Short thesis for the degree of doctor of philosophy (PhD)

Domino cyclization reactions for the stereoselective preparation of chiral, condensed derivatives with new heterocyclic scaffolds

Mihály Kajtár

Supervisor: Dr. Tibor Kurtán

Industrial consultant : Dr. Attila Paczal

UNIVERSITY OF DEBRECEN

Doctoral School of Chemistry

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1. Introduction and objectives

Chiral condensed heterocycles are common structural motifs in nature, the biological activities of which show a great diversity. Although individual 3,4-dihydro-2*H*-pyrane, furan, thiophene and pyrrole motifs are relatively rare in nature, their benzene-fused or saturated forms are much more common. Natural products and their derivatives can serve as a basis of drug development and discovery. In the case of natural metabolites, it is not only the uneconomical procedure of the isolation that implies a problem but also the difficulty of the further synthetic modifications of these compounds, which can be a challenging task due to the complexity of the derivatives. If the heterocyclic skeleton or the substitution pattern need to be changed to improve the activity of the molecule, the synthetic preparation is inevitable. Our research group has been working on the preparation of novel condensed chiral *O,N*-heterocycles with domino cyclization reactions and identification of anti-proliferative activity for several years.

2. Applied methods

During the synthetic work, the semi-micro- and micro-methods of modern preparative organic chemistry were used. Thin-layer chromatography was used to monitor the reactions and to control the purity of the products. The reactions were purified by column chromatography and/or crystallization. The prepared novel compounds were characterized by classical analytical methods (melting point, retention factor), and their structures were elucidated by 1D- and 2D-NMR methods, infrared spectroscopy, mass spectrosmetry and single-cystal X-Ray diffraction analysis. The relative configuration of the products were assigned using their characteristic NOE effects. The antiproliferative activities were tested on A2780 (ovarian carcinoma), U87 (glioblastoma), HepG2 (liver carcinoma), HL60 (lukemia), HELA (cervical cancer) and BEL-7402 (liver carcinoma) cell lines using MTT method.

3. New scientific results of the dissertation

a. **By using *domino Knoevenagel-hetero Diels-Alder reaction of N-arylcinnamylamine substrates and cyclic β-dicarbonyl reagents, we prepared six novel chiral condensed heterocyclic derivatives and optimized the reaction conditions.***

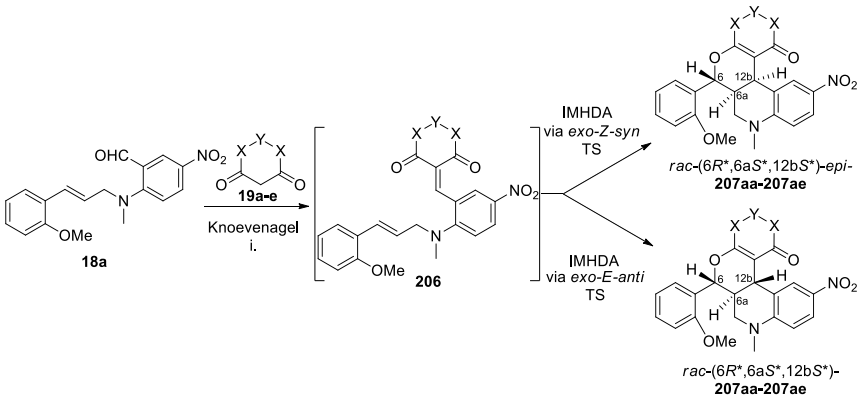


Table 1. DK-IMHDA reactions of **18a** and β-dicarbonyl derivatives.

Entry	**19a-e**	X	Y	Products	Earlier yields (%)	New vields (%)	dr.
1.	**19a**	CH_2	CH_2	**207aa**/*epi*-**508aa**	51	50	1:1[a]
2.	**19b**	CH_2	-	**207ab**/*epi*-**508ab**	13	60	3:1[a]
3.	**19c**	NMe	C=O	**207ac**/*epi*-**207ac**	37	69	2:1[a]
4.	**19d**	NEt	C=S	**207ad**/*epi*-**207ad**	75	85	2:1[a]
5.	**19e**			**207ae**/*epi*-**207ae**	30	92	3:2[b]

i. piperidine, ethanol, room temperature. a) ratio of *rac*-(6*R**,6a*S**,12b*R**) and *rac*-(6*R**,6a*S**,12b*S**) according to ^{1}H-NMR spectra b) isolated diastereomeric ratio.

The domino Knoevenagel intramolecular hetero-Diels-Alder (DK-IMHDA) reactions of cyclic β-dicarbonyl derivatives and **18a** had been already examined in our research group. The yields of the domino reactions were quite low and products were isolated as diastereomeric mixtures. During my Ph.D. research work, the solvent was changed from toluene to ethanol and the reflux temperature to room temperature. Thus the yields were improved but the diastereoselectivity could not be improved by either changing the solvent or the temperature.

In the DK-IMHDA reactions with cyclic β-dicarbonyl reagents, the chiral condensed heterocycles **207aa**/*epi*-**207aa**–**207ad**/*epi*-**207ad** were synthesized as mixtures of diastereomers. The mixtures of diastereomers were initially isolated by crystallization from the reaction mixture and then column chromatography was tested for further separation. The diastereomers were successfully separated in the case of **207ae**/*epi*-**207ae**. The separation was challenging because of the low solubility of the diastereomers, which could be improved by the addition of 10-50% of CH_2Cl_2 or chloroform to the eluent, which usually caused the poor separation of the diastereomers. The formation of two regioisomers were possible with the 4-hydroxy-6-methyl-2*H*-pyrone (**19e**) but

in the domino reaction only the mixture of **207ae**/*epi*-**207ae** was detected. In this case, the more reactive ketone carbonyl moiety reacted in the cycloaddition step.

The relative configurations of the products were determined in each case by their three bond coupling constants between the 12b-H and 6a-H protons and their characteristic NOE correlations.

3.2 Novel chiral condensed hexahydrobenzo[j]phenantridine and cyclobutane derivatives were synthesized by diastereoselective competing Knoevenagel-intramolecular styryl- Diels-Alder and ionic [2+2] cycloaddition reactions.

In our research work, a domino Knoeveangel-intramolecular Diels-Alder reaction was identified between the **18a-f** and malonitrile, during which the benzene ring of the substrate acted as part of the diene in the cycloaddition. In order to improve the yields of the reactions, the previously used toluene solvent was replaced by ethanol and the reflux temperature were changed to room temperature excep for one case.

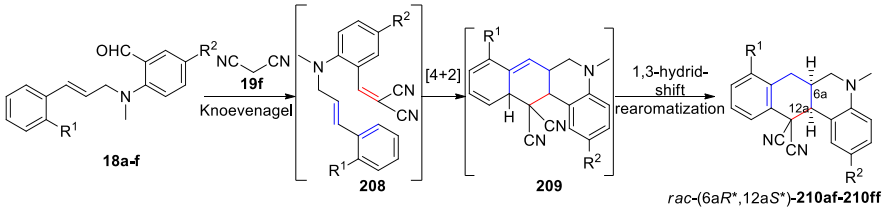


Table 2. Stepwise domino Knoevenagel Diels-Alder cycloaddition with malonitrile.

Entry	**18a-f**	R^1	R^2	Products	Yields (%)	dr.
1.	**18a**	OMe	NO_2	**210af**	65	1:0
2.	**18b**	H	NO_2	**210bf**	64	1:0
3.	**18c**	NO_2	NO_2	**210cf**/epi-**210cf**	57	1:1[a,b]
4.	**18d**	OMe	CF_3	**210df**	92	1:0
5.	**18e**	H	CF_3	**210ef**	61	1:0
6.	**18f**	OMe	H	**210ff**	33	1:0

a) Ratio of *rac*-(6a*R**,12a*S**) and *rac*-(6a*R**,12a*R**) according to ^{1}H-NMR spectra b) 60 °C.

The improvement of the yields was observed in all the cases with the modified reaction conditions. The relative configurations were assigned by means of the three-bonding coupling constants of the 6a-H and 12a-H, which were $^3J_{6a\text{-}H\text{-}12a\text{-}H}$ = 2,4 - 4,0 Hz. In the case of **210bf** and **210df**, the relative configurations and the planar structure were also confimed by single crystal X-ray diffraction analysis. The domino Knoevenagel-intramolecular styryl Diels-Alder reaction of **18c** and malonitrile were accomplished at 60 °C and C-12a epimers were isolated. The presence

of the *epi*-**210cf** in the diastereomeric mixture was proved by the 12a-H doublet proton signal in the ^{1}H-NMR spectrum, which had the value $^3J_{6a\text{-}H\text{-}12a\text{-}H}$ = 12.1 Hz. Different diastereoselectivity was observed because of the nitro group of the aromatic ring "A". Due to the electron withdrawing effect, the concerted asynchronous mechanism turned to a stepwise ionic one. During the rearomatization in the ionic mechanism, the formation of both diastereomers were feasible.

In the reaction of the **18a-c** starting materials with other nitrile derivatives, competing domino Knoevangel-[2+2] and [1,5]-hydride-shift-6-endo cyclization occurred depending on the substitution pattern.

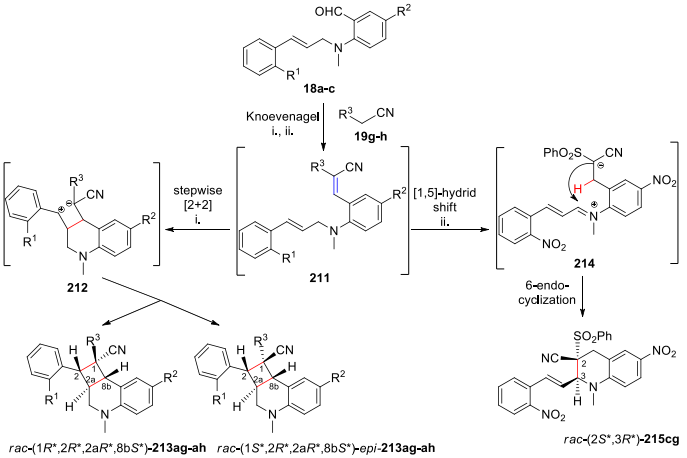


Table 3. Domino Knoevenagel-[2+2] and [1,5]-hydride shift-6-endo-cyclizations.

Entry	**18a-d**	R^1	R^2	**19g-h**	R^3	Products (yields (%))	dr.
1.	**18a**	OMe	NO_2	**19g**	SO_2Ph	**213ag**/*epi*-**213ag** (31)	1:1[a]
2.	**18c**	NO_2	NO_2	**19g**	SO_2Ph	**215cg** (28)	1:0[a]
3.	**18d**	OMe	CF_3	**19g**	SO_2Ph	**213dg** (30)	1:0[a]
4.	**18a**	OMe	NO_2	**19h**	2-(pyiridyl)-	**213ah**/*epi*-**213ah** (39)	1:1[b]

i.: Piperidine, AcOH, reflux, or room temperature, ii.: piperidine, AcOH, dry EtOH, 4 h reflux, a) reflux, b) room temperature.

Domino Knoevenagel-[2+2] cycloadditions were observed in the reactions of **18a** and **19g-h** when an electron-donating group was present on the benzene ring of the starting material. The tetrahydroquinoline derivative **215cg** was isolated in the reaction of **18c** and **19g**, which formed in a diastereoselective domino Knoevenagel [1,5]-hydride-shift-6-endo cyclization. Any other

cyclization mechanisms were unfavourable due to the presence of the electron-withdrawing nitro group on the styrene moiety.

The competition reaction mechanisms were also detected with 2-cyanoacetic acid reagents, which were tuneable with the substitution pattern of the starting materials and the reagents.

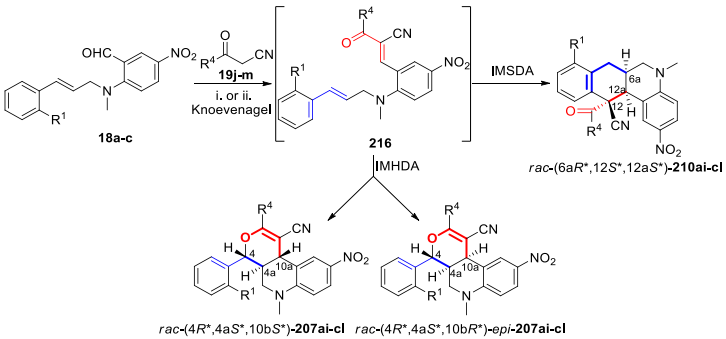


Table 4. Domino Knoevenagel-hetero and -styryl-Diels-Alder reactions.

Entry	**18a-c**	R^1	**19j-m**	R^3	Products	Yields (%)	dr.
1.	**18a**	OMe	**19i**	OEt	**207ai**	50[ii.]	1:0
					210ai	18[ii.]	1:0
2.	**18b**	H	**19i**	OEt	***epi*-207bi**	46[ii.]	1:0
					210bi	36[ii.]	1:0
3.	**18c**	NO_2	**19i**	OEt	**207ci**/***epi*-207ci**	26[ii.]	3:1
					210ci/***epi*-210ci**	33[ii.]	3:1
4.	**18a**	OMe	**19j**	NH_2	**210aj**	66[i.]	1:0
5.	**18b**	H	**19j**	NH_2	**210bj**	74[i.]	1:0
6	**18c**	NO_2	**19j**	NH_2	**207cj**	60[ii.]	1:0
7	**18a**	OMe	**19k**	NHMe	**210ak**	67[i.]	1:0
8	**18b**	H	**19k**	NHMe	**210bk**	76[i.]	1:0
9	**18c**	NO_2	**19k**	NHMe	no reaction[ii.]		
10	**18a**	OMe	**19l**	NMe_2	**207al**	50[i.]	1:0
					210al	20[i.]	1:0
11	**18b**	H	**19l**	NMe_2	decomposition[i.]		
12	**18c**	NO_2	**19l**	NMe_2	**207cl**/***epi*-207cl**	24/24[ii.]	1:1[b]
					210cl	17[ii.]	1:0

i. Piperidine, AcOH, dry EtOH 16 h room temperature, ii. piperidine, AcOH, dry EtOH 4 h reflux, a) ratio of the epimers according to the ^{1}H-NMR spectrum b) ratio of the isolated epimers.

Two different products were formed in the diastereoselective DK-IMHDA and DK-IMDA reactions between ethyl 2-cyanoacetate and substrates **18a-b**. When nitro groups were present on the benzene rings (**18c**), loss of diastereoselectivity and chemoselectivity was observed, which were probably caused by the previously shown stepwise mechanism. With 2-cyanoacetamide (**19j**) the chemoselectivity was higher and only the SDA products were isolated in the case of

18a-b, and HDA product with the **18c**. Transformations with the **19k** was very similar to the 2-cyanoacetamide (**19j**) with the exception of **18c**, when there was no reaction at all. If **19l** was the reagent, both of the cyclization mechanisms were observed and it afforded the condensed chiral heterocycles with low chemoselectivity. Due to the low stability of the intermediates and the products in the stepwise [2+2] cycloaddition, only decomposition products were identified in the reaction of **18b** and **19l**. Considering the results the substituent pattern of the starting material can tune the reaction mechanism and the electron donating group in the styryl and electron withdrawing at the aniline ring are the favourable.

3.3 Condensed heterocyclic derivatives with novel skeletons were prepared in multi-step domino Knoevenagel-cyclization reactions of styryl derivatives and Meldrum's acid, which occurred diastereoselectively with the formation of four new chirality centers.

A multi-step domino Knoevenagel cyclization sequence was carried out between substrate **18a** and Meldrum's acid (**28**) in presence of different aliphatic primary and secondary amines. The amines reacted in a domino reaction with the IMHDA intermediate **222** to afford condensed heterocyclic products with four contiguous chirality centers with remarkable diastereoselectivity.

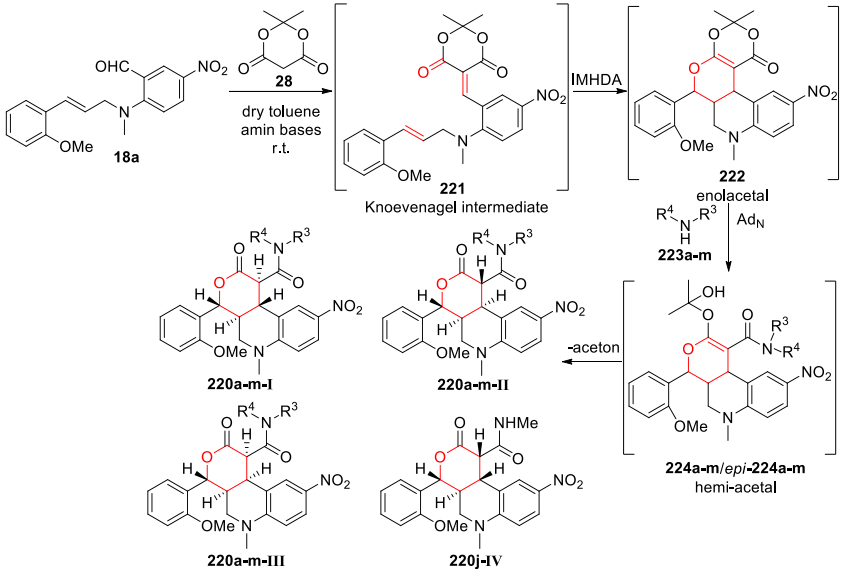


Table 5. Domino Knoevenagel-IMHDA-Ad_N-acetone loss sequences with different amines.

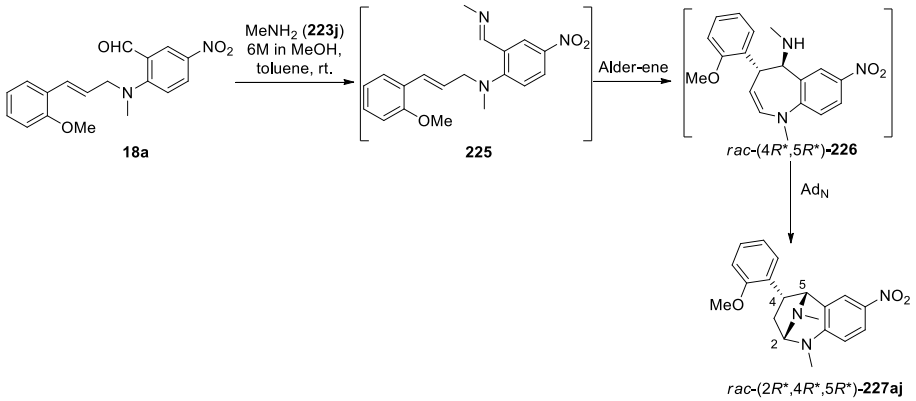
Entry	Amines	Products	Yields (%)	d.r.	Relative configuration (**I-IV**)

1	**223a** piperidine	**220a-I** **220a-II**	65	5:4:0:0[a]	*rac*-(1*R**,4*R**,4a*S**,10b*R**) *rac*-(1*S**,4*R**,4a*S**,10b*S**)
2	**223b** pyrrolidine	**220b-I** **220b-II**	3 18	1:6:0:0	*rac*-(1*R**,4*R**,4a*S**,10b*R**) *rac*-(1*S**,4*R**,4*a*S*,10b*S**)
3	**223c** morpholine	**220c-I** **220c-II**	16 9	7:4:0:0	*rac*(1*R**,4*R**,4a*S**,10b*R**) *rac*-(1*S**,4*R**,4a*S**,10b*S**)
4	**223d** *N*-methylpiperazin	**220d**	decomposition	-	-
5	**223e** dimethyilamine	**220e-II**	38	0:1:0:0	*rac*-(1*S**,4*R**,4a*S**,10b*S**)
6	**223f** diisopropylamine	**220f**	decomposition	-	-
7	**223g** Et_3N	**220g** *epi*-**220g**	46	2:1[a]	*rac*-(4*R**,4a*S**,10b*S**) *rac*-(4*R**,4a*S**,10b*R**)
8	**223h** ethanolamine	*epi*-**220h**	46	0:1	*rac*-(4*R**,4a*S**,10b*R**)
9	**223i** benzylamine	**220i-I** **220i-II**	21 4	5:1:0:0	*rac*-(1*R**,4*R**,4a*S**,10b*R**) *rac*-(1*S**,4*R**,4a*S**,10b*S**)
10	**223j** methylamine	**220j-III** **220j-IV**	24 24	1:0:0:1	*rac*-(1*R**,4*R**,4a*S**,10b*S**) *rac*-(1*S**,4*R**,4a*S**,10b*R**)
11	**223k** ciklohexylamine	**220k**	decomposition	-	-
12	**223l** allylamine	**220l-III**	37	-	*rac*-(1*R**,4*R**,4a*S**,10b*S**)
13	**223m** propargylamine	**220m**	decomposition	-	-

a) Ratio of the diastereomers according to ^{1}H-NMR spectra.

Mixtures of diastereomers were produced in the reactions with the cyclic secondary amines **223a-d**, which were successfully separated by flash column chromatography except for **220a-I-II.** Only decomposition products were isolated with the *N*-methylpiperazine additive. In the case of the acyclic amines the decomposition reactions were more favourable implying decarboxylation after the cyclization, which had been also observed with triethylamine. Reactions with ethanolamine (**223h**), dimethylamine (**223e**), methylamine (**223j**), allylamine (**223l**) and benzylamine (**223i**) were successful and the *epi*-**220a**, **220l-III** and **220e-II** products were isolated as a single diastereomer, respectively. Different cyclization mechanisms were induced if methylamine were added directly to the reaction mixture as a solution in methanol or when it formed *in situ* in the reaction mixture from the hydrochloride salt with potassium carbonate. The domino sequence observed with previous amines occurred if the methylamine formed *in situ* in the reaction mixture affording **220j-III** and **220j-IV**. Participation of the Meldrum's acid was not observed if the methylamine was added to the mixture as a solution in methanol. The initially formed imine **225**

reacted in Alder-ene cyclization and with a nucleophilic addition, the **227aj** bridged heterocycle was established.



Scheme 1. Domino Ad_N-Alder-ene-Ad_N reaction.

The emergence of this pathway was caused by the methanol, which changed the polarity of the medium. Therefore the imine formation was more favourable than the Knoevenagel condensation. Probably the prevalence of this alternative reaction pathway caused the generation of the highly polar decomposition products with certain amines discussed above. Besides decomposition, the other main reason of the lower yields is the formation of the **220g**/*epi*-**220g**, which were not isolated in a pure form.

3.4 We prepared novel chiral condensed heterocyclic scaffolds by diastereoselective domino Knoevenagel-intramolecular hetero Diels-Alder reactions of furan, thiophene and pyrrole subunits.

The domino cyclization reactions were also carried out with substrates, which contained a furan, thiophene or pyrrole subunit instead of the styryl moiety. The domino Knoevenagel-intramolecular hetero Diels-Alder reactions (DK-IMHDA) with cyclic β-dicarbonyl reagents afforded the condensed heterocyclic products **230aa**-**230do** with complete diastereoselectivity. The relative configurations were assigned by 2D-NOESY/ROESY measurements, while the planar structures were confirmed with 2D-NMR techniques (COSY, HSQC, HMBC). According to the literature, the five-membered heteroarene moieties usually react in a cycloaddition as a diene but in our cases they acted as a dienophile.

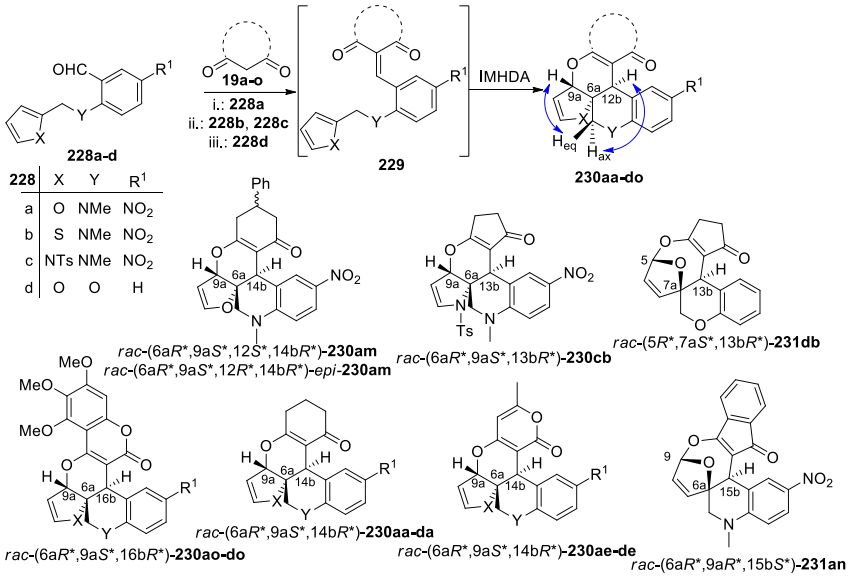
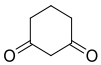
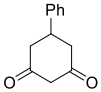
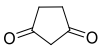
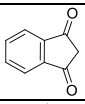
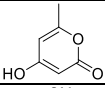
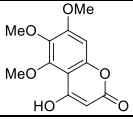


Table 6. DK-IMHDA reactions with furan, thiophene and pyrrole derivatives.

Entry	228a-d	19a-o	Structure of the reagents	Product	Yields (%)
1	**228a**	**19a**		**230aa**	87
2	**228b**			**230ba**	62
3	**228c**			**230ca**	83
4	**228d**			**230da**	82
5	**228a**	**19m**		**230am**	68
6	**228a**	**19b**		-	0
7	**228c**			**230cb**	41
8	**228d**			**231db**	12
9	**228a**	**19n**		**231an**	60
10	**228a**	**19e**		**230ae**	55
11	**228c**			**230ce**	77
12	**228d**			**230ce**	97
13	**228a**	**19o**		**230ao**	44
14	**228c**			**230co**	69
15	**228d**			**230do**	35

i. Et_3N, dry $ClCH_2CH_2Cl$, room temperature ii. piperidine dry EtOH, room temperature, iii. AcOH, CH_2Cl_2, room temperature.

The cyclic β-dicarbonyl reagent cyclohexane-1,3-dione reacted with all the substrates regardless the type of heteroaromatic subunit or the heteroatoms in the chains. All the active methylene reagents were tested with the thiophene-containing substrate **228b** but only the cyclohexane-1,3-dione showed sufficient reactivity to form the desired product. The microwave activation or Lewis-acid catalysis did not help the situation either. A desymmetrization was attempted in the domino reaction of **19m** and **228a** but the phenyl moiety of the reagent was not close enough sterically to induce diastereoselectivity, and thus C-12 epimers were isolated. In the domino reactions of indane-1,3-dione, only the furan-containing substrate **228a** reacted and a stepwise [4+4] cycloaddition occurred affording the oxygen-bridged **231an**. With non-symmetrical reagents such as 4-hydroxy-6-methyl-2*H*-pirane-2-on (**19e**) and 4-hydroxy-5,6,7-trimethoxykumarine (**19o**), two cyclization products could form from the Knoevenagel intermediate with the participation of the lactone or ketone carbonyl groups. The cyclizations were completely chemoselective and only the ketone carbonyl group took part in it. In the reactions with cyclopentane-1,3-dione, the **228c-d** substrates reacted but two different cyclization mechanisms were observed. With **228c**, DK-IMHDA reaction was observed and a stepwise [4+4] cycloaddition occurred with **228d** affording the product **231db**. According to the FMO theory, the [4+4] cycloaddition is only allowed under photocatalytic conditions, and thus in our case, a multistep ionic mechanism was feasible. The yield of the domino Knoevenagel-[4+4] cyclization reaction was quite low (12%), which required optimization of the reaction conditions.

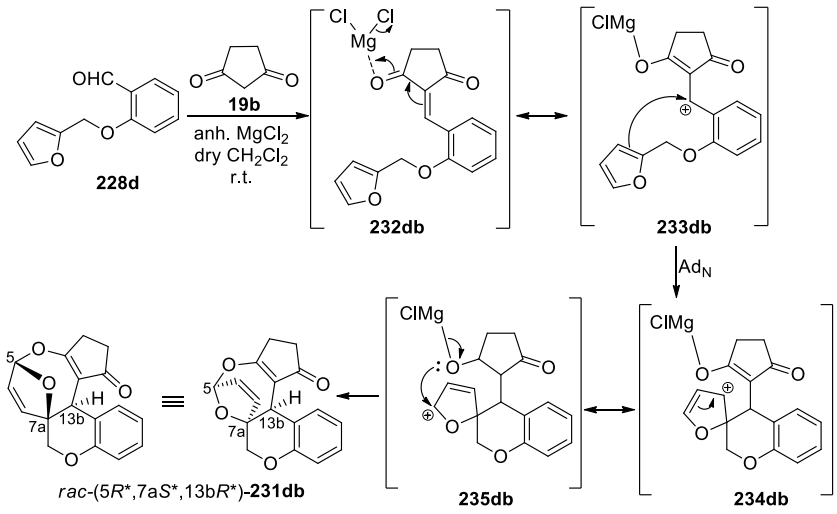


Table 7. Formation of the product **231db** and optimization of the reaction conditions.

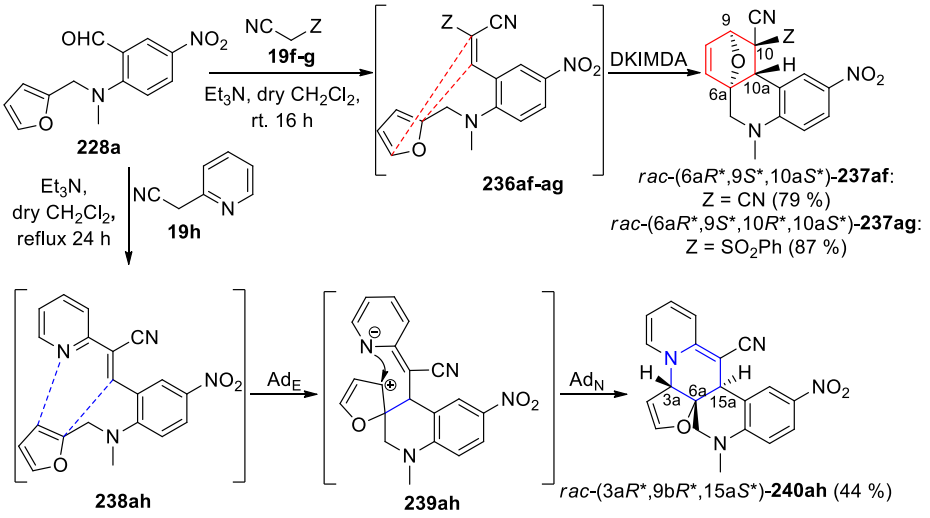
Entry	Catalyst	Solvent	isolated yields (%)
1	*rac*-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (0,5 eq.)	dry toluol	18
2	*rac*-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (0,5 eq.)	dry CH_2Cl_2	decomposition
3	*rac*-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (0,5 eq.)	dry ACN	no reaction
4	*rac*-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (0,5 eq.)	dry DMSO	no reaction
5	TFA (1,2 ekv.)	dry CH_2Cl_2	decomposition
6	pTSA (1,2 ekv.)	dry toluol	35
7	pTSA (1,2 ekv.)	dry ACN	17
8	TFMSA (1,0 ekv.)	dry toluol	decomposition
9	TFMSA (1,0 ekv.)	dry ACN	decomposition
10	Amberlyst 15 (20 mg)	dry CH_2Cl_2	no reaction
11	NH_4Cl (1 ekv.)	dry CH_2Cl_2	31
12	NaH_2PO_4 (1 ekv.)	dry CH_2Cl_2	42
13	anhydrous $MgCl_2$ (1,5 ekv.)	dry CH_2Cl_2	47
14	anhydrous $NiCl_2$ (1,5 ekv.)	dry CH_2Cl_2	29
15	anhydrous $InCl_3$ (1,5 ekv.)	dry CH_2Cl_2	23
16	anhydrous $Sc(OTf)_3$ (1,5 ekv.)	dry CH_2Cl_2	decomposition

Since the substrate **228d** was sensitive to the presence of Brönsted- or Lewis-acids, in most cases only decomposition was observed. The best result was achieved with the reaction condition of entry 13 (Table 7.). A possible reaction mechanism was proposed, which starts with the Knoevenagel condensation affording intermediate **230db**. The $MgCl_2$ coordinates to this intermediate and an Ad_N reaction occurred at the α-position of the heteroaromatic ring in the intermediate **233db** affording the arenium ion **234db**. Finally the tautomerization of the **234db** afforded the intermediate **235db**, which could readily cyclize to the final product **231db**. This mechanism was observed in the reaction of **228a** and **19n**, which afforded **231an** with good yields without any optimization. According to the ^{1}H- and ^{13}C-NMR chemical shifts, the relative configurations of the chirality centers were same and only the numbering of the products was different.

During the [4+2] cycloadditions, three contiguous chirality centers formed diastereoselectively, from which the C-6a is a quaternary spiro carbon atom. In order to assign the relative configuration of this center, 2D-NOESY and 2D-ROESY experiments were measured. The relative configuration was confirmed by NOE correlations and by X-ray diffraction analysis of **230ba** and **230ce**. The planar structure of the **231db**, which formed in the stepwise [4+4] cycloaddition was determined by 1- and 2D-NMR spectra but the assignment of the relative configuration was not possible by using only NMR experiments. Thus the relative configuration was determined by single crystal X-ray analysis, which also confirmed its planar structure.

3.5 In competing domino Knoevenagel cyclization reactions of furan and pyrrole, we prepared novel condensed and bridged heterocycles with acyclic active methylene reagents.

Acyclic active methylene reagents lacking the carbonyl moiety such as malonitrile and 2-(phenylsulphonyl)acetonitrile were tested in the domino reactions. In these domino reactions, an intramolecular Diels-Alder reaction occurred and the furan acted as a diene, which is the usual reactivity described well in the literature, and it afforded the oxygen-bridged products **237af** and **237ag**.



Scheme 2. Domino Knoevenagel intramolecular Diels-Alder (DK-IMDA) and aza-Diels-Alder cyclizations.

The relative configurations of the two products (**237af** and **237ag**) forming in the DA reaction were also confirmed by single crystal X-ray analysis. In order to prepare further bridged heterocycles, 2-(2-pyridyl)-acetonitrile (**19h**) were utilized in the cyclization reaction but after the Knoevenagel condensation, an unexpected condesnd product **240ah** was isolated as a single diastereomer. The formation of **240ah** could be explained by a two-step formal aza-Diels-Alder reaction, in which the pyridine ring was part of the heterodiene. Besides 2D-NMR experiments, the structure and relative configuration of the **240ah** were also determined by X-ray diffraction analysis.

This reaction was also carried out with further acyclic active methylene reagents and the competition of HDA and DA reactions was observed depending on the type of heteroarene subunit and the reagents.

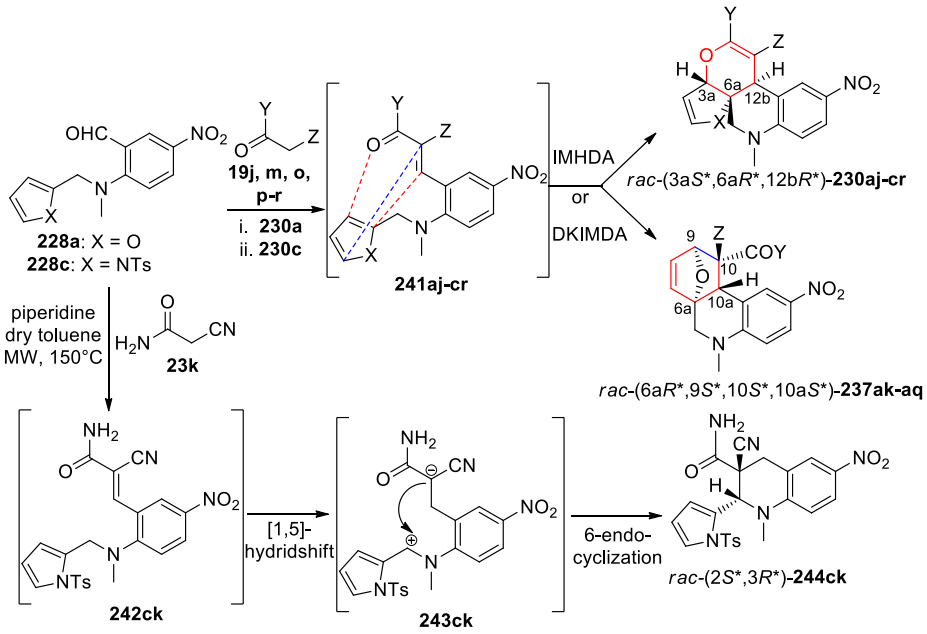
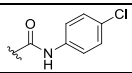


Table 8. Competing domino Knoevenagel hetero-Diels-Alder and Diels-Alder reactions with furan and pyrrole subunits.

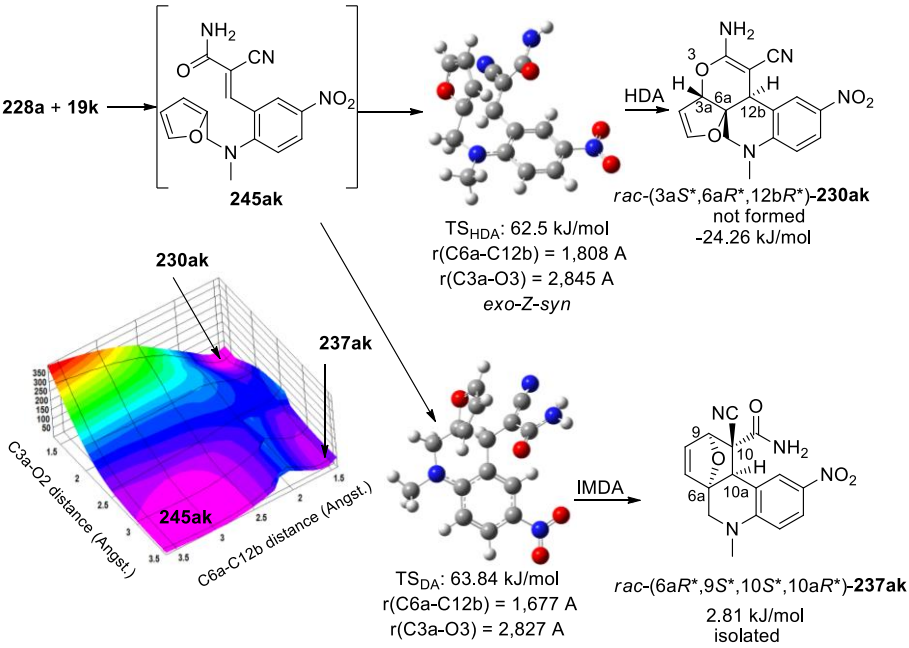
Entry	228a-c	19j-r	Y	Z	Products	Yields (%)[a]
1	**228a**	**19p**	Ph	CN	**230ap**	80
2	**228c**				**230cp**	85
3	**228a**	**19k**	NH_2	CN	**237ak**	93
4[b]	**228c**				**244ck**	39
5	**228a**	**19m**	NMe_2	CN	**237am**	73
6	**228a**	**19j**	OEt	CN	**237aj**	84
7	**228a**	**19q**	Me	COOMe	**237aq**	23
8	**228c**				**230cq**	63
9	**228c**	**19r**	Me		**230cr**	55
10	**228c**	**19s**	Me	SO_2Ph	**230cs**	81

a) Isolated yields, b) piperidine, dry toluene MW and 150 °C.

DK-IMHDA reactions took place between reagents containing a ketone carbonyl group and substrates **228a** and **228c** affording the products **230aj-cs**. In the cases when the reagents (**19k-q**) contained an ester or amide carbonyl group with lower reactivity, a DK-IMDA reactions prevailed affording the bridged products **237ak-aq** diastereoselectively. The relative

configuration of the products **237ak** and **237am** was determined by the characteristic NOE correlations of NH/9 and NMe/9 as *rac*-(6a*R**,9*S**,10*S**,10a*S**). A microwave-assisted cyclization was attempted in the reaction of cyanoacetamide (**19k**) and the pyrrole-containing substrate **228c** but the pyrrole ring did not participate in the cyclization and instead a [1,5]-hydride shift-6-endo cylization occurred affording the terahydroquinoline derivative **244ck**. The reagents containing an amide (**19r**) or sulphonyl side-chain (**19s**) reacted in a DK-IMHDA reaction with the pyrrole-containing substrate **228c**, while there was no reaction with the substrate **228a**. The planar structure and the relative configuration of the **230ap** were also confirmed with single crystal X-ray analysis.

The mechanism of the domino Knoevenagel-cyclization reaction was examined by quantum chemical calculations in the reaction of **228a** and **19k** (Scheme 43).



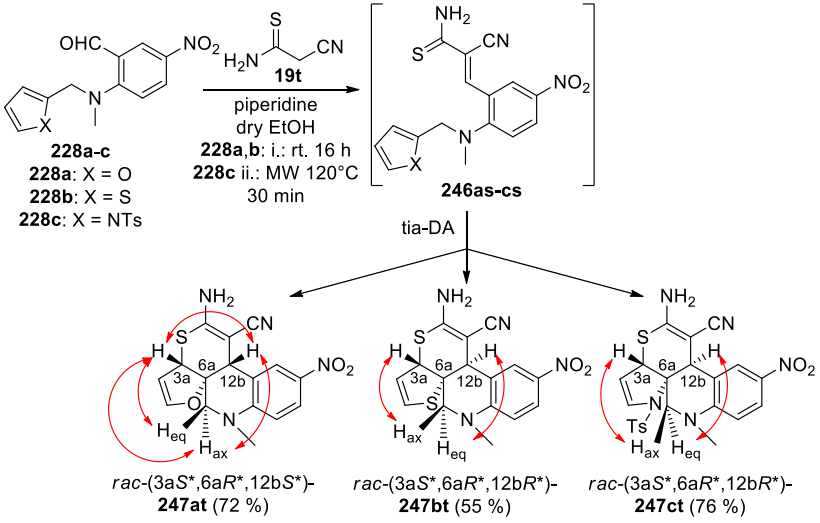
Scheme 3. Mechanism calculation.

Before the calculations, we supposed that the product **230ak** formed diastereoselectively since there are very similar ^{1}H- and ^{13}C-NMR chemical shifts and multiplicity for the two possible products. Thus the calculations were performed for the Knoevenagel intermediate (**245ak**) and produc **230ak** t, and the potential energy hypersurface was computed by the increasing the

distance of the newly formed bonds C-3a–O-3 and C-6a–O-12b with 0.1 Å intervals at B3LYP/6-31G (d) level in the gas phase. By optimizing the transition states at M06-2X/TZVP PCM 1,2-dichloroethane level, and the energies of the transition states were determined for both reaction pathways, which was found 62.5 kJ/mol for the IMHDA reaction and 63.84 kJ/mol for the IMDA cyclization. The relative energies of the HDA (**230ak**) and DA (**237ak**) products were –24.26 kJ/mol and 2.82 kJ/mol, respectively, on the basis of which the formation of **230ak** was thermodynamically more favourable than that of the bridged DA product. On the basis of our subsequent HMBC and NOESY experiments, we could reveal that the *rac*-(6a*R**,9*S**,10*S**,10a*S**)-**237ak** was the product, which required revision of our previous hypothesis. The formation of this product can be explained by the different (*E*) configuration of the double bond produced in the Knoevenagel condensation.

3.6 Condensed chiral 3,4-dihydro-2H-thiopyrane derivatives were prepared by diastereoselective domino Knoevenagel-intramolecular thia-Diels-Alder reaction of the furan, thiophene and pyrrole subunits.

We modified heterocyclic core by the replacing the oxa-Diels-Alder reaction to a thia-Diels-Alder one. For this modification, the 2-cyanothioacetamide (**19t**) was utilized as a reagent.

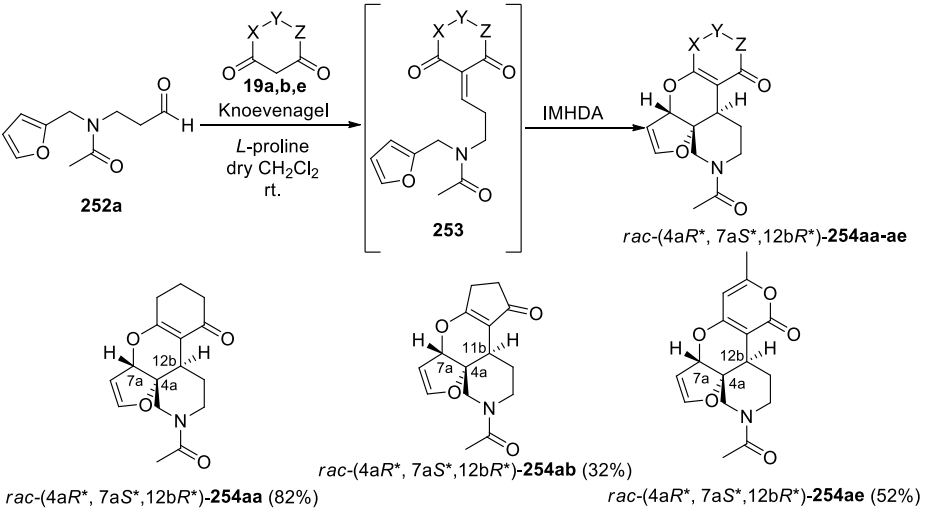


Scheme 4. Domino Knoevenagel-thia-Diels-Alder reactions with **228a-c**.

Regardless the type of the heteroarene subunit, the products **247at-247ct** were prepared with good yields. The thia-Diels-Alder cyclization was diastereoselective in all the cases but a different stereoisomer was isolated with furan from those with pyrrole and thiophene. The relative configuration was assigned by the characteristic NOE correlations, which are highlighted with red arrows on Scheme 44 and in the case of **247bt** it was also confirmed by single crystal X-ray analysis. The different diastereoselectivity can be attributed to the different size of the heteroatoms or their secondary orbital interactions, because the tether of the molecules are the same.

3.7 We performed the diastereoselective synthesis of simplified chiral condensed heterocycles via domino Knoevenagel hetero-Diels-Alder reactions utilizing furan and styrol subunits.

In order to prepare condensed, chiral heterocycles with more favourable pharmacological parameters, we synthesized heterocycles, which did not contain the condensed ring "E". The optimizations of the domino reactions were carried out with each the starting materials and cyclohexane-1,3-dione. Products **254aa-ae** were prepared in a DK-IMHDA reaction diastereoselectively and the structure of the **254ae** was confirmed with single crystal X-ray analysis and NMR measurements.



Scheme 5. Domino Knoevenagel hetero-Diels-Alder reactions between **252a** and cyclic β-dicarbonyl reagents.

The DK-IMHDA reactions were successful with the styrene derivative **252b** as well. The reactivity of the substrate was decreased dramatically, which was caused by the lack of the

conjugating benzene ring. Due to the decreased reactivity, the reactions could be only achieved with microwave irradiation.

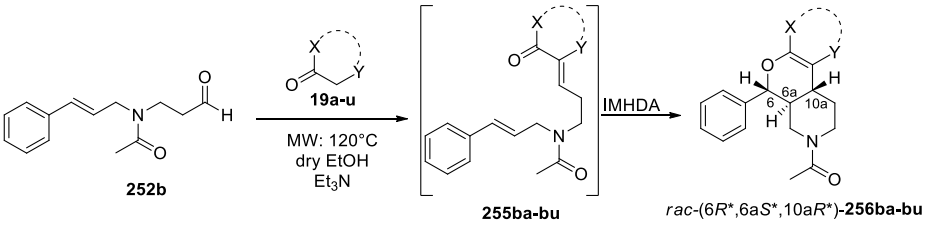
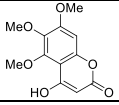


Table 9. DK-IMHDA reactions of the **252b**.

Entry	**19a-u**	Reagents	Products	Yields (%)
1	**19a**		**256ba**	92
2	**19b**		**256bb**	42
3	**19c**		**256bc**	16
4	**19e**		**256be**	37
5	**19o**		**256bo**	21
6	**19p**		**256bp**	48
7	**19u**		**256bu**	63

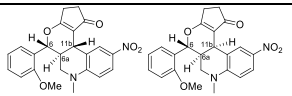
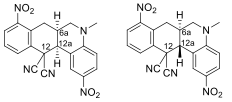
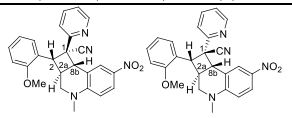
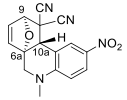
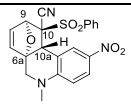
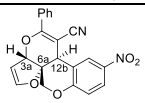
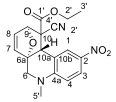
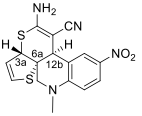
The domino reactions were performed with all of the previously utilized active methylene reagents but the desired products were only observed in the seven shown examples. With the rest of the reagents, only decomposition was observed. The structures and relative configuration of the formed products were assigned with 1- and 2D-NMR techniques and in the case of **256ba**, it was also confirmed by single crystal X-ray analysis.

3.8 The antiproliferative effect of the products prepared by the domino Knoevenagel-cyclization reactions were tested on human cancer cell lines and we identified antiproliferartive activity of several target compounds with low micromolar IC_{50} values for different cell lines.

The antiproliferative activity of the compounds prepared during my Ph.D. research work were tested on U87 (glioblastoma) and HepG2 (liver carcinoma) cell lines in the Research Group of Peptide Chemistry at the Eötvös Loránd University. The products from the earlier stage of my

Ph.D. work were tested on HL60 (leukemia), A2780 (ovarian cancer), HELA (cervical cancer) and BEL-7402 (liver carcinoma) cell lines in the Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, where doxorubicin was used as a positive control.

Table 10. IC_{50} values of the prepared molecules on the tested human cancer cell lines.

	IC_{50} (μM)					
Molecules	U87	HepG2	HL60	A2780	HELA	BEL-7402
 *rac*-(6*R**,6a*S**,12b*S**)-**207ab** *rac*-(6*R**,6a*S**,12b*S**)-*epi*-**207ab**	-	-	10,3	18,5	>30	>30
 *rac*-(6a*R**,12a*S**)-**210cf** *rac*-(6a*R**,12a*S**)-*epi*-**210cf**	-	-	>30	6,0	6,9	6,1
 *rac*-(1*R**,2*S**,2a*R**,8b*S**)-**213ah** *rac*-(1*S**,2*S**,2a*R**,8b*S**)-*epi*-**213ah**	33,7	-	-	-	-	-
 *rac*-(6a*R**,9*S**,10a*S**)-**237af**	-	9,8	-	-	-	-
 *rac*-(6a*R**,9*S**,10*R**,10a*S**)-**237ag**	1,8	4,1	-	-	-	-
 *rac*-(3a*S**,6a*R**,12b*R**)-**230dp**	9,9	-	-	-	-	-
 *rac*-(6a*R**,9*S**,10a*S**)-**237aj**	8,3	12,9	-	-	-	-
 (3a*S**,6a*R**,12b*R**)-**250bt**	45	-	-	-	-	-

Furan derivatives showed the best activities, and promising antiproliferative candidates with low micromolar IC_{50} values were identified among both the bridged (**237**) and condensed (**230**) heterocycles. The corresponding pyrrole and thiophene derivatives were found inactive on the tested cell lines (IC_{50} > 50 μM). Among the molecules prepared with DK-IMHDA reactions, the **230dp** was selective for the U87 cell line. From the products obtained in thia-DA reactions, the **247bt** had moderate activity. The best antiproliferative activities were identified for the bridged furan derivatives **237af**, **237ag** and **237aj**. The IC_{50} value of **237ag** was 1.8 μM on U87 cell line, which is similar to that of the most potent heterocyclic compound from our research group. Temozolomide was the positive control on the U87 and etoposide (IC_{50} = 30.50 μM) was on Hep2G cell line. Temozolomide is a chemotherapeutic active pharmacological ingredient against glioblastoma, which has an IC_{50} value of 100-250 μM. Recently there are no effective drugs available for the treatment of glioblastoma and thus the preparation and discovery of the new effective skeletons or lead compounds are essential.

4. Application potential of the research results

In the frame of my Ph.D. research work, domino Knoevenagel-cyclization sequences of subtrates containing styrene, furan, thiophene or pyrrole subunit were discovered and optimized. Eight different cyclization mechanisms were identified, which afforded nine novel condensed chiral heterocyclic series. The prepared heterocyclic scaffolds have not been reported in the literature. The scope and limitations of the reactions and the favourable substitution patterns were identified in the case of the different heterocyclic skeletons and reagents. The structures and the relative configurations of the novel compounds and skeletons were confirmed with single crystal X-ray analysis and NMR techniques.

In eight cases, antiproliferative activity with low micromolar IC_{50} values against human cancer cell lines was identified. By considering the optimized reaction conditions and the substitution patterns of the pharmacologically active molecules, there will be a possible ways to improve the activity of our heterocyclic compounds further.

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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Kajtár, M.**, Király, S. B., Bényei, A., Kiss-Szikszai, A., Ábrahám, A., Zhang, N., Horváth, L. B., Bősze, S., Li, D., Kotschy, A., Paczal, A., Kurtán, T.: Competing Domino Knoevenagel-Cyclization Sequences with N-Arylcinnamylamines.
   J. Org. Chem. 89 (10), 6937-6950, 2024. ISSN: 0022-3263.
   DOI: http://dx.doi.org/10.1021/acs.joc.4c00299
   IF: 3.3 (2023)

2. **Kajtár, M.**, Király, S. B., Bényei, A., Kiss-Szikszai, A., Ábrahám, A., Horváth, B. L., Bősze, S., Kotschy, A., Paczal, A., Kurtán, T.: Knoevenagel-IMHDA and -IMSDA sequences for the synthesis of chiral condensed O,N-, S,N- and N-heterocycles.
   RSC Advances. "Accepted by Publisher" (-), [1-19], 2024. ISSN: 2046-2069.
   IF: 3.9 (2023)

Total IF of journals (all publications): 7,2
Total IF of journals (publications related to the dissertation): 7,2

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

09 January, 2025

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5. List of publications

Publications in the subject of the thesis

1. M. Kajtár, S. B. Király, A. Bényei, A. Kiss-Szikszai, A. Kónya-Ábrahám, N. Zhang, L. B. Horváth, Sz. Bősze, D. Li, A. Kotschy, A. Paczal, and T. Kurtán: Competing Domino Knoevenagel-Cyclization Sequences with *N*-Arylcinnamylamines, *J. Org. Chem*. **2024**, *89* (10), 6937-6950.
2. M. Kajtár, S. B. Király, A. Bényei, A. Kiss-Szikszai, A. Kónya-Ábrahám, L. B. Horváth, Sz. Bősze, A. Kotschy, A. Paczal, T. Kurtán: Knoevenagel-IMHDA and -IMSDA sequences for the synthesis of chiral condensed *O*,*N*-, *S*,*N*- and *N*-heterocycles, RSC Advances; **2025**, accepted for publication, manuscript ID: RA-ART-11-2024-008353.R1.
3. M. Kajtár, S. B. Király, Sz. Bősze, A. Paczal, A. Kotschy, T. Kurtán: Domino Knoevenagel-intramolecular cyclization reactions using five-membered aromatic heterocycles with one heteroatom as a dienophile, patent priority claim, **2024**, 138028-1423 TEP.
4. M. Kajtár, S. B. Király, A. Mándi, Sz. Bősze, A. Paczal, A. Kotschy, T. Kurtán: Domino Knoevenagel-intramolecular cyclization reactions using five-membered aromatic heterocycles with one heteroatom as a diene, patent priority claim, **2024**, 138029-1423 TEP.
5. M. Kajtár, S. B. Király, A. Rimóczi, A. Mándi, A. Bényei, A. Kiss, L. B. Horváth, Sz. Bősze, A. Paczal, A. Kotschy, T. Kurtán: The Janus-faced reactivity of π-excessive five-membered heterocycles in domino Knoevenagel-cyclization reactions, **2025**, before publication.

Publications in other subjects

1. S. B. Király, M. Kajtár, A. Bényei, E. Lisztes, B. I. Tóth, T. Bíró, G. Vasvári, M. Vecsernyés, T. Kurtán: Regio- and diastereoselectivity of domino Knoevenagel-hetero Diels-Alder reactions with 4-hydroxycoumarins and related derivatives as reagents, **2025**, before publication.

Lectures in the subject

1. Kajtár Mihály, Király Sándor Balázs, Mándi Attila, György Péter Vendel, Kurtán Tibor: Domino-Knoevenagel gyűrűzárási reakciók vizsgálata szubsztituált N-(3-arilprop-2-én-1-il)anilin származékokkal, MTA Alkaloid- és Flavonoidkémiai Munkabizottság Ülése (Mátrafüred, 2020.10.01-02).
2. Kajtár Mihály, Király Sándor Balázs, Kurtán Tibor: Domino gyűrűzárási reakciók vizsgálata kondenzált heterociklusok diasztereoszelektív előállítására, MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság Patonay Tamás-díj emlékülése (Budapest, 2021.09.02).
3. Kajtár Mihály, Király Sándor Balázs, Mándi Attila, Kovács Tibor, Kurtán Tibor: Domino gyűrűzárási reakciók új alapvázat képviselő kondenzált heterociklusok előállítására, Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium (Herceghalom, 2021.09.20-21.).
4. Kajtár Mihály, Király Sándor Balázs, Vajnay Dorottya, Kurtán Tibor: Domino gyűrűzárási reakciók vizsgálata kondenzált heterociklusok diasztereoszelektív előállítására, MTA Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2021.10.07-08).

5. Kajtár Mihály, Király Sándor Balázs, Kurtán Tibor: Királis, Kondenzált Heterociklusok Előállítása Domino Gyűrűzárási Szekvenciákkal, Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése (Balatonszemes, 2022.05.23-25).

6. Kajtár Mihály, Király Sándor Balázs, Kurtán Tibor: Királis, kondenzált heterociklusok előállítása domino gyűrűzárási szekvenciákkal. MKE Vegyészkonferencia (Eger, 2022.06.15-17.).

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