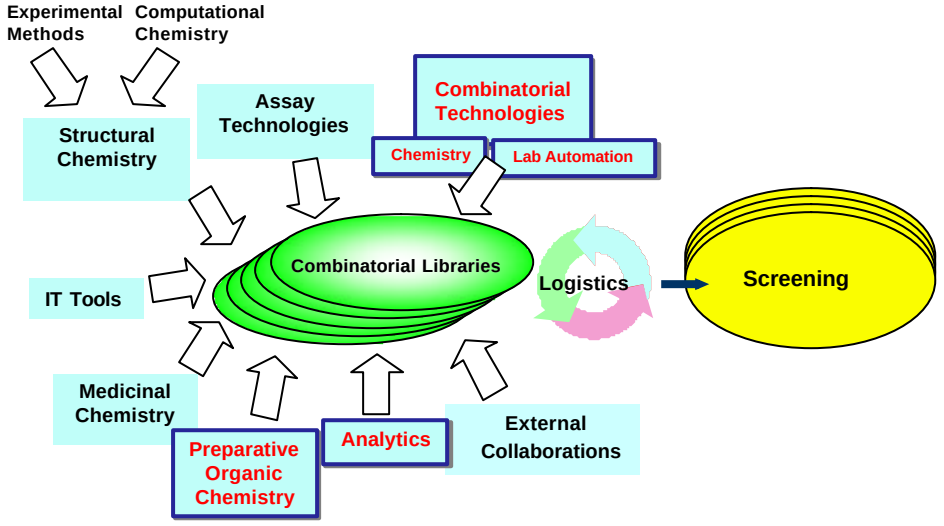
High-throughput synthesis on solid phase

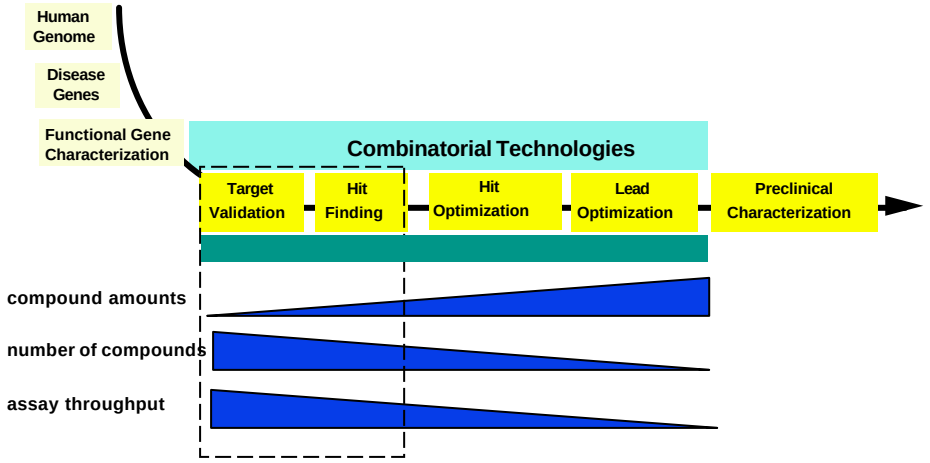
Towards rigorous quality standards and quantitative characterization without compromising efficiency

Eduard Felder *et al.*
Dept. of Chemistry - Discovery Research - Pharmacia Italia

Network of Activities



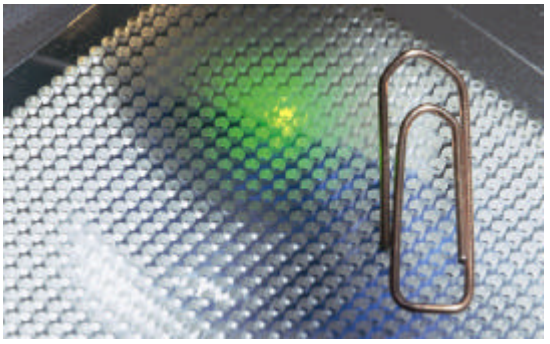
Drug discovery process



Provide high-quality compounds / high-quality data

Assay Sensitivity and Throughput

UHTS Plate:	> 2000 wells / plate
Assay Volume:	**1-2 µl**
Microtiter Plate:	96 wells/ plate
Assay Volume:	200µl



Process Development Areas

LEAD FINDING | **LEAD OPTIMIZATION**

Hit Generation | Hit Optimization | Parallel Medicinal Chemistry

Avenues:

- 'Brute Force'
  - cmpd # - - (combinatorial)
  - screening data - - (Ultra-HTS)
- Screening by NMR (novel scaffolds)
- Combinatorial target-guided ligand assembly

Technical Development Areas:

Synthesis ® Automation

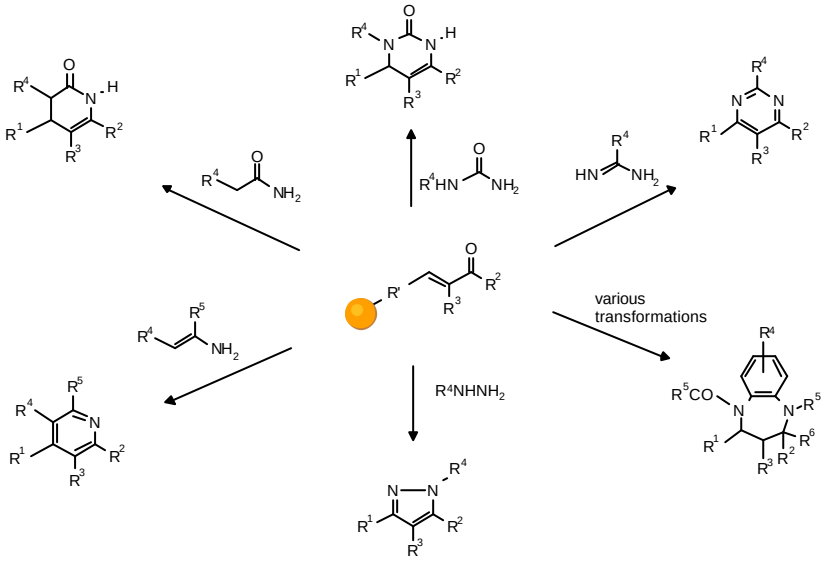
Separations ® High resolution / throughput
MS-directed fraction collection
Logistics

Analytics ® Process control analytics in 'new formats':
- on solid phase: IR / NMR
- in microtiterplates: LC-MS; NMR, quant. N-detection

Chemistry Progress: Solid Phase

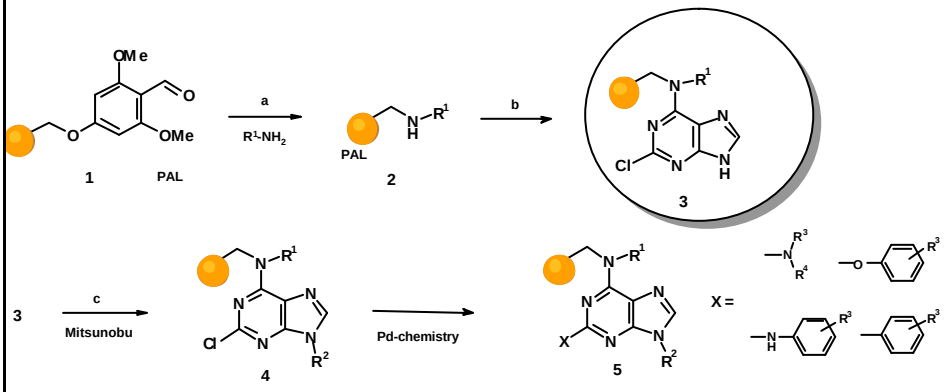
Diversity Platform

NOVARTIS



A.L. Marzinzik and E.R. Felder, J. Org. Chem. 63, 723 (1998).

Derivatizing solid supported core scaffolds with common reactions

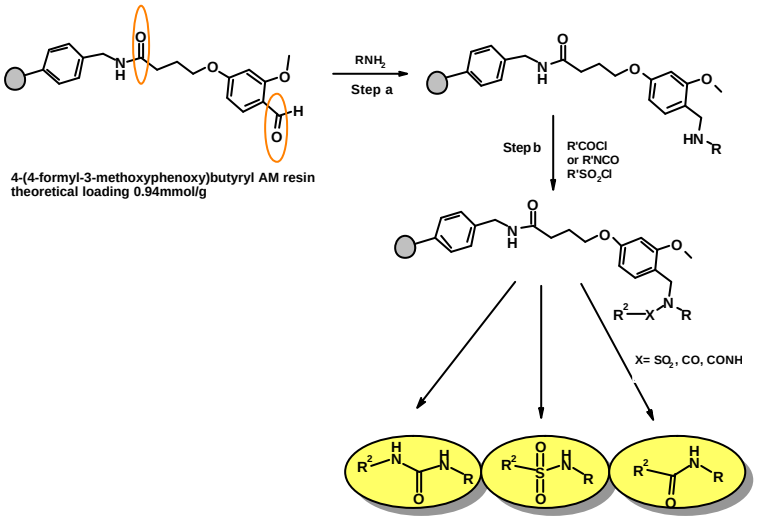


S. Ding et al. J. Am. Chem. Soc. 124, 1594 (2002).

Solid Phase Synthesis - Process Control

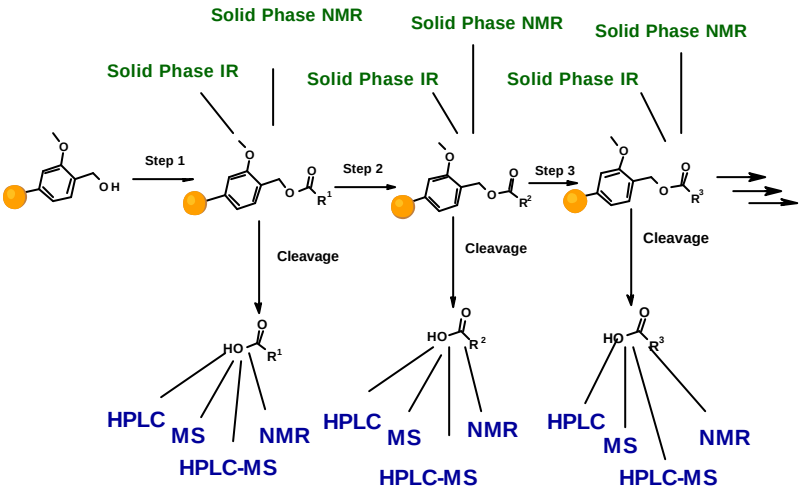
- Single Bead IR
- Magic Angle Spinning NMR
- Quantitation by NMR
- Direct-Injection NMR for High-Throughput Spectroscopy
- LC-MS
- HPLC Online Nitrogen Detection

Synthesis Pathway (Example)

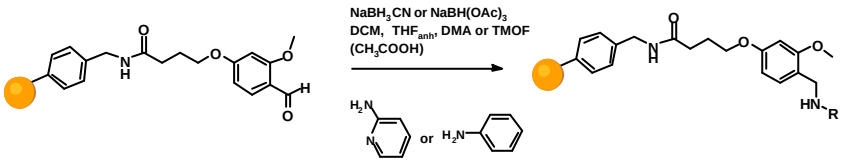


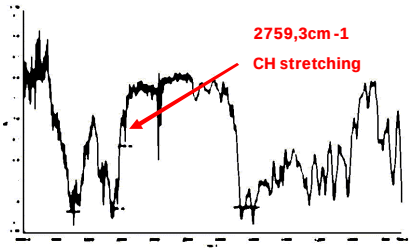
4-(4-formyl-3-methoxyphenoxy)butyryl AM resin
theoretical loading 0.94mmol/g

Solid Phase Synthesis: Monitoring progress



Reductive Amination - Feasibility Study





STARTING RESIN IR

Different analytical techniques were compared

solid phase IR,

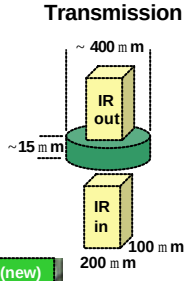
MAS NMR,

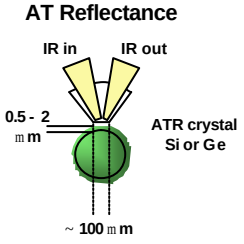
HPLC

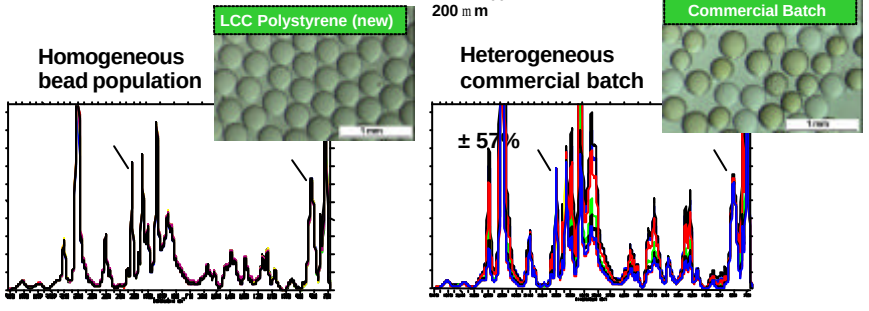
On-bead IR

NOVARTIS

The uniformity of bead loading is measured with microscope IR spectroscopy.
The information obtained is unaffected by cleavage efficiency and post-cleavage handling.

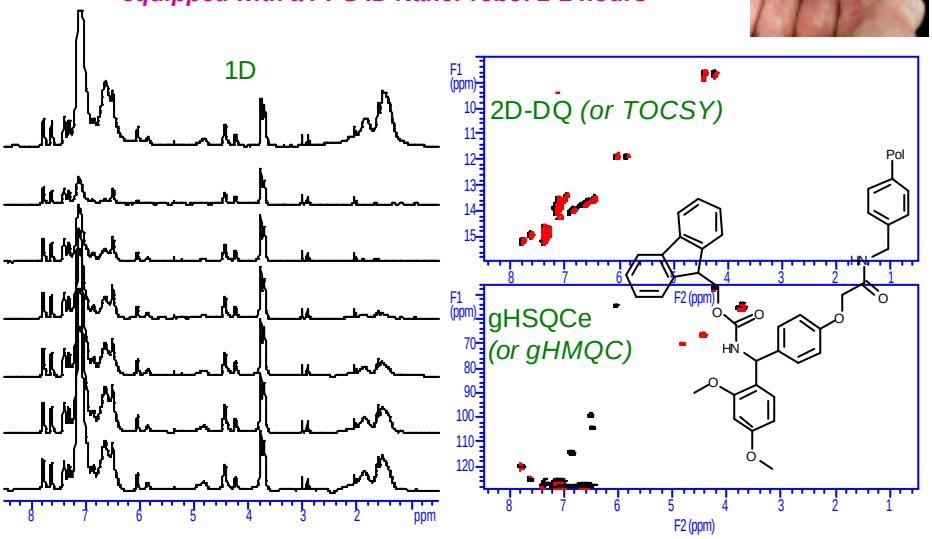




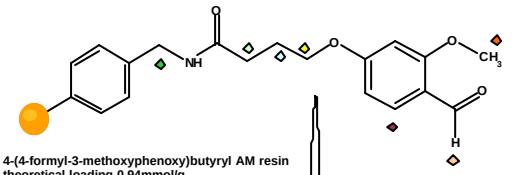


NMR: Our Procedure for SPS-bound cpds

1-3 mg of resin in 40 µl of CD_2Cl_2, Inova-500 equipped with a PFG-ID NanoProbe: 1-2 hours

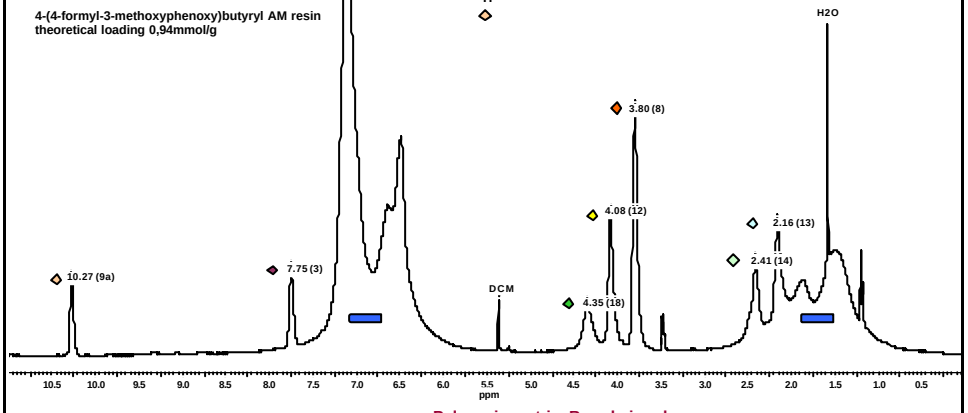


1H MAS NMR Spectra of the formyl resin



4-(4-formyl-3-methoxyphenoxy)butyryl AM resin
theoretical loading 0,94mmol/g

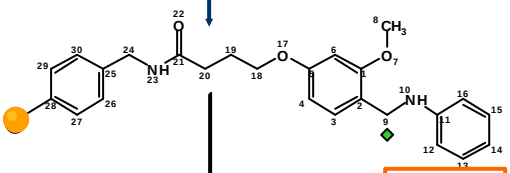
Instrument VARIAN INOVA 500
nano probe 40µl
1-3 mg of resin swollen in CD_2Cl_2
scan # 16 t=10ms 3 min exp

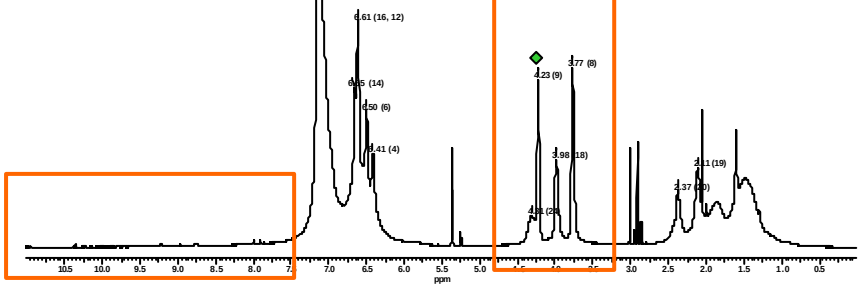


Polymeric matrix: Broad signals

Aniline derivative

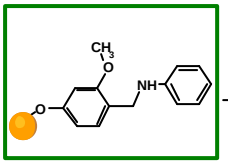
Reaction one pot
aniline 5eq., CH_3COOH 1 eq.,
$NaBH(OAc)_3$ 3 eq, in THF o.n.



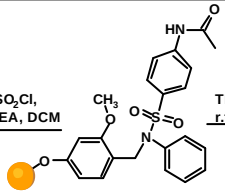


Aniline derivative: Solid phase IR and HPLC after cleavage

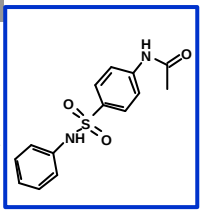
aniline,
CH_3COOH, $NaBH(OAc)_3$
THF o.n.

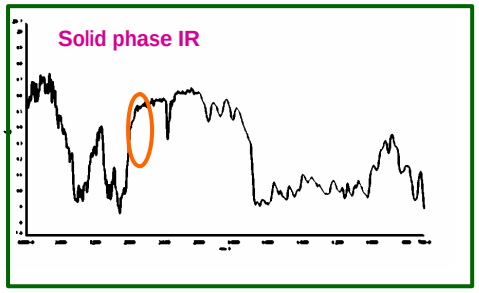


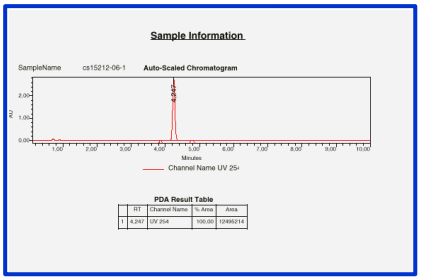
RSO_2Cl,
DIEA, DCM



TFA 30%
r.t. 2h.



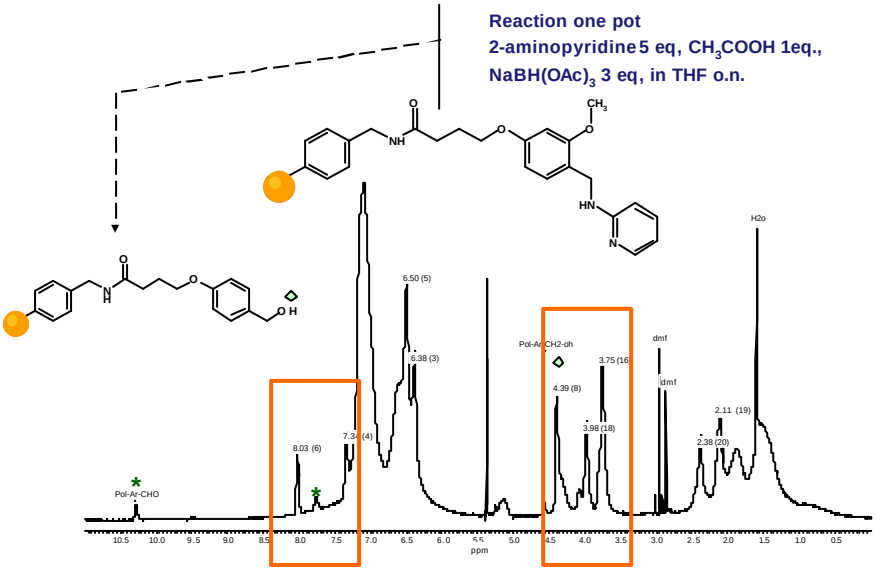




HPLC @254 A=12495214 after reaction with RSO_2Cl and cleavage

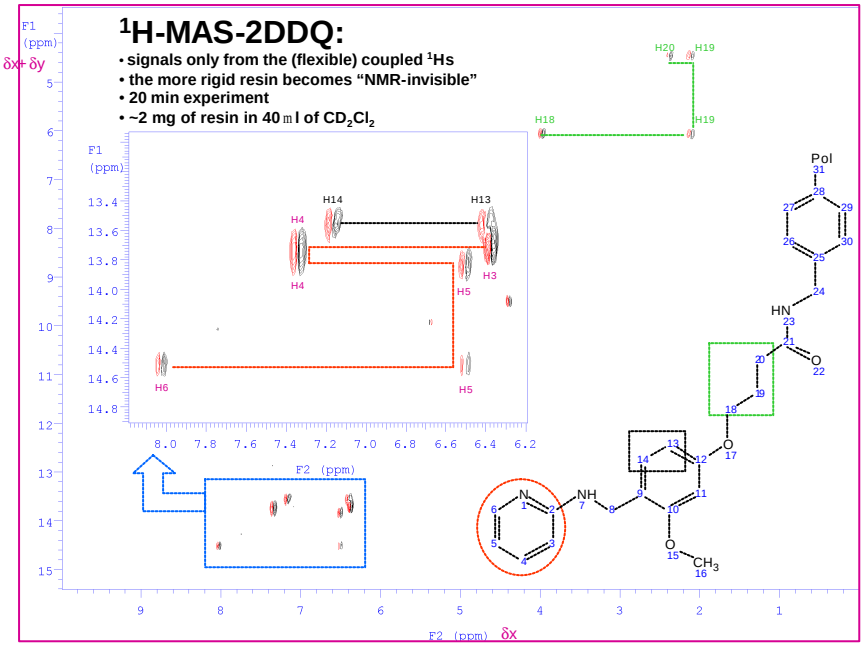
Pyridylamine: MAS NMR of a loaded resin

Reaction one pot
2-aminopyridine 5 eq, CH_3COOH 1eq.,
$NaBH(OAc)_3$ 3 eq, in THF o.n.

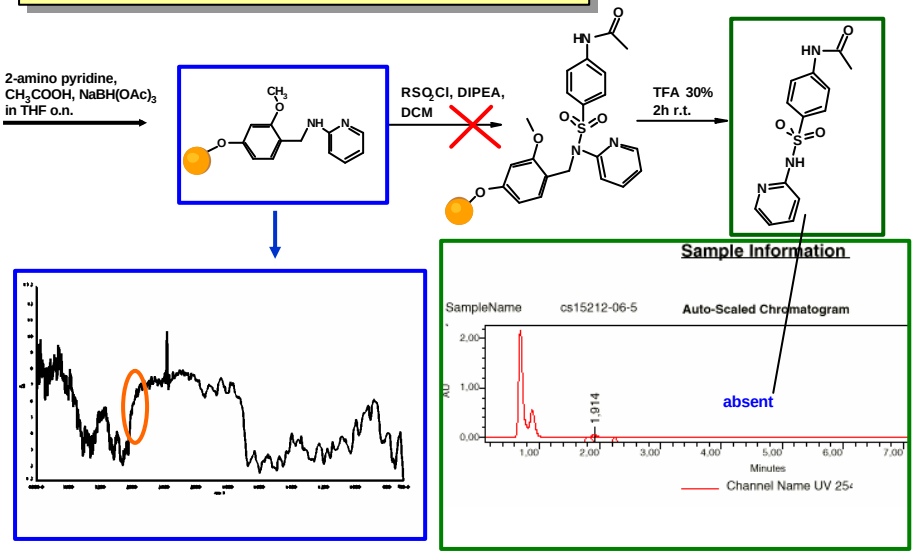


^{1}H-MAS-2DDQ:

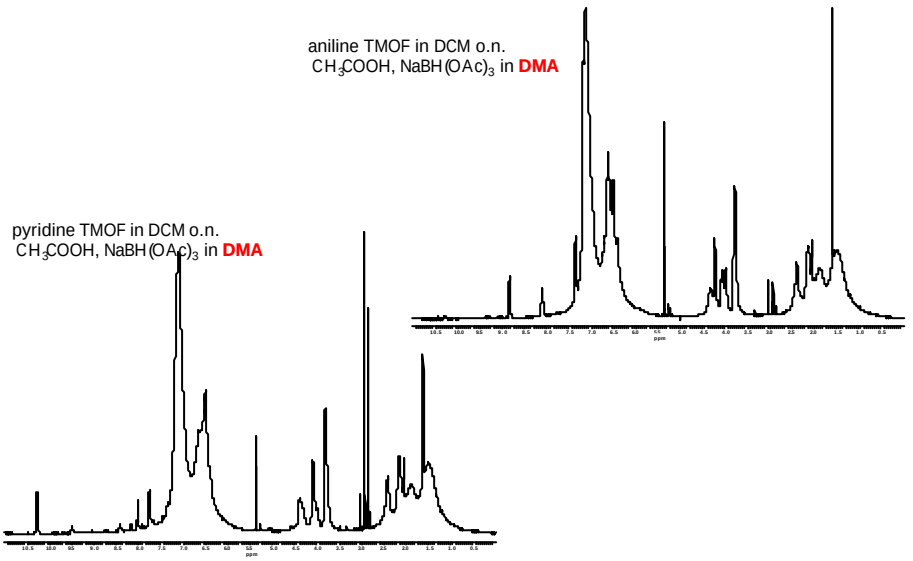
- signals only from the (flexible) coupled ^{1}Hs
- the more rigid resin becomes "NMR-invisible"
- 20 min experiment
- ~2 mg of resin in 40 µl of CD_2Cl_2



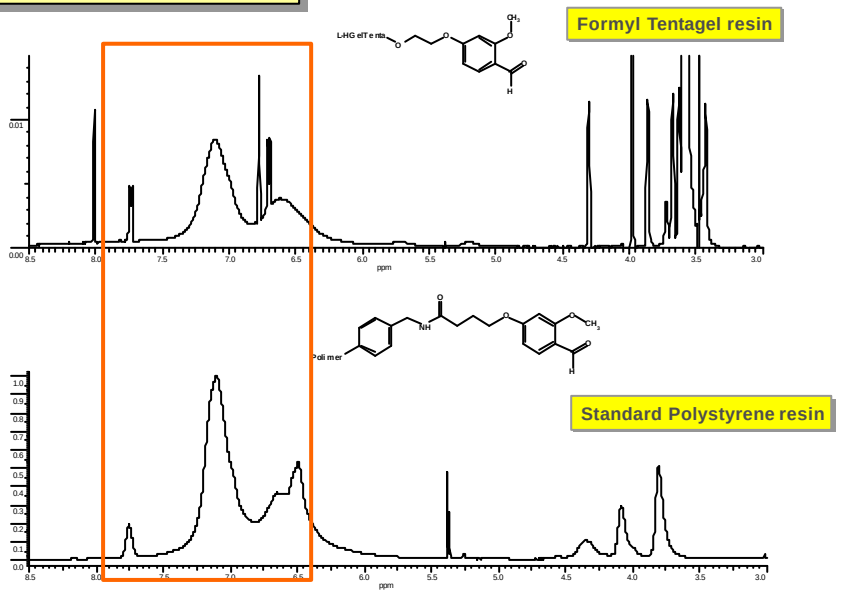
Pyridylamine: MAS NMR of a loaded resin
Solid phase IR and HPLC of the cleaved product



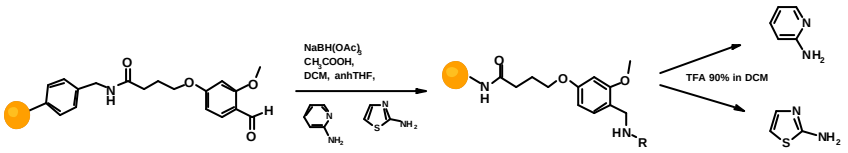
Some non obvious examples



High mobility solid support



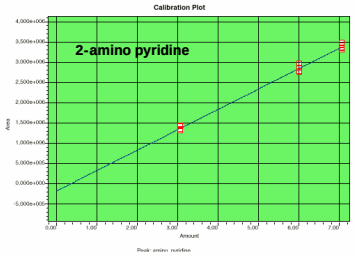
Yield calculation

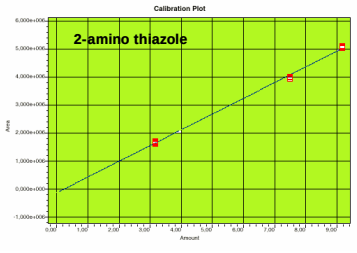


We compared:

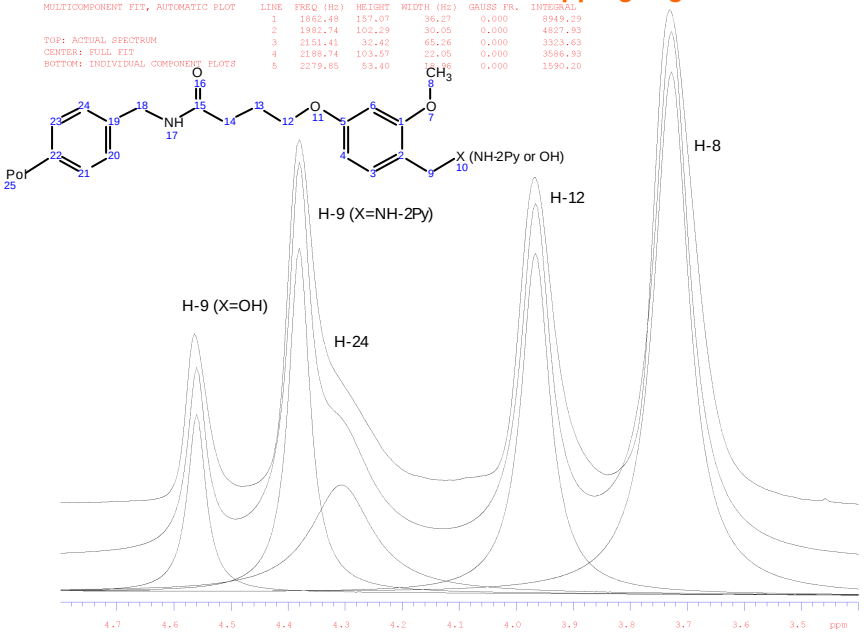
1. Weight
2. HPLC against standard,
3. Deconvoluted 1H MAS spectra

HPLC:
calibration curve





^{1}H-HRMAS-NMR: Deconvolution of Overlapping Signals

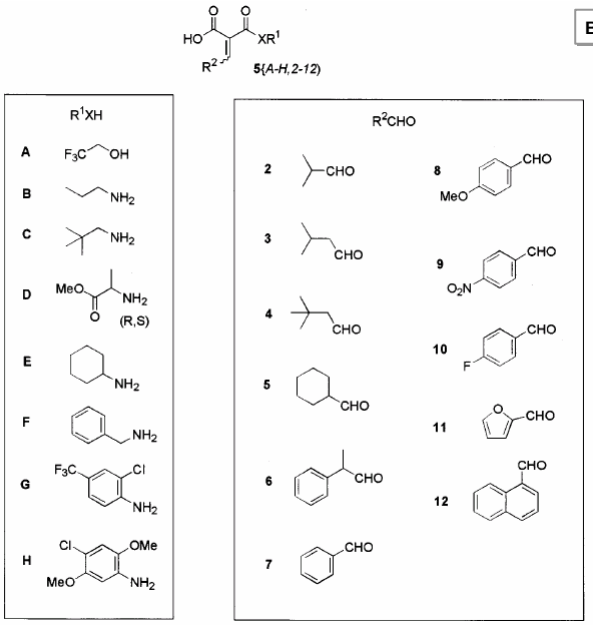


Yield evaluation by weight, HPLC against standard curve, deconvoluted NMR

entry	amine	resin mg (01.94m mol/g)	TMOF 10eq	CH_3COOH 1 eq	$NaBH(OAc)_3$ 3eq	$NaCNBH_3$ 3eq	solvent	NMR yield	WEIGHT Yield(mg)	HPLC Yield against Standard (%)
1	Thiaz	50		X	X		DCM	43%	5,9 (59%)	23
2	Thiaz	50	X			X	DCM	34%	10,6 (100%)	27
3	Thiaz	50	X			X	THF	n.d.	6,1 (60%)	2
4	Thiaz	50	X		X		DCM	40%	13,2 (131%)	32
5	Pyr	50		X	X		DCM	48%	11,8 (121%)	40
6	Pyr	50	X			X	DCM	53%	12,7 (123%)	43
7	Pyr	50	X			X	THF	35%	12,4 (121%)	27
8	Pyr	50	X		X		DCM	n.d.	6,2 (63%)	10

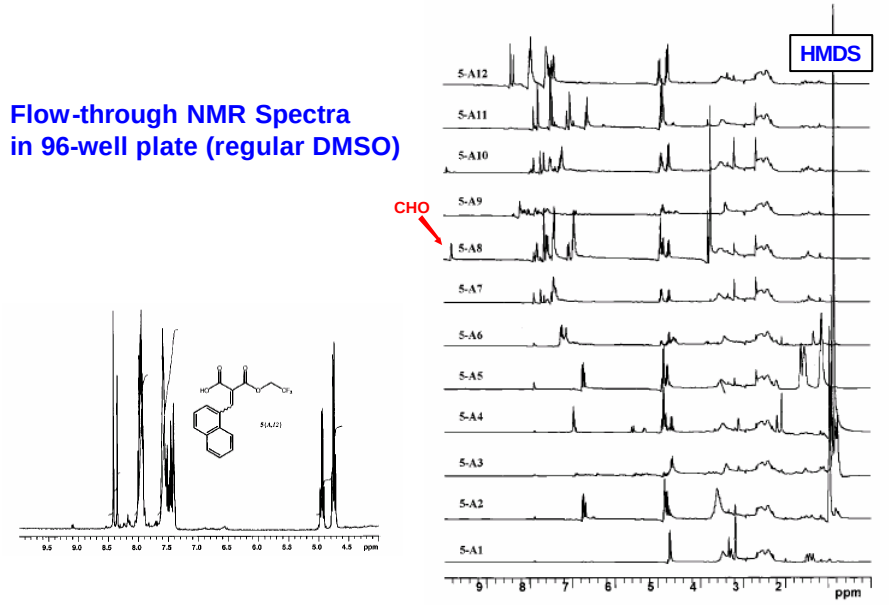
First implementations of rapid NMR quality assessments from titerplates

B. Hamper *et al.*



Flow-through NMR Spectra in 96-well plate (regular DMSO)

B. Hamper *et al.*



Complementary HPLC Analysis

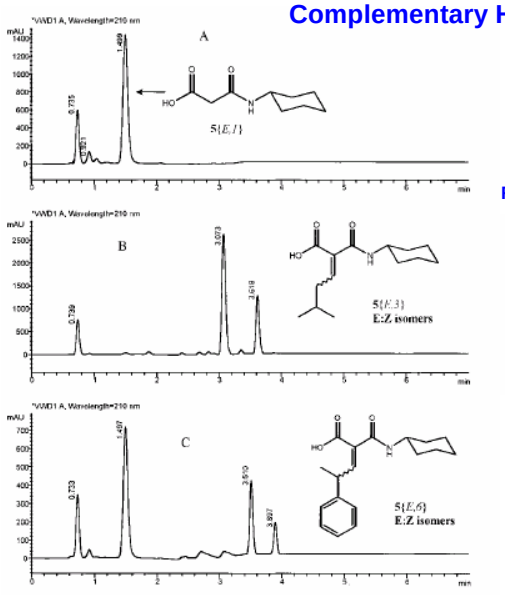


Plate View

Residual malonamide intermediates (%)

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	0	0	0	0	0	10	5	0	15	5	0
B	100	0	0	0	0	100	0	0	0	0	0	0
C	100	19	0	0	7	94	6	5	0	6	0	0
D	100	0	0	0	0	90	0	0	0	0	0	0
E	100	7	3	0	4	49	0	0	0	2	2	2
F	100	14	11	4	12	100	11	8	3	3	5	5
G	100	13	8	5	16	24	8	10	5	8	0	5
H	100	35	22	22	20	100	18	16	12	21	0	22

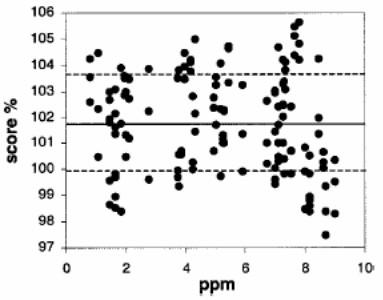
Reference intermediates

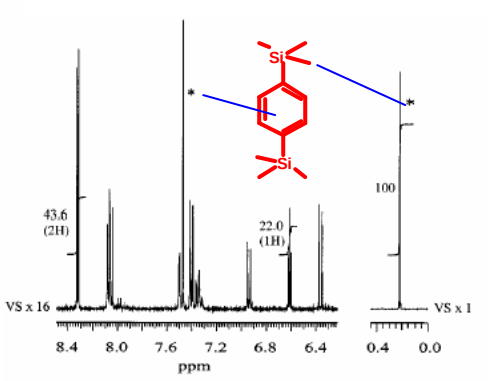
Malonamides

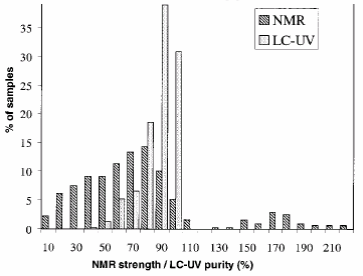
Development of accurate NMR quantitation with BTMSB standard

V. Pinciroli et al. J.Comb.Chem. 3, 434 (2001)

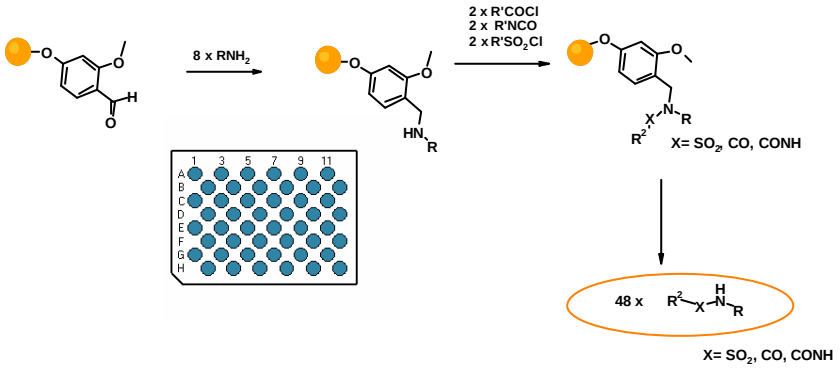
V. Pinciroli *et al.*





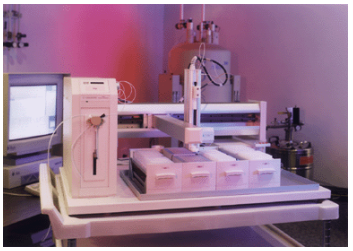


Application example (small compound array)



After cleavage the products were analysed by:

- **HPLC-MS (identity, purity)**
- **1H NMR against standard (identity, purity, amount)**
- **1H NMR Vast System against standard (identity, purity, amount)**

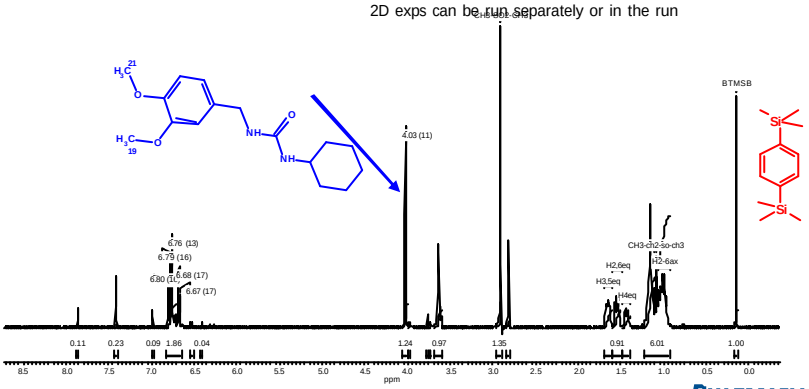


Direct-Injection NMR for High-Throughput Spectroscopy

VAST= Versatile Automatic Sample Transfer

Experimental

4mM DMSO 400ml *(H_2O presence does not matter)*
60 scans plus scout scan each sample
20 min each sample *(including temperature stabilization, gradient shimming on the first sample & 2 rinsing cycles per sample)*
Virtually no contamination between samples
Reliable automation
2D exps can be run separately or in the run



NMR Routines on CombiChem Libraries

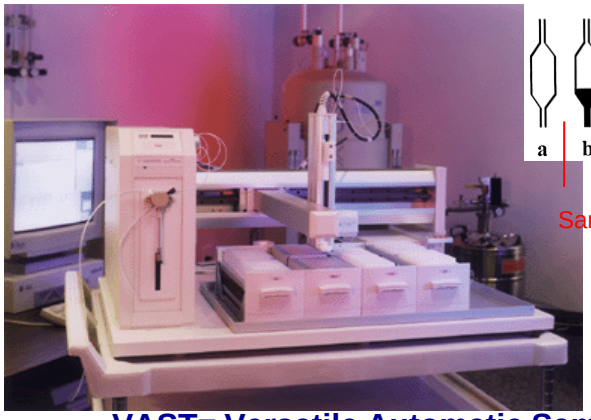
PHARMACIA

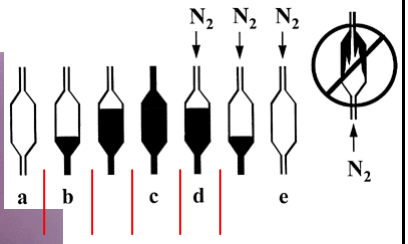
M. Tatò *et al.*

Direct-Injection NMR for High-Throughput Spectroscopy

- **96 wells plate run in nearly 16 hours**
  - 0.5-2 mg / 200 µl $DMSO_{-H6}$ *(H_2O does not matter)*
  - 32-128 scans plus **scout scan** each sample
  - 8 -12 min each sample *(including temperature stabilization, gradient shimming on the first sample and 1 rinsing cycle per sample)*
  - virtually no contamination between samples
  - reliable automation
  - 2D exps can be run separately or in the run

Direct-Injection NMR for High-Throughput Spectroscopy



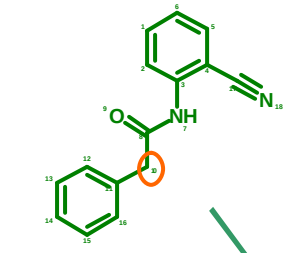


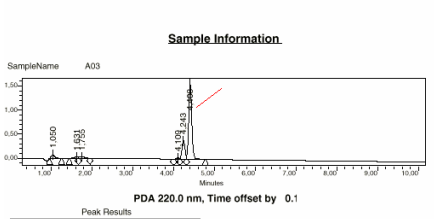
Sample in $DMSO_{H6}$

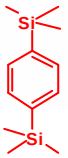
VAST= Versatile Automatic Sample Transfer (Varian)

Ideal situation

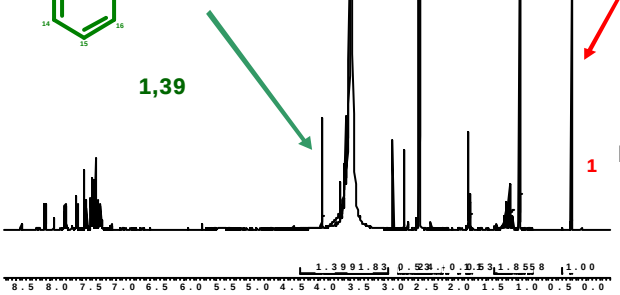
HPLC A %@220 = 72%







1,39

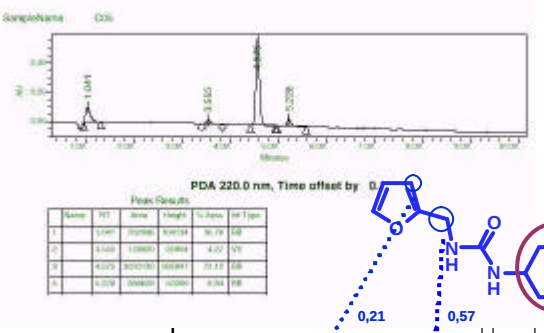


NMR sample strength 70 %

Flow NMR strength: 70%

V. Pinciroli et al. J.Comb.Chem. 3, 434 (2001)

If the starting material is not UV visible



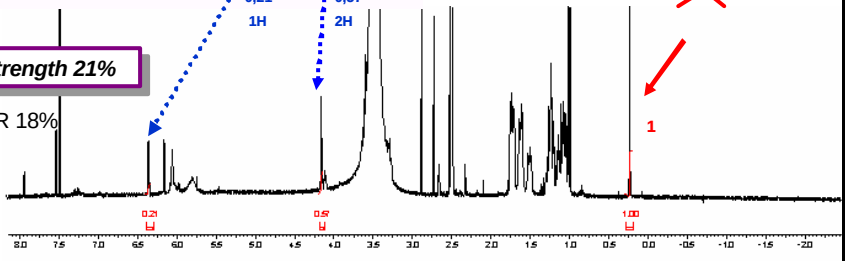
HPLC A% @220 = 72%

0,21 1H

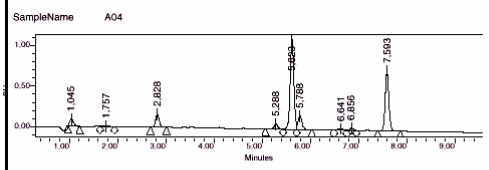
0,57 2H

NMR strength 21%

Flow NMR 18%



If there are no diagnostic NMR signals

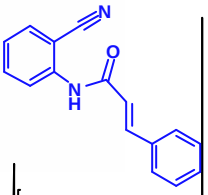


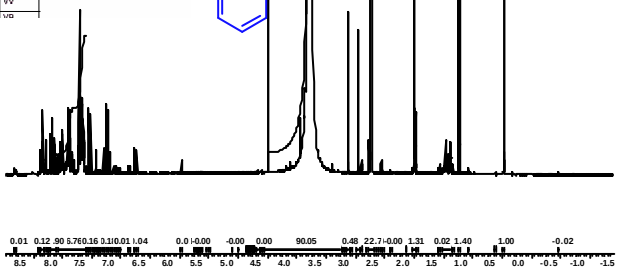
HPLC A% @220= 46%

PDA 220.0 nm, Time offset by 0.1

Peak Results

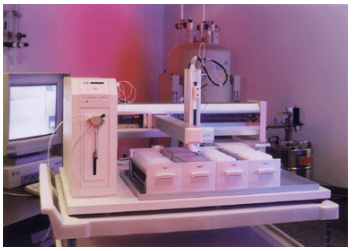
	Name	RT	Area	Height	% Area	Int Type
1		1.045	481021	86252	3.79	BB
2		1.757	120679	9637	0.95	VV
3		2.828	813403	160792	6.41	BB
4		5.288	353014	62733	2.78	BV
5		5.623	5840944	1095191	46.07	VV





NMR strength not easily measurable

Flow NMR not measurable



Direct-Injection NMR for High-Throughput Spectroscopy

VAST= Versatile Automatic Sample Transfer

Experimental

4mM DMSO 400ml *(H_2O presence does not matter)*

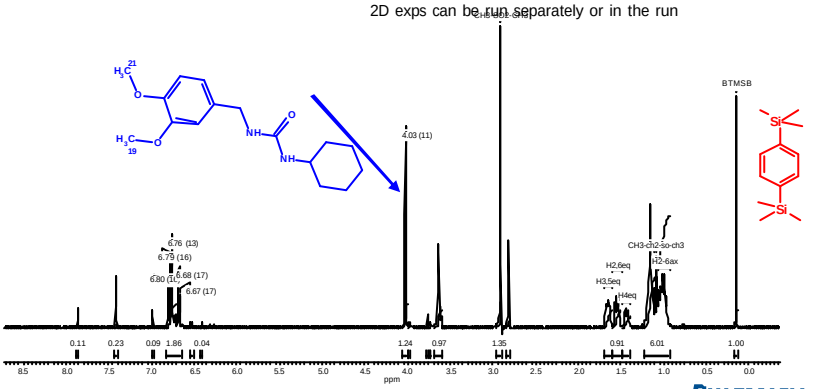
60 scans plus scout scan each sample

20 min each sample *(including temperature stabilization, gradient shimming on the first sample & 2 rinsing cycles per sample)*

Virtually no contamination between samples

Reliable automation

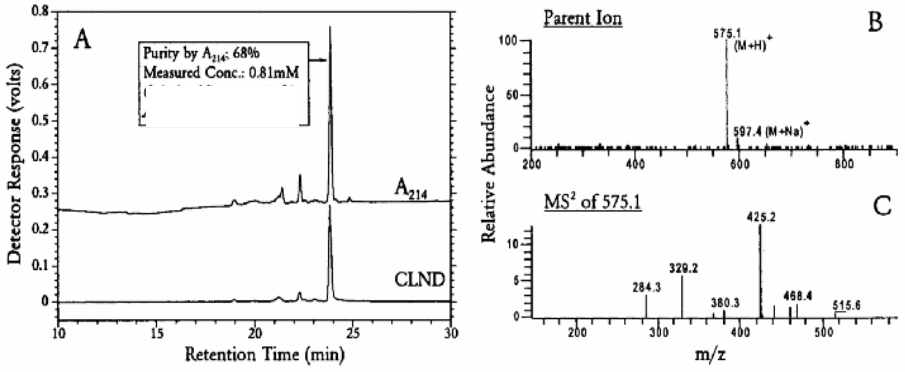
2D exps can be run separately or in the run



Minimal Sample Consumption: Including Nitrogen Detection

HPLC/N/UV/MS^n detection platform

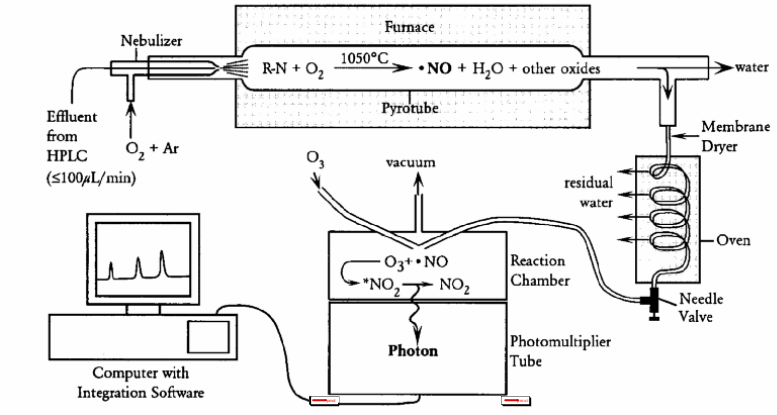
Identity-Quantity-Purity



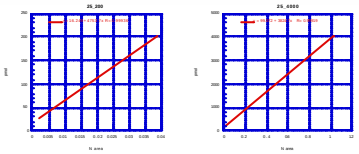
Integrated Analytics

CHIRON TECHNOLOGIES
NOVARTIS

Nitrogen detection



Nitrogen Content Calibration



Online N-Detection: Standard Sample (200 pmol)

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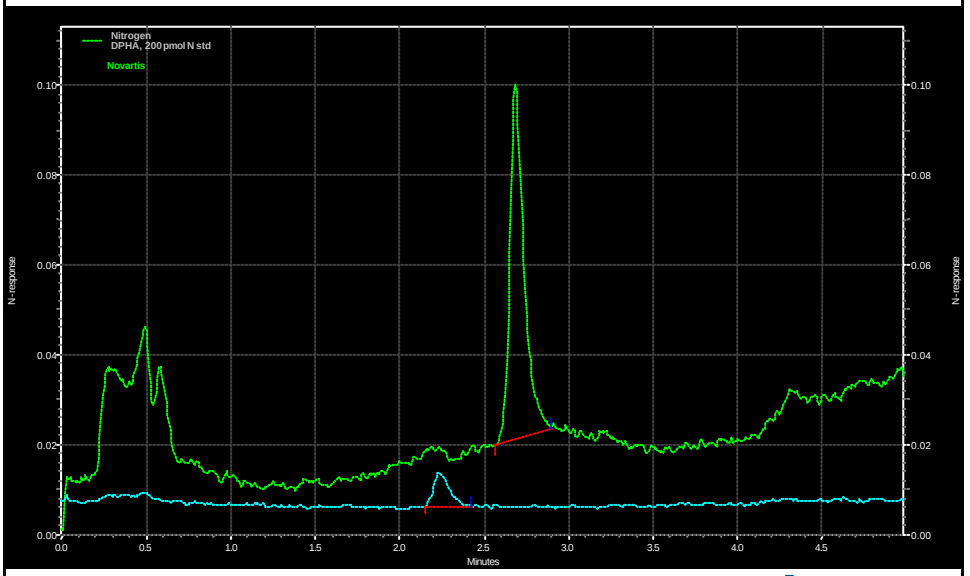
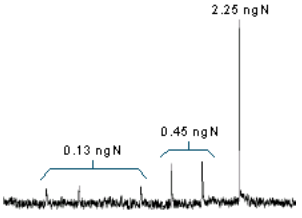
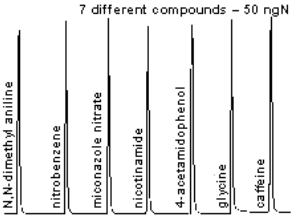


Nitrogen detection





Linear standard calibration curves over a $>10^4$ range of analyte concentrations.
HPLC-CLND is compatible with reversed phase, size exclusion, and ion chromatography.
Sensitivity is 0.5 ppm Gly-Gln peptide (0.1 ppm N) in water, 4x > absorbance at 215 nm.

Chemiluminescent reaction between ozone generated from oxygen and nitric oxide via high temperature pyrolysis of nitrogen-containing compounds

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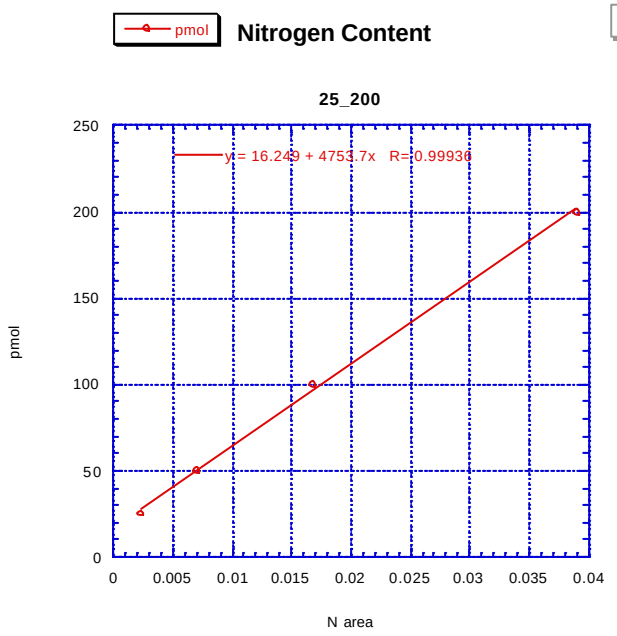

Concluding Remarks

- Derivatizing novel scaffolds on solid phase with standard reactions is an efficient, compound prolific approach
- Ample choice of techniques for in-process control of solid phase syntheses (on and off beads)
- No significant bottlenecks
- Beware quantitation by weighing of non-crystalline microsamples (< 5mg)
- NMR quantitation against standard is the most accurate system however ... it is relatively slow. Impractical for samples < 1mg
- The MAS NMR on solid phase gives good qualitative information, the quantitative evaluations may be less precise and more demanding in terms of interpretation (spectra deconvolution)
- Nitrogen detection (HPLC online) has excellent throughput and is the method of choice for the quantitation of microsamples of large libraries
- Initial concerns on analytical limitations of solid phase chemistry have been overcome

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