Electronic Supplementary Information

Silver(I) complexes with different pyridine-4,5-dicarboxylate ligands as efficient agents for the control of cow mastitis associated pathogens

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Abstract

Infections of the cow udder leading to mastitis and lower milk quality are one of the biggest problems in the dairy industry worldwide. Unfortunately, therapeutic options for the treatment of cow mastitis are limited as the consequence of pathogens resistance development to conventionally used antibiotics. In a search for the agents, which will be active against cow mastitis associated pathogens, in the present study, five new silver(I) complexes with different chelating pyridine-4,5-dicarboxylate type of ligands, $[Ag(NO_3)(py\text{-}2py)]_n$ (**1**), $[Ag(NO_3)(py\text{-}2metz)]_n$ (**2**), $[Ag(CH_3CN)(py\text{-}2py)]BF_4$ (**3**), $[Ag(py\text{-}2tz)_2]BF_4$ (**4**) and $[Ag(py\text{-}2metz)_2]BF_4$ (**5**), py-2py is dimethyl 2,2'-bipyridine-4,5-dicarboxylate, py-2metz is dimethyl 2-(4-methylthiazol-2-yl)pyridine-4,5-dicarboxylate and py-2tz is dimethyl 2-(thiazol-2-yl)pyridine-4,5-dicarboxylate, were synthesized, structurally characterized and assessed for *in vitro* antimicrobial activity using both standard bioassay and clinical isolates from the contaminated milk sample originating from the cow with mastitis. These complexes showed remarkable activity against the standard panel of microorganisms as well as selection of clinical isolates from the milk of cow diagnosed with mastitis. With the aim to determine the therapeutic potential of the silver(I) complexes, their toxicity *in vivo* against the model organism, *Caenorhabditis elegans* (*C. elegans*) was investigated. The complexes which had the best therapeutic profile, **2** and **5**, induced bacterial membrane depolarization, production of the reactive oxygen species (ROS) in *Candida albicans* cells and inhibited the hyphae, as well as the biofilm formation. Taken together, the presented data suggest that the silver(I) complexes with pyridine ligands could be considered for the treatment of microbial pathogens, which are causative agents of cow mastitis.

Keywords: Silver(I) complexes; Pyridine-4,5-dicarboxylates; Cow mastitis; Antimicrobial activity; *Caenorhabditis elegans*

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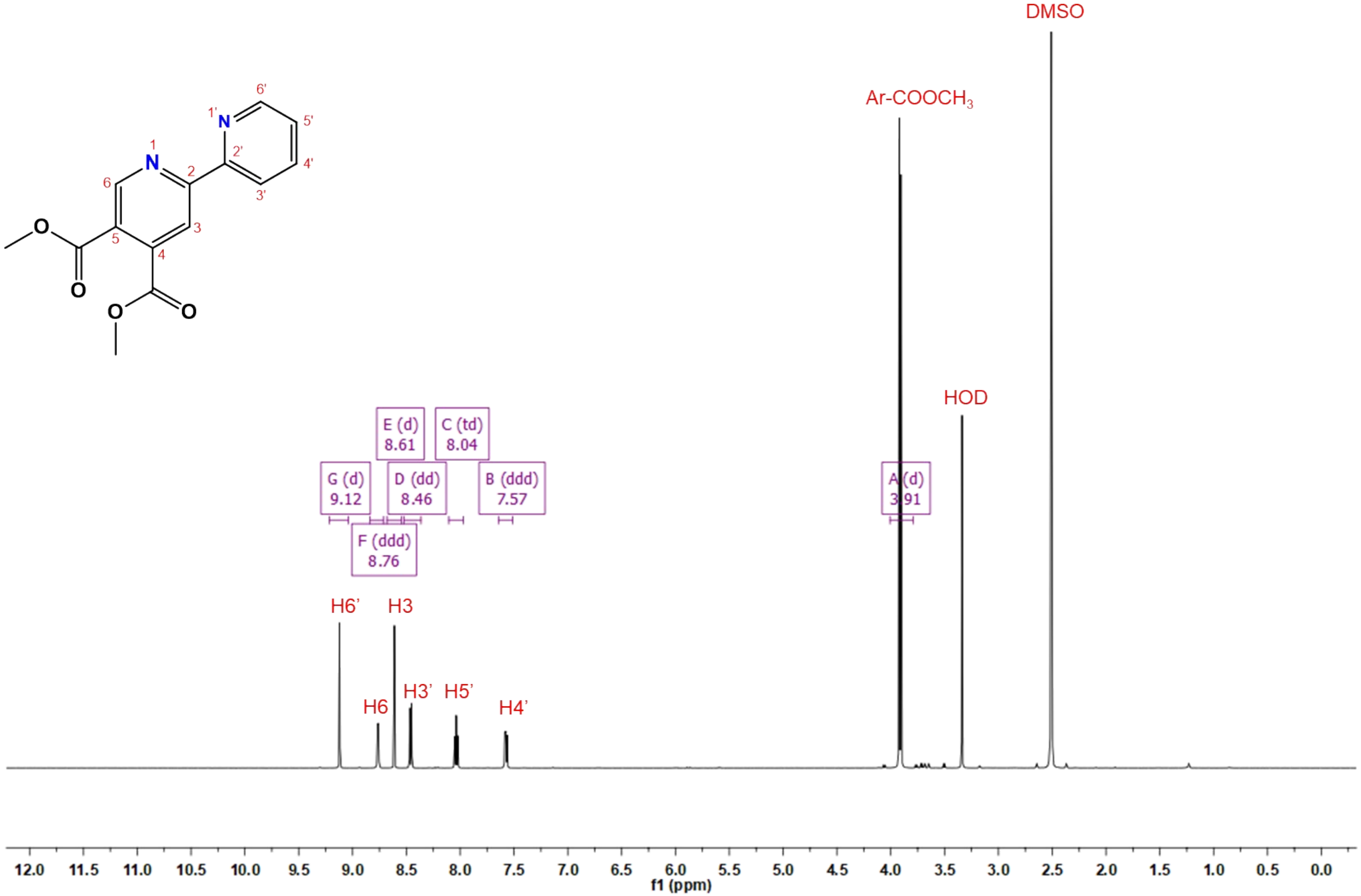
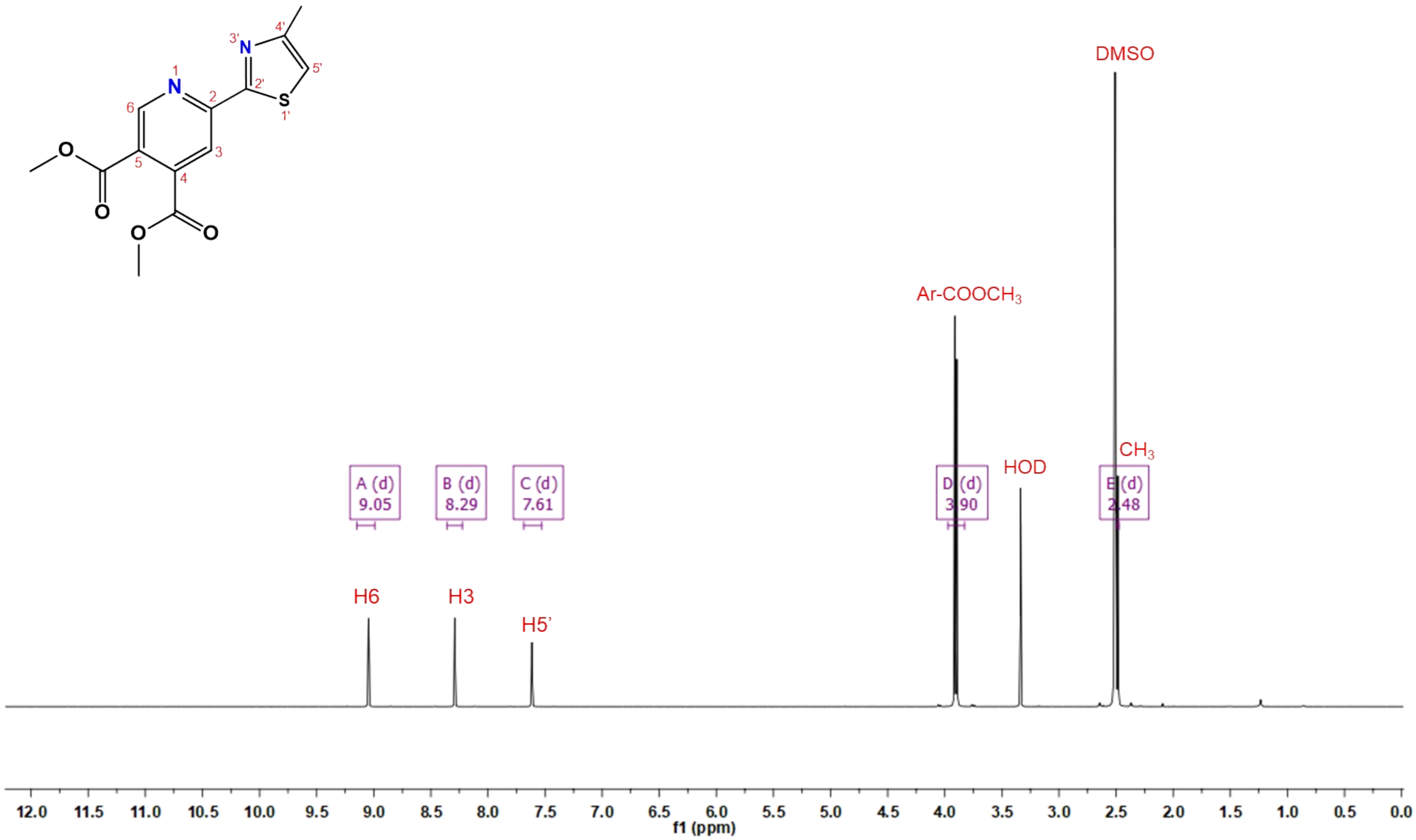
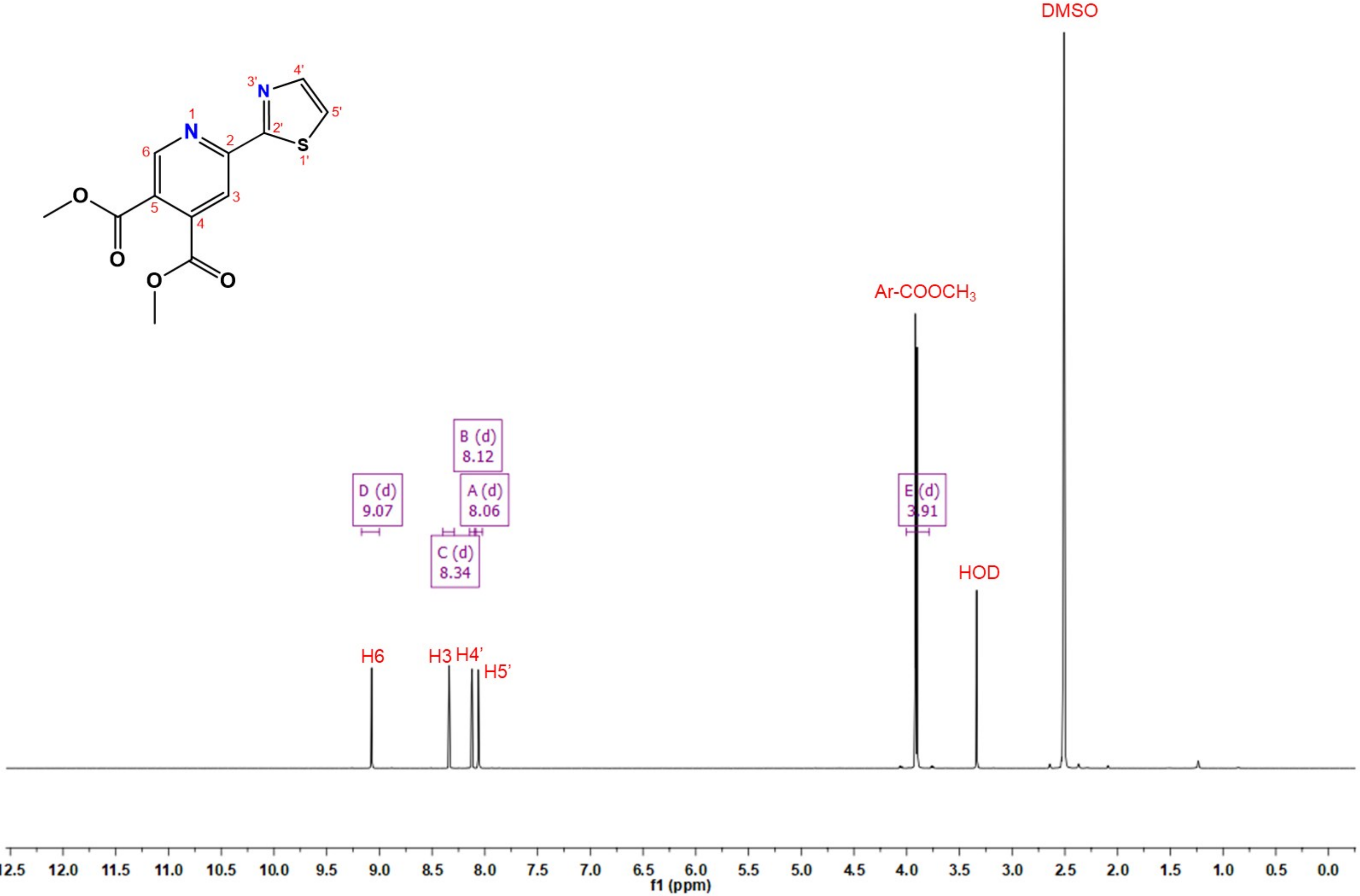
[1]H NMR spectrum of dimethyl 2,2'-bipyridine-4,5-dicarboxylate (py-2py)



[1]H NMR spectrum of dimethyl 2-(4-methylthiazol-2-yl)pyridine-4,5-dicarboxylate (py-2metz)



[1]H NMR spectrum of 2-(thiazol-2-yl)pyridine-4,5-dicarboxylate (py-2tz)



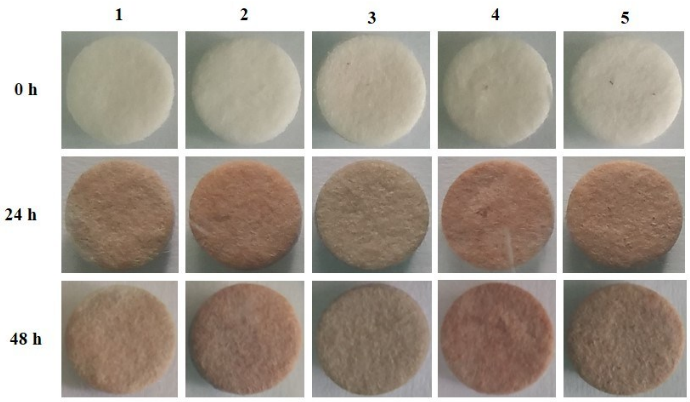


Fig. S1 Air/light stability of silver(I) complexes **1 – 5**.

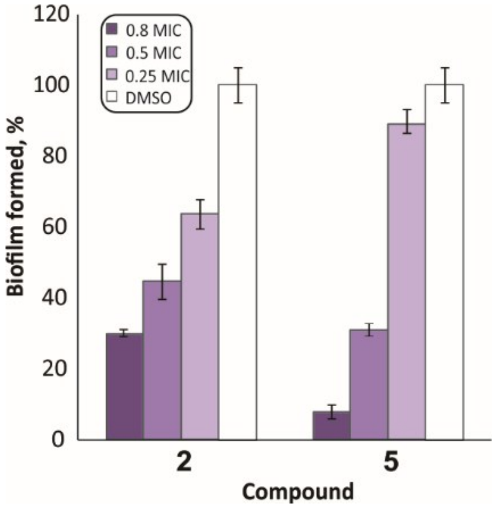


Fig. S2 Antibiofilm activity of **2** and **5** against *S. aureus* biofilms formed in the presence of sub-inhibitory concentrations of complexes for 24 h. The amount of DMSO was 0.1% (v/v).

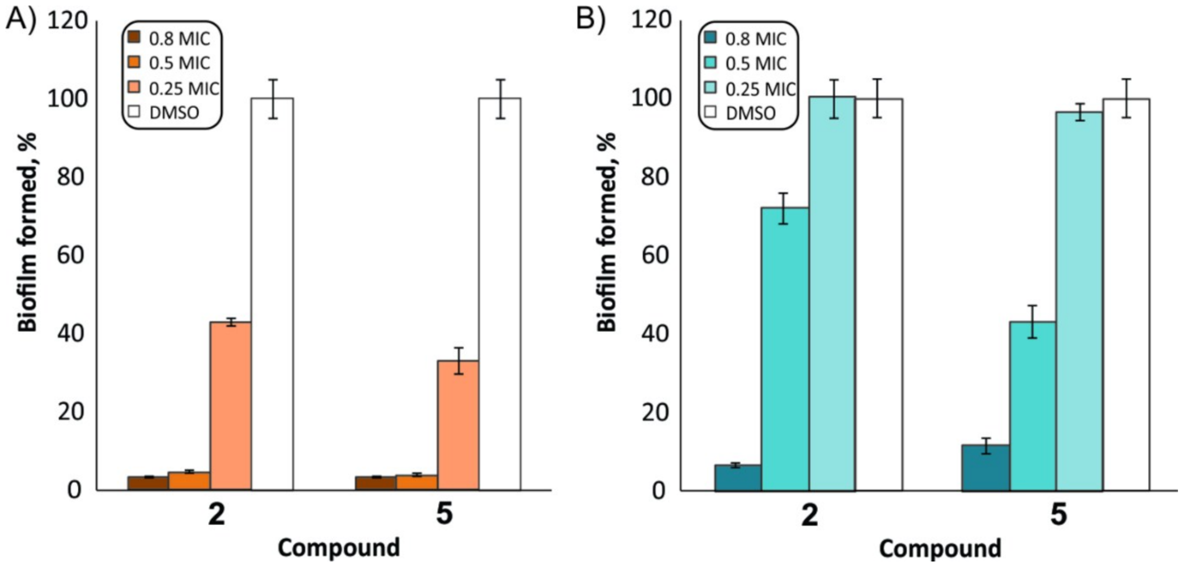


Fig. S3 Antibiofilm activity of **2** and **5** against *C. albicans* biofilms formed in the presence of sub-inhibitory concentrations of complexes for A) 24 h and B) 48 h. The amount of DMSO was 0.1% (v/v).

Table S1 ROS positive *C. albicans* cells detected by flow cytometry (fluorescence due to DCFH-DA (2',7'-dichlorofluorescin diacetate) probe oxidation)

Treatment	ROS^+, %[a]
Control (DMSO)	3.5
2	9.1
5	18.7
AmB	23.0

[a]percent of ROS^+ cells was determined by Partec FloMax software, whereby the baseline of zero (background level of fluorescence) was set based on the maximum value of the control sample without the ROS indicator. The percentage of ROS-positive cells was determined by counting cells with the higher ROS level than background and conversion into percentage.

Table S2 Crystallographic data for silver(I) complexes **1 – 5**

Compound	**Ag1**	**Ag2**
Empirical formula	$C_{14}H_{12}AgN_3O_7$	$C_{13}H_{12}AgN_3O_7S$
Formula weight	442.14	462.19
Temperature/K	150.00(10)	150.00(10)
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$
a/Å	12.3460(6)	11.8454(6)
b/Å	7.4026(4)	7.2758(4)
c/Å	16.9382(7)	18.8865(10)
α/°	90	90
β/°	104.169(4)	107.701(5)
γ/°	90	90
Volume/Å^3	1500.93(13)	1550.67(15)
Z	4	4
ρ_{calc}g/cm^3	1.957	1.980
μ/mm^{-1}	1.390	1.479
F(000)	880.0	920.0
Crystal size/mm^3	0.1 × 0.1 × 0.03	0.2 × 0.1 × 0.1
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data coll./°	5.284 to 54.968	4.858 to 54.962
Index ranges	$-16 \le h \le 13$, $-9 \le k \le 6$, $-19 \le l \le 21$	$-15 \le h \le 11$, $-9 \le k \le 6$, $-24 \le l \le 24$
Reflections collected	7600	7288
Independent reflections	3438 [R_{int} = 0.0261, R_{sigma} = 0.0381]	3560 [R_{int} = 0.0275, R_{sigma} = 0.0400]
Data/restraints/parameters	3438/0/228	3560/0/229
Goodness-of-fit on F^2	1.070	1.042
Final R indexes [I>=2σ (I)]	R_1 = 0.0292, wR_2 = 0.0620	R_1 = 0.0297, wR_2 = 0.0707
Final R indexes [all data]	R_1 = 0.0400, wR_2 = 0.0694	R_1 = 0.0382, wR_2 = 0.0773
Largest diff. peak/hole / eÅ^{-3}	0.41/-0.66	0.44/-1.08

Compound	**Ag3**	**Ag4**
Empirical formula	$C_{32}H_{30}Ag_2B_2F_8N_6O_8$	$C_{24}H_{20}AgBF_4N_4O_8S_2$
Formula weight	1015.98	751.24
Temperature/K	150.00(10)	150.00(10)
Crystal system	triclinic	monoclinic
Space group	P-1	$P2_1/n$
a/Å	6.8388(6)	12.2044(5)
b/Å	11.9872(9)	7.9645(3)
c/Å	12.9686(9)	29.6530(9)

$\alpha/°$	113.877(7)	90
$\beta/°$	93.318(6)	97.886(3)
$\gamma/°$	100.412(7)	90
Volume/Å^3	945.94(14)	2855.07(18)
Z	1	4
ρ_{calc}g/cm^3	1.783	1.748
μ/mm^{-1}	1.132	0.934
F(000)	504.0	1504.0
Crystal size/mm^3	0.4 × 0.1 × 0.1	0.3 × 0.1 × 0.1
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data coll./°	6.122 to 54.968	5.3 to 54.97
Index ranges	$-7 \leq h \leq 8$, $-12 \leq k \leq 15$, $-16 \leq l \leq 12$	$-15 \leq h \leq 15$, $-10 \leq k \leq 8$, $-28 \leq l \leq 38$
Reflections collected	6739	13853
Independent reflections	4334 [R_{int} = 0.0195, R_{sigma} = 0.0403]	6545 [R_{int} = 0.0235, R_{sigma} = 0.0365]
Data/restraints/parameters	4334/0/293	6545/0/438
Goodness-of-fit on F^2	1.067	1.019
Final R indexes [I>=2σ (I)]	R_1 = 0.0391, wR_2 = 0.0878	R_1 = 0.0293, wR_2 = 0.0641
Final R indexes [all data]	R_1 = 0.0539, wR_2 = 0.0986	R_1 = 0.0391, wR_2 = 0.0688
Largest diff. peak/hole / eÅ^{-3}	1.02/-1.45	0.40/-0.51

Compound	**Ag5**
Empirical formula	$C_{26}H_{24}AgBF_4N_4O_8S_2$
Formula weight	779.29
Temperature/K	150.00(10)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	10.5822(5)
b/Å	24.4148(8)
c/Å	12.6708(5)
$\alpha/°$	90
$\beta/°$	114.002(6)
$\gamma/°$	90
Volume/Å^3	2990.6(2)
Z	4
ρ_{calc}g/cm^3	1.731
μ/mm^{-1}	0.895
F(000)	1568.0
Crystal size/mm^3	0.1 × 0.1 × 0.05
Radiation	MoKα (λ = 0.71073)
2Θ range for data coll./°	4.85 to 54.958

Index ranges	$-13 \leq h \leq 13$, $-31 \leq k \leq 23$, $-16 \leq l \leq 11$
Reflections collected	13217
Independent reflections	6852 [R_{int} = 0.0260, R_{sigma} = 0.0401]
Data/restraints/parameters	6852/0/421
Goodness-of-fit on F^2	1.037
Final R indexes [I>=2σ (I)]	R_1 = 0.0325, wR_2 = 0.0758
Final R indexes [all data]	R_1 = 0.0442, wR_2 = 0.0832
Largest diff. peak/hole / eÅ^{-3}	0.63/-0.53