Bachelorarbeit

Synthese und Evaluierung ^{18}F-markierter Bausteine zur Peptid-/Proteinkonjugation

Mathematisch-Naturwissenschaftliche Fakultät
der Universität zu Köln

Studiengang B. Sc. Chemie

vorgelegt von
Katharina Lauchner

Köln, September 2018

Eidesstattliche Erklärung

Hiermit erkläre ich, Katharina Lauchner, dass ich die vorliegende Arbeit mit dem Thema

"Synthese und Evaluierung ^{18}F-markierter Bausteine zur Peptid-/Proteinkonjugation"

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Inhalt

1. Introduction

1.1.General remarks

Nuclear medicine is a fast developing and highly promising field dedicated to the detection of metabolic pathways and the treatment of diseases. The radiotracer method is one of the most frequently used techniques in nuclear medicine and was developed by George de Hevesy who first researched the chemical processes of the plant *Vicia faba* with the ^{212}Pb isotope.[1] This was the groundwork for scintigraphy, the visualization of tissue by the Anger or gamma camera, which results in two-dimensional images.[2] In 1975, the positron emission tomography (PET) was developed to visualize the structure of interest three-dimensionally as a *non-invasive* imaging technique.[3] PET uses radiotracers, which consist of a positron-emitting nuclide bound to a specific probe. Those probes are usually small molecules which either bind to a target (e.g. a peptide) or are metabolized in a specific way. PET-tracers visualize where the target molecule or the metabolic processes are localized in the body.[4] Nowadays combined PET/CT systems are routinely used in the clinic to detect tumors in cancer patients or to evaluate the severity of neurodegenerative diseases. As the knowledge about the pathomechanisms constantly increases, new radiotracers have to be developed to address specific details or subtypes of diseases.

The probes which are radiolabeled often consist of a protein and a prosthetic group. The prosthetic group is a molecule which is tightly bound to the protein and is required for its biological function. The radiolabeling process takes advantage of the specific binding between the protein and prosthetic group so that the latter can be used as an exchangeable component for radiolabeling of the protein. The synthesis and radiolabeling of new prosthetic groups is therefore an important task to manage.

The first step is the synthesis of a precursor of the prosthetic group, where the positions of the later ^{18}F-labeling are occupied by leaving groups.

In this work, the following prosthetic groups were examined: [^{18}F]fluorobenzaldehyde, 6-[^{18}F]fluoronicotinaldehyde, 6-[^{18}F]fluoro-2-methoxy-nicotinaldehyde and *N*-methyl 6-[^{18}F]fluoro-3-azaisatoic anhydride, as well as an attempted prosthetic group synthesis of 4-[^{18}F]fluorophthalaldehyde with various analogs. These prosthetic groups bind to an amine group of the peptide as the labeling agents belong to the amino reactive prosthetic groups. Additionally, the leaving groups trimethylamine and diazabicyclo[2.2.2]octane were compared in consideration of

precursor synthesis, stability, and reactivity. Lastly, the imidazolesulfonate group was introduced as another potential leaving group for labeling agents.

Kommentiert [BD1]: Gleichzeitig reaktiv und stabil wiederspricht sich doch. Meinen Sie die metabolische Stabilität des Radiotracers und die Reaktivität seines Vorläufers?

1.2.Positron Emission Tomography

Positron Emission Tomography (PET) uses positron-emitting radionuclides to create a three-dimensional image of the body. Radiotracers are injected into the patients to visualize tumors and metastases. Other research and clinical purposes for PET are found in neurology as well as in cardiology. [5]

Kommentiert [BD2]: Sie sollten noch erwähnen, dass PET nicht nur für onkologische, sondern auch für neurologische und kardiologische Fragestellungen verwendet wird.

The technique behind the PET uses coincidence detectors to detect gamma photons emitted during positron-electron annihilation. Positrons are the result of β^+ decay and are the antiparticles of electrons.

$$^1_1p \longrightarrow ^1_0n + \beta^+ + \nu_e$$

Equation 1: β^+ decay.

Electrons and positrons have the same mass and charge. During decay, kinetic energy is transferred to the emitted positron. This kinetic energy is lost afterwards partly or completely through collisions with electrons in the surrounding tissue. The complete loss of energy follows from the combination of the positron with an electron forming a short-lived composition, the positronium. The positronium is annihilated and converts all its mass into energy by emitting simultaneously two gamma photons in opposite directions. These photons each have an energy of 511 keV which results from the resting energy of the electron or the positron and can be detected scintillator crystals of the PET scanner.

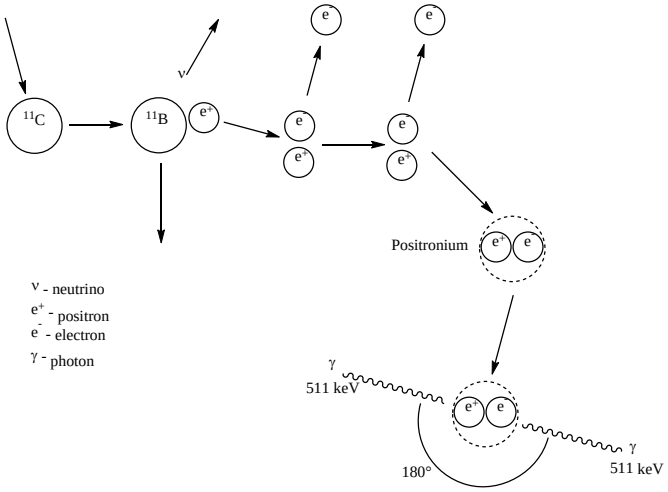


Figure 1: Schematic diagram of the positron annihilation.

Scintillation detectors coupled to photomultiplier tubes are arranged in a number of rings which surround the patient. The detection of a coincident event involves two detectors which both register a gamma photon within a fixed coincidence window (1–10 ns). It is then assumed that an annihilation event has occurred somewhere along the line connecting the two detectors. For the simplest method of image reconstruction, the filtered back projection a medium grey level is assigned to the whole line of response. Many lines with different angles will intersect in spots with high concentration of the radionuclide and add up to a high intensity grey level. A final filtering step further attenuates the lines in the periphery. The resulting 3D data set can be used e.g. to localize a tumor and to generate assumptions about its viability.

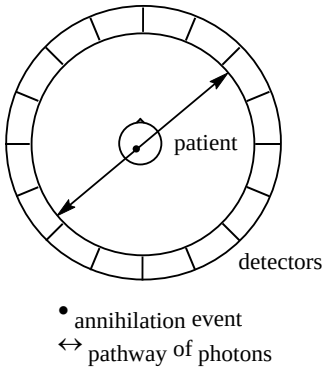


Figure 2: Schematic diagram of a PET scanner.

1.3.Radionuclides

The most commonly used positron emitters in PET are ^{11}C, ^{13}N, ^{15}O, ^{68}Ga, ^{124}I, and ^{18}F. Radionuclides in PET-chemistry have short half-life and have to be chosen properly for their specific purpose.[5]

^{11}C, ^{13}N, and ^{15}O for example are used for fast biochemical processes, as they have a shorter half-life and can substitute stable isotopes in simple molecules like water or ammonia. $[^{15}O]H_2O$ and $[^{13}N]NH_3$ are usually used as blood flow tracers[6], whereas[^{11}C]methionine[7] and [^{11}C]choline[8] are used in oncological examinations, as ^{11}C (20 min) provides a slightly longer half-life than ^{15}O (122.24 s) or ^{13}N (9.96 min).

^{68}Ga, ^{124}I, and ^{18}F, on the other hand, have a longer half-life and can be used for time-intensive experiments but are also limited, as pharmacological relevant molecules with these substituents are rare and the development of analog tracers as well as facile radiolabeling methods is required. Numerous radiolabeling methods for PET probe production have been developed, unfortunately, almost all suffer from

different challenges: poor functional group tolerance, low yields, hardly accessible or insufficiently stable radiolabeling precursors or reagents, harsh or strictly controlled reaction conditions, complicated purification, and formulation.[9]

^{18}F is ideally suitable for PET-chemistry, with a half-life of 109.6 min allowing multi-step radio syntheses and purification as well as longer PET-examinations. The

1.4.Radiolabeling strategies

1.5.Prosthetic groups

Carboxylic acid, amino and thiol reactive groups.
Acylation, oxime ligation

1.6.Nucleophilic aromatic substitution (S_NAr)

2. Goals

Three main points were elaborated/covered in this work:

- Synthesis of precursors of the known radiolabeled building blocks, 6-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA), 6-[^{18}F]fluoronicotinaldehyde ([^{18}F]FNA) as well as the novel radiolabeled synthon 6-[^{18}F]fluoro-2-methoxy-nicotinaldehyde and their preparation using simplified radiolabeling procedures
- Preparation of radiolabeling precursors for and synthesis of radiofluorinated *o*-phthalaldehyde(s) as a novel building block(s) for indirect radiolabeling of biopolymers.
- Preparation of ^{18}F-fluorinated *N*-methyl 6-fluoro-3-azaisatoic anhydride as a novel amine reactive prosthetic group for the introduction of 6-fluoro-2-methylaminopyridine-3-carboxamide fragment.

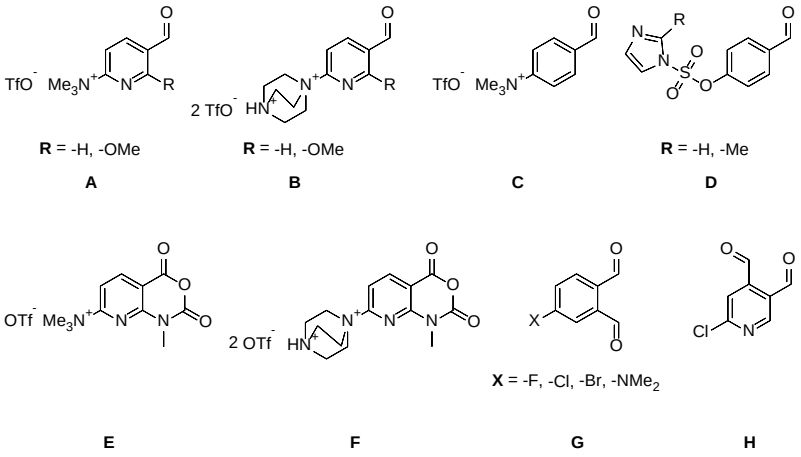


Figure 3: Investigated precursors of 6-[^{18}F]FNA (**A**, **B**), 6-[^{18}F]Fluoro-2-methoxynicotinaldehyde (**A**, **B**), 6-[^{18}F]FBA (**C**), 7-[^{18}F]Fluoro-*N*-methyl-3-azaisatoic anhydride (**E**, **F**) and *o*-phthalaldehyde analogs (**D**, **G**, **H**).

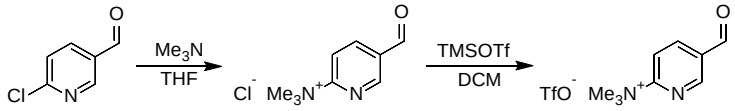
3. Results and Discussion

3.1.Synthesis of precursors with TMA and DABCO

The synthesis of the 6-[^{18}F]fluoronicotinaldehyde precursor was performed based on the procedure described by Basuli *et al*.[10] [^{18}F]FNA is an interesting alternative for [^{18}F]FBA due to its more hydrophilic composition and it is assumed to have a positive influence on pharmacokinetics.

Kommentiert [KL3]: Gehört das schon eigentlich nicht eher in die conclusion? Ich bin mir bei vielen Dingen, die ich schreibe unsicher, was ich wo hinpacken soll.

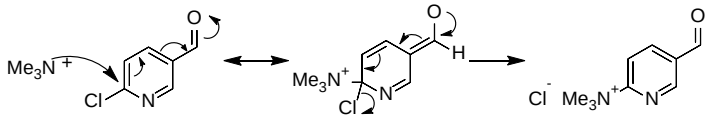
6-Chloropyridine-3-carbaldehyde (**1**) was treated with trimethylamine (TMA) resulting in the quaternarization of TMA by forming 6-(*N*,*N*,*N*-trimethylammonium)pyridine-3-carbaldehyde chloride(**2**) in 55 % yield**,** followed by a salt metathesis with TMSOTf giving 6-(*N*,*N*,*N*-trimethylammonium)pyridine-3-carbaldehyde triflate (**3**) in 70 % yield (Scheme 1). The overall yield of **3** is 20 %.



1 **2** (55 %) **3** (70 %)

Scheme 1: Synthesis route of 6-[^{18}F]fluoronicotinaldehyde precursor with trimethylammonium as a leaving group.

The first step of the synthesis of **3** is a nucleophile aromatic substitution (S_NAr). TMA is a nucleophile and undergoes a conjugate addition to the sixth position of **1**. The quaternarization of TMA takes place resulting in an enolate transition state. The challenging step here is the elimination of a leaving group, which results in a competition between TMA and chloride. As chloride (pKa = −7) has a lower acid dissociation constant (pKa) than TMA (pKa ≈ 9–10), chloride will dissociate more readily than TMA.[11] In consequence, a chloride ion is eliminated from the transition state forming a salt. As the salt is precipitated, there is no need for further purification and **2** can be used for the following ion metathesis.



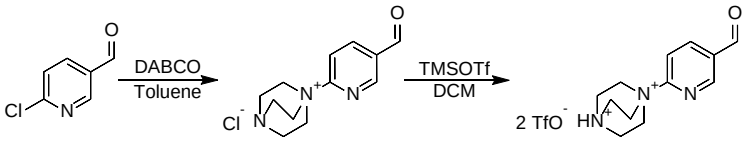
Scheme 2: S_NAr-mechanism with TMA as the nucleophile.

The metathesis is the counter ion exchange and substitutes the chloride ion by a triflate ion resulting in **3** and is then ready to be used as a 6-[^{18}F]fluoronicotinaldehyde precursor for radiolabeling.

Kommentiert [KL4]: Klingt das zu informell?

As trimethylammonium salts are already established precursors for radiolabeling, diazabicyclo[2.2.2]octane (DABCO) was additionally investigated as another possible leaving group. The synthesis was based on For this purpose, DABCO and **1** were dissolved in toluene at room temperature giving 6-(1,4-diazabicyclo[2.2.2]octan-1-ium)pyridine-3-carbaldehyde chloride (**4**) in 84 % yield, which precipitated and was only washed and dried for further use. The salt metathesis to 6-(1,4-diazabicyclo[2.2.2]octan-1-ium)pyridine-3-carbaldehyde triflate (**5**) results in 97 % yield (Scheme 3).

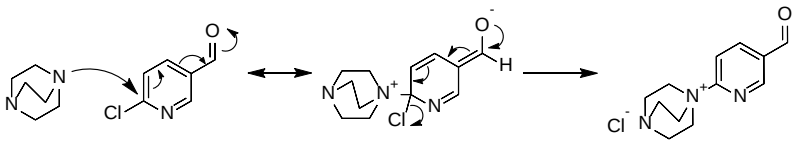
Kommentiert [KL5]: Ich will schreiben, dass die trimethylammonium salze gute precursor darstellen und deswegen DABCO als Abgangsgruppe für einen weiteren Onium-Salz-Precursor untersucht werden soll.



1 **4** (84 %) **5** (97 %)

Scheme 3: Synthesis route of the 6-[^{18}F]fluoronicotinaldehyde precursor with 1,4-diazabicyclo[2.2.2]octan-1-ium as a leaving group.

The mechanism of the quaternarization is similar to the TMA compound. DABCO acts as a nucleophile and conjugates in para-position to the carbonyl group. In this transitional state, an enolate is formed, which eliminates the chloride in the same manner as the above described TMA analog transition state (Scheme 4). This results in **4** and can undergo a salt metathesis with TMSOTf to **5**.



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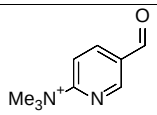
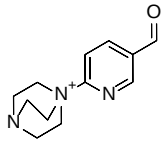
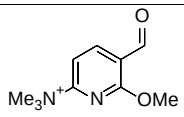
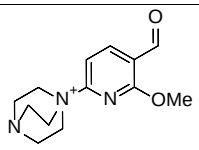
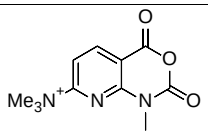
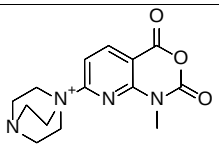
Scheme 4: S_NAr-mechanism with DABCO as the nucleophile.

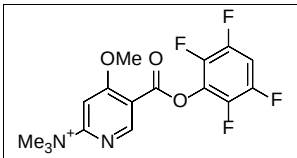
DABCO and TMA are interesting leaving groups, both are tertiary amines and good nucleophiles. The difference between TMA and DABCO is the cyclic structure of DABCO. The cyclic structure withholds the alkyl substituents and does not hinder the nucleophilic lone pair of the nitrogen to approach an electrophile. This effect influences the relative rate of reaction, which is approximately 40 times higher for the cyclic amine than for the alkylated amine. Both reagents are often used as catalysts, as they are regained completely at the end of the reaction.[11]

In this synthesis, the stability of the onium salts is a huge benefit. The precursor can still be used for radiolabeling after a period of time and its reactivity is sufficient to eliminate the leaving group in the process of labeling.

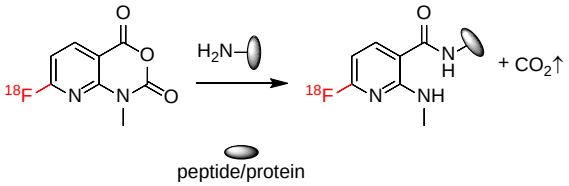
Alongside the [^{18}F]FNA precursor, analog compounds with different substituents were also synthesized. The overall yields of the analogs amount to over 20 % (table 1). Starting molecules 6-chloro-2-methoxypyridine-3-carbaldehyde, *N*-methyl 6-fluoro-3-azaisatoic anhydride and 2,3,5,6-tetrafluorophenyl 6-chloro-4-methoxypyridine-3-carboxylate were kindly provided by Elizaveta A. Urusova.

Table 1: Actual Yields of precursors.

	Yield in %		
	Cl^-	OTf^-	Overall
	55	70	20
	84	97	65
	82	71	48
	75	53	32
	97	96	90
	98	81	72

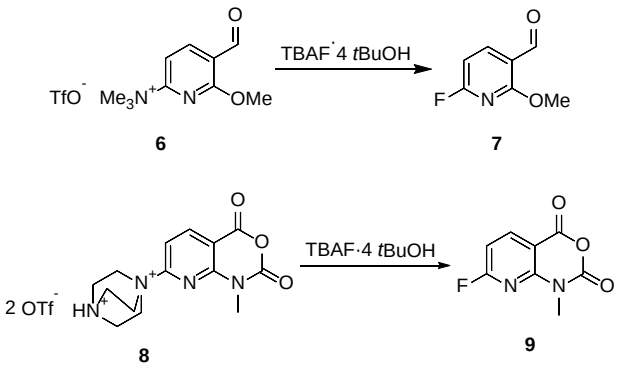
	30	92	23

Different prosthetic groups induce different pharmacokinetics of the tracers *in vivo*. Adding a methoxy group in the second position of [^{18}F]FNA makes the molecule more hydrophilic and enhances the solubility in aqueous solutions. 6-[^{18}F]Fluoro-2-methoxy-nicotinaldehyde is therefore less volatile and can be better isolated. Other substituents in the analogs of [^{18}F]FNA allow different conjugation strategies. The peptide conjugation of [^{18}F]FNA and 6-[^{18}F]fluoro-2-methoxy-nicotinaldehyde occurs in an oxime ligation at the N-terminal end of the peptide, while the ^{18}F-labeled analogs of *N*-methyl 6-fluoro-3-azaisatoic anhydride and 2,3,5,6-tetrafluorophenyl 6-chloro-4-methoxypyridine-3-carboxylate can undergo an acylation with the peptide. *N*-methyl 6-fluoro-3-azaisatoic anhydride is also interesting for peptide conjugation because of its novel acylation product: labeling a peptide and inserting a 2-methylaminopyridine-3-carboxamide fragment takes place in one step (Scheme 5).



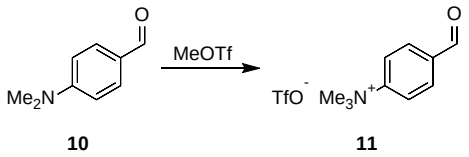
Scheme 5: Potential acylation of a peptide/protein with 6-[^{18}F]fluoro-3-azaisatoic anhydride introducing a 6-[^{18}F]fluoro-2-methylaminopyridine-3-carboxamide fragment to the peptide or protein.

Besides the precursors, also cold standards were synthesized for compounds which fluorinated standards were not available for purchase. 6-Fluoro-2-methoxy pyridine-3-carbaldehyde (**7**) and *N*-methyl 6-fluoro-3-azaisatoic anhydride (**9**) were synthesized by treating their triflate precursors with TBAF·4 *t*BuOH. The standards could not be completely purified which is why the actual yield could not be determined.



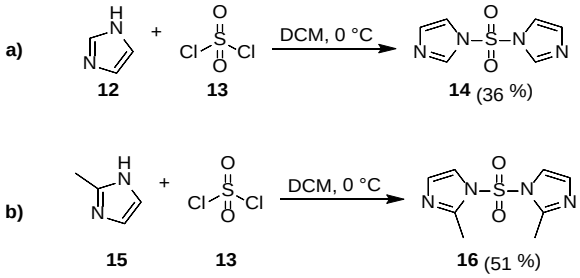
Scheme 6: Cold standard synthesis of 6-[^{18}F]fluoro-2-methoxy pyridine-3-carbaldehyde and 7-[^{18}F]fluoro-1-methyl-1H,2H,4H-pyrido[2,3-d][1,3]oxazine-2,4-dione.

Additionally to the [^{18}F]FNA and analog precursors, some [^{18}F]FBA precursors were tried to synthesize to compare labeling properties with [^{18}F]FNA as well as introducing a novel leaving group of sulfuryldiimidazoles. 6-(*N*,*N*,*N*-trimethylammonium)benzaldehyde triflate (**11**) was successfully synthesized in 35 % yield, by quaternarization of 6-(*N*,*N*-dimethylamino)benzaldehyde with methyltriflate (MeOTf), a strong methylation agent.[12]



Scheme 7: Synthesis of [^{18}F]FBA precursor **11** for radio-labeling.

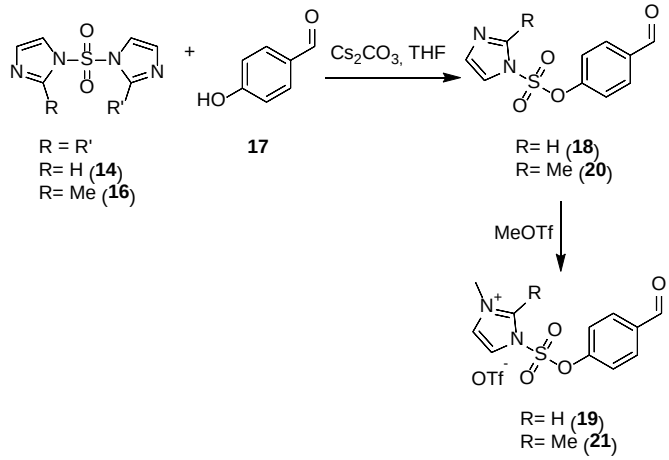
To introduce sulfuryldiimidazoles as a leaving group, *N*,*N'*-sulfuryldiimidazole (**14**) and 2-methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole (**16**) were synthesized. Here, imidazole (**12**) and 2-methylimidazole (**15**) were treated with sulfurylchloride (**13**) at 0 °C. The yield of **14** adds up to 36 %, **16** amounts to 51 %.



Scheme 8: Syntheses of *N,N'*-sulfuryldiimidazole and 2-methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole for further [^{18}F]FBA precursor synthesis.

The next step to the [^{18}F]FBA precursor would be an "imidazolsulfonation" of *p*-methoxybenzaldehyde with ceasiumcarbonate under mild conditions following the procedure of Civicos *et al*.[13] The resulting [^{18}F]FBA precursors 4-formylphenyl - 1H-imidazole- 1- sulfonate (**18**) and 4-formylphenyl 2- methyl- 1H- imidazole- 1-sulfonate (**19**) could undergo a quaternarization with MeOTf to enhance the leaving group ability of the imidazolesulfonate group. Unfortunately, the reactions could not be carried out due to time limitations.

Kommentiert [KL6]: Ist sulfonation hier richtig? Die Definition von Sulfonation ist laut wiki die S_EAr von Sulfonsäure mit einem Aromaten. Was könnte man hier stattdessen schreiben?

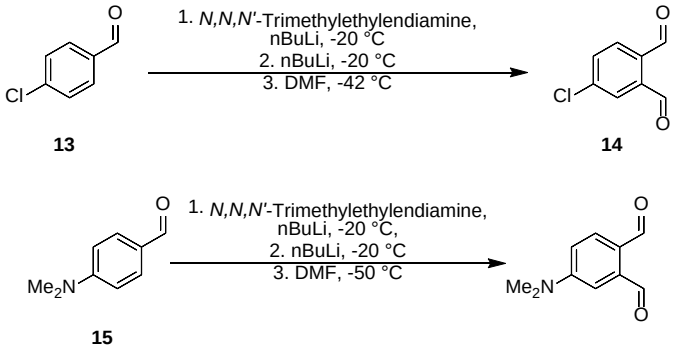


Scheme 9: Possible synthesis routes for potential [^{18}F]FBA precursors **18**, **19**, **20** and **21**.

The imidazolesulfonate compounds are also interesting for another synthesis route: the *o*-phthalaldehydes, which are also part of this work and the upcoming topic.

3.2.Synthesis of *o*-phthalaldehyde analogs

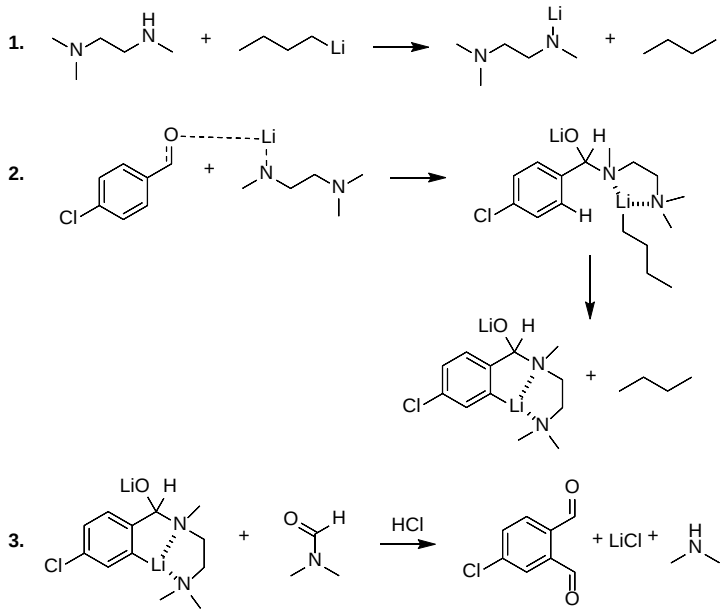
Another interesting molecule group are *o*-phthalaldehydes which can undergo a phthalimidine formation already described by D'Hollander *et al.* and Tung *et al*.[14, 15] To avoid harsh reaction conditions, two *o*-phthalaldehyde analogs were synthesized following the procedure described by Comins and Brown beginning from para-substituted benzaldehydes.[16] 4-Chloro-*o*-phthalaldehyde was obtained in 64 % yield, 4-dimethyl-*o*-phthalaldehyde was obtained in 39 % yield.



Scheme 10: Synthesis routes of 4-chloro-*o*-phthalaldehyde and 4-dimethylamino-*o*-phthalaldehyde.

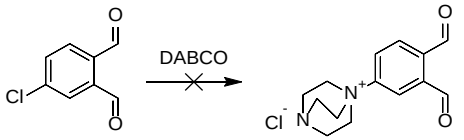
N,*N*,*N*'-Trimethylethylenediamine (TriMEDA) is activated by *n*-butyllithium (*n*BuLi), a strong base used for the carbonyl addition in this synthesis (Scheme 11, step 1), and conjugates to the carbonyl group of the para-substituted benzaldehyde. Next, TriMEDA directs *n*BuLi to the ortho position after the carbonyl conjugation (Scheme 11, step 2). In both steps *n*-butane is a by-product. The former benzaldehyde can act as an organolithium reagent by adding the carbonyl of dimethylformamide (DMF) to the ortho-position and complete the reaction by the addition of hydrochloric acid (Scheme 11, step 3). As scheme 11 suggests, this synthesis gives a lot of by-products, like *n*-butane, lithium chloride and dimethylamine as well as decomposition products. This leads to challenging work-ups, as the desired product can not be completely isolated due to the decomposition.

Kommentiert [KL7]: Ich brauch eine bessere Formulierung.



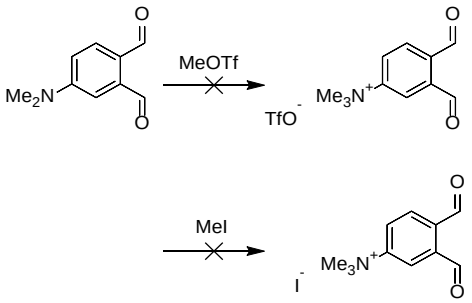
Scheme 11: Synthesis route and mechanism to 4-chloro-*o*-phthalaldehyde.

The further procedure planned for the *o*-phthalaldehyde analogs was the introduction of a leaving group to attempt a synthesis of 4-[^{18}F]fluoro-*o*-phthalaldehyde precursors. 4-Chloro-*o*-phthalaldehyde was treated with DABCO by the already described procedure, but the synthesis was not successful (Scheme 12).



Scheme 12: Attempted synthesis of 4-[^{18}F]fluoro-o-phthalaldehyde precursor.

An alternative for a 4-[^{18}F]fluoro-*o*-phthalaldehyde precursor was the quaternarization of 4-dimethyl-*o*-phthalaldehyde with MeOTf or methyliodide (MeI) to obtain an onium salt (Scheme 13). Unfortunately, both attempted synthesis routes did not result in the desired product or the product could not be isolated and purified. The unsuccessful attempt could be ascribed due to the decomposition as well as the problem of the isolation and purification of the phthalaldehyde.



Scheme 13: Attempted synthesis of 4-(*N*,*N*,*N*-trimethylammonium)phthalaldehydes.

As an alternative, bromo and fluoro phthalaldehyde analogs were attempted to synthesize by the already described procedure.

The Miyaura borylation is an established procedure to introduce a leaving group/Cu vermittelte fluorierungsmethode/ A popular precursor for r/Miyaura borylation/4-Bromo-*o*-phthalaldehyde and 4-fluoro-*o*-phthalaldehyde could not be obtained by the procedure of Comins and Brown.

4-Fluoro-*o*-phthalaldehyde → Isotopenaustausch → different approach, procedure by

3.3.Radiosynthesis

3.3.1. Minimalist

3.3.2. Basuli

4. Conclusion

- The minimalist fluorination method performed with EtOH is a feasible alternative to MeOH or the procedure by Basuli *et al.* and opens a broad field of application in radiolabeling. Further research to this topic is promising. [DATEN ERGÄNZEN, VERGLEICH ZW. EtOH und BASULI]
- The bioconjugation of *N*-methyl 6-[^{18}F]fluoro-3-azaisatoic anhydride is an interesting aspect of acylation and contributes to the functionalization of biomolecules. With the successful labeling of *N*-methyl 6-[^{18}F]fluoro-3-azaisatoic anhydride, a potential prosthetic group was obtained for bioconjugation through acylation and concurrent functionalization by addition of a 6-[^{18}F]fluoro-2-methylaminopyridine-3-carboxamide fragment.
- Unfortunately, the synthesis of ^{18}F-phthalaldehyde precursors for radiolabeling was unsuccessful. Although the synthesis of *o*-phthalaldehydes is challenging, the interest of ^{18}F-fluorinated *o*-phthalaldehydes as a prosthetic group for bioconjugation via e.g. phthalimidine formation is high. Other possible precursors of ^{18}F-fluorinated *o*-phthalaldehyde could be synthesized via imidazolesulfonates and are interesting components for further research into this topic.
- Imidalzolesulfonates are potential leaving groups for radiofluorination, but due to time limitation further research to this topic is remaining. Taking the topics of this work into account, the field of application of this newly introduced leaving group varies, starting from [^{18}F]FBA precursors synthesis to potential *o*-phthaladehydes.

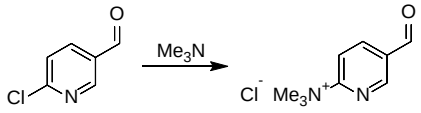
5. Experimental Section

5.1.General experimental conditions

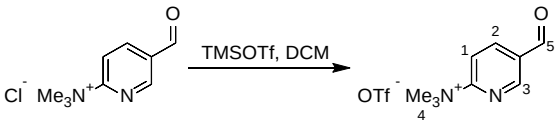
5.2.Analytical methods

5.3. Cold synthesis

5.3.1. 6-(*N*,*N*,*N*-Trimethylamino)nicotinaldehyde triflate



6-Chloronicotinaldehyde (1.50 g, 10.59 mmol, 1.00 eq.) was dissolved in 2M trimethylamine in THF (18.50 ml, 37.00 mmol, 3.50 eq) and stirred for 2 h at room temperature. The precipitation was filtered and washed with DCM, ether, and pentane. The solid residue was dried under reduced pressure. 6-(*N*,*N*,*N*-Trimethylamino)nicotinaldehyde chloride (1.17 g, 5.86 mmol) was obtained as a colorless solid in 55 % yield.

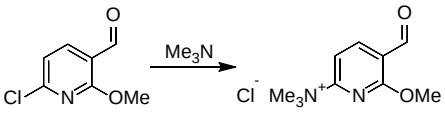


6-(*N*,*N*,*N*-Trimethylamino)nicotinaldehyde chloride (0.60 g, 2.97 mmol, 1.00 eq.) was suspended with DCM and cooled in an ice bath. TMSOTf (1.61 ml, 8.93 mmol) was added to the suspension, which dissolved the salt. The mixture was stirred for 1 h before the solvent was evaporated under vacuum. The solid was taken up in acetonitrile, filtered and the filtrate was again treated under reduced pressure. The solid was precipitated in diethyl ether and filtered before drying under high vacuum. 6-(*N*,*N*,*N*-Trimethylamino)nicotinaldehyde triflate (0.65 g, 2.08 mmol) was obtained as a colorless solid in 70 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.21 (s, 1H, H-5), 9.17 – 9.13 (m, 1H, H-2), 8.67 (dd, *J* = 8.6, 2.2 Hz, 1H, H-1), 8.30 (d, *J* = 8.6 Hz, 1H, H-3), 3.63 (s, 9H, H-4, H-4', H-4'').

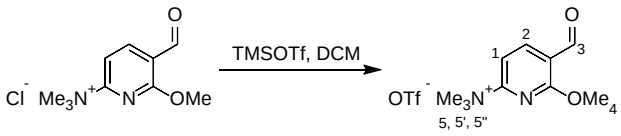
^{13}C NMR (101 MHz, DMSO) δ (ppm) = 191.02 (s), 150.22 (s), 141.11 (s), 132.78 (s), 116.17 (s), 54.65 (s).

5.3.2. 6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde triflate



To 6-chloro-2-methoxy-3-pyridinecarboxaldehyde (3.03 g, 17.65 mmol, 1.00 eq.) a 2M solution of trimethylamine in THF (30.80 ml, 61.78 mmol, 3.50 eq.) was added

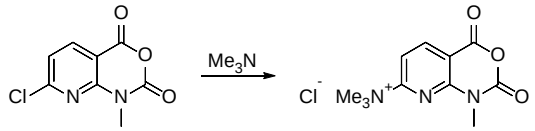
and stirred for 2 h. The precipitation was filtered and washed with DCM and ether. The solid residue was dried under reduced pressure. 6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde chloride (3.34 g, 14.48 mmol) was obtained as a colorless solid in 82 % yield.



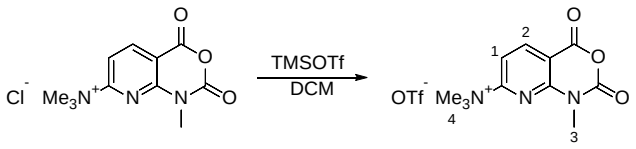
6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde chloride (2.74 g, 11.88 mmol, 1.00 eq.) was suspended in DCM. To the suspension, TMSOTf (6.47 ml, 35.64 mmol, 3.00 eq.) was added and stirred overnight. The precipitant was filtered on the next day, washed with ether and dried under reduced pressure. 6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde triflate (2.92 g, 8.49 mmol) was obtained as a colorless solid in 71 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.24 (s, 1H, H-3), 8.47 (d, *J* = 8.0 Hz, 1H, H-2), 7.81 (d, *J* = 8.0 Hz, 1H, H-1), 4.12 (s, 3H, H-4), 3.65 (s, 9H, H-5, H-5', H-5''). **^{13}C NMR** (101 MHz, DMSO) δ (ppm) = 187.70 (s), 143.05 (s), 108.15 (s), 55.05 (s), 54.36 (s).

5.3.3. *N*,*N*,*N*,1-tetramethyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d][1,3]oxazin-7-aminium triflate



7-Chloro-1-methyl-1H,2H,4H-pyrido[2,3-d][1,3]oxazine-2,4-dione (0.50 g, 2.35 mmol, 1.00 eq.) was dissolved in 2M trimethylamine in THF (10.00 ml, 20.00 mmol, 8.51 eq.) and stirred for 1 h. The suspension was filtered and washed with DCM, diethyl ether and *n*-pentane before drying under reduced pressure. *N*,*N*,*N*,1-tetramethyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d][1,3]oxazin-7-aminium chloride (0.62 g, 2.29 mmol) was obtained as a colorless solid in 97 % yield.

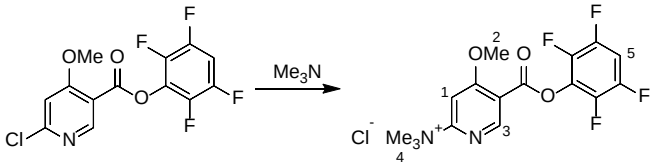


N,*N*,*N*,1-tetramethyl- 2,4- dioxo- 1H,2H,4H- pyrido[2,3- d] [1,3]oxazin-7-aminium chloride (0.60 g, 2.21 mmol, 1.00 eq.) was suspended in DCM before TMSOTf (1.00 ml, 5.52 mmol, 2.50 eq.) was added and stirred overnight. On the next day the solid residue was filtered, washed with diethyl ether and EtOAc and dried under reduced pressure. *N*,*N*,*N*,1-tetramethyl- 2,4- dioxo- 1H,2H,4H- pyrido[2,3-d] [1,3]oxazin-7- aminium triflate (0.81 g, 2.12 mmol) was obtained as a colorless solid in 96 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.79 (d, *J* = 8.4 Hz, 1H, H-1), 7.92 (d, *J* = 8.4 Hz, 1H, H-2), 3.65 (s, 9H, H-4), 3.55 (s, 3H, H-3).

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 159.56 (s), 156.92 (s), 151.73 (s), 147.40 (s), 143.70 (s), 110.20 (s), 110.13 (s), 54.64 (s), 30.47 (s).

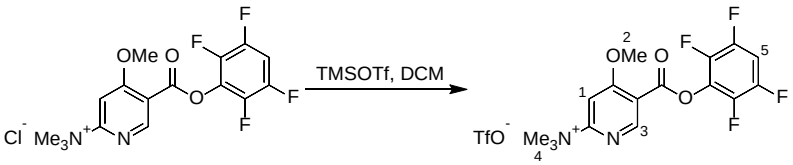
5.3.4. 4-Methoxy- *N*,*N*,*N*-trimethyl- 5- [(2,3,5,6-tetrafluorophenoxy)carbonyl]-pyridin-2- aminium triflate



2,3,5,6-tetrafluorophenyl 6-chloro- 4- methoxypyridine- 3- carboxylate (1.50 g, 4.47 mmol, 1.00 eq.) was dissolved in 2M trimethylamine in THF (20.00 ml, 40.00 mmol, 9.00 eq.) and stirred for 2 h. The suspension was filtered, and the solid residue was washed with DCM and ether before drying under reduced pressure. 4-methoxy- *N*,*N*,*N*-trimethyl- 5- [(2,3,5,6- tetrafluorophenoxy)carbonyl] pyridine-2-aminium chloride (0.52 g, 1.31 mmol) was obtained as a colorless solid in 30 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 9.11 (s, 1H, H-1), 8.04 (tt, *J* = 10.9, 7.4 Hz, 1H, H-5), 7.91 (s, 1H, H-3), 4.17 (s, 3H, H-2), 3.65 (s, 9H, H-4, H-4', H-4'').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 168.53 (s), 161.93 (s), 158.78 (s), 151.59 (s), 147.01 – 142.91 (m), 142.01 – 137.39 (m), 114.30 (s), 105.08 (t, *J* = 23.8 Hz), 101.51 (s), 58.19 (s), 54.76 (s).

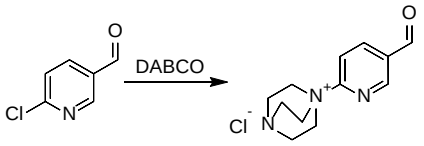


4-methoxy-*N*,*N*,*N*-trimethyl-5-[(2,3,5,6-tetrafluorophenoxy)carbonyl]pyridin-2-aminium chloride (0.45 g, 1.14 mmol, 1.00 eq.) was suspended in DCM and TMSOTf (0.70 ml, 3.86 mmol, 3.39 eq.) was added. The resulting suspension was filtered and washed with ether and EtOAc before drying under reduced pressure. 4-methoxy-*N*,*N*,*N*-trimethyl-5-[(2,3,5,6-tetrafluorophenoxy)carbonyl]pyridin-2-aminium triflate (0.53 g, 1.05 mmol) was obtained as a colorless solid in 92 % yield.

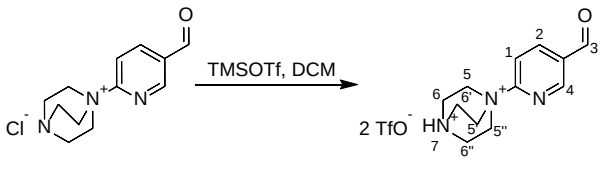
^{1}H NMR (400 MHz, DMSO) δ (ppm) = 9.11 (s, 1H, H-1), 8.03 (tt, *J* = 10.8, 7.4 Hz, 1H, H-5), 7.92 (s, 1H, H-3), 4.17 (s, 3H, H-2), 3.65 (s, 9H, H-4, H-4', H-4'').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 168.33 (s), 161.73 (s), 158.57 (s), 151.38 (s), 147.70 – 142.77 (m), 141.66 – 136.73 (m), 114.08 (s), 104.86 (t, *J* = 23.8 Hz), 101.31 (s), 59.02 (s), 57.98 (s), 54.55 (s).

5.3.5. 1-(5-formylpyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium triflate



6-Chloronicotinaldehyde (1.25 g, 8.83 mmol, 1.00 eq.) and DABCO (2.97 g, 26.50 mmol, 3.00 eq.) were dissolved with 30 ml toluene and stirred for 2.5 h. The suspension was filtered, washed with DCM, diethyl ether and EtOAc before drying under reduced pressure. 1-(5-formylpyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium chloride (1.90 g, 7.50 mmol) was obtained as an off-white solid in 84 % yield.

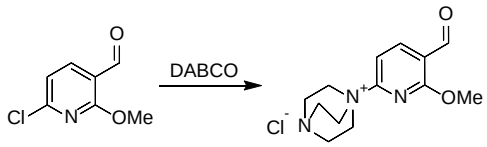


1-(5-formylpyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium chloride (1.50 g, 5.92 mmol, 1.00 eq.) was suspended in DCM. TMSOTf (4.00 ml, 22.10 mmol, 3.71 eq.) was added to the suspension and stirred for 1 h. The suspension was filtered and washed with diethyl ether and EtOAc before drying under reduced pressure. 1-(5-formylpyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium triflate (2.98 g, 5.77 mmol) was obtained as a colorless solid in 97 %yield.

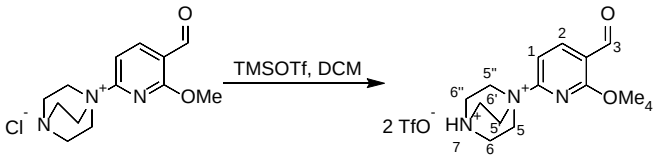
^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.22 (s, 1H, H3), 9.20 (d, *J* = 2.1 Hz, 1H, H-4), 8.72 (d, *J* = 6.4 Hz, 1H, H-2), 8.27 (d, *J* = 8.6 Hz, 1H, H-1), 4.32 – 4.15 (m, 6H, H-6, H-6', H-6''), 3.79 – 3.59 (m, 6H, H-5, H-5', H-5'').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 191.00 (s), 157.57 (s), 150.45 (s), 141.50 (s), 133.16 (s), 117.16 (s), 52.91 (s), 43.97 (s).

5.3.6. 1-(5-formyl-4-methoxypyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2] octan-1-ylium triflate



6-Chloro-2-methoxy-nicotinaldehyde (1.50 g, 8.77 mmol, 1.00 eq.) and DABCO (2.95 g, 26.30 mmol, 3.00 eq.) were dissolved with 30 ml toluene and stirred for 1 h. The suspension was filtered, washed with DCM, diethyl ether and pentane before drying under reduced pressure. 1-(5-formyl-4-methoxypyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium chloride (1.86 g, 6.56 mmol) was obtained as an off-white solid in 75 % yield.

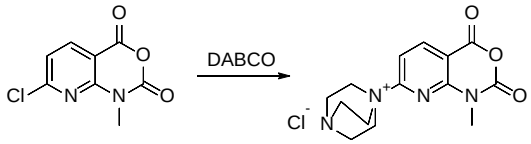


1-(5-formyl-4-methoxypyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium chloride (1.50 g, 5.28 mmol, 1.00 eq.) was suspended in DCM. TMSOTf (4.00 ml, 22.10 mmol, 4.19 eq.) was added to the suspension and stirred for 2 h. The suspension was filtered, washed with diethyl ether and EtOAc and dried under reduced pressure. 1-(5-formyl-4-methoxypyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]-octan-1-ylium triflate (1.55 g, 2.84 mmol) was obtained as an off-white solid in 53 %yield.

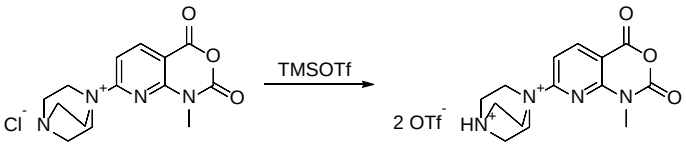
^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.22 (s, 1H, H-3), 8.46 (d, *J* = 8.2 Hz, 1H, H-1), 7.74 (d, *J* = 8.1 Hz, 1H, H-2), 4.10 (s, 3H, H-4), 3.97 – 3.87 (m, 6H, H-6, H-6', H-6''), 3.25 – 3.16 (m, 6H, H-5, H-5', H-5'').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 187.79 (s), 162.17 (s), 156.63 (s), 143.04 (s), 119.97 (s), 109.32 (s), 55.08 (s), 53.62 (s), 45.06 (s).

5.3.7. 1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda-5,4-diazabicyclo-[2.2.2]octan-1-ylium triflate



7-Chloro-1-methyl-1H,2H,4H-pyrido[2,3-d] [1,3]oxazine-2,4-dione (0.52 g, 2.44 mmol, 1.00 eq.) and DABCO (0.80 g, 7.13 mmol, 3.00 eq.) were dissolved with 30 ml toluene and stirred for 1 h. The suspension was filtered, washed with DCM, diethyl ether and pentane before drying under reduced pressure. 1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda-5,4-diazabicyclo[2.2.2]-octan-1-ylium chloride (0.77 g, 2.39 mmol) was obtained as an off-white solid in 98 % yield.

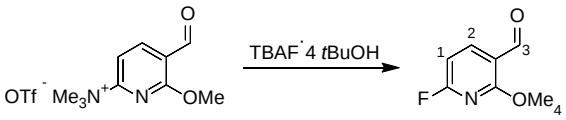


1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda5,4-diazabicyclo[2.2.2]octan-1-ylium chloride (0.70 g, 2.15 mmol, 1.00 eq.) was suspended in DCM. TMSOTf (1.56 ml, 8.62 mmol, 4.00 eq.) was added to the suspension and stirred for 2 h. The suspension was filtered and washed with diethyl ether and EtOAc before drying under reduced pressure. 1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda5,4-diazabicyclo-[2.2.2]octan-1-ylium triflate (1.04 g, 1.75 mmol) was obtained as an off-white solid in 81 %yield.

Kommentiert [KL8]: NMR

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.83 (d, *J* = 8.4 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 3.68 (t, 6H), 3.60 (t, *J* = 6.2 Hz, 6H), 3.44 (s, 3H).

5.3.8. 6-Fluoro-2-methoxy pyridine-3-carbaldehyde



6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde triflate (0.56 g, 1.63 mmol, 1.00 eq.) and TBAF·4 *t*BuOH (2.27 g, 4.07 mmol, 2.5 eq.) were dissolved in acetonitrile. After 2 h the solution was diluted with ether and washed with water and brine. The combined organic phases were dried with $MgSO_4$, filtered and the solvents were removed under reduced pressure. 6-Fluoro-2-methoxy pyridine-3-carbaldehyde was obtained as a yellow solid.

Kommentiert [KL9]: yield

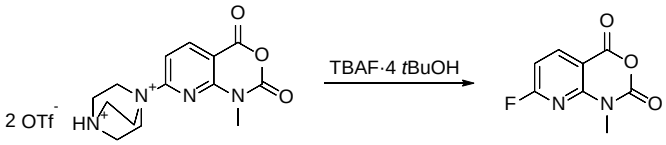
^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.17 (s, 1H), 8.38 – 8.17 (m, 1H), 6.96 – 6.85 (m, 1H), 4.01 (s, 3H).

CNMR

Kommentiert [KL10]: NMR seltsam

^{19}F NMR (376 MHz, DMSO) δ (ppm) = -60.00 (s).

5.3.9. 7-F luoro-1- methyl- 1H,2H,4H- pyrido[2,3- d][1,3]oxazine- 2,4- dione

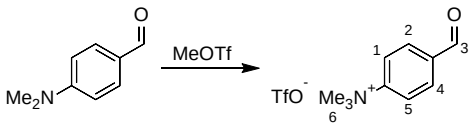


1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda5,4-diazabicyclo[2.2.2]octan-1-ylium triflate (0.25 g, 0.42 mmol, 1.00 eq.) and TBAF·4 *t*BuOH (0.58 g, 1.05 mmol, 2.50 eq.) were dissolved in AcCN and stirred for 10 min. The solution was diluted with diethyl ether, washed with water and brine, dried over $MgSO_4$ and filtrated. The solvent was removed under reduced pressure. 7-Fluoro-1- methyl- 1H,2H,4H- pyrido[2,3- d][1,3]oxazine- 2,4- dione was obtained as a mixture of product and byproducts.

NMR

Kommentiert [KL11]:

5.3.10. 4-Trimethylammoniumbenzaldehyde triflate

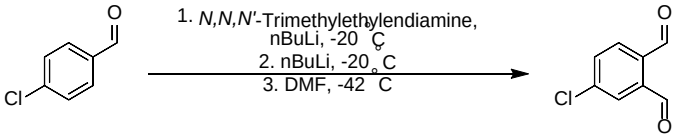


4-Dimethylaminobenzaldehyde (5.18 g, 34.73 mmol, 1.00 eq.) was dissolved in DCM and MeOTf (6.00 ml, 54.94 mmol, 1.58 eq.) was added to the solution. The suspension was filtered, washed with diethyl ether and dried under reduced pressure. 4-Trimethylammoniumbenzaldehyde triflate (3.87 g, 12.36 mmol) was obtained as a colourless solid in 35 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.11 (s, 1H, H-3), 8.21 (d, *J* = 8.8 Hz, 2H, H-1, H-5), 8.15 (d, *J* = 8.4 Hz, 2H, H-2, H-4), 3.66 (s, 9H, H-6).

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 192.13 (s), 151.06 (s), 136.80 (s), 130.90 (s), 121.71 (s), 56.41 (s).

5.3.11. 4-Chloro-*o*-phthalaldehyde

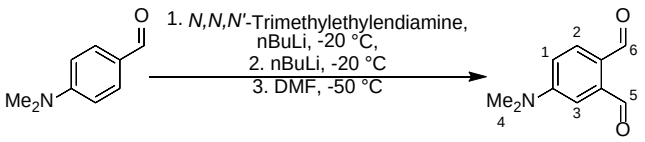


N,*N*,*N*'-Trimethylethylendiamine (0.43 ml, 3.04 mmol, 1.07 eq) was diluted with THF and cooled to -20 °C before dropwise addition of nBuLi (1.30 ml, 2.94 mmol, 1.03 eq.). The mixture was stirred for 30 min after which 4-chlorobenzaldehyde (0.40 g, 2.85 mmol, 1.00 eq.) dissolved in THF (6 ml) was added dropwise. The mixture was stirred for 15 min before nBuLi (4.00 ml, 8.55 mmol, 3.00) was added dropwise. After stirring for 3 h at −20 °C, DMF (1.50 ml, 17.10 mmol, 6.00 mmol) was added dropwise at −42 °C. The mixture was stirred for 5 min before the cooling bath was removed and left stirring overnight to warm up to room temperature. 30 ml of 2N HCl were added, extracted with EtOAc, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was purified via flash chromatography on silica (Hexane/EtOAc 1:10/1:3). After recrystallisation, 4-chloro-*o*-phthalaldehyde (0.31 g, 1.84 mmol) was obtained as yellow crystals in 64 %.

NMR

Kommentiert [KL12]: NMR sieht schlecht aus, leider keine Probe mehr zur Verfügung für ein neues.

5.3.12. 4-Dimethylamino-*o*-phthalaldehyde

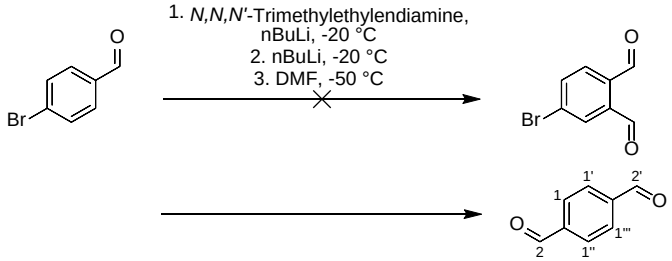


N,N,N'-Trimethylethylendiamine (1.85 ml, 14.35 mmol, 1.07 eq) was diluted with THF and cooled to -20 °C for 15 min before dropwise addition of nBuLi (5.50 ml, 13.81 mmol, 1.03 eq.). The mixture was stirred for 15 min after which 4-dimethylamino benzaldehyde (2.10 g, 13.41 mmol, 1.00 eq.) in THF (6 ml) was added dropwise. The mixture was stirred for 15 min before nBuLi (16.00 ml, 40.23 mmol, 3.00 eq.) was added dropwise. The reaction was allowed to stir at −20 °C overnight. DMF (6.50 ml, 80.46 mmol, 6.00 eq.) was added dropwise at −50 °C. The mixture was stirred for 5 min before the cooling bath was removed and left stirring to warm up to room temperature. 20 ml of cold 2N HCl were added, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was purified via flash chromatography on silica (Hexane/EtOAc 1:3). After recrystallisation, 4-dimethylamino-*o*-phthalaldehyde (0.93 g, 5.25 mmol) was obtained as yellow crystals in 39 %.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.50 (s, 1H, H-6), 10.16 (s, 1H, H-5), 7.84 (d, *J* = 8.8 Hz, 1H, H-2), 7.10 (d, *J* = 2.8 Hz, 1H, H-3), 6.99 (dd, *J* = 8.8, 2.8 Hz, 1H, H-1), 3.09 (s, 6H, H-4, H-4').

^{13}C NMR (101 MHz, DMSO) δ 194.01 (s), 190.22 (s), 153.26 (s), 138.29 (s), 133.47 (s), 123.41 (s), 114.27 (s), 111.86 (s), 39.65 (s).

5.3.13. *p*-Phthalaldehyde



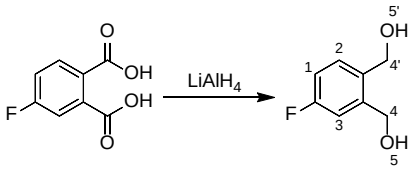
N,N,N'-Trimethylethylendiamine (1.48 ml, 11.63 mmol, 1.07 eq.) was diluted with THF and cooled to -20 °C before dropwise addition of nBuLi (4.50 ml, 11.20 mmol, 1.03 eq.). The mixture was stirred for 15 min after which 4-bromobenzaldehyde (2.00 g, 10.87 mmol, 1.00 eq.) in THF (10 ml) was added dropwise. The mixture was stirred for 15 min before nBuLi (13.00 ml, 32.61 mmol, 3.00 eq.) was added dropwise and stirred overnight at −20 °C. DMF (5.00 ml, 65.22 mmol, 6.00 eq.) was added dropwise at −42 °C and stirred for 15 min before the

cooling bath was removed and left to warm up to room temperature. 30 ml of 5N HCl were added, extracted with EtOAc,, washed with water and brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was purified via flash chromatography on silica (Hexane/EtOAc 1:3). After recrystallisation, *p*-phthalaldehyde (0.33 g, 2.45 mmol) was obtained as a yellow solid as ^{1}H-NMR and ^{13}C-NMR suggest.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 10.13 (s, 2H, H-2, H-2'), 8.10 (s, 4H, H-1, H-1', H-1'', H-1''').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 193.56 (s), 140.21 (s), 130.47 (s).

5.3.14. [5-Fluoro-2-(hydroxymethyl)phenyl]methanol

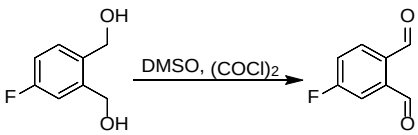


4-Fluorobenzene-1,2-dicarboxylic acid (2.50 g, 15.05 mmol, 1.00 eq.) was dissolved in THF and added dropwise to a solution of $LiAlH_4$ (1.48 g, 39.13 mmol, 2.60 eq.) in THF at 0 °C. The ice bath was removed and the mixture was refluxed for 2 h at 50 °C. After removing the oil bath and cooling to room temperature, 10 ml of water were added dropwise at 0 °C. 20 ml of 15 % (w/v) NaOH were added dropwise and the resulting suspension was filtrated. The organic phase was extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure. [5-Fluoro-2-(hydroxymethyl)phenyl]methanol (1.48 g, 9.47 mmol) was obtained as yellow oil in 61 % yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.29 – 7.23 (m, 1H, H-2), 7.04 (dd, *J* = 9.3, 2.7 Hz, 1H, H-3), 6.96 (td, *J* = 8.4, 2.7 Hz, 1H, H-1), 4.59 (d, *J* = 6.1 Hz, 4H, H-4, H-4'), 3.74 – 3.70 (m, 1H, H-5'), 1.87 – 1.83 (m, 1H, H-5).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) = 162.53 (d, *J* = 247.1 Hz), 141.92 (d, *J* = 6.8 Hz), 134.89 (d, *J* = 3.5 Hz), 131.35 (d, *J* = 8.1 Hz), 116.25 (d, *J* = 21.7 Hz), 114.67 (d, *J* = 20.9 Hz), 63.13 (d, *J* = 19.8 Hz).

5.3.15. 4-Fluoro-*o*-phthalaldehyde



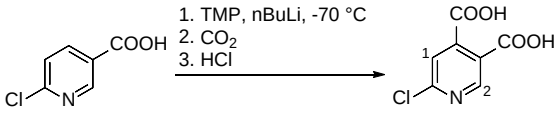
DMSO (3.26 ml, 45.91 mmol, 5.20 eq.) was added dropwise to $(COCl)_2$ (1.96 ml, 22.95 mmol, 2.60 eq.) at −78 °C and stirred for 10 min. [5-Fluoro-2-(hydroxymethyl)phenyl]methanol (1.38 g, 8.83 mmol, 1.00 eq.) in DCM was added to the mixture and stirred for 1 h before the cooling bath was removed and the reaction was warmed up to room temperature. Trimethylamine (13.00 ml, 1.40 ml/mmol diol) was added dropwise and the mixture was stirred at room temperature overnight. Water was added to the reaction, followed by extraction with DCM, washing with water and brine, drying with $MgSO_4$ and filtering. The solvent was removed under reduced pressure. 4-Fluoro-*o*-phthalaldehyde (0.89 g, 5.87 mmol) was obtained as a dark yellow solid in 66 % yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 10.54 (d, *J* = 1.9 Hz, 1H), 10.36 (s, 1H), 7.98 (dd, *J* = 8.5, 5.2 Hz, 1H), 7.60 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.40 (ddd, *J* = 8.4, 7.7, 2.6 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) = 190.84 (s), 190.72 (s), 165.70 (d, *J* = 259.1 Hz), 139.67 – 138.94 (m), 134.84 (d, *J* = 9.1 Hz), 133.32 – 132.55 (m), 120.66 (d, *J* = 21.9 Hz), 117.32 (d, *J* = 23.1 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -101.70 (s).

5.3.16. 6-Chloropyridine-3,4- dicarboxylic acid

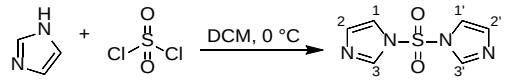


nBuLi (20.30 ml, 50.76 mmol, 4.00 eq.) was added dropwise to TMP (6.45 ml, 38.07 mmol, 3.00 eq.) at −70 °C. 6-Chloronicotincarboxylic acid (2.00 g, 12.69 mmol, 1.00 eq.) was added dropwise and the mixture was stirred for 2 h. The solution was poured on to freshly crushed dry ice and left overnight to react and warm up to room temperature. The precipitant was dried under reduced pressure and the residue was washed with Hexane/Ether (30:70), filtered and dried again under reduced pressure. The dried solid was dissolved in water and acidified to pH 4, extracted with DCM and the aqueous phase was acidified to pH 1. The aqueous phase was evaporated under reduced pressure, filtrated and washed with little water. 6-Chloropyridine-3,4-dicarboxylic acid (1.56 g, 7.74 mmol) was obtained as colourless solid in 61 %.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.82 (s, 1H, H-1), 7.79 (s, 1H, H-2).

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 166.73 (s), 165.88 (s), 153.77 (s), 151.44 (s), 145.63 (s), 125.32 (s), 122.84 (s).

5.3.17. *N,N'*-Sulfuryldiimidazole

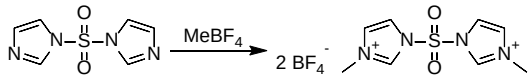


Imidazole (3.23 g, 47.50 mmol, 4.75 eq.) in DCM was added to a solution of sulfuryl chloride (0.81 ml, 10 mmol, 1.00 eq.) in DCM at 0 °C and stirred overnight. The suspension was filtered and the solvent was removed under reduced pressure. The crude product was recrystallized with isopropanol. *N,N'*-Sulfuryldiimidazole (0.71 g, 3.58 mmol) was obtained as a colourless solid in 36 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.50 (dd, *J* = 1.3, 0.9 Hz, 2H, H-3, H-3'), 7.95 – 7.86 (m, 2H, H-2, H-2'), 7.23 (dd, *J* = 1.7, 0.8 Hz, 2H, H-3, H-3').

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 137.76 (s), 132.00 (s), 118.56 (s).

5.3.18. 1-Methyl-3-(3-methylimidazol-3-ium-1-ylsulfonyl)imidazol-1-ium tetrafluoroborate



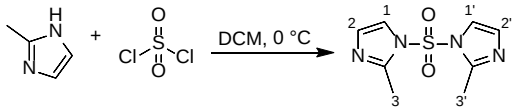
N,N'-Sulfuryldiimidazole (1.00 g, 5.05 mmol, 1.00 eq.) and Me_3OBF_4 (1.53 g, 10.34 mmol, 2.05 eq.) were dissolved in DCM at 0 °C and stirred overnight. The suspension was filtered on the next day, washed with diethyl ether and EtOAc and dried under removed pressure. 1-Methyl-3-(3-methylimidazol-3-ium-1-ylsulfonyl)imidazol-1-ium tetrafluoroborate (2.02 g, 5.02 mmol) was obtained as a colourless solid in 99 % yield.

H NMR zeigt viel zu viele unreinheiten

Kommentiert [KL13]:

^{13}C NMR (101 MHz, DMSO) δ 123.34 (d, *J* = 32.0 Hz), 119.52 (d, *J* = 40.7 Hz), 60.96 (d, *J* = 241.2 Hz), 35.57 (d, *J* = 26.3 Hz).

5.3.19. 2-Methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole



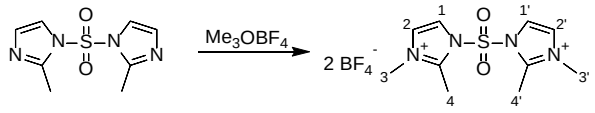
2-Methylimidazole (20.30 g, 246.80 mmol, 4.00 eq.) was suspended in DCM and cooled to 0 °C. Sulfuryl chloride (5.00 ml, 61.70 mmol, 1.00 eq.) was added dropwise and stirred for1 h, then warmed up to room temperature and left stirring overnight. Water (100 ml) was added and the organic phase was extracted with DCM; washed with water and brine, dried over $MgSO_4$ and filtered. The solvent was removed

under reduced pressure. 2-Methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole (7.12 g, 31.49 mmol) was obtained as a colourless solid in 51 % yield.

^{1}H NMR (400 MHz, DMSO) δ (ppm) = 7.82 (d, *J* = 1.9 Hz, 2H, H-1, H-1‘), 7.03 (d, *J* = 1.9 Hz, 2H, H-2, H-2‘), 2.49 (s, 6H, H-3, H-3‘).

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 145.54 (s), 128.65 (s), 120.63 (s), 14.76 (s).

5.3.20. 3-(2,3- dimethylimidazol- 3- ium- 1- yl sulfonyl)-1,2-dimethylimidazol-1- ium tetrafluoroborate



2-Methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole (0.60 g, 2.65 mmol, 1.00 eq.) and Me_3OBF_4 (0.80 g, 5.43 mmol, 2.05 eq.) were dissolved in DCM at 0 °C and stirred overnight. The suspension was filtered on the next day, washed with diethyl ether and EtOAc and dried under removed pressure. 3-(2,3- dimethylimidazol- 3- ium-1- yl sulfonyl)-1,2- dimethylimidazol- 1- ium tetrafluoroborate (0.51 g, 1.18 mmol) was obtained as a colourless solid in 45 % yield.

^{1}H NMR (400 MHz, DMSO) δ 7.55 (d, *J* = 1.9 Hz, 2H, H-1, H-1‘), 7.50 (d, *J* = 1.9 Hz, 2H, H-2, H-2‘), 3.72 (s, 6H, H-3, H-3‘), 3.19 (s, 6H, H-4, H-4‘).

^{13}C NMR (101 MHz, DMSO) δ (ppm) = 123.02 (s), 118.65 (s), 117.60 (s), 33.88 (s), 10.24 (s).

5.4.Radiosynthesis

6. Appendix

6.1.Abbreviations

6.2.NMR spectra

6.2.1. 6-(*N*,*N*,*N*-Trimethylamino)nicotinaldehyde triflate

^{1}H NMR

^{13}C NMR

6.2.2. 6-(*N*,*N*,*N*-Trimethylamino)-2-methoxy-nicotinaldehyde triflate

^{1}H NMR

^{13}C NMR

6.2.3. *N*,*N*,*N*,1-tetramethyl- 2,4- dioxo- 1H,2H,4H- pyrido[2,3- d] [1,3]oxazin-7- aminium triflate

^{1}H NMR

^{13}C NMR

6.2.4. 4-Methoxy- *N*,*N*,*N*-trimethyl- 5- [(2,3,5,6- tetrafluorophenoxy)carbonyl]-pyridin-2- aminium triflate

^{1}H NMR

^{13}C NMR

6.2.5. 1-(5-formylpyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2]octan-1-ylium triflate

^{1}H NMR

^{13}C NMR

6.2.6. 1-(5-formyl-4-methoxypyridin-2-yl)-1-lambda-5,4-diazabicyclo[2.2.2] octan-1-ylium triflate

^{1}H NMR

^{13}C NMR

6.2.7. 1-(1-methyl-2,4-dioxo-1H,2H,4H-pyrido[2,3-d] [1,3]oxazin-7-yl)-1lambda-5,4-diazabicyclo-[2.2.2]octan-1-ylium triflate

^{1}H NMR

^{13}C NMR

6.2.8. 6-Fluoro-2-methoxy pyridine-3-carbaldehyde

^{1}H NMR

^{13}C NMR

6.2.9. 7 Fluoro-1- methyl- 1H,2H,4H- pyrido[2,3- d][1,3]oxazine- 2,4-dione

^{1}H NMR

^{13}C NMR

6.2.10. 4-Trimethylammoniumbenzaldehyde triflate

^{1}H NMR

^{13}C NMR

6.2.11. 4-Chloro-*o*-phthalaldehyde

^{1}H NMR

^{13}C NMR

6.2.12. 4-Dimethylamino-*o*-phthalaldehyde

^{1}H NMR

^{13}C NMR

6.2.13. *p*-Phthalaldehyde

^{1}H NMR

^{13}C NMR

6.2.14. [5-Fluoro-2-(hydroxymethyl)phenyl]methanol

^{1}H NMR

^{13}C NMR

6.2.15. 4-Fluoro-*o*-phthalaldehyde

^{1}H NMR

^{13}C NMR

6.2.16. 6-Chloropyridine-3,4-dicarboxylic acid

^{1}H NMR

^{13}C NMR

6.2.17. *N,N'*-Sulfuryldiimidazole

^{1}H NMR

^{13}C NMR

6.2.18. 1-Methyl-3-(3-methylimidazol-3-ium-1-ylsulfonyl)imidazol-1-ium tetrafluoroborate

^{1}H NMR

^{13}C NMR

6.2.19. 2-Methyl-1-(2-methylimidazol-1-yl sulfonyl)imidazole

^{1}H NMR

^{13}C NMR

6.2.20. 3-(2,3- dimethylimidazol- 3- ium- 1- yl sulfonyl)-1,2-dimethylimidazol-1- ium tetrafluoroborate

^{1}H NMR

^{13}C NMR

6.3.HPLC chromatograms

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Danksagung