

Orthoformimycin, a selective inhibitor of bacterial translation elongation from *Streptomyces* containing an unusual orthoformate

Sonia I. Maffioli^{*1}, Attilio Fabbretti², Letizia Brandi², Andreas Savelsbergh³, Paolo Monciardini¹, Monica Abbondi⁴, Rossana Rossi⁵, Stefano Donadio¹, Claudio O. Gualerzi^{*2}

¹ *Naicons, 20138 Milano, Italy;* ² *Laboratory of Genetics, Department of Biosciences and Biotechnology, University of Camerino, 62032 Camerino, Italy;* ³ *Institut für Medizinische Biochemie, Universität Witten/Herdecke, 58453 Witten, Germany;* ⁴ *Vicuron Pharmaceuticals, 21040 Gerenzano, Italy;* ⁵ *ITB-CNR, 20090 Segrate, Italy.*

Supporting Information

Supplementary figures and tables:

Supplementary Figure S1 ^1H -NMR spectrum of Orthoformimycin in CD_3CN .

Supplementary Figure S2 COSY spectrum of Orthoformimycin in CD_3CN .

Supplementary Figure S3 TOCSY spectrum of Orthoformimycin in CD_3CN .

Supplementary Figure S4 HSQC spectrum of Orthoformimycin in CD_3CN .

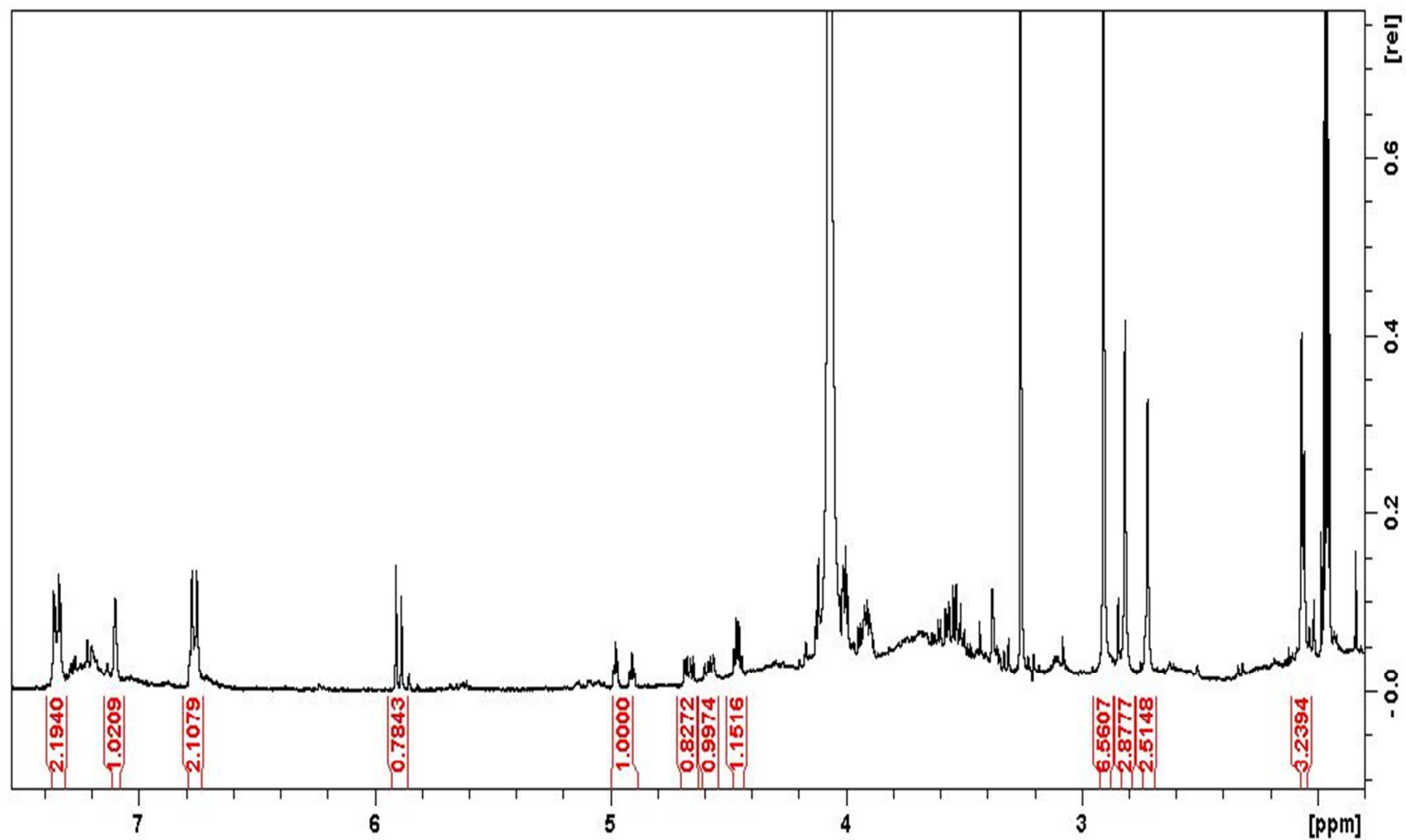
Supplementary Figure S5 HMBC spectrum of Orthoformimycin in CD_3CN .

Supplementary Figure S6 NOESY spectrum of Orthoformimycin in CD_3CN .

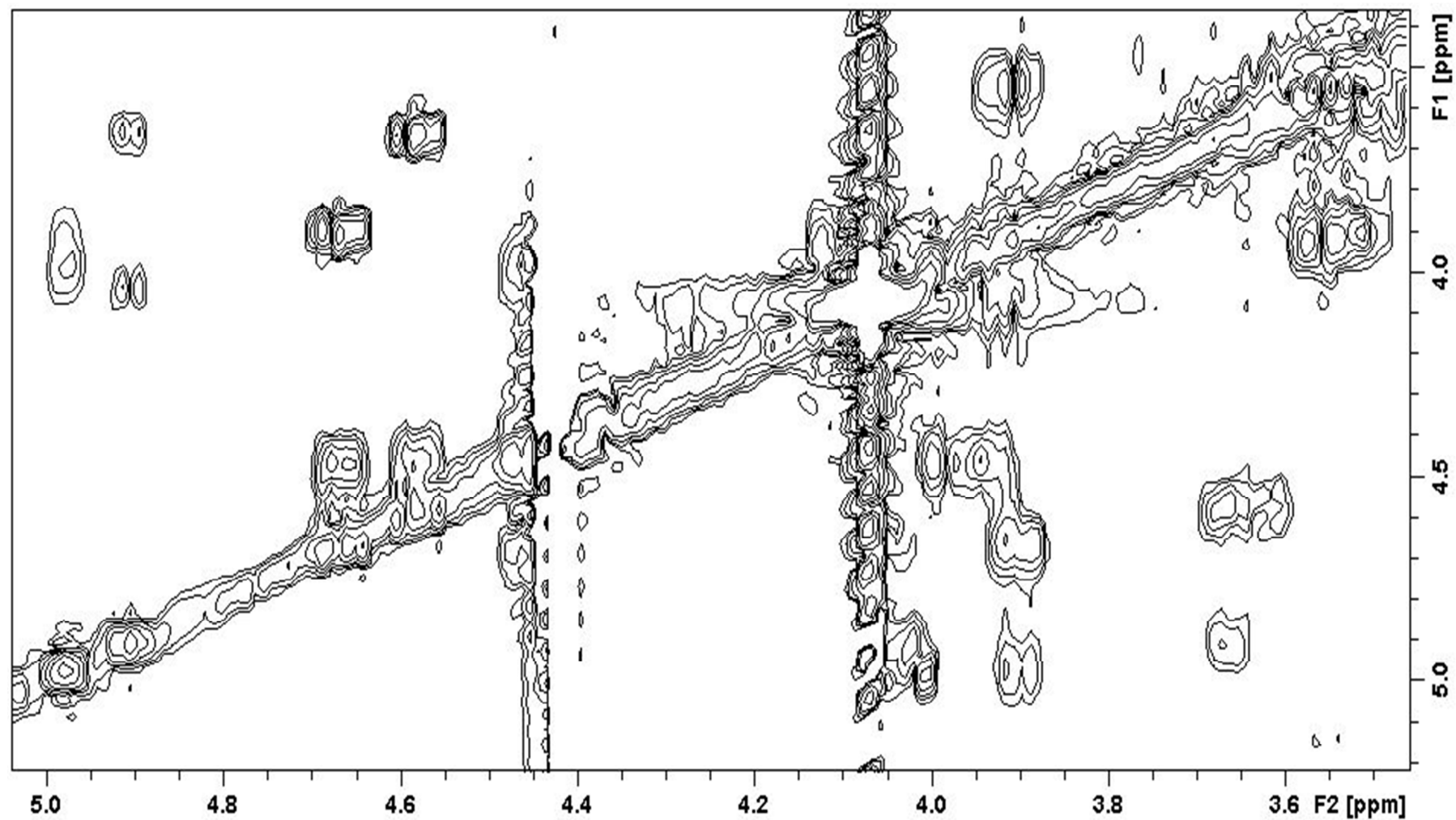
Supplementary Figure S7 Effect of orthoformimycin and other antibiotics on P-site and A-site decoding activity

Supplementary Figure S8 Effect of orthoformimycin and other antibiotics on various translocation steps

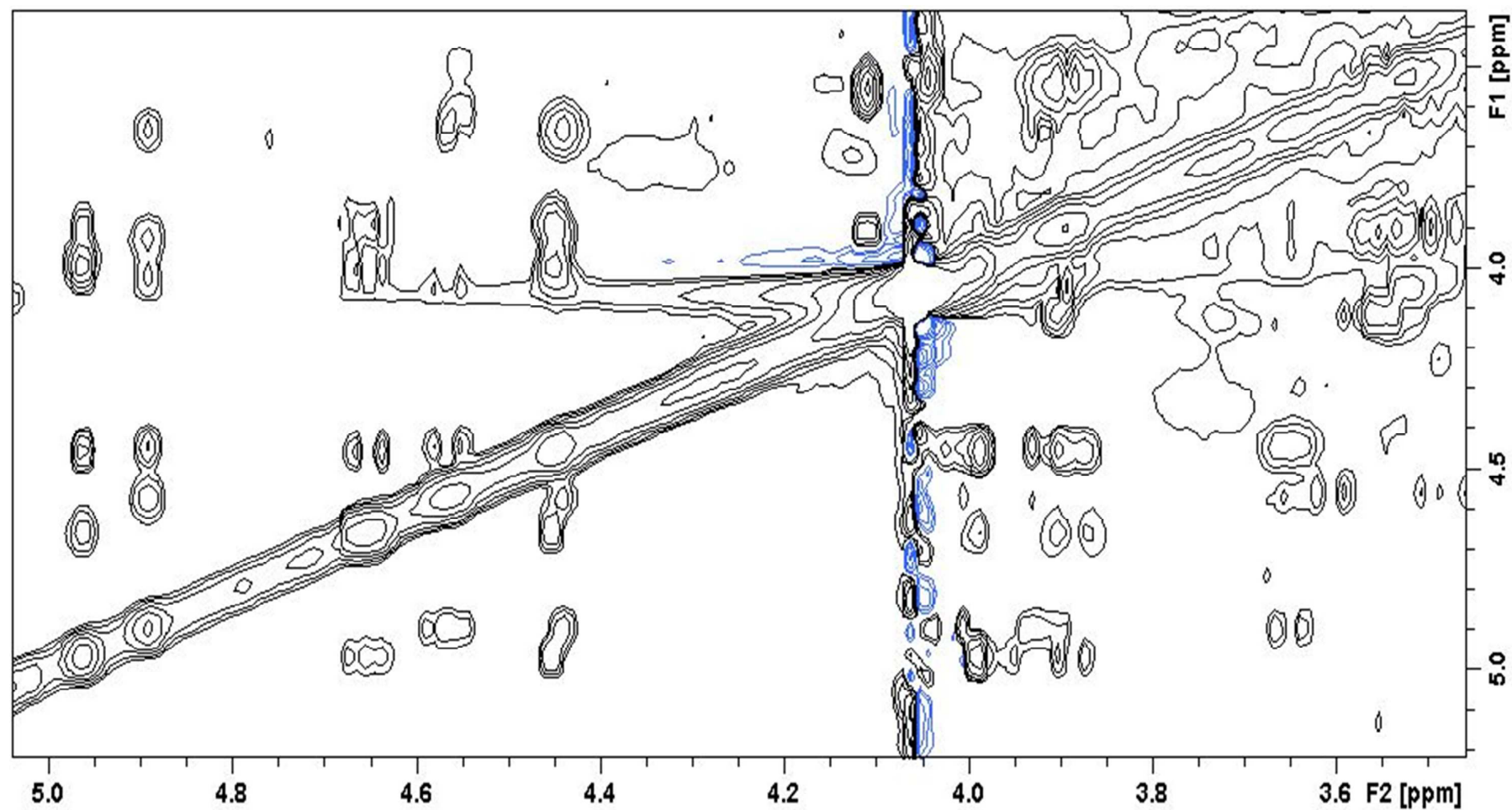
Supplementary Table S1 HR-MS and MS^n data for Orthoformimycin (m/z 540) and its acetylated derivative (m/z 680) (See Fig.3)



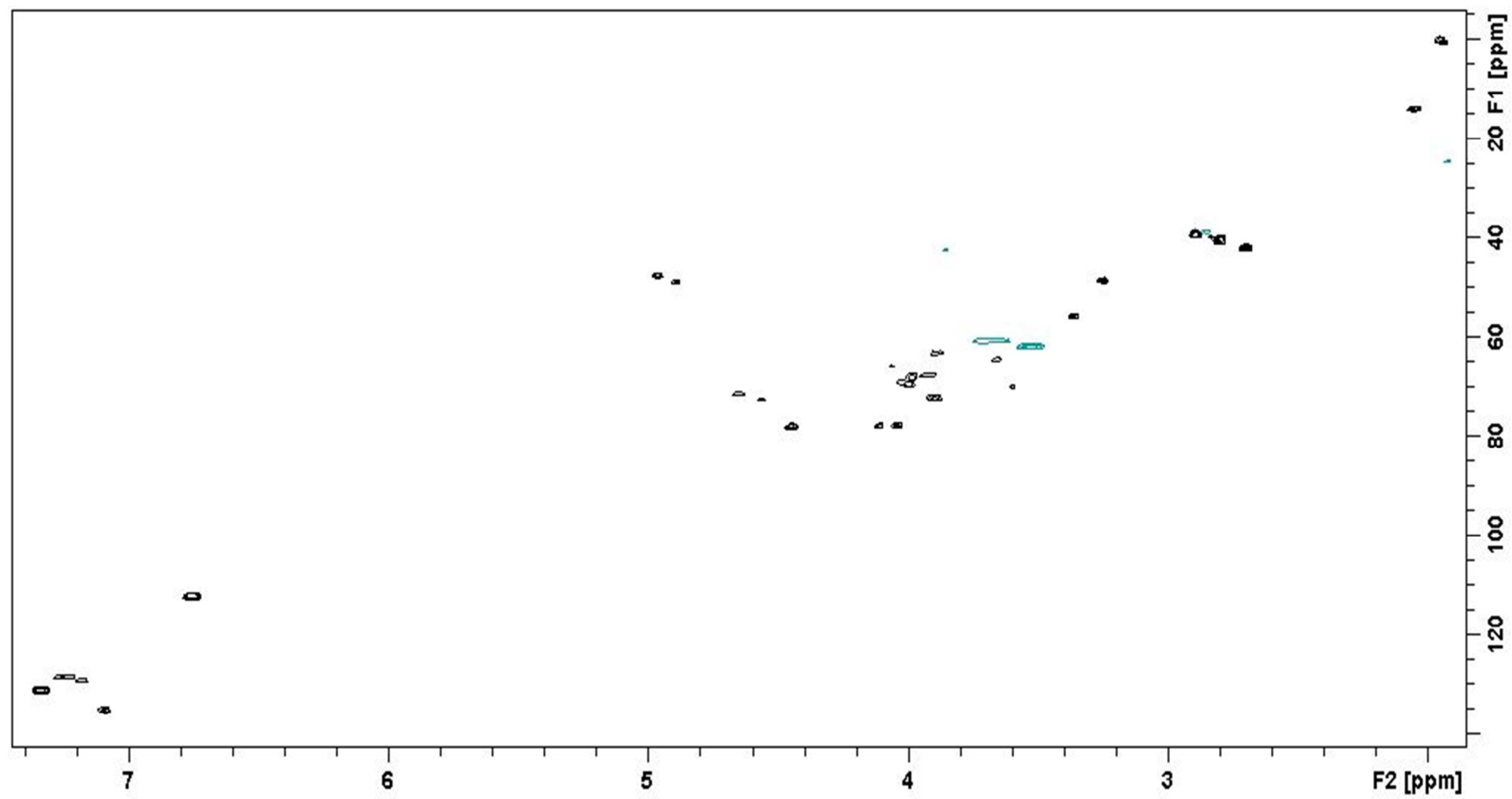
Supplementary Figure S1 ^1H -NMR spectrum of Orthoformimycin in CD_3CN .



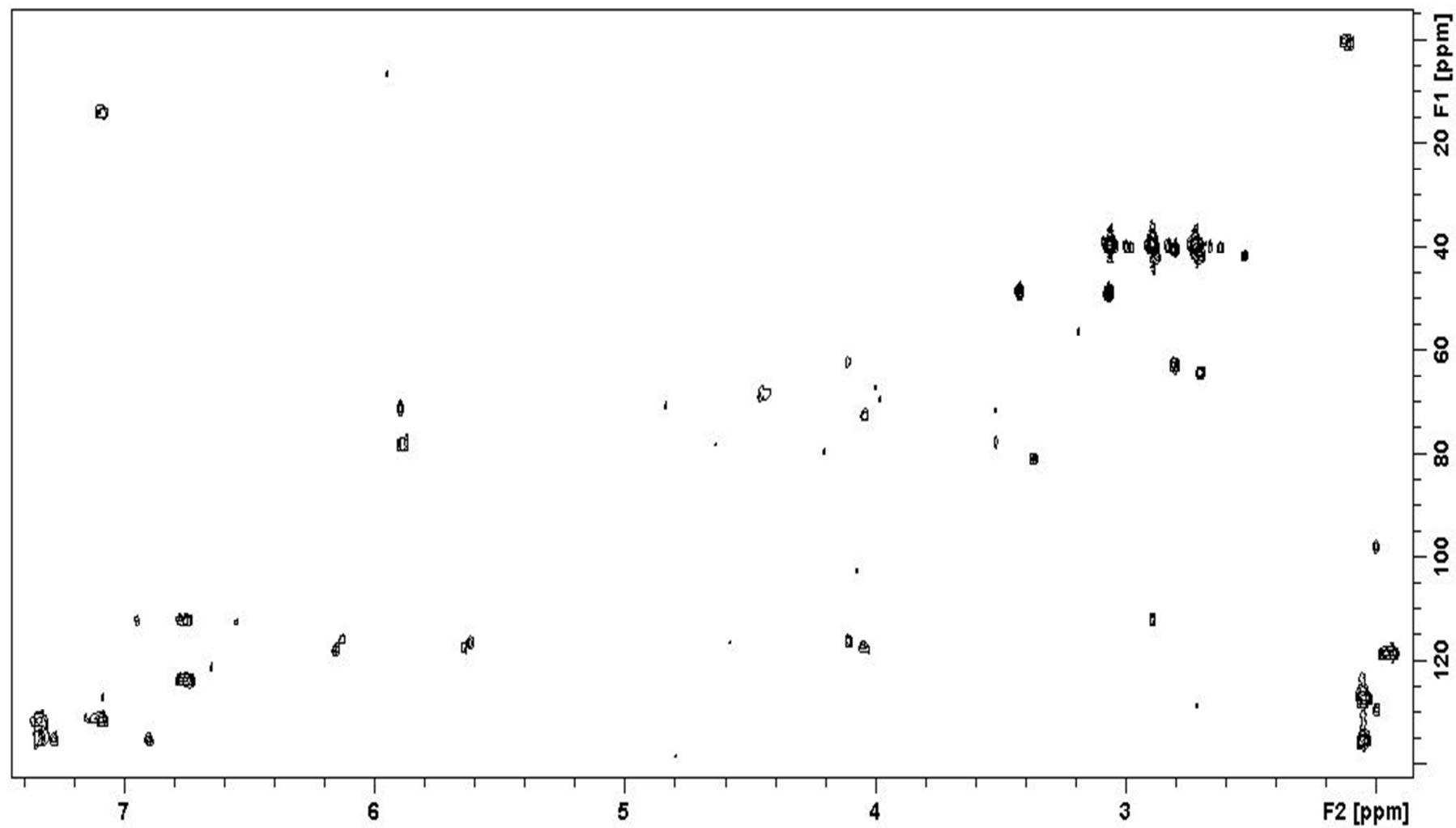
Supplementary Figure S2 COSY spectrum of Orthoformimycin in CD₃CN.



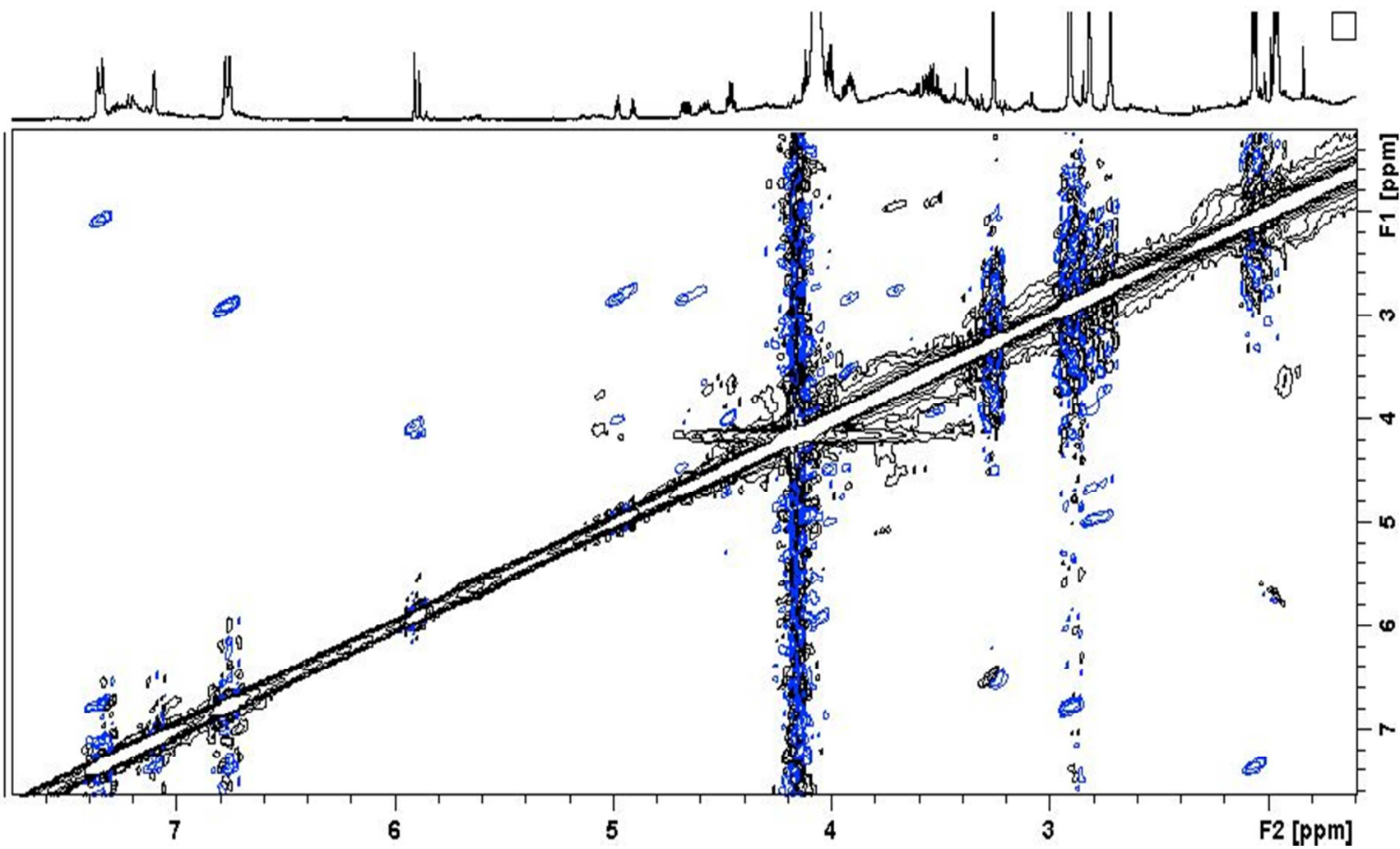
Supplementary Figure S3 TOCSY spectrum of Orthoformimycin in CD₃CN.



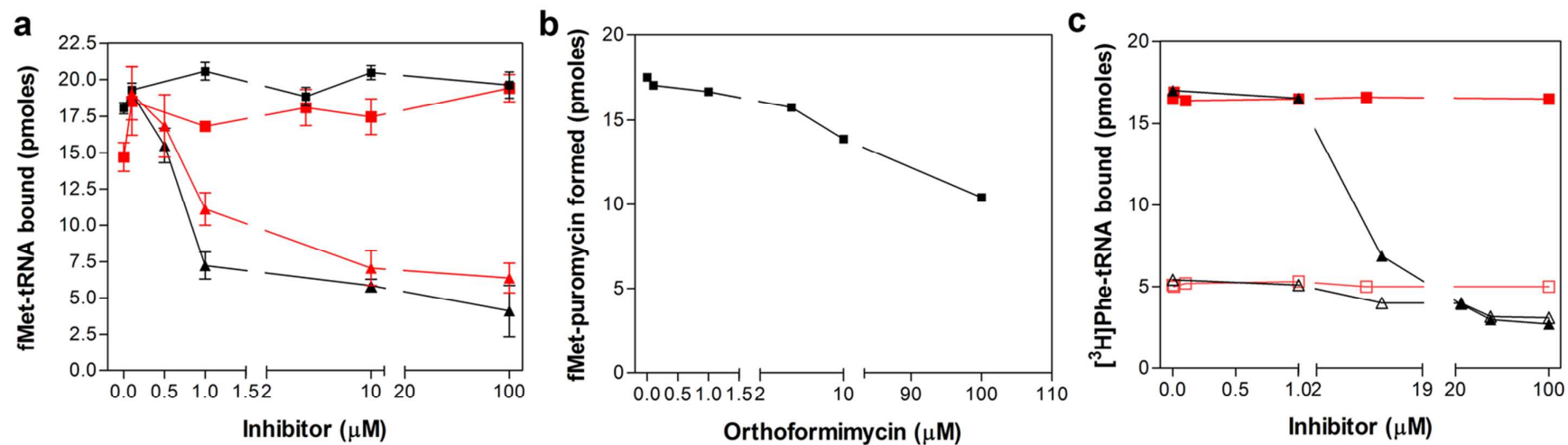
Supplementary Figure S4 HSQC spectrum of Orthoformimycin in CD₃CN.



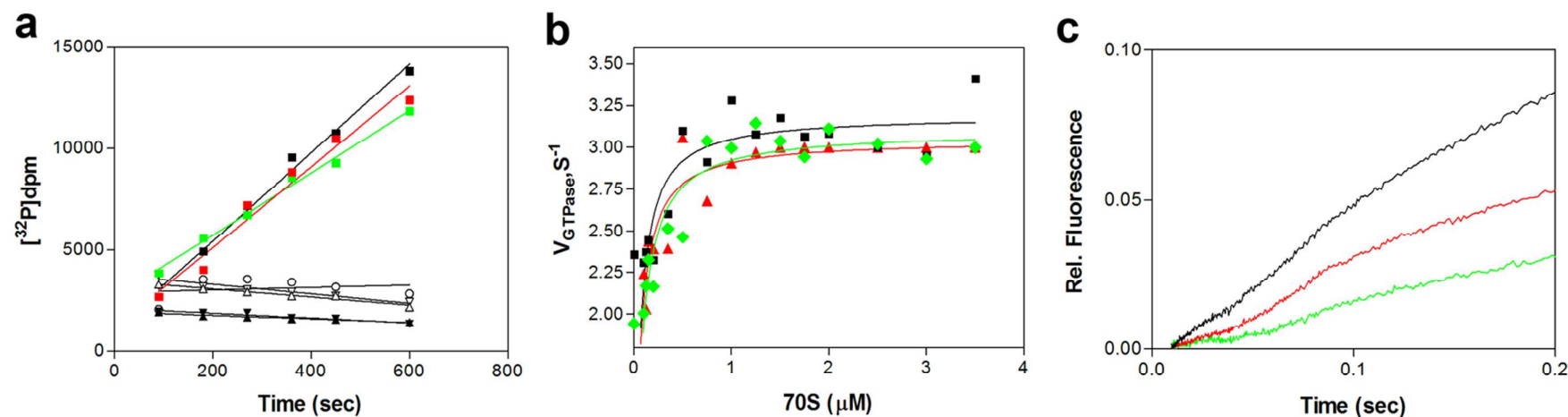
Supplementary Figure S5 HMBC spectrum of Orthoformimycin in CD₃CN.



Supplementary Figure S6 NOESY spectrum of Orthoformimycin in CD₃CN.



Supplementary Figure S7 Effect of orthoformimycin and other antibiotics on P-site and A-site decoding activity. a) Effect of the indicated orthoformimycin (■, ■) and GE81112⁷ (▲, ▲) concentrations on the P-site fMet-tRNA binding to 30S (red) or 70S (black) ribosomes to yield 30S and 70S initiation complexes (ICs), respectively; b) effect of the indicated concentrations of orthoformimycin on the formation of fMet-puromycin upon addition of 50S subunits and puromycin to the 30S IC formed as in panel a); c) effect of the indicated orthoformimycin (■, □) and tetracycline (▲, △) concentrations on the EF-Tu-dependent (■, ▲) and EF-Tu-independent (□, △) A-site binding of Phe-tRNA to poly(U)-programmed 30S ribosomal subunits.



Supplementary Figure S8 Effect of orthoformimycin and other antibiotics on various translocation steps. a) Effect of 20 μM of orthoformimycin (\blacksquare) or GE82832/dityromycin⁹ (\blacksquare) on the multiple turnover EF-G-dependent GTP hydrolysis; the positive control is the complete system without inhibitors (\blacksquare) whereas negative controls are a system containing orthoformimycin (\triangle, ∇) or GE82832/dityromycin ($\blacktriangle, \blacktriangledown$) but lacking either ribosomes ($\triangle, \blacktriangle$) or EF-G ($\nabla, \blacktriangledown$) or lacking ribosomes, EF-G and antibiotics (O); b) fast kinetics of 70S-dependent and EF-G-dependent GTP hydrolysis in the absence of antibiotics (\blacksquare) or in the presence of 20 μM of either orthoformimycin (\blacktriangle) or GE82832/dityromycin (\blacklozenge); c) P_i release from EF-G-GDP- P_i in the absence (black tracing) or in the presence of 20 μM of either orthoformimycin (red tracing) or GE82832/dityromycin (green tracing).

<i>m/z</i>			R₁^a	R₂^a	ion	formula	dbe^c
nominal	calculated	experimental					
540	540,2552	540,2582	H	H	[M+H] ⁺	C ₂₅ H ₃₈ N ₃ O ₁₀	9
680	680,3031	680,3051	Ac	Me	[M+H] ⁺	C ₃₂ H ₄₆ N ₃ O ₁₃	12
404	404,2180	404,2212	H	-	M ⁺ ^b	C ₄ H ₈ O ₅	9
488	488,2396	488,2389	Ac	-	M ⁺ ^b	C ₇ H ₁₂ O ₆	11
136	136,0372	136,0370	H	H	Neutral loss	C ₂₀ H ₃₁ N ₃ O ₅	1
394	394,2337	394,2357	H	-	[M+H] ⁺	C ₂₀ H ₃₁ N ₃ O ₅	7
206	206,1266	206,1271	H	-	Neutral loss	C ₈ H ₁₈ N ₂ O ₄	1
188	188,1070	188,1086	-	-	M ⁺ ^b	C ₁₂ H ₁₄ NO	7
(a) as defined in figure 3 (b) not revealed in negative ion mode (c) double bond equivalents							

Supplementary Table S1 HR-MS and MSⁿ data for Orthoformimycin (*m/z* 540) and its acetylated derivative (*m/z* 680) (See Fig.3)