HANDY ADVICE HOPEFULLY

This is a collection of my thoughts and advice. I know there is a lot of stuff to digest here and some of it may seem a bit precise/'retentive' but I hope it's a useful guide to some of my philosophy. I hope to play an active role as your supervisor and getting some insight into my (possibly idiosyncratic) approach will probably save us all hassle in the long term. If you think it's poor or I've missed something that those starting in the future will find useful and I've missed out then let me know – I see the group as an ever-changing thing and the process that we adopt together should be fluid and responsive to the state of the group (within the important constraints of degree courses, safety etc).

General

- Read Good Laboratory Practice (you should know these inside out)
- Read the Roles of Supervisor and Student (if appropriate) – this is a great guide.
- Read the Safety Rules – you need to do this before you can start work.
- Buy and read Harwood and Moody's practical book (quite large but should be plenty of copies in the 2nd-hand bit of Blackwells) – it's full of laboratory class experiments but there also a good section with advice on lab techniques.
- Buy and read "Advanced Practical Organic Chemistry" by J. Leonard, B. Lygo and G. Proctor published by Blackie Academic – there's a copy in the lab but try to leave it there so that everyone can read it.
- Get Collins and Ferrier "Monosaccharides" and Fersht "Enzyme Structure and Mechanism" – again there are copies in the lab.
- Maybe get Corey "The Logic of Chemical Synthesis"
- For those of you who will be touching on Mol Biol at all Sambrook & Fritsch is *the* almanac. There are several other good starter books as well.
- Have a look at the evolving Amazon wish list on the website.
- Look at all of the documentation on our Group Activities link – another great source (http://users.ox.ac.uk/~dplb0149/activities/index.html)
- I love the job that I have and working with you all as collaborators makes that the case. However, as PI I take ultimate responsibility for the safety of all of you. As such, at times it is necessary for me to make decisions regarding aspects of safety or group management (which may not always meet with universal approval). We can always discuss such issues but I will need to make the final call.
- The laboratory environment should be one of mutual respect and trust. To foster such an environment we should be honest and transparent with one another.
- Please do feel free to speak to me openly and directly at any time if you have any issues with aspects of lab management, project direction/ideas, running of the group or anything else related to you time in the group.

Safety

Obviously a key thing. Some of the following points are repeated but better to be safe than sorry.

- Safety Clobber – if you're doing an expt you need to wear goggles, gown and gloves – no ifs, no buts. No gowns in the write-up room – no-one in the lab without a gown.
- Risk Assessments (RAs) – these are key and need to contain the following: 1. hazard, 2. risk, 3. unusual protective gear (ie other than coat, goggles, gloves which you should always wear), 4. emergency procedures, 5. your signature. These are checked by me, the lab, and the university – make sure you do them. 6. PDRA signature.
- Part IIs/Students need to get all RAs signed by me and a designated Postdoc. PhDs can sign their own and then get them signed by a Postdoc after 3 months on present policy. Whatever happens you need to sign it and your lab manager should be aware of what you are doing. They and I can often be a form of useful advice in planning and helping (we hope!)
- Be clear about the relative nature of Hazard, Risk and Exposure. This means that things change when you scale-up and change conditions. Never just refer to another Risk Assessment. It's the process of doing it that helps to focus the mind in a safe way.
- Something to think about when you're doing your RA – "is their some less risky alternative to this ..?"
- Recently we've been making sure that the lab managers for each unit are also signing everyone's RA also – this is great at allowing others to know what you're doing. This helps not just with safety but also with science.
- Scale-up: If you scale up a reaction (you should think twice/ask if you scale up by more than a factor of 3) then you must do a new RA – most bad accidents happen on scale-up. If you think you want to scale-up more than this – you must ask & check safety first.
- The core hours of the laboratory buildings are 8am-6pm Mon-Fri – although you should always be working in the lab only when there is someone else there, it is particularly important to bear this in mind when you are working outside of these core hours. Do not forget that you need to log your presence outside of these hours using the sign out system (Out-of-Hours book) The key quote is that you must be satisfied that "adequate numbers of personnel are available to deal with any emergency that may arise."
- Tidiness – keep things trim – this is not an issue of being retentive – it influences very strongly how safely you work. Paperwork should not be piled up in the lab as it's a fire hazard. Try to keep your own space (fumehood and benchspace) free from too much clutter. The more mess, the more stuff you will lose and the more mistakes you will make – trust me. Try to extend tidiness in the lab to your write-up desk space. *All* communal areas must be constantly free from any junk.
- Needles and glassware – there is no excuse for ever leaving a needle or a sharps hazard on any surface. Do not re-use them – dispose of them as soon as you have used them – *before you even think of putting it down*.
- Overnight forms – these must be done and done properly: proper signatures, correct contact details, a new form for each date (don't just cross it out and write another).
- Laboratory Books – these need to be kept up to date (see below) as a safety guide and for good scientific practice. This is a safety obligation on your part.
- If in doubt, ask – cheesy but true
- We have a book of departmental procedures and Risk Assessments. You need to read these and be aware of these also.
- Please also use the safety books that we have bought and placed in the safety bookshelf.
- You may find that I remind you of these key rules. Please don't take this as being a personal 'go' at you should I do so. It's my job to try and keep you as safe as possible and I take personal responsibility for that. I know it may seem a bit like being nagged but it's vitally important – complacency leads to errors. I hope you will do give me the respect of being the person who has to be 'in charge' in this respect. As adults, I hope we would all do the same

for each other. You may see lax practice, on occasions, but let us all work together to prevent this and help increase the quality and safety of what we do, whenever we can.

General Approach

Gloves - don't hesitate about buying any safety stuff
- Disposable gloves - use nitrile (purple ones) - latex can give you allergies
- double up layers if you can still move your hands
- get some marigolds for toxic, smelly and hazardous work
- vinyl ones should *only* be used for aqueous solvents (enzymes/buffersetc) as they let organics straight through.

Ordering - check with me to get big stuff signed - try to get the cheapest option but don't worry too much about costs – your time is the most valuable thing.
- most stuff will be VAT free but those in stores will know this probably
- don't hesitate to nag to get things delivered quickly or to chase stuff up – the system can slip up and occasionally needs a kick
- sometimes things will be delayed because I haven't signed a VAT free or "Big Order" (> £100) slip – nag me….if I am away a lot for a period or if things get v. slow then we should try to get someone else authorized to sign. Often the Lab Technician (Amy) or my PA (Jo/Sarah) can help.
- if things don't come quickly then call, nudge, prod. Please don't feel that you are being rude should you do so. Your time is valuable and waiting for things should not be a part of it. We expect orders back within 24 to 48 hours and things are going wrong, if not.

Timekeeping /Time Management - Try to get into the labs between 8-30 and 9-00 am - this is the time that you are expected to get in and if you do it will mean that your day becomes less stressed /less frantic. Remember it looks quite lazy and privileged if we all stroll in at 9:30am when all of the superb support staff have been in since 8:00am.

- Normal laboratory hours are 8:00 am until 6:00pm - outside of these you have to sign in at the front entrance.
- Safety dictates that you shouldn't work in the labs unless someone is "within shouting distance" but feel free to use the write-up room. Theirs is often a list in the write-up room of times so that you can sign up and agree to meet up over weekends, holidays etc. but verbal communication works well too. Those of you doing degrees can consider the weekend to be a very useful time, just as it was during your undergraduate days. Often people find this to be the most productive 'uncluttered' time.
- Try not to take too much time-off in the middle of the day (unless around lunchtime) this will only mean you having to stay later and it may irritate those looking for you or trying to organise duties e.g. solvents etc
- Don't worry too much/overly about timekeeping though – you are doing your project for your own sake and to learn. As long as you feel satisfied with your progress then all is well. Remember though it is easy to get behind so try not to let this happen. It's much more about efficiency and completing experiments in a productive way than being seen to be in the lab or 'clockwatching'.
- My old boss use to say "each month is 3% of your PhD" – which is sort of about 6 pages of your thesis – it's a bit strict to think of it like that but gives you a bit of a guide.
- Some form of plan (GANTT, list) outlining goals and objectives is key but this is just a guide rather than a strict timetable. It is essential that we keep using this as a guide at our weekly meetings. Its purpose is not to hold anyone to account but

simply to ensure, steady, unstressed progress. Nothing is more stressful than realizing that you have planned badly. Without efficient timekeeping time can 'slip away' and this is a skill that you may have to work at.

- As a good golden rule ***A reaction a day keeps the doctor away*** – this is clearly phrased partly in (bad) jest but it is also a good pneumonic / guide. You should expect to do at least 6-700 experiments in an entire PhD, normally up to 1000 – this works out at ~ 5-7 per week). That said if you do 4 in one week and then 6 the next this keeps the average the same. It is much more sensible to do 4 good, complete experiments that you could put in a thesis rather than 'bang on' 10 experiments that you don't follow properly or that do not give rise to useful outcomes.
- As an overall point don't forget that a PhD is not a job but a degree. Just like any other degree there is a certain standard that you need to aim for and a certain amount of content that you need to cover. It's not and never has been just putting the time in a leaving after a certain number of years with a piece of paper but rather the quality, amount and rate of your work will determine entirely when you will be in a position to submit either your transfer application (end of 1st year) or your final thesis. My role is to act as your 'tutor' to get you to this point. Sometimes I may point out where you are in respect of these goals or standards and I would be letting you down if this was not as accurate and transparent as I can be – please don't take this personally. This type of 'peer review' will be a key part of your ongoing lives, if you choose to be a scientist.

Absence

- Holiday are always a fine plan. The EPSRC, BBSRC and the University of Oxford contract all set down 6 weeks, including bank holidays, as the maximum period (ie 30 days). Most of you will be covered by this but even if you're not I think it's fair that everyone gets the same. When and how to take them is up to you. It's probably best to split them and to use them when the lab is officially shut (Easter and Christmas) but if you want to take a long period off then be aware that it wouldn't be fair on anyone else for me to let you have more than 6 weeks in total.
- We have a system run via the office that allows you to plan absences in advance and then we post who is away each month on the wall for communal information. Feel free to do this well in advance but do make sure that you do it in the month before any planned trips. If plans change at the last minute then we understand but do try to aim for this notice period (ie let the office know a week before the month in which you want to take time off).
- whenever you are off from the lab (even if it's just for a day) you *must* let me know – notice is useful (see above for holiday notice). This is a safety as well as a planning issue as if there was a fire we would assume that you are in and someone would go risk their neck to look for you.
- If you're ill there will obviously be times when you can't make contact but please do try if you can (phone, email, text – whichever). For long periods of illness (more than a couple of days) then get a note from the doctors, please.
- With regard to being ill – there's no point being overly brave – recover back to full strength before you come back in – it'll save you time in the long run.

Conferences

- Most of you will have some money associated with your grants for conferences and for me these are a top thing to go to as they help to place your work in context. Plan to go to one "big" conference e.g, an ACS or GRC meeting in the USA or EUROCARB or ICS and several more local ones e.g. Pfizer poster competition, RSC Bioorganic or Carbohydrate Symposia. Wherever possible it's important that if

you go you also present something (talk much-preferred over poster). “Time off”-wise the EPSRC considers days at the conference not to be "time off" - which is only fair really, but time either side is.

Reactions

- Get these on as early as possible - but carefully - this will allow you to keep an eye on them all day and work them up in the afternoon or leave them overnight, accordingly. Don’t come in and start checking email for an hour, or go to tea! – make the most of your time in the lab to do ‘lab work’ – be engaged, efficient, excited.
- If your reaction appears to have stopped then you should either work them up or use more strenuous conditions (e.g. warming, refluxing, adding more reagent, more catalyst) - the application of these will be limited when the reaction that you are carrying out is selective. Do not just leave it hanging around if nothing is happening. Remember that the formation of products happens initially more rapidly (this is basic kinetics – so if nothing is happening after 2h - then do something).
- Most reactions are done within a few hours. 16 hours in an experimental is a clear sign that someone put a reaction on when they left the lab and just hoped it would work without following it in real-time. This approach is anti-scientific and quite lazy and will ultimately cost you time rather than save it. It is usually possible to get most experiments done within a day start-to-finish although some things (bacterial or cell growth may have their own kinetics).
- Think of optimization strategies but be logical. Remember controls, change variables logically and think of parallel and serial approaches e.g. ‘Design of Experiment’.
- **Anything either smelly or volatile or vaguely dangerous must be kept in the fumehood at all times** - this includes gloves that may have pyridine on and the like and volatile solvents like ether, chloroform etc
- The Group/Lab - remember they are all doing the same thing as you - they are there because they enjoy chemistry
- they are very experienced so ask them - but don't forget that they are busy as well
- make sure you do your group chores – e.g., solvents whenever we are out - emptying waste - looking after the stills, getting more Winchesters etc.).

There is no real excuse for not doing them no matter how busy – you’re part of a community and others are relying on you - (people will willingly let you do them half an hour later if you are in the middle of something)

- On the last Thursday of each month there is a group tidy up – a list of duties placed on the board in the write-up rooms is one way this has been done, although distribution of jobs on the day works too. Please work with people. No-one is so important that their experiments justify them doing nothing to help the community.
- Try to tidy up any communal areas that you use as you go along - ie the fume-hoods, instrument areas. As the person who takes the safety role, I reserve the right to get you (or others) to tidy up at short notice. So keep things that you don’t want cleared away in a safe place – locker/ filing cabinet. You don’t want to lose it!
- The same goes for communal kit – not emptying the trap or letting one of the Büchis or pumps run with a cack vacuum is just plain slack – if you’re not sure how to do/mend something then ask – leaving it is dumb: e.g., I’ve known people lose 2 hours waiting for a the high vac büchi to take off 30mL of DMF if the vacuum is poor (it should do 30mL in 10-15min)
- Be aware that some compounds may be unstable at room temperature especially if they are oils or liquids [this is effectively a very high concentration solution of compound and therefore able to react at very high rates] and so it is often prudent to store these under nitrogen in the freezer. Get yourself a slot and box but don’t block

the freezer up with round bottom flasks – if you are going to store something in the freezer long term put it into a smaller vial (e.g. using the Genevac).

Tidying-Up - keep your bench free of debris even if this means temporarily placing it in a bucket
- try to wash-up at least once a day - I know this will seem a pain but it will avoid grief in the long-run
- the safety people can just drop in any time - so you can guarantee that if on one day you do something dicky they'll turn up – so *don't*.

Literature - You should always know every detail of the background to our project. Reading papers will keep you up to date but this should be augmented by database searches on a regular basis.
- You should look at: SciFinder, Beilstein, W of K/S, F1000, Scopus as a bare minimum. If you haven't got access or don't know how – get passwords and go on the deptl training courses asap.
- Every compound and transformation should be checked on at least SciFinder, Beilstein, before you do it. There is almost no greater blunder that not knowing that something has been done before. Finding out 3 months too late that something has already been done is a guaranteed way of losing 3 months of your life – not good. Also related work can really help give you insight into reactivity profiles.

Attitude - it should be fun and enjoyable.
- Don't get into the position with yourself that you hate going in to the lab.
- the more you share with the others the more will enjoy this.
- pop up to the library after hours just to keep an eye on bits of chemistry – I reckon that "reading" 20-30 papers a week is well healthy but this doesn't necessarily mean absorbing every proton chemical shift – you should learn to skim read. There is little point knowing one paper really well if the one that you get asked about in your viva or an interview or talk was one in the other 29 that you would have read. Reading has a profound effect on developing not only your intellect and knowledge but also your logic, writing style and English.
- make sure you understand the theory behind every reaction that you are doing.
- don't forget chemistry is an art – you are crafting beautiful things which have never been seen before.
- Remember that blasting out an email will mean on occasions that you will say some things more forcefully than you meant to and that you might regret. Please don't send communal emails unless they are just for information. Wait until group meetings (see 'Griping' below).
- Try also not to take things too personally. No-one is trying to 'have a go'. All of us are here to help each other and do the best science that we can. Peer review, including during science meetings, progress meetings ('Hands' meetings), group meetings and lectures with me and others may sometimes involve direct comments that 'cut to the chase' and these may highlight errors or alternative ways of viewing things. This should be viewed as professional, impersonal advice to help improve your science.
- Motivation is a key thing but this comes from your own self-discipline, belief, method, care and precision as much as it does from those around you. Try not to take criticism and failure as condemnation but a constructive opportunity to improve. Sometimes experiments will not give you an expected outcome A->B but find out what they do do! Often this is more exciting. There is not really such a thing as "It

didn't work" in science; there is almost always an outcome – hunt it down and be excited by the hunt.
- Feel free to come and chat about any aspect of this with me or the others around you (Postdocs, Co-supervisors, College Advisors etc). We are all working together to help you.

Laboratory Notebook

- Get duplicate ones - don't forget to shift the carbon copy and every month put the copies somewhere safe and separate

Guide for Layout:

Top of the Page -

Then -

Your Name Date
Expt No. BGD

A diagram showing interconversion of structural formulae
- Label the arrow with all conditions
- Record relative molecular mass of structures and your compound code (if you have one)

Reference to Previous Experiment or Literature Paper

Risk Assessment

Compound	Amount	Hazard No. of equiv., mass, volume

Method

Record *all* information about the experiment ie all method, all observations, t.l.c. sketches, results, yields and even your thoughts as to why a reaction was more or less successful

Until you are told otherwise come and see me (or perhaps your postdoc supervisor) before you bang a reaction on - just to make sure all's cool with the risk assessments etc.

• Keep the lab book up to date at all times. Keep it with you during your experiments and make frequent clear notes.
• Don't use other notebooks and transfer at a later date. Put all in your lab book.
• The lab book is a legal document for safety and intellectual property. It belongs to the university (not you!) and is, in essence, a log-book of what you are doing and have done. Use it to show us (and future generations) what you have done.
• In disputes, exams, papers, court cases your lab book is the final and true record of what happened/what you did.
• There will be times (present and future) where your lab book will provide key information, data and guidance. Think about those in the future reading it. When you are out of the lab make sure that your lab book is at hand ie. on your write-up space for those who might need it e.g. me, safety people should there be an incident, fire-service etc. No-one is trying to 'snoop' – we are all working together so don't be protective or paranoid about hiding your book – be proud of it!

Reaction/Experiment Itself

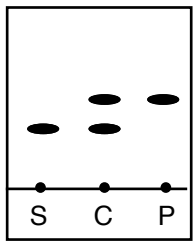
Plan Ahead - write your reaction entry in your notebook the night before
- if you need dry glassware then put it in the oven the night before (it needs to be dried for about 24h) as well as drying any needles or syringes you may need
- try to get the reaction on first thing. There is no point coming in and going for tea and not getting going until 10/11am. This will make you late for the rest of the day and is poor time management – relish your time in the lab and make it efficient. Bang experiments on early following planning from the night before. Monitor intensely for the first key period.

Pure starting materials - make sure the stuff that you use is pure and characterized. Don't assume that it will be pure just because you bought it! You may need to purify it yourself. Make sure you check all SMs before you use by characterizing in some way. If it is your own sample but you haven't used for a while then it may have gone off so check again.

Solvents - make sure you allow time to distil the solvents you need before your reaction and that you have a good sense of dryness. The canisters can get wet, you may (rarely) need to distil or you may need to buy dry. Plan this through.

Dry reactions - as well as requiring dry equipment you may need to dry molecular sieves (3Å) - flaming for 2h then cooling under nitrogen
- ensure an atmosphere of nitrogen over reactants at all times

Monitoring - t.l.c - find a system that displays all reactants and products
- there are many suggested systems on the group website
- you can never take too many t.l.c's
- you should use a three lane system (reactant, mixed spot, product)
- take t.l.c's frequently (every 5/10 min) - especially at the start of a reaction
- you can develop them in more than one way (e.g molybdate normally, ninhydrin for amines, potassium permanganate, iodine, UV etc)



- IR - spot onto the crude NaCl plate
- NMR - if necessary take a small aliquot from the solution and run an NMR of it once dried
- Small molecule MS – MS on small molecules is very misleading so don't use this as an active part of reaction monitoring for such reactions since it is poor in its relationship to concentration
- Protein MS – this is quite the opposite as global pI will change little from reaction SM to product. This makes it the ideal way and our 'protein reaction TLC'. Again try to use this is real time. It is a false economy to stack up samples; you need to know what is going on as it happens. This may require careful set-up of the MS machines but it will pay dividends.

Work Up - learn the ideas behind work-up - these will tend to involve partitioning between organic solvents and an aqueous layers
- brine is used to partially dry
- anhyd. magnesium sulphate completes drying (anhydr sodium sulphate sometimes for amines)

- acids or bases are used to neutralize accordingly
- pH 7 buffer (0.1M phosphate based is good) can be used for sensitive substrates

Flash Chromatography
- run a t.l.c in the system you intend to use so that you can see all the compounds that you need to isolate
- run a 2D t.l.c. to see if your compounds are stable on silica
- it is often worth running several to get the best separation (if ethyl acetate/hexane systems fail - try ethanol/chloroform - or acetone/toluene)
- even very polar things (e.g., deprotected sugars / peptides) can be columned – try MeOH/EtOAc (you can go up to 15-20% MeOH before you start dissolving silica) or CMAW (chloroform, methanol, acetic acid, water) or conc ammonia in MeOH.
- use the guide table posted on the benches (a copy is in Harwood & Moody too) shows you what size column to use - a test-tube is 20ml, a boiling-tube is 50ml.
- The spot to be recovered should be done so using a solvent where the spot is R_f 0.3
- you must recover *all* compounds from the column - this may involve "flushing" the column with a more polar solvent system (normally 10 column volumes needed for a thorough flush).
- think too about alternative chromatography if things get tricky – reverse phase silica (for v polar compounds); alumina for acid sensitive (sometimes just putting in 0.5% Et_3N works instead); ion exchange media (dowex); size exclusion media (sephadex or carbon:celite)
- we have a list of solvents for TLC and columns on the website.

Recrystallization
- will give you a much purer sample than flash chromatography
- you may need to re - recrystallize the mother liquor in order to recover all material
- it may become a question of trial and error
- there is an art to recrystallization – don't be discouraged – it *is* worth persevering as it's easier, quicker and better than flash chromatography.
- There are many different ways of doing it – ask other people –everyone has their favourite – the books I recommend at the top have their recommendations.
- If you get any vaguely good looking crystals – go get a crystal structure (you can get training to run these on a DIY basis – see the Xtallography website)

Always NMR your products and file them – even the crude rubbish looking ones – you never know when you will want them. Record all details.

IF IN DOUBT - ASK

<u>Reaction Data</u>

All products of reactions should be characterized – we now have an excel spreadsheet checklist – use it!

Known Compounds

- proton NMR
- optical rotation $[\alpha]_D$
- mp

- IR (if informative)

Unknown Compounds

- proton NMR
- carbon 13 NMR
- optical rotation $[\alpha]_D$
- mp
- IR (KBr disc if solid; film from volatile solution if oil)
- mass spec
- microanalysis (the most important within 0.3% for solids and 0.4% for oils)

High Resolution Mass Spec (HRMS) can serve as a poor alternative to Microanalysis but ONLY when several attempts at microanalysis have failed (re-purifying between each submission)

To keep track of characterization you should construct a data sheet for each compound.

In addition you should keep a table that displays which you have characterized and which you have not (use the lab excel spreadsheet). There is a useful ACS Characterization Checklist as an excel file that I can let you have.

Keep all of your data in a well organised form. e.g. ring-binder or document holder or cardboard folders - file your information as soon as you get it - if not you will lose it.

A good idea is to have two filing systems:
1) a folder or file per compound that contains 'good' NMRs/data ie your characterization data that could be asked for by examiners and that will be scanned for any papers
AND
2) and a separate ring-binder to keep the NMRs for day-to-day experiment-by-experiment work, even stuff that you might think is rubbish at the time

This means that when I speak to you I will expect you to be able to have at hand a folder which contains all of the data for a given experiment (eg crude NMR, grubby MS etc) and one that contains the really good characterization data that you use for your written characterization. Be clear that if you cannot retrieve the exact data in your viva examination then this is quite rightly grounds to fail you. The same can be said for manuscripts/papers. Having this data is part your ethical responsibilities and is one of the bedrocks of science.

All of this data should be in good order and available.

My main advice is do not let this slip – it is so hard to catch up missed characterization and you're work is ***a. not good enough to pass a thesis standard*** *and* ***b. unpublishable*** *without full characterization.*

<u>Mol Bio Data</u>

The same precision goes for Mol Bio or more Biological work too:

- sufficient duplicates on runs to created errors
- copies of all gels (protein and DNA - electronic and hard), blots etc

- sequencing data
- protocols, batch numbers of kits/reagents
- precise conc and volumes

You should ensure that you have deposited plasmid (several copies) and glycerol (ditto) for all key recombinant work in the storage/library -80 freezers. This is a key legacy and needs to be accurate.

Reports

- Those of you who are on CASE awards or industrially-sponsored projects will need to prepare a progress report every quarter or so anyway (1st Jan, 1st Apr etc..). But I think they are useful for everyone – so I'd like you all to pop them in – I certainly found that it made writing up my first-year report and final thesis much easier having had the head start of the reports. It is also really useful, so that we can sort of take stock of progress and direction – it often provides you with a cracking opportunity to shape the project in the way you want it to go.
- Always put experimental procedures in – this is the real grind of writing up your thesis but its is the bit that examiners go through with a fine tooth comb. Most importantly this is your legacy and the methods that you invent will be used by many to come so write them in as much detail as possible.
- If you give them to me as Word files and pdfs too this will make my life much easier.

Chemicals, Reagents and COSHH forms

- As we're split across several labs, then we'll all have to be flexible. Clearly the chemical store will be in labelled cupboards & there is a chemical but this system needs to be shared and we all need to take responsibility for keeping it up-to-date. The -20 freezers (Chemicals and Biological) and Fridges will hold other key stuff. Most mol. bio. stuff will need to used responsibly as contamination or improper handling can destroy biocatalysts and fragile protein reagents and their ineffectiveness will not be realized until the expt is run on many occasions – please treat these communal stocks with respect.
- If you do whip stuff or lend it out please change the location box on the computer catalogue so that someone else can find it.
- In case the computer that we use for the catalogue gets knackered back up the chemical catalogue needs to be up to date every time we have a lab tidy up – this should be being automatically backed up onto Victoria (or another server) but please do check.
- If you find a chemical that's in the wrong place or not on the computer then just put it back or change the details as appropriate.
- COSHH assessment is part of your exptl write up – to make life easier a file should contain safety assessments or data sheets of all the chemicals we have but there may be some missing – if there's no sheet fill in/ print off the web a new one – otherwise we will get a shed load of grief.
- remember our biological stuff will need BIOCOSHH forms too – typically Level I containment (if in doubt check with the Biol. Safety Officer). Please save these to the BIOCOSHH folder, once signed.

Your samples

-Keeping these in the freezer/fridge under nitrogen makes sense if you're going to store them unless you know that they are stable enough to be left on your bench
- Label them so that someone doesn't lob them away or in case you forget about them and they're there for years. Marker pens tend to only last a short time so cardboard tags are the best even if they are a bit of a hassle (pencil lasts longer than some pens on these cardboard tags)

- With samples that you are sure that you aren't going to use again/for a while vac them down into small vials and put them in a box (e.g. labelled lunchbox type thing) for yourself in the storage freezer. You'll notice a tray in there full of my junk that I've accumulated over the years. It's well possible that someone will want them in the future including you when you are writing up. You'll need to do this as part of your tidying up for leaving anyway so you might as well start now.
- There is a checklist for leaving the group that will help you with this.
- Remember plasmids too.

Group Meetings

Twice a week we get together as a group for 2 types of meetings:
• Research Presentation [usually Thurs 8-9] – talk for 30 min about your results since you last talked. This doesn't have to be just about what worked but should also give everyone else an idea of what you're doing, the background etc. It's also a great place to discuss things that haven't worked as hopefully you'll be sitting in a room full of big brains. On this basis there will *always* be something to discuss [unless you have genuinely done no expts for 6 weeks, in which case you are a). in trouble or b). have used up all your holiday or c.) both]. Feel free to use board, overheads, powerpoint…whatever – as long as you feel happy you can get the info across.
• Problem / Literature Meetings [usually Tues morning 8.30am-9.30am] covering one or more of:
a. Lit. presentation – talk about some work of note that you've seen published in the last 6 months to a year. Don't forget to tell us why you think it's great (or not).
b. Problems – sometimes set earlier (sometimes not) - all take turns to go through them – it doesn't matter if you can't do them as we all chip in and try to help – don't forget it's good to practice youre general understanding of chemistry in this way (sometimes up to 90% of time in a *viva* can be spent on it). We normally split into smaller groups for this.
c. Tips – some form of handy method/lab technique that you've stumbled upon – no matter how simple
• Griping sessions – these discussions about how things are running are well acceptable and in fact often very necessary to keep things ticking along – the first 10 mins of either group meeting are fair game for these – just chip in, no matter how small it might seem (if it's worrying you then it isn't small) – anything is a fair topic of discussion.

Who does what when will be put out in a timetable every quarter or so. There is also a guidance sheet on the website that will give you more details and up-to-date methods for how we hold these as they change.

Being late for Group Meetings can happen, especially when this is in the morning but I think you should show sufficient respect for those presenting to be there on time. They (like you) will work hard on presentations and they want you to be there. Strolling in late is a very clear way of saying "This meeting and your work was not important enough for me to be here on time". Yes, there may be mishaps that make you late on rare occasion (no problem – just let us know by text / call) but if you are consistently 5 min late then this is just a lack of respect and shows that you don't value your colleagues – horrible!

Papers and Reading

- Learning stuff is always good so try to find time to go to the library or online to look up new stuff as well as papers that you need. If you find stuff that you think others will find useful then whack it into paper boxes in the write-up room or on the journal table
- Boxes can be labelled with various headings usually according to loose project areas. I will bung papers and journals that I think are useful into them now and then. I'm not allowed by copyright laws to give you all copies of papers so the copy in the box will probably be the only one. I will

also send by email. You are all allowed to copy a copy (I know it makes no sense) – but if you do, don't forget to put it back so that others (including me) can find it (if the box is not there then someone has nicked it – go find it).
- We also one copy of each thesis and QR in bound form on the shelves (again if not there then someone has nicked it).
- These boxes should be a good place not only to find background literature but nitty-gritty papers that you've just photocopied for their experimental method.
- Don't forget to keep your own file of papers too.
- Think about using a database programme like Papers or EndNote. We tend to use .enl files when writing papers to keep things all together.

The Write Up Room

- This is your communal area to do with as you like; feel free to use it as your base.
- Desk space and computers may need to be shared but it's good to have somewhere to dump your stuff so grab one of the desk bays each. This will make a good place for me to dump stuff for you as well.
- There are communal books, the boxes of papers, computers, a coffee maker, fridge – if you want more stuff ie books, new computers, a microwave - let me know and we can probably arrange it
- I'll occasionally bung things up on the notice boards that I think might be useful.
- There are lockers in the lab for keeping your stuff safe (Get the keys from buildings staff (Kim or Joy in 2014) – these are first come-first serve so don't hang about).
- Also make use of the filing cabinets.
- Make sure to lock up the computers (esp. laptops) so that they don't get tea-leafed.
- Old stuff can be chucked or stored if you need to make space. Ask if you are unsure.
- Overall keep the place relaxed (our group 'spirit') but tidy and safe.

Closing-Up

If you're the last one out of the lab then just do a few things before you bog off
- switch off non-permanent kit
- make sure there are cards and stuff on overnight reactions
- check taps and ting – floods are a real killer and can be caused by only a wee flow
- lights

HPLC & GC

HPLC is a good but somewhat special method. In principle - anything can be run - any system, solvent etc - the limitation is usually finding a method of detecting your compound/molecule - most of all 3 things look possibly very useful and you should consider using them when necessary:

(i) Preparative HPLC - for very tricky to separate samples this will allow the isolation of upto say 30 mg (enough for characterization) of pure compound - normally analytical HPLC (the standard method) only provides a few mg.
(ii) Chiral HPLC - chiral columns are available which allow the determination of enantiomeric excesses in racemic mixtures - very useful for resolutions.
(iii) M/z detection - normally HPLC is limited by the detection method and until recently this has usually meant one of the following:

a. UV/vis/fluorescence - you need a chromo/fluorophore - not always an option unless one present in say a protecting group or residue

b. refractometry/light scattering
c. conductimetry
d. turbidity
e. Polarimetry - detection of anything that has an optical rotation (the greater the rotation - the greater the sensitivity) - this is obviously a winner for sugars

Obviously if large amounts are needed then HPLC will not be the answer - but if you are having hassles analysing/separating samples - please do make use of this.

There is also communal gas chromatography kit – same room as polarimeter.

Keep and treat columns with care. It is easy to 'strip', ruin, contaminate them and they are not cheap so we may run out of one way of solving your experimental problems if a column is 'lost' too many times.

Me

Any hassles *at all* then let me know – especially if they're to do with me being useless/unhelpful – we are all learning all the time – that's what life's all about. Don't forget that at the end of your project your should be much more of an expert than me – I'll be disappointed if you're not.

Above all, communication is key. Talking is always the best way (face-to-face, phone, Skype). Many 'modern' methods lack the nuance and sophistication of the personal touch. I think that email is pretty poor on the whole – it is good for transferring data but not much else. Don't assume that people always will have read things or know things. Ask, discuss, learn.

AN EXAMPLE OF EXPERIMENTAL STYLE

*4.2. 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl methanethiosulfonate **1***

Acetobromoglucose (**13**) (1 g, 2.43 mmol) was added to a solution of $NaSSO_2CH_3$ (380 mg, 2.84 mmol) in ethanol (4 ml) at 90° C under N_2. After 20 min. the resulting suspension was cooled and the solvent removed. The residue was purified by flash chromatography (EtOAc : hexane, 9:11) and the resulting solid recrystallized from ether to give **1** (674 mg, 63%) as a white solid; mp 151-152° C melts then decomp. (ether); $[\alpha]^{27}_{D}$ = -19.0 (*c* 1.24, $CHCl_3$); IR (KBr) 1749 cm^{-1} (C=O), 1333, 1140 cm^{-1} (S-SO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00, 2.04, 2.06, 2.07 (s × 4, 3H × 4, Ac × 4), 3.44 (s, 3H, CH_3SO_2-), 3.82 (ddd, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 5.9 Hz, $J_{5,6'}$ 2.2 Hz, 1H, H-5), 4.08 (dd, $J_{5,6}$ 5.9 Hz, $J_{6,6'}$ 12.5 Hz, 1H, H-6), 4.31 (dd, $J_{5,6'}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, 1H, H-6'), 5.05 (t, *J* 9.8 Hz, 1H, H-4), 5.07 (dd, $J_{1,2}$ 10.5 Hz, $J_{2,3}$ 9.4 Hz, 1H, H-2), 5.25 (d, $J_{1,2}$ 10.5 Hz,1H, H-1), 5.29 (t, *J* 9.3 Hz, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5, 20.7 ($\underline{C}H_3COO$- × 4), 52.8 (CH_3SO_2-), 61.8, 68.0, 68.7, 73.3, 76.6 (C-2, C-3, C-4, C-5, C-6), 86.4 (C-1), 169.3, 169.3, 169.7, 170.1 ($CH_3\underline{C}OO$- × 4); HRMS m/z (EI+): Found 443.0636 ($M+H^+$); $C_{15}H_{23}O_{11}S_2$ requires 443.0682.

Flash Chromatography:

Flash chromatography, also known as medium pressure chromatography, was popularized several years ago by Clark Still of Columbia University, as an alternative to slow and often inefficient gravity-fed chromatography. Flash chromatography differs from the conventional technique in two ways: first, slightly smaller silica gel particles (250-400 mesh) are used, and second, due to restricted flow of solvent caused by the small gel particles, pressurized gas (*ca.* 10-15 psi) is used to drive the solvent through the column of stationary phase. The net result is a rapid ("over in a flash") and high resolution chromatography.

Selecting a Solvent System

The compound of interest should have a TLC R_f of ≈0.15 to 0.20 in the solvent system you choose. Binary (two component) solvent systems with one solvent having a higher polarity than the other are usually best since they allow for easy adjustment of the average polarity of the eluent. The ratio of solvents determines the polarity of the solvent system, and hence the rates of elution of the compounds to be separated. *Higher polarity of solvent increases rate of elution* for ALL compounds. Common binary solvent systems in order of increasing polarity are dichloromethane/hexane, ether/hexane, hexane/ethyl acetate, and dichloromethane/methanol. Hexane/ethyl acetate can be used on the bench, all other solvents should be used in the hood. If your R_f is a ≈0.2, you will need a volume of solvent ≈5X the volume of the dry silica gel in order to run your column.

Determining the Quantity of Silica Gel Required

The amount of silica gel depends on the R_f difference of the compounds to be separated, and on the amount of sample. For *n* grams of sample, you should use 30 to 100 *n* grams of silica gel. For easier separations, ratios closer to 30 : 1 are effective, for difficult separations, more silica gel is often required. However, by using more silica gel, the length of time required for the chromatography is extended. The density of powdered silica gel is about 0.75 g per mL.

Packing the Column

Obtain a glass column and make sure that it has either a glass frit or a plug of cotton wool directly above the stopcock to prevent the silica gel from escaping from the column through the stopcock. (IF it doesn't have either, you will have to put in a somewhat loosely stuffed plug of cotton wool; if you stuff it too much, solvent flow becomes painfully slow even with air pressure above the column). Next, put a ~1/2 in. layer of clean sand above the plug of glass wool. Use only as much as is necessary to obtain a flat surface, with the same diameter as that of the body of the column. Make sure the surface is flat. Then pour in the silica gel using a funnel. DO THIS STEP

IN THE HOOD! Silica gel is chemically similar to asbestos, and is a known carcinogen. Manipulations with dry silica should be done only in the hood.

Solvating the Silica Gel Column

Next, tap gently and evenly the sides of the column with a piece of rubber tubing to settle the silicagel. Pour a good amount of your elution solvent onto the silica gel. Use pressurized gas to force the solvent through the silica. As you force through a few hundred milliliters, you should see the top part of the silica become more homogeneous. This is because you are forcing out air that was entrapped in the silica gel. Continue to flush solvent through the silica gel until the entire silica plug becomes homogeneous in appearance. You may have to recycle the solvent coming through the column onto the top of the column several times before all the silica gel is solvated. Do not let the top of the column run dry, otherwise you will force air back into the top of the silica, and you will be back where you started.

Applying the Sample

Allow the solvent which remains above the silica to drain down until it is flush with the surface of the silica. If the top surface of the silica gel is not flat, gently tap the side of the column until it is. Dissolve your sample into the minimum volume of the elution solvent. Apply this to the top of the column, being careful not to disturb the top of the silica. Allow the sample to soak into the silica. Next, rinse the sides of the column with as **few** as possible milliliters of the elution solvent. Let this soak into the silica. After the rinsings have soaked into the silica gel, add a small amount of sand to protect the top surface of the silica when you add more solvent.

Eluting the Sample

Add a good part of your elution solvent to the column. Apply pressure to force the solvent through the column. The pressure should be the minimum necessary to keep a steady stream coming out of the column. Be aware that if you have chosen your solvent properly, it will take a little while before your compound of interest begins to elute. This means that the solvent, at first, contains none of your compound and can be discarded. If the R_f of your compound is 0.33 or less, you should be safe discarding an amount of solvent equal to the volume of the dry silica you used for the column. When you have collected this much solvent, begin collecting the eluted solvent into separate test tubes (fractions). When you have used all of your solvent, your sample should have finished eluting into the test tubes you collected. To maximize the efficiency of your chromatography, the fractions you collect should be no more than about one tenth of the column volume. For example, if you use 25 g of silica gel you should collect fractions of about 3 mL.

Locating the Sample

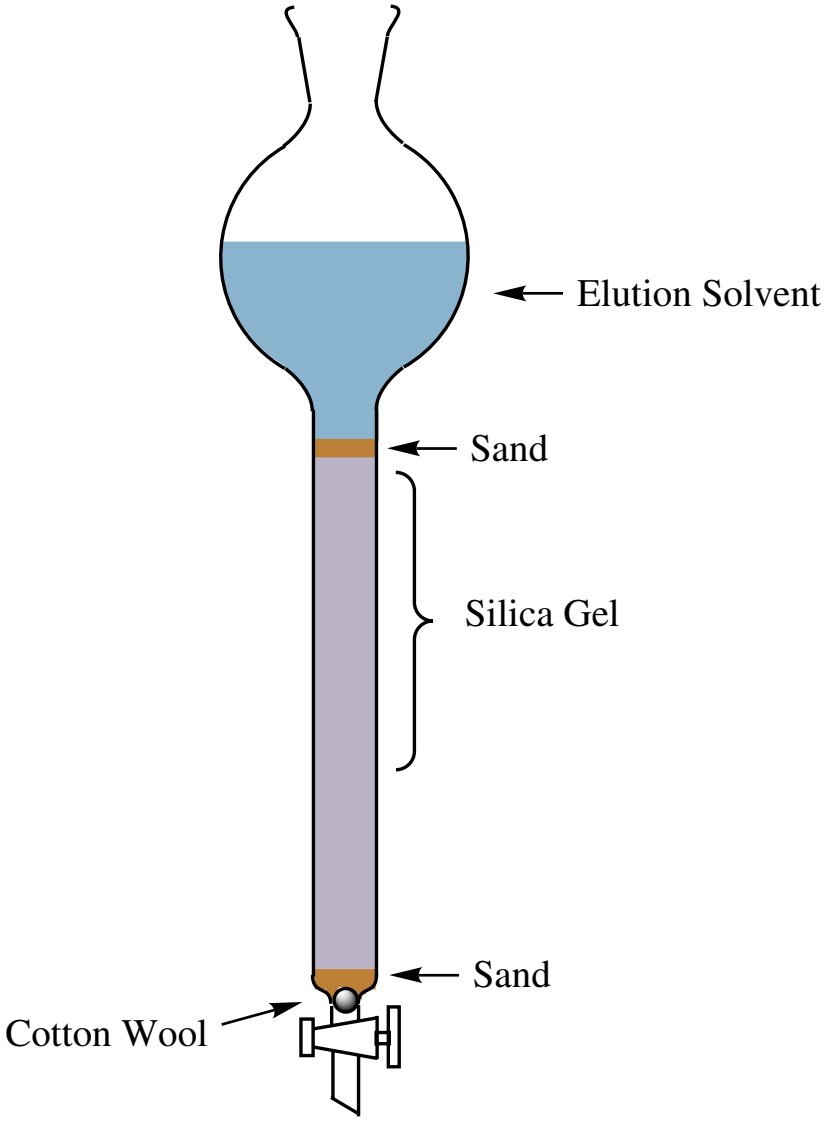
Use TLC to determine which fractions contain your compound. As the fractions fill, you should analyze each using TLC. It is **best to spot 10 fractions on one TLC plate and elute that 10 lane plate once**, rather than conducting individual analyses for each fraction. Combine the fractions that contain your sample together in a flask, then concentrate the sample on the rotavap (rotary evaporator).

Cleaning the Column

Flush all the remaining solvent out of the column using pressurized gas. When all liquid solvent has bee removed from the reservoir, remove the last remnants of solvent by applying a vacuum (from aspirator) to the bottom of the column.

Dispose of the used silica gel in a special collection container!!

Flash Chromatography Column



Original paper for Clark Still's

flash chromatography technique

Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution

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We wish to describe a simple absorption chromatography technique for the routine purification of organic compounds. Large scale preparative separations are traditionally carried out by tedious long column chromatography. Although the results are sometimes satisfactory, the technique is always time consuming and frequently gives poor recovery due to band tailing. These problems are especially acute when samples of greater than 1 or 2 g must be separated. In recent years several preparative systems have evolved which reduce separation times to 1–3 h and allow the resolution of components having $\Delta R_f \geq 0.05$ on analytical TLC. Of these, medium pressure chromatography[1] and short column chromatography[2] have been the most successful in our laboratory. We have recently developed a substantially faster technique for the routine purification of reaction products which we call flash chromatography. Although its resolution is only moderate ($\Delta R_f \geq 0.15$), the system is extremely inexpensive to set up and operate and allows separations of samples weighing 0.01–10.0 g[3] in 10–15 min.[4]

Flash chromatography is basically an air pressure driven hybrid of medium pressure and short column chromatography which has been optimized for particularly rapid separations. Optimization studies were carried out under a set of standard conditions[5] using samples of benzyl alcohol on a 20 mm × 5 in. column of silica gel 60 and monitoring the column output with a Tracor 970 ultraviolet detector. Resolution is measured in terms of the ratio of retention time (r) to peak width (w,$w/2$) (Figure 1), and the results are diagrammed in Figures 2–4 for variations in silica gel particle size, eluant flow rate, and sample size.

A number of interesting facts emerge from these data. First, we find that one of the most popular grades of silica gel 60, 70–230 mesh (63–200 μm), gives the poorest resolution of any gel studied under our standard conditions. Second, particle sizes less than 40 μm offer no improvement in resolution with our method of packing.[7] Column performance is quite sensitive to the rate of elution and is best with relatively high eluant flow rates. The solvent head above the adsorbent bed should drop 2.0 ± 0.1 in./min for optimum resolution with mixtures of ethyl acetate/petroleum ether (30–60 °C).[8] Finally, the peak width shows the expected increase with the sample size. Sample recovery was ≥95%.

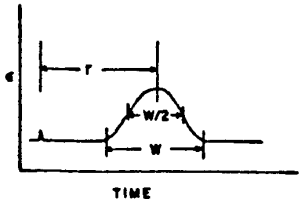


Figure 1. Typical chromatogram.

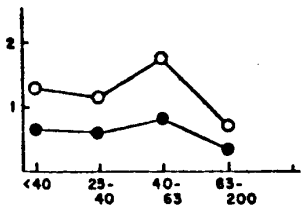


Figure 2. Silica gel particle size[6] (μm): (●) r/w; (○) $r/(w/2)$.

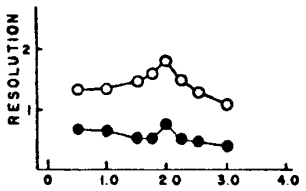


Figure 3. Eluant flow rate (in./min).

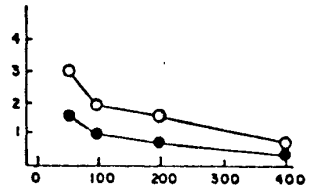


Figure 4. Sample size (mg).

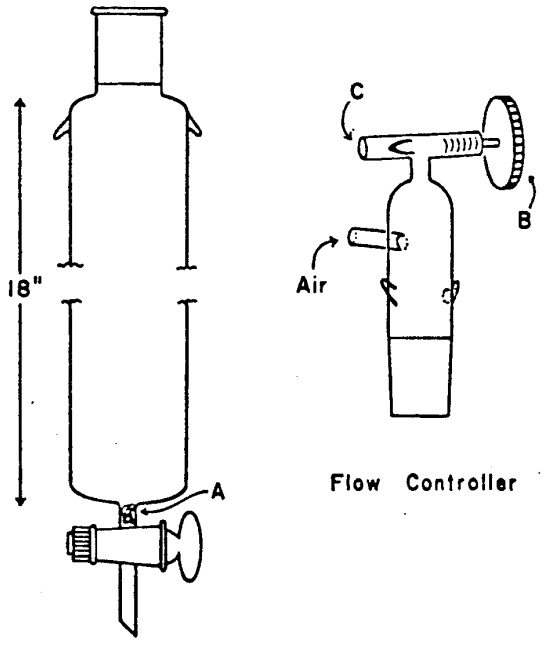


Figure 5.

The apparatus required for this technique consists of a set of chromatography columns and a flow controller valve (below). The column is a flattened bottom 18 in. glass tube fitted with a Teflon stopcock and topped with a 24/40 glass joint. Columns without fritted glass bed supports are generally preferred since they have significantly less dead volume than the standard fritted round-bottom variety. The flow controller

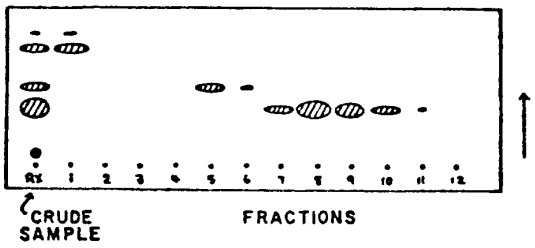


Figure 6.

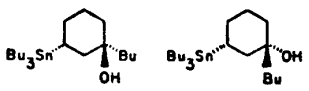
valve is a simple variable bleed device for precise regulation of the elution rate and is constructed from a glass/Teflon needle valve (Ace Glass Co. No. 8193-04 or equivalent) and a standard 24/40 joint.

A detailed procedure is presented in the experimental section and is summarized as follows: (1) A solvent is chosen which gives good separation and moves the desired component to R_f = 0.35 on analytical TLC (E. Merck No. 5765).[9] (2) A column of the appropriate diameter (see Table I) is selected and filled with 5–6 in. of dry 40–63 μm silica gel (E. Merck No. 9385).[10] (3) The column is filled with solvent and pressure is used to rapidly push all the air from the silica gel. (4) The sample is applied and the column is refilled with solvent and eluted at a flow rate of 2 in./min.

The time required to elute the desired components from the column is generally so fast (5–10 min) that we have abandoned automatic fraction collectors in favor of a simple rack holding forty 20 × 150 mm test tubes. Small fractions are typically collected early in the elution with larger ones being collected toward the end of the chromatography. Separated components are conveniently detected by spotting ~5 μL of each fraction along the long side of 7 cm × 2.5 cm TLC plate and then by developing the plate sideways. Heavier spotting may be required for small samples or highly retentive components. A typical separation is shown in Figure 6.

Over the past year we have run many hundreds of these columns. In every case we have been able to effect clean separation of compounds having $\Delta R_f \geq 0.15$ in less than 15 min and in many cases separations at $\Delta R_f \simeq 0.10$ were possible. The amount of sample used on a given column is proportional to its cross-sectional area and Table I can serve as a guide to column selection.

The sample size may increase substantially if less resolution is required; we have used a 50-mm column for the purification of up to 10 g of compound having impurities at $\Delta R_f \geq 0.4$. Resolution is maintained even with large diameter columns. For example the epimeric alcohols 1 and 2 have an R_f of 0.34



1 2

and 0.25, respectively, in 5% ethyl acetate/petroleum ether. A 1.0-g mixture of 1 and 2 (ΔR_f = 0.09) easily separated with only a 65-mg mixed fraction in 7 min on a 40-mm diameter column (500 mL of 5% EtOAc/petroleum ether).

If the components to be separated are closer on TLC than ΔR_f 0.15, increased resolution may be achieved by using a longer (e.g., 10 in.) column of gel alternatively a less polar solvent can be used. Such a solvent can be selected to move the desired components on TLC to R_f = 0.25 without increasing the elution times too drastically. In either case, the column should be only lightly loaded with sample and a rapid flow rate of 2 in./min should be maintained. Slower flows clearly give poorer resolution with ethyl acetate/petroleum ether mixtures.

Table I

column diameter, mm	vol of eluant,[a] mL	sample: typical loading (mg) $\Delta R_f \geq 0.2$	sample: typical loading (mg) $\Delta R_f \geq 0.1$	typical fraction size, mL
10	100	100	40	5
20	200	400	160	10
30	400	900	360	20
40	600	1600	600	30
50	1000	2500	1000	50

[a] Typical volume of eluant required for packing and elution.

In conclusion, flash chromatography provides a rapid and inexpensive general method for the preparative separation of mixtures requiring only moderate resolution. Even in cases where high resolution is required, preliminary purification by the flash technique allows simplified high-resolution separations without contamination of expensive HPLC columns. Finally, we would like to stress the facts that use of the 40–63 μm silica gel and a pressure- (and not vacuum-) driven flow rate of 2.0 in./min are crucial for successful separations by this method.

Experimental Section

Chromatography columns and the flow controller valve were assembled as described in the text. The silica gel used was 40–63 μm (400–230 mesh) silica gel 60 (E. Merck No. 9385).[10] Solvents were distilled prior to use. Thin layer chromatograms (TLC) were run on glass supported silica gel 60 plates (0.25-mm layer, F-254) (E. Merck No. 5765).

Flash Chromatography. General Procedure. First a low viscosity solvent system (e.g., ethyl acetate/30–60 °C petroleum ether)[8] is found which separates the mixture and moves the desired component on analytical TLC to an R_f of 0.35.[9] If several compounds are to be separated which run very close on TLC, adjust the solvent to put the midpoint between the components at R_f = 0.35. If the compounds are widely separated, adjust the R_f of the less mobile component to 0.35. Having chosen the solvent, a column of the appropriate diameter (see text, Table I) is selected and a small plug of glass wool is placed in the tube connecting the stopcock to the column body (A in the diagram above). Two telescoping lengths of glass tubing (6 and 8 mm o.d.) make placement of the glass wool plug easy. Next a smooth ⅛ in. layer of 50–100 mesh sand is added to cover the bottom of the column and dry 40–63 μm silica gel is poured into the column in a single portion to give a depth of 5.5–6 in. With the stopcock open, the column is gently tapped vertically on the bench top to pack the gel. Next a ⅛ in. layer of sand is carefully placed on the flat top of the dry gel bed and the column is clamped for pressure packing and elution. The solvent chosen above is then poured carefully over the sand to fill the column completely. The needle valve (B) of the flow controller is opened all the way and the flow controller is fitted tightly to the top of the column and secured with strong rubber bands. The main air line valve leading to the flow controller is opened slightly and a finger is placed fairly tightly over the bleed port (C). This will cause the pressure above the adsorbent bed to climb rapidly and compress the silica gel as solvent is rapidly forced through the column. It is important to maintain the pressure until *all* the air is expelled and the lower part of the column is cool; otherwise, the column will fragment and should be repacked unless the separation desired is a trivial one. Particular care is necessary with large diameter columns. The pressure is then released and excess eluant is forced out of the column above the adsorbent bed by partially blocking the bleed port (C). The top of the silica gel should not be allowed to run dry. Next the sample is applied by pipette as a 20–25% solution in the eluant to the top of the adsorbent bed and the flow controller is briefly placed on top of the column to push all of the sample into the silica gel.[11] The solvent used to pack the column is ordinarily reused to elute the column. The walls of the column are washed down with a few milliliters of fresh eluant, the washings are pushed into the gel as before, and the column is carefully filled with eluant so as not to disturb the adsorbent bed. The flow controller is finally secured to the column and adjusted to cause the surface of the solvent in the column to fall 2.0 in./min. This seems to be an optimum value of the flow rate for most low viscosity solvents for any column diameter with the 40–63 μm silica gel. Fractions are

collected until all the solvent has been used (see Table I to estimate the amount of solvent and fraction size). It is best not to let the column run dry since further elution is occasionally necessary. Purified components are identified as described in the text by TLC. If the foregoing instructions are *followed exactly, there is little opportunity* for the separation to fail.

Although we generally pack fresh columns for each separation, the expense of large-scale separations makes it advantageous to reuse large diameter columns. Column recycling is effected by first flushing (rate = 2 in./min) the column with approximately 5 in. of the more polar component in the eluant (generally ethyl acetate or acetone) and then with 5 in. of the desired eluant. If the eluant is relatively nonpolar (e.g., ≤10% EtOAc/petroleum ether), it may be more advisable to use a flushing solvent (e.g., 20–50% EtOAc/petroleum ether) which is somewhat less polar than the pure high polarity component.

Registry No.—1, 66417-28-5; 2, 66417-27-4.

References and Notes

(1) Such units have been described and used extensively by J. M. McCall, R. E. TenBrinkt, and C. H. Lin at the Upjohn Company and A. I. Meyers at Colorado State University.
(2) B. J. Hunt and W. Rigby, *Chem. Ind. (London)*, 1868 (1967).
(3) *This is not a limitation but is merely the scale range which we have used.*
(4) This is the total time required for column packing, sample application, and complete elution.
(5) Standard conditions: 5 in. high bed of 40–63 μm silica gel 60 in a 20 mm diameter column packed as described in text, 2.0 in. of solvent flow/min, 200 mg of benzyl alcohol, 25% ethyl acetate/petroleum ether eluant.
(6) These gels are manufactured by E. Merck and are the following grades: <40 μm (silica gel H, No. 7736), 25–40 μm (LiChroPrep Si60, No. 9390), 40–63 μm (silica gel 60, No. 9385), 63–200 μm (silica gel 60, No. 10180).
(7) Slurry packing, incremental dry packing, or single portion dry packing gave identical results with the 40–63 μm gel. Since the last technique was the simplest, it was employed in all our studies.
(8) This is a particularly good general solvent system. For extremely polar compounds, acetone/petroleum ether or acetone/methylene chloride mixtures are often useful. Significantly higher viscosity solvents will require slower optimum resolution flow rates.
(9) If this R_f is given by a solvent having <2% of the polar component, a slightly less polar eluant is desirable. Thus if 1% ethyl acetate/petroleum ether gives a compound an R_f of 0.35 on TLC, the column is run with 0.5% ethyl acetate.
(10) 40–63 μm gel is also used for medium pressure chromatography[1] and is available from MCB in 1 kg ($45/kg) or 25 kg ($16/kg) lots.
(11) If the sample is only partially soluble in the eluant, just enough of the more polar component is added to give complete dissolution. Large quantities of very polar impurities are best removed prior to chromatography so that excessive quantities of solvent or large increases in solvent polarity will be unnecessary for sample application.

Homo-*C*-nucleosides. The Synthesis of Certain 6-Substituted 4-Pyrimidinones[1]

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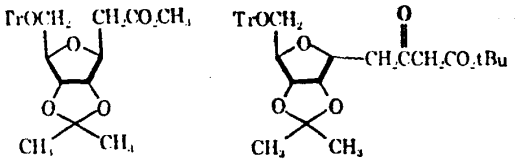
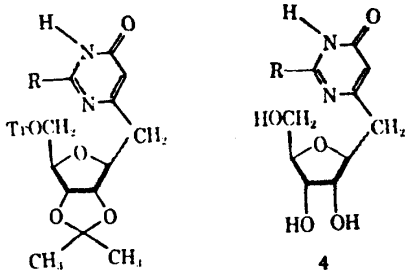
The chemistry of *C*-nucleosides has received considerable attention recently due to the biological activities of naturally occurring compounds such as showdomycin, formycin, and oxazinomycin.[2] Though synthetic methodology has evolved for the preparation of a number of *C*-nucleoside analogues,[2] only one investigation has dealt with the synthesis of homo-*C*-nucleosides,[3] compounds with a methylene unit between a carbon of the nitrogen base and the standard D-ribose moiety. This note describes the facile synthesis of a series of 6-substituted 4-pyrimidinone homo-*C*-nucleosides from the ester 1, which is available in three steps from D-ribose.[4,5]

Treatment of 1 with lithio-*tert*-butyl acetate[6] in toluene at 0 °C for several hours affords an anomeric mixture (ca. 3:1, β/α) of the β-keto ester 2 in 75% yield. The assignment of β to the major anomer was made on the basis of ^{13}C NMR data. In particular, the isopropylidene methyls of the major anomer occur at δ 25.66 and 27.54, within the range strongly indicative of a β configuration (25.5 ± 0.2 and 27.5 ± 0.2).[7,8]



1 2

Tr = trityl



3 4

a, R = NH_2
b, R = CH_3
c, R = SH
d, R = phenyl
e, R = H

It has been shown that the α-anomer of 1 is more stable than the β,[4] and recently a rationalization for this seemingly unusual behavior has been presented.[9] On this basis it seems likely that the α anomer of 2 is also more stable than the β. The conditions involved in the preparation of 2 (low-temperature, aprotic solvent) probably do not allow equilibration, though there is some leakage to the α-anomer. Further support for these postulates is provided by the finding that β-2 is isomerized readily under basic conditions to an α/β mixture which is predominantly α.

Condensation of 2 with guanidine, acetamidine, thiourea, and benzamidine under basic conditions afforded the protected nucleosides 3a–d as anomeric mixtures (ca. 3:1, α/β) which were chromatographically inseparable. That the major anomers after condensation are all α is also indicated by the chemical shifts of the isopropylidene methyls. For example, the shifts of the methyls in 3a are at δ 25.09 and 26.33, clearly in the α range (24.9 ± 0.3 and 26.3 ± 0.2).[7,8] In view of the ready isomerization of β-2 to a mixture of anomers containing predominantly α-2, it seems likely that equilibration is occurring prior to cyclization, and that the anomeric composition of 2 after equilibration dictates the ratio of α- and β-homo-*C*-nucleosides. Desulfurization of 3c with Raney Nickel in refluxing 95% ethanol provided the hydrogen-substituted compound 3e. Interestingly, while both urea and formamidine reacted with 2, neither led to the formation of cyclized material under a variety of conditions. The free nucleosides 4a–e were obtained by treatment of 3a–e with either methanolic hydrogen chloride or aqueous trifluoroacetic acid for several hours. These acidic conditions, even over longer periods of time (2 days), caused no change in the α/β ratio of the nucleosides. Chromatographic separation of the free nucleoside anomers was once again not possible. 4e was also available by desulfurization of 4c.

The ^{13}C NMR spectra of the free nucleosides contained characteristic signals for the five compounds, and all values are reported in the Experimental Section. Salient ^1H NMR values are the methyl singlet of 4b at δ 2.28 and the pyrimidine C_2H singlet of 4e at δ 8.92, as well as the pyrimidine C_5 signal of all five nucleosides in the neighborhood of δ 6.0.

Davis Group Biological Safety

All items relating to biological safety can be found in the 'safety' folder on the Davis group server, and in the 'Biological Safety' folder in the safety area.

Before you begin any biological work:

Obtain a copy of the 'Good Microbiological Practice and Containment' document. Read and familiarise yourself with document, and sign, and get a supervisor to counter-sign.

Obtain a copy of the relevant COSHH assessments from the 'general COSHH assessments for biological work in the Davis Group' document. Read, familiarise, sign and get counter-signed.

Read the risk assessments contained in the folder for any equipment you will use. This currently covers centrifuges, glassware, FPLC, HPLC, the laminar flow hood, autoclaves, and the freeze dryer. Sign the table after the risk assessment to say you have read it.

Before every experiment:

University regulations require that a COSHH assessment be carried out before every experiment that you do. It is acceptable to refer to the generic risk assessments in the 'general COSHH assessments for biological work in the Davis Group', provided that these have been signed off, and provided that the risk assessments are kept with your lab book.

Before undertaking any genetic modification of an organism:

A 'Risk Assessment made under the Genetically Modified Organisms (Contained Use) Regulations 2000' must be completed, and submitted to the department GMO Safety Committee (via Zhihong Zhang). The assessment can be made very generic, so that as many modifications are covered (*for example, cloning a variety of glycosyltransferases into E. coli could be covered by one risk assessment*). No work should be undertaken until approval has been granted by the committee.

The form, and guidelines for its completion are available with the other biological safety material. A copy of the completed form, with relevant signatures, should be obtained from the committee, and placed in the biological safety folder.

Before obtaining any new microorganisms:

If you wish to work with a microorganism that has not been used before in the department, a 'Work with Microorganisms' form must be completed, and submitted to the department Biological Safety Committee (via Zhihong Zhang). The microorganism may not be received in the department until approval has been granted by the committee.
The form is available with the other biological safety material Guidelines for the completion of the form are available at
http://www.admin.ox.ac.uk/safety/oxonly/s195/s195.shtml
A copy of the completed form, with relevant signatures, should be obtained from the committee, and placed in the biological safety folder.

Risk Assessment Procedures

Procedure	Reference
Heated Reaction	BGD 1
Ultraviolet Light Sources	BGD 2
Electrophoresis	BGD 3
Electrical Equipment	BGD 4
DNA Preparation	BGD 5
HPLC	BGD 6
Laminar Flow Hood	BGD 7
Bacterial Growth – category 1	BGD 8
Unltrafiltration – concentration protein	BGD 9
Use of Fume Hoods	BGD 10
Autoclave Use	BGD 12
Handling Glassware	BGD 13
FPLC	BGD 14
Microwave	BGD 15
Disposal of sharps	BGD 17
Washing up Glassware	BGD 18
Sonicator	BGD 19

Those of you who are on CASE awards or industrially-sponsored projects will need to prepare a progress report every quarter or so anyway (1st Jan, 1st Apr etc..). But I think they are useful for everyone – so I'd like you all to pop them in – I certainly found that it made writing up my first-year report and final thesis much easier having had the head start of the reports. It is also really useful, so that we can sort of take stock of progress and direction – it often provides you with a cracking opportunity to shape the project in the way you want it to go.

Dont forget to put in experimental procedures - the more the merrier at this stage - you can always cut this out when you come to write your thesis – this is the real grind of writing up your thesis but its is the bit that examiners go through with a fine tooth comb. Most importantly this is your legacy and the methods that you invent will be used by many to come so write them in as much detail as possible.

- If you give them to me as a pdf and then as a Word and rtf (in case of PC to Mac trouble) files too this will make my life much easier - pretty please.

In terms of format for your reports put in four sections

1.INTRO + BACKGROUND
- dont feel that you have to limit this - put in as many refs as you like - the more you analyze now the better - the report is going to go out to everyone so this will help explain the background to your work to everyone else
As a guide a thesis chapter 1 introduction will have approx 100 refs to key papers so its good to start collecting and reading these now.

2.RESULTS AND DISCUSSION
- analyze all the experiments youve done - even the failed ones - this is important for you
- dont decide which are good and which are bad now - leave that until the end of 3 years - in the meantime bung it all in
- also speculate as to the meaning of the results whether the expt worked or not
- put in weird / small observations (within reason) e.g. if something went off in one solvent or if an NMR looked weird – it might make sense when you come to look back it at it

3.EXPERIMENTAL
- put ALL your experiments in experimental format - ive put at the bottom of this examples of a chemical experimental but all experiments can be written in this way – if you would like examples of our biological exptl (methods section) then ask me please but the distinction in style is pretty arbitrary
- for those of you doing more biological experiments, a standard operating procedure (SOP) AS WELL as the details of a particular experiment should be recorded - this will be the recipe for future people to follow
- put all of the characterizations that you have in

- if the compound is known put this in too
- again you cant put too much in
- even highlighting gaps in you're exptl "i.e. NEED mp" is useful for both you and me

4.FUTURE
- write out bullets as to what you feel you want/have to do in the next quarter
- mundane things such as "get a melting point on compound 6" are just as useful as sweeping goals such as "cure all the world's boils"

LAB BOOKS
- going through these to write your experimental will help you to check that these are all in good nick - examiners do ask to see these and they should all be up to date and ting - and as well filed as all your characterization data

LAB REPORT
- each quarter I'll collect everyone's report together to make a big report that will be hard bound and placed in the write-up area for everyone to have access to - this will give us each a record of everyone else's way of doing things -which is well handy
- to help this can you bung me your reports as word documents, please? – ta
Please don't nick the hard copy as it's the only one.

I hope all of this doesnt see too excessive but starting this now will really save you time most of all even just writing down what you have done and need to do gives you a cool perspective on your project especially the future bit which i found helped to tie up the loose ends in my head and stopped them bugging me so much - catching up missed characterizations and experiments at the end of your 3rd year/Trinity term is no fun - trust me

Aim for the 1st day of the quarter but in case of delay - can i have everyone's report by the 5th day of each quarter at the latest, please?

any questions just give me a shout

ta

ben

E.g. of experimental style for QR (if you are writing for a paper check the particular journal style)

*4.2. 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl methanethiosulfonate **1***

Acetobromoglucose (**13**) (1 g, 2.43 mmol) was added to a solution of $NaSSO_2CH_3$ (380 mg, 2.84 mmol) in ethanol (4 ml) at 90° C under N_2. After 20 min. the resulting suspension was cooled and the solvent removed. The residue was purified by flash chromatography (EtOAc : hexane, 9:11) and the resulting solid recrystallized from ether to give **1** (674 mg, 63%) as a white solid; mp 151-152° C melts then decomp. (ether); $[\alpha]^{27}_D$ = -19.0 (*c* 1.24, $CHCl_3$); IR (KBr) 1749 cm^{-1} (C=O), 1333, 1140 cm^{-1} (S-SO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00, 2.04, 2.06, 2.07 (s × 4, 3H × 4, Ac × 4), 3.44 (s, 3H, CH_3SO_2-), 3.82 (ddd, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 5.9 Hz, $J_{5,6'}$ 2.2 Hz, 1H, H-5), 4.08 (dd, $J_{5,6}$ 5.9 Hz, $J_{6,6'}$ 12.5 Hz, 1H, H-6), 4.31 (dd, $J_{5,6'}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, 1H, H-6'), 5.05 (t, J 9.8 Hz, 1H, H-4), 5.07 (dd, $J_{1,2}$ 10.5 Hz, $J_{2,3}$ 9.4 Hz, 1H, H-2), 5.25 (d, $J_{1,2}$ 10.5 Hz,1H, H-1), 5.29 (t, J 9.3 Hz, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5, 20.7 ($\underline{C}H_3COO$- × 4), 52.8 (CH_3SO_2-), 61.8, 68.0, 68.7, 73.3, 76.6 (C-2, C-3, C-4, C-5, C-6), 86.4 (C-1), 169.3, 169.3, 169.7, 170.1 ($CH_3\underline{C}OO$- × 4); HRMS m/z (EI+): Found 443.0636 ($M+H^+$); $C_{15}H_{23}O_{11}S_2$ requires 443.0682.

*4.3. 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl methanethiosulfonate **2***

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride 3,4,6-triacetate **14** (2 g, 5.722 mmol) was added to a solution of $NaSSO_2CH_3$ (900mg, 6.72 mmol) in DMF (35 ml) under N_2 and stirred for 72 h. The reaction course was followed by t.l.c. (EtOAc:Hexane, 70:30) which showed the consumption of starting material (R_f 0.2) and the formation a major product (R_f 0.4). The solvent was removed and the residue purified by flash chromatography (EtOAc:Hexane, 70:30) to give an off-white powder. The powder was recrystallised (EtOAc/hexane) to give **2** as a white crystalline powder (0.584 g, 25%); mp 139-140° C; $[\alpha]^{20}_D$ = - 33.0 (*c* 0.06, $CHCl_3$); IR (KBr) 3400 (br, N-H), 1748 (C=O), 1657 (amide I), 1542 (amide II), 1311, 1139 cm^{-1} (S-SO_2); ^{1}H NMR δ (300 MHz, CD_3CN) δ 1.84, 1.96, 1.99, 2.01 (s x 4, 3H x 4, Ac x 4), 3.46 (s, 3H, $SSO_2C\underline{H}_3$), 3.9-3.96 (ddd, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 6.2 Hz, $J_{6,6'}$ 10.0 Hz, 1H, H-5), 4.05-4.15 (m, 2H), 4.20 (dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.4, 1H, H-6), 5.00 (pt, J 9.8 Hz, 1H, H-2), 5.28 (pt, J 9.8 Hz, 1H, H-3), 5.40 (d, $J_{1,2}$ 10.8Hz, 1H, H-1), 6.6 (d, J 10Hz, 1H, N$\underline{H}$Ac); ^{13}C NMR (50.3 MHz, CD_3CN) δ 20.1, 20.2 ($\underline{C}H_3COO$- x 3), 22.2 ($\underline{C}H_3CONH$-), 51.8 ($SSO_2\underline{C}H_3$), 52.2, 62.4, 68.6, 72.8, 76.2 (C-2,3,4,5,6), 87.62 (C-1), 169.8, 170.4, 170.4, 170.6 ($CH_3\underline{C}OO$ x 3, $CH_3\underline{C}ONH$);); m/z (ES): 464 (14, $M+Na^+$), 352 (100%, oxazoline + Na^+). Found: C, 41.22; H, 5.43; N, 2.90%; $C_{15}H_{23}O_{10}NS_2$ requires C, 40.81; H, 5.25; N, 3.17%.

Leaving The Group and Tidying-Up

Thanks for all you have done.
Before you leave there are a few things that will make everyone else's life easier after you have gone:

- Reports - we've been doing this as things go along but if there is *anything* that you think you haven't included in what you have given me then let bung it down into a Word document (rtf format please) or PDF and give it to me. This includes things like: Standard Operating Protocols for Bits of Kit, Suggestions, Future Ideas, Drafts of Papers, Things you wanted to Finish but didn't have time. The best way of doing this is probably a collected final report of everything.
- Samples - The compounds that you have leftover will be useful to us. Could you
  a). vac them down into the smallest vials you can easily get them into for storage
  b). label them (pencil on a card tag is best as it won't run or wipe off in the freezer)
  c). get a sandwich/tuppaware box and put them in that in the freezer - labelling the box with your name will help too. If you have a library use the number box system (this may be better in other cases)
  d). if you have been making libraries, in particular, you will need to leave a clear record of any numbering system that you have used
  e). enzymes – depending on the quantity these should be in ependorfs or rbfs labelled in the biological freezer (-20˚C) and in a dessicator as appropriate. Again – the use of good labelling that will last is important here.
  f). bugs – as glycerols - these should be in cryo boxes in the –78˚C freezer – labelled of course
  g) plasmids – all constructs should also be stored as plasmids for longer lasting use – again -80˚ freezer
- Lab Books - These are legal documents for patent discovery etc and belong to the group/university so you must leave these behind (feel free to photocopy them if you want to). Please bring to me before you leave.
- Lockers – we have lockers on the ground floor for storing spectra. Space is limited so when you use these please be efficient – store only essential data ie paper spectra. We do not need copies of papers etc as long as you have referenced these in your reports correctly. Place what you can on CD. Please use box files and boxes to make best use of this space. Please add your name to a label on the outside of the locker.
- Spectra/Characterization/Data - This is key stuff too. Can you make up folders of
  a) "best data" ie the spectra that you used to write you characterisation and label this quite carefully. This is best housed in the same box file as your lab books. This is the stuff that your characterization hinges on.
  b) "day-to-day" data ie nmrs for each expt - this doesn't have to be pristine just easy enough to get to so that we can revisit stuff in the near future Could you get a box file from stores to put them in and then get them put away in the lockers that we have for storage.
- Electronic Files - If you have (or can get) electronic files of any of the following could you email them to me and/or bung them on a CD [if in doubt include it]
  a) papers, reports etc
  b) Xray data and files or pictures
  c) NMR data (FIDs, NUTS files)
  d) IR data
  e) Mass spec data – raw files and processed files with details of history
  f) Kinetic data etc from the UV Kinetics machine
  g) **Scans of key compound data e.g. spectra – [remember that if we publish in an ACS journal we'll need scans of clean spectra for all compounds that don't have microanalysis]**
  h) Scans of gels – these are often vital for theses and papers and should be of as high a quality as possible.
  i) In addition these should be deposited wherever possible in the deptl ACD database.
- Paper drafts – I am always happy to receive any suggestions for papers based on your work at any stage. This will be made easier if all of the above is in place and if we have discussed the initial ideas and stages prior to you leaving.
- Thesis copies – in addition to the copies that you need for the university could you also provide the following copies (I am happy to pay for the costs involved):
  a) 1 extra copy of your softbound submitted copy.
  b) 3 extra copies of your final hardbound: 1 for me, 1 for the DP library, 1 for the group
  c) CD copy of both the thesis prior to submission and then after corrections.

Thanks for doing this, I know it seems highly retentive but without it stuff just gets lost and its the only proper way of making sure you get proper recognition (especially if your work hasn't yet been published) for what you've done and will let us develop the stuff you've done yet further. This is our legacy to science and it should be done properly.
Finally, I owe you multiple gifts/ales/chocolate after this - don't forget to remind me. Ta.

CHECKLIST:

☐Final Report(BGD)	☐Chem Samples in Box&Freezer	☐Protein/MolBiol Samples in Box&Freezer(s)
☐Lab Books(BGD)	☐Spectra in Files&Box in Lockers	☐Scans of Spectra/Gels
☐CD+ACD files(BGD)	☐Paper drafts(if applicable)	☐Thesis Copies (if applicable)

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