How good is ASV*?

Theory and Practice

Richard Lewis
ACD User Meeting 2014

***Automated Structure Verification**

AstraZeneca

Outline

Introduction

- What is automated structure verification?
- Why do we need **NMR** for ASV?

ASV performance in Theory

- Distinguishing pairs of compounds: How well does it perform compared to a chemist?

ASV performance in Practice

- How well does it perform on real samples?

What does ASV by NMR do?

Compares a proposed structure with NMR data and makes a judgement whether the structure is correct

- Does anything suggest that the structure might be wrong?
  - Coupling patterns
  - Integration
  - Chemical shifts
  - Extra or missing peaks

It is not a perfect process and *not a proof of structure*

- For use when there is no particular reason to suspect that a compound might be wrong
- Probably not enough information to prove the structure
- We have always accepted the uncertainty / errors
- The chemist uses other information to guide (*e.g.* synthetic route)
- *We are trying to automate what synthetic chemists do every day*

What do we want to use ASV for?

Finding the needles in the haystack

A small fraction of content of compound collections have the wrong structure

- Unexpected synthesis products
- Wrong starting material
- Degradation
- Manual errors when registering compound

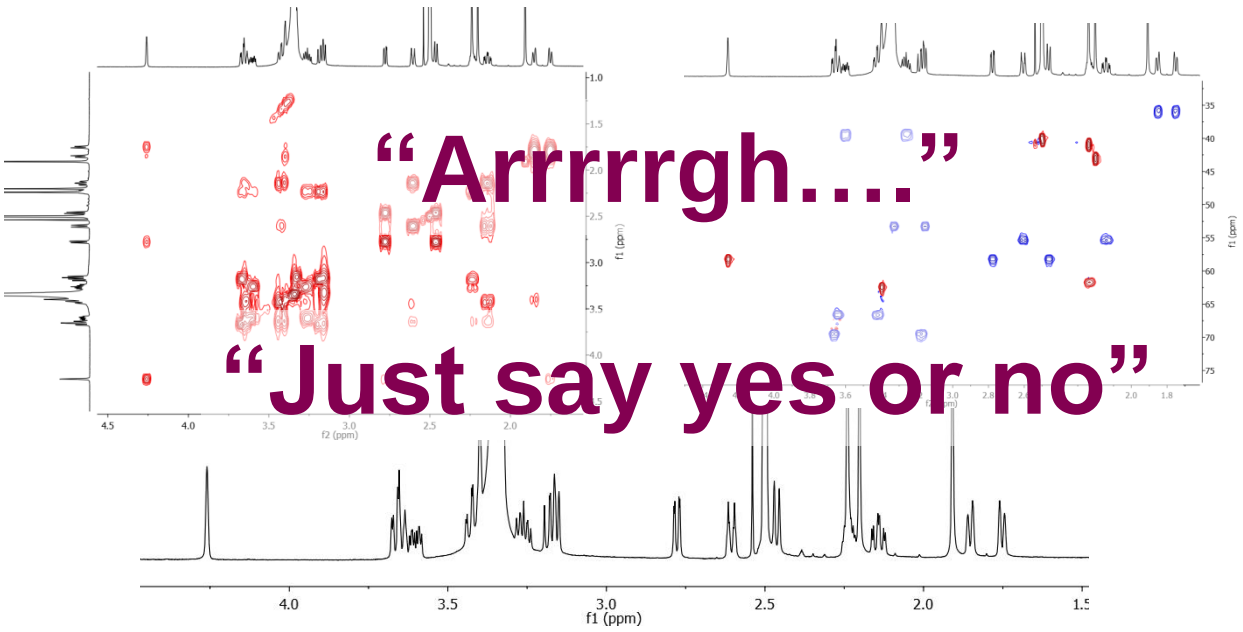
Wrong structures can lead to a lot of wasted effort

Why NMR?

Compound collections usually checked by LC/MS

- Get two numbers – mass and purity
- Easy to interpret data!

NMR is the most powerful structure elucidation technique but has been ignored for high-throughput work



Wrong compound: worst case senario

C&EN news of the week

MAY 26, 2014 EDITED BY WILLIAM G. SCHULZ & CRAIG BETTENHAUSEN

TUG-OF-WAR OVER PROMISING CANCER DRUG CANDIDATE

DRUG DISCOVERY: Structure error threatens existing patent and clinical trials

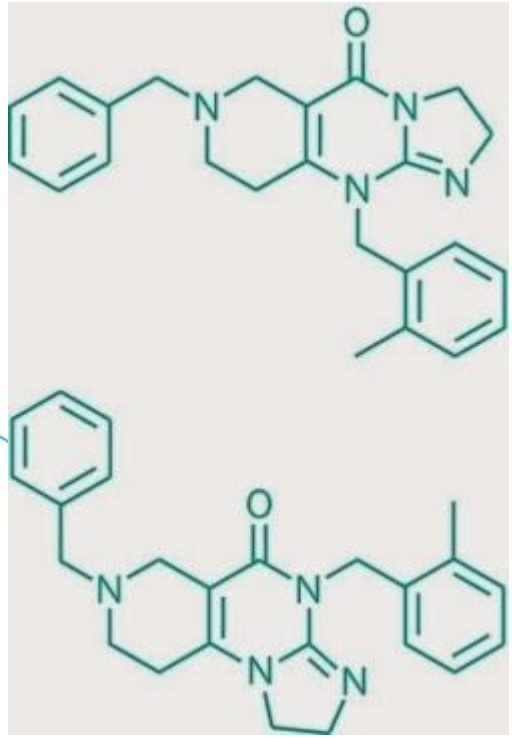
A PROMISING ANTICANCER agent about to enter human clinical trials is on the hook because of a chemical structure error discovered by scientists at Scripps Research Institute California. The patented compound, known as TIC10 or ONC201, is owned by the biotech firm Oncoceutics. However,

ture, they found it to be biologically inactive. But when they obtained TIC10 from NCI, it was bioactive. They used X-ray crystallography and total synthesis to confirm that bioactive TIC10 has a different structure than that shown in the patent (*Angew. Chem. Int. Ed.* 2014, DOI: 10.1002/anie.201402133).

Janda concluded that Oncoceutics and the other research institutions had been working on the bioactive compound but had patented the inactive structure. Scripps applied for a patent on the correct structure and licensed it exclusively to Sorrento, where Janda is a director.

When C&EN informed Jerry M. Collins, an administrator of the NCI chemical library that provided TIC10, about the misassignment problem, he said the facility's lead chemist would look into the structure and correct it if warranted.

The structures of compounds have posed



C&EN 26th May 2014

How can you Evaluate ASV Software?

Output from ASV

- Number between 0 and 1; closer to 1 equals better fit
- User defined threshold converts number to answer
  - > 0.70 = **YES**
  - < 0.70 = **NO**

How can the Software be Evaluated ?

Two Important Questions

- How good is the **Yes** / **No** answer?
- Does it help us find wrong compounds?

Not so easy answer....but we can ask

- How good is it at distinguishing between right and wrong compounds compared to a human?
- How good is it in real life?

Evaluation of ASV Software at AstraZeneca

Choice of test data

- Over 100 datasets
- Wide range of mw and structures
- Proton and HSQC data used

One false structure generated per dataset

- Incorrect structure of same mw
- Around 50% represented real problems
- Problems are probably harder than average
- Only ACD built-in shift database used

Compare scores for right and wrong compound

- To help us answer the question: "*How good is the software*?"
- Can't do this in real life
- Software "helped" to very limited extend – peak picking but **not** interpreting of spectrum

Same Problems give to a chemist

- Given both structures, decide which is right, or can't tell?

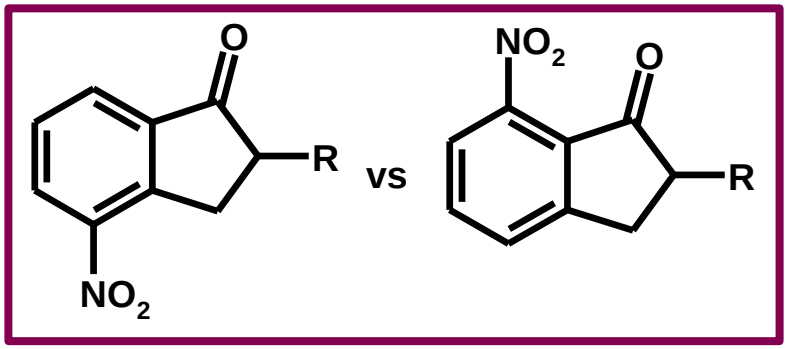
Comparing ASV to a Real Person

Our Volunteer Chemist

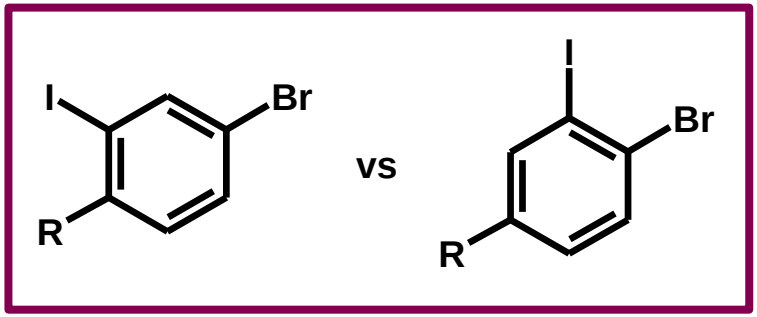
- Good NMR skills
- Not familiar with particular compounds

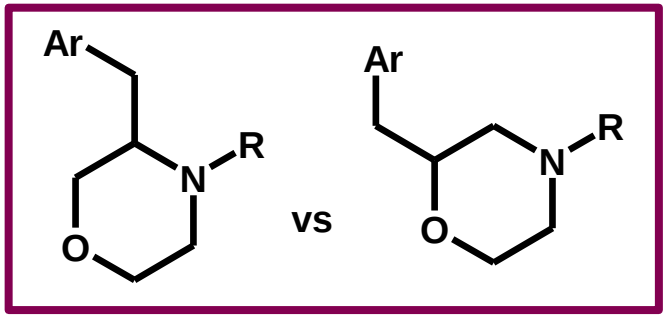
- Given proton and HSQC data, was asked to decide which of the two structures was correct.
- Can’t decide also an option
- Allowed to use chemical shift prediction, data books etc
- Emphasis on **making a decision** using available data
- Emphasis on **working quickly**

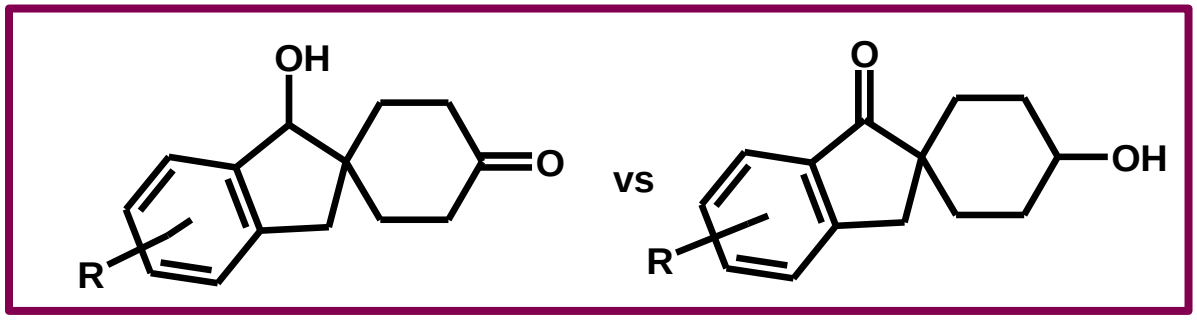
Examples of Typical Challenges



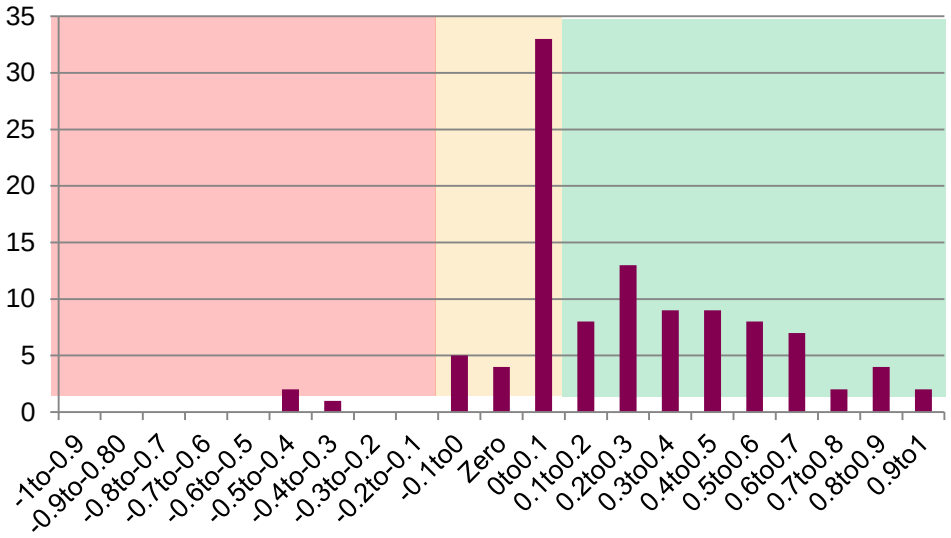
Because these are only partial structures, bear in mind that the spectra will be more complicated that they might appear.







Results – Comparison to Chemist

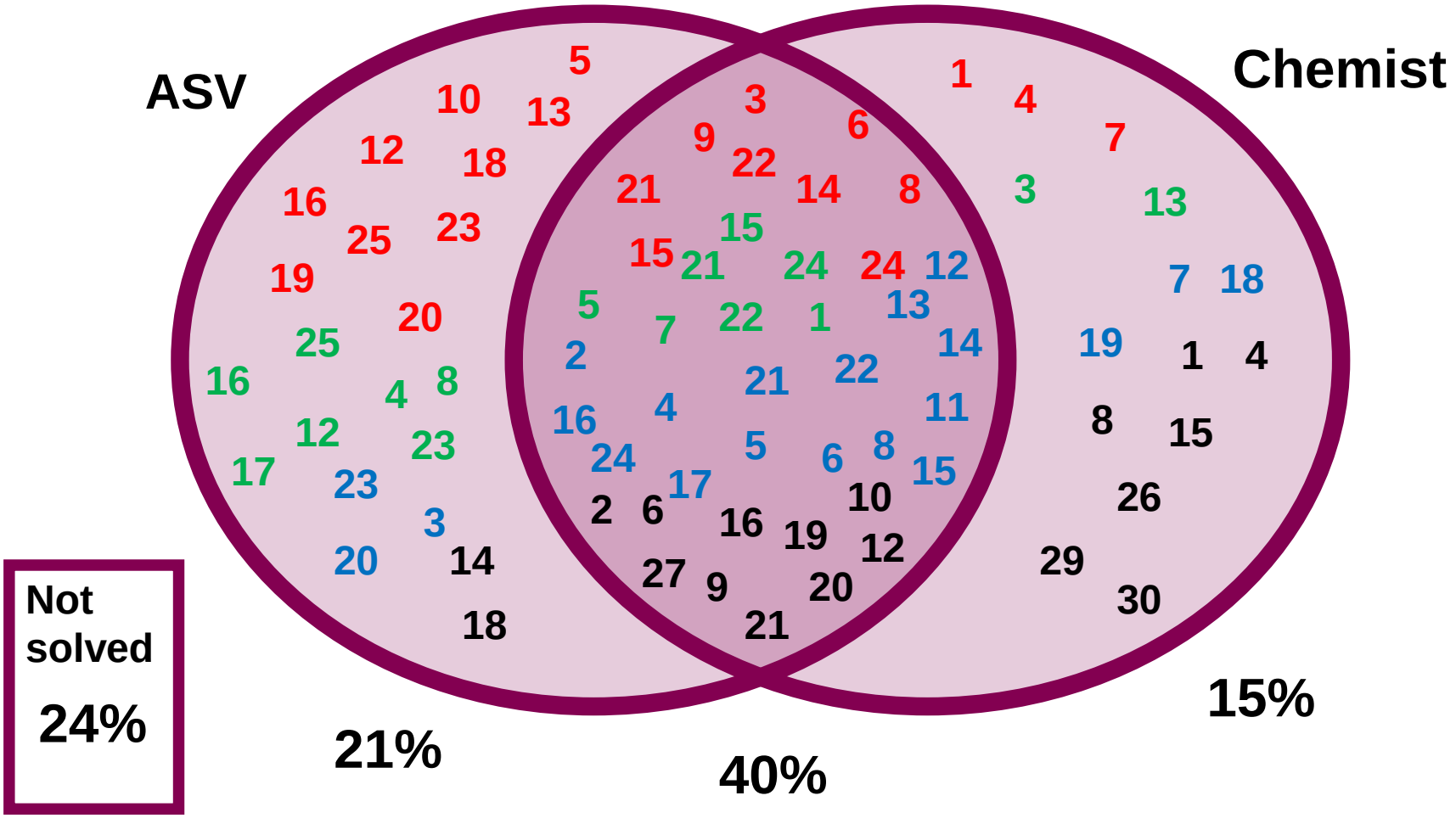


Use difference between scores of two structures

If difference <0.1 (10%) then not distinguished, otherwise correct or incorrect depending on which structure is favoured

Category	# Structures 10% diff criterion	Chemist	# Structures 4% diff criterion
Correct	63/103 (61%)	56/103 (54%)	74/103 (72%)
Incorrect	2/103 (2%)	6/103 (6%)	2/103 (2%)
Not distinguished	38/103 (37%)	41/103 (40%)	27/103 (26%)

Did ASV and the Chemist Solve the Same Problems?



Any Patterns in the Solved Compounds?

What did chemist solve when ASV failed?

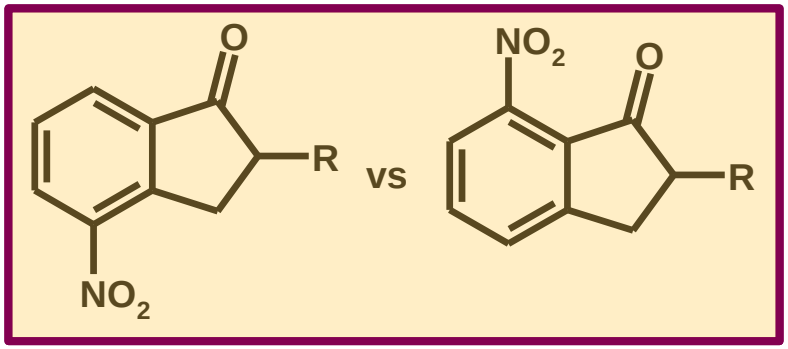
What did ASV solve when chemist failed?

Not easy to see the logic for either ASV or the chemist!

Generalisations:

- Compounds that ASV successfully distinguished, but Chemist failed to are all chemical shift related
- Compounds with which the chemist succeeded but ASV failed are a mix of chemical shift, coupling pattern and symmetry.
- Not easy to see why the chemist was happy with chemical shift distinction for some compounds but not others.

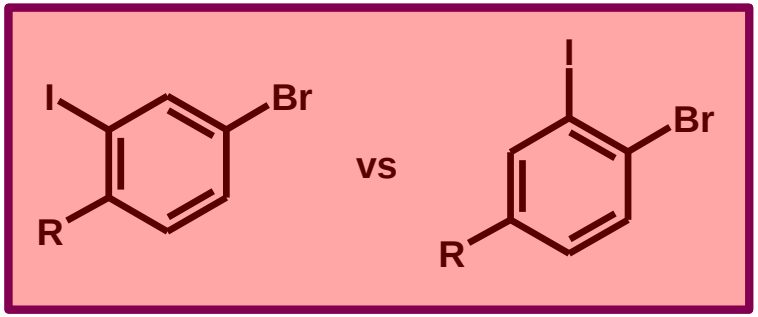
Were these Structures Solved?

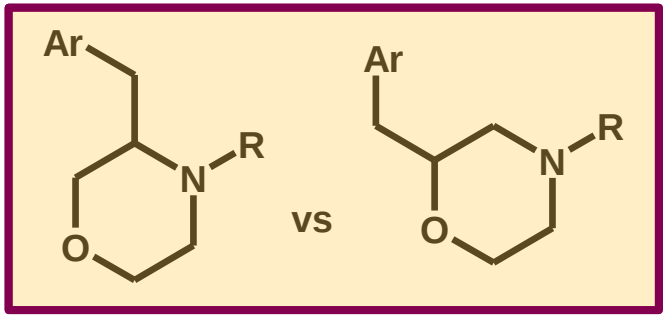


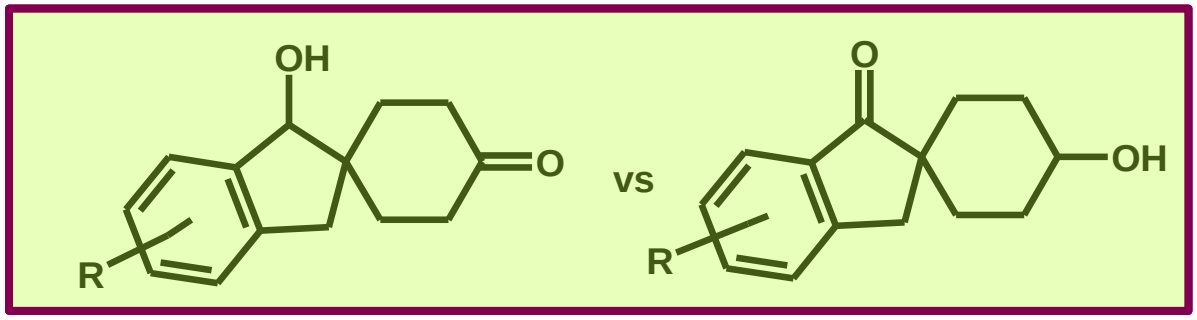
Solved by both ASV and Chemist

Solved by ASV only

Not solved







Evaluating ASV in Practice

Preliminary results

Currently evaluating using compounds which have been purified

- Purified on the basis of mass – so should be right.....or an isomer
- How many false negatives do we get?
- Do we find wrong Compounds?
- Also checking historical “wrong compounds” to see if they would have been discovered

False negatives

- Like a piece of straw attracted to the magnet
- Because there is so much “straw” even a low rate of false negatives can be a problem

ASV in Practice – False Negatives

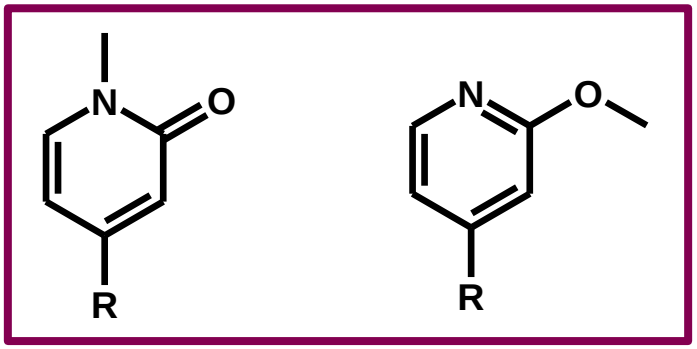
False negative rate too high

Reasons for ASV score < 0.7

- Failure to pick peaks in 1D or 2D spectrum
- Failure to correctly interpret 1D or 2D spectrum – eg multiplicity
- Compound shows rotamers in NMR
- Poor spectrum quality – eg broad lines, hidden or weak signals
- Unexpected / unusual chemical shifts
- Wrong compound

ASV in Practice – Wrong Compounds I

Two of the wrong compounds that ASV has found.
To be fair, the chemists also spotted them



Should be
Score 0.08
Fails

Actually
Score 0.90
Passes

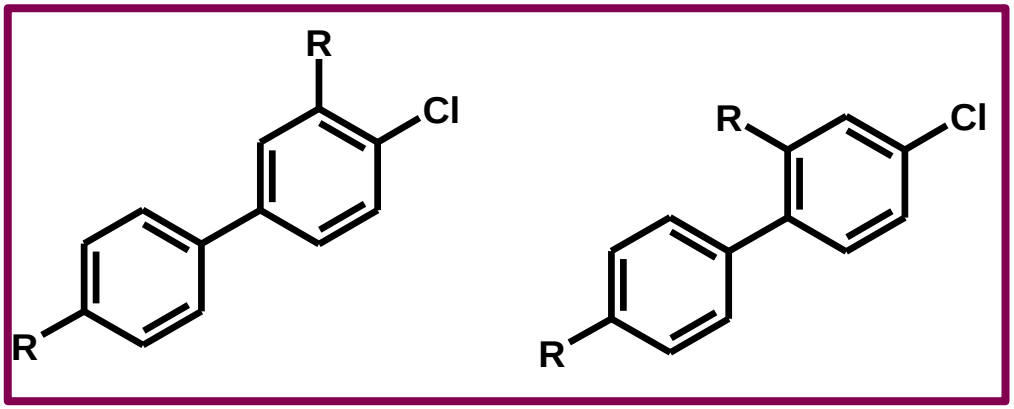
Should be
Score 0.19
Fails

Actually
Score 0.94
Passes

Probably the wrong starting material was supplied or bottle incorrectly labelled

ASV in Practice – Wrong Compounds II

A historical wrong compound that ASV *might* find



Should be
Score 0.87
Passes

Actually
Score 0.92
Passes

- Both pass the 0.70 threshold, but the correct compound passes with a better score
- Earlier work suggests that a difference in score of 0.05 *is* significant
- The correct structure can be generated automatically by the “concurrent verification” procedure.

Musings on Concurrent Verification

What is concurrent verification

- A procedure that generates *some* isomers of the proposed structure and scores them alongside the proposed structure
- Gives some confidence about how discriminating the score is.

Potential of concurrent verification

- If your structure is wrong, and you are unlucky, it might still pass the threshold
- But if you are lucky the right structure might be generated by concurrent verification
- Our earlier work shows that there is a very good chance that the correct structure will have a higher score than the wrong one
- If so, you are not reliant on correctly picking a rather arbitrary threshold (eg 0.70)
- A difference in score is a lot more revealing than an absolute value

If concurrent verification has generated all possible (reasonable) answers, including the correct structure, then you can use Bayesian statistics to give a probability that the structure is correct

Conclusions and Questions

The intrinsic ability of ASV seems as good or perhaps better than a chemist.

The false negative rate is high, but improving

We can find wrong compounds

Is ASV best suited as a stand-alone application or as a tool for the chemist?

- Chemical intuition of a chemist is hard to replicate
- Abilities seem complimentary

Can we build chemical understanding into ASV?

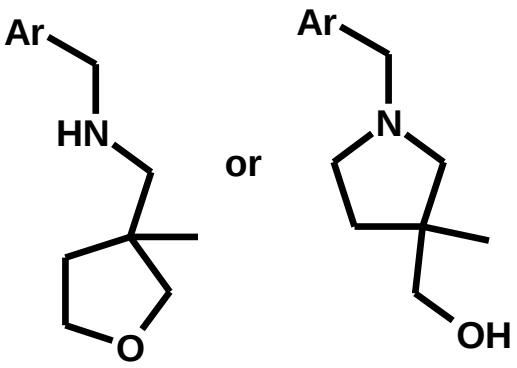
- Is this a good idea?

Acknowledgements

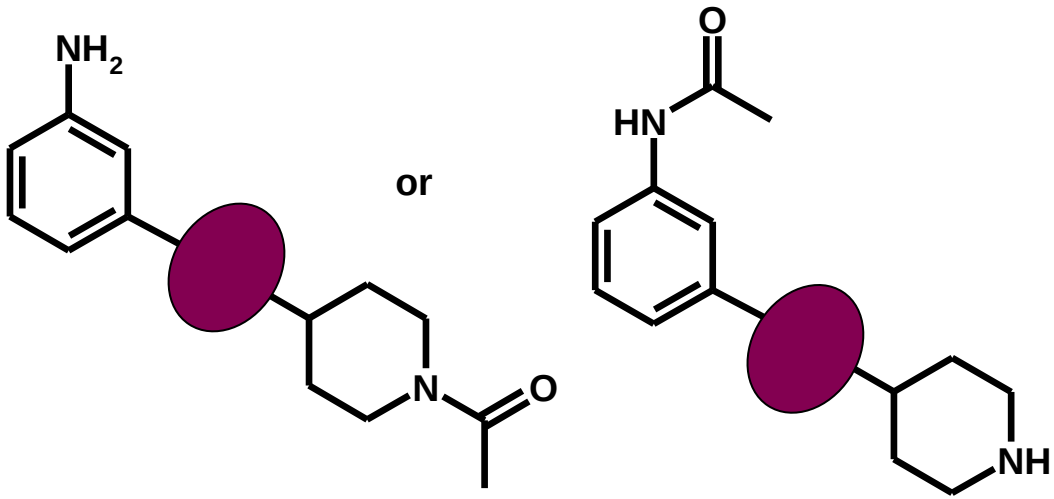
Alexandra Bernlind
Sarah-Kate Cantlie
Steve Coombes
Gunnar Grönberg
Jesse Laurila
Tineke Papavoine
Andy Phillips

Albert Van Wyk (ACD)
Ryan Sasaki (ACD)
Patrick Wheeler (ACD)

Problems Solved by ASV but not by Chemist



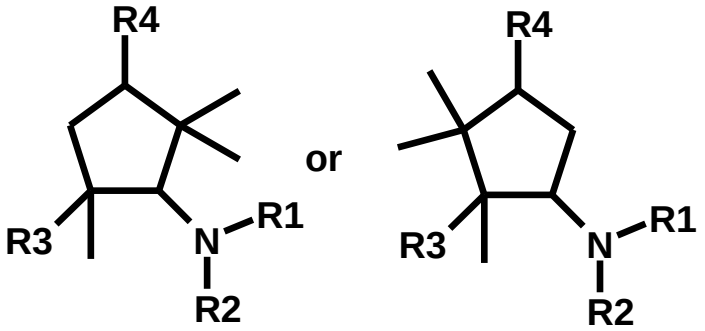
Solvable, but all methylene groups show AB patterns and come at similar shifts. Need confidence in assignments to distinguish and experience interpreting HSQC

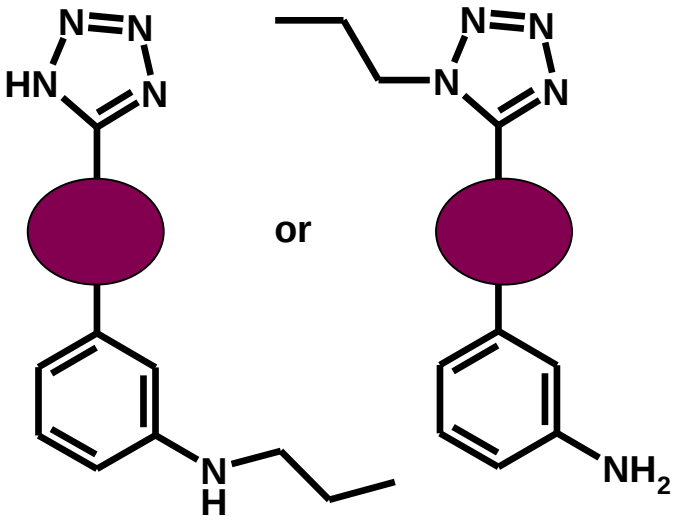


Changes in proton shift caused by the anisotropy of the carbonyl. We teach chemists not to rely on proton shifts

Problems Solved by Chemist but not by ASV

Chemist spotted that multiplicity of proton on carbon next to N is key. ASV passed both with no difference in scores





Chemist identified multiplicity and integral of NH group. ASV failed both with no difference in scores