Bettina Grumm BSc

Inverse Electron Demand Diels-Alder Reactions (iEDDA) of 1,2,4,5-Tetrazines and Cyclic Enols

MASTERARBEIT

zur Erlangung des akademischen Grades

Diplom-Ingenieurin

Masterstudium Technische Chemie

eingereicht an der

Technischen Universität Graz

Betreuer

Assoc. Prof. Dipl.-Ing. Dr. techn. Christian Slugovc

Institut für Chemische Technologie von Materialien

Dr. Astrid-Caroline Knall

Graz, August 2014

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Acknowledgement

I would like to thank everyone who encouraged me during my studies.

I want to thank Prof. Christian Slugovc and Dr. Astrid Knall for providing an interesting topic. Both supported me during my master thesis and helped me whenever problems arose. They pointed out new aspects and assisted in adding new facets to my work. Although Astrid was in England the whole time, she was always contactable.

Additionally I want to thank my colleagues at work for their support. Especially my working group, Anita, Chrissi, Eva, Simone and Sarah helped me whenever I needed them and we had a lot of fun together. I also want to acknowledge the help of Dr. Petra Kaschnitz regarding NMR-spectroscopy, Prof. Robert Saf for the MS-spectroscopy, the Baumgartner/Marschner group for reactants and crystal structure analysis, Oliver Hofmann for DFT-calculations and Birgit Ehmann for providing all the chemicals needed and helping with hints and tricks.

I also want to thank my friends who not only helped me during the last six months but the last years. They stood by me, supported my decisions and had always a sympathetic ear. Especially my best friend, Yassi, was very important for me, because she knew exactly when to motivate and when to distract me regarding my work.

Furthermore I want to thank my parents, Christine and Maximilian, and my sister Sandi to have supported me in any way possible during the last 23 years. They always motivated me and without them, my studies would not have been possible. I also want to acknowledge Gisi and Hans, because they encouraged me during the last 4 years and always had a sympathetic ear.

Most importantly, thanks go to my boyfriend Luki. Without his complete support and his ability to always calm me down, I would not have reached the goals I wanted.

Abstract

1,2,4,5-tetrazines (or s-tetrazines) are a group of aromatic heterocyclic compounds which are coloured in different shades of red and pink. Due to their diversity concerning different substituents in 3- and 6-position, they are regarded as interesting reactants in organic chemistry. One reaction, where *s*-tetrazines are frequently used, are inverse electron demand Diels-Alder reactions (iEDDA) with dienophiles like cyclic enol ethers or norbornenes. Especially with electron withdrawing groups on the tetrazine, this reaction can achieve high reaction rates. For this thesis, various tetrazines and three different enols (2,3-dihydrofuran, 3,4-dihydro-2*H*-pyran and 3,4-dihydro-2-methoxy-pyran) were used. Initially, various reaction conditions were tested for the reaction between3,6- di(pyridin-2-yl9-1,2,4,5-tetrazine and 2,3-dihydrofuran. Thereby, two side products – 3,6-di(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazine and 4,7-di(pyridin-2-yl)-2,3-dihydrofuro[2,3-d]pyridazin, could be identified. Subsequently, different tetrazines and the aforementioned cyclic enol ethers were tested and compared concerning reaction rate and yield. For closer investigations regarding the reaction rate, the fading of the red colour as a consequence of iEDDA was measured using UV-Vis spectroscopy. Additionally, the reaction between the dipyridinyl-substituted tetrazine and 2,2'-((3aR,4R,7R,7aS)-3,3a,7,7a- tetrahydro-4H- 4,7- methanoindene -2,4- diyl)bis(1,1,1,3,3,3 – hexamethyl-2-(trimethylsilyl) trisilane was carried out and the resulting product was characterised through NMR, crystal structure analysis and exact mass analysis. To gain more information about the reaction mechanism of the iEDDA and possible stable intermediates, some reactions were monitored *in situ* in NMR-tubes. Regarding this information, different rate-determining steps for the reactions of the five- and six-membered cyclic enol ethers were defined.

Kurzfassung

1,2,4,5-Tetrazine sind eine Gruppe aromatischer Heterozyklen, die in verschiedenen rot bis pink Tönen gefärbt sind. Wegen ihrer großen Vielfalt durch unterschiedliche mögliche Substituenten in 3- und 6-Position sind sie interessante Moleküle für die organische Chemie. Sie werden beispielsweise in Diels-Alder Reaktionen mit inversem Elektronenbedarf eingesetzt. Als Dienophile werden zum Beispiel zyklische Enolether oder Norbornene eingesetzt. In dieser Arbeit wurden unterschiedliche Tetrazine, sowie drei verschiedene Olefine (2,3-Dihydrofuran, 3,4-Dihydro-2*H*-pyran und 3,4-Dihydro-2-methoxy-pyran) eingesetzt. Zu Beginn wurde eine Reaktion zwischen 3,6-Di(pyridin-2yl)-1,2,4,5-tetrazin und 2,3-Dihydrofuran, unter verschiedenen Reaktionsbedingungen durchgeführt. Dabei wurden zwei Nebenprodukte gefunden, die als 3,6-Di(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin und 4,7-Di(pyridin-2-yl)-2,3-dihydrofuro[2,3-d]pyridazin identifiziert werden konnten. Darüber hinaus wurden weitere Tetrazine mit den drei oben genannten Olefinen umgesetzt und ihre Reaktionsgeschwindigkeiten und Ausbeuten verglichen. Zur genaueren Untersuchung der Reaktionsgeschwindigkeit wurde das Verblassen der roten Farbe durch die iEDDA Reaktion mittels UV-Vis Spektroskopie untersucht. Zusätzlich wurde die Reaktion zwischen 3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazin und 2,2'-((3aR,4R,7R,7aS)-3,3a,7,7a-Tetrahydro-4H-4,7-methanoindene-2,4-diyl)bis (1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilane durchgeführt und das Produkt mittels NMR, Kristallstrukturanalyse und exakter Massenbestimmung analysiert. Um genauere Informationen über den Reaktionsmechanismus der iEDDA und möglichen Zwischenprodukten zu erhalten, wurden Reaktionen *in situ* mittels NMR-Spektroskopie verfolgt. Unter Einbeziehung dieser Informationen wurden unterschiedliche geschwindigkeitsbestimmende Schritte für Reaktionen mit 5- bzw. 6-Ringen definiert.

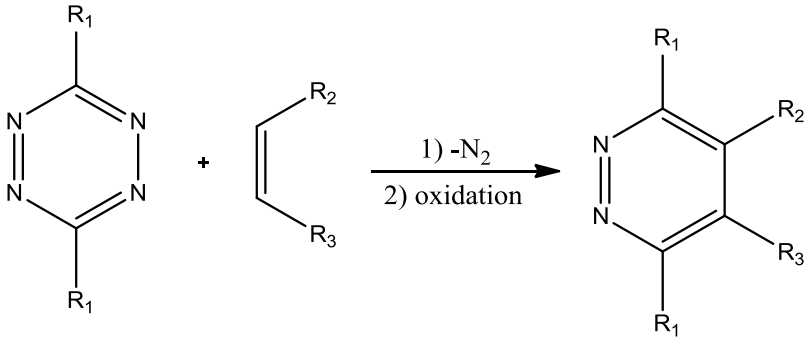
Abbrevations

Å	Angstrom
aq	aqueous
$CDCl_3$	deuterated chloroform
CH	cyclohexane
DCM	dichloromethane
DCPD	dicyclopentadiene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHF	2,3-dihydrofuran
DHP	3,4-dihydro-2*H*-pyran
DMSO	dimethylsulfoxide
EA	ethylacetate
EtOH	ethanol
HAc	acetic acid
HCl	hydrochloric acid
H_2O	water
H_2SO_4	sulfuric acid
iEDDA	inverse electron demand Diels-Alder reaction
k	rate constant
mDHP	3,4-dihydro-2-methoxy-2H-pyran
MeOH	methanol
NaCl	sodium chloride
$NaNO_2$	sodium nitrite
NMR	nuclear magnetic resonance
NO_x	nitrous gases
O	olefin
Ph	phenyl
Py	pyridinyl
THF	tetrahydrofuran
TLC	thin layer chromatography/chromatogram
Tz	tetrazine
UV-Vis	ultra violet – visible light

Table of content

1 Introduction

The concept of click chemistry, initially introduced by Sharpless et al. in 2001[1], becomes more and more important in today's chemistry. In contrast to THE click reaction, the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC), there are nowadays many different click reactions which do not require catalysts.[2] A main advantage for catalyst free reactions is the easier adaptation in living organisms. Inverse electron demand Diels-Alder reactions (iEDDA) between tetrazines and olefins are already used for live-cell imaging[3] and as a synthesis pathway to generate ligands for metal complexes.[4] Also the use of iEDDA in the preparation of ^{18}F-labeled tracers for positron emission tomography has been published.[5] The ^{18}F labelled molecule reacts as a dienophile with tetrazines connected to drugs or aminodextrane to generate radiolabeled compounds. For both strategies, synthesising molecules used in labelling experiments or applying the reaction directly (in vivo/vitro), selectivity, bioorthogonality and fast reaction rates are important.[6]



Scheme 1: iEDDA of tetrazine and olefin

The reactant of choice for the mentioned and many other applications are 1,2,4,5-tetrazines as dienes and different dienophiles, for example olefins as depicted in Scheme 1. Especially for symmetrically substituted tetrazines there, is a variety of published substituents, which are usually electron withdrawing groups like pyridines, esters or pyrazoles.[7,8] Well-suited dienophiles typically feature an easily accessible multiple bond in addition to electron donating groups. Also cyclic educts react well, especially those with a high degree of ring strain. Additionally to high reactivity in iEDDA reactions, tetrazines prove to have interesting spectroscopic properties and some are used as fluorogenic markers.[3]

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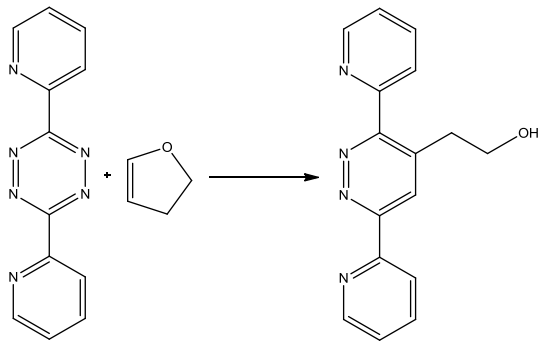
[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.,* 2001, **40**, 2004.
[2] C. R. Becer, R. Hoogenboom, U. S. Schubert, *Angew. Chem. Int. Ed.,* 2009, **48**, 4900.
[3] J. Yang, J. Šečkutė, C. M. Cole, N. K. Devaraj, *Angew. Chem. Int. Ed*., 2012, **51**, 7476.
[4] R. Hoogenboom, G. Kickelbick, U. S. Schubert, *Eur. J. Org. Chem*., 2003, **24**, 4887.
[5] R. Selvaraj, S. Liu, M. Hassink, C. Huang, L. Yap, R. Park, J. M. Fox, P. S. Conti, *Bioorg. Med. Chem. Letters*, 2011, **21**, 5011.
[6] A. C. Knall, C. Slugovc, *Chem. Soc. Rev.*, 2013, **42**, 5131.
[7] N. Saracoglu, *Tetrahedron*, 2007, **63**, 4199 and references therein.
[8] Y. H. Gong, F. Miomandre, R. Meallet-Renault, S. Badre, L. Galmiche, J. Tang, P. Audebert, G. Clavier, Eur. *J. Org. Chem.*, 2009, **35**, 6121.

In literature many different dienophiles were researched, especially their reactivity with 3,6-dipyridin-2-yl-1,2,4,5-tetrazine. For applications in polymer and material science[9] the reaction with different norbornenes was observed.[10,11,12] Less reactive, but nonetheless also very important are both linear and cyclic enols. For this master thesis attention shall be focused on cyclic olefins, especially 2,3-dihydrofuran (DHF) and 3,4-dihydro-2*H*-pyran (DHP).

Starting with one reaction (see Scheme 2) the impact of temperature, ratio of both educts and the used solvent was investigated. Different reactions were compared by reaction time and yield/purity of the crude, and later on the purified product. Bearing the results of these tests in mind, suitable reaction conditions were defined and all subsequent iEDDA reactions were carried out at these optimised conditions.



Scheme 2: iEDDA from 3,6-(dipyridin-2-yl)-1,2,4,5-tetrazine and DHF

For a better understanding how various substituents and olefins react, different molecules were tested. Since the electronegativity of tetrazine substituents was expected to play an important role, dienes which differ in this property were chosen. Additionally the effect of substituents at the dienophile and the importance of ring strain were investigated. Linear alcohols containing a double or triple bond were also tested and compared afterwards.

Further insight about the reaction mechanism was achieved by performing kinetic measurements. Nuclear magnet resonance spectroscopy (NMR) and UV/Vis-spectroscopy were used for those measurements. The iEDDA was performed directly in NMR-tubes and checked periodically to identify possible side reactions and intermediates more precisely. Furthermore, the colour change during iEDDA was measured and first order reaction rates were computed using the spectral data.[13] Again, different reaction partners were analysed and compared.

All in all the mechanism of the iEDDA between tetrazines and cyclic olefins was examined more closely through different analytical methods. Additionally, the gained knowledge through synthetic work was summarised and applied for different reaction partners.

[9] A. C. Knall, S. Kovačič, M. Hollauf, D. Reishofer, R. Saf, C. Slugovc, *Chem. Commun.*, 2013, **49**, 7325.
[10] C. F. Hansell, A. Lu, J. P. Patterson, R. K. O'Reilly, *Nanoscale*, 2014, **6**, 4102.
[11] C. F. Hansell, P. Espeel, M. M. Stamenovic, I. A. Barker, A. P. Dove, F. E. Du Prez, R. K. O'Reilly, *J. Am. Chem. Soc*., 2011, **133**, 13828.
[12] M. Vrabel, P. Kölle, T. Carell, *Chem. Eur. J*., 2013, **19**, 13309.
[13] A. C. Knall, M. Hollauf, C. Slugovc, *Tetrahedron Lett*., 2014, **55**, 4763.

2 Theoretical Part

2.1 Tetrazines

Tetrazines consist of four nitrogen and two carbon atoms in an aromatic six-membered ring. The 1,2,3,5-tetrazine (2) is the least explored one. There is some literature for 1,2,3,4- tetrazine (3)[14] but 1,2,4,5-tetrazine, also called s-tetrazine, (4) is certainly the most examined one.[7] The so called parent compound without any substituents was initially synthesised in 1900 by Hantzsch and Lehmann.[15]

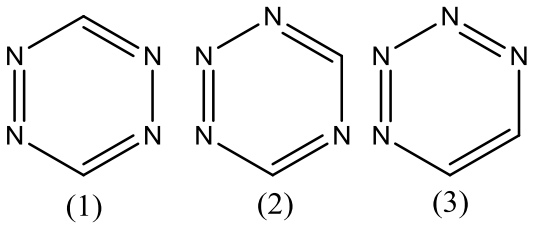
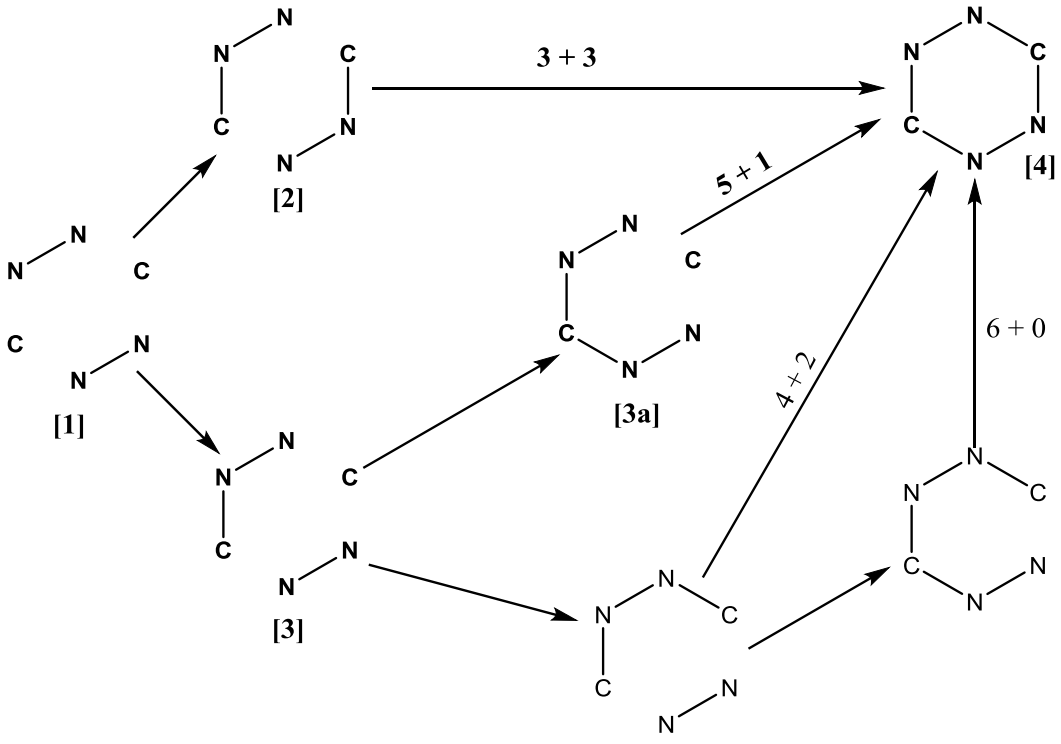


Figure 1: Tetrazine isomers

S-tetrazines are normally synthesized via dihydro-tetrazines as intermediates, and subsequently oxidised to yield the required tetrazines. There are many different ways to prepare dihydro-tetrazines:



Scheme 3: Possible ways to synthesize dihydrotetrazines[16]

The reaction pathways with bold structures (depicted in Scheme 3) were used to prepare products used in this master thesis. While there are many ways to synthesize dihydrotetrazines, only the [5+1] and the [3+3] cyclic addition are described more closely.

[14] A. M. Churakov, V. A. Tartakovsky, *Chem. Rev*, 2004, **104**, 2601 and references therein.
[15] A. Hantzsch, M. Lehmann, *Ber. Dtsch. Chem. Ges.*, 1900, **33**, 3668.
[16] M. Bohle, G. Boyd, *Houben-Weyl*, 1998, **E9c**, 870.

Tetrazines with carboxy groups in 3- and 6-position, are synthesised via dimerization of diazoacetate esters in presence of a base, using two N-N-C-fragments [2]. It is one of the oldest methods for preparation of s-tetrazines in general.[15] Depending on the exact method and kind of ester the yield ranges from 92 % to complete conversion. When using this method for different substituents the yield decreases to 4-84 % depending on the substituent. The pathway to generate symmetrical tetrazines with mostly arylic substituents starts with [1]. Initially the according nitriles react with hydrazine monohydrate to generate N-N-C fragments [2], which subsequently generate dihydrotetrazines.[16] The [5+1] pathway is used to synthesise tetrazines from hydrazide-hydrazones with various substituents. Depending on those, the yield differs from below 10 % (imidates or amidines) to over 70 % when sulfurmethyl is used as the substituent. Additionally, 3,6-bis-(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine can be synthesised when guanidine-1,2,3-triamine is used.[16]

In a second step, the dihydrotetrazines obtained from these reactions need to be oxidised to the corresponding tetrazines. Most commonly, nitrous gases are used as oxidation agents, which are typically generated *in situ* using sodiumnitrite or isopentyl nitrite and a strong acid (e. g. hydrochloric or acetic acid).[17] Depending on the substituents some dihydrotetrazines may be oxidised with air or organic oxidation agents like 2,3-dichlor-5,6-dicyano-1,4-benzoquinone (DDQ)[11] and meta-chloroperoxybenzoic acid (m-CPBA)[18]. Iron(III)-chloride is also used for oxidation.[19]

The reactivity of tetrazines in different reactions, in particular inverse electron demand Diels-Alder reactions (iEDDA), is strongly influenced by the substituents in 3- and 6-position. The greater the electron withdrawing character of the substituents, the better the reactivity in iEDDA reactions is.[6] With chloride or 3,5-dimethylpyrazole as substituents, selective nucleophilic substitutions on tetrazines are possible. Both substituents act as leaving groups and generate new asymmetric and symmetric tetrazines.[20,21] Another possibility to synthesise asymmetric tetrazines would be through cross-coupling reactions.[22]

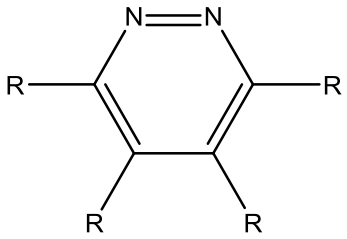
Regardless of the substitutions in 3- and 6-position all s-tetrazine compounds are coloured. Depending on the substituents the colours range from pink over violet to red. The reason for the absorption between 520 and 570 nm is a low energy π^* orbital. The energy of the corresponding n-> π^* transition lies within the visible spectrum. Another absorption maximum is between 250 and 300

[17] J. Sauer, D. K. Heldmann, J. Krauthan, J. Schuster, *Eur. J. Org. Chem*., 1998, **12**, 2885.
[18] R. Selvaraj, J. M. Fox, *Tetrahedron Letters*, 2014, **55**, 4795.
[19] D. R. Soenen, J. M. Zimpleman, D. L. Boger, *J. Org. Chem.*, 2003, **68**, 3593.
[20] Z. Novák, B. Bostai, M. Csékei, K. Lőrincz, A. Kotschy, *Heterocycles*, 2003, **60**, 2653.
[21] R. I. Ishmetova, N. I. Latosh, I. N. Ganebnykh, N. K. Ignatenko, S. G. Tolshchina, G. L. Rusinov, *Russian J. Org. Chem*., 2009, **45**, 1102.
[22] Z. Novák, A. Kotschy, *Org. Letters*, 2003, **5**, 3495.

nm and can be explained by a π -> π* transition. Even fluorescence and phosphorescence properties of for instance the parent 1,2,4,5-tetrazine have been reported.[8,16]

Depending on the amount of nitrogen atoms in substituted s-tetrazines they are sometimes explosive and can be used as ingredients of explosives and pyrotechnic. Especially when two s-tetrazines are connected through an azo-group the possibility to get an explosive by pure impact increases with each nitrogen atom in line.[23,24] Therefore it was important to sustain a balance between nitrogen and non-nitrogen atoms in the synthesised tetrazines.

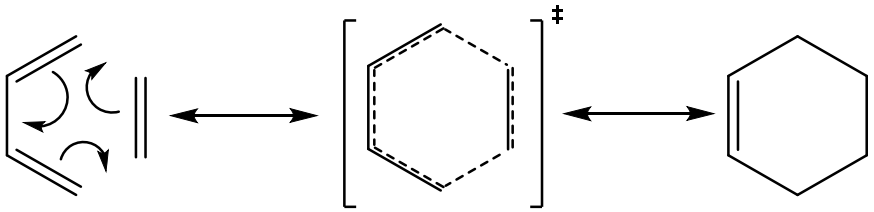
2.1.1 Pyridazines



Similar to tetrazines they are a group of aromatic six membered rings, but in contrast to those they contain two nitrogen atoms. Pyridazines can be synthesised by iEDDA from the corresponding tetrazines. Depending on the used olefin different substituents can be introduced in 4- and 5-position. They can be used as ligands for different metals and sometimes even form [2x2] grid-like metal complexes with the right substituents and metals.[4,25,26]

2.2 Inverse electron demand Diels-Alder reaction (iEDDA)

The iEDDA and, more generally, all Diels-Alder reactions are [4 + 2] cycloadditions. Those pericyclic reactions do not have any charges on intermediates. The transition state has a delocalised, pseudo aromatic character, as in Scheme 4, and therefore is the reason why this kind of reactions occurs.



Scheme 4: Mechanism of a Diels-Alder reaction

As depicted in Scheme 4, the reaction takes place between a conjugated diene and a dienophile, normally an alkene. When the energy gap is not minimised by suitable substituents, the forth and back reaction are usually in equilibrium. These neutral Diels-Alder reactions are reversible, but with suitable substituents the equilibrium can be shifted to the product side. Therefore, electron-withdrawing or – donating groups are important to minimise the activation barrier of the reaction.

[23] D. E: Chavez, M. A. Hiskey, *J. Energ. Materials*, 1999, **17**, 357.
[24] D. E. Chavez, M. A. Hiskey, R. D. Gilardi, *Angew. Chem. Int. Ed.*, 2000, **39**, 1791.
[25] R. Hoogenboom, B. C. Moore, U. S. Schubert, *J. Org. Chem.*, 2006, **71**, 4903.
[26] W. Kain, *Coordination Chem. Rev.*, 2002, **230**, 127.

For the normal electron demand Diels-Alder reaction dienophiles have electron withdrawing groups that lower the LUMO of the molecule. Electron donating groups on the conjugated system of the diene raise its HOMO (see Figure 2). The smaller the resulting energy gap the likelier and easier the reaction occurs.[27]

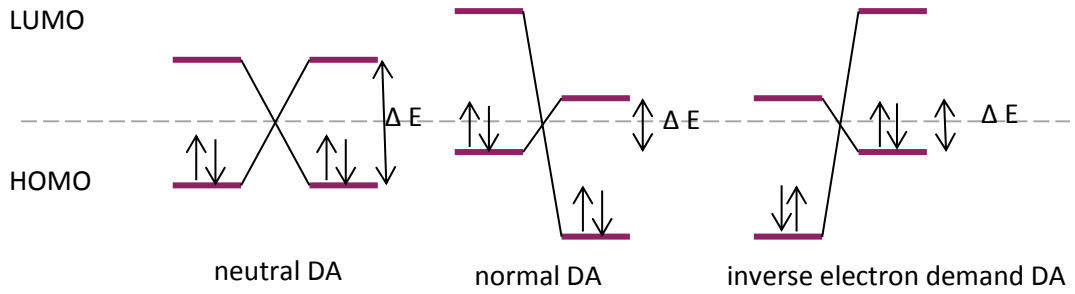


Figure 2: Frontier orbital models of different Diels-Alder reactions

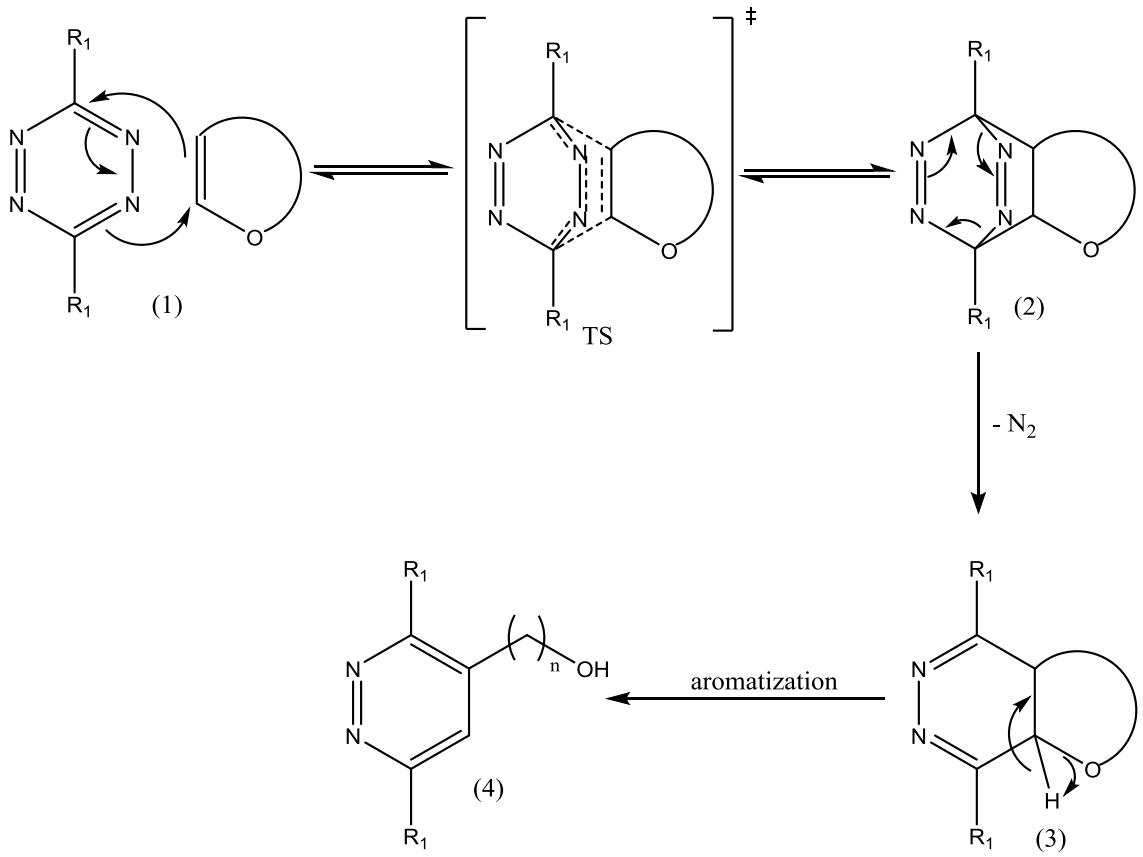
The aforementioned substituent effects are also important for inverse electron demand Diels-Alder reactions, however in this case electron withdrawing groups are at the diene and vice versa. This results in a reduction of the $HOMO_{dienophile}$ – $LUMO_{diene}$ – gap which should be as small as possible.[28] Like in normal electron-demand Diels-Alder reactions, the regioselectivity plays an important role.

Since 2008, when iEDDA was initially mentioned as a possible click reaction by Blackman et al. and Devaraj et al., this type of reaction has certainly obtained the status to be "clickable".[29,30]

The term "Click Chemistry" was defined by Sharpless et al. in 2001.[31] In the same review he defined a few criteria a click reaction has to follow. The requirements contain high yields, purification without chromatographic methods, simple reaction conditions, benign solvents (if any) and easily accessible starting materials. Additionally a high driving force of more than 20 kcal/mol is required by Sharpless' criteria. The first click reaction was the copper-catalysed azide-alkyne Huisgen 1,3-dipolar cycloaddition (CuAAC).[32]

In the following especially click reactions between tetrazines as dienes and enol ethers as dienophiles are being explained more closely.

[27] J. Clayden, N. Greeves, S. Warren, P. Wothers, *Oxford University Press*, Organic Chemistry, 2001.
[28] A. M. Prokhorov, D. N. Kozhevnikov, *Chem. Heterocyclic Compounds*, 2012, **48**, 1153.
[29] M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc*., 2008, **130**, 13518.
[30] N. K. Devaraj, G. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem*., 2008, **19**, 2297.
[31] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2001, **40**, 2004.
[32] M. Meldal, C. W. Tornøe, *Chem. Rev*., 2008, **108**, 2952 and references therein.



Scheme 5: Mechanism of iEDDA between tetrazines and olefins

Initially, tetrazine and olefin undergo the iEDDA reaction (1), which results in a high strained bicyclic intermediate (2). Similar to the normal Diels-Alder reaction the transition state has an aromatic character. The intermediate undergoes a retro Diels-Alder reaction, nitrogen is released and the 4,5-dihydropyridazin is obtained (3). The intermediate is directly oxidised by the oxygen within the cyclic dienophile and the aromatised product is formed (4). [6,33]

In the big pool of different click reactions the iEDDA stands out because of many advantages and a few disadvantages. It was observed that a tetrazine can react as an oxidizing agent in the DA reaction and therefore the corresponding dihydrotetrazine can occur as a side product. [28,34,35] Hence, the purification is more time consuming und normally a column chromatography is necessary to separate product and by product. However this is not always the case and there are also iEDDA reactions which are quick, have excellent yields and occur with N_2 as the only by-product.[2]

There are no catalysts or initiators necessary to start or accelerate the reaction. Those also have to be separated in the workup process and would hinder the reaction to be applied in living organisms. This application is already workable as Hildebrand et al. already used iEDDA with tetrazines in

[33] R. A. A. Foster, M. C. Willis, *Chem. Soc. Rev.*, 2013, **42**, 63.
[34] Z. K. Wan, G. H. C. Woo, J. K. Snyder, *Tetrahedron*, 2001, **57**, 5497.
[35] N. Rahanyan, A. Linden, K. K. Baldridge, J. S. Siegel, *Org. Biomol. Chem*., 2009, **7**, 2082.

imaging living cells.[30] The iEDDA belongs to the quickest click-reactions, when highly reactive tetrazines and dienophiles are used.[36] A big advantage is that both the diene and the dienophile can be modified to adjust the reactivity. Also an increase in temperature improves the reaction rate and for very slow reaction partners microwave assisted reaction conditions are possible.[25] Furthermore it is also possible to combine iEDDA with other click reactions as mutual orthogonality can be achieved. There are no protecting groups necessary when the reaction is combined with CuAAc[37] or thiol-ene[29] click chemistry.

Figure 3: colour change and nitrogen evaporation during an iEDDA

When using, as shown in Scheme 5, tetrazines as dienes, the end of the reaction can be detected by naked eye. As tetrazines are coloured from pink to red, the reaction with olefins is finished as soon as the colour changes during the reaction. Therefore there is not any difficult reaction control necessary. Additionally, it is possible to determine the reaction rate by UV-Vis-spectroscopy under pseudo first order conditions. Also monitoring by NMR-spectroscopy is possible to get insight information how this reaction occurs and which intermediates are at least stable for a short time.

In material science iEDDA in combination with tetrazines gets increasingly important, for instance to modify materials.[6] In polymer chemistry those click reactions with tetrazines, especially 3,6-dipyridin-2-yl-1,2,4,5-tetrazines, are applied to functionalise macroporous foams consisting of poly(dicyclopentadiene) [38] or synthesise block copolymers.[11,39]

[36] C.-H. Wong, S. C. Zimmerman, *Chem. Commun*., 2013, **49**, 1679.
[37] C. F. Hansell, R. K. O'Reilly, *ACS Macro Lett*., 2012, **1**, 896.
[38] A. C. Knall, S. Kovačič, M. Hollauf, D. Reishofer, R. Saf, C. Slugovc, Slugovc, *Chem. Commun*., 2013, **49**, 7325.
[39] N. K. Devaraj, G. M. Thurber, E. J. Keliher, B. Marinelli, R. Weissleder, *Proc. Natl. Acad. Sci. U.S.A*., 2012, **109**, 4762.

3 Results and Discussion

3.1 Tetrazine synthesis

As discussed in the previous chapter, there are various methods to synthesise tetrazines. In the following section, different ways, depending on desired substituents and reactivity, are presented.

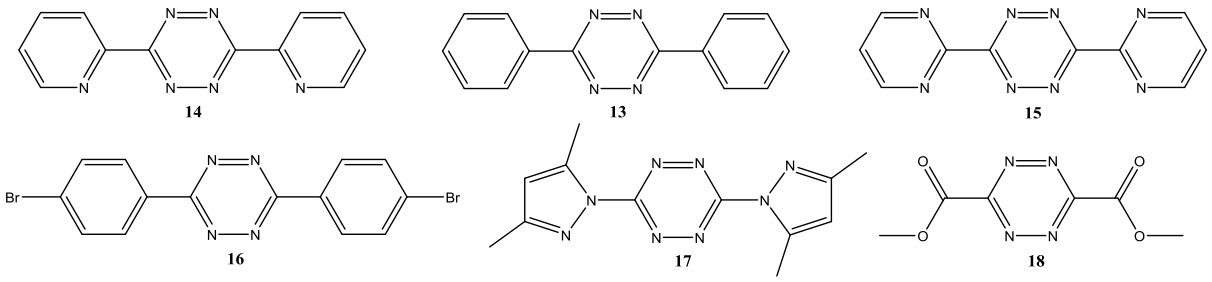
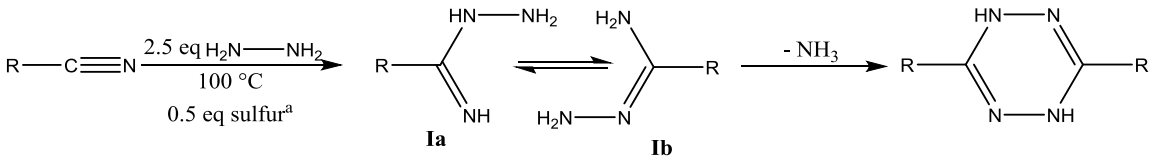


Figure 4: Different tetrazines synthesised for this master thesis

For the synthesis of the tetrazines depicted in Figure 4, the dihydrotetrazine is generated in the first step and subsequently oxidised to generate the desired products.

3.1.1 Formation of 1,4-dihydrotetrazines

3.1.1.1 Dihydrotetrazines from aromatic nitriles



Scheme 6: Synthetic pathway of dihydrotetrazines with aromatic substituents

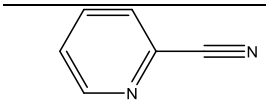
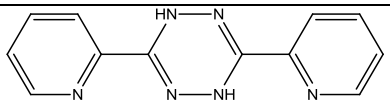
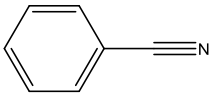
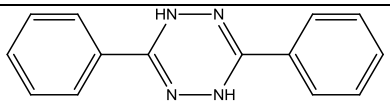
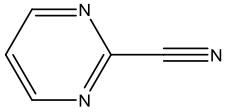
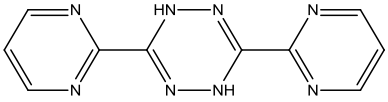
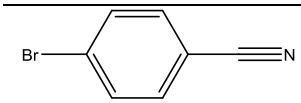
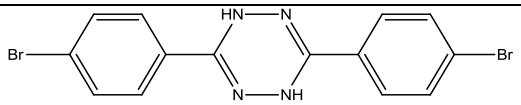
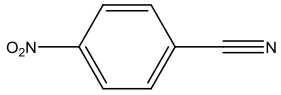
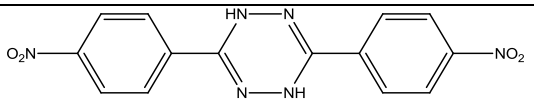
Mechanistically, at first each cyano-group reacts with one hydrazine monohydrate to form intermediate **Ia** which then can isomerise (**Ib**). Without any special treatment isomer **Ib** is favoured and reacts immediately with a second intermediate to form the desired product. Therefore the lone-pair of the hydrazine-nitrogen attacks the quaternary carbon atom and ammoniac is released. The second C-N bond is formed in a similar fashion to obtain the substituted dihydrotetrazine derivative.

As depicted in Scheme 6, many different symmetrical tetrazines can be synthesized by using aromatic nitriles and hydrazine monohydrate. The latter is added as a 50% aqueous solution, because of security concerns (pure hydrazine was forbidden by REACH regulations). Hydrazine is toxic, flammable, environmentally harmful and corrosive. For the least reactive nitriles (**1b, d**) sulphur had to be added additionally as a catalyst.[40] In contrast, 4-nitrobenzonitrile (**1e**) was very reactive, so that

[40] E. Kurach, D. Djurado, M. Zagorska, A. Pron, *Phys. Chem. Chem. Phys*., 2011, **13**, 2690.

the heating had to be stopped in between, because the reaction led to a rapid gas evolution. In general electron poor nitriles seemed to be more reactive than electron rich ones.

Table 1: Exact information about the dihydrotetrazine synthesis, [a]4 had been synthesized beforehand and was only oxidized, [b] used in crude form for oxidation

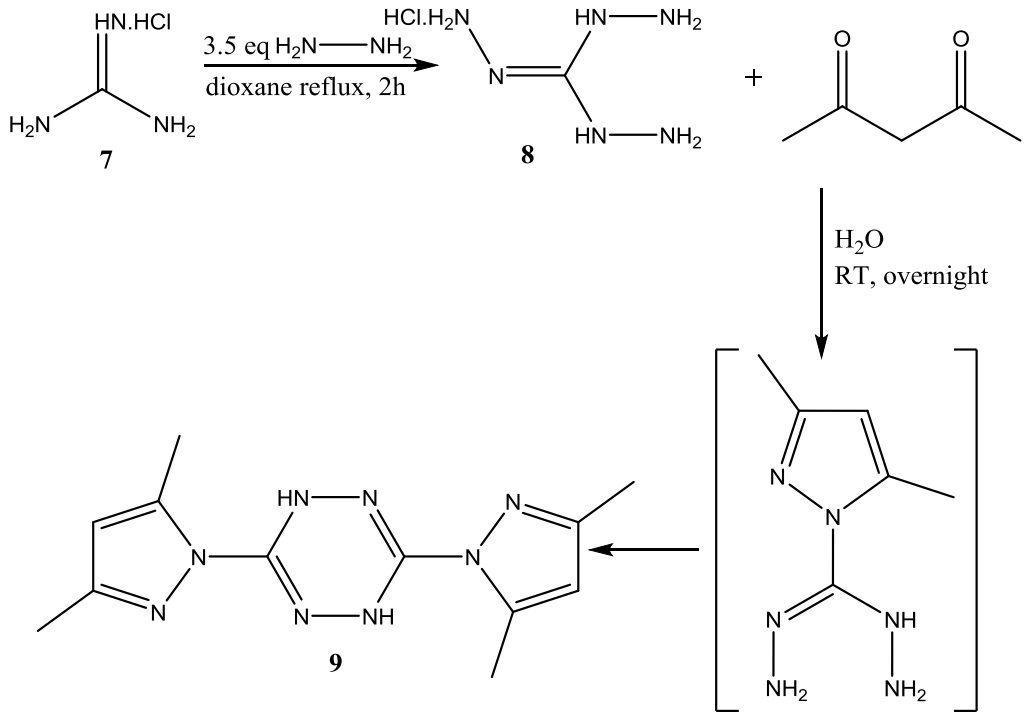
nitrile	reagent	product	structure	yield
	**1a**	**2**		46.6 %
	**1b**	**3**		-[b]
	**1c**	**4**		-[a]
	**1d**	**5**		-[b]
	**1e**	**6**		48.3 %

The reactions usually took four to five hours until conversions were complete, which was determined by TLC (verification of complete consumption of **1**). A big advantage of this reaction is the convenient workup. The obtained products are virtually insoluble in water and precipitated after the reaction mixture is cooled to room temperature. The orange to light red solids could be collected in sufficient purities.

As shown in Table 1, typical product yields of about 50 % were achieved. In literature, the yields of derivative **2** range from 58 %[41] over 65 %[42] to nearly 100 %[43]. Possible reasons for higher yields could be the use of inert condition (Versteegen et al.), simply evaporating everything volatile instead of filtrating it (Klingele et al.) or cooling the mixture to 0 °C before obtaining the product (Dubreuil et al.). Some product could be lost during the filtration process. If the ethanol is not cold enough it will dissolve small parts of the dihydrotetrazine. Due to the fact of only being able to test product and reactant via TLC but not the intermediates, it is also possible that there was still Ia/b in the reaction mixture. This could be soluble in ethanol and therefore been removed. Although the hydrazine was added in high excess it is possible that it decomposes before reacting.

[41] M. Klingele, P. D. W. Boyd, B. Moubaraki, K. S. Murray, S. Brooker, *Eur. J. Inorg. Chem*. 2006, **3**, 573.
[42] R. M. Versteegen, R. Rossin, W. ten Hoeve, H. M. Janssen, M. S. Robillard, *Angew. Chem. Int. Ed*. 2013, **52**, 14112, supporting information.
[43] H. Bakkali, C. Marie, M. Evain, D. Dubreuil, *Eur. J. Org. Chem.* 2008, **12**, 2156.

3.1.1.2 3,6-Bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,4-dihydro-1,2,4,5-tetrazine synthesis



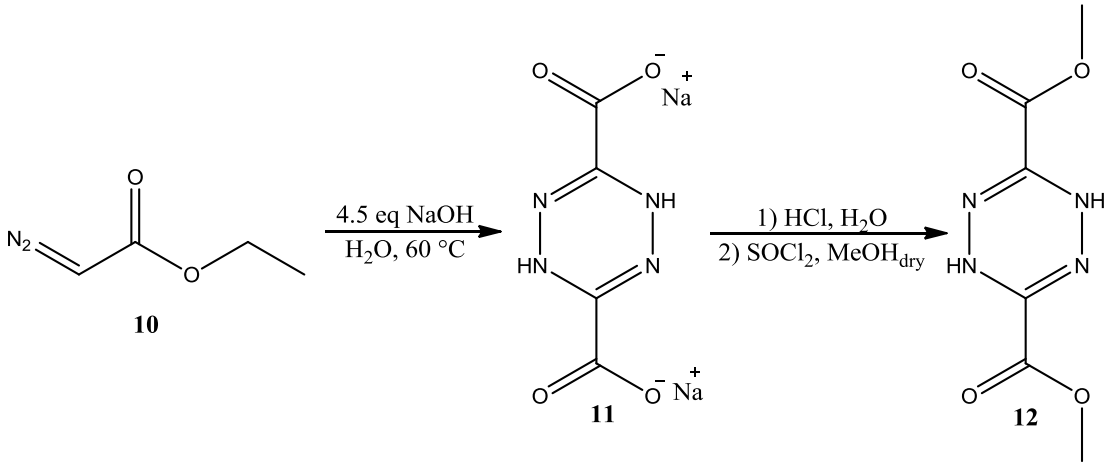
Scheme 7: Synthetic pathway of dihydro-pyridazine with pyrazol substituents[44]

To synthesize 1 H-pyrazol-1-yl-tetrazines, firstly triaminoguanidine (**8**) has to be prepared. Therefore, guanidine hydrochloride is heated to reflux for 2 hours in dioxane with excess hydrazine monohydrate. At least three equivalents were needed to aminate the guanidine as desired, to be on the safe side, 3.5 equivalents were used. As the reagent was soluble in hot dioxane and the product precipitated, it could be obtained by filtration. Possible unreacted reagents were removed by washing with dioxane. After drying the colourless solid, a yield of 88 % was achieved.

The pyrazole substituents for the dihydrotetrazines were generated in a second step. **8** is dispersed in water and 2,4-dipentanone is slowly added via a dropping funnel. Initially, 1-H-pyrazole is formed and water is released. Subsequently, two intermediates generate one dihydrotetrazine. The solution turned yellow and overnight a bright yellow precipitate formed which was recovered by filtration. Again the only purification step was washing the product with cold water yielding 89 % of **9** as a yellow solid.

[44] M. D. Coburn, G. A. Buntain, B. W. Harris, M. A. Hiskey, K. Y. LEA, D. G. Ott, *Heterocyclic Chem.,* 1991, **28**, 2049.

3.1.1.3 Dimethyl-1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate



Scheme 8: Synthetic pathway of dihydrotetrazine with carboxylate substituents

Ethyl diazoacetate (**10**) is hazardous and can only be purchased in solution with a minimum content of 13 wt% dichloromethane (DCM) Therefore the exact concentration of diazoacetate was unknown and exactly 13 wt% DCM were assumed. Due to the dilution the reaction was not as exothermic as expected and was heated to 60 °C while adding **10** to a solution of sodium hydroxide in water. After the solvent was removed, the slurry was poured in ethanol, mixed and the liquid decanted. This procedure was repeated three times. The yellow solid was finally obtained by filtration. Based on a DCM content of 13 wt% in the starting material **10**, sodium-1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**11**) was obtained in 66 % yield.

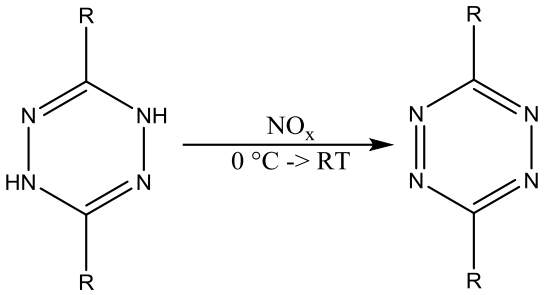
Then, the corresponding acid of **11** was generated temporarily. Therefore derivative **11** was dispersed in a water/ice mixture and cooled with an ice/NaCl bath. After adding hydrochloric acid, the acid was simply obtained by filtration and stirred in an ice-bath for half an hour to remove the excess HCl. Then the obtained solid was dried on high vacuum at 60 °C to remove remaining water.

The formation of the methyl-ester was achieved through an acid-chloride. To inhibit hydrolysis the following reactions were performed under inert conditions and exposure to air was minimized as much as possible.[45] Thionylchloride was dissolved in dry methanol and cooled to – 30 °C. The free acid was then added through a dropping funnel and the reaction mixture was stirred at room temperature for 2.5 hours, as the thionylchloride attached to the acid and hydrochloric acid was set free simultaneously. The resulting sulfurochloridous anhydride intermediate is unstable and quickly reacts with the carbonyl group to generate the acid chloride, hydrochloric acid and sulphur dioxide. The acid chloride derivative reacts with methanol to form the desired product **12**.[46] The product was washed with water and subsequently, the ester was dissolved in dichloromethane. After the solvent

[45] D. L. Boger, R. S. Coleman, J. S. Panek, *J. Org. Chem*., 1985, **50**, 5377.
[46] J. Clayden, N. Greeves, S. Warren, P. Wothers, *Oxford University Press*, Organic Chemistry, 2001.

was removed, **12** was dried and stored in high vacuum. As contact with air should be prevented the yield was not determined.

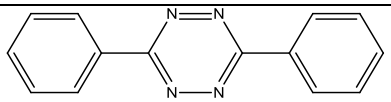
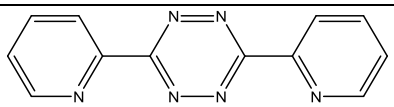
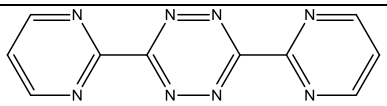
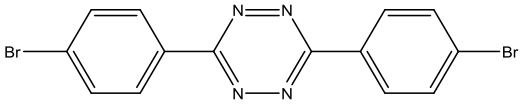
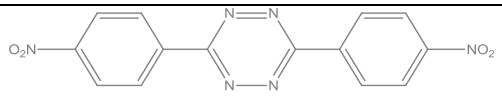
3.1.2 Oxidation to 1,2,4,5-tetrazines



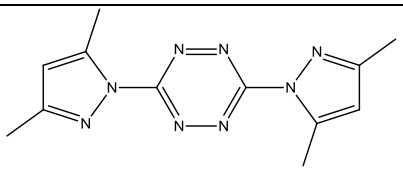
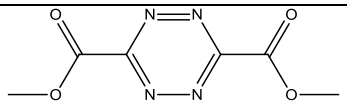
Scheme 9: General pathway for oxidation with nitrous gases and colour difference between dihydrotetrazine and tetrazine

Dihydrotetrazines are in most cases oxidized by nitrogen oxides (NO_x), which are generated in situ upon the reaction of a NO_2-source such as $NaNO_2$ and glacial acetic acid.

Table 2: Tetrazine synthesis, [a] yield of both synthesis and subsequent oxidation, [b] NO_x was generated in a separate vessel and passed through using a nitrogen flow and HCl was used instead of HAc

react.	NO_x source	pr.	structure	$yield_{th}$	$yield_{lit}$
2	$NaNO_2$ in H_2O	**13**		36.1 %[a]	98.2 %[47]
3	$NaNO_2$ in H_2O	**14**		45.3 %	50.9 %[47]
4	isopentyl nitrite in H_2O	**15**		82.2 %	249 %[48]
5	$NaNO_2$ in H_2O	**16**		59.4 %[a]	75 %[49]
6	-	**-**		-	-

[47] W. Chen, D. Wang, C. Dai, D. Hamelberg, B. Wang, *Chem. Commun*., 2012, **48**, 1736.
[48] V. P. Krivopalov, M. B. Bushuev, Y. V. Gatilov, O. P. Shkurko, *Russian Chem. Bulletin*, 2010, **59**, 1808.
[49] L. I. Robins, R. D. Carpenter, J. C. Fettinger, M. J. Haddadin, D. S. Tinti, M. J. Kurth, *J. Org. Chem*., 2006, **71**, 2480, supporting information.

react.	NO_x source	pr.	structure	$yield_{th}$	$yield_{lit}$
9	$NaNO_2$ in H_2O	**17**		20.4 %	21.5 %[50]
12	$NaNO_2$ in H_2O[b]	**18**		9.4 %[a]	29.8 %[51]

As shown in Table 2, nitrogen oxides were usually generated through sodium nitrite and glacial acetic acid. In general, the dihydrotetrazine was dispersed in acetic acid and cooled to 0 °C. Then sodium nitrite, dissolved in water, was added drop wise, producing nitrous acid and sodium acetate in situ. The nitrous acid spontaneously decomposes to nitrogen oxides, which oxidise the dihydrotetrazine yielding the desired 1,2,4,5-tetrazine. Due to the toxic nature of the gas, the reaction vessel was directly connected to the vent to prevent gas effusion. The reaction progress could be visually observed, as the suspension changes from yellow/orange to dark red/violet/pink. To reach full conversion, three equivalents sodium nitrite were added and the reaction mixture was subsequently stirred for another two hours.

Diphenyl-tetrazine (**13**), dipyridinyl-tetrazine (**14**) and di(para-bromo-phenyl)tetrazine (**16**) were oxidized as described in the previous paragraph including the workup. As they were insoluble in the water/acetic acid mixture, the products could be obtained by filtration. The crude products were recrystallized from suitable solvents to remove impurities. Depending on the solubility, ethanol (**14**), dichloromethane (**13**) or dimethylformamide (**15**) were used, respectively.

[50] J. Zhu, J. Hiltz, R. B. Lennox, R. Schirrmacher, *Chem. Commun*., 2013, **49**, 10275, supporting information.
[51] *Organic Synthesis*, 1992, **70**, 79.

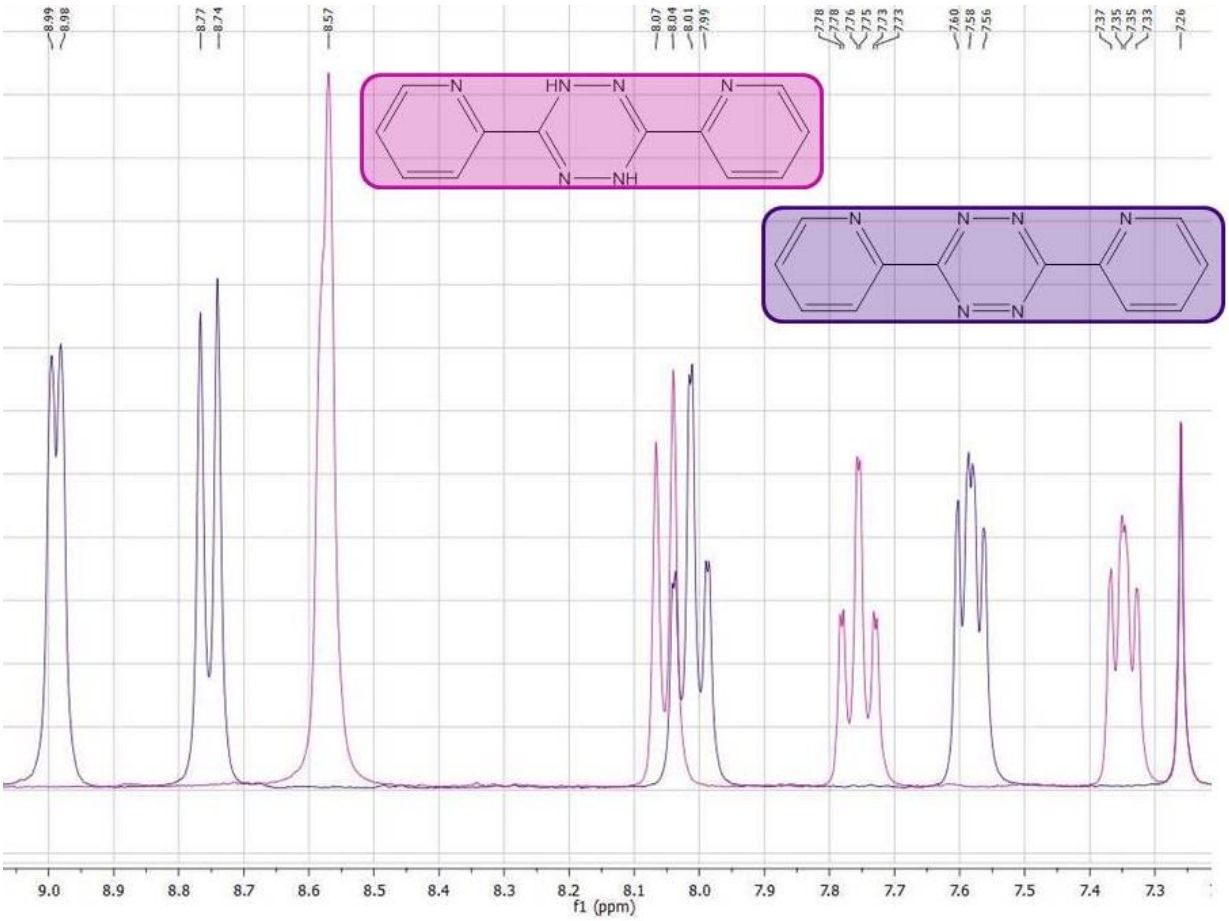


Figure 5: NMR-spectrum of 14 and 3 in $CDCl_3$

Using NMR spectroscopy full conversion of **3** to **14** can be easily confirmed. Most peaks simply have a higher chemical shifted but otherwise stay the same. This is due to the conjugation of the product and therefore higher electron distribution. The broad singlet at 8.57 ppm, which is assigned to the hydrogens in the dihydrotetrazine ring and the hydrogens connected to the C^6 of the pyridine rings, integrates to 4 hydrogens, respectively. After full conversion, the pyridine hydrogens correspond to a doublet at 8.99 ppm and the HN vanished.

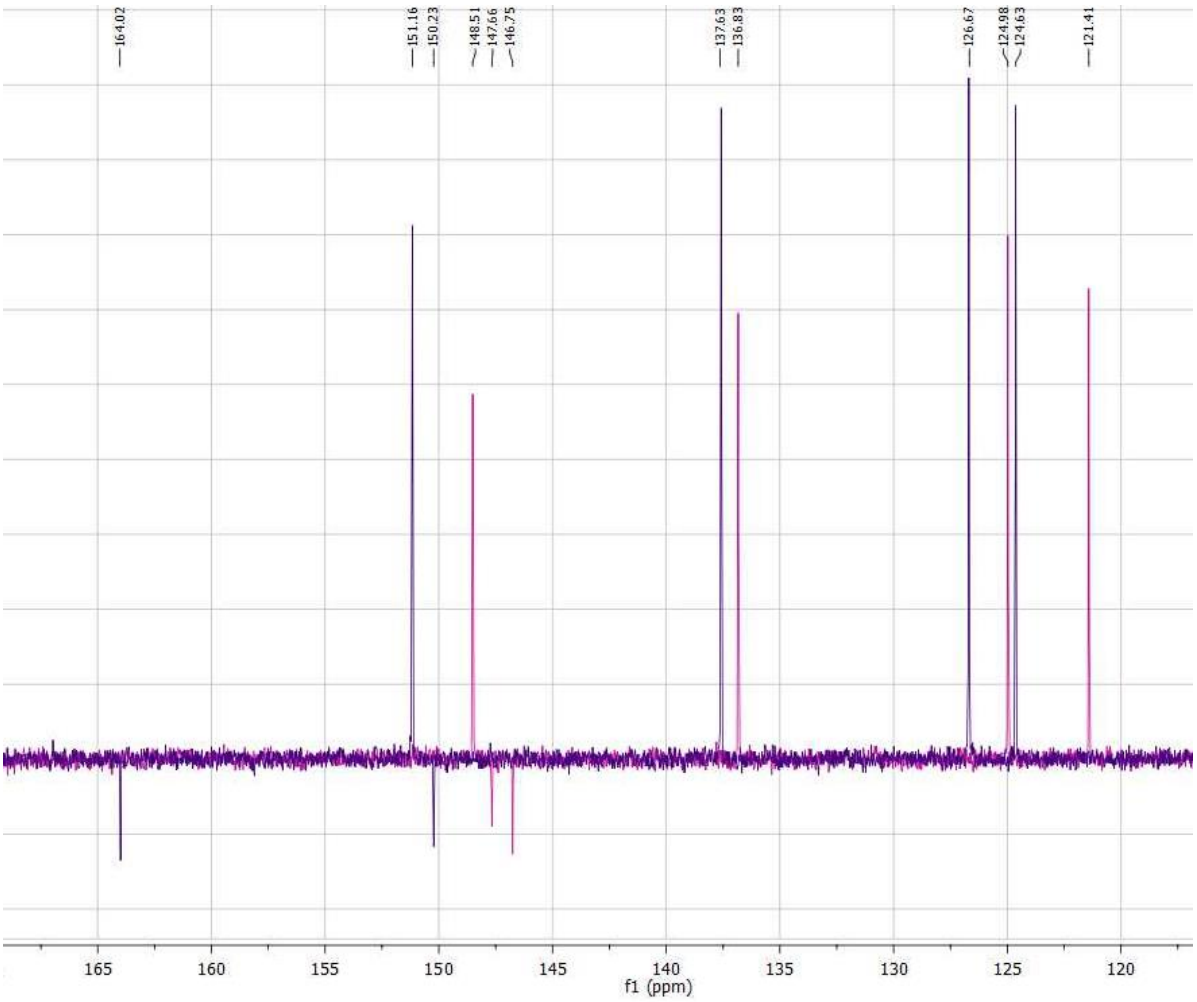


Figure 6: ^{13}C - spectrum of dihydrotetrazine 3 (pink) and tetrazine 14 (violet) ($CDCl_3$)

Similarly to the ^{1}H-spectrum, the tetrazine is shifted more to the downfield of the spectrum in the carbon NMR. The oxidation is best depicted in the shift of the C_q of the tetrazine-ring which changes from 146.8 ppm to 164.0 ppm, respectively. The other synthesized compounds behaved similarly and therefore, their NMR spectra are only discussed in the experimental part.

Another possibility to generate NO_x is using isopentyl nitrite as a nitrogen-oxide source. Similar to the other oxidation pathway, glacial acetic acid was used to generate nitrous acid. In this case, however, the reaction was performed at room temperature and stirred overnight. Dipyrimidinyl-tetrazine (**15**) was oxidized in a similar fashion. Subsequently the product was isolated through filtration.

Di-(dimethyl-pyrazole)-tetrazine (**17**) was obtained via a similar procedure. Dihydrotetrazine **9** was dissolved in dichloromethane and then sodium nitrite, dissolved in water, was added to the reaction mixture. Afterwards glacial acetic acid was added drop wise through a septum. Both the dihydrotetrazine and the product **17** were soluble in the organic layer. Therefore, the phases had to be separated and subsequently, the solvent was removed to isolate the crude product. Since recrystallization was insufficient to yield pure **17**, the product was purified using column, resulting in

a rather low yield. In future experiments, column chromatography should be employed right away, as the separating problem was simple.

As an acid is essential to generate nitrous gases in this setup and dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**18**) is sensitive to hydrolysis, the apparatus for the oxidation of **12** was more elaborate. Two three necked round-bottomed flasks were used, both prepared by evacuating and flushing with nitrogen. The first one was filled with concentrated hydrogen chloride and equipped with gas inlet and outlet. The tube for the outgoing gas was attached to the second flask, where **12** was dissolved in dry dichloromethane. An aqueous solution of sodium nitrite was slowly added to the first flask and the evolving gas was flushed through the solution using a syringe needle, resulting in an immediate colour change from yellow to red. As in the usual setup, the gases afterwards were directly let into the vent. The workup in this case was to remove the solvent and dry the product as quickly as possible.[52] Due to the limited stability of **18**, the following reactions were done immediately after its preparation and the remaining solid was stored under nitrogen. However, the yield over the last few steps was low. The source of the losses could not be determined.

Figure 7: Reaction vessel for the oxidation of 18

In case of di(para-nitro-phenyl)-1,4-dihydro-1,2,4,5-tetrazine (**6**), oxidation with $NaNO_2$/HAc failed. Although a colour change from orange to brown/red was noted, NMR characterisation of the putative product revealed the presence of several products, which might include multiply nitrated derivatives (which might be explosive). No attempts to separate the product mixture were carried out. Instead alternative oxidation agents were tried.

The reaction of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, Figure 8) with **6** was employed following the work of Hansell et al..[53] In a Schlenk flask, flushed with nitrogen, **6** was dissolved in toluene and two equivalents of DDQ were added. The mixture was heated to reflux and stirred overnight. Again, a dark brown solid formed which was insufficiently soluble for characterisation via NMR spectroscopy. Since TLC revealed consumption of **6**, the desired product

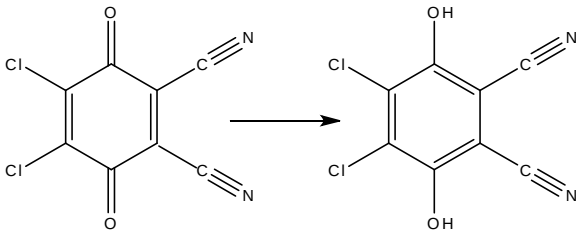


Figure 8: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

[52] *Org. Synth*. 1992, **70**, 79.
[53] C. F. Hansell, P. Espell, M. M. Stamenović, F. E. Du Prez, R. K. O'Reilly, *J. Am. Chem. Soc*., 2011, **133**, 13828.

might have formed, but as mentioned low solubility (DMSO, $CHCl_3$) impeded further characterisation (moreover low solubility of the tetrazine is not desired in the research carried out in this work). As a third option the oxidation with $Fe(III)Cl_3$ in water/ethanol at 75 °C was tested.[54] Compound **6**, dissolved in alcohol, was heated up and the iron-chloride solution was added drop wise. No colour change was observed, nevertheless workup as described in literature was carried out. NMR spectroscopy revealed recovery of the starting material and it can be concluded that $Fe(III)Cl_3$ is not a suitable oxidation agent for this reaction. Finally, meta-chloroperoxybenzoic acid (m-CPBA) was used for the oxidation.[55] The reaction was carried out in a NMR-vial ($CDCl_3$) using an excess of m-CPBA and the reaction progress was monitored by repeated measurements. Additional peaks appeared in the spectra. However, product peaks could not be identified with absolute certainty. After two days, derivative **6** vanished, but the peaks could not be assigned to a structure. However two sets of signals for the nitrophenyl-substituents could be identified ruling out the formation of the corresponding symmetric tetrazine derivative.

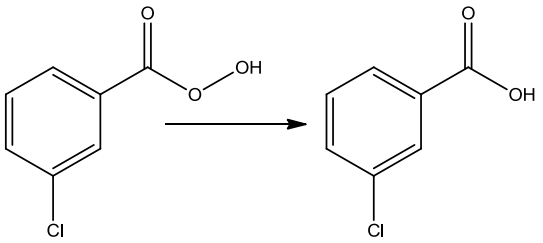


Figure 9: Meta-chloroperoxybenzoic acid

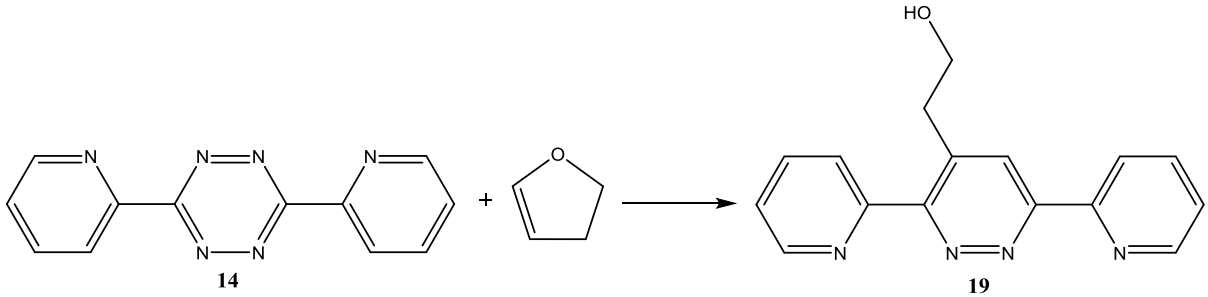
[54] D. R. Soenen, J. M. Zimpleman, D. L. Boger, *J. Org. Chem.*, 2003, **68**, 3593.
[55] R. Selvaraj, J. M. Fox, *Tetrahedron Lett*., 2014, **55**, 4795.

3.2 Inverse electron demand Diels-Alder reaction

The major part of this work was dedicated to gaining a better understanding of the iEDDA reaction of tetrazines with cyclic enol ethers, examining different influences (reaction conditions) and investigating the underlying kinetics.

3.2.1 General tests

To establish a general understanding how this [4+2] cycloaddition works, at first the same two reactants (see Scheme 10) were investigated under different reaction conditions. Therefore, small batches - about 50 mg tetrazine - were used and the yield was determined by NMR spectroscopy of the crude product.



Scheme 10: General synthesis of 2-(3,6-di(pyridine-2-yl)pyridazin-4-yl)ethanol

Table 3: Reaction times for iEDDA (cf. Scheme 10) under different conditions; [a] measured by TLC, [b] another 0.5 eq were added to complete the reaction

	eq DHF	LM	additives	T	t[a]	NMR	
						by-product	other impurities
I	1 + 0.5[b]	DCM	-	RT	2 days	2 %	no
II	2	DCM	-	RT	2 h	2 %	no
III	5	DCM	-	RT	15 min	1 %	yes (DHF)
IV	10	DCM	-	RT	10 min	by-product detected by TLC	
V	1.2	THF	-	60 °C	15 min	4 %	yes (DHF)
VI	1.2	dioxane	-	60 °C	15 min	3.5 %	yes
VII	1.2	toluene	-	60 °C	25 min	4 %	no
VIII	1.2	DCM	-	40 °C	1.1 h	1.5 %	yes
IX	1.2	toluene	-	100 °C	15 min	4 %	yes
X	1.2	DMSO	1 eq NaOH	80 °C	30 min	decomposition	
XI	1.2	DMSO	1 eq KOH	80 °C	30 min	decomposition	
XII	1.2	THF	0.5 µL H_2O	60 °C	15 min	2 %	no

To be competitive with the many existing click reactions, a high reaction speed is an important prerequisite. Therefore this was the first point investigated. Firstly, the impact of olefin

concentration in the solution was studied. 2,3-Dihydro-furan (DHF) was added equimolar (**I**) and in two (**II**), five (**III**) and tenfold (**IV**) excess, with respect to the tetrazine concentration and dichloromethane was used as solvent. To confirm the end of the reaction the obvious colour change observed and additionally, thin layer chromatography was used. High concentrations, 5 and 10 equivalents, accelerated the reaction, although the quantitative difference was hard to determine. However, raising the concentration from equimolar to 2 equivalents of DHF already caused a significant increase in reaction time to about two hours. Tests with equimolar amounts of both reagents were rather challenging to accomplish. For the reactions only small amounts of olefin were needed and it was difficult to exactly match the amounts of tetrazine and DHF, due to the low boiling point of the olefin. Therefore the reaction times of more than one day were not totally accurate as usually an additional amount of olefin had to be added after some time, because there was only diene but no dienophile left in the solution . On this account always at least 1.2 equivalents were used to have enough for completing the reaction. Summarizing it could be said that when quick reaction rates are the only goal, high excess of the olefin should be added. If however the olefin is very expensive/ difficult to obtain, then also 1:1.2 for small batches and even equimolar concentrations for bigger ones are suitable for the reaction to work.

In that case other points have to be found to accelerate the reaction rate. Therefore, secondly, also a change in temperature was investigated. Here, finding a good compromise between a solvent with a high boiling point and one which is still easy removable is the most important point (**V**-**IX**). Rising the temperature only a little bit already has a big impact on the reaction rate. While stirring at room temperature with a ratio of 1:1.2 took several hours until the reaction was finished, heating to just 40 °C already decreased the time to a little bit more than an hour (**VIII**). When the reaction took place at 100 °C, there was not any tetrazine left after 10 minutes (**IX**). At high temperatures the reaction vessel had to be closed tightly, because DHF has a very low boiling point (55 °C) and the reaction mixture had to cool down before a sample for reaction control could be taken. Additionally, when such a high reaction temperature has to be achieved, the solvent must be chosen appropriately. Toluene and DMSO can be heated to 100 °C without any problem, also THF and dioxane can be used at higher temperatures around 60 °C which already speed up the reaction. However the solvent had to be removed after the reaction was finished. For many tests the product was easily isolated by evaporating the solvent under reduced pressure. Although removing the solvent took longer than when dichloromethane was used, the preparation altogether was briefer. Even higher temperatures than 100 °C would be possible when using DMSO, but it was very hard to remove the solvent afterwards. The best way was to perform a more complicated workup. Through applying the fact that DMSO is soluble in water, the product could be extracted with dichloromethane. Then, the organic solvent was dried over sodium sulphate and removed under

reduced pressure. Doing that, only a little bit of solvent was left in the obtained product. There was not any great difference in difficulty noted for the other three solvents. So all in all, toluene became the solvent of choice. The solubility of the tetrazine was lower in toluene compared to dichloromethane, which, in spite of reduced reaction rates had no further negative effects on the reaction as such.

In different papers it is noted that there are also ways to catalyse the iEDDA in different ways. Some propose to add bases like sodium or potassium hydroxide to quicken the reaction between tetrazines and alkynes.[56] The base shall either be attached to the quaternary carbon atom of the tetrazine cycle and remove the aromatisation of it or deprotonate the attacking olefin. When tried out with the reactants in

Scheme 10 (**X, XI**) the reaction was not notable quicker and the mixture turned all shades of brown to black instead of yellow. DMSO had to be used as solvent because of base-catalysed tetrazine hydrolysis in the presence of water. As a workup a solvent extraction had to be done. However no product could be identified in the NMRs recorded which indicated decomposition of the tetrazine. Catalytic amounts of water (50 µL water in 1 mL solvent) were reported to accelerate the reaction.[57] Using the miscibility of water and THF, it was the solvent of choice in this case (**XII**). Under the same reaction conditions the reaction time was about the same as without using the water. Maybe more difference would have been noticed if a slower reaction was examined or different reaction conditions like an increase in the amount of water were used. Altogether it can be said that bases at least not catalyse the reaction if cyclic alkenes are used instead of alkynes and water could accelerate the reaction but it was not observed under the conditions examined.

3.2.1.1 Closer investigation of the reaction between 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazin and DHF

While repeatedly performing the same reaction under varying reaction conditions the final product was analysed using NMR spectroscopy. Eventually it became clear that the side products were identical, which then could be identified as depicted in Scheme 11 and Figure 10.

[56] B. A. Trofimov, D. A. Shabalin, I. A. Ushakov, A. I. Mikhaleva, *Adv. Synth. Catal*., 2013, **355**, 1535.
[57] J. W. Wijnen, S. Zavarise, J. B. F. N. Engberts, *J. Org. Chem.*, 1996, **61**, 2001.

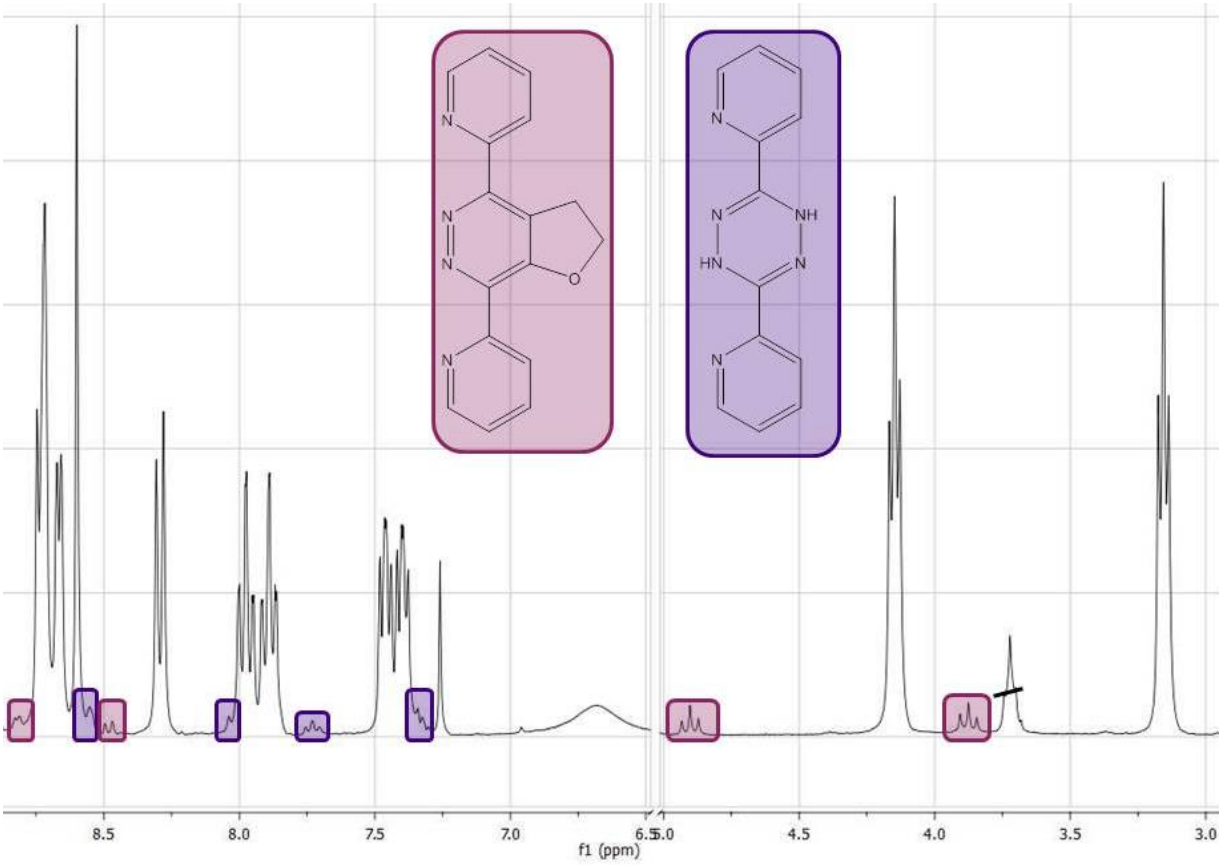


Figure 10: NMR of a crude product of iEDDA of the model reaction ($CDCl_3$)

Using TLC two side products could be identified. One was less abundant, the other one slightly more polar than the product. Dihydrotetrazine **3** was easily identified since the yellow dots on the TLC plate turned pink overnight. Thus it was proven that the tetrazine was reduced during the reaction and re-oxidized in the presence of air. Referring to simple electrochemistry, something has to be oxidized if small amounts of the tetrazine are being reduced during the reaction. The bicyclic by-product could have been oxidised by the tetrazine, a side reaction already experienced a few times.[58,59,60]

The peaks related to product **19** are visible in Figure 10, but for a better assignability there is a spectrum of the pure iEDDA product depicted in Figure 11. A distinctive peak is the singlet at 8.6 ppm, which correlates to the only hydrogen of the pyridazine ring. The other peaks shifted in the aromatic section belong to both pyridinyl-rings. The broad singlet at 6.65 ppm comes from the alcohol group. Both triplets in the high field correspond to the aliphatic chain. The NMR-spectrum of the dihydrotetrazine (Figure 12) has two triplets belonging to the hydrogens of the C^4/C^5 atoms of the pyridinyl-rings. Both NH hydrogens of the dihydrotetrazine ring, in addition to the hydrogens of the C^6 atom of the pyridinyl-rings, correspond to the broad peak at 8.57 ppm.

[58] A. M. Prokhorov, D. N. Kozhevnikov, *Chemistry of Heterocyclic Compounds*, 2012, **48**, 1153.
[59] Z. K. Wan, G. H. C. Woo, J. K. Snyder, *Tetrahedron*, 2001, **57**, 5497.
[60] N. Rahanyan, A. Linden, K. K. Baldridge, J. S. Siegel, *Org. Biomol. Chem*., 2009, **7**, 2082.

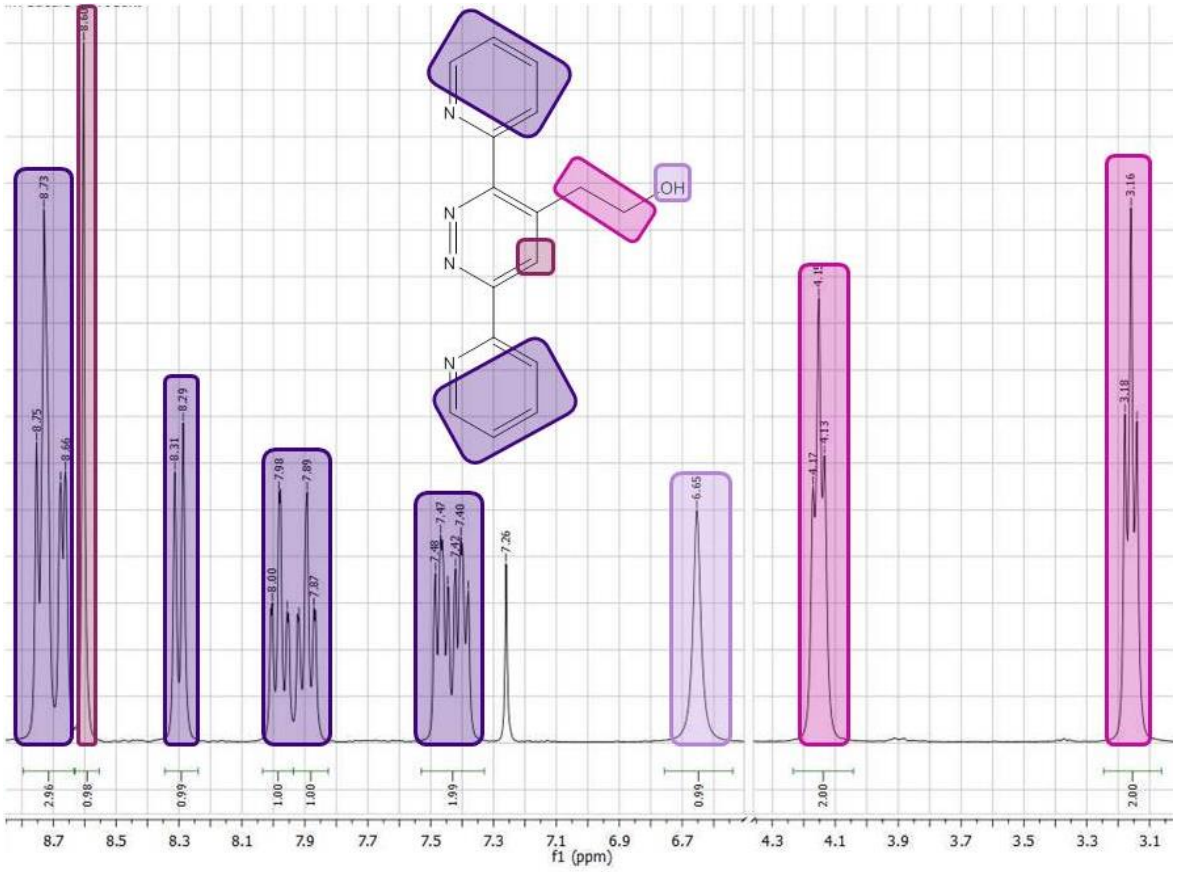


Figure 11: NMR-spectra of product 19 ($CDCl_3$)

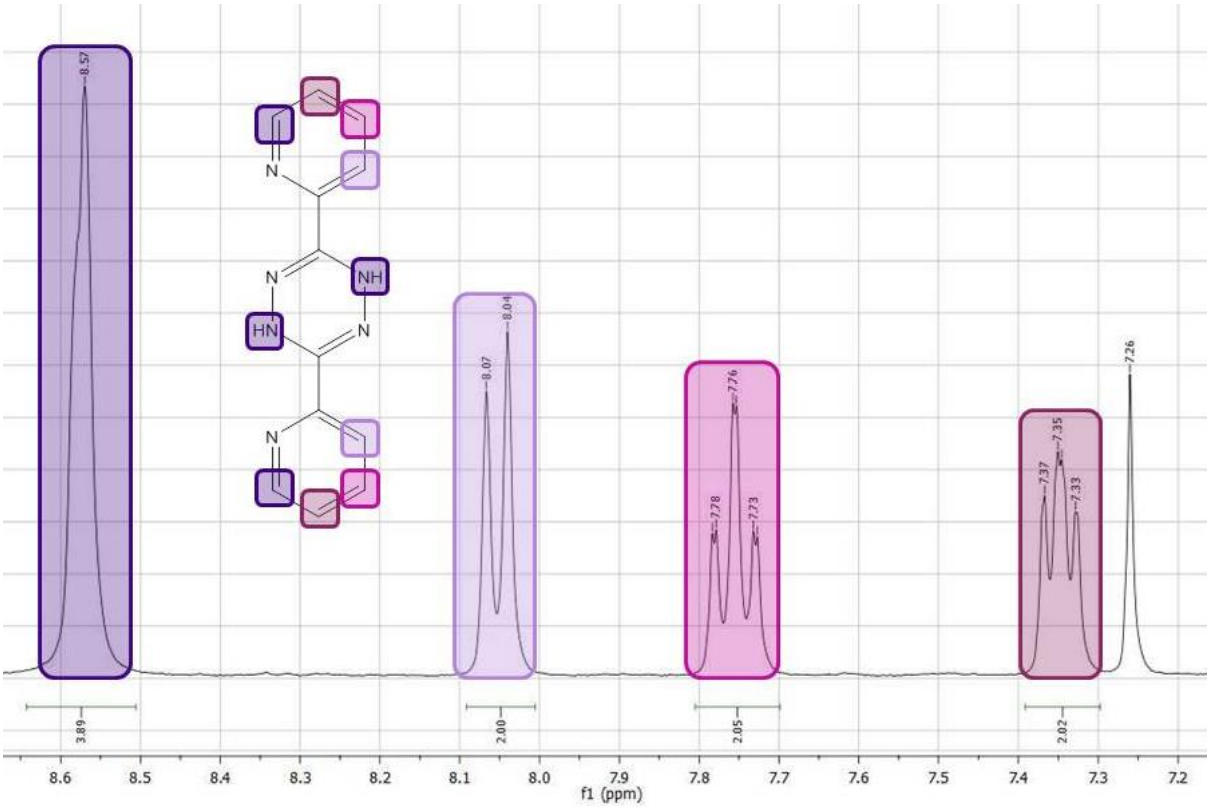


Figure 12: NMR-spectra of by-product 3 ($CDCl_3$)

However, in contrast to **3** and **15** (which were easily separable by column chromatography), **16** was challenging to isolate purely. The main problem was that, although it had a lower R_f value on TLC, **16** eluted alongside the product, or even before when performing column chromatography. This led to the assumption that the polarity is quite similar for both molecules. After a second column where the mixed fractions were separated again and only small fractions were collected, the second by-product could be identified by NMR spectroscopy.

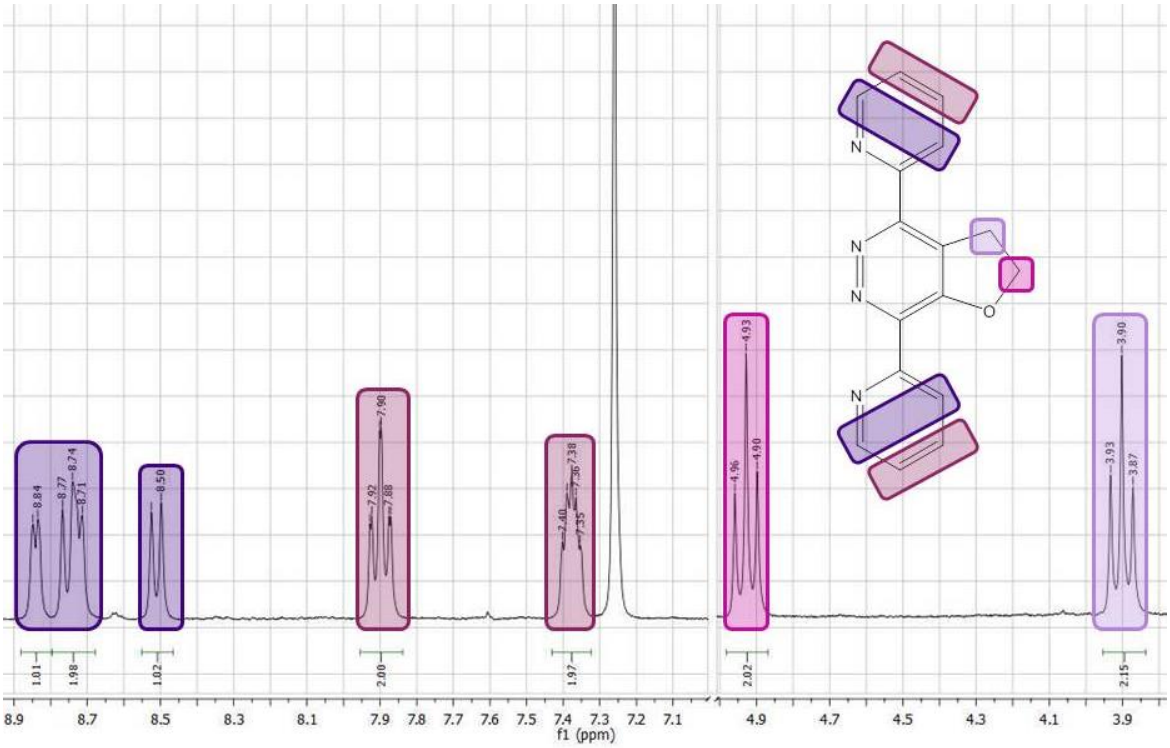
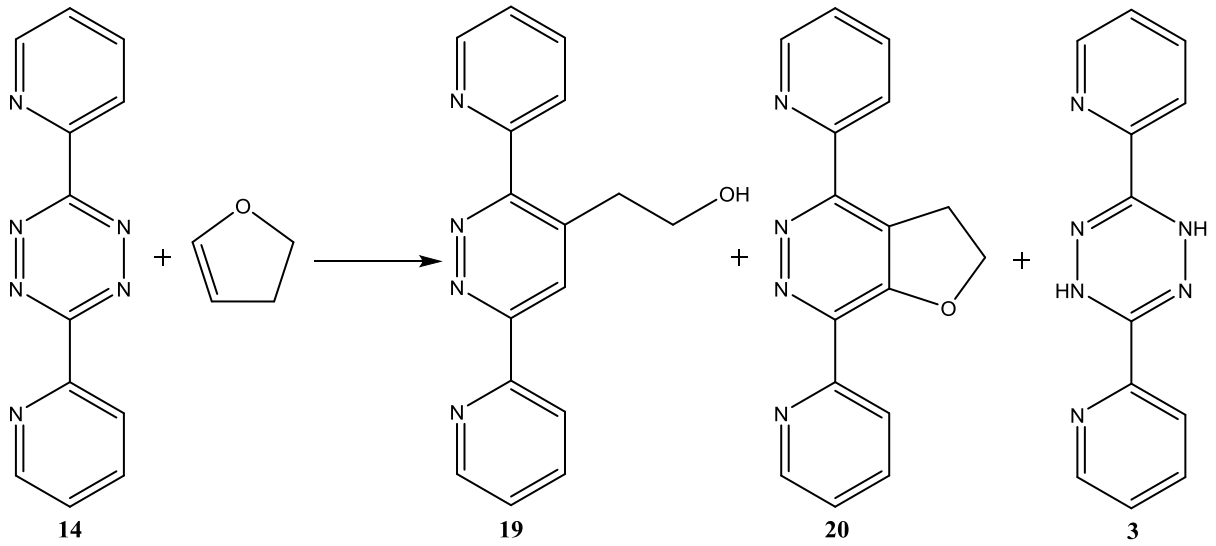


Figure 13: NMR-spectra of the by-product 20 ($CDCl_3$)

Initially, the aromatic peaks did not meet our expectations in the context of an iEDDA reaction. Therefore the assumption arose that the by-product might be symmetrical. All products with an aliphatic chain on the 5th carbon atom of the pyridazine have different environments in both pyridinyl-cycles and thus split up. Additionally, all integrals in the low field amount only integrate to eight aromatic hydrogen atoms. As nothing had changed with the pyridinyl-cycles, the hydrogen of the pyridazine ring did not exist in this molecule. All those ideas resulted in identifying the second by-product and modifying the reaction equation in the beginning of this chapter (Scheme 11, compared to Scheme 10).



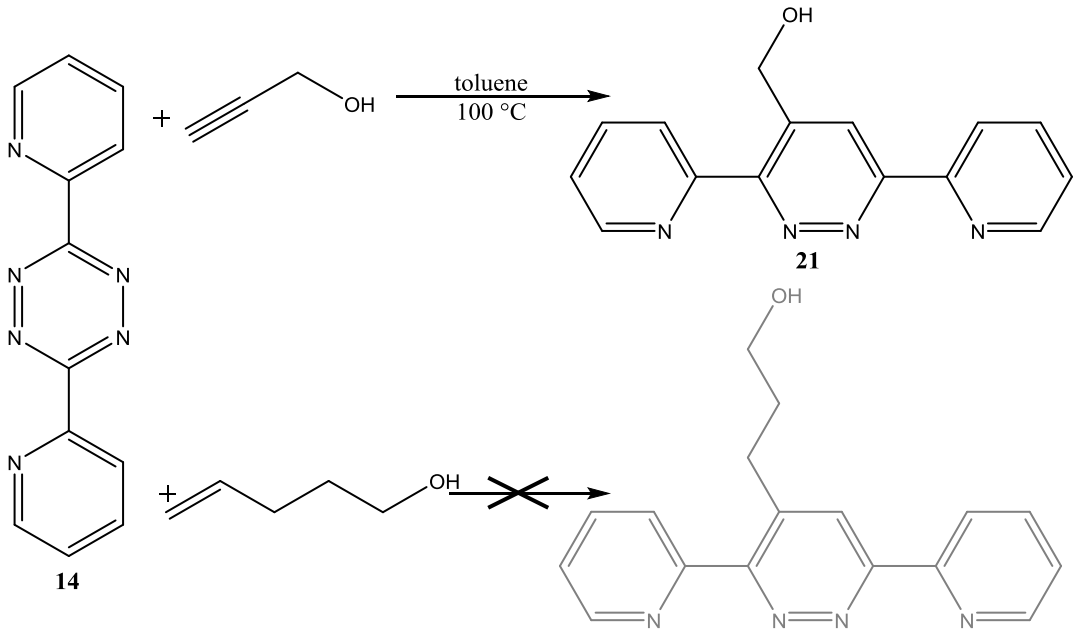
Scheme 11: Modified reaction Scheme for the iEDDA between dipyridyl-tetrazine and dihydro-furan

Different reaction conditions were employed to minimise the formation of the closed by-product **20**, which complicated the purification, lowering the yield of pure **15** to roughly 60%. However, neither a higher reaction rate nor inert reaction conditions brought an improvement. The first option was attempted by employing 10 equivalents of olefin and elevated temperatures. The second one was done under an inert atmosphere, giving comparable yields and roughly 3% by-product **20**. As the oxygen concentration seemed unimportant, it was presumed that on the other hand a pure oxygen atmosphere would not be beneficial and the tetrazine **14** is the oxidation agent in this reaction.

3.2.2 Comparison between different olefin substrates

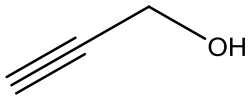
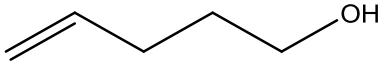
Since the only requirements for dienophiles in iEDDA are at least one multiple bond and electron donating groups, a huge variety of substrates can be theoretically reacted with the diene. In this thesis, the attention was focused on cyclic molecules with a double bound adjacent to oxygen. Additionally these substrates were compared to linear dienophiles with multiple bonds in terminal position.

3.2.2.1 Cyclic or linear - the differences in iEDDA



Scheme 12: Reaction pathway of linear olefins with dipyridyl-tetrazine

Table 4: Comparison between different tetrazine/olefin ratios under the same reaction conditions (toluene, 100 °C)

	equivalents	t (measured by TLC)
	1.2	15 min
	5	7 min
	10	5 min
	1.2	overnight
	5	175 min
	10	155 min
	1.2	5 h
	5	15 min
	10	< 15 min

The products of linear dienophiles differ only slightly from the reaction between DHF and dipyridinyl-tetrazine, making a comparison of different dienophiles easier. As reagents at hand were used, they have a different amount of carbon atoms than DHF.

Propargyl alcohol reacted very slowly. Therefore no conversion was observed at room temperature. Upon heating, product **21** was formed slowly. Again the correlation between temperature and reaction rate was obvious, as it took 26 days at 40 °C, 9 days at 60 °C and only overnight at 100 °C until full conversion. When the reaction was performed with higher concentrations of dienophile the reaction rate was accelerated. However, it was, compared to the dihydro-furan, still significantly slower (see Table 4). When **14** was dispersed in 1 mL neat propargyl alcohol and heated to 100 °C the reaction was complete after one hour. Workup und purification were similar to the already tested reaction. A column chromatography was necessary to remove the again present dihydrotetrazine **2**. The NMR-spectrum (Figure 14) naturally has many similarities, only the peak of the alcohol group (6.52 ppm) is much less distinct and therefore not marked additionally.

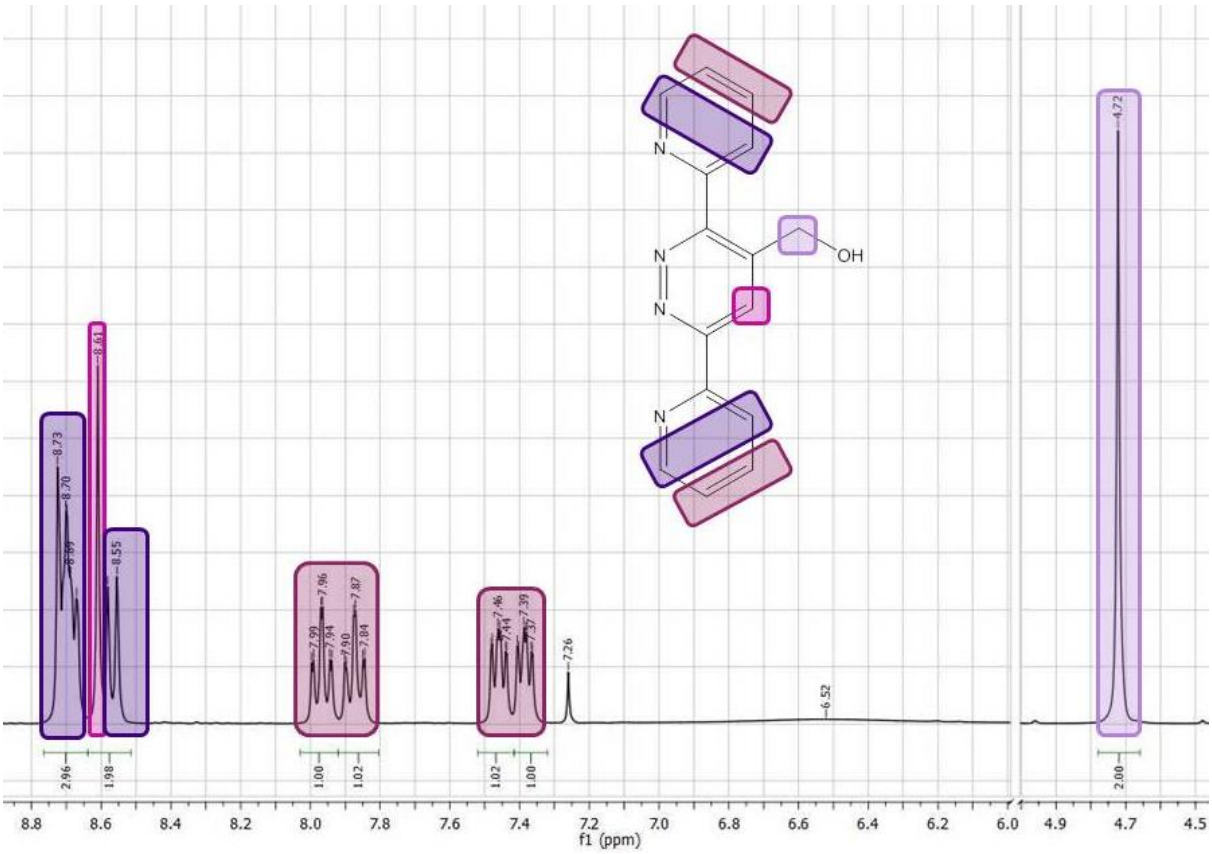


Figure 14: NMR spectrum of 21 ($CDCl_3$)

The yield was also comparable, if not a bit higher (75%), because no closed by-product formed. Due to the triple bond the resulting product is aromatic, removing the need for an oxidation agent. Base catalysis[61], although more efficient than in the model reaction, did not result in significant improvements. The reactions were, as predicted, much quicker (about one hour at 60 °C), but the main product was decomposed tetrazine. In the NMR spectrum of the reaction with propargyl alcohol there was a pronounced singlet around eleven and no aliphatic peaks to confirm a successful

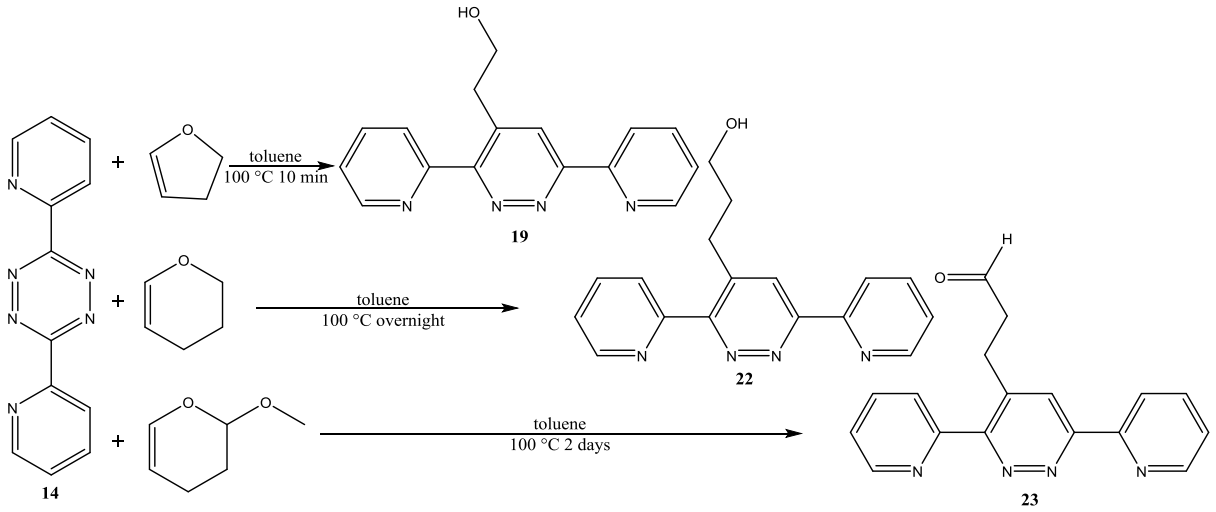
[61] B. A. Trofimov, T. E. Glotova, D. A. Shabalin, M. Y. Dvorko, I. A. Ushakov, E. Y. Schmidt, A. V. Kuzmin, A. I. Mikhaleva, *Adv. Synth. Catal*., 2013, **355**, 1535.

reaction. Therefore using base is not the method of choice to accelerate the reaction. Instead, high reaction temperatures and a large excess of alcohol increased the reaction speed dramatically, compensating for the lack of ring strain.

As another substrate pent-1-ene-5-ol was tested. After a successful iEDDA reaction the product has to be further oxidized by external oxidation agents like the used DDQ, because it neither has a triple bond nor oxygen which abstracts a hydrogen atom to form a terminal alcohol. Therefore, a more complex workup was necessary as the oxidation agent had to be removed after the reaction. To not influence the iEDDA, DDQ was added after the reaction mixture turned yellow and no reactant **14** was observed by TLC. After adding the oxidation agent, the mixture turned black immediately and was stirred for a few hours. DDQ was removed using aluminium oxide filtration. However no product could be identified via NMR. Also an NMR spectrum of the reaction mixture before the addition of DDQ was recorded, but also there, no intermediate product could be identified.

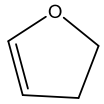
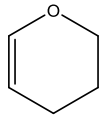
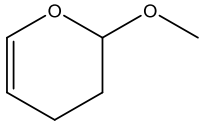
3.2.2.2 Investigation of the reactivity of different cyclic dienophiles

Additionally to linear dienophiles, also different cyclic ones were tested and compared to the model reaction. As shown in Scheme 13, DHP and 2-methoxy-2,3-dihydro-pyran (mDHP) were employed.



Scheme 13: Synthetic pathway of 3,6-dipyridin-2-yl-1,2,4,5-tetrazine with cyclic olefins

Table 5: Exact information about reactions with different cyclic dienophiles

	eq	T	t (measured by TLC)	by-product(s)
	1.2	100 °C	15 min	Dihydrotetrazine, **20**
	2	RT	2 h	Dihydrotetrazine, **20**
	5	100 °C	7 min	Dihydrotetrazine, **20**
	1.2	100 °C	16 hours	Dihydrotetrazine
	2	RT	three weeks	Dihydrotetrazine
	5	100 °C	5 hours	Dihydrotetrazine
	1.2	100 °C	24 hours	Dihydrotetrazine

In the beginning the reaction between dipyridyl-tetrazine and 3,4-dihydro-2*H*-pyran was carried out at room temperature. It took about three weeks until the desired product **22** was obtained. Therefore both ways to accelerate this reaction, high excess of the olefin and high temperatures, were attempted. At 100 °C the equimolar reaction (1:1.2) took overnight and with the fivefold amount of dienophile the reaction was finished after 5 hours. The mDHP was even slower as it took 24 hours until the equimolar reaction was finished at 100 °C. Besides the lower reactivity, both 6-membered rings behaved similarly. Because of the reduced reactivity of 6-membered cyclic olefins, the following reactions with less reactive tetrazines had to be performed with the respective dienophiles in higher excess or even in neat olefin solution.

NMR spectra of crude **22** and **23** showed that the only detectable by-product is the dihydrotetrazine **3** which was found in each reaction. Due to the former coexistence of both side products, the crude mixture was analysed concerning a closed by product, which could not be identified. The acetal reacted in a similar fashion as only by-product **3** could be detected in addition to product **23**. Therefore the purification through column chromatography for both reactions was straightforward and pure products could be obtained in respectable yields of 69.5 % (**22**) and 53.4 % (**23**).

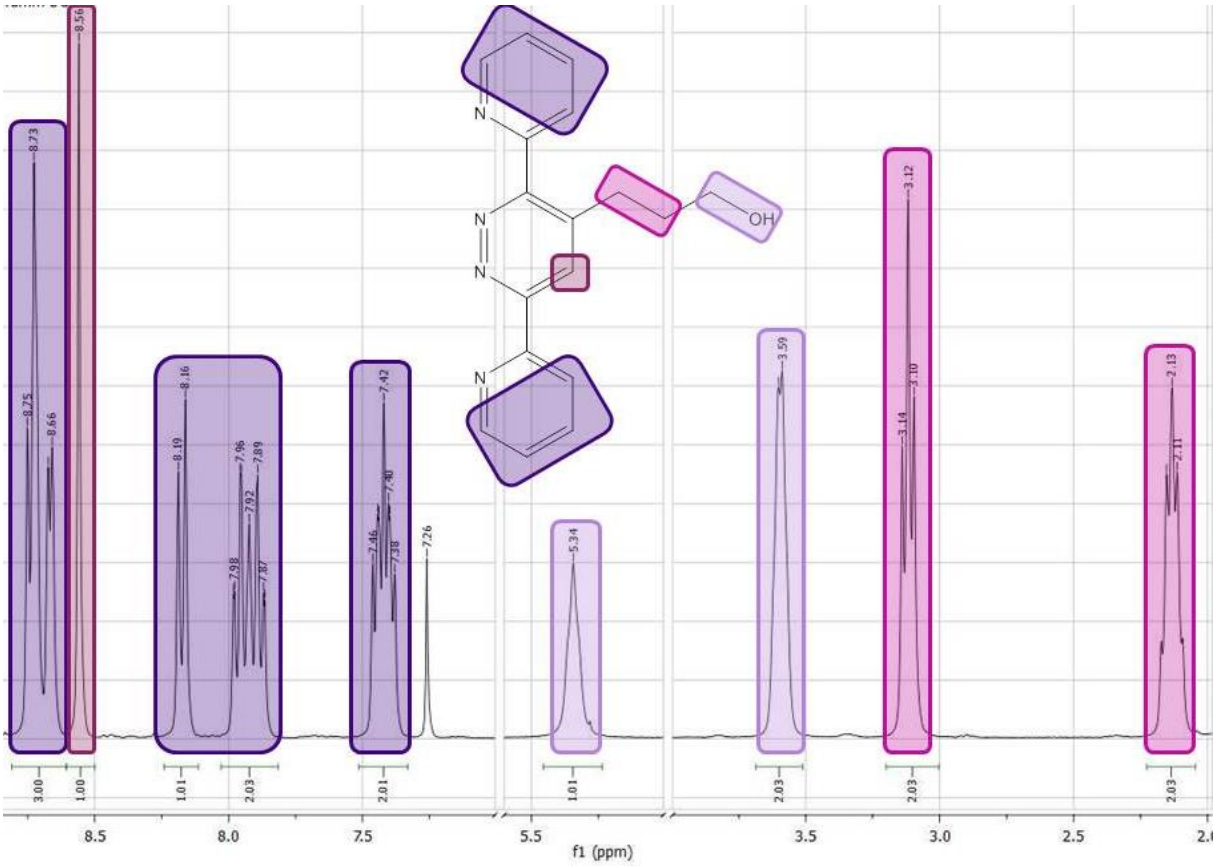


Figure 15: NMR-spectrum of 22 ($CDCl_3$)

In Figure 15 the NMR spectrum of **22** is depicted. The singlet at 8.56 ppm confirms the formation of the iEDDA product. Alongside the aliphatic peaks split according to their chemical surroundings. The CH_2 in the middle of the aliphatic chain has the lowest chemical shift and can be identified by its multiplet.

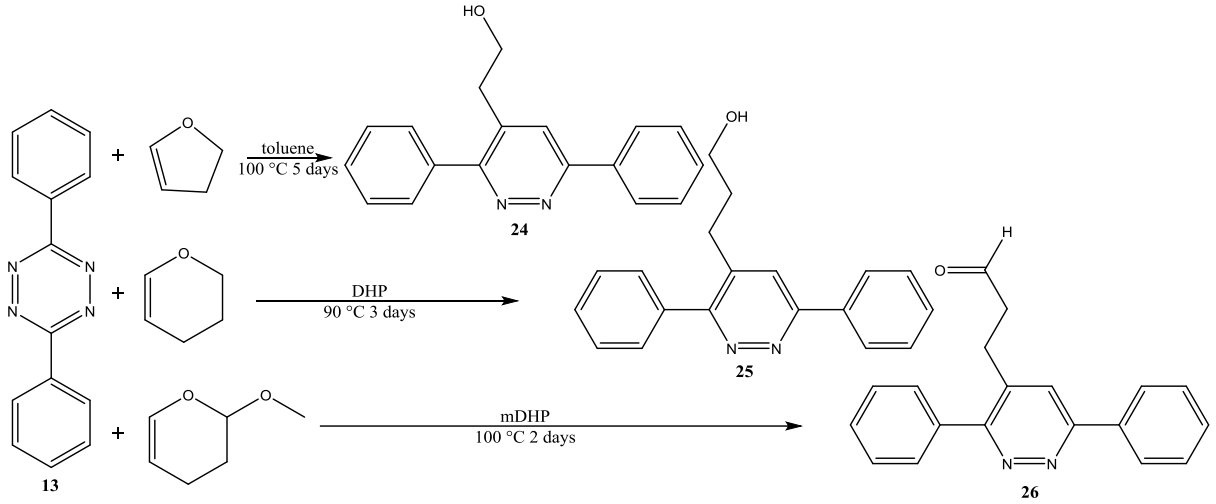
3.2.3 Closer study concerning different tetrazines

Similar to the investigation of different dienophiles, various tetrazines were tested as dienes. Normally only small amounts of the diene (around 50 mg) were used for the reactions. In contrast to the tests regarding the reaction between DHF and dipyridinyl-tetrazine **14**, the reaction products were isolated by column chromatography.

Table 6: Summarised data for the reactions with different tetrazines

tetrazine	olefin	product	t (by TLC)	T	solvent	yield
DipyTz **14**	DHF	**19**	15 min	100 °C	toluene	60.3 %
	DHP	**22**	16 h	100 °C	toluene	69.5 %
	mDHP	**23**	2 days	100 °C	toluene	53.4 %

tetrazin	olefin	product	t (by TLC)	T	solvent	yield
DiphTz **13**	DHF	**24**	5 days	100 °C	toluene	66.4 %
	DHP	**25**	3 days	90 °C	olefin	52.9 %
	mDHP	**26**	2 days	100 °C	olefin	38.7 %
DiprTz **15**	DHF	**27**	2 hours	100 °C	toluene	81.4 %
	DHP	**28**	40 min	90 °C	olefin	53.4 %
	mDHP	**29**	80 min	100 °C	olefin	72.3 %
MexTz **17**	DHF	**30**	< 1 min	RT	DCM	7.0 %
	DHP	**31**	1 min	RT	DCM	21.7 %



Scheme 14: Synthetic pathway to generate diphenyl-pyridazines

Because electron withdrawing functionalities at the tetrazine promote the reaction, and phenyl rings only consist of carbon and hydrogen atoms (and therefore are electron donating), the reaction was presumed to be much slower. This assumption was confirmed by the results. Only product **24** could be obtained by using the previously tested 1:1.2 ratio. The other two dienophiles had to be employed as the solvent for the reaction to be finished after an adequate period of time. The reason for a slower conversion for **25** in contrast to **26** could have been the slightly lower reaction temperature.

For all three reactions purification through column chromatography was necessary to obtain the pure products in acceptable yields. The lowest amount was achieved for **26** with 38.7 %, while the others yielded 52.9 % (**25**) and 66.4 % (**24**), respectively. In contrast to the reaction of dipyridinyl-tetrazine with 2,3-dihydro-furan, no closed side product could be identified in the separation process. Dihydrotetrazine **2** could not be identified either. The resulting NMR spectra were similar to those of the dipyridinyl-pyridazine-derivatives. Only in the aromatic area there were fewer defined peaks but two multiplets for all hydrogens on the phenyl groups. Only the pyridazine-hydrogen resulted in a distinct signal. Figure 16 exemplarily shows the spectrum of **26**, where the aldehyde-hydrogen is

represented by another well-defined singlet. Both aliphatic peaks are easily assignable due to their triplet-splitting. The unidentified multiplet (at around 3.5 ppm) could be from the polymerized olefin. An excess of mDHP was used for the reaction and under acidic conditions (such as when a column chromatography is done), the additional amount could have partially polymerized.

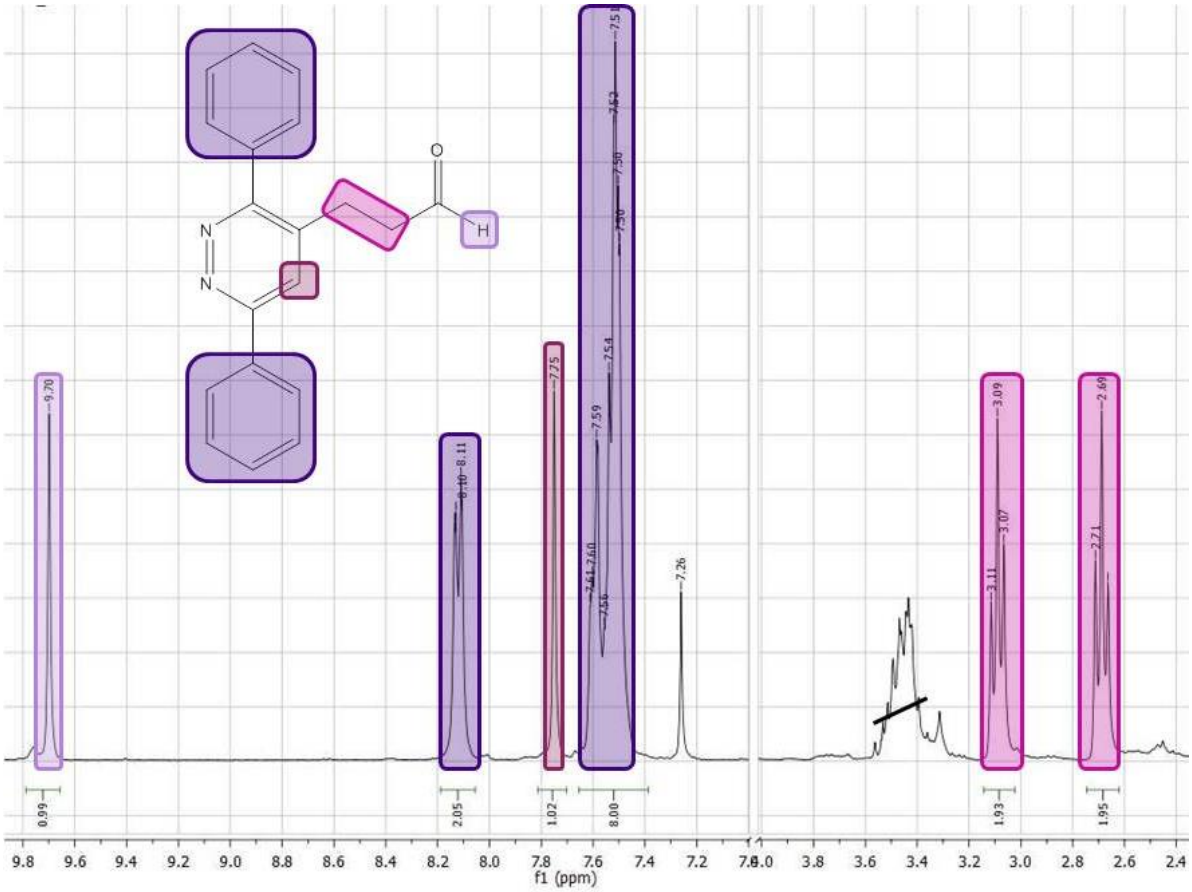
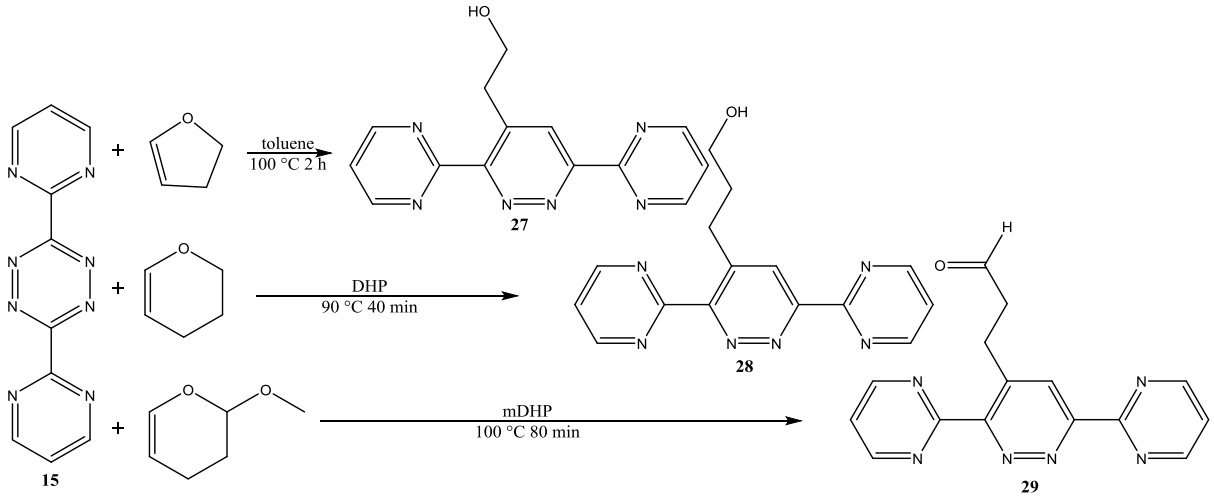


Figure 16: NMR-spectrum of product 26 ($CDCl_3$)

The same conditions were applied to synthesize the dipyrimidinyl-pyridazine derivatives. The arylic-substituents containing two nitrogen atoms lead to a more electron poor character compared to the pyridine-substituted tetrazines. Therefore, iEDDA is favoured for this tetrazine and the reaction should be quicker than in the model reaction. To further accelerate the reactions for both six-membered rings, neat dienophile was used as the solvent (shown in Scheme 15).



Scheme 15: Synthetic pathway to generate dipyrimidinyl-pyridazines

A disadvantage was the poor solubility of the tetrazine **15** in toluene. Thus, despite the stronger electron withdrawing groups, dipyrimidinyl-tetrazine was slower than dipyridinyl-tetrazine. As the tetrazine was better soluble in the olefins used than in toluene, the reaction times for **28** and **29** confirm the higher reactivity of **15**. Nonetheless valid statements concerning the reaction rate only can be done investigating the kinetics. Not only should the reactants be quicker employing an adequate solvent, but also the purification is easier to handle. Only **28** and **29** had to be purified through column chromatography, **27** could be used without further purification.

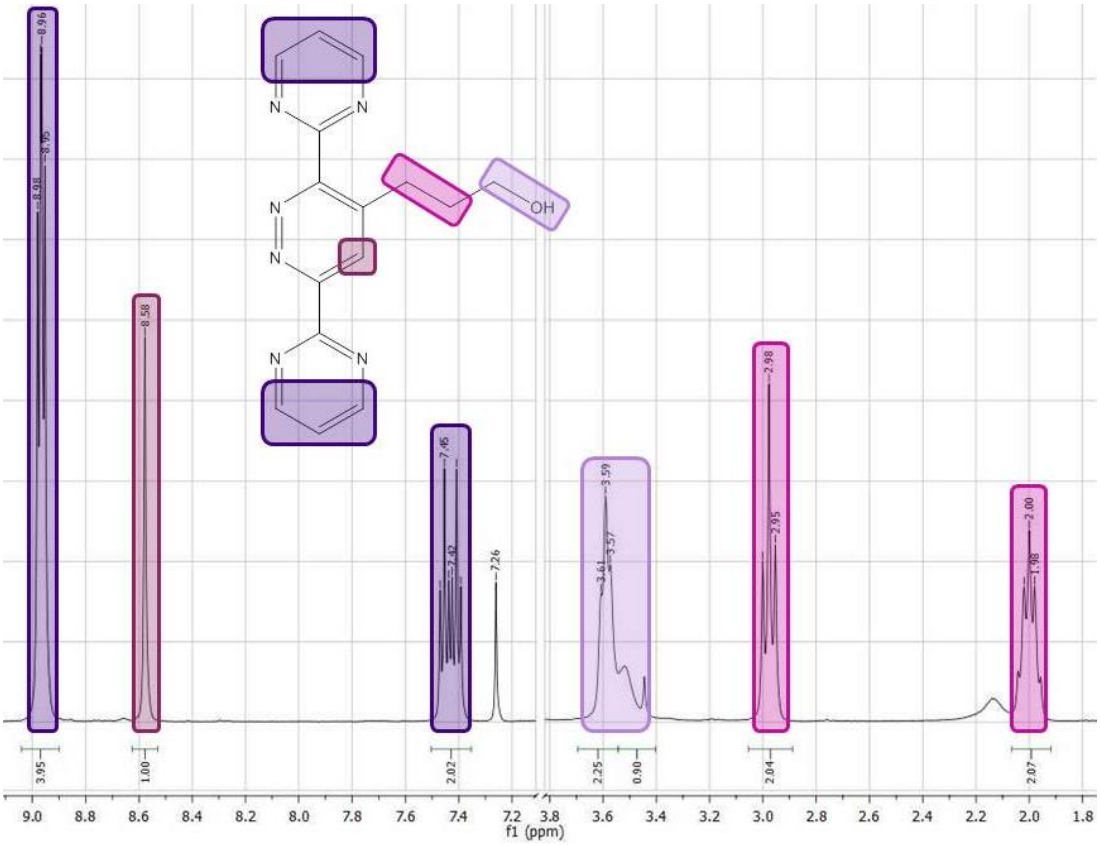
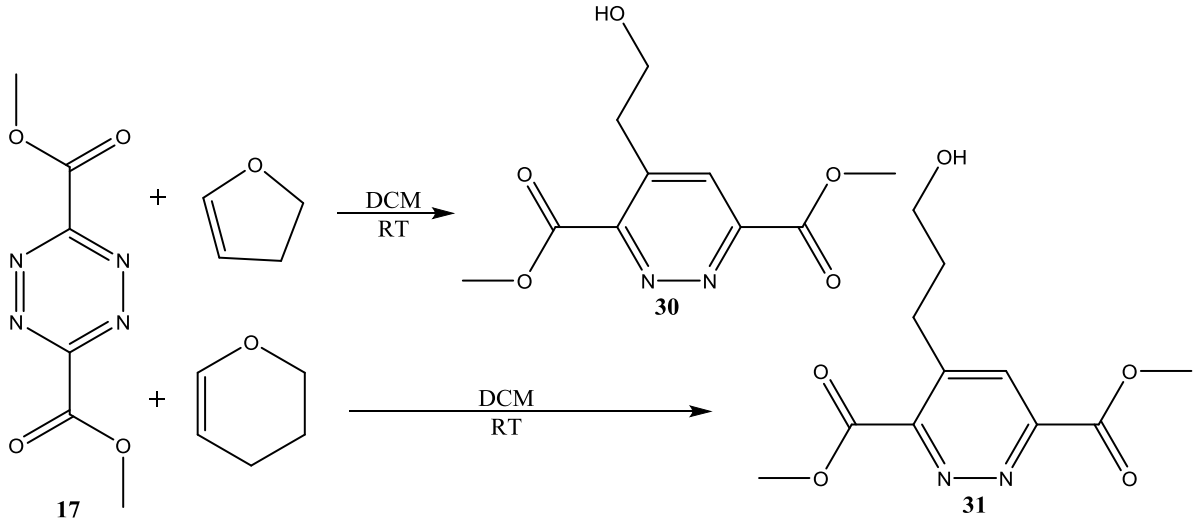


Figure 17: NMR-spectrum of product 28 ($CDCl_3$)

As shown in Figure 17, the spectrum of a pyrimidine-substituted has many analogies to those having pyridinyl-substituents. In the aromatic region there is a well-defined singlet of the pyridazine-hydrogen which confirms, together with the peaks in the high field, the product identity. In this case there is no defined alcohol peak visible, because it overlaps with the CH_2-peak at 3.5 ppm. Similar to the spectrum of **22**, the aromatic hydrogen atoms of both rings split slightly differently, which can be observed at the signal at 7.42 ppm.

The reactions with dimethyl-pyridazine-dicarboxylates operated as assumed. Due to the strong electron withdrawing substituents both reactions were finished after a few minutes at room temperature. Since **17** could only be obtained on a milligram scale, only the un-substituted olefins could be tested (see Scheme 16).



Scheme 16: Synthetic pathway to generate dimethyl-pyridazine-dicarboxylates

When performing those two reactions, the release of nitrogen could be observed as a strong gas evolution. As mentioned in the section about synthesizing the reactant **17**, dry solvents were used under inert conditions. After the iEDDA was performed those precautions were no longer necessary and both products were purified via column chromatography. Again, the singlet of the pyridazine-ring was easily visible in the NMR spectrum. Due to the de-symmetrisation of the tetrazine through the iEDDA reaction the two methyl groups show slightly different chemical shifts.

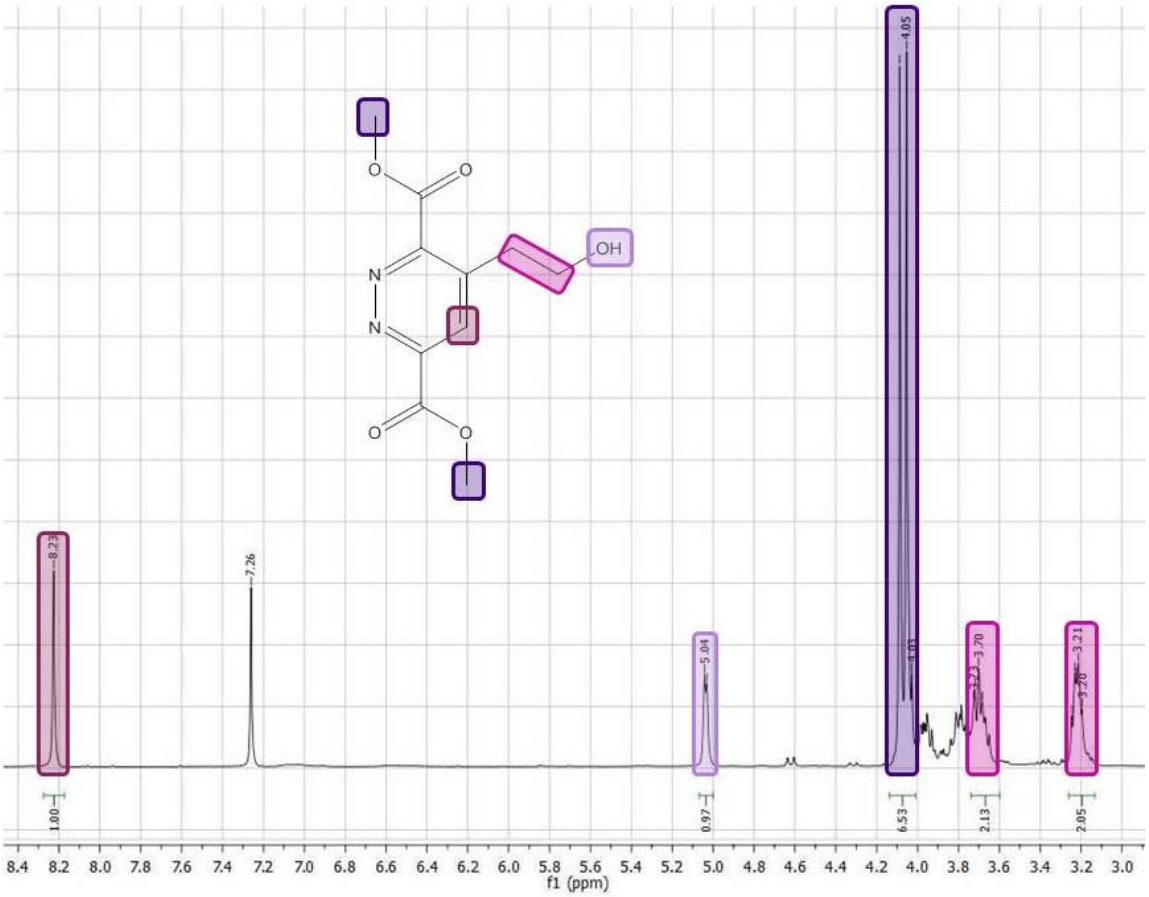


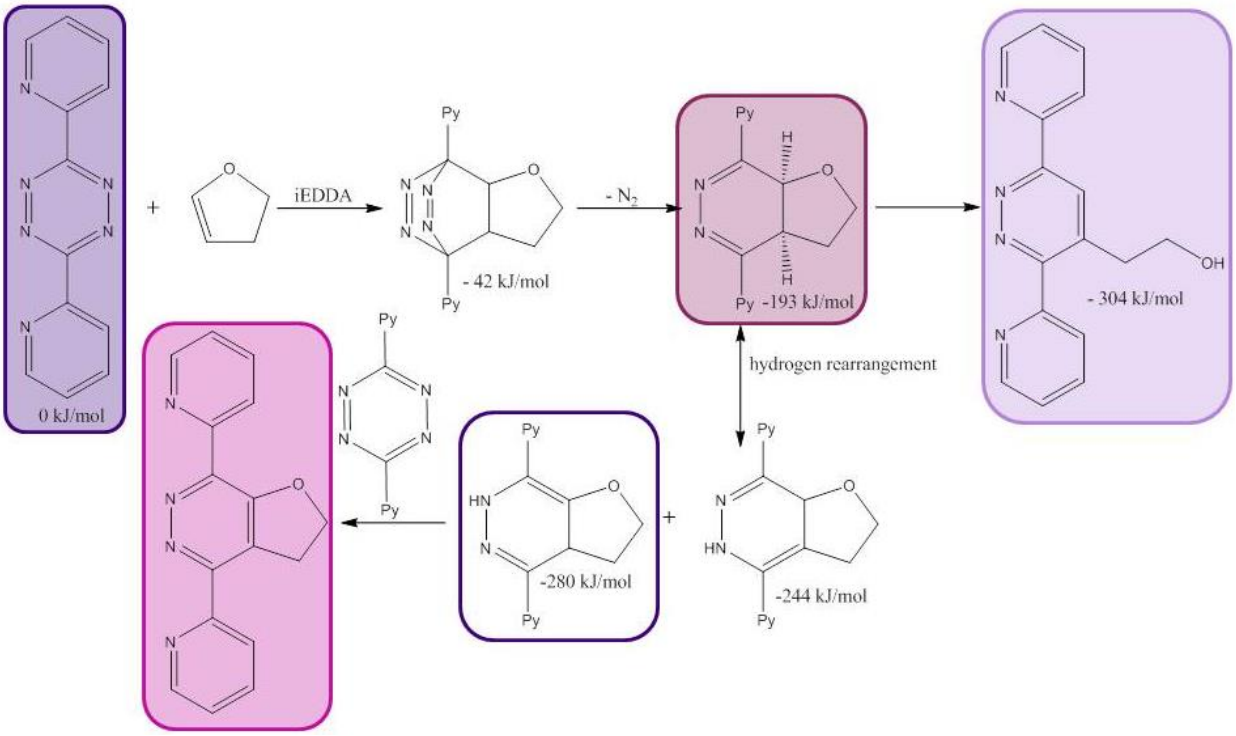
Figure 18: NMR-spectrum of 30 ($CDCl_3$)

In both cases dihydrotetrazine (**12**) could be observed, which originates from the self-oxidation during the iEDDA reaction of from an impurity in the previous step. Due to the fact that **17** decomposed easily, both reactions were performed before the purity of the reactant was checked. A subsequent, more detailed characterization of **17** revealed that only around 80 % of the dihydrotetrazine precursor had been oxidised. This could be one reason why both products yielded rather low with 21.7 % (**31**) and 7.0 % (**30**).

3.3 Kinetic studies

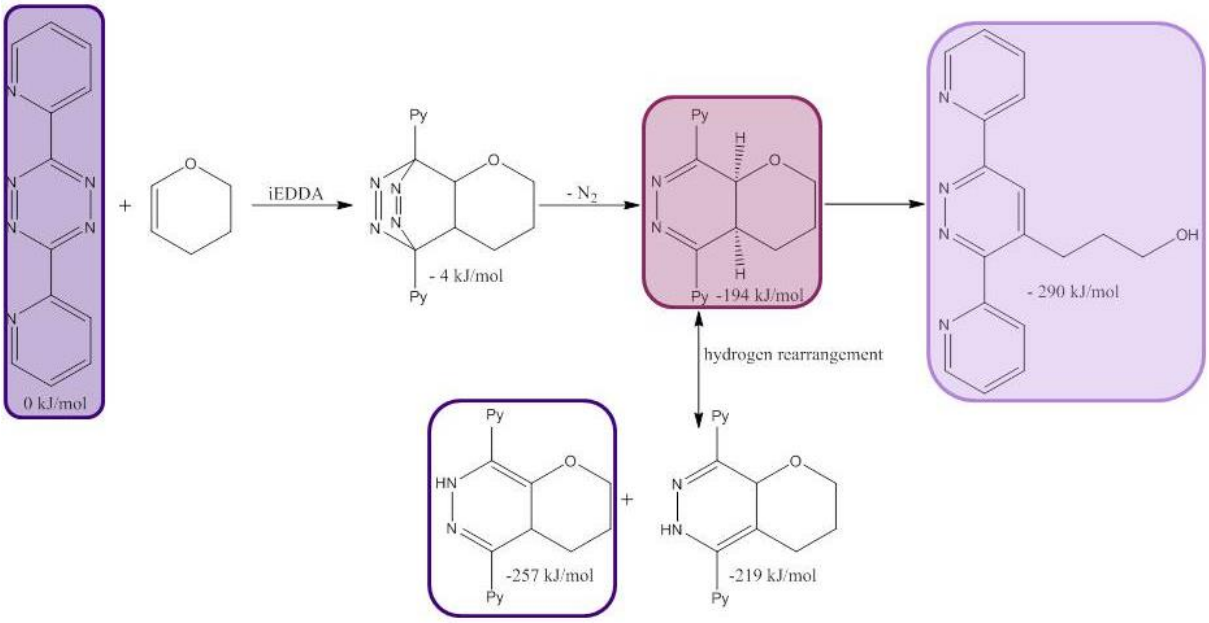
3.3.1 Nuclear Magnet Resonance and theoretical calculations

Our knowledge of the iEDDA was further expanded by reactions between the dipyridinyl-tetrazine and DHP/DHF, monitored using NMR-spectroscopy and theoretical computations done by Oliver Hofmann (institute of solid state physics). Thus, it was attempted to obtain more profound information about possible stable intermediates.



Scheme 17: Possible intermediates and theoretically calculated energies in the reaction between DHF and dipyridinyl-tetrazine

In Scheme 17 the presumed mechanism of the iEDDA reaction between 2,3-dihydrofuran and 3,6-dipyridin-2-yl-1,2,4,5-tetrazine is shown with the energies calculated. The reaction using DHP as dienophile should work analogously (see Scheme 18).



Scheme 18: Reaction mechanism and theoretically calculated energies of dipyridinyl-tetrazine and DHP

However, as mentioned before, the bicyclic by-product could not be obtained. DHF has a higher ring strain[62,63] and therefore reacts much quicker than the other olefins in this study. Probably this results in different rate-determining steps. For the reaction with DHP, the addition to the tetrazine is assumed to be rate-determining. The corresponding energy is -4 kJ/mol compared to -42 kJ/mol for the five-membered ring (see Figure 19). The transition state is very tense, as the energetically favoured chair conformation cannot be formed and the oxygen is even closer to the nitrogen atoms compared to the case when DHF is used as dienophile. After the removal of nitrogen, both reactions form similar intermediates with hardly any energy difference. Subsequently, the pyran-cycle opens. Oxidation and alcohol formation are taking place accordingly. In this mechanism, no intermediate can be detected throughout the reaction, as it immediately reacts onwards and therefore hydrogen rearrangement does not take place. In contrast, the addition of the dienophile proceeds easier in the model reaction. It is presumed that, as the ring opening is rate determining, traces of the intermediate are present in the solution until the product is formed and the cycle opens. During this period the hydrogen atoms may undergo an 1,3-rearrangement, rendering product formation

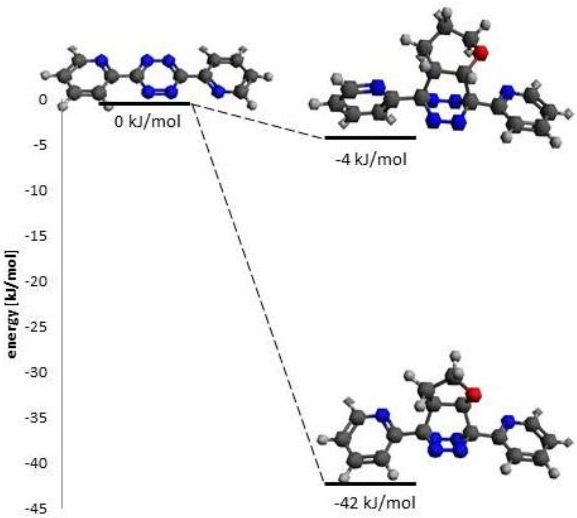


Figure 19: Energy diagram of the transition states of both reactions

[62] O. Nuyken, R. B. Raether, C. E. Spindler, Macromol. *Chem. Phys*., 1998, **199**, 191.
[63] E. Taskinen, *J. Phys. Org. Chem*., 2009, **22**, 42.

impossible, because the hydrogens are on the wrong positions. However, the cyclic by-product can be formed by oxidation with dipyridyl-tetrazine. For both reactions one re-arranged intermediate (framed ones in the Schemes) is energetically favoured and more likely to be formed. In the model reaction the energy difference between the product (-304 kJ/mol) and the rearranged intermediate (-257 kJ/mol) is smaller than for the six-membered ring (-290 kJ/mol to – 257 kJ/mol).

This thesis of having a measurable amount of the intermediate in the reaction vessel was confirmed by analysing the NMR spectra of the model reactions more closely. The NMR spectrum shown in Figure 20 was taken about 5 minutes after both reactants had been added to the vial. Coloured peaks are coloured identically as in Scheme 17 and the other refer to both reactants and some product.

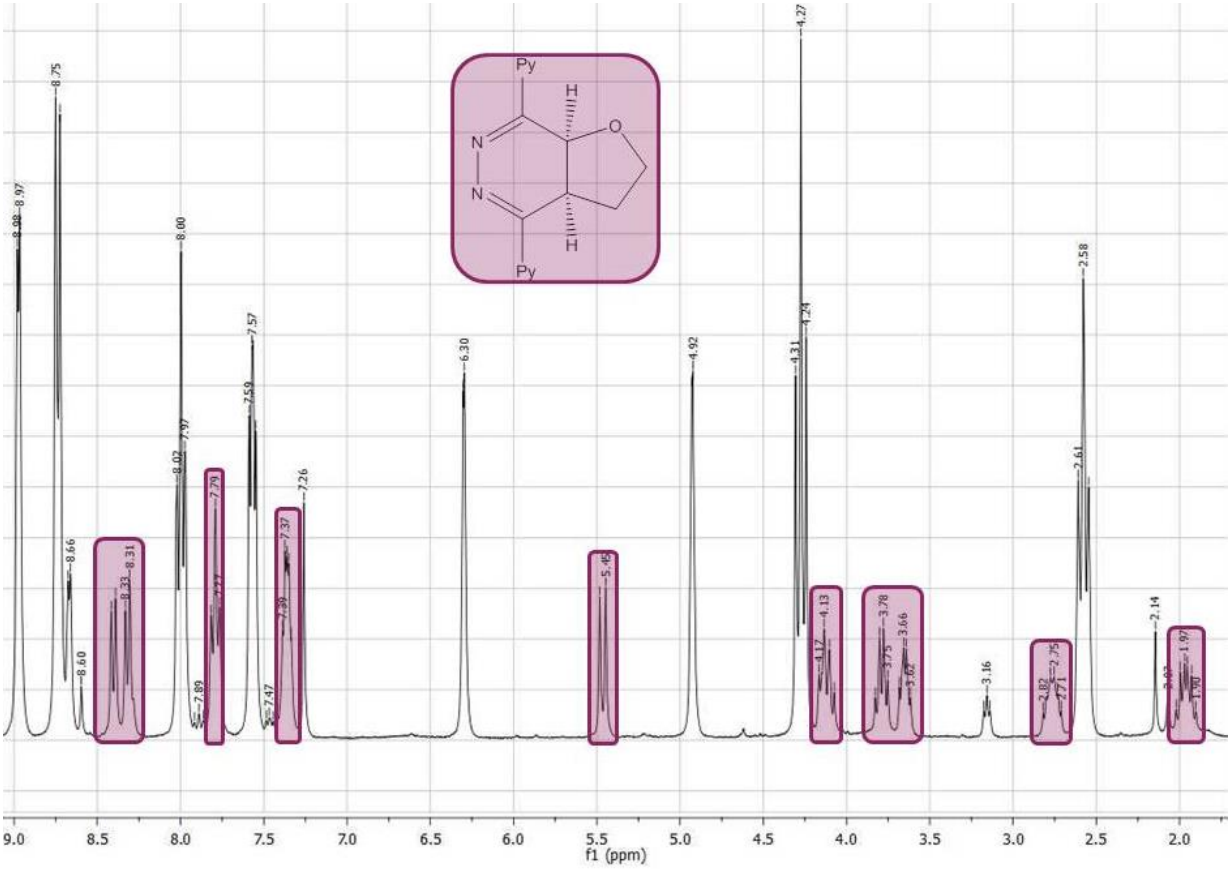


Figure 20: NMR spectrum of the iEDDA of the model reaction; peaks of the intermediate are coloured accordingly ($CDCl_3$)

In the aromatic section the spectrum is similar, only the peaks at 8.4 ppm and 7.79 ppm differ slightly. In the aliphatic part of the spectrum all six hydrogen atoms can be detected. Also both CH_2 groups of the ring split up due to different chemical surroundings. Therefore six peaks are visible that integrate to one. Closer investigation of the spectrum confirmed to have observed this intermediate instead of the other two. Additionally, when, using increments, calculated peak areas were compared (see Figure 21), the data of **III** was the one closest to the measured spectrum. Derivative **II** has one hydrogen atom next to the oxygen too, and the shift would suit even better to the spectrum, however in this case only a singlet would be observable. In intermediate **I**, the hydrogen between the

two rings would split up (into a double-doubled), however the shift would be more in the high field. The doublet at 5.45 ppm therefore stems from the hydrogen atom next to the oxygen in the pyridazine – ring.

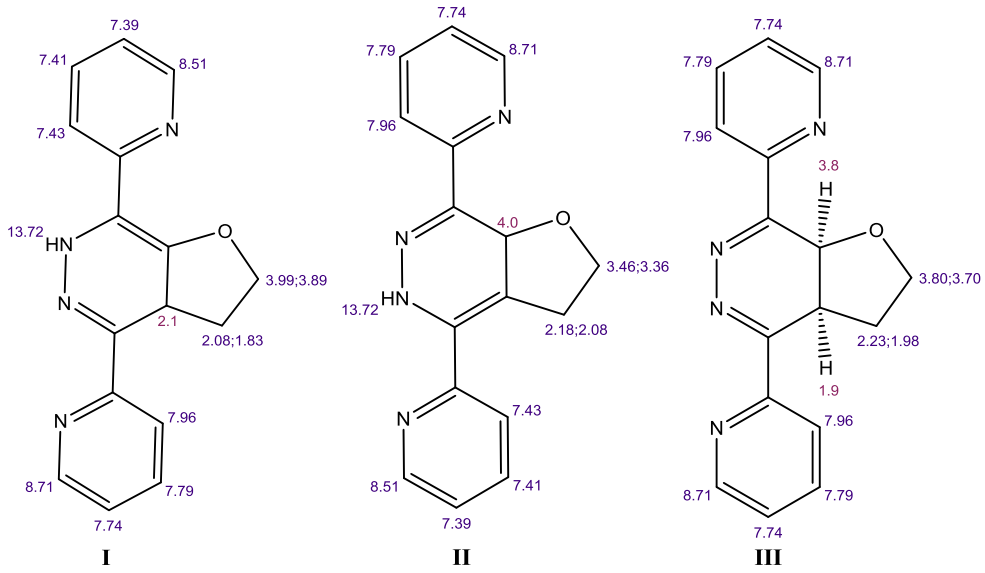


Figure 21: Calculated peak areas (using increments) for all three possible intermediates of the model reaction; lighter numbers are not completely computable

Following the reaction progress over time using NMR spectroscopy, it gets obvious, that those peaks are only present at the beginning of the reaction.

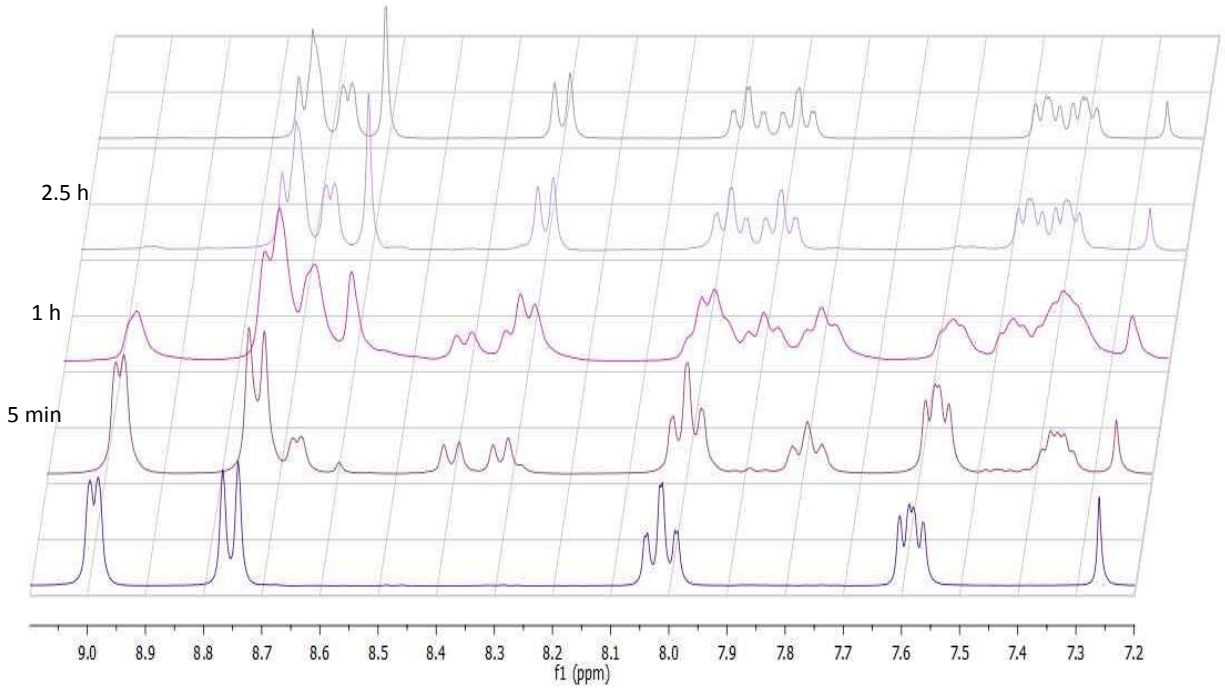


Figure 22: Reaction progress of the model reaction from the start (front) until the end (back); showing the changes in downfield ($CDCl_3$)

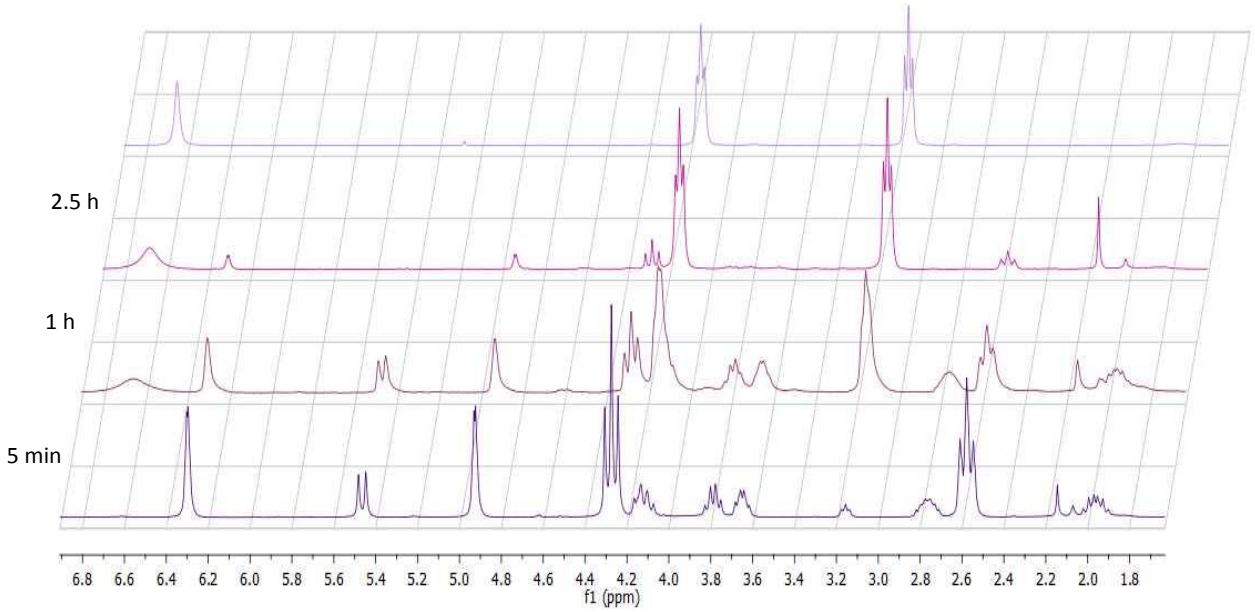


Figure 23: Reaction progress of the model reaction from the start (front) until the end (back); showing the changes in upfield ($CDCl_3$)

The spectrum of dipyridinyl-tetrazine **14** is not depicted because all peaks are in the aromatic region. After the reaction was finished the excess of DHF and both side products were removed to obtain a spectrum of the pure product. Both side products were visible in the NMR beforehand, but the peaks were too small to be visible in the spectra of the reaction process.

Also the reaction between dipyridinyl-tetrazine and DHP was analysed using NMR spectroscopy. Similar to the other reaction, the NMR-tube was equipped with both reactants in $CDCl_3$. Due to the reaction proceeding slower, the measurements were recorded after days instead of minutes/hours to be able to observe a difference. In contrast to the reaction with DHF, no intermediate was visible. This supports the theory of different rate-determining steps. The formation of the intermediate through cycloaddition is the slowest step in this reaction pathway and therefore the formed intermediate immediately reacts to the desired product. Hence, a 1,3 rearrangement of hydrogen does not take place and the formation of a cyclic side product is impossible.

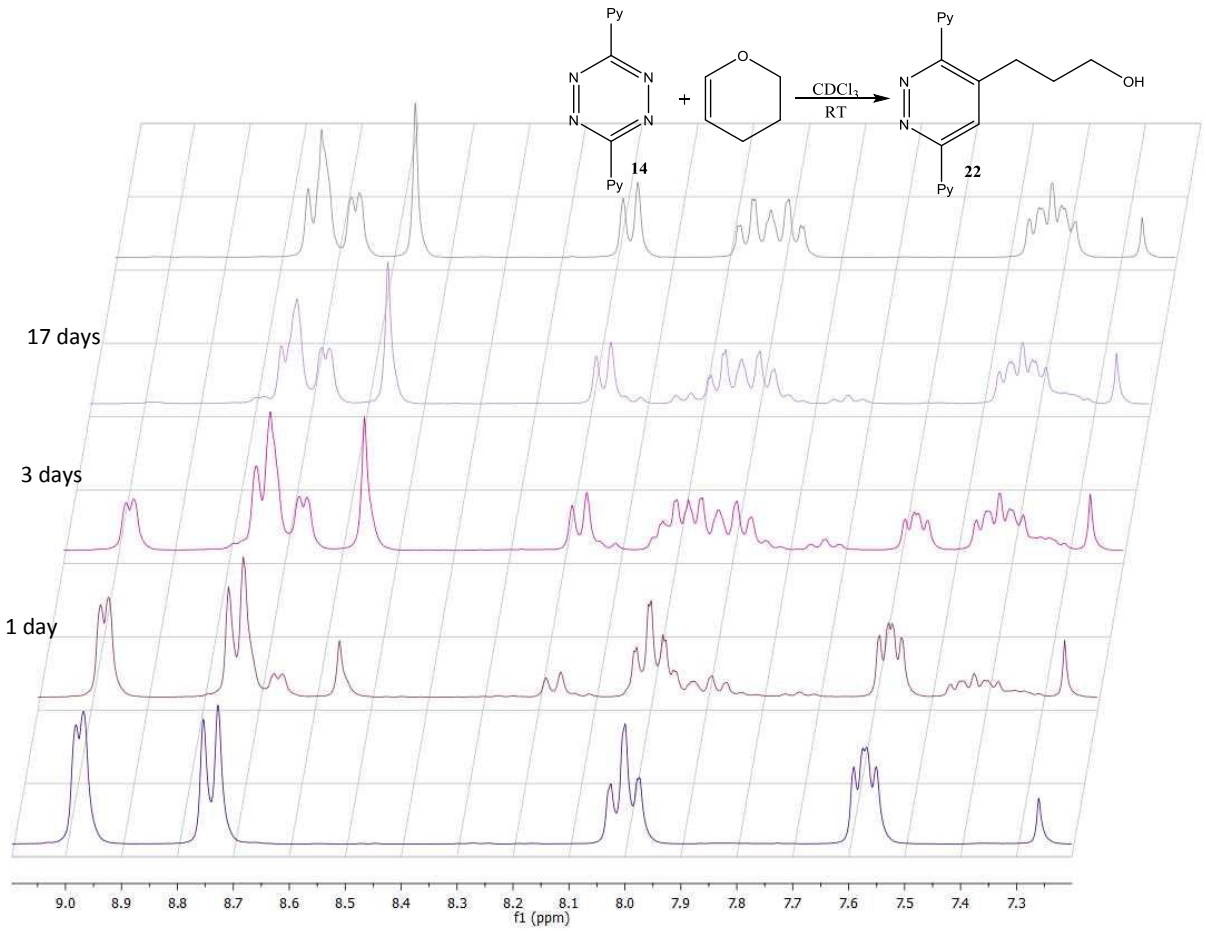


Figure 24: Reaction progress of the model reaction from the start (front) until the end (back); showing the changes in downfield ($CDCl_3$)

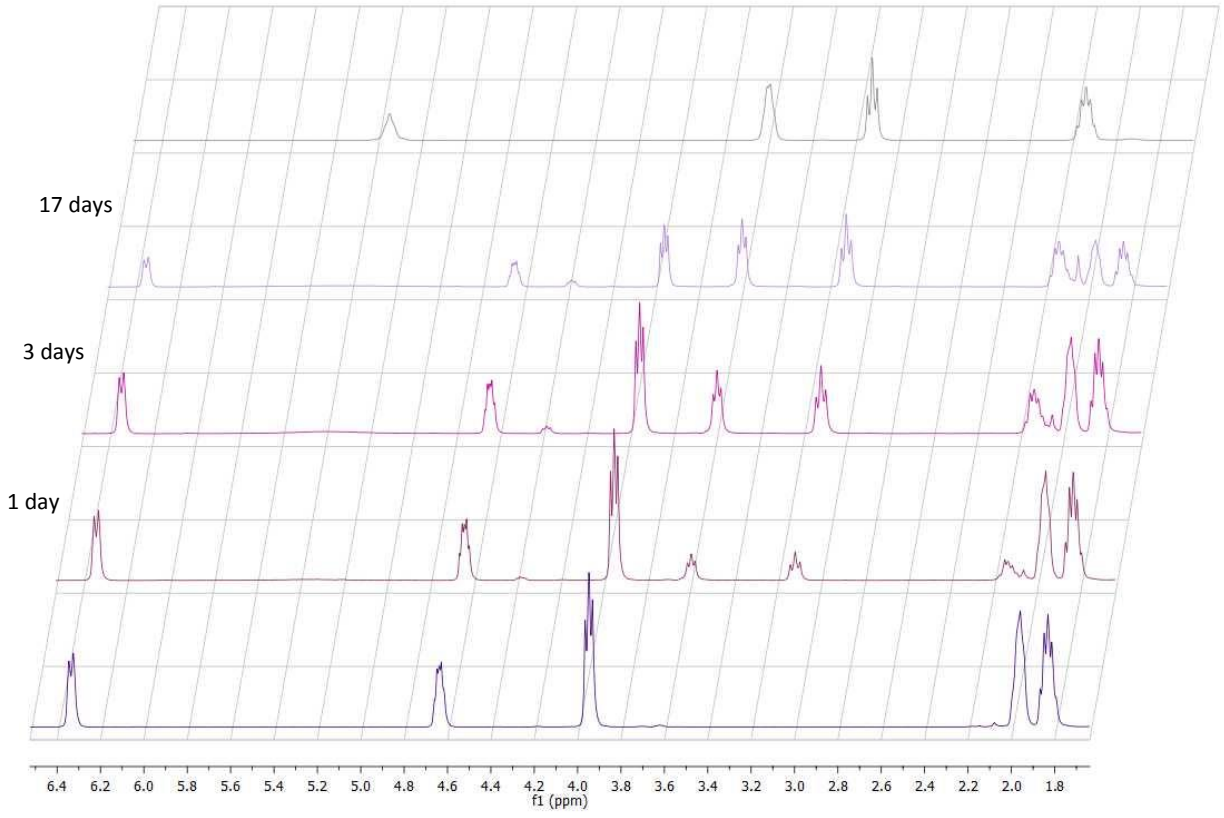


Figure 25: Reaction progress of the model reaction from the start (front) until the end (back); showing the changes in upfield ($CDCl_3$)

Similar to the other experiment, the crude product was purified to measure the spectrum in Figure 25.

3.3.2 UV-Vis spectroscopy

To compare diene-dienophile pairs pseudo first order rate constants were measured by UV-Vis spectroscopy. Both investigated tetrazines were analysed with all three cyclic dienophiles at five different concentrations. The suitable wavelength for the measurements was determined beforehand by recording an absorption spectrum of the tetrazine in pure solvent. The reaction progress was determined at around 550 nm, depending on the exact maxima (see Figure 26) and then the reaction rates were computed accordingly.

Table 7: First order rate constants of the iEDDA reactions measured by UV/Vis-spectroscopy (14 measured in MeOH, 15 measured in $CHCl_3$)

	wavelength	DHF $[\mathrm{mol \cdot L^{-1}s^{-1}}]$	DHP $[\mathrm{mol \cdot L^{-1}s^{-1}}]$	Acetal $[\mathrm{mol \cdot L^{-1}s^{-1}}]$
Pytz **14**	545 nm	$0.03 \pm 1.1*10^{-4}$	$1.65*10^{-3} \pm 9*10^{-5}$	$4.2*10^{-5} \pm 3*10^{-6}$
Prtz **15**	537 nm	0.114 ± 0.016	$1.65*10^{-3} \pm 1.0*10^{-4}$	$3.60*10^{-4} \pm 1.0*10^{-5}$

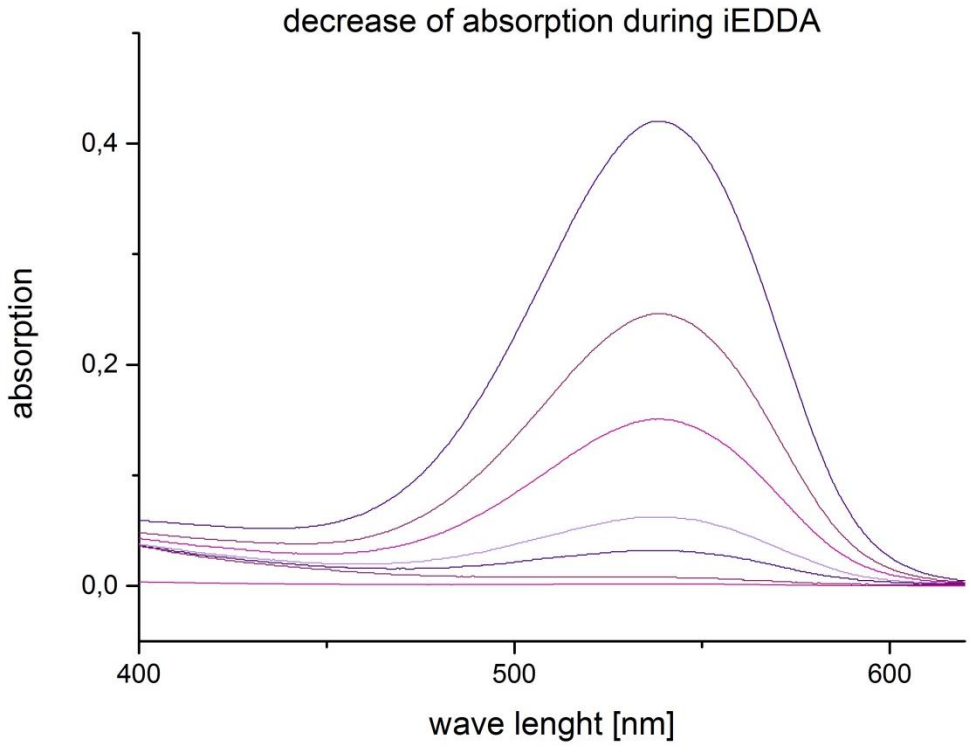


Figure 26: Decrease of tetrazine concentration (15) during iEDDA corresponding to the absorption at 537 nm

Due to the low reaction speed with diphenyl-tetrazines, only the reactions rates of dipyridinyl-tetrazines and dipyrimidinyl-tetrazines were tested. At first k' was calculated for different concentration of each reaction pair by plotting the results like shown in Figure 27.

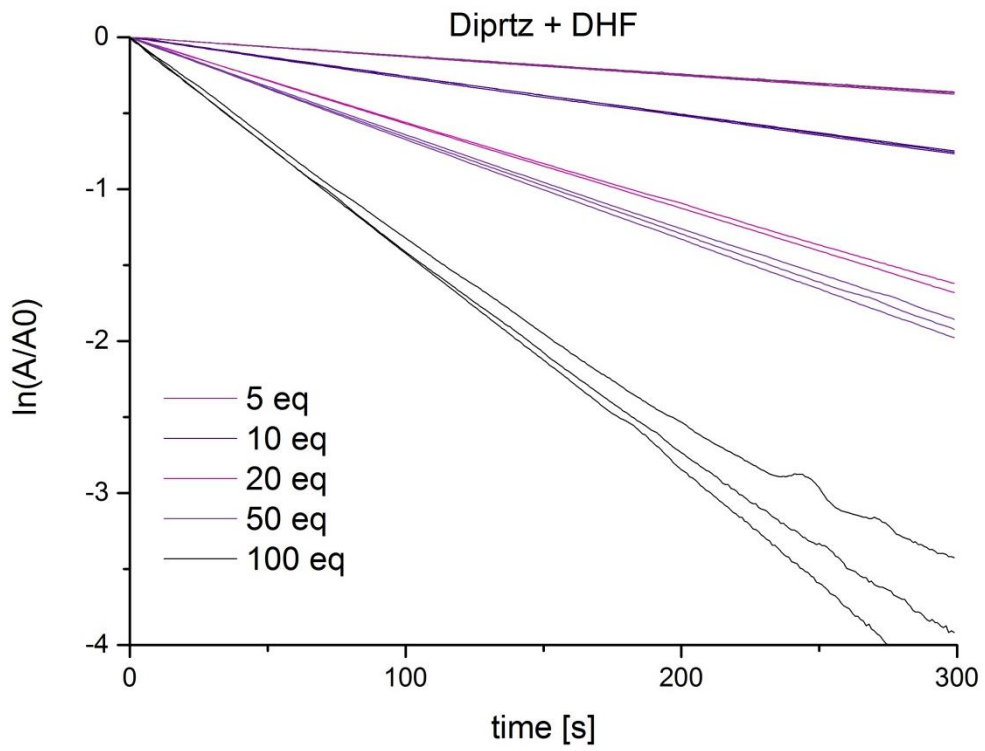


Figure 27: ln(A/A$_0$) against the time for different equivalents of DHF with dipyrimidinyl-tetrazine as diene

Subsequently, k' was plotted versus the corresponding concentration to gain comparable pseudo first order reaction rates of the reactions. Normally the dienophile was added in 10-, 20-, 50- and 100-fold excess. However, the reaction between dipyrimidinyl-tetrazine and DHF was too quick finishing before the measuring time of five minutes was over. Therefore 5 eq of dienophile were measured additionally for this reaction. For slower reactions the measurement time had to be adjusted appropriately to monitor the concentration decreasing at least by 0.01. In those cases the cyvette was closed to prevent the solvent from evaporating and allering the concentration. For all measurements with dipyridinyl-tetrazine, methanol was employed due to its low vapour volatility. Dipyrimidinyl-tetrazine is only sparsely soluble in methanol and therefore chloroform was used. For better comparability the reaction with DHF was recorded in chloroform, too.

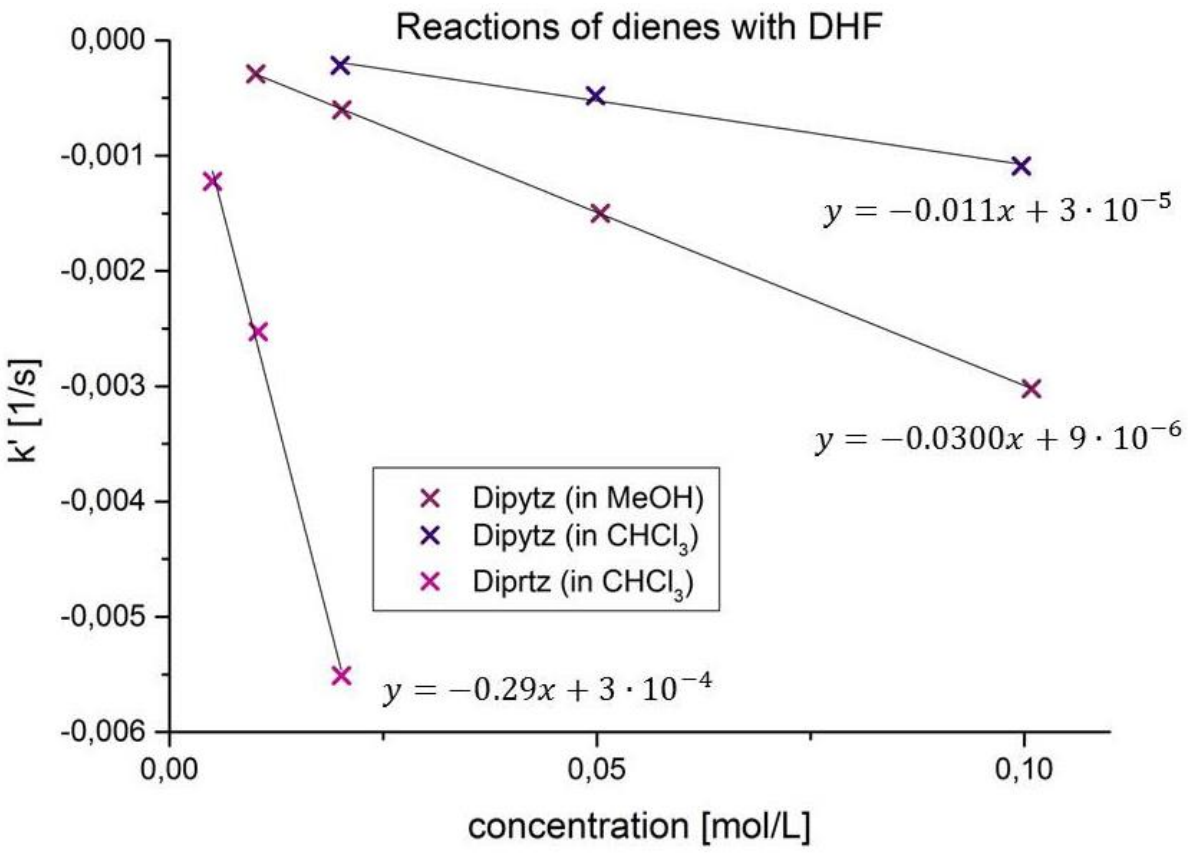


Figure 28: Rate constants of DHF and both tested tetrazines

As shown in Figure 28 dipyrimidinyl-tetrazine reacts roughly tenfold faster with olefinic dienophiles compared to with dipyridinyl-tetrazine (0.29 $L*mol^{-1}*s^{-1}$ versus 0.03004 $L*mol^{-1}*s^{-1}$). At first this extreme difference was attributed to the different solvents used. Therefore the reaction rate for the dipyridyl-tetrazine was also tested in chloroform where it proved to be even slower with 0.0111 $L*mol^{-1}*s^{-1}$ than in MeOH. This could be due to the fact that protic solvents promote the transition states of the iEDDA reaction. Due to the fact that the reactions with 6-membered-rings are much slower, it was not expedient to plot all reaction rates in one figure.

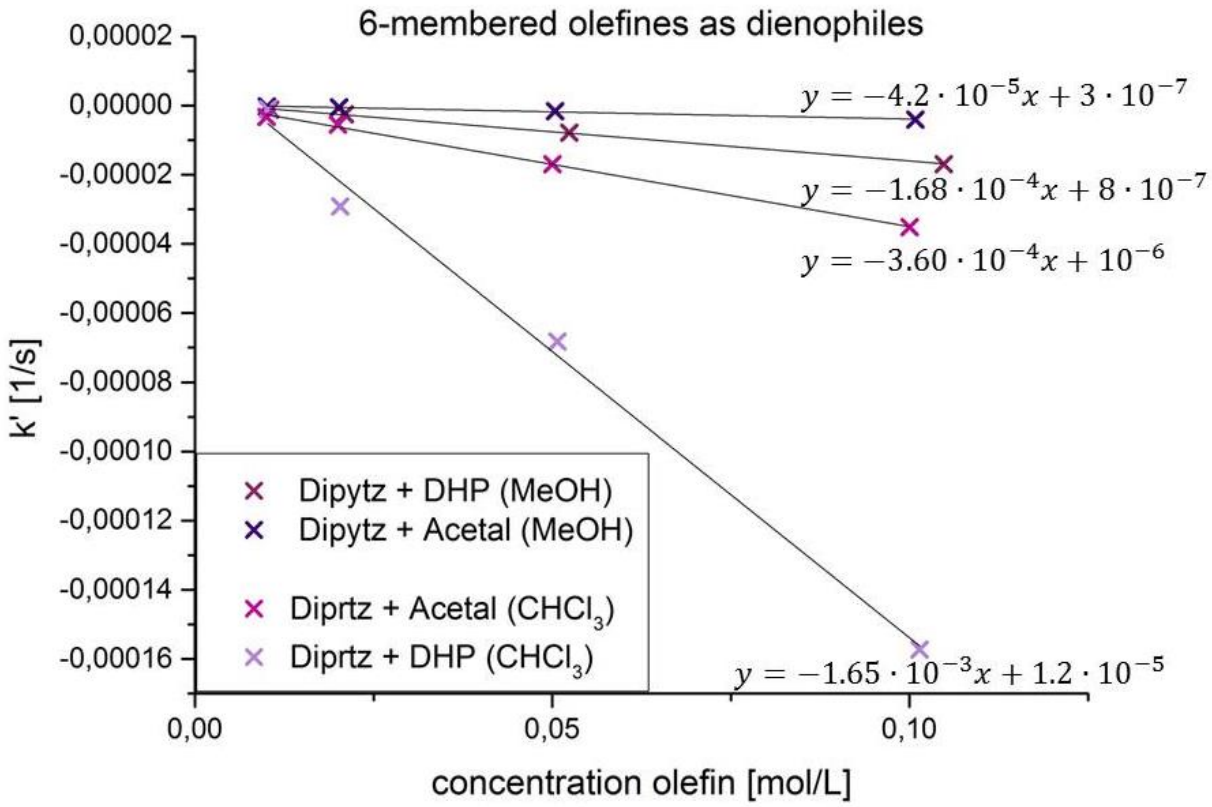


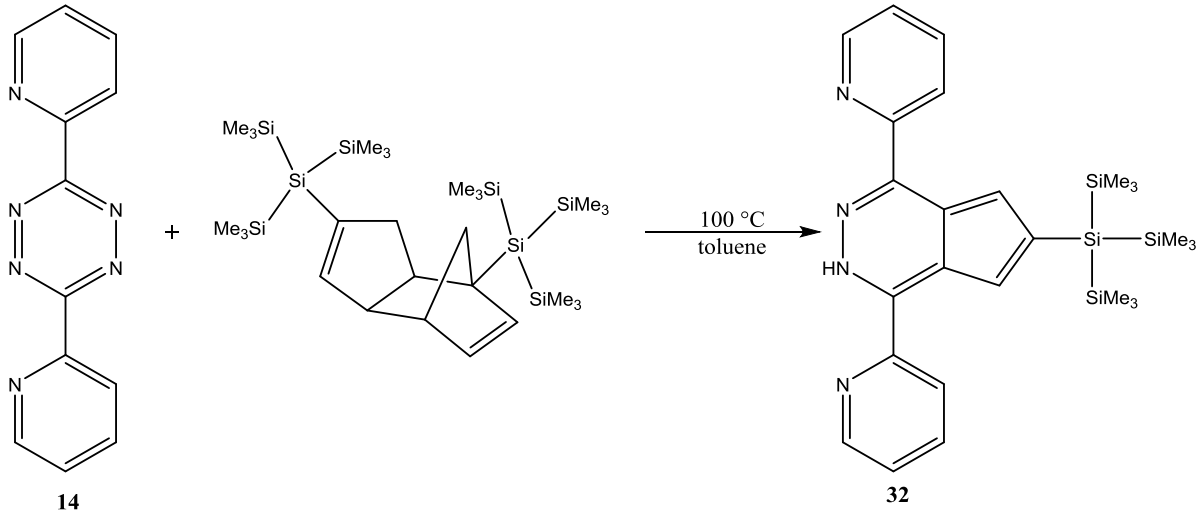
Figure 29: Rate constants of the six-membered-rings reacting with both tetrazines

Similarly, also reactions with the other two olefins were significantly quicker when dipyrimidine-tetrazine was used as the diene. The differences between DHP and mDHP were even higher with $1.69*10^{-4}$ L*mol^{-1}*s^{-1} to $4.2*10^{-5}$ L*mol^{-1}*s^{-1} for the dipyridinyl-tetrazine. With the dipyrimidinyl-tetrazine as diene, the rate constants were 0.00165 L*mol^{-1}*s^{-1} for the DHP and $3.60*10^{-4}$ L*mol^{-1}*s^{-1} for the mDHP (for more exact information, see page 62).

Altogether, the kinetic measurements supported the reaction times observed in the synthesis with different olefins. Due to the more electron withdrawing character of the pyrimidine compared to pyridine, the higher reaction rates of **27** are no surprise. They confirm the thesis that the dipyrimidinyl-tetrazine was only slower in the synthetic tests with iEDDA because of its low solubility regarding the used solvent.

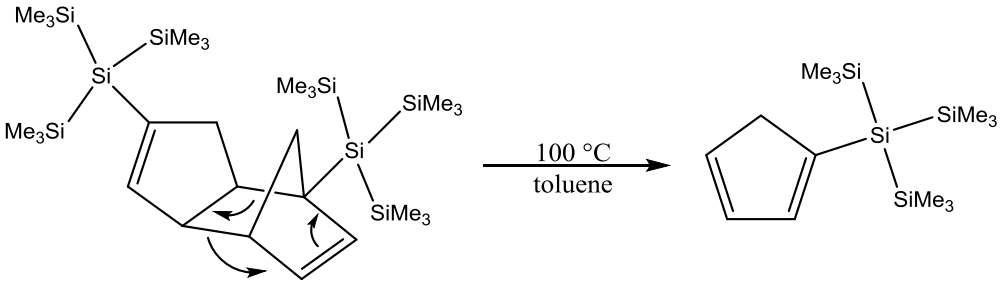
3.4 3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazine and Hypersilyl-DCPD

As the reactions between tetrazines and norbornenes are very common, it was also attempted to react dipyridyl-tetrazine with a very particular dicylcopentadien derivative provided by the Baumgartner/Marschner group. As shown in Scheme 19, a reactant with hypersilyl- substituents (2,2'-((3aR,4R,7R,7aS)-3,3a,7,7a-tetrahydro-4H-4,7-methanoindene-2,4-diyl)bis(1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilane)) was chosen.



Scheme 19: Reaction pathway of pyridinyl-tetrazine and polysilyl-DCPD

At the reaction conditions used for iEDDA, hypersilyl-DCPD spontaneously undergoes a retro Diels-Alder reaction. Based on this knowledge, only half of the normal amount of olefin had to be added to accomplish the reaction, as one DCPD gives two dienophiles (see Scheme 20).



Scheme 20: Retro Diels-Alder reaction of polysilyl-DCPD

After both reactants were dissolved in toluene at 100 °C the reaction was stirred for six hours. The completion of the reaction was determined by TLC. Once again dihydrotetrazine **2** could be identified as a side product. At first it was tried to separate it from the product by dissolving **32** in pentane. Due to the trimethyl-silyl-groups the product is soluble in pentane and dihydrotetrazine could be removed by filtration. However the product was not completely pure afterwards and a column chromatography had to be done additionally. With this method product could be isolated, although

the majority was still contaminated with the reduced tetrazine. The pure product decomposes slowly over a period of two or three weeks, resulting in a colour change from dark red to a brown/blackish. Fortunately, the product contaminated with dihydrotetrazine did not decompose. It could be almost completely purified by another column chromatography and was stored under nitrogen.

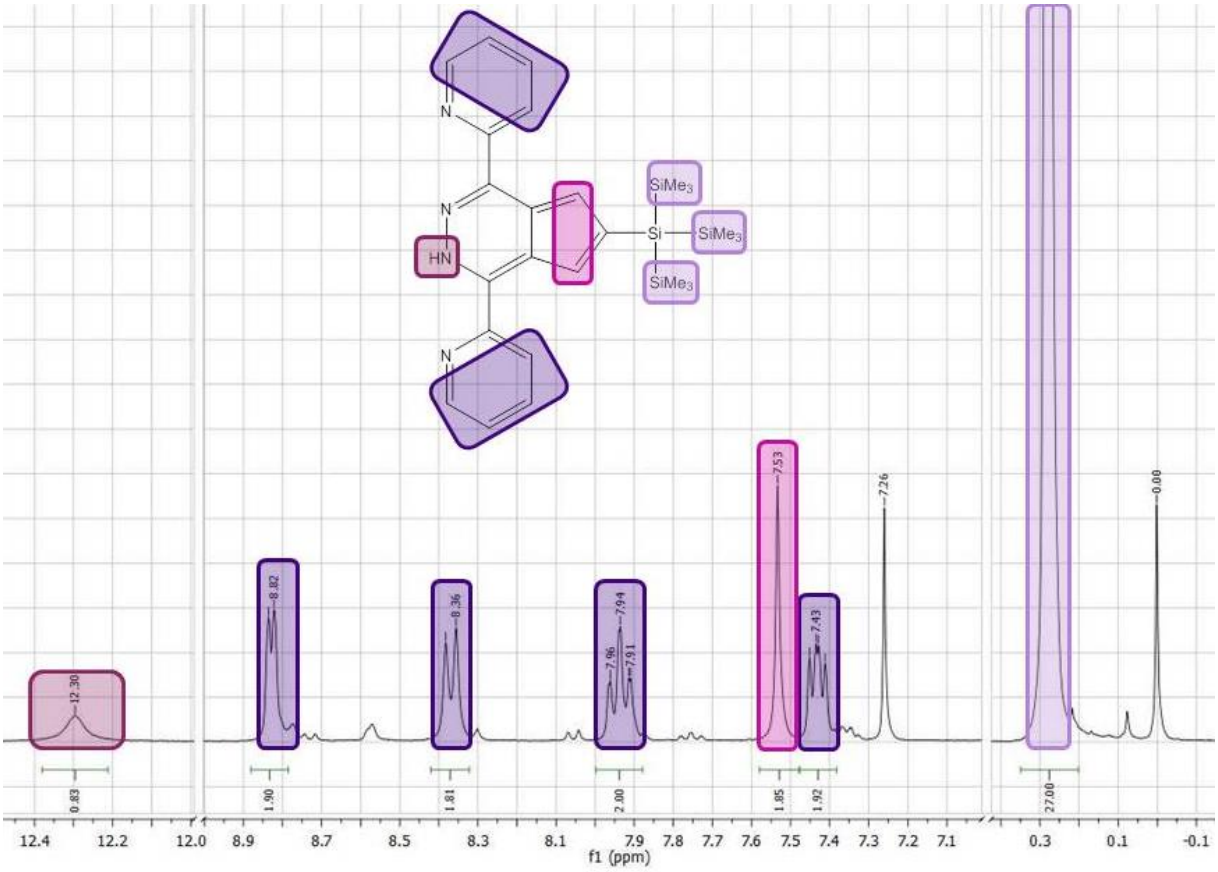


Figure 30: NMR spectrum of 32 containing minor amounts of 2 before the second purification

The spectra depicted in Figure 30 shows product **32** before the second column chromatography. About 7 % of dihydrotetrazine **2** are visible in the aromatic area. The pure product was examined more closely. As the structure of the molecule could only be guessed by NMR-interpretation, its crystal structure was investigated. The spatial arrangement of product **32** and its exact structure is shown in Figure 31.

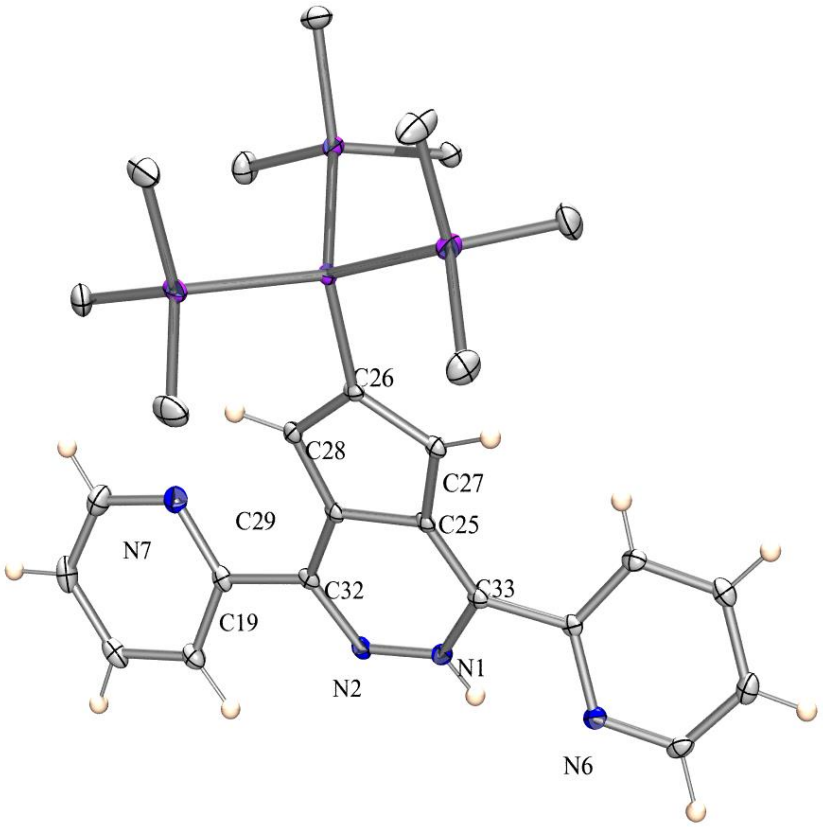


Figure 31: Crystal structure of product 32

Regarding the N_1-C_{33} bond, (Figure 31), which is a single bond lengths are 1.349(3) Å compared to 1.47 Å. The theoretical bond length of a N=C double bond would be 1.30 Å. Bond lengths between single and double bonds were also observed in the five-membered ring, for example, the C_{28}-C_{26} bond in Scheme 19, which is drawn as a single bond. The theoretical lengths for carbon-carbon bonds are 1.54 Å (C-C) and 1.34 Å (C=C). Compared to those the one between C_{28} and C_{26} is with 1.421(4) Å again in the middle. Both examples for bond lengths implicate an aromatic character for the product. The π-electrons are not stationary but distributed equally over the whole molecule. All four cycles are almost planar, although they are all strongly twisted relative to each other. The hypersilyl-substituent is sterically demanding because all the methyl-groups are distorted against each other.

For further analysis **32** was subjected to mass spectroscopy. The sample was ionized by electron impact (EI) and detected using a time of flight detector (TOF). The calculated molecular mass of 518.2173 g/mol matched the exact mass of 518.2161 for $C_{26}H_{38}N_4Si_4$.

Due to the bright red colour of the solid also the absorption-spectrum was done. Therefore the molar extinction coefficient was determined at different absorption maxima of the spectrum.

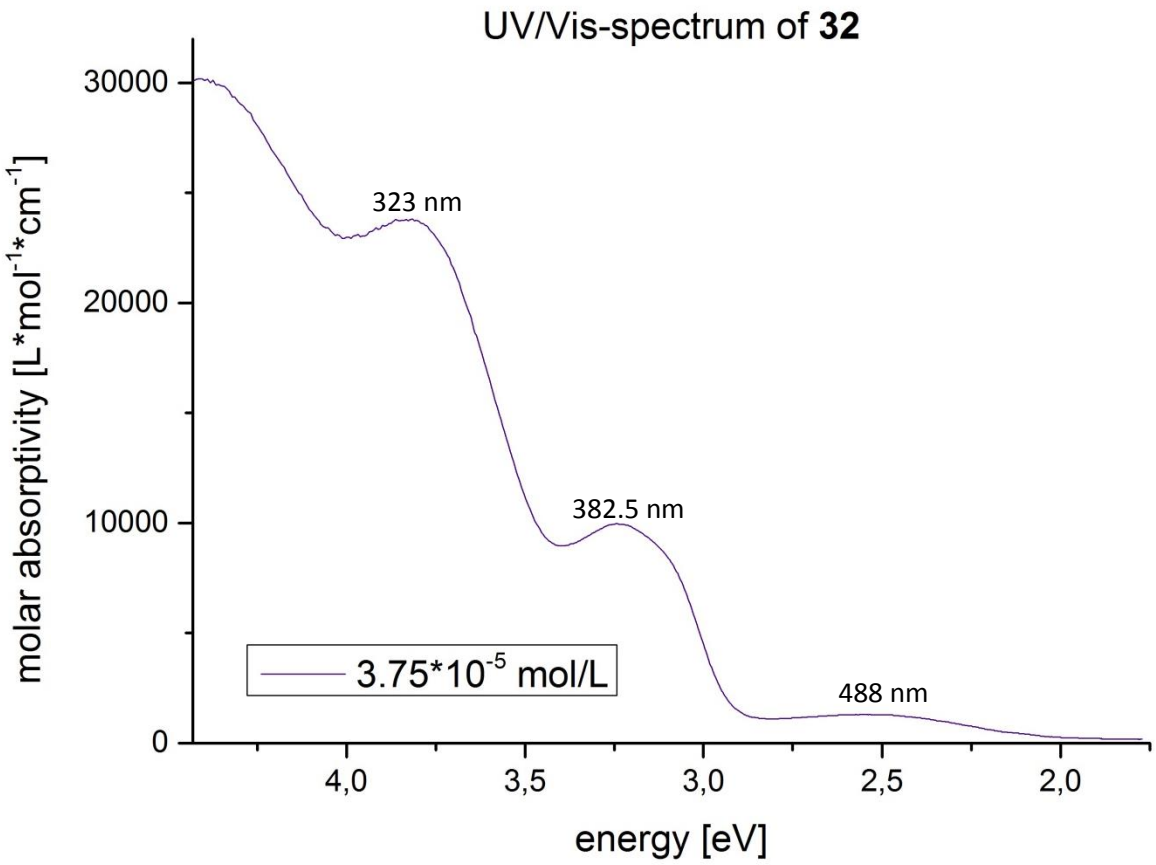


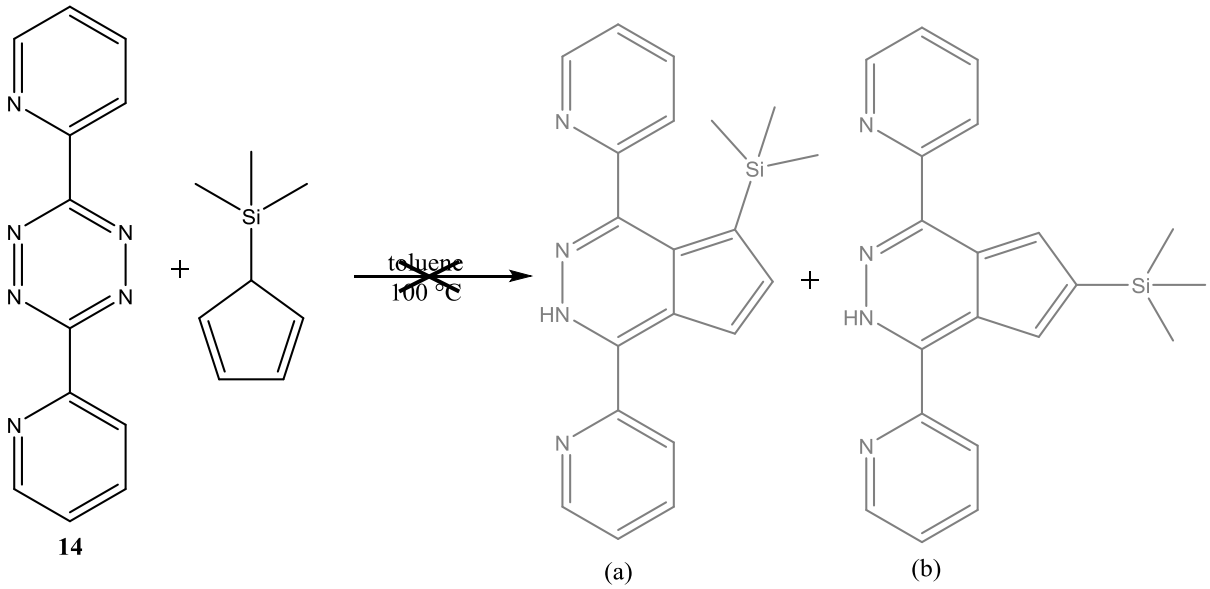
Figure 32: Absorption spectrum of 32, absorption against energy [eV], corresponding wavelengths of the three maxima are depicted

The measurements were performed as quickly as possible to avoid decomposition. Using different concentrations, interferences could be eliminated. As depicted in Figure 32, high concentrations resulted in saturation of the detector. All different concentrations measured could only be used for calculating the maxima around 500 nm. Due to the fact of higher absorption at lower wave lengths for the other two maxima lower concentrations were used for computation. The absorption coefficient was calculated using the Beer-Lambert law (shown in Table 8).

Table 8: Molar absorptivity of 32 at different wave lengths

wave length	molar absorptivity
323 nm	23339 L*mol^{-1}*cm^{-1}
382.5 nm	8145 L*mol^{-1}*cm^{-1}
488 nm	905 L*mol^{-1}*cm^{-1}

Another reaction with a similar compound was tried out to investigate these kinds of dienophiles more closely.



Scheme 21: Expected possible products of 14 and trimethylsilyl-cyclopentadiene

The reaction was performed analogous to the other iEDDA reactions and 1.2 equivalents of cylcopenta-2,4-dien-1-yltrimethylsilane were added. After stirring it overnight at 100 °C, conversion of **14** was completed. The products appearing on TLC were separated by column chromatography. However neither (a) nor (b) could be detected by NMR. The only identified product was dihydrotetrazine **2**. The decomposition at room temperature in air was much quicker compared to product **32**. The colours of the collected fractions already changed during the removal of the solvent. Due to the slightly different arrangement of double bonds regarding polysilyl-DCPD, the formation of (a) would be favoured. However, no product could be identified via NMR spectroscopy. It was not possible to investigate the products more closely because the decomposition was too quick, despite of storage under nitrogen after solvent removal.

4 Summary & Outlook

There are diverse options to synthesise tetrazines, depending on the desired substituents, like nitriles and hydrazine or ethyl diazoacetate. All reaction methods had in common that, usually, at first dihydrotetrazines are synthesised which are subsequently oxidised to tetrazines, using NO_x. The yields for both reaction steps differ greatly, but can be improved by applying a few tricks like cooling the reaction vessel before obtaining the product by filtration. The synthesised di-1,3-dimethylpyrazol-tetrazine can be used in nuclear substitution reactions to generate asymmetric tetrazines, which are rather difficult to synthesise. Using nucleophiles with two functional groups could result in possible monomers for a polymer built of tetrazines.

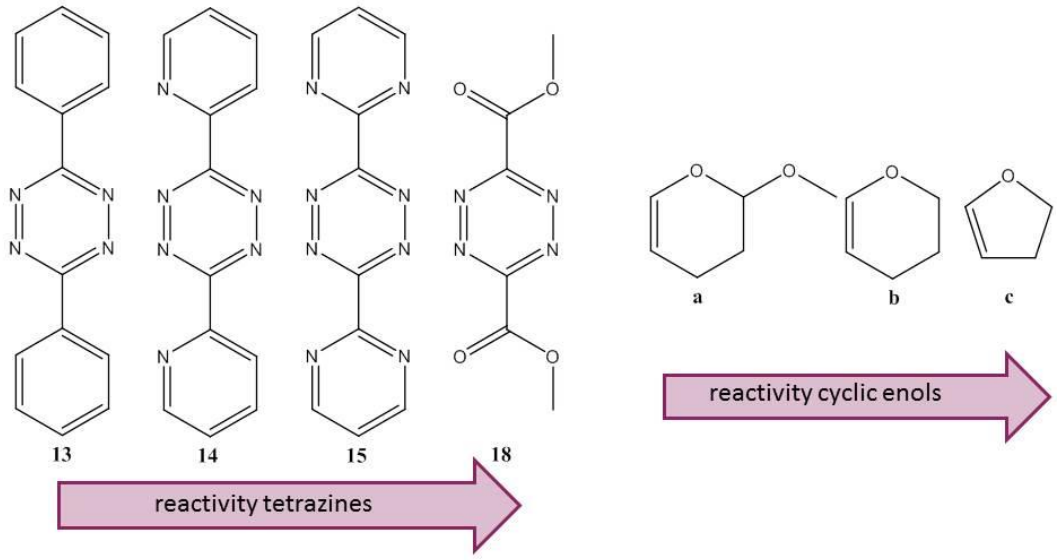


Figure 33: reactivity of different reactants in iEDDA

The synthesised tetrazines were used as dienes in inverse electron demand Diels-Alder reactions. Electron withdrawing substituents on the tetrazines, ring strain and electron donating groups on the dienophile accelerated the reaction (see Figure 33). Additional acceleration was achieved by high reaction temperatures and excess of the dienophile.

Table 9: First order rate constants of the iEDDA reactions measured by UV/Vis-spectroscopy (14 measured in MeOH, 15 measured in $CHCl_3$)

	wavelength	DHF $[mol \cdot L^{-1}s^{-1}]$	DHP $[mol \cdot L^{-1}s^{-1}]$	Acetal $[mol \cdot L^{-1}s^{-1}]$
Pytz **14**	545 nm	$0.03 \pm 1.1*10^{-4}$	$1.65*10^{-3} \pm 9*10^{-5}$	$4.2*10^{-5} \pm 3*10^{-6}$
Prtz **15**	537 nm	0.114 ± 0.016	$1.65*10^{-3} \pm 1.0*10^{-4}$	$3.60*10^{-4} \pm 1.0*10^{-5}$

Using UV/Vis-spectroscopy, rate constants of pseudo first order reactions were calculated. With this methode the fading of the red colour was measured to gain exact information of the different reaction rates (depicted in Table 9).

Closer investigations concerning the reaction mechanism showed the oxidising character of dipyridin-tetrazines **14** in iEDDA. The dihydrotetrazine-derivative **2** was obtained as a by-product. Additionally also a bicyclic pyridazine (4,7-di(pyridin-2-yl)furo[2,3-d]pyridazine) was found when the reaction was performed with dihydro-furan. Regarding the mechanism of **14** with **b/c** different rate determining steps are most likely the reason for the formation of the closed by-product merely in this reaction. For tetrazine **14** and enol **c** the rate determining step is the oxidation of the pyridazine-ring, whereas when **b** is used, the addition of the dienophile to the tetrazine is rate determining. For an even better understanding regarding the mechanism and kinetics of this reaction, further measurements at different temperatures (NMR-spectroscopy and UV/Vis-spectroscopy) are necessary.

5 Experimental Part

5.1 General

5.1.1 Chemicals

All chemicals were purchased from Sigma Aldrich, Alfa Aesar, Acros Organics, Fluka or ABCR and used as received. Solvents used for reaction workup and purification were generally of analytical grade and normally used as received. Only cyclohexane had to be distilled before usage due to impurities. If not mentioned differently, distilled water was always used for synthesis.

When experiments had to be performed under inert conditions, the reaction vessel was evacuated, heated and flushed with nitrogen three times. Dichloromethane was distilled over P_2O_5 and stored over molecular sieves. Also methanol was dried by storing it over molecular sieves, which were previously activated by heating them at 200 °C in high vacuum for a few hours.

5.1.2 Analytical methods

For thin layer chromatography, silica-gel plates on aluminium foil from Merck (TLC silicagel 60 F_{254}) were used. If spots were not visible to the naked eye, a UV-lamp or dipping solutions were used. Potassium permanganate solution was prepared by dissolving 1 w% $KMnO_4$ in water. For CAM a solution of 10 w% $(NH_3)_3MO_4.4H_2O$ in H_2SO_4 (10 %) was mixed with 10 w% cerium(IV)-sulfate in H_2SO_4 (10%) in a ratio of 12.5 to 1.

As a stationary phase for column chromatography silica gel was used. The used amount was depending on the separation problem at hand and also determined the size of the column used. As solvent either a mixture of CH and EA or DCM and MeOH were used. The separation was performed using a rubber hand-pump to generate pressure.

For nuclear magnet resonance spectroscopy (NMR) a Bruker Avance III 300 spectrometer with auto sampler was used for measurement of ^1H spectra with 300 MHz and ^{13}C spectra with 75 MHz. Applied solvents were deuterated chloroform and d-DMSO with chemical shifts at δ = 7.26 ppm (^1H), 77.16 ppm (^{13}C) and δ = 2.50 ppm (^1H), 39.52 ppm (^{13}C). The residual peaks were used as reference for the chemical shifts.

5.2 Kinetics

To get a general overview concerning the reaction mechanism and impacts of temperature, ratios and various reaction partners, reactions were performed directly in UV-Vis cuvettes and NMR-tubes.

5.2.1 NMR

To find out about possible stable transition states, which cannot be observed in the final product, a few reactions were started in NMR-tubes. Therefore about seven to ten milligram of the respective tetrazine were dissolved in d-chloroform and about one equivalent of the olefin was added. The reaction progress was monitored by recording five to ten spectra at different degrees of conversion whereby the exact intervals depended on the individual reaction rate of each experiment.

5.2.2 UV-Vis spectroscopy

For all measurements stock solutions with 2 mmol/L for the tetrazine and 200 mmol/L for the olefin were prepared beforehand and then mixed to get the final concentration in a volume of 2 mL (see Table 10). The decrease in absorption at a fixed wave length was determined three times each at four different concentrations.

Table 10: Exact mixing rations at different concentrations

ratio Tz:O	tetrazine [µL]	olefin [µL]	solvent [µL]
1:5	1000	50	950
1:10	1000	100	900
1:20	1000	200	800
1:50	1000	500	500
1:100	1000	1000	-

As the olefin was added in high excess, it was possible to generate rate constants with pseudo first order kinetics. Using the following formulas firstly k' was computed for different olefin/tetrazine ratios and afterwards the according average values were plotted against the olefin concentrations to get the second order rate constant k for each tetrazine-olefin couple.

$$ln\left(\frac{A}{A_0}\right) = k' \cdot t \qquad k' = k \cdot c$$

A… absorption at time t A_0…absorption at t = 0 t…time [s]

c…concentration [mol/L] k…rate constant $\left[\frac{mol}{L \cdot s}\right]$

5.2.2.1 3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazine

The absorption was measured at 545 nm corresponding to the absorption of the tetrazine and MeOH was used as a solvent. Approximately 240 to 300 points were recorded depending on the measured time period. The exact method settings are summarised in Table 11.

Table 11: Method settings of 3,6-di(pyridine-2-yl)-1,2,4,5-tetrazine

		1:10	1:20	1:50	1:100
DHF	total time [min]	5	5	5	5
	time between measuring points [s]	1	1	1	1
DHP	total time [min]	480	240	120	60
	time between measuring points [s]	120	60	30	15
Acetal	total time [min]	480	240	120	60
	time between measuring points [s]	120	60	30	15

For exact computations of the rate constant the "real" concentrations instead of the theoretical ones were used for the calculations of k.

Table 12: Ratio constants of tests with 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (in methanol)

olefin	ratio Tz:O	average k' [s^{-1}]	k $\left[\mathbf{mol \cdot L^{-1} s^{-1}}\right]$
DHF	1:10.1	$2.9*10^{-4} \pm 2*10^{-5}$	$0.03 \pm 1.1*10^{-4}$
	1:20.3	$6.02*10^{-4} \pm 5*10^{-6}$	
	1:50.7	$0.0015 \pm 8*10^{-6}$	
	1:101.4	$0.0030 \pm 1*10^{-4}$	
DHP	1:10.5	$1.18*10^{-6} \pm 6*10^{-8}$	$1.65*10^{-3} \pm 9*10^{-5}$
	1:21.0	$2.48*10^{-6} \pm 9*10^{-8}$	
	1:52.6	$7.9*10^{-6} \pm 2*10^{-7}$	
	1:105.1	$1.7*10^{-5} \pm 2*10^{-6}$	
Acetal	1:10.0	$3.0*10^{-7} \pm 5*10^{-8}$	$4.2*10^{-5} \pm 3*10^{-6}$
	1:20.1	$5.35*10^{-7} \pm 8*10^{-9}$	
	1:50.2	$1.6*10^{-6} \pm 1.8*10^{-7}$	
	1:100.4	$4.0*10^{-6} \pm 5*10^{-7}$	

5.2.2.2 3,6-Di(pyrimidin-2-yl)-1,2,4,5-tetrazine

All reactions were monitored at the absorption maximum of 3,6-di(pyrimidin-2-yl)-1,2,4,5-tetrazine at 537 nm and chloroform was used as a solvent. Following methods were used for measuring:

Table 13: Method settings of 3,6-di(pyrimidin-2-yl)-1,2,4,5-tetrazine

		1:10	1:20	1:50	1:100
DHF	total time [min]	5	5	5	5
	time between measuring points [s]	1	1	1	1
DHP	total time [min]	40	30	20	10
	time between measuring points [s]	8	6	4	2
Acetal	total time [min]	50	40	30	20
	time between measuring points [s]	10	8	6	4

Additionally to the ratios mentioned in Table 13 also 1:5 was measured with DHF and 5 to 50 eq were used for calculating k.

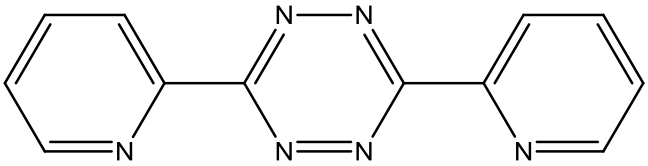
Table 14: Ratio constants of the tests with 3,6-di(pyrimidin-2-yl)-1,2,4,5-tetrazine (in chloroform)

olefin	ratio Tz:O	average k' [s^{-1}]	k $\left[\mathbf{mol \cdot L^{-1} s^{-1}}\right]$
DHF	1:5.0	$1.23*10^{-3} \pm 3*10^{-5}$	0.114 ± 0.016
	1:10.1	$2.53*10^{-3} \pm 4*10^{-5}$	
	1:20.1	$5.51*10^{-3} \pm 1.4*10^{-4}$	
	1:50.3	$6.4*10^{-3} \pm 3*10^{-4}$	
DHP	1:10.1	$1.10*10^{-6} \pm 5*10^{-8}$	$1.65*10^{-3} \pm 1.0*10^{-4}$
	1:20.1	$2.91*10^{-5} \pm 1.3*10^{-6}$	
	1:50.3	$6.8*10^{-5} \pm 6*10^{-6}$	
	1:100.6	$1.57*10^{-4} \pm 3*10^{-6}$	
Acetal	1:9.9	$3.3*10^{-6} \pm 3*10^{-7}$	$3.60*10^{-4} \pm 1.0*10^{-5}$
	1:19.9	$5.5*10^{-6} \pm 1*10^{-6}$	
	1:50.6	$1.7*10^{-5} \pm 1.1*10^{-6}$	
	1:101.2	$3.5*10^{-5} \pm 1.3*10^{-6}$	

5.3 Synthesis

5.3.1 Tetrazines

5.3.1.1 3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazine[64]

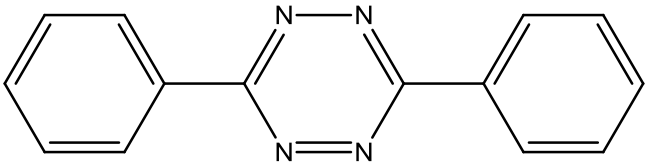


10 g (96 mmol, 1.0 eq) 2-cyanopyridine was dissolved in 15 mL (240 mmol, 2.5 eq) hydrazine hydrate (50 % aq. solution) and the mixture was heated to 100 °C for 4.5 hours. The resulting orange solid was obtained by filtration and washed with cold ethanol (2 x 20 mL). The solid was suspended in 13 mL of glacial acetic acid and 5 mL of water and cooled to 0 °C. Then 9.94 g (144 mmol, 3.0 eq) $NaNO_2$, dissolved in 20 mL water, were added drop wise through a septum. After 2 hours, the formed solid was obtained by filtration, purified by recrystallization in EtOH and dried in high vacuum.

$C_{12}H_8N_6$ [236.2]

Yield	5.14 g pink solid (45.3 %)
R_f (DCM/MeOH 10:1)	0.67
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.97 (d, 2H, $^3J_{HH}$ = 4.1 Hz, py^6), 8.74 (d, 2H, $^3J_{HH}$ = 8.2 Hz, py^3), 8.00 (dt, 2H, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.5 Hz, py^4), 7.57 (m, 2H, py^5).
^{13}C{^{1}H}-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 148.5 (2C, py^6), 147.7 (2C, q, py^2), 146.8 (2C, q, tz^{3,6}), 136.8 (2C, py^4), 125.0 (2C, py^5), 121.4 (2C, py^3).

5.3.1.2 3,6-Diphenyl-1,2,4,5-tetrazine[65]



10 g (97 mmol, 1.0 eq) benzonitrile was dissolved in 15 mL (240 mmol, 2.5 eq) hydrazine hydrate (50 % aq. solution), 1.55 g (48.5 mmol, 0.5 eq) sulfur was added and the mixture was heated to 100 °C for 17 hours. The resulting yellow solid was obtained by filtration. The solid was suspended in 13 mL of glacial acetic acid and a few mL of water and cooled to 0 °C. Then 9.94 g (144 mmol, 3.0 eq) $NaNO_2$

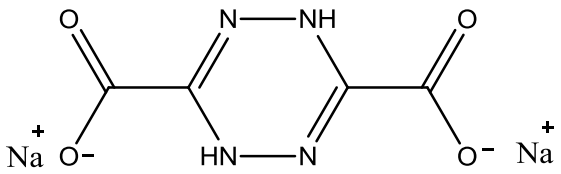
[64] M. H. Klingele, P. D. W. Boyd, B. Moubaraki, K. S. Murray, S. Brooker, *Eur. J. Inorg. Chem.*, 2006, **3**, 573.
[65] W. Chen, D. Wang, C. Dai, D. Hamelberg, B. Wang, *Chem. Commun*., 2012, **48**, 1736. (supporting information)

dissolved in 20 mL of water were added drop wise through a septum. After 2 hours, the formed solid was obtained by filtration and purified by recrystallization in DCM and dried in high vacuum.

$C_{14}H_{10}N_4$ [234.3]

Yield	4.099 g dark pink/red solid (36.1 %)
R_f (CH/EA 10:1)	0.63
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.66 (dd, 4H, $^3J_{HH}$ = 7.82 Hz, $ph^{2,6}$), 7.67-7.59 (m, 6H, $ph^{3,4,5}$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 164.1 (q, 2C, $tz^{3,6}$), 132.8 (2C, ph^4), 131.9 (q, 2C, ph^1), 129.5 (4C, $ph^{3,5}$), 128.1 (4C, $ph^{2,6}$).

5.3.1.3 Sodium 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate

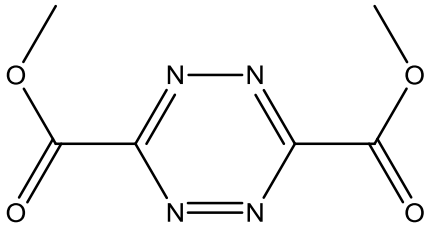


6.862 g (171 mmol, 4.5 eq) NaOH was dissolved in distilled water and about 4.35 g (38.1 mmol, 1.0 eq) ethyl diazoacetate (≥ 13 w% DCM; assumption of exact 13 % DCM) was added drop-wise. The reaction mixture was kept at 60 °C during the addition. Then, the dichloromethane was removed and the resulting slurry was poured on 40 mL EtOH. After 10 minutes of stirring the solid was collected by decanting. This procedure was repeated five times using 20 mL of EtOH each. The solid was obtained by filtration, washed with EtOH and Et_2O and dried in high vacuum.

$C_4H_2N_4O_4Na_2$ [216.06]

Yield	2.714 g yellow solid (65.9 % if DCM = 13 w%)

5.3.1.4 Dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate[66]



2.4 g (11.11 mmol, 1.0 eq) sodium 1,4,-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate was suspended in 3 mL water and 3 g crushed ice. The reaction mixture was cooled using an ice/NaCl-bath. 4.9 mL HCl conc. was added drop wise and the forming solid was filtrated immediately. Subsequently the solid was dispersed in an ice/water mixture for 30 minutes, gained by filtration and dried in high vacuum.

[66] D. L. Boger, R. S. Coleman, J. S. Panek, F. X. Huber, J. Sauer, *J. Org. Chem*., 1985, **50**, 5377.

In a 100 mL 3-necked round bottom flask 2 mL thionylchloride dissolved in 10 mL dry MeOH were cooled to – 30 °C using an acetone/dry ice mixture. 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylic acid was suspended in 10 mL dry MeOH. The mixture was added to the cooled thionylchloride/methanol mixture over 30 minutes using a dropping funnel. Afterwards the orange solution was stirred for another 2 hours at room temperature. Then the solvent was removed under reduced pressure, dissolved in water and extracted four times with DCM. The combined organic layers were dried over Na_2SO_4 and the solvent was removed.

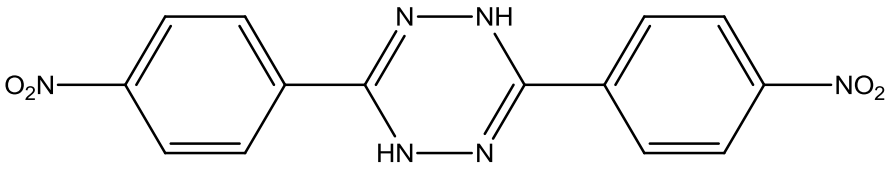
Dry dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate was dissolved in dry DCM and cooled to 0 °C in an ice bath. In a second 100 mL 3-necked round-bottomed flask containing 5 mL HCl conc. slowly 8 mL of an 6 N $NaNO_2$ solution was added drop wise through a septum. Emerging brown gases were bubbled into the reaction vessel using a constant N_2 stream. After the mixture had turned from orange to red the solvent was removed and the product dried in high vacuum.

$C_6H_6N_4O_4$ [198.14]

Yield 207 mg red solid (9.4 %)

^{1}H-NMR (DMSO, 25 °C, 300 MHz): δ (ppm) = 4.22 (s, 6H, COOC*H*$_3$).

5.3.1.5 3,6-Bis(4-nitrophenyl)-1,4-dihydro-1,2,4,5-tetrazine



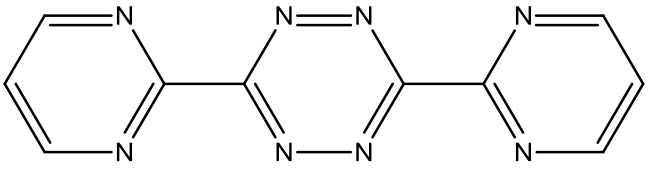
5.012 g (33.8 mmol, 1.0 eq) 4-nitrobenzonitrile and 4.12 mL (84.4 mmol, 2.5 eq) hydrazine monohydrate were combined in a 100 mL flask. During heating the mixture to 100 °C a strong gas evolution was observed. Heating was stopped till the gas emergence ended and the stirring continued overnight. After cooling the solution down to room temperature an orange precipitate formed. To isolate the product, the solid was washed with cold EtOH and dried in high vacuum.

$C_{14}H_{10}N_6O_4$ [326.27]

Yield 2.66 g orange solid (48.3 %)

^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz): δ (ppm) = 7.41 (d, 4H, $^3J_{HH}$ = 8.06 Hz, ph^{2,6}), 6.64 (d, 4H, $^3J_{HH}$ = 8.06 Hz, ph^{3,5}).

5.3.1.6 3,6-Di(pyrimidin-2-yl)-1,2,4,5-tetrazine[67]

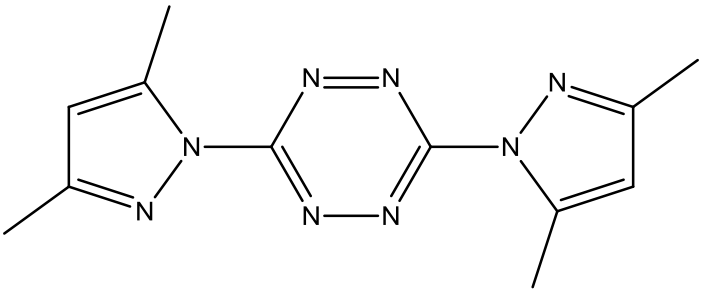


1.024 g (4.23 mmol, 1.0 eq) 3,6-di(pyrimidin-2-yl)-1,4-dihydro-1,2,4,5-tetrazine was suspended in 20 mL acetic acid. 860 µL (6.297 mmol, 1.5 eq) isopentyl nitrite was added drop wise over half an hour and the colour of the suspension changed from orange to violet. After stirring overnight the violet solvent was obtained by filtration and dried in high vacuum.

$C_{10}H_6N_8$ [238.21]

Yield	823 mg violet solid (82 3%)
R_f (DCM/MeOH 10:1)	0.60
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 9.18 (d, 4H, $^3J_{HH}$ = 4.85 Hz, $pr^{4,6}$), 7.63 (t, 2H, $^3J_{HH}$ = 4.85 Hz, pr^5).

5.3.1.7 3,6-Bis(3,5-dimethyl-1*H*-pyrazol-1-yl)-1,2,4,5-tetrazine[68]



2.797 g (29.28 mmol, 1 eq) guanidine hydrochloride and 5 mL (102.5 mmol, 3.5 eq) hydrazine monohydrate were dissolved in 15 mL 1,4-dioxane and heated to reflux for 2 hours. A colourless precipitate formed and was obtained by filtration. The solid was dispersed in 25 mL water in a 3-necked flask and a reflux condenser was added. 5.9 mL (57.8 mmol, 2.0 eq) 2,4-dipentanone were added using a dropping funnel. After 20 minutes the addition was finished and the reaction mixture had turned orange. Stirring was continued overnight, after which an orange precipitate formed. After obtaining it by filtration it was dried on high vacuum.

3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,4-dihydro-1,2,4,5-tetrazine was dissolved in 30 mL DCM and cooled to 0 °C. 5.27 g (76.38 mmol, 3.0 eq) $NaNO_2$ dissolved in 30 mL water were added. Additionally, 3.8 mL acetic acid were added drop by drop through a septum. After four hours the aqueous layer

[67] V. P. Krivopalov, M. B. Bushuev, Y. V. Galitov, O. P. Shkurko, *Russian Chem. Bulletin*, 2010, **59**, 1808.
[68] M. D. Coburn, G. A. Buntain, B. W. Harris, M. A. Hiskey, K. Y. LEA, D. G. Ott, *Heterocyclic Chem*., 1991, **28**, 2049.

was washed with DCM five times. The combined organic phases were washed with 5 % K_2CO_3, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was recrystallized with ethanol and purified by column chromatography (CH/EA 1:1 -> CH/EA 1:5).

$C_{12}H_{14}N_8$ [270.29]

Yield	1.42 g red solid (20.4 %)
R_f (CH/EA 1:1)	0.44
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 6.19 (s, 2H, pz^4), 2.71, 2.39 (2 s, 12H, $pz^{3,5}$-*Me*).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 159.4 (q, 2C, $tz^{3,6}$), 154.6 (q, 2C, pz^5), 143.9 (q, 2C, pz^3), 112.0 (2C, pz^4), 14.8, 14.0 (4C, $pz^{3,5}$-*Me*).

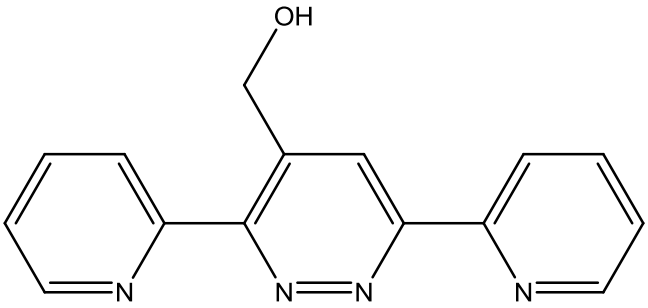
5.3.2 Pyridazines

5.3.2.1 General procedure

If not mentioned otherwise, all pyridazines were synthesised in the same way.

The according tetrazine was dissolved or suspended in toluene and 1.2 eq of the olefin were added. Subsequently the vial or Schlenk flask (dependable on the batch size) was heated to 100 °C until the red to pink colour disappeared. After the solvent was removed the crude product normally was purified by column chromatography.

5.3.2.2 (3,6-Di(pyridin-2-yl)pyridazin-4-yl)methanol[69]



50 mg (0.212 mmol, 1 eq) 3,6-di(pyridine-2-yl)-1,2,4,5-tetrazine was dissolved in 1 mL propargyl alcohol. The crude product was purified by column chromatography (CH/EA 1:1).

$C_{15}H_{12}N_4O$ [264.28]

Yield	42 mg slightly yellow solid (75.0 %)
R_f (CH/EA 1:1)	0.18
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.75-8.64 (m, 3H, $py^{3,6}$), 8.61 (s, 1H, pz^5), 8.57 (d, 1H, $^3J_{HH}$ = 8.0 Hz, py^3), 7.97 (dt, 1H, $^3J_{HH}$ = 7.7 Hz, ^4J_H = 1.6

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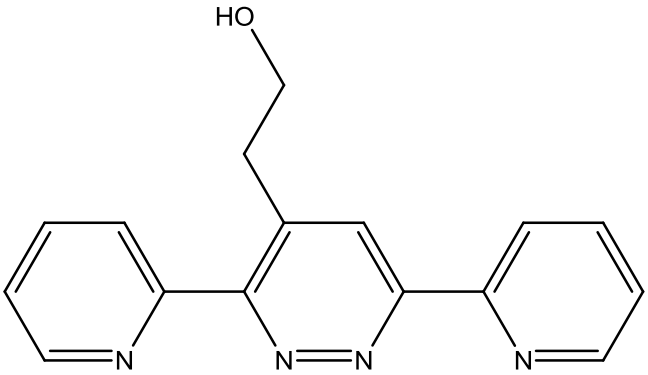
	Hz, py[4]), 7.87 (dt, 1H, $^3J_{HH}$ = 7.7 Hz, $^4J_{HH}$ = 1.5 Hz, py[4]), 7.46 (dt, 1H, $^3J_{HH}$ = 6.3 Hz, $^4J_{HH}$ = 1.7 Hz, py[5]), 7.38 (dt, 1H, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 1.5 Hz, py[5]), 6.5 (bs, 1H, C*H*$_2$OH), 4.72 (s, 2H, C*H*$_2$OH).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	158.3, 158.2, 155.3, 153.2 (4C, q, py[2], pz[3,6]), 149.6, 148.2 (2C, py[6]), 140.0 (1C, q, pz[4]), 138.1, 137.3 (2C, py[4]), 126.0, 125.1, 125.0, 124.6 (4C, py[3,5]), 121.8 (1C, pz[5]), 63.5 (1C, *C*H$_2$OH).

5.3.2.3 2-(3,6-Di(pyridin-2-yl)pyridazin-4-yl)ethanol[70]



500 mg (2.12 mmol, 1.0 eq) 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine and 192 µL (2.54 mmol, 1.2 eq) 2,3-dihydrofuran were dissolved in 10 mL toluene and heated to 100 °C 10 minutes. The crude product was purified by flash chromatography (DCM/MeOH 50:1).

$C_{16}H_{14}N_4O$ [278.31]

Yield	361 mg beige solid (60.3 %)
R_f (DCM/MeOH 20:1)	0.21
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.77-8.69 (m, 2H, py[3,6]), 8.67 (d, 1H, $^3J_{HH}$ = 5.0 Hz, py[6]), 8.60 (s, 1H, pz[5]), 8.30 (d, 1H, $^3J_{HH}$ = 8.0 Hz, py[3]), 7.97 (m, 1H, py[4]), 7.89 (m, 1H, py[4]), 7.46 (m, 1H, py[5]), 7.40 (m, 1H, py[5]), 6.7 (bs, 1H, -OH), 4.15 (t, 2H, CH_2CH_2OH), 3.16 (t, 2H, C*H*$_2$$CH_2$OH).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 159.0, 157.3, 155.0, 153.4 (4C, q, py[2], pz[3,6]), 149.6, 147.5 (2C, py[6]), 140.6 (1C, q, pz[4]), 138.2, 137.3 (2C, py[4]), 126.7, 125.9, 124.9, 124.2, 122.0 (5C, py[3,5], pz[5]), 63.6 (1C, CH_2*C*H$_2$OH), 34.7 (1C, *C*H$_2$$CH_2$OH).

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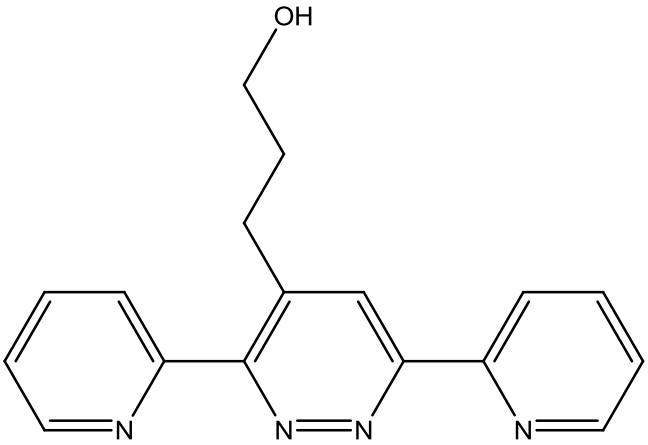
5.3.2.4 3-(3,6-Di(pyridine-2-yl)pyridazin-4-yl)propanol[71]



500 mg (2.12 mmol, 1.0 eq) 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine and 230 µL (2.54 mmol, 1.2 eq) 2,3-dihydropyran were dissolved in 10 mL toluene and heated to 100 °C overnight. The crude product was purified by flash chromatography (DCM/MeOH 50:1).

$C_{17}H_{16}N_4O$ [292.34]

Yield	430 mg beige solid (69.5 %)
R_f (DCM/MeOH 10:1)	0.71
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.78-8.69 (m, 2H, $py^{3,6}$), 8.67 (d, 1H, $^3J_{HH}$ = 4.8 Hz, py^6), 8.56 (s, 1H, pz^5), 8.18 (d, 1H, $^3J_{HH}$ = 8.1 Hz, py^3), 7.93 (m, 2H, py^4), 7.42 (m, 2H, py^5), 5.3 (s, $CH_2CH_2CH_2O\mathit{H}$), 3.60 (m, 2H, $CH_2CH_2C\mathit{H_2}OH$), 3.12 (t, 2H, $C\mathit{H_2}CH_2CH_2OH$), 2.13 (qt, 2H, $CH_2C\mathit{H_2}CH_2OH$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 159.1, 157.4, 155.8, 153.4 (4C, q, py^2, $pz^{3,6}$), 149.6, 147.9 (2C, py^6), 141.7 (1C, q, pz^4), 137.9, 137.4 (2C, py^4), 126.2, 125.9, 124.9, 124.1, 122.0 (5C, $py^{3,5}$, pz^5), 60.0 (1C, $CH_2CH_2\mathit{C}H_2OH$), 33.1 (1C, $\mathit{C}H_2CH_2CH_2OH$), 27.2 (1C, $CH_2\mathit{C}H_2CH_2OH$).

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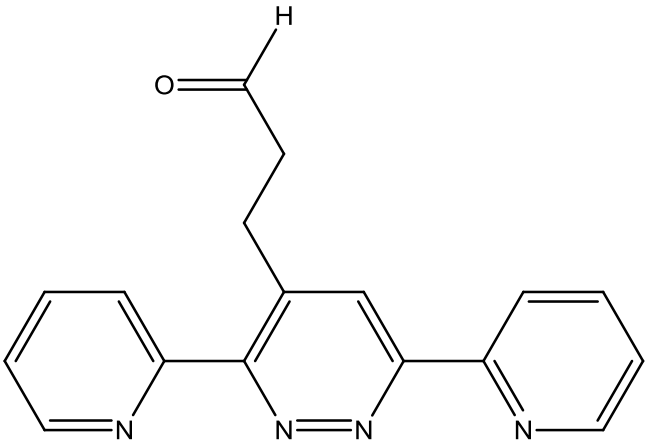
5.3.2.5 3-(3,6-Di(pyridin-2-yl)pyridazin-4-yl)propanal

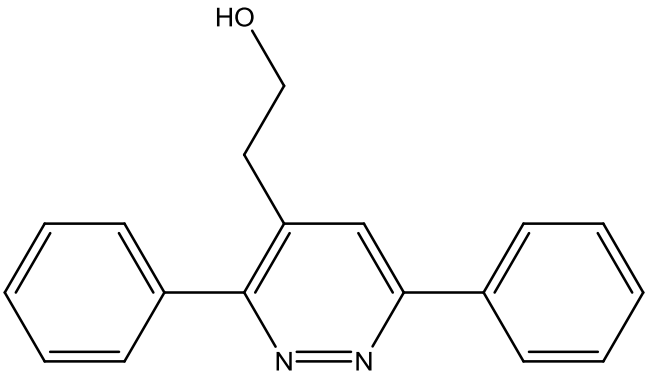


500 mg (2.12 mmol, 1.0 eq) 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine and 290 mg (2.54 mmol, 1.2 eq) 2-methoxy-3,4-dihydro-2H-pyran were dissolved in 10 mL toluene and heated to 100 °C for two days. Purification was achieved by column chromatography (CH/EA 1:5).

$C_{17}H_{14}N_4O$ [290.32]

Yield	328 mg brown solid (53.4 %)
R_f (CH/EA 1:5)	0.48
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 9.8 (s, 1H, CO*H*), 8.74-8.61 (m, 3H, $py^{3,6}$), 8.50 (s, 1H, pz^5), 8.23 (d, 1H, $^3J_{HH}$ = 7.6 Hz, py^3), 7.86 (m, 2H, py^4), 7.35 (m, 2H, py^5), 3.37 (m, 2H, C*H*$_2$CH$_2$COH), 2.98 (t, 2H, CH$_2$C*H*$_2$COH).
^{13}C{^{1}H}-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 200 (C, CH$_2$CH$_2$*C*OH), 158.2, 156.9, 155.5, 152.8 (4C, q, py^2, $pz^{3,6}$), 149.1, 148.2 (2C, py^6), 140.5 (1C, q, pz^4), 136.8, 136.6 (2C, py^4), 125.7, 124.4, 124.3, 123.4, 121.3 (5C, $py^{3,5}$, pz^5), 43.7 (1C, CH$_2$*C*H$_2$COH), 25.3 (1C, *C*H$_2$CH$_2$COH).

5.3.2.6 2-(3,6-Diphenylpyridazin-4-yl)ethanol

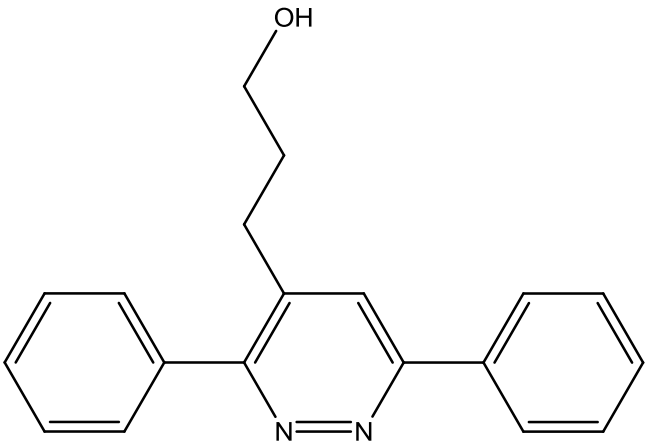


500 mg (2.15 mmol, 1.0 eq) 3,6-diphenyl-1,2,4,5-tetrazine and 195 µL (2.59 mmol, 1.2 eq) 2,3-dihydrofuran were dissolved in 10 mL toluene and heated to 100 °C for 2 weeks. Then another 30 µL of DHF were added as everything was used up. Normally the reaction would have been finished after 5 days. The crude product was purified by column chromatography (CH/EA 5:1 -> CH/EA 1:1).

$C_{18}H_{16}N_2O$ [276.33]

Yield	395 mg colourless solid (66.4 %)
R_f (CH/EA 10:1)	0.2
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.02-7.93 (m, 2H, $ph^{2,6}$), 7.81 (s, 1H, pz^5), 7.49-7.42 (m, 2H, $ph^{2,6}$), 7.36 (m, 6H, $ph^{3,4,5}$), 4.14 (bs, 1H, $CH_2CH_2O\mathit{H}$), 3.65 (t, 2H, $CH_2C\mathit{H}_2OH$), 2.81 (t, 2H, $C\mathit{H}_2CH_2OH$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 160.8, 157.3 (2C, q, $pz^{3,6}$), 137.8, 136.9, 136.2 (3C, q, pz^4, ph^1), 129.6, 129.1, 128.7, 128.5, 128.2, 126.9 (10C, $ph^{2,3,4,5,6}$), 125.0 (1C, pz^5), 60.5 ($CH_2\mathit{C}H_2OH$), 34.7 (1C, $\mathit{C}H_2CH_2OH$).

5.3.2.7 3-(3,6-Diphenylpyridazin-4-yl)propan-1-ol



50 mg (0.215 mmol, 1.0 eq) 3,6-diphenyl-1,2,4,5-tetrazine was dissolved in 1 mL 2,3-dihydropyran and heated to 85 °C for 3 days. Column chromatography was done to purify the product (CH/EA 5:1 -> CH/EA 1:1).

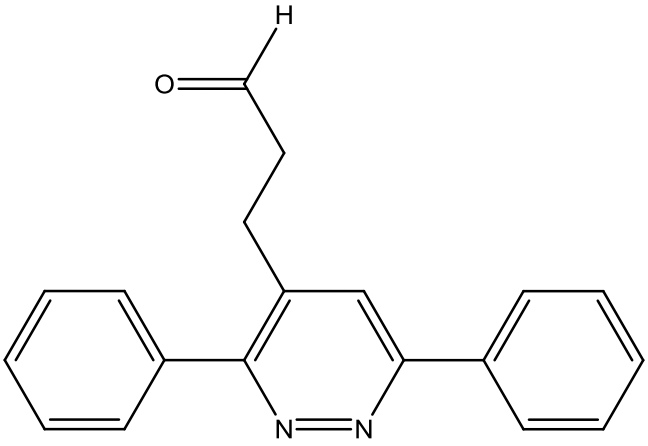
$C_{19}H_{18}N_2O$ [290.36]

Yield	33 mg beige solid (52.9 %)
R_f (CH/EA 1:1)	0.22
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.14 (m, 2H, $ph^{2,6}$), 7.78 (s, 1H, pz^5), 7.65-7.58 (m, 2H, $ph^{2,6}$), 7.58-7.44 (m, 6H, $ph^{3,4,5}$), 3.59 (t, 2H, $C\mathit{H}_2CH_2CH_2OH$), 2.86 (t, 2H, $CH_2CH_2C\mathit{H}_2OH$), 1.81 (m, 2H,

$CH_2C\mathit{H}_2CH_2OH$).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz): δ (ppm) = 160.9, 157.9 (q, 2C, $pz^{3,6}$), 140.2, 137.2, 136.5 (q, 3C, pz^4, ph^1), 130.0, 129.3, 129.1, 129.0, 128.6, 127.2 (10C, $ph^{2,3,4,5,6}$), 124.4 (1C, pz^5), 61.7 (1C, $\mathit{C}H_2CH_2CH_2OH$), 32.4 (1C, $CH_2CH_2\mathit{C}H_2OH$), 28.6 (1C, $CH_2\mathit{C}H_2CH_2OH$).

5.3.2.8 3-(3,6-Diphenylpyridazin-4-yl)propanal

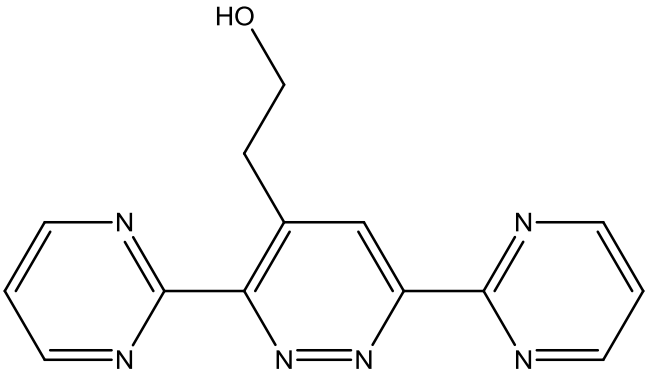


50 mg (0.215 mmol, 1.0 eq) 3,6-diphenyl-1,2,4,5-tetrazine was dissolved in 1 mL 2-methoxy-3,4-dihydro-2H-pyran and heated to 85 °C for 2 days. A flash chromatography was done to purify the product (CH/EA 5:1 -> CH/EA 1:1).

$C_{19}H_{16}N_2O$ [288.34]

Yield	24 mg light brown solid (38.7%)
R_f (CH/EA 1:1)	0.57
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 9.70 (s, 1H, $CH_2CH_2C\mathit{H}O$), 8.12 (m, 2H, $ph^{2,6}$), 7.8 (s, 1H, pz^5), 7.63-7.45 (m, 8H, $ph^{2,3,4,5}$), 3.10 (t, 2H, $C\mathit{H}_2CH_2CHO$), 2.69 (t, 2H, $CH_2C\mathit{H}_2CHO$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 199.6 (1C, $CH_2CH_2\mathit{C}HO$), 160.6, 158.0 (q, 2C, $pz^{3,6}$), 138.7, 137.0, 136.3 (q, 3C, pz^4, ph^1), 130.1, 129.2, 129.1, 128.8, 127.2 (10C, $ph^{2,3,4,5,6}$), 124.4 (1C, pz^4), 43.0 (1C, $CH_2\mathit{C}H_2CHO$), 24.7 (1C, $\mathit{C}H_2CH_2CHO$).

5.3.2.9 2-(3,6-Di(pyrimidin-2-yl)pyridazin-4-yl)ethanol

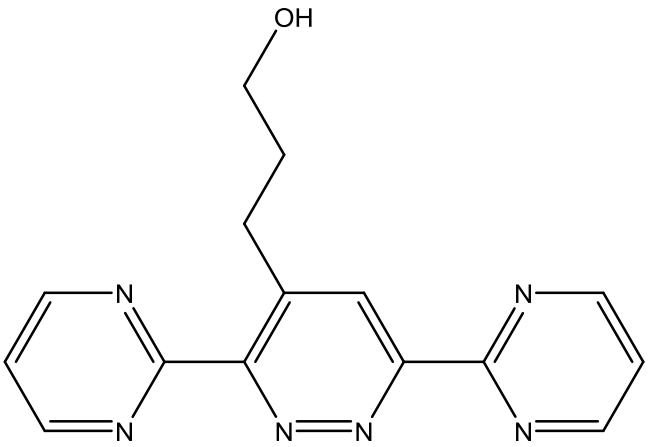


30.2 mg (0.127 mmol, 1.0 eq) 3,6-di(pyrimidin-2-yl)pyridazin and 11.5 µL (0.152 mmol, 1.2 eq) 2,3-dihydrofuran were dispersed in 1 mL toluene for 2 hours. The product could be used without further purification.

$C_{14}H_{12}N_6O$ [280.28]

Yield	29 mg slightly pink solid (81.4 %)
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.95 (d, 4H, $^3J_{HH}$ = 4.7 Hz, $pr^{4,6}$), 8.62 (s, 1H, pz^5), 7.45 (t, 1H, $^3J_{HH}$ = 4.7 Hz, pr^5), 7.39 (t, 1H, $^3J_{HH}$ = 4.9 Hz, pr^5), 4.04 (t, 3H, CH_2CH_2OH), 3.08 (t, 2H, CH_2CH_2OH).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 163.3, 161.9 (2C, q, pr^2), 159.0, 157,0 (2C, q, $pz^{3,6}$), 157.9, 157.5 (4C, $pr^{4,6}$), 139.8 (1C, q, pz^4), 128.6 (1C, pz^5), 121.3, 120.8 (2C, pr^5), 62.7 (1C, CH_2CH_2OH), 34.7 (1C, CH_2CH_2OH).

5.3.2.10 3-(3,6-Di(pyrimidin-2-yl)pyridazin-4-yl)propan-1-ol

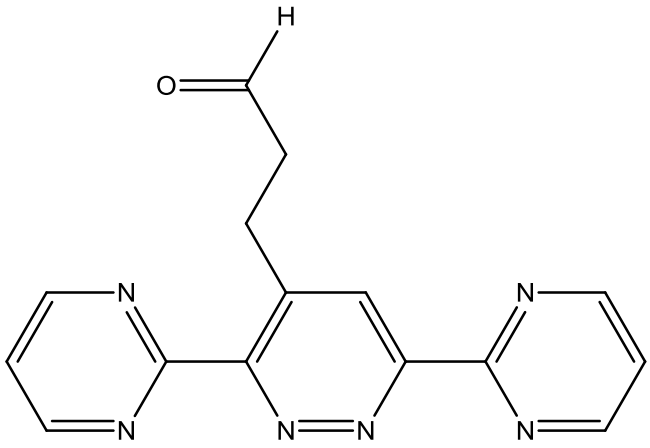


100 mg (0.426 mmol, 1.0 eq) 3,6-di(pyrimidin-2-yl)-1,2,4,5-tetrazine was dispersed in 1 mL 3,4-dihydro-2*H*-pyran and stirred at 90 °C for 40 minutes. The crude product was purified using column chromatography (DCM/MeOH 20:1).

$C_{15}H_{14}N_6O$ [294.32]

Yield	67 mg brown solid (53.4 %)
R_f (DCM/MeOH 10:1)	0.25
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.97 (m, 4H, $pr^{4,6}$), 8.58 (s, 1H, pz^5), 7.45 (t, 1H, $^3J_{HH}$ = 4.94 Hz, pr^5), 7.41 (t, 1H, $^3J_{HH}$ = 4.94 Hz, pr^5), 3.59 (t, 2H, $CH_2CH_2CH_2OH$), 3.5 (bs, 1H, $CH_2CH_2CH_2OH$), 2.98 (t, 2H, $CH_2CH_2CH_2OH$), 2.00 (m, 2H, $CH_2CH_2CH_2OH$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 163.9, 162.0 (q, 2C, pr^2), 159.1, 157.0 (q, 2C, $pz^{3,6}$), 158.0, 157.5 (4C, $pr^{4,6}$), 141.4 (q, 1C, pz^4), 127.8 (1C, pz^5), 121.3, 120.8 (2C, pr^5), 60,8 (1C, $CH_2CH_2CH_2OH$), 32.6 (1C, $CH_2CH_2CH_2OH$), 27.6 (1C, $CH_2CH_2CH_2OH$).

5.3.2.11 3-(3,6-Di(pyrimidin-2-yl)pyridazin-4-yl)propanal



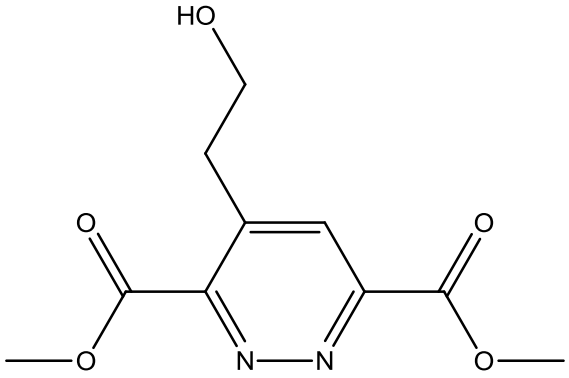
51 mg (0.213 mmol, 1.0 eq) 3,6-di(pyrimidin-2-yl)-1,2,4,5-tetrazine was dispersed in 1 mL 2-methoxy-3,4-dihydro-2*H*-pyran and stirred at 90 °C for 80 minutes. The crude product was purified using column chromatography (DCM/MeOH 20:1).

$C_{15}H_{12}N_6O$ [292.30]

Yield	45 mg yellow/brown solid (72.3 %)
R_f (DCM/MeOH 10:1)	0.36
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 9.81 (s, 1H, CH_2CH_2COH), 8.99 (m, 4H, $pr^{4,6}$), 8.58 (s, 1H, pz^5), 7.44 (m, 2H, pr^3), 3.22 (t, 2H, CH_2CH_2COH), 2.96 (t, 2H, CH_2CH_2COH).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 199.9 (1C, CH_2CH_2COH), 163.9 (q, 2C, pr^2), 158.0, 157.6 (4C, $pr^{4,6}$), 157.1 (q, 2C, $pz^{3,6}$), 140.5 (q, 1C, pz^4), 128.0 (1C, pz^5), 121.4, 120.8 (2C, pr^5), 44.0 (1C, CH_2CH_2COH), 24.9

(1C, *C*H_2CH_2COH).

5.3.2.12 Dimethyl 4-(2-hydroxyethyl)pyridazine-3,6-dicarboxylate

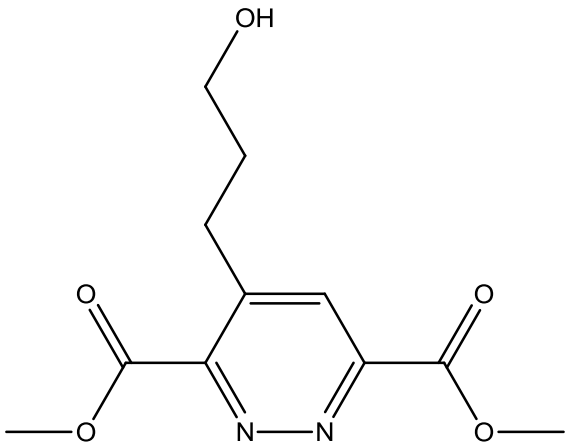


72.4 mg (0.365 mmol, 1.0 eq) dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate were dissolved in 1 mL dry DCM and then 33.1 µL (0.438 mmol, 1.2 eq) 2,3-dihydrofuran were added and the reaction immediately turned from red to yellow. Purification was achieved by column chromatography (CH/EA 5:1 -> 1:1 -> 1:5).

$C_{10}H_{12}N_2O_5$ [240.21]

Yield	6 mg colourless solid (7.0 %)
R_f (CH/EA 1:1)	0.43
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.23 (s, 1H, pz[5]), 5.4 (m, 1H, $CH_2CH_2CH_2O$*H*), 4.09 (s, 3H, COOC*H*$_3$), 4.06 (s, 3H, COOC*H*$_3$), 3.76-3.64 (m, 2H, CH_2C*H*$_2$OH), 3.22 (m, 2H, C*H*$_2CH_2$OH).
^{13}C{^{1}H}-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) =

5.3.2.13 Dimethyl 4-(3-hydroxypropyl)pyridazine-3,6-dicarboxylate[72]



61 mg (0.308 mmol, 1.0 eq) dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate was dissolved in 1 mL DCM_{dry} in an evacuated and with N_2 flushed Schlenk tube. After addition of 33.5 µL (0.369 mmol, 1.2

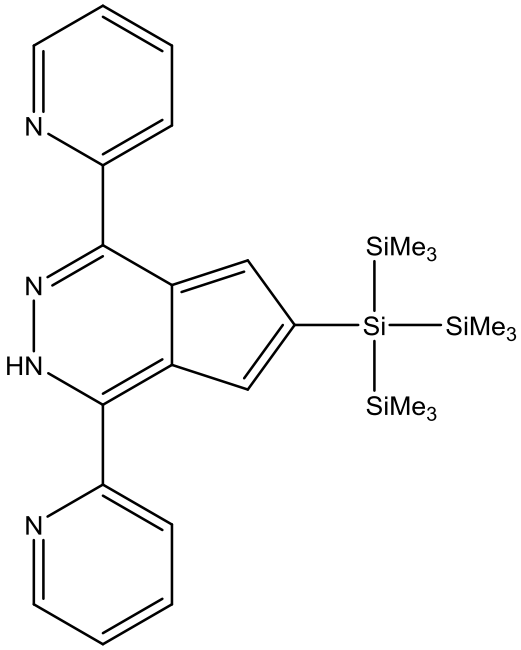
[72] D. L. Boger, M. Patel, J. Org. Chem., 1988, 53, 1405.

eq) 3,4-dihydro-2*H*-pyran there was a strong gas evolution and the reaction mixture nearly immediately turned yellow. The crude product was purified via column chromatography (CH/EA 1:1).

$C_{11}H_{14}N_2O_5$ [254.24]

Yield	17 mg beige product (21.7 %)
R_f (CH/EA 1:1)	0.42
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.16 (s, 1H, pz^5), 4.5 (bs, 1H, $CH_2CH_2CH_2OH$), 4.07 (s, 3H, $COOCH_3$), 4.04 (s, 3H, $COOCH_3$), 3.86-3.73 (m, 2H, $CH_2CH_2CH_2OH$), 3.04 (t, 2H, $CH_2CH_2CH_2OH$), 1.96 (m, 2H, $CH_2CH_2CH_2OH$).
$^{13}C\{^1H\}$-NMR ($CDCl_3$, 25°C, 75 MHz):	δ (ppm) = 165.2, 164.3 (q, 2C, $COOCH_3$), 154.3, 151.7 (q, 2C, $pz^{3,6}$), 143.3 (q, 1C, pz^4), 129.4 (1C, pz^5), 62.7 (1C, $CH_2CH_2CH_2OH$), 30.7 (1C, $CH_2CH_2CH_2OH$), 29.0 (1C, $CH_2CH_2CH_2OH$), 53.6, 53.4 (2C, $COOCH_3$).

5.3.3 6-(1,1,1,3,3,3-Hexamethyl-2-(trimethylsilyl)trisilan-2-yl)-1,4-di(pyridin-2-yl)-2H-cyclopenta[d]pyridazine



660.1 mg (1.058 mmol, 1.0 eq) 2,2'-((3aR,4R,7R,7aS)-3,3a,7,7a-tetrahydro-4H-4,7-methanoindene-2,4-diyl)bis(1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilane and 500 mg (2.117 mmol, 2.0 eq) 3,6-di(pyridine-2-yl)-1,2,4,5-tetrazine were dissolved in 10 mL toluene and heated to 100 °C. After 6 hours the reaction was finished, and the solvent was removed. For purification the crude product was dissolved in pentane, by-products removed by filtration and the product obtained by evaporation of the solvent. Additionally a column chromatography was performed (CH/EA 10:1).

$C_{26}H_{38}N_4Si_4$ [518.96]

Yield	257 mg red product (46.8 %)
R_f (CH/EA 10:1)	0.2
^{1}H-NMR ($CDCl_3$, 25 °C, 300 MHz):	δ (ppm) = 8.83 (d, 2H, $^3J_{HH}$ = 3.9 Hz, py^6), 8.37 (d, 2H, $^3J_{HH}$ = 7.58 Hz, py^3), 7.94 (t, 2H, $^3J_{HH}$ = 7.77 Hz, $^4J_{HH}$ = 1.35 Hz py^4), 7.53 (s, 2H, N*H*), 7.43 (t, 2H, $^3J_{HH}$ = 6.08 Hz, py^5), 0.28 (s, 27H, Me-*H*).

5.3.3.1 Molar absorption coefficient

For the measurement 5 different stock solutions (10^{-2} mmol/L – 10^{-5} mmol/L) were prepared and measured in a range of 280 nm to 650 nm. As solvent chloroform with suitable purity for spectroscopy was used. The measurements were performed as quickly as possible to avoid decomposition.

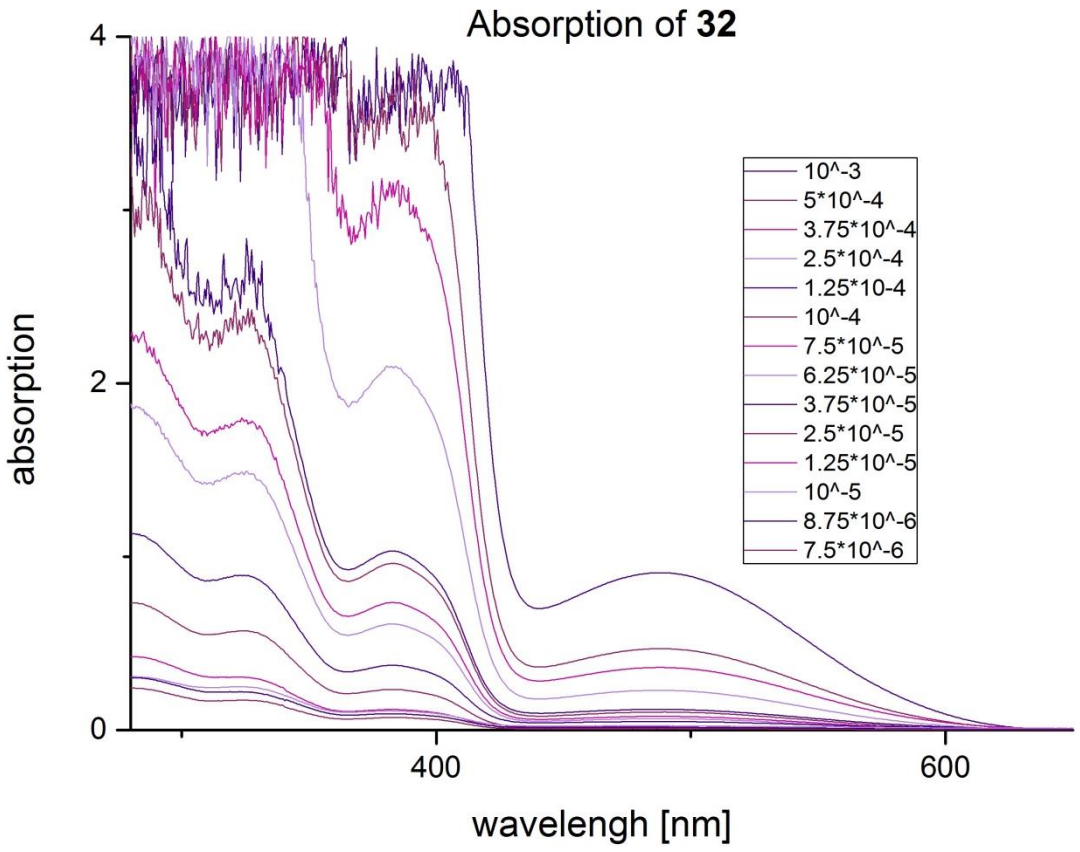


Figure 34: Absorption spectrum of the dilution series of 32; concentrations are in mol/L

All different concentrations measured could only be used for calculating the maxima around 500 nm. Due to the fact of higher absorption at lower wave lengths for the other two maxima lower concentrations had to be used for computation.

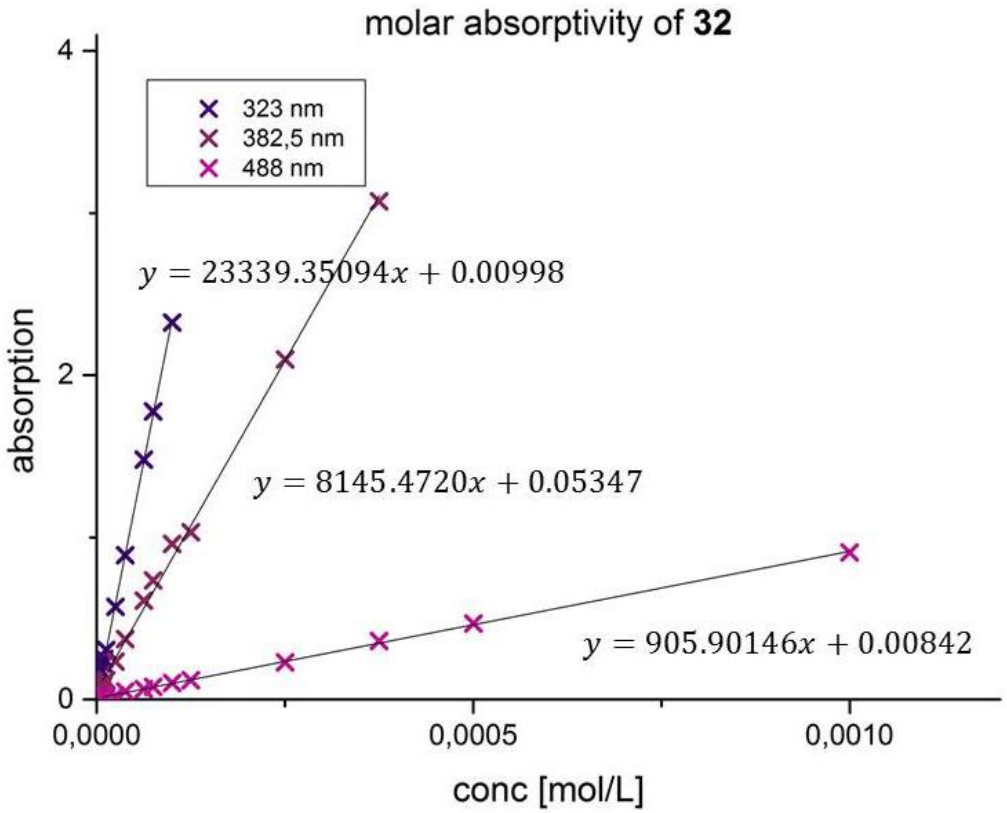


Figure 35: Calculation of the molar absorptivity of 32 at the three different maxima

5.3.3.2 Further characterisation

The exact mass was determined by time of flight mass spectroscopy. The sample was ionised by direct ionisation-electron impact (DI-EI-TOF-MS).

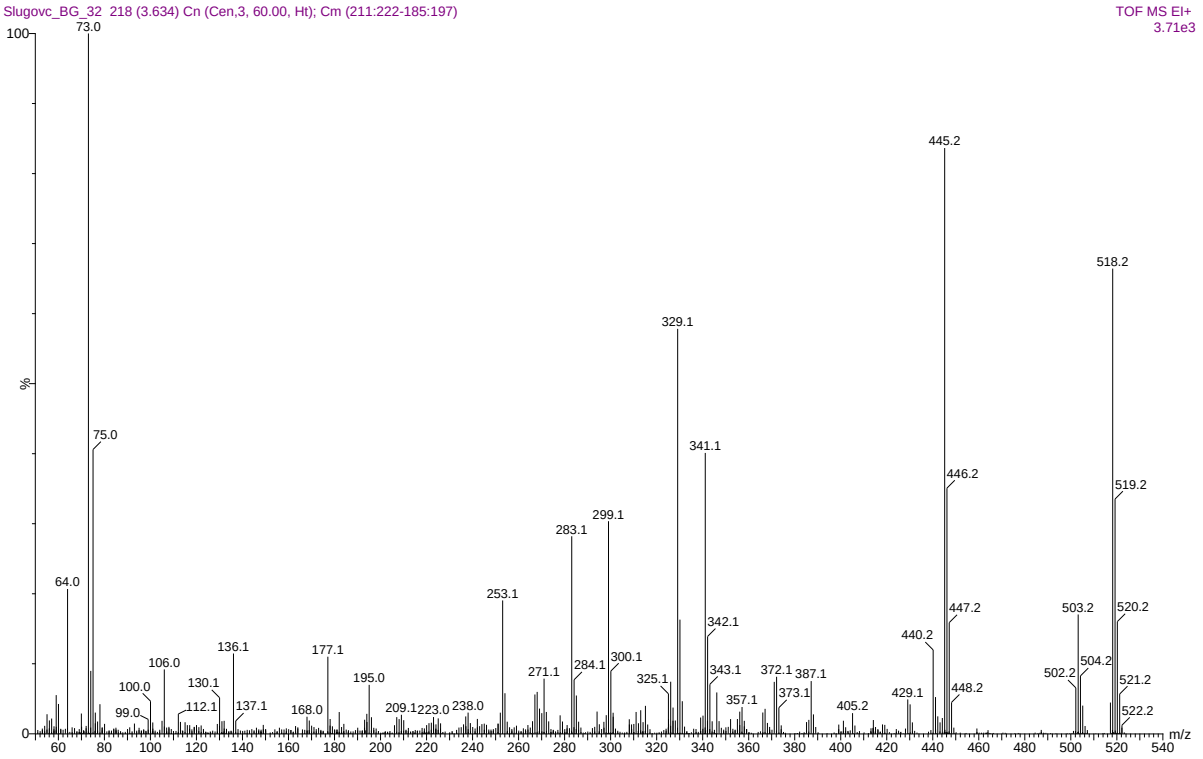


Figure 36: mass spectrum of 32

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7 List of Schemes

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