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Titel der Diplomarbeit

Syntheses of new adenosine A_3 receptor antagonists

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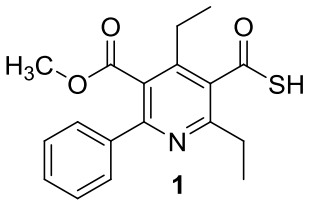
ABSTRACT

In the course of the development of the first adenosine A_3 receptor PET tracer [18F]FE@SUPPY, SUPPY:0 (4,6-diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid) (**4**) was reported as versatile synthesis precursor for SUPPY-type radiotracers. Thus, the aim of this thesis was the preparation of several SUPPY:0 derivatives, which were required as precursors for radiolabeling and as reference compounds for HPLC analysis. With few exceptions microwave-assisted synthesis was used to prepare each target compound in one to two steps starting from compound (4). SUPPY:0 was alkylated with different alkyl halides and alkyl triflates under yielding in most cases O/S - regioselective products.

In conclusion, one new compound (**6**) has been developed and nine new synthetic pathways for derivatives already known (**1-3**, **5**, **7**) were established.



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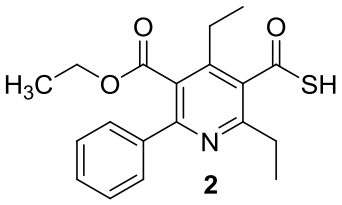

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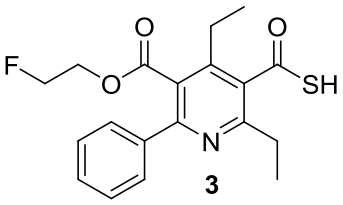

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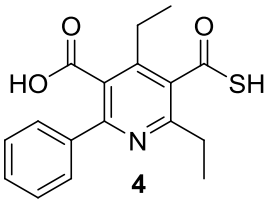

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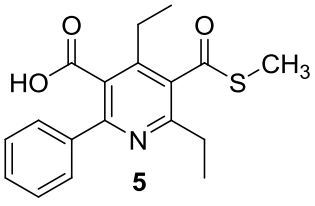

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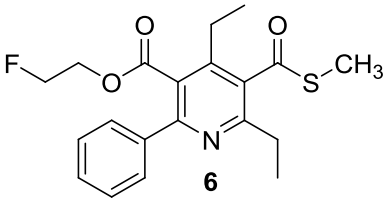

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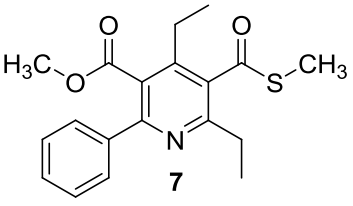

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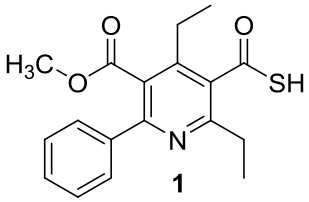
EXPOSÉ

Im Rahmen der Entwicklung des ersten Adenosin-A_3-Rezeptor-PET-Tracers, [18F]FE@SUPPY wurde SUPPY:0 (4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridin-3-carbonsäure) (**4**) als ein vielseitiger Synthesepräcursor für Radiotracer dieser Art beschrieben.

Das Ziel der vorliegenden Arbeit war daher die Herstellung mehrere SUPPY:0 Derivate, welche als Precuror für Radiosynthesen und als Referenzsubstanzen für HPLC-Analysen dienen sollen. Mit einigen Ausnahmen wurden die Zielverbindungen durch mikrowellensynthesen-assistierte organische Synthese in einem oder zwei Schritten hergestellt. Die für alle Mikrowellenversuche verwendete Ausgangsverbindung, SUPPY:0 (**4**) wurde mit verschiedenen Alkylhalogeniden und Alkyltriflaten unter Mikrowellenbedingungen alkyliert, wobei in den meisten Fällen O/S-regioselektive Produkte entstanden.

Im Rahmen dieser Arbeit wurde eine neue Verbindung (**6**) hergestellt und außerdem neun neue Synthesewege für bereits bekannte Verbindungen (**1**-**3**, **5**, **7**) erschlossen.



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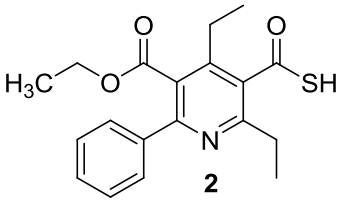

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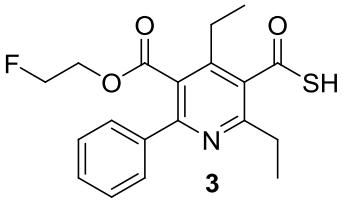

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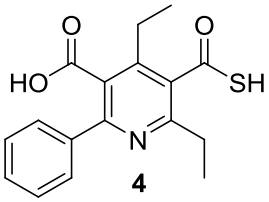

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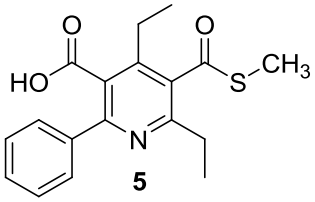

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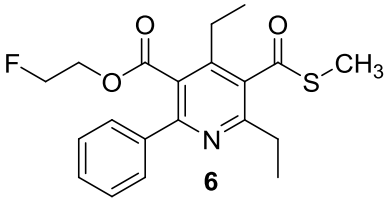

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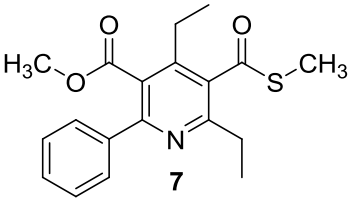

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1 INTRODUCTION

1.1 Adenosine and adenosine receptors

1.1.1 Adenosine

As a nucleoside, adenosine (fig. 1) is an important molecule for biochemical processes as well as for signal transduction. It is formed by the purine base adenine which is attached to the monosaccharide β-D-ribose by a β-N^9 - glycosidic bond.[1,2]

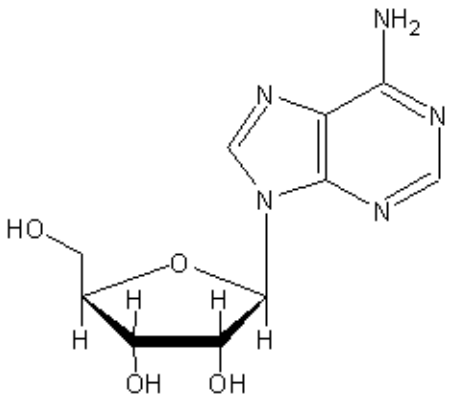


Figure 1:[3] Adenosine

Adenosine is released from metabolically stressed or active cells and binds specifically to A_1, A_2 and A_3 cell surface receptors, and acts as a regulatory molecule.[4]

It is omnipresent in body fluids and plays an important role in the energy transfer in all cells in form of adenosine triphosphate (ATP). Furthermore, adenosine has enormous effects on the cardiovascular as well as the neurological system, the gastrointestinal tract, and the immune system. By inhibiting the release of excitatory neurotransmitters like acetylcholine, dopamine and noradrenalin, adenosine causes vasodilatation and thereby reduces blood pressure.[5]

In spite of this fact, adenosine is not used clinically as a vasodilator, due to its short acting. In human blood, its half-life is only ten seconds. In addition, it can cause coronary vascular steal syndrome.[6] However, adenosine is used as an antiarrhythmic drug for the rapid treatment of supraventricular tachycardia.[6,7]

In principle, there are two metabolic pathways for adenosine in the human body. The first one is its degradation. Adenosine is metabolized in red blood cells by adenosine kinase and also, but to a smaller degree, by adenosine deaminase to adenosine monophosphate (AMP) and inosine, which again is catabolized to hypoxanthine, xanthine, and eventually to uric acid (fig. 2).[8] Its second pathway is the chemical recovery, where the nucleoside can be rephosphorylated to AMP by the adenosine kinase.[6]

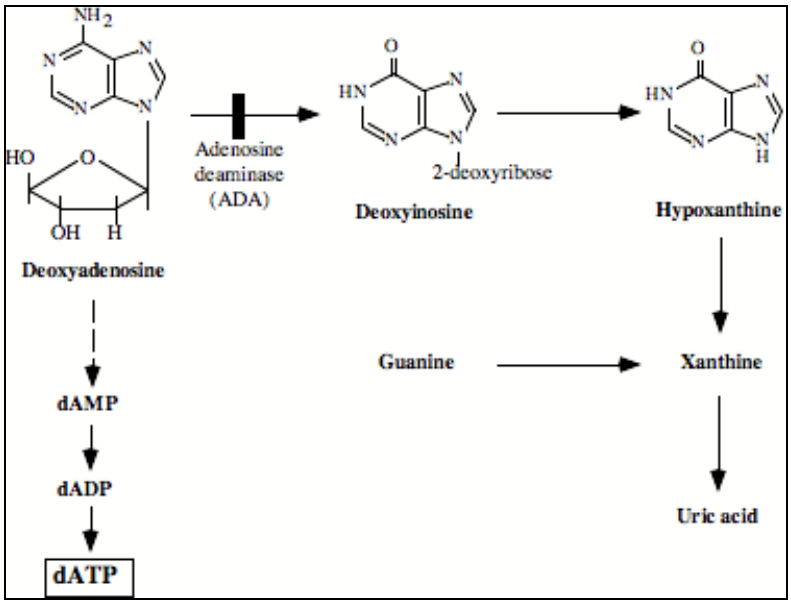


Figure 2:[9] Adenosine metabolism

Adenosine is also part of energy rich compounds such as nucleic acids (DNA, RNA), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP).[10] As mentioned above the triphosphate unit, ATP is needed for the cellular energy transfer. The stored energy of the two reactive phosphoanhydride bonds is released by hydrolysation and so available for cellular processes or chemical reactions. Thereby ATP is transformed to ADP.[11]

1.1.2 Adenosine receptors

The three different adenosine receptor subtypes are called A_1, A_2 and A_3 adenosine receptors according to the International Union of Pharmacology (NC-IUPHAR).[12] These receptor subtypes are G-protein-coupled receptors, consisting of seven transmembrane domains with common signal transduction pathways.[13] The receptors either lead to inhibition (A_1 and A_3) or to stimulation (A_2) of the enzyme adenylate cyclase[14] and differ in their pharmacological profile as well as in the tissue distribution (fig.3).[2]

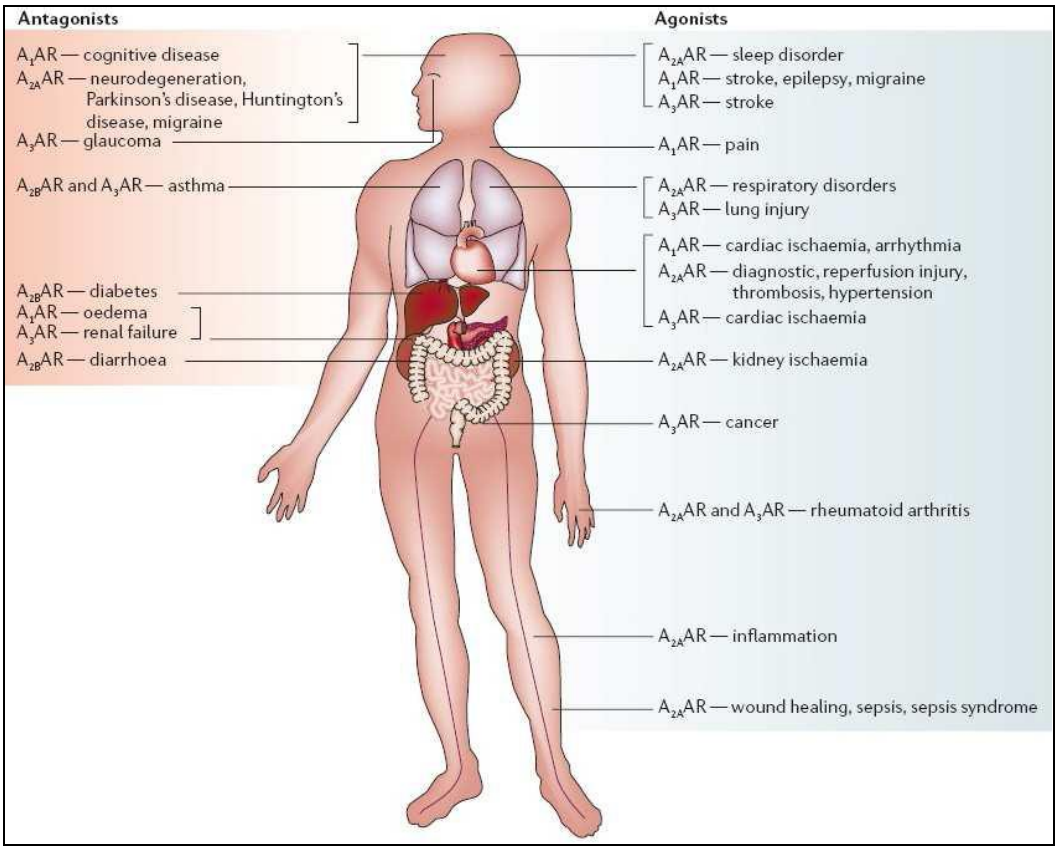


Figure 3:[14] Distribution of adenosine receptors in the human body

1.1.2.1 The adenosine A_1 receptor

High expression rates of A_1 receptors in the human body were found in the brain, heart, stomach, kidneys, liver, immune system and in blood vessels amongst others. Agonists cause negative inotropic and chronotropic effects.[15, 16]

Pharmacological data show that the A_1 receptor is important for activating the mitochondrial ATP-depending K^+-channels, which results in protection of the heart from ischemia-reperfusion injury.[12] Furthermore, A_1 receptors have neuroprotective effects on the brain. By stimulation of this receptor subtype, the release of glutamate is inhibited causing a decrease of excitatory effects.[17]

1.1.2.2 The adenosine A_2 receptor

This receptor subtype can be classified into the adenosine A_{2A} receptor and the adenosine A_{2B} receptor.

The A_{2A} receptor is expressed in a few regions of the human brain such as the striatum and the nucleus accumbens. It plays a pivotal role in neurodegeneration and migraine and could be involved in Parkinson's and Huntington's disease.[15] Furthermore, the activation of A_{2A} receptor in blood vessels causes vasodilatation and consequently decreases blood pressure.[16,18] This receptor type also occurs on immune cells, including monocytes, lymphocytes and macrophages. Thus, the activation of this receptor depending on adenosine involves anti-inflammatory effects,[19-21] which can terminate chronic inflammatory processes.

The A_{2B} receptor is widespread on almost every cell type.[22] The stimulation results in the degranulation of human mast cells and causes bronchoconstrictory effects.[23-25] Additionally, the A_{2B} receptor is located on cells of the intestinal tract where it is involved in the regulation of chloride channels.[26]

1.1.2.3 The adenosine A_3 receptor

The A_3 receptor subtypes were not only detected in high concentrations in human lung and liver cells, but also in the heart, kidneys, brain and spleen.[15] In rats adenosine A_3 receptor mRNA was located in testes, heart and brain.[27] However, the A_3 receptor subtype has been found to be involved in a variety of diseases, such as epilepsy,[28] stroke,[29] cardiac,[30] and cerebral ischemia.[31] Furthermore, the receptors play an important role in the cell cycle.[32] Activation of A_3 adenosine receptors lead to deceleration of the cell cycle[33] as well as to changes in the cytoskeleton.[34] Studies have shown that A_3 receptors are expressed on the cell

surface of many tumor cell lines and could therefore serve as target for tumor imaging and inhibition of tumor growth.[35]

In recent years, many compounds were identified as potential A_3 receptor antagonists such as flavonoids, xanthenes, thiazol pyridines and 6-phenyl-pyridines.[36] Li *et al*[37] evaluated a series of molecules, investigating their affinity and selectivity towards the adenosine A_3 receptor. Among these compounds, the fluoroethylester (2-fluoroethyl-4,6-diethyl-5-[(ethylsulfanyl)carbonyl]-2-phenylpyridine-3-carboxylate, fig. 4) exhibited promising results regarding the affinity and the selectivity towards A_3 receptors.[2]

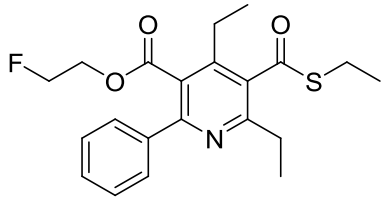


Figure 4: 2-Fluorethyl 4,6-diethyl-5-[(ethylsulfanyl)carbonyl]-2-phenylpyridine-3-carboxylate

1.2 Radioactivity

The term "radioactivity" was first coined by Marie Curie, who discovered the spontaneous disintegration of elements into smaller pieces.[38] Several atomic nuclei, also called parent nuclides, have the ability to emit ionizing particles or gamma radiation by releasing energy and turning into stable chemical elements, the daughter nuclides. These stable chemical molecules have less energy content than the parent nuclides.[39]

In general, radioactivity can be divided into two types:[40]

1) natural or spontaneous radioactivity, defined as spontaneous disintegration of an unstable atomic nucleus and the emission of particles or electromagnetic radiation and

2) artificial or induced radioactivity which is produced by bombarding naturally occurring isotopes with subatomic particles or with high levels of x-rays or gamma rays.

Radiation can be measured by decay rate, also called activity, which is defined as the number of transforming atomic nuclei of a radioactive nuclide during a certain period of time. The SI unit for radioactivity is Becquerel (Bq) according to the General Conference on Weights and Measures (CGPM). It is defined as one decay per second.[41]

1.2.1 Forms of decay

Radioactive nuclei decay in different modes depending on their composition concerning protons and neutrons. Important modes of decay are alpha decay, where two protons and two neutrons are emitted as a helium nucleus and beta decay, differentiating beta plus and beta minus decay. In this mode of decay an electron or positron, also called beta particle, is emitted. The third mode of decay is gamma decay, which is a form of high frequent electromagnetic radiation.[39, 42]

1.2.1.1 Alpha decay

Alpha decay, a corpuscular form of radiation, preferentially occurs in nuclei with a high mass number. During the decay, an alpha particle consisting of two neutrons and two protons (fig. 5), is emitted with high energy from the mother nuclide with almost 10% of speed of light.[39]

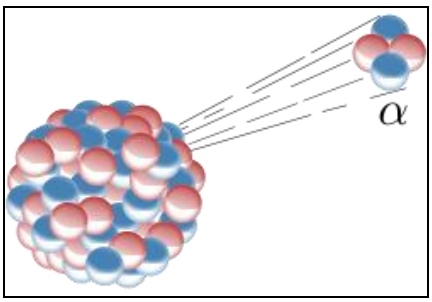


Figure 5:[43] Alpha particle consisting of two neutrons and two protons

Generally speaking, a nucleus (X) converts into a new atom (Y) and a ^{4}He nucleus, the alpha particle. The newly formed atom exhibits a mass number A minus 4 and an atomic number Z minus 2 (fig. 6).[40]

$$ {}^{A}_{Z}\mathrm{X} \rightarrow {}^{A-4}_{Z-2}\mathrm{Y} + {}^{4}_{2}\mathrm{He} $$

Figure 6[44]

Alpha particles possess a huge amount of binding energy, which promotes spontaneous alpha decay.[41] The typical kinetic energy varies between 2 and 5 MeV. However, numbers can rise even to 10 MeV, when creating such particles artificially.

Uranium, radium and thorium, so called heavy elements, undergo alpha decay, because of their energy rich structure. Nevertheless, also light elements have the ability to emit alpha rays like nuclides of tellurium, with mass numbers 100 to 106. As mentioned above, alpha particles exhibit high mass and low velocity, due to interaction with the surrounding matter, which causes only low depth of penetration. Depending on their energy, the mean free path of alpha particles under normal conditions amounts to 10 cm. Thus, a decrease of air pressure, and therefore fewer collision partners results in an increase of the mean free path. Thus, alpha radiation can easily be shielded by a sheet of paper or by creating a rather short distance to the source of radiation.

Alpha rays are relatively harmless to the human body if exposure occurs externally because of their inability to penetrate the skin. Nevertheless, the intake through inhalation, wounds, mucosa or even oral intake is considered dangerous and can cause serious damage to living cells. Due to the long half life and strong ionizing effects of alpha radiation, it is not used for diagnostic purposes.[41, 42]

1.2.1.2 Beta decay[41, 42]

Beta decay can be divided into beta plus (β^+) and beta minus (β^-)-decay.

Beta minus radiation is a corpuscular radiation consisting of electrons. Especially nuclides with high amounts of neutrons undergo this type of decay.

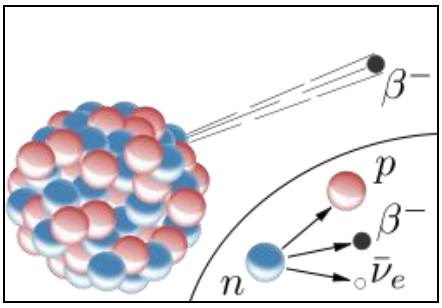


Figure 7:[43] Beta minus particle

During this mode of decay, an electron (e^-) as well as an electron antineutrino (ν_e), together called beta minus particles, are emitted, while conversion of a neutron into a proton occurs, which results in the transformation of the mother nuclide into a daughter nuclide (Y), with the mass number A and atomic number Z plus 1 (fig. 8).

$$ {}^{A}_{Z}\mathrm{X} \rightarrow {}^{A}_{Z+1}\mathrm{Y} + \mathrm{e}^- + \overline{\nu}_\mathrm{e} $$

Figure 8[44]

Beta plus radiation, which provides the basis for positron emission tomography, also belongs to corpuscular radiation. During this decay, a nuclide high in protons transforms a proton into a stable neutron, while emitting a positron (e^+) and an electron neutrino (ν_e). Thereby, the decay energy is distributed equally as kinetic energy between both, the neutrino and positron.

The newly formed atom (Y) shows an unchanged mass number A but an atomic number Z minus 1 (fig. 9).

$$ {}^{A}_{Z}\mathrm{X} \rightarrow {}^{A}_{Z-1}\mathrm{Y} + \mathrm{e}^{+} + \nu_e $$

Figure 9[44]

As antimatter positrons possess the characteristic of being instable when in contact with matter, which results in annihilation. Thereby, a positron colliding with an electron causes the conversion of their mass-energy into electromagnetic radiation. Annihilation radiation is emitted as two gamma quanta in an angle of 180 degrees with an energy of 511 keV, which is exploited in positron emission tomography (PET) (fig. 10).

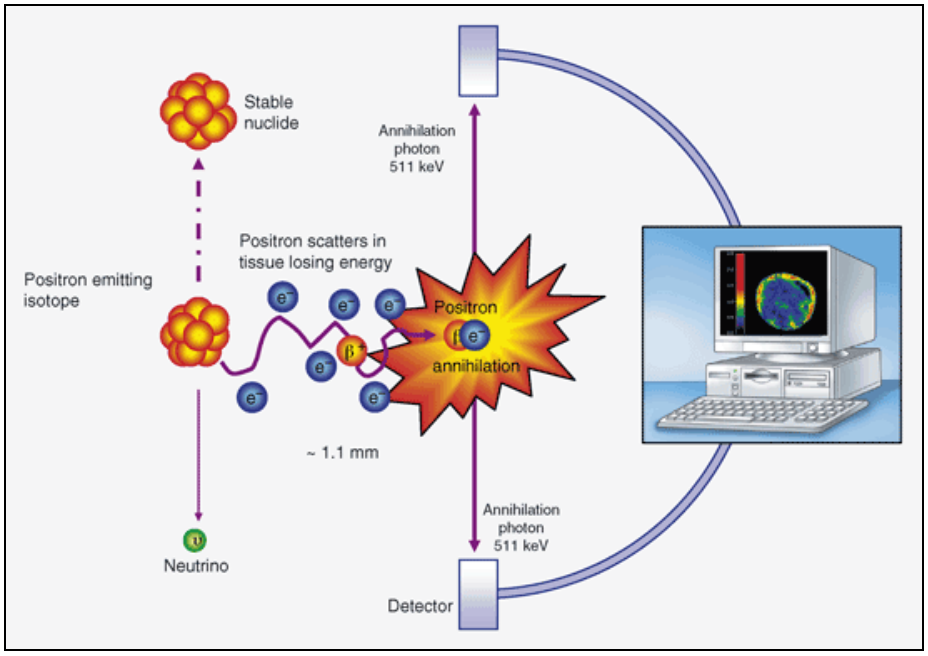


Figure 10:[45] Annihilation and detection via PET

Beta rays interact with matter to a lesser extent than alpha rays. For this reason beta radiation exhibits a longer mean free path than alpha radiation. Exposed to the human body, this type of radiation can damage several skin layers resulting in radiation burns and can cause long-term consequences, such as skin cancer. In case of intake, this ionizing radiation severely affects the surrounding tissue by causing mutation of cells However, beta radiation is exploited therapeutically for several diseases, such as colon cancer, hepatic cancer and bone cancer.[46-48]

Another field of application is medical imaging with positron emission tomography (PET), which uses beta plus emitter labeled molecules as tracer molecules.

Lower-atomic-number materials are commonly used to shield beta rays, such as aluminum or plastic materials.

1.2.1.3 Gamma decay[41, 42, 49]

Gamma radiation is a form of electromagnetic radiation of high frequency. In contrast to alpha and beta rays, gamma rays have a large depth of penetration and are referred to as indirectly ionizing radiation since having no charge, they mostly pass through matter until they interact with a particle. When such collision occurs, gamma radiation transfers energy to the particle, such as an electron. The excited electron can then directly interact with other electrons or atoms and cause ionization, which is why the process is called indirect ionization. Gamma radiation needs to be shielded appropriately with higher-atomic-number materials of high density such as lead and concrete.

Gamma decay occurs in natural and artificially produced radioactive isotopes. In case of a nuclear transformation through alpha or beta decay, the daughter nuclide remains in an excited state. Even though further emission of particles is not possible, excess energy of the nucleus is transformed into electromagnetic radiation and emitted as gamma quanta with the speed of light (fig. 11).

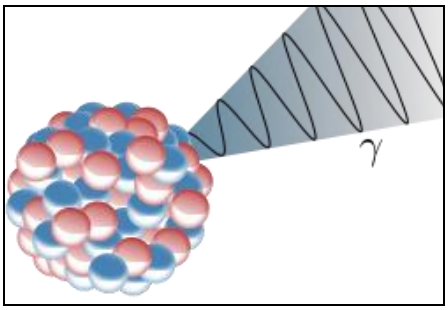


Figure 11:[43] Gamma quanta

Mass number A as well as the atomic number Z of a nuclide do not change as a result of gamma decay. It merely results in the loss of binding energy of the nucleus (fig. 12). This energy of gamma quanta varies between 0.1 and 20 MeV.

$$ {}^{A}_{Z}\mathrm{X}^{**} \rightarrow {}^{A}_{Z}\mathrm{X}^{*} + \gamma $$

Figure 12[44]

1.2.2 Background radiation

Natural radiation reaches the earth from everywhere and living organisms are persistently exposed. When life on earth began about 3.5 billion years ago, the natural level of radiation was approximately three to five times higher than today.[50] It could be possible that the high radiation was needed in former times to initiate life and it still could be needed to sustain these species, as studies with bacteria and protozoa have shown.[51]

Background radiation can be divided into cosmic and terrestrial radiation. Cosmic radiation increases with magnetic latitude and altitude and bombards the earth continually with high-energy particles, originating in outer space. Interacting with the nuclei of atmospheric constituents, these rays produce secondary reaction products and a cascade of interactions, known as radioactive nuclei like ^{3}H and ^{14}C.[52]

Terrestrial radiation can be classified as external and internal radiation. As for external radiation there is radioactive content in soil, building material or the environment.[53] This kind of radiation, originates from common elements like carbon (^{14}C), potassium (^{40}K), uranium (^{238}U) and thorium (^{232}Th) or their decay products radium and radon, which have existed since the beginning of the earth. Internal exposure arises from the intake of terrestrial radionuclides by ingestion or inhalation.[52]

The individual ratio of natural radiation for human beings is about 2.2 mSv per year. This amount varies from location to location on earth. In some regions, such as Brazil, India or Iran, the natural dose rate is much higher than in other parts of the world. Nevertheless, people, animals or plants living there have never shown malign effects despite this high radiation.[54]

1.2.3 Artificial radiation

Artificial radiation emanates exclusively from man-made production. Its principles are the very same as of natural radiation. By bombardment of atomic nuclei with either alpha or gamma rays as well as neutrons, protons or other atomic nuclei, such radionuclides can be produced. Due to their short half-life, they have to be created in-situ for each application purpose. Only long-lived radionuclides or stable nuclides can be used as parent nuclides.[41]

In 1919, the first artificial nuclear transformation was accomplished by E. Rutherford.[55] Irène Joliot-Curie, daughter of Marie Curie, first coined the term "artificial radiation" in the place. Together with her husband, Frederic Joliot-Curie she discovered artificial radioactivity and was therefore awarded the Nobel Prize in 1935.[56]

In general, there are three different ways of producing radioactive nuclides:[41]

- Neutron-rich radionuclides are produced by nuclear fission or by neutron irradiation of the parent nuclide in a nuclear reactor, whereas
- neutron-poor radionuclides are produced by using a generator or cyclotron.

1.2.3.1 Radionuclide production by nuclear reactor

Nuclear fission, also just called fission,[57] is defined as a process in which highly excited and instable nuclides of high atomic mass decay into several fragments, showing medium atomic mass while releasing energy.[41]

Before producing radionuclides in nuclear reactors, nuclear fission (spontaneous fission) was detected in uranium by Flerov and Petrzhak.[58]

Nuclear fission is usually carried out in a nuclear reactor. It can be divided into distinct stages. The first stage consists of a division of the excited, original nucleus through a neutron flux with no loss of energy or particles. These primary fission fragments emit energy in form of photons, neutrons and sometimes alpha particles. The final process in nuclear fission is the beta decay, accompanied by photon emission which results in stable nuclei (fig. 13).[59]

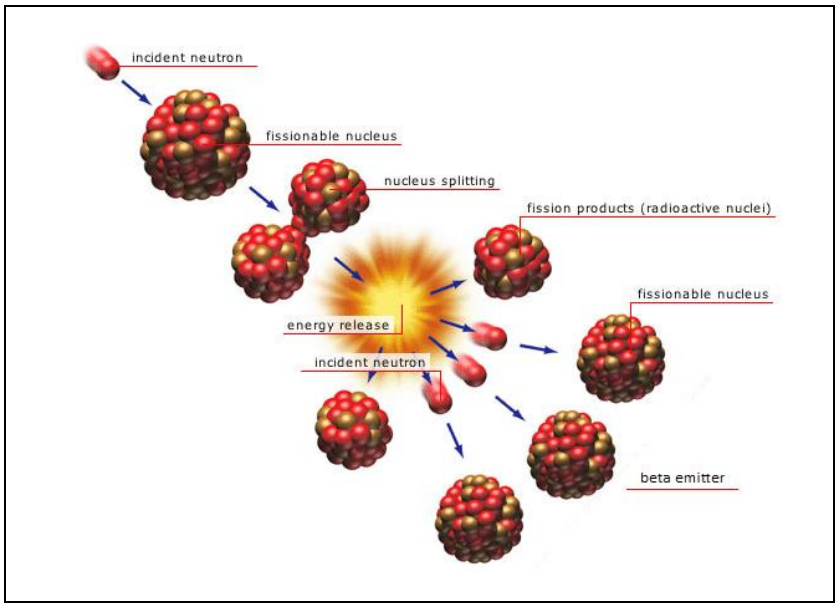


Figure 13:[60] Nuclear fission (modified)

Such nuclei are ^{3}H ($t_{1/2}$ = 12.3 a), ^{14}C ($t_{1/2}$ = 5730 a), ^{33}P ($t_{1/2}$ = 25.3 d), ^{35}S ($t_{1/2}$ = 87.5 d), and ^{125}I ($t_{1/2}$ = 59.4 d). They are mostly used for scientific and medical purposes.[41]

1.2.3.2 Radionuclide production by radionuclide generator

In general, this system consists of a column containing absorbent material, such as aluminum, an inlet and an outlet. It is shielded against radioactive radiation by lead (fig. 14).

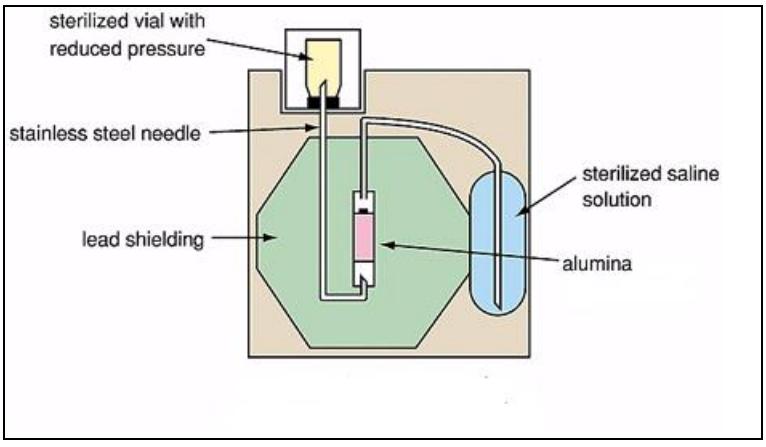


Figure 14:[61] Radionuclide generator (modified)

Long-lived parent nuclides, such as ^{99}Mo, can be absorbed onto the supporting material. The decay of the nuclide results in the formation of the daughter nuclide. In case of ^{99}Mo, ^{99m}Tc is

formed, which can be obtained from the generator column by eluting with a washing solution.[62] Especially ^{99m}Tc is a commonly used nuclide since its application possibilities in single photon emission computed tomography (SPECT) are widespread.[55]

1.2.3.3 Radionuclide production by cyclotron

In 1929, E. Lawrence concluded that raising the energy of ions in small steps using low voltages would be the simplest way of accelerating ions to high enough energies to induce nuclear reactions. He was the first one, who elaborated a concept for a cyclotron by using a magnetic field to bend the ion's paths into circular arcs. Thereby, they could be repeatedly accelerated by the same electrodes as well as excited by an alternating voltage resulting in an energy gain at each gap crossing. Based on these facts, four different accelerator types have been developed (chronological order):[63]

- **Synchrocyclotrons** with energy rates of 1 GeV allowing artificial production of pions and muons.
- **Synchrotrons**, where a magnetic field is added.
- **Isochronous cyclotrons**, where the magnet is split into several sectors to create extra focusing forces.
- **Fixed-field alternating-gradient** (FFAG) **accelerators** with split magnets and modulated frequency.

Today's cyclotrons mainly consist of an electromagnet around a flat vacuum chamber between two dipoles. They can produce high-energy ion beams without the problems associated with high voltages, resulting in artificially produced isotopes (fig. 15). This concept is based on the early experiments of N. Edlefsen and his follower M. Stanley Livingston, both graduate students of E. Lawrence.[63]

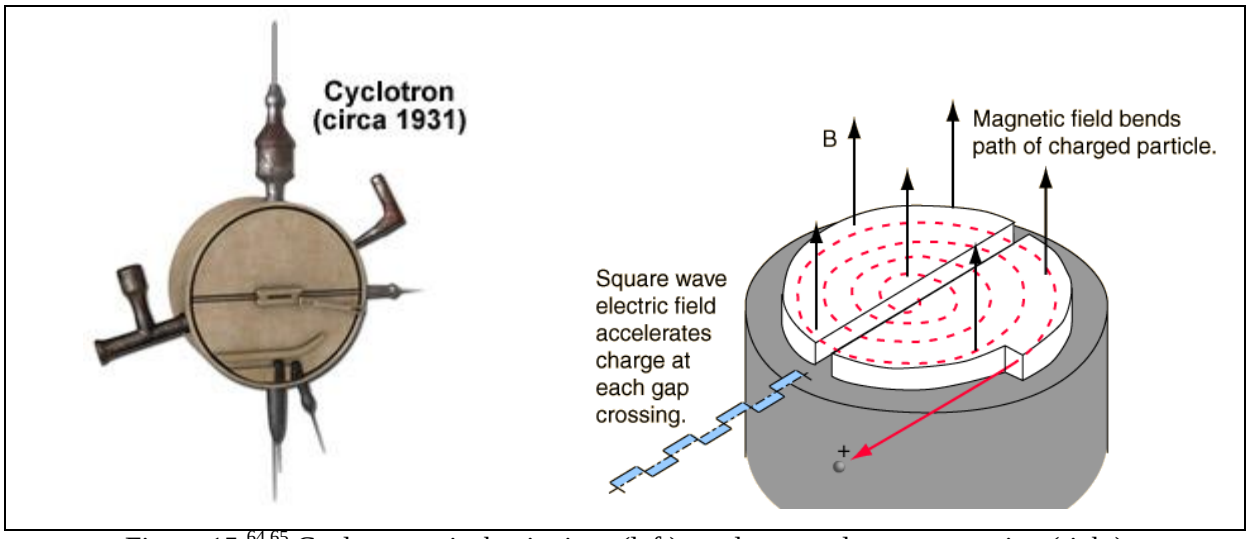


Figure 15:[64,65] Cyclotron at its beginnings (left), cyclotron today, cross section (right)

New isotopes were formed and soon used in studies of biological and chemical processes. Also medicine profited from the discovery of these isotopes, such as ^{14}C, ^{24}Na, ^{32}P, ^{59}Fe and ^{131}I. Especially ^{32}P was proven beneficial for diseases like leukemia and polycythemia.[63]

Today, cyclotron-produced beta minus emitters, ^{67}Cu ($t_{1/2}$ = 2.6 d) and ^{186}Re ($t_{1/2}$ = 3.7 d), are used for radiosyntheses of tumor affine substances, while alpha emitter, ^{211}At ($t_{1/2}$ = 7.2 h) and ^{255}Ac ($t_{1/2}$ = 10.0 d), mainly find their use in the treatment of thyroid carcinoma.[55]

1.3 Nuclear medicine and radiopharmaceuticals

Since radioactivity was first discovered at the end of the 19th century, the history of nuclear medicine has been rather short. In the 1940s, the first nuclear medical examinations of the thyroid were carried out, and it was not until 1985 that the first clinical application of a positron emitting nuclide was described.[66] When significant improvement in the instrumentation was made, positron emission tomography (PET) became important as a diagnostic tool. Investigating potential radiotracers for PET, Ido *et al*[67] developed [^{18}F]2-fluoro-2-deoxy-D-glucose, which in fact was the first established radionuclide. Hamacher and Coenen improved the synthesis of this compound, which led to continued success of [^{18}F]FDG.[68] In the following years, further studies concerning PET and PET-tracers were performed in order to develop more selective and specific tracers. Thus, more information and better knowledge about neurological, cardiological and oncological diseases can be obtained today.[69]

1.4 Positron emission tomography (PET)

Positron emission tomography, is finding its use in nuclear medicine and is an important non-invasive, scientific and clinical tool for probing biochemical processes in the human body. This powerful imaging technique is mainly used for quantitative measurement such as metabolic levels and biological processes *in vivo*.[70] Due to its sensitivity and versatility, it has become an important tool in clinical research as well as in routine diagnostics.

In general, PET consists of two merged techniques, both awarded with the Nobel Prize. The first one is the tracer principle, which was invented by de Hevesy in 1943, and the second technique is the tomographic principle, devised by Houndsfield and Cormack in 1979.[71]

1.4.1 Tracer principle

Radionuclides used for PET are pure positron emitters and mostly short-lived. Such nuclides are ^{11}C ($t_{1/2}$ = 20 min), ^{18}F ($t_{1/2}$ = 110 min), ^{13}N ($t_{1/2}$ = 10 min) and ^{15}O ($t_{1/2}$ = 2 min). The advantage of the nuclides above is that they can be introduced directly into biomolecules. Time is a critical parameter in the production of radiotracers. These short-lived radioisotopes need to be produced and used for imaging within a time compatible with the half-life of the PET nuclides.[70] However, also long-lived nuclides, such as ^{129}I, are getting more popular for the use in PET due to the resulting extension of the physiological time window of PET.[71]

Producing radiotracers for PET requires at the preparation of appropriate nuclides, which are produced in a cyclotron. This method affords the labeling nuclide in an unreactive form, which has to be chemically activated (e. g. reduction of $^{11}CO_2$ to methyl iodide).[2, 70]

A variety of synthetic methods and techniques are available for the labeling of biomolecules with a positron emitter, such as standard procedures of labeling carboxylic acids with [^{18}F]bromofluoroethan.[72, 73] Fast and effective PET radiochemistry is necessary for the production of specifically positron-emitter-labeled tracer molecules with high specific activity. On the one hand, the timeframe, as explained above, plays a crucial role, that is why the synthesis of a radiotracer should be accomplished within three half lives. On the other hand, the use of very small amounts of reactants in radiosynthesis significantly increases radiochemical yields.[74]

For a PET scan only nanomolar amounts of a radiotracer are required to be injected in the human body averting any pharmacological effects of the radiotracer. Kinetic modeling and mathematical characterization of the data acquired in a PET scan allows the measurement of several parameters like enzyme activity, biosynthesis rate and receptor density. An important prerequisite for optimal binding of radioligands to their targets is the fast penetration of the blood-brain barrier. Furthermore, polar properties of the tracer are required for a fast clearance from the brain.[74]

1.4.2 Tomographic principle

When emitted positrons lose kinetic energy through collision with electrons in surrounding tissue, so called short-lived positroniums are created. By annihilation, their mass is converted into energy, which is emitted in form of two 511 keV gamma photons in an angle of 180 degrees.[75] The annihilation radiation can be detected with coincidence detection, a rather novel technique in nuclear medicine.[76] Thereby, scintillation detectors, containing bismuth germanate (BGO) or lutetium oxyorthosilicate (LSO) crystals, and photomultiplier tubes, are placed opposite to the source of positron emitters. By measuring a coincidence event the location of the annihilation event can be calculated along a line joining these two detectors (called line of response or LOR).

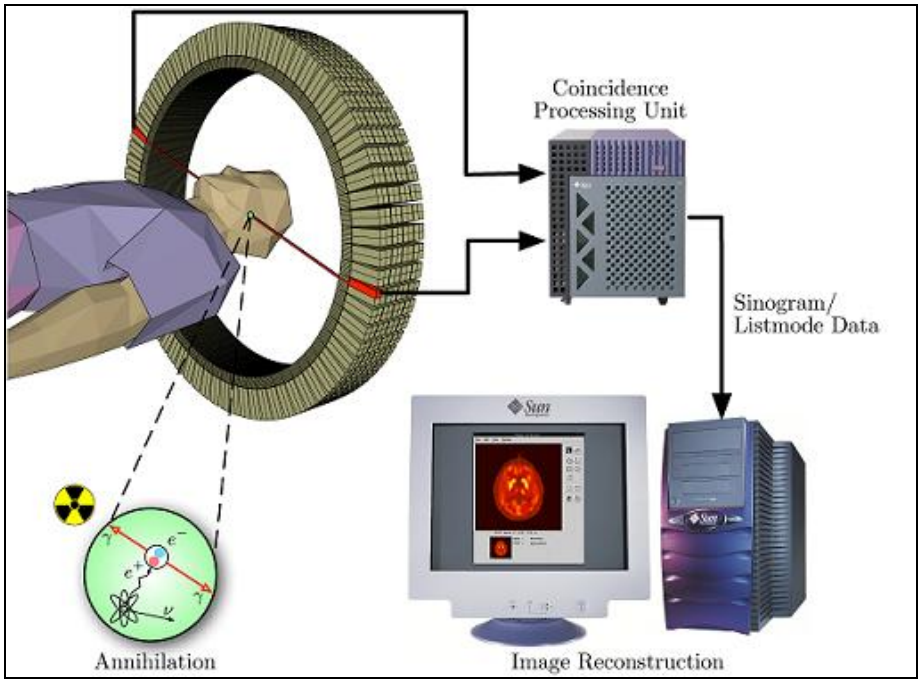


Figure 16:[77] Positron emission tomography - data evaluation

Each pair of parallel and opposite detectors is able to produce a coincidence line. Thus the position of an annihilation event can be localized. This line is unique in terms of location and direction. Many coincidence lines form a data set also called sinogram. These data however need to be corrected for tissue alternations and detector non-uniformity. Thereafter, the sinogram data can be used to reconstruct the image by the use of iterative technique or filtered back projection (fig. 16). Today a patient, subjected to a PET-scan, is surrounded by hundreds of detectors in form of a ring. Coincidence events are detected and yield information about the concentration and distribution of the positron emitter within the patient.[75]

1.4.3 2-[^{18}F]-fluoro-2-deoxy-D-glucose

2-[^{18}F]-fluoro-2-deoxy-D-glucose (fig. 17) is not only a widespread PET tracer, but also one of the earliest PET tracer molecules.[66] It is a D-glucose analog and is converted in cells into [^{18}F]FDG-6-phosphate ([^{18}F]FDG-6-PO_4) by the enzyme hexokinase.

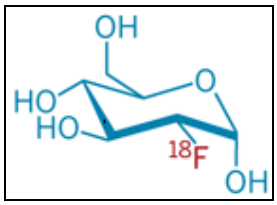


Figure 17:[78] Fluorine-18 labeled D-glucose

By being metabolically trapped, [^{18}F]FDG-6-PO_4 accumulates in tissues, since [^{18}F]FDG is lacking the 2'-hydroxyl group further metabolization is not possible. Its accumulation rate is proportional to the phosphorylation rate of exogenous glucose as well as the D-glucose utilization of the tissue. [^{18}F]FDG accumulates mainly in the brain and the heart of the human body, because these tissues have a high glucose turnover rate Due to increased glycolysis [^{18}F]FDG also accumulates in malign tissue. With its half life of only 110 min, fluorine-18 labeled D-glucose is especially useful in positron emission tomography, due to the short exposure to radiation of the human body.[79]

1.4.4 Development of new PET radiotracers

For the development of new PET pharmaceuticals several aspects must be, considered:[80]

- choice of labeling position in the tracer molecule
- stereochemical properties
- choice of the cyclotron produced primary precursors and online production of the secondary precursors
- chemical properties concerning mode of the precursor molecule and choice of solvent
- choice of solvent for final formulation
- expected clinical result for the nuclear medicinal outcome

Synthesis of all radiotracers in PET first starts with small cyclotron-produced precursors. The number of chemical modes of the commonly used tracer nuclides (^{11}C, ^{18}F, ^{13}N and ^{15}O) is limited due to extreme conditions in the cyclotron. By the end of a radiation process, stable and rather unreactive molecules are formed, which is related to thermodynamic determination of the chemical mode through high energy. ^{11}C and ^{18}F as well as ^{13}N and ^{15}O can be developed by bombardment with protons, deuterons, ^{3}H or ^{4}H causing a multitude of nuclear reactions. Nevertheless, just one or two of the above reactions are applied to every isotope. Mostly, protons are used as projectiles because of their effortless availability and their low price.[70]

Another important characteristic of radiotracers is their specific activity, meaning the ratio of radiolabeled molecules to the total number of molecules. The major aim of PET is the analysis of physiological processes without interference. Thus, the lower the amount of radiotracers relative to native molecules caused by high specific activity, the less interference and disturbance of analyzed processes. In measuring receptor density and enzyme concentration, this knowledge is of great significance.[81]

1.4.5 Application field of PET

Positron emission tomography is mainly used in the neurological and cardiological field. Neurological diseases like Morbus Alzheimer, Parkinson, epilepsy or Chorea Huntington can be diagnosed and analyzed. In cardiology, PET is useful for studying atherosclerosis and related vascular diseases.[82]

Today, about 70% of clinical PET use affects oncologic diseases.[83] Due to high glycolytic rate, [^{18}F] FDG-6-PO_4 is trapped in cancer cells and can be monitored easily via positron emission tomography. Lymphoma, melanoma and breast cancer are just a few forms of cancer, which can be detected. By measuring the metabolic activity a differentiation between malignant and normal tissue can be made.[82]

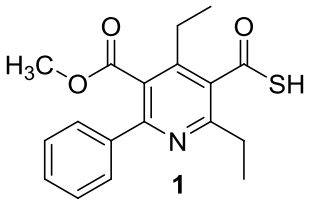
Furthermore, monitoring the response to treatment and establishing prognostic information are advantages of PET today.[83] Amongst other techniques in nuclear medicine, PET became an important tool of modern molecular imaging. Thus three - dimensional graphical representation of regional and timely distribution of radiopharmaceuticals in the human body became possible. In the future, positron emission tomography will especially focus on brain research and still on cancer. Faster reacting detector crystals with higher density and more light output are in development in order to achieve better results. Hence the use of PET-CT-scanner combination gains popularity due to the advantage of combining the functional data of a PET scan with the anatomical accuracy of a CT scan.[71]

2 RESULTS AND DISCUSSION

2.1 Aim

The adenosine A_3 receptor subtype (A_3AR) is involved in a variety of pathologies, especially neurological and psychiatric diseases (cerebral ischemia, glaucoma, stroke, and epilepsy). Thus, Wadsak *et al* recently reported on the preparation and evaluation of [^{18}F]FE@SUPPY (2-**F**luor**e**thyl-4,5-diethyl-5-[(ethyl**su**lfanyl)carbonyl]-2-**p**henyl**py**ridin-3-carboxylat),[84] the first PET radiotracer for the adenosine A_3 receptor and on SUPPY:0 (4,6-diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid)[85] a versatile precursor for a variety of metabolites and derivatives of SUPPY-type PET radiotracers.

Hence, the aim of this thesis was the preparation of several SUPPY:0 derivatives. One new compound (**6**) has been developed and nine new synthetic pathways for already known derivatives (**1**-**3**, **5**, **7**) were established.



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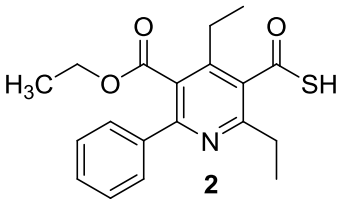

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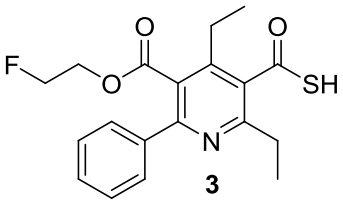

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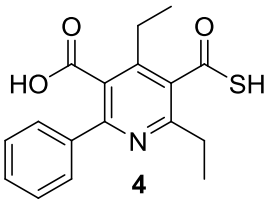

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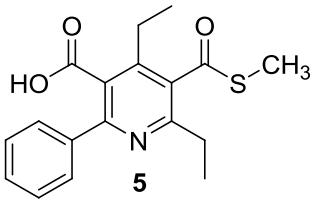

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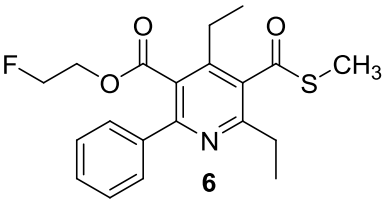

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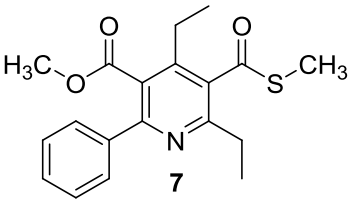


7

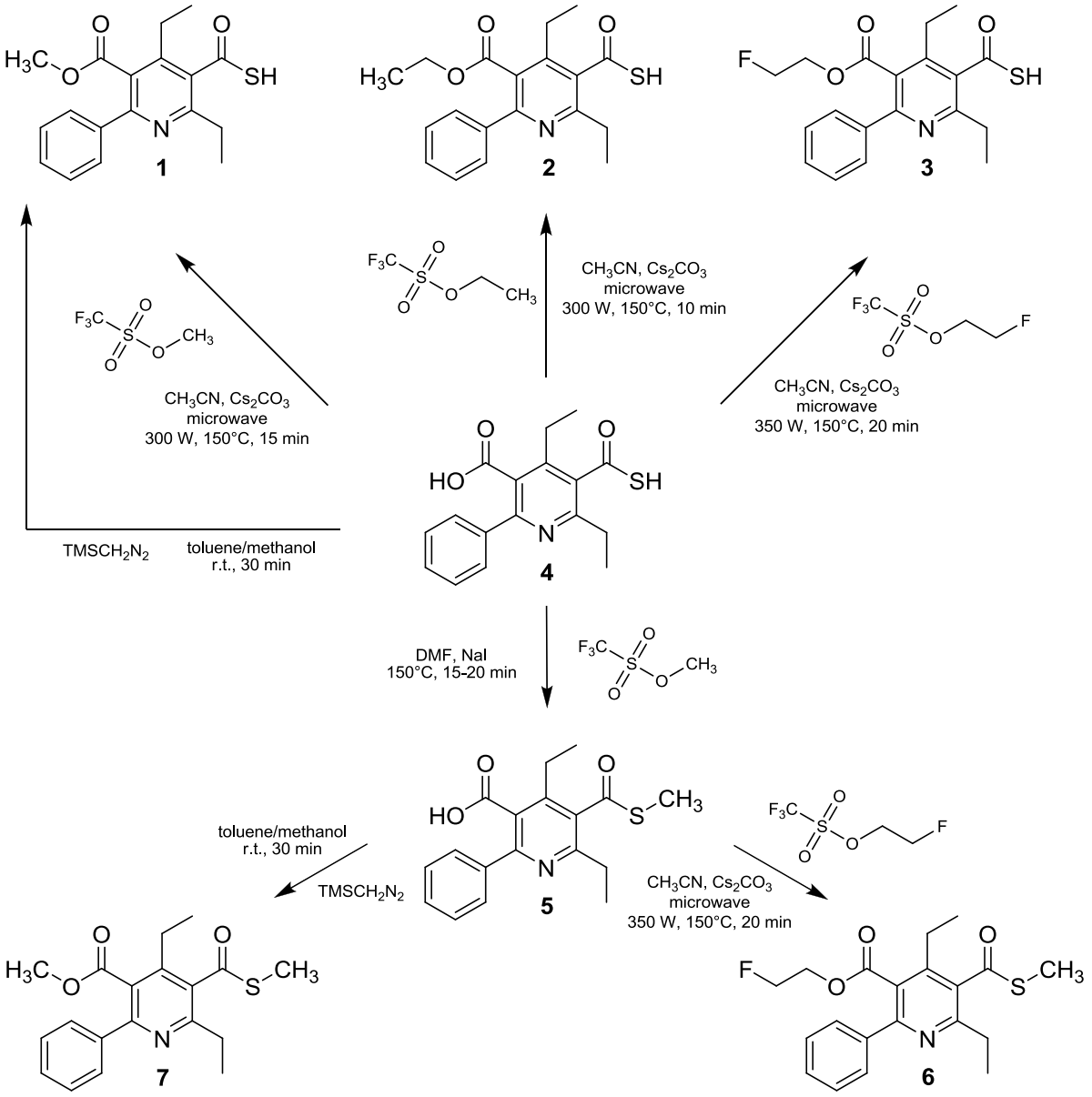
With a few exceptions microwave-assisted synthetic techniques were used, which allowed the synthesis of each target compound in one to two steps starting from SUPPY:0 (scheme 1).

SUPPY:0 was alkylated with different alkyl halides and alkyl triflates under microwave conditions yielding in most cases O/S - regioselective products.

In conclusion, a straightforward synthetic method was established to compounds required as precursors for radiolabeling and as reference compounds for HPLC analysis of labeled SUPPY-type PET tracers.

2.2 Reaction schemes:

Scheme 1:



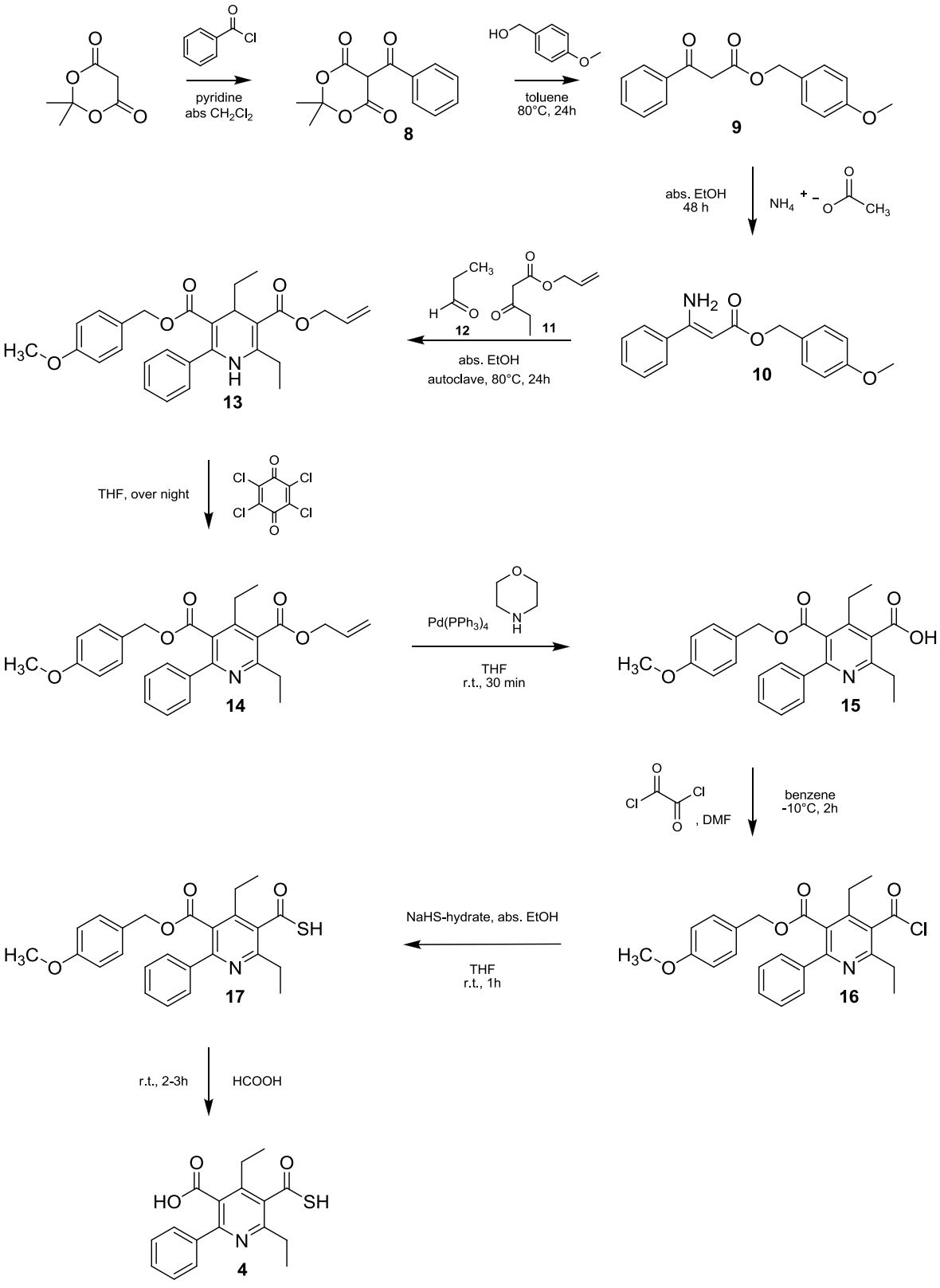
2.3 Syntheses

2.3.1 Preparation of 4,6-diethyl-2-phenyl-5-thiocarboxy-pyridine-3-carboxylic acid, SUPPY:0 (4)

This compound was prepared according to Shanab[2] deviating in a few synthetic improvements, regarding reaction solvents and the eluting mixtures for purification, to increase yields (scheme 2).

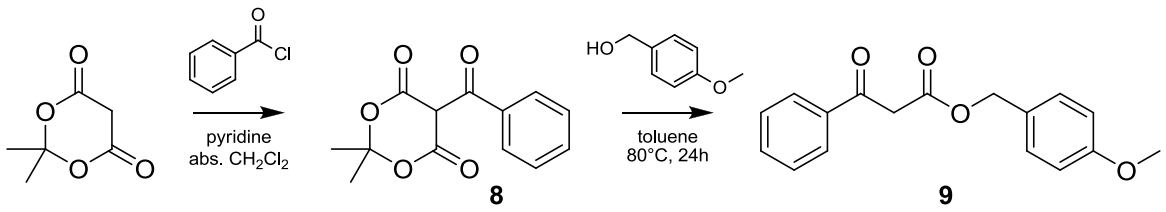
Furthermore, 4,6-diethyl-2-phenyl-5-thiocarboxy-pyridine-3-carboxylic acid (SUPPY:0) (**4**) was used as starting material for four compounds (**1-3**, **5**). While mono-substituted products (**1-3**, **5**) can serve as precursor for further radiosynthetic experiments, bis-substituted compounds (**6**, **7**) serve as references for HPLC analyses.

Scheme 2:


pyridine
abs CH_2Cl_2
8
toluene
80°C, 24h
9
abs. EtOH
48 h
10
12
11
abs. EtOH
autoclave, 80°C, 24h
13
THF, over night
14
$Pd(PPh_3)_4$
THF
r.t., 30 min
15
, DMF
benzene
-10°C, 2h
16
NaHS-hydrate, abs. EtOH
THF
r.t., 1h
17
r.t., 2-3h
HCOOH
4

2.3.1.1 4-Methoxybenzyl 3-oxo-3-phenylpropanoate (9)

In order to obtain the pyridine derivative SUPPY:0, the preparation of β-ketoester (**9**) was required. This procedure was carried out in two reaction steps (scheme 3).

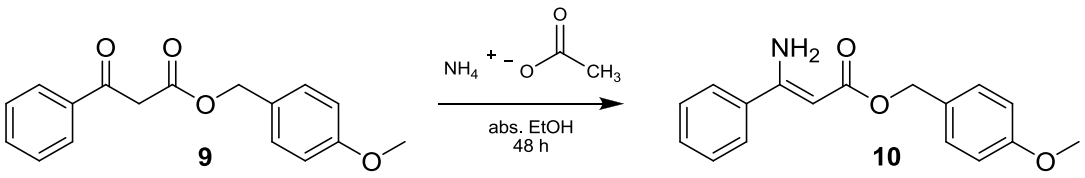


Scheme 3

For the first reaction step 2,2-dimethyl-1,3-dioxane-4,6-dione was reacted with benzoyl chloride and pyridine in absolute dichloromethane under argon atmosphere. By acylation of meldrum's acid, 5-benzoyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**8**) was prepared in good yields.

The second reaction step was necessary to afford β-ketoester (**9**) by treatment *p*-methoxybenzyl alcohol in toluene. Ring opening occurred under decarboxylation to give compound **3**. The crude product was purified several times by column chromatography using a mixture of petrolether and ethyl acetate as eluent. Compared to Shanab[2], who used petrolether/ethyl acetate 1:1, the ratio of petrolether to ethyl acetate was 8:2, which led to improved separation.

2.3.1.2 4-Methoxybenzyl 3-amino-3-phenylacrylate (10)



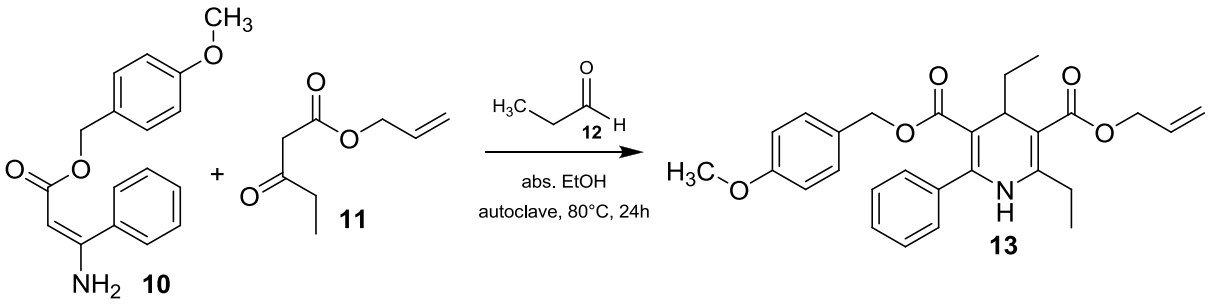
Scheme 4

The required enamine (**10**) was obtained by adding ammoniumacetate to a mixture of 4-methoxybenzyl 3-oxo-3-phenylpropanoate (**9**) and tetraethyl orthosiliate in absolute ethanol. This mixture was stirred for 48 h under argon atmosphere to afford the product (**10**) in

excellent yields (scheme 4). The completion of the reaction was monitored by thin layer chromatography. After removal of the solvent under reduced pressure the brownish oily residue was purified by column chromatography.

2.3.1.3 3-Allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenyl-1,4-dihydropyridine-3,5 dicarboxylate (13)

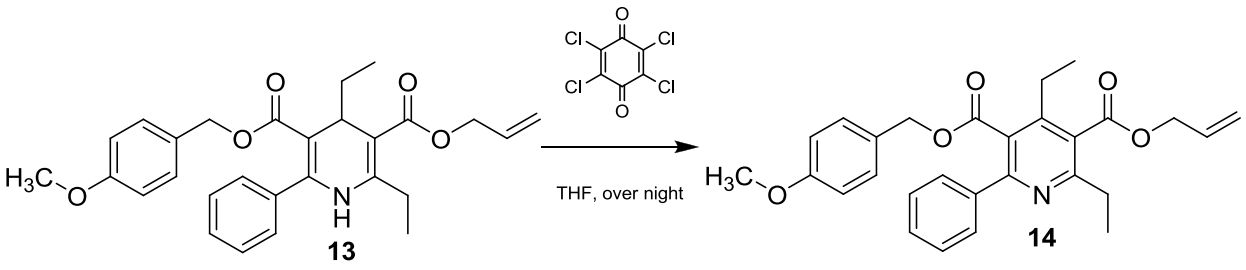
The product obtained from the previous reaction step (**10**) was subjected to the following reaction to give the desired 1,4-dihydropyridine (**13**) (scheme 5).



Scheme 5

Enamine (**10**), the β-ketoester (**11**) and propionaldehyde (**12**) were heated in an ethanolic solution in an autoclave at 80°C for 24 h to form the desired product (**13**) in a Hantzsch condensation. The following thin layer chromatography showed typical fluorescence of the product (**13**) at 366 nm. Column chromatography afforded the pure final product as yellow-greenish oil.

2.3.1.4 3-Allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate (14)



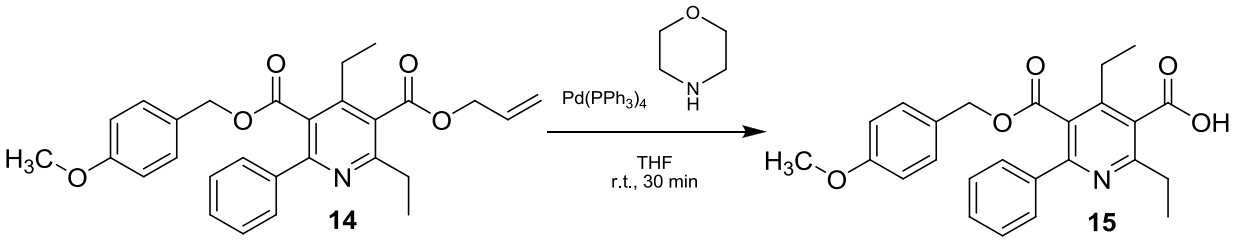
Scheme 6

Oxidation of dihydropyridine (**13**) to pyridine (**14**) was carried out with 2,3,5,6-tetrachlorobenzo-1,4-quinone in tetrahydrofuran (scheme 6). The mixture was heated to reflux overnight to assure completion of the reaction which was detected by thin layer chromatography. In comparison to the starting material the product (**14**) did not show fluorescence at 366 nm. The crude product was purified by column chromatography to give a colorless-reddish oil.

2.3.1.5 2,4-Diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carboxylic acid (15)

The subsequent step allowed selective cleavage of the allyl protecting group by using a tetrakis(triphenylphosphine)palladium catalyst and morpholine as nucleophile (scheme 7).

Resonance stabilization of the carboxylate anion activates the allyl ester for nucleophilic attack by the palladium catalyst, which results in the formation of a π-allyl-palladium complex. The nucleophile (e.g. morpholine, pyrrolidine, or dimedone) traps this very complex to liberate the carboxylic acid.

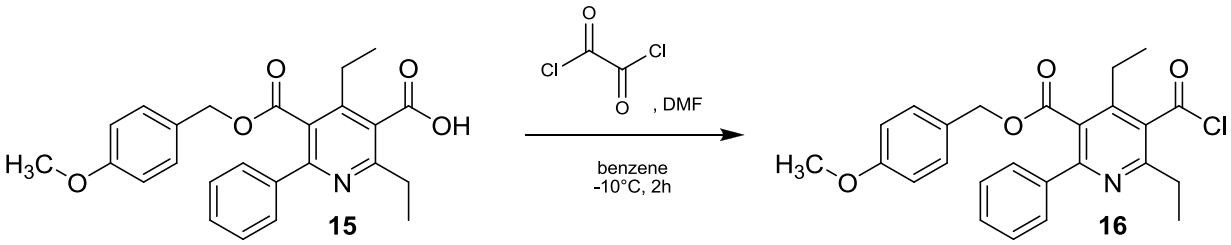


Scheme 7

The reaction was carried out under argon atmosphere. Carboxylic acid (**15**) was obtained by treating the allyl ester (**14**) with the reagents depicted in scheme 7. The obtained product required extensive purification. The catalyst was removed by suction and the crude product was purified by silica gel column chromatography. Due to remaining impurities purification was repeated several times until the product (**15**) was obtained as light orange crystals.

2.3.1.6 4-Methoxybenzyl 5-(chlorocarbonyl)-4,6-diethyl-2-phenylpyridine-3-carboxylate (16)

The next step consisted of the conversion of the carboxylic acid (**15**) into a thiocarboxylic acid via acid chloride (**16**) by treatment with oxalylchloride and dimethylformamide as catalyst in benzene (scheme 8).

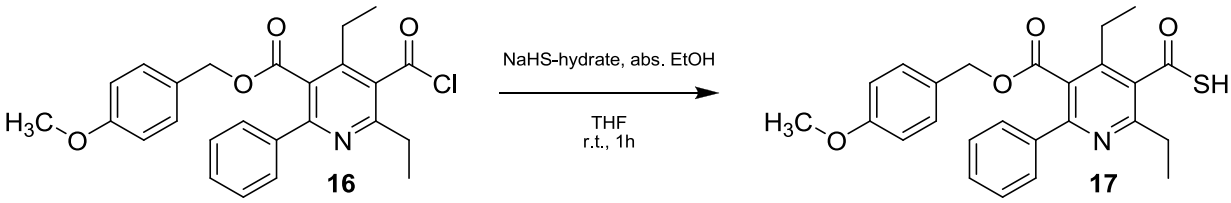


Scheme 8

In order to increase yields, reaction mixture was cooled to a temperature of 0°C during addition of the reagents. Then the solution was stirred at room temperature for 2 hours. After the reaction had reached completion, the solvent was removed under reduced pressure and to remove residues of benzene the crude product was co-evaporated several times with toluene. Acid chloride (**16**) was directly used for the next reaction step (2.3.1.7) without further purification.

2.3.1.7 2,4-Diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carbothioic *S*-acid (17)

In order to maximize yields 4-methoxybenzyl 5-(chlorocarbonyl)-4,6-diethyl-2-phenylpyridine-3-carboxylate (**16**) was dried under vacuum prior to the subsequent reaction (scheme 9).



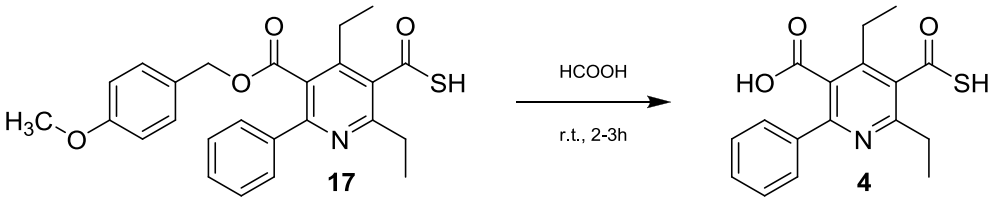
Scheme 9

Before the addition of the reagents the reaction mixture was cooled to -10°C. Sodium hydrosulfide serves as SH^- ion donor which is substituting the chlorine atom by nucleophilic attack following a S_N2t mechanism.

Cooling was stopped to allow warming of the reaction mixture to room temperature. Stirring was continued for one additional hour to obtain **17** in moderate yields. The purification of thiocarboxylic acid (**17**) was rather tedious due to formation of by-products. At this point of the synthesis reversed phase chromatography was utilized for purification to separate by-products efficiently. Purification was repeated several times to meet purity requirements for NMR spectroscopy.

2.3.1.8 4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (4)

The final step of the SUPPY:0 synthesis required cleavage of the p-methoxybenzyl protecting group



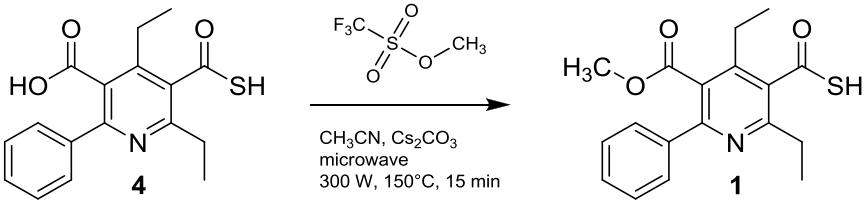
Scheme 10

The cleavage was performed by treating (**17)** with formic acid at room temperature (scheme 10). After stirring for 3 hours, the formic acid was evaporated. The crude product was purified by reversed phase column chromatography (acetonitrile/water) several times to give the crude product as white crystals in moderate yield.

2.3.2 Preparation of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (1)

Preparation of product **1** was realized in two different pathways:

2.3.2.1 Microwave assisted synthesis

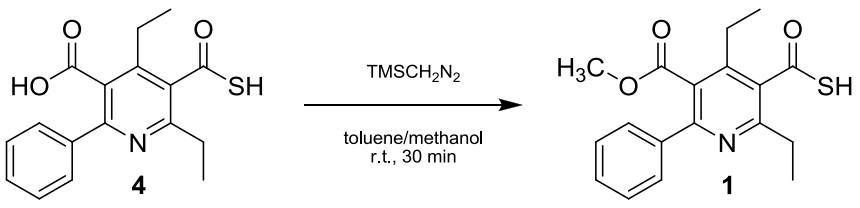


Scheme 11

For this pathway, the starting material, SUPPY:0 (**4**), was treated with methyl trifluoromethanesulfonate and caesium carbonate (scheme 11), which served as an ionic additive to improve the microwave absorbtion of the solvent on the one hand and as a base on the other hand. Acting as a base, caesium carbonate was used to deprotonize carboxylic acid **4** to activate it for the nucleophilic attack of methyl trifluoromethanesulfonate. This reaction afforded white crystals in moderate yields.

2.3.2.2 Preparation with diazomethane

The second pathway was carried out with diazomethane (scheme 12). In comparison to the microwave assisted approach, this method provided better yields.

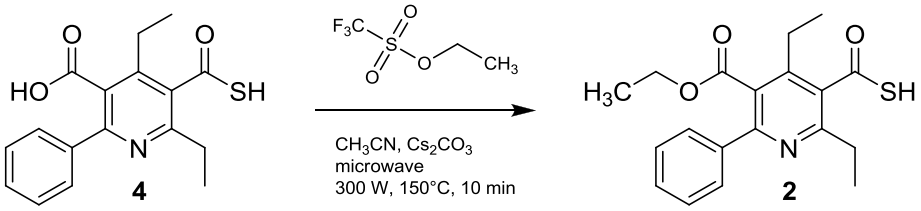


Scheme 12

For the preparation of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (**1**), SUPPY:0 was treated with trimethylsilyl diazomethane in a mixture of toluene and

methanol as solvent. This solution was stirred at room temperature for 30 min. Finally, white crystals were obtained in good yield.

2.3.3 Preparation of 5-(ethoxycarbonyl)-2,4-diethyl-6-phenylpyridine-3-carbothioic *S*-acid (2)

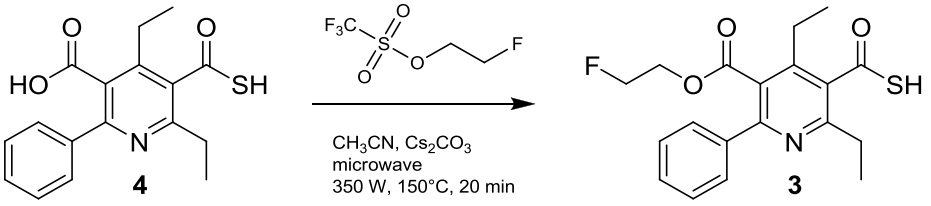


Scheme 13

The synthesis of derivative **2** was achieved by treating SUPPY:0 (**4**) with the appropriate triflate under microwave conditions which allow rapid heating of the reactants up to 150 °C in low boiling solvents (acetonitrile) (scheme 13). Cesium ions (caesium carbonate) were added to improve the absorption and conversion of the microwave energy into heat by an ionic conduction mechanism. Under the investigated conditions the alkylation always occurred on the carboxylic acid and not on the thiocarboxylic moiety. Compound **2** was obtained in good yield prior to several purification steps.

2.3.4 Preparation of 2,4-diethyl-5-[(2-fluoroethoxy)carbonyl]-6-phenylpyridine-3-carbothioic *S*-acid (3)

For the synthesis of compound **3** 2-fluoroethyl trifluoromethanesulfonate was prepared following a procedure of Chi *et al.*[86] In a microwave assisted synthesis 2,4-diethyl-5-[(2-fluoroethoxy)carbonyl]-6-phenylpyridine-3-carbothioic *S*-acid (**3**) was obtained by treating the starting material (**4**) with the depicted reagent (scheme 14).



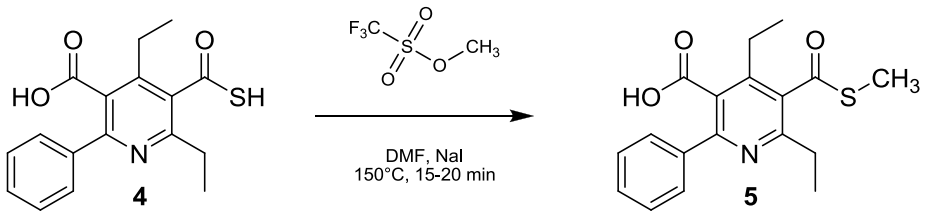
Scheme 14

Preparing compound **3** under microwave conditions had the advantage of the reaction mixture heating up quickly to 150°C in low boiling solvents, such as acetonitrile. Consequently, the use of high boiling solvents such as dimethylformamide and dimethyl sulfoxide could be avoided, which are usually difficult to remove.

As mentioned above (2.3.2 and 2.3.3), cesium ions were added to improve the absorption and the conversion of microwave energy into heat.

2.3.5 Preparation of 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (5)

Product **5** was synthesized using the conventional heating method with SUPPY:0 (**4**) as starting material (scheme 15).



Scheme 15

After addition of methyl trifluoromethanesulfonate and heating to 150°C the solution turned yellow. Thin layer chromatography and a reversed phase thin layer chromatography were performed to monitor the progress of the reaction. The latter showed that the addition of more methyl trifluoromethanesulfonate was required. After stirring another 15-20 min the solvent was removed by Kugelrohr distillation.

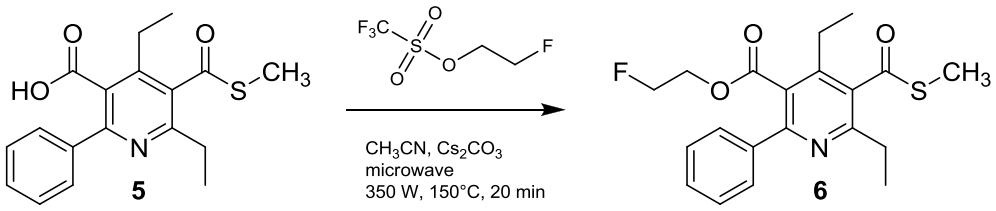
Due to formation of many by-products compound **5** was purified several times. The utilization of reversed phase column chromatography was necessary to eliminate very non-polar impurties and silica gel 60 column chromatography was necessary for the final purification of compound **5**.

The purity of product **5** was increased by additional extraction with ethyl acetate following column chromatography.

The addition of sodium thiosulfate a solution reduced elemental iodine and decolorized the crude product but also resulted in a decreased yield.

2.3.6 Preparation of 2-fluoroethyl-4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (6)

For this microwave assisted synthesis, 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (**5**) was used as starting material.



Scheme 16

Compound **5** was treated with 2-fluoroethyl trifluoromethanesulfonate and cesium ions as cesium carbonate in acetonitrile to give product **6** in moderate yield (scheme 16). Thin layer chromatography showed the consumption of the starting material and a significant amount of the assumed product (**6**). Compound **6** was purified several times by column chromatography to be finally isolated as colorless oil.

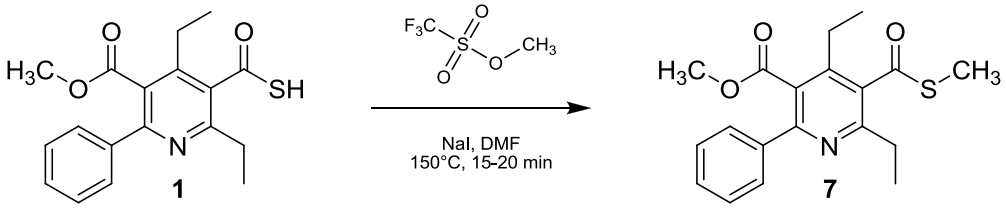
2.3.7 Preparation of methyl 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (7)

The synthesis of product **7** was attempted by 3 different methods:

1. Methylation with methyl trifluoromethanesulfonate
2. Methylation with methyl iodide and sodium hydride
3. Methylation with diazomethane

2.3.7.1 Methylation of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (1) with methyl trifluoromethanesulfonate

This reaction was carried out by treating starting material **1** with sodium iodide and methyl trifluoromethanesulfonate in dimethylformamide (scheme 17).

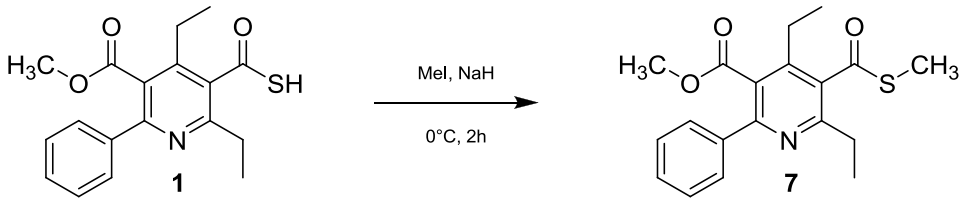


Scheme 17

Thin layer chromatography was carried out after completion of the reaction time, but no product could be detected. Even addition of excess methyl trifluoromethanesulfonate and prolonged reaction times did not afford the desired product.

2.3.7.2 Methylation of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (1) via methyl iodide and sodium hydride

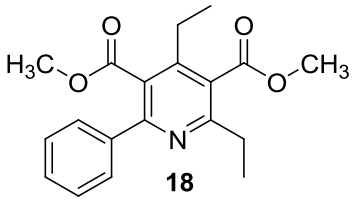
For this reaction, compound **1** served also as starting material.



Scheme 18

The reaction was carried out with sodium hydride in tetrahydrofurane and methyl iodide (scheme 18). The mixture was stirred at 0°C and every 30 min. thin layer chromatography was performed. Since no conversion was detected, the solution was stirred at room temperature for 72 h. The crude product was purified by column chromatography, but only minimal amounts of a product were obtained.

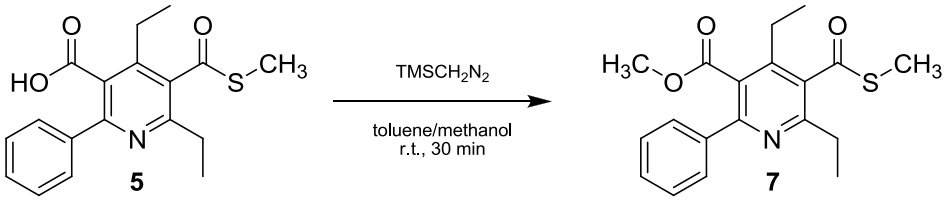
NMR spectra were recorded which revealed the loss of the sulfur atom indicated by the shift of the signal at about 190.0 ppm (thiocarboxyl carbon) to about 170.0 ppm (carboxyl carbon). Thus, it was concluded that product **18** had formed instead of desired compound **7**.



18

2.3.7.3 Methylation of 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (5) with diazomethane

This reaction was carried out using conventional heating with compound **5** as starting material, which led successfully to product **7** (scheme 19).



5 **7**

Scheme 19

The reaction was carried out under argon atmosphere. An excess amount of diazomethane in hexane was added since thin layer chromatography showed only low conversion with with equimolar amounts. Subsequently, the crude product was purified by column chromatography to afford compound **7** as light yellow crystals.

3 EXPERIMENTAL

3.1 General

Recording of spectra:

Infrared spectra: Perkin-Elmer FTIR spectrometer Spectrum 1000 (KBr pellets).

^{1}H, ^{13}C NMR Spectra: Bruker Avance DPX-200 spectrometer at 27°C (200.13 MHz for ^{1}H, 50.32 MHz for ^{13}C). The solvent signal is used as internal reference standard related to TMS, occurring at δ = 7.26 ppm (^{1}H in $CDCl_3$), δ = 2.49 ppm (^{1}H in DMSO-d_6), δ = 77.0 ppm (^{13}C in $CDCl_3$) and δ = 39.5 ppm (^{13}C in DMSO-d_6).

Mass spectra: Shimadzu QP 1000 (EI, 70 eV), direct inlet

High resolution mass spectra: Finnigan MAT 8230 (EI, 70 eV) and Finnigan MAT 900 S (ESI, 4 kV, 3 μA CH_3CN/MeOH).

Chromatographic separation:

Thin layer chromatography: Merck TLC Silica Gel 60 F254 aluminum sheets, layer thickness 0.2 mm; Merck TLC Silica Gel 60 RP-18 F254s aluminum sheets, layer thickness 0.2 mm.

Column chromatography: Merck Silica Gel 60 F254; Merck Silica Gel 60 RP-18 F254s

Other:

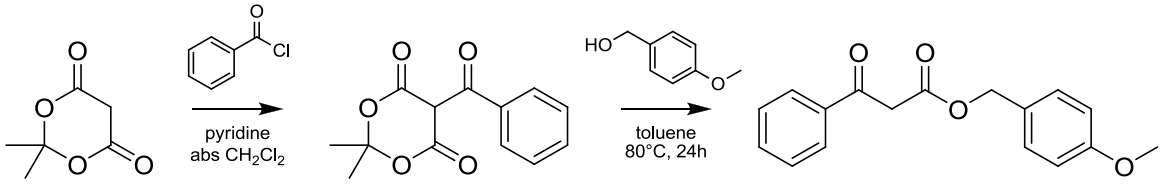
Microwave: Synthos 3000 microwave oven with a SXQ80 rotor of Anton Paar

Melting points: Reichert-Kofler hot-stage microscope

3.2 Syntheses

3.2.1 Preparation of 4,6-diethyl-2-phenyl-5-thiocarboxy-pyridine-3-carboxylic acid (4)

3.2.1.1 4-Methoxybenzyl 3-oxo-3-phenylpropanoate (9)

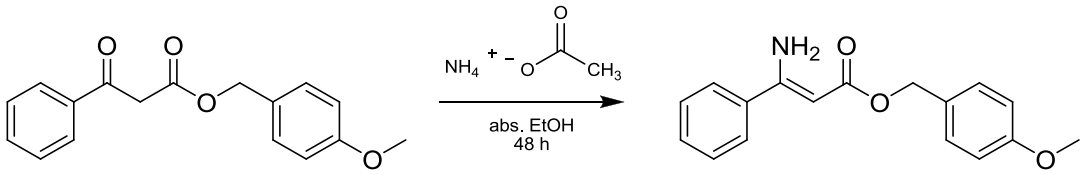


2,2-Dimethyl-1,3-dioxane-4,6-dione (40.00 g, 227.53 mmol) and benzoyl chloride (35.5 ml, 0.28 mmol) were dissolved in dichloromethane (400 ml) and stirred under argon atmosphere. The solution was cooled to -40°C and pyridine was added. After reaching 0°C the reaction mixture was stirred for 1 hour and one additional hour after reaching room temperature.
The reaction mixture was then extracted with 1N HCl (200 ml) and water (115 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was directly used for the next step, without further purification.
The crude product and 4-methoxybenzylalcohol (115 g, 832.37 mmol) was dissolved in toluene and stirred for 24 h at 80°C. After removal of the solvent, the oily product **9** was purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2).

Yield: 43.02 g (67%) reddish oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.2 4-Methoxybenzyl 3-amino-3-phenylacrylate (10)

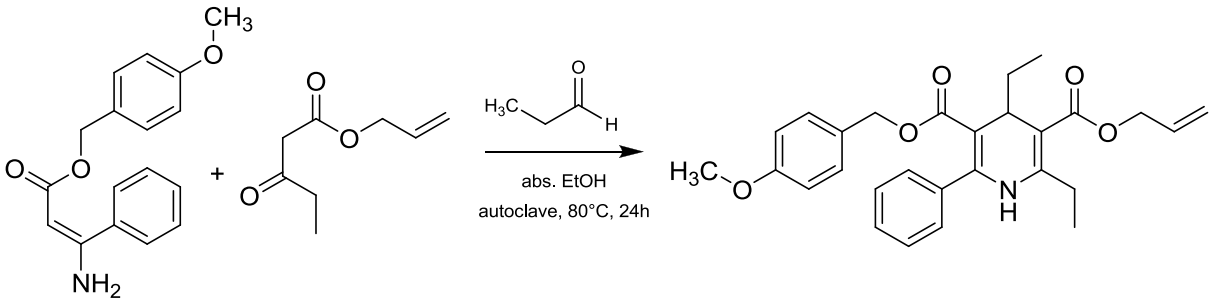


To a solution of 4-methoxybenzyl 3-oxo-3-phenylpropanoate (42.00 g, 148.24 mmol) and tetraethyl orthosilicate (61.77 g, 229.50 mmol) dissolved in absolute ethanol (600 ml), ammoniumacetate (57.13 g, 741.18 mmol) was added. The resulting solution was heated to reflux and argon atmosphere for 48 hours. Thereafter the solvent was removed under reduced pressure and product **10** was purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2).

Yield: 38.02 g (91 %) brownish oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.3 3-Allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (13)

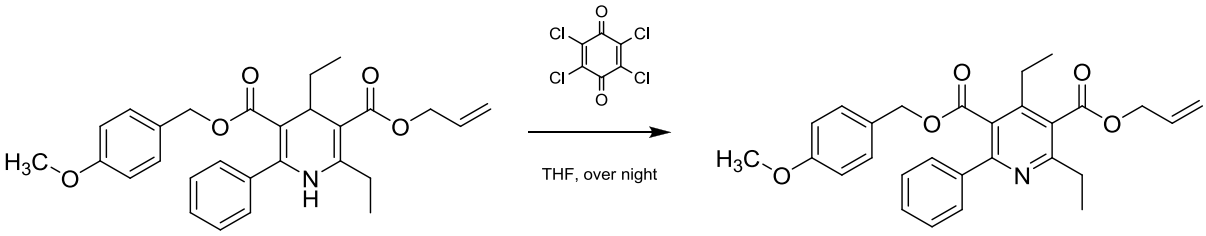


4-Methoxybenzyl 3-amino-3-phenylacrylate (43.02 g, 151.84 mmol), propionaldehyde (8.82 g, 151.84 mmol) and allyl-3-oxopentanoate (23.71 g, 151.84 mmol) were dissolved in absolute ethanol and stirred in an autoclave for 24h at 80°C. After cooling to room temperature, the solvent was removed and the residue was purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2).

Yield: 36.54 g (52%) yellow-greenish oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.4 3-Allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate (14)

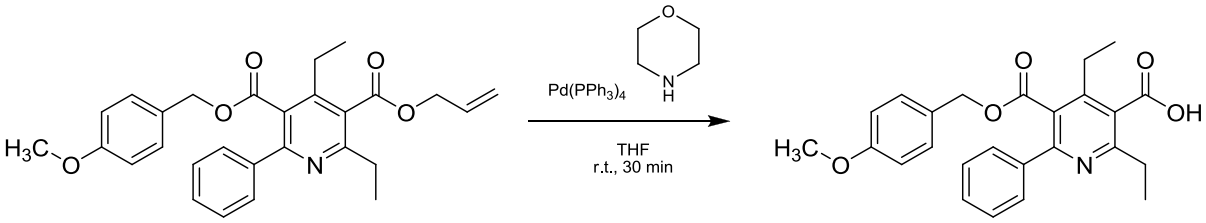


3-Allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (36.54 g, 79.27 mmol) and 2,3,5,6-tetrachloro-1,4-benzoquinone (21.44 g, 87.23 mmol) in THF (600 ml) were heated to reflux overnight. The reaction mixture was cooled to room temperature and the solvent evaporated prior to the purification by column chromatography (silica gel 60, petrolether/EtOAc 9+1).

Yield: 34.03 g (94%) red-brownish oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.5 2,4-Diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carboxylic acid (15)

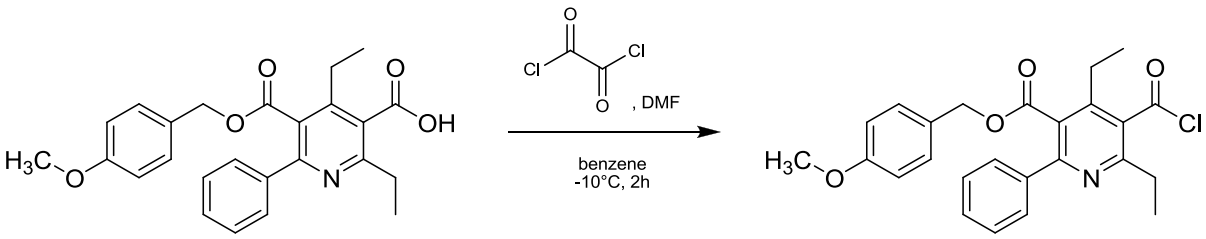


Tetrakis(triphenylphosphine)palladium (9.16 g, 7,93 mmol) and morpholine (82.7 ml, 950.81 mmol) were added to a stirred solution of 3-allyl 5-(4-methoxybenzyl)-2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate (56.03 g, 146.11 mmol) in THF (588 ml) under argon atmosphere at room temperature. The mixture was cooled and stirred for 30 min. After removal of the solvent under reduced pressure at room temperature, ethyl acetate (300 ml) was added until the residue was dissolved. The solution was then extracted three times with 1N HCl and the catalyst removed by suction. After drying over Na_2SO_4, the solvent was removed under reduced pressure and product **15** was purified by column chromatography (silica gel 60, EtOAc/MeOH 8+2).

Yield: 48.92 g (96 %) light orange crystals, mp. 137-139° C

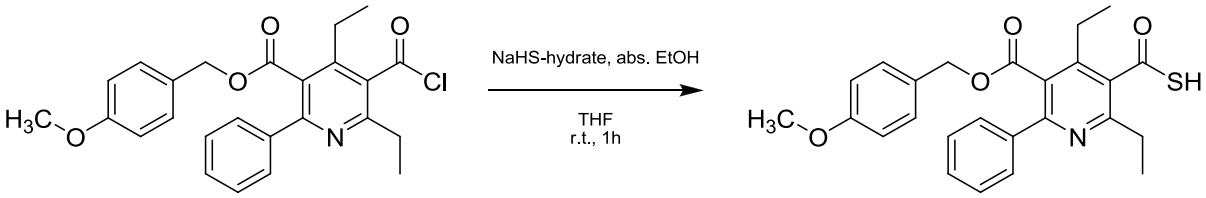
Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.6 4-Methoxybenzyl 5-(chlorocarbonyl)-4,6-diethyl-2phenylpyridine-3-carboxylate (16)



To a solution of 2,4-diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carboxylic acid (16.92 g, 40.34 mmol) in benzene (170 ml), oxalyl chloride (17 ml, 0.20 mmol) and DMF (1 ml) were added dropwise at -10°C. After reaching room temperature, the solution was stirred for 2h. Then the solvent was removed under reduced pressure and the oily product was co-evaporated with toluene several times. The resulting product **16** was used immediately for the next reaction step (3.2.1.7) without further purification.

3.2.1.7 2,4-Diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carbothioic-*S* acid (17)

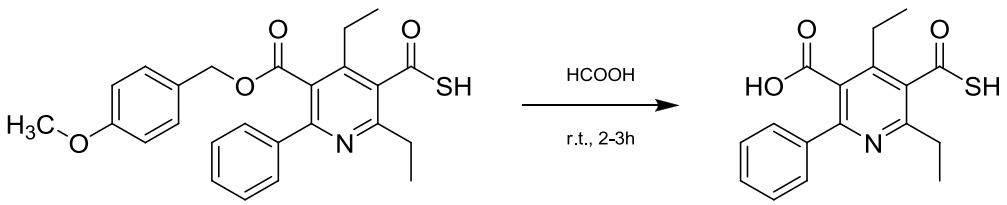


The product (**16**) of the previous reaction step was dissolved in THF (10 ml) and added dropwise to a solution of NaHS hydrate (2.26 g, 40.31 mmol) in absolute ethanol (100 ml) at -10°C. After reaching room temperature, the mixture was stirred for one hour and then acidified with 2N HCl. The aqueous layer was extracted with EtOAc. The organic layer was washed twice with a saturated NaCl solution and dried over Na_2SO_4. Then the solvent was removed under reduced pressure and the crude product **17** was purified by reversed - phase column chromatography (RP-18 silica gel, CH_3CN/H_2O 8+2)

Yield: 8.14 g (46%) colorless crystals, mp. 59-60° C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.1.8 4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (4)



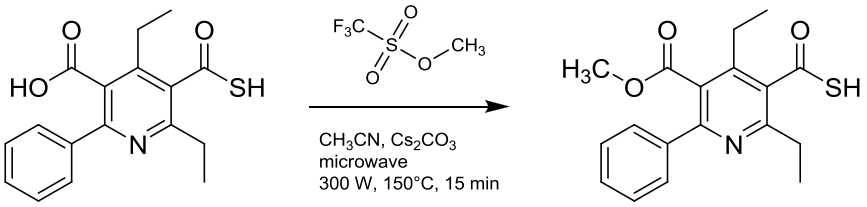
Formic acid (80 ml) was added to 2,4-diethyl-5-[(4-methoxybenzyloxy)carbonyl]-6-phenylpyridine-3-carbothioic-*S*-acid (8 g, 18.37 mmol) and stirred at room temperature for 3 hours. Then formic acid was removed under reduced pressure and the product **4** was purified by reversed phase column chromatography (RP-18 silica gel, CH_3CN/H_2O 9.5+0.5).

Yield: 2.41 g (42%) colorless crystals, mp. 191° C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.2 Preparation of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (1)

3.2.2.1 Microwave assisted synthesis

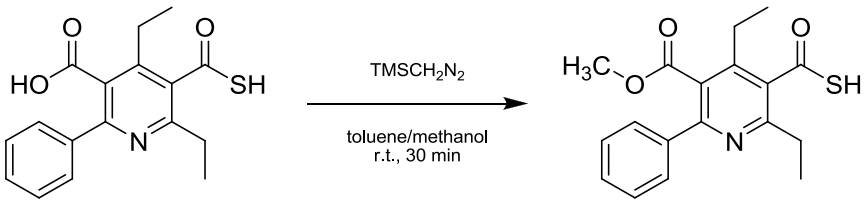


4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (0.10 g, 0.33 mmol), Cs_2CO_3 (0.22 g, 0.66 mmol) and methyl trifluoromethanesulfonate (0.11g, 0.69 mmol) were suspended in CH_3CN (10 ml). The reaction mixture was heated in a microwave oven to 150°C at 300 W for 15 min. Then the solvent was removed under reduced pressure and the product purified by column chromatography (silica gel 60, CH_3CN).

Yield: 30 mg (29%) white crystals, mp. 161-162° C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.2.2 Preparation with diazomethane

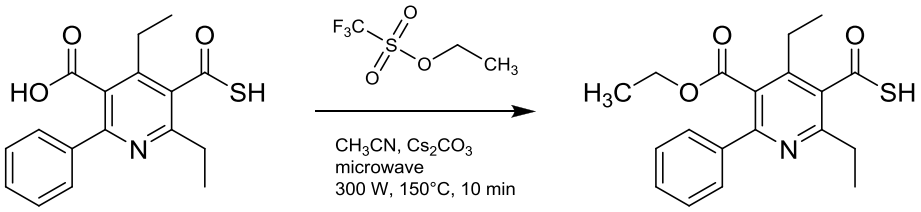


To a stirred solution of 4,6-diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (0.20 g, 0.63 mmol) in toluene/MeOH (9+6), trimethylsilyldiazomethane(0.4 ml, 0.63 mmol) was added dropwise until the yellow color of the mixture persisted. Thereafter, the solution was stirred for another 30 min at room temperature. The solvent was removed by reduced pressure and the product **1** purified by reversed phase column chromatography (RP-18 silica gel, CH_3CN/H_2O 9+1).

Yield: 141 mg (68%) white crystals, mp. 161-162° C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.3 Preparation of 5-(ethoxycarbonyl)-2,4-diethyl-6-phenylpyridine-3-carbothioic *S*-acid (2)



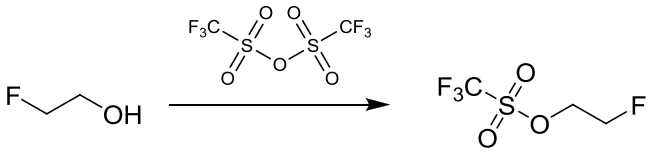
To a solution of 4,6-diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (0.22 g, 0.70 mmol) and Cs_2CO_3 (0.42 g, 1.29 mmol) in CH_3CN (15 ml), ethyl trifluoromethanesulfonate (0.42 g, 2.36 mmol) was added. The mixture was heated in the microwave oven to 150°C at 300 W for 10 min. Then the solvent was evaporated and crude product **2** purified by reversed phase column chromatography (RP-18 silica gel, CH_3CN/H_2O 9+1).

Yield: 120 mg (50%) yellow oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.4 Preparation of 2,4-diethyl-5-[(2-fluoroethoxy)carbonyl]-6- phenylpyridine-3-carbothioic *S*-acid (3)

3.2.4.1 2-Fluoroethyl trifluoromethanesulfonate

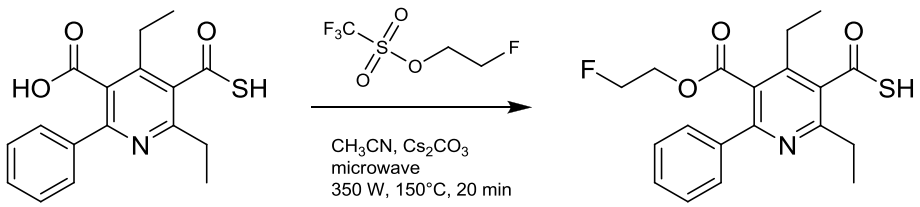


To a solution of 2-fluoroethanol (10.0 g, 156.10 mmol) in lutidine (20 ml) CH_2Cl_2 (100 ml) was added after cooling the mixture below 0°C. After adding trifluoromethanesulfonic anhydride (55 ml, 327.81 mmol) dropwise, the solution was stirred for 30 min at 0°C. Finally, a mixture of 10% EtOAc in hexane (50 ml) was added. For purification, the solution was passed through a silica gel cartridge. After removal of the solvent the product (**3**) was purified by Kugelrohr distillation.

Yield: 29.08 g (95%) colorless fluid

Spectroscopic data were in complete accordance with literature values.[86]

3.2.4.2 2,4-Diethyl-5-[(2-fluoroethoxy)carbonyl]-6-phenylpyridine-3-carbothioic *S*-acid (3)

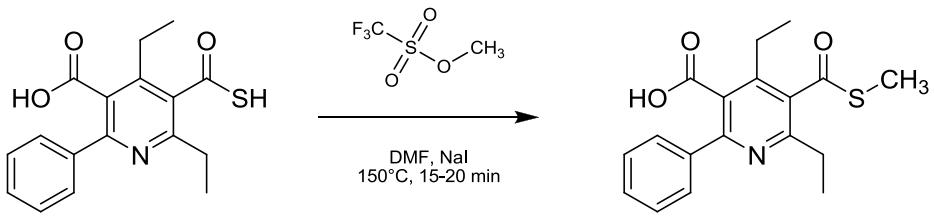


4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (0.25 g, 0.79 mmol), Cs_2CO_3 (0.52 g, 1.59 mmol) and 2-fluoroethyl trifluoromethanesulfonate (0.34 g, 1.74 mmol) were suspended in CH_3CN (45 ml). The reaction mixture was heated in a microwave oven at 350 W to 150°C for 20 min. Thereafter, the solvent was evaporated and the resulting product **3** purified by column chromatography (silica gel 60, petrolether/EtOAc 9+1).

Yield: 87 mg (30%) light brown oil

Spectroscopic data were in complete accordance with literature values.[87]

3.2.5 Preparation of 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (5)

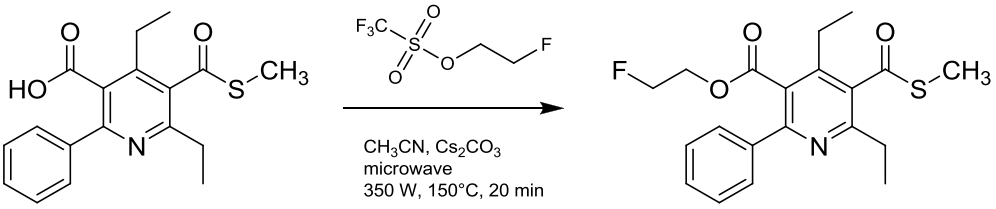


4,6-Diethyl-2-phenyl-5-(sulfanylcarbonyl)pyridine-3-carboxylic acid (0.10 g, 0.32 mmol) and NaI (0.48 g, 3.17 mmol) were dissolved in DMF (10 ml) to stir the solution at room temperature. Then the reaction mixture was heated to 155°C and methyl trifluoromethanesulfonate (0.12 g, 0.71 mmol) was added. The solution was stirred for another 15-20 min. Thereafter, DMF was removed by Kugelrohr distillation and the product **5** was purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2). The orange-red solution was alkalized with NaOH and extracted with EtOAc. The aqueous layer was acidified with 2N HCl and extracted again with EtOAc. Finally, the combined organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure.

Yield: 52 mg (49%) white crystals, mp. 243-244° C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.6 Preparation of 2-fluoroethyl 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (6)



To a solution of 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (0.05 g, 0.15 mmol) and Cs_2CO_3 (0.99 g, 0.30 mmol) in CH_3CN (15 ml), 2-fluoroethyl trifluoromethanesulfonate (0.63 g, 0.33 mmol) was added. The reaction mixture was heated in a microwave oven at 350 W to 150°C for 20 min. Then the solvent was removed under reduced pressure and the crude product **5** purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2).

Yield: 18 mg (32%) colorless oil

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm) 7.59 (m, 2H), 7.43 (m, 3H), 4.42 (m, 1H), 4.33 (m, 1H), 4.31 (q, *J* = 7.5 Hz, 2H), 4.19 (s, 2H), 2.72 (q, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 1.33 (t, *J* = 7.4 Hz, 3H), 1.23 (t, *J* = 7.6 Hz, 3H)

^{13}C-NMR (50 MHz, $CDCl_3$): δ (ppm) 195.6, 168.2, 159.8, 157.3, 148.2, 139.8, 133.1, 128.9, 128.4, 128.3, 125.9, 82.2, 78.8, 64.3, 63.9, 29.2, 24.2, 15.7, 14.1, 12.7

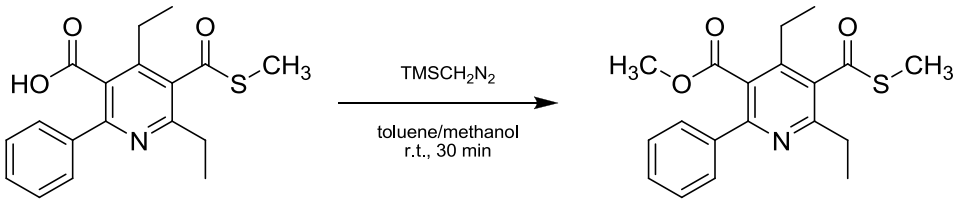
IR (KBr): ν (cm^{-1}) 3664, 2932, 2926, 2852, 1732, 1668, 1556, 1496, 1460, 1406, 1376, 1266, 1252

MS: m/z (%) 375.25 (M^+ + H, 1), 330.40 (2), 329.35 (21), 328.25 (100), 282.10 (6), 254.10 (5), 236.15 (5), 208.20 (3)

HRMS: m/z calculated for $C_{20}H_{22}FNSO_3H$: 376.1383. Found: 367.1385

3.2.1 Preparation of methyl 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (7)

3.2.7.1 Preparation via diazomethane

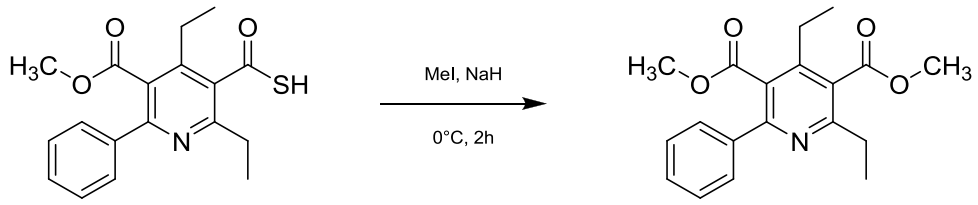


To a stirred solution of 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylic acid (0.08 g, 0.26 mmol) in toluene/MeOH (10 ml, 6+4) under argon atmosphere, trimethylsilyldiazomethane (0.44 g, 0.38 mmol) was added and stirred for 30 min. After evaporation of the solvent, the crude product (**7**) was purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2)

Yield: 57 mg (64%) light yellow crystals, mp. 77°C

Spectroscopic data were in complete accordance with literature values.[87]

3.2.7.2 Preparation of dimethyl 2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate (18)



A 60% NaH dispersion in mineral oil (0.01 g, 0.23 mmol) was washed with hexane (3 ml) which was then replaced by THF (5 ml) prior to the slow addition of a solution of 2,4-diethyl-5-(methoxycarbonyl)-6-phenylpyridine-3-carbothioic *S*-acid (0.07 g, 0.21 mmol) in THF (2 ml). Thereafter, the mixture was cooled to 0°C and MeI (0.04 g, 0.25 mmol) was added carefully.

After two hours, thin layer chromatography (silica gel 60, petrolether/EtOAc 9+1) was carried out, but no product could be detected. Thus, the mixture was stirred for another 48 hours. After completion of the reaction, the solvent was evaporated and the crude product (**18**) purified by column chromatography (silica gel 60, petrolether/EtOAc 8+2).

Yield: 30 mg (43%) violet-reddish oil

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm) 7.60 (m, 2H), 7.42 (m, 3H), 3.96 (s, 3H), 3.62 (s, 3H), 2.83 (q, *J* = 7.5 Hz, 2H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.32 (t, *J* = 7.4, 3H), 1.22 (t, *J* = 7.6, 3H)

^{13}C-NMR (50 MHz, $CDCl_3$): δ (ppm) 169.1, 169.0, 160.3, 156.7, 148.6, 139.7, 128.8, 128.4, 128.2, 127.3, 126.2, 52.4, 52.3, 29.8, 24.9, 15.2, 13.7
IR (KBr): ν (cm^{-1}) 3446, 2974, 2948, 2878, 2854, 1730, 1562, 1496, 1448, 1436, 1410, 1376, 1308, 1268, 1252

MS: m/z (%) 328.30 (M^+ + H, 15), 312.25 (100), 296.25 (27), 280.25 (22), 254.10 (15), 236.00 (11), 207.95 (14)

HRMS: m/z calculated for $C_{19}H_{21}NO_4H$: 328.1549. Found: 328.1554

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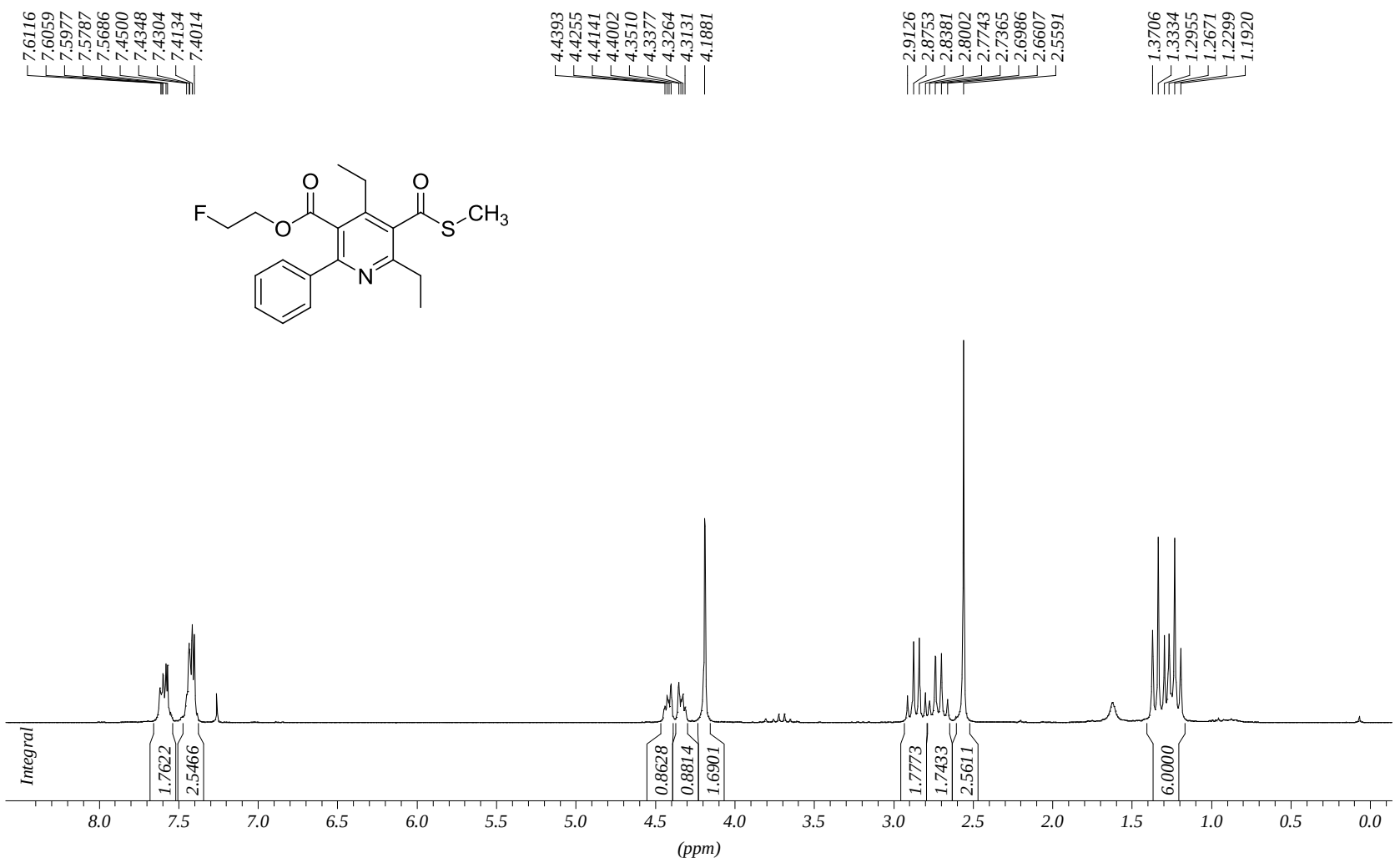
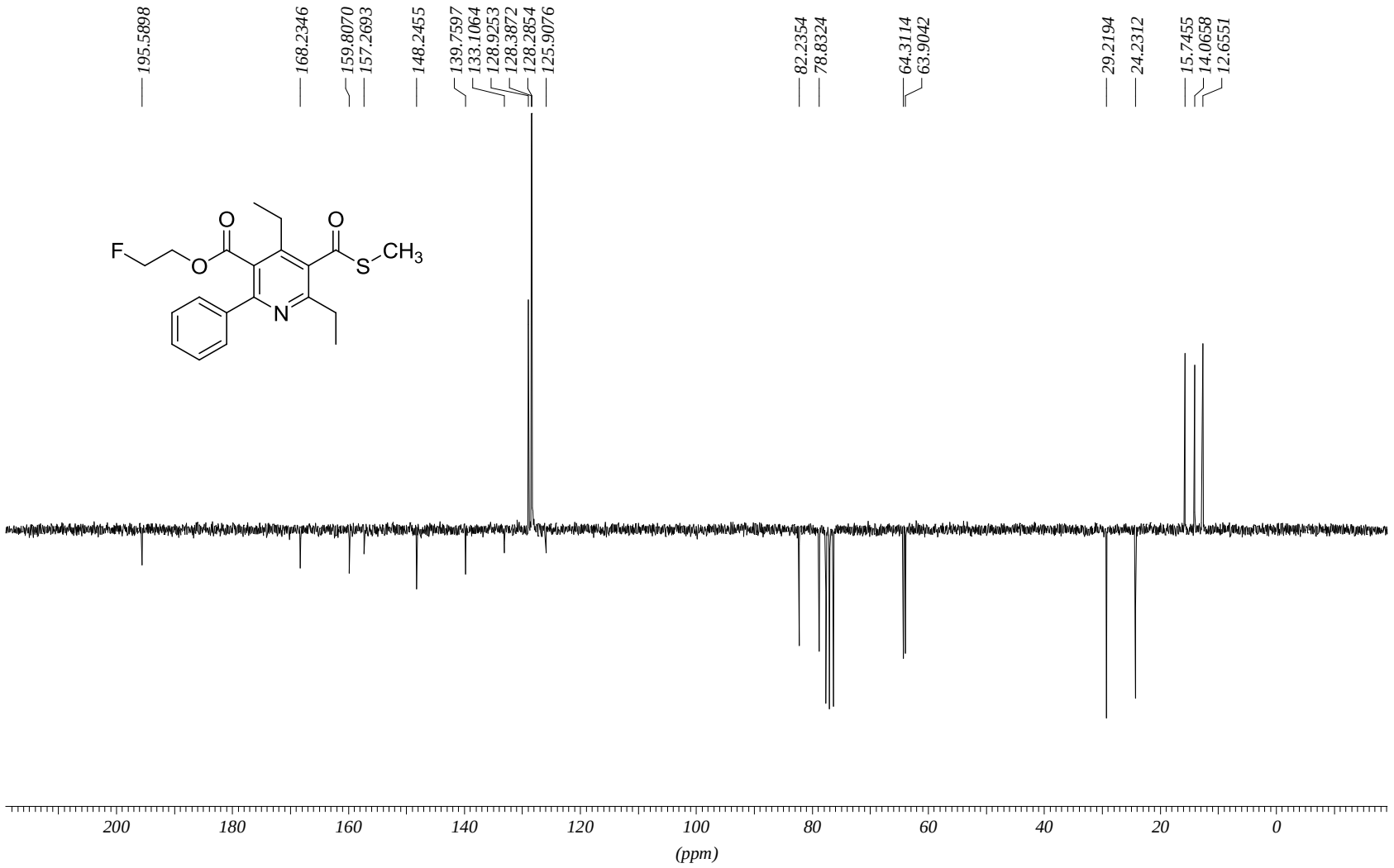
5 APPENDIX

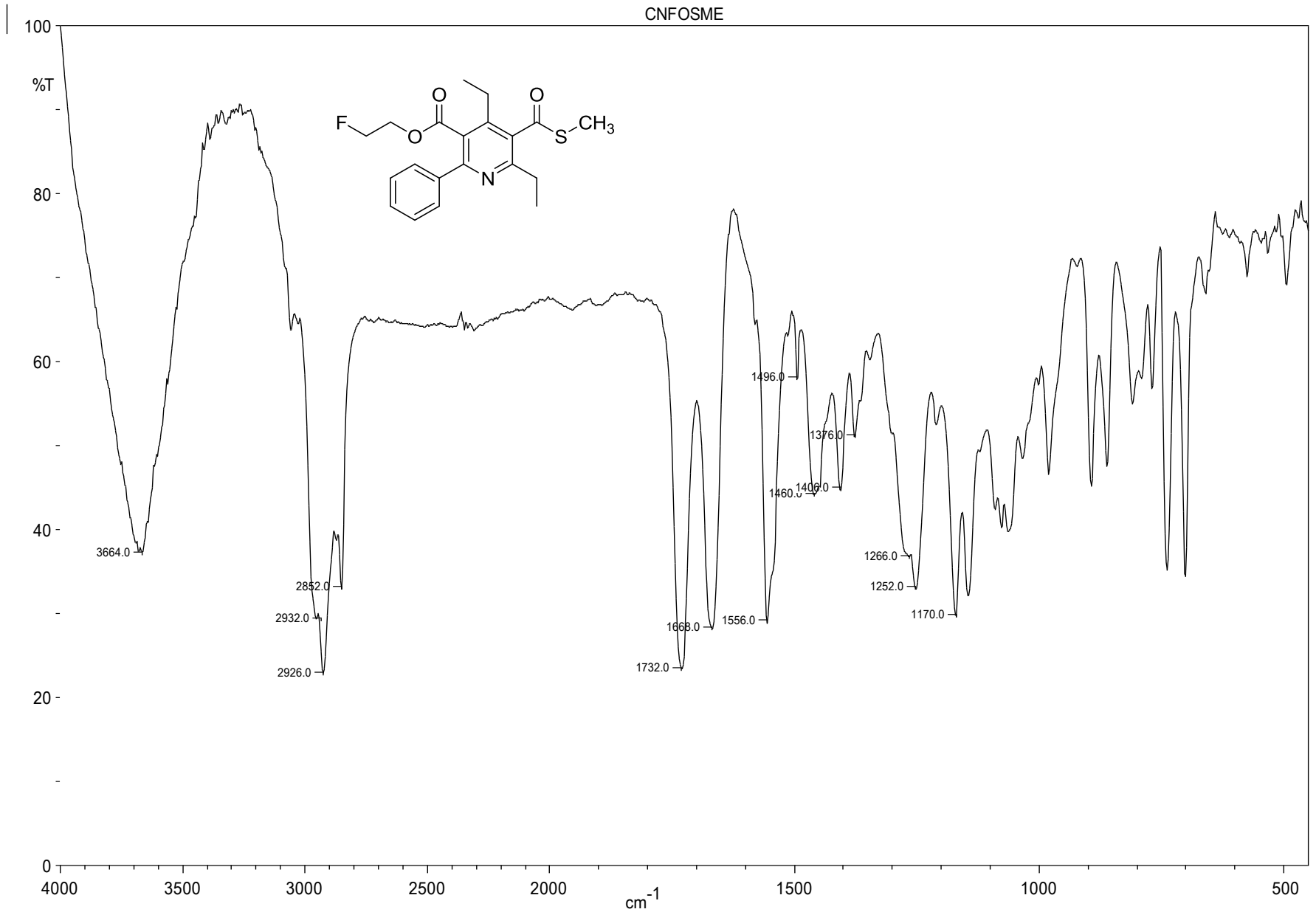
5.1 Spectra

cnfosme, 2-fluoroethyl 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylate, Proton in CDCl3



cnfosme, 2-fluoroethyl 4,6-diethyl-5-(methylthiocarbonyl)-2-phenylpyridine-3-carboxylate, C13APT in CDCl3




CNFOSME
100
%T
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
cm-1
3664.0
2932.0
2852.0
2926.0
1732.0
1668.0
1556.0
1496.0
1460.0
1406.0
1376.0
1266.0
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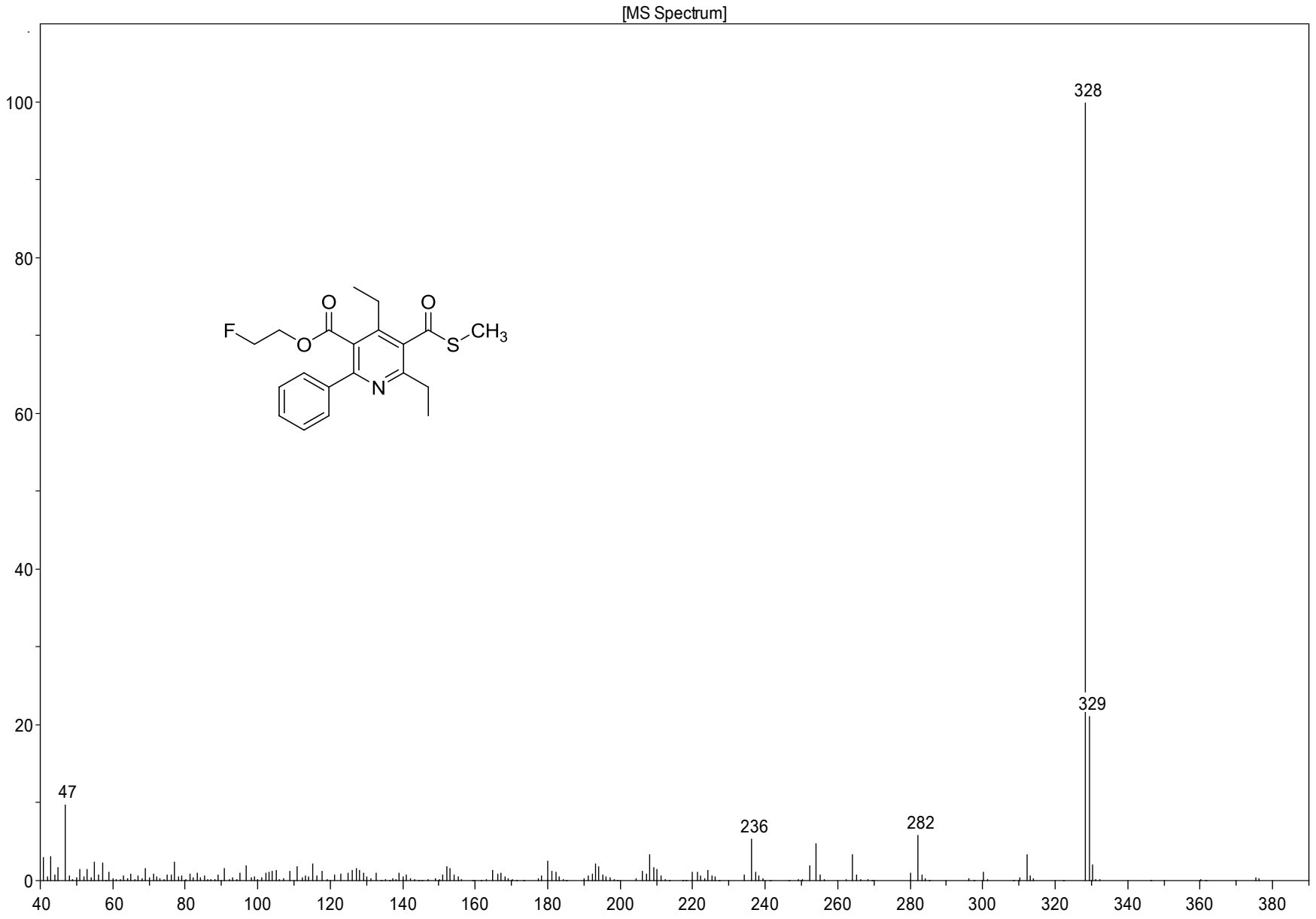
1170.0
F
O
O
O
S
CH3
N


[MS Spectrum]
328
329
47
236
282
100
80
60
40
20
0
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
F
O
O
O
S
CH3
N

cnome2, dimethyl 2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate in CDCl3

7.6229
7.6002
7.5838
7.5743
7.4500
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7.4216
7.4058
7.3957

3.9628
3.6182

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2.8097
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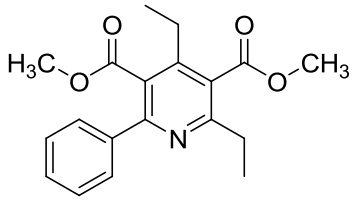
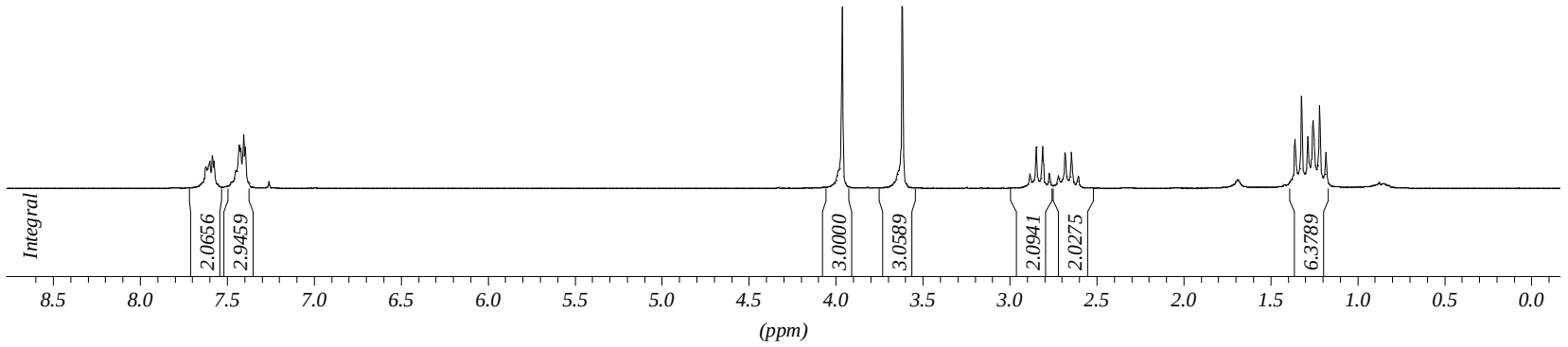
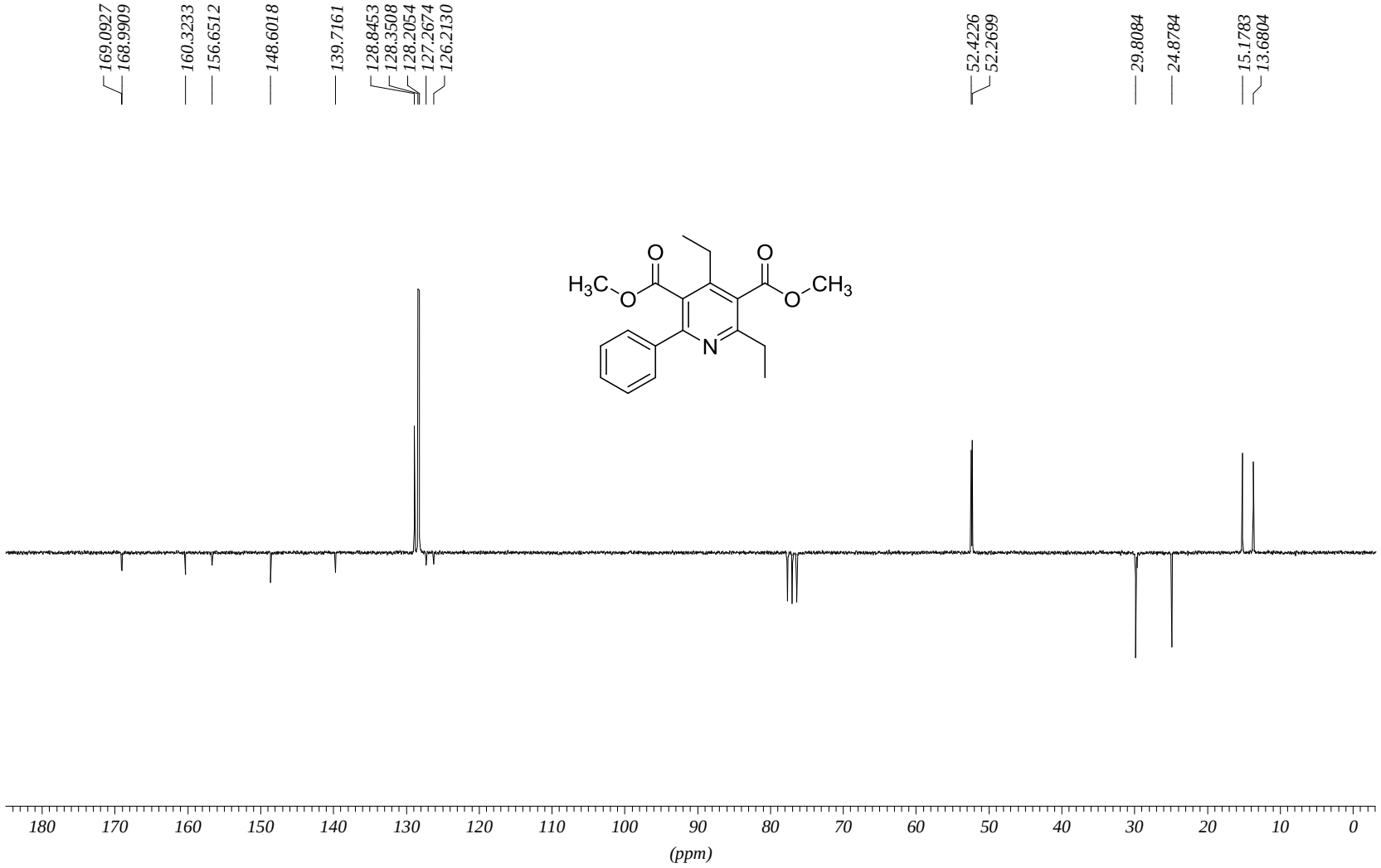
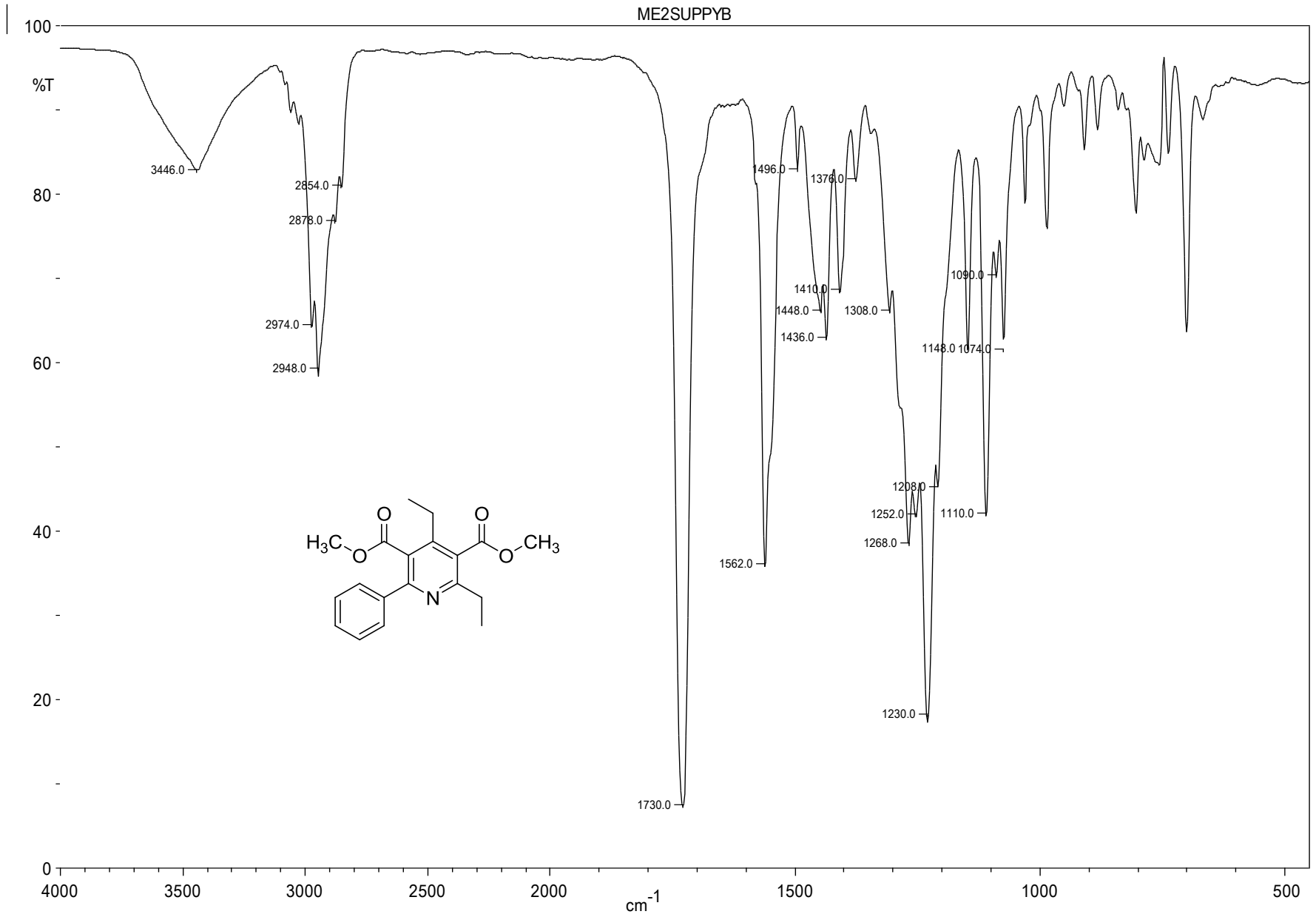




cnome2, dimethyl 2,4-diethyl-6-phenylpyridine-3,5-dicarboxylate in CDCl3

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%T
80
60
40
20
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1562.0
1496.0
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1410.0
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1268.0
1230.0
1148.0
1090.0
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CH3
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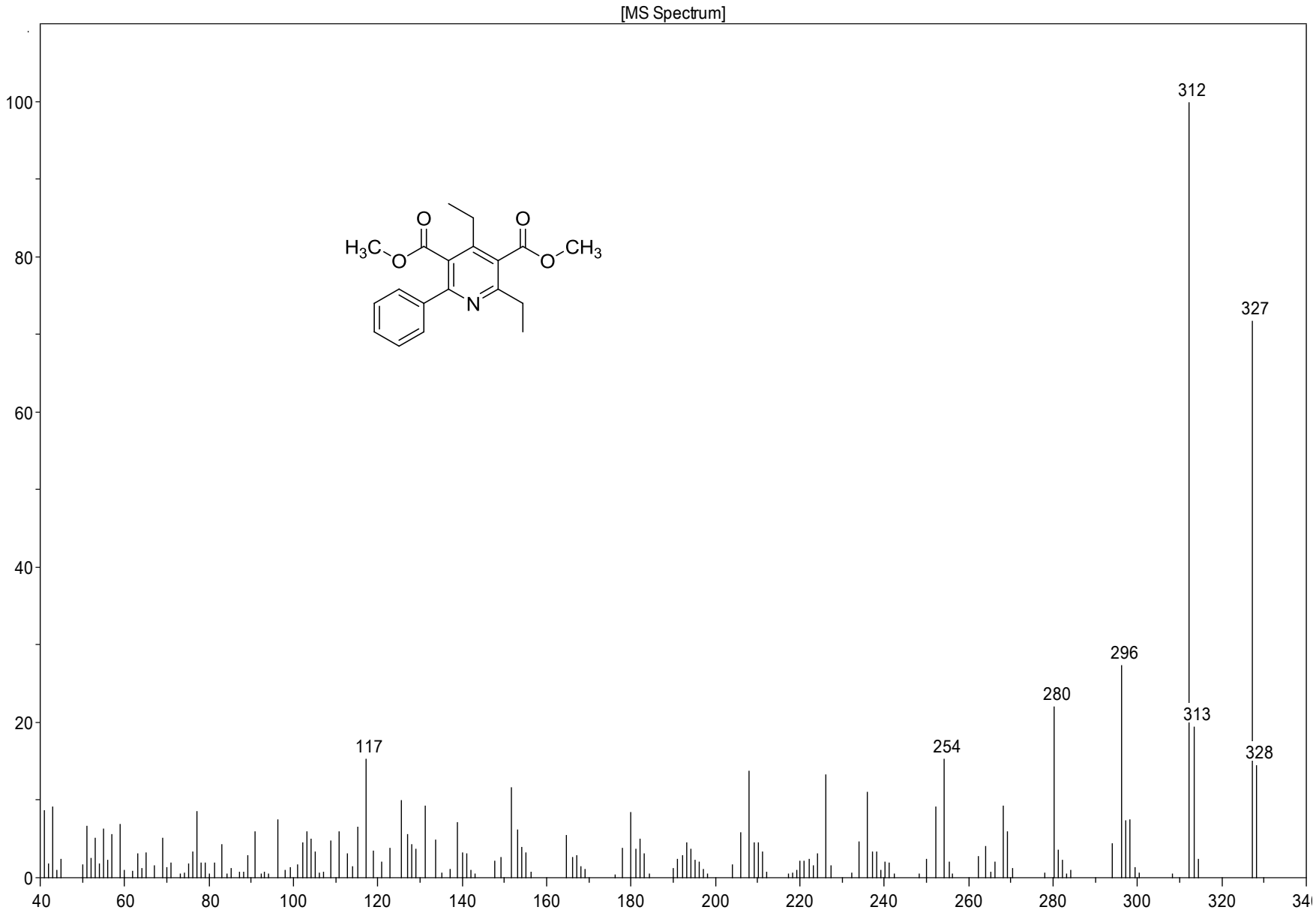
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280
313
328
254
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80
100
120
140
160
180
200
220
240
260
280
300
320
O
O
H_3C
O
O
CH_3
N

5.2 Curriculum vitae

Name:	Catharina Neudorfer
Date of birth:	31.3.1988
Marital status:	Single
Nationality:	Austrian
Parents:	Dipl. Ing. Wolfgang Neudorfer
	Judith Neudorfer
Siblings:	Clemens Neudorfer
	Carolina Neudorfer
	Christoph Neudorfer

Education:

2010	Diploma thesis at the Department for Drug and Natural Product Synthesis, University of Vienna
2009 and 2010	Tutor in practical courses of general microbiology at the Department of Pharmacognosy, University of Vienna
2006 – 2010	Undergraduate study of pharmacy, University of Vienna
Summer 2006	Internship at the pharmacy: Apotheke zum Weinstock, 1210 Wien
1998 - 2006	Secondary school Gymnasium Sacré - Coeur 1030 Wien
1994 - 1998	Primary school Volksschule Jochbergengasse 1210 Wien