

β -Alanyl aminopeptidase-activated fluorogenic probes for the rapid identification of *Pseudomonas aeruginosa* in clinical samples

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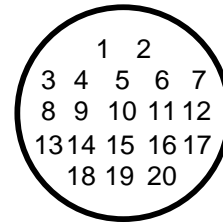
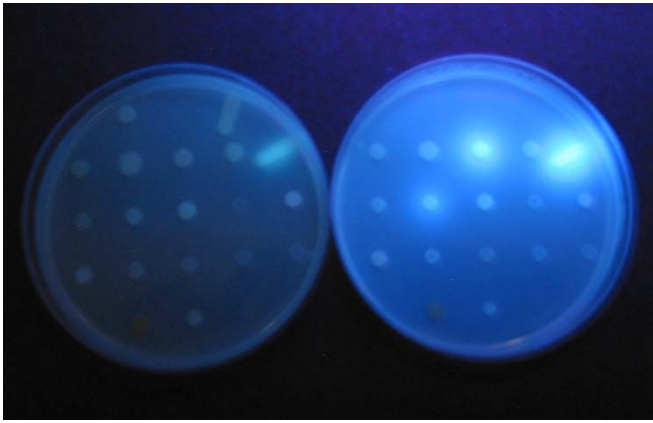
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Table S1. List of microorganisms inoculated onto 96-well plate for the evaluation of **8a,b** and **9**

Species	bioMérieux strains collection number	ATCC / NCTC reference number
<i>E. coli</i>	API 00 08 013	
<i>P. aeruginosa</i>	API 77 05 034	NCTC 10662
<i>S. marcescens</i>	API 04 04 009	
<i>S. marcescens</i>	API 75 08 042	ATCC 264
<i>S. marcescens</i>	API 95 05 151	ATCC 43861
<i>P. aeruginosa</i>	API 10 11 314	
<i>P. aeruginosa</i>	API 14 02 224	ATCC 27853
<i>P. aeruginosa</i>	API 10 06 028	ATCC 10145
<i>S. marcescens</i>	API 92 11 027	
<i>P. aeruginosa</i>	API 08 04 064	
<i>P. aeruginosa</i>	API 14 02 100	
<i>P. aeruginosa</i>	API 14 02 103	

*:API xx xx xxx = bioMérieux strains collection number



- | | | | |
|----|--------------------------------------|----|----------------------------------|
| 1 | <i>Escherichia coli</i> | 11 | <i>Streptococcus pyogenes</i> |
| 2 | <i>Klebsiella pneumoniae</i> | 12 | MRSA |
| 3 | <i>Providencia rettgeri</i> | 13 | <i>Staphylococcus aureus</i> |
| 4 | <i>Enterobacter cloacae</i> | 14 | <i>Streptococcus epidermidis</i> |
| 5 | <i>Serratia marcescens</i> | 15 | <i>Listeria monocytogenes</i> |
| 6 | <i>Salmonella typhimurium</i> | 16 | <i>Enterococcus faecium</i> |
| 7 | <i>Pseudomonas aeruginosa</i> | 17 | <i>Enterococcus faecalis</i> |
| 8 | <i>Yersinia enterocolitica</i> | 18 | <i>Bacillus subtilis</i> |
| 9 | <i>Burkholderia cepacia</i> | 19 | <i>Candida albicans</i> |
| 10 | <i>Acinetobacter baumannii</i> | 20 | <i>Candida glabrata</i> |

Figure S1. Clinically relevant Gram positive, Gram negative, and yeast microorganisms (1.5×10^5 CFU/spot) grown for 18h on an agar medium containing substrate **8b**; fluorescence corresponds only to the known BAP producers, *Pseudomonas aeruginosa* (spot 7), *Burkholderia cepacia* (spot 9) and *Serratia marcescens* (spot 5)[see Table 1 for identity of spots].

Figure S2. Readings from wells in the absence of microorganisms containing no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a)** absorption at 660 nm; **b)** fluorescent signal intensities at 365 / 440 nm; **c)** fluorescent intensities at 375 / 445 nm.

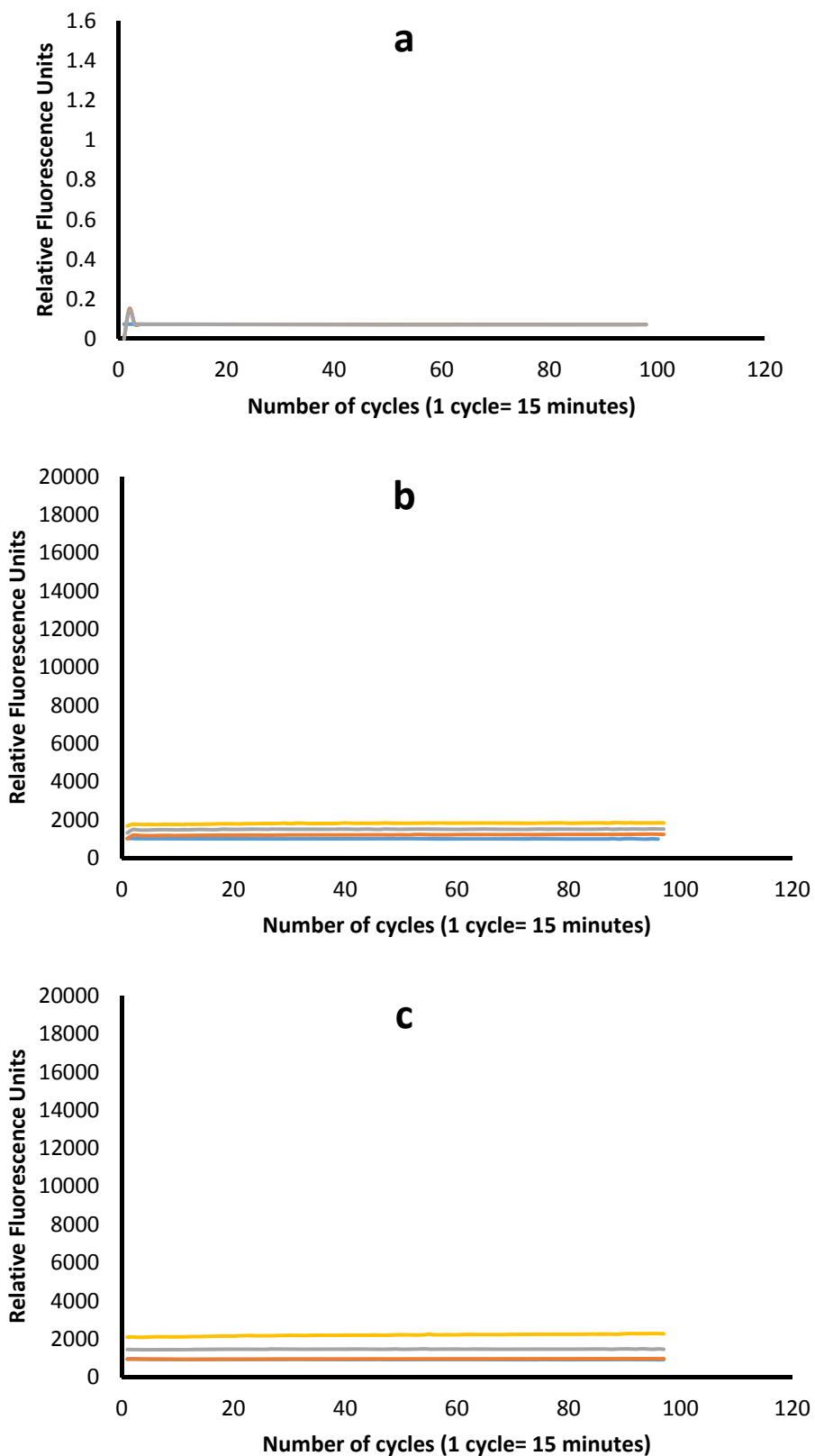


Figure S3. Readings from wells containing *E. coli* (API 00 08 013) in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

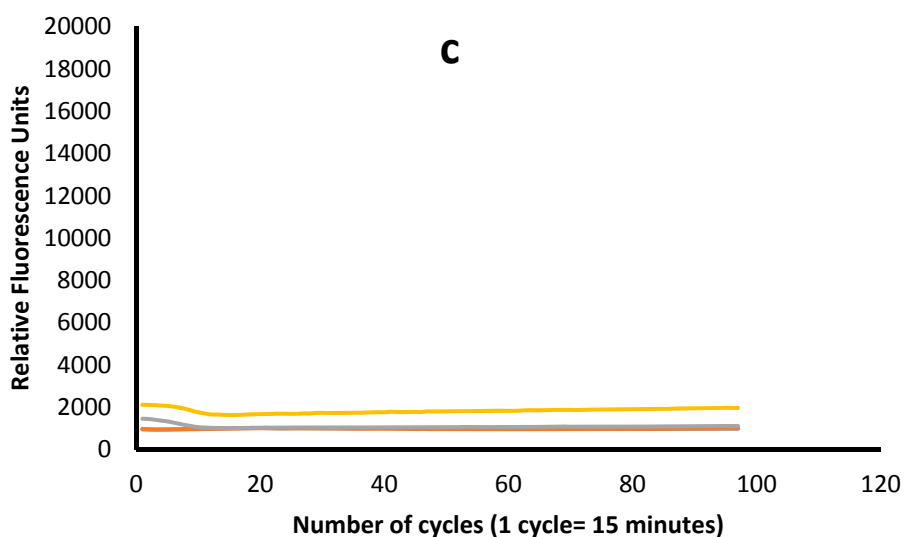
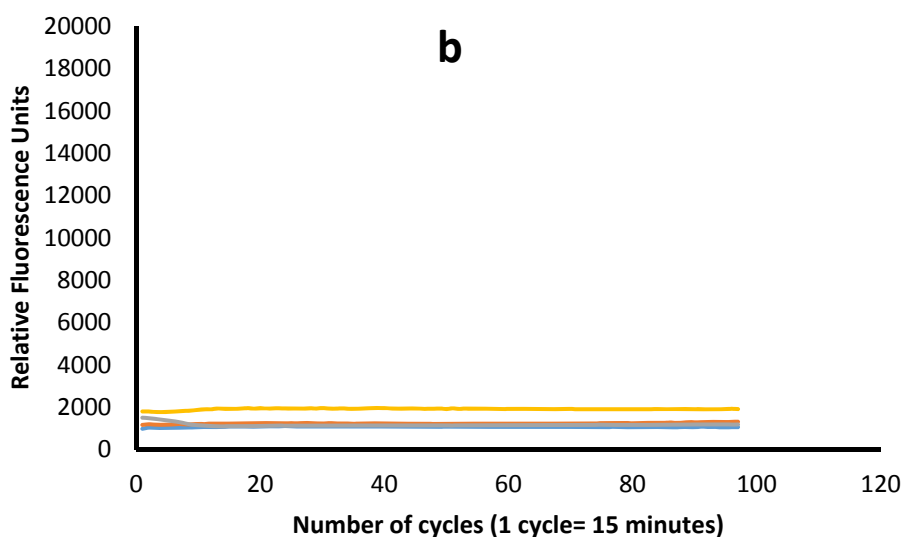
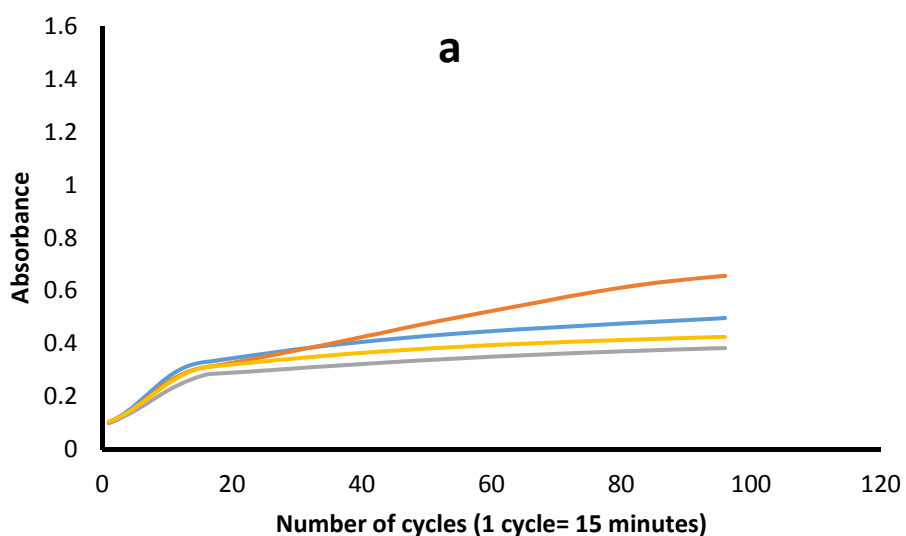


Figure S4. Readings from wells containing *P. aeruginosa* NCTC 10662 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

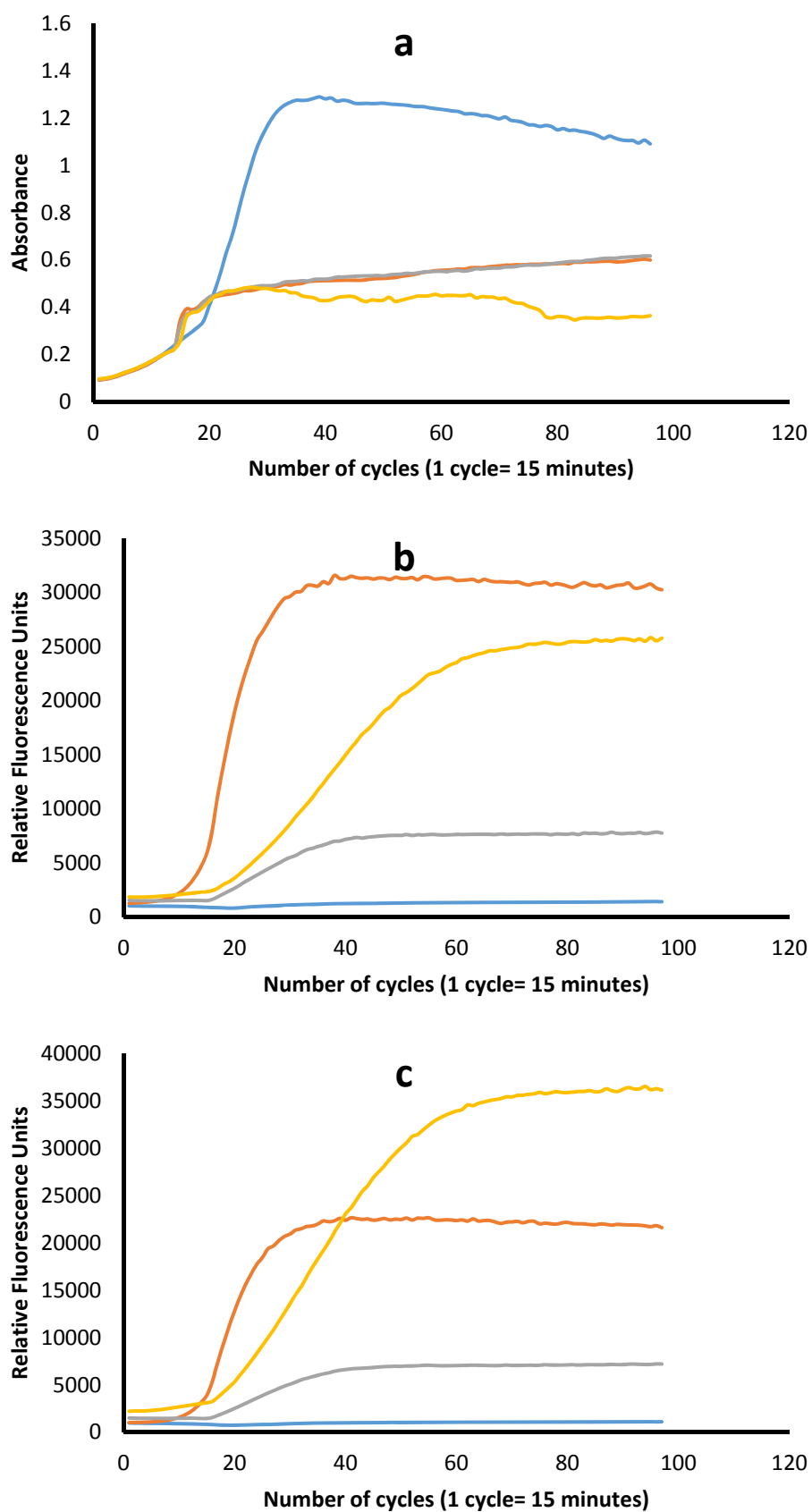


Figure S5. Readings from wells containing *S. marcescens* API 04 4 009 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

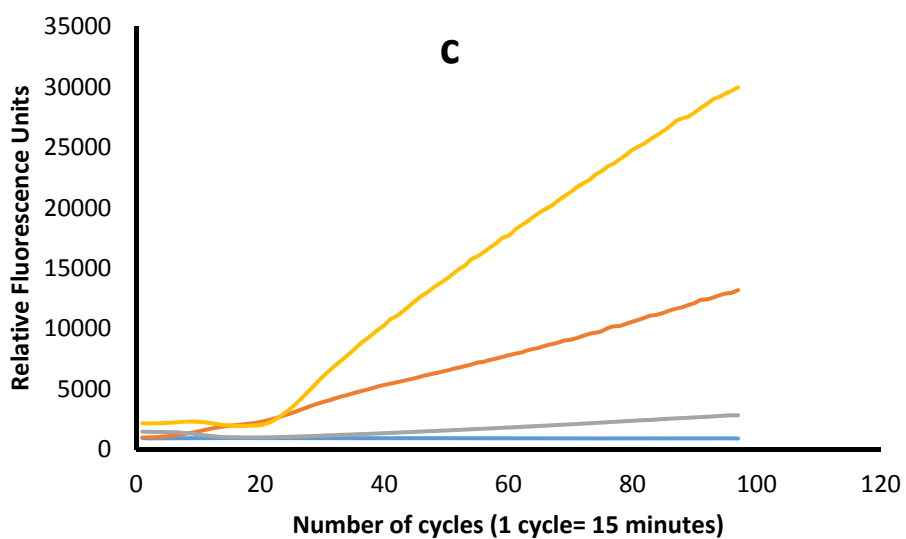
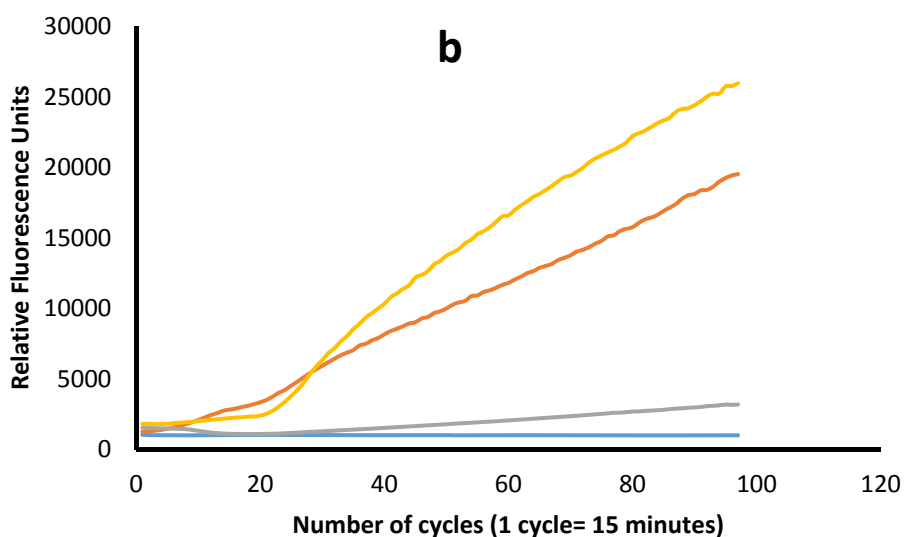
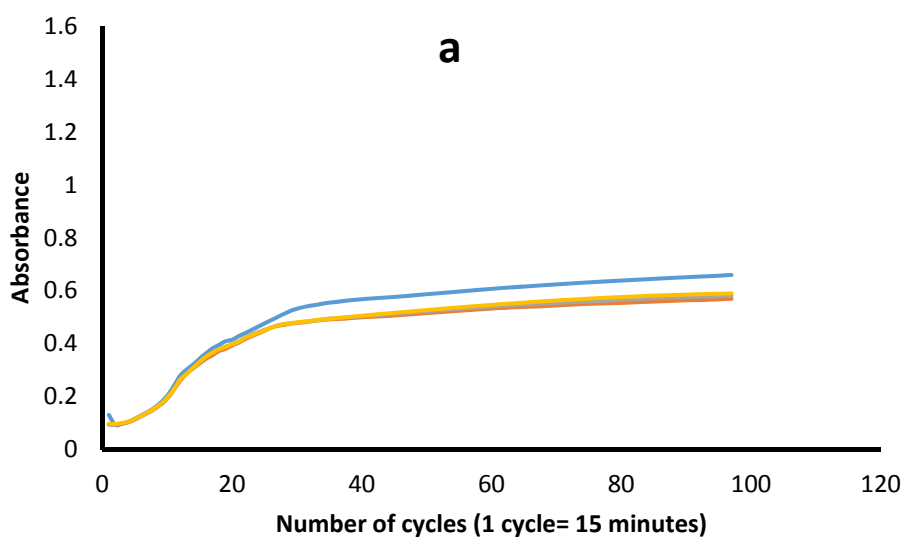


Figure S6. Readings from wells containing *S. marcescens* ATCC 264 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

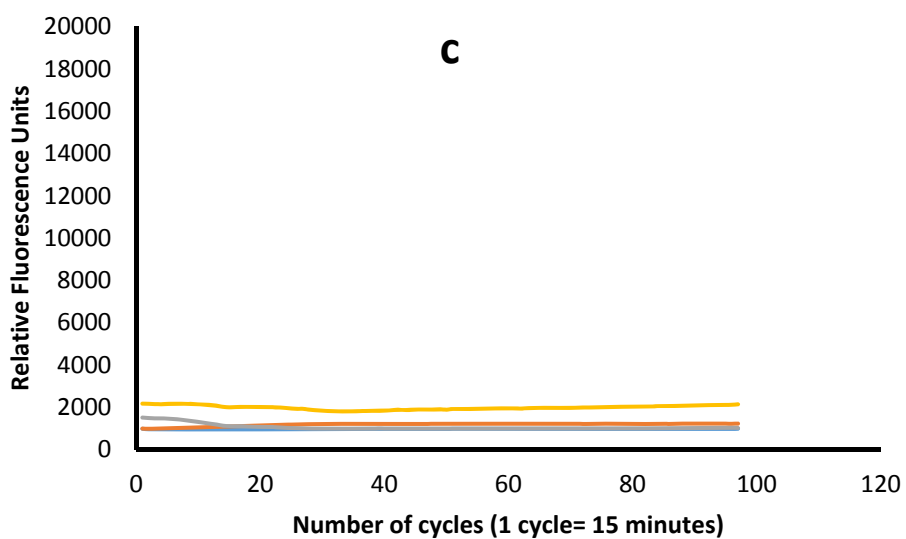
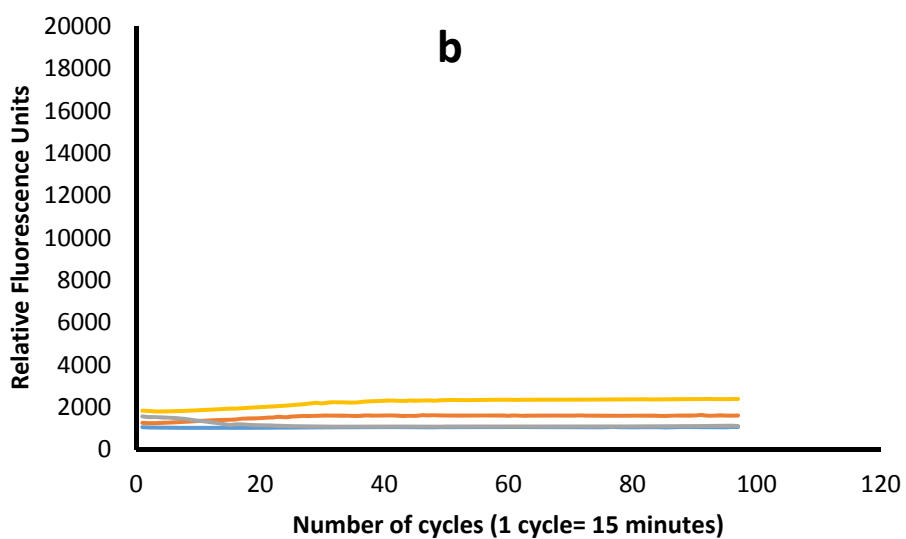
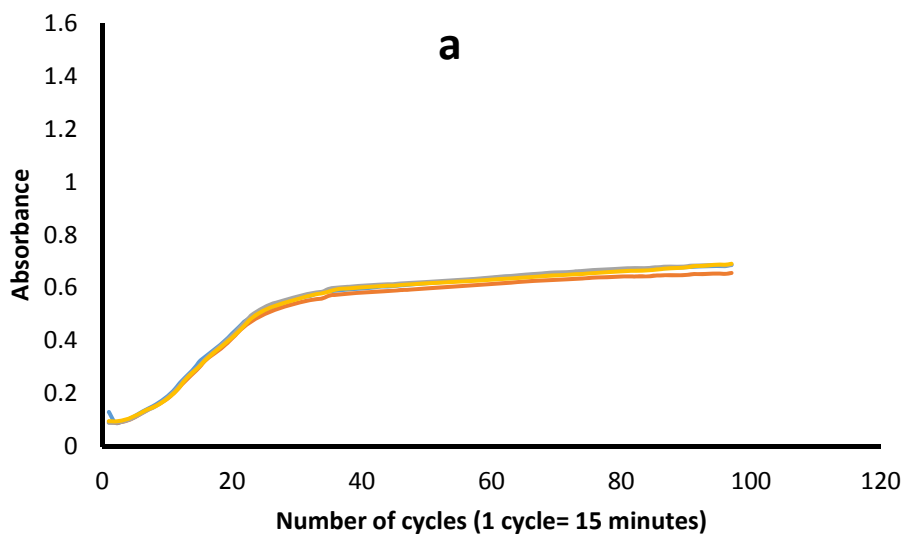


Figure S7. Readings from wells containing *S. marcescens* ATCC 43861 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

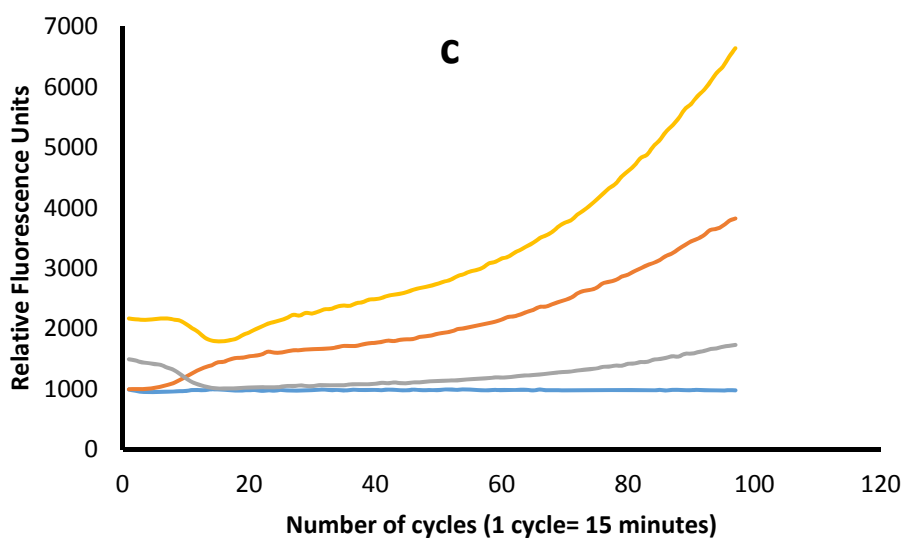
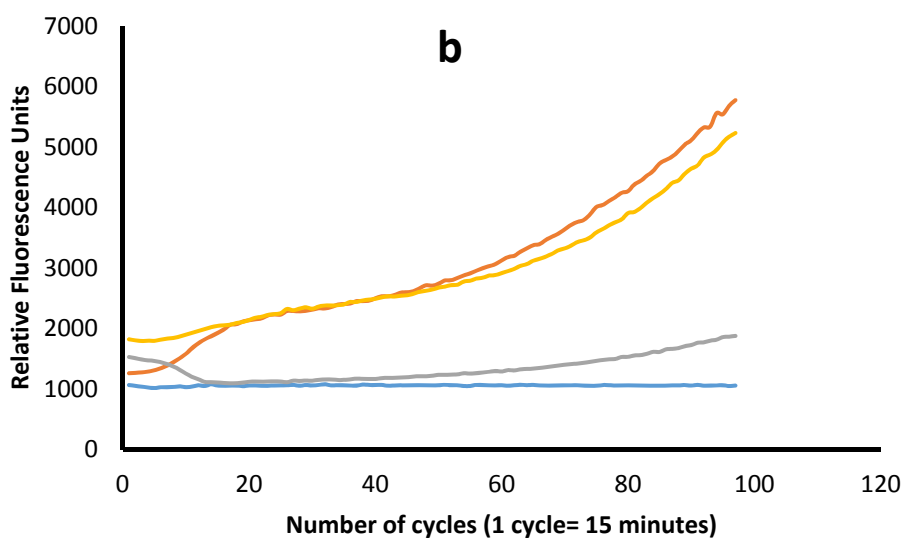
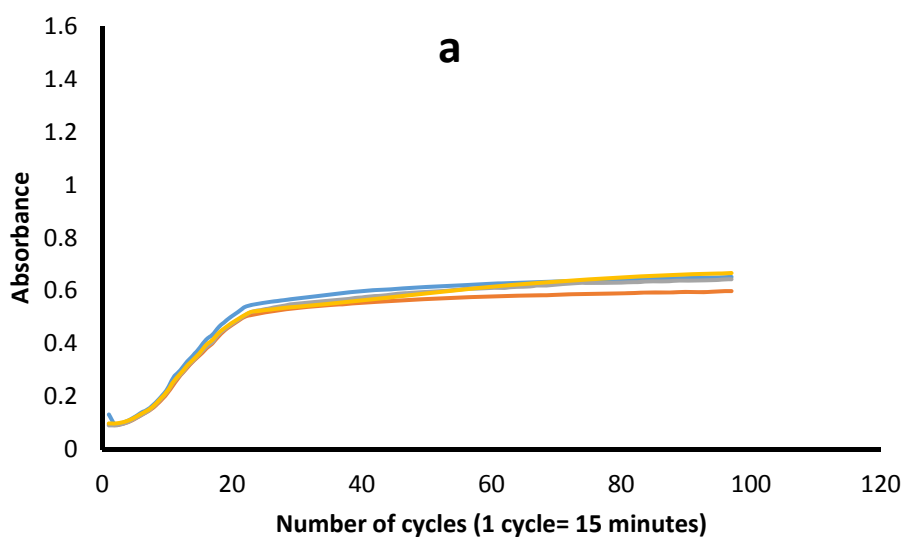


Figure S8. Readings from wells containing *P. aeruginosa* API 10 11 314 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively: **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

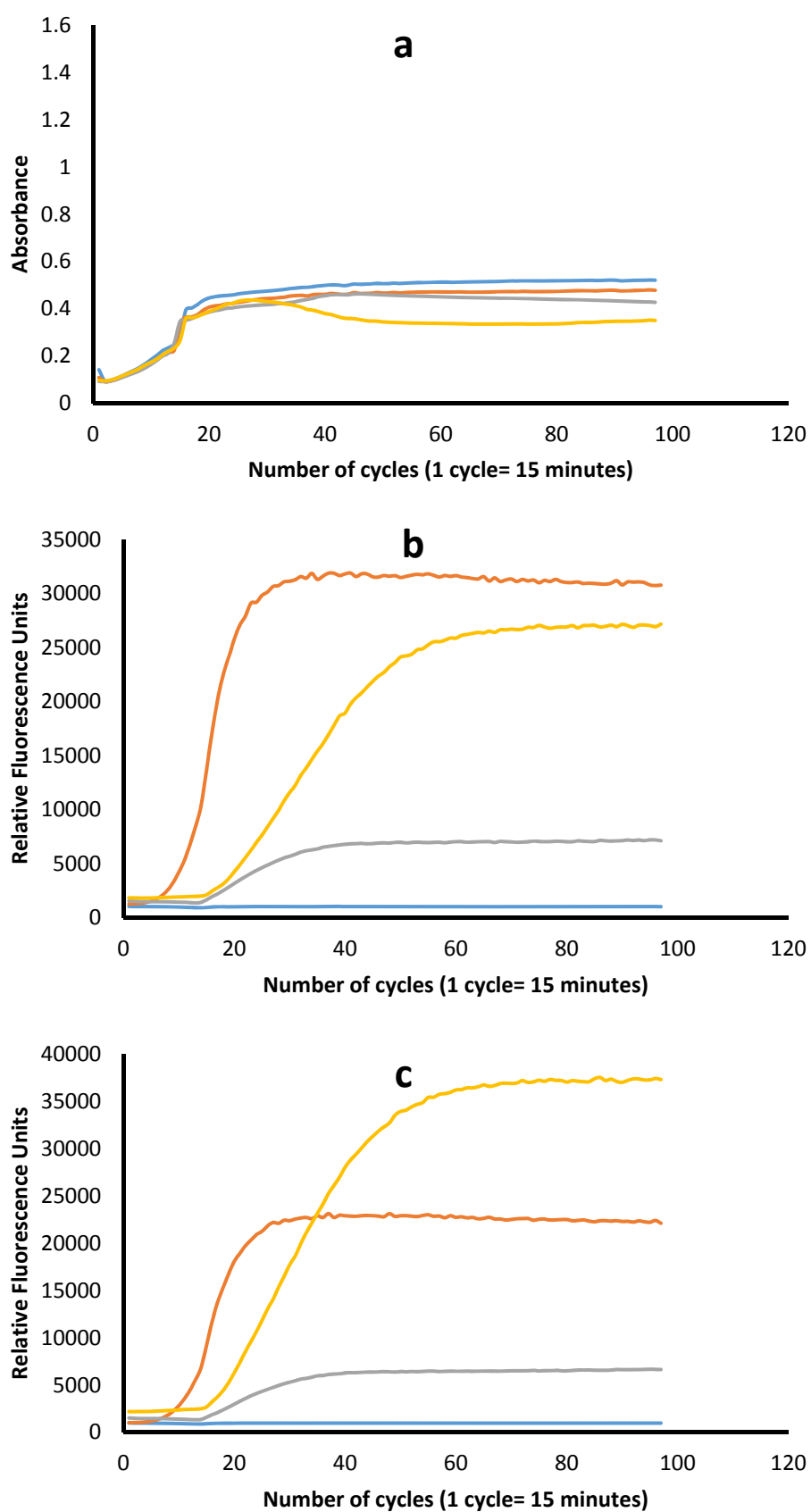


Figure S9. Readings from wells containing *P. aeruginosa* ATCC 27853 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

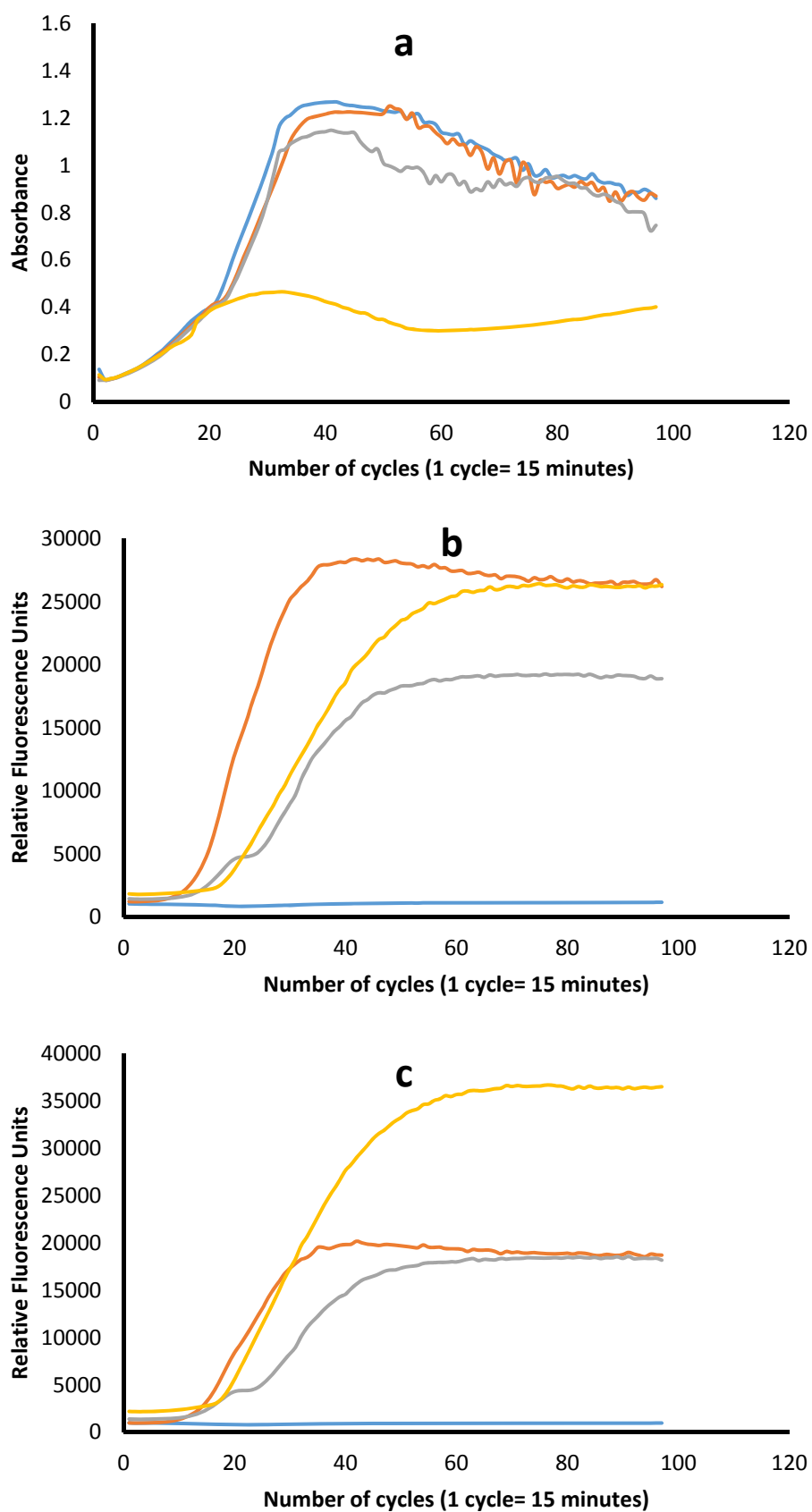


Figure S10. Readings from wells containing *P. aeruginosa* ATCC 10145 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

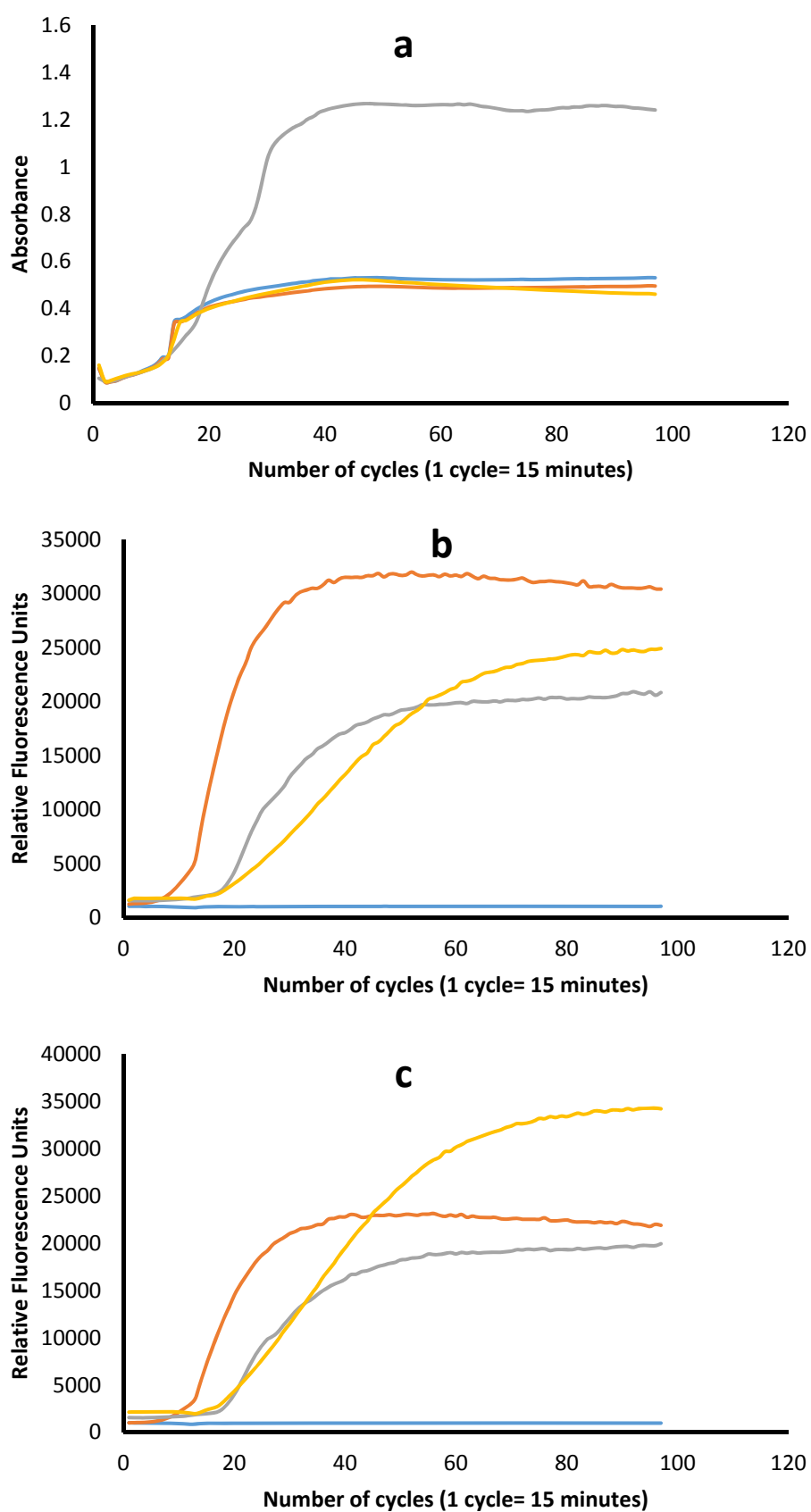


Figure S11. Readings from wells containing *S. marcescens* API 92 11 027 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

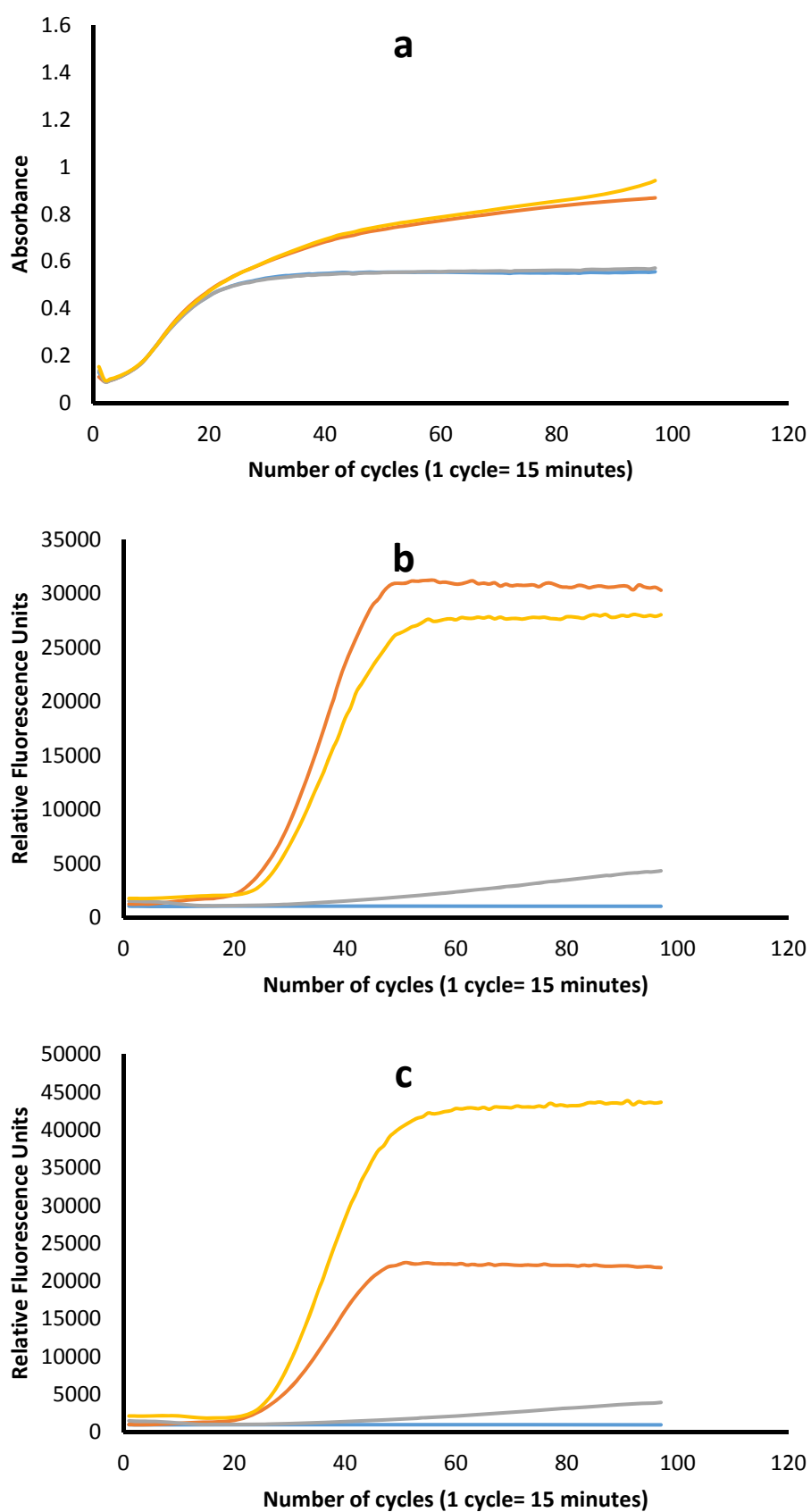


Figure S12. Readings from wells containing *P. aeruginosa* API 08 04 064 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

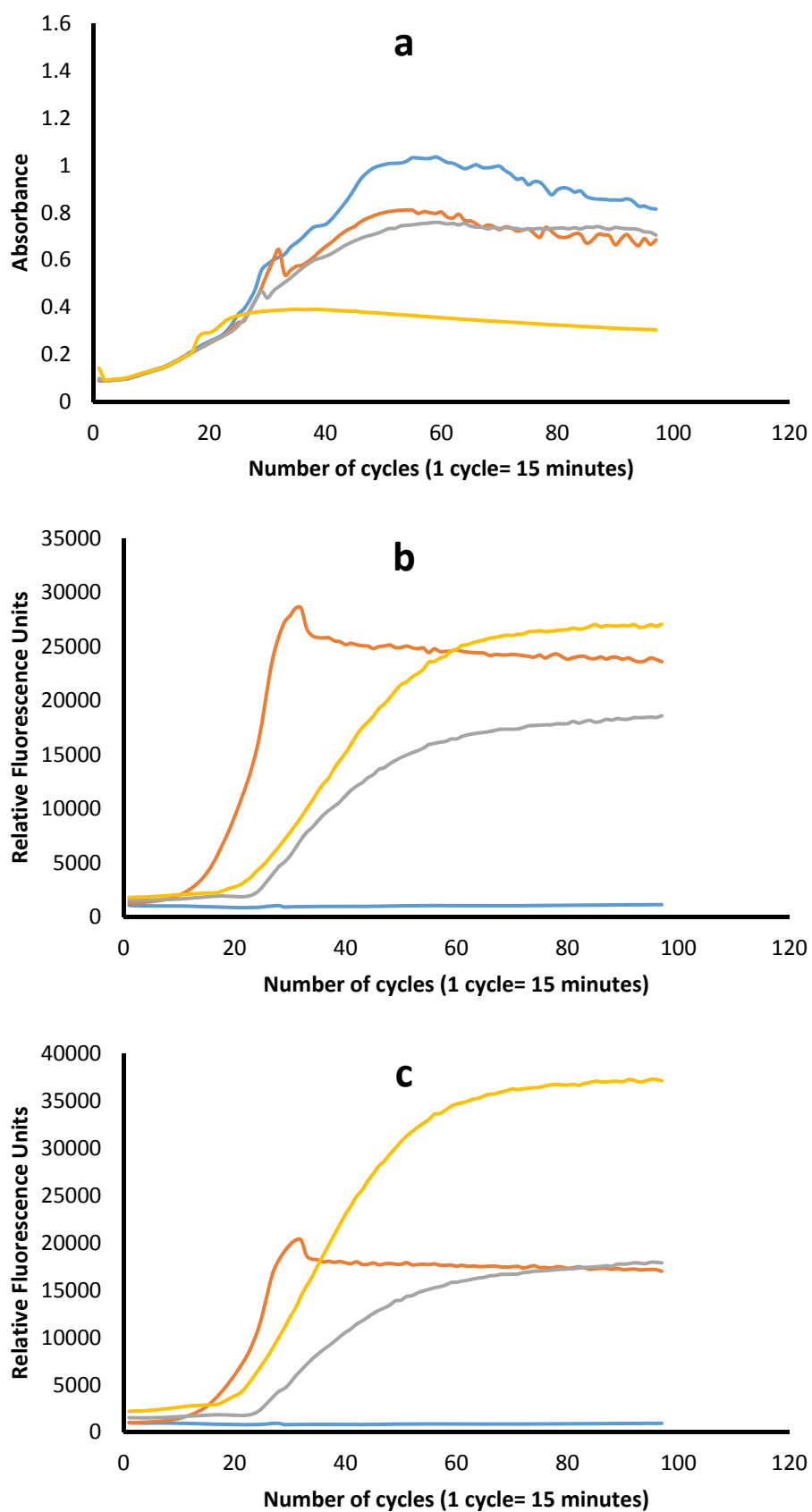


Figure S13. Readings from wells containing *P. aeruginosa* API 14 02 100 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

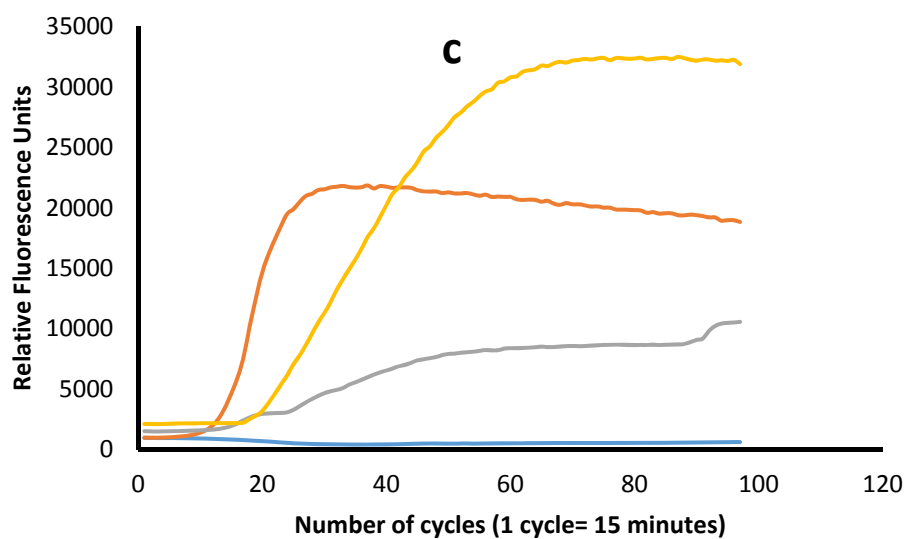
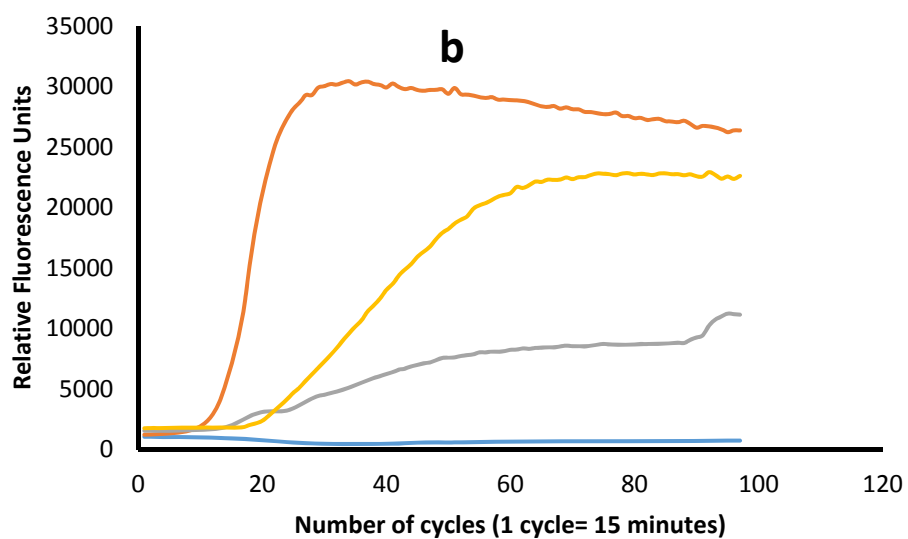
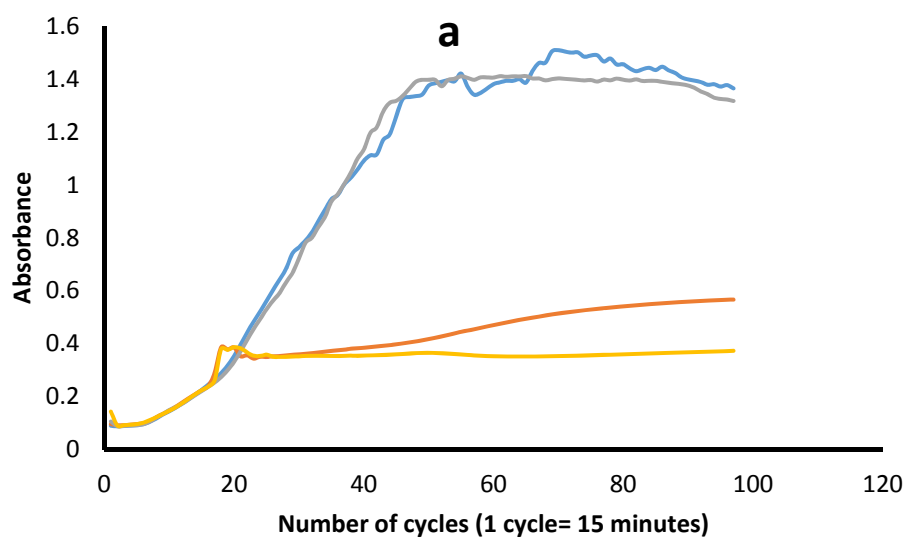
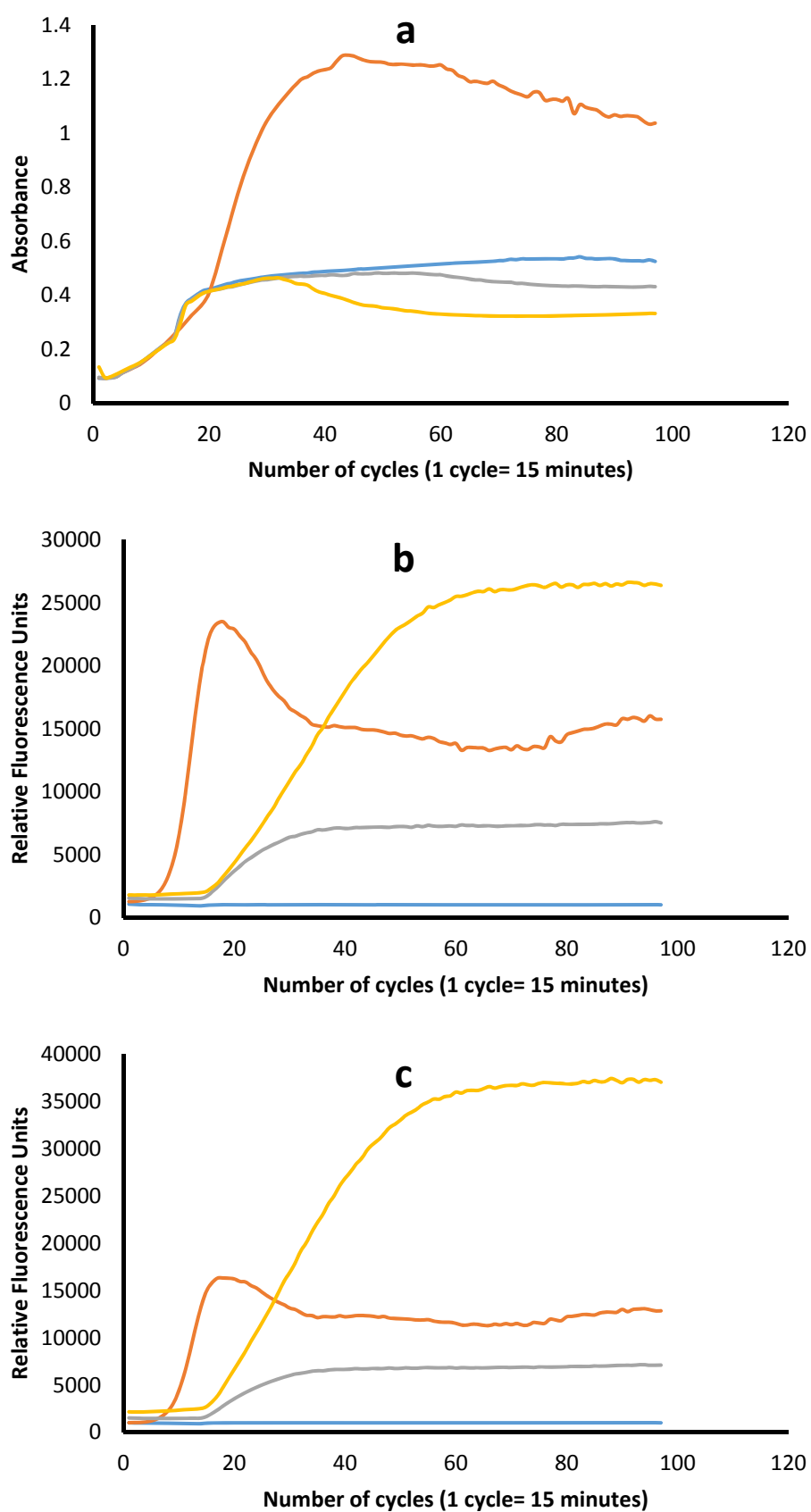
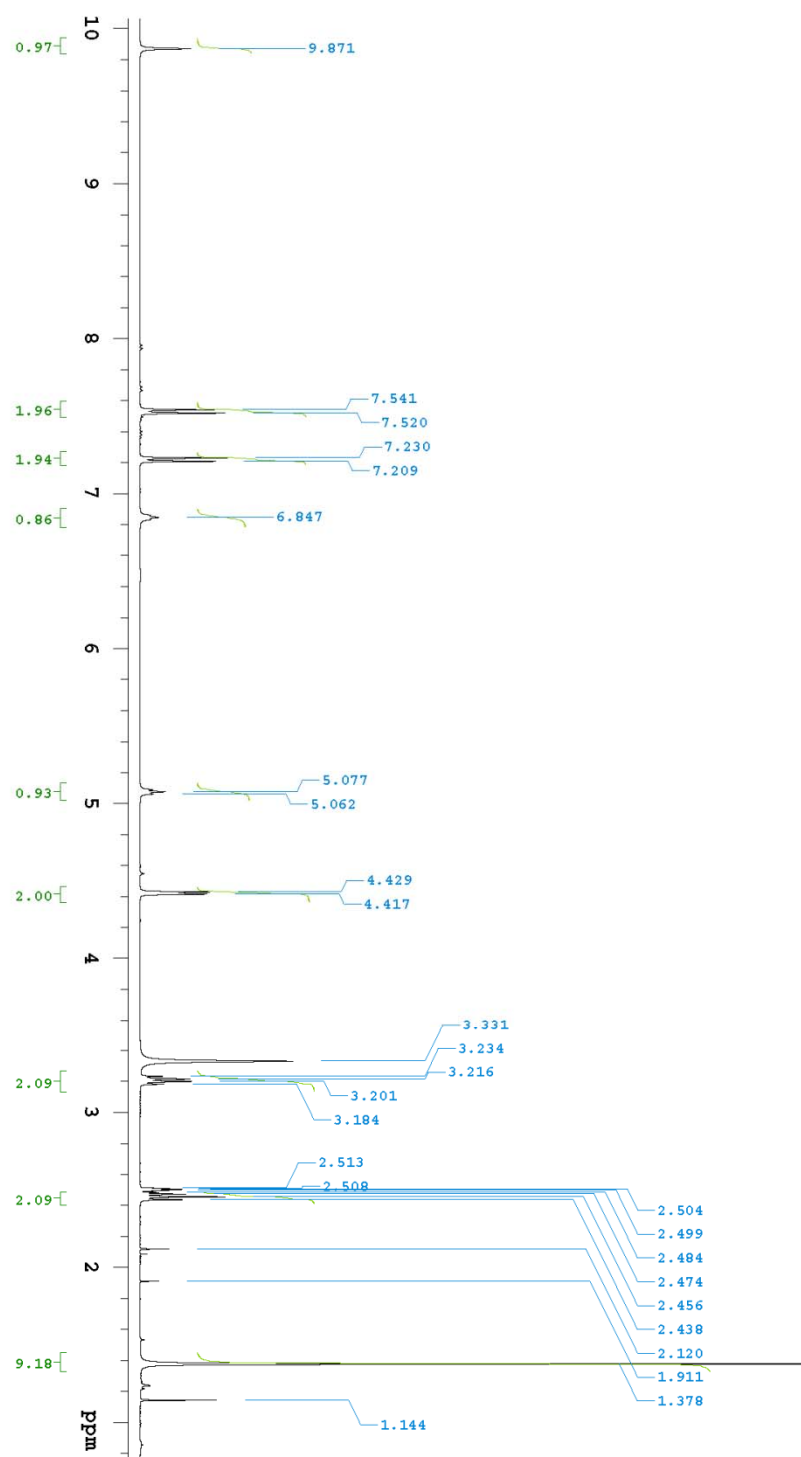


Figure S14. Readings from wells containing *P. aeruginosa* API 14 02 103 in the presence of no substrate (blue line), substrate **8a** (grey line), substrate **8b** (yellow line) or substrate **9** (orange line), respectively; **a**) absorbance at 660 nm; **b**) fluorescent signal intensities at 365 / 440 nm; **c**) fluorescent intensities at 375 / 445 nm.

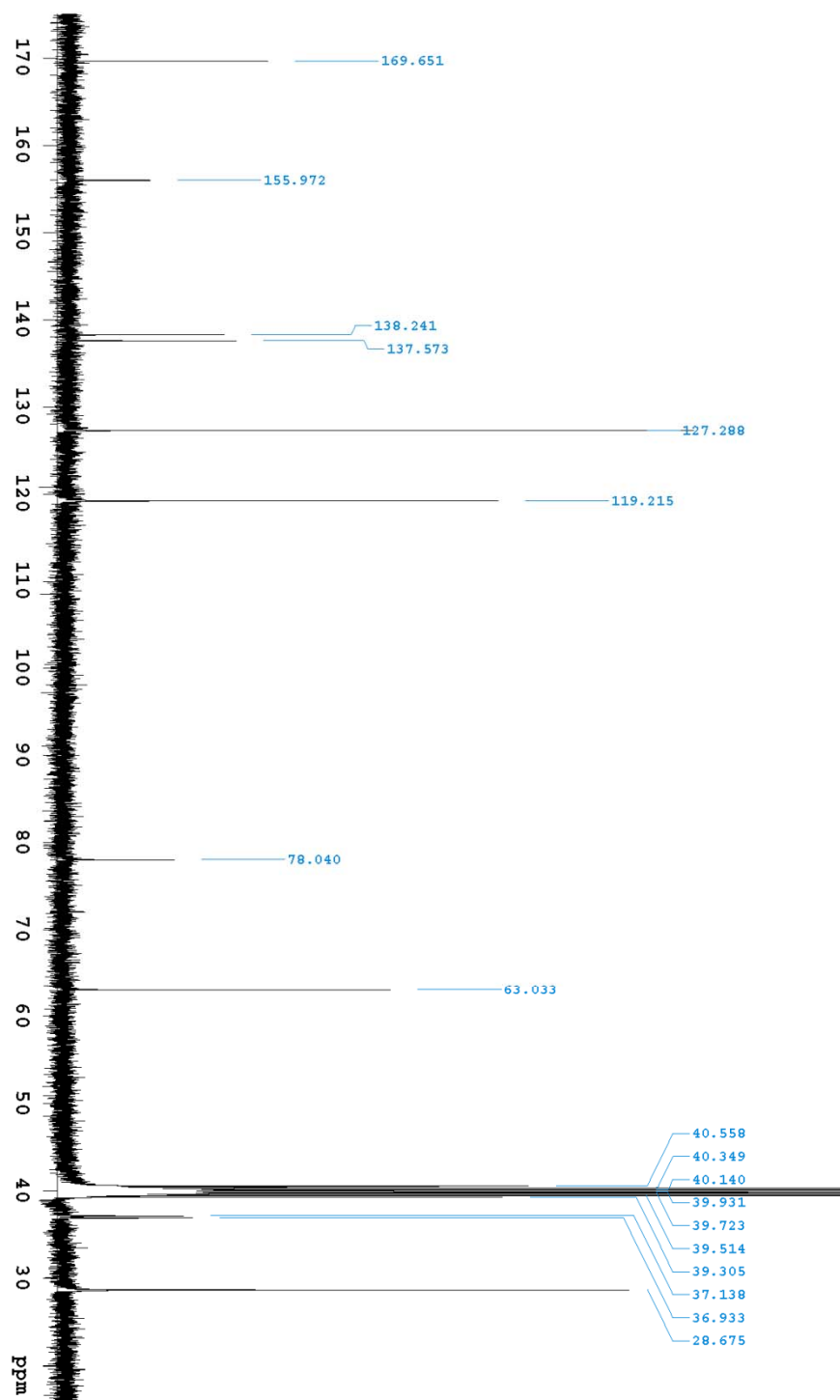


NMR Spectra

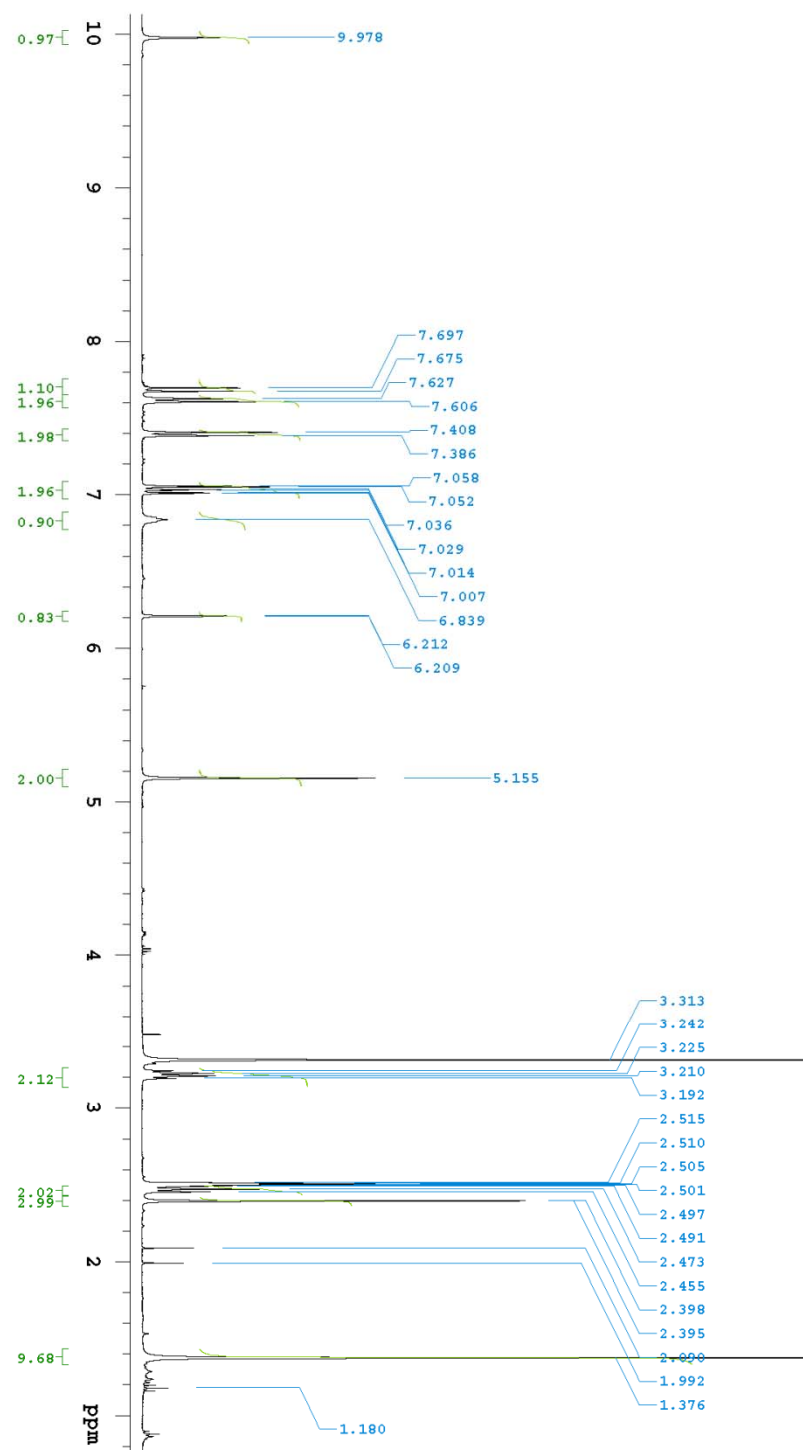
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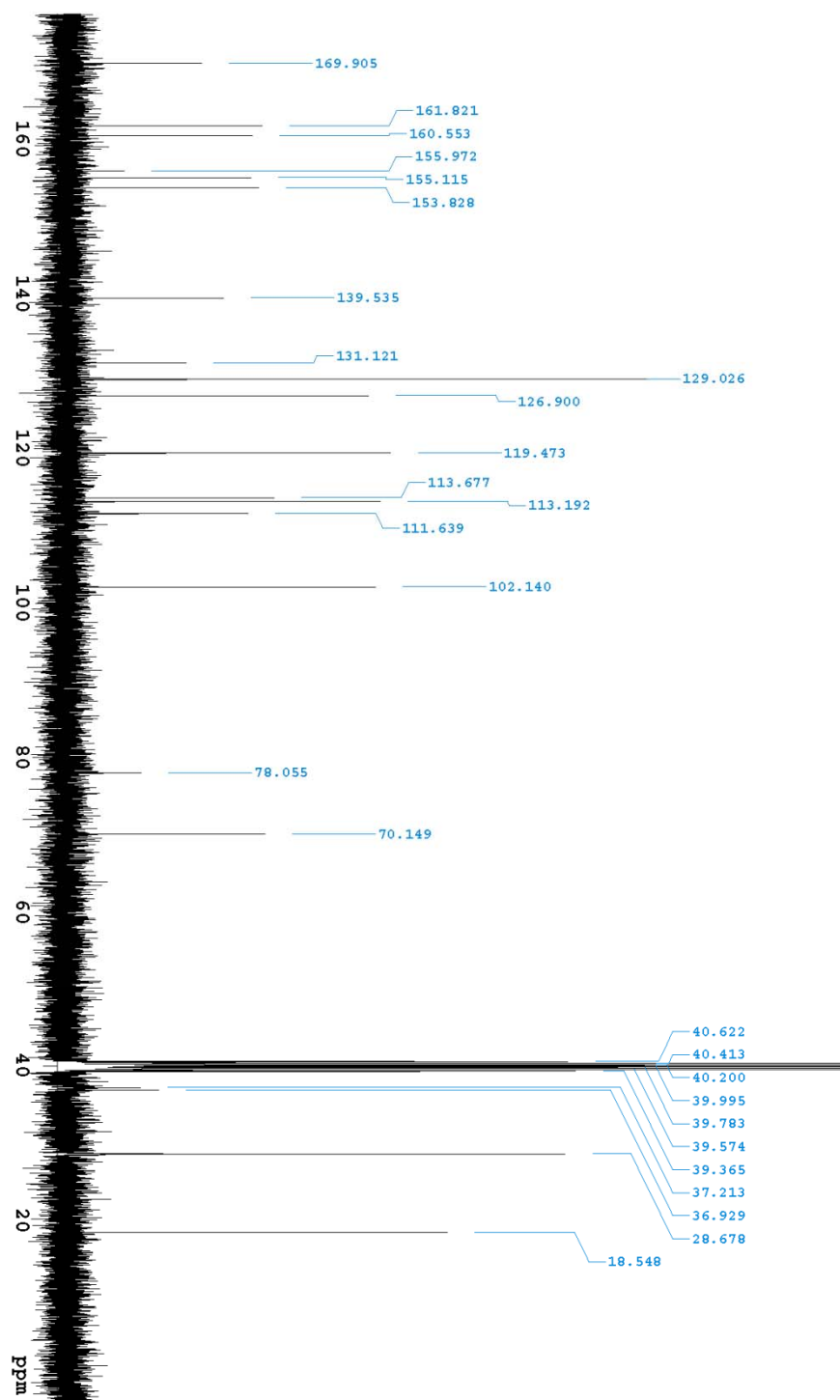
4-(Boc-β-Alanyl)benzyl alcohol ¹³C NMR



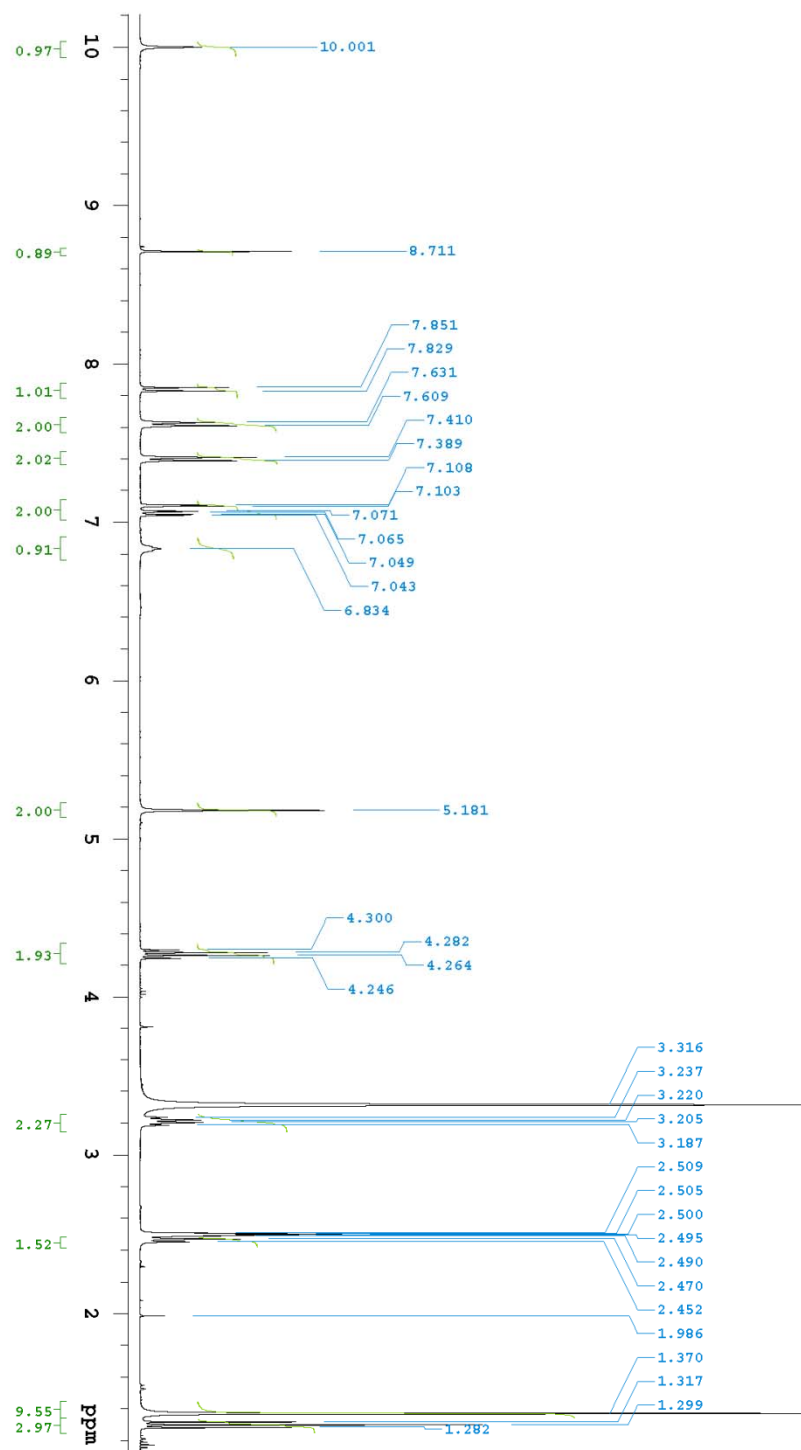
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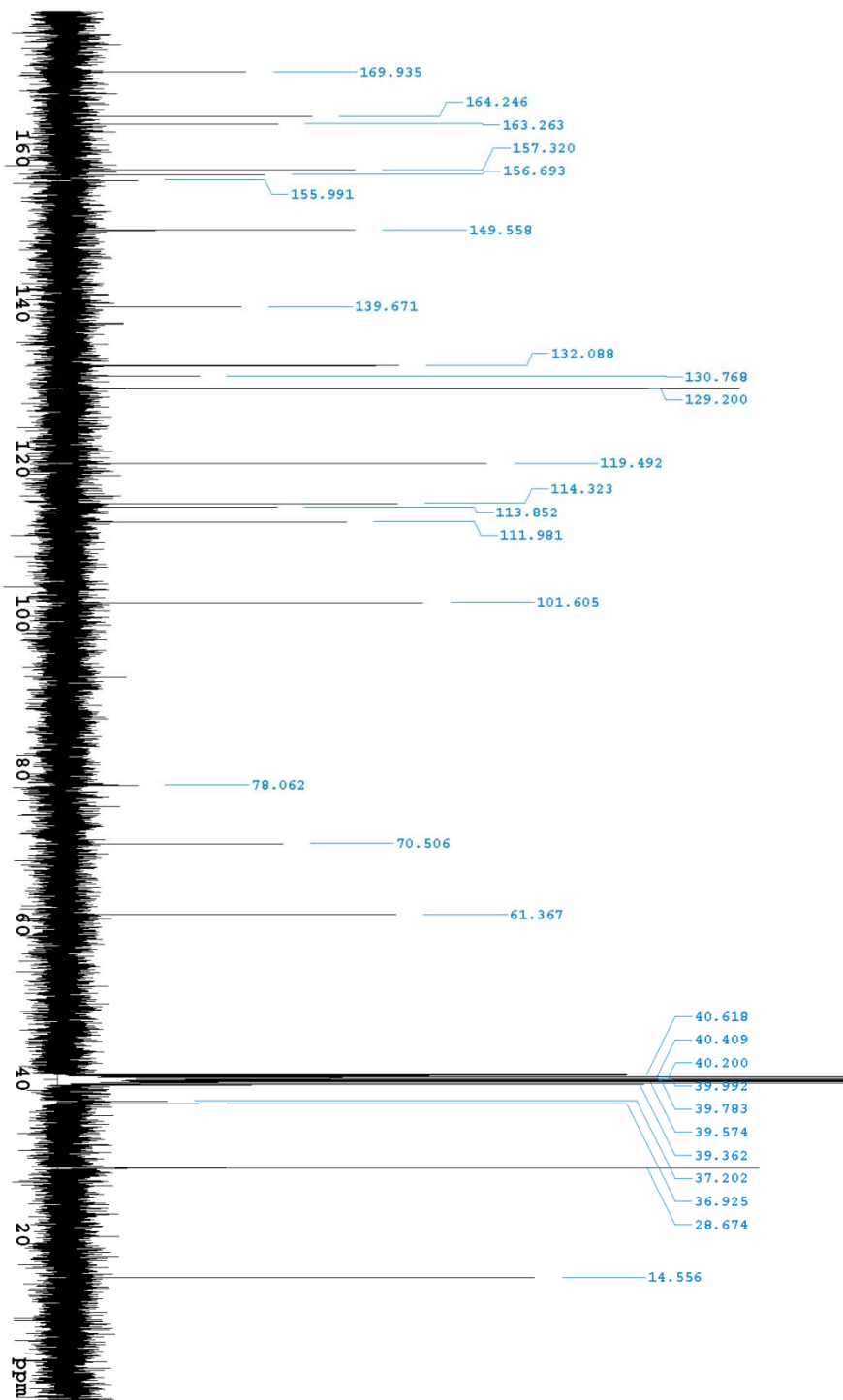
7-{4-(Boc-β-Alanyl)amino}benzyloxy-4-methylcoumarin 7a ¹³C NMR



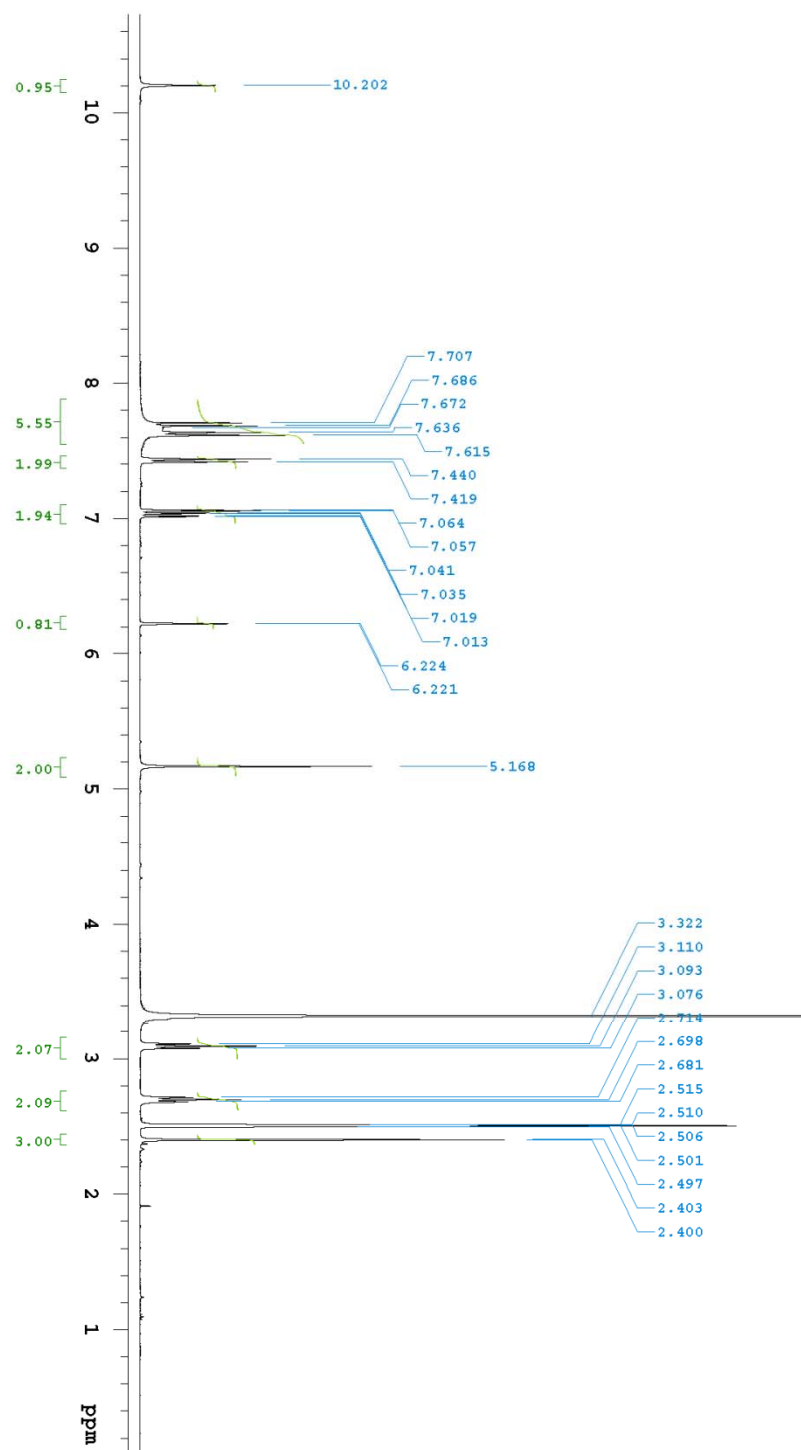
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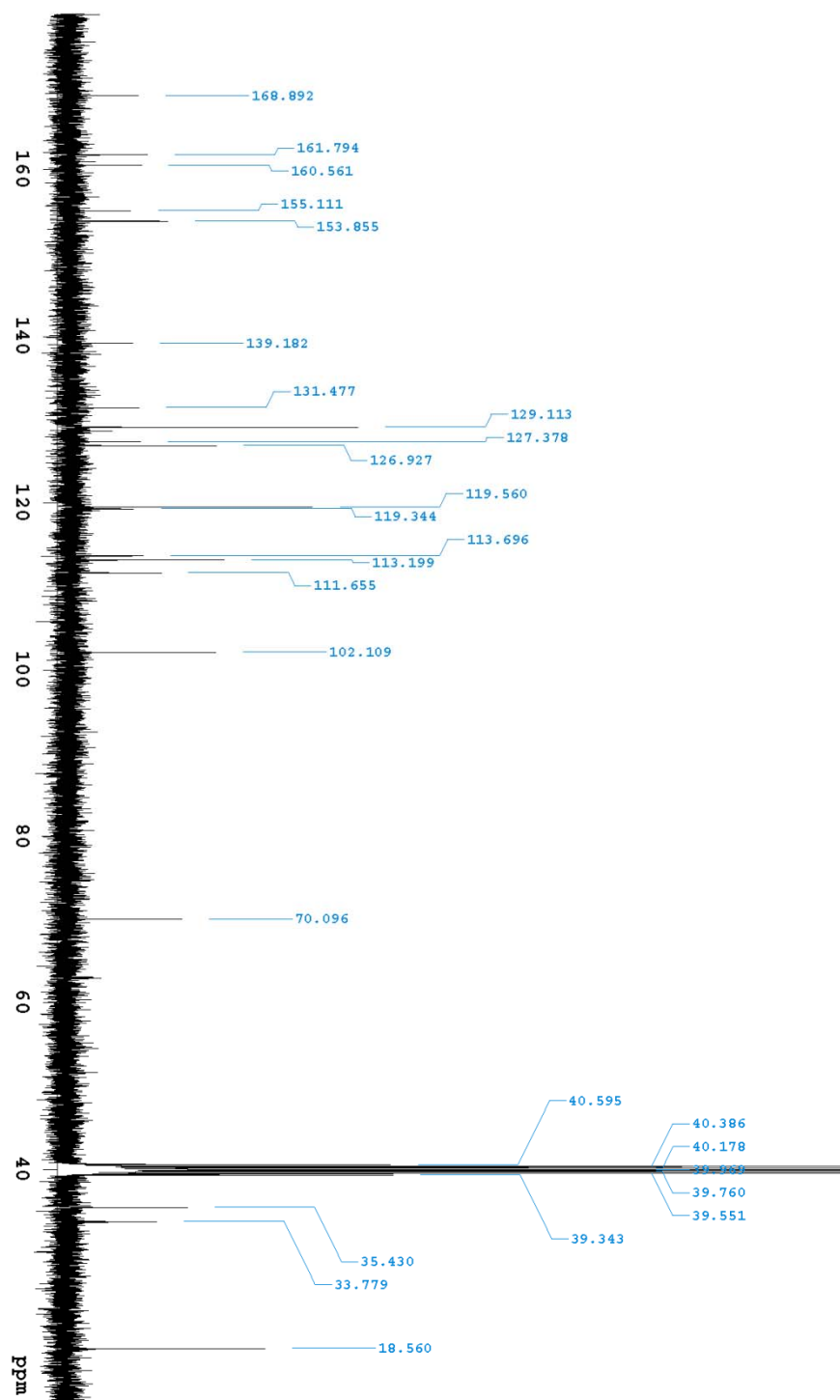
7-{4-(Boc-β-Alanyl-amino)}benzyloxy-3-ethoxycarbonylcoumarin 7b ¹³C NMR



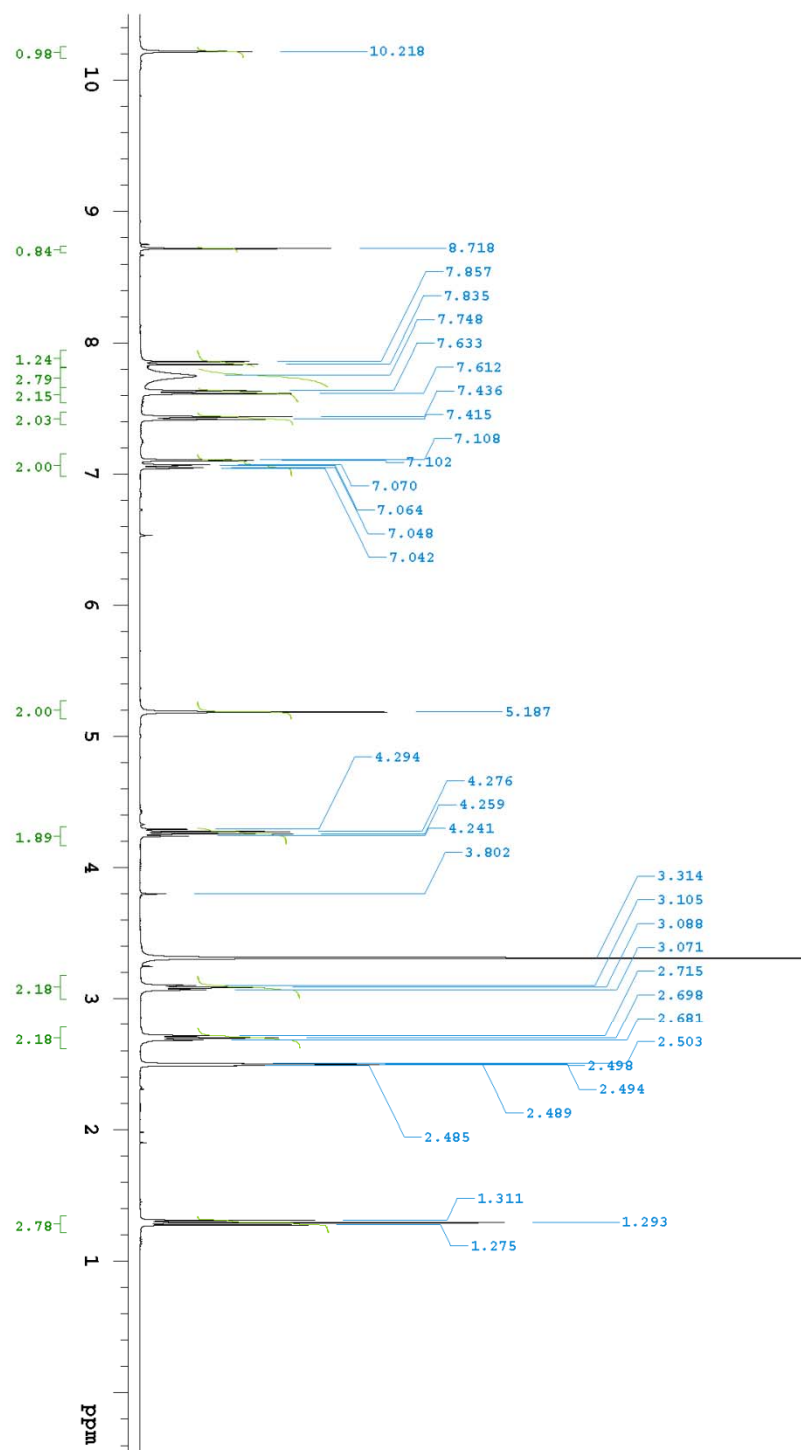
7-{4-(β -Alanylamino)}benzyloxy-4-methylcoumarin trifluoroacetate 8a ^1H NMR



7-{4-(β-Alanyl-amino)}benzyloxy-4-methylcoumarin trifluoroacetate 8a ¹³C NMR



7-{4-(β-Alanylamino)}benzyloxy-3-ethoxycarbonylcoumarin trifluoroacetate 8b ¹H NMR



7-{4-(β-Alanylamino)}benzyloxy-3-ethoxycarbonylcoumarin trifluoroacetate 8b ¹³C NMR

