UNIVERSITA' DEGLI STUDI DI CATANIA
FACOLTA' DI FARMACIA

DOTTORATO DI RICERCA IN SCIENZE DEL FARMACO

Antonio Leonardi

DESIGN, SYNTHESIS OF SUBSTITUTED PURINES, PHENYLPYRROLIDINE DERIVATIVES, CYCLIC AND ACYCLIC BIS(*p*-NITROBENZENESULFONAMIDES), AND *IN VITRO* ANTI-CANCER ACTIVITY AGAINST LUNG, BREAST AND PROSTATE CELL LINES

Relatore:
Prof. **J.M. Campos**
Correlatore:
Prof. **O. Prezzavento**

Ciclo XXIV (2008-2011)

Per mio nonno

Abbreviations

- **5-FU** 5-fluorouracil
- **ATM** Ataxia telangiectasia mutated
- **AZT** 3'-azide-2',3'-dideoxythymidine
- **Bcl-2** B cell lymphoma gene-2
- **Cdk** Cyclin-dependent kinase
- **Cyc** Cyclins
- **DBAD** Di-*tert*-butyl azodicarboxylate
- **ddNS** 2',3'-dideoxynucleosides
- **DEPT** Distorsionless Enhancement by Polarization Transfer
- **DIPAD** Diisopropyl azodicarboxylate
- **DMF** Dimethylformamide
- **dNTPs** deoxynucleoside-5'-triphosphate
- **DRG** Delayed Response Genes
- **ERG** Early Response Genes
- **EtOAc** Ethyl acetate
- **FBS** Fetal bovine serum
- **FdUMP** Fluorodeoxyuridine monophosphate
- **FUTP** 5-fluorouridine-5-triphosphate
- **HMQC** Heteronuclear Multiple-Quantum Correlation
- **HMDS** Hexamethyldisilazane
- **HSQC** Heteronuclear Single Quantum Coherence
- **IRF-1** Interferon regulatory factor
- **KO*t*Bu** Potassium *tert*-butoxide
- **MTT** (3-{4,5-Dimethylthiazol-2-yl}-2,5-diphenyltetrazolium) bromide
- **$NaBH_4$** Sodium borohydride
- **NMR** Nuclear Magnetic Resonance
- **PPh_3** Triphenylphospine
- **ppm** Parts per million
- **pRb** Retinoblastoma
- **TCS** Trimethylchlorosilane
- **TFA** Trifluoroacetic acid

- **THF** Tetrahydrofurane
- **TMSOTf** Trimethylsilyl trifluoromethanesulfonate
- **TLC** Thin layer chromatography
- **TS** Thymidylate synthase

INDEX

1. DEFINITION OF TUMOUR

A tumour is commonly used as a synonym for a neoplasm (a solid or fluid-filled lesion that could or could not be formed by an unusual growth of neoplastic cells) that appears enlarged in size. The term tumour is derived from the Latin word for "swelling": tumour. In its medical sense it has traditionally meant an abnormal swelling of the flesh. In contemporary English, the word tumour is often used as a synonym for a cystic (liquid-filled) growth or solid neoplasm, with other forms of swelling often referred to merely as swellings. Related terms are common in medical literature, where the nouns “tumefaction and tumescence” (derived from the adjective tumefied), are current medical terms for non-neoplastic swelling. This type of swelling is most often caused by inflammation caused by trauma, infection, etc. Tumours may be caused by conditions other than an overgrowth of neoplastic cells, however. Cysts (such as sebaceous cysts) are also referred to as tumours, even though they have no neoplastic cells. This is standard in medical billing terminology (especially when billing for a growth whose pathology has yet to be determined).

1.1 Difference between Tumour and Cancer

A tumor is basically an abnormal growth or swelling of something. Tumours can be benign, that is, non-cancerous. An example of this would be a “lipoma”, which often manifest as fatty encapsulated lumps beneath the skin of the arms, neck or back. They are unsightly, but although they do not harm, some benign tumours can compromise health, if they grow large enough to compress or obstruct other organs, like the adrenal gland.

Cancer is a malignant growth, and this can be detected it it grows in an “aggressive” manner, invades surrounding tissues and metastasizes to other areas of the body. Cancer is literally runaway cell growth that the cellular replication machinery is unable to shut down. Cancer cells can spread to other parts of the body through the blood and lymph systems. Cancer is not just one disease but many diseases. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the colon is called colon cancer; cancer that begins in basal

cells of the skin is called basal cell carcinoma. Cancer types can be grouped into broader categories. The main categories of cancer include:

Carcinoma - Cancer that begins in the skin or in tissues that line or cover internal organs.

Sarcoma - Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

Leukemia - Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

Lymphoma and myeloma - Cancers that begin in the cells of the immune system.

Central nervous system cancers - They begin in the tissues of the brain and spinal cord.

All cancers begin in cells, the body's basic units of life. To understand cancer, it is helpful to know what happens when normal cells become cancer cells. The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are

needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells.

1.2. Common Carcinomas

The most frequent types of cancer in the carcinoma class are the cancers of lung, prostate and breast. Worldwide, lung cancer is the most common cancer in terms of both incidence and mortality (1.1 million new cases per year and 0.95 million deaths in males and 0.51 million new cases per year and 0.43 million deaths in females). Prostate cancer is the second most common cancer in men worldwide. Around 910.000 cases of prostate cancer were recorded in 2008. In the same year, breast cancer caused 458.503 deaths worldwide. Many studies were done and are being developed today to understand, prevent and treat these classes of cancers. Many results were obtained and the different cancer therapies have decreased the mortality index worldwide. However these cancers are not extinguished and there is still much to do. In cancer therapy the isolation of pure cancer cell lines was a crucial point that has permitted the development of new selective drugs. Pure cancer cell lines allow *in vitro* studies. The molecular mechanisms of anticancer activity could be investigated and the

understanding of cancer is facilitated. In the cases of lung, prostate and breast cancers the pure cell lines most used are the following:

- A549 cells are adenocarcinomic human alveolar basal epithelial cells that were developed in 1972 by D. J. Giard, et al. through the removal and culturing of cancerous lung tissue in the explanted tumour of a 58-year-old Caucasian male.

- PC3 cells were originally derived from advanced androgen independent bone metastasized prostate cancer.

- MCF-7 is a cell line that was first isolated in 1970 from the breast tissue of a 69-year old Caucasian woman.

2. EPIDEMIOLOGY

The most significant risk factor is age. According to cancer researcher Robert A. Weinberg, "if we live long enough, sooner or later we would all get cancer".[1] Essentially the increase in cancer rates between prehistoric times and people who died in England between 1901 and

1905 is due to increased life spans. Since then, some other factors, especially the increased use of tobacco, have further raised the rates.[1]

Over a third of cancer deaths worldwide are due to potentially modifiable risk factors. The leading modifiable risk factors worldwide are:

- Tobacco smoking, which is strongly associated with lung cancer, mouth, and throat cancer.
- Drinking alcohol, which is associated with a small increase in oral, esophageal, breast, and other cancers.
- A diet low in fruit and vegetables.
- Physical inactivity, which is associated with increased risk of colon, breast, and possibly other cancers.
- Obesity, which is associated with colon, breast, endometrial, and possibly other cancers.
- Sexual transmission of human papillomavirus, which causes cervical cancer and some forms of anal cancer.

Men with cancer are twice as likely as women to have a modifiable risk factor for their disease.[2]

Other lifestyle and environmental factors known to affect cancer risk (either beneficially or detrimentally) include the use of exogenous

hormones (e.g., hormone replacement therapy causes breast cancer), exposure to ionizing radiation and ultraviolet radiation, and certain occupational and chemical exposures.

Every year, at least 200,000 people die worldwide from cancer related to their workplace.[3] Millions of workers run the risk of developing cancers such as lung cancer and mesothelioma from inhaling asbestos fibers and tobacco smoke, or leukemia from exposure to benzene at their workplaces.[3] Currently, most cancer deaths caused by occupational risk factors occur in the developed world.[3] It is estimated that approximately 20,000 cancer deaths and 40,000 new cases of cancer each year in the U.S. are attributable to occupation.[4]

The development and growth in the field of vital statistics made it possible to study the patterns of cancer mortality. Unfortunately, mortality data published nowadays by the World Health Organization (WHO) are of different quality and may have several biases:

- Coverage of the population is incomplete as mortality rates are implausibly low in some countries.
- Validity of the cause of death information is low in some countries.

The need for more accurate data on cancer patients and the relatively clear-cut pathological case confirmation led to the development of hospital- and population-based cancer registries.

- Hospital-based registries are concerned with the recording of information on cancer patients seen in a particular hospital. The main purpose of such registries is to contribute to patient care by providing readily accessible information on all cancer patients, seen in this hospital, the treatment they had received, and its result. These registries cannot provide measures of the occurrence of cancer in a defined population, because their catchments population, for instance the population from which all cases arise, is unknown.

- Population-based registries collect data on all new cases of cancer occurring in a well-defined population over a given period of time. The first population-based cancer registry was established in Connecticut in the United States in 1940 with cases registered retrospectively to 1935. In Europe, the first registry started to operate in Denmark in 1942; others were set up in subsequent decades, so that by 1955, almost 20 existed in various regions and countries (England and Wales, Slovenia, Finland and other Nordic countries, and a few others). In some

countries cancer registration is nationwide; in others, cancer registries cover only a proportion of the population. The main role of these registries is the provision of data on cancer incidence and the prevalence of some, also survival and mortality.[5]

3.1. CELL PROLIFERATION AND ITS REGULATION: THE CELL CYCLE

Most eukaryotic cells follow a process of growth and division called cell cycle. These events include a growth stage, mitosis or nuclear division and cytokinesis or division of the cytoplasm. The end of the cell cycle coincides with the duplication of the cell structures and the subsequent division into two daughter cells genetically identical to each other (Fig. 1). In multicellular organisms, cell division as well as for reproduction, it is also necessary to allow the body growth during development, and to replace cells that have been damaged or who have died for necrosis or apoptosis processes. Order to maintain within specified physiological limits the development, organization and structure of multicellular organisms, it is essential that cell proliferation be controlled and limited by a complicated system of regulatory mechanisms. For most of the

time cells can be found in a resting state (G0 phase, G= *gap*), during which they can perform all those functions for which they are differentiated and specialized. To leave this temporary resting state external signals that stimulate the cell are required to start a new cell cycle.

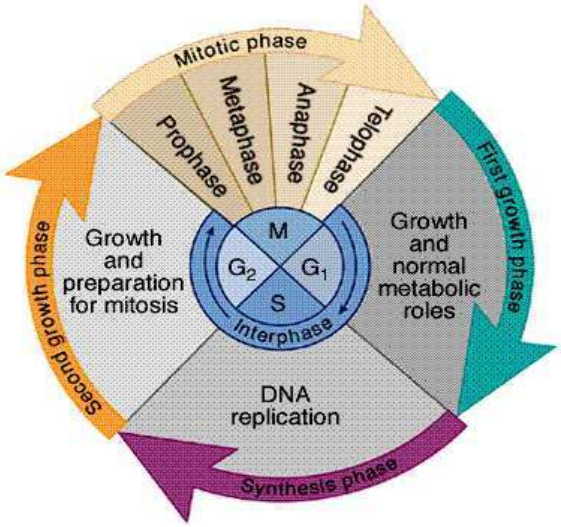


Figure 1. The Cell cycle

The cell cycle consists of an ordered series of stages during which, firstly the cell duplicates its contents and then divides into two daughters cells. Usually cell cycle consists of four distinct phases:

- **G1:** the cell controls its size and the external environment, and synthesizing RNA molecules and proteins grows.
- **S:** is the "synthesis" phase, in which the replication of genetic material takes place.
- **G2:** is the phase where all replicated chromosomes are checked and it occurs before the cell begins mitosis. The above three steps are generally called interphase.
- **M:** is the "mitosis", which includes prophase, metaphase, anaphase and telophase and concludes with cytokinesis (physical segregation of cellular material).

During a standard cell cycle there are 3 checkpoints. In G1 phase the cell increases its size and prepares to divide. At this stage there is the first control point: the **G1 checkpoint**, also called the **"restrict" point** (point R). This controls cell size and ensures the presence of a positive environment, and at the same time possible damaged DNA is checked.[6] After the first control point, S phase begins and the cell duplicates its genetic material, and then there is the G2 phase where

the cell grows again.[7] At the end of this phase there is the **G2 / M checkpoint** that controls cell size again and checks the complete duplication and integrity of all the genetic material. During this checkpoint damaged DNA will be repaired.

Subsequently, M phase begins and we find the last checkpoint, **checkpoints M**, that controls the correct chromosomes assembly on spindle apparatus.

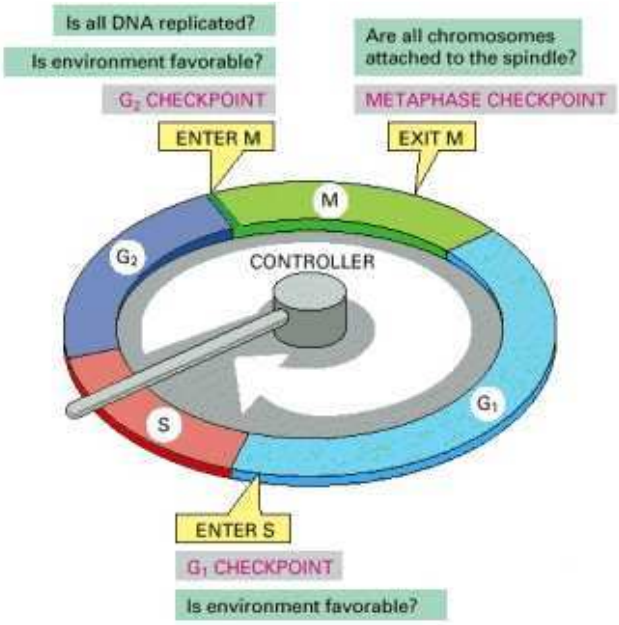


Figure 2. Cell cycle and checkpoints

Before proceeding, the cell ensures that the previous stages of the cell cycle have been carried out. At point R the cell integrates information

received during the first half of G1 phase and decides whether to complete the G1 phase and the cell cycle or to interrupt the cell cycle and to enter in G0 phase or in a greater spindle differentiation.[8]

At molecular level, we can say that the formation, the activation and the demolition of **cyclin-Cdk** (cyclin-dependent kinase) **complex** is the key event that drives the cell into the cell cycle. Initially the activity of Cdk kinase increases until the cells progresses on the cell cycle. When mitosis starts an enhanced activity of Cdks leads to an increase of phosphorylation of proteins that control: the chromosomes condensation, the cell membrane breaking and the spindly apparatus assembly. Cdks activity depends on association with other proteins: **the cyclins** (Cyc), for which there is a synthesis and degradation cycle in each cell division.

The key that allows passing through the R point would seem precisely the hyper-phosphorylation and the **retinoblastoma (pRb)** inactivation. The retinoblastoma is a tumour-suppressor that can be present in two forms: hypophosphorylated (active) or hyperphosphorylated (inactive). In the active form the pRb has the **transcription factor E2F** complexed in its structure, for this cell cycle is inhibited. However, when the cell enters S-phase, pRb is phosphorylated by the cyclin-Cdk complex and E2F is detached; in this way it becomes active. The

phosphorylation takes place in two stages: the first is mediated by the Cdk4/Cyc-D, Cdk6/Cyc-D complexes, and the second by the Cdk2/Cyc-E, Cdk2/Cyc-A complexes. The **type-1 phosphatase** dephosphorylates the retinoblastoma and its activity is inhibited by the complexes Cdk/Cyc. Cyclins activity is induced by the presence of growth factors and is suppressed in the first half of the G1 phase by cell proliferation inhibitors. The link between growth factors and membrane receptors stimulates a cascade of intracellular translation (Ras-Raf-MAP kinase) that activates the **Early Response Genes (ERG)** and the **Delayed Response Genes (DRG)**. The Cdk-4/Cyc-D, Cdk-6/Cyc-D complexes can induce the Cdk inhibitor p27Cip1 destruction, enabling the cell to pass through the point R. Additionally, through various negative feedback mechanisms in G1 phase the cyclins limit their formation: CycA-Cyc-B, through phosphorylation, inactivates E2F and Cyc-E is degraded by ubiquitin and probably by the same complex Cdk2/Cyc-E.[8,9] The checkpoint G2/M has the objective of verifying the duplicated DNA status in S phase. Checkpoint G2/M is regulated by CDK1 (or Cdk-2)/B-Cyc complex. CDK1 accumulates during the S/G2/M phases, and is degraded by ubiquitin during the mitosis metaphase-anaphase stages. *Anaphase promoter complex* (APC) transfers a ubiquitin residue to CDK1 and makes it

sensitive to proteolysis by proteasomes. The inactivation of Cdk activity is necessary to finish mitosis.[9]

3.2. APOPTOSIS: CELLULAR MECHANISMS OF PROTECTION WHEN DNA CHANGES

Cells can respond to damaged DNA by stopping the cell cycle, repairing any errors or activating the mechanisms of cell death. The cell may die because of irreparable damages that impede growth and cell division, or through a programmed cell death: **the apoptosis.**[10] In recognition and appropriate response to any damaged DNA, PI3 kinase proteins, which include **ATM** (*ataxia telangiectasia mutated*), ATR, DNA-PK, FRAP, are very important.

Several types of DNA lesions activate ATM that, through the **Chk1** and/or **Chk2** kinases, generates chain reactions that conclude with **tumour suppressor genes** induction. The tumour suppressor genes are important in cell cycle regulation, are activated by DNA damage and through the transcription of Cdk $p21^{Cip1}$ inhibitor, induct cell cycle block in G1/S checkpoint. This inhibitor binds the Cdk4/Cyc-D

Cdk2/Cyc-E complexes blocking their activities and therefore, Cdk's cannot phosphorylate the Retinoblastoma and cell cycle stops.

p53 is a tumour suppressor gene that is activated by the direct phosphorylation of ATM kinase or through the Chk1/2 kinases. In order to be activated p53 needs to be associated with **IRF-1 (interferon regulatory factor)**, and this activation mechanism is not yet clear. On the other hand, the Chk1/2 kinases can inactivate the Cdc25 phospatase that eliminates inhibitor phosphate groups by the CycB/Cdc2 and Cdk2/Cyc-A complexes; these complexes are activated and can stimulate mitosis. In this way, the block of G2/M checkpoint starts and to keep it blocked, p53 and $p21^{Cip1}$ are required.

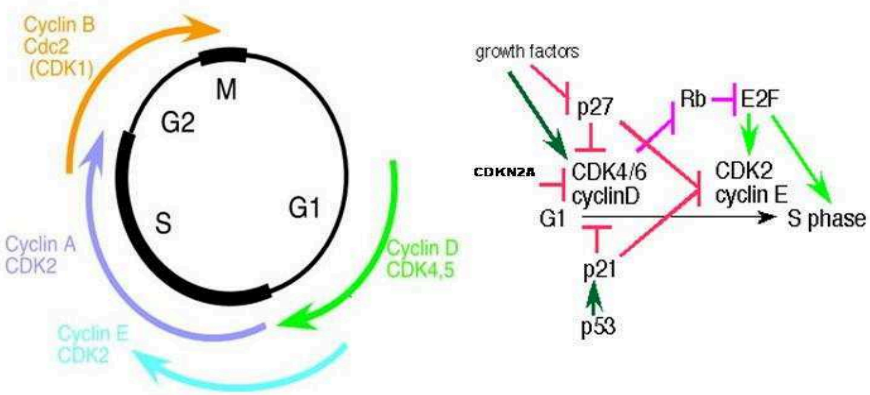


Figure 3. DNA repair processes

3.2.1. Apoptosis

Apoptosis plays an important biological role in the regulation of many important physiological processes, involving differentiation, homeostasis, and the removal of abnormal cells in all tissues. The body is thus protected from the presence of potentially dangerous mutated cells. In humans, apoptosis is essential in embryonic development, in maturation of the central nervous system, in endocrine-dependent tissue atrophy and in a tissue cell turnover. Even at immune-system level, apoptosis plays an important role, for example during the elimination of virus-infected cells or tumour cells. Therefore it is easy to understand how a dysfunction or deregulation of the apoptotic process may be the cause of a wide variety of pathological conditions, that often result in serious cancer or autoimmune and neurodegenerative diseases.[10] Apoptosis can be divided in two phases: the induction of the same process and the implementation of macrophages activity.

Apoptosis activation depends on endogenous and exogenous pathways. An important endogenous apoptosis inducer is the p53 protein, the product of the p53 gene.

Apoptosis execution takes place through a series of chain enzymatic reactions that lead the cell to cell death. In execution phase the **Caspase enzymes** play an important role; their catalytic activity depends on the presence in the active site (QACRG) of a highly conserved cysteine residue and an aspartic acid residue where the proteolytic cleavage occurs.

Caspases stay in the cytosol like procaspases, proteic inactive zymogens that are activated by a catalytic process or by a proteolytic cleavage. The ultimate aim of caspases is the deactivation of all the enzymes involved in the cellular systems maintenance (such as DNA replication and reparation, RNA splicing and cytoskeletal structure preservation), to induce cell death.

Programmed cell death involves two different pathways.

- the extrinsic or receptor-dependent pathway, in which the death signal is provided by the oligomerization of death receptors, expressed on the cell surface, though ligand binding
- the intrinsic pathway that depends on procaspase proteins

The first way (execution phase) has the initiator caspase-8, while the intrinsic pathway uses caspase-9. The two paths converge in active

caspases (caspases-3, -6 and -7) activation, which execute the cell death programme.

The final step of apoptosis involves fragmentation of the whole cell in apoptotic bodies, which will be rapidly recognized and removed by macrophages to prevent possible inflammatory responses and secondary inflammatory processes.

Alterations of apoptotic pathways are involved in a variety of physio-pathological processes, including cancerous diseases. In tumour cells apoptosis decreases or disappears. However, cell cycle deregulation can directly influence cell susceptibility to pro-apoptotic stimulation. Recent studies have shown that in the early stages of neoplastic progression, tumour cells are more sensitive to chemotherapeutic agents. Instead in advanced stages cells develop resistance.[10]

3.3. ALTERATION OF PROLIFERATION AND CANCER DEVELOPMENT

Six basic alterations lead to the transformation of normal cells into cancer cells: self-sufficiency in growth signals, insensitivity to the inhibition of cell growth signals, evasion of the cell death programme

(apoptosis), unlimited replicative potential, sustained angiogenesis and metastasis. Each physiological change corresponds to the loss of one or more defense mechanisms. It is the result of genome instability, caused by defective repair systems. Cancer cells that undergo this transformation are able to avoid the various control points of the cycle. They become self-sufficient and insensitive to all the stop signals that allow the cell to repair any damages or to go into apoptosis. Thus, they begin to metastasize and angiogenesis starts. At molecular level, tumour development is based on the accumulation of genes mutations that produces alterations in the cell cycle, in DNA repair mechanisms and in apoptosis. These genes are called proto-oncogenes and when they change become oncogenes. At the same time, tumour suppressor genes play a critical role in cell proliferation inhibition. The knowledge of the mechanisms and relationships between growth, differentiation, neoplastic transformation and gene expression is the basis for a possible pharmacological approach in cancer therapy.

3.4. CANCER THERAPY

Nowadays there are three different strategies used in cancer therapy:

3.4.1. Differentiation therapy

Differentiation therapy is based on the demonstration that a tumour is a reversible state in which the transformed phenotype can be suppressed through the use of cytostatic agents. A tumour is thus directed towards benign forms with no proliferative potential. Conceptually, when cell cycle and differentiation are been restored, uncontrolled proliferation stopping could be possible[11]. The advantages of this strategy are mainly the decrease of adverse and toxic effects in normal tissues. In this therapy a wide range of agents are used, characterized by chemical structures and different mechanisms of action:

- Histone deacetylases inhibitors.
- Retinotids.
- Cyclin-dependent kinase inhibitors.
- Cyclins inhibitors.
- Cytotoxic agents in differentiation therapy.

3.4.2. Apoptosis induction therapy

In cancer therapy it is very important to improve the activity of apoptosis-inducing compounds. By using DNA damaging agents, for example, it is possible to activate the tumour suppressor genes, stimulating growth arrest and apoptosis. When the repair process is inefficient, the accumulation of errors in the double helix, can lead to cell death and to a transformed phenotype. It is resistant to drug treatment and has an increased metastatic potential.

3.4.3. Cytotoxic therapy

The cytotoxic drugs have represented the most effective strategy in cancer treatment, even if their use involves a number of side effects. They also act also on healthy cells, especially in rapidly proliferating tissues, Furthermore, they bring the cells resistant to treatment. Drugs of this class are the alkylating agents, whose mechanism of action is the alteration or the interruption of cell replication causing direct DNA damages.

3.4.3.1. Antimetabolites

Antimetabolites are drugs that block DNA synthesis by inhibiting enzymes involved in its repair[12]. An example is the 5-fluorouracil (5-FU) (Fig. 4), used in the treatment of solid tumours, such as the gastrointestinal tumour. As a competitive antagonist of 2- deoxyuridilic acid, it blocks the thymidylate synthase (TS) enzyme that catalyzes the synthesis of thymine from uracil. It uses the *N*5, *N*9-methylenetetrahydrofolate acid, as a methyl-group-donor cofactor. The 5-FU, in vivo is activated in fluorodeoxyuridine monophosphate (FdUMP), enters in the synthetic route and creates a stable drug-enzyme cofactor. The enzyme is irreversibly inhibited and DNA synthesis stops for lack of thymine, leading to cell death. Furthermore, 5-FU is enzymatically converted in fluorouridine and later, in 5-fluorouridine-5-triphosphate (FUTP), which incorporates RNA and acts as the antimetabolite analogue, uridine triphosphate (Fig. 4).

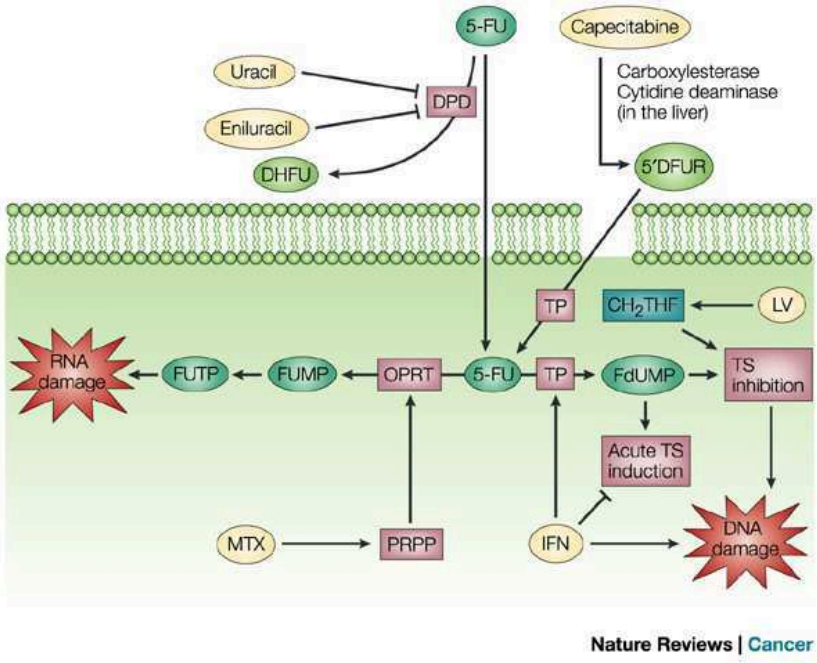


Figure 4. 5-FU mechanism of action

5-FU causes severe toxic effects resulting from its distribution in bone marrow, the gastrointestinal tract, central nervous system and skin. To Increase the selectivity and to decrease its side effects, was the aim for which many derivatives have been designed with several radical groups that act as carriers to the site of action. Our research group has a broad background in the preparation of compounds considered as 5-FU prodrugs (more details in section 4.5).

3.4.3.1a. Nucleosides analogues drugs

Nucleoside analogues are an antimetabolites class able to be phosphorylated to nucleotides and to be incorporated into the DNA strand by the DNA polymerases action. Their usefulness has been demonstrated both in cancer and antiviral therapy, although the latter is limited by liver and bone toxicity and by the appearance of resistance. They can be divided into two main groups: acyclic nucleosides and nucleotides.

In acyclic nucleosides, the sugar is replaced by a linear chain. Their development occurred after the success of acyclovir, an anti-herpes drug. Acyclic nucleosides with unnatural bases, such as 5-FU (Fig. 7) have also been synthesized. In this area, our group has collaborated with the preparation of numerous structures with interesting activity.

The 2',3'-dideoxynucleosides (ddNS), competitors of the 2'-deoxynucleoside-5'-triphosphate (dNTPs) are remarkable among nucleosides. Typically they incorporate modifications in carbon atoms, 2' or 3', of sugar. Some of them have more than one heteroatom in the carbohydrate nucleus. The 3'-azide-2',3'-dideoxythymidine (AZT) and dioxolane-T are examples of cyclic nucleosides with antiviral activity

(Fig. 5). The ddNS toxicity is associated with myelosuppression, anemia and neutropenia.

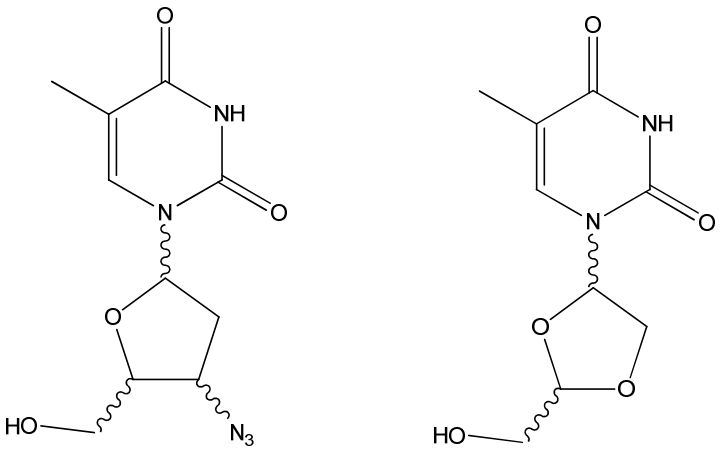


Figure 5.

3.5. BACKGROUND IN CANCER THERAPY

The development of the structures described in this thesis, purines linked to phenylpyrrolidines and to benzo-fused seven- and eight-membered heterocycles, is the result of the long experience of our group in the preparation of related compounds able to induce cell differentiation and cell cycle arrest.

3.5.1. 5-Fluorouracil prodrugs

With the aim of diminishing the toxicity and obtaining biologically active derivatives of 5-FU suitable for oral administration, great effort has been made in medicinal chemistry for the preparation of 5-FU prodrug derivatives.[13] Prodrugs are described as biologically inactive molecules which undergo chemical changes when administered to the body. It usually happens by the action of enzymes, leading to the release of the active substance. The prodrug is thus a precursor of the active substance.[14] Going through the literature a review about 5-FU prodrugs was noticed.[15] In Fig. 6 there are some examples of 5-FU prodrugs, converted in 5-FU, passing the gastro-intestinal tract. In particular ftorafur and doxifluridine are activated through hydrolysis of the *O,N*-Acetalic bond.

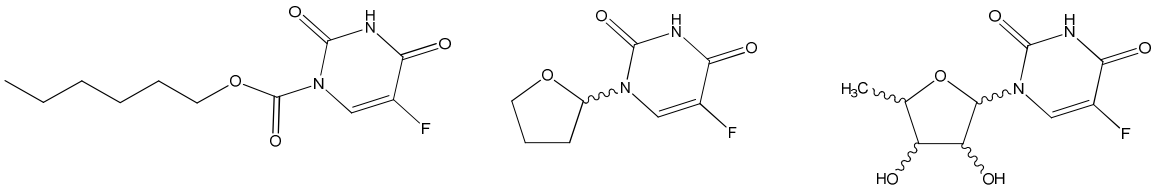
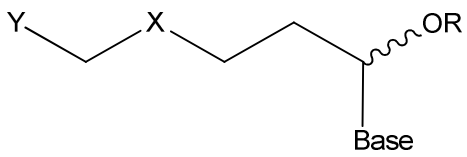


Figure 6. Some 5-FU prodrugs

3.5.2. Structural evolution of O,N-Acetals

The first *O,N*-acetals synthesized were acyclic nucleosides with the general structure 1-[3-(2-hydroxyethylhetero)-1-alkoxypropyl]-5-fluorouracil[16] (Fig. 7). Against different tumour lines, compounds **1** and **2**, show better *in vitro* activity compared with 5-FU. The former has an IC_{50} of 22 μM against HEp-2 (human larynx carcinoma). The latter has an IC_{50} of 9.4, 3.9 and 5.8 μM against the tumour lines CX-1 (human colon carcinoma), MX-1 (human breast carcinoma) and LZ-1 (human lung carcinoma), respectively. In order to increase power and inducing differentiation, 3-hydroxyethoxypropyl chain was changed. Some examples are shown in Figure 7.



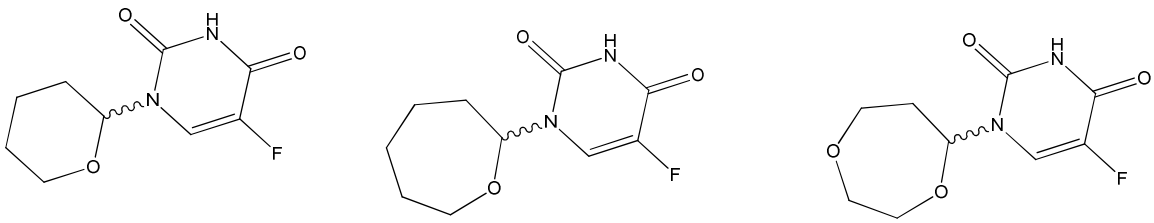
	R	X	Y	Base
1	iPr	O	CH_2OH	5-FU
2	iPr	S	CH_2OH	5-FU
3	Me	O	CH_2OTs	5-FU
4	Me	O	CH_2Cl	5-FU
5	iPr	O	CO_2Me	5-FU
6	iPr	O	$CH=CH_2$	Adenine
7	iPr	O	$CH=CH_2$	Uracil
8	iPr	O	$CH=CH_2$	5-FU
9	iPr	O	$CH(OH)CH_2OH$	5-FU

10	Me	O	$CH(OH)CH_2Cl$	5-FU
11	Me	O	CH(OMe)5-FU	5-FU

Figure 7.

The compounds with the hydroxyl group blocked and therefore not phosphorylated, were designed to act as 5-FU prodrugs. Their hydrolysis in the tumour tissues releases two cytotoxic substances, 5-FU and acrolein. Cytotoxic activity assays of these compounds on HT-29 cells (human colon carcinoma) showed the compound **10** as the more interesting (IC_{50} = 70 μM). Even so, it is eight times less active than 5-FU. In RD cell culture (human rhabdomyosarcoma), studies of differentiating capacity, show that both **10** and 5-FU produce an increase of various differentiation markers (such as desmin, α-actinin and tropomyosin). Compound **10** showed better differentiating capacity and less toxicity than 5-FU.

With the aim to increase activity, it was thought of boosting lipophilicity. An initial strategy was to cyclisize the acyclic 5-FU *O,N*-acetals (with an OH group) to give the cyclic ones **12-14**. In this way the following compounds were synthesized:[17]

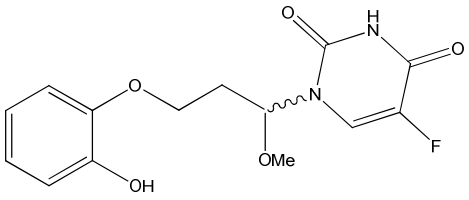


12 **13** **14**

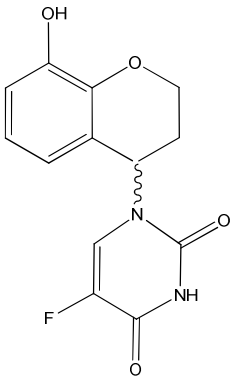
Figure 8.

As was expected, it was noted that on augmenting the ring size the activity also increased. Following this strategy, a further approach was to increase lipophilicity by incorporation of aromatic rings in certain positions of structures **1**-**11** and fusing a benzene ring to the saturated heterocyclic ring of **12**-**14** (Fig. 8).[18,19,20]

Family I

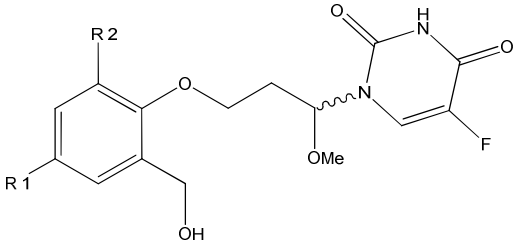


15

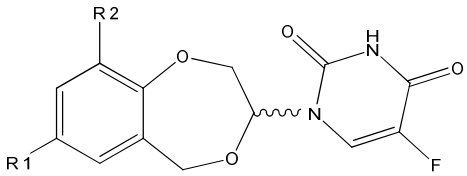


16

Family II

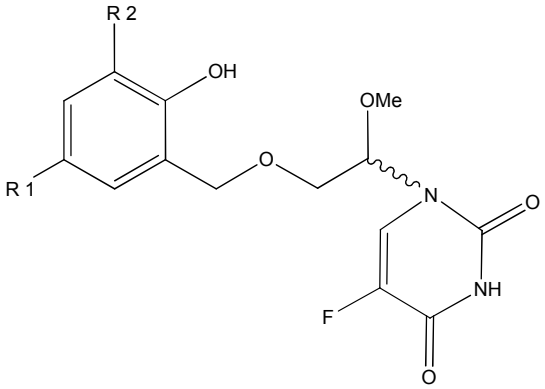


17



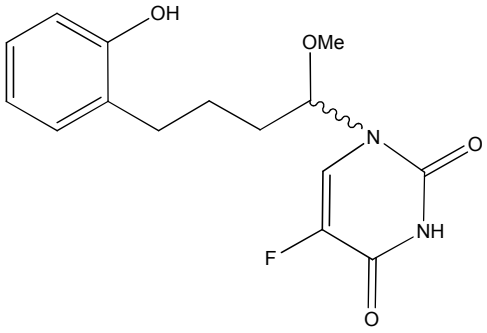
18

Family III

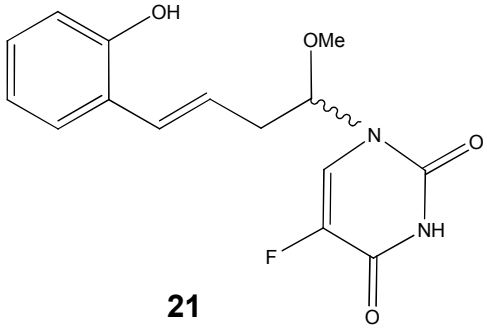


19

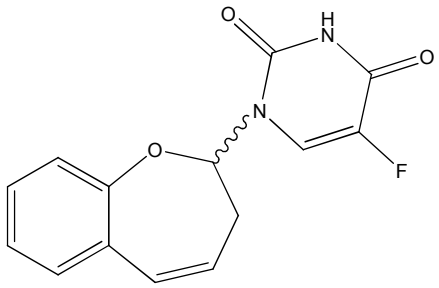
Family IV



20



21



22

Cyclic Compounds	R_1	R_2	Base	IC_{50}(μM)	Acyclic Compounds	R_1	R_2	IC_{50}(μM)
5-FU				2.8	**17a**	H	H	18.5
Ftorafur				3	**17b**	Cl	H	18
16				69	**17c**	Br	H	16
18a	H	H	5-FU	7	**17d**	NO_2	H	5.4
18b	H	H	Uracil	5				
18c	OCH_3	H	5-FU	4.5				
18d	H	OCH_3	5-FU	22				
22	H	H	5-FU	14				

Figure 8. General structures of the benzo-fused *O,N*-acetals and some examples that have been prepared and tested on MCF-7 tumour cells

The increase of lipophilicity resulted in an improvement of the IC_{50} values against breast carcinoma human cells MCF-7. It is evidenced by comparing the activity of **14** (IC_{50} = 23 μM) and of compounds **18a**, **18b**, **18c** and **22**. Studies of inducing apoptosis ability were carried out on these products. Paclitaxel (Taxol), 43% induction in breast cancer cell cultures, is one of the few known examples of apoptosis inducers. Among the compounds developed in our laboratory, there are various that exceeded the value of paclitaxel at equivalent IC_{50} concentrations. In the human MCF-7 tumour cell line, the best apoptosis inducers were compounds **16** (54.33 and 35.49% at 24 and 48 h respectively), **18d** (40.08 and 46.73%), **22** (57.33 and 51.37%) and **17a** (59.90% at 24h). Further, compared to 5-FU and ftorafur, these compounds showed

their ability to accumulate cells in different cell cycle phases. Inhibiting the thymidylate synthase enzyme, 5-FU and ftorafur retain cells in the S-phase, whereas compounds **16**, **18a-d**, **22** do so in the G0/G1 phase and compounds **17b** and **17c** in the G2/M phase. This suggests the ability of these compounds to be drugs *per se* (except **17d**, with a similar pattern to 5-FU in cell cycle arrest).[21]

The most active compounds did not show any toxicity when intravenously administered to mice, twice a week, for a month (50 mg/kg).[21] However after 3 weeks of administration of equivalent doses of 5-FU resulted in excessive weight loss and increased mortality in mice.

3.5.2.1. Modification of molecular markers caused by cyclic 5-FU *O,N*-acetals[22]

Cyclin inhibition studies on compounds **16**, **18a-d** and **22** show that there is a halting in the G1 phase of the cell cycle, as a consequence of a decrease of Cyc-D1, even leading to its disappearance. Compounds **18a** and **18c** also decrease the Cdc-2 activity and, consequently, mitosis begins.

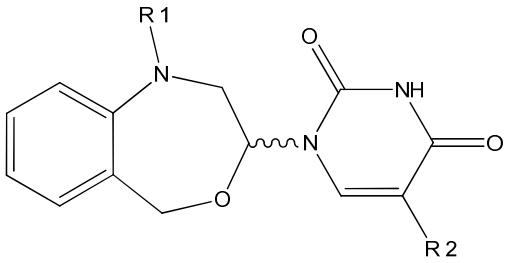
These are contrary to the 5-FU effects that increase the Cyc-D1 or Cdc-2 levels and accumulate cells in the S phase. Evidently, it indicates that the activity of these compounds is independent from the mechanism of action of 5-FU. Thus, these compounds in MCF-7 cells increase the expression of tumour suppressor gene **p53** (mainly **22**) and the decrease of Bcl-2 (B cell lymphoma gene-2) levels, which explains the apoptosis induction.

3.5.2.2. Modification of molecular markers caused by acyclic 5-FU *O,N*-acetals[22]

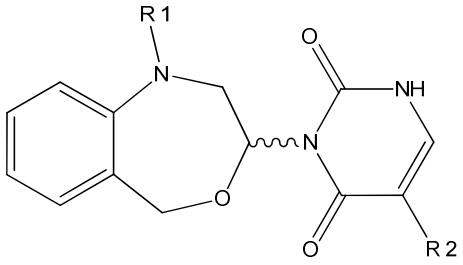
On MCF-7 cells, the compounds **17b** and **17d** decrease Cyc-D1 and Cdc-2 expression and increase the inhibitor Cdk p21. However, these two compounds operate in different ways. On one hand, compound **17b** decreases Bcl-2 expression and does not alter p53 levels. On the other, **17d** does not alter Bcl-2 expression and increases p53.

3.5.3. THE *N*-ISOSTERIC REPLACEMENT

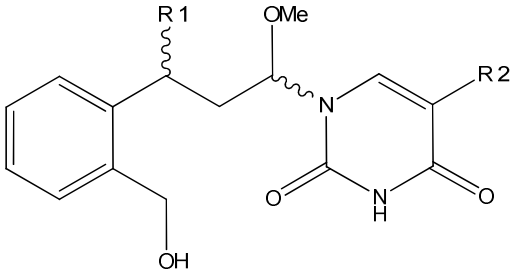
One of the most useful tools for medicinal chemistry is the isosteric replacement. Bioisosteres represent an approach used for the rational modification of lead compounds into safer and more clinically effective agents. The objective of a bioisosteric replacement is to create a new compound with similar biological properties to the parent compounds. In the compounds previously described it was thought that the isosteric replacement of oxygen by a nitrogen atom could help to increase the activity of compounds. Indeed, nitrogen atom provides a new substitution positions, thus, by an appropriate selection of nitrogen substituents, the modulation of lipophilicity could be possible.[23] Therefore, new classes of *O,N*-acetals were synthesized. Using the general structures of cyclic 5-FU *O,N*-acetals **18**, and acyclic 5-FU *O,N*-acetals **17**, the series of the benzoxepines (**23**, **24**) and **25** were developed[24] (Fig. 9).



23



24



25

Cyclic compounds	R_1	R_2	IC_{50} (µM)	Acyclic compounds	R_1	R_2	IC_{50} (µM)
23a	H	F	> 100	**25a**	H	F	35.97 ± 0.40
23b	SO_2-C_6H_4-*p*NO_2	F	19.33 ± 1.04	**24b**	CO-C_6H_5	F	16.14 ± 0.77
23c	SO_2-C_6H_4-*p*NH_2	F	14.37 ± 0.67	**25c**	SO_2-C_6H_4-*p*NO_2	F	52.22 ± 12.1
23d	SO_2-C_6H_4-*o*NO_2	F	19,.70 ± 0.15	**25d**	SO_2-C_6H_4-*o*NO_2	F	90.99 ± 6.06
23e	SO_2-C_6H_4-*o*NH_2	F	54.82 ± 1.04	**25e**	SO_2-C_6H_4-*o*NH_2	F	> 100
23f	SO_2-C_6H_4-*p*NO_2	H	39.78 ± 2.60	**25f**	SO_2-C_6H_4-*p*NO_2	H	45.76 ± 2.45
23g	SO_2-C_6H_4-*o*NO_2	H	45.17 ± 0.48	**25g**	SO_2-C_6H_4-*p*NO_2	H(N_3)	55.22 ± 12.1
24a	H	F	72.40 ± 11.30	**25h**	SO_2-C_6H_4-*o*NO_2	H	64.81 ± 0.05
24b	SO_2-C_6H_4-*p*NO_2	F	19.81 ± 0.08				
24c	SO_2-C_6H_4-*o*NO_2	F	22.63 ± 0.11				
24d	SO_2-C_6H_4-*o*NH_2	F	43.70 ± 0.09				

24e	SO_2-C_6H_4-*p*NO_2	H	44.28 ± 4.65
24f	SO_2-C_6H_4-*o*NO_2	H	50.90 ± 3.87

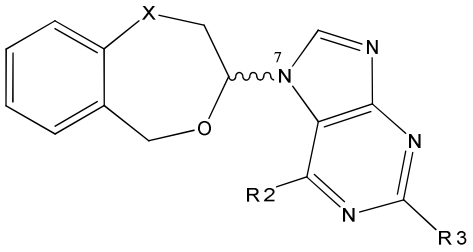
Figure 9. Benzoxepine *O,N*-acetals containing purine rings (**23a-g** and **24a-f**) and acyclic *O,N*-acetals containing pyrimidine (**25a-h**). IC_{50} values are referred to MCF-7 cell line.

The anti-proliferative activities of these new classes of *O,N*-acetals have been studied on MCF-7 human breast cancer cells and the IC50 values obtained are shown in Figure 9. Among the benzoxepine derivatives **23**–**24**, those containing 5-fluorouracil (R_2 = F) show better activity than those derived from uracil (R_2 = H). The *N*1 or *N*3 pyrimidine bond slightly affects the activity. Both of these benzoxepine derivatives are more potent when R_1 is not hydrogen. The lipophilic character of R_1 increases the activity, and no limit of volume has been observed for the studied groups. The electron-withdrawing character of R_1 could help to increase the activity.[23] When R_1= benzenesulfonamide it can be observed that the *para*-substitution on R_1 is preferred to the *ortho*-one, and that the nitro group renders better results than the amino one. IC_{50} values of acyclic compounds **25** show again, that the *para*-substitution on R_1 is preferred to *ortho*-substitution. Comparing

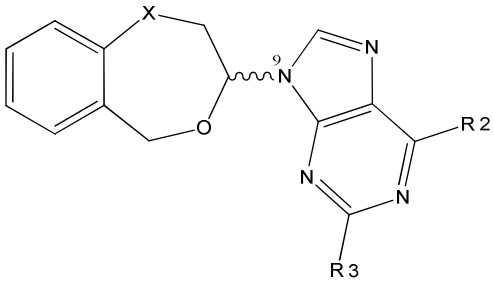
acyclic compounds and benzoxepine derivatives it is evident that good activity values are correlated to the benzoxepine semi-rigid conformation. The change to 4,1-benzoxepine rings has afforded an enhancement of the anti-proliferative activity on MCF-7 cells in relation to the previous 1,4-benzodioxepines,[13,21,25] with an improvement on the values of IC_{50} from 22 μM (for **18d**) to 14.37 μM (for **23c**).

3.5.4. FROM PYRIMIDINES TO PURINES: NEW CLASSES OF *O,N*-ACETALS

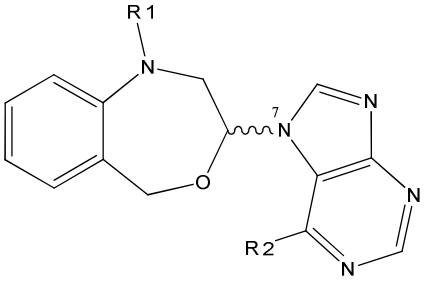
In all the compounds previously described, cyclic and acyclic *O,N*-acetals, the *O,N*-acetalic bond is linked to pyrimidine bases. In all these compounds, the increase of lipophilicity is correlated with good activity values. With the aim to further increase lipophilicity and to obtain more structural diversity, our research group substituted the pyrimidine base for the purine one.[13] Observing the best IC_{50} values of the previous molecules, the series of compounds **17**, **18**, **23** and **25** were taken as *leads* for the synthesis of the new *O,N*-acetals **26**, **27**, **28**, **29** and **30**[13,24,26] (Fig.10). The heterocyclic bases used were 6-substituted- and 2,6-disubstituted-purines, that were linked through their *N-7* and *N-9* atoms of the purine moiety.



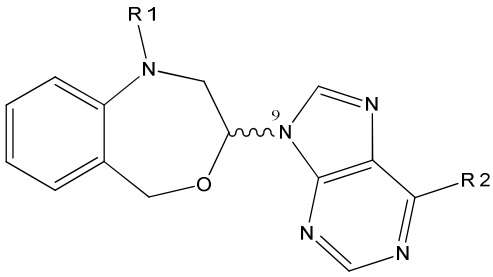
26



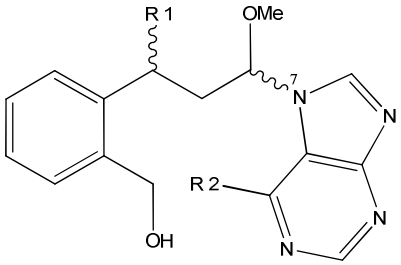
27



28



29



30

Compd.	X	R_2	R_3	IC_{50}	Compd.	X	R_2	R_3	IC_{50}
26a	O	Cl	H	2.74 ± 0.31	**27a**	O	Cl	H	4.63 ± 0.02
26b	O	I	H	4.01 ± 0.33	**27b**	O	I	H	6.75 ± 0.23
26c	O	Cl	Cl	1.28 ± 0.61	**27c**	O	Cl	Cl	4.40 ± 0.88
26d	O	NH_2	H	27.1 ± 4.93	**27d**	O	NH_2	H	18.4 ± 3.16
26e	O	NMe_2	H	22.2 ± 3.80	**27e**	O	H	H	44.9 ± 9.83

26f	O	H	H	40.4 ± 7.85	**27f**	O	CF_3	H	3.21 ± 0.40
26g	O	CF_3	H	27.9 ± 2.93	**27g**	O	Ph	H	16.4 ± 0.26
26h	O	Ph	H	22.8 ± 0.19	**27h**	O	Oph	H	24.7 ± 3.82
26i	O	Oph	H	21.1 ± 2.93	**27i**	O	$OCH_2CH=CH_2$	H	5.04 ± 1.68
26l	O	$OCH_2CH=CH_2$	H	20.9 ± 1.24	**27l**	S	Cl	H	3.55 ± 1.10
26m	S	Cl	H	5.46 ± 0.02	**27m**	SO2	Cl	H	2.58 ± 0.08
26n	SO2	Cl	H	16.5 ± 1.59	**5-FU**				4.32 ± 0.02

Compd.	**R_1**	**R_2**	**IC_{50}**	**Compd.**	**R_1**	**R_2**	**IC_{50}**
28a	SO_2-C_6H_4-*p*NO_2	Cl	2.73 ± 0.17	**30a**	SO_2-C_6H_4-*p*NO_2	Cl	18.70 ± 0.08
28b	SO_2-C_6H_4-*o*NO_2	Cl	2.10 ± 0.69	**30b**	SO_2-C_6H_4-*o*NO_2	Cl (*N-7*)	3.25 ± 0.23
28c	Fmoc	Cl	0.67 ± 0.18	**30c**	SO_2-C_6H_4-*o*NO_2	Cl	11.30 ± 1.27
29a	SO_2-C_6H_4-*p*NO_2	Cl	1.22 ± 0.12				
29b	SO_2-C_6H_4-*o*NO_2	Cl	0.92 ± 0.01				
29c	SO_2-C_6H_4-*o*NH_2	Cl	9,14 ± 1,24				
29d	Fmoc	Cl	0.88 ± 0.04				
29e	SO_2-C_6H_4-*p*NO_2	OH	> 100				
29f	SO_2-C_6H_4-*o*NO_2	OH	19.66 ± 5.27				
29g	Fmoc	OH	53.57 ± 13.1				
29h	H	SPh	48.92 ± 9.89				
29i	SO_2-C_6H_4-*p*NO_2	SPh	0.86 ± 0.12				
29l	SO_2-C_6H_4-*o*NO_2	SPh	2.59 ± 0.57				

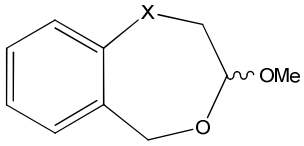
Figure 10. Some examples of *O,N*-acetals containing purines and their respective IC_{50} values on the MCF-7 cell line

3.5.4.1. (6'-Substituted)-7- or 9-(2,3-dihydro-5*H*-1,4-benzodioxepine-3-yl)-7*H*- or 9*H*-purines

The two isomeric classes of (6'-substituted)-[7- or 9-(2,3-dihydro-5*H*-1,4-benzodioxepine-3-yl)-7*H*- or 9*H* purines (**26a**-**26l** and **27a**-**27i**) were developed through the synthesis described and recently published[13] for compounds **26a**-**26h** and **27a**-**27g**, and for compounds **26i**-**26l** and **27h**-**27i**.[27] Adenine derivatives (**26d** and **27d**) and the *N,N*-dimethyladenine structure (**26e**) have shown no interesting anti-proliferative activity against the MCF-7 human brest cancer cell line, in contrast to the uracil derivative **18b**. It is also evident that to improve the activity, the presence of at least one halogen atom on the purine skeleton (**26a**-**26c** and **27a**-**27c**) or in a 6'-methyl group (**27f**) is necessary. Among the synthesized compounds, the most active is the *N-7*-dichloro derivatives (**26c**) that is 3,4-fold more active than 5-FU. Its *N-9* isomer (**27c**) is equipotent to 5-FU.[26]

3.5.4.2. 6'-Chloro-7- or 9-(2,3-dihydro-5*H*-4,1-benzoxathiepine-3-yl)-7*H*- or 9*H* purines

The synthesis of this class of compounds (**26m**-**26n** and **27l**-**27m**) was performed through a condensation reaction between the seven-membered *O,O*-acetals (**31** and **32**) and 6-chloropurine.



31 X=S
32 X=SO_2

Reactions were accomplished using trimethylsilyl trifluoromethanesulfonate (TMSOTf), trimethylchlorosilane (TCS) and hexamethyldisilazane (HMDS) in dry acetonitrile for 24h.[26] Analyzing anti-proliferative activity of these compounds on MCF-7 human brest cancer cell line (Fig.10), it is notable that the presence of the sulfur atom on the heterocyclic seven-membered structure increases the activity. *N-7'* purine derivatives (**26m**-**26n**) have a better activity than their *N-9'* regioisomers (**27l**-**27m**).[26] In this class of compounds, the most active compound is the *N-7'* -6'-chloro-substituted purine **26n**, 2-fold more potent than 5-FU.

3.5.4.3. (1,2,3,5-Tetrahydro-4,1-benzoxazepine-3-yl)purine derivatives

The bioisosteric classes of (1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)purine derivatives (**28a**-**28c** and **29a**-**29l**) were synthesized via the procedure previously described.[28,29] Compounds **28c**, **29d** and **29g** present in their structures the 9-fluorenylmethoxycarbonyl group (Fmoc), a protecting group extensively used in peptide chemistry.

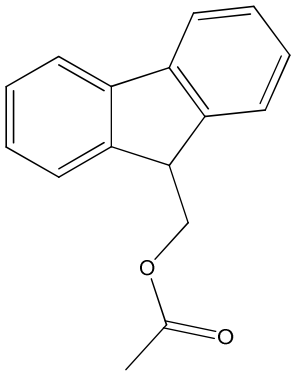


Figure 11. The Fmoc group.

As the pyrimidine derivatives (**23a**-**23g** and **24a**-**24f**), purine derivatives, are more potent when R_1 is not hydrogen. The lipophilic character of R_1 increases the activity even more and no limit of volume in relation to the biological activity has been observed for the studied groups. The electron-withdrawing character of R_1 could help to

increase the activity.[23] Bulky and lipophilic groups (such as -Cl and –SPh) on the C-6'' purine atom afforded the best values of IC_{50}, while 6''-purinone compounds (**29e**-**29g**) gave activities similar to the pyrimidine analogues.[26] The most potent molecules are the compounds **28c**, **29b**, **29c** and **29i** that present IC_{50} values below 1 μM.

Compounds **28c** and **29i** were selected to identify the molecular key targets of their anti-cancer activity. The up-regulated and the down-regulated genes include those that encode for signal molecules, response to stress, cellular development process, regulation of the cell cycle and apoptosis, etc. Analysis of the mRNAs, which are deregulated (up-regulated or down-regulated) at least 2-fold in treated cells, showed these results: 26 genes up-regulated and 59 genes down-regulated in **28c**-MCF-7 treated cells; and 26 genes up-regulated and 17 genes down-regulated in **29i**- MCF-7 treated cells. One of the more important results in this study was the ability of **28c** to modulate the expression of genes involved in apoptosis or its delay of mitosis. This effect can be explained by the accumulation of cells in the G2/M checkpoint of the cell cycle, particularly GP132, the receptor for an unknown ligand, which activates a G2a protein. This is transcriptionally up-regulated by stress-inducing and cell-damaging agents and is involved in caspase-mediated apoptosis.[30] Similarly, the ERN1 gene

that belongs to the Ser/Thr protein kinase family is a potent unfolded-protein response transcriptional activator and acts by triggering growth arrest and apoptosis.[31] However, **29i** induced the down-regulation of a gene involved in the metastatic progression of cancer such as RAC1, a Ras-like protein member of the Rho family of the GTPase key downstream target in Ras signaling.[24,32]

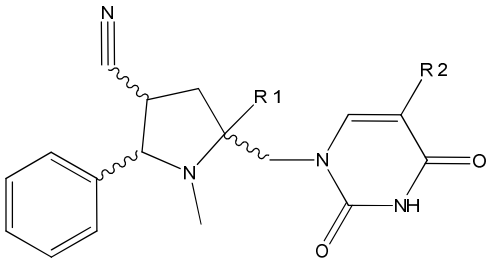
Considering what has been previously written, the change to 4,1-benzoxazepine rings afforded an enhancement of the anti-proliferative activity on MCF-7 cells, with an improvement in the values of IC_{50} from 14.37 μM (for **23c**) to 0.67 μM (for **28c**).

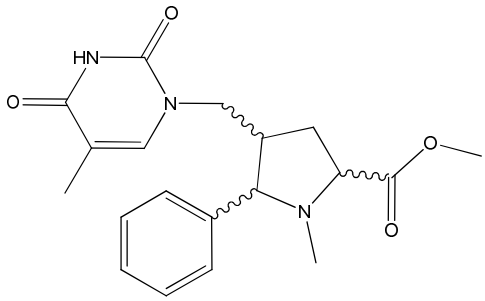
3.5.4.4. Acyclic purine *O,N*-acetals

Acyclic purine *O,N*-acetals (**30a**-**30c**) show higher potency than the pyrimidine acyclic derivatives (**25a**-**25h**). The *N7*-alkylated purine **30b** shows an excellent value of IC_{50}. In contrast to the cyclic analogues, the presence of an *o*NO_2 or *p*NO_2 group does not modify the activity of the *N*9-isomers (**30a** and **30c**).[24,26]

4. PHENYLPYRROLIDINE FAMILY

Interesting anti-tumours belong to the class of phenylpyrrolidine. The synthesis of some of them, and in particular the pyrimidine-containing phenylpyrrolidine derivatives **KUD327, KUD526, KUD600** and **KUD533** (Fig.12) are described.[33] These structures present the residue of 1-methyl-pyrimidine at positions 2 or 4 of the pyrrolidine ring. Varying the 1-methyl-pyrimidine position (from position -*5'* to position -*2'*) these compounds are active against different tumour cell lines. This evidences that the position of the 1-methyl-pyrimidine residue is crucial for the anticancer activity. In particular compound **KUD533** is active against three different cancer cell lines: lung (AC49), breast (MCF-7) and prostate (PC-3). **KUD533** shows its best activity against the prostate cancer cell line.





KUD 533

	R_1	R_2
KUD327	Me	H
KUD526	Me	Me
KUD600	H	Me

Figure 12. Phenylpyrrolidine derivatives

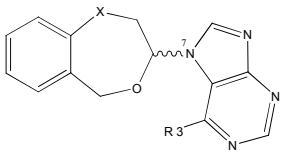
5. AIM

The aim of this thesis is the drug design, synthesis and biological evaluation (against three different tumour cell lines) of three new classes of anti-tumour drugs. The research project is focused on the development of new anticancer molecules, originated from a series of compounds (described in the introduction section) with high apoptotic activity, also capable of blocking the cell cycle. The synthesized molecules will then be tested against the following cancer cell lines: lung (AC49), breast (MCF-7) and prostate (PC-3). The final compounds are grouped into libraries according to their structural characteristics.

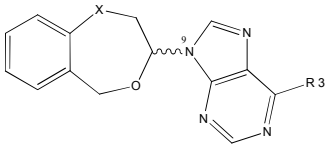
This work was prompted by the following considerations:

- The first one was based on compounds of Fig.13. These derivatives present the 1,4–benzodioxepine and 4,1-benzoxazepine rings linked to purine bases at positions *N-9* (**27**, **29**) or *N-7* (**26**, **28**)[13] (Fig.12). Derivatives 9-(2,3-dihydro-1,4-benzoxathiine-3-yl-methyl)-9*H*-purine (**33**)[24] (Fig.12) were also investigated for the same activity profile. SAR studies have shown the importance of the aromatic lipophilic group on the 1,4-

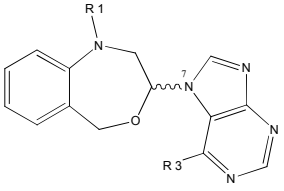
dioxepane and 4,1-oxazepine nucleus. In fact, its removal dramatically lowers the anti-tumour activity. Most derivatives of these classes show a notable anti-proliferative activity and they are also drugs *per se* and, accordingly, are not prodrugs.[21] Attention has been drawn to the presence of the *O,N*-acetal bond in series **26**, **28**, **27** and **29**. Indeed, the *O,N*-acetal bond hydrolysis do not break and do not cause the release of the active compound; in other words, such compounds are not prodrugs. For this reason the presence of this bond is not essential for the anti-cancer activity.



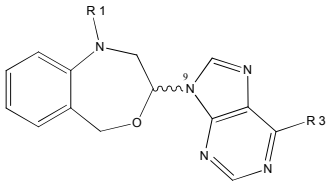
26



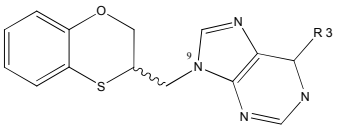
27



28



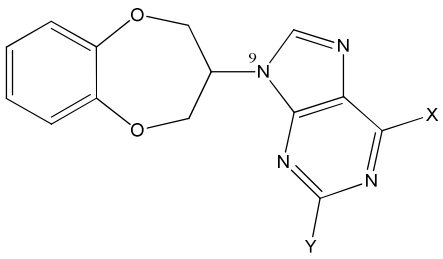
29



33

Fig. 13

For this reason, the design and synthesis of (6'-substituted)-7-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines (**34**) was carried out (Fig. 14). Compounds **34** do not show an *O,N*-acetal bond and, in contrast to derivatives of series **26**, **28**, **27** and **29**, they present a symmetry plane. This fact avoids the presence of chirality in compounds **34**.

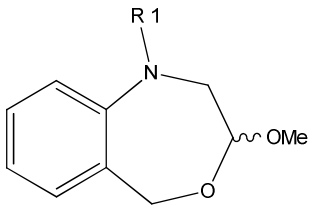


34

	X	**Y**
34a	Cl	Cl
34b	I	H
34c	Cl	H
34d	H	H
34e	Br	H

Fig. 14

- The second one affects compounds **35**, that are intermediates in the preparation of derivatives **28** and **29** (Fig. 15).



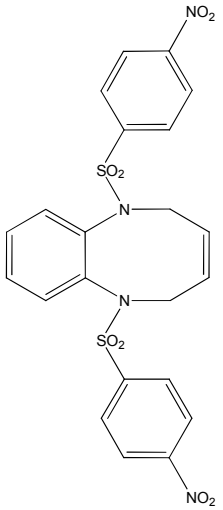
35

	R1
35a	SO_2-C_6H_4-*o*NO_2
35b	SO_2-C_6H_4-*p*NO_2
35c	H
35d	$CO(CH_2)_2CH_3$
35e	$COCF_3$
35f	COPh

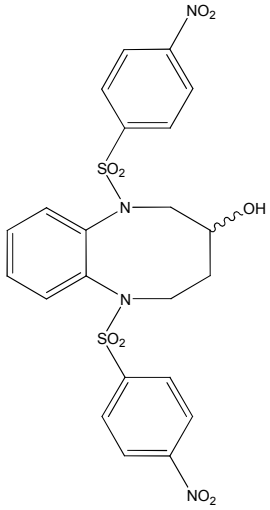
Fig.15

The anti-proliferative activity of this family against the MCF-7 tumour cell line[23] suggests that the *p*-nitrobenzenesulfonamide group should be important for the activity profile. Based on this fact we propose herein the design and synthesis of the bis(*p*-nitrobenzenesulfonamide) derivatives **36** (Fig. 16). If the *p*-nitrobenzenesulfonamide group is crucial in the anticancer activity, these new families should be even more potent than the parent compounds **35**. Accordingly cyclic (**36** and **37**) and acyclic bis(*p*-nitrobenzenesulfonamides) **38** have been proposed. The acyclic bis(*p*-nitrobenzenesulfonamides) were designed to understand if

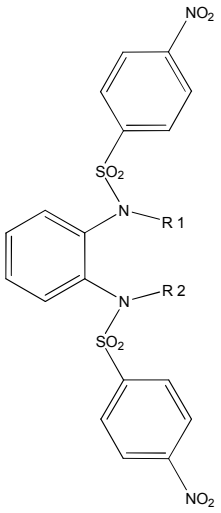
rigidification is also essential for activity. Rigidification has been a popular tactic used to increase the activity of a drug or to reduce its side-effects.



36



37

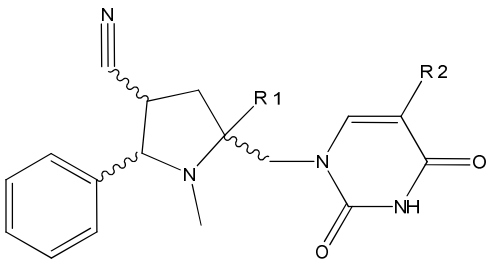


38

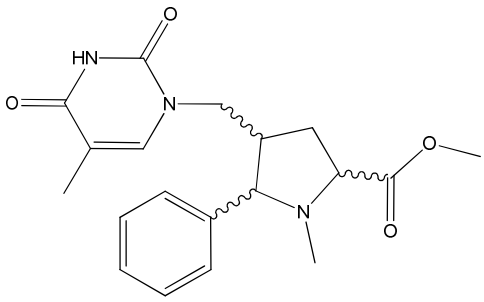
	R_1	R_2
38a	CH_2COOH	H
38b	H	H
38c	CH_2CO_2Et	H
38d	CH_2CO_2Et	CH_2CO_2Et

Fig. 16

- The third consideration was based on the activity profiles of derivatives **KUD327, KUD526, KUD600** and **KUD533** (see section 5). In particular, it was noted that anti-tumour activity is absent if the pyrimidine ring is located in position *5′* of the pyrrolidine cycle (**KUD327**, **KUD526** and **KUD600**). Anti-tumour activity is present only when pyrimidine is located in position *3′* of the pyrrolidine cycle (**KUD533**).[33]

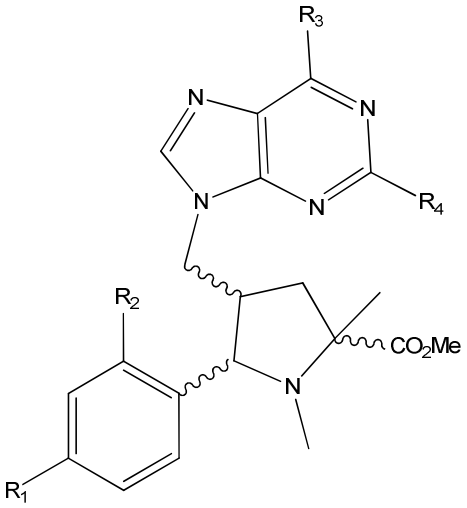


KUD327 KUD526 KUD600



KUD533

Thus, with the objective of increasing both the lipophilicity and the structural diversity of the target molecules, we propose herein to change the heterocyclic base of **KUD 533**, from the pyrimidine to the purine moiety. To this end the new class of compounds **39** (Fig.17) has also been designed and synthesized.



39

Fig. 17

Except for compounds **36**, **37** and **38**, the synthesis of the designed derivatives was performed via the Mitsunobu reaction. This procedure allows the conversion of primary or secondary alcohols in esters, amines and other compounds. A very frequent problem of this type of reaction is the difficulty of removing by-products, such as the reduced

form of diisopropyl azodicarboxylate (DIPAD) and triphenyl phosphine oxide. In the work described in this thesis, this problem has been solved by using diphenyl (2-pyrildyl) phosphine (instead of triphenyl phosphine) and di-*tert*-butyl azodicarboxylate (DBAD) (instead of DIPAD).[34] All these changes have facilitated the work-up. Indeed, the by-products can be removed easily by a simple water extraction, avoiding the tedious chromatographic purification process.

6. THEORETICAL PART

Eighteen final products have been prepared and the general structures are shown in Figure 18.

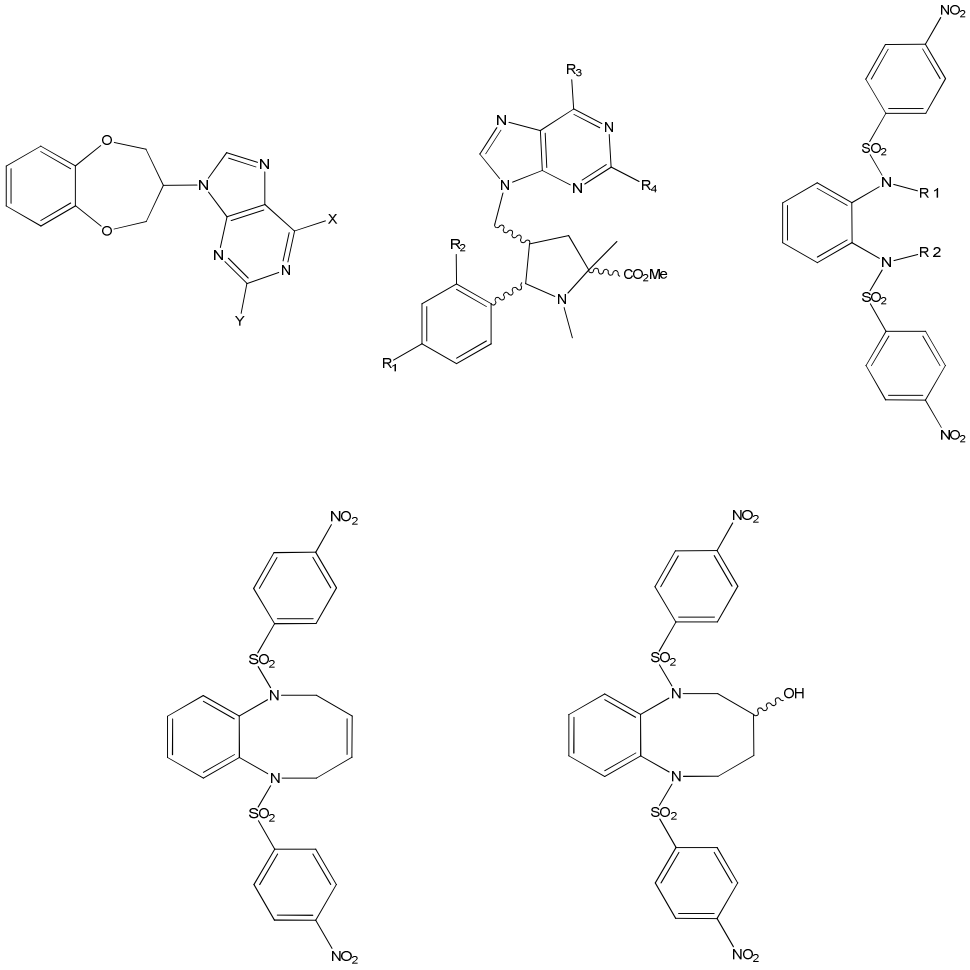


Fig. 18. Structures synthesized in this thesis.

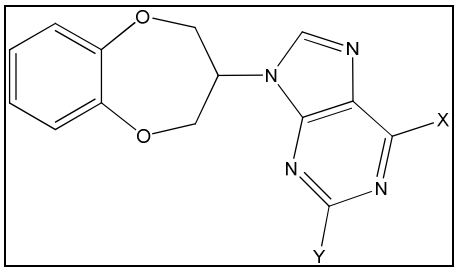
These compounds are divided in three different classes.

- **Family 1** (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl))-9*H*-purines.
- **Family 2** Phenylpyrrolidine derivatives
- **Family 3** Cyclic and acyclic bis(*p*-nitrobenzenesulfonamides)

In families 1 and 2, purine bases are linked through their *N-9'* atoms. The purine rings present substituents of a different nature at positions 6' and 2'.

The substituents of the structures of the final compounds are shown in Tables 1, 2 and 3.

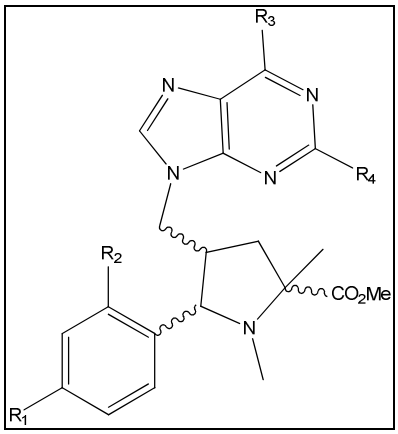
- **(6'-Substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines**



		X	Y
AL-300311-f1		Cl	Cl
AL-240311		Br	H
AL-150210-f3		Cl	H
AL-260510-f3		H	H
AL-110411-f1		I	H

Table 1. Family 1: (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines.

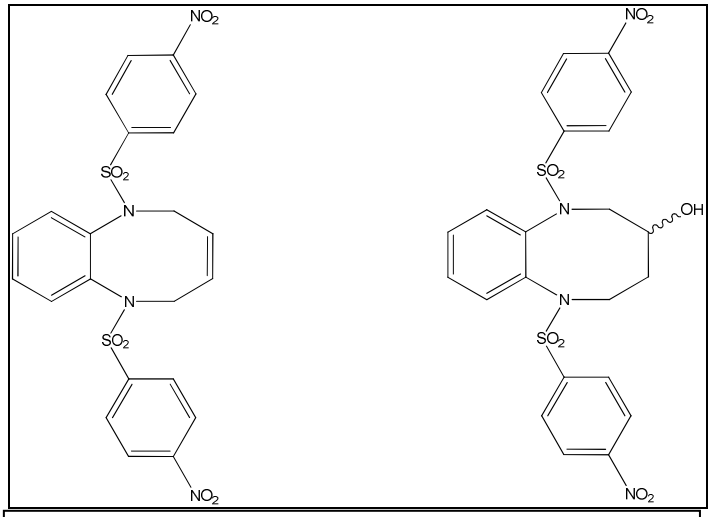
- **Phenylpyrrolidine derivatives**



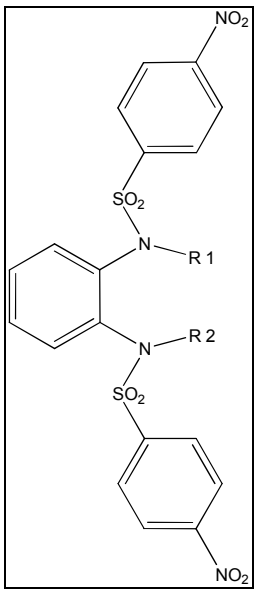
	R_1	R_2	R_3	R_4
AL-120411-f1	H	Cl	Cl	H
AL-250511-f3	H	Cl	Br	H
AL-310511-f1(Cl)	H	H	Cl	Cl
AL-310511-f1(Br)	H	H	Br	H
AL-260511-f2	H	Cl	Cl	Cl
AL-080611-f2(Br)	F	H	Br	H
AL-080611-f2(2,6 diCl)	F	H	Cl	Cl

Table 2. Family 2: Phenylpyrrolidines.

- **Cyclic and acyclic bis(*p*-nitrobenzenesulfonamides)**



AL-091210(2) **AL-110111-f3**



	R_1	R_2
AL-010311	CH_2COOH	H
AL-200910	H	H
AL-220910-f2	CH_2CO_2Et	H
AL-220910-f4	CH_2CO_2Et	CH_2CO_2Et

Table 3. Family 3: bis(*p*-nitrodiphenylsulfonamides)

6.1. SYNTHESIS

6.1.1. Synthesis of (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines

The synthesis of the 1,5-benzodioxepine derivatives is depicted in Fig. 19.

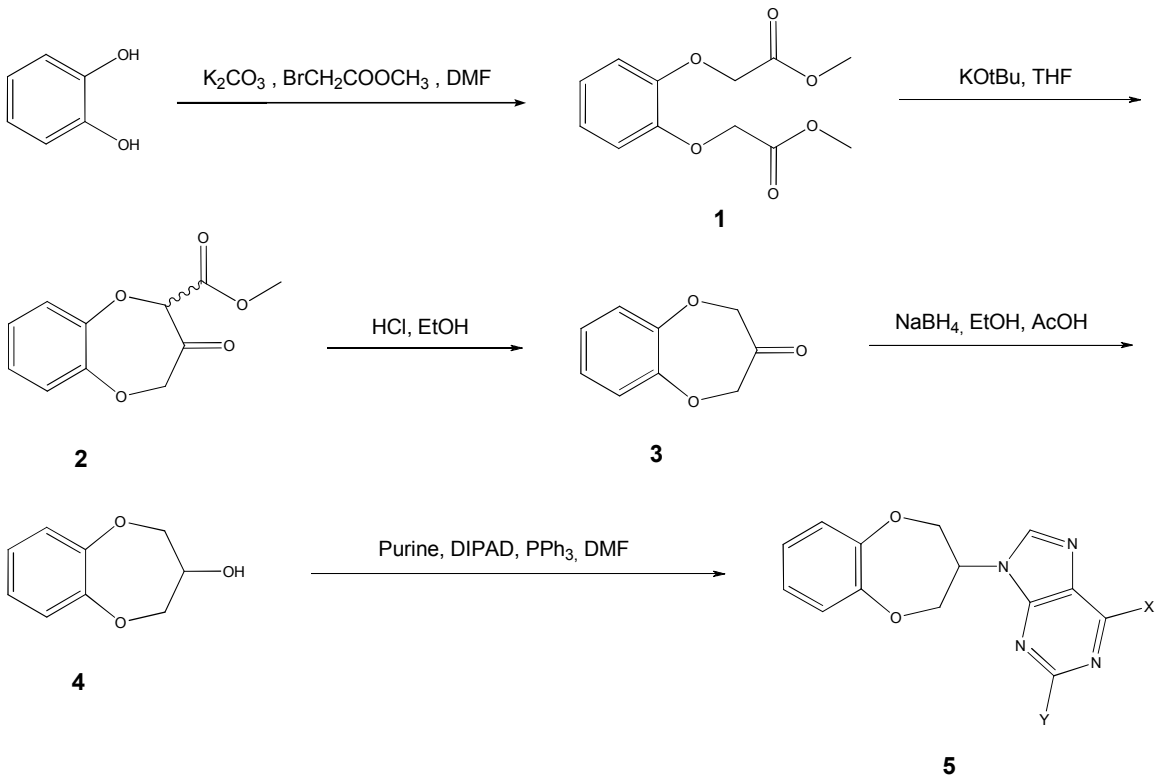


Fig. 19. Synthetic route for the preparation of the purine target molecules.

The first problem solved was the synthesis of the 3,4-dihydro-2*H*-1,5-benzodiaxepine-3-ol (**4**), summarized by the following procedure:

a) Reaction between catechol and two moles of methyl bromoacetate using K_2CO_3 as base.[35]

b) The Dieckmann condensation of the corresponding diester (**1**) under basic conditions. The Dieckmann condensation works well to produce 5- or 6-membered cyclic β-ketoesters. Because we wanted to produce a 7-membered cyclic β-ketoester the reaction was forced using the strong base potassium *tert*-butoxide (KO*t*Bu) and increasing temperatures.

c) Acid decarboxylation of the resulting β-ketoester (**2**), using aqueous hydrochloric acid 2M, produces 3,4-dihydro-2*H*-1,5-benzodioxepine-3-one (**3**).[35]

d) The reduction of 3,4-dihydro-2*H*-1,5-benzodioxepine-3-one to the corresponding secondary alcohol (**4**) was carried out using sodium borohydride ($NaBH_4$).[36]

The conventional Mitsunobu conditions employing diisopropyl azocarboxilate (DIPAD) and triphenylphospine (PPh_3) in anhydrous dimethylformamide (DMF) were used between the secondary alcohol (**4**) and various *6*- and *2,6*- mono- and di-substituted purines.

Triphenylphosphine reacts with DIPAD to generate a triphenylphosphonium intermediate that binds to the alcohol oxygen, activating it as a good leaving group. Its substitution by the purine derivatives completes the process. Syntheses of **AL-150210-f3** (22% yield), **AL-240311** (8% yield), **AL-300311-f1** (15% yield) and **AL-260510-f3** (10% yield), were carried out at 165°C for 10 min under microwave irradiation.[37] The final products were purified by flash chromatography and preparative TLC.

A very frequent problem of the Mitsunobu reaction is the difficulty of removing the by-products, such as the reduced form of diisopropyl azodicarboxylate (DIPAD) and triphenylphosphine oxide, as well as the unreacted starting materials (Fig. 20).

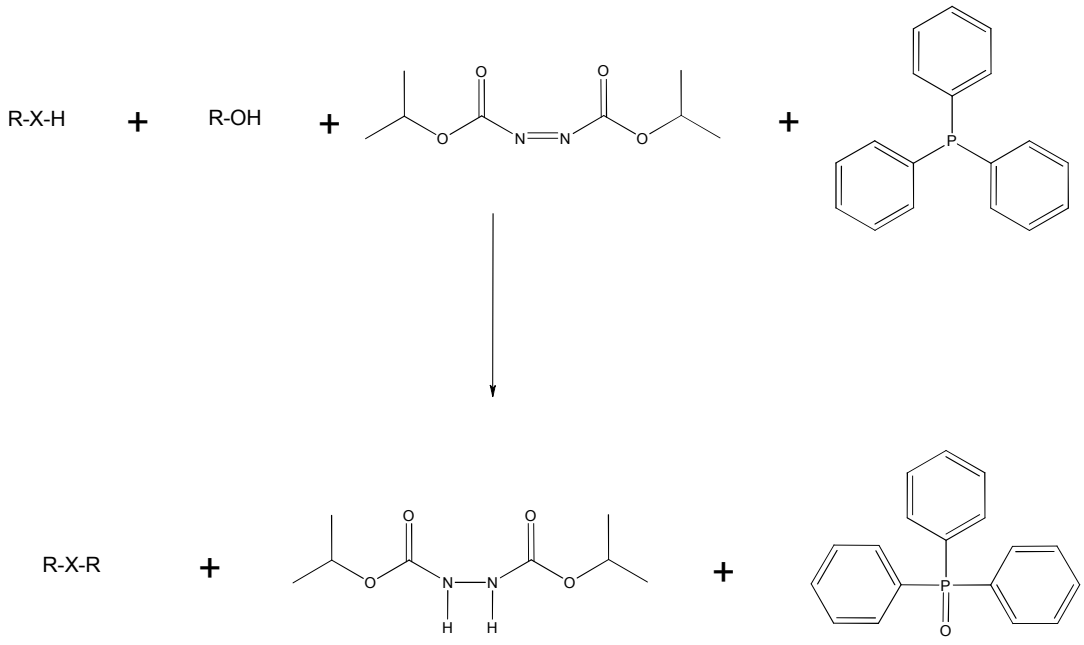


Fig.20. Standard Mitsunobu conditions

This problem has been solved by using diphenyl (2-piridyl) phosphine (instead of triphenylphosphine) and di*tert*-butyl azodicarboxylate (DBAD) (instead of DIPAD).[34]

As shown in Fig. 21, the reagents themselves and their respective oxidation or reduction products, are either directly soluble in aqueous acid or are converted to gaseous byproducts and water soluble materials on treatment with acid.[34] These changes have facilitated the work-up of the reaction, favouring the elimination of by-products, and

thus avoiding the very time-consuming chromatographic purification process.

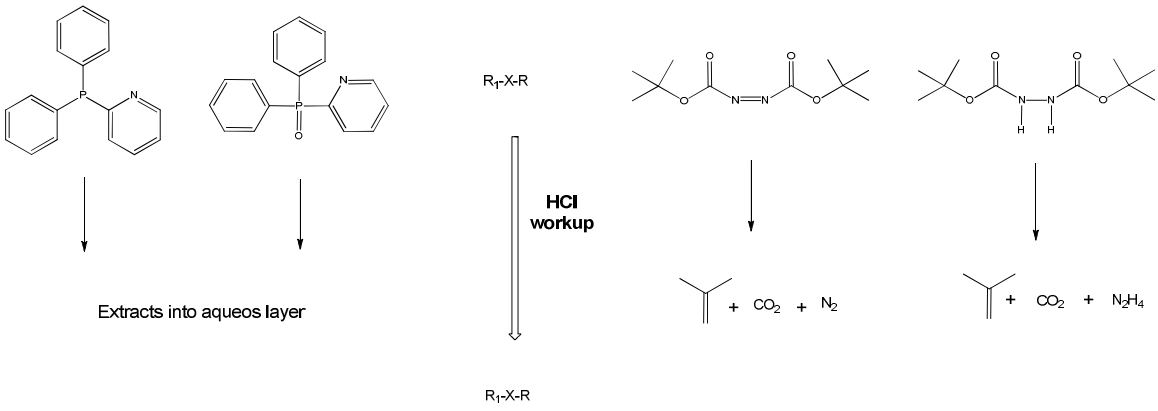


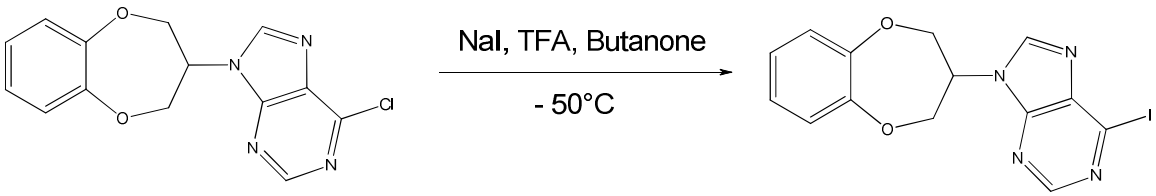
Fig. 21. Modified Mitsunobu conditions

Applying these new conditions, the final products previously described, **AL-150210-f3**, **AL-240311**, **AL-300311-f1** and **AL-260510-f3,** were again synthesized and the yields of their synthetic preparations were increased notably: **AL-150210-f3** (80% yield), **AL-240311** (56% yield), **AL-300311-f1** (70% yield) and **AL-260510-f3** (63% yield).

Under the Mitsunobu conditions, two different regioisomers could have been generated, namely the *N-9'* and *N-7'* isomers. The structures of the final compounds have been determined by 1H and 13C NMR,

DEPT, HMBC (Heteronuclear Multiple Quantum Connectivity) experiments. The discrimination between *N-9'* and *N-7'* purines relies on the correlation between H-3 of the seven-membered moiety and C-4' and C-5' , respectively. Very important is the correlation between H-3 (~δ 5.30 ppm) and the quaternary carbon at ~δ 152.00 ppm, which has the following two consequences: (a) this signal can be assigned to C-4' and (b) this correlation proves unequivocally that the linkage between the seven-membered moiety and the purine base takes place through *N-9'*. Moreover, assignment of *N-9'* versus *N-7'* isomers can be readily carried out from the ^{13}C-NMR signals of the C4' peaks: the signal ~δ = 152 ppm is characteristic of the C4' atom of *N-9'* regioisomers, whilst the signals ~δ = 160 ppm is characteristic of the C4' atom of *N-7'* regioisomers.

The 6-Iodo derivative **AL-110411-f1** (23% yield), was obtained through the Finkelstein reaction (Fig. 22) by substituting the chlorine atom of **AL-150210-f3** with iodine. This reaction was carried out at -50°C using sodium iodide (NaI) and trifluoroacetic acid (TFA) in butanone. These conditions have been successfully used to perform the same process in nucleosides.[38]

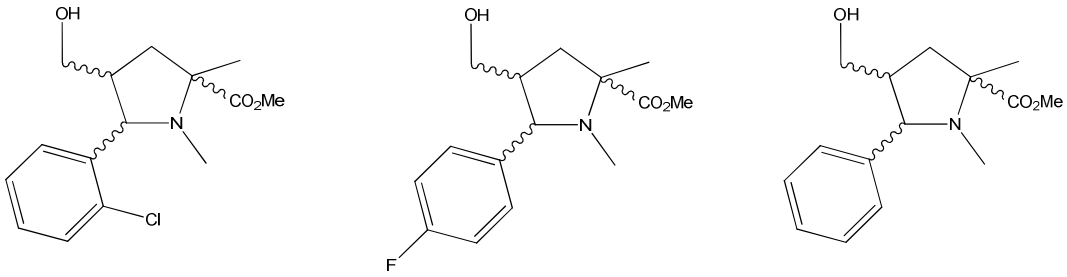


AL-150210-f3 **AL-110411-f1**

Fig. 22. Synthesis of the iodine derivative **AL-110411-f1**.

6.1.2. Synthesis of phenylpyrrolidine derivatives

The starting materials **KUD169**, **KUD223** and **KUD225** (Fig.23) used for the synthesis of this class have been provided by the *"Department of Chemistry, Moscow state University, Leninskie Gory".* Their preparations have been previously described.[33]



KUD 225 **KUD223** **KUD169**

Fig. 23

The conventional Mitsunobu conditions employing DIPAD and PPh_3 in anhydrous DMF were used between the primary alcohols (**KUD169**, **KUD223** and **KUD225**) and various *6*- and *2,6*- mono- and di-substituted purines (Fig. 24).

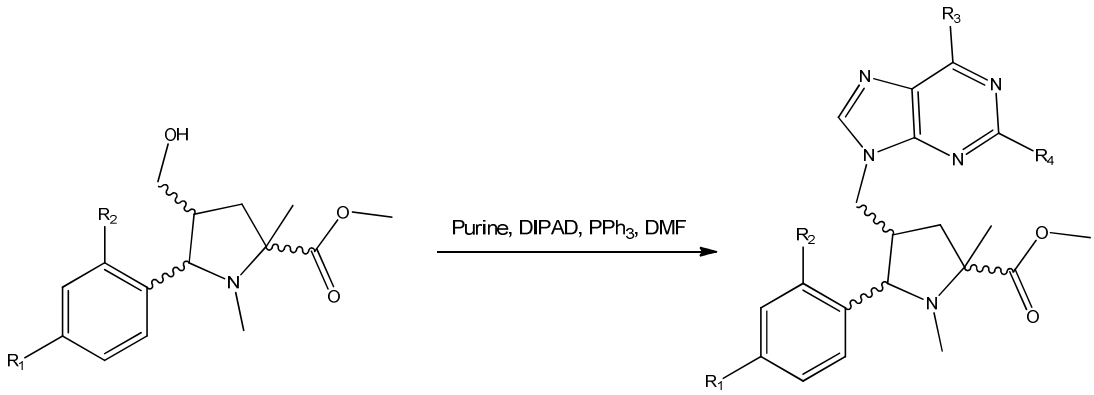


Fig. 24. Synthesis of phenylpyrrolidine derivatives.

For each primary alcohol, three different purine bases (*6*-chloro-, *6*-bromo- and *2,6*-dichloro-purines) were used and the following seven final compounds were obtained: **AL-120411-f1** (94% yield), **AL-250511-f3** (71% yield), **AL-310511-f1(Cl)** (11% yield), **AL-310511-f1(Br)** (95% yield), **AL-260511-f2** (88% yield), **AL-080611-f2(Br)** (97% yield), and **AL-080611-f2(2,6 diCl)** (95% yield). All reactions were carried out at 165°C for 10 min. under microwave irradiation.[37] Final products were purified by flash chromatography and preparative TLC.

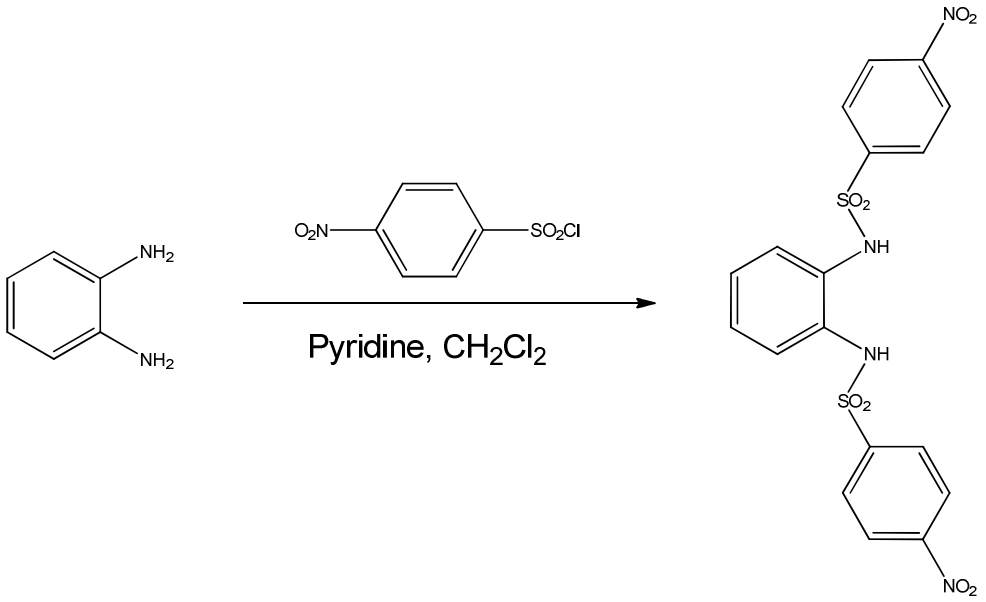
The modified Mitsunobu conditions, using diphenyl (2-piridyl) phosphine (instead of triphenylphosphine) and di*tert*-butyl azodicarboxylate (DBAD) (instead of DIPAD),[34] were also used in the synthesis of the phenylpyrrolidine derivatives. Nevertheless, these conditions have not produced the desired results. No final products were obtained using the modified Mitsunobu conditions.

Two different regioisomers could have been generated, namely the *N-9'* and *N-7'* isomers. The discrimination between the *N-9'* and *N-7'* derivatives was clarified through the NMR experiment (see "experimental part") and only the *N-9'* regioisomers have been identified. Assignment of *N-9'* versus *N-7'* isomers can be readily done through the ^{13}C-NMR signals of the C4' peaks: the signal ~δ = 129 ppm is characteristic of the C4' atom of *N-9'* regioisomers, whilst the signals ~δ = 140 ppm is characteristic of the C4' atom of *N-7'* regioisomers.

6.1.3. Synthesis of cyclic and acyclic bis(*p*-nitrobenzenesulfonamides)

The first step in the synthesis of both cyclic and acyclic bis(*p*-nitrobenzenesulfonamides) is the synthesis of *N,N'*-(1,2-

phenylene)bis(*p*-nitrobenzenesulfonamide) (**AL-200910**) (89% yield) (Fig. 25).

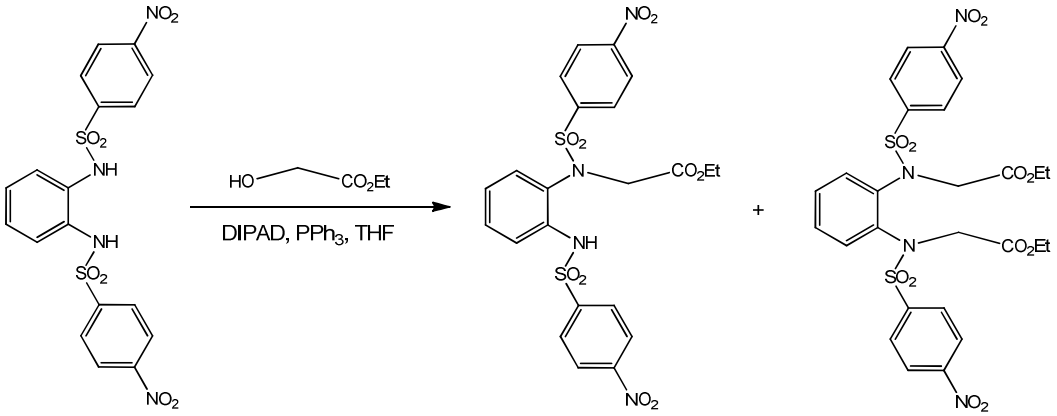


AL-200910

Fig. 25. Synthesis of *N,N'*-(1,2-phenylene)bis(*p*-nitrobenzenesulfonamide)

The *o*-phenylenediamine was dissolved in cold dichloromethane (CH_2Cl_2, 0°C) and treated sequentially with pyridine (2.0 equiv.) and *p*-nitrobenzenesulfonyl chloride (2.0 equiv.). The reaction mixture was then warmed to room temperature (rt) until thin layer chromatography (TLC) showed the total disappearance of the starting materials.[39]

The conventional Mitsunobu conditions employing diisopropyl azocarboxylate (DIPAD) and triphenylphospine (PPh_3) in anhydrous tetrahydrofurane (THF) were used between ethyl glicolate and **AL-200910** (Fig. 26). Triphenylphosphine reacts with DIPAD to generate a triphenylphosphonium intermediate, activating the hydroxyl group as a good leaving group. The synthesis of **AL-220910-f2** (mono-substitution product, 30% yield) and **AL-220910-f4** (di-substitution product, 60% yield) was carried out at 30°C for 21h. These products were purified by flash chromatography (EtOAc/Hexane, 0.1:1→ 0.7:1).

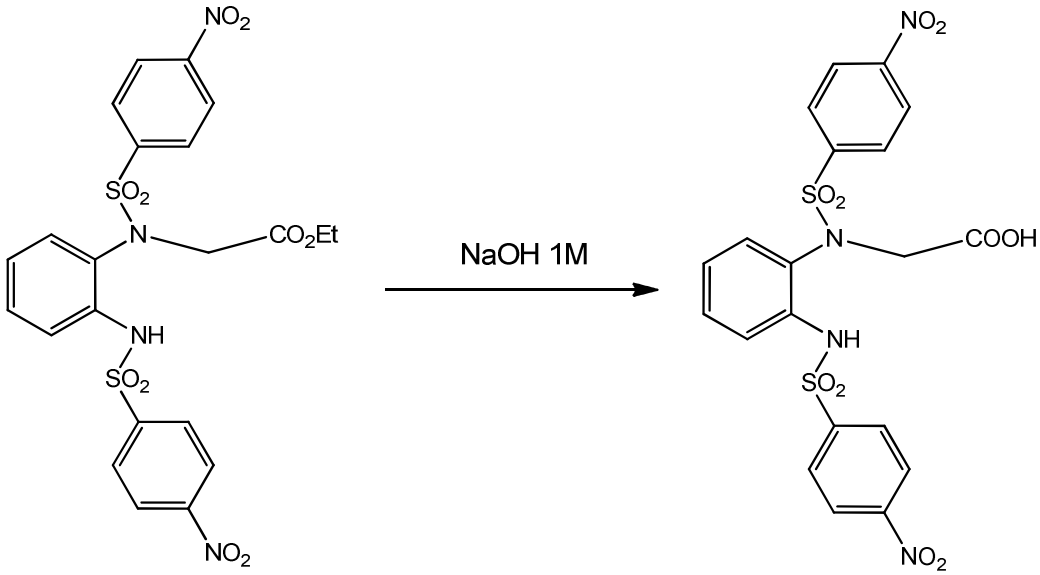


AL-200910 **AL-220910-f2** **AL-220910-f4**

Fig. 26. Synthesis of acyclic bis(*p*-nitrobenzenesulfonamides)

For the preparation of the 2-{*p*-nitro-*N*-[2-(*p*-nitrobenzenesulfonamido)benzene]benzenesulfonamido}acetic acid

AL-010311, the ethyl 2-{*p*-nitro-*N*-(2-(*p*-nitrobenzenesulfonamido)benzene]benzene-sulfonamido}acetate **AL-220910-f2** was treated with NaOH 1M until its complete hydrolysis (Fig. 27).

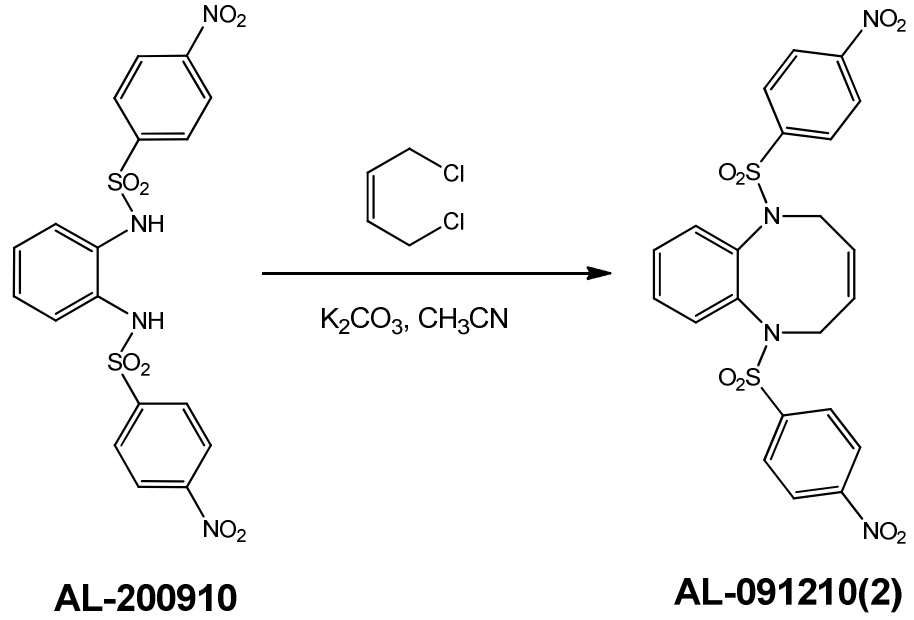


AL-220910-f2 **AL-010311**

Fig. 27. Synthesis of 2-{*p*-nitro-N-[2-(*p*-nitrophenylsulfonamido)benzene]benzene-sulfonamido}acetic acid

For the preparation of the benzodiazocine **AL-091210(2)**, the *N,N'*-(1,2-phenylene)bis(*p*-nitrobenzenesulfonamide **AL-200910** was dissolved in acetonitrile (CH_3CN), and K_2CO_3 and *cis*-1,4-dichloro-2-butene were

added, and the mixture heated at reflux overnight[39] (Fig. 28). Flash chromatography on silica gel (EtOAc/Hexane, 0.5:1) afforded pure **AL-091210(2)**.



AL-200910 **AL-091210(2)**

Fig. 28. Synthesis of benzodiazocine **AL-091210(2)**

The two-step reaction, hydroboration-oxidation, was used to produce the alcohol **AL-110111-f3**. In the first step, the alkene **AL-091210(2)** was subjected to hydroboration with the borane-tetrahydrofurane complex, 1.0M solution in THF (BH_3·THF) followed by oxidation with trimethylamine *N*-oxide (Me_3NO) to give the secondary alcohol **AL-110111-f3**[40,41] (Fig. 29).

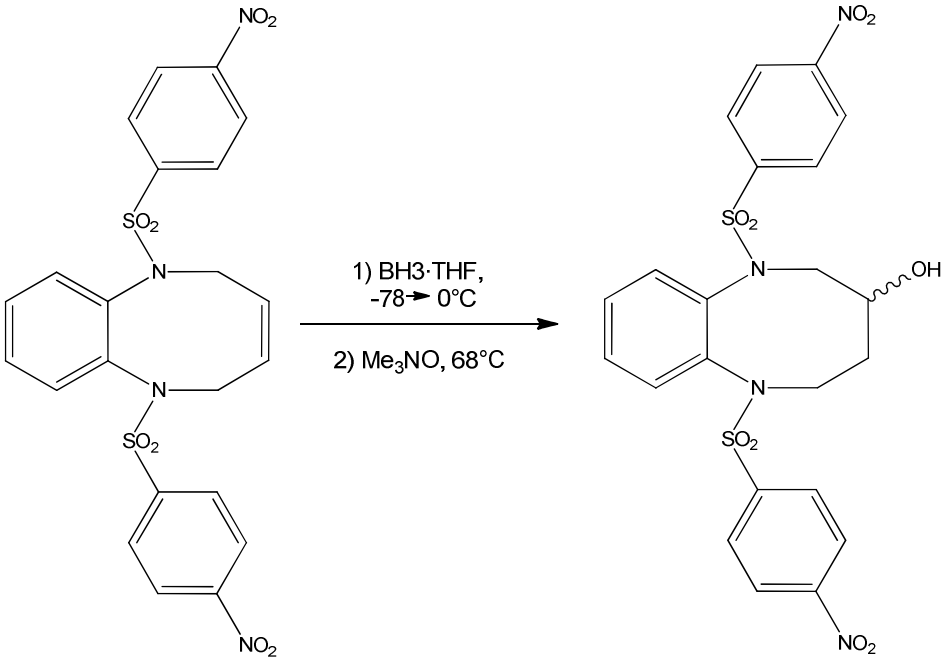


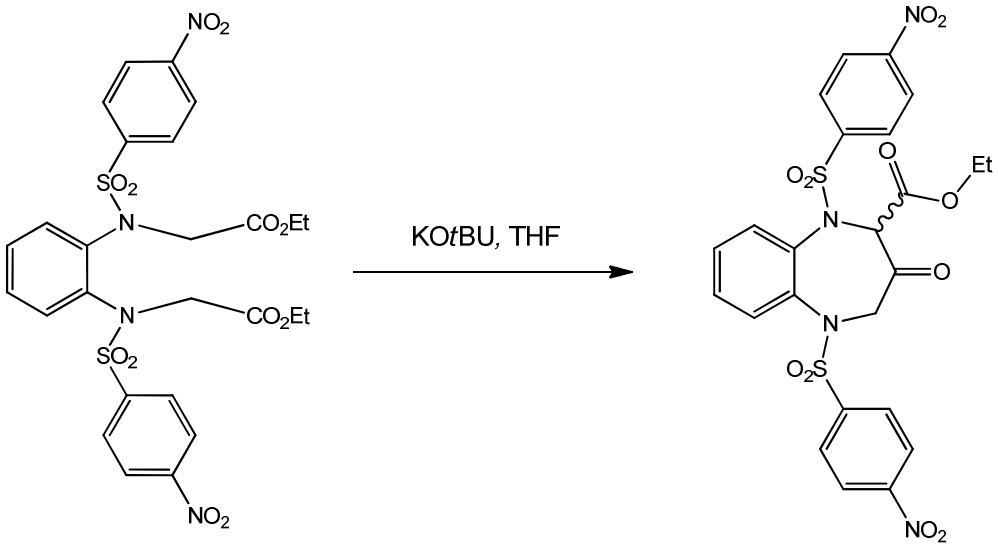
Fig. 29. Synthesis of the secondary alcohol **AL-110111-f3.**

6.2. FAILED REACTIONS

Different reactions in the synthesis project did not occur. This has caused changes in the synthetic strategies and different intermediates

and final products have not been obtained. To avoid this, many attempts have been made without any successful results.

As in the synthesis of (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines (see 7.1.1) we thought on making the Dieckmann reaction on compound **AL-220910-f4** to obtain the corresponding β-ketoester (Fig. 30).



AL-220910-f4

Fig. 30. Attempted preparation of the seven-membered β-ketoester.

The resulting β-ketoester could have been saponified, treated with an acid to produce the β-ketoacid, and subsequently decarboxylated to render a symmetrical ketone. This could have been reduced to the secondary alcohol to allow a Mitsunobu reaction with various *6*- and

2,6- mono- and di-substituted purines (Fig. 31). Unfortunately, the first step (Dieckmann reaction) did not come out and the multi-step synthetic pathway was abandoned. Many attempts have been made on the first step without any successful results. Because the Dieckmann condensation works well to produce 5- or 6-membered cyclic β-ketoesters and we pretended to produce a cyclic 7-membered β-ketoester, we forced the reaction conditions using more equivalents of the strong base potassium *tert*-butoxide (KO*t*Bu) and increasing the reaction temperature.

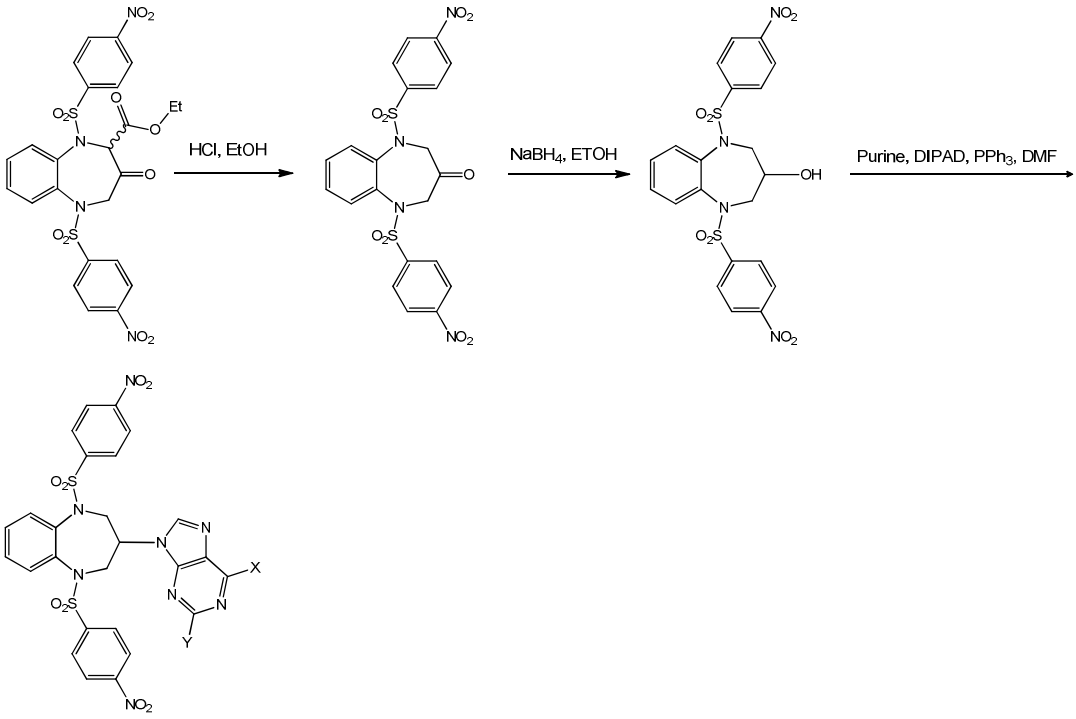
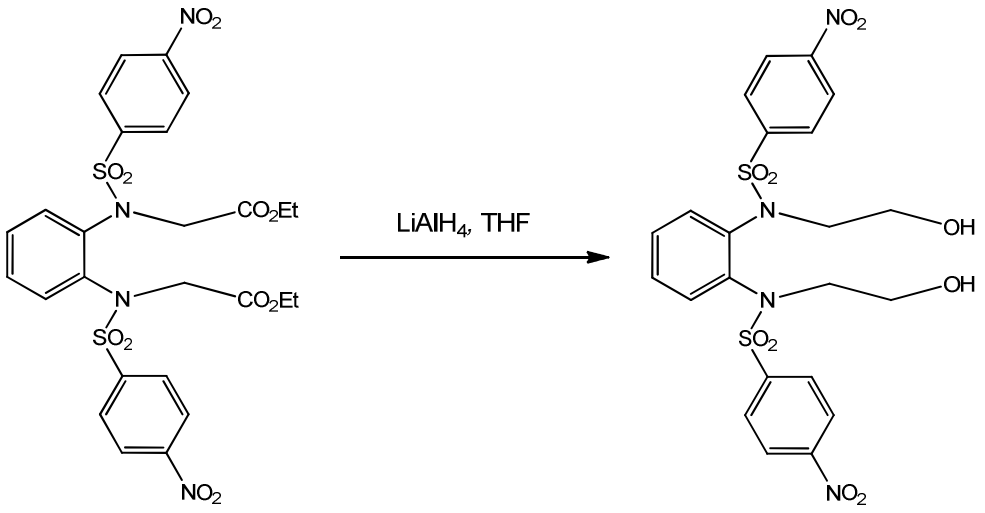


Fig. 31.

As no results were obtained with this strategy, we thought that the problem could have been due to the steric hindrance caused between KO*t*Bu and the bulky *p*-nitrobenzenesulfonamide group of the β-ketoester. We thus changed the base, from KO*t*Bu to sodium ethoxide, a non-bulky base. Despite this change the reaction was not achieved.

In the synthesis of acyclic bis(*p*-nitrobenzenesulfonamide) the reduction of **AL-220910-f4** to the corresponding diol (Fig. 32) did not take place.



AL-220910-f4

Fig. 32.

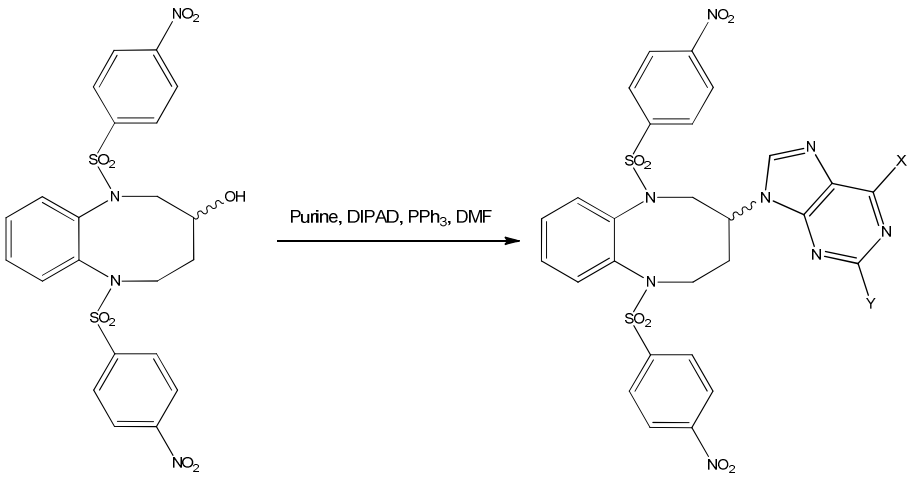
Lithium aluminum hydride ($LiAlH_4$) did not reduce the di-esters to diol but broke down he sulfonamide bond.

- **Failed Mitsunobu reactions**

Many Mitsunobu reactions were done in this project (see above), most of them were reached successfully and many final products were obtained. Unfortunately some of them have not given the desired final products.

In the synthesis of phenylpyrrolidine derivatives the reactions between 6-chloro-Purine and **KUD 223** and **KUD 169** did not take place. Lack of time did not allow the repetition of these reactions.

In the synthesis of a new purine family the reactions between **AL-110111-f3** and various *6*- and *2,6*- mono- and di-substituted purines did not occur (Fig. 33).



AL-110111-f3

Fig. 33.

Many fruitless attempts, such as the use of modified Mitsunobu conditions (see above) were made in this case. Probably the unsuccessful reaction is due to the electron-withdrawing electronic effect of the *p*-nitrosulfonamide group. It does not allow the alcohol oxygen to be bound to triphenylphosphine to form the triphenylphosphonium intermediate.

6.3. BIOLOGICAL ACTIVITY

6.3.1. Cell lines and growth conditions

A549 bronchoalveolar carcinoma, MCF-7 breast carcinoma and PC-3 prostate cancer cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These cells were maintained in a complete medium, consisting of RPMI 1640 growth medium (Invitrogen) with Glutamax® (A549 and PC-3) or of DMEM:F12 medium (Lonza, Basel, Switzerland) (MCF-7), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (both antibiotics from Invitrogen). Cells were grown at 37°C in a 5% CO_2 atmosphere.

6.3.2. Cell proliferation assay (MTT)

Cells were seeded in 96-well plates at a density of 1000 cells/well. After 24 h to allow for attachment, cells were treated with 0.05, 0.5, 1, 2, 5, 10, 20, 50 and 100μM synthesized and purified compounds or remained untreated (controls) during 72h. Cell proliferation or growth inhibition was measured using the MTT (3-{4,5-Dimethylthiazol-2-yl}-2,5-diphenyltetrazolium) bromide, Sigma-Aldrich, USA) assay following

the MTT Cell Proliferation Kit I (Roche, Mannheim, Germany) and according to the manufacturer's recommendations. Absorbance readings at 540/690 nm were realized using the Sunrise ELISA plate reader (Tecan Austria GmbH, Salzburg, Austria). Wells containing only complete medium were used as controls.

6.3.3. Activities

Results of the biological assays at the 0.05, 0.5, 1, 2, 5, 10, 20, 50 and 100 µM of the screened compounds are shown. The more interesting values are highlighted in yellow.

1) AL-250511-f3

	0	0,05	0,5	1	2	5	10	20	50	100
	Control	**0,05µM**	**0,5µM**	**1µM**	**2µM**	**5µM**	**10µM**	**20µM**	**50µM**	**100µM**
A549	100	99,4884287	86,3824604	95,6881851	98,9768575	94,0316687	98,2704019	89,5736906	86,3093788	83,361754
MCF-7	100	93,5907539	92,4349882	93,7483583	93,4068821	90,333596	95,7184134	90,7538744	88,1271342	79,6164959
PC-3	100	101,019208	101,019208	98,9807918	90,3567229	99,2159937	100,980008	95,374363	63,8965112	40,4155233

2) AL-310511-f1(Cl)

	Control	**0,05µM**	**0,5µM**	**1µM**	**2µM**	**5µM**	**10µM**	**20µM**	**50µM**	**100µM**
A549	100	82,0379323	91,0561547	89,6801785	110,933433	95,7419115	91,1305318	96,2439569	81,8891781	79,3417627
MCF-7	100	89,2461782	95,1502372	93,5951502	118,371112	95,3874539	95,6246705	93,4633632	87,8228782	84,6072746
PC-3	100	91,1408144	93,472179	94,5601492	98,5700964	101,585328	101,336649	100,870376	74,0752254	40,441405

3) AL-310511-f1(Br)

	Control	**0,05µM**	**0,5µM**	**1µM**	**2µM**	**5µM**	**10µM**	**20µM**	**50µM**	**100µM**
A549	100	88,5750729	93,7864431	96,1005831	100,145773	91,1989796	90,6705539	86,2062682	80,5029155	68,7682216
MCF-7	100	96,5900846	96,2756953	91,7775091	91,076179	91,1245466	94,5586457	91,9951632	82,853688	68,923821
PC-3	100	104,481793	107,563025	106,162465	103,781513	100	103,781513	99,3347339	57,1078431	30,952381

4) AL-220910-f4

	Control	**0,05µM**	**0,5µM**	**1µM**	**2µM**	**5µM**	**10µM**	**20µM**	**50µM**	**100µM**
A549	100	94,6247068	100,351837	98,4753714	98,3580923	98,1626271	88,7216575	76,3682565	76,7982799	67,7677873
MCF-7	100	96,2602842	92,9194715	93,5926203	95,0137123	94,5150835	89,8030416	81,2515582	78,1849913	71,7277487
PC-3	100	93,9533544	90,5845091	93,0607544	95,709761	89,3175929	88,3961993	82,2919666	80,8810826	65,7644688

5) AL-300311-f1

	Control	**0,05µM**	**0,5µM**	**1µM**	**2µM**	**5µM**	**10µM**	**20µM**	**50µM**	**100µM**
A549	100	96,8099592	100,291772	97,1211826	97,5296635	95,4483564	92,3944758	89,2822408	86,3061661	77,3390391
MCF-7	100	92,7175844	85,812611	92,2513321	93,0950266	87,3667851	88,7211368	81,0612789	85,2353464	80,2619893
PC-3	100	100,766667	99,6666667	97,7333333	96,1333333	97,9333333	97,9	97,4333333	100,4	97,8666667

6) AL-010311

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	98,8742236	98,9906832	100,989907	97,0496894	99,6506211	99,1459627	98,0784161	92,2166149	64,2468944
MCF-7	100	89,0382387	90,9154114	94,2526072	89,3858633	92,9316338	95,6431054	97,0799537	101,274623	97,5434531
PC-3	100	88,3051907	78,4865541	76,641651	77,9862414	81,3320826	83,9899937	93,5272045	87,6172608	43,4333959

7) AL-240311

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	94,8468249	94,1164536	94,1367417	97,4639886	92,6557111	90,5254616	90,3022926	77,6628119	65,6522621
MCF-7	100	82,89801	79,7470978	76,8242123	81,0323383	77,7363184	77,238806	73,6525705	67,8897181	63,6401327
PC-3	100	97,4184783	94,1915761	89,2663043	91,9497283	89,1983696	81,7595109	72,7581522	45,2785326	37,0244565

8) AL-220910-f2

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	97,6648914	95,7189676	98,1360098	95,4321999	96,7226546	98,2179435	97,8082753	83,3879558	32,3637853
MCF-7	100	88,8963812	88,6491346	87,7950101	85,6821758	88,0647336	84,0863115	87,5702405	82,2881546	43,8300742
PC-3	100	98,1108702	94,9829669	90,4304738	90,7401672	90,6162899	91,0188913	86,714153	84,4224218	44,1932487

9) AL-150210-f3

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	103,694538	103,659183	103,977373	99,6994874	95,0857345	90,41895	80,9439632	82,2874315	66,9259325
MCF-7	100	92,4328723	89,9511798	90,3173312	83,5842148	87,8763222	88,0593979	82,2213181	83,7876322	78,3360456
PC-3	100	104,094666	88,4673178	98,8354621	97,9338843	100,075131	88,2419234	86,2885049	76,296018	45,0413223

10) AL-200910

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	108,278906	96,9285084	100,776699	108,331862	100,529568	96,8049426	103,759929	98,0052957	92,7096205
MCF-7	100	95,8235753	86,5534738	90,6323185	89,9297424	85,3044496	95,5503513	90,1249024	89,4613583	89,6955504
PC-3	100	92,0623671	106,484763	87,9518072	81,644224	85,0106308	93,2317505	91,318214	88,518781	82,9907867

11) AL-091210 (2)

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	87,7177231	99,1201293	98,02478	92,1350332	99,8922607	79,5295385	84,3598492	68,1630454	51,6968935
MCF-7	100	76,8262586	111,056269	116,263574	108,563672	112,314906	83,242843	96,2981244	86,6485686	53,8252715
PC-3	100	96,9536424	97,615894	109,624724	98,1456954	98,1456954	87,4172185	86,3576159	82,6490066	57,8366446

12) AL-260510-f3

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	94,8622222	94,08	91,1466667	97,3688889	91,6088889	93,0666667	95,7511111	85,9555556	74,6666667
MCF-7	100	90,0132861	88,6403897	88,3968113	86,1603189	83,3923826	83,9238264	84,3224092	75,8635961	67,3383525
PC-3	100	104,096386	101,927711	100,963855	104,016064	92,4497992	86,8674699	68,2329317	41,0843373	35,060241

13) AL-110111-f3

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	96,2837838	96,5682788	97,2439545	97,101707	90,3271693	94,0789474	93,2610242	83,9082504	80,6365576
MCF-7	100	85,2678571	86,1383929	83,28125	79,8883929	79,1964286	83,8392857	82,7232143	83,6607143	84,5089286
PC-3	100	96,4259396	97,1260133	88,8725129	90,0147384	93,8098747	95,6890199	99,3367723	95,3205601	95,357406

14) KUD 327

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	98,9906273	94,6467195	92,4837779	94,9891853	95,8002884	94,0519106	93,5472242	84,1204037	75,1261716
MCF-7	100	89,6466905	86,627907	87,5670841	83,6538462	79,3157424	82,2450805	79,0026834	77,3703041	70,6395349
PC-3	100	92,7457381	99,5647443	95,1759159	95,3210011	88,1392818	91,4036997	94,4142184	89,4087777	82,3721436

15) KUD 526

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	103,115638	104,533653	96,3051728	93,9285001	95,0469343	89,5546235	90,6331136	84,3618933	78,8496105
MCF-7	100	82,15973	82,4521935	90,1687289	79,7075366	83,5545557	86,6591676	84,071991	81,1248594	78,807649
PC-3	100	97,3486206	94,9122178	93,9806521	92,547474	93,0490863	92,7982802	96,0587603	91,9742028	85,7757076

16) KUD 600

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	99,7183665	99,6781332	100,160933	95,272581	94,2466305	89,0162945	84,6509757	78,5556226	66,1436331
MCF-7	100	89,3559489	87,4877089	89,0609636	84,7590954	90,2409046	82,9891839	86,7256637	85,0786627	80,3834808
PC-3	100	103,637124	108,277592	99,7491639	92,2240803	95,6103679	90,7608696	96,4046823	92,1404682	99,1638796

17) KUD 533

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	100	100,25878	100,831793	96,8207024	100,147874	93,4565619	84,103512	58,5582255	44,8613678
MCF-7	100	85,6465302	87,1442836	85,9710434	86,7698452	91,6375437	89,091363	85,4468298	59,1612581	47,3290065
PC-3	100	99,776086	98,1191223	97,5817286	112,091357	103,224362	100,895656	95,7456337	63,546798	55,4858934

18) AL-080611-f2 (Br)

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	105,260024	107,622072	101,409289	102,441445	100,258039	97,697499	96,7645891	87,8324732	76,0619293
MCF-7	100	86,3793957	82,4665676	83,7543338	82,4418029	85,2402179	83,3580981	89,252105	86,4041605	78,7766221
PC-3	100	86,9799692	85,5161787	84,6302003	87,8659476	93,1047766	87,3651772	86,2865948	91,3328197	95,7241911

19) AL-080911-f2 (2,6diCl)

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	94,4707091	95,971223	97,5128469	98,4378212	101,952724	100,24666	101,418294	93,7307297	97,1839671
MCF-7	100	91,6626155	90,2041808	92,0758386	88,5026738	89,6937287	91,4924648	87,9193	90,1312591	91,7112299
PC-3	100	106,669179	102,298417	97,2494348	97,4378297	102,411454	101,770912	102,373775	102,034665	109,231349

20) AL-260511-f2

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	97,2013524	99,6431255	107,006011	91,1157025	98,703982	88,1667919	93,5762585	77,1036814	79,2073629
MCF-7	100	81,3658978	86,1148801	84,418815	79,6924469	78,3808232	77,9285391	82,0217096	72,7951153	81,2302126
PC-3	100	95,7575758	95,6439394	92,2348485	93,7878788	95,0757576	99,3939394	96,780303	81,7424242	86,25

21) AL-120411-f1

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	80,6835999	85,2461587	92,6622766	88,8679837	82,925682	79,4763249	82,6904986	78,3788021	63,750392
MCF-7	100	82,8180212	81,9567138	81,4487633	78,2022968	76,5238516	79,1740283	79,6157244	77,5176678	72,4823322
PC-3	100	93,8526155	92,9744177	91,4471172	90,1489118	85,2615502	87,2088583	89,8434517	92,8598702	81,2905689

22) AL-110411-f1

	Control	0,05µM	0,5µM	1µM	2µM	5µM	10µM	20µM	50µM	100µM
A549	100	93,3578657	89,6044158	85,4461822	86,1453542	83,4038638	82,6126955	70,873965	69,2732291	54,2594296
MCF-7	100	80,738673	82,1773674	77,41035	71,4193687	74,5973803	74,8550569	69,873309	64,3547348	65,6216448
PC-3	100	95,5911824	92,7187709	93,4869739	90,7815631	86,1055444	70,4074816	51,3026052	37,3413494	32,49833

The IC_{50} values were calculated from semi-logarithmic dose-response curves by linear interpolation. All of the experiments were plated in triplicate wells and were carried out thrice.

Compounds	A549	MCF-7	PC-3
1) AL-250511-f3	>100	>100	87
2) AL-310511-f1(Cl)	>100	>100	50-100
3) AL-310511-f1(Br)	>100	>100	50-100
4) AL-220910-f4	>100	>100	>100
5) AL-300311-f1	>100	>100	>100
6) AL-010311	>100	>100	50-100
7) AL-240311	>100	>100	31
8) AL-220910-f2	50-100	50-100	50-100
9) AL-150210-f3	>100	>100	50-100
10) AL-200910	>100	>100	>100
11) AL-091210 (2)	100	100	100
12) AL-260510-f3	>100	>100	27
13) AL-110111-f3	>100	>100	>100
14) KUD 327	>100	>100	>100
15) KUD 526	>100	>100	>100
16) KUD 600	>100	>100	>100
17) KUD 533	50-100	50-100	100
18) AL-080611-f2 (Br)	>100	>100	>100
19) AL-080911-f2 (2,6diCl)	>100	>100	>100
20) AL-260511-f2	>100	>100	>100
21) AL-120411-f1	>100	>100	>100
22) AL-110411-f1	100	>100	20

7. CONCLUSIONS

1. Three families of anti-tumour drugs (18 compounds) have been synthesized to appraise the differences of anticancer activity against the A549 bronchoalveolar carcinoma, MCF-7 breast carcinoma and PC-3 prostate cancer cell lines:

- **Family 1**: (6'-Substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines (**AL-150210-f3**, **AL-240311**, **AL-300311-f1**, **AL-260510-f3** and **AL-110411-f1**)

- **Family 2:** Phenylpyrrolidine derivatives (**AL-120411-f1**, **AL-250511-f3**, **AL-310511-f1(Cl)**, **AL-310511-f1(Br)**, **AL-260511-f2**, **AL-080611-f2(Br)**, and **AL-080611-f2(2,6 diCl)**).

- **Family 3:** Cyclic and acyclic bis(*p*-nitrobenzenesulfonamides) (**AL-200910**, **AL-220910-f2**, **AL-220910-f4**, **AL-010311**, **AL-091210(2)** and **AL-110111-f3**).

2. All the final target purine derivatives (those linked to the 2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl and to the phenylpyrrolidine moieties) were obtained *via* the Mitsunobu reaction under microwave-assisted irradiation, which has provided faster access to the target compounds.

In all the cases the *N*-9' regioisomers of the purine moiety have only been formed.

3. The use of a modified Mitsunobu reaction, that corresponds to the last step in the synthetic route of (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines has considerably increased the yield of this series.

4. The ^{1}H and ^{13}C signals of the title compounds have been fully assigned using one and two-dimensional NMR techniques including DEPT (Distorsionless Enhancement by Polarization Transfer), HMQC (Heteronuclear Multiple-Quantum Correlation) and HSQC (Heteronuclear Single Quantum Coherence).

5. AI-110411-f1 [9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-6'-iodo-9*H*-purine], **AL260510-f3** [9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purine] and **AL240311** [6'-bromo-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purine] are the three most active compounds against PC-3 with IC_{50} = 20, 27 and 31 μM, respectively. On the other hand, **AL-250511-f3** is the most active derivative of the phenylpyrrolidine family with an IC_{50} = 87 μM against the PC-3 cell line.

8. FUTURE WORK

(6'-Substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines are key compounds for further development of anti-cancer drugs. One of the oxygen atoms of the seven-membered scaffold will be changed for its isostere atoms such as sulfur and nitrogen. In the latter case, and according to the experience of the Granada's research group, the presence of a strong electron-withdrawing group linked to the nitrogen of the seven-membered ring is of paramount importance in relation to the potential anti-cancer activity of such molecules. Such isosteric changes will break the symmetry of the resulting molecules and accordingly, the new carbon 3 will be a stereogenic center. Both enantiomers are being stereo-specifically synthesized at the University of Granada and once prepared, they will be screened for their anti-cancer activity.

On the other hand, cyclic and acyclic bis(*p*-nitrobenzenesulfonamides) are additionally modified and will be screened for biological activity in a different therapeutic area., such as anti-parasitical molecules. The structural modifications previously commented are under way. In short, the present thesis constitutes the basis an inspiration for the development and improvement of even more active anti-cancer and

anti-leishmania drugs, the latter field in collaboration with the *Instituto de Parasitología López-Neira* of Granada.

9. EXPERIMENTAL PART

9.1. GENERAL CONDITIONS

The melting points were determined using open capillaries in electro-thermic systems used for melting point calculation. The thin-layer chromatography (TLC) was performed using aluminum sheets Merck Kieselgel 60 F_{254}. TLC stains were detected through UV light (254 nm). All the evaporations were carried out under vacuum by Buchi rotavapors and the pressure controlled by Vacucbran CVC^{II} devices. Flash chromatography was performed using the silica gel Merck 60 with 0040-0063 mm size (230-400 mesh ASTM). The nuclear magnetic resonance (NMR) spectra were performed in *Centro de Instrumentación Cientifica (CIC)/Universidad de Granada.* They were developed and registered at 300 MHz ^{1}H and 100 MHz ^{13}C using NMR Varian NMR-system-TM spectrometer, or at 400 o 300 MHZ ^{1}H and 75 MHz ^{13}C NMR using Varian Inova-TM spectrometer, at room temperature. The chemical shift (δ) was reported in parts per million (ppm) and referenced to the residual solvent peak. The coupling constants (*J*) are given in Hertz (Hz). The spin multiplicity is given by s (singlet), d (doublet), t (triplet),

dd (double doublet), dt (double triplet), m (multiplet). The high resolution mass spectroscopy (HR-LSIMS) was performed on a high-resolution mass spectrometer: VG Auto Spec Q (Fisons Instruments). The HMBC spectra were measured using a pulse sequence of 10 Hz (delay between pulses for the evolution of long-range couplings: 50 ms) and a gradient combination of 5:3:4. In this way, direct responses (^1J coupling) are not completely removed. Delay between pulses of 3.2 ms and a gradient combination of 5:3:4 were used for the HMQC spectra. Microwave syntheses were performed with a Biotage initiator apparatus 2.0 which produces controlled irradiation till 2,450 GHz (Biotage AB, Uppsala). The reaction time refers to the time at 140/160°C (reaction temperature), and not to the total time of irradiation. The temperature is measured with an IR sensor on the outside side of reaction vessel. 6-Chloropurine, 6-bromopurine and 2,6-dichloropurine were purchased from Aldrich. Anhydrous solvents (DMF and THF) were purchased from VWR International Eurolab. Reactions were performed under inert gas (Ar) unless specified.

Synthesis of dimethyl 2,2'-[1,2-phenylenebis(oxy)] diacetate (1).[35]

Ground over-dried K_2CO_3 (4 eq.) and methyl bromoacetate (4 eq.) were added sequentially to a solution of catechol (1 eq.) in anhydrous DMF. The solution was heated at 120°C for 2h, then the hot mixture was poured into ice/H_2O and extracted with CH_2Cl_2 (3 ×). The combined organic phase was washed with 5% NaOH followed by H_2O (2 ×), dried ($MgSO_4$) and concentrated. Flash chromatography (EtOAc/Hexane, 1:1) afforded **1** (52%, R_f = 0.50) as a white solid: Mp =43-44°C.

^{1}H-NMR (300MHz, $CDCl_3$): δ (ppm) 6.94-6.86 (*m*, 4H), 4.71 (*s*, 4H), 3.77 (*s*, 6H). ^{13}C-NMR: (75 MHz, $CDCl_3$): δ (ppm) 169.3 (2 C-O), 147.9 (C_1-C_2), 122.6 (C_4- C_5), 115.3 (C_3-C_6), 66.5 (2 CH_2), 52.1 (2 CH_3).

Synthesis of methyl 3-oxo-3,4-dihydro-2*H*-1,4-benzodioxepine-2-carboxylate (2).[35]

Under an inert atmosphere a solution of **1** (1 eq.) in anhydrous THF was added within 5 min to a stirred solution of KO*t*Bu (2 eq.) in anhydrous THF with cooling in a ice/H_2O bath. The solution was then transferred to an oil bath at 70°C and heated for 30 min. before quenching in a stirred mixture of 0.2 M HCl in ice. The acid solution

was extracted with CH_2Cl_2 (3 ×) washed with H_2O (2 ×), dried ($MgSO_4$) and concentrated. Flash chromatography (EtOAc/Hexane, 3:2) afforded **2** (36%, R_f = 0.78) as a pale brown oil.

^{1}H-NMR (300MHz, $CDCl_3$): δ (ppm) 7.11-6.95 (*m*, 4H), 5.36 (*s*, 1H), 4.82 (*d, J*=16.7, 1H), 4.56 (*d, J*=16.7, 1H), 3.85 (*s*, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 199.7 (C_3), 164.5 (CO_2), 147.1 (C_{9a}), 145.9 (C_{5a}), 130.7 (C_6), 125.2 (C_7), 124.0 (C_8), 118.1 (C_9), 81.8 (C_2), 76.4 (C_4), 52.9 (CH_3).

Synthesis of 3,4-dihydro-2*H*-1,5-benzodioxepine-3-one (3).[35]

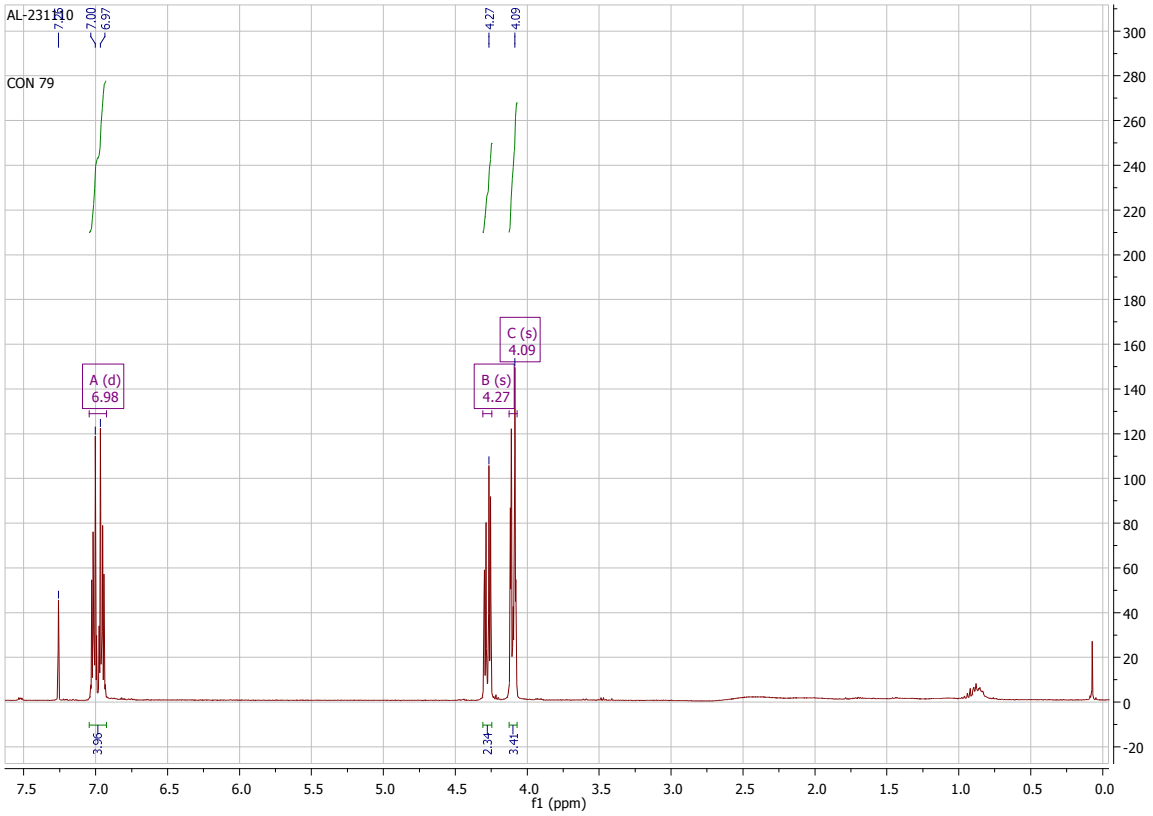
To a solution of **2** (0.4 g) in EtOH (5 mL) was added 2M HCl (21,3 mL) and the solution was heated at 90°C while stirring in an oil bath. After 2h, the mixture was poured into ice/H_2O and extracted with CH_2Cl_2. The combined organic phase extract was washed with H_2O (2 ×), dried ($MgSO_4$) and concentrated. The obtained brown semi-solid was subjected to bulb-to-bulb distillation to obtain **3** (79.5% yield).

^{1}H-NMR (300MHz, $CDCl_3$): δ (ppm) 6.86 (*m*, 4H), 4.74 (*s*, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 205.1 (C_3), 148.4 (C_{9a}-C_{5a}), 130.4 (C_6-C_9), 125.4 (C_7-C_8), 75.6 (C_4-C_2).

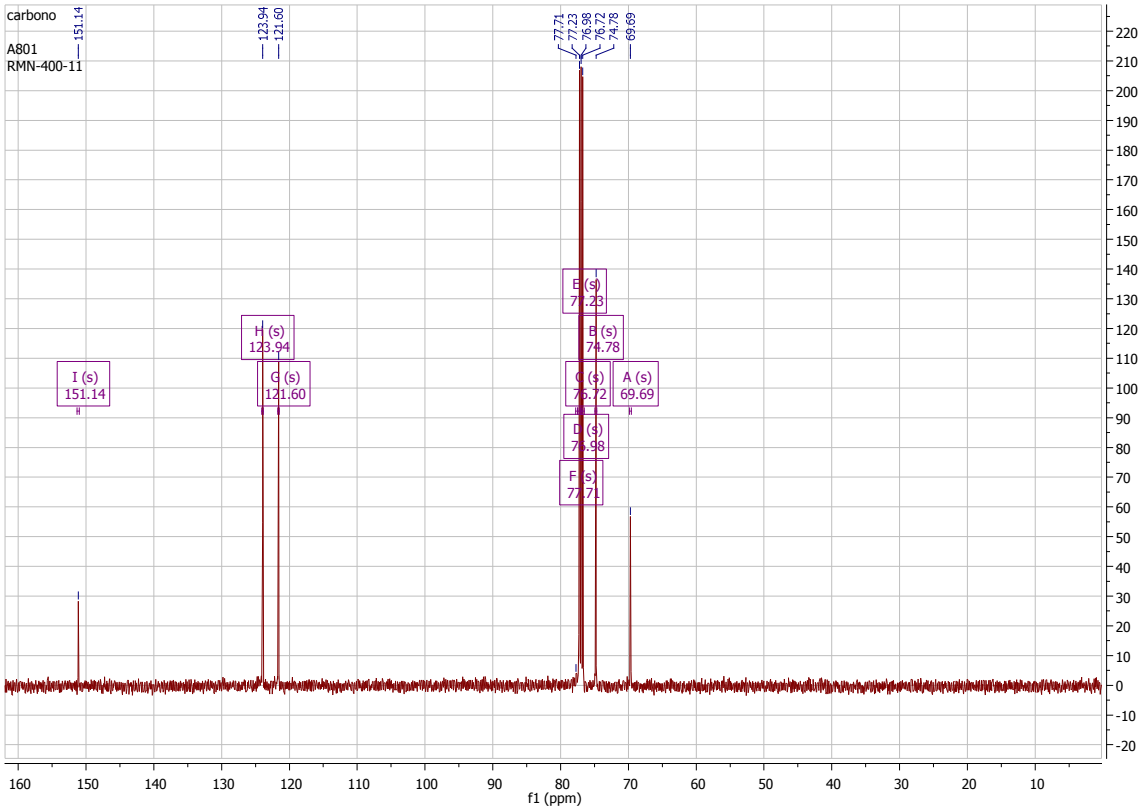
Synthesis of 3,4-dihydro-2*H*-1,5-benzodiaxepine-3-ol (4).

To a solution of **3** (1 eq.) in EtOH at room temperature was added portion wise $NaBH_4$ (1.2 eq.) and the mixture stirred at room temperature during 3h. AcOH was added and the solvent was evaporated. The residue was dissolved in Et_2O, washed with H_2O (2 ×), dried ($MgSO_4$) and concentrated. The residue was crystallized from Et_2O and a white solid (**4**) was obtained (72.2% yield).

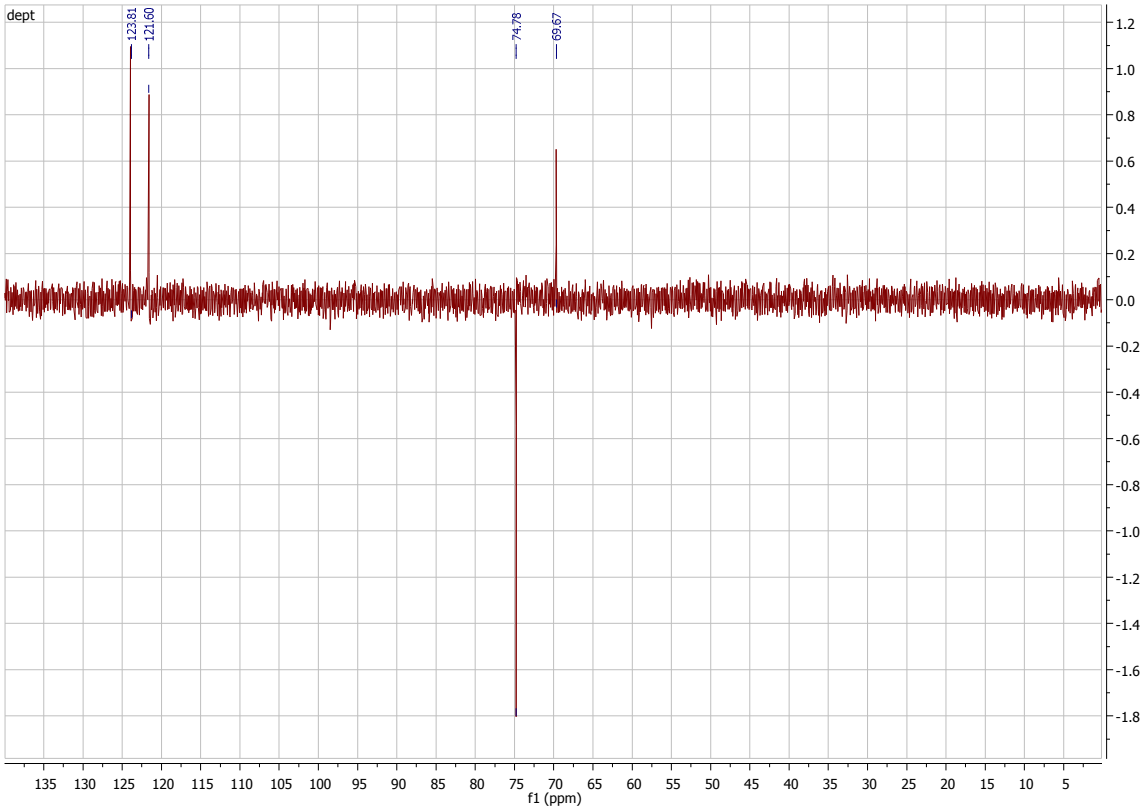
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.98 (*m*, 4H), 4.27 (*s*, 4H), 4.09 (*s*, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 151.14 (C_{5a}-C_{9a}), 123.94 (C_6-C_9), 121.60 (C_7-C_8), 74.78 (C_2-C_4), 69.69 (C_3). Anal. for $C_9H_{10}O_3$: Calcd.: C 65.05; H 6.07. Found: C 64.98; H 5.99.


AL-231110
CON 79
7.00
6.97
4.27
4.09
A (d)
6.98
B (s)
4.27
C (s)
4.09
3.96
2.34
3.41
f1 (ppm)

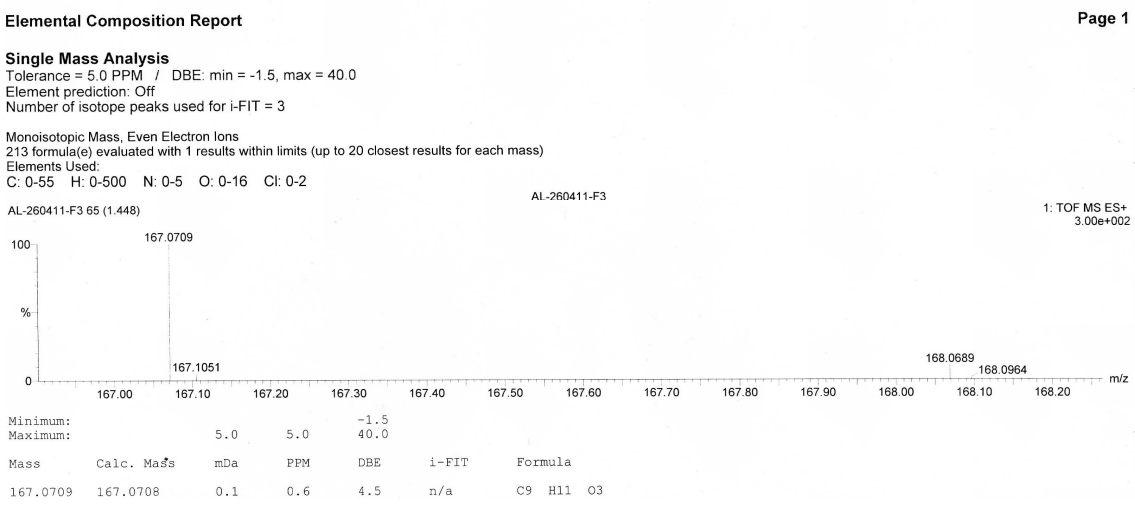
^{1}H-NMR of (4)


carbono
A801
RMN-400-11
151.14
123.94
121.60
77.71
77.23
76.98
76.72
74.78
69.69
I (s)
151.14
H (s)
123.94
G (s)
121.60
E (s)
77.23
B (s)
74.78
C (s)
76.72
A (s)
69.69
D (s)
76.98
F (s)
77.71
f1 (ppm)

^{13}C-NMR of (4)


dept
123.81
121.60
74.78
69.67
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

DEPT of (4)



Mass analysis of (4)

Synthesis of (6'-substituted)-9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-9*H*-purines (AL-150210-f3, AL-240311, AL-300311-f1, AL-260510-f3).

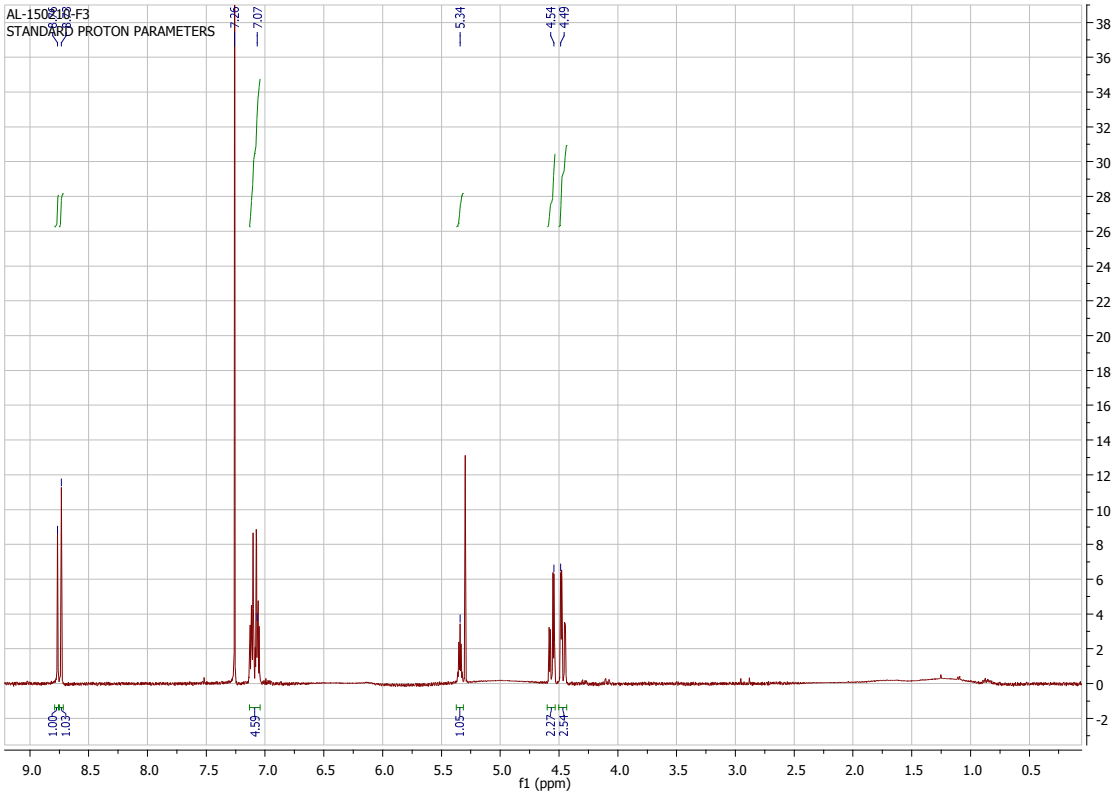
Standard conditions: A microwave vial (2-5mL) equipped with a magnetic stirrer bead was charged with **4** (50mg, 1eq.), triphenylphosphine (1.5 eq.), and the corresponding purine derivative (1.2 eq.) in dry DMF (3.5mL), cooled in an CO_2/acetone bath (-20°C) and DIPAD (1.5 eq.) was slowly added. The vial was sealed and microwave irradiated at a temperature of 165°C for 10 min. After

completion of the irradiation time, the reaction mixture was cooled to room temperature through rapid pressurized air supply gas–jet cooling. The solvent was evaporated, and the crude product was added onto a silica column and purified firstly by flash chromatography (CH_2Cl_2/MeOH, 9.8:0.2) and then by preparative TLC (CH_2Cl_2/MeOH, 9.8:0.2) to afford **AL-150210-f3** (22% yield, R_f = 0.60), **AL-240311** (8% yield, R_f = 0.68), **AL-300311-f1** (15% yield, R_f = 0.65) and **AL-260510-f3** (10% yield, R_f = 0,70) as white solids.

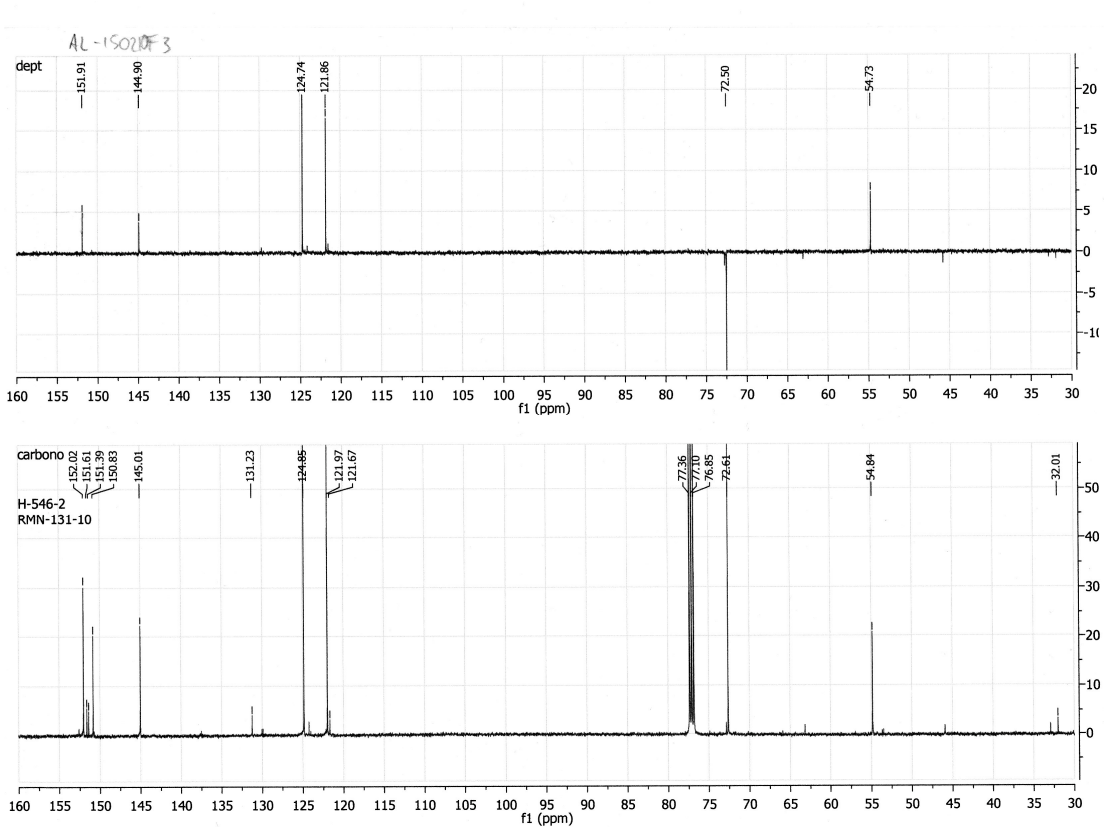
Modified conditions: A microwave vial (2-5mL) equipped with a magnetic stirrer bead was charged with **4** (50mg, 1eq.), diphenyl (2-piridyl) phosphine (1.5 eq.), and the corresponding purine derivative (1eq.) in dry DMF (3.5mL), cooled in a CO_2/acetone bath (-20°C) and DBAD (1.5 eq.) was slowly added. The vial was sealed and microwave irradiated at a set temperature of 165°C for 10 min. After completion of the irradiation time, the reaction mixture was cooled to room temperature through rapid pressurized air supply gas–jet cooling. A solution of HCl 4 M in dioxane (3 mL) was added to the mixture and after stirring for 1.5 h the excess solvent was evaporated. The residue was dissolved in Et_2O or CH_2Cl_2 and shaken vigorously with 4M aqueous HCl (2 ×), dried ($MgSO_4$) and concentrated to gave the

desired products **AL-150210-f3** (80% yield), **AL-240311** (56% yield), **AL-300311-f1** (70% yield) and **AL-260510-f3** (63% yield).

AL-150210-f3: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.76 (*s*, 1H), 8.73 (*s*, 1H), 7.07 (*m*, 4H), 5.34 (*s*, 1H), 4.56 (*dd*, *J* = 12.6, 3.9 Hz, 2H), 4.47 (*dd*, *J* = 12.6, 3.9 Hz, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm) 152.02 ($C_{2'}$-$C_{6'}$), 151.61 (C_{5a}-C_{9a}), 151.39 ($C_{4'}$), 131.23 ($C_{8'}$), 124.85 (C_7-C_8), 121.96 (C_9-C_6), 72.61 (C_2-C_4), 54.84 (C_3). Anal. for $C_{14}H_{11}ClN_4O_2$: Calcd.: C 55.55; H 3.66; N 18.51. Found: C 55.65; H 3.78; N 18.78.

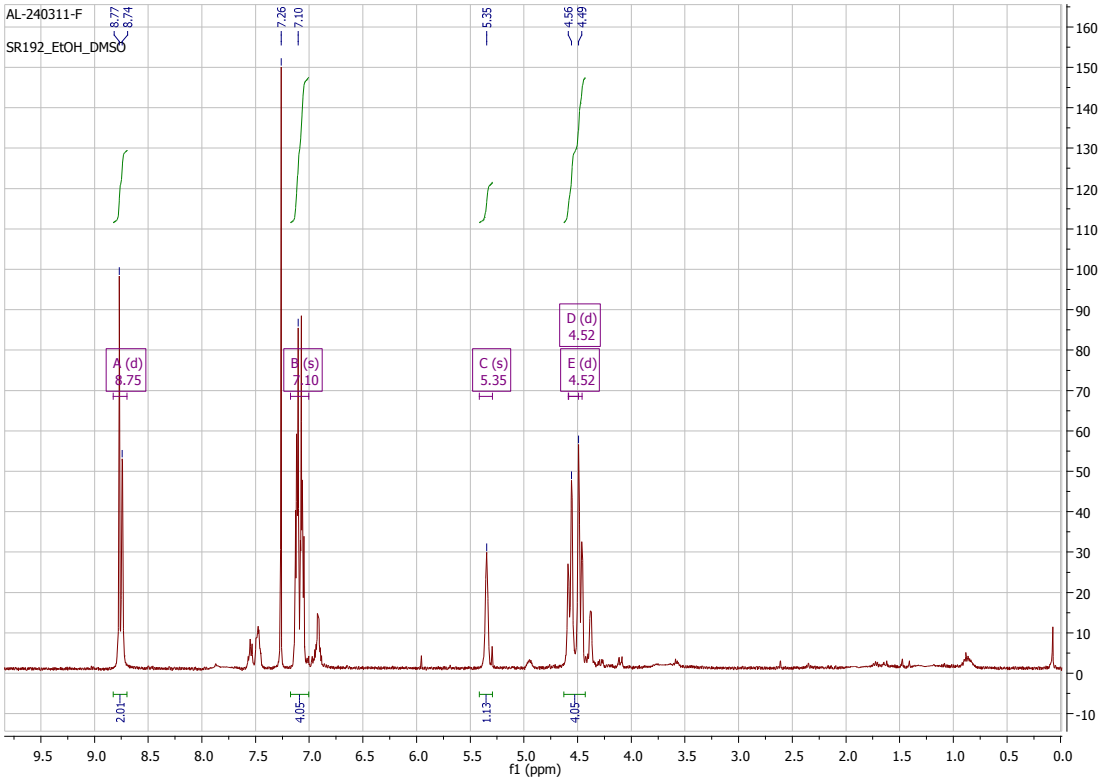


^{1}H-NMR of AL-150210-f3

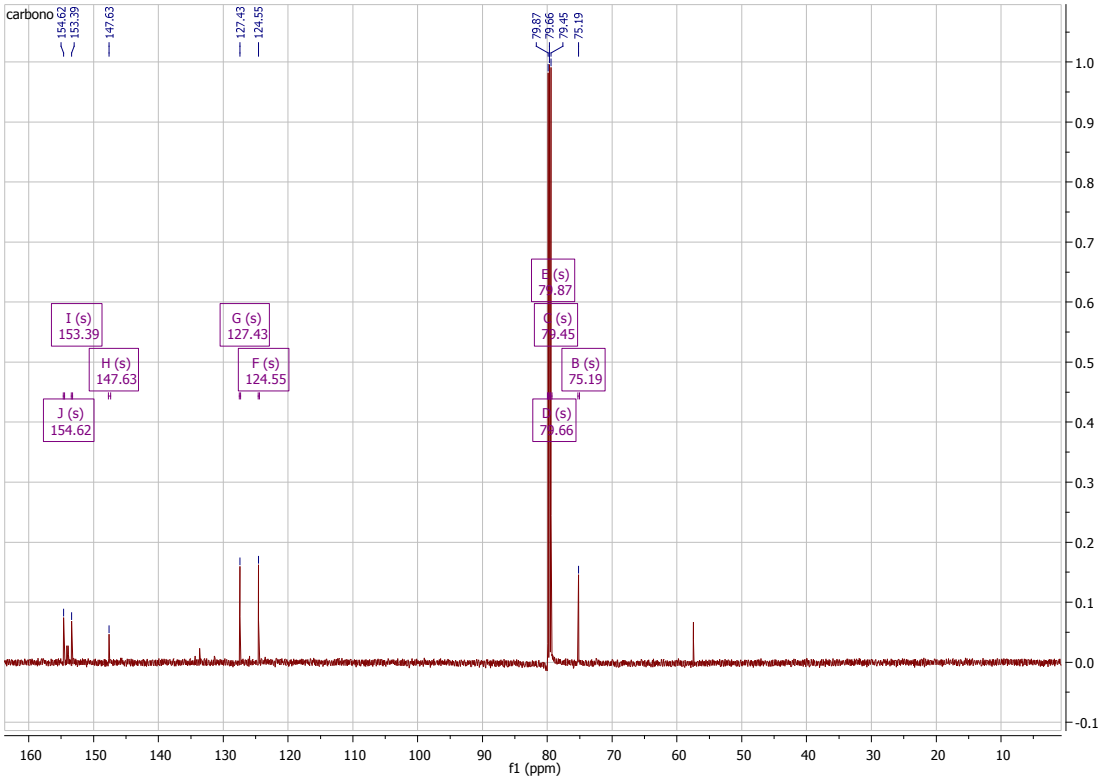


^{13}C-NMR and DEPT of AL-150210-f3

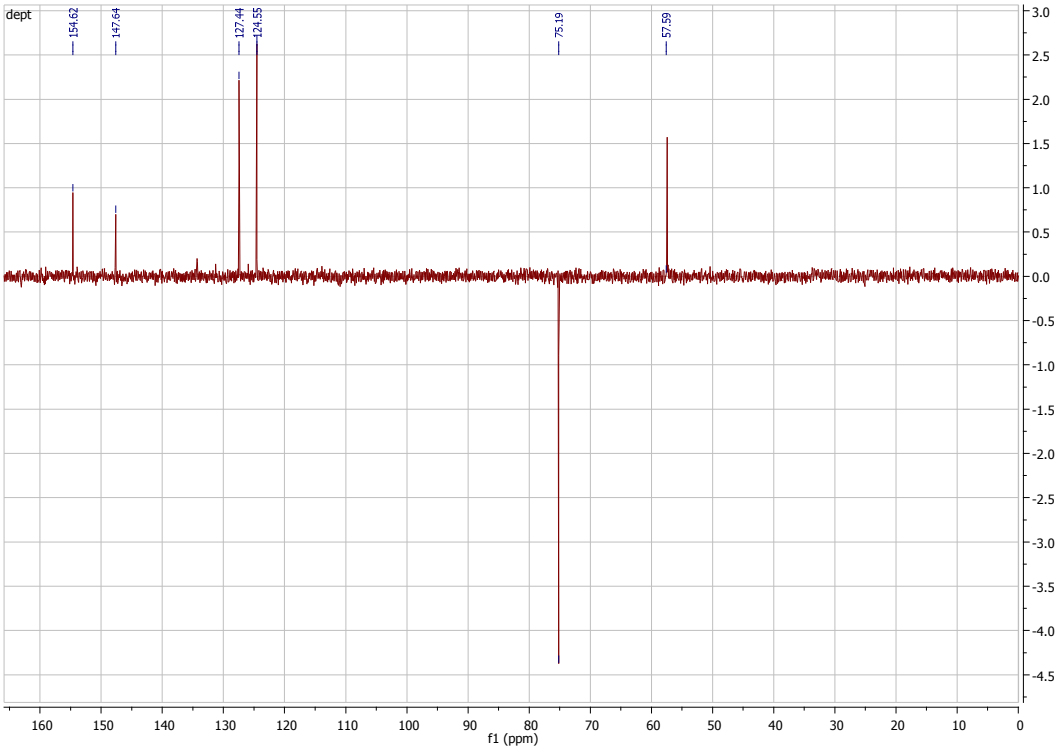
AL-240311: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.77 (*s*, 1H), 8.74 (*s*, 1H), 7.10 (*m*, 4H), 5.35 (*s*, 1H), 4.52 (*ddd*, *J* = 14.7, 13.0, 2.5 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm) 153.62 ($C_{2'}$), 153.12 ($C_{6'}$), 152.92 (C_{9a}-C_{5a}), 152.39 ($C_{4'}$), 146.63 ($C_{8'}$), 132.63 ($C_{5'}$), 126.36 (C_7-C_8), 123.55 (C_9-C_6), 74.19 (C_2-C_4), 56.44 (C_3). Anal. for $C_{14}H_{11}BrN_4O_2$: Calcd.: C 48.43; H 3.19; N 16.14. Found: C 48.59; H 2.95; N 16.15.



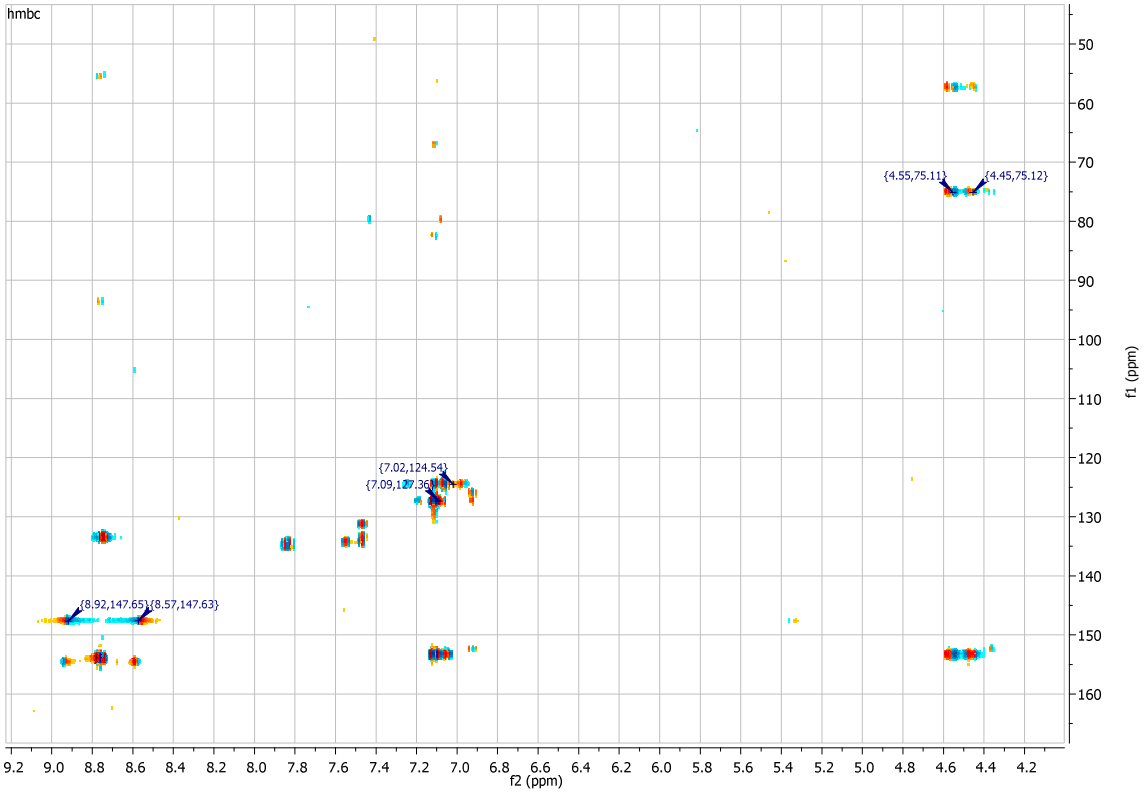
^{1}H-NMR of AL-240311



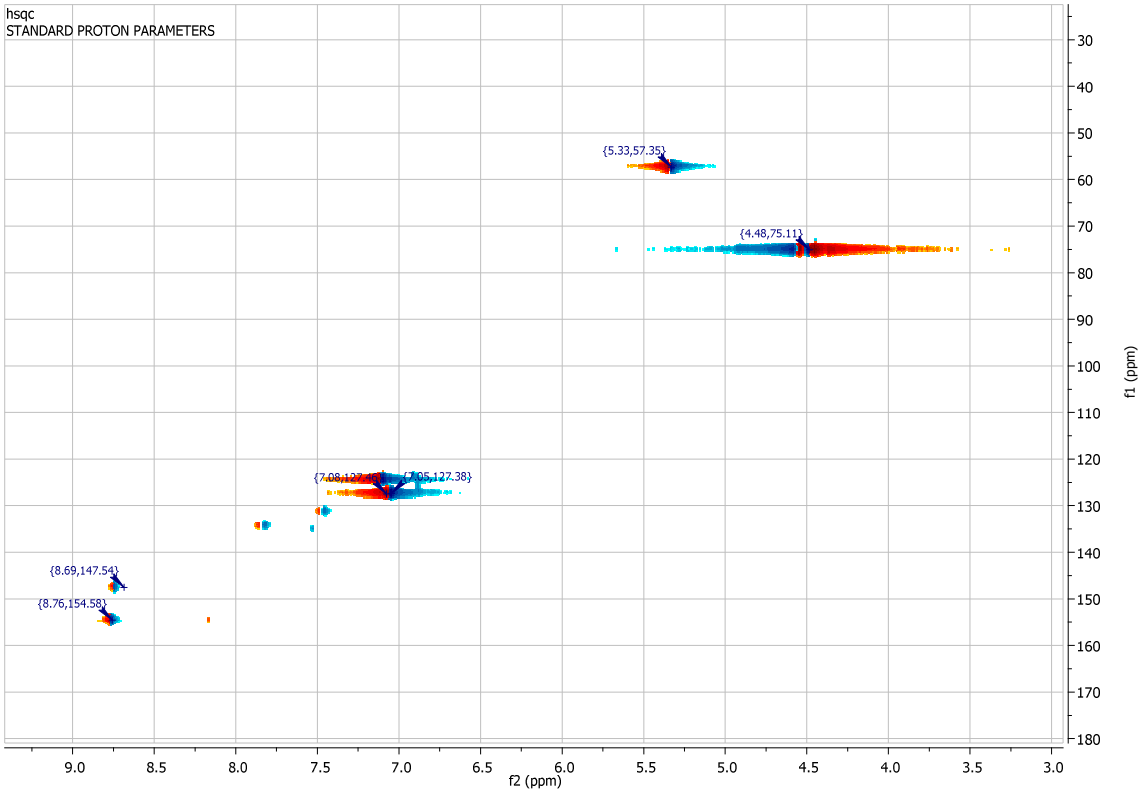
^{13}C-NMR of AL-240311



DEPT of AL-240311



HMBC of AL-240311

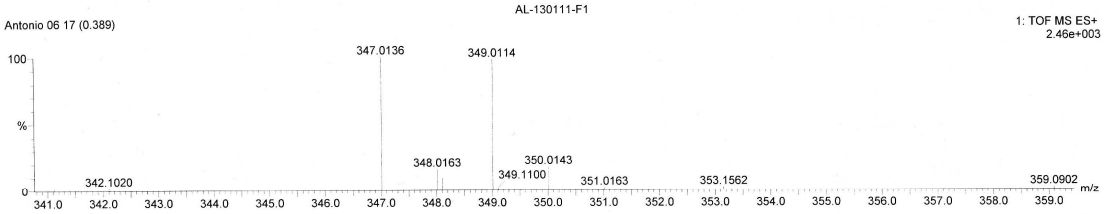


HSQC of AL-240311

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 40.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

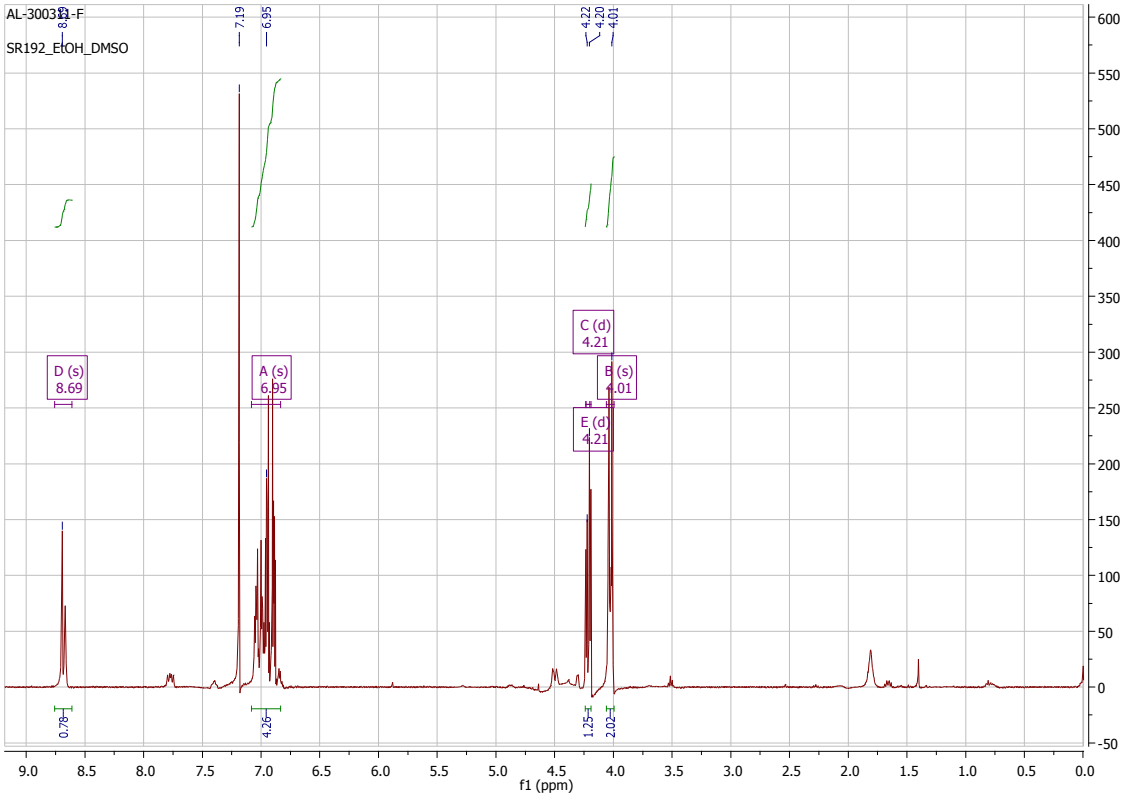
Monoisotopic Mass, Even Electron Ions
865 formula(e) evaluated with 6 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-300 N: 0-5 O: 0-10 Na: 0-1 Br: 0-1



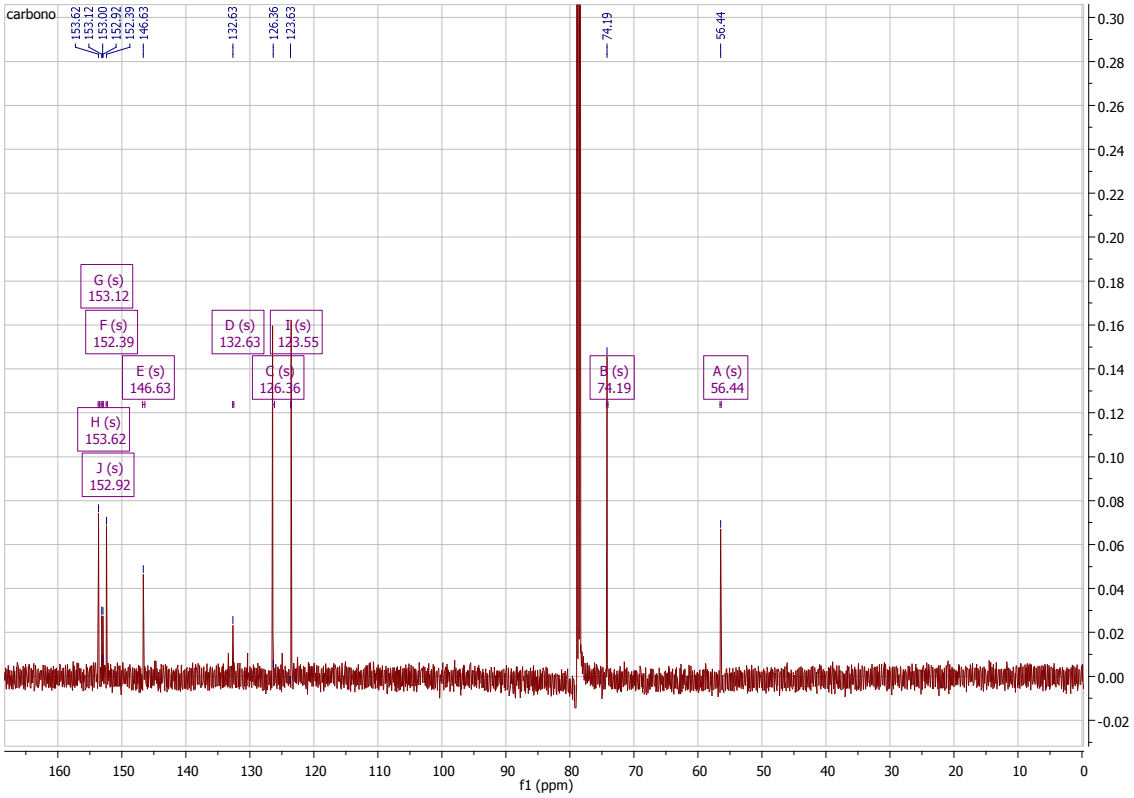
Minimum:				-1.5		
Maximum:		5.0	5.0	40.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
347.0136	347.0144	-0.8	-2.3	10.5	1.2	C14 H12 N4 O2 Br
	347.0120	1.6	4.6	7.5	1.0	C12 H13 N4 O2 Na Br
	347.0130	0.6	1.7	5.5	1.2	C13 H16 O6 Br
	347.0127	0.9	2.6	9.5	1137.1	C12 H8 N2 O9 Na
	347.0152	-1.6	-4.6	12.5	1131.5	C14 H7 N2 O9
	347.0133	0.3	0.9	25.5	1200.0	C26 H3 O2

Mass Analysis of AL-240311

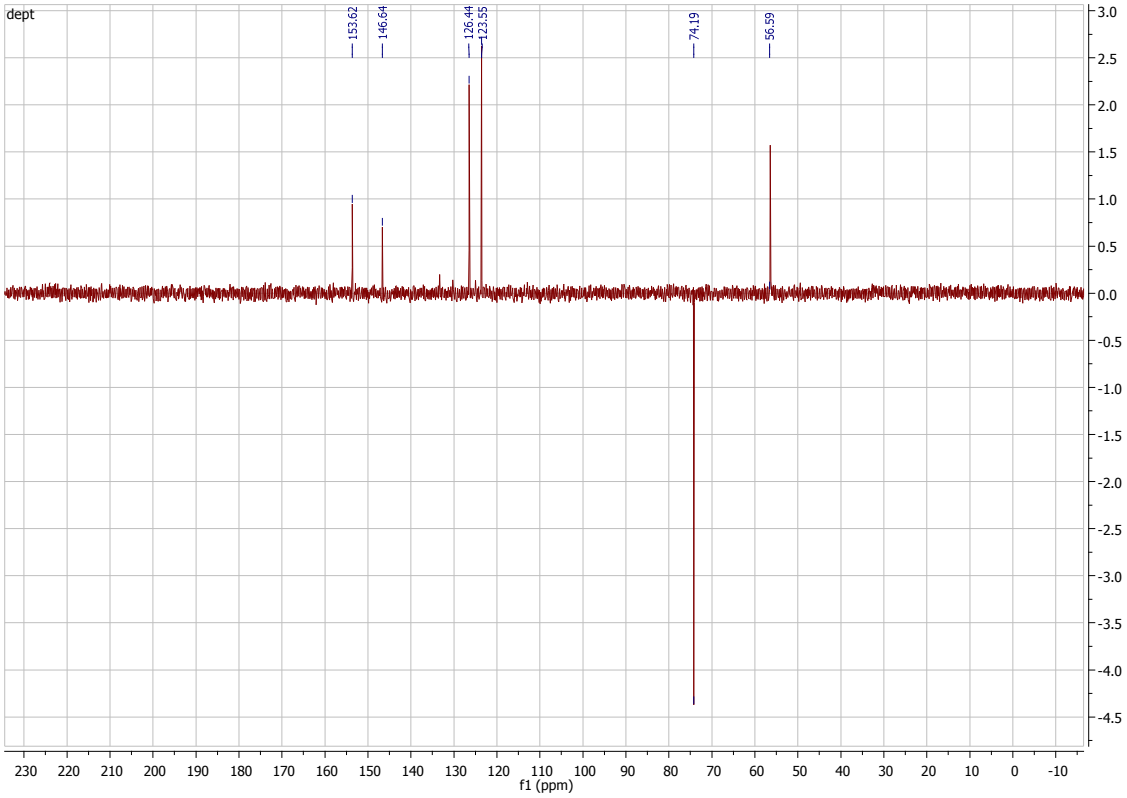
AL-300311-f1: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.73 (*s*, 1H), 8.69 (*s*, 1H), 6.95 (*m*, 4H), 4.19 (*dd*, *J* = 12.6, 3.9 Hz, 4H), 4.01 (*s*, 1H). ^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm) 153.62 ($C_{2'}$-$C_{6'}$), 152.39 (C_{5a}-C_{9a}), 146.63 ($C_{4'}$), 126.43 ($C_{8'}$), 123.55 (C_7-C_8), 78.87 (C_9-C_6), 78.66 (C_2-C_4), 78.45 (C_3). Anal. for $C_{14}H_{10}Cl_2N_4O_2$: Calcd.: C 49.87; H 2.99; N 16.62. Found: C 50.03; H 2.89; N 16.54.



^{1}H-NMR of AL-300311-f1

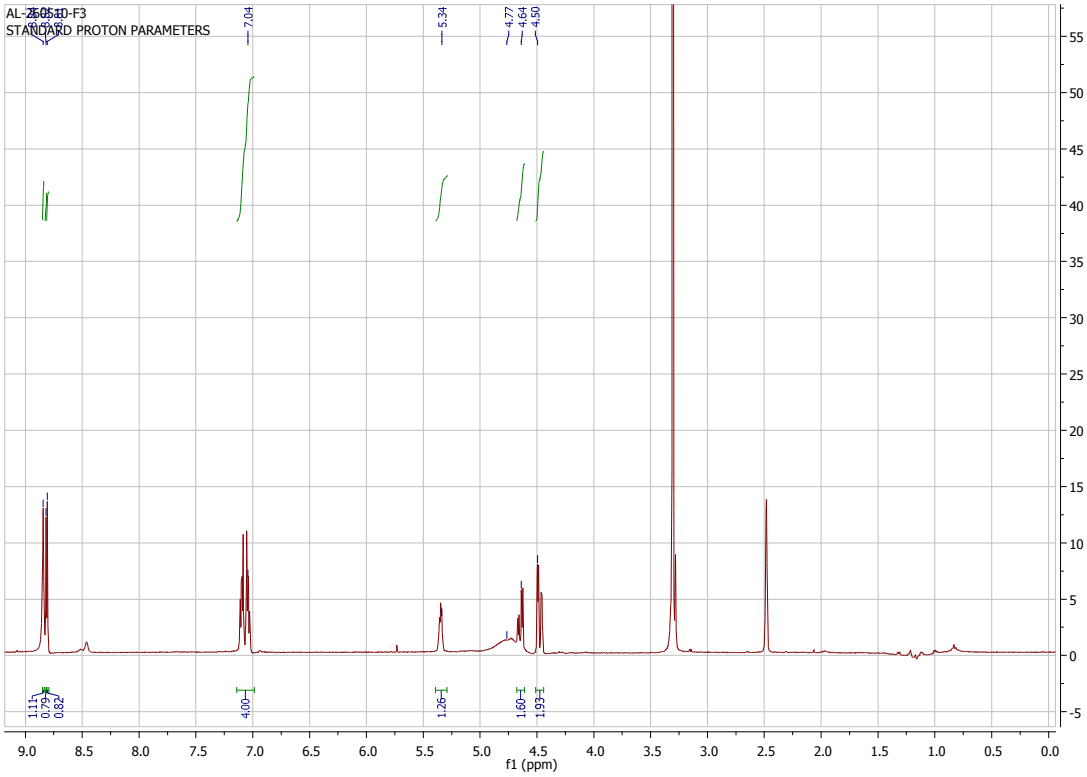


^{13}C-NMR of AL-300311-f1



DEPT of AL-300311-f1

AL-260510-f3: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.84 (*s*, 1H), 8.82 (*s*, 1H), 8.81 (*s*, 1H), 7.04 (*m*, 4H), 5.34 (*s*, 1H), 4.65 (*dd*, J = 12.8, 4.7 Hz, 2H), 4.48 (*dd*, J = 12.8, 3.5 Hz, 2H). Anal. for $C_{14}H_{12}N_4O_2$: Calcd.: C 62.68; H 4.51; N 20.88. Found: C 62.50; H 4.74; N 20.79.



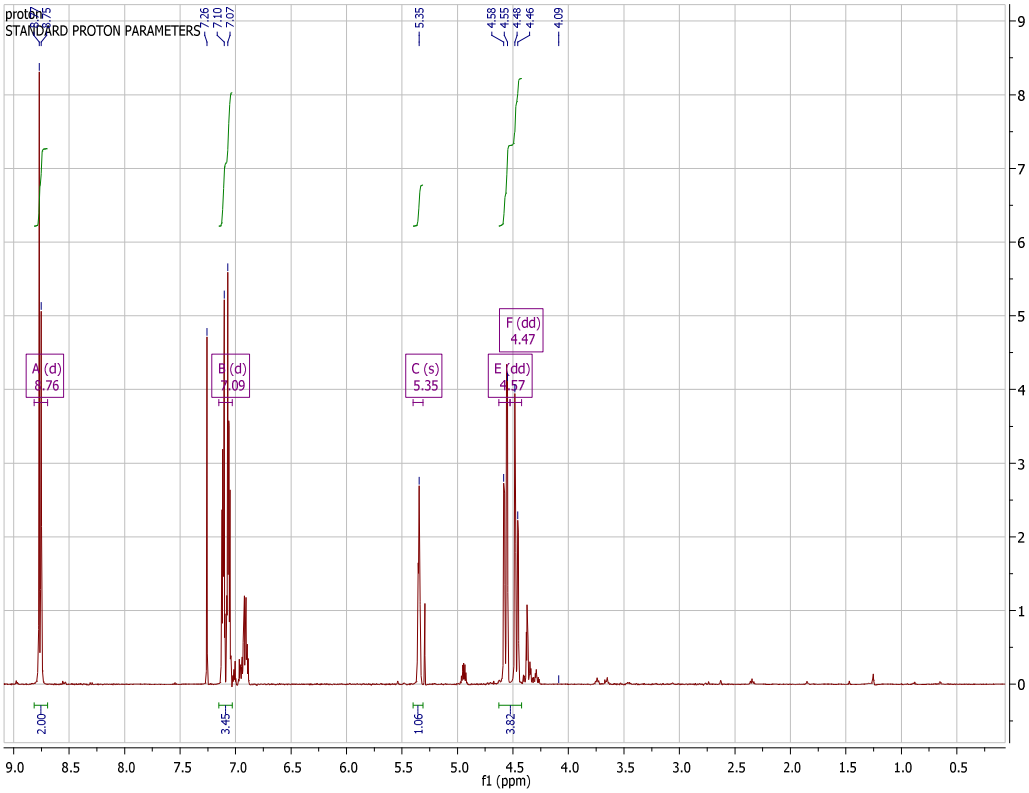
^{1}H-NMR of AL-260510-f3

Synthesis of 9-(2,3-dihydro-5*H*-1,5-benzodioxepine-3-yl)-6-iodo-9*H*-purine AL-110411-f1.

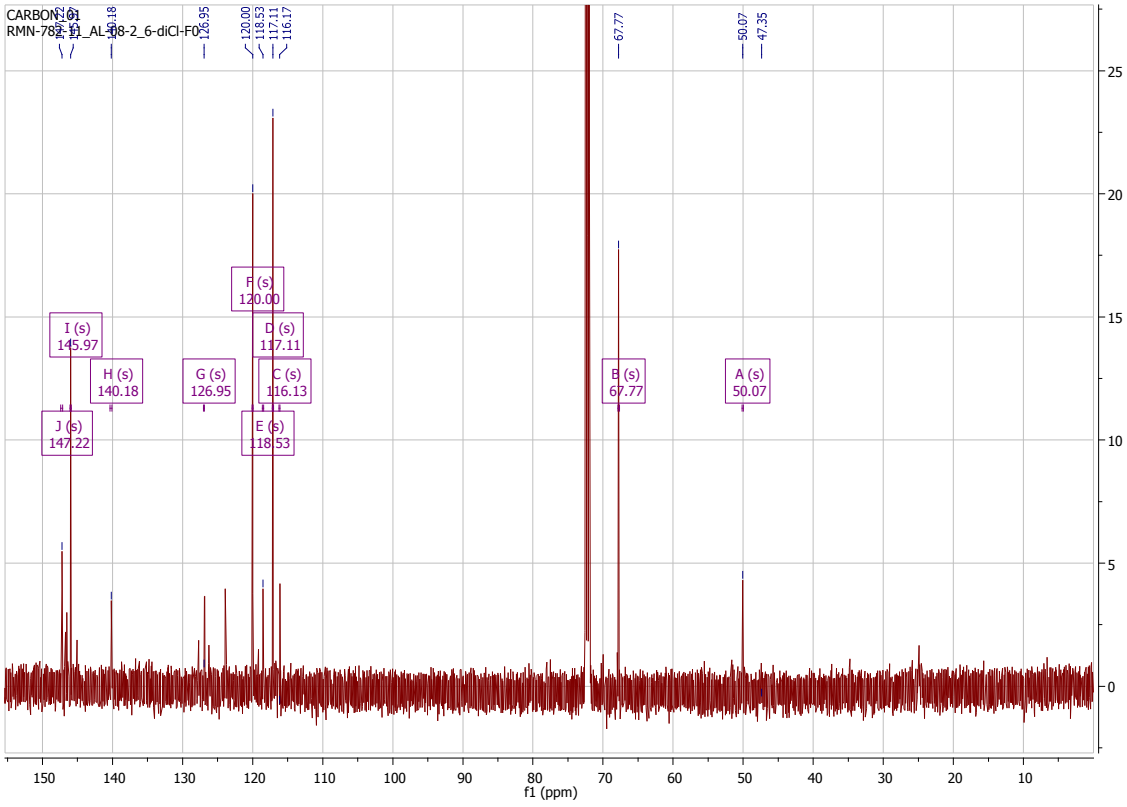
AL-150210-f3 (1 eq.), trifluoroacetic acid (5 eq.) and NaI (20 eq.) in butanone were stirred at -40° to -50°C for 5 h. The reaction mixture was poured into saturated $NaHCO_3/H_2O$ solution and extracted with

CH_2Cl_2. The organic layer was washed ($NaHSO_3$/H_2O, brine) and dried (Na_2SO_4). Volatiles were evaporated and the residue was purified by flash chromatography (EtOAc/Hexane, 0.5:1) afforded **110411-f1** (23%, R_f = 0.48) as a white solid.

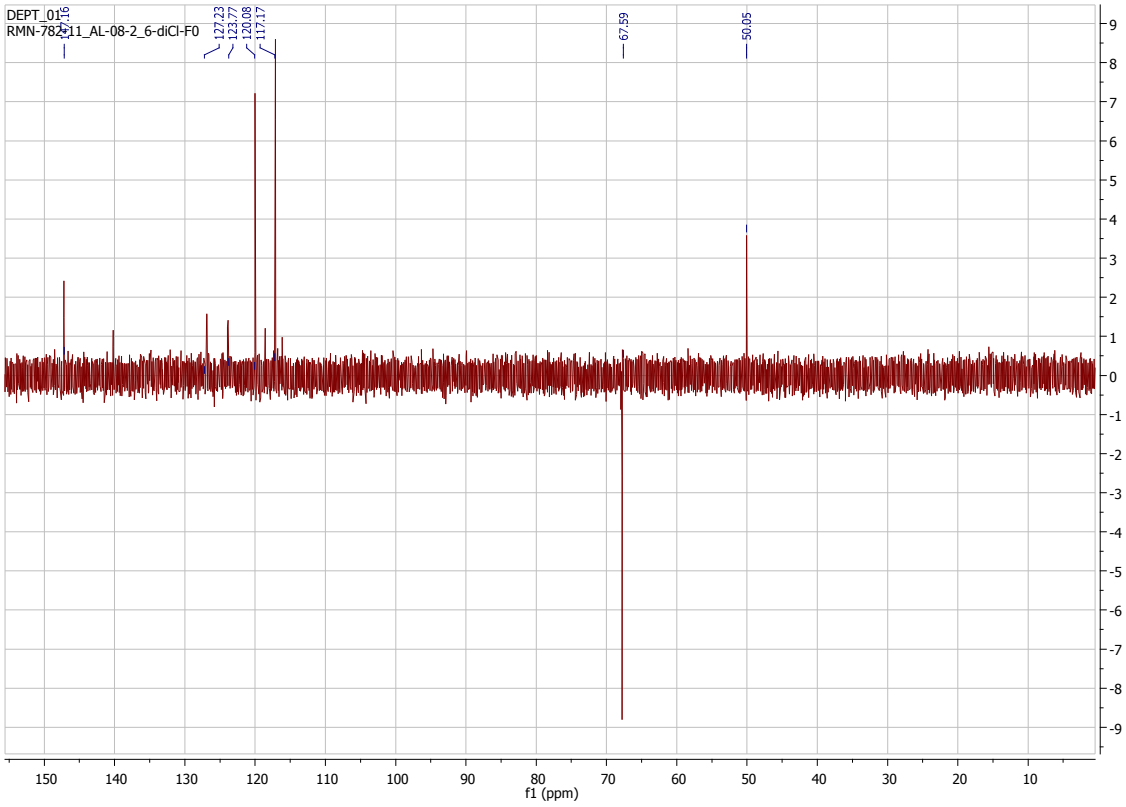
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.77 (*s*, 1H), 8.75 (*s*, 1H), 7.09 (*m*, 4H), 5.35 (*s*, 1H), 4.57 (*dd*, *J* = 12.8, 3.8 Hz, 2H), 4.47 (*dd*, *J* = 12.8, 3.2 Hz, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 147.22 ($C_{2'}$-$C_{6'}$), 145.97 (C_{9a}-C_{5a}), 140.18 ($C_{4'}$), 126.95 ($C_{8'}$), 120.00 ($C_{5'}$), 118.53 (C_7-C_8), 117.11 (C_9-C_6), 67.77 (C_2-C_4), 50.07 (C_3). Anal. for $C_{14}H_{11}IN_4O_2$: Calcd.: C 42.66; H 2.81; N 14.21. Found: C 42.63; H 2.65; N 14.29.



^{1}H-NMR of AL-110411-f1



^{13}C-NMR of AL-110411-f1

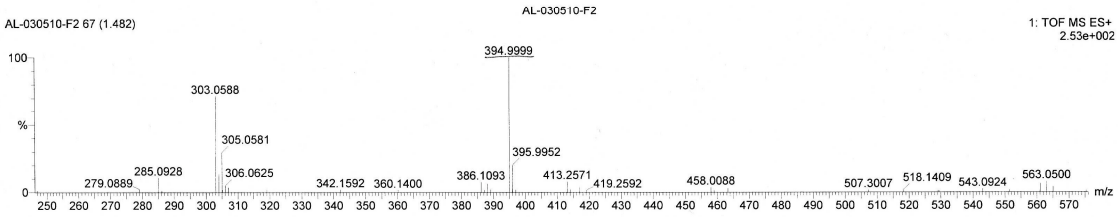


DEPT of AL-110411-f1

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 20.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
135 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-300 N: 0-4 O: 0-2 127I: 0-1



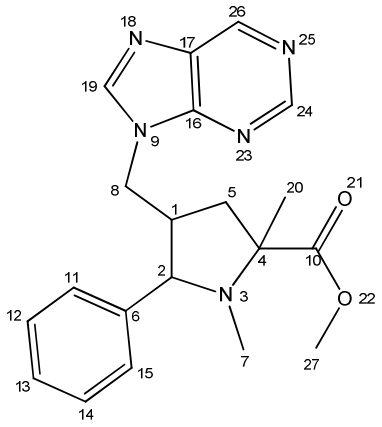
Minimum:		5.0	5.0	-1.5		
Maximum:				20.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
394.9999	395.0005	-0.6	-1.5	10.5	0.5	C14 H12 N4 O2 127I

Mass Analysis of AL-110411-f1

Synthesis of phenylpyrrolidines AL-120411-f1, AL-250511-f3, AL-310511-f1(Cl), AL-310511-f1(Br), AL-260511-f2, AL-080611-f2(Br), AL-080611-f2(2,6 diCl).

A microwave vial (2-5mL) equipped with a magnetic stirrer bead was charged with **KUD169**, **KUD223** or **KUD225** (50mg, 1eq.), triphenylphosphine (1.5 eq.), and the corresponding purine derivative (1.2 eq.) in dry DMF (3.5mL), cooled in an CO_2/acetone bath (-20°C) and DIPAD (1.5 eq.) was slowly added. The vial was sealed and

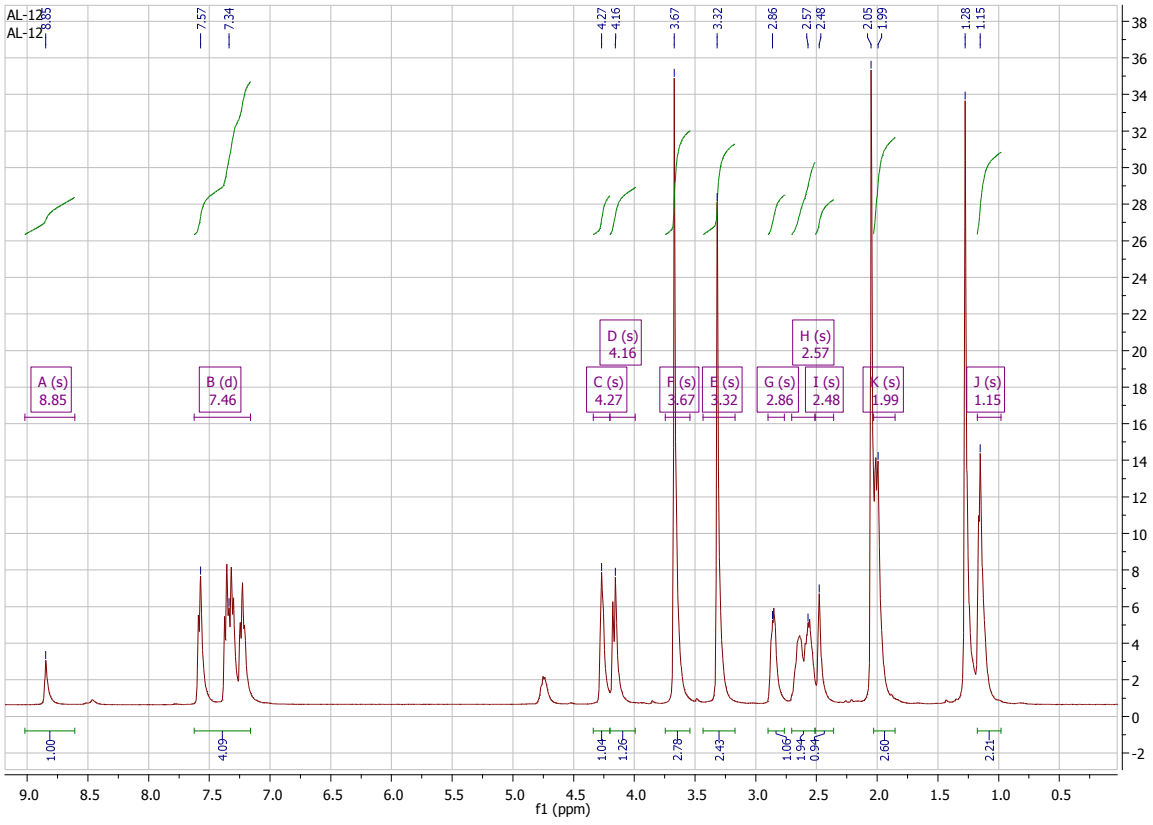
microwave irradiated at a set temperature of 165°C for 10 min. After completion of the irradiation time, the reaction mixture was cooled to room temperature through rapid pressurized air supply gas–jet cooling. The solvent was evaporated, and the crude product was added onto a silica column and purified firstly by flash chromatography (CH_2Cl_2/MeOH, 9.95:0.05 → 9.8:0.2) and then by preparative TLC (CH_2Cl_2/MeOH, 9.8:0.2) to afford **AL-120411-f1** (94% yield, R_f = 0.38), **AL-250511-f3** (71% yield, R_f = 0.34), **AL-310511-f1(Cl)** (11% yield, R_f = 0.35), **AL-310511-f1(Br)** (95% yield, R_f = 0.39), **AL-260511-f2** (88% yield, R_f = 0.36), **AL-080611-f2(Br)** (97% yield, R_f = 0.38), and **AL-080611-f2(2,6 diCl)** (95% yield, R_f = 0.30) as white solids.



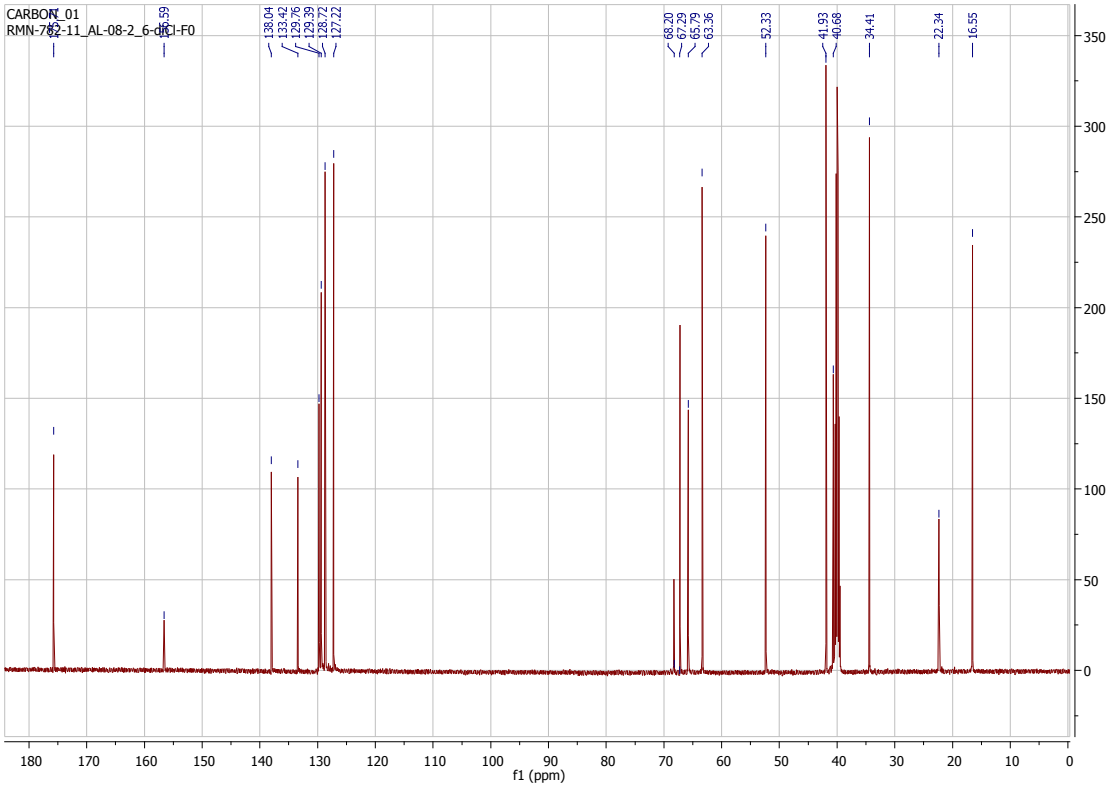
Phenylpyrrolidines atomic number

AL-120411-f1: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.85 (*s*, 2H), 7.46 (*m*, *J* = 93.4 Hz, 4H), 4.27 (*s*, 1H), 4.16 (*s*, 1H), 3.67 (*s*, 3H), 3.32 (*s*, 3H),

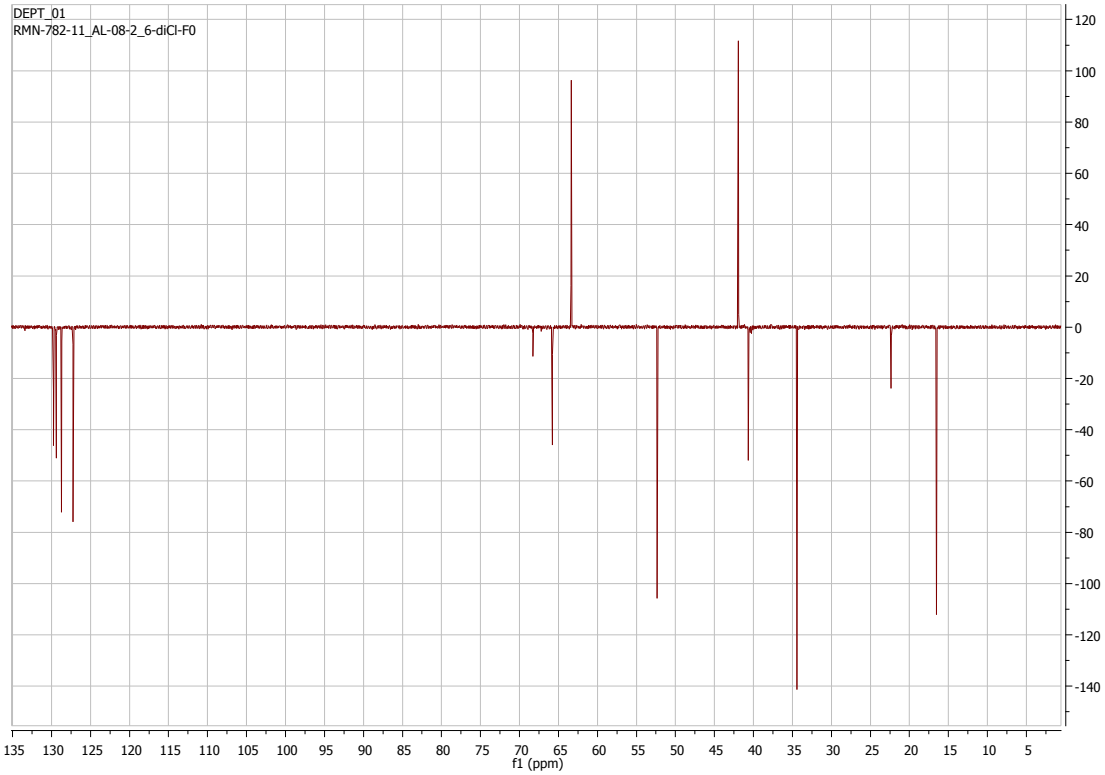
2.86 (*s*, 1H), 2.57 (*s*, 2H), 2.48 (*s*, 1H), 1.99 (*s*, 3H), 1.15 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 175.71 (C_{10}), 156.59 (C_{24}), 138.04 (C_{16}), 133.42 (C_{26}), 129.76 (C_{19}), 129.39 (C_6), 128.72 (C_{17}), 127.22 (C_{12}), 68.20 (C_{14}), 67.22 (C_{11}), 65.79 (C_{15}), 63.36 (C_{13}), 52.33 (C_4), 41.93 (C_2), 40.68 (C_{27}), 34.41 (C_8), 22.34 (C_7), 16.55 (C_1). Anal. for $C_{20}H_{21}Cl_2N_5O_2$: Calcd.: C 55.31; H 4.87; N 16.12. Found: C 55.42; H 5.05; N 16.17.



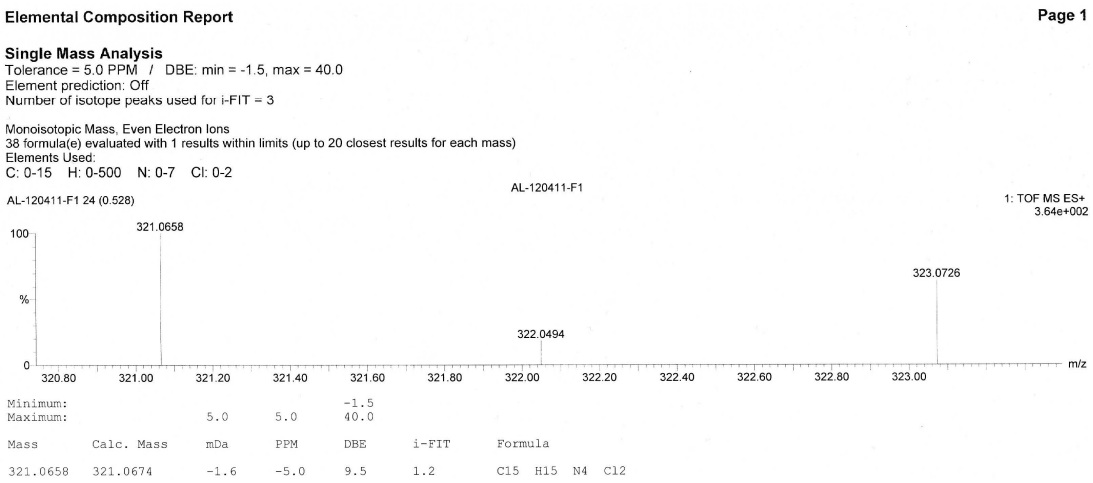
^{1}H-NMR of AL-120411-f1



^{13}C-NMR of AL-120411-f1

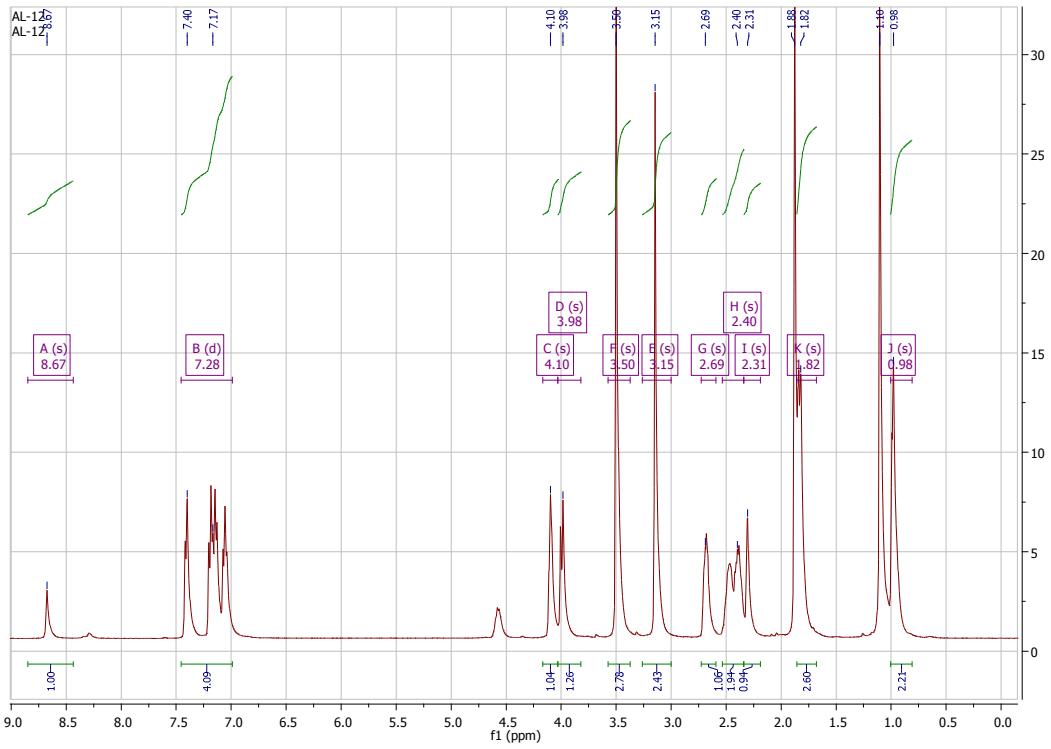


DEPT of AL-120411-f1

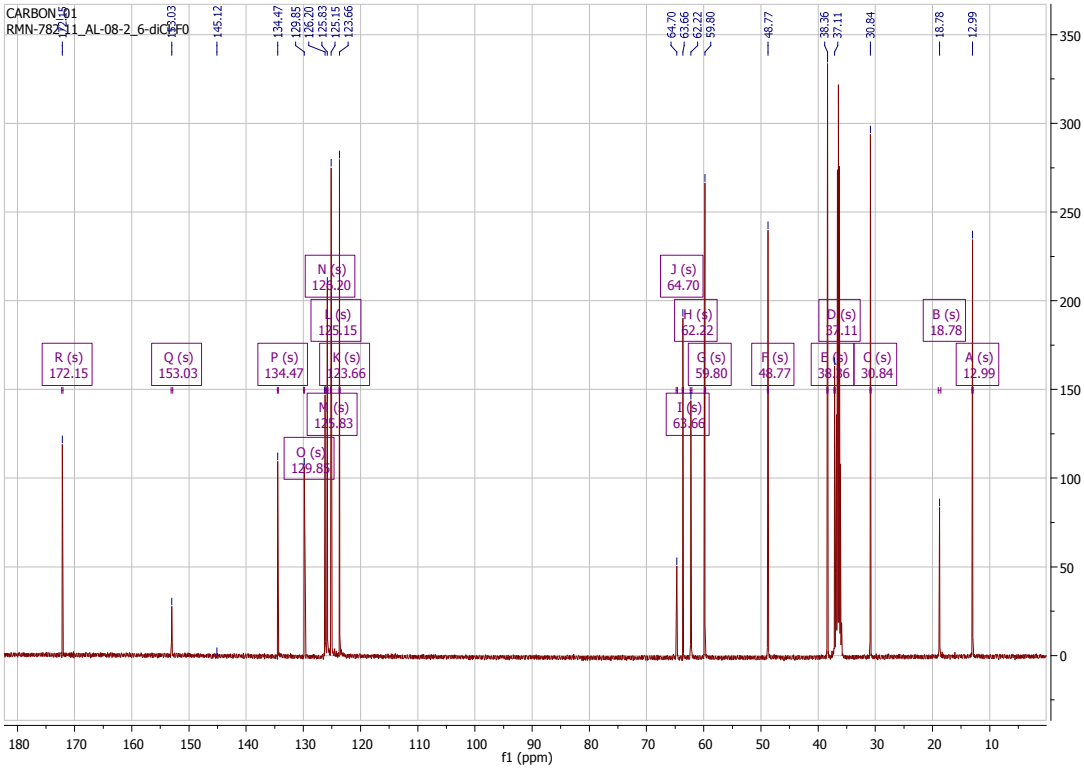


Mass Analysis of AL-120411-f1

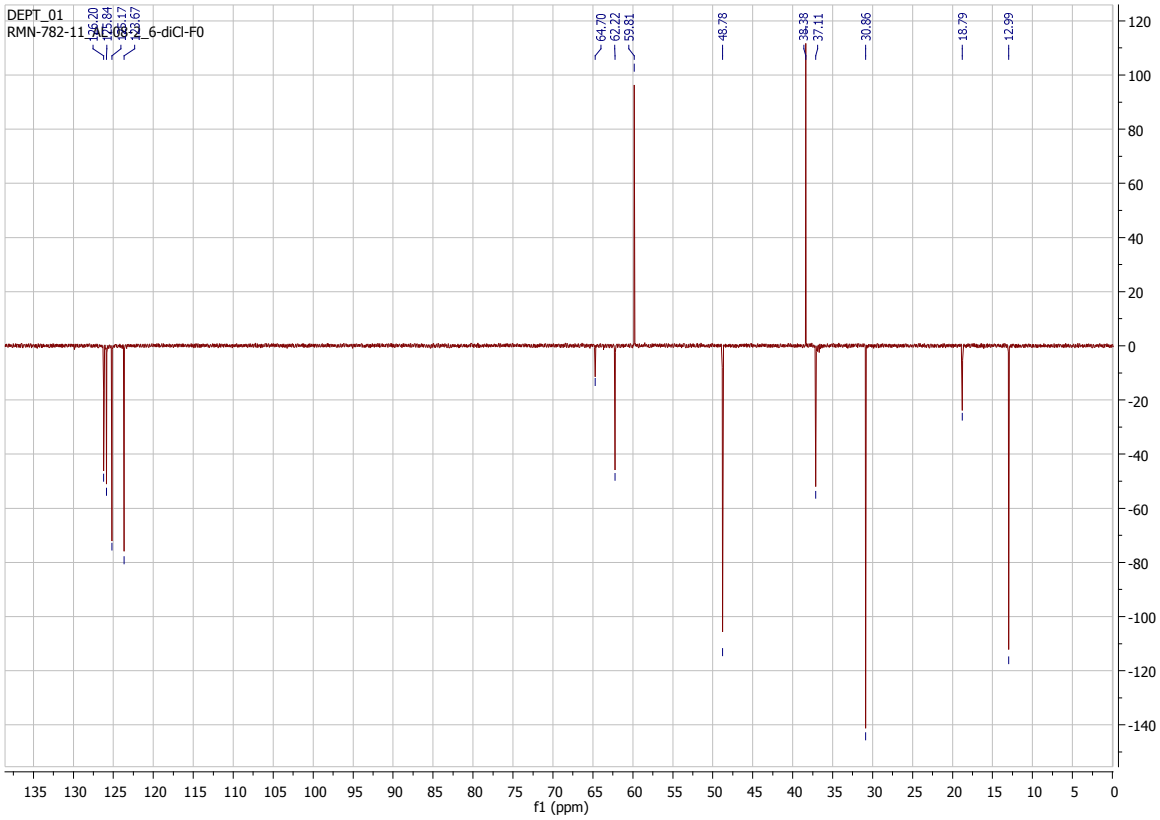
AL-250511-f3: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.67 (*s*, 2H), 7.28 (*m*, *J* = 93.7 Hz, 4H), 4.10 (*s*, 1H), 3.98 (*s*, 1H), 3.50 (*s*, 3H), 3.15 (*s*, 3H), 2.69 (*s*, 1H), 2.40 (*s*, 2H), 2.31 (*s*, 1H), 1.82 (*s*, 3H), 0.98 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 172.15 (C_{10}), 153.03 (C_{24}), 134.47 (C_{16}), 129.85 (C_{26}), 126.20 (C_{19}), 125.83 (C_6), 125.15 (C_{17}), 123.66 (C_{12}), 64.70 (C_{14}), 63.66 (C_{11}), 62.22 (C_{15}), 59.80 (C_{13}), 48.77 (C_4), 38.36 (C_2), 37.11 (C_{27}), 30.84 (C_8), 18.78 (C_7), 12.99 (C_1). Anal. for $C_{20}H_{21}BrClN_5O_2$: Calcd.: C 50.17; H 4.42; N 14.63. Found: C 50.27; H 4.33; N 14.38.



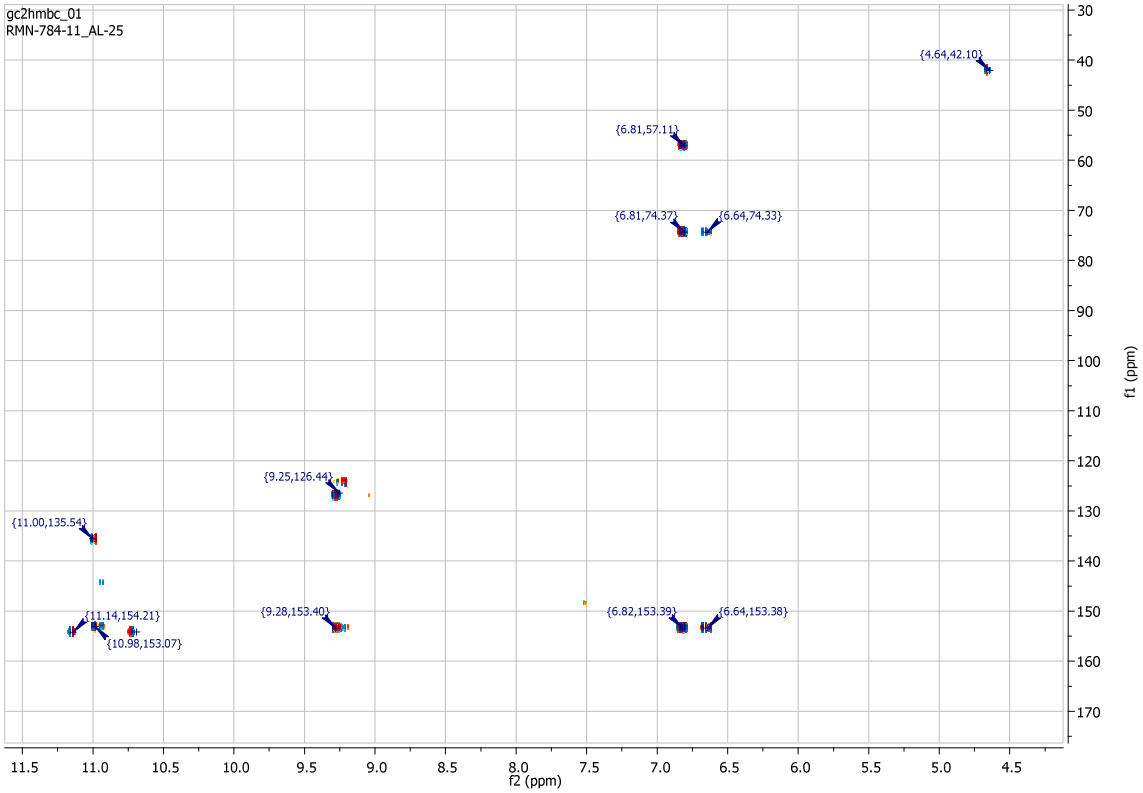
^{1}H-NMR of AL-250511-f3



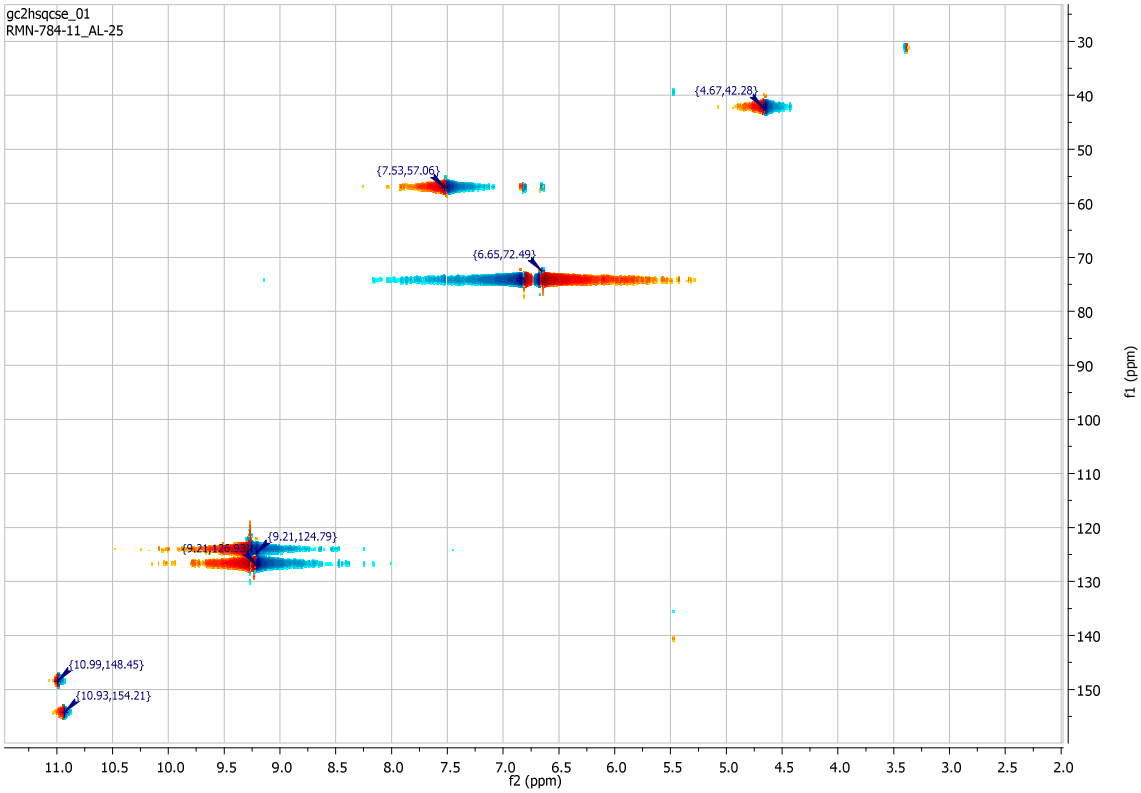
^{13}C-NMR of AL-250511-f3



DEPT of AL-250511-f3



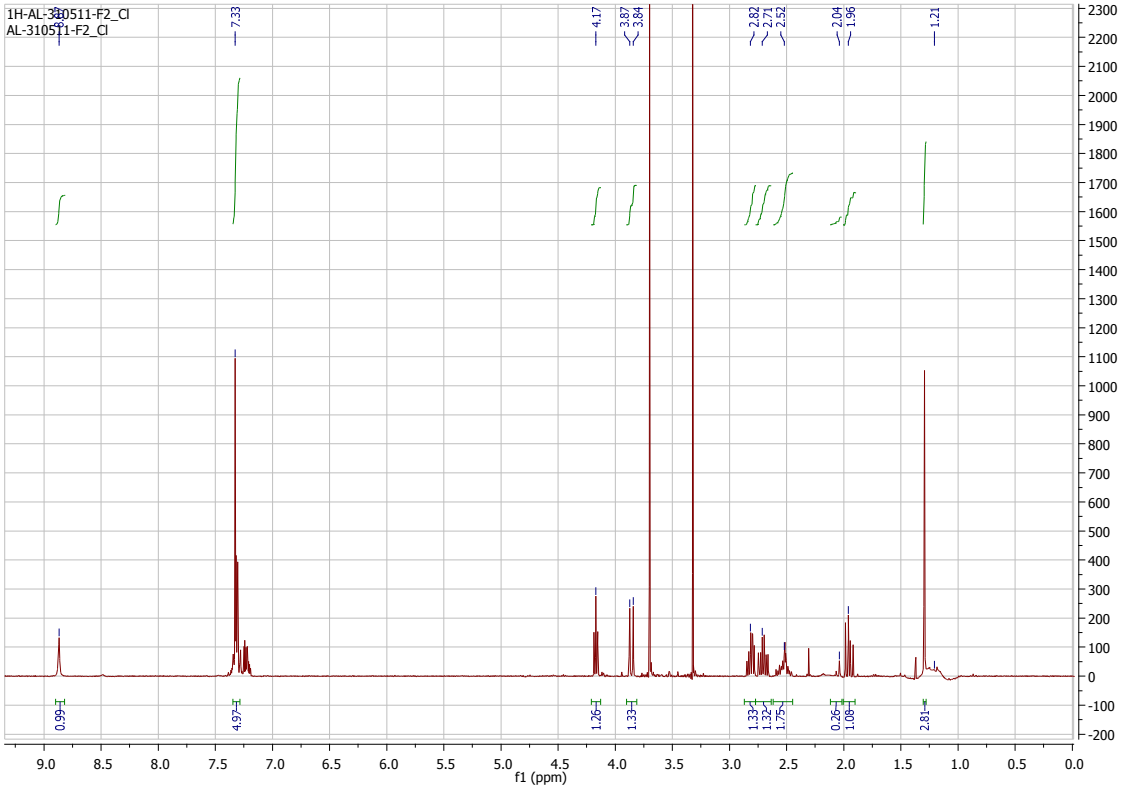
HMBC of AL-250511-f3



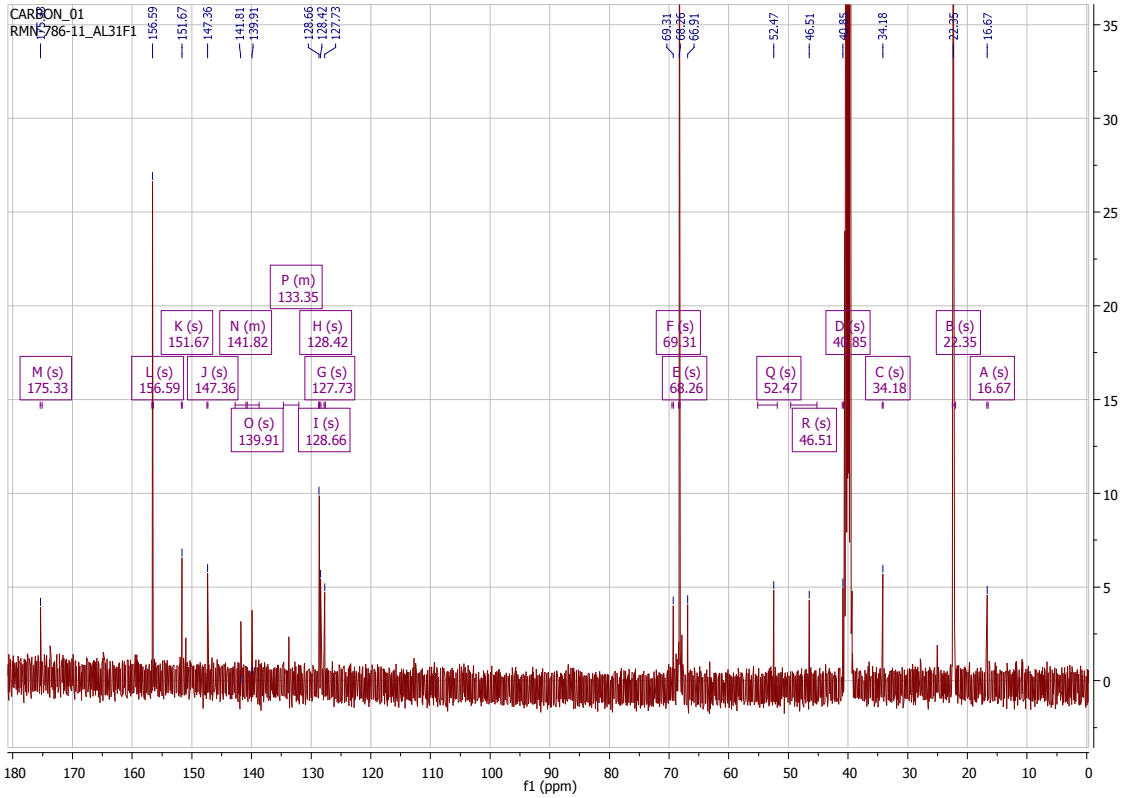
HSQC of AL-250511-f3

AL-310511-f1(Cl): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.87 (*s*, 2H), 7.33 (*m*, *J* = 93.7 Hz, 5H), 4.17 (*s*, 1H), 3.86 (*d*, J = 9.1 Hz, 1H), 2.82 (*s*, 3H), 2.71 (*s*, 3H), 2.52 (*s*, 1H), 2.04 (*s*, 2H), 1.96 (*s*, 1H), 1.29 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 175.33 (C_{10}), 156.59 (C_{24}), 151.67

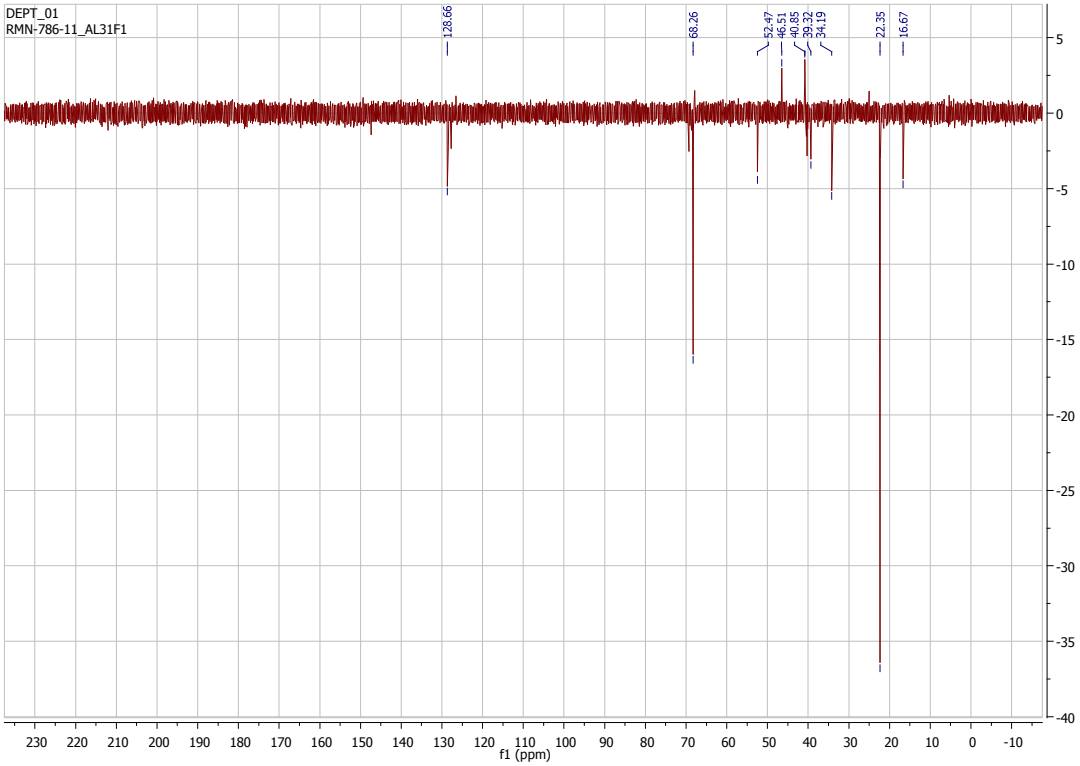
(C_{16}), 147.36 (C_{26}), 142.74 (C_{19}), 140.89 (C_6), 134.63 (C_{17}), 132.06 (C_{12}), 128.66 (C_{14}), 128.42 (C_{11}), 127.73 69.31 (C_{15}), 68.26 (C_{13}), 52.47 (C_4), 46.51 (C_2), 40.85 (C_{27}) 34.18 (C_8), 22.35 (C_7), 16.67 (C_1). Anal. for $C_{20}H_{21}Cl_2N_5O_2$: Calcd.: C 55.31; H 4.87; N 16.12. Found: C 55.02; H 4.95; N 16.07.



^{1}H-NMR of AL-310511-f1(Cl)



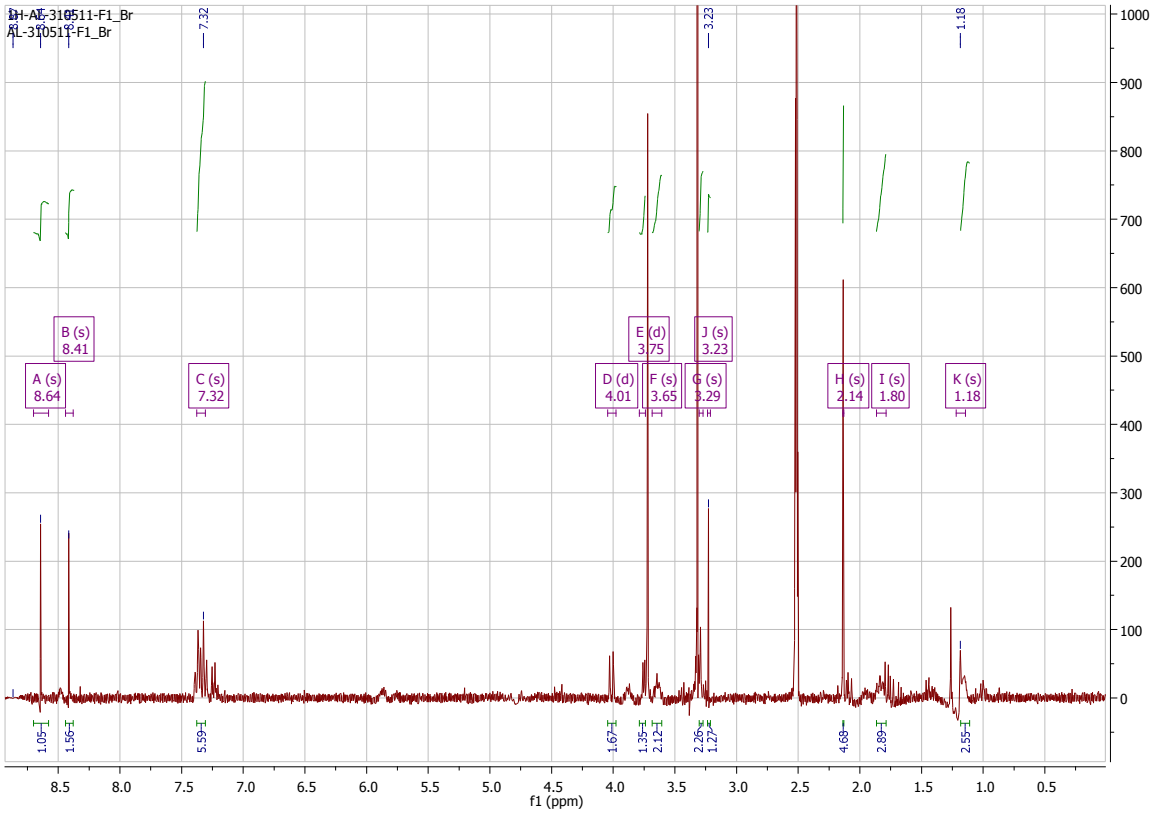
^{13}C-NMR of AL-310511-f1(Cl)



DEPT of AL-310511-f1(Cl)

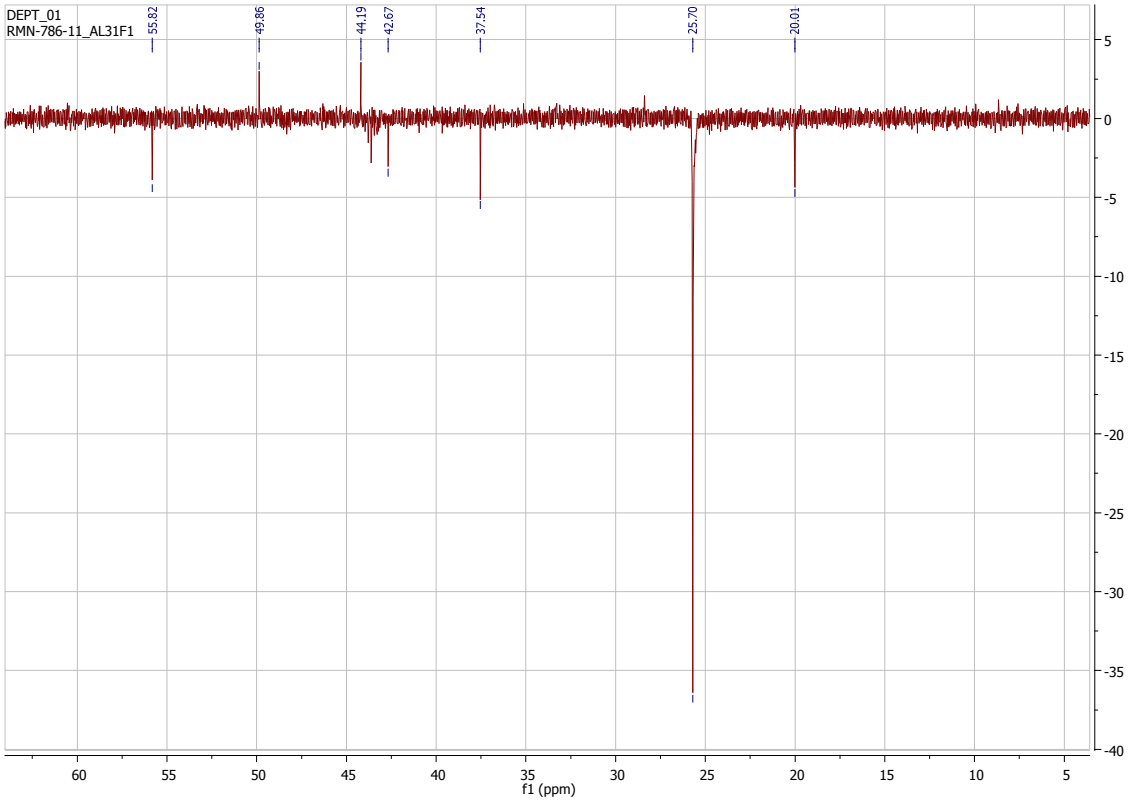
AL-310511-f1(Br): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.64 (*s*, 2H), 8.41 (*s*, 2H), 7.32 (*m*, 5H), 4.01(*s*, 1H), 3.75 (*d*, J = 9.1 Hz, 1H), 3.65 (*s*, 3H), 3.29 (*s*, 3H), 3.23 (*s*, 1H), 2.14 (*s*, 2H), 1.80 (*s*, 1H), 1.18 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 178.58 (C_{10}), 159.96 (C_{24}), 155.04 (C_{16}), 150.74 (C_{26}), 145.48 (C_{19}), 143.76 (C_6), 137.12 (C_{17}), 132.04 (C_{12}), 131.80 (C_{14}), 131.10 (C_{11}), 72.68 (C_{15}), 71.64 (C_{13}), 55.84 (C_4), 49.89

(C_2), 44.23 (C_{27}) 37.56 (C_8), 25.73 (C_7), 20.05 (C_1). Anal. for $C_{20}H_{22}BrN_5O_2$: Calcd.: C 54.06; H 4.99; N 15.76. Found: C 54.03; H 5.07; N 16.00.

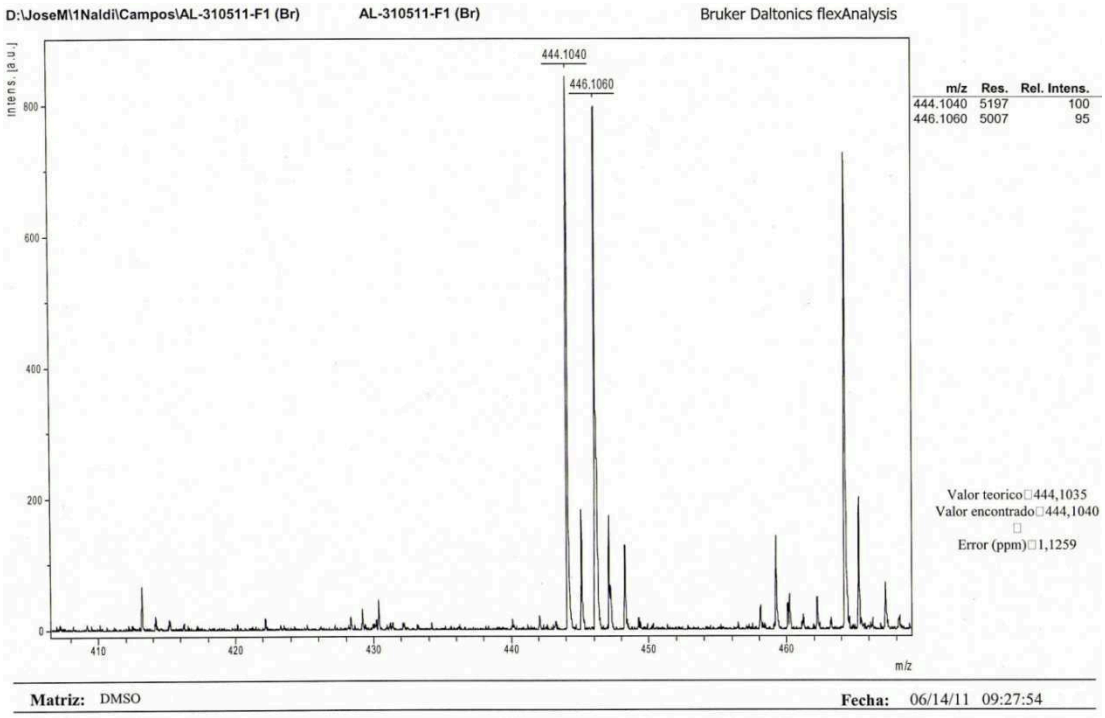


^{1}H-NMR of AL-310511-f1(Br)

^{13}C-NMR of AL-310511-f1(Br)



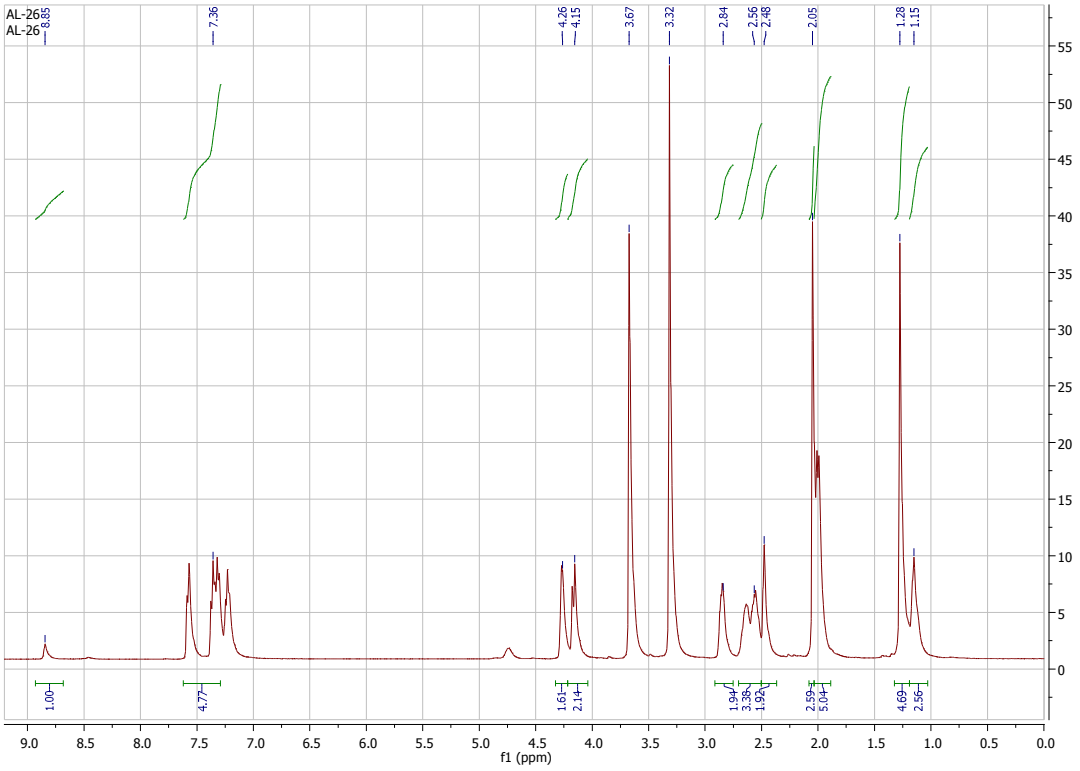
DEPT of AL-310511-f1(Br)



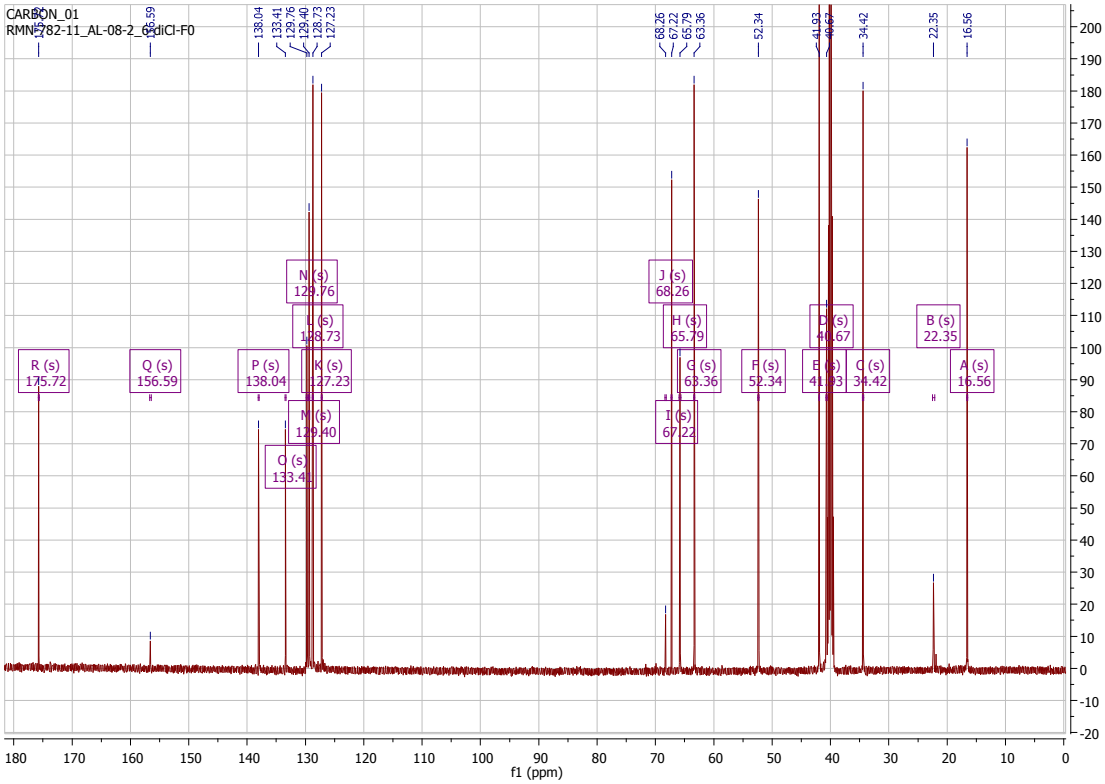
Mass Analysis of AL-310511-f1(Br)

AL-260511-f2: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.85 (*s*, 1H), 7.45 (*m*, 4H), 4.26 (*s*, 1H), 4.15 (*s*, 1H), 2.84 (*s*, 3H), 2.56 (*s*, 3H), 2.48 (*s*, 1H), 2.05 (*s*, 2H), 2.00 (*s*, 1H), 1.28 (*s*, 3H), 1.15 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 175.72 (C_{10}), 156.59 (C_{24}), 138.04 (C_{16}), 133.41 (C_{26}), 129.76 (C_{19}), 129.40 (C_6), 128.73 (C_{17}), 127.23 (C_{12}), 68.26 (C_{14}), 67.22 (C_{11}), 65.79 (C_{15}), 63.36 (C_{13}), 52.34 (C_4), 41.93 (C_2), 40.67

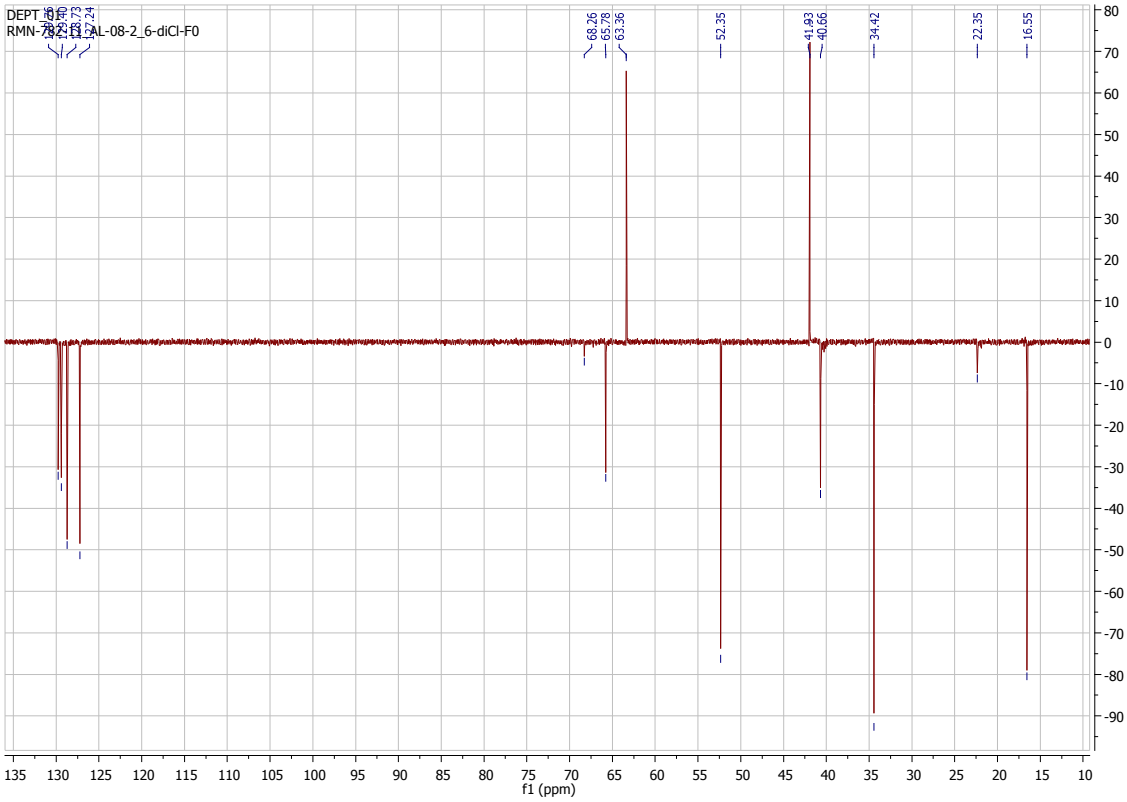
(C_{27}), 34.42 (C_8), 22.33 (C_7), 16.55 (C_1). Anal. for $C_{20}H_{20}Cl_3N_5O_2$: Calcd.: C 51.24; H 4.30; N 14.94. Found: C 51.22; H 4.17; N 14.99.



^{1}H-NMR of AL-260511-f2



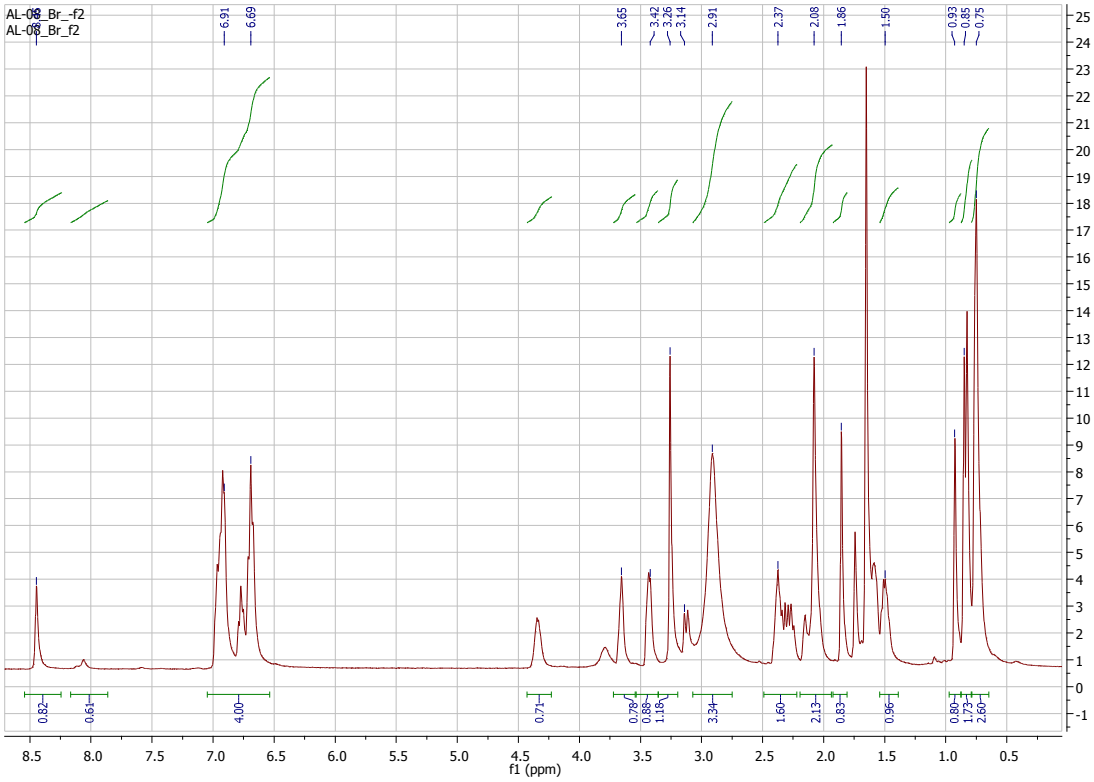
^{13}C-NMR of AL-260511-f2



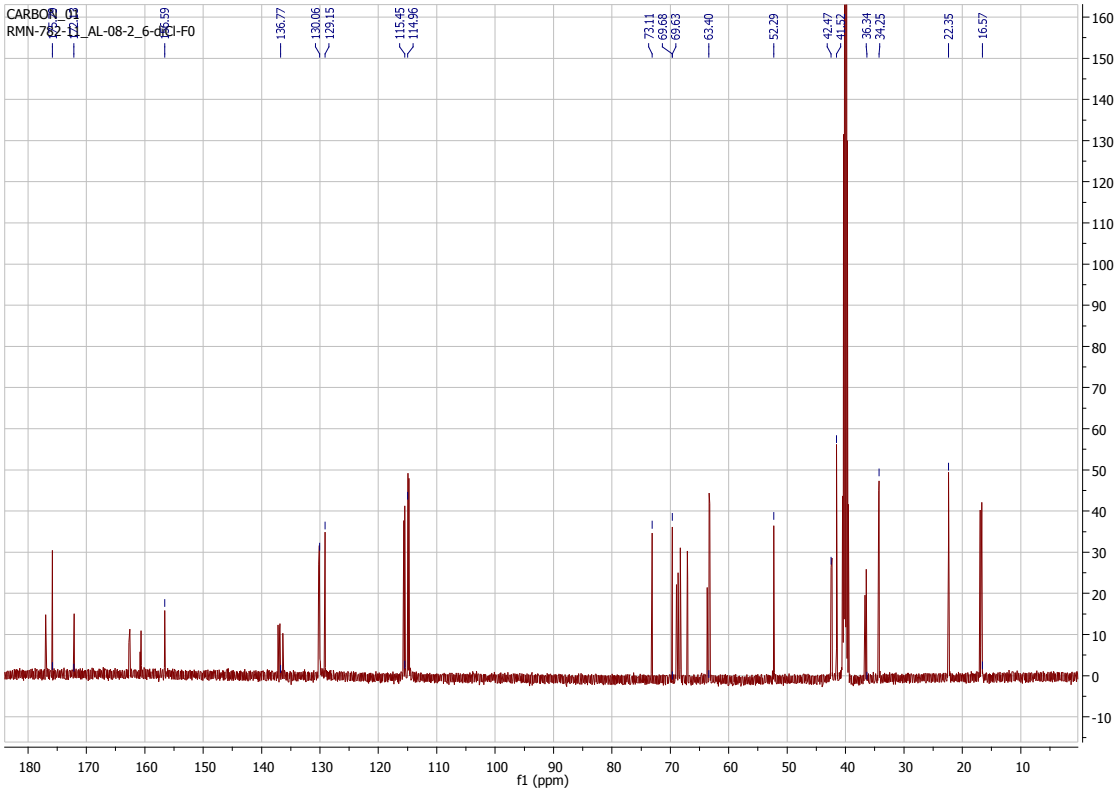
DEPT of AL-260511-f2

AL-080611-f2(Br): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.45 (*s*, 1H), 8.06 (*s*, 1H) 6.80 (*m*, *J* = 93.4 Hz, 4H), 3.65 (*s*, 1H), 3.42 (*s*, 1H), 2.91 (*s*, 3H), 2.37 (*s*, 3H), 2.08 (*s*, 1H), 1.86 (*s*, 2H), 0.93 (*s*, 1H), 0.85 (*s*, 3H), 0.75 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 175.79 (C_{10}), 172.13 (C_{24}), 156.59 (C_{16}), 136.77 (C_{26}), 130.06 (C_{19}), 129.15 (C_6), 115.45

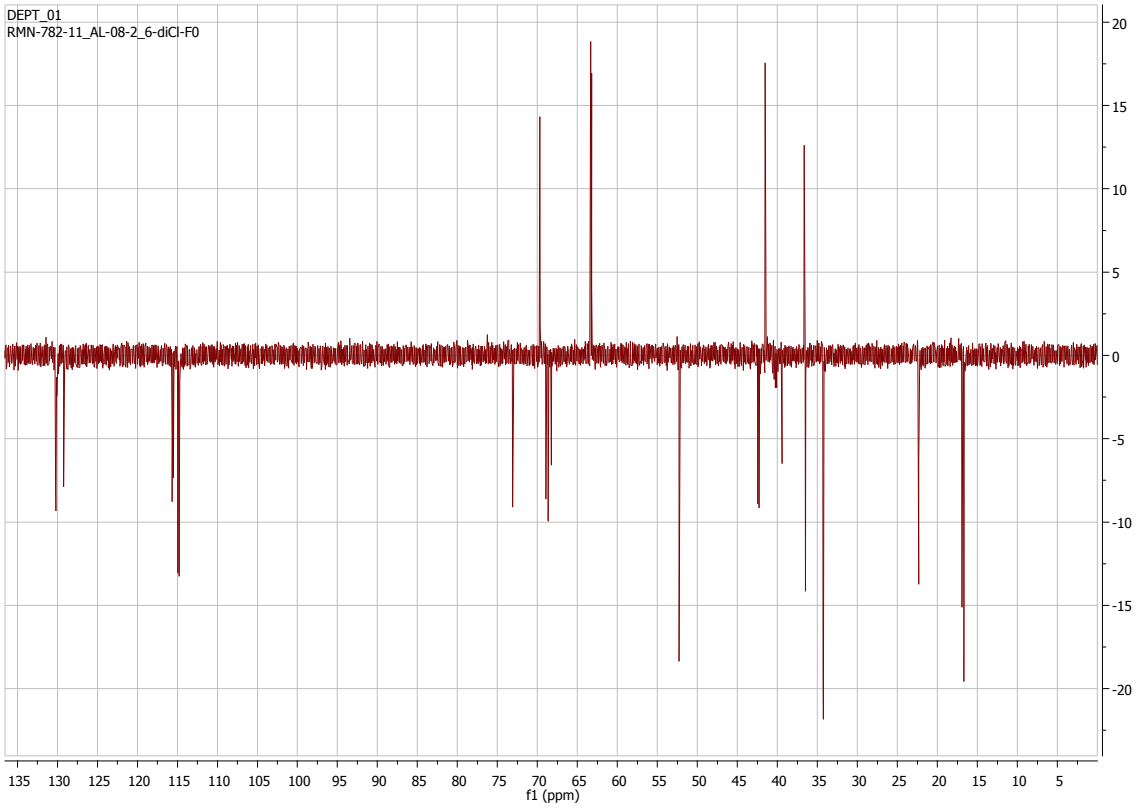
(C_{17}), 114.96 (C_{12}), 73.11 (C_{14}), 69.68 (C_{11}), 63.35 (C_{15}), 52.29 (C_{13}), 42.47 (C_4), 41.52 (C_2), 36.37(C_{27}), 34.25 (C_8), 22.35 (C_7), 16.57 (C_1). Anal. for $C_{20}H_{21}BrFN_5O_2$: Calcd.: C 51.96; H 4.58; N 15.15. Found: C 52.00; H 4.78; N 15.05.



^{1}H-NMR of AL-080611-f2(Br)



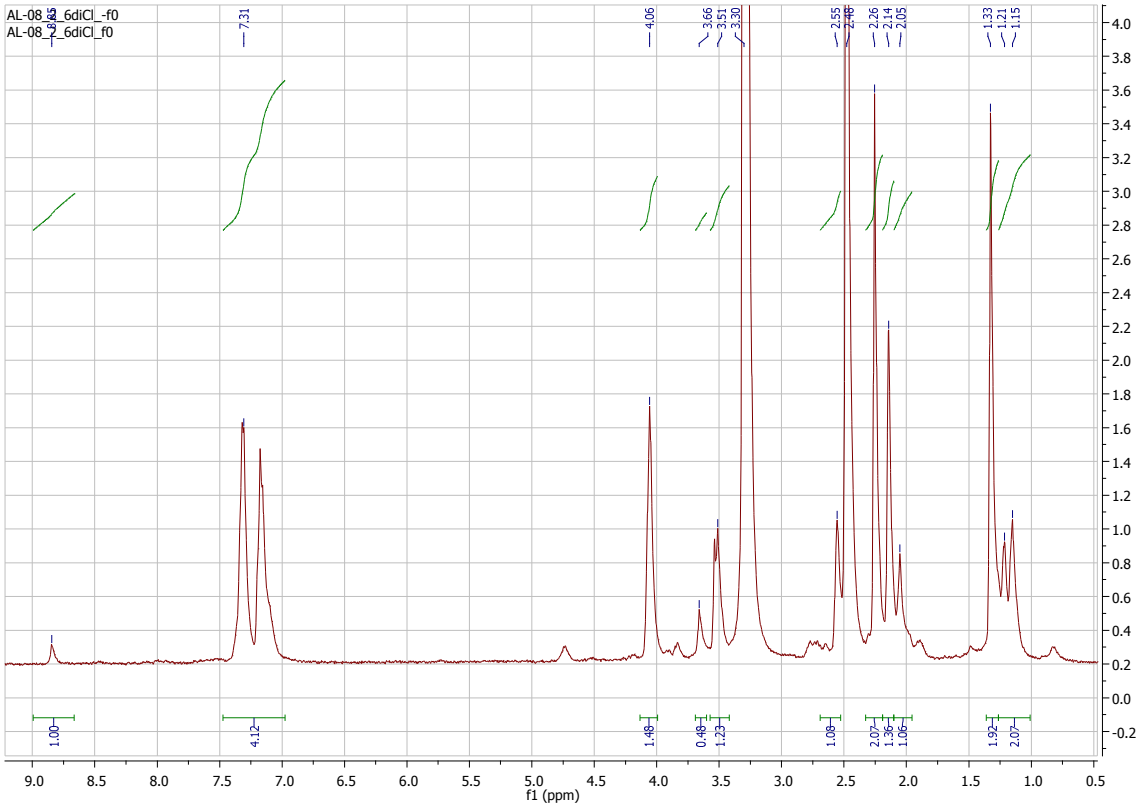
^{13}C-NMR of AL-080611-f2(Br)



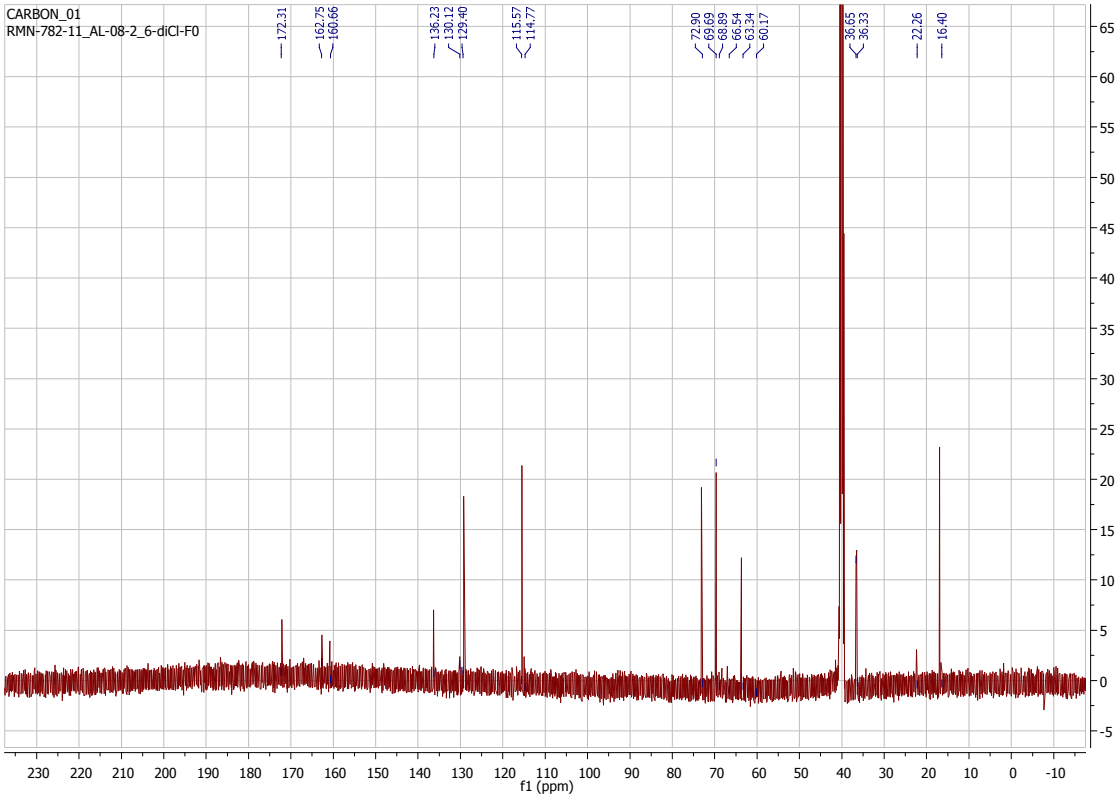
DEPT of AL-080611-f2(Br)

AL-080611-f2(2,6 diCl): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.85 (*s*, 2H), 7.31 (*m*, *J* = 93.4 Hz, 4H), 4.06 (*s*, 1H), 3.66 (*s*, 1H), 3.51 (*s*, 3H), 2.55 (*s*, 3H), 2.26 (*s*, 1H), 2.14 (*s*, 2H), 2.05 (*s*, 1H), 1.33 (*s*, 3H), 1.18 (*s*, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 172.31 (C_{10}), 162.75 (C_{24}), 160.74 (C_{16}), 136.23 (C_{26}), 130.12 (C_{19}), 129.1 (C_6), 115.49 (C_{17}),

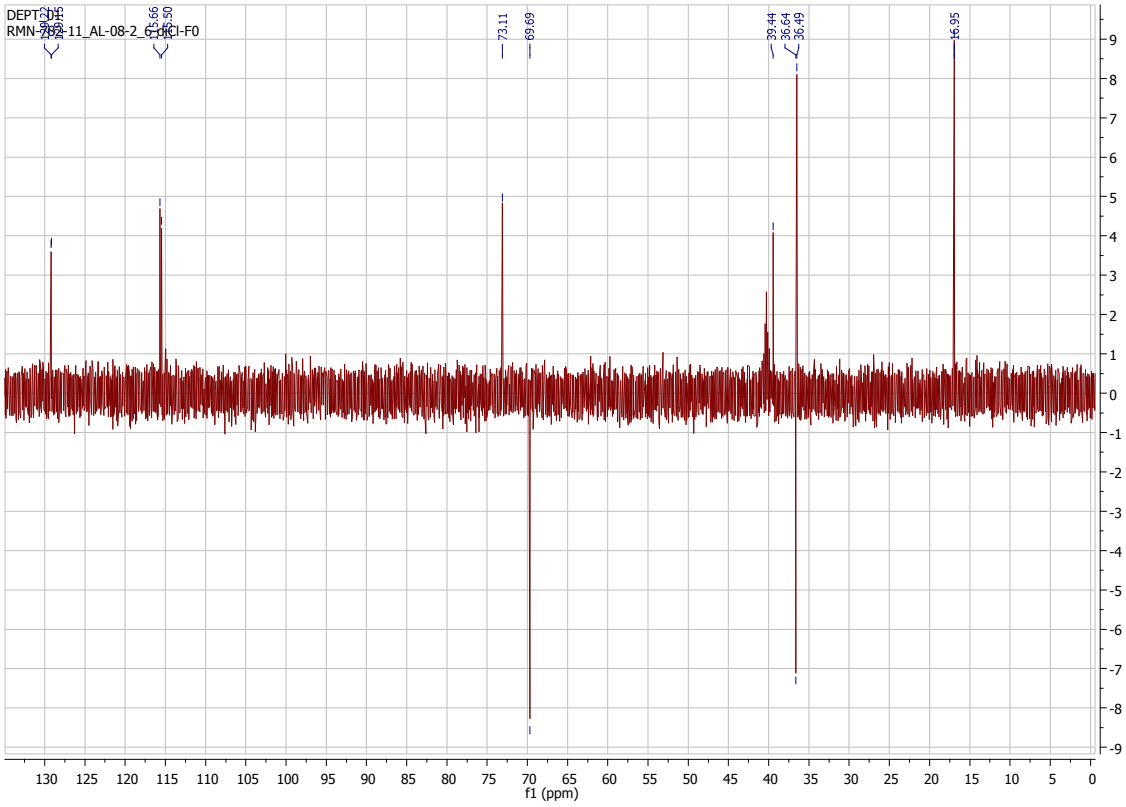
115.33 (C_{12}), 73.11 (C_{14}), 69.69 (C_{11}), 68.88 (C_{15}), 67.83 (C_{13}), 66.55 (C_4), 63.71 (C_2), 36.65(C_{27}), 34.25 (C_8), 22.75 (C_7), 16.95 (C_1). Anal. for $C_{20}H_{21}Cl_2FN_5O_2$: Calcd.: C 53.11; H 4.46; N 15.48. Found: C 53.12; H 4.33; N 15.50.



^{1}H-NMR of AL-080611-f2(2,6 diCl)



^{13}C-NMR of AL-080611-f2(2,6 diCl)



DEPT of AL-080611-f2(2,6 diCl)

Elemental Composition Report Page 1

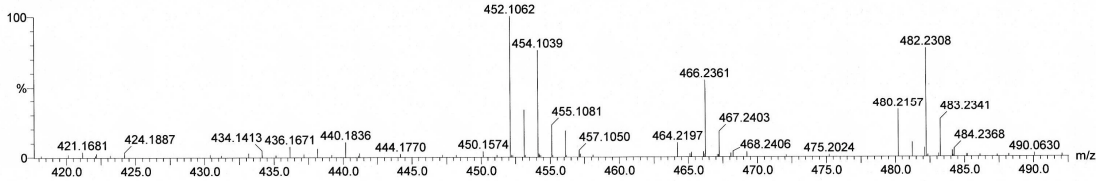
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3507 formula(e) evaluated with 20 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 0-5 O: 0-10 P: 0-1 Cl: 0-2 F: 0-1

AL-080611-F1-2-6Cl

AL-080611-F1-2-6Cl 7 (0.123)

1: TOF MS ES+
1.17e+004



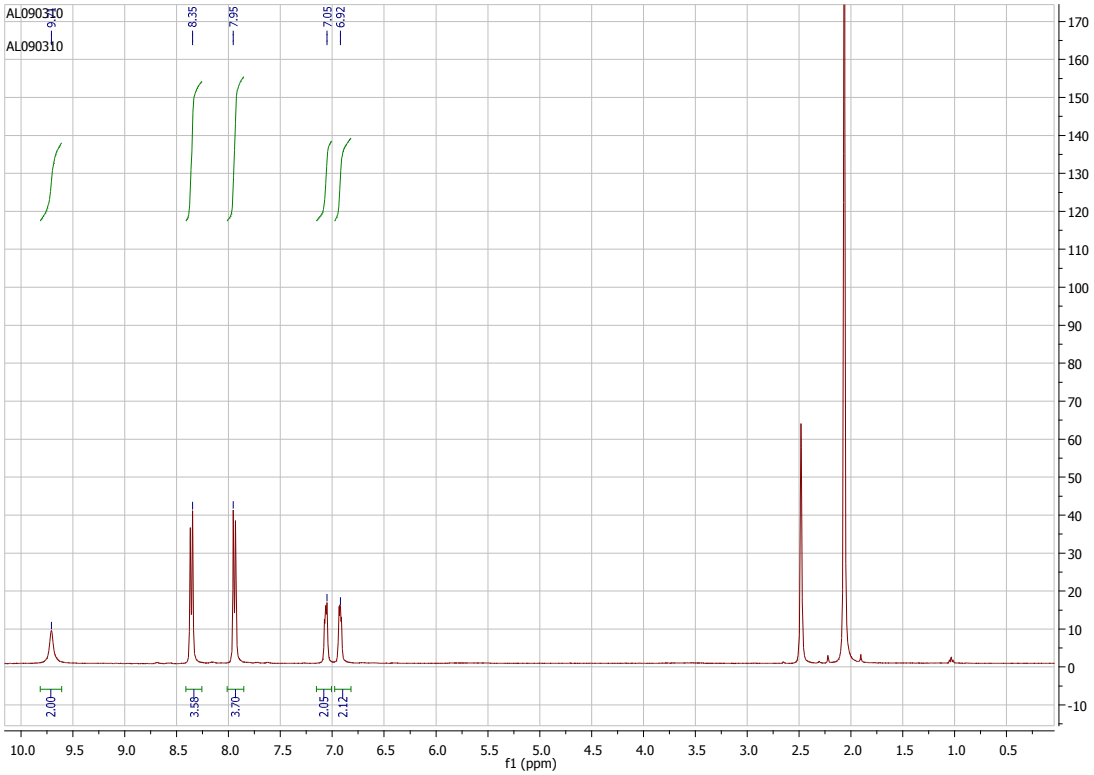
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
452.1062	452.1061	0.1	0.2	10.5	77.2	0.7	C21 H25 N3 O2 P Cl2
	452.1045	1.7	3.8	15.5	78.1	1.6	C23 H20 N5 O Cl2
	452.1056	0.6	1.3	11.5	78.3	1.8	C20 H21 N5 O2 Cl2 F
	452.1073	-1.1	-2.4	6.5	78.7	2.2	C18 H26 N3 O3 P Cl2 F
	452.1043	1.9	4.2	6.5	80.3	3.9	C19 H25 N O6 Cl2 F
	452.1065	-0.3	-0.7	15.5	82.1	5.7	C25 H20 N O4 Cl F
	452.1053	0.9	2.0	19.5	82.8	6.4	C28 H19 N O3 Cl
	452.1072	-1.0	-2.2	6.5	82.8	6.4	C16 H23 N3 O10 Cl
	452.1055	0.7	1.5	11.5	83.2	6.7	C19 H21 N5 O3 P Cl F
	452.1078	-1.6	-3.5	20.5	83.7	7.2	C26 H16 N5 Cl F
	452.1083	-2.1	-4.6	19.5	84.1	7.7	C27 H20 N3 P Cl
	452.1041	2.1	4.6	6.5	84.2	7.8	C18 H25 N O7 P Cl F
	452.1043	1.9	4.2	15.5	84.3	7.0	C22 H20 N5 O2 P Cl
	452.1054	0.8	1.8	11.5	92.9	16.4	C17 H18 N5 O10
	452.1047	1.5	3.3	20.5	93.1	16.6	C26 H15 N3 O4 F
	452.1052	1.0	2.2	19.5	93.2	16.7	C27 H19 N O4 P
	452.1065	-0.3	-0.7	24.5	93.3	16.8	C28 H15 N5 P
	452.1063	-0.1	-0.2	15.5	93.3	16.8	C24 H20 N O5 P F
	452.1077	-1.5	-3.3	20.5	93.3	16.9	C25 H16 N5 O P F
	452.1075	-1.3	-2.9	28.5	93.4	16.9	C34 H14 N O

Mass Analysis of AL-080611-f2(2,6 diCl)

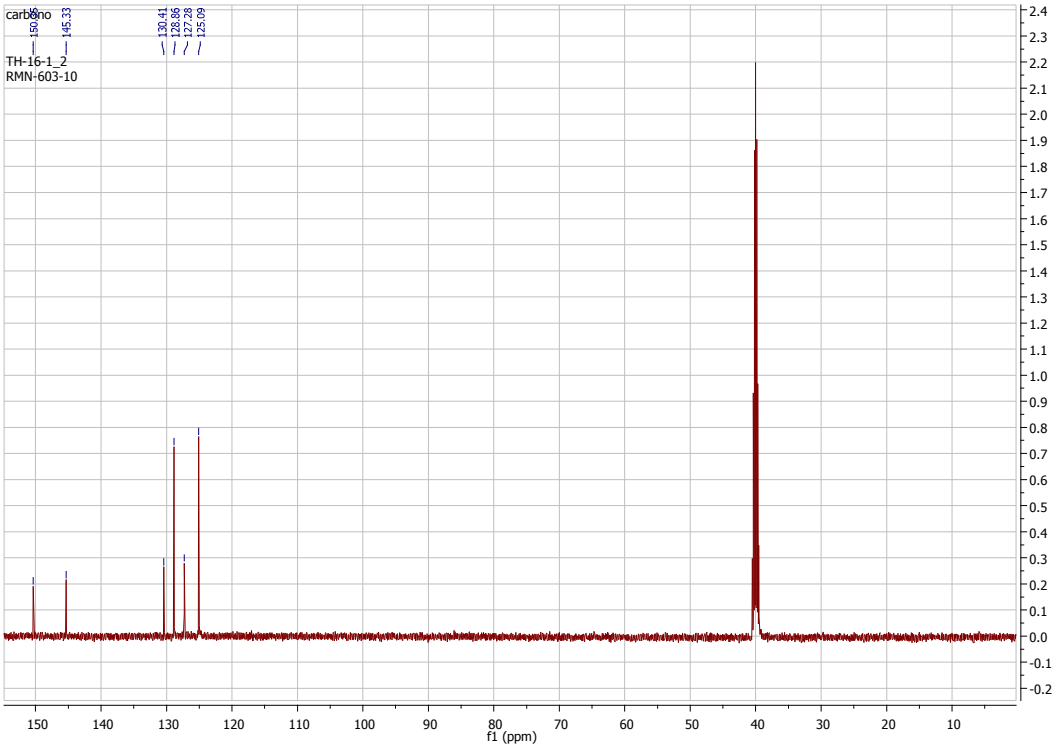
Synthesis of *N,N'*-(1,2-phenylene)bis(*p*-nitrobenzenesulfonamide) AL-200910.

To an ice-cold solution of *o*-phenylenediamine (2g, 1 eq.) and pyridine (2 eq.) in CH_2Cl_2 was added portion wise the *p*-nitrobenzenesulfonyl

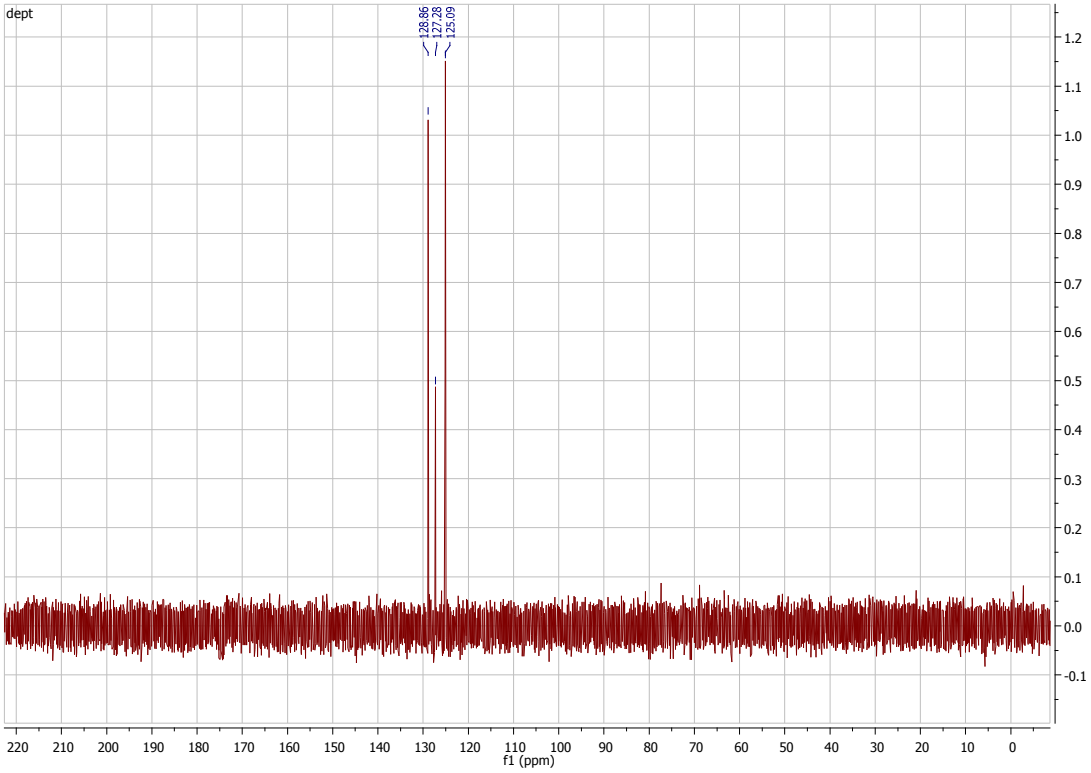
chloride (2 eq.). The reaction mixture was warmed and maintained at room temperature until completion of the reaction (TLC analysis). Water (50mL) was next added, causing the product to precipitate. The reaction mixture was filtered and the cake was washed with H_2O. The orange-red solid so obtained was recrystallized from EtOH to afford **AL-200910** (89% yield) as a pale-yellow crystalline product. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.71 (*s*, 2H), 8.35 (*m*, 4H), 7.95 (*m*, 4H), 7.05 (*m*, 2H), 6.92 (*m*, 2H). ^{13}C-NMR (75 MHz, DMSO-d_6): 150.35 (C_2-C_5), 145.33 (C_3-C_4), 130.41 (C_7-C_{7a}), 128.86 (C_8-C_{12}-C_{8a}-C_{12a}), 127.28 (C_{10}-C_{10a}), 125.09 (C_9-C_{11}-C_{9a}-C_{11a}). Anal. for $C_{18}H_{14}N_4O_8S_2$: Calcd.: C 45.19; H 2.95; N 11.71; S 13.40. Found: C 45.20; H 3.08; N 11.69; S 13.31.



^{1}H-NMR of AL-200910



^{13}C-NMR of AL-200910



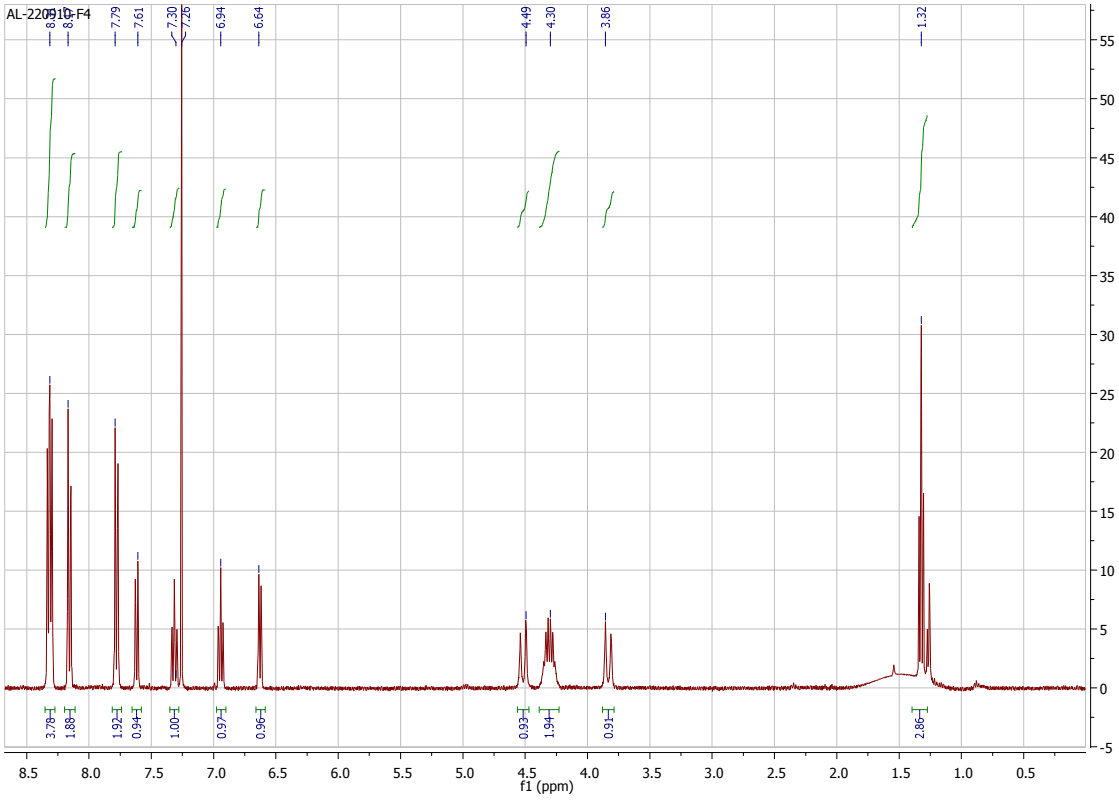
DEPT of AL-200910

Synthesis of ethyl 2-{*p*-nitro-*N*-[2-(4-nitrobenzenesulfonamido)benzene]benzenesulfonamido}acetate AL-220910-f2 and diethyl 2,2'-{1,2-phenylenebis[(*p*-nitrobenzene)sulfonyl]-azadiyl}diacetate AL-220910-f4.

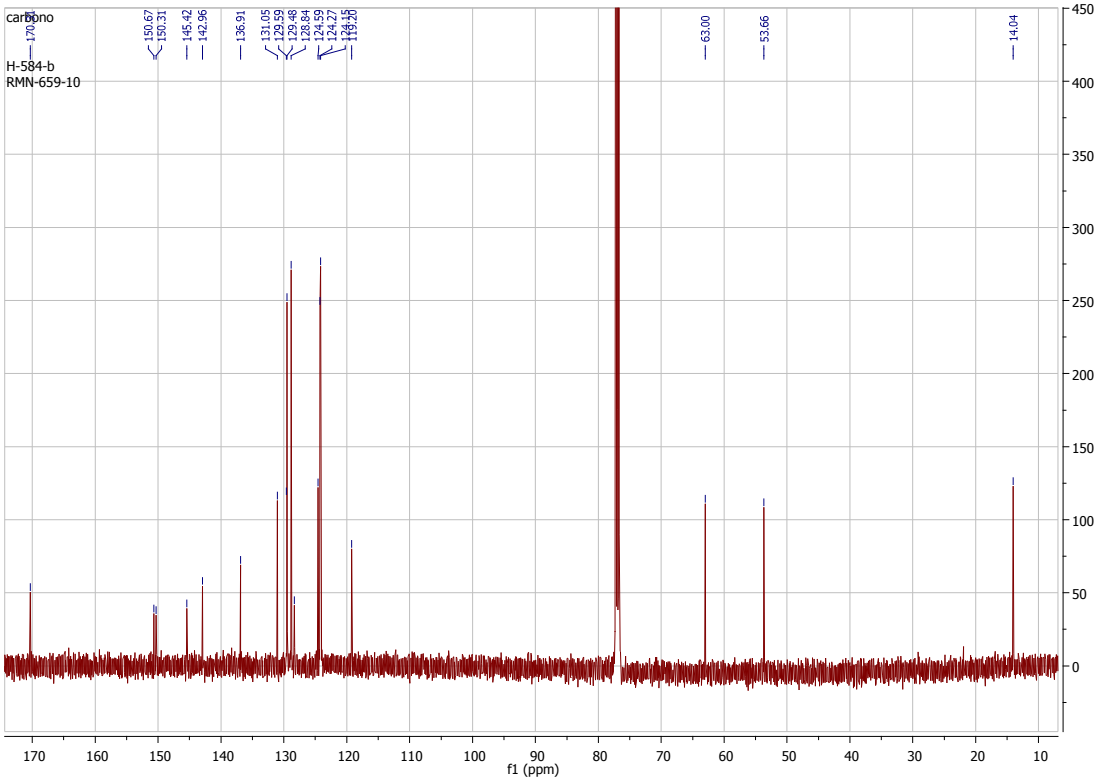
A solution of **AL-200910** (3g, 1 eq.), Ethyl glycolate (2 eq.) and triphenylphosphine (2.4 eq.) in dried THF was prepared under argon atmosphere. To this solution, DIPAD (2.2 eq.) was added dropwise at -20°C and temperature was then allowed to rise to 5°C before warming to 30°C. The solution was stirred for 21h under argon atmosphere. Then, the solvent was evaporated, and the crude product was added onto a silica column and purified by flash chromatography (EtOAc/Hexane, 0.1:1→ 0.7:1) to afford both **AL-220910-f2** (30%, R_f =0.46) as a white solid, and **AL-220910-f4** [(60%, R_f =0.28 (EtOAc/Hexane 0.5:1)] as a pale yellow solid.

AL-220910-f2: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.31 (*m*, 4H), 8.16 (*d*, J= 8.8 Hz, 2H), 7.78 (*d*, J= 8.8 Hz, 2H), 7.62 (*d,* J= 8.4 Hz, 2H), 7.30 (*t*, J= 7.7 Hz, 1H), 6.94 (*t*, J = 7.7 Hz, 1H), 6.63 (*d*, J= 7.7 Hz, 1H), 4.52 (*d*, J = 18.3 Hz, 1H), 4.30 (*q*, J = 34.3 Hz, 1H), 3.83 (*d*, J = 19.2 Hz, 1H), 1.32 (*t*, J = 7.2 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$): 170.31 (C_1), 150.67 (C_6), 150.31 (C_5), 145.42 (C_4), 142.96 (C_3), 136.91 (C_2), 131.05 (C_1), 129.53 (C_{11}), 128.84 (C_{12}), 128.35 (C_{13}), 124.59 (C_{14}), 124.21

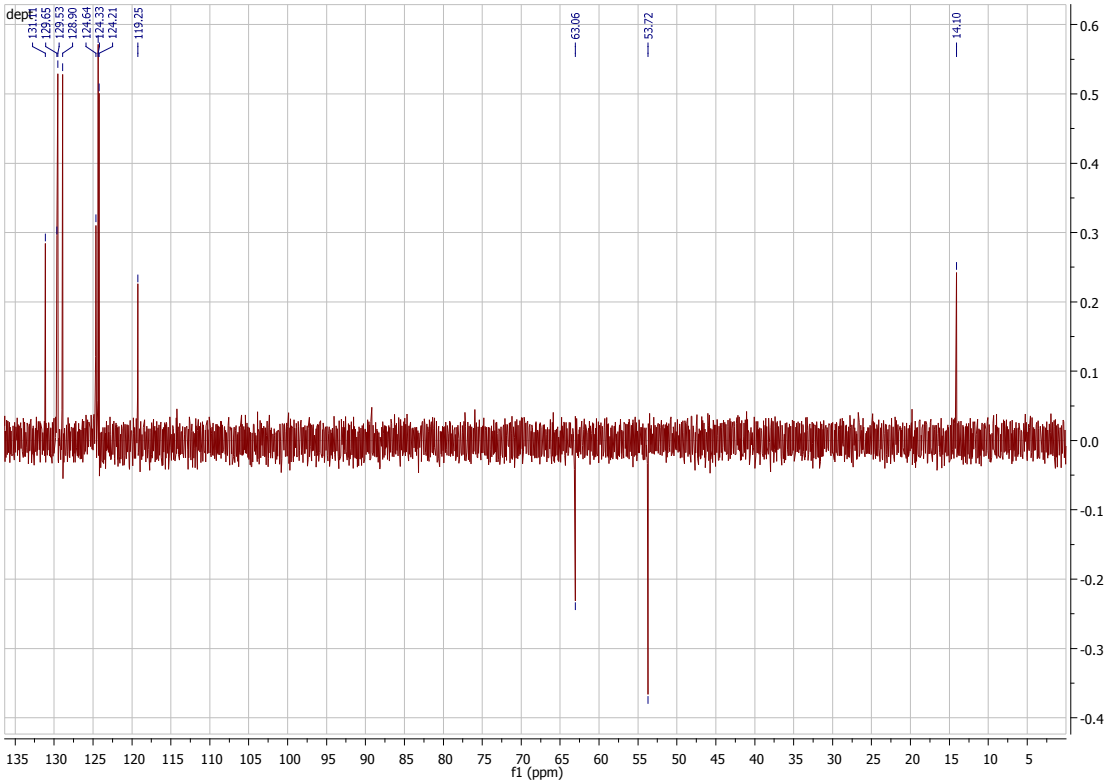
(C_7), 119.20 (C_8), 63.00 (C_{15}), 53.66 (C_{17}), 14.04 (C_{18}). Anal. for $C_{22}H_{20}N_4O_{10}S_2$: Calcd.: C 46.80; H 3.57; N 9.92; S 11.36. Found: C 46.89; H 3.24; N 10.06; S 11.45.



^{1}H-NMR of AL-220910-f2



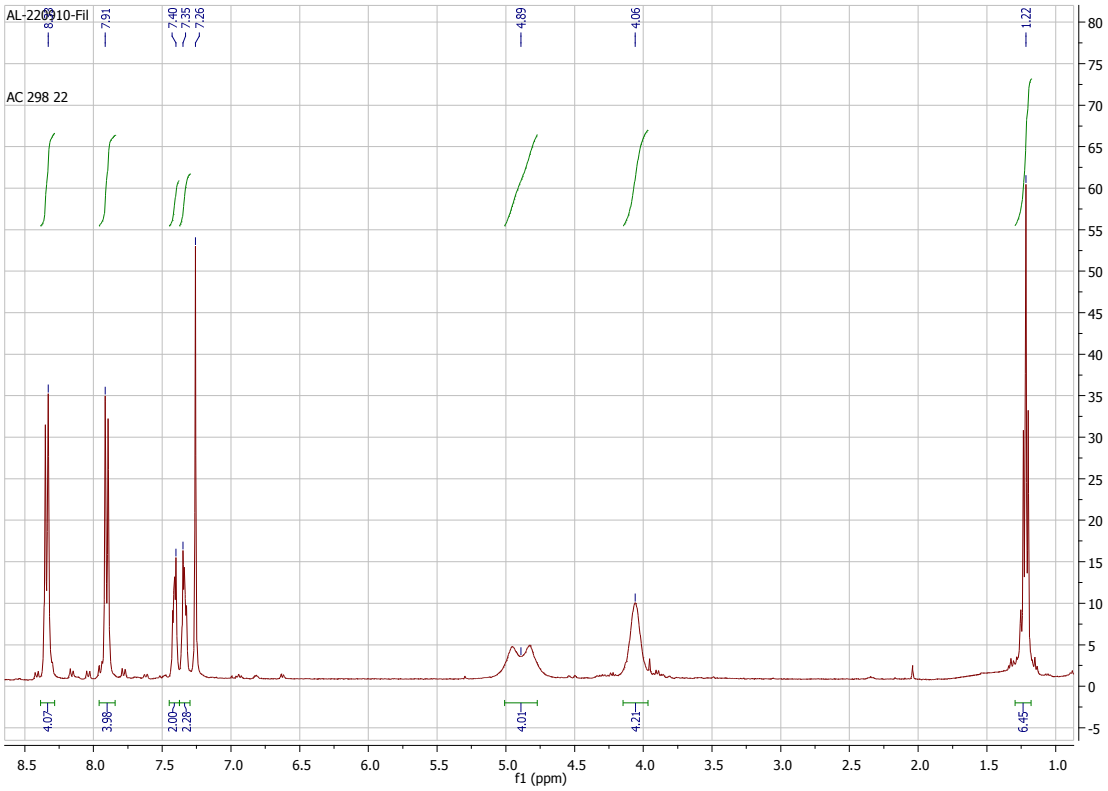
^{13}C-NMR of AL-220910-f2



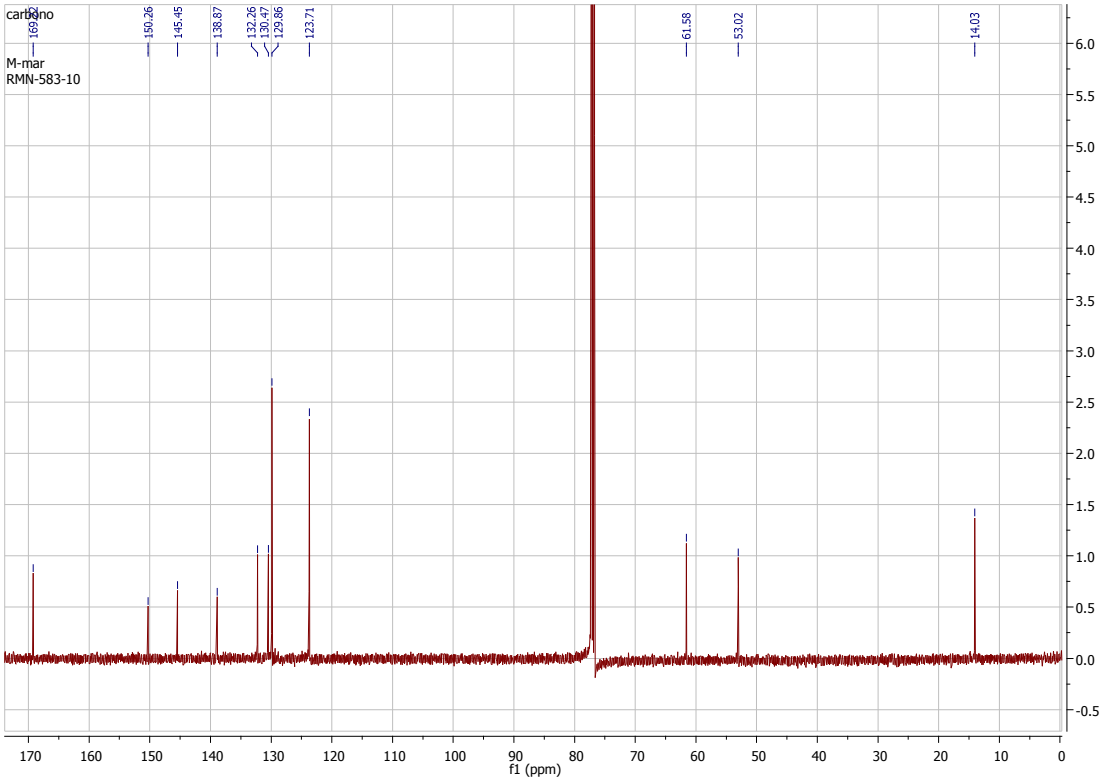
DEPT of AL-220910-f2

AL-220910-f4: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.34 (d, *J* = 8.7 Hz, 4H), 7.90 (d, *J* = 8.7 Hz, 4H), 7.40 (*m*, 2H), 7.35 (*m*, 2H), 4.89 (*m*, 4H), 4.06 (*s*, 4H), 1.22 (*t*, *J*= 7.1, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$): 169.22 (C_{14}-C_{14a}), 150.26 (C_{12}-C_{12a}), 145.45 (C_6-C_1), 138.87 (C_7-C_{7a}), 132.26 (C_2-C_5), 130.47 (C_3-C_4), 129.86 (C_{10}-C_{11}-C_{10a}-C_{11a}), 123.71 (C_8-C_9-C_{8a}-C_{9a}), 61.58 (C_{13}-C_{13a}), 53.02 (C_{15}-C_{15a}), 14.03 (C_{16}-C_{16a}). Anal. for

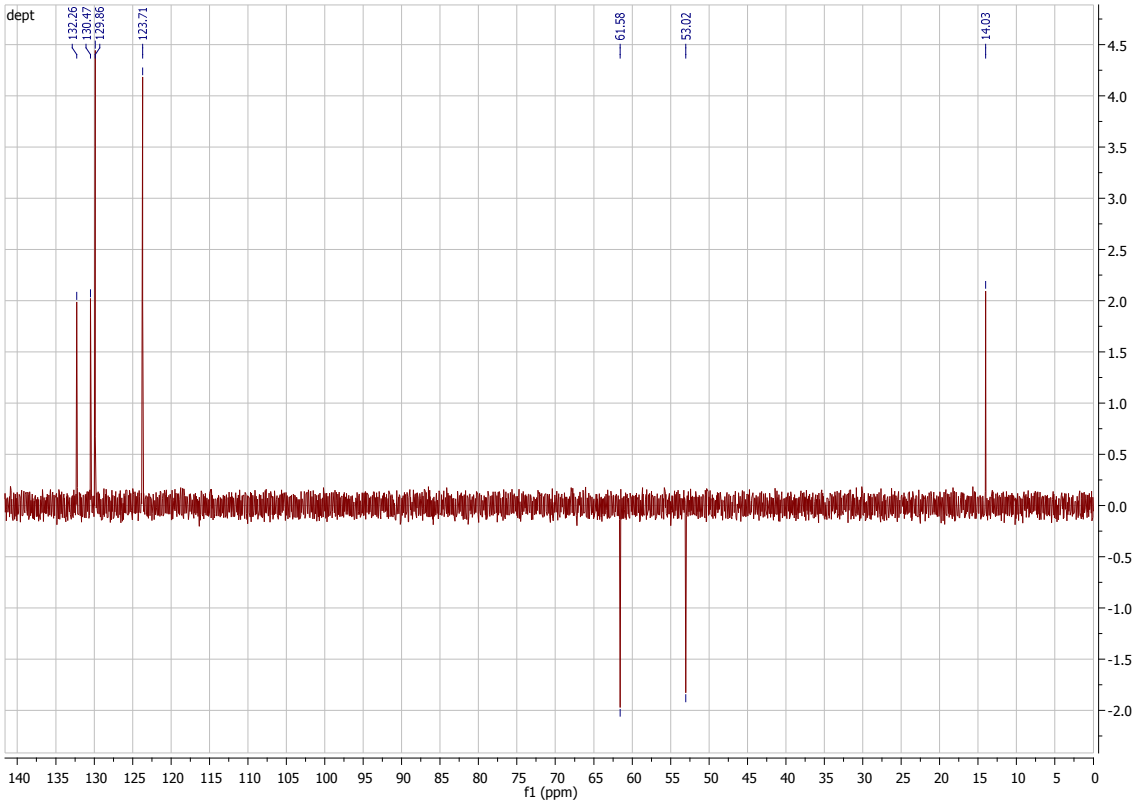
$C_{26}H_{26}N_4O_{12}S_2$: Calcd.: C 48.00; H 4.03; N 8.61; S 9.86. Found: C 47.84; H 4.03; N 8.61; S 9.65.



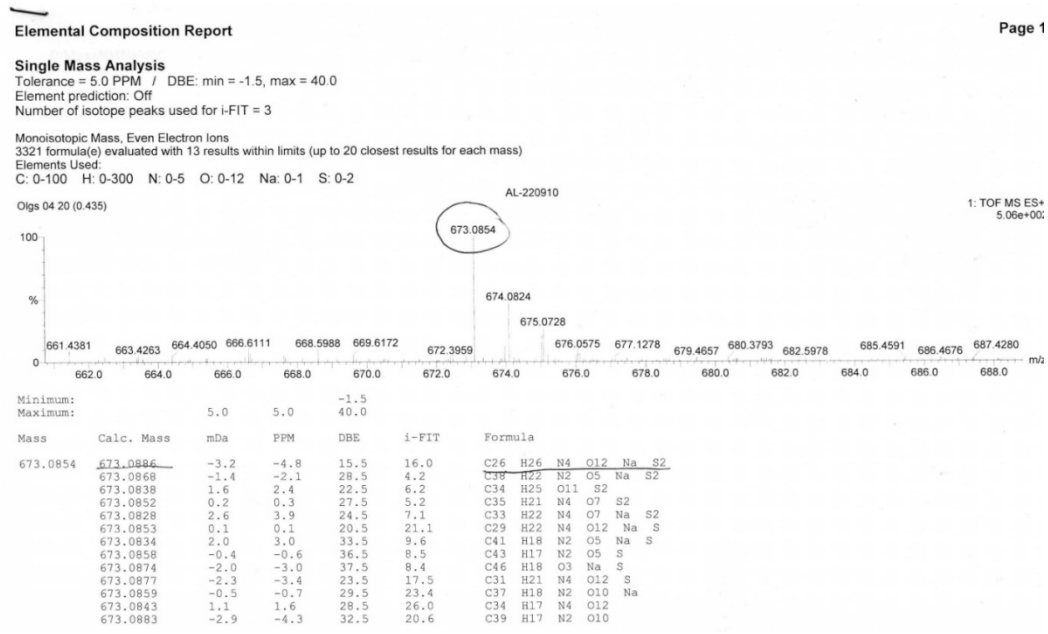
^{1}H-NMR of AL-220910-f4



^{13}C-NMR of AL-220910-f4



DEPT of AL-220910-f4



Elemental Composition Report

Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 40.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3321 formula(e) evaluated with 13 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-100 H: 0-300 N: 0-5 O: 0-12 Na: 0-1 S: 0-2

AL-220910

Olgs 04 20 (0.435)

1: TOF MS ES+
5.06e+00

Minimum:				-1.5		
Maximum:		5.0	5.0	40.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
673.0854	673.0886	-3.2	-4.8	15.5	16.0	C26 H26 N4 O12 Na S2
	673.0868	-1.4	-2.1	28.5	4.2	C38 H22 N2 O5 Na S2
	673.0838	1.6	2.4	22.5	6.2	C34 H25 O11 S2
	673.0852	0.2	0.3	27.5	5.2	C35 H21 N4 O7 S2
	673.0828	2.6	3.9	24.5	7.1	C33 H22 N4 O7 Na S2
	673.0853	0.1	0.1	20.5	21.1	C29 H22 N4 O12 Na S
	673.0834	2.0	3.0	33.5	9.6	C41 H18 N2 O5 Na S
	673.0858	-0.4	-0.6	36.5	8.5	C43 H17 N2 O5 S
	673.0874	-2.0	-3.0	37.5	8.4	C46 H18 O3 Na S
	673.0877	-2.3	-3.4	23.5	17.5	C31 H21 N4 O12 S
	673.0859	-0.5	-0.7	29.5	23.4	C37 H18 N2 O10 Na
	673.0843	1.1	1.6	28.5	26.0	C34 H17 N4 O12
	673.0883	-2.9	-4.3	32.5	20.6	C39 H17 N2 O10

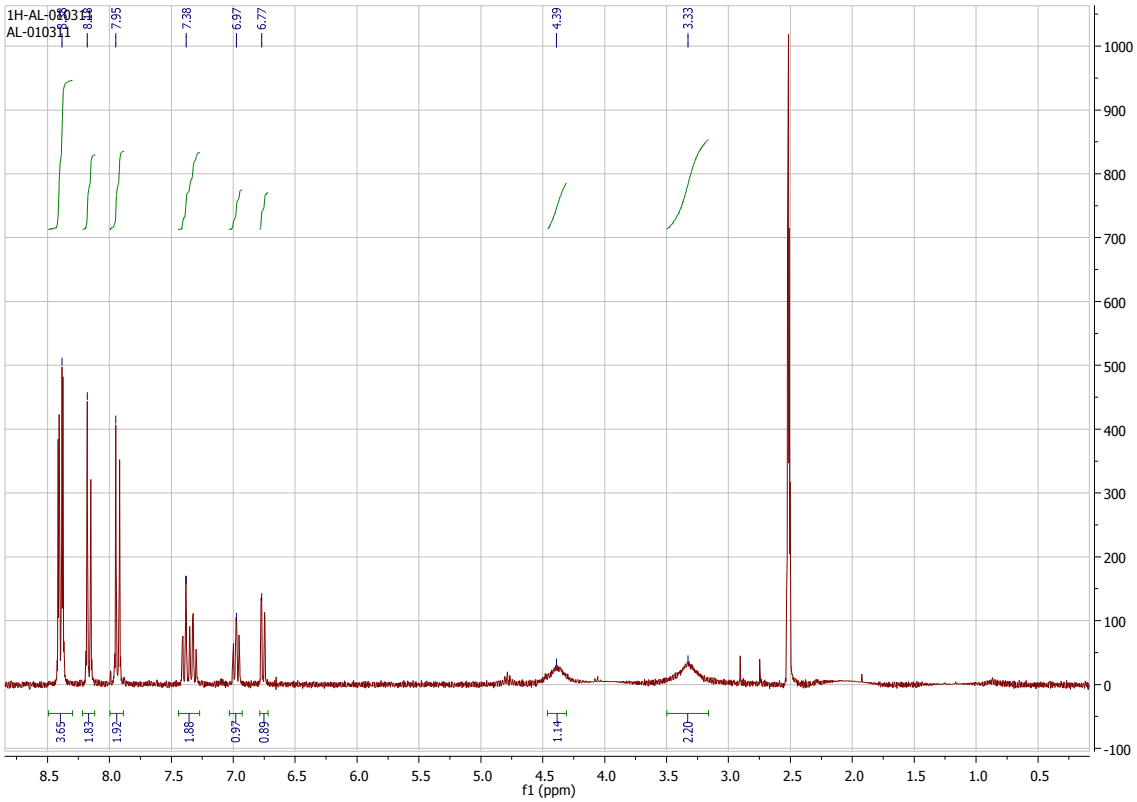
Mass Analysis of AL-220910-f4

Synthesis of 2-{*p*-nitro-*N*-[2-(*p*-nitrobenzenesulfonamido)benzene]-benzenesulfonamido}acetic acid AL-010311

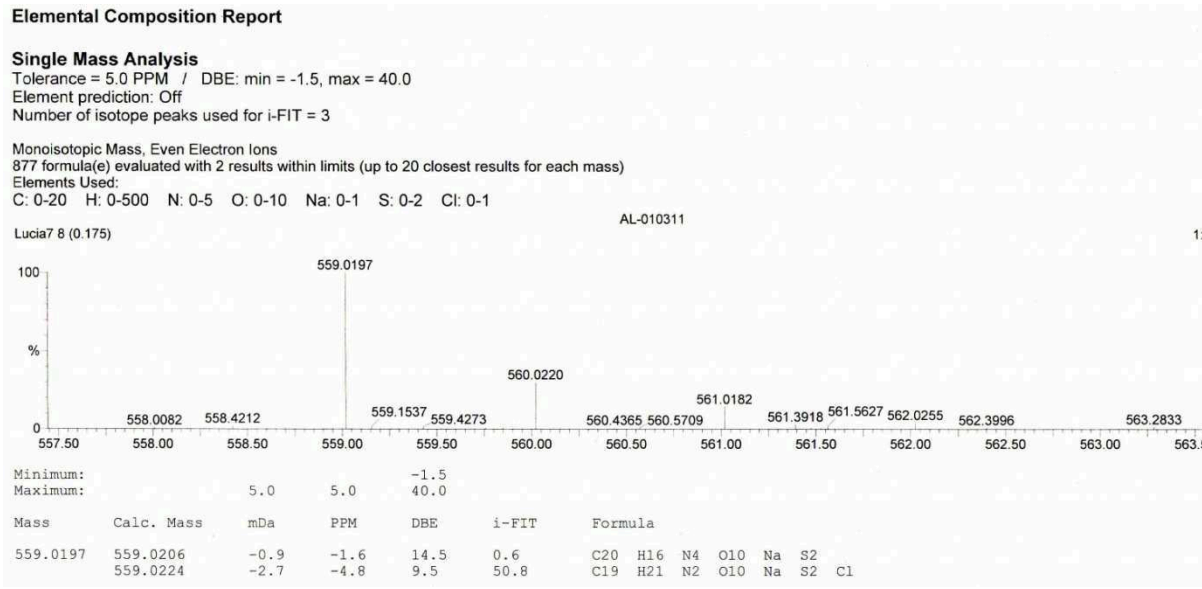
A mixture of **AL-220910-f2** and NaOH 1M was stirred at room temperature until TLC analysis showed the starting materials disappeared. The solution was washed with ethyl acetate. The aqueous layer was then acidified with 1M HCl and extracted with ethyl

acetate (2 ×), dried ($MgSO_4$) and concentrated to afford **AL-010311**(94% yield) as a white solid.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.38 (*m*, 4H), 8.18 (*m*, 2H), 7.95 (*m*, 2H), 7.38 (*m*, 2H), 6.97 (*m*, 1H), 6.77 (*m*, 1H), 4.39 (*s*, N-H), 3.33 (*s*, 2H). Anal. for $C_{20}H_{16}N_4O_{10}S_2$: Calcd.: C 44.77; H 3.01; N 10.44; S 11.95. Found: C 45.01; H 2.99; N 10.31; S 12.03.



^{1}H-NMR of AL-010311



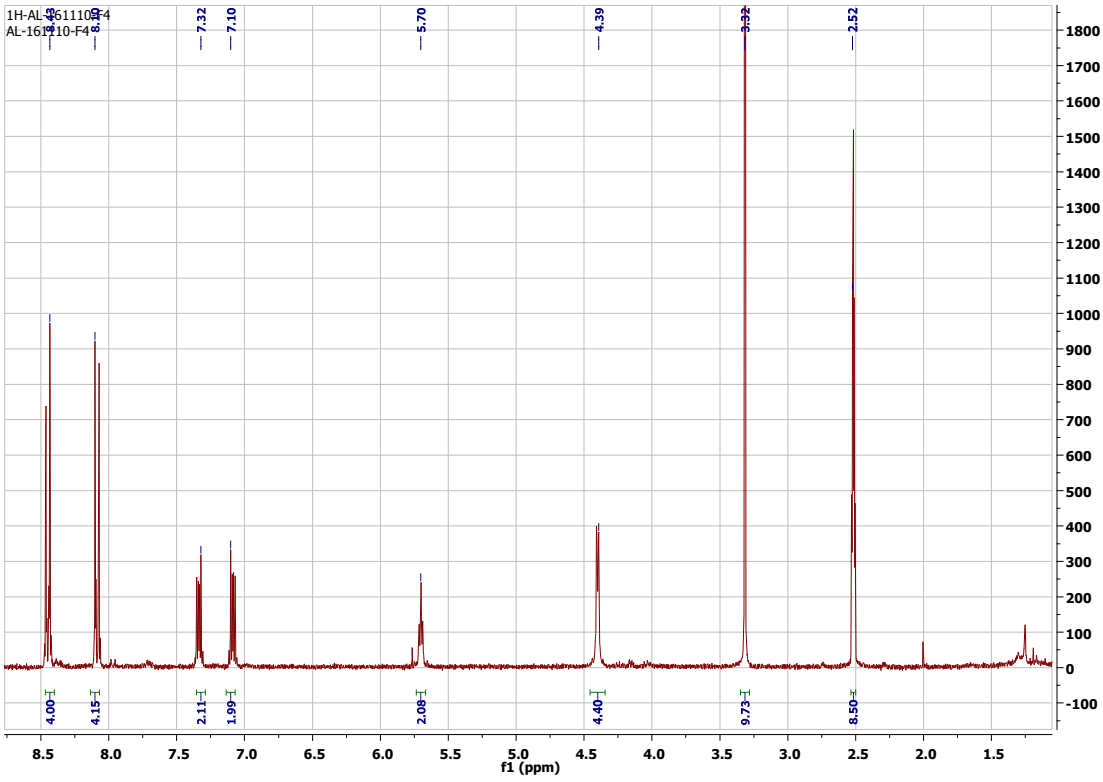
Mass Analysis of AL-010311

Synthesis of (*Z*)-1,6-bis[(*p*-nitrobenzene)sulfonyl]-1,2,5,6-tetrahydro-1,4-benzodiazocine AL-091210(2).

A solution of **AL-200910** (1g, 1 eq.) and *cis*-1,4-dichloro-2-butene (1 eq.) in acetonitrile over K_2CO_3 (5 eq.) was heated at reflux for 24 h. At the end of this period, the reaction mixture was cooled at room temperature, filtered and freed of solvent under reduced pressure. The resulting pale yellow solid was purified by flash chromatography

(CH_2Cl_2/MeOH, 9.8:0.2) to afford **AL-091210(2)** (41% yield, R_f = 0.37) as a white solid.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.43 (*m*, 4H), 8.10 (*m*, 4H), 7.32 (*m*, 2H), 7.10 (*m*, 2H), 5.72 (*t*, *J*= 3.9, 4H), 4.41 (*d*, *J*= 3.9, 2H). Anal. for $C_{22}H_{18}N_4O_8S_2$: Calcd.: C 49.81; H 3.42; N 10.56; S 12.09. Found: C 49.98; H 3.53; N 10.35; S 12.01.

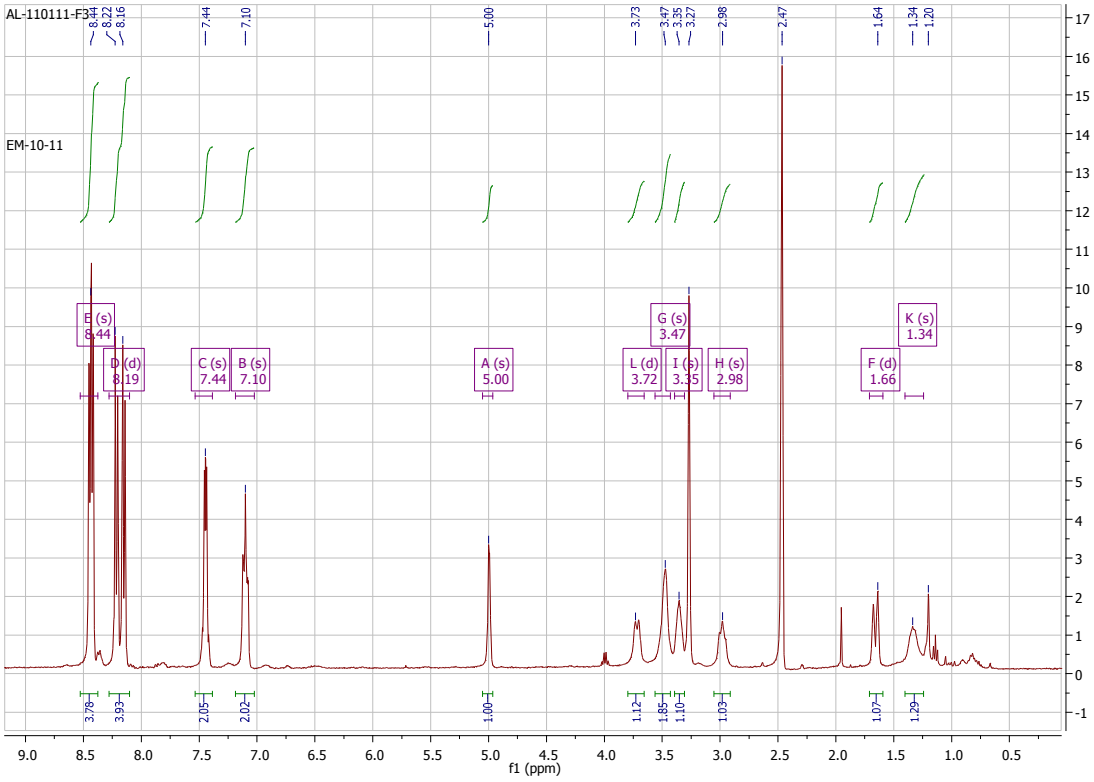


^{1}H-NMR of AL-091210(2)

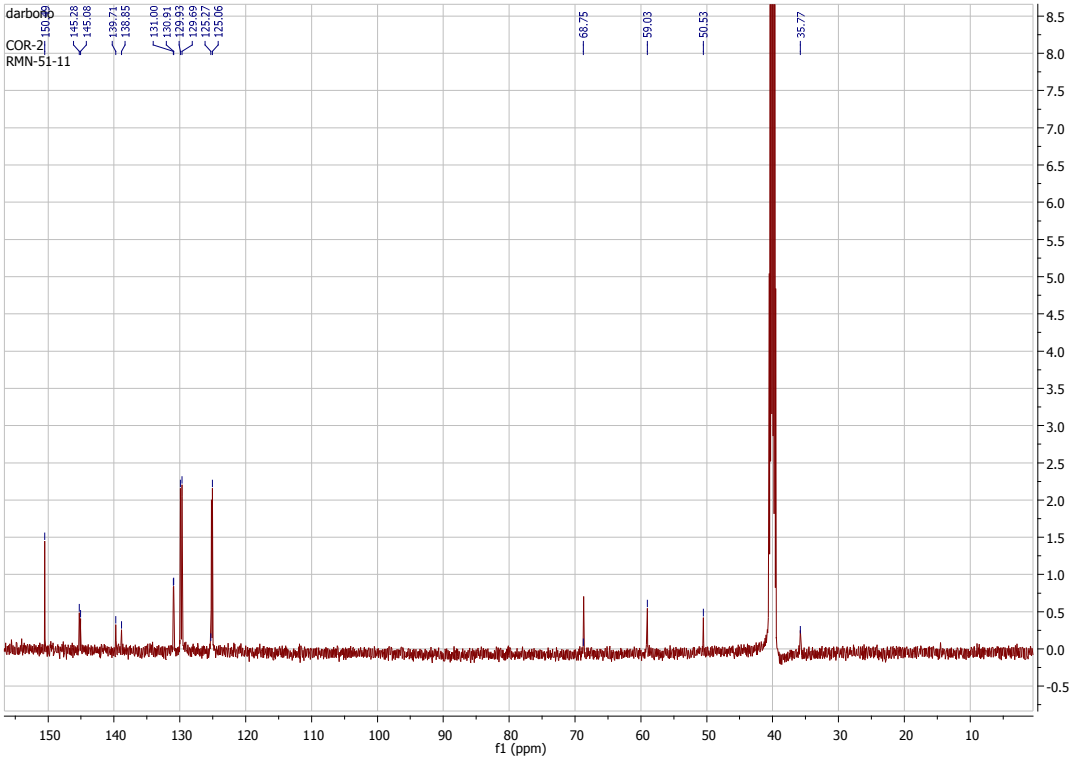
Synthesis of 1,6-bis[(*p*-nitrobenzene)sulfonyl]-1,2,3,4,5,6-hexahydro-1,4-benzodiazocine-3-ol AL-110111-f3.

To a stirred solution of **AL-091210(2)** (200mg, 1eq.) in anhydrous THF, under Argon atmosphere and cooled at -78°C, was added a solution of BH_3·THF (3.42 eq.). After 5 min the flask was submerged in an ice bath and left at 0°C for 20 h. Then, under vigorous stirring, trimethylamine *N*-oxide (12 eq.) was added and, after mounting a condenser, the reaction was heated at 65°C for 2 h. After cooling in an ice bath, EtOAc was added and the organic layer was washed with brine (3 ×), dried ($MgSO_4$) and concentrated. The crude product was added onto a silica column and purified by flash chromatography (EtOAc/Hexane, 0.1:1→ 1.5:1) to afford **AL-110111-f3** as a white solid [58%, R_f =0.27 (EtOAc/Hexane 0.5:1)].

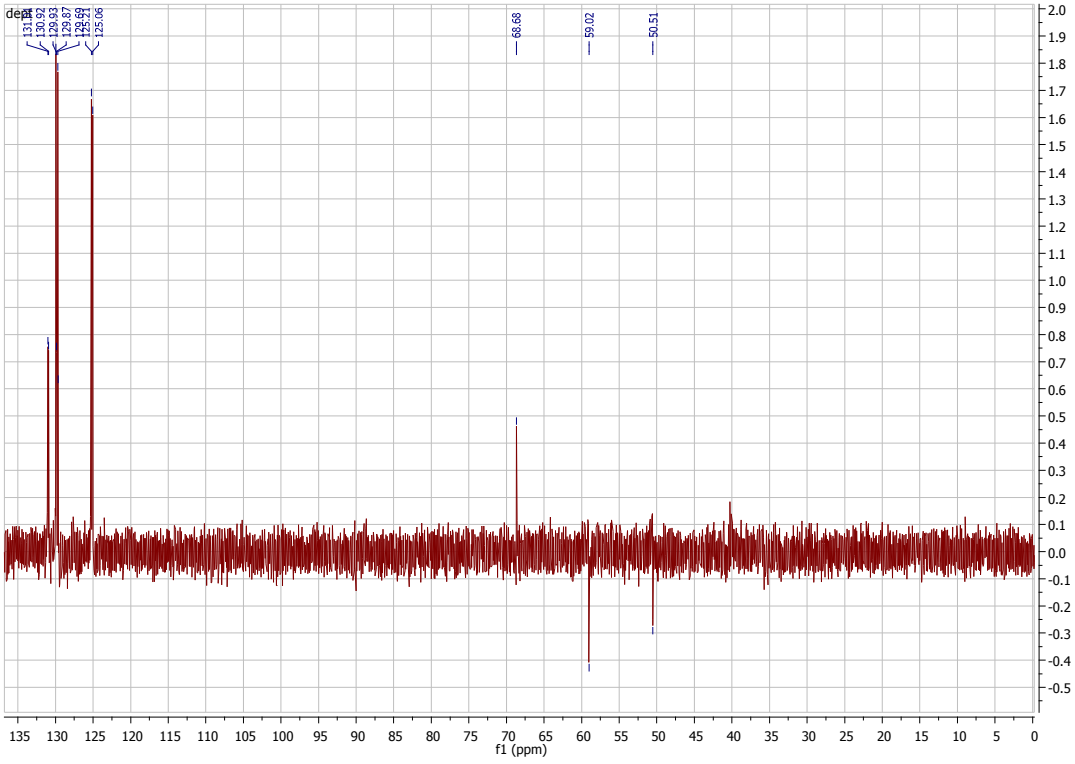
^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.44 (*m*, 4H), 8.19 (*m*, 4H), 7.44 (*m*, 2H), 7.10 (*m*, 2H), 5.00 (*s*, 1H), 3.72 (*d*, *J* = 10.9 Hz, 1H), 3.47 (*s*, 2H), 3.35 (*s*, 1H), 2.98 (*s*, 1H), 1.66 (*d*, *J* = 15.1 Hz, 1H), 1.34 (*s*, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 150.49 (C_{10}-C_{16}), 145.28 (C_7), 145.08 (C_8), 139.71 (C_{11}), 138.85 (C_{10}), 131.00 (C_9), 129.93 (C_{15}-C_{17}), 129.69 (C_9-C_{11}), 125.27(C_8-C_{12}), 125.06 (C_{14}-C_{18}), 68.75 (C_3), 59.03 (C_2), 50.53 (C_4), 35.77 (C_5). Anal. for $C_{22}H_{20}N_4O_9S_2$: Calcd.: C 48.17; H 3.67; N 10.21; S 11.69. Found: C 48.22; H 3.57; N 10.09; S 11.70.



^{1}H-NMR of AL-110111-f3



^{13}C-NMR of AL-110111-f3



DEPT of AL-110111-f3

10. REFERENCES

1) Johnson, George. "Unearthing Prehistoric Tumors, and Debate". The New York Times. 28-12-2010.

2) Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M (2005). "Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors". Lancet 366 (9499): 1784–93. doi:10.1016/S0140-6736(05)67725-2. PMID 16298215.

3) WHO calls for prevention of cancer through healthy workplaces (Press release). World Health Organization. 13-10-2007.

4) "National Institute for Occupational Safety and Health- Occupational Cancer". United States National Institute for Occupational Safety and Health. 13-10-2007.

5) M Primic-Zakelj Institute of Oncology Ljubljana Epidemiology and Cancer Registry 12-12-2007

6) Pardee, A. B:, A restriction point for control of normal animal cell proliferation, Proc Natl Acad Sci U S A, 1974 , 71, 1286-1290.

7) Planas-Silva, M.D.; Weinberg, R.A., The restriction point and control of cell proliferation, Curr. Opin. Cel. Biol., 1997, 9, 768-772.

8) Novák, B.; Tyson, J.J., Modelling the controls of the eukaryotic cell cycle, Biochem. Soc. Trans., 2003, 31, 1526-1529.

9) Coultas, L.; Strasser, A.; The molecular control of DNA damage-induced cell death. Apoptosis, 2000, 5, 491- 507.

10) Bold, J.R.; Termuhlen, P.M., Mc Conkey, D.J.; Apoptosis, cancer and cancer therapy, Surg. Oncol., 1997, 6, 133-142.

11) Díaz Gavilán M.; Diseño y Síntesis de Bezoxazepinas como Fármacos Antitumorales. Incremento de la Diversidad Estructural. Tesis Doctoral, Departamento de Química Farmacéutica y Orgánica, Universidad de Granada. 2005.

12) Campos, J. M.; Saniger, E.; Marchal, J. A.; Aiello, S.; Suárez, I.; Boulaiz, H.; Aránega, A.; Gallo M. A.; Espinosa A., New Medium Oxacyclic O,N -Acetals and Related Open Analogues: Biological Activities, Curr. Med. Chem., 2005, 12, 1423-1438.

13) M. C. Nunez et al. Tetrahedron 62 (2006) 11724-11733

14) G. J. Friis, H. Bundgaard; *A textbook of Drug design and Development.*

15) M. Malet-Martino et al.; *Curr. Med. Chem. Med.: Anticancer Agents* 2002, 2, 267.

16) J.A Gómez, et. al. Tetrahedron 1997, 53(21), 7319-7334.

17) Trujillo, M.A.; Gómez, J.A.; Campos, J.; Espinosa, A.; Gallo, M.A. Tetrahedron 2001, 57, 3951-3961.

18) Saniger Bernal, E. Nuevos Profármacos de 5-Fluorouracilo con Restos de Dihidrobenzodioxepinos y Compuestos Relacionables con Actividad Diferenciadora Celular y Antiproliferativa. Tesis Doctoral, Departamento de Química Farmacéutica y Orgánica, Universidad de Granada. Granada, 2002.

19) Saniger, E.; Campos, J.M.; Entrena, A.; Marchal, J.A.; Suárez, I.; Aránega, A.; Choquecillo, D.; Niclós, J.; Gallo, M.A.; Espinosa, A. Tetrahedron 2003, 59, 5457-5467.

20) Saniger, E.; Campos, J.M.; Entrena, A., Marchal, J.A.; Boulaiz, H.; Aránega, A.; Gallo, M.A.; Espinosa, A. Tetrahedron 2003, 59, 8017-8026.

21) Saniger, E.; Campos, J.M.; Entrena, A.; Marchal, J.A.; Suárez, I.; Aránega, A.; Choquecillo, D.; Niclós, J.; Gallo, M.A.; Espinosa, A. Tetrahedron 2003, 59, 5457-5467.

22) Campos, J.; Saniger, E.; Marchal, J.A.; Aiello, S; Súarez, I.; Boulaiz, H.; Aránega, A.; Gallo, M.A.; Espinosa, A. Current Medicinal Chemistry 2005, 12, 887-916.

23) Diaz-Gavillan M. Tetrahedron, 60, 2004, 11547-11557

24) Dıaz-Gavilan M. et al. Bioorg. Med. Chem. Lett. 18 (2008) 1457–1460

25) Campos, J. M.; Espinosa, A.; Gallo, M. A.; Gomez-Vidal, J. A.; Nunez, M. C.; Aranega, A.; Marchal, J. A.; Rodrıguez-Serrano, F. Spanish Patent, P200601538, 2006.

26) M. C. Nunez et al., Curr Med Chem, (2008), 15, 2614-2631.

27) Conejo-Garcia, A et al., Eur. J. Med. Chem., 2008, 43(8):1742-1748

28) Dıaz-Gavilan M. et al., J. Org. Chem., 2006, 71, 1043.

29) Dıaz-Gavilan M. et al., Tetrahedron, 2007, 63, 5274.

30) Lin P. et al., J. Biol. Chem., 2003, 278, 14379

31) Yoneda T. et al., J. Biol. Chem., 2001, 276, 13935

32) Baugher, P. J.; Krishnamoorthy, L.; Price, J. E.; Dharmawardhane, S. F. Breast Cancer Res. 2005, 7, R965.

33) K. V. Kudryavtsev, M. Y. Tsentalovich, A. S. Yegorov, and E.L. Kolychev. J. Heterocyclic Chem. 43, 1461, 2006.

34) Kiankiarimi M. et al., Tetrahedron Letters, 40, 1999, 4497-4500

35) Gaudin J.M. et al., Helvetica Chimica Acta, 90, 2007, 1245-1265

36) Naimi-Jamal M.R. et al., Eur. J. Org. Chem. 2009, 3567-3572

37) Diaz.Gavillan et al., Chem Med Chem, 2008, 3, 127-135.

38) Liu, J. et al., J. Org. Lett. 2004, 6, 2917

39) Proust, N. et al., J. Org. Chem., 2009, 74, 2897-2900

40) Bartali L. et al., Eur J. Org. Chem., 2010, 5831-5840

41) Savoia D. et al., Org. Lett., Vol. 12, No. 21, 2010, 4964-4967