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Synthesis and Reactivity of Spirocarbocycles as Scaffolds for Nucleoside Analogues

Jonas Verhoeven,* Xavier Deraet, Vineet Pande, Weimei Sun, Mercedes Alonso, Frank De Proft, Lieven Meerpoel, Jan Willem Thuring, and Guido Verniest

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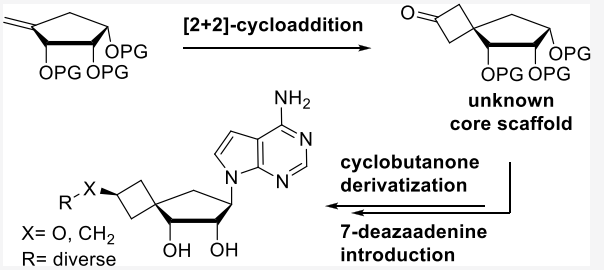
ABSTRACT: A novel class of substituted spiro[3.4]octanes can be accessed via a [2 + 2]-cycloaddition of dichloroketene on a readily prepared *exo*-methylene cyclopentane building block. This reaction sequence was found to be robust on a multigram scale and afforded a central spirocyclobutanone scaffold for carbocyclic nucleosides. The reactivity of this constrained building block was evaluated and compared to the corresponding 4′-spirocyclic furanose analogues. Density functional theory calculations were performed to support the observed selectivity in the carbonyl reduction of spirocyclobutanone building blocks. Starting from novel spirocyclic intermediates, we exemplified the preparation of an undescribed class of carbocyclic nucleoside analogues and provided a proof of concept for application as inhibitors for the protein methyltransferase target PRMT5.



INTRODUCTION

The term carbocyclic nucleoside is used to refer to structural analogues of naturally occurring and synthetic nucleosides in which the oxygen atom of the sugar ring is replaced by carbon. These nucleoside isosteres are characterized by an increased metabolic stability when compared to the riboside analogues as they are not recognized by, for example, nucleoside phosphorylases and hydrolases that otherwise can cleave the glycosidic bond.[1] Consequently, the synthetic design[2] and biological properties[3] of various carbocyclic nucleosides have been well documented, with applications of this compound class in the therapeutic areas of antiviral and anticancer drug discovery research. Important examples of carbocyclic nucleosides that have been marketed include the antiviral drug abacavir (**1**),[4] which is a highly potent nucleoside reverse transcriptase inhibitor used as an anti-HIV medication, and entecavir (**2**),[5] used in the treatment of patients suffering from hepatitis B infection (Figure 1). In a recent example, the carbocyclic analogue **3b** of the anticancer drug decitabine (**3a**) was found to be significantly more stable toward hydrolysis while maintaining activity toward DNA methyltransferases.[6] Also in the field of anticancer agents, the carbocyclic analogue **4b** of the potent DOT1L histone methyltransferase inhibitor pinometostat (**4a**) is currently undergoing clinical trials for the treatment of acute leukemia and has demonstrated an improved metabolic stability compared to **4a** while desirable target activity and selectivity are conserved.[7] Furthermore, compound **5** is shown to be a highly potent *S*-adenosylmethionine (SAM)-competitive methyltransferase inhibitor for the PRMT5:MEP50 multimer complex (IC_{50} = 0.14 nM), displaying a high selectivity and favorable pharmacokinetic profile in different *in vitro* and cellular assays, and is efficacious *in vivo* in certain tumor xenograft models.[8]

As depicted in Scheme 1, we previously developed the synthesis of conformationally constrained 4′-spirocyclobutyl nucleoside analogues (type **7**) using a [2 + 2]-cycloaddition of dichloroketene on 4′-*exo*-methylene furanose substrates (type **6**), followed by Vorbrüggen nucleobase introduction.[9a] However, a synthetic method that includes the corresponding carbocyclic analogues of **7** has not been described to date.[9b−f] The development of a strategy that allows access to carbocyclic core scaffolds of type **9** would result in a valuable expansion with the potential to discover biologically active constrained nucleoside analogues of type **10**. Indeed, building block **9** can serve as a modular scaffold in the preparation of carbocyclic nucleoside mimetics, as well as for the introduction of various pharmacophore substituents at the cyclobutanone moiety. Replacing the endocyclic furanose oxygen in **6** by a carbon atom, however, is expected to alter the reactivity of the double bond, so that the question whether in this case a similar cycloaddition strategy would be possible and hence whether spirocarbocyclic nucleoside **10** can be accessed remained to be investigated.

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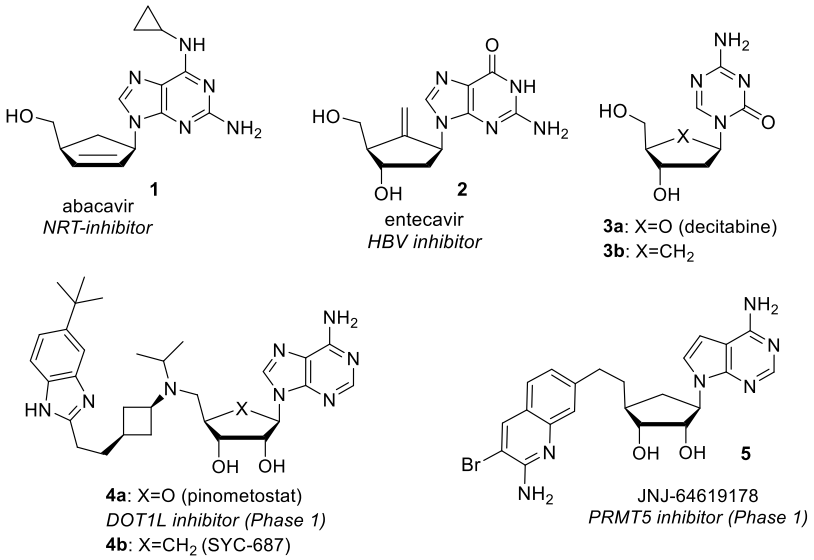
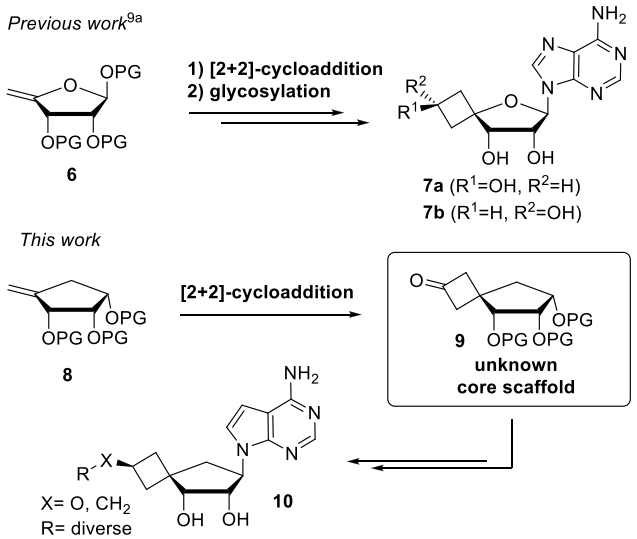


Figure 1. Examples of carbocyclic nucleoside analogues in medicinal chemistry.

Scheme 1. Synthesis of 4′-Spirocyclic Nucleoside Analogues of Type 7 (Previous Work[9a]) and Envisioned Preparation of Carbocyclic Nucleoside Analogues of Type 10 (This Work)



RESULTS AND DISCUSSION

Commercially available cyclopentenone **11**, a frequently used synthon in the synthesis of carbocyclic nucleoside analogues,[10] was used as a chiral building block in the preparation of *exo*-methylene substrate **15**. The conversion of **11** toward alcohol **14** has been reported in the literature, starting with a Michael addition on cyclopentenone **11** via rhodium-catalyzed addition of potassium vinyltrifluoroborate[8b] or by using vinylmagnesium bromide as a nucleophile,[10a] affording substrate **12**. Sequentially, stereoselective reduction of ketone **12** and silylation of the resulting alcohol was performed to afford cyclopentane **13** (Scheme 2) in 76% overall yield from **12**.[8b,10a,11]

The oxidation of **13** to the intermediate aldehyde has been described using catalytic amounts of OsO_4 in combination with co-oxidant $NaIO_4$, followed by reduction to afford the corresponding alcohol (**14**).[11] However, when we used these oxidation conditions, inconsistent conversions from the starting material (**13**) were observed on a small scale (0.5−1.0 mmol), and the formation of side products was noticed. Aiming to improve the reproducibility of the reaction on a multigram scale, we evaluated the oxidation of **13** using ozone as a milder alternative that does not require the use of transition metals and simplifies the reaction work-up. To facilitate this, ozone was electrochemically generated from oxygen and bubbled through a solution of **13** in dichloromethane at −78 °C. Applying these reaction conditions, we observed complete conversion from the starting material (**13**), and upon treatment of the intermediate aldehyde with an excess of $NaBH_4$ after a solvent switch to aqueous methanol, the desired alcohol (**14**) was obtained in high yield (Scheme 2).

To obtain key *exo*-methylene intermediates of type **8** (Scheme 1), an Appel reaction was performed on alcohol **14** and the iodomethyl intermediate was treated with DBU in tetrahydrofuran (THF) to afford **15** (Scheme 2). The synthesis of building block **15** was successfully repeated on a 25 g scale in an excellent overall yield of 67% starting from ketone **12**, requiring only a single purification via silica gel chromatography after the final elimination step toward alkene **15**.

Having access to multigram quantities of building block **15**, the [2 + 2]-cycloaddition reaction of dichloroketene was attempted using standard reaction conditions as performed previously on 4′-*exo*-methylene substrates of type **6**, that is, the dropwise addition of trichloroacetyl chloride in anhydrous diethyl ether to a mixture of substrate **15** with activated zinc powder.[9a,12] We were pleased to observe that by using this protocol, cycloadduct **16** was obtained as a single diastereoisomer in an almost quantitative amount (Scheme 3). The structure of **16** was elucidated via NOE nuclear magnetic resonance (NMR) analysis, which confirmed that dichloroketene reacts with *exo*-methylene substrate **15** from the least sterically hindered *β*-face (Scheme 3). It was noticed that the purification of **16** via normal-phase silica gel chromatography was not efficient and resulted in a low recovery, presumably because of the degradation via ring opening of dichlorocyclobutanone. Given the high purity in which **16** was obtained after the [2 + 2]-cycloaddition reaction, no further attempts were made to purify the cycloadduct (**16**) at this stage. Reductive

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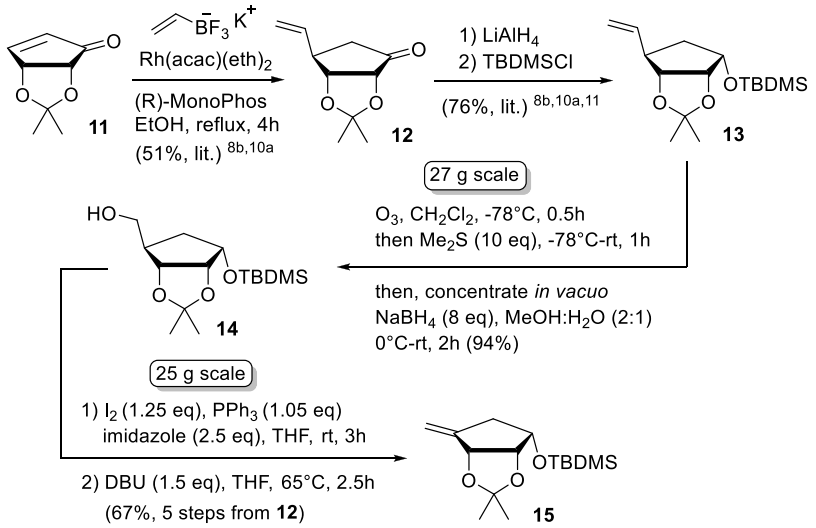
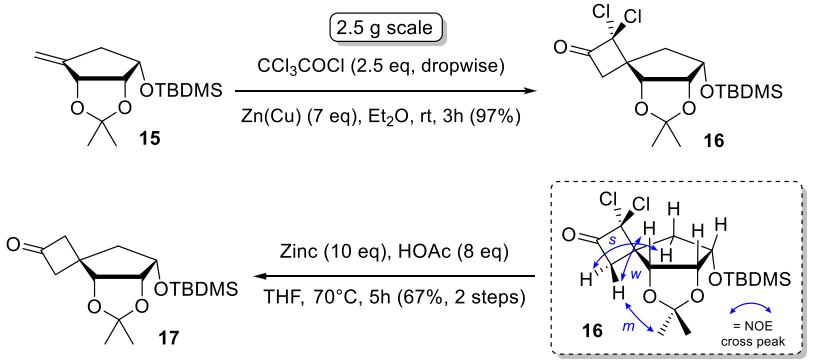
Scheme 2. Synthesis of *exo*-Methylene 15 from Cyclopentenone 11



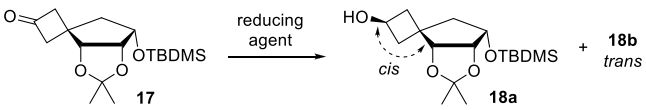
Scheme 3. [2 + 2]-Cycloaddition of Dichloroketene on *exo*-Methylene 15 and Reductive Dechlorination toward Cyclobutanone 17



dechlorination was performed via treatment of **16** with zinc and acetic acid, which readily afforded the stable cyclobutanone product **17** in good overall yield after purification via silica gel chromatography (Scheme 3).

Next, the selectivity of the carbonyl reduction was investigated for spirocyclobutanone **17**. An initial screening with commonly used reducing agents, including $NaBH_4$, $LiBH_4$, or $LiAlH_4$, all resulted in the formation of 1:1 mixtures of alcohols **18a** (*cis*) and **18b** (*trans*). As depicted in Table 1, only a slight preference for *cis*-isomer **18a** (56%) was observed when L-selectride (entry 4) was used as a reducing agent. A modest selectivity was noticed when borane reductions were used in combination with chiral Corey–Itsuno (Corey–Bakshi–Shibata, CBS) oxazaborolidine catalysts.[13] Here, a preference of 67% for *cis*-alcohol **18a** (entry 5, Table 1) and 58% for *trans* alcohol **18b** (entry 6) could be obtained at best depending on the chirality of the CBS catalyst used. Apart from the rather moderate selectivity, longer reaction times were required and generally incomplete conversion from the starting material (**17**) was obtained. These results, together with the observation that diastereomers **18a** and **18b** could not easily be separated via normal-phase silica gel chromatography or preparative high-performance liquid chromatography (HPLC) separation but required the use of supercritical fluid chromatography (SFC) instead, showed that this method of reduction is of limited synthetic value.

Table 1. Screening of Reducing Agents on Cyclobutanone 17 (0.15 mmol Scale)



entry	reducing agent	solvent	time/h	conv./%	ratio **18a**:**18b**
1	$NaBH_4$	MeOH	1	100	50:50
2	$LiBH_4$	THF	1.5	100	50:50
3	$LiAlH_4$	THF	1.5	100	50:50
4	L-selectride	THF	1.5	100	56:44
5	BH_3/(*R*)-MeCBS	THF	20	64	67:33
6	BH_3/(*S*)-MeCBS	THF	20	100	42:58
7	BH_3/(*R*)-BuCBS	THF	21	52	67:33
8	BH_3/(*S*)-BuCBS	THF	21	51	42:58
9	BH_3/(*R*)-*o*-tolyl CBS	THF	21	31	60:40
10	BH_3/(*S*)-*o*-tolyl CBS	THF	21	34	50:50

The results listed in Table 1 are in sharp contrast when compared to previous carbonyl reductions on ribose-derived cyclobutanones **19** and **20** (Scheme 4), where a high selectivity for both alcohols **21a** (94%, *cis*) and **22a** (88%, *cis*) was observed.[9a] We hypothesized that this selectivity could be attributed to a repulsive electrostatic interaction of the incoming hydride with the endocyclic furanose oxygen,

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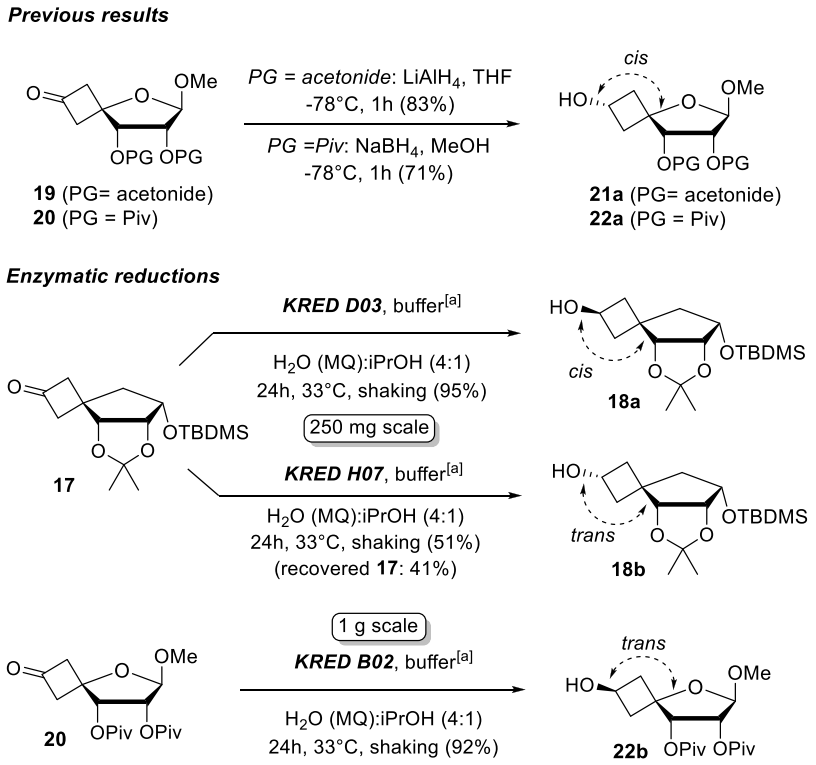
Scheme 4. Carbonyl Reductions of Cyclobutanones 17 and 19−20



[a]Buffer: Na_3PO_4 (128 mM), $MgSO_4$ (1.7 mM), and $NADP^+$ (1.1 mM) at pH 7.0 dissolved in H_2O (MQ).

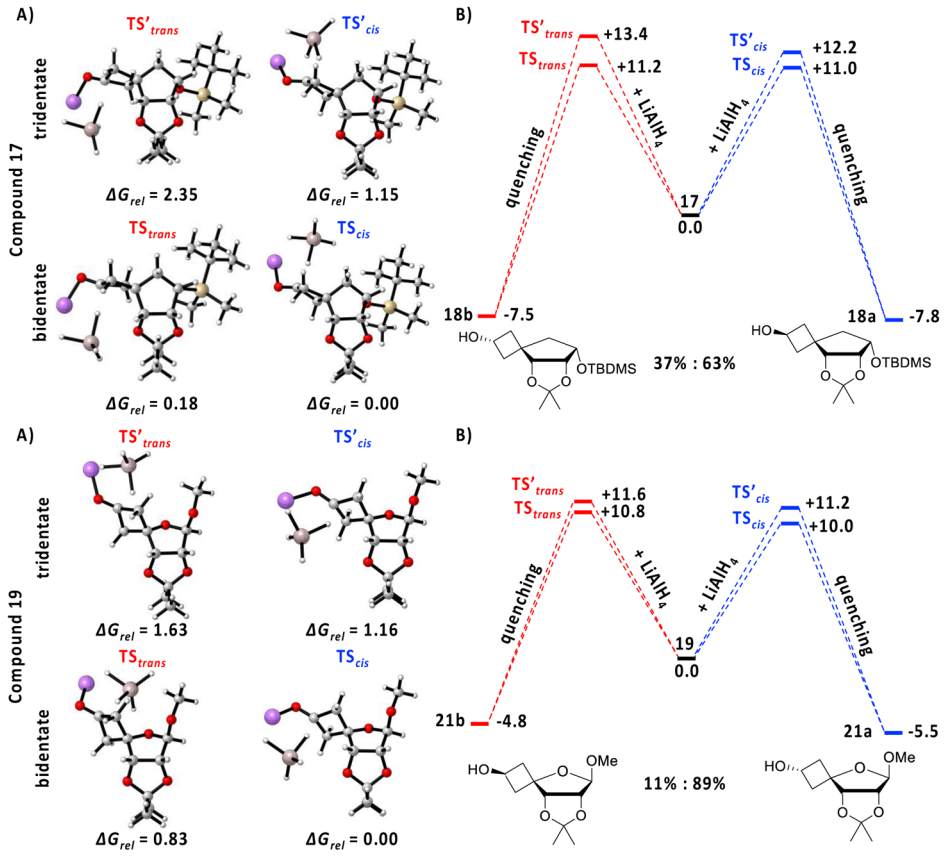


Figure 2. (A) Optimized geometries for the various transition states involved in the reduction of 4′-spirocyclobutanones **17** and **19** with $LiAlH_4$. (B) Gibbs free reaction profiles (in kcal mol^{-1}) for the different pathways considered in the hydride reduction of **17** and **19** in THF at 195.15 K. The quenching process refers to the addition of water and subsequent removal of the reducing agent.

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Table 2. Relative Gibbs Free Energies (in kcal mol^{-1}) Calculated in THF at 195.15 K Together with Selected Torsional Parameters (ϕ and D in °) for the Different Transition States Involved in the Reduction of 4′-Spirocyclobutanones 17 and 19 with $LiAlH_4$

	compound 17					compound 19				
	ref[a]	TS_{cis}	TS_{trans}	TS'_{cis}	TS'_{trans}	ref[a]	TS_{cis}	TS_{trans}	TS'_{cis}	TS'_{trans}
ΔG_{rel}		0.00	0.18	1.15	2.35		0.00	0.83	1.16	1.63
ϕ	14.2	15.2	17.9	16.6	19.7	15.8	19.4	17.0	19.0	15.5
D	79.3	80.9	72.1	82.2	73.8	82.8	73.6	77.4	77.2	83.1

[a]The reference values correspond to the cyclobutanone ring in the reactant structures of 17 and 19.

which directs the approach to the opposite (*trans*) side, leading to a preferred formation of *cis*-isomers **21a** and **22a**. These results are indeed in agreement with the literature precedent on the reduction of 3-alkoxycyclobutanones, affording *cis*-3-alkoxycyclobutanols as major reaction products (9:1 ratio).[14] Because this electrostatic repulsive effect does not exist for cyclopentane analogue **17**, and because no additional steric differences are present at both faces of the cyclobutanone, no significant selectivity is expected in the carbonyl reduction of **17**. To validate our hypothesis for the significant selectivity differences in the carbonyl reductions of substrates **17** and **19**, density functional theory (DFT) calculations were performed.

Based on our previous computational study,[15] the reduction of 4′-spirocyclobutanones with $LiAlH_4$ was described using four different transition states in which the lithium counterion coordinates to either one (bidentate) or two hydrogen atoms (tridentate). The *cis*-alcohol products of both reductions (**18a** and **21a**) always arise from a pathway involving the bidentate TS_{cis} transition state or its tridentate analogue TS'_{cis}, while the remaining transition states TS_{trans} and TS'_{trans} yield the trans isomers **18b** and **21b**. Consequently, the *cis*-isomer **18a** is obtained from a syn-facial approach of the reducing agent with respect to the cyclopentane ring of compound **17**, while the *trans* cyclobutanol **18b** results from an anti-face attack via TS_{trans} and TS'_{trans}. In the case of the ribose-derived spirocyclobutanone **19**, the opposite face approach of $LiAlH_4$ relative to the endocyclic furanose oxygen atom yields *cis*-cyclobutanol **21a**, whereas alcohol **21b** arises from the syn-facial hydride attack. The calculated Gibbs free reaction profiles for these competing transition states (Figure 2) indicate that the pathway involving the bidentate transition state TS_{cis} exhibits the lowest energy barrier for both 4′-spirocyclobutanones, and hence, the *cis*-cyclobutanols **18a** and **21a** should be obtained as major isomers. The syn-facial approach of the hydride toward cyclopentane analogue **17** involves an energy barrier of 11.0 kcal mol^{-1}, while the computed activation barrier for the reduction of **19** through TS_{cis} is 10.0 kcal mol^{-1} at 195.15 K. However, the bidentate TS_{trans} pathways resulting in isomers **18b** and **21b** have activation barriers that are only 0.2 and 0.8 kcal mol^{-1}, respectively, higher than the most favorable pathways. On the other hand, the activation barriers involving tridentate transition states turn out to be systematically 1−2 kcal mol^{-1} higher in energy than the bidentate pathways, though the preference for the *cis*-isomer is still retained. Despite the preference for TS_{cis} pathways, a clear difference in selectivity between both reductions is noted from the estimated Boltzmann populations. While the reduction of spirocyclobutanone **19** yields 89% of *cis* product **21a**, which is in close agreement with the experimental value (94%), the reduction of compound **17** results in 63% of *cis*-alcohol. These findings fully support the previously mentioned experimental observations.

To assess the driving forces behind the distinct stereoselectivity of these reductions, we have analyzed the structural changes in the cyclobutanone ring arising from the hydride approach. The puckering angle (ϕ), defined as the angle formed by the intersection between the $C_2-C_1-C_4$ and $C_2-C_3-C_4$ planes,[16] and the dihedral angle $D(O-C_1-C_2-H'_2)$ are two geometrical parameters that allow to quantify, respectively, the torsional strain and eclipsed interactions relative to the initial reagents (Figure S1). On the one hand, an increase in ϕ with respect to the reference values indicates a relief in torsional strain, while the cyclobutanone ring becomes more puckered. On the other hand, a decrease in D denotes less eclipsed interactions between the carbonyl bond and the neighboring C−H bond. Both parameters have been previously used to establish the role of torsional effects on the stereoselectivity of cyclobutanone reductions.[15,16] The relative Gibbs free energies and structural parameters for the different transition states together with the reference values for the reactants **17** and **19** are summarized in Table 2.

From Table 2, it is clear that both anti- and syn-facial attacks of the hydride involve a reduction in torsional strain because ϕ increases with respect to the reactants, except for TS'_{trans} of **19**. In the case of spirocyclobutanone **19**, the preference for TS_{cis} can be attributed to the largest increase in ring puckering ($\Delta\phi$ = +3.6°) as well as the relief of eclipsed interactions (ΔD = −9.2°). By contrast, the preference for the reduction pathway involving TS_{cis} for **17** cannot be ascribed to torsional effects because the antifacial attack via TS_{trans} and TS'_{trans} entangles a larger increase in puckering angle than the syn-facial hydride approach. Consequently, we further investigated the role of the noncovalent interactions (NCIs) using the NCI index.[17]

The two-dimensional plots of the reduced density gradient (s) with respect to the electron density (ρ) multiplied by the sign of the second eigenvalue of the electron density Hessian matrix (λ_2) allow to discern attractive interactions ($\lambda_2 < 0$) from the repulsive ($\lambda_2 > 0$) ones. Furthermore, the visualization of the NCIs in real space is possible by representing the reduced gradient isosurfaces in three dimensions using a RGB color scale, with red isosurfaces corresponding to repulsive interactions, the green ones to weak van der Waals interactions, and attractive interactions appearing as blue isosurfaces. The isosurfaces of the four different transition states involved in the reduction of compounds **17** and **19** are shown in Figures S2 and S3, respectively. The NCI plots (Figure S3) show a repulsive electrostatic interaction (marked with a circle) involving the endocyclic furanose oxygen atom that hampers the syn-facial approach of $LiAlH_4$ toward the ribose-derived spirocyclobutanone **19** via TS_{trans} and TS'_{trans}. It can therefore be concluded that the pronounced stereoselectivity for the *cis*-cyclobutanol **21a** is driven by both the relief of repulsive interactions of the incoming hydride with the furanose oxygen atom and the largest decrease in torsional

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J. Org. Chem. XXXX, XXX, XXX−XXX

strain. On the other hand, repulsive interactions are present for both the syn- and anti-facial approach of the hydride toward spirocyclobutanone **17**, though these interactions are more pronounced between the acetate protective group and the anti-facially attacking hydride in TS_{trans} (red circle in Figure S2). Consequently, we hypothesize that the poor selectivity of the reduction of **17** is due to a competition between a hydride approach delivering maximal torsional strain relief or minimal repulsive interactions.

To improve the poor stereoselectivity in the reduction of cyclobutanone **17**, we investigated biocatalytic ketone reductions, as various ketoreductases (KREDs) are commercially available, and these enzymes are typically characterized by a broad substrate scope.[18] To this end, a screening kit[19] containing 19 engineered KRED enzymes derived from *Lactobacillus kefir* and *Lactobacillus brevis* wild-type enzymes was evaluated. A small-scale screening using the different enzymes on spirocyclobutanone substrate **17** revealed five KREDs, which showed an excellent selectivity exceeding 95% for both diastereoisomers **18a** and **18b**, combined with high conversions from ketone substrate **17**, as depicted in Table 3

Table 3. Screening Enzymatic Carbonyl Reductions of Cyclobutanones 17 and 20

entry	KRED	ketone	conv./%	ratio a/b[a]
1	B05	**17**	100	95:5
2	B02	**17**	80	≥99% **18a**
3	D03	**17**	100	≥99% **18a**
4	A12	**17**	95	3:97
5	H07	**17**	77	≥99% **18b**
6	C01	**20**	65	2:98
7	C02	**20**	73	≥99% **22b**
8	B02	**20**	93	≥99% **22b**
9	A12	**20**	12	90:10

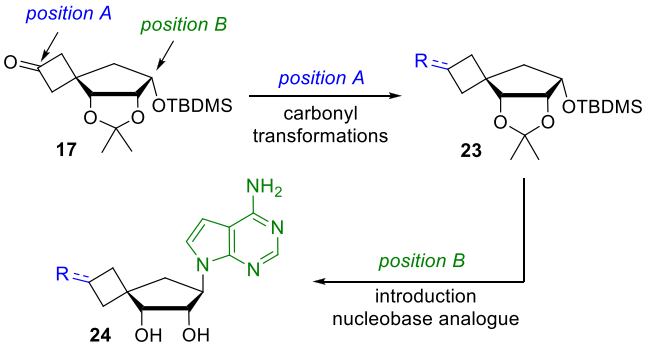
[a]**18a**:**18b** (substrate **17**) or **22a**:**22b** (substrate **20**).

(entries 1−5). Consequently, the enzymes that displayed the most promising outcome based on small-scale screening were selected to evaluate the stereoselective synthesis of alcohols **18a** (*cis*) and **18b** (*trans*). Cyclobutanone **17** dissolved in isopropyl alcohol was mixed with enzyme D03 (entry 3, Table 3), and the aqueous buffer solution containing the cofactor was moderately shaken for 24 h at 33 °C. After a short elution over the silica gel column, we observed that alcohol **18a** was obtained as a single isomer in excellent isolated yield (95%) (Scheme 4). Likewise, alcohol **18b** (*trans*) could be obtained in a diastereoselective way using a different KRED (entry 5, Table 3). Because of the partial conversion in the latter reduction, **18b** was obtained in a lower yield; however, the unreacted ketone (**17**) could be efficiently recovered via silica gel chromatography.

The successful stereoselective transformation of ketone **17** to alcohols **18a** (*cis*) and **18b** (*trans*) prompted us to repeat the screening with the different KREDs on ribose-derived cyclobutanone **20**, where previously a maximum chemoselectivity of 88% for isomer **22a** (*cis*) was obtained using hydride reducing agents (Scheme 4). Also in this case, several enzymes were identified for the diastereoselective reduction of spirocyclobutanone **20** to alcohol **22b** (*trans*), of which a selection is listed in Table 3 (entries 6−9). Encouraged by these results, the enzyme that resulted in a diastereoselective reduction combined with the highest conversion from ketone **20** (entry 8, Table 3) was used for scale-up, which resulted in the development of a reproducible, high yielding protocol to transform ketone **20** to alcohol **22b** (*trans*) on 1 g scale. Consequently, this biocatalytic reduction using a green solvent mixture became the preferred method to obtain alcohol **22b** (*trans*), thereby avoiding the cumbersome isolation of this isomer from a cis−trans mixture of diastereoisomers **22a** and **22b**. Noteworthy, only one enzyme (entry 9, Table 3) was found to be partly (90%) selective for alcohol **22a** (*cis*), previously identified as the major reaction product in the screening of chemical carbonyl reductions (Scheme 4). Overall, this enzymatic reduction protocol affords a highly efficient approach for the stereoselective synthesis of alcohols **18a**, **18b**, and **22b**, making use of commercially available KREDs.

Having established a robust protocol toward the central carba-nucleoside core scaffold **17**, we embarked on the introduction of functional group handles and key pharmacophores at two relevant positions in **17** (positions A and B, Scheme 5). Specifically, we attempted to (1) introduce a

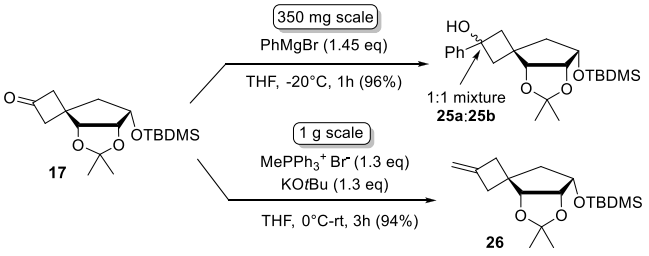
Scheme 5. Functionalization of Core Scaffold 17



tertiary phenyl carbinol (toward a constrained mimetic of the methyltransferase inhibitor LLY-283[20]); (2) introduce an exocyclic alkene functionality at the cyclobutanone position (as a precursor for the synthesis of spirocyclic analogues of PRMT5 inhibitor **5**[8a−c]); and (3) install a nucleobase analogue at the relevant position toward compounds of type **24**, as depicted in Scheme 5. Moreover, we investigated the differences in chemical reactivity between the novel spirocarbocyclic building block **17** and the corresponding furanose analogues **19** and **20**.

The reactivity evaluation of ketone **17** was initiated by treatment with phenylmagnesium bromide at low temperatures, which readily afforded alcohols **25a** and **25b** in a 1:1 ratio (Scheme 6). This Grignard addition displayed no stereoselectivity, similar to the carbonyl reduction of **17** as

Scheme 6. Grignard Addition and Wittig Reaction on Cyclobutanone 17



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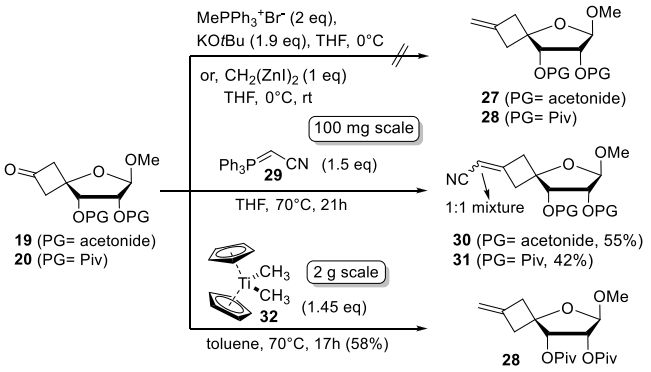
listed in Table 1. Nonetheless, both tertiary alcohols **25a** and **25b** could be separated in an efficient manner via SFC purification and were obtained in good isolated yields. As stated previously, these intermediates could be used as building blocks for the preparation of constrained, carbocyclic analogues of the PRMT5 inhibitor LLY-283.[20] To further explore the synthetic potential of ketone **17**, a Wittig reaction was attempted in order to prepare *exo*-methylene **26** (Scheme 6). Therefore, methyltriphenyl phosphonium bromide was treated with KO*t*Bu to generate the corresponding Wittig ylide *in situ*, which was then added in portions to a solution of ketone **17** in THF. This reaction efficiently afforded alkene **26** in excellent isolated yield after a short elution over the silica gel column. The terminal alkene moiety in **26** can serve as a useful functional handle for further derivatization via, for example, hydroboration reactions or transition-metal-catalyzed cross-couplings.

Surprisingly, when the same Wittig reaction was attempted on ribose analogues **19** and **20** to prepare alkenes **27** and **28**, respectively, an instantaneous degradation of the ketones was observed during the addition of the Wittig ylide, resulting in the formation of a dark-yellow-colored mixture (Scheme 7).

Scheme 7. Evaluation of Olefination Reactions on Cyclobutanones 19 and 20



The instability of the latter ketone substrates under basic reaction conditions was confirmed by the addition of different bases (KO*t*Bu, DBU, and NaH) to substrates **19** and **20** in THF, showing fast decomposition on ^{1}H NMR.[21] It is very likely that ketones **19** and **20** are readily enolized at the cyclobutanone when subjected to a strong base and undergo fragmentation through ring opening of the furanose ring. This hypothesis was further confirmed when the deactivated and less basic phosphonium ylide **29** was found to successfully convert ketones **19** and **20** to alkenes **30** and **31**, resulting in isomeric mixtures on both substrates (Scheme 7). The instability of **19** and **20** toward basic reaction conditions clearly does not apply to cyclopentane analogue **17**, as both Grignard addition and Wittig reaction are compatible and afford the desired products in high yields (Scheme 6). The absence of the endocyclic oxygen atom in ketone **17** thus prevents the decomposition of the spirocyclic scaffold after α-deprotonation of the cyclobutanone.

In order to synthesize alkene **28**, an alternative approach was followed that uses the bis(iodozincio)methane reagent, which is reported to have a reduced basicity as compared to the previously employed Wittig ylide (Scheme 7).[22] Unfortunately, no conversion was observed from ketones **19** or **20** toward the desired products, and decomposition of the starting material was observed instead. In a final attempt, the Petasis reagent (**32**) was tested to facilitate the olefination of ketone **20** (Scheme 7).[23] To this end, commercially available dimethyltitanocene **32** was mixed with ketone **20** in toluene and heated to 70 °C to generate the active methylene species *in situ*. Gratifyingly, this reaction successfully resulted in a clean conversion from spirocyclobutanone **20** toward the desired alkene **28**, which was isolated in 58% yield on a 2 g scale after silica gel chromatography.

Next, we evaluated if the obtained novel spirocarbocyclic scaffold structures can be used as building blocks for the preparation of the corresponding nucleoside analogues. To deliver a proof of concept for this transformation, alcohol **18b** was selected as a substrate in the synthesis of pyrrolopyrimidine nucleoside analogue **38** (Scheme 8). In analogy to the potent methyltransferase inhibitor **5** (Figure 1), we planned the introduction of a quinoline pharmacophore at the cyclobutyl alcohol, as this can potentially result in biologically active nucleoside analogues as mimetics of SAM. Therefore, prior to the introduction of the pyrrolopyrimidine moiety, the cyclobutyl alcohol **18b** was transformed to **33** via a Mitsunobu reaction with inversion of stereochemistry. Subsequently, the treatment of **33** with tetrabutylammonium fluoride (TBAF) efficiently removed the silyl protecting group to afford alcohol **34** as a suitable substrate for the introduction of a heterocyclic pyrrolopyrimidine base. Using 6-chloro-7-deazapurine (**35a**) as a reagent, initial attempts were made to introduce this heterocyclic moiety via a Mitsunobu reaction on alcohol **34** (Scheme 8); however, this approach turned out to be not synthetically useful and resulted in the formation of numerous side products. Therefore, this strategy was not investigated further, and focus was directed to the nucleophilic substitution with deazapurine **35b** on *O*-triflated substrate **34**, as described for related cyclopentanols.[8c,24] First, **34** was treated with triflic anhydride to prepare the intermediate triflate, followed by a nucleophilic substitution using an excess of preformed potassium salt **35b**. A complete conversion toward the desired product **36** was observed after 4 h, alongside a fraction of elimination side product **37** in a 3:1 ratio, respectively. After an efficient removal of **37** via silica gel chromatography, the desired intermediate (**36**) was isolated in a 52% overall yield from **33**.

Finally, substitution of the chlorine atom in **36** was carried out via treatment with aqueous ammonia in dioxane at elevated temperature, followed by acidic hydrolysis using a mixture of aqueous HCl in ethanol to deprotect the acetal group. This reaction sequence successfully afforded carbocyclic nucleoside analogue **38** in good overall yield (Scheme 8). This example validates the compatibility of the synthetic methodology to access a novel class of pharmacologically relevant carbospirocyclic nucleoside analogues.

To demonstrate that *exo*-methylene **26** offers a useful handle for the late-stage functionalization of the cyclobutyl ring, we attempted the preparation of the derived nucleoside analogue **39** (Scheme 9). To facilitate this, the silyl protecting group in **26** was removed, followed by treatment with triflic anhydride and substitution of the triflate with pyrrolopyrimidine **35b**, which showed an improved selectivity for the desired substitution product over the elimination side product (82:18 ratio, respectively). In analogy to substrate **36** (Scheme 8), treatment of the corresponding intermediate with ammonia successfully afforded building block **39** in an excellent overall yield. We were pleased to find that the treatment of **39** with 9-

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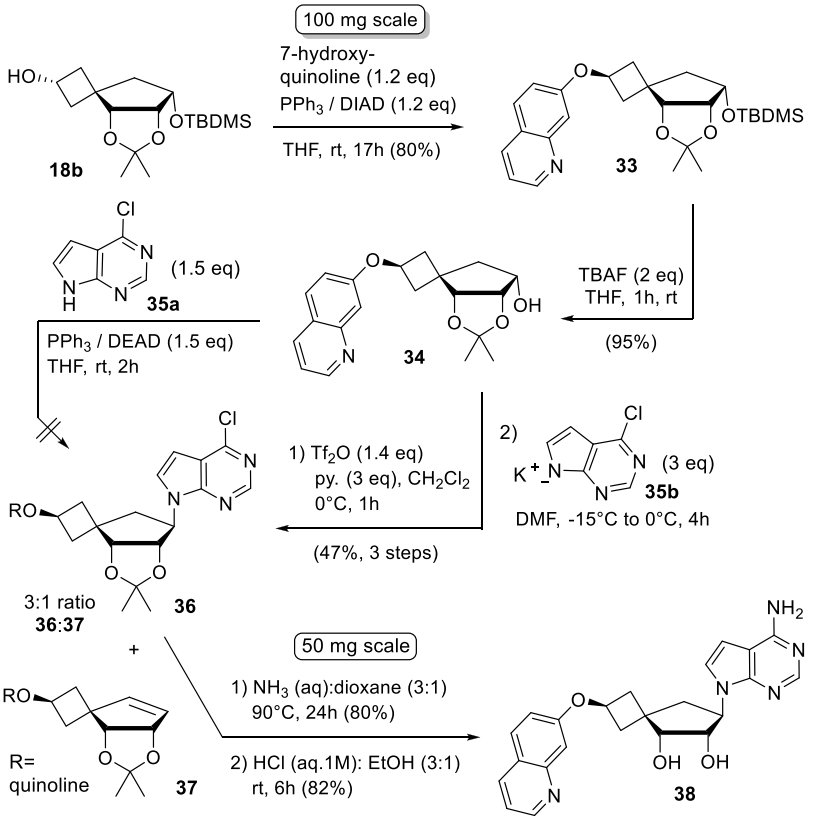
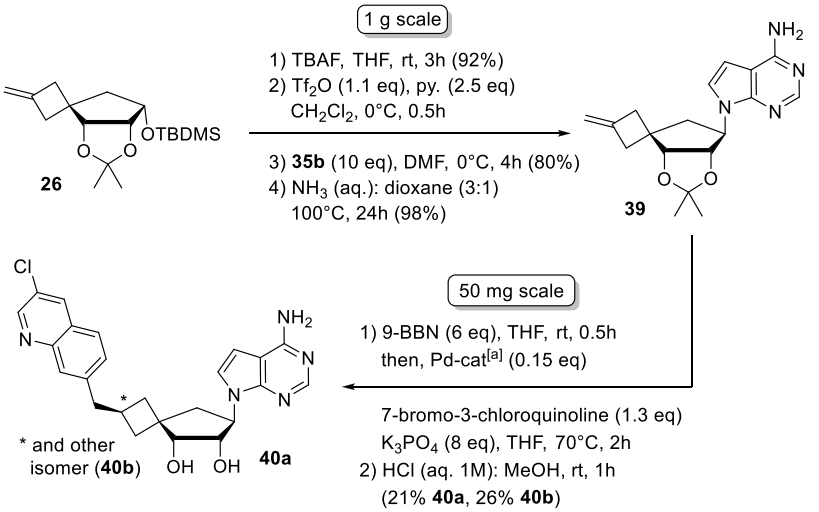
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 8. Synthesis of Carbocyclic Nucleoside Analogue 38 from Spirocyclic Building Block 18b



Scheme 9. Synthesis of Carbocyclic Nucleoside Analogues 40a and 40b from Spirocyclic Building Block 26



[a]Pd-cat: 1,1′-bis(di*tert*-butylphosphino)-ferrocene palladium dichloride.

BBN readily afforded the intermediate borane and could successfully be converted toward the corresponding cross-coupled products as exemplified by the introduction of a 3-chloroquinoline moiety, which is a highly potent PRMT5 pharmacophore, at the cyclobutyl ring.[25] As depicted in Scheme 9, final deprotection of the acetonide allowed the isolation of spirocarbocyclic nucleoside analogues **40a** and **40b** in a 1:1 ratio. The above synthetic sequence thus demonstrates to be compatible with the novel spirocarbocyclic nucleoside building blocks of type **39**, enabling access to a variety of constrained mimetics of the potent PRMT5 inhibitor **5**.

The examination of the *in vitro* activity of novel 4′-spirocarbocyclic nucleoside analogues **40a** and **40b** in the PRMT5/MEP50 multimer complex indicates promising micromolar inhibitions of 2.00 and 1.09 μM (IC_{50} values), respectively. Furthermore, we observed that compound **38** (Scheme 8), bearing a nonfunctionalized quinoline pharmacophore attached to the cyclobutyl moiety via an oxygen linker, resulted in a 10-fold increase in potency compared to **40b**, showing a sub-micromolar inhibition value of 0.09 μM in the PRMT5/MEP50 assay. These biological results clearly indicate that novel spirocarbocycles of types **18** and **26** are valuable

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J. Org. Chem. XXXX, XXX, XXX−XXX

building blocks for the synthesis of a variety of potential competitive PRMT5 inhibitors.

CONCLUSIONS

We have achieved the synthesis of a novel carbospirocyclic core scaffold (**17**) on a multigram scale, starting from commercially available cyclopentenone **11**, and demonstrated that the derived nucleosides analogues could be accessed from this key building block. *exo*-Methylene **15** was found to be an excellent substrate for the [2 + 2]-cycloaddition of dichloroketene, affording cycloadduct **16** in a stereoselective way. An evaluation of the reactivity of the derived cyclobutanone (**17**) revealed significant differences when compared to previously prepared spirocyclic ribose analogues **19** and **20**. We supported the experimentally observed differences in carbonyl reduction selectivity between substrates **17** and **19** by means of DFT calculations and found that chemoenzymatic reductions of ketone **17** were successful to selectively prepare a single stereoisomer (**18a** or **18b**), whereas conventional carbonyl reductions proved to be significantly inferior. Also, cyclobutanone **17** was found to be compatible with conditions involving basic reagents, such as Grignard and Wittig reactions. In contrast, ketones **19** and **20** derived from ribose were unstable under such conditions, requiring a different synthetic approach. We showed that core scaffold **17** can be efficiently transformed into carbospirocyclic nucleoside analogues, either after derivatizations of the cyclobutanone functional group handle or prior to the introduction of desired pharmacophore groups, allowing late-stage functionalization of intermediate **39**. Finally, we delivered a proof of concept that spirocarbocycles of type **18** and **26** are modular building blocks for the preparation of potent inhibitors for methyltransferase PRMT5.

EXPERIMENTAL SECTION

General Information. Structural assignments were made with additional information from gCOSY, gHSQC, and gHMBC experiments.

Materials and Instrumentation: NMR. For synthesized compounds, ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-360 operating at 360 MHz for ^{1}H NMR and 91 MHz for ^{13}C NMR, on a Bruker Avance 400 operating at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR, or on a Bruker Avance III 400 operating at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR. Alternatively, ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR on a Bruker Avance II 500 console or at 250 MHz for ^{1}H NMR and 63 MHz for ^{13}C NMR on a Bruker Avance DRX 250 console. Tetramethylsilane (TMS) was used as an internal standard. The used solvent and frequency are mentioned before every analysis. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. Coupling constants (J) are given in hertz (Hz). The following abbreviations are used in the description of spectra: singlet (s), doublet (d), triplet (t), quadruplet (q), quintet (qn), multiplet (m), doublet of doublets (dd), triplet of doublets (td), doublet of triplets (dt), doublet of doublet of doublets (ddd), and pseudo (ps).

Liquid Chromatography−Mass Spectroscopy. HPLC measurement was performed using an LC pump, a diode-array UV detector, and a column as specified in the respective methods (entries 1−4, Table S1, Supporting Information). Flow from the column was brought to the mass spectrometer, which was configured with an atmospheric pressure ion source. Data acquisition was performed with MassLynx software from Waters. Intermediates and final compounds are described by their experimental retention times (R_t) and ions. Electrospray ionization (ES+ and/or ES−) was used as an ionization method. If not specified differently in the table of data, the reported molecular ion corresponds to the $[M + H]^+$ (protonated molecule) and/or [M − H]— (deprotonated molecule). If the compound was not directly ionizable, the type of adduct is specified (i.e., $[M + NH_4]^+$, $[M + HCOO]^-$, etc.). For molecules with multiple isotopic patterns (Br and Cl), the values are reported for the different isotopes with corresponding intensities, or the reported value is the one obtained for the lowest monoisotopic mass.

Gas Chromatography−Mass Spectroscopy. The gas chromatography (GC) measurement was carried out on a J&W HP-5MS column (20 m × 0.250 mm, 0.25 mm) from Agilent Technologies (instrument: GC6890-MSD5973N) with a constant pressure of 12.97 psi. The temperature gradient applied was as follows: initial temperature 50 °C, held for 0.1 min, then a 24 °C/min ramp applied for 9.58 min until 280 °C, and held for 5.0 min in a 14.68 min run. The front inlet temperature was 250 °C. The split injection mode was used, 1 mL injection volume, with a 10/1 ratio into the GC−mass spectroscopy (MS) system and a flow of 2.3 mL/min. Electron ionization was used as the ionization mode.

Supercritical Fluid Chromatography−Mass Spectrometry. SFC purifications were performed on an Acquity UPC2 system from waters equipped with a UV, QDA, and evaporative light scattering detector (ELSD). The SFC measurement was performed using an analytical SFC system composed by a binary pump for delivering carbon dioxide (CO_2) and a modifier, an autosampler, a column oven, and a diode array detector equipped with a high-pressure flow cell standing up to 400 bars. If configured with a mass spectrometer, the flow from the column was brought to the mass spectrometer. Data acquisition was performed with MassLynx software from Waters. The analytical method used for SFC−MS is detailed in Table S2 (Supporting Information)

High-Resolution Mass Spectrometry. Ultra-high-performance liquid chromatography (UHPLC)−high-resolution mass spectrometry (HRMS) analyses were performed using a Dionex Ultimate 3000 UHPLC system coupled to a Thermo Orbitrap Fusion Lumos Tribrid mass spectrometer. High-resolution accurate mass data were acquired in the positive ion mode using an electrospray ionization source. Note: for the following new molecules listed, HRMS data could not be provided as a result of poor ionization: **15**−**17**, **18a**, **18b**, **25a**, **25b**, **26**, **28**, **30**, **31**, and **42**. The identity of aforementioned compounds has been unambiguously confirmed by NMR analysis (see below). For all the other intermediates prepared via the linear synthesis strategy, HRMS data is included for at least every third compound in a row. This applies to intermediates **33**, **37**, **39**, **41**, and **43** (see experimental procedures and characterization below).

Silica Gel Chromatography. Purifications were conducted manually using synthetic amorphous silica gel Silica gel 60 (Merck Millipore, 0.040−0.063 mm) or automatically using a Reveleris X2 flash chromatography system with integrated ELS/UV/UV−vis detection and prepacked silica gel cartridges (Reveleris SRC Silica Flash Cartridges, 40 μm) from Grace Reveleris or silica gel cartridges (puriFlash, 25 μm) from Interchim. Alternatively, a Biotage SP4 flash chromatography system was used with integrated UV detection and prepacked silica cartridges (SNAP ultra, 25 μm) from Biotage.

Experimental Procedures and Characterization. *Synthesis of (3aR,6R,6aR)-2,2-Dimethyl-6-vinyltetrahydro-4H-cyclopenta[d]-[1,3]-dioxol-4-one* ***12****.*[8b] Acetylacetonatobis(ethylene)rhodium(I) (837 mg, 3.24 mmol, 0.02 equiv) and (*R*)-*N*,*N*-dimethyldinaphtho-[2,1-*d*:1′,2′-F][1,3,2]dioxaphosphepin-4-amine (2.91 g, 8.11 mmol, 0.05 equiv) were dissolved in EtOH (625 mL) under the nitrogen atmosphere. The mixture was stirred at room temperature and flushed with nitrogen for 15 min. Then, (−)-(3a*R*,6a*R*)-3a,6a-dihydro-2,2-dimethyl-4*H*-cyclopenta-1,3-dioxol-4-one (**11**, 25.0 g, 162.16 mmol, 1.00 equiv) and potassium vinyltrifluoroborate (45.7 g, 324 mmol, 2.00 equiv) were added and the reaction mixture was stirred at reflux using an oil bath for 4 h. The mixture was cooled to room temperature, filtered over a path of Celite, and rinsed with *n*-heptane. A total volume of 500 mL of *n*-heptane was added, followed by water (300 mL), and the product was extracted in *n*-heptane (3 × 500 mL). The combined organic layers were washed with NH_4OH (aq 12%, 3 × 250 mL) and brine (2 × 250 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent was removed by distillation at

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J. Org. Chem. XXXX, XXX, XXX−XXX

atmospheric pressure. *n*-Heptane (200 mL) was added to the residue, and the solids were removed via decantation of the liquids. Further removal of residual solvent was performed via distillation at atmospheric pressure to afford the title compound **12**. Dark red oil. Yield: 51% (16.2 g, crude). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.83 (ddd, J = 17.2, 10.7, 6.3 Hz, 1H), 5.05−5.20 (m, 2H), 4.64 (d, J = 4.9 Hz, 1H), 4.20 (d, J = 4.9 Hz, 1H), 3.07−3.15 (m, 1H), 2.84 (dd, J = 18.3, 8.5 Hz, 1H), 2.29 (br d, J = 18.3 Hz, 1H), 1.45 (s, 3H), 1.36 ppm (s, 3H). The spectroscopic results are consistent with the reported literature data.[8b,10a,11]

Synthesis of tert-Butyl(((3aR,4S,6R,6aR)-2,2-dimethyl-6-vinyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)oxy)dimethylsilane ***13***. [8b,10a,11,26] A nitrogen-flushed four-necked 1 L flask was equipped with a pressure-equalized dropping funnel, a thermometer, two septa, and subsequently charged with THF (174 mL) and lithium aluminum hydride (5.71 g, 143 mmol, 1.50 equiv), respectively. The mixture was cooled to 0 °C using an ice bath, and (3a*R*,6*R*,6a*R*)-2,2-dimethyl-6-vinyltetrahydro-4*H*-cyclopenta[*d*][1,3]-dioxol-4-one **12** (17.4 g, 95.5 mmol, 1.00 equiv) dissolved in THF (87 mL) was added dropwise to the mixture over a period of 1 h during which a temperature between 0 and 5 °C was maintained. After addition, the mixture was stirred for an additional 45 min at 0 °C. Subsequently, the mixture was carefully quenched at 0 °C with water (5.5 mL, dropwise over 15 min) and NaOH (15% in H_2O, 5.5 mL) was added. The mixture was filtered over Celite and rinsed with THF (300 mL). The mixture was concentrated *in vacuo* and the residue (16.6 g) was dissolved in pyridine (55 mL), cooled to 0 °C, and *tert*-butyldimethylsilyl chloride (14.9 g, 98.8 mmol, 1.00 equiv) was added portionwise over 15 min. After addition, the mixture was warmed to room temperature and stirred for 22 h. Subsequently, *n*-heptane (300 mL) was added to the solution, followed by $NaHCO_3$ (aq sat. 100 mL). The product was extracted in *n*-heptane (3 × 300 mL) and the combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 99:1 to 9:1). The fractions containing the product were collected and the solvent was evaporated to afford the title compound **13**. Slight yellow oil. Yield: 76% (19.8 g, 66.4 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (ddd, J = 17.3, 10.6, 6.5 Hz, 1H), 4.88−5.02 (m, 2H), 4.24−4.32 (m, 2H), 3.93−4.03 (m, 1H), 2.52−2.63 (m, 1H), 1.95 (ddd, J = 12.3, 9.2, 7.3 Hz, 1H), 1.57−1.69 (m, 1H), 1.40 (s, 3H), 1.22 (s, 3H), 0.82 (s, 9H), 0.00 ppm (s, 6H). GC−MS m/z: [M-TBDMSi] found 183.0, rt = 5.55 min. The spectroscopic results are consistent with the reported literature data.[26]

Synthesis of ((3aR,4R,6S,6aR)-6-((tert-Butyldimethylsilyl)oxy)-2,2-dimethyltetra-hydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-methanol ***14***. *tert*-Butyl(((3a*R*,4*S*,6*R*,6a*R*)-2,2-dimethyl-6-vinyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)oxy)dimethylsilane **13** (27.5 g, 92.1 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (800 mL) and the mixture was cooled to −78 °C using a cooling bath containing dry ice in acetone. Ozone was generated from oxygen gas with an ozone generator (Fischer OZS00/5 apparatus) and bubbled through the cooled solution via a glass pipet. A blue color was observed after 1.5 h, and ozone was added for an additional 20 min at −78 °C. Subsequently, the mixture was flushed with nitrogen for 5 min until a colorless solution was obtained, and dimethyl sulfide (40.6 mL, 553 mmol, 6.00 equiv) was added at −78 °C. The flow of nitrogen gas was stopped, and the mixture was stirred for 1 h, while the temperature gradually increased to −40 °C. The mixture was concentrated *in vacuo* at 30 °C to a minimal volume and the resulting yellow oil was dissolved in methanol (460 mL) and water (230 mL). The solution was cooled to 0 °C using an ice bath, and sodium borohydride (31.3 g, 829 mmol, 9.00 equiv) was added portionwise. The ice bath was removed after 1.5 h and stirring was continued at room temperature. After 4 h, the mixture was diluted in CH_2Cl_2 (500 mL) and NH_4Cl (aq sat. 150 mL) was added. The product was extracted in CH_2Cl_2 (3 × 500 mL), combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo* to afford the title compound **14**. An analytical sample was purified by column chromatography over silica gel for characterization (gradient elution: *n*-heptane/EtOAc from 1:0 to 0:1). Colorless oil. Yield: 94% (26.3 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.37−4.42 (m, 2H), 4.10−4.15 (m, 1H), 3.55−3.62 (m, 1H), 3.45−3.53 (m, 1H), 2.16−2.26 (m, 1H), 2.01 (dt, J = 12.7, 8.2 Hz, 1H), 1.86 (br s, 1H), 1.57−1.66 (m, 1H), 1.49 (s, 3H), 1.31 (s, 3H), 0.91 (s, 9H), 0.09 ppm (d, J = 2.9 Hz, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 111.7, 82.0, 80.9, 72.7, 64.3, 44.7, 34.4, 26.5, 26.0, 24.9, 18.4, −4.5 ppm. GC−MS m/z: [M-TBDMSi] found 187.1, rt = 6.55 min. The spectroscopic results are consistent with the reported literature data.[11]

Synthesis of tert-Butyl(((3aR,4S,6aR)-2,2-dimethyl-6-methylenetetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)oxy)dimethylsilane ***15***. ((3a*R*,4*R*,6*S*,6a*R*)-6-((*tert*-Butyldimethylsilyl)oxy)-2,2-dimethyltetra-hydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)methanol **14** (26.3 g, 86.9 mmol, 1.00 equiv) was dissolved in THF (450 mL). Imidazole (14.8 g, 217 mmol, 2.50 equiv) and triphenylphosphine (26.4 g, 95.6 mmol, 1.10 equiv) were added, followed by the portionwise addition of iodine (27.9 g, 108.7 mmol, 1.25 equiv) at room temperature over 20 min. After 30 min of completing the addition, the mixture was concentrated to a minimal volume *in vacuo* and *n*-heptane (500 mL) was added. Triphenylphosphine oxides were precipitated and the mixture was sonicated for 30 min. The solids were separated by filtration and rinsed with *n*-heptane (100 mL). To the filtrate was added sodium thiosulfite (aq sat. 150 mL), and the product was extracted in *n*-heptane (3 × 400 mL). Combined organic fractions were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue (30.6 g) was dissolved in THF (500 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 11.9 mL, 79.3 mmol, 1.10 equiv) was added. The mixture was heated to 65 °C using a heating mantle for 1.5 h, after which an additional portion of DBU (2.16 mL, 14.4 mmol, 0.20 equiv) was added and heating was continued for 1.5 h. Precipitated salts were removed via filtration at room temperature and the solids were rinsed with THF. The filtrate was concentrated to a minimal volume *in vacuo* and *n*-heptane (500 mL) and brine (150 mL) were added. The organic layer was washed with brine (3 × 150 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 1:1). The fractions containing the product were collected and the solvent was evaporated to afford the title compound **15**. Colorless liquid. Yield: 67% (five steps from **12**, 18.3 g, 64.5 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.14−5.20 (m, 1H), 5.11 (dd, J = 2.6, 1.0 Hz, 1H), 4.62 (d, J = 5.7 Hz, 1H), 4.46 (t, J = 5.1 Hz, 1H), 3.90 (ddd, J = 11.2, 6.5, 4.7 Hz, 1H), 2.67 (ddtd, J = 13.9, 11.1, 2.7, 1.2 Hz, 1H), 2.29−2.35 (m, 1H), 1.50 (s, 3H), 1.35 (s, 3H), 0.92 (s, 9H), 0.11 ppm (d, J = 2.8 Hz, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 145.6, 113.6, 111.2, 80.8, 80.4, 72.3, 37.5, 26.4, 26.0, 24.7, 18.4, −4.5, −4.6 ppm. GC−MS m/z: [M-TBDMSi] found 169.1, rt = 5.22 min.

Synthesis of (1R,3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2,2-dichloro-2′,2′-dimethyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-one ***16***. Activation of zinc:[27] zinc powder (25.0 g, 0.380 mol) was added to a two-necked round-bottomed flask (500 mL) containing demineralized water (100 mL) and the solution was degassed with nitrogen for 15 min. Subsequently, copper(II) sulfate (1.85 g, 11.5 mmol) was added and the stirring solution was degassed for 45 min. The mixture was filtered, and the solids were washed with degassed water (250 mL) and degassed acetone (250 mL), respectively. The zinc−copper couple was dried *in vacuo* for 12 h. [2 + 2]-Cycloaddition: *tert*-butyl(((3a*R*,4*S*,6a*R*)-2,2-dimethyl-6-methylenetetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-oxy)dimethylsilane **15** (2.50 g, 8.79 mmol, 1.00 equiv) was dissolved in anhydrous diethyl ether (70 mL) and dried over molecular sieves and Zn(Cu) (7.93 g, 61.5 mmol, 7.00 equiv) was added. Trichloroacetyl chloride (2.45 mL, 22.0 mmol, 2.50 equiv), dissolved in anhydrous diethyl ether (20 mL) was charged in a glass syringe and added dropwise (6.5 mL/h) at room temperature over a period of 3 h. The mixture was filtered over Celite to remove the solids and rinsed with diethyl ether (400 mL). The organic layer was washed with $NaHCO_3$ (aq sat. 3 × 200 mL) and brine (2 × 150 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated *in vacuo* to afford the title compound **16**. Yellow oil. Yield: 97% (3.36 g,

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J. Org. Chem. XXXX, XXX, XXX−XXX

intermediate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.79 (dd, J = 5.8, 1.0 Hz, 1H), 4.55 (t, J = 5.3 Hz, 1H), 4.11 (dt, J = 9.8, 5.1 Hz, 1H), 3.65 (d_{AB}, J = 18.3 Hz, 1H), 3.12 (d_{AB}, J = 18.3 Hz, 1H), 2.36 (dd, J = 12.9, 5.4 Hz, 1H), 2.15 (dd, J = 12.9, 9.8 Hz, 1H), 1.48 (s, 3H), 1.37 (s, 3H), 0.91−0.93 (m, 9H), 0.12 ppm (d, J = 2.2 Hz, 6H). ^{13}C{1H} NMR ($CDCl_3$, 101 MHz): δ 191.8, 112.3, 90.5, 80.7, 80.1, 71.5, 51.7, 49.2, 39.6, 26.1, 25.9, 24.7 18.4, −4.6, −4.9 ppm.

Synthesis of (3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro-[cyclobutane-1,4′-cyclopenta[d]-[1,3]dioxol]-3-one ***17***. (1*R*,3a′*R*,6′*S*,6a′*R*)-6′-((*tert*-Butyldimethylsilyl)oxy)-2,2-dichloro-2′,2′-dimethyltetrahydrospiro-[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-3-one **16** (3.36 g, 8.50 mmol, 1.00 equiv) was dissolved in THF (80 mL) and zinc (5.56 g, 85.0 mmol, 10.0 equiv), followed by glacial acetic acid (3.89 mL, 68.0 mmol, 8.00 equiv) were added. The mixture was heated to 70 °C for 5 h using an oil bath and filtered over Celite, rinsed with THF (100 m), and concentrated to a minimal volume *in vacuo*. The residue was dissolved in EtOAc (300 mL) and washed with $NaHCO_3$ (3 × 100 mL) and brine (3 × 100 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified via silica gel chromatography (gradient elution: *n*-heptane/EtOAc: 1/0 to 2/3). Fractions containing the product were combined and the solvent was removed *in vacuo* to afford the title compound **17**. Colorless oil. Yield: 67% (two steps from **15**, 1.90 g, 5.82 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.49 (t, J = 5.1 Hz, 1H), 4.29−4.34 (m, 1H), 3.88 (dt, J = 10.9, 5.3 Hz, 1H), 3.36 (ddd, J = 18.3, 4.1, 2.4 Hz, 1H), 2.89−2.99 (m, 1H), 2.79−2.87 (m, 1H), 2.68−2.75 (m, 1H), 2.19 (t, J = 11.4 Hz, 1H), 1.82 (dd, J = 11.8, 5.7 Hz, 1H), 1.58 (s, 1H), 1.48 (s, 3H), 1.34 (s, 3H), 0.90−0.94 (m, 9H), 0.11 ppm (d, J = 2.8 Hz, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 206.0, 111.1, 85.2, 80.2, 72.3, 56.7, 52.8, 41.3, 34.4, 26.0, 26.0, 24.5, 18.4, −4.4, −4.7 ppm. GC−MS *m*/*z*: [M-TBDMSi] found 211.1, rt = 6.91 min.

General Procedure 1: Enzymatic Cyclobutanone Reduction. *Synthesis of (2S,4r,6R,7R,8S)-2-Hydroxy-6-methoxy-5-oxaspiro-[3.4]octane-7,8-diyl Bis(2,2-dimethylpropanoate)* ***22b***. The Codex KRED screening kit was purchased from Codexis. Detailed protocols for the initial enzyme screening can be found in the Supporting Information. (6*R*,7*R*,8*S*)-6-Methoxy-2-oxo-5-oxaspiro[3.4]octane-7,8-diyl bis(2,2-dimethylpropanoate) **20** (1.00 g, 2.81 mmol, 1.00 equiv) was dissolved in *i*PrOH (2.1 mL) in a falcon tube. The crude enzyme (B02, 50 mg) was added to a well-mixed solution of H_2O (Milli-Q, 12.6 mL) and KRED recycle mix P buffer (100 mg) [Na_3PO_4 (128 mM), $MgSO_4$ (1.7 mM), and $NADP^+$ (1.1 mM) at pH 7.0]. The aqueous solution was gently shaken to avoid foam formation until a homogeneous solution was obtained and was subsequently added all at once to ketone **20** dissolved in *i*PrOH. The mixture was shaken at 33 °C for 24 h using a shaking plate equipped with heating block. To the falcon tube was added EtOAc (10 mL) and the mixture was mixed vigorously and centrifuged (4000 rpm, 2 min). The organic layer was collected, and this extraction with EtOAc was repeated twice. Combined organic fractions were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 4:1 to 0:1). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **22b**. White solid. Yield: 92% (910 mg, 2.59 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.30 (d, J = 4.5 Hz, 1H), 5.13−5.17 (m, 1H), 4.84 (d, J = 1.6 Hz, 1H), 4.46−4.60 (m, 1H), 3.39 (s, 3H), 2.54−2.63 (m, 1H), 2.39−2.50 (m, 2H), 2.20−2.33 (m, 2H), 1.25 (s, 9H), 1.20 ppm (s, 9H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 177.6, 177.0, 105.5, 80.6, 75.2, 74.8, 62.6, 55.3, 44.3, 41.6, 39.0, 38.8, 27.2, 27.1 ppm. LC−MS (method 3, ESI^+) *m*/*z*: $[M + H]^+$ found 359.3, rt = 2.06 min. The spectroscopic results are consistent with the reported literature data.[9a]

(1s,3R,3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro-[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-ol ***(18a)***. The reaction was performed according to general procedure 1 by employing KRED enzyme D03 (65 mg), KRED recycle mix P buffer (150 mg), Milli-Q H_2O (9 mL), and *i*PrOH (1.45 mL). Colorless viscous oil. Yield: 95% (240 mg, 0.73 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.32 (t, J = 5.17 Hz, 1 H), 4.22−4.30 (m, 1 H), 4.18 (d, J = 5.28 Hz, 1 H), 3.77−3.85 (m, 1 H), 2.31 (br s, 1 H), 2.02−2.25 (m, 3 H), 1.85−1.93 (m, 1 H), 1.62−1.72 (m, 2 H), 1.42 (s, 3 H), 1.29 (s, 3 H), 0.90 (s, 9 H), 0.08 ppm (d, J = 1.76 Hz, 6 H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 110.42, 87.35, 79.59, 72.09, 63.86, 43.13, 40.97, 39.08, 35.41, 25.98, 24.48, 18.41, −4.4, −4.6 ppm. GC−MS *m*/*z*: [M-TBDMSi] found 213.1, rt = 6.07 min.

(1r,3S,3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro-[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-ol ***(18b)***. The reaction was performed according to general procedure 1 by employing KRED enzyme H07 (155 mg), KRED recycle mix P buffer (150 mg), Milli-Q H_2O (9 mL), and *i*PrOH (1.45 mL). Colorless viscous oil. Yield: 51% (129 mg, 0.39 mmol, 41% of **17** recovered). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.37 (t, J = 5.17 Hz, 1 H), 4.28 (quin, J = 7.32 Hz, 1 H), 4.16 (d, J = 5.28 Hz, 1 H), 3.76 (dt, J = 11.17, 5.53 Hz, 1 H), 2.59 (ddd, J = 11.99, 6.93, 5.28 Hz, 1 H), 2.22 (br s, 1 H), 2.13 (ddd, J = 11.55, 6.71, 5.28 Hz, 1 H), 1.93 (t, J = 11.44 Hz, 1 H), 1.84 (dd, J = 11.99, 7.59 Hz, 1 H), 1.59−1.71 (m, 2 H), 1.43 (s, 3 H), 1.30 (s, 3 H), 0.89 (s, 9 H), 0.07 ppm (d, J = 1.98 Hz, 6 H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 110.50, 84.90, 79.94, 71.62, 63.20, 43.28, 43.01, 37.64, 35.55, 25.98, 25.97, 24.49, 18.39, −4.4, −4.7 ppm.

Synthesis of tert-Butyl(((3a′R,6′S,6a′R)-2′,2′-dimethyl-3-methyleneteterahydrospiro-[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-6′-yl)oxy)dimethylsilane ***26***. Methyltriphenylphosphonium bromide (1.45 g, 3.98 mmol, 1.30 equiv) was weighed in an oven-dried vial and THF (12.0 mL) was added. The heterogeneous solution was cooled to 0 °C using an ice bath, and potassium *tert*-butoxide (3.98 mL, 1 M in THF, 3.98 mmol, 1.30 equiv) was added dropwise. The mixture was stirred at 0 °C for 20 min. The freshly prepared Wittig reagent was added dropwise via a syringe to (3a′*R*,6′*S*,6a′*R*)-6′-((*tert*-butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro-[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-3-one **17** (1.00 g, 3.06 mmol, 1.00 equiv) dissolved in THF (12.0 mL) at 0 °C. The yellow mixture was stirred for 1.5 h at 0 °C, followed by 1.5 h stirring at room temperature. The mixture was concentrated to a minimal volume *in vacuo* and redissolved in *n*-heptane (300 mL). Triphenylphosphine oxide was precipitated, and the mixture was sonicated for 5 min and filtered, and the filtrate was washed with NH_4Cl (aq sat. 2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 7:3). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **26**. Colorless oil. Yield: 94% (931 mg, 2.88 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.81 (quin, J = 2.4 Hz, 1H), 4.78 (quin, J = 2.4 Hz, 1H), 4.40 (t, J = 5.1 Hz, 1H), 4.26 (dd, J = 5.5, 0.9 Hz, 1H), 3.80 (dt, J = 11.2, 5.5 Hz, 1H), 2.87 (dd, J = 16.1, 2.2 Hz, 1H), 2.54 (dq, J = 15.9, 2.4 Hz, 1H), 2.31−2.46 (m, 2H), 1.95 (t, J = 11.4 Hz, 1H), 1.76 (dd, J = 11.7, 5.7 Hz, 1H), 1.45 (s, 3H), 1.32 (s, 3H), 0.91 (s, 9H), 0.10 ppm (d, J = 2.2 Hz, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 144.7, 110.4, 106.8, 85.3, 79.9, 72.0, 42.5, 41.2, 39.2, 36.8, 26.0, 26.0, 24.5, 18.4, −4.4, −4.6 ppm.

Synthesis of (6R,7R,8S)-6-Methoxy-2-methylene-5-oxaspiro[3.4]-octane-7,8-diyl Bis(2,2-dimethylpropanoate) ***28***. (6*R*,7*R*,8*S*)-6-Methoxy-2-oxo-5-oxaspiro[3.4]octane-7,8-diyl bis(2,2-dimethylpropanoate) **20** (2.00 g, 5.50 mmol, 1.00 equiv) was weighed in a three-neck 100 mL flask equipped with a reflux condenser, thermometer, and $CaCl_2$ tube. To this flask was added a solution of bis-(cyclopentadienyl)dimethyltitanium (**32**, 39.4 mL, 5 wt % in toluene, 7.97 mmol, 1.45 equiv, CAS: 1271-66-5). The flask was covered from light with aluminum foil and heated to 70 °C using an oil bath. The reaction was stirred for 17 h after which full conversion was observed. The mixture was concentrated to a minimal volume *in vacuo* and to the residue was added *n*-heptane (100 mL). The solids were sonicated for 5 min and removed via filtration over Celite (rinsed with *n*-heptane). The organic layer was concentrated to a minimal volume *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 3:7). Fractions containing the product were collected, and the solvent was evaporated to afford the title compound **28**. Colorless oil. Yield: 58% (1.14 g,

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J. Org. Chem. XXXX, XXX, XXX−XXX

3.19 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.42 (d, J = 4.5 Hz, 1H), 5.17, (dd, J = 4.5, 1.7 Hz, 1H), 4.85−4.83 (m, 3H), 3.39 (s, 3H), 3.09−3.05 (m, 2H), 2.94−2.85 (m, 2H), 1.21 (s, 9H), 1.20 ppm (s, 9H). ^{13}C{1H} NMR (125 MHz, $CDCl_3$): δ 177.4, 177.2, 139.7, 107.6, 105.7, 80.6, 75.6, 74.5, 55.6, 44.8, 41.5, 39.2, 39.0, 27.4, 27.3 ppm. LC−MS (method 1, ESI$^+$) m/z: [M + Na]$^+$ found 376.86.

Synthesis of (3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2′,2′-dimethyl-3-phenyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-ol ***25***. (3a′*R*,6′*S*,6a′*R*)-6′-((*tert*-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-3-one **17** (350 mg, 1.07 mmol, 1.00 equiv) was dissolved in anhydrous THF (5.50 mL) and cooled to −20 °C. Subsequently, phenylmagnesium bromide (0.44 mL, 2.9 M in THF, 1.29 mmol, 1.45 equiv) was added dropwise, and the mixture was stirred at −10 °C for 1 h using an ice/NaCl bath. The mixture was quenched at −10 °C by adding NH_4Cl (aq sat. 20 mL), and the product was extracted in CH_2Cl_2 (1 × 70 mL, 2 × 50 mL). Combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 2:3). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **25** as a 1:1 mixture of diastereoisomers. An analytical sample was purified via column chromatography for characterization of **25a** and **25b**. Colorless oil. Yield: 96% (417 mg, 1.03 mmol, combined). **25a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.35 (m, 2H), 7.22−7.29 (m, 2H), 7.12−7.19 (m, 1H), 4.22 (t, J = 5.1 Hz, 1H), 3.96 (d, J = 5.3 Hz, 1H), 3.69−3.82 (m, 1H), 2.87 (dd, J = 13.2, 1.4 Hz, 1H), 2.23 (dd, J = 12.2, 2.0 Hz, 1H), 2.15 (dd, J = 13.0, 2.8 Hz, 1H), 2.06 (dd, J = 12.2, 2.8 Hz, 1H), 1.94−2.01 (m, 2H), 1.34 (s, 3H), 1.14 (s, 3H), 0.81 (s, 9H), 0.00 ppm (s, 6H). ^{13}C{1H} NMR (101 MHz, CDCl3): δ 146.6, 128.5, 127.4, 125.0, 110.4, 85.9, 79.8, 73.7, 71.8, 46.9, 42.7, 40.7, 35.8, 26.1, 26.0, 24.5, 18.5, −4.4, −4.5 ppm. LC−MS (method 2, ESI$^+$) m/z: [M + H]$^+$ found 405.4 (R_t: 1.37 min, poor mass response). **25b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35−7.46 (m, 4H), 7.26−7.32 (m, 1H), 4.61 (d, J = 5.5 Hz, 1H), 4.44 (t, J = 5.2 Hz, 1H), 3.81 (dt, J = 10.7, 5.4 Hz, 1H), 2.62−2.69 (m, 1H), 2.53−2.61 (m, 1H), 2.27−2.36 (m, 1H), 2.18−2.27 (m, 1H), 2.13 (s, 1H), 1.94 (t, J = 11.4 Hz, 1H), 1.59 (dd, J = 11.7, 5.5 Hz, 1H), 1.48 (s, 3H), 1.36 (s, 3H), 0.90 (s, 9H), 0.08 ppm (d, J = 4.4 Hz, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 146.6, 128.5, 127.4, 125.0, 110.7, 86.5, 80.0, 74.4, 71.8, 46.6, 42.1, 40.9, 36.6, 26.1, 26.0, 24.6, 18.5, −4.4, −4.6 ppm. LC−MS (method 2, ESI$^+$) m/z: [M + H]$^+$ found 405.4 (R_t: 1.39 min, poor mass response).

General Procedure 2: Wittig Reaction. *Synthesis of 2-((3a′S,6′R,6a′R)-6′-Methoxy-2′,2′-dimethyldihydro-6′H-spiro [Cyclobutane-1,4′-furo[3,4-d][1,3]dioxol]-3-ylidene)acetonitrile* ***30***. (3a′*S*,6′*R*,6a′*R*)-6′-Methoxy-2′,2′-dimethyldihydro-6′*H*-spiro-[cyclobutane-1,4′-furo[3,4-*d*][1,3]dioxol]-3-one **19** (100 mg, 0.44 mmol, 1.00 equiv) was dissolved in THF (1.5 mL) and cyanomethyltriphenylphosphonium ylide **29** (191 mg, 0.66 mmol, 1.50 equiv) was added. The mixture was heated to 65 °C for 21 h using an oil bath. The mixture was concentrated to a minimal volume *in vacuo*, and the residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 99:1 to 1:9). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **30** as a 1:1 mixture of diastereoisomers. An analytical sample was purified by silica gel chromatography for the characterization of both diastereoisomers **30a** and **30b**. Colorless oil. Yield: 55% (60 mg, 0.24 mmol, combined). **30a**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.28−5.27 (m, 1H), 4.88 (s, 1H), 4.61 (d, J = 5.8 Hz, 1H), 4.56 (d, J = 5.8 Hz, 1H), 3.34 (s, 3H), 3.34−3.26 (m, 4H), 3.13−3.10 (m, 1H), 3.01−3.00 (m, 2H), 1.39 (s, 3H), 1.30 ppm (s, 3H). ^{13}C{1H} NMR (125 MHz, $CDCl_3$): δ 162.6, 116.0, 112.9, 108.4, 93.9, 85.2, 83.9, 81.7, 55.2, 46.3, 40.3, 26.5, 25.3 ppm. **30b**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.26−5.24 (m, 1H), 4.89 (s, 1H), 4.60 (d, J = 5.8 Hz, 1H), 4.55 (d, J = 5.8 Hz, 1H), 3.48−3.42 (m, 1H), 3.34 (s, 3H), 3.22−3.17 (m, 1H), 2.97−2.93 (m, 1H), 2.89−2.84 (m, 1H), 1.41 (s, 3H), 1.31 ppm (s, 1H). ^{13}C{1H} NMR (125 MHz, $CDCl_3$): δ 162.6, 116.0, 113.0, 108.5, 93.9, 85.2, 83.9, 81.8, 55.3, 46.5, 40.2, 26.5, 25.4 ppm.

(6R,7R,8S)-2-(Cyanomethylene)-6-methoxy-5-oxaspiro[3.4]-octane-7,8-diyl Bis(2,2-dimethylpropanoate) ***(31)***. The reaction was performed according to general procedure 2. Colorless oil. Yield: 42% (22 mg, 0.059 mmol, combined). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.42 (d, J = 4.46 Hz, 1H), 5.41 (d, J = 4.46 Hz, 1H), 5.24−5.22 (m, 2H), 5.19−5.17 (m, 2H), 4.84 (s, 1H), 4.82 (s, 1H), 3.43−3.33 (m, 7H), 3.32−3.24 (m, 4H), 3.21−3.16 (m, 1H), 3.10−2.98 (m, 4H), 1.21 (s, 18H), 1.20 ppm (s, 18H). ^{13}C{1H} NMR (125 MHz, $CDCl_3$): δ 177.5, 177.2, 177.1, 177.0, 161.9, 161.8, 116.0, 115.8, 105.8, 105.7, 93.5, 93.3, 79.7, 79.6, 75.1, 74.2, 74.0, 55.7, 55.6, 45.5, 45.1, 42.7, 42.6, 39.2, 39.1, 39.1, 39.0, 27.3 ppm.

Synthesis of 7-(((1s,3R,3a′R,6′S,6a′R)-6′-((tert-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydro-spiro-[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-yl)oxy)quinoline ***33***. (1*r*,3*S*,3a′*R*,6′*S*,6a′*R*)-6′-((*tert*-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-3-ol **18b** (111 mg, 0.34 mmol, 1.00 equiv) was dissolved in THF (3 mL), and triphenylphosphine (106 mg, 0.41 mmol, 1.20 equiv), 7-hydroxyquinoline (58.8 mg, 0.41 mmol, 1.20 equiv), and diisopropyl azodicarboxylate (0.08 mL, 0.41 mmol, 1.2 equiv) were added at room temperature. The mixture was stirred for 17 h at room temperature, and additional portions of triphenylphosphine (53.0 mg, 0.21 mmol, 0.6 equiv), 7-hydroxyquinoline (29.4 mg, 0.21 mmol, 0.6 equiv), and diisopropyl azodicarboxylate (0.04 mL, 0.21 mmol, 0.6 equiv) were added. After 7 h, the mixture was concentrated *in vacuo*, and the residue was purified via silica gel chromatography (gradient: *n*-heptane:EtOAc 1:1 to 1:4). Fractions containing the product were combined, and the solvent was removed *in vacuo* to afford the title compound **33**. Colorless oil. Yield: 80% (123 mg, 0.27 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (dd, J = 4.4, 1.8 Hz, 1H), 7.93 (dd, J = 8.3, 1.4 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.12−7.15 (m, 1H), 7.11 (s, 1H), 7.07−7.14 (m, 1H), 7.04 (dd, J = 8.8, 2.4 Hz, 1H), 4.72 (quin, J = 6.8 Hz, 1H), 4.25 (t, J = 5.1 Hz, 1H), 4.14 (d, J = 5.3 Hz, 1H), 3.76 (dt, J = 10.9, 5.6 Hz, 1H), 2.31−2.44 (m, 2H), 2.17−2.31 (m, 1H), 1.85−1.94 (m, 2H), 1.70 (dd, J = 11.8, 5.6 Hz, 2H), 1.31 (s, 3H), 1.18 (s, 3H), 0.81 (s, 9H), 0.00 ppm (s, 6H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 158.4, 150.5, 149.9, 135.7, 128.9, 123.5, 120.2, 119.0, 110.6, 108.8, 87.1, 79.8, 72.1, 68.8, 41.5, 40.7, 37.0, 35.9, 26.1, 24.6, 18.5, −4.4, −4.5 ppm. LC−MS (method 3, ESI$^+$) m/z: [M + H]$^+$ found 456.3 (R_t: 2.76 min).

Synthesis of 7-(((1s,3R,3a′R,6′R,6a′S)-6′-(4-Chloro-7H-pyrrolo-[2,3-d]pyrimidin-7-yl)-2′,2′-dimethyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-3-yl)oxy)-quinoline ***36***. 7-(((1*s*,3*R*,3a′*R*,6′*S*,6a′*R*)-6′-((*tert*-Butyldimethylsilyl)oxy)-2′,2′-dimethyltetrahydro-spiro-[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-3-yl)oxy)quinoline **33** (123 mg, 0.27 mmol, 1.00 equiv) was dissolved in THF (3.50 mL) and *tert*-butylammonium fluoride (0.54 mL, 1 M in THF, 2 equiv) was added. The mixture was stirred for 1 h at room temperature and concentrated to a minimal volume *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with $NaHCO_3$ (1 × 25 mL) and brine (3 × 25 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to a minimal volume *in vacuo*. The residue was dissolved in anhydrous CH_2Cl_2 (2.00 mL) and anhydrous pyridine (0.07 mL, 0.84 mmol, 3.00 equiv) was added. The solution was cooled to 0 °C using an ice bath, and trifluoromethanesulfonic anhydride (0.07 mL, 0.39 mmol, 1.40 equiv) was added dropwise. The mixture was stirred for 30 min at 0 °C and then diluted in CH_2Cl_2 (25 mL), and $NaHCO_3$ (aq 5 mL) was added. The product was extracted in CH_2Cl_2 (3 × 25 mL), combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The orange residue was used immediately further. 4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**35b**, 206 mg, 1.07 mmol, 4.00 equiv) was dissolved in anhydrous dimethylformamide (DMF) (2.0 mL), and the crude triflate residue dissolved in DMF (1.5 mL) was added dropwise at −10 °C for 15 min. The mixture was stirred for 1.5 h at −15 °C in an ice/NaCl bath and then 30 min at room temperature. The reaction was quenched with NH_4Cl (aq sat. 50 mL), and the product was extracted with CH_2Cl_2 (3 × 80 mL).

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J. Org. Chem. XXXX, XXX, XXX−XXX

Combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. A mixture of the desired substitution product **36** (75%) and elimination side product **37** (25%) was obtained, as evidenced via NMR analysis. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 0:1). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **36**. White solid. Yield: 47% (three steps, 59 mg, 0.12 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (dd, J = 4.3, 1.8 Hz, 1H), 8.66 (s, 1H), 8.06 (dd, J = 8.1, 1.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.15−7.36 (m, 5H), 6.64 (d, J = 3.3 Hz, 1H), 5.10 (dd, J = 6.7, 3.9 Hz, 1H), 4.89−5.01 (m, 1H), 4.69−4.85 (m, 2H), 2.78 (dd, J = 12.0, 7.1 Hz, 1H), 2.34−2.63 (m, 6H), 1.86 (br s, 1H), 1.52 (s, 3H), 1.31 ppm (s, 3H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 158.3, 152.4, 150.9, 150.6, 150.6, 149.8, 135.7, 129.0, 127.8, 123.6, 120.1, 119.1, 118.1, 113.2, 108.7, 100.0, 85.7, 83.9, 67.9, 61.5, 42.8, 40.3, 39.8, 36.6, 26.6, 24.8 ppm. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{26}H_{26}ClN_4O_3$, 477.1693; found, 477.1691 (R_t: 10.96 min). 7-(((1*s*,3*S*,3a′*R*,6a′*S*)-2′,2′-Dimethyl-3a′,6a′-dihydrospiro[cyclobutane-1,4′-cyclopenta-[*d*][1,3]dioxol]-3-yl)oxy)quinoline **37**: Colorless oil. Yield: 10% (three steps, 9 mg, 0.03 mmol), colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (dd, J = 4.3, 1.8 Hz, 1H), 8.06 (dd, J = 8.1, 1.6 Hz, 1H), 7.69 (d, J = 9.0 Hz, 1H), 7.22−7.30 (m, 2H), 7.18 (dd, J = 8.7, 2.6 Hz, 1H), 5.97 (d, J = 5.7 Hz, 1H), 5.79 (dd, J = 5.7, 1.6 Hz, 1H), 5.13 (dd, J = 5.7, 1.2 Hz, 1H), 4.92 (t, J = 6.9 Hz, 1H), 4.51 (d, J = 6.1 Hz, 1H), 2.77 (dd, J = 12.2, 6.9 Hz, 1H), 2.49−2.62 (m, 2H), 2.41 (dd, J = 11.8, 6.9 Hz, 1H), 1.36 (s, 3H), 1.35 ppm (s, 3H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 158.3, 150.5, 149.8, 140.4, 135.7, 129.5, 128.9, 123.5, 120.2, 119.0, 111.1, 108.7, 86.4, 84.6, 68.0, 46.9, 40.7, 36.7, 27.5, 26.2 ppm. LC−MS (method 2, ESI$^+$) m/z: [M + H]$^+$ found 324.2 (R_t: 1.12 min).

Synthesis of 7-((1s,3R,3a′R,6′R,6a′S)-2′,2′-Dimethyl-3-(quinolin-7-yloxy)tetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]-dioxol]-6′-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine ***41***. 7-(((1*s*,3*R*,3a′*R*,6′*R*,6a′*S*)-6′-(4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2′,2′-dimethyltetrahydrospiro[cyclobutane-1,4′-cyclopenta[*d*]-[1,3]dioxol]-3-yl)oxy)-quinoline **36** (46.0 mg, 0.10 mmol, 1.00 equiv) was dissolved in 1,4-dioxane (10 mL) and NH_3 (28% in H_2O, 30 mL) was added. The mixture was heated to 90 °C in a pressure reactor in a heating block for 17 h. The mixture was concentrated to a minimal volume *in vacuo*, and CH_2Cl_2 (30 mL) and brine (15 mL) were added. The product was extracted in CH_2Cl_2 (3 × 30 mL), combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified via silica gel chromatography (gradient: CH_2Cl_2/MeOH from 99:1 to 85:15), fractions containing the product were collected, and the solvent was removed *in vacuo* to afford the title compound **41**. Slight orange oil. Yield: 80% (35.5 mg, 0.08 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.80 (dd, J = 4.29, 1.65 Hz, 1H) 8.34 (s, 1H) 8.05 (dd, J = 8.25, 1.21 Hz, 1H) 7.68 (d, J = 9.02 Hz, 1H) 7.21−7.28 (m, 2H) 7.16 (dd, J = 8.91, 2.53 Hz, 1H) 6.90 (d, J = 3.74 Hz, 1H) 6.37 (d, J = 3.52 Hz, 1H) 5.36 (br s, 2H) 5.09 (dd, J = 6.71, 3.41 Hz, 1H) 4.91 (td, J = 7.26, 3.30 Hz, 1H) 4.77 (quin, J = 7.04 Hz, 1H) 4.68 (d, J = 6.60 Hz, 1H) 2.75 (dd, J = 12.21, 7.15 Hz, 1H) 2.29−2.52 (m, 5H) 1.52 (s, 3H) 1.33 ppm (s, 3H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 158.4, 156.8, 151.8, 150.5, 150.5, 149.8, 135.7, 128.9, 123.5, 123.3, 120.2, 119.0, 112.8, 108.8, 103.7, 97.9, 86.0, 84.2, 68.1, 60.8, 42.9, 40.3, 39.9, 36.6, 26.5, 24.8 ppm. LC−MS (method 2, ESI$^+$) m/z: [M + H]$^+$ found 458.3 (R_t = 0.92 min).

Synthesis of (2R,4s,5R,6S,7R)-7-(4-Amino-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-(quinolin-7-yloxy)spiro[3.4]octane-5,6-diol ***38***. 7-((1*s*,3*R*,3a′*R*,6′*R*,6a′*S*)-2′,2′-Dimethyl-3-(quinolin-7-yloxy)-tetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-6′-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **41** (35 mg, 0.08 mmol, 1.00 equiv) was dissolved in EtOH (1 mL) and HCl (0.77 mL, 1 M in H_2O, 0.76 mmol, 10.0 equiv) was added. The mixture was stirred at room temperature for 4 h and HCl (0.77 mL, 1 M in H_2O, 0.76 mmol, 10.0 equiv) was added. After 3 h, the mixture was diluted with water (8 mL) and lyophilized to afford the title compound **38**. Slight orange oil. Yield: 82% (28.5 mg, 0.06 mmol, HCl salt). An analytical sample was purified via Prep SCF (stationary phase: CHIRALCEL Daicel OJ 20 × 250 mm, mobile phase: CO_2, EtOH + 0.4 *i*PrNH$_2$) to afford the title compound **38**. Slight orange solid. Yield: 31% (10.0 mg, 0.025 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (dd, J = 4.3, 1.7 Hz, 1H), 8.26 (dd, J = 8.1, 1.3 Hz, 1H), 8.06 (s, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.36 (dd, J = 8.1, 4.2 Hz, 1H), 7.21−7.30 (m, 3H), 6.91 (s, 2H), 6.57 (d, J = 3.5 Hz, 1H), 4.80−4.99 (m, 4H), 4.25 (q, J = 5.7 Hz, 1H), 3.82 (t, J = 5.0 Hz, 1H), 2.54−2.71 (m, 1H), 2.43−2.49 (m, 1H), 2.26−2.37 (m, 1H), 2.21 (dd, J = 11.2, 7.0 Hz, 1H), 2.06 ppm (dd, J = 13.1, 8.9 Hz, 1H). ^{13}C{1H} NMR (101 MHz, DMSO-d_6): δ 157.9, 157.4, 151.3, 150.6, 149.8, 149.4, 135.6, 129.3, 123.0, 122.5, 119.5, 119.2, 108.6, 102.8, 98.6, 77.2, 75.3, 67.8, 59.8, 40.3, 39.6, 39.2, 36.2 ppm. HRMS (ESI$^+$) m/z: [M + H]$^+$ calcd for $C_{23}H_{24}N_5O_3$, 418.1879; found, 418.1873 (R_t: 6.73 min).

Synthesis of (3a′R,6′S,6a′S)-2′,2′-Dimethyl-3-methylenetetrahydrospiro[cyclobutane-1,4′-cyclopenta-[d][1,3]-dioxol]-6′-ol ***42***. *tert*-Butyl(((3a′*R*,6′*S*,6a′*R*)-2′,2′-dimethyl-3-methylenetetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]-dioxol]-6′-yl)oxy)dimethylsilane **26** (931 mg, 2.87 mmol, 1.00 equiv) was dissolved in THF (2.00 mL), and TBAF (10.0 mL, 1 M in THF, 10.0 mmol, 3.50 equiv) was added. The mixture was stirred at room temperature for 3 h. The mixture was concentrated to a minimal volume *in vacuo*, dissolved in EtOAc (250 mL), and washed with NH_4Cl (aq sat. 3 × 50 mL) and brine (3 × 50 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 0:1). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **42**. Colorless oil. Yield: 92% (556 mg, 2.64 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.83 (quin, J = 2.3 Hz, 1H), 4.80 (quin, J = 2.4 Hz, 1H), 4.46−4.49 (m, 1H), 4.36−4.39 (m, 1H), 3.81 (br s, 1H), 2.82−2.88 (m, 1H), 2.60 (dq, J = 16.1, 2.4 Hz, 1H), 2.38−2.46 (m, 2H), 2.27−2.38 (m, 1H), 1.97 (dd, J = 12.0, 5.9 Hz, 1H), 1.74 (t, J = 11.4 Hz, 1H), 1.47 (s, 3H), 1.36 ppm (s, 3H). ^{13}C{1H} NMR (101 MHz, $CDCl_3$): δ 144.1, 110.6, 107.2, 85.4, 78.7, 70.8, 41.7, 41.2, 39.5, 36.6, 25.9, 24.3 ppm.

Synthesis of 4-Chloro-7-((3a′R,6′R,6a′S)-2′,2′-dimethyl-3-methylenetetrahydrospiro-[cyclobutane-1,4′-cyclopenta[d][1,3]dioxol]-6′-yl)-7H-pyrrolo[2,3-d]pyrimidine ***43***. (3a′*R*,6′*S*,6a′*S*)-2′,2′-Dimethyl-3-methylenetetrahydrospiro[cyclobutane-1,4′-cyclopenta-[*d*][1,3]-dioxol]-6′-ol **42** (647 mg, 3.08 mmol, 1.00 equiv) was dissolved in anhydrous CH_2Cl_2 (20.0 mL), and pyridine (0.62 mL, 7.69 mmol, 2.50 equiv) was added. The mixture was cooled to 0 °C using an ice bath, and trifluoromethanesulfonic anhydride (0.57 mL, 3.39 mmol, 1.10 equiv) was added dropwise. The mixture was stirred for 30 min at 0 °C, diluted in CH_2Cl_2 (100 mL), and $NaHCO_3$ (aq sat. 40 mL) was added. The product was extracted in CH_2Cl_2 (3 × 100 mL), combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in anhydrous DMF (8 mL) and added dropwise over 15 min to a mixture of **35b** (5.90 g, 30.8 mmol, 10.0 equiv) in anhydrous DMF (35.0 mL), which was previously stirred for 30 min at 0 °C using an ice bath. After addition, the mixture was stirred for 2 h at 0 °C, then warmed to room temperature, and stirred for an additional 2 h. The mixture was poured in NH_4Cl (aq sat. 50 mL), and the product was extracted in EtOAc (3 × 100 mL). Combined organic layers were washed with brine (3 × 100 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated *in vacuo* to a minimal volume. To the resulting powder, *n*-heptane (100 mL) was added and the mixture was sonicated for 10 min. The solids were filtered and rinsed with *n*-heptane, and the filtrate was concentrated to a minimal volume *in vacuo*. The residue was purified by column chromatography over silica gel (gradient elution: *n*-heptane/EtOAc from 1:0 to 0:1). The fractions containing the product were collected, and the solvent was evaporated to afford the title compound **43**. Off-white powder. Yield: 80% (two steps, 856 mg, 2.46 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64−8.65 (m, 1H), 7.18 (d, J = 3.7 Hz, 1H), 6.61 (d, J = 3.7 Hz, 1H), 5.10 (dd, J = 6.3, 3.1 Hz, 1H), 4.96 (td, J = 6.8, 3.1 Hz, 1H), 4.82 (quin, J = 2.3 Hz, 1H), 4.78 (quin, J = 2.3 Hz, 1H), 4.69 (d, J = 6.5 Hz, 1H), 3.18 (dd, J = 15.5, 2.4 Hz, 1H), 2.75 (dd, J = 15.3, 2.6

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J. Org. Chem. XXXX, XXX, XXX−XXX

Hz, 1H), 2.48−2.58 (m, 2H), 2.33−2.44 (m, 2H), 1.55 (s, 3H), 1.35 ppm (s, 3H). $^{13}C\{1H\}$ NMR (101 MHz, $CDCl_3$): δ 152.3, 150.6, 143.4, 127.5, 117.9, 112.7, 107.0, 99.8, 85.7, 84.9, 61.5, 43.0, 42.3, 42.2, 38.3, 26.6, 24.7 ppm. LC−MS (method 3, ESI^+) m/z: $[M + H]^+$ found 346.2 (R_t: 2.32 min).

Synthesis of 7-((3a′R,6′R,6a′S)-2′,2′-Dimethyl-3-methylenetetrahydrospiro[cyclobutane-1,4′-cyclopenta[d][1,3]-dioxol]-6′-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine ***39***. 4-Chloro-7-((3a′*R*,6′*R*,6a′*S*)-2′,2′-dimethyl-3-methylenetetrahydrospiro-[cyclobutane-1,4′-cyclopenta[*d*][1,3]dioxol]-6′-yl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine **43** (850 mg, 2.46 mmol, 1.00 equiv) was dissolved in 1,4-dioxane (25 mL) and NH_3 (aq 28%, 60 mL) was added. The mixture was heated in a sealed pressure reactor to 100 °C in a heating block for 24 h. Then, the solution was concentrated to a minimal volume *in vacuo* and coevaporated twice with toluene. The residue was purified via column chromatography over silica gel (gradient elution: CH_2Cl_2/MeOH from 1:0 to 4:1). Fractions containing the product were combined to afford the title compound **39**. White solid. Yield: 98% (790 mg, 2.41 mmol). ^1H NMR (400 MHz, $CDCl_3$): δ 8.33 (s, 1H), 6.89 (d, J = 3.7 Hz, 1H), 6.34 (d, J = 3.5 Hz, 1H), 5.17 (br s, 2H), 5.08 (dd, J = 6.4, 2.9 Hz, 1H), 4.92 (td, J = 6.7, 2.9 Hz, 1H), 4.80 (quin, J = 2.4 Hz, 1H), 4.76 (quin, J = 2.4 Hz, 1H), 4.67 (d, J = 6.4 Hz, 1H), 3.16 (dq, J = 15.5, 2.4 Hz, 1H), 2.67−2.78 (m, 1H), 2.45−2.55 (m, 2H), 2.29−2.44 (m, 2H), 1.35 ppm (s, 3H). $^{13}C\{1H\}$ NMR (101 MHz, $CDCl_3$): δ 156.6, 151.7, 150.5, 143.8, 123.0, 112.4, 106.8, 103.5, 97.6, 85.9, 85.1, 60.8, 43.1, 42.5, 42.3, 38.3, 26.6, 24.7 ppm. LC−MS (method 2, ESI^+) m/z: $[M + H]^+$ found 327.3 (R_t: 0.89 min).

Synthesis of (2S,4r,5R,6S,7R)-7-(4-Amino-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-((3-chloroquinolin-7-yl)methyl)spiro[3.4]octane-5,6-diol ***40a*** *and (2R,4s,5R,6S,7R)-7-(4-Amino-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-((3-chloroquinolin-7-yl)methyl)spiro[3.4]octane-5,6-diol* ***40b***. 7-((3a′*R*,6′*R*,6a′*S*)-2′,2′-Dimethyl-3-methylenetetrahydrospiro[cyclobutane-1,4′-cyclopenta[*d*][1,3]-dioxol]-6′-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **39** (52.0 mg, 0.16 mmol, 1.00 equiv) was dissolved in 9-borabicyclo[3.3.1]nonane (0.5 M in THF, 1.91 mL, 0.96 mmol, 6.00 equiv) at room temperature. The mixture was stirred for 30 min. Subsequently, potassium phosphate (271 mg, 1.28 mmol, 8.00 equiv) dissolved in water (0.50 mL, 27.7 mmol, 173 equiv) was degassed with nitrogen for 10 min and added to the reaction mixture. The solution was stirred for 10 min at room temperature while degassing, and 7-bromo-3-chloroquinoline (49.8 mg, 0.207 mmol, 1.30 equiv) and 1,1′-bis(di*tert*-butylphosphino)ferrocene palladium dichloride (15.7 mg, 0.03 mmol, 0.15 equiv) dissolved in THF (2.00 mL) were added to the mixture. Degassing with nitrogen was continued for 15 min before the mixture was heated to 70 °C using an oil bath. After 2 h, the dark brown solution was cooled to room temperature, diluted with EtOAc (90 mL), and washed with NH_4OH (25% in H_2O, 2 × 30 mL) and brine (2 × 30 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue (78.1 mg) was dissolved in EtOH (2 mL), and HCl (aq 1 M, 3 mL) was added. The mixture was stirred at room temperature for 1 h. The solution was diluted with water (20 mL), frozen, and lyophilized to afford a solid residue. Purification was performed via Prep SFC (stationary phase: CHIRALCEL Daicel OJ 20 × 250 mm, mobile phase: CO_2, EtOH + 0.4 *i*PrNH_2). Fractions containing the products were combined to afford the title compounds **40a** and **40b**. **40a**: White solid. Yield: 21% (two steps, 10.5 mg, 0.02 mmol). ^1H NMR (400 MHz, DMSO-d_6): δ 8.84 (d, J = 2.6 Hz, 1H), 8.51 (d, J = 2.4 Hz, 1H), 8.00 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 7.52 (dd, J = 8.4, 1.5 Hz, 1H), 7.15 (d, J = 3.5 Hz, 1H), 6.86 (s, 2H), 6.52 (d, J = 3.5 Hz, 1H), 4.79−4.85 (m, 1H), 4.75 (dd, J = 10.1, 5.5 Hz, 2H), 4.17−4.24 (m, 1H), 3.71 (t, J = 4.8 Hz, 1H), 2.90 (d, J = 7.7 Hz, 2H), 2.53−2.67 (m, 1H), 2.27−2.40 (m, 1H), 2.14 (dd, J = 11.2, 8.8 Hz, 1H), 2.00−2.10 (m, 1H), 1.91 (dd, J = 13.2, 8.6 Hz, 1H), 1.85 (dd, J = 10.8, 8.6 Hz, 1H), 1.73 ppm (ddd, J = 11.2, 7.9, 3.5 Hz, 1H). $^{13}C\{1H\}$ NMR (101 MHz, DMSO-d_6): δ 157.9, 151.7, 150.3, 149.5, 146.5, 143.8, 134.4, 129.9, 127.7, 127.7, 127.2, 127.0, 122.8, 103.2, 99.1, 78.4, 76.0, 60.0, 42.8, 42.7, 41.7, 38.5, 34.7, 30.8 ppm. HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{24}H_{25}ClN_5O_2$, 450.1697; found, 450.1693 (R_t: 8.33 min). **40b**: White powder. Yield: 26% (two steps, 13.2 mg, 0.03 mmol). ^1H NMR (400 MHz, DMSO-d_6): δ 8.84 (d, J = 2.4 Hz, 1H), 8.51 (d, J = 2.2 Hz, 1H), 8.01 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.82 (s, 1H), 7.52 (dd, J = 8.4, 1.3 Hz, 1H), 7.11 (d, J = 3.5 Hz, 1H), 6.87 (s, 2H), 6.54 (d, J = 3.5 Hz, 1H), 4.73−4.88 (m, 3H), 4.23−4.32 (m, 1H), 3.78 (t, J = 4.1 Hz, 1H), 2.89 (d, J = 7.0 Hz, 2H), 2.51−2.58 (m, 1H), 2.42−2.48 (m, J = 3.7 Hz, 1H), 2.29 (dd, J = 13.6, 10.3 Hz, 1H), 2.09−2.18 (m, 1H), 1.76−1.91 (m, 2H), 1.54 ppm (br dd, J = 10.5, 8.0 Hz, 1H). $^{13}C\{1H\}$ NMR (101 MHz, DMSO-d_6): δ 157.9, 151.7, 150.5, 149.5, 146.5, 143.8, 134.4, 129.9, 127.7, 127.2, 127.0, 122.4, 103.2, 99.1, 77.9, 76.2, 59.3, 43.1, 43.0, 42.4, 40.8, 34.5, 30.7 ppm. HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{24}H_{25}ClN_5O_2$, 450.1697; found, 450.1692 (R_t: 8.31 min).

Computational Details. The reduction of 4′-spirocyclobutanones **17** and **19** with the $LiAlH_4$ reducing agent was investigated using the Gaussian quantum chemistry package (version G09.D01).[28] In line with our previous works on the facial selectivity of hydride reductions on 2-substituted cyclohexanones[29] and 3-substituted cyclobutanones,[15] all quantum-chemical calculations were performed using a two-step computational protocol. In our recent benchmark of DFT approximations,[29] single-point energy calculations with the double-hybrid B2PLYP-D3[30] functional on the ωB97X-D[31] optimized geometries were shown to provide very accurate transition-state energies with respect to the canonical gold standard CCSD(T) energies, while providing cis/trans ratios in good agreement with the experimental observations.

All the transition states were optimized and characterized by means of harmonic vibrational frequency calculations using a large "ultrafine" integration grid at the ωB97X-D/cc-pVDZ[32] level of theory. The characteristic single negative eigenvalue of the Hessian matrix, coinciding with a transition state, was identified as the transfer of the hydride toward the carbonyl functionality. The thermodynamic corrections to the enthalpy (ΔH) and Gibbs free energy (ΔG) were computed at 1 atm for both room (298.15 K) and reaction (195.15 K) temperature. Subsequently, single-point energy calculations using the double-hybrid B2PLYP-D3 functional and aug-cc-pVTZ[33] basis set were performed on the ωB97X-D geometries. The DFT-D3 empirical dispersion correction of Grimme together with the Becke−Johnson damping function was included because it improves the description of noncovalent interactions and reaction barrier heights.[34] Implicit solvent effects for THF were assessed on the gas-phase geometries by means of the polarizable continuum model with radii and nonelectrostatic terms from Truhlar and co-workers' SMD model[35] at the same level of theory. The stereoselectivity of the reductions (cis/trans ratios) was determined based on the Gibbs free energies of the different transition states ($\Delta G^{\neq}$) using a Maxwell−Boltzmann distribution at 298.15 and 195.15 K.

The effect of NCIs on stereoselectivity was established using the NCI index,[17] as implemented in the NCIPLOT program,[36] starting from the ωB97X-D/cc-pVDZ wave functions of the optimized geometries of the transition states. The NCI index allows to visualize the noncovalent interactions by plotting the reduced density gradient (s) versus the electron density (ρ) multiplied by the sign of the second eigenvalue of the electron density Hessian matrix [$\mathrm{sign}(\lambda_2)$]. The latter is used to distinguish between attractive [$\mathrm{sign}(\lambda_2) < 0$] and repulsive [$\mathrm{sign}(\lambda_2) > 0$] interactions.[17] A very important tool consists of visualizing the reduced gradient isosurfaces in real space. The value of the $\mathrm{sign}(\lambda_2)\,\rho$ is used to color the different isosurfaces using a RGB (red−blue−green) scale: red isosurfaces indicate repulsive interactions, blue stands for attractive interactions, and green for very weak van der Waals-type interactions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01825.

Mass chromatography methods, NCI analysis of 4′-spirocyclobutanones **17** and **19**, DFT selectivity assess-

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ment of 3- and 3,3-(di)substituted cyclobutanone reductions, KRED screening results, method for *in vivo* inhibition studies, and copies of ^{1}H and ^{13}C NMR spectra for all the new compounds (PDF)

AUTHOR INFORMATION

Corresponding Author

Jonas Verhoeven − *Research Group of Organic Chemistry (ORGC), Department of Chemistry and Department of Bio-Engineering Sciences, Faculty of Science and Bio-Engineering Sciences, Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium; Janssen Pharmaceutica NV, 2340 Beerse, Belgium;* orcid.org/0000-0002-4125-8759; Email: jverhoe9@its.jnj.com

Authors

Xavier Deraet − *Research Group of General Chemistry (ALGC), Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium;* orcid.org/0000-0002-5296-857X

Vineet Pande − *Janssen Pharmaceutica NV, 2340 Beerse, Belgium*

Weimei Sun − *Janssen Pharmaceutica NV, 2340 Beerse, Belgium*

Mercedes Alonso − *Research Group of General Chemistry (ALGC), Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium;* orcid.org/0000-0002-7076-2305

Frank De Proft − *Research Group of General Chemistry (ALGC), Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium*

Lieven Meerpoel − *Janssen Pharmaceutica NV, 2340 Beerse, Belgium*

Jan Willem Thuring − *Janssen Pharmaceutica NV, 2340 Beerse, Belgium;* orcid.org/0000-0003-4154-1586

Guido Verniest − *Research Group of Organic Chemistry (ORGC), Department of Chemistry and Department of Bio-Engineering Sciences, Faculty of Science and Bio-Engineering Sciences, Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium; Janssen Pharmaceutica NV, 2340 Beerse, Belgium*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.0c01825

Notes

The authors declare no competing financial interest.

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