Supporting Information

Precursor Manipulation in Glycopeptide Antibiotic Biosynthesis: Are β-Amino Acids Compatible with the Oxidative Cyclization Cascade?

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I. Synthesis routes for CoA-peptides (1-7)

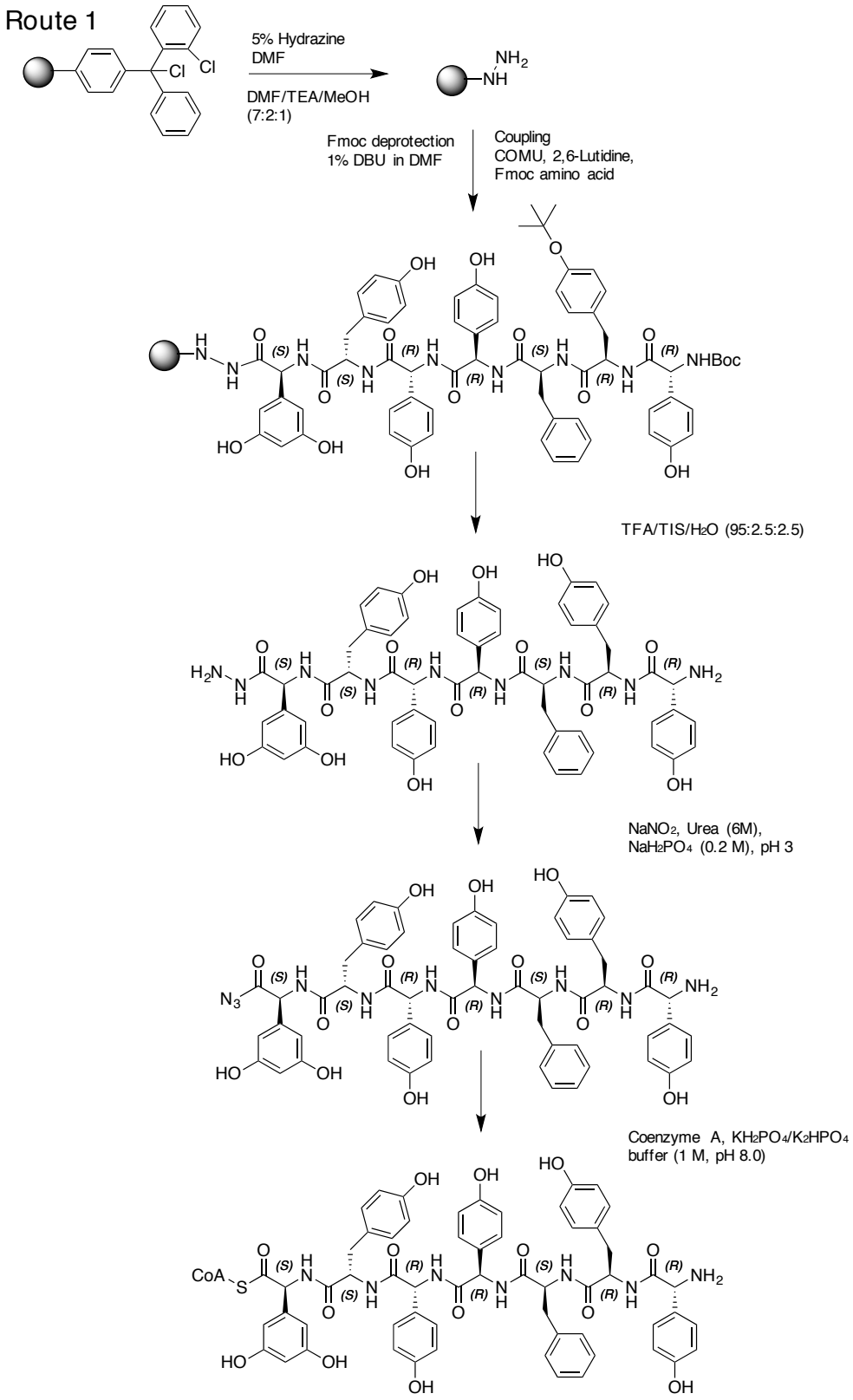


Figure S1 For the synthesis of CoA-peptide **1-5** and **7**, route 1 was performed providing hydrazide peptides **1a-5a** and **7a** as intermediates.

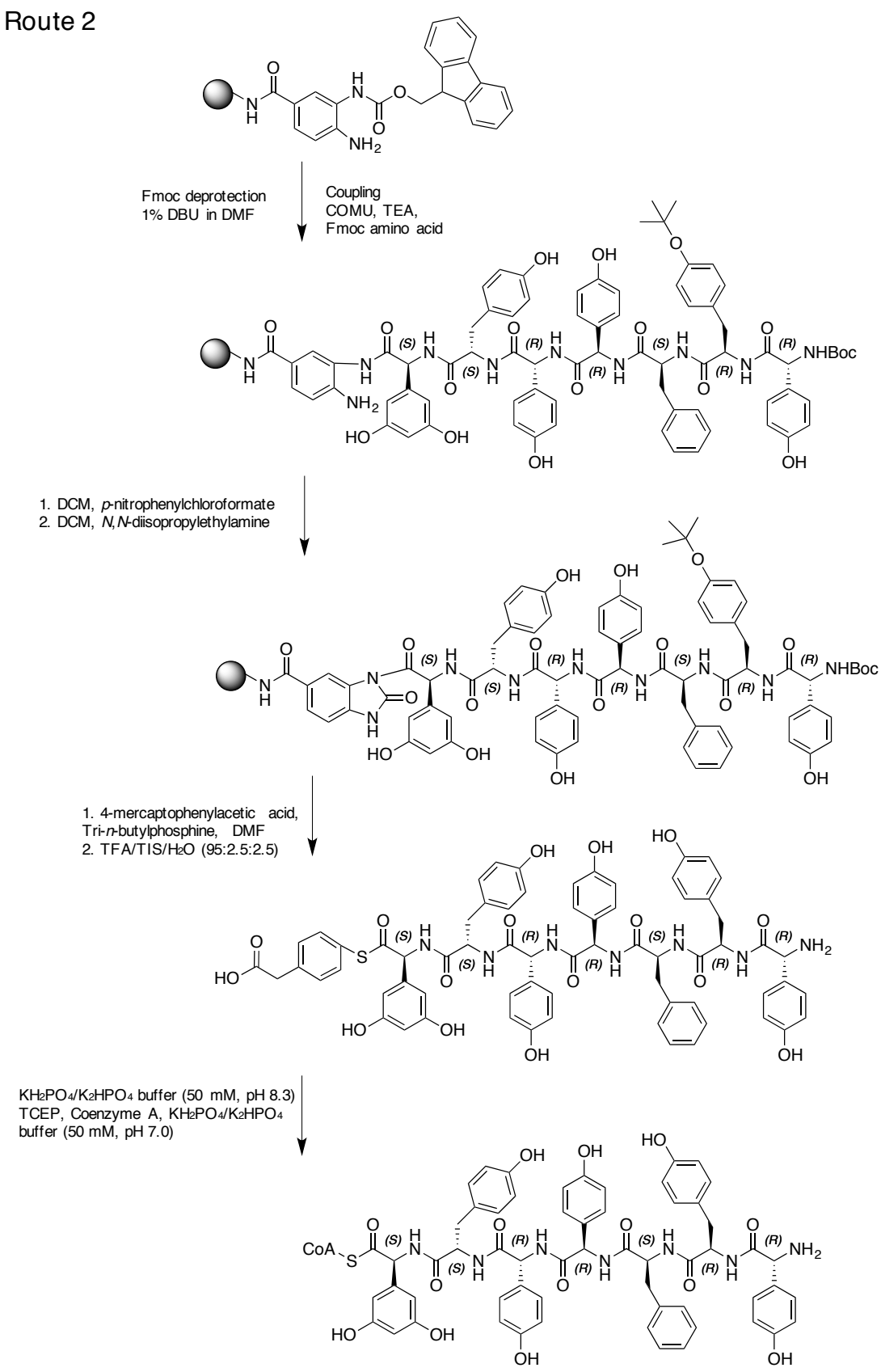
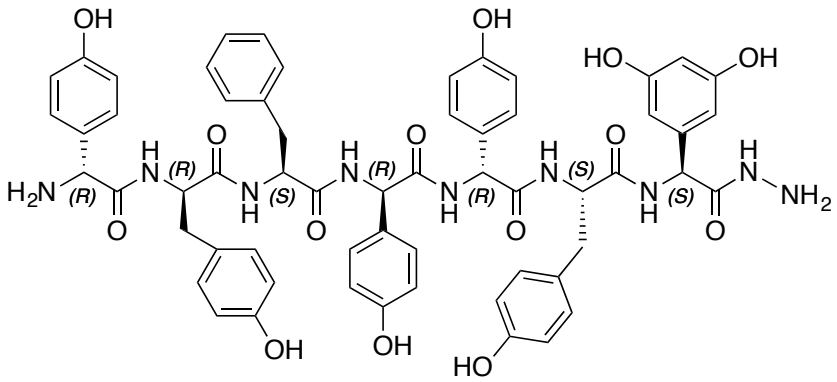


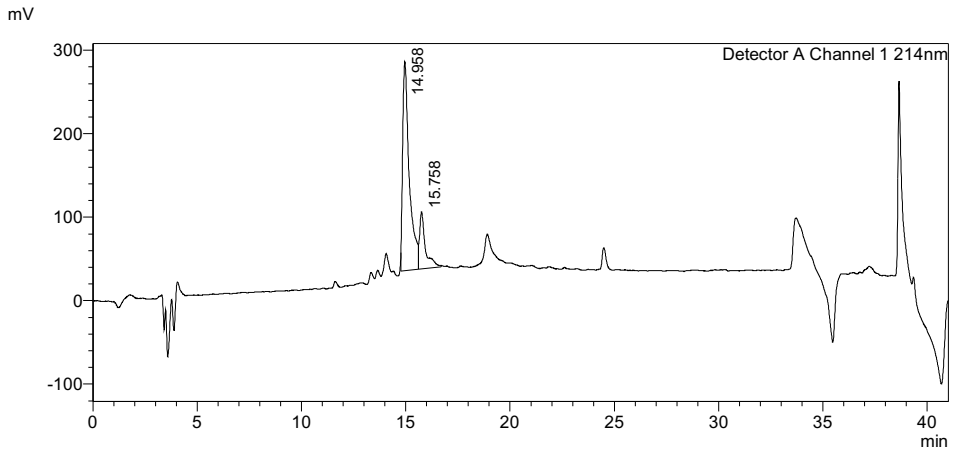
Figure S2 For CoA-peptide **6**, route 2 was performed that did not result in isolation of a peptide hydrazide intermediate.

II. MS (ESI) data for peptide hydrazide intermediates

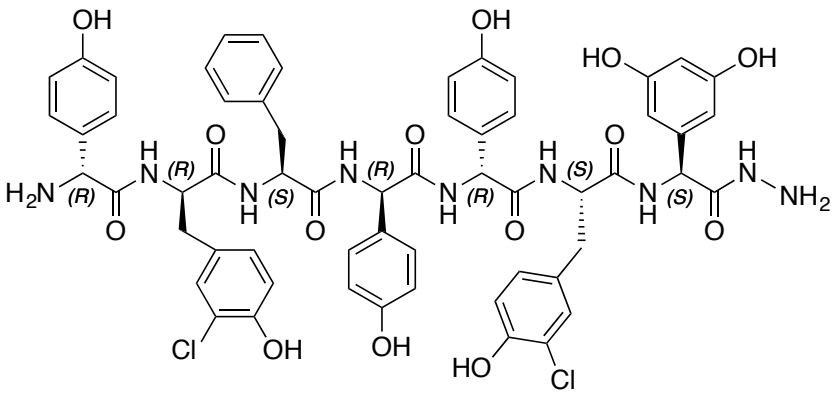
Actinoidin-hydrazide (1a)



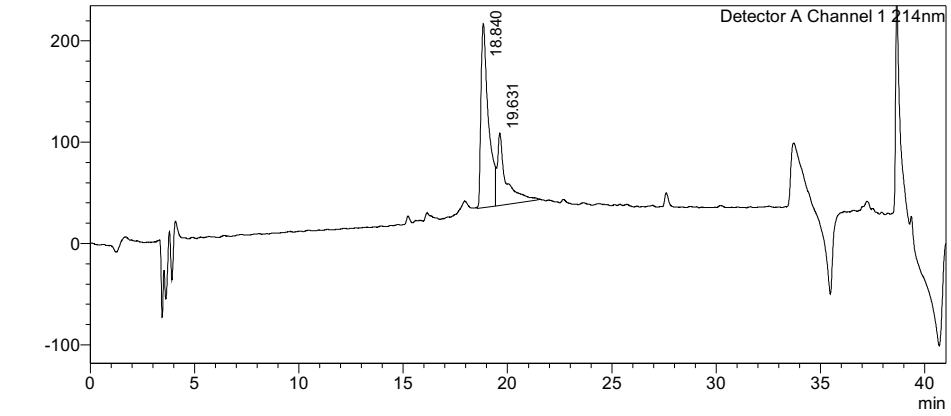
Hydrazide HPLC trace:



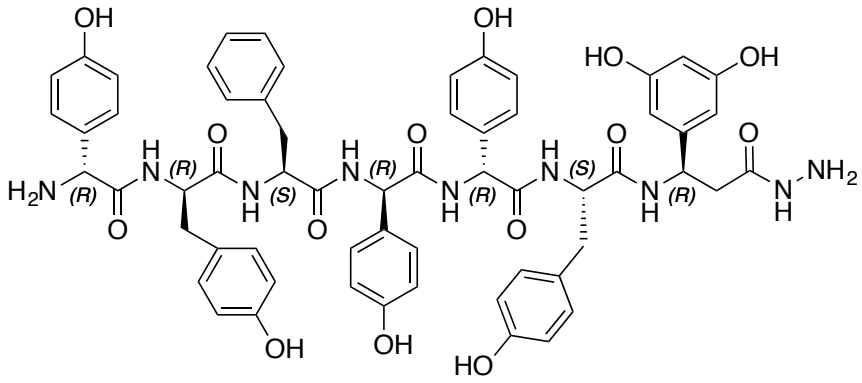
Actinoidin-(Cl)-hydrazide (2a)



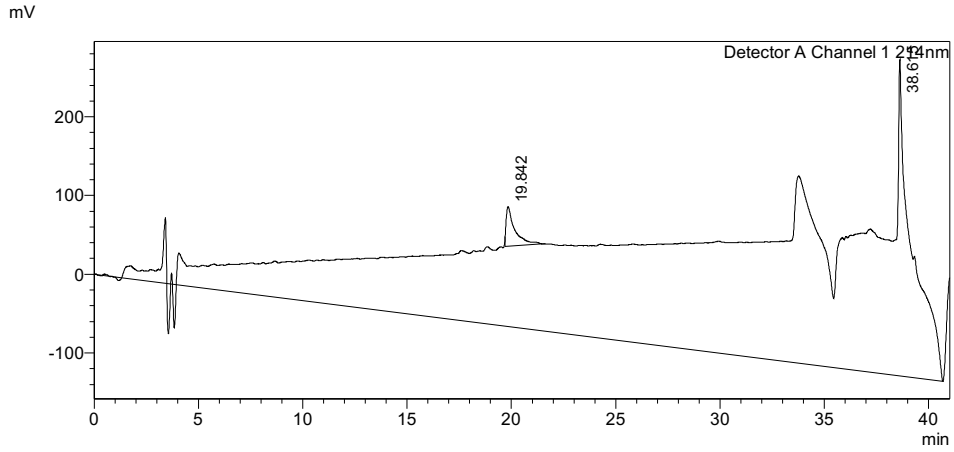
Hydrazide HPLC trace:



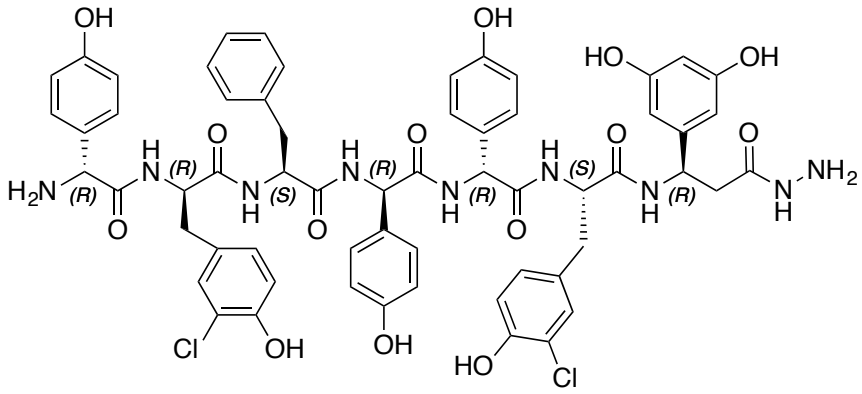
Actinoidin-7-(β-3,5-Dpg)-hydrazide (3a)



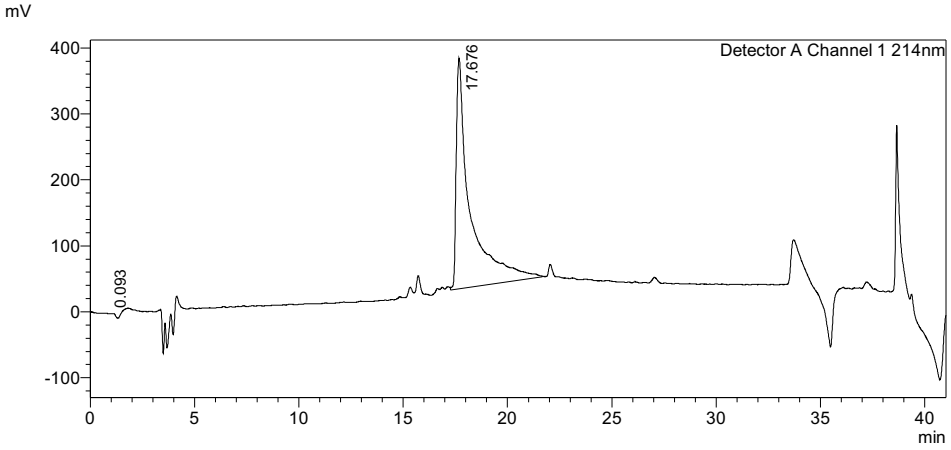
Hydrazide HPLC trace:



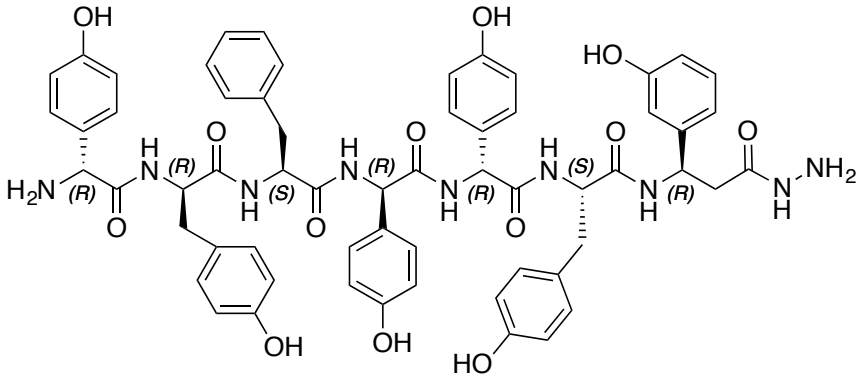
Actinoidin-(Cl)-7-(β-3,5-Dpg)-hydrazide (4a)



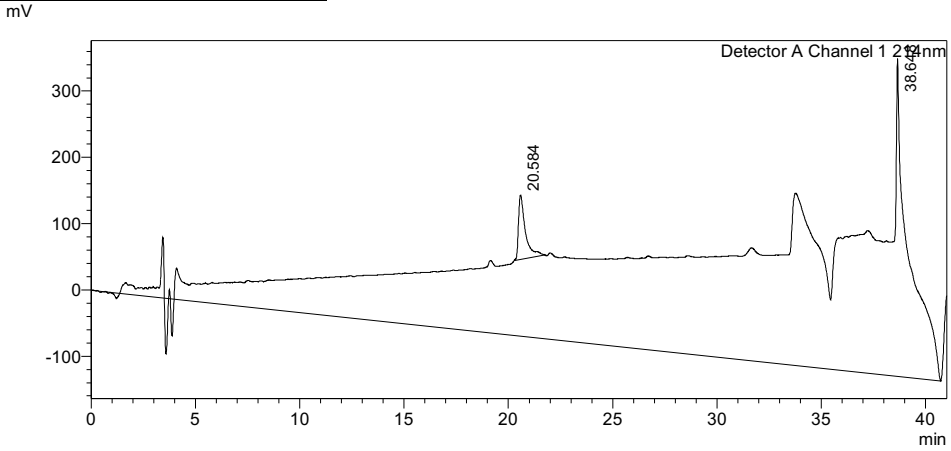
Hydrazide HPLC trace:



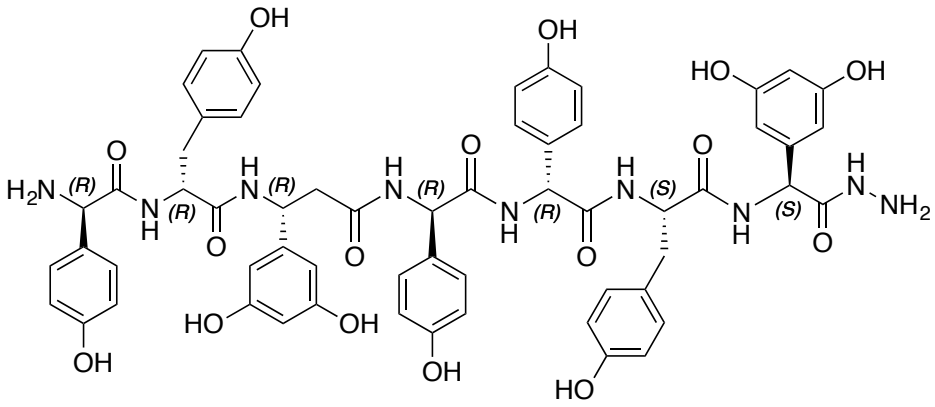
Actinoidin-7-(β-3-Hpg)-hydrazide (5a)



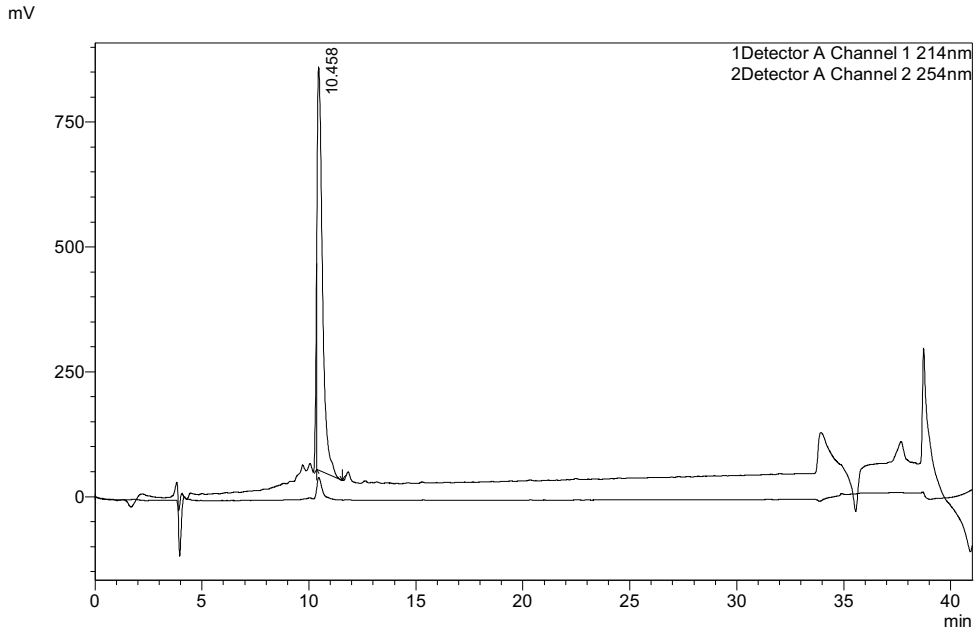
Hydrazide HPLC trace:



Actinoidin-3-(β-3,5-Dpg)-hydrazide (7)

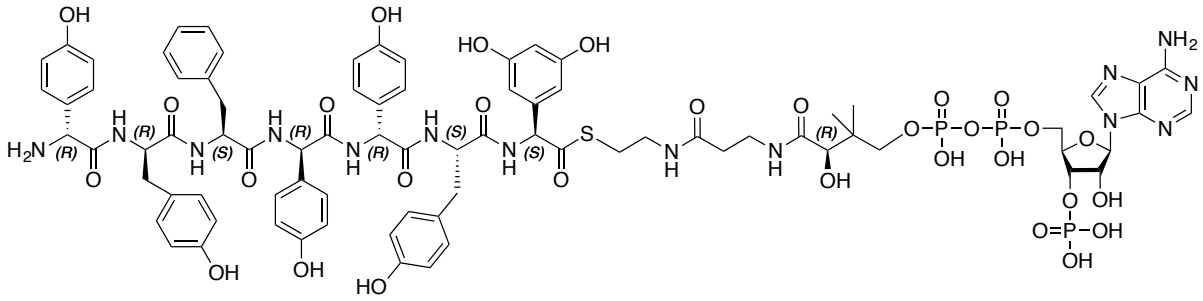


Hydrazide HPLC trace:

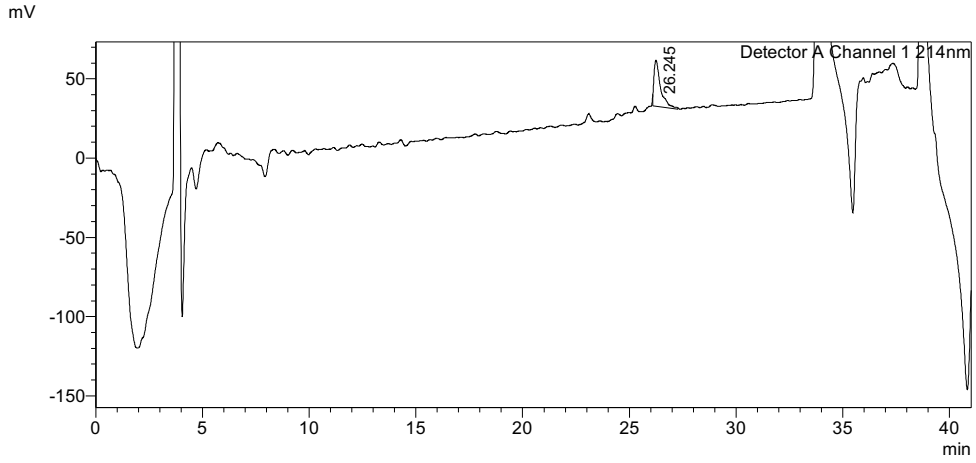


III. HPLC traces, ^{1}H NMR and HRMS spectra for CoA-Peptides

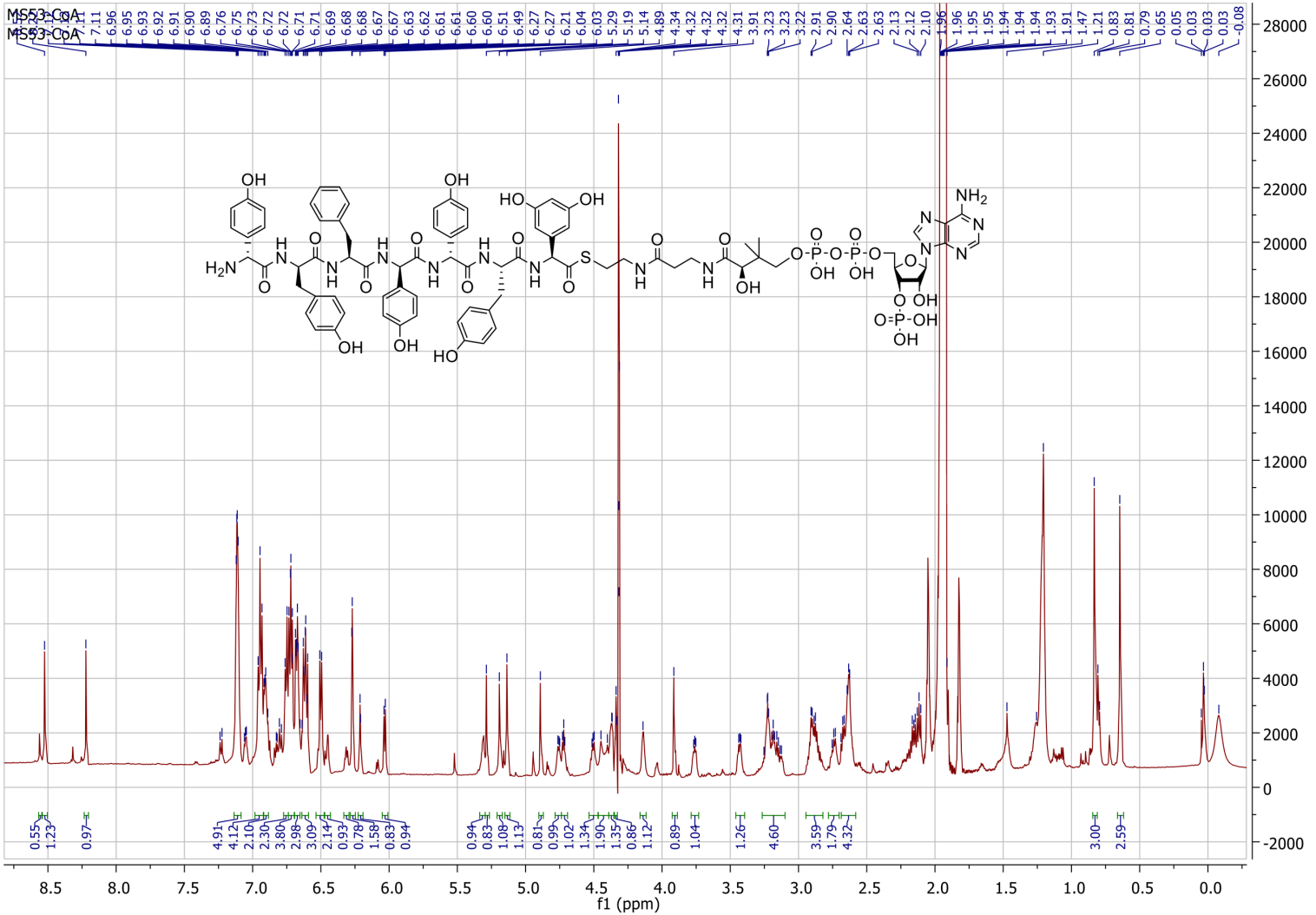
Actinoidin-CoA (1)



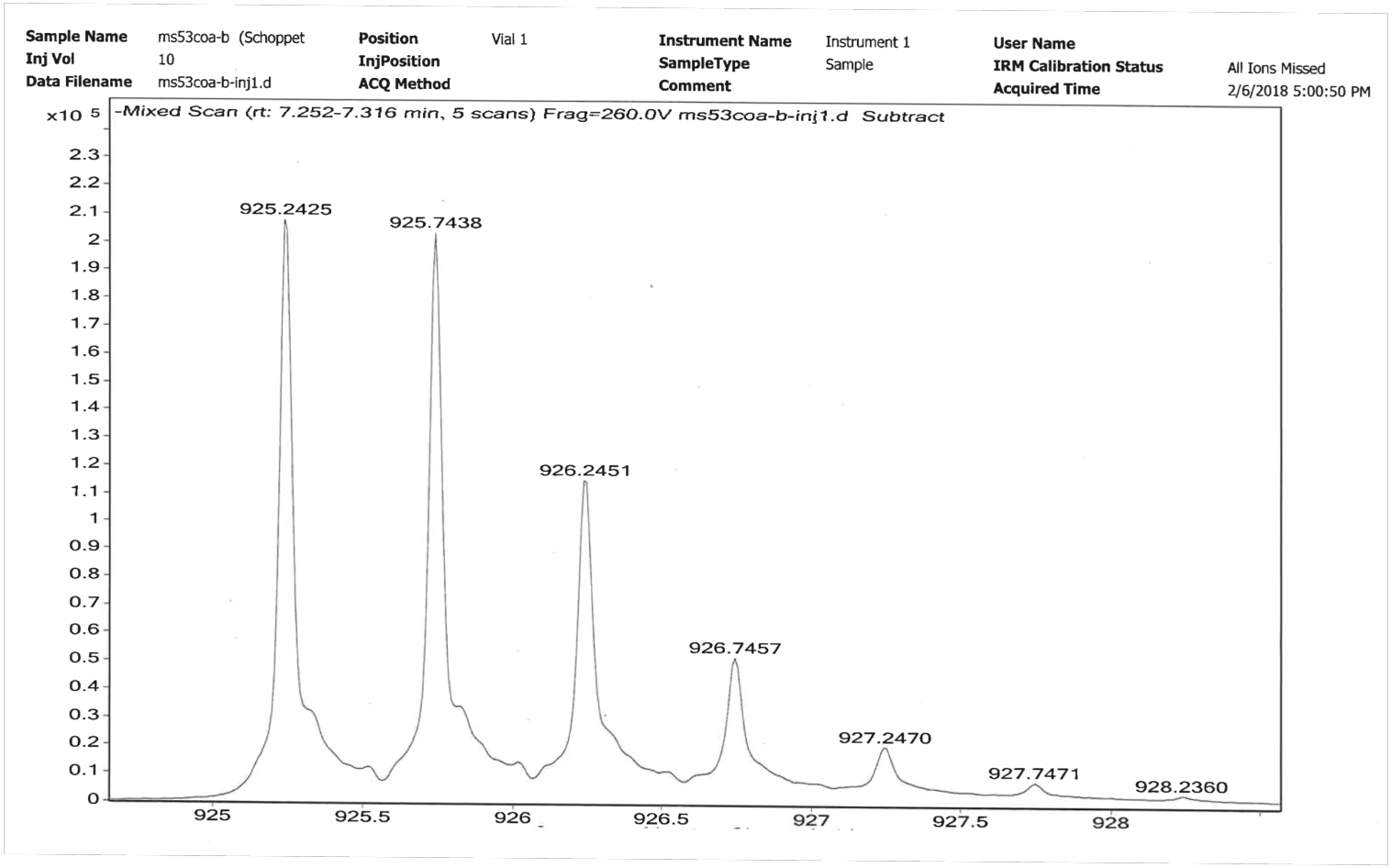
HPLC trace



^{1}H NMR spectrum



HRMS trace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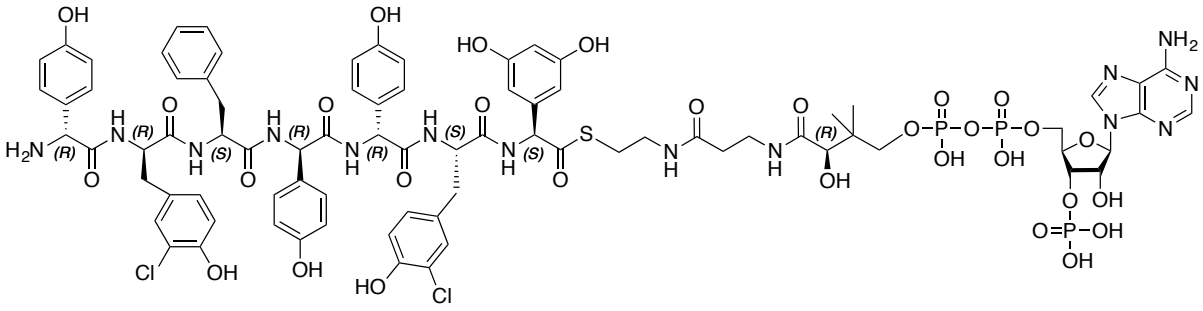
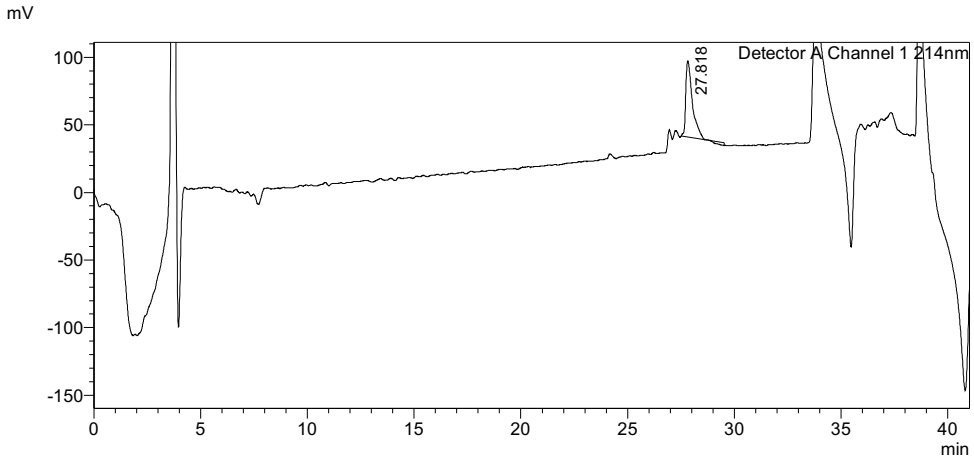
Inj Vol 10
Data Filename ms53coa-b-inj1.d
Position Vial 1
InjPosition
ACQ Method
Instrument Name Instrument 1
SampleType Sample
Comment
User Name
IRM Calibration Status All Ions Missed
Acquired Time 2/6/2018 5:00:50 PM
-Mixed Scan (rt: 7.252-7.316 min, 5 scans) Frag=260.0V ms53coa-b-inj1.d Subtract
x10 5
925.2425
925.7438
926.2451
926.7457
927.2470
927.7471
928.2360
925
925.5
926
926.5
927
927.5
928

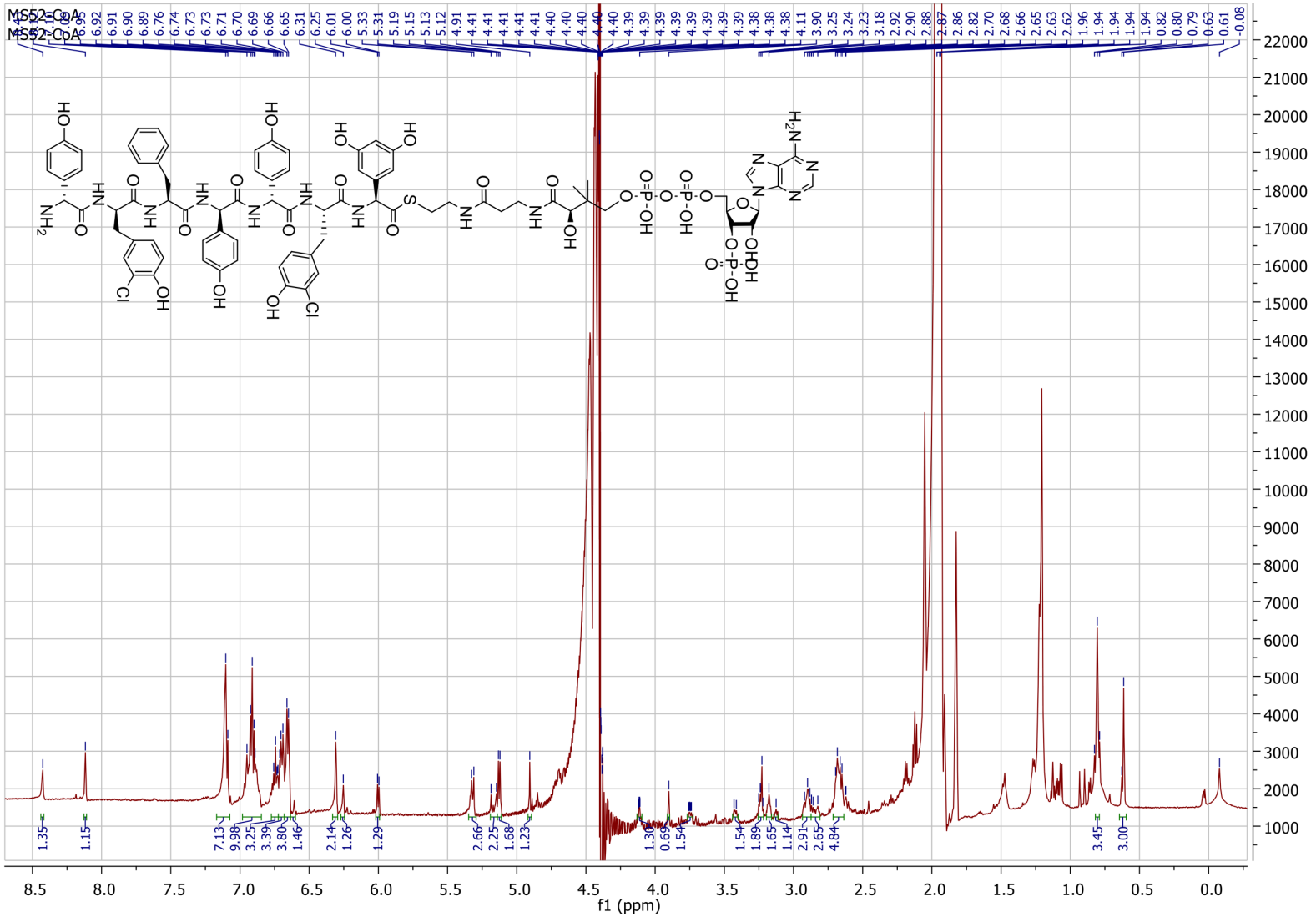
Actinoidin-(Cl)-CoA (2)



HPLC trace



^{1}H NMR spectrum



HRMS trace

Sample Name	ms52coa- (Schoppet)	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
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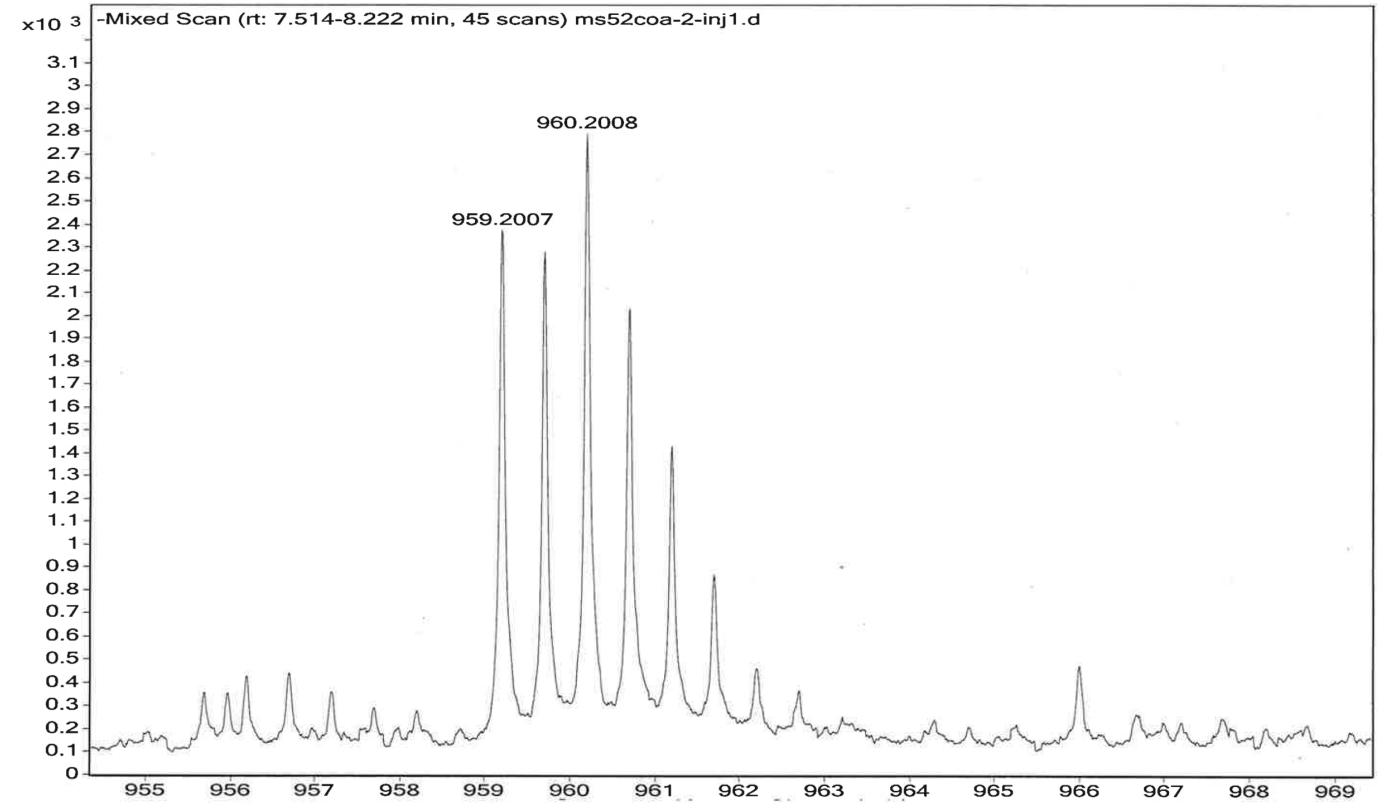
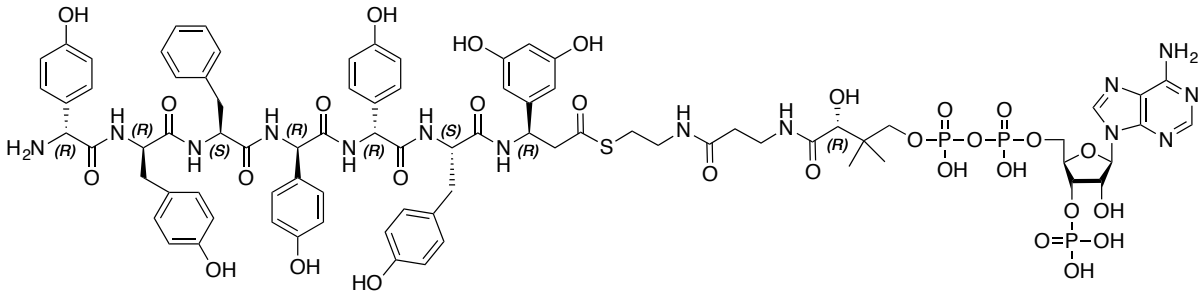
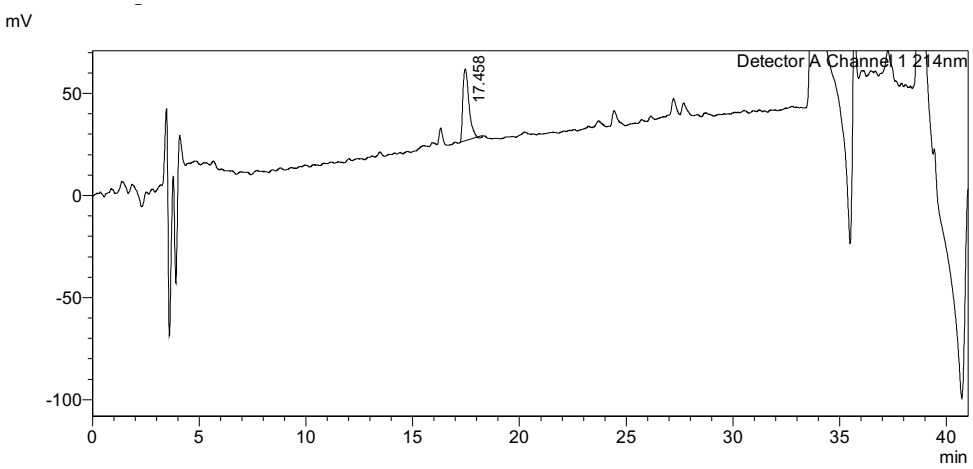


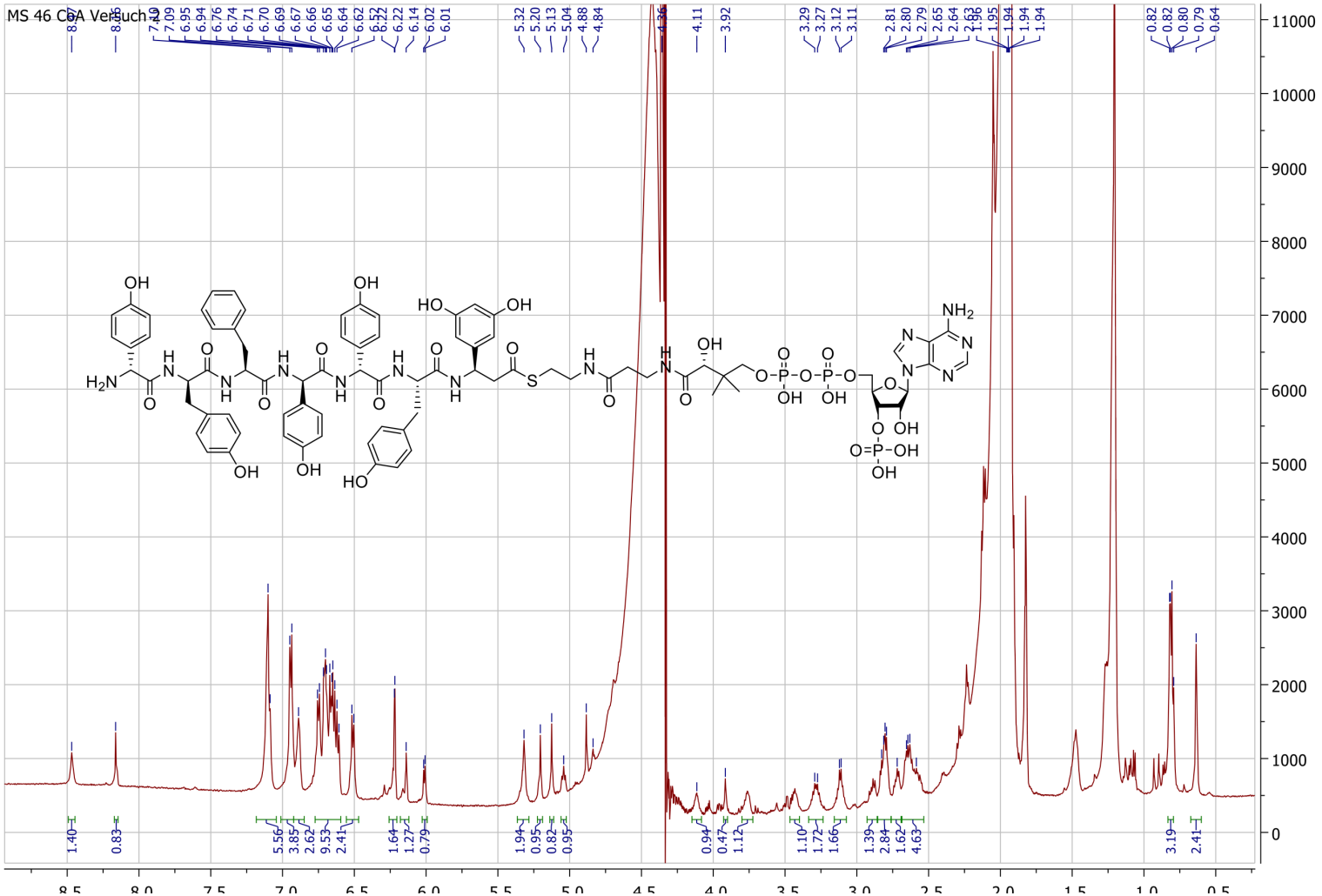
Actinoidin-7-(β-3,5-Dpg)-CoA (3)



HPLC trace



^{1}H NMR spectrum



HRMS trace

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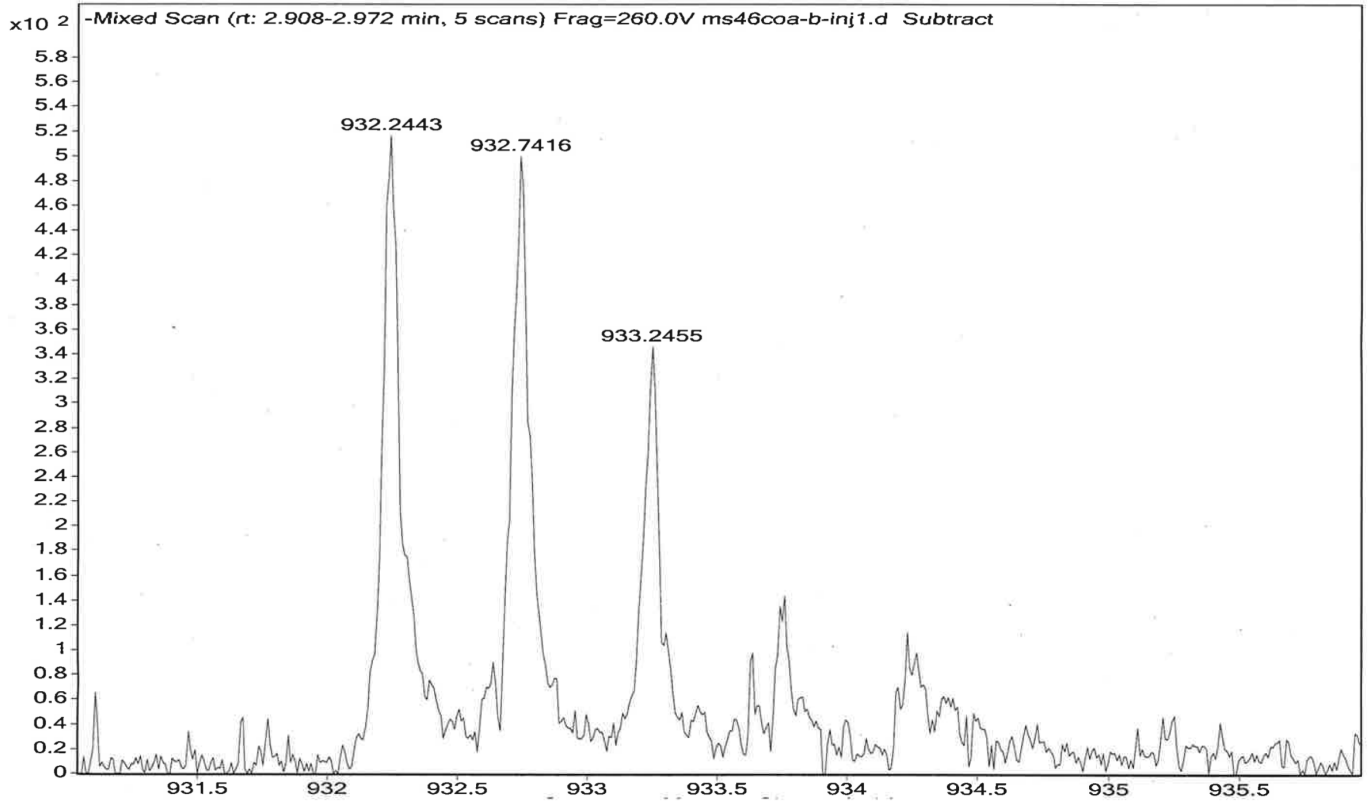
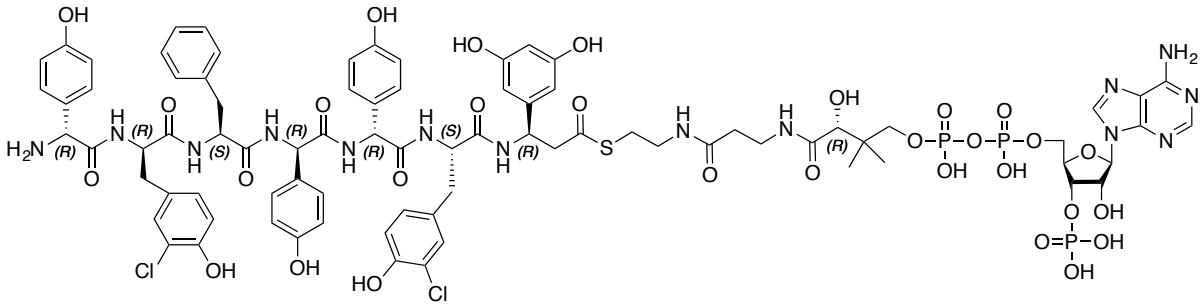
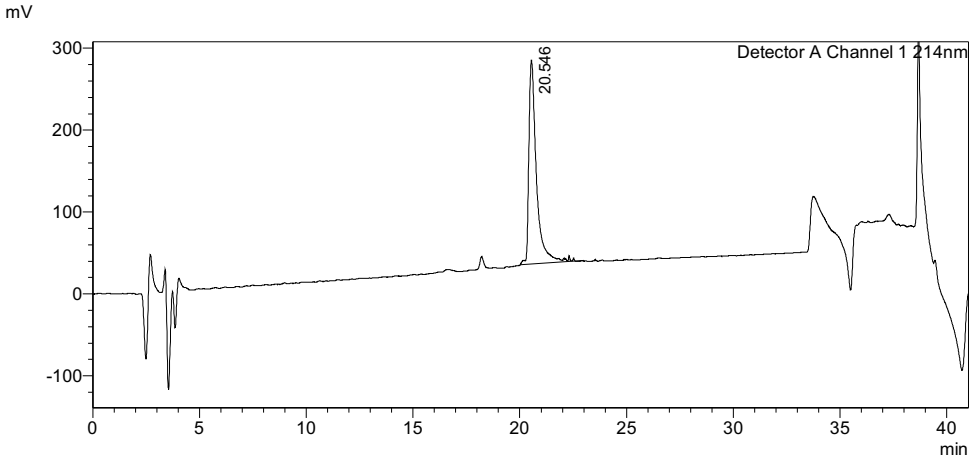


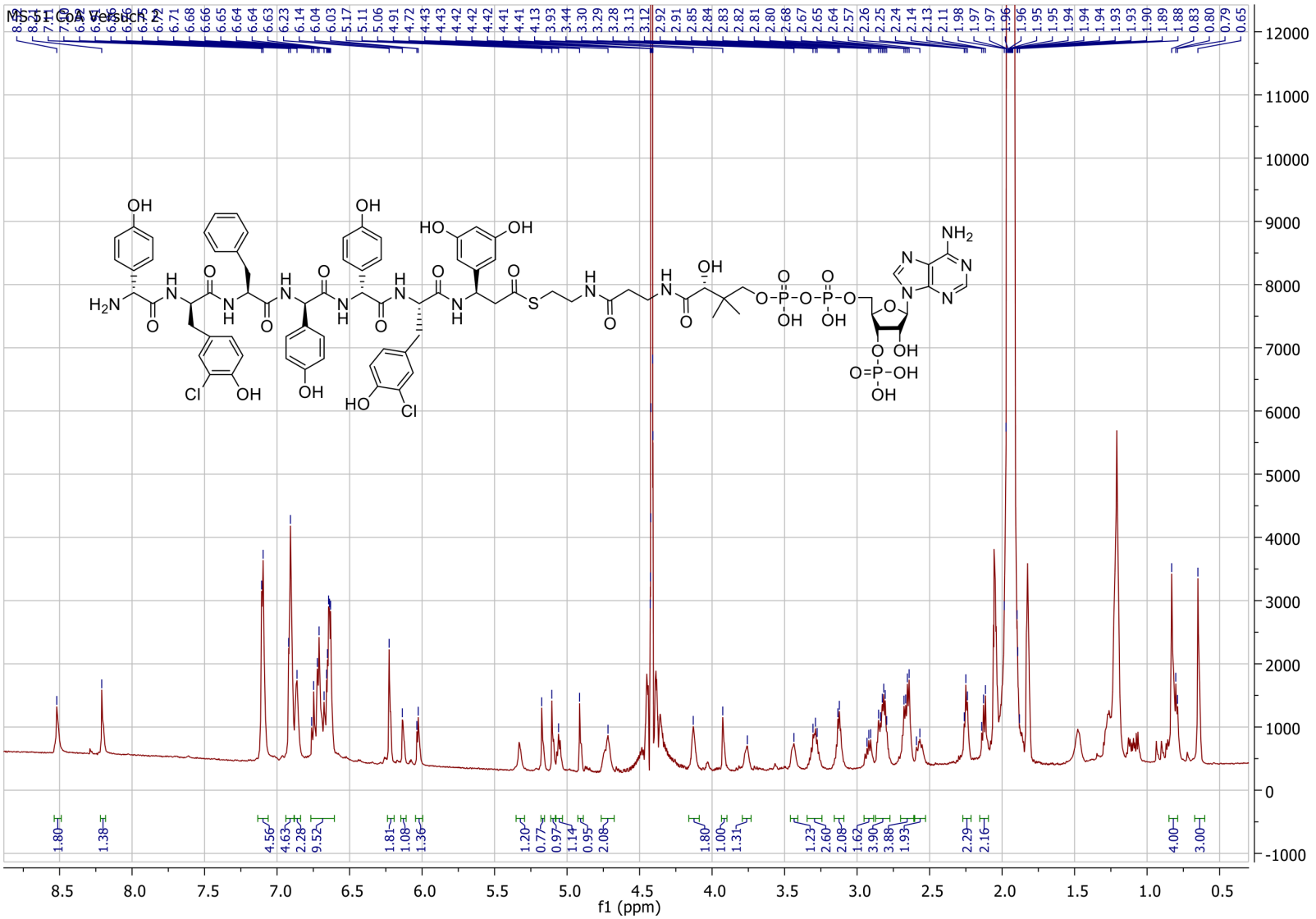
Actinoidin-(Cl)-7-(β-3,5-Dpg)-CoA (4)



HPLC trace



^{1}H NMR spectrum



HRMS trace

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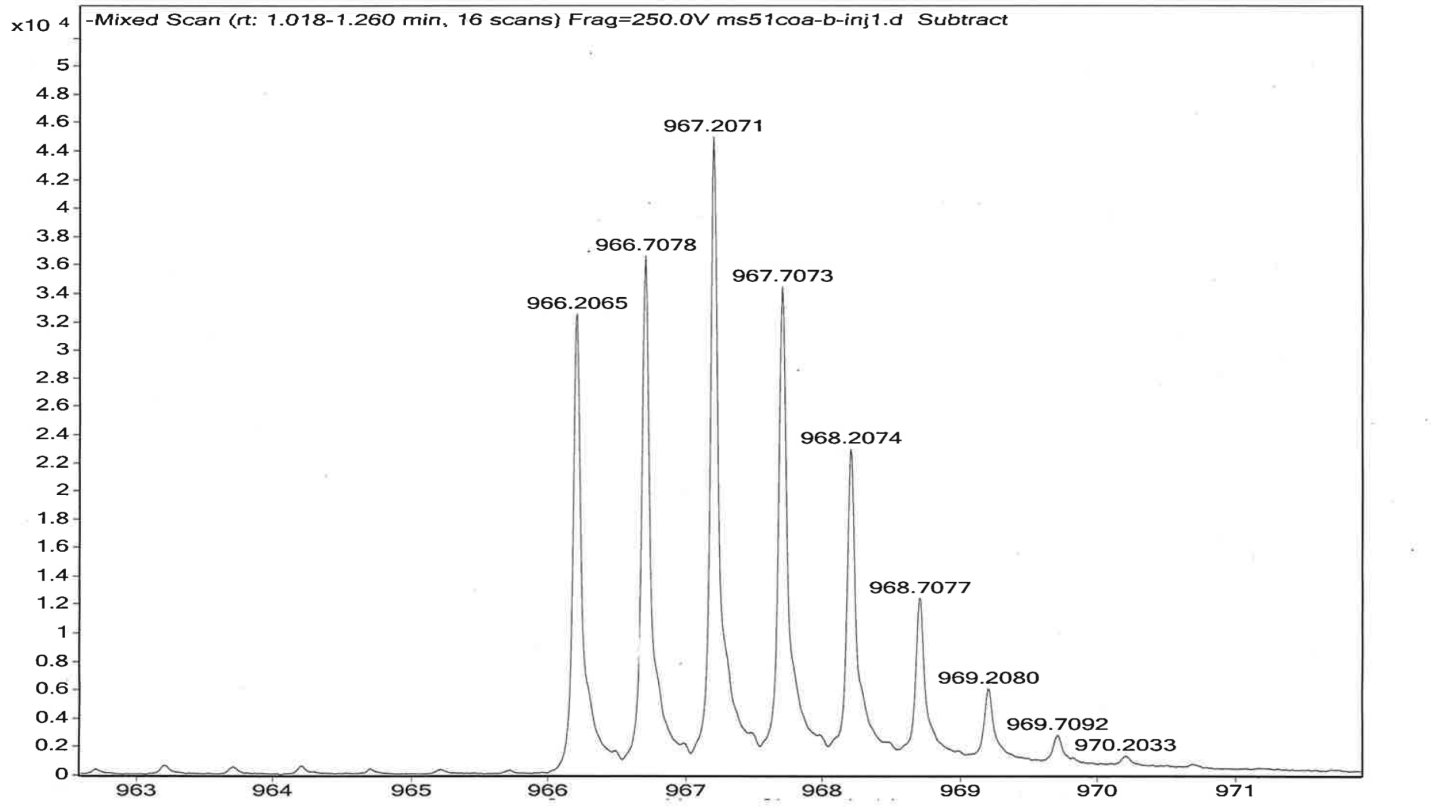
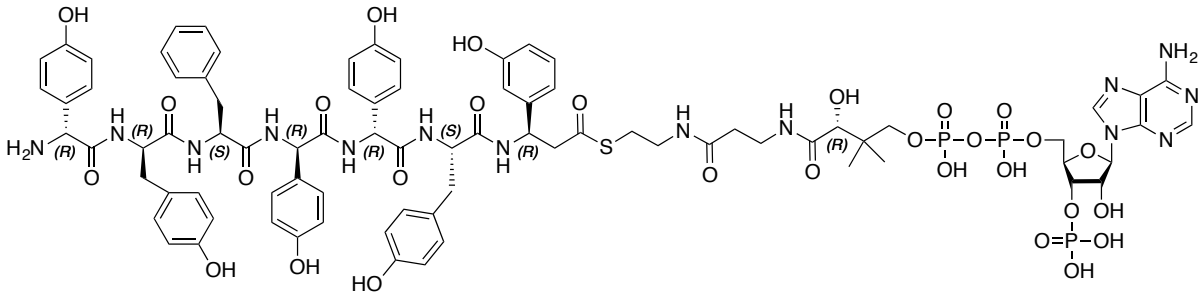
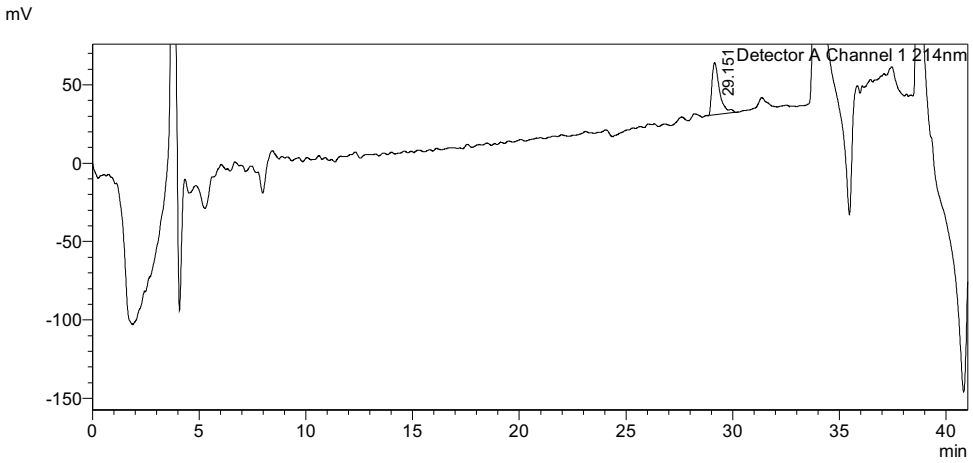


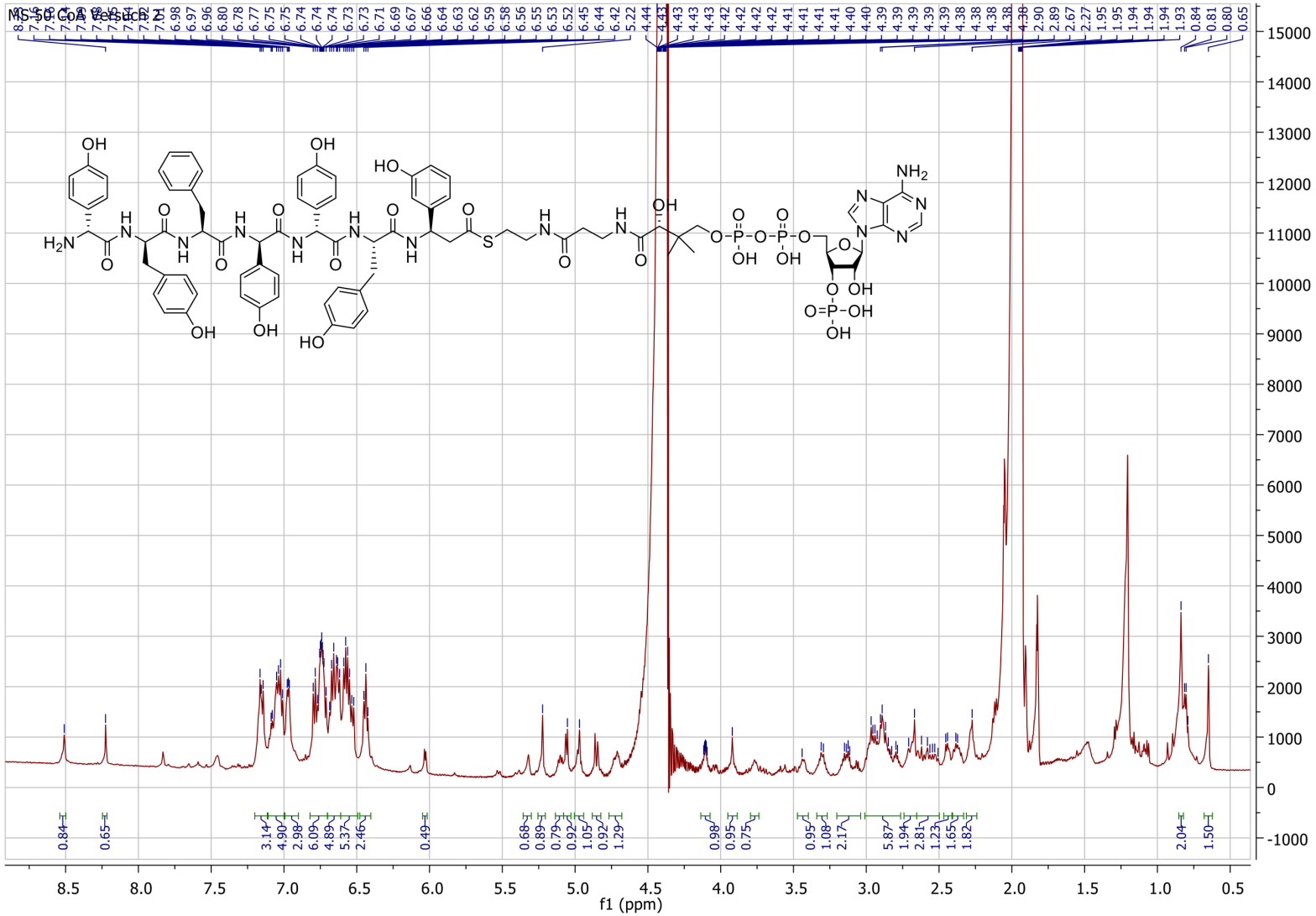
Actinoidin-7-(β-3-Hpg)-CoA (5)



HPLC trace



^{1}H NMR spectrum



HRMS trace

Sample Name	sm50coa	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
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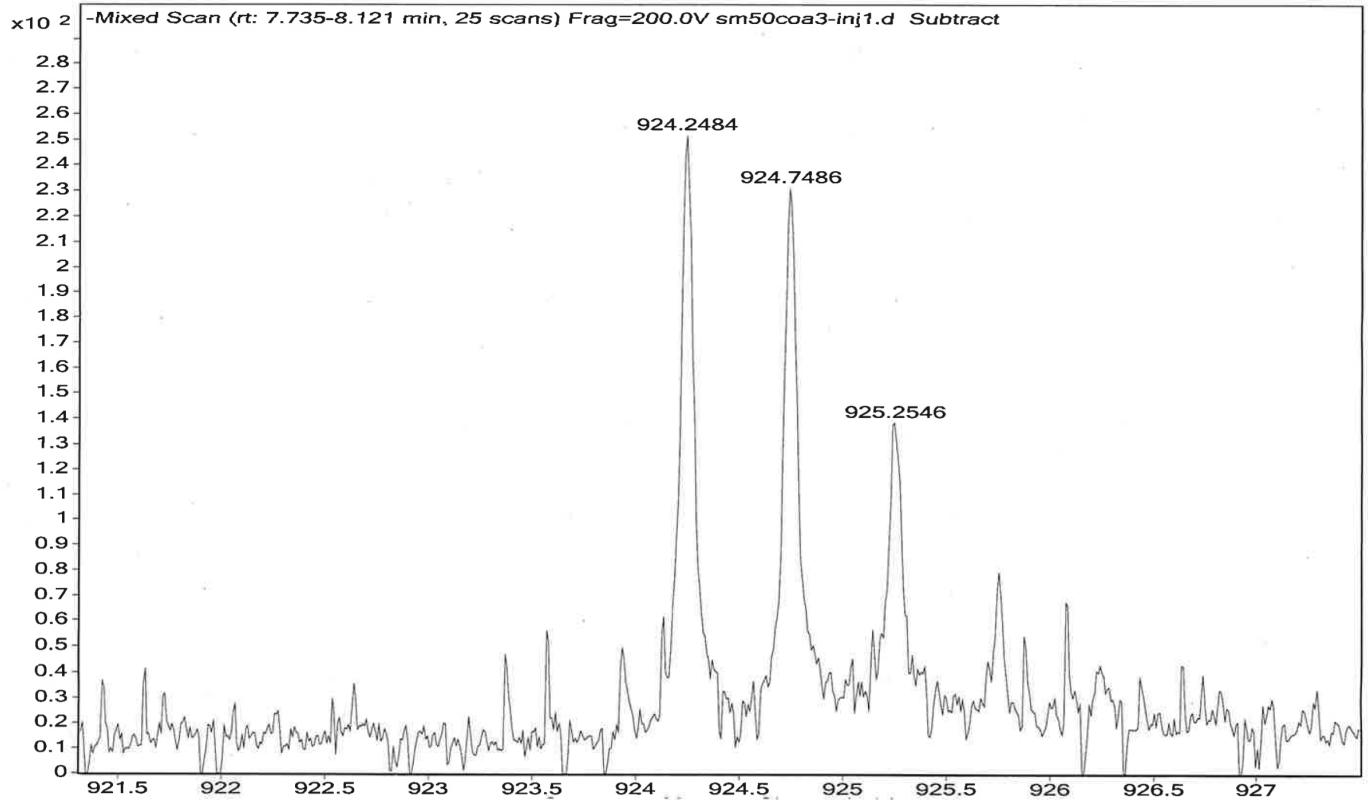
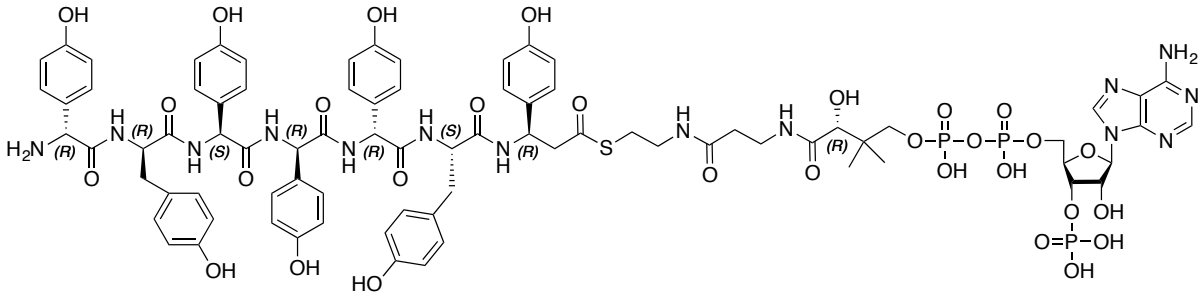
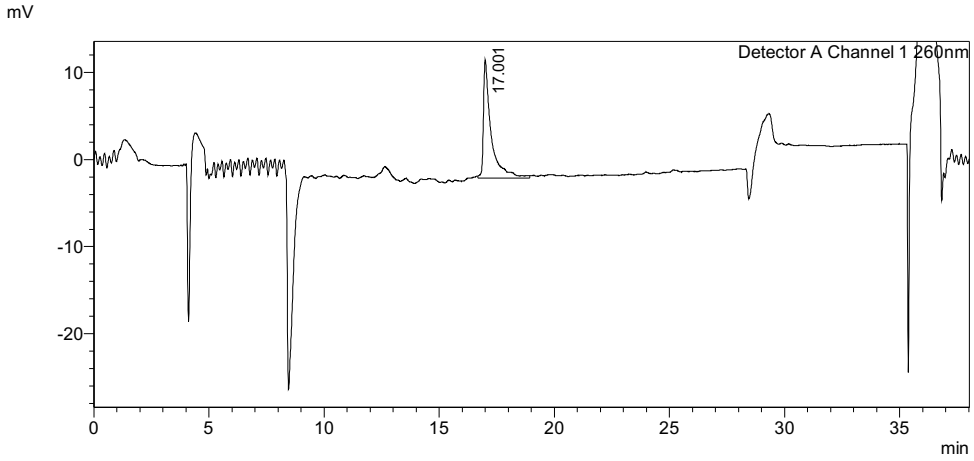


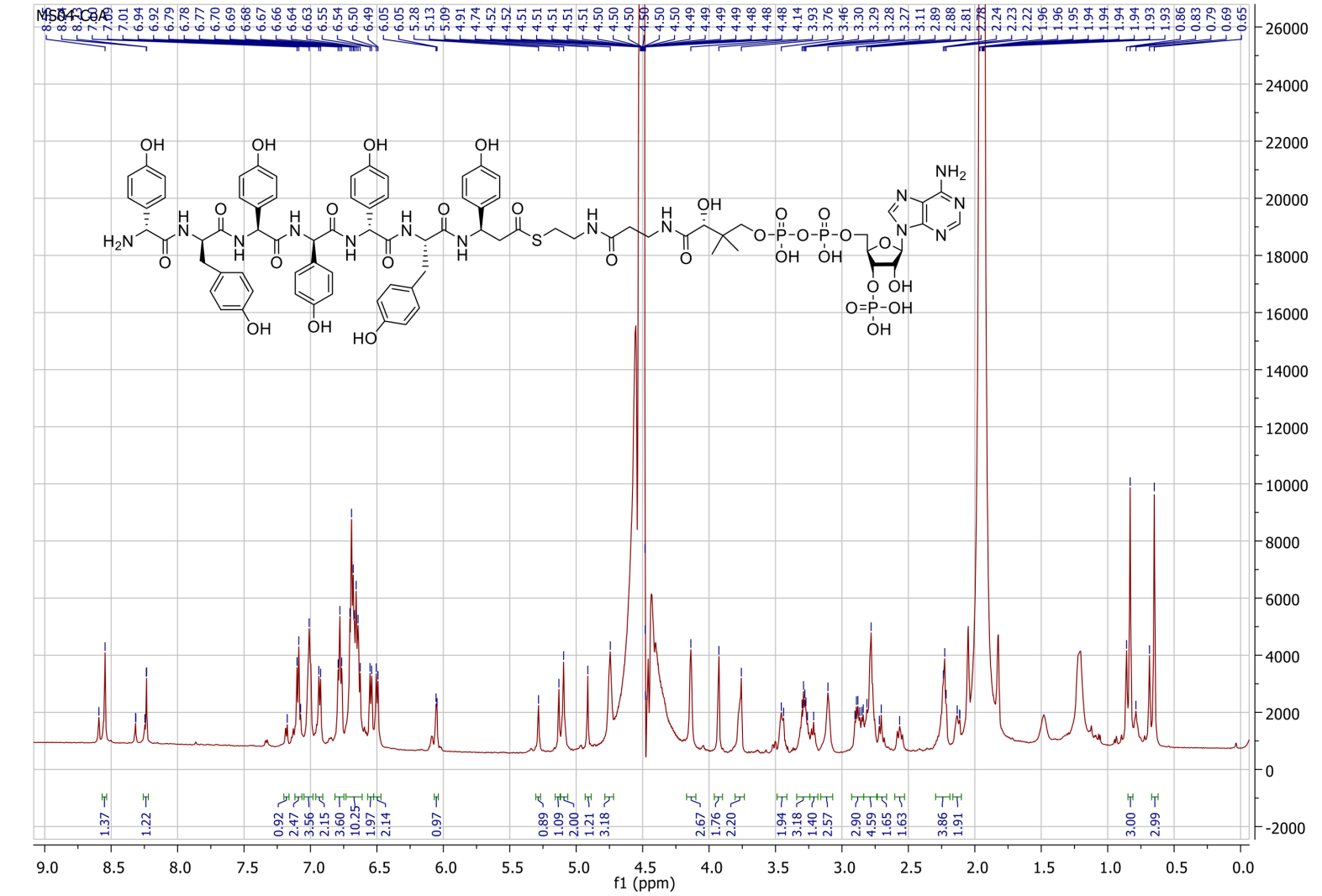
Teicoplanin-7-(β-4-Hpg)-CoA (6)



HPLC trace



^{1}H NMR spectrum



HRMS trace

Sample Name	ms-04Coa	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
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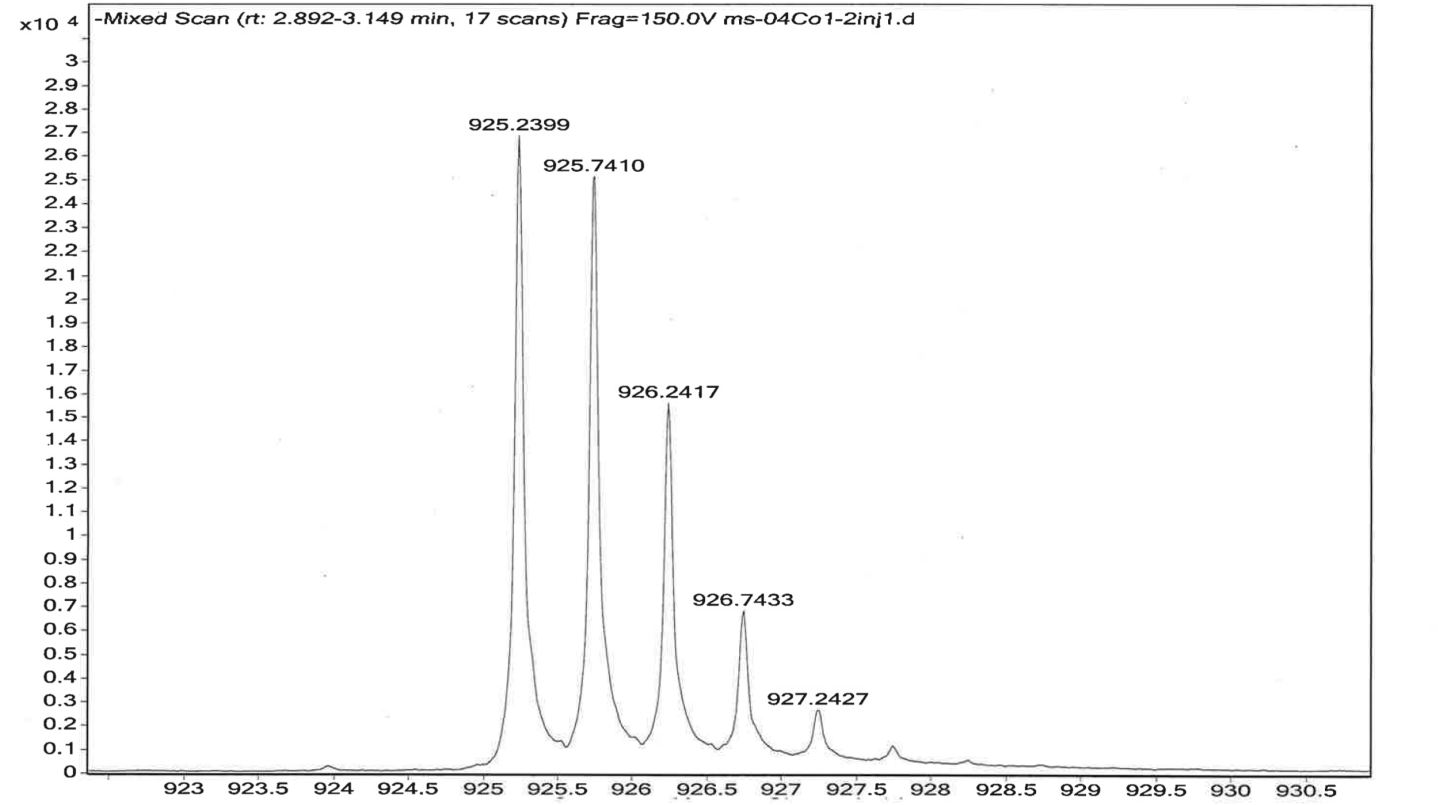
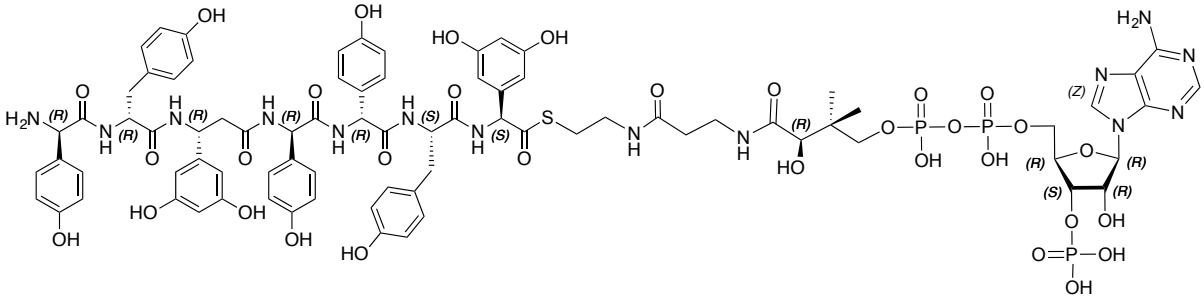
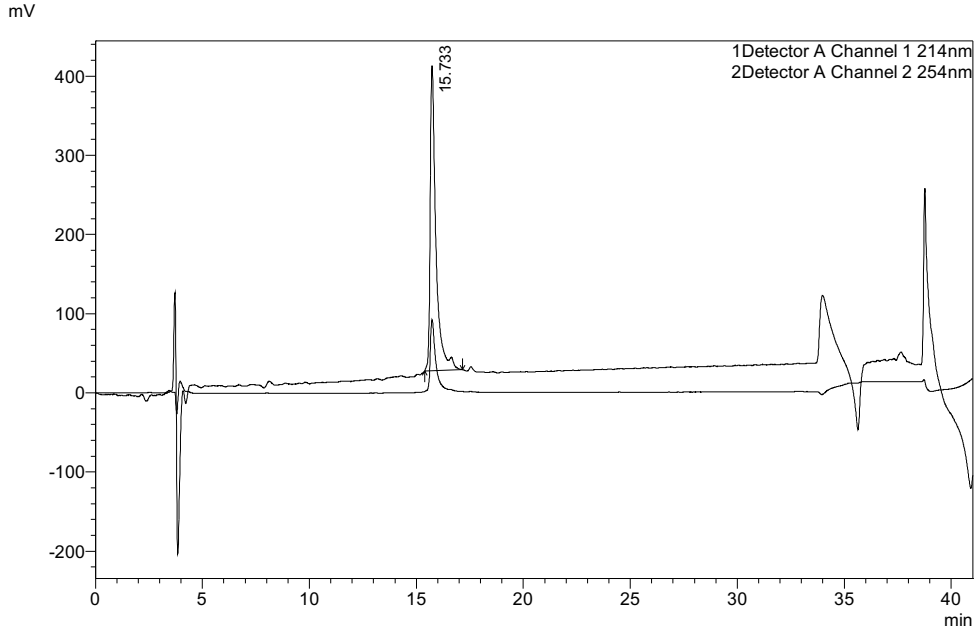


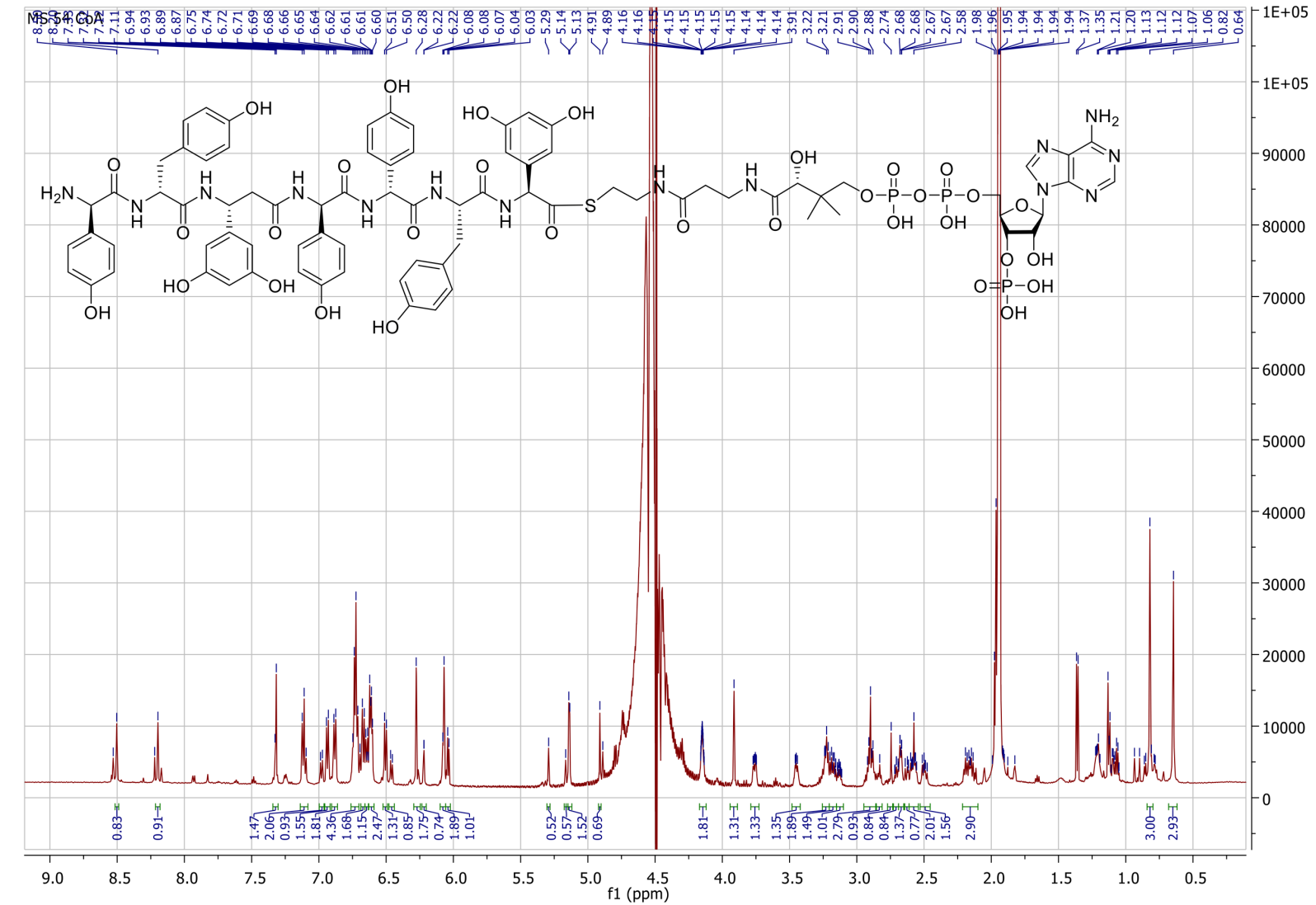
Actinoidin-3-(β-3,5-Dpg)-CoA (7)



HPLC trace



^{1}H NMR spectrum



HRMS trace

Sample Name	ms-54	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
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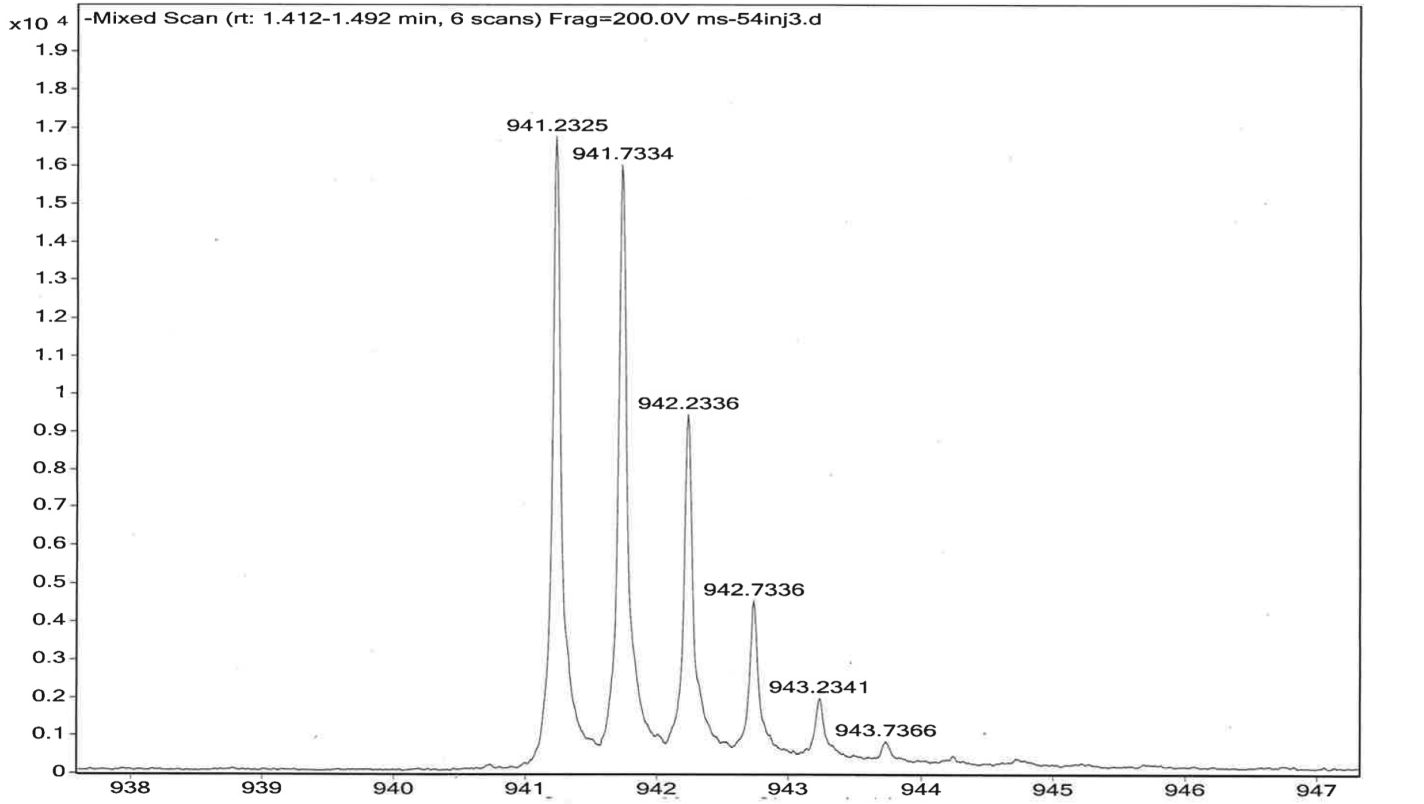

IV. Calculation of turnover results

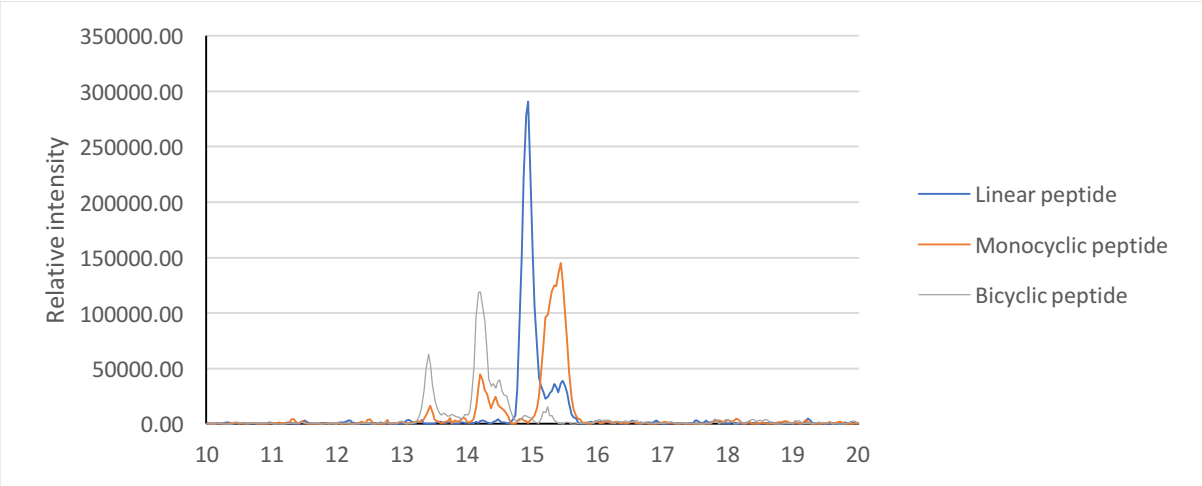

Figure S3 Example for turnover with compound **1** using $OxyB_{van}$ and $OxyA_{ris}$.

Calculation of turnover yields in percent (%)

The results are presented as percentage of specific peptide relative to the total amount of peptide that was detected. The yields were calculated as in the following example of determining the yield of monocyclic peptide:

$(\Sigma_{monocyclic})/(\Sigma_{linear}+\Sigma_{monocyclic}+\Sigma_{bicyclic})*100$.

Thus, OxyA activity is only expressed as a percentage of the total peptide, rather than as a percentage of the OxyA substrate that is the monocyclic peptides produced by OxyB.

All experiments were performed as triplicates followed by calculation of average and standard deviation.