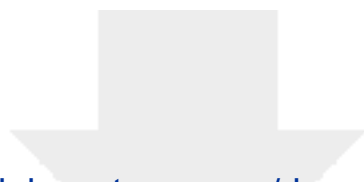# ChemBioChem

Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential
--Manuscript Draft--

|	
Manuscript Number:	cbic.201900472R1
Article Type:	Communication
Corresponding Author:	Tobias Alexander Marius Gulder TU München Garching, GERMANY
Corresponding Author E-Mail:	tobias.gulder@ch.tum.de
Order of Authors (with Contributor Roles):	Anna Sib
	Tobias M Milzarek
	Alexander Herrmann
	Lila Oubraham
	Jonas I Müller
	Andreas Pichlmair
	Ruth Brack-Werner
	Tobias Alexander Marius Gulder
Keywords:	biocatalysis * IAV * HIV * sorbicillinoids * anti-viral bioactivity
Abstract:	The sorbicillinoids are fungal polyketides that are characterized by highly complex and diverse molecular structures with considerable stereochemical intricacy combined with a high degree of oxygenation. Many sorbicillinoids possess promising biological activities. An interesting member of this natural product family is sorbicatechol A (7a), reported to have anti-viral activity, particularly against influenza A virus (H1N1). Utilizing a straightforward, one-pot chemo-enzymatic approach with the oxidoreductase SorbC that was recently developed in our group, we set out to structurally diversify the characteristic bicyclo[2.2.2]octane core of sorbicatechol by variation of its natural 2-methoxyphenol substituent. This facilitated the preparation of a focused library of structural analogs bearing substituted aromatic systems, alkanes, heterocycles and ethers. The fast access to this structural diversity provided an opportunity to explore the anti-viral potential of the sorbicatechol family.
Response to Reviewers:	The changes suggested by the review were all minor changes to the manuscript text. We have followed all suggestions and highlighted all changes by using track-changes in the doc file.
Section/Category:	ChemBioTalents-by invitation only
Additional Information:	
Question	**Response**
Submitted solely to this journal?	Yes
Has there been a previous version?	Yes
Please state previous 1) Manuscript ID and 2) journal. 3) If the paper was reviewed, please include a point-by-point response to the reviewer comments. as follow-up to "Has there been a previous version?"	cbic.201900170 The response to the reviewers can be found within cover letter.
Do you or any of your co-authors have a	No. The authors declare no conflict of interest.

conflict of interest to declare?	
Animal/tissue experiments?	No

Click here to access/download

Supporting Information

Gulder_Catechols_RevisedESI.docx

WILEY-VCH
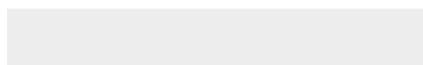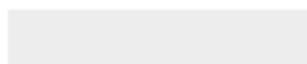
COMMUNICATION

Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential

Anna Sib,[‡][a] Tobias M. Milzarek,[‡][a,b] Alexander Herrmann,[c] Lila Oubraham,[d] Jonas I. Müller,[b] Andreas Pichlmair,[d] Ruth Brack-Werner,[c] and Tobias A. M. Gulder*[a,b]

Dedication ((optional))

Abstract: The sorbicillinoids are fungal polyketides ~~that are~~ characterized by highly complex and diverse molecular structures with considerable stereochemical intricacy combined with a high degree of oxygenation. Many sorbicillinoids possess promising biological activities. An interesting member of this natural product family is sorbicatechol A (7a), reported to have anti-viral activity, particularly against influenza A virus (H1N1). Utilizing a straightforward, one-pot chemo-enzymatic approach with the oxidoreductase SorbC that was recently developed in our group, we set out to structurally diversify the characteristic bicyclo[2.2.2]octane core of sorbicatechol by variation of its natural 2-methoxyphenol substituent. This facilitated the preparation of a focused library of structural analogs bearing substituted aromatic systems, alkanes, heterocycles and ethers. The fast access to this structural diversity provided an opportunity to explore the anti-viral potential of the sorbicatechol family.

Sorbicillinoids are a large polyketide natural product family consisting of more than 50 members.[1] They can be isolated from a diverse set of marine and terrestrial fungi and can be categorized into four different groups according to their molecular structures: the monomeric, dimeric, trimeric and the further functionalized sorbicillinoids. Biosynthetically, all sorbicillinoids derive from stereo- and regioselective oxidative dearomatization of sorbicillin (1) to the highly reactive sorbicillinol (2) by the oxidoreductase SorbC (Figure 1).[2] The dimeric/trimeric sorbicillinoids result from subsequent dimerization/trimerization of 2 with 1 or 2 by Michael addition or Diels-Alder cycloaddition due to the inherent respective reactivit~~iesy~~ of 2, leading to beautiful molecular architectures such as bisorbicillinol (3, resulting from a dimerization by Diels-Alder reaction)[3] or trichodimerol (4, double Michael addition/ketalization)[4] (Figure 1). Further functionalized sorbicillinoids are likewise formed by these transformations, instead of a simple dimerization, however, involving non-sorbicillinoid nucleophiles or dienophiles. Structurally and biomedically interesting examples of this class of sorbicillinoids include sorbicillactone A (5),[5] spirosorbicillinol A (6)[6] and sorbicatechol A (7a).[7]
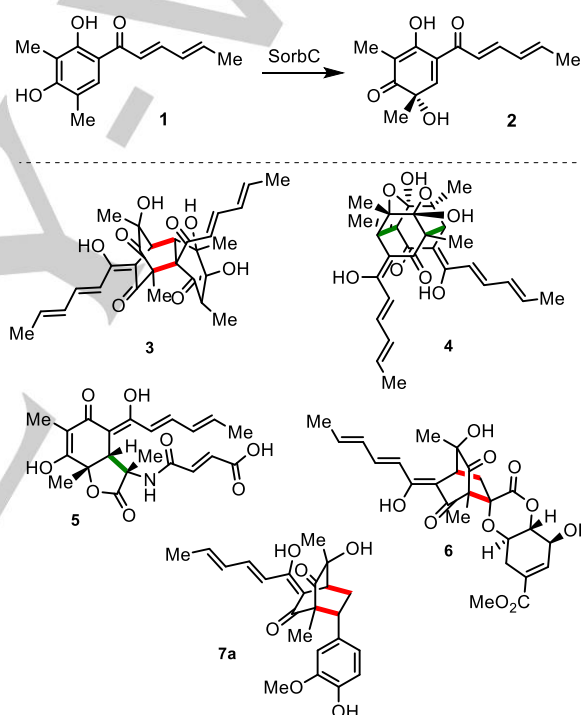

Figure 1. Top: Oxidative dearomatization of sorbicillin (1) to sorbicillinol (2). Bottom: Examples of dimeric sorbicillinoid natural products bisorbicillinol (3) and trichodimerol (4) and of further functionalized congeners formed by Mich~~aeea~~l addition (green bonds), e.g., sorbicillactone A (5), or Diels-Alder cycloaddition chemistry (red bonds), e.g., spirosorbicillinol A (6) and sorbicatechol A (7a).

The ~~reported~~ biological activities of the functionalized sorbicillinoids are as diverse as their structures. Sorbicillactone A (5) is active against murine leukemic lymphoblast cell line L5178y with an IC_{50} of 2.2 mg/mL and protects human T cells from HIV-1 in a concentration range of 0.3 and 3.0 mg/mL.[5] Spirosorbicillinol A (6) shows DPPH-radical scavenging activity,[6] a property also typical for dimeric sorbicillinoids such as 3.[3] While currently far less studied when compared to the above-mentioned compounds, sorbicatechol A (7a) was reported to exhibit promising anti-viral

[a]   A. Sib, T.M. Milzarek, Jonas I. Müller, Prof. Dr. T.A.M. Gulder
        Biosystems Chemistry, Department of Chemistry and Center for
        Integrated Protein Science Munich (CIPSM)
        Technical University of Munich
        Lichtenbergstraße 4, 85748 Garching (Germany)
        E-mail: tobias.gulder@ch.tum.de
[b]   T.M. Milzarek, Jonas I. Müller, Prof. Dr. T.A.M. Gulder
        Chair of Technical Biochemistry
        Technical University of Dresden
        Bergstraße 66, 01069 Dresden (Germany)
        E-mail: tobias.gulder@tu-dresden.de
[c]   A. Herrmann, Prof. Dr. R. Brack-Werner
        Helmholtz Zentrum München
        German Research Center for Environmental Health (GmbH)
        Institute of Virology
        Ingolstädter Landstraße 1, 85764 Neuherberg (Germany)
[d]   Dr. L. Oubraham, Prof. Dr. A. Pichlmair
        Immunopathology of Virus Infections Laboratory
        Institute of Virology
        Technical University of Munich
        Schneckenburgerstr. 8, 81675 Munich (Germany)
[‡]   These authors have contributed equally to this work.

activity against H1N1.[7] With an IC$_{50}$ value of 85 μM, **7a** shows identical potential as the antiviral drug ribavirin (IC$_{50}$ of 84 μM).

As the structural variability of any given type of natural sorbicillinoid core is rather limited and as the isolation of these compounds from the complex metabolic matrix of the fungal producer strains is tedious, synthetic strategies are required to facilitate biomedical studies on these fascinating molecules. While a number of elegant total synthetic routes towards dimeric sorbicillinoids such as **3** and **4** do exist, most of these are not stereoselective and/or rather lengthy.[8] In case of the sorbicatechol core structure, only a single total synthetic approach exists. This led to the synthesis of *ent*-rezishanone bearing an ethoxy- instead of the 2-methoxyphenol substituent when compared to **7a**. Overall, the synthetic route contains >20 individual steps with a combined yield of less than 3%,[9] clearly evidencing the need for improved synthetic strategies towards the sorbicillinoids. To this end, we have recently developed chemo-enzymatic approaches for the efficient one-pot synthesis of dimeric soribcillinoids such as **3** and **4**, utilizing synthetically readily available sorbicillin (**1**) that gets oxidatively dearomatized employing SorbC to give sorbicillinol (**2**) enantioselectively. The latter can be dimerized in a controlled manner depending on the organic cosolvent employed during the biocatalytic reaction.[10] This methodology was further extended to enable functionalization of **2** with external nucleophiles, e.g., leading to the first stereoselective total synthesis of epoxysorbicillinol, and dienophiles, yielding to the first synthetic access to rezishanones and sorbicatechol A (**7a**).[11] We herein present the application of this chemo-enzymatic toolkit featuring a facile intramolecular Diels-Alder reaction for the straightforward preparation, of a focused library of sorbicatechol-type structural analogs to enable the evaluation of their anti-viral potential.

The required synthetic starting material **1** for the biocatalytic reaction was prepared in three-steps starting from 2-methylresorcinol following our published procedure.[11] In a regio- and stereoselective oxidative dearomatization reaction using SorbC, **1** was transformed into **2**, the reactive precursor of all sorbicatechol derivatives. This oxidative dearomatization reactions proceeds with perfect stereocontrol, exclusively delivering the desired (S)-**2**.10 Quenching of the reaction solution containing **2** with a diverse set of dienophiles readily delivered the target compounds in yields ranging from 21% to 32% (Scheme 1), irrespective of the substitution of the ene-function. This is in the range of the typical product yields for all sorbicillinoids following this chemo-enzymatic strategy and can be explained by unreacted starting material **1** as well as by the formation of dimeric side-products, particularly **3**, that cannot be fully suppressed due to the intrinsic high reactivity of **2**. Besides the synthesis of sorbicatechol A (**7a**), sets of styrenes substituted at the aromatic portion (**9a-e**), of vinyl ethers (**9f-k**), ethyl vinyl ketone (**9l**) and heteroaromatic building blocks (**9m-o**) were employed to give the respective sorbicatechol analogs **7a-o**. All compounds were isolated by semi-preparative HPLC giving pure and stereochemically defined material (Figure S33).

In the original isolation paper, the absolute configuration of the biologically more active *endo* Diels-Alder product sorbicatechol A (**7a**) and its likewise isolated, minor *exo* congener was thoroughly

investigated by a combination of DFT-calculations, 2D NOESY measurements and analysis of experimental *versus* calculated CD spectra, allowing for an unambiguous assignment of the absolute configuration to both compounds.[7] Comparison of the NMR data of compound **7a** produced by our approach with the data reported for the respective *endo* and *exo* products clearly revealed our material to correspond to the *endo* product. This can most convincingly be deduced from chemical shift analysis of the ^{13}C data, most importantly at C2 (**7a**: 198.0, reported for *endo*: 197.9, reported for *exo*: 199.7), C3 (112.2, 112.0, 110,5), C7 (47.8, 47.8, 49,9), C8 (31.5, 31.4, 30,2), C16 (110.4, 110.2, 111.2) and C5-methyl (24.4, 24.3, 25.2) as well as from difference in ^{1}H chemical shifts, particularly at H8 (1.84 and 3.05, 1.84 and 3.05, 2.25 and 2.57), H16 (6.44, 6.45, 6.71) and H20 (24.4, 24.3, 25.2). In addition, NOESY interactions between the protons of C1-methyl at the sorbicillin-derived core structure with H16 and C17-OMe can only be seen in the *exo* analog,[7] and are consequently also absent in our product **7a** (Figure S4), in agreement with above *endo* assignment. Most importantly, the large chemical shift difference of the geminal protons attached to C8 as typical for the *endo* product **7a** (difference 1.21 ppm) when compared to *exo* (0.32), can also be found in all other compounds presented
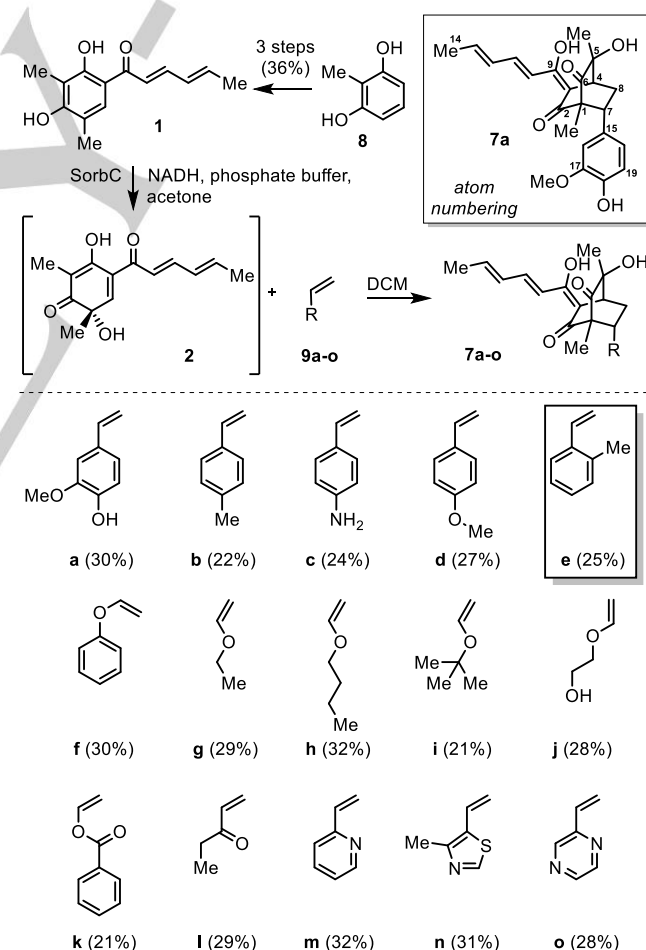
Scheme 1. Synthesis of sorbicatechol A (**7a**) and 14 structural analogs **7b–o**. Product yields for each analog are given in brackets.

in this work (**7b**: 1.15; **7c**: 1.23, **7d**: 1.16, **7e**: 1.24, **7f**: 1.22, **7g**: 1.10, **7h**: 1.09, **7i**: 1.17, **7j**: 1.12, **7k**: 1.42, **7l**: 1.46, **7m**: 1.53, **7n**: 1,45, **7o**: 0.89), thus corroborating all products to be *endo*. The

COMMUNICATION

regioselectivity of the Diels-Alder cycloadditions can generally be explained by the directing effect of the C1-methyl group (see numbering of **7a** in Scheme 1, *ortho*-rule).

Because of the reported anti-viral activity of **7a** against H1N1, a set of the prepared sorbicatechol analogs **7a-o** was evaluated for its anti-viral activity against influenza A virus (IAV). Initial tests were performed using the gaussia luciferase reporter virus.[12] Abundance of gaussia luciferase activity that accumulates in the supernatant of cells infected with this virus can be used as a proxy for virus replication. A resazurine conversion assay was also performed in order to assess the cytotoxic effect of the compounds on the cells.

Unfortunately, the tested compounds exhibited cytotoxicity to the viral host cells at concentrations below the observation of anti-viral effects. This effect thus not only made it impossible to assess any anti-viral effects against IAV, but also raises the question if the initially reported anti-viral activity of **7a**[7] is a true anti-viral effect or rather the result of the cytotoxicity of the compound to the host cells. To further broaden our anti-viral screening we thus decided to expand from only testing *Orthomyxoviridae* (IAV) to *Retroviridae* by including tests against HIV-1. This system was also chosen due to the significant need for finding compounds active against HIV. Despite the fact that current anti-HIV drugs can minimize virus replication and thereby prevent the outbreak of AIDS, a series of severe problems remain, including the rapid emergence of resistant viruses, high virus variability, high costs and adverse side-effects.[13] As test system for anti-HIV activity, the EASY-HIT assay[14] was performed, which is based on the reporter cell line LC5-RIC that contains a stably integrated fluorescent reporter gene. Upon HIV infection these cells express the fluorescent marker dsRed which can be directly connected to the anti-HIV activity of a compound when reduced during treatment.[14] We used the resazurin conversion assay, which is based on enzymatic reduction of resazurin,[15] to check for any signs of negative effects on the vitality and metabolism of the LC5-RIC cells that could appear during the course of the EASY-HIT assay. Out of the 15 sorbicatechol analogs evaluated for anti-HIV properties, six compounds showed activities beyond their respective cytotoxicity values as determined by the viability assay.[15] Interestingly, all active compounds were either derived of a styrene (**9a-e**) or equipped with an aromatic portion in close proximity to the ene functionality in the substrate, as in phenyl vinyl ether **9f**. All other compounds did not exhibit a measureable activity, including all additional ethers and all heteroaromatic systems as well as the benzoylated derivate **7k** with only one additional keto function in between the alkene and the aromatic moiety when compared to **7f**. These results strongly indicate that an aromatic portion in close proximity to the bicyclo[2.2.2]octane core structure of the sorbicatechols is required for activity. The selectivity indices (equaling the CC_{50}/IC_{50}) for the active compounds are mostly in the range of 1.37 to 1.72 (Table 1). In this range, cytotoxic effects on the host cells might indeed influence the anti-viral activity values. For the *ortho*-methyl substituted analog **7e**, however, a significant increase in activity can be observed, along with a slight decrease of cytotoxicity. Overall, this leads to a selectivity index of 3.49 for **7e**. Interestingly, a simple repositioning of the methyl substituent from the *para* (as in **7b**) to the *ortho* position led to a 2 fold increase in activity.

Table 1. Anti-HIV activity given as IC_{50} values and cytotoxicity determined as CC_{50} in the viability assay.[15] The selectivity index is calculated as CC_{50}/IC_{50}. Emtricitabine (FTC) was used as HIV inhibition control.

7x	Anti-HIV activity IC_{50} [µM]	Viability assay CC_{50} [µM]	Selectivity index (CC_{50}/IC_{50})
a	65.9 ± 4.68	*102**	1.55
b	68.8 ± 6.97	*105.2**	1.53
c	75.9 ± 7.37	*125.2**	1.65
d	62.7 ± 10.08	*108.4**	1.72
e	32.2 ± 2.52	*112.3**	3.49
f	76.6 ± 4.28	*105.2**	1.37
FTC	0.7 ± 0.22	*>100.0*	>140

Taken together, we have synthesized 15 sorbicatechol derivatives **7a**-**o** by application of a chemo-enzymatic synthesis of sorbicillinol (**2**) followed by quenching of **2** with a diverse set of dienophiles. Evaluation of their activity against IAV has revealed their cytotoxicity against the host cell system rather than a true anti-viral effect. Screening of the compound library against HIV-1 showed that aromatic substitution as the bicyclo[2.2.2]octane substructure is required to obtain anti-viral activity, although it remains weak. The strongest activity was obtained with **7e** with an IC_{50} value of approx. 32 µM and a selectivity index of 3.49. The activity is thus about 50-fold lower when compared to the commercial virostatic drug emtricitabine (FTC) with a >40-fold decreased selectivity index. In contrast to previous reports, our findings did ~~thus~~ not reveal antiviral activity of the sorbicatechols in a range promising for further antiviral lead optimization.

Experimental Section

Experimental Details.

The synthesis of sorbicatechol derivatives follows a two-phase procedure, which is described here exemplary for the production of the most active derivative **7e**. First, sorbicillin (**1**) is dissolved in acetone and added to phosphate buffer (50 mM, pH = 8) with the enzyme SorbC. The reaction starts upon addition of NADH and is incubated for 6 h at room temperature. Second, the produced sorbicillinol (**2**) is extracted with organic solvent and *o*-methylstyrene (**9e**) is added. After slow evaporation of the organic solvent under reduced pressure, to increase the concentration of the dienophile slowly over time, the crude product is purified by ~~preperative~~ preparative HPLC.

Acknowledgements

[1] A. M. Harned, K. A. Volp, *Nat. Prod. Rep.* **2011**, *28*, 1790-1810.

[2] A. Al-Fahad, A. Abood, K. M. Fisch, A. Osipow, J. Davison, M. Avramovic, C. P. Butts, J. Piel, T. J. Simpson, R. J. Cox, *Chem. Sci.* **2014**, *5*, 523-527.

[3] N. Abe, T. Murata, A. Hirota, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 661-666.

[4] R. Andrade, W. A. Ayer, P. P. Mebe, *Can. J. Chem.* **1992**, *70*, 2526-2536.

[5] a) G. Bringmann, G. Lang, T. A. M. Gulder, H. Tsuruta, J. Mühlbacher, K. Maksimenka, S. Steffens, K. Schaumann, R. Stöhr, J. Wiese, J. F. Imhoff, S. Perovic-Ottstadt, O. Boreiko, W. E. G. Müller, *Tetrahedron* **2005**, *61*, 7252-7265. b) G. Bringmann, T. A. M. Gulder, G. Lang, S. Schmitt, R. Stöhr, J. Wiese, K. Nagel, J. F. Imhoff, *Mar. Drugs* **2007**, *5*, 23-30.

[6] K. Washida, N. Abe, Y. Sugiyama, A. Hirota, *Biosci. Biotechnol. Biochem.*, **2009**, *73*, 1355-1361.

[7] J. Peng, X. Zhang, L. Du, W. Wang, T. Zhu, Q. Gu, D. Li, *J.Nat. Prod.*, **2014**, *77*, 424-428.

[8] a) K. C. Nicolaou, R. Jautelat, G. Vassilikogiannakis, P. S. Baran, K. B. Simonsen, *Chem. Eur. J.* **1999**, *5*, 3651–3665; b) K. C. Nicolaou, G. Vassilikogiannakis, K. B. Simonsen, P. S. Baran, Y.- L. Zhong, V. P. Vidali, E. N. Pitsinos, E. A. Couladouros, *J. Am.Chem. Soc.* **2000**, *122*, 3071–3079. b) R. Hong, Y. Cheng, L. Deng, *Angew. Chem. Int. Ed.* **2005**, *44*, 3478-3481. c) D. Barnes-Seeman, E. J. Corey, *Org. Lett.* **1999**, *1*, 1503-1504.

[9] Q. Yan, M. G. Banwell, M. L. Coote, R. Lee, A. C. Willis, *Chem. Asian J.* **2017**, *12*, 1480-1484.

[10] A. Sib, T.A.M. Gulder, *Angew. Chem. Int. Ed.* **2017**, *56*, 12888-12891.

[11] A. Sib, T.A.M. Gulder, *Angew. Chem. Int. Ed.* **2018**, *57*, 14650-14653.

[12] N. Eckert, F. Wrensch, S. Gärtner, N. Palanisamy, U. Goedecke, N. Jäger, S. Pöhlmann, M. Winkler, *PLoS One*, **2014**, *9*, 1-11.

[13] A. A. Waheed, G. Tachedjian, *Curr. Top. Med. Chem.* **2016**, *16*, 1343-1349.

[14] S. Kremb, M. Helfer, W. Heller, D. Hoffmann, H. Wolff, A. Kleinschmidt, S. Cepok, B. Hemmer, J. Durner, R. Brack-Werner, *Antimicrob. Agents Chemother.* **2010**, *54*, 5257-5268.

[15] T. L. Riss, R. A. Moravec, A. L. Niles, S. Duellman, H. A. Benink, T. J. Worzella, L. Minor in *Assay Guidance Manual*, Vol. 1 (Eds: G. S. Sittampalam, N. P. Coussens, K. Brimacombe, A. Grossman, M. Arkin, D. Auld, C. Austin, J. Baell, B. Bejcek, J. M. M. Caaverio, T. D. Y. Chung, J. L. Dahlin, V. Devanaryan, T. L. Foley, M. Glicksman, M D. Hall, J. V. Haas, J. Inglese, P. W. Iversen, S. D. Kahl, S. C. Kales, M. Lal-Nag, Z. Li, J. McGee, O. McManus, T. Riss, O. J. Trask, J. R. Weidner, M. J. Wildey, M. Xia, X.Xu), Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda, **2004**, pp. 305-335.

Entry for the Table of Contents (Please choose one layout)

Layout 2:

COMMUNICATION
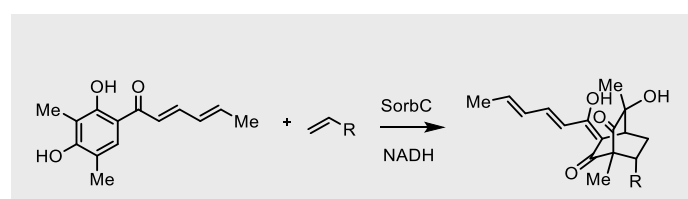
Stereoselective oxidative dearomatization using the enzyme SorbC facilitated the chemo-enzymatic total synthesis of a focused sorbicatechol library that allowed for the assessment of the anti-viral properties of this class of natural products against IAV and HIV.

Anna Sib, Tobias M. Milzarek, Alexander Herrmann, Lila Oubraham, Jonas I. Müller, Andreas Pichlmair, Ruth Brack-Werner, and Tobias A. M. Gulder*

Page No. – Page No.

Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential