

Multifaceted Domino Knoevenagel-Cyclization Reactions; Four Movements for 2*H*-Chromenes and Chromans

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Dedicated to the memory of professor Sándor Antus.



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Abstract: Domino Knoevenagel-cyclization reactions of 2*H*-chromene and chroman derivatives containing *o*-formylaryl amine or ether side-chain was carried out to produce four series of chiral condensed heterocycles representing four novel skeletons and exhibiting antiproliferative activity. The cyclization step occurred with four different mechanisms: a concerted intramolecular hetero Diels-Alder reaction (IMHDA), a stepwise polar [2 + 2] cycloaddition, a [1,5]-hydride shift-6-*endo* cyclization or a multi-step nitro hetero Diels-Alder-ring-opening-Cadogan-type cyclization sequence. The latter reaction provided a new route to hydroxyindoles by an inverse Cadogan-type cyclization, in which the nitro group is deoxygenated by a nitro IMHDA-ring-opening sequence. The cyclization mechanisms and their stereoselectivity were studied by DFT calculations, based on which we proposed a mechanism for the multi-step cyclization to hydroxyindoles and explained the observed diastereoselectivity.

Keywords: Cycloaddition; Configuration determination; Diastereoselectivity; Domino reactions; Heterocycles; Molecular diversity

Introduction

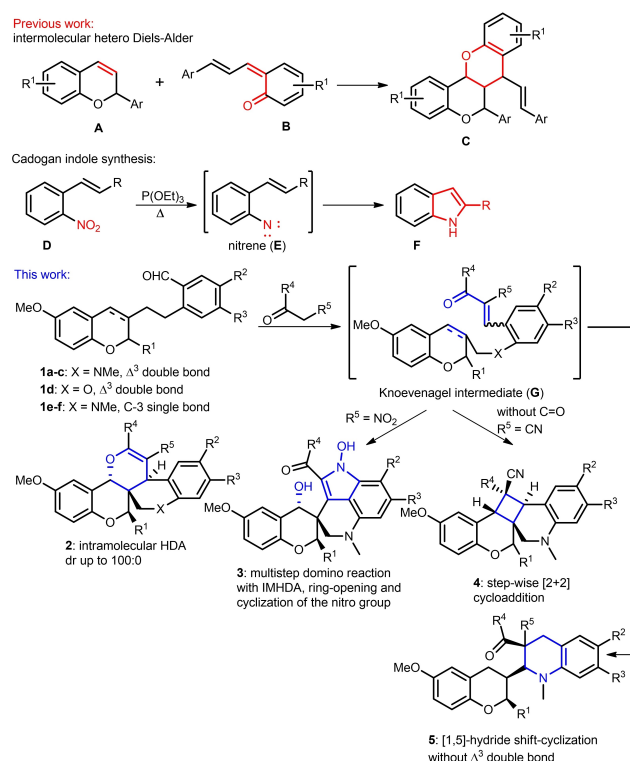
Chiral benzene-condensed *O*- and *O,N*-heterocyclic systems containing tetrasubstituted annulation points are common structural features in anticancer natural or synthetic derivatives as exemplified by omacetaxine,^[1] exiguamine A,^[2] lapidilectine B,^[3] and cytosporolides A–C.^[4] The synthesis of complex condensed chiral heterocycles is usually a challenging task often requiring multi-step sequences.^[5] Intra- and intermolecular Diels-Alder or hetero Diels-Alder reactions are frequently utilized by both Nature^[6] and biomimetic synthetic approaches^[7] to assemble chiral condensed polycyclic skeletons. Substituted 2*H*-chromene natural products, represented by the general formula **A**, are biosynthetic precursors of condensed polycyclic chroman dimers **C** such as dependensin^[8] and kamalachalcone A,^[9] which are obtained by hetero Diels-Alder reaction-mediated dimerization between the electron-rich Δ^3 double bond of the 2*H*-chromene **A** and the *ortho*-quinone methide heterodiene **B** produced by an acid-catalyzed ring opening of **A** (Scheme 1). Although several recent synthetic works have described the biomimetic dimerization of 2*H*-chromenes through the hetero Diels-Alder reaction with *ortho* quinone methides^[10] and there are reports for the reaction with other *in situ* generated quinone methides,^[11] the synthetic potential of 2*H*-chromenes to react with regular heterodienes is clearly underutilized. In this work, we envisioned that 2*H*-chromene derivatives containing an *ortho*-formyl-arylamine or ether side-chain, as shown in **1a–d**, can undergo three different domino Knoevenagel-cyclization sequences with active methylene reagents including a Knoevenagel-intramolecular oxa-hetero-Diels-Alder (oxa-IMHDA) domino reaction to assemble diastereoselectively three heterocycles with three new contiguous chirality centers, fused by a chiral quaternary annulation point (**1a–d**→**G**→**2**, Scheme 1). Knoevenagel-HDA reactions developed by Tietze and co-workers were reported to be useful in the step-, time- and atom-efficient synthesis of condensed chiral dihydropyran derivatives in regio- and diastereoselective manner.^[12] By using nitroacetic acid derivatives as a reagent, we identified a novel multistep domino cyclization reaction (**1a**→**G**→**3**), which consisted in a Knoevenagel-nitro IMHDA-ring-opening-Cadogan cyclization sequence producing condensed *N*-hydroxyindole derivatives **3** diastereoselectively with a spiro chirality center.

The formation of the *N*-hydroxyindole moiety of **3** from the nitro group of the Knoevenagel intermediate **G** ($R^5 = \text{NO}_2$) showed distant analogy with the Cadogan reaction,^[13] in which the nitro group of *ortho*-nitrostyrenes (**D**) cyclizes with the β -carbon of the alkenyl side-chain to form an indole derivative **F** through a nitrene intermediate **E** when refluxing with

trialkylphosphite. However, in our domino reaction the nitrogen atom of the nitro group cyclizes with the arene unit (**1a**→**G**→**3**) instead of the sp^2 carbon of the alkenyl side-chain (**D**→**E**→**F**), which may be considered an inverse Cadogan pathway. While the Cadogan reaction requires a deoxygenating reagent such as triethyl phosphite to remove one of the oxygens of the nitro group, in our sequence the nitro group was activated under mild conditions by an initial nitro IMHDA reaction providing a 1,2-oxazine ring, in which the cleavage of the N–O bond provided the nitroso derivative for the Cadogan cyclization. When malonitrile or (phenylsulfonyl)acetonitrile was used as an active methylene reagent, the Knoevenagel intermediate **G** reacted with a step-wise [2+2] cycloaddition^[14] resulting in the diastereoselective formation of a condensed cyclobutane derivative **4**, in which the cyclobutane ring connected a chromane and a tetrahydroquinoline unit with four contiguous chirality centers (Scheme 1).

In the absence of the Δ^3 double bond, the initially formed Knoevenagel intermediate (**1e**→**G**) reacted in a [1,5]-hydride shift-cyclization cascade affording chroman-tetrahydroquinoline hybrids **5**, in which the two subunits were attached by a C-3-C-2' linkage.

The heterocyclic scaffolds of the condensed target molecules **2–5** represent new molecular entities, which were readily accessed by the four different mechanisms



Scheme 1. Previous related work and domino Knoevenagel-cyclization pathways.

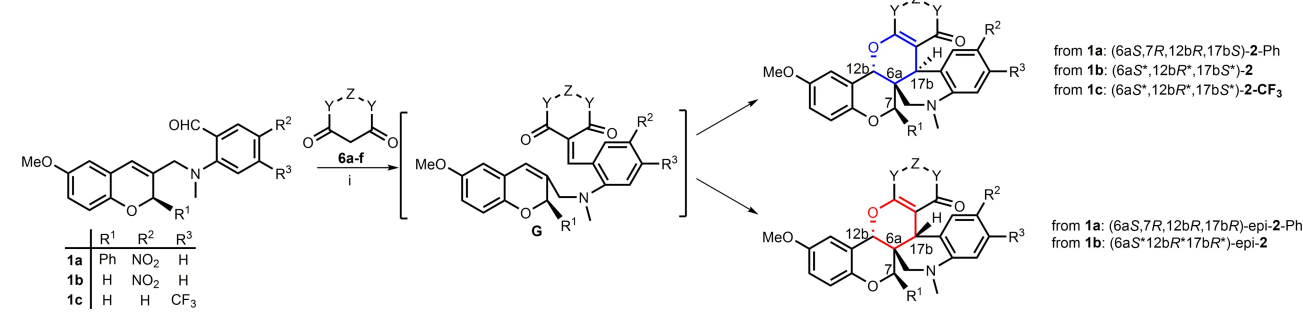
of the domino reactions. We investigated which domino mechanism is preferred by the different combinations of substrate, reagent and reaction conditions. The planar structure and stereochemistry of the products were confirmed and characterized by NMR measurements, DFT calculations of ^{13}C NMR chemical shifts, ECD and HPLC-ECD measurements, TDDFT-ECD calculations and single crystal X-ray geometries in four cases. The mechanism and diastereoselectivity of the different routes were studied by DFT calculations of the transition states and activation parameters. Antiproliferative assays of the chiral condensed molecular entities identified low micromolar cytostatic effect for several derivatives against A2780 and WM35 human cancer cell lines.

Results and Discussion

Knoevenagel-IMHDA Reactions with Cyclic Active Methylene Reagents

The 2*H*-chromene derivatives **1a–c** (inlet scheme of Table 1), starting materials of the domino Knoevenagel-IMHDA reactions containing an *o*-formyl-arylamine side-chain, were prepared in three steps from 5-methoxysalicylaldehyde (Scheme S1 and S3). Compound (*R*)-**1a** was prepared in optically active form with 88% enantiomeric excess, since the initial (*R*)-2-phenyl-3-formyl-2*H*-chromene derivative was assembled by following a reported organocatalytic enantioselective domino oxa-Michael-aldol reaction (Scheme S2).^[15] (*R*)-**1a** was reacted with cyclic active methylene reagents producing the Knoevenagel intermediate **G**, which underwent a substrate-controlled diastereoselective IMHDA reaction generating three

Table 1. Domino Knoevenagel-IMHDA reactions of 2*H*-chromenes derivatives **1a–c** containing an *o*-formyl-arylamine side-chain with cyclic active methylene reagents **6a–f**. i: toluene, reflux for entry 1–4; i: toluene, piperidine (1 eq.), reflux for entry 5–13.

 from 1a: (6<i>aS</i>, 7<i>R</i>, 12<i>bR</i>, 17<i>bS</i>)-2-Ph from 1b: (6<i>aS</i>*, 12<i>bR</i>*, 17<i>bS</i>*)-2 from 1c: (6<i>aS</i>*, 12<i>bR</i>*, 17<i>bS</i>*)-2-CF₃ from 1a: (6<i>aS</i>, 7<i>R</i>, 12<i>bR</i>, 17<i>bR</i>)-<i>epi-2-Ph</i> from 1b: (6<i>aS</i>* 12<i>bR</i>* 17<i>bR</i>*)-<i>epi-2</i>							
entry	1a–c	6a–f	Y	Z	product	yield ^[a]	2/ <i>epi-2</i> ^[b]
1	(<i>R</i>)- 1a	6a	NMe	C=O	2a-Ph	84%	100:0
2	1b	6a	NMe	C=O	2a	75%	100:0
3	(<i>R</i>)- 1a	6b	NEt	C=S	2b-Ph	96%	100:0
4	1b	6b	NEt	C=S	2b	79%	100:0
5	(<i>R</i>)- 1a	6c	CH ₂	CH ₂	2c-Ph , <i>epi-2c-Ph</i>	92%	58:42
6	1b	6c	CH ₂	CH ₂	2c	64%	100:0
7	1c	6c	CH ₂	CH ₂	2c-CF₃	32%	100:0
8	(<i>R</i>)- 1a	6d	CH ₂	CH-Ph	2d-Ph , ^[g] <i>epi-2d-Ph</i> ^h	98%	56:44
9	1b	6d	CH ₂	CH-Ph	2d ^[i]	54%	100:0
10	(<i>R</i>)- 1a	6e	CH ₂	—	2e-Ph , ^[j] <i>epi-2e-Ph</i> ^j	72%	52:48
11	1b	6e	CH ₂	—	2e , ^[j] <i>epi-2e</i> ^[j]	67%	53:47
12	(<i>R</i>)- 1a	6f ^e	1,2-phenylene ^[c]	—	2f-Ph , ^[d]	77%	100:0
13	1b	6f ^e	1,2-phenylene ^[c]	—	2f , ^[e] <i>epi-2f</i> ^[f]	69%	78:22

^[a] Isolated yield.

^[b] Ratio of epimers as determined from NMR.

^[c] **6f**: 1,3-indandione reagent.

^[d] (6*aS*, 7*R*, 12*bR*, 18*bS*) absolute configuration.

^[e] (6*aR**, 12*bS**, 18*bR**) relative configuration.

^[f] (6*aR**, 12*bS**, 18*bS**) relative configuration.

^[g] ~2:1 Mixture of unseparated C-15 epimers.

^[h] Separated C-15 epimers with ~3:2 ratio.

^[i] C-15 epimers with 65:35 ratio.

^[j] Numbering of the C-17b chirality center changes to C-16b.

new contiguous chirality centers including a tetrasubstituted carbon (Table 1).

With *N,N*-dimethylbarbituric acid (**6a**, entry 1, Table 1), the IMHDA reaction afforded a single stereoisomer **2a-Ph** with (6*aS*,7*R*,12*bR*,17*bS*) absolute configuration (AC) (Table 1, Figure 1a). The AC of the three new chirality centers was correlated with that of C-7 and determined by the characteristic 7*a*-H_{ax}/17*b*-H_{ax} (3.03 Å), 6*a*-H_{ax}/17*b*-H_{ax} (2.61 Å), 6*a*-H_{eq}/12*b*-H_{eq} (3.02 Å) NOE correlations aided by the geometries of the computed low-energy DFT conformers (Figure 1d, Figure S18). The AC was also confirmed independently by TDDFT-ECD calculations^[16] producing good agreement of the experimental ECD spectrum of **2a-Ph** with the computed TDDFT-ECD spectrum of the (6*aS*,7*R*,12*bR*,17*bS*) stereoisomer (Figure 1e).

The formation of the C-17*b*–C-6*a* and O-13–C-12*b* σ-bonds took place from the face opposite to the orientation of the *equatorial* C-2 phenyl group. In

order to reveal the details of the IMHDA mechanism, the potential energy surface was computed for the cyclization step by systematically changing the C-17*b*–C-6*a* and O-13–C-12*b* bond lengths with 0.1 Å intervals, starting from the B3LYP/6-31G(d)-optimized geometry of (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** (Figure 1b). The calculations confirmed the asynchronous concerted mechanism of the IMHDA cyclization step with a single transition state (TS1: 23.56 kcal/mol), in which the formation of the C-17*b*–C-6*a* single bond is ahead of that of the O-13–C-12*b* one. In the transition state (TS1-**2a-Ph**, Figure 1d), the C-17*b*–C-6*a* bond length (1.98 Å) is at 128% of the corresponding one (1.55 Å) in the product, while the O-13–C-12*b* bond length (2.70 Å) is at 184% of the final bond length (1.47 Å). An *exo-Z-syn* transition state was identified according to the nomenclature introduced by Tietze *et al.*^[17] However, in the transition state, the barbituric acid moiety was oriented above the hetero ring of the

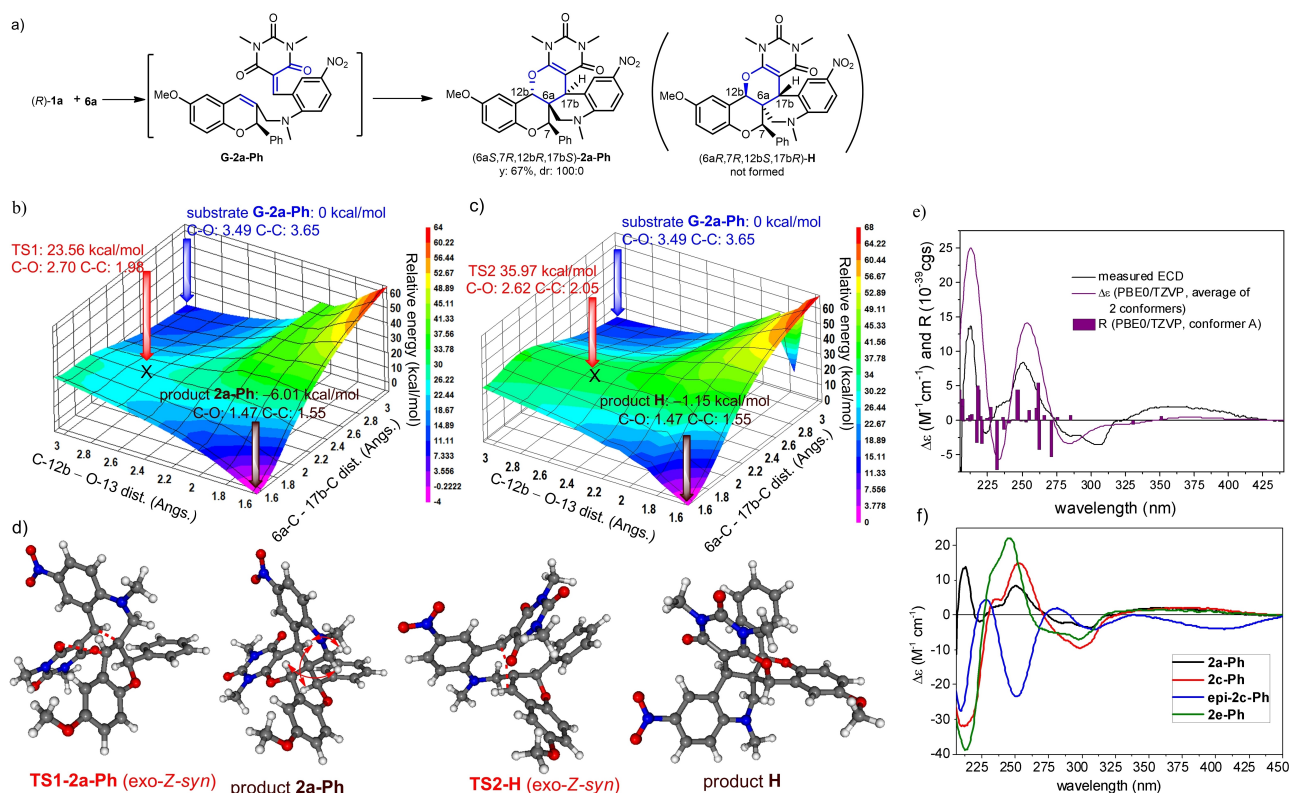


Figure 1. a) Formation of (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** in the reaction of (R)-**1a** with **6a**; reaction conditions: toluene, reflux. b) DFT potential energy hypersurface computed for the **G-2a-Ph** → (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** cyclization step by systematically changing the C-6*a*–C-17*b* and C-12*b*–O-13 bond lengths of the Knoevenagel intermediate **G-2a-Ph** with 0.1 Å intervals. The position of the TS, relative energies and characteristic bond lengths (Å) are disclosed c) DFT potential energy hypersurface computed for the **G-2a-Ph** → (6*aR*,7*R*,12*bS*,17*bR*)-**H** (theoretical product) cyclization step. d) Computed DFT geometries of the products (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** and (6*aR*,7*R*,12*bS*,17*bR*)-**H**; red arrows on **2a-Ph** represent characteristic experimental NOE correlations. e) Comparison of the experimental ECD spectrum of (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** with the Boltzmann-averaged PBE0/TZVP/MeCN ECD spectrum of (6*aS*,7*R*,12*bR*,17*bS*)-**2a-Ph** computed for the ωB97X/TZVP PCM/MeCN conformers. Bars represent the rotational strength values of the lowest-energy conformer. f) Comparison of the experimental ECD spectra of **2a-Ph**, **2c-Ph**, *epi*-**2c-Ph** and **2e-Ph** recorded in MeCN.

2*H*-chromene subunit enabling favorable interactions of the orbitals, which would suggest an *endo* TS if the overlapping of the orbitals is considered. The (6*aR*,7*R*,12*bS*,17*bR*) stereoisomer of **2a-Ph** with opposite AC for the new chirality centers (product **H**) was selected arbitrarily for potential energy surface calculations to explain the diastereoselectivity preferring the exclusive formation of the (6*aS*,7*R*,12*bR*,17*bS*) stereoisomer (Figure 1a). The DFT calculations of the unfavorable stereoisomer (6*aR*,7*R*,12*bS*,17*bR*)-**H** (theoretical product) showed higher activation energy for the transition state (TS2: 35.97 kcal/mol versus TS1: 23.56 kcal/mol) and higher relative potential energy for the product (−1.15 kcal/mol versus −6.01 kcal/mol), which suggested that the formation of the isolated (6*aS*,7*R*,12*bR*,17*bS*) stereoisomer of **2a-Ph** was preferred by both kinetic and thermodynamic control (Figure 1c and 1d). According to Tietze's terminology, *exo-Z-syn* transition state (TS2) belonged also to the unfavorable (6*aR*,7*R*,12*bS*,17*bR*) stereoisomer, in which the barbituric acid moiety containing the heterodiene was pointing away from the hetero ring of the 2*H*-chromene unit and there was π -stacking with the C-2 phenyl ring forced in an *axial* orientation (Figure 1f). The *axial* orientation of the C-2 phenyl group in the TS2 and the (6*aR*,7*R*,12*bS*,17*bR*) configuration of the product decreased the steric crowding with the newly formed σ -bonds but it elevated the energy of TS2 and the product and resulted in a folded geometry.

The IMHDA reaction of (*R*)-**1a** was also diastereoselective with 1,3-diethyl-2-thiobarbituric acid (**6b**, entry 3) and 1,3-indandione (**6f**, entry 12) resulting in the formation of **2b-Ph** and **2f-Ph**, respectively (Table 1). These products showed the same characteristic NOE correlations as **2a-Ph** and thus they had the same configuration (Figure S24 and S87). In contrast, the reaction of (*R*)-**1a** with 1,3-cyclohexanedione (**5c**, entry 5) and 1,3-cyclopentanedione (**6e**, entry 10) provided two stereoisomers with 70:30 and 56:44 ratio, respectively, from which the major products **2c-Ph** and **2e-Ph** had the same AC as that of **1a-Ph**, while the minor ones, *epi*-**2c-Ph** and *epi*-**2e-Ph**, were proved to be the C-17*b* (C-16*b* in *epi*-**2e-Ph**) epimers. The ACs of the *epi*-**2c-Ph** and *epi*-**2e-Ph** were determined on the basis of the observed 6- H_{ax} /12*b*- H_{ax} , 6- H_{ax} /17*b*- H_{ax} and 12*b*- H_{ax} /17*b*- H_{ax} NOE correlations, which suggested that the three correlated axial protons are pointing toward the same face of the molecule (Figure S36 and S72). Moreover, the measured ECD spectra of the major stereoisomers **2c-Ph** and **2e-Ph** had the same ECD profile as that of **2a-Ph** with negative Cotton effects (CEs) at 405 nm and 250 nm, while the minor stereoisomers *epi*-**2c-Ph** and *epi*-**2e-Ph** had opposite CEs for these bands, which derived from the opposite AC of the C-17*b* (C-16*b*) bridgehead atom (Figure 1g). The opposite AC of this carbon atom

determined the relative orientation of the nitroaryl and enone chromophores, which was reflected in the characteristic ECD bands. In the reaction of (*R*)-**1a** with 5-phenyl-1,3-cyclohexanedione (**6d**), a desymmetrization reaction was attempted at C-15 parallel with the diastereoselective IMHDA step (entry 8, Table 1). However, the two usual stereoisomers **2d-Ph** and *epi*-**2d-Ph** were obtained in comparable ratio and isolated as 1:2 and 3:2 mixtures of the C-15 epimers, respectively. The C-15 epimers of *epi*-**2d-Ph** could be separated but the AC of C-15 was not assigned, since they showed identical ECD spectra (Figure S223) and NOE correlations (Figure S54 and S57).

The domino reaction of the achiral starting material **1b**, lacking the C-2 phenyl substituent, yielded the **2a**, **2b**, **2c** racemates diastereoselectively (entry 2, 4, 6, Table 1) with the substituted barbituric acid reagents **6a**, **6b** and the 1,3-cyclohexanedione (**6c**), respectively, while the C-15 epimeric mixture of **2d** was produced with the reagent 5-phenyl-1,3-cyclohexanedione (**6d**) (entry 9). The reaction of **1b** with 1,3-cyclopentanedione (**6e**) gave a mixture of the two diastereomers (6*aS**,12*bR**,16*bS**)- and (6*aS**,12*bR**,16*bR**)-*epi*-**2e** with comparable ratio (53:47), which prompted us to check the activation parameters for the formation of the two diastereomers by DFT calculations (Figure 2). The near identical activation energy values (24.35 versus 24.49 kcal/mol) of the computed *exo-Z-syn* and *exo-E-anti* transitions states for (6*aS*,12*bR*,16*bS*)-**2e** and (6*aS*,12*bR*,16*bR*)-*epi*-**2e** were in full accordance with the comparable isolated yields of the two diastereomers (Figure 2) and suggested kinetic control for the IMHDA step. These results can be also used to explain the diastereoselectivity of the cyclization reactions with non-symmetric acyclic active methylene reagents (*vide infra*), since the (*Z*)- and (*E*)-diastereomers of Knoevenagel intermediate **G** afforded epimeric products through the *exo-Z-syn* and *exo-E-anti* transitions states, respectively.

The Knoevenagel-IMHDA reaction was also extended to the **1d** substrate, containing an *o*-formyl-aryl ether moiety instead of the *tertiary* amine one (Table 2). The IMHDA cyclization resulted in the (6*aS**,12*bR**,17*bR**)-**2a-O**, **2b-O** and **2f-O** racemates of a single diastereomer with the reagents **6a**, **6b** and **6f**, respectively (entry 14,15 and 19, Table 2). The planar structure and relative configuration of **2f-O** were also confirmed by single crystal X-ray diffraction analysis (Figure S307, CCDC deposition no.: 2210411). The (6*aS**,12*bR**,17*bR**) relative configuration derived from *exo-Z-syn* transition state and represented the same geometry as those of the major products **2a-e** for the *N*-methyl analogues but the CIP priority order changed for the C-17*b*. With the reagents **6c-e**, the (6*aS**,12*bR**,17*bR**) diastereomers **2c-O**, **2e-O** were obtained as the major products and the C-17*b* epimers as the minor ones through the *exo-E-anti*

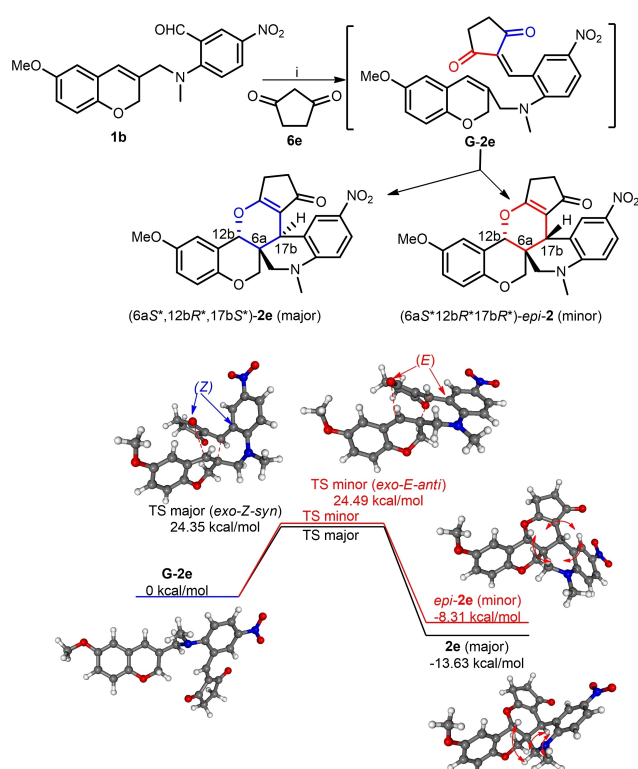


Figure 2. DFT computed Gibbs free energies and transition state structures for the IMHDA step ($G-2e \rightarrow 2e$ and $epi-2e$) of the domino reaction of **1b** with **6e**. Red curly arrows on the structures of the products represent characteristic NOE correlations for the assignment of relative configuration.

transition state. Low micromolar IC_{50} values were identified for the antiproliferative activity of **2a-O**, **epi-2c-O** and **epi-2e-O** against A2780 and WM35 human cancer cell lines (*vide infra*), which prompted us to prepare further derivatives (Figure S243–244, S248–249 and S257–258).

When reacting (*R*)-**1a** with Meldrum's acid (**6g**) in the presence of piperidine, the nucleophilic piperidine attacked the lactone carbonyl of the IMHDA intermediate (**I**), opened the ring and the hemiacetal moiety was lost in the form of an acetone molecule to produce the optically active amide **2g-Ph** with five contiguous chirality centers (Table 3, entry 20). If triethyl amine was used as a base in the same reaction (entry 21), the ring-opening of the lactone ring was performed with a water molecule and the resultant carboxyl group was lost in a decarboxylation step (**I** $\rightarrow$ **2g-Ph** $\rightarrow$ **2g-Ph-dec**). A single product (3*aS*,9*aS*,10*R*,15*bR*)-**2g-Ph-dec** was isolated deriving from the *exo-Z-syn* transition state of the IMHDA step. With the achiral substrate **1b**, reaction with piperidine provided diastereomeric amides (3*S**,3*aR**,9*aS**,15*bR**)-**2g** and (3*R**,3*aS**,9*aS**,15*bR**)/(3*S**,3*aS**,9*aS**,15*bR**)-**epi-2g** in ~1:1 ratio (entry 22), while triethyl amine afforded the 63:37 diastereomeric mixture of **2g-dec** and **epi-**

2g-dec (entry 23). In **epi-2g**, the C-3 center did not form selectively, the product was isolated as a mixture of *trans* and *cis* C-3 epimers. The **1d** substrate yielded the (3*aR**,9*aS**,15*bR**)-**2g-O-dec** diastereoselectively in the presence of triethyl amine (entry 24). Analogous three- or four-component multistep Knoevenagel-IMHDA reactions with Meldrum's acid were utilized by Tietze and co-workers for the synthesis of hirsutin B^[12d] and (+)-camptothecin,^[18] in which an electron-rich dienophile was one of the components.

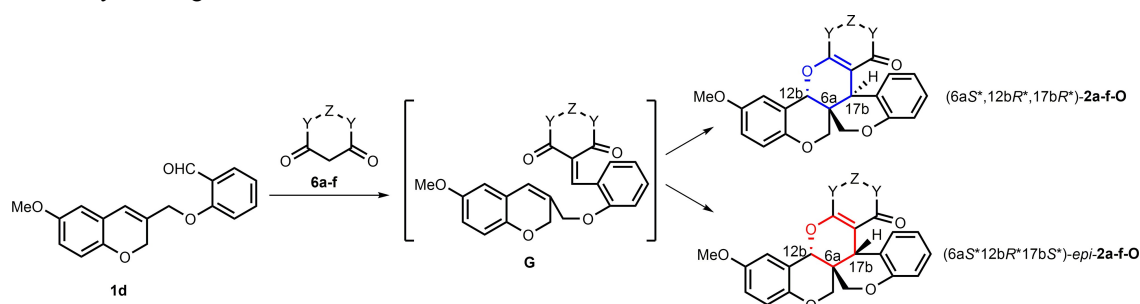
Knoevenagel-Nitro IMHDA-Ring-Opening-Cadogan Cyclization Sequence with Nitroacetic Acid Derivatives

With acyclic active methylene reagents such as nitroacetic acid derivatives, the reaction conditions had to be modified to refluxing in an acetic acid/piperidine buffer in EtOH to promote the domino reaction. In the diastereoselective multistep domino reaction of achiral **1b** with methyl nitroacetate (**6h**), an unexpected condensed hydroxyindole derivative **3h** was obtained with the molecular formula $C_{22}H_{21}N_3O_8$ as determined from HRESIMS data (entry 25, Table 4).

The product **3h** contained a chromene and a dihydro-pyrrolo[4,3,2-*de*]quinoline subunit attached by a 3,3' spiro linkage. The regular Knoevenagel-IMHDA reaction with the participation of the ester carbonyl in the heterodiene moiety would have resulted in the formation of product **K**, which however could not be observed. The Δ^3 double bond of the 2*H*-chromene was oxygenated at C-4 and a new carbon-carbon bond formed at C-3 indicating an IMHDA reaction but the methoxycarbonyl group was intact ($\delta_{C=O}$: 161.9 ppm) and C-2a' was found a quaternary sp^2 carbon giving HMBc correlations with both 4'-H protons. Moreover, the 8'a-H aromatic proton of the nitroaryl moiety was missing indicating that an additional cyclization took place at the C-8'a position with either the oxygen (**J** $\rightarrow$ **L**) or nitrogen atom (**J** $\rightarrow$ **3h**) of the original nitro group.

The presence of the hydroxyindole subunit was confirmed by ^{15}N - ^1H HMBC experiment showing four-bond correlation between the indole nitrogen and 7'-H (Figure S121). This also excluded the possibility of the alternative 1,2-oxazine derivative **L**, in which the corresponding five-bond ^{15}N -H correlation could not have been observed. Besides the ^{13}C - and ^{15}N - ^1H HMBC, ^{13}C - ^1H HSQC and ^1H - ^1H COSY experiments, the planar structure of **5h** was also confirmed by the mPW1PW91/6-311+(2d,p)/B3LYP/6-31+G(d,p) calculations of the ^{13}C chemical shifts (Table S11).^[19] The product was isolated as a racemate of a single diastereomer with 55% yield and the *trans* relative configuration of the 4-OH and the C-3'-C-4' bond [(3*R**,4*S**)] was determined by the 4-H/4'-H NOE correlation (Figure S120). The domino reaction must

Table 2. Domino Knoevenagel-IMHDA reactions of 2*H*-chromenes derivative **1d** containing an *o*-formyl-arylether side-chain with cyclic active methylene reagents **6a–f**.



entry	6 a–f	Y	Z	product	yield ^[a]	2:epi-2 ^[b]
14	6 a	NMe	C=O	2 a-O	76%	100:0
15	6 b	NEt	C=S	2 b-O	88%	100:0
16	6 c	CH ₂	CH ₂	2 c-O , <i>epi-2 c-O</i>	83%	77:23
17	6 d	CH ₂	CH–Ph	2 d-O , ^[f] <i>epi-2 d-O</i> ^g	82%	85:15
18	6 e	CH ₂	–	2 e-O , ^[d] <i>epi-2 e-O</i> ^[d]	46%	60:40
19	6 f ^[c]	1,2-phenylene ^[c]	–	2 f-O ^[e]	42%	100:0

^[a] Isolated yield.

^[b] Ratio of epimers as determined from NMR.

^[c] **6 f**: 1,3-indandione reagent.

^[d] Numbering of the 17b chirality center changes to 16b.

^[e] Numbering of the C-17b chirality center changes to C-18b.

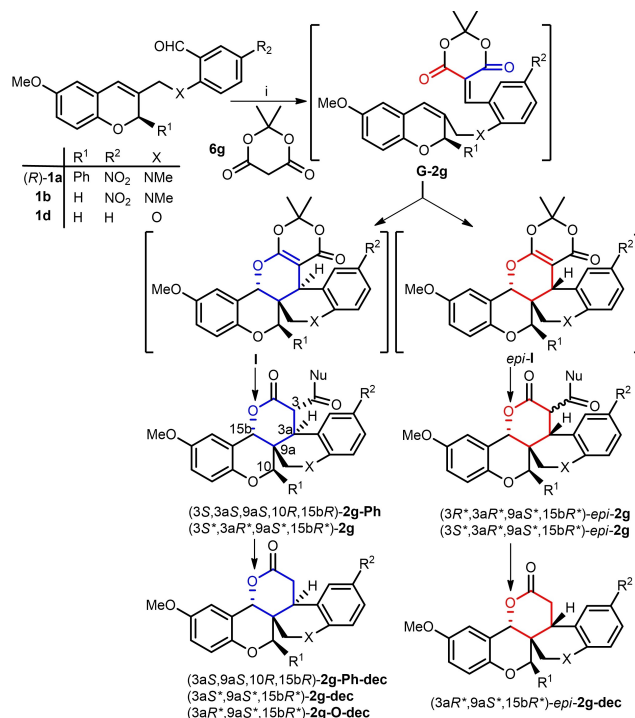
^[f] 3:2 Mixture of C-15 epimers.

^[g] Only one C-15 epimer isolated.

have been promoted by an aza-IMHDA reaction of the initial **G-3h** Knoevenagel intermediate, with the nitroalkene moiety acting as an azadiene (**G-3h**→**M**), during which the C-4 was oxygenated and the stereogenic tetrasubstituted C-3 spiro center was established by forming a cyclic nitronate moiety (**M**) (Scheme 2). On the basis of our B3LYP/6-31G(d) and M06-2X/TZVP calculations (Figure S305), the aza-IMHDA step took place with a highly asynchronous concerted mechanism, in the TS of which the formation of the C–C bond was much more advanced (1.70 Å in the **TS1-M** and 1.55 Å in the intermediate **M**; 109.7%) than that of the C–O one (3.01/1.46 Å for the **TS1-M** and **M**; 206.2%) (Figure S285–S286). The polar *exo-Z-anti* **TS1-M** produced diastereoselectively the 5,6-dihydro-4*H*-1,2-oxazine-2-oxide (cyclic nitronate) intermediate (3*aS**,9*aR**,15*bS**)-**M** from the (*Z*)-**G-3h** Knoevenagel intermediate, with 39.72 kcal/mol activation energy in an exergonic step. The IRC analysis confirmed that there were no other TSs and thus it followed a highly asynchronous concerted mechanism as reported previously in the computational studies of HDA reactions of nitroalkenes and alkenes.^[20] Besides the major product (*Z*)-**G-3h**, the Knoevenagel condensation of **1b** and **6h** is expected to afford (*E*)-**G-3h**

as well, since similar condensation reactions of nitro compounds reportedly have low (*E*)/(*Z*) selectivities.^[21] The (*E*)-**G-3h** either isomerizes to the (*Z*) isomer under the reaction conditions, or it can also undergo the aza-IMHDA cyclization to afford the epimeric (3*aR**,9*aR**,15*bS**)-**M**. Since the C-3*a* chirality center of **M** (C-2*a*' in the product) is lost in the deprotonation step **N**→**O**, this epimer would also lead to the same product **3h**. The formation and isolation of cyclic chiral nitronates had been reported in intra- and intermolecular HDA reactions of nitroalkenes and dienophiles^[22] and they were also utilized as reactive intermediates for further transformations.^[23] In accordance with literature data,^[24] our calculations showed that the nitronate ring is quite stable and the spontaneous cleavage of the N–O single bond has too high activation energy (> 68 kcal/mol). Thus the protonation of the 1,2-oxazine oxygen is required to promote the ring-opening and computational analysis confirmed that spontaneous cleavage of the O–N bond occurred upon protonation (**M**→**N**, Scheme 2). On the basis of our N-1'–C-8*a*' scan, the cyclizations of the positively charged high-energy intermediate **N** and its neutral derivative obtained by deprotonation at C-2*a*' was not feasible, since instead of the nitrogen, the NO oxygen

Table 3. Domino Knoevenagel-IMHDA reactions of 2*H*-chromene derivatives (*R*)-**1a**, **1b** and **1d** with Meldrum's acid (**6g**) followed by ring-opening and decarboxylation. i: piperidine, toluene, reflux or toluene, NEt₃, reflux.



entry	1 a,b,d	X	HNu	product ^[a]	yield ^[b]	2:epi-2^[c]
20	(<i>R</i>)- 1a	NMe	pip. ^[d]	2g-Ph	66%	100:0
21	(<i>R</i>)- 1a	NMe	H ₂ O ^[e]	2g-Ph-dec	50%	100:0
22	1b	NMe	pip. ^[d]	2g, epi-2g^[f]	42%	48:52
23	1b	NMe	H ₂ O ^[e]	2g-dec, epi-2g-dec	85%	63:37 ^g
24	1d	O	H ₂ O ^[e]	2g-O-dec	26%	100:0

^[a] Configuration is indicated in the Scheme.

^[b] Isolated yield.

^[c] Ratio of epimers as determined from NMR.

^[d] Reaction conditions: toluene, piperidine (1 eq.), Δ.

^[e] Reaction conditions: toluene, NEt₃ (1 eq.), Δ.

^[f] Isolated as a 2:1 mixture of C-3 epimers.

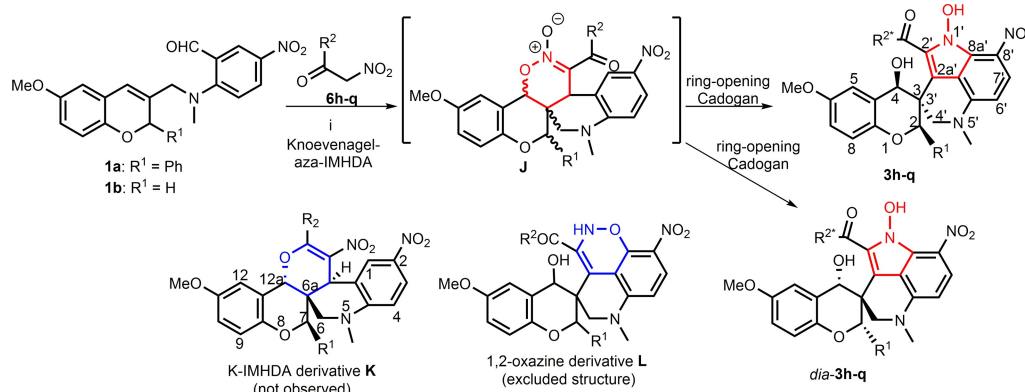
^[g] Isolated as unseparated mixture.

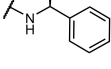
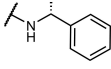
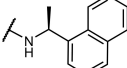
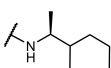
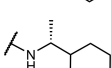
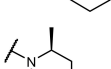
cyclized with the 8b' and 8a' aromatic carbons forming a 1,2-oxazole and a 1,2-oxazine ring, respectively (Figure S306). The protonation of the nitroso oxygen is clearly needed to activate the nitrogen for the cyclization. However, the direct protonation of intermediate **N** is highly unfavored due to the formation of the resulting high-energy dication (Figure S306). Thus the deprotonation at the benzylic C-2a' took place first (**N**→**O**) followed by protonation of the NO oxygen (**O**→**P**), which are exergonic steps assisted by the acetic acid/piperidine buffer. In the following step, the nitrogen atom of the protonated nitroso group of **P** attacked the C-8a' aromatic carbon as an electrophile resulting in the arenium ion intermediate **R**, which rearomatized upon deprotonation to afford the final

product with $-39.02 \text{ kcal mol}^{-1}$ computed ΔG_{rel} compared to the Knoevenagel intermediate **G-3h**.

One of the closest analogies of our cyclization mechanism has been reported recently by Kürti and Ess who suggested stepwise electrophilic aromatic mechanism for the intramolecular amination of arene C(*sp*²)-H bonds of 2-nitrobiaryls resulting in carbazoles.^[25] Another analogous example for the last cyclization step was reported in the tandem [3+2] cycloaddition/reductive cyclization of nitrochalcones with activated methylene isocyanides, in which the nitro group was also converted to an electrophilic nitroso group, which underwent an intramolecular C-N bond formation with the α carbon of a pyrrole subunit at 140 °C.^[26] The multistep conversion of **G-3h**

Table 4. Multistep domino Knoevenagel-nitro DA-ring-opening-cyclization reactions of **1 a,b** with nitroacetic acid derivatives **6 h–s**. i: piperidine/AcOH, EtOH, reflux.



entry	1 a–b	6 h–q	R²	product (yield) ^[a]	3/dia-3 ^[c]
25	1 b	6 h	OMe	3 h^b (55%)	50:50 ^f
26	1 b	6 i	Ph	3 i^{b,c} (70%)	50:50 ^f
27	<i>rac</i> - 1 a	6 h	OMe ^[g]	3 h-Ph^[d] (49%)	50:50 ^f
28	<i>rac</i> - 1 a	6 i	Ph	3 i-Ph^[d] (34%)	50:50 ^f
29	1 b	6 j	NH ₂	3 j^[b] (65%)	50:50 ^f
30	1 b	6 k		3 k^[b] (74%)	50:50 ^f
31	1 b	6 l		3 l^[b] (58%)	50:50 ^f
32	1 b	6 m		3 m^[b] (91%)	50:50 ^f
33	1 b	(<i>S</i>)- 6 n		(<i>3R,4S,3''S</i>)- 3 n (<i>3S,4R,3''S</i>)- 3 n (68%)	51:49
34	1 b	(<i>R</i>)- 6 n		(<i>3R,4S,3''R</i>)- 3 n (<i>3S,4R,3''R</i>)- 3 n (36%)	53:47
35	1 b	(<i>S</i>)- 6 o		(<i>3R,4S,3''S</i>)- 3 o (<i>3S,4R,3''S</i>)- 3 o (18%)	54:46
36	1 b	(<i>S</i>)- 6 p		(<i>3R,4S,3''S</i>)- 3 p (<i>3S,4R,3''S</i>)- 3 p (40%)	51:49
37	1 b	(<i>R</i>)- 6 p		(<i>3R,4S,3''R</i>)- 3 p (<i>3S,4R,3''R</i>)- 3 p (54%)	51:49
38	1 b	(<i>S</i>)- 6 q		(<i>3R,4S,3''S</i>)- 3 q (<i>3S,4R,3''S</i>)- 3 q (59%)	n.d.

^[a] Isolated yield.

^[b] Racemate of the (*3R**,*4S**) diastereomer.

^[c] Duplication of ¹H-NMR signals was observed with a ratio of 54:46 deriving from rotamers of the acyl group.

^[d] Racemate of the (*2R**,*3S**,*4S**) diastereomer.

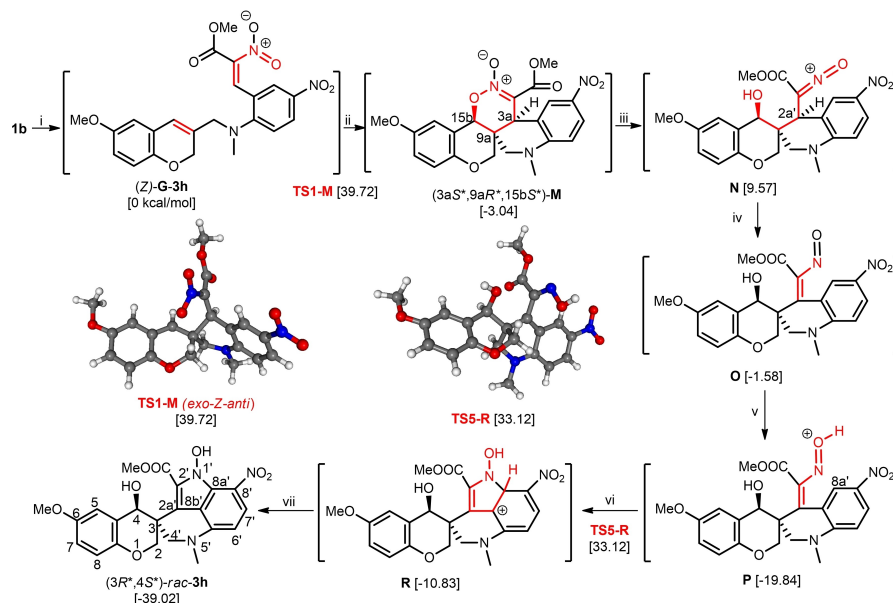
^[e] Ratio of epimers as determined from NMR.

^[f] Pair of enantiomer.

^[g] Isolated and characterized as a mixture of ethyl and methyl esters due to transesterification with the ethanol solvent.

to the hydroxyindole derivative (*3R**,*4S**)-*rac*-**3 h** can be considered an inverse Cadogan-type reaction, in which the nitro group is deoxygenated and activated

for the cyclization by a nitro IMHDA reaction, acid-catalysed ring-opening and proton shift sequence. Our multistep domino reaction took place under mild



Scheme 2. Proposed mechanism for the formation of (3*R**,4*S**)-*rac*-**3h** in the reaction of **1b** with **6h** (ΔG_{rel} energies in kcal mol⁻¹). i: piperidine/AcOH, EtOH, reflux, Knoevenagel reaction. ii: aza-IMHDA reaction. iii: protonation of the oxygen of the heteroring followed by spontaneous ring-opening. iv: deprotonation at the benzylic position. v: protonation of the nitroso oxygen. vi: cyclization. vii: deprotonation.

reaction conditions, in refluxing ethanol with AcOH/piperidine buffer, which contrasts the triethyl phosphite or triphenylphosphine/high temperature conditions of regular Cadogan reactions required for the deoxygenation.^[13j] Recently, as an alternative of the classical Cadogan conditions, the deoxygenation of the nitro group could be also achieved by the palladium-catalyzed reductive cyclization of β -nitrostyrene derivatives to indoles using carbon monoxide as reductant.^[13i,k]

In order to test the scope and limitations, the domino reaction was performed with different active methylene reagents containing a nitro group such as benzoylnitromethane (**6i**, entry 26), 2-nitroacetamide (**6j**, entry 29), achiral (**6k–m**, entry 30–32) and chiral non-racemic 2-nitroacetamide derivatives (**6n–q**, entry 33–38) and with the chiral racemic substrate *rac*-**1a** (entry 27, 28, Table 4). Interestingly, the reaction of **1b** with benzoylnitromethane (**6i**) also followed the same domino sequence with high yield (70%), although the reagent had a ketone carbonyl group to react with an intramolecular oxa-HDA reaction (entry 26, Table 4). With achiral reagents (*R*² does not contain a chirality center, entry 25–32), all the reactions produced a single diastereomer as a racemate and the products **3h–m** and *dia*-**3h–m** represent enantiomers of the racemates. IMHDA products, corresponding to the structure of derivative **K** and supposing the involvement of the amide carbonyl group in the heterodiene, could not be observed with acetamide reagents **6j–q**. When using chiral non-racemic 2-nitroacetamide reagents **6n–q**,

the products were isolated as a 1:1 diastereomeric mixture of **3n–q** and *dia*-**3n–q**, which suggested that there was no chiral induction by the amide moiety during the cyclization step. The optically active diastereomers **3n–p** and *dia*-**3n–p** were separated by HPLC using chiral stationary phase and online HPLC-ECD spectra were recorded, which allowed assigning their AC (Figure S229–S236). The diastereomers (3*R*,4*S*,3''*S*)-**3n** and (3*S*,4*R*,3''*S*)-**3n** showed near mirror-image HPLC-ECD spectra, which suggested that the remote C-3'' chirality center of the *N*-alkyl residue did not have significant contribution to the ECD (Figure 3). Thus TDDFT-ECD spectra were computed for the CAM-B3LYP/TZVP PCM/CHCl₃ conformers of the truncated model compound (3*R*,4*S*)-**S** lacking the C-3'' chirality center (Figure 3).

The Boltzmann-weighted BH&HLYP/TZVP ECD spectrum of (3*R*,4*S*)-**S** reproduced well the experimental HPLC-ECD spectrum of the first-eluted diastereomer of **3n** with known (3''*S*) AC, for which the (3*R*,4*S*,3''*S*)-**3n** AC could be assigned. Based on the similar HPLC-ECD pattern, the high-wavelength Cotton effects (CEs) were used to assign the AC of the separated diastereomers **3n–p** and *epi*-**3n–p**. The antiproliferative activity of the hydroxyindol derivatives **3h–q** was tested against human cancer cell lines (Figure S238 and S241) and some derivatives showed low-micromolar IC₅₀ values (*vide infra*) (Figure S259–S261).

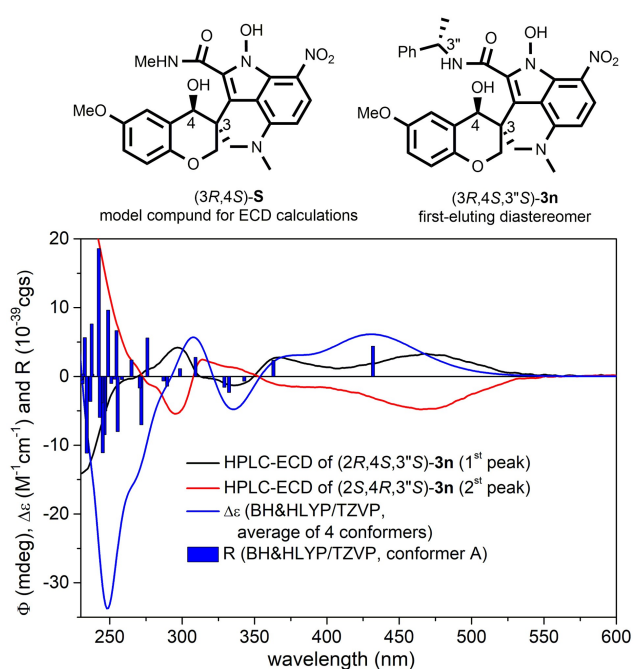


Figure 3. Structures of (3*R*,4*S*,3''*S*)-**3n** and its truncated model compound used for the ECD calculation. Overlapped HPLC-ECD spectra of the first- (black) and second-eluting diastereomers (red) of (3*R*,4*S*,3''*S*)-**3n** compared to that computed for CAM-B3LYP/TZVP PCM/CHCl₃ conformers of (3*R*,4*S*)-**S** (blue). Bars represent the rotational strength values of the lowest-energy conformer.

Knoevenagel-IMHDA with Acyclic Active Methylene Reagents

When the acyclic active methylene reagents (**2s–y**) did not contain a nitro group but they had at least one carbonyl group, the Knoevenagel-IMHDA sequence proceeded with the substrates *rac*-**1a**, **1b** and **1d** analogously to reactions with cyclic reagents (Table 5, 1 and 2). The α,β -unsaturated carbonyl moiety of the initial Knoevenagel intermediate **G** acted as the heterodiene in the IMHDA reaction, affording the condensed *O*- and *O,N*-heterocyclic products **2s–y** (Table 5).

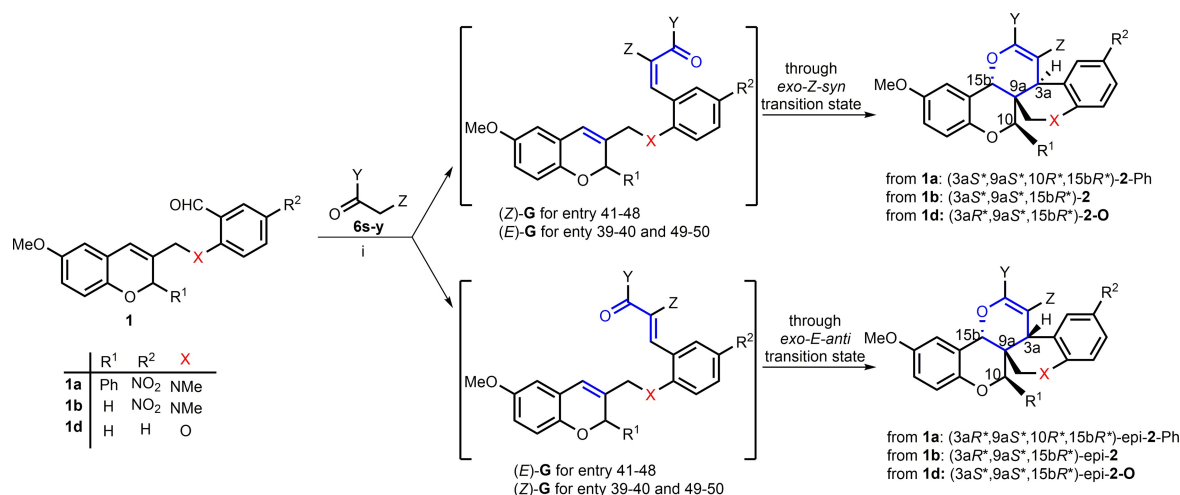
The domino reactions with acyclic reagents required the use of piperidine/AcOH buffer in refluxing ethanol, since no reaction occurred under the conditions applied with cyclic reagents. In most cases (entry 39, 41–43, 46, 48–50, Table 5), a single product was isolated with good or high yield (52–88%), which formed diastereoselectively from the *exo-Z-syn* transition state of the IMHDA reaction. The *exo-Z-syn* transition state derived from the Knoevenagel isomer **G**, in which the interacting carbonyl and the aryl moiety had *cis* orientation along the double bond, which stands for (*Z*)-**G** for entry 41–48 and (*E*)-**G** for entry 39, 40, 49 and 50. In entry 40, 44, 45 and 47, the *epi-2* product formed through the *exo-E-anti* transition

state, which derived from *anti* orientation of the interacting carbonyl and the aryl moiety in the Knoevenagel intermediate **G**. This corresponds to the (*E*)-**G** diastereomer for entry 41–48 and the (*Z*)-**G** one for entry 39, 40, 49 and 50.

Interestingly, in the reaction of benzoylacetonitrile (**6v**) and *rac*-**1a** (entry 44), the other diastereomer with (3*aS**,9*aS**,10*R**,15*bR**) relative configuration formed exclusively, which derived from the *exo-E-anti* transition state. In entry 40, 45, and 47, the two diastereomers formed with comparable ratio and they were analyzed in their diastereomeric mixture. In the reaction of *rac*-**1a** with acetoacetic ester (**6s**), the α,β -unsaturated ketone subunit of the Knoevenagel intermediate **G** acted as the heterodiene, and the reaction afforded the product (3*aS**,9*aS**,10*R**,15*bR**)-**2s-Ph** diastereoselectively (entry 39). In contrast, the reaction of **6s** with the achiral substrate **1b** produced a diastereomeric mixture of (3*aS**,9*aS**,15*bR**)-**2s** and (3*aR**,9*aS**,15*bR**)-*epi-2s* (entry 40). The amide reagent **6t** contained both amide and ketone carbonyl groups but the corresponding Knoevenagel intermediate **G** reacted with the less reactive α,β -unsaturated amide moiety in the IMHDA step to yield **2t-Ph** or **2t** as the only product (entry 41 and 42).

While HDA reactions involving the amide carbonyl unit of cyclic α,β -unsaturated amides such as that of barbituric acid^[12a,27] or 4-hydroxyquinolin-2(1*H*)-one^[28] derivatives are known, there is no example for HDA reactions with acyclic amides. The unexpected reactivity was attributed to the intramolecular hydrogen bond between the amide N–H and the ketone oxygen, which were present in the intermediate **G** and the products **2t** and **2t-Ph**, as suggested by the high chemical shift of the N–H proton (13.53 and 13.85 ppm respectively). This intramolecular hydrogen bond forces the α,β -unsaturated ketone in an *s-trans* conformation, hence preventing it to participate in the HDA reaction. With the benzoylacetonitrile reagent (**6v**), different diastereoselectivity of the IMHDA step was observed with the different substrates *rac*-**1a**, **1b** and **1d** (entry 44–46). Cyclization with *rac*-**1a** and **1d** was fully diastereoselective, but afforded different diastereomers (3*aR**,9*aS**,10*R**,15*bR**)-*epi-2v-Ph* and (3*aS**,9*aS**,15*bR**)-**2v-O**, respectively, while the achiral derivative **1b** produced an equimolar diastereomeric mixture of **2v** and *epi-2v* in an excellent yield. The ethyl cyanoacetate reagent (**6w**) behaved in a different way (entry 45 and 46) and showed different diastereoselectivity from those of **6v**. With the achiral substrate **1b**, it gave (3*aS**,9*aS**,15*bR**)-**2w** diastereoselectively, while with *rac*-**1a** it afforded a 1:1 diastereomeric mixture of (3*aS**,9*aS**,10*R**,15*bR**)-**2w-Ph** and (3*aR**,9*aS**,10*R**,15*bR**)-*epi-2w-Ph*. The relative configuration of the chirality centers was determined by the characteristic NOE correlations in the NOESY spectra and it was also confirmed by

Table 5. Domino Knoevenagel-IMHDA reactions of 2*H*-chromenes derivatives **1a,b** and **1d** with acyclic active methylene reagents **6s–y**. i: piperidine/AcOH, EtOH, reflux.



Entry	1 a–d	6 s–y	Y	Z	Product (Yield) ^[a]	2/ <i>epi</i> -2 ^[b]
39	<i>rac</i> - 1 a	6 s	CH ₃	COOMe	2 s-Ph (67%)	100:0
40	1 b	6 s	CH ₃	COOMe	2 s , <i>epi</i> - 2 s (30%)	64:36
41	<i>rac</i> - 1 a	6 t		Ac	2 t-Ph (52%)	100:0
42	1 b	6 t		Ac	2 t (47%)	100:0
43	1 b	6 u	OEt	COOEt	2 u (64%)	100:0
44	<i>rac</i> - 1 a	6 v	Ph	CN	<i>epi</i> - 2 v-Ph (77%)	0:100
45	1 b	6 v	Ph	CN	2 v , <i>epi</i> - 2 v (92%)	51:49
46	1 d	6 v	Ph	CN	2 v-O (53%)	100:0
47	<i>rac</i> - 1 a	6 w	OEt	CN	2 w-Ph , <i>epi</i> - 2 w-Ph (63%)	51:49
48	1 b	6 w	OEt	CN	2 w (88%)	100:0
49	1 b	6 x	OMe	SO ₂ Ph	2 x (75%)	100:0
50	1 b	6 y	Me	SO ₂ Ph	2 y (76%)	100:0

^[a] iIsolated yield.

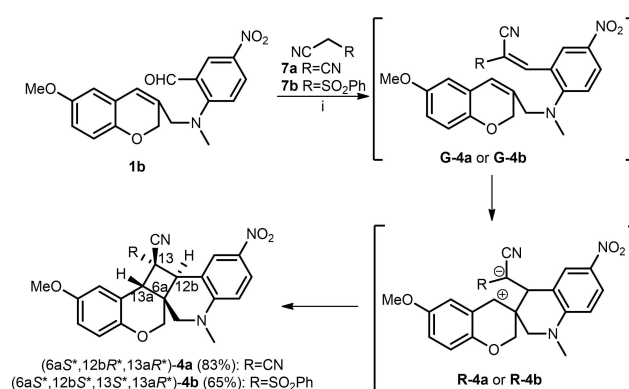
^[b] Ratio of epimers as determined from NMR.

single crystal X-ray diffraction analysis for **2 w** (CCDC no. 2210408) and **2 x** (CCDC no. 2210409) (Figure S308 and S309).

Knoevenagel-Polar [2 + 2] Cycloaddition with Nitrile Derivatives

When using malonitrile (**7a**) or 2-(phenylsulfonyl)acetonitrile (**7b**) as active methylene reagent of the domino sequence with the substrate **1b**, a different cyclization mechanism was identified (Scheme 3).

In the lack of the carbonyl group, which can contribute to the heterodiene moiety, the initial Knoevenagel intermediates **G-4a** or **G-4b** underwent a formal [2 + 2] cycloaddition resulting in condensed chiral cyclobutane derivatives *rac*-**4a** and *rac*-**4b** diastereoselectively (Scheme 3).



Scheme 3. Domino Knoevenagel-[2 + 2] cycloaddition reaction of 2*H*-chromene substrate **1b** with malonitrile (**7a**) and 2-(phenylsulfonyl)acetonitrile (**7b**). i: Me₂NH₂Cl, toluene, reflux.

In order to explore the reaction mechanism, the potential energy hypersurface was computed for the **G-4a**→**4a** cyclization step by systematically changing the C-6a–C-12b and C-13–C-13a bond lengths with 0.1 Å intervals, starting from the B3LYP/6-31G(d)-optimized geometry of (6a*S*,12b*R*,13a*R*)-**4a** (Figure 4).

The calculations confirmed that first the C-6a–C-12b sigma bond forms resulting in the **R-4a** zwitterionic intermediate, which is stabilized by the benzylic position of the positive charge and electron-withdrawing nitrile groups attached to negatively charged C-13 carbon atom. In the next step, the oppositely charged carbon centers form a sigma bond establishing the condensed chiral cyclobutane ring. Thus the [2+2] cycloaddition follows a step-wise polar mechanism involving a zwitterionic intermediate. Similar mechanism was reported earlier in the intermolecular reaction of electron deficient alkenes such as di-^[14a,29] or tetracyanoalkenes^[30] or dialkyl

alkydenemalonates^[14b,c,31] with enol ethers and enamines but intramolecular version has not been reported yet. The (6a*S**,12b*S**,13a*R**) relative configuration of *rac*-**4a** was determined on the basis of the 12b-*H*_{ax}/6-*H*_s and 13a-*H*_{ax}/7-*H*_s NOE correlations (Figure S192), and it was also confirmed independently by the single crystal X-ray diffraction analysis of *rac*-**4a** (CCDC no. 2210410, Figure S310). The relative configuration of the additional C-13 stereogenic center in **4b** was determined by the NOE correlation observed between the *ortho*-hydrogens of the phenyl group and 12b-*H*, affording the (6a*S**,12b*S**,13*S**,13a*R**) relative configuration (Figure S195). It is most likely the stability of product that governs the (6a*S**,12b*R**,13a*R**) diastereoselectivity for the formation of *rac*-**4a** with the symmetric malonitrile reagent (**7a**), which provides a low-energy extended geometry for the condensed ring system with less steric hindrance. The corresponding chirality centers of (6a*S**,12b*S**,13*S**,13a*R**)-**4b** forms with the same diastereoselectivity but it has an additional C-13 chirality center due to the asymmetric (phenylsulfonyl)acetonitrile reagent (**7b**). According to reported data, the Knoevenagel condensations with sulfonyl-acetonitriles usually have (*E*) diastereoselectivity.^[32] Provided that there is no significant C–C bond rotation in the zwitterionic **R-4b** intermediate, the (*E*) configuration of the double bond is retained in the (13*S**) relative configuration of (6a*S**,12b*S**,13*S**,13a*R**)-**4b**. Moreover, with this relative configuration the bulky phenylsulfonyl group is pointing away from the condensed ring system to minimize steric interactions.

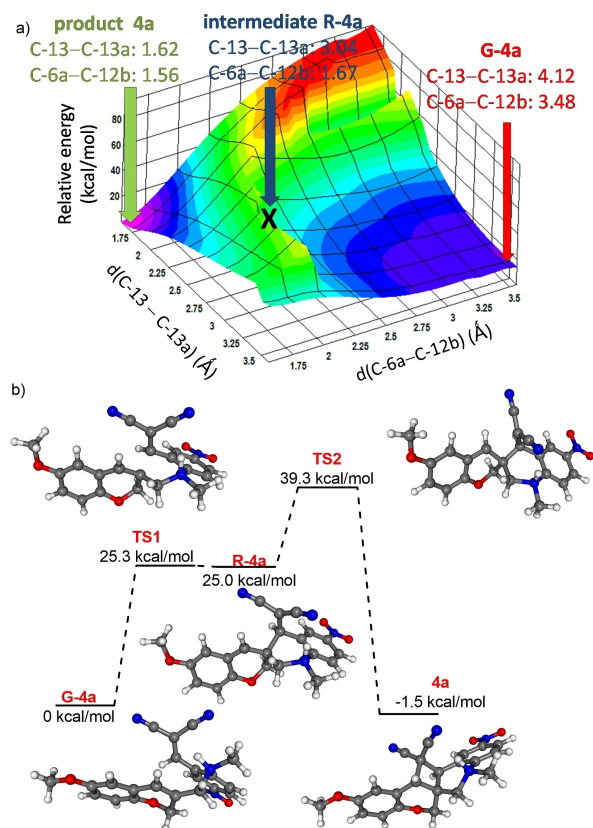


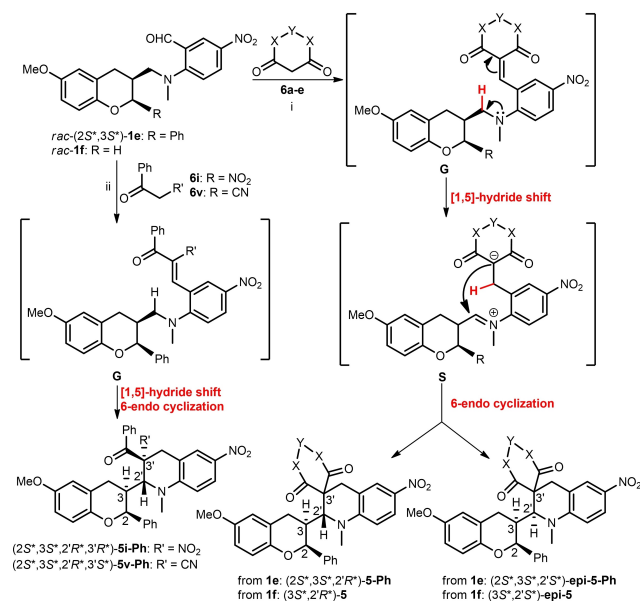
Figure 4. a) DFT potential energy hypersurface computed for the **G-4a**→(6a*S*,12b*R*,13a*R*)-**4a** cyclization step by systematically changing the C-6a–C-12b and C-13–C-13a bond lengths of the Knoevenagel intermediate **G-4a** with 0.1 Å intervals. The position of the zwitterionic intermediate **R-4a** and characteristic bond lengths (Å) are disclosed. b) Computed DFT geometries and relative energies of the Knoevenagel intermediate **G-4a**, zwitterionic intermediate **R-4a**, the product (6a*S*,12b*R*,13a*R*)-**4a** and the corresponding transition states.

Knoevenagel-[1,5]-Hydride-Shift-Cyclization of Chroman Derivatives

In order to exclude the hetero Diels-Alder cycloaddition as a possible mechanism for the cyclization step of the domino sequence, the Δ^3 double bond of the 2*H*-chromen moiety was removed and chroman derivatives *rac*-**1e** and **1f**, starting materials of the domino sequence, were prepared from 5-methoxyisalicylaldehyde in four steps (Scheme S5).

The *cis*-(2*S**,3*S**) relative configuration of the 2-phenyl derivative *rac*-**1e** was assigned on the basis of the small value of the $^3J_{2-H,3-H}$ coupling constant (1.1 Hz). In the absence of the Δ^3 double bond, the substrates *rac*-**1e** and **1f** could not undergo hetero Diels-Alder reaction or polar [2+2] cycloaddition after the Knoevenagel condensation, and thus a [1,5]-hydride shift took place (**G**→**S**) facilitated by the presence of the tertiary amine moiety (Table 1). The resultant zwitterionic intermediate **S** reacted in a 6-endo cyclization (**S**→**5**) to afford products **5**, in which the chiral chromane and tetrahydroquinoline scaffolds were linked through a C-3–C-2' sigma bond. Except for entry 55 (Table 6), the domino reactions of *rac*-**1e**

Table 6. Domino Knoevenagel-[1,5]-hydride shift-6-endo cyclization cascade with chromane derivatives **1e–f**: i: Me₂NH₂Cl, toluene, reflux; ii: Me₂NH₂Cl, *n*-BuOH, reflux.



Entry	1	6	X	Y	Product (Yield)	5/epi-5
51	1e	6a	NMe	C=O	5a-Ph (78%)	100:0
52	1f	6a	NMe	C=O	5a , epi-5a (42%)	n.d.
53	1e	6b	NEt	C=S	5b-Ph (83%)	100:0
54	1f	6b	NEt	C=S	5b , epi-5b (69%)	n.d.
55	1e	6c	CH ₂	CH ₂	5c-Ph , epi-5c-Ph (62%)	58:42
56	1e	6i	see scheme		5i-Ph (59%)	100:0
57	1e	6v	see scheme		5v-Ph (80%)	100:0

was diastereoselective and provided single diastereomers as products **5a-Ph**, **5b-Ph**, **5i-Ph** and **5v-Ph** with good yields (entry 51, 53, 56 and 57). With symmetric active methylene reagents **6a** and **6b**, the domino cyclization established one new chirality center at C-2'. Due to the relatively free rotation about the C-3–C-2' sigma bond, the relative configuration of the C-2' chirality center had to be determined by computing the low-energy B97D/TZVP PCM/CHCl₃ and B3LYP/TZVP PCM/CHCl₃ conformers of both (2*S**,3*S**,2'*R*')-**5a-Ph** and (2*S**,3*S**,2'*S*')-**epi-5a-Ph** and correlating the geometries with the experimental NMR data (entry 51). In the low-energy computed conformers of (2*S**,3*S**,2'*R*')-**5a-Ph**, the 3-H and 2'-H methine protons adopted near *trans*-periplanar orientation (Figure S299 and S301), which agreed well the large ³J_{3-H,2'-H} coupling constant (8.0 Hz) and the lack of NOE correlation between 3-H and 2'-H. The C-2 phenyl group adopted a parallel orientation with the benzene ring of the tetrahydroquinoline unit enabling favorable π–π stacking interaction, which is expected to have a decisive role in the diastereoselectivity. In contrast, *gauche* orientation of 3-H and 2'-H was identified in the two low-energy computed con-

formers of (2*S**,3*S**,2'*S*')-**epi-5a-Ph** (Figure S300 and S302), which would suggest a smaller ³J_{3-H,2'-H} coupling constant and the presence of NOE correlation. The enantiomers of (2*S**,3*S**,2'*R*')-**5a-Ph** were separated by chiral HPLC and mirror image online HPLC-ECD spectra of the enantiomers were recorded (Figure S235). In the reaction of **rac-1e** with non-symmetric reagents benzoyl-nitromethane (**6i**) and benzoyl-acetonitrile (**6v**), the solvent had to be changed to *n*-butanol, which yielded **5i-Ph** and **5v-Ph** with full diastereoselectivity (entry 56 and 57). The additional C-3' chirality center also formed diastereoselectively (Figure S304).

The domino reactions of substrate **1f**, lacking the C-2 chirality center, afforded inseparable mixtures of the two diastereomers with low selectivity (entry 52 and 54). Exact ratio of the diastereomers were not determined due to signal overlaps in the ¹H-NMR spectrum.

Antiproliferative Activity

In order to explore the bioactivity for the products of the domino reactions containing novel condensed heterocyclic scaffolds, antiproliferative activities were evaluated initially against A2780 ovarian and WM35 melanoma cancer cell lines at 50 μM concentration. IC₅₀ values were determined for the most active derivatives by MTT assay and some selected results are listed in Table 7.

Several products obtained by IMHDA reaction with cyclic (entry 58–64) or acyclic (entry 66, 67) active methylene reagents and containing either an *N*-Me (entry 58, 59, 66 and 67) or an oxygen linkage (entry

Table 7. *In vitro* antiproliferative activity of the products of the domino reactions against A2780 and WM35 human cancer cell lines and HaCaT cell line reported as IC₅₀ values.

entry	Compound	Cell lines/IC ₅₀ (μM)		
		A2780	WM35	HaCaT
58	rac-2e	9.94	> 50	2.95
59	rac-epi-2e	1.28	14.3	18.7
60	rac-2e-O	34.76	32.93	nd
61	rac-epi-2e-O	37.68	35.25	nd
62	rac-2a-O	1.62	6.29	3.29
63	rac-2c-O	18.70	7.41	nd
64	rac-epi-2c-O	4.76	5.99	nd
65	(3'' <i>S</i>)- 3p ^[b]	3.96	2.28	5.29
66	rac-2s/rac-epi-2s	6.17	6.36	nd
67	rac-2w	4.48	9.27	nd
68	rac-4a	5.98	23.7	18.8
69	doxorubicin ^[a]	0.07	0.14	0.03

^[a] Positive control;

^[b] 51:49 Mixture of (3*R*,4*S*,3''*S*)-**3p** (3*S*,4*R*,3''*S*)-**3p**; nd: not determined.

60–64) showed low micromolar IC_{50} values down to 1.28 μ M value (entry 59). Antiproliferative activity was also identified for the hydroxyindole derivative (3''S)-**3p** (entry 65) obtained in a Knoevenagel-nitro IMHDA-ring-opening-Cadogan cyclization sequence and for the condensed cyclobutane derivative *rac*-**4a** (entry 68) produced by a Knoevenagel-polar [2+2] cycloaddition.

Conclusion

We exploited the reactivity of the Δ^3 double bond of 2*H*-chromene derivatives containing *o*-formylaryl amine or ether side-chain, used also in HDA dimerization reaction of natural 2*H*-chromene, in domino Knoevenagel-cyclization reactions and a series of chiral condensed heterocycles was produced representing novel skeletons. The cyclization step occurred with three different mechanisms; a concerted intramolecular hetero Diels-Alder reaction (IMHDA), a stepwise polar [2+2] cycloaddition affording condensed cyclobutane derivatives and a multi-step nitro hetero Diels-Alder-ring-opening-Cadogan-type cyclization sequence. The latter reaction is a new route to condensed hydroxyindoles, in which an “inverse” Cadogan-type cyclization of the deoxygenated nitro group took place as part of the multi-step sequence. While the nitrogen of the nitroarene moiety cyclizes with the double bond of the side-chain in the regular Cadogan reaction, in our case the nitrogen of the deoxygenated nitro group cyclizes with the aromatic ring. The deoxygenation was initiated by a nitro IMHDA reaction followed by the acid-catalyzed ring-opening sequence of the nitronate intermediate. The mechanism and stereoselectivity of the cyclization steps were studied by DFT calculations. We determined the transition states and activation parameters of the elementary steps and proposed a mechanism for the multi-step cyclization to hydroxyindoles and confirmed step-wise mechanism for the polar [2+2] cycloaddition. With chroman substrates lacking the Δ^3 double bond, a Knoevenagel-[1,5]-hydride shift-6-*endo* cyclization sequence prevailed affording 3,2'-linked chroman-tetrahydroquinoline conjugates. The four domino cyclization mechanisms provided four series of novel condensed chiral heterocyclic scaffolds, in which the chroman and tetrahydroquinoline moieties were attached by either a spiro carbon atom or a rotatable sigma bond. The structural elucidation including the assignment of the configuration was aided by DFT conformational analysis, ^{13}C DFT-NMR and TDDFT-ECD calculations and single crystal X-ray diffraction. Antiproliferative assays of the products identified low micromolar cytostatic effect for several derivatives against A2780 and WM35 human cancer cell lines.

Experimental Section

Chemicals were purchased puriss p.a. from commercial suppliers, and solvents were purified by distillation before use. For thin-layer chromatography (TLC), silica gel plates Merck 60 F254 were used and compounds were visualized by irradiation with UV light. Column chromatography was performed using silica gel Merck 60 (particle size 0.063–0.200 mm). Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The NMR spectra were recorded on Bruker Avance II 400 (^1H : 400 MHz, ^{13}C : 100 MHz) and Bruker Avance II 500 MHz (^1H : 500 MHz, ^{13}C : 125 MHz) spectrometers using TMS as internal standard. Chemical shifts were reported as δ in ppm and $^3J_{HH}$ coupling constants in Hz. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer and absorption bands are presented as wavenumber in cm^{-1} . The optical rotations of the compounds were measured by a Perkin Elmer (Norwalk, CT) 341 polarimeter. ECD spectra were recorded on a JASCO J-810 spectropolarimeter. Electrospray Quadrupole Time-of-Flight HRMS measurements were performed with a MicroTOF-Q type QqTOF MS instrument equipped with an ESI source from Bruker (Bruker Daltoniks, Bremen, Germany).

Chiral HPLC separation and ECD measurement: Separation of the enantiomers of **3n-p** and **5a-Ph** were performed on a JASCO HPLC system with Chiralpak-IA column (3 μ m, 250 $\times$ 4.6 mm, hexane/dichloromethane or hexane/2-propanol eluent, 1 mLmin $^{-1}$ flow rate) and HPLC-ECD spectra were recorded in stopped-flow mode on a JASCO J-810 spectropolarimeter equipped with a 10 mm HPLC flow cell. ECD ellipticity (ϵ) values were not corrected for concentration. For an HPLC-ECD spectrum, three consecutive scans were recorded and averaged with 2 nm bandwidth, 1 s response, and standard sensitivity. The HPLC-ECD spectrum of the eluent recorded in the same way was used as background. The concentration of the injected sample was set so that the HT value did not exceed 500 V in the HT channel down to 230 nm.

MTT assay: WM35 and A2780 cells were cultured in RPMI1640 Medium (ThermoFisher, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, ThermoFisher) and antibiotics penicillin and streptomycin (Pen-Strep, ThermoFisher), whereas HaCaT keratinocytes were cultured in DMEM medium (ThermoFisher) with the same supplementation. The number of viable cells was indirectly determined by measuring the conversion of the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich] to formazan by mitochondrial dehydrogenases. Cells were plated in 96-well multi-titer plates (10,000 cells per well density) in quadruplicates and were cultured for 3 days and treated by the compounds daily. Negative control group was treated with equal amount of vehicle solvent (DMSO) (green line on graphs) and the positive control group was treated with 1 μ g/ml doxorubicin (blue line on graphs). Cells were then incubated with 5 mg/ml MTT for 3 hours, precipitated formazan crystals were dissolved in acidic isopropanol (10% 1 M HCl in isopropanol supplemented with 10% Triton X 100) and concentration of formazan was assessed colorimetrically by measuring absorbance at 565 nm.

Determination of IC_{50} : Logistic dose-response curves were fitted using the equation $y = A_2 + (A_1 - A_2)/(1 + (x/x_0)^p)$ where

the parameters are the following: A1: initial value ($y_{\min}$), A2: final value ($y_{\max}$), x0: center (EC/IC₅₀) and p is the calculated power. Fittings were carried out and parameters were calculated using Origin 8.6 (OriginLab Corporation, Northampton, MA, USA). Data are presented as Mean $\pm$ SEM, N=4 at each data point.

Computational methods: For the generation of the initial conformers, mixed torsional/low-frequency mode conformational searches were carried out by means of the MacroModel 10.8.011 software using the Merck Molecular Force Field (MMFF) with an implicit solvent model for CHCl₃.^[33] The geometry re-optimizations of the initial conformers, transition state calculations, DFT-NMR and TDDFT-ECD calculations were performed with the Gaussian 09 software package.^[34] Visualization of the results was performed by the MOLEKEL software package.^[35]

TDDFT-ECD calculations: The geometry re-optimizations were performed at the CAM-B3LYP/TZVP^[36] PCM/CHCl₃ and the ω B97X/TZVP^[37] PCM/MeCN levels, while the ECD calculations were performed at the B3LYP/TZVP, BH&HLYP/TZVP, CAM-B3LYP/TZVP and PBE0/TZVP levels with the same solvent model as for the preceding DFT optimization step. ECD spectra were produced as the sum of Gaussians with 3000 and 3600 cm⁻¹ half-height widths using dipole-velocity computed rotational strengths.^[38]

DFT-NMR calculations: The geometry re-optimizations were performed at the B3LYP/6-31 + G(d,p) level, while the chemical shifts were calculated at the mPW1PW91/6-311 + G(2d,p)^[39] with the GIAO method.^[40] Computed ¹³C NMR chemical shift data were corrected for DMSO with I=185.2853 and S=−1.0267.^[41]

Mechanistic studies: Geometry optimizations of the minima and transition states were performed at the B3LYP/6-31G(d), CAM-B3LYP/6-31G(d) and M06-2X/TZVP^[42] levels. In most cases, 2D scans of the potential energy surface of the reaction were used to find the region of the TSs with a 0.1 Å step size, refined by 0.05 or 0.02 Å steps around the supposed TS if it was necessary. TS calculations were started from the points of the TS region of the surface. In some cases, assumed bond lengths were set to start the TS calculations. The nature of the obtained stationary points was verified by making sure that the Hessian had no negative eigenvalues for minima, and a single one for transition states. The identified TSs were examined and confirmed by IRC calculations. Relative enthalpies (ΔH), entropies (ΔS), and Gibbs free energies (ΔG) were calculated with standard statistical thermodynamics at 298.15 K. For the mechanism described in Scheme 2, the relative energies were normalized with the computed DFT energy values of pyridine or pyridinium cation to estimate the effect of the buffer and to have the same net charges throughout the multistep cyclization mechanism.

DFT re-optimizations for comparison with the NOE measurements: The geometry re-optimizations were performed at the B3LYP/6-31 + G(d,p), B3LYP/TZVP PCM/CHCl₃, CAM-B3LYP/TZVP PCM/CHCl₃ and the B97D/TZVP PCM/CHCl₃ levels of theory.

Crystal and molecular structure analysis: X-ray quality crystals were grown by slow evaporation of the solvent from a saturated

solution of the sample in acetone/chloroform for **2f-O**, DMSO/acetone for **2w**, hexane/dichloromethane for **2x** or toluene/methanol for **4a**. A suitable crystal was fixed under microscope onto a Mitegen MicroMesh loop using high-density oil. Diffraction intensity data collection was carried out using a Bruker-D8 Venture diffractometer equipped with INCOATEC I μ S 3.0 dual (Cu and Mo) sealed tube micro sources and Photon II Charge-Integrating Pixel Array detector (for **2f-O**, **2w** and **2x**) or an Enraf Nonius MACH3 diffractometer (for **4a**) using Mo K α (λ =0.71073 Å) (for **2w**, **2x** and **4a**) or Cu K α (λ =1.54178 Å) (for **2f-O**) radiation. The structures were determined using room temperature data collection. High multiplicity data collection and integration was performed using the APEX3 (Ver. 2017.3-0, Bruker AXS Inc., 2017.) software. Data reduction and multi-scan absorption correction was performed using SAINT (Ver. 8.38A, Bruker AXS Inc., 2017). The structures could be solved using direct methods and refined on F2 using the SHELXL program^[43] incorporated into APEX3 suite. Refinement was performed anisotropically for all non-hydrogen atoms. Hydrogen atoms were placed into geometric positions. The CIF files were merged and manually edited using the PubCif software.^[44] Results of X-ray diffraction structure determinations were good according to the Checkcif of PLATON software^[45] and structural parameters such as bond length and angle data are in the expected range. The racemates crystallized in the centrosymmetric triclinic space group of P-1 for **2x**, monoclinic P2₁/c for **2w**, monoclinic P2₁ for **2f-O** and monoclinic P2₁/a for **4a**. The crystallographic and refinement details can be seen in Tables S12, S14, S17 and S20, while geometric parameters are compiled in Tables S13, S15, S18 and S21. The structures of **2w** and **2x** are also stabilized with weak C–H–O intermolecular hydrogen bonds (Table S16 and S19).

General Procedures for the Domino Knoevenagel-Cyclization Reactions

Method A: In a round-bottom flask, 2*H*-chromene derivatives **1a–d** (100 mg) and barbiturates **6a–b** (1.5 equivalent) were dissolved in toluene (5 ml), and the solution was refluxed for three hours. The solution was then allowed to cool to room temperature, the precipitate filtered and washed with cold ether. If no precipitate formed, the solution was concentrated *in vacuo* and purified by column chromatography (hexane/ethyl acetate eluent).

Method B: In a round-bottom flask, 2*H*-chromene derivatives **1a–d** (100 mg) and active methylene compound **6c–g** (1.2 equivalent) were dissolved in toluene (5 ml). Piperidine (1 equivalent) was added to the solution, and it was refluxed for three hours. The solution was then allowed to cool to room temperature, the precipitate filtered and washed with cold ether. If no precipitate formed, the solution was concentrated *in vacuo* and purified by column chromatography (hexane/ethyl acetate eluent).

Method C: In a round-bottom flask, 2*H*-chromene derivatives **1a–d** (100 mg) and active methylene compound **6s–y** (1.2 equivalent) were dissolved in ethanol (3 ml). Piperidine (1 equivalent) and acetic acid (1 equivalent) were added to the solution, and it was refluxed for five hours. The solution was then allowed to cool to room temperature, the precipitate filtered and washed with cold ether. If no precipitate formed,

the solution was concentrated *in vacuo*, and purified by column chromatography (hexane/ethyl acetate eluent).

Method D: In a round-bottom flask, 2*H*-chromene derivatives **1a–d** (100 mg) and nitro derivative **6h–q** (1.2 equivalent) were dissolved in ethanol (3 ml). Piperidine (1 equivalent) and acetic acid (1 equivalent) were added to the solution, and it was refluxed for five hours. The solution was then allowed to cool to room temperature, the precipitate filtered and washed with cold ether. The filtrate was concentrated *in vacuo*, and purified by column chromatography (hexane/acetone eluent).

Method E: In a round-bottom flask, chroman derivative **2e–f** (100 mg), active methylene reagent **6a–c**, **6e**, **6i** or **6v** (1.2 equivalent), and dimethylamine hydrochloride (1.5 equivalent) were dissolved in toluene (5 ml), and the solution was refluxed for five hours. The solution was then allowed to cool to room temperature, the precipitate filtered and washed with cold ether. If no precipitate formed, the solution was concentrated *in vacuo*, and purified by column chromatography (hexane/ethyl-acetate eluent).

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