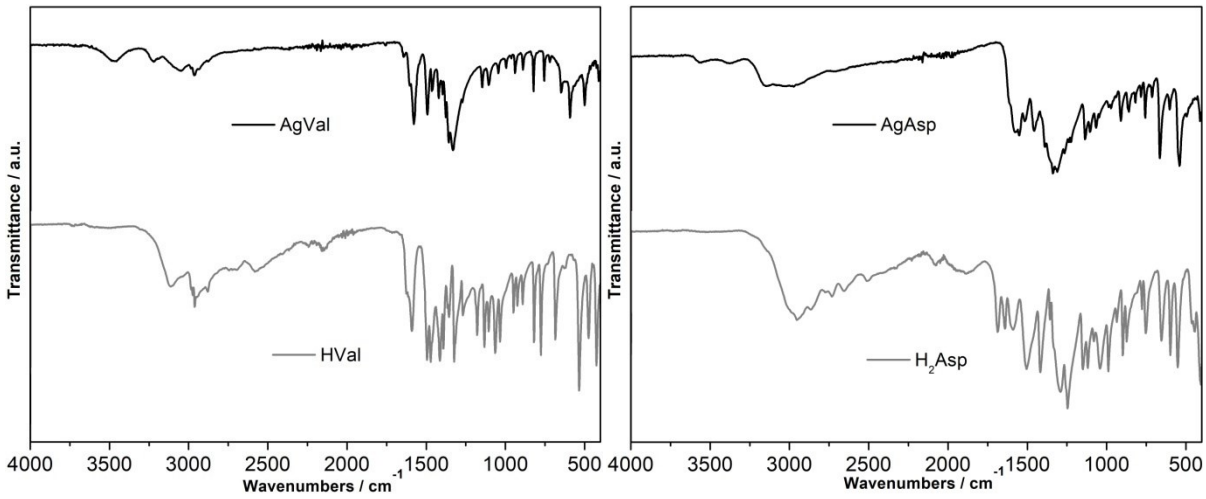


Fig. S1 IR spectra of ligand HVal and its AgVal complex (left); ligand H_2Asp and its AgAsp complex (right).

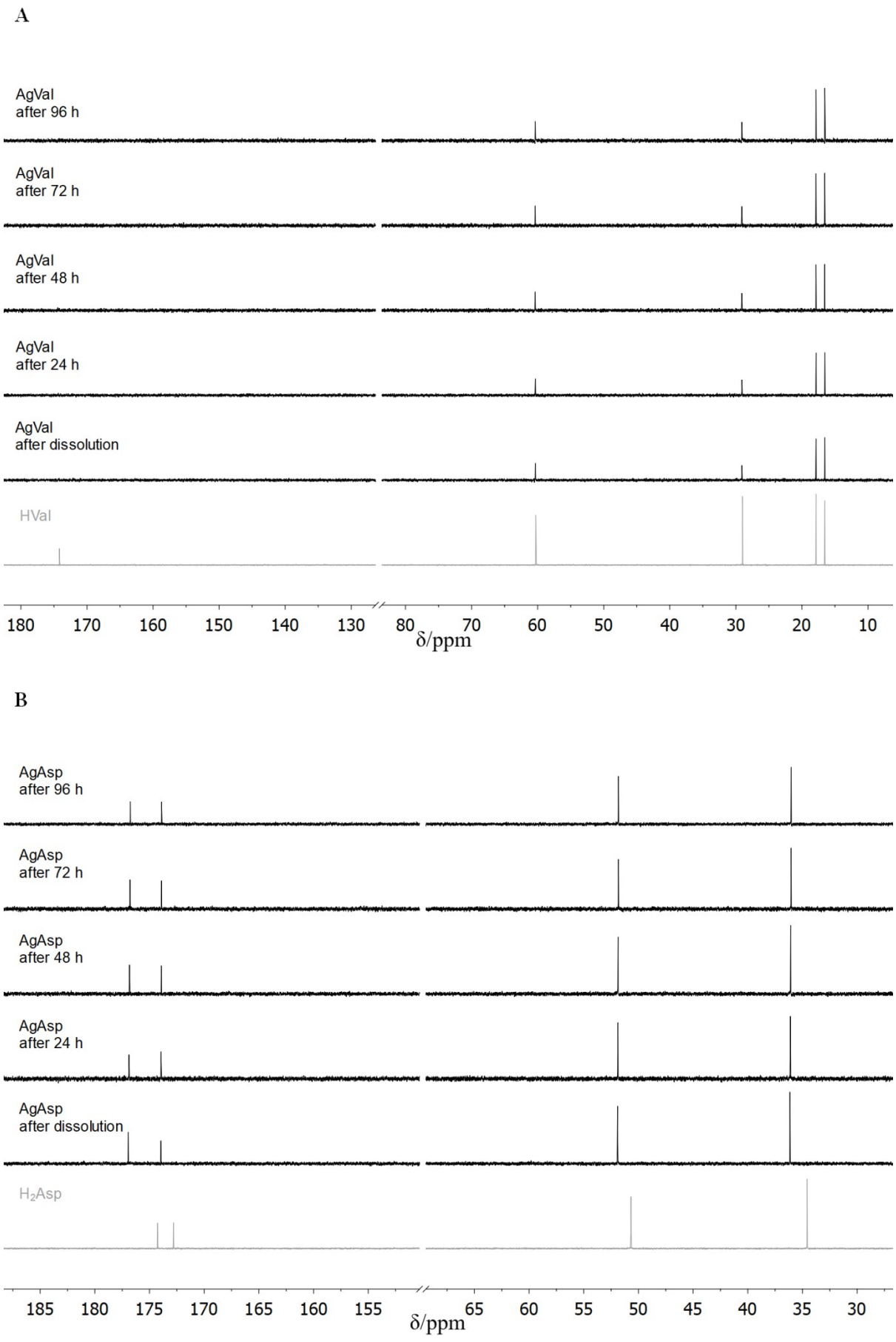
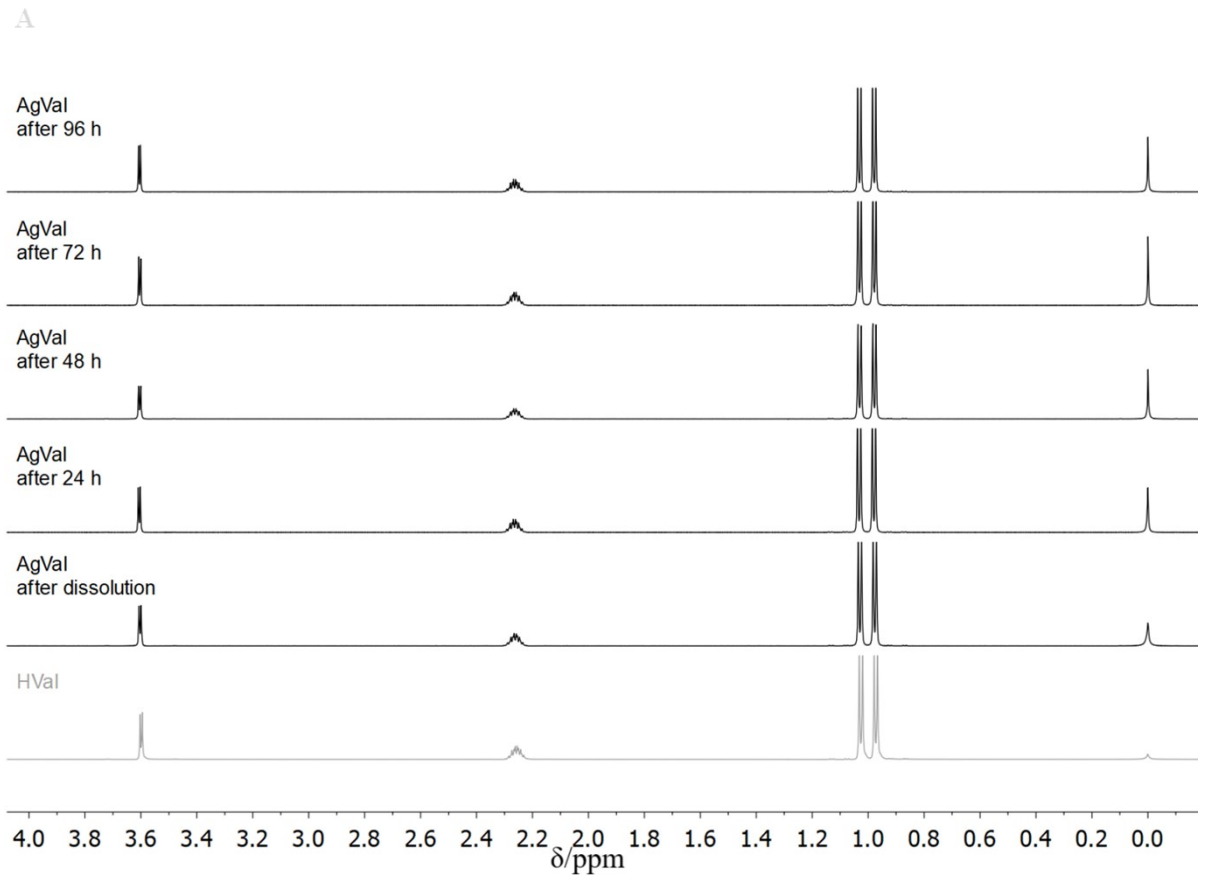


Fig. S2 ^{13}C spectrum of HVal and time-dependent spectra of AgVal (**A**); ^{13}C spectrum of H_2Asp and time-dependent spectra of AgAsp (**B**), measured in D_2O.



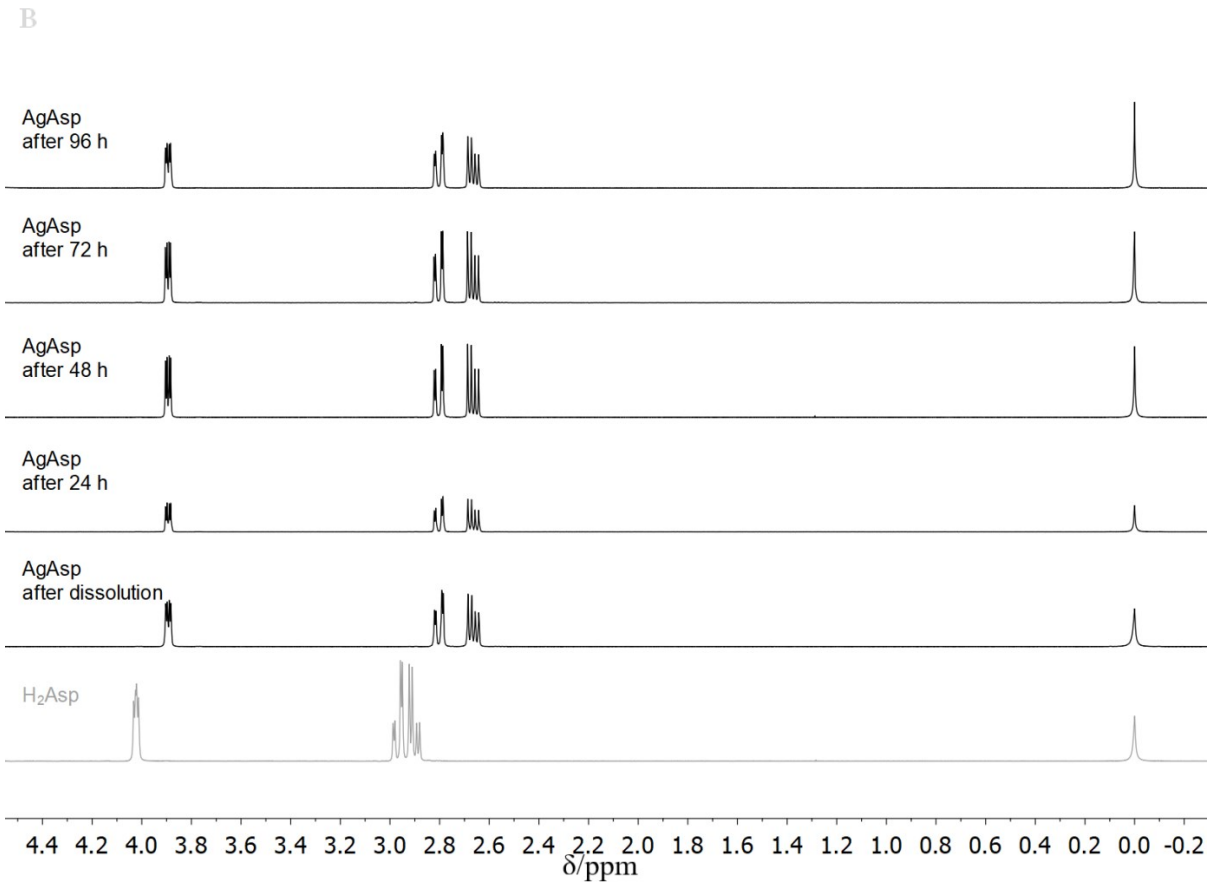


Fig. S3 ^{1}H spectrum of HVal and time-dependent spectra of AgVal (**A**); ^{1}H spectrum of H_2Asp and time-dependent spectra of AgAsp (**B**), measured in PBS buffer; ref. to TSP signal (0.00 ppm).

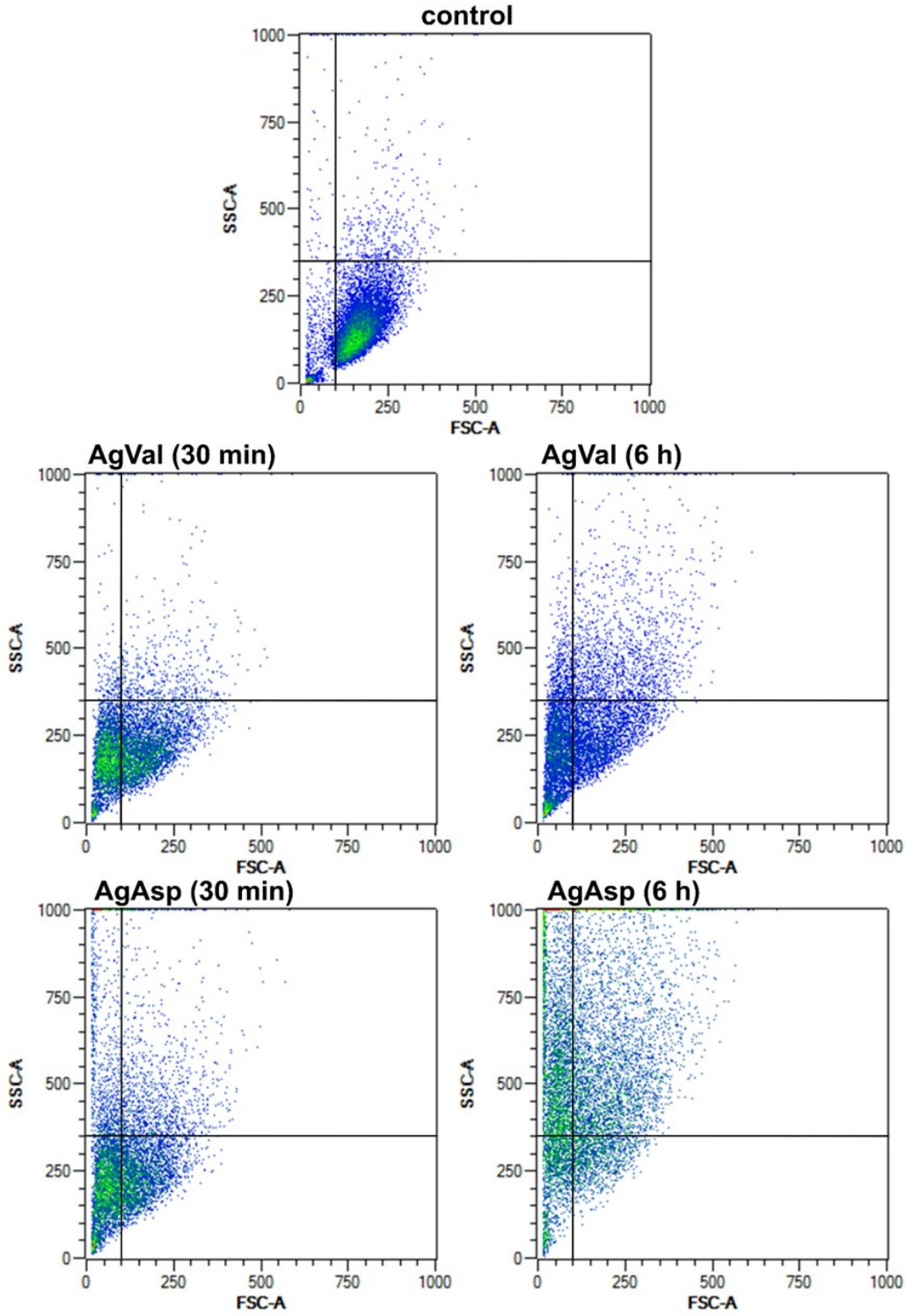


Fig. S4 SSC vs. FSC characteristics of human dermal fibroblasts in the absence and presence of 400 μM AgVal and AgAsp. Flow-cytometry was performed 30 min and 6 h after the administration. Number of events was color coded (from blue up to red).

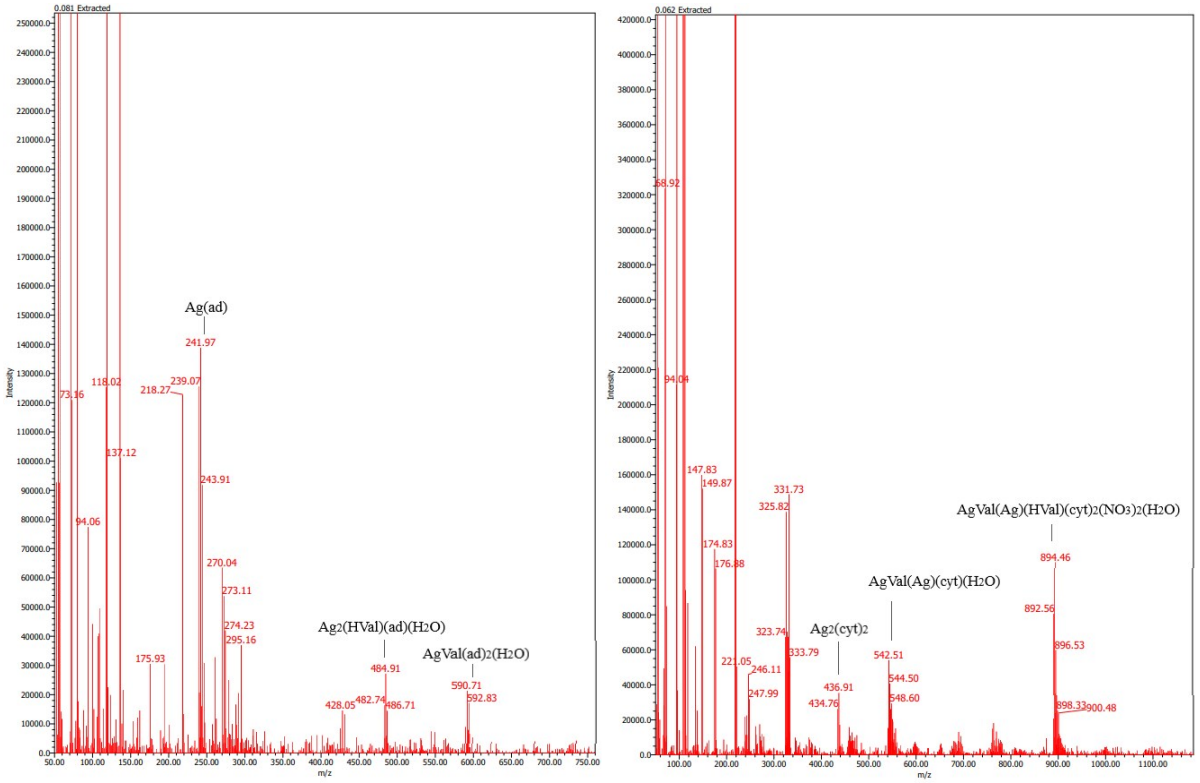


Fig. S5 Mass spectra of equimolar aqueous solution mixture of AgVal and adenine (left); AgVal and cytosine (right).

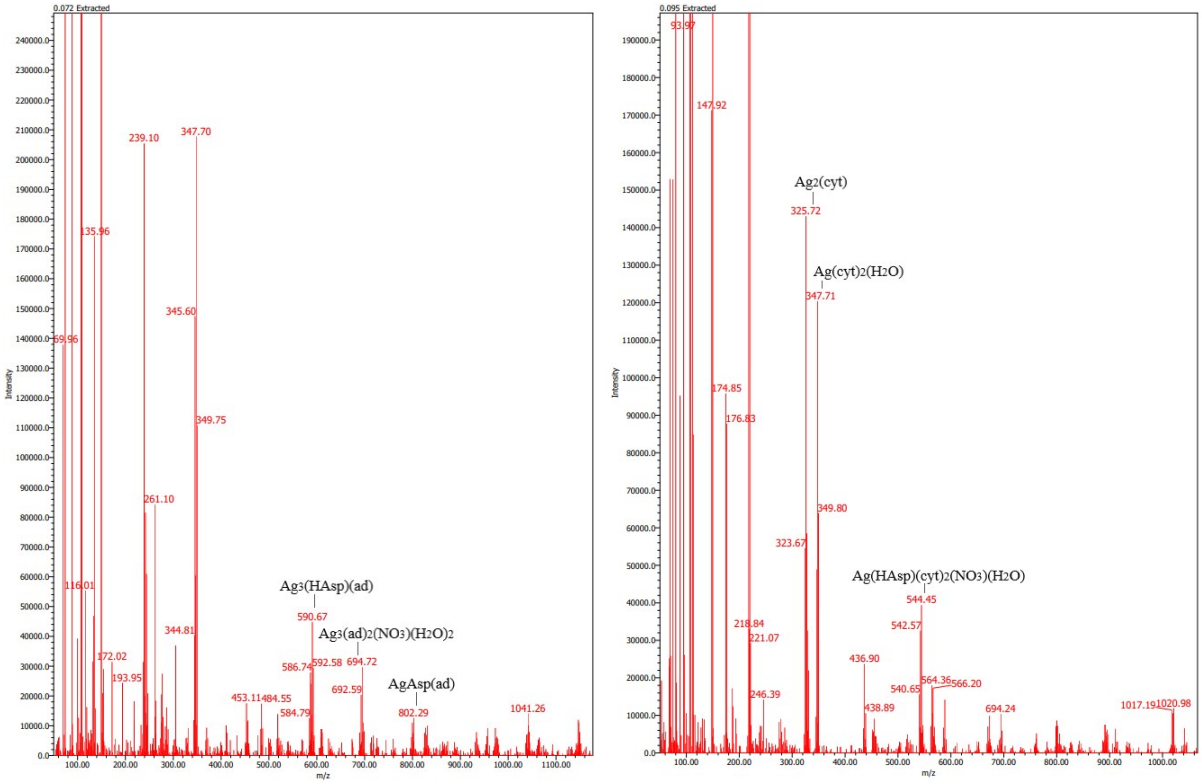


Fig. S6 Mass spectra of equimolar aqueous solution mixture of AgAsp and adenine (left); AgAsp and cytosine (right).

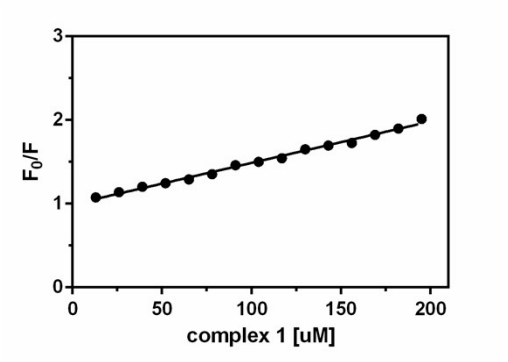


Fig. S7 Stern–Volmer plot for DNA-HO quenching by AgVal. *Ksv* was calculated from the slope of plot F_0/F vs. AgVal concentration.

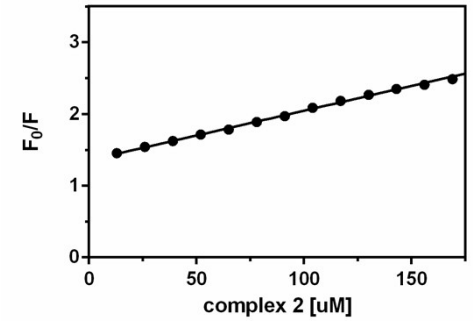


Fig. S8 Stern–Volmer plot for DNA-HO quenching by AgAsp. *Ksv* was calculated from the slope of plot F_0/F vs. AgAsp concentration.

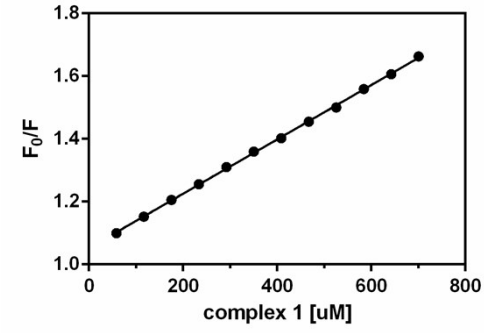


Fig. S9 Stern–Volmer plot for DNA-EB quenching by AgVal. *Ksv* was calculated from the slope of plot F_0/F vs. AgVal concentration.

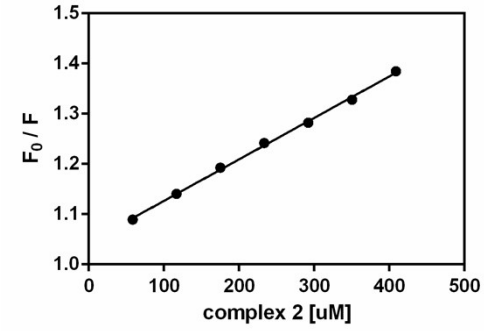


Fig. S10 Stern–Volmer plot for DNA-EB quenching by AgAsp. *Ksv* was calculated from the slope of plot F_0/F vs. AgAsp concentration.

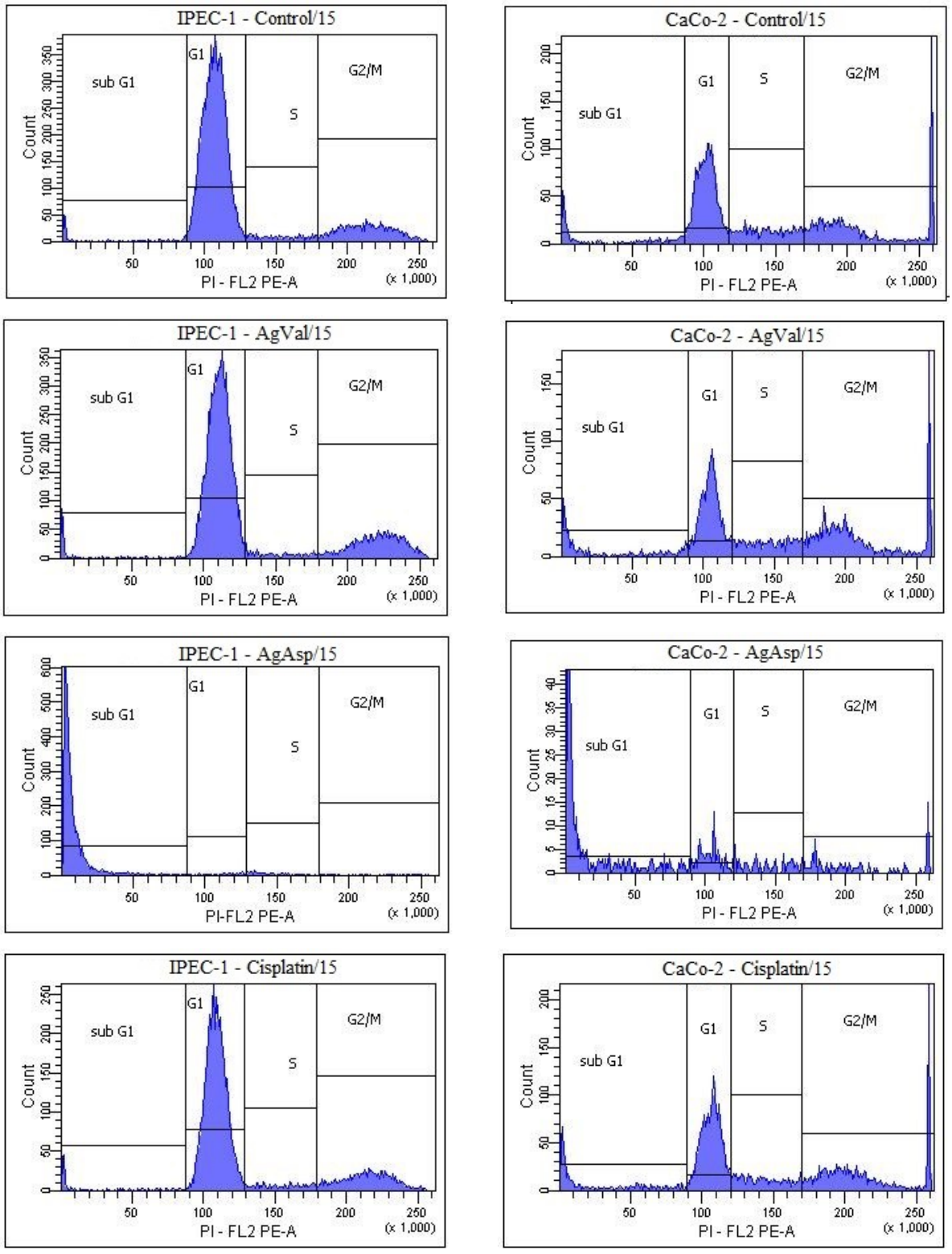


Fig. S11 Effect of AgVal, AgAsp and cisplatin (at 15 μM) on cell cycle against IPEC-1 (left column) and CaCo-2 cells (right column). The relative number of cells within each cell cycle was determined by flow cytometry.

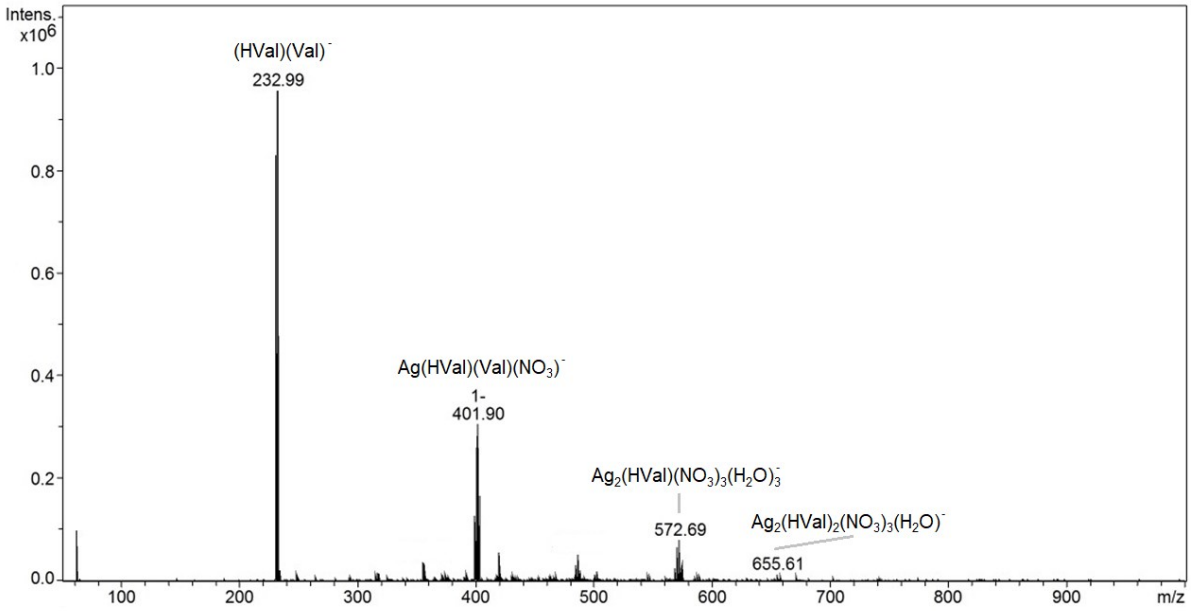


Fig. S12 Mass spectra of the acetonitrile solution of complex AgVal measured in negative mode.

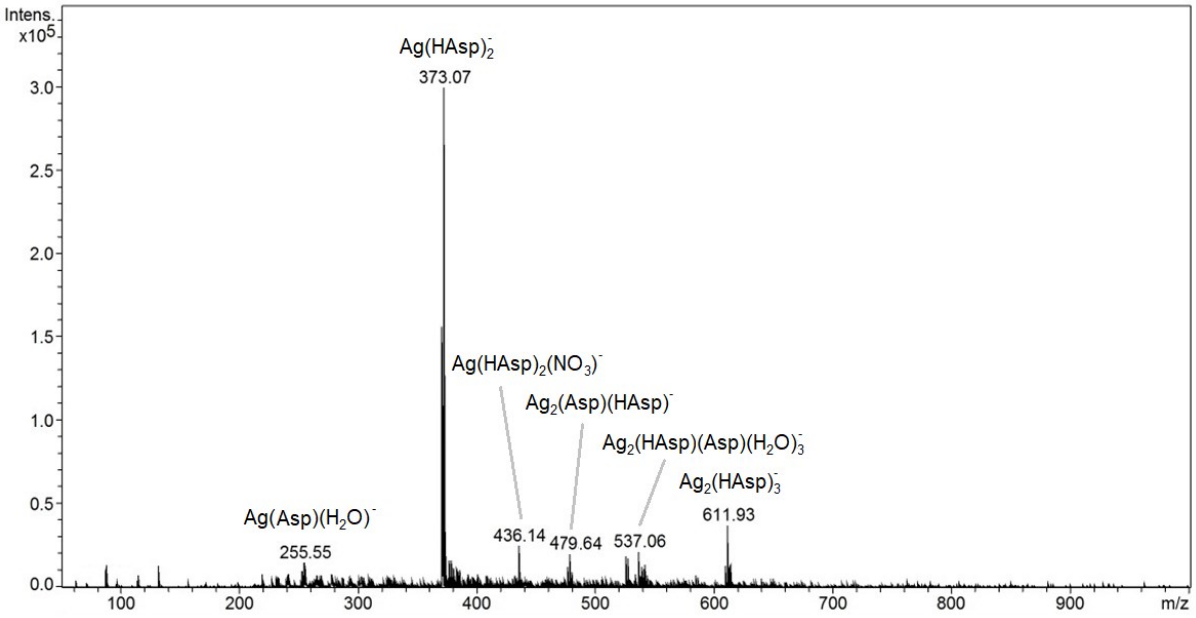


Fig. S13 Mass spectra of the methanol/water (v:v / 1:1) solution of complex AgAsp measured in negative mode.

Tab. S1 Selected bond distances (Å) and angles (°) for **AgVal**.

Bond distances			
Ag(1)—O(2)^{i}	2.2398(10)	C(4)—H(4A)	0.9800
Ag(1)—O(1)^{ii}	2.3161(10)	C(4)—H(4B)	0.9800
Ag(1)—O(6)	2.4458(12)	C(4)—H(4C)	0.9800
Ag(1)—O(3)	2.6757(11)	C(5)—H(5A)	0.9800
Ag(1)—Ag(1)^{iii}	2.9724(2)	C(5)—H(5B)	0.9800
O(1)—C(1)	1.2489(17)	C(5)—H(5C)	0.9800
C(1)—O(2)	1.2620(17)	N(1)—H(1A)	0.91(2)
C(1)—C(2)	1.5269(18)	N(1)—H(1B)	0.86(2)
C(2)—N(1)	1.4952(17)	N(1)—H(1C)	0.82(2)
C(2)—C(3)	1.5372(19)	N(2)—O(5)	1.2457(16)
C(2)—H(2)	1.0000	N(2)—O(3)	1.2498(16)
C(3)—C(5)	1.528(2)	N(2)—O(4)	1.2613(17)
C(3)—C(4)	1.529(2)	O(6)—H(6A)	0.81(2)
C(3)—H(3)	1.0000	O(6)—H(6B)	0.85(3)
Bond angles			
O(2)^{i}—Ag(1)—O(1)^{ii}	148.81(4)	C(3)—C(4)—H(4B)	109.5
O(2)^{i}—Ag(1)—O(6)	120.78(4)	H(4A)—C(4)—H(4B)	109.5
O(1)^{ii}—Ag(1)—O(6)	89.97(4)	C(3)—C(4)—H(4C)	109.5
O(2)^{i}—Ag(1)—Ag(1)^{iii}	82.70(3)	H(4A)—C(4)—H(4C)	109.5
O(1)^{ii}—Ag(1)—Ag(1)^{iii}	74.79(3)	H(4B)—C(4)—H(4C)	109.5
O(6)—Ag(1)—Ag(1)^{iii}	141.23(3)	C(3)—C(5)—H(5A)	109.5
C(1)—O(1)—Ag(1)^{ii}	122.68(9)	C(3)—C(5)—H(5B)	109.5
O(1)—C(1)—O(2)	126.70(13)	H(5A)—C(5)—H(5B)	109.5
O(1)—C(1)—C(2)	118.54(12)	C(3)—C(5)—H(5C)	109.5
O(2)—C(1)—C(2)	114.76(12)	H(5A)—C(5)—H(5C)	109.5
C(1)—O(2)—Ag(1)^{iv}	119.54(9)	H(5B)—C(5)—H(5C)	109.5
N(1)—C(2)—C(1)	108.99(11)	C(2)—N(1)—H(1A)	110.2(12)
N(1)—C(2)—C(3)	112.20(11)	C(2)—N(1)—H(1B)	110.8(12)
C(1)—C(2)—C(3)	112.24(11)	H(1A)—N(1)—H(1B)	106.7(16)
N(1)—C(2)—H(2)	107.7	C(2)—N(1)—H(1C)	113.9(15)
C(1)—C(2)—H(2)	107.7	H(1A)—N(1)—H(1C)	103.6(18)
C(3)—C(2)—H(2)	107.7	H(1B)—N(1)—H(1C)	111.1(19)
C(5)—C(3)—C(4)	111.48(13)	O(5)—N(2)—O(3)	120.47(13)
C(5)—C(3)—C(2)	110.70(12)	O(5)—N(2)—O(4)	119.66(12)
C(4)—C(3)—C(2)	113.06(12)	O(3)—N(2)—O(4)	119.87(12)
C(5)—C(3)—H(3)	107.1	Ag(1)—O(6)—H(6A)	108.9(16)
C(4)—C(3)—H(3)	107.1	Ag(1)—O(6)—H(6B)	96.7(16)
C(2)—C(3)—H(3)	107.1	H(6A)—O(6)—H(6B)	101(2)
C(3)—C(4)—H(4A)	109.5		

Symmetry codes: (i) x−1, y, z; (ii) -x+2, -y, -z+1; (iii) -x+1, -y, -z+1; (iv) x+1, y, z.

Tab. S2 Possible hydrogen bonds (Å, °) for **AgVal**.

D—H···A	**d(D—H)**	**d(H···A)**	**d(D···A)**	**<(DHA)**
N(1)—H(1A)...O(4)^{ii}	0.91(2)	2.568(19)	3.2440(17)	131.7(15)
N(1)—H(1A)...O(5)^{iii}	0.91(2)	2.254(19)	2.9143(17)	129.3(15)
N(1)—H(1B)...N(2)^{vi}	0.86(2)	2.63(2)	3.4808(18)	172.5(16)
N(1)—H(1B)...O(4)^{vi}	0.86(2)	2.09(2)	2.8953(17)	156.3(17)
N(1)—H(1B)...O(5)^{vi}	0.86(2)	2.46(2)	3.2081(17)	146.1(16)
N(1)—H(1C)...O(2)^{i}	0.82(2)	2.03(2)	2.8393(16)	167(2)
O(6)—H(6A)...O(3)^{iv}	0.81(2)	2.04(2)	2.8444(16)	170(2)
O(6)—H(6B)...O(4)	0.85(3)	2.11(3)	2.9423(16)	164(2)

Symmetry codes: (i) x−1, y, z; (ii) -x+2, -y, -z+1; (iii) -x+1, -y, -z+1; (iv) x+1, y, z; (vi) x, y, z+1.

Tab. S3 Selected bond distances (Å) and angles (°) for **AgAsp**.

Bond distances			
Ag(1)—O(5)	2.189(3)	Ag(2)—O(6)	2.194(3)
Ag(1)—O(5)^{i}	2.189(3)	Ag(2)—O(9)^{viii}	2.732(3)
Ag(1)—O(1)	2.579(3)	O(5)—Ag(4B)^{v}	2.703(3)
Ag(1)—O(1)^{i}	2.579(3)	O(5)—C(5)	1.253(4)
Ag(1)—Ag(1)^{ii}	2.9254(8)	O(6)—C(5)	1.267(4)
Ag(1)—Ag(2)^{iii}	2.9307(4)	C(5)—C(6)	1.525(5)
Ag(1)—Ag(2)	2.9307(4)	C(6)—C(7)	1.535(5)
O(1)—C(1)	1.258(4)	C(6)—H(6A)	0.9900
O(1)—Ag(4A)^{iv}	2.228(6)	C(6)—H(6B)	0.9900
O(1)—Ag(4B)^{iv}	2.244(3)	C(7)—N(2)	1.491(4)
C(1)—O(2)	1.245(4)	C(7)—C(8)	1.535(5)
C(1)—C(2)	1.539(5)	C(7)—H(7)	1.0000
C(2)—N(1)	1.491(4)	N(2)—H(2A)	0.9100
C(2)—C(3)	1.531(4)	N(2)—H(2B)	0.9100
C(2)—H(2)	1.0000	N(2)—H(2C)	0.9100
N(1)—H(1A)	0.9100	C(8)—O(8)	1.240(4)
N(1)—H(1B)	0.9100	C(8)—O(7)	1.269(4)
N(1)—H(1C)	0.9100	O(7)—Ag(4B)	2.212(3)
C(3)—C(4)	1.531(5)	O(7)—Ag(4A)	2.271(6)
C(3)—H(3A)	0.9900	O(7)—Ag(3)	2.561(2)
C(3)—H(3B)	0.9900	O(9)—N(3)	1.263(4)
C(4)—O(4)^{v}	1.253(4)	N(3)—O(10)	1.240(5)
C(4)—O(3)	1.259(4)	N(3)—O(11)	1.249(5)
O(3)—Ag(3)	2.250(2)	O(11)—Ag(3)	2.555(3)
O(4)—Ag(3)	2.224(3)	Ag(3)—Ag(3)^{v}	2.8441(6)
O(4)—Ag(4A)	2.560(6)	Ag(3)—Ag(3)^{vi}	2.8942(6)
Ag(2)—O(6)^{ii}	2.194(3)		

Bond angles			
O(5)—Ag(1)—O(5)^{i}	173.74(14)	O(6)^{ii}—Ag(2)—Ag(1)	83.42(7)
O(5)—Ag(1)—O(1)	91.71(9)	O(6)—Ag(2)—Ag(1)	80.66(7)
O(5)^{i}—Ag(1)—O(1)	84.51(9)	Ag(1)^{ii}—Ag(2)—Ag(1)	59.881(15)
O(5)—Ag(1)—O(1)^{i}	84.51(9)	C(5)—O(5)—Ag(1)	125.4(2)
O(5)^{i}—Ag(1)—O(1)^{i}	91.71(9)	C(5)—O(6)—Ag(2)	123.9(2)
O(1)—Ag(1)—O(1)^{i}	105.85(13)	O(5)—C(5)—O(6)	126.4(3)
O(5)—Ag(1)—Ag(1)^{ii}	93.13(7)	O(5)—C(5)—C(6)	117.6(3)
O(5)^{i}—Ag(1)—Ag(1)^{ii}	93.13(7)	O(6)—C(5)—C(6)	116.0(3)
O(1)—Ag(1)—Ag(1)^{ii}	127.08(6)	C(5)—C(6)—C(7)	112.5(3)
O(1)^{i}—Ag(1)—Ag(1)^{ii}	127.07(6)	N(2)—C(7)—C(8)	109.1(3)
O(5)—Ag(1)—Ag(2)^{iii}	102.84(7)	N(2)—C(7)—C(6)	112.2(3)
O(5)^{i}—Ag(1)—Ag(2)^{iii}	80.34(7)	C(8)—C(7)—C(6)	110.2(3)
O(1)—Ag(1)—Ag(2)^{iii}	163.76(6)	O(8)—C(8)—O(7)	126.6(3)
O(1)^{i}—Ag(1)—Ag(2)^{iii}	69.00(6)	O(8)—C(8)—C(7)	118.6(3)
Ag(1)^{ii}—Ag(1)—Ag(2)^{iii}	60.059(8)	O(7)—C(8)—C(7)	114.9(3)
O(5)—Ag(1)—Ag(2)	80.35(7)	C(8)—O(7)—Ag(4B)	119.1(2)
O(5)^{i}—Ag(1)—Ag(2)	102.84(7)	C(8)—O(7)—Ag(4A)	115.5(3)
O(1)—Ag(1)—Ag(2)	69.00(6)	C(8)—O(7)—Ag(3)	115.3(2)
O(1)^{i}—Ag(1)—Ag(2)	163.76(6)	Ag(4B)—O(7)—Ag(3)	94.44(10)
Ag(1)^{ii}—Ag(1)—Ag(2)	60.059(8)	Ag(4A)—O(7)—Ag(3)	88.56(15)
Ag(2)^{iii}—Ag(1)—Ag(2)	120.119(15)	O(10)—N(3)—O(11)	120.0(3)
C(1)—O(1)—Ag(4A)^{iv}	112.8(2)	O(10)—N(3)—O(9)	120.3(3)
C(1)—O(1)—Ag(4B)^{iv}	122.4(2)	O(11)—N(3)—O(9)	119.7(3)
Ag(4A)^{iv}—O(1)—Ag(4B)^{iv}	9.65(9)	N(3)—O(11)—Ag(3)	107.8(2)
C(1)—O(1)—Ag(1)	139.5(2)	O(4)—Ag(3)—O(3)	164.36(9)
Ag(4A)^{iv}—O(1)—Ag(1)	105.81(13)	O(4)—Ag(3)—O(11)	120.83(10)
Ag(4B)^{iv}—O(1)—Ag(1)	96.29(10)	O(3)—Ag(3)—O(11)	74.38(10)
O(2)—C(1)—O(1)	126.4(3)	O(4)—Ag(3)—O(7)	83.25(9)
O(2)—C(1)—C(2)	118.5(3)	O(3)—Ag(3)—O(7)	97.62(9)
O(1)—C(1)—C(2)	115.1(3)	O(11)—Ag(3)—O(7)	77.82(9)
N(1)—C(2)—C(3)	109.4(3)	O(4)—Ag(3)—Ag(3)^{v}	82.53(7)
N(1)—C(2)—C(1)	109.9(3)	O(3)—Ag(3)—Ag(3)^{v}	82.10(6)
C(3)—C(2)—C(1)	110.7(3)	O(11)—Ag(3)—Ag(3)^{v}	156.40(8)
C(4)—C(3)—C(2)	114.3(3)	O(7)—Ag(3)—Ag(3)^{v}	103.81(6)
O(4)^{v}—C(4)—O(3)	126.8(3)	O(4)—Ag(3)—Ag(3) ^{vi}	104.66(8)
O(4)^{v}—C(4)—C(3)	115.5(3)	O(3)—Ag(3)—Ag(3)^{vi}	77.21(7)
O(3)—C(4)—C(3)	117.7(3)	O(11)—Ag(3)—Ag(3)^{vi}	90.42(7)
C(4)—O(3)—Ag(3)	123.8(2)	O(7)—Ag(3)—Ag(3)^{vi}	168.11(6)
C(4)^{v}—O(4)—Ag(3)	124.7(2)	Ag(3)^{v}—Ag(3)—Ag(3)^{vi}	86.225(5)

C(4)[v]—O(4)—Ag(4A)	123.2(3)	O(1)[vii]—Ag(4A)—O(7)	163.90(19)
Ag(3)—O(4)—Ag(4A)	89.63(15)	O(1)[vii]—Ag(4A)—O(4)	98.5(2)
O(6)[ii]—Ag(2)—O(6)	161.62(13)	O(7)—Ag(4A)—O(4)	82.4(2)
O(6)[ii]—Ag(2)—Ag(1)[ii]	80.66(7)	O(7)—Ag(4B)—O(1)[vii]	176.37(12)
O(6)—Ag(2)—Ag(1)[ii]	83.42(7)		

Symmetry codes: (i) -x, y, -z, (ii) -x, -y+1, z; (iii) x, -y+1, -z; (iv) x-1, y, z; (v) -x+1, y, -z; (vi) -x+1, -y, z; (vii) x+1, y, z; (viii) x-1/2, -y+1/2, -z+1/2.

Tab. S4 Possible hydrogen bonds (Å, °) for **AgAsp**.

D—H···A	**d(D—H)**	**d(H···A)**	**d(D···A)**	**<(DHA)**
N(1)—H(1A)...O(2)[ix]	0.91	1.97	2.855(4)	164
N(1)—H(1B)...O(8)[x]	0.91	2.22	2.998(4)	144
N(1)—H(1C)...O(3)	0.91	2.13	2.812(4)	131
N(1)—H(1C)...O(10)[vi]	0.91	2.37	2.980(4)	125
N(2)—H(2A)...O(8)[xi]	0.91	1.90	2.798(4)	170
N(2)—H(2B)...O(2)[xii]	0.91	2.13	2.979(4)	154
N(2)—H(2C)...O(6)	0.91	2.40	2.846(4)	110
N(2)—H(2C)...O(9)[x]	0.91	2.06	2.955(4)	170
N(2)—H(2C)...O(10)[x]	0.91	2.59	3.095(4)	116

Symmetry codes: (vi) -x+1, -y, z; (ix) -x, -y, z; (x) x-1/2, -y+1/2, -z+1/2; (xi) -x+1, -y+1, z; (xii) x+1/2, -y+1/2, -z+1/2.

Tab. S5 Crystal data and structure refinement summary for prepared complexes.

	AgVal	**AgAsp**
Empirical formula	$C_5H_{13}Ag_1N_2O_6$	$C_8H_{14}Ag_3N_3O_{12}$
Formula weight	305.04	667.83
Temperature	120(2) K	120(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Triclinic	Orthorhombic
Space group	*P*-1	*I*222
Unit cell dimensions	a = 5.3842(3) Å	a = 11.7530(7) Å
	b = 9.5109(6) Å	b = 12.4417(7) Å
	c = 10.1930(6) Å	c = 20.3709(11) Å
	α = 75.554(2)°	α = 90°
	β = 82.498(2)°	β = 90°
	γ = 74.559(2)°	γ = 90°
Volume	486.07(5) Å^3	2978.8(3) Å^3
Z	2	8
Density (calculated)	2.084 g/cm^3	2.978 g/cm^3
Absorption coefficient	2.082 mm^{-1}	3.983 mm^{-1}
F(000)	304	2560
Crystal size	0.290 x 0.205 x 0.200 mm^3	0.206 x 0.128 x 0.063 mm^3
θ range for data collection	2.713 to 27.523°	3.112 to 27.590°
Index ranges	$-6\leq h\leq 6$, $-12\leq k\leq 12$, $-13\leq l\leq 13$	$-15\leq h\leq 15$, $-16\leq k\leq 16$, $-26\leq l\leq 26$
Reflections collected/independent	19763/2218 [R(int) = 0.0243]	95745/3455 [R(int) = 0.0366]
Completeness to θ = 25.242°	99.8%	99.6%
Max. and min. transmission	0.68 and 0.60	0.73 and 0.37
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	2218 / 0 / 149	3455 / 0 / 239
Goodness-of-fit on F^2	1.192	1.211
Final R indices [$I>2\sigma(I)$]	$R1$ = 0.0142, $wR2$ = 0.0356	$R1$ = 0.0152, $wR2$ = 0.0389
R indices (all data)	$R1$ = 0.0151, $wR2$ = 0.0359	$R1$ = 0.0153, $wR2$ = 0.0389
Largest diff. peak and hole	0.378 and -0.606 e.Å^{-3}	0.377 and -0.816 e. Å^{-3}