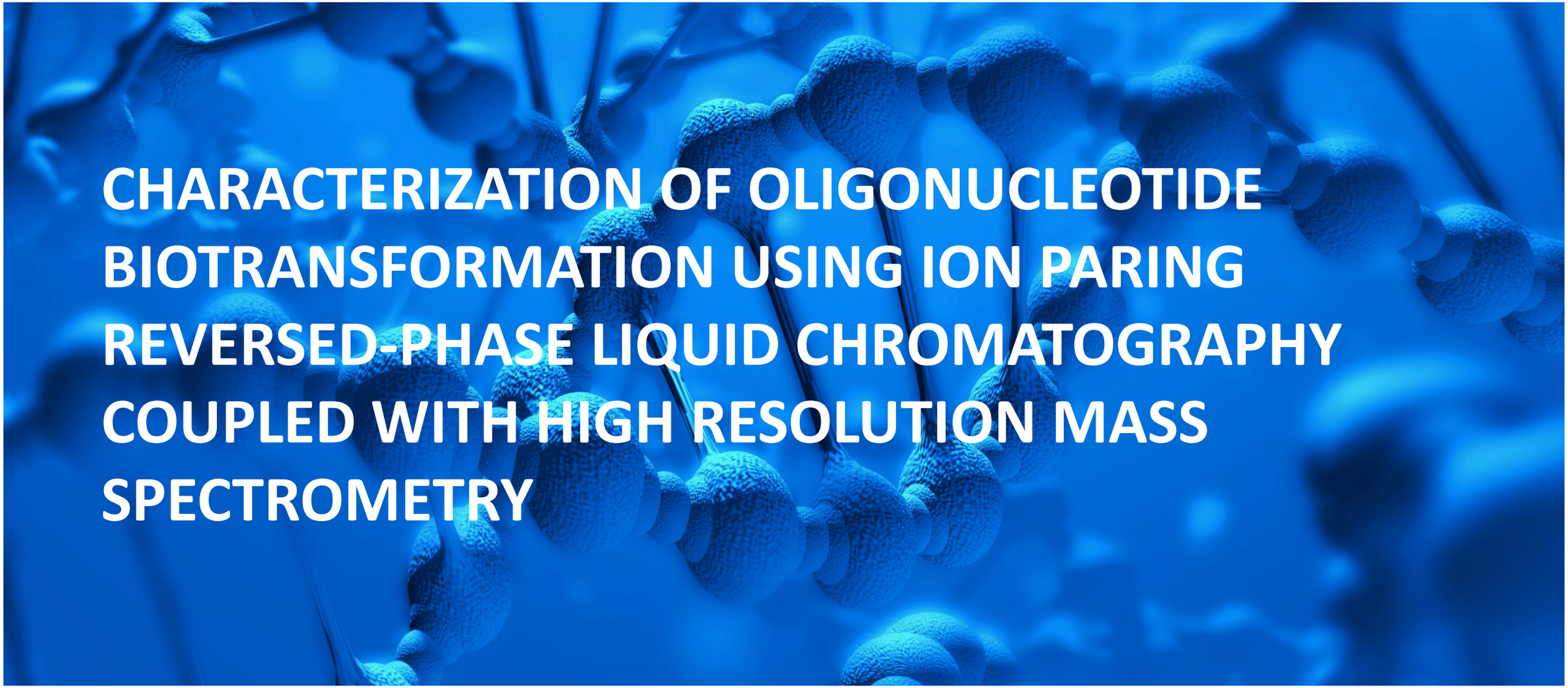


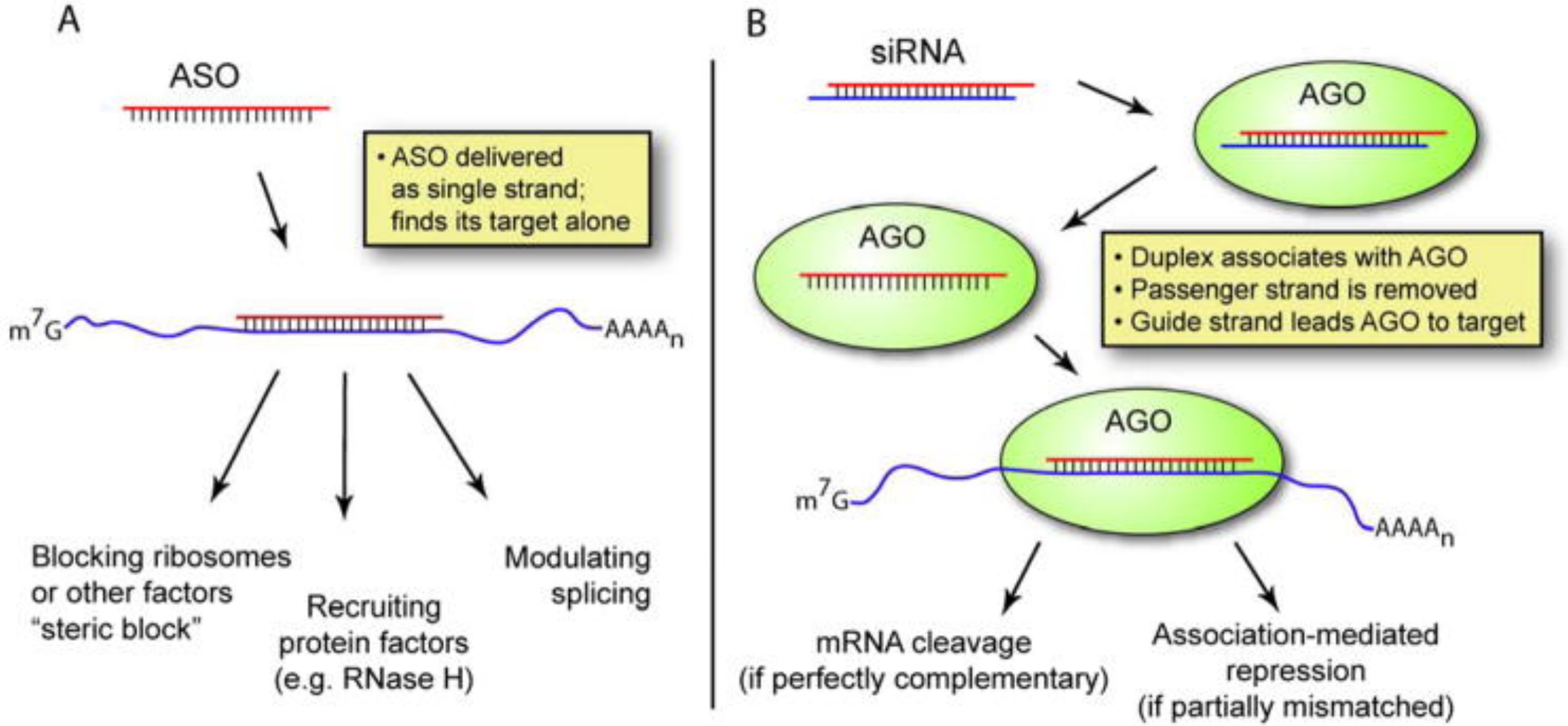
CHARACTERIZATION OF OLIGONUCLEOTIDE BIOTRANSFORMATION USING ION PARING REVERSED-PHASE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY

BABAK BASIRI

2019 STANFORD UNIVERSITY MS RESEARCH APPLICATIONS SYMPOSIUM
OCTOBER 10, 2019

AMGEN®

OLIGONUCLEOTIDE THERAPEUTICS HAVE TWO MAJOR MECHANISMS OF ACTION:



J Pathol. 2012 Jan; 226(2): 365–379.

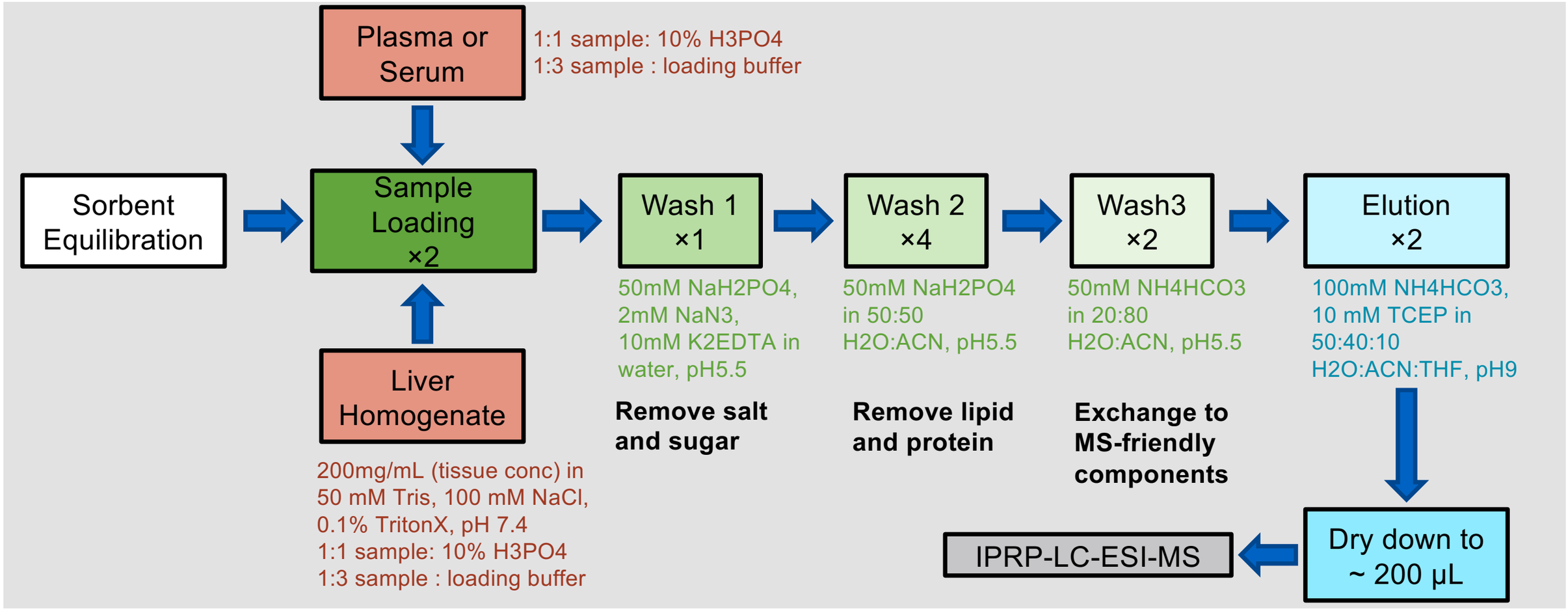
AMGEN

WORKFLOW OF LC-MS BASED siRNA MET ID

- **Incubation of siRNA in liver homogenate**
  - siRNAs were incubated in liver homogenate (200 mg/mL tissue in 100 mM Tris and 1 mM magnesium chloride in water, pH=6.0) for up to 48 hours.
- **Extraction of siRNA from biological matrices**
  - Phenomenex Clarity OTX SPE plates
  - Optimized SPE protocol
- **Ion-pairing RP LC**
  - Waters Oligonucleotide BEH C_{18} (130 Å, 1.7 µm, 2.1x50mm), 80°C
  - 15 mM TEA, 400 mM HFIP in H_2O (Solvent A) or MeOH (Solvent B)
- **MS**
  - Negative mode (-3000V), full scan MS, 60K resolution

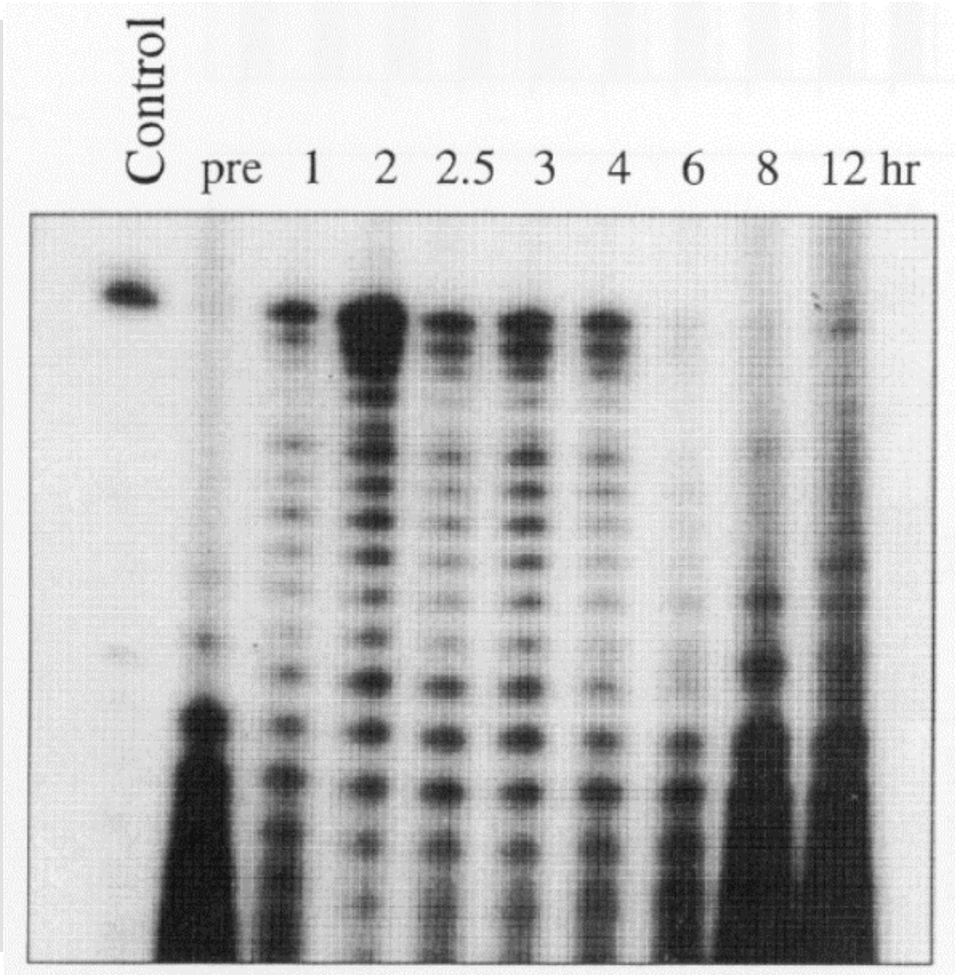
AMGEN

OPTIMIZED SPE PROTOCOL



AMGEN

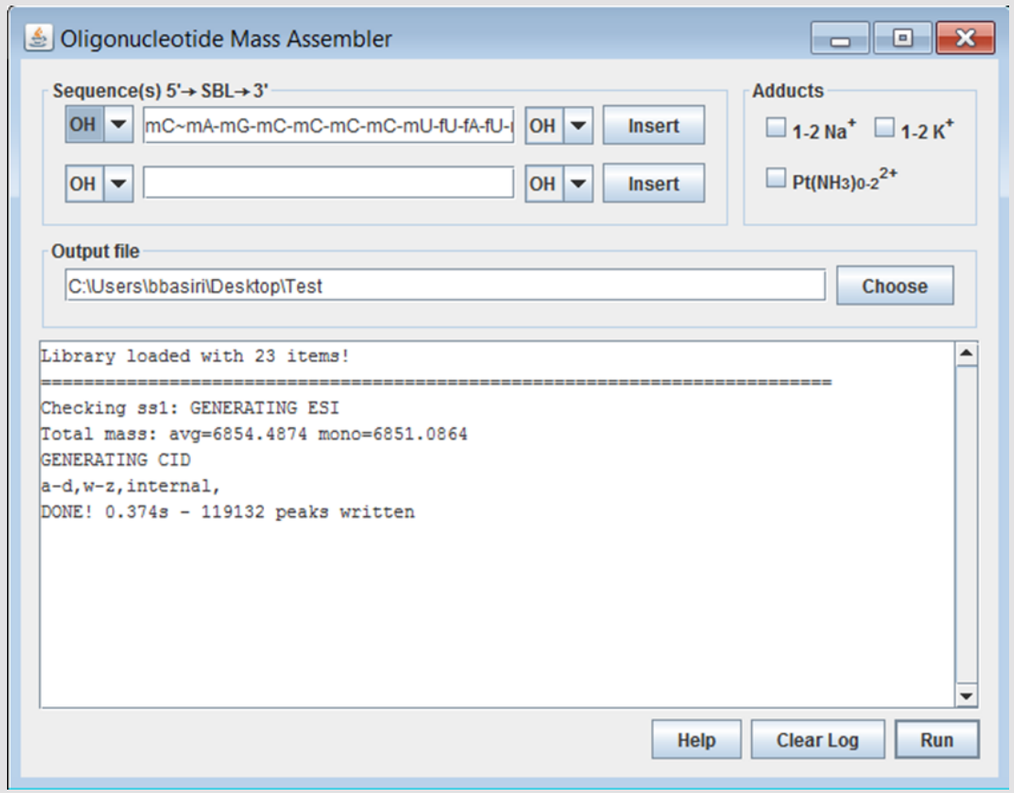
THE BIOTRANSFORMATION OF OLIGONUCLEOTIDE DRUGS IS PRIMARILY MEDIATED BY NUCLEASES:

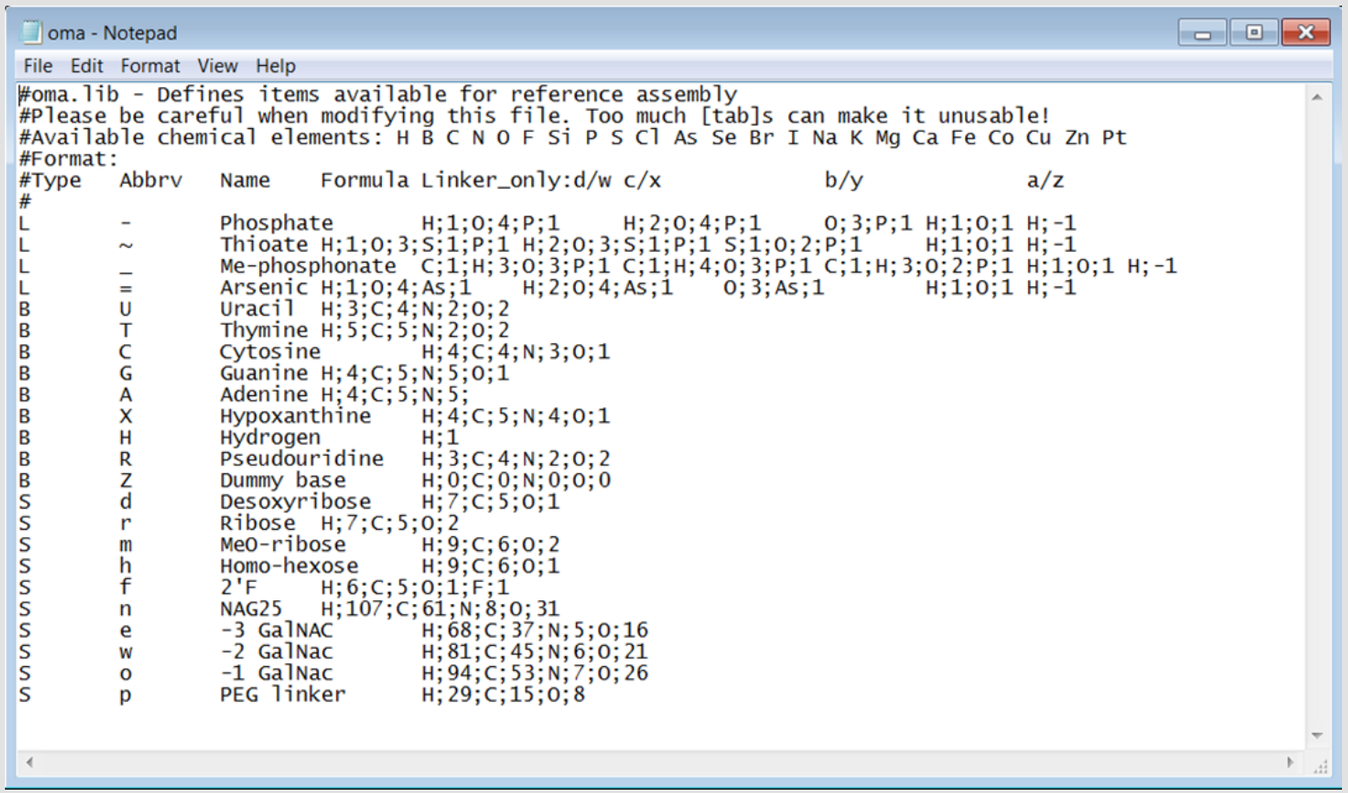


Antisense Nucleic Acid Drug Dev 8: 43-52 (1998)

AMGEN

OMA AND OPA SOFTWARE PACKAGE

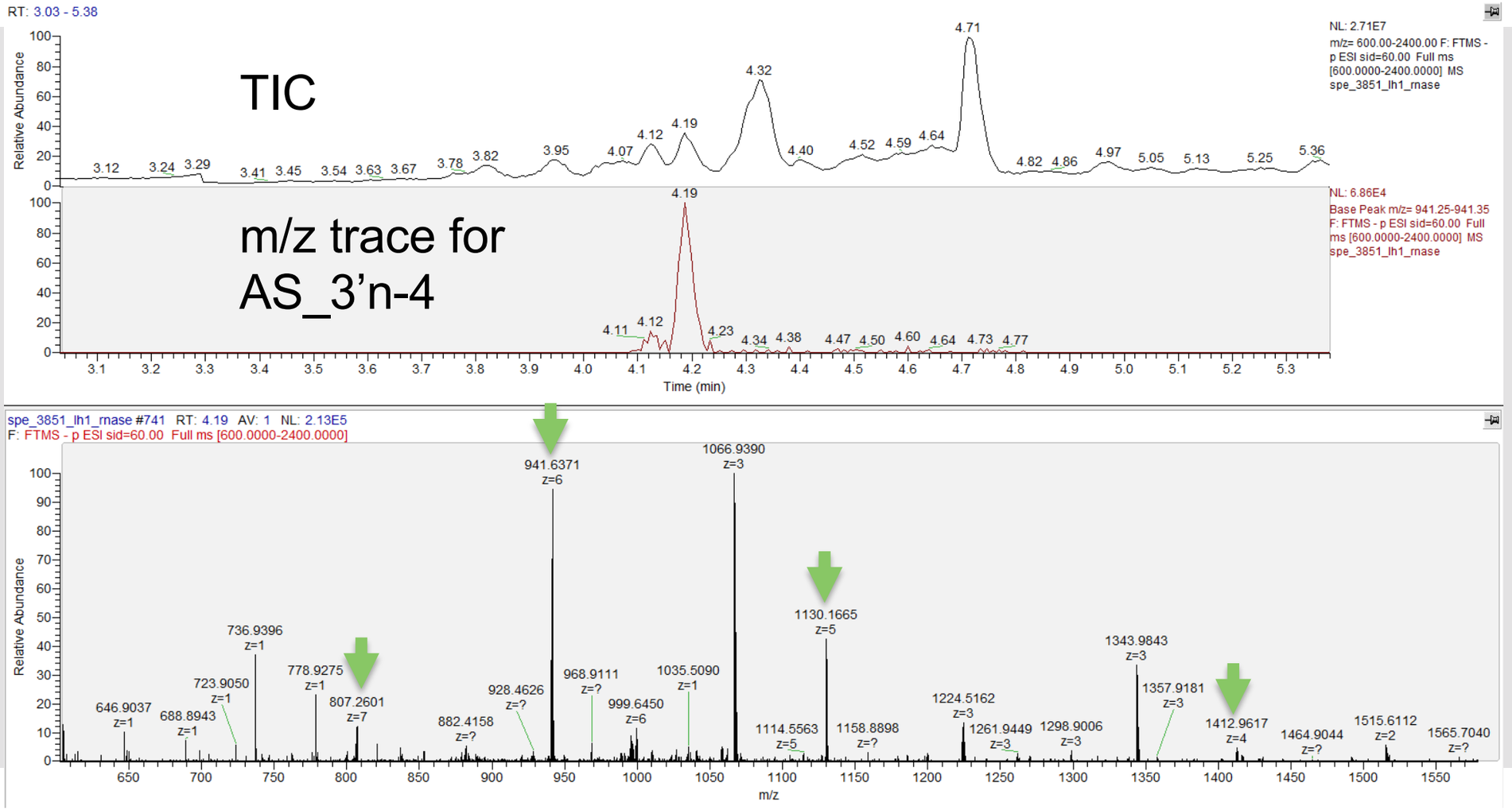


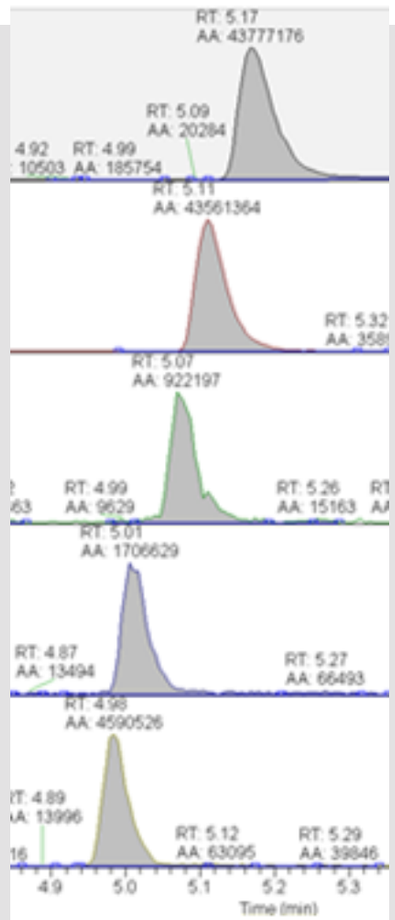


JASMS (2013) 24: 249-256

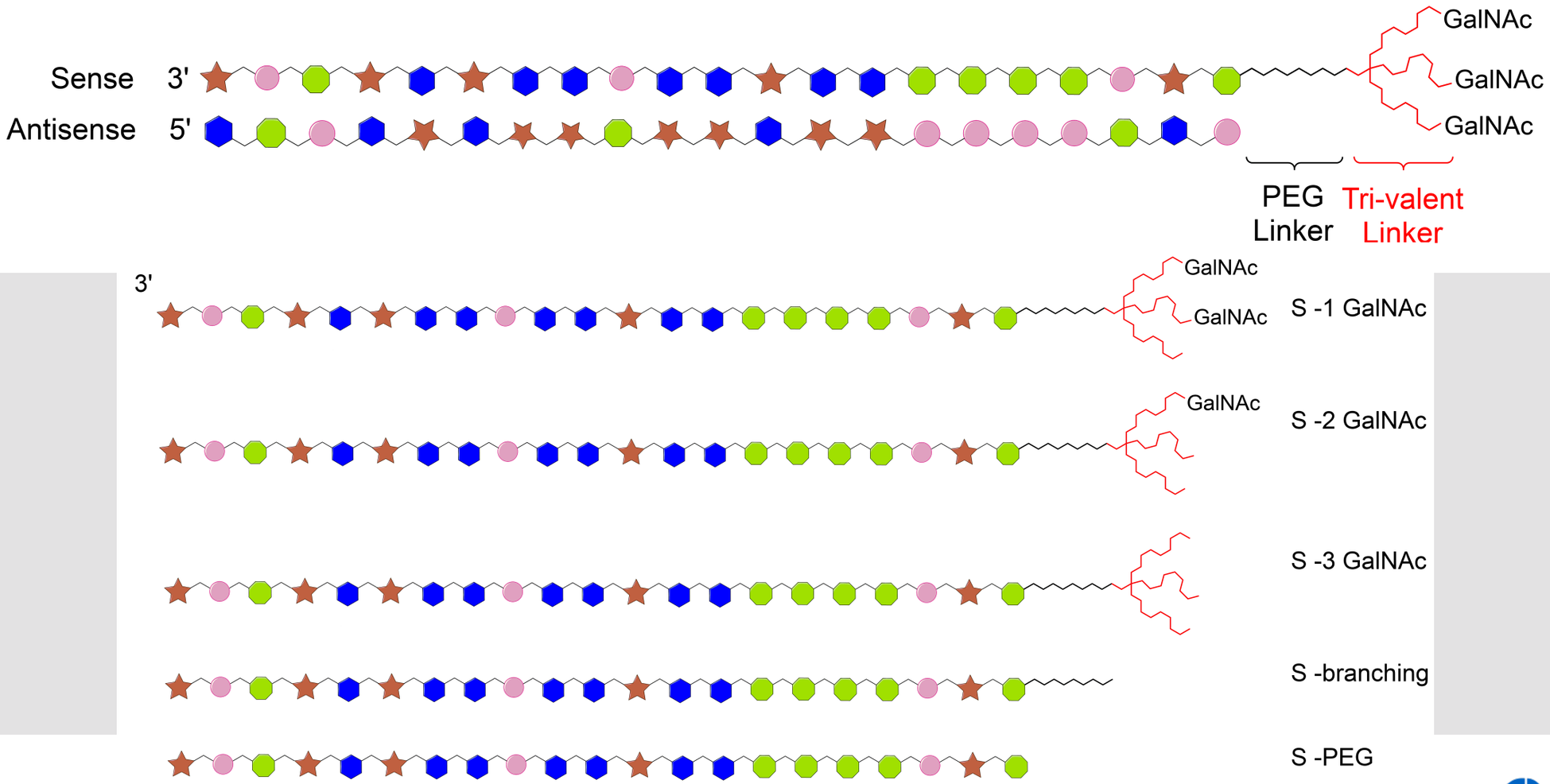
AMGEN

METABOLITES CAN BE CONFIDENTLY ANNOTATED FROM THE FULL-SCAN MS

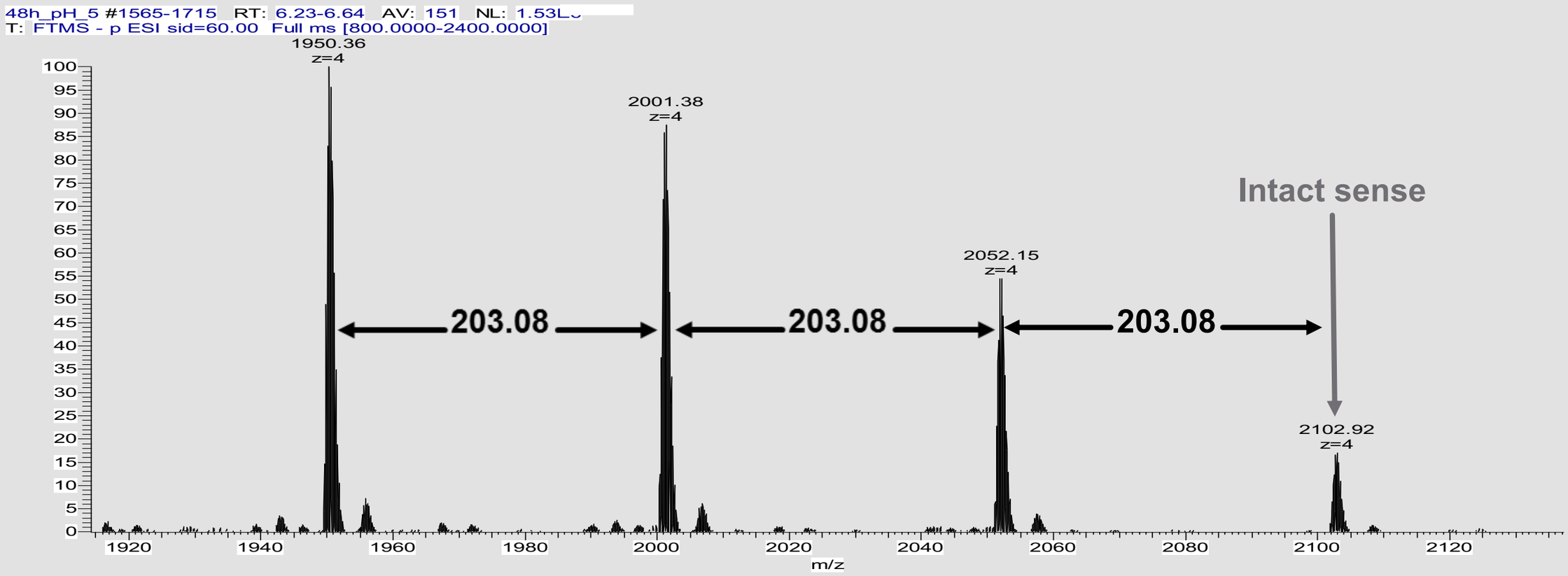




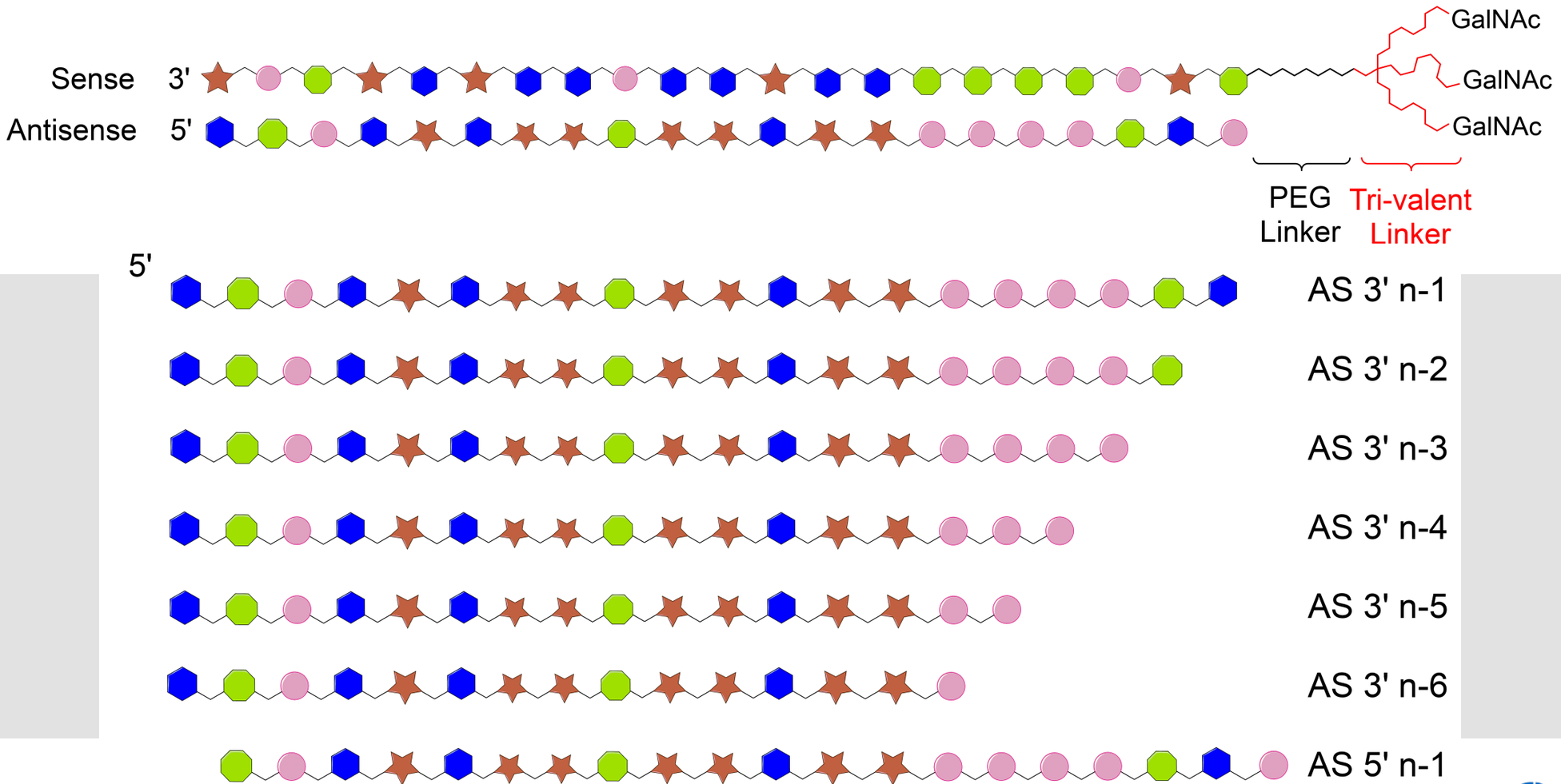
SENSE (PASSENGER) STRAND METABOLITES



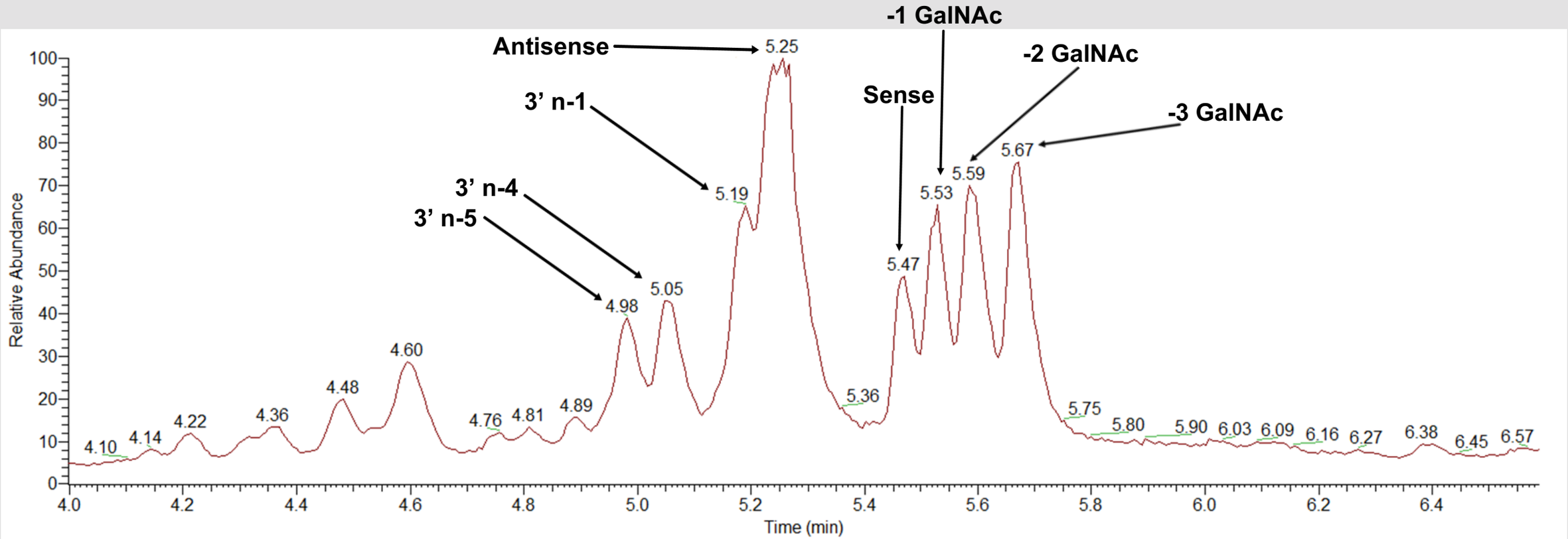
SENSE STRAND IS MAINLY METABOLIZED VIA THE SUCCESSIVE REMOVAL OF ITS *GalNAc* RESIDUES *in vitro*



ANTISENSE (GUIDE) STRAND METABOLITES

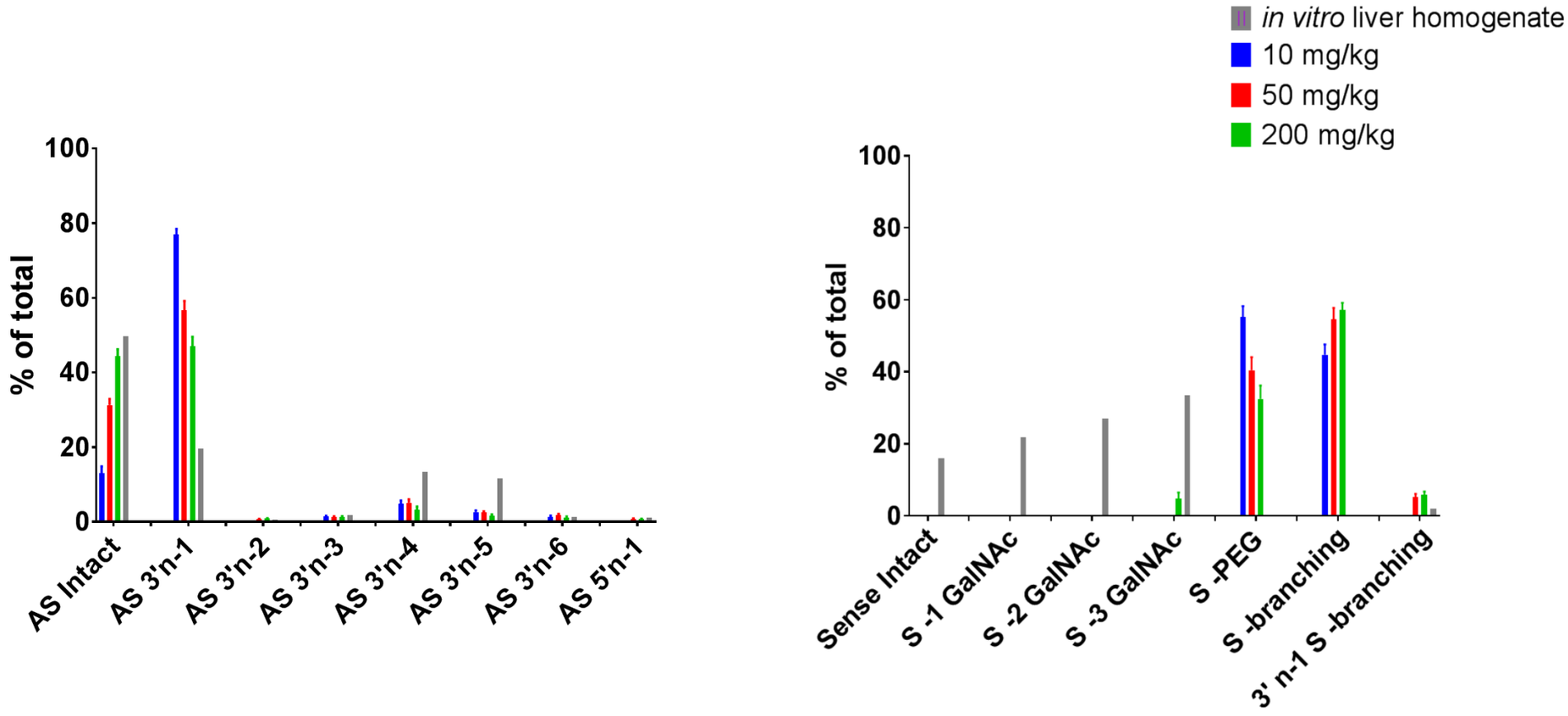


REPRESENTATIVE CHROMATOGRAM



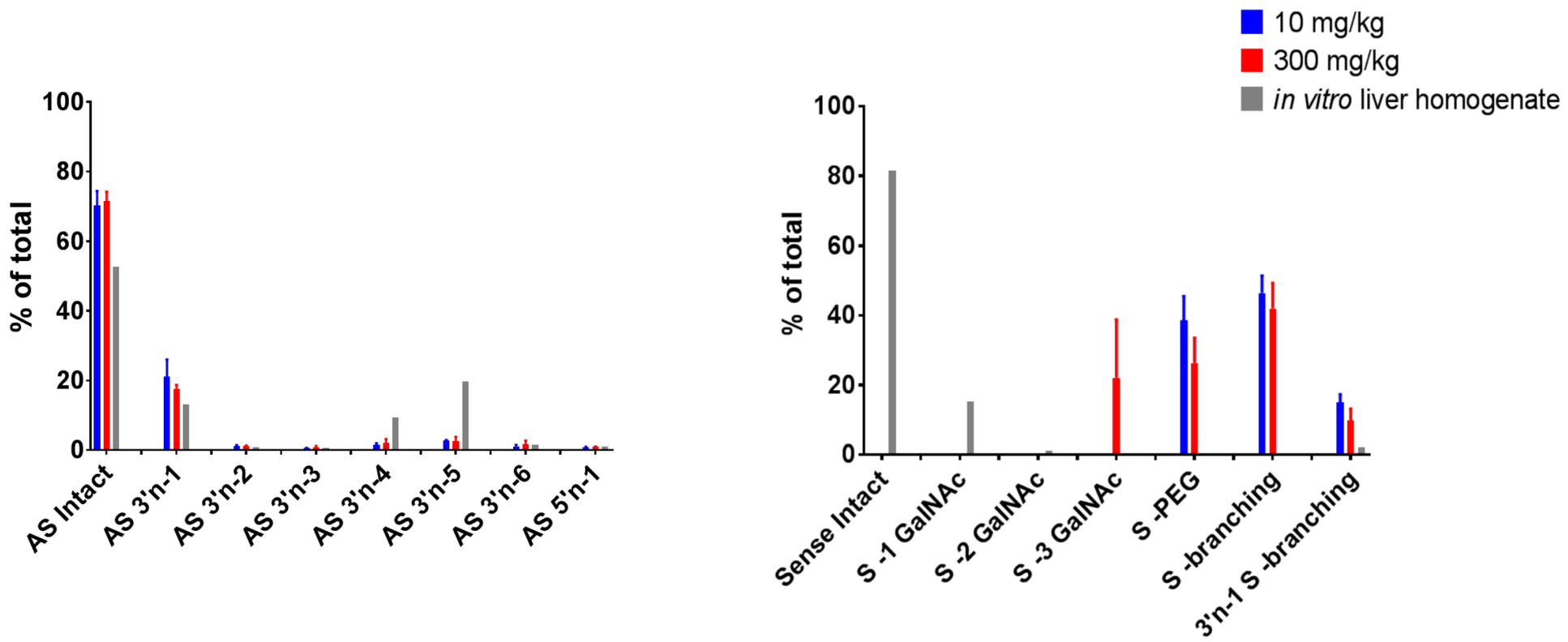
IN VITRO/IN VIVO CORRELATION

a) Rat



IN VITRO/IN VIVO CORRELATION

b) NHP



ACKNOWLEDGEMENTS

- Fang Xie
- Jianxia Shi
- Julie Lade
- Sara Humphreys
- Mai Thayer
- David Doherty
- Thuy Tran
- Li Li
- Mei Han
- Bin Wu
- Omar Barnaby
- John Harrold
- Brooke Rock
- Dan Rock

AMGEN