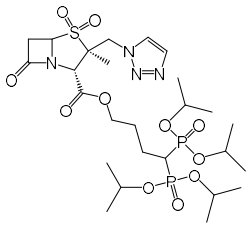
Synthesis and Biological Evaluation of a Bisphosphonate–Tazobactam Conjugate for Bone-Targeted β-Lactamase Inhibition

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Supplementary Materials



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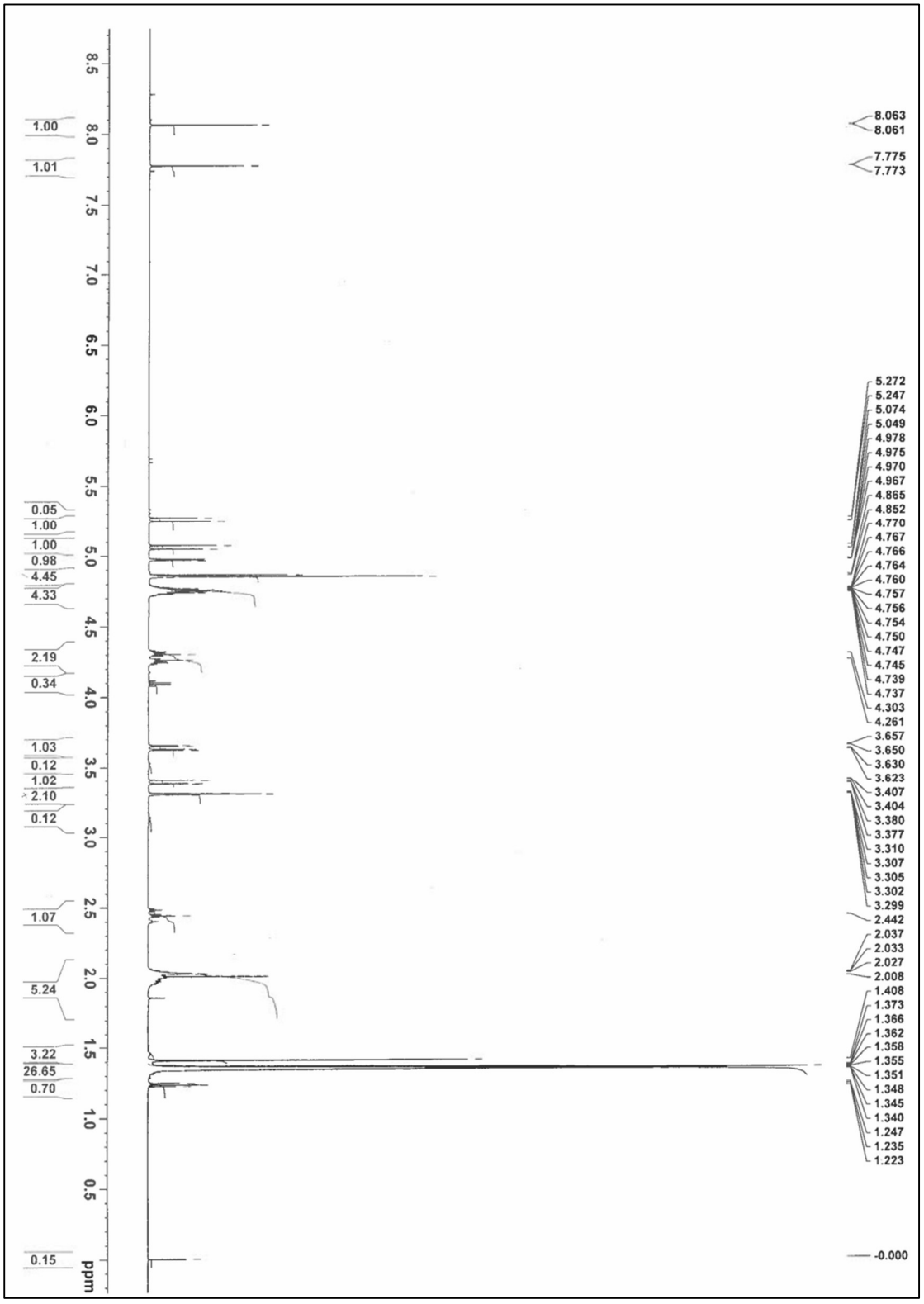


Figure S1: ^{1}H NMR spectrum of compound 5;

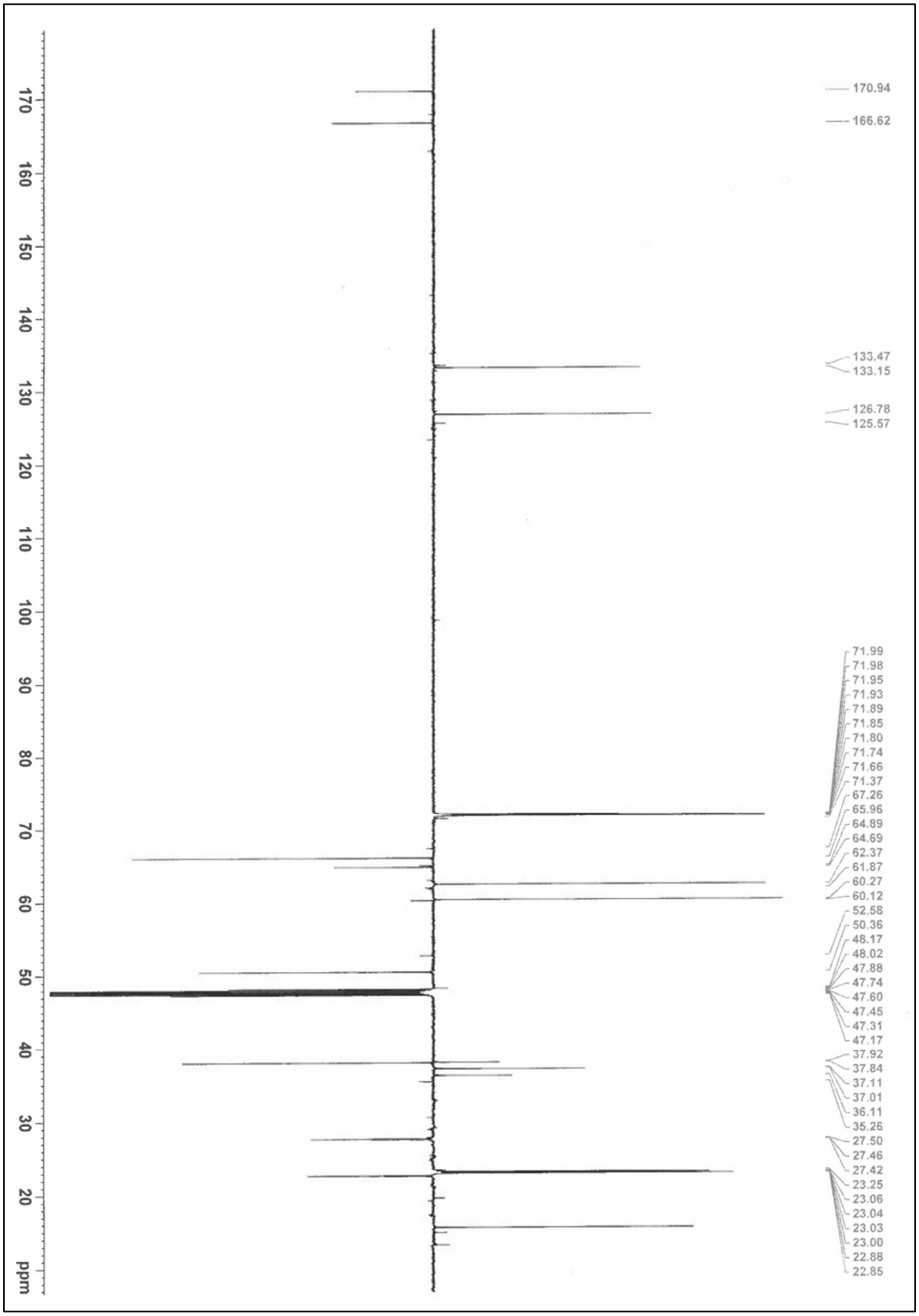


Figure S2: ^{13}C NMR spectrum of compound 5;

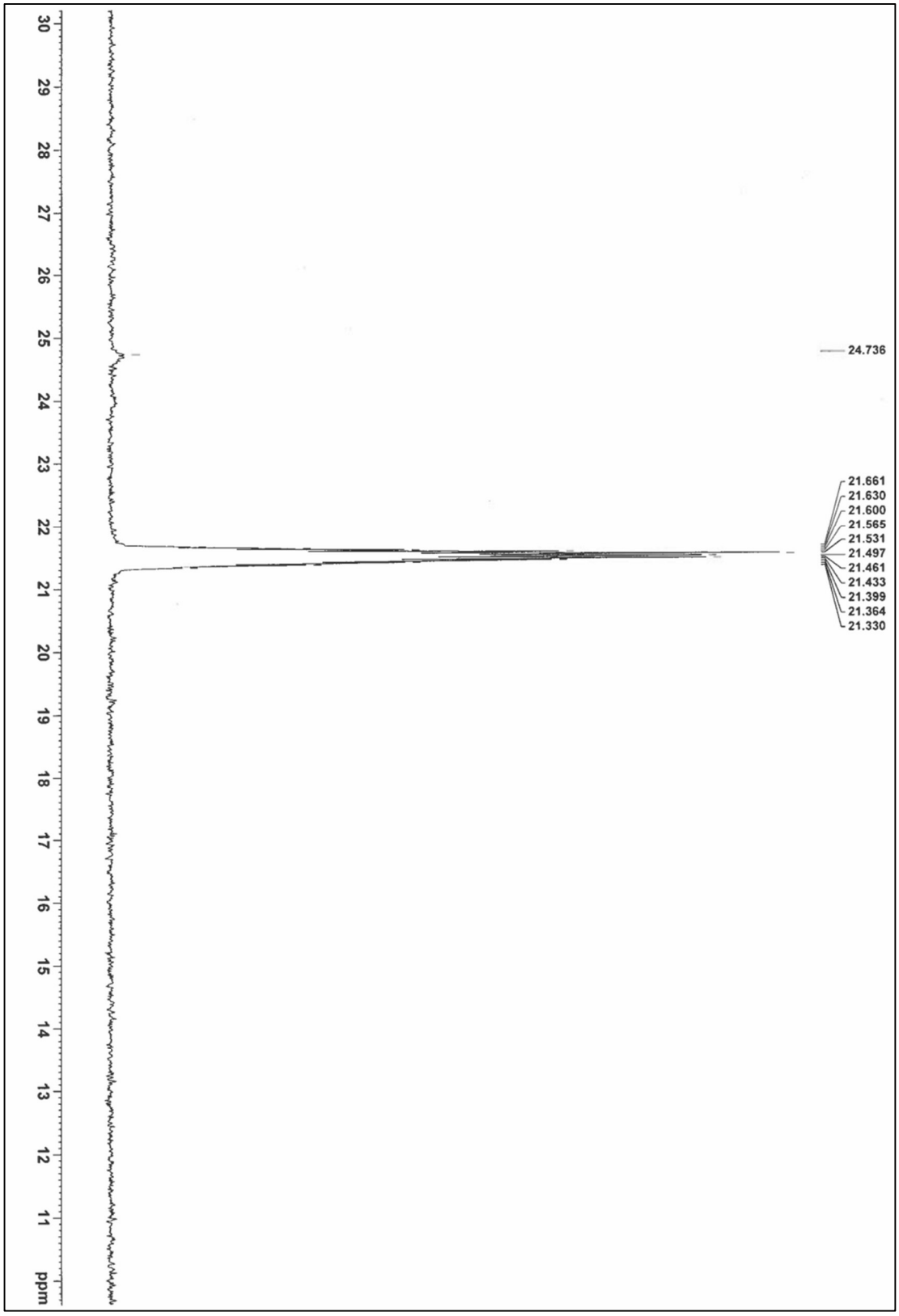


Figure S3: ^{31}P NMR spectrum of compound 5;

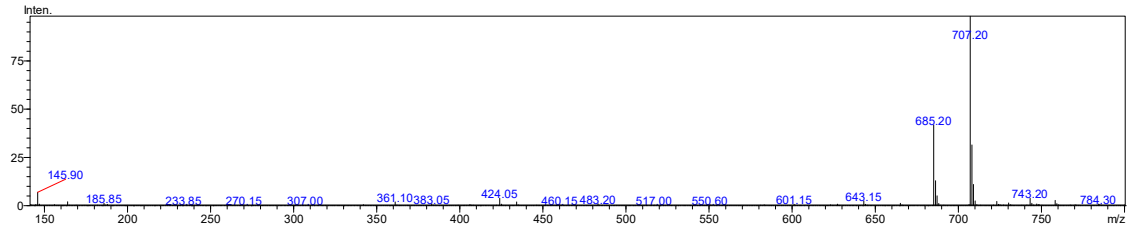


Figure S4: ESI–MS spectrum of compound 5;

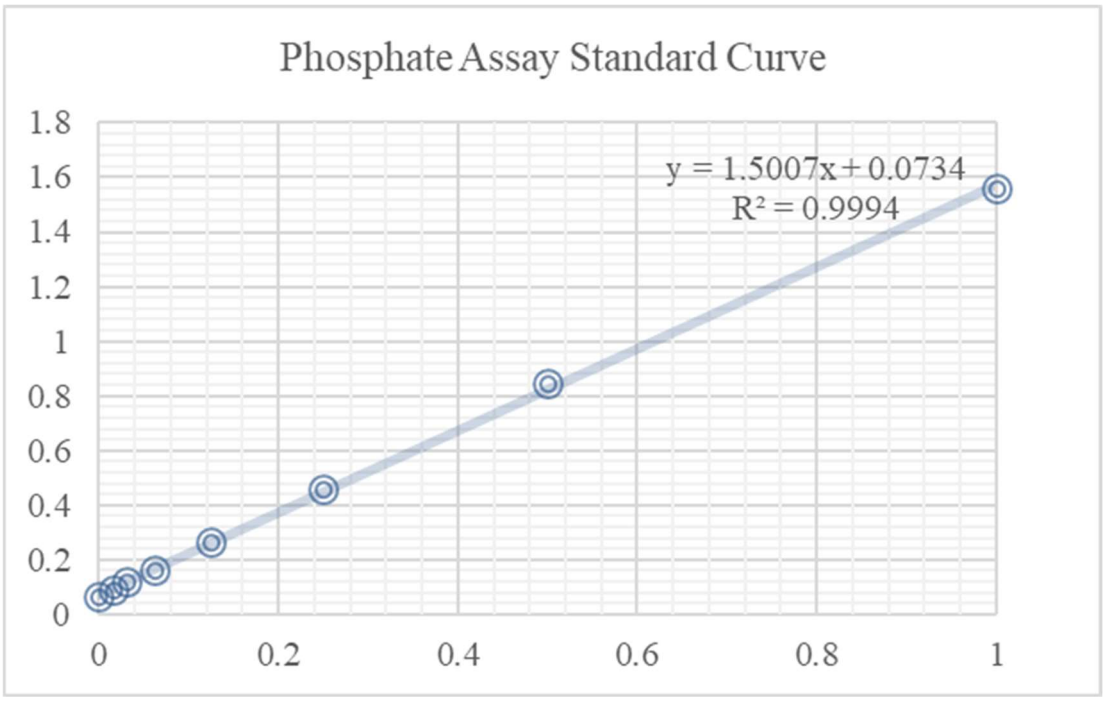


Figure S5: phosphate-assay calibration curve;

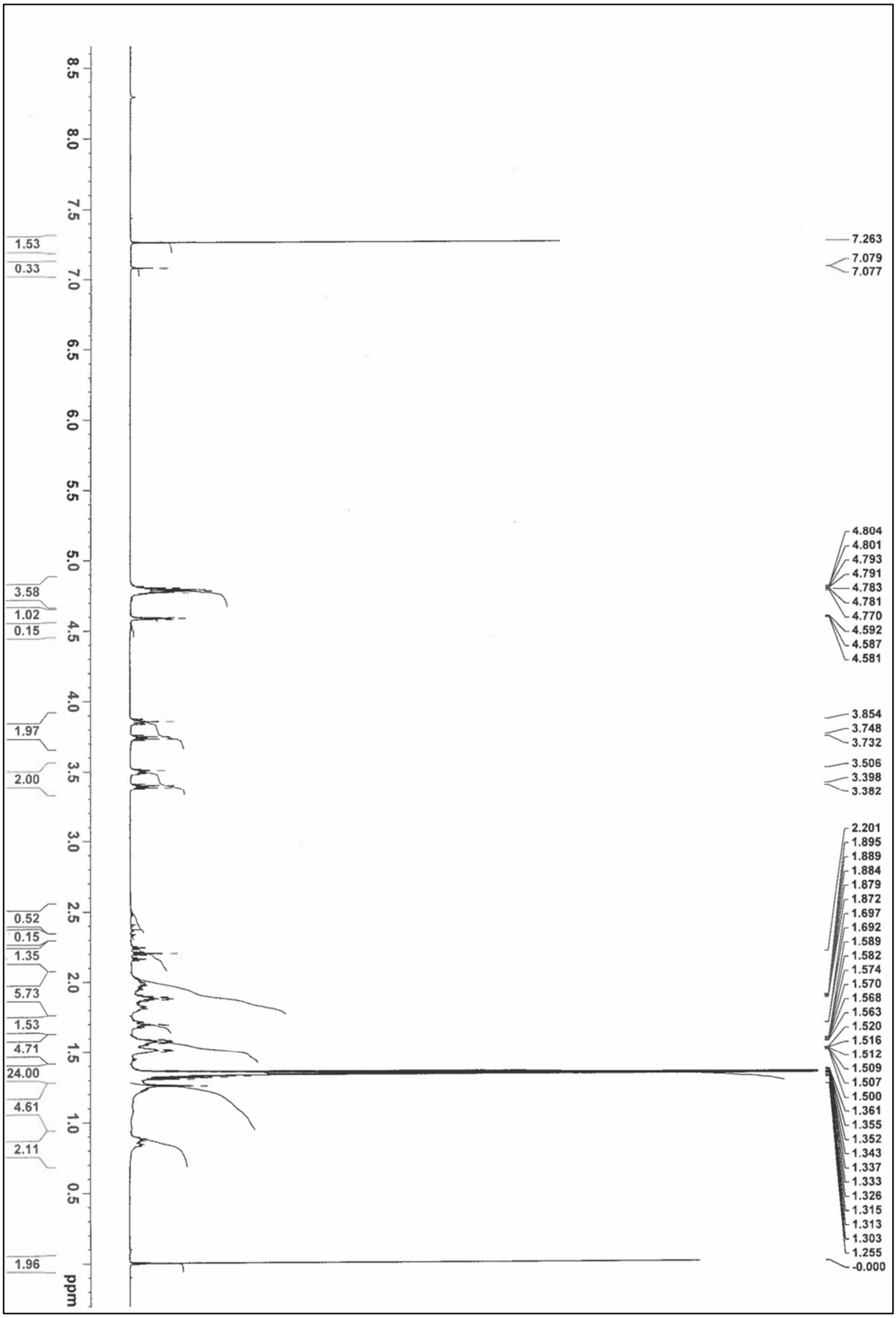


Figure S6: ^{1}H NMR spectrum of compound 3;

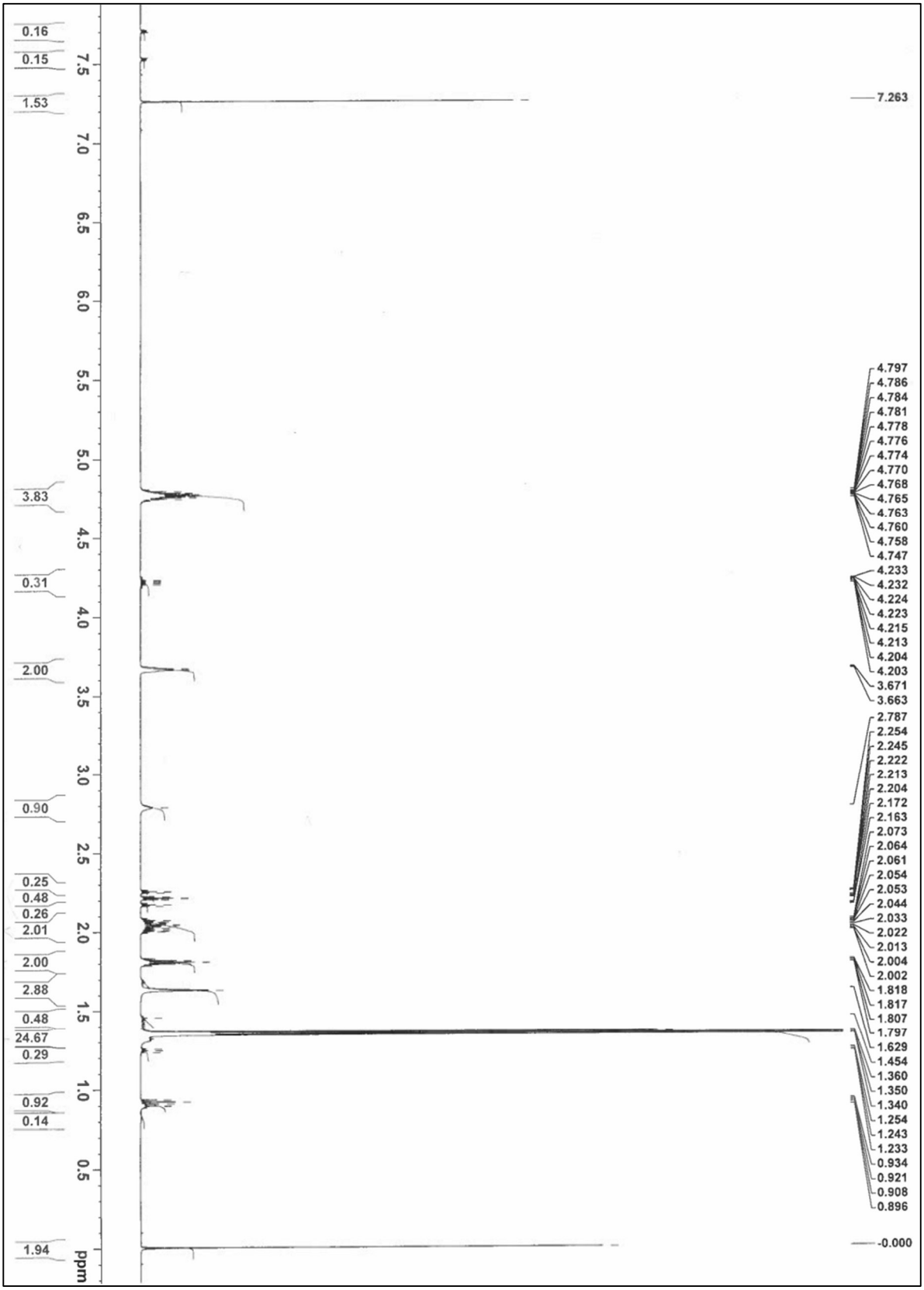


Figure S7: ^{1}H NMR spectrum of compound 4;

Table S1. Complete antimicrobial concentration matrix and raw OD_{660} values.

Treatment	Nominal concentration (μg/mL)	OD_{660} Replicate 1	OD_{660} Replicate 2	OD_{660} Replicate 3	Mean raw OD_{660}	Antimicrobial-only OD_{660}
Piperacillin	250	0.153	0.111	0.101	0.122	0.036
Piperacillin	125	0.289	0.282	0.266	0.279	0.036
Piperacillin	62.5	0.408	0.455	0.380	0.414	0.036
Piperacillin	31.25	0.403	0.668	0.402	0.491	0.036
Piperacillin	15.62	0.459	0.460	0.692	0.537	0.036
Piperacillin	7.81	0.464	0.476	1.000	0.647	0.036
Piperacillin	3.9	1.000	1.000	1.000	1.000	0.036
Piperacillin	1.95	1.000	1.000	1.000	1.000	0.036
Piperacillin + tazobactam	250	0.041	0.042	0.040	0.041	0.036
Piperacillin + tazobactam	125	0.042	0.043	0.043	0.043	0.036
Piperacillin + tazobactam	62.5	0.043	0.043	0.042	0.043	0.037
Piperacillin + tazobactam	31.25	0.564	0.429	0.492	0.495	0.037
Piperacillin + tazobactam	15.62	0.767	0.841	0.876	0.828	0.040
Piperacillin + tazobactam	7.81	0.491	0.846	0.802	0.713	0.036

Treatment	Nominal concentration (μg/mL)	OD_{660} Replicate 1	OD_{660} Replicate 2	OD_{660} Replicate 3	Mean raw OD_{660}	Antimicrobial-only OD_{660}
Piperacillin + tazobactam	3.9	0.902	0.930	0.847	0.893	0.036
Piperacillin + tazobactam	1.95	1.000	1.000	1.000	1.000	0.036
Piperacillin + compound 5	250	0.041	0.040	0.041	0.041	0.036
Piperacillin + compound 5	125	0.042	0.041	0.042	0.042	0.036
Piperacillin + compound 5	62.5	0.243	0.290	0.123	0.219	0.036
Piperacillin + compound 5	31.25	0.639	0.640	0.653	0.644	0.036
Piperacillin + compound 5	15.62	0.881	0.903	0.871	0.885	0.036
Piperacillin + compound 5	7.81	0.842	0.831	0.853	0.842	0.036
Piperacillin + compound 5	3.9	0.866	0.892	0.908	0.889	0.036
Piperacillin + compound 5	1.95	1.000	1.000	1.000	1.000	0.036

Note: OD_{660} values are uncorrected raw plate-reader measurements; mean values are arithmetic means of the three treatment replicates. The antimicrobial-only column gives the corresponding drug-background reading used in the source calculations. The piperacillin + tazobactam and piperacillin + compound 5 series were prepared as fixed 8:1 (w/w) piperacillin:inhibitor mixtures (222.2 μg/mL piperacillin and 27.8 μg/mL inhibitor at the nominal total concentration of 250 μg/mL), followed by twofold serial dilution. Raw untreated *E. coli* control OD_{660} readings were 0.873, 0.968, 1.000; raw blank readings were 0.047, 0.041, 0.041, 0.042, 0.042, 0.042, 0.043, 0.047.

Table S2. Individual bone-slice CFU measurements.

Treatment	Bone-slice replicate	Colonies counted	CFU/mL	$\log_{10}$ CFU/mL	Detection status
Infected, untreated bone-slice control	1	90	900,000	5.954	Detected
Infected, untreated bone-slice control	2	87	870,000	5.940	Detected
Infected, untreated bone-slice control	3	112	1,120,000	6.049	Detected
Ciprofloxacin (0.5 μg/mL)	1	52	520,000	5.716	Detected
Ciprofloxacin (0.5 μg/mL)	2	38	380,000	5.580	Detected
Ciprofloxacin (0.5 μg/mL)	3	62	620,000	5.792	Detected
Piperacillin (250 μg/mL)	1	1	10,000	4.000	Detected
Piperacillin (250 μg/mL)	2	1	10,000	4.000	Detected
Piperacillin (250 μg/mL)	3	0	0	N/A	No colonies detected
Piperacillin + tazobactam (62.5 μg/mL)	1	0	0	N/A	No colonies detected
Piperacillin + tazobactam (62.5 μg/mL)	2	0	0	N/A	No colonies detected
Piperacillin + tazobactam (62.5 μg/mL)	3	0	0	N/A	No colonies detected
Piperacillin + compound 5 (62.5 μg/mL)	1	0	0	N/A	No colonies detected
Piperacillin + compound 5 (62.5 μg/mL)	2	0	0	N/A	No colonies detected

Treatment	Bone-slice replicate	Colonies counted	CFU/mL	log_{10} CFU/mL	Detection status
Piperacillin + compound 5 (62.5 μg/mL)	3	0	0	N/A	No colonies detected
Piperacillin + compound 5 (125 μg/mL)	1	0	0	N/A	No colonies detected
Piperacillin + compound 5 (125 μg/mL)	2	0	0	N/A	No colonies detected
Piperacillin + compound 5 (125 μg/mL)	3	0	0	N/A	No colonies detected
Sterile, non-infected bone-slice control	1	0	0	N/A	No colonies detected
Sterile, non-infected bone-slice control	2	0	0	N/A	No colonies detected
Sterile, non-infected bone-slice control	3	0	0	N/A	No colonies detected

Note: Each row represents an individual bone-slice replicate from the source workbook. For all samples, the recorded dilution factor was 1000 and the plated volume was 0.1 mL. CFU/mL was calculated as colony count × dilution factor / plated volume. For zero-colony plates, the source workbook records 0 CFU/mL and "N/A" for log_{10} CFU/mL; these observations are reported here as "No colonies detected" and should be interpreted according to the assay detection limit defined in the Methods.

Table S3. Additional structural and drug-likeness descriptors for tazobactam and compound 5.

Descriptor	Tazobactam	Compound 5 (BP-Tazo-ester)	Source / interpretation
Heavy atom count	20	44	Structure-derived; tazobactam cross-checked against PubChem

Stereocenter count	3	3	Compound 5 retains the tazobactam (2S,3S,5R) stereocenters
β-Lactam moiety	Present	Present	Structure-derived
Triazole moiety	Present	Present	Structure-derived
Bisphosphonate functionality	Absent	Two phosphonate groups, present as isopropyl-protected esters	Structure-derived
Ester linker	Absent	Present	Structure-derived
Lipinski Rule-of-Five violations	0	2 (molar mass >500 g/mol; HBA >10)	SwissADME
Veber criteria	Compliant	Non-compliant (RotB >10; TPSA >140 Å^2)	SwissADME;
Predicted BBB permeability	Low/non-permeant	Low/non-permeant	SwissADME

Note: Parameters in Table S3 are supplementary descriptors and are not experimental measures of pharmacokinetics, biodistribution, bone affinity, or antimicrobial efficacy.

Table S4. Comparison of the ^{1}H NMR data for compounds 3 and 4 with the corresponding literature values reported by Tanaka et al. [20].

Assignment	This work (600 MHz, $CDCl_3$)	Tanaka et al. [20] (400 MHz, $CDCl_3$)
Compound 3 (this work) vs. compound 5c [20]		
P–CH–P (methine)	δ 2.20 (tt, *J* = 24.3, 5.9 Hz, 1H)	δ 2.19 (tt, *J* = 24.3, 5.9 Hz, 1H)
$OCH(CH_3)_2$, CH_3	δ 1.30–1.36 (m, 24H)	δ 1.33–1.36 (m, 24H)
$OCH(CH_3)_2$, CH	δ 4.79 (m, 4H)	δ 4.67 (m, 4H)
THP O–CH–O	δ 4.59 (m, 1H)	δ 4.59 (m, 1H)
CH_2–O (×2)	δ 3.45 (m, 2H), 3.80 (m, 2H)	δ 3.51 (m, 2H), 3.86 (m, 2H)
Alkyl/THP CH_2	δ 1.50–1.90 (m, 10H)	δ 1.45–2.06 (m, 8H), 2.13 (m, 2H)
Total integration	44H	44H
Compound 4 (this work) vs. compound 6c [20]		
P–CH–P (methine)	δ 2.21 (tt, *J* = 24.6, 5.4 Hz, 1H)	δ 2.21 (tt, *J* = 24.8, 5.4 Hz, 1H)

Assignment	This work (600 MHz, $CDCl_3$)	Tanaka et al. [20] (400 MHz, $CDCl_3$)
$OCH(CH_3)_2$, CH_3	δ 1.34–1.36 (m, 24H)	δ 1.33–1.36 (m, 24H)
$OCH(CH_3)_2$, CH	δ 4.75–4.80 (m, 4H)	δ 4.70–4.83 (m, 4H)
CH_2–OH	δ 3.66 (m, 2H)	δ 3.66 (t, *J* = 5.9 Hz, 2H)
Alkyl CH_2 and OH*	δ 1.80–1.82 (m, 2H), 2.00–2.07 (m, 2H), 2.79 (m, 1H, OH)	δ 1.77–1.83 (m, 1H), 1.96–2.10 (m, 2H), 2.31–2.42 (m, 2H)
Total integration	36H	36H

**The hydroxyl proton is exchangeable, and its chemical shift depends on concentration and residual water; the OH resonance was not separately assigned in ref. [20]. The total integration of this region (5H) is identical in both data sets.*

Table S5. Minimum inhibitory concentration (MIC) results against *S. aureus* CCUG 25925.

Treatment	MIC (μg/mL)	Assay / concentration convention
Piperacillin (PIP)	7.81	Piperacillin alone
PIP–Tazo (8:1, w/w)	0.98	PIP–Tazo combination; total nominal concentration
PIP–BP–Tazo (8:1, w/w)	0.98	PIP–BP–Tazo combination; total nominal concentration
BP–Tazo-ester (compound 5) alone	>250	Compound 5 alone; single-agent concentration

All four treatment groups belong to the same continuous *S. aureus* CCUG 25925 MIC study and were tested in consecutive group-specific dilution series. PIP–Tazo and PIP–BP–Tazo used the same 8:1 (w/w) piperacillin:inhibitor ratio, and their MICs are expressed as total nominal formulation concentrations. The MIC for compound 5 alone is expressed as its single-agent concentration. MICs are discrete twofold-dilution endpoints.

Table S6. Raw OD_{660} readings and blank-corrected growth-inhibition estimates for BP–Tazo-ester (compound 5) alone against *S. aureus* CCUG 25925.

Compound 5 (μg/mL)	OD_{660} rep. 1	OD_{660} rep. 2	OD_{660} rep. 3	Mean OD_{660}	Corrected inhibition (%)
250	0.332	0.321	0.320	0.324	24.8
125	0.332	0.324	0.323	0.326	24.3
62.5	0.395	0.350	0.350	0.365	14.0
31.25	0.352	0.381	0.381	0.371	12.4
15.62	0.343	0.380	0.323	0.349	18.4
7.812	0.366	0.370	0.375	0.370	12.6
3.906	0.366	0.366	0.326	0.353	17.3
1.953	0.347	0.347	0.333	0.342	20.0
0.9766	0.389	0.366	0.389	0.381	9.7
0.4883	0.353	0.387	0.348	0.363	14.7

Bacteria-only controls (n = 9): 0.443, 0.450, 0.443, 0.443, 0.390, 0.420, 0.399, 0.387, and 0.388 (mean = 0.4181). Media-only blank: 0.040. Blank-corrected inhibition (%) = 100 × [1 − (mean sample OD − 0.040)/(0.4181 − 0.040)]. Each sample concentration was tested in three technical replicate wells. The highest concentration tested was 250 μg/mL; complete inhibition was not observed.