Technische Universität Wien

DISSERTATION

Die Synthese deuterierter Analoga, Referenz Standards und metaboliten der Pharmazeutika.

Ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der technischen Wissenschaften unter der Leitung von

Ao.Univ.Prof. Dipl.-Ing. Dr.techn. **Ulrich Jordis**
Institutsnummer: E163
Institut für Angewandte Synthesechemie

eingereicht an der Technischen Universität Wien
Fakultät für Technische Chemie

von

Dipl.-Ing. **Petr Knesl**
Matrikelnummer: 0226289
Gessayova 16, 85103, Bratislava

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Thanks to all my friends and colleagues from our group not only for their kind cooperation, support, valuable suggestions and advices for my work but also for their presence and amicable words in the hard hours met by everyone at times in life.

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I would like to thank my parents, grand parents and all the family, especially my mother for her support and patience in the process of my growth and education.

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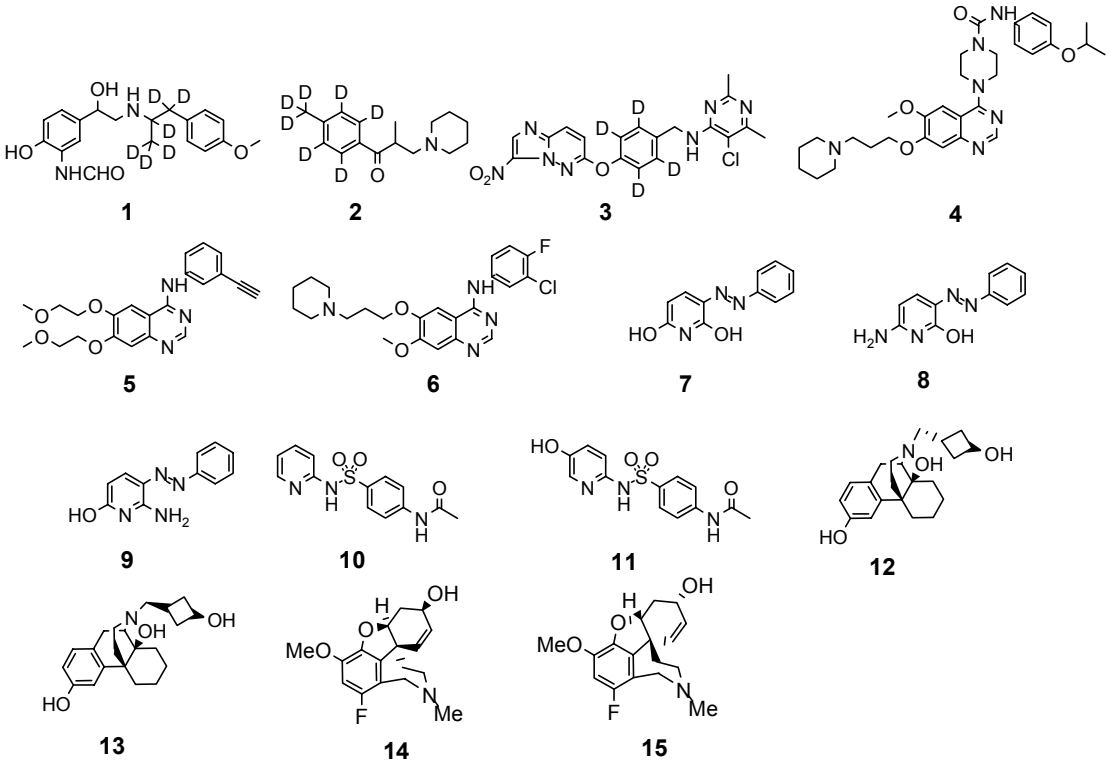
Die Synthese deuterierter Analoga, Referenz Standards und metaboliten der Pharmazeutika.

Das Ziel des ersten Teils der vorliegenden Arbeit war die Synthese deuterierter Analoga pharmazeutisch relevanten Verbindungen, um diese als interne analytische Standards für die LC/MS/MS Analyse zu verwenden. In diesem Teil der Arbeit ist die Darstellung von Formoterol-d_6 (**1**), Tolperison-d_7 (**2**) und BY-170424-d_4 (**3**) beschrieben.

Im zweiten Teil wird deutlich verbesserte Synthesen von Tandutinib (**4**), Erlotinib (**5**) und Gefitinib (**6**) einschließlich einer vollständigen Charakterisierung aller Intermediate präsentiert. Diesen Verbindungen dienen als Referenzproben im rahmen des Drug Matrix Chemogenomics Programms.

Um notwendige Standards für die Zuführung zur NDA (new drug application), wurden im dritten Teil der Arbeit Nebenprodukte der Phenazopyridine (**7**, **8**, **9**) Synthese als API (active pharmaceutical ingrediet) dargestellt und vollständig charakterisiert. Die Hauptmetaboliten von Sulfapyridine, N-Acetyl-sulfapyridine (**10**) und 5-Hydroxy-sulfapyridine (**11**) und beide Isomeren vom Butorphanol Metabolit, trans- (**12**) und cis- hydroxy-butorphanol (**13**), wurden Synthetisiert.

Im vierten Teil ist die Synthese von 8-Fluoro-galanthamin und Trennung der beiden Stereoisomere (**14**, **15**) beschrieben. Die Struktur wurde mittels Röntgenstrukturanalyse bestimmt. Diese Verbindungen sollen hinsichtlich ihrer physiko-chemischen Eigenschaften als Acetylcholinesterase Inhibitoren studiert werden.



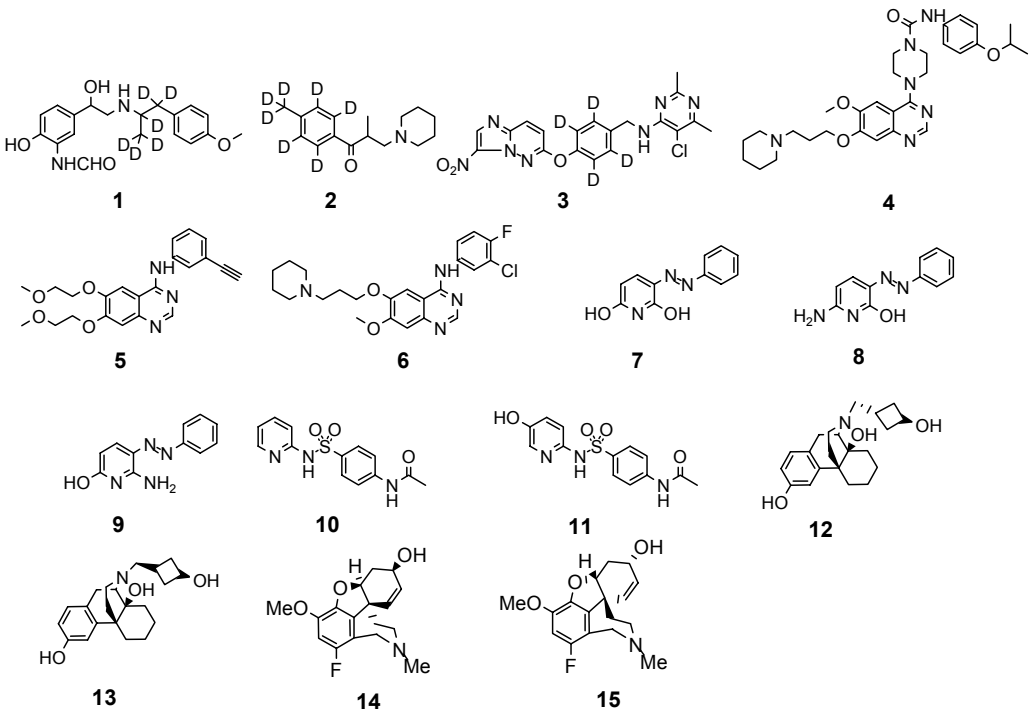
Synthesis of deuterated analogs, reference standards and metabolites of pharmaceuticals.

The aim of the work was the synthesis of deuterated analogs of pharmaceuticals in order to provide an internal analytical standard suitable for LC/MS/MS analysis of very low concentrations of these drugs in body fluids. In this part of the work deuterated formoterol-d_6 (**1**), deuterated tolperison-d_7 (**2**) and deuterated analog of BY-170424 (**3**) were prepared.

Tandutinib (**4**), Erlotinib (**5**) and Gefitinib (**6**) were prepared with substantial improvement and full characterization of the intermediates to be used as reference compounds for the Drug Matrix Chemogenomics program.

In order to provide the analytical standards necessary for NDA submission, the by-products of phenazopyridines (**7**, **8**, **9**) (were synthesized and fully characterized as API (active pharmaceutical ingredient). The main metabolites of sulphapyridine, N-acetyl-sulfapyridin (**10**) and 5-hydroxy-sulfapyridine (**11**) as well as both isomers of oxygenated butorphanol metabolite, trans- (**12**) and cis-hydroxybutorphanol (**13**), were synthesized.

8-Fluoro-galanthamine was prepared and separated into the stereoisomers (**14**, **15**) and the structure confirmed by X-ray analysis in order to be able to study the pharmacological and physical properties of this compound as acetylcholin esterase inhibitor.



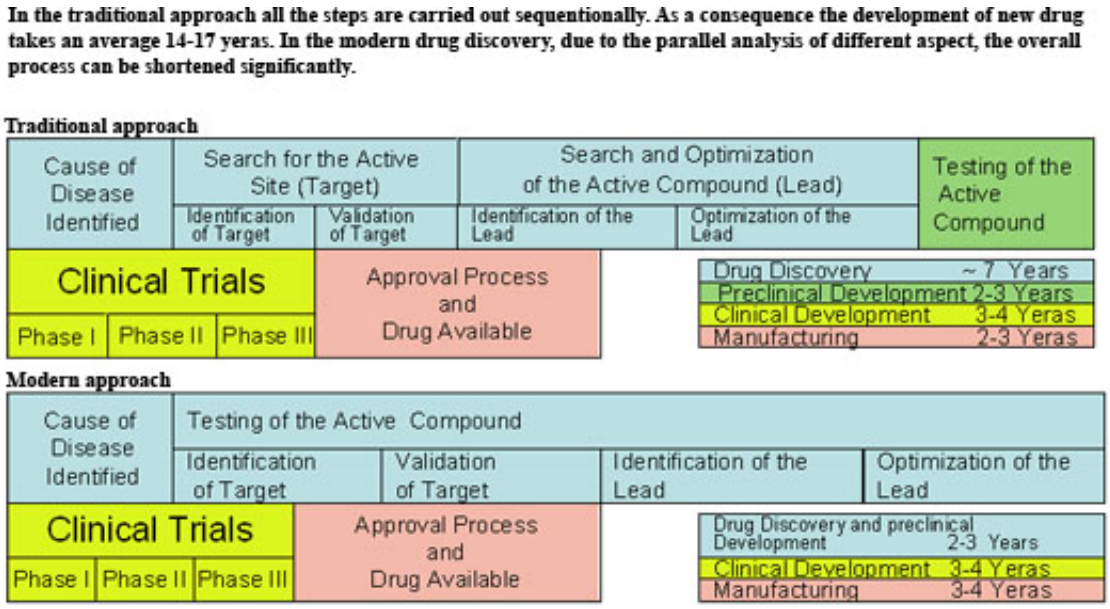
CONTENT

III. Publications

Introduction to drug development:

Drug development can be defined as a series of specialized events performed to satisfy internal and external criteria in order to yield a novel drug. Compound libraries are screened, then hits are selected and modified to get new leads. The leads are optimized until a compound emerges that can be developed into a drug candidate. The process consists of four distinct stages as demonstrated in *Fig. 1*:

FIG. 1.



Drug discovery

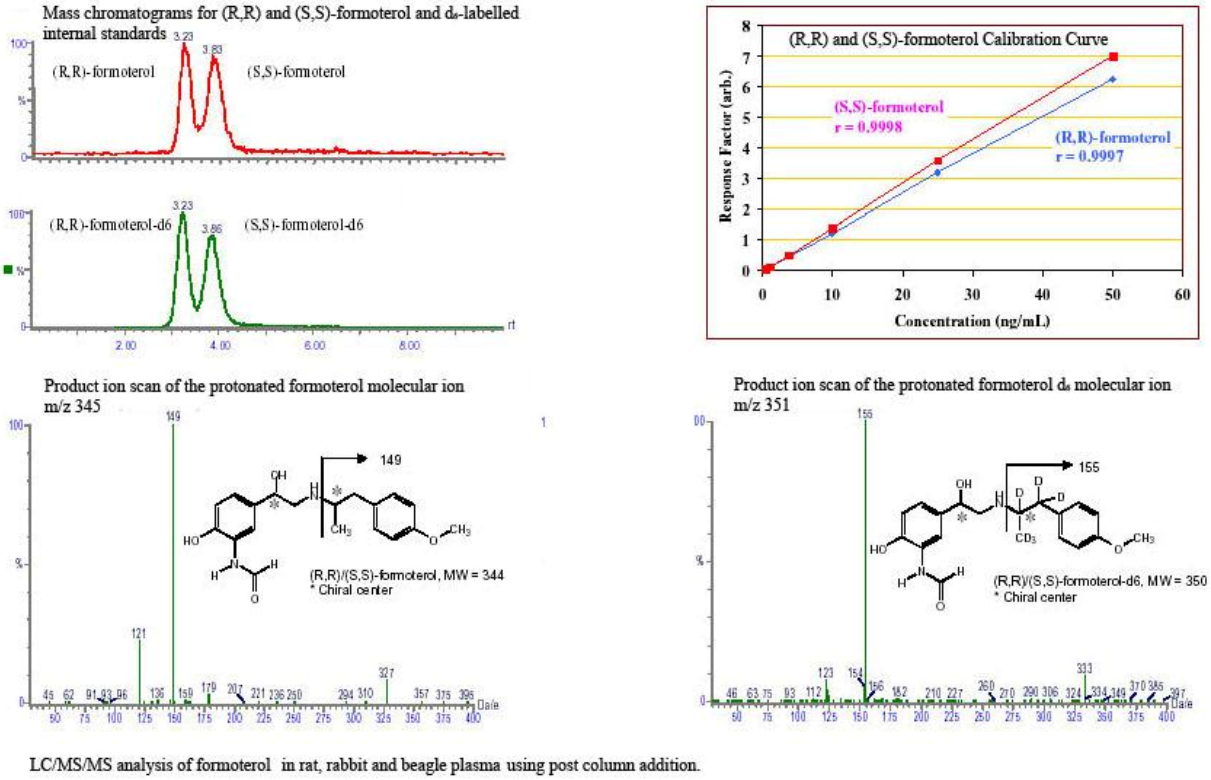
The goal of drug discovery is to generate a novel lead candidate with suitable pharmaceutical properties (i.e., efficacy, bioavailability, toxicity) for preclinical evaluation. A successful lead drug candidate development program often requires consideration of chemical and biochemical assays in the identification of active "hit" compounds. This early hit compound identification stage is commonly followed by *in vitro* and *in vivo* studies, conducted in parallel, to permit further selection of lead candidates with desirable absorption, distribution, metabolism, excretion and toxicology as well as pharmakokinetics (***ADMET-PK***) properties.

During these early stages of drug discovery, applications of liquid chromatography mass spectrometry (***LC/MS***) and liquid chromatography tandem mass spectrometry (***LC/MS/MS***) assays have become extremely useful analytical chemistry tools to accelerate the transition from discovery to preclinical development[1].

Preclinical development

Preclinical development is focused on activities which are necessary for filling an IND/CTA (investigational new drug/clinical trial application). The completed IND/CTA contains information that details the drug's composition and the synthetic processes utilized for its production as well as animal toxicity data, protocols for early phase clinical trials, and an outline of specific details and plans for evaluation. Process research, formulation, metabolism, and toxicity are the major areas of responsibility in this development stage. Appropriate bioanalytical methods are developed for the evaluation of pharmacokinetics, typically a series of studies focused on absorption, distribution, metabolism, and excretion (***ADME***). The use of an ***LC/MS*** or ***LC/MS/MS*** assay method in support of ***ADMET*** investigations can generate significant benefit. Samples such as serum, plasma, urine, tissue or *in vitro* assay samples are commonly presented to the analytical laboratory in 50 µl to 100 µl aliquots. Additionally, an analytical method must be able to detect drug in levels concentrations as low as nanogram. The application of ***LC/MS*** or ***LC/MS/MS*** assays, using simultaneous online radiometric (RM) (^{14}C/ ^{3}H) measurement is increasingly useful in support of *in vivo* metabolism and tissue distribution studies, particularly for drugs candidates exhibiting low plasma concentrations. Online radiometric/mass spectrometry detection involves splitting the HPLC column effluent for simultaneous online detection of a $^{14}C/^{3}H$-labelled drug using a flow-through liquid scintillation detector, operating in parallel with a mass spectrometer. The use of radiometric detection provides assay sensitivity, often in the low picogram range. On *Fig. 2* the analytical method of detection of Formoterol is depicted, showing very low levels in serum and plasma. This sensitive assay is based on the use of a deuterated internal standard, which was mixed with the serum. Formoterol and the standard were subsequently extracted using solid phase extraction and detected by ***LC/MS/MS***[2].

FIG. 2.[2]



LC/MS/MS analysis of formoterol in rat, rabbit and beagle plasma using post column addition.

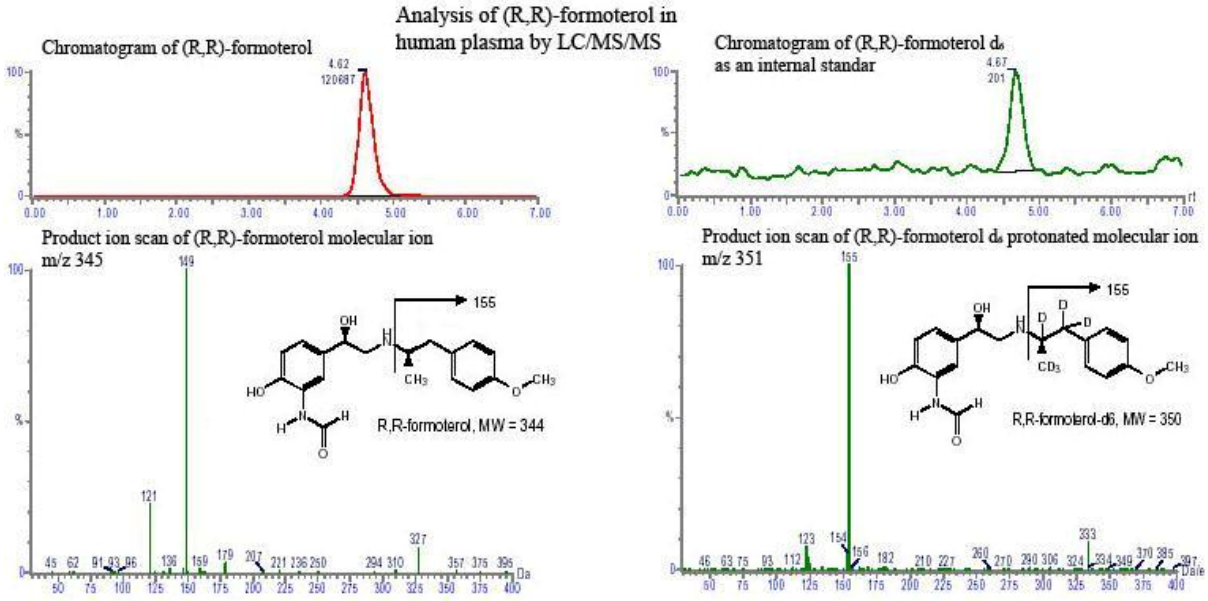
1) Sample, Calibrants,QCs **2)** Add internal standard to 1 mL of sample, vortex **3)** Place on Spec MPI cartridge, condition. **4)** Load sample, sample rinse with 1 mL of acetic acid, methanol, elute 2% NH_3, H_2O in IPA **5)** Salt washing with ACN **6)** Evaporate under nitrogen, reconst. With mobil phase 2 **7)** Analysed by LC/MS/MS

Clinical development

Clinical development comprises three distinct components (phase I-III). During phase I of the clinical development, the compound's safety and pharmacokinetic profile is defined. The determination of Cmax (maximum drug concentration in blood), AUC*, elimination half-life, volume of distribution, clearance and excretion, and potential for drug accumulation are made in addition to studies which provide estimates of efficacious doses. The goal of phase II is to determine the effective dose range and obtain safety and tolerability data. The goal of phase III is to complete the human safety and efficacy programs and to secure approval. *Fig. 3* describes the analytical method of detection of Formoterol in human plasma at very low concentrations, achieved after administration of low therapeutic doses. This method uses the deuterated internal standard to increase the sensitivity of the analytical assay. The internal standard was mixed with a sample of human plasma, worked up using solid phase extraction and finally analyzed by ***LC/MS/MS***[3].

* See chapter: Introduction to Pharmacokinetics on page. 37.

FIG. 3.[3]



1) Sample, Calibrants, QCs **2)** Add internal standard to 1 mL of sample **3)** Place on Spec MPI cartridge and condition. **4)** Load sample, sample rinse with 1 mL of acetic acid, methanol, elute NH_3, H_2O in IPA **5)** Salt washing with ACN **6)** Evaporate under nitrogen, reconst. With mobil phase 2 **7)** Analysed by LC/MS/MS

Manufacturing

As with earlier stages of drug development, the transition to the manufacturing stage begins while the previous clinical development activities are moving toward its milestone "New Drug Application/Marketing Authorization Application (NDA/MAA)". Once formulated, the drug is packaged and readied for distribution to pharmacies. Manufacturing processes and facilities undergo a pre-approval regulatory review and periodic inspections, once production is in progress. Analytical procedures and information databases are formalized into standard operating procedures (SOPs) and product specifications. This information and technology are formally transferred for routine monitoring and release by quality control (QC) scientists in manufacturing groups.

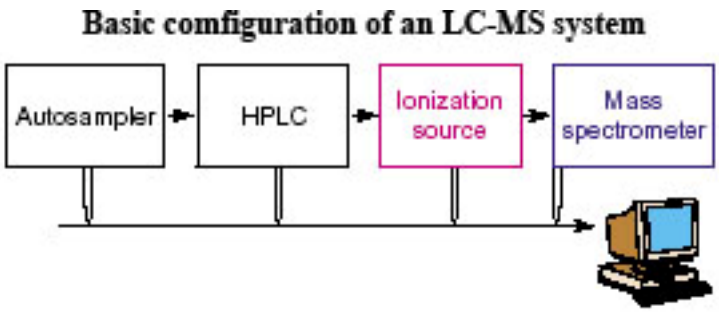
The use of LC/MS and LC/MS/MS in drug development:

The major applications of ***LC/MS*** in pharmaceutical analysis have been in drug metabolism studies[4], analysis and identification of impurities and degradation products of pharmaceuticals and in the isolation and characterization of potential drug substances from natural or synthetic sources.

Application of LC/MS and LC/MS/MS in drug discovery and preclinical development

LC/MS is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection[5]. The basic configuration of an ***LC/MS*** system is depicted in *Fig. 4*. The accelerated pace of contemporary drug discovery and development in the pharmaceutical industry has generated increasing demands for early information on the metabolic fate of candidate drugs to guide the selection of new compounds for clinical evaluation. ***LC/MS*** has been the most prominent technique, with its high sensitivity and specificity, for most assays used in various stages of new drug discovery[6].

FIG. 4.



Drug metabolism studies:

The metabolism of a potential pharmaceutical should be investigated before it is submitted for further development into a therapeutic agent. A good drug candidate should be metabolically stable and should show a good pharmacokinetic profile. A more complete characterization of pharmacokinetic properties is performed in animals (typically, rats and dogs) during this stage. Some of the metabolites can have higher biological activity or they can be more toxic than the parent drug. The drug metabolism can be studied *in vitro* with liver microsomal preparations or hepatocytes[7] or *in vivo*, involving analysis of drugs and metabolites in blood, urine and faeces[8]. The extensive development of combinatorial chemistry has brought the

demand for "accelerated drug discovery methods". This led to the development of high-throughput methods of analysis in the biological screening stage where drugs and metabolites are analysed in *in vivo* and *in vitro* biological samples[9]. The identification of metabolite structures with ***LC/MS*** and ***LC/MS/MS*** techniques is an effective approach due to their ability to analyze trace mixtures from complex samples of urine, bile, and plasma[10].
Recently, Kerns et al. demonstrated the application of ***MS*** and ***LC/MS/MS*** standard method approaches in preclinical development for the metabolite identification of buspirone, a widely used anxiolytic drug [11]. The success of this method relies on the performance of the ***LC/MS*** interface and the ability to generate abundant ions which correspond to the molecular weight of the drug and drug metabolites. A disadvantage of the use of constant neutral loss scans and parent ion scans is that any change in the structure which results in a change in the mass of the neutral loss or the *m/z* of the product ion monitored, may result in major metabolites not being detected[12]. To overcome this limitation, drug metabolism scientists predict likely metabolic transformations and use full-scan liquid chromatography with mass spectrometry (***LC/MS***) data to search for predicted products of metabolism[13]. With the demand for metabolism data on larger numbers of compounds, the need to increase the pace of analysis for biotransformation products has come. Recently Fernandez-Metzler et. al. described a procedure for the characterization of biotransformation products from a mixture of substrates[7]. This procedure has the potential of significantly increasing the number of compounds which can be analyzed at one time.

Application of LC/MS and LC/MS/MS in clinical development:

LC/MS analysis plays a major role in the success, efficiency, and timeliness of clinical development. The main application of ***LC/MS/MS*** in the course of clinical development is summarized in the *table 1*. The widespread acceptance of ***LC/ MS*** in the area of pharmacokinetics has led to major investments within pharmaceutical companies and contract analytical laboratories. ***LC/MS*** has demonstrated a clear advantage to HPLC for quantitative pharmacokinetic studies, in terms of method development time, cost, sample throughput, and turn-around[14]. Structure identification has also played a major role in accelerating clinical development. The rapid availability of structural information provides an improved understanding, in real-time, as processes and formulations are developed and metabolism is investigated. The quantitative analysis of targeted components in physiological fluids is a major requirement in the clinical development stage. Quantitative ***LC/MS*** assays generally involve four steps: sample preparation, assay calibration, sample analysis, and data management.

TABLE 1: Application of ***LC/MS*** in clinical development.

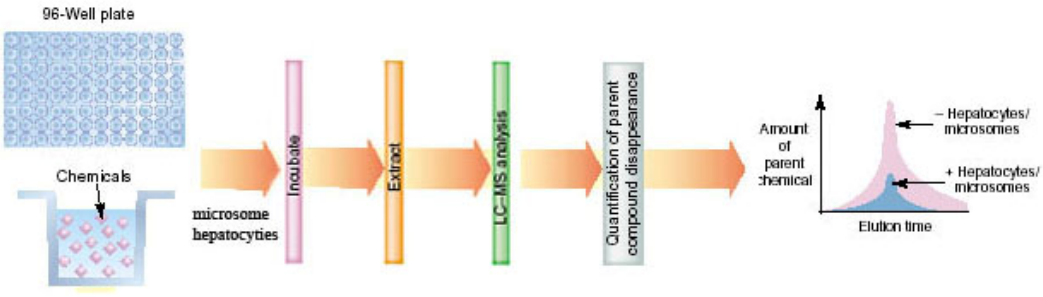
Clinical development activity	Analysis	LC/MS application
Pharmacokinetics	Quantitative bioanalysis	Selected ion monitoring
		Selected reaction monitoring
		Automated off-line SPE extraction
		Automated on-line extraction
Metabolism	Metabolite identification	Template structure identification
Long-term stability	Degradant identification	Stand. method LC/MS protocol

1. Quantitative Bioanalysis- Selected Ion Monitoring[15] (SIM). In the ***LC/MS*** analysis involves the use of SIM the molecular ions $[M-H]^-$ which correspond to the drug m/z and internal standard m/z is monitored. In this ***LC/MS*** application, the negative ion mode is highly sensitive for the class of compound.

2. Quantitative Bioanalysis- Selected reaction monitoring (SRM)[16]. The SRM experiment provides a high degree of selectivity and better limit of detection (LOD) than full-scan or SIM experiments for the analysis of complex mixtures.

3. Quantitative Bioanalysis-Automated Solid-Phase Extraction[17]: The analysis strategy uses ***LC/MS/MS*** in combination with a 96-well disk plate SPE, *Fig. 5*. The activities associated with sample preparation and analysis are decoupled to simplify troubleshooting and to allow a greater flexibility. The benefit of this off-line approach is the ability to simultaneously prepare 96 samples with SPE in 1 h.

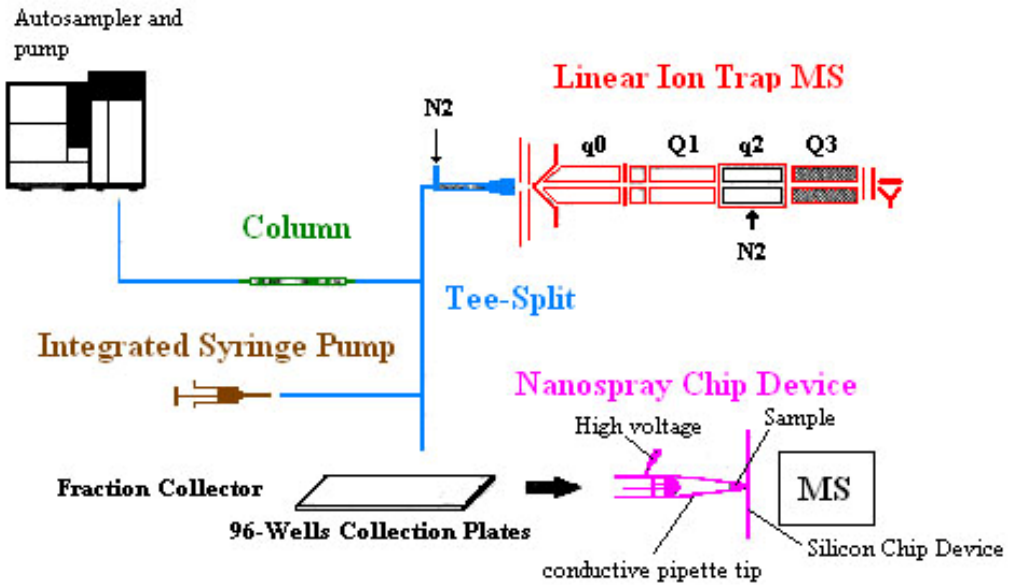
FIG. 5.[26]



The use of SPE in Metabolic stability screening assay. The test compounds after incubation with liver microsomes or hepatocytes are extracted and analyzed using LC/MS resp. LC/MS/MS.

4. Quantitative Bioanalysis–Automated On-Line Extraction[18]: This quantitative process approach eliminates the time-consuming sample extraction step and provides an opportunity to perform an integrated on-line automated method for extraction and analysis. *Fig. 6* shows on-line ***LC/MS/MS*** configuration included software-based tools for metabolite identification, detection and localization, complemented by fraction collection with chip-based MS-infusion. One part of the ***LC*** effluent is used for the mass spectrometer and the remaining part is used for a 96-well collection plate[19].

FIG. 6.



Configuration of the on-line LC/MS with fraction collection and off-line chip-based infusion

Drug Metabolism and Degradant Identification studies:

During the course of clinical development, it is often important to identify the structures of metabolites. This information provides an opportunity to better understand interpatient variability in pharmacokinetics and toxicity. Samples are collected by extraction from plasma, urine and bile involving normally the SPE procedure[20]. The resulting extracts are profiled with ***LC/MS*** and ***LC/MS/MS***. The analysis involves the use of the product-ion spectrum as the structural template for the identification of metabolite structures. A four step strategy has been proposed to characterize metabolites[21]:1) screening for potential metabolites by precursor ion scan and neutral loss scan, 2) product ion analysis of metabolites, 3) further structural elucidation of metabolites by ***MS***, 4) accurate mass measurement. Careful monitoring of degradant formation is an important aspect in determining the stability of the drug. Identification of the degradants is useful to determine potency and to provide insight into improved formulations.

Application of LC/MS and LC/MS/MS in impurities and degradant identification:

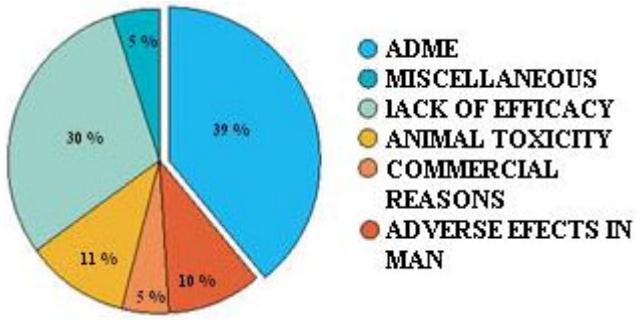
Synthetic impurities are of particular concern during process research and safety evaluation activities. Often, impurities are the result of synthetic by-products or starting materials of the scale-up process. Impurities provide a comprehensive indicator of the chemical process and are diagnostic of overall quality. The resulting information is used by process chemists to

guide process optimization. Knowledge of the identity and relative amount of impurities is used to diagnose process reactions so that changes in reagents and reaction conditions lead to better yields and higher quality material. With an increasing number of novel lead candidates which enter into preclinical development, considerable resources are needed to identify impurities. During the course of drug development, the bulk drug and drug formulation are studied under a variety of stress conditions such as temperature, humidity, acidity, basicity, oxidization, and light[22]. The strategy for impurity and degradant identification subjects lead candidates to various development conditions, followed by ***LC/MS*** and ***LC/MS/MS*** analysis[23]. The procedure incorporates qualitative and quantitative process changes for analysis. The structural information, necessary for successful drug development, is emphasized. The corresponding structural information provides insight for decisions, based on which leads are to be developed for clinical testing. The early structural information on degradants of a drug candidate offers a unique capability for synthetic modification to minimize degradation.

Introduction to the absorption, distribution, metabolism, elimination and toxicology of drugs (ADMET)

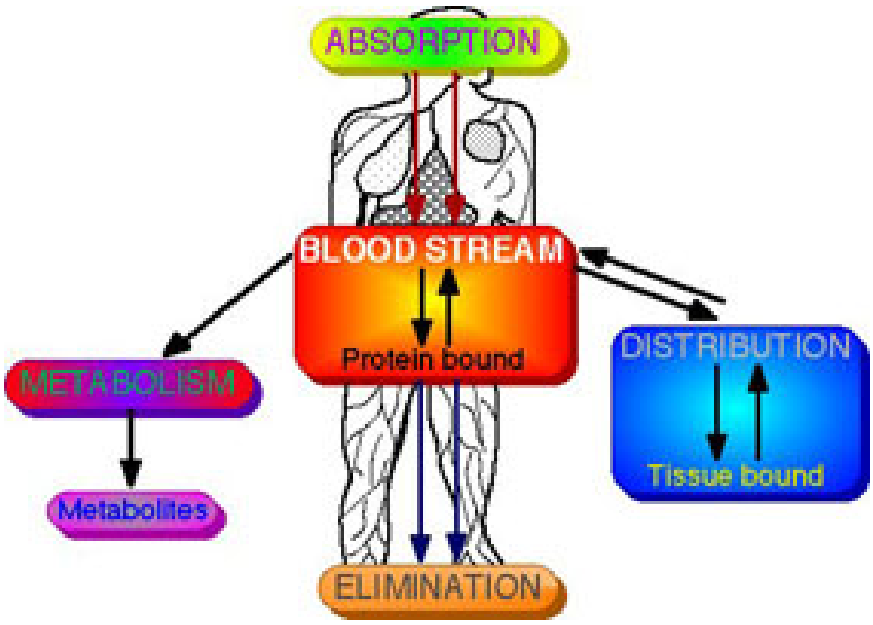
The success of a drug's journey through the body is measured in the dimensions of absorption, distribution, metabolism, and elimination (***ADME***), *Fig. 8a* The ***ADME*** properties of a drug, together with its pharmacological properties, are conventionally viewed as part of the drug development. Toxicology is the way to make sure that the molecule causes no harm, regardless of what good is does[24]. Around half of all drugs in development fail to make it to the market because of ***ADME*** deficiencies (*Fig. 7*), and 50% of the drugs which do make it to market still have some ***ADME*** or toxicological problems[25].

FIG. 7 [25]



In the traditional drug-development model, ***ADMET*** issues were addressed in preclinical development. The focus onto the early identification of ***ADME*** properties has changed in the last years. Molecular and cellular biology techniques have meant that compounds, selected initially for selectivity against a specific pharmacological target, and for potency and aqueous solubility, can be subsequently subjected to further screens which explore some dimensions of their ***ADMET*** properties. Physicochemical measurements of a compound's ionizability (*pKa*), and lipophilicity (*log P* or *log D*), provide an indication of its likely absorption in the gut. In the metabolic studies (*in vitro)*, the use of cloned cytochrom P450 (CYPs) as an early screen for lead compounds is now also a well-established part of the lead optimization.

FIG. 8a



In *Fig. 8b* a chart flow summarizing the process of drug discovery is depicted, including major contributions from chemistry, biology and ***ADME-Tox***[24].

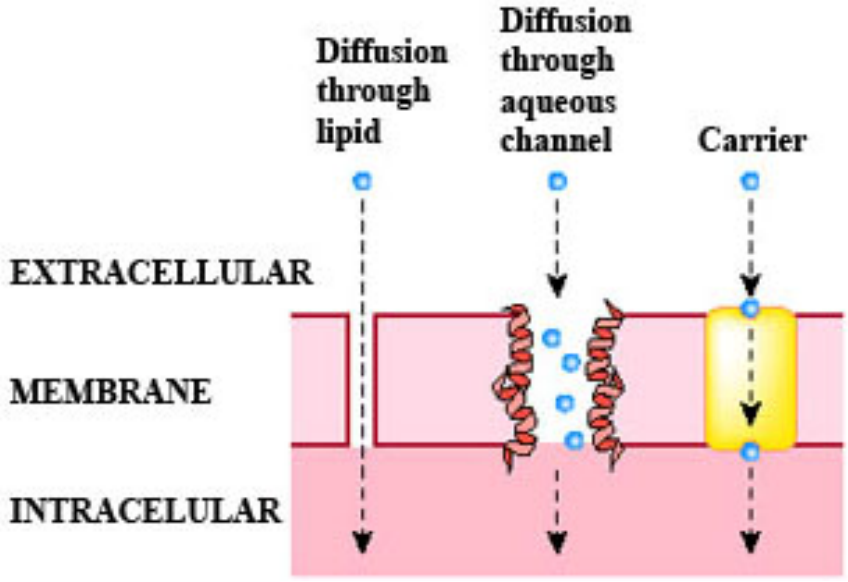
FIG. 8b[24]

Target indentification and verification	***Hit identification***	***Lead identification***	***Lead optimization***
	CHEMISTRY .Virtual library .Chemical libraries .Medicinal cheistry	CHEMISTRY .Combinatorial chemistry .Medicinal chemistry	
BIOLOGY .Genomics .Proteomics .Biotransformation	BIOLOGY .Ultra HTS .Hit confirmation	BIOLOGY .Hit confirmation .Further screening .Affiniti Selectivity assays	BIOLOGY .Affinity Sellectivity assays .Efficiacy assays .Disease models
	ADMET .In silico .(emp. model)	ADMET .In vitro (primary assays) .In silico (emp./mechan. mod.)	ADMET .In vitro (secondary assays) .In silico (mechanistic models) .In vivo

Absorbtion and Distribution:

Absorption is defined as the passage of a drug from its site of administration into the plasma. It is, therefore, important for all routes of administration, except intravenous injection. The main routes of administration are oral, sublingual, rectal, application to other epithelial surfaces (e.g. skin, cornea, vagina and nasal mucosa), inhalation and injection (subcutaneous, intramuscular, intravenous). Oral delivery is the most desirable route of drug administration. Therefore, it is important to develop drugs that can be absorbed effectively through the intestinal epithelium (mucosa)[26]. The major mechanism for drug uptake through the intestinal epithelium is passive diffusion that is driven by a concentration gradient. Passive diffusion can occur between cell junctions (paracellular transport) or through the cytoplasm (transcellular transport). Lipophilic compounds, such as testosterone, are mainly transported transcellularly. Hydrophilic compounds, such as mannitol, are transported predominantly via the paracellular route. The main routes how the molecules can cross the membrane are depicted in the *Fig. 9*.

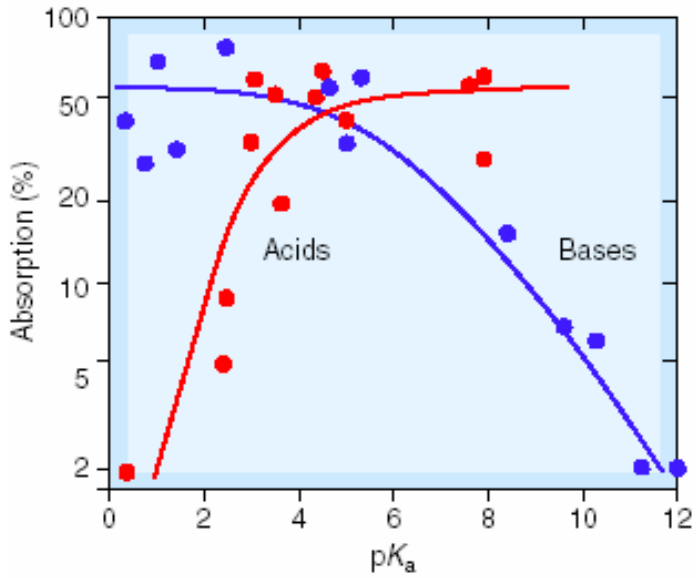
FIG. 9.



The rate of passive transfer is determined by the ionisation and lipid solubility of the drug molecules. Strong bases of p*K*a 10 or higher are poorly absorbed, as are strong acids of p*K*a less than 3, because they are fully ionised[27], as illustrated in *Fig. 10*. In addition to passive diffusion, some substances, such as amino acids and glucose, are actively transported by specific transporters. An important feature of the intestinal epithelium is that some substances which enter the cytoplasm of mucosal cells can be transported back to the intestinal lumen (efflux). This efflux process is mainly a function of a transporter in the plasma membrane

called P-glycoprotein (P-gp). P-glycoprotein is associated with the plasma membrane of intestinal mucosal epithelium which actively pumps some drugs back into the intestinal lumen after they are absorbed by the cells. P-gp substrates, such as vinblastine, enter the intestinal mucosal cells via passive diffusion, and a portion of the substrate is transported out of the cell and into the intestinal lumen by P-gp[28].

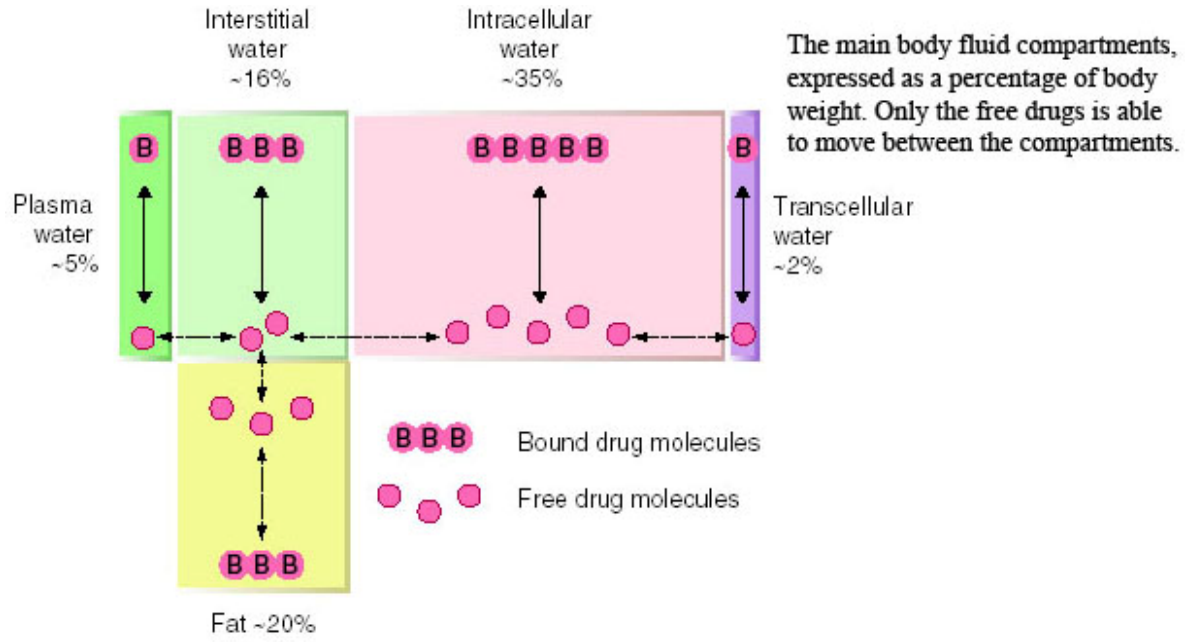
FIG. 10.[27]



Drug distribution refers to the movement of drugs to and from the blood and various tissues of the body (for example, fat, muscle, and brain tissue) and the relative proportions of drugs in the tissues[29] (*Fig. 11*). After a drug is absorbed into the bloodstream, it rapidly circulates through the body; the average circulation time of blood is 1 minute. Drugs which dissolve in water (water-soluble drugs), tend to stay within the blood and the fluid that surrounds cells (interstitial space). Drugs that dissolve in fat (fat-soluble drugs), tend to concentrate in fatty tissues. Other drugs concentrate mainly in only one small part of the body, because the tissues there have a special attraction for and ability to retain (affinity) the drug. Fat-soluble drugs can cross cell membranes more quickly than water-soluble drugs can. Some drugs leave the bloodstream very slowly, because they bind tightly to proteins circulating in the blood. Others quickly leave the bloodstream and enter other tissues, because they are less tightly bound to blood proteins. Some drugs accumulate in certain tissues, which can also act as reservoirs of extra drugs. These tissues slowly release the drug into the bloodstream, keeping blood levels of the drug from decreasing rapidly and thereby prolonging the effect of the drug. Extracellular fluid comprises the blood plasma, interstitial fluid and lymph. Intracellular fluid is the sum of the fluid contents of all cells in the body. Transcellular fluid includes the

cerebrospinal, intraocular, peritoneal, pleural and synovial fluids and digestive secretions. Within each of these aqueous compartments, drug molecules usually exist both in free solution and in bound form, furthermore, drugs which are weak acids or bases will exist as an equilibrium mixture of the charged and uncharged forms[30].

FIG. 11.



Drug Metabolism:

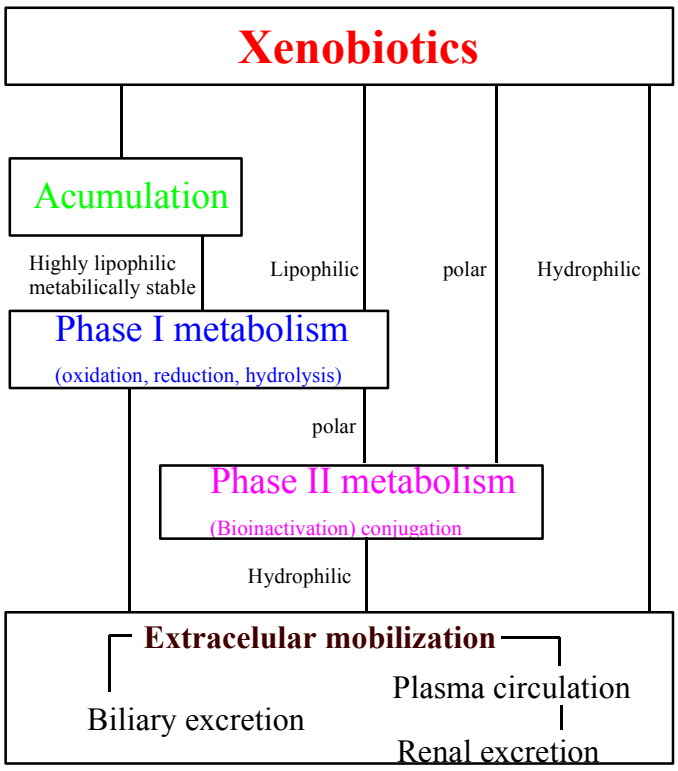
Drugs may be metabolised and biotransformed by many routes which include such a chemical reaction as oxidation, reduction, hydrolysis, hydratation, conjugation or condensation. The metabolism of a drug is divided into two phases. Phase I is considered as a preparation of the substances for the phase II reaction by producing a chemically reactive group (deprotection or uncovering) in which the phase II reaction will take place[31]. Common chemical reactions associated with phase I and phase II are given in Tab.2.

TAB 2.: Reaction of phase I and phase II drug metabolism

Phase I reactions	**Phase II reaction**
Oxidation	
Cytochrom P-450 dependent momooxydase	***Glucuronidation***
Xanthin oxidase	Glucuronyltransferases
Peroxidase	***Sulfation***
Amine oxidase	Sulfotransferases
Monoamine oxidase	***Methylation***
Dioxygenase	Methyltransferases
Reduction	***Acetylation***
Cytochrom P-450 dependent reductase	N-Acetyltransferase
Ketoreductase, Glutathion peroxidases	Acyltransferases
Hydratation	***Gluthation conjugation***
Epoxide hydrolase	Gluthation-S-transferase
Ester hydrolysis	***Amino acid conjugation***
Carboxylesterases	Transacylase
Amidases	

In fact there is a close relationship between normal chemical processes running in the human body and drug biotransformation. Therefore many of enzymes involved in the drug metabolism are principally responsible for the transformation of endogenous compounds and the metabolization of drugs due to their similarity whith natural substances.

FIG. 12.: The concept of Xenobiotic disposition in the body



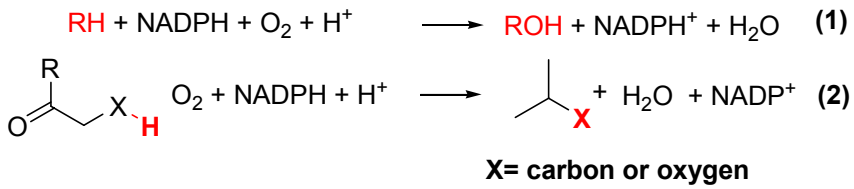
Phase I metabolism:

Oxidation performed by the microsomal mixed-function oxidase system (cytochrom P450-dependent) as well as the other oxidation types, reduction, hydrolysis, hydration, isomerization and other miscellaneous reactions are considered as phase I reactions.

Oxidation by Cytochrom P-450:

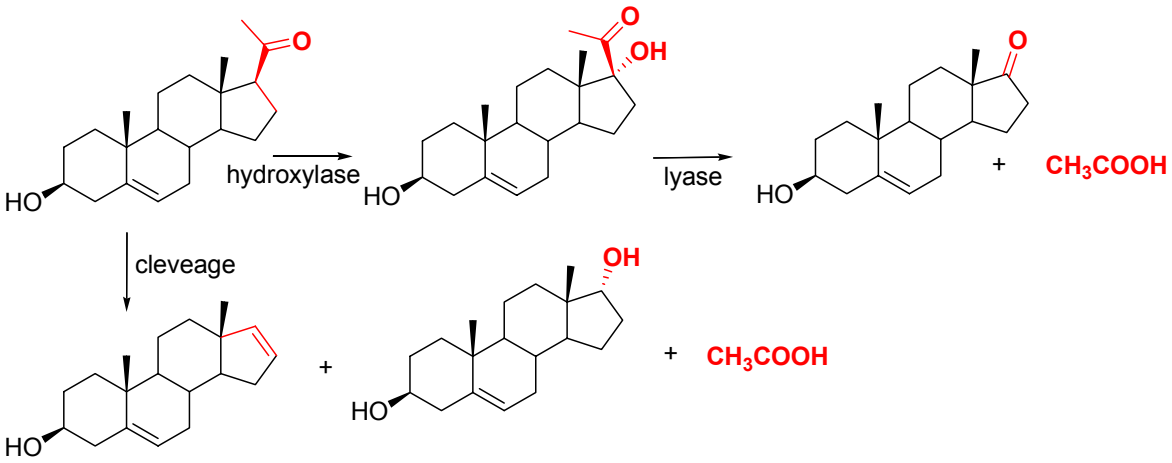
Many cells (particularly the cells of liver, kidney, lung and intestines) are equipped with a mixed function oxidase system (P-450) located in the microsomes of the endoplasmatic reticulum, where the following reactions are performed: aromatic hydroxylation, aliphatic hydroxylation, epoxidation, N-dealkylation, O-dealkylation, S-dealkylation, oxidative deamination, N-oxidation, S-oxidation, phosphothionate oxidation dehalogenation or alcohol oxygenation. Cytochrome P-450s are a large group of enzymes which have been historically associated with hydroxylation reactions[32] . Later on it was shown, that these enzymes catalyze not only hydroxylation but also oxidation reactions and more importantly acyl-carbonyl bond cleveage reactions[33, 34]. Cytochrome P-450s constitute a superfamily of isozymes which contain a ferroprotoporphyrin heme prosthetic group and a polypeptide, encoded by a single gene. The heme moiety acts as an oxidation reaction center, and the apoprotein determines the substrate specificity and binding affinity of individual isozymes[35, 36].

Scheme 1



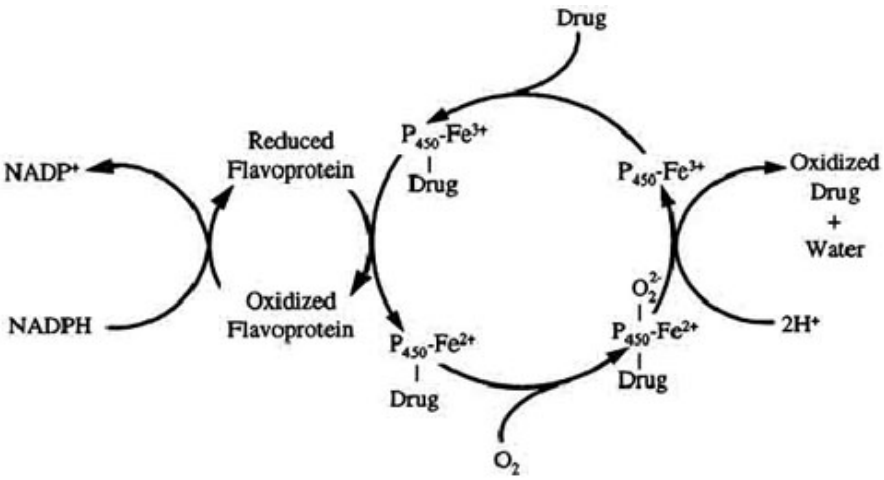
Examples of reactions performed by cytochrom P-450 are given below:

Scheme 2



The mechanism of CYP catalysis is complex and some co-enzymes and co-factors are required for the oxidation reaction. *See Fig. 13*.

FIG. 13.



In the catalysis term, the catalytic cycle of CYP450 enzymes consists of the following steps: 1) binding the substrate reversibly to the active domain of CYP enzyme, 2) reducing the haem group in the active domain from the ferric to the ferrous state by an electron generated from NADPH by the coenzyme CYP reductase, 3) binding the oxygen molecule to the ferrous CYP-substrate complex, 4) cleaving the O-O bond by a second electron provided by NADPH via CYP reductase or cytochrome b_5, 5) oxidating the substrate, 6) releasing the product from the CYP enzyme[37, 38].

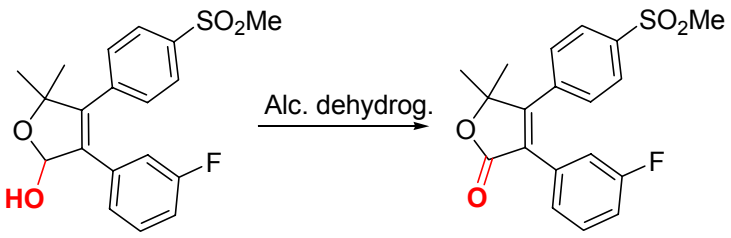
Other oxidations (not related with cytochrom P-450):

A number of enzymes which are involved in endogenous metabolism can be included in drug oxidation. Especially alcohol dehydrogenase, aldehyde dehydrogenase, xanthine oxidase, amine oxidase, aromatases and alkylhydrazine oxidase are particularly important enzymes in the non-microsomal mixed-function oxidase processes[39].

Alcohol oxidation:

Alcohol dehydrogenase is localised in liver, kidney and lung cells and is responsible for the oxidation of alcohols to aldehydes. This enzyme uses NAD^+ as a cofactor and therefore in fact dehydrogenase. Even though alcohol dehydrogenase is the major metaboliser of ethanol, this process can be particularly carried out by the microsomal ethanol oxidazing system. Human alcohol dehydrogenase (*Hs*ADH) comprises class I (α,β,γ), class II (π), and class IV (σ) enzymes[40].

Scheme 3: oxidation of DFU-lactol to DFU (cyclooxygenase 2-inhibitor)[41]

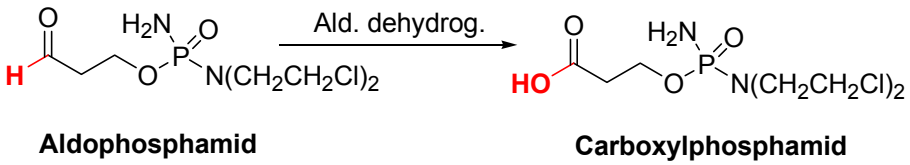


DFU-lactol **DFU**

Aldehyd oxidation:

Aldehyde dehydrogenase (utilizing NAD^+ as a cofactor), aldehyde oxidase and xanthin oxidase are the major enzymes taking part in the conversion of aldehydes into corresponding carboxylic acids in human body. Cytosolic aldehyde dehydrogenase is a key factor in the detoxication of the cytotoxic drug, cyclophosphamide, and other related oxazaphosphorines[42].

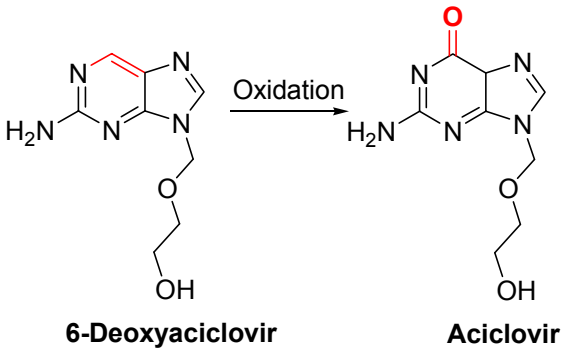
Scheme 4: Oxidation of Aldophosphamid (derived from cyclophosphamid) to carboxylphosphamid[42, 43].



Xanthin oxidation:

Xanthin containing drugs such as caffein or theobromin are oxidized to corresponding derivates of uric acid. This process is carried out by the xanthin-oxydase enzyme.

Scheme 5: Activation of Aciclovir by oxidation catalyzed by Xanthin oxydase enzymes[44]



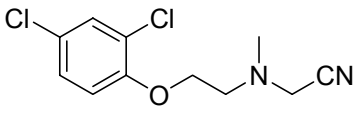
Amin oxidation:

The oxidation of amines takes place with a group of enzymes which can be divided into the following subgroups (in general amine oxidases):

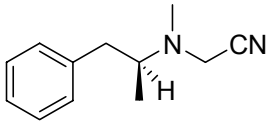
1. monoamine oxidases
2. diamine oxidases
3. flavoprotein N-oxidases and N-hydroxylases

The monoamine oxidase is a flavin-adenosin-dinucleotid (FAD) containing enzyme which exist in two isozymic forms (MAO-A, MAO-B)[45]. Selective Inhibotors of MAO-A such a clorgylin exhibit antidepressant activity[46] whereas selective inhibitors of MAO-B such a (R)-deprenyl exhibit antiparkinsonian activity[47].

Scheme 6: Selective inhibitors of MAO-A and MAO-B enzymes



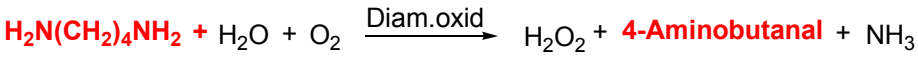
Clorgyline



(R)-Deprenyl

The diamine oxidase, primary involved in the endogenous metabolism, is of little relevance for the drug metabolism. The diamine oxidase catalyses the oxidative deamination of the diamines Putrescine and Cadaverine, producing the corresponding aminoaldehyde, ammonia and H_2O_2[48].

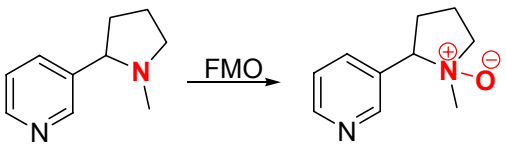
Scheme 7: Oxidation of Putrescine by diamine oxydase



Putrescine

N-oxidases, located in the liver cells, convert tertiary amines in to the corresponding N-oxides, are of great importance for the drug metabolism. These enzymes are in general flavoproteins and require NADPH and oxygen for their function. In general this enzyme is known as a flavin-containing-monooxygenase (FMO).

Scheme 8: Oxidation of nicotin by FMO



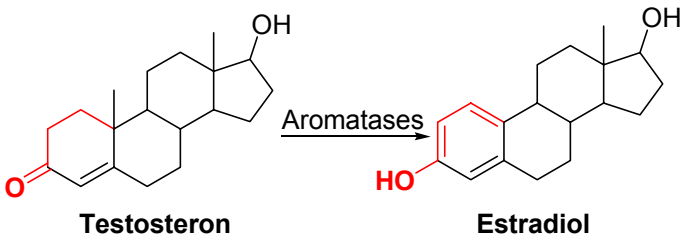
Nicotine **Nicotine 1-N-oxide**

Aromatization:

Aromatases are enzymes responsible for the conversion of xenobiotics, containing cyclohexancarboxylic acid groups, into the corresponding benzoic acids. These enzymes, located in the liver and kidney cells, require co-enzym A of the acid as a substrate, FAD as a co-factor and oxygen for their function. Aromatase is the enzyme complex which catalyses

the synthesis of oestrogens from androgens. Both aromatase cytochrome P450 and NADPH-cytochrome P450 reductase, the two essential components of the enzyme complex, are highly conserved among mammals and vertebrates[49].

Scheme 9: Aromatization of testosterone to estradiol



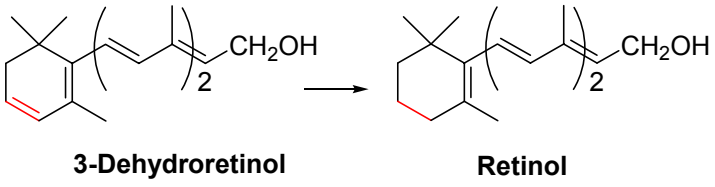
Alkylhydrazine oxidation:

Drugs, containing hydrazin groups, can be biotransformed by the alkyhydrazine oxidases enzymes by oxidation of the nitrogen and subsequently decomposition of the intermediate.

Reductive metabolism:

Azo-compounds, Nitro-compounds, epoxides, heterocyclic ring compounds or halogenated hydrocarbons undergo reductive metabolism. These reactions can be catalysed by the hepatic microsomes or the mixed-function oxidase enzymes. Azo- and nitro-reductions can be catalyzed by the cytochrom P450 or by NADPH-cytochrom c (P450) reductase[50]. Epoxides are converted into the hydrocarbons and further undergo rearrangement and hydrolysis.

Scheme 10: Reductive degradation of 3-Dehydroretinol to Retinol[51]



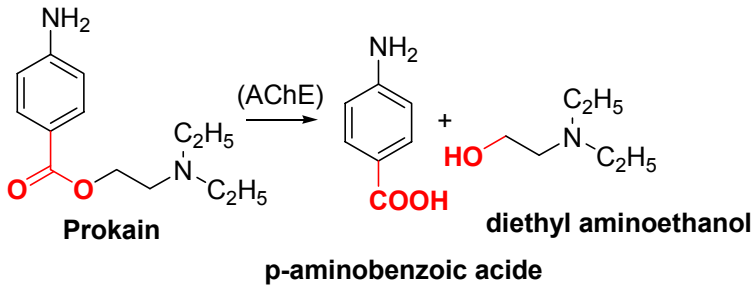
Hydrolysis:

A number of compounds like esters, amides, hydrazides or carbamates can by hydrolysed by a variety of enzymes.

Ester hydrolysis:

A variety of enzymes in the plasma, such as non-specific acetylcholine esterases or pseudocholin esterases, or in the liver can readily converted esters to corresponding alcohols and carboxylic acids[52].

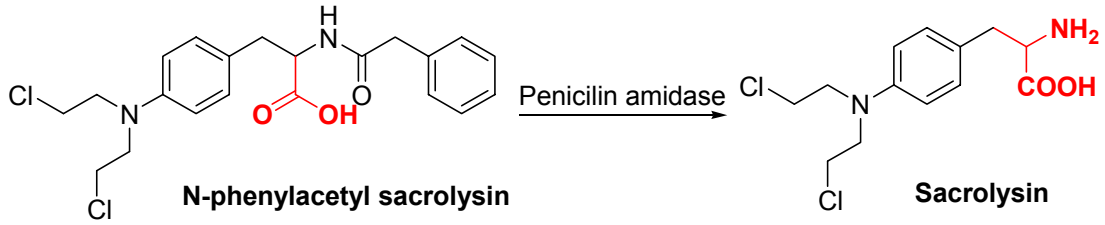
Scheme 11: Hydrolysis of procaine



Amide hydrolysis:

Amides are metabolised to the corresponding amines and carboxylic acids by the liver amidases or by the non-specific plasma esterases.

Scheme 12: Activation of prodrug N-phenylacetyl sacrolysin by amidase[53]



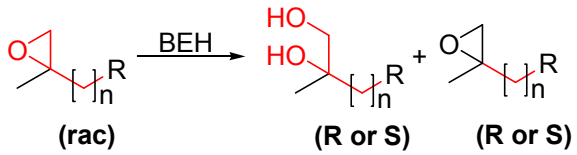
Hydrazide and carbamate hydrolysis:

Drugs containing hydrazin groups can undergo as well hydrolysis, except oxidative metabolism, mentioned in previous chapter.

Hydration:

Hydration is considered as a special type of hydrolysis whereas water is added without splitting the substrate. Good examples are epoxides: They are predisposed to water addition catalyzed by enzyme epoxid hydratase.

Scheme 13: Biocatalytic hydrolysis of 2,2-disubstituated oxiranes[54]



Except these very common phase I reactions there are many other possible routes of metabolism as ring closure, N-carboxylation, dimerisation, isomerisation, decarboxylation which have been found for specific drugs. Both phase I and phase II metabolic reactions are compiled in the MDL metabolic database.

Phase II metabolism:

This phase is considered as the true detoxification, whereas the aim of phase I is to prepare the compounds for phase II. Products of this phase are generally good water soluble and thus can be easily excreted. In Tab.3 major phase II reactions related to groups on which the reactions takes place, are summarized. Phase II enzymes are conjugating enzymes which increase hydrophilicity, thereby enhancing excretion in the bile or urine and consequently affecting detoxification[55]. Phase II consists of many superfamilies of enzymes, including sulfotransferase (SULT)[56], Glutathione Stransferase[57], UDP-glucuronosyltransferase[58] , N-acetyltransferase[59], and epoxide hydrolase[55].

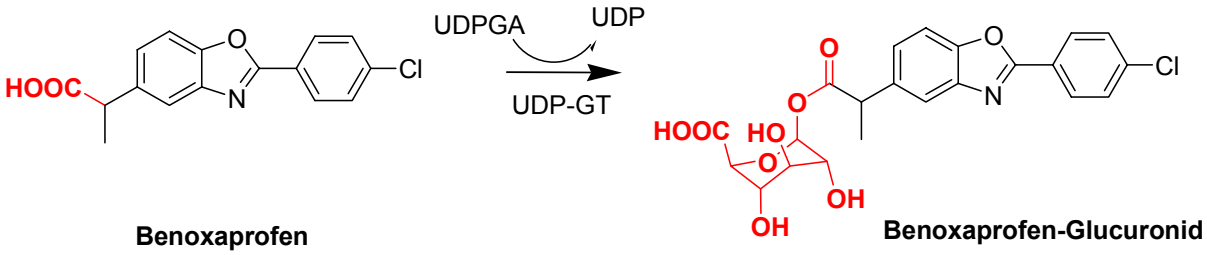
TAB.3: Conjugation reaction

Conjugation reaction	**Functional group**
Glucuronic acid conjugation	-OH, -COOH, -NH_2, -NH, -SH, -CH
Sulfate conjugation	Aromatic –OH, aromatic –NH_2, alcohols
Glycine conjugation	Aromatic –NH_2, -COOH
Acetylation	Aromatic –NH_2, aliphatic –NH_2, hydrazines
Methylation	Aromatic –OH, -NH_2, -NH, -SH
Gluthation conjugation	Epoxide, organic halides

Glucuronidation:

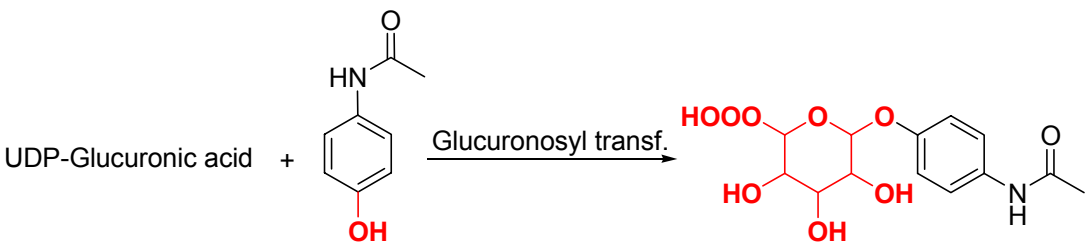
The glucuronidation is the most widespread conjugation reaction and the glucuronide formation is the most important form of conjugation of drugs and endogenous compounds. The glucuronidation occurs with alcohol, phenols, hydroxylamines, carboxylic acids, amines, sulfonamides and thiols. In this sense O-Glucuronides are formed from phenols, alcohols (ether-glucuronides), and carboxylic acids (esters glucuronides). N-Glucuronides are formed from amines, amides and sulfonamides and S-Glucuronides formed from thiols.

Scheme 14: Glucuronidation of Benoxaprofen[60]



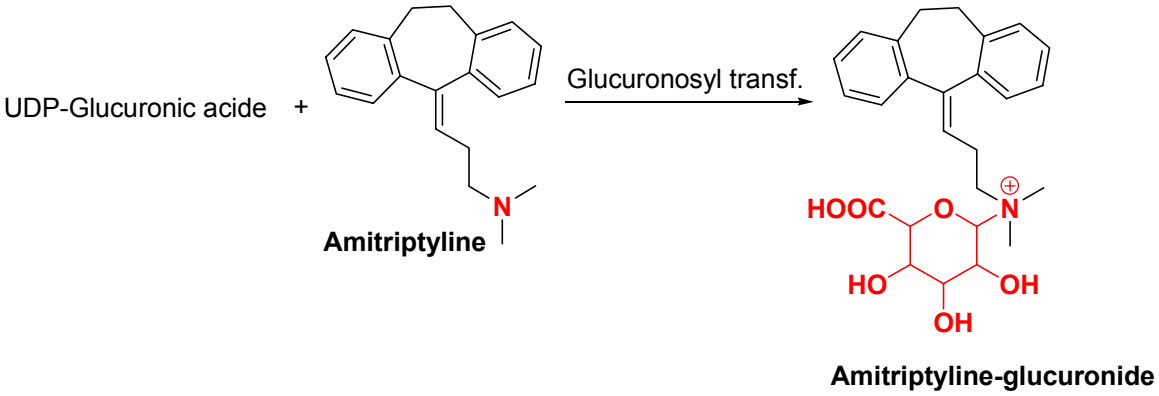
Benoxaprofen **Benoxaprofen-Glucuronid**

Scheme 15: Formation of acetaminophen-O-glucuronide as a major maetabolite of p-aminophenol[61]



acetaminophenol **acetaminophen-O-glucuronide**

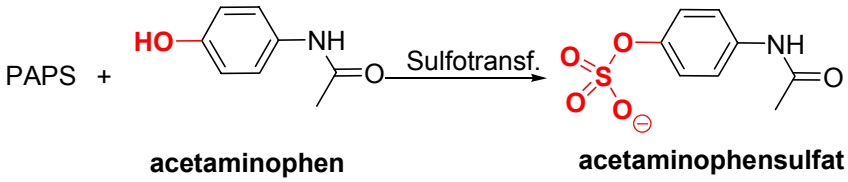
Scheme 16: Metabolism of Aminotriptyline, Glucuronidaction by UGT[62]



Sulfation:

The sulfation occurs by alcohols, amines and thiols but is a major conjugation reaction for phenols. Sulfonations require the interaction of the drug and PAPS (3'-phosphoadenosine-5'-phosphosulfonate) in presence of a cytosolic enzyme, sulfotransferase. Most of the drugs which can be glucuronidated can be sulfonated as well which can lead to a competitive metabolism.

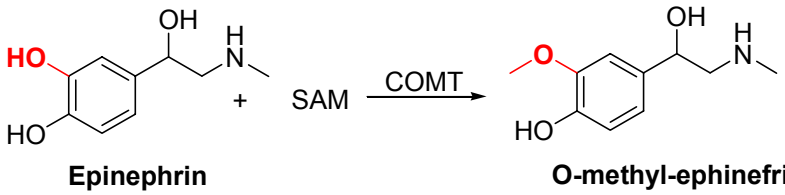
Scheme 17: Formation of Acetaminophensulphate, metabolite of p-aminophenol[63]



Methylation:

Some drugs can undergo methalation reactions by non specific methyltransferases found in the lung or by the physiological methyltransferases. The methylation requires a co-factor, S-adenosylmethionin (SAM), which is formed from L-methionine and ATP under the influence of the enzyme, L-methionine adenosyltrensferase.

Scheme 18: Methylation of ephinefrin by Catechol-O-metyltransferase (COMT)[64]

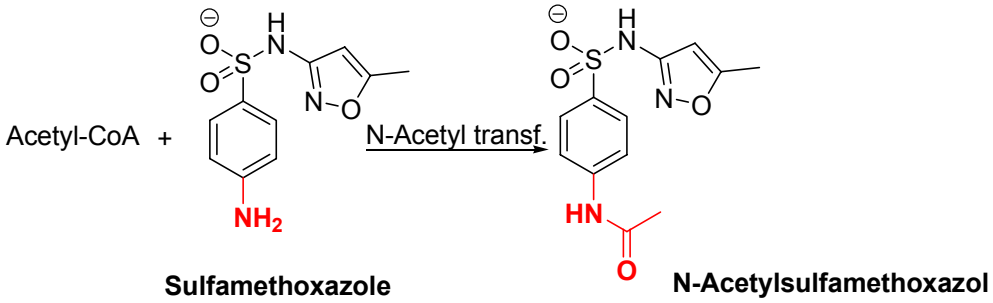


Epinephrin **O-methyl-ephinefrin**

Acetylacion:

Acetylation reactions take place in the liver or reticuloendothelial cells of the spleen, lung and gut and require the enzyme reffered to as N-acetyltransferase and co-factor (acetyl-CoA). The acetylation is a common metabolic pathway for aromatic amines.

Scheme 19: Acetylation of Sulfamethoxazol by N-acetyl transferase[65]

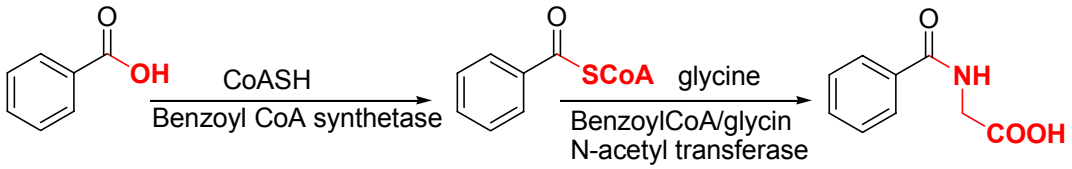


Sulfamethoxazole **N-Acetylsulfamethoxazol**

Amino acid conjugation:

Carboxylic acids can form CoA in the body and afterwards react with diverse amines, such as amino acids or with drugs instead of endogenous amino compounds. An amino acid conjugation is a special form of a N-Acetalation.

Scheme 20: Glycine conjugation to Benzoic acid in rats catalyzed by mitochondrial enzymes[66]

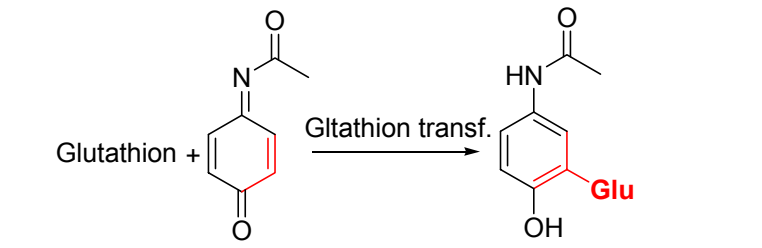


Benzoic acide **BenzoylCoA** **Benzoylglycine**

Gluthation conjugation:

Many copmpounds such as epoxides, haloalkanes, nitroalkanes, alkenes and aromatic halo or nitro compounds can be conjugated to glutathion. Many drugs can be converted by phase I metabolisms to strong electrofiles, and these can later on react with gluthation in order to form conjugates. The enzymes catalyzed by this transformation are gluthation-S-transferases and are located in the kidney, liver and gut.

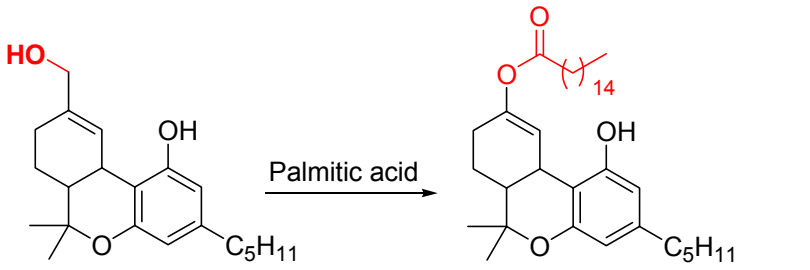
Scheme 21: Glutathion conjugation of N-acetyl-p-benzoquinoneimine (a cytochrome P-450 dependent metabolite of Acetaminophen)[67].



Fatty acid conjugation and cholesteryl ester conjugation:

Drugs containing carboxylic acid groups, can be esterified as a part of a mixed triglyceride with fatty acid or as a cholesteryl ester. The fatty acids involved are stearic or palmitic acids. A fatty acid conjugation has been shown to occur for 11-hydroxy-Δ^9-tetrahydro-cannabinol[68].

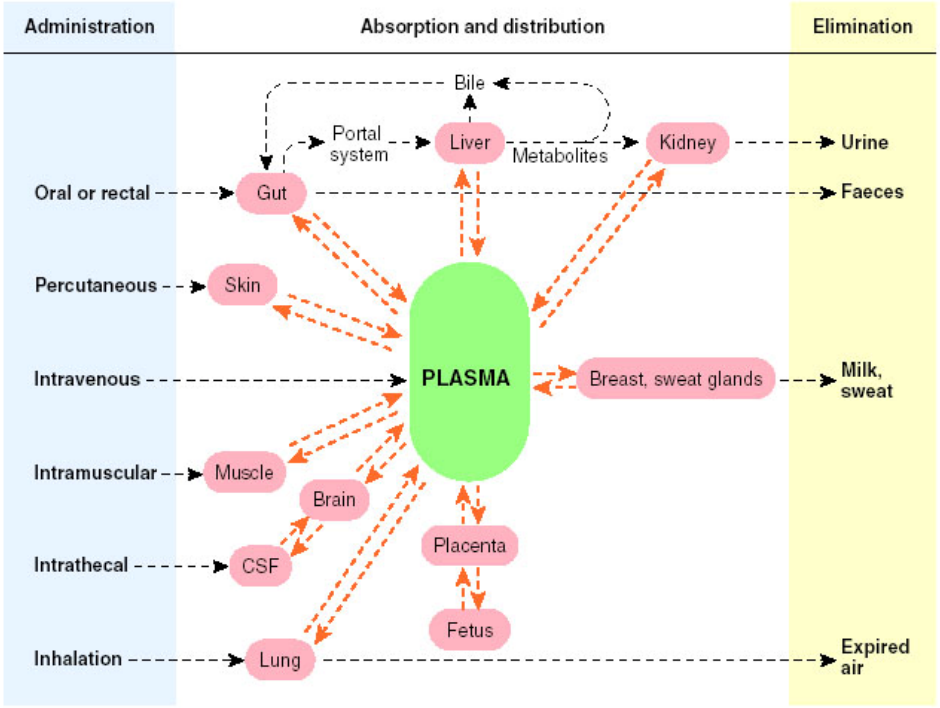
Scheme 22: The conjugation of 11-hydroxy-Δ^9-tetrahydro-cannabinol to palmitic acid[68]



Elimination:

Drug elimination is the removal of drugs from the body. All drugs are eliminated from the body in a chemically altered (metabolized) form or by excretion. The main administration and elimination routes are depicted on *Fig. 14*. Most drugs, particularly water-soluble drugs and their metabolites are eliminated largely by the kidneys in urine. To be extensively excreted in urine, a drug or metabolite must be water soluble and must not be bound too tightly to proteins in the bloodstream. The acidity of urine, which is affected by diet, drugs, and kidney disorders, can affect the rate at which the kidneys excrete some drugs. The kidneys' ability to excrete drugs also depends on urine flow, blood flow through the kidneys, and the condition of the kidneys. Some drugs pass through the liver and are excreted unchanged in the bile. The bile then enters the digestive tract. From there, drugs are eliminated in feces or reabsorbed into the bloodstream and thus recycled. Other drugs are converted to metabolites that are excreted in the bile. The metabolites may be excreted in the feces or converted back to the drug, which is then reabsorbed into the bloodstream and recycled. Some drugs are excreted in saliva, sweat, breast milk, and even exhaled air. Most are excreted in small amounts. The excretion of drugs in breast milk is significant only because the drug may affect the infant. Excretion in exhaled air is the main way that inhaled anesthetics are eliminated.

FIG. 14.

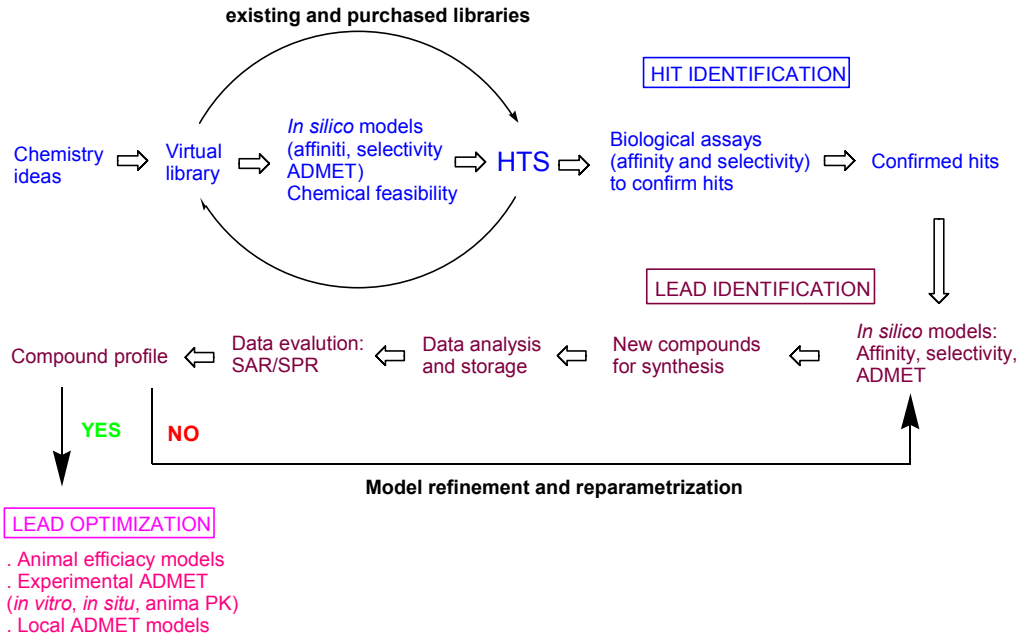


The main routes of grug administration and elimination

In silico ADME-Tox.

As mentioned at the beginning of this chapter, drug discovery and development is a costly process, taking an average of 15 years to generate a new drug. It has been estimated that nearly 50% of drugs fail because of unacceptable efficacy, which includes poor bioavailability as a result of ineffective intestinal absorption and undesirable metabolic stability[69]. It is becoming obvious that in addition to pharmacological properties, ADME/Tox properties are crucial determinants of the ultimate clinical success of a drug. This realization has led to the early introduction of ADME/Tox screening during the drug discovery process, in an effort to select against drugs with problematic ADME/Tox profiles. The goal of *in vitro* ADME–Tox characterization is to provide a preliminary prediction of the *in vivo* behaviour of a compound to assess its potential to become a drug. The need for increased ADME–Tox throughput to fully meet the demands of discovery has led to increasing interests in computational or *in silico* models that make predictions based on chemical structures[70]. The optimal approach for ADME–Tox support of discovery will be one that uses both *in vitro* and *in silico* ADME–Tox in a complementary way and ensures that ADME–Tox is used and considered at almost every stage of the discovery process. Fig. 22 shows the integrated use of computational and experimental technologies in the assessment and selection of compounds.

Fig. 22 [24]

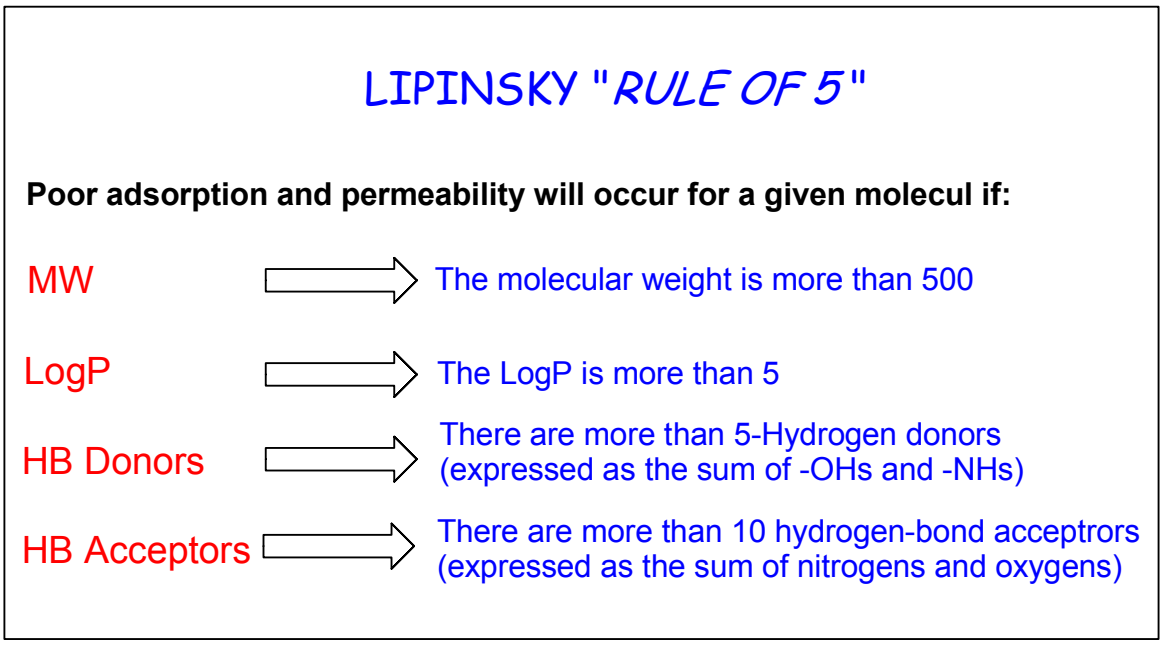


The *In silico* models have several advantages in comparison to classical *in vitro* approach[71]:

1. The ability to screen more compounds in less time, thereby accelerating the drug discovery process.
2. The ability to run these models on virtual compounds, so that an appropriate balance of various properties can be chosen from the large virtual libraries for chemical synthesis.
3. An understanding of the relationship between these ADMET properties and the structure and physiochemical properties of a compound can be gained.

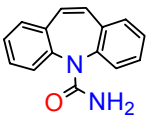
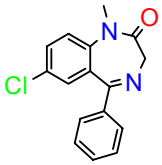
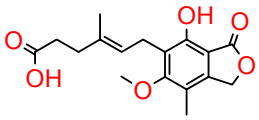
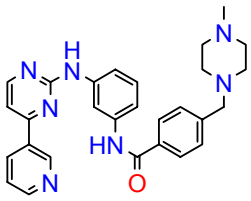
The descriptors used for most methods of correlation and prediction of *in vitro* or *in vivo* ADME data range from simple descriptors such as lipophilicity, hydrogen bonding, flexibility, molecular size and VolSurf approaches to more complex ones, such as topological descriptors. Based on the analysis of the World Drug Index (WDI) Lipinsky developed the now popular "Lipinsky rule of five" (depicted in the Fig.23) which predicts in a simple manner the drug likeness of a molecule.

Fig.23



In *Table 4* the properties of 4 drugs are compared with regards to the Lipinsky "rule of 5".

Table 4.

	MW	LogP	HB Don.	HB Acc.
 Targetol *Anticonvulsant*	236.3	2.5	1	3
 Diazepam *Anxiolytic*	284.7	2.7	0	3
 Melbex *Antiviral*	320.3	2.0	2	6
 Gleevec *Anticancer*	493.6	4.2	2	8

Models of ADME properties are most commonly based on empirical approaches such quantitative structure activity relationship (QSAR). These techniques can be applied with great efficiency to large numbers of molecules, but require a significant quantity of high quality data that allows to deduce a relationship between structure and activity. Several approaches can be used to train a QSAR model[70].

In addition to the well-know Lipinsky Rule of Five the following filters have been reported and are used in in silico-programs like ilib diverse[72], a program for creating libraries of drug-like organic molecules suitable for rational lead structure discovery.

Filters for High drug-likeness:

High drug-likeness: Ghose filter[73]

More than 80% of the compounds of the Comprehensive Medicinal Chemistry database Ver. 97.1 were found with the filter developed by Ghose. The following drug-likeness constraints are applied to ilib diverse's Ghose filter:

MW: 160-480

Number of atoms: 20-70

logP: -0,4 to +5,6

High drug-likeness: Lee filter[74]

Lee developed a filter for high drug-likeness by the analysis of natural products and trade drugs. The following constraints are applied to ilib diverse's Lee drug-likeness filter:

MW: mean 356

logP: mean 2,1

High drug-likeness: Mozziconacci filter

Mozziconacci developed a filter for drug-likeness by analyzing 15 commercially or freely available chemical libraries. The drug-likeness of these compounds was then investigated using common chemical features such as the Rule-of-5, the flexibility, the atom types and the functional groups. Based on this information, successive filters were designed to extract a drug-like subset of compounds. The following drug-likeness constraints are applied to ilib diverse's Mozziconacci filter:

RB: max. 15

Rings: max. 6

Oxygens: min. 1

Nitrogens: min. 1

Halogens: max. 7

High drug-likeness: Oprea filter[75]

Oprea analyzed property distributions of physicochemical descriptors and properties in many databases containing drug-like compounds to identify optimal ranges. MDDR, Current Patents Fast-alert, CMC, Physician Desk Reference and New Chemical Entities were used as drug-like references and ACD as non drug-like data pool. The following drug-likeness constraints are applied to ilib diverse's Oprea filter:

HDO: 0-2

HAC: 2-9

RB: 2-8

High drug-likeness: Walters & Murcko filter[76]

Walters and Murcko developed an extensive filter system for high drug-likeness. The following drug-likeness constraints are applied to ilib diverse's Walters & Murcko filter:

MW: 200-500

HDO: 0-5

HAC: 0-10

PSA: 0-120

RB: 0-8

Heavy atoms: 20-70

Charge: -2 to +2

Filters for orally bio-available drugs:

Orally bio-available: Fichert filter[77]

The Fichert setting was developed using a Caco-2 model and 41 compounds. This cell model is widely used as an indicator of oral drug absorption. The following drug-likeness constraints are applied to ilib diverse's Fichert filter:

MW: max. 500

logD: 0-3

Lipinski filter[78]

Lipinski's rule of 5 is the most approved filter for discrimination between drug-like and non drug-like molecules. It was developed analyzing the physicochemical properties of 2,245 compounds of the World Drug Index. Only compounds with INN (International Non proprietary Name) or USAN (United States Adopted Name) are included in the study.

MW: max. 500

HAC: max. 10

HDO: max. 5

logP: max. 5

Orally bio-available: Palm filters[79, 80]

This settings are based on the first study in which dynamic surface properties of drug molecules are correlated with drug absorption. The results suggest that the PSA (Polar Surface Area) is a better descriptor of intestinal drug absorption than log P.

Drugs with PSA > 139 A^2 will be < 10% absorbed while drugs with PSA < 63 A^2 will be completely absorbed.

PSA max. 140 A^2: orally bioavailable

PSA max. 63 A^2: strictly orally bioavailable

Orally bio-available: Enhanced Palm filters[81, 82]

This settings are based on the first study in which dynamic surface properties of drug molecules are correlated with drug absorption. The results suggest that the PSA (Polar Surface Area) is a better descriptor of intestinal drug absorption than log P.

Drugs with PSA > 139 A² will be < 10% absorbed while drugs with PSA < 63 A² will be completely absorbed.
PSA max. 140 A²: orally bioavailable
PSA max. 63 A²: strictly orally bioavailable

Orally bio-available: Veber filter[83]

The VEBER filter was developed by the analysis of 1,100 drug candidates. It seems that molecules fitting to these two properties have a high probability of good oral bioavailability in the rat.
RB: max. 12
PSA: max. 140 A²

Filters for blood brain barrier permeable drugs:

Blood brain barrier permeable: Murcko filter[84, 85]

Murcko used 1D and 2D descriptors based on Lipinski's Rule-of-5 and 2D fingerprints to determine CNS activity. The following constraints are applied to ilib diverse's Murcko filter:
MW: 200-450
logP: 0-5,2
HAC: max. 4
HDO: max. 3
RB: max. 7

Blood brain barrier permeable: Van de Waterbeemd filter[86]

In contrast to drug-likeness, logP as descriptor of the blood-brain barrier is not an effective classifier of CNS (Central Nervous System)-likeness. Van de Waterbeemd developed a filter for CNS-likeness with PSA (Polar Surface Area) and MW (Molecular Weight) as descriptors.
MW: < 450
PSA: < 90 A²

Filters for for lead-likeness:

Lead-likeness: Oprea filter[87]

Oprea distinguished the lead-like chemical space from the drug-like space. The following lead-likeness constraints are applied to ilib diverse's Oprea lead-likeness filter:

MW: max. 450

logP: -3,5 to +4,5

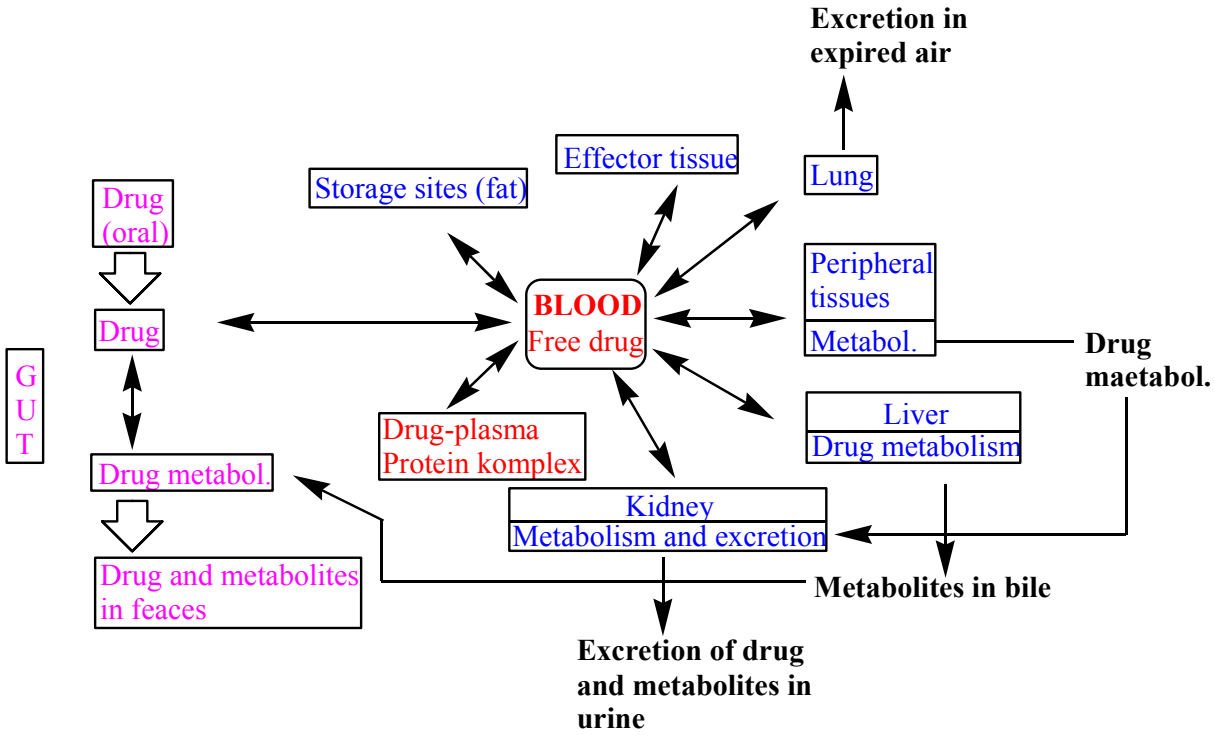
HAC: max. 8

HDO: max. 5

Introduction to Pharmacokinetics.

Pharmacokinetics (*PK*) is the study of the uptake, distribution and clearance of drugs with respect to time. The **PK** profile of a drug, in which the absorption, distribution, metabolism and excretion processes following *in vivo* administration are mathematically described, is derived from the plot of the systemic drug concentration versus time for that compound[88]. On *Fig.15* the main patterns involved in the process of determination of drug pharmacokinetics, are depicted.

FIG. 15.



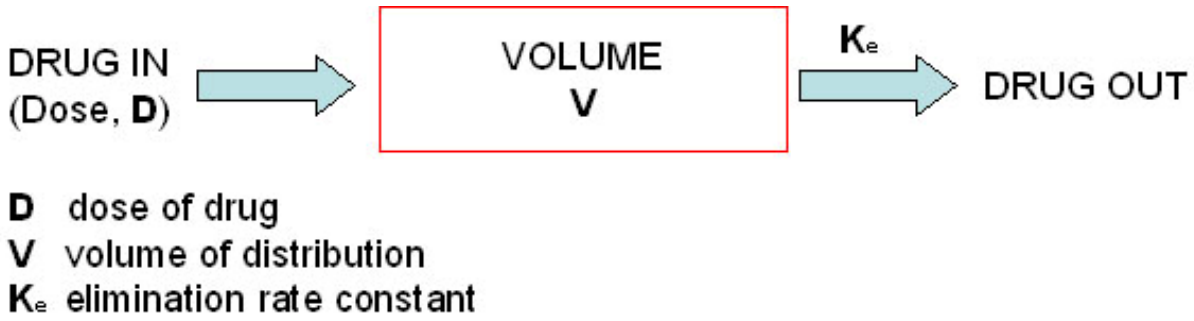
In order to interpret pharmacokinetic data, it is necessary to set up certain models of the body so that the mathematical equations describing the movement of the drugs can be formulated.

Very simple mathematical models describing the concentration changes of the drug in the body are: 1) the one compartment model and 2) the two compartment model.

One compartment model:

In this model it is assumed, that all body compartments are in rapid equilibrium with a central compartment and therefore the drug concentration is constant in each single compartment. The corresponding pharmacokinetic model is depicted in *Fig. 16*.

FIG. 16.



The concentration of the drug can be expressed:

EQUATION 1.

$$C_0 = D/V \text{ resp. } V = D/C_0$$

C_0 concentration at the time 0 s.

The clearance of the drug from the compartment depends off the elimination rate constant (k_e). The half time for the drug ($t_{1/2}$) in one compartment model is always the same, due to the first order kinetics. All the parameters which can be measured in the one compartment model are: V- volume of distribution, C_0- concentration of drug at the time 0 s, C_t- concentration of the drug at various times, $t_{1/2}$- half-life of elimination, k_e- elimination rate constant, D- dose of drug given. The elimination rate constant is mathematically defined:

EQUATION 2.

$$dC/dt = -k_e.C_0$$

Using this equation and measurements of plasma drug concentration at various times the elimination rate constant can be calculated. The combination of *equation 1 and equation 2* gives the *equation 3* for the overall rate at which the drug can be cleared from the body.

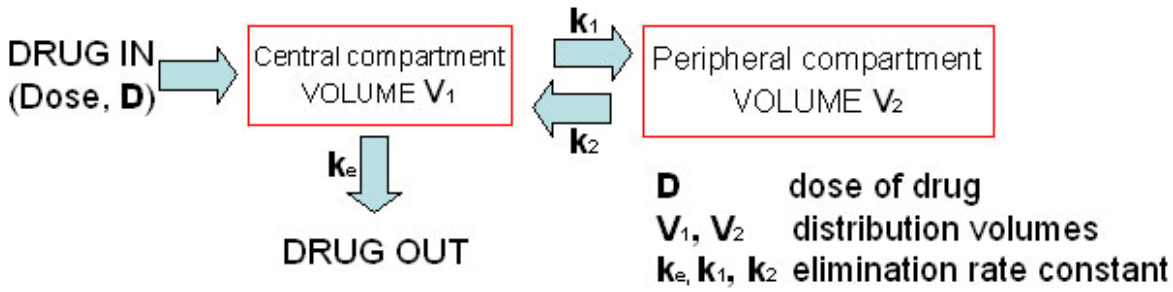
EQUATION 3.

$$dC/dt = k_e.D/V$$

Two compartment model:

In the two compartment model one non-excreting compartment is added to the single compartment in order to correct the fact, that many peripheral tissues can not excrete directly and excretion takes place mainly from the blood. (*Fig. 17*).

FIG. 17.



All parameters calculated from one compartment model can be calculated from two-compartment model as well, although the mathematical analysis is more complex. One more parameter appears in the two- compartment model: the area under the concentration times curve (AUC), which expresses the total body load of drug and is a direct indication of the therapeutic value of the drug. AUC can be measured experimentally and used to calculate the clearance *equation 4*. Clearance is defined as the volume of the central compartment which is cleared in unit time.

EQUATION 4.

$$\text{clearance} = D/AUC \quad \text{or} \quad \text{clearance} = k_e.V_1$$

More complicated models can be constructed by modification of the one and two compartment model, for example by adding absorption compartments and other compartments.

FIG. 20.

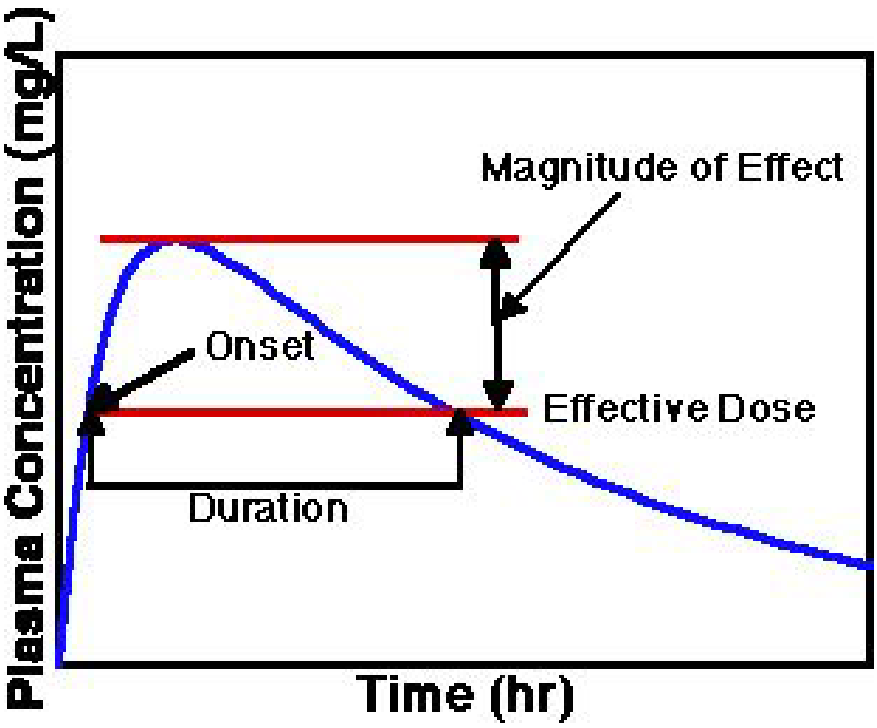


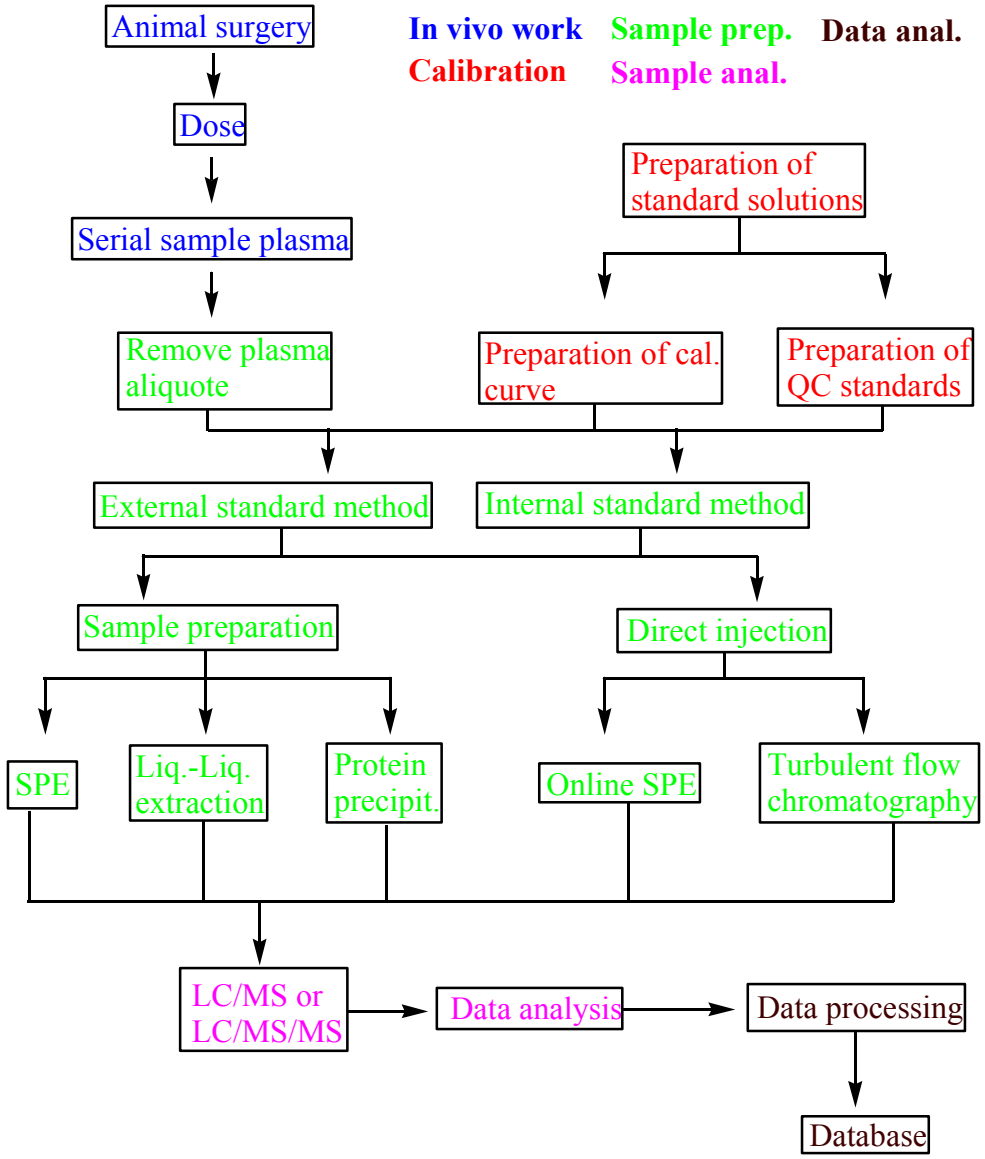
Fig. 20 shows the relationship between drug concentration and drug effect as a function of time. If a drug has to reach an effective concentration at a receptor site, this will be reflected as a required blood concentration.

Practical pharmacokinetics measurements:

In practise, after parental or oral administration, a series of blood samples is collected as a function of the time and then analyzed for the drug content. The complete process consists of five stages[89]: 1) The *in vivo* work 2) The calibration of an analytical method 3) The preparation of samples 4) The quantitative analysis 5) Data reporting. The gravity point is on the steps 2,3 and 4. The use of LC coupled with ***(API)-MS*** or ***MS/MS*** for the analysis of bio

fluids increased rapidly in the last teen years[90, 91]. *Fig. 18* shows the process of pre-clinical pharmacokinetic studies in the flow- diagram.

FIG. 18.[89]



The use of stable isotopes in clinical pharmacology:

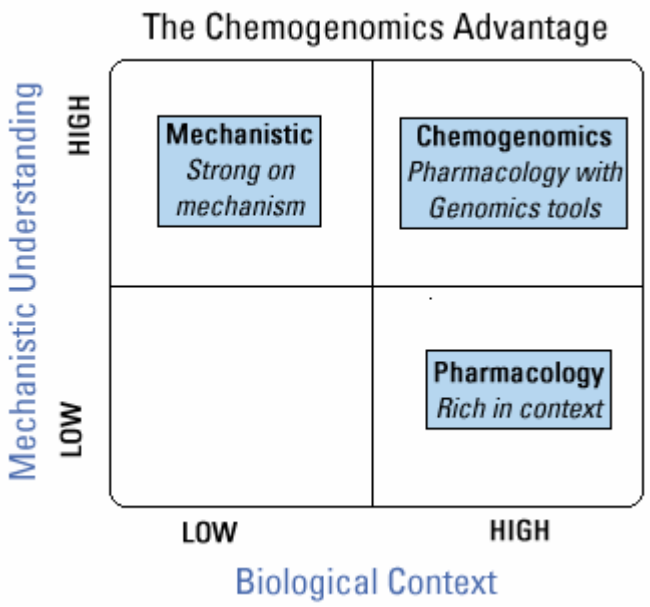
Stable isotopes can be incorporated into a molecule, producing a stable-isotope-labeled (***SIL***) molecule with a higher or lower molecular weight than that of the unlabeled molecule. The natural ***SIL*** of molecules is proportional to the natural occurrence of the considered stable isotope and yields a low rate of labeling. Artificial ***SIL*** allows higher rates of labeling. The customary and ***SIL*** drug forms are identical, except that the ***SIL*** form differs by one or more atomic mass units. These two forms cannot be distinguished using analytical techniques such as high-pressure liquid chromatography or gas chromatography, since the change in molecular

weight by itself does not cause a measurable change in retention time. However, the customary drug form can be distinguished from the ***SIL*** form by gas chromatography (***GC***)/mass spectroscopy (***MS***), because molecular mass differences can be separated from the corresponding fragments of the two drug forms[92]. ***SIL*** methodology can substantially extend the power of clinical pharmacokinetics[93, 94]. Bioequivalence studies traditionally require a cross-over design, in which the same drug is administered to the same individual on separate occasions. One trial involves dosage with the "reference" compound, in the other trial, the dosage form being tested is administered. Plasma drug concentrations are measured at multiple time points after drug administration in each trial, and bioequivalence is evaluated by comparison of variables such as peak plasma concentration (C_{max}), time of peak concentration (T_{max}), elimination half-life, and area under the plasma concentration curve (AUC). With ***SIL*** methodology, bioequivalence studies can be done with a single exposure trial per subject[95]. One dosage form is the conventional drug, the other is the ***SIL*** form. Both are administered at the same time. The ***GC/MS*** analytical procedure can simultaneously quantitate concentrations of each drug form in each plasma sample. This approach saves time and reduces drug exposure and blood sampling requirements for human volunteers. ***SIL*** is useful in different types of studies[96] such as studies on the fate of drugs (drug metabolic pathways and pharmacokinetics), determination of compliance, and assessment of therapeutic and unwanted effects of drugs. The stable-isotope-labeled methodology can also be used to study definitively the single-dose pharmacokinetics of a drug in a patient already receiving the same drug on a chronic basis[97]. Using stable-isotope tracer methods, it is possible to perform pharmacokinetic studies which measure steady-state values and evaluate time-dependent and dose-dependent pharmacokinetic changes without exposing the subject to radiation, without creating radioactive waste, and without withholding necessary medication[98]. ***SIL*** methodology has drawbacks as well as benefits. Substantial synthetic and medicinal chemistry resources are needed to prepare ***SIL*** drugs and formulate them for human administration. ***GC/MS*** facilities must be available for analysis of all biological samples. These analyses are much more difficult and expensive than usual high-performance liquid chromatographic and ***GC*** procedures.

Chemogenomics and DrugMatrix

Chemogenomics can be defined as the use of genomics to measure the broad effect of a compound on all pathways in an intact biological system. The goal is to define an organism's biological response to treatment with a compound, rather than looking at each component, be it an individual receptor, cell type, or tissue, individually. As such, chemogenomics is related to pharmacology, which measures the *in vivo* physiological response to drug treatment, but uses genomic tools to measure biological responses. The historic approach to in vivo pharmacology was highly relevant from a biological perspective because it looked at the pharmacology of the intact system, but provided little knowledge about what was going on at the mechanistic level. The reductionist approach brought about by the genomics revolution went far in compensating for the deficiencies of old pharmacology by providing a better mechanistic description but lost the advantage of the biological context of being in a living system, as targets are looked at in isolation. Chemogenomics combines the strengths both approaches, by looking at the mechanistic approach, but on a genome-wide scale (depicet in the *Fig.24*). Chemogenomics can change the drug discovery process by increasing efficiencies and by weeding out failures early.

Fig.24:



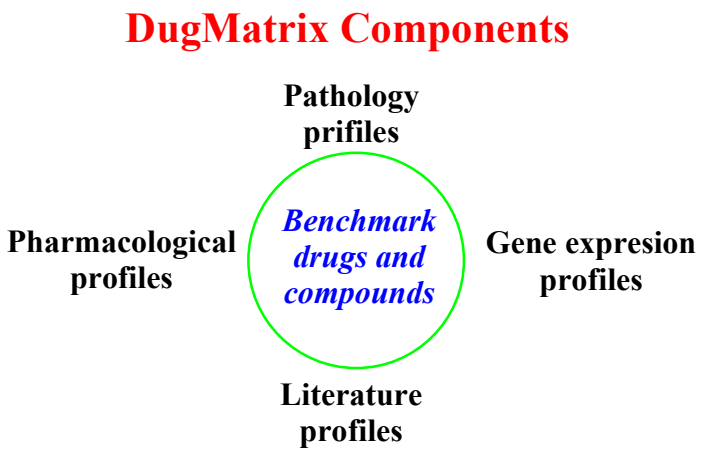
Applying chemogenomics to the understanding of the broad spectrum of effects that compounds exert in a living system result in the following possibilities:

1. Characterize the mechanistic profile of a compound.
2. Assess the on-target and off-target effects of compounds (not just the adverse effects, but also to identify potential alternative therapeutic applications of compounds).

3. Allow prioritization of candidates and removal of failures early in the process.
4. Identify surrogate markers of the biological effects of compounds (again, for both positive efficacious effects, and negative effects).
5. Lead to a better understanding of which collected data are the most informative.
6. Ultimately, this may also assist in the design of clinical studies.

DrugMatrix is a comprehensive research tool in the new field of chemogenomics. It enables to select the highest quality leads and drug candidates at the earliest stages of drug discovery and development and eliminate failures. The DrugMatrix informatics system is a reference system that allows to explore the effects and properties of well understood drugs and structurally related failures, and reference compounds such as standard toxicants and biochemical standards. It combines data access with content consisting of several dimensions of data on whole biological systems that have been treated in vivo and in vitro with chemical compounds. The data integrated by DrugMatrix consist of data from molecular pharmacology profiles, gene expression profiles, and literature profiles, extracted from primary, secondary, and on-line sources. The DrugMatrix components are schematically depicted in the *Fig.25*.

Fig.25



- The literature annotation has a primary focus on consolidating all the available data on clinical studies, in vivo and in vitro pharmacology and toxicology, and associated physiology, into the database.
- The pharmacological profiles are made up of data generated in a standard panel of ~130 assays from MDS Pharma Services. It includes receptors, ion channels, kinases, P450,s etc, selected partly on the amount of use of the assays by pharmaceutical companies, and on the mode of action of the compounds being studied.

- The gene expression profiles are being generated on 10,000 gene arrays, with an average of 24 arrays per tissue per compound.

DrugMatrix can be applied to understanding the broad spectrum of effects of chemical compounds on a living system to characterize, evaluate, and prioritize compounds for further optimization.

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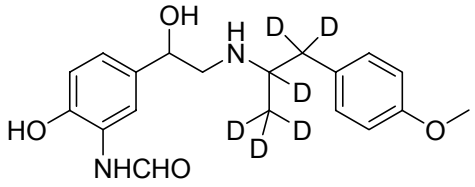
Special part and discussion

As the results from this dissertation have been submitted as 8 separate papers, the manuscripts are summarized here:

1. Synthesis of standards of biological active compounds

1.1. Deuterated analogs

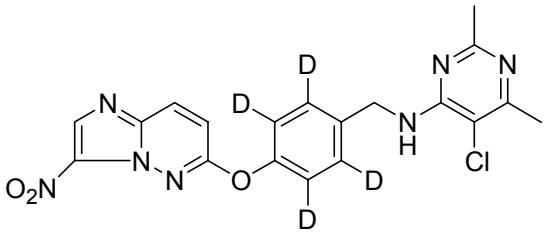
1.1.1. Synthesis of d_6-formoterol for use as internal standard in a LC/MS/MS method developed for quantification of the drug in human serum.



For the new drug application (NDA) of formoterol as a selective *β*-adrenoreceptor a deuterated analog was prepared to serve as an internal standard for the LC/MS/MS analysis of this drugl in human plasma at very low concentrations (0.40 – 100.24 pg/ml) following administration of low therapeutical doses (9 – 36 μg).

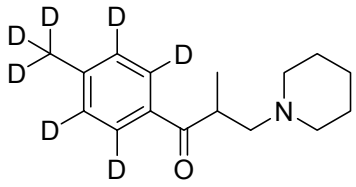
This paper has been submitted to the Journal of Labelled Compounds and Radiopharmaceuticals. Posters with various aspects of this work were presented at the 9th Blue Danube Symposium on Heterocyclic Chemistry (BDSHC) (Tatranska Lomnica, 16-20. 6. 2002) and at the Joint Meeting on Medicinal Chemistry (Vienna, 20-20. 6. 2005).

1.1.2. Synthesis of d_4 BY-170424 (5-chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo [1,2-b]pyridazin-6-yl)oxy]-2,3,5,6-d4-phenyl]methyl]-4-pyrimidinamine) as internal standard for LC/MS/MS determination in human serum.



A deuterated analog of BY-170424 was prepared as an internal standard for the LC/MS/MS analysis of low concentrations of BY-170424 in body fluids. The poster was presented at the Joint meeting on Medicinal Chemistry (Vienna, 20-20. 6. 2005) and this paper has been submitted to Journal of Labelled Compounds and Radiopharmaceuticals.

1.1.3. Synthesis of d_7-tolperisone for use as LC/MS/MS standard

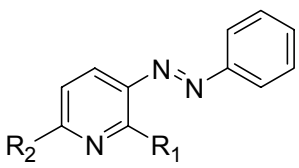


Deuterated tolperison-d_7 was synthesized in order to provide an internal analytical standard suitable for LC/MS/MS analysis of very low concentrations of tolperisone in body fluids. Additionally, it has been claimed*, that deuterated analogs may exhibit improved pharmacokinetics as well as pharmacodynamic properties. The poster was presented at the Joint meeting on Medicinal Chemistry (Vienna, 20-20. 6. 2005). This paper has been submitted to Journal of Labelled Compounds and Radiopharmaceuticals.

* Alken RG, Stabingis T, PCT Int. Appl. WO 2002088100, 2002.

1.2. *Reference standards*

1.2.1. Synthesis of by-products of Phenazopyridines as API (active pharmaceutical ingredient) standards.



R_1= R_2= NH_2 **1**

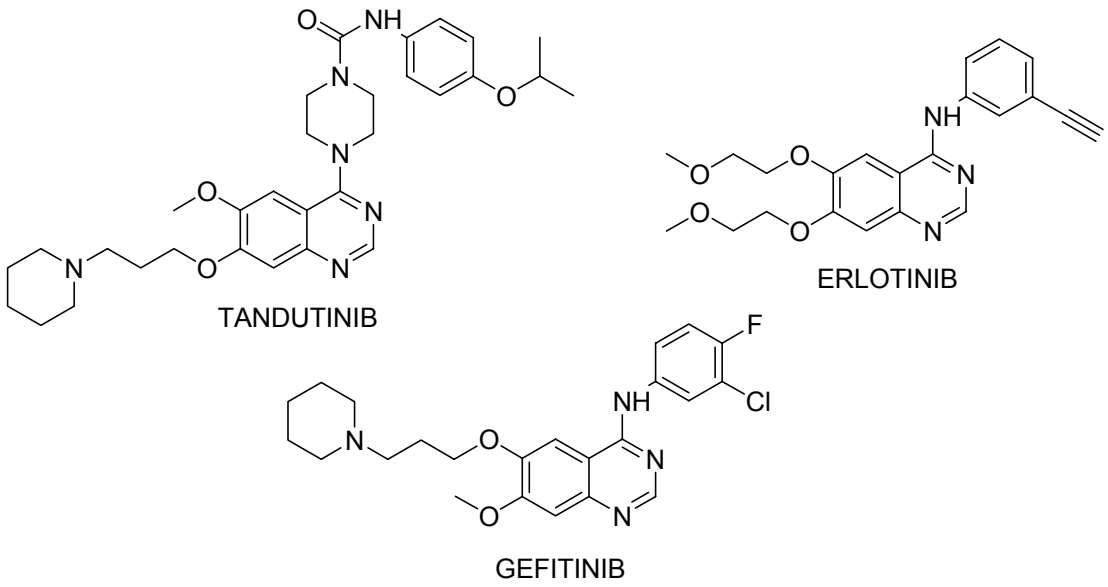
R_1= NH_2, R_2= OH **2**

R_1= OH, R_2= NH_2 **3**

R_1= R_2= OH **4**

In the course of the NDA or re-registration of a new or known drug reference standards of all impurities of the API that are present in >0.5% have to be prepared in order to fully characterized the product. To full fill this need the reference standards **1**-**4** were prepared. This paper has been submitted to Molecules.

1.2.2. Improved Synthesis of Substituted 6,7-Dihydroxy-4-quinazolines: Tandutinib, Erlotinib, Gefitinib.

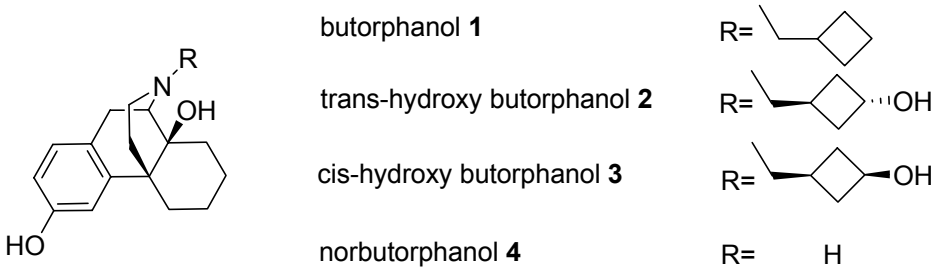


These compounds were prepared with substantial improvement and full characterization of the intermediates to be used as reference compounds for the Drug Matrix. This work was presented as a poster at the 10th Blue Danube Symposium on Heterocyclic Chemistry (Vienna 3-6. 9. 2003) and the paper has been submitted to Molecules, and has already appeard: http://www.mdpi.org/molecules/list06.htm#issue4

2. Synthesis of metabolites and antimetabolites of biological active compounds

2.1. *Metabolites*

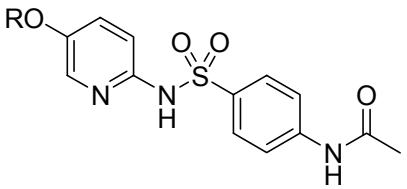
2.1.1. Synthesis of oxygenated Butorphanol metabolites.



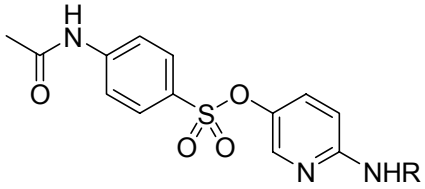
Butorphanol (**1**) is a synthetic opioid analgesic used in the treatment of post-surgical and dental pain. Trans-hydroxybutorphanol (**2**) was identified as major urinary metabolite of **1.** We synthesised both isomers, **2** and **3,** in order to provide an analytical standard necessary for NDA submission. The posters with various aspect of this work were presented at the 11th Blue Danube Symposium on Heterocyclic Chemistry (Brno, 28. 8.- 1. 9. 2005) and on the 9th

International Electronic Conference on Synthetic Chemistry (1- 30. 11. 2005). This paper has been submitted to the European Journal of Organic Chemistry.

2.2.2. Synthesis of Sulfapyridine metabolites



R=H, Ac

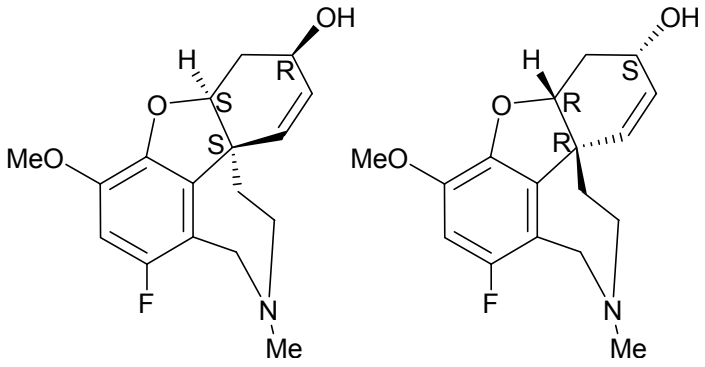


R=H, Ac

Sulfapyridine (**SP**) and sulfasalazine (**SASP**) are used for the treatment of Crohn`s disease, rheumatoid arthritis and ulcerative colitis. **SP** is the therapeutically active moiety of **SASP** and in the body undergoes further degradation. The main metabolites were reported to be the N-acetylsulfapyridin (**Ac-SP**) and 5-hydroxy-sulfapyridine (**OH-SP**). We have synthesised N-acetyl-5-OH-sulphapyridine (**Ac-OH-SP**), 4-acetylamino-benzenesulfonic acid 6-amino-pyridin-3-yl ester and two more potential metabolites of **SP**, N-[4- (1-oxy-pyridin-2-yl-sulfamoyl)-phenyl]-acetamide and N-[4- (hydroxyl-piridine-2-yl-sulfamoyl)-phenyl]-acetamide having the same molecular mass as **Ac-OH-SP**. This work has been presented as a poster in the year 2004 on the 21th European Colloqium on Heterocyclic Chemistry (Sopron, 12-15. 9. 2004) and on the 7th International Electronic Conferences on Synthetic Organic Chemistry (1-30. 11. 2003) and published as a special issue devoted to this event.

2.2. *Antimetabolites*

2.2.1. Synthesis of (-)- and (+)-8-fluoro-galanthamin:



The synthesis and chiral separation of 8-fluoro-galanthamine on a preparative scale was achieved. The structure was verified by X-ray and found to be almost identical to galanthamine. The poster was presented on 19- ÖPhG- Tagung (Innsbruck, 20- 22 april 2006). This paper has been accepted by Tetrahedron Letters as a short note and a full paper has been submited to Tetrahedron.

Synthesis of d_6-formoterol for use as internal standard in a LC/MS/MS method developed for quantification of the drug in human serum.

Petr Knesl and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology

Getreidemarkt 9, 1060 Vienna, Austria

Summary

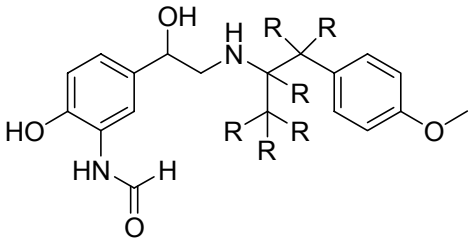
The synthesis of hexadeuterated formoterol (**1**) to be used as internal standard for LC/MS/MS determination of **2** in human serum is described.

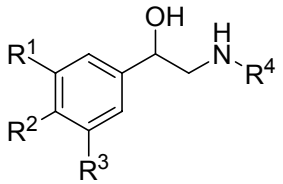
Key Words: Formoterol d_6; deuterium exchange; reductive amination

Introduction

Bronchodilators, in particular *β*-adrenoceptor agonists, are recognized as effective drugs to treat asthma and other bronchospastic conditions.[1] A variety of *β*-adrenoceptor agonists, exhibiting a *β*-phenyl-ethylamine substructure, such as isoproterenol[2] (**3**), salbutamol (**4**) and terbutaline[3] (**5**), are known. *See Fig. 1*. Formoterol (**2**) is a selective *β*-adrenoreceptor featuring rapid onset and long duration of action.[4] The synthesis of deuterated ($^{+}/_{-}$) formoterol (**1**) was required to provide an internal standard suitable for LC/MS/MS determination of formoterol (**2**) in biological samples.[5] To the best of our knowledge no preparation of deuterated formoterol (**1**) has been published. The use of formoterol d_6 was mentioned in web publications describing the LC/MS/MS analysis of formoterol in human plasma at 0.4-100 pg/ml after administration of low therapeutic doses of about 4.5-18 μg[6,7,8] and determination of formoterol in rat, rabbit and beagle plasma using post column addition.[9] Here we describe the preparation of formoterol d_6 (**1**) via the deuterated intermediates 4-methoxy-
Fig. phenylacetone d_5 (**8**) and N-benzyl-2-amino-1(-4-methoxyphenyl)-propane d_6 (**9**).

Fig.1: Known *β*-adrenoreceptors





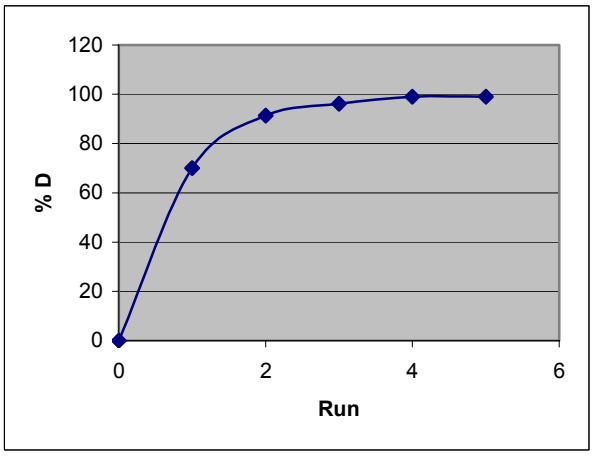
1: R= D
2: R= H

Isoproterenol **3**: R^1= OH, R^2= H, R^3= i-propyl
Salbutamol **4**: R^1= OH, R^2= CH_2OH, R^3= t-butyl
Terbutaline **5**: R^1= OH, R^2= H, R^3= t-butyl

Results and discussion:

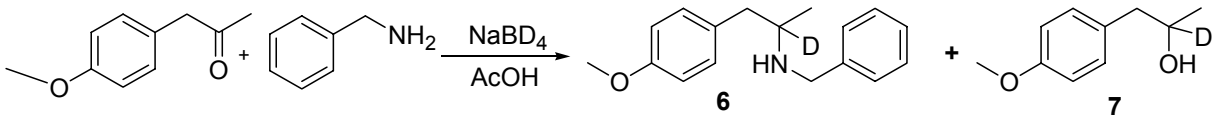
The key step in the described synthesis of formoterol d_6 (**1**) was the preparation of N-benzyl-2-amino-1(-4-methoxyphenyl)-propane d_6 (**9**), obtained from the reaction of 4-methoxy-phenyl(1,1,3,3,3-D_5)- acetone (**8**) and deuterated benzylamine. For the conversion of 4-methoxy-phenyl-acetone to the corresponding 4-methoxy-phenyl(1,1,3,3,3-D_5)-acetone (**8**) D_2O/ MeOD and K_2CO_3 was used as a base. This procedure was repeated six times and the progress was monitored by using ^{1}H-NMR. *See Fig. 2.*

Fig. 2.



Reductive amination using a non deuterated starting material and $NaBD_4$ in AcOH afforded N-benzyl-2-amino-1(-4-methoxyphenyl)-propane d_1 (**6**) in 27% yield. 4-Methoxyphenyl-propane-2-ol (**7**) was isolated as a side product. *See Scheme 1.*

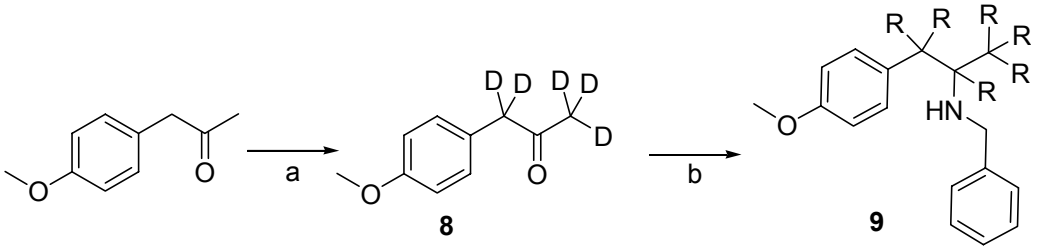
Scheme 1.



Using this method with the deuterated ketone **8** resulted in the loss of deuterium and afforded N-benzyl-2-amino-1-(-4-methoxyphenyl)-propane d_6 (**9**) with only 20% deuterium content as determined from ^{1}H-NMR. Applying Ti(O-i-PrOH)$_4$ and $NaBD_3CN$ in the reductive amination with benzylamine in MeOD allowed us to improve the yield (50%) as well as the deuterium content to 88% as determined by ^{1}H-NMR. This reaction was repeated under the same conditions using benzylamine d_2 and MeOD as a solvent affording N-benzyl-2-amino-1-(-4-methoxyphenyl)-propane d_6 (**9**) in 50% yield, a HPLC purity of >99% and deuterium

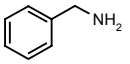
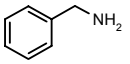
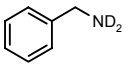
content 96% based on ^{1}H-NMR. *See Scheme 2*. The results of the reductive amination at diverse conditions are summarized in *Table.1*. Benzylamine d_2 was prepared from benzylamine by deuterium exchange with MeOD. The content of deuterium as determined by ^{1}H-NMR was 92% after repeating the procedure seven times.

Scheme 2.



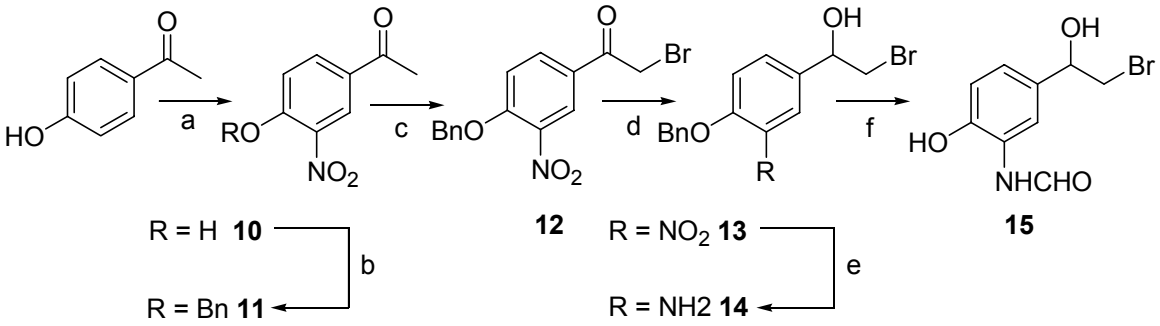
a) K_2CO_3, D_2O, MeOD b) see table 1.

Table 1.

Reactant 2	Conditions	Yield%	HPLC%	D-content%
	$NaBD_4$/AcOH	25	98	20
	Ti(o-iPrO)$_4$/ NaBD3CN MeOD	51	97	88
	Ti(o-iPrO)$_4$/ $NaBD_3CN$ MeOD	52	99	96

The synthesis of the second key intermediate **15** started from 4-hydroxy acetophenone which was nitrated followed by protection of the hydroxy group with benzyl chloride. The bromination of the methyl ketone **11** was carried out with bromine in dioxane as a solvent. The HBr formed was removed by a stream of nitrogen and the product **12** was reduced to **13** using a borane-dimethylsulfide complex. This reaction afforded bromohydrin **13** in 75% yield and 97% HPLC purity.

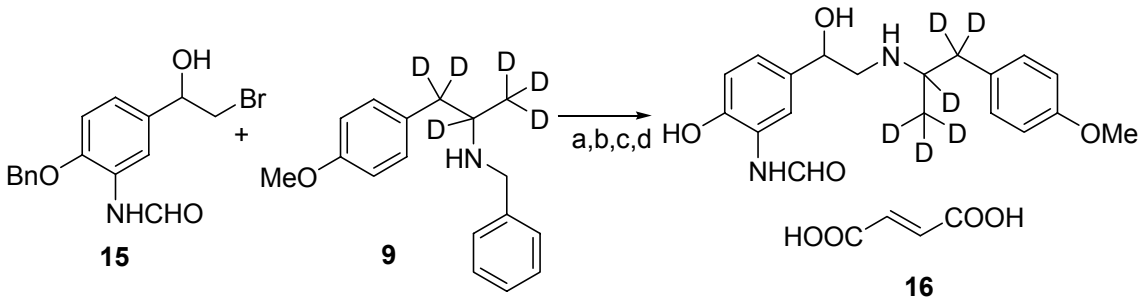
Scheme 3:



a) HNO_3, AcOH b) BnCl, K_2CO_3 c) Dioxane d) $(CH_3)_2S$/THF e) PtO_2, H_2 f) HCOOH, Ac_2O

Reduction of the nitro group via catalytic hydrogenation followed by formylation using in situ prepared formic-acetic-anhydride gave the intermediate **15** in an overall yield of 20% and a HPLC purity of >99%. *See Scheme 3*. The final step was accomplished by following the procedure of Hett and Gao[10]: reacting the formamide **15** with potassium carbonate results in formation of an epoxide which reacted with **9** at 120°C without additional solvent. Catalytic N-debenzylation generated formoterol d_6 as a free base which was transformed to the fumarate **16**. *See Scheme 4.*

Scheme 4.



a) K_2CO_3, MeOH, THF b) neat, 120 °C c) Pd/C, H_2 d) fumaric acid, i-PrOH

Experimental part

Melting points were measured on a Büchi B-545 melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO-d_6. For thin layer chromatography (TLC) Merck TLC aluminum sheets silica 60 F_{254} were used. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). MPLC (medium pressure liquid chromatography) was performed using silica gel (VWR 40-63 μm), a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument with Merck Chromolith RP_{18} columns and a

gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 1.0 to 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

4-Methoxy-α-methyl-N-(phenylmethyl)- benzeneethanamine (1-d_1) **(6)** *and 1-(4-methoxy-phenyl)-propane-2(d_1),2-ol* **(7)**

A solution of benzylamine (0.3 g, 2.8 mmol) in acetic acid (5 mL) was added drop wise to the 4-methoxyphenylacetone (0.38 g, 2.31 mmol) and the resulting mixture was stirred at room temperature under nitrogen for 60 min. Sodium borodeuteride (1.2 g, 28.8 mmol) was added in small portions in the course of 90 min. and the mixture stirred at room temperature under nitrogen for 12 h. The pH of the solution was adjusted to 10 using sodium hydroxide (2 M water solution) and subsequently extracted with dichloromethane (5x20 mL). The organic layers were collected, dried (Na_2SO_4), decolorized (charcoal) and volatiles removed to afford the crude product (380 mg) which was purified by column chromatography using (dichloromethane/methanol 5%) to afford two products, **6** (165 mg, yield 28%, HPLC 98%) as an colourless oil and **7** (158 mg, HPLC 99%) as an colourless oil.

^{1}H-NMR ($CDCl_3$) **6**: 1.07 (s, 3H), 1.53 (w, 1H), 2.54-2.73 (dd, 2H, J_2= 13.59 Hz), 3.68-3.88 (dd, 2H, J_2= 13.3 Hz), 3.78 (s, 3H), 6.78-6.85 (d, 2H, J_3= 8.7 Hz), 7.03-7.09 (d, 2H, J_3= 8.7 Hz), 7.16-7.33 (m, 5H),

^{1}H-NMR ($CDCl_3$) **7**: 1.21 (s, 3H), 1.63 (w, 1H), 2.57-2.76 (dd, 2H, J_2= 13.5 Hz), 3.79 (s, 3H), 6.83-6.87 (d, 2H, J_3= 8.7), 7.10-7.14 (d, 2H, J_3= 8.7).

1-(4-Methoxy-phenyl)-2-propanone-(1,1,3,3,3-d_5) **(8)**

A suspension of 4-methoxyphenylaceton (10.0 g,61 mmol) and potassium carbonate (0.177 g, 1.3 mmol) in D_2O (7.7 g, 38 mmol) and MeOD (2.0 mL) was stirred vigorously and heated to reflux for 16 h. under a atmosphere of dry argon. Then the mixture was cooled and extracted with CH_2Cl_2 (4x50 mL), the organic layers were collected, decolorized (charcoal) and dried (Na_2SO_4). Volatiles were removed to afford a yellow liquid which was analyzed by ^{1}H-NMR. After repeating the procedure six times the deuterium content was determined to be >98% via ^{1}H-NMR, and the product obtained as yellow liquid (8.2 g, yield 80%, HPLC 96%). ^{1}H-NMR ($CDCl_3$) : 2.07-2.11 (m, 0.014H), 3.79 (s, 3H), 6.84-6.90 (d, 2H, J_3= 8.65 Hz), 7.09-7.14(d, 2H, J_3= 8.65).

4-Methoxy-α-methyl-N-(phenylmethyl)- benzeneethanamine (1,2,2,α,α,α-d_6) **(9)**

A mixture of **8** (1.69 g, 9.99 mmol), benzylamine d_2 (1.1 g, 10 mmol) and titanium ortho-isopropoxide (3.55 g, 12.5 mmol) was stirred at room temperature under argon for 3 h. Deuterated methanol (10 mL) and sodium cyanoborodeuteride (0.42 g, 6.7 mmol) were added at once and the mixture was stirred under the same conditions for further 48 h. Deuterium oxide (5 mL) was added and the mixture stirred vigorously for 10 min followed by addition of dichloromethane (50 mL). The white precipitate obtained was filtered and washed with dichloromethane (2x50 mL). The organic layers were collected, dried (Na_2SO_4), decolorized (charcoal) and evaporated to dryness to afford the crude product (1.95 mg) as a yellowish oil which was purified by column chromatography using dichloromethane/methanol 97:3 to afford (1.26 mg, yield 52%, HPLC 99%, 96% D according to ^{1}H-NMR) of the product as a colourless oil.

^{1}H-NMR ($CDCl_3$) 1.07 (w, 0.1H), 1.70 (w, 1H), 2.57-2.66 (d, 0.08H), 3.68-3.88 (dd, 2H, J_2= 13.2 Hz), 3.78 (s, 3H), 6.80-6.84 (d, 2H, J_3= 8.8 Hz), 7.04-7.09 (d, 2H, J_3= 8.8 Hz), 7.19-7.34 (m, 5H).

1-(4-Hydroxy-3-nitrophenyl)-ethanone **(10)**

A solution of 4-hydroxyacetophenone (40.0 g, 294 mmol) in glacial acetic acid (200 mL) was stirred mechanically and heated to 60°C. HNO_3 conc. (21.6 mL) was added drop wise while the temperature was kept at 60-70 °C and subsequently this mixture was maintained for another 30 min at 60 °C. The reaction mixture was poured in ice/water solution, the yellow precipitate was filtered and crystallized from ethanol to afford the product (33.2 g, yield 62%, HPLC 98%, m.p.:132-134 °C, Lit.m.p.[11]:134 °C) as a yellow crystals.

1-(4-Benzyloxy-3-nitrophenyl)-ethanone **(11)**

A suspension of **10** (33.0 g, 182 mmol), benzyl chloride (28.0 g, 219 mmol), potassium carbonate (30.0 g, 219 mmol) and sodium iodide (1.37 g, 0.05 mmol) in a mixture of acetone/water 1:1 (600 mL) was heated to reflux. Reaction progress was monitored by HPLC and the starting material was found to be consumed after 48 h. The reaction mixture was allowed to cool overnight and **11** was obtained as white crystalline material (32.1 g, yield 65%, HPLC 98%, m.p.:136-138 °C, Lit.m.p.[12]: 138-140 °C) was obtained after filtration.

^{1}H-NMR ($CDCl_3$): 2.50 (s, 3H), 5.33 (s, 2H), 7.20-7.24 (d, 2H, J_3= 8.90 Hz), 7.41-7.48 (m, 5H), 8.15-8.20 (dd, 1H, J_3= 8.90 Hz, J_4= 2.05 Hz), 8.36-8.41 (dd, 1H, J_4= 2.05 Hz).

2-Bromo-1-(3-nitro-4-benzyloxyphenyl) ethanon **(12)**

To a solution of **11** (10.1 g, 37 mmol) in dry dioxan (80 mL) bromine (1.93 mL, 36.9 mmol) dissolved in dry dioxan (80 mL) was added drop wise at R.T. over a time period of 30 min. while a stream of nitrogen was bubbled trough the reaction mixture in order to remove the HBr formed. The reaction mixture was stirred overnight at room temperature and the reaction progress monitored by HPLC. The organic solvent was then removed and the brownish oil obtained was dissolved in methylene chloride (150 mL), washed with sodium hydrogen carbonate (100 mL of 10% water solution) and brine (100 mL). The organic layer was decolorized (charcoal), dried (Na_2SO_4) and the volatiles were removed to afford a yellowish oil which was crystallized from ethyl acetate and petrol ether to give the product (10.0 g, yield 78%, HPLC 98%, m.p.: 138-140 °C, Lit.m.p.[13]: 141-143 °C) as yellowish crystals. ^{1}H-NMR ($CDCl_3$): 4.37 (s, 2H), 5.33 (s, 2H), 7.19-7.24 (d, 2H, J_3= 9.00 Hz), 7.37-7.44 (m, 5H), 8.11-8.17 (dd, 1H, J_3= 9.00 Hz, J_4= 2.15 Hz), 8.46-8.47 (dd, 1H, J_4= 2.15 Hz).

α-(Bromomethyl)-3-nitro-4-(phenylmethoxy)- benzenemethanol **(13)**

To a solution of **12** (12.0 g, 34 mmol) in dry THF (100 mL), $BH_3{\cdot}(SCH_3)_2$ (12 mL, 24 mmol, 2 M solution in THF) was added drop wise while the temperature was kept at room temperature. The reaction mixture was stirred at room temperature for further 1h and the reaction progress was followed by HPLC. Then another portion of $BH_3(SCH_3)_2$ (6 mL, 2 M solution in THF) was added and the reaction was continued at room temperature. The conversion was monitored by HPLC and found to be complete after 16 h. Methanol (25 mL) was added drop wise at 20 °C and nitrogen was bubbled trough the reaction mixture for 60 min. Volatiles were evaporated, and the oil obtained was dissolved in toluene (60 mL, washed with H_2SO_4 (20 mL, 0.2 M water solution) and water (20 mL). The organic layer was dried (Na_2SO_4), decolorized (charcoal) and evaporated to dryness to give the crude product (10.2 g, yield 80%, HPLC 96%) as a colourless oil which was purified by column chromatography (SiO_2, ethyl acetate / petrol ether 1:3) to afford the product (8.7 g, yield 69%, HPLC 98%, m.p. 59-61 °C) as a colourless crystals.

^{1}H-NMR ($CDCl_3$): 3.43-3.53 (dd, 1H, J_2=10.5 Hz, J_3= 8.21 Hz), 3.58-3.65 (dd, 1H, J_2= 10.5 Hz, J_3= 3.71 Hz), 4.88-4.94 (dd, 1H, J_3= 3.71 Hz, J_3 = 8.21 Hz), 5.23 (s, 2H), 7.25-7.47 (m, 5H), 7.09-7.14 (d, 1H, J_3= 8.7 Hz), 7.49-7.54 (dd, 1H, J_3= 8.7 Hz, J_4= 2.4 Hz), 7.88-7.89 (d, 1H, J_4= 2.4 Hz).

3-Amino-α-(bromomethyl)- 4-(phenylmethoxy)-benzenemethanol **(14)**

A solution of **13** (5.5 g, 16 mmol) in a mixture of THF/toluene 1:1 (40 mL) was hydrogenated in a Parr apparatus with PtO_2 (300 mg) at room temperature and 40- 45 p.s.i. for 48 h.

resulting in a constant pressure and monitored by HPLC. The catalyst was filtered and volatiles were removed under reduced pressure to afford the crude product (4.6 g, yield 91%, HPLC 98%) as an amber oil which was used as such in the next step.
^{1}H-NMR ($CDCl_3$): 3.34-3.50 (m, 2H), 4.16 (w, 3H), 4.65-4.71 (m, 1H), 4.97 (s, 2H), 6.6-6.8 (m, 3 H), 7.23-7.32 (m, 5H).

N-[5-[2-Bromo-1-hydroxyethyl]-2-(phenymethoxy)phenyl]-formamide **(15)**
To a solution of **14** (4.55 g, 14.10 mmol) in a mixture of THF/toluene 1:1 (40 mL) a mixture of formic acid (1.18 g, 25.6 mmol) and acetic anhydride (1.85 g, 18.1 mmol) was added drop wise at a temperature of 10-15 °C and the resulting mixture was stirred at the same temperature. Reaction progress was followed by HPLC and found to be complete after 30 min. Volatiles were removed under reduced pressure to afford the crude product (6.2 g) as an amber oil which was purified using column chromatography (SiO_2, ethyl acetate/petrol ether 1:3) to afford the product (4.6 g, yield 93%, HPLC 99%, m.p.:97- 100 °C) as a white crystalline compound.
^{1}H-NMR ($CDCl_3$): 3.48-3.57 (dd,1H, J_2= 10.41 Hz, J_3= 8.41 Hz), 3.6-3.67 (dd, 1H, J_2= 10.41 Hz J_3= 3.81 Hz), 4.84-4.90 (dd, 1H, J_3= 3.81 Hz, J_3= 8.41 Hz), 6.94-7.00 (d, 1H, J_3= 8.61), 7.11-7.16 (dd, 1H, J_3= 8.61 Hz, J_4= 2.00 Hz), 7.38-7.41 (m, 5H,), 7.84(w, 1H), 8.38-8.39 (d, 1H, J_4= 2.00 Hz), 8.41 (s, 1H), 8.72-8.78 (d, 1H).

3-Formamido-4-hydroxy- [[N-(4-methoxy- methylphenethyl)amino]methyl]benzyl alcohol (Formoterol) d_6 **(16)** *fumarate*
A suspension of **9** (1.0 g, 3.83 mmol), **15 (**1.4 g, 4.02 mmol) and potassium carbonate (1.42 g, 10.3 mmol) in a mixture of dry THF/MeOD 1:1 (20 mL) was stirred at room temperature for 2 h. Volatiles were removed and the residue portioned between a mixture of toluene/D_2O 1:1 (20 mL). The toluene layer was separated and the aqueous layer extracted with toluene (15 mL). The organic layers were collected, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The clear oil obtained was heated without solvent at 120°C for 20 hours argon and the reaction progress was followed by HPLC. The reaction mixture was cooled to 75-80 °C, MeOD (15 mL) was added followed by addition of Pd/C 10% (0.2 g) and the resulting mixture was hydrogenated at room temperature and 45 p.s.i.. The reaction progress was followed by HPLC and the reaction found to be complet after 3 h. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to afford the crude mixture (1.2 g) as a yellowish oil which was purified using column chromatography (60 g SiO_2, UV det. 262 nm, dichloromethane/ 7N NH_3 in methanol) to afford 440 mg (yield 33%, HPLC 99%, 85% D according to NMR) of the product as an oil of the free base. The oil

was dissolved in methanol and a solution of fumaric acid (73 mg, 0.63 mmol) in methanol (10 mL) was added drop wise. The solution was stirred and heated to reflux for 5 min, than cooled to room temperature and subsequently added drop wise to vigorously stirred diethyl ether (500 mL). The white precipitate obtained was filtered and dried under reduced pressure to afford the fumarate (466 mg, yield 79%, HPLC 99%) as a white powder. ^{1}H-NMR (DMSO) : 1.08-1.18 (m, 0.44 H,), 2.63-2.75 (m, 2H), 3.77 (s, 3H), 4.46-4.52 (m, 1H), 6.81-6.94 (m, 4H), 7.07-7.15 (m, 2H), 8.06 (s, 1H), 8.33 (s, 1H), 9.58 (w, 1H). $C_{23}H_{22}D_6O_8$: calc. C 59.34, H 6.06, N 6.02, found C 59.29, H 6.36, N 6.11.

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Synthesis of d_4 BY-170424 (5-chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo [1,2-b]pyridazin-6-yl)oxy]-2,3,5,6-d4-phenyl]methyl]-4-pyrimidinamine) as internal standard for LC/MS/MS determination in human serum.

Petr Knesl and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology

Getreidemarkt 9, 1060 Vienna, Austria

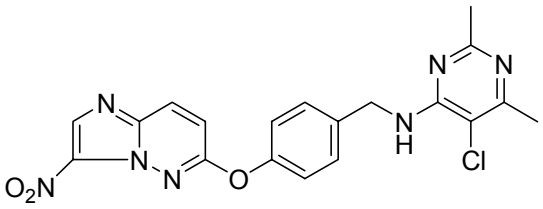
Summary:

We report the synthesis of 5-chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo[1,2-b]pyridazin-6-yl)oxy]-2,3,5,6-d_4-phenyl]methyl]-4-pyrimidinamine (**14**).

Key Words: deuterated labelled analog, analytical standards.

Introduction:

5-Chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo[1,2-b]pyridazin-6-yl)oxy]phenyl]methyl]-4-pyrimidinamine (BY-170424, BYK-170424) (**1**) is an investigational drug targeted for the treatment of Heliobacter pylori infections that can cause peptic ulcers.[1] For further pharmacological studies a deuterated standard was needed. Additionally, it has been shown, that some deuterated drug analogs exhibit improved pharmacokinetic as well as pharmacodynamics properties.[2]

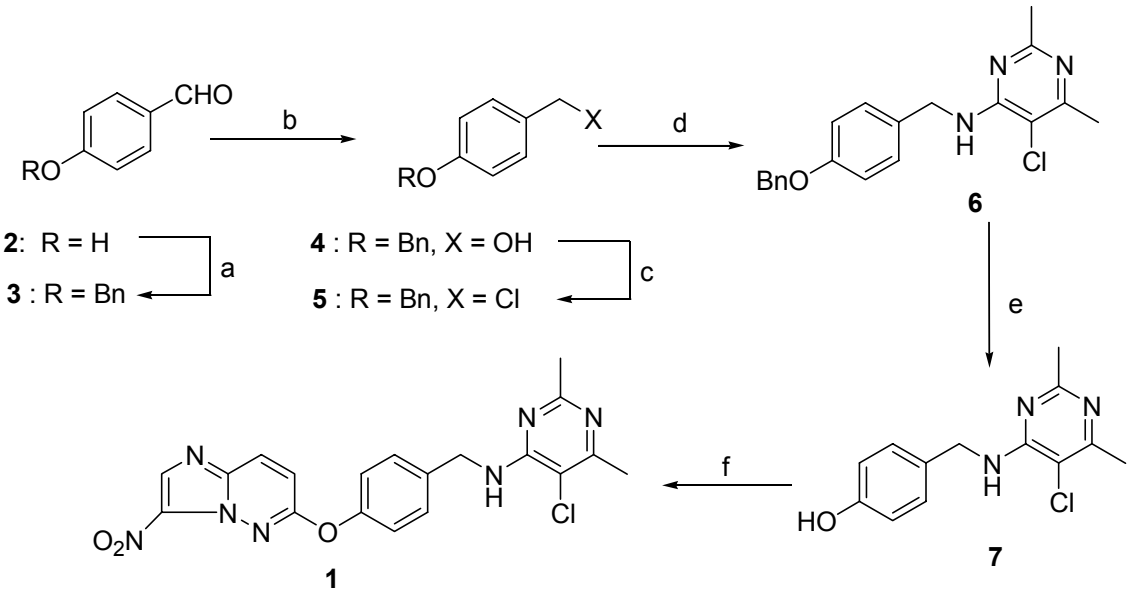


1

Results and discussion:

The synthesis of **1** is described in the patent literature[3] however, the synthesis of the deuterated analog was not known. *Scheme 1*.

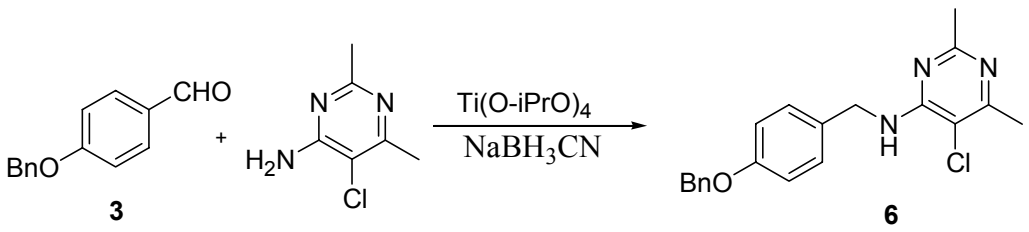
Scheme 1:



a) Benzyl chloride, K_2CO_3 b) $NaBH_4$ c) $SOCl_2$, benzotriazole d) 5-amino-4-chloro-2,6-dimethylpyrimidine e) Pd/C, H_2 f) 6-chloro-3-nitro-imidazolo[1,2-b]pyridazin, K_2CO_3

We decided to optimize this sequence using non- deuterated starting materials. Thus we first tried to simplify the scheme via reductive amination using **3** and 4-amino-5-chloro-2,6-dimethylpyrimidine in the presence of titanium ortho-titanate followed by a reduction with sodium cyanoborohydride (*Scheme 2*).

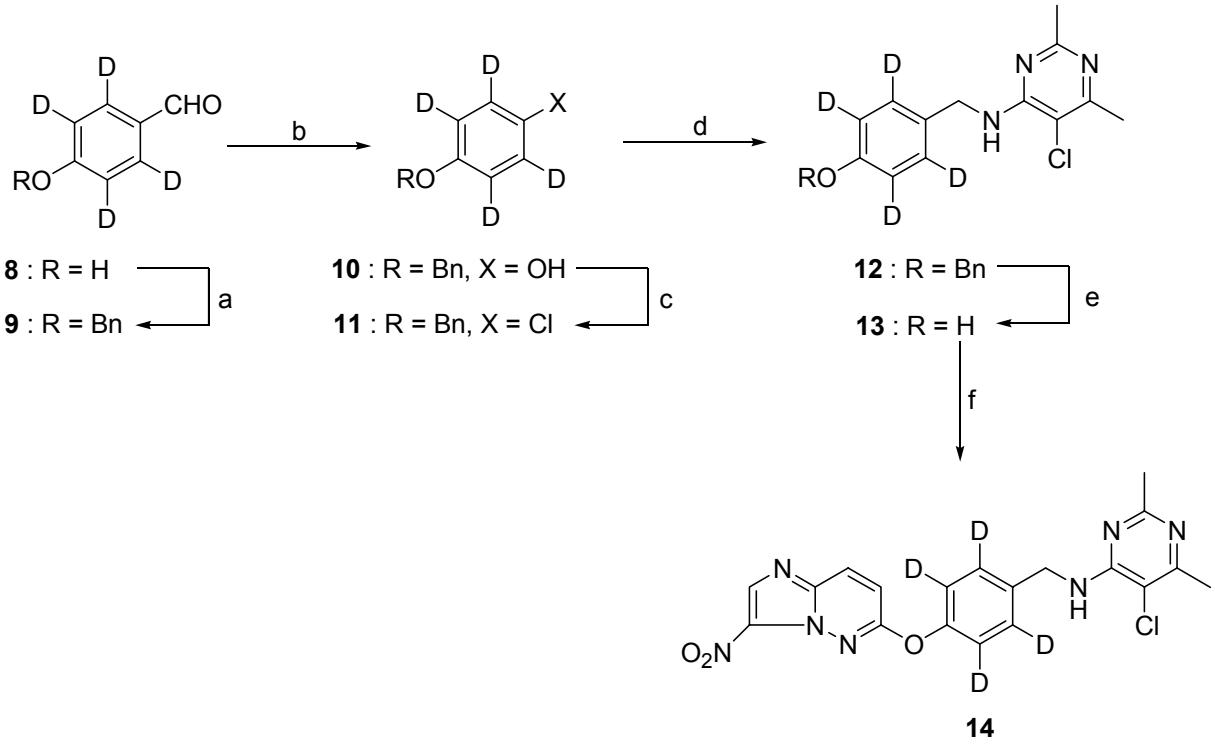
Scheme 2:



As this reaction did not give satisfactory results under the chosen conditions we had to follows the longer pathway. Protection of 2,3,5,6-d_4-4-hydroxy-benzaldehyde (**8**) using benzyl chloride followed by borohydride reduction gave the tetradeuterated alcohol **10** in 98% yield. The crude chloride **11** obtained by using a $SOCl_2$/benzotriazol complex was reacted

with 4-amino-5-chloro-2,6-dimethylpyrimidine to afford **12** in 85% yield. The deprotection of the benzyl group and the reaction with 6-chloro-3-nitroimidazo[1,2-b]pyridazine[4] gave the desired product **14** in a 45% yield and a purity of >99% a with deuterium content of >98% as determined by ^{1}H-NMR. *See scheme 3*.

Scheme 3:



a) Benzyl chloride, K_2CO_3 b) $NaBH_4$ c) $SOCl_2$, benzotriazole d) 5-amino-4-chloro-2,6-dimethylpyrimidine e) Pd/C, H_2 f) 6-chloro-3-nitro-imidazolo[1,2-b]pyridazin, K_2CO_3

Experimental

Melting points were measured on a Büchi B-545 melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO-d_6. For thin layer chromatography (TLC) Merck TLC aluminum sheets silica 60 F_{254} were used. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). MPLC (medium pressure liquid chromatography) was performed using silica gel (VWR 40-63 μm), a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument with Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 1.0 to 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

4-(Phenylmethoxy)-benzaldehyde-2,3,5,6-d_4 **(9)**

A mixture of 4-hydroxybenzaldehyde-2,3,5,6-d_4 (**8**) (0.5 g, 4.0 mmol), benzyl chloride (0.64 g, 5.06 mmol, 0.58 mL), potassium carbonate (0.78 g, 5.64 mmol) and potassium iodide (1.01 g, 6.11 mmol) in dry acetone (10 mL) and heated to reflux under stirring. The reaction was followed by HPLC and found to be complete after 12 h. Acetone was rotoevaporated and the solid residue dissolved in water (20 mL) and extracted with dichloromethane (4x30 mL). The organic fractions were collected, dried (Na_2SO_4), decolorized (charcoal) and volatiles were removed under reduced pressure to give the product (0.82 g, yield 96%, HPLC 97%, deuterium content >98% as determined by ^{1}H-NMR) as an colourless oil. ^{1}H-NMR ($CDCl_3$): 5.09 (s, 2H), 7.21- 7.39 (m, 5H), 9.81 (s, 1H).

4-(Benzyloxy)-benzylalcohol-2,3,5,6-d_4 **(10)**

A solution of 4-(phenylmethoxy)-benzaldehyde-2,3,5,6-d_4 (**9**) (0.81 g, 3.71 mmol) in dry methanol (15 mL) was cooled to 0 °C and sodium borohydride (0.43 g, 11.4 mmol) was added in three portions over a time period of 1 h. The reaction mixture was stirred overnight to room temperature. Reaction progress was monitored by TLC and the reaction was found to be complet after this time. Methanol was removed under reduced pressure, the residue dissolved in water (15 mL) and acidified using 2 N HCl to pH 2-3. The aqueous phase was extracted with ethyl acetate (5x50 mL), organic fractions were collected, dried (Na_2SO_4), decolorized (charcoal) and solvent was removed under reduced pressure to dryness to afford the product (0.80 g, yield 99%, HPLC 95%, deuterium content >98% as determined by ^{1}H-NMR) as a white powder. ^{1}H-NMR ($CDCl_3$): 4.52 (s, 2 H), 4.99 (s, 2H), 7.17- 7.37 (m, 5H).

p-(Benzyloxy)-benzyl chloride-2,3,5,6-d_4 **(11)**

Benzotriazole (BTA, 8.93 g, 75 mmol) was added in portions to the $SOCl_2$ (5.46 mL, 75 mmol) and this mixture was diluted with dry dichloromethane to a volume of 50 mL. A part of this solution (3.2 mL, 4.8 mmol of the $SOCl_2$/BTA complex) was added drop wise to a solution of 4-(benzyloxy)-benzylalcohol-2,3,5,6-d_4 (**10**) (0.8 g, 3.7 mmol) in dry dichloromethane (50 mL) and this mixture was stirred at room temperature and a white precipitate was formed. Reaction development was followed by GC and the reaction was found to be complete after 1 h. The white precipitate was filtered and the organic solution rotoevaporated to dryness to afford a colourless oil (0.75 g, yield 86%, GC 90%, deuterium content >98% as determined by ^{1}H-NMR). A small sample was crystallized from petrol ether to give the product as a white crystalline solid with a GC purity of >99%. ^{1} H-NMR (DMSO): 4.70 (s, 2H), 5.10 (s, 2H), 7.31-7.47 (m, 5H).

5-Chloro-2,6-dimethyl-N-[[4-(phenylmethoxy)-2,3,5,6-d_4-phenyl]methyl]-4-pyrimidinamine d_4 **(12)**

To a solution of 5-amino-4-chloro-2,6-dimethylpyrimidine (0.49 g, 3.13 mmol) in dry t-butanol (20 mL), t-BuOK (0.56 g, 4.99 mmol) was added and this mixture was stirred for 1 h at room temperature under nitrogen. 4-(Benzyloxy)-benzyl chloride-2,3,5,6-d_4 (**11**) (0.74 g, 3.13 mmol) was added and the solution heated to reflux for 3 h and monitored by TLC. The reaction mixture was cooled to room temperature, poured into water (100ml) and extracted with ethyl acetate (5x50 mL). The combined organic layers were washed with acetic acid (50 mL, 2 N solution), sodium bicarbonate (50 mL) and brine (50 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure to give a yellowish oil (1.10 g, yield 98%, HPLC 84%) which was chromatographed on silica using dichloromethane/methanol 98:2 as mobile phase to afford the product (0.93 g, 83%, HPLC 97%, deuterium content >98% as determined by ^{1}H-NMR) as a white solid. ^{1}H-NMR (DMSO): 2.50 (s, 3H), 2.56 (s, 3H), 4.63-4.66 (d, 2H, J_2= 6.06 Hz), 5.07 (s, 2H), 7.29-7.43 (m, 5H), 9.45-9.51 (m, 1H).

4-[[(5-Chloro-2,6-dimethyl-4-pyrimidinyl)amino]methyl]-2,3,5,6-d_4-phenol **(13)**

Pd/C (0.1 g, 10%) was suspended in methanol (30 mL) and pre-hydrogenated for 30 min at room temperature and 40 p.s.i. A solution of 5-chloro-2,6-dimethyl-N-[[4-(phenylmethoxy)-2,3,5,6-d_4-phenyl]methyl]-4-pyrimidinamine (**12**) (0.83 g, 2.32 mmol) in methanol (40 mL) was added to the pre-hydrogenated catalyst and hydrogenated for 1 h at room temperature whereby the reaction progress was followed by HPLC. The catalyst was removed by filtration trough a pad of celite, washed with methanol and the solvent was removed under reduced pressure to afford (0.81 g, HPLC 60%) of the crude product, which was purified using reverse phase chromatography (gradient H_2O: CH_3CN 5:95 to 15:95) to afford the product (0.45 g, yield 72%, HPLC 93%, deuterium content >98% as determined by ^{1}H-NMR). ^{1}H-NMR (DMSO): 2.53 (s, 3H), 2.57 (s, 3H), 4.70-4.73 (d, 2H, J_2= 6.03 Hz), 9.30-9.33 (s, 1H).

5-Chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo[1,2-b]pyridazin-6-yl)oxy]-2,3,5,6-d4-phenyl]methyl]-4-pyrimidinamine **(14)**

To a solution of 4-[[(5-chloro-2,6-dimethyl-4-pyrimidinyl)amino]methyl]-2,3,5,6-d_4-phenol (**13**) (0.43 g, 1.61 mmol) in dry dimethyl formamide (15 mL) potassium carbonate (1.1 g, 8.0 mmol) was added and this suspension was stirred for 0.5 h at room temperature under nitrogen . Subsequently a solution of 6-chloro-3-nitro-imidazo[1,2-b]pyridazine (0.32 g, 1.61 mmol) in dry dimethyl formamide (15 mL) was added drop wise at 60 °C and this solution was stirred under nitrogen for another 0.5 h. Then the temperature was increased to 120 °C and stirring was continued at this temperature for further 0.5 h. To complete the conversion,

the reaction was stirred overnight at 50 °C. The reaction mixture was poured into water (200 mL) and extracted with dichloromethane (4x100 mL). The collected organic layers were washed with brine (2x100 mL), dried (Na_2SO_4), decolorized (charcoal) and the solvent was removed under reduced pressure to afford the crude product (0.45 g, yield 66%, HPLC 95%) as a brown oil which was chromatographed on silica using petrol ether: ethyl acetate 1:1 to afford the product (0.31 g, yield 45%, HPLC 99%, deuterium content >98% as determined by ^{1}H-NMR) as colorless solid.

^{1}H-NMR ($CDCl_3$): 2.44 (s, 3H), 2.50 (s, 3H), 4.76-4.79 (d, 2H, J= 5.87 Hz), 5.66 (w, 1H), 7.21- 7.26 (d, 1H, J_3= 9.78 Hz), 8.05- 8.10 (d, 1H, J_3= 9.78 Hz), 8.48 (s, 1H).

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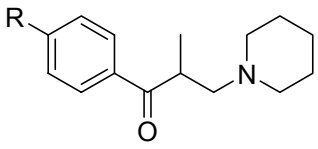
Synthesis of d_7-tolperisone for use as LC/MS/MS standard

Petr Knesl and Ulrich Jordis*
Institute of Applied Synthetic Chemistry, Vienna University of Technology
Getreidemarkt 9, 1060 Vienna, Austria

Summary:
The preparation of 2-methyl-1-[4-(methyl-d3)phenyl-2,3,5,6-d4]-3-(1-piperidinyl)-1-propanone (d7-tolperisone, deutolperisone) is described.

Key Words: deuterium labelled analog, analytical standard, deutolperisone.

Introduction:
Some 2-methyl-3-aminoprophiophenones like tolperisone (**1)** and eperisone (**2**) exhibit muscle relaxant activity[1] and are being developed for the treatment of back pain. Among them **1** is of special interest because L-tolperisone has bronchodilatory and peripheral vasodilatory activity while the D-tolperisone acts as a muscle relaxant.[2] The metabolic pathway of tolperisone is known and 11 metabolites are present in the human urine.[3] The mechanism of action as a muscle relaxant remains unclear.[4] To aid the analysis of low concentrations of **1** in body fluids we synthesised a deuterated tolperisone analog. Additionally, it has been claimed[5], that deuterated analogs may exhibit improved pharmacokinetics as well as pharmacodynamic properties.



1: R = Me **tolperisone**

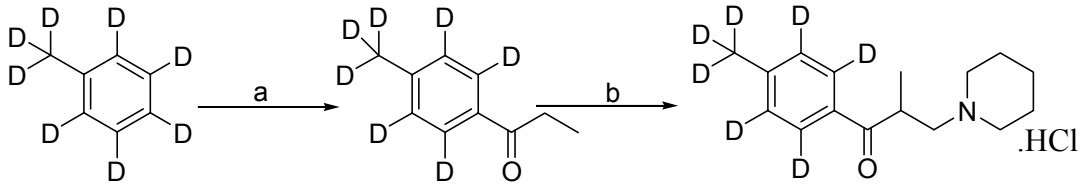
2: R = Et **eperisone**

Results and discussion:

We first performed model reactions using toluene for the Friedel-Crafts acylation with propionic anhydride and carbon disulfide as a solvent in analogy to the procedure of Lee and Roseman[6] and obtained the desired 4-methyl-propiophenone in 90% yield and 99% HPLC purity. The following Mannich reaction using 1,4-dioxolane[7] instead of the widely used para-formaldehyde gave **1** in 90% yield and >99% HPLC purity.

Applying this reaction sequence to toluene-d_8 we obtained tolperison-d_7 in an overall yield of 75% and >99% HPLC purity with a deuterium content of >98% as determined from ^{1}H- NMR (*Scheme 1*).

Scheme 1:



3 **4**.HCl

a) propionic anhydride, CS_2 b) 1,3-dioxolane, piperidine hydrochloride C) HCl

Experimental:

Melting points were measured on a Büchi B-545 melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or D_2O. For thin layer chromatography (TLC) Merck TLC aluminum sheets silica 60 F_{254} were used. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). MPLC (medium pressure liquid chromatography) was performed using silica gel (VWR 40-63 μm), a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument with Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 1.0 to 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

1-(4-Methyl-d_3-2,3,5,6-phenyl-d_4)-1-propanone **(3)**

To a solution of toluene-d_8 (1.0 g, 11 mmol) in carbon disulfide (4.0 mL) anhydrous aluminium chloride (3.0 g, 22.3 mmol) was added and this mixture was magnetically stirred and heated to reflux. Propionic anhydride (1.14 g, 8.76 mmol) was added drop wise and

reflux continued for additional 1.5 h followed by distillation of carbon disulfide. The residue was treated with a mixture of ice/water (100 mL) and conc. HCl (5 mL). The resulting aqueous solution was extracted with diethyl ether (5x50 mL). The combined organic layers were dried ($MgSO_4$) and volatiles were rotoevaporated to afford the crude material (2.5 g) as a yellowish oil which was distilled (101-106 °C, 1 torr) to give the product **3** (1.26 g, yield 85%, HPLC >99%). ^{1}H-NMR ($CDCl_3$): 1.18-1.25 (t, 3 H, J_3= 7.23 Hz), 2.91-3.02 (kv, 2 H, J_3= 7.23 Hz).

2-Methyl-1-(4-methyl-d_3- phenyl-2,3,5,6-d4)-3-(1-piperidinyl)-1-propanone **(4)** *hydrochloride*
A suspension of 1-(4-methyl-d_3-2,3,5,6-phenyl-d_4)-1-propanone (**3**) (1.1 g, 7.1 mmol), 1,3-dioxolane (2.1 g, 28 mmol), piperazine hydrochloride (1.06 g, 8.7 mmol) and conc. HCl (0.04 mL) was stirred and heated to 90 °C. The reaction progress was followed by HPLC and found to be complete after 8 h. The reaction mixture was cooled to room temperature, diluted with water (25 mL) and washed with diethyl ether (2x25 mL). The aqueous layer was adjusted to pH 12-13 by using 2 N NaOH and extracted with diethyl ether (4x50 mL). The combined ether layers were washed with water (25 mL), dried ($MgSO_4$) and volatiles were removed under reduced pressure to afford 1.97 g of the product as a yellow oil with a purity of >99% (HPLC) and 93% (GC). A solution of the oil in dry diethyl ether (10 mL) was added to a saturated solution of HCl in the same solvent (25 mL) at 0 °C. This mixture was stirred for 0.5 h. Volatiles were removed under reduced pressure and the obtained white semisolid material was crystallized using acetonitrile to afford **4** . HCl (1.8 g, yield 88%, >99% HPLC purity, deuterium content >??% according to NMR, GC of the free base >99%) as colourless crystals. ^{1}H-NMR (D_2O) **4**.HCl: 1.19-1.23 (d, 3 H, J_3 = 7.24 Hz), 1.38-1.88 (m, 6 H), 2.77-3.03 (m, 2 H), 3.11- 3.19 (dd, 1 H, J_2 = 13.3 Hz, J_3 = 9.39 Hz), 3.31- 3.52 (m, 2 H), 3.60- 3.72 (dd, 1 H, J_2 = 13.3 Hz, J_3 = 3.91 Hz). ^{1}H-NMR ($CDCl_3$) **4**: 1.08- 1.12 (d, 3 H, J_3= 6.85 Hz), 1.28- 1.47 (m, 6 H), 2.26- 2.35 (m, 5 H), 2.68- 2.78 (dd, 1 H, J_2= 12.72 Hz, J_3= 7.43 Hz), 3.53- 3.70 (m, 1H). $C_{16}H_{17}NOD_7Cl$: calc. C 66.53, H 8.37, N 4.85, Cl 12.27, found C 66.54, H 8.30, N 4.89, Cl 12.23.

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Synthesis of Sulfapyridine metabolites

Petr Knesl and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology,
http://phoenix.tuwien.ac.at
Getreidemarkt 9, 1060 Vienna, Austria, ujordis@pop.tuwien.ac.at

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Abstract: N-acetyl-5-OH-sulphapyridine (**Ac-OH-SP**, **4**) was prepared as the reported metabolite of sulphapyridine (**SP**). Additionally, 4-acetylamino-benzenesulfonic acid 6-amino-pyridin-3-yl ester (**5**) and two more potential metabolites of **SP**, N-[4- (1-oxy-pyridin-2-yl-sulfamoyl)-phenyl]-acetamide (**9**) and N-[4- (hydroxyl-piridine-2-yl-sulfamoyl)-phenyl]-acetamide (**10**) having the same molecular mass as **4**, were prepared.
Keywords: metabolites, N-oxid, isomers.

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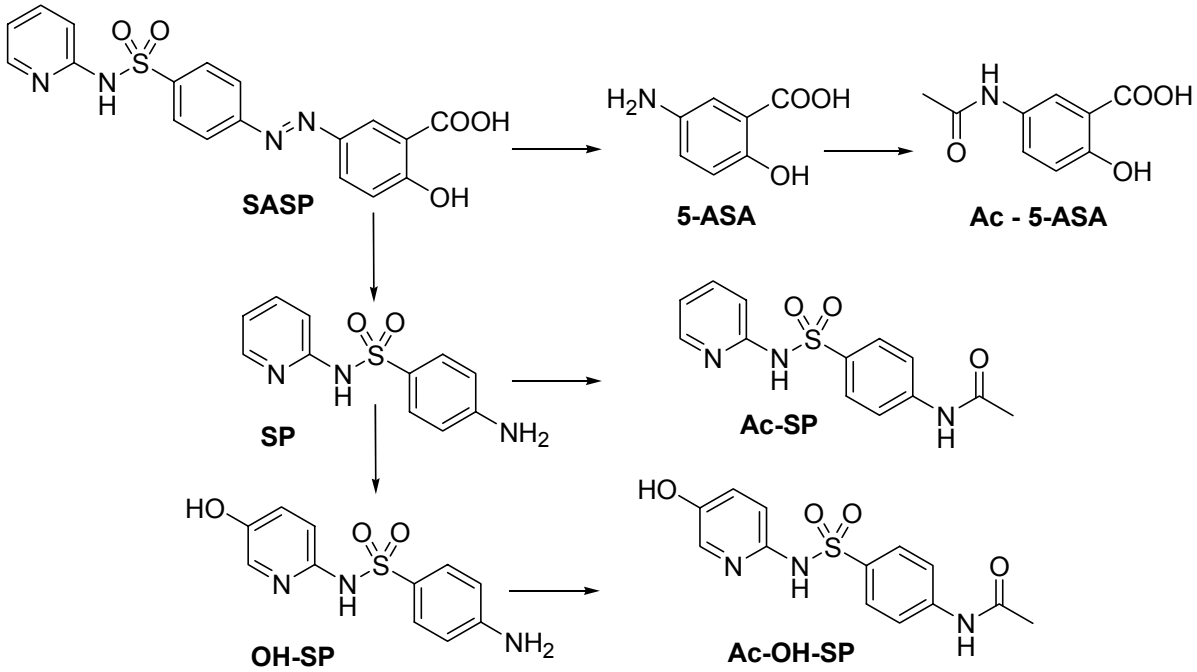
1. Introduction

Sulfapyridine (**SP**) and sulfasalazine (**SASP**) are used for the treatment of Crohn`s disease, rheumatoid arthritis and ulcerative colitis. When **SASP** is given orally it is partly absorbed unchanged and partly cleaved to aminosalicylic acid (**5-ASA**) and **SP** by bacterial azo reductase[1]. **SP** is the therapeutically active moiety of **SASP** and undergoes further acetylation, hydroxylation and subsequent glucoronidation[2, 3, 4]. The main metabolites were reported to be the N-acetylsulfapyridin (**Ac-SP**) and 5-hydroxy-sulfapyridine (**OH-SP**). the formation of **Ac-SP** and **OH-SP** is under genetic control and the proportion of acetylated and hydroxylated **SP** in serum and urine depends on the genetically determined acetylation and hydroxylation phenotypes of the patients[5].

The proposed metabolic pathway is given in Scheme 1.

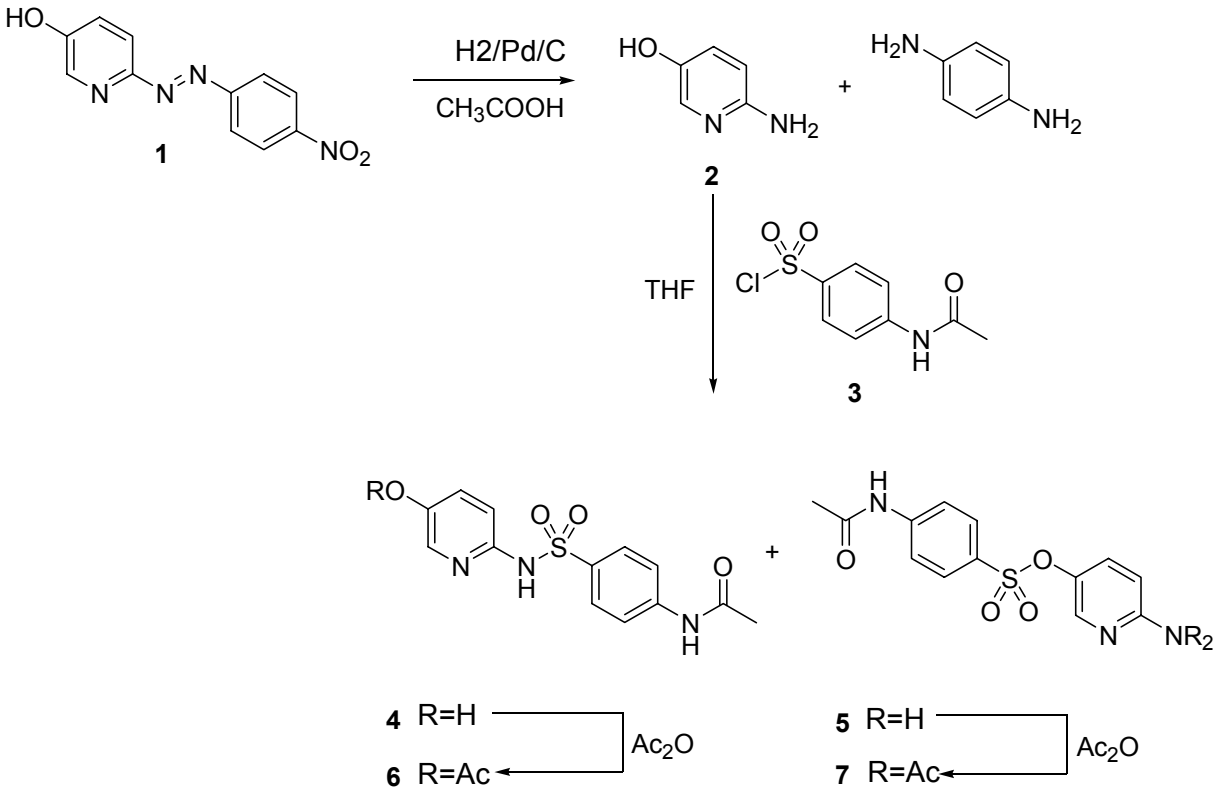
Scheme 1: Reported[4] metabolism of **SASP**



2. Results and Discussion

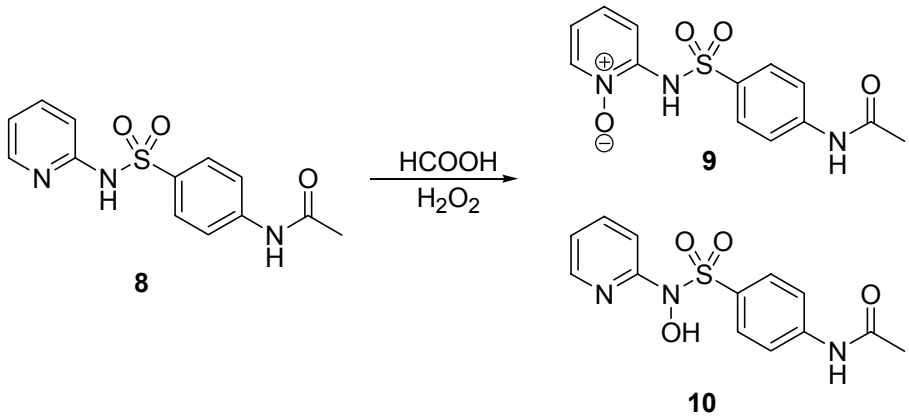
To the best of our knowledge no papers describing the synthesis of **SASP** metabolites have been reported, although several papers regarding analytical separation[5, 6] and metabolism[1, 2, 3] including **OH-SP** and **Ac-OH-SP** were published. As starting material for our synthesis we used 6- (4-nitro-phenylazo)-pyridin-3-ol[7] **1** which was reduced with H_2/Pd to the highly unstable 5-hydroxy-2-aminopyridine **2** and immediately treated with **3** to give a mixture of products which was separated by column chromatography (Scheme 2). From the isomers **4** and **5** the acetylated compounds **6** and **7** were prepared. Structures of the isomeric compounds **4** and **5** were deducted from the structures of the acetylated compounds **6** and **7**. The structure of the latter was confirmed by ^{1}H, ^{13}C NMR and MS.

Scheme 2: Synthesis of AC-OH-SP (**4**)



As neither **4** nor **6** showed LC/MS behavior identical to the expected metabolites in the urine of persons treated with **SP**, two additional known[8, 9, 10] oxygenated **SP** derivatives, *N*-[4-(1-oxy-pyridin-2-ylsulfamoyl)-phenyl]-acetamide (**9**) and *N*-[4-(hydroxy-pyridin-2-yl-sulfamoyl)-phenyl]-acetamide, (**10**) with the same m/e ratio as **Ac-OH-SP** were synthesized (Scheme 3). N-acetylsulfapyridine (**8**) was oxidized to afford a mixture of both products[10] which was separated and characterized by ^{1}H and ^{13}C NMR. Neither **9** nor **10** could be detected in the urine of persons treated with **SP**.

Scheme 3: Synthesis of potential metabolites



3. Experimental Section

Melting points were measured on a Büchi melting point apparatus B-545. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO-d_6. Thin layer chromatography (TLC) was performed on Merck TLC aluminum sheets silica 60 F_{254}. Visualization was carried out with UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). Column chromatography was performed using silica gel (Baker 40-60 μm). MPLC (medium pressure liquid chromatography) was performed using a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument and Merck Chromolith RP_{18} columns, standard method Gradient: 3% to 60% acetonitrile/water (0.1% TFA), Flow 3 ml/min The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium Software with the Maxplot option for the UV maximum of the corresponding peak.

N-4-Acetyl-5'-OH-sulphapyridine (AcOH-SP) 4 and 4-acetylamino-benzenesulfonic acid 6-amino-pyridin-3-yl ester 5:

A solution of 1 (5.0 g, mmol) in glacial acetic acid (50 ml) was hydrogenated in a Parr apparatus with Pd/C (0.1 g, 10%) for 30 min at 50-55 psi. The hydrogen atmosphere was replaced by argon and HBr (9.6 ml, 48% solution) was added and the resulting precipitate was filtered and washed with acetic acid (10 ml). The filtrate was evaporated and the residue was taken up in water and made basic by addition of natrium hydrogen carbonate. To this was added a solution of 3 (5.6 g, 24 mmol) in tetrahydrofuran (100 ml) and the mixture was shaken at room temp. for 30 min. The reaction mixture was concentrated on the rotavap to remove most of the tetrahydrofuran and then extracted with ethyl acetate (5x100 ml). The combined organic layers were dried (sodium sulphate), cleaned (charcoal) and filtered. The filtrate was evaporated to the dryness to yield crude product (5 g) showing 4 spots on the TLC. 2g of this mixture was chromatographed on a silica column using petrol ether / ethylacetate 1:1 and methanol: chloroform 5:95 resulting in the isolation of two compounds:

N-4-Acetyl-5'-OH-sulphapyridine (4) (m.p.: 217-219°C, 0.89 g, yield 35.2%, HPLC 98.5%, M+= 307.9).

^{1}H-NMR, (200 MHz; DMSO): 2.09 (s, 3H), 6.10 (s, 2H), 6.33-6.37 (d, 1H), 7.00-7.05 (dd, 1H), 7.43-7.44 (d, 1H), 7.7-7.85 (k, 4H), 10.47 (s, 1H), ^{13}C NMR, (200 MHz, DMSO): 24.13, 108.06, 118.62, 126.62, 129.71, 131.45, 137.06, 140.95, 144.82, 158.51, 169.33, and 4-Acetylamino-benzenesulfonic acid 6-amino-pyridin-3-yl ester (5) (m.p.:167-172 °C, 100 mg, yield 4%, HPLC 97.6%, M+= 307.9), ^{1}H-NMR, (200 MHz, DMSO): 2.09 (s, 3H), 6.10 (s,

2H), 6.33-6.37 (d, 1H), 7.00-7.05 (dd, 1H), 7.43-7.44 (d, 1H), 7.7-7.85 (k, 4H,), 10.47 (s, 1H), ^{13}C NMR, (200 MHz, DMSO): 24.1, 108.06, 118.62, 126.62, 129.71, 131.45, 137.06, 140.95, 144.82, 158.51, 169.33.

N-[4-[[[5-(Acetyloxy)-2-pyridinyl] amino] sulfonyl] phenyl]-acetamide 6:

A solution of 4 (100 mg, 33.0 mmol) in acetic anhydride (5 ml) was heated to reflux for 3 h. After completion of the reaction (control by HPLC) the solvent was evaporated and the crude product chromatographed on silica using chloroform / methanol 97:3 giving the product 6 (60 mg, yield 53%, HPLC >98%) as a colorless crystals, ^{1}H-NMR, (200 MHz, DMSO): 2, 06 (s, 3H), 2.10 (s, 3H), 7.42-7.48 (dd, 1H), 7.73-7.86 (k, 4H), 7.91-7.92 (d, 1H), 8.02-8.06 (d, 1H), 10.5 (s, 1H), 10.61 (s, 1H), ^{13}C NMR, (200 MHz, DMSO): 23.73, 24.15, 113.7, 118.71, 126.35, 129.84, 132.11, 141.44, 141.56, 145.14, 150.65, 169.25, 169.35.

4-Acetylamino-benzenesulfonic acid 6-diacetylamino-pyridin-3-yl ester 7:

A solution of 5 (100 mg, 33.0 mmol) in acetic anhydride (5 ml) was heated to reflux for 3 hours. After completion of the reaction (control by HPLC) the solvent was evaporated and the crude product chromatographed on silica using chloroform / methanol 97:3 giving the product 7 (m.p.: 141-143 °C, 64 mg, yield 51%, HPLC >97%) as of colorless crystals, ^{1}H-NMR, (200 MHz, $CDCl_3$): 2.12 (s, 3H), 2.27 (s, 6H), 7.36-7.43 (dd, 1H), 7.58-7.62 (dd, 1H), 7.69-7.81 (k, 4H), 8.46-8.49 (dd, 1H), 8.57 (s, 1H), ^{13}C NMR, (200 MHz, CDCl3): 24.12, 25.81, 118.93, 125.27, 128.25, 129.2, 131.57, 142.00, 144.26, 145.61, 147.03, 168.95, 172.01.

N-[4-(1-Oxy-pyridin-2-ylsulfamoyl)-phenyl]-acetamide 9 and N-[4- (hydroxy-pyridin-2-yl-sulfamoyl)-phenyl]-acetamide 10:

To a solution of 8 (5.0 g, 17.2 mmol) in formic acid (30 ml), hydrogen peroxide (1.68 ml, 30% solution) was added, resulting in an exothermic reaction to 58 °C. It was stirred without heating for 30 min. and another portion of hydrogen peroxide (1.68 ml, 30% solution) was added, the mixture was heated to 58°C for 2 h. and stirring continued for another 12 h without heating. Volatiles were evaporated and co-evaporated with methanol. The residue was recrystallized from acetic acid (10% solution). The crystalline material was filtered and stirred for 1 h with a solution of natrium hydrogen carbonate (0, 67g in 15 ml of water), filtered and the filtrate was acidified with HCl (conc.) to pH 2-3 resulting in a precipitate. This precipitate was filtered and recrystallized from ethanol to give 9 (m.p.: 219-220°C, 1.50 g, yield 28.8%, HPLC 98.9%, Lit.[12] m.p.: 216-218°C, M+= 307, 8) as a yellowish crystals, ^{1}H-NMR, (200 MHz, DMSO): 2.05 (s, 3H), 6.75-6.83 (m, 1H), 7.4-7.5 (m, 1H), 7.51-7.59 (m, 1H), 7.66-7.83 (m, 4H) 8.21-8.25 (m, 1H), 10.23 (s, 1H), ^{13}C NMR, (200 MHz, DMSO): 24.08, 116.74,

117.94, 122.1, 127.11, 130.24, 138.12, 144.06, 147.73, 155.22, 169.132. The natrium hydrogen carbonate-insoluble precipitate was recrystallized from ethanol to give 10 (m.p.: 179-182°C , 2.2g, yield 42.3%, HPLC 99%, Lit.[12]m.p.: 178°C, M+= 307, 8) as a white crystals, ^{1}H-NMR, (200 MHz, DMSO): 2.06 (s, 3H), 7.04-7.08 (m, 1H), 7.2-7.26 (m, 1H), 7.46-7.75 (m, 4H), 7.74-7.79 (m, 1H), 8.33-8.36 (m, 1H), 10.35 (s, 1H), 11.01 (s, 1H), ^{13}C NMR, (200 MHz, DMSO) 24.00, 113.14, 115.8, 118.43, 127.34, 135.64, 136.09, 137.33, 142.33, 149.67; 168.88.

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Synthesis of standard reference materials occurring as reaction byproducts in the manufacture of phenazopyridine

Petr Knesl and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria. ujordis@pop.tuwien.ac.at

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Abstract: We report the synthesis, separation and identification of three major impurities that occur as side products during the manufacture of phenazopyridine.

Keywords: byproducts, isomers, separation, chromatography.

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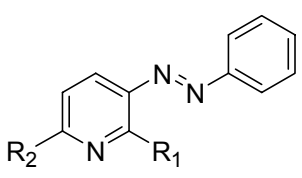
1. Introduction

Phenazopyridine (**1**), first synthesized by Chichibabin and Zeide[1], is an azo dye and its hydrochloride salt is widely used in medicine as urinary antiseptic and as an adjunct to the therapy in pain treatment. Phenazopyridine exerts an analgesic effect on the urinary tract mucous and provides symptomatic relief of burning, urgency, frequency, pain, and other discomforts[2a]. Recently a combination of pentamidine and phenazopyridine was reported for the treatment of fungal infections[2b].

The process of preparation of **1** encompasses the coupling benzenediazonium chloride with 2,6-diaminopyridine in acidic media[1, 3]. It is known[4], that under these conditions one or both amino groups of 2,6-diaminopyridine are hydrolyzed to the corresponding hydroxyl groups, resulting in the formation of the by-products **2**, **3** and **4.** (Fig. 1)Although this process to prepare **1** is well established in an industrial scale and the potential side-reactions are well known, the preparation of the compounds **2**, **3** have not been described[7]. The preparation of **4** has been described before[6,8] and was included in this study for the sake of completeness.

Figure 1:



$R_1 = R_2 = NH_2$ **1**

$R_1 = NH_2$, $R_2 = OH$ **2**

$R_1 = OH$, $R_2 = NH_2$ **3**

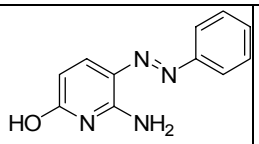
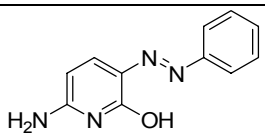
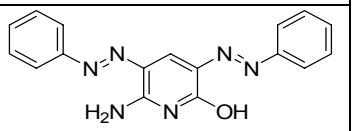
$R_1 = R_2 = OH$ **4**

Note: only one of the many possible tautomeric structures for structures 1-4 is displayed

2. Results and Discussion

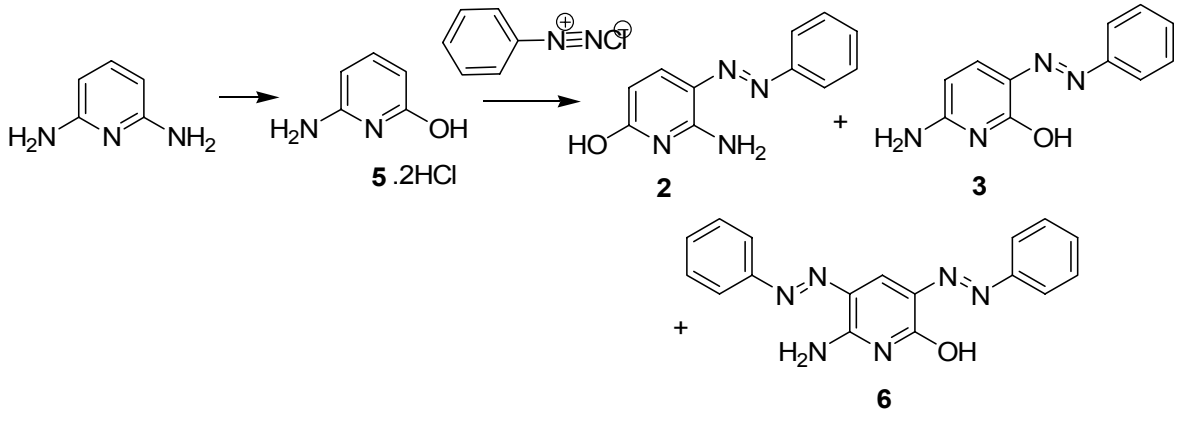
First 2,6-diaminopyridine was hydrolyzed with conc. hydrochloric acid to give **5.HCl** [10a, 10b] which was coupled with benzene diazonium chloride under acidic as well as under basic conditions to afford a mixture of the products **2**, **3**, **6** (Table 1).

Table 1: Products obtained from the coupling of **5** under basic and acidic conditions

	% yield (HPLC)		
	 **2**	 **3**	 **6**
Coupling in 6N-NaOH	5	44	45
Coupling in 2N-HCl	53	41	2

Chromatographic separation of the product mixtures obtained from the coupling in basic and acidic media afforded compounds **2**, **3**, **6** in HPLC-purities of 98- >99%. *Scheme 1*.

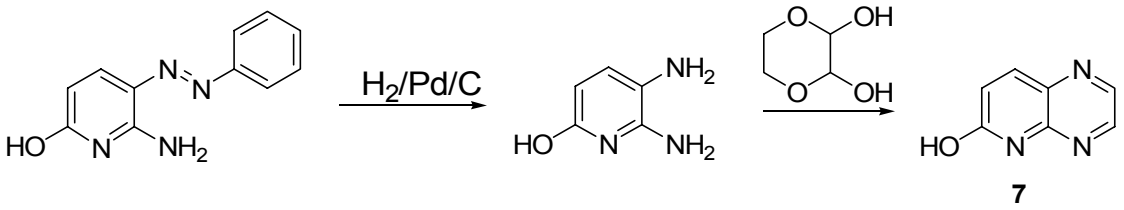
Scheme 1:



As it was not possible to assign unequivocally the structures to the two isomers **2** and **3** based on the ^{1}H and ^{13}C NMR spectral data, we subjected each isomer to catalytic hydrogenation to reduce the azo structure to the corresponding amine and reacted it with 2,3-

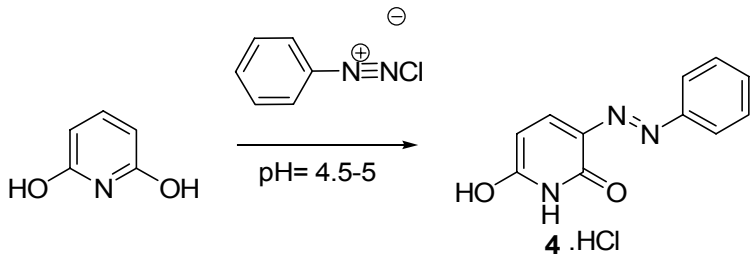
dihydroxy-1,4-dioxane[5]. Thus we obtained 6-hydroxy-pyrido[2,3:b]pyridazine (**7**)[11, 12] from one of the isomers thus proving it to be **2**. (*Scheme 2)*.

Scheme 2:



For the sake of completion we also prepared 6-hydroxy-3-(phenylazo)2-(1H)-pyridinone (**4**) (*Scheme 3)*.

Scheme 3:



3. Experimental

Melting points were measured on a Büchi B-545 melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO. For thin layer chromatography (TLC) Merck TLC aluminum sheets silica 60 F_{254} were used. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). MPLC (medium pressure liquid chromatography) was performed using silica gel (VWR 40-63 μm), a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument with Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 1.0 to 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

6-Hydroxy-3- (phenylazo)-2 (1H)-pyridinone 4:

To a solution of aniline hydrochloride (24.4, 0.19 mol) in 2N-HCl (300 mL) a solution of sodium nitrite (13.0 g, 0.19 mol) in water (100 mL) was added drop wise at -5 to 0 °C. This mixture was stirred for 30 min. at the same temperature followed by addition of a suspension of 2,6-dihydroxypyridine hydrochloride (15.0 g, 0.10 mol) in water. The pH value of the resulting mixture was adjusted to 4.5- 5.0 by using ammonium acetate and kept at range 4.5-5 at a temperature -5 to0 °C. The reaction mixture was stirred to room temperature for additional 1h, the precipitate filtered off, washed with water (2x500 mL), methanol (3x250 mL) and dried to afford the product **4** (m.p.:216-219 °C, 14.6 g, yield 67%, HPLC >99%, Lit.[6] m.p.: 218-220 °C) as a yellow powder, ^{1}H-NMR (DMSO, 200 MHz): 6.07-6.12 (d,1H), 7.15-7.18 (d,1H), 7.35-7.45 (m,5H), 11.5 (s,1H), 14.06 (s,1H), ^{13}C NMR (DMSO, 200 MHz): 115.87, 117.18, 124.9, 125.11, 129.53, 141.29, 141.45, 162.16, 164.09.

Azo-coupling of 6-amino-2(1H)-pyridion.2HCl (5)

a) Coupling under acidic conditions: To a solution benzene diazonium chloride prepared from 0.165 mol of aniline hydrochloride as described for **4** a solution of 2-amino-6-hydroxypyridine dihydrochloride (20.0 g, 0.11 mol) in 2N-HCl (300 mL) was added drop wise at the same temperature. This mixture was stirred overnight while the temperature rose to RT. The red precipitate obtained was filtered, washed with water (2x500 mL), acetone (250 mL) and methanol (250 mL) to obtain a crude orange-red material (15.2 g) which was purified by using column chromatography (700g basic alumina, chloroform/ 2 N ammonia in methanol). The first pure product that was eluted was isolated as an orange powder. It was identified as 6-amino-5-(phenylazo)-2 (1H)-pyridinone (**2**) (m.p.: 240-245 °C, 3.5 g, yield 15%, HPLC 99%) ^{1}H-NMR (TFA, 200 MHz): 7.71-7.76 (d,1H), 8.63-8.83 (m,5H), 9.11-9.15 (d,1H), ^{13}C NMR, $CDCl_3$, 200 MHz: 98.28, 120.48, 124.74, 127.62, 127.95, 140.70, 150.83, 151.91, 158.08. Additionally 6-amino-3- (phenylazo)-2(1H)-pyridinone (**3**) (m.p.: 234-238 °C, 1.0 g, yield 4.2%, HPLC 98.6%) was isolated as an orange powder. ^{1}H-NMR (DMSO+TFA, 200 MHz): 6.21-6.26 (d, 1H), 7.19-7.80 (m, 7H), 8.10-8.16 (d, 1H), 9.50 (s, 1H); ^{13}C NMR (DMSO+TFA, 200 MHz): 116.83, 117.20, 119.62, 126.15, 129.15, 129.48, 142.29, 157.43, 161.51.

b) Coupling under basic conditions: The reaction was performed as before with exception that the diazonium salt was added to the suspension of 2-amino-6-hydroxypyridine dihydrochloride (20.0 g, 0.11 mol) in sodium hydroxide (300 mL, 6 N solution) at -5 to 0 °C and shaken overnight at room temperature. The obtained red precipitate (20.1 g) was boiled

with methanol (3x100 mL). The insoluble, deep red material was identified as a 6-amino-3,5-di-(phenylazo)-2(1H)-pyridinone (**6**), (5.1 g, HPLC 98%). ^{1}H-NMR (DMSO, 200 MHz): 7.2-7.46 (m,6H),7,66-7,74 (t,4H),8.11 (s,1H),8.82 (s,1H) ^{13}C NMR (DMSO, 200 MHz): 121.27, 121.76, 122.00, 128.12, 128.74, 129.02, 131.52, 152.74, 153.24, 156.54, 167.12.

From the methanol washings another sample of pure **3** (0.96 g, HPLC 99.4%) was isolated by chromatography.

6-Hydroxy-pyrido[2,3:b]pyridazin (7):

A suspension of **2** (200 mg, 0.93 mmol) in ethanol (40 mL) was reduced in the Parr hydrogenation apparatus using Pd/C (50 mg, 10%) at room temperature and H_2 (20 p.s.i) until the yellow color had not yet disappeared completely (15 min.). 2,3-Dihydroxy-1,4-dioxane (230 mg, 2.4 mmol) was added and the mixture stirred for 1 h. at room temperature under nitrogen. The reaction progress was monitored by HPLC. The catalyst was filtered trough a pad of celite and washed with ethanol (2x10 mL). Volatiles were removed and the dark oil obtained was washed with diethyl ether. The insoluble precipitate was filtered and dried to afford the product (180 mg, ???% crude yield) as a dark oil which was purified by flash chromatography. Thus **7** was obtained as an amber solid. ^{1}H-NMR, $CDCl_3$, 200 MHz: 6.89-6.94 (d,1H, ^{3}J= 9.8), 7.90-7.95 (d,1H, ^{3}J= 9.8), 8.45-8.49 (dd,2H, ^{3}J= 2.34).

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Improved Synthesis of Substituted 6,7-Dihydroxy-4-quinazolineamines: Tandutinib, Erlotinib and Gefitinib.

Petr Knesl, Dirk Röseling and Ulrich Jordis*

Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria.

* Author to whom correspondence should be addressed; E-mail: ujordis@pop.tuwien.ac.at

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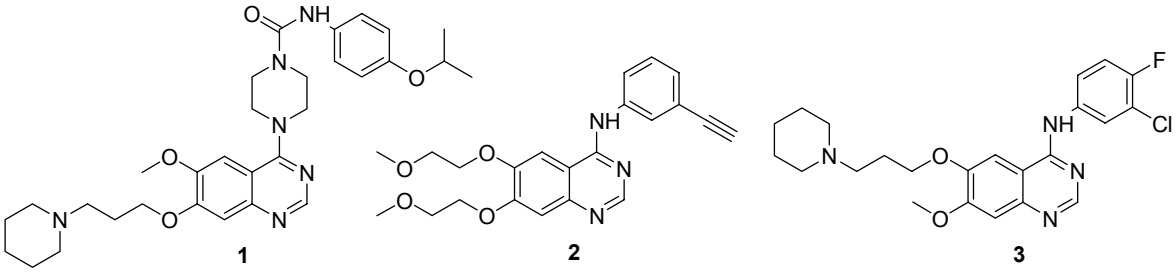
Abstract: The synthesis of three substituted 6,7-dihydroxy-4-quinazolineamines: tandutinib (**1**), erlotinib (**2**) and gefitinib (**3**) in improved yields is reported. The intermediates were characterized by NMR and the purities determined by HPLC.

Keywords: Quinazolines, tyrosine kinase inhibitors.

Introduction

Many signal transduction pathways are governed by protein kinases regulating various aspects of cell functions. Mutations which deregulate their expression, their function or both can result in cancers. Protein kinase inhibitors, particularly phosphorylation inhibitors, have become important targets for selective cancer therapies. The genome project has identified more than 500 protein kinases and 16 protein tyrosine kinases (PTKs) are possible therapeutic targets. The epidermal growth factor receptor (EGFR), a cell membrane receptor, plays a key role in cancer development and progression [1]. Tyrosine kinase inhibitors erlotinib (**2**, Tarceva®, OSI-774) and gefitinib (**3**, Iressa®) have been launched for the treatment of non-small-cell lung cancer (NSCLC) and tandutinib (**1**, MLN-518, CT-53518) is in phase II clinical trials for myeloid leukemia (ML) or advanced myelodysplasia (MDS). Several substances that inhibit platelet-derived growth factor receptor (PDGFR) phosphorylation via competitive binding of adenosine 5´-triphosphate [2,3] have been reported previously. Furthermore gefitinib (**3**) and erlotinib (**2**) inhibit tyrosine kinase activity and restrict the receptor's catalytic activity, autophosphorylation, and its engagement with signal transducers [4]. Gefitinib (**3**) is the first EGFR-targeting agent to be registered as an anticancer drug in Japan, in Australia and in the US for the third-line treatment of chemo-resistant NSCLC patients [1]. Tandutinib (**1**) selectively inhibits FLT3 and PDGFR, while other tyrosine or serine/threonine kinases are not significantly inhibited [5]. Here we report at the improved synthesis of these three PTK inhibitors: tandutinib (**1**), erlotinib (**2**) and gefitinib (**3**) (Figure 1).

Figure 1

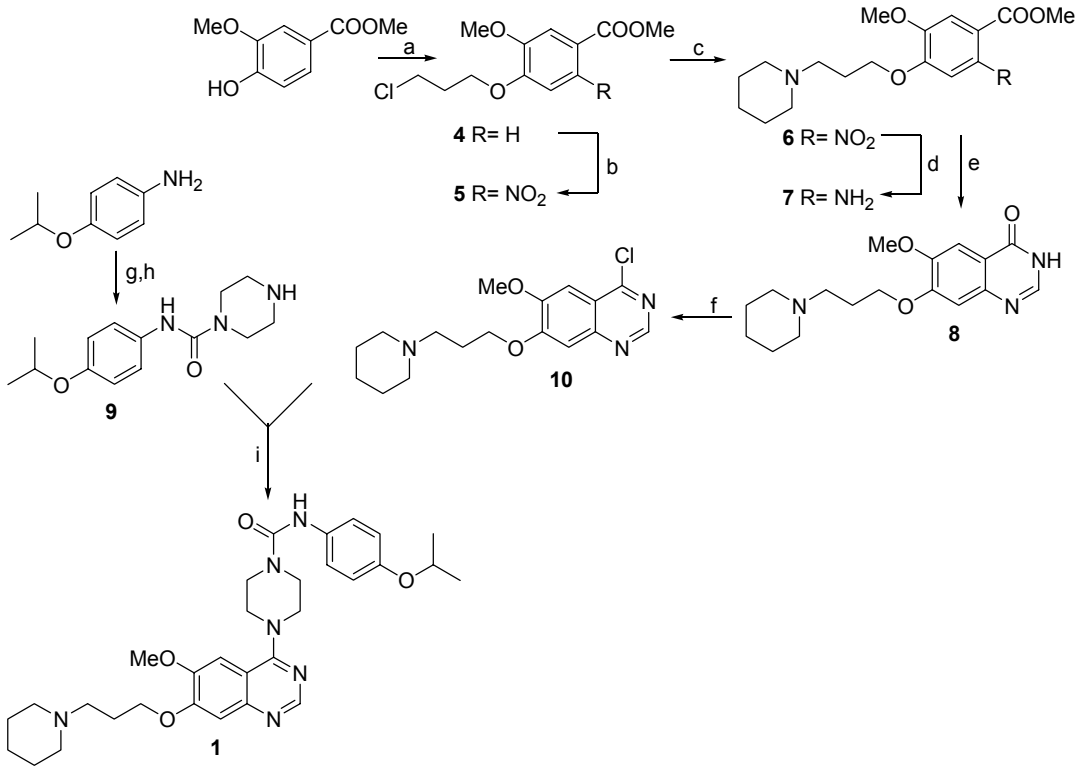


1 TANDUTINIB 2 ERLOTINIB 3 GEFITINIB

Results and Discussion

For tandutinib (**1**) the synthesis of the key intermediate **8** starts from 4-hydroxy-3-methoxy-benzoic acid, which is reacted with 1-bromo-3-chloro-propane followed by nitration, yielding the desired regioisomer in 75% yield. These steps, as well as the following substitution with piperidine and subsequent catalytic hydrogenation were performed according to the published procedures [6], however all intermediates were purified and fully characterized (Scheme 1).

Scheme 1: Synthesis of tandutinib



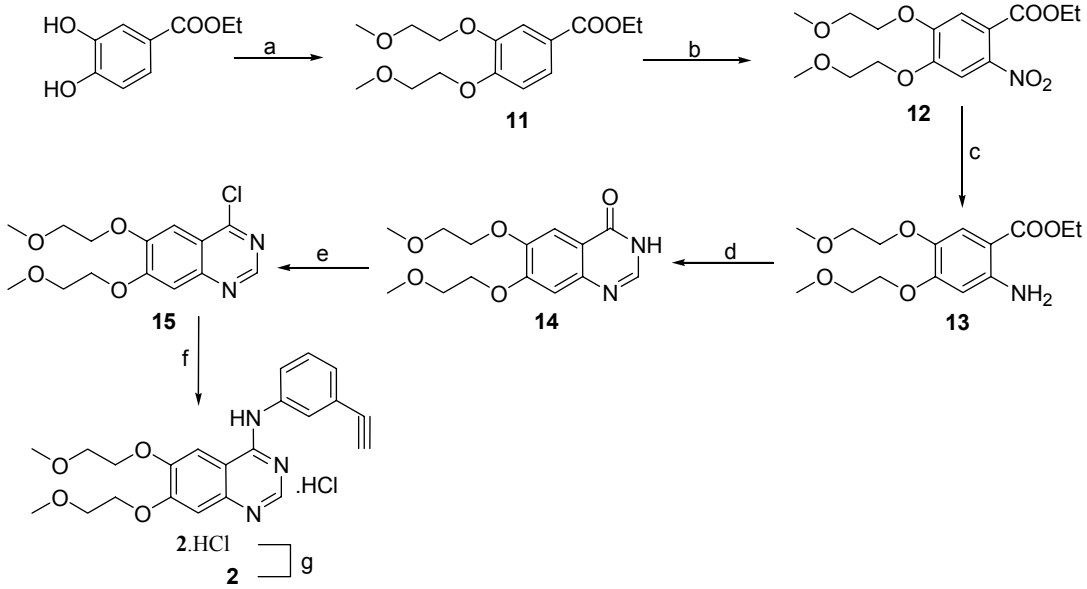
a) 1-bromo-3-chloropropane, K_2CO_3,$Bu_4N^+Br^-$ b) AcOH, HNO_3, 0-5 °C c) piperidine, K_2CO_3, NaI, $Bu_4N^+Br^-$ d) $PtO_2.H_2O$, H_2, MeOH, 40 p.s.i. e) formamidine acetate, 2-methoxyethanol, reflux f) $POCl_3$, *N,N*-diethylaniline g) trichloromethyl chloroformate h) piperazine, toluene, 90 °C i) K_2CO_3, DMF, 50 °C

In our hands the reported cyclization of **7** to **8** using either formamide or formamide with addition of ammonium formiate resulted in yields of only 10-20 %. Using formamidine acetate and 2-methoxy-ethanol as a solvent, we obtained **8** in 85 % yields and >98 % HPLC purity. For the chlorination step we found that the use of thionyl chloride and DMF [6] gave

less reliable results than our method using phosphoryl chloride and *N,N*-diethylaniline [7] where we obtained 70-86 %. The preparation of **9** is not mentioned in the literature. We prepared it by reaction of 4-isopropoxyphenylamine first with trichloromethyl chloroformate followed by reaction with excess of piperazine. A close example starting from benzylpiperazine and 4-methoxyphenylisocyanate is described in literature [5]. The final convergent coupling of **9** with **10** gave 95 % of **1** in >90 % HPLC purity. Tandutinib (**1**) was obtained after recrystallization in >99 % HPLC purity. The synthesis of tandutinib **1** was also described by Scarborough *et al.* but without experimental details [2].

The synthesis of erlotinib (**2**, Scheme 2) starts with the *O*-alkylation of methyl 3,4-dihydroxy-benzoate using either 1-chloro-2-methoxyethane or 1-bromo-2-methoxyethane. In both cases the yield was >90 %, with the chlorine compound requiring longer reaction times. Nitration of **11** gave the desired regioisomer on 92% yield and was followed by catalytic hydrogenation and cyclization with formamide performed according to the patent literature [8]. Again, our preferred reagent for the chlorination was phosphoryl chloride and *N,N*-diethylaniline [7], giving **15** in 89 % yield and 96 % HPLC purity. The final product was obtained as the hydrochloride salt **2·HCl** and converted to **2.** Erlotinib (**2**) was thus obtained in 56 % overall yield, each step being improved substantially. All intermediates were fully characterized.

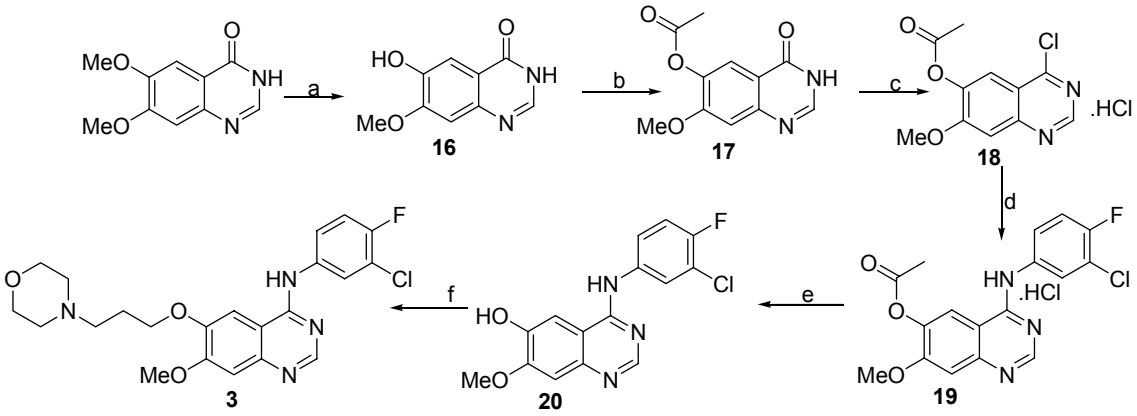
Scheme 2: Synthesis of elotinib



a) 1-chloro-2-methoxyethane or 1-bromo-2-methoxyethane , K_2CO_3, $Bu_4N^+Br^-$, Acetone b) AcOH, HNO_3, 0-5 °C c) $PtO_2.H_2O$, H_2 d) Formamide, 165-170 °C e) $POCl_3$, *N,N*-diethylaniline f) 3-ethynylaniline, pyridine, i-PrOH g) NH_4OH/H_2O

The synthesis of gefitinib (**3,** Scheme 3) starts with regioselective demethylation of 6,7-dimethoxy-3H-quinazolin-4-one followed by *O*-protection. Attempts to prepare the desired chloro compound using thionyl chloride and DMF [9] gave only unsatisfactory yields. Similar to the syntheses described above, phosphoryl chloride and *N, N*-diethylaniline [7] proved to be advantageous. Each of the following steps could be improved substantially and the overall yield from **16** to **3** was 33 % as compared to 20 % previously [9].

Scheme 3: Synthesis of gefitinib



a) Methansulfonic acid, L-methionine, reflux b) d) Ac_2O, pyridine, DMAP c) $POCl_3$, DEA d) 3-chloro-4-fluoro phenylamine, i-PrOH e) NH_3/MeOH, H_2O f) 4-(3-chloropropyl)morpholine, K_2CO_3, DMF

Experimental

General

Melting points were measured on a Büchi melting point apparatus B-545. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO-d_6. Thin layer chromatography (TLC) was performed on Merck TLC aluminum sheets silica 60 F_{254}. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). Column chromatography was performed using silica gel (Baker 40-60 µm). MPLC (medium pressure liquid chromatography) was performed using a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument and Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium Software with the Maxplot option for the UV maximum of the corresponding peak.

Methyl 4-(3-chloropropoxy)-3-methoxybenzoate (**4**):

A solution of methyl 3-methoxy-4-hydroxybenzoate (1, 87.0 g, 478 mmol), 1-bromo-3-chloro-propane (200.0 g, 1.270 mmol), potassium carbonate (350.0 g, 2.533 mol) and tetrabutylammonium iodide (9.0 g, 27 mol) in acetone (900 mL) was heated to reflux for 2 h. Reaction progress was monitored by HPLC and the reaction was found to be complete after this time. The reaction mixture was cooled to room temperature, solid material was filtered and washed with acetone. The combined filtrates and washings were evaporated under reduced pressure to give a yellow, solid product (110.6 g) which was crystallized from acetonitrile (500 mL) to afford the title compound (m.p.: 111-113 °C, 93.5 g, 76%, 94% HPLC purity). 1H-NMR (CDCl3): 2.25-2.42 (tt, 2H, -CH2CH2CH2-, 3Ja= 6.26 Hz, 3Jb= 6.06 Hz), 3.74-3.80 (t, 2H, CH2Cl, 3Ja= 6.26 Hz), 3.89 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 4.19-4.25 (t, 2H, CH2O, 3Jb=6.06 Hz), 6.89-6.93 (d, 1H, HAr, 3J= 8.41 Hz), 7.54-7.55 (d,

1H, HAr, 4J= 1.96 Hz), 7.63-7.68 (dd, 1H, HAr, 3J= 8.41 Hz, 4J=1.96 H); 13C-NMR (CDCl3): 32.52, 40.99, 50.06, 68.73, 116.13, 116.05, 123.23, 123.12, 148.63, 149.25.

Methyl 4-(3-chloropropoxy)-5-methoxy-2-nitrobenzoate (**5**):

A solution of methyl 4-(3-chloropropoxy)-3-methoxybenzoate (**4**, 93.0 g, 128 mmol) in acetic acid (350 mL) was added dropwise to nitric acid (84.5 mL, 66%) at 0-5 °C and this mixture was stirred at room temperature for 1 h and than for 2 h at 50 °C. According to HPLC the reaction was complete. The reaction mixture was poured on ice/water (1300 mL) and extracted with ethyl acetate (6 x 200 mL). The combined organic phases were collected, washed with saturated sodium bicarbonate (2 x 150 mL), brine (500 mL), dried (Na_2SO_4) and decolorized (charcoal). Ethyl acetate was then removed under reduced pressure to give a yellow oil (130.3 g) which was crystallized from ethyl acetate/petroleum ether to afford the product as light yellow crystals (m.p.: 54-56 °C, 81.3 g, 75%, 97% HPLC purity). ^{1}H-NMR ($CDCl_3$): 2.26-2.38 (tt, 2H, -$CH_2CH_2CH_2$-, ^3J_a= 6.16 Hz, ^3J_b= 5.87 Hz), 3.74-3.80 (t, 2H, CH_2Cl, ^3J_a= 6.16 Hz), 3.90 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.22-4.28 (t, 2H, CH_2O, ^3J_b=5.87 Hz), 7.07 (s, 1H, H_{Ar}), 7.48 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 32.95, 42.33, 50.03, 58.54, 70.02, 112.22, 115.62, 120.16, 143.15, 150.05, 154.87, 169.87.

Methyl 5-methoxy-2-nitro-4-(3-piperidin-1-yl-propoxy)benzoate (**6**):

A solution of methyl 4-(3-chloropropoxy)-5-methoxy-2-nitrobenzoate (**5**, 110.0 g, 0.362 mol), potassium carbonate (200.0 g, 1.447 mol), sodium iodide (110 g, 0.734 mol) and tetrabutylammonium iodide (5.7 g, 10.7 mmol) in acetonitrile (900 mL) was stirred for 5-10 min at room temperature. Piperidine (95 g, 1.116 mol) was added and this mixture heated to reflux for 3 h. The reaction was found to be complete after this time (HPLC). Solid material was removed by filtration and washed with acetone. The combined filtrates were evaporated and the dark product obtained dissolved in dichloromethane (650 mL) and extracted with water (4 x 250 mL). The organic phase was dried (Na_2SO_4), decolorized (charcoal), filtered and evaporated to afford the product (110.0 g, 86%, 98% HPLC purity) as an amber oil. ^{1}H-NMR ($CDCl_3$): 1.41-1.67 (m, 6H, 3 x CH_2pip.), 2.26-2.38 (m, 2H, -$CH_2CH_2CH_2$) 2.48-2.62 (m, 6H, 3 x CH_2N), 3.95 (s, 3H, OCH_3), 4.22 (s, 3H, OCH_3), 4.28-4.39 (t, 2H, CH_2O), 7.13 (s, 1H, H_{Ar}), 7.62 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 24.32, 26.25, 32.33, 49.95, 51.15, 53.03, 71.23, 111.12, 116.26, 117.87, 141.15, 150.07, 153.02, 170.08.

Methyl 2-amino-5-methoxy-4-(3-piperidin-1-yl-propoxy)benzoate (**7**):

A solution of methyl 5-methoxy-2-nitro-4-(3-piperidin-1-yl-propoxy)benzoate (**6**, 90.0 g, 0.25 mol) in methanol (700 mL) was hydrogenated with $PtO_2 \cdot H_2O$ (1.0 g) at room temperature and an initial pressure of 40 p.s.i. for 48 h until no further hydrogen uptake was noted. The catalyst was filtered, washed with methanol and volatiles evaporated to give a dark oil, which was dissolved in dichloromethane (400 mL), extracted with a saturated solution of sodium carbonate (1 x 100 mL) and brine (1 x 100 mL), dried (Na_2SO_4) and decolorized (charcoal). Dichloromethane was removed on the rotavapor and the brown, semisolid residue

triturated with diisopropyl ether to afford the product as a light brown powder (m.p.: 96-98 °C, 63.4 g, 77%, 97% HPLC purity). ^{1}H-NMR ($CDCl_3$): 1.35-1.47 (m, 2H, CH_2pip.), 1.52-1.63 (m, 4H, 2 x CH_2pip.), 1.94-2.08 (tt, 2H, -$CH_2CH_2CH_2$-, ^{3}J= 6.85 Hz), 2.35-2.48 (m, 6H, 3 x CH_2N), 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.00-4.06 (t, 2H, CH_2O, ^{3}J=6.85 Hz), 5.56 (w, 2H, NH_2), 6.16 (s, 1H, H_{ard}), 7.29 (s, 1H, H_{ard}); ^{13}C-NMR ($CDCl_3$): 25.07, 27.06, 30.99, 50.25, 51.65, 53.80, 73.06, 101.65, 110.20, 118.85, 141.18, 136.65, 149.07, 153.02, 167.88.

6-Methoxy-7-(3-piperidin-1-yl-propoxy)quinazoline-4(3H)-one (**8**):

A solution of methyl 2-amino-5-methoxy-4-(3-piperidin-1-yl-propoxy)benzoate (**7**, 25.0 g, 78 mol) and formamidine acetate (10.4 g, 100 mmol) in 2-methoxyethanol (250 mL) was heated to reflux for 4 h. To complete the reaction another portion of formamidine acetate (2.00 g, 19.2 mmol) was added and heating continued for further 2 h. The reaction progress was monitored by HPLC and found to be complete after this time. Volatiles were removed under reduced pressure and the orange, sticky residue obtained triturated with diethyl ether (4 x 150 mL) to afford the product as a white powder which was collected by filtration and air dried (m.p.: 218-219 °C, 20.9 g, 85%, 99% HPLC purity). ^{1}H-NMR ($CDCl_3$): 1.32-1.40 (m, 2H, CH_2pip.), 1.43-1.53 (m, 4H, 2 x CH_2pip.), 1.83-1.97 (tt, 2H, -$CH_2CH_2CH_2$-, ^{3}J= 6.26 Hz), 2.29-2.41 (m, 6H, 3 x CH_2N), 3.86 (s, 3H, OCH_3), 4.09-4.15 (t, 2H, CH_2O, ^{3}J=6.26 Hz), 7.10 (s, 1H, H_{Ar}), 7.43 (s, 1H, H_{Ar}), 7.97 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 27.02, 29.34, 31.37, 51.25, 53.78, 55.23, 71.19, 109.78, 115.52, 120.57, 142.07, 146.36, 150.15, 165.28, 176.32.

4-Chloro-6-methoxy-7-(3-piperidin-1-yl-propoxy)quinazoline (**10**):

Phosphoryl chloride (80 mL) was added to *N,N*-diethylaniline (6.2 mL) with magnetic stirring followed by addition of 6-methoxy-7-(3-piperidin-1-ylpropoxy)quinazoline-4(*3H*)-one (**8**, 10.0 g, 31.51 mmol) and the reaction flask was immersed in a preheated oil bath (70 °C).The temperature was increased to 90 °C over a period of 10 min. and kept at 80-90 °C for another 30 min. Most of the excess of phosphoryl chloride was then removed under reduced pressure and the resulting dark oil triturated with toluene (3 x 40 mL). The residue was dissolved in water (20 mL) and extracted with ethyl acetate (2 x 15 mL). The pH of the aqueous phase was adjusted to 9-10 with sodium bicarbonate and it was extracted with dichloromethane (3x150 mL), dried (Na_2SO_4), decolorized (charcoal) and the organic solvent was removed to afford the product as a yellowish oil (9.1 g, yield 86%, 99% HPLC purity). ^{1}H-NMR ($CDCl_3$): 1.30-1.39 (m, 2H, CH_2pip.), 1.41-1.50 (m, 4H, 2 x CH_2pip.), 1.82-1.96 (tt, 2H, -$CH_2CH_2CH_2$-), 2.16-2.35 (m, 6H, 3 x CH_2N), 3.80 (s, 3H, OCH_3), 4.00-4.09 (t, 2H, CH_2O), 7.41 (s, 1H, H_{Ar}), 7.45 (s, 1H, H_{Ar}), 8.76 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 26.22, 27.94, 30.27, 51.45, 52.97, 56.54, 70.03, 101.88, 108.52, 121.57, 147.07, 155.26, 160.95, 157.28, 159.32.

N-(4-Isopropoxyphenyl)-4-[6-methoxy-7-(3-piperidin-1-yl-propoxy)quinazolin-4-yl]piperazine-1-carboxamide (**1**):

A suspension of *N*-(4-isopropoxyphenyl)piperazine-1-carboxamide (**9**, 6.90 g, 88%, 23 mmol) and potassium carbonate (12.7 g, 96.2 mmol) in dry DMF (60 mL) was stirred at room temperature for 20 min, then 4-chloro-6-methoxy-7-(3-piperidin-1-yl-propoxy)quinazoline (**10**, 7.83 g, 23 mmol, 98.8%) was added and this mixture was heated at 50 °C for 18 h. The reaction was monitored by HPLC and found to be complete. The reaction mixture was poured on water (500 mL) and extracted with dichloromethane (4 x 150 mL). The combined phases were washed with brine (2 x 80 mL), dried (Na_2SO_4), decolorized (charcoal) and the solvent was removed under reduced pressure to dryness to give a crude yellow, sticky material (12.4 g, yield 96%, 95% HPLC purity) which was crystallized from ethyl acetate/petroleum ether to afford the product >99% HPLC purity. ^{1}H-NMR ($CDCl_3$): 1.29-1.32 (d, 6H, 2 x CH_3, ^3J=6.06 Hz) 1.39-1.50 (m, 2H, CH_2pip.), 1.53-1.64 (m, 4H, 2 x CH_2pip.), 2.03-2.17 (tt, 2H, -$CH_2CH_2CH_2$-, ^3J_a= 6.84 Hz, ^3J_b= 6.65 Hz), 2.38-2.43 (m, 4H, 2 x CH_2N), 2.47-2.54 (t, 2H, CH_2N, ^3J_a= 6.84 Hz), 3.71 (w, 8H, 4 x CH_2N), 3.96 (s, 3H, OCH_3), 4.19-4.25 (t, 2H, CH_2O, ^3J_b=6.65 Hz), 4.38-4.56 (m, 1H, $(CH_3)_2$CH), 6.50 (s, 1H, NH), 6.81-6.86 (m, 2H, H_{Ar}) 7.09 (s, 1H, H_{Ar}), 7.22-7.27 (m, 3H, H_{Ar}), 8.67 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 23.02, 25.65, 28.82, 31.11, 49.89, 52.20, 54.70, 56.76, 58.89, 72.31, 73.76, 103.11, 108.99, 114.45, 118.25, 122.43, 131.32, 148.87, 150.12, 155.67, 158.87, 160.76, 161.73, 165.75, 180.11.

N-(4-Isopropoxyphenyl)piperazine-1-carboxamide (**9**):

A solution of 4-isopropoxyphenylamine (1.0 g, 6.61 mmol) in dry toluene (10 mL) was added drop- wise to a solution of trichloromethyl chloroformate (13.0 g, 9.94 mmol) in dry toluene (10 mL) at 0-5 °C and the mixture was stirred at the same temperature for 20 min. The reaction mixture was then stirred and heated to 100 °C over 1h and kept at this temperature for 1 h. Reaction progress was monitored by HPLC and found to be complete by this time. The reaction mixture was cooled to room temperature and extracted with saturated solution of sodium bicarbonate (1 x 10 mL). The toluene solution was separated and aqueous phase extracted with toluene (5 mL). The combined toluene layers were added dropwise to a solution of piperazine (2.80 g, 32.5 mmol) in toluene (10 mL) and this mixture was heated at 90 °C for 3 h, cooled and washed with water (2 x 15). The toluene layer was dried (Na_2SO_4) and evaporated to afford the product (0.91 g, 52%, 95% HPLC purity). ^{1}H-NMR (DMSO): 1.20-1.23 (d, 6H, 2 x CH_3, ^3J= 6.06), 2.73-2.78 (m, 4H, 2 x CH_2N), 3.15 (w, 1H, NH), 3.39-3.48 (m, 4H, 2 x CH_2N), 4.41-4.53 (s, 1H, ^3J=6.06), 6.75-6.80 (d, 2H, H_{Ar}, ^3J=8.9), 7.31-7.36 (d, 2H, H_{Ar}, ^3J=8.9), 8.37 (s, 1H, NH); ^{13}C-NMR (DMSO): 20.28, 49.05, 55.63, 68.82, 111.33, 120.20, 126.35, 155.05, 160.01.

Ethyl 3,4-bis(2-methoxyethoxy)benzoate (**11**):

A suspension of ethyl 3,4-dihydroxybenzoate (40.0 g, 220 mmol), potassium carbonate (70.0 g, 507 mmol) and tetrabutylammonium iodide (2.80 g, 7.6 mmol) in acetone (300 mL) was stirred for 20 min at room temperature. 1-Chloro-2-methoxyethane (158.0 g, 1.671 mol)

was added and the solution stirred and heated to reflux for 5 days. Reaction progress was monitored by HPLC and the reaction was found to be complete after this time.The reaction mixture was cooled to room temperature and diethyl ether (250 mL) was added. Inorganic salts were filtered off and washed with diethyl ether (2 x 250 mL). The combined organic layers were dried (Na_2SO_4), decolorized (charcoal) and volatiles evaporated under reduced pressure to afford the product as light oil (72.5 g) which was dissolved in petrol ether (250 mL) and cooled to 0-5 °C. The resulting white precipitate was collected by filtration and dried on the air (m.p.: 55-58 °C, 61.8 g, yield: 94 %, 97% HPLC purity). ^{1}H-NMR ($CDCl_3$): 1.27-1.34 (t, 3H, CH_3CH_2, ^{3}J= 7.10 Hz), 3.38 (s, 6H, 2 x OCH_3), 3.69-3.74 (m, 4H, 2 x CH_2O), 4.11-4.15 (m, 4H, 2 x CH_2O), 4.21-4.32 (q, 2H, CH_2, ^{3}J=7.10 Hz), 6.82-6.86 (d, 1H, $H_{Ar,}$ ^{3}J=8.41 Hz), 7.51-7.52 (d, 1H, $H_{Ar,}$ ^{4}J=1.96 Hz), 7.57-7.62 (dd, 1H, $H_{Ar,}$ ^{3}J=8.41 Hz, ^{4}J=1.96 Hz); ^{13}C-NMR ($CDCl_3$): 15.08, 50.25, 61.62, 70.76, 73.89, 111.54, 116.27, 121.83, 124.35, 139.12, 152.55, 165.13.

Ethyl 4,5-bis(2-methoxyethoxy)-2-nitrobenzoate (**12**):

A solution of ethyl 3,4-bis(2-methoxyethoxy)benzoate (**11**, 67.0 g, 0.23 mol) in AcOH (230 mL) was added dropwise to nitric acid (60 mL, 65%) at 0-5 °C, stirred at the same temperature for 30 min and than for 24 h at room temperature. Reaction progress was monitored by HPLC and it was found to be complete after this time. The reaction mixture was poured on ice/water (1000 mL) followed by extraction with ethyl acetate (5 x 250 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 x 250 mL), brine (3 x 250 mL), dried (Na_2SO_4), decolorized (charcoal) and evaporated to give the product (71.30 g, 92%, 95% HPLC purity) as an amber oil. ^{1}H-NMR ($CDCl_3$): 1.35-1.43 (t, 3H, CH_3CH_2, ^{3}J= 7.20 Hz), 3.35 (s, 6H, 2 x OCH_3), 3.72-3.85 (m, 4H, 2 x CH_2O), 4.20-4.26 (m, 4H, 2 x CH_2O), 4.31-4.42 (q, 2H, CH_2, ^{3}J=7.20 Hz), 7.52 (s, 1H, $H_{Ar,}$), 7.82 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 20.12, 51.55, 59.92, 71.13, 76.18, 115.54, 118.03, 120.16, 142.24, 149.86, 150.12, 167.23.

Ethyl 2-amino-4,5-bis(2-methoxyethoxy)benzoate (**13**):

A solution of ethyl 4,5-bis(2-methoxyethoxy)-2-nitrobenzoate (**12**, 38.0 g, 0.11 mmol) in methanol (250 mL) was hydrogenated in a Parr apparatus with $PtO_2 \cdot H_2O$ (0.5 g) at room temperature and 50 p.s.i. pressure. Reaction development was monitored by HPLC and the reduction continued until no more hydrogen was consumed. The catalyst was filtered off and methanol removed to afford a brown slurry (39.2 g) which was triturated with diethyl ether (500 mL). The light brown precipitate was filtered trough a glass funnel and dried on the air (34.2 g, 99%, 94% HPLC purity). ^{1}H-NMR ($CDCl_3$): 1.30-1.37 (t, 3H, CH_3CH_2, ^{3}J= 7.10 Hz), 3.30 (s, 6H, 2 x OCH_3), 3.70-3.81 (m, 4H, 2 x CH_2O), 4.16-4.21 (m, 4H, 2 x CH_2O), 4.25-4.34 (q, 2H, CH_2, ^{3}J=7.10 Hz), 5.25 (br, 2H, NH_2), 6.38 (s, 1H, $H_{Ar,}$), 7.42 (s, 1H, H_{Ar}); ^{13}C-MR ($CDCl_3$): 18.54, 52.45, 60.92, 74.13, 77.33, 101.24, 108.76, 116.53, 133.55, 141.20, 150.05, 167.23.

6,7-Bis-(2-methoxyethoxy)-quinazolin-4(3H)-one (**14**):

A solution of ethyl 2-amino-4,5-bis(2-methoxyethoxy)benzoate (**13**, 4.0 g, 109 mmol) in formamide (50 mL) was heated to 165-170 °C under N_2 for 12 h., when HPLC indicated the absence of starting material. The reaction mixture was cooled and the amber sticky precipitate triturated with diethyl ether (250 mL), filtered and triturated in boiling acetonitrile (100 mL) for 30 min, than cooled to 5 °C and diethyl ether (300 mL) was added. The resulting white powder was filtered using a sintered glass frit and dried under reduced pressure. (m.p.: 183-184 °C, 26.8 g, 84%, 98% HPLC purity). ^{1}H- NMR (DMSO): 3.32 (s, 6H, OCH_3), 3.67-3.73 (m, 4H, 2 x OCH_2), 4.16-4.26 (m, 4H, 2 x OCH_2), 7.13 (s, 1H, H_{Ar}), 7.45 (s, 1H, H_{Ar}), 7.97 (s, 1H, H_{Ar}); ^{13}C-NMR (DMSO): 52.26, 70.13, 73.15, 110.44, 115.23, 121.22, 139.79, 140.12, 143.56, 150.22, 165.56, 172.23.

4-Chloro-6,7-bis-(2-methoxyethoxy)-quinazoline (**15**):

Phosphoryl chloride (30 mL) was added to *N,N*-diethylaniline (5.8 g, 38.9 mmol) with magnetic stirring followed by addition of 6,7-bis-(2-methoxyethoxy)-quinazolin-4(*3H*)-one (**14**, 10.0 g, 34 mmol) and the reaction flask was immersed in oil bath preheated to 70 °C. The temperature was increased to 90 °C over a period of 10 min. and kept at 80-90 °C for additional 30 min. Most of the excess of phosphoryl chloride was removed under reduced pressure and the dark oil triturated with toluene (3 x 150 mL). Oil residue was poured on crushed ice/water mixture (200 mL) and extracted with ethyl acetate (4 x 150 mL). The combined organic layers were dried (Na_2SO_4) and decolorized (charcoal). The volatiles were evaporated under reduced pressure to give the product as yellowish crystals (m.p.:105-107 °C, 10.8 g, 89%, 96% HPLC purity). ^{1}H-NMR ($CDCl_3$): 3.49 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.87-3.91 (m, 4H, 2 x OCH_2), 4.30-4.36 (m, 4H, 2 x OCH_2), 7.33 (s, 1H, H_{Ar}), 7.42 (s, 1H, H_{Ar}), 8.85 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 50.34, 69.99, 71.46, 102.72, 110.33, 122.24, 146.98, 150.42, 143.56, 158.87, 160.30, 163.33.

N-(3-ethynylphenyl)-6,7-Bis-(2-methoxyethoxy)-quinazolin-4-amine hydrochloride (**2·HCl**) *and free base* **2**:

A solution of 4-chloro-6,7-bis-(2-methoxyethoxy)-quinazoline (**15**, 20.30 g, 65 mmol) in i-PrOH (100 mL) was added drop wise to a solution of pyridine (5.90 g, 75 mmol) and 4-ethynylphenylamine (8.80 g, 75 mmol) in i-PrOH (260 mL). This mixture was stirred and heated at reflux for 4 h under argon resulting in the precipitation of an orange solid. After stirring at room temperature overnight the precipitate was filtered, washed with hot i-PrOH and dried on the air to afford the crude **2·HCl** product (26.4 g, 95%, 95% HPLC purity) which was crystallized from methanol to afford a yellowish powder (m.p.: 219-221 °C, > 99% HPLC purity). To isolate the free base 0.5 g of **2·HCl** was dissolved in water (10 mL) and basified using conc. aq. ammonia (5 mL). Extraction with dichloromethane (4 x 25 mL), gave after drying and evaporation, **2** as yellow crystals. (m.p.: 159-160 °C, 425 mg, 92%, 99% HPLC purity). ^{1}H-NMR ($CDCl_3$): 3.08 (s, 1H, CH), 3.40 (s, 3H, OCH_3), 3.73-3.78 (m, 4H, 2 x CH_2O), 4.13-4.21 (m, 4H, 2 x CH_2O), 7.12 (s, 1H, H_{Ar}), 7.42-7.36 (m, 3H, H_{Ar}), 7.70-7.76

(m, 1H, H_{Ar}), 7.85 (s, 1H, H_{Ar}), 7.96 (w, 1H, NH), 8.60 (s, 1H, H_{Ar}); ^{13}C-NMR ($CDCl_3$): 51.25, 73.52, 75.78, 80.63, 86.76, 100.97, 108.83, 114.15, 117.65, 122.34, 124.35, 125.76, 130.89, 142.31, 147.28, 151.20, 155.07, 159.11, 170.22.

6-Hydroxy-7-methoxyquinazolin-4(3H)-one (**16**):

A mixture of 6,7-dimethoxyquinazolin-4(3H)-one (64.7 g, 0.31 mol) and L-methionine (53.7 g, 0.34 mol) was dissolved in methanesulfonic acid (425 mL) and heated to reflux for 12 h. Reaction was monitored by HPLC and no starting material was indicated after this time. Crushed ice/water mixture (200 mL) was added and the solution was cooled to 0 °C. Then NaOH (40% water solution) was added slowly (pH~7) resulting in precipitation of a white deposit. This product was collected by filtration using a sintered glass funnel, washed with water and dried at 50 °C and 23 mbar to afford the product (58.3 g, 98%, 90% HPLC purity) which was used without further purification.

7-Methoxy-4-oxo-3,4-dihydroquinazolin-6-yl acetate (**17**):

A suspension of 6-hydroxy-7-methoxyquinazolin-4(*3H*)-one (**16**, 36.7 g, 191 mmol) in acetic anhydride (300 mL), pyridine (41.2 mL) and *N,N*-dimethyl-4-aminopyridine (100 mg, 0.819 mmol) was stirred and heated to 100 °C under Ar atmosphere for 6 h. Reaction progress was monitored by HPLC. Crushed ice/water solution (300 mL) was added and the resulting white deposit filtered, washed with water and dried over P_2O_5 to afford the product (25.05 g, 56%, 95% HPLC purity). ^{1}H-NMR (DMSO): 2.29 (s, 3H, CH_3CO), 3.90 (s, 3H, OCH_3), 7.26 (s, 1H, $H_{Ar.}$), 7.74 (s, 1H, $H_{Ar.}$), 8.07 (s, 1H, $H_{Ar.}$); ^{13}C-NMR (DMSO): 21.22, 57.31, 110.12, 114.65, 120.82, 136.65, 144.99, 146.22, 164.23, 166.81, 170.25.

4-Chloro-7-methoxyquinazolin-6-yl acetate (**18**):

A solution of 7-methoxy-4-oxo-3,4-dihydroquinazolin-6-yl acetate (**17**, 30.0 g, 0.128 mmol) and *N,N*-diethylaniline (28.5 mL) in phosphoryl chloride (114 mL) was immersed in a preheated oil bath (100 °C) and at this temperature stirred for 30 min. The reaction mixture was cooled to 80 °C and stirred for further 30 min. Phosphoryl chloride was removed and the obtained crude material triturated with abs. toluene (3 x 150 mL). Crushed ice/water was added (200 mL), the light brown precipitate filtered and washed with ice water (500 mL). The brownish precipitate was dried over P_2O_5 to give the product (33.90 g, 92%, 99% HPLC purity). ^{1}H-NMR (DMSO): 2.34 (s, 3H, CH_3CO), 3.97 (s, 3H, OCH_3), 7.48 (s, 1H, $H_{Ar.}$), 7.86 (s, 1H, $H_{Ar.}$), 8.76 (s, 1H, $H_{Ar.}$); ^{13}C-NMR (DMSO): 20.29, 56.66, 114.53, 119.99, 108.82, 139.66, 148.09, 156.76, 158.42, 164.32, 168.39.

4-[(3-Chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-yl acetate (**19**):

A solution of 4-chloro-7-methoxyquinazolin-6-yl acetate (**18**, 27.96 g, 0.111 mol) and 3-chloro-4-fluorophenylamine (16.12 g, 0.111 mol) in i-PrOH (500 mL) was stirred and heated to 90 °C under Ar atmosphere for 5 h. Reaction progress was monitored by HPLC and no

starting materials were detected after this time. The reaction mixture was cooled to room temperature and the obtained precipitate was filtered trough a glass funnel, dried under reduced pressure (50.8 g) and crystallized from methanol to afford **19** (42.2 g, 96%, 96% HPLC purity) as a white powder. ^{1}H-NMR (DMSO): 2.27 (s, 3H, CH_3CO), 3.90 (s, 3H, OCH_3), 7.38-7.46 (m, 1H, $H_{Ar.}$), 7.63-7.67 (m, 1H, $H_{Ar.}$), 7.92-7.97 (m, 1H, $H_{Ar.}$), 8.75 (s, 1H, $H_{Ar.}$), 8.84 (s, 1H, $H_{Ar.}$), 11.55 (w, 1H, NH); ^{13}C-NMR (DMSO): 20.55, 57.11, 110.22, 112.25, 112.82, 114.46, 116.29, 118.52, 119.30, 122.34, 141.35, 143.77, 147.82, 153.76, 158.82, 161.01, 169.23, 170.11.

4-[(3-Chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-ol (**20**):

A solution of 4-[(3-chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-yl acetate (**19**, 35.0 g, 97 mmol) in NH_3/MeOH (350 mL, 7 N solution)/H_2O (200 mL) was stirred at room temperature for 24 h. No starting material was present after this time indicated by HPLC. The white precipitate was filtered off and washed with small amount of water. The product was dried at 50 °C to afford **20** as a white solid (27.8 g, 90%, 98.5% HPLC purity).

N-(3-Chloro-4-fluorophenyl)-7[methoxy-6-[(3-morpholin-4-yl)propoxy]-quinazolin-4-yl]amine (**3**):

A solution of 4-[(3-chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-ol (**20**, 27.8 g, 87 mmol) and potassium carbonate (24.1 g, 175 mmol) in DMF (500 mL) was stirred at 40 °C for 20 min and than 4-(3-chloropropyl)morpholine was added. This mixture was stirred and heated at 80 °C for 12 h under an Ar atmosphere. Reaction development was monitored by HPLC and reaction found to be complete after this time.The reaction mixture was poured on crushed ice/water mixture and extracted with ethyl acetate (4 x 150 mL). Organic layers were combined, extracted with brine (2 x 100 mL), dried (Na_2SO_4) and solvent was removed. The crude material was crystallized from toluene to afford the product (29.5 g, 76%, > 99% HPLC purity). ^{1}H-NMR (DMSO): 1.93-2.00 (m, 2H, $CH_2CH_2CH_2$), 2.34-2.51 (m, 6H, $N(CH_3)_3$), 3.54-3.59 (m, 4H, $O(CH_2)_2$), 3.92 (s, 3H, OCH_3), 4.12-4.18 (m, 2H, $ArOCH_2$), 7.16 (s, 1H, $H_{Ar.}$), 7.35-7.45 (m, 1H, $H_{Ar.}$),7.45-7.82 (m, 2H, $H_{Ar.}$), 8.08-8.13 (m, 1H, $H_{Ar.}$), 8.48 (s, 1H, $H_{Ar.}$), 9.51 (s, 1H, $H_{Ar.}$); ^{13}C-NMR (DMSO): 25.84, 38.20, 38.62, 39.04, 39.46, 39.87, 40.29, 40.71, 53.35, 54.90, 55.69, 66.11, 67.10, 102.53, 107.11, 108.74, 116.02, 116.45, 118.45, 118.81, 122.02, 122.16, 123.29, 136.78, 136.84, 146.86, 148.22, 150.58, 152.42, 154.36, 155.40, 155.87.

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Synthesis of oxygenated butorphanol metabolites

Petr Knesl and Ulrich Jordis
Institute of Applied Synthetic Chemistry, Vienna University of Technology
Getreidemarkt 9, 1060 Vienna, Austria, ujordis@pop.tuwien.ac.at

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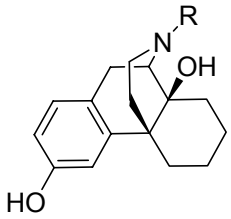
Abstract:
The synthesis of the the main butorphanol (**1**) metabolite, [17(*trans*)]-17-[(3-hydroxy cyclobutyl) metyl]-morphinan-3, 14 diol (**2**) as well as the corresponding *cis*-isomer **3** is described.

Introduction:
Butorphanol (**1**) is a synthetic opioid analgesic of the phenanthrene series with affinity to μ and κ-opioid receptor sites.[1, 2] It is used in the treatment of post-surgical and dental pain as well as migraine.[3] *Trans*-hydroxybutorphanol (**2**) was identified as major urinary metabolite of **1** but no synthesis has been reported so far, except a short note without any experimental details in a paper describing the metabolism of tritiated **1**.[4] In the present work we report the synthesis of [17(*trans*)]-17-[(3-hydroxy cyclobutyl) metyl]-morphinan-3, 14 diol (**2**) and the *cis*-isomer **3** (*Figure 1*).

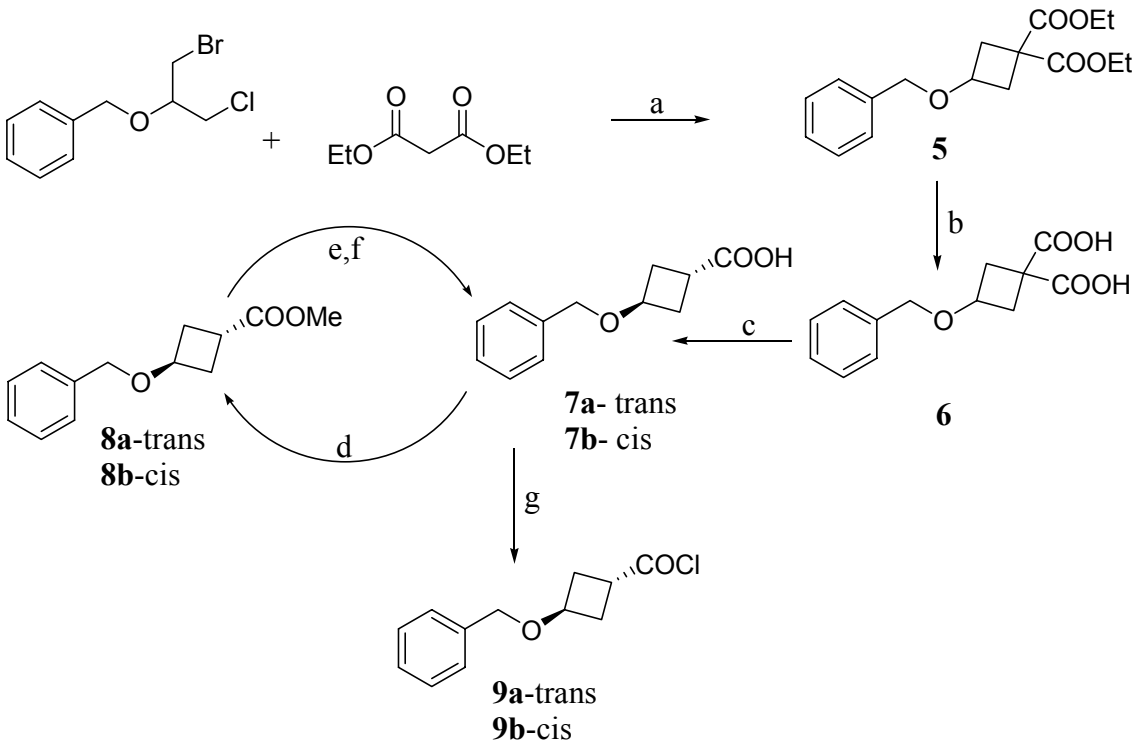


1: butorphanol R=
2: *trans*-hydroxybutorphanol R= OH
3: *cis*-hydroxybutorphanol R= OH
4: norbutorphanol R= H

Results and Discussion :
The synthesis sequence starts with the reaction of [[2-bromo-1-(chloromethyl)ethoxy]methyl] benzene with diethyl malonate to give **5** which was hydrolyzed to **6.** Decarboxylation of **6** gave a mixture of *cis* and *trans* 3-benzyloxy-cyclobutanecarboxylic acid (**7**). Attempts to separate this mixture according to a patent[5] failed in our hands thus verifying this fact.[6] The

separation of the methyl esters by column chromatography was successful and comparable to the previous separation of the ethyl esters.[6] The pure isomers **7a** and **7b** were then converted to the corresponding acid chlorides **9a** and **9b** (*Scheme 1*).

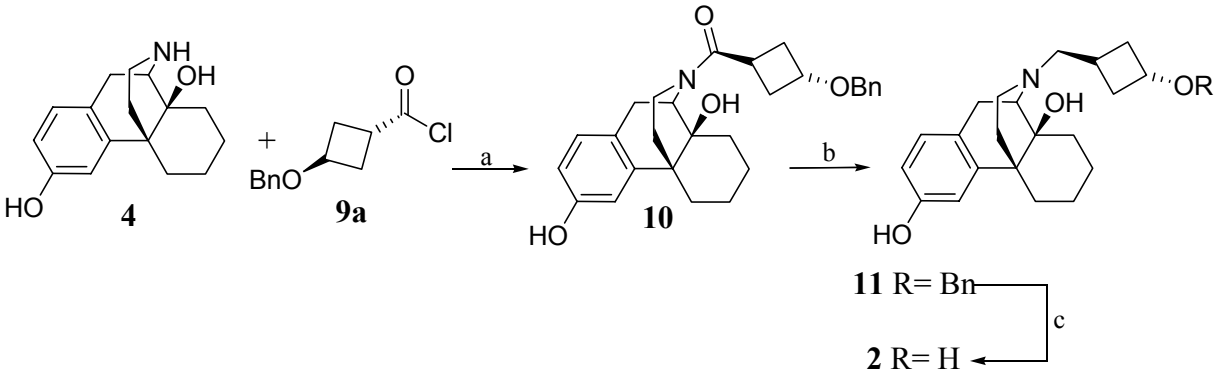
Scheme 1:



a) diethyl malonate, NaH, dioxane b) KOH, ethanol/water c) 175 °C, 11 mbar, 1h and 150-165 °C, 0.5 mbar. d) MeOH, $SOCl_2$ e) chromatographic separation (pentane/ EtOAc 99:1) f) NaOH, ethanol/ water g) $SOCl_2$, cat. DMF.

Our first attempt to prepare **2** started from norbutorphanol (**4)** which was acylated with **9a**. The subsequent reduction of the carbonyl group using diborane followed by reduction of benzyl group utilizing catalytic hydrogenation resulted in the anticipated product **2**, however the yield obtained in the last step was not satisfactory (*Scheme 2)*.

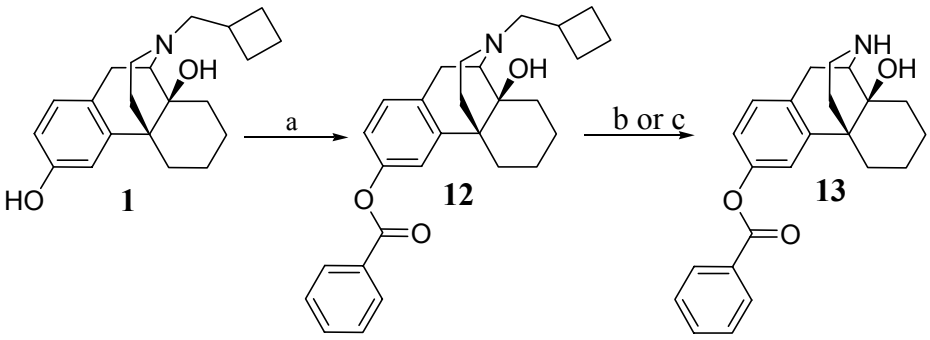
Scheme 2:



a) **9a**, Hünig's base, CH_2Cl_2 b) B_2H_6.THF c) Pd/C, H_2

The overall yield of the synthesis was improved by protection of the phenol group as the benzoate followed by Polonowsky N-dealkylation of the N-oxide by ferrous chloride.[7a, 7b] Replacing dichloromethane against methanol for the N-oxide formation resulted an increase of the yield from 30% to 60% for the conversion of **12** to **13** (*Scheme 3*).

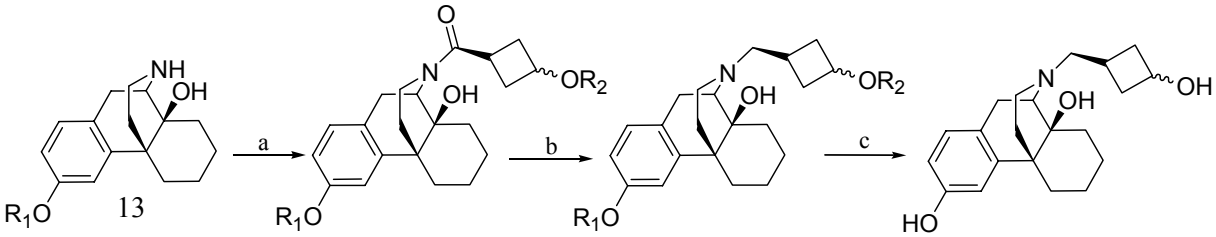
Scheme 3:



a) Benzoyl chloride, N_3Et, CH_2Cl_2 b) MCPBA, CH_2Cl_2 C) MCPBA, methanol d) $FeCl_2.4H_2O$, water

The target compounds **2** and **3** were prepared by reacting **13** with either **9a** or **9b** followed by catalytic debenzylation to **15** and **17** and amide reduction using diborane (*Scheme 4*).

Scheme 4:



14 trans **15** trans **2** trans

16 cis **17** cis **3** cis

R1= Benzoyl, R2= Benzyl

a) **9a** or **9b**, Hünig's base, CH_2Cl_2 b) Pd/C 10 %, methanol, H_2 c) B_2H_6.THF

Experimental Section:

General remarks: Melting points were measured on a Büchi B-545 melting point apparatus. ^1H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or DMSO-d_6. Thin layer chromatography (TLC) was performed on Merck TLC aluminum sheets silica 60 F_{254}. Visualization was by UV light at

254 and 366 nm or spray reagents (molybdophosphoric acid and heating). Column chromatography was performed using silica gel (VWR 40-63 μm). MPLC (medium pressure liquid chromatography) was performed using a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument and Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium Software with the Maxplot option for the UV maximum of the corresponding peak.

3-Benzyloxy-cyclobutane-1, 1-dicarboxylic acid diethyl ester (5): A 2 l three-necked flask was charged with dry dioxane (375.0 mL) and sodium hydride (60%, 24.0g, 0.6 mol, freed from mineral oil by washing with petrol ether) was added. Under mechanical stirring diethyl malonate (96.1 g, 0.6 mol) was added under nitrogen over a period of 20 min. followed by the addition of [[2-bromo-1-(chloromethyl)ethoxy]methyl]-benzene (158.0 g, 0.6 mol) over a period of 20 min. The mixture was heated under reflux for 44 hours, then the reaction mixture was allowed to cool to room temperature and subsequently NaH (60%, 24.0 g, 0.6 mol) was added in portions followed by the addition of dry dioxane (50 mL).The mixture was heated to reflux for another 60 hours. The major amount of the solvent was evaporated, water (300 mL) was added and the mixture extracted with diethyl ether (4 × 150 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to give the crude product as orange oil, which was distilled using 20 cm Vigreaux column. The fraction boiling at 158-160 °C / 0.5 mbar (lit.[8]b.p. 174-176 °C / 0.9 mbar) 73.2 g, 40 % yield, 92% GC purity, was used in the next step.

3-Benzyloxy-cyclobutane-1, 1-dicarboxylic acid (6): A mixture of **5** (73.00 g, 0.24 mol) and KOH (85%, 60.0 g, 1.1 mol) in ethanol (150 mL) and water (35 mL) was heated to reflux for two hours under nitrogen. Most of the ethanol was then evaporated, water (100 mL) was added and extracted with diethyl ether (2 × 50 mL, discarded).The aqueous phase was cooled to 0 °C and adjusted to pH 2.5 using 6N HCl. The resulting white precipitate was stirred for 30 min at 0 °C, filtered and recrystallized from hot water (100 mL) to afford the product (33.2 g, yield 56%, HPLC >99%) as colourless crystals with m.p. 164-166 °C, lit.m.p.[8] 160-162 °C. Workup of the mother liquor gave a second fraction (16.0 g, m.p.162-168 °C, additional yield 27%, HPLC >95%) as colourless crystals. Only the purer fraction was used in the next step.

3-Benzyloxy-cyclobutanecarboxylic acid (7): 3-Benzyloxy-cyclobutane-1,1-dicarboxylic acid (**6**) (10.10 g, 0.04 mol) was heated at 175 °C and 11 mbar for 1 hour and the decarboxylation product was then Kugelrohr distilled at 150-165 °C and 0.5 mbar to obtain

the product (7.8 g, yield 95%, Lit. yield[9]: 83%) as a mixture of the *cis*- and *trans*-isomers with a combined GC purity of >99%.

***cis*- and *trans*- 3-Benzyloxy-cyclobutanecarboxylic acid methyl ester (8a and 8b):** To a solution of **7** (7.80 g, 0.04 mol) in dry methanol (50 mL) thionyl chloride (3.0 mL, 40 mmol) was added drop wise at 0°C over a period of 10 min. Stirring was continued without cooling for 30 min. and the mixture was evaporated to give the product as a mixture of *cis*- and *trans*-isomers **8** (8.15 g, yield 97%, GC purity 99%) as an oil. The obtained yellow oil was then subjected to a preparative HPLC separation of the isomers using a radially compressed Waters preparative SiO_2-column (PrepPak module 1000 , 30×6 cm) and pentane/ ethylacetate as mobil phase. Batches of 1.0g could be separated into 370 mg *trans*-product (**8a**) and 300 mg *cis*-product (**8b**) each in 95-97% GC purity and a mixed fraction that was recycled. **8a**: ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 2.4-2.8 (m, 2 H), 2.7-2.9 (m, 2 H), 3.0-3.14 (m, 1 H), 3.7 (s, 3 H), 4.27-4.34 (q, 1 H), 4.43 (s, 2 H), 7.27-7.32 (m, 5 H). **8b**: ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 2.11-2.27 (m, 2 H), 2.34-2.64 (m, 3 H), 3.6 (s, 3 H), 3.81-3.95 (q, 1 H), 4.35 (s, 2 H), 7.19-7.27 (m, 5 H).

***trans*-3-(Phenylmethoxy)-cyclobutanecarbonyl chloride (9a): 8a** (1.0 g, 4.5 mmol) in ethanol (10 mL) was saponified using 1N-NaOH (5 mL) at reflux for 2 h. Ethanol was evaporated, the residue diluted with water (30 mL) and extracted with diethyl ether (50mL, discarded).The solution was then acidified at 0°C with conc. HCl and extracted with dichloromethane (4 × 50 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to afford the crude acid (830 mg, yield 93%) which was subsequently heated to reflux for 2 hrs. in thionyl chloride (10 mL) and DMF (5 drops). The volatiles were evaporated and the residue was Kugelrohr-distilled at 105-110 °C and 0.5 mbar to afford the product (0.86 g, yield 84%, GC purity 95% Lit. yield[8]: 99%) as an colourless oil. ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 2.28-2.43 (m, 2 H), 2.51-2.63 (m, 2 H), 3.03-3.17 (m, 1 H), 4.26-4.44 (q, 1 H), 4.45 (s, 2 H), 7.3-7.34 (m, 5 H).

***cis*-3-(Phenylmethoxy)-cyclobutanecarbonyl chloride (9b):** Was prepared following the procedure for the preparation of **9a**. ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 2.19-2.36 (m, 2 H), 2.46-2.60 (m, 2 H), 2.92-3.09 (m, 1 H), 3.82-3.96 (m, 1 H), 4.34 (s, 2 H), 7.19-7.28 (m, 5 H).

[17(*trans*)]-17-[(3-benzyloxycyclobutyl)carbonyl]-morphinan-3,14-diol (10): A mixture of norbutorphanol (**4**) (100 mg, 0.347 mmol) and diisopropylethylamine (50 mg, 0.387 mmol) in 10 mL of dry dichloromethane was cooled to 0-5 °C and a solution of **9a** in dry dichloromethane (86 mg, 0.387 mmol) was added drop wise under nitrogen. This mixture was stirred for 1 hour at the same temperature and then washed with water (10 mL), 2N HCl (10 mL) and 2N $NaHCO_3$ (10 mL). The organic phase was then dried (Na_2SO_4) and evaporated to

give the crude product (145 mg, yield 94%) as colourless oil which was used without purification in the next step.

[17(*trans*)]-17-[(3-benzyloxycyclobutyl)methyl]-morphinan-3,14-diol (11): A solution of **10** (145mg, 0. 324 mmol) in dry THF (10 mL) was cooled to 0 °C, B_2H_6.THF (278mg, 3.24 mmol, 1N solution) was added drop wise and stirred for 30 min. Then the reaction mixture was heated for two hours to reflux and the reaction was complete as monitored by TLC (dichloromethane/ methanol 5%).The reaction mixture was cooled to room temperature and methanol (5 mL) was added drop wise. Volatiles were evaporated and methanol (10 mL) was added and co-evaporated. This co-evaporation was repeated four times to give the crude product (140 mg) which was purified by column chromatography on SiO_2 utilizing dichloromethane/ methanol (98:2) as eluent to afford the anticipated product (75 mg, yield 53%) as colourless oil.

^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 0.90-0.97 (m, 1 H), 1.2-1.38 (m, 6 H), 1.57-1.64 (m, 1 H), 1.82-2.1 (m, 8 H), 2.25-2.73 (m, 2 H), 2.88-2.97 (m, 1 H), 3.53-3.63 (m, 1 H), 4.0-4.12 (m, 1 H), 4.02 (s, 2 H), 6.52-6.57 (dd, 1 H), 6.6-6.68 (m, 1 H), 6.83-6.87 (m, 1 H), 7.18-7.22 (m, 5 H), ^{13}C NMR (200 MHz, $CDCl_3$, 25 °C) δ = 21.69, 25.05, 25.86, 29.86, 30.24, 31.54, 32.88, 33.10, 36.95, 41.44, 44.50, 59.82, 61.61, 62.68, 69.98, 70.21, 72.04, 111.97, 113.20, 127.63, 127.86, 128.39, 138.13, 142.59, 154.75.

3-Benzoyl-17-(cyclobutylmethyl)-morphinan-14-ol (12): An ice-cold solution of butorphanol tartrate (5.00 g, 0.01mol) in deionized water (150 mL), was basified with 25% ammonia (5 mL) and the resulting suspension extracted with dichloromethane (6 × 70 mL) The organic phases were combined, dried (Na_2SO_4) and evaporated to afford the free base **1** (3.10 g) as a white powder. To the solution of this base in dry dichloromethane (180 mL), triethylamine (1.01 g, 10 mmol) dissolved in dry dichloromethane (10 mL) was added and benzoyl chloride (1.76 g, 12.52 mmol) in dry dichloromethane (10 mL) was added drop wise at 0- 5 °C and stirring continued at 5-10 °C for 2 hrs. The reaction mixture was washed with water (3 × 50 mL) and saturated Na_2CO_3 (2 × 50 mL). The organic phase was dried (Na_2SO_4) and evaporated to afford crude material (4.6 g, HPLC- purity 86%) which was chromatographed on SiO_2 (pentane/ ethyl acetate, 85:15) to afford the product (2.93 g, yield 66%, HPLC >99%) as a white powder. ^{1}H-NMR (200 MHz, DMSO, 25 °C) δ = 1.08-1.46 (m, 5 H), 1.58-2.26 (m, 10 H), 2.32-2.5 (m, 1 H), 2.74-3.21 (m, 4 H), 3.35 (s, 3 H), 5.94 (s, 1 H), 7.16-7.32 (m, 3 H), 7.55-7.74 (m, 3 H), 8.10-8.13 (m, 2 H), 8.95 (s, 1 H). ^{13}C NMR (200 MHz, DMSO, 25 °C) δ = 18.06, 20.07, 20.80, 24.42, 24.74, 25.33, 26.70, 28.38, 30.45, 33.41, 46.33, 57.27, 59.96, 68.48, 118.65, 119.86, 128.89, 129.04, 129.69, 131.48, 133.96, 140.62, 149.63, 164.62.

3-Benzoyl-morphinan-14-ol (13): To a solution of **12** (1.50 g, 3.5 mmol) in methanol (10 mL), 3-chloroperbenzoic acid (1.60 g, 6.96 mmol, 75%) was added in several portions at room temperature over a time period of 0.5 h. The reaction progress was monitored by TLC (dichloromethane/ methanol 5%) and reaction found to be complete after this time. Than an aqueous solution of $FeCl_2.4H_2O$ (0.69 g, 3.48 mmol, 10 ml) was added at 0 °C and stirring continued at room temperature for 2 h. Methanol was evaporated, the residue diluted with water (20 ml) and extracted with dichloromethane (4 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to afford the crude product as a brown oil which was purified on a SiO_2 column using dichloromethane/ 7 N NH_3 solution in methanol (95:5) as an eluent to afford the product as an amber oil (0.75 g, 60% yield, HPLC >99%). $C_{23}H_{25}NO_3$ (363.5): calcd. C 76.01 H 6.93 N 3.85; found C 76.21 H 7.01 N 3.87. ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 0.9-0.99 (m, 1 H), 1.09-1.47 (m, 6 H), 1.62-2.0 (m, 4 H), 2.55-2.68 (m, 2 H), 2.82-2.98 (m, 2 H), 3.16-3.45 (m, 1 H), 6.91-7.11 (m, 3 H), 7.38-7.6 (m, 3 H), 8.1-8.15 (m, 2 H), ^{13}C NMR (200 MHz, $CDCl_3$, 25 °C) δ = 20.12, 20.28, 29.02, 30.16, 33.49, 35.00, 36.50, 40.76, 54.45, 68.01, 117.23, 117.60, 126.89, 127.14, 128.31, 128.72, 132.10, 132.76, 141.53, 148.30, 163.91.

[17(*trans*)]-3-Benzoyl-17-[(3-benzyloxycyclobutyl)carbonyl]-morphinan-14-ol (14): A solution of **13** (0.79 g, 2.17 mmol) and diisopropylethylamine (0.31 g, 2.4 mmol) in dry dichloromethane (60 mL) was cooled to -5 to -10 °C and a solution of **9a** (0.54 g, 2.40 mmol) in dichloromethane was added drop wise under argon. This mixture was stirred for one hour at -10 °C and subsequently washed with water (60 mL), 2N HCl (100 mL) and saturated $NaHCO_3$ (100 mL). The organic phases was dried (Na_2SO_4) and rotoevaporated to give the product as a foam (1.19 g, yield 99%, HPLC >99%).

^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 0.92-0.99 (m, 1 H), 1.08-1.16 (m, 1 H), 1.25-1.71 (m, 5 H), 1.73-1.94 (m, 3 H), 2.04-2.34 (m, 4 H), 2.38-2.61 (m, 2 H), 2.71-2.86 (m, 2 H), 3.12-4.18 (m, 3 H), 4.03-4.21 (m, 1 H), 4.26-4.41 (m, 2 H), 4.66-4.78 (m, 1 H) 6.94-7.11 (m, 3 H), 7.24-7.26 (m, 5 H), 7.39-7.6 (m, 3 H), 8.1-8.14 (m, 2 H). ^{13}C NMR (200 MHz, $CDCl_3$, 25 °C) δ = 21.13, 29.54, 31.14, 32.15, 32.55, 32.77, 33.01, 34.91, 35.57, 39.32, 41.81, 52.61, 70.22, 71.17, 118.67, 119.62, 127.62, 127.81, 128.40, 128.57, 128.90, 129.57, 130.13, 132.86, 133.61, 138.17, 141.88, 149.99, 165.27, 174.98.

[17(*trans*)]-3-Benzoyl-17-[(3-hydroxycyclobutyl)carbonyl]-morphinan-14-ol (15): In a Parr apparatus Pd/C 10% (200 mg) was suspended in dry methanol (30 mL) and pre-hydrogenated at 40 psi at room temperature for 1 hour. A solution of **14** (1.12 g, 2.03 mmol) in dry methanol (80 mL) was added and the mixture hydrogenated at 40-50 psi at room temperature for 3 hours. Additional Pd/C (300 mg) 10% was added and the hydrogenation continued for another 14 h. until completion as monitored by TLC. The catalyst was filtered

using a pad of celite, washed with methanol and the filtrate evaporated to afford the product (0.75 mg, yield 80%, HPLC 97%) as colourless oil. ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 1.01-1.18 (m, 1 H), 1.33-1.62 (m, 5 H), 1.8-1.94 (m, 3 H), 2.16-2.28 (m, 2 H), 2.41-2.69 (m, 3 H), 2.8-2.94 (m, 3 H), 3.26-3.36 (m, 4 H), 3.69-3.72 (m, 1 H), 4.37-4.54 (m, 1 H), 4.76-4.79 (m, 1 H), 7.03-7.19 (m, 3 H), 7.47-7.68 (m, 3 H), 8.18-8.21 (m, 2 H), ^{13}C NMR (200 MHz, $CDCl_3$, 25 °C) δ = 21.11, 21.32, 30.47, 32.13, 33.05, 35.14, 35.61, 35.98, 39.38, 41.74, 52.67, 64.90, 70.11, 118.67, 119.60, 128.57, 128.91, 129.54, 130.12, 132.84, 133.60, 141.90, 149.97, 165.30, 175.09.

trans*-Hydroxybutorphanol (2):** ***Path 1: Pd/C-10% (50 mg) was suspended in dry methanol (15.0 mL) and pre-hydrogenated for 30 min with H_2 at 40 p.s.i. Then **11** (75 mg, 0.173 mmol) dissolved in dry methanol (15 mL) was added to the pre-hydrogenated catalyst and hydrogenated at 40 psi over night. TLC control (dichloromethane/ methanol 10%) showed complete conversion. The catalyst was filtered and washed with methanol. The filtrate was evaporated to give crude product that was chromatographed on a SiO_2 column (dichloromethane: methanol 94:6 to dichloromethane: methanol 88:12) to afford 18 mg of product **2** identical to the one obtained by path 2.

Path 2: To a solution of **15** (0.67 g, 1.44 mmol) in dry THF (40 mL) B_2H_6 .THF (0.40 g, 14.4 mmol, 14.4 mL of 1 N solution) was added drop wise at 0°C under argon, stirred for 30 min. at room temperature and heated to reflux for 12 hrs. Dry methanol (20 mL) was added to the cooled reaction mixture and volatiles were evaporated. Dry methanol (5 × 50 mL) was added and co-evaporated. The crude product (798 mg) was obtained as clear transparent oil that was purified by MPLC column chromatography (60 g SiO_2, dichloromethane: 7N NH_3 in methanol 98:2) affording product **2** (300 mg, yield 61%, HPLC >99%) as a white powder. $C_{21}H_{29}NO_3$ (343.5): calcd. C 71.56, H 8.58, N 3.90; found C 71.35, H 8.65, N 3.94. ^{1}H-NMR (200 MHz, DMSO, 25 °C) δ = 0.84-0.9 (m, 1 H), 1.25-1.44 (m, 5 H), 1.6-2.04 (m, 9 H), 2.17-2.6 (m, 5 H), 2.66-2.69 (m, 1 H), 2.89-2.98 (m, 1 H), 3.32 (s, 2 H), 4.13-4.26 (m, 2 H), 4.86-4.89 (m, 1 H), 6.48-6.53 (dd, 1 H), 6.6-6.61 (m, 1 H), 6.85-6.89 (m, 1 H), ^{13}C NMR (200 MHz, DMSO, 25 °C) δ = 21.43, 22.72, 24.40, 24.57, 29.73, 31.34, 35.68, 35.91, 36.60, 44.06, 59.00, 60.67, 64.35, 68.84, 111.25, 112.99, 126.21, 127.98, 141.78, 155.59.

[17(*cis*)]-3-Benzoyl-17-[(3-benzyloxycyclobutyl)carbonyl]-morphinan-14-ol (16): Following the procedure for the preparation of **14** but starting from **13** (0.243 g, 0.669 mmol) and **9b** (0.166 g, 0.74 mmol) the product **16** was obtained (0.356 g, yield 97%, HPLC 91%) as a white foam. ^{1}H-NMR (200 MHz, $CDCl_3$, 25 °C) δ = 0.92-0.98 (m, 1 H), 1.01-1.22 (m, 1 H), 1.30-1.53 (m, 5 H), 1.65-1.94 (m, 3 H), 2.00-2.47 (m, 6 H), 2.59-2.84 (m, 2 H), 3.16-3.67 (m, 2 H), 3.81-3.96 (m, 1 H), 4.31-4.37 (m, 2 H), 4.67-4.70 (m, 1 H), 6.94-7.01 (m, 3 H), 7.14-7.36 (m, 5 H), 7.38-7.62 (m, 3 H), 8.10-8.16 (m, 2 H), ^{13}C NMR (200 MHz, $CDCl_3$, 25 °C) δ

= 21.12, 28.97, 29.53, 29.86, 32.11, 33.02, 33.65, 33.88, 35.63, 39.27, 41.79, 52.71, 68.44, 69.81, 70.12, 118.64, 119.57, 127.59, 127.79, 128.34, 128.57, 129.56, 130.12, 132.95, 133.60, 138.16, 141.91, 149.97, 165.27, 174.04.

[17(*cis*)]-3-Benzoyl-17-[(3-hydroxycyclobutyl)carbonyl]-morphinan-14-ol (17): Following the procedure for the preparation of **15** but starting from **16** (0.69 g, 1.25 mmol) the product **17** was obtained (0.46 mg, yield 80%, HPLC 96%) as colourless oil. ^{1}H-NMR (200 MHz, DMSO, 25 °C) δ = 0.73-1.10 (m, 1 H), 1.11-1.58 (m, 5 H), 1.61-1.99 (m, 3 H), 2.06-2.24 (m, 2 H), 2.28- 2.65 (m, 3 H), 2.67-2.91 (m, 3 H), 3.13-3.49 (m, 4 H), 4.00-4.15 (m, 1 H), 4.70-4.74 (m, 1 H), 6.90-7.12 (m, 3 H), 7.39-7.60 (m, 3 H), 8.11-8.15 (m, 2 H). ^{13}C NMR (200 MHz, DMSO, 25 °C) δ = 21.11, 21.37, 28.09, 29.49, 32.13, 33.13, 35.61, 36.06, 37.60, 39.49, 41.67, 41.76, 52.84, 63.22, 70.01, 118.69, 128.57, 129.56, 130.13, 132.88, 133.60, 141.92, 149.98, 165.28, 174.49.

***cis*-Hydroxybutorphanol (3):** Following the procedure for the preparation of **2** (*path 2*) but starting from **17** (0.369 g, 0.80 mmol) the product **3** was obtained (0.176 g, yield 64%, HPLC >99%) as a white solid. $C_{21}H_{29}NO_3$ (343,5): calcd. C 73.44 H 8.51 N 4.08; found: C 73.24 H 8.50 N 4.02. ^{1}H-NMR (200 MHz, DMSO, 25 °C) δ = 0.83-0.89 (m, 1 H), 1.24-1.49 (m, 7 H), 1.60-2.03 (m, 6 H), 2.17-2.68 (m, 4 H), 2.89-2.97 (m, 1 H), 3.80-3.98 (m, 1 H), 4.26 (s, 1 H), 4.89-4.92 (m, 1 H), 6.49-6.62 (m, 2 H), 6.87-6.91 (m, 1 H), 8.98 (s, 1 H), ^{13}C NMR (200 MHz, DMSO, 25 °C) δ = 21.09, 21.43, 23.82, 24.31, 29.71, 31.37, 36.60, 38.19, 38.34, 40.77, 44.30, 60.32, 60.67, 62.36, 68.79, 111.24, 113.00, 126.27, 128.05, 141.80, 155.59.

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Synthesis of (+)- and (-)-8-Fluorogalanthamine

Petr Knesl, Ulrich Jordis and Behrooz H. Yousefi
Institute of Applied Synthetic Chemistry, Vienna University of Technology
Getreidemarkt 9, 1060 Vienna, Austria, ujordis@pop.tuwien.ac.at

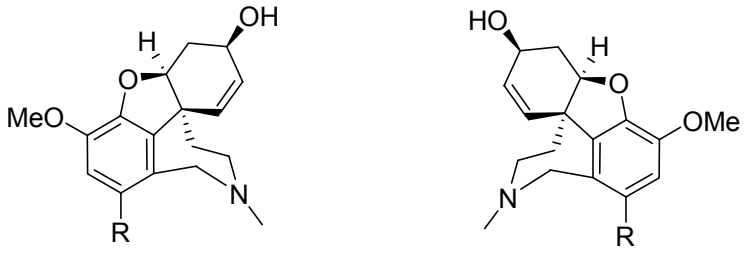
Abstract:

We report the synthesis of both enantiomers of 8-fluorogalanthamine [4aS-(4aα,6β,8aR*)]-4a,5,9,10,11,12-hexahydro-1-fluor-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol (**1)** and [4aR-(4aα,6β,8aS*)]-4a,5,9,10,11,12-hexahydro-1-fluor-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol (**2**).

Introduction:

Galanthamine (**3**), an Amaryllidaceae alkaloid, has been used clinically for the treatment of neurological illnesses such as myasthenia gravis[1] or poliomyelitis[2], as an anti-curare agent[3] and as a parasympathomimetic[4]. Later studies proved that Galanthamine acts as a selective, reversible and competitive acetylcholinesterase (AChE) inhibitor[5] as well as an allosteric ligand of nicotine acetylcholine receptors (nAChRs) [3]. It is an approved drug for the treatment of Alzheimer's disease. The synthesis and pharmacology of galanthamine have been reviewed recently and our previous approaches to this molecule and derivatives thereof are summarized therein[6]. Here we report the synthesis of both pure enantiomers of 8-fluorogalanthamine **1** and **2** using the phenolic oxidative coupling approach.

Fig. 1:



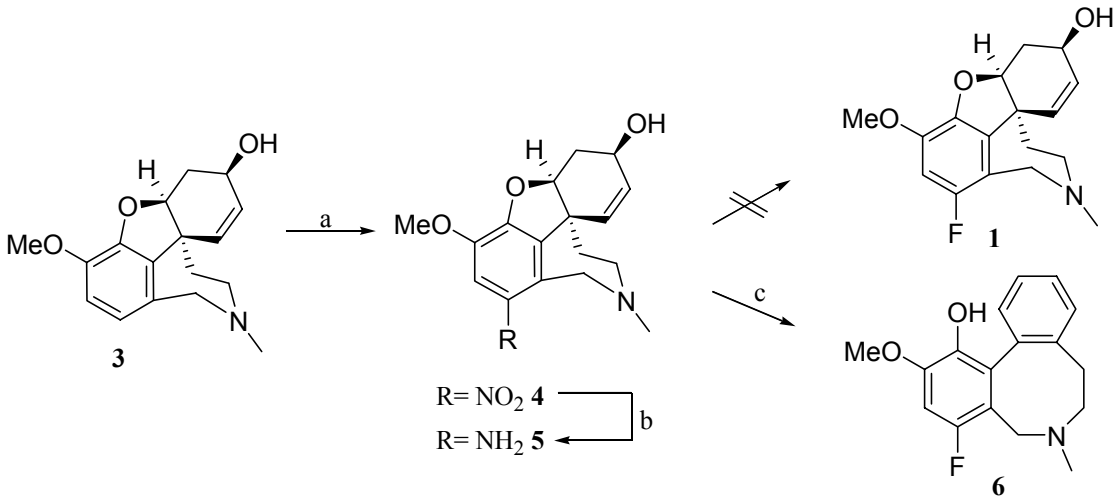
R= F **1**
R= H **3**

R= F **2**

Results and discussion:

Our effort to prepare enantiomerically pure (-) 8-fluorogalanthamin (**2**) started from (-) galanthamine (**3**) which was converted to the corresponding nitro derivate **4** by reacting with fuming HNO_3 in acetic acid media. Subsequently the nitro group was reduced to the amine **5** using sodium dithionit and we tried to achieve the final product via diazotation followed by reaction with HBF_4 in the presence of MgO. Instead of the expected (-) 8-fluorogalanthamine (**1**) we obtained the rearrangement product **6**. The structure of **6** was determined by ^{1}H-NMR, ^{13}C NMR, ^{13}C NMR DEPT, H-C COSY H-H COSY and F-NMR experiments *(Scheme 1)*.

Scheme 1:

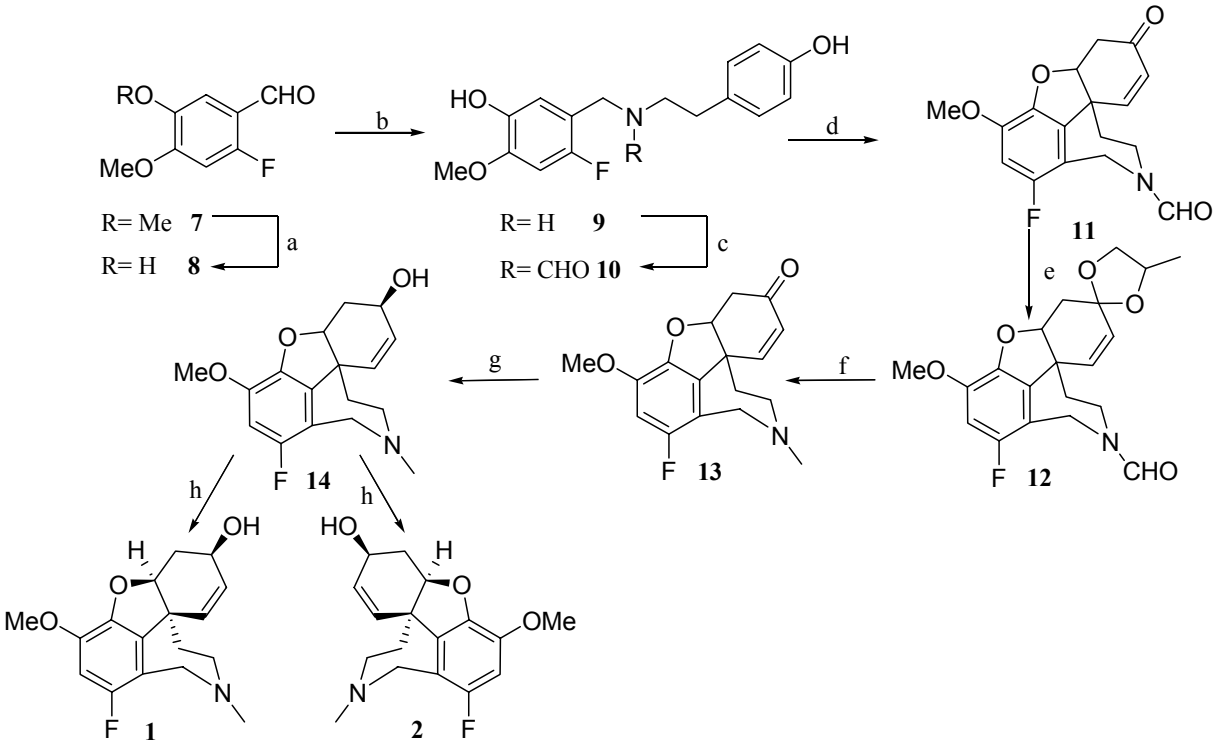


a) HNO_3 (fum.), AcOH b) Fe powder, NH_4Cl, c) $NaNO_2$, HBF_4, MgO, 170 °C

After this unsuccessful attempt to prepare **1** starting from **3** we decided to follow our previously developed method for the preparation of (-)-Galanthamine on the kilogram scale[7], based on the phenolic oxidative intramolecular coupling of **10**, thereby, enantioselectivity was achieved by a crystallization induced chiral conversion via Michael-retro-Michael addition on the stage of ($^{+}$/$_{-}$)-Narwedine. This approach started from 2-fluoro-4,5-dimethoxy benzaldehyde (**7**) which was selectively demethylated to the 2-fluoro-5-hydroxy-4-methoxy benzaldehyde (**8**) by a treatment with sulphuric acid. **8** was subsequently reacted with 4-hydroxy-phenylethyl amine and reduced to **9** with $NaBH_4$. By using ethyl formiate, DMF and formic acid we were able to prepare **10** in a 81% yield, however, the utilization of a mixture of acetic anhydride and formic acid allowed us to increase the yield in this step up to 93%. The key step of the synthesis, phenolic oxidative coupling of **10** to **11,** was carried out under the previously developed and optimized conditions to afford the intermediate **11** in a 40% yield. Our attempts to improve the yields by using detergents for a better phase transfer between aqueous and organic layer finished without significant success. In the next step the

present carbonyl group was protected by using 1,2-propanediol to afford **12** and subsequently the N-formyl group was reduced using $LiAlH_4$. Surprisingly a considerable loss of fluorine was observed, which is not fully explained. Moisture and presence of oxygen as reasons for this outcome could be excluded. Thus we obtained **13** in only 40% yield with HPLC purity of 98% after column chromatography as an colourless oil which was crystallized from ethanol. Based on the crystal structure of ($^{+}$/-) 8-fluoro-narwedine (**13**), it was found, that crystallisation-induced chiral conversion could not be achieved. Therefore we continued with the reduction of the carbonyl group by using L-selectride and obtained ($^{+}$/-) 8-fluorogalanthamine (**14**) in 90% yield and HPLC purity of >99%. ($^{+}$/-) 8-Fluorgalanthamine (**14**) was analyzed by chiral column chromatography (Chiracel I OD-H, 80% n-Heptan + 0.1% DEA/ 20% i-PrOH) and then separated by using chiral preparative column chromatography (Chiracel OD, 5µm, 5 x50 cm, 80% n-Heptan/ 20% i-PrOH) to afford the products **1** and **2** which were converted to the corresponding hydrobromid salts[8]. The optically more pure compound was analyzed by X-Ray analysis and the crystals found identical with the non-natural form of (+)-galanthamine regarding the absolute configuration of 4aR-6S-8aR. For both enantiomers optical rotation was recorded. Whereby, obtained values were similar to the values obtained for the corresponding enantiomers of galanthamine. For the better illustration of the crystal structure of (-)-8-Fluor-galanthamine (**1**) the recorded structure was converted computationally to the corresponding mirror image and is depicted together with the structure of ($^{+}$/-)-8-Fluoro-narwedine in *Fig.2*.

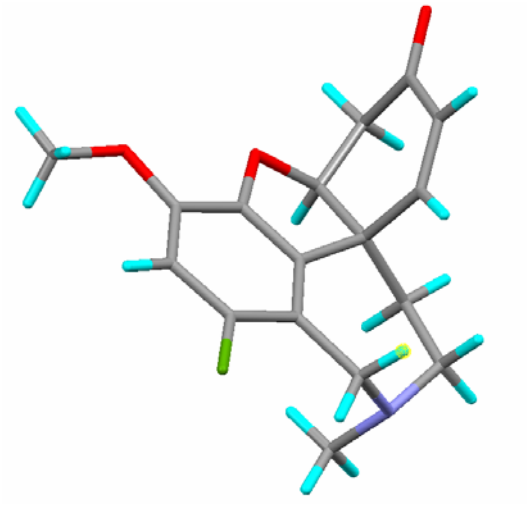
Scheme 2:



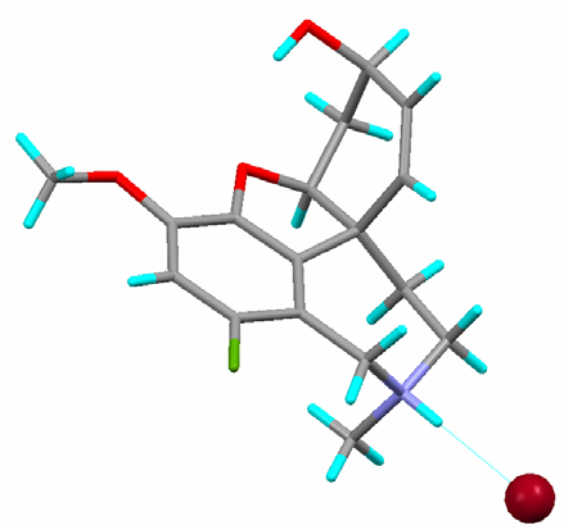
a) H_2SO_4, 90 °C b) 4-hydroxy-phenylethyl amine c) ethylformiate, formic acid d) $K_3[Fe(CN)_6]$, K_2CO_3 e) 1,2-propanediol, PTSA f) $LiAlH_4$, THF g) L-selectride, THF

Fig.2:

(+/-)-8-fluoro-narwedine(**10**)



(-)-8-fluoro-galanthamine (**2**)·HBr



Experimental part:

Melting points were measured on a Büchi B-545 melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$, DMSO or MeOD. For thin layer chromatography (TLC) Merck TLC aluminum sheets silica 60 F_{254} were used. Visualization was by UV light at 254 and 366 nm or spray reagents (molybdophosphoric acid and heating). MPLC (medium pressure liquid chromatography) was performed using silica gel (VWR 40-63 µm), a LC-8A pump (Shimadzu), a SPD-6AV UV-detector (Shimadzu) and Büchi preparative columns. HPLC was performed using a Waters 2695 instrument with Merck Chromolith RP_{18} columns and a gradient of 3 % to 60 % acetonitrile/water (0.1 % TFA) at a flow of 1.0 to 3.0 ml/min. The HPLC purity reported is the number generated for the peak area as calculated using the Waters Millennium software with the Maxplot option for the UV maximum of the corresponding peak.

(-)-8-Nitro-galanthamine (4):

To a solution of (-)-Galanthamine (1.0 g, 3.48 mmol) in glacial acetic acid (10 ml), a mixture of fuming HNO_3 (2 ml) and AcOH (8 ml) was added drop wise and the mixture was stirred at a temperature of 15-20 °C for 1h. followed by addition of a mixture of fuming HNO_3 (1 ml) and AcOH (1 ml). The reaction progress was followed by TLC ($CHCl_3$/MeOH 6:4) and found to be completed after 3h. Water (300 ml) was added to the reaction mixture, which was basified with NaOH (40% aqueous solution) to pH 12-14 and extracted with EtOAc (4x100

ml). Organic phases were collected washed with brine (1x100 ml), dried (Na_2SO_4) and decolorized (charcoal). Volatiles were rota evaporated to afford the product (1.12 g, 87%, HPLC 98%) as a yellow solid. ^{1}H-NMR ($CDCl_3$, 200 MHz) 1.58- 1.65 (m, 1H), 1.95-2.04 (m, 4H), 2.37 (s, 3H), 2.57- 3.20 (m, 4H), 3.80 (s, 3H), 3.89- 4.33 (m, 5 H), 4.63 (w, 1H), 5.93-6.10 (m, 2H), 7.29 (s, 1H); ^{13}C NMR ($CDCl_3$, 200 MHz) 29.75, 33.27, 48.55, 53.45, 54.26, 56.24, 61.40, 89.65, 109.03, 126.01, 128.50, 134.90, 143.21, 149.96

(-)-8-Amino-galanthamine (5):

To a solution of **4** (1.1 g, 3.3 mmol) in EtOH (20 ml), Fe powder (0.6 g, 20 mmol) and a solution of ammonium chloride (1.5 g, 27 mmol) in water (6 ml) was added and the reaction mixture was stirred and heated to reflux. The reaction progress was monitored by TLC ($CHCl_3$/MeOH 6:4) and the reaction was found to be completed after 16 h. The mixture was filtered and the obtained clear solution was concentrated under reduced pressure. Water (100 ml) was added and the aqueous solution was basified by using conc. ammonia to pH 12.After extraction with dichloromethane (5x100 ml) the organic layers were collected, washed with brine, dried (Na_2SO_4) and volatiles were rota evaporated to afford crude product (1.2 g) as an amber oil. The crude material was purified chromatographically by using $CHCl_3$/MeOH 75:25 as an eluent to afford the product (0.76 g, 76%, HPLC 98%) as a colourless oil.

4-Fluor-5,6,7,8-tetrahydro-2-methoxy-6-methyl-dibenz[c,e]azocin-1-ol (6):

To a solution of sodium nitrite (163 mg, 2.3 mmol) in water (2 ml) cooled to 0°C, an ice cooled suspension of **5** (0.7 g, 2.3 mmol) in HBF_4 (2 ml, 50% water solution) was added in three portions and this mixture was stirred without external cooling for additional 2 h. The mixture was than frozen and dried under high vacuum till dryness. MgO (2 g, 0.05 mol) was added and the mixture heated under occasional stirring on an oil bath at 170 °C for 2h under reduced pressure. After this time the reaction mixture was cooled, dissolved in HCl (10 ml, 1 N aqueous solution) and subsequently basified by using NaOH (10% aqueous solution) to pH 9 and extracted with $CHCl_3$ (3x100 ml). The organic layers were collected, washed with brine (50 ml), dried (Na_2SO_4), decolorized (charcoal) and volatiles were rota evaporated to afford crude product as an amber oil which was purified by column chromatography ($CHCl_3$/MeOH 95:5) to afford **6** (0,105 g, 17%, mp.: 207-208 °C, HPLC >99%). ^{1}H-NMR ($CDCl_3$, 400 MHz) 2.34-2.52 (m, 5H), 2.59- 2.69 (m, 2H), 3.07- 3.12 (m, 1H), 3.69- 3.88 (m, 5H), 6.63-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 400 MHz) 33.15, 45.78, 50.44, 56.74, 59.49, 98.92, 99.21, 126.04, 127.20, 129.19, 130.41, 130.66, 133.87, 138.62, 142.51, 146.36, 154.41, 156.78.

2-fluoro-5-hydroxy-4-methoxy benzaldehyde (8):

Sulfuric acid (50 ml, 95-98% p.a.) was stirred and heated to 90-95 °C under the stream of dry nitrogen followed by addition of 6-F-veratraldehyde (10.09 g, 54,81 mmol) in one portion. The reaction was followed by TLC (EtOAc/PE 3:7) and found to be finished after 3 h. The hot reaction mixture was poured on crushed ice (150 g), the formed white slurry was heated on the oil bad to 60-65 °C for 5 min. and cooled overnight in the fridge. The obtained precipitate was filtered off over a glass frit and the water was extracted with methylenechloride (3x150 ml). The wet product was dried in the desiccator to give (8.8 g, 82%, HPLC 94%, m.p.: 146.1-146.5, Lit. m.p.[8]: 133-136 °C) the product as a white powder.

4-Fluoro-5-[[[2-(4-hydroxyphenyl)ethyl]amino]methyl]-2-methoxy-phenol (9):

A solution of **8** (7.6 g, 45 mmol) and tyramine (6.7 g, 49 mmol, Aldrich 99%) in dry toluene (250 ml) and n-Butanol (250 ml) was heated and stirred under reflux utilizing a dean-stark trap. Reaction progress was monitored by TLC (MeOH:CH_2Cl_2 1:9) and reaction found to be completed after 5h. Solvents were evaporated and the residue was dissolved in dry MeOH (500 ml). $NaBH_4$ (1.8 g, 45 mmol) was added at a temperature of 0-5 °C and the resulting mixture was stirred overnight while the temperature rised to room temperature and a white solid precipitated from the reaction mixture. The obtained solid was filtered on the sintered glass of funnel and washed with cold methanol (2x50 ml). The white wet cake was dried in the desiccator under reduced pressure to give the product (9.6 g, 74%, HPLC >99%) as a white powder. The filtrate was rota evaporated to give a brown slurry (3.6 g), which was chromatographed on the silica (dichloromethane/methanol, gradient 0-10%) to give another portion (2.5 g, 19%, HPLC >99%) of product as a off white powder (total yield 93%, m.p.: 160-162 °C). *^{1}H-NMR (MeOD, 200 MHz):* 2.69 (s, broad, 4H), 3.66 (s, 2H), 3.80 (s, 3H), 6.66-6.77 (m, 4H), 6.96-7.00 (m, 2H)

N-[(2-fluoro-5-hydroxy-4-methoxyphenyl)methyl]-N-[2-(4-hydroxyphenyl)ethyl]-Formamide (10):

A mixture of acetic anhydride (9.3 ml) and formic acid (4.00 ml) was stirred and heated to 60 °C for 1 h. After this time the mixture was cooled to room temperature, added to **9** (9.0 g, 3.4 mmol) and stirred at 0-5 °C. The reaction was followed by TLC (dichloromethane/ methanol 97:3) and found to be completed after 10-15 min. The reaction mixture was poured into water (150 ml) and extracted with dichloromethane (3x100 ml). Organic solvents were collected and washed with $NaHCO_3$ (3x50 ml) and brine (1x50 ml), decolorized (charcoal), dried (Na_2SO_4) and volatiles were rota evaporated to give **10** (9.1g, 92%, HPLC 98% for both rotamers, m.p.: 153-168 °C) as a white powder. *^{1}H-NMR (DMSO, 200 MHz):* 2.49-2.67 (m,

2H), 3.15-3.29 (m, 2H), 3.75 (s, 3H), 4.28-4.35 (d, 2H, J_2= 13.89 Hz), 6.64-6.95 (m, 6H), 7.84 (s, 0.5 H), 8.20 (s, 0.5 H), 8.95-9.00 (d, 1H, 10.17 Hz), 9.18-9.20 (d, 1H, J= 2.44 Hz)

4α,5,9,10,11,12-hexahydro-1-fluoro-3-methoxy-11-formyl-6H-benzofuro[3a,3,2-ef] benzazepine-6-one (11):

The vigorously stirred mixture of potassium carbonate (19 g, 0.14 mol) and potassium hexacyanoferrate (40 g, 0.12 mol) in toluene (800 ml) and water (180 ml) was heated to 50 °C and fine pulverized **10** (7.91 g, 24.8 mmol) was added in one portion. This suspension was kept at 50-60 °C and intensively stirred for 1 h. After this time the reaction mixture was filtered trough a pad of celite. The organic was separated and the aqueous layer was extracted with toluene (2x200 ml). The organic phases were collected, dried (Na_2SO_4) and evaporated under reduced pressure to afford product (3.2 g, 40%, HPLC 99%) as a white foam.
^{1}H-NMR (DMSO, 200 MHz): 1.75-1.93 (m, 1H), 2.15-2.30 (m, 1H), 2.73-2.83 (m, 1H), 3.00-3.12 (m, 1H), 3.40 (s, 4H), 3.98-4.13 (m, 1H), 4.28-4.35 (m, 0.5 H), 4.51-4.97 (m, 2H), 5.27-5.34 (d, 0.5 H, J= 15,45 Hz), 5.94-6.00 (d, 1H, J= 10.37 Hz), 6.77-6.86 (m, 1H), 7.15-7.26 (m, 1H), 8.10-8.15 (d, 1H, J= 8.99 Hz)
^{13}C NMR (DMSO, 200 MHz): 34.02, 37.21, 37.32, 45.45, 49.33, 49.53, 56.05, 87.29, 100.20, 100.34, 100.77, 100.90, 114.55, 114.93, 115.08, 126.66, 130.83, 130.93, 143.12, 143.43, 143.64, 143.76, 144.29, 144.52, 162.39, 162.62, 194.77

4α,5,9,10,11,12-hexahydro-1-fluoro-3-methoxy-11-formyl-6H-benzofuro[3a,3,2-ef] benzazepine-6-(1,2-propanediol)-ketal (12):

To a solution of **11** (3.05 g, 9.61 mmol) in toluene (200 ml) a solution of PTSA (0.1 g, 0.58 mmol) in propylene glycol (2 ml, 27.6 mmol) was added and the resulting mixture heated to reflux for 1 h while the water was removed. Another solution of PTSA (0.05 g, 0.29 mmol) dissolved in propylene glycol (1.5 ml, 20.8 mmol) was added and the mixture heated to reflux. The reaction was followed by TLC (CH_2Cl_2/MeOH 98:2) and after additional 3 h. completed. The reaction was cooled to room temperature and washed with acetic acid (2x50 ml, 10% in water), sodium hydrogen carbonate (2x50 ml, water solution) and brine (1x50 ml). The organic layer was dried (Na_2SO_4) and evaporated to give a crude product (3.2 g) as an amber oil. This was crystallized using i-Propanol to afford the product (2.6 g, 72%, HPLC 98%) as colourless crystals. *^{1}H-NMR ($CDCl_3$, 200 MHz):* 0.74-2.66 (m, 10 H), 2.98-4.86 (m, 8 H), 5.44-5.74 (m, 1H), 6.34-6.39 (m, 1H), 7.98-8.03 (m, 1H), ^{13}C NMR ($CDCl_3$, 200 MHz): 33.42, 37.22, 43.18, 49.43, 51.95, 54.13, 56.20, 88.12, 99.98-100.58 (d), 114.98-115.34 (d), 127.31, 131.33-131.44 (d), 142.84-142.89 (d), 143.51-143.71 (d), 144.11, 152.42-157.18 (d), 194.14

(+/-)-8-Fluoro-narwedine (13):

To a solution of **12** (3.38 g, 9.00 mmol) in dry THF (50 ml) a suspension of Lithium aluminium hydride (8.0 ml, 16.0 mmol, 2 N solution in THF) was added at -5-0 °C and this mixture was stirred for 1 h. under a stream of dry nitrogen. Reaction development was controlled by HPLC and no starting material was detected after this time. The reaction mixture was quenched with Water/THF 1:1 (50 ml) and volatiles removed under reduced pressure. The residue was dissolved in 2N-HCl (25 ml) and stirred at room temperature for 30 min. The clear solution was basified using conc. ammonia to pH 12 and extracted with ethylacetate (4x75 ml). The organic phases were collected, dried (Na_2SO_4), decolorized (charcoal) and rota evaporated to give the crude product (2.8 g) as an amber oil which was chromatographed by using CH_2Cl_2/MeOH (gradient 0-15% of MeOH) to give the product (1.1 g, 40% yield, HPLC 97%) as an colourless oil. After crystallization from Ethanol the product was obtained (0.65 mg, 24%, HPLC >99%) as a crystalline solid from which X-Ray was recorded. *^{1}H-NMR ($CDCl_3$, 200 MHz):* 1.77-1.84 (m, 1H), 2.09-2.24 (m, 1H), 2.38 (s, 3H), 2.60-2.71 (m, 1H), 2.98-3.11 (m, 3H), 3.64-3.72 (m, 4H), 4.03-4.11 (d, 1H, J= 15.65 Hz), 4.65 (s, 1H), 5.93-5.98 (d, 1H, J= 10.56 Hz), 6.40-6.46 (d, 1H, J= 11.34 Hz), 6.84-6.89 (m, 1H), *^{13}C NMR ($CDCl_3$, 200 MHz):* 33.42, 37.22, 43.18, 49.43, 51.95, 54.13, 56.20, 88.12, 99.98-100.58 (d), 114.98-115.34 (d), 127.31, 131.33-131.44 (d), 142.84-142.89 (d), 143.51-143.71 (d), 144.11, 152.42-157.18 (d), 194.14

(+/-)-8-F-galanthamine (14):

To a solution of (+/-) Narwedine (320 mg, 1.05 mmol) in abs. THF (20 ml) L-Selectride (1.6 ml, 1 M solution in THF) was added drop wise at a temperature of -5-0 °C. The resulting mixture was stirred under the stream of dry nitrogen while the temperature was kept at 0 °C. The reaction was monitored by TLC (CH2Cl2/MeOH 97:3) and found to be finished after 30 min. The solution was quenched with a Water/THF mixture 2:1 (30 ml) and volatiles were removed under reduced pressure. The residue was dissolved in HCl (15 ml, 2N aqueous solution) and washed with diethyl ether (3x25 ml). The aqueous solution was basified by using conc. ammonia to pH 12 and extracted with ethyl acetate (4x50 ml). The organic layers were collected, washed with brine (30 ml), dried (Na_2SO_4) and rota evaporated to afford the crude product (310 mg) as an yellowish oil. The crude product was purified by column chromatography (isocratic, using EtOAc as a solvent) to afford **14** (290 mg, 90%, HPLC >99%) as a white foam. The mixture of (+)- and (-)-8-Fluorogalanthamin was analyzed by chiral LC-chromatography (Chiracel I OD-H, 80% n-Heptan + 0.1% DEA/ 20% i-PrOH) showing the ratio for both enantiomers of 1:1. *^{1}H-NMR ($CDCl_3$, 400 MHz):* 1.25 (s, 1H), 1.55-1.67 (m, 1 H), 1.92-2.10 (m, 2 H), 2.41 (s, 4H), 2.62-2.70 (m, 1H), 2.98-3.29 (m, 2H),

3.72-3.78 (d, 1H), 3.81 (s, 3H), 4.07-4.20 (m, 2H), 4.60 (s, 1H), 6.03 (s, 2H), 6.47-6.49 (d, 1H), *^{13}C NMR* (*$CDCl_3$, 400 MHz):* 30.11, 30.27, 34.31, 43.10, 49.21, 52.15, 54.32, 56.55, 62.37, 89.29, 99.86, 100.16, 126.89, 128.53, 134.25, 134.30, 142.09, 144.23, 144.33, 154.31, 156.69

(+)-8-F-galanthamin (2) and (-)-8-F-galanthamin (1) and their HBr Salts :

The mixture of ($^{+}$/$_{-}$)-8-Fluoro-galanthamine (290 mg) was separated on the chiral preparative HPLC system (Chiracel OD, 5x50 cm, 80% n-Heptan/ 20% i-PrOH) to afford two fractions. 52 mg, 34%, HPLC >99%, EE 92.7% (**2**), and 123 mg, 85%, HPLC >99%, EE 77.8% (**1**). Both of this fractions were dissolved in abs. Ethanol (1.5 ml), conc. HBr (60 µl for **2** and 130 µl for **1**) was added and this mixture stirred for 0.5 h. at the room temperature. Ethyl acetate was added (5 ml) and the mixtures were allowed to crystallized in the fridge overnight to give the **2**.HBr (32 mg, 49%) as a white crystalline material and **1.**HBr (82 mg, 49%, α [D, 20]= -72.40) as a white crystalline material). The fraction with higher EE was recrystallized from DMF to give white crystals suitable for X-Ray analysis.

Conclusion:

In conclusion, we have proved, that the enantiomeric mixture of **13** does not crystallize as a conglomerate. Therefore, there is no place for utilization of crystallization induced resolution of enantiomeric mixtures at this stage of the synthesis. The compounds **1** and **2** were finally obtained in the mixture and separated using chiral preparative chromatography. The absolute configurations of the obtained enantiomers were confirmed by the X-ray analysis.

Acknowledgement:

We would like to thank to the Institute of Chemical Technology and Analysis of Technical University of Vienna , especially to the Ao. Univ. Prof. Dr. phil. Kurt Mereiter for his valuable helps with crystallization and X-ray structure analysis.

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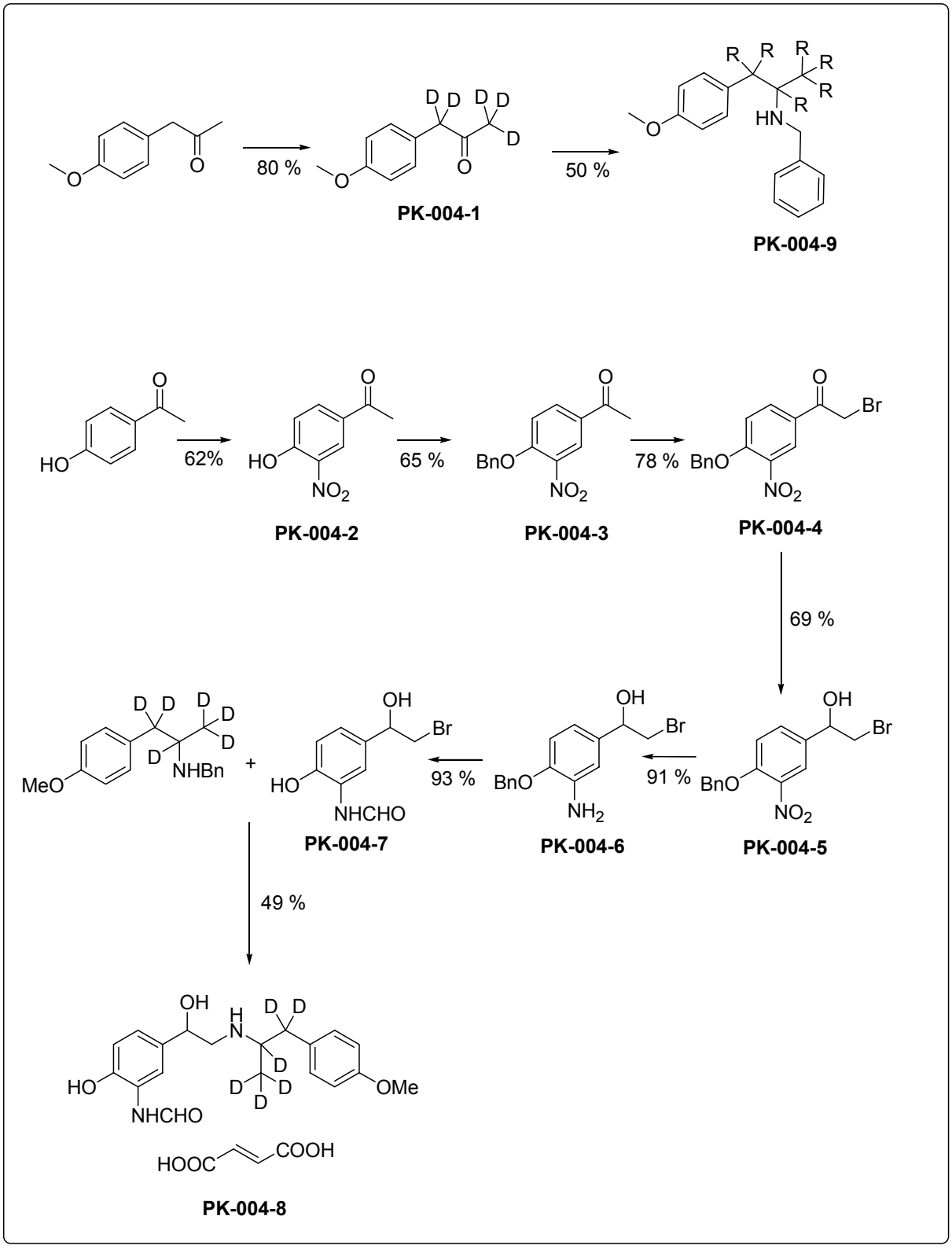
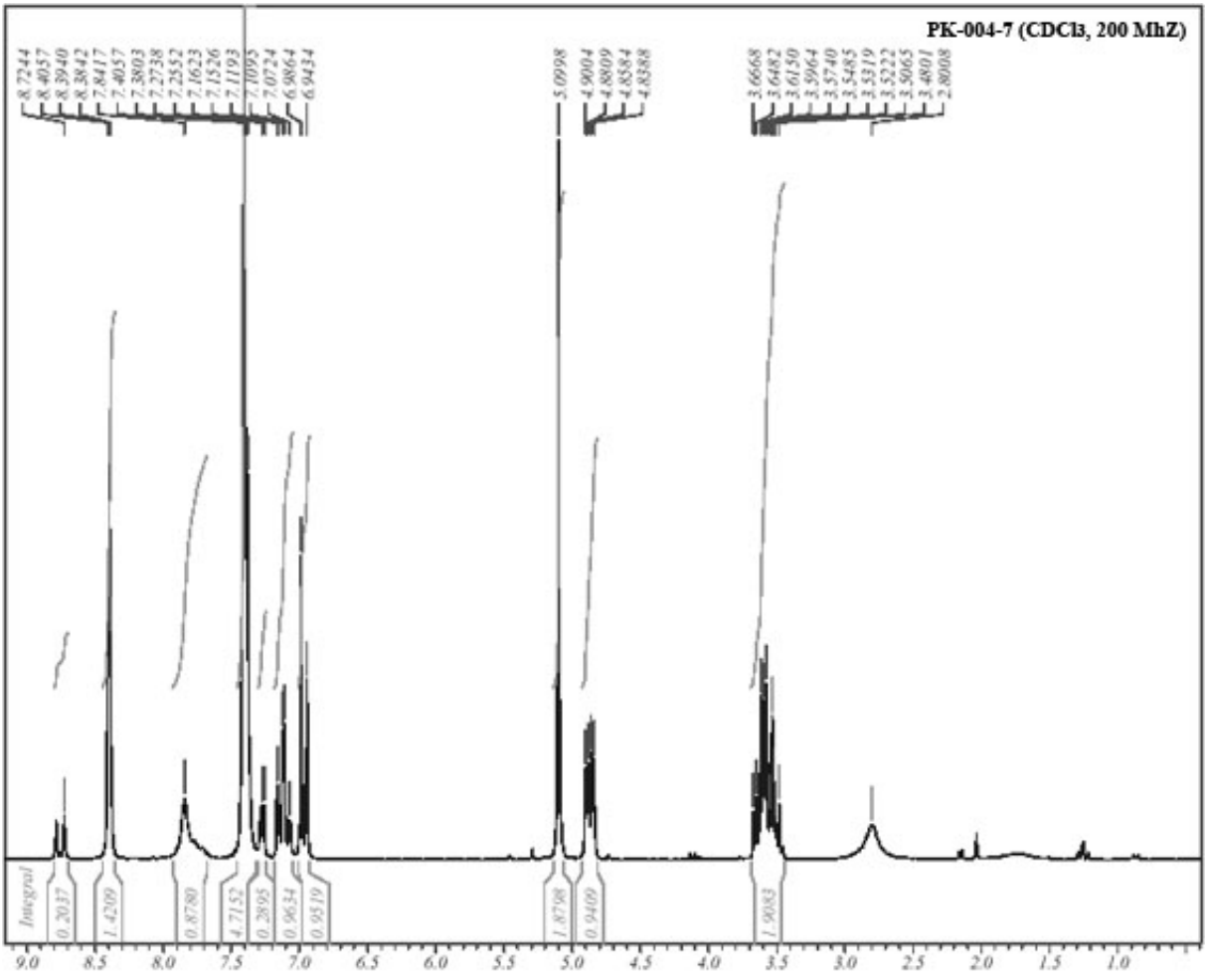
Synthesis of d_6-formoterol for use as internal standard in a LC/MS/MS method developed for quantification of the drug in human serum.

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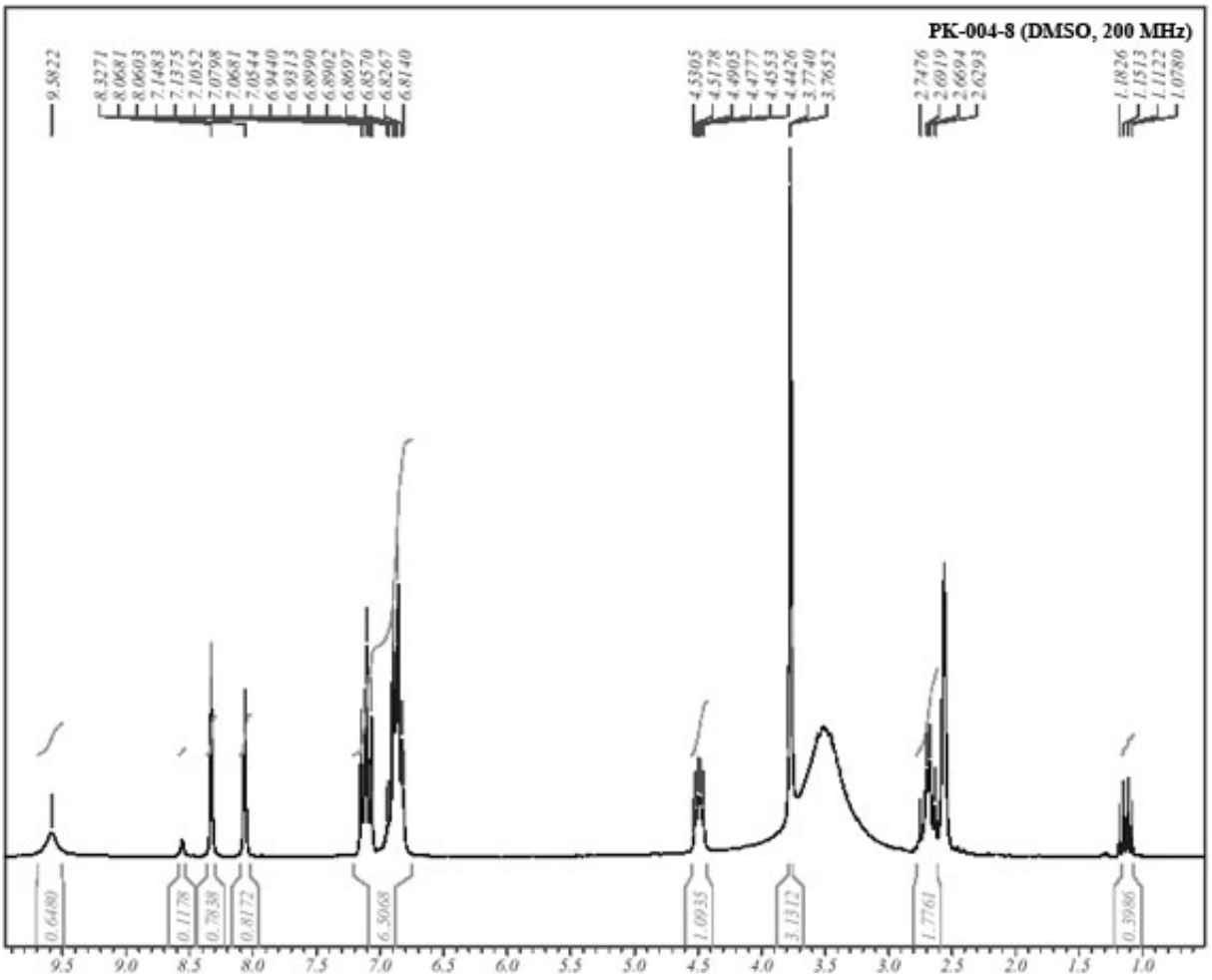
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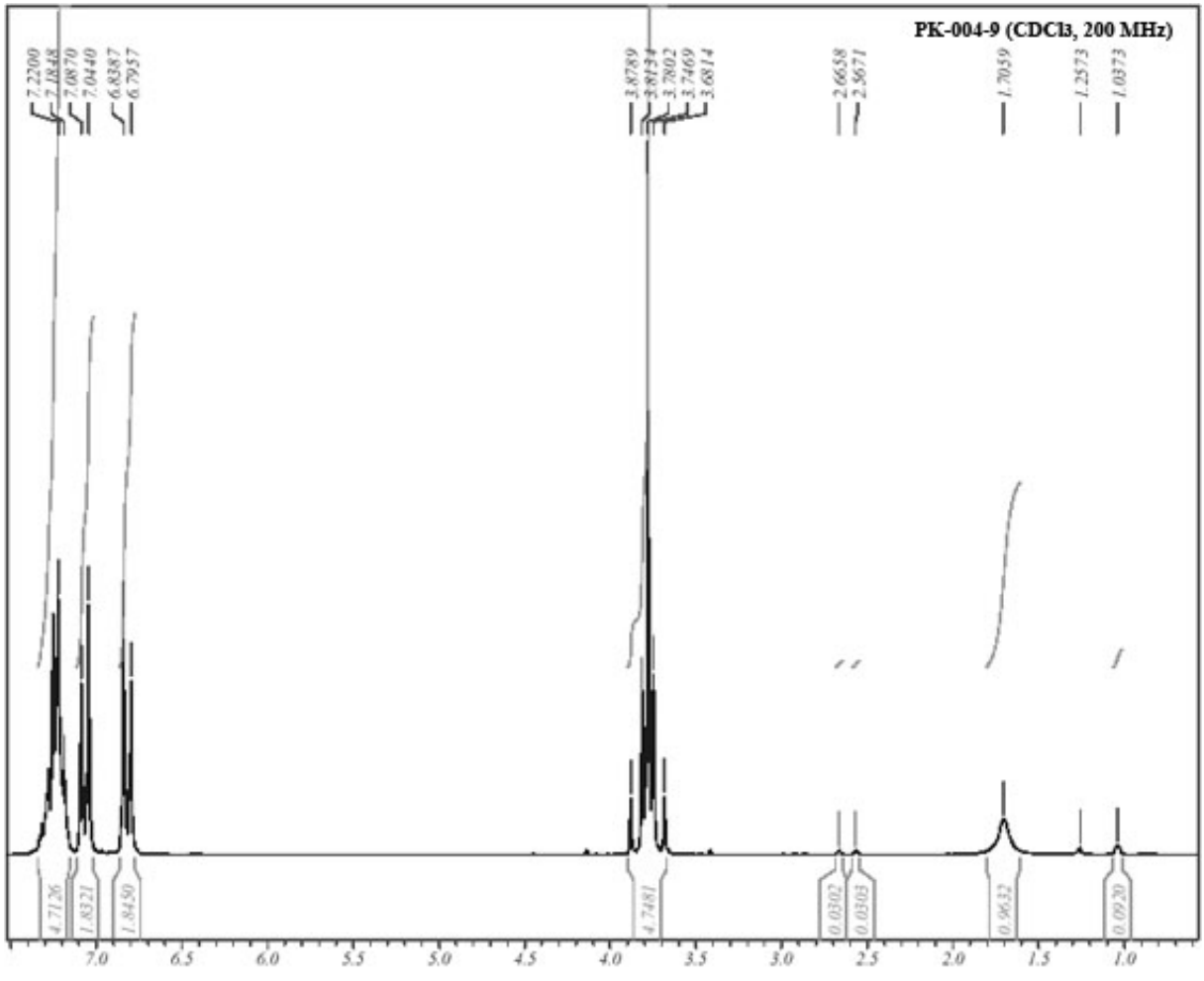
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2.0
1.5
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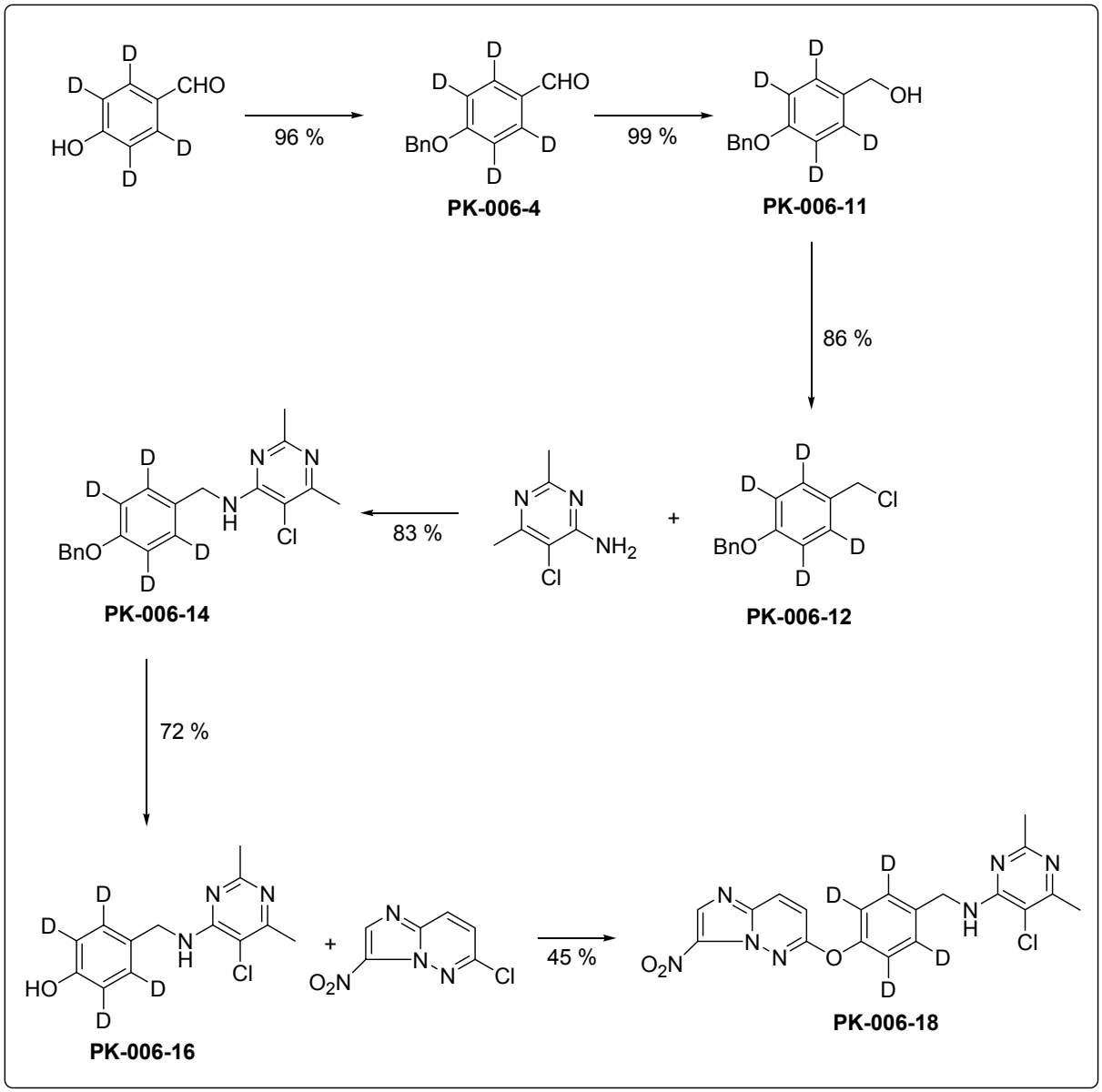
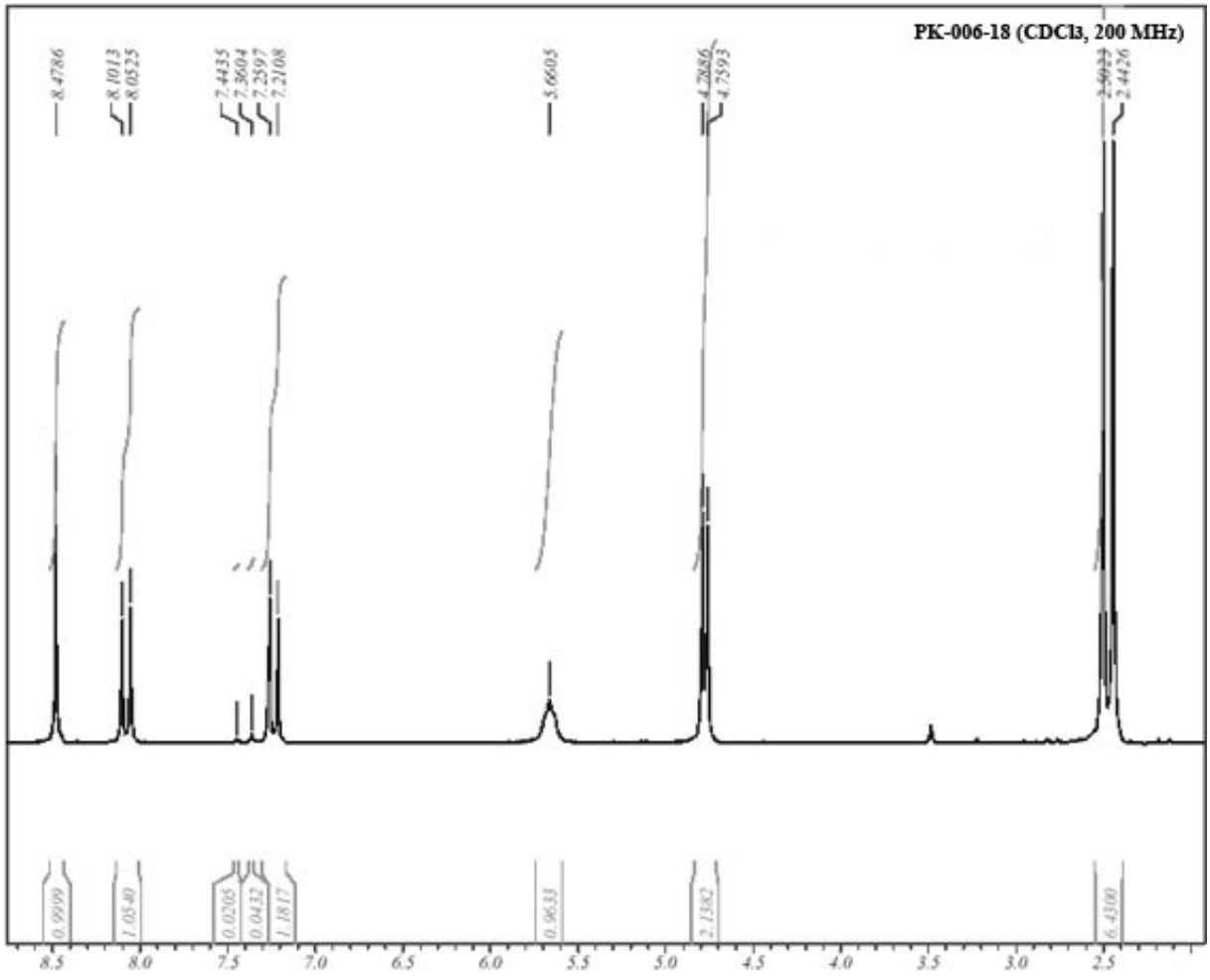
Synthesis of d_4 BY-170424 (5-chloro-2,6-dimethyl-N-[[4-[(3-nitroimidazo [1,2-b]pyridazin-6-yl)oxy]-2,3,5,6-d4-phenyl]methyl]-4-pyrimidinamine) as internal standard for LC/MS/MS determination in human serum.

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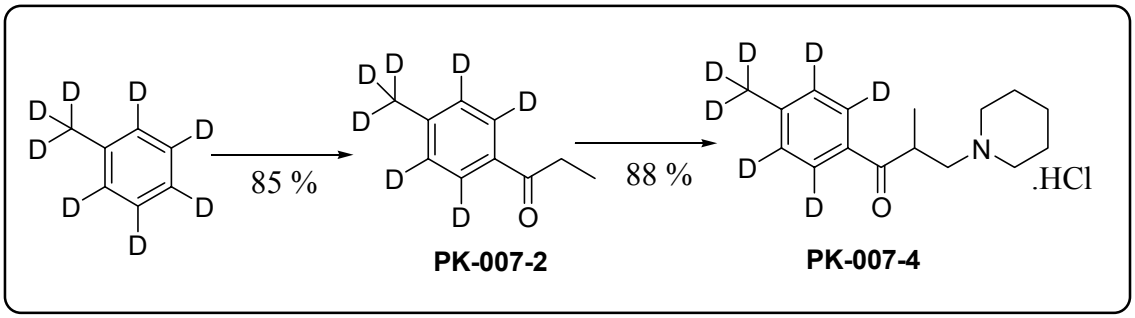
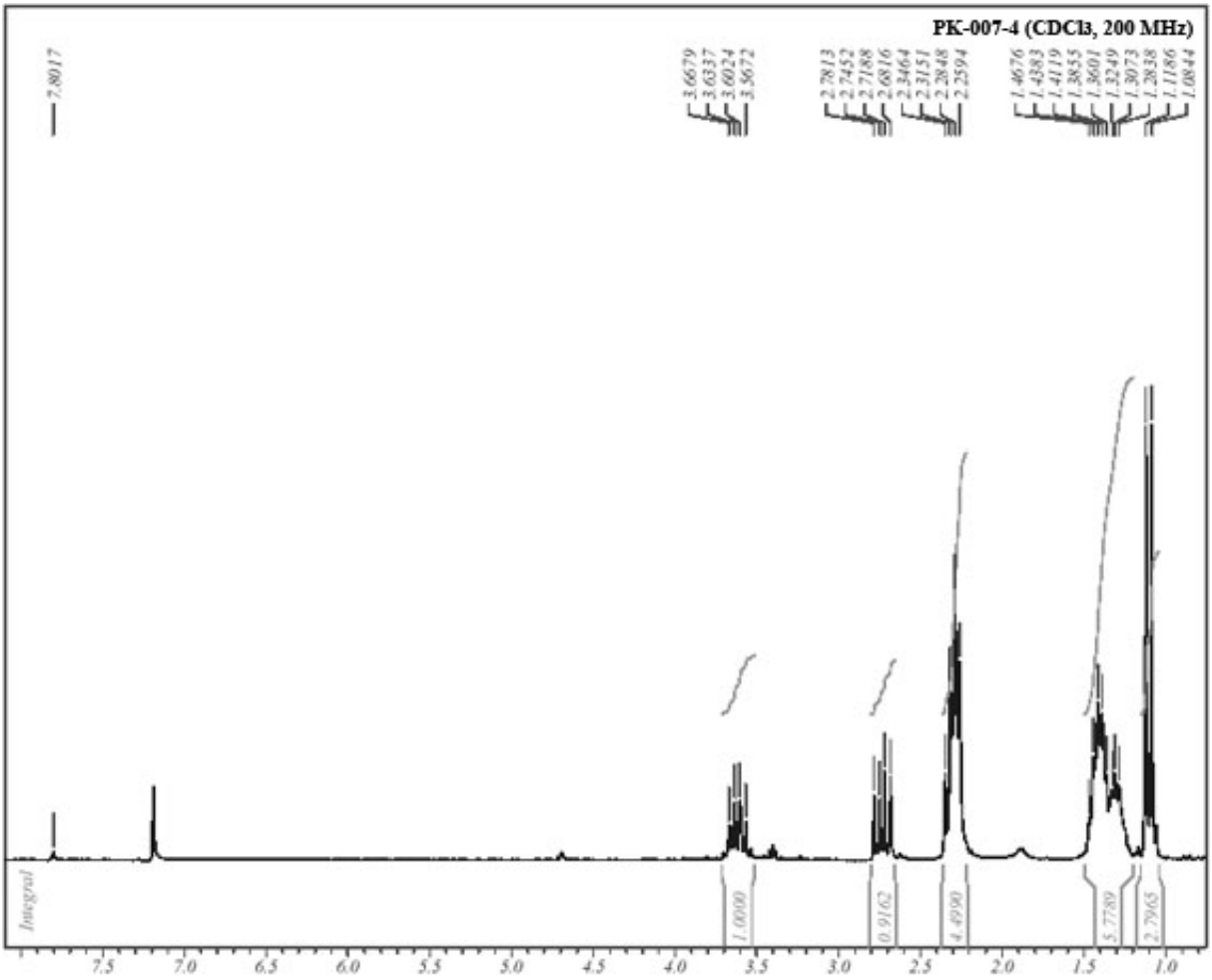
2.5

Synthesis of d_7-tolperisone for use as LC/MS/MS standard.

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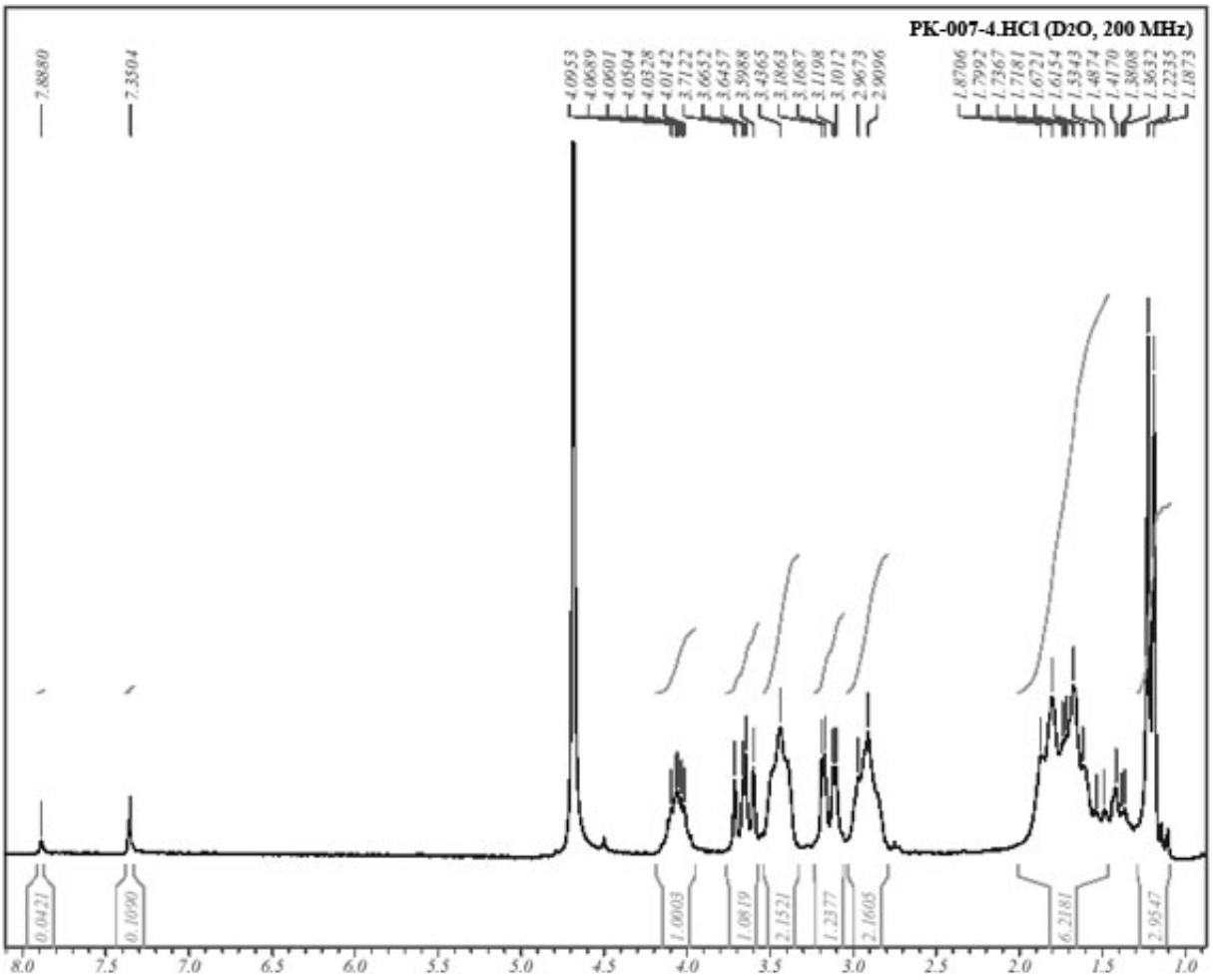
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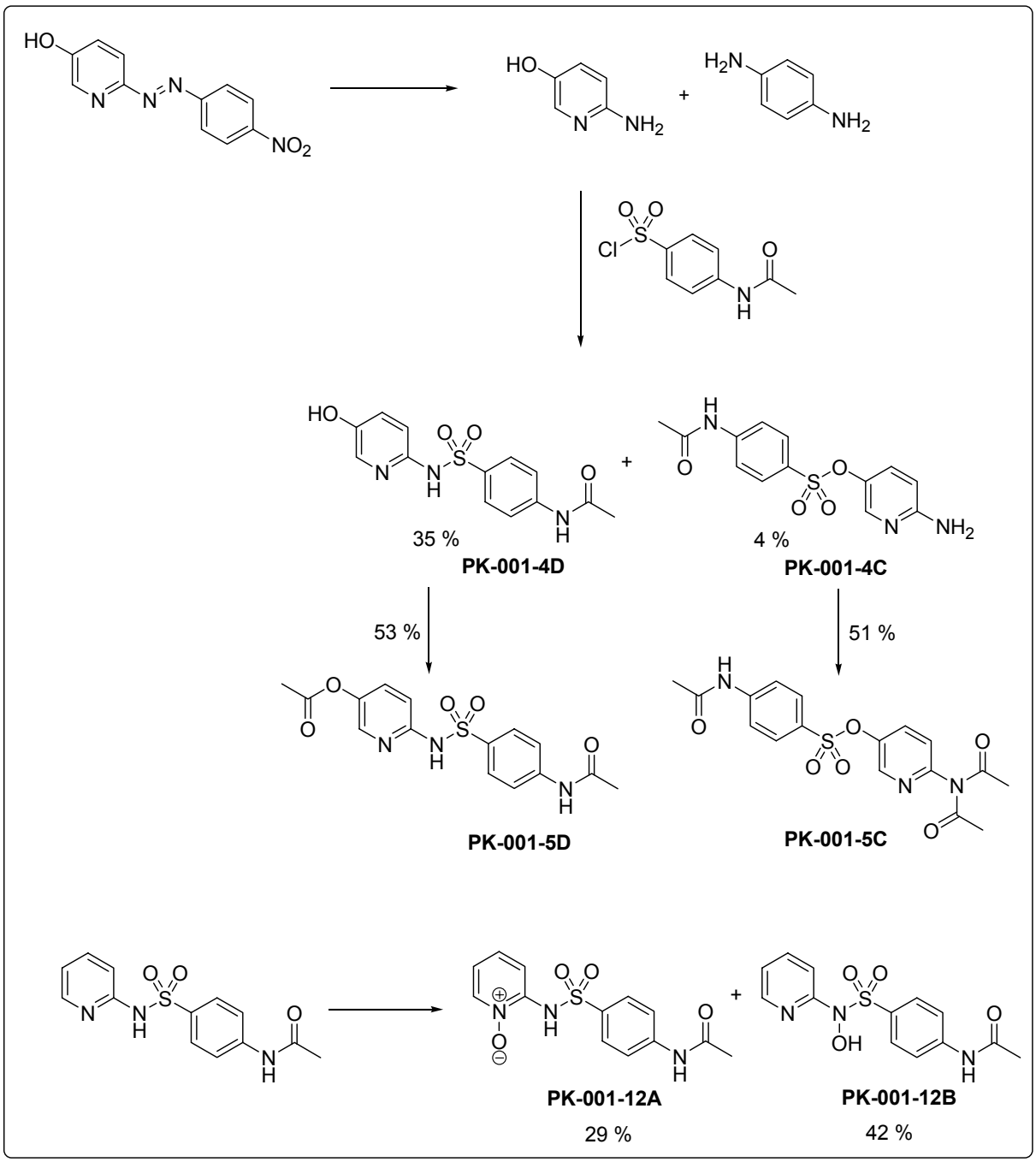
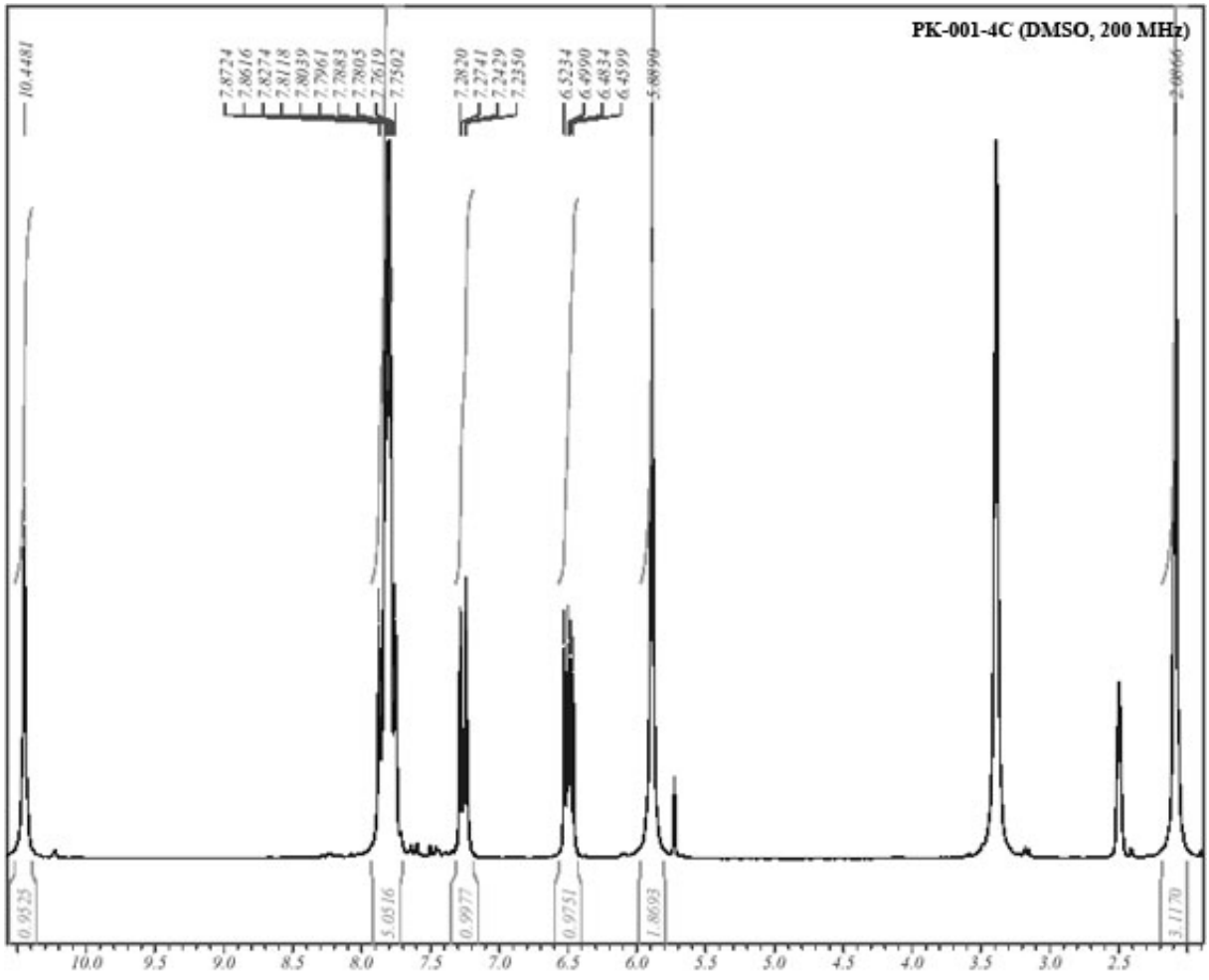
Synthesis of Sulfapyridine metabolites.

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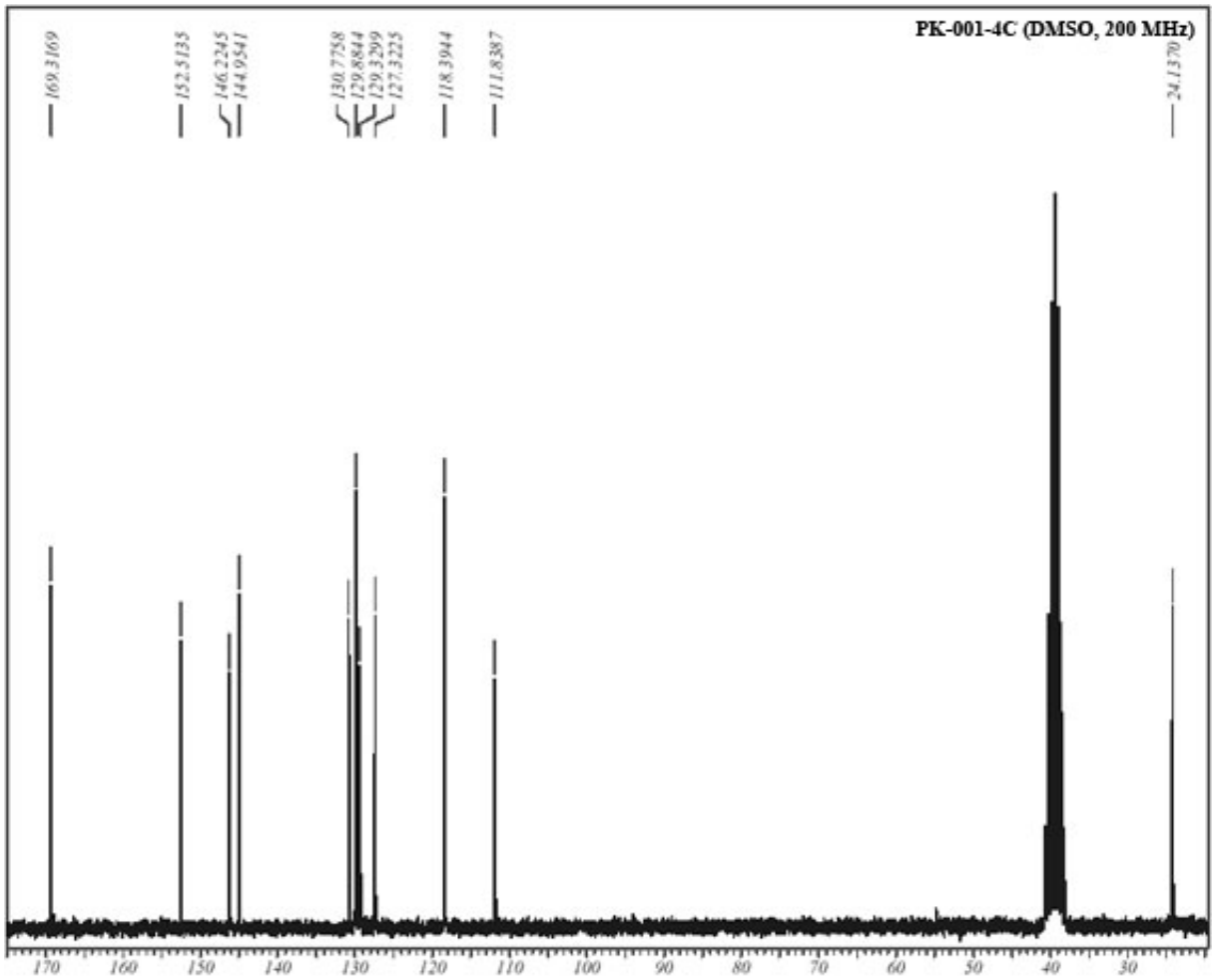
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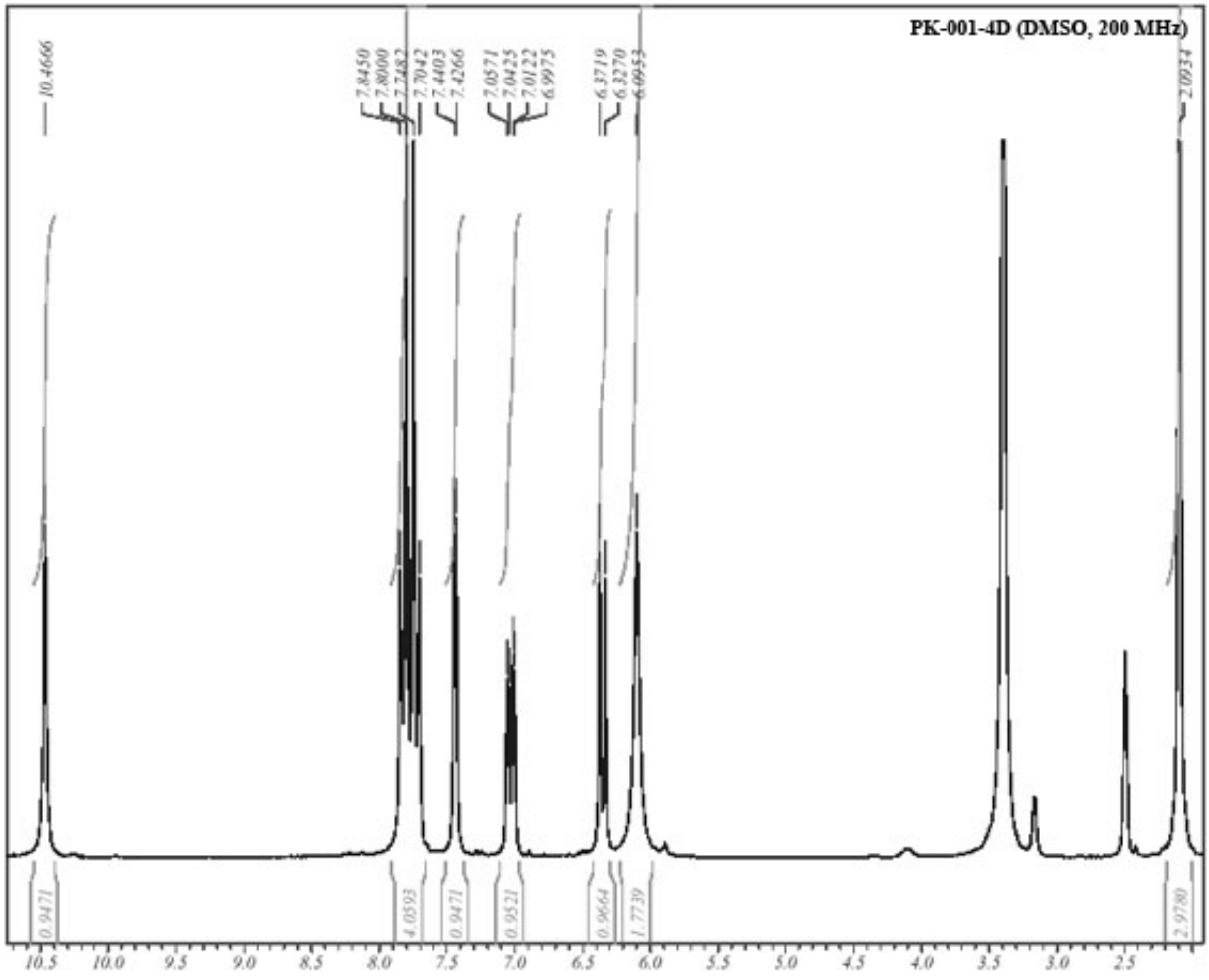
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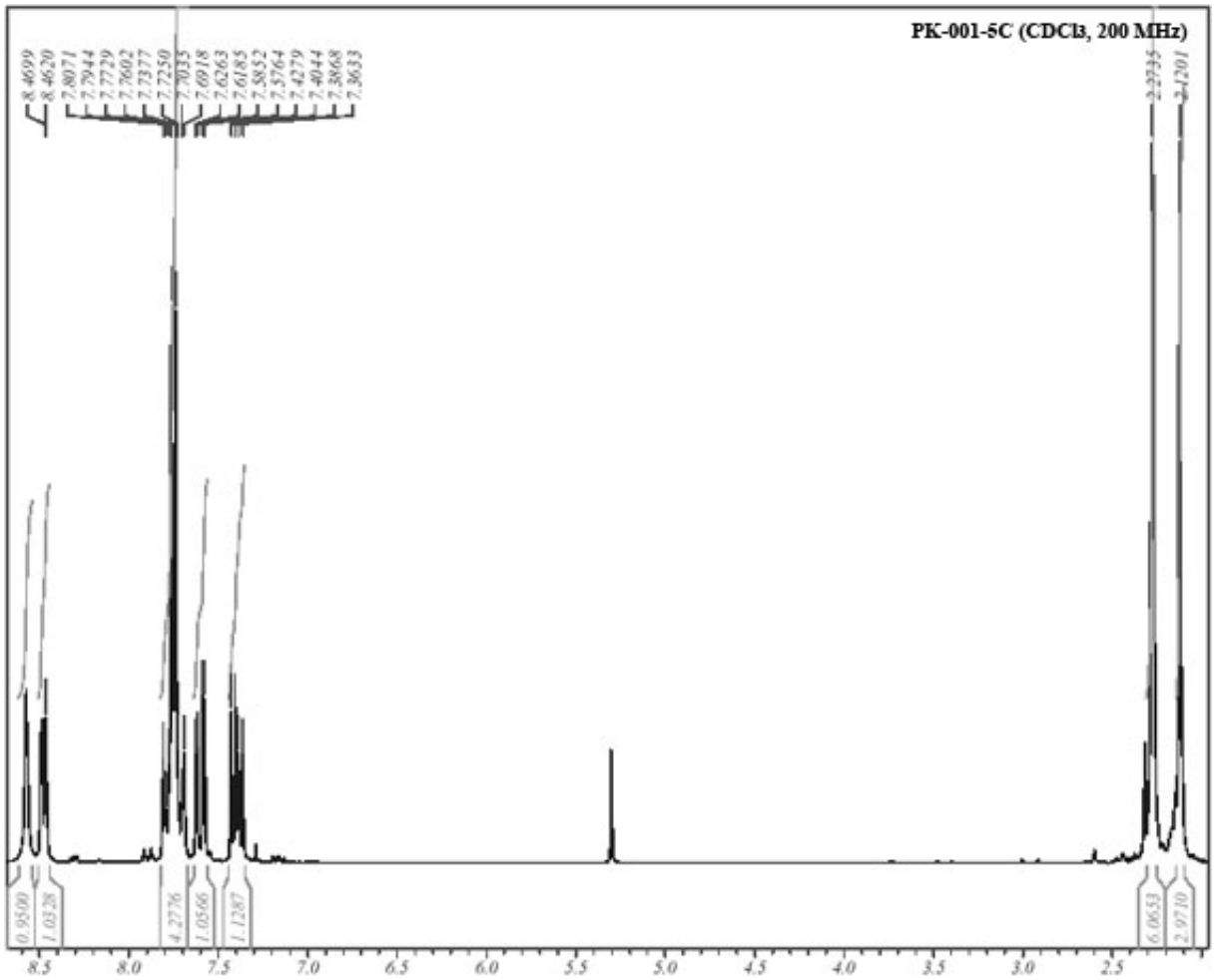
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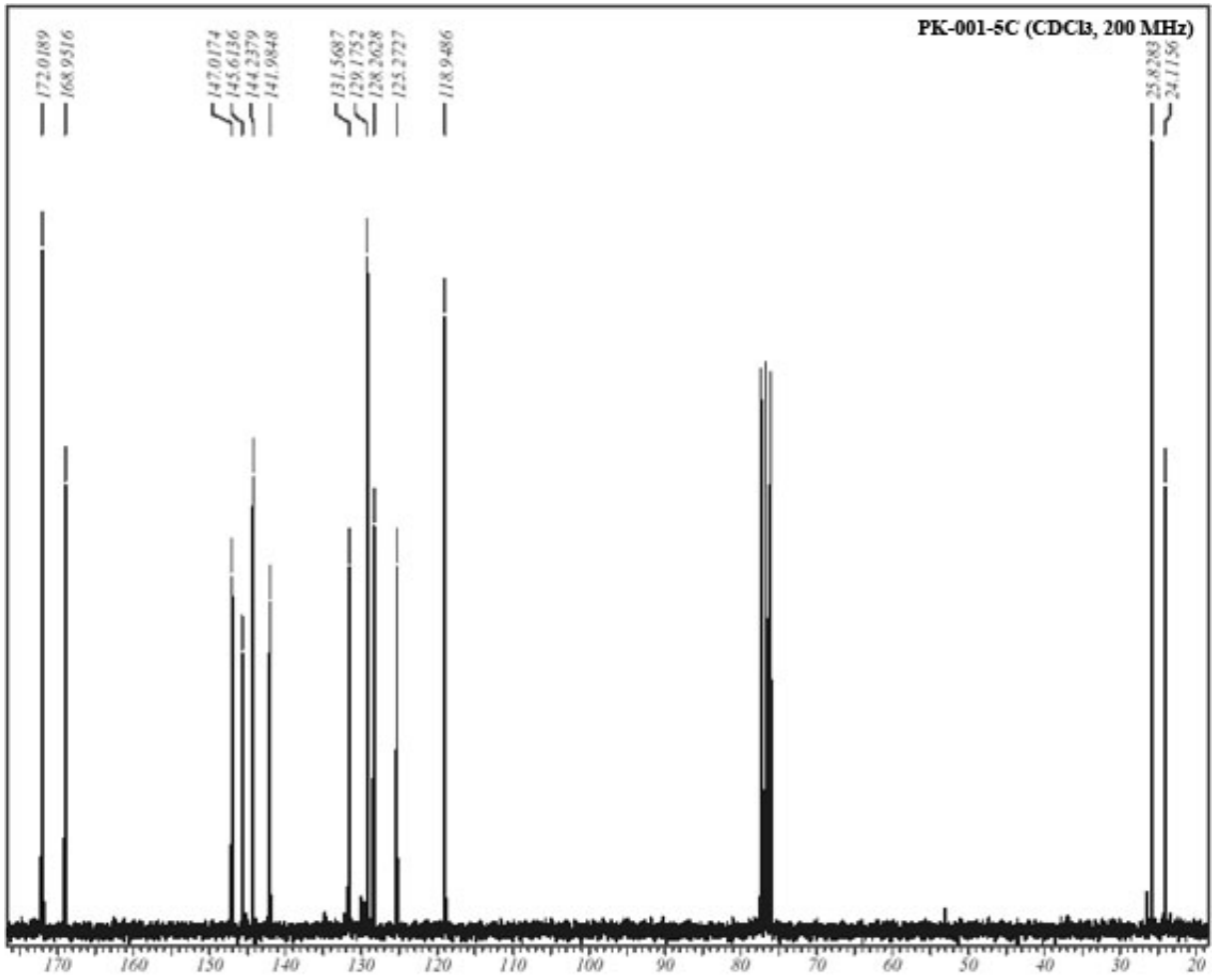
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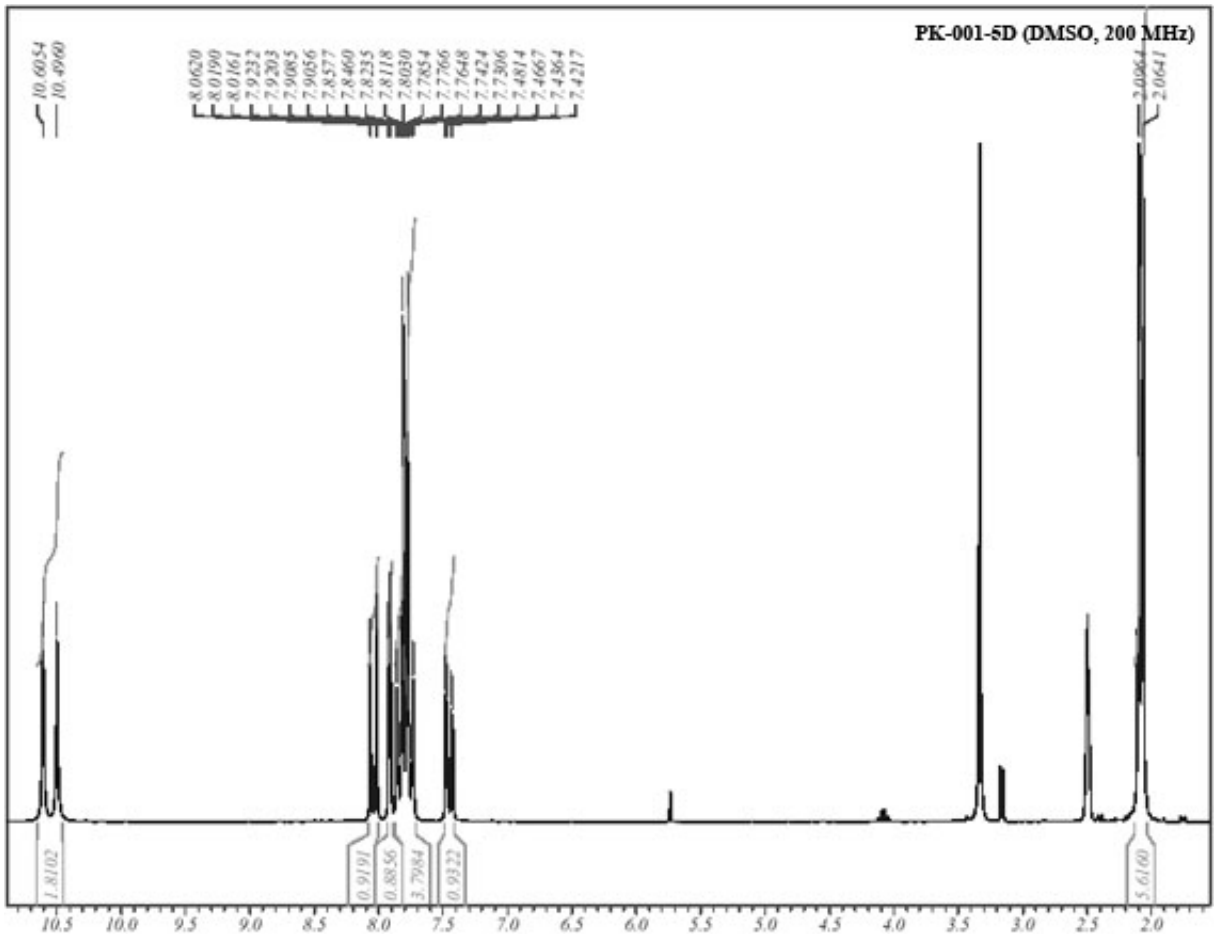
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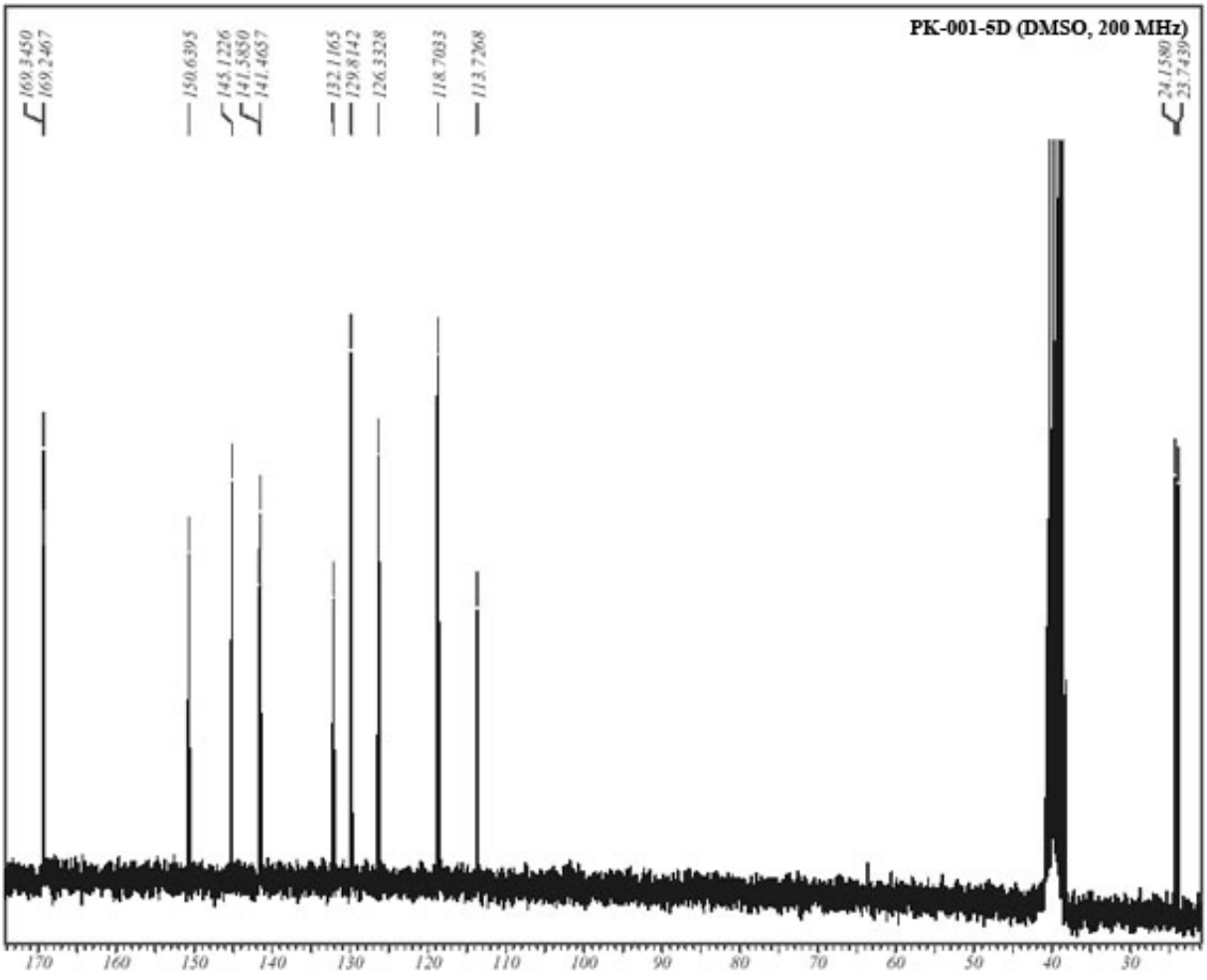
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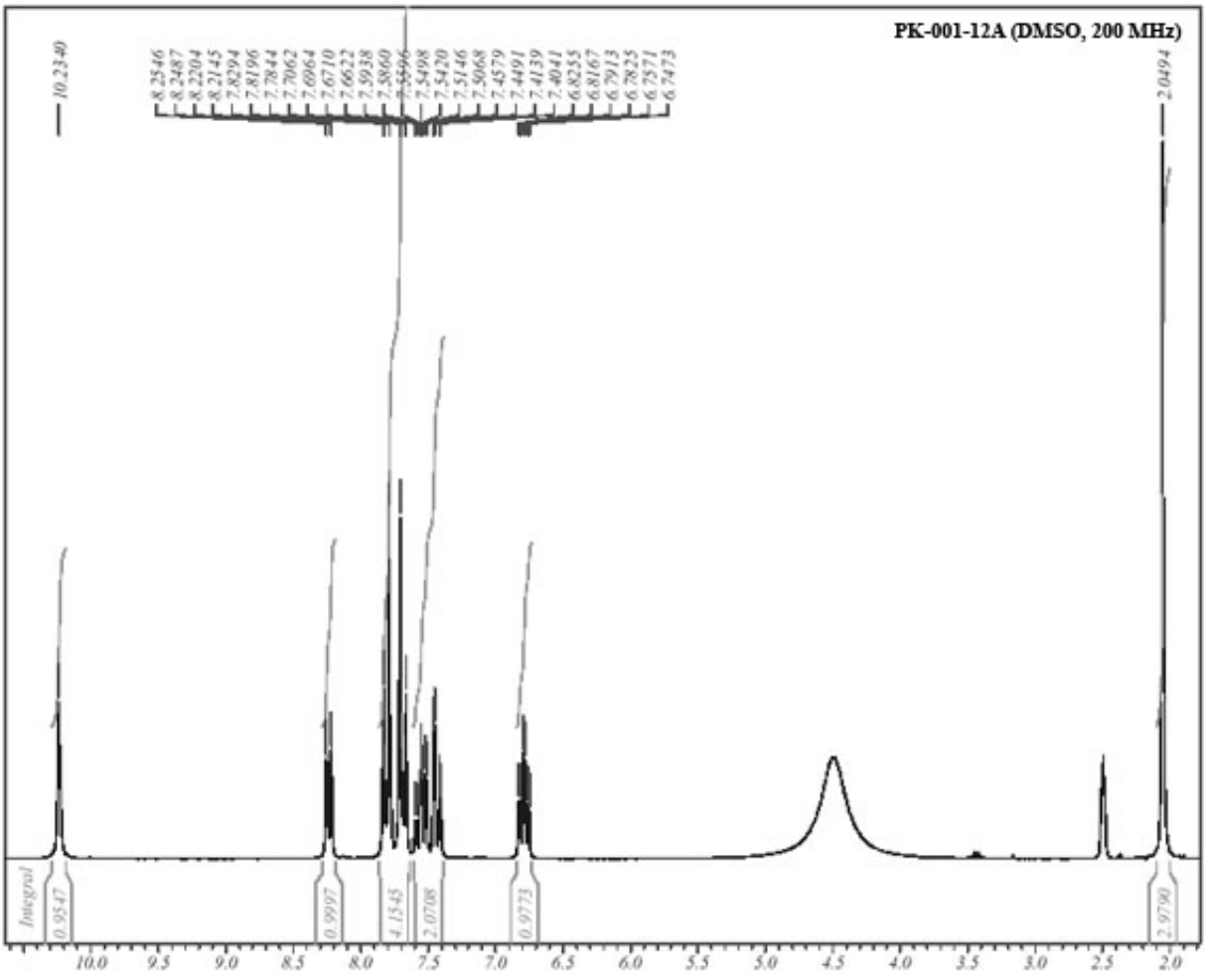
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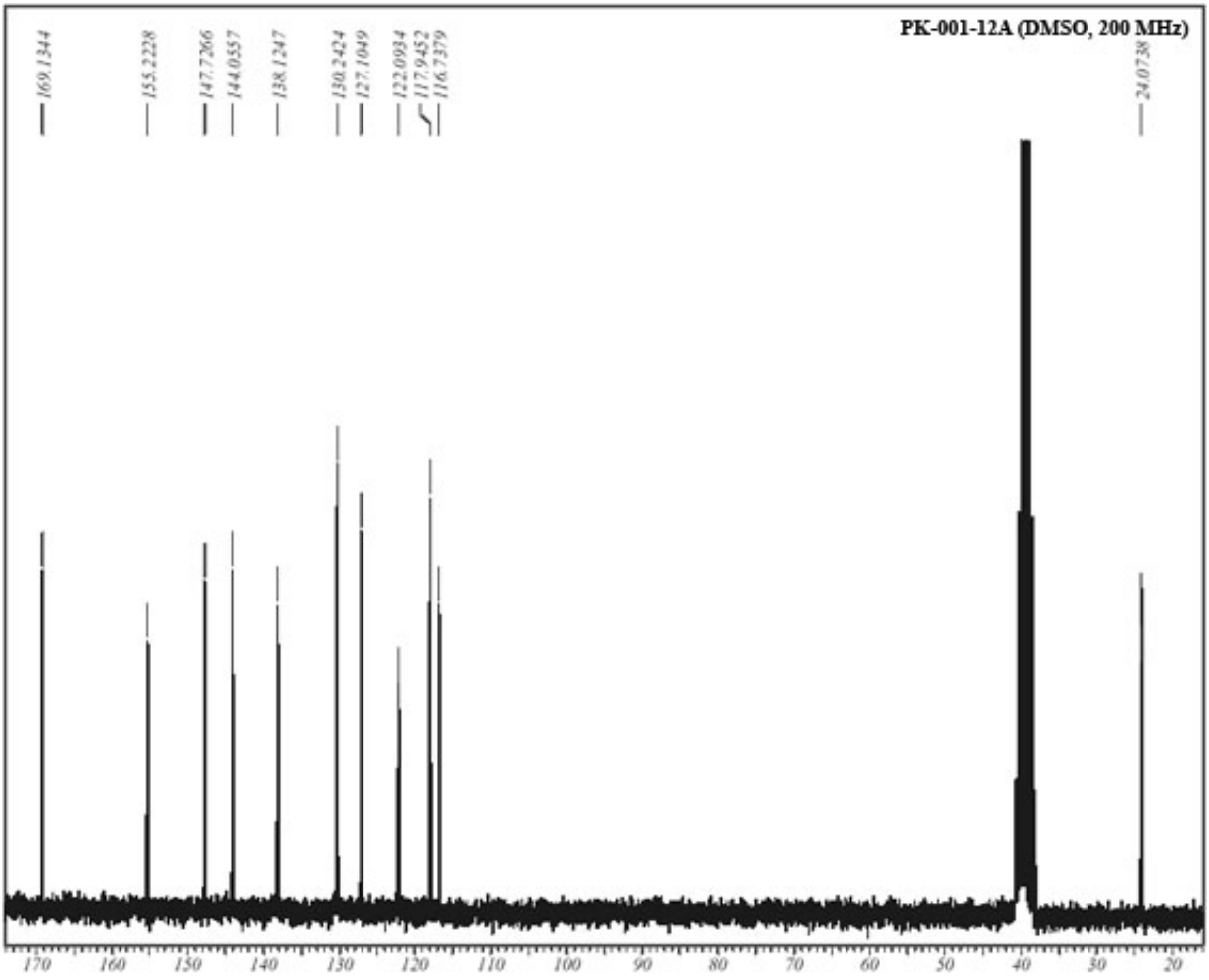
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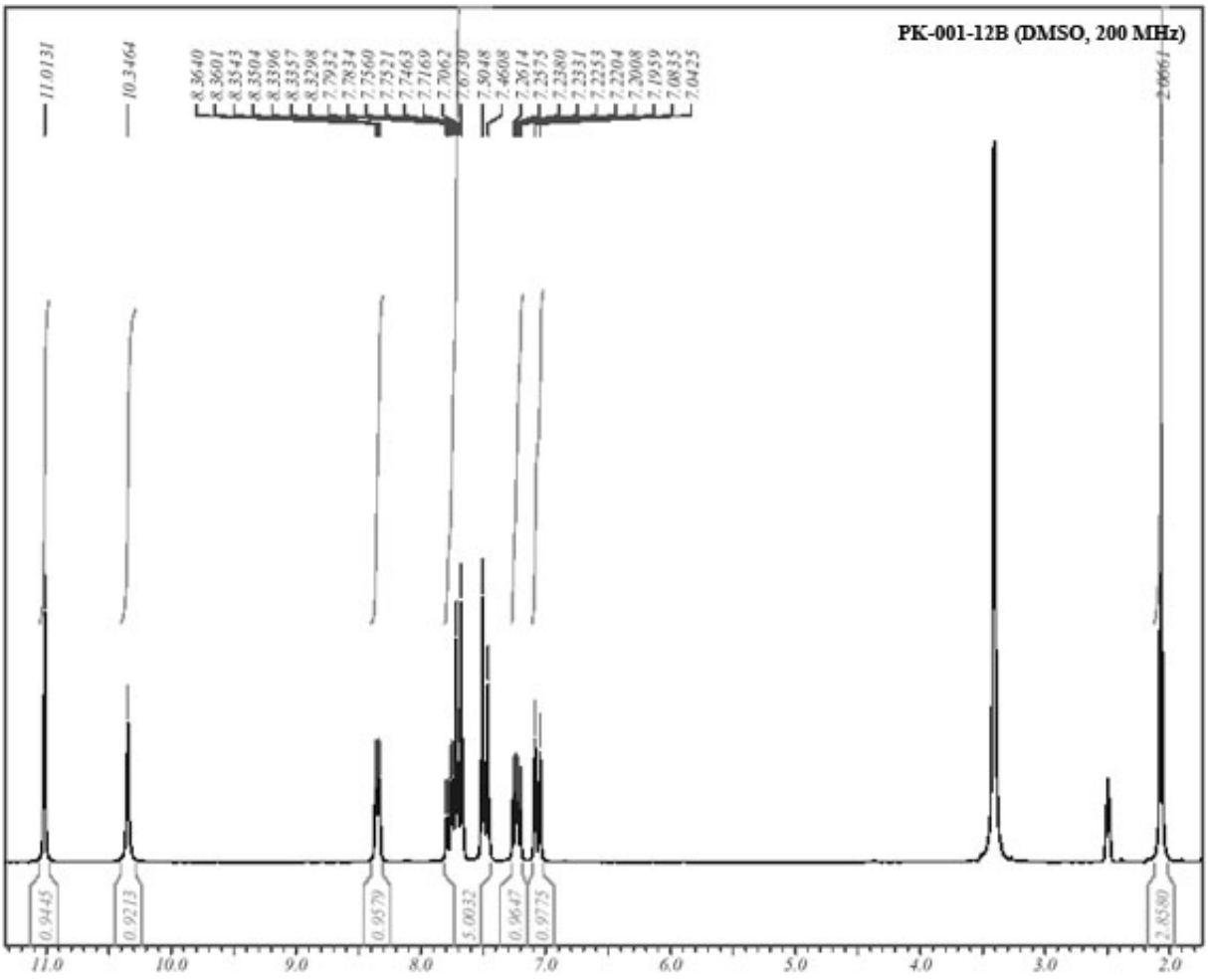
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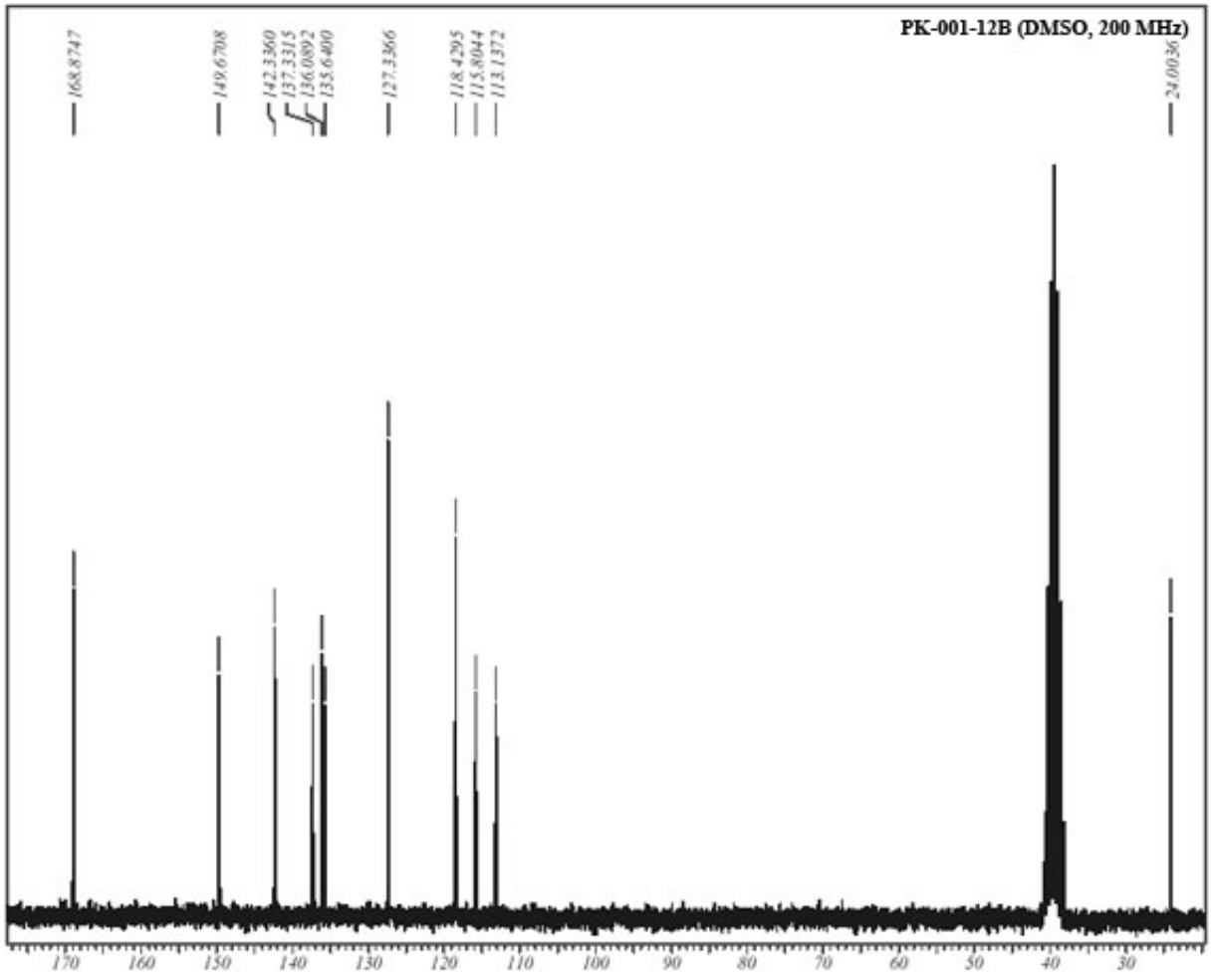
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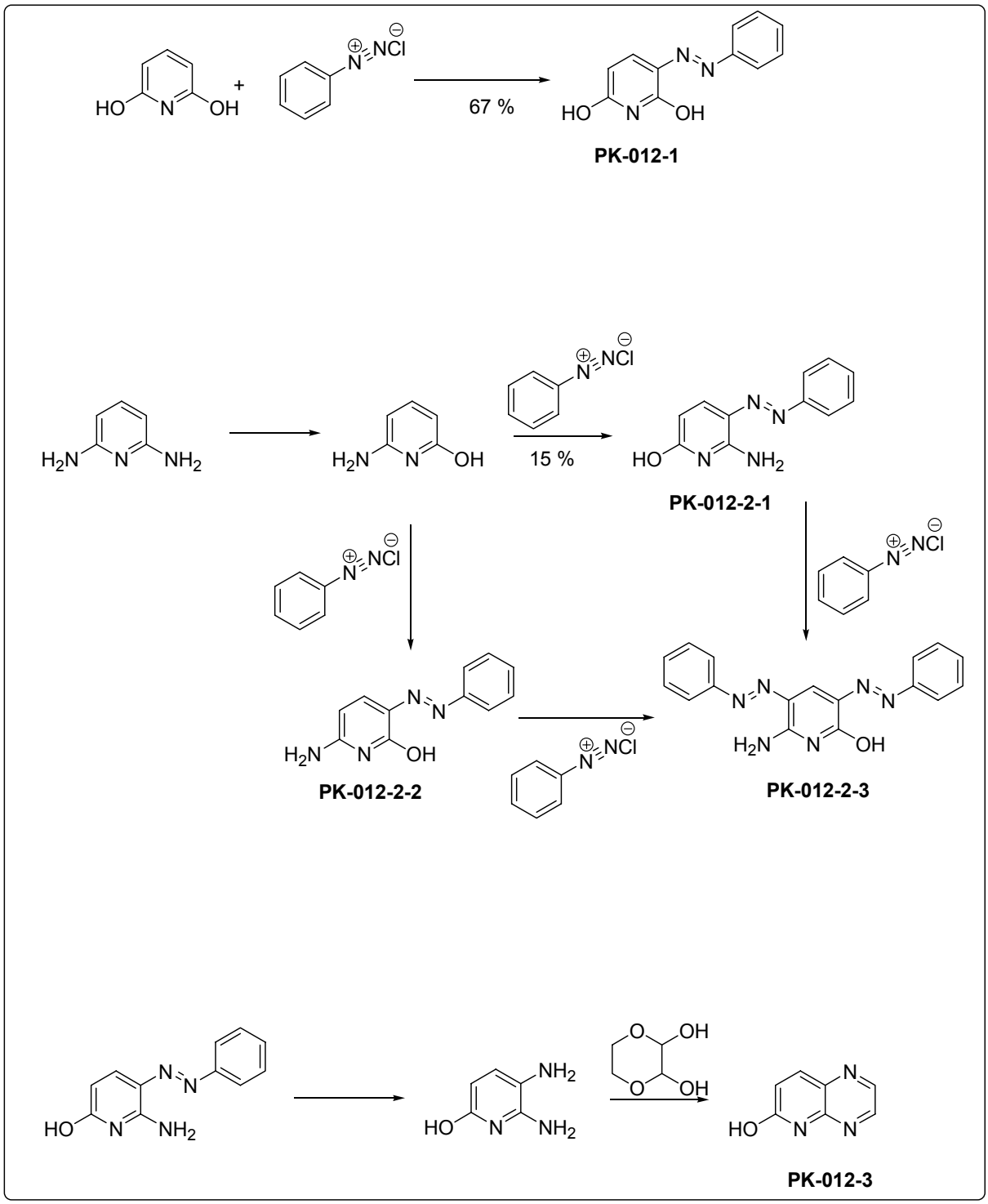
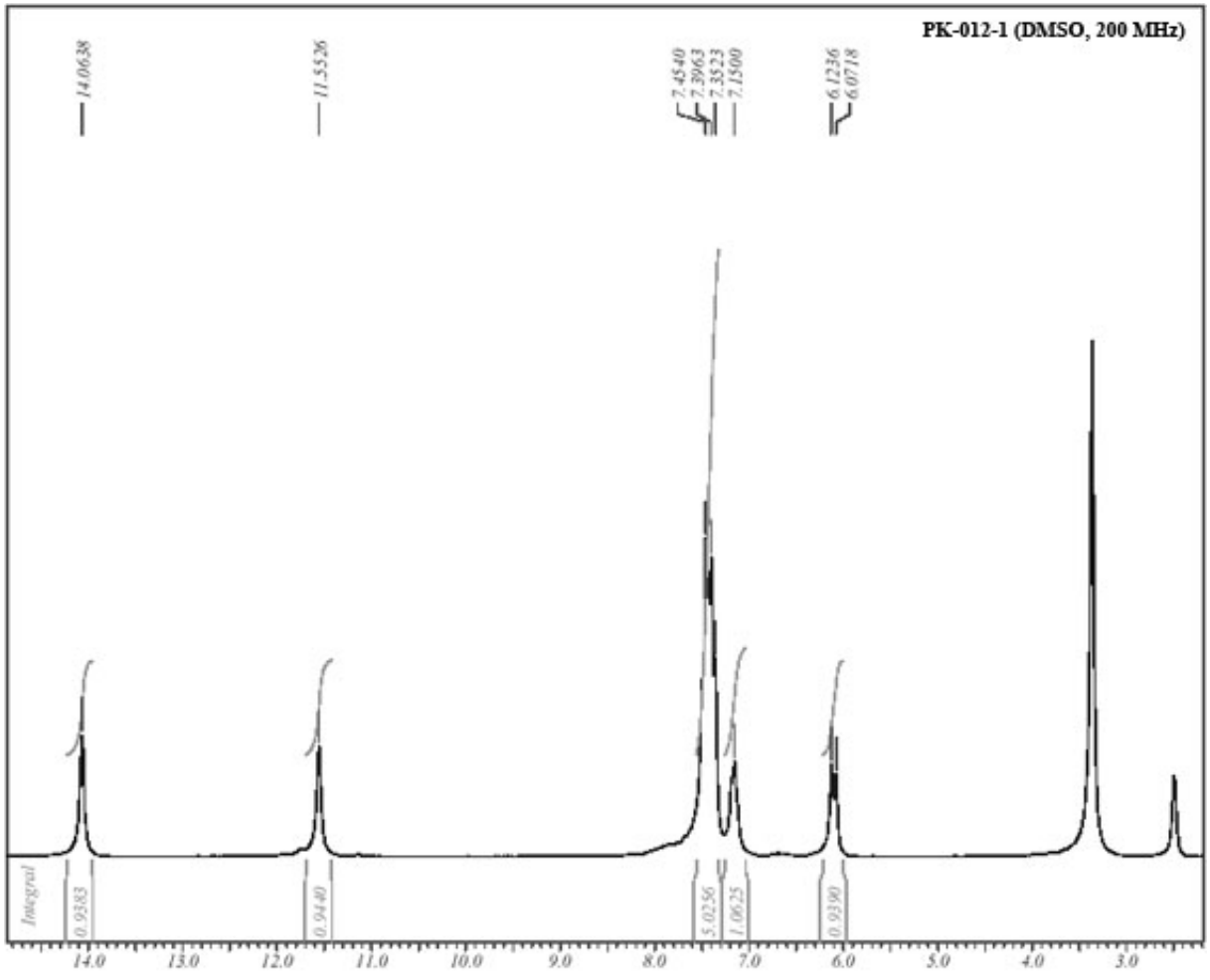
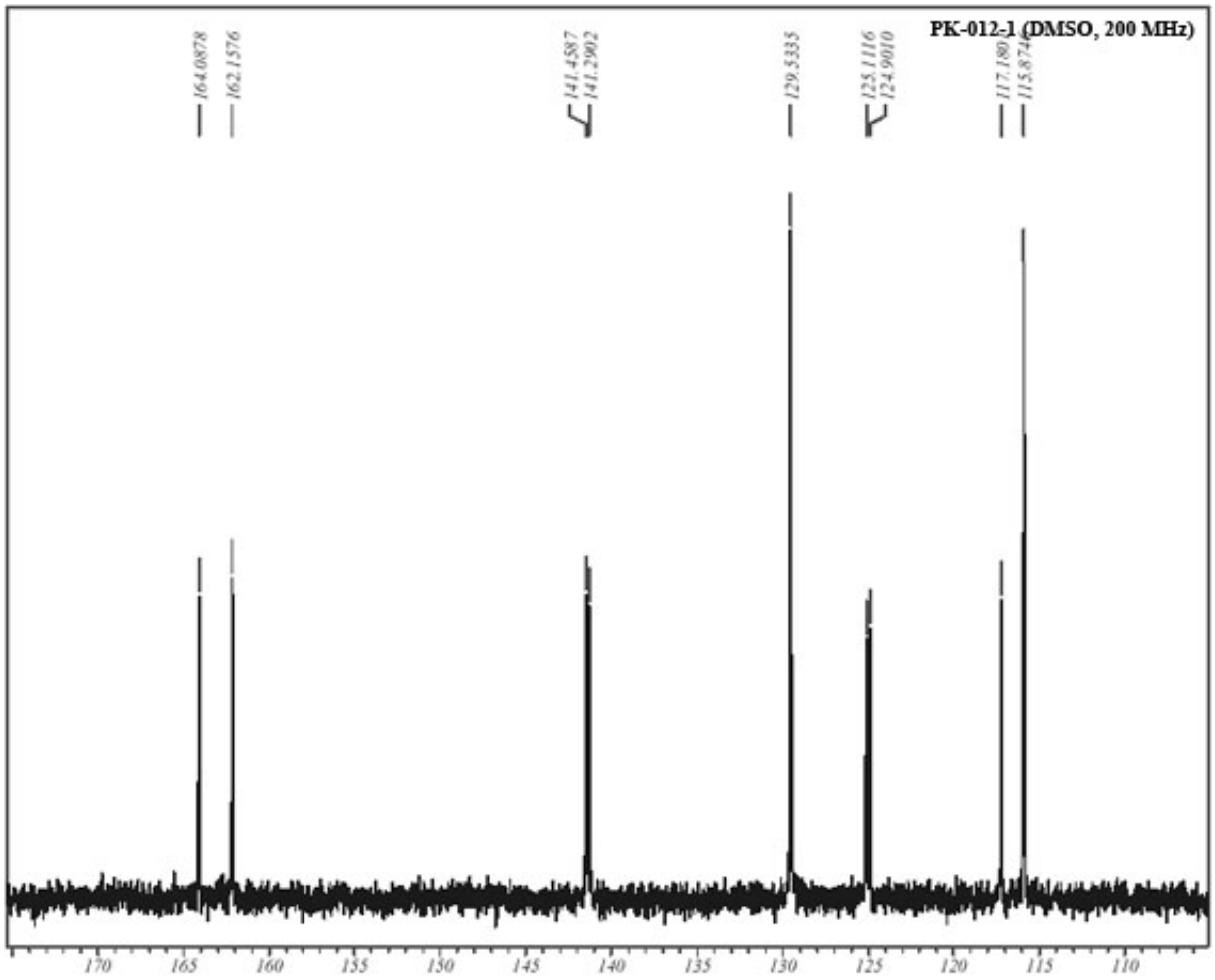
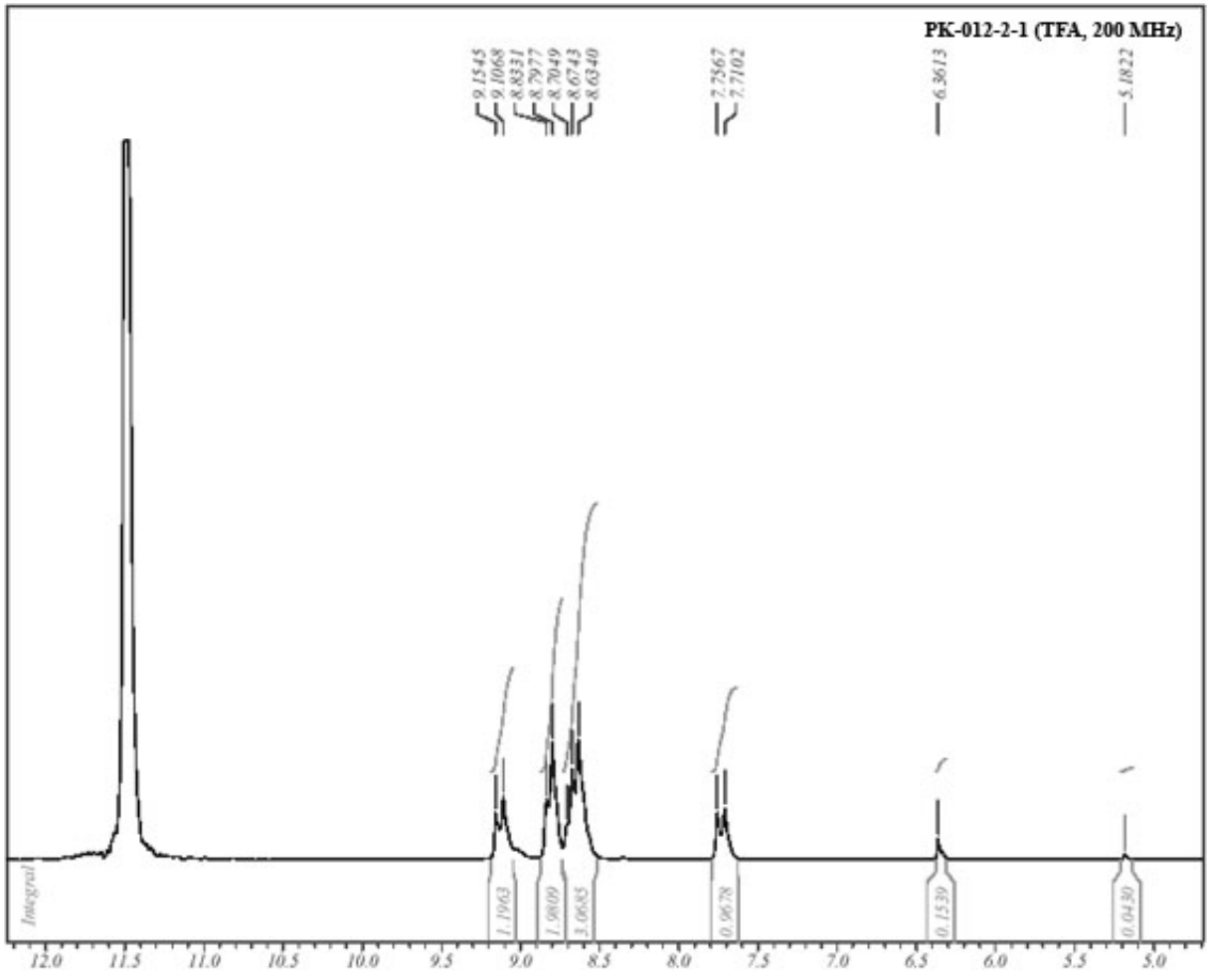
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

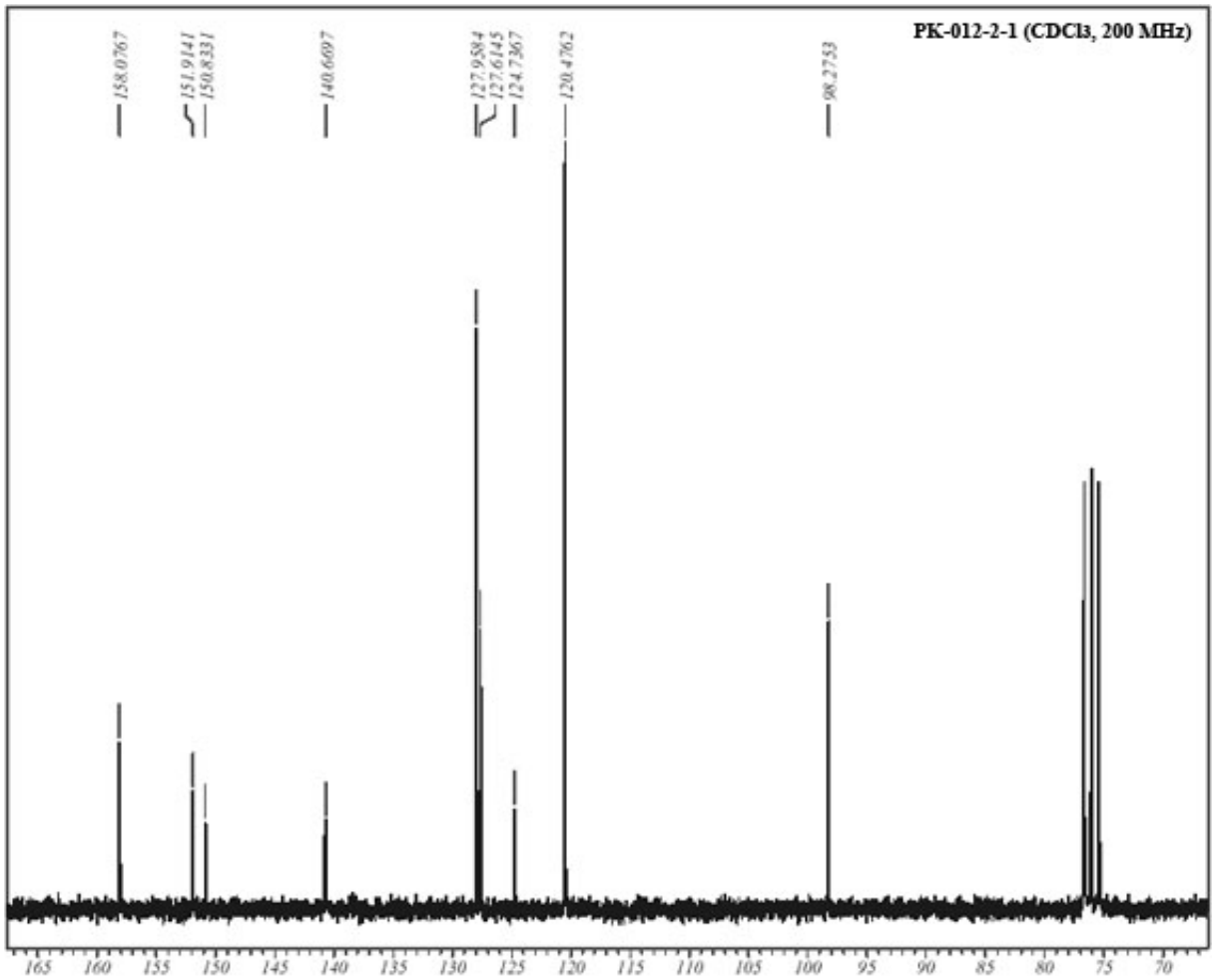
Synthesis of standard reference materials occurring as reaction byproducts in the manufacture of phenazopyridine.

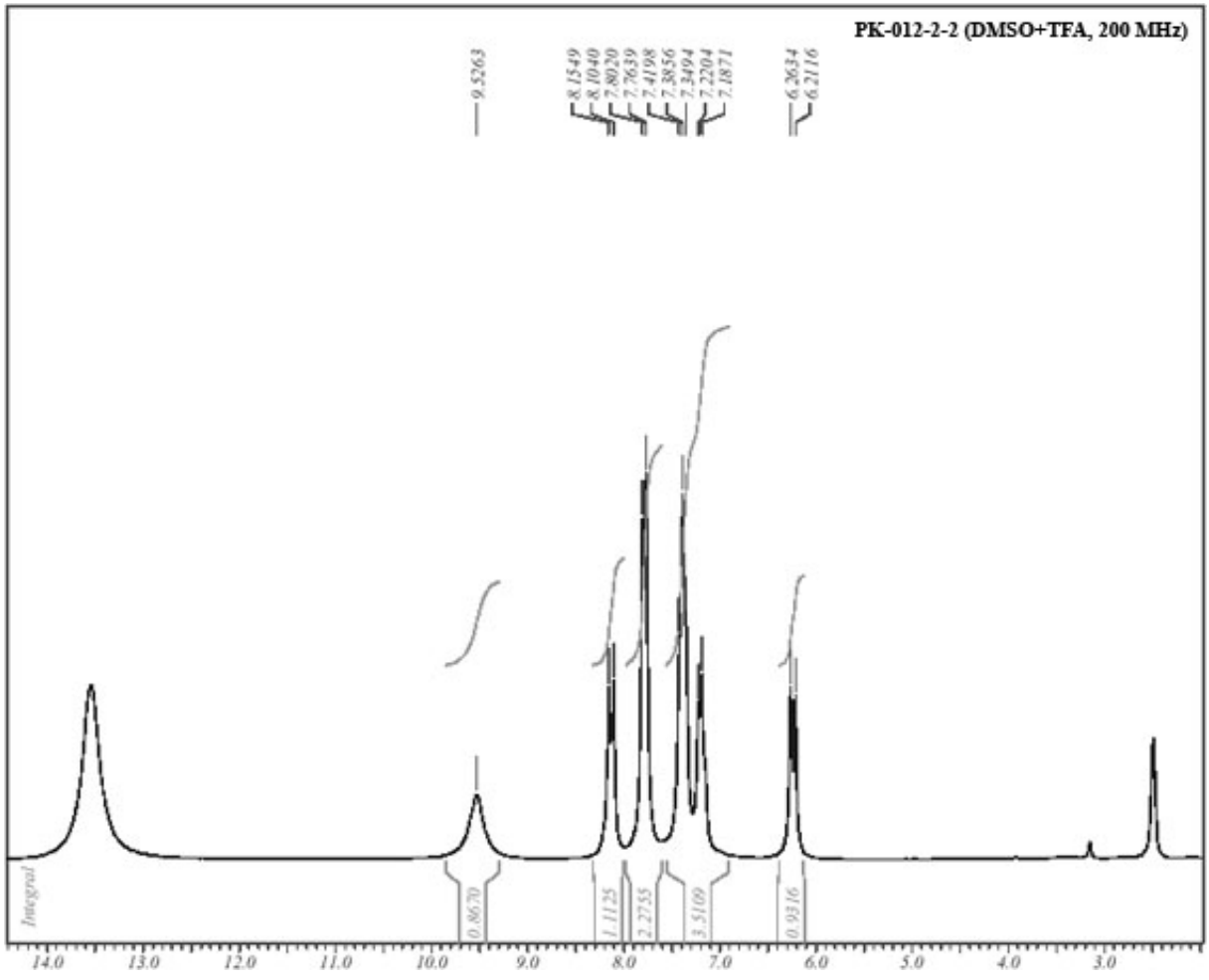


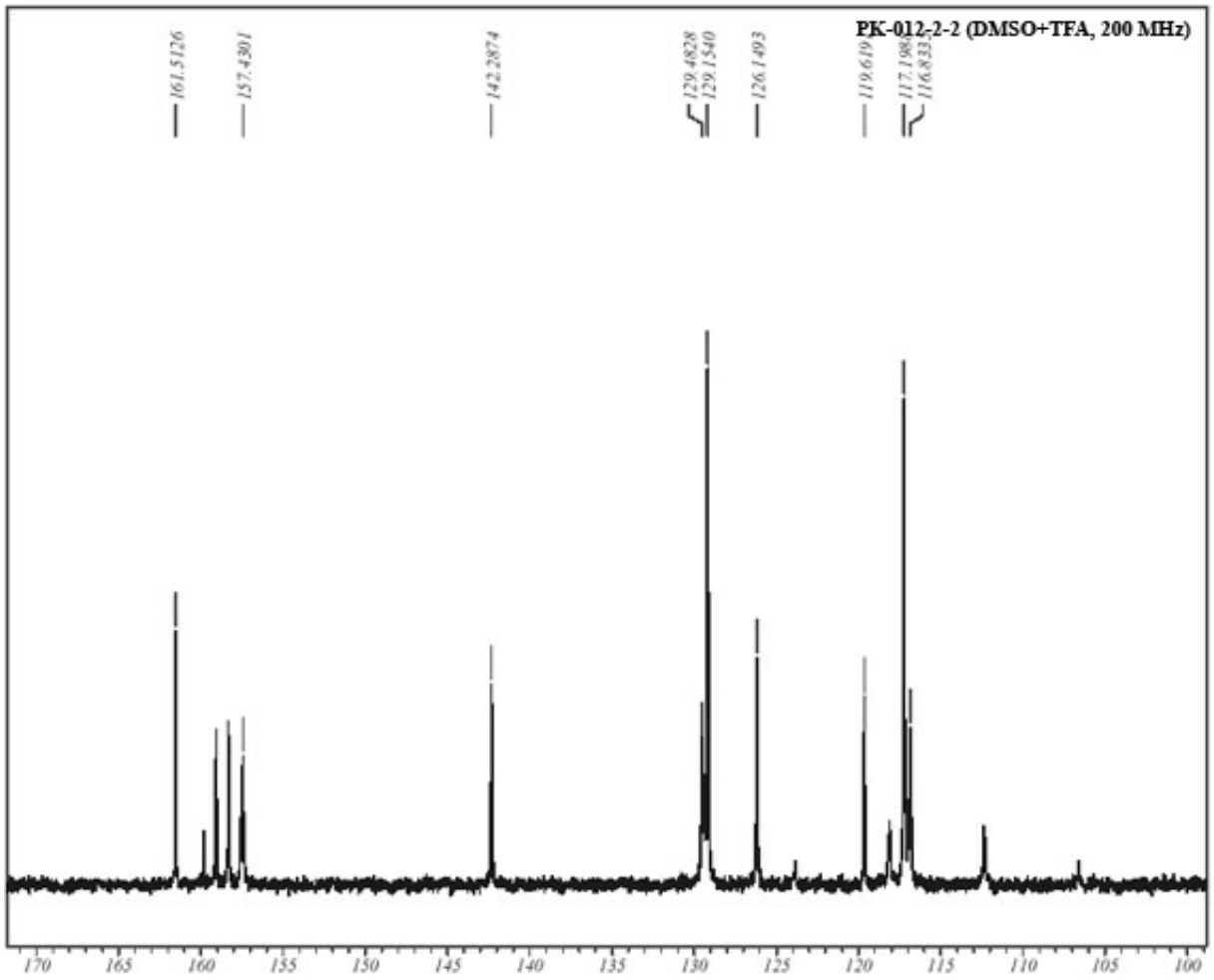

PK-012-1 (DMSO, 200 MHz)
14.0638
11.5526
7.4540
7.3963
7.3523
7.1500
6.1236
6.0718
Integral
0.9383
0.9440
5.0256
1.0625
0.9390
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0

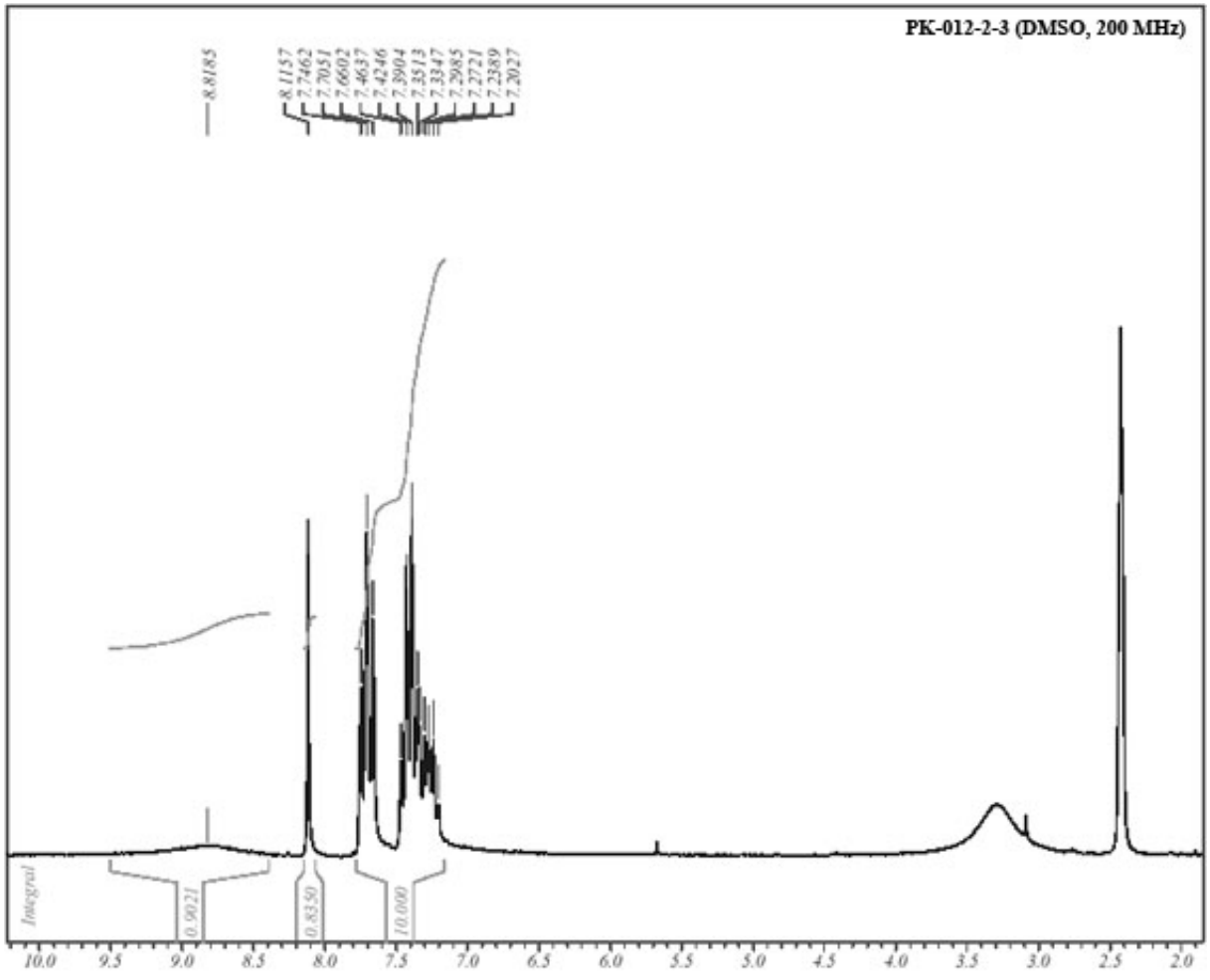

PK-012-1 (DMSO, 200 MHz)
164.0878
162.1576
141.4587
141.2902
129.5335
125.1116
124.9010
117.1801
115.8747
170
165
160
155
150
145
140
135
130
125
120
115
110

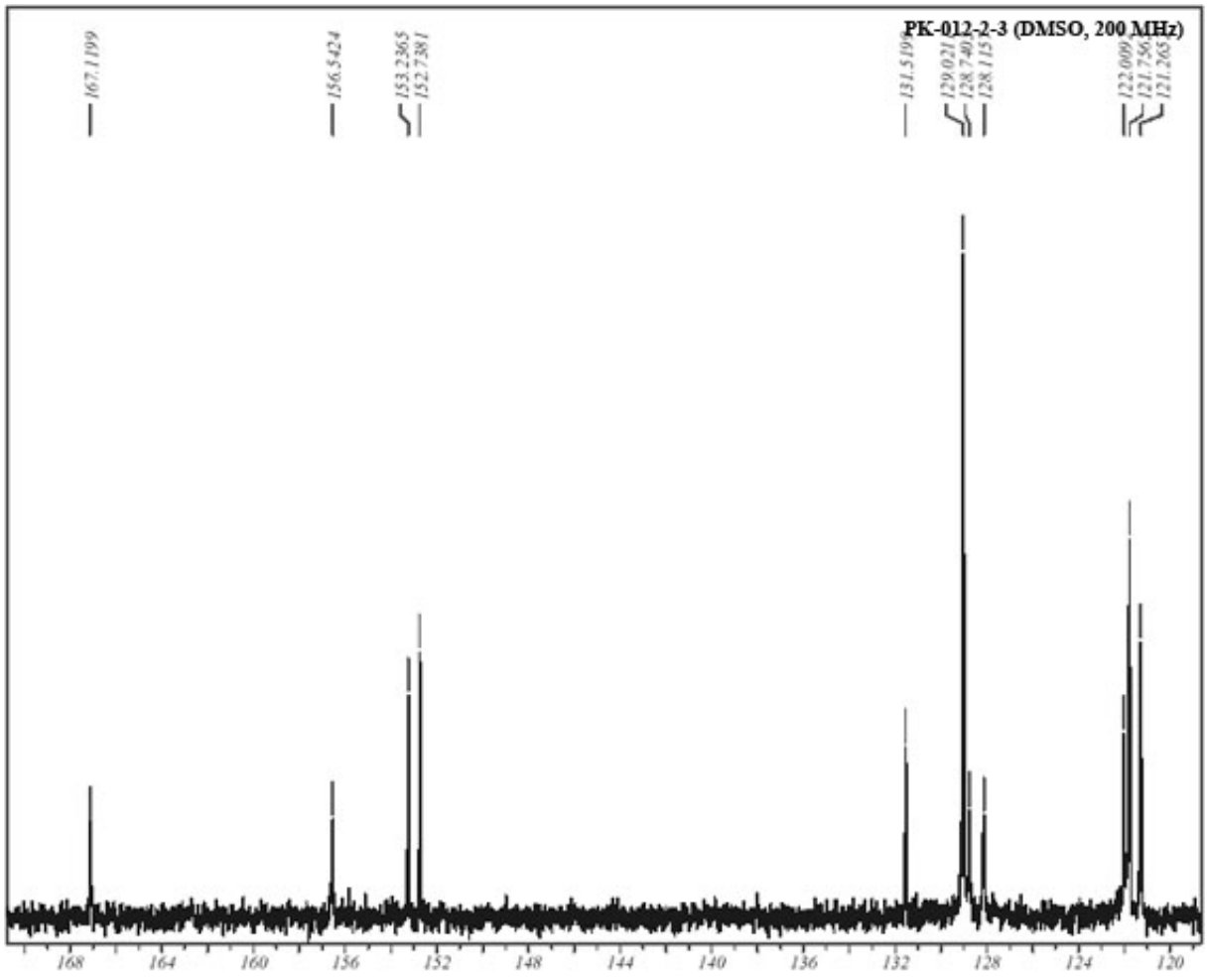

PK-012-2-1 (TFA, 200 MHz)
9.1545
9.1068
8.8331
8.7977
8.7049
8.6743
8.6340
7.7567
7.7102
6.3613
5.1822
Integral
1.1963
1.9809
3.0685
0.9678
0.1539
0.0430
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0

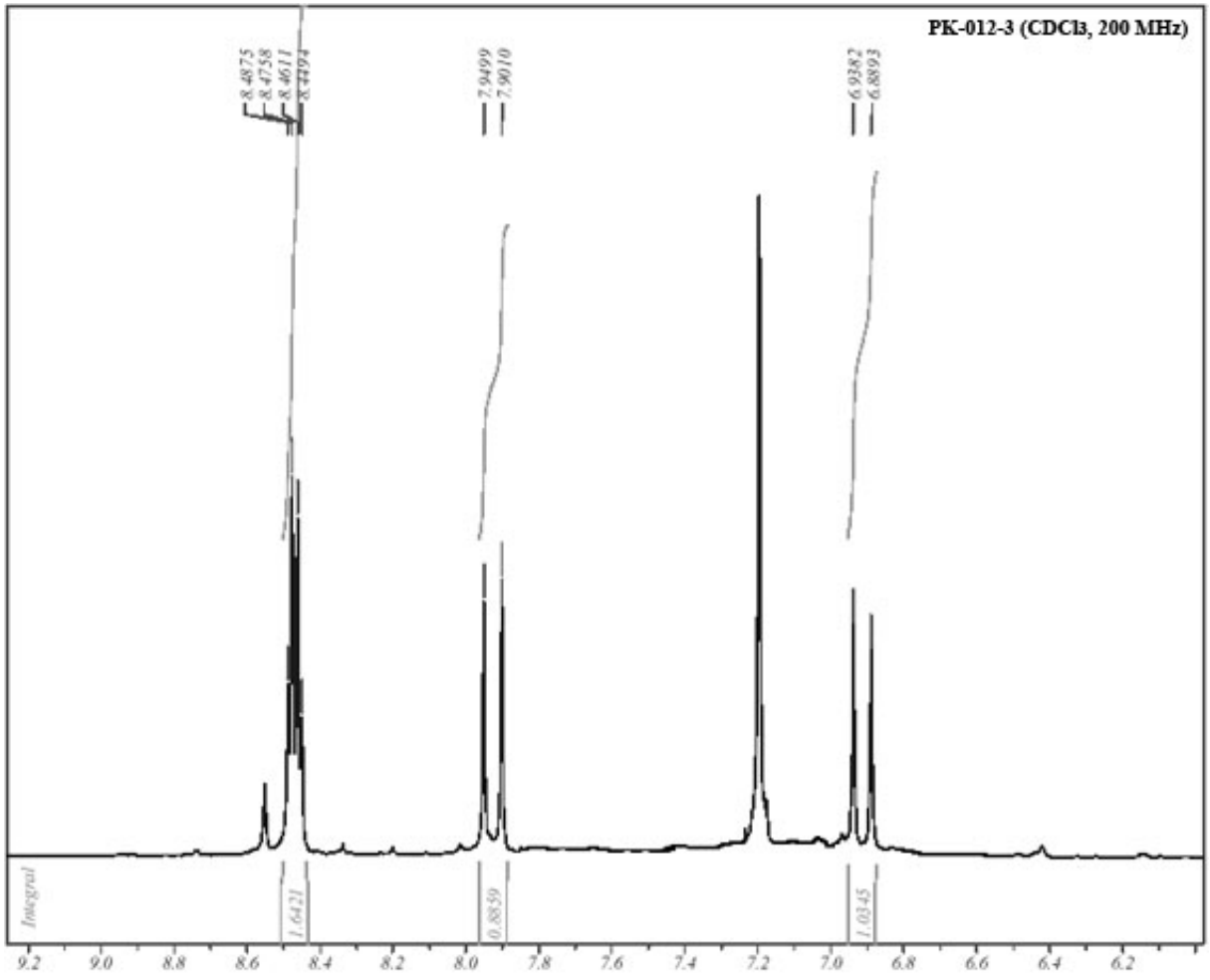

PK-012-2-1 (CDCl3, 200 MHz)
158.0767
151.9141
150.8331
140.6697
127.9584
127.6145
124.7367
120.4762
98.2753
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70


PK-012-2-2 (DMSO+TFA, 200 MHz)
9.5263
8.1549
8.1040
7.8020
7.7639
7.4198
7.3856
7.3494
7.2204
7.1871
6.2634
6.2116
Integral
0.8670
1.1125
2.2755
3.5109
0.9316
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0

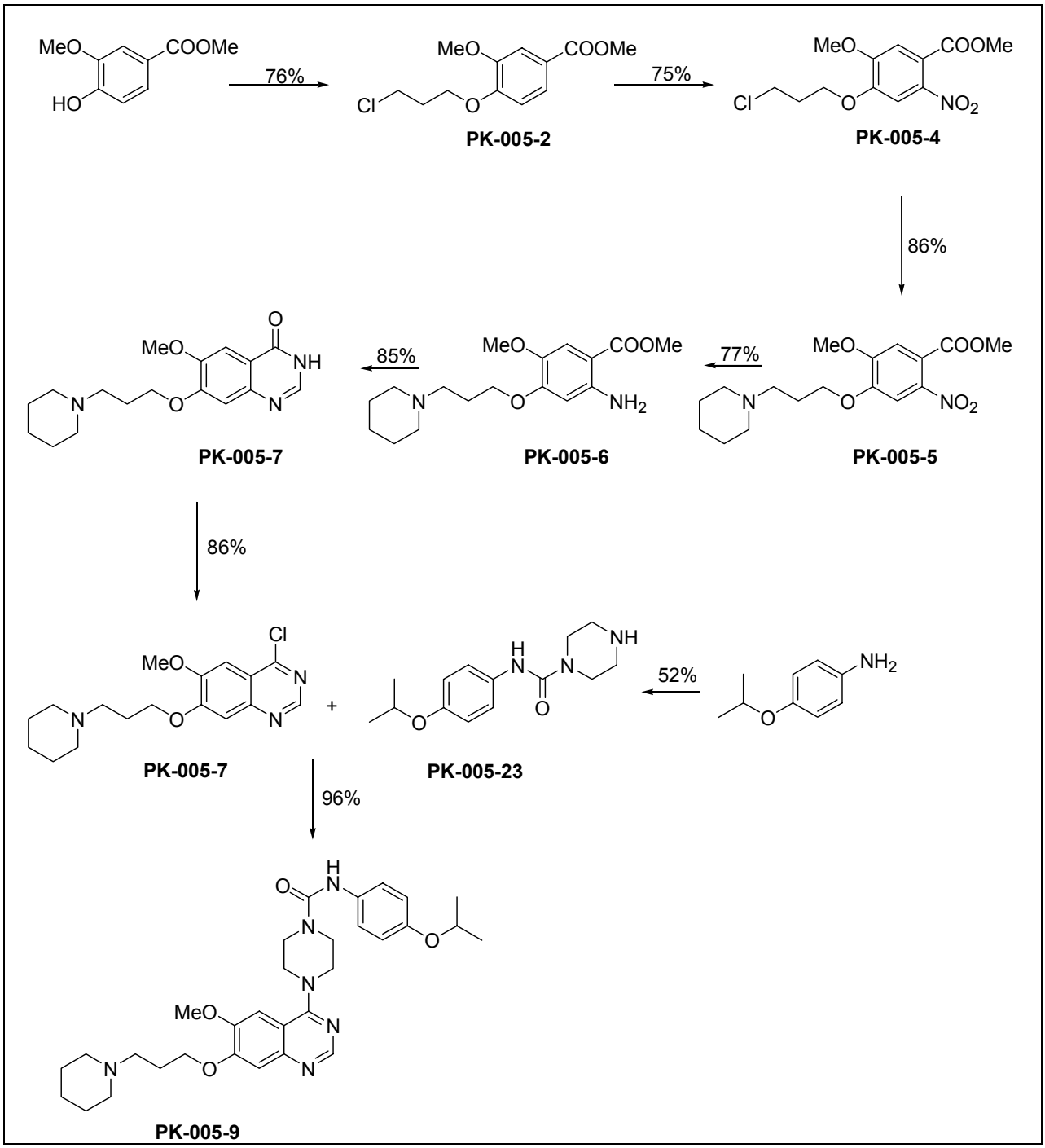

PK-012-2-2 (DMSO+TFA, 200 MHz)
161.5126
157.4301
142.2874
129.4828
129.1540
126.1493
119.6191
117.1985
116.8331
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100

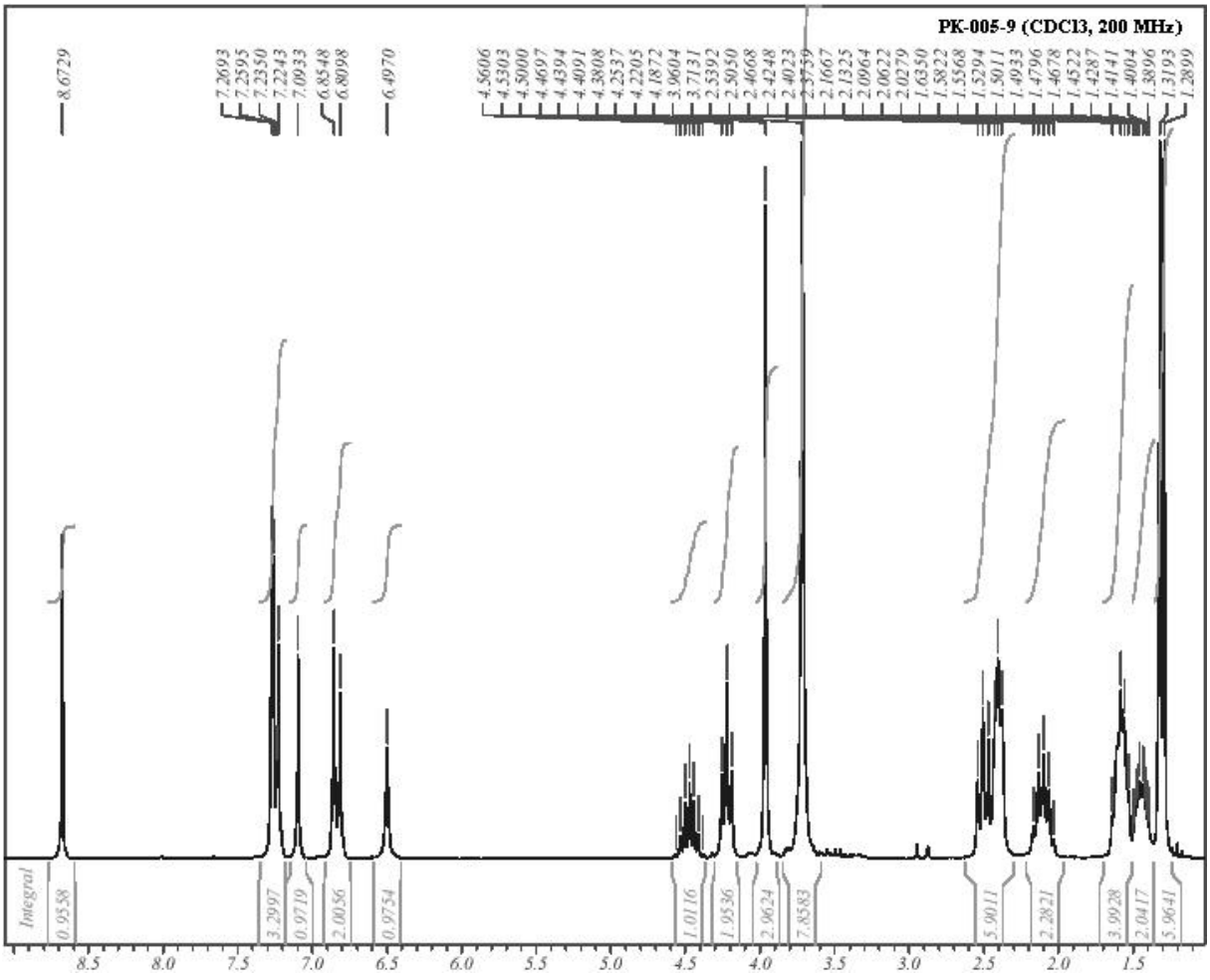

PK-012-2-3 (DMSO, 200 MHz)
8.8185
8.1157
7.7462
7.7051
7.6602
7.4637
7.4246
7.3904
7.3513
7.3347
7.2985
7.2721
7.2389
7.2027
Integral
0.9021
0.8350
10.000
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0


PK-012-2-3 (DMSO, 200 MHz)
167.1199
156.5424
153.2365
152.7381
131.5197
129.0211
128.7401
128.1151
122.0097
121.7562
121.2652
168
164
160
156
152
148
144
140
136
132
128
124
120

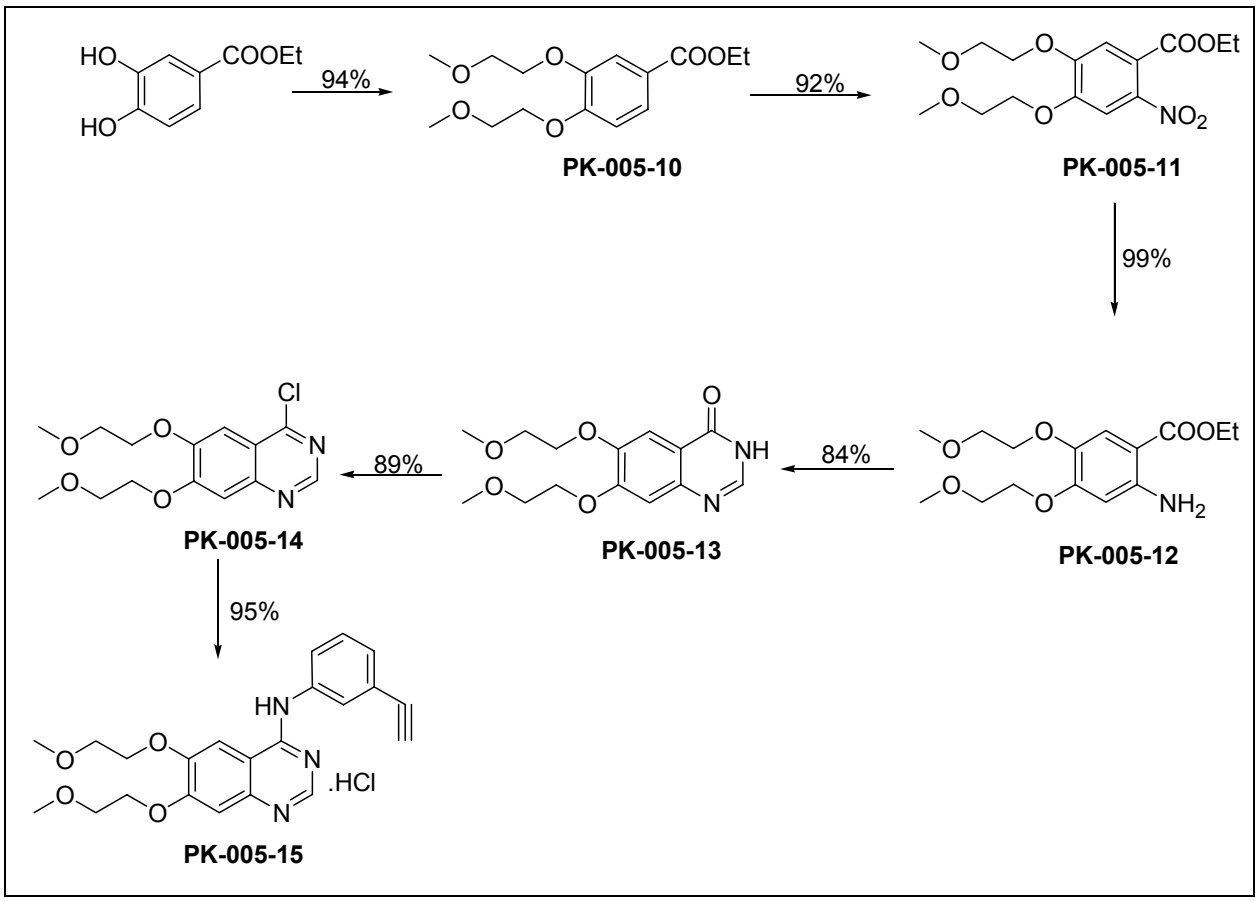

PK-012-3 (CDCl3, 200 MHz)
8.4875
8.4758
8.4611
8.4494
7.9499
7.9010
6.9382
6.8893
Integral
1.6421
0.8859
1.0345
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2

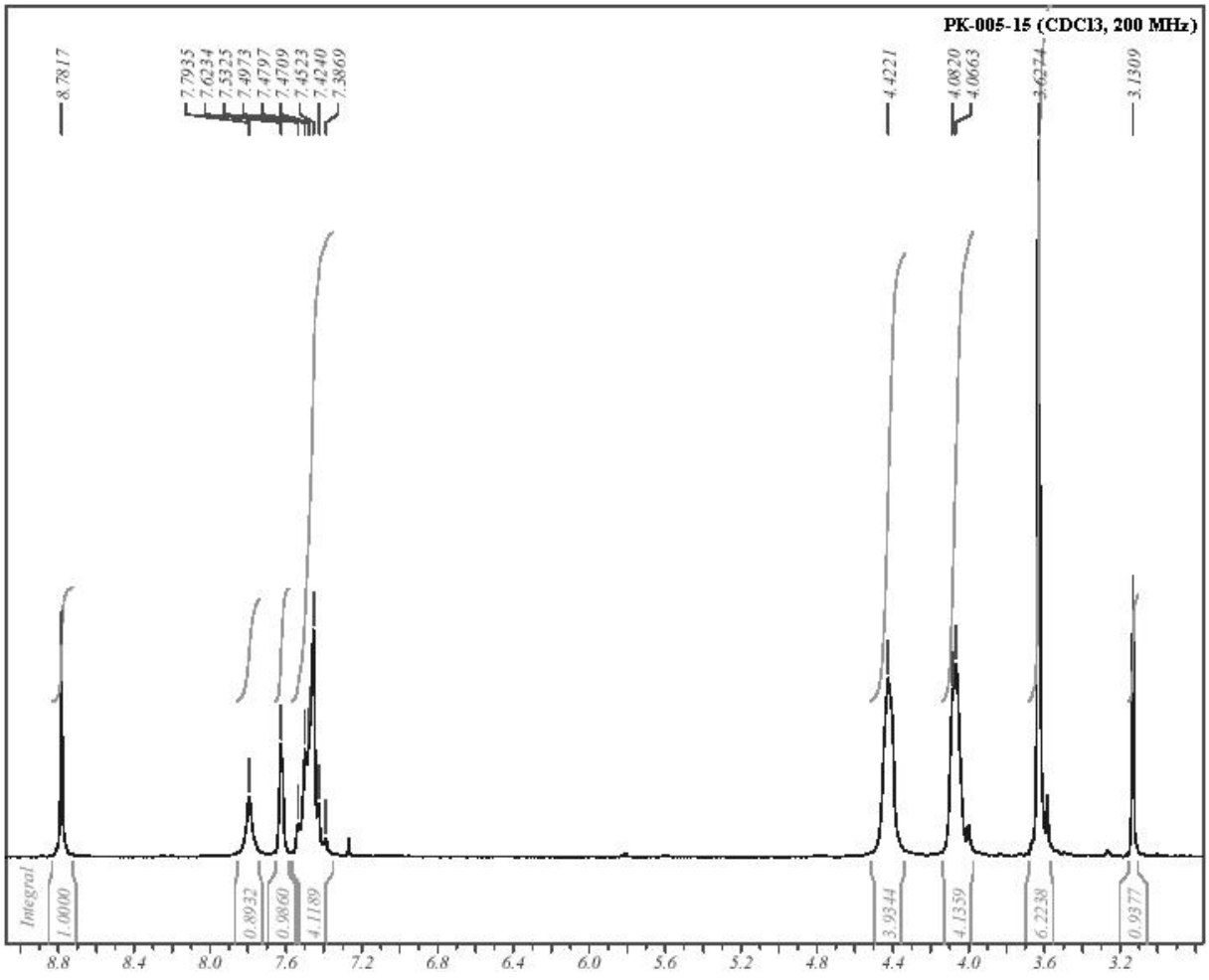
Synthesis of 4-[6-Methoxy-7-(3-piperidin-1-yl-propoxy)-quinazolin-4-yl]-piperazine-1-carboxylic acid (4-isopropoxy-phenyl)-amide (TANDUTINIB).

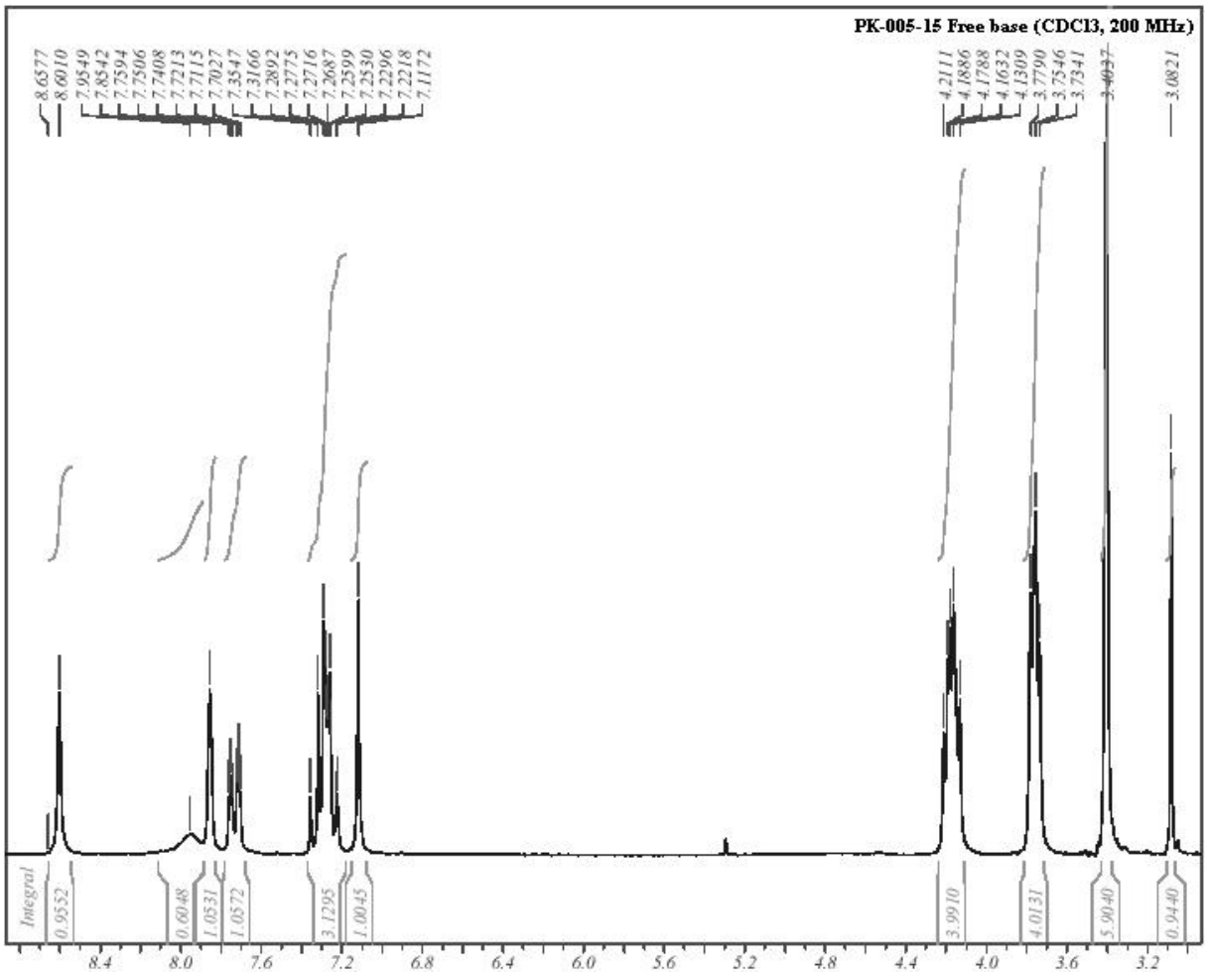



PK-005-9 (CDCl3, 200 MHz)
8.6729
7.2693
7.2595
7.2350
7.2243
7.0933
6.8548
6.8098
6.4970
4.5606
4.5303
4.5000
4.4697
4.4394
4.4091
4.3808
4.2537
4.2205
4.1872
3.9604
3.7131
3.5392
2.5050
2.4668
2.4248
2.4023
2.3759
2.1667
2.1325
2.0964
2.0622
2.0279
1.6350
1.5822
1.5568
1.5294
1.5011
1.4933
1.4796
1.4678
1.4522
1.4287
1.4141
1.4004
1.3896
1.3193
1.2899
Integral
0.9558
3.2997
0.9719
2.0056
0.9754
1.0116
1.9536
2.9624
7.8583
5.9011
2.2821
3.9928
2.0417
5.9641
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

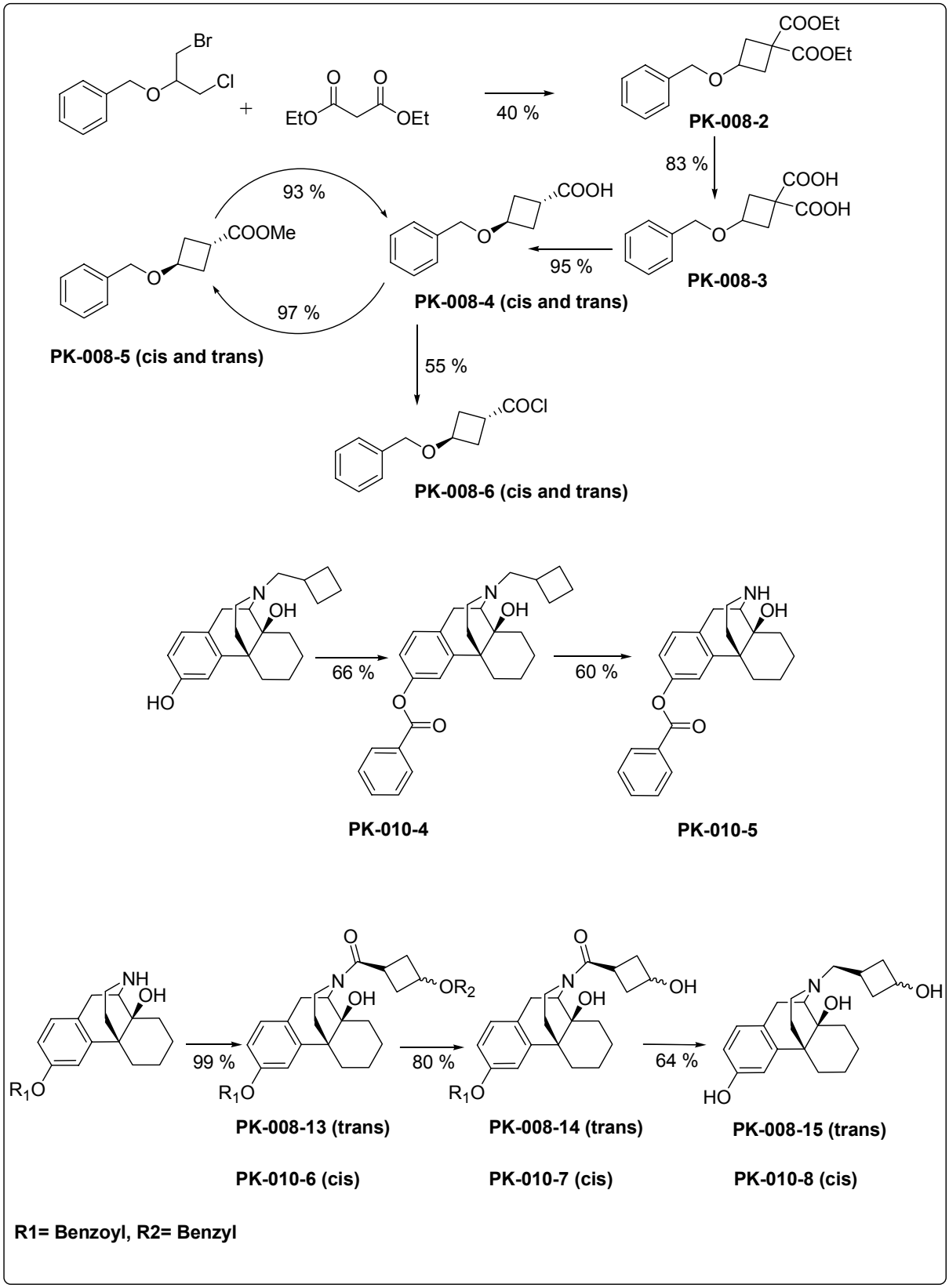
Synthesis of [6,7-Bis-(2-methoxy-ethoxy)-quinazolin-4-yl]-(3-ethynyl-phenyl)-amine hydrochloride (ERLOTINIB).

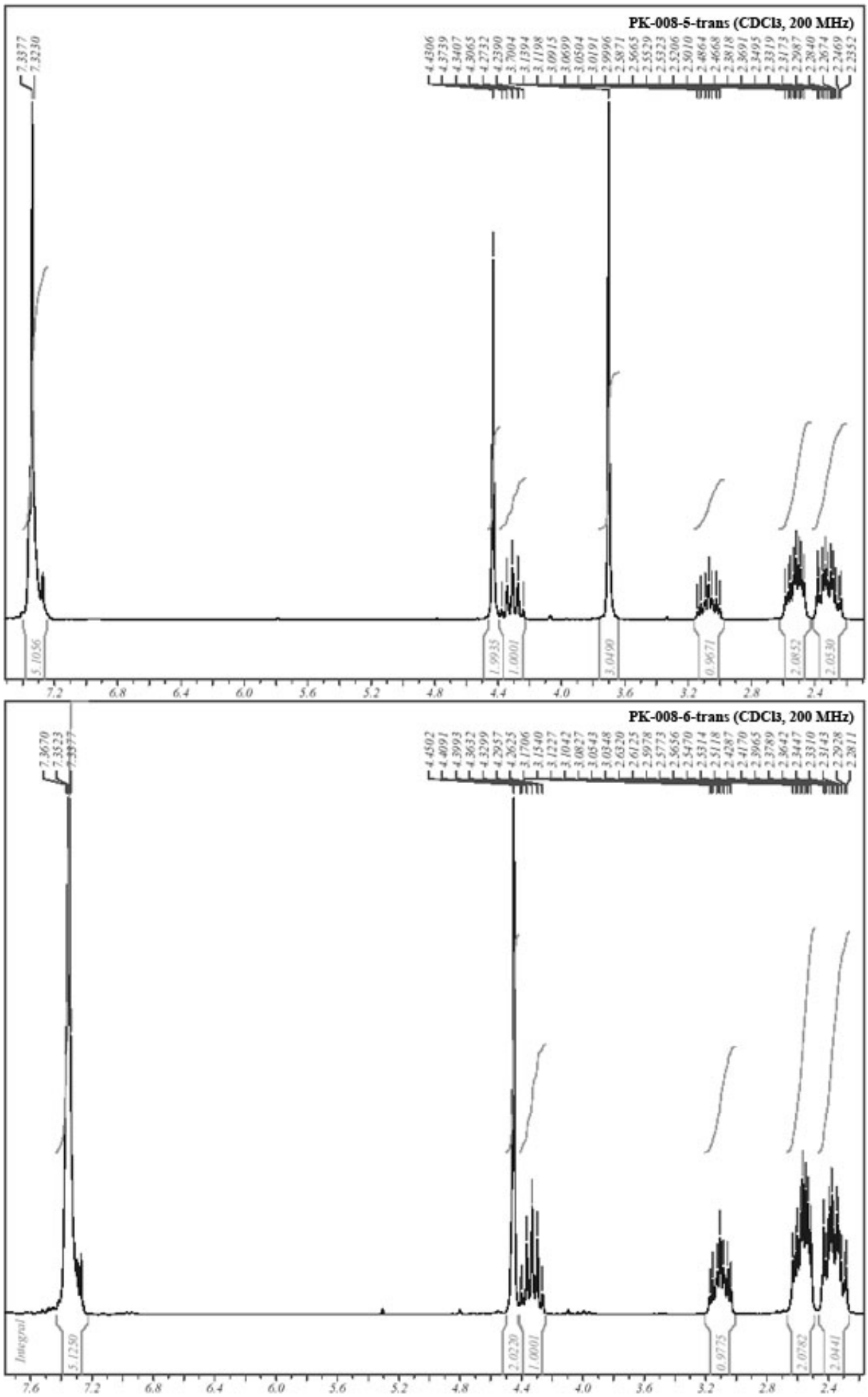


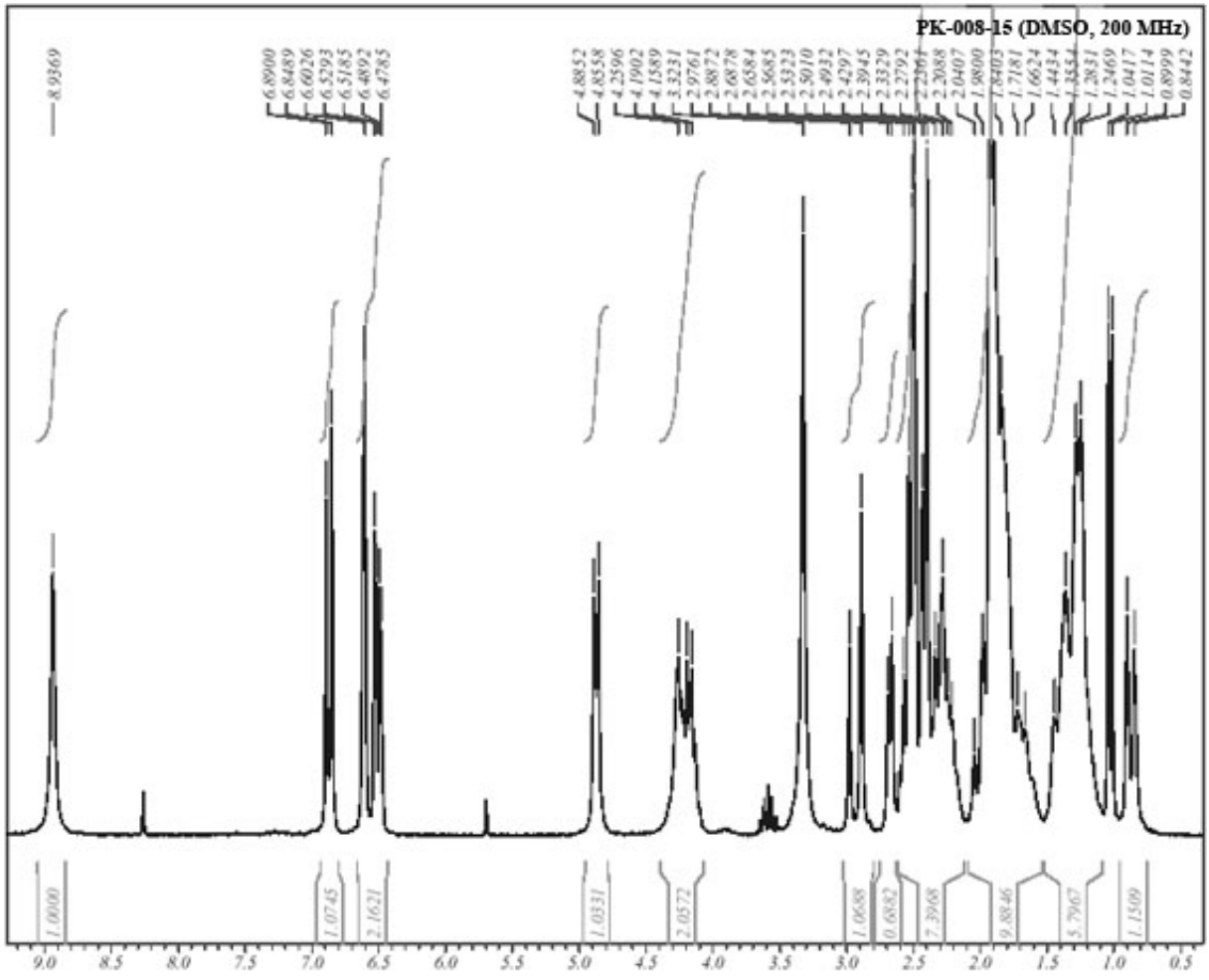

PK-005-15 (CDCl3, 200 MHz)
8.7817
7.7935
7.6234
7.5325
7.4973
7.4797
7.4709
7.4523
7.4240
7.3869
4.4221
4.0820
4.0663
3.6274
3.1309
Integral
1.0000
0.8932
0.9860
4.1189
3.9344
4.1359
6.2238
0.9377

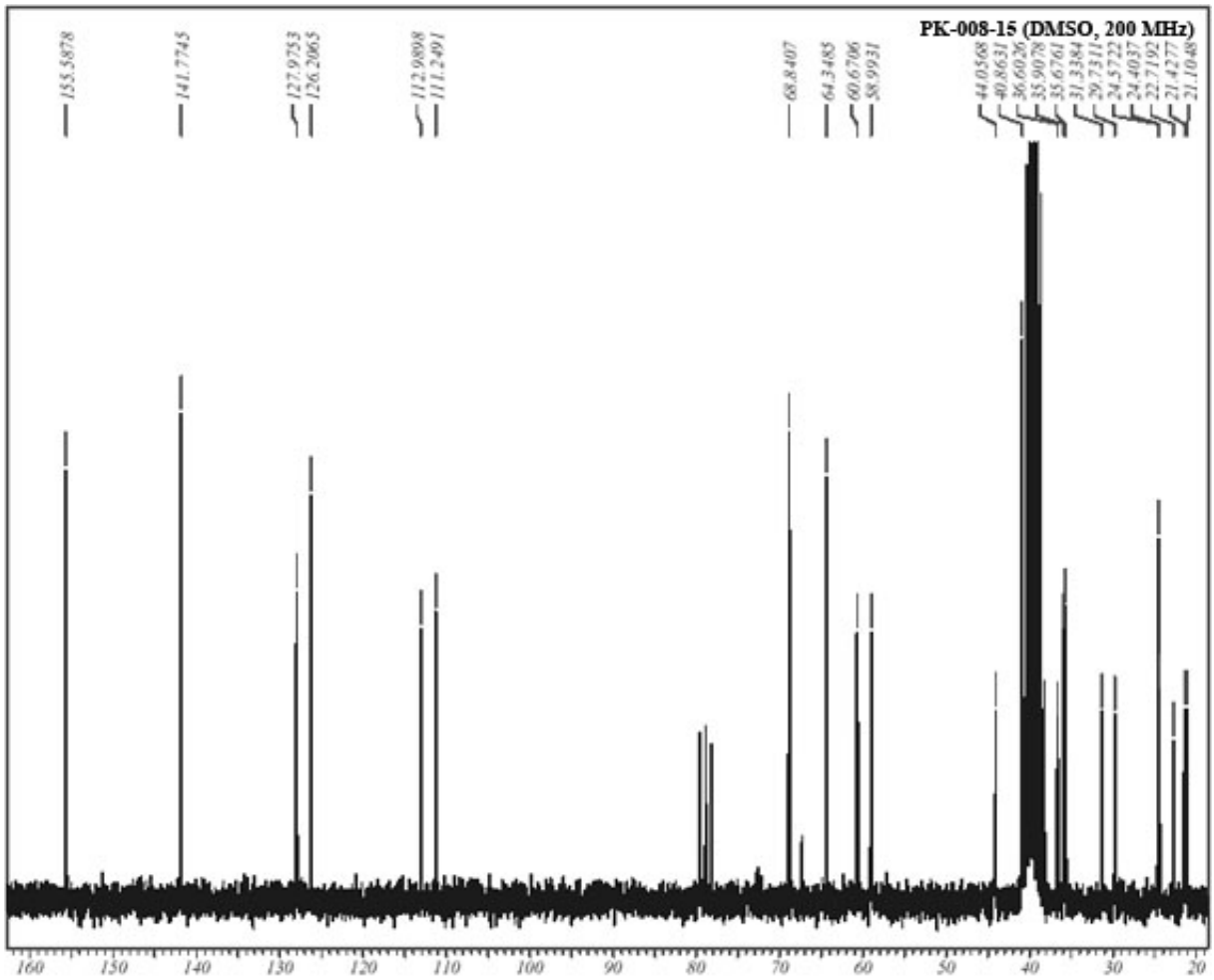

PK-005-15 Free base (CDCl3, 200 MHz)
8.6577
8.6010
7.9549
7.8542
7.7594
7.7506
7.7408
7.7213
7.7115
7.7027
7.3547
7.3166
7.2892
7.2775
7.2716
7.2687
7.2599
7.2530
7.2296
7.2218
7.1172
4.2111
4.1886
4.1788
4.1632
4.1309
3.7790
3.7546
3.7341
3.4037
3.0821
Integral
0.9552
0.6048
1.0531
1.0572
3.1295
1.0045
3.9910
4.0131
5.9040
0.9440

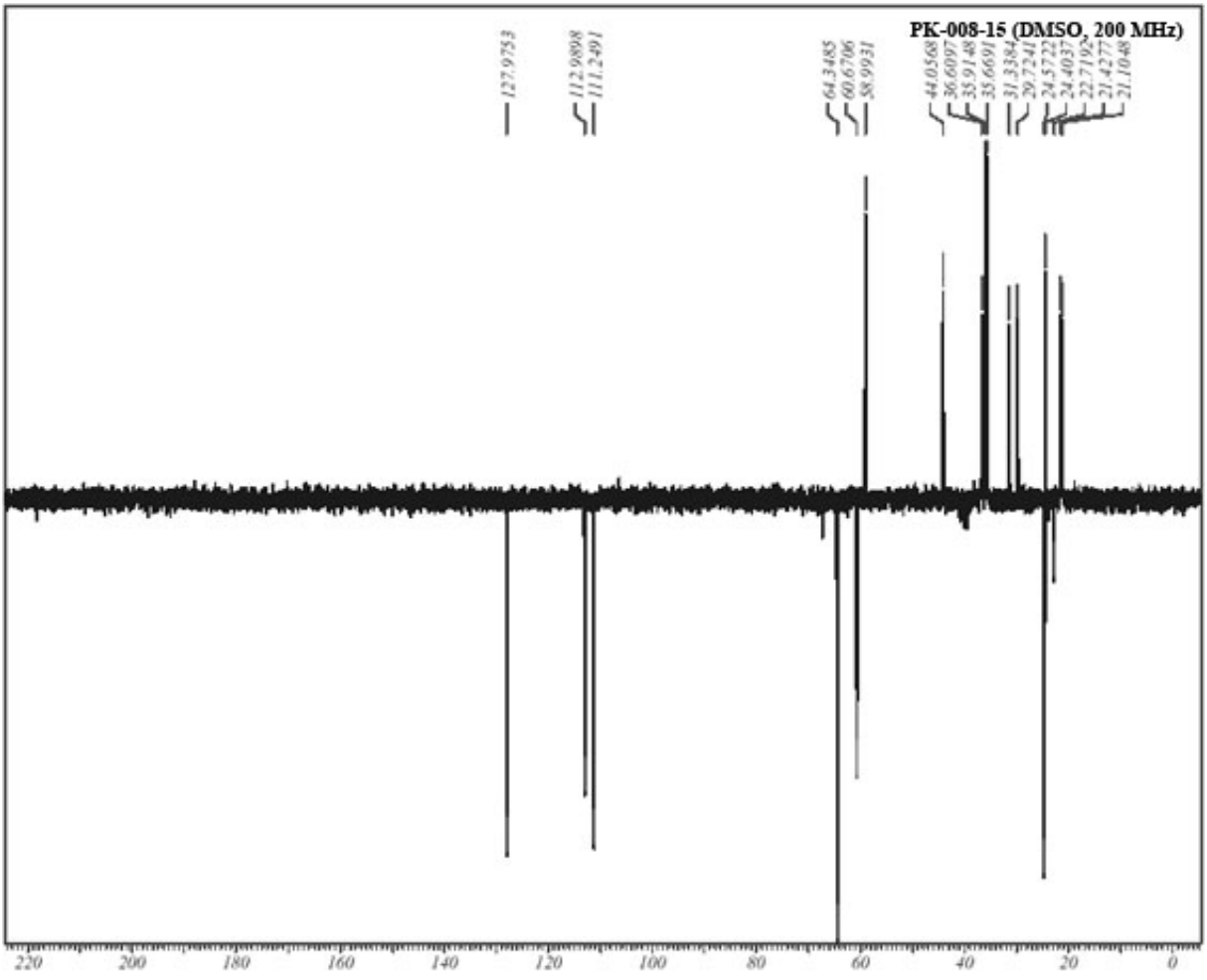
Synthesis of 17(*trans*)]-17-[(3-hydroxy cyclobutyl) metyl]-morphinan-3, 14 diol and 17(*cis*)]-17-[(3-hydroxy cyclobutyl) metyl]-morphinan-3, 14 diol

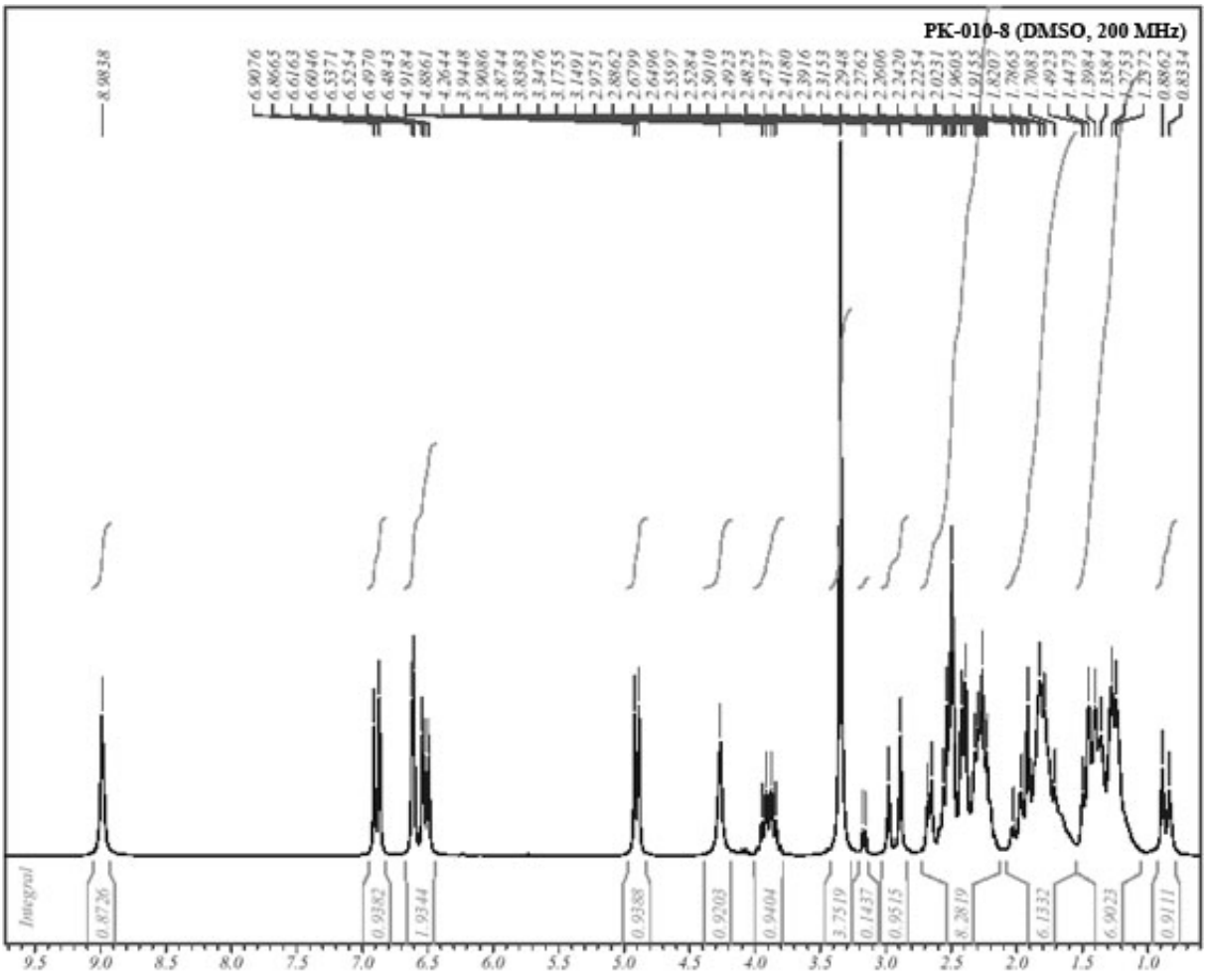


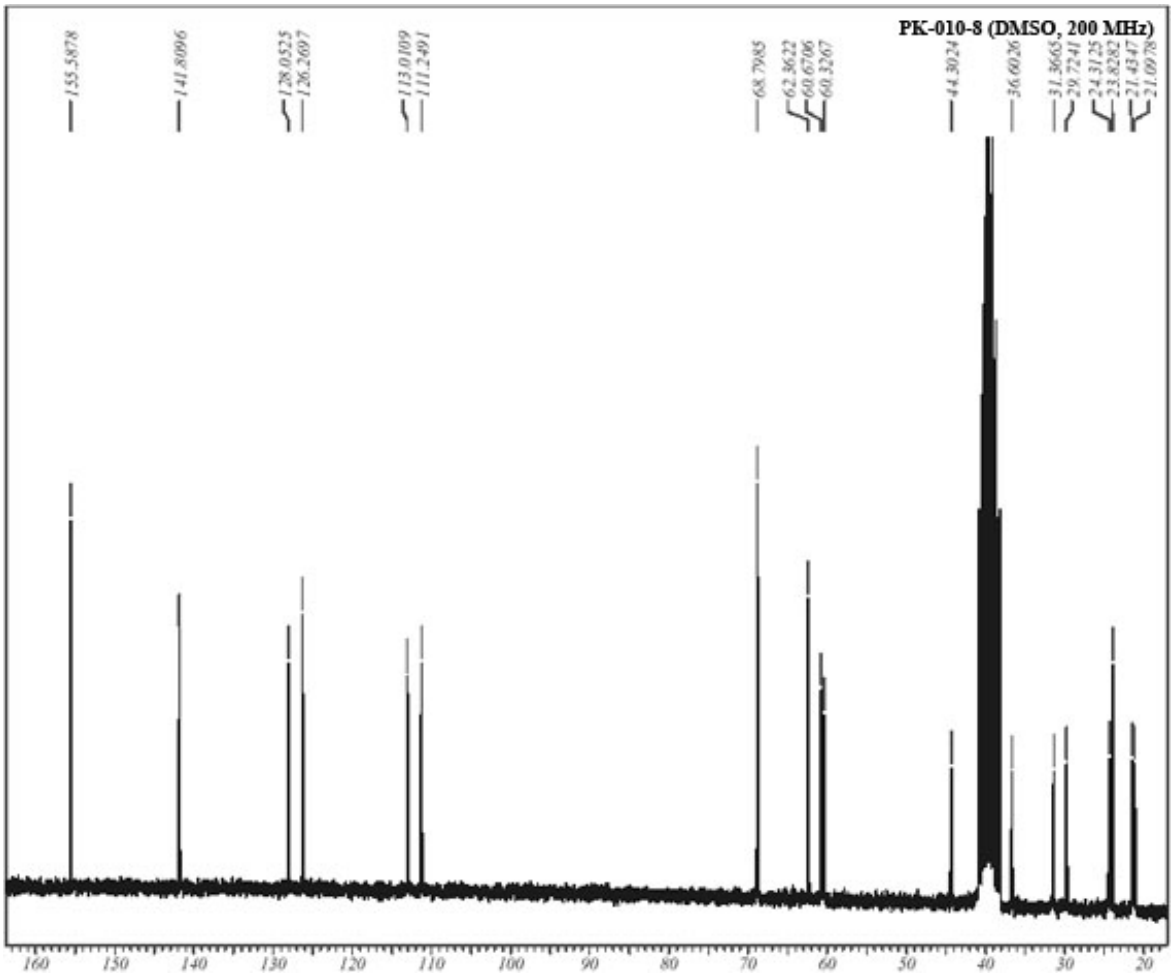

PK-008-5-trans (CDCl3, 200 MHz)
PK-008-6-trans (CDCl3, 200 MHz)

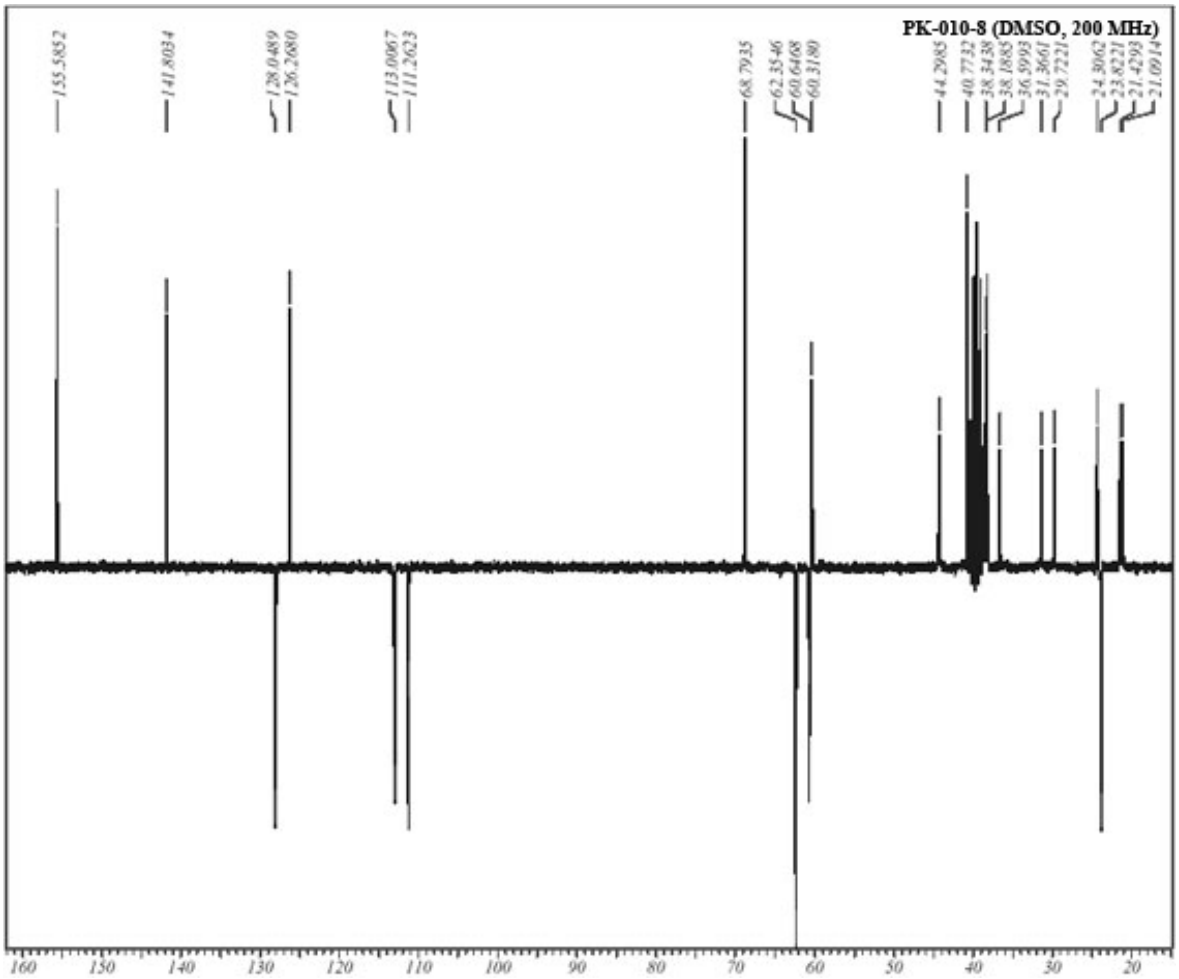

PK-008-15 (DMSO, 200 MHz)
8.9369
6.8900
6.8489
6.6026
6.5393
6.5185
6.4892
6.4785
4.8852
4.8558
4.2596
4.1902
4.1589
3.3231
2.9761
2.8872
2.6878
2.6684
2.5685
2.5323
2.5010
2.4932
2.4297
2.3945
2.3329
2.2792
2.2361
2.2088
2.0407
1.9800
1.8403
1.7181
1.6624
1.4434
1.3834
1.2831
1.2469
1.0417
1.0114
0.8999
0.8442
1.0000
1.0745
2.1621
1.0331
2.0572
1.0688
0.6882
7.3968
9.8846
5.7967
1.1509
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


PK-008-15 (DMSO, 200 MHz)
155.5878
141.7745
127.9753
126.2065
112.9898
111.2491
68.8407
64.3485
60.6706
58.9931
44.0568
40.8631
36.6026
35.9078
35.6761
31.3384
29.7311
24.5722
24.4037
22.7192
21.4277
21.1048
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

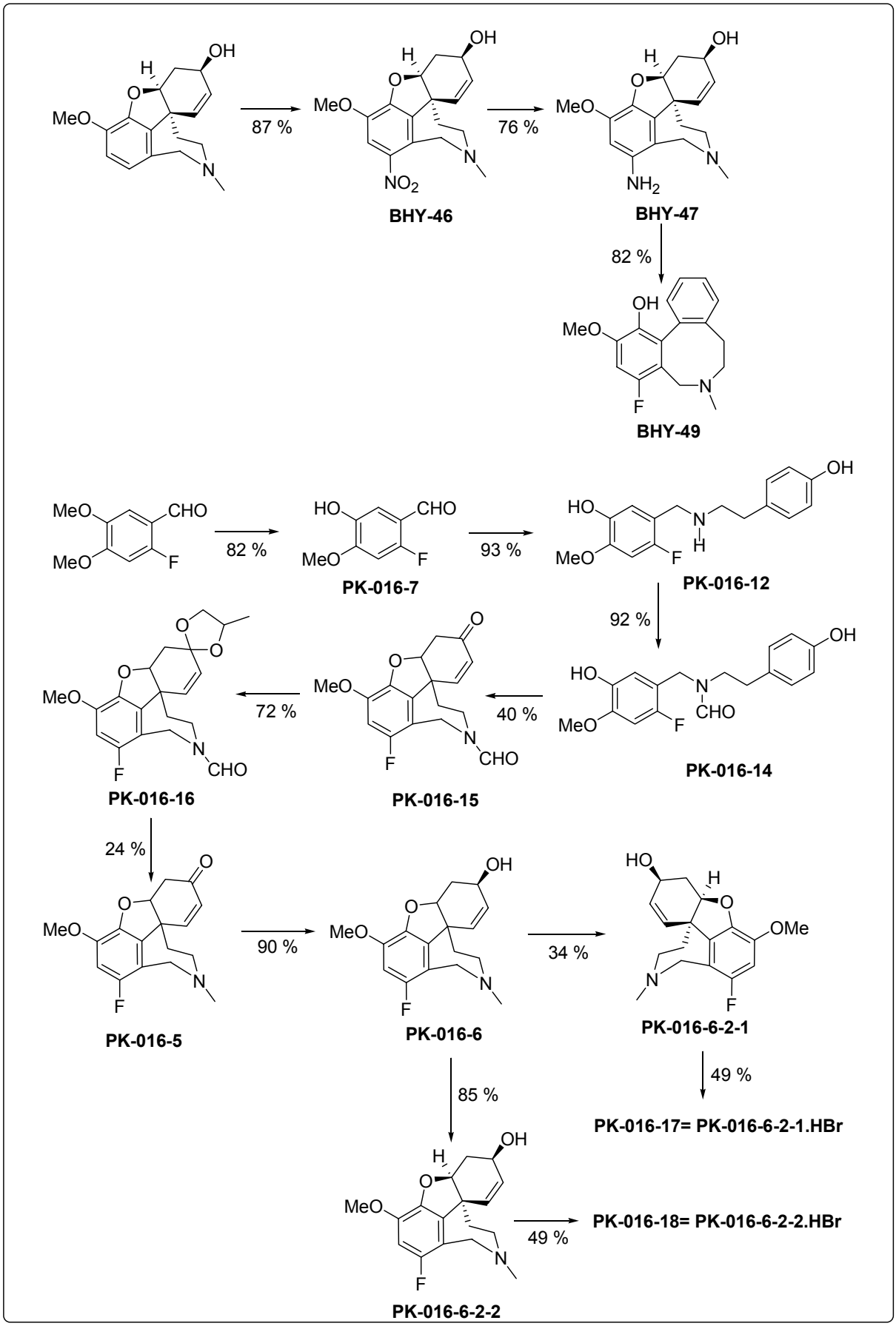

PK-008-15 (DMSO, 200 MHz)
127.9753
112.9898
111.2491
64.3485
60.6706
58.9931
44.0568
36.6097
35.9148
33.6691
31.3384
29.7241
24.5722
24.4037
22.7192
21.4277
21.1048

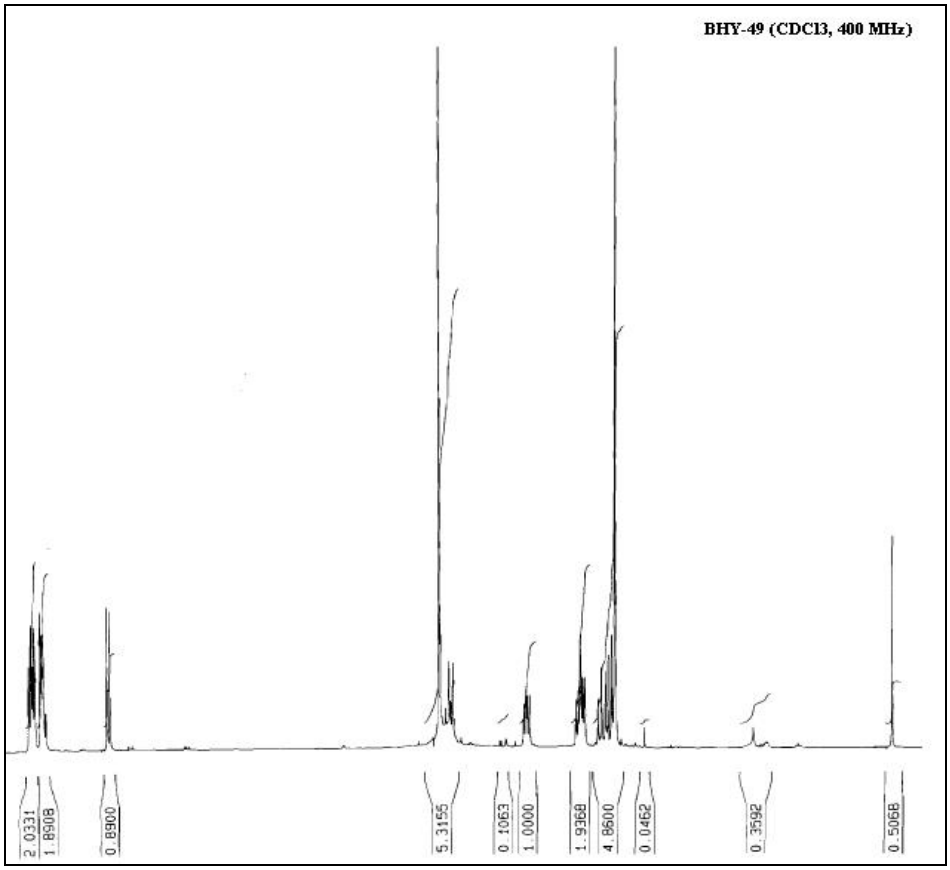

PK-010-8 (DMSO, 200 MHz)
8.9838
6.9076
6.8665
6.6163
6.6046
6.5371
6.5254
6.4970
6.4843
4.9184
4.8861
4.2644
3.9448
3.9086
3.8744
3.8383
3.3476
3.1755
3.1491
2.9751
2.8862
2.6799
2.6496
2.5597
2.5284
2.5010
2.4923
2.4825
2.4737
2.4180
2.3916
2.3153
2.2948
2.2762
2.2606
2.2420
2.2254
2.0231
1.9605
1.9155
1.8207
1.7865
1.7083
1.4923
1.4473
1.3984
1.3584
1.2753
1.2572
0.8862
0.8334
Integral
0.8726
0.9382
1.9344
0.9388
0.9203
0.9404
3.7519
0.1437
0.9515
8.2819
6.1332
6.9023
0.9111

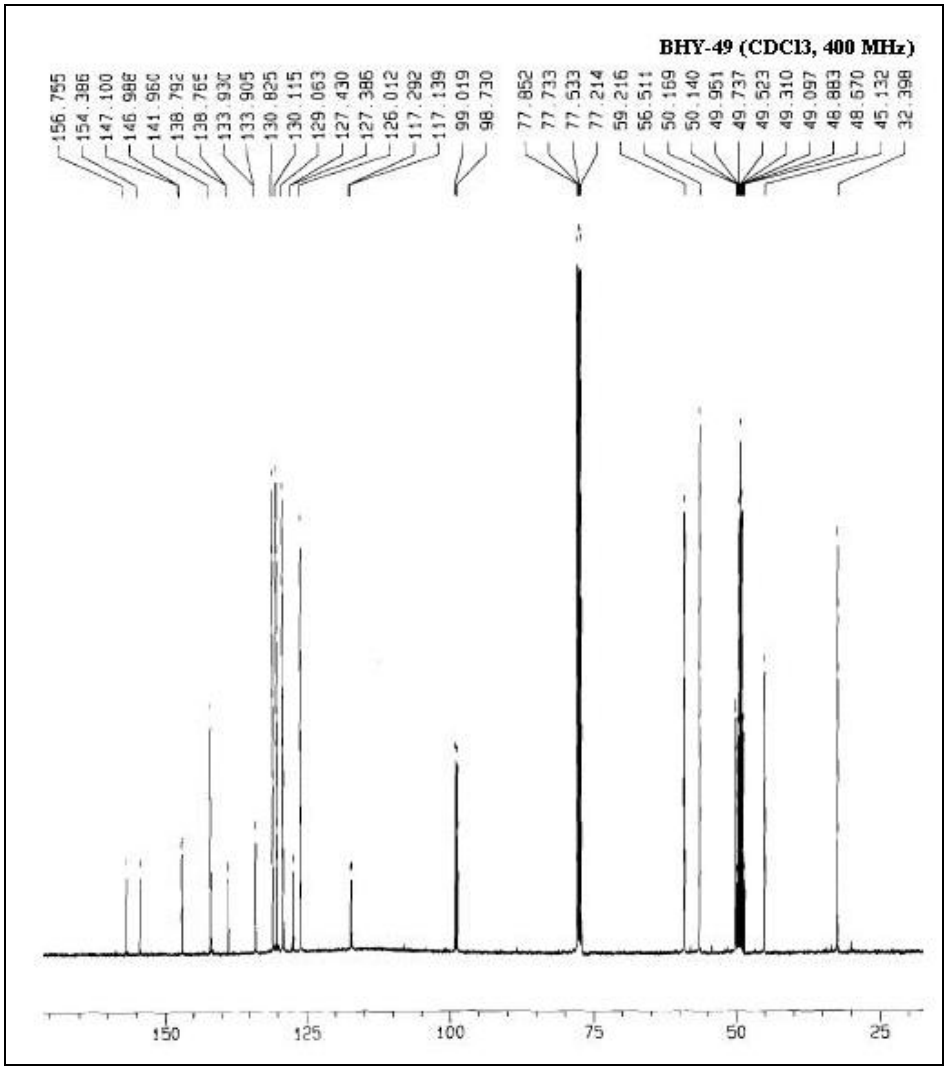

PK-010-8 (DMSO, 200 MHz)
155.5878
141.8096
128.0525
126.2697
113.0109
111.2491
68.7985
62.3622
60.6706
60.3267
44.3024
36.6026
31.3665
29.7241
24.3125
23.8282
21.4347
21.0978
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

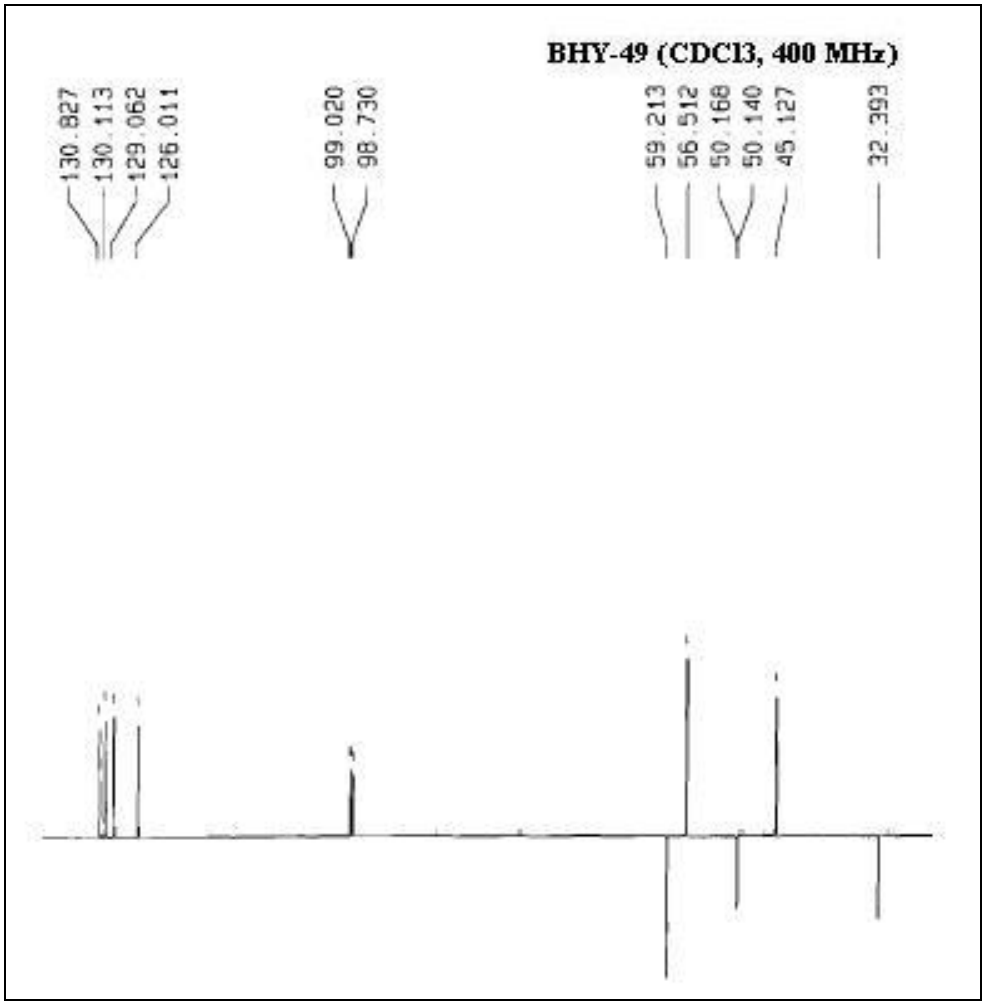

PK-010-8 (DMSO, 200 MHz)
155.5852
141.8034
128.0489
126.2680
113.0067
111.2623
68.7935
62.3546
60.6468
60.3180
44.2985
40.7732
38.3438
38.1885
36.5993
31.3661
29.7221
24.3062
23.8221
21.4293
21.0914
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

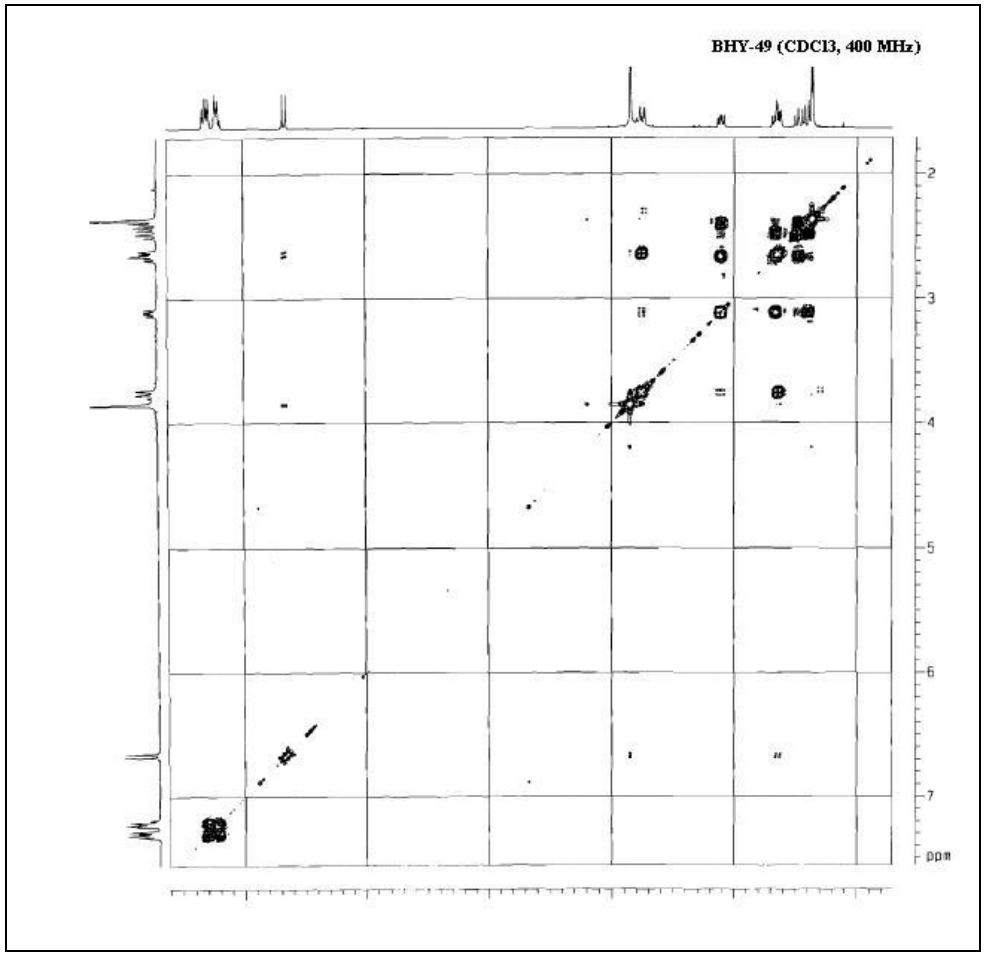
Synthesis of (+)- and (-)-8-Fluorogalanthamine

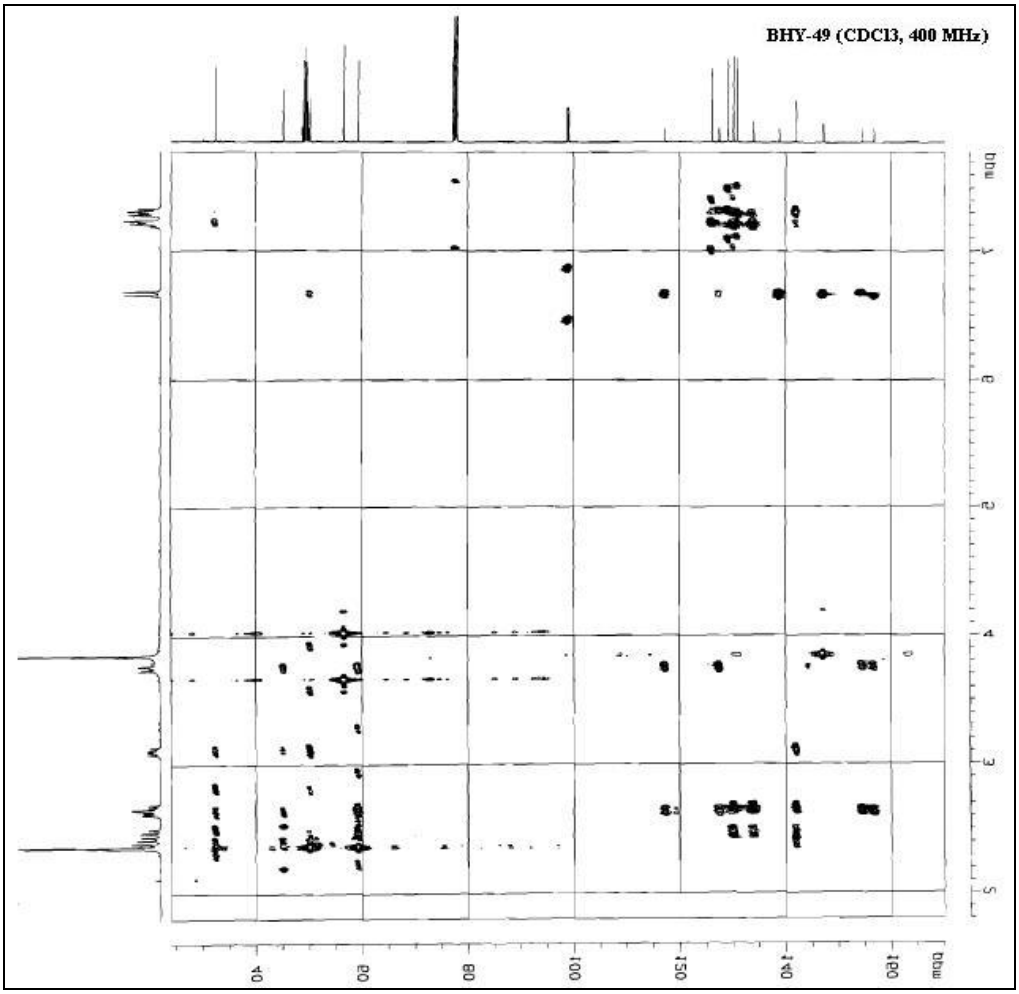


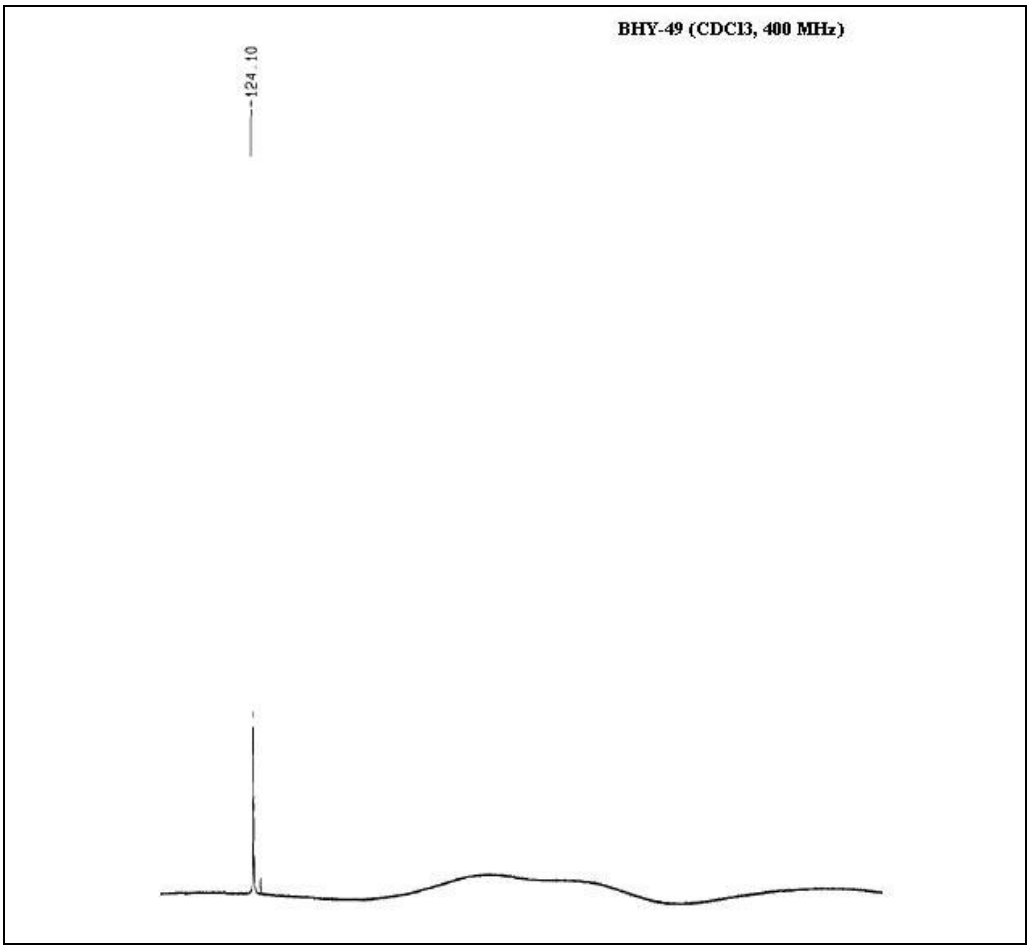

BHY-49 (CDCl3, 400 MHz)
2.0331
1.8908
0.8900
5.3155
0.1063
1.0000
1.9368
4.8600
0.0462
0.3592
0.5068

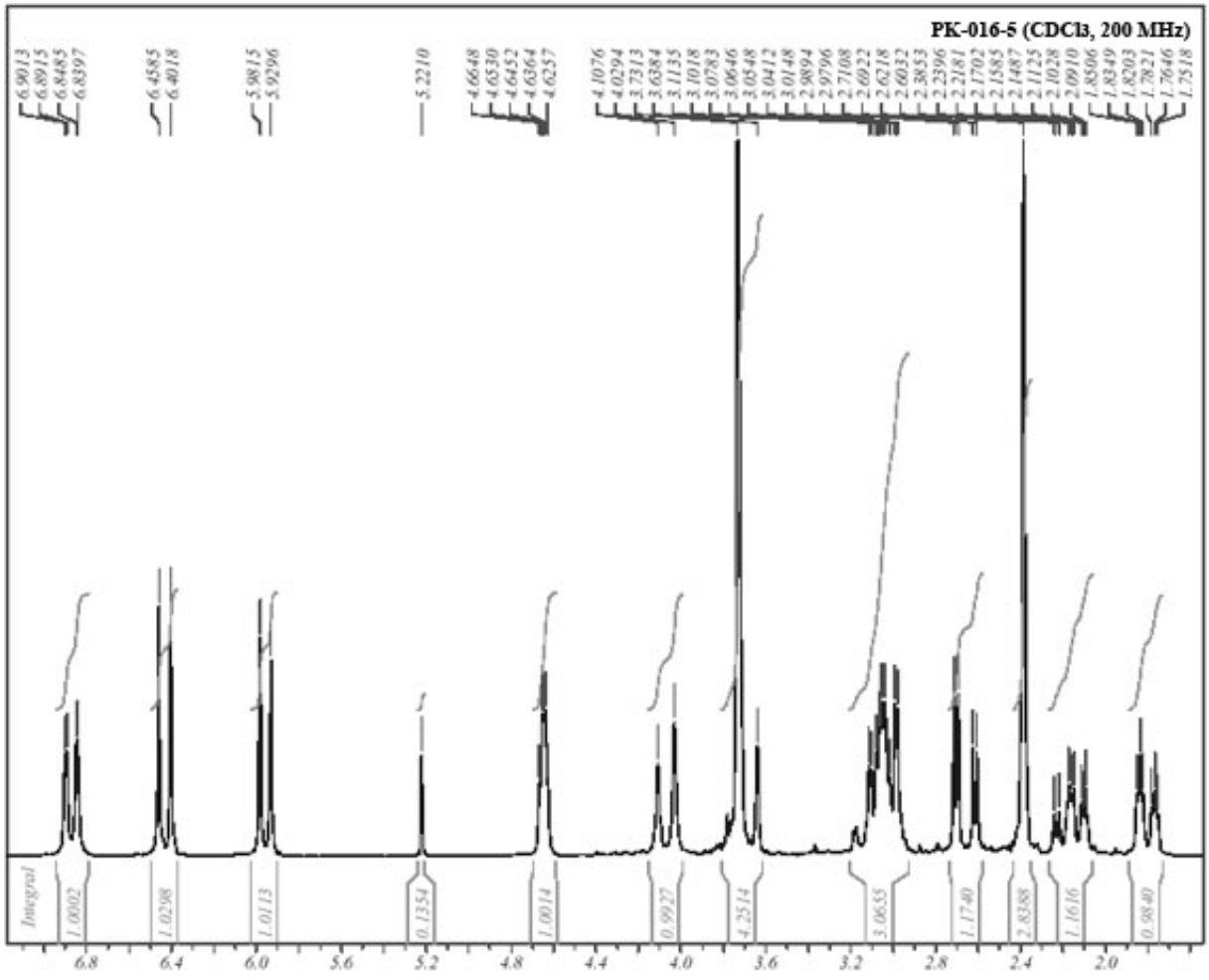

BHY-49 (CDCl3, 400 MHz)
156.755
154.386
147.100
146.988
141.960
138.792
138.765
133.930
133.905
130.825
130.115
129.063
127.430
127.386
126.012
117.292
117.139
99.019
98.730
77.852
77.733
77.533
77.214
59.216
56.511
50.169
50.140
49.951
49.737
49.523
49.310
49.097
48.883
48.670
45.132
32.398
150
125
100
75
50
25

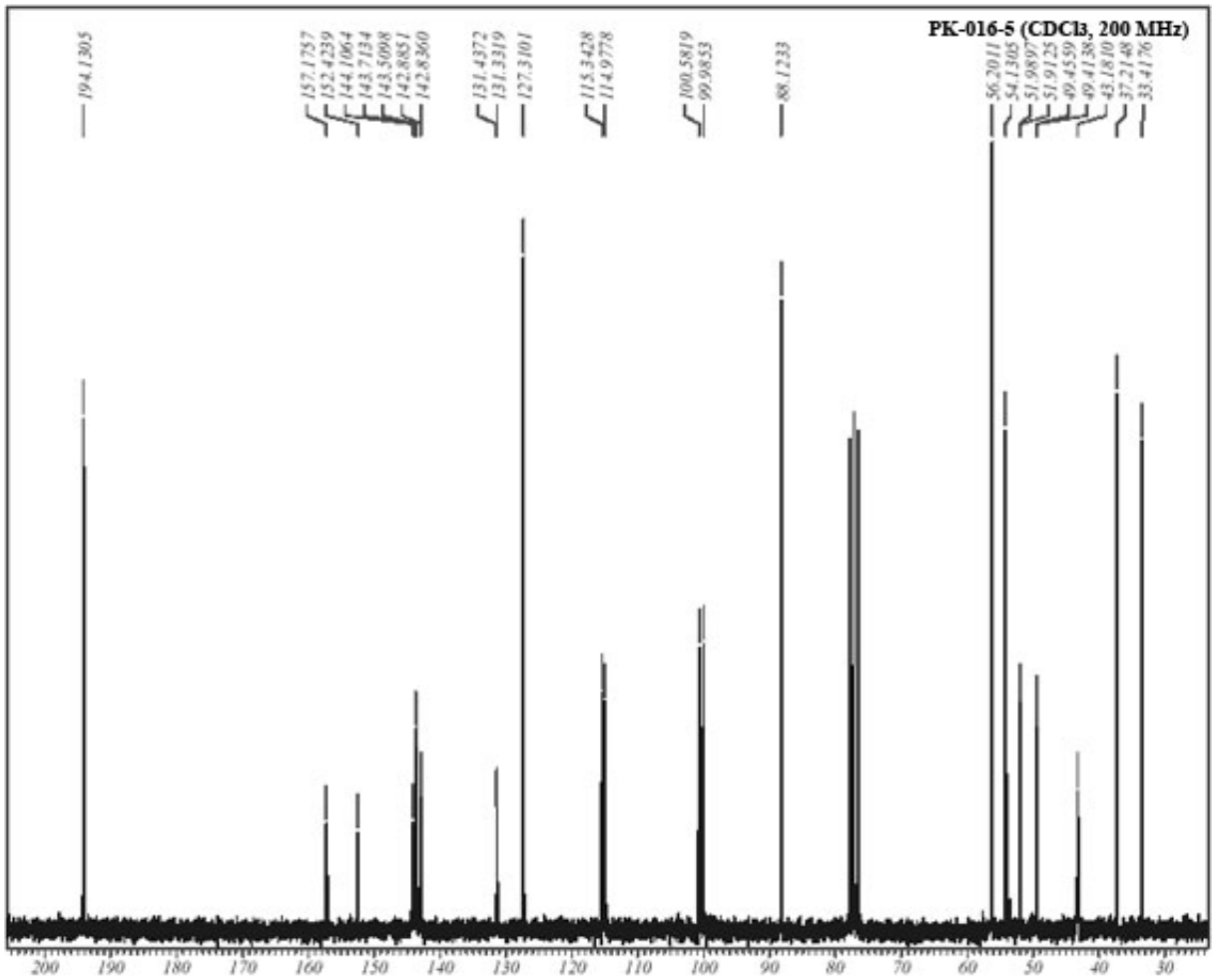

BHY-49 (CDCl3, 400 MHz)
130.827
130.113
129.062
126.011
99.020
98.730
59.213
56.512
50.168
50.140
45.127
32.393


BHY-49 (CDCl3, 400 MHz)
2
3
4
5
6
7
ppm

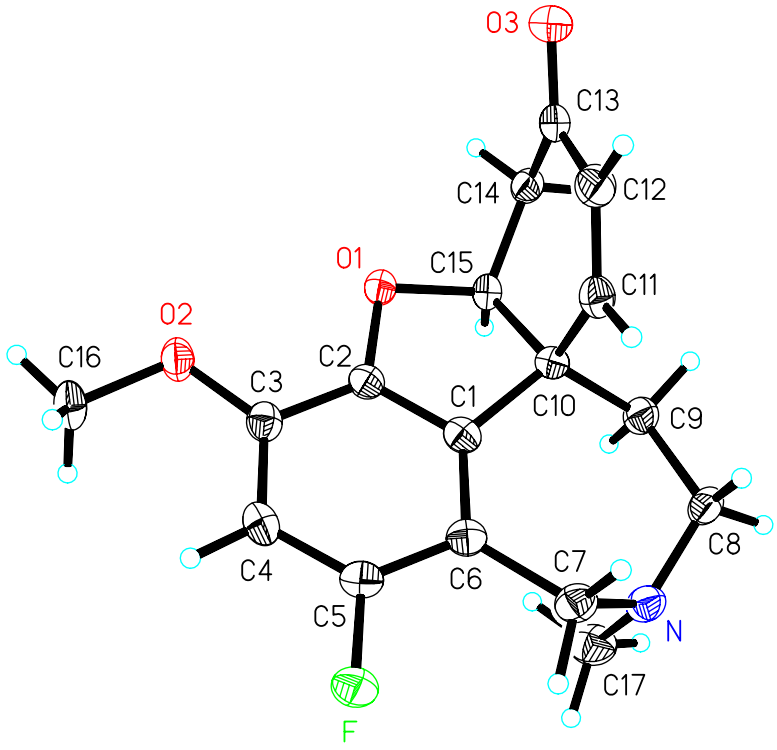

BHY-49 (CDCl3, 400 MHz)
ppm
ppm

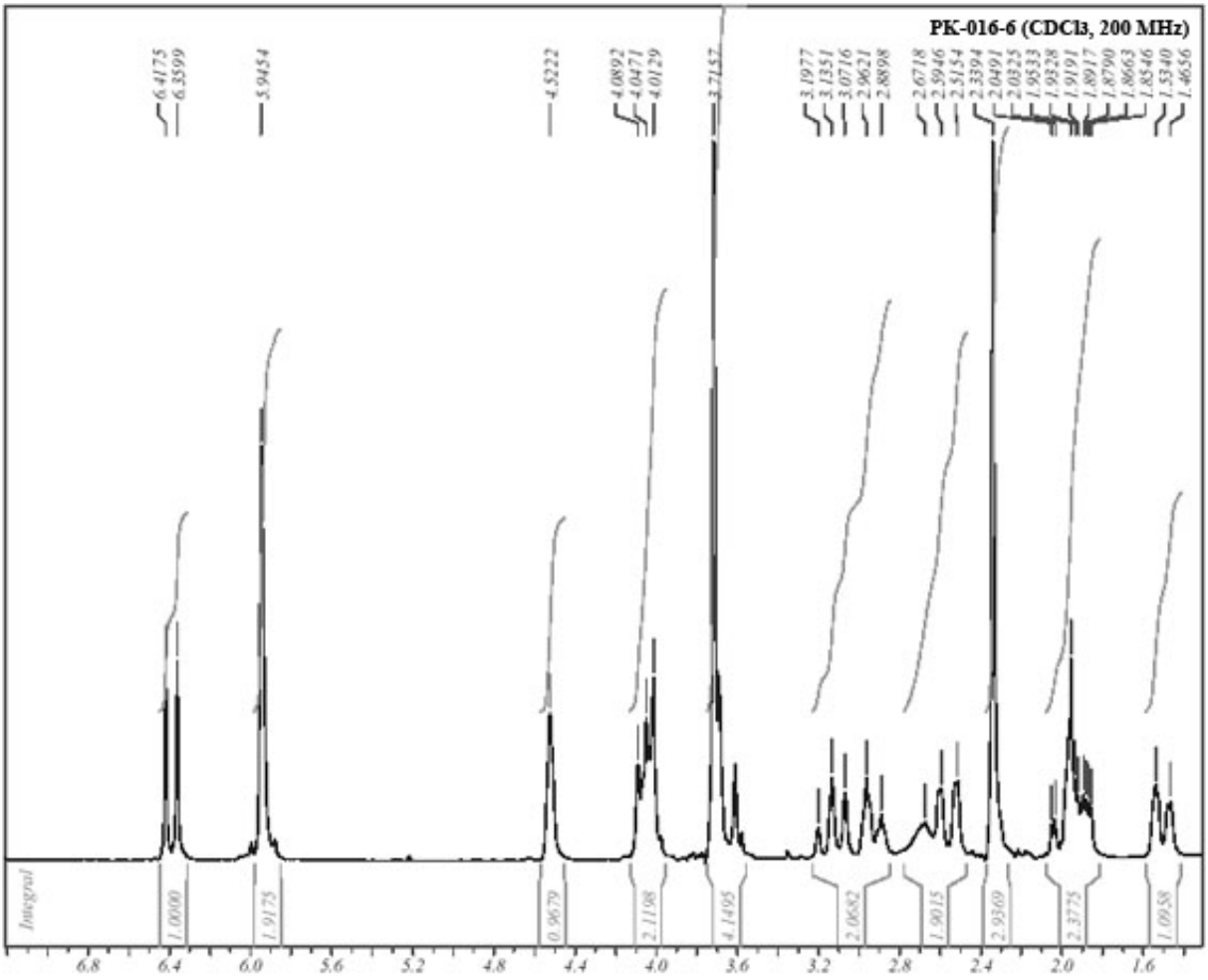

BHY-49 (CDCl3, 400 MHz)
124.10

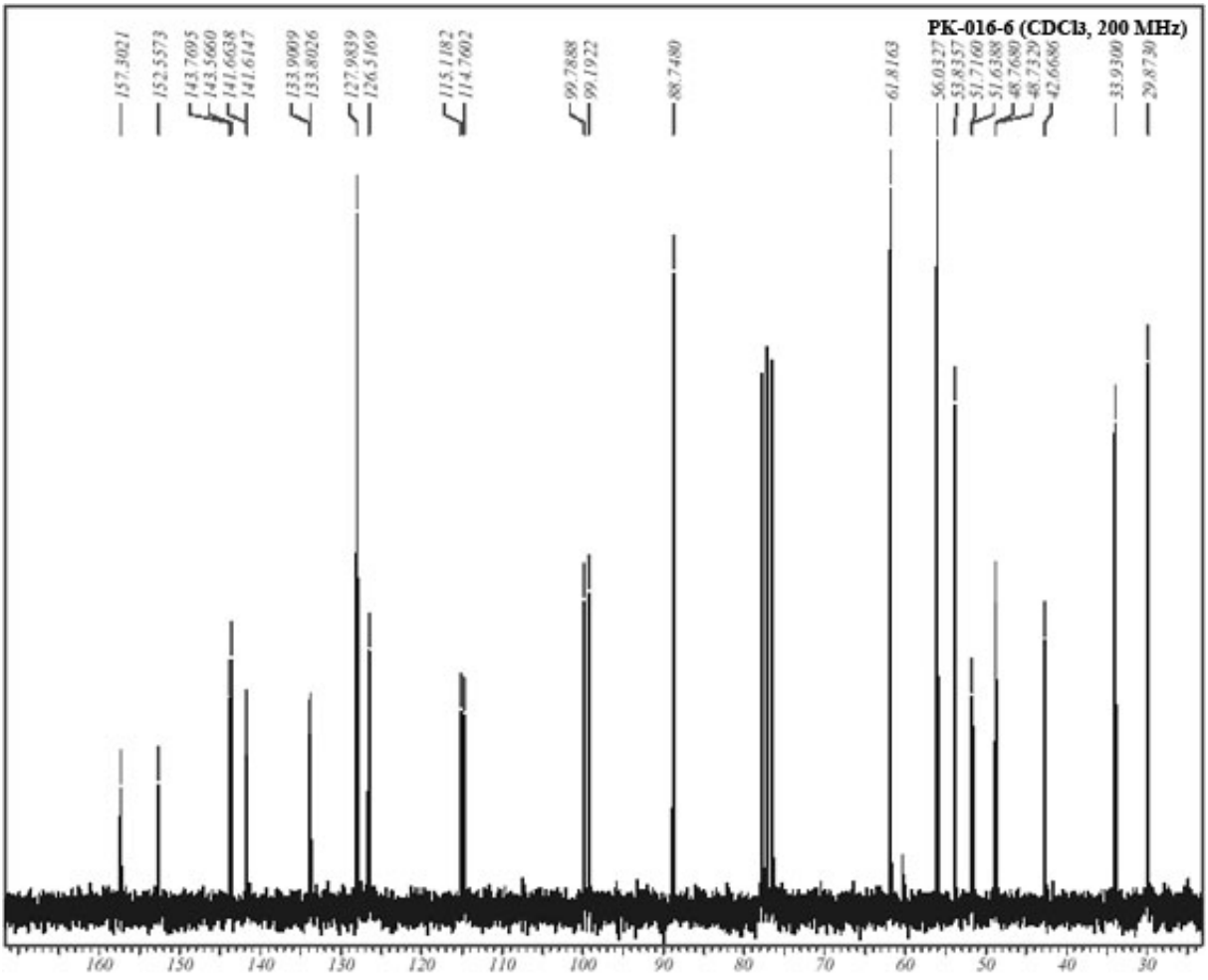

PK-016-5 (CDCl3, 200 MHz)
6.9013
6.8915
6.8485
6.8397
6.4585
6.4018
5.9815
5.9296
5.2210
4.6648
4.6530
4.6452
4.6364
4.6257
4.1076
4.0294
3.7313
3.6384
3.1135
3.1018
3.0783
3.0646
3.0548
3.0412
3.0148
2.9894
2.9796
2.7108
2.6922
2.6218
2.6032
2.3853
2.3596
2.2181
2.1702
2.1585
2.1487
2.1125
2.1028
2.0910
1.8506
1.8349
1.8203
1.7821
1.7646
1.7518
Integral
1.0002
1.0298
1.0113
0.1354
1.0014
0.9927
4.2514
3.0655
1.1740
2.8388
1.1616
0.9840


PK-016-5 (CDCl3, 200 MHz)
194.1305
157.1757
152.4239
144.1064
143.7134
143.5098
142.8851
142.8360
131.4372
131.3319
127.3101
115.3428
114.9778
100.5819
99.9853
88.1233
56.2011
54.1305
51.9897
51.9125
49.4559
49.4138
43.1810
37.2148
33.4176

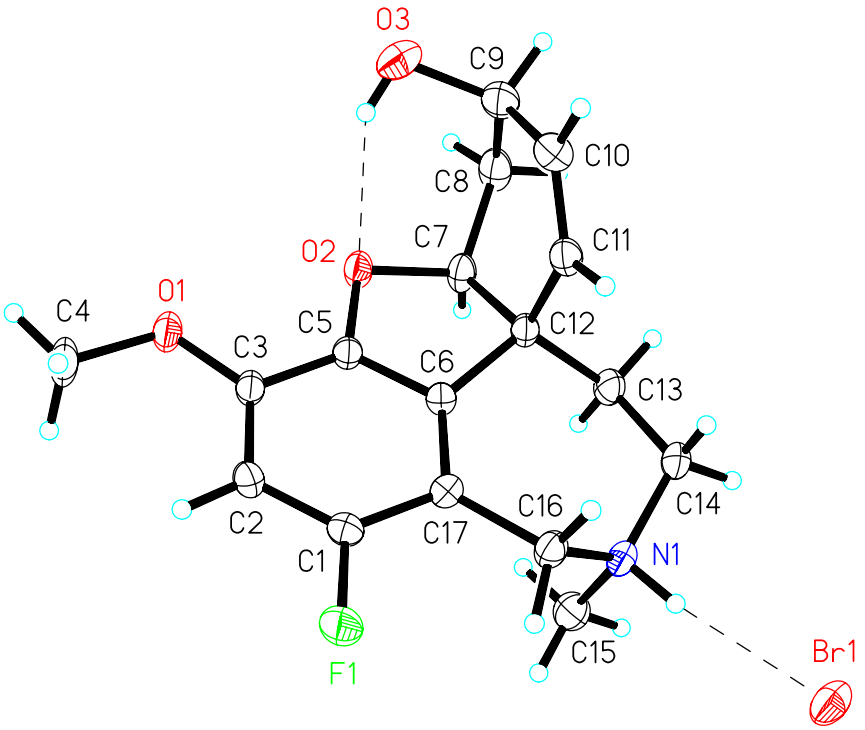
PK-016-5


O3
C13
C14
C12
O1
C15
C11
O2
C16
C2
C3
C1
C10
C9
C8
C4
C6
C7
C5
N
C17
F


PK-016-6 (CDCl3, 200 MHz)
6.4175
6.3599
5.9454
4.5222
4.0892
4.0471
4.0129
3.7157
3.1977
3.1351
3.0716
2.9621
2.8898
2.6718
2.5946
2.5154
2.3394
2.0491
2.0325
1.9533
1.9328
1.9191
1.8917
1.8790
1.8663
1.8546
1.5340
1.4656
Integral
1.0000
1.9175
0.9679
2.1198
4.1495
2.0682
1.9015
2.9369
2.3775
1.0958
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6


PK-016-6 (CDCl3, 200 MHz)
157.3021
152.5573
143.7695
143.5660
141.6638
141.6147
133.9009
133.8026
127.9839
126.5169
115.1182
114.7602
99.7888
99.1922
88.7480
61.8163
56.0327
53.8357
51.7160
51.6388
48.7680
48.7329
42.6680
33.9300
29.8730
160
150
140
130
120
110
100
90
80
70
60
50
40
30

PK-016-18


O3
C9
C10
C8
C7
C11
O2
O1
C4
C5
C12
C3
C6
C13
C14
C2
C16
C17
N1
C1
C15
Br1
F1

Dipl. Ing. Knesl Petr, Gessayova 16 Bratislava 85103, Slovak Republic

Curriculum Vitae

Personal Date:

Date of Birth	20.04.1974.
Place of Birth	Uherske Hradiste, Czech Republik.
Nationality	Czech Republic, Slovak Republic

2004	TU Wien, Institute for applied Organic Synthesis. Dissertation, Topic: Synthesis of deuterated analogs, reference standards and metabolites of pharmaceuticals.
2000-2003	Q-chem l.t.d., Nobelova 9, Bratislava, Chemist.
1998-2000	Slovak Academy of Science, Institute of experimental Pharmacology. Scientific assistant. Focus: Synthesis of Stobadine derivates as potential antioxidants.
25.5.1998	State exam on the Institute of Organic Chemistry (cum laude), Faculty of Chemistry and Technology, Slovak Technical University.
1993-1998	Faculty of Chemistry and Technology, Slovak Technical University. Diploma thesis: Synthesis of new 1,4-dihydropyridine derivates.
1.6.1992	University-entrance diploma (cum laude), Gymnasium L.Saru, Bratislava.
1988-1992	Gymnasium in Samorin and Bratislava.
1980-1988	Primary school in Uherske Hradiste and Dunajska Luzna.