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A β-lactam-azasugar hybrid as a competitive potent galactosidase inhibitor

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Abstract—A β-lactam-azasugar hybrid (polyhydroxylated carbacephem) has been designed and synthesized as a potent glycosidase inhibitor.

The design and synthesis of hybrid molecules, that is, structural motifs developed through domain assimilation of two or more different classes of biologically active compounds of natural and/or synthetic origin has attracted the attention of synthetic chemists in the past few years owing to the enhanced possibility of discovering new biologically active therapeutic agents.[1] In this context, several hybrid molecules of natural products such as steroids, taxoids, carbohydrates and peptides with counterparts such as β-lactams, C_{60}-fullerenes, anthraquinones, enediyne and porphyrin have been synthesized and their properties evaluated.[2]

Azasugar inhibitors of glycosidases and related enzymes are the subject of intense current research interest due to their potential clinical applications as anti-diabetic,[3] anti-cancer,[4] anti-HIV[5] and anti-influenza[6] agents. These low molecular weight entities are believed to exhibit their inhibitory activities due to their binding with glycosidases by mimicking the shape and charge of the postulated oxo-carbenium ion intermediate for the glycosidic bond cleavage reaction.[7] Some of the potent azasugar based glycosidase inhibitors (Fig. 1), such as **1**,[8] **2**,[9] **3**,[10] **4**,[11] and **5**[12], which become positively charged on protonation due to the presence of basic amino, amidine and hydrazine moieties, are suggested to derive their inhibitory activities either by mimicking the charge or shape, or both, of the glycosidase transition state. In contrast, neutral glyconolactams,[13] such as **6** (K_i = 85 μM, β-glucosidase), where the glycosidic oxygen is replaced by a pseudo sp^2 ring nitrogen, was originally believed to inhibit glycosidases by involving a tautomeric iminol form. However, Withers and co-workers[14] have suggested that glycosidase inhibition by **6** and similar other compounds such as **7** and **8** may in fact be caused by H-bonding of the lactam carbonyl moiety with the enzyme as the tautomerization energy for the amide–iminol conversion is of the order of 11 kcal mol^{-1},[15] indicating the concentration of the corresponding iminol form in solution at any given time to be very low.

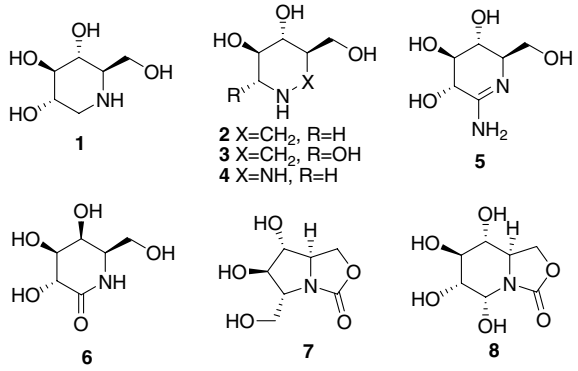

Figure 1. Structures of some potent glycosidase inhibitors.

Keywords: Glycosidase inhibitor; β-Lactam-azasugar; Hybrid molecules; β-Galactosidase.
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Recently, research activity in this field has evolved to evaluate hybrid molecules as glycosidase inhibitors and in this context hybrids of D-glucose with several heterocycles,[16] D-galactose with 1-deoxynojirimycin and a few more related structures[17] have been synthesized and evaluated. The possibility of developing new glycosidase inhibitors through this approach is gaining appreciable importance for the future developments in this area.

Based on the aforementioned, we postulated that a β-lactam-azasugar hybrid molecule of type **9** (Fig. 2), which can also be referred to as polyhydroxylated carbacephem, may function as a potent glycosidase inhibitor due to its conformationally constrained structural features: (a) β-lactam ring compelling the polyhydroxylated piperidine ring to adopt a nearly half-chair conformation mimicking the shape of the glycosidase inhibition transition state, (b) the carbonyl group in the β-lactam ring may provide an additional hydrogen bonding site for specific enzyme–substrate interactions.

Thus, we have synthesized β-lactam-azasugar hybrid **9**, its enantiomer **23** and another related structure **26** and have evaluated their glycosidase inhibitory activities. Herein, we disclose our preliminary results in this letter. To the best of knowledge, there are no other such studies in the literature.

The synthesis of **9** was pursued through the retrosynthetic analysis shown in Scheme 1. We first synthesized the key precursor **16** in a 71% yield by coupling **12** and **13** via reductive amination using sodium triacetoxyborohydride (2 equiv) as the reducing agent.[18] Compound **12** was obtained by IBX oxidation of the corresponding alcohol **14**, prepared from L-(+)-tartaric acid following the reported procedure.[19] Alcohol **13** was obtained by the α-metalation of the *N*-Boc protected cyclic aminoacetal **15** using *s*-BuLi/TMEDA in THF at −78 °C followed by the trimethylsilyl chloride addition and acidic hydrolysis (Scheme 2). Although, the cyclization of **16** would have given the corresponding piperidine derivative, our previous experience of a poor diastereoselectivity in such cyclizations led us to transform it into cyclic 1,3-oxazine **11** for a better diastereoselectivity.[20b]

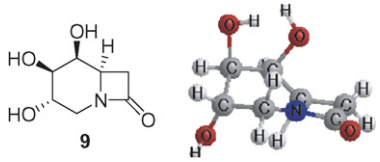
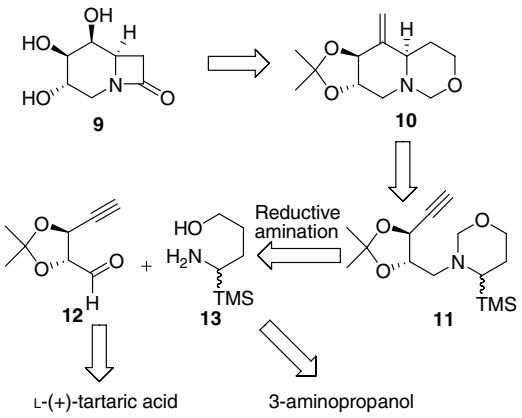
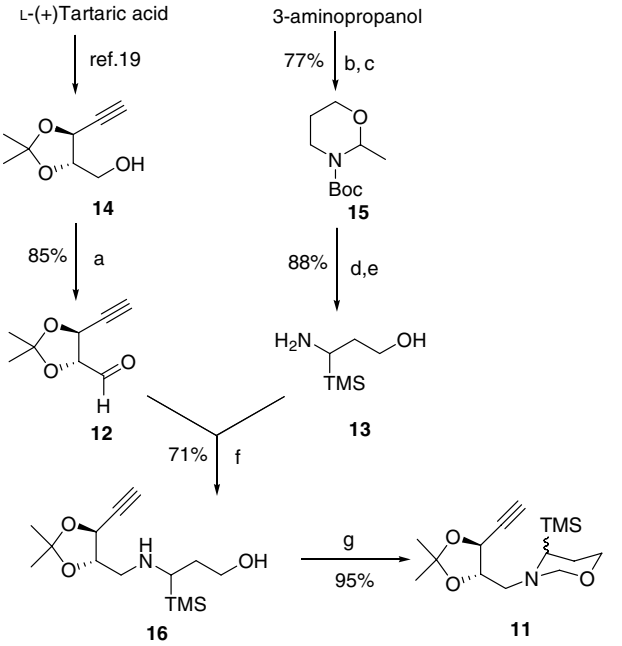


Figure 2. β-Lactam azasugar.

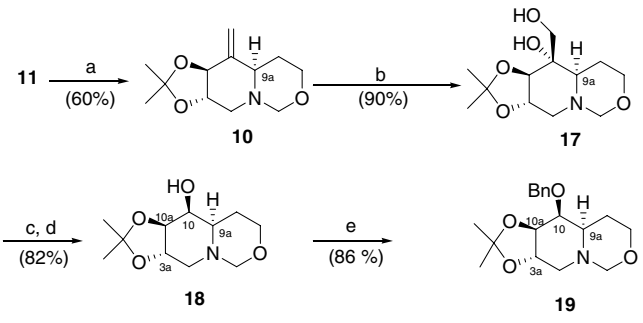


Scheme 1. Retrosynthetic analysis.

Substrate **11** was cyclized, employing a protocol reported from our group[20] by irradiating a dilute solution of **11** (3 mmol) and 1,4-dicyanonaphthalene (0.4 mmol) in a mixture of acetonitrile:*iso*-propanol (3:1, 250 mL) in a Pyrex vessel using a 450 W Hanovia medium pressure lamp, to give **10** as a single diastereomer in a 60% yield (Scheme 3). The cyclized product **10** was fully char-



Scheme 2. Synthesis of **11** via reductive amination of **12** and **13**: Reagents and conditions: (a) IBX, EtOAc, reflux, 9 h; (b) $(Boc)_2O$, TEA, 18 h; (c) $CH_3CH(OEt)_2$, PPTS, benzene, reflux, 24 h; (d) *s*-BuLi, TMEDA, −78 °C, 3 h, then TMSCl, −78 °C to rt, 3 h (e) 2 N HCl, dioxane, 80 °C, 45 min; (f) $NaBH(OAc)_3$, 1,2-dichloroethane, 12 h, then 2 N NaOH, 2 h and (g) $(CH_2O)_n$, benzene, Dean–Stark, 4 h.



Scheme 3. Reagents and conditions: (a) h*v*, 450 W, lamp, CH_3CN: *i*-PrOH (3:1), 4 h; (b) OsO_4, $K_3Fe(CN)_6$, K_2CO_3, py, *t*-BuOH/H_2O (1:1), rt, 16 h; (c) $NaIO_4$, silica gel, 15 min; (d) $NaBH_4$, MeOH, rt, 4 h and (e) BnBr, NaH, THF, reflux, 12 h.

acterized by ^{1}H NMR, ^{13}C NMR, 2D COSY and NOESY spectral analyzes. The dihydroxylation of **10** using OsO_4 produced **17** in a 90% yield. Single crystal X-ray diffraction analysis[21] unequivocally confirmed the stereochemistry of **10** at H-9a. The diol, upon sodium periodate oxidation afforded the corresponding ketone, which was immediately subjected to sodium borohydride reduction to afford **18** in a 82% yield as the exclusive diastereomer. The stereochemistry of **18** was also confirmed from 1D as well as 2D ^{1}H NMR spectroscopy of the corresponding benzylated derivative **19**. The stereochemistry at C-10 of **19** was ascertained by analyzing the coupling constants for H-3a (δ 4.24, dt, $J = 4.2$, 9.8 Hz), H-10a (δ 3.40, dd $J = 2.2$, 9.3 Hz), H-10 (δ 3.84, t, $J = 2$ Hz) and H-9a (δ 2.24, t, $J = 3.3$ Hz), which suggested orientations H-3a-axial, H-10a-axial, H-10-equatorial and H-9a-axial (Fig. 3). This stereochemical analysis was further confirmed by X-ray crystallography.[21]

A selective deprotection of the acetonide moiety of **19** and benzyl protection of the resultant diol gave the corresponding tribenzylated molecule **20** (Scheme 4). Subsequently, the 1,3-oxazine moiety of **20** was ring opened by refluxing with 6 N HCl in dioxane–methanol for 48 h. The resultant secondary amine was re-protected as its *N*-Boc derivative prior to PDC oxidation to the corresponding acid **21**. The deprotection of the *N*-Boc moiety of **21** by stirring with TFA in DCM at 0 °C for 3 h followed by the treatment with 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent)[22] in the presence of excess triethylamine afforded β-lactam **22** in a 53% yield. The removal of the *O*-benzyl protecting groups by hydrogenation at 60 psi afforded β-lactam-azasugar hybrid molecule **9** in a 95% yield.[23] In order to correlate the enzyme specific inhibition property of **9**, we also synthesized its (L-galacto configured) enantiomer **23** (*ent*-**9**) in a similar manner starting from D-(−)-tartaric acid.

Since, we had earlier observed[24] that 1-*N*-iminosugar **25** showed better inhibitory activity for the β-glucosidase ($K_i = 30$ μM) than **24** ($K_i = 90$ μM), we thought it would be interesting to evaluate the enzyme inhibition activity of **26** as well (Fig. 4). In this context, we synthesized compound **26**[25] following an analogous route to that described for **9**, starting from alcohol **27**[24] as shown in Scheme 5. The stereochemistry at C-10 and C-9a of **29** was ascertained by analyzing the coupling constants for H-3a (δ 3.57, ddd, $J = 4.1$, 7.3, 10.4 Hz), H-10a (δ 2.96, dd, $J = 8.7$, 10.5 Hz) and by ^{1}H–^{1}H NOESY spectroscopy.

The inhibitory activities of **9**, **23** and **26** were assessed against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase/β-mannosidase (almonds), α-glucosidase (yeast) and α-mannosidase (jack beans). The results are summarized in Table 1.

From the above results, it is apparent that the D-galacto-configured β-lactam **9** exhibited competitive and specific inhibition only against β-galactosidase. It inhibited α-galactosidase inhibition very poorly and showed no inhibition against α-/β-glucosidase and α-/β-mannosidase. This enzyme specific inhibition of **9** is in good agreement with its D-galacto-configured structure. Similarly, compound **23**, which is L-galacto/L-fuco-configured, showed no inhibition against any of the enzymes studied suggesting that it might be specific to fucosidase. Furthermore, β-lactam **26** which lacks the hydroxy func-

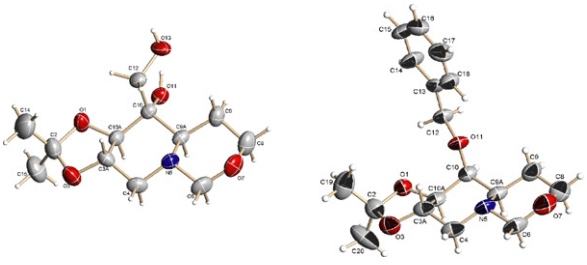


Figure 3. ORTEP diagrams of **17** and **19**.

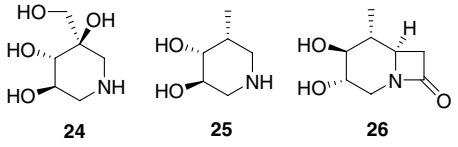
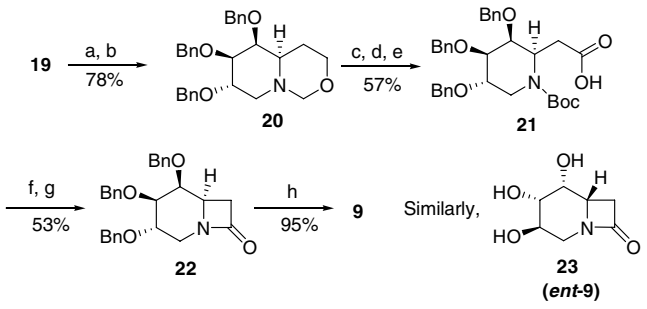
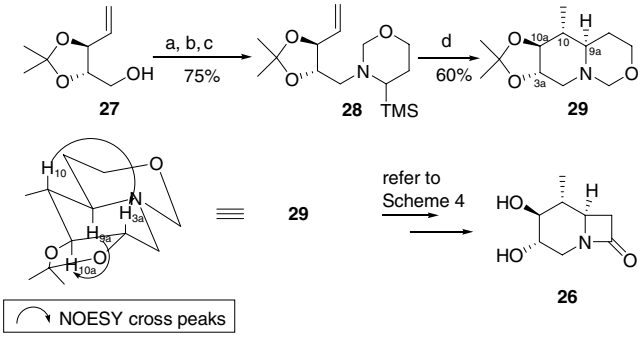


Figure 4. β-Lactam-azasugar hybrid **26** and comparative structures.



Scheme 4. Reagents and conditions: (a) 1 N HCl, MeOH, rt, 4 h; (b) BnBr, NaH, TBAI, THF, reflux, 24 h; (c) 6 N HCl, dioxane–MeOH, reflux, 48 h; (d) $(Boc)_2O$, TEA, rt, DCM, 8 h; (e) PDC, DMF, rt, 8 h; (f) TFA, DCM, 0 °C, 3 h; (g) 2-chloro-1-methylpyridinium iodide, TEA, CH_3CN, 60 °C–rt 32 h and (h) H_2, Pd/C, 60 psi, MeOH, 6 h.



Scheme 5. Reagents and conditions: (a) IBX, EtOAc, reflux, 9 h; (b) **13**, $NaBH(OAc)_3$, 1,2-dichloroethane, 12 h, then 2 N NaOH, 2 h; (c) $(CH_2O)_n$, benzene, Dean–Stark, 4 h and (d) hν, 450 W lamp, CH_3CN:*i*-PrOH (3:1), 4 h.

Table 1. Enzyme inhibition (K_i in μM) data

Enzyme	9	23	26
β-Galactosidase	172	n.i.	n.i.
α-Galactosidase	900	n.i.	n.i.
β-Glucosidase	n.i.	n.i.	n.i.
α-Glucosidase	n.i.	n.i.	n.i.
β-Mannosidase	n.i.	n.i.	n.i.
α-Mannosidase	n.i.	n.i.	n.i.

n.i = no inhibition up to 1 mM.

tionality at C-5 (loss of polarity and a binding site), unfortunately, did not inhibit any of the enzymes studied.

The fairly good and specific glycosidase inhibition exhibited by neutral β-lactam-azasugar hybrid molecule **9** points towards the possibility of improving its potency further by incorporating minor structural variations. We are probing this aspect, currently, by incorporating a hydroxymethylene functionality at C-7 of the β-lactam ring with the hope that it may provide an additional H-bonding site for recognition and would also increase the polarity of the molecule. Furthermore, we are also synthesizing other possible stereoisomeric analogues of **9** and the results will be disclosed appropriately in a full letter.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.09.005.

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21. CCDC-607587 and CCDC-607588 contain Supplementary crystallographic data for compounds **17** and **19**, respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.ac.uk/data_request/cif.
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23. Data for compound **9**: $[\alpha]_D^{27}$ +19.7 (*c* 0.25, MeOH); ^{1}H NMR (500 MHz, D_2O) δ 2.82 (app t, 1H, $J = 11.2$, 11.6), 3.05–3.12 (br m, 2H), 3.76 (dd, 1H, $J = 2.3$, 9.7), 3.81–3.85 (m, 1H), 3.90–3.96 (m, 1H), 4.70 (dd, 1H, $J = 6.8$, 13.0), 4.24 (aap t, 1H, $J = 1.9$, 2.3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 37.3 (CH_2), 43.2 (CH_2), 50.3 (CH), 64.6 (CH), 68.2 (CH), 73.4 (CH), 169.5 (C); MS: 196 ($M+Na^+$, 100%), 174 (MH^+, 20%), 155 (18%).
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25. Data for compound **26**: $[\alpha]_D^{27}$ +15.8 (*c* 0.18, MeOH); IR (in $CHCl_3$): 3440, 1750, 1212. cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 0.84 (d, 3H, $J = 6.6$), 1.27–1.36 (m, 1H), 2.46–2.59 (m, 2H), 2.96 [two sets of dd, like ddd, 1H, $J = (2.2, 4.4)$, (1.6, 4.4) and 14.8], 3.03 (dd, 1H, $J = 4.4$, 9.9), 3.08 (app t, 1H, $J = 9.3$, 10.4), 3.30–3.36 (m, 1H), 3.78 (dd, 1H, $J = 6.0$, 13.2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.2 (CH_3), 41.7 (CH), 42.2 (CH_2), 43.8 (CH_2), 52.2 (CH), 70.5 (CH), 76.2 (CH), 169.8 (C); MS: 194 ($M+Na^+$, 100%), 172 (MH^+, 15%).