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Semi-Synthetic Development of Mithramycin Analogues

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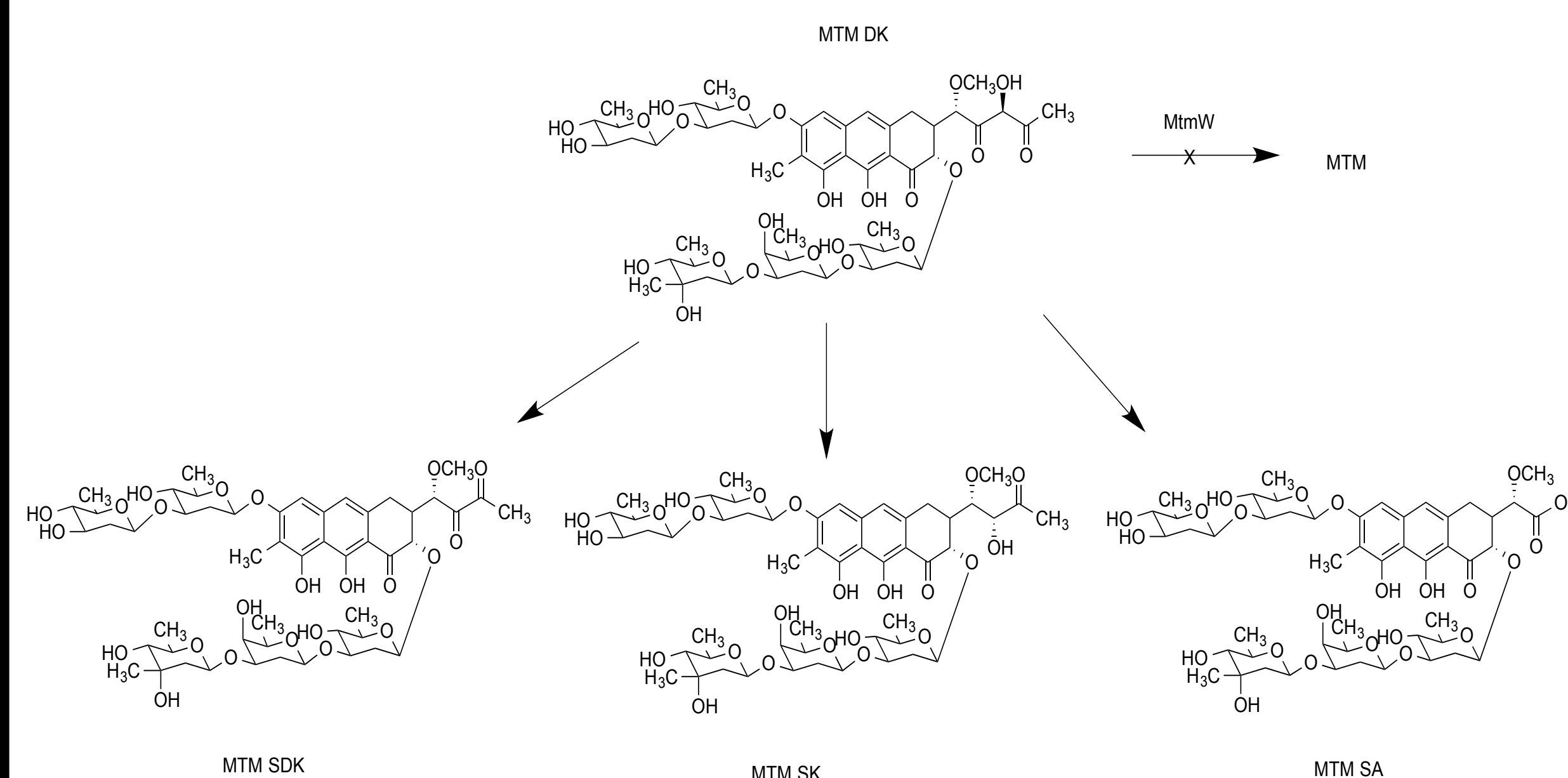
Introduction

Mithramycin (MTM) is an aureolic acid-type polyketide antibiotic produced naturally by the soil bacteria from the *Streptomyces* genus. This compound gained popularity in the 1960's, exhibiting anti-cancer activity by cross-linking GC-rich DNA as a divalent cation-coordinated MTM dimer complex, shutting down proto-oncogenes and up-regulating anti-angiogenic proteins such as c-Myc and p53.

MTM has been used clinically in the past to treat testicular carcinoma, chronic and acute myeloid leukemias, and hypercalcemia, but its use was discontinued due to poor selectivity and extreme side effects. However, MTM has recently garnered renewed attention as a candidate for Ewing family sarcomas and for combinational therapies. Previous research by this lab has shown that a combinational approach of biosynthetic analogue generation followed by synthetic manipulation has the ability to create MTM derivatives with further improved anti-cancer activity. By inactivating the *mtmW* gene, the gene encoding the last acting enzyme in the MTM biosynthetic pathway, the three analogues MTM SK (SK), MTM SDK (SDK), and MTM SA (SA) are produced.

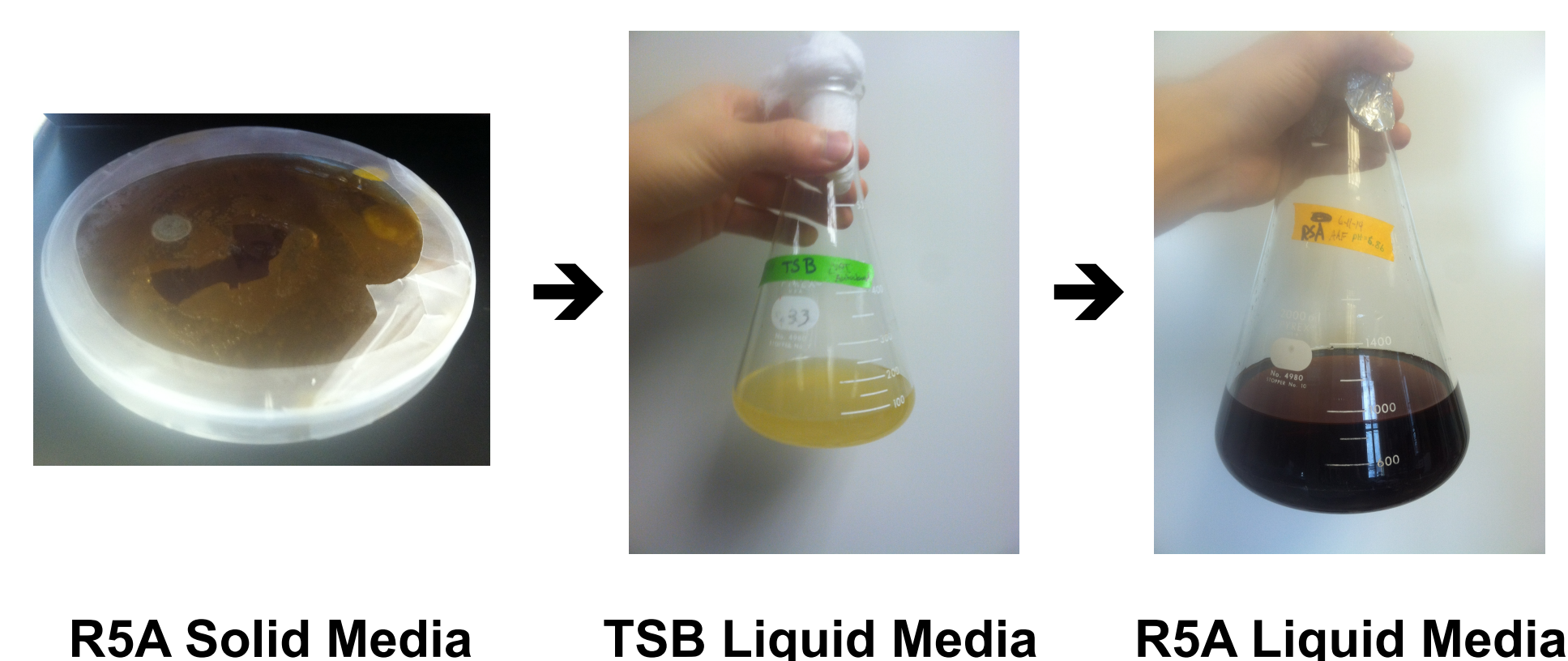
SK and SDK showed improved anti-cancer activity compared to native MTM, but SA was largely discarded due to its decreased anti-cancer activity. The decreased activity is likely due to a negative charge at physiological pH, which inhibits the binding of MTM to DNA. Recently, derivatives of SA have shown improved anti-cancer activity compared to SK and SDK. To that end, our objectives are to design an improved isolation procedure for SA, to further derivatize SA, and to evaluate the new analogues with regard to their anti-cancer activity.

Mithramycin Analogue Biosynthesis

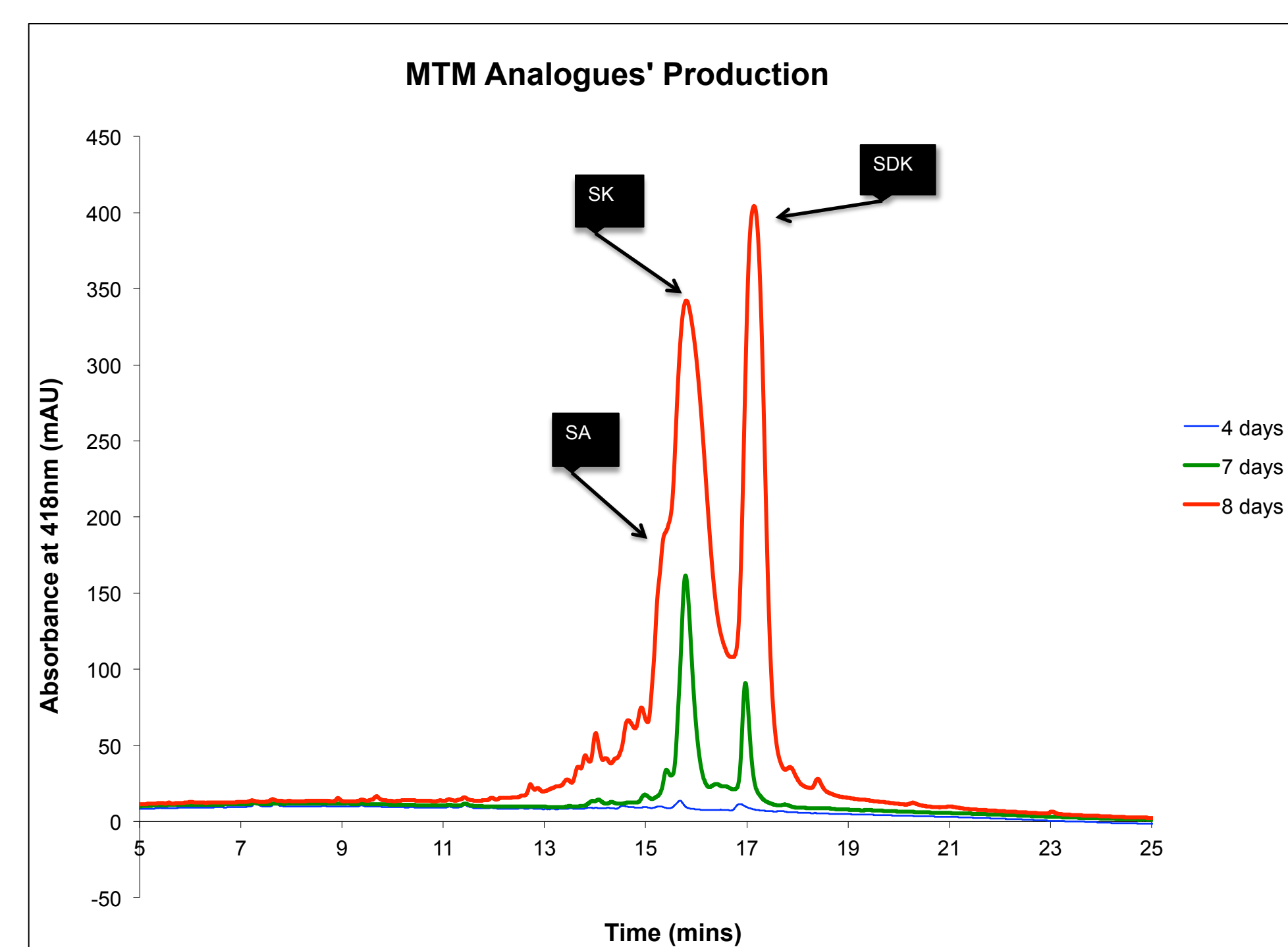


The MTM analogues SA, SK, and SDK are produced by the inactivation of the *mtmW* gene responsible for the last enzyme in the biosynthetic pathway of MTM.

Bacteria Growth and Drug Production



S. Argillaceus is grown on R5A solid media for 5 days. A portion is cut out and transferred to liquid TSB media for two days of further promoted growth. A sample of this is transferred to R5A liquid media for 5 or more days in order to promote drug production.

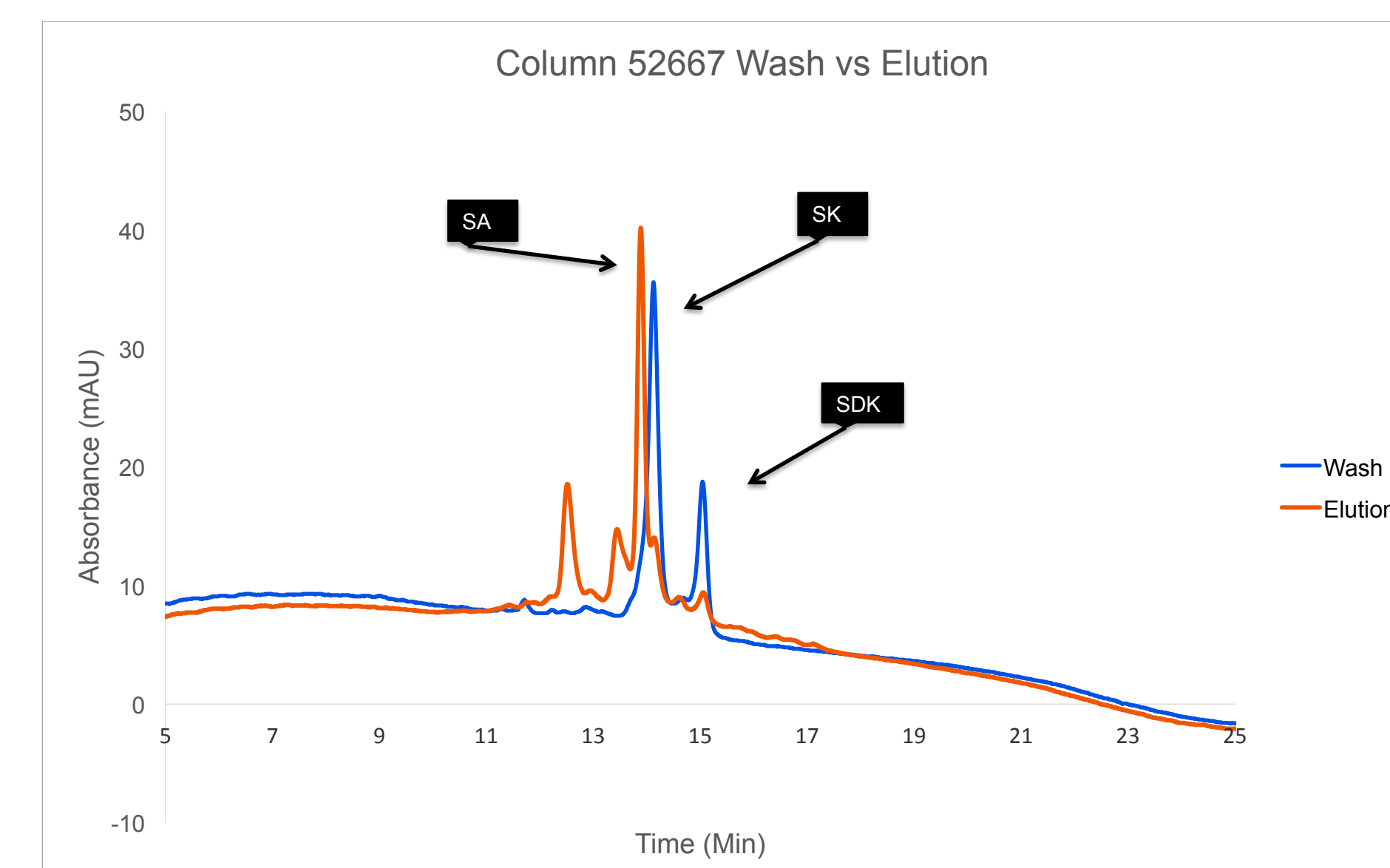


HPLC data of relative drug production by *S. argillaceus* over several days. Peaks for SA, SK, and SDK are labeled.

Isolation of SA

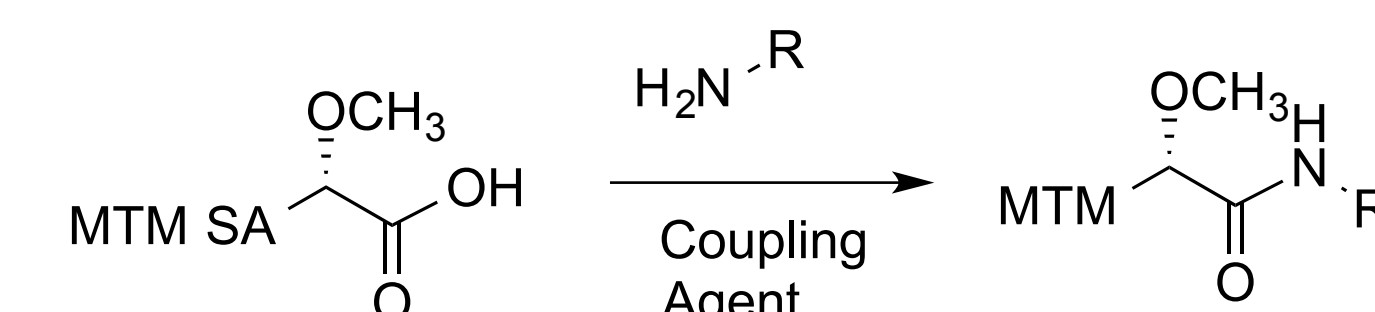
Column	Binds SA	Binds SK	Binds SDK	Washes off SA	Washes off SK	Washes off SDK	Elutes off SA	Elutes off SK	Elutes off SDK	Separates Analogues
C-18	X	X	X				X	X	X	No
52640	X	X	X				X	X	X	No
Amberlite										No
52579										No
52667	X	X	X		X	X	X			Yes

Column characteristics for isolation of SA. Column 52667, a strong anion exchange column with quaternary amines, was able to separate SA from other analogues.

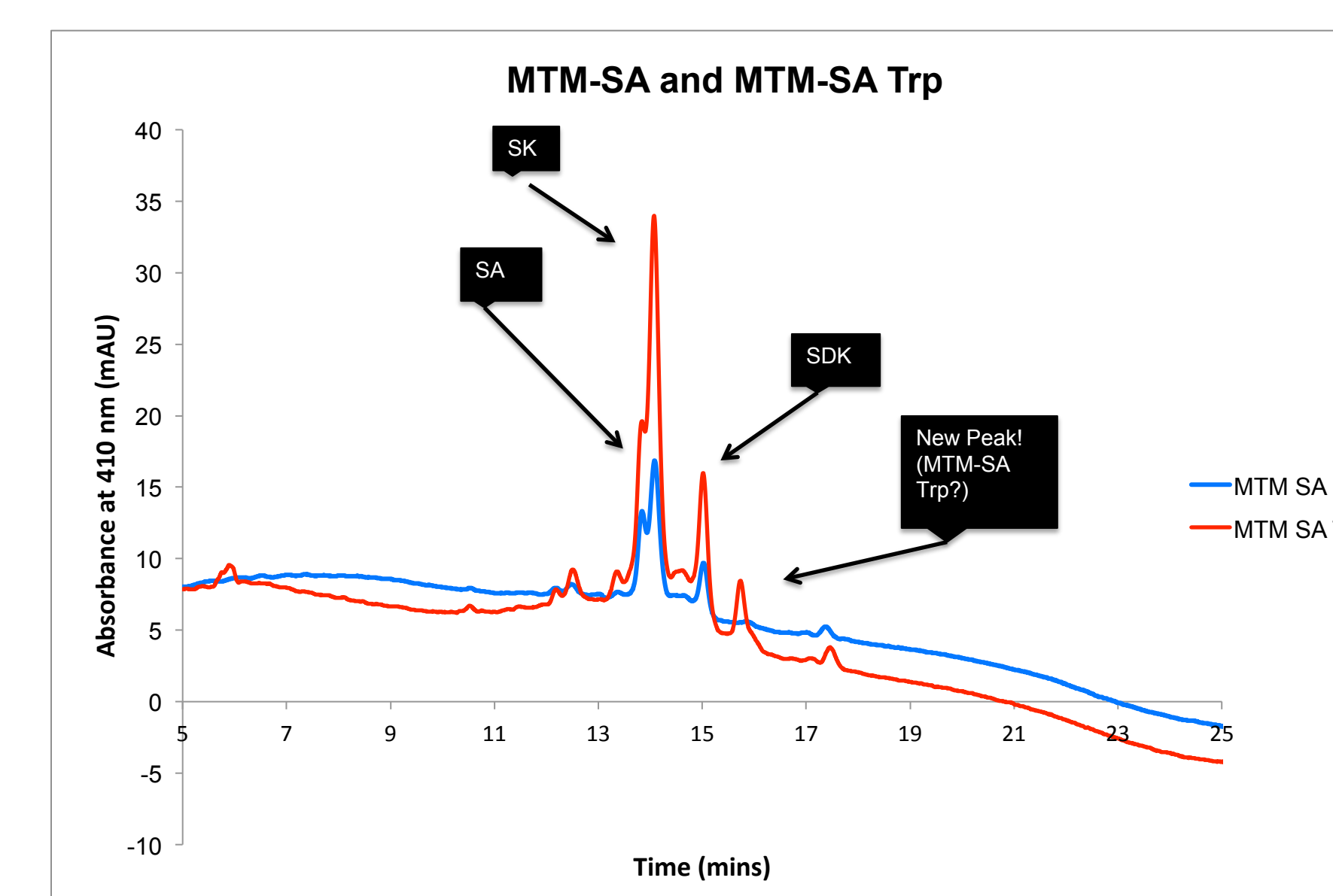
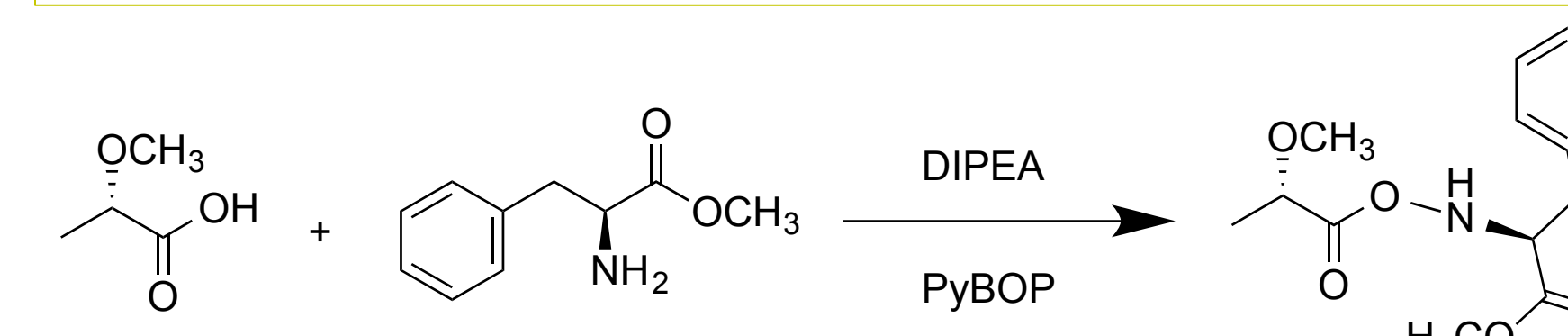


Column 52667 was the first major isolation of SA. Only SK and SDK came off in washes as shown in wash 2 above, while mostly SA came off in elution 1. A second pass through the column fully isolates SA from the analogues..

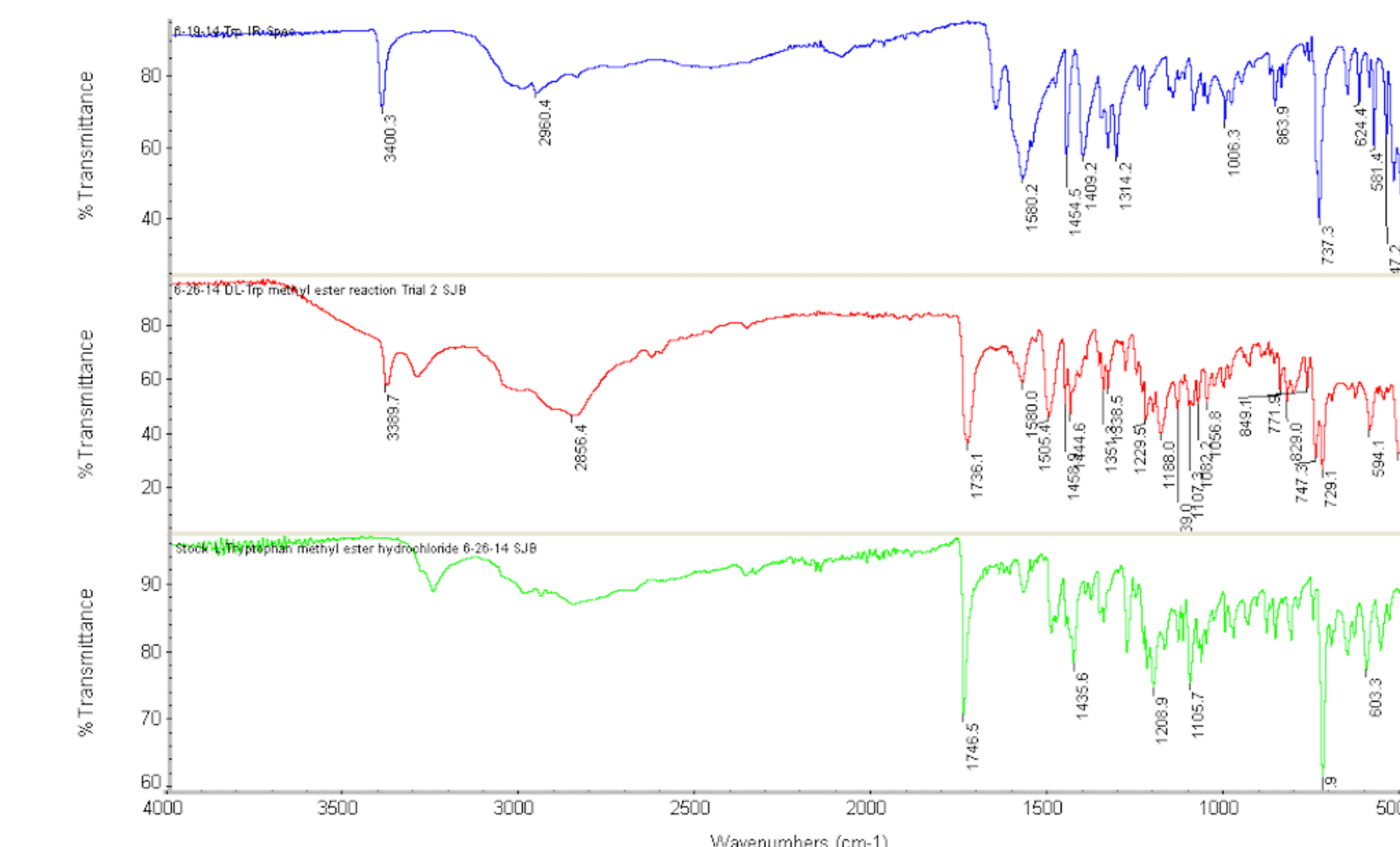
Derivatization of SA



A side chain containing a primary amine is coupled to the carboxylic acid of SA to create the new derivatives. We used tryptophan, histidine, and phenylalanine as side chain additions. An example reaction with tryptophan is shown below.

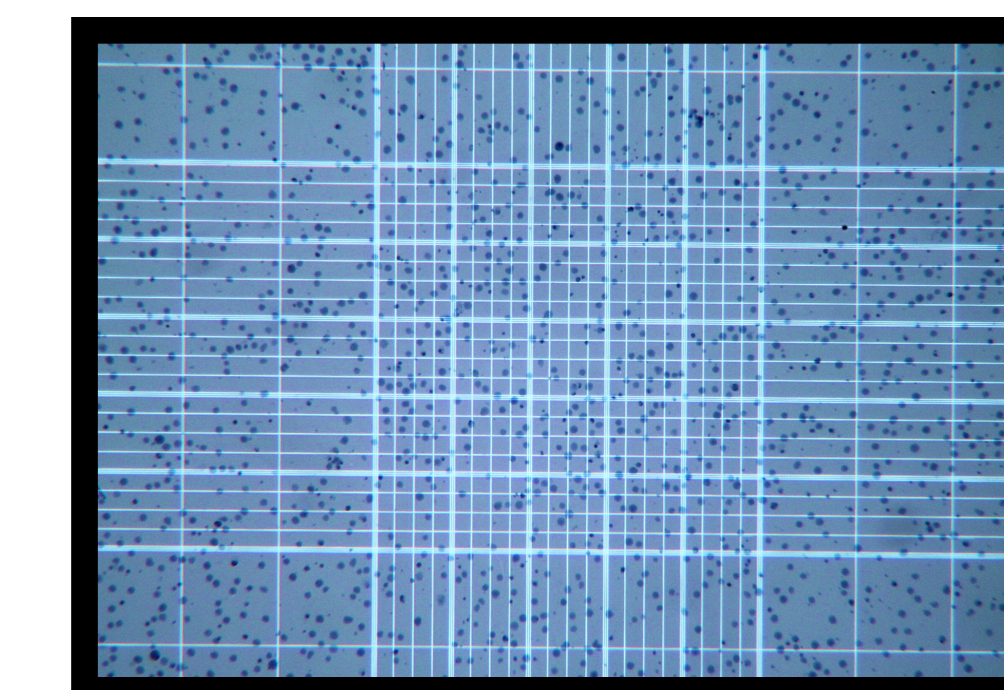


HPLC spectral analysis revealed a new peak in the MTM-SA Trp compound compared to SA (impure). The histidine and phenylalanine reactions yielded several new peaks and require additional testing.



Certain side chain additions require methylation in order to prevent peptide chain formation. We developed a methylation procedure, the success of which is shown above in the IR data of Trp (top), a Trp methylation reaction (middle), and stock Trp methyl ester (bottom).

Jurkat Cell Culturing



Jurkat leukemia cells are being culture for cytotoxicity assays to evaluate the anticancer activity of the SA derivatives.

Conclusions

- SA was successfully isolated using a strong anion exchange column with a quaternary amine and specialized wash and elution solutions.
- SA derivatization is accomplished by the coupling of a primary amine of a desired side chain and the carboxylic acid of SA.
- A methylation procedure was established for side chains requiring modification to prevent multiple additions.
- SA derivatives were synthesized from tryptophan, phenylalanine, and histidine methyl esters, but will require further work to confirm and isolate the products.
- Anti-cancer activity of the new SA derivatives will be evaluated using leukemia and lung cancer cell lines. SK will be evaluated with both cell lines as a benchmark.

Acknowledgments

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