C-(α-D-Glucopyranosyl)-phenyldiazomethanes—irreversible inhibitors of α-glucosidase

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A B S T R A C T

Several *C* (α D glucopyranosyl) phenyldiazomethanes, with different substituent groups at the *para* position of the phenyl ring, were prepared. The stabilities of these diazo compounds were investigated through NMR and UV monitoring. The *para* cyano substituted diazo compound was found to be stable in neutral media (pH 7.0 buffer) and could be isolated. Inhibitory activity investigations indicated that this compound is an irreversible inhibitor against α glucosidase from *Saccharomyces cerevisiae*.

Keywords:
α-Glucosidase
Irreversible inhibition
Diazo compounds
Carbohydrate
Synthesis

1. Introduction

Irreversible inhibitors of glycosidases are powerful tools for structural and functional studies, particularly when they bind at the active site of the enzyme.[1–4] To this end, generally substrate analogues are chosen possessing functional groups that can be activated by the catalytic center of the enzyme, leading to ensuing covalent bond generation and thus to enzyme inactivation. The presence of acidic groups within the active site of glycosidases suggests the attachment of acid sensitive moieties for such inhibitors, thus, yielding on protonation eventually electrophilic intermediates that generate a covalent bond with nucleophilic amino acid residue in the active site.[2–7] Examples of such potential mechanism based inhibitors include epoxide and aziridine moiety containing inositols and carbohydrates and derivatives.[1,4,8–13] Also the inhibition by glycosyl diazomethane,[14] 1 *N* glycosyl 3 *N* aryl triazenes[2,15] and *C* glycosyldiazoketones[16] has been discussed along these lines. Other types of covalent mechanism based inhibitors are compounds either fluorinated in the aglycon[4,17] or, even more interesting, in the carbohydrate residue,[4,18] generating on activation relatively stable covalent adducts with the enzyme. Related compounds with other reactive groups (azido, diazirino, isothiocyanato, bromoacetyl or quinonyl groups) have been used as affinity labels.[4,19–22] The reported work was mainly focused so far on irreversible inhibition of β glycosidases, however, irreversible inhibition of α glycosidases is more challenging.[4]

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The discussion on covalent mechanism based glycosidase inhibition is not without ambiguity. For instance, *C* glycosyl diazomethanes and derivatives are expected to be protonated in the active site, generating under nitrogen loss an alkylating agent that forms a covalent bond with the enzyme.[4,14] However, the parent *C* glycosyl diazomethanes, introduced by the Lehmann group,[14] possess even at pH 7.0 very low stability, hence their chance to reach the enzyme active site before decomposition is low. The Sinnott group introduced 1 *N* glycosyl 3 *N* aryl triazenes.[15] As they are stable at pH 7.0 and lead after protonation at low pH to the same intermediate as generated from *C* glycosyl diazomethanes, their glycosidase inhibition properties were studied. Yet, finally it was concluded 'some optimization ... may be necessary before unambiguous active site directed irreversible inhibition is seen'.[15b] Possibly, in these enzyme catalyzed triazene decompositions further products, for instance by reaction with the released anilines, were formed, that were not considered in the studies.

Generally, aryl glycosides are good substrates for glycosidases and C aryl substitution greatly stabilizes diazomethanes, hence, we turned to *C* (α D glucosyl) phenyldiazomethane (Fig. 1, **1a**) as potential mechanism based inhibitor of α glucosidases.[23] Yet, also **1a** could be only used in methanolic solution for enzyme studies and it still decomposed quite rapidly at pH 7.0.[23] Thus, in a quantitative reaction from **1a** exclusively the methyl ether and in aqueous solution the hydroxy compound was obtained. Hence, it was the aim to introduce based on this structure design a shelf stable molecule that could be readily investigated in enzyme inhibition studies. In this paper, we report on the synthesis of *C* (α D glucopyranosyl) phenyldiazomethanes **1b 1e** (Fig. 1) with different

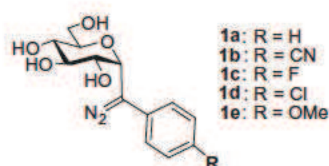


Figure 1. *C*-(α-D-Glucopyranosyl)-phenyldiazomethanes **1a–1e**.

substituents at the *para* position of the phenyl ring, and on the investigation of their stabilities, as well as on the inhibition prop erties towards α glucosidase from yeast.

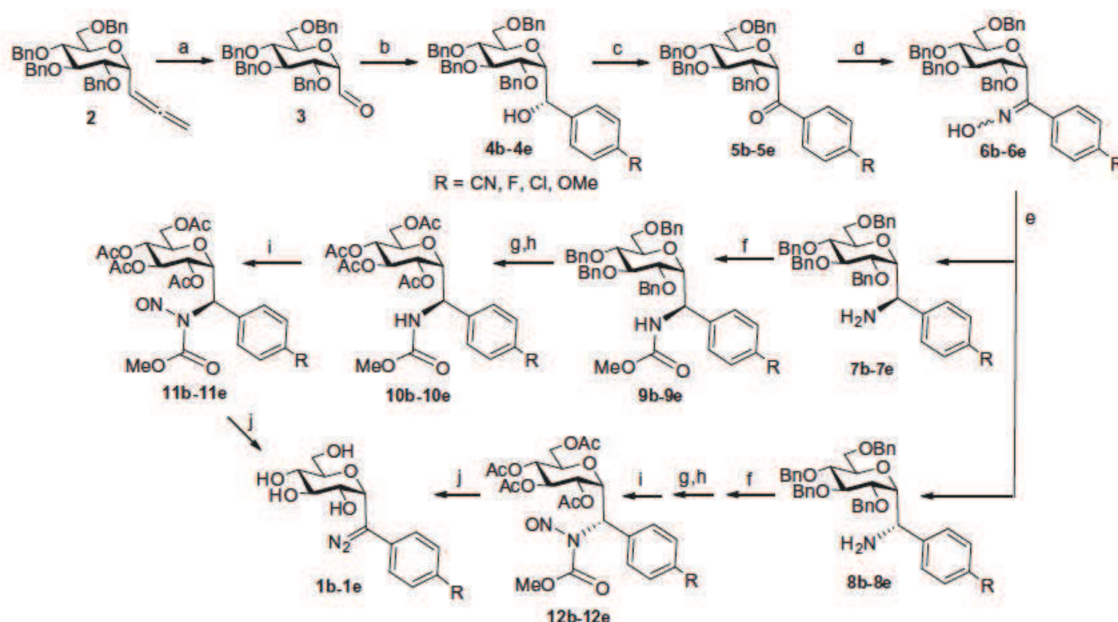
2. Results and discussions

2.1. Synthesis of *C*-(α-D-glucopyranosyl)-phenyldiazomethanes

All the target molecules **1b 1e** were prepared from the same readily available starting material, namely α linked *C* glucosyl al lene **2** (Scheme 1).[24] Ozonolysis of compound **2** gave the *C* glucosyl aldehyde **3**[25] which, without isolation, was immediately reacted with different Grignard reagents to afford the corresponding *C* glucosyl benzylic alcohols **4b 4e**, with different substituents at the *para* position of the phenyl ring, including the electron with drawing cyano, fluorine and chlorine groups, as well as the elec tron donating methoxy group. In each case, the configuration of the newly formed stereocenter at the benzyl carbon was assigned following our previous work.[26] For the introduction of the amino groups, **4b 4e** were oxidized to phenyl ketones **5b 5e**, which were treated with hydroxylammonium chloride to afford the diastereo meric oximes **6b 6e**. The (*E*, *Z*) configuration of the oximes was not established since a mixture was sufficient in the following step. The oximes were then reduced to diastereomeric amines **7b 7e** and **8b 8e**. The configuration of the diastereomers was differenti ated following the assignment in our previous work.[26] For the sub strates without cyano group, the reduction reactions were conducted under standard conditions by using $LiAlH_4$ in THF, while the cyano substituted compound **6b** was reduced under the milder conditions of Zn/$HCOONH_4$ in MeOH.[27] Treatment of **7b 7e** with methyl chloroformate and triethylamine furnished the desired ure thanes **9b 9e**. Hydrogenolytic O debenylation followed by acetyla tion under standard conditions gave the O acetylated derivatives **10b 10e**, which were treated with $NaNO_2$ in a mixture of Ac_2O and HOAc to give the *N* nitroso urethanes **11b 11e**.[28] The other diastereomers **12b 12e** were prepared following the same route. Both diastereomers can be used as the precursors for generating the desired phenyldiazomethane derivatives **1b 1e** by adding 2 equiv of sodium methanolate in MeOH as the solvent.[23]

2.2. Characterizations and stability investigations of the diazo compounds

The in situ prepared diazo compounds were characterized by the NMR monitored reactions in CD_3OD, as well as by their typical UV and IR absorptions. First, with the purpose of investigating the products of step j in Scheme 1, that generates the diazo compounds from the *N* nitroso urethanes, the reactions were performed in small scale (0.015 mmol) in the NMR tube by using CD_3OD (0.15 mL) as the solvent and 2 equiv of sodium methanolate as the base (Fig. 2) and the NMR spectra of the reaction mixtures were recorded. It was found that the electronic properties of the substi tuent on the phenyl ring had a strong influence on the reaction products. As shown, with the strong electron withdrawing cyano group at the *para* position of the phenyl ring, the in situ prepared glucosyl phenyldiazomethane **1b** was stable enough to resist the solvolysis by CD_3OD (Fig. 2a). On the other hand, the electron donating methoxy group destabilized the diazo compound **1e**, that was trapped by the CD_3OD immediately after its generation. Hence, only diastereomeric solvolysis products **13e** were detected (Fig. 2d). The effects of the other two electron withdrawing groups fluorine and chlorine are in between; both diazo compounds **1c** and **1d** as well as solvolysis products **13c** and **13d** were detected during the reactions (Fig. 2b and c).



Scheme 1. Preparation of diazo compounds **1b–1e**. Reagents and conditions: (a) O_3, DCM, −78 °C, 100%; (b) for **4b**, 4-bromobenzonitrile, *i*PrMgCl·LiCl, THF, 0 °C for 2 h, then **3**, −10 to 0 °C, THF, two steps, 65%; for **4c**, 1-bromo-4-fluorobenzene, Mg, THF, reflux for 2 h, then **3**, 0 °C–rt, THF, two steps, 81%; for **4d**, 1-bromo-4-chlorobenzene, Mg, THF, reflux for 2 h, then **3**, 0 °C to rt, THF, two steps, 75%; for **4e**, 4-bromanisol, Mg, THF, reflux for 2 h, then **3**, 0 °C–rt, THF, two steps, 80%; (c) PCC, DCM, rt, 71–85%; (d) $NH_2OH{\cdot}HCl$, Py, rt, 92–98%; (e) for **7b**, Zn, $HCOONH_4$, MeOH, reflux, 79%; for **7c–7e**, $LiAlH_4$, THF, reflux, 86–93%; (f) Cl-COOMe, Et_3N, DCM, rt, 88–95%; (g) for **10b** and **12b**, Pd/C, H_2, EtOAc, rt, 90%; for **10c–10e** and **2c–12e**, EtOAc/MeOH (v/v, 1:1), rt, 100%; (h) Ac_2O, DMAP, Py, 100%; (i) $NaNO_2$, Ac_2O/HOAc (v/v, 2:1), 83–88%; (j) MeONa, MeOH.

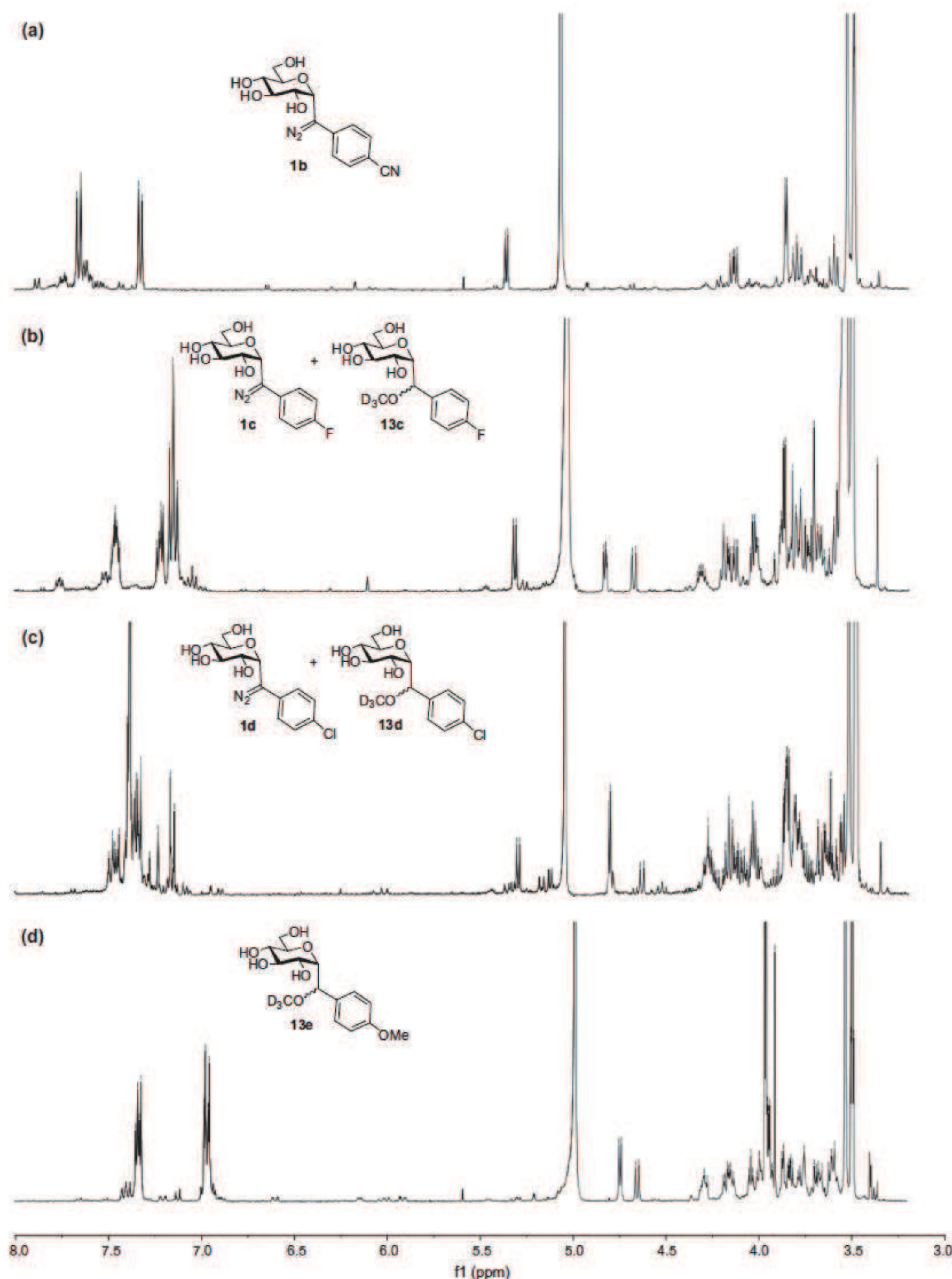
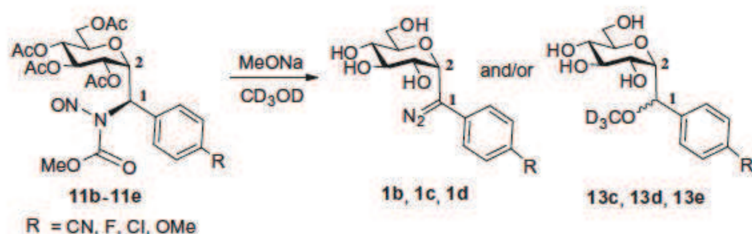


Figure 2. Effects of different substituents on the stabilities of the diazo products through NMR-monitored generating reactions.

Other characterization data of the reaction mixture recorded by UV and IR spectra supported the conclusions (Table 1). In all cases, except for the methoxy substitution, the typical absorptions for diazo compounds (2000 2100 cm $^{-1}$) in the IR spectra were de tected, indicating the generation of the expected diazo molecules. In the UV spectra, the absorption of the phenyl ring (normally at 254 nm) showed a big redshift in phenyldiazomethane, due to the negative charge at the benzyl carbon. The pure glucosyl phenyldiazomethane **1b** gave a λ_{max} of 317 nm, the pure solvolysis products **13e** gave a λ_{max} of 247 nm, while the mixtures of the dia zo compounds and the solvolysis products gave the λ_{max} in be tween, which were 280 nm in the fluorine substituted case and 281 nm in the chlorine substituted case.

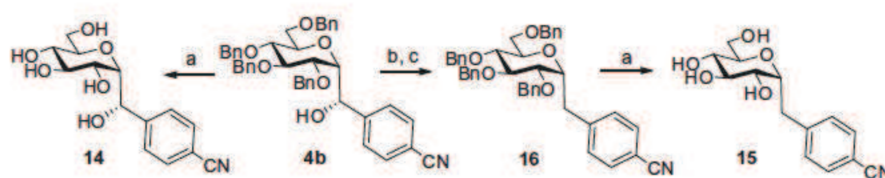
After investigating the NMR, UV and IR spectra of the generated reaction mixtures, it was obvious, the electron withdrawing groups, especially the cyano group, stabilized the diazo compound,

Table 1
Characterization data of the reaction mixture by NMR, UV and IR spectra



Starting material	11b	11c	11d	11e
R	CN	F	Cl	–OMe
Products	**1b**	**1c**, **13c**	**1d**, **13d**	**13e**
$\delta_{2\,H}$ and $J_{2,3}$ of the diazo compound (ppm, Hz)	5.28 (6.4 Hz)	5.22 (6.4 Hz)	5.20 (6.4 Hz)	–
$\delta_{1\,H}$ and $J_{1,2}$ of the solvolysis products (ppm, Hz)	–	4.71 (3.6 Hz) 4.55 (8.4 Hz)	4.68 (4.4 Hz) 4.50 (8.4 Hz)	4.61 (4.8 Hz) 4.51 (8.4 Hz)
Ratio of the diazo compound (%)	>95	48	32	–
Ratio of the sovolysis compounds (%)	–	52	68	>95
λ_{max} in MeOH (nm)	317	280 (broad)	281 (broad)	247
Typical absorption in IR (cm^{-1})	2072	2057	2072	–

Figure 3. Decomposition behavior of compound **1b** at different pH values. (a) UV-monitored decomposition experiment at pH 6.0. (b) UV-monitored decomposition experiment at pH 5.0. (c) Decomposition rate at different pH values, fitting equation: $A = A_1\exp(-t/t_1) + A_0$, $t_{1/2} = t_1\ln 2$.



Scheme 2. Preparation of compounds **14** and **15**. Reagents and conditions: (a) Pd/C, H_2, ethyl acetate, rt, 87% for **14**, 85% for **15**; (b) PTC-Cl, DMAP, CH_3CN, 86%; (c) Bu_3SnH, AIBN, toluene, reflux, 76%.

Figure 4. Time and concentration-dependent inhibition of α-glucosidase from *Saccharomyces cerevisiae* by **1b**. (a) Non-linear plot of residual enzyme activity versus time at different inhibitor concentrations fitted to an exponential decay equation. (b) Pseudo-first-order rate constants (k_{obs}) at each concentration. (c) Re-plot of the reciprocal of the observed rate constants (k_{obs}) versus the reciprocal of the inhibitor concentration. (d) Protective effect of reversible inhibitor **17** on the inhibition of α-glucosidase by **1b**.

while the electron donating methoxy group destabilized the diazo compound. Thus, a real stable diazo compound **1b** could be obtained that can even be purified through column chromatography.

2.3. pH-dependent stability of the cyano-substituted glucosyl phenyldiazomethane 1b

In order to get more precise data about the stability of the diazo compound **1b**, we investigated the decomposition kinetics of **1b** in buffers with different pH values. The isolated pure compound **1b** was dissolved in 0.2 mL buffer in a cuvette, with the concentration of 50 μM. The UV spectra of the compound were scanned in the range of 190 350 nm every 30 s at room temperature until there was no change. As shown in Figure 3a and b, at pH 6.0 or pH 5.0, the λ_{max} of the compound was processing slowly a blue shift, indicating the diazo compound was decomposing along with time; at pH 7.0, the UV spectra of 1b did not change at all for even several hours, indicating **1b** is stable in neutral media; while at pH 4.0 or pH 3.0, the blue shift processed too fast to be recorded in details. With the original UV spectra, we can also get the decomposition

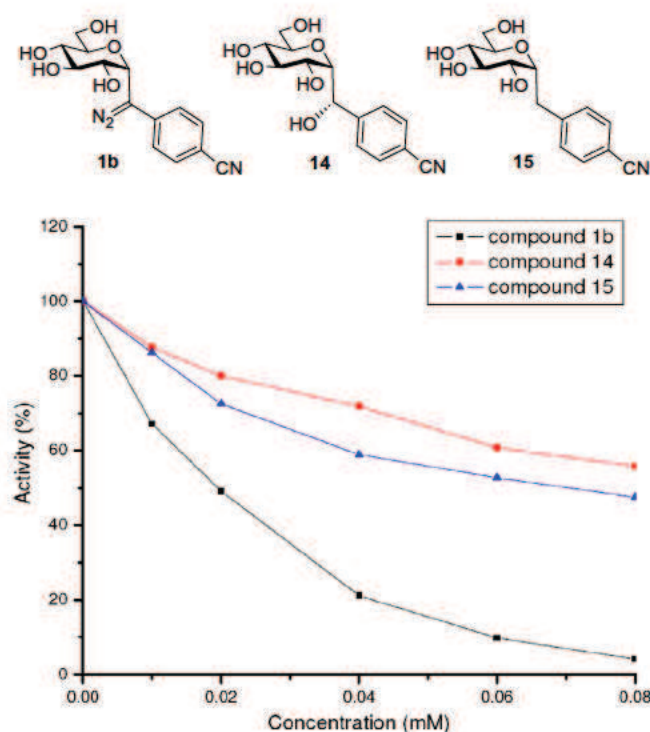


Figure 5. Inhibiting property comparisons of irreversible inhibitor **1b** with reversible inhibitors **14** and **15**.

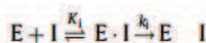
kinetic curves (absorption vs time) of the diazo compound **1b** by extracting the absorption values at 315 nm, which is the λ_{max} at pH 7.0, at each time interval (Fig. 3c). The decomposition behavior of **1b** at every pH value from 7.0 to 3.0 followed perfectly an exponential decay with time. After fitting to an equation, we easily got the $t_{1/2}$ at different pH values, $t_{1/2}$ at pH 6.0 is 4.2 min, $t_{1/2}$ at pH 5.0 is 141 s, $t_{1/2}$ at pH 4.0 is 29 s, and $t_{1/2}$ at pH 3.0 is 11 s.

2.4. Synthesis of compounds 14 and 15 for comparison

For comparison purpose, we prepared for the glucosidase inhibition studies *C* glucosides **14** and **15** from compound **4b** (Scheme 2). Hydrogenolytic O debenzylation of **4b** with Pd/C as catalyst in ethyl acetate for 6 h afforded compound **14** in good yield. Compound **4b** was then transformed to compound **16** by using the standard conditions of the Barton McCombie deoxygenation.[29,30] Hydrogenolytic O debenylation of **16** gave compound **15**.

2.5. Inhibiting property of compound 1b towards α-glucosidase from *Saccharomyces cerevisiae*

With the stable glucosyl phenyldiazomethane 1b in hand, the inhibiting activity and the type of inhibition towards α glucosidase from *Saccharomyces cerevisiae* was investigated.[31] The enzyme assay method was based on measuring the release of *p* nitrophenol from *p* nitrophenyl α D glucopyranoside (PNPG).[32] Different concentrations of inhibitor **1b** in pH 7.5 Tris HCl buffer were incubated with the enzyme, at different time intervals, aliquots were taken from the incubation assay and added to the PNPG solution in pH 6.8 buffer for investigating the remaining activity. Results are expressed as percentage activities relative to the corresponding enzyme controls. The inhibition was found to follow the kinetic scheme shown below: the inhibitor (I) first binds reversibly to the enzyme (E), with an equilibrium binding constant K_i, then reacts covalently, with a rate constant k_i:



$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} E \cdot I \xrightarrow{k_i} E\ \ I$$

with incubation of diazo compound **1b**, an exponential decay of α glucosidase activity with time was observed, which is concentration dependent (Fig. 4a), indicating the inhibitor behaved in an irreversible manner. Pseudo first order rate constants (k_{obs}) for different inactivation concentrations were received by fitting the plots of residual rates versus incubation time to first order curves (Fig. 4b). By plotting the reciprocal of the observed rate constants (k_{obs}) as the function of the reciprocal of the inhibition concentration according to the method of Kitz and Wilson,[33] a straight line was obtained (Fig. 4c). This line did not pass through the origin but through the positive *y* axis. Thus, a saturation is effective that exhibits the participation of the active site in the enzyme inhibition. Kinetic constants of the equilibrium binding constant (K_i) and the inactivation rate constant (k_i) for irreversible inhibitors were obtained by fitting the equation: $k_{obs} = k_i[I]/(K_i + [I])$ (Fig. 4c). In our case, the K_i and k_i values were calculated to be 2.16 mM and 2.14 min^{-1}, respectively. A protective effect of the known reversible α glucosidase inhibitor **17** (K_i = 1.1 μM)[26] on the inactivation of α glucosidase by **1b** was found when both of the compounds were incubated with the enzyme (Fig. 4d).

Under the conditions described above, using an incubation time of 40 min, the concentration dependence of inhibiting activity of compound **1b** was also compared to the reversible inhibitors **14** and **15** (Fig. 5). As shown, **1b** performed a better inhibition property than the other two compounds: incubation of α glucosidase with 60 μM **1b** for 40 min resulted in 90% inactivation of the enzyme, while **14** and **15** only afforded 40 48% inactivation. For compounds **14** and **15** dialysis of the inactivated enzyme for 24 h at 4 °C fully restored enzyme activity whereas, the same treatment for **1b** did not regenerate enzyme activity. Thus a covalent linkage of the inhibitor **1b** to the enzyme active site is strongly supported. The IC_{50} value of compound **1b** with an incubation time of 40 min is 20.6 μM.

Analysis of the pure α glucosidase by MALDI TOF MS showed a molecular weight of 62385 Da, which is in agreement with the reported data.[31,34] Similar analysis of the inactivated enzyme revealed a broad peak with a maximum at 64492 Da, thus corresponding to binding of up to several inhibitor molecules.

3. Conclusion

Among the α D glucopyranosyl phenyldiazomethanes with different substituent groups at the *para* position of the phenyl ring, *p* cyano substituted compound **1b**, with a strong electron withdrawing group to stabilize the negative charge, was found to be stable in neutral or basic media. The characterization data including NMR, UV, IR and MS can be obtained after column chromatography. The stability of **1b** depends on the pH values. Inhibitory activity investigations indicated that this compound is an irreversible inhibitor (eventually a suicide substrate) of α glucosidase. The kinetic constants for the irreversible inhibition and the IC_{50} value of **1b** are promising. Compound **1b** should be useful for further structural and mechanistic studies of α glucosidases.

4. Experimental

4.1. General procedure

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Solvents were purified by standard procedures. All reactions were carried out under anhydrous conditions with freshly distilled solvents, unless otherwise noted. Reactions were monitored by analytical thin layer chromatography on silica gel 60 F254 precoated on

aluminum plates (E. Merck). Spots were detected under UV (254 nm) and/or by staining with a solution of $(NH_4)_6Mo_7O_{24}$ $4H_2O$ (24 g) and $Ce(NH_4)_2(NO_3)_6$ (0.5 g) in sulfuric acid (5%, 500 mL). Solvents were evaporated under reduced pressure and below 40 °C (bath). Organic solutions of crude products were dried over anhydrous $MgSO_4$. Column chromatography was performed on silica gel (200 300 mesh). ^{1}H NMR spectra were recorded on an Advance DRX Bruker 400 spectrometer at 25 °C. Chemical shifts (in ppm) were referenced to resonance of the undeuterated solvent (solvent $CDCl_3$, δ = 7.26 ppm). ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with the undeuterated solvent (solvent $CDCl_3$, δ = 77.00 ppm). Mass spectra were recorded using a Bruker esquire 3000+ ESI MS mass spectrometer. Optical rotations were measured at 25 °C using sodium D line light. UV spectra were taken by a CARY 100 UV vis spectrophotometer and IR spectra were taken by a Perkin Elmer spectrum100 FT IR spectrometer at 25 °C. MeOH was used as the solvent in both cases.

4.2. Preparation of C-(α-D-glucopyranosyl)-phenyldiazomethanes

4.2.1. Compound 3

A solution of 1.4 g (2.5 mmol) of allene **2**[24] in CH_2C1_2 (50 mL) was cooled to 78 °C and ozone was bubbled through the solution until it was saturated. The excess ozone was eliminated by bubbling nitrogen through the solution. The solution was warmed to rt, the solvent was removed in vacuo without heating and the crude product of **3** was used directly in the next step. The ^{1}H NMR data were identical to the literature.[25]

4.2.2. Compound 4b

A dry and nitrogen flushed 25 mL flask, was charged with *i*PrMgCl LiCl (4.9 mL, 14% solution in THF, 5.0 mmol), and 4 bromobenzonitrile (1.36 g, 7.5 mmol) in degassed THF (8 mL) was added at 0 °C. The reaction mixture was stirred at the same temperature under nitrogen for 4 h to complete the Br/Mg exchange.[35] Then the solution was cooled to 20 °C and the in situ prepared aldehyde **3** (2.5 mmol) in THF (10 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h until the aldehyde was consumed completely, satd NH_4Cl solution was added to quench the reaction. The mixture was extracted three times with ethyl acetate, the combined organic layer was dried, filtered and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 3:1) to afford **4b** (1.06 g, 65%) as a colorless oil: R_f = 0.3 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D$ +29.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.40 7.27 (m, 16H), 7.22 7.16 (m, 4H), 5.17 (d, *J* = 8.4 Hz, 1H), 4.85 (d, *J* = 11.6 Hz, 1H), 4.85 (s, 2H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.64 (d, *J* = 11.2 Hz, 1H), 4.51 (d, *J* = 11.2 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.29 (d, *J* = 11.6 Hz, 1H), 4.07 4.01 (m, 2H), 3.97 3.94 (m, 2H), 3.78 (ddd, *J* = 8.8, 4.4, 2.4 Hz, 1H), 3.64 (dd, *J* = 8.8, 7.6 Hz, 1H), 3.53 (dd, *J* = 10.4, 4.4 Hz, 1H), 3.40 (dd, *J* = 10.4, 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.34, 138.05, 137.76, 137.72, 136.77, 131.93, 128.77, 128.52, 128.46, 128.37, 128.34, 127.95, 127.91, 127.89, 127.79, 127.75, 118.86, 111.53, 80.98, 79.37, 74.93, 74.85, 74.57, 74.43, 73.91, 73.38, 71.68, 68.79; HRMS (ESI) Calcd for $C_{42}H_{41}NO_6Na$ $[M+Na]^+$: 678.2832. Found: 678.2837.

4.2.3. Compound 5b

To a vigorously stirred solution of compound **4b** (1.05 g, 1.6 mmol) in anhydrous CH_2C1_2 (20 mL), activated 4 Å molecular sieves (1.5 g) and pyridinium chlorochromate (1.73 g, 8.0 mmol) were added in one portion. The reaction mixture was stirred at rt for 5 h, then concentrated in vacuo and purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to afford ketone **5b** (0.74 g, 71%) as a colorless oil: R_f = 0.6 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D$ +37.4 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.39 7.14 (m, 18H), 7.11 (d, *J* = 6.6 Hz, 2H), 5.06 (d, *J* = 6.0 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.50 (d, *J* = 10.4 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.39 (t, *J* = 8.0 Hz, 1H), 3.95 (dd, *J* = 8.4, 6.0 Hz, 1H), 3.83 (ddd, *J* = 9.6, 3.6, 2.2 Hz, 1H), 3.71 3.65 (m, 2H), 3.56 (dd, *J* = 10.4, 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.37, 139.38, 138.47, 138.10, 137.82, 137.68, 132.20, 129.10, 128.44, 128.43, 128.34, 127.98, 127.89, 127.81, 127.74, 127.72, 127.68, 117.90, 116.24, 81.33, 78.73, 75.07, 74.59, 74.52, 74.29, 73.89, 73.37, 68.62; HRMS (ESI) Calcd for $C_{42}H_{39}NO_6K$ $[M+K]^+$: 692.2414. Found: 692.2420.

4.2.4. Compound 6b

To a solution of **5b** (0.71 g, 1.09 mmol) in dry pyridine (20 mL) was added hydroxylammonium chloride (0.38 g, 5.44 mmol). After stirring for 4 h at rt, the mixture was concentrated in vacuo and purified by column chromatography (petroleum ether/ethyl acetate, 4:1) to give *E*/*Z* (1:1) isomers **6b*h*,*l*** (0.67 g, 92%) as colorless oils. Compound **6b*h***: R_f = 0.5 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D$ +40.3 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.53 7.51 (m, 2H), 7.44 7.42 (m, 2H), 7.37 7.21 (m, 18H), 7.12 7.10 (m, 2H), 5.02 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.83 (d, *J* = 12.4 Hz, 1H), 4.78 (d, *J* = 10.8 Hz, 1H), 4.60 (t, *J* = 8.8 Hz, 1H), 4.56 (d, *J* = 6.4 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.50 (d, *J* = 11.2 Hz, 1H), 4.45 (d, *J* = 12.0 Hz, 1H), 4.33 (d, *J* = 12.0 Hz, 1H), 3.88 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.83 (ddd, *J* = 10.0, 4.0, 2.0 Hz, 1H), 3.60 3.50 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.56, 138.81, 138.05, 137.75, 137.46, 131.76, 128.79, 128.51, 128.41, 128.34, 128.31, 128.29, 128.16, 127.99, 127.77, 127.76, 127.62, 118.48, 112.44, 82.78, 80.19, 78.39, 75.59, 75.39, 75.03, 74.64, 73.41, 72.90, 68.99; HRMS (ESI) Calcd for $C_{42}H_{40}N_2O_6Na$ $[M+Na]^+$: 691.2784. Found: 691.2791. Compound **6b*l***: R_f = 0.3 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D$ +12.6 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.34 7.14 (m, 20H), 5.66 (d, *J* = 3.2 Hz, 1H), 4.60 4.52 (m, 5H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 2H), 4.40 (t, *J* = 3.2 Hz, 1H), 4.35 (d, *J* = 12.0 Hz, 1H), 3.94 3.90 (m, 1H), 3.87 (dd, *J* = 4.4, 3.2 Hz, 1H), 3.81 (dd, *J* = 8.0, 4.4 Hz, 1H), 3.55 (dd, *J* = 10.4, 4.4 Hz, 1H), 3.47 (dd, *J* = 10.8, 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.96, 138.48, 138.07, 138.03, 137.82, 137.45, 131.23, 130.09, 128.46, 128.33, 128.29, 128.26, 128.09, 127.87, 127.82, 127.76, 127.71, 127.62, 118.87, 112.14, 78.74, 76.00, 75.77, 73.78, 73.16, 72.92, 72.66, 72.26, 70.05, 69.14; MS (ESI) 669 $[M + H]^+$.

4.2.5. Compound 7b and 8b

To a solution of a mixture of **6b*h*,*l*** (0.67 g, 1.0 mmol) in methanol (10 mL) was added ammonium formate (0.63 g, 10.0 mmol) and zinc dust (0.33 g, 5.0 mmol). The mixture was stirred under reflux for 6 h, and then filtered through Celite. The filtrate was evaporated under vacuum and purified by column chromatography (petroleum ether/ethyl acetate, 1:1.5 1:5) to afford **7b** (233 mg, 35%) as a white solid and **8b** (286 mg, 44%) as a colorless oil. Compound **7b**: R_f = 0.4 (dichloromethane/methanol, 30:1); $[\alpha]_D$ +26.1 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.36 7.26 (m, 16H), 7.19 7.16 (m, 4H), 4.79 (d, *J* = 11.6 Hz, 1H), 4.77 (d, *J* = 12.0 Hz, 1H), 4.74 (d, *J* = 11.6 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.61 (d, *J* = 11.2 Hz, 1H), 4.49 (d, *J* = 11.2 Hz, 1H), 4.41 (d, *J* = 9.2 Hz, 1H), 4.34 (d, *J* = 12.0 Hz, 1H), 4.23 (d, *J* = 12.0 Hz, 1H), 4.07 (dd, *J* = 8.8, 4.0 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 1H), 3.89 (dd, *J* = 6.4, 4.0 Hz, 1H), 3.73 3.69 (m, 1H), 3.65 (dd, *J* = 8.0, 6.4 Hz, 1H), 3.46 (dd, *J* = 10.4,

4.4 Hz, 1H), 3.27 (dd, J = 10.4, 2.8 Hz, 1H), 1.79 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.10, 137.92, 137.91, 137.48, 131.92, 128.59, 128.48, 128.40, 128.29, 128.14, 127.98, 127.84, 127.77, 127.62, 127.58, 118.92, 110.98, 79.53, 77.70, 76.48, 75.80, 73.98, 73.96, 73.57, 73.19, 73.06, 68.78, 54.92; HRMS (ESI) Calcd for $C_{42}H_{42}N_2O_5$-Na [M+Na]$^+$: 677.2991. Found: 677.2989. Compound **8b**: R_f = 0.1 (dichloromethane/methanol, 30:1); $[\alpha]_D$ +13.5 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.4 Hz, 2H), 7.40 7.35 (m, 2H), 7.36 7.25 (m, 15H), 7.24 7.18 (m, 5H), 7.03 7.00 (m, 2H), 4.68 (d, J = 11.2 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.58 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 10.4 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.53 (d, J = 11.2 Hz, 1H), 4.40 (d, J = 8.0 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.14 4.07 (m, 1H), 3.99 (dd, J = 9.2, 4.0 Hz, 1H), 3.92 (d, J = 11.2 Hz, 1H), 3.89 (t, J = 5.6 Hz, 1H), 3.75 3.68 (m, 2H), 3.63 (dd, J = 7.6, 5.6 Hz, 1H), 3.34 (dd, J = 6.0, 4.4 Hz, 1H), 1.95 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.01, 138.10, 137.96, 137.87, 137.34, 131.95, 128.88, 128.43, 128.38, 128.24, 127.90, 127.79, 127.72, 127.64, 118.85, 110.98, 78.25, 77.15, 76.28, 76.20, 73.56, 73.55, 73.52, 73.38, 72.16, 69.29, 54.72; HRMS (ESI) Calcd for $C_{42}H_{42}N_2O_5Na$ [M+Na]$^+$: 677.2991. Found: 677.3005.

4.2.6. Compound 9b

To a solution of **7b** (233 mg, 0.36 mmol) in CH_2Cl_2 (7 mL) was added methyl chloroformate (55 µL, 0.71 mmol) and triethylamine (0.2 mL, 1.43 mmol). The mixture was stirred at room temperature for 2 h, then evaporated under vacuum and purified by column chromatography (petroleum ether/ethyl acetate, 2.5:1) to afford **9b** (224 mg, 88%) as a colorless glassy solid: R_f = 0.4 (petroleum ether/ethyl acetate, 1.5:1); $[\alpha]_D$ +12.8 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 8.4 Hz, 2H), 7.36 7.24 (m, 18H), 7.22 7.18 (m, 4H), 6.19 (br s, 1H), 5.14 (t, J = 6.8 Hz, 1H), 4.69 (d, J = 11.6 Hz, 1H), 5.66 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 10.4 Hz, 1H), 4.54 (d, J = 11.2 Hz, 1H), 4.52 (d, J = 11.2 Hz, 1H), 4.50 (d, J = 12.0 Hz, 1H), 4.40 (d, J = 12.0 Hz, 1H), 4.33 (d, J = 11.2 Hz, 1H), 4.09 (br s, 1H), 4.00 3.96 (m, 1H), 3.87 (t, J = 5.6 Hz, 1H), 3.72 (dd, J = 8.0, 6.0 Hz, 1H), 3.63 (dd, J = 10.4, 4.4 Hz, 1H), 3.60 (s, 3H), 3.58 3.52 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.42, 145.77, 138.02, 137.91, 137.68, 137.00, 132.11, 128.63, 128.51, 128.40, 128.36, 128.23, 128.18, 127.98, 127.88, 127.81, 127.71, 127.68, 127.58, 118.77, 111.06, 77.20, 76.31, 73.98, 73.58, 73.40, 73.34 (2C), 72.91, 68.95, 56.11, 52.22; HRMS (ESI) Calcd for $C_{44}H_{45}N_2O_7$ [M+H]$^+$: 713.3227. Found: 713.3218.

4.2.7. Compound 10b

To a solution of **9b** (142 mg, 0.2 mmol) in ethyl acetate (10 mL) was added palladium on carbon (10%, 108 mg, 0.1 mmol). After hydrogenolysis for 5 h at room temperature, the mixture was fil tered and concentrated in vacuo. The residue was resolved in dry pyridine (4 mL), Ac_2O (0.4 mL, 4.0 mmol) was added then to the solution. After stirring overnight at room temperature, the mixture was coevaporated with toluene and purified by column chroma tography (petroleum ether/ethyl acetate, 1:1) to afford **10b** (92 mg, 90%) as a colorless glassy solid: R_f = 0.2 (petroleum ether/ ethyl acetate, 1:1.5); $[\alpha]_D$ +4.0 (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 5.28 (d, J = 9.2 Hz, 1H), 5.17 4.99 (m, 3H), 4.77 (t, J = 4.0 Hz, 1H), 4.42 (dd, J = 11.2, 8.8 Hz, 1H), 4.27 (dd, J = 8.8, 2.4 Hz, 1H), 4.10 4.00 (m, 1H), 3.87 (dd, J = 12.4, 4.0 Hz, 1H), 3.61 (s, 3H), 2.10 (s, 3H), 2.08 (s, 6H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.40, 169.66, 169.45, 168.60, 155.84, 144.37, 132.23, 128.40, 118.44, 111.70, 73.04, 69.99, 67.91, 66.53, 66.19, 60.13, 54.15, 52.58, 20.79, 20.77, 20.69, 20.52; MS (ESI) 543 [M+Na]$^+$.

4.2.8. Compound 11b

Compound **10b** (104 mg, 0.2 mmol) was dissolved in Ac_2O/ HOAc (6 mL, v/v, 2:1), $NaNO_2$ (276 mg, 4.0 mmol) was added in four portions at 0 °C. The mixture was warmed to rt and stirred for 0.5 h, then poured into ice, extracted with ethyl acetate for three times, the combined organic layer was washed with satd $NaHCO_3$ solution, dried, filtered and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 1.5:1) to afford **11b** (91 mg, 83%) as a yellow foam: R_f = 0.3 (petroleum ether/ethyl acetate, 1:1); $[\alpha]_D$ 33.1 (c 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 7.57 (m, 2H), 7.48 (d, J = 8.4 Hz, 2H), 6.12 (d, J = 8.0 Hz, 1H), 5.26 (dd, J = 10.0, 2.4 Hz, 1H), 5.11 (t, J = 4.8 Hz, 1H), 4.86 (t, J = 4.8 Hz, 1H), 4.81 (br s, 1H), 4.28 (dd, J = 12.0, 7.2 Hz, 1H), 4.12 3.98 (m, 5H), 2.13 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.20, 169.30, 169.20, 168.74, 154.18, 132.15, 129.73, 120.28, 118.14, 112.45, 77.20, 72.57, 68.02, 66.73, 66.36, 60.91, 55.30, 20.70, 20.70, 20.54, 20.43; HRMS (ESI) Calcd for $C_{24}H_{27}N_3O_{12}Na$ [M+Na]$^+$: 572.1492. Found: 572.1498; IR (MeOH, cm ^{1}): 1443 (ν_{NO}); UV/Vis (MeOH): max 243 nm.

4.2.9. Compound 12b

Compound **12b** was prepared from **8b** following the same procedure as **11b**. The residue was purified by column chroma tography (petroleum ether/ethyl acetate, 1.2:1) to afford **12b** (75%) as a yellow oil: R_f = 0.25 (petroleum ether/ethyl acetate, 1:1); $[\alpha]_D$ +10.1 (c 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 6.21 (d, J = 7.2 Hz, 1H), 5.39 (dd, J = 11.2, 4.8 Hz, 1H), 5.22 (t, J = 6.8 Hz, 1H), 4.96 4.85 (m, 2H), 4.13 4.04 (m, 5H), 3.50 (br s, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ170.20, 170.16, 169.33, 169.17, 153.92, 139.77, 131.99, 129.22, 118.21, 112.12, 77.20, 75.79, 74.20, 72.07, 71.24, 68.30, 62.14, 55.10, 20.55, 20.52, 20.49, 20.37; HRMS (ESI) Calcd for $C_{24}H_{27}N_3O_{12}Na$ [M+Na]$^+$: 572.1492. Found: 572.1502.

4.2.10. Compound 1b

To the solution of **11b** (22 mg, 0.04 mmol) in anhydrous MeOH (1 mL), was added MeONa (100 µL, 2 M solution in MeOH, 0.2 mmol). The mixture was stirred at room temperature for 5 min, and then concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/methanol, 8:1) to afford **1b** (11.5 mg, 95%) as an orange foam: R_f = 0.4 (ethyl acetate/meth anol, 5:1); $[\alpha]_D$ +6.1 (c 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.64 7.62 (m, 2H), 7.31 7.26 (m, 2H), 5.24 (d, J = 6.4 Hz, 1H), 3.98 (dd, J = 10.0, 6.4 Hz, 1H), 3.71 3.66 (m, 2H), 3.62 (dd, J = 9.6, 8.4 Hz, 2H), 3.41 (t, J = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, MeOD) δ 139.22, 133.54, 123.23, 113.84, 107.17, 76.02, 75.66, 74.14, 72.69, 72.08, 63.39, 62.63; HRMS (ESI) Calcd for $C_{14}H_{14}N_3O_5$ [M H] : 304.0939. Found: 304.0951; IR (MeOH, cm ^{1}): 2071 ($\nu_{C=N=N}$); UV/vis (MeOH): max 317 nm.

4.2.11. Compound 14

To a solution of **4b** (65.5 mg, 0.1 mmol) in ethyl acetate (5 mL) was added palladium on carbon (10%, 53 mg, 0.05 mmol). After hydrogenolysis for 6 h at rt, the mixture was filtered and concen trated in vacuo. The residue was purified by column chromatogra phy (ethyl acetate/methanol, 4:1) to afford **14** (25.5 mg, 87%) as a glassy solid: R_f = 0.25 (ethyl acetate/methanol, 5:1); ^{1}H NMR (400 MHz, CD_3OD) 7.70 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.0 Hz, 2H), 5.17 (d, J = 5.2 Hz, 1H), 4.16 (td, J = 6.4, 2.8 Hz, 1H), 4.03 (t, J = 6.8 Hz, 1H), 3.91 (t, J = 4.8 Hz, 1H), 3.77 (dd, J = 12.0, 6.4 Hz, 1H), 3.64 (dd, J = 12.0, 2.4 Hz, 1H), 3.45 (dd, J = 4.8, 2.8 Hz, 2H), 3.41 (t, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.67, 132.95, 130.05, 129.93, 129.31, 119.88, 111.90, 79.63, 77.80, 74.29, 73.91, 72.08, 71.34, 62.33, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36; HRMS (ESI) Calcd for $C_{14}H_{16}NO_6$ [M H]$^-$: 294.0978. Found: 294.0989.

4.2.12. Compound 16

To a solution of **4b** (131 mg, 0.2 mmol) in anhydrous CH_3CN (4 mL) was added DMAP (122 mg, 1.0 mmol) and phenyl chloroth ionocarbonate (PTC Cl, 41 μL, 0.3 mmol). The mixture was stirred at room temperature for 3 h, concentrated in vacuo, purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to af ford a colorless oil (136 mg, 0.17 mmol, 86%) that was dissolved in toluene (3 mL). Tributyltin hydride (0.23 mL, 0.86 mmol) and AIBN (6 mg, 0.04 mmol) were added to the solution. After refluxing for 6 h, the mixture was concentrated, the residue was purified by col umn chromatography (petroleum ether/ethyl acetate, 6:1) to af ford 16 (83.5 mg, 76%) as a white solid: R_f = 0.5 (petroleum ether/ ethyl acetate, 3:1); $[\alpha]_D$ +76.1 (*c* 1.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 7.46 (m, 2H), 7.38 7.24 (m, 20H), 7.19 7.16 (m, 2H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.51 (d, *J* = 10.4 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.19 (dt, *J* = 8.8, 5.6 Hz, 1H), 3.88 3.78 (m, 3H), 3.67 3.58 (m, 3H), 3.06 3.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.72, 138.52, 138.05, 137.96, 137.87, 132.06, 129.85, 128.53, 128.43, 128.33, 128.00, 127.95, 127.84, 127.79, 127.72, 127.69, 119.01, 110.00, 82.09, 80.09, 78.07, 75.44, 75.25, 75.10, 73.62, 73.48, 71.79, 69.12, 31.28; MS (ESI) 674 $[M+Cl]$.

4.2.13. Compound 15

To a solution of **16** (80 mg, 0.125 mmol) in ethyl acetate (5 mL) was added palladium on carbon (10%, 66 mg, 0.06 mmol). After hydrogenolysis for 5 h at rt, the mixture was filtered and concen trated in vacuo to afford **15** (41 mg, 85%) as a glassy solid: R_f = 0.4 (ethyl acetate/methanol, 5:1); ^1H NMR (400 MHz, CD_3OD) 7.63 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 4.15 4.10 (m, 1H), 3.77 3.58 (m, 6H), 3.07 (br s, 1H), 3.06 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 147.38, 133.09, 131.46, 130.15, 129.87, 119.98, 110.74, 78.67, 75.03, 75.00, 72.82, 72.14, 62.83, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 31.82; HRMS (ESI) Calcd for $C_{14}H_{17}ClNO_5$ $[M+Cl]$: 314.0795. Found: 314.0802.

4.2.14. *N*-Nitroso-urethanes 11c–11e and 12c–12e

The other *N* nitroso urethanes, with fluorine, chlorine or meth oxy substituted at the *para* position of the phenyl ring, were pre pared from the corresponding benzylic alcohols **4c** **4e**.

4.2.15. Generating glucosyl phenyldiazomethanes in CD_3OD and recording the UV and IR spectra of the reaction mixture

N Nitroso urethane precursor **11b** or **12b** (8.0 mg, 0.015 mmol), or any other *N* nitroso urethane **11c**, **11d**, **11e**, **12c**, **12d**, **12e**, was dissolved in CD_3OD (0.15 mL) in a dried NMR tube, 15 μL of MeONa (0.03 mmol, 2.0 M solution in CD_3OD) was added to the solution. After shaking the solution for 1 min, the orange color appeared, indicating the generation of the diazo compound. The NMR tube was loaded to NMR spectrometer, and the ^1H NMR was recorded immediately. After the NMR experiment, aliquot (10 μL) was taken to be diluted to 1 mL methanol solution and 10 μL of the solution was added to 0.4 mL cuvette containing 190 μL MeOH (concentra tion = 50 μM). The UV spectra of the solution were scanned in the range of 190 350 nm. Then the diluted methanol solution of the reaction mixture (around 50 μL) was transferred to a cuvette for the IR spectrometer. The IR spectra of the compound were scanned in the range of 900 4000 $cm^{\ 1}$.

4.3. Glycosidase inhibiting studies

4.3.1. Enzyme assays for investigating the inhibiting property of compound 1b towards α-glucosidase from *Saccharomyces cerevisiae*

With different concentration (*c* = 2.5, 5, 10, 15, 20 mM; to give the final assay concentrations of 0.01, 0.02, 0.04, 0.06, 0.08 mM, respectively), 20 μL solution of **1b** in pH 7.5 Tris HCl buffer was added to a 180 μL solution of α glucosidase (110 μg/mL in pH 7.5 Tris HCl buffer; to give the final assay concentration of 4 μg/mL, 0.064 μM). The solution was incubated at 30 °C. After 5, 10, 15, 20, 25, 30 and 40 min, aliquots (20 μL) were taken from the incu bation assay and added to 480 μL PNPG solutions (2 mM in pH 6.8 buffer) and incubated for a further 5 min at 30 °C. Residual en zyme activity was determined via absorption of *p* nitrophenolate (at 400 nm) after quenching the reactions with 0.5 mL Na_2CO_3 solution. Results are expressed as percentage activities relative to the corresponding enzyme controls (with no inhibitor present).

4.3.2. Enzyme assays for comparison of compound 1b with 14 and 15

10 μL solutions of compound **1b**, **14** or **15** (*c* = 2.5, 5, 10, 15, 20 mM in pH 7.5 buffer; to give the final assay concentrations of 0.01, 0.02, 0.04, 0.06, 0.08 mM, respectively) was added to a 90 μL solution of α glucosidase (110 μg/mL in pH 7.5 buffer; to give final assay concentration of 4 μg/mL, 0.064 μM), the solution was incubated at 30 °C. After 40 min, 20 μL was taken from the incubation assay and added to 480 μL PNPG solution (2 mM in pH 6.8 buffer) and incubated for a further 5 min at 30 °C. Residual enzyme activity was determined via absorption of *p* nitropheno late (at 400 nm) after quenching the reactions with 0.5 mL Na_2CO_3 solution. Results are expressed as percentage activities relative to the corresponding enzyme controls.

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