RSC Advances

PAPER

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Cite this: *RSC Adv.*, 2016, **6**, 115058

Reduction of Biginelli compounds by $LiAlH_4$: a rapid access to molecular diversity†

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Received 2nd October 2016
Accepted 25th November 2016

DOI: 10.1039/c6ra24535h

www.rsc.org/advances

Herein, the reduction of Biginelli compounds by $LiAlH_4$ was investigated for the first time. The reduction of urea-derived dihydropyrimidinones yielded 80–95% of hydrogenolysis products (4-aryl-5-(m)ethyl-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones; 11 examples), while the reduction of *N*-1-methylated Biginelli compounds gave the corresponding alcohols (4-aryl-5-((1-)hydroxy(m)ethyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones; 4 examples) in 70–80% yield. The obtained alcohols could be readily dehydrated to vicinal bis(*exo*-methylene) derivatives (4-aryl-1-methyl-5-(m)ethylene-6-methylene-tetrahydropyrimidin-2(1*H*)-ones; 4 examples) or isomerized to acyclic compounds (1-methyl-3-(1-aryl-2-methylene-3-oxobutyl)ureas; 2 examples) under mildly acidic conditions. The outcome of the reduction also depended on other structural features and reaction conditions such as: urea/thiourea and the type of 1,3-dicarbonyl compound, order of reagent addition, *etc.* $LiAlH_4$-reduction of Biginelli compounds affords a rapid approach to a library of diverse compounds of apparent synthetic utility and possible biological interest. The mechanism of this reduction was discussed and additionally elucidated through deuteration experiments and pK_a measurements.

Introduction

The Biginelli reaction is a one-pot multicomponent reaction allowing the synthesis of functionalized 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) by the condensation reaction of urea, a 1,3-dicarbonyl compound and an aldehyde.[1] The reaction was discovered by Pietro Biginelli in 1883 (ref. 1) and the products are commonly referred to as Biginelli compounds.[2] Only few papers dealing with the reaction were published until the 1970s when the interest in the reaction was slowly renewed.[3] Today, the Biginelli reaction is widely utilized to yield new biologically active compounds and Biginelli products attract equally the attention of both academia and the pharmaceutical industry.[3]

There are several reasons why this is so. First and foremost, DHPMs are known to possess a wide range of therapeutic and pharmacological properties (*e.g.* antiviral, antibacterial, antitumor, *etc.* For a detailed review of biologically active DHPMs, see Kappe).[4] Furthermore, DHPM scaffold was also found to be a structural part of several (biologically active) natural compounds and Biginelli condensation has been employed as a key step in a number of natural product total syntheses (Li, 2005 (ref. 1) and references cited therein). The third reason, not to be overlooked, is the simplicity of the one-pot high-yielding Biginelli protocol with a straightforward workup and purification and readily available starting materials.[3] Biginelli reaction is also quite robust and the reaction tolerates the presence of a variety of functional groups.[1] And the last reason is the variety of the available building blocks – each of the three-components can be significantly varied and this offers a high level of product diversity which is especially useful in combinatorial chemistry.[3] According to SciFinder®, in September 2016, there were more than 70 000 different Biginelli-like products published to date.

Most of the papers published on the reaction after the elucidation of the Biginelli mechanism[5] dealt with the synthetic methodology.[1] Almost all DHPMs reported in the literature were prepared directly using Biginelli reactions; however, many DHPM analogues can be prepared through synthetic functionalization of the dihydropyrimidinone core. Each of the six centres in the heterocyclic ring can be modified; for an exhaustive literature overview of the synthetic manipulations of Biginelli products, the reader is referred to the review of Singh and Singh.[6] These reactions include manipulations at the C-6 methyl group (such as halogenation), alkylation/acylation at the heteroatoms or modification of the ester group attached at C-5.[6]

Although there were several attempts to reduce Biginelli products, interestingly, they all involved desulfurization; *e.g.* sulfur-containing DHPMs and their *S*-methylated derivative were reduced using RANEY®-Ni to C-2 unsubstituted 1,4-dihydropyrimidines.[7] Reductive dethionation of 3,4-dihydropyrimidin-2-thiones under flow conditions (RANEY®-

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† Electronic supplementary information (ESI) available: The spectral data of the synthesised compounds. See DOI: 10.1039/c6ra24535h

Ni, H_2 at 1–2 bars in acetonitrile) yielded the same products.[8] However, a detailed literature survey revealed a complete lack of research that dealt with the reduction of DHPMs using other reducing agents, even common ones, such as complex metal hydrides. This was very surprising considering that a successful reduction of both the ester and urea units in a chiral DHPM molecule could lead to the formation of chiral diamino alcohols, potentially useful chiral auxiliaries in organic synthesis. Still, no such reaction was published.

In this work, a number of Biginelli compounds were prepared (varying the structure in several ways: the identity of the aromatic ring, the substitution at N-1 atom, urea/thiourea used and the substitution at C-5) and, for the first time, the reactions with several reducing agents (such as lithium aluminium hydride, sodium borohydride, alane and borane) were investigated under different reaction conditions. Interestingly, structural variations of the Biginelli compounds led to the formation of different reduction products significantly broadening the range of available and synthetically useful Biginelli-related compounds. A proposition of the likely mechanisms of $LiAlH_4$ reduction was given and, in some cases, substantiated by isotopic labeling experiments and pK_a measurement.

Results and discussion

Synthesis of Biginelli compounds 1–19

Although several procedures were initially attempted, Biginelli products **1–19** were prepared according to the protocol developed by Jin *et al.* (2002)[9] – *p*-toluenesulfonic acid was employed as the acid catalyst and the expected products were obtained in excellent yields in short reaction times. The crude products precipitated readily from the reaction mixtures and were easily purified by recrystallization (no chromatographic purification was needed). The identity of the compounds was confirmed by GC-MS, IR, UV and 1D and 2D NMR. The obtained experimental data was in accordance with the published spectral data (*cf.* Experimental section). The complete assignment of ^{1}H and ^{13}C NMR signals (verified by HSQC and HMBC experiments) is given in ESI.† Since these compounds were already thoroughly investigated, herein only general features of the NMR spectra of the prepared Biginelli products are summarized: (1) H-7 methyl group protons coupled to H-4 proton with a homoallylic coupling constant of $^5J = 0.6$ Hz (as indicated by the corresponding cross-peaks in ^{1}H–^{1}H COSY spectra); (2) H-4 proton additionally coupled with NH-3 ($^3J = 3.4$ Hz) and appeared as a doublet of quartets. Quartet lines were, however, in most cases, poorly resolved, but clearly visible after irradiation of NH-3 (in a selective ^{1}H homodecoupling experiment); (3) NH-1 and NH-3 protons showed a "W" coupling with ^4J value of around 2.1 Hz (if both present in the molecule); (4) C-2 (urea C=O) displayed a three-bond HMBC correlation with H-4, but it did not show correlations with any of the NH-protons; (5) NH-1 coupled with the upfield double bond carbon (C-5), but did not couple with C-6 *via* two bonds. The Biginelli products were much less soluble in deuterated chloroform when compared to deuterated dimethyl sulfoxide, hence their spectra were mostly recorded in CD_3SOCD_3; the change from CD_3SOCD_3 to $CDCl_3$ led to an upfield shift and broadening of NH protons (*cf.* the spectra of compound **5**). Many of the mentioned features reappeared also in the reduction products (see below), thus facilitating the NMR elucidation of these compounds.

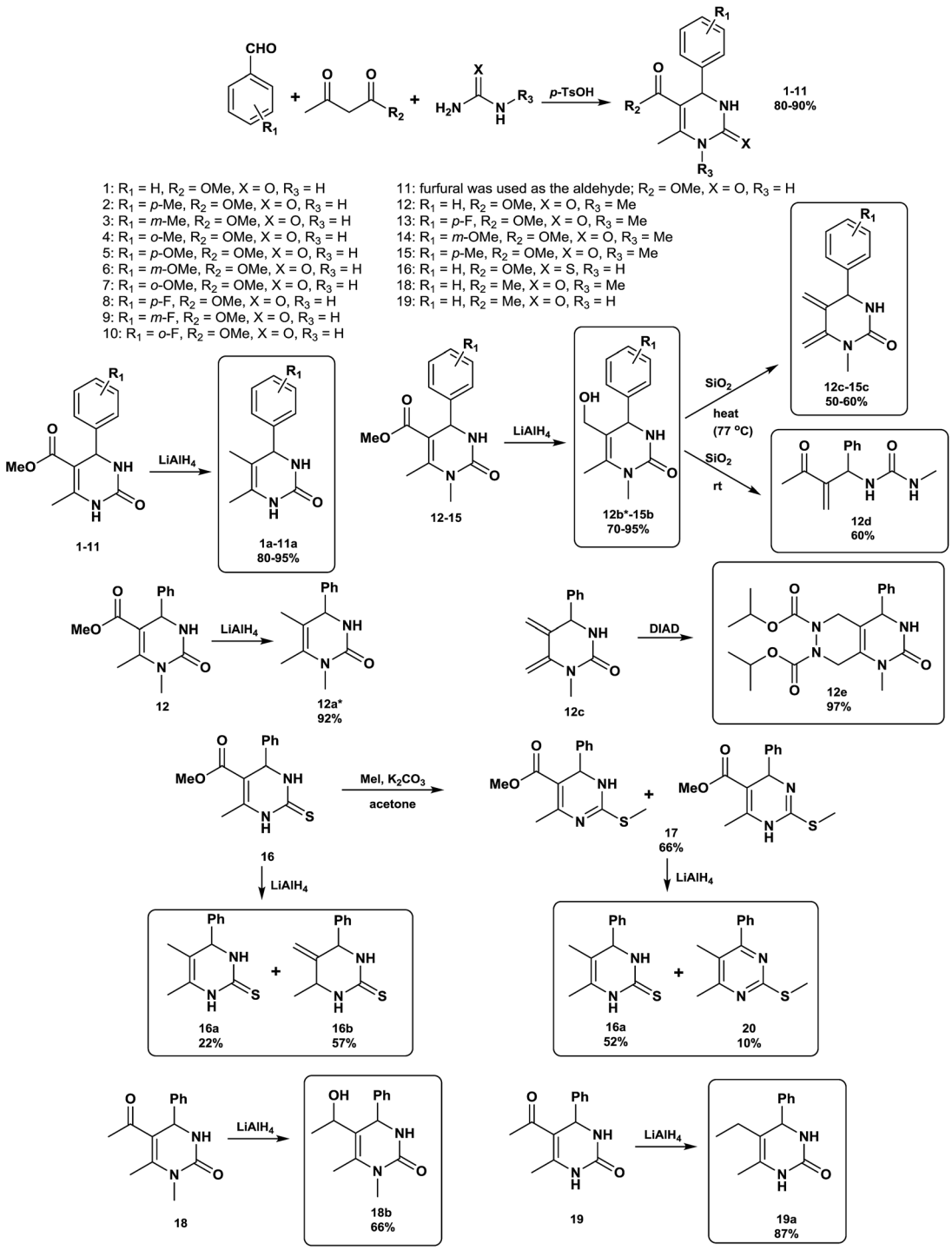
Reductions of Biginelli compounds 1-19 by $LiAlH_4$

A reduction of compounds **1–11** (Biginelli products derived from urea and methyl acetoacetate) by an excess of $LiAlH_4$ in dry refluxing diethyl ether led to an almost quantitative formation of compounds **1a–11a** (Scheme 1). A detailed account of the spectral data interpretation that allowed us to determine the structure of these compounds (and all other mentioned below) are presented in the following section. The observed behaviour is somewhat reminiscent of $LiAlH_4$ reductions of indol-3-yl- or pyrrol-3-yl carbonyl derivatives.[10] For example, 3-formylindole and 3-acetylindole were reduced to 3-methyl- and 3-ethylindoles, respectively.[10]

The outcome of the $LiAlH_4$ reduction of N-1 methylated Biginelli compound **12** was heavily influenced by the order of reactant addition. A dropwise addition of an Et_2O suspension of compound **12** to $LiAlH_4$ slurry yielded exclusively compound **12a** in almost quantitative yield. On the other hand, a slow addition of the reducing agent to the diethyl ether suspension of compound **12**, followed by reflux gave the alcohol **12b** as the major (74% yield) product. This was in agreement with the fact that the deprotonation of the acidic NH group during the $LiAlH_4$ reduction of indole-3-carboxaldehyde is of great importance in the facilitation of the hydrogenolysis step – an attempted reduction of the methylated derivative (1-methyl-1*H*-indole-3-carboxaldehyde) gave only 3-hydroxymethyl-1-methyl-1*H*-indole.[10] In this case, the hydrogenolysis (formation of compound **12a**) occurred to a very minor extent (less than 1% yield). When the same order of reagent addition was applied, compounds **13–15** were reduced to alcohols **13b–15b**. Almost all of the starting material was consumed after the first 30 minutes of reflux and longer reaction times had little effect on the composition and the yield of the products. The reaction also proceeded smoothly at room temperature; albeit with a longer reaction time (*ca.* 5 hours were necessary for completion, *i.e.* for the disappearance of the TLC spot of the starting Biginelli compound).

The reduction of compounds **18** and **19** (Biginelli compounds derived from acetylacetone) was analogous to the reduction of compounds **1–15** described above. N–Me derivative **18** yielded a single diastereomer of the alcohol **18b**. Although we did not ascertain the relative stereochemistry of **18b** from spectral data, due to the lack of useful data from the NOESY spectrum, the *in silico* modelling allowed us to propose the likely relative stereochemistry – *S**, *R**. Compound **19** gave the hydrogenolysis product **19a**.

Diene **12c** was initially isolated *via* a chromatographic separation of the raw product obtained by the $LiAlH_4$ reduction of **12**. It proved difficult to propose a reasonable mechanism for the formation of diene **12c**. We discarded the possibility that the diene formed during the reduction and alkaline reaction



Scheme 1 Access to structurally diverse products (framed compounds) by a $LiAlH_4$ reduction of Biginelli compounds (*$LiAlH_4$ reduction of compound **12** gave either **12a** or **12b** as the major products, depending on the order in which the reagents were added).

workup – ^1H NMR spectrum of the raw unchromatographed product showed no sp^2 proton shifts of the terminal $=CH_2$ atoms and was dominated by the signals of the alcohol **12b**. As it turned out, **12c** formed during the dry loading of the sample to silica gel – a suspension of SiO_2 and the raw reaction product in EtOAc was heated at 77 °C in order to remove the solvent. However, when the crude product was loaded on a SiO_2 column as a CH_2Cl_2 solution, only alcohol **12b** eluted from the column. This was additionally confirmed when the alcohols **12b–15b** were refluxed in EtOAc in the presence of silica gel and compounds **12c–15c** were obtained in high yields (50–60%). This turned out to be a very convenient method for the preparation of dienes **12c–15c**, as other attempts to dehydrate alcohols **12b–15b** using catalytic amounts of strong acids (*p*-TsOH,

BF_3, HCl) failed to give any product. It is also important to note that alcohol **15b** (containing a MeO– group on the aromatic ring in the position 4) was much less stable than compounds **13b** and **14b** – these two compounds decomposed significantly over the course of several days at room temperature but were perfectly stable when kept in a freezer at −20 °C. Some degradation of **15b** was observed in the NMR tube after only few hours of recording in acid-free $CDCl_3$.

Interestingly, when the dehydration of **12b** and **15b** was attempted at room temperature, a completely different outcome was noted. An isomerization took place that led to the fission of the dihydropyrimidinone ring and formation of an acyclic conjugated ketone **12d**. Compounds **12d** and **15d** were isolated in *ca.* 60% yield after column chromatography on SiO_2, but their solutions in $CDCl_3$ were stable for some 24 h before substantial degradation occurred.

The reduction of the Biginelli product (**16**) derived from thiourea yielded two major isomeric products (**16a** and **16b**), differing only in the position of the double bond. The expected *endo*-alkene (**16a**) amounted to only half of the yield of the *exo*-alkene (**16b**), while the total yield of the two alkenes was relatively high (almost 80%). This result revealed a profound effect of the interchange of C=O for C=S on the outcome of the $LiAlH_4$ reduction. We extended the investigation of the scope of this reaction by reducing *S*-methylated **17** (two compounds existing in tautomeric equilibrium). The major isolated product, formed by demethylation, ester group reduction and hydrogenolysis, was compound **16a**. A side product of the reaction was identified as a pyrimidine derivative **20** (4,5-dimethyl-2-(methylthio)-6-phenylpyrimidine).

Diene **12b** reacted readily at room temperature with diisopropyl azodicarboxylate producing a Diels–Alder adduct **12e** (Scheme 1) in almost quantitative yield (97%). This reaction demonstrates the reactivity and possible utility of the dienes further enlarging the chemical diversity of organic compounds that are accessible from Biginelli products.

Table 1 ^{13}C chemical shifts (ppm) of compounds **1a–11a** in $CDCl_3$ or CD_3SOCD_3 (at 100.6 MHz)

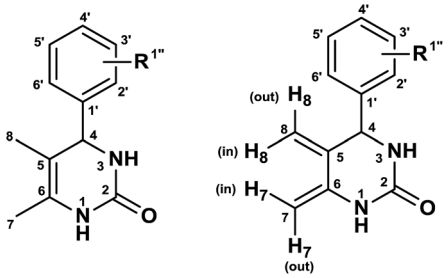
Assignment	**1a** ($CDCl_3$)	**2a** ($CDCl_3$)	**3a** ($CDCl_3$)	**4a** ($CDCl_3$)	**5a** ($CDCl_3$)	**6a** ($CDCl_3$)	**7a** ($CDCl_3$)	**8a** ($CDCl_3$)	**9a** ($CDCl_3$)	**10a** ($CDCl_3$)	**11a** (CD_3SOCD_3)
C-2	153.6	153.7	154.2	153.8	154.0	153.4	154.4	154.1	153.8	154.3	153.0
C-4	61.8	61.4	61.6	58.4	61.0	61.7	54.1	60.8	61.1	53.8	53.1
C-5	102.8	102.8	102.5	101.9	102.8	102.6	101.1	102.4	102.1	101.1	98.6
C-6	125.2	125.2	125.5	126.3	125.3	125.4	127.5	125.8	126.0	126.9	127.3
C-7	15.7	15.6	15.5	15.5	15.5	15.7	15.6	15.5	15.6	15.5	14.8
C-8	14.3	14.3	14.3	14.1	14.3	14.3	14.8	14.3	14.3	14.2	14.0
C-1′	143.2	140.4	143.4	140.1	135.6	144.8	129.3	139.2	145.9	129.7	155.7
C-2′	127.0	127.0	127.7	135.6	128.2	113.2	157.1	128.7	114.0	160.2	105.7
C-3′	128.8	129.4	138.4	130.6	114.0	160.0	110.4	115.6	163.1	115.5	110.1
C-4′	128.1	137.7	128.5	128.3	159.0	112.8	120.8	162.4	115.0	129.5	142.1
C-5′			128.7	126.7		129.8	127.5		130.3	124.7	
C-6′			124.2	127.8		119.4	129.0		122.7	128.8	
C-1″		21.1	21.4	19.0	55.3	55.3	55.3				

Table 2 ^{1}H chemical shifts (ppm) of compounds **1a–11a** in $CDCl_3$ or CD_3SOCD_3 (at 400 MHz)

Assignment	**1a** ($CDCl_3$)	**2a** ($CDCl_3$)	**3a** ($CDCl_3$)	**4a** ($CDCl_3$)	**5a** ($CDCl_3$)	**6a** ($CDCl_3$)	**7a** ($CDCl_3$)	**8a** ($CDCl_3$)	**9a** ($CDCl_3$)	**10a** ($CDCl_3$)	**11a** (CD_3SOCD_3)
H-2	^{13}C										
H-4	4.77	4.78	4.74	5.12	4.74	4.75	5.16	4.74	4.76	5.19	4.69
H-5											
H-6											
H-7	1.80	1.79	1.81	1.81	1.81	1.80	1.87	1.78	1.80	1.83	1.67
H-8	1.44	1.43	1.45	1.39	1.44	1.44	1.56	1.41	1.44	1.51	1.47
H-1′	7.27–7.39 (5H)		7.03–7.14 (4H)	7.11–7.20 (4H)		6.80–6.95 (4H)			6.90–7.10 (4H)		
H-2′		7.20			7.23			7.25			6.18
H-3′		7.17			6.88		6.88	7.00		7.05	6.37
H-4′							7.14			7.35	7.57
H-5′							6.92			7.16	
H-6′							7.25			7.27	
H-1″		2.34	2.36	2.38	3.82	3.80					
NH-1	6.10	6.78	7.51	—	7.18	6.43	6.59	7.47	7.20	7.27	8.02
NH-3	4.99	5.17	5.49	5.60	5.36	5.09	5.28	—	5.53	5.34	7.01

Structural elucidation of $LiAlH_4$-reduction products of Biginelli compounds

Compounds 1a–11a. As inferred from their MS, their molecular weights were 44 amu lower than the starting dihydropyrimidinones. The proton spectra of the products showed much similarity to the ^{1}H NMR spectra of the starting Biginelli compounds. Namely, the only striking change was the loss of the ester MeO group (H-9 at *ca.* 3.50 ppm for the Biginelli compounds). This signal was replaced by another methyl group at around 1.50 ppm. All other proton signals were accounted for with only slight differences in their chemical shifts (Tables 1 and 2, see Scheme 2 for the numbering system that was used in this work). The ester carbonyl signal was missing from the ^{13}C NMR spectra and the corresponding IR band, as well. The urea portion of the molecule turned out to be resistant to $LiAlH_4$ as the ^{13}C resonance of C-2 could still be observed following the reduction. The proton shift value of the newly appeared methyl group (H-8) indicated that it was attached to a double bond – this was corroborated by the observed HMBC correlations. H-8 correlated not only with the double bond carbons (C-5 and C-6) but also with C-4, thus implying that the reduction led to a deoxygenation of the ester group and the formation of **1a–11a**. In this way, we concluded that $LiAlH_4$ reduction of urea-acetoacetate derived Biginelli compounds yielded 4-aryl-5,6-dimethyl-3,4-dihydropyrimidin-2(1*H*)-ones.



Scheme 2 A numbering scheme used in the NMR assignment.

Compounds 12a–d. The reduction of *N*-methyldihydropyrimidinone **12** gave two products. The identity of the minor product **12a** (yield = 14%) was determined in a straightforward manner since the structure of this compound was analogous to the structures of **1a–11a**. The additional MeN– signal at δ 3.18 replaced the signal of the proton attached to N-1 which was usually found around δ 6.10 in **1a–11a**. The major product of the reaction (yield = 74%) turned out to be the alcohol **12b**. Two diastereotopic protons appeared at δ 4.22 and 3.52, and both were coupled to the OH– proton (δ 4.65, dd, J = 6.3, 4.5 Hz, CD_3SOCD_3). Dehydration of alcohol **12b** gave the diene **12c**, containing two *exo* double bonds. NOESY spectrum proved very useful for the shift assignment of the four =CH_2 protons. The observed nOe cross-peak between NMe protons (at δ 3.16) and the doublet (J_{gem} = 1.5 Hz) at δ 4.22 allowed the assignation of this signal to H-7_{out} (see Table 3). This signal was split by H-7_{in}, which showed a nOe cross-peak to H-8_{in}. H-8_{in} coupled geminally to H-8_{out} and both of these protons coupled additionally

Table 3 ^{1}H and ^{13}C chemical shifts (ppm) of compounds **12c–15c** in $CDCl_3$ (at 400 and 100.6 MHz respectively)

	12c ($CDCl_3$)		**13c** ($CDCl_3$)		**14c** ($CDCl_3$)		**15c** ($CDCl_3$)	
Assignment	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
C-2	153.9		153.7		153.8		153.9	
C-4	58.2	5.00	57.5	5.01	58.1	4.98	57.9	4.98
C-5	139.4		139.3		139.3		138.0	
C-6	142.9		142.7		142.9		140.3	
C-7	89.2	4.20 (H-7_{OUT}, d, J = 1.5 Hz) 4.57 (H-7_{IN}, d, J = 1.5 Hz)	89.4	4.24 (H-7_{OUT}, d, J = 1.5 Hz) 4.59 (H-7_{IN}, d, J = 1.5 Hz)	89.2	4.21 (H-7_{OUT}, d, J = 1.5 Hz) 4.58 (H-7_{IN}, d, J = 1.5 Hz)	89.1	4.20 (H-7_{OUT}, d, J = 1.5 Hz) 4.55 (H-7_{IN}, d, J = 1.5 Hz)
C-8	113.3	4.84 (H-8_{OUT}, d, J = 0.7 Hz) 5.57 (H-8_{IN}, d, J = 0.7 Hz)	113.4	4.84 (H-8_{OUT}, d, J = 0.6 Hz) 5.59 (H-8_{IN}, d, J = 0.6 Hz)	113.4	4.80 (H-8_{OUT}, d, J = 0.7 Hz) 5.57 (H-8_{IN}, d, J = 0.7 Hz)	113.2	4.81 (H-8_{OUT}, d, J = 0.7 Hz) 5.55 (H-8_{IN}, d, J = 0.7 Hz
C-1′	139.9	7.27–7.40 (5H)	135.6 (d, J = 3.0 Hz)		141.5	6.80–6.95 (4H)	139.6	
C-2′	126.6		128.3 (d, J = 8.1 Hz)	7.28 (2H)	113.5		126.5	7.17 (4H)
C-3′	128.8		115.8 (d, J = 21.9 Hz)	7.08 (2H)	160.0		129.5	
C-4′	128.1		162.5 (d, J = 247.0 Hz)		112.3		136.8	
C-5′					129.9			
C-6′					118.9			
C-1″					55.3	3.80	21.1	2.34
N–Me	30.4	3.16	30.4	3.18	30.4	3.17	30.4	3.17
NH-3		—		5.38		5.30		5.24

through four bonds with H-4. The position of the two double bonds was also concluded from the HMBC spectrum: protons attached to C-8 correlated with C-6 and protons attached to C-7 correlated with C-5. Due to a delocalization of the nitrogen electron pair, C-7 signal was significantly shifted upfield by almost 25 ppm compared to C-8. Isomerization of **12b** gave the acyclic compound **12d**. A ketone carbon (δ 199.7) showed HMBC correlations with C-7 methyl protons and two protons at sp^2 C-8 (see Scheme 2 for numbering scheme). H-8 protons coupled with C-4, H-4 coupled with NH-3 ($J = 8.4$ Hz), the urea carbon C-2 coupled to H-1 protons, while NH-1 and NH-3 showed a correlation in the NOESY spectrum. These allowed us to assign the structure of **12d** as that of 1-methyl-3-(2-methylene-3-oxo-1-phenylbutyl)urea.

Compounds 16a–b. Two products were isolated following the reduction of dihydropyrimidinethione **16**. Product **16a** (obtained in 22% yield) was straightforwardly identified since its NMR spectra contained the same features as compounds **1a–11a**. The main reaction product **16b** (yield: 57%), although isomeric to **16a**, was found to contain a single *exo*-methylene group (two sp^2 protons, δ_H 5.15 and 4.68, attached to the same carbon, δ_C 113.2). The exact position of the double bond was established based on the analysis of its HMBC spectrum: protons attached to the double bond correlated, across three bonds, with both CH carbons at δ 61.0 (C-4) and at δ 51.5 (C-6), which was only possible if the double bond formed between C-5 and C-8. It is interesting to note that, except for the vicinal coupling between H-7 methyl group and H-6 proton, $^3J = 6.6$ Hz, and within the phenyl group, all other coupling constants in the proton spectrum were equal in value – the geminal coupling of H-8 protons (pro-E and pro-Z), allylic couplings between H-6 and both H-8, as well as H-4 and both H-8, and a "W" coupling between H-4 and H-6 had the same value of the constant – $J = 1.5$ Hz. The mentioned "W" coupling can only exist if the relative stereochemistry of the formed 4-methyl-5-methylene-6-phenyltetrahydropyrimidine-2(1*H*)-thione was 4*S**, 6*R**, *i.e.* if the methyl and phenyl groups were on the same side of the heterocyclic ring.

Methylation of compound **16** gave an equilibrating mixture of *S*-methylated tautomers **17** (methyl 6-methyl-2-(methylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate, and methyl-4-methyl-2-(methylthio)-6-phenyl-1,6-dihydropyrimidine-5-carboxylate). $LiAlH_4$ reduction of the mixture yielded two compounds, **16a** and **20**; the chemical shifts of carbons C-2, C-4, C-5 and C-6 were characteristic for a pyrimidine derivative[11] and the connectivity was confirmed using HMBC and NOESY correlations of the three present methyl groups. The methyl group attached to sulfur showed only one CH coupling (to C-2), H-7 coupled with C-5 and C-6 (through three and two bonds, respectively), while H-8 similarly showed correlations with C-4 and C-6 (through three bonds) and C-5 (through two bonds). These spectral data led us to assign the structure of 4,5-dimethyl-2-(methylthio)-6-phenylpyrimidine to **20a**.

Acidity of Biginelli compounds (compound 1)

The observed drastic difference in the $LiAlH_4$-reduction of the N-1 methylated and non-methylated Biginelli compounds pointed to the importance of the H-atom at N-1. Since being unavailable in the literature, the acidity constant of a urea-derived (N-1 unsubstituted) Biginelli compound (**1**) was determined for the first time in this work. UV spectra of compound **1** in the pH region 13.20–13.59 is shown on Fig. 1. A clearly visible isosbestic point means that the interconversion between compound **1** and its conjugated base was the only process occurring in the solution, and served to confirm the validity of the spectrophotometric method for pK_a measurement. The acidity constant value was calculated according to transformed forms of the classical spectrophotometric equation (see Experimental section for more details). The determined value (pK_a = 13.52) showed a dramatic influence of additional conjugation (within the 3-aminoacrylate moiety) on the acidity of NH-1 proton; when compared to unsubstituted urea [pK_a(urea) = 27], the acidity of NH increased by a factor of 10^{13}.

Mechanism of the reactions

To investigate the proposed mechanism, we also performed deuterium labeling experiments. The reductions of compounds **1** and **12** were carried out using lithium aluminium deuteride (see Scheme 3). Carbon C-8 in compound **1** was completely deuterated, as inferred from the appearance of a septuplet at 13.5 ppm in the ^{13}C NMR spectrum, disappearance of the H-8 signal and simplification of H-7 and H-4 multiplets (in addition to an isotope shift). The number of incorporated deuterium atoms and their position was corroborated by the *m*/*z* value of the molecular ion and from the occurrence of $[M - CD_3]^+$ fragment ion in the mass spectrum, respectively. Similarly, the presence of a quintet at 58.6 ppm indicated that two deuterium atoms were bonded to C-8 carbon during the $LiAlD_4$ reduction of compound **12**. From these experiments, we can conclude that the transformations (given in Schemes 3–5) consist of the following steps:

(1) In the first step, $LiAlH_4$ attacks the ester/ketone carbonyl group of the Biginelli compound. This step is predated by the deprotonation of the relatively acidic (pK_a = 13.52, as we determined in this work) NH-1 nitrogen atom, if the Biginelli compound is derived from urea. This deprotonation decreases the electrophilicity of the ester/ketone carbonyl as evidenced from the failed attempts to hydrolyse compound **1** by a strong

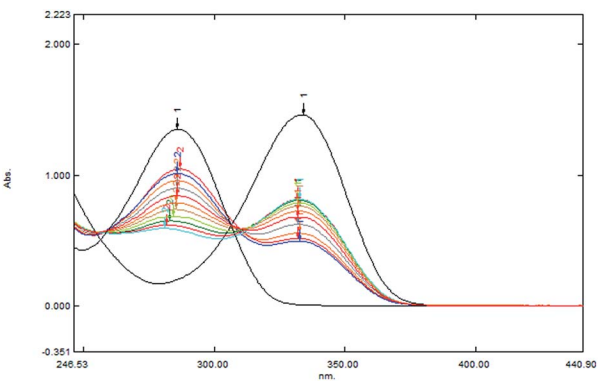
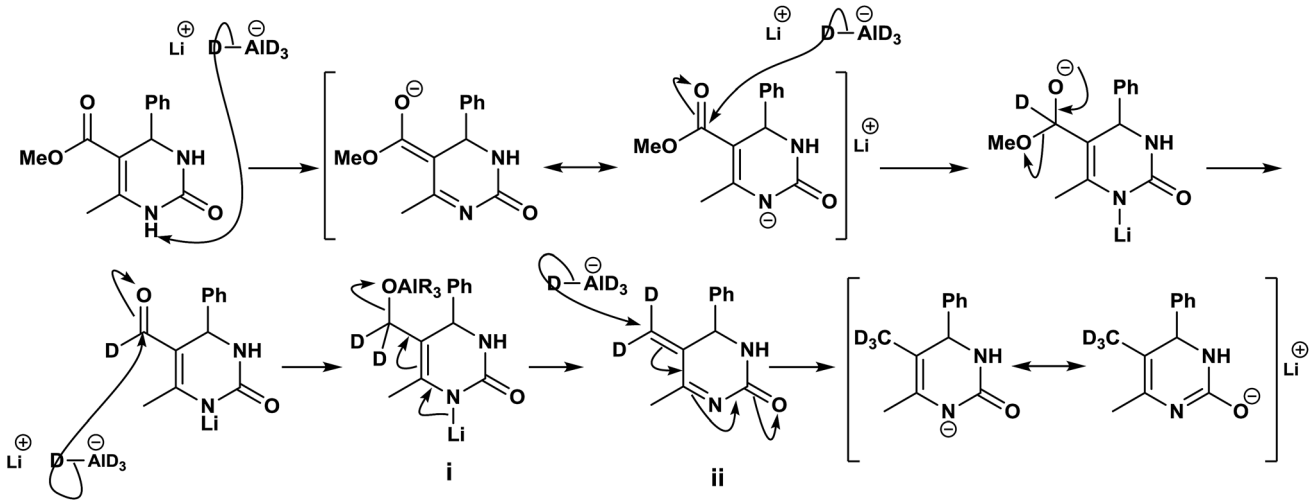


Fig. 1 UV spectra of compound **1** in the pH region 13.20–13.59.



Scheme 3 A proposed mechanism of $LiAlD_4$ reduction of compound **1** (and other Biginelli compounds lacking N-1–Me) leading to deoxygenated products **1a–11a**.

alkali (as opposed to facile hydrolysis of the *N*-methylated counterpart).[6]

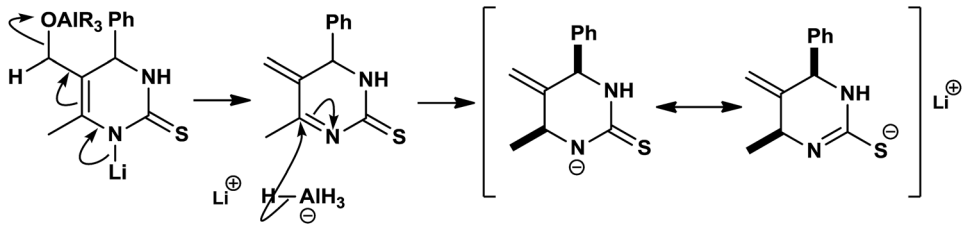
(2) The generated tetrahedral intermediate collapses to an aldehyde which is attacked by the second hydride equivalent giving the alkoxide (more precisely aluminate) species **i** in Scheme 3. In the case of compounds **18** and **19**, this intermediate is formed in step 1. For N-1 methylated Biginelli derivatives, the reaction ends here, and, after aqueous alkaline work-up, alcohols **12b–15b** were isolated.

(3) In the case of urea-derived Biginelli compounds, formally, the free electron pair from the negatively charged nitrogen atom (formed in step 1) expels (*via* C-5–C-6 double bond) the leaving group at C-8 (presumably an aluminate) from the molecule and intermediate **ii** is formed. Intermediate **ii** (a conjugated imine) is expected to be highly electrophilic (at positions C-6 and C-8) due to additional linear conjugation with the carbonyl group, C-2.

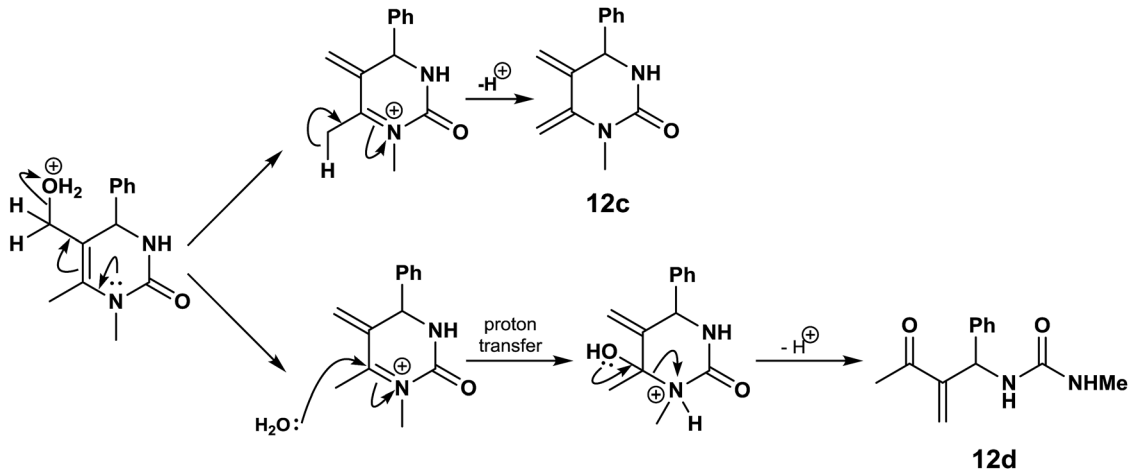
(4) Products **1a–11a** are formed by the attack of a third hydride equivalent on the conjugated imine **ii** in a Michael-type manner, followed by protonation at N-1 nitrogen during the reaction work-up. An alternative mechanism is also possible: a hydride attack at C-6 of intermediate **ii** would lead to the formation of an *exo* double bond and the resulting compound could then isomerize to the more stable product **1a** during the work-up. This potential mechanism was, however, discarded. $LiAlD_4$ reduction of **1** gave exclusively the product with completely deuterated C-8 atom. If the two proposed mechanisms were actually competing, deuteration percentage on C-8 would be less than 100% (since a proton (protium) from H_2O would bind at C-8 during the work-up).

(5) The mechanism for the formation of **16b** includes a direct hydride nucleophilic attack at the imine carbon, C-6, of intermediate **ii** (see Scheme 4). The observed stereoselectivity (hydride predominantly attacks from the less hindered face of the heterocyclic ring, opposite to the phenyl/aryl group) confirms that **14b** forms directly by the nucleophilic addition to C=N. The difference in the regiochemistry of the hydride attack appears to have its origin in the lack of the extended delocalization in the thiourea derived Biginelli compounds (sulfur carrying negative charge as opposed to oxygen carrying it).

(6) Heating of the alcohol **12b** in the presence of weakly acidic silica gel led to dehydration; however, at room temperature ring opening isomerization occurred. The formation of compounds **12c** and **12d** most likely involves the same intermediate – conjugated iminium ion (see Scheme 5). Higher temperature gave the entropy-favoured diene **12c** and at room temperature **12d** was formed probably as a thermodynamically-favoured product (conjugated ketone).



Scheme 4 A proposed mechanism of $LiAlH_4$ reduction of compound **16** (and a thiourea-derive Biginelli compound lacking N-1–Me) leading to compound **16b**.



Scheme 5 A proposed mechanism of the alternative transformation pathways, dehydration (up) and isomerisation (down), of compound **12** (and other alcohols obtained in the reduction of Biginelli compounds possessing a N-1–Me group) leading to compounds **12c** and **12d**, respectively.

Conclusions

$LiAlH_4$-reduction of Biginelli compounds turned out to be a remarkable reaction for molecular diversification. Reduction of urea-derived compounds **1–11** led to the formation of hydrogenolysis products **1a–11a**; the outcome of the reduction of *N*-methylurea-derived Biginelli compounds depended on the order of reactant addition as both hydrogenolysis products (**12a**) or alcohols **12b–15b** could be obtained as the major products. Reduction of thiourea-derived compound **16** showed a different regioselectivity compared to the urea analogues – compound **16b**, containing an *exo* double bond, was isolated as the major product. Either dienes **12c–15c** or an acyclic compound **12d** could be prepared from alcohols **12b–15c**; refluxing in EtOAc in the presence of silica gel resulted in dehydration, while isomerization was favoured at room temperature. The reactions presented in this work proceeded through a common intermediate, conjugated iminium species **ii** (Scheme 5). From this point, hydrogenolysis products **1a–11a**, by the hydride attack in a Michael-type manner, and the *exo*-methylene product **16b**, by the hydride attack at the imine carbon, were formed. The standard ester reduction products, alcohols **12b–14b**, could readily be additionally diversified to an acyclic compound **12d** through an isomerization at room temperature, or to vicinal bis(*exo*-methylene) derivatives **12c–15c** through dehydration at higher temperatures. Access to these reduction products is expected to impact both synthetic (electron-rich dienes **12c–15c** appear to be excellent candidates for Diels–Alder cycloadditions, as demonstrated by the formation of **12e**) and biological (combinatorial) chemistry.

Materials and methods

General

All chemicals were obtained from Sigma-Aldrich (St. Louis, USA), Fluka (Neu-Ulm, Germany) or Carl Roth (Karlsruhe, Germany) and were used as purchased, with the exception of solvents that were additionally dried and purified by conventional methods prior to use. All reactions were performed in oven-dried (120 °C) glassware under an atmosphere of dry nitrogen. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer (Bruker, Fällanden, Switzerland) operating at 400 and 100.6 MHz, respectively.

2D experiments (NOESY, and gradient (edited or non-edited) HSQC, HMBC and ^1H–^1H COSY), as well as DEPT-90, DEPT-135 and selective homonuclear decoupling experiments, were run on the same instrument with the built-in Bruker pulse sequences. NMR spectra were measured at 25 °C in $CDCl_3$ and CD_3SOCD_3 with tetramethylsilane (TMS) as an internal reference. Chemical shifts (δ) are reported in parts per million and referenced to tetramethylsilane ($\delta_H = 0$ ppm) in ^1H NMR spectra and/or to solvent signals (residual $CHCl_3$: $\delta_H = 7.26$ ppm, and $^{13}CDCl_3$: $\delta_C = 77.16$ ppm; CHD_2SOCD_3: $\delta_H = 2.50$ ppm, and $^{13}CD_3SOCD_3$: $\delta_C = 39.52$ ppm) in heteronuclear 2D spectra. Scalar couplings are reported in hertz (Hz). Microanalysis of carbon and hydrogen were carried out with a Carlo Erba 1106 microanalyser (Carlo Erba, Milan, Italy) and their results agreed favourably with the calculated values. UV spectra (in acetonitrile) were measured using a UV-1800 Shimadzu spectrophotometer (Tokyo, Japan). pH values were measured using a HI2020-edge® Multiparameter pH Meter (Woonsocket, Rhode Island, USA). IR measurements (neat, ATR-attenuated total reflectance) were carried out using a Thermo Nicolet model 6700 FT-IR instrument (Waltham, USA). Mass spectra were recorded on a Hewlett Packard 5975B mass selective detector coupled with a Hewlett Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA). The injector and interface were operated at 250 °C and 320 °C, respectively. Oven temperature was raised from 70 °C to 310 °C at a heating rate of 5 °C min^{-1} and then isothermally held for 10 min. As a carrier gas, He at 1.0 mL min^{-1} was used. The samples (1 mg per 1 mL) were injected in a pulsed-split mode (the flow was 1.5 mL min^{-1} for the first 0.5 min and then set to 1.0 mL min^{-1} throughout the rest of the analysis; split ratio, 40 : 1). MS Conditions: ionization voltage,

70 eV, acquisition mass range, 35–650 amu, scan time, 0.32 s. Preparative medium-pressure liquid chromatography (MPLC) was performed with a pump module C-601 and a pump controller C-610 Work-21 pump (Bóchi, Flawil, Switzerland) and was carried out on pre-packed column cartridges (40 mm × 75 mm, silica-gel 60, particle size distribution 40–63 μm, Büchi, Flawil, Switzerland). Precoated Al silica gel plates (Kieselgel 60 F_{254}, 0.2 mm, Merck, Germany) were used for analytical TLC analyses. The spots on TLC plates were readily visualized by UV light (254 nm) and by spraying with vanillin-sulphuric acid reagent (6% vanillin [w/v] and 1% H_2SO_4 [v/v] in ethanol) followed by a short, gentle heating.

Synthesis of dihydropyrimidinones (Biginelli compounds 1–19)

Starting DHPMs were prepared according to Jin *et al.*[9] with the use of *p*-toluenesulfonic acid as the acid catalyst. A mixture of 2 mmol of an aromatic aldehyde (see Scheme 1 for a list of the employed aldehydes), 2 mmol of a dicarbonyl compound (methyl acetoacetate or acetylacetone), 3 mmol of a urea (urea, methyl urea or thiourea) and 50 mg of *p*-toluenesulfonic acid was refluxed in 20 mL of ethanol (95%, v/v) for 2–4 h. Reaction progress was followed by TLC and after the completion of the reaction the reaction mixture was cooled down to 0 °C. In most cases the products precipitated readily, otherwise nucleation was promoted by scratching the surface of the flask with a spatula. The solids were collected by filtration, washed with water and ice-cold ethanol and recrystallized from hot ethanol (95%, v/v). The obtained products (the yields were in the range of 80–90%) were judged pure by TLC, GC-MS and NMR analyses. Complete proton and ^{13}C data for compounds **1–19** (including the complete shift assignment), as well as IR and MS, are given in the ESI.† The obtained spectral data were in general agreement with the literature data.[9]

Methylation of 3,4-dihydropyrimidin-2-(1*H*)-thione (16)

Methylation of 3,4-dihydropyrimidin-2-(1*H*)-thione **16** was performed following the procedure of Wang *et al.*[12] Mixture of compound **16** (1 mmol), methyl iodide (1.1 mmol) and K_2CO_3 (2.0 mmol) was stirred overnight in acetone (10 mL) at room temperature. The mixture was then poured into water (50 mL). The obtained solids were filtered and, after drying, the product turned out to be a mixture (**17**) of methyl-6-methyl-2-(methylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate and its tautomer methyl-4-methyl-2-(methylthio)-6-phenyl-1,6-dihydropyrimidine-5-carboxylate in a ratio of 2.2 : 1. Their spectral data correlated well with the published values.[12,13] The complete NMR data are given in ESI.†

Determination of the acidity constant of the urea-derived Biginelli product (compound 1)

Acidity constant and isosbestic point of compound **1** were determined by UV-VIS spectrophotometry. A stock solution of compound **1** was prepared by dissolving 10.0 mg of compound **1** in 10 mL of CH_3SOCH_3; three solutions containing the same concentration of compound **1** ($c = 1.63 \times 10^{-4}$ M) were then prepared. Solution 1: 2.0 mL of the stock solution were diluted to 50 mL with 10 mM phosphate buffer solution (pH 1.80, 0.1 M NaCl). Solution 2: 2.0 mL of the stock solution were diluted to 50 mL with 0.3 M NaOH. Solution: 2.0 mL of the stock solution were diluted to 50 mL with 5 M NaOH. Solutions 1 and 3 were used to record UV spectra of the solutions in which only compound **1** (solution 1) or its conjugated base (solution 3) existed. Solution 2 was titrated with 10 M NaOH, the pH value of the resulting solution was continuously measured during the titration and UV spectra were recorded after equilibration. pK_a was determined according to the equation: $pK_a = pH_i - \log[(A_{HA} - A_i)/(A_i - A_{A^-})]$, where A_{HA} was the absorbance of solution 1, A_{A^-} was the absorbance of solution 3 and A_i was the intermediate absorbance recorded at 331.5 nm (the wavelength where the difference between the absorbance curves of the solutions 1 and 3 was the greatest).

Lithium aluminium hydride reduction of compounds 1–19

Compounds **1–19** (1 mmol) were suspended in 10 mL of dry diethyl ether at 0 °C and lithium aluminium hydride (5 mmol, 190 mg) was slowly added in the course of 5 min. The mixture was then refluxed for 3 h, cooled to 0 °C and quenched by successive addition of water (1 mL), 15% aqueous (w/v) sodium hydroxide (1 mL), and water (1 mL). The mixture was extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$ and evaporated *in vacuo*. The products (see Scheme 1) were purified by MPLC (ethyl acetate: hexane 1 : 1 [v/v]). Yields of the products, as well as proton and ^{13}C spectral data can be found in Scheme 1 or ESI.† Full assignment of chemical shifts was performed by various 1D and 2D NMR studies.

Reductions of compounds **1** and **12** using lithium aluminium deuteride were performed in an analogous way. Also, several additional $LiAlH_4$ reductions of compound **12** were tried:

(1) Reaction duration was varied between 30 and 90 min to investigate its effect on the yield and the composition of the product; (2) effect of the inverse order in which the reagents were mixed was also studied – a fine suspension of **12** was added in small portions to a $LiAlH_4$ slurry. Otherwise, these reductions followed the general procedure given above.

Dehydration of alcohols 12b–15b

Compounds **12b–15b** (0.05 mmol) were dissolved in 5 mL of ethyl acetate, 5 g of silica gel were added and the slurry was refluxed for 15 min. Afterwards, 5 mL of methanol were added, silica was filtered and washed with 10 mL of MeOH–CH_2Cl_2 (1 : 1 [v/v]). The combined filtrates were evaporated *in vacuo* and the residue was purified by MPLC (ethyl acetate : hexane 1 : 1 [v/v]). Dienes **12c–15c** were obtained as the only products (yield 50–60%).

Isomerization of alcohols 12b and 14b

A mixture of an alcohol (**12b** or **14b**, 0.05 mmol), silica gel (5 g) and dichloromethane (5 mL) was stirred at room temperature for 48 h. The mixture was then diluted with 5 mL of MeOH, filtered and the solvent removed *in vacuo*. The resulting solid was purified by MPLC using pure EtOAc as the eluent.

Acyclic compounds **12d** and **14d** were isolated as the only products (*ca.* 60%).

Diels–Alder reaction of diene 12c with diisopropyl azodicarboxylate (DIAD)

Diene **12c** (0.1 mmol, 21.4 mg) and diisopropyl azodicarboxylate (0.3 mmol, 60.7 mg) were stirred in dichloromethane/diethyl ether mixture (1 : 3, v/v, 2 mL) until TLC showed a complete consumption of the starting material (1 hour). The reaction was also monitored by NMR and the reaction yield (97%) was calculated by qNMR analysis (the yield was calculated by internal standardization). NMR spectrum of the crude product showed the presence of several diastereotopic, slowly interconverting conformers of the adduct **12e**, and one of these was successfully isolated by MPLC in pure form (ethyl acetate was used as the eluent). The obtained spectral data are given in ESI.†

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of Serbia [Project no. 172061]. This study is a part of the Ph.D. thesis of Dragan B. Zlatković under the supervision of Niko S. Radulović.

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