Check for updates

EurJOC
European Journal of Organic Chemistry

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: Organocatalytic synthesis and anti-trypanosomal activity evaluation of L-pentofuranose-mimetic iminosugars

Authors: Juan Manuel Mesa, Marcelo Alberto Comini, Estefania Dibello, and Daniela Gamenara

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Eur. J. Org. Chem.* **2022**, e202200636

Link to VoR: https://doi.org/10.1002/ejoc.202200636

WILEY-VCH

WILEY-VCH

RESEARCH ARTICLE

Organocatalytic synthesis and anti-trypanosomal activity evaluation of L-pentofuranose-mimetic iminosugars

Juan M. Mesa,[a] Marcelo A. Comini,[b] Estefanía Dibello*[a,b] and Daniela Gamenara*[a]

[a] Mr. J. M. Mesa, Dr. E. Dibello, Dr. D. Gamenara
Organic Chemistry Department
Facultad de Química, Universidad de la República (UdelaR)
Av. Gral. Flores 2124. 11800, Montevideo, Uruguay
E-mail: edibello@fq.edu.uy; dgamenar@fq.edu.uy
[b] Dr. Marcelo A. Comini, Dr. E. Dibello
Group Redox Biology of Trypanosomes
Institut Pasteur de Montevideo
Mataojo 2020. 11400, Montevideo, Uruguay

Supporting information for this article is given via a link at the end of the document.

Abstract: A series of L-*lyxo* and L-*xylo*- pentofuranose-mimetic iminosugars and derivatives were efficiently synthesized in a stereoselective manner, through a concise strategy which involves an organocatalyzed Mannich reaction of an imine and a protected dioxanone as key step, using D-proline as catalyst. A prelimiary evaluation of their activity as potential anti-trypanosomal agents was studied. One of the synthesized compounds showed an interesting activity against bloodstream *Trypanosoma brucei brucei* (EC_{50} = 3.8 ± 1.0 μM).

Introduction

Iminosugars are natural occurring sugar mimetics[1] in which the oxygen atom of the hemiacetal carbohydrate ring is replaced by a basic nitrogen atom.[2] Their structural similarity to carbohydrates turns them potential competitive inhibitors of enzymes acting on sugar substrates.[3] They are, for example, suitable for being explored as antivirals,[4] acting as potential inhibitors of ER (Endoplasmic Reticulum) alpha-glucosidases I and II.[5] Iminosugars showed antiviral activity against a range of viruses including influenza,[6] HCV (Hepatitis C Virus),[7] and HIV (Human Immunodeficiency Virus).[8] Additionally, their therapeutic potential also includes antitumor[9] and immunosuppressive activity,[10] treatment of Gaucher's disease,[11] type II diabetes,[11c,12] as well as leishmaniasis.[13] Within this group of glycomimetics, the focus has been mainly placed on 1-deoxy-iminosugars, since it has been shown that the hemiaminal function confers iminosugars an undesired instability.[14]
Recently, sp^2-iminosugars, in which the nitrogen atom is amide-type (sp^2), were also described. These compounds have shown remarkable discrimination of glycosidase isoenzymes. The use of this type of glycomimetic has also being studied for the treatment of various diseases as Gaucher,[15] Fabri,[16] and in the search of anti-leishmania activity.[17] Indeed, the current chemotherapy used to treat leishmaniasis (cutaneous, mucocutaneous, or visceral) and trypanosomiasis (that include African trypanosomiasis and Chagas disease) need to be improved in order to overcome the toxicity, administration, low efficacy, and resistance issues.[18] The carbohydrate and nucleotide metabolism of African trypanosomes (*Trypanosoma brucei* spp. and related species) is considered an attractive drug target.[19] At variance with other trypanosomatids, bloodstream stage of *T. brucei* was a very high metabolic demand for carbohydrates and nucleotides, which are important bioenergetic substrates, DNA/RNA and glycosylation precursors.[20] In fact, this stage of the parasite outstands for being highly proliferative (doubling time ~5-6 hours), and for having the highest glycolytic and glycoprotein biosynthesis rates ever reported for a unicellular eukaryote.[20,21]
Most of the published works are focused on iminosugars of the D-series, due to their widespread availability. However, interesting activities have been recently described for L-iminosugars, sometimes higher than those of their enantiomers, which has led to focus the interest on this type of compounds as promising drug candidates.[22] Therefore, the development of efficient synthetic methodologies for the preparation of these compounds, appear as a challenge of wide interest to the synthetic chemistry community.
Since the first description on the use of small organic molecules as enantioselective catalysts, by Eder and Hajos in the 1970s,[23] and later in 2000, with the works of List and Barbas III, and MacMillan,[24] organocatalysis has become an excellent strategy for enantioselective synthesis. The seminal work of MacMillan and Northrup showed organocatalysis as a key tool for carbohydrate synthesis[25] and it quickly became a widely applicable strategy for the preparation of carbohydrates and glycomimetics, in particular, iminosugars.[26]
In the context of organocatalyzed formation of C-C bonds, the aldol and Mannich reactions are arguably the most studied, and this strategy has been repeatedly used in the synthesis of different natural products.[27]
Based on our experience in the use of organocatalytic aldol reactions as key step for the synthesis of carbohydrates and derivatives,[28] we are now focused on the organocatalyzed synthesis of iminosugars with potential biological activity. In this context, herein we describe the synthesis of a series of five L-pentose-mimetic iminosugars, using an organocatalyzed Mannich reaction as the key step. The synthetic strategy is based on a D-proline-catalyzed Mannich reaction between an imine and a protected dihydroxyacetone, followed by an enantioselective reduction and further deprotection steps, to achieve two of the possible diastereomers of L-imino-pentose derivatives, those with L-*lyxo* and L-*xylo* configurations. Also, a preliminary evaluation of their anti-*T. brucei brucei* activity was carried out.

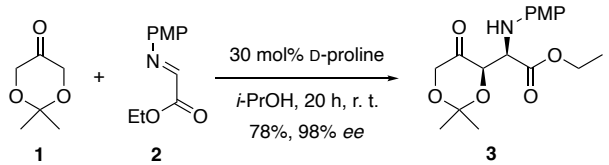
Results and Discussion

The first step in the proposed synthetic route for both L-*lyxo* and L-*xylo* pentofuranose-mimetic iminosugars, was the D-proline catalyzed Mannich reaction between the dioxanone **1** and the *N*-*p*-methoxyphenyl imine **2**, (Scheme 1) in which the *syn*-configured product **3** was obtained in 78% yield with 98% *ee*. Imine **2** was chosen as the acceptor in the Mannich reaction because it allows to obtain the corresponding products with

This article is protected by copyright. All rights reserved.

Accepted Manuscript

optimal *ee*'s, and, additionally, the introduced *p*-methoxyphenyl (PMP) group can be easily removed in the final product under oxidative conditions.[29] The starting materials **1** and **2** were prepared according to known literature procedures.[30]



1 **2** **3**

Scheme 1. Preparation of intermediate **3** through an organocatalyzed Mannich reaction as key step in the designed synthetic strategy.

The reaction was initially carried out under the conditions described by Enders[31] and Córdova,[32] for the preparation of the enantiomer of **3** (they use L-proline as catalyst to obtain *ent*-**3**). This strategy involved a 'one pot two step' reaction, in which the imine is formed *in situ* from ethyl glyoxalate and *p*-anisidine, and then reacted with the dioxanone **1** as donor. Unfortunately, we could not reproduce the yields reported in the literature for (2*S*,3*S*)-**3**, obtaining compound (2*R*,3*R*)-**3** in only 43%, and with low enantiomeric excesses (up to 50%). In view of these results, we decided to prepare and isolate first the imine **2**, and then proceed with the organocatalytic Mannich reaction, as also described.[33] The procedure was optimized by varying equivalents of **1**, amount of catalyst, solvents, temperature, and reaction times (See most significant results in Table 1). Thus, the amount of D-proline was 10 or 30% mol, the temperature was 2 ºC or room temperature, and the reaction times ranged from 2 to 5 days. As solvents, DMF or DMSO were used, anhydrous or mixed with water, since it's reported that the addition of 1 to 10 equivalents or water led to an increase of the stereoselectivity, independently of the solvent employed (although decreasing the reaction rate),[31a] and also isopropanol, dioxane, and acetonitrile.

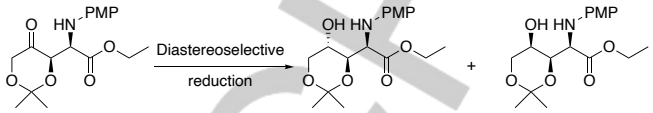
Table 1. Optimization of organocatalytic Mannich reaction of **1** and **2**.

Entry	**1** (eq)	Solvent	H_2O (eq)	Time (h)	Yield (%)	*ee*[a] (%)
1[b]	2	DMF	3	120	21	-
2	2	DMSO	5	48	47	50
3	4	DMSO	5	48	53	-
4	6	DMSO	5	48	65	-
5	2	DMSO	0	24	29	91
6	2	DMF	0	24	26	94
7	2	*i*-PrOH	0	20	78	98
8	2	Dioxane	0	24	44	88
9	2	MeCN	0	24	39	94

[a] Determined by HPLC on chiral stationary phase Lux Cellulose-1-Phenomenex. (See Supporting Information). All reactions were carried out at room temperature, and using 30 mol% D-proline, except for entry 1: [b] 10 mol% D-proline was used, and the reaction was carried out at 2 ºC.

Best results were obtained when using *i*-PrOH as solvent, two equivalents of dioxanone **1**, and surprisingly, without addition of water, yielding **3** after 20 h in 78% and 98% *ee* (Entry 7). To the best of our knowledge, this is the first report of the synthesis of (2*R*,3*R*)-**3**, through a Mannich reaction, using D-proline as catalyst for the required stereochemistry.

The next step in the synthetic sequence was the diastereoselective reduction of **3** to obtain the corresponding *anti*- and *syn*- β-aminoalcohols, (Scheme 2, Table 2).



3 **4** **5**

Scheme 2. Diastereoselective reduction of **3**.

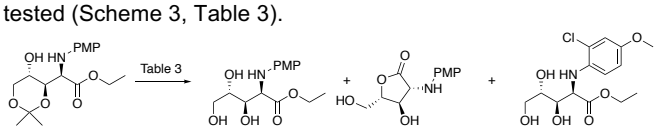
Table 2. Diastereoselective reduction of **3**.

Entry	Reducing agent	Solvent	Temperature (ºC)	Yield (%**4**/%**5**)[a]
1	$NaBH_4$	MeOH	0	48/38
2	$NaBH_4$	MeOH	-40	64/19
3	$NaBH_4$	MeOH	-70	71/19
4	$LiEt_3BH$	THF	-70	n.d./49

n.d.: Not detected by ^1H NMR of the crude. [a] Diastereomeric mixtures could be successfully separated using SiO_2 column chromatography.

Initially, classical conditions were assayed using $NaBH_4$/MeOH at 0 ºC, and an easily separable mixture of isomers **4** (*anti*-) and **5** (*syn*-) was obtained, being **4** the major product (Entry 1). Then, the reaction temperature was decreased, attempting to enhance the selectivity towards product **4**, considering a kinetic control of the reaction, according to the torsional strain model (Entries 2 and 3).[34] Compound **4** was then obtained with 71% yield and good selectivity at -70 ºC (Entry 3). In order to obtain the *syn*-reduced product, the bulkier reducing agent $LiEt_3BH$ was chosen, and the *syn*-product **5** was obtained as a sole diastereomer, in 49% yield, in agreement with the literature (Entry 4).[35] The absolute stereochemistry of **4** was confirmed by preparing the corresponding (*R*)- and (*S*)-Mosher's derivatives (see supporting information).[36] It could be appreciated that for the (*S*)-Mosher's derivative, the anisotropic effect of the phenyl group shields the protons of the methylene in C5, while for the (*R*)-Mosher's derivative, the same effect shields protons in C2 and C3. This fact could be confirmed by analysis of the δ values in the ^1H RMN spectra for both derivatives, which confirmed the (*S*)-configuration at C4 (the carbon atom bearing the free hydroxyl group).

Once the preparation of either **4** or **5** was optimized, we proceeded to remove the protective acetonide group. In the case of **4**, this removal was not easy, and several conditions were tested (Scheme 3, Table 3).



4 **6** **7** **6a**

Scheme 3. Deprotection of acetonide group in **4**.

This article is protected by copyright. All rights reserved.

Accepted Manuscript

Table 3. Deprotection of acetonide group in **4**.

Entry	Reactant	Solvent	Temperature (ºC)	Yield (%)
1	Dowex[a]	MeOH	RT to 50	-- [b]
2	Yb(OTf)$_3$	MeCN	RT to 50	-- [b]
3	$CuCl_2$	MeCN	RT	**6a**, 51%
4	I_2 (2%)	MeOH	RT to 50	**6**,**7** (1:1), 86%[c]

[a] Dowex 50W-X8. [b] Results not reproducible. [c] Compounds **6** and **7** were separated only for analytical purposes (NMR experiments) using SiO_2 column chromatography.

Initial deprotection attempts using acidic resin in MeOH or Yb(OTf)$_3$, afforded mixtures of the desired triol **6** and γ-lactone **7**, in variable yields (Entries 1 and 2). Reaction with copper (II) chloride at room temperature was then carried out, expecting that a milder Lewis acid could prevent the unwanted lactonization. Unfortunately, this treatment promoted not only the removal of the acetonide group, but also the chlorination of the aromatic ring, giving the undesired product **6a** in 51% yield (Entry 3), according to literature.[37]

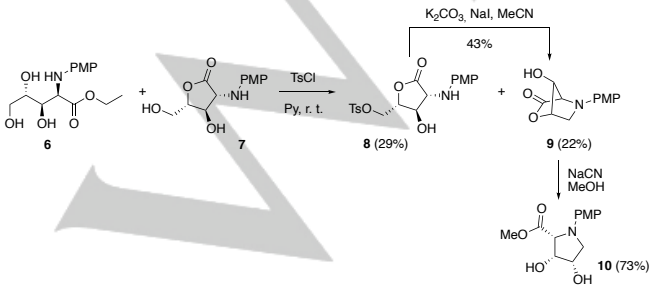
Finally, treatment with a 2% I_2 in MeOH at 50 ºC for 2 hours,[38] consistently gave an equimolecular mixture of the deprotected product **6** and the cyclization product **7** in high yield (Entry 4).

In view of these results, protection of the free hydroxyl group in **4** was conducted, to prevent the formation of **7**. Several conditions were tested, using TBSCl, TMSOTf, Ac_2O or BnBr as protecting agents. The protection with TBSOTf proceeded in good yield, but when deprotecting the acetonide group, the concomitant removal of the TBS group took place, obtaining again an equimolecular mixture of **6** and **7**. Other protections were not efficient.

Since this strategy failed, we decided to continue working with the 1:1 mixture of **6** and **7**, expecting that in the following steps of the synthetic sequence, both could be converted to the corresponding iminosugar.

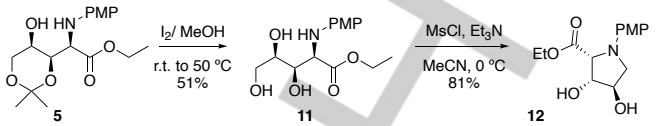
As last steps in the synthetic sequence, the mixture of **6** and **7** was reacted with TsCl in pyridine at room temperature for two hours, yielding a separable mixture of **8** (29%) and **9** (22%), resulting from base-induced lactonization of **6** and further selective tosylation of the primary alcohol in **7** to give **8**, which could suffer cyclization to **9**. In turn, **8** could be converted into **9** with 43% yield, using K_2CO_3, and NaI as nucleophilic catalyst. As a result, compound **9** could be obtained from the mixture of **6** and **7** in 35% yield, in a two-step sequence.

Finally, bicycle **9** was opened through transesterification, to obtain the iminosugar derivative **10**, using NaCN in MeOH, with 73% yield (Scheme 5).



Scheme 4. Final steps to the iminosugar derivative **10**.

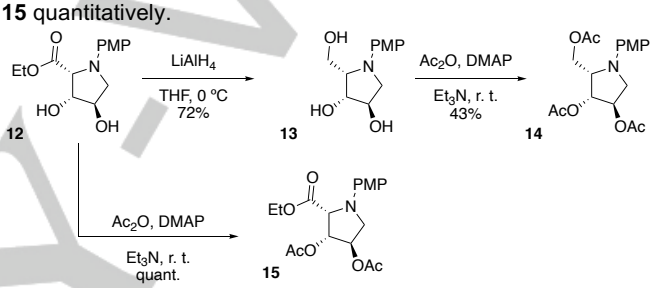
Back to compound **5**, to remove its acetonide group we went straight to use 2% I_2 in MeOH, as it was optimized for deprotecting the diastereomer **4**. To our delight, in this case we obtained triol **11** as a sole product, in 51% yield (Scheme 6).



Scheme 5. Deprotection of acetonide group in **5** and formation of iminosugar **12**.

The final step involved the reaction of **11** with MsCl in MeCN, and the iminosugar **12** was obtained in 81%, through spontaneous cyclization (Scheme 5).

With the iminosugar **12** in hand, we prepared its reduced and protected derivatives (Scheme 6), in order to explore the potential biological activity. First, we reduced the ester function, yielding **13** in 72%. Then, the hydroxyl groups were esterified as acetates, and **14** was obtained in 43% yield. Finally, acetylation of **12** gave **15** quantitatively.



Scheme 6. Synthesis of reduced and protected iminosugar derivatives.

As part of our search for candidate compounds to develop novel antiparasitic agents, a preliminary characterization of the biological activity against bloodstream *T. brucei brucei* was investigated for compounds: **3-5**, **6a**, **9-15**. First, a screening assay was performed at 10 μM, which identified compound **10** as the only one able to reduce parasite viability to <50% (Table 4). Worth noting, compound **10** is the only furanose-mimetic of the L-*lyxo* series tested.

Table 4. *In vitro* activity of compounds **3-5**, **6a**, **9-15** against bloodstream *T. b. brucei* and murine macrophages (cell line J774).

Entry	Compound	*T. brucei* viability at 10 μM (%) / EC_{50} (μM)	Macrophage cytotoxicity (CC_{50}, μM) / *Selectivity index* (CC_{50}/EC_{50})
1	**3**	89.0 ± 3.2	
2	**4**	93.1 ± 7.1	
3	**5**	98.1 ± 11.7	
4	**6a**	90.6 ± 6.5	
5	**9**	93.5 ± 6.9	
6	**10**	4.4 ± 2.5 / *3.8 ± 1.0*	11.6 ± 1.1 / *3*
7	**11**	*96.7* ± 7.2	
8	**12**	87.5 ± 4.1	
9	**13**	100.2 ± 10.9	

This article is protected by copyright. All rights reserved.

Accepted Manuscript

10	**14**	103.3 ± 21.5	
11	**15**	94.1 ± 15.0	
12	**Nfx.** (6 μM)	61.2 ± 3.7 / *6.0 ± 0.4*[a]	140.0 ± 2.0 / *23* [a]

Negative control: DMSO. Positive control: Nifurtimox (Nfx.). [a] Data from literature.[39]

Compound **10** displayed a potency (EC_{50} = 3.8 μM) similar to Nifurtimox (EC_{50} = 6.0 μM) but a comparatively lower selectivity (SI = 3) than this reference drug (SI = 23). This result highlights the importance of the configuration of the stereocenter on bioactivity, and taking it into account, future studies will address modifications of this hit, to improve its potency and selectivity. In this regard, precursors bearing different substituents in the nitrogen or the adjacent carbon, will be explored.

Conclusion

In summary, a series of five iminosugars and derivatives, mimetics of L-pentofuranoses, were prepared in a stereoselective fashion, using an organocatalyzed Mannich reaction as key step. Additionally, this is the first report of the D-proline-catalyzed Mannich reaction of the dioxanone **1** and the *N*-*p*-methoxyphenyl imine **2**, to give the (2*R*,3*R*)-stereochemistry in the Mannich adduct **3**. Furthermore, compound **10** emerged as a new head-of-series, to be optimized for its selectivity.

Experimental Section

General Experimental Remarks: All non-hydrolytic reactions were carried out in a nitrogen atmosphere by using standard techniques for the exclusion of air. All solvents were distilled prior to use. Starting materials and reagents were purchased from commercial suppliers and were used without further purification, unless otherwise stated. Melting point of compound **11** was determined on a Gallenkamp capillary melting point apparatus and was uncorrected. High-Resolution Mass Spectra (HRMS) were performed on an Agilent Technologies LC/MS 6210 model (ESI+ mode). Analytical HPLC analyses were performed on a Shimadzu LC-20 Prominence designed with diode array detector and using a stationary chiral phase Lux Cellulose-1-Phenomenex. Infrared spectra were recorded on NaCl disks on a Shimadzu DR-8100 FTIR spectrometer. NMR spectra were acquired in a Bruker Ascend 400 MHz or in a Bruker Avance 400 MHz instruments. All experiments were taken at 30 ºC and using $CDCl_3$ or MeOD as solvents, as indicated in each case. Proton chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS as internal reference, and carbon chemical shifts are reported in ppm relative to the center line of the $CDCl_3$ triplet (δ = 77.0 ppm). Optical rotations were measured with a Zuzi 412 polarimeter using a 0.5 dm cell. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC were performed on Silica gel 60F-254 plates and visualized with UV light (245 nm) and/or *p*-anisaldehyde in acidic ethanolic solution. Flash column chromatography was performed using silica gel (Kieselgel 60, EM reagent, 230-400 mesh).

Ethyl (2*R*,3*R*)-3,5-isopropylidendioxy-2-((*p*-methoxyphenyl)amino)-4-oxopentanoate (3): To a solution of **1** (5.07 g, 39 mmol) in *i*-PrOH (15 mL), a solution of **2** (4.03g, 19.5 mmol) and D-proline (0,67 g, 30 mol%) in *i*-PrOH (15 mL) was added. The reaction was maintained under magnetic stirring for 20 hours, then quenched with saturated aqueous NH_4Cl (50 mL) and extracted with AcOEt (3 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvent was distilled under reduced pressure and the residue was purified by column chromatography using an hexane:AcOEt 9:1 mixture to afford **3** (5.16 g, 15.4 mmol, 78%) as a pale-oil. 98% *ee.*

Ethyl (2*R*,3*R*,4*S*)-4-hydroxy-3,5-isoprolylidendioxy-2-((*p*-methoxyphenyl)amino)pentanoate (4): To a solution of **3** (0.181 g, 0.54 mmol) in MeOH (3 mL), $NaBH_4$ (0.025 g, 0.67 mmol) was added under N_2 atmosphere and the mixture was stirred at -70 ºC. After 15 minutes, AcOEt (20 mL) and saturated aqueous NH_4Cl solution (10 mL) were added and stirred for 20 minutes. The cooling was interrupted, and the reaction stirred until reached room temperature. The aqueous layer was extracted with AcOEt (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was distilled under reduced pressure and the crude was purified by column chromatography using gradient hexane:AcOEt 8:2 to 7:3 to afford **4** (0.131 g, 0.38 mmol, 71%) as a colorless oil.

Ethyl (2*R*,3*R*,4*R*)-4-hydroxy-3,5-isoprolylidendioxy-2-((*p*-methoxyphenyl)amino)pentanoate (5): To a solution of **3** (0.877 g, 2.6 mmol) in THF (30 mL) a 1M solution of $LiEt_3BH$ (Superhydride®) in THF was added, under N_2 atmosphere and at -70 ºC. The reaction was stirred for 25 minutes. Then, AcOEt (50 mL) and saturated aqueous NH_4Cl solution (20 mL) were added and stirred until the reaction reached room temperature. The organic layer was washed with saturated aqueous NaCl solution (3 x 20 mL) and dried over anhydrous Na_2SO_4. The solvent was distilled under reduced pressure, and the crude was purified by column chromatography using an hexane:AcOEt 7:3 mixture to afford **5** (0.431 g, 1.27 mmol, 49%) as a colorless oil.

Ethyl (2*R*,3*R*,4*S*)-3,4,5-trihydroxy-2-((*p*-methoxyphenyl)amino)pentanoate (6): To a 2% solution of I_2 in MeOH (10 mL), at room temperature, **4** (0.500 g, 1.47 mmol) was added under N_2 atmosphere. The reaction was warmed to 50 ºC and stirred for 2 hours. The heating was stopped, the reaction allowed to reach room temperature and $Na_2S_2O_3.5H_2O$ (0.470 g, 3 mmol) was added. After 15 minutes, the solvent was distilled under reduced pressure and the crude was purified by column chromatography using gradient hexane:AcOEt 3:7 to 2:8, to afford a 1:1 mixture of **6** and **7** (86%) as a colorless oil.

(2*R*,3*R*,4*S*)-3-hydroxy-2-((*p*-methoxyphenyl)amino)-4-tosyloxymethyl-γ-butyrolactone (8): To a 1:1 mixture of **6** and **7** (0.096 g, 0.35 mmol) in anhydrous pyridine (1 mL) TsCl (0.073 g, 0.30 mmol) was added at room temperature. After stirring for 2 hours, AcOEt was added (20 mL). The solution was washed with $CuSO_4$ (3 x 10 mL) and NaCl (2 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was distilled under reduced pressure. The crude was purified by column chromatography using a hexane:AcOEt 1:1 mixture to afford **8** (0.041 g, 0.10 mmol, 29%) and **9** (0.19 g, 0.08 mmol, 22%) as a yellow oil.

Conversion of 8 to 9: To a solution of **8** (0.046 g, 0,11 mmol) in MeCN (3 mL) K_2CO_3 (0.031 g, 0.22 mmol) and NaI (catalytic quant.) were added under N_2 atmosphere. The reaction was stirred at room temperature for 14 hours. The solvent was distilled under reduced pressure, and the crude was purified by column chromatography using a hexane:AcOEt 1:1 mixture, to afford **9** (0,011 g, 0.047 mmol, 43%) as a yellow oil.

Methyl (2*R*,3*R*,4*S*)-3,4-dihidroxy-1-(*p*-methoxyphenyl)pyrrolidine-2-carboxylate (10): To a solution of **9** (0.016 g, 0.068 mmol) in MeOH (1 mL) NaCN (catalytic quant.) was added under N_2 atmosphere, and the reaction mixture was stirred for 1 hour at 50 ºC. The solvent was distilled under reduced pressure, and the crude was purified by column chromatography using a hexane:AcOEt 3:7 mixture, to afford **10** (0.013 g, 0.049 mmol, 73%) as a yellow oil.

Ethyl (2*R*,3*R*,4*R*)-3,4,5-trihydroxy-2-((*p*-methoxyphenyl)amino)pentanoate (11): To a 2% solution of I_2 in MeOH (10 mL), **5** (0.405 g, 1.19 mmol) was added at room temperature, under N_2 atmosphere. The reaction was warmed to 50 ºC and stirred for 2 hours. The heating was then stopped, the reaction allowed to reach room

Accepted Manuscript

This article is protected by copyright. All rights reserved.

temperature, and $Na_2S_2O_3.5H_2O$ (0.564 g, 3.57 mmol) was added. After 15 minutes, the solvent was distilled under reduced pressure and the crude was purified by column chromatography using a hexane:AcOEt 4:6 mixture, to afford **11** (0.181 g, 0.61 mmol, 51%) as pale yellow crystals.

Ethyl (2*R*,3*R*,4*R*)-3,4-dihydroxy-1-((*p*-methoxyphenyl)pyrrolidine-2-carboxylate (12): To a solution of **11** (0.145 g, 0.48 mmol) in MeCN (20 mL) at 0 ºC, Et_3N (0.133 mL, 0.087 g, 0.96 mmol) and MsCl (0.044 mL, 0.065 g, 0.57 mmol) were added under N_2 atmosphere. The reaction was stirred for 30 minutes. The solvent was distilled under reduced pressure and the crude was purified by column chromatography using a hexane:AcOEt 1:1 mixture, to afford **12** (0.116 g, 0.39 mmol, 81%) as a pale yellow oil.

(2*S*,3*R*,4*R*)-2-(hydroxymethyl)-1-(*p*-methoxyphenyl)pyrrolidine-3,4-diol (13): To a solution of **12** (0.030 g, 0.107 mmol) in THF (2 mL) at 0 ºC and under N_2 atmosphere, a 1M solution of $LiAlH_4$ in THF (0.321 mL, 0.321 mmol) was added. The reaction was allowed to reach room temperature and stirred for 30 minutes. AcOEt (5 mL) was added and after 5 minutes, H_2O (0.5 mL). The solvent was distilled under reduced pressure, and the crude was purified by column chromatography using a hexane:AcOEt 1:9 mixture, to afford **13** (0.018 g, 0.077 mmol, 72%) as a colorless oil.

(2*S*,3*R*,4*R*)-2-(acetoxymethyl)-1-(*p*-methoxyphenyl)pyrrolidine-3,4-diol diacetate (14): To a solution of **13** (0.017 g, 0.071 mmol) in MeCN (2 mL) at room temperature and under N_2 atmosphere, Et_3N (0.177 mL, 0.129 g, 1.28 mmol), DMAP (catalytic quant.) and Ac_2O (0.060 mL, 0.065 g, 0.640 mmol) were added. The reaction was stirred for 30 minutes. AcOEt (10 mL) was then added and the mixture was washed with $CuSO_4$ (2 x 10 mL) and NaCl (2 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was distilled under reduced pressure. The crude was purified by column chromatography using a hexane:AcOEt 8:2 mixture, to afford **14** (0.011 g, 0.030 mmol, 43%) as a pale yellow oil.

(2*R*,3*R*,4*R*)-3,4-diacetoxy-1-((*p*-methoxyphenyl)pyrrolidine-2-carboxylate (15): To a solution of **12** (0.012 g, 0.043 mmol) in MeCN (2 mL) at room temperature and under N_2 atmosphere, Et_3N (0.071 mL, 0.052 g, 0.256 mmol), DMAP (catalytic quant.) and Ac_2O (0.024 mL, 0.026 g, 0.256 mmol) were added. The reaction was stirred for 30 minutes. AcOEt (10 mL) was then added, and the mixture was washed with $CuSO_4$ (2 x 10 mL) and NaCl (2 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was distilled under reduced pressure. The crude was purified by column chromatography using a hexane:AcOEt 7:3 mixture, to afford **15** (0.014 g, 0.043 mmol, quantitative) as a pale yellow oil.

Viability Assays for *T. brucei brucei*: the anti-trypanosomal activity of all compounds was evaluated against the bioluminescent cell line of the bloodstream stage of *Trypanosoma brucei brucei* as previously described.[40] Briefly, to a 96-well culture plate containing 2.2 µL/well DMSO (negative control) or compounds dissolved in DMSO (1% final concentration), 220 µL/well of a suspension of 1×10^5 parasites/mL was added. The plates were incubated at 37 °C and 5% CO_2 for 24 h. Next, each well was transferred to a 96-well black plate and 20 µL of a solution containing D-Luciferin (1.5 mg/mL in PBS glucose 1% w/v) and Triton X-100 (0.05% vol/vol) was added. Bioluminescence signal was measured in a LUMIstar OPTIMA Microplate luminometer using the following settings: 10 s shaking, 5 s/well acquisition, 0.2 s measurement delay, maximum gain, and 37 °C. For the bioluminescence assay, parasite viability was calculated according to the following formula: Viability (%) = $(BL_{cpd} - BL_{blank})/(BL_{neg} - BL_{blank}) \times 100$, where BL refers to the mean of bioluminescence signal corresponding to the tested compound (cpd), the blank (blank, complete media containing 1% v/v DMSO), or the negative control (neg, parasites treated with 1% v/v DMSO). EC_{50} value was determined from concentration-response curve fitted to a four-parameter sigmoid equation using the GraphPad Prism software (version 6.0). All errors are expressed as one SD.

Cytotoxicity assays on murine macrophages: Mouse macrophages from the cell line J774 (ATCC® TIB-67TM) were cultivated under a humidified 5% CO_2/95% air atmosphere at 37 ºC in Dubelcco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) FBS, 10 U/mL penicillin and 10 µg/mL streptomycin. The experimental protocol for determination of cytotoxicity at 100 µM was essentially the same as that previously described, except that 200 µL/well of a cell suspension at 6 x 10^4 cells per mL was added in a 96-well culture plate and washes were made with 150 µL of DMEM.[41] The cytotoxicity of **10** was evaluated (1:3 serial dilutions starting from 100 µM) in triplicate using the WST-1 reagent (Roche). The control treatment included cells cultured in the presence of 1% DMSO (v/v). Absorbance at 450 nm, corresponding to the formazan dye produced by metabolically active cells, was measured with an EL 800 or Varioskan-Flash microplate reader. The corrected absorbance values at 450 nm were obtained by subtracting the corresponding absorbance value at 630 nm and the blank average (e.g., $Ai^{c450nm} = Ai^{450} - Ai^{630nm} - Ablank^{450nm}$).

Acknowledgements

The authors wish to thank Prof. Robert Britton for HRMS ESI TOF-MS analyses, Dr. Agustina Vila, for HPLC analyses and Dr. G. Moyna (Departamento de Química del Litoral, CENUR Litoral Norte, Paysandú, UdelaR) for performing some of the NMR experiments. Support for this work from CSIC-UdelaR (Comisión Sectorial de Investigación Científica, Grupos I+D 2001), ANII (Agencia Nacional de Investigación e Innovación, FCE_1_2019_1_156376) and PEDECIBA (Programa de Desarrollo de las Ciencias Básicas) are gratefully acknowledged. M. Comini acknowledges the support from FOCEM (MERCOSUR Structural Convergence Fund, COF 03/11). E. Dibello thanks CAP-UdelaR (Comisión Académica de Posgrado) for a postdoctoral fellowship, and J. M. Mesa thanks ANII and CAP-UdelaR for a Master scholarship.

Keywords: Drug discovery • Glycomimetics • Iminosugars • Mannich reaction • Organocatalysis

[1] a) N. Asano, in *Iminosugars: From Synthesis to Therapeutic Applications* (Eds.: P. Compain, O.R. Martin), John Wiley & Sons. Inc., Chichester, UK, **2007**, Ch. 2. b) N. Asano, in *Carbohydrate Chemistry: The State of the Art and Challenges in Drug Development.* (Ed.: L. Cipolla), Imperial College Press, London, UK, **2015**, Ch. 11.

[2] a) H. Paulsen, *Angew. Chem. Int. Ed.* **1966**, *5*, 495–510. b) N. G. Ramesh, in *Carbohydrates in Drug Discovery Development.* (Ed.: V.K. Tiwari), Elsevier Ltd, **2020**, Ch. 8.

[3] a) S. Compain, P.; Desvergnes, V.; Liautard, V.; Pillard, C.; Toumieux, in *Iminosugars: From Synthesis to Therapeutic Applications.* (Eds.: P. Compain, O.R. Martin), John Wiley & Sons. Inc., Chichester, UK, **2007**, Ch. 14. b) S. Sattin, A. Bernardi, in *Carbohydrate Chemistry*. Vol. 41 (Eds.: Rauter, A. P., Lindhorst, T., Queneau, Y.), The Royal Society Of Chemistry, **2016**, Ch. 1. c) G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams, R. Storer, *Drug Discov. Today* **2011**, *16*, 107–118. d) R. J. Nash, A. Kato, C. Y. Yu, G. W. Fleet, *Future Med. Chem.* **2011**, *3*, 1513–1521.

[4] D. S. Alonzi, K. A. Scott, R. A. Dwek, N. Zitzmann, *Biochem. Soc. Trans.* **2017**, *45*, 571–582.

[5] a) C. Hammond, I. Braakman, A. Helenius, *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 913–917. b) K. Shailubhai, B. S. Pukazhenthi, E. S. Saxena, G. M. Varma, I. K. Vijay, *J. Biol. Chem.* **1991**, *266*, 16587–16593. c) G. B. Karlsson, T. D. Butters, R. A. Dwek, F. M. Platt, *J. Biol. Chem.* **1993**, *268*, 570–576. d) W. Chen, J. Helenius, I. Braakman, A. Helenius, *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 6229–6233. e) J. J. Caramelo, A. J. Parodi, *J. Biol. Chem.* **2008**, *283*, 10221–10225. f) S. O'Keefe, Q. P. Roebuck, I. Nakagome, S. Hirono, A. Kato, R. Nash, S. High, *Glycobiology* **2019**, *29*, 530–542.

[6] a) S. Hussain, J. L. Miller, D. J. Harvey, Y. Gu, P. B. Rosenthal, N. Zitzmann, J. W. McCauley, *J. Antimicrob. Chemother.* **2015**, *70*, 136–152. b) K. L. Warfield, D. L. Barnard, S. G. Enterlein, D. F. Smee, M. Khaliq, A. Sampath, M. V. Callahan, U. Ramstedt, C. W. Day, *Viruses* **2016**, *8*, 1–9. c) B. E. Tyrrell, A. C. Sayce, K. L. Warfield, J. L. Miller, N.

This article is protected by copyright. All rights reserved.

Accepted Manuscript

Zitzmann, *Crit. Rev. Microbiol.* **2017**, *43*, 521–545.

[7] a) C. Chapel, C. Garcia, B. Bartosch, P. Roingeard, N. Zitzmann, F. L. Cosset, J. Dubuisson, R. A. Dwek, C. Trépo, F. Zoulim, D. Durantel, *J. Gen. Virol.* **2007**, *88*, 1133–1143. b) E. Steinmann, T. Whitfield, S. Kallis, R. A. Dwek, N. Zitzmann, T. Pietschmann, R. Bartenschlager, *Hepatology* **2007**, *46*, 330–338.

[8] a) H. L. Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; de Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, *Nature* **1987**, *330*, 74–77. b) G. W. J. Fleet, A. Karpas, R. A. Dwek, L. E. Fellows, A. S. Tyms, S. Petursson, S. K. Namgoong, N. G. Ramsden, P. W. Smith, J. C. Son, F. Wilson, D. R. Witty, G. S. Jacob, T. W. Rademacher, *FEBS Lett.* **1988**, *237*, 128–132.

[9] T. Wrodnigg, A. Steiner, B. Ueberbacher, *Anticancer. Agents Med. Chem.* **2008**, *8*, 77–85.

[10] M. Liu, S. Wang, Y. D. Zhou, T. Xiang, H. Dong, K. Yang, X. L. Zhang, *Bioorganic Med. Chem. Lett.* **2012**, *22*, 564–570.

[11] a) F. M. Platt, G. R. Neises, R. A. Dwek, T. D. Butters, *J. Biol. Chem.* **1994**, *269*, 8362–8365. b) T. Cox, R. Lachmann, C. Hollak, J. Aerts, S. Van Weely, M. Hrebícek, F. Platt, T. Butters, R. Dwek, C. Moyses, I. Gow, D. Elstein, A. Zimran, *Lancet* **2000**, *355*, 1481–1485. c) M. B. Haarr, O. Lopeź, L. Pejov, J. G. Fernańdez-Bolaños, E. Lindbačk, M. O. Sydnes, *ACS Omega* **2020**, *5*, 18507–18514.

[12] P. H. Joubert, C. P. Venter, H. F. Joubert, I. Hillebrand, *Eur. J. Clin. Pharmacol.* **1985**, *28*, 705–708.

[13] D. Ruhela, P. Chatterjee, R. A. Vishwakarma, *Org. Biomol. Chem.* **2005**, *3*, 1043–1048.

[14] a) A. Kaddour, S. Toumieux, A. Wadouachi, *Synlett* **2017**, *28*, 2174–2178. b) M. Y. Kawamura, A. G. Talero, J. V. Santiago, E. Garambel-Vilca, I. G. Rosset, A. C. B. Burtoloso, *J. Org. Chem.* **2016**, *81*, 10569–10575. c) K. I. Kondo, H. Adachi, E. Shitara, F. Kojima, Y. Nishimura, *Bioorganic Med. Chem.* **2001**, *9*, 1091–1095. d) V. Kumar, N. G. Ramesh, *Tetrahedron* **2006**, *62*, 1877–1885. e) A. Soler, X. Garrabou, K. Hernández, M. L. Gutiérrez, E. Busto, J. Bujons, T. Parella, J. Joglar, P. Clapés, *Adv. Synth. Catal.* **2014**, *356*, 3007–3024. f) A. H. Viuff, L. M. Besenbacher, A. Kamori, M. T. Jensen, M. Kilian, A. Kato, H. H. Jensen, *Org. Biomol. Chem.* **2015**, *13*, 9637–9658.

[15] T. Mena-Barragán, M. I. García-Moreno, A. Sevšek, T. Okazaki, E. Nanba, K. Higaki, N. I. Martin, R. J. Pieters, J. M. García Fernández, C. O. Mellet, *Molecules* **2018**, *23*, 1–18.

[16] a) E. M. Sánchez-Fernández, R. Rísquez-Cuadro, C. Ortiz Mellet, J. M. García Fernández, P. M. Nieto, J. Angulo, *Chem. Eur. J.* **2012**, *18*, 8527–8539. b) I. Sylte, R. Dawadi, N. Malla, S. von Hofsten, T. M. Nguyen, A. I. Solli, E. Berg, O. A. Adekoya, G. Svineng, J. O. Winberg, *PLoS One* **2018**, *13*, DOI 10.1371/journal.pone.0200237.

[17] E. M. Sánchez-Fernández, V. Gómez-Pérez, R. García-Hernández, J. M. García Fernández, G. B. Plata, J. M. Padrón, C. Ortiz Mellet, S. Castanys, F. Gamarro, *RSC Adv.* **2015**, *5*, 21812–21822.

[18] F. Altamura, R. Rajesh, C. M. C. Catta-Preta, N. S. Moretti, I. Cestari, *Drug Dev. Res.* **2020**, 225–252.

[19] a) M. Gualdrón-López, P. A. M. Michels, W. Quiñones, A. J. Cáceres, L. Avilán, J. L. Concepción, in *Trypanosomatid Diseases: Molecular Routes to Drug Discovery.* (Eds.: T. Jäger, O. Koch, L. Flohé), Wiley-Blackwell, Weinheim, Germany, **2013**, Ch. 7. b) D. J. Hammond, W. E. Gutteridge, *Mol. Biochem. Parasitol.* **1984**, *13*, 243–261. c) M. A. Comini, C. Ortíz, J. J. Cazzulo, in *Trypanosomatid Diseases: Molecular Routes to Drug Discovery.* (Eds.: T. Jäger, O. Koch, L. Flohé), Wiley-Blackwell, Weinheim, Germany, **2013**, Ch. 16. d) S. Pomel, P. M. Loiseau, in *Trypanosomatid Diseases: Molecular Routes to Drug Discovery.* (Eds.: J. Timo, O. Koch, L. Flohé), Wiley-Blackwell, Weinheim, Germany, **2013**, Ch. 17.

[20] P. A. M. Michels, O. Villafraz, E. Pineda, M. B. Alencar, A. J. Cáceres, A. M. Silber, F. Bringaud, *Exp. Parasitol.* **2021**, *224*, DOI 10.1016/j.exppara.2021.108102.

[21] P. T. Manna, C. Boehm, K. F. Leung, S. K. Natesan, M. C. Field, *Trends Parasitol.* **2014**, *30*, 251–258.

[22] a) D. D'Alonzo, A. Guaragna, G. Palumbo, *Curr. Med. Chem.* **2009**, *16*, 473–505. b) M. De Fenza, D. D'Alonzo, A. Esposito, S. Munari, N. Loberto, A. Santangelo, I. Lampronti, A. Tamanini, A. Rossi, S. Ranucci, I. De Fino, A. Bragonzi, M. Aureli, R. Bassi, M. Tironi, G. Lippi, R. Gambari, G. Cabrini, G. Palumbo, M. C. Dechecchi, A. Guaragna, *Eur. J. Med. Chem.* **2019**, *175*, 63–71.

[23] a) B. U. Eder, G. Sauer, R. Wiechert, **1971**, *10*, 496–497. b) Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **1974**, *39*, 1615–1621.

[24] a) B. List, R. A. Lerner, C. F. Barbas, *J. Am. Chem. Soc.* **2000**, *122*, 2395–2396. b) K. A. Ahrendt, C. J. Borths, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2000**, *122*, 4243–4244.

[25] a) A. B. Northrup, D. W. C. MacMillan, *Science (80-.).* **2004**, *305*, 1752–1755. b) A. B. Northrup, I. K. Mangion, F. Hettche, D. W. C. MacMillan, *Angew. Chem. Int. Ed.* **2004**, *43*, 2152–2154.

[26] a) M. Bergeron-Brlek, J. Goodwin-Tindall, N. Cekic, C. Roth, W. F. Zandberg, X. Shan, V. Varghese, S. Chan, G. J. Davies, D. J. Vocadlo, R. Britton, *Angew. Chem. Int. Ed.* **2015**, *54*, 15429–15433. b) M. Bergeron-Brlek, M. Meanwell, R. Britton, *Nat. Commun.* **2015**, *6*, 1–6. c) M. Meanwell, G. Fehr, W. Ren, B. Adluri, V. Rose, J. Lehmann, S. M. Silverman, R. Rowshanpour, C. Adamson, M. Bergeron-Brlek, H. Foy, V. R. Challa, L. C. Campeau, T. Dudding, R. Britton, *Commun. Chem.* **2021**, *4*, 1–9.

[27] a) M. Waser, *Asymmetric Organocatalysis in Natural Products Synthesis*, Springer-Verlag, Vienne, Austria, **2012**. b) E. Dibello, D. Gamenara, G. Seoane, *Curr. Organocatal.* **2015**, *2*, 124–149.

[28] a) E. Dibello, D. Gamenara, G. A. Seoane, *Synth.* **2017**, *49*, 1087–1092. b) E. Dibello, L. Suescun, G. A. Seoane, D. Gamenara, *Tetrahedron: Asymmetry* **2017**, *28*, 344–348.

[29] B. List, P. Pojarliev, W. T. Biller, H. J. Martin, *J. Am. Chem. Soc.* **2002**, *124*, 827–833.

[30] a) D. Hoppe, H. Schmincke, H. W. Kleemann, *Tetrahedron* **1989**, *45*, 687–694. b) R. C. Simon, E. Busto, J. H. Schrittwieser, J. H. Sattler, J. Pietruszka, K. Faber, W. Kroutil, *Chem. Commun.* **2014**, *50*, 15669–15672.

[31] a) D. Enders, C. Grondal, M. Vrettou, G. Raabe, *Angew. Chem. Int. Ed.* **2005**, *44*, 4079–4083. b) D. Enders, C. Grondal, M. Vrettou, *Synthesis (Stuttg).* **2006**, 3597–3604.

[32] I. Ibrahem, W. Zou, Y. Xu, A. Córdova, *Adv. Synth. Catal.* **2006**, *348*, 211–222.

[33] B. Westermann, C. Neuhaus, *Angew. Chem. Int. Ed.* **2005**, *44*, 4077–4079.

[34] a) M. Chérest, H. Felkin, *Tetrahedron Lett.* **1968**, *9*, 2205–2208. b) M. Chérest, H. Felkin, **1971**, *12*, 383–386. c) G. J. Tanoury, S. Roeper, *Tetrahedron* **2018**, *74*, 7103–7110.

[35] a) R. A. Pilli, D. Russowsky, L. C. Dias, *J. Chem. Soc., Perkin Trans. 1* **1990**, 1213–1214. b) F. A. Davis, P. M. Gaspari, B. M. Nolt, P. Xu, *J. Org. Chem.* **2008**, *73*, 9619–9626.

[36] a) G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem.* **1973**, *38*, 2143–2147. b) J. M. Seco, E. Quiñoá, R. Riguera, *Chem. Rev.* **2004**, *104*, 17–118.

[37] X. Y. Yang, H. Y. Zhao, S. Mao, S. Q. Zhang, *Synth. Commun.* **2018**, *48*, 2708–2714.

[38] M. Fujii, T. Miura, T. Kajimoto, Y. Ida, *Synlett* **2000**, *6*, 1046–1048.

[39] C. Ortiz, F. Moraca, M. Laverriere, A. Jordan, N. Hamilton, M. A. Comini, *Molecules* **2021**, *26*, 1–21.

[40] D. Benítez, E. Dibello, M. Bonilla, M. A. Comini, *Drug Dev. Res.* **2020**, *83*, 253–263.

[41] B. Demoro, C. Sarniguet, R. Sánchez-Delgado, M. Rossi, D. Liebowitz, F. Caruso, C. Olea-Azar, V. Moreno, A. Medeiros, M. A. Comini, L. Otero, D. Gambino, *Dalton Trans.* **2012**, *41*, 1534-1543.

Accepted Manuscript

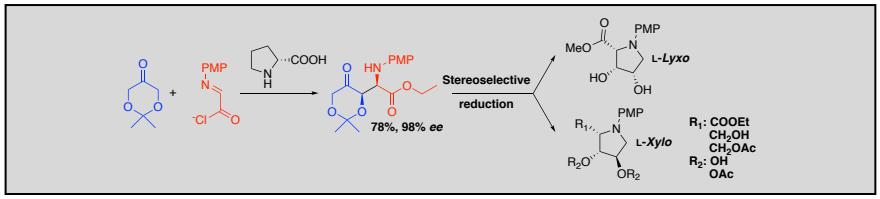
This article is protected by copyright. All rights reserved.

10.1002/ejoc.202200636

WILEY-VCH

RESEARCH ARTICLE

Entry for the Table of Contents



The D-proline-catalyzed Mannich reaction of an imine and a protected dioxanone is described as the key step for the stereoselective synthesis of a series of L-*lyxo* and L-*xylo*- pentofuranose-mimetic iminosugars and derivatives. The L-*lyxo* derivative **12**, has emerged as head of series, seeking for the development of novel glycomimetics with potential activity towards pathogenic trypanosomatids.

WILEY-VCH

Accepted Manuscript

This article is protected by copyright. All rights reserved.